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FACOLTA' DI SCIENZE MATEMATICHE, FISICHE E NATURALI

DOTTORATO DI RICERCA IN SCIENZE CHIMICHE

XIX CICLO

SYNTHESIS AND ELABORATION OF MONO- AND OLIGO-SACCHARIDES

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Table of Contents

Preface

In this thesis several synthetic aspects of carbohydrate chemistry are explored with a particular emphasis on the development and implementation of mild glycosylation procedures.

The use of $Yb(OTf)_3$ and acid washed molecular sieves as alternative feasible promoters for trihaloacetimidate method is proposed. Their effectiveness is confirmed by the assembly of biologically relevant oligosaccharide structures in stepwise as well as in one-pot fashion.

The extreme mildness of the above mentioned promoting systems is demonstrated by the syntheses of novel glycoconjugates with pharmacological activity. 17β-Estradiol and derivatives thereof are regioselectively glycosylated in order to improve their pharmacokinetic and pharmacodinamic properties. Whereas, a peculiar β-lactam structure is variously conjugated to saccharidic structures with the aim of generating a novel class of antibiotics.

Novel procedures are developed for the synthesis of synthetically useful glycosyl iodides and glycosyl phosphates, rendering the access to these classes of molecules more straightforward and experimentally convenient.

Portions of this work have been adapted form the following articles that were co-written by the author:

A. Ravidà, X. Liu, L. Kovacs, P. H. Seeberger "Synthesis of Glycosyl Phosphates from 1,2-Orthoesters and Application to in Situ Glycosylation Reactions" *Organic Letters* **2006**, *9*, 1815-1818.

M. Adinolfi, A. Iadonisi, A. Ravidà "Tunable Activation of Glycosyl Trichloro- and (N-phenyl)Trifluoroacetimidates with Ytterbium(III) Triflate: One-pot Synthesis of Trisaccharides under Catalytic Conditions" *Synlett* **2006**, 583-586.

M. Adinolfi, P. Galletti, D. Giacomini, A. Iadonisi, A. Quintavalla, A. Ravidà "Toward Novel Glycoconjugates: Efficient Synthesis of Glycosylated 4-Alkylidene-β-lactams" *European Journal of Organic Chemistry* **2005**, 69-73.

M. Adinolfi, A. Iadonisi, A. Pezzella, A. Ravidà "Regioselective Phenol or Carbinol Glycosidation of 17β-Estradiol and Derivatives Thereof" *Synlett* **2005**, 1848-1852.

M. Adinolfi, A. Iadonisi, A. Ravidà, M. Schiattarella "Versatile Use of Ytterbium(III) Triflate and Acid Washed Molecular Sieves in the Activation of Glycosyl Trifluoroacetimidate Donors. Assemblage of a Biologically Relevant Tetrasaccharide Sequence of Globo H" *Journal of Organic Chemistry* **2005**, *13*, 5316-5319.

M. Adinolfi, A. Iadonisi, A. Ravidà, M. Schiattarella "Effect of 1,2-Dimethoxyethane in $Yb(OTf)_3$ Promoted Glycosidations" *Tetrahedron Letters* **2004**, *23*, 4485-4488.

M. Adinolfi, A. Iadonisi, A. Ravidà, M. Schiattarella "Moisture Stable Promoters for Selective α-Fucosylation Reactions: Synthesis of Antigen Fragments" *Synlett* **2004**, 275-278.

M. Adinolfi, A. Iadonisi, A. Ravidà, M. Schiattarella "Efficient and Direct Synthesis of Saccaridic 1,2-Ethylidenes, Orthoesters, and Glycals from Peracetylated Sugars *via* the *in Situ* Generation of Glycosyl Iodides with I_2/Et_3SiH" *Tetrahedron Letters* **2003**, *44*, 7863-7866.

List of Abbreviations

)))	Sonication
^{13}CNMR	Carbon nuclear magnetic resonance
^{1}HNMR	Proton nuclear magnetic resonance
Ac	Acetyl
Ala	Alanine
All	Allyl
Alloc	Allyloxycarbonyl
AW 300 MS	Acid washed molecular sieves 4Å
AW 500 MS	Acid washed molecular sieves 5Å
AW MS	Acid washed molecular sieves
Bn	Benzyl
BSA	Bovine serum albumin
BSP	1-Benzenesulfinyl piperidine
Bz	Benzoyl
Bzd	Benzylidene
Cbz	Benzyloxycarbonyl
CIP	Contact ion pair
Cp	Cyclopentenyl
CSA	(±)-Camphor-10-sulfonic acid
CyHex	Cyclohexane
DAST	(Diethylamino)sulphur trifluoride
DBTO	Dibenzothiophene-5-oxide
DBU	Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DIPEA	*N*-Ethyldiisopropylamine
DMAP	4-Dimethylaminopyridine
DMDO	Dimethyldioxyrane
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMT	4,4′-Dimethoxytrityl (=4,4′-Dimethoxytriphenylmethyl)
DMTST	Dimethyl(methylthio)sulfonium triflate
DNA	2′-Deoxyribonucleic acid
DPC	*N*,*N*′-Diisopropylcarbodiimide
DTBP	2,6-Di-*tert*-butylpyridine
Fmoc	9-Fluorenylmethyl-carbonate
Gal	Galactose
GalNAc	*N*-Acetyl galactosamine
Gly	Glycine
GSLs	Glycosphingolipids
HPLC	High performance liquid chromatography
HRMS	High resolution mass spettrometry
IAD	Intramolecular aglycon delivery
IDCP	Iodonium dicollidine perchlorate
IDCT	Iodonium dicollidine triflate
ImH	Imidazole
KLH	Keyhole limphet hemocyanin
Lev	Levulinoyl
Le$^{x/y/a/b}$	Lewis$^{x/y/a/b}$ antigen
mAb	Monoclonal antibody
MALDI	Matrix assisted laser desorption ionization
MBz	4-Methoxybenzoyl
*m*DAP	*meso*-Diaminopimelic acid
MIC	Minimum inhibition concentration
mRNA	Messenger ribonucleic acid
Mur	Muraminic acid
NAG	*N*-Acetyl glucosamine
NAM	*N*-Acetyl muraminic acid
NBS	*N*-bromo succinimide
NDPs	Nucleotide 5′-diphospho sugars
NIS	*N*-iodo succinimide
NISac	*N*-Iodosaccharin
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PE	Phytoalexin elicitor
PEG	Poly(ethylene glycol)
Pent	Pentenyl
PEP	Phosphoenolpyruvate
Ph	Phenyl
PhH	Benzene
Phth	Phtaloyl
Piv	Pivaloyl
*p*MBn	*para*-Methoxybenzyl
ppm	Parts per million
Py	Pyridine
RRVs	Relative reactivity values
RT	Room tempreature
Box	Benzoxazoyl
S_N1	Unimolecular nucleophilic substitution
S_N2	Bimolecular nucleophilic substitution
SSIP	Solvent separed ion pair

TBAB	Tetrabutylammonium bromide
TBAC	Tetrabutylammonium chloride
TBAI	Tetrabutylammonium iodide
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl
TBS	Tributylsilyl
TCA	Trichloroacetyl
TCL	Thin layer chromatography
TES	Triethylsilyl
Tf	Trifluoromethansulfonyl (= Triflyl)
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TMEDA	*N*,*N*,*N*′-Trimethylethylenediamine
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl triflate
TOF	Time of flight
Tr	Triphenylmethyl (= Trityl)
Troc	Trichloroethoxycarbonyl
Ts	Toluensulfonyl (=Tosyl)
TTBP	2,4,6-Tri-*tert*-butylpyridine
UDP	Uridine 5′-diphosphate
WHO	World Health Organisation

Chapter 1

Introduction

General Introduction

Carbohydrates do represent a third class of bio-informative macromolecules, together with nucleic acids and proteins. This concept has strongly emerged only recently, although sugars have already been considered for very long time as one of the most important class of bio-molecules, because of their essential role in metabolism and as important structural components of biological systems.[1]

The idea of carbohydrates, one of the most abundant macromolecules in nature, "just" as energy source or building block for cell walls, is pretty limiting. Furthermore it's very improbable that nature didn't use the huge bio-informative potential of carbohydrates, far greater than that of nucleic acids or proteins. Abundant and diverse glycans cover all the cells of all living organisms. Carbohydrate chains are prominent components at the surfaces of mammalian cells, occurring as N- and O-glycans on glycoproteins, glycosaminoglycan chains on proteoglycans, and oligosaccharides of glycolipids. Carbohydrate chains also occur on many secreted and extra cellular glycoproteins. Some carbohydrate sequences are widely distributed in different cell types, while others, such as the blood group antigens,[2] are genetically determined. Still others have a regulated expression at different stages of embryonic development and cellular differentiation.[3]

A very different portrait of sugars has been emerging due to new rising fields of research gathered under the very general name of glycoscience.

The study of the structure-function relation of carbohydrates and, especially, of oligosaccharide chains of carbohydrate-decorated molecules (also known as glycoconjugates) evidenced that the saccharidic moieties are very often involved in cell communication, signaling and trafficking processes, playing key roles in immune response, viral replication, cell-cell adhesion, fertilisation, parasitic infection, cell growth

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[1] Taylor M. E., Drickamer K. in *Introduction to Glycobiology*, Oxford University Press: New York, **2006**.

[2] Lowe, J. B., in *The molecular basis of blood diseases*, Eds.: Stamatoyannopolous, G., Nienhuis, a. W., Majerus, P. W., Varmus, H., Saunders, Philadelphia, **1987**, chap 8.

[3] Lehmann J. in *Carbohydrates-Structure and Biology-*, Thieme, Stuttgart ·New York: **1998**.

and differentiation, and inflammation.[4] Cancer is another area where carbohydrates turn out to play a big role, helping to transmit the signals that trigger unchecked cell growth.[5] Obviously the involvement of carbohydrates in so many crucial biological processes suggested their use in medicinal chemistry. Several carbohydrate-based drugs have been developed in several different fields.[6]

The multiplicity of signal trasduction processes involving specific oligosaccharide sequences put strongly in evidence the bio-informative nature of carbohydrates. But, in contrast to the two major classes of bio-informative macromolecules, in the case of sugars, scientists haven't cracked the code yet. The pivotal, but still not well understood, role of sugars in so many and different cellular processes has been driving the scientific community to investigate the glycome -all the glycan molecules synthesized by an organism- in the same systematic way than for DNA and proteins. A new field of research has been opened named glycomics, in analogy with genomics and proteomics, with the aim of creating a cell-by-cell catalogue of glycan structures and glycosyltransferases involved in their bio-synthesis.

In 1958 F. Crick referred to the "central dogma" as a universally accepted rule for the direction of genetic information (DNA to mRNA, and mRNA to protein). Almost fifty years ago the central dogma ended with proteins, nowadays we should reconsider it, including the post-translational modifications of the proteins, terminating the cascade of the genetic information with glycans.

Post-translational glycosylation of proteins is a non-template-driven synthesis, a glycan structure does not mimic the sequences of the proteins that produced it, this means that at the sugars point nature gives a new challenge to scientists: a code-break. The task of the glycoscientists is to decipher the "sweet-code".

From this point of view, glycomics is far behind genomics and proteomics, not only because the youngest subject, but also for the great structural complexity carbohydrates exhibit. While DNA and proteins have essentially linear sequences, sugars branch; DNA has just four basic building blocks and proteins have 20, but sugars have more than 30. The number of all linear and branched isomers of an hexasaccharide is calculated to be over $1x10^{12}$. Even a simple disaccharide composed of two glucose units can be represented by 19 different structures.

Because of this great structural diversity between carbohydrates and the others bio-informative molecules glyco-scientists have to face a completely different scenario, "the languages of both nucleic acids and proteins can be compared to the Western phonogram (alphabet); and that of glycans, to the Eastern ideogram (kanji)".[7]

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[4] a) Rudd, P. M.; Elliot, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. S*cience* **2001**, *291*, 2370; b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683; c) Feizi, T. *Curr. Opin. Struct. Biol.* **1993**, *3*, 701; d) Rosen, S. D.; Bertozzi, C. R. *Curr. Biol.* **1996**, *6*, 261; e) Varki, A. *Glycobiology* **1993**, *3*, 97; f) Philips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1130; g) Lasky, L. A. *Science* **1992**, *258*, 964; Miller, D. J.; Macek, M. B.; Schur, B. D. *Nature* **1992**, *357*, 589; h) Feizi, T. *Nature* **1985**, *314*, 53.

[5] a) Treves, A. J.; Carnaud, C.; Tranin, N.; Feldman, M.; Cohen, I. R. *Eur. J. Immunol.* **1974**, *4*, 722; b) Fujimoto, S.; Greene, M. I.; Sehon, A. H. *J. Immunol.* **1976**, *116*, 791; c) Naor, D. *Adv. Cancer. Res.* **1979**, *29*, 45; d) Dye, E. S.; North, R. J. *J. Exp. Med.* **1981**, *154*, 1033; e) Kirchner, H.; Chused, T. M.; Herberman, R. B.; Holden, H. T.; Lavrin, D. H. *J. Exp. Med.* **1974**, *139*, 1473.

[6] *Carbohydrate-based Drug Discovery*, Wong C. –H., Ed., Wiley-VCH: Weinheim, **2003**.

[7] Hirabayashi, J.; Kasai, K.-I. *Trends in Glycoscience and Glycotechnology*, **2000**, 63, 1.

Clearly, for unravelling the mysteries of sugars, elucidating their biological functions, pure oligosaccharides of defined structure are required in sufficient amounts for biophysical and biochemical studies. But these biomolecules are often found in nature in low concentrations and in microheterogeneous forms. Most glycoproteins are not uniform, but consist of various "glycoforms" differing in type, length, branching and terminal decoration of oligosaccharides, and number or site of glycosylation. The isolation of oligosaccharides in a pure form from natural sources is therefore extremely challenging, when at all possible.

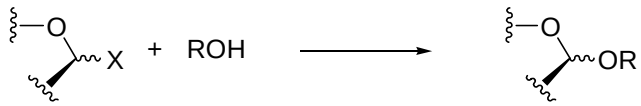
Access to pure carbohydrates for biological studies relies on chemical or enzymatic synthesis. No amplification methods analogous to the polymerase chain reaction (PCR) for DNA are available for carbohydrates, nor a fully developed automated synthesis technology as in the case of oligopeptides and oligonucleotides. Recently automated[8] and computer-assisted[9] synthetic approaches have been developed to boost the raising field of glycomics. To date these technologies are not yet diffused and in the glycolabs the synthesis of target molecules is still mainly practised by chemical synthesis.

Enzymatic or chemoenzymatic approaches are certainly promising, but of the vast array of glycosyltranferases involved in biosynthetic pathways of carbohydrates, only a small fraction has been overexpressed and few of these are commercially available.[10] Additionally, glycosyltranferases have narrow substrate specificities, being able to catalyse the formation of single types of glycosidic linkages. The extreme specificity exhibited by enzymes is the dream of every synthetic chemist, guaranteeing the desired glycosylation outcomes in terms of regio- and stereo-selectivity, without any previous chemical modification of the substrates. But, since every type of linkage requires a specific enzyme, the synthesis of target oligosaccharides *via* enzymatic catalysis is still expensive, every lab needing a wide library of glycosyltransferases.

In conclusion, although lots of promising approaches for the synthesis of oligosaccharide have been emerging as the future, unfortunately we are still in the present and the chemical approach is still more reliable and cheap.

Glycosylation Reaction

The key step in oligosaccharide synthesis is surely the glycosylation reaction. The majority of glycosylations are performed by nucleophilic substitution reaction at the anomeric carbon (*Scheme 1.1*).



SCHEME 1.1 Glycoside Synthesis by Nucleophilic Substitution at the Anomeric Carbon.

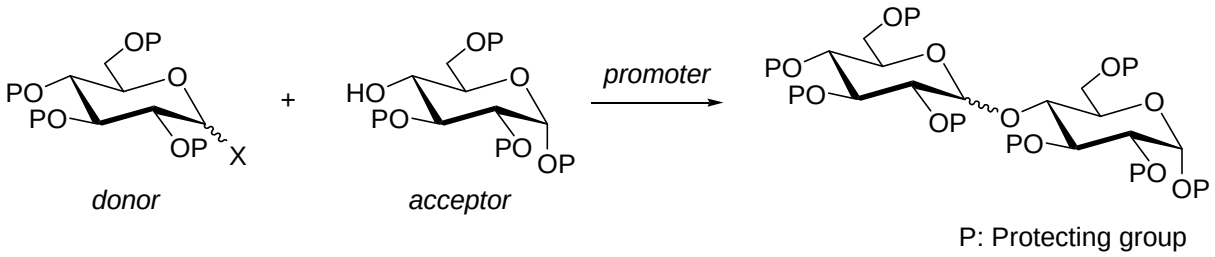
The glycosylating agent, in the vocabulary of carbohydrate chemists, is called *glycosyl donor*. Generally it carries a leaving group at the anomeric position, that can be activated by an appropriate promoter. Several different glycosylation methods exist whose classification relies on the nature of the leaving groups. Every class of leaving groups

[8] Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523.

[9] Zhang, Z.; Ollmann, I. R.; Ye, X. –S.; Wishnat, R.; Baasov, T.; Wong, C. –H. *J. Am. Chem. Soc.* **1999**, *121*, 734.

[10] *Carbohydrates in Chemistry and Biology*, Part II Vol.2, Ernst B., Hart G. W., Sinaÿ P., Eds, Wiley-VCH: Weinheim, **2000**.

requires specific promoting systems. Since a universal glycosylation method - generally applicable - hasn't been developed yet, several different methods are used in the glycolabs, and the choice depends on the specific reaction to be performed and on the planned synthetic strategy.
The nucleophile species is generally called *glycosyl acceptor* (*Scheme 1.2*).



SCHEME 1.2 Glycosylation Reaction.

An ideal glycosylation reaction should be fast, high yielding, experimentally simple and completely regio- and stereo-selective.
The regioselectivity of glycosidation is generally controlled by the use of protection strategies. Both glycosyl donor and acceptor have several nuclephilic functionalities (mostly hydroxyl and amino groups) that are able to compete each other in the reaction with the glycosylating agent. The regioselective protection of all the potentially reactive moieties, but the one desired, allows the regiochemical outcome to be perfectly controlled.
Stereoselectivity is one of the major challenge in glycosylation reactions. The anomeric carbon on the glycosyl donor is a chiral centre, thus the nucleophilic attack on it generates two diasteroisomers. If the newly formed glycosidic bond is axially oriented (on the saccharidic backbone of the former glycosyl donor), the resulting sugar is called α anomer; β anomer is formed when equatorial orientation is obtained (*Figure 1.1*).

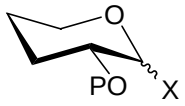
FIGURE 1.1 Anomeric Configurations of D-Glucopyranose.

Several approaches are used to achieve the desired stereoselectivity. The most effective method involves the use of neighboring group participation. Installation of an acyl protecting group at the donor O-2 generally results in excellent stereoselectivities for 1,2-*trans*-glycoside syntheses. When formation of 1,2-*cis*-glycosides is required, different strategies have to be used. Very common is the use of solvent effect: coordinating solvents such as ethers or nitriles influence the stereochemical outcome of the reaction via the formation of intermediate carbocation-solvent adducts.
Further aspects have to be considered when approaching to the synthesis of structures longer than a disaccharide. The synthesis of oligosaccharides requires a careful planning of the protection strategy and differentiation between permanent and temporary protecting groups is crucial. Thus, the building blocks synthesis becomes more complicate and time consuming. Also the synthetic strategy is a matter of choice, often driven by the

intrinsic nature of the target molecules. The oligosaccharide chain can be elongated following stepwise or convergent approaches, or more straightforwardly in one-pot fashion.
Insights in all these aspects of the glycosylation reactions are the object of next paragraphs and represent the topic of several excellent work.[11]

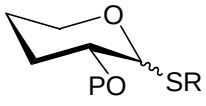
Glycosylation Methods

As anticipated in the previous paragraph, glycosylations are mostly carried out by nucleophilic substitution at the anomeric carbon of the glycosyl donor. Other approaches are used such as the nucleophilic substitution at the aglycone carbon or addition reactions to 1,2-unsaturated sugars.
Most of the methods belong to the first class, they are classified on the nature of the leaving group on the glycosyl donor and can be grouped according to the heteroatom attached to the anomeric center (*Figure 1.2*).



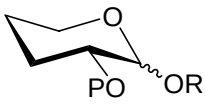
Glycosyl halides

Bromides and Chlorides
Fluorides
Iodides



Thio derivatives

Thioglycosides
Sulfoxides, Sulfones
Others



O-derivatives

Imidates
1,2-Anhydro derivatives
Phosphates, Phosphites
Others

FIGURE 1.2 Types of Glycosyl Donors.

The departure of the leaving group is generally activated by the use of specific promoters. The choice of the glycosylation method implies the use of its specific promoter, this means that also the synthetic strategy has to be planned considering the compatibility of all protecting groups in the glycosylation conditions.
Glycosyl halogenides (especially bromides and chlorides) were the first type of glycosyl donors used for the synthesis of complex oligosaccharides.
Glycosylations with glycosyl **bromides and chlorides** in the presence of stoichiometric amounts of silver salts (Ag_2O, Ag_2CO_3), known as the Koenigs-Knorr reaction,[12] is one of the oldest glycosylation methods. Over the years a wide variety of promoters to activate glycosyl halides in glycosylation reactions have been developed. The heavy metal salts frequently used as promoters include silver salts of hydroxyl carboxylic acids, such as silver salicilate,[12b] silver imidazolate,[13] $Hg(CN)_2$ and $HgBr_2$,[14] HgO and $HgBr_2$,[15] HgI_2[16] and

[11] a) *Carbohydrates in Chemistry and Biology*, Part II Vol.2, Ernst B., Hart G. W., Sinaÿ P., Eds, Wiley-VCH: Weinheim, **2000**; b) *The Organic Chemistry of Sugars*, Levy D. E., Fügedi, P., Eds., Taylor & Francis: Boca Raton, **2006**.
[12] a) Koenigs, W.; Knorr, E. *Ber. Dtsch. Chem. Ges*. **1901**, *34*, 957; b) Wulff, G.; Röhle, G. *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157; c) Igarashi, K. *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 243.
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[14] a) Helferich, B.; Wedemeyer, K. -F. *Liebigs Ann. Chem.* **1949**, *563*, 139; b) Helferich, B.; Jung, K. -H. *Liebigs Ann. Chem.* **1954**, *589*, 77; c) Helferich, B.; Berger, A. *Chem. Ber.* **1957**, *90*, 2492.

$CdCO_3$.[17] $AgClO_4$[18] and AgOTf[19] are the most efficient among the heavy metal promoters, and the latter is the most frequently used. Heterogeneous catalysts such as silver silicate,[20] silver zeolite[21] and silver silica-alumina[22] were developed particularly for the synthesis of otherwise hard to synthesize β-mannosidic and β-rhamnosidic linkages.
Besides heavy metal salts, Lewis acids such as $SnCl_4$, $BF_3 \cdot Et_2O$, [23] or $ZnCl_2$[24] have also been introduced as promoters.
The use of acid scavengers and drying agents in the reaction mixture in combination with the promoters is advantageous, and often necessary. Metal carbonates and oxides (Ag_2O, Ag_2CO_3, HgO, $CdCO_3$) not only serves as promoters, but they also neutralize the acid released in the reaction.
It should be noted that glycosylation reactions of glycosyl halides with participating groups at O-2 in the presence of excess base lead to 1,2-orthoesters instead of glycosides. Therefore the amount of base in the reaction mixture should be limited.
A different type of activation was introduced by Lemieux: converting α-glycosyl bromides into glycosides *via* their highly reactive β-anomers.[25] In these cases, promoters such as Bu_4NBr or Et_4NBr in combination with DIPEA (Hünig's base) are used. These halide ion-catalyzed glycosylations give 1,2-*cis*-glycosides in excellent stereoselectivity. Unfortunately, the scope of the method is limited to reactive glycosyl halides and acceptors.
Glycosyl halides, for a long time pratically the only type of glycosyl donors, have now lost their predominant role in glycosylations. This is due to attempts to avoid the use of stoichiometric amounts of toxic, expensive and often light- and moisture-sensitive heavy metal salts for their activation. Additionally, glycosyl halides are moderately stable. In the synthesis of glycosyl halogenides with non trivial protecting group patterns, the introduction of the halide is usually the last step of the synthetic pathway. The chemical elaboration of the non-anomeric regions can be hardly performed without damaging the highly reactive halide at the anomeric position. Furthermore, purification of the halides from these reactions is often problematic.
Installation of the halide at the anomeric centers is generally performed by treatment of 1-*O*-Ac-sugars with the desired hydrogen halide in acetic acid.
Of course, this procedure is not compatible with acid-labile protecting groups, several different approaches have been developed, but for the synthesis of peracetylated glycosyl halogenides this represents still one of the easiest and most reliable procedures.
Glycosyl fluorides are more stable than glycosyl bromides and chlorides, but this has an effect also on their reactivity. Infact they are too unreactive to be activated under standard

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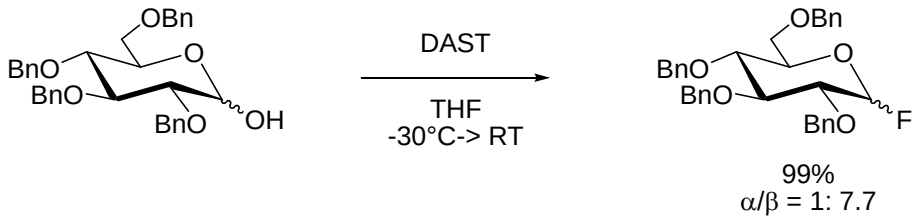
[17] Conrow, R. B.; Bernstein, S. *J. Org. Chem.* **1971**, *36*, 863.
[18] a) Bredereck, H.; Wagner, A.; Faber, G.; Ott, H. *Chem. Ber.* **1959**, *92*, 1135; b) Bredereck, H.; Wagner, A.; Kuhn, H.; Ott, H. *Chem. Ber.* **1960**, *93*, 1201; c) Bredereck, H.; Wagner, A.; Geissel, D.; Gross, P.; Hutten, U.; Ott, H. *Chem. Ber.* **1962**, *95*, 3056; d) Bredereck, H.; Wagner, A.; Geissel, D.; Ott, H. *Chem. Ber.* **1962**, *95*, 3064.
[19] a) Lemieux, R. U.; Takeda, T.; Chung, B. Y. *ACS. Symp. Ser.* **1976**, *39*, 90; b) Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, C13.
[20] Paulsen, H.; Lockhoff, O. *Chem. Ber.* **1981**, *114*, 3102.
[21] Garegg, P. J.; Ossowski, P. *Acta Chem. Scand. B* **1983**, *37*, 249.
[22] van Boeckel, C. A. A.; Beetz, T. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 596.
[23] Ogawa, T.; Matsui, M. *Carbohydr. Res.* **1976**, *51*, C13.
[24] Higashi, K.; Nakayama, K.; Soga, T.; Shioya, E.; Uoto, K.; Kusama, T. *Chem. Pharm. Bull.* **1990**, *38*, 3280.
[25] Leimieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056.

Koenigs-Knorr conditions, and, for this reason, their use as glycosyl donors was explored relatively late. Their use was introduced in 1981 by Mukaiyama and coworkers,[26] and glycosyl fluorides are currently among the most frequently used glycosyl donors.
Several methods for the preparation of glycosyl fluorides are known.[27] They are commonly prepared by the reaction of a protected sugar with a free anomeric hydroxyl group with diethylaminosulfur trifluoride (DAST) (*Scheme 1.3*).[28]

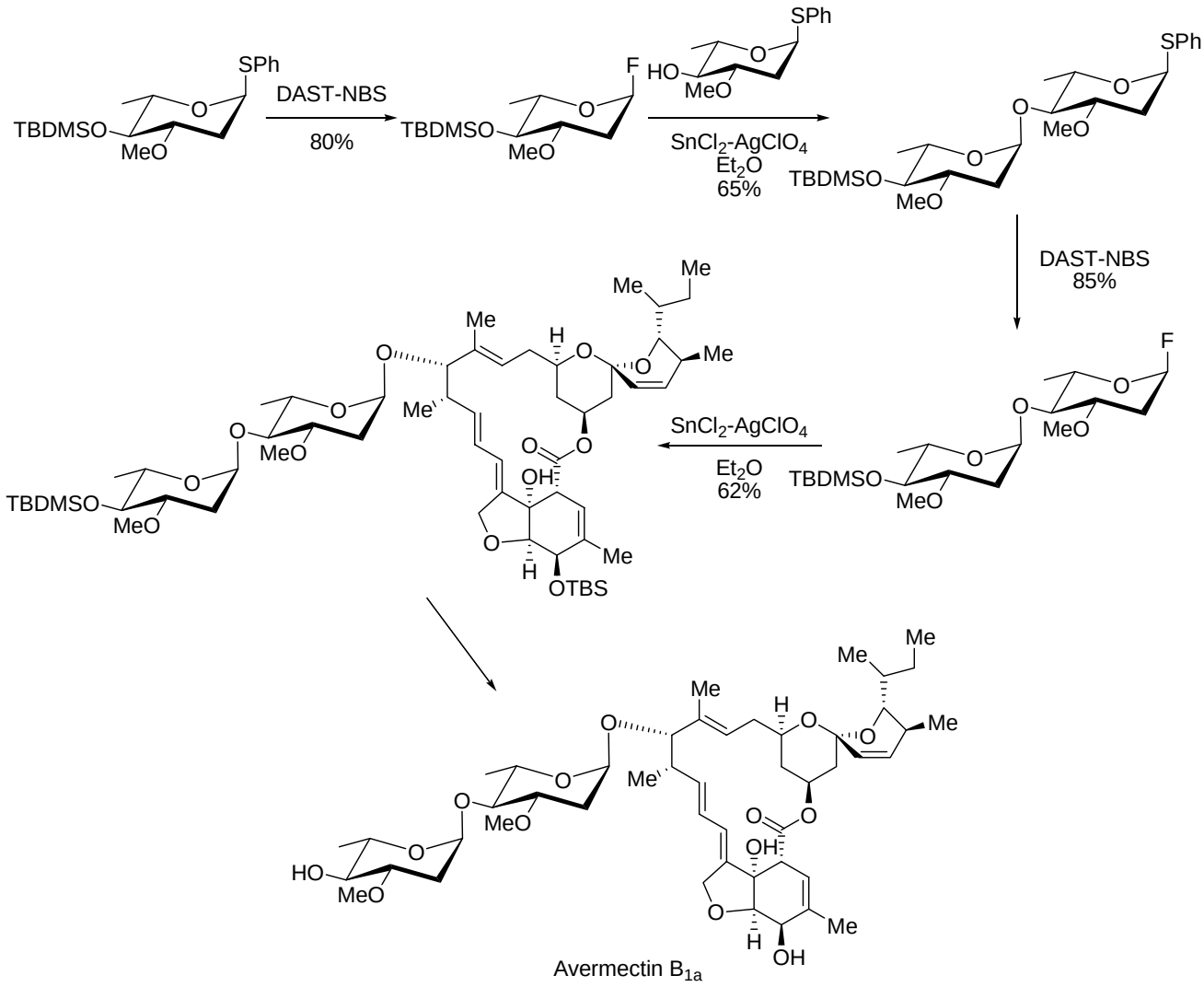


SCHEME 1.3 Synthesis of Glycosyl Fluorides.

Most importantly, glycosyl fluorides can be activated under specific conditions that most protecting groups survive. The first useful activation of glycosyl fluorides for glycosylation was introduced by Mukaiyama[27] using a combination of $SnCl_2$ with $AgClO_4$ (often replaced by AgOTf).[29] Noyori and coworkers introduced the use of SiF_4 and TMSOTf,[30] whereas $BF_3 \cdot Et_2O$ was introduced independently by Nicolau and Kunz.[31] Additionally, Wessel et al. introduced Tf_2O as a powerful glycosylaton promoter for glycosyl fluoride donors.[32] The sequence TMSOTf < $SnCl_2$-AgOTf <Tf_2O was suggested for the relative reactivity of different promoters, all used in stoichiometric amounts. Catalytic amounts of the protic acid TfOH were recently used by Mukaiyama and coworkers.[33]
Glycosyl fluorides have found use in the synthesis of a wide range of complex natural products. In *Scheme 1.4* is reported the Nicolaou's synthesis of avermectin B_{1a}, where glycosyl fluorides are prepared from thioglycosides by reaction with NBS and DAST.[34]
Although emphasis is placed on the donor properties of glycosyl fluorides, it should be mentioned that, in contrast to glycosyl bromides, partially unprotected glycosyl fluorides could also serve as glycosyl acceptors in oligosaccharides synthesis, acting as latent

[26] Mukaiyama, T.; Murai, Y.; Shoda, S. -Y. *Chem. Lett.* **1981**, 431.
[27] a) Shimizu, M.; Togo, H.; Yokoyama, M. *Synthesis* **1998**, 799; b) Toshima, K. In *Glycoscience – Chemistry and Chemical Biology*, Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds., Springer Verlag, Berlin, Heidelberg, 2001, pp. 584-625.
[28] Posner, G. H.; Haines, S. R. *Tetrahedron Lett.* **1985**, *26*, 5.
[29] a) Ogawa, T.; Takahashi, Y. *Carbohydr. Res.* **1985**, *138*, C5; b) Takahashi, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *164*, 277.
[30] Hashimoto, S.; Hayashi, M.; Noyori, R. *Tetrahedron Lett.* **1984**, *25*, 1379.
[31] a) Nicolaou, K. C.; Chucholowski, A.; Dolle, R. E.; Randall, J. L. *J. Chem. Soc. Chem. Commun.* **1984**, 1155; b) Kunz, H.; Sager, W. *Helv. Chim. Acta* **1985**, *68*, 283; c) Kunz, H.; Waldmann, H. *J. Chem. Soc. Chem. Commun.* **1985**, 638.
[32] a) Dobarro-Rodriguez, A.; Trumtel, M.; Wessel, H. P. *J. Carbohydr. Chem.* **1992**, *11*, 255; b) Wessel, H. P. *Tetrahedron Lett.* **1990**, *31*, 6863; c) Wessel, H. P.; Ruiz, N. *J. Carbohydr. Chem.* **1991**, *10*, 901.
[33] a) Jona, H.; Mandai, H.; Chavasiri, W.; Takeuchi, K.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 291; b) Mukaiyama, T.; Jona, H.; Takeuki, K. *Chem. Lett.* **2000**, 696; c) Jona, H.; Takeuki, K.; Mukaiyama, T. *Chem. Lett.* **2000**, 1278.
[34] Nicolaou, K.C.; Dolle, R.E.; Papahatjis, D. P. *J. Am. Chem. Soc.* **1984**, *106*, 4189.

glycosylating agents. They can be glycosylated by a variety of methods including thioglycoside and trichloroacetimidate donors.[35]



SCHEME 1.4 Synthesis of Avermectin B_{1a}.

Glycosyl iodides have been known for a long time.[36] However, because of their instability, they have been used only occasionally as glycosyl donors. Since the introduction of the halide ion-catalysed glycosylation method,[25] a number of glycosylations actually proceeded through *in situ* generated glycosyl iodides intermediates by activating glycosyl bromides with tetraalkylammonium iodides. Recently improved methods for the preparation of glycosyl iodides have been developed, and several glycosyl iodides were prepared and isolated. Although benzylated glycosyl iodides are very unstable and should be used directly, acyl protected derivatives are considerably less labile, and stable crystalline glycosyl iodides can be prepared. [37]

[35] Barena, M. I.; Echarri, R.; Castillon, S. *Synlett* **1996**, 675.
[36] Fischer, E.; Fischer, H. *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 2521.
[37] Glycosyl iodides are object of *Chapter 2*, for more insights in their preparation and use in glycosylation reactions see pp 51-63.

Thioglycosides are the most used type of donors in oligosaccharide synthesis. The first thioglycoside was synthesized in 1909,[38] but it is only recently that their excellent glycosyl donor qualities have been recognized. The strength of thioglycosides as glycosylating agents is their easy synthesis combined with their stability, and, at the same time, their effective activation using chemoselective thiophilic promoters.
Thioglycosides show remarkable stability: not only they do have long shelf lives, but they also tolerate very diverse chemical manipulations leaving the thioglycoside function intact. Importantly, most of the common carbohydrate protecting group manipulations can be performed on thioglycosides, a feature making the preparation of highly functionalized thioglycoside donors possible. Moreover, they are inert under several glycosylation conditions, so thioglycosides can serve as glycosyl acceptor in the assembly of oligosaccharide blocks. In addition, thioglycosides can also directly be converted into other types of donors, making them a very versatile class of compounds (*Figure 1.3)*.

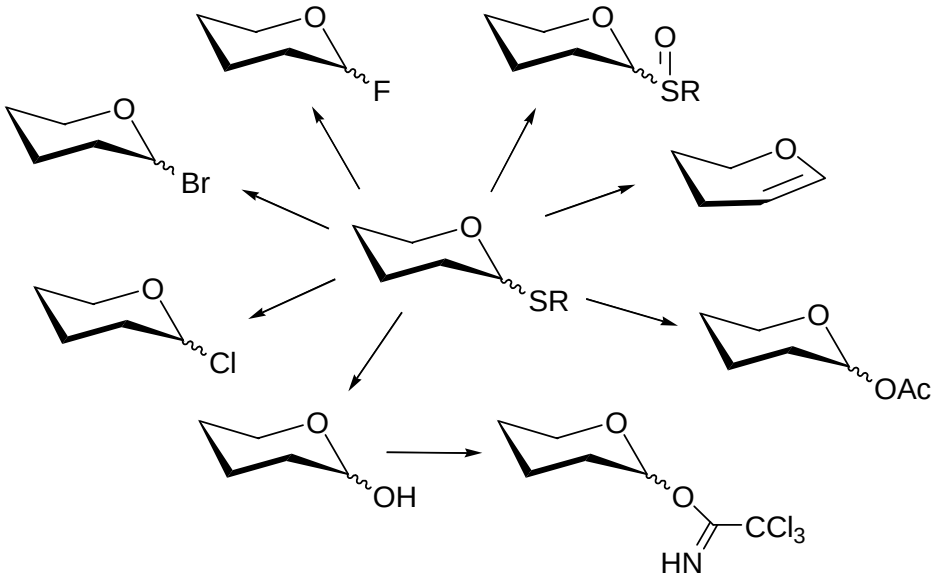
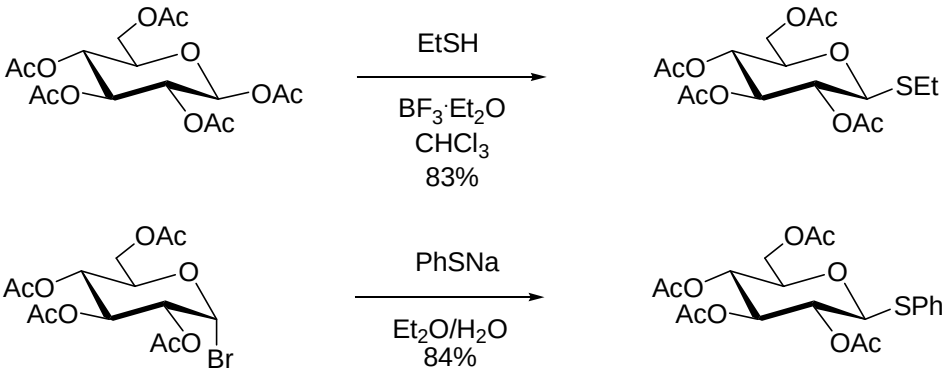


FIGURE 1.3 Conversion of Thioglycosides into Other Types of Donors.

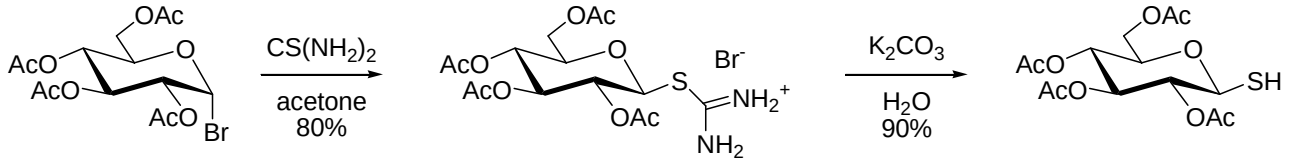
The synthesis of the most used types of thioglycoside donors, mainly simple alkyl and aryl glycosides, is straightforward and can be performed on a large scale using cheap chemicals. Often the product is crystalline and can be purified without chromatography. Numerous ways to prepare thioglycosides have been described,[39] but for the easy production of thioglycosides mainly two synthetic methods are commonly employed, namely the raction of peracetylated glycosides with a thiol in the presence of a Lewis acid[40] and the reaction of an acylated glycosyl halide with a thiolate (*Scheme 1.5)*.[38]

[38] Fisher, E.; Delbrück, K. *Ber*. **1909**, *42*, 1476.
[39] a) Horton, D.; Hutson, D. H. *Adv. Carbohydr. Chem.* **1963**, *18*, 123; b) Norberg, T. in *Modern methods in Carbohydrate Synthesis*, Khan, S. H., O'Neill, R. A., Eds., Harwood Academic Publishers, **1995**, pp 82-106; c) Garegg, P. J. *Adv. Carbohydr. Chem. Biochem*. **1997**, *52*, 179.
[40] a) Ferrier, R. J.; Furneaux, R. H. *Carbohydr. Res.* **1976**, *52*, 63; b) Dasgupta, F.; Garegg, P. J. *Acta Chem. Scand.* **1989**, *43*, 471.



SCHEME 1.5 Synthesis of Thioglycosides.

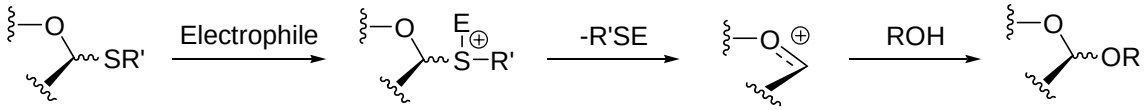
Indirect formation of thioglycosides can also be performed from glycosyl halides. Reaction with thiourea yields a pseudothiouronium salt, which can be hydrolyzed under mild, selective conditions to give the anomeric thiol, which then can be alkylated in an efficient, non-smelling procedure to produce thioglycosides (*Scheme 1.6*).[41]



SCHEME 1.6 Alternative non-Smelling Procedure for Thioglycosides Generation.

All common protecting groups such as esters, ethers, acetals and orthoesters can effectively be introduced, manipulated and removed. Problems encountered are the removal of benzyl groups using catalytic hydrogenolysis, since the sulfur generally contaminates the catalyst.

Despite their stability, thioglycosides can be easily activated with thiophilic reagents, typically soft electrophilic reagents, under mild conditions (*Scheme 1.7*). Treatment of a thioglycoside with a thiophilic electrophile leads to the formation of a sulfonium ion species, that, with the assistance of the ring oxygen or a neighbouring group, evolves to the common intermediate of glycosylation reactions, the oxycarbenium ion depicted in the scheme, which then reacts with the nucleophile to afford the desired glycoside.



SCHEME 1.7 Activation of Thioglycosides for Glycosylations by Electrophilic Reagents.

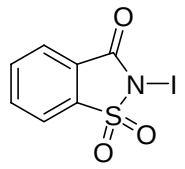
The first efforts to use thioglycosides directly as donors were performed mainly with mercury (II) salts as promoters. It was early recognised that mercury had high affinity for the sulfur functionality. In the first successful attempt $HgSO_4$ was employed.[42] Other

[41] Horton, D. *Methods Carbohydr. Chem.* **1963**, *2*, 433.
[42] Ferrier, R. J.; Hay, R. W.; Vethaviyasar, N. A. *Carbohydr. Res.* **1973**, *27*, 55.

mercury salts, such as $Hg(OAc)_2$, $HgCl_2$, $Hg(OBz)_2$, and PhHgOTf, were later tried.[43] These activations gave moderate yields, and were not powerful enough to be of general use. Starting from the mid-1980s, however, a series of highly powerful activation methods of thioglicosides was developed. Starting with NBS,[44] and especially with MeOTf[45] and dimethyl(methylthio)sulfonium triflate (DMTST)[46] a seemingly never ending spectrum of new promoters has been reported, but also electrochemical and radical activation have been described.[47]

Iodine-containig compounds are also used as promoters. Iodonium dicollidine perchlorate (IDCP) was introduced first as iodonium source,[48] later on the corresponding triflate (IDCT) started to be effectively used.[49] A very efficient promoter system, *N*-iodosuccinimide in the presence of a catalytic amount of triflic acid, was introduced independently by van Boom[50] and Fraser-Reid.[51] Glycosylations promoted by NIS-TfOH are frequently used as they proceed at low temperatures within a short time, and are capable of activating a wide variety of glycosyl donors with various acceptors. Triflic acid is frequently replaced by different Lewis acids such as TMSOTf, TESOTf, AgOTf or $BF_3 \cdot Et_2O$.

N-iodosaccharin (NISac) (*Figure 1.4*) was recently proposed as a replacement of NIS to reduce occasional by-products caused by the nucleophilicity of succinimide generally obtained as by-product in NIS promoted reactions.[52]



NISac

FIGURE 1.4 *N*-iodosaccharin.

[43] a)Tsai, T. Y. R.; Jin, H.; Wiesner, K. A. *Can. J. Chem.* **1984**, *62*, 1403; b) van Cleve, J. W. *Carbohydr. Res.* **1979**, *70*, 161; c) Garegg, P. J.; Henrichson, C.; Norberg, T. *Carbohydr. Res.* **1983**, *116*, 162.

[44] a) Hanessian, S.; Bacquet, C.; Lehong, N. *Carbohydr. Res.* **1980**, *80*, C17; b) Nicolaou, K. C.; Seitz, S. P.; Papahatjis, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 2430.

[45] a) Lönn, H. *Carbohydr. Res.* **1985**, *139*, 105; b) Lönn, H. *Carbohydr. Res.* **1985**, *139*, 115; c) Lönn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301.

[46] a) Andersson, F.; Fügedi, P.; Garegg, P. J.; Nashed, M. *Tetrahedron Lett.* **1986**, *27*, 3919; b) Fügedi, P. in *e-EROS, Electronic Encyclopedia of Reagents for Organic Synthesis*, Paquette, L. A., Ed., Wiley Interscience, New York, **2002**, http://www.mrw.interscience.wiley.com/eros/eros_articles_fs.html.

[47] a) Griffin, G. W.; Bandara, N. C.; Clarke, M. A.; Tsang, W. –S.; Garegg, P. J.; Oscarson, S.; Silwanis, B. A. *Heterocycles* **1990**, *30*, 939; b) amatore, C.; Jutand, A.; Mallet, J. –M.; Meyer, G.; Sinaÿ, P. *J. Chem. Soc. Chem. Commun.* **1990**, 718; c) Balavoine, G. Greg, A.; Fischer, J. –C.; Lubineau, A. *Tetrahedron Lett.* **1990**, *31*, 5761; d) Marra, A.; Amatore, J. –M.; Amatore, C.; Sinaÿ, P. *Synlett* **1990**, 572.

[48] a) Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275; b) Zuurmond, H. M.; van der Laan, S. C.; van der Marel, G. A.; van Boom, J. H. *Carbohydr. Res.* **1991**, *215*, C1; c) Smid, P.; de Ruiter, G. A.; van der Marel, G. A.; Rombouts, F. M.; van Boom, J. H. *J. Carbohydr. Chem.* **1991**, *10*, 833.

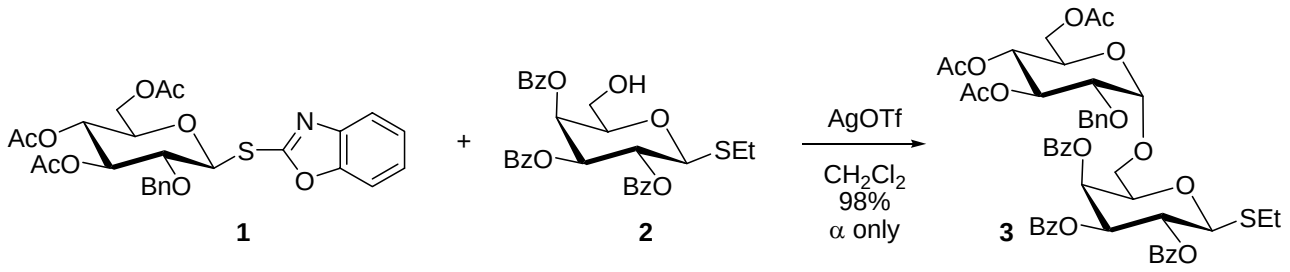
[49] Veeneman, G. H.; van Leeuwen, S. H.; Zuurmond, H.; van Boom, J. H. *J. Carbohydr. Chem.* **1990**, *9*, 783.

[50] Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.*. **1990**, *31*, 1331.

[51] a) Konradsson, P.; Mootoo, D. R.; McDevitt, R. E.; Fraser-Reid, B. *J. Chem. Soc. Chem. Commun.* **1990**, 270; b) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.

[52] Aloui, M.; Fairbanks, A. J. *Synlett* **2001**, 797.

Numerous highly complex structures have been synthesized using the here cited promoting systems, verifying the impressive utility of the method.
These promoters also complement each other nicely regarding reactivity, from the least reactive IDCP and MeOTf through the intermediate DMTST up to the most reactive NIS/TfOH. This promoter reactivity difference, in combination with the different reactivity of donors and acceptors, allows many practical orthogonal couplings between thioglycosides. Furthermore, the thioglycosides reactivity can also be tuned by the choice of the aglycone.[53] Bulky or electron withdrawing groups on the sulphur decrease the reactivity of thioglycoside donors. Furthermore, several heterocyclic aglycones, such as benzothiazol-2-yl,[54] pyridine-2-yl,[44a] pyrimidin-2-yl[44a] and, most recently, benzoxazol-2-yl[55] derivatives, have been installed on the sulfur atom.
These heterocyclic thioglycosides can be activated by the remote activation concept by reagents, which do not necessarily activate common alkyl- and aryl-thioglycosides. Thus, pyrimidinyl thioglycosides, for example, were activated by TMSOTf, which does not activate alkyl-/aryl-thioglycosides, to give glycosides in good yields.[56] AgOTf or $ZrCl_4$-Ag_2CO_3 proved to be effective promoters for the recently introduced benzoxazolyl thioglycosides (SBox glycosides).[55b]
As shown in *Scheme 1.8*, the thioglycoside acceptor **2** could be glycosylated by SBox glycoside **1** in excellent yield as the promoter does not activate the ethylthio-function of the acceptor **2**.[55b]

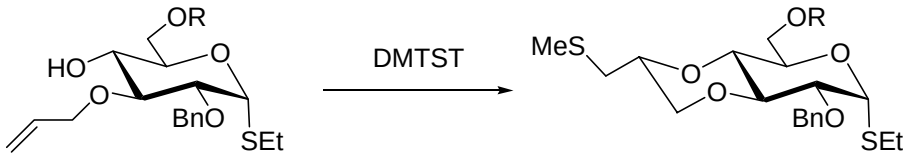


SCHEME 1.8 Glycosylation with SBox Thioglycoside.

Notwithstanding the efficiency of thioglycosides as donors, problems can, of course, be encountered in various applications. The problems are connected both to the type of thioglycoside used as well as to the promoter employed. Regarding the promoter, MeOTf can, if the acceptor is unreactive, give methylation instead of glycosylation of the acceptor hydroxyl group.[57] As previously reported, *N*-succinimide glycosides can be formed as major product in NIS-promoted glycosylations with unreactive acceptors.[58] Soft nucleophilic centers, such as double bonds, could compete with the sulfur in its reaction with the electrophilic promoter, but allyl and pentenyl groups proved to be compatible

[53] The effect of protecting groups on the reactivity of both glycosyl donors and acceptors is discussed in the following paragraphs.
[54] a) Mukaiyama, T.; Nakatsuka, T.; Shoda, S. *Chem. Lett.* **1979**, 487; b) Gama, Y.; Yasumoto, A. *Chem. Lett.* **1993**, 319.
[55] a) Demchenko, A. V.; Kamat, M. N.; De Meo, C. *Synlett* **2003**, 1287; b) Demchenko, A. V.; Malysheva, N. N.; De Meo, C. *Org. Lett.* **2003**, *5*, 455.
[56] Chen, Q.; Kong, F. *Carbohydr. Res.* **1995**, *272*, 149.
[57] Garegg, P. J.; Oscarson, S.; Szönyi, M. *Carbohydr. Res.* **1990**, *205*, 125.
[58] a) Oscarson, S.; Tedebarck, D.; Turek, D. *Carbohydr. Res.* **1997**, *159*, 159; b) Wang, L. –X.; Sakiri, N.; Kezukura, H. *J. Chem. Soc. Perkin I* **1990**, 1677; c) Krog-Jensen, C.; Oscarson, S. *J. Org. Chem.* **1996**, *61*, 1234.

with thioglycoside method performing glycosidation under controlled conditions.[59] Problems have been encountered when the acceptor hydroxyl group is adjacent to an allyl protecting group, which have resulted in internal addition to an activated double bond being the major reaction (*Scheme 1.9*).[48a]

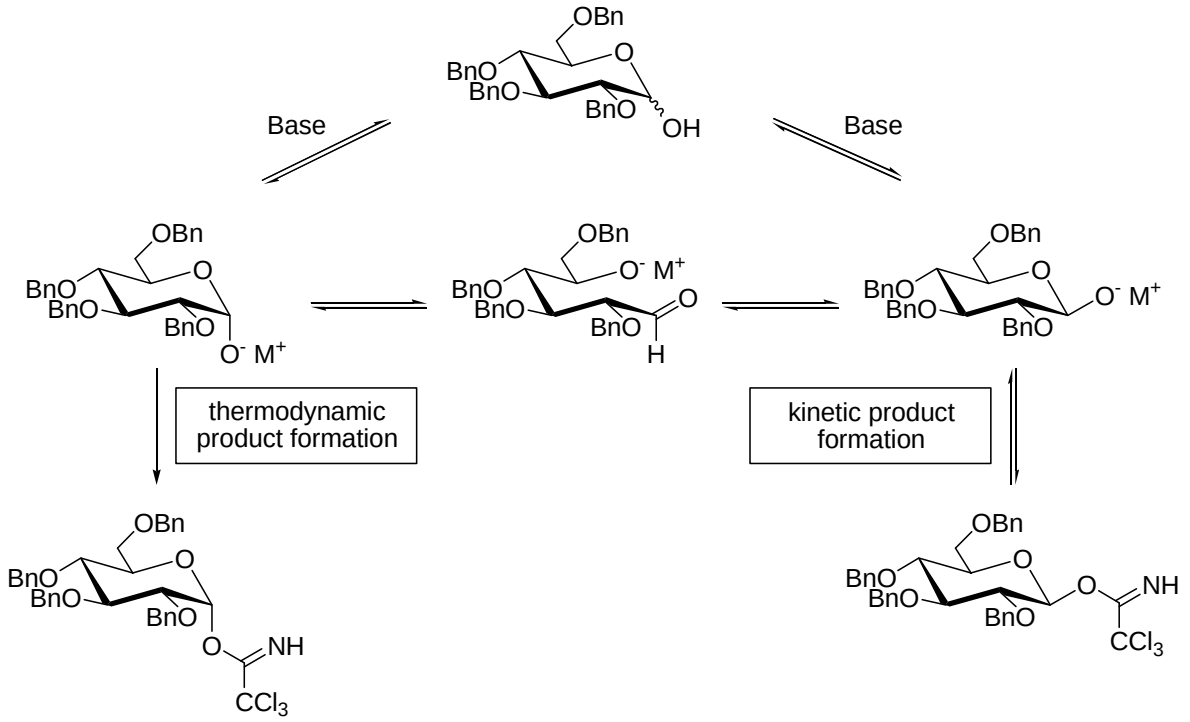


SCHEME 1.9 Side-product of Thioglycosides Activation.

The use of thioglycosides in glycosylations has been reviewed.[60]

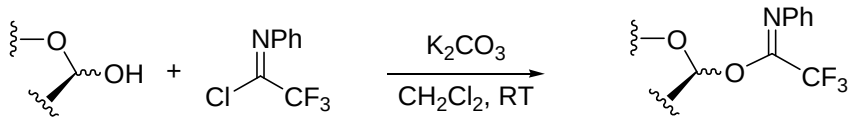
Thioglycoside method is surely the most exploited in oligosaccharide syntheses, another very versatile and widely used approach is represented by **glycosyl trichloroacetimidate** method. Although the first paper on this method was published as recently as 1980,[61] it has already become a classical approach. This is essentially because only catalytic amounts of promoter are required to provide very high glycosyl donor properties whereas the here so far described methods generally require at least equimolar amounts of promoting system, which is obviously often associated with disadvantages of various kind.
Glycosyl trichloroacetimidates are synthesized by reacting the free hydroxyl of lactols with trichloroacetonitrile under basic conditions. This reaction is generally high-yielding and, because of its reversibility, high anomeric control can often be achieved. In the presence of a weak base, such as potassium carbonate, the β-imidate can be isolated as the kinetic product, whereas the use of strong bases, such as sodium hydride or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), results in the formation of the thermodynamically more stable α-trichloroacetimidates (*Scheme 1.10*).[62]
Controlling the anomeric configuration of glycosyl trichloroacetimidates isn't only of academic interest. The two diasteromers exhibit different reactivity, α anomers are generally more stable, thus less reactive, and require slightly harsher conditions to be activated. Furthermore, the use of diasteroisomerically pure glycosyl donors is of utmost importance when a SN_2 mechanism in the glycosylation medium is invoked.
Recently, Yu and coworkers introduced a class of fluorinated analogues of the Schmidt's donors: **glycosyl *N*-phenyl trifluoroacetimidates**.[63] Schmidt and co-workers also pioneered the preparation of glycosyl trifluoroacetimidates, whereupon the toxic CF_3CN with low boiling point (-63.9 °C) was used.[64]

[59] a) Das, S. K.; Ghosh, R.; Roy, N. *J. Carbohydr. Chem.* **1993**, *12*, 693; b) Zuurmond, H. M.; van der Laan, S. C.; van der Marel, G. A.; van Boom, J. H. *Carbohydr. Res.* **1991**, *215*, c1.
[60] a) Fügedi, P.; Garegg, P. J.; Lönn, H.; Norberg, T. Glycoconjugate J. **1987**, 4, 97; b) Garegg, P. J. Adv. Carbohydr. Chem. Biochem. **1997**, 52, 179.
[61] Schmidt, R. R.; Michel, J. Angew. Chem. Int. Ed. Engl. **1980**, 19, 731.
[62] Schmidt, R. R.; Michel, J. Tetrahedron Lett. **1984**, 25, 821.
[63] a) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405; b) Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099.
[64] Schimdt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984**, 1343.



SCHEME 1.10 Anomeric Control in Trichloroacetimidate Synthesis.

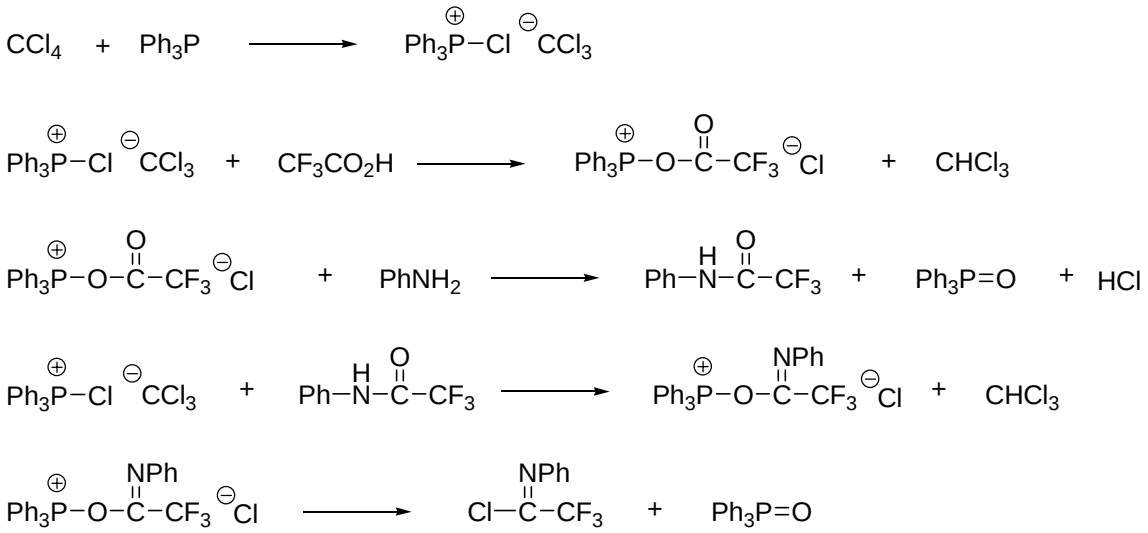
Glycosyl *N*-phenyl trifluoroacetimidates are synthesized in high yield by treatment of the corresponding 1-hydroxyl sugar with *N*-phenyl trifluoroacetimidoyl chloride in the presence of K_2CO_3 in lab grade non-anhydrous CH_2Cl_2 or acetone at room temperature (*Scheme 1.11)*.



SCHEME 1.11 Synthesis of *N*-Phenyl Trifluoroacetimidates.

It should be mentioned that moisture in the solvent was found to be important, it might increase the solubility of K_2CO_3 and/or result in the presence of HO^- to facilitate the deprotonation of 1-OH sugars, otherwise the reaction was found sluggish.[65] Furthermore, termodinamically favoured α anomers were produced predominantly due to the anomeric effect of the corresponding 1-*O*-potassium sugars. When DIPEA is used as base in anhydrous dichloromethane almost exclusively β anomers were obtained.[66]
Unlike trichloroacetonitrile, *N*-phenyl trifluoroacetimidoyl chloride is not commercially available and it has to be synthesized by reacting trifluoroacetic acid with aniline in a PPh_3-Et_3N-CCl_4 system (*Scheme 1.12)*.[67]

[65] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Synlett* **2002**, 269.
[66] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *J. Org. Chem.* **2005**, *70*, 5316.
[67] Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. *J. Org. Chem.* **1993**, *58*, 32.



SCHEME 1.12 Synthesis of *N*-phenyl Trifluoroacetimidoyl Chloride.

Aniline can easily be replaced by other nucleophiles, thus variously *N*-substituted trifluoroacetimidate donors might be prepared, providing an additional element for tuning the reactivity of the glycosyl trifluoroacetimidate donors.[68]
The presence of electron-withdrawing fluorine atoms on the leaving group greatly influences both reactivity and stability of *N*-phenyl trifluoroacetimidate donors. As a matter of fact, if compared with the chlorinated analogues, they are less prone to undesired degradation reactions during glycosylations and exhibit better storability properties. Like glycosyl halides, trichloro- and trifluoro-acetimidate moieties are too labile to survive protecting group manipulations. The installation of the imidate leaving group at the anomeric centre is the last step of multistep glycosyl donor syntheses. Generally in the early steps, a temporary protecting group is installed at the anomeric centre, in order to safely manipulate the other hydroxyl groups on the saccharide backbone. Both glycosyl trichloro- and *N*-phenyl trifluoro-acetimidates are relatively stable under basic or neutral conditions, but react readily in presence of catalytic amounts of Brønsted or Lewis acids. Originally, *p*TsOH and $BF_3 \cdot Et_2O$ were used,[61] while the latter together with TMSOTf[69] are currently the most frequently employed promoters. Glycosylations with these promoters take place at low temperatures and in high yields. Besides the activators mentioned thus far, several additional promoters have been introduced, these include strong protic and Lewis acids.
One of the aim of our lab is the development of new mild, moisture-stable, non-corrosive glycosylation promoting systems for the trihaloacetimidate methods. Several mild promoters have been proposed during the last years, such as the system I_2/Et_3SiH, as source of *in situ* generated HI,[70] and lanthanide salts such as $Sm(OTf)_3$[71] or $Yb(OTf)_3$.[72] More recently, commercially available acid washed molecular sieves (AW 300 MS) proved

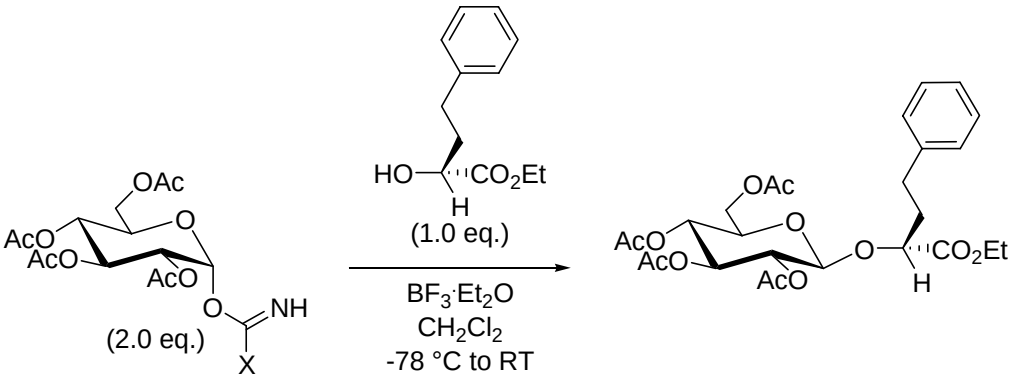
[68] Cai, S.; Yu, B. Org. Lett. 2003, 5, 3827.
[69] Schmidt, R. R.; Grundler, G. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 781.
[70] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Synlett* **2002**, 269.
[71] Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **2000**, *41*, 9005.
[72] a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Mangoni, L.; Schiattarella, M. *Tetrahedron Lett.* **2001**, *42*, 5967; b) Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573.

to effectively activate trihaloacetimidates without any further promoter.[73] The last and very powerful promoting system developed in our lab concerns the use of catalytic amounts of $Bi(OTf)_3$.[74] Especially in the cases of $Yb(OTf)_3$, AW 300 MS and $Bi(OTf)_3$ the glycosylation conditions proved to be extremely mild and compatible with a number of acid sensitive protecting groups. In the case of $Yb(OTf)_3$-catalyzed glycosylations, it has been proved the stability of the extremely labile dimethoxytrityl group (DMT), never used before in glycosylation media.[75]

The higher stability of *N*-phenyl trifluoro- compared with that of trichloro-acetimidates has also an effect on the reactivity. Generally, Schmidt's donors are activated at lower temperatures and by smaller amounts of promoter than those required by the fluorinated-analogues. These observations allowed the development of an effective one-pot procedure in which trichloro-acetimidates first, and then *N*-phenyl trifluoro-acetimidates are sequentially activated by catalytic amounts of $Yb(OTf)_3$, adjusting reaction temperature and promoter amounts in due course.[76]

It should be observed that the presence of three fluorine atoms is responsible by itself of the higher amount of promoter required for effectively activate glycosyl trifluoro-acetimidates, instead the presence of a bulky group on the nitrogen might entail the better yields often registered with *N*-phenyl trifluoroacetimidates, if compared with trichloro-acetimidate donors.

In *Table 1.1* is shown a comparative study on $BF_3 \cdot Et_2O$-catalyzed glycosylations with glycosyl trichloro- *vs* trifluoro-acetimidate donors.[77] As evident from the table, best results are achieved with 0.25 equivalents of promoter for trichloroacetimidate donor and with 0.5 equivalents in the case of the fluorinate-analogue, in both cases the desired product was isolated in 73% yield.

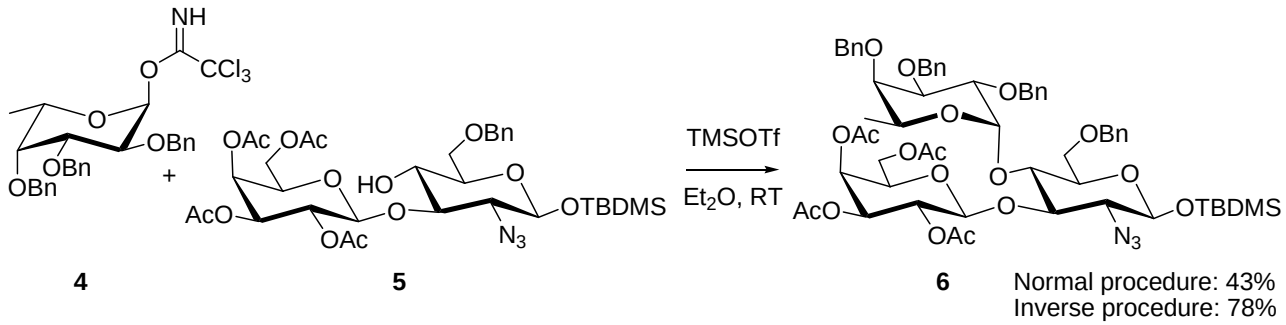


X	*$BF_3 \cdot Et_2O$ (equiv.)*	*Yield (%)*
CCl_3	0.25	73
CCl_3	0.5	67
CF_3	0.25	65
CF_3	0.5	73

TABLE 1.1 $BF_3 \cdot Et_2O$-catalyzed Glycosylation: Glycosyl Trichloroacetimidates *vs* Trifluoroacetimidates.

[73] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Org. Lett.* **2003**, *5*, 987.
[74] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Valerio, S. *Tetrahedron Lett.* **2006**, *47*, 2595.
[75] Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479.
[76] Adinolfi, M.; Iadonisi, A.; Ravidà, A. *Synlett* **2006**, 583. The results of this work are described with more details in *Chapter 7*.
[77] Nakajima, N.; Saito, M.; Kudo, M.; Ubukata, M. *Tetrahedron* **2002**, *58*, 3579.

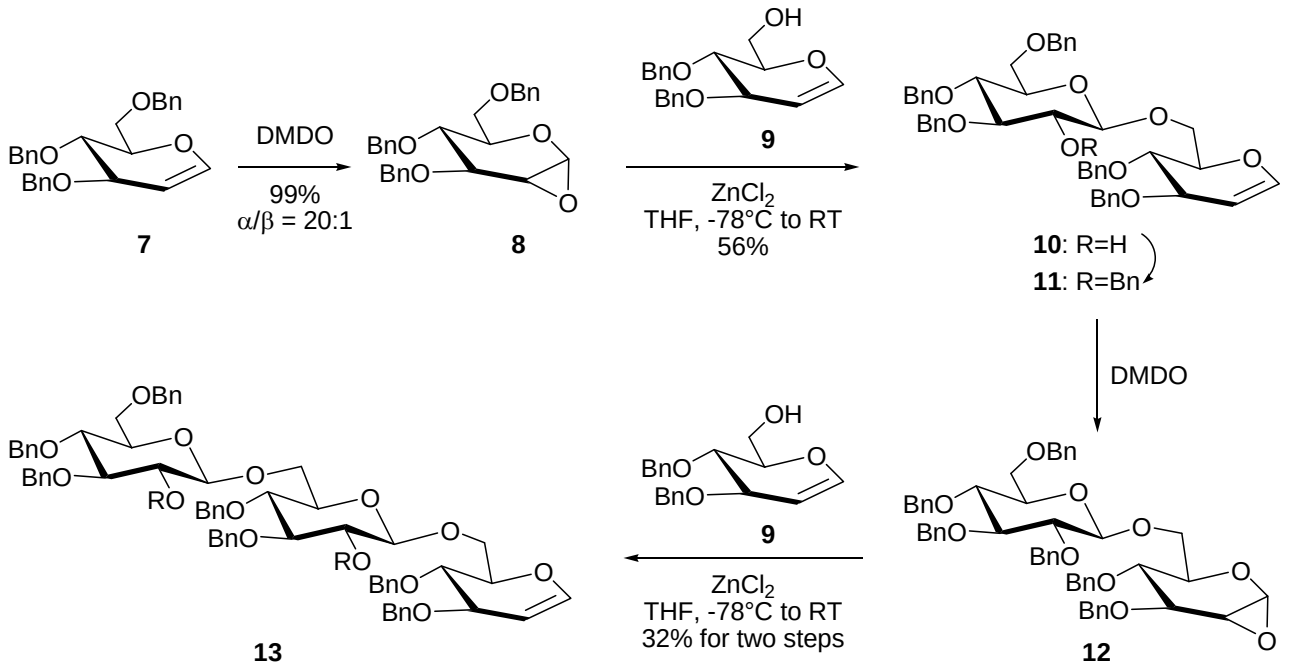
The effect of the phenyl group in *N*-phenyl trifluoroacetimidate donors is generally related to the prevention of undesired side-reactions, that often take place with Schmidt's donors, such as β-elimination[78] or rearrangement to unreactive glycosyl trichloroacetamides.[79]
N-phenyl trifluoroacetimidate donors proved to be very effective glycosylating agents in several different syntheses sometimes furnishing better results than the Schimidt's donors, but are not yet as diffused as the tricholoro-analgues.
Besides the introduction of new promoters, another technical development related to glycosyl trichloroacetimidates as glycosyl donors was the introduction of the *inverse procedure*.[80] In the original procedure, the promoter is added to a mixture of glycosyl donor and acceptor. It was assumed that highly reactive glycosyl trichloroacetimidates might partially decompose prior to being exposed to the glycosyl acceptor. In support of this hypothesis, adding the glycosyl donor to a mixture of the acceptor and promoter resulted in increased yields (*Scheme 1.13)*.



SCHEME 1.13 The Normal and the Inverse Procedure.

Glycosylation of the disaccharide acceptor **5** with the reactive fucosyl imidate **4** gave the trisaccharide **6** in a 43% yield by the normal procedure, whereas **6** was obtained in a 78% yield by the inverse procedure.
A completely different glycosyl donor's family is represented by **1,2-anhydro sugars**. Glycals recently came into the limelight because of the work of Danishefsky and co-workers, who developed a highly efficient procedure for their oxidation to 1,2-anhydro sugars with dimethyldioxirane (DMDO).[81] Reaction of the epoxides **8** and **12**, promoted by $ZnCl_2$ in tetrahydrofuran or dichloromethane, produced 1,2-*trans*-glycosides (*Scheme 1.14*). Glycosylation of glycals by this method gives a reiterative glycosylation strategy for the synthesis of oligosaccharides. Unfortunately, it was established that the glycosylation reaction is not always stereospecific.[82] A potentially useful feature of the glycosides formed in glycosylations with 1,2-anhydro sugars is that they possess a free 2-OH ready for further transformations, for example, into 2-deoxy glycosides,[83] or for configurational inversion as in the synthesis of β-mannosides from β-glucosides.[84] 1,2- anhydro sugars are very versatile because they can also be readily converted into other types of glycosyl donors, such as thioglycosides, glycosyl fluorides and phosphates.

[78] Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
[79] a) Cramer, F.; Hennrich, N. *Chem. Ber.* **1961**, *94*, 976; b) Zhu, T.; Boons, G. –J. *Carbohydr. Res.* **2000**, *329*, 709.
[80] Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353.
[81] Halcomb, R. L.; Danishefsky, S. J.; *J. Am. Chem. Soc.* **1989**, *111*, 6661.
[82] Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380.
[83] Gervay, J.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*, 5448.
[84] Liu, K. K. C.; Danishefsky, S. J. *J. Org. Chem.* **1994**, *59*, 1892.



SCHEME 1.14 Iterative Glycosylation with Glycals.

Glycosyl phosphate triesters can be considered the nature's glycosyl donors. Nucleotide 5′-diphospho sugars (NDPs) (*Figure 1.5*) are the natural substrates of glycosyl trasferases, that are the enzymes responsible for the biosynthesis of oligosaccharides in living systems.[85]

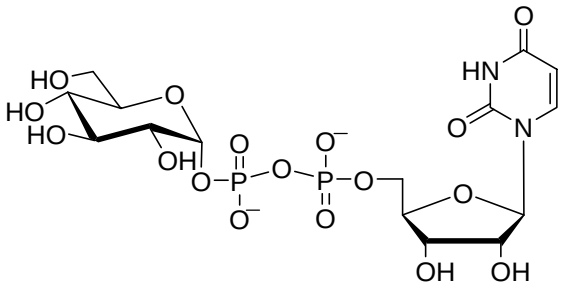


FIGURE 1.5 Nucleotide 5′-Diphospho Sugar.

Despite this strong suggestion from nature, glycosyl phosphate triesters were introduced as glycosyl donors in the scenario of saccharides synthesis only relatively recently by Ikegami and co-workers.[86] Stoichiometric amounts of TMSOTf proved to efficiently activate glycosyl phosphates at low temperatures. A variety of other Lewis acids, such as $SnCl_2$, ZnI_2, $Zn(OTf)_2$ and $BF_3 \cdot Et_2O$, was screened achieving in all cases moderate results.[87]

[85] a) Bayer, T. A.; Sadler, J. E.; Rearick, J. I.; Paulson, J. C.; Hill, R. L. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1981**, *52*, 23; b) Heidlas, J. E.; Williams, K. W.; Whitesides, G. M. *Acc. Chem. Res.* **1992**, *25*, 307.
[86] Hashimoto, S.; Honda, T.; Ikegami, S. *J. Chem. Soc. Chem. Commun.* **1989**, 685.
[87] Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2001**, *123*, 9545.

Most frequently, diphenyl and di-*n*-butyl phosphates are used. Recently, Singh and co-workers introduced the cyclic propane-1,3-diyl phosphates (*Figure 1.6*).[88]

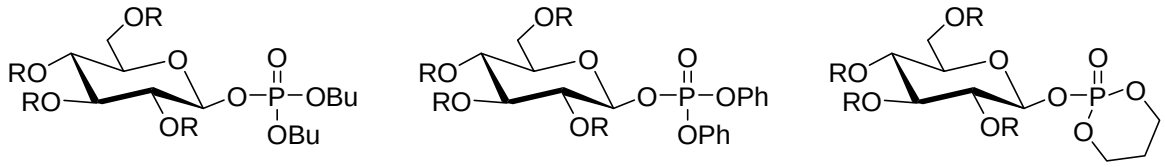
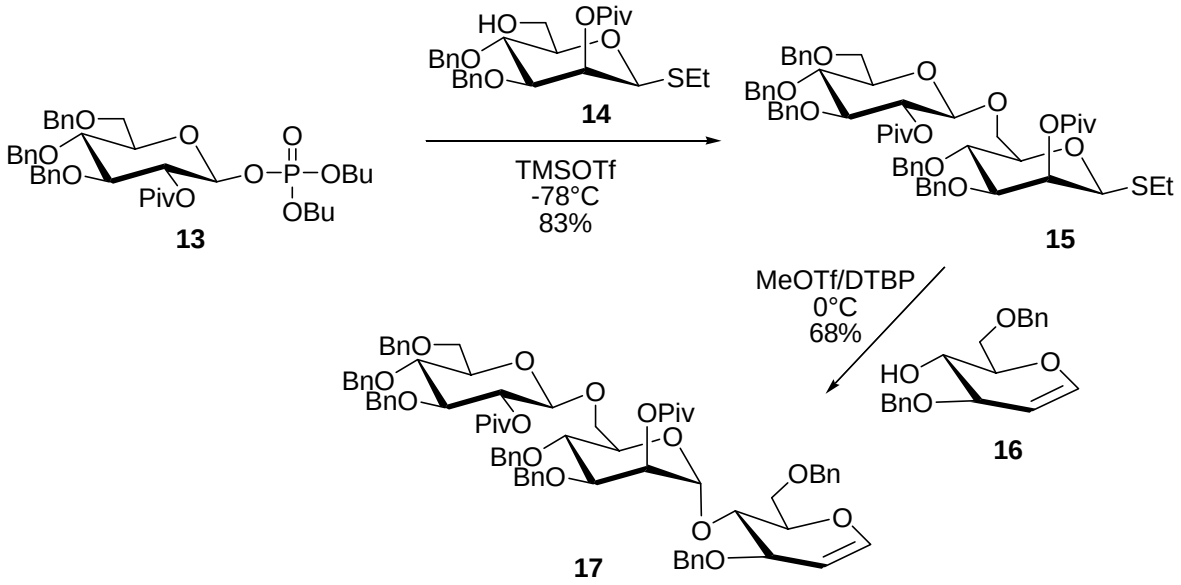


FIGURE 1.6 Most Commonly Used Glycosyl Phosphates.

Synthesis of glycosyl phosphates is the subject of *Chapter 8*, there the variety of approaches proposed for their preparation is described.
Glycosyl phosphates proved to be very effective glycosylating agents in the syntheses of various biologically relevant oligosaccharides both in solution and on solid-support.[89] Furthermore, their versatility was demonstrated in orthogonal and regioselective glycosylation strategies.[87]
Glycosyl phosphate **13** could be activated in the presence of thioglycoside-acceptor **14** at very low temperature to furnish the desired disaccharide **15** in good yield (*Scheme 1.15*). The disaccharide donor thus obtained was further activated by the use of MeOTf/DTBP system for the coupling with the glycal **16**, affording the trisaccharide **17** in 68% yield.

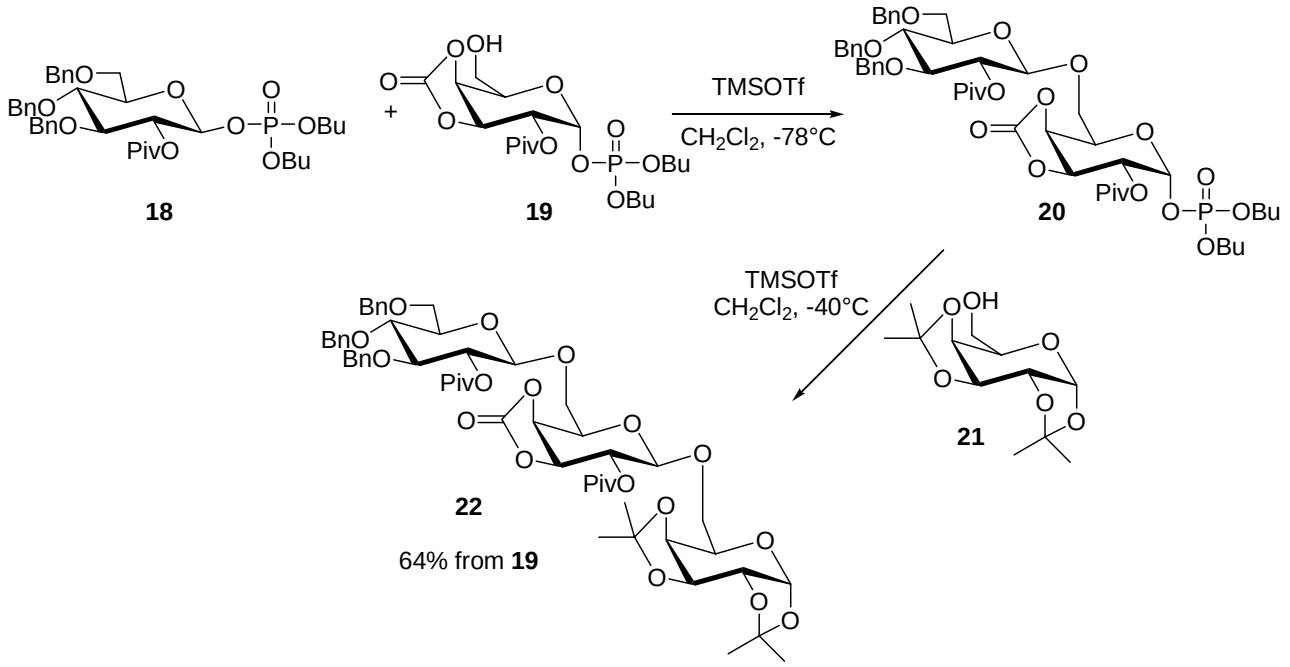


SCHEME 1.15 Orthogonal Glycosylation with Glycosyl Phosphates and Thioglycosides.

Orthogonal glycosylation strategies, similar to that described above, had previously been established for glycosyl donors other than glycosyl phosphates. Control of donor

[88] a) Hariprasad, V.; Singh, G.; Tranoy, I *Chem. Commun.* **1998**, 2129; b) Singh, G.; Vankayalapati, H. *Tetrahedron: Asymmetry* **2000**, *11*, 125; c) Vankayalapati, H.; Singh, G. *J. Chem. Soc. Perkin. Trans.1* **2000**, 2187; d) Vankayalapati, H.; Singh, G.; Tranoy, I. *Tetrahedron: Asymmetry* **2001**, *12*, 1373.
[89] a) Love, K. R.; Andrade, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001**, *66*, 8165; b) Bosse, F.; Marcaurelle, L. A.; Seeberger, P. H. *J. Org. Chem.* **2002**, *67*, 6659; c) Swanson, E. R.; Seeberger, P. H. *Org. Lett.* **2003**, *24*, 4717; d) Love, K. R.; Seeberger, P. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 602.

reactivity *via* anomeric configuration was, instead, never explored previously. The latter strategy is based on the reactivity differences of α- and β-glycosyl phosphates (*Scheme 1.16*).



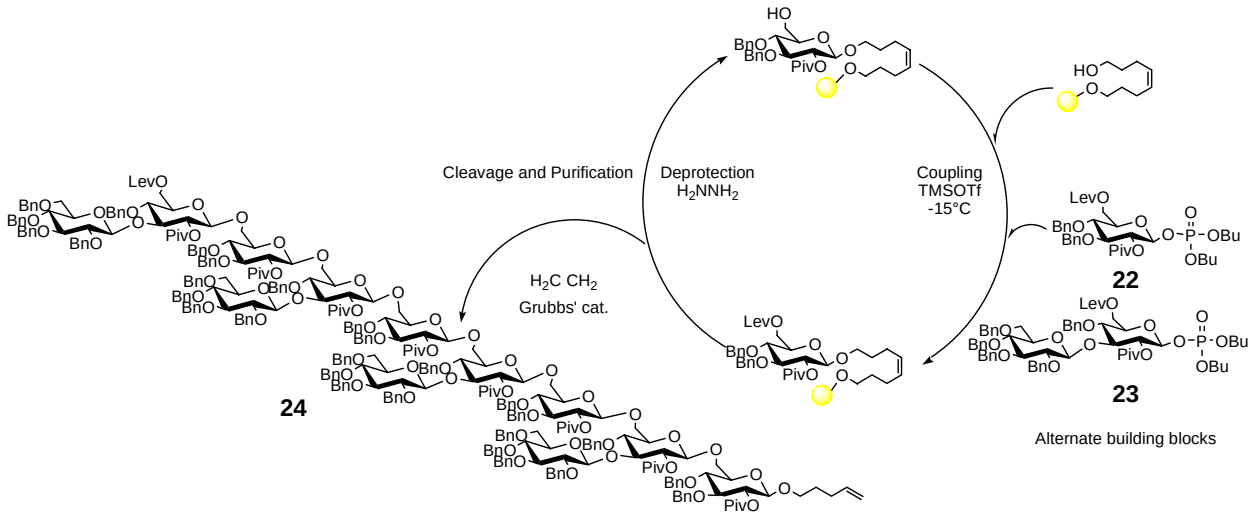
SCHEME 1.16 Orthogonal Glycosylation Based on Anomeric Configuration.

Glycosylation of α-galactosyl phosphate **19** with β-glucosyl phosphate **18** at -78°C resulted exclusively in β-phosphate activation and afforded β-(1→6)-linked disaccharide α-phosphate **20**. Disaccharide **20** was coupled with **21** at -40 °C to afford trisaccharide **22** in two steps and good overall yield (64%).

One of the reason of the growing interest toward glycosyl phosphates is their compatibility with the automated solid-phase approach. Recently, Seeberger and co-workers developed the first automated solid-phase oligosaccharide synthesizer.[8]

A peptide synthesizer was adapted to carbohydrate synthesis. Specific coupling cycles were designed for glycosyl trichloroacetimidate and phosphate methods. The success of the approach was demonstrated by the assembly of the phytoalexin elicitor (PE) dodecasaccharide in automated fashion (*Scheme 1.17*).

Two different glycosyl phosphate donors, **22** and **23**, were synthesized. Levulinoyl esters were employed as 6-*O* temporary protecting groups and the 2-*O*-pivaloyl group was used to control the stereochemical outcome of the glycosylation reaction *via* neighbouring participation effect. Each cycle incorporated double glycosylations (at -15 °C) and double deprotections to ensure high yielding steps (*Table 1.2*). A branched hexasaccharide was constructed in ten hours in over 80% yield, whereas dodecasaccharide **24** was prepared in 17 hours and in 50% yield using the same cycle.



SCHEME ***1.17*** Automated Solid Phase Synthesis of Protected Phytoalexin Elicitor β-Glucan.

Step	*Function*	*Reagent*	*Time (min)*
1	Couple	5 equiv. building block and 5 equiv. TMSOTf	30
2	Wash	Dichloromethane	6
3	Couple	5 equiv. building block and 5 equiv. TMSOTf	30
4	Wash	1:9 Methanol:Dichloromethane	4
5	Wash	Tetrahydrofuran	4
6	Wash	3:2 Pyridine:Acetic Acid	3
7	Deprotection	2 x 20 equiv. Hydrazine (3:2 Pyridine:Acetic Acid)	30
8	Wash	3:2 Pyridine:Acetic Acid	3
9	Wash	1:9 Methanol:Dichloromethane	4
10	Wash	0.2 M Acetic Acid in Tetrahydrofuran	4
11	Wash	Tetrahydrofuran	4
12	Wash	Dichloromethane	6

TABLE ***1.2*** General Cycle Used with Glycosyl Phosphate Donors.

Despite the efficiency and versatility of glycosyl phosphate donors, their use isn't widely spread. This can be attributed to different reasons. First of all, their synthesis often requires the use of moisture-sensitive, expensive, or non-commercially available reagents.[90] Furthermore glycosyl phosphates are unstable toward prolonged time purifications on silica gel. Synthetic procedures requiring only fast filtration of the reaction crude are generally preferable. Once in pure form, α- and β-glycosyl phosphates were found to be completely stable to storage for several months at 0 °C.
The use of stoichiometric amounts of promoter implies strong acidic condition in the glycosylation medium. This limits the number of protecting group compatible with the method. Acid-labile protecting groups have to be avoided, but an efficient protection strategy has been developed, using base-labile and UV-detectable 9-fluorenylmethyl-carbonate (Fmoc) as temporary group.[91]

[90] See *Chapter 8*.
[91] Love, K. R.; Seeberger, P. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 602.

Besides glycosyl phosphates, a variety of other phosphorus containing leaving groups have been designed and proposed. **Glycosyl phosphites** were introduced by Schmidt,[92] Wong[93] and Watanabe.[94] They react with alcohols at low temperatures in the presence of catalytic amounts of Lewis acid promoters such as TMSOTf, $BF_3 \cdot Et_2O$ and $Sn(OTf)_2$, thus creating glycosides. The typical activators are $ZnCl_2$, $ZnCl_2$-$AgClO_4$ and $BiCl_3$.[87, 95] Glycosyl thio compounds with phosphorus in the leaving group, such as **dithiophosphates**[96,87] or **phosphorodiamidimidothioates**,[97] have also been studied (*Figure 1.7*).

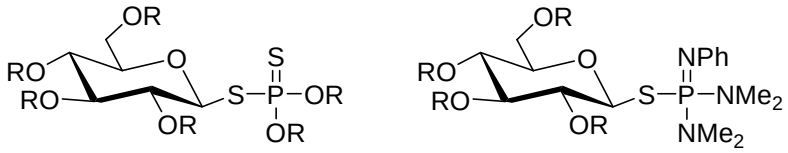
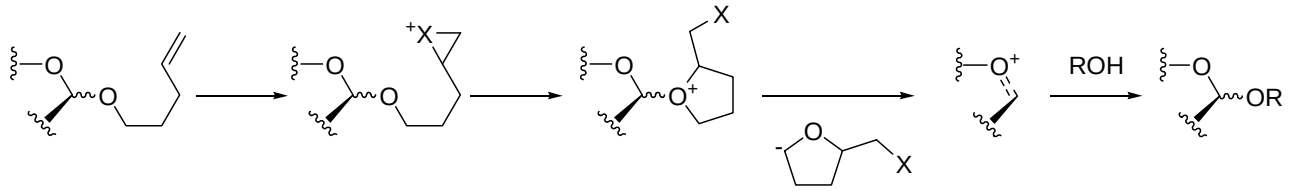


FIGURE 1.7 Glycosyl Dithiophosphate and Phosphorodiamidimidothioate Donors.

Despite their efficiency, none of the glycosylation methods described so far is generally applicable. The choice of the suitable glycosylation method for a given glycosidation depends on several parameters, among the others the specific linkage to be formed and the nature of the coupling partners. Generally the best results cannot be predicted in advance and come after several experimental attempts. Often a complementarity is found among the methods. For this reason many new glycosylation methods have been being developed, in order to achieve the universal one. Although we are still far to it, very efficient methodologies have been described and, besides the ones already illustrated, some others are worth mentioning. ***n*-Pentenyl glycosides**, introduced by Fraser-Reid,[98] provide excellent stability under a variety of protecting group manipulations. These glycosyl donors are activated by halogenation of the double bond, which results in cyclization and the release of the aglycone, thus providing active glycosylating carbocation (*Scheme 1.18*).



SCHEME 1.18 Glycosylation with *n*-Pentenyl Glycosides.

[92] a) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123; b) Müller, T.; Schneider, R.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 4763.

[93] a) Kondo, H.; Ichikawa, Y.; Wong, C. –H. *J. Am. Chem. Soc.* **1992**, *114*, 8748; b) Sim, M. M.; Kondo, H.; Wong, C –H.; *J. Am. Chem. Soc.* **1993**, *115*, 2260; c) Kondo, H.; Aoki, S.; Ichikawa, Y.; Halcomb, R. L.; Ritzen, H.; Wong, C. –H. *J. Org. Chem. Soc.* **1994**, *59*, 864.

[94] a) Watanabe, Y.; Nakamoto, C.; Ozaki, S. *Synlett* **1993**, 115; b) Watanabe, Y.; Nakamoto, C.; Yamamoto, T.; Ozaki, S. *Tetrahedron* **1994**, *50*, 6523.

[95] Corey, E. J.; Wu, Y. –J. *J. Am. Chem. Soc.* **1993**, *115*, 8871.

[96] a) Lauplicher, L.; Sajus, H.; Thiem, J. *Synthesis* **1992**, 1133; b) Bielawska, H.; Michalska, M. *Tetrahedron Lett.* **1998**, *39*, 9761.

[97] a) Hashimoto, S.; Honda, T.; Ikegami, S. *Tetrahedron Lett.* **1990**, *31*, 4769; b) Hashimoto, S.; Sakamoto, H.; Honda, T.; Ikegami, S. *Tetrahedron Lett.* **1997**, *38*, 5181.

[98] Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodung, U. *J. Chem. Soc. Chem. Commun.* **1988**, 823.

These glycosylation reactions are promoted by NBS, iodonium dicollidine perchlorate (IDCP) or the corresponding triflate (IDCT). NIS in combination with catalytic amounts of protic or Lewis acids such as TfOH[99] or TESOTf,[100] was introduced as a more active catalyst.
The *n*-pentenyl glycosylation was used in the synthesis of biologically important molecules.[101]
Glycosyl sulfoxides as glycosyl donors were introduced by Kahne.[102] When activated by triflic anhydride or catalytic amounts of triflic acid, glycosyl sulfoxides react with unreactive substrates giving glycosides in good yields. The reaction is reported to proceed through the glycosyl α-triflate, which forms the basis of a very useful method for the synthesis of β-mannopyranosides.[103] Compared with glycosyl sulfoxides, **glycosyl sulfones** are far less reactive. Neverthless, activation of sulfones of some 2-deoxy sugars has been successfully used in glycosylations using $MgBr_2 \cdot Et_2O$.[104]

Stereoselectivity of Glycosidation Reactions

The effectiveness of a glycosylation doesn't relies only on the coupling yield. The stereoselectivity is a crucial issue in glycosylation reactions. Problems of stereocontrol arise as result of difficulties in accomplishing clean S_N2 reactions at the anomeric center. The ready assistance of the endocyclic oxygen in the glycosyl donor's leaving group diparture and subsequent stabilisation of the carbocation gives a considerable S_N1 component to these reactions rendering stereocontrol difficult.
Depending on the spatial orientation of the substituent on the anomeric center, the newly formed glycosidic linkage can either be α or β, but it can also be referred as 1,2-*trans* or 1,2-*cis*, highlighting the relation with the substituent at the neighbouring carbon (*Figure 1.8*).

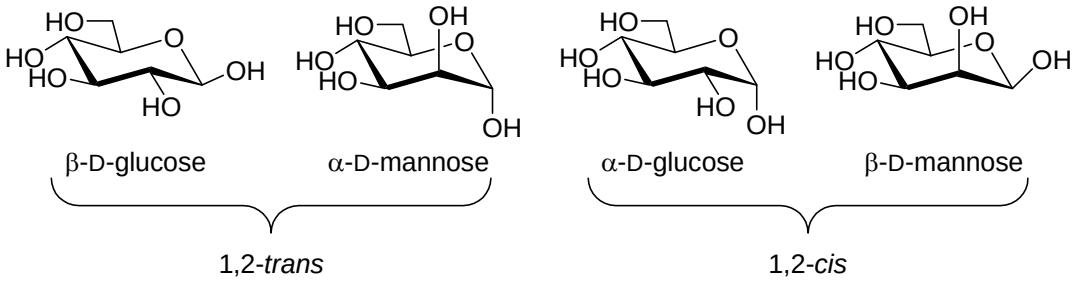
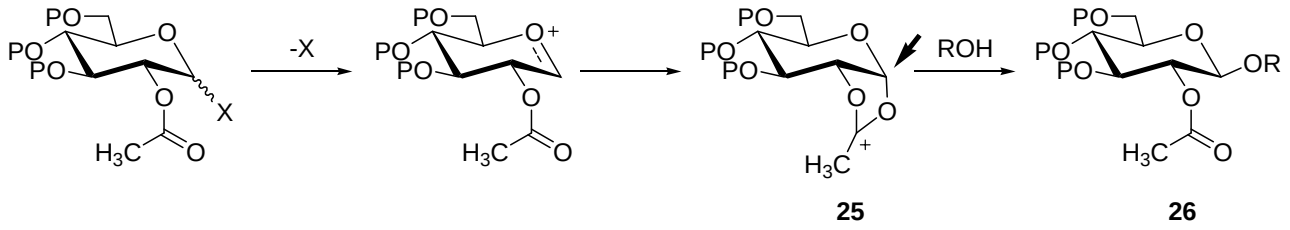


FIGURE 1.8 Anomeric Configurations of D-Glucose and D-Mannose.

The latter description is rather diffused, because it furnishes an easy way to understand the difficulty of making a determinate glycosidic bond. 1,2-*trans*-Glycosides can be easily synthesized exploiting **neighbouring group participation**. When a glycosyl donor is equipped at O-2 position with an acyl group, the departure of the leaving group on the

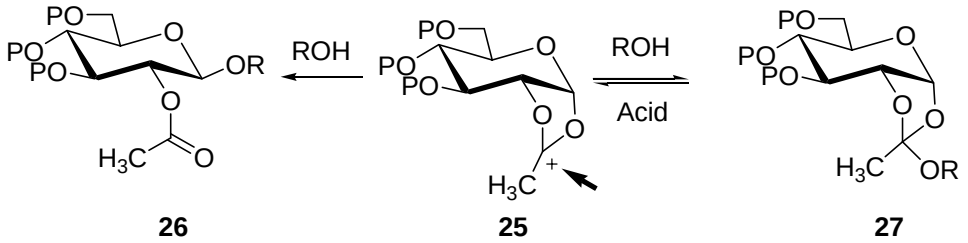
[99] Konradsson, P.; Mootoo, D. R.; McDevitt, R. E.; Fraser-Reid, B. *J. Chem. Soc. Chem. Commun.* **1990**, 270.
[100] Fraser-Reid, B.; Udodung, U.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927.
[101] Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1989**, *111*, 8540.
[102] Kahne, D.; Walzer, S.; Cheng, Y.; van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881.
[103] Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321.
[104] Brown, D. S.; Ley, S. V.; *Tetrahedron Lett.* **1988**, *29*, 4873.

anomeric position is anchimerically assisted by the carbonyl moiety and the acyloxonium ion **25** is readily formed (*Scheme 1.19*).



SCHEME 1.19 Neighbouring Group Participation.

Reaction of the nucleophile with the acyloxonium ion **25** takes place from the upper face of the ring and therefore results in the formation of 1,2-*trans*-glicoside **26** with regeneration of the acyloxy group.
The nucleophile might also react with the other electrophile carbon, thus forming the undesired orthoester **27** (*Scheme 1.20*).

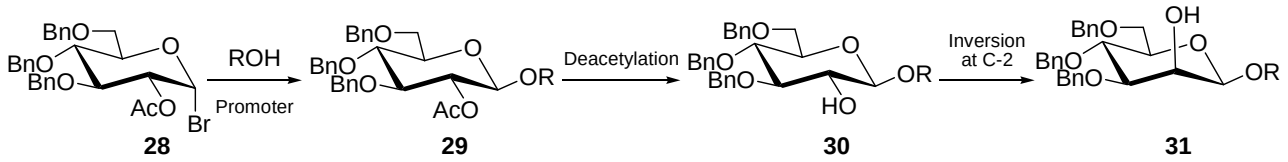


SCHEME 1.20 1,2-Orthoester Formation.

In the most commonly used glycosylation methods, strong Lewis and Brønsted acids are used as promoters and the orthoester formation is a reversible process. When mild promoters are used, such as lanthanide salts for the trihaloacetimidate methods,[71,72] glycosyl orthoesters become non-reactive side-products that negatively affect the glycosylation yield. Electrophilicity of the carbonyl carbon can be decreased in order to avoid this side-reaction. Sterically hindered pivaloyl groups are often used, but their cleavage require harsher conditions than those used for the common acetyl and benzoyl groups. Recently, methoxycarbonyl group was introduced as neighbouring participating group.[105] The use of this protecting group guarantees complete 1,2-*trans*-stereoselectivities, orthocarbonate side-products being not detected even in glycosylations assisted by very mild promoters. Furthermore, cleavage of methoxycarbonyl moieties requires the same conditions as for acetyl and benzoyl groups.
Neighbouring group participation represents the most reliable tool to control the stereoselectivity of glycosylation reactions, providing excellent stereo-selectivities with various types of glycosyl donors. However the neighbouring group participation is limited to the synthesis of 1,2-*trans*-glycosides, but, since its effectiveness, it is sometimes used for the indirect synthesis of 1,2-*cis*-mannosides (*Scheme 1.21*). In this approach, advantage is taken of the ready formation of 1,2-*trans*-glucoside **29** by neighbouring group

[105] a) Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **2000**, *41*, 9005; b) Adinolfi, M.; Barone, G.; Iadonisi, A.; Mangoni, L.; Schiattarella, M. *Tetrahedron Lett.* **2001**, *42*, 5967.

participation from glucosyl donor **28**, which possesses orthogonally removable acetyl at O-2. After selective removal of this protecting group, the C-2 configuration of the resulting **30** is inverted by nucleophilic displacement or oxidation-reduction type reactions, thus creating the 1,2-*cis*-mannoside **31**.



28 **29** **30** **31**

SCHEME 1.21 Synthesis of β-D-Mannopyranosides by Configurational Inversion at C-2.

Other approaches to achieve stereoselective glycosylations have been developed. Obviously, the major challenge is represented by the formation of 1,2-*cis*-glycosides, but it's useful when not necessary to rely on alternative methods for the control of the stereochemistry of glycosylations also for 1,2-*trans*-linkage formation. Participating groups are generally acyl or carbonyl moieties. These groups don't influence only the stereochemical outcome of the reactions but also the reactivity of glycosyl donors. Their electron-withdrawing nature has a destabilizing effect on the carbocation intermediate that is formed after the departure of the glycosyl donor leaving group. Glycosyl donors equipped with electron-withdrawing protecting groups are called *disarmed* and exhibit decreased reactivity.[106] Depending on specific synthetic demands, this effect might be undesired and also 1,2-*trans*-selectivity has to be achieved in a different fashion.

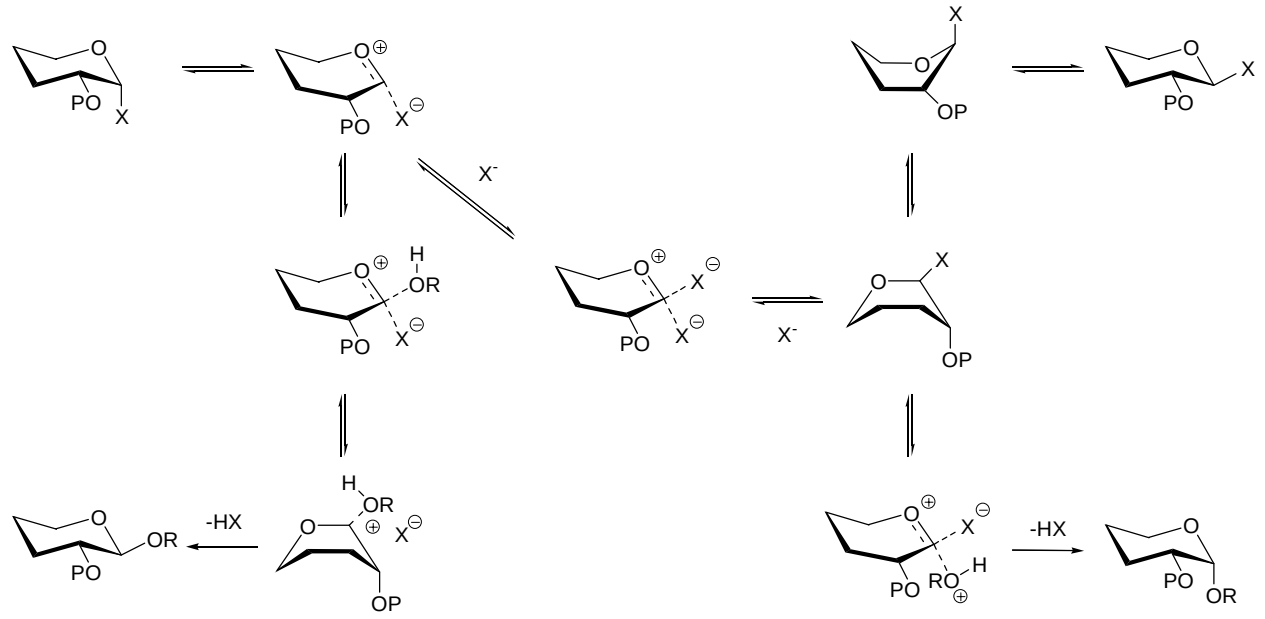
The **solvent effect** is widely used for controlling the stereoselectivity of glycosylation reactions. A prerequisite for the use of the solvent effect is the avoidance of participating groups on C-2 heteroatom of glycosyl donors. Ether-type protecting groups have a low tendency to participate and therefore are commonly used for this purpose. In these cases, benzyl ethers are the most frequently used derivatives for hydroxyl groups, whereas for amino sugars, the azido function serves as an excellent non-participating masked form of the amino group.

In non-coordinating solvents, such as toluene and CH_2Cl_2, glycosylation reactions proceed through a tight ion pair, favouring an S_N2-like mechanism with inversion of configuration at the anomeric carbon (*Scheme 22*).[107]

The accomplishment of a pure S_N2 reaction is very difficult. The ready participation of the endocyclic oxygen in leaving group displacement gives a considerable S_N1 component and, generally, anomeric mixtures, enriched in the inversion product, are obtained.

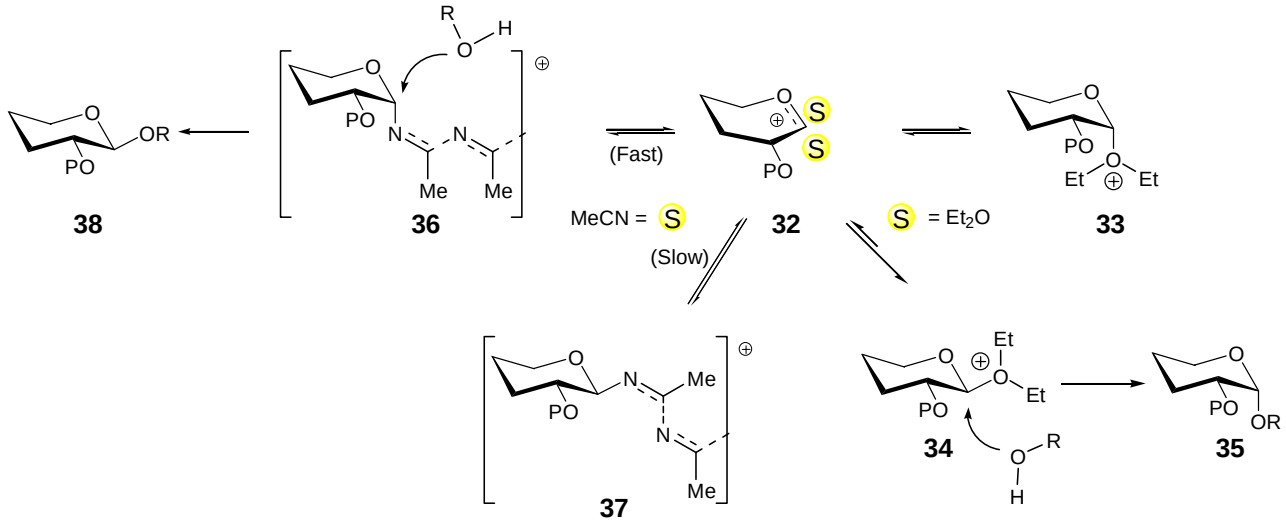
When glycosylation reactions are conducted in coordinating solvents, the stereoselectivity is altered by the direct involvement of the solvent in the reaction mechanism. Experimental evidence has shown that ether-type solvents favour the formation of α-glycosides, alternatively, the use of nitrile-type solvents shifts the selectivity toward the preferential formation of β-anomers.

[106] Mootoo, D. R.; Konradsson, P.; Udodung, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583.
[107] Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056.



SCHEME 1.22 Halide Ion-Catalized Glycosylation.

This effect on the stereoselectivity was rationalized invoking an S_N1-type reaction mechanism in which a sugar-solvent adduct is formed as intermediate.[108] After the departure of the leaving group from the glycosyl donor, the oxocarbenium ion **32** is solvated by the coordinating solvent (*Scheme 1.23*).



SCHEME 1.23 Solvent Participation in Glycosylations.

In the case of ether-type solvents, the kinetic formation of the α-adduct **33** is rapidly followed by its conversion into the termodinamically favoured β-oxonium intermediate **34**. The β-adduct **34** is more stable than the axial oxonium **33** because of reverse anomeric

[108] a) Wulff, G.; Röhle, G. *Angew. Chem. In. Ed. Eng.* **1974**, *3*, 157; b) Schmidt, R. R.; Rüker, E. *Tetrahedron Lett.* **1980**, *21*, 1421; c) Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694; d) Demchenko, A.; Stauch, T.; Boons, G. –J. *Synlett* **1997**, 818.

effect. The presence of a positive charge on the exocyclic heteroatom, favours the equatorial displacement of the aglycone because of dipolar effects (*Figure 1.9*).

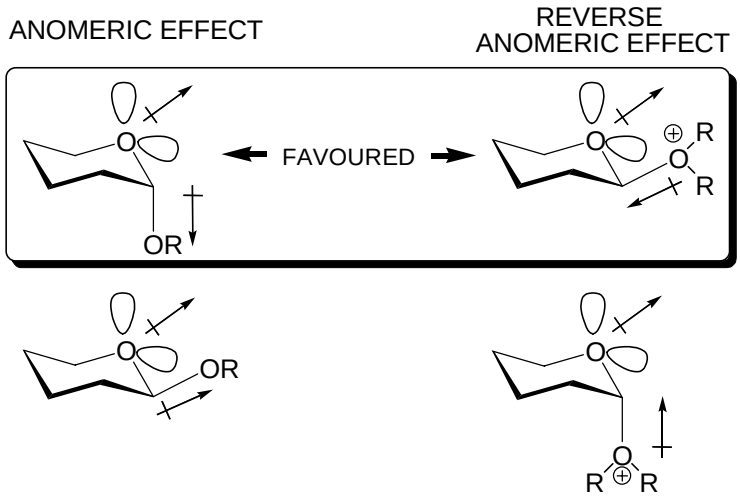
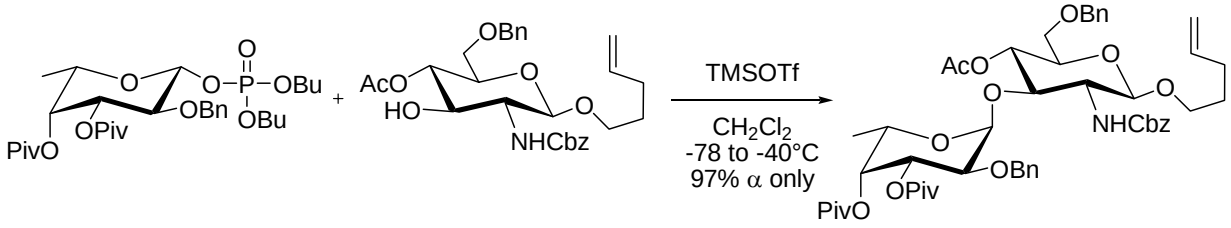


FIGURE 1.9 Dipole-Dipole Interaction in Direct and Reverse Anomeric Effects.

Intermediate **34** then reacts with the nucleophile to form the axial glycoside **35**, thus achieving the desired α-stereoselectivity. When nitrile-type solvents are used, the same model can be applied. In this case, the conversion of the α-adduct **36** into the termodinamically more stable β-nitrilium ion **37** is slower than in the case of ether-type solvents. This is due to the formation of nitrilium-nitrile-conjugate species that, stabilized by polydispersion of the positive charge, evolves to the corresponding β-adduct very slowly. The nucleophile, in this case, attaks the α-adduct with an equatorial approach leading to the formation of the desired β-glycoside **38**. This model is well established and the solvent effect is widely recognized. It had been supported by several experimental evidences, collected in the course of the years. Reaction at different temperatures and rates were conducted and the nitrilium ion was also trapped.[109]

Although the solvent and the neighbouring participation effect are the most used methods, other approaches have been developed in order to control the sereoselectivity of glycosylation reactions. Protecting groups on position other than O-2 can influence the stereochemistry of glycosylations exploiting long range participation or sterical and torsional effects.

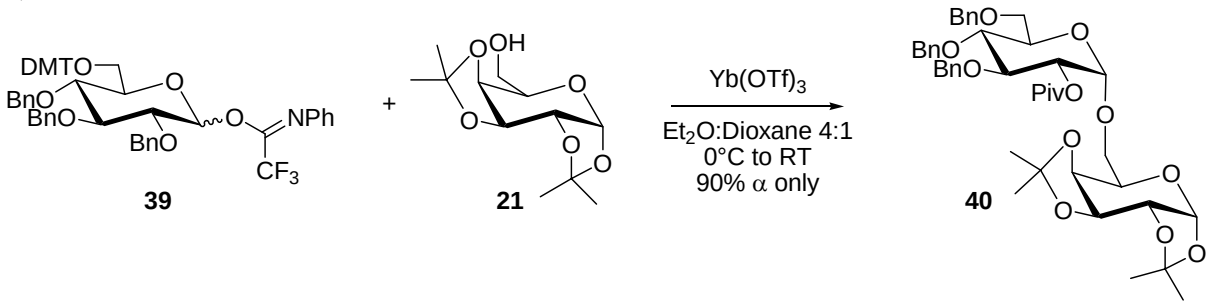
The use of an acyl protection on O-4 of galactose and fucose donors proved to improve the α-selectivity of glycosylation reactions via **long range participation effect** (*Scheme 1.24*).[87]



SCHEME 1.24 Long-Range Participation Effect.

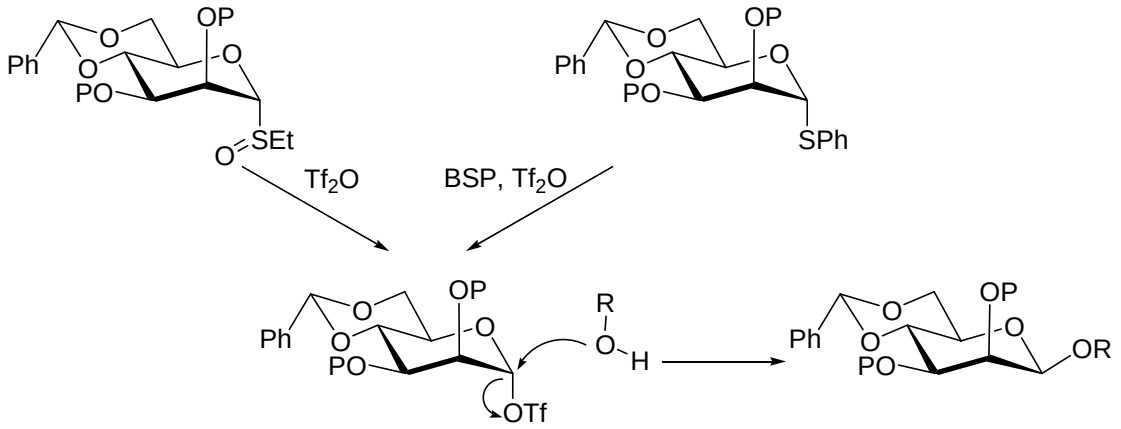
[109] a) Pougny, J. –R.; Sinaÿ, P. *Tetrahedron Lett.* **1976**, *45*, 4073; b) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc. Perkin Trans. I* **1990**, 747.

Furthermore, α-selectivity can also be enhanced by the use of **bulky protecting groups** on the primary hydroxyl of glycosyl donors. As shown in *Scheme 1.25*, the presence of the bulky dimethoxytrityl group on the O-6 of glucosyl *N*-phenyl trifluoroacetimidate **39** guaranteed the exclusive formation of the desired α-glycoside **40** in high yield (*Scheme 1.25*).[75]



SCHEME 1.25 Effect of the donor 6-*O*-substituents on α-selectivity of glycosylations.

The use of **4,6-*O*-benzylidene protection** on mannosyl donors is one of the keys to the efficient 1,2-*cis*-selectivity reported by Crich and coworkers in their work.[110] 4,6-*O*-Benzylidene-protected mannosyl sulfoxides are first activated with triflic anhydride to give a covalent triflate, that is then displaced by the acceptor to give the β-mannoside with excellent yield and selectivity. In a more recent version, the α-mannosyl triflate is generated from a mannosyl thioglycoside by the combination of triflic anhydride and 1-benzenesulfinyl piperidine (BSP) before addition of the acceptor (*Scheme 1.26*).[111]

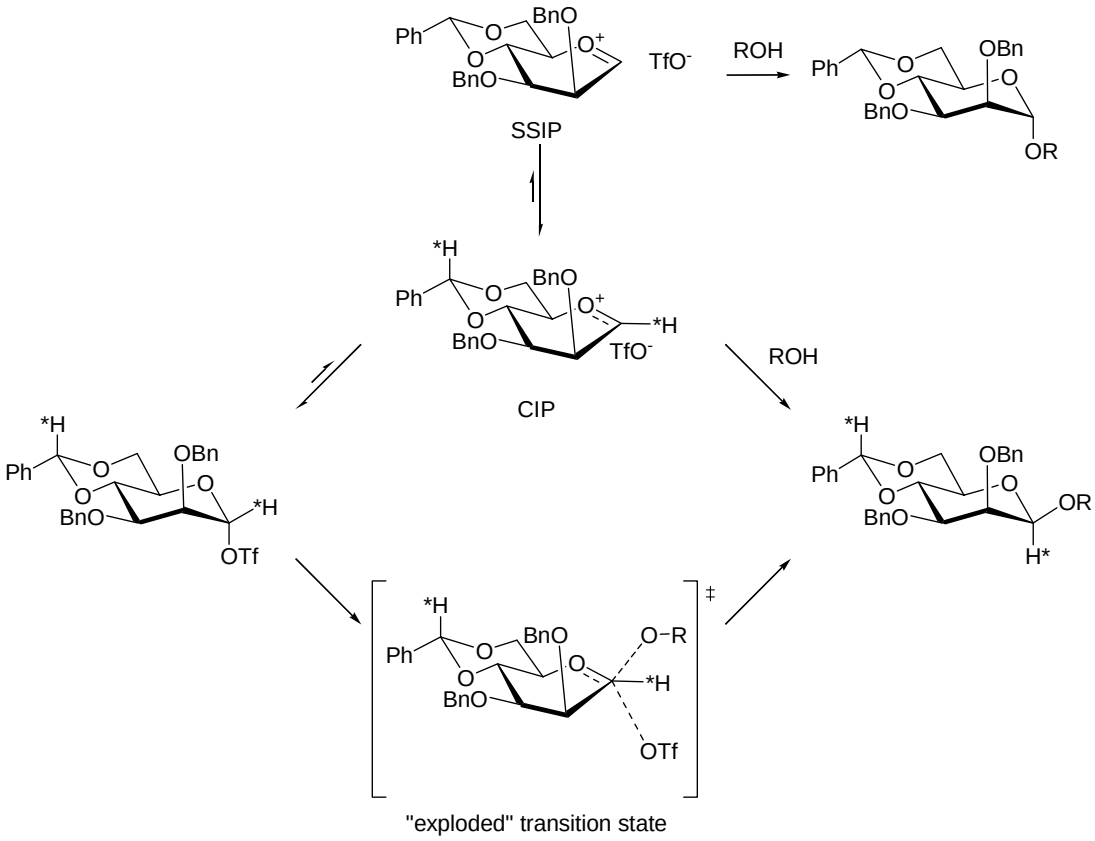


SCHEME 1.26 Synthesis of β-Mannosides via α-Triflate Formation.

The mechanism proposed and verified by Kinetic Isotope Effect invokes the formation of a transient contact ion pair (CIP) or a functionally equivalent "exploded" transition state (*Scheme 1.27*).[112] In the CIP mechanism the triflate anion is necessarily closely associated with the face of the oxacarbenium ion from which it has just departed and shields that face against attack by the incoming alcohol. In the alternative mechanism there is a loose association of the nucleophile with the anomeric center as the leaving group departs. The minor amount of α-mannosides formed in these reactions most likely arise through the

[110] Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217.
[111] Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015.
[112] Crich, D.; Chandrasekera, N. S. *Angew. Chem. Int. Ed.*. **2004**, *43*, 5386.

intermediacy of a looser, perhaps solvent-separated, ion pair (SSIP), which is in equilibrium with an initial CIP. The benzylidene group plays an important role in this equilibrium. It opposes to rehybridisation at anomeric carbon because of torsional effect and, so, shifts the complete set of equilibria toward the covalent triflate and away from the SSIP, thereby minimizing α-glycoside formation.

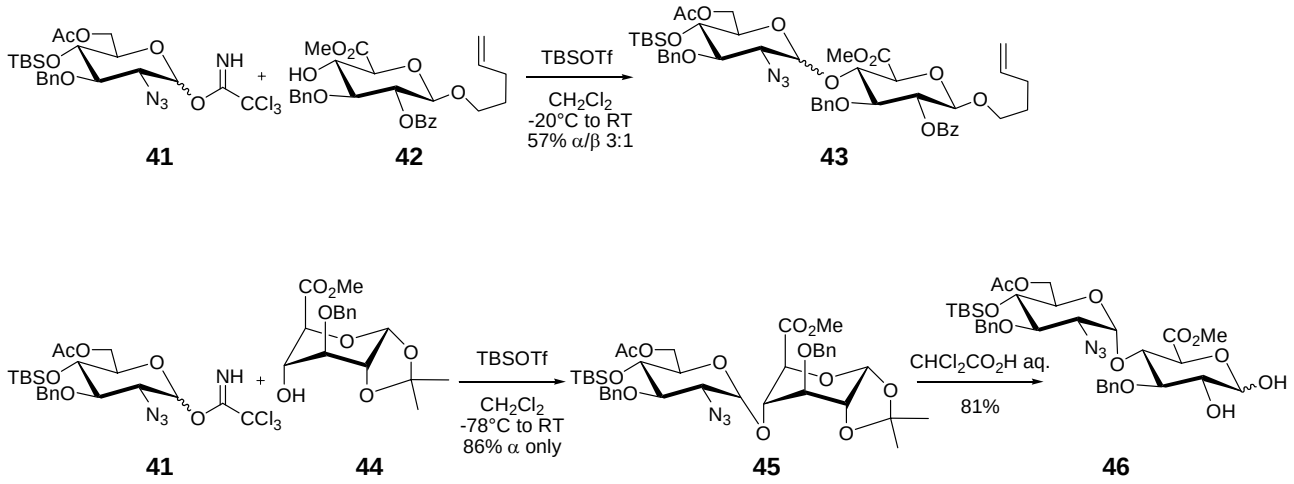


SCHEME 1.27 Proposed Glycosylation Mechanism for Crich Method.

In most of the approaches described so far, the steric and electronical nature of the glycosyl donor is tuned to direct the stereochemistry of glycosylation reactions. Manipulation of the protection pattern of the glycosyl acceptor to control the stereochemical outcome of coupling ractions has received less attention. Two features of the acceptor are known to influence the streochemical course of the reaction: the intrinsic reactivity of the hydoxy groups that function as nucleophile (axial hydroxyl groups are generally less reactive than equatorial hydroxyl groups)[113] and steric factors, which result in matched/mismatched pairs of glycosyl dononors and acceptors.[114] Recently, a new concept for stereochemical control of glycosylation reaction has been developed by **locking the conformation of the monosaccharide acceptor**.[115]

In the synthesis of key disaccharide building blocks for the assembly of the heparin backbone, the reaction of C-2-azido glucose trichloroacetimidate **41** with glucuronic acid acceptor **42** yielded anomeric mixture of disaccharide **43** (*Scheme 1.28*).

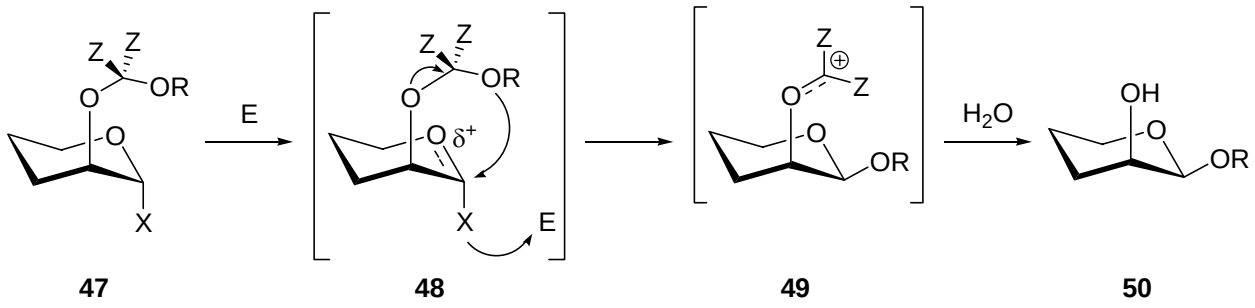
[113] Haines, A. H. Adv. *Carbohydr. Chem. Biochem*. **1976**, *33*, 11.
[114] Spijker, N. M.; van Boeckel, C. A. A. *Angew. Chem. Int. Ed.* **1991**, *30*, 180.
[115] Orgueira, H. A.; Bartolozzi, A.; Schell, P. Seeberger, P. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 2128.



SCHEME 1.28 Improvement of α-Selectivity by Conformational Locking of Glycosyl Acceptor.

Glucuronic acid **42** adopts a $^4C_1$ conformation with the C-4 hydroxyl group equatorially oriented. Locking the conformation of the glycosyl acceptor in a $^1C_4$ conformation allowed the completely selective preparation of the desired *cis*-glycoside. Coupling glycosyl tricholoroacetimidate **41** with acceptor **44** resulted exclusively in formation of the α-linked disaccharide **45** in very good yield.

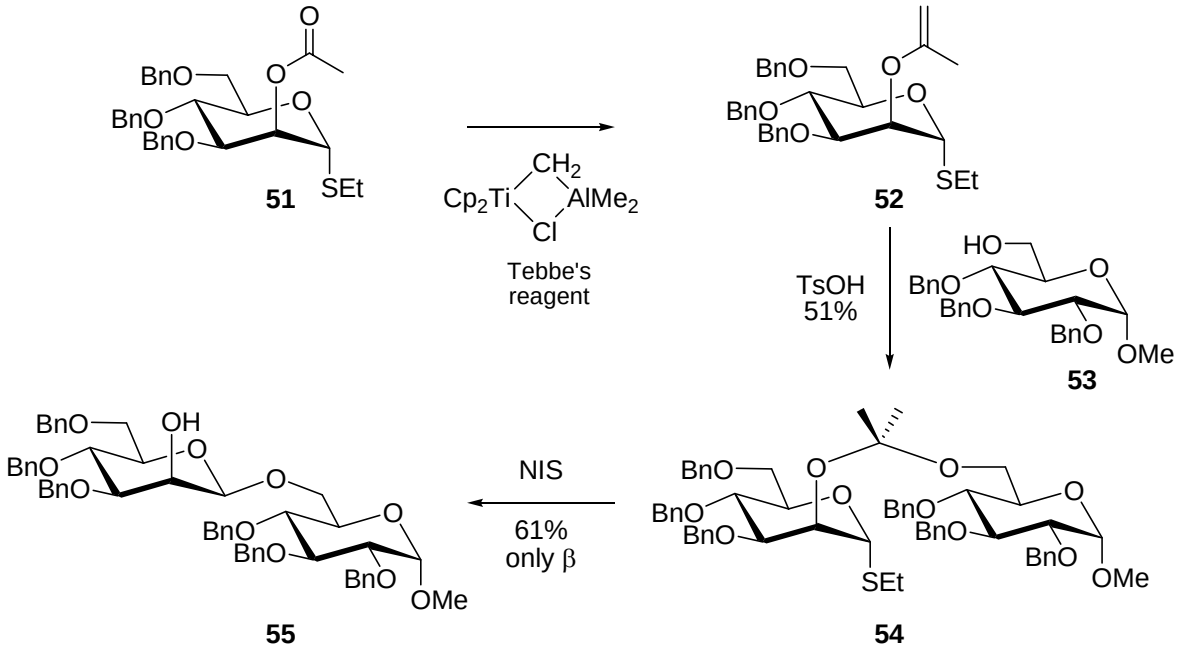
The **intramolecular aglycone delivery (IAD)** method is a new and effective strategy developed to improve stereocontrol and yields in difficult glycosylations. It was introduced by Hindsgaul and coworkers for the preparation of β-D-mannopyranosides.[116] The strategy involves the initial covalent attachment of the glycosyl acceptor to a group on O-2 of the glycosyl donor (*Scheme 1.29*). Activation of the anomeric leaving group in the tethered derivative **47** by an electrophile is then expected to proceed intramolecularly *via* a concerted mechanism. Thus, the derivative **47** results in formation of **49** via **48**. On quenching with water, **49** releases O-2 and yields the glycoside **50**.



SCHEME 1.29 Intramolecular Aglycone Delivery.

This concept was implemented using isopropylidene ketal type tethering. As shown in *Scheme 1.30*, the 2-*O*-acetyl thioglycoside **51** was transformed into the isoprenyl ether **52** using Tebbe's reagent. Acid-catalyzed addition of the glycosyl acceptor **53** afforded the tethered derivative **54**. Glycosylation by activation of the thioglycoside with NIS afforded the β-mannopyranoside **55** with excellent stereoselectivity.

[116] Barresi, F.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 9376.



SCHEME 1.30 Intramolecular Aglycone Delivery Using Isopropylidene Ketal Tethering.

The method worked well on simple molecules; however its exstension to higher oligosaccharides and complex structures had limited success.[117] Numerous variations of the above intramolecular glycosylation were studied, including changes in the position of linkers and variations in the length, the type and the rigidity of the linker.[118]

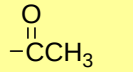
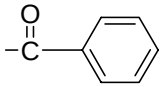
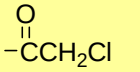
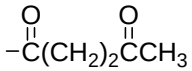
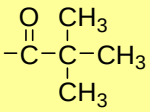
Regioselectivity of Glycosidation Reactions

Glycosylation reactions have to fulfil the essential requirement of regioselectivity. This issue is generally settled by the use of protection strategies. Protecting group manipulations represent an essential aspect of all total syntheses of organic molecules. This is particularly true in oligosaccharide synthesis, because of the multifunctional nature of carbohydrates. Most of the functional groups present on these molecules are of the same sort - hydroxyl groups – and necessitate regioselective protection strategies. As anticipated previously, all the functional groups that could potentially compete with the desired nucleophile in the attack on glycosyl donor are, generally, suitably protected.[119] When a simple disaccharide has to be synthesized, theoretically, only one kind of protecting group can be used for both reaction partners. Even in this simple case, however, regioselective protecting group manipulations have to be exerted on both molecules, in order to differentiate the anomeric center from the other hydroxyl groups of glycosyl donor and regioselectively protect all the nucleophilic groups on the acceptor but the one desired. All the protecting groups used in carbohydrate chemistry have to be stable in glycosylation conditions and must be installed and removed without compromising the stability of both saccharide ring and the sensitive glycosidic bond.

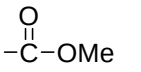
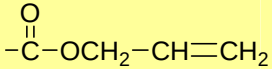
[117] Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447.
[118] a) Ito, Y.; Ogawa, T. *Angew. Chem. Int. Ed.* **1994**, *33*, 1765; b) Steward, C. M. P.; Cumpstey, I.; Aloui, M.; Ennis, S. C.; Redgrave, A. J.; Fairbanks, A. J. *Chem. Commun.* **2000**, 1409; c) Jung, K. –H.; Müller, M.; Schmidt, R. R. *Chem. Rev.* **2000**, *100*, 4423.
[119] See *Scheme 1.2,* page 5.

HYDROXYL PROTECTING GROUPS

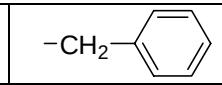
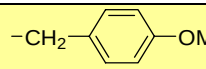
Esters

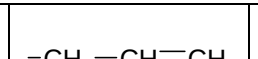
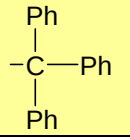
ACETYL		Ac
BENZOYL		Bz
CHLORO-ACETYL		ClAc
LEVULINOYL		Lev
PIVALOYL		Piv

Carbonates

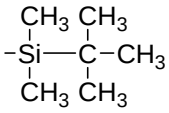
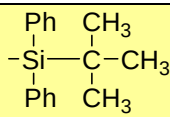
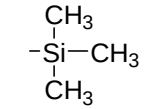
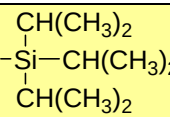
METHOXY-CARBONYL		
ALLYLOXY-CARBONYL		Alloc

Ethers

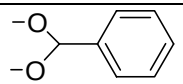
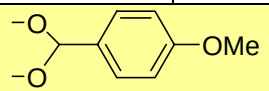
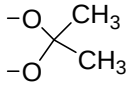
BENZYL		Bn
P-METHOXY-BENZYL		*p*MBn

ALLYL		All
TRITYL		Tr

Silyl ethers

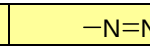
*TERT*BUTYL DIMETYLSILYL		TBDMS
*TERT*BUTYL DIPHENYLSILYL		TBDPS
TRIMETHYLSILYL		TMS
TRIISOPROPYL SILYL		TIPS

Acetals

BENZYLIDENE		Bzd
P-METHOXY-BENZYLIDENE		
ISOPROPYLIDENE		

AMINO PROTECTING and MASKING GROUPS

Masking Groups

AZIDO		

Protecting Groups

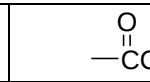
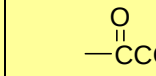
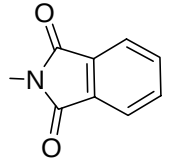
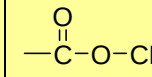
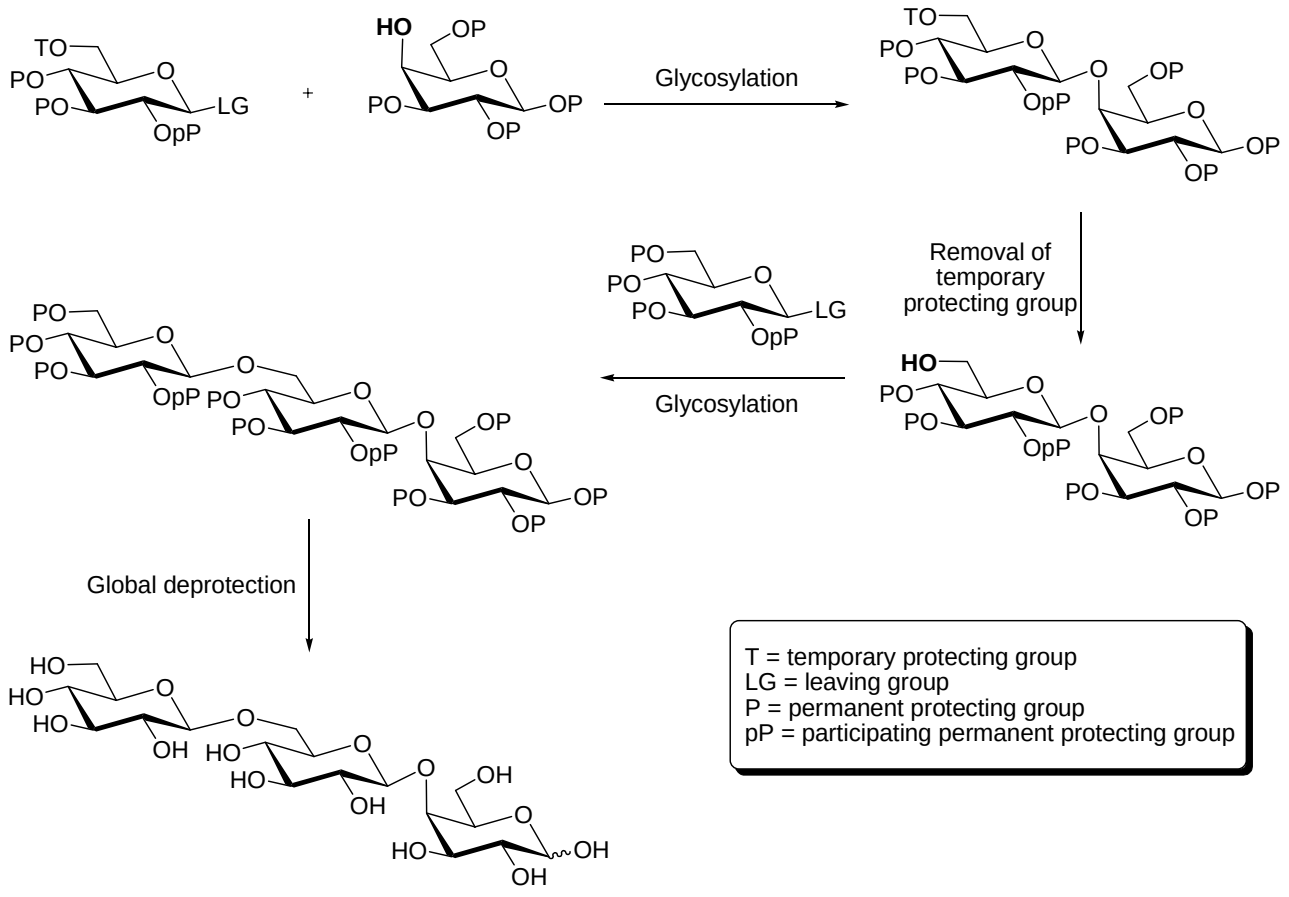
ACETAMIDO		Ac
TRICHLORO-ACETAMIDO		TCA
PHTALIMIDO		NPhth
TRICHLORO-ETHYL-CARBAMATE		Troc

TABLE 1.3 Protecting Groups Most Commonly Employed in Carbohydrate Chemistry.

These requirements limit the number of protecting groups that are used in carbohydrate chemistry, if compared with those used in other fields of organic chemistry. On the other hand, like in any other area of organic synthesis, in oligosaccharide syntheses it's extremely crucial to rely on a wide range of protecting groups, in order to project efficient synthetic strategies.

By far, the most important protecting groups in carbohydrate chemistry are those used for the protection of hydroxyl groups, including the anomeric hemiacetal. Amino-protecting groups (for amino-deoxy sugars) and carboxyl protection (for uronic acids) are also of interst. In *Table 1.3* the protecting groups most frequently used in carbohydrate chemistry are illustrated. Several procedures can be followed for their installation and cleavage also in regioselective fashion.[120]

In oligosaccharide synthesis beyond the level of disaccharides, **temporary and permanent** protecting groups must frequently be distinguished. Permanent protecting groups will remain through all synthetic steps until the liberation of final target oligosaccharide. The removal of temporary protecting groups during the synthetic sequence will reveal a free hydroxyl group, most often to produce a glycosyl acceptor ready for glycosylation (*Scheme 1.31*).



SCHEME 1.31 Schematic Synthesis of a Trisaccharide.

The two different types of protecting groups require different qualities. It must be possible to introduce and remove permanent groups in bulk with regiocontrol and high efficiency.

[120] a) Green, T.; Wuts, P. G. M., *Protective Groups in Organic Synthesis*, 3rd ed., Wiley, New York, **1999**; b) Kocienski, P. *Protecting Groups*, Georg Thieme Verlag, Stuttgart, **2000**.

Obviously, they should be stable to conditions used for the removal (and introduction) of temporary groups. In the synthesis of branched structures or structures with substituents (phosphates, sulfates, acetates, etc.), it might be required the preparation of building blocks whose hydroxyl groups have to be differentiated from each other by orthogonal sets of temporary protecting groups. The idea of **orthogonal protection** was defined by Baranay and Mettifield as "a set of completely independent classes of protection groups, such that each class can be removed in any order and in the presence of all other classes".[121] As summarized in *Figure 1.10*, when individual hydroxyl groups are protected with A, B, C, and D, respectively, and individual protecting groups can be removed in any order under certain conditions, the protecting groups can be said to be in an orthogonal relationship. A representative set of orthogonal hydroxyl protecting groups successfully used in carbohydrate chemistry is A: chloroacetyl (a: $NaHCO_3$/MeOH/H_2O), B: methoxybenzyl (b: TFA/CH_2Cl_2), C: levulinoyl (c: NH_2NH_2/AcOH/THF/MeOH), and D: TBDPS (d: HF/Py/AcOH/THF).[122]

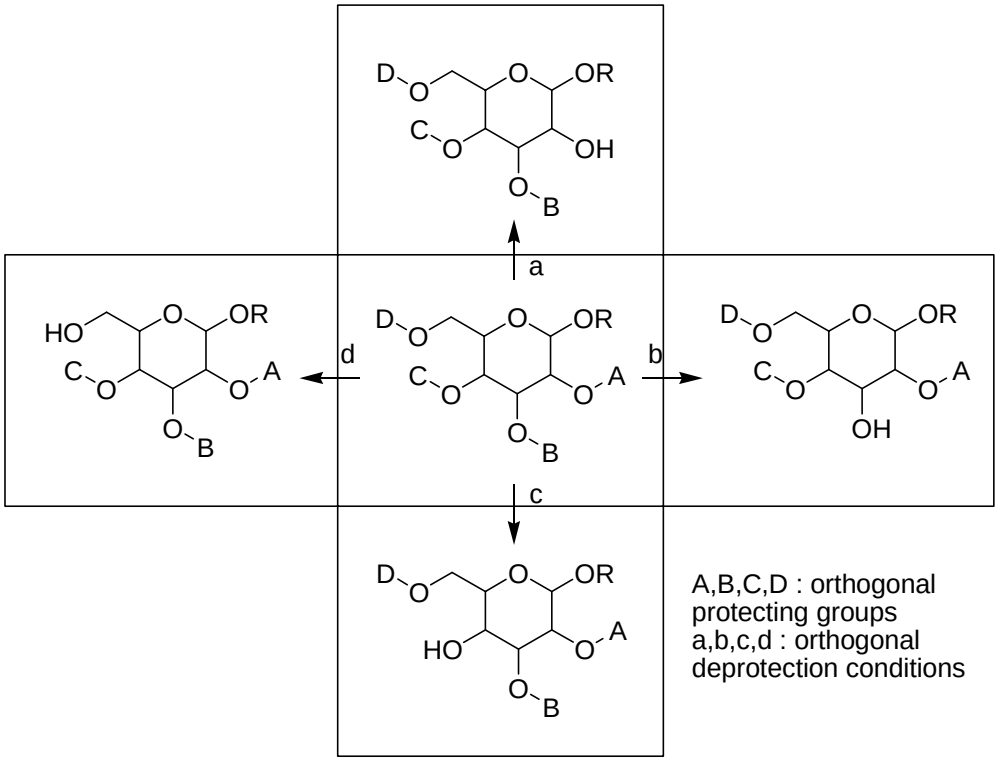
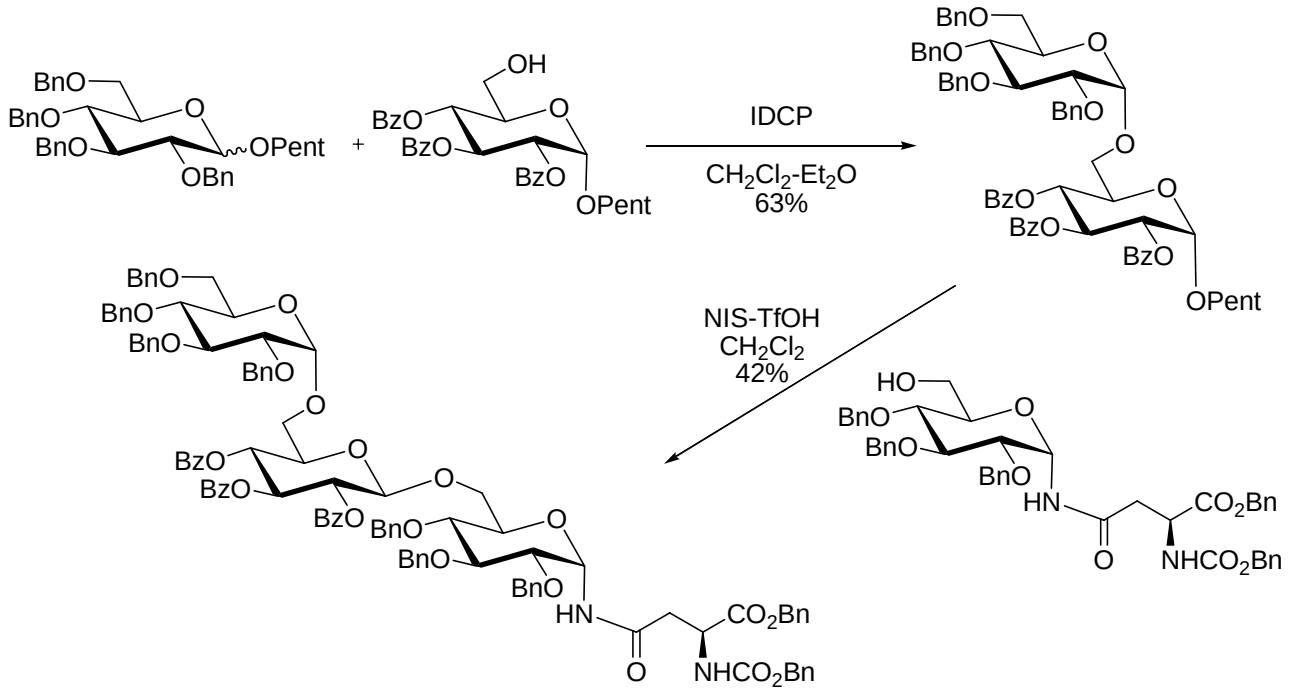


FIGURE 1.10 Orthogonal Protecting Group Manipulations.

Preferably only one type of permanent protecting group should be used, to allow only one final deprotection step, an aspiration seldom possible to realize. Very few of the large numbers of protecting groups available fulfil the criteria for permanent protecting groups. More or less only acetates, benzoates, benzyl ethers, benzylidene and isopropylidene acetals have the stability and, at the same time, the efficient introduction/deprotection properties needed to make them suitable for this purpose.
When planning the protection strategy, several issues have to be considered, first of all it has to be taken into account that protecting groups can modify the reactivity of the

[121] Barany, G.; Merrifield, R. B.; *J. Am. Chem. Soc.* **1977**, *116*, 7363.
[122] Wong, C. –H.; Ye, X. –S.; Zhang, Z. *J. Am. Chem. Soc.* **1998**, *120*, 7137.

molecules they are installed on. As examined in details in the previous paragraph, some protecting groups can direct the stereochemical outcome of glycosylations *via* neighbouring or long range participation effects or because of torsional constraints.
Furthermore, the intrinsic reactivity of glycosyl donors during glycosylations is strictly dependent by the nature of the protecting groups used for their protection. A major difference in reactivity between perbenzylated and peracylated derivatives was first observed in the case of *n*-pentenyl glycosides. Specifically, the acylated glycosyl donors react at much slower rates. This observation resulted in the development of the ***armed-disarmed*** concept, now widely extended also to other glycosyl donor classes.[106] A disarmed (acylated) *n*-pentenyl glycoside with a free hydroxyl group could be glycosylated by an armed (benzylated) *n*-pentenyl glycoside without self-condensation of the previous one (*Scheme 1.32*).[123]



SCHEME 1.32 Oligosaccharide Synthesis by Armed-Disarmed Glycosylations.

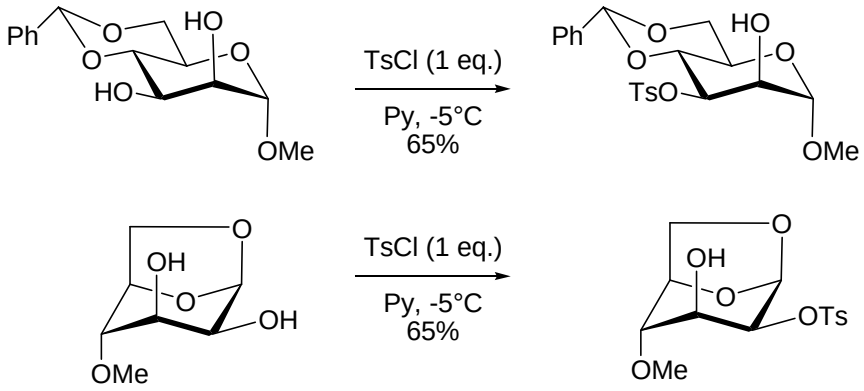
A rationalization of this effect can be advanced considering the effects that ether- and acyl-type protecting groups have on the carbocationic intermediates **56** and **57**, that are formed in the glycosylation medium after the departure of the leaving group from the glycosyl donor (*Figure 1.11*).

FIGURE 1.10 Carboxonium Ion Intermediates.

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

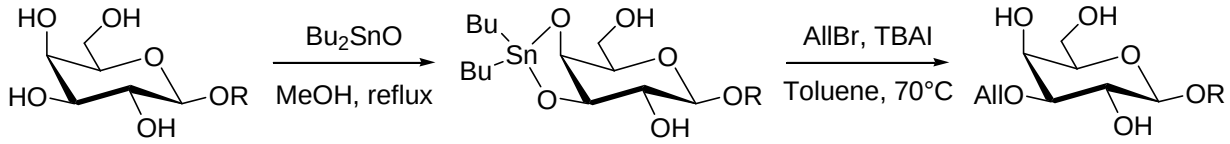
The electron-withdrawing acyl groups destabilize the cationic intermediate **57**. The parent glycosyl donor is, thus, less prone to be activated and converted into **57** with the overall effect of deactivation of the glycosylating capability. In the case of ether-type protecting groups, the destabilizing effect is less strong and the corresponding glycosyl donors are more reactive (armed).
The arming-disarming effect of protecting groups is also used for tuning the reactivity of glycosyl donors. Deoxy sugars, such as fucose or rhamnose, when equipped with a suitable leaving group on the anomeric center, are extremely reactive glycosyl donors. Glycosylations involving these kind of donors generally lead to formation of considerably amounts of lactols as products of hydrolysis side-reaction. In order to decrease their reactivity and, thus, the amount of degradation by-products, acyl protecting groups can be used.
So far, it has been described the crucial role played by protecting groups in the synthesis of saccharides and the accurate study required for planning a protection strategy. It's clear that for effectively synthesize complex oligosaccharidic targets differently protected precursors have to be prepared. For this scope, it's essential to dispose of synthetic methodologies for distinguishing between the several hydroxyl groups present on the saccharidic backbone in order to regioselectively install a given protection on a determinate position.[124]
General considerations on the reactivity of the various hydroxyl groups present on the sugar ring have to be discussed. In aldo-hexo-pyranoses three different kind of hydroxyl groups can be distinguished: the hemiacetalic at C-1, the primary at C-6, and three secondary hydroxyl groups at C-2, C-3 and C-4. The reactivity of the hemiacetalic OH is clearly different from the others. Unlike the other positions, the anomeric center in acidic conditions undergoes nucleophilc attack and, because of this, can be easily differentiated.
Installation of a temporary protecting group at the anomeric position in Fischer conditions is generally the first operation of a multi-step building block synthesis.
Most of the procedures for protecting groups installation exploit the nucleophilicity of the hydroxyl functions of the sugars. In these cases the primary position is appreciably more reactive than the other non-anomeric positions. The primary alchool can be effectively chemoselectively functionalized with a bulky protecting group (such as DMT, TBDMS, TIPS, etc.) in the presence of free secondary hydroxyl groups.
The ability of distinguishing between the three secondary position is strongly dependent by their configuration. Consistent with well-known generalizations from cyclohexane chemistry, equatorial secondary hydroxyl groups of pyranoid compounds tend to react more readily than axial groups as illustrated in *Scheme 1.33*, which involves two D-mannopyranosyl compounds in the $^4C_1$ and $^1C_4$ conformations, whose hydroxyl groups at C-2, C-3 thus are axial, equatorial and equatorial, axial, respectively.[125]
Further generalizations are difficult to make, although it is frequently noted that the hydroxyl group at C-2 of glucopyranosides is the most reactive of the secondaries while that at C-4 is least so.

[124] For a wide treatment of selective manipulations on saccharide backbone: Collins, P., Ferrier, R. In *Monosaccharides -Their Chemistry and Their Role in Natural Products-*, John Wiley & Sons: Chichester, **1995**.
[125] Aspinall, G. O.; Zweifel, G. *J. Chem. Soc.* **1957**, 2271.



SCHEME 1.33 Regioselective Protection of Equatorial Hydroxyl Groups.

Formation of trialkylstannyl ethers or cyclic stannylidene derivarives enhances the nucleophilicity of the oxygen atoms involved and thus activates the hydroxyl groups towards electrophilic reagents.[126] Selective stannylation or stanniylidene formation can consequently alter relative reactivities within polyhydroxyl compounds, and practical use can be made of this finding. Cyclic stannylidene acetals are generally formed by reaction of dibutyltin oxide with a *cis* vicinal diol. The stannylidene generation is generally followed by the *in situ* addition of an electrophile that preferentially reacts with only one of the two oxygen atoms involved in the acetal cycle. The tin chemistry is effectively used for the regioselective protection of the C-3 equatorial hydroxyl groups that are in a *cis* relationship with the axial C-4 and C-2 hydroxyl groups of galacto- and manno-pyranoses, respectively (*Scheme 1.34*).



SCHEME 1.34 Generic Regioselective 3-*O*-Allylation *via* Stannilidene Acetal Intermediate Formation.

A very useful approach for the differentiation of the several alcohol moieties of monosaccharides is the use of cyclic protecting groups for diols, such as benzylidene and isopropilidene acetals, orthoesters and orthocarbonates. These strategies rely on strict spatial relationships that have to be fulfilled by the hydroxyl groups to be protected. Benzylidene acetals are generally regioselectively installed on C-4 and C-6 position, thus forming a stable six-memberd pyranose ring; isopropylidene acetals as well as carbonates require a 1,2-*cis* relationship between the two hydroxyl groups; orthoesters are often formed involving C-1 and C-2 positions (*Figure 1.11*).
As evident from *Figure 1.11*, blocking at least two out of five hydroxyl groups enormously simplifies the further manipulations required for the completion of the synthetic pathway. It has also to be considered that the use of benzylidene acetals and orthoesters allows further differentiation of the two oxygen atoms involved in the protection.

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

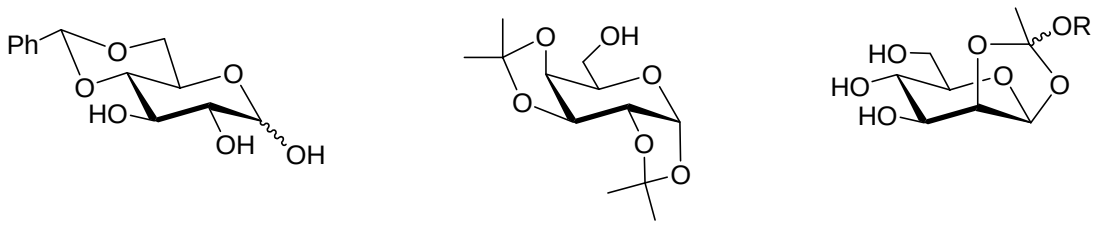
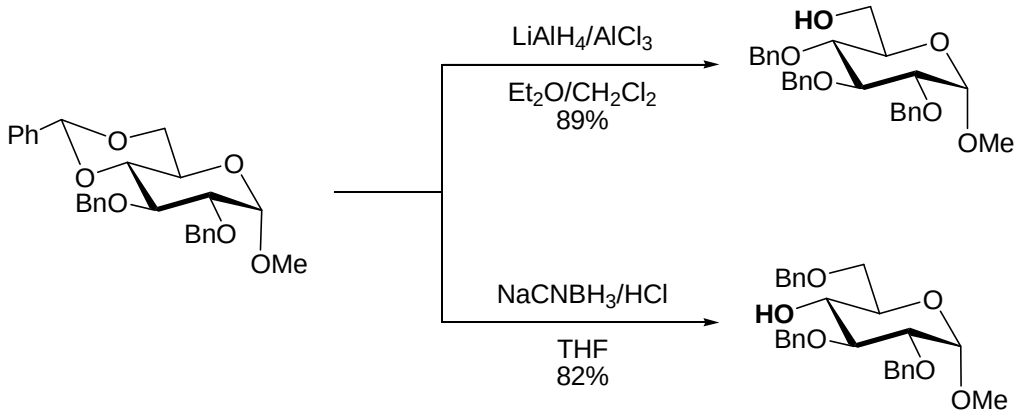


FIGURE 1.11 4,6-*O*-Benzylidene-D-Glucopyranose, 1,2:3,4-Di-*O*-Isopropylidene-D-Galactopyranoside and D-Mannopyranose 1,2-Orthoacetate.

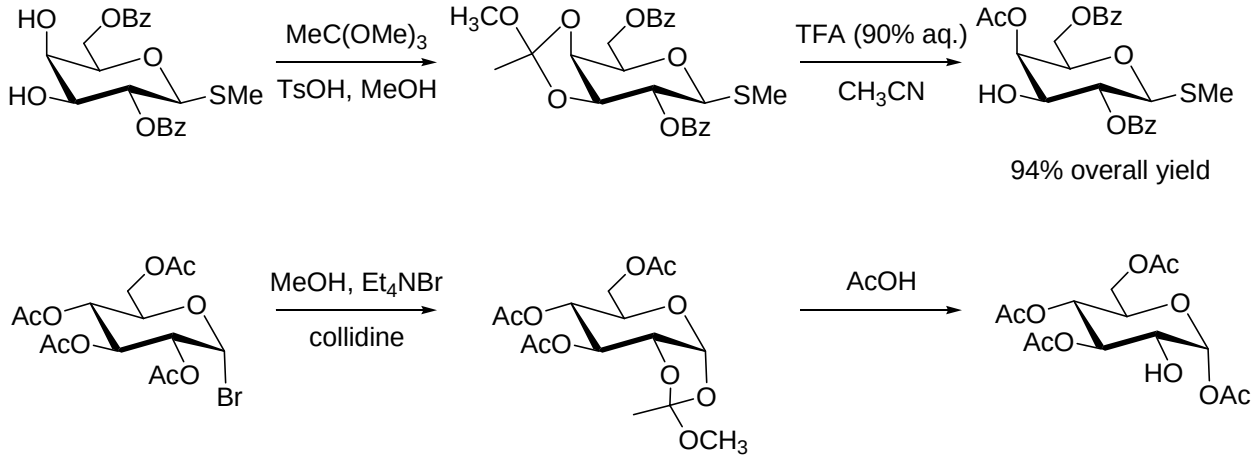
In the case of 4,6-*O*-benzylidene acetals, efficient methods have been developed for the regioselective reductive opening of the acetal to yield benzyl ethers and a free hydroxyl group. The control of the regioselectivity of the reaction is exerted by the choice of the suitable reducing system (*Scheme 1.35*).[127]



SCHEME 1.35 Reductive Cleavage of Benzylidene Acetals.

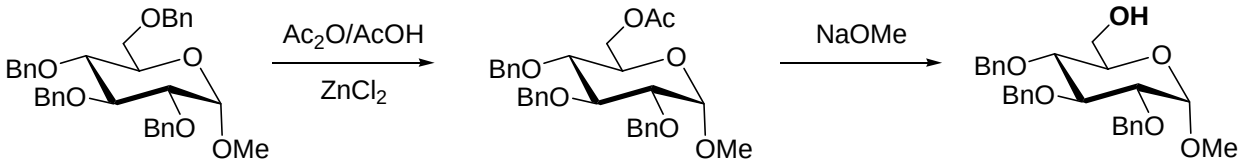
Orthoesters undergo ring opening by mild acidic hydrolysis to yield the corresponding ester derivative exposing a hydroxyl for further reactions.[128] Interestingly, the selectivity is opposite to many other methods, since opening of an orthoester protecting a vicinal *cis*-diol gives the ester on the axial hydroxyl group (*Scheme 1.36*).
Care has to be taken to avoid acyl migration to the uncovered equatorial hydroxyl group, using, for example, orthobenzoates instead of orthoacetates, since benzoates migrate more slowly than acetates.

[127] 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.
[128] Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *15*, 4069.



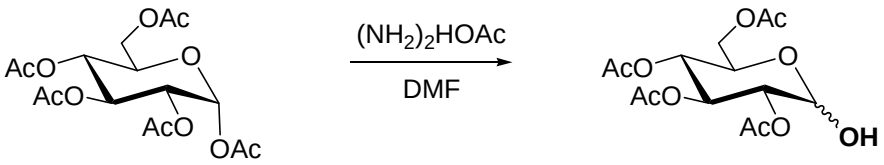
SCHEME 1.36 Orthoester Formation and Opening.

The methods to achieve regioselectively protected derivatives by selective deprotection are less common, however, a few standard procedures utilize this approach.[129] The rate difference in acetolysis of primary (as compared with secondary) benzyl ethers is high enough to allow selective removal. The obtained 6-*O*-acetate can then be removed to expose the 6-hydroxyl group (*Scheme 1.37*).[130]



SCHEME 1.37 Regioselective Removal of Primary Benzyl Group.

Furthermore, anomeric esters are more labile than other esters and can be removed selectively by mild base treatment (*Scheme 1.38*).

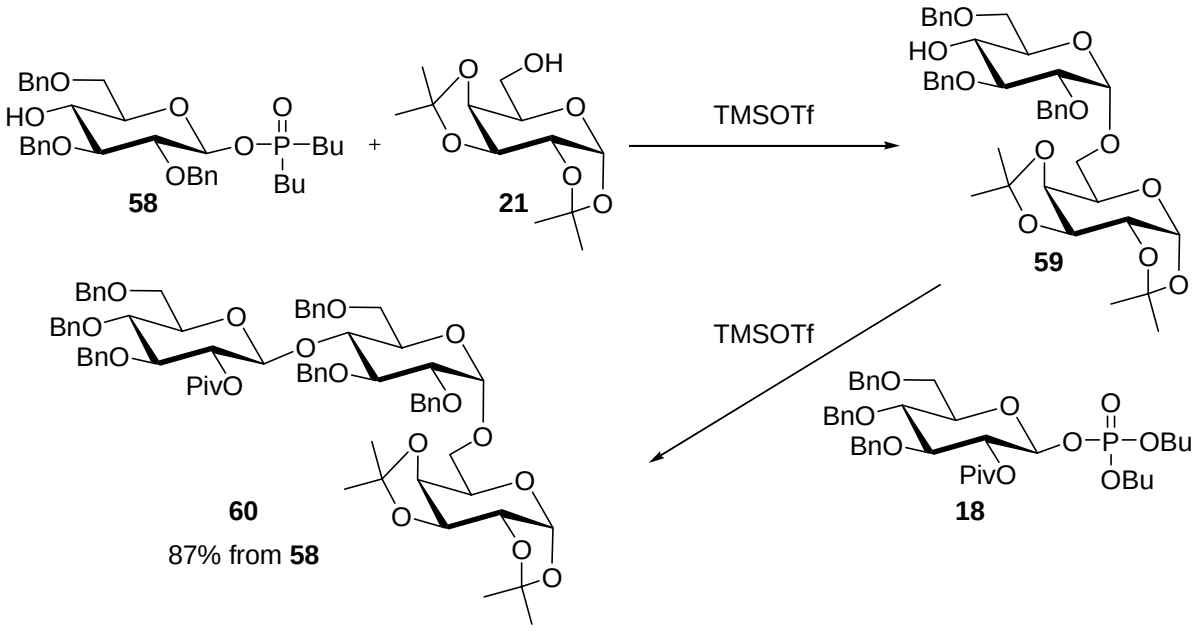


SCHEME 1.38 Regioselective Removal of Anomeric Acetate.

All the so far described protection strategies are applied in the syntheses of the variously functionalized building blocks that are then used in the oligosaccharide assembly. Often several consecutive protection-deprotection steps are required to obtain the desired monosaccharidic targets. Generally, the preparation of the monomeric precursors represents the most time-consuming and, at the same time, challenging issue in oligosaccharide syntheses. The excellence of a synthetic plan is strongly dependent by the

[129] Haines, A. *Adv. Carbohydr. Chem. Biochem*. **1981**, *39*, 13.
[130] Yang, G.; Ding, X.; Kong, F. *Tetrahedron Lett.* **1997**, *38*, 6725.

protection strategies adopted. Of course, the fewer protecting group manipulations, the better the synthesis is valued.
An elegant approach in this sense is represented by regioselective glycosylation strategies, where the intrinsic differences in nucleophilicity of different hydroxyl groups are used to drive the regiochemistry of glycosylation reactions. Very often a primary hydroxyl group is glycosylated in the presence of a less reactive secondary position, thus building a disaccharide with an unprotected secondary hydroxyl that can be further glycosylated. In *Scheme 1.39* the regioselective glycosylation of acceptor **21** using C4-OH glucosyl phosphate **58** as glycosylating agent is reported.[87]
Fundamental to this approach was the use of the central building block **58** capable of displaying both donor and acceptor properties. Activation of donor **58** at -78 °C in the presence of the primary alcohol **21** afforded β-(1→6)-linked disaccharide **59** bearing a C-4 hydroxyl group in excellent yield (94%) as the only coupled product. Subsequent glycosylation of disaccharide **59** containing a unique C-4 hydroxyl with glucosyl phosphate **18** provided trisaccharide **60** in 72% yield in a one-pot fashion.
This strategy rely on the huge difference in reactivity between the primary position and the C-4 hydroxyl (the less reactive on the glucosidic backbone).
However the usual regioselectivity can be increased or even reversed manipulating the protection pattern of the positions that surround the hydroxyl group directly involved in the glycosylation reaction. The absence of a protecting group in a neighbour position generally induces an improvement in the coupling yields.



SCHEME 1.39 Regioselective Glycosylation with C4-OH Glucosyl Phosphate **58**.

On the contrary, the use of a bulky protecting group next to a nucleophilic center reduces its reactivity favouring the involvement of positions otherwise less prone to be glycosylated. A renowned example is given by the synthesis of Lewis^x trisaccharide (*Figure 1.12*).

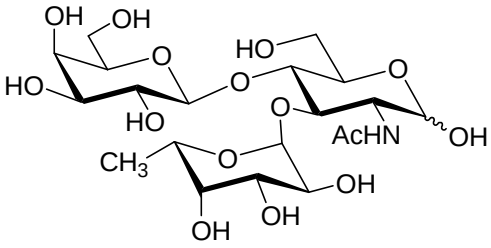
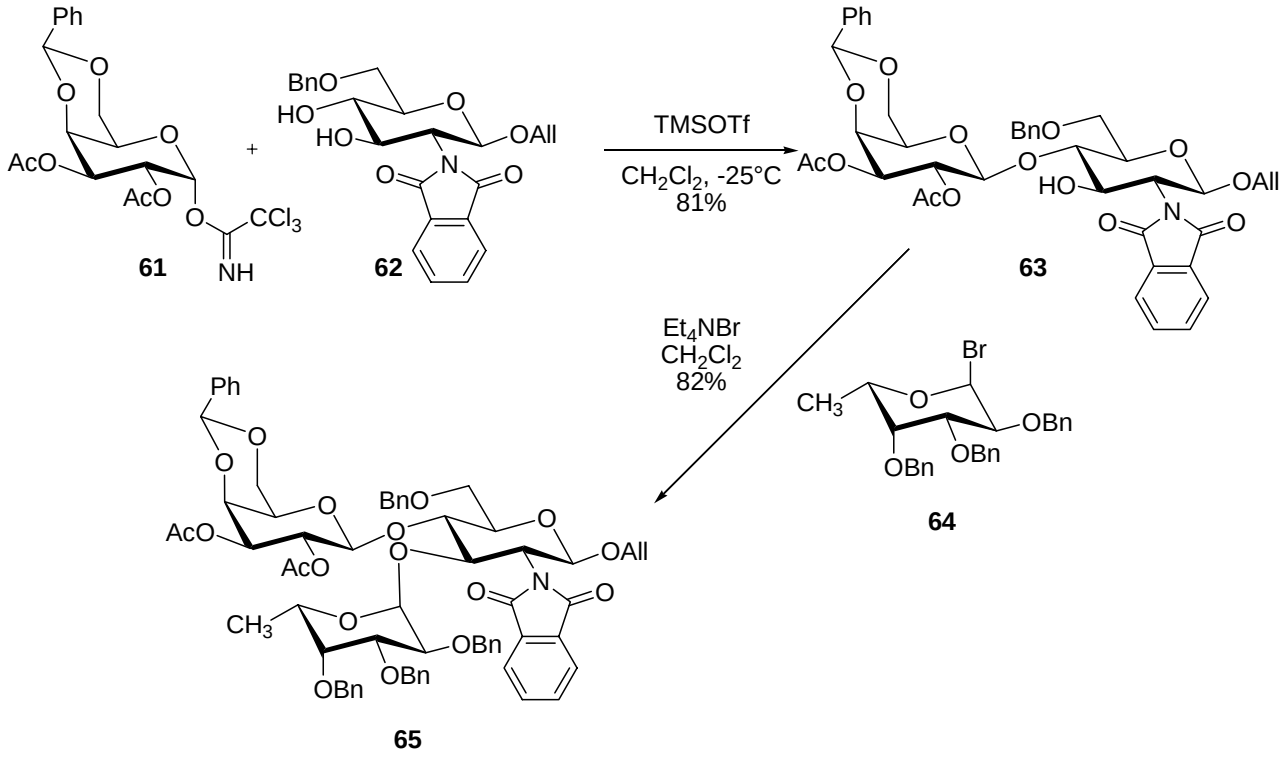


FIGURE 1.12 Lewis^x Trisaccharide.

Several straightforward strategies have been developed making use of regioselective glycosylation process at crucial steps.[131] Most of them rely on the possibility to reverse the usual reactivity of a 3,4-diol in a glucosamine acceptor by the use of phtalimido function at C-2.[132]

In *Scheme 1.40* the synthesis of the protected form of Lewis^x trisaccharide proposed Verez-Bencomo and coworkers is reported.[133] Complete regiospecific galactosylation of diol acceptor **62** bearing a phtalimido protecting groups was attained. The presence of a *cis*-decalin bicyclic system on galactosyl donor **61** had already shown to have a profound effect on the donor properties.[134]



SCHEME 1.40 Regioselective Lewis^x Trisaccharide Synthesis.

[131] a) Nunomura, S.; Iida, M.; Numata, M.; Sugimoto, M.; Ogawa, T. *Carbohydr. Res.* **1994**, *263*, C1-C6; b) Jain, R. K.; Vig, R.; Locke, R. D.; Mohammad, A.; Matta, K. L. *Chem. Commun.* **1996**, 65; c) Xia, J.; Alderfer, J. L.; Piskorz, C. F.; Matta, K. L. *Chem. Eur. J.* **2000**, *18*, 3442.

[132] a) Ehara, T.; Kameyama, A.; Yamada, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1996**, *281*, 237; b) Zou, W.; Jennings, H. *Biorg. Med. Chem. Lett.* **1997**, *7*, 647; c) Huang, B. G.; Locke, R. D.; Jain, R. K.; Matta, K. L. *Biorg. Med. Chem. Lett.* **1997**, 1157.

[133] Figueroa-Pérez, S.; Verez-Bencomo, V. *Tetrahedron Lett.* **1998**, *39*, 9143.

[134] Qui, D.; Ganghi, S. S.; Koganty, R. R. *Tetrahedron Lett.* **1997**, *37*, 595.

The β-(1→4) disaccharide **63** was obtained in a 81% yield with complete regio- and stereoselectivity. Bromide ion catalyzed fucosylation of disaccharide **63** proceeded with complete stereoselectivity to provide the trisaccharide **65** in 82% yield.

Oligosaccharide Synthesis

For the successful synthesis of oligosaccharides, both chemical reactions and tactics are important concerns. All the methodologies developed for effectively promote, stereo- and regio-chemically direct glycosylation reactions have to be skilfully combined in order to achieve the desired oligosaccharide targets in high yields and reasonable time.
For the efficient synthesis of oligosaccharides, **stepwise** or **convergent** methods can be employed (*Figure 1.13*).

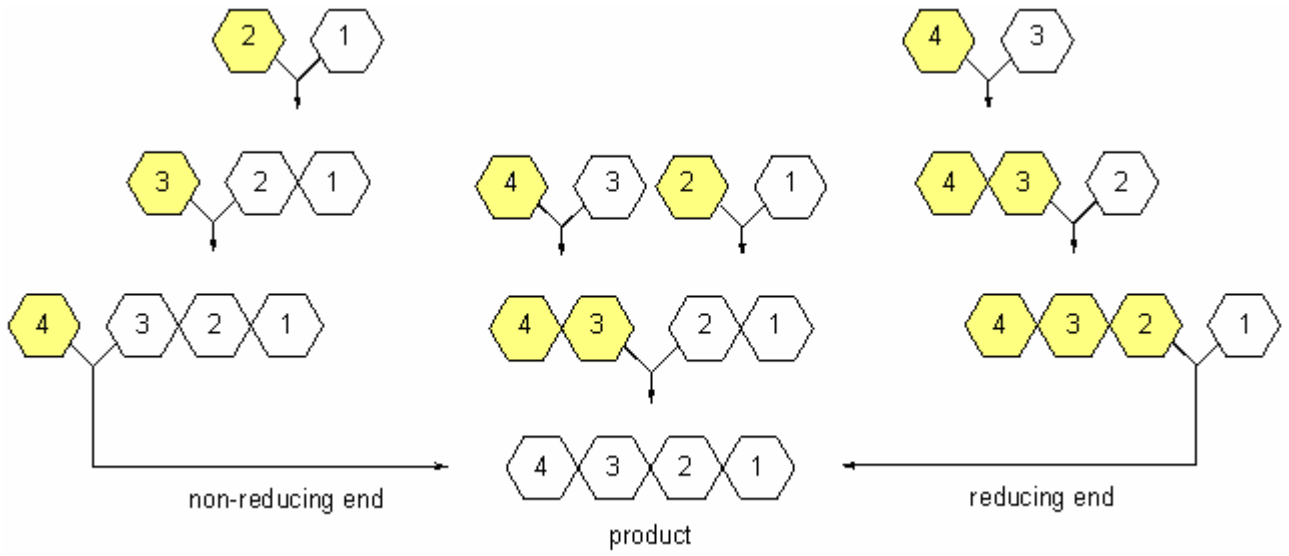
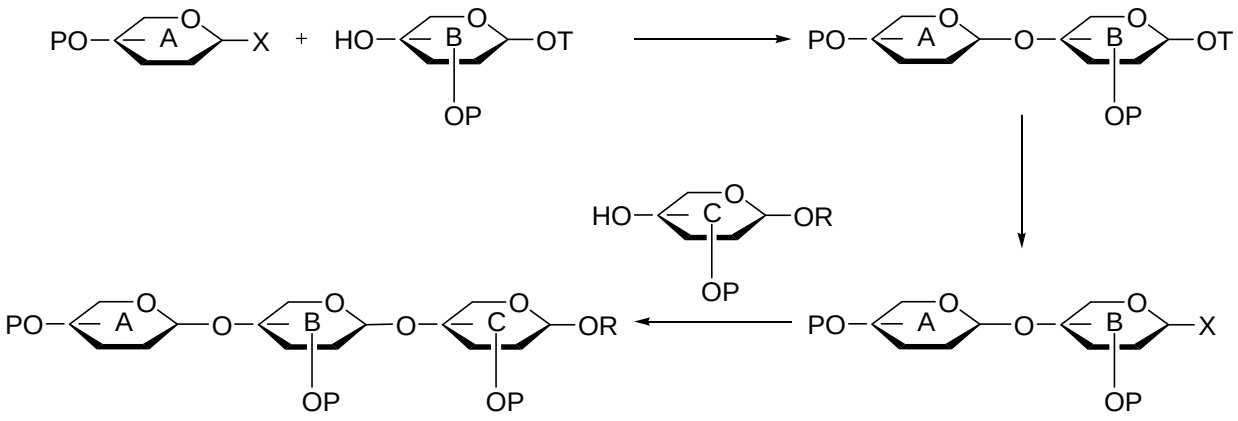


FIGURE 1.13 Stepwise and Convergent Methods in the Synthesis of Oligosaccharides.

In the stepwise format the synthesis might start either from the reducing or the non-reducing end. The first approach is generally more convenient. Because of their reactivity, during glycosylations most glycosyl donors often undergo side-reactions (hydrolysis, rearrangement, etc.). Thus, in order to maximize coupling yields, they are used in slight excess compared with glycosyl acceptors. Starting from the non-reducing end implies the use of oligosaccharide donors and, therefore, the waste of precious oligosaccharide structures in case of decomposition. The use of monosaccharide donors and oligosaccharide acceptors is preferable instead. Unreacted glycosyl acceptors can be easily recovered from the glycosylation medium and directly reused. Hydrolysis or decomposition products require more synthetic steps to be converted into the parent glycosyl donors. The recent development of new anomeric protecting groups and some substituent groups that can be easily converted into leaving groups, however, has enabled the alternative format (starting from the non-reducing end) to be used.
The concept of stepwise synthesis is especially important for the construction of relatively small oligomers, but the convergent format is preferably employed for the synthesis of larger saccharides, especially in the syntheses of oligosaccharides possessing repeating

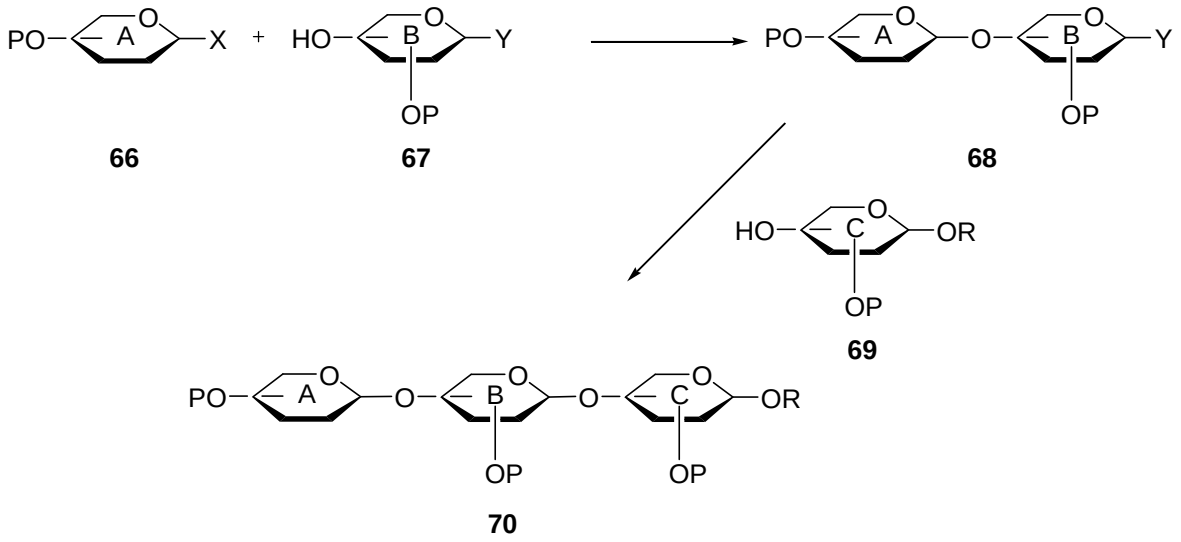
units in their structures. Furthermore, block syntheses reduce the overall number of steps and the convergent nature of this strategy makes it more efficient. Also, block syntheses afford greater flexibility in synthetic design. Specifically critical steps can be performed at early stages on smaller molecules, thereby facilitating chromatographic separations.
In block syntheses, the reducing end unit of a block has to fulfil opposite requirements. It should be stable enough to serve as a glycosyl acceptor in the synthesis of the block and, at the same time, it should be reactive enough to serve as glycosyl donor in the assembly of the oligosaccharide from the blocks. Several strategies have been adopted to settle this issue.
One solution to this problem is to **exchange the anomeric substituent** after the synthesis of the block. During the synthesis of the block, the reducing end is protected by a temporary protecting group (T), that is then removed converting the block into a glycosyl donor with a leaving group (X) at the reducing end (*Scheme 1.41)*.



SCHEME 1.41 Block Synthesis by Reactivation via Exchange of the Anomeric Substituent.

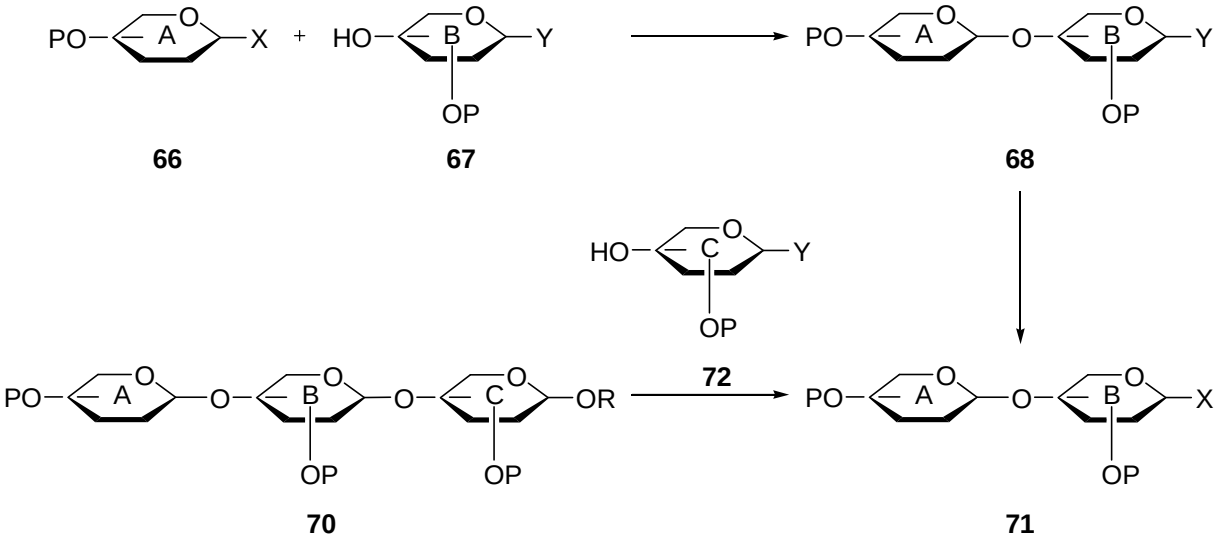
A drawback of this strategy is that the conversion of the block into a glycosyl donor requires several steps, which are especially undesirable in the case of larger fragments.
An alternative approach uses **different types of glycosyl donors** in sequential glycosylation steps. As *Scheme 1.42* shows, the key intermediate **67** carries an unprotected hydroxyl and has an anomeric substituent (Y) that withstand glycosylation with donor **66**. After glycosylation, the newly generated block **68** can be used directly as a donor, without any replacement of the anomeric substituent, to provide the oligosaccharide **70**. An example of this strategy is reported in *Scheme 1.15*.[135]

[135] See page 19.



SCHEME 1.42 Block Synthesis by Sequential Glycosylations with Different Types of Glycosyl Donors.

Nicolaou and coworkers proposed the **two-stage activation** strategy,[34] where two types of anomeric substituents are used. One type, having Y at the anomeric center, serves as glycosyl acceptor (**67**), the other one, possessing X, is used as glycosyl donor **66** (*Scheme 1.43*).

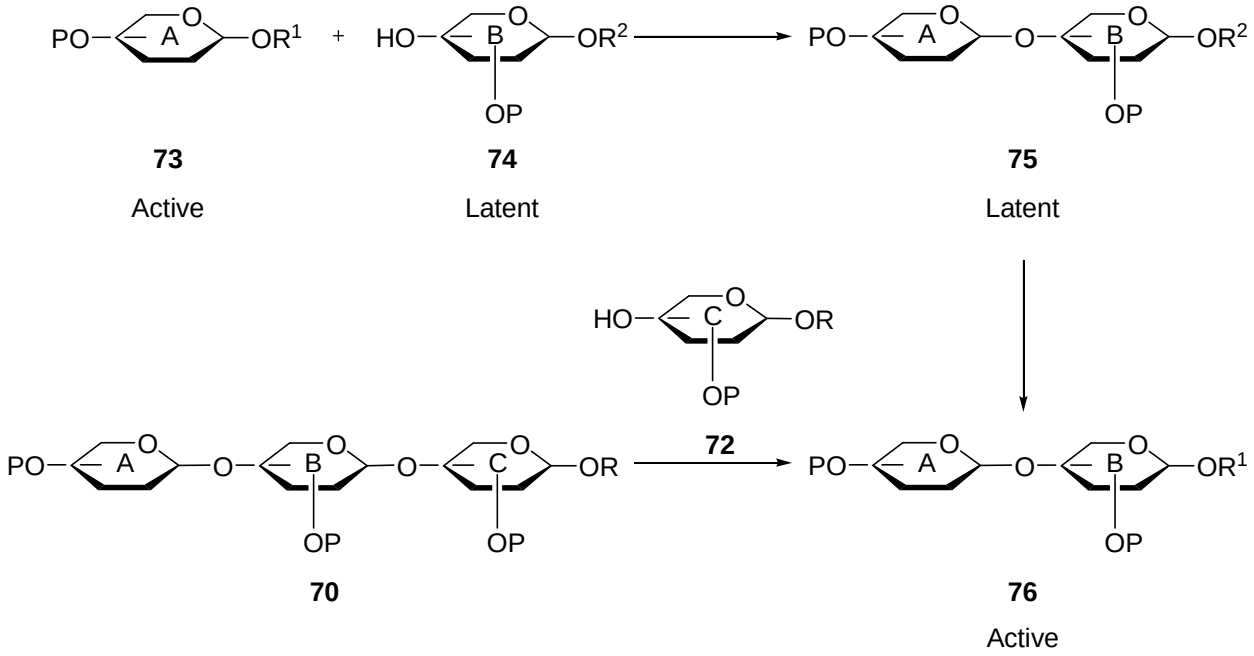


SCHEME 1.43 Block Two-Stage Activation.

After glycosylating **67** with **66**, the anomeric substituent Y in the coupling product **68** is converted back into X (**71**), so the resulting block can be used as a donor in further glycosylations. Using acceptor **72** of the same type as before makes the process suitable for further reiteration. Nicolaou used glycosyl fluorides as glycosyl donors and thioglycosides as acceptors and the procedure took advantage of the ready conversion of thioglycoside into glycosyl fluorides with NBS and DAST, as also shown in *Scheme 1.4*.[136]

[136] See page 8.

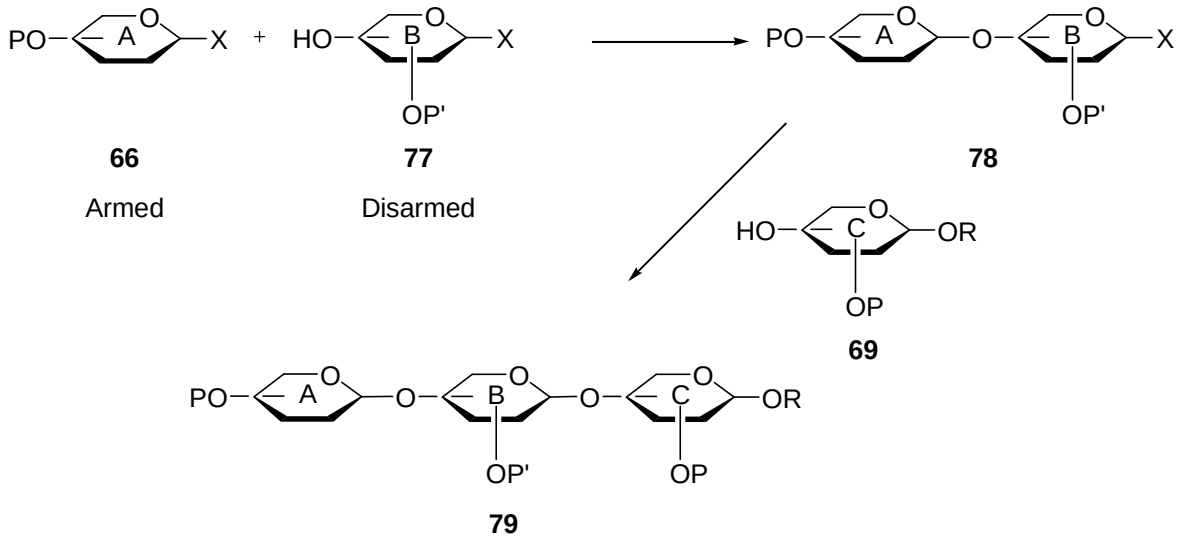
In ***active-latent*** glycosylation strategy, the glycosyl donor capability of an acceptor is turned on by a slight chemical modification in its aglycone. Glycosylation of the latent compound **74** by the active donor **73** affords the latent disaccharide **75** (*Scheme 1.44*).



SCHEME 1.44 Active-Latent Glycosylation.

Conversion of disaccharide aglycone (R^2) into an active form (R^1), transforms **75** into active glycosyl donor **76** that can be used in further chain elongations.
This approach has been used by Danishefsky in iterative glycosylations using glycals as latent compounds and 1,2-anhydro sugars as active glycosyl donors, as depicted in *Scheme 1.14*.[137]
The ***armed-disarmed*** concept[138] can also be counted among the several glycosylation strategies used in block syntheses. As shown in *Scheme 1.45* , the armed glycosyl donor **66** can be coupled with the disarmed **77** without self condensation of the latter. The resulting disarmed compound **78** can be used as a glycosyl donor in further coupling, either by arming it, by exchanging the protecting groups, or by using a more powerful promoter capable of activating the disarmed compound.
Besides general synthetic strategies, some new methods and techniques are also worthy of discussion.
In the traditional approaches of oligosaccharide synthesis, the product of a glycosylation reaction had to be isolated and it required some chemical transformations to make it suitable for the next glycosylation reaction.

[137] See page 18.
[138] See page 35.



SCHEME 1.45 Block Armed-Disarmed Glycosylation.

In some of the synthetic strategies herein described, such as in the sequential and in the armed-disarmed glycosylations, the product of one glycosylation reaction is used directly in the next coupling reaction. This opened the way for omitting the isolation step and performing multiple glycosylations in a **one-pot** fashion (*Figure 1.14*).

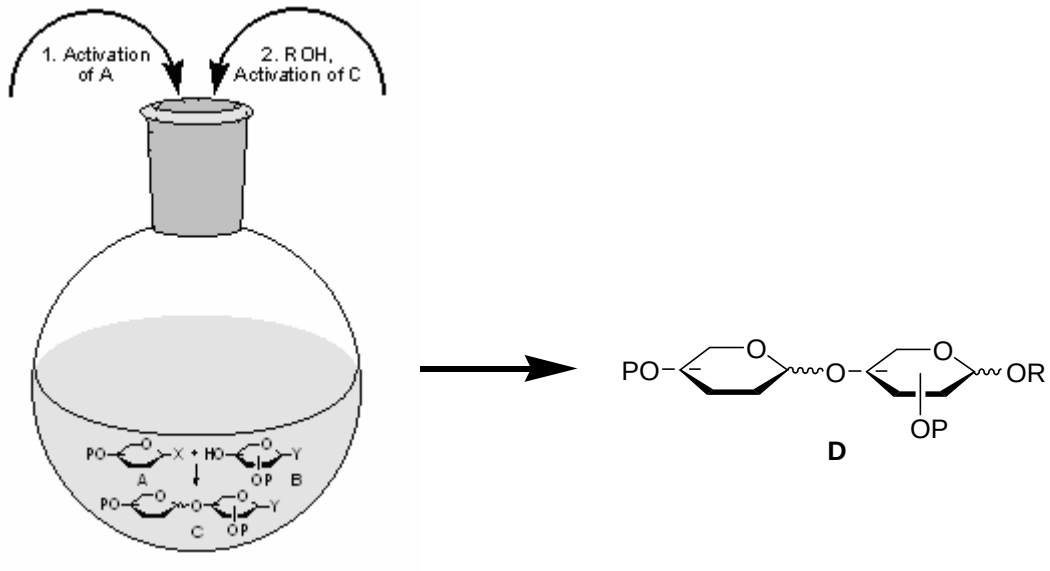
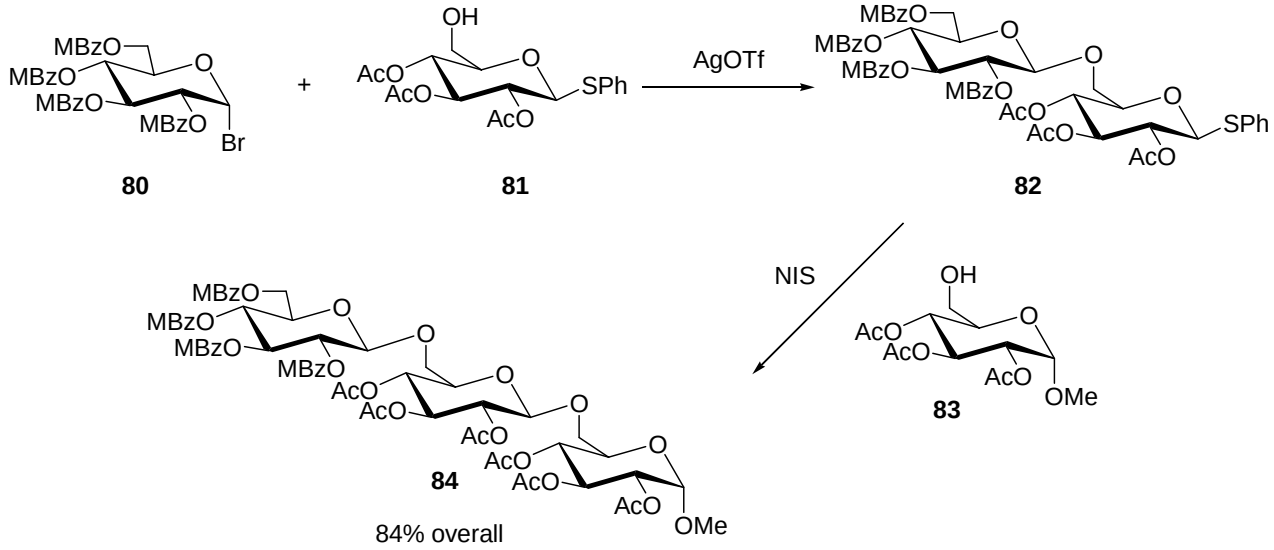


FIGURE 1.14 General Scheme of One-Pot Glycosylation.

Several glycosylation strategies can be performed in a one-pot manner. Often different types of glycosyl donors are employed in one-pot sequential glycosylations. Generally, the orthogonality between the different classes of the used donors is not required.
Glycosylation are performed starting from the non reducing end, sequentially activating glycosyl donors with increasing stability. The first one-pot glycosylation sequence using this approach performed the progressive glycosylation of thioglycoside acceptor **81** by

glycosyl bromide **80**, and then of *O*-glycoside **83** by the newly formed thioglicoside donor **82** (glycosyl bromide → thioglycoside → *O*-glycoside) (*Scheme 1.46*).[139]



SCHEME 1.46 One-pot Sequential Glycosylation Using Different Types of Glycosyl Donors.

Similar one-pot glycosylation sequences were performed using glycosyl trichloroacetimidate → thioglycoside → *O*-glycoside; glycosyl fluoride → thioglycoside → *O*-glycoside or glycosyl phosphate → thioglycoside → glycal.[140]

Armed-disarmed type glycosylations can also be readily performed in a one-pot fashion. In this case the leaving group on the glycosyl donors is always the same. The control of the chemoselectivity is exerted by tuning the glycosyl donor reactivities through suitable protection strategies. The ability to control glycosyl donor reactivity by careful selection of hydroxyl protecting groups is one of the underlying principles of **programmable one-pot oligosaccharide syntheses**. Wong and coworkers set up a procedure for determining glycosyl donor reactivity by a competitive HPLC experiment.[9] Donors and acceptors with donor capability (thioglycosides with one hydroxyl group exposed) with various protecting group patterns provided a set of building blocks with diverse reactivities. The relative reactivity values (RRVs), as observed by HPLC, were tabulated in a database, from which the computer program 'Optimer' was created. After the user has selected an oligosaccharide structure, the program lists the best combination of building blocks for its preparation (*Figure 1.15*).

With this strategy, oligosaccharides containing three to six monosaccharides are rapidly assembled in minutes or hours by mixing the selected building blocks in sequence, with the most reactive first. 'Optimer' has been successfully applied to the synthesis of linear and branched oligosaccharide structures, as well as to the construction of a 33-membered oligosaccharide library.[141]

[139] Yamada, H.; Harada, T.; Miyazaki, H.; Takahashi, T. *Tetrahedron Lett.* **1994**, *35*, 3979.
[140] The latter case is depicted in *Scheme 1.15*, page 19.
[141] Ye, X. –S.; Wong, C. –H. *J. Org. Chem.* **2000**, *65*, 2410.

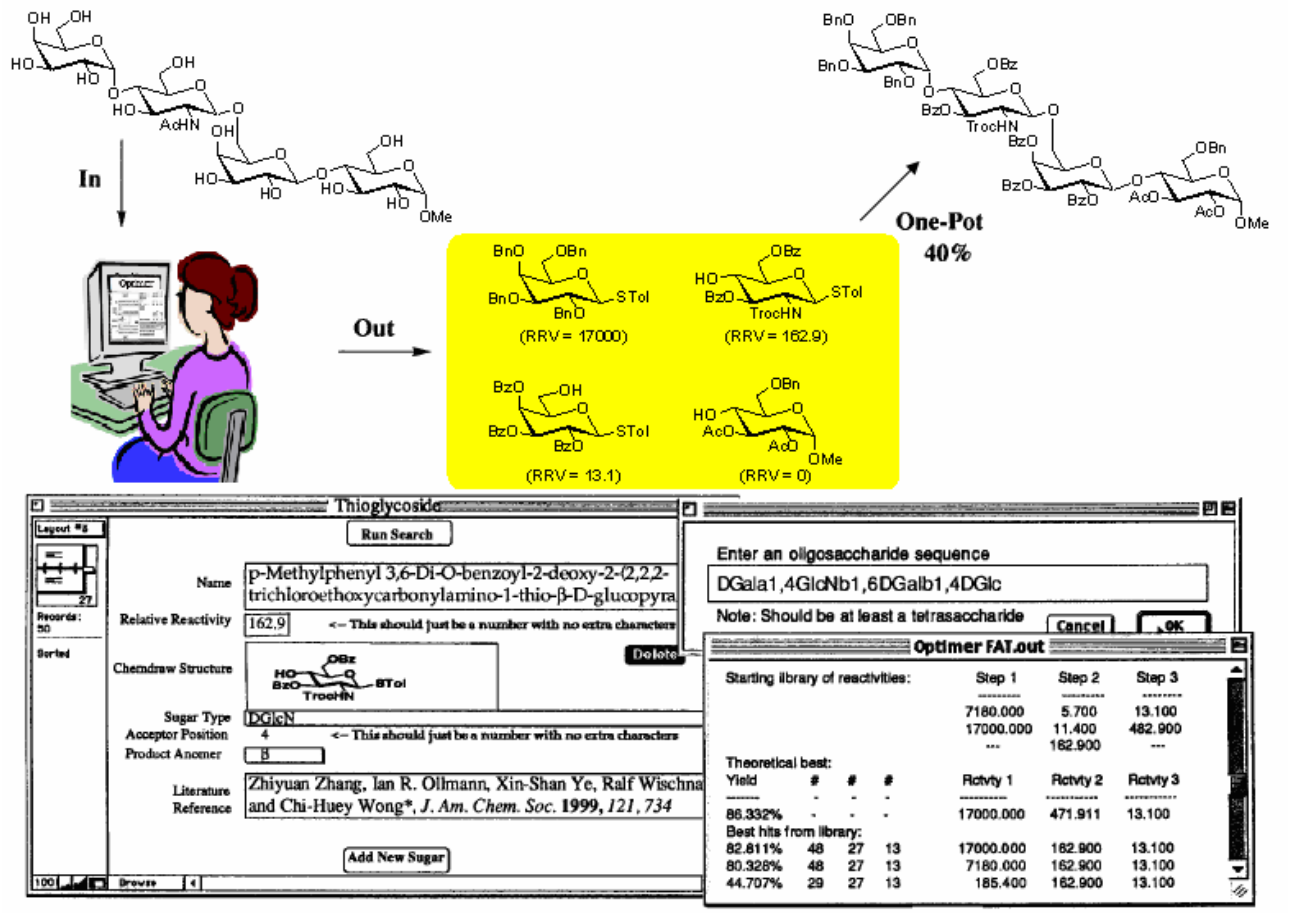


FIGURE 1.15 Programmable One-Pot Oligosaccharide Synthesis with the Aid of 'Optimer' Computer Program.

Solid-phase synthesis proved to be a very effective technique in several fields of the organic chemistry. The advantage of solid-phase reactions is the simple and quick workup process. Because only the growing molecule is attached on the support, a simple filtration is enough to wash away all the other reagents used in the reaction. Higher reaction yields can generally be achieved by use of excess amounts of reagents.

Solid-phase oligosaccharide synthesis has been extensively studied, especially since the advent of effective glycosylation methods in the 1980s.[142] As support matrices, Merrifield's resin (polystyrene cross-linked with 1% divinylbenzene), controlled pore glass or PEG grafted on polystyrene (TentaGel, ArgoGel) are commonly employed. Various linkers have been applied to attach the carbohydrate to the solid support, including silyl ethers and acid- or base-labile linkers. The recently introduced octanediol linker is very versatile as it provides *n*-pentenyl glycosides after cleavage by olefin metathesis.[143]

A great variety of glycosyl donors and glycosylation methods have been tried in solid-phase synthesis, including glycals,[144] glycosyl sulfoxides,[145] thioglycosides,[146] glycosyl

[142] a) Osborn, H. M. I.; Khan, T. H. *Tetrahedron* **1999**, *55*, 1807; b) Seeberger, P. H.; Haase, W. C. *Chem. Rev.* **2000**, *100*, 4349; c) Seeberger, P. H. *J. Carbohydr. Chem.* **2002**, *21*, 613.

[143] Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seebereger, P. H. *Org. Lett.* **1999**, *1*, 1811.

[144] Danishefsky, S. J.; McClure, K. F.; Randolph, J. T.; Ruggeri, R. B. *Science* **1993**, *260*, 1307.

[145] Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520.

trichloroacetimidates,[147] *n*-pentenyl glycosides[148] and glycosyl phosphates.[149] Oligosaccharides up to the size of a dodecamer have been synthesized.[146b,8]
Recently solid-phase oligosaccharide synthesis has been automated by the use of a modified peptide synthesizer (*Figure 1.16*).[8]
The effectiveness of the automated approach has been confirmed by the successful assembly of a host of biologically relevant oligosaccharides using glycosyl trichloroacetimidate and glycosyl phosphate methods. As an example of automated oligosaccharide synthesis, in *Scheme 1.17*[150] is reported the synthesis of dodecamer phytoalexin elicitor β-glucan. Automated solid-phase oligosaccharide synthesis shows great promise as a future technology to fuel the growing need for defined oligosaccharide structures as glycomics efforts gather momentum.

FIGURE 1.16 The First Oligosaccharide Automated Synthesizer.

Aim and Outline of the Thesis

In this work several synthetic aspects of carbohydrate chemistry have been explored with a particular emphasis on the development and implementation of mild glycosylation procedures. As described above, the most versatile glycosylation methods, thioglicosydes, glycosyl trihaloacetimidates and phosphate triesters, although very efficient, suffer from the use of strong Lewis and Brønsted acids as promoters (such as $BF_3 \cdot Et_2O$, TMSOTf, NIS

[146] a) Nicolaou, K. C.; Wissinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449; b) Nicolaou, K. C.; Watanabe, N.; Li, J.; Pastor, J.; Wissinger, N. *Angew. Chem. Int. Ed.* **1998**, *37*, 1559.
[147] Wu, X.; Grathwohl, M.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2002**, 4489.
[148] Rodebaugh, R.; Joshi, S.; Fraser-Reid, B.; Geysen, H. M. *J. Org. Chem.* **1997**, *62*, 5660.
[149] Palmacci, E. R.; Plante, O. J.; Seeberger, P. H. *Eur. J. Org. Chem.* **2002**, 595.
[150] See page 21.

and TfOH). These reagents not only complicate the experimental procedures because of their corrosiveness, moisture sensitiveness and difficulty to be handled, but, because of their strong acidity, they also limit the pattern of protecting groups to be used. In the last years in our laboratory a great effort has been addressed to the development of novel mild protocols for the activation of glycosyl trichloroacetimidates and *N*-phenyl trifluoroacetimidates, of more recent introduction. In this thesis a particular interest is addressed to the use of $Yb(OTf)_3$ and AW MS in performing glycosylation reactions. The experimental advantages connected with the use of these cheap and easy to handle reagents had been evidenced by previous work.

In *Chapter 2* a novel approach for the synthesis of glycosyl iodides is reported. The effective protocol, that takes advantage of the *in situ* generation of HI with the I_2/Et_3SiH combined system, is applied to directly access to glycosyl 1,2-orthoesters, 1,2-ethylidenes and 1,2-glycals.

In *Chapter 3* the effective use of $Yb(OTf)_3$ and AW MS was exerted in the synthesis of complex oligosaccharide antigen portions, testing their effectiveness and, at the same time, furnishing an alternative feasible synthetic strategy for the obtainment of pharmacological active molecules.

In *Chapter 4* the use of dimethoxyethane (DME) as a novel α-stereodirecting cosolvent is proposed. The beneficial effect of DME in α-selectivity improvement was demonstrated in both $Yb(OTf)_3$ and TMSOTf promoted glycosylations.

The mildness of these promoting systems was also effectively exerted in the preparation of novel glycoconjugates. In *Chapter 5* 17β-estradiol and derivatives thereof were regio-selectively glycosylated in order to improve the pharmacokinetic and pharmacodinamic properties of the studied drugs. While in *Chapter 6*, two different classes of glycosyl β-lactams are synthesized with the aim of producing novel carbohydrate-containing antibiotics active against resistant bacteria.

In *Chapter 7* a novel one-pot approach for the synthesis of oligosaccharides is reported. Glycosyl trichloro- and *N*-phenyl trifluoro-acetimidates are sequentially activated in the glycosylation medium by tuning the $Yb(OTf)_3$ activation conditions.

In the last project a highly effective protocol to convert 1,2-glycosyl orthoesters, 1,2-oxazolines and 1,2,6 mannosyl orthoester to C2-acyl glycosyl phosphates is illustrated (*Chapter 8*). Furthermore, the protocol is conveniently applied to the synthesis of a biologically relevant trisaccharide antigen exerting the *in situ* generation of the anomeric phosphates.

Chapter 2

Synthesis of Glycosyl Iodides with I_2/Et_3SiH Combined System. Some Applications

Introduction

Glycosyl iodides have been known for a long time, but, due to their instability, they have been considered unpractical reagents in carbohydrate chemistry. Their application was mostly restricted to halide ion-catalysed glycosylations, where reactive β-glycosyl iodides were *in situ* generated by treatment of more stable α-glycosyl bromides with tetraalkykammonium iodides.[1] Procedures for directly synthesizing these derivatives were known since 1929 (reaction of glycosyl bromides with sodium iodide in acetone[2] or treatment of anomeric acetates with hydroiodic acid in glacial acetic acid[3]), but difficulties in isolation and purification limited their use. α-D-Glycosyl iodides have served as glycosyl donors in only a few cases,[4] and the general consensus has been that these compounds are too reactive to be synthetically useful.[5]

However, in the last years glycosyl iodides have attracted some interest and a variety of approaches have been published for their synthesis. For example, glycosyl iodides have been prepared by treatment of the corresponding hemiacetals with iodoenamines[6] or with a complex of polystyryl phosphane and iodine[7] (*Scheme 2.1)*.

In addition, these compounds can also be prepared from glycosyl acetates with catalytic BiI_3 with an excess of alkyl silyl iodides.[8]

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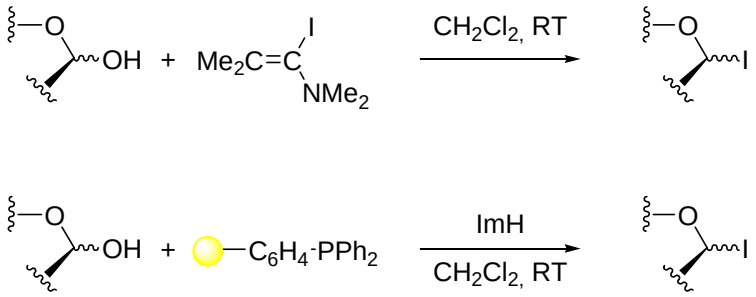
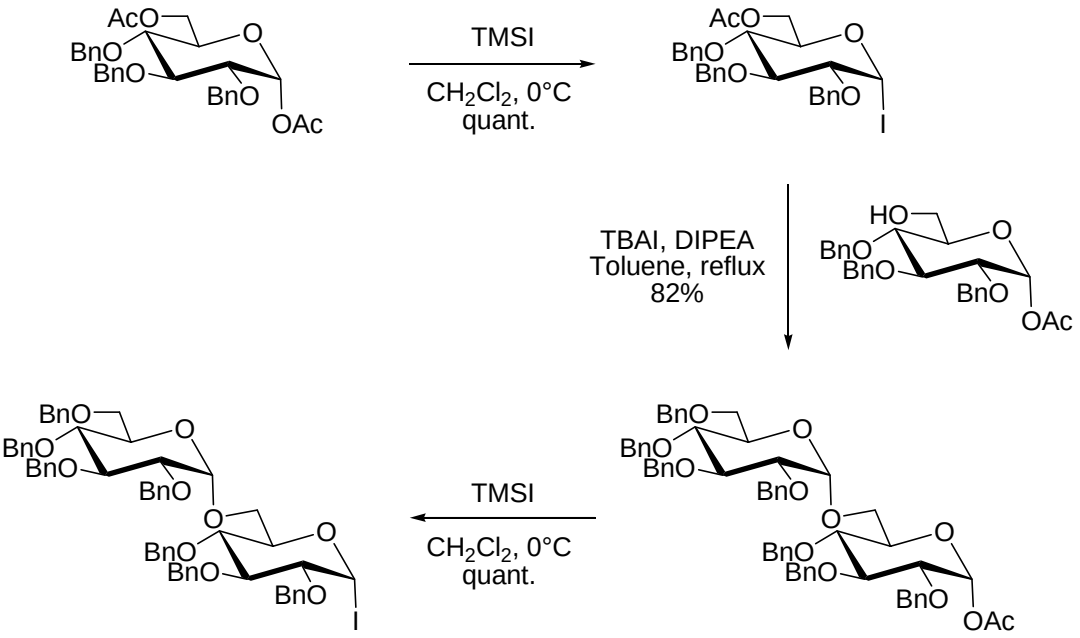
[1] Leimieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056.
[2] Helferich, B.; Gootz, R. *Chem. Ber.* **1929**, *62*, 2788.
[3] Ness, R. K.; Fletcher, H. G.; Hudrson, C. S. *J. Am. Chem. Soc.* **1950**, *72*, 2200.
[4] a) Kronzer, F. J.; Schuerch, C. *Carbohydr. Res.* **1974**, *34*, 71; b) Araki, Y.; Endo, T.; Tanji, M.; Nagasama, J.; Ispido, Y. *Tetrahedron Lett.* **1987**, *28*, 5853.
[5] Schmidt, R. R. *Angew. Chem. Int. Ed. Eng.* **1986**, *25*, 212.
[6] Ernst, B.; Winkler, T. Tetrahedron Lett. 1989, 30, 3081.
[7] Caputo, R.; Kunz, H.; Mastroianni, D.; Palumbo, G.; Pedatella, S.; Solla, F. *Eur. J. Org. Chem.* **1999**, 3147.
[8] Montero, J.-L.; Winum, J.-Y; Leydet, A.; Kamal, M.; Pavia, A. A.; Roque, J.-P. Carbohydr. Res. 1997, 297, 175.



SCHEME 2.1 Glycosyl Iodides Synthesis from Hemiacetals.

Quite recently, a practical access to glycosyl iodides from the corresponding 1-*O*-acetylated derivatives has been described by Gervay and coworkers.[9] This procedure is based on the use of TMSI and takes advantage of the easy removal of volatile by-products. The same research group has shown the feasible utilization of the obtained donors in the synthesis of *O*-, *C*-, and *N*-glycosides exploiting either a mechanism of direct displacement[10] or an α-selective glycosidation based on the *in situ* anomerization promoted by tetrabutylammonium iodide. As depicted in *Scheme 2.2*, this procedure found straightforward application in iterative iodination/glycosylation steps leading to the fast assembly of exasaccharide structures.[11]

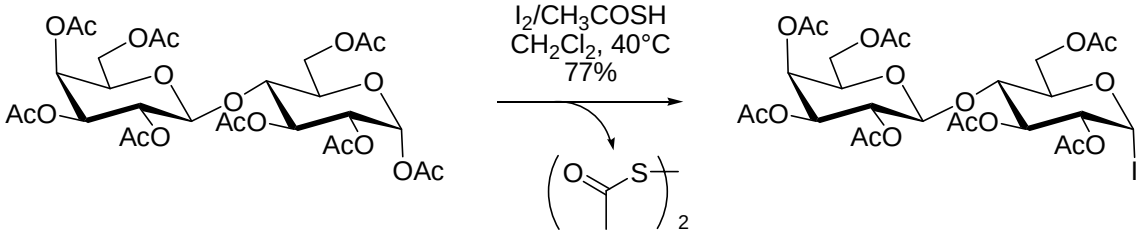


SCHEME 2.2 Iterative Oligosaccharide Synthesis Using Glycosyl Iodides.

In order to find an alternative to the unstable and expensive TMSI, Koreeda has reported a protocol for converting glycosyl acetates into iodides by *in situ* generation of anhydrous

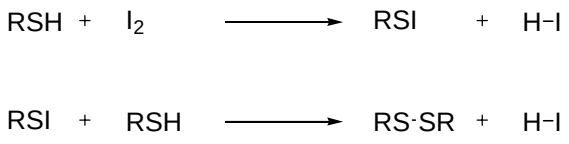
[9] Gervay, J.; Nguyen, T. N.; Hadd, M. J. Carbohydr. Res. **1997**, 300, 119.
[10] a) Gervay, J.; Hadd, M. J. *J. Org. Chem.* **1997**, *62*, 6961; b) Bhat, A. S.; Gervay-Hague, J. *Org. Lett.* **2001**, *3*, 2081; c) Ying, L.; Gervay-Hague, J. *Carbohydr. Res.* **2003**, *338*, 835.
[11] a) Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61; b) Lam, S. N.; Gervay-Hague, J. *Org. Lett.* **2002**, *4*, 2039; c) Lam, S. N.; Gervay-Hague, J. *Carbohydr. Res.* **2002**, *337*, 1953.

HI through the combination of cheap and stable co-reagents such as iodine and thiolacetic acid (or 1,3-propanedithiol) (*Scheme 2.3*).[12]



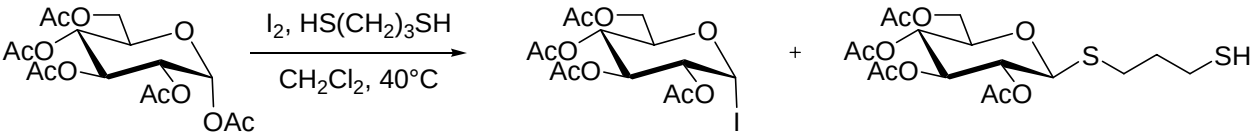
SCHEME 2.3 Synthesis of Glycosyl Iodides with *in Situ* Generated HI.

Investigation into this reagent mixture indicated that the source of the HI was likely the result of the oxidation of the thiol in the presence of iodine. In the general scheme depicted below, a thiol molecule reacts with molecular iodine to form an intermediate sulfenyl iodo species and one molecule of HI (*Scheme 2.4*). The sulfenyl iodide reacts with the second thiol molecule to yield the corresponding disulfide and a second molecule of HI.



SCHEME 2.4 HI Generation *via* Thiol Oxidation by Iodine.

More recently, the use of the I_2/Et_3SiH reagent as glycosidation promoter[13] highlighted as an ancillary result that this combined system could be a feasible alternative to the latter approach avoiding the use of malodorous thiols, whose nucleophilic character proved to give undesired interferences. As a matter of fact, in the Koreeda method the use of 1,3-propanedithiol in combination with iodine, although proved to be an effective source of HI in addition reactions to alkenes, in the case of glycosyl iodides led to the production of β-thioglycosides as side-products (*Scheme 2.5*).



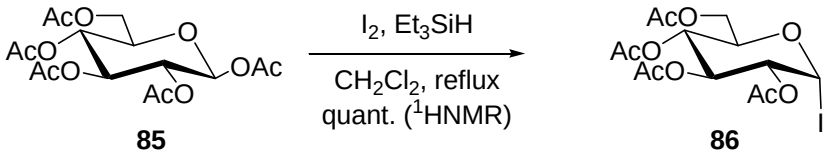
SCHEME 2.5 Thioglycoside Side-Product Formation Using $I_2/HS(CH_2)_3SH$ System.

This thioglycoside may result from the nucleophilic capture of the oxonium ion intermediate or direct displacement of the α-glycosyl iodide product.

[12] Chervin, S. M.; Abada, P.; Koreeda, M. *Org. Lett.* **2000**, *2*, 369.
[13] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Synlett*, **2002**, 269.

Results and Discussion

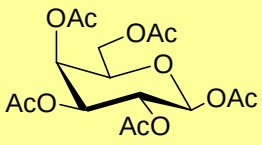
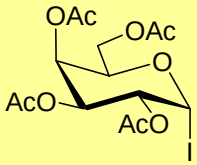
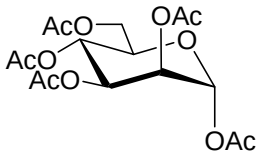
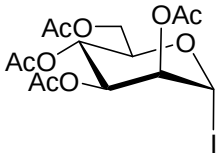
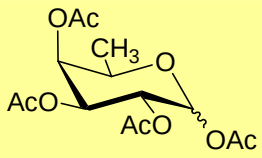
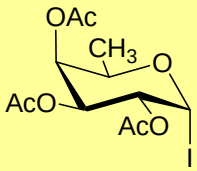
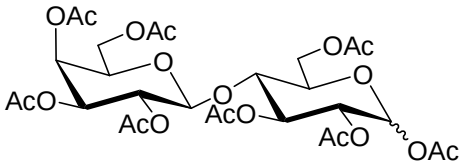
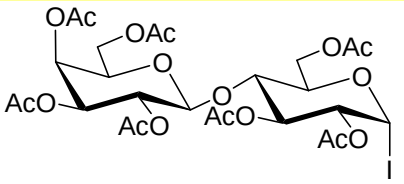
On the basis of our experience with I_2/Et_3SiH system as a source of HI, we set up a new protocol for the convenient conversion of peracetylated sugars into the corresponding glycosyl iodides. Treatment of peracetylated sugar **85** in dry dichloromethane with a slight stoichiometric excess of iodine and triethylsylane led to the quantitative formation of the desired glycosyl iodide **86** within 10-20 minutes under reflux (*Scheme 2.6*).



85 **86**

SCHEME 2.6 Effective Preparation of Glycosyl Iodide **86** Using I_2/Et_3SiH system.

This procedure was effectively applied to the preparation of several derivatives (*Table 2.1*).

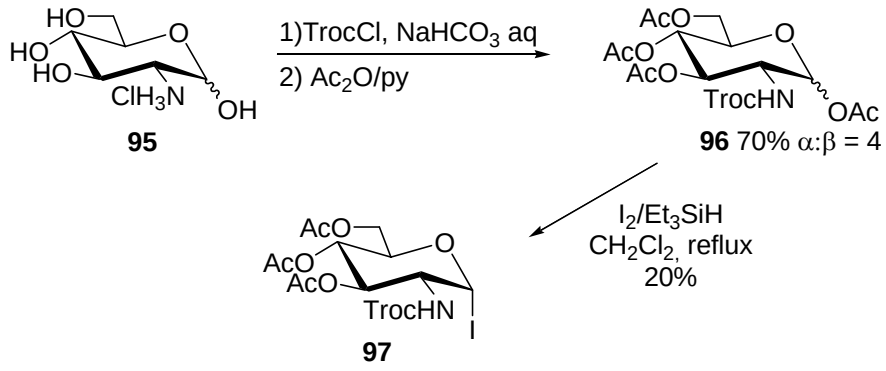
Entry	*Reagent*	*Product*
1	 **87**	 **88**
2	 **89**	 **90**
3	 **91** (β:α = 1.3)	 **92**
4	 **93**(α:β = 5)	 **94**

Procedure: I_2 (1.4 eq.), Et_3SiH (1.4 eq.), CH_2Cl_2, reflux, 10-20 mins, yields > 90% (^{1}HNMR)

TABLE 2.1 Quantitative Conversion of Peracetylated Sugars into Corresponding Glycosyl Iodides.

In all cases extractive work-up was sufficient to furnish in excellent yields the desired products contaminated only by traces of non-reactive silyl ether side-products. Noteworthy, the stability of the glycosidic linkage of lactose **93** was not compromised by the stoichiometric amounts of HI *in situ* generated. Of particular interest is also the generation in high yield of fucosyl iodide **92**, since the well-known deoxy-sugars aptitude

for easily undergoing hydrolysis reaction. Furthermore, it has to be observed that the effectiveness of the procedure isn't affected by the anomeric configuration of starting materials. Pure β anomers were used in the case of glucose and galactose **85** and **87**, respectively, whereas in the cases of mannose, fucose and lactose, pure α or variously enriched anomeric mixtures furnished the corresponding iodides in high yields. These observations are in contrast with the results obtained with glucosamine derivatives. Iodination of 2-deoxy aminosugars was reported to be a challenging issue. Peracetylated *N*-acetyl glucosamine proved to react scarcely with the effective TMSI procedure. From Lay and co-workers work emerged that the nitrogen protecting groups play a crucial role in the iodination reaction.[14] In fact *N*-dimethyl-maleimido or *N*-phtalimido protection were required for the effective conversion of glucosamine derivatives into the corresponding iodides by treatment with TMSI. We intended to test the effectiveness of the I_2/Et_3SiH system in this difficult task, setting as starting material the previously unexplored 1-*O*-Acetyl glucosamine **96** (*Scheme 2.7*).

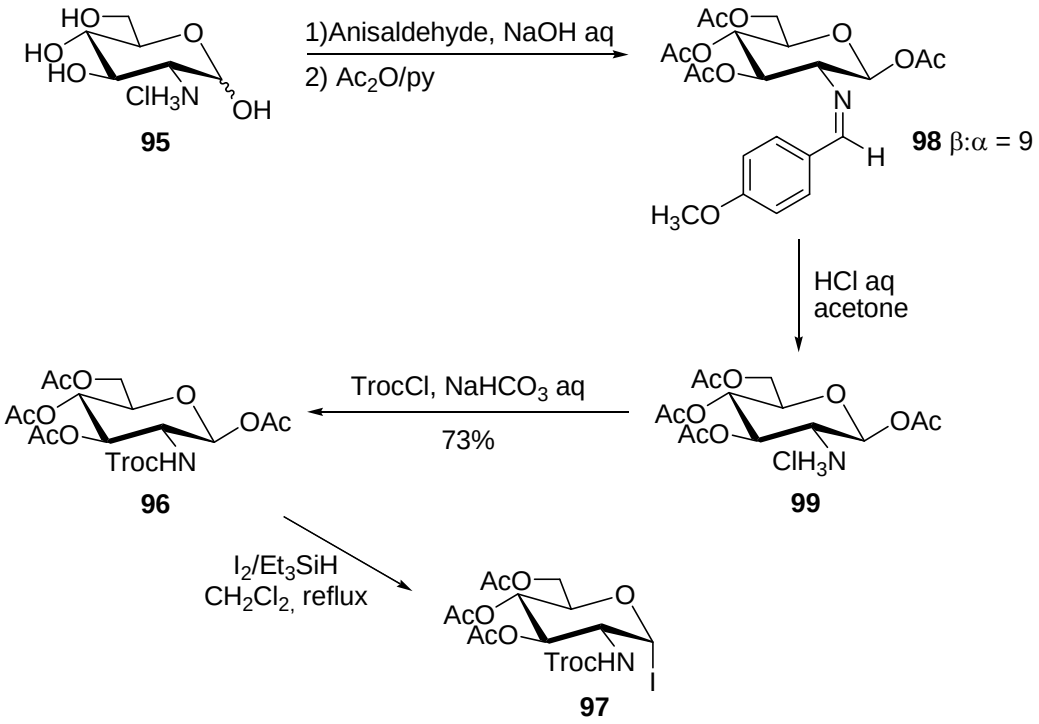


SCHEME 3.7 Synthesis of Glucosamine Iodides **97**.

Trichloroethoxycarbonyl protection for amino groups is very common in carbohydrate chemistry, therefore the development of an effective procedure for the synthesis of a thus functionalized iodide is very useful and of practical interest. Glucosamine hydrochloride **95** was transformed into Troc-protected derivative **96** using a standard procedure that calls for Troc installation followed by peracetylation. Derivative **96** was then treated with I_2/Et_3SiH system, but only a partial conversion (ca. 20%) into the desired iodide **97** was observed. By accurate NMR analysis of the crude, it turned out that the sole β-anomer of the anomeric mixture **96** was consumed during the iodination step. As a matter of fact 1,3,4,6-tetra-*O*-acetyl *N*-Troc glucosamine **96** was obtained as an α/β 4:1 mixture after py/Ac_2O treatment. Consequently, a stereocontrolled route to the sole β-anomer of **96** was pursued following a versatile strategy that may also be easily extended to nitrogen protections other than Troc (*Scheme 2.8*). Amino group of glucosamine hydrochloride **95** was first protected as imine by treatment with anisaldehyde and subsequently acetylated to furnish derivative **98** in a β/α ratio of 9.[15] After acidic hydrolysis of derivative **98** any protecting group can be installed on the amine. In our case, Troc-protection was performed and glucosamine **96** was then exposed to I_2/Et_3SiH system eventually

[14] Miquel, N.; Vignando, J.; Russo, G.; Lay, L. *Synlett* **2004**, 275.
[15] Domingos, J. S.; Wang, H.; Allanson, N. M.; Jain, R. K.; Sofia, M. J. *J. Org. Chem.* **1999**, *64*, 5926.

furnishing the corresponding iodide **97** slightly contaminated by a small amount (less than 10%) of unreacted 1α-acetyl glucosamine.



SCHEME 3.8 Improved Synthesis of Glucosamine Iodide **97**.

Set up this effective procedure for glycosyl iodides preparation, we exploited it for the one-pot conversion of easily prepared and commercially available peracetylated sugars into broadly used saccharidic building blocks such as 1,2-orthoesters, 1,2-ethylidenes and 1,2-glycals.

All these intermediates are typically prepared from the corresponding glycosyl bromides, whose synthesis from the corresponding 1-*O*-acetylated precursors requires quite tedious experimental conditions, especially in large scale preparations, either for the necessity to neutralize strongly acidic solutions (HBr in acetic acid) or for the adoption of moisture sensitive reagents (for example $TiBr_4$).[16]

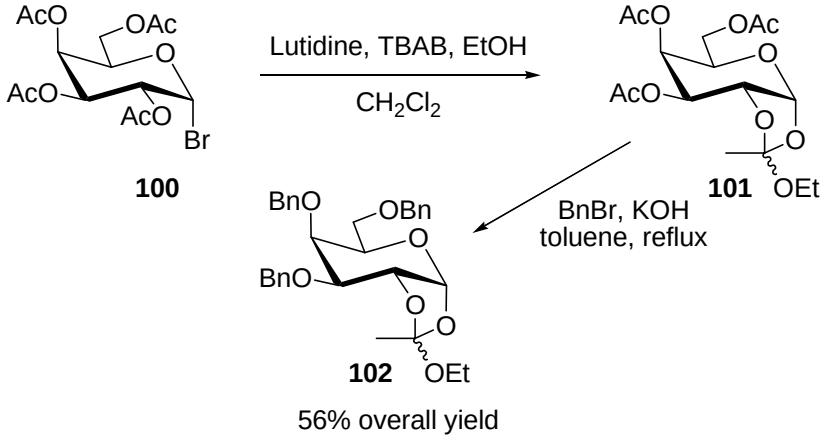
The whole investigation on glycosyl iodides synthesis and application was inspired by a practical problem we met in the preparation of intermediate **102** (*Scheme 2.9*), useful for preparation of galactosyl donors equipped with a selectively removable group at C-2 position.[17] The preparation of **102** had first been accomplished exploiting the standard procedure with glycosyl bromides. Acetobromo galactose **100** was initially converted into the acetylated orthoester **101** under standard conditions for the halide promoted anomerization (lutidine, ethanol and tetrabutylammonium bromide),[18] the concentrated crude mixture containing the orthoester **101** was then treated with KOH in toluene and subsequent addition of benzyl bromide afforded compound **102** in good overall yield (56%

[16] For a discussion on the problems associated to synthesis of glycosyl bromides and related references: Franz, A. H.; Wei, Y. Q.; Samoshin, V. V.; Gross, P. H. *J. Org. Chem.* **2002**, *67*, 7662.

[17] An example is represented by galactosyl donor **129**, page 77.

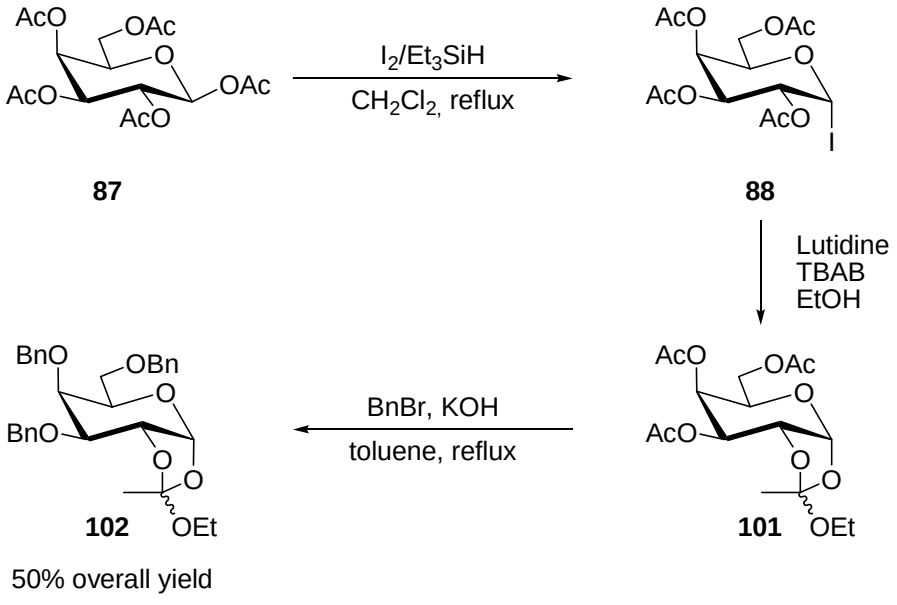
[18] Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2199.

for three synthetic operations). Although efficient, the procedure suffered from the use of the relatively expensive acetobromo galactose **100** as a precursor, and therefore we searched for a more practical alternative approach.



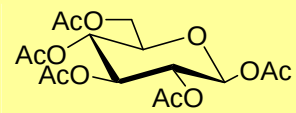
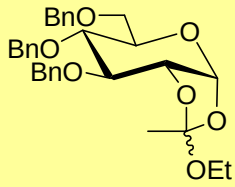
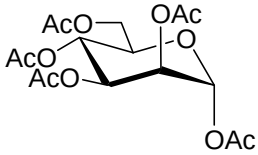
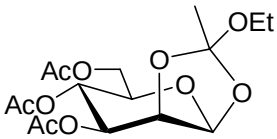
SCHEME 2.9 Synthesis of Orthoester **102** Starting from Acetobromo Galactose **100**.

Therefore, a novel synthesis of **102** has been attempted starting from the cheaper pentaacetyl galactose **87** (*Scheme 2.10*). Treatment of derivative **87** with 1.4 equivalents of I_2 and Et_3SiH in refluxing dichloromethane produced the fast formation of the corresponding α-iodide **88** (TLC and NMR analysis of an aliquot of the crude reaction mixture). Lutidine, ethanol, and tetrabutylammonium bromide were then simply added and the mixture was left under stirring overnight. NMR analysis of the crude material evidenced the high yielding formation of the desired orthoester derivative **101**. Without purification, the crude mixture was subjected to the one-pot deacetylation-benzylation sequence to afford compound **102** in a 50% overall yield over four synthetic operations and a single chromatographic purification.



SCHEME 2.10 One-Pot Synthesis of Orthoester **102** Starting from Peracetylated Galactose **87**.

Encouraged by these results, the synthesis of a variety of useful saccharidic building-blocks commonly prepared from peracetylated glycosyl bromides was investigated. For example, the same synthetic sequence of *Scheme 2.10* was also applied on the gluco precursor **85** with a good overall yield (*Table 2.2*, entry 1).

Entry	*Reagent*	*Procedure*	*Product*	*Overall Yield (%)*
1	 **85**	A	 **103**	58
2	 **89**	B	 **104**	71

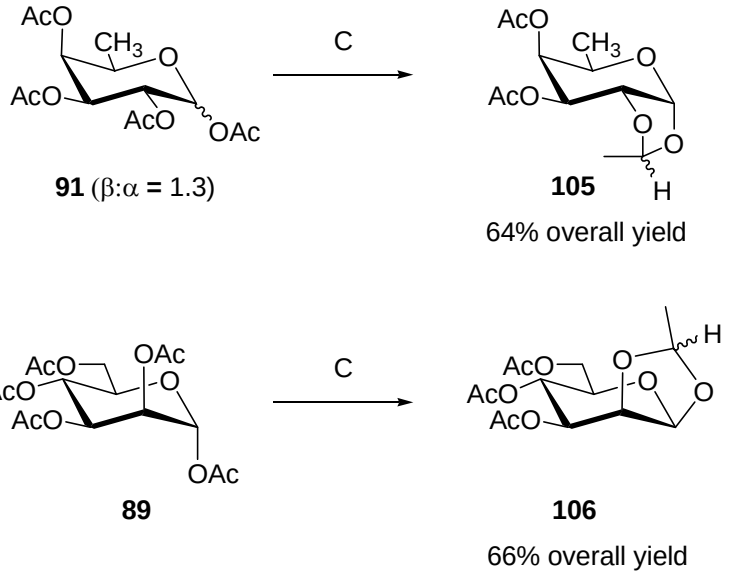
Procedure A: See *Scheme 2.10.*
Procedure B: I_2 (1.4 eq), Et_3SiH (1.4 eq.), CH_2Cl_2, reflux, 30 mins; lutidine (4 eq), EtOH (6 eq.), TBAB (0.4 eq.), overnight, RT.

TABLE 2.2 One-Pot Conversion of Peracetylated Sugars into 1,2-Glycosyl Othoesters.

It should be noted that the orthoesterification reaction was accomplished through a one pot procedure without any work-up of the iodination mixture, differently from the case of the corresponding synthesis via glycosyl bromides. In addition, the efficacy of the whole synthetic sequence was not compromised by the use of unpurified intermediates. The acetylated orthoester **104** was prepared from the corresponding peracetylated *D*-mannose derivative **89** with an analogous one-pot sequence of anomeric iodination and orthoesterification (entry 2). The sequence afforded the product as a single diastereoisomer in a 71 % overall yield after the final chromatographic purification.

Further interesting application of the protocol is represented by the synthesis of 1,2-ethylidenes, another class of very useful precursors in carbohydrate chemistry. These derivatives are routinely prepared by treating glycosyl bromides with excess of $NaBH_4$, and (for *gluco*- and *galacto*- derivatives) catalytic tetrabutylammonium bromide in acetonitrile.[19] The feasible synthesis of these compounds directly from peracetylated precursors has been demonstrated starting from mannose and fucose derivatives (*Scheme 2.11*).
After glycosyl iodide generation the initial solvent (dichloromethane) was removed to be replaced by acetonitrile, sodium borohydride and (only for the fucose) tetrabutylammoniun bromide were then added. Also in these cases the one-pot sequence gave the useful advantage of experimental operations minimization. In addition the generation of 1,2-ethylidenes from glycosyl iodide intermediates turned out to be a faster process than in the case of brominated analogues.

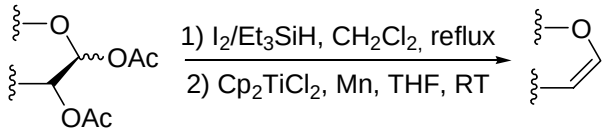
[19] Betaneli, V. I.; Ovchinnicov, M. V.; Backinowsky, L. L.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *107*, 285.

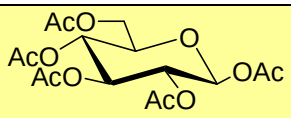
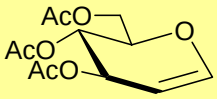
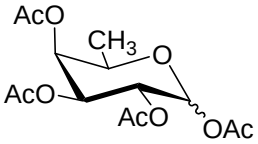
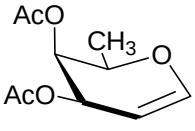
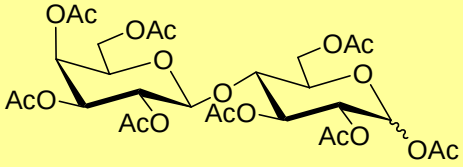
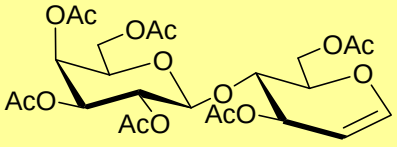


Procedure C: : I_2 (1.4 eq), Et_3SiH (1.4 eq.), CH_2Cl_2, reflux, 30 mins; then removal of the solvent and addition of $NaBH_4$, CH_3CN, RT, 2-4 h.

SCHEME 2.11 One-Pot Conversion of Peracetylated Sugars into 1,2-Ethylidenes.

A further application has been evaluated in the synthesis of 1,2-glycals (*Table 2.3*).

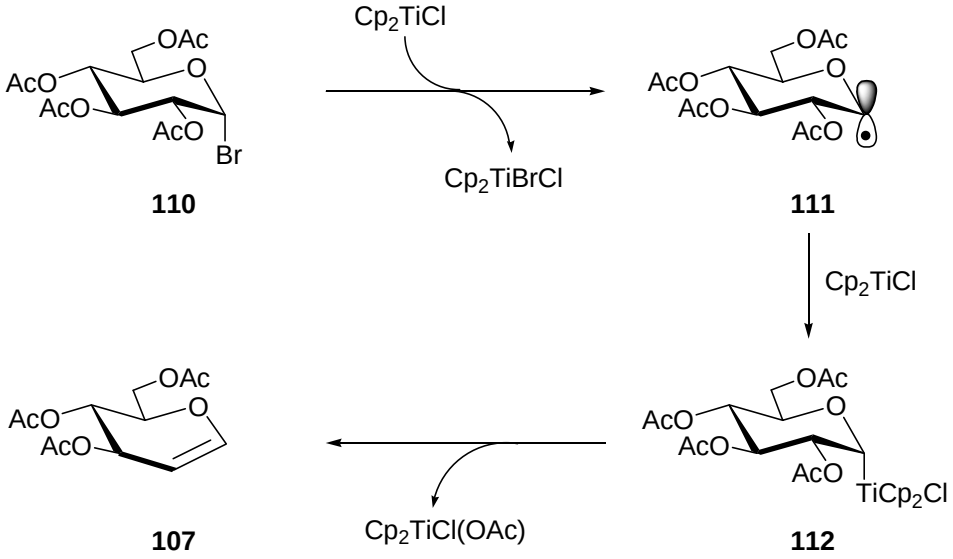


Entry	*Reagent*	*Procedure*	*Product*	*Overall Yield (%)*
1	 **85**	D	 **107**	81
2	 **91** (β:α = 1.3)	D	 **108**	47
3	 **93** (α:β = 5)	D	 **109**	71

Procedure D: I_2 (1.4 eq), Et_3SiH (1.4 eq.), CH_2Cl_2, reflux, 30 mins; then extractive work-up and addition of Cp_2TiCl_2 (2.5 eq.), Mn (5 eq.), THF, RT, 2-5 h.

TABLE 2.3 One-Pot Conversion of Peracetylated Sugars into 1,2-Glycals.

In this case the iodination mixture was worked up by a simple extraction and the crude product was directly subjected to the elimination conditions described by Skrydstrup and coworkers (Cp_2TiCl_2 and manganese in THF).[20] The original procedure was developed for anomeric bromides reduction. In the proposed mechanism the reactive species is Ti(III) that is *in situ* generated (as Cp_2TiCl) by reaction of Cp_2TiCl_2 with a metal reductant (Mn). Ti(III) promotes a fast electron transfer to the bromide to give an anomeric radical species **111** which is subsequently reduced by another Cp_2TiCl to the corresponding anion **112** that evolves eliminating the C-2-acetate to give the corresponding glycal **107** (*Scheme 2.*12).



SCHEME 2.11 One-Pot Conversion of Peracetylated Sugars into 1,2-Ethylidenes.

Also in this case the elimination step required typically much shorter times than with the corresponding glycosyl bromides (2-4 hours instead of more than 10 hours).[19] A relevant applications of this approach was performed in the synthesis of the expensive lactal derivative **109** (entry 3).

Conclusion

In conclusion, an efficient approach for the synthesis of glycosyl iodides based on the use of cheap and easily handled reagents has been described. These intermediates can be efficiently converted into 1,2-orthoesters and 1,2–ethylidenes with a one-pot approach, while 1,2-glycals can be readily obtained after a simple extractive work-up of the iodination mixture. The protocol was efficiently applied also to the difficult case of 2-deoxy glucosamine obtaining the desired iodide in high yield.

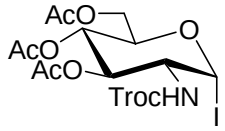
[20] Hansen, T.; Krintel, S. L.; Daasbjerg, K.; Skrydstrup, N. *Tetrahedron Lett*. **1999**, *40*, 6087

Experimental Section

General methods and Material

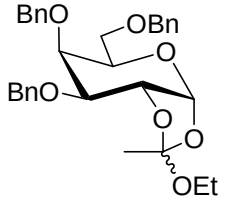
Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian spectrometers at 200 or 300 MHz and 50 or 75 MHz, respectively; or on a Bruker Avance 500 spectrometer at 500 MHz. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Analytical thin layer chromatography (TLC) was performed on E. Merck pre-coated (25 nm) silica gel 60F-254 plates. Column chromatography was performed using Carlo Erba 0.06-0.20 silica gel. When specified, anhydrous solvents stored over molecular sieves (purchased from Aldrich) were used.

General procedure for the synthesis of glycosyl iodides: the peracetylated sugar (2 mmol) is coevaporated with dry toluene, and then dissolved in anhydrous dichloromethane (6 mL). To the solution are added I_2 (711 mg, 2.8 mmol) and triethylsilane (450 μL, 2.8 mmol). The mixture is refluxed until TLC analysis displays the complete consumption of the peracetylated sugar (the glycosyl iodides are partially unstable on TLC, especially in the case of the fucose derivative), and then submitted to further reactions.



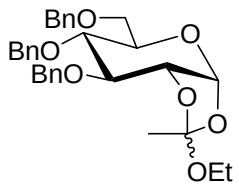
97. ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.91 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1), 5.53 (1H, d, $J_{2,NH}$ = 8.6 Hz, NH), 5.30-5.20 (2H, m, H-3 e H-4), 4.76 e 4.64 (2H, AB, J_{gem} = 12.0 Hz, -OC$\underline{H}_2$CCl$_3$), 4.31 (1H, dd, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ = 12.6 Hz, H-6$_a$), 4.07 (1H, bd, H-6$_b$), 3.99 (1H, m, H-5), 3.42 (1H, m, H-2), 2.06, 2.02, 2.00 (9H, 3xs, 3x CH_3 acetyls).^{13}C NMR and DEPT (50 MHz, $CDCl_3$) δ 171.0, 170.4, e 169.2 (3 x -$\underline{C}$OCH$_3$), 153.8 (-NH$\underline{C}$O$_2$CH$_2$CCl$_3$), 95.0 (-NHCO$_2$CH$_2\underline{C}$Cl$_3$), 79.0 (C-1), 75.2 , 72.1, 66.4 (C-3, C-4, C-5), 74.6 (-NHCO$_2\underline{C}$H$_2$CCl$_3$), 60.7 (C-6), 55.3 (C-2), 20.4 (-CO$\underline{C}$H$_3$) .

General procedure for preparation of 1,2-orthoesters. To the iodination mixture are sequentially added lutidine (930 μL, 8 mmol), ethanol (680 μL, 12 mmol) and tetrabutylammonium bromide (258 mg, 0.8 mmol). The mixture is left under stirring overnight at rt (in the case of *galacto*- and *manno* derivatives) or refluxed for 4 hours (*gluco* derivative). When the reaction is complete (TLC analysis), the mixture is concentrated and chromatographed on silica gel (*Table 2.*2, entry 2) or directly submitted to the deacetylation-benzylation one pot procedure (*Scheme 2.10* and *Table 2.2,* entry 1).

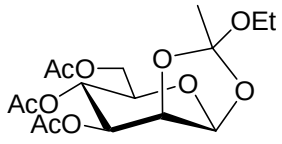


102. (ca 4:1 mixture of diastereoisomers): ^{1}H NMR major diastereoisomer (300 MHz, $CDCl_3$) δ 7.40-7.15 (aromatic protons), 5.74 (1H, d, $J_{1,2}$ = 4.8 Hz, H-1), 4.96-4.40 (7H, m, 3x benzyl CH_2 and H-2), 4.15-4.05 (3H, m, H-3, H-4 and H-5), 3.74-3.55 (4H, m, 6-CH_2 and –OC$\underline{H}_2$CH$_3$), 1.64 (3H, s, orthoester CH_3), 1.24 (3H, t, J_{vic} = 6.9 Hz, -OCH$_2$C$\underline{H}_3$).

^{13}C NMR major diastereoisomer (50 MHz, $CDCl_3$) δ 138.1, 137.9, and 137.7 (aromatic C), 128.3-127.5 (aromatic CH), 121.6 (quaternary C orthoester), 97.6 (C-1), 80.2, 79.4, 74.4, 73.4, 73.0, 72.8, 71.3, 67.9, 57.7 (C-2, C-3, C-4, C-5, C-6, 3x -$\underline{C}$H$_2$Ph, and O$\underline{C}$H$_2$CH$_3$), 24.7 (orthoester CH_3), 15.1 (-OCH$_2\underline{C}$H$_3$).

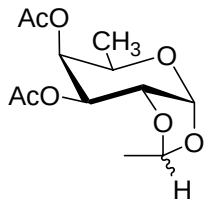


103. (ca 12:1 mixture of diastereoisomers): ^{1}H NMR major diastereoisomer (200 MHz, $CDCl_3$) δ 7.40-7.10 (aromatic protons), 5.76 (1H, d, $J_{1,2}$ = 5.2 Hz, H-1), 4.74-4.36 (7H, m, 3x benzyl CH_2 and H-2), 3.87 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 4.0 Hz, H-3), 3.80-3.62 (4H, m, H-4, H-5, and 6-CH_2), 3.60-3.49 (2H, m, –O$\underline{C}H_2CH_3$), 1.56 (3H, s, orthoester CH_3), 1.19 (3H, t, J_{vis}= 7.2 Hz, -OCH$_2$C$\underline{H}_3$). ^{13}C NMR major diastereoisomer (50 MHz, $CDCl_3$) δ 138.0, 137.8, and 137.6 (aromatic C), 128.2-126.7 (aromatic CH), 120.8 (quaternary C orthoester), 97.6 (C-1), 78.6, 75.6, 74.8, 73.2, 72.7, 71.8, 70.4, 69.1, 58.5 (C-2, C-3, C-4, C-5, C-6, 3x -$\underline{C}H_2$Ph, and O$\underline{C}H_2CH_3$), 21.7 (orthoester CH_3), 15.2 (-OCH$_2\underline{C}H_3$).

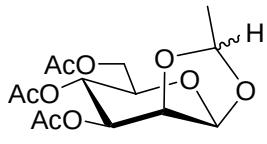


104. ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.43 (1H, d, $J_{1,2}$ = 2.6 Hz, H-1), 5.24 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.10 (1H, dd, $J_{2,3}$ = 4.0 Hz, H-3), 4.54 (1H, dd, H-2), 4.24-4.02 (2H, m, 6-CH_2), 3.64 (1H, m, H-5), 3.58-3.42 (2H, m, –O$\underline{C}H_2CH_3$), 2.06, 2.01, and 1.99 (9H, 3xs, 3x acetyl CH_3), 1.69 (3H, s, orthoester CH_3), 1.12 (3H, t, J_{vis}= 7.2 Hz, -OCH$_2$C$\underline{H}_3$).^{13}C NMR (50 MHz, $CDCl_3$) δ 170.5, 170.2, and 169.3 (3x -$\underline{C}OCH_3$), 124.0 (quaternary C orthoester), 97.2 (C-1), 76.3, 71.1, 70.5, 65.4, 62.2, and 58.0 (C-2, C-3, C-4, C-5, C-6, and O$\underline{C}H_2CH_3$), 24.6 (orthoester CH_3), 20.6 (3x -CO$\underline{C}H_3$), 14.9 (-OCH$_2\underline{C}H_3$).

General procedure for preparation of 1,2-ethylidenes. After the above described synthesis of the glycosyl iodide intermediate, dichloromethane is removed under reduced pressure. The residue is dissolved in acetonitrile, and then sodium borohydride (378 mg, 10 mmol) and tetrabutylammonium bromide (258 mg, 0.8 mmol) (only for fucose) are sequentially added (exothermic reaction). After reaction completion (TLC analysis), the mixture is diluted with dichloromethane and washed with water. Concentration of the organic phase affords a residue that is purified by silica gel chromatography.

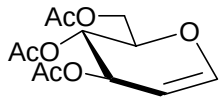


105. (ca 1.2:1 mixture of diastereoisomers) ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.57 (1H, t, $J_{1,2}$ = 4.8 Hz, H-1 minor), 5.50 (1H, t, $J_{1,2}$ = 4.8 Hz, H-1 major), 5.44 (1H, q, J_{vic} = 4.8 Hz, –C$\underline{H}CH_3$ minor), 5.30-5.04 (m, H-4 major and minor, –C$\underline{H}CH_3$ major), 5.04 (1H, dd, $J_{2,3}$ = 7.6 Hz, $J_{3,4}$ = 3.4 Hz, H-3 minor), 4.97 (1H, dd, $J_{2,3}$ = 7.6 Hz, $J_{3,4}$ = 3.4 Hz, H-3 major), 4.35-4.10 (m, H-5 major and minor, H-2 minor), 3.99 (1H, dd, H-2 major), 2.13, 2.11, 2.04, and 2.04 (4x acetyl CH_3), 1.43 (3H, d, J_{vic} = 4.8 Hz, –CHC$\underline{H}_3$ major), 1.36 (3H, d, J_{vic} = 4.8 Hz, –CHC$\underline{H}_3$ minor), 1.17 (3H, d, $J_{5,6}$ = 6.6 Hz, 6-CH_3 major), 1.15 (3H, d, $J_{5,6}$ = 6.6 Hz, 6-CH_3 minor). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.3 (4x -$\underline{C}OCH_3$), 100.4, 99.2, 98.6, 97.5 (–$\underline{C}HCH_3$ major and minor, C-1 major and minor), 73.2, 72.7, 70.6, 69.3, 69.3, 68.8, 67.2, 66.8 (C-2, C-3, C-4, C-5 major and minor), 21.1 and 21.0 (–CH$\underline{C}H_3$ mjor and minor), 20.8 and 20.6 (4x -CO$\underline{C}H_3$), 16.1 (6-CH_3 major and minor).

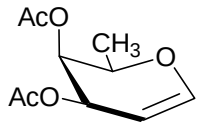


106. (ca 5:1 mixture of diatereoisomers) ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.32-5.10 (4H, m, H-1, H-3, H-4, and –C<u>H</u>CH_3), 4.19 (1H, t, $J_{2,3}$ = $J_{2,3}$ = 3.0 Hz, H-2), 4.15-4.00 (2H, m, 6-CH_2), 3.63 (1H, m, H-5), 2.03, 1.99, and 1.97 (9H, 3xs, 3x acetyl CH_3), 1.45 (3H, d, J_{vic} = 5.0 Hz, –CHC<u>H</u>$_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.5, 170.1, and 169.3 (3x -<u>C</u>OCH_3), 104.5 (–<u>C</u>HCH_3), 96.3 (C-1), 77.2, 71.4, 70.4, 65.9, 62.3 (C-2, C-3, C-4, C-5, C-6), 21.4 (–CH<u>C</u>H_3), 20.6 (3x -CO<u>C</u>H_3), 14.9 (-OCH_2<u>C</u>H_3).

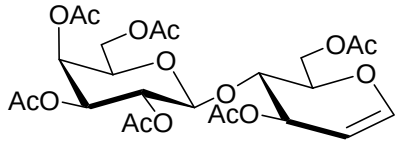
General procedure for preparation of 1,2-glycals. After the above described synthesis of the glycosyl iodide intermediate, the mixture is diluted with dichloromethane and washed with a solution of sodium bicarbonate containing sodium thiosulfate. The organic phase is dried and concentrated. The residue is dissolved in THF (5 mL), Cp_2Cl_2Ti (1.25 g, 5 mmol) and manganese (50 mesh, 550 mg, 10 mmol) are added at room temperature under argon. After completion of the reaction (TLC) the mixture is concentrated and the residue chromatographed on silica gel.



107. ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.44 (1H, dd, $J_{1,2}$ = 6.2 Hz, $J_{1,3}$ = 1.4 Hz, H-1), 5.31 (1H, ddd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 5.8 Hz, H-3), 5.21 (1H, dd, $J_{4,5}$ = 7.4 Hz, H-4), 4.81 (1H, dd, H-2), 4.44-4.13 (3H, m, H-5 and 6-CH_2), 2.06, 2.05, and 2.02 (9H, 3xs, 3x acetyl CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.5, 170.4, 169.6 (3x -<u>C</u>OCH_3), 145.6 (C-1), 98.9 (C-2), 73.9, 67.4, 67.1, 61.3 (C-3, C-4, C-5, and C-6), 20.9, 20.8, and 20.7 (3x -CO<u>C</u>H_3).



108. ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.40 (1H, dd, $J_{1,2}$ = 6.4 Hz, $J_{1,3}$ = 1.8 Hz, H-1), 5.52 (1H, m, H-3), 5.23 (1H, bd, $J_{3,4}$ = 4.6 Hz, H-4), 4.58 (1H, dt, $J_{2,4}$ = $J_{2,3}$ = 1.8 Hz, H-2), 4.16 (1H, bq, $J_{5,6}$ = 6.8 Hz, H-5), 2.10 and 1.96 (6H, 2xs, 2x acetyl CH_3), 1.22 (3H, d, 6-CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.5, 170.2 (2x -<u>C</u>OCH_3), 146.0 (C-1), 98.1 (C-2), 71.4, 66.1, 64.9 (C-3, C-4, C-5), 20.7 and 20.5 (2x -CO<u>C</u>H_3), 16.4 (6-CH_3).



109. ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.39 (1H, bd, $J_{1,2}$ = 6.2 Hz, H-1), 5.41-5.34 (2H, m, H-3 Glc and H-4 Gal), 5.18 (1H, dd, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 10.4 Hz, H-2 Gal), 4.98 (1H, dd, $J_{3,4}$ = 3.4 Hz, H-3 Gal), 4.83 (1H, dd, $J_{2,3}$ = 3.4 Hz, H-2 Glc), 4.65 (1H, d, H-1 Gal), 4.45-3.99 (6H, 6-CH_2 Gal and Glc, H-5 Glc, and H-4 Glc), 3.89 (1H, bt, $J_{5,6}$ = 6.8 Hz, H-5 Gal), 2.14, 2.10, 2.07, 2.05, 2.04, 1.97 (18H, 6xs, 6x acetyl CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.2, 170.2, 170.0, 169.9, 169.7, 169.1 (6x -<u>C</u>OCH_3), 145.3 (C-1 Glc), 100.8 (C-1 Gal), 98.8 (C-2 Glc), 74.4, 74.0, 70.6, 70.5, 68.7, 68.7, 66.6, 61.6, an 60.8 (C-2 Gal, C-3, C-4, C-5, C-6 Gal and Glc), 20.8, 20.6, and 20.4 (6x -CO<u>C</u>H_3).

Chapter 3

Synthesis of Oligosaccharide Antigen Fragments

Introduction

Carbohydrates mediate a variety of biological functions including inflammation, control of growth and differentiation, cell-cell adhesion, as well as are involved in cell communication, signalling and trafficking processes.[1] These tasks are generally accomplished by specific oligosaccharide structures exposed on the cell surface. Very often these saccharidic moieties play their biological roles in conjugated form with bio-molecules such as proteins, lipids or sphingolipids. The latter class of glycoconjugates, glycosphingolipids (GSLs), is essential for cellular adhesion and recognition.[2]

An interesting paradigm is offered by the human blood group antigens. Erythrocyte membranes are characterized by the presence of genetically determined oligosaccharide structures (occurring conjugated either *via N*- or *O*-linkages to proteins or *via* a ceramide linkage to lipids).[3] Antigen specificity is inherited and three specific alleles are involved at the ABO locus. The ABO related antigens are the terminal carbohydrate portions of glycan chains located on the erythrocyte surface. The core pentasaccharide, called H antigen, is expressed on the surface of red blood cells of O blood-type individuals. In individuals of type A blood group, an *N*-acetyl galactosamine (GalNAc) is also added to the terminal galctose residue, whereas in B-type individuals a Gal residue is appended (*Figure 3.1*).

Closely related to the ABO(H) blood groups are the Lewis blood group antigens (Le), which refer to the family name of individuals suffering from a red blood cell incompatibility problem that led to the discovery of this group of antigens. Lewis oligosaccharides are, actually, secretory substances adsorbed onto erythrocytes and are complexly related to blood-group type.

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[1] a) Rudd, P. M.; Elliot, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. S*cience* **2001**, *291*, 2370; b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683; c) Feizi, T. *Curr. Opin. Struct. Biol.* **1993**, *3*, 701; d) Rosen, S. D.; Bertozzi, C. R. *Curr. Biol.* **1996**, *6*, 261; e) Varki, A. *Glycobiology* **1993**, *3*, 97; f) Philips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1130; g) Lasky, L. A. *Science* **1992**, *258*, 964; Miller, D. J.; Macek, M. B.; Schur, B. D. *Nature* **1992**, *357*, 589; h) Feizi, T. *Nature* **1985**, *314*, 53.

[2] Vankar, Y. D.; Schmidt, R. R. *Chem. Soc. Rev.* **2000**, *29*, 201.

[3] a) Lowe, J. B., in *The molecular basis of blood diseases*, Eds.: Stamatoyannopolous, G., Nienhuis, a. W., Majerus, P. W., Varmus, H., Saunders, Philadelphia, **1987**, chap 8; b) Greenwell, P. *Glycoconj. J.* **1997**, *14*, 159.

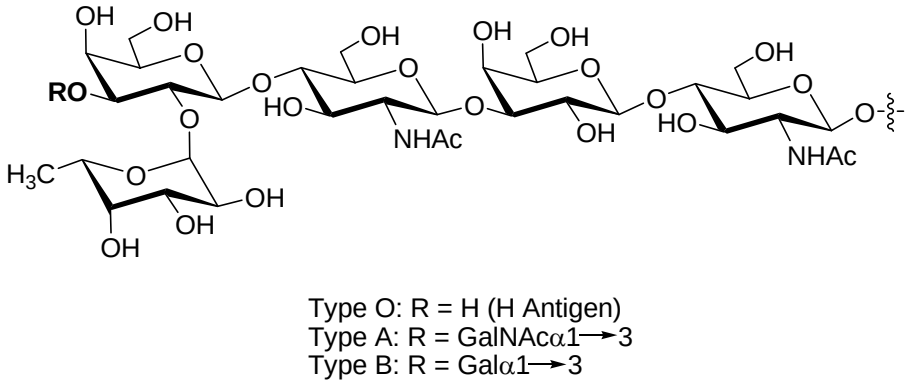

FIGURE 3.1 Structure of Type ABO(H) Carbohydrate Determinats.

The Lewis blood group oligosaccharides (fucosylated, ceramide-containing GSLs) are composed of a reducing end lactose β-(1→3) linked to a central *N*-acetyl glucosamine unit. The structures differ in the arrangement of galactose and fucose residues about this core glucosamine (*Figure 3.2*).

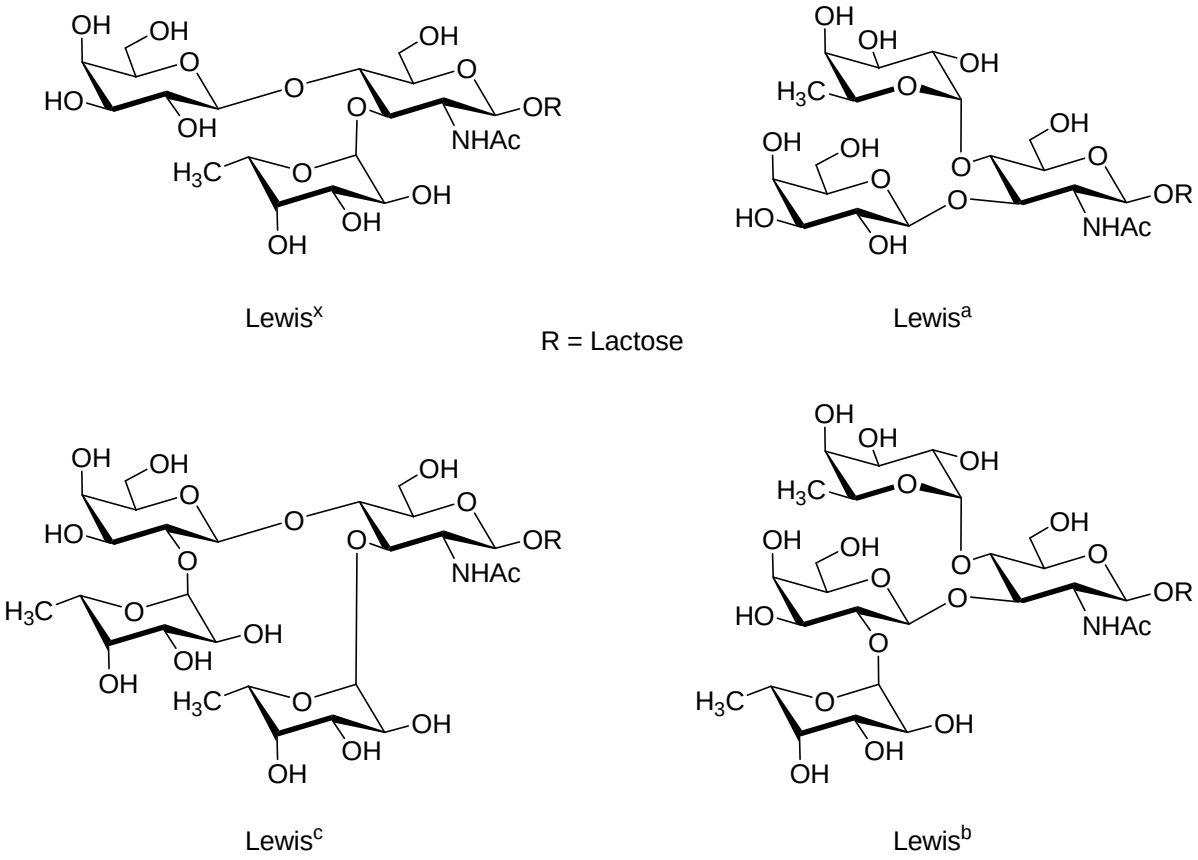

FIGURE 3.2 Structure of Lewis Blood Group Antigens.

These ABO(H) and Lewis structures are not restricted to the surface of erythrocytes. They are also present in secretions and ectodermally derived tissues (digestive and respiratory mucosa, salivary glands).[4] The Lewis antigens have widespread implications in both

[4] a) Hakomori, S. *Semin. Hemat.* **1981**, *18*, 39; b) Oriol, L. in *Blood Cell Biochemistry*, vol. 6; Plenum Press: New York, **1995**, 37-73.

normal cellular adhesion processes like inflammatory response,[5] as well as adhesion associated with disease states, including microbial infections[6] and metastatic cancers.[7]
The role played by Lewis oligosaccharides in inflammatory response is of utmost importance, being the principal responsible for white blood cells adhesion to endothelial tissues. In order to repair and defend damaged tissues against possible microbial infection, circulating leucocytes (white blood cells) must interact with endothelial cells lining blood vessel to reach the underlying site of injury (*Figure 3.3*).

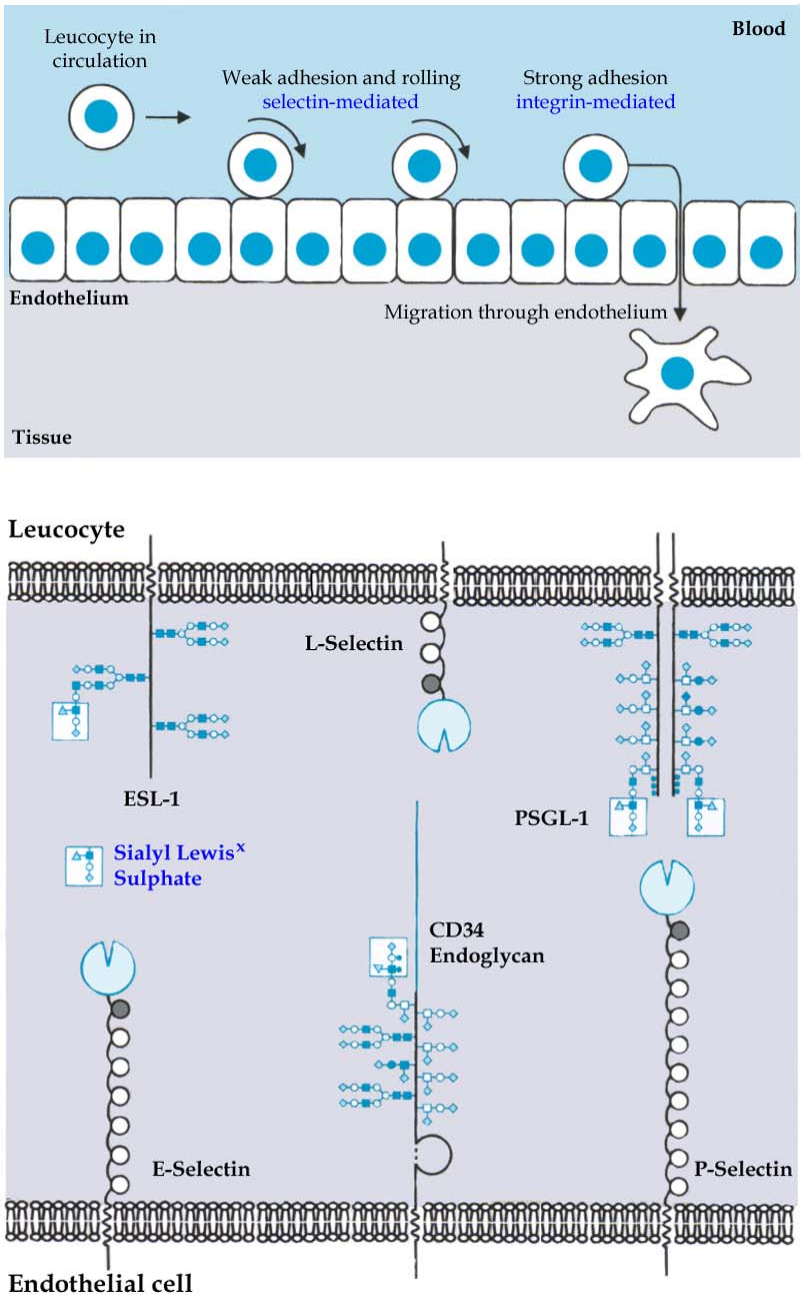

FIGURE 3.3 Leucocyte Interactions with Endothelial Cells.

[5] Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C. H. *Chem. Rev.* **1998**, *98*, 833.
[6] a) Karlsson, K. A. *Curr. Opin. Struct. Biol.* **1995**, *5*, 622.; b) Ilver, D.; Arnqvist, A.; Ögren, J.; Frick, I. –M.; Kersulyte, D.; Incecik, E. T.; Berg, D. E.; Covacci, A.; Engstrand, L.; Borén, T. *Science* **1998**, *279*, 373.
[7] Hakomori, S.; Zhang, Y. *Chem. Biol.* **1997**, *4*, 97.

This complex process takes place in several steps. First damaged tissue releases cytokines that trigger the expression of two proteins on the endothelium surface, E- and P-selectin. These selectins recognize Le^x and Le^a sequences (including sialylated and sulphated congeners) exposed on leukocyte surfaces, mediating the initial rolling phase. The rolling process is then followed by integrin-mediated extravasation into tissue where the white blood cells eventually perform their protective response.[8]

The key role played by this class of antigens in adhesion events explains their frequent involvement in diseases such as microbial infections or cancer metastatic processes where adhesion is a crucial issue. In a manner analogous to migration of leukocytes through the endothelium adjacent to a site of injury, *Helicobacter pylori* infects host organisms by recognition of Le^b antigen exposed on the gastric epithelium. Furthermore, *Helicobacter* is also able to bind sialyl-$Lewis^x$ with a different protein. Expression of sialyl-$Lewis^x$ is not common in normal stomach tissue, but it's greatly increased during inflammation, including inflammation caused by *H. pilori* infection. It is thought that adherence of *Helicobacter* to sialyl-$Lewis^x$ contributes to virulence and persistence of infection leading to a variety of diseases including gastric ulcers and gastric adenocarcinoma.[6b,9]

Lewis antigens, like other glycosphingolipids, were also found on the surface of different cancerous cells.[10] Although GSLs are present in both normal and cancerous cellular tissues, the latter are characterized by aberrant glycosylation, thus making these oligosaccharide structures markers for various human cancers. Carcinomas, including colon, breast, and lung tumours, derive from epithelial cells and are particularly prone to metastasis. The ability of cells released from a primary tumour to travel through the blood and enter and colonize distant organs is reminiscent of the way leukocytes enter tissues at sites of inflammation using selectins to adhere to the endothelium before extravasation into the tissue. There is a good correlation between the degree of expression of sialyl-$Lewis^x$ and sialyl-$Lewis^a$ antigens on carcinomas and their metastatic potential. Furthermore, Le^y determinant has also recently been implicated as a marker in metastatic prostate cancer and was found to be overexpressed in ovarian tumors.[11]

Overexpression of Lewis antigens isn't the only cancer-associated change in glycosylation patterns of tumour cells. Among the others, changes in glycosaminoglycans structures were also detected. Since glycosamino-glycans attached to cell-surface proteoglycans play an important part in the action of growth factors at the surface of cells, it's reasonable that changes in the structures of the glycosaminoglycans would affect the response of cells to growth factors and thus their capacity for replication.

Moreover, it was found that specific type of cancer cells expose on their surface peculiar oligosaccharide motifs in the form of glycoproteins or glycolipids, such as the MBr1 antigen Globo-H or the KH-1 antigen (*Figure 3.4*).

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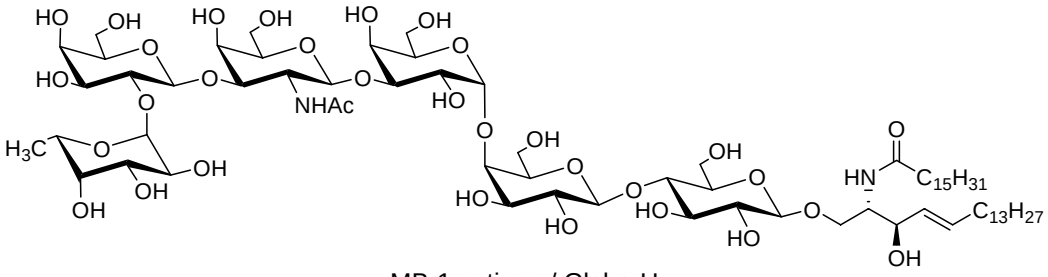
[8] Taylor, M. E., Drickamer, K. In *Introduction to Glycobiology*, Second Ed., Oxford University Press: New York, **2006**, 131-136.

[9] a) Boren, T.; Falk, P.; Roth, K. A.; Larson, G.; Normark, S. *Science* **1993**, *262*, 1892; b) Appelmelk, B. J.; Monteiro, M. A.; Martin, S. L.; Moran, A. P.; Vandenbroucke-Grauls, C. M. J. E. *Trends in Microbiology* **2000**, *8*, 565; c) Wang, G.; Ge, Z. M.; Rasko, A.; taylor, D. E. *Mol. Microbiol.* **2000**, *36*, 1187.

[10] a) Hakomori, S. I. *Adv. Cancer. Res.* **1989**, *52*, 257; b) Brockhausen, I. *Biochimica et Biophys Acta* **1999**, *1473*, 67; c) Kim, Y.J.; Varki, A. *Glycoconj. J.* **1997**, *14*, 569.

[11] Yin, B. W.; Finstad, C. L.; Kitamura, K.; Federici, M. G.; Welshiner, M.; Kudryashov, V.; Hoskins, W. J.; Welt, S.; Lloyd, K. O. *Int. J. Cancer* **1996**, *65*, 406.



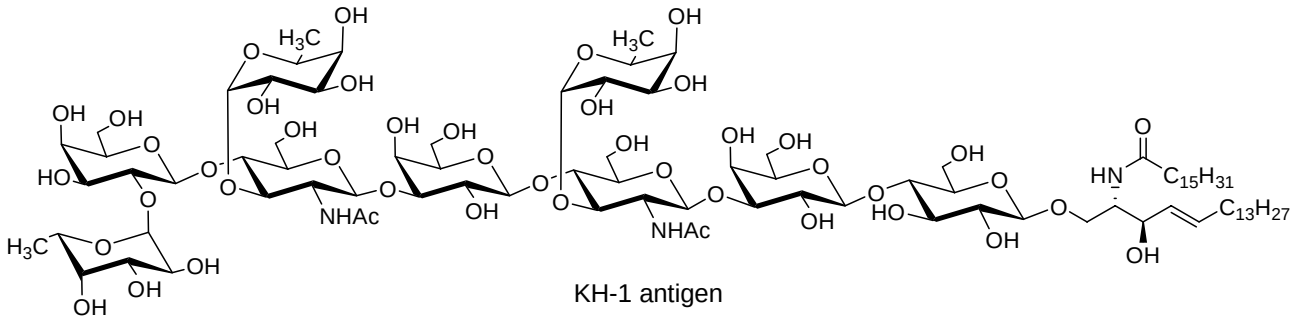


FIGURE 3.4 Globo-H and KH-1 Antigens.

Globo-H is a hexasaccharide isolated in submilligram quantities as a ceramide-liked glycolipid from the human breast cancer cell line MCF-7 by Hakomori *et al.*[12] Subsequent immunohistological analysis with monoclonal antibody (mAb) MBr1[13] found that the antigen is also expressed in other type of carcinomas including colon, lung, ovary, and small cell lung cancers.[14] Globo-H has also been detected in the majority of the carcinomas of the pancreas, stomach, uterine endometrium, and, in particular, was found to be espressed in both primary and metastatic prostate cancer specimens.

The glycolipid KH-1 is perhaps the most complex carbohydrate-based tumour antigen thus far characterized.[15] The antigen was isolated from human colonic adenocarcinoma cells by using antibodies generated against the classical Le^y determinant (*Figure 3.2*). KH-1 has been present on the cell surface of all adenocarcinoma cells thus far studied.

Monoclonal antibodies were raised against this antigen and found to bind specifically to KH-1 antigen. Based on these studies, Hakomori *et al.* postulated that the KH-1 antigen is a highly specific marker for malignancy and premalignancy involving colonic adenocarcinoma.[16]

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[12] a) Kannagi, R.; Levery, S. B.; Ishijamik, F.; Hakomori, S.; Schevinsky, L. H. *J. Biol. Chem.* **1983**, *258*, 8934; b) Bremer, E. G.; Levery, S. B.; Sonnino, S.; Ghidoni, R.; Canevari, S.; Kannagi, R.; Hakomori, S. *J. Biol. Chem.* **1984**, *259*, 14773.

[13] The antibody had been obtained from mice immunized with intact MCF-7 cell lines.

[14] a) Livingston, P. O. *Cancer. Biol.* **1995**, *6*, 357; b) Zhang, S.; Cordon-Cardo, C.; Zhang, H. S.; Reuter, V. E.; Adluri, S.; Hamilton, W. B.; Lloyd, K. O.; Livingston, P. O. *Int. J. Cancer* **1997**, *3*, 42.

[15] Nudelman, E.; Levery, S. B.; Kaizu, T.; Hakomori, S. *J. Biol. Chem.* **1986**, *261*, 11247.

[16] a) Kaizu, T.; Levery, S. B.; Nudelman, E.; Stenkamp, R. E.; Hakomori, S. *J. Biol. Chem.* **1986**, *261*, 11254; b) Kim, S. Y.; Yuan, M.; Itzkowitz, S. H.; Sun, Q., Kaizu, T.; Palekar, A.; Trump, B. F.; Hakomori, S. *Cancer Res.* **1986**, *46*, 5985.

Even in the absence of a complete understanding of how changes in glycosylation relate to tumour progression, these changes provide important avenues to cancer detection and treatment. As a matter of fact high levels of expression of specific types of glycolipids or glycoproteins on tumour cells cause an antibody response, consequently rendering the cell-surface glycoconjugate recognizable as a tumour-associated antigen. The idea of such glycoconjugates as tumour-associated antigens is the basis of Danishefsky group intuition of using carbohydrates in the development of anticancer vaccines.[17] The underlying strategy is the targeted killing of cancer cells by stimulating an immune response directed towards antigen specific to tumour cells. Thus immunogens based on tumour-specific glycans can be used to stimulate a cytotoxic response by the host immune system. The carbohydrate-based vaccines are meant not to prevent tumour occurrence but to provide enhanced protection against tumour relapse and metastasis when the tumour burden has been rendered minimal through surgery, radiation, or chemotherapeutic treatment.

Tumour immunotherapy is based on the theory that tumours possess specific antigens that can be recognized when presented to or processed by a properly trained immune system. A conceptual drawback of this strategy raises observing that these antigens in vaccines should trigger the immune machinery, while in their natural form on tumour cells they don't induce any effective immune response. The goal in the development of anticancer vaccine is to break the tolerance the immune system has developed for antigens expressed mainly or exclusively by the tumour. This aim can be pursued suitably conjugating synthetically derived, cell-free antigens with immunogenic molecules, such as keyhole limphet hemocyanin, KLH, bovine serum albumin, BSA, or ceramides. Obviously, one of the most critical issues to settle is the obtainment of discrete amounts of antigens in pure and homogeneous form. The immense difficulties associated with isolation and purification of these molecules from natural source make the synthesis the only feasible way to follow. Moreover, chemistry plays a major role also in the conjugation phase, which is decisive in upgrading a synthetic antigen to a vaccine.

In *Figure 3.5* it is depicted the general approach to synthetic carbohydrate vaccines performed by Danishefsky and co-workers. In their elegant work the several oligosaccharide antigens were synthesized exploiting the glycal assembly method, developed over many years in the same laboratory.[18,19] The conjugation was performed reductively aminating the oligosaccharide, terminating in a glycoaldehyde, with a lysine residue of the appropriate carrier protein.[20]

Preclinical and clinical studies with synthetic conjugates carbohydrate vaccines (containing the MBr-1 antigen Globo-H, the adenocarcinoma antigen KH-1, the blood group determinant and ovarian cancer antigen Le^y) show induction of IgM and IgG antibody responses. Noteworthy, The Globo-H-KLH conjugate is poised to enter phase II and phase III human clinical trials for the treatment of progressive and reoccurring prostate cancer, and it has also been administrated to breast cancer patients in a phase I clinical trial. These first results raise hopes of effectively using carbohydrate-based vaccines to enable a more favourable survival and "quality of life" prognosis.

[17] For a review on the subject: Danishefsky, S. J.; Allen, J. R. *Angew. Chem. Int. Ed.* **2000**, *39*, 836.
[18] Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380.
[19] See *Chapter 1*.
[20] Bernstein, M. A.; Hall, L. D. *Carbohydr. Res.***1980**, *78*, C1.

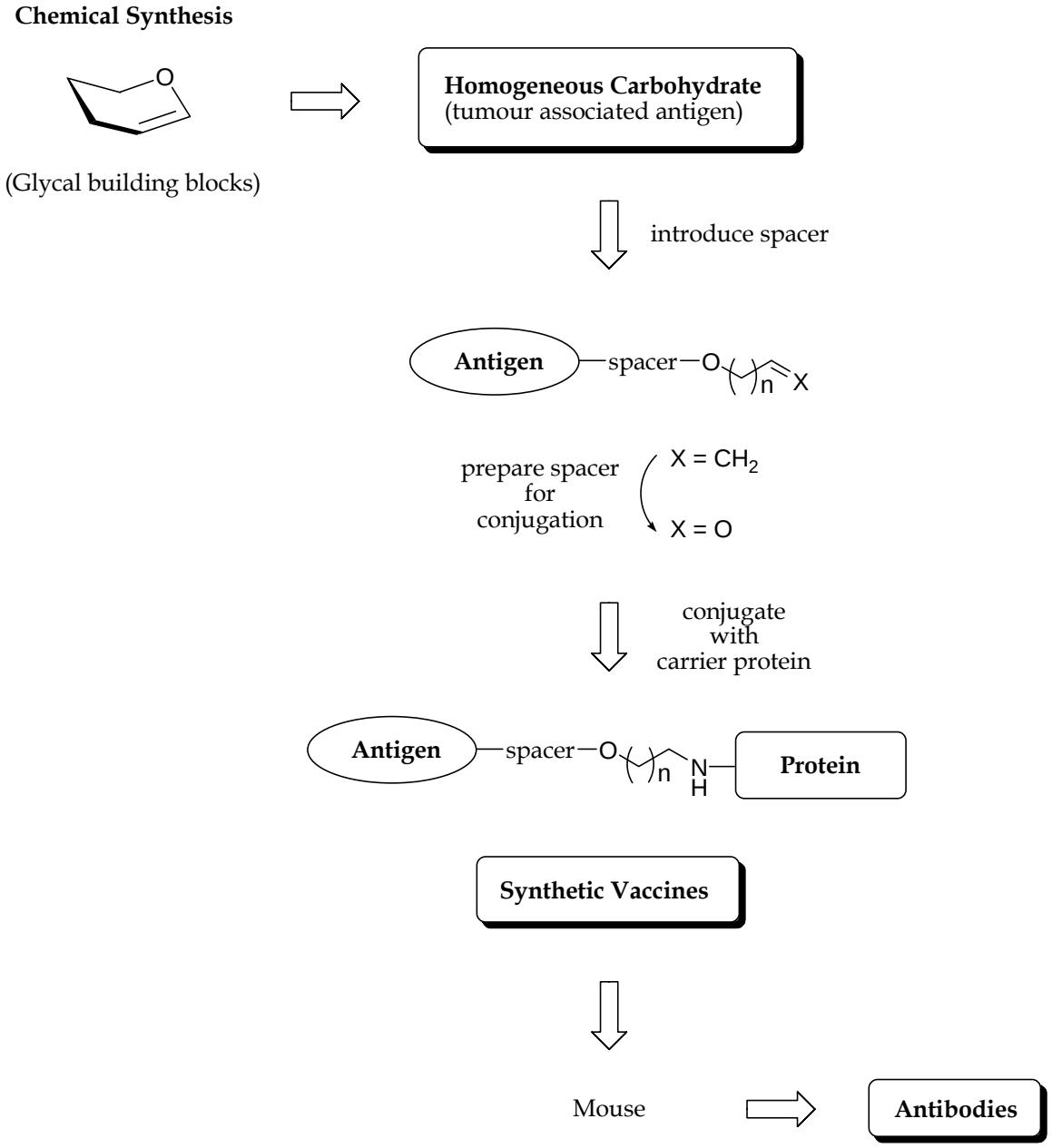


FIGURE 3.5 General Approach to Synthetic Carbohydrate Vaccines.

The only limit in this new field of research is represented by the synthetic ability to assemble such and even more complex glycoconjugate structures in sufficient amounts for immunological studies.
Several research groups have been addressing their efforts to the development of effective alternative synthetic strategies for the obtainment of these and other oligosaccharide antigen structures.
One of the main interest of our research group is the development of alternative glycosylation procedures relying on the use of mild and moisture stable promoters, featuring undoubted experimental advantages if compared with the acidic agents employed in the standard procedures. Recently, the feasible use of catalytic $Yb(OTf)_3$ for the activation of both armed and disarmed glycosyl trichloro-[21] and *N*-

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

phenyltrifluoroacetimidates[22] was reported.[23] The extremely mildness of $Yb(OTf)_3$–promoted glycosylation conditions was confirmed by the use of the very acid-labile dimethoxytrityl as protecting group, never reported before to withstand the strong acidic conditions imposed on the glycosylation medium by other promoters.[24]

More recently, 4Å acid washed molecular sieves (commercially known as AW 300 MS) were also found to efficiently activate glycosyl trihaloacetimidates, although in this case glycosidations did not exhibit a satisfying stereocontrol with donors devoid of participating groups.[25] The experimental advantages of using these promoters are a matter of fact, as they are cheap, very ordinary reagents (always present on the shelf of every synthesis lab), insoluble (thus, easily removable from the reaction medium by simple filtration), non toxic and very mild.
In order to expand the scope of these approaches to the synthesis of complex oligosaccharide sequences and contextually test their effectiveness, we investigate the feasible application of such promoters in the difficult task of Le^x trisaccharide and Globo-H tetrasaccharide fragment synthesis.

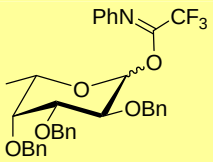
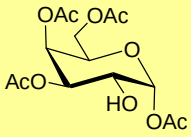
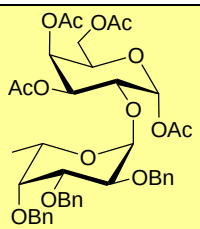
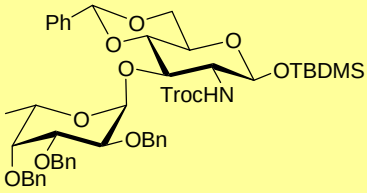
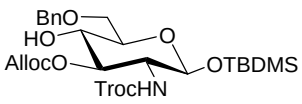
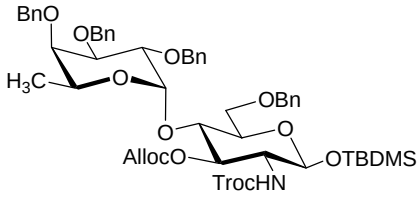
Results and Discussion

α-L-Fucosylation: a common synthetic challenge
As it can be observed in *Figures 3.2* and *3.4* all the depicted antigen structures (with a particular interest in Le^x and Globo-H) are characterized by the presence of one or more L-fucose units with α-anomeric configuration. The stereoselective construction of α-fucosidic linkages is a very challenging issue to perform.[26] In fact, fucosyl donors are quite reactive and amenable to decomposition, so that excess amounts are often required to achieve high glycosidation yields, especially with poorly reactive glycosyl acceptors.[27,28a] Additionally, α-fucosylation reactions lead to 1,2-*cis* glycosides whose stereoselective construction can not be guaranteed by an approach as efficient as the neighbouring participation effect exerted by acyl protecting groups on C-2 position in the stereo-controlled synthesis of 1,2-*trans* glycosides.[29]
To face these problems several tactics were described over the last years. For example, Schmidt has reported the use of the inverse procedure (slow addition of a fucosyl trichloroacetimidate to a mixture containing the promoter and the acceptor) to minimize

[22] Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405.
[23] a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Mangoni, L.; Schiattarella, M. *Tetrahedron Lett.* **2001**, *42*, 5967; b) Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573.
[24] Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479.
[25] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Org. Lett.* **2003**, *5*, 987.
[26] For a discussion on the problems related to α-fucosylations and pertinent references see: (a) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2001**, *123*, 9545; (b) Love, K. R.; Andrade, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001**, 66, 8165.
[27] For other recent examples: (a) Manzoni, L.; Lay, L.; Schmidt, R. R. *J. Carbohydr. Chem.* **1998**, *17*, 739; (b) Xia, J.; Alderfer, J. L.; Piskorz, C. F., Matta, K. L. *Chem. Eur. J.* **2000**, *6*, 3442; (c) Söderman, P.; Larsson, E. A.; Wilman, G. *Eur. J. Org. Chem.* **2002**, 1614; (d) Ando, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **2003**, *338*, 503; (e) Xia, J.; Alderfer, J. L.; Locke, R. D.; Piskorz, C. F.; Matta, K. L. *J. Org. Chem.* **2003**, 68, 2752.
[28] a) Schmid, U.; Waldmann, H. *Chem. Eur. J.* **1998**, *4*, 494; b) Böhm, G.; Waldmann, H. *Tetrahedron Lett.* **1995**, *36*, 3843.
[29] For a discussion on 1,2-*cis*-glycosides synthesis see pp. 23-31.

the fall in yields due to donor degradation.[30] An alternative and effective approach, used also with other galactose-type donors, relies on the long range participation effect performed by acyl protection on C-4 position of fucosyl donors.[26, 31] In order to avoid 1,2-*trans*-fucosides formation, C-2 hydroxyl group has to be functionalized with a non-participating protecting group, most commonly a benzyl group. Obviously, this entails an increased number of steps in the building block preparation, since a fine differentiation in the protection pattern of secondary hydroxyls is required, but glycosidation yields are generally improved as the partially acylated donors appear relatively less prone to degradation than their perbenzylated counterparts.[32]

With our target molecules in mind, we first investigated the feasible synthesis of α-fucosides by adopting the readily prepared perbenzylated donor **113** (*Table 3.1*). *N*-phenyltrifluoroacetimidate donors were chosen for their lower propensity to give undesired side products in the course of glycosidations,[33] and their higher stability in storage than the corresponding trichloroacetimidate analogues.[22] Furthermore, in order to reconcile this methodological investigation with the possibility to prepare useful disaccharide building blocks to be elaborated into the antigenic structures of our interest (preparing also useful precursors for Lewis[a,b] and Le[y] sequences), secondary model acceptors **114-116** were chosen.

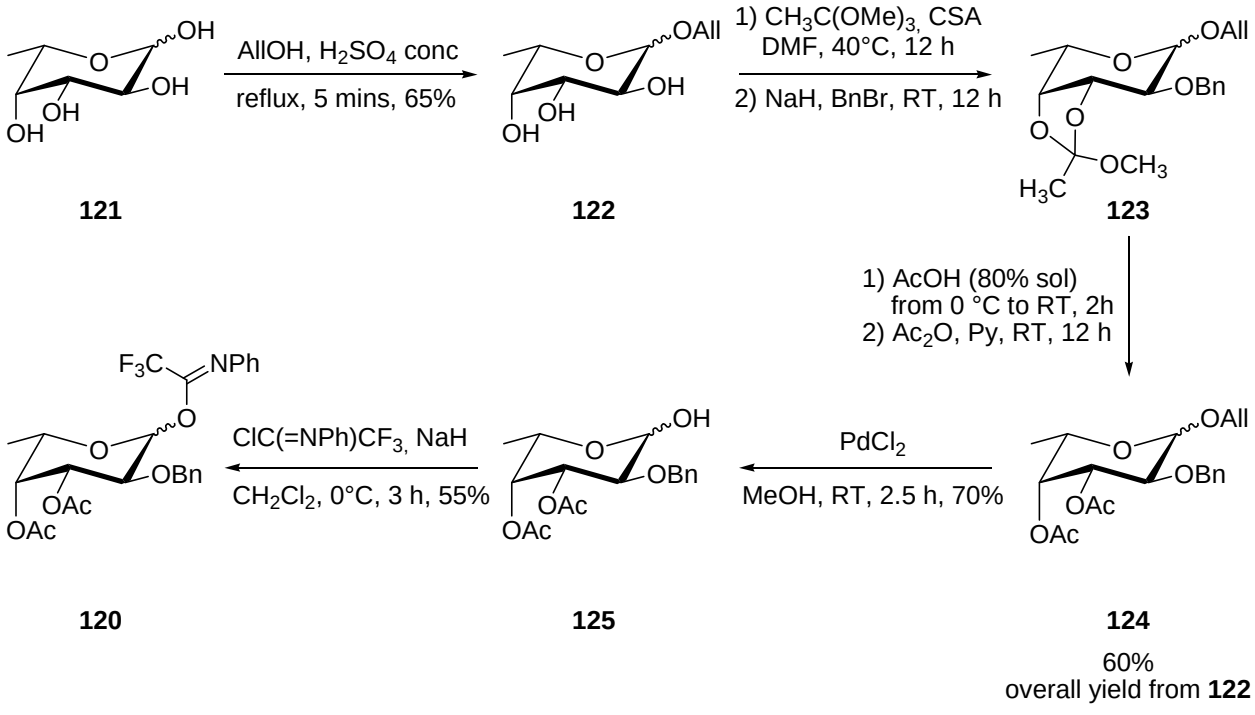
Entry	*Donor (equiv.)*	*Acceptor*	*Product*	*Yield[a] (α:β)*
1	 **113** (1.4)	 **114**	 **117**	66%[b] (9:1)
2	**113** (1.4)	**114**	**117**	79% (8:1)
3	**113** (3.0)	 **115**	 **118**	83% (>10:1)
4	**113** (2.5)	 **116**	 **119**	75% (>10:1)

[a] Procedure: $Yb(OTf)_3$ (0.1 equiv.), AW 300 MS, CH_2Cl_2/Et_2O/dioxane 4:1:1, -30 °C, 1-3 h; [b] Toluene/ Et_2O/dioxane 4:1:1 was used as solvent mixture.

TABLE 3.1 α-Selective Fucosylation of Acceptors **114-116** under the Agency of $Yb(OTf)_3$.

[30] Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353. A paradigm is also depicted in *Scheme 1.13* (page 17).
[31] See *Scheme 1.24* (page 27).
[32] Flowers, H. M. *Carbohydr. Res.* **1983**, *119*, 75.
[33] Tanaka, H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T. *J. Am. Chem. Soc.* **2005**, *127*, 1630-1631.

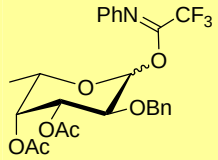
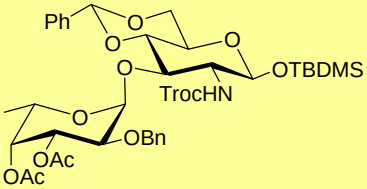
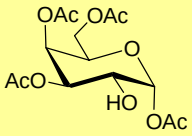
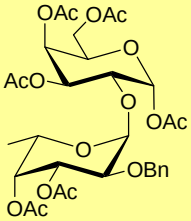
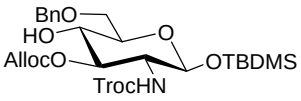
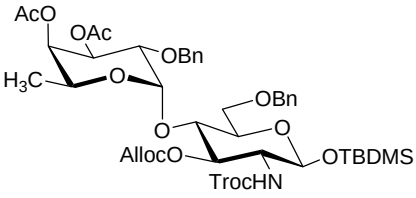
Noteworthy donor **113** is devoid of acyl protecting group on C-4 position, therefore the stereoselectivity of the reaction had to be controlled exploiting the α-directing effect of ether-type solvents. Since the AW 300 MS protocol proved to be incompatible with the solvent effect, for these couplings, only activation of $Yb(OTf)_3$ was examined. Thus, several conditions were tested for the coupling of **113** with acceptor **114**, starting from those previously reported for a perbenzylated glucosyl donor (-10 °C to r.t, 0.1 eq of $Yb(OTf)_3$, toluene/Et_2O/dioxane 4:1:1 as the solvent).[23b] As shown in *Table 3.1* (entries 1 and 2) fucosyl donor **113** proved to be reactive even at -30 °C in the presence of 0.1 eq of $Yb(OTf)_3$, while the ternary mixture dichloromethane/Et_2O/dioxane 4:1:1 represented the solvent of choice due to the best solubility of the acceptor at the low reaction temperature. Under these conditions a good yield was achieved for disaccharide **117** together with a good control of stereoselectivity. The established conditions of activation were then tested in the α-fucosylation of the glucosamine acceptors **115** and **116**, and also in these cases synthetically useful results in terms of both yield and selectivity were smoothly obtained (entries 3 and 4). Encouraged by these results, some effort was dedicated to ascertain whether α-selective fucosylations might be achieved with the simple activation of acid washed molecular sieves taking advantage of a long range participation effect.
For this purpose fucosyl *N*-penyl trifluoroacetimidate **120** was prepared following the synthetic pathway illustrated in *Scheme 3.1*.



SCHEME 3.1 Synthesis of Fucosyl Donor **120**.

The synthetic strategy used for the obtainment of donor **120** proved to be very straightforward. In fact, after allylation of fucose **121** in strongly acidic conditions, derivative **124** was obtained in four steps without any chromatographical purification of the intermediates. Allyl-fucoside **122** was one-pot converted into derivative **123**, exploiting an *in situ* benzylation after the regioselective installation of orthoacetate moiety on C-3 and C-4 positions. The orthoester ring was then opened in acidic condition, leaving the hydroxyl on C-3 position unprotected. Standard acetylation in pyridine furnished

derivative **124** in 60% yield over four steps. Fucosyl donor **120** was obtained by Pd(II)-catalyzed deallylation followed by treatment of the thus obtained lactol **125** with *N*-phenyl trifluoroaceimidoyl chloride in presence of a base.
The activation of **120** was initially tested in the attempted fucosylation of acceptor **115** (*Table 3.2*). The reaction proceeded at room temperature in 24-36 hours to afford the desired disaccharide **126** in good yield and high α-selectivity. Both toluene and dichloroethane proved to be suitable solvents for this reaction, comparable results being obtained (entries 1 and 2). The procedure was then tested on acceptors **114** and **116** to produce the corresponding disaccharides in good yield and complete α-selectivity (entries 4 and 5).
Having demonstrated the applicability of these alternative fucosylation protocols to the synthesis of several disaccharides, their extension to Le^x and Globo-H structures was attempted.

Entry	*Donor (equiv.)*	*Acceptor*	*Product*	*Yield[a] (α:β)*
1	 **120** (2.0)	 **115**	 **126**	61% (10:1)
2	**120** (2.0)	**115**	**126**	66%[b] (10:1)
3	**120** (2.0)	 **114**	 **127**	78%[b] (only α)
4	**120** (2.0)	 **116**	 **128**	58% (only α)

[a] Procedure: AW 300 MS, toluene, from 0 °C to RT, 24-36 h; [b] Dichloroethane was used as solvent.

TABLE 3.2 α-Selective Fucosylation of Acceptors **114-116** under the Agency of AW 300 MS.

Lewis^x trisaccharide assembly

Due to the central role played by Le^x motif in several biological processes and to its structural complexity, several strategies have been developed for its synthesis.[34] Although

[34] For some examples of syntheses of Lewis X derivatives: a) Jacquinet, J.-C.; Sinaÿ, P. *J. Chem. Soc., Perkin Trans. 1* **1979**, 314; b) Hindsgaul, O.; Norberg, T.; Pendu, J. L.; Lemieux, R. U. *Carbohydr. Res.* **1982**, *109*, 109; c) Lonn, H. *Carbohydr. Res.* **1985**, *139*, 115; d) Nillsson, M.; Norberg, T. *Carbohydr. Res.* **1988**, *183*, 71; e) Sato, S.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 5267; f) Classon, B.; Garegg, P. J.; Helland, A.-C. *J. Carbohydr. Chem.* **1989**, *8*, 543; g) Nillsson, M.; Norberg, T. *J. Carbohydr. Chem.* **1989**, *8*, 613; h) Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J.; Wong, C.H. *Chem. Commun.* **1991**, 870; i) Toepfer, A.; Schmidt, R. R. *Tetrahedron Lett.*

it's just a trisaccharide, Le^x offers several synthetic challenges. The central glucosamine unit has to be sequentially glycosylated on vicinal secondary hydroxyls (C-3 and C-4). This implies a fine differentiation in secondary hydroxyls protection has to be performed. Furthermore, the C-4 hydroxyl group is notoriously the less nucleophile on the saccharidic backbone. Thus, in order to maximize coupling yields and minimize the steric hindrance in the vicinity of the OH-4, galactosylation of glucosamine has to be attained in the first step. This reasonable choice affects, on the other hand, the following α-fucosylation step, making this already difficult task even more complicate.

Our synthetic strategy relies on the use of glucosamine acceptor **116** as key intermediate (*Scheme 3.3*). Derivative **116** posses on the C-4 a free OH, ready for the first galactosylation step, and carries on the C-3 position the selectively removable allyloxycarbonyl group. Since in the first glycosylation the formation of a β- linkage is required, derivative **129** was the donor of choice. The stereoselectivity of the reaction could be controlled by the neighbouring participating effect exerted by methoxycarbonyl group at C-2 hydroxyl of the donor. The choice of this unusual protecting group was supported by our previous observations that 2-*O*-methoxycarbonylated donors display less propensity to yield orthoester-like coupling products than the more canonical acetylated or benzoylated counterparts, especially when glycosidations are conducted under very mild activation conditions.[23a] Furthermore, chemical conditions for the removal of this group are comparable to those required by usual *O*-deacylations (see below).

The need to effectively synthesizing a such functionalized galactosyl donor (C-1 and C-2 hydroxyls are differentiated from the other positions and from each other) stimulated the development of a convenient alternative route to the one-pot preparation of glycosyl 1,2-orthoesters *via* glycosyl iodides.[35] As a matter of fact, galactosyl orthoester **122** (*Scheme 3*.2) represents an ideal precursor for galactosyl donor **129**, since C-1 and C-2 positions are already differentiated from the others, and the further differentiation from each other can be easily performed in acidic conditions. Therefore an effective strategy for the synthesis of galactosyl donor **129** was pursued. Orthoester intermediate **122** (diastereoisomeric mixture) was accessed starting from peracetylated galactopyranose **107** through a one pot sequence of anomeric iodination, halide promoted orthoesterification, deacetylation, and benzylation followed by a chromatographical purification (50-56 % overall yield).[36] **122** was then exposed to allyl alcohol at 70 °C in the presence of *in situ* generated HCl to achieve introduction of the anomeric allyl group and simultaneous deprotection of the 2-OH. Intermediate **130** (anomeric mixture) was readily purified by chromatography and then protected with a methoxycarbonyl group. The product of the TMEDA based methoxycarbonylation procedure[37] **131** was recovered pure in quantitative yield after a very short reaction time by simple extractive work-up. Compound **131** was subjected to

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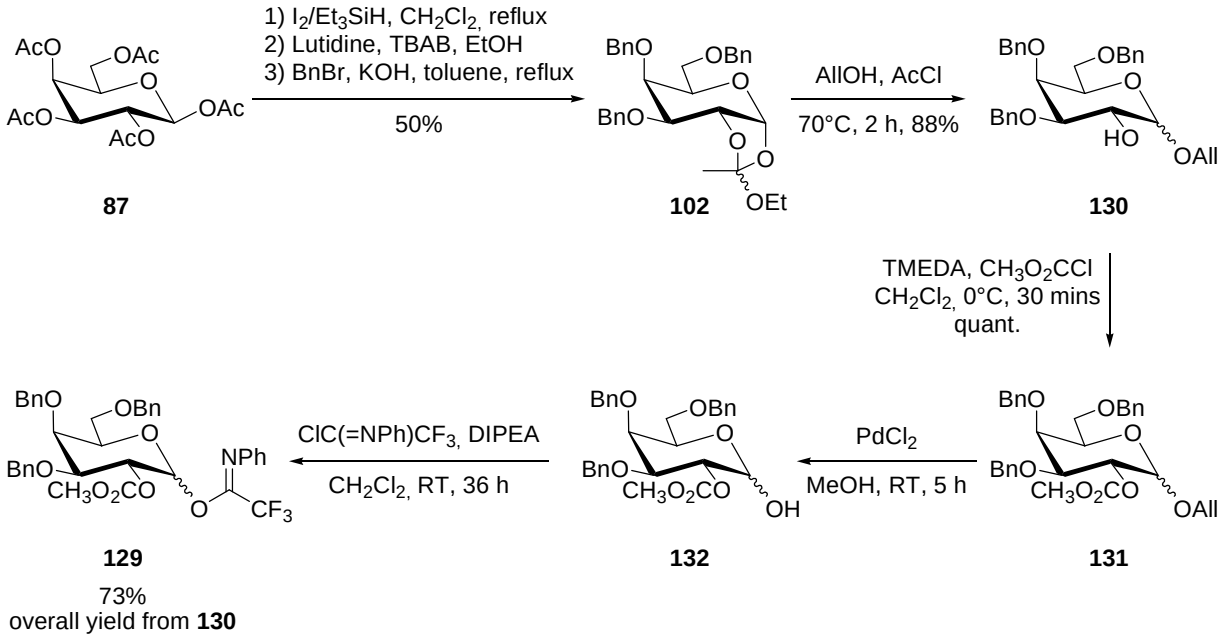
1992, *33*, 5161; j) Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R. *J. Am. Chem. Soc.* **1993**, *115*, 8843; k) Numomura, S.; Iida, M.; Numata, M.; Sugimoto, M.; Ogawa, T. *Carbohydr. Res*. **1994**, *263*, C1; l) vom de Brook, K.; Kunz, H. *Angew. Chem. Int. Ed. Eng*. **1994**, *33*, 101; m) Jain, R. K.; Vig, R.; Locke, R. D.; Mohammad, A.; Matta, K. L. *Chem. Commun*. **1996**, 65; n) Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239; o) Hummel, G.; Schmidt, R. R. *Tetrahedron Lett.* **1997**, *38*, 1173; p) Figueroa-Perez, S.; Verez-Bencomo, V. *Tetrahedron Lett.* **1998**, *39*, 9143; q) Ellervik, U.; Magnusson, G. *J. Org. Chem.* **1998**, *63*, 9314; r) Gege, C.; Vogel, J.; Bendas, G.; Rothe, U.; Schmidt, R. R. *Chem. Eur. J.* **2000**, *6*, 111; s) Gege, C.; Oscarson, S.; Schmidt, R. R. *Tetrahedron Lett.* **2001**, *42*, 377.;t) Majumdar, D.; Zhu, T.; Boons, G.-J. *Org. Lett.* **2003**, *5*, 3591.

[35] See *Chapter 2*.

[36] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 7863-7866.

[37] Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **2000**, *41*, 9305-9309.

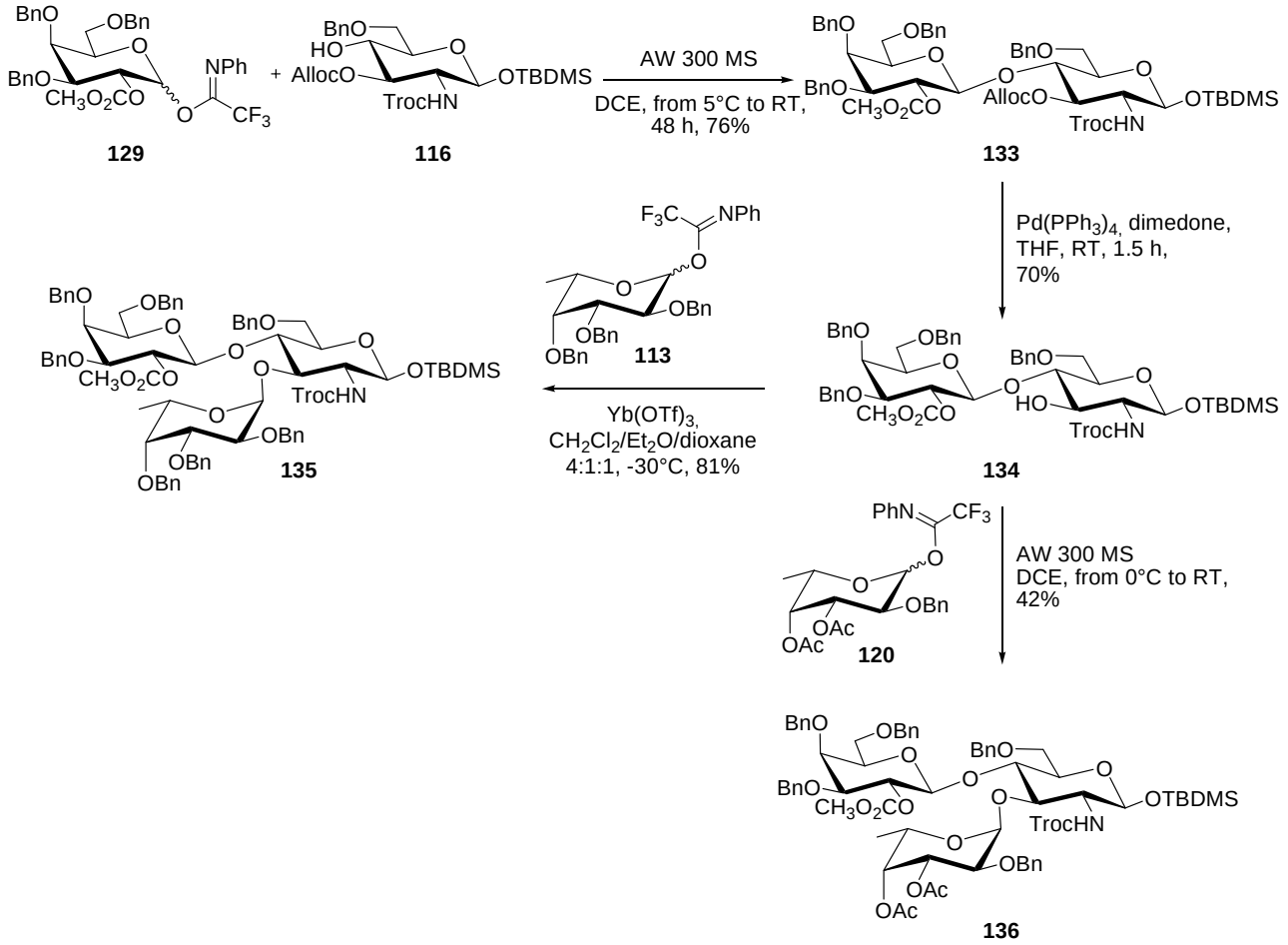
anomeric deallylation with catalytic $PdCl_2$. Crude compound **132**, isolated by a simple filtration, was directly converted into the corresponding trifluoroacetimidate **129**. It should be noted that the whole synthetic sequence to donor **129** requires eight chemical transformations but only three chromatographical purifications.



SCHEME 3.2 Synthesis of Galactosyl donor **129**.

With galactosyl donor **129** in the hands, glycosylation of glucosamine acceptor **116** was performed under the activation of acid washed molecular sieves (*Scheme 3.3*).
The choice of the promoter is strongly dependent by the nature of the linkage to be formed. Acid washed molecular sieves are generally preferable, because of the extreme simplification of the experimental procedure, but, as anticipated previously, their use is limited to 1,2-*trans*-glycosides formation with donors bearing participating groups at the C-2 position or alternatively to α-fucosides formation *via* C-4 acyl long range participation. In the absence of such a group, either 1,2-*cis* or 1,2-*trans* selectivity can be attained by the use of $Yb(OTf)_3$ and the suitable choice of the reaction solvent. Since, in this case, the formation of a β-linkage was required, exclusively acid washed molecular sieves were used as promoters in the coupling, furnishing disaccharide **133** in good yield (65-76%). Disaccharide **133** was smoothly deprotected at C-3[38] position of the glucosamine to provide acceptor **134** that was submitted to the fucosylation procedures previously established. The coupling with excess of **113** in the presence of catalytic $Yb(OTf)_3$ afforded trisaccharide **135** in high yield and complete α-selectivity (81%). Notably, the synthesis of the Lewis^x trisaccharide **136** based on the exclusive activation with AW MS in all the glycosidation steps turned out to be feasible although the final fucosylation proceeded in average yield (42%) but with complete selectivity.
The reported results demonstered the enforceability of our approaches to complex structures synthesis, thus encouraging to extend our methodologies to the synthesis of a tetrasaccharide fragment of Globo-H.

[38] Tanaka, H.; Amaya, T.; Takahashi, T. *Tetrahedron Lett.* **2003**, *44*, 3053.



SCHEME 3.3 Synthesis of Lewis^x Trisaccharides **135** and **136**.

Globo-H tetrasaccharide fragment assembly

The very promising results registered using Globo-H-KLH coniugate as anticancer vaccin stimulated the implementation of effective and high yielding synthetic strategies for the obtainment of this molecule.[39] Several truncated versions of Globo-H have been prepared and biologically evaluated in order to define synthetically simpler candidates as anticancer vaccines.[40] These investigations led to the disclosure of a relevant immunogenic activity associated to the tetrasaccharidic non reducing end of Globo-H (*Figure 3.6*).[41]

[39] For other syntheses of the complete sequence: a) Park, T. K.; Kim, I. J.; Hu, S.; Bilodeau, M. T.; Randolph, J. T.; Kwon, O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 11488-11500. b) Lassaletta, J. M.; Schmidt, R. R. *Liebigs Ann.* **1996**, 1417-1423. c) Zhu, T.; Boons, G.-J. *Angew. Chem. Int. Ed. Eng.* **1999**, *38*, 3495-3497. d) Burkhart, F.; Zhang, Z.; Wacowich-Sgarbi, S.; Wong, C.-H. *Angew. Chem. Int. Ed. Eng.* **2001**, *40*, 1274-1277.

[40] a) Lay, L.; Nicotra, F.; Panza, L.; Russo, G.; Adobati, E. *Helv. Chim. Acta* **1994**, *77*, 509-514. b) Lay, L.; Panza, L.; Russo, G.; Colombo, D.; Ronchetti, F.; Adobati, E.; Canevari, S. *Helv. Chim. Acta* **1995**, *78*, 533-538. c) Toma, L.; Colombo, D.; Ronchetti, F.; Panza, L.; Russo, G. *Helv. Chim. Acta* **1995**, *78*, 636-646. d) Kim, I. J.; Park, T. K.; Hu, S.; Abrampah, K.; Zhang, S.; Livingston, P. O.; Danishefsky, S. J. *J. Org. Chem.* **1995**, *60*, 7716-7717. e) Adobati, E.; Panza, L.; Russo, G.; Colnaghi, I.; Canevari, S. *Glycobiology* **1997**, *7*, 173-178.

[41] a) Panza, L.; Poletti, L.; Prosperi, D.; Canevari S.; Perico, M. E. *Eur. J. Org. Chem.* **2001**, 4331-4336. b) Perico, M. E.; Mezzanzanica, D.; Luison, E.; Alberti, P.; Panza, L.; Russo, G.; Canevari S. *Cancer Immunol. Immunother*. **2000**, *49*, 296-304.

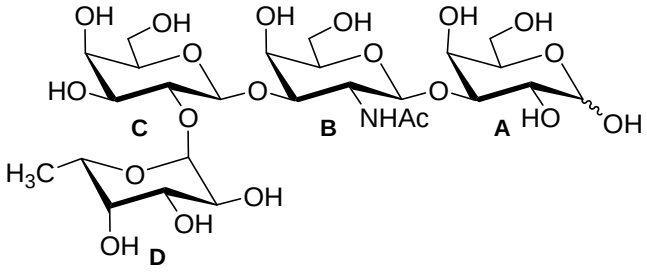


FIGURE 3.6 Biologically Active Tetrasaccharidic non-Reducing End of Globo-H Antigen.

Tetrasaccharide **137** was our target molecule and monosaccharide building blocks **138**, **139**, **129** and **113** were used as precursors of residues A-D, respectively (*Figure 3.7*).

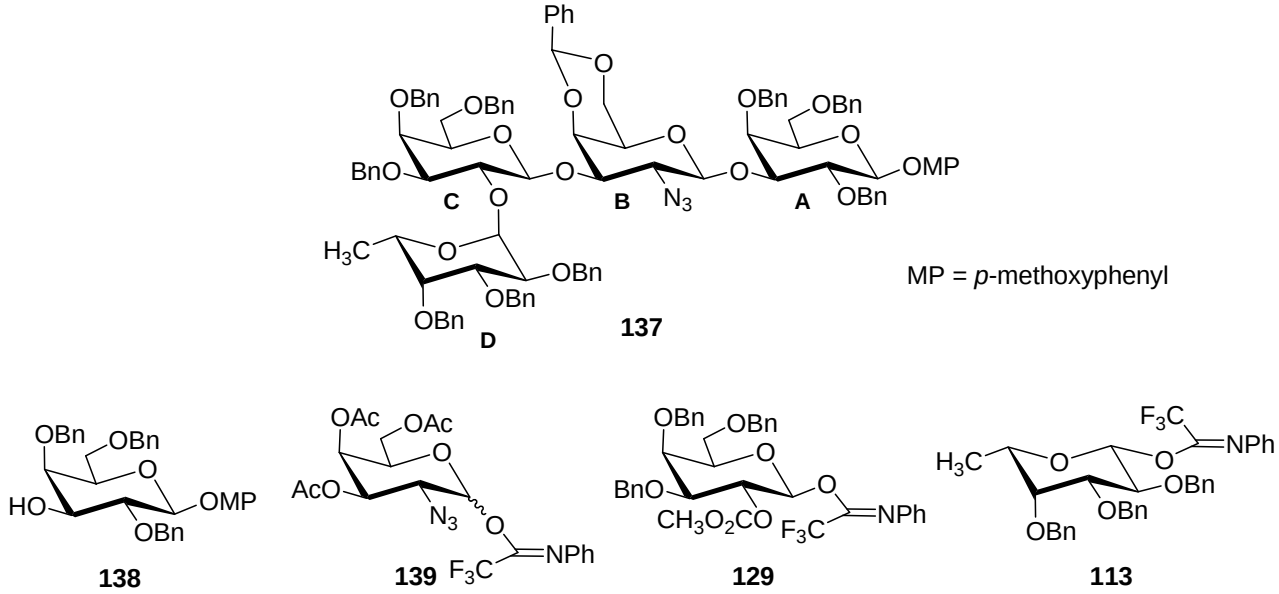
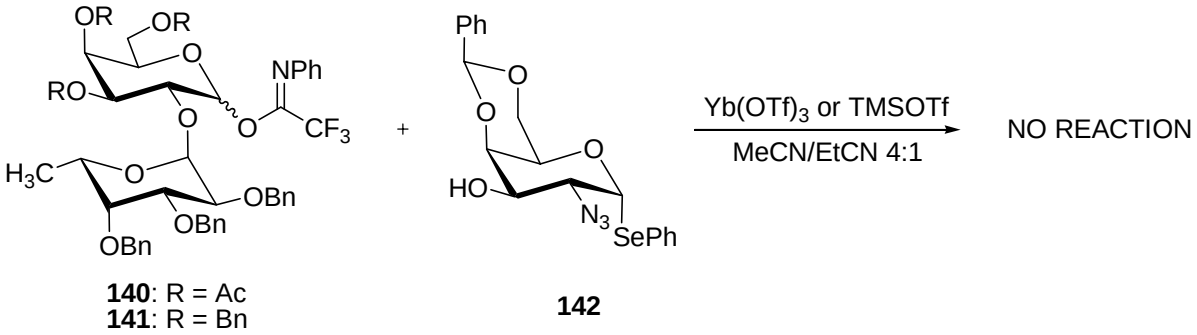


FIGURE 4.7 Target Compound and Building Blocks.

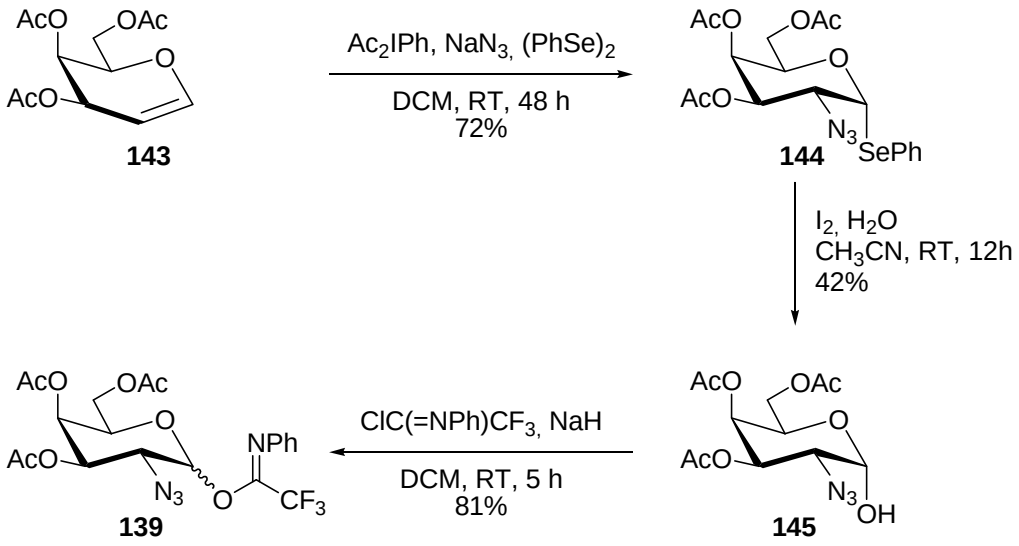
A linear approach was followed, because preliminary studies revealed the unfeasibility of a convergent approach (AB+CD). In fact, extreme poor reactivity was exhibited by CD residue precursors **140** and **141** in model couplings with monosaccharide acceptor **142** even under the stronger promoting action of TMSOTf (*Scheme 3.4*).[42]



SCHEME 3.4 Preliminary Studies for the Convergent Approach.

[42] Unpublished results.

The whole synthetic sequence proposed relies on the complemetary use of acid washed molecular sieves and $Yb(OTf)_3$ in stereocontrolled construction of three strategically different typologies of glycosidic linkages.
The linear construction of the tetrasaccharide started with the coupling (*Scheme 3.6*) of the known acceptor **138**[43] with donor **139** (anomeric mixture), equipped with a 2-azido functionality, whose synthesis was conducted following the synthetic pathway depicted in *Scheme 3.5*.



SCHEME 3.5 Synthesis of Galactosyl Donor **139**.

In the first step, the relatively cheap tri-*O*-acetyl-D-galactal **143** was effectively converted into galactosazide derivative **144**. Azido-phenylselenylation was reported to occur with exclusive regio-selectivity providing the installation of the azido-moiety on the C-2 and the phenylselenide on the anomeric center.[44] Furthermore, in the case of galacto-series the reaction exhibits also a regarding stereoselectivity: the azido function attacks the C-2 position with an equatorial orientation, providing thus only the galacto-derivative **144**. In the case of gluco-series a lower stereoeselctivity is observed and the azidoselenylation yield is decreased by the formation of undesired mannose-type derivative. Selenoglycoside **144** was hydrolized *via* iodine-mediated activation of the selenide moiety[45] and then efficiently converted into galactosyl donor **139** by treatment with *N*-phenyl trfluoroacetimidoyl chloride and sodium hydride.
Despite the lack of participating ability of the azide group, the reaction gave excellent results thanks to the activation of catalytic ytterbium(III) triflate (0.1 eq) and the β-directing effect exerted by the acetonitrile solvent.[23b,46] As a matter of fact, the β-linked disaccharide **146** was obtained in high yield (70-77%) and traces of the α-linked disaccharide could be monitored only by a careful inspection of the NMR spectrum of the crude reaction mixture. Interestingly, this result was achieved without resorting to the low

[43] Bazin, H. G.; Du, Y. ; Polat, T.; Linhardt, R. J. *J. Org. Chem.* **1999**, *64*, 7254-7259.
[44] Czerniecki, S.; Randriamandimby, D. *Tetrahedron Lett.* **1993**, *34*, 7915.
[45] Kartha, K. P. R.; Karkkainen, T. S.; Marsh, S. J.; Field, R. A. *Synlett* **2001**, 260.
[46] Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694-696.

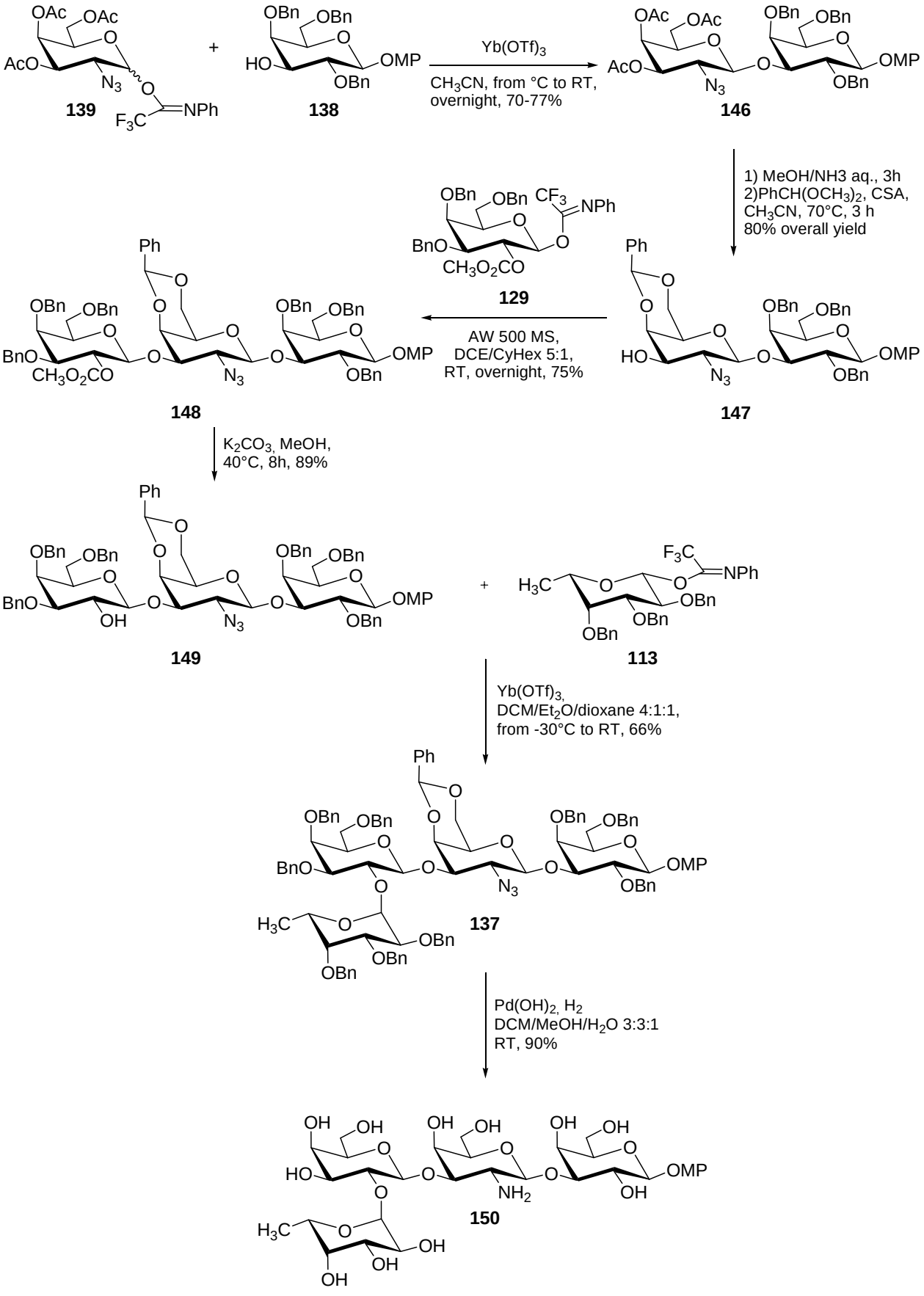
temperatures required for the corresponding TMSOTf promoted reactions of 2-azido trichloroacetimidates.[47] Moreover, 2-azido-3,4,6-*O*-acetylated trichloroacetimidates were recently reported to provide disappointing results in TMSOTf promoted glycosidatons in nitrile solvents.[47]

Disaccharide **146** was submitted to a deacetylation-benzylidenation sequence that readily provided the disaccharide acceptor **147** (80% yield over two steps) that was then coupled with the galactose donor **129**. In initial attempts the use of commercially available 4Å acid washed molecular sieves in the double role of activators and drying agents led to satisfying yields (61-64 %).[25] Replacement of the 4Å with the 5Å AW MS afforded slightly higher yields (65-70%) within a sensibly shorter reaction time (*ca*. 24 h *vs* 48 h). A further improvement (75% yield) was registered with a modified procedure that entails the slow addition of donor **129** to a solution of acceptor **147** in a dichloroethane/cyclohexane mixture containing the 5Å sieves.

The resulting trisaccharide **148** was easily deprotected with K_2CO_3 in methanol at 40°C to yield acceptor **149** (89%). The final sterecontrolled α-*L*-fucosylation of the sterically encumbered 2-OH was achieved by means of the previously described procedure which combines the efficient activation of catalytic ytterbium(III) triflate with the α-directing solvent mixture 4:1:1 dichloromethane/dioxane/diethyl ether. Due to the high reactivity of the perbenzylated fucosyl donor **113**, the reaction was conducted at low temperature (-30°C) to give the desired α-anomer **137** (66% yield).

Derivative **137** is expected to be a useful building-block for the planned synthesis of novel *N*-derivatized analogues, the 2-azido group functionality representing a useful handle to this purpose. However, in order to verify the feasibility of block deprotection, hydrogenolysis of **137** led to the removal of benzyl and benzylidene groups and the concomitant reduction of the azide functionality furnishing **150** tetrasaccharide in good yield.

[47] Tsuda, T.; Nakamura, S.; Hashimoto, S. *Tetrahedron* **2004**, *60*, 10711-10737.



SCHEME 3.6 Globo-H Tetrasaccharide Assembly.

Conclusion

In conclusion, we have reported the use of two alternative moisture stable and mild activating systems of *N*-phenyltrifluoroacetimidate donors for the stereocontrolled synthesis of α-fucosides. In a first approach catalytic $Yb(OTf)_3$ was found to provide good yields and α-selectivity in short reaction times when used in combination with solvent mixtures containing diethyl ether and dioxane. In an alternative approach, synthetically useful results were achieved by activating a partially acylated fucosyl donor with AW 300 MS. In this case a high stereocontrol could be obtained exploiting a long-range participation effect of the acyl groups installed on the fucose residues. Both these approaches were used in the synthesis of several fragments contained in biologically interesting sequences, including the Lewis^x trisaccharide and the tetrasaccharide extremity of Globo-H. In the latter case, complementary use of both promoting system was achieved. In the absence of 2-*O*-participating groups on the donor, ytterbium(III) triflate proved efficient in promoting the synthesis of either 1,2-*cis* or 1,2-*trans* glycosides, depending on the nature of the adopted solvents. With the donor equipped with appropriate participating group even the sole acid washed molecular sieves could be used to conveniently perform the required 1,2-*trans* glycosidation. This work demonstrates that $Yb(OTf)_3$ and acid washed molecular sieves represent a practical alternative to the harsh and sensitive agents adopted in standard glycosylation protocols even for the assemblage of non trivial oligosaccharide sequences.

Experimental Section

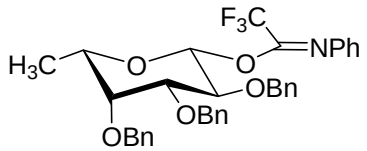
General Methods and Material

Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian spectrometers at 200 or 300 MHz and 50 or 75 MHz, respectively; or on a Bruker Avance 500 spectrometer at 500 MHz. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Analytical thin layer chromatography (TLC) was performed on E. Merck pre-coated (25 nm) silica gel 60F-254 plates. Column chromatography was preformed using Carlo Erba 0.06-0.20 silica gel. Anhydrous solvents stored over molecular sieves (purchased from Aldrich) were used for performing the reactions.

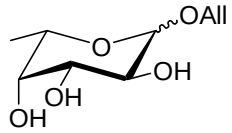
α-L-Fucosylations

General procedure for the synthesis of N-phenyl trifluoroacetimidate armed donors.

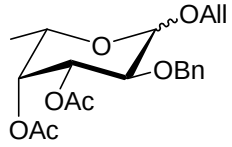
0.192 mmol of lactol are fluxed under argon and then dissolved at room temperature in 3 mL of dry CH_2Cl_2. The system is cooled down to 0°C and 32 μL (0.250 mmol) of *N*-phenyl trifluoroacetimidoyl chloride and 6 mg (0.250 mmol) of NaH (60%) are added. After 2 hours stirring the mixture is concentrated and the crude is purified by alumina column chromatography (petrol ether/ethyl acetate 9:1).



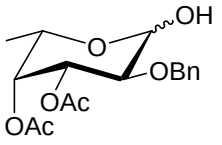
113. Donor **113** was prepared from the commercially available hemiacetal (purchased from CMS Chemicals) following the above described procedure. In this case the chromatographical purification was conducted on neutral alumina (eluent petroleum ether/ethyl acetate 95:5 with a few drops of TEA) to yield **113** (99%) as a white solid. $[\alpha]_D$ -92.4 (c 0.7 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-6.75 (aromatic protons), 5.58 (1H, bs, H-1), 5.04-4.64 (6H, 3xAB, 3x- benzyl CH_2), 4.04 (1H, bq, $J_{5,6}$ = 6.8 Hz, H-5), 3.70-3.50 (3H, H-2, H-3, and H-5), 1.17 (3H, d, 6-CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.5, 138.1, 138.1, and 138.0 (aromatic C), 128.5-127.5, 124.0, and 119.2 (aromatic CH), 97.4 (C-1), 16.5 (C-6). Other signals at 82.3, 77.9, 75.8, 75.4, 74.7, 73.0 and 71.5. $C_{35}H_{34}F_3NO_5$: calcd C 69.41, H 5.66; found C 69.29, H 5.38.



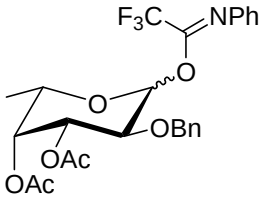
122. 40 mL of allyl alchool were added at room temperature to 2.105 g (12.8 mmol) of L-fucose **121**. The system was refluxed until the complete solubilization of the sugar. 320 μL of conc. H_2SO_4 were added dropwise. After 5 minutes, the mixture was neutralized with K_2CO_3 aq. After filtration, the crude was purified by silica gel column chromatography (CH_2Cl_2/MeOH 94:6) furnishing 1.693 g of **122** (65% yield).



124. 1.693 g (8.289 mmol) of **122** were coevaporated with dry toluene, fluxed under argon and dissolved into 15 mL of dry DMF at room temperature, then 16.1 mL (124.335 mmol) of methylorthoacetate and 192 mg (0.829 mmol) of canphorsulforic acid were added. The mixture was stirred for 12 h at 40 °C. Then 3.9 mL (0.0331 mmol) of benzyl bromide and 2.487 g of NaH (60%) were admixed. After 5 hours stirring, 2mL of MeOH were added to quench the unreacted traces of benzyl bromide. The mixture was then diluted with CH_2Cl_2 and the solvent removed under reduced pressure. The thus obtained crude **123** was dissolved in 20 mL of acetic acid (80% solution) at 0 °C. After 1.5 hours stirring the mixture was diluted with CH_2Cl_2 and washed with Na_2CO_3 aq. to neutrality. The organic phase was then concentrated. In order to achieve the final acetylation, the crude was dissolved in pyridine (10 mL) and 5 mL of Ac_2O were added. After reaction completion (12 hours at room temperature) MeOH was added to quench the unreacted anhydride, the mixture was diluted with CH_2Cl_2 and washed with water. The organic phase was concentrated and purified by silica gel column chromatography (petrol ether/ethyl acetate 8:2) obtaining **124** (1.867 g) in 60% yield. ^{1}HNMR ($CDCl_3$, 300 MHz) significative signals at δ : 7.40-7.20 (aromatic), 5.90 (1H, m, C<u>H</u>=CH_2), 5.40-5.10 (5H, m, H-3 α, H-4 α and β, 2H CH=C<u>H</u>$_2$), 5.00 (1H, dd, $J_{3,2}$= 9.37 Hz, $J_{3,4}$= 3.5 Hz, H-3 β), 4.88 (1H, d, $J_{1,2}$= 3.0 Hz, H-1 α), 4.72 (4H, 2xAB, CH_2 benzyl), 4.50 (1H, d, $J_{1,2}$= 7.5 Hz, H-1 α), 4.20-4.10 (3H, m, H-5 α and 2H C<u>H</u>$_2$-CH=CH_2), 3.86 (1H, dd, $J_{1,2}$= 3.5 Hz, $J_{2,3}$= 10.54 Hz, H-2 α), 3.76 (1H, m, H-5 β), 3.64 (1H, dd, $J_{1,2}$= 7.0 Hz, $J_{2,3}$= 10.54 Hz, H-2 β), 2.14 (6H, 2xs, CH_3 acetyl α and β), 1.997 (3H, s, CH_3 acetyl α), 1.966 (3H, s, CH_3 acetyl β), 1.217 (3H, d, ^{3}J= 7.3 Hz, 3H-6 β), 1.112 (3H, d, ^{3}J= 7.3 Hz, 3H-6 α)



125. To a solution of **124** (1.073 g, 2.840 mmol) in of MeOH (20 mL) 0.284 mmol of $PdCl_2$ were added. After 3 hours stirring, the mixture was diluted with CH_2Cl_2 and filtered through a small pad of silica gel (CH_2Cl_2/MeOH 9:1). The concentrated residue was purified by silica gel column chromatography furnishing **125** (649 mg, 70% yield). ^{1}HNMR ($CDCl_3$, 200 MHz) significative signals at δ : 7.50-7.20 (aromatic), 5.40-5.22 (3H, m, H-3 α, 2H-4 α and β), 5.15 (1H, d, ^{3}J= 3.8 Hz, H-1 α), 5.00-4.59 (6H, m, H-3 β, 2xCH_2 benzyls α and β and H-1 β), 4.31 (1H, q, ^{3}J= 7.7 Hz, H-5 α), 3.90-3.70 (2H, m, H-2 α and H-5 β), 3.55 (1H, t, ^{3}J= 9.6 Hz, H-2 β), 2.20-1.80 (12H, 4xs, 4xCH_3 acetyls α and β), 1.20-1.00 (6H, m, 2x 3H-6 α and β).

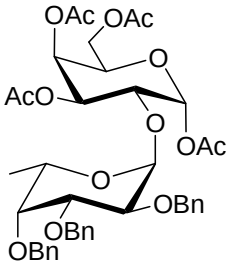


120. (α/β 1:1.6): ^{1}HNMR ($CDCl_3$, 200 MHz) significative signals at δ: 7.40-7.20 (aromatic), 6.90 (2H, d, ^{3}J= 7.7 Hz, H-orto Ph, β), 6.75 (2H, d, ^{3}J= 7.2 Hz, H-orto Ph, α), 6.60 (1H, bs, H-1 α), 5.46-5.36 (2H, M, H-3 and H-4), 5.26 (1H, bs, H-1 β), 4.90-4.65 (4H, 2x AB, CH_2 benzyls α and β), 4.35 (1H, m, H-5 α), 4.10-3.80 (3H, m, H-2 α and β and H-5 β), 2.22 (3H, s, CH_3 acetyl β), 1.23-1.21 (6H, m, 3xH-6 α and β).

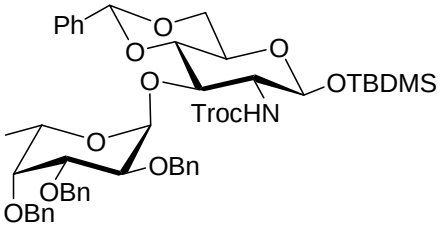
Procedure with Yb(OTf)$_3$: A mixture of acceptor (0.2 mmol) and donor **113** (see *Table 3.1* for relative amounts) were coevaporated three times in anhydrous toluene and the residue was kept under vacuum for 1 hour. Acid washed molecular sieves (4 Å AW 300 MS, pellets, 200 mg) were then added and the mixture dissolved at 0°C with dichloromethane (2.8 mL), and diethyl ether (700 μL). After cooling at -30 °C, a solution of $Yb(OTf)_3$ (12.5 mg, 0.02 mmol) in dioxane (700 μL) was added drop-wise. The mixture was kept under stirring at this temperature until complete consumption of the fucosyl donor (1-3 h, TLC) and then few drops of triethylamine were added. The mixture was filtered on a short pad of silica gel, concentrated, and the residue purified by silica gel chromatography (eluent: hexane/ethyl acetate mixtures).

Procedure with AW 300 MS: A mixture of acceptor (0.2 mmol) and donor **120** (see *Table 3.2* for relative amounts) were coevaporated three times in anhydrous toluene and the residue was kept under vacuum for 1 hour. Acid washed molecular sieves (4 Å AW 300 MS, pellets, 1.5-2 g) were then added and the solvent (dichloroethane or toluene, 2-4 mL) was added at 0 °C. The mixture was kept at 0 °C under stirring for 30 minutes and then temperature was left to raise spontaneously. After complete consumption of the donor (24-36 hours), the mixture was filtered through a cotton pad and concentrated. The residue was purified by silica gel chromatography (eluent: hexane/ethyl acetate mixtures).

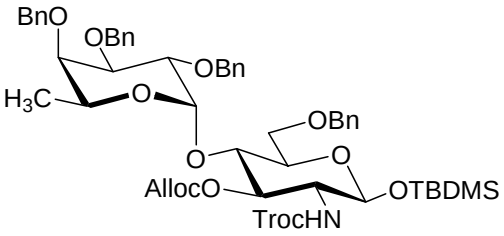
In the following list of selected NMR data, the saccharidic residue derived from the donor is indicated as A, while the residue derived from acceptor is indicated as B.



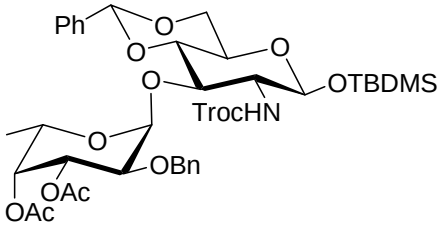
117. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40-7.20 (aromatic protons), 6.28 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1 B), 5.48 (1H, bd, $J_{3,4}$ = 3.2 Hz, H-4 B), 5.35 (1H, dd, $J_{2,3}$ = 10.6 Hz, H-3 B), 5.05 (1H, d, $J_{1,2}$ = 3.4 Hz, H-1 A), 5.00-4.60 (6H, 3x benzyl CH_2), 4.29 (1H, bd, $J_{5,6}$ = 6.6 Hz, H-5 B), 4.20-3.95 (4H, m, H-2 A, H-2 B and H_2-6 B), 3.83 (1H, bq, $J_{5,6}$ = 6.6 Hz, H-5 A), 3.77 (1H, dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.8 Hz, H-3 A), 3.63 (1H, bd, H-4 A), 2.16, 2.10, 2.03, 1.73 (12H, 4xs, 4x acetyl CH_3), 1.11 (3H, d, $J_{5,6}$ = 6.6 Hz, H_3-6 A). ^{13}C NMR (50MHz, $CDCl_3$) δ 170.4, 170.0, 170.0, 169.6 (4x -$\underline{C}OCH_3$), 138.6, 138.6, and 138.5 (aromatic C), 128.3 - 127.2 (aromatic CH), 99.6 and 91.3 (C-1 A and B), 20.9, 20.6, 20.6, 20.4 (4x -CO$\underline{C}H_3$), 16.5 (6-CH_3 A); other signals at δ 78.5, 77.2, 75.7, 74.7, 72.7, 72.6, 71.4, 69.3, 68.3, 67.6, 67.2, 61.2.



118. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.50-7.20 (aromatic protons), 5.50 (1H, s, benzylidene acetal CH), 5.17 (1H, d, $J_{1,2}$ = 3.4 Hz, H-1 A), 4.98 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1 B), 4.96-4.50 (8H, m, Troc CH_2 and 3x benzyl CH_2), 4.30 (1H, dd, $J_{5,6eq}$ = 4.5 Hz, $J_{6ax,6eq}$ = 10.2 Hz, H-6_{eq} B), 4.25 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3 B), 4.14-4.04 (2H, m, H-2 and H-5 A), 3.96 (1H, dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.6 Hz, H-3 A), 3.77 (1H, t, H-6_{ax} B), 3.62 (1H, t, H-4 B), 3.57 (1H, bd, H-4 A), 3.47 (1H, m, H-5 B), 3.25 (1H, m, H-2 B), 0.87 (9H, s, -SiC$(CH_3)_3$), 0.82 (3H, d, $J_{5,6}$ = 6.6 Hz, H_3-6 A), 0.09 and 0.06 (6H, 2xs, -Si $(CH_3)_2$).^{13}C NMR (50MHz, $CDCl_3$) δ 153.8 (-NH-$\underline{C}$O-OCH_2CCl_3), 138.5, 138.4, 138.2, and 137.1 (aromatic C), 129.0-126.2 (aromatic CH), 101.6, 97.8, and 95.6 (benzylidene acetal CH, C-1 A and B), 25.5 (-SiC($\underline{C}H_3)_3$), 17.8 (-Si$\underline{C}(CH_3)_3$), 16.2 (6-CH_3 A), -4.3 and -5.4 (-Si ($\underline{C}H_3)_2$); other signals at δ 80.5, 79.4, 74.9, 74.8, 74.6, 74.3, 73.6, 72.9, 71.6, 69.7, 66.7, 66.1, 61.1.

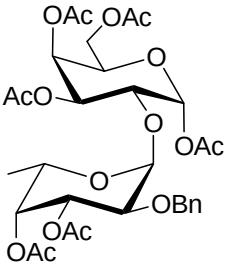


119. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.20 (aromatic protons), 5.92-5.78 (1H, m, CH_2=C$\underline{H}$-CH_2-), 5.28 (1H, bd, J_{trans} = 17.1 Hz, C$\underline{H}_{trans}$=CH-CH_2-), 5.20 (1H, bd, J_{cis} = 9.3 Hz, C$\underline{H}_{cis}$=CH-CH_2-), 5.08 (1H, d, $J_{1,2}$ = 8.7 Hz, H-1 B), 5.04 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 A), 4.94 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 10.2 Hz, H-3 B), 4.96-4.38 (12H, Troc CH_2, 4x benzyl CH_2, and CH_2=CH-C$\underline{H}_2$-), 4.20 (1H, bq, $J_{5,6}$ = 6.4 Hz, H-5 A), 4.01 (1H, dd, $J_{2,3}$ = 10.5 Hz, H-2 A), 3.88-3.50 (7H, H-3 A, H-4 A, H-2 B, H-4 B, H-5 B, H_2-6 B), 1.08 (3H, d, H_3-6 A), 0.87 (9H, s, -SiC$(CH_3)_3$), 0.12 and 0.08 (6H, 2xs, -Si$(CH_3)_2$). ^{13}C NMR (50MHz, $CDCl_3$) δ 155.2 and 154.0 (-NH-$\underline{C}$O-OCH_2CCl_3 and -O-$\underline{C}$O-All), 138.7, 138.6, 138.5, 138.4 (aromatic C), 131.3 (CH_2=$\underline{C}$H-CH_2-),128.4-127.4 (aromatic CH), 119.2 ($\underline{C}H_2$=CH-CH_2-), 99.4 and 95.9 (C-1 A and B), 25.6 (-SiC($\underline{C}H_3)_3$), 17.9 (-Si$\underline{C}(CH_3)_3$), 16.5 (6-CH_3 A), -4.1 and -5.3 (-Si($\underline{C}H_3)_2$); other signals at δ 79.4, 79.2, 77.6, 76.3, 75.2, 74.8, 74.2, 73.2, 72.8, 68.8, 67.4, 66.8, 58.7.

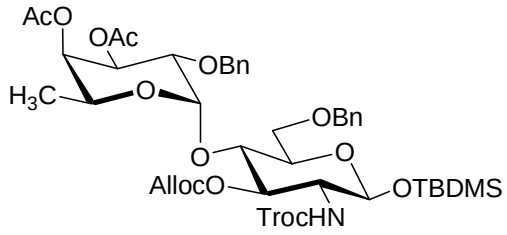


126. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.50-7.20 (aromatic protons), 5.50 (1H, s, benzylidene acetal CH), 5.34-5.26 (2H, m, H-3 and H-4 A), 5.24 (1H, $J_{NH,2}$ = 7.2 Hz, NH-2 B), 5.10 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1 A), 5.08 (1H, d, $J_{1,2}$ = 7.6 Hz, H-1 B), 4.90-4.44 (4H, m, Troc CH_2 and benzyl CH_2), 4.38-4.20 (3H, H-5 A, H-6_{eq} and H-3 B), 3.82 (1H,

dd, $J_{2,3}$ = 10.2 Hz, H-2 A), 3.78 (1H, t, $J_{3,4} = J_{4,5}$ = 10.2 Hz, H-4 B), 3.59 (1H, t, $J_{5,6ax} = J_{6ax,6eq}$ = 9.2 Hz, H-6_{ax} B), 3.48 (1H, m, H-5 B), 3.07 (1H, m, H-2 B) 2.07, 1.95 (6H, 2xs, 2x acetyl CH_3), 0.85 (9H, s, -SiC$(CH_3)_3$), 0.57 (3H, d, $J_{5,6}$ = 6.6 Hz, H_3-6 A), 0.06 and 0.04 (6H, 2xs, -Si$(CH_3)_2$). ^{13}C NMR (50MHz, $CDCl_3$) δ 170.6, 170.1, (2x -COCH$_3$), 153.7 (-NH-CO-OCH_2CCl_3), 138.7, 137.1 (aromatic C), 129.2 - 126.4 (aromatic CH), 102.0, 97.9, and 95.0 (non aromatic CH benzylidene, C-1 A and B), 25.5 (-SiC$(CH_3)_3$), 20.9, 20.6 (2x -COCH$_3$), 17.8 (-SiC$(CH_3)_3$), 15.1 (6-CH_3 A), -4.3 and -5.4 (-Si$(CH_3)_2$); other signals at δ 80.4, 77.1, 74.9, 74.5, 74.2, 73.1, 71.6, 70.1, 68.8, 66.1, 64.4, 61.4.

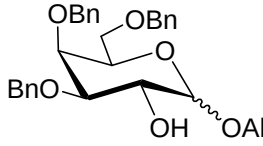


127. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40-7.20 (aromatic protons), 6.34 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 B), 5.47 (1H, bd, $J_{3,4}$ = 2.8 Hz, H-4 B), 5.34 (1H, dd, $J_{2,3}$ = 10.4 Hz, H-3 B), 5.26-5.14 (2H, m, H-3 and H-4 A), 5.07 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 A), 4.59 (2H, s, benzyl CH_2), 4.29 (1H, bt, $J_{5,6}$ = 7.0 Hz, H-5 B), 4.18-4.04 (4H, H-5 A, H-2 B, and H_2-6 B), 3.82 (1H, dd, $J_{2,3}$ = 9.6 Hz, H-2 A), 2.21, 2.15, 2.11, 2.03, 1.93, and 1.74 (18H, 6xs, 6x acetyl CH_3), 1.09 (3H, d, $J_{5,6}$ = 6.6 Hz, H_3-6 A). ^{13}C NMR (50MHz, $CDCl_3$) δ 171.3, 170.9, 170.9, 170.7, 170.7, 170.3 (6x -COCH$_3$), 138.6 (aromatic C), 128.9, 128.6, and 128.3 (aromatic CH), 100.0 and 91.1 (C-1 A and B), 20.1-19.8 (6x -COCH$_3$), 15.1 (6-CH_3 A); other signals at δ 73.2, 72.6, 71.3, 69.4, 69.1, 68.2, 65.3, and 61.0.



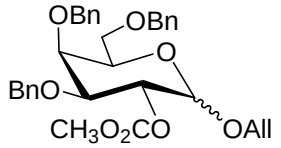
128. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40-7.20 (aromatic protons), 6.00-5.80 (1H, m, CH_2=CH-CH_2-), 5.40-5.14 (5H, CH_2=CH-CH_2-, H-3 A, H-4 A, and H-1 B), 5.11 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 A), 4.93 (1H, t, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3 B), 4.88-4.36 (8H, Troc CH_2, 2x benzyl CH_2, and CH_2=CH-CH_2-), 4.11 (1H, bq, $J_{5,6}$ = 6.4 Hz, H-5 A), 3.96-3.45 (6H, H-2 A, H-2 B, H-4 B, H-5 B, and 6-CH_2 B), 2.11 and 1.96 (6H, 2xs, 2x acetyl CH_3), 1.04 (3H, d, H_3-6 A), 0.87 (9H, s, -SiC$(CH_3)_3$), 0.12 and 0.08 (6H, 2xs, -Si$(CH_3)_2$). ^{13}C NMR (50MHz, $CDCl_3$) δ 170.5, 170.0 (2x -COCH$_3$), 155.2 and 154.0 (-NH-CO-OCH_2CCl_3 and -O-CO-All), 138.7, 137.1 (aromatic C), 131.1 (CH_2=CH-CH_2-), 128.4-127.4 (aromatic CH), 119.4 (CH_2=CH-CH_2-), 99.2 and 96.1 (C-1 A and B), 25.5 (-SiC$(CH_3)_3$), 20.8 and 20.7 (2x -COCH$_3$), 17.9 (-SiC$(CH_3)_3$), 15.7 (6-CH_3 A), -4.2 and -5.4 (-Si$(CH_3)_2$); other signals at δ 76.0, 75.0, 74.6, 73.9, 73.7, 73.3, 71.6, 70.2, 69.0, 68.2, 65.4, 58.7.

Lewis^x assembly

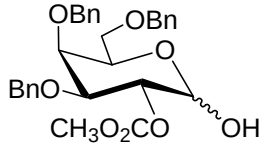


130. Acetyl chloride (530 μL, 6.75 mmol) was added to a solution of compound **122** (1.312 g, 2.5 mmol) in allyl alcohol (11 mL). The mixture was heated at 70°C for two hours and then concentrated under vacuum. The residue was purified on a short silica gel column (eluent petroleum ether/ethyl acetate 9:1) to afford **130** (1.09 g, 88% yield) as a white solid. (α:β ca 3.5) ^{1}H NMR of the major anomer (300 MHz, $CDCl_3$): δ 7.40-7.20 (aromatic protons), 6.00-5.84 (1H, m, CH_2-CH=CH_2), 5.26 (1H, bd, CH_2-CH=CH_{trans}, J_{trans} = 17.1 Hz), 5.17 (1H, bd, CH_2-CH=CH_{cis}, J_{cis} = 12.0 Hz), 4.98 (1H, d, $J_{1,2}$ = 3.9 Hz, H-1), 4.90-4.39 (6H, 3xAB, 3x benzyl CH_2), 4.24-4.20 (3H, overlapped signals, H-2 and CH_2-CH=CH_2), 3.98 (1H, bd, $J_{3,4}$ = 2.4 Hz, H-4), 3.94 (1H, bt, $J_{5,6a} = J_{5,6b}$ = 6.6 Hz, H-5), 3.71 (1H,

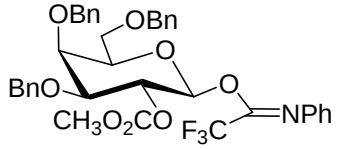
dd, $J_{2,3}$ = 9.6 Hz, H-3), 3.65-3.50 (2H, m, 6-CH_2). Significative signals of the δ anomer at δ 4.27 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1), 3.42 (1H, bd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 2.4 Hz, H-3). ^{13}C NMR major anomer (50 MHz, $CDCl_3$) δ 138.3, 138.1, 137.7 (aromatic C), 133.6 (-CH_2-$\underline{C}$H=CH_2), 128.2-127.4 (aromatic CH), 117.6 (-CH_2-CH=$\underline{C}H_2$), 97.6 (C-1). Other signals at δ 79.5, 74.5, 73.9, 73.3, 72.3, 69.5, 68.8, 68.7, 68.3. Significative signals of the β anomer at δ 133.8 (-CH_2-$\underline{C}$H=CH_2), 101.9 (C-1). $C_{30}H_{34}O_6$: calcd C 73.45, H 6.99; found C 73.21, H 6.81.



131. TMEDA (290 μL, 1.9 mmol) and methylchloroformate (200 μL, 2.6 mmol) were sequentially added at 0°C to a solution of **130** (672 mg, 1.4 mmol) in anhydrous dichloromethane (8 mL). After 30′ the mixture was diluted with dichloromethane and washed with water and the aqueous phase extracted with dichloromethane. The collected organic phases were concentrated to yield pure **131** (765 mg, quantitative yield) as an oil. (α:β ca 3.5:1) ^{1}H NMR of the major anomer (200 MHz, $CDCl_3$): δ 7.45-7.20 (aromatic protons), 6.05-5.80 (1H, m, -CH_2-$\underline{C}$H=CH_2), 5.40-5.16 (4H, overlapped signals, H-1, H-2, and -CH_2-CH=C$\underline{H}_2$), 5.04-4.40 (6H, 3xAB, 3x benzyl CH_2), 4.28-3.96 (5H, overlapped signals, H-3, H-4, H-5, and -$C\underline{H}_2$-CH=CH_2), 3.81 (3H, -OCH_3), 3.70-3.55 (2H, m, 6-CH_2). Significative signals of the β anomer at δ 4.45 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1), 3.82 (3H, -OCH_3). ^{13}C NMR major anomer (50 MHz, $CDCl_3$) δ 155.2 (-$\underline{C}O_2CH_3$), 138.4, 138.2, 137.9 (aromatic C), 133.7 (-CH_2-$\underline{C}$H=CH_2), 128.3-127.3 (aromatic CH), 117.4 (-CH_2-CH=$\underline{C}H_2$), 95.4 (C-1), 54.7 (OCH_3). Other signals at 74.6, 74.5, 73.3, 72.9, 69.4, 68.7, 68.3. Significative signals of the β anomer at δ 155.0 (-$\underline{C}O_2CH_3$), 116.9 (-CH_2-CH=$\underline{C}H_2$), 100.0 (C-1). $C_{32}H_{36}O_8$: calcd C 70.06, H 6.61; found C 70.19, H 6.52.

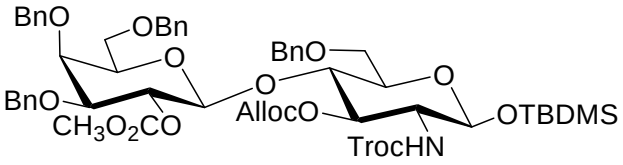


132. $PdCl_2$ (51 mg, 0.28 mmol) was added to a solution of **131** (759 mg, 1.4 mmol) in non anhydrous methanol (10 mL). After stirring for 5 hours at room temperature the mixture was concentrated and the residue filtrated on a short silica gel plug (eluent dichloromethane/methanol 95/5) to remove the palladium by products. The filtrated was concentrated to yield crude **132** (715 mg) in a satisfying purity to be directly used in the following step. (α:β ca 1.5:1) ^{1}H NMR of the major anomer (200 MHz, $CDCl_3$): δ 7.45-7.20 (aromatic protons), 5.51 (1H, t, $J_{1,2}$ = $J_{1,OH}$ = 3.4 Hz, H-1), 5.21 (1H, t, $J_{2,3}$ = 9.6 Hz, H-2), 4.96-4.38 (6H, 3xAB, 3x benzyl CH_2), 4.17 (1H, bt, $J_{5,6a}$ = $J_{5,6b}$ = 6.0 Hz, H-5), 3.99 (1H, dd, $J_{3,4}$ = 2.8 Hz, H-3), 3.93 (1H, bd, H-4), 3.78 (3H, s, -OCH_3), 3.65-3.40 (2H, m, 6-CH_2), 2.55 (1H, bd, 1-OH). Significative signals of the β anomer at δ 5.01 (1H, dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 4.68 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1), 3.81 (3H, -OCH_3). ^{13}C NMR major anomer (50 MHz, $CDCl_3$) δ 155.1 (-$\underline{C}O_2CH_3$), 138.1, 138.1, 137.3 (aromatic C), 128.2-127.3 (aromatic CH), 90.4 (C-1), 54.5 (OCH_3). Other signals at 76.3, 74.6, 74.4, 73.2, 72.8, 69.0. Significative signals of the β anomer at δ 155.7 (-$\underline{C}O_2CH_3$), 95.4 (C-1), 54.9 (-OCH_3).

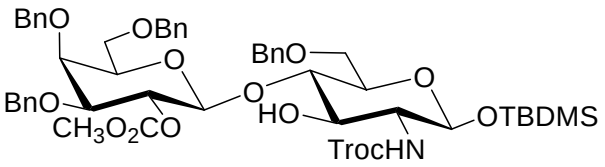


129. Crude compound **132** (715 mg, 1.4 mmol) was dissolved in anhydrous dichloromethane (7 mL). DIPEA (890 μL, 5.0 mmol) and *N*- (phenyl)trifluoroacetimidoyl chloride (560 μL, 4.2 mmol) were added at 0°C. The mixture was stirred for 36 hours at room temperature and then concentrated. The residue was chromatographed on a silica

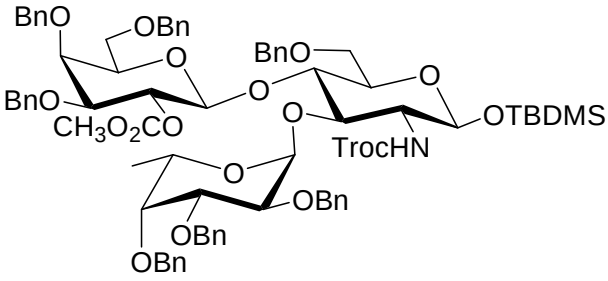
gel column eluted with petroleum ether/ethyl acetate 95:5 (with a few drops of TEA) to yield **129** (658 mg, 73% yield from 150) as an oil. $[\alpha]_D$ + 53.6 (c 0.9 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–6.80 (aromatic protons), 5.77 (1H, bs, H-1), 5.48 (1H, bt, $J_{1,2} = J_{2,3}$ = 9.6 Hz, H-2), 5.04-4.46 (6H, 3x AB, 3x benzyl CH_2), 4.05 (1H, bd, $J_{3,4}$ = 2.4 Hz, H-4), 3.88 (3H, s, OCH_3), 3.60-3.85 (4H, overlapped signals, H-3, H-5 and 6-CH_2). ^{13}C NMR (50 MHz, $CDCl_3$) δ 154.7 ($O\underline{C}O_2CH_3$), 143.2, 138.0, 137.6, and 137.4 (aromatic C), 128.6-127.4, 124.2, and 119.2 (aromatic CH), 95.1 (C-1), 55.1 (OCH_3). Other signals at 79.8, 74.6, 74.5, 73.4, 72.4, 67.8. ESI-MS for $C_{37}H_{36}F_3NO_8$ (m/z): M_r (calcd) 679.24, M_r (found) 701.94 $(M+Na)^+$. $C_{37}H_{36}F_3NO_8$: calcd C 65.38, H 5.34; found C 65.43, H 5.39.



133. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50-7.20 (aromatic protons), 5.82-5.66 (1H, m, CH_2=C<u>H</u>-CH_2-), 5.18 (1H, bd, J_{trans} = 17.2 Hz, C<u>H</u>$_{trans}$=CH-CH_2-), 5.09 (1H, bd, J_{cis} = 9.2 Hz, C<u>H</u>$_{cis}$=CH-CH_2-), 5.05 (1H, dd, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 10.2 Hz, H-2 A), 4.95 (1H, t, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3 B), 4.95-4.24 (12H, Troc CH_2, 4x benzyl CH_2, and CH_2=CH-C<u>H</u>$_2$-), 4.69 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1 B), 4.52 (1H, d, H-1 A), 3.96 (1H, bd, $J_{3,4}$ = 2.4 Hz, H-4 A), 3.87 (1H, t, H-4 B), 3.75 (3H, s, -OCH_3), 3.90-3.45 (6H, H-5 A, H_2-6A, H-2 B, H-5 B, H_2-6 B), 3.42 (1H, dd, H-3 A), 0.88 (9H, s, -SiC(CH_3)$_3$), 0.13 and 0.08 (6H, 2xs, Si(CH_3)$_2$). ^{13}C NMR (50MHz, $CDCl_3$) δ 154.9, 154.6, and 154.0 (-NH-<u>C</u>O-OCH_2CCl_3, -O-<u>C</u>O-OMe, -O-<u>C</u>O-OAll), 138.5, 138.3, 137.8, and 137.8 (aromatic C), 131.4 (CH_2=<u>C</u>H-CH_2-), 128.4-127.2 (aromatic CH), 118.3 (<u>C</u>H_2=CH-CH_2-), 101.1 and 96.1 (C-1 A and B), 95.3 (-NH-CO-OCH_2<u>C</u>Cl_3), 54.9 (-OCH_3), 25.5 (-SiC(<u>C</u>H_3)$_3$), 17.8 (-Si<u>C</u>(CH_3)$_3$), -4.2 and -5.4 (-Si(<u>C</u>H_3)$_2$); other signals at δ 80.4, 76.1, 75.8, 74.6, 74.5, 74.4, 73.4, 73.1, 72.5, 71.9, 68.5, 68.0, 67.6, and 58.1.

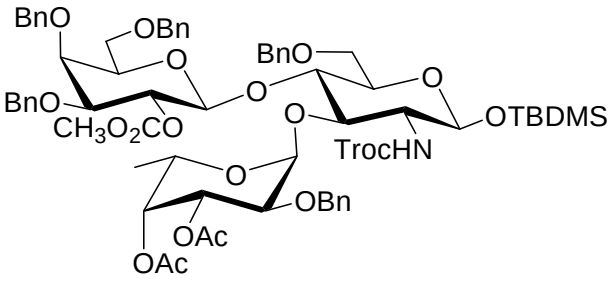


134. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.20 (aromatic protons), 5.15 (1H, dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.2 Hz, H-2 A), 4.98 (1H, bd, NH), 4.94-4.36 (10 H, Troc CH_2, 4x benzyl CH_2), 4.74 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1 B), 4.41 (1H, d, H-1 A), 3.88 (1H, bd, $J_{3,4}$ = 3.0 Hz, H-4 A), 3.78 (3H, s, -OCH_3), 3.82-3.44 (9H, H-3 A, H-5 A, H_2-6 A, H-3 B, H-4 B, H-5 B, H_2-6 B), 3.36 (1H, m, H-2 B), 0.89 (9H, s, -SiC(CH_3)$_3$), 0.13 and 0.09 (6H, 2xs, Si(CH_3)$_2$). ^{13}C NMR (50MHz, $CDCl_3$) δ 155.0 and 154.0 (-NH-<u>C</u>O-OCH_2CCl_3, -O-<u>C</u>O-OMe), 138.4, 137.9, 137.5, and 137.3 (aromatic C), 128.4-127.2 (aromatic CH), 101.7 and 95.8 (C-1 A and B), 95.3 (-NH-CO-OCH_2<u>C</u>Cl_3), 55.1 (-OCH_3), 25.6 (-SiC(<u>C</u>H_3)$_3$), 17.9 (-Si<u>C</u>(CH_3)$_3$), -4.2 and -5.3 (-Si(<u>C</u>H_3)$_2$); other signals at δ 81.8, 80.2, 75.5, 74.6, 74.5, 73.9, 73.7, 73.6, 73.1, 72.5, 72.3, 71.9, 68.5, 68.1, 59.5.



135. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50-7.20 (aromatic protons), 5.10 (1H, d, $J_{1,2}$ = 3.9 Hz, H-1 Fuc), 5.07 (1H, d, $J_{1,2}$ = 8.2 Hz, H-1 GlcN), 5.03 (1H, dd, $J_{1,2}$ = 7.4 Hz, $J_{2,3}$ = 10.2 Hz, H-2 Gal), 4.62 (1H, d, H-1 Gal), 4.90-4.34 (17 H, Troc CH_2, 7x benzyl CH_2, and H-5 Fuc), 4.18 (1H, t, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3

GlcN), 4.04-3.26 (12H, H-3 Gal, H-4 Gal, H-5 Gal, H_2-6 Gal, H-4 GlcN, H-5 GlcN, H_2-6 GlcN, H-2 Fuc, H-3 Fuc, and H-4 Fuc), 3.81 (3H, s, -OCH_3), 3.03 (1H, m, H-2 GlcN), 1.13 (3H, d, $J_{5,6}$ = 6.2 Hz, H_3-6 Fuc), 0.86 (9H, s, -$SiC(CH_3)_3$), 0.08 and 0.03 (6H, 2xs, -$Si(CH_3)_2$). ^{13}C NMR (50MHz, $CDCl_3$) δ 155.0 and 153.4 (-NH-CO-OCH_2CCl_3, -O-CO-OMe), 139.3, 139.2, 138.8, 138.6, 138.4, 137.9, and 137.8 (aromatic C), 128.8-127.0 (aromatic CH), 99.5, 97.3, and 94.4 (C-1 Gal, GlcN, Fuc), 95.1 (-NH-CO-OCH_2CCl_3) 55.0 (-OCH_3), 25.6 (-$SiC(CH_3)_3$), 17.9 (-$SiC(CH_3)_3$), 16.2 (C-6 Fuc), -4.2 and -5.3 (-$Si(CH_3)_2$); other signals at δ 80.9, 79.6, 78.8, 76.6, 76.0, 75.4, 75.0, 74.7, 73.8, 73.4, 73.2, 72.8, 72.4, 72.3, 68.2, 67.6, 66.4, 61.8.

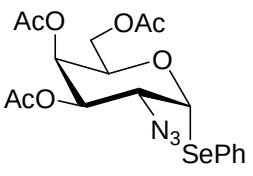


136. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.15 (aromatic protons), 5.27 (1H, dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.2 Hz, H-3 Fuc), 5.21 (1H, bd, H-4 Fuc), 5.15 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 Fuc), 5.11 (1H, d, $J_{1,2}$ = 7.8 Hz, H-1 GlcN), 5.00-4.96 (2H, m, H-2 Gal and H-5 Fuc), 4.59 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 Gal), 4.72-4.40 (12 H, Troc CH_2, 5 x benzyl CH_2), 4.20 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3 GlcN), 3.98-3.28 (10H, H-3 Gal, H-4 Gal, H-5 Gal, H_2-6 Gal, H-4 GlcN, H-5 GlcN, H_2-6 GlcN, and H-2 Fuc), 3.78 (3H, s, -OCH_3), 2.91 (1H, m, H-2 GlcN), 2.09 and 1.98 (6H, 2xs, 2x acetyl CH_3), 0.93 (3H, d, $J_{5,6}$ = 6.2 Hz, H_3-6 Fuc), 0.84 (9H, s, -$SiC(CH_3)_3$), 0.06 and 0.01 (6H, 2xs, -$Si(CH_3)_2$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.4 and 169.4 (2x -$COCH_3$), 155.1 and 154.0 (-NH-CO-CH_2CCl_3, -O-CO-OMe), 138.6, 138.3, 138.3, 138.1, and 138.1 (aromatic C), 129.0-127.2 (aromatic CH), 99.4, 97.5, 93.9 (C-1 Gal, GlcN, and Fuc), 55.0 (-OCH_3), 25.6 (-$SiC(CH_3)_3$), 20.9 and 20.7 (2x -$COCH_3$), 17.9 (-$SiC(CH_3)_3$), 15.2 (C-6 Fuc), -4.2 and -5.3 (-$Si(CH_3)_2$); other signals at δ 80.5, 74.8, 74.6, 74.4, 73.6, 73.2, 73.1, 72.3, 72.0, 7

Globo-H Assembly

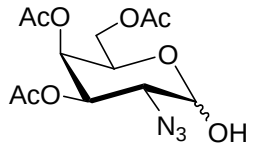
Preparation of 5Å acid washed molecular sieves

Commercial 5Å molecular sieves (UOP Type 5A, 1/8" rods, purchased from Fluka) were treated three times with a saturated aqueous solution of ammonium chloride for 20 minutes. The sieves were then repeatedly washed with distilled water until neutralization of the washing liquid. The sieves were left to dry at room temperature and then were heated at 200°C under vacuum for 5 hours. The latter procedure was repeated until no further generation of moisture was observed from the sieves upon this treatment.

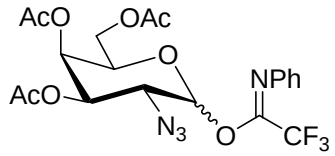


144. 1.617 g (5.94 mmol) of commercially available 3,4,6-tri-*O*-acetyl-D-galactal **143**, 1.147 mg (3.6 mmol, 97%) of PhSeSePh and 927.0 mg (14.3 mmol) of NaN_3 were fluxed under argon athmosphere and dissolved in 20.0 mL of dry CH_2Cl_2. 2.679 g (8.3 mmol) of $(AcO)_2PhI$ were added to the solution and the resulting mixture was stirred at room temperature for 48 h. The completion of the reaction was monitored *via* TLC analysis. The reaction mixture was then extracted in CH_2Cl_2/$NaHCO_3$ (aq.). The organic phases were collected and concentrated under reduced pressure. The

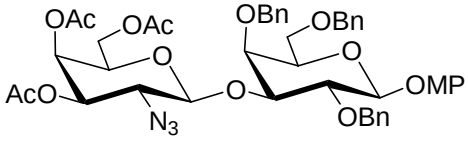
crude was purified by silica gel column chromatography (petroleum ether/ethyl acetate 7:3) and 2.040 g of **144**[44] were obtained in 73% yield.



145. 2.021 g of **144** (4.30 mmol) were dissolved in 12 mL of MeCN. 1.637 g (6.45 mmol) of I_2 and 60 μL of distilled H_2O were admixed and the solution was stirred at room temperature. After completion (5 h) the mixture was concentrated and the crude was purified by silica gel column chromatography (Petroleum ether/ethyl acetate 8:2) and **145** was obtained in 42% yield. (α:β ratio = 2:1) ^{1}H NMR (200 MHz, $CDCl_3$) α anomer: δ 5.46 (1H, bd, $J_{3,4}$ = 3.2 Hz, H-4), 5.45 (1H, d, $J_{1,2}$ = 3.4 Hz, H-1), 5.40 (1H, bd, $J_{2,3}$ = 9.8 Hz, H-2), 4.46 (1H, bt, $J_{5,6a} = J_{5,6b}$ = 6.8 Hz, H-5), 4.18-4.06 (2H, m, 6-CH_2), 3.75 (1H, dd, H-2), 3.10 (1H, bs, 1-OH), 2.15, 2.07, 2.05 (3x3H, 3xs, 3x $COCH_3$).

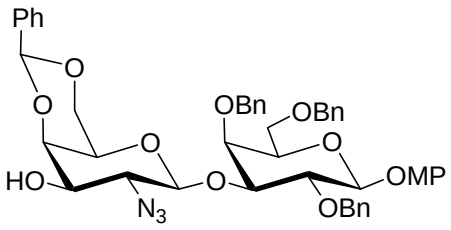


139. 417 mg (1.33 mmol) of **145** were fluxed under argon atmosphere and then dissolved into 3 mL of dry CH_2Cl_2. To the solution 250 μL (2.00 mmol) of *N*-phenyl trifluoroacetimidoyl chloride and 69 mg (1.73 mmol) of NaH were admixed at 0°C. After 1.5 h stirring, the reaction was warmed up to room temperature, concentrated under reducer pressure and purified by Et_3N-neutralized silica gel column chromatography (petroleum ether/ ethyl acetate 8:2). 496 mg of **139** were obtained in 74% yield. (β:α mixture = 2.5:1) ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40–6.80 (aromatic), 6.46 (1H, bs, H-1α), 5.60 (1H, bs, H-1β), 5.53 (1H, bd, H-4 α), 5.42-5.32 (overlapped signals, H-4 β and H-3 α), 4.88 (1H, dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.2 Hz, H-3 β), 4.63 (1H, bt, $J_{5,6a} = J_{5,6b}$ = 6.8 Hz, H-5α), 4.30-4.18 (6-CH_2 α and β), 4.05-3.60 (overlapped signals, H-5 β, H–2 α and β), 2.17, 2.07, 2.00 (3x3H, 3xs, 3x $COCH_3$ β), 2.16, 2.08, 2.06 (3x3H, 3xs, 3x $COCH_3$ α).

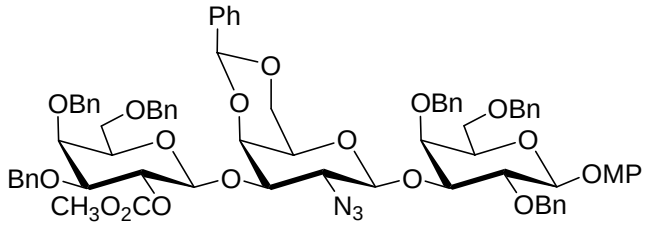


146. Donor **139** (246 mg, 0.49 mmol) and acceptor **138** (194 mg, 0.35 mmol) were coevaporated three times with anhydrous toluene and kept for an our hour under vacuum. After the addition of freshly activated 4Å AW 300 MS (ca 400 mg in pellets), the mixture was dissolved under argon in anhydrous acetonitrile (1.8 mL) at 0°C. After 15 minutes a solution of $Yb(OTf)_3$ (21.7 mg, 0.035 mmol) in acetonitrile (1.1 mL) was added. The mixture was allowed to warm to room temperature and left overnight under stirring to ensure complete glycosidation. The reaction was quenched with a few drops of pyridine and the mixture filtered on a short plug of silica gel eluted with 9:1 dichloromethane/methanol (with a few drops of pyridine). The residue was then chromatographed on a silica gel column eluted with petroleum ether/ethyl acetate (from 8:2 to 7:3) to yield pure disaccharide **146** (211 mg, 70%). $[\alpha]_D$ -32.6 (c 0.5 in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.40–6.80 (aromatic protons), 5.33 (1H, bd, J = 3.4 Hz), 5.11-4.36 (6H, 3x AB, 3x benzyl CH_2), 4.86 (2H, 2xd, J = 7.6 and 8.0 Hz), 4.76 (1H, dd, J = 7.6 and 11.0 Hz), 4.22-4.06 (3H), 3.98-3.90 (2H), 3.78 (3H, s, -OCH_3), 3.74-3.56 (5H), 2.16, 2.07, 2.00 (3x3H, 3xs, 3x -$COCH_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.2, 170.2, 169.4, 155.3, 151.5, 138.5, 138.5, 137.8, 128.5-127.8, 118.5, 114.5, 103.1, 102.7, 80.4, 79.2, 75.7, 75.3, 74.8, 73.7,

73.6, 70.9, 70.6, 68.8, 66.4, 61.4, 61.1, 55.6, 20.6. $C_{46}H_{51}N_3O_{14}$: calcd C 63.51, H 5.91; found C 63.23, H 5.68.

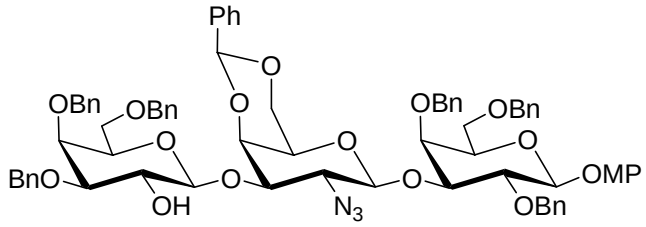


147. Disaccharide **146** (199 mg, 0.23 mmol) was dissolved in MeOH (9.8 mL). Ammonium hydroxide (32% aqueous solution, 1.1 mL) was then added and the mixture was left under stirring at room temperature. After 3 hours the mixture was concentrated under vacuum and the residue coevaporated with anhydrous toluene. The crude triol was then dissolved in anhydrous acetonitrile (4 mL), and dimethoxytoluene (60 μL, 0.40 mmol) and camphorsulfonic acid (6 mg, 0.026 mmol) were added. The mixture was kept for 3 hours at 70°C and then diluted with ethyl acetate. The organic phase was washed with saturated aq bicarbonate. The residue from the organic phase was chromatographed on a silica gel column (eluent dichloromethane and 98:2 dichloromethane/methanol) to give pure compund **147** (152 mg, 80%) as an oil.
$[\alpha]_D$ -24.9 (c 1 in CH_2Cl_2). ^{1}H NMR and COSY (500 MHz, $CDCl_3$): δ 7.50–6.80 (aromatic protons), 5.58 (1H, s, CH benzylidene), 4.87 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 B), 4.74 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 A), 5.11-4.37 (6H, 3 x AB, 3x benzyl CH_2), 4.32 (1H, d, $J_{6a,6b}$ = 12.0 Hz, H-6a A), 4.19-4.15 (2H, overlapped signals, H-2 B and H-4 B), 4.10 (1H, d, $J_{3,4}$ = 2.5 Hz, H-4 A), 4.06 (1H, d, H-6b A), 3.90 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.0 Hz, H-3 B), 3.77 (3H, s, -OCH_3), 3.70-3.52 (overlapped signals, H-2 A, H-3 A, H-5 B, and 6-CH_2 B), 3.38 (1H, s, H-5 A). ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.3 and 151.5 (oxygenated aromatic C), 138.4, 138.3, 138.3, and 137.3 (non oxygenated aromatic C), 129.3-126.3 (aromatic CH of benzyl and benzylidene), 118.4 and 114.4 (aromatic CH methoxyphenol), 103.0, 102.8, and 101.4 (C-1 and benzylidene non aromatic C), 55.6 (OCH_3). Other signals at 81.0, 79.1, 75.5, 75.4, 74.7, 74.5, 73.9, 73.5, 71.2, 69.0, 68.9, 66.3, 64.6. $C_{47}H_{49}N_3O_{11}$: calcd C 67.86, H 5.94; found C 67.68, H 5.99.

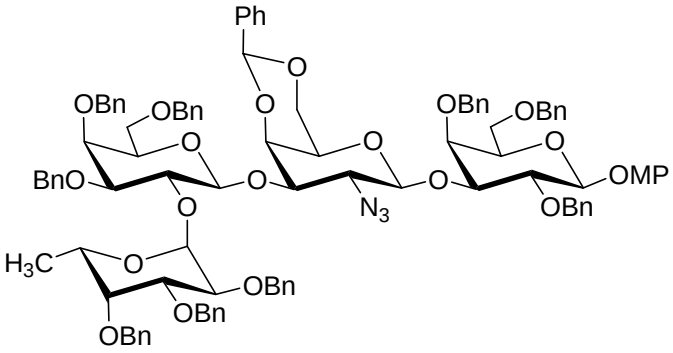


148. A solution of donor **129** (86 mg, 0.12 mmol) in 5:1 dichloroethane/cyclohexane (720 μL) was added in 6 hours at room temperature by a syringe pump to a solution of acceptor **147** (53 mg, 0.063 mmol) in 5:1 dichloroethane/cyclohexane (1.2 mL) containing freshly activated 5Å AW molecular sieves in pellets (1.1 g). After completion of the addition the mixture was left under overnight stirring to ensure complete consumption of the donor. The mixture was then filtered on a cotton plug washed repeatedly with 9:1 dichloromethane/methanol (with drops of pyridine). Silica gel chromatography of the residue from the organic phase (eluent: petroleum ether/ethyl acetate from 8:2 to 65:35) afforded pure trisaccharide **148** (63 mg, 75%) as an oil. $[\alpha]_D$ -12.3 (c 1.2 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–6.80 (aromatic protons), 5.51 (1H, s), 5.23 (1H, dd, J = 7.8 and 9.6 Hz), 5.10-4.30 (12H, 6x AB, 6x benzyl CH_2), 4.84 (1H, d, J = 7.5 Hz), 4.71 (1H, d, J = 7.8 Hz), 4.68 (1H, d), 4.26-4.20 (2H), 4.12-4.04 (2H), 3.94-3.78 (4H), 3.77 and 3.74 (2x 3H, 2x s, 2x -OCH_3), 3.70-3.40 (7H), 3.24 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.1, 155.0, 151.6, 138.6, 138.5, 138.3, 138.0, 137.8, 137.8, 137.4, 128.6-126.3, 118.4, 114.4,

103.1, 103.0, 102.4, 100.6, 81.0, 80.5, 79.1, 78.1, 75.8, 75.6, 75.2, 74.7, 74.5, 73.9, 73.4, 73.0, 72.6, 69.2, 69.0, 66.5, 62.9, 55.6, 55.0. MALDI-TOF MS for $C_{76}H_{79}N_3O_{18}$ (m/z): M_r (calcd) 1321.54, M_r (found) 1344.80 $(M+Na)^+$.$C_{76}H_{79}N_3O_{18}$: calcd C 69.02, H 6.02; found C 68.88, H 6.21.

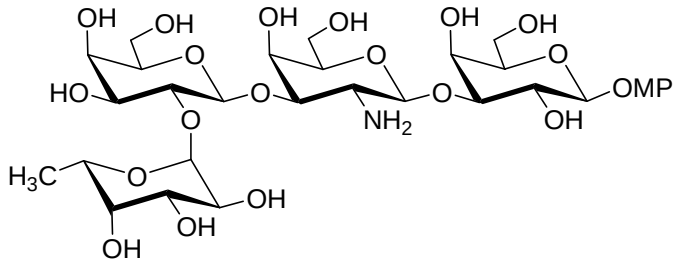


149. Trisaccharide **148** (119 mg, 0.090 mmol) was dissolved with a saturated solution of K_2CO_3 in MeOH (12 mL, prepared suspending 5 mg of K_2CO_3 per mL of MeOH). The solution was stirred at 40 °C for 8 hours and then diluted with dichloromethane. The organic phase was washed with water and the residue purified through a short silica gel chromatography (eluent dichlormetane: methanol 9.1) to afford acceptor **149** (107 mg, 89%).
$[\alpha]_D$ -14.1 (c 0.5 in CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 7.50–6.80 (aromatic protons), 5.49 (1H, s, benzylidene non aromatic CH), 5.10-4.54 (12H, 6 x AB, 6x benzyl CH_2), 4.93 (1H, d, $J_{1,2}$ = 7.6 Hz, H-1 C), 4.84 (2x 1H, 2x d, $J_{1,2}$ = 7.6 Hz, H-1 A and C), 4.30-4.20 (2H, H-6a and H-4 B), 4.15-4.05 (3H, H-2 A and C, H-4 C), 3.94-3.80 (4H, H-3 A and C, H-4 A, H-6b B), 3.76 (3H, s, -OCH_3), 3.70-3.45 (H-3 B, H-5 A and C, 6-CH_2 A and C), 3.24 (1H, s, H-5 A). ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.0 and 151.6 (oxygenated aromatic C), 138.6, 138.5, 138.5, 137.8, 137.7, 137.7, and 135.9 (non oxygenated aromatic C), 128.9-126.3 (aromatic CH of benzyl and benzylidene), 118.4 and 114.4 (aromatic CH of methoxyphenol), 105.1, 103.0, 103.0 and 101.0 (C-1 and non aromatic benzylidene CH), 55.5 (OCH_3). Other signals at 81.8, 81.1, 79.0, 77.9, 75.7, 75.2, 75.1, 74.7, 74.6, 74.0, 73.9, 73.4, 73.0, 71.4, 69.1, 68.0. $C_{74}H_{77}N_3O_{16}$: calcd C 70.29, H 6.14; found C 70.39, H 6.01.



137. Trisaccharide **149** (69 mg, 0.055 mmol) and the fucosyl donor **113** (99 mg, 0.16 mmol) were coevaporated three times in anhydrous toluene. After adding 4Å AW 300 MS, the mixture was dissolved under argon in 4:1 dichloromethane/diethyl ether (1.5 mL) and immediately cooled to -30 °C. After stirring for 15 minutes, a solution of ytterbium triflate (3.4 mg, 5.5 μmol) in dioxane (300 μL) was added dropwise. After three hours at -30 °C the mixture was allowed to warm to room temperature to ensure the consumption of residual amounts of the donor and the reaction was then quenched with pyridine. The mixture was filtered on a short plug of silica gel washed with 9:1 dichloromethane/methanol (with drops of pyridine). The residue was then purified on a silica gel column eluted with toluene/ethyl acetate (from 5:1 to 3:1) to yield tetrasaccharide **137** (61 mg, 66%) as the only detectable anomer. $[\alpha]_D$ -38.6 (c 0.5 in CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 7.50–6.80 (aromatic protons), 5.61 (1H, d, J = 3.2 Hz), 5.18-4.40 (18 H, 9x AB, 9x benzyl CH_2), 5.54 (1H, s), 4.89 (1H, d, J = 7.6 Hz,), 4.78 (1H, d, J = 8.0 Hz), 4.75 (1H, d, J = 7.6 Hz), 4.34 (1H, bq, J = 6.8 Hz), 4.28-4.15 (5H), 4.10-3.90

(4H), 3.79 (3H, s, -OCH_3), 3.80-3.50 (9H), 3.26 (1H, s), 0.69 (3H, d, J = 6.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.2, 151.6, 139.0, 139.0, 138.9, 138.6, 138.4, 138.3, 138.2, 138.0, 137.9, 137.9, 128.5-126.3, 118.5, 114.4, 103.6, 103.3, 102.9, 101.2, 97.8, 84.0, 81.2, 79.9, 79.1, 78.4, 76.2, 75.5, 75.4, 75.3, 74.9, 74.5, 74.0, 73.5, 73.0, 72.8, 72.6, 72.4, 71.4, 69.1, 68.9, 66.7, 66.4, 55.6, 16.1. MALDI-TOF MS for $C_{101}H_{105}N_3O_{20}$ (m/z): M_r (calcd) 1679.72, M_r (found) 1702.40 $(M+Na)^+$. $C_{101}H_{105}N_3O_{20}$: calcd C 72.17, H 6.30; found C 71.90, H 6.45.



150. $Pd(OH)_2/C$ (68 mg) was added to a solution of tetrasaccharide **137** (44 mg, 0.026 mmol) in 3:3:1 DCM/MeOH/H_2O (15 mL) and the mixture was hydrogenolized for 72 h at room temperature. The mixture was then filtered on a Celite pad that was washed with 18:13.5:3 MeOH/H_2O/DCM. The residue from the evaporation of the filtrate was treated with water and the soln was lyophilized to give **150** (18 mg, 90%). [α]D - 27.9 (c 0.5 in MeOH). ^1H NMR (500 MHz, D_2O): δ 6.98-6.83 (aromatic protons), 5.14 (1H, d, $J_{1,2}$ = 3.5 Hz), 4.90 (1H, d, $J_{1,2}$ = 8.0 Hz), 4.88 (1H, d, $J_{1,2}$ = 8.0 Hz), 4.69 (1H, d, $J_{1,2}$ = 7.5 Hz), 4.24 (1H, bd, $J_{3,4}$ = 3.0 Hz), 4.19 (1H, bq, $J_{5,6}$ = 6.5 Hz), 4.14 (1H, bd, $J_{3,4}$ = 3.0 Hz), 3.90-3.50 (overlapped signals), 3.70 (1H, s, -OCH_3), 3.43 (1H, t, $J_{1,2}$ = $J_{2,3}$ = 8.0 Hz), 1.16 (3H, d, $J_{5,6}$ = 6.5 Hz).

^{13}C NMR (125 MHz, D_2O): δ 154.9 e 151.1 (oxygenated aromatic C), 118.4 and 115.2 (aromatic CH of methoxyphenol), 103.2, 101.7, 100.6, 100.2 (4x C-1), 56.0 (OCH_3), 52.8 (C-2 C), 15.9 (6-CH_3 A). Other signals at 81.7, 80.0, 78.2, 75.2, 75.0, 72.0, 71.8, 70.0, 69.6, 69.2, 68.7, 68.6, 68.5, 67.7, 61.0, 60.9. MALDI-TOF MS for $C_{30}H_{47}NO_{21}$ (*m/z*): M_r (calcd) 757.26, M_r (found) 780.21 $(M+Na)^+$.

Chapter 4

1,2-Dimethoxyethane: Novel α-Stereodirecting Solvent for Glycosylation Reactions

Introduction

One of the most relevant problems associated with oligosaccharide synthesis is the stereochemical control of the glycosidation reaction, especially in the synthesis of 1,2-*cis* glycosides. As described in the previous chapter, a very efficient approach for the stereocontrolled synthesis of 1,2-*trans* glycosides can be achieved by using 2-*O*-acylated glycosyl donors, taking advantage of a neighbouring participation mechanism.[1] In contrast, complete stereocontrol in the synthesis of 1,2-*cis* glycosides is considerably a more difficult task. Several tactics have been proposed for solving this problem.[2] The use of ether-type solvents in glycosylations conducted with perbenzylated donors represent one of the most reliable method for obtaining the predominance of α-linked glycosides.
Very recently, our interest was attracted by the development of glycosylation promoters featuring convenient advantages such as chemical mildness and moisture stability in contrast to the acidic agents employed in the standard procedures. Among others, ytterbium(III) triflate proved[3] to be an interesting promoter for glycosylations performed with glycosyl trichloro-[4] and *N*-phenyl trifluoroacetimidates.[5] In the course of this research, use of solvent mixtures containing diethyl ether and dioxane was found to give good α-selectivity in the glucosylation of secondary acceptors with a perbenzylated glucosyl imidate (with α/β ratios ranging from 3 to 4).[3] Unfortunately, poor selectivity was achieved by coupling a more reactive primary acceptor with a perbenzylated glucosyl trifluoroacetimidate (α/β 1.7:1, *Table 4.1*, entry 1), while no selectivity was registered when using the corresponding glucosyl trichloroacetimidate as donor (*Table 4.1*, entry 3).

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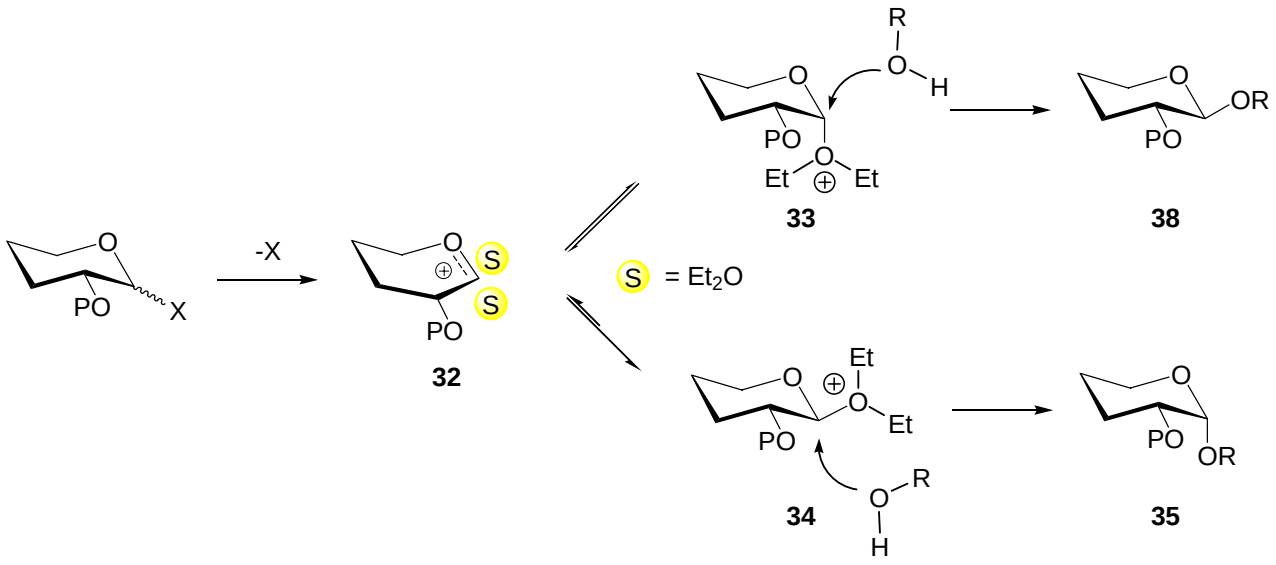
[1] For recent reviews on oligosaccharide synthesis: a) Boons. G. J. *Tetrahedron* **1996**, *52*, 1095; b) Seeberger, P. H.; Haase, W. *Chem. Rev.* **2000**, *100*, 4349.
[2] For a discussion on 1,2-*cis*-glycosides synthesis see pp. 23-31.
[3] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573.
[4] Schmidt; R. R.; Kinzy, W. *Adv. Carohydr. Chem. Biochem.* **1994**, *50*, 21.
[5] Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405-2407; *J. Org. Chem.* **2002**, *67*, 9099.

Results and Discussion

In an attempt to improve these disappointing results further investigations were dedicated to the stereo-directing capability of coordinating solvents. The α-stereo-directing behaviour of ether-type solvents is commonly explained assuming the conversion of the activated donor into glycosyl cation **32**, the initial attack of an ether solvent molecule occurring at the α-side, and the fast conversion of this kinetic α-adduct **33** into the thermodynamically more stable (due to the inverse anomeric effect) β-adduct **34**.[6] Finally, nucleophilic attack of the alcoholic acceptor on this latter intermediate can account for the α-selectivity, especially in the case of less reactive secondary acceptors (*Scheme 4.1*).[7]



SCHEME 4.1 α-Stereodirecting Effect of Ether-Type Solvents.

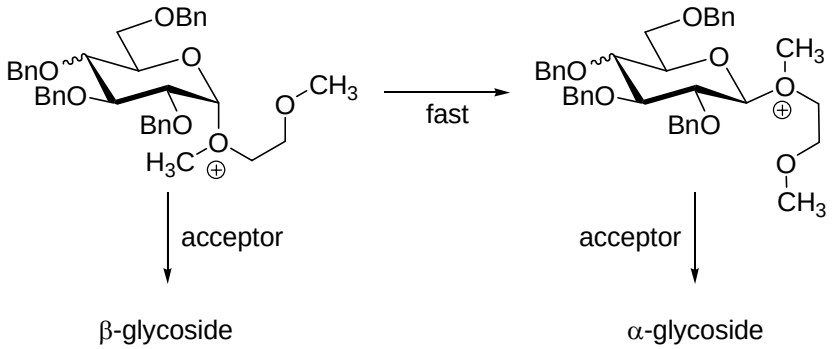
Reasoning on the conjectured mechanism, we hypothesized that an improved α-selectivity would be achieved by speeding the conversion of the kinetic α-linked solvent-glycosyl cation adduct (affording the undesired β-glycoside **38**) into the corresponding β-adduct (providing the desired α-glycoside **35**).

On this regard, use of a bidentate ether as the solvent could appear beneficial, as the desired anomerization would be kinetically favoured by entropic factors as depicted in *Scheme 4.2*. 1,2-Dimethoxyethane (DME) appeared as an especially interesting option, because it is sufficiently cheap and volatile (b. p. 85 °C) to be practically used as a solvent.

In a first experiment the coupling between donor **151** and primary acceptor **21** was attempted under the activation of ytterbium(III) triflate in DME (*Table 4.1*, entry 2). The choice of extremely reactive **21** as model acceptor in the preliminary reaction is due to the poor selectivities registered for this acceptor in glycosylations with standard solvents.[3]

[6] a) Wulff, G.; Rohle, G. *Angew. Chem., Int. Ed. Eng.* **1974**, *13*, 157; b) Schmidt; R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694; c) Demchenko, A.; Stauch, T.; Boons. G. J. *Synlett* **1997**, 818.

[7] For further details see pp. 26-27.

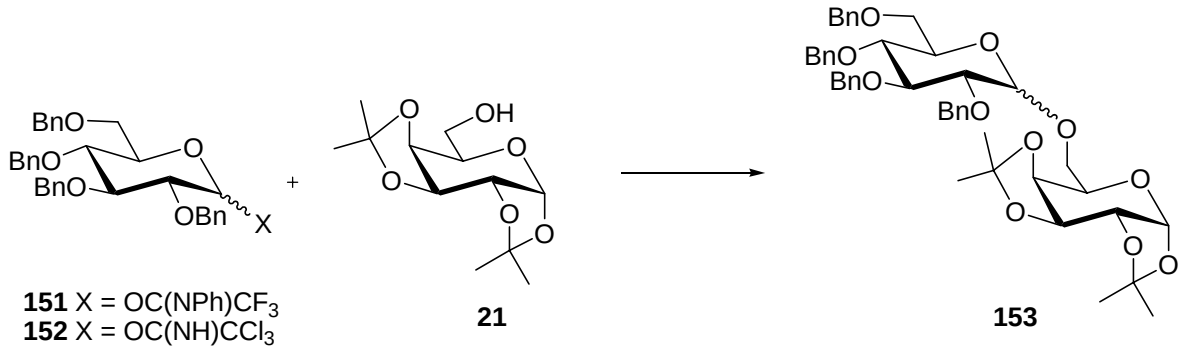


SCHEME 4.2 Hypothesized Intramolecular Mechanism of Anomerization of the Glycosyl Cation-Solvent Adduct with DME.

In DME the coupling proceeded in high yield with a sensible improvement of α-selectivity (3.5:1). Higher temperature conditions (starting from 0 °C to reach spontaneously room value) were needed to achieve reaction rates comparable with those registered when using dioxane/diethyl ether solvent mixture (reaction conducted at -10 °C) (*Table 4.1*, entry 1). At -10 °C the glycosylation in DME proved to be very sluggish. This slowing down effect could be ascribed to the ability of DME to depress the activity of the promoter by chelation at the metal site. Several commercially available lanthanide triflates were also tested, in order to verify their ability to promote α-glycosylations using DME as stereodirecting solvent, possibly improving the results registered with $Yb(OTf)_3$. Good to excellent coupling yields were registered (entries 5-10). The α/β ratios were comparable ranging from 2.0 (with $Sc(OTf)_3$) to 3.0 (with $Sm(OTf)_3$, $Y(OTf)_3$ and $Eu(OTf)_3$). In all cases reaction rates were very sluggish, up to 36 hours (entry 5) were required to reaction completion. In the light of these experiments, $Yb(OTf)_3$ proved to be the best compromise in terms of experimental results and also considering the non secondary economic aspect ($Yb(OTf)_3$ is the cheapest among the others).

In an attempt to improve both selectivity and rate of $Yb(OTf)_3$-promoted reactions, the use of co-solvents was tested (entries 11-15). For this issue toluene and dioxane were chosen, because of the good results previously obtained by their use in binary and ternary solvent mixtures in $Yb(OTf)_3$-promoted α-glycosylations.[3] Even improved stereoselectivities were registered by using dioxane/DME mixtures (entries 12-13). The best result in terms of stereocontrol was registered by adopting the ternary mixture dioxane /DME/toluene 4:1:1 which produced a very good stereoselectivity (α/β 4.3:1) (entry 15). Interestingly, use of diethy ether as a cosolvent, commonly used in TMSOTf activation of trichloroacetimidates,[8] here resulted in very sluggish reactions.

[8] Schmidt, R. R:; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 5573.



151 X = $OC(NPh)CF_3$
152 X = $OC(NH)CCl_3$

21

153

Entry	*Donor*	*Promoter (0.1 eq.)*	*Solvent*	*Temp (°C)*	*Time (h)*	*Yield[a] of **153**(%) (α/β)[b]*
1	**151**	$Yb(OTf)_3$	Et_2O/Dioxane 4:1	-10	4	86 (1.7)
2	**151**	$Yb(OTf)_3$	DME	RT	4	80 (3.5)
3	**152**	$Yb(OTf)_3$	Et_2O/Dioxane 4:1	-10	2	81 (1.0)
4	**152**	$Yb(OTf)_3$	DME	-15	2	90 (1.7)
5	**151**	$Sm(OTf)_3$	DME	0 to RT	36	70[d] (3.0)
6	**151**	$Y(OTf)_3$	DME	0 to RT	24	90[d] (3.0)
7	**151**	$Gd(OTf)_3$	DME	0 to RT	48	95[d] (2.7)
8	**151**	$Eu(OTf)_3$	DME	0 to RT	48	90[d] (3.0)
9	**151**	$La(OTf)_3$	DME	0 to RT	48	60[d] (2.7)
10	**151**	$Sc(OTf)_3$	DME	0 to RT	5	95[d] (2.0)
11	**151**	$Yb(OTf)_3$	Dioxane/DME 1:4	RT	6	76 (3.1)
12	**151**	$Yb(OTf)_3$	Dioxane/DME 1:1	0 to RT	6	79 (4.0)
13	**151**	$Yb(OTf)_3$	Dioxane/DME 4:1	0 to RT	4	84 (3.9)
14	**151**	$Yb(OTf)_3$	Toluene/DME 4:1	0 to RT	6	73 (3.0)
15	**151**	$Yb(OTf)_3$	Dioxane/DME/Toluene 4:1:1	0 to RT	8	79 (4.3)

[a] Isolated yield. [b] Measured by ^{1}HNMR. [c] With 0.03 eq of $Yb(OTf)_3$. [d] ^{1}HNMR yield.

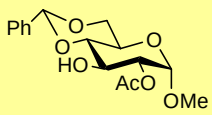
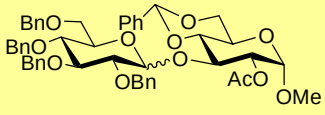
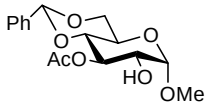
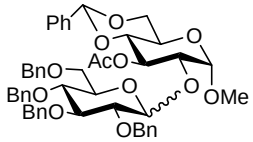
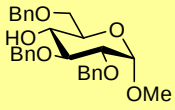
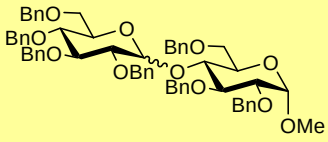
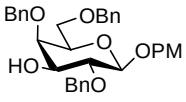
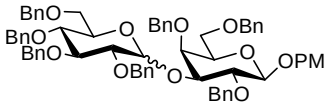
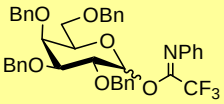
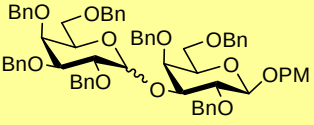
TABLE 4.1 α-Selective Glycosidation of Acceptor **21** with Glucosyl Imidate Donors **151** and **152** Promoted by Lanthanide Triflates.

An interesting experimental evidence is the strong dependence of the reaction rate on the solvent used for dissolving the promoter. In $Yb(OTf)_3$-promoted glycosylations, the promoter is generally added as solution to the mixture of glycosyl donor and acceptor. In the case of binary and ternary mixtures containing dioxane and DME, when the promoter is first dissolved in DME and then added to the reaction mixture (containing the other solvents) the reaction rate values are those reported in *Table 4.1*. If $Yb(OTf)_3$ is first dissolved in dioxane and then added to the reaction medium, glycosylations result to be very sluggish. This difference in reactivity is a sign of the formation of solvent-promoter complexes of different stability. An excessive stability of the complex $Yb(OTf)_3$-dioxane would make the promoter less prone to coordinate the glycosyl donor, explaining thus the decreased reactivity exhibited by the lanthanide salt when dissolved first in dioxane.[9]

The preliminary results obtained with acceptor **21**, led us to reconsider the α-selective glycosylation of a range of secondary glycosyl acceptors. In these cases, use of DME as the sole solvent often resulted in lengthy reactions. In contrast, use of binary and ternary solvent mixtures with dioxane and toluene provided appreciable glycosidation yields within a few hours. Good to excellent stereoselectivities were registered, in all cases the

[9] Further studies are required to validate this hypothesis, the results will be reported in due course.

beneficial effect of DME was confirmed by comparison with the previously[3] reported results (*Table 4.2*). Glycosylations with secondary glycosyl acceptors **154**, **156**, **158** and **160** are intrinsically more difficult than those with the primary **21**. The alcoholic functions of **154** and **156** are made less nucleophile by the presence of neighbour electron-withdrawing acyl groups. Whereas acceptor **158** exhibits a low reactivity because of the sterical hindrance of the C4 hydroxyl group.

Entry	*Donor*	*Acceptor*	*Product*	*Solvent*	*Yield[a] (α/β)[b]*
1	**151**	 **154**	 **155**	Dioxane/DME 4:1	75% (7.4)
2	**151**	**154**	**155**	Dioxane/DME 1:1	77% (7.2)
3	**151**	**154**	**155**	Dioxane/DME/Toluene 4:1:1	70% (8.2)
4	**151**	 **156**	 **157**	Dioxane/DME 4:1	82% (8.0)
5	**151**	**156**	**157**	Dioxane/DME 1:1	51% (7.5)
6	**151**	**156**	**157**	Dioxane/DME/Toluene 4:1:1	80% (7.6)
7	**152**	 **158**	 **158**	Dioxane/DME 4:1	63% (6.3)
8	**151**	**158**	**158**	Dioxane/DME 4:1	55% (6.0)
9	**151**	**158**	**158**	Dioxane/DME/Toluene 4:1:1	51% (5.8)
10	**151**	 **160**	 **161**	Dioxane/DME/Toluene 4:1:1	65% (only α)
11	 **162**	**160**	 **163**	Dioxane/DME/Toluene 4:1:1	81% (only α)

[a] Isolated yield. [b] Measured by ^{1}HNMR.

TABLE 4.2 α-Selective Glycosidation of Secondary Acceptors Promoted by $Yb(OTf)_3$ (0.1 eq).

Acceptors **154** and **156** were glycosylated in high yields and improved α-selectivities were registered, if compared with the results previously obtained with $Yb(OTf)_3$ in other ether-type solvent mixtures.[3] Because of the low reactivity of acceptor **158**, the coupling with glycosyl donor **151** yielded the desired disaccharide **159** in good stereoselectivity (α/β = 6.0) but poor yield (55%). In this case better results were achieved using the more reactive

trichloroacetimidate donor **152** achieving disaccharide **159** improving both yield (63%) and α/β ratio (6.3) (entry 7).
In some cases, exclusive formation of α-linked disaccharides was observed as in the preparation of the protected disaccharide fragment **163** representing the extensively studied epitope involved in the antibody-mediated hyperacute rejection in xenotransplantation.[10]

Having established the conditions for achieving good α-selectivities in glycosylations with a variety of glycosyl acceptors, α-glycosylation of a non-saccharidic nucleophile was studied. In many *O*-linked glycoproteins the conjugation between the glycan and the peptide moieties is characterized by α-linkages involving serine or threonine residues. A relevant paradigm is given by mucins, a family of cell-surface glycoproteins often associated, in aberrant glycoforms, with tumors of epithelial tissues. Isolation and characterization of the oligosaccharide structures over-expressed on the malignant cells led to interesting application in medicinal chemistry.
Clustered motifs of Tn and TF glycoepitopes (*Figure 2.1*), suitably conjugated with immunogenic carriers, proved to provoke robust production of antibodies, with promising cell-surface reactivity for those tumors expressing the respective antigen.
These preliminary studies constitute the base for the development of a glycopeptide-based vaccine against prostate cancer, now under clinical trials.[11]

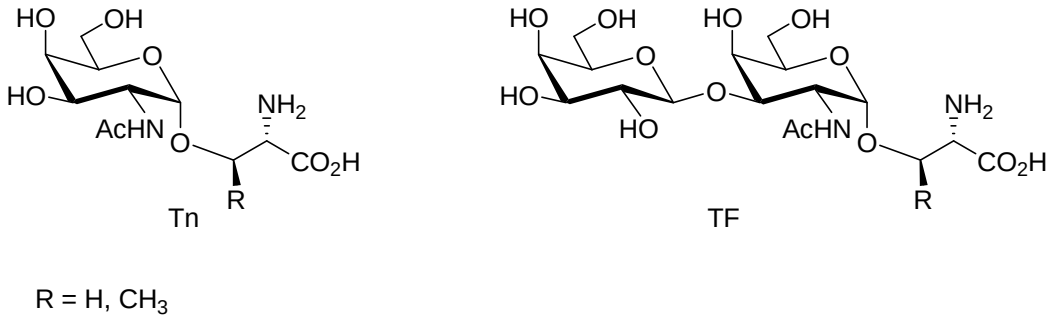
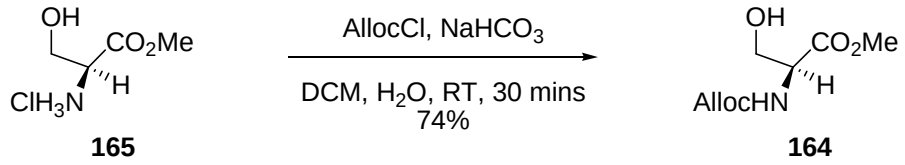


***FIGURE* 2.1** Tn and TF Antigens.

The crux of the difficulty is the problematic character of synthesizing carbohydrate domains *O*-linked to the key amino acids, serine and threonine, with strong stereochemical control in the formation of the α-glycosidic linkage.
Since improved α-selectivities were registered in glycosylations of primary hydroxyl functions using DME-based solvent mixtures, we decided to verify the versatility of our protocol in the difficult task of serine α-glycosylation.

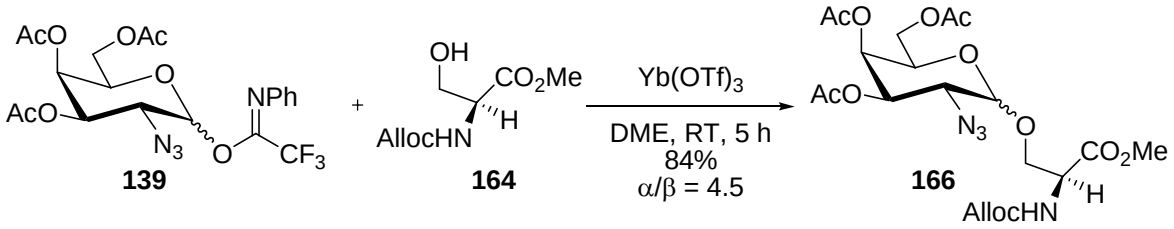
[10] a) Galili, U. *Sci. Med.* **1998**, *5*, 28; b) Cooper, D. K. C.; Good, A. H.; Koren, E.; Oriol, R.; Malcolm, A. J.; Ippolito, R. M.; Neethling, F. A.; Ye, Y.; Romano, E.; Zhudi, N. *Transplant Immunol.* **1993**, 198; c) Boons, G.-J.; Zhu, T. *J. Chem. Soc., Perkin Trans.1*, **1998**, 857; d) Janczuk, A. J.; Zhang, W.; Andreana, P. R.; Warrick, J.; Wang, P. G. *Carbohydr. Res.* **2002**, *337*, 1247; e) Zhang, Y.; Telyatnikov, V.; Sathe, M.; Zeng, X.; Wang, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 9292.
[11] a) Kuduk, S. D.; Schwarz, J. B.; Chen, X. –T.; Glunz, P. W.; Sames, D.; Ragupathi, G.; Livingston, P. O.; Danishewsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 12474; b) Schwarz, J. B.; Kuduk, S. D.; Chen, X. –T.; Sames, D.; Glunz, P. W.; Danishewsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 2662.

Our synthetic target was the protected precursor of Tn antigen, chacacterized by the challenging α-linkage between a galactosamine and the hydroxyl group of serine.
Glucosamine **139** was chosen as model donor, devoid of participating group at C-2 position. Amino acid nucleophile **164** was prepared treating the amino group of commercially available methyl ester **165** with allyloxycarbonyl chloride in order to protect the amino function otherwise competing with the alcoholic group of serine side-chain in the glycosidation reaction (*Scheme 4.4*).



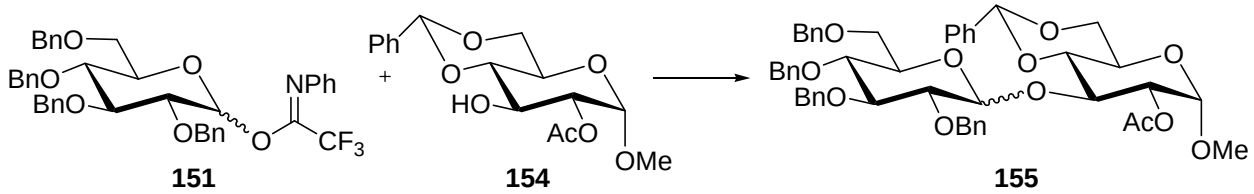
SCHEME 4.4 Alloc Protection of Serine **165**.

Galactosyl donor **139** and the thus obtained serine **164** were coupled in $Yb(OTf)_3$-promoted glycosylation in sole DME. The reaction proceeded at room temperature in 5 hours furnishing the desired galactoside **166** in 84% yield and 4.5 α/β ratio (*Scheme 4.5*).



SCHEME 4.5 Synthesis of Tn Antigen Precursor **166**.

Having established the ability of DME to improve the α-selectivity of $Yb(OTf)_3$ promoted glycosidations, some efforts were dedicated to establish the feasible use of DME as α-directing solvent in glycosidations performed with the more usual TMSOTf promoting system. As a matter of fact, the stereocontrolled synthesis of α-glucosides and α-galactosides with the recently introduced *N*-phenyl trifluoroacetimidate donors has not yet been explored as extensively as with standard trichloroacetimidate donors. Therefore, the model coupling between donor **151** (1.4 eq) and acceptor **154** was examined under the activation of TMSOTf (0.05 eq) at 0°C with four different solvents (*Table 4.3*).
Under Yu's conditions (dichloromethane as the solvent)[5] the reaction proceeded in high yield but poor selectivity (entry 1). In diethyl ether, commonly used to induce α-selectivity with glucosyl and galactosyl trichloroacetimidate donors,[4] the coupling proceeded with high stereocontrol but moderate yield (entry 2). In DME (entry 3) the disaccharide was obtained with both high yield and selectivity, although a slightly lower α/β ratio than in the case of the coupling in diethyl ether was obtained. A comparable yield and an improved α-selectivity was registered with the ternary mixture dioxane/toluene/DME 4:1:1 (entry 4) consistently with the trend observed also with $Yb(OTf)_3$.



Entry	*Solvent*	*Yield[a] of **155** (α/β)[b]*
1	DCM	76% (1.8)
2	Et_2O	53% (8.8)
3	DME	83% (6.5)
4	Dioxane/DME/Toluene 4:1:1	84% (7.2)

[a]Isolated yield. [b]Measured by ^{1}H NMR.

TABLE 4.3 TMSOTf (0.05 eq) Promoted Glycosylation of Acceptor **154** with Donor **151** (1.4 eq) at 0 °C .

Conclusion

In conclusion, DME has been shown to be a suitable cosolvent for achieving high α-selectivity in glycosidations catalytically promoted by Ytterbium(III) triflate. Under these conditions good results can be obtained even with reactive primary acceptors and amino acid residues. These results expand the potential of this moisture stable promoter in oligosaccharide synthesis. In addition, DME proved to be an interesting alternative to conventional ether solvents even in glycosidations performed with the standard TMSOTf activation of trifluoroacetimidates.

Experimental Section

General Methods and Material

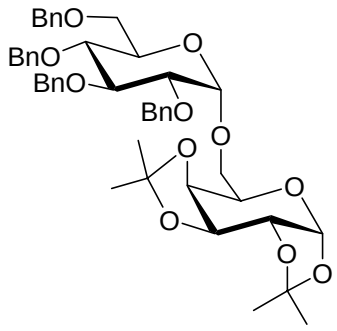
Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian spectrometers at 200 or 300 MHz and 50 or 75 MHz, respectively; or on a Bruker Avance 500 spectrometer at 500 MHz. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Analytical thin layer chromatography (TLC) was performed on E. Merck pre-coated (25 nm) silica gel 60F-254 plates. Column chromatography was preformed using Carlo Erba 0.06-0.20 silica gel. Anhydrous solvents stored over molecular sieves (purchased from Aldrich) were used for performing the reactions. All reactions were conducted under argon atmosphere.

General procedure of glycosidation with $Yb(OTf)_3$

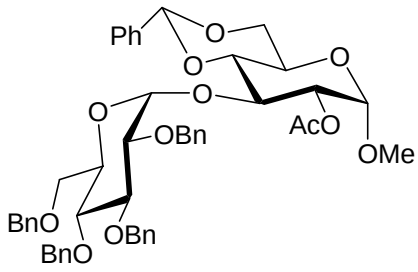
A mixture of acceptor (0.10 mmol) and donor (0.13-0.15 mmol) are coevaporated three times in anhydrous toluene. After adding freshly activated 4 Å acid washed molecular sieves (AW 300 MS) in pellets, the mixture is dissolved at 0 °C under argon in 1:4 toluene-dioxane (1.5 mL). After stirring for 20 minutes, a DME solution of $Yb(OTf)_3$ (0.05 M, 200 μL, 0.01 mmol) is added dropwise. The temperature is then allowed to raise to rt. After completion of the reaction (TLC analysis), a few drops of pyridine are added, the mixture is filtered through a short pad of silica gel and then evaporated. The residue is purified by silica gel chromatography (petroleum ether-ethyl acetate mixtures).

General procedure of glycosidation with TMSOTf

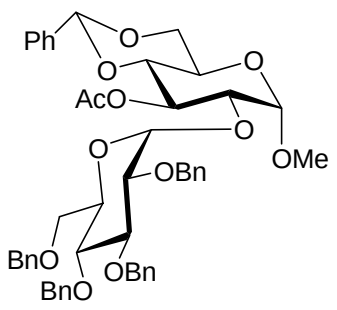
A mixture of acceptor **154** (11 mg, 35 μmol) and donor **151** (32 mg, 45 μmol) are coevaporated three times in anhydrous toluene. After adding freshly activated 4 Å molecular sieves in pellets, the mixture is dissolved in dichloromethane, or diethyl ether, or dimethoxyethane, or 4:1 dioxane/toluene (0.8 mL). After stirring for 20 minutes, a solution of TMSOTf in the reaction solvent (for entries 1-3 of *Table 4.3*) or dimethoxyethane (for entry 4) (30 μL, 1.7 μmol) is added dropwise to the mixture at 0°C. After completion of the reaction (1-3 hours, TLC analysis), a few drops of pyridine are added and the mixture is concentrated. The disaccharide **155** is purified by PLC (petroleum ether-ethyl acetate, 7:3).



153. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.50-7.10 (aromatic protons), 5.52 (1H, d, $J_{1,2}$ = 5.2 Hz, H-1 B), 5.05-4.10 (8H, 4xAB, CH_2 benzyl), 5.00 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1 A), 4.58 (1H, m, H-3 B), 4.34 (1H, dd, $J_{4,3}$ = 8.4 Hz, $J_{4,5}$ = 2.2 Hz, H-4 B), 4.30 (1H, dd, $J_{2,3}$ = 2.8 Hz, H-2 B), 4.10 (1H, t, H-5 B), 3.98 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.0 Hz, H-3 A), 3.88-3.64 (6H), 3.58 (1H, dd, H-2 A), 1.53, 1.45, 1.32 and 1.31 (12H, 4xs, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 138.9, 138.3, 138.3 and 138.0 (aromatic C), 128.6 - 127.5 (aromatic CH), 109.2 and 108.5 (-$\underline{C}(CH_3)_2$), 97.0 and 96.3 (C-1 A and B), 26.1, 26.0, 24.9, and 24.6 (-C($\underline{C}H_3)_2$). Other signals at δ 81.9, 79.8, 75.6, 74.9, 73.4, 72.3, 70.8, 70.6, 70.6, 70.2, 68.3, 66.2 and 65.7.

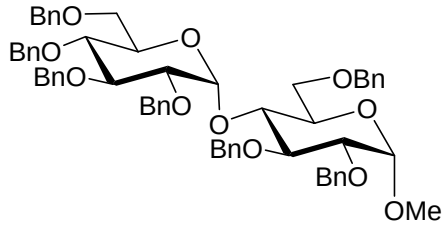


155. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45-6.95 (aromatic protons), 5.58 (1H, d, $J_{1,2}$ = 3.9 Hz, H-1 A), 5.46 (1H, s, non aromatic CH benzylidene), 4.99 (1H, d, $J_{1,2}$ = 3.9 Hz, H-1 B), 4.92 (1H, dd, $J_{2,3}$ = 9.9 Hz, H-2 B), 5.00-4.33 (8 H, 4xAB, 4x-CH_2Ph), 4.27 (1H, dd, $J_{6eq,\ 6ax}$ = 9.9 Hz, $J_{6eq,\ 5}$ = 3.9 Hz, H-6eq B), 4.03 (1H, m, H-5 A), 3.95-3.70 (7H), 3.62 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4 A), 3.46 (1H, dd, $J_{2,3}$ = 9.9 Hz, H-2 A), 3.40 (3H, s, -OCH_3), 2.03 (3H, s, -$COCH_3$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 170.2 (acetyl CO), 138.8, 138.6, 137.9, 137.8, 136.9 (aromatic C), 128.4-126.3 (aromatic CH), 102.1 (benzylidene benzyl CH), 97.4 and 96.0 (C-1 A and B), 55.3 (OCH_3), 20.9 (acetyl CH_3). Other signals at δ 82.6, 81.4, 78.6, 77.2, 75.6, 74.7, 73.5, 72.2, 71.2, 70.5, 70.3, 69.0, 68.3, 61.9.

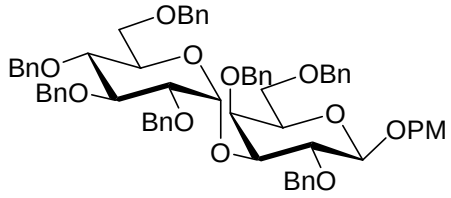


157. H NMR ($CDCl_3$, 300 MHz): δ 7.50-7.05 (aromatic protons), 5.61 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3 B), 5.46 (1H, s, non aromatic CH benzylidene), 4.97-4.43 (8 H, 4xAB, 4x-CH_2Ph), 4.90 (1H, d, $J_{1,2}$ = 3.9 Hz, H-1 B), 4.80 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 A), 4.29 (1H, dd, $J_{6eq,\ 6ax}$ = 10.2 Hz, $J_{6eq,\ 5}$ = 4.8 Hz, H-6eq B), 3.98-3.82 (3H, overlapped signals, H-3 A, H-5 A, and H-5 B), 3.79 (1H, dd, H-2 B), 3.76-3.57 (4H), 3.53 (1H, t, $J_{4,5}$ = 9.6 Hz, H-4 B), 3.52 (1H, dd, $J_{2,3}$ = 9.9 Hz, H-2 A), 3.44 (3H, s, -OCH_3), 2.00 (3H, s, -$COCH_3$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 169.6 (acetyl CO), 138.7, 138.5, 138.4, 137.9,

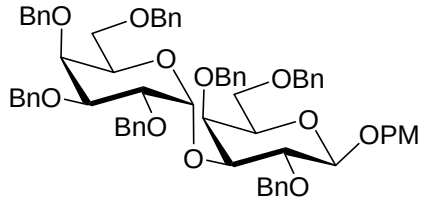
137.0 (aromatic C), 129.0-126.2 (aromatic CH), 101.5 benzylidene benzyl CH), 97.7 and 96.0 (C-1 A and B), 55.3 (OCH_3), 21.0 (acetyl CH_3). Other signals at δ 81.6, 79.5, 79.3, 77.2, 75.6, 74.7, 73.4, 73.1, 70.7, 69.9, 69.0, 68.2, 62.3.



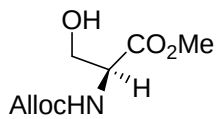
158. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.05 (aromatic protons), 5.70 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 A), 5.06-4.28 (14H, 7xAB, 7x -CH_2Ph AB), 4.60 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 B), 4.10 (1H, dd, t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3 B), 4.05 (1H, t, $J_{4,5}$ = 9.0 Hz, H-4 B), 3.95-3.83 (3H, overlapped signals, H-3 A, H-4 A, and H-5 B), 3.75-3.62 (3H, overlapped signals, H-5 A, H_2-6 B), 3.60 (1H, dd, H-2 B), 3.54-3.49 (2H, overlapped signals, H-2 A, H-6_a A), 3.40 (1H, bd, $J_{6a,6b}$ = 10.5 Hz, H-6_b A), 3.39 (3H, s, -OCH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 138.9, 138.7, 138.5, 138.1, 137.9, 138.0 and 138.0 (aromatic C), 128.2-126.7 (aromatic CH), 97.7 and 96.6 (C-1 A and C-1 B), 55.1 (1-OCH_3). Other signals at δ 82.0, 80.2, 79.4, 75.5, 74.9, 74.4, 73.3, 73.1, 72.3, 71.0, 69.5, 69.0, 68.1.



161. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40-6.75 (aromatic protons), 5.14 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 A), 5.15-4.29 (14H, 7xAB, 7x-CH_2Ph), 4.79 (1H, d, $J_{1,2}$ = 7.4 Hz, H-1 B), 4.17 (1H, m, H-5 A), 4.09 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3 A), 4.05 (1H, dd, $J_{2,3}$ = 10.0 Hz, H-2 B), 3.93 (1H, bd, $J_{3,4}$ = 2.2 Hz, H-4 B), 3.81 (1H, dd, H-3 B), 3.76 (3H, s, -OCH_3), 3.70-3.20 (7H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.0 and 151.6 (oxygenated aromatic C), 138.8, 138.7, 138.6, 138.0, 138.0, 138.0, and 138.0 (other aromatic C), 128.8-127.2, 118.2, and 114.4 (aromatic CH), 103.2 (C-1 B), 95.8 (C-1 A), 55.6 (OCH_3). Other signals at δ 82.2, 79.8, 78.7, 78.0, 75.5, 75.3, 74.9, 74.7, 74.2, 73.6, 73.5, 73.2, 72.9, 70.2, 68.8, 68.0.

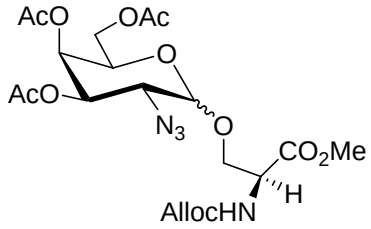


163. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40-6.75 (aromatic protons), 5.22 (1H, d, $J_{1,2}$ = 3.3 Hz, H-1 A), 5.08-4.35 (12H, 6xAB, 6x-CH_2Ph), 4.74 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1 B), 4.32 (1H, bt, $J_{5,6a}$ = $J_{5,6b}$ = 6.0 Hz, H-5 B), 4.25 (2H, s, -CH_2Ph), 4.14 (1H, dd, $J_{2,3}$ = 9.6 Hz, H-2 A), 4.06 (1H, dd, $J_{2,3}$ = 9.9 Hz, H-2 B), 3.97 (1H, dd, $J_{3,4}$ = 1.8 Hz, H-3 A), 3.92 (1H, bd, $J_{3,4}$ = 1.6 Hz, H-4 B), 3.84 (1H, dd, H-3 B), 3.77 (3H, s, -OCH_3), 3.70 (1H, bs, H-5 B), 3.60-3.25 (5H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.0 and 151.7 (oxygenated aromatic C), 138.7, 138.6, 138.6, 138.6, 138.3, 138.3, and 138.0 (other C), 129.0-126.5, 118.3, and 114.4 (aromatic CH), 103.2 (C-1 B), 95.7 (C-1 A), 55.6 (OCH_3). Other signals at δ 79.1, 78.1, 77.5, 75.2, 75.0, 74.7, 74.3, 73.7, 73.4, 72.7, 72.5, 69.1, 68.9.



164. To a solution of serine methyl ester **165** (373 mg, 2.4 mmol) in CH_2Cl_2 (3 mL) 1 mL of aqueous solution of $NaHCO_3$ (210 mg, 2.5 mmol) and 300 μL of allylchloroformiate were added. After 30 minutes stirring at room temperature, the organic phase was diluted with CH_2Cl_2, washed with water and concentrated to furnish pure **164** (370 mg) in 74% yield.

^{1}HNMR ($CDCl_3$, 200 MHz) δ 5.95 (1H, m, C<u>H</u>=CH_2), 5.73 (1H, bs, N-<u>H</u>), 5.53 e 5.50 (2H, dd, CH=C<u>H_2</u>, J_{cis} = 10.0 Hz ,J_{trans} = 14.2 Hz), 4.64 (2H, d, -C<u>H_2</u>CH=CH_2), 4.44 (1H, bt, $HOCH_2$-C-<u>H</u>), 4.02 (2H, m, C<u>H_2</u>OH), 3.85 (3H, s, $COOCH_3$), 2.32 (1H, t, CH_2O<u>H</u>).



166. ^{1}HNMR ($CDCl_3$, 200 MHz) δ 5.86 (1H, m, C<u>H</u>=CH_2), 5.72 (1H, bs N-H), 5.53 (2H, dd, J_{cis} = 10 Hz, J_{trans} = 14 Hz, CH=C<u>H_2</u>,), 5.35-5.19 (2H, m, H-3, H-4, A), 4.95 (1H,dd, H-1, $J_{1,2}$ =3.8 Hz) 4.61 (1H, bt, B), 4.09-3.87 (5 H, H-5, 2xH-6, CH_2, allyl), 3.60 (1H, dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 11 Hz, H-2), 2.11, 1.99, 1.97 (9H, 3xs, 3xCH_3 acetyl). ^{13}C NMR ($CDCl_3$, 50 MHz) significative signals at δ 170.2, 170.0, 170.0, 169.9 (C=O acetyl and methyl ester), 153.6 (C=O allyloxycarbonyl), 132.3 (-CH_2<u>C</u>H=CH_2), 117.9 (CH_2CH=<u>C</u>H_2), 99.0 (anomeric carbon), 20.5 (3xCH_3). Other signals at 69.5, 67.7, 67.3, 66.0, 63.2, 61.5, 57.2, 54.2, 52.8.

Chapter 5

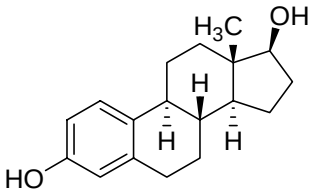
Regioselective Glycoconjugation of 17β-Estradiol and Derivatives Thereof

Introduction

Mono- and oligo-saccharide structures are often found as decoration of many natural compounds such as proteins, lipids or other cellular secondary metabolites. As described in previous chapters, recent studies have been proving the crucial role played by the carbohydrate portions in the several processes involving these bioactive glycoconjugates. Specific action of saccharidic moieties is often exerted in trafficking and signalling processes, where structural diversity of oligosaccharide chains is the key element of recognition events. However, carbohydrates accomplish also less specific but, at the same time, extremely important tasks. Saccharidic portions, when don't take directly part in the process, often improve the bioactivity of the molecules they decorate affecting, for example, the stability. Glycosides can be, in fact, considered nature's protecting groups, they often prevent hydrolysis of the biomolecules which are conjugated to by shielding labile sites with their encumbrance. An example of glycans-dependent proteolysis modulation is represented by tissue plasmogenin activation.[1] Furthermore, conjugation with deoxygenated and/or functionalized sugars represents a powerful tool used by nature to influence biomolecules absorption, distribution and excretion by a fine tuning of their hydrophobic/hydrophilic features. Moreover, saccharide-decorations proved to have a strong effect in protein folding processes, driving the formation of peculiar threedimensional structures macromolecule activity is strictly related to. All these findings stressed the interest of the scientific community towards the application of oligosaccharides and oligosaccharides-decorated molecules in medicinal chemistry. Conjugation of active principles with saccharide moieties proved to influence their pharmacodynamic and pharmacokinetic features and has become an usual strategy for improving drug properties. Obviously, this adds a further challenge to the often not easy task of synthesizing the sole active principle. For the efficient total or partial synthesis of mono- and oligo-saccharide-containing complex products there are several issues to overcome. The first one is the moment of glycosylation within the planned sequence so as

[1] Wittwer, A.; Howard, S. C. *Biochemistry* **1990**, *29*, 4175.

to optimize efficiency. The second issue relates to the choice of the glycosylation method to be used with regard to the yield and stereoselectivity and, first of all, the withstanding of the non-saccharidic portion. The third issue is associated with the choice of appropriate protecting groups, whose cleavage must not compromise the stability of the rest of the molecule.
On the basis of our experience in carbohydrates elaboration, with a particular attention in mild glycosylation procedures, we set up a collaboration with dr. Pezzella, Univeristy of Napoli, for the feasible glycoconjugation of 17β-estradiol (*Figure* 5.*1*) and derivatives thereof.



167

FIGURE 5.1 17β-Estradiol.

17β-Estradiol functionalization has attracted considerable attention over the past years because of the wide pharmacological use of this hormone in substitutive therapies.[2] As previously anticipated, in medicinal chemistry drugs implementation is often achieved introducing chemical modification on lead compounds with the overall effect of altering both pharmacodynamic and pharmacokinetic features. This strategy was also applied to 17β-estradiol as well as to some related derivatives i.e. diethylstilbestrol, 17α-ethynylestradiol, 2-methoxyestradiol, fulvestrant. As witnessed by the case of 2-methoxyestradiol,[3] modifications at the aromatic moiety of the hormone affect the biological activity by modulating its affinity of for estrogen receptors,[4] whereas the pharmacokinetic properties of these hydrophobic compounds are tightly connected with water solubility. A paradigm of what has been just described is offered by the case of fulvestrant (*Figure 5.2*), a 7α substituted estradiol derivative approved for the treatment of advanced postmenopausal breast cancer,[5] whose glycosylation either at the phenol or the carbinol site proved to improve its water solubility and bioavailability.[6] These transformations were accomplished adopting fulvestrant derivatives protected at either of the OH groups to guarantee complete regioselectivity in the glycoconjugation.

[2] Nilsson, S.; Koehler K.F. *Basic Clin. Pharmacol. Toxicol.* **2005**, *1*, 15.
[3] Leese, M. P.; Newman, S.P.; Purohit, A.; Reed, M. J.; Potter, B. V. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 3135.
[4] a) Pribluda, V. S.; Gubish, E. R. Jr.; La Vallee, T. M.; Treston, A.; Swartz, G. M.; Green, S. J. *Cancer Metastasis Rev.* **2000**, *19*, 173; b) Edsall, A. B.; Mohanakrishnan, A. K.; Yang, D.; Fanwick, P. E.; Hamel, E.; Hanson, A. D.; Agoston, G. E.; Cushman, M. *J. Med. Chem.* **2004**, *47*, 5126 and references therein.
[5] Osborne, C. K.; Pippen, J.; Jones, S. E.; Parker, L.M.; Ellis, M.; Come, S.; Gertler, S. Z.; May, J.; Burton, G.; Dimery, I.; Webster, A.; Morris, C.; Elledge, R.; Buzdar, A. *J. Clin. Oncol.* **2002**, *20*, 3386.
[6] a) Thompson, M. J.; Hutchinson, E. J.; Stratford, T. H.; Bowler, W. B.; Blackburn, G. M. *Tetrahedron Lett.* **2004**, *45*, 1207; b) Ferguson, J. R.; Harding, J. R.; Lumbard, K. W.; Schneimann, F.; Stachulski, A. V. *Tetrahedron Lett.* **2000**, *41*, 389.

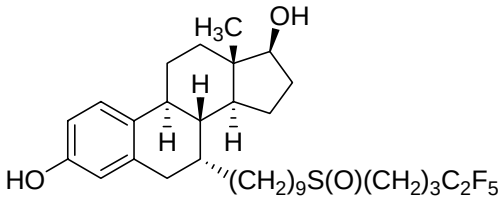
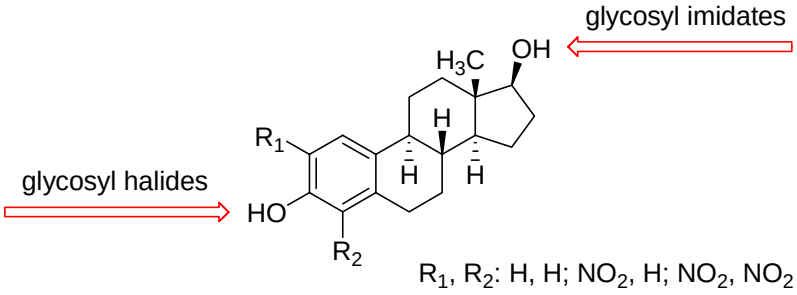


FIGURE 5.2 Fulvestrant.

The glucuronidation of estradiol was also reported starting from a precursor protected at the carbinol site, while regioselectivity was achieved only by resorting to enzymatic approaches.[7] The carbinol glucuronidation of estradiol has also been very recently reported still adopting an acceptor protected at the other potentially nucleophilic site.[8] Programs aimed at the preparation and biological evaluation of glycosylated derivatives of steroids are in progress.[9] On this regard, the search for the regioselective glycosidation of 17β-estradiol was pursued to accelerate the whole synthetic procedure by avoiding preventive protection steps. In our approach we wanted to develop feasible protocols for regioselective glycosidation of estradiol and derivatives thereof either at the phenol or the carbinol site (*Scheme 5.1*).



SCHEME 5.1 17β-Estradiol Regioselective Glycosylation Strategy.

Results and Discussion

It's well known that phenols can be functionalized by reactions conducted under double phase conditions in the presence of a base and a suitable phase-transfer catalyst, generally a tetraalkylammonium salt. This strategy enabled the glycosidation of simple phenols with glycosyl halides (especially bromides or chlorides) under non anhydrous conditions,[10] in contrast to other conventional glycosidation procedures. The regioselective application of this approach to unprotected estradiol derivatives appeared viable by virtue of the relevantly lower acidity of the 17-OH functionality. In addition, we decided to test the

[7] For chemical approaches: a) Werschkun, B.; Gorziza, K.; Thiem, J. *J. Carbohydr. Chem.* **1999**, *18*, 629; b) Conrow, R. B.; Bernstein, S. *J. Org. Chem.* **1971**, *36*, 863; c) Elce, J. J.; Carpenter, J. G. D.; Kellie, A. E. *J. Chem. Soc. (C)* **1967**, 542. For one example of enzymatic glucuronidation at the 3-OH: Werschkun, B.; Wendt, A.; Thiem, J. *J. Chem. Soc., Perkin Trans. I* **1998**, 3021.

[8] Harding, J. R.; King, C. D.; Perrie, J. A.; Sinnott, D.; Stachulski, A. V. *Org. Biomol. Chem.* **2005**, *3*, 1501.

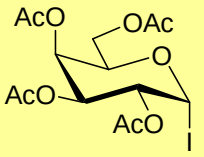
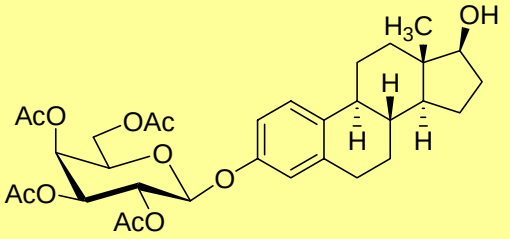
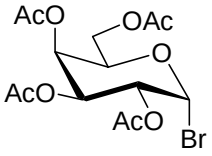
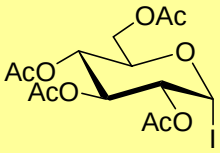
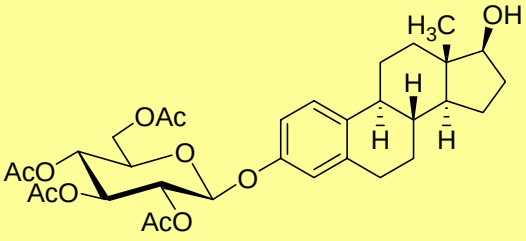
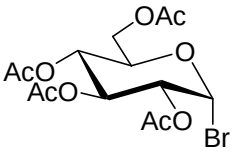
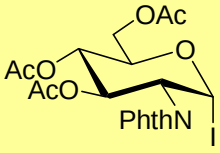
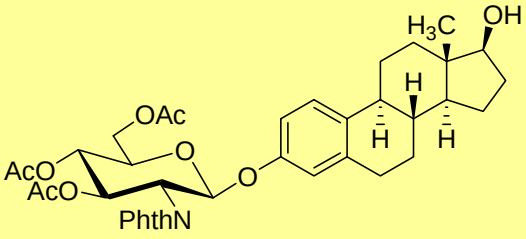
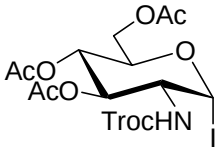
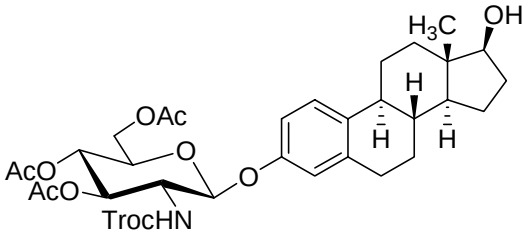
[9] For a review on the glycosidation of steroids: Pellissier, H. *Tetrahedron* **2004**, *60*, 5123.

[10] Jensen, K. J. *J. Chem. Soc., Perkin Trans. I* **2002**, 2219 and references therein.

reactivity of glycosyl iodides for these transformations, as we recently[11] disclosed a very efficient approach for their preparation. Such a procedure entails the short exposure (less than one hour) of a peracetylated sugar to iodine and triethylsilane in refluxing dichloromethane.[12] The produced anomeric iodides are rapidly isolated by a simple extractive work-up. They are contaminated by a side product from triethylsilane (presumably the corresponding bis(silyl)ether) that is however expected to be unreactive under the conditions of the ensuing transformation. On the other hand, we had previously found that these crude iodides can be transformed into the corresponding glycals, ethylidenes and orthoesters in high yields without any apparent interference of the triethylsilyl impurity.[11]

The first experiments for the present work were conducted by coupling 17β-estradiol with tetra-*O*-acetylated galactosyl iodide **88** (*Table 5.1*). The reaction was tested under a wide range of conditions by varying the amounts and the nature of the base (NaOH, KOH, sodium or potassium carbonate) and the phase-transfer catalyst (TBAB, TBAC, TBAI, cetyltrimethylammonium bromide). The best result in terms of yield and reaction rate (75% yield of glycoside **168**) was obtained by using NaOH as the base and tetrabutylammonium bromide as the phase-transfer catalyst (*Table 5.1*, entry 1). In all cases 2-*O*-acetyl 1,2-galactal represented the main by-product. For comparison purposes, the coupling was also tried under analogous conditions with the corresponding galactosyl bromide **169** to obtain **168** in a slightly improved yield (entry 2). However, it should be noted that the use of the crude galactosyl iodide could be advantageous in terms of rapidity of preparation. Coupling of 17β-estradiol with tetra-*O*-acetylated glucosyl iodide **86** or bromide **171** gave poor to modest yields, the elimination process being favoured (entries 3 and 4). The higher trend of glucosyl halides to afford elimination products than the galactosyl counterparts has also been observed in other investigations.[13] However, slightly improved yields were obtained when the more expensive cesium hydroxide was used as the base (entry 5 and 6).
The protocol was also extended to the use of glucosamine iodide donors. A recent work of Lay and coworkers[14] evidenced the difficulty in performing glucosamine iodination with the effective Gervay's protocol by the use of TMSI with anomeric acetates.[15] In these conditions, the preparation of anomeric iodides proved to require *N*-phtalimido or *N*-dimethylmaleimido protection of the glucosamine precursors. In the course of the present investigation the application of the iodine/triethylsilane system in refluxing DCM proved equally efficient for the synthesis of the *N*-phtalimido and *N*-Troc protected donors **172** and **97** from the corresponding 1-*O*-acetylated β-anomers. However, glycosylation of 17β-estradiol with **172** did not provide any detectable amount of the corresponding glycoside **173** (entry 7), while a very satisfying yield was registered when using glucosamine donor **97** (entry 8).

[11] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 7863.
[12] For further approaches towards the synthesis of glycosyl iodides see *Chapter 2*.
[13] Dess, D.; Kleine, H. P.; Weinberg, D. V.; Kaufman, R. J.; Sidhu, R. *Synthesis* **1981**, 883.
[14] Miquel, N.; Vignando, S.; Russo, G.; Lay, L. *Synlett* **2004**, 341.
[15] Gervay, J.; Nguyen, T. N.; Hadd, M. J. Carbohydr. Res. **1997**, 300, 119.

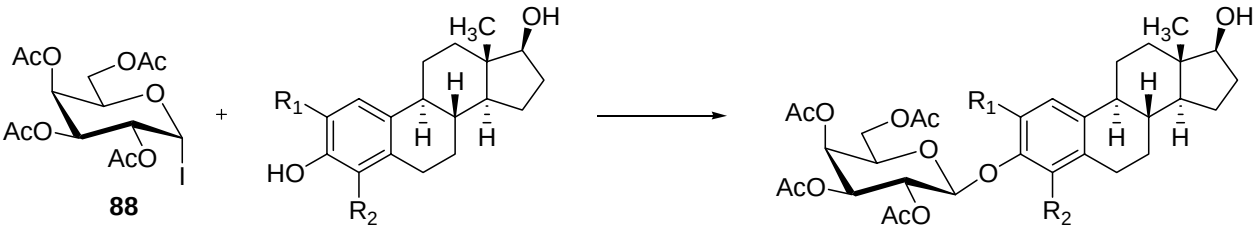
Entry[a]	*Donor*	*Base*	*Product*	*Yield (%)*
1	 **88**	NaOH	 **168**	75
2	 **169**	NaOH	**168**	84
3	 **86**	NaOH	 **170**	11
4	 **171**	NaOH	**170**	38
5	**86**	CsOH	**170**	31
6	**171**	CsOH	**170**	42
7	 **172**	NaOH	 **173**	N.d.
8	 **97**	NaOH	 **174**	81

[a]Conditions: donor (1.5 eq.), 17β-estradiol **167** (1 eq.), TBAB (1 eq.), base (5 eq.), H_2O, CH_2Cl_2, RT, 2 h.

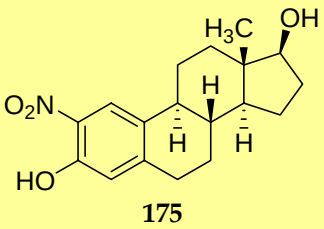
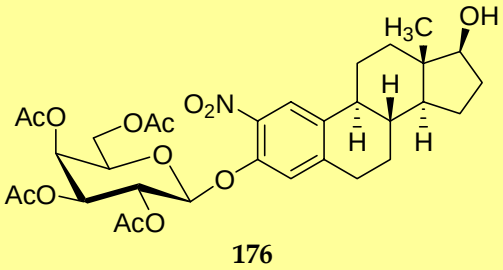
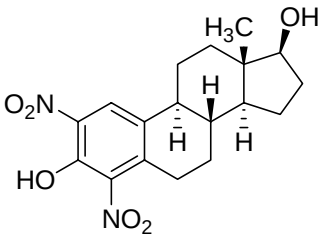
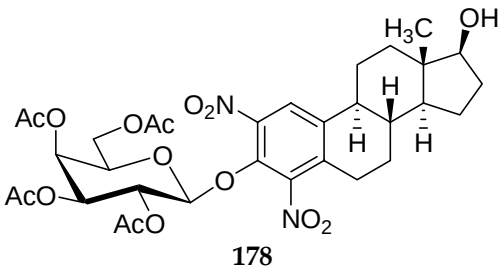
TABLE 5.1 Regioselective Phenolic Glycosylation of 17β-Estradiol **167**.

Due to the biological interest of some estradiol derivatives containing further substituents at the aromatic A ring (for example, 2-methoxyestradiol),[2] we have examined the

glycosylation of the easily accessible 2-nitro- and 2,4-dinitroestradiol derivatives.[16] As a matter of fact, these acceptors were both β-galactosylated in excellent yields (*Table 5.2*). The observed yields of coupling reflected the increased acidity of the phenolic hydroxyl of these derivatives due to the presence of the electron-withdrawing nitro groups. These latter not only allowed better coupling yields but their versatile reactivity should also enable the synthesis of a small library of glycoconjugate-estradiol derivatives variously substituted on the aromatic cycle.



R_1,R_2: NO_2, H; NO_2, NO_2

Entry	*17β-Estradiol Derivatives*	*Base*	*Product*	*Yield (%)*
1[a]	 **175**	NaOH	 **176**	75
2[b]	 **177**	NaOH	 **178**	84

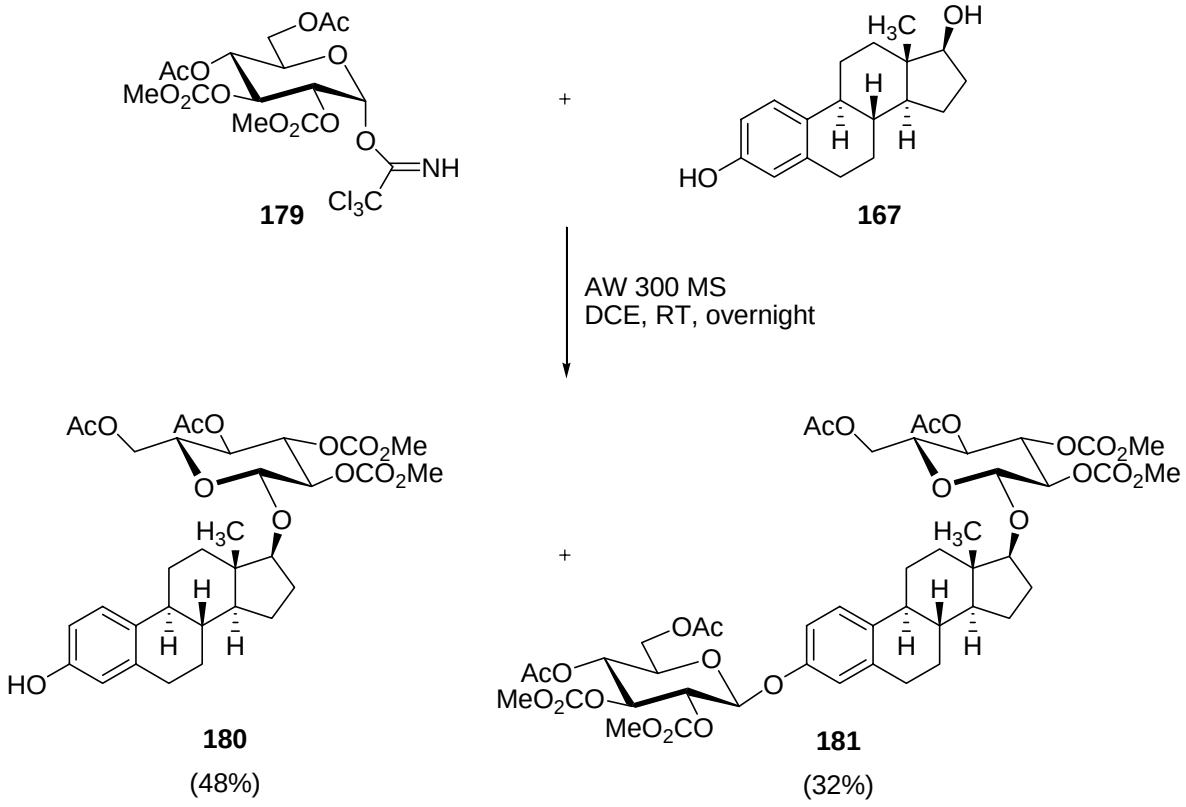
[a]Conditions: donor **88** (1 eq.), **175** (2 eq.), TBAB (2 eq.), base (10 eq.), H_2O, CH_2Cl_2, RT, 2 h; [b]Conditions: donor **108** (1 eq.), **177** (2 eq.), TBAB (2 eq.), base (5 eq.), H_2O, CH_2Cl_2, RT, 20 h.

***TABLE* 5.2** Regioselective Phenolic Glycosylation of 17β-Estradiol Derivatives with Galactosyl Iodide **88**.

After exploring the glycosidation at the phenol site, we attempted to attain the complementary selectivity adopting non basic glycosylation conditions. In absence of the base the carbinol function was expected to be more nucleophilic because of the higher electronic delocalization occurring at the phenol oxygen. The carbinol glycosidation of both 3-*O*-protected fulvestrant and estradiol was reported to be rather difficult under the acid promoted activation of glycosyl trichloroacetimidates owing to the generation of undesired orthoester coupling products and the occurrence of an acyl transfer process yielding the 17-*O*-acylated product.[5,7] These problems could be partly circumvented by

[16] Pezzella, A.; Manini, P.; Di Donato, P.; Boni, R.; Napolitano, A.; Palumbo, A.; D'Ischia, M. *Biorg. Med. Chem.* **2004**, *12*, 2927.

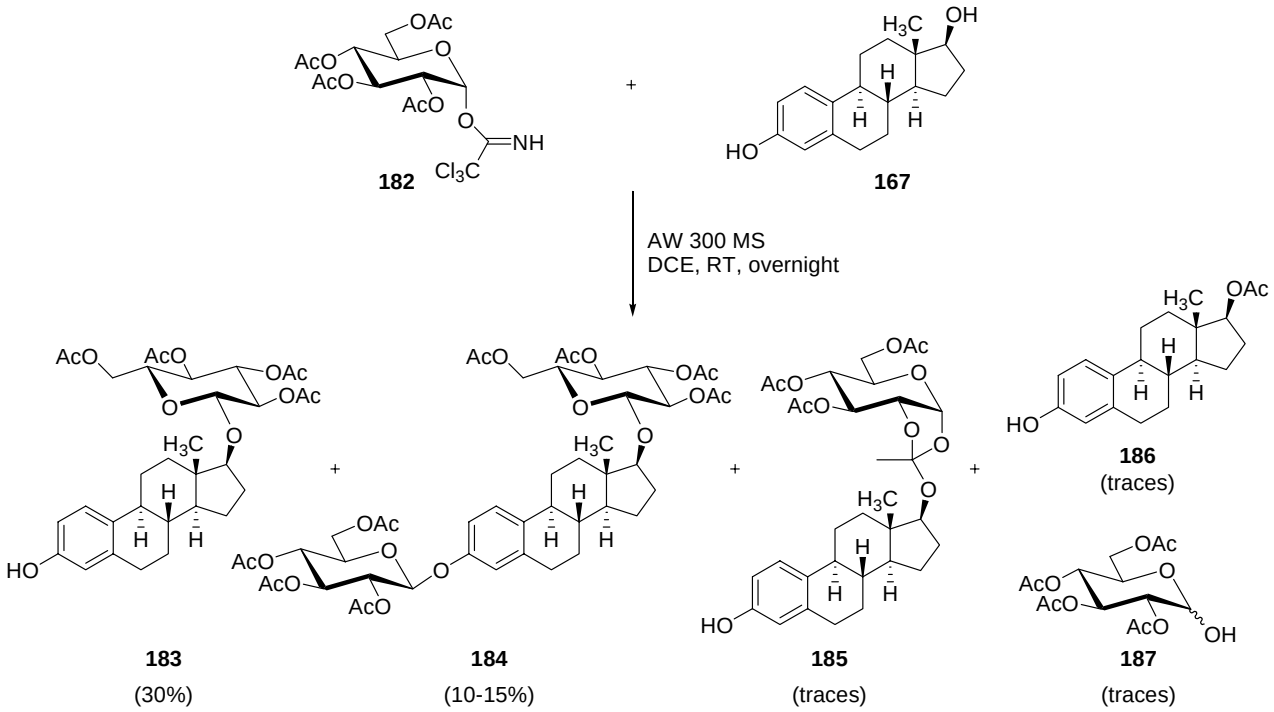
adopting trichloroacetimidate donors equipped with sterically bulky acyl groups (pivaloyl or isobutyryl) and following an "inverse addition" protocol (slow addition of the donor to a mixture of the steroidal alcohol and the acid promoter).[5,7] Recently our group reported the convenient use of 4Å acid washed molecular sieves (4Å AW 300 MS) in the dual role of promoters and drying agents in glycosidations with trihaloacetimidate donors.[17] The mildness of such procedure prompted us to test its applicability to the difficult regioselective carbinol glycosidation of 17β-estradiol. On the bases of our previous experience, a 2-*O*-methoxycarbonylated donor was expected to be more efficient than a 2-*O*-acetylated congener because of the lower propensity to yield undesired orthoester-like coupling products.[18] As a matter of fact, when trichloroacetimidate **179**[18] and a slight stoichiometric excess of 17β-estradiol were stirred in the presence of commercially available 4Å AW 300 MS in dichloroethane, the desired carbinol glycoside **180** (*Scheme 5.2*) was obtained in the appreciable yield of 47% along with the di-glycosylated derivative **181** (32% yield). Interestingly, the product of mono glycosylation at the phenol site was not detected from the reaction mixture, thus suggesting a sequential order of glycosylation with the carbinol site occourring first. It is worth of note that acetyl and methoxycarbonyl groups can be readily removed from **180** and **181** under standard Zemplen conditions whereas time-consuming procedures may be needed for the removal of pivaloyl groups from the above mentioned glycosylated steroids.[5,7]



SCHEME 5.2 Regioselective Carbinol Glycosylation of 17β-Estradiol with Glycosyl Donor **179**.

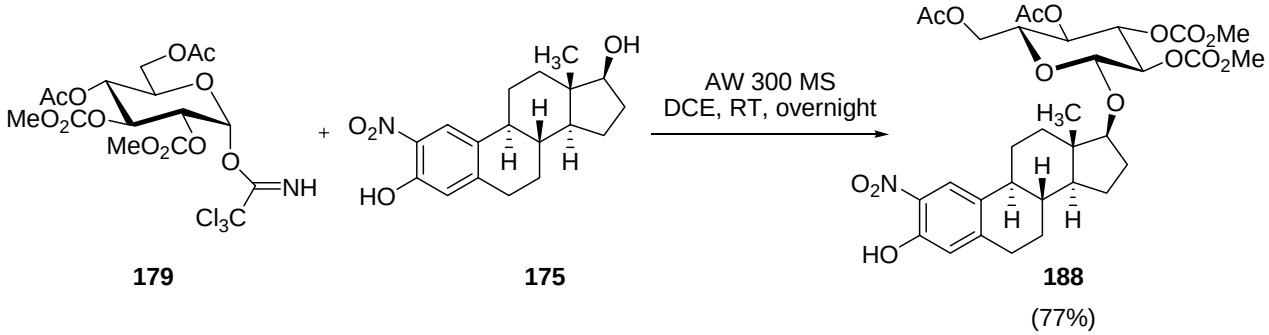
[17] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Org. Lett.* **2003**, *5*, 987.
[18] Adinolfi, M.; Barone, G.; Iadonisi, A.; Mangoni, L.; Schiattarella, M. *Tetrahedron Lett.* **2001**, *42*, 5967.

Under analogous conditions the corresponding tetra-*O*-acetylated donor **182** afforded 17-*O*-glucosylated estradiol **183** in lower yield (30%) along with the di-*O*-glucosylated product **184** (estimated yield 10-15%, the product being recovered contaminated). NMR analysis of the crude reaction mixture revealed the formation of 2,3,4,6-tetra-*O*-acetyl glucose as the main by-product from the donor, and lower amounts (5-10%) of the 1,2-orthoester coupling product and of 17-*O*-acetyl estradiol (*Scheme 5.3*).



SCHEME 5.2 Regioselective Carbinol Glycosylation of 17β-Estradiol with Glycosyl Donor **182**.

When 2-nitro-17β-estradiol was coupled with donor **179**, the mono glycosidation of the carbinol site was achieved in very good yield (77%) without appreciable detection of any other glycosylation product (*Scheme 5.3*).



SCHEME 5.3 Regioselective Carbinol Glycosylation of 17β-Estradiol Derivative **175** with Glycosyl Donor **179**.

Conclusion

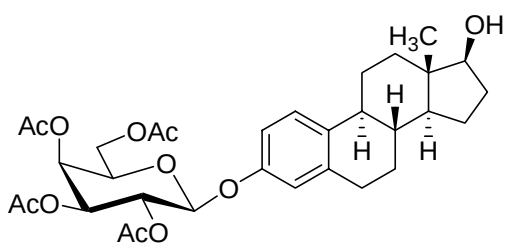
In conclusion, we have demonstrated that selective glycosidation of estradiol derivatives can be achieved either at the phenol or at the carbinol centre without extra steps for preventive protection of the estrogen derivative. The regioselective glycosidation at the phenolic site can be achieved adopting experimentally simple reactions conducted under two-phase conditions and good results were obtained with a variety of glycosyl bromides and iodides as the donors. The alternative regioselective glycosidation at the carbinol site can instead be obtained by the mild activation of trichloroacetimidates by acid washed molecular sieves. In both cases especially high yields were obtained in the glycosidation of estradiols bearing aromatic nitro groups. Work is in progress for the exploitation of the proposed methodologies for the synthesis of novel glycosylated derivatives of estradiol and for the chemical elaboration of the obtained glycosyl nitroestradiols. The complete library of deprotected compounds will be then submitted to microbiological tests for evaluation of the biological activity.

Experimental Section

General Methods and Material

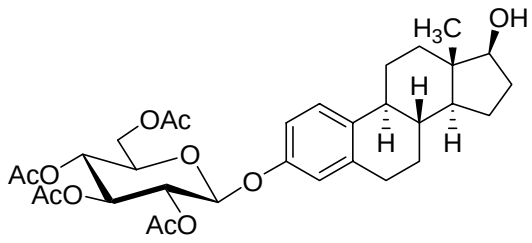
Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian spectrometers at 200 or 300 MHz and 50 or 75 MHz, respectively; or on a Bruker Avance 500 spectrometer at 500 MHz. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Analytical thin layer chromatography (TLC) was performed on E. Merck pre-coated (25 nm) silica gel 60F-254 plates. Column chromatography was preformed using Carlo Erba 0.06-0.20 silica gel. Mass spectra were recorded in a reflection positive mode on a MALDI-TOF spectrometer. Acid washed molecular sieves were dried by overnight heating at 200 °C under vacuum before their use in glycosidations. Anhydrous solvents stored over molecular sieves (purchased from Aldrich) were used for performing the reactions.

Typical procedure of phenol glycosidation of estradiol: An aqueous solution of NaOH (5%, 4 mL) was added at room temperature to a suspension of 17β-estradiol (27 mg, 0.1 mmol) and TBAB (32 mg, 0.1 mmol) in dichloromethane (2 mL) and the system kept under stirring for ten minutes. A solution of crude iodide[11] **88** (0.15 mmol) in dichloromethane (2 mL) was then added. After the consumption of the donor (ca 4 hours, TLC analysis), the mixture was repeatedly extracted with dichloromethane and the collected organic phases dried and concentrated. The residue was purified by silica gel chromatography (eluent petroleum ether/ethyl acetate from 7:3 to 6:4) to yield pure glycoside **168** (45 mg, 75% yield).

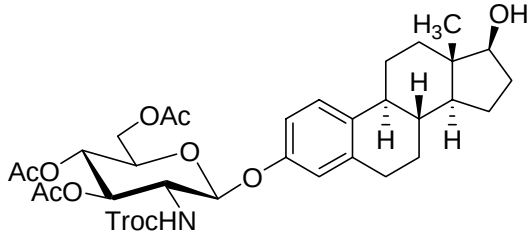


168. $[\alpha]_D$ +51.9 (c 0.7, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) significative signals at δ 7.21 (1H, d, $J_{1,2}$ = 8.2 Hz, H-1 estradiol), 6.77 (1H, dd, $J_{2,4}$ = 2.4 Hz, H-2 estradiol), 6.72 (1H, d, H-4 estradiol), 5.47 (1H, dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.4 Hz, H-2 Gal), 5.44 (1H, bd, $J_{3,4}$ = 3.6 Hz, H-4 Gal), 5.08 (1H, dd, H-3 Gal), 4.99 (1H,

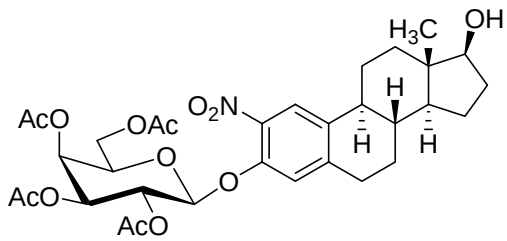
d, H-1 Gal), 4.30-4.08 (2H, m, H_2-6 Gal), 4.04 (1H, bt, $J_{5,6a} = J_{5,6b} = 6.4$ Hz, H-5 Gal), 3.73 (1H, t, J = 8.0 Hz, H-17 estradiol), 2.18, 2.06, 2.06, 2.01 (12H, 3xs, 4x -$COCH_3$), 0.77 (3H, s, CH_3 estradiol). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.3, 170.1, and 169.4 (-$\underline{C}OCH_3$), 154.9, 138.3, 135.5 (aromatic C), 126.4, 116.9, and 114.3 (aromatic CH), 99.9 (C-1 Gal), 81.8 (C-17 estradiol), 70.9, 70.9, 68.7, 67.0 (C-2, C-3, C-4, and C-5 Gal), 61.4 (C-6 Gal), 20.6 (4x -CO$\underline{C}H_3$), 11.0 (CH_3 estradiol). Other signals of estradiol at δ 50.0, 44.0, 43.2, 38.7, 36.7, 30.6, 29.7, 27.1, 26.2, 23.1. MALDI-TOF MS for $C_{32}H_{42}O_{11}$ (M 602.3), m/z 625. 5 $[M+Na]^+$.



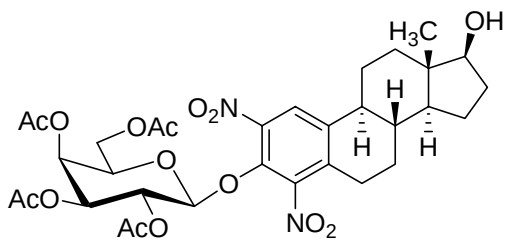
170. ^{1}H NMR (200 MHz, $CDCl_3$) significative signals at δ 7.21 (1H, d, J_{ab} = 8.4 Hz, H-1 estradiol), 6.78 (1H, dd, J_{bd} = 3.4 Hz, H-2 estradiol), 6.73 (1H, d, H-4 estrdiol), 5.36-5.15 (3H, overlapped signals, H-2, H-3, and H-4 Glc), 5.04 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 Glc), 4.26 (1H, dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 5.6 Hz, H-6 Glc), 4.16 (1H, dd, $J_{5,6b}$ = 2.4 Hz, H-6 Glc), 3.84 (1H, m, H-5 Glc), 3.73 (1H, t, J = 8.0 Hz, H-17 estradiol), 2.08, 2.05, 2.04, 2.03 (12H, 4xs, 4xCH_3 acetyls), 0.77 (3H, s, CH_3 estradiol).



174. ^{1}H NMR (300 MHz, $CDCl_3$) significative signals at δ 7.17 (1H, d, J_{orto} = 8.7 Hz, H-1 estradiol), 6.77 (1H, dd, J_{meta} = 2.7 Hz, H-2 estradiol), 6.71 (1H, d, H-4 estrdiol), 5.40 (1H, d, $J_{2,NH}$ = 9.3, NHTroc), 5.39 (1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4 GlcNHTroc), 5.17 (1H, d, $J_{1,2}$ = 8.4 Hz, H-1 GlcNHTroc), 5.12 (1H, t, $J_{2,3}$ = 9.3 Hz, H-3 GlcNHTroc), 4.72 (2H, bs, -OC$\underline{H}_2CCl_3$), 4.28 (1H, dd, J_{gem} = 12.6 Hz, $J_{5,6a}$ = 5.4 Hz, H-6 GlcNHTroc), 4.17 (1H, dd, $J_{5,6b}$ = 2.4 Hz, H-6 GlcNHTroc), 3.95-3.70 (2H, m, H-2 and H-5 GlcNHTroc), 3.73 (1H, t, J = 8.1 Hz, H-17 estradiol), 2.08, 2.05, 2.04 (9H, 3xs, 3xCH_3 acetyls), 0.74 (3H, s, CH_3 estradiol).



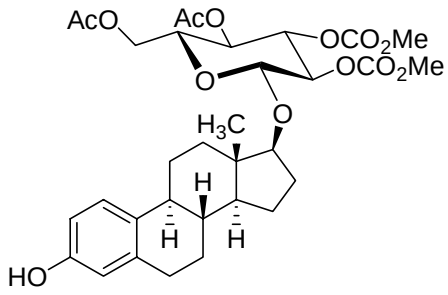
176. ^{1}H NMR (200 MHz, $CDCl_3$) significative signals at δ 7.78 (1H, s, H-1 estradiol), 7.04 (1H, s, H-4 estradiol), 5.53 (1H, dd, Hz, $J_{1,2}$ = 8.0 Hz, H-2 Gal), 5.46 (1H, d, $J_{3,4}$ = 3.0 Hz, H-4 Gal), 5.08 (1H, dd, $J_{2,3}$ =10.2 Hz, H-3 Gal), 5.01 (1H, d, H-1 Gal), 4.23 (1H, dd, $J_{5,6a}$ = 6.8 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6 Gal), 4.17 (1H, dd, $J_{5,6b}$ =7.6 Hz, H-6 Gal), 4.05 (1H, t, H-5 Gal), 3.74 (1H, t, $J_{s,r}$= 8.2 Hz, H-17 estradiol), 2.98 (2H, m, H-9 and H-6 estradiol), 2.18, 2.12, 2.08 and 2.01 (4 x s, CH_3CO), 0.78 (3H,s, CH_3 estradiol).



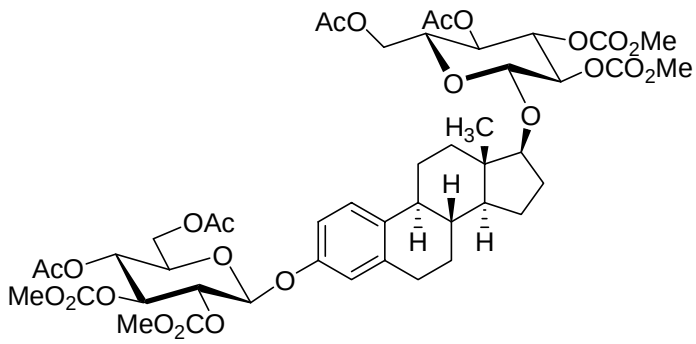
178. ^{1}H NMR (200 MHz, $CDCl_3$) significative signals at δ 7.83 (1H, s, H-1 estradiol), 5.39 (1H, dd, $J_{1,2}$ =8.0 Hz, $J_{2,3}$ =10.6 Hz, H-2 Gal), 5.35 (1H, d, $J_{3,4}$ =3.4 Hz, H-4 Gal), 5.01 (1H, dd, H-3 Gal), 4.91 (1H, d, H-1 Gal), 4.09 (1H, dd, $J_{5,6a}$ =6.4 Hz, $J_{6a,6b}$ =11.2 Hz, H-6 A Gal), 4.00 (1H, dd, $J_{5,6}$=8.4 Hz, H-6 B Gal), 3.81 (1H, t, H-5 Gal), 3.74 (1H, t, J =8.0 Hz, H-17 estradiol), 2.98-2.62

(2H, m, H-9 e 2xH-6 estradiol), 2.19, 2.14, 2.01 e 1.98 (4 x CH_3CO), 0.79 (3H, s, CH_3 estradiol).

Typical procedure of carbinol glycosidation of estradiol: Freshly activated 4Å AW 300 MS (ca 500 mg in pellets) were added to a mixture of donor **179** (28 mg, 0.053 mmol) and 17β-estradiol (16 mg, 0.059 mmol). The mixture was suspended under argon with anhydrous dichloroethane (1 mL) at 0 °C and then the temperature was allowed to raise spontaneously. The mixture was left overnight under stirring at room temperature and then filtered on a short plug of silica gel. The residue was purified by silica gel chromatography (eluent toluene/ethyl acetate 8:2) to afford monoglycoside **180** (16 mg, 47% yield), unreacted estradiol, and then diglycoside **181** (9 mg, 32%).

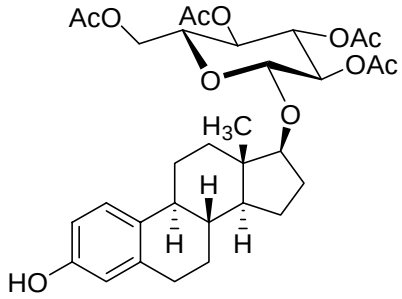


180. $[\alpha]_D$ +16.4 (c 0.6, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) significative signals at δ 7.14 (1H, d, $J_{1,2}$ = 8.4 Hz, H-1 estradiol), 6.63 (1H, dd, $J_{2,4}$ = 2.4 Hz, H-2 estradiol), 6.56 (1H, d, H-4 estradiol), 5.13 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-4 Glc), 5.06 (1H, t, $J_{2,3}$ = 9.6 Hz, H-3 Glc), 4.85 (1H, dd, $J_{1,2}$ = 8.0 Hz, H-2 Glc), 4.58 (1H, d, H-1 Glc), 4.26 (1H, dd, $J_{6a,5}$ = 5.2 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a Glc), 4.15 (1H, dd, $J_{6b,5}$ = 2.8 Hz, H-6b Glc), 3.81 and 3.78 (6H, 2xs, 2x -OCH_3), 3.70 (1H, m, H-5 Glc), 3.63 (1H, t, J = 8.4 Hz, H-17 estradiol), 2.10 and 2.05 (6H, 2xs, 2 -$COCH_3$), 0.75 (3H, s, CH_3 estradiol). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.6 and 169.4 (2x -$\underline{C}OCH_3$), 155.1 and 154.5 (2x –$O\underline{C}O_2CH_3$), 153.3, 138.2, 126.5 (aromatic C), 126.4, 115.2 and 112.6 (aromatic CH), 101.6 (C-1 Glc), 90.7 (C-17 estradiol), 76.6, 75.2, 71.6, 68.7 (C-2, C-3, C-4, and C-5), 62.1 (C-6), 55.3 and 55.2 (–$OCO_2\underline{C}H_3$), 20.8 and 20.6 (2x -$CO\underline{C}H_3$), 11.5 (CH_3 estradiol). Other signals of estradiol at δ 49.6, 43.9, 43.3, 38.5, 37.2, 29.5, 28.8, 27.1, 26.3, 23.1. MALDI-TOF MS for $C_{32}H_{42}O_{13}$ (M 634.3), m/z 657.4 $[M+Na]^+$.

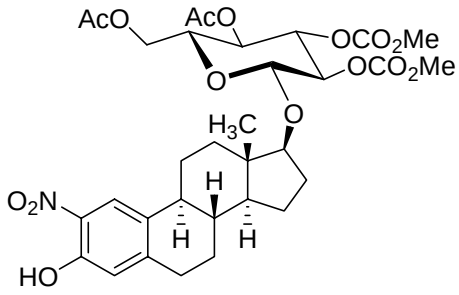


181. $[\alpha]_D$ +26.6 (c 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) significative signals at δ 7.18 (1H, d, $J_{1,2}$ = 8.4 Hz, H-1 estradiol), 6.75 (1H, dd, $J_{2,4}$ = 2.4 Hz, H-2 estradiol), 6.71 (1H, d, H-4 estradiol), 5.20-5.00 (6H, overlapped signals, H-3 and H-4 phenol and carbinol Glc, H-1 and H-2 phenol Glc), 4.84 (1H, dd, $J_{1,2}$ = 8.0 Hz, H-2 Glc), 4.57 (1H, d, H-1 Glc), 4.28-4-10 (4H, overlapped signals, H_2-6 carbinol and phenol Glc), 3.84 (1H, m, H-5 phenol Glc), 3.82, 3.80, 3.78, and 3.77 (12H, 4xs, 4x -OCH_3), 3.70 (1H, m, H-5 carbinol Glc), 3.64 (1H, t, J = 8.4 Hz, H-17 estradiol), 2.09, 2.07, 2.05 and 2.04 (12H, 4xs, 4x-$COCH_3$), 0.73 (3H, s, CH_3 estradiol). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.6 and 169.3 (-$\underline{C}OCH_3$), 155.1 and 154.5 (–$O\underline{C}O_2CH_3$ and C-3 estradiol), 138.2, 135.4 (aromatic C), 126.4, 117.2, and 114.5 (aromatic CH), 101.6 (C-1 carbinol Glc), 99.1 (C-1 phenol Glc), 90.6 (C-17 estradiol), 76.6, 75.2, 74.8, 71.6, 71.5, 68.7, 68.3 (C-2, C-3, C-4, and C-5, phenyl and carbinol Glc), 61.9 (C-6, phenyl and carbinol Glc), 55.3 (–$OCO_2\underline{C}H_3$), 20.6 and 20.5 -$CO\underline{C}H_3$), 11.4 (CH_3 estradiol). Other signals

of estradiol at δ 49.6, 43.9, 43.2, 38.3, 37.1, 29.6, 28.8, 27.0, 26.1, 23.1. MALDI-TOF MS for $C_{46}H_{60}O_{24}$ (M 996.4), m/z 1019.3 $[M+Na]^+$.



183. ^{1}H NMR (300 MHz, $CDCl_3$) significative signals at δ 7.17 (1H, d, H-1 estradiol), 6.68-6.52 (2H, m, H-2 and H-4 estradiol), 5.30-4.96 (3H, m, H-2, H-3 and H-4 Glc), 4.56 (1H, d, $J_{1,2}$= 7.8 Hz, H-1 Glc), 4.26 (1H, dd, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6A Glc), 4.13 (1H, dd, $J_{5,6b}$ =2.8 Hz, H-6 B Glc), 3.72-3.59 (2H, m, H-5 Glc and H-17 estradiol), 2.79 (3H, m, H-9 and 2 x H-6 estradiol), 2.09, 2.06, 2.02 and 2.01 (12H, 4xs, 4xCH_3 acetyls), 0.74 (3H, s, CH_3 estradiol).



188. ^{1}H NMR (400 MHz, $CDCl_3$) significative signals at δ 10.40 (1H, s, OH phenolic estradiol), 7.97 (1H, s, H-1 estradiol), 6.84 (1H, s, H-4 estradiol), 5.11-5.06 (2H, m, overlapped signals H-4 and H-3 Glc), 4.85 (1H, t, $J_{1,2}$ = 8.4 Hz, H-2 Glc), 4.56 (1H, d, H-1 Glc), 4.25 (1H, dd, $J_{6a,5}$ = 5.1 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a Glc), 4.13 (1H, dd, $J_{6b,5}$ = 2.4 Hz, H-6b Glc), 3.82 and 3.78 (6H, 2xs, 2x -OCH_3), 3.70 (1H, m, H-5 Glc), 3.61 (1H, t, J = 7.8 Hz, H-17 estradiol), 2.09 and 2.04 (6H, 2xs, 2 -$COCH_3$), 0.75 (3H, s, CH_3 estradiol). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.6 and 169.4 (2x -$\underline{C}OCH_3$), 155.1 and 154.6 (2x –$O\underline{C}O_2CH_3$), 152.8 (C-2 estradiol), 149.1 (C-3 estradiol), 133.6 and 131.7 (C-5 and C-6 estradiol), 121.5 and 118.9 (C-4 and C-1 estradiol), 101.8 (C-1 Glc), 90.7 (C-17 estradiol), 76.6, 75.2, 71.6, 68.7 (C-2, C-3, C-4, and C-5 Glc), 62.1 (C-6 Glc), 55.3 and 54.9 (2x –$OCO_2\underline{C}H_3$), 20.7 and 20.6 (2x -$CO\underline{C}H_3$), 11.4 (CH_3 estradiol). Other signals of estradiol at δ 49.5, 43.4, 43.2, 39.7, 37.8, 29.7, 28.8, 26.5, 26.0, 23.1.

Chapter 6

Toward Novel Antibiotics: Glycosyl 4-Alkilidene-β-Lactams

Introduction

Antibiotics are natural or synthetic compounds that inhibit the growth of bacteria. Natural sources of antibiotic substances are micro-organisms and moulds. Among these, *Penicillum* and *Cephalosporium* are very well known because produce, as secondary metabolites, penicillins and cephalosporins, β-lactam-containing antibacterial molecules. Since penicillins, the first major class of antibiotics, were introduced in the 1940s and 1950s, the number of antibiotic agents available had been rising steeply. Nevertheless as result of saturation of the market, many pharmaceutical companies lost interest in the development of antibiotic drugs and instead focused on chronic diseases.[1] Nowadays a new emergency has to be faced: sixty years of use and misuse of such drugs resulted in an impressive rise in bacterial resistance not only to single but also to multiple antibiotics exposure.[2] Many factors have contributed to the development and spread of antibiotic resistance, including the expanding population of immuno-compromised patients, the increased use of invasive medical procedures, and the inappropriate use of antibiotics in both medicine and agriculture. One of the main causes is represented by the non-compliance to the course of treatment by patients. Both the long-term exposure to low doses and the failure to finish a prescription encourage more resistant bacterial strains to flourish. Furthermore, despite the wealth of treatment options, a limited variety of antibiotic mechanisms are implemented. The various antibiotics target few vital processes, whose tampering results in micro-organism death. Five types of cellular processes inhibition are generally performed:

- Protein synthesis
- Bacterial cell wall synthesis
- DNA synthesis
- RNA synthesis
- Folic acid metabolism.

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[1] Shales, D. M. *Curr. Opinion in Pharmacology* **2003**, *3*, 470.
[2] Gold, H. S.; Moellering, R. C. *N. Engl. J. Med.* **1996**, *335*, 1445.

The bacterial adaptation to these ways of action has been so successful that some infections resulted untreatable with any antibiotic.
Obviously, the widespread occurrence of resistance mechanisms has renewed the industrial interest in this field. World Health Organisation (WHO) encourages the research community to have a better insight into the mechanism of bacterial resistance with the aim of developing new effective drugs.[3]
Bacterial resistance to antibiotic drugs is mediated by one or more of the following processes:[4]

- Prevention of the drug from reaching its target either by active efflux from or by reduced uptake into the cell, as well as by sequestration of the antibiotic by protein binding.
- Deactivation of the antibiotic by enzymatic modification.
- Modification of the drug's target, thereby eliminating or reducing the binding of the antibiotic.
- Metabolic bypass of the inhibited reaction.
- Overproduction of the antibiotic target.

Of these mechanisms, the first three represent the most common processes that enhance bacterial resistance to antibiotics (*Figure 6.1*).[5]

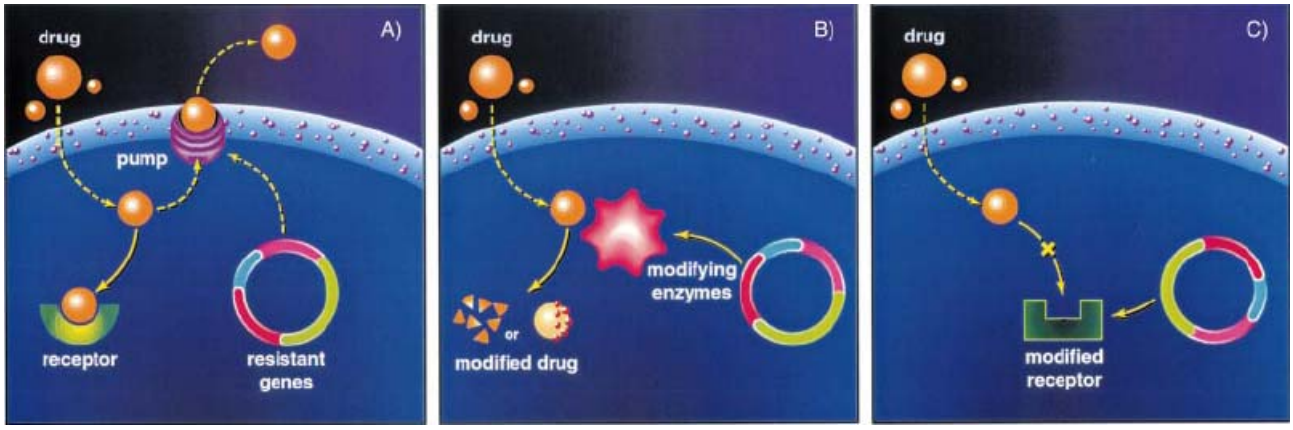


FIGURE ***6.1*** Mechanisms of Bacterial Drug Resistance: A) Active Reflux; B) Enzymatic Modification of the Drug; C) Modification of Target Receptor or Enzymes.

To efficiently overcome resistance problems, the identification of new targets as well as the improvement of the existing drugs will be necessary.
For several years our group has been part of a multidisciplinary team whose expertise in various field was addressed to the development of novel glycoconjugates with antibiotic activity against resistant bacteria. The aim of the project, supported by Ministero dell'Istruzione, dell'Università e della Ricerca as a "Research Programme of Prominent National Interest", is the design, synthesis and screening of β-lactam-glycoconjugates. Three research groups are responsible for the synthesis and conjugation of novel β-lactam-containing molecules, whose activity is then microbiologically tested on clinical isolates belonging to both Gram-positive and Gram-negative bacteria, including antibiotic

[3] World Organization Report of Infection Diseases 2000 Overcoming Antimicrobial Resistance, www.who.int/infectious-disease-report/2000/index.html.
[4] Davies, J. *Science* **1994**, *262*, 375.
[5] Walsh, C. *Nature* **2000**, *406*, 775.

resistant strains. The study and design phase as well as the microbiological tests analysis are assisted by chemoinformatic means. Molecular modelling is applied to correlate the changes in chemical structures with antibiotic activities in order to better understand the drug-receptor molecular interactions and therefore predict feasible modifications on the structures to be synthesized. The mechanism of action of β-lactam antibiotics, such as penicillins and cephalosporins, targets the peptidoglycan biosynthesis. In the cell envelope of both Gram-positive and Gram-negative bacteria the peptidoglycan layer provides the strength and rigidity, which allows them to live in a hypotonic environment and gives them their characteristic shape (*Figure 6.2*).

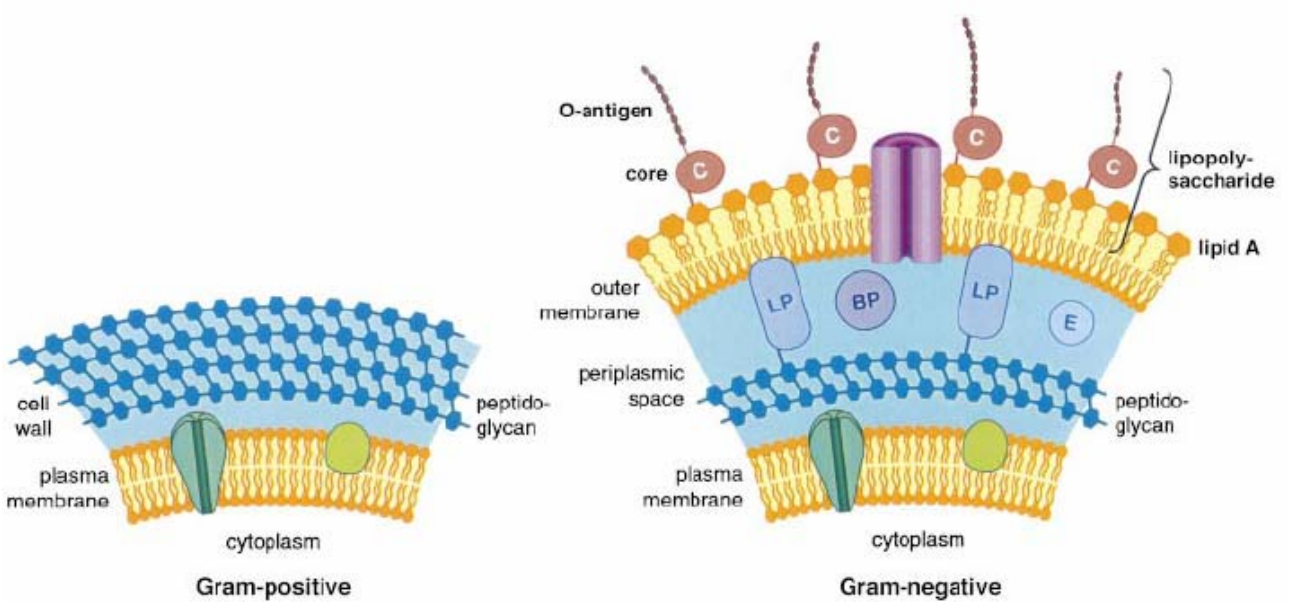


FIGURE 6.2 The Cell Envelope of Gram-Positive and Gram-Negative Bacteria.

Antibiotic-induced defects lead to cell lysis as a result of the inability of the bacteria to cope with the internal osmotic pressure.

Peptidoglycan is a polymer composed by polysaccharide chains of two sugars, *N*-acetyl glucosamine (NAG) and *N*-acetyl muramic acid (NAM) linked through β(1→4) glycosidic bond, that in turn are crosslinked by pentapeptide-pentaglycine bridges (*Figure 6.3*).

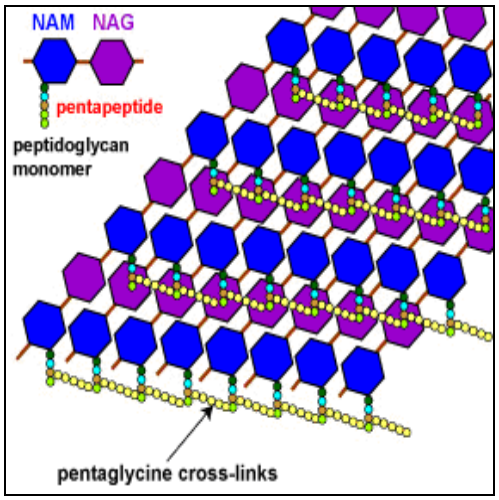


FIGURE 6.3 Structure of Peptidoglycan.

Peptidoglycan biosynthesis[6] commences with the transformation of one unit of UDP-*N*-acetylglucosamine (UDP-GlcNAc) into UDP-*N*-acetylmuramic acid (UDP-MurNAc) *via* phosphoenolpyruvate (PEP) transfer followed by reduction of the resulting unsaturated acid (*Figure 6.4*). Three amino acid residues are then attached sequentially in ATP-dependent reactions to the lactic acid residue of UDP-MurNAc. This is followed by the coupling of a preformed dipeptide, usually D-Ala-D-Ala, to the peptide terminus. The presence of unusual D-amino acid residues confers to peptidoglycan a better stability against proteolysis. In the next step, MurNAc-pentapeptide phosphate is transferred from its UDP derivative to undecaprenyl phosphate, a carrier molecule embedded in the cytoplasmic membrane. A second GlcNAc residue is then coupled at the C-4 position of MurNAc-pentapeptide. At this point, the GlcNAc-MurNAc peptide is translocated across the cytoplasmic membrane to the cell surface by an unknown mechanism. Here, the disaccharide units are polymerized by the action of several transglycosylases. Finally, transpeptidases catalyze the attack of the terminal side-chain of *meso*-diaminopimelic acid (*m*DAP, Gram-negative bacteria) or the amino terminus of the oligo-Gly linker (Gram-positive bacteria) at the peptide bond between the two D-Ala residues of a neighbouring peptide chain, resulting in crosslinking with a concomitant loss of a D-Ala.

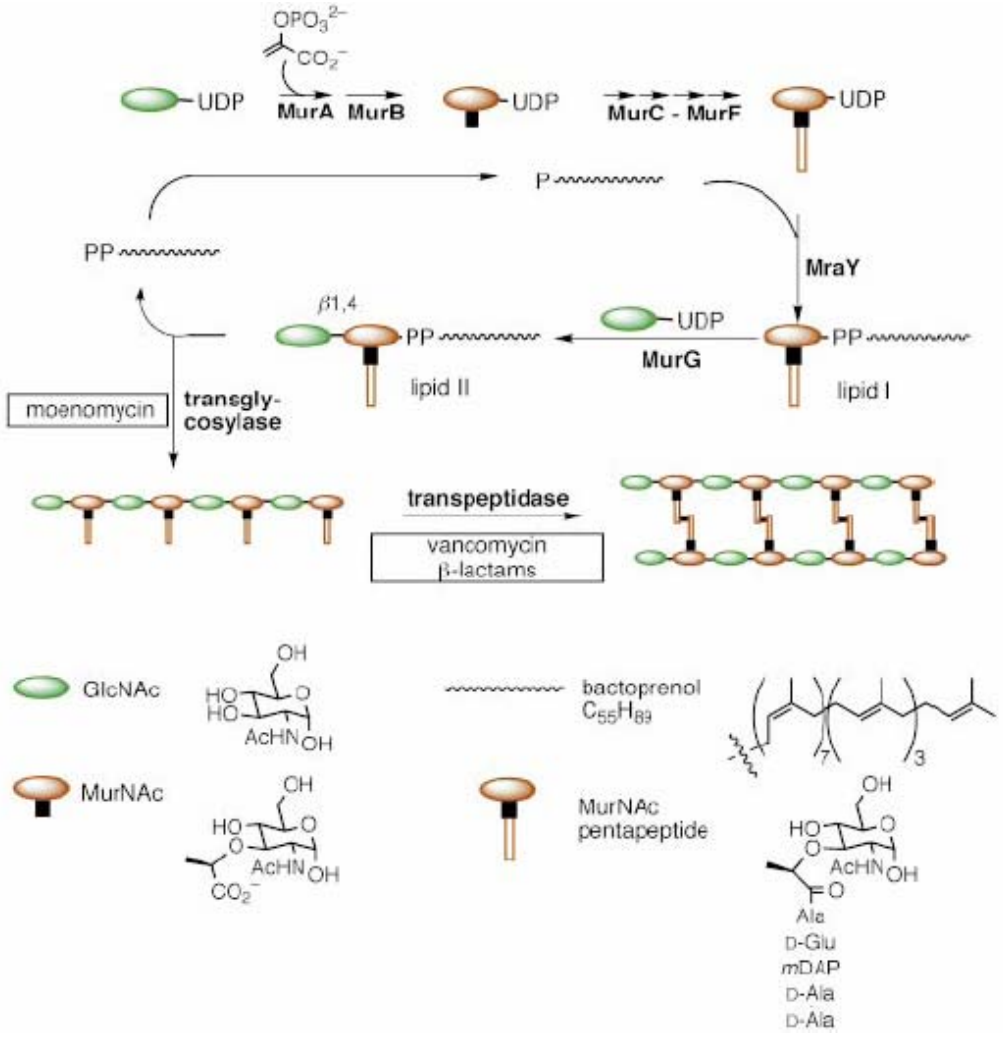
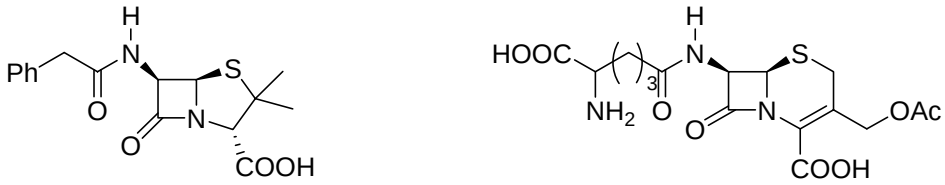


FIGURE 6.4 Biosynthesis of Peptidoglycan (UDP = uridine diphosphate, Mur = muraminic acid, P = phosphate substituent, MraY = translocase).

[6] van Heijenoort, J. *New. Compr. Biochem.* **1994**, *27*, 39.

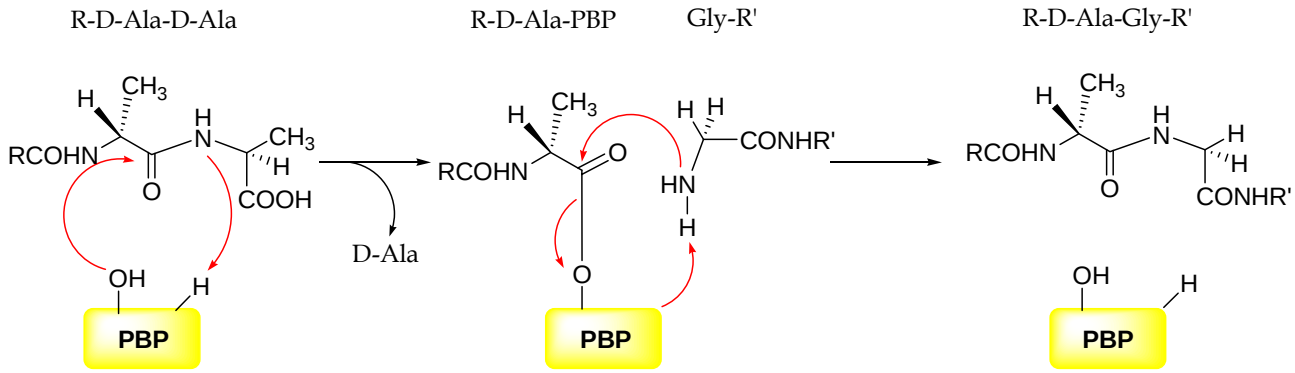
Each of these enzymes represents a target for antibiotic development.
Penicillins and cephalosporins (*Figure 6.5*) target the enzyme transpeptidase that catalyze the crosslinking step.[7] The pharmacological activity of these molecules is mainly due to the presence of the four membered lactam ring (azetidinone) that mimics the D-Ala-D-Ala extremity of peptide chains to be crosslinked.



Penicillin G Cephalosporin C

FIGURE 6.5 Penicillin G and Cephalosporin C Structures.

During the crosslinking step a serine-carboxypeptidase, named Penicillin Binding Protein (PBP), hydrolyzes the peptide bond between the two D-Ala residues generating a temporary ester linkage with the peptide chain to be crosslinked. After the departure of the terminal D-Ala residue from the active site of the enzyme, the amino group of a neighbouring peptide chain (*m*DAP or oligo-Gly) interacts with the PBP-D-Ala adduct completing the crosslinking (*Scheme 6.1*).



SCHEME 6.1 Penicillin Binding Protein Mechanism of Action.

Penicillins, and, more generally, β-lactam-based antibiotics effectively interact with PBPs because of their structural resemblance with D-alanyl-D-alanine (*Figure 6.6*).

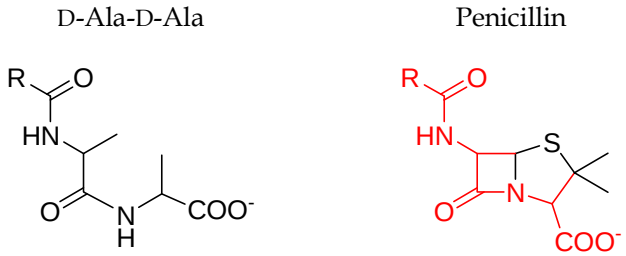
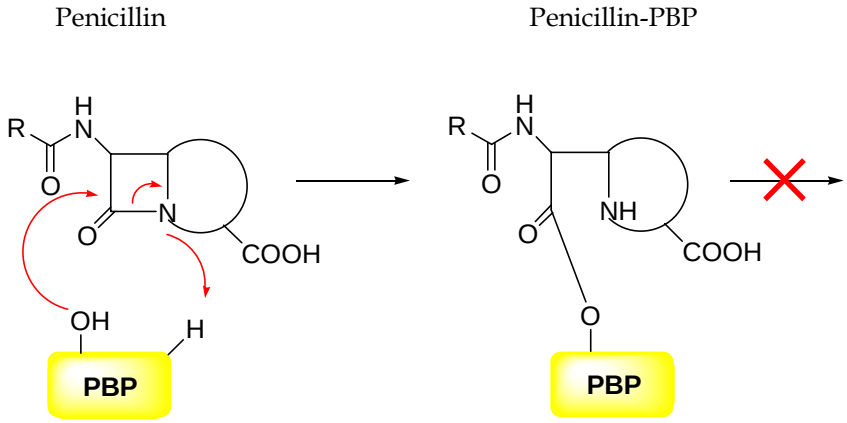


FIGURE 6.6 Structural Resemblance between D-Alanyl-D-Alanine and Penicillins.

[7] Beadle, B. M.; Nicholas, R. A.; Shoichet, B. K. *Science* **2001**, *10*, 1254.

Penicillin inhibits the PBP's crosslinking capabilities by acylating the enzyme and binding irreversibly to its catalytic site (*Scheme 6.2*).[8] The great stability of the PBP-Penicillin adduct is due to the steric hindrance that occurs at the enzyme active site. Despite the D-Ala-D-Ala case, because of the cyclic nature of penicillin no release of encumbrance is registered after acylation of the enzyme. The PBP-penicillin adduct is thus inaccessible to oligo-Gly chains for the crosslinking step or even to water for hydrolysis reaction.



***SCHEME* 6.2** Penicillin Binding Protein Acylation.

The overall effect is the peptidoglycan reticulation inhibition that means cell wall weakening and results in bacteria death by lysis. The effectiveness of β-lactam antibiotics made them a widely used class of antibacterial drugs. However, some bacteria developed specific resistance mechanisms against β-lactam-based drugs such as (*Figure 6.7*):

- producing enzymes (zinc- or serine-dependent β-lactamases) that inactivate the drug
- synthesizing modified PBPs with a low affinity for the drug
- altering their permeability for the drug or providing efficient extrusion of it by efflux pumps.

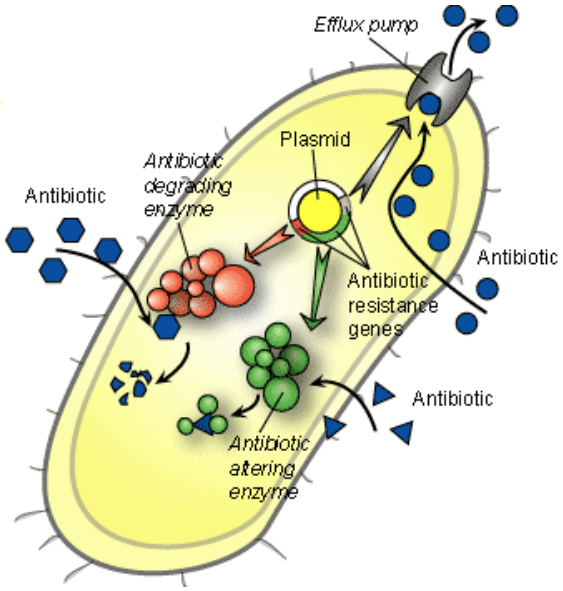


***FIGURE* 6.7** Major Bacterial β-Lactam Resistance Mechanisms.

[8] Blumberg, P. M.; Strominger, J. L. *Bacteriological Rev.* **1974**, *38*, 291.

The most important mechanism of bacterial resistance to β-lactams is represented by expression of β-lactamases, enzymes able to hydrolyze the β-lactam ring causing loss of activity of these drugs (*Figure 6.8*). These enzymes, which make up a large heterogeneous group, are found widely among Gram-positive and Gram-negative bacteria.

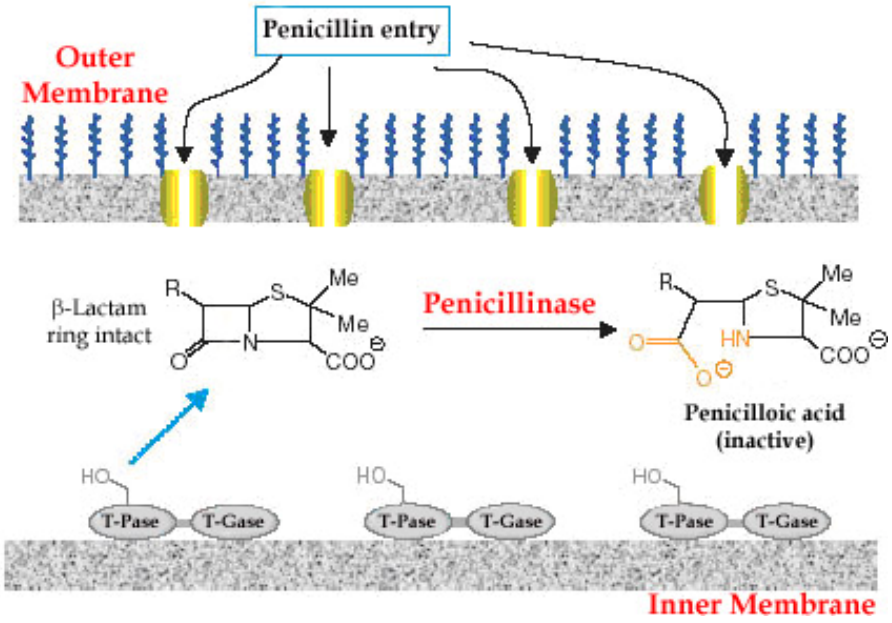


FIGURE ***6.8*** β-Lactamase Action.

Of particular interest for our studies is the resistance mechanism relevant to the synthesis of modified PBPs. A recent study on methicillin resistant *Staphylococcus aureus* (MRSA) revealed that in the presence of classical β-lactam antibiotics the biosynthesis of bacterial cell wall can be regularly fulfilled, because resistant strains codify a new modified protein (PBP2A) with low-affinity for these types of drugs.[9] This investigation, developed on a molecular level, demonstrated that PBP2A couples with PBPs, whose active site have been locked by β-lactams, and restores the trans-peptidic domain, thus bypassing the antibiotic effect of the drugs (*Figure 6.9*).

More recently, the combined administration of a β-lactam drug (niotrocephin) together with short synthetic fragments of peptidoglycan increased the affinity of PBP2A for these kind of antibiotics, suggesting a feasible inhibition of this protein as well.[10] The short saccharidic structures, interacting with the transglycosylase domain of PBPs/PBP2A, likely promote conformational changes in the protein hence enabling the β-lactam to access the inhibition site.

These latter results together with the consideration that none of the other classes of antibiotics is featured by as high efficiency and low toxicity as β-lactams are, suggested the idea of synthesizing novel β-lactam-based glycoconjugates. A few number of reports describe the synthesis of β-lactams conjugated to carbohydrates, and in many cases the

[9] Pinho, M. G.; de Lencastre, H.; Tomasz, A. *Proc. Natl : Acad. Sci. USA* **2001**, *98*, 10886.
[10] Fuda, C.; Hesek, D.; Lee, M.; Morio, K. –I.; Nowak, T.; Mobashery, S. *J. Am. Chem. Soc.* **2005**, *127*, 2056.

saccharidic moiety plays the role of chiral auxiliary for the stereocontrolled construction of the lactam ring.[11]

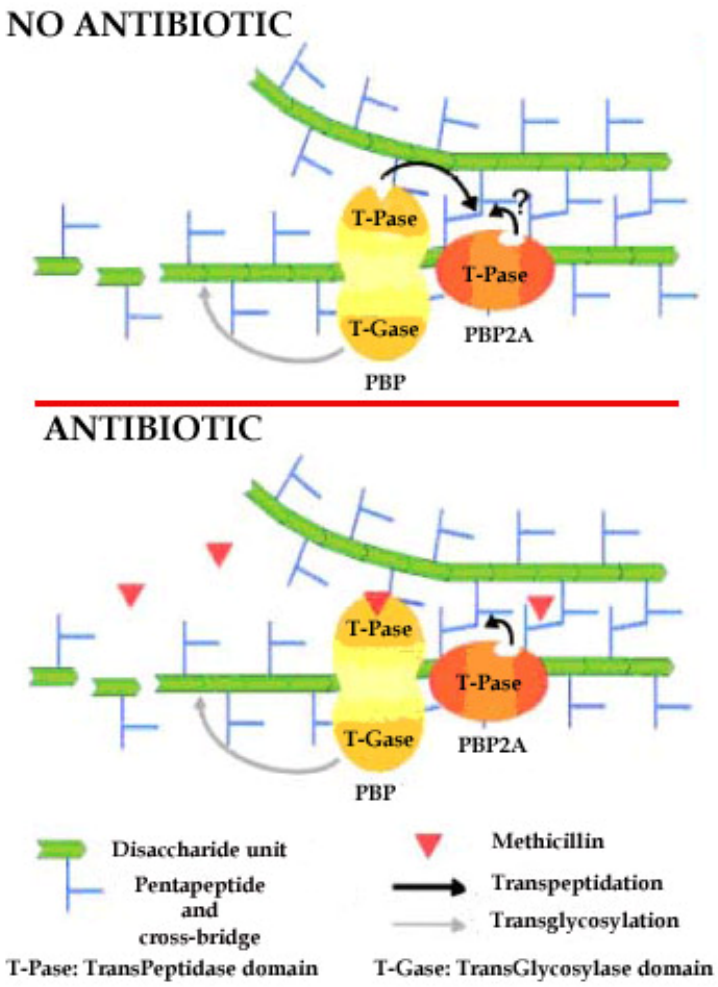


FIGURE 6.9 PBP2A Mechanism of Action.

Isolated examples are instead focused on the biological properties of such conjugates[11] even though this strategy offers several theoretical advantages, among the others an improved resemblance of the drug with the natural substrates of PBPs. Furthermore, the saccharidic portion of these molecules is supposed not only to improve the farmacokinetic properties of the drugs, as already proved by other studies,[12] but also might play an important role in the inhibition process as well as the β-lactam moiety. Recently, several carbohydrate-based antibiotics, exploiting various mechanisms of action, have been developed.[13] A significant paradigm is offered by vancomycin (*Figure 6.10*), whose

[11] a) I. Izquierdo, M. T. Plaza, R. Robles, A. J. Mota, *Tetrahedron: Asymmetry* **2000**, *11*, 4509; b) T. B. Durham, M. J. Miller, *Org. Lett.* **2002**, *4*, 135; c) G. I. Georg, E. Akgün, P. M. Mashava, M. Milstead, H. Ping, Z.-J. Wu, D. V. Velde, *Tetrahedron Lett.* **1992**, *33*, 2111; d) B. C. Borer, D. W. Balogh, *Tetrahedron Lett.* **1991**, *32*, 1039; e) A. K. Bose, B. K. Banik, C. Mathur, D. R. Wagle, M. S. Manhas, *Tetrahedron* **2000**, *56*, 5603; f) A. Dondoni, A. Massi, S. Sabbatini, V. Bertolasi, *Adv. Synth. Catal.* **2004**, *346*, 1355.

[12] a) Toth, I. R. A.; Hughes, G.; Dekany, R. S.; Hillery, A. M.; Ward. P. *Liebigs Ann. Chem.* 1994, 685; b) Molina, L.; Gerardin-Charbonnier, C.; Selve, C.; Stebe, M. –J.; Maugras, M.; Infante, M. –R.; Torres, J. –L.; Manresa, M. –A.; Vinardell, P. *New J. Chem.* **1997**, *21*, 1027; c) Shibata, H.; Nagaoka, M.; Takagi, I.; Hashimoto, S. *European Patent* n° WO 2000020009.

[13] Ritter, T. K.; Wong, C. –H. *Angew. Chem. Int. Ed.* **2001**, *40*, 3508.

disaccharide and cyclopeptide moieties proved to separately exert an antibiotic effect by interfering with different processes of the peptidoglycan biosynthesis.[14]
In the light of these data, a favourable synergy is expected by the coupling between carbohydrates and β-lactams, because these novel glycoconjugates might potentially be able to simultaneously lock both transpeptidase (with the β-lactam core) and trans-glycosylase domains (with the saccaridic portion) of resistant bacteria PBPs.

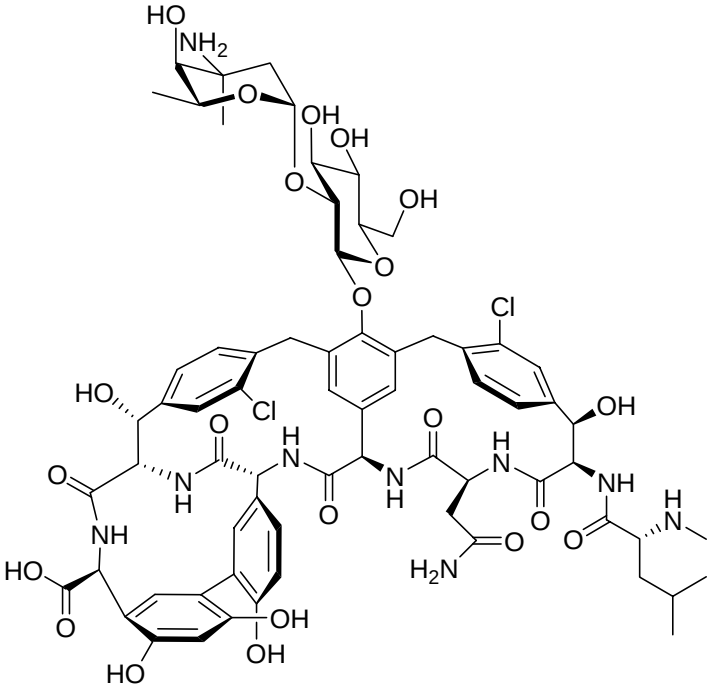
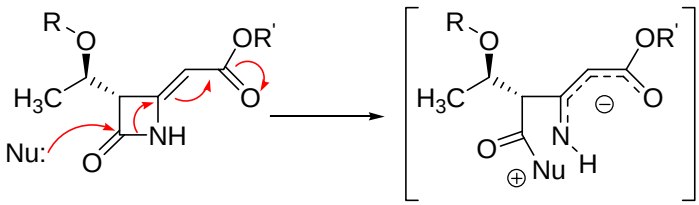


FIGURE ***6.10*** Vancomycin.

Results and Discussion

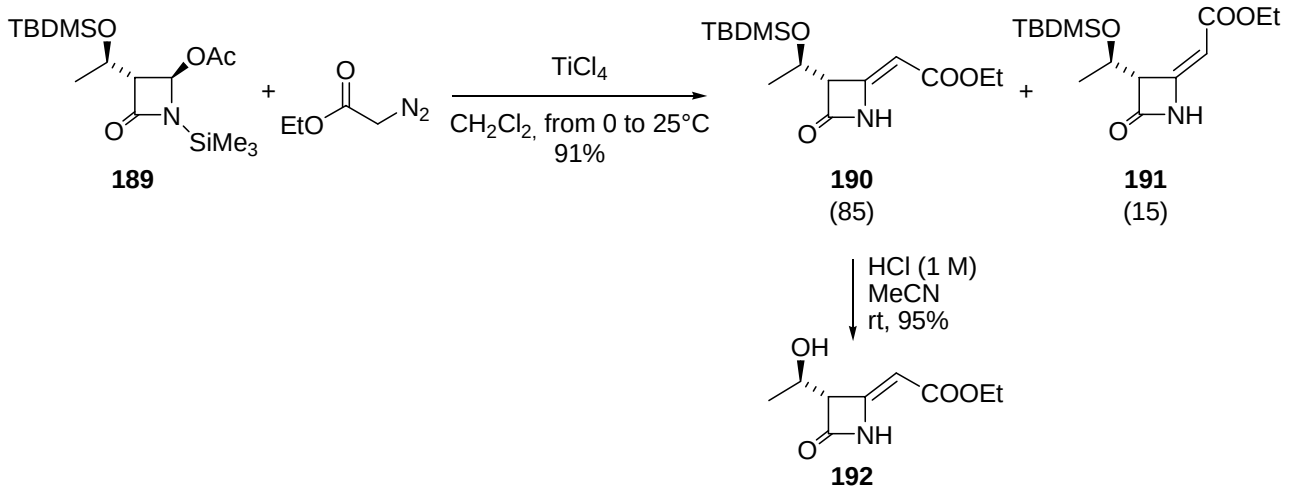
In the first phase of our multidisciplinary project, the effective synthesis of the β-lactam portion has been pursued by Cainelli's group, University of Bologna. 4-Alkylidene-β-lactams were chosen as synthetic target because of encouraging preliminary results registered with some derivatives belonging to this class in the inhibition of serine proteases such as leuckocyte elastase and gelatinase.[15] The promising reactivity of these molecules is strictly related to the conjugation of the exocyclic double bond with the lactam carbonyl group, that therefore exhibits an increased electrophilic character (*Scheme 6.3*).



SCHEME ***6.3*** Effective Delocalization of the Charge after Nucleophilic Attack on 4-Alkylidene-β-Lactams.

[14] Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. *Science* **1999**, *284*, 507.
[15] G. Cainelli, P. Galletti, S. Garbisa, D. Giacomini, L. Sartor, A. Quintavalla, *Bioorg. Med. Chem.* **2003**, 11, 5391.

4-Alkylidene-azetidinone precursors were effectively synthesized by exploiting as the key step the Lewis acid-mediated reaction of 4-acetoxy-azetidinones with α-diazo carbonyls.[16] 4-Alkyliden-azetidin-2-one **192** was obtained in excellent yield by reaction of the *N*-trimethylsilyl derivative of commercially available (3*R*,4*R*)-4-acetoxy-3-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one **189** and ethyldiazoacetate in presence of $TiCl_4$ (*Scheme 6.4*).



SCHEME 6.4 Synthesis of 4-Alkylidene-β-Lactam **192**.

The reaction proceeded smoothly, to yield an 85:15 mixture of the *Z* and *E* isomers **190** and **191**. Critical to the success of the reaction were the stoichiometric amount of $TiCl_4$ and the excess of the diazo-compound associated with the requirement for trimethylsilyl protection of the β-lactam nitrogen atom. The diastereomeric products *E* and *Z* were easily separated by column chromatography, allowing access to stereochemically pure **190**. Treatment of **190** with HCl in acetonitrile produced the deprotected derivative **192**.

The presence of a free hydroxyl in compound **192** and the positive inhibitory effects associated to its functionalization[15,17] prompted us to explore an initial glycoconjugation strategy based on a direct connection mediated by a glicosidic bond.

The sensitivity of **192** toward both acidic and basic conditions posed some concerns in the choice of a suitable glycosidation approach and of the protection pattern for the saccharidic moiety, since both glycosylation and final deprotection steps had to be compatible with the whole structure of the glycosylated β-lactam. As the extreme lability of **192** in mild basic conditions was revealed in preliminary tests,[18] acyl protection for the saccharidic moiety was ruled out. On the other hand, the observed survival of the alkylidene-β-lactams under mild hydrogenation conditions drove our choice towards benzyl groups.[19]

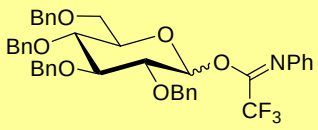
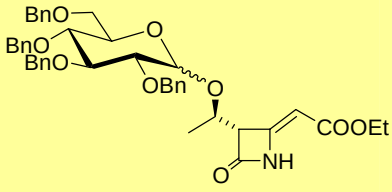
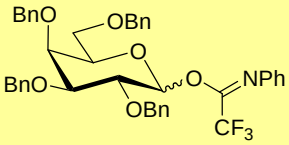
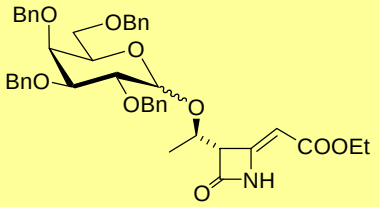
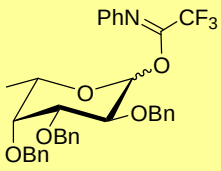
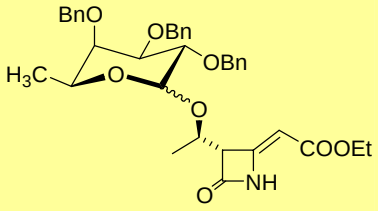
[16] a) G. Cainelli, P. Galletti, M. Gazzano, D. Giacomini, A. Quintavalla, *Tetrahedron Lett.* **2002**, *43*, 233; b) G. Cainelli, D. Giacomini, P. Galletti, A. Quintavalla, *Eur. J. Org. Chem.* **2003**, 1765.

[17] G. Cainelli, P. Galletti, S. Garbisa, D. Giacomini, L. Sartor, A. Quintavalla, *Bioorg. Med. Chem.* **2005**, *13*, 6120.

[18] After 1 minute treatment with K_2CO_3 in MeOH at 0 °C complete degradation of **192** was observed.

[19] 4-Alkylidene-β-lactams are stable towards under pression (7 bar) hydrogenation with Pd on charcoal as the catalyst.

As for the glycosylation approach, the choice was addressed to the catalytic activation of glycosyl *N*-phenyl trifluoroacetimidates[20] with $Yb(OTf)_3$ which had proved compatible with a wide range of functional groups,[21] including extremely acid labile ones.[22] The unviability of using acyl groups also led to the necessity of controlling the stereochemical outcome with a mechanism other than the neighbouring participation that typically operates when 2-O-acylated donors are used. In previous reports, the suitable choice of the solvent was shown to tune the anomeric selectivity of ytterbium(III) triflate-promoted glycosylations with (*N*-phenyl)trifluoroacetimidates (nitrile-type solvents favour β-selectivity, whereas ether mixtures display α-selectivity).[21,23]
For this investigation *N*-phenyl trifluoroacetimidate donors **151**, **162** and **113** (*Table 6.1*) of perbenzylated D-glucose, D-galactose, and L-fucose were prepared as previously reported (treatment of the commercially available hemiacetals in anhydrous dichloromethane with *N*-phenyl trifluoroacetimidoyl chloride and sodium hydride as the base).[21,22] Under these conditions all the donors were obtained with a large predominance of the β-anomer and were used in the ensuing glycosidation steps as anomeric mixtures.

Entry[a]	*Donor*	*Solvent and Temperature [°C]*	*Product*	*Yield [%] (α:β)*
1	 **151**	DME/dioxane 1:1 0 to RT	 **193**	70 (3.9:1)
2	**151**	CH_3CN -15 to RT	**193**	73 (1:2.4)
3	 **162**	DME/dioxane 1:1 0 to RT	 **194**	90 (4.3:1)
4	**162**	CH_3CN -15 to RT	**194**	55 (1:1.4)
5[b]	 **113**	CH_2Cl_2/Et_2O/dioxane 4:1:1 -30 to RT	 **195**	88 (7.4:1)

[a]Conditions: donor (1.3-1.5 eq.), **192** (1 eq.), $Yb(OTf)_3$ (0.1 eq.), AW MS; [b]donor (2.5 eq.), $Yb(OTf)_3$ (0.05 eq.).

TABLE 6.1 $Yb(OTf)_3$-Promoted Glycosylations of 4-Alkylidene-β-Lactam **192**.

[20] B. Yu, H. Tao, *Tetrahedron Lett.* **2001**, *42*, 2405.
[21] M. Adinolfi, G. Barone, A. Iadonisi, M. Schiattarella, *Tetrahedron Lett.* **2002**, *43*, 5573.
[22] M. Adinolfi, A. Iadonisi, M. Schiattarella, *Tetrahedron Lett.* **2003,** *44*, 6479.
[23] a) M. Adinolfi, A. Iadonisi, A. Ravidà, M. Schiattarella, *Tetrahedron Lett.* **2004,** *45*, 4485; b) M. Adinolfi, A. Iadonisi, A. Ravidà, M. Schiattarella, *Synlett* **2004**, 1645; c) M. Adinolfi, A. Iadonisi, A. Ravidà, M. Schiattarella, *J. Org. Chem.* **2005**, *70*, 5316.

In the first attempts the syntheses of α-linked glycosides of glucose and galactose were investigated taking advantage of the α-directing ability of dimethoxyethane/dioxane solvent mixtures.[23a] In both cases, the glycosylations produced the desired glycosides in high yields (70 and 90%, respectively) and satisfying stereocontrol (α/β ≈ 4:1 in both cases). The anomers could be separated by silica-gel chromatography and characterized by NMR spectroscopy, which also clearly indicated the integrity of the alkylidene-β-lactam skeleton. Glycoconjugation of compound **192** was also attempted through a α-L-fucosidation reaction which represents an important task in oligosaccharide synthesis because of the frequent occurrence of α-L-fucosyl residues in important antigen sequences.[24] In the present study the reaction might have allowed the access to glycosyl-conjugated products of reduced polarity. Owing to the high reactivity of fucosyl donors, the glycosylation with **113** could be performed at low temperature. Consistently with previous findings[23b,c] a solvent mixture containing dioxane and diethyl ether led to desired product in very good yield and α-selectivity (88%, α/β ≈ 7.5:1).
β-Selective glycosylations of **192** with donors **151** and **162** were then attempted by catalytic activation with ytterbium(III) triflate in nitrile-type solvents. In both cases, lower yields and selectivities were registered if compared with the results previously obtained with ether solvents (*Table 6.1*, entries 2 and 4). Neverthless, the obtained β-glycosides **193β** and **194β** were easily separated from the corresponding α-anomers by chromatography. It should be noted that acceptors bearing hydroxyl groups in the proximity of rigid cyclic structures appear to be glycosylated in lower yield and β-selectivity when using nitrile solvents.[20,25] The origin of such behaviour will be further explored.
α-Glycosides **193α**, **194α**, and **195α** were deprotected by transfer-hydrogenolysis in methanol/formic acid (9:1) mixture under sonication.[26] Careful control of temperature was found to play a decisive role in obtaining the desired products **196α–198α** in high yields (> 90%) (*Figure 6.12*). Noteworthy the exocyclic double bond on the β-lactam backbone appeared to be completely unaffected by this reaction.

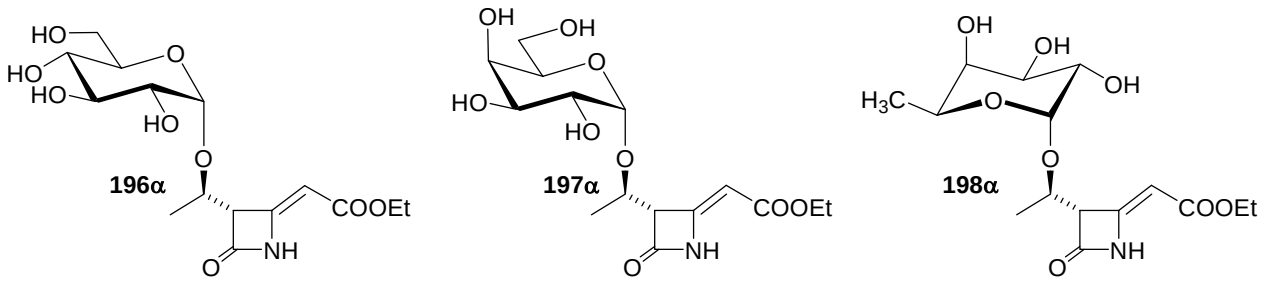


FIGURE 6.11 Deprotected Derivatives **196α-198α**.

A second approach was also followed for the conjugation of alkylidene-β-lactam **192** with the saccharide portion. A different class of glycoconjugated was generated interposing a succinyl spacer between the β-lactam and the carbohydrate moieties. Despite the previous strategy, in this case the conjugation calls for the formation of an ester linkage. Derivatives **199** and **200** represented our synthetic targets (*Figure 6.12*). The choice of C-3 position for

[24] See *Chapter 3*.
[25] R. R. Schmidt, M. Behrendt, M. Toepfer, *Synlett* **1990**, 694.
[26] V. S. Rao, A. S. Perlin, *Carbohydr. Res.* **1980**, *83*, 175.

the linker installation and therefore β-lactam conjugation is intended to mimic the natural PBPs substrates. In fact, the pentapeptide chain terminating with the D-Ala-D-Ala residue naturally occurs on C-3 position of MurNAc units. Moreover, in order to further improve the biomimetic feature of these glycoconjugates, the use of a glucosamine residue was also investigated.

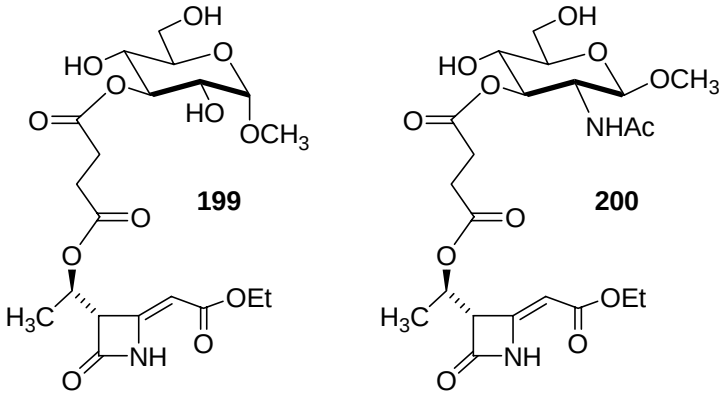
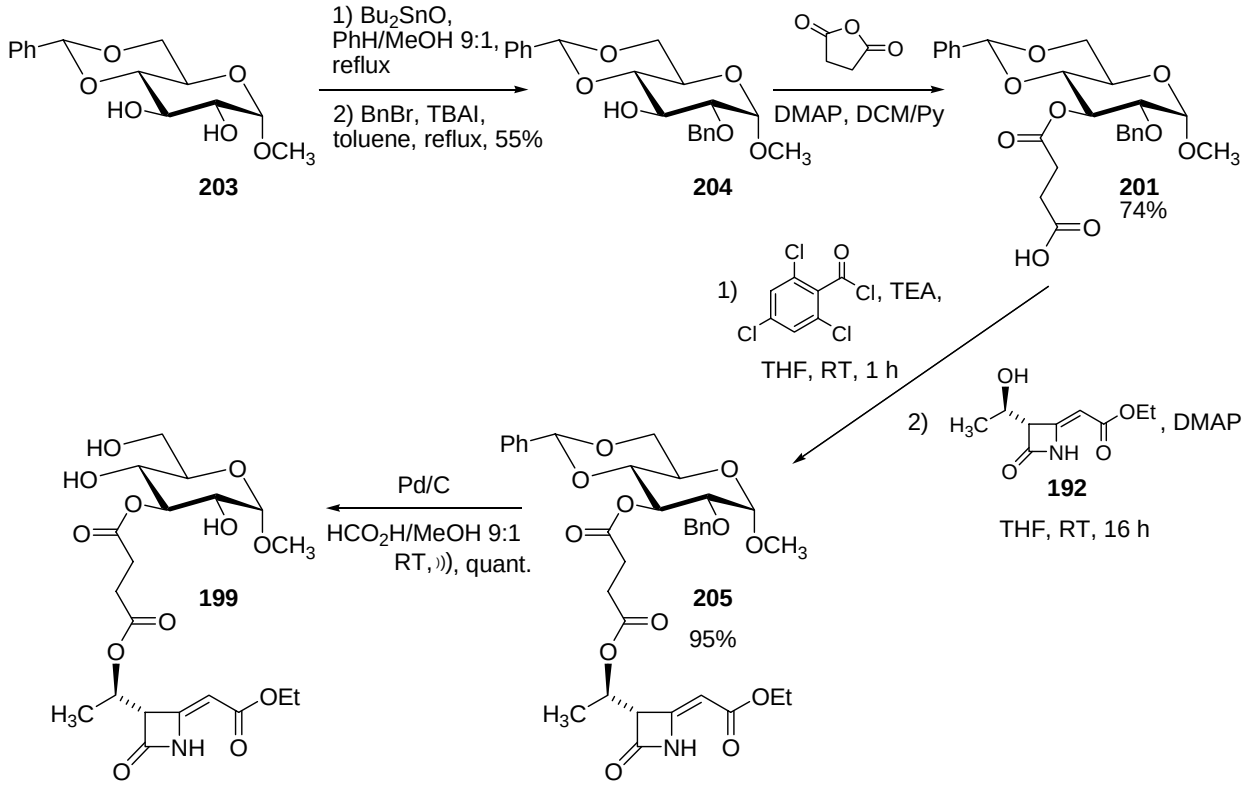


FIGURE 6.12 Synthetic Targets.

Therefore, suitably protected succinyl derivatives **201** and **202** were synthesized and effectively conjugated with 4-alkyliden-β-lactam **192 (***Schemes 6.5* and *6.6*).
Derivative **201** was prepared using as starting material the commercially available 4,6-benzylidene-protected methyl glucoside **203** (*Scheme 6.5*). Derivative **203** was regioselectively benzylated at C-2 position by a one-pot procedure taking advantage of the formation of a temporary stannilidene acetal as intermediate. The benzylation proceeded with a good overall yield (92%), but the regiomeric mixture of products furnished the desired regioisomer in 55% yield.
Monobenzylated derivative **204** was then treated with succinic anhydride and base to yield **201** in excellent yield. Several approaches have been followed for effectively achieving the conjugation with the β-lactam moiety. Activation of carboxylic function of derivative **201** proved to be not trivial. Low yields and/or reaction rates were registered exploiting classical procedures for example using carbodimides (DCC and DPC) or generating the acyl chloride intermediate by the use of Ph_3P/CH_3CN system.[27] Excellent results were instead obtained using the Yamaguchi protocol, widely used to perform macrolactonization reactions.[28] The mixed anhydride intermediate, formed by treating the succinyl group with 2,4,6-trichlorobenzoyl chloride, effectively reacted with nucleophile **192** in the presence of dimethylaminopyridine (DMAP) furnishing glycoconjugate **205** in excellent 95% yield. As previously described, block deprotection was perfomed by Perlin hydrogenolisis[26] and desired derivative **199** was isolated in good yield. Noteworthy, also in this case the chioice of protecting groups was driven by the stability of the β-lactam ring and the mild deprotection step allowed the effective cleavage of both benzyl group and benzylidene acetal without affecting the labile azetidinone moiety.

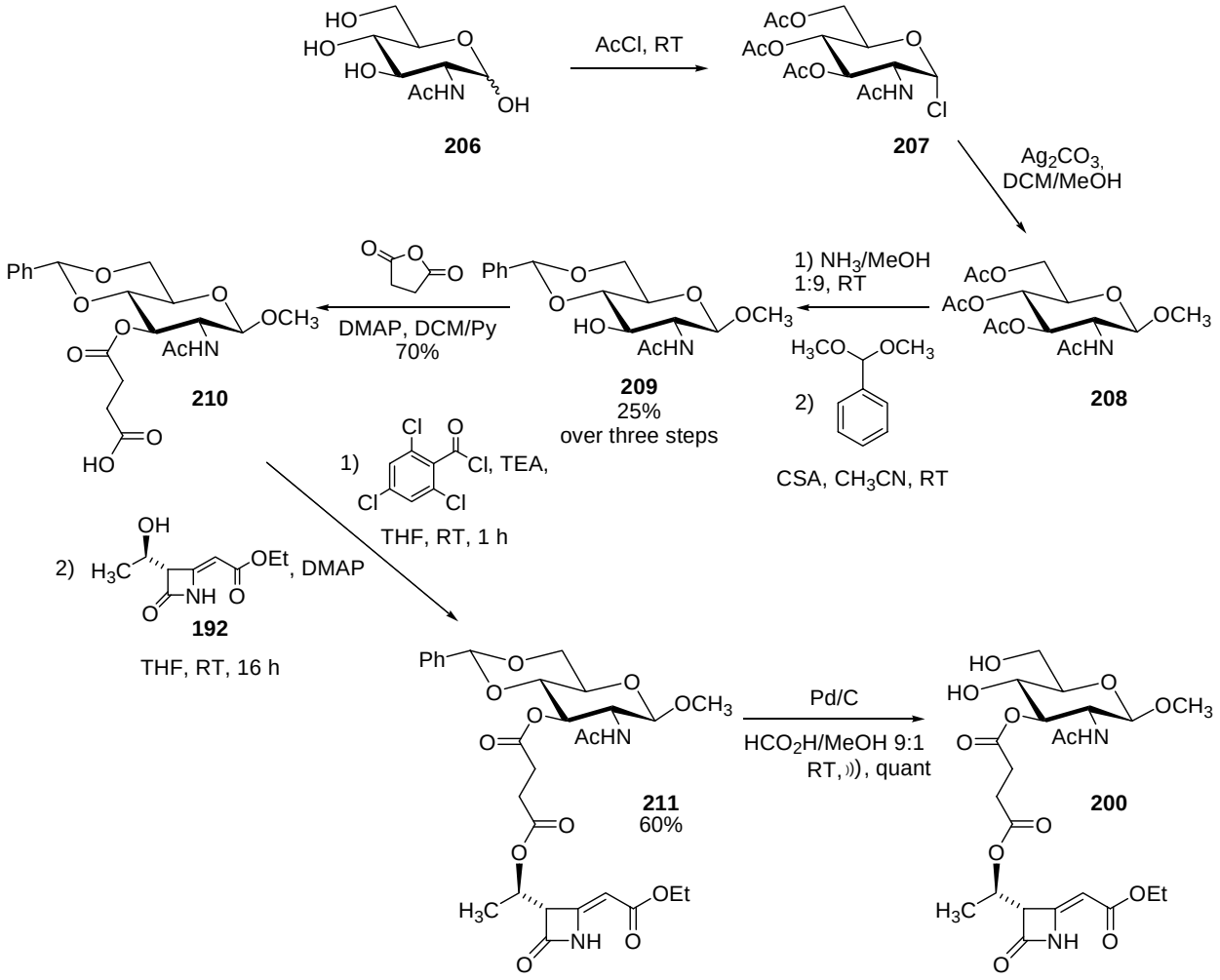
[27] Jang, D. O.; Park, D. J.; Kim, J. *Tetrahedron Lett.* **1999,** *40*, 5323.
[28] Inanaga, J. ; Hirata, K. ; Saeki, H. ; Katsuki, T. ; Yamaguchi, M. *Bull. Chem. Soc. Jap.* **1979**, *52*, 1989.



SCHEME 6.5 Synthesis of Derivative **199**.

The synthetic strategy used for the obtainment of glucosamine derivative **200** is very similar to that just described for glucose derivative **199**. As evident from *Scheme 6.6*, a further manipulation was required to convert *N*-acetyl glucosamine **206** into derivative **209** that was then subjected to the succinylation-conjugation-hydrogenation sequence already performed on glucose.

Treatment of commercially available *N*-acetyl glucosamine **206** with an excess of acetyl chloride allowed the straightforward installation of a chlorine atom at the anomeric position and the simultaneous acetylation of the other hydroxyl groups. Glucosamine derivative **207** was then glycosidated by methanol exerting the silver-promoted activation of the anomeric chloride. Conversion of **208** into benzilidene protected **209** was accomplished by cleavage of ester acetyls by ammonia treatment and ensuing installation of the acetal protection catalytic acidic conditions. Noteworthy, here Yamaguchi esterification protocol furnished the desired glycoconjugate **211** in lower yield than that registered in the case of glucose derivative. However, derived **211** was subjected to Perlin hydrolysis yielding the final target **200**.

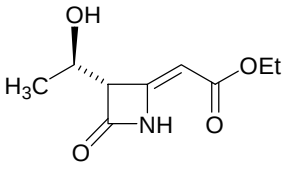


SCHEME 6.6 Synthesis of Derivative **200**.

The small library of β-lactam-glycoconjugates thus synthesized (*Figure 6.13*) was tested by Milanese group of prof. Cocuzza for evaluating the activity against more than 100 Gram-positive and Gram-negative bacteria, including antibiotic resistant strains.
Unfortunately, the β-lactam **192** itself, despite the positive preliminary tests on leukocyte elastase and gelatinase, proved to be ineffective as antibiotic, exhibiting in all cases Minumum Inhibition Concentration (MIC) values never lower than 128 mg/L. Results as bad as in the case of the sole **192** were registered for glycosides **196α-198α** directly linked to the β-lactam moiety. On the contrary, promising results were obtained using the second class of glycoconjugates, especially in the case of glucosamine derivative **200**. This latter showed antibiotic activity against several antibiotic resistant bacteria strains. The MIC values observed are still higher than those exhibited by the commercially available antibiotics used as control (such as Imipenem, Meropenem), but validate the principle that drove our investigation. Conjugation with glucosamine and interposition of the succinyl spacer drastically modified the antibiotic activity of 4-alkylidene-β-lactam **192**. Furthermore, the conjugation through the linker seems to positively influence the antimicrobial activity in a more specific fashion than the generic improvement of drug solubility and/or stability. This observation is prompted by the different behaviour exhibited by the two classes of glycoconjugates. Both classes of molecules are supposed to have similar properties in terms of stability and solubility, but the second, especially in the

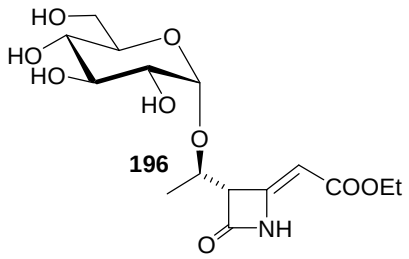
case of glucosamine derivative, is characterized by a far higher similarity to the natural substrates of the target protein, thus suggesting a specific involvement of the saccharidic portion in the overall antibiotic action. Further studies are required to verify these hypotheses, that, for the moment, lie over on a speculative level.

From an applicative point of view, novel β-lactam structures have been designed and synthesized by Cainelli and coworkers with the support of the chemoinformatic means of prof. Musumarra group of University of Catania. The molecules exhibiting the best antibiotic activities will be subjected to conjugation. A second generation of glycoconjugates will be prepared, exploiting the already performed interposition of a spacer between the β-lactam and the saccharide portions. Furthermore more bio-mimetic structures will be synthesized using glucosamine di- and oligo-saccharides and also varying the nature and the length of the spacer.



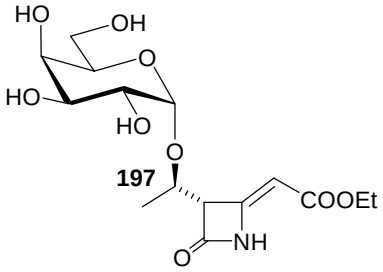
192

MIC: >128 mg/L



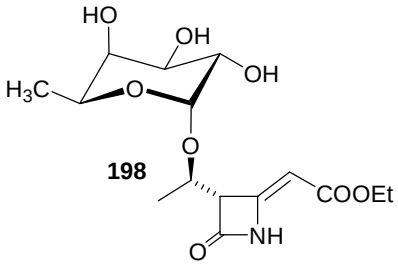
196

MIC: >128 mg/L



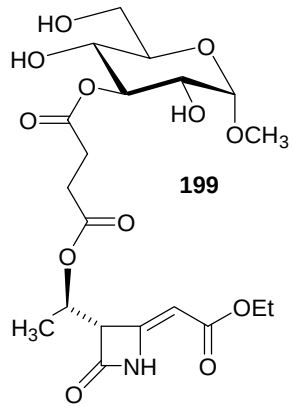
197

MIC: >128 mg/L



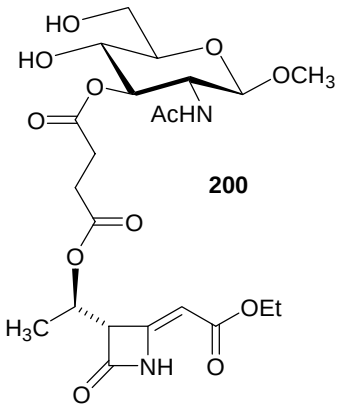
198

MIC: >128 mg/L



199

S. pyogenes EryR 6 (RC) MIC: 64 mg/L
S. pneumoniae PEN S (Lecco 48) MIC: 64 mg/L



200

S. pyogenes EryR 6 (RC) MIC: 64 mg/L
S. pneumoniae PEN S (Lecco 48) MIC: 64 mg/L
MRSA A931 MIC: 64 mg/L
S. epidermidis MR MZ31 MIC: 16 mg/L
S. epidermidis C416 MIC: 32 mg/L
S. epidermidis MR C440 MIC: 32 mg/L

FIGURE 6.13 Glycoconjugates Tested for Antibiotic Activity.

Conclusion

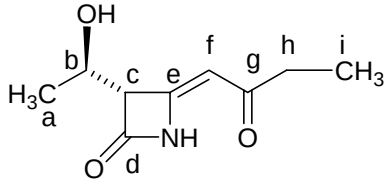
In conclusion, two class of novel β-lactam-glycoconjugates were synthesized. The extreme sensitivity of the 4-alkylidene-β-lactam **192** called for the exploitation of very mild protocols for performing glycosylation and deprotection reactions. In the first class of glycoconjugates the generation of a direct linkage between the β-lactam and the saccharidic moieties was performed. The glycosylation of the sensitive β-lactam acceptor **192** exploited the mild activation of *N*-phenyl trifluoroacetimidate donors with ytterbium(III) triflate. Satisfying yields were obtained in the synthesis of both α- and β-linked glycosides, the best results being registered in the former case. In the second approach a succinyl spacer was interposed between the two cyclic molecules. The conjugation in this case was performed by an esterification reaction, effectively accomplished exerting the Yamaguki protocol. The thus obtained glycoconjugates underwent hydrogenolytic deprotection of the saccharidic portions without the β-lactam moiety being affected. Evaluation of these unprecedented derivatives for antibiotic activity against resistant bacteria furnished promising results for the second type of glycoconjugates. Further studies are in progress. New β-lactam structures have been screened and will be conjugated to suitable saccharidic structures generating a second generation of antibiotic β-lactam-glycoconjugates.

Experimental Section

General Methods and Material

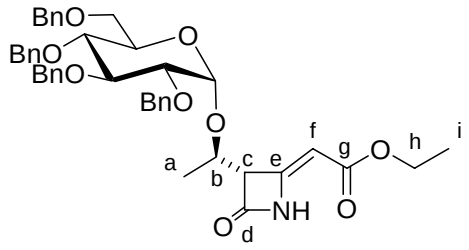
Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian spectrometers at 200 or 300 MHz and 50 or 75 MHz, respectively; or on a Bruker Avance 500 spectrometer at 500 MHz. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Analytical thin layer chromatography (TLC) was performed on E. Merck pre-coated (25 nm) silica gel 60F-254 plates. Column chromatography was preformed using Carlo Erba 0.06-0.20 silica gel. Mass spectra were recorded in a reflection positive mode on a MALDI-TOF spectrometer. Acid washed molecular sieves and Ytterbium(III) triflate were dried by overnight heating at 200 °C under vacuum before their use in glycosidations. Anhydrous solvents stored over molecular sieves (purchased from Aldrich) were used for performing the reactions.

Starting materials: Compounds **190**, **191**, and **192** were prepared accordingly to reference 16. *N*-Phenyl trifluoroacetimidates **151**, **162**, and **113** were prepared from the corresponding commercially available hemiacetals as reported in references 20 and 22.

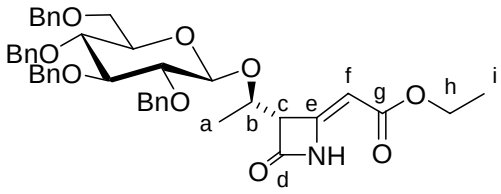


192. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.37 (1H, bs, N-H, H_f), 5.27 (1H, s, H_e), 4.20 (3H, m, H_b e 2xH_g), 3.74 (1H, d, J_{bd} = 5.7 Hz, H_d), 2.13 (1H, bs, H_c), 1.39 (3H, d, J_{ab} = 6.6 Hz, H_a), 1.29 (3H, t, J_{gh} = 7.2 Hz, H_h).^{13}C NMR ($CDCl_3$, 50 MHz) significative signals at δ 166.9 and 166.2 (NH<u>C</u>=O, C=C-<u>C</u>=O), 152.2 (<u>C</u>=C-C=O), 90.8 (C=<u>C</u>-C=O), 64.8, 64.0, 60.2 (C_b, C_d, C_g), 21.4 and 14.3 (C_a, C_h).

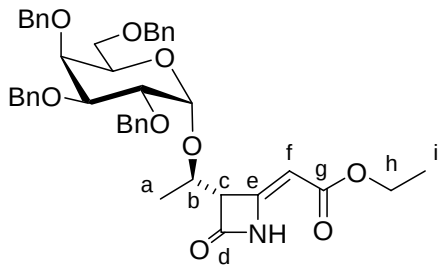
General procedure for glycosylations: A mixture of **192** (0.10 mmol) and the glycosyl donor (0.14 mmol) was dissolved under argon in anhydrous 1,2-dimethoxyethane (0.75 mL) and dioxane (1 mL) in the presence of freshly activated 4Å acid washed molecular sieves (AW 300 MS). A solution of $Yb(OTf)_3$ (6.2 mg, 0.01 mmol) in DME (0.25 mL) was then added at 0 °C. The mixture was allowed to gradually warm to room temperature. After consumption of the donor (TLC analysis, generally ca 5 hours) a few drops of pyridine were added and the mixture was filtered on a short plug of silica gel eluted with dichloromethane/methanol 9:1. The residue was then purified on a short silica gel column eluted with toluene/ethyl acetate mixtures.



193α. (40 mg, yield 56%) $[\alpha]_D$ +40.3 (c 1, CH_2Cl_2). ^1H NMR (200 MHz, $CDCl_3$): δ = 8.39 (bs, 1H, NH), 7.40–7.05 (aromatic protons), 5.55 (bs, 1H, –C=C*H*–CO_2Et), 4.96 (d, $J_{1,2}$ = 3.6 Hz, 1H, 1-H Glc), 5.00–4.42 (4 × AB, 8H, 4 × CH_2 benzyl protons), 4.13 (q, *J* = 7.0 Hz, 2H, –O*C*H_2CH$_3$), 4.00 (m, 1H, CH_3–C*H*(OGlc)CH–), 3.96 (t, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, 1H, 3-H Glc), 3.88 (m, 1H, 5-H Glc), 3.75 (bd, *J* = 7.0 Hz, 1H, CH_3CH(OGlc)C*H*–), 3.75–3.55 (overlapped signals, 3H, 4-H and CH_2-6 Glc), 3.51 (dd, 1H, 2-H Glc), 1.42 (d, *J* = 6.4 Hz, 3H, C*H*$_3$CH(OGlc)CH–), 1.21 (t, *J* = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 167.3, 165.6, 152.3, 138.8, 138.3, 138.2, 138.0, 128.4–127.7, 98.7 (C-1 Glc), 91.4, 81.7, 79.5, 75.5, 75.1, 73.8, 73.5, 73.1, 70.9, 68.4, 62.6, 60.0, 17.0, 14.3. MALDI-TOF MS: for $C_{43}H_{47}NO_9$ (721.3) m/z = 744.4 $[M+Na]^+$.

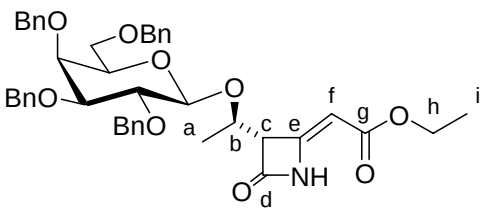


193β. (37 mg, yield 51%) ^1H NMR (200 MHz, $CDCl_3$): δ = 8.45 (bs, 1H, NH), 7.40–7.05 (aromatic protons), 5.31 (bs, 1H, –C=CH–CO_2Et), 4.95–4.52 (4 × AB, 8H, 4× CH_2 benzyl protons), 4.47 (d, $J_{1,2}$ = 7.6 Hz, 1H, 1-H Glc), 4.31 (quintuplet, *J* = 6.2 Hz, 1H, CH_3C*H*(OGlc)CH–), 4.20–4.05 (m, 2H, –O*C*H_2CH$_3$), 3.87 (bd, *J* = 5.8 Hz, 1H, CH_3CH(OGlc)C*H*–), 3.80–3.53 (overlapped signals, 4H, 3-H, 4-H and CH_2-6 Glc), 3.50–3.38 (overlapped signals, 2H, 2-H and 5-H Glc), 1.37 (d, *J* = 6.4 Hz, 3H, C*H*$_3$CH(OGlc)CH–), 1.22 (t, *J* = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 167.2, 165.5, 152.5, 138.6, 138.4, 138.3, 138.1, 128.4–127.7, 100.6 (C-1 Glc), 90.7, 84.7, 81.9, 75.3, 75.1, 75.0, 73.5, 70.1, 69.0, 62.8, 60.1, 17.5, 14.2. MALDI-TOF MS: for $C_{43}H_{47}NO_9$ (721.3) m/z = 744.3 $[M+Na]^+$.

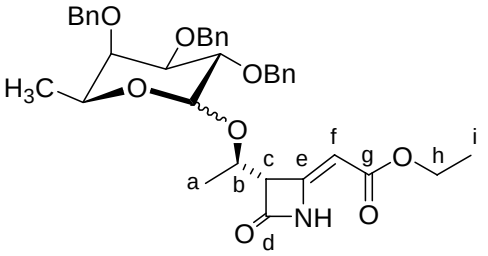


194α. (58 mg, yield 81%) $[\alpha]_D$ +54.9 (c 1, CH_2Cl_2). ^1H NMR (200 MHz, $CDCl_3$): δ = 8.37 (bs, 1H, NH), 7.40–7.20 (aromatic protons), 5.57 (bs, 1H, –C=C*H*–CO_2Et), 4.98 (d, $J_{1,2}$ = 3.6 Hz, 1H, 1-H Glc), 4.95–4.36 (4 × AB, 8H, 4 × CH_2 benzyl protons), 4.14 (q, *J* = 7.2 Hz, 2H, –O*C*H_2CH$_3$), 4.04–3.95 (overlapped signals, 4H, 2-H, 4-H, 5-H Gal, and CH_3C*H*(OGal)CH–), 3.75 (bd, *J* = 8.0 Hz, 1H, CH_3CH(OGal)C*H*–), 3.56–3.42 (m, 2H, CH_2-6 Gal), 1.41 (d, *J* = 6.2 Hz, 3H, C*H*$_3$CH(OGal)CH–), 1.23 (t, *J* = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 167.3, 165.6, 152.5, 138.6, 138.5, 137.9, 128.4–127.4, 99.2 (C-1 Gal), 91.3,

79.0, 75.6, 74.9, 74.7, 73.5, 73.4, 72.7, 69.9, 69.0, 62.7, 60.0, 20.2, 14.3. MALDI-TOF MS: for $C_{43}H_{47}NO_9$ (721.3) m/z = 744.4 $[M+Na]^+$.



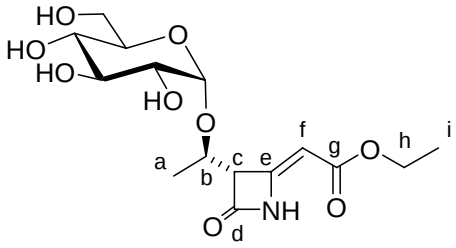
194β. (23 mg, yield 32%) ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.41 (bs, 1H, NH), 7.40–7.20 (aromatic protons), 5.31 (bs, 1H, –C=CH–CO_2Et), 4.97–4.36 (4 × AB, 8H, 4 × CH_2 benzyl protons), 4.42 (d, $J_{1,2}$ = 7.8 Hz, 1H, 1-H Gal), 4.25 (quintuplet, J = 6.4 Hz, 1H, CH_3CH(OGal)CH–), 4.15–4.10 (m, 2H, –OCH_2CH_3), 3.87–3.84 (overlapped signals, 2H, 4-H Gal and CH_3CH(OGal)C*H*–), 3.78 (dd, $J_{2,3}$ = 9.6 Hz, 1H, 2-H Gal), 3.60–3.48 (overlapped signals, 4H, 3-H, 5-H, and CH_2-6 Gal), 1.34 (d, J = 7.2 Hz, 3H, CH_3CH(OGal)CH–), 1.23 (t, J = 7.2 Hz, 3H, –OCH_2CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 167.2, 165.5, 152.4, 138.7, 138.6, 138.5, 138.0, 128.4–127.5, 101.2 (C-1 Gal), 90.8, 82.2, 79.3, 77.1, 75.2, 74.5, 73.7, 73.5, 73.1, 70.5, 68.9, 62.8, 60.0, 17.4, 14.3. MALDI-TOF MS: for $C_{43}H_{47}NO_9$ (721.3) m/z = 744.3 $[M+Na]^+$.



195α. (48 mg, yield 78%) $[\alpha]_D$ –94.6 (c 1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.43 (bs, 1H, NH), 7.40–7.20 (aromatic protons), 5.21 (bs, 1H, –C=C*H*–CO_2Et), 4.95 (d, $J_{1,2}$ = 3.8 Hz, 1H, 1-H Fuc), 5.00–4.59 (3 × AB, 6H, 3 × CH_2 benzyl protons), 4.18 (q, J = 7.2 Hz, 2H, –OCH_2CH_3), 4.15–4.05 (overlapped signals, 2H, 2-H Fuc and CH_3CH(OFuc)CH–), 3.93 (bq, $J_{5,6}$ = 6.6 Hz, 1H, 5-H Fuc), 3.90–3.85 (overlapped signals, 2H, 3-H Fuc and CH_3CH(OFuc)C*H*–), 3.66 (bd, $J_{3,4}$ = 2.4 Hz, 1H, 4-H Fuc), 1.32 (d, J = 6.2 Hz, 3H, CH_3CH(OFuc)CH–), 1.29 (t, 3H, –OCH_2CH_3), 1.10 (d, 3H, CH_3-6 Fuc). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 167.0, 165.8, 152.6, 138.8, 138.6, 128.5–127.5, 94.8 (C-1 Fuc), 90.7, 78.9, 76.1, 74.8, 73.1, 72.9, 68.5, 67.0, 62.7, 60.1, 16.8, 16.6, 14.3. MALDI-TOF MS: for $C_{36}H_{41}NO_8$ (615.3) m/z = 638.3 $[M+Na]^+$.

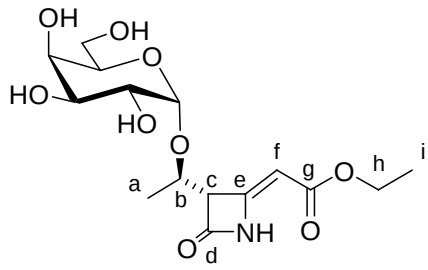
General debenzylation procedure

35 mg of Pd/C 10% were suspended in methanol/formic acid 9:1 (500 μL) under argon atmosphere, the glycoconjugated was then admixed as solution in the same solvent mixture (1 mL) under argon atmosphere. The reaction mixture was sonicated keeping the temperature ≤ 15°C. After reaction completion (TLC analysis), the mixture was filtered on Celite and the resulting mixture was eluted on mixed-bed ionic exchange resin DOWEX MR-3 or purified by preparative thin layer chromatography.

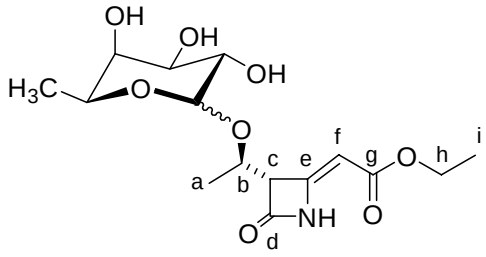


196α. (13 mg, yield 92%) $[\alpha]_D$ +49.6 (c 1, CH_3OH).^{1}H NMR (200 MHz, D_2O): δ = 5.40 (bs, 1H, –C=C*H*–CO_2Et), 5.04 (d, $J_{1,2}$ = 3.8 Hz, 1H, 1-H Glc), 4.26 (m, 1H, CH_3CH(OGlc)CH–), 4.21 (q, J = 7.2 Hz, 2H, –OCH_2CH_3), 4.07 (d, J = 5.4 Hz, 1H, CH_3CH(OGlc)C*H*–), 3.87–3.62 (overlapped signals, 4H, 3-H, 5-H and CH_2-6 Glc), 3.49 (dd, $J_{2,3}$ = 10.0 Hz, 1H, 2-H Glc), 3.37 (t, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, 1H, 4-H Glc), 1.38 (d, J = 6.4 Hz, 3H,

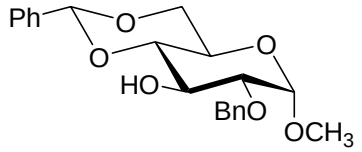
C*H*$_3$CH(OGlc)CH–), 1.27 (t, 3H, –OCH$_2$C*H*$_3$). ^{13}C NMR (50 MHz, D$_2$O): δ = 167.7, 167.7, 150.4, 96.5 (C-1 Glc), 89.1, 70.6, 69.9, 69.6, 69.2, 67.4, 59.2, 59.0, 58.3, 16.2, 11.3. MALDI-TOF MS: for $C_{15}H_{23}NO_9$ (361.2) m/z = 384.3 $[M+Na]^+$.



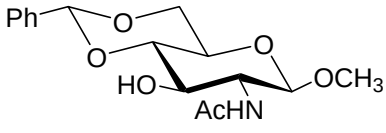
197α. (15 mg, yield 94%) $[\alpha]_D$ +60.7 (c 1, CH$_3$OH). ^{1}H NMR (200 MHz, D$_2$O): δ = 5.38 (bs, 1H, –C=C*H*–CO$_2$Et), 5.03 (d, $J_{1,2}$ = 3.2 Hz, 1H, 1-H Glc), 4.23 (m, 1H, CH$_3$C*H*(OGal)CH–), 4.17 (q, *J* = 7.0 Hz, 2H, –OC*H*$_2$CH$_3$), 4.12–3.90 (overlapped signals, 4H, 3-H, 4-H, 5-H Gal and CH$_3$CH(OGal)C*H*–), 3.80–3.65 (overlapped signals, 3H, 2-H, and CH$_2$-6 Gal), 1.36 (d, *J* = 6.4 Hz, 3H, C*H*$_3$CH(OGlc)CH–), 1.24 (t, *J* = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$). ^{13}C NMR (50 MHz, D$_2$O) δ 168.0, 166.3, 150.5, 96.9 (C-1 Gal), 89.2, 69.5, 69.0, 67.1, 66.2, 59.4, 59.1, 16.2, 13.4. MALDI-TOF MS: for $C_{15}H_{23}NO_9$ (361.2) m/z = 384.2 $[M+Na]^+$.



198α. (15 mg, yield 91%) ^{1}H NMR (500 MHz, D$_2$O): δ = 5.38 (s, 1H, –C=CH–CO$_2$Et), 5.02 (d, $J_{1,2}$ = 3.5 Hz, 1H, 1-H Fuc), 4.25 (q, *J* = 7.0 Hz, 2H, –OC*H*$_2$CH$_3$), 4.12 (d, *J* = 7.0 Hz, 1H, CH$_3$CH(OFuc)C*H*–), 4.05 (bd, $J_{5,6}$ = 6.5 Hz, 1H, 5-H Fuc), 3.85–3.75 (overlapped signals, 3H, 2-H, 3-H and 4-H Fuc), 1.35 (d, *J* = 6.0 Hz, 3H, C*H*$_3$CH(OFuc)CH–), 1.31 (t, 3H, –OCH$_2$C*H*$_3$), 1.21 (3H, CH$_3$-6 Fuc). ^{13}C NMR (50 MHz, D$_2$O): δ = 168.4, 166.2, 150.4, 94.1 (C-1 Fuc), 90.7, 89.2, 69.7, 68.7, 67.4, 66.8, 65.6, 65.0, 59.7, 59.1, 14.0, 13.1, 11.4. MALDI-TOF MS: for $C_{15}H_{23}NO_8$ (345.1) m/z = 368.2 $[M+Na]^+$.



204. Diol **203** (846 mg, 3 mmol) and dibutyltinoxide (846 mg, 3 mmol) were suspended in a benzene/methanol 10:1 mixture (22 mL). The system was refluxed and after three hours the resulting mixture was concentrated and coevaporated three times with dry toluene. To the crude were added sequentially dry toluene (15 mL), benzyl bromide (2 mL) and tetrabutylammonium iodide (1.1g, 3 mmol). The reaction mixture was stirred at 90 °C overnight and then purified on silica gel by column chromatography (petrol ether/ethyl acetate 85:15). The purification yielded **204** (614 mg, 55%) together with a smaller amount of the other monobenzylated regioisomer (412 mg, 37%).^{1}H NMR ($CDCl_3$ 200 MHz) significative signals at δ 5.10 (1H, s, CH benzylidene), 4.75 (2H, AB, J_{gem} =12.4 Hz, CH_2 benzyl), 4.62 (1H, d, $J_{1,2}$ =3.8 Hz, H-1), 4.26 (1H, dd, $J_{5,6eq}$ =3.2 Hz, $J_{6ax,6eq}$ =10.2 Hz, H-6 eq.), 4.16 (1H, t, $J_{2,3}$ = $J_{3,4}$=10.2 Hz, H-3), 3.81 (1H, td, $J_{4,5}$= $J_{5,6ax}$ =10.2 Hz, H-5), 3.70 (1H, t, H-4), 3.48 (2H, m, H-2 e H-6 ax.), 3.38 (3H, s, OCH_3).

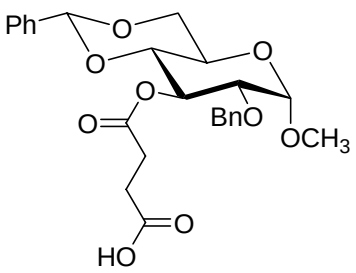


209. Derivative **207** (774 mg, 2.1 mmol) was dissolved into a dichloromethane/methanol 2:1 mixture (7.7 mL). To this solution freshly activate 4Å molecular sieves and Ag_2CO_3 (583 mg, 2.1 mmol) were admixed at room temperature under argon atmosphere. The reaction mixture was stirred overnight at room temperature and then filtered on a pad of Celite. The crude was then treated with a methanol/ammonia 9:1 mixture (8mL). After 6 hours stirring at room temperature the

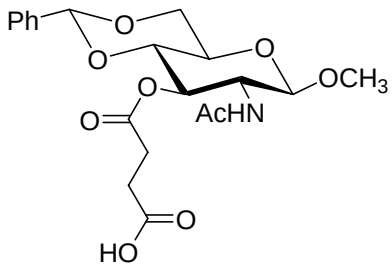
mixture was concentrated under reduced pressure and the residue was coevaporated three times with dry toluene. 4Å Molecular sieves, camphorsulfonic acid (43 mg, 0.19 mmol) and dry acetonitrile were added under argon atmosphere. After 5 minutes stirring, dimethoxytoluene (411 µL, 2.7 mmoli) was admixed and the mixture was stirred at room temperature for 12 hours. The resulting mixture was diluted with dichloromethane and washed with $NaHCO_3$ aq. The organic phase was purified by column chromatography (dichloromethane/methanol from 98:2 to 95:5) that yielded the desired **209** (170 mg, 25% yield over three steps). ^{1}H NMR (200 MHz, $CDCl_3$) significative signals at δ 5.95 (1H, bd, $J_{2,NH}$ =8.2 Hz, NH), 5.56 (1H, s, H benzylidene), 4.64 (1H d, $J_{1,2}$ =8.0 Hz, H-1), 4.36 (1H, dd, $J_{6ax,6eq}$ =10.4 Hz, $J_{5,6eq}$ =4.8 Hz, H-6 eq.), 4.16 (1H, t, $J_{3,4}$ =9.6 Hz, H-3), 3.79 (1H, t, H-6 ax.), 3.60-3.47 (2H, m, H-2 e H-5), 3.53 (3H, s, -OCH_3), 2.06 (3H, s, $C\underline{H}_3CONH$).

General succinylation procedure

The saccaridi derivative (0.34 mmol) and succinic anhydride (52 mg, 0.52 mmol) were dissolved in a dichloromethane/pyridine 1:1 mixture (2mL). To the mixture DMAP (67 mg, 0.55 mmol) was added under argon atmosphere. After stirring overnight at room temperature the mixture was condentrated under reduced pressure and purified on siliga gel by column chromatography (dichloromethane/methanol mixtures).



201. ^{1}H NMR (200 MHz, $CDCl_3$) significative signals at δ 7.23-7.10 (H aromatics), 5.57 (1H, t, $J_{3,4}$ =9.8 Hz, H-3), 5.45 (1H, s, H benzylidene), 4.64 (2H, AB, J_{gem}= 12.4 Hz, CH_2 benzyl), 4.65 (1H, d, $J_{1,2}$ = 3.2 Hz, H-1), 4.26 (1H, dd, $J_{6ax,6eq}$ = 9.6 Hz, $J_{5,6eq}$ =4.4 Hz, H-6 eq.), 3.88 (1H, td, $J_{4,5}$=$J_{5,6ax}$ =9.6 Hz, H-5), 3.69 (1H, t, H-6 ax.), 3.59 (1H, dd, H-2), 3.54 (1H, t, H-4), 3.45 (3H, s, OCH_3), 2.62 (4H, s, 2xCH_2 succinate). ^{13}C NMR ($CDCl_3$, 50 MHz) significative signals at δ 175.9, 171.1 (CO succinate), 138.4, 137.8 (C aromatics), 128.9, 128.5, 128.2, 128.0, 127.9, 126.1 (CH aromatics), 101.3 (CH del benzylidene), 98.8 (C anomeric), 55.4 (CH_3O), 29.0, 28.9 (CH_2 succinate). Other signals at δ 79.4, 77.2, 73.1, 70.9, 68.9, 62.3.

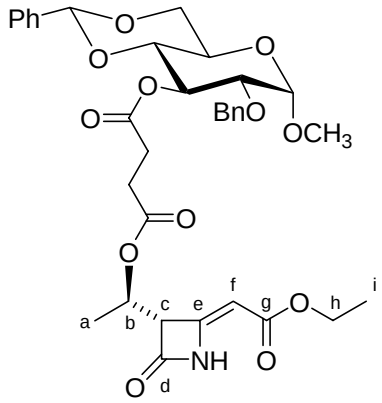


210. ^{1}H NMR (200 MHz, $CDCl_3$) significative signals at δ 7.30-7.50 (5H, m, H aromatics), 5.57 (1H, s, H benzylidene), 5.27 (1H, t, $J_{2,3}$= 9.8 Hz, H-3), 4.54 (1H, d, $J_{1,2}$= 8.4 Hz, H-1), 4.31 (1H, dd, $J_{5,6eq}$ =5.2 Hz, $J_{6ax,6eq}$ =10.4 Hz, H-6 eq.), 3.93 (1H, dd, H-2), 3.83 (1H, t, $J_{5,6ax}$ = 10.4 Hz, H-6 ax.), 3.75 (1H, t, $J_{4,5}$ =9.4 Hz, H-4), 3.52 (1H, td, H-5), 3.46 (3H, s, OCH_3), 2.58 (4H, s, 2 x CH_2 succinate), 1.92 (3H, s, CH_3CONH).

General Yamaguki esterification

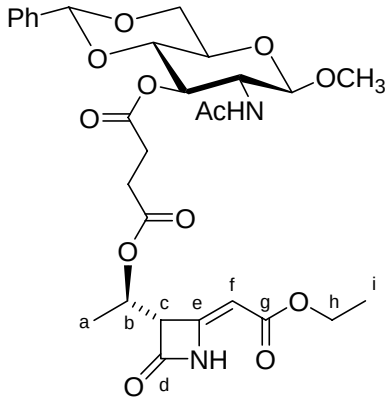
Derivative **201** (14 mg, 0.03 mmol) was coevaporated three times with dry toluene and then dissolved in 600 µL of dry THF under argon atmosphere. To this solution 4Å molecular sives were added followed by triethylamine (10 µL, 0.08 mmol) and 2,4,6-trichlorobenzoyl chloride (5 µL, 0.03 mmol). After two hours stirring, derivative **192** was added as THF solution (500 µL) and followed by DMAP (9 mg, 0.075 mmol). After reaction completion (overnight at room temperature) the mixture was purified on silica gel by

preparative thin layer chromatography (toluene/ethyl acetate 7:3) furnishing 16 mg of **205** (95% yield).

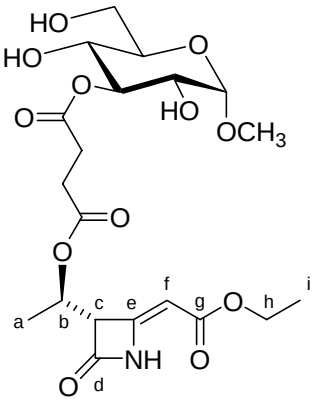


201. ^{1}H NMR ($CDCl_3$ 200 MHz)) δ 8.47 (1H, bs, N-H), 7.34-7.26 (aromatics), 5.56 (1H, t, $J_{2,3}$= $J_{3,4}$= 9.8 Hz, H-3), 5.45 (1H, s, H benzylidene), 5.23-5.10 (2H, m, H_b e H_f), 4.65 (2H, AB, J_{gem} = 12.4 Hz, CH_2 benzyl), 4.67 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1), 4.26 (1H, dd, $J_{5,6eq}$ =4.4 Hz, $J_{6ax,6eq}$ =10.0 Hz, H-6 eq.), 4.17 (2H, q, J_{hi} =7.2 Hz, H_h), 3.94-3.81 (1H, m, H-5), 3.80 (1H, d, J_{bc}= 6.8 Hz, H_c), 3.70 (1H, t, $J_{5,6ax}$ =10.0 Hz, H-6 ax.), 3.55 (1H, t, $J_{4,5}$=9.8 Hz, H-4), 3.48 (3H, s, -OCH_3), 2.60 (4H, s, 2 x CH_2 succinate), 1.33 (3H, d, J_{ab} =6.2 Hz, H_a), 1.29 (3H, t, H_i). ^{13}C NMR ($CDCl_3$, 50 MHz) significative signals at δ 171 e 170.8 (CO succinate), 166.9 and 164.5 (NH<u>C</u>=O, C=C-<u>C</u>=O), 151.2 (<u>C</u>=C-C=O), 137.9 and 137.0 (C aromatics), 129.0-126.2 (CH aromatics), 101.5 (C acetal benzylidene), 98.8 (C_1 anomeric), 91.0 (C=<u>C</u>-C=O), 79.4 (C_2), 73.1, 71, 69 67.1 (carbinolic carbons), 62.3, 61.2, 60.3 (CH_2-6, -O<u>CH_2</u>CH_3, CH_2 benzyl), 55.4 (OCH_3), 29.1 and 14.3 (2 x CH_2 succinate), 17.8 and 14.3 (CH_3 a, -OCH_2<u>CH_3</u>).

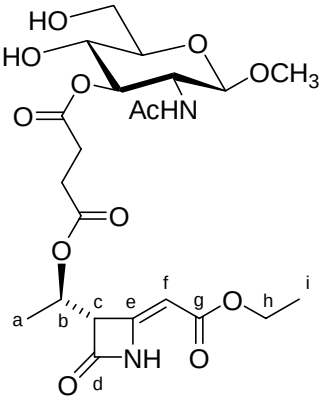
211. ^{1}H NMR ($CDCl_3$ 200 MHz) δ 8.55 (1H, s, NH), 7.50-7.12 (aromatics), 5.91 (1H, d, $J_{2,NH}$ =8.8 Hz, NH-Ac), 5.50 (1H, s, H benzylidene), 5.36 (1H, t, $J_{2,3}$= $J_{3,4}$ = 9.4 Hz, H-3), 5.23-5.13 (2H, m, H_b and H_f), 4.63 (1H, d, $J_{1,2}$ = 8.4 Hz, H-1), 4.36 (1H, dd, $J_{5,6eq}$ =4.6 Hz, $J_{6ax,6eq}$ = 10.2 Hz, H-6 eq.), 4.19 (2H, q, J_{hi} = 7.0 Hz, H_i), 3.98-3.81 (3H, m, H-2, H_c, H-6 ax.), 3.70 (1H, t, $J_{4,5}$ =9.4 Hz, H-4), 3.52 (1H, td, H-5), 3.50 (3H, s, -OCH_3), 2.61 (4H, bs, 2 x CH_2 succinate), 1.97 (3H, s, NHCO<u>CH_3</u>), 1.35 (3H, d, J_{ab} =6.4 Hz, H_a), 1.30 (3H, t, H_i).



199. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.32-5.19 (2H, m, H_b e H_f), 5.13 (1H, bt, $J_{3,4}$ =10.2 Hz, H-3), 4.79 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1), 4.19 (2H, q, $J_{h,i}$ = 7.2 Hz, H_h), 3.89-3.86 (3H, m, H_c and 2xH-6), 3.67-3.57 (2H, m, H-2 and H-4), 3.49 (3H, s, -OCH_3), 2.69 (4H, s, 2xCH_2 succinate), 1.40 (3H, d, J_{ab} = 6.4 Hz, H_a), 1.30 (3H, t, H_i). ^{13}C NMR ($CDCl_3$, 50 MHz) significative signals at δ 176.5 e 173.3 (CO succinate), 171.5 and 167.5 (NH<u>C</u>=O, C=C-<u>C</u>=O), 154.5 (<u>C</u>=C-C=O), 99.4 (C_1 anomeric), 91.2 (C=<u>C</u>-C=O), 71.3, 70.7, 69.3, 67.3 (carbinolic carbons), 55.5 (OCH_3), 29.7 e 29.4 (CH_2 succinate).



200. ^{1}H NMR ($CDCl_3$ 200 MHz) δ 5.93 (1H, d, $J_{NH,2}$ = 8.6 Hz, NHAc), 5.33-5.19 (2H, m, H_b and H_f), 5.10 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 4.51 (1H, d, $J_{1,2}$= 8.2 Hz, H1), 4.19 (2H, q, $J_{h,i}$ =7.0 Hz, H_h), 3.95-3.87 (3H, m, 2xH-6, H-2), 3.77 (1H, t, $J_{4,5}$ =9.6 Hz, H-4), 3.49-3.42 (4H, m, -OCH_3 and H-5), 2.64 (4H, bs, 2xCH_2 succinate), 1.95 (3H, bs, NHAc), 1.39 (3H, d, $J_{a,b}$= 6.4 Hz, H_a), 1.30 (3H, t, H_i). ^{13}C NMR ($CDCl_3$, 50 MHz) significative signals at δ 172.9 and 171.6 (2C, succinate), 170.7 and 166.8 (NHC=O, C=C-C=O), 150.9 (C=C-C=O), 102.1 (C_1 anomeric), 91.4 (C=C-C=O), 75.4, 69.2, 67.1 (carbinolic carbons C-3, C-4 and C-5), 56.9 (carbinolic C-2), 54.0 (OCH_3), 29.4 and 29.4 (CH_2 succinate), 23.4 (CH_3 NHAc), 17.6 and 14.3 ($CH_{3\ a}$, $CH_{3\ i}$). Other signals at δ 62.1, 61.2, 60.4.



Chapter 7

One-Pot Trisaccharides Synthesis

Introduction

One of the most important recent advances in oligosaccharide synthesis is represented by the development of synthetic procedures enabling the construction of multiple glycosidic bonds in a one-pot fashion. These advances were strongly elicited by the recognition of the dramatic influence exerted by protecting groups on the reactivity of the glycosyl donors, an observation that has been elaborated in the "armed" and "disarmed" concept.[1] This tunable reactivity may be exploited in the sequential connection of several building-blocks all bearing an identical leaving group.[2,3] Recently this approach culminated in the development of a computer-assisted planning of oligosaccharide synthesis based on the preliminary assessment of the relative reactivity for a great number of protected or partially protected thioglycoside donors.[2]
In an alternative conceptual approach, the one-pot sequential multiglycosidation process can also take advantage of an available set of glycosyl donors activated under orthogonal conditions.[4]

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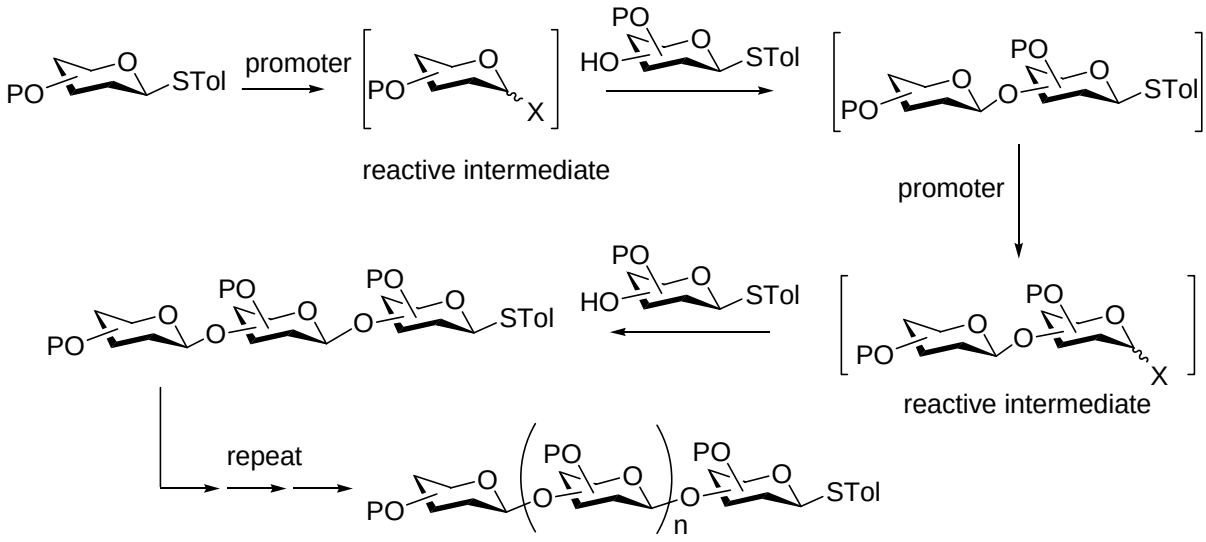
[1] Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583.
[2] a) Zhang, Z.; Ollman, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734; b) Ye, X.-S.; Wong, C.-H. *J. Org. Chem.* **2000**, *65*, 2410; c) G. H Burkhart, F.; Zhang, Z.; Wacowich-Sgarbi, S.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2001**, *40*, 1274; d) Mong, T. K.- K.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2002**, *41*, 4087; e) Mong, T. K.- K.; Lee, H.-K.; Durón, S. G.; Wong, C.-H. *Procl. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 797; f) Mong, T. K.- K.; Lee, H.-K.; Durón, S. G.; Wong, C.-H. *J. Org. Chem.* **2003**, *68*, 2135; g) Durón, S. G.; Polat, T.; Wong, C.-H. *Org. Lett.* **2004**, *6*, 839; h) Lee, H.-K.; Scanlan, C. N.; Huang, C.-Y.; Chang, A. Y.; Calarese, D. A.; Dwek, R. A.; Rudd, P. M.; Burton, D. R.; Wilson, I. A.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2004**, *43*, 1000.
[3] a) Ley, S. V.; Priepke, H. W. M. *Angew. Chem. Int. Ed.* **1994**, *33*, 2292; b) Douglas, N. L.; Ley, S. V.; Lücking, U.; Warriner, S. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51; c) Fridman, M.; Solomon, D.; Yogev, S.; Baasov, T. *Org. Lett.* **2002**, *4*, 281; d) Wang, Y.; Huang, X.; Zhang, L.-H., Ye, X.-S. *Org. Lett.* **2004**, *6*, 4415.
[4] a) Grice, P.; Ley, S. V.; Pietuszka, J.; Priepke, H. W. M.; Walther, E. P. E. *Synlett* **1995**, 781; b) Cheung, M.-K.; Douglas, N.; Hinzen, B.; Ley, S. V.; Pannecouncke, X. *Synlett* **1997**, 257; c) Grice, P.; Ley, S. V.; Pietuszka, J.; Osborn, H. M. I.; Priepke, H. W. M.; Warriner, S. L. *Chem. Eur. J.* **1997**, *3*, 431; d) Green, L.; Hinzen, B.; Ince, S. J.; Langer, P.; Ley, S. V.; Warriner, S. L. *Synlett* **1998**, 440; e) Langer, P.; Ince, S. J.; Ley, S. V. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3913; f) Tanaka, H.; Adachi, M.; Tsukamoto, H.; Ikeda, T.; Yamada, H.; Takahashi, T. *Org. Lett.* **2002**, *4*, 4213; g) Hashihayata, H.; Ikegai, K.; Takeuchi, K.; Jona, H.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 1829; h) Mukaiyama, T.; Kobashi, Y. *Chem. Lett.* **2004**, *33*, 10; i) Tanaka, H.; Adachi, M.; Takahashi, T. *Tetrahedron Lett.* **2004**, *45*, 1433.

A further option is represented by preactivating a thioglycoside building block (donor) with a stoichiometric promoter and subsequently adding a partially protected thioglycoside which is intended to act at this stage as the acceptor. The sequence is thus iterated until the desired elongation is achieved (*Scheme 7.1*).[5]



SCHEME 7.1 Oligosaccharide One-pot Synthesis *via* Thioglycoside Donors Preactivation.

This approach allows to circumvent the normal reactivity of the building blocks, in fact following this strategy a disarmed thioglycoside can be selectively activated in the coupling with an armed thioglycoside.[5] A less common approach contemplates the use of residues equipped with analogous but differentiated leaving groups (for example thioaryl and thioethyl glycosides) whose selective activation can be tuned by the proper choice of experimental conditions.[3c,6]

The one-pot multiglycosidation procedures are more commonly accomplished with thioglycosides.[7] This is not surprising since partially non protected thioglycosides, the necessary building blocks for this purpose, can be routinely prepared. Glycosyl fluorides, bromides,[8] selenides, sulfoxides[9] and underivatized hemiacetals[10] have also been used, especially in synthetic schemes relying on orthogonal activation.[11] These mentioned methodologies are all based on activation systems entailing the use of stoichiometric amounts of promoter or coreagent (NIS, triflic anhydride, phenyl sulfoxide, silver triflate, Cp_2HfCl_2, $BF_3{\cdot}OEt_2$, etc). In contrast, use of glycosyl trichloroacetimidates[12] in multiglycosidations approaches is seriously restricted by the difficult preparation of partially unprotected saccharidic derivatives equipped with the trichloroacetimidate leaving group at the anomeric position. Indeed, the installation of the trichloroacetimidate leaving group is commonly carried out by

[5] Huang, X.; Huang, H.; Wang, H.; Ye, X.-S. *Angew. Chem. Int. Ed.* **2004**, *43*, 5221.
[6] Lahmann, M.; Oscarson, S. *Org. Lett.* **2001**, *3*, 4201.
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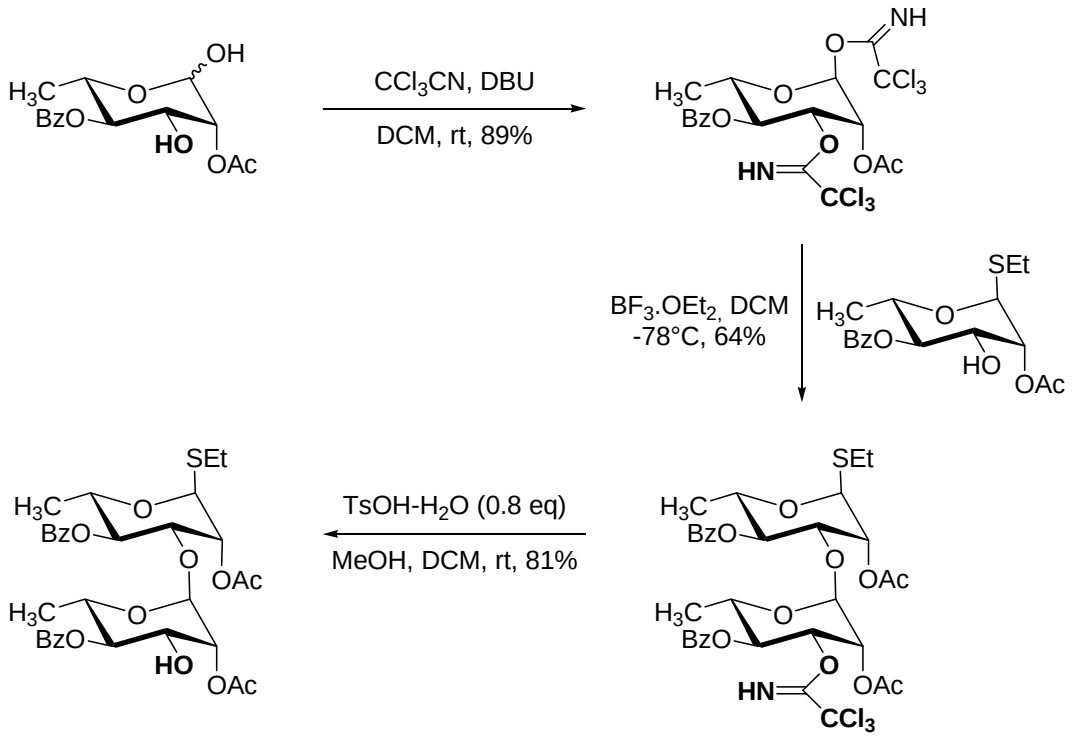
[8] Yamada, H.; Kato, T.; Takahashi, T. *Tetrahedron Lett.* **1999**, *40*, 4581.
[9] Raghavan, S.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 1580.
[10] Codée, J. D. C.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1947.
[11] See also *Chapter 1*.
[12] Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

reacting the sugar hemiacetal with trichloroacetonitrile in the presence of catalytic amounts of base. Under similar conditions non anomeric hydroxyl groups of sugars are also known to react, so that the trichloroacetimidate functionality has also beeen exploited for protection of non anomeric alcohols (*Scheme 7.2*).[13]



SCHEME **7.2** Trichloroacetimidate as non-Anomeric Hydroxyl Protecting Group.

As a matter of fact, use of glycosyl trichloroacetimidates in multiglycosidation approaches has been limited to the attachment of fragments at the non reducing terminus of the targets and in combination with donors orthogonally activated under stoichiometric conditions, such as thio- and pentenyl-glycosides.[14]

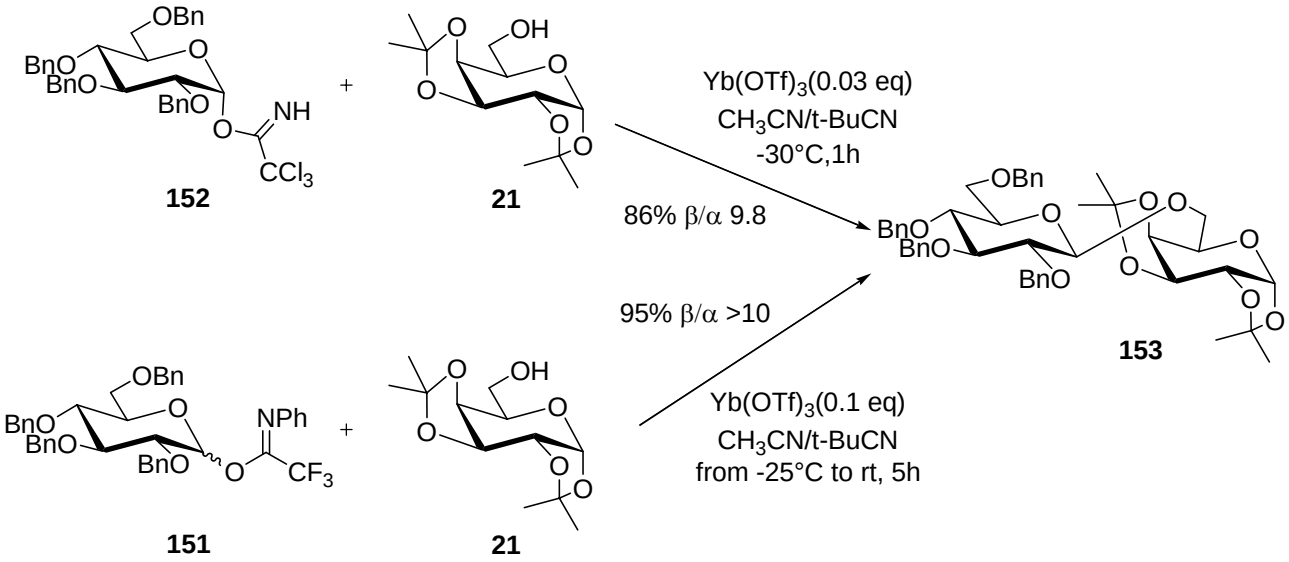
Recently, Yu and coworkers have introduced glycosyl (*N*-phenyl)trifluoracetimidates as a class of analogues of trichloroacetimidate donors.[15] On the other hand, in the course of our investigation aimed at establishing the development of glycosidation procedures relying on moisture stable promoters such as Ytterbium(III) triflate,[16] we have realized that the activation of these novel donors is entailing relatively more forced conditions than their trichloroacetimidate congeners. For example, the coupling in nitrile solvents between the trichloroacetimidate donor **152** (1.4 eq) and acceptor **21** (1 eq) proceeds at -30 °C and requires a very low amount of catalyst (3%) whereas higher temperatures and amounts of promoter (10%) are needed with the fluorinated donor **151** (*Scheme 7.3*).

[13] a) Qiu, D.; Koganty, R. R. *Tetrahedron Lett*. **1997**, *38*, 961; b) Yu, B.; Yu, H.; Hui, Y. ; Han, X. *Synlett* **1999**, 753; c) Dowlut, M.; Hall, D. G.; Hindsgaul, O. *J. Org. Chem.* **2005**, *70*, 9809.

[14] a) Yamada, H.; Harada, T.; Takahashi, T. *J. Am. Chem. Soc.* **1994**, *116*, 7919; b) Jayaprakash, K. N.; Fraser-Reid, B. *Org. Lett*. **2004**, *6*, 4211.

[15] Yu, B.; Tao, H. *Tetrahedron Lett*. **2001**, *42*, 2405.

[16] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett*. **2002**, *43*, 5573.



SCHEME 7.3 Comparative Glycosylation of Acceptor **21** with Glycosyl Trichloro- and *N*-Phenyl Trifluoroacetimidate Donors **152** and **151**.

In both these experiments the lanthanide salt was added as a solution in pivalonitrile, the beneficial effect of such a cosolvent on both rate and β-selectivity having been recently disclosed.[17] This different behaviour suggested the feasible development of a one-pot multi-glycosidation procedure based on: *i*) selective activation of a trichloroacetimidate donor in the presence of a (*N*-phenyl)trifluoroacetimidate derivative bearing a free hydroxyl functionality (acting as the acceptor in the first glycosidation step) and *ii*) the subsequent addition of a further new acceptor and the adjustment of the conditions to achieve the activation of the less reactive trifluoroacetimidate leaving group.

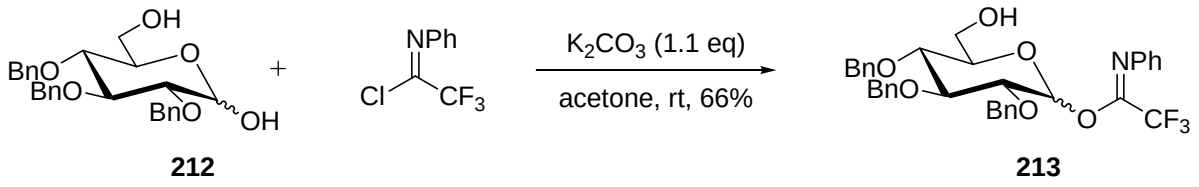
Results and Discussion

For the accomplishment of the planned one-pot glycosylation strategy, the synthetic access to a partially protected glycosyl (*N*-phenyl)trifluoroacetimidate is necessary. In contrast to trichloroacetimidates, these derivatives appear less difficult to be prepared. Indeed, the installation of (*N*-phenyl)trifluoroacetimidate group entails a substitution reaction with (*N*-phenyl)trifluoroacetimidoyl chloride in the presence of a stoichiometric amount of a mild base (for instance K_2CO_3). Use of one equivalent of the base should allow the selective functionalization of the anomeric hydroxyl group in the presence of a second alcoholic function owing to the higher acidity of the former. Actually, in a recent report Yu and coworkers attained this kind of selective functionalization on a disaccharide substrate.[18] However in that example the non anomeric hydroxyl was barely accessible so that the high regioselectivity observed may be ascribed to steric crowding. To demonstrate the generality of this selectivity the readily accessible 2,3,4-tri-*O*-benzyl glucopyranose[19] was reacted with (*N*-phenyl)trifluoroacetimidoyl chloride in the presence of a slight excess of K_2CO_3 in acetone to yield the desired derivative **213** in a satisfying isolated yield (*Scheme 7.4*).

[17] Communication at 13th European Carbohydrate Symposium, Bratislava, Slovakia, August 22-26, 2005, abstract OP 48.

[18] Sun, J.; Han, X. ; Yu, B. *Synlett* **2005**, 437.

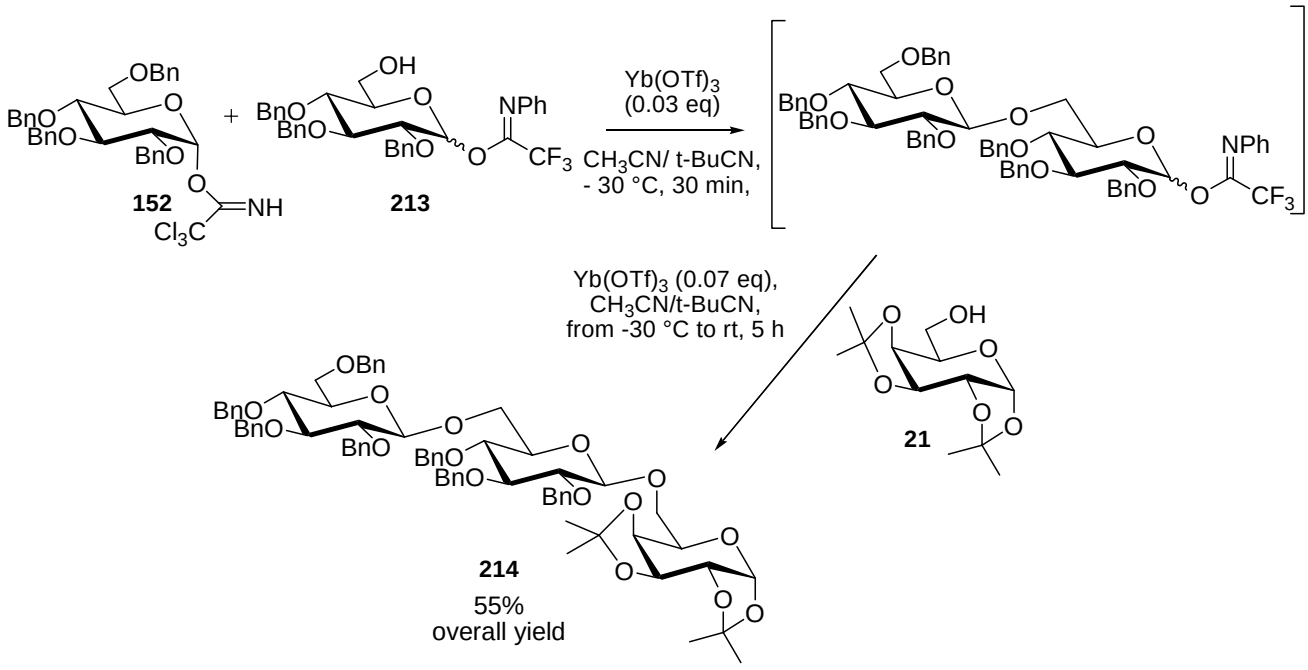
[19] This compound was readily accessed by Zemplen deacetylation of the corresponding 1,6-di-*O*-acetylated precursor obtained as described in: Lam, S. N.; Gevay-Hague, J. *Carbohydr. Res.* **2002**, *337*, 1953.



212 **213**

SCHEME ***7.4*** Synthesis of 6-OH Glucosyl Donor **213**.

The successful access to this building block prompted us to test the "one-pot" synthesis of the model trisaccharide **214** (*Scheme 7.5*). Initial mixing of **152** (1.4 eq) and **213** (1 eq) in acetonitrile at -30 °C in the presence of a low amount of $Yb(OTf)_3$ (0.03 eq) led to the consumption of the more reactive compound **152** in less than one hour (TLC). Then acceptor **21** (1.4 eq) was added together with a further amount of lanthanide triflate (0.07 eq) and the mixture was allowed to slowly warm up to room temperature. The desired trisaccharide was thus obtained in 55% yield slightly contaminated by anomeric by-products containing α-glycosidic bonds.

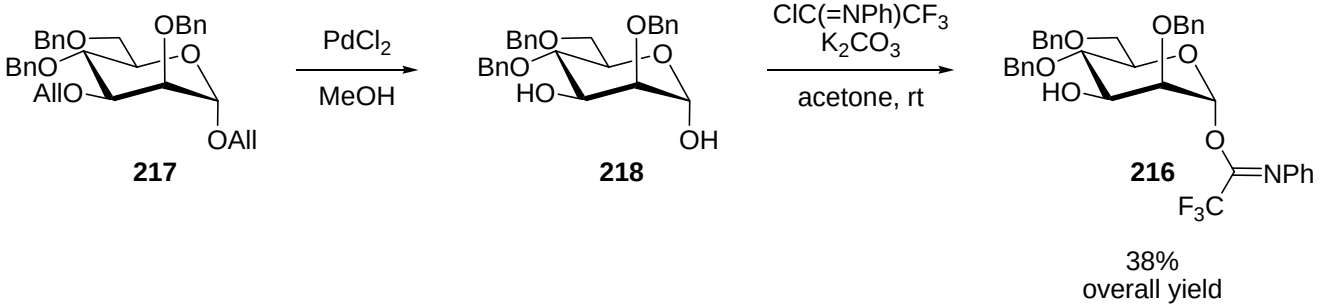


SCHEME ***7.5*** One-Pot Synthesis of Trisaccharide **214** *via* Sequential Activation of Donors **152** and **213**.

To demonstrate the applicability of the approach also in ether solvents, generally adopted for obtaining the preferential generation of α-glycosides in the absence of a participating effect on the donor, the protocol was examined for the synthesis of trisaccharide **215** (*Scheme 7.7*) representing the protected form of an important epitope of mannans from *Saccharomyces cerevisiae*.[20] To this aim, trifluoroacetimidate derivative **216**, with the free 3-OH, was readily prepared according to the sequence illustrated in *Scheme 7.6*. Known allyl 3-*O*-allyl-2,3,6-tri-

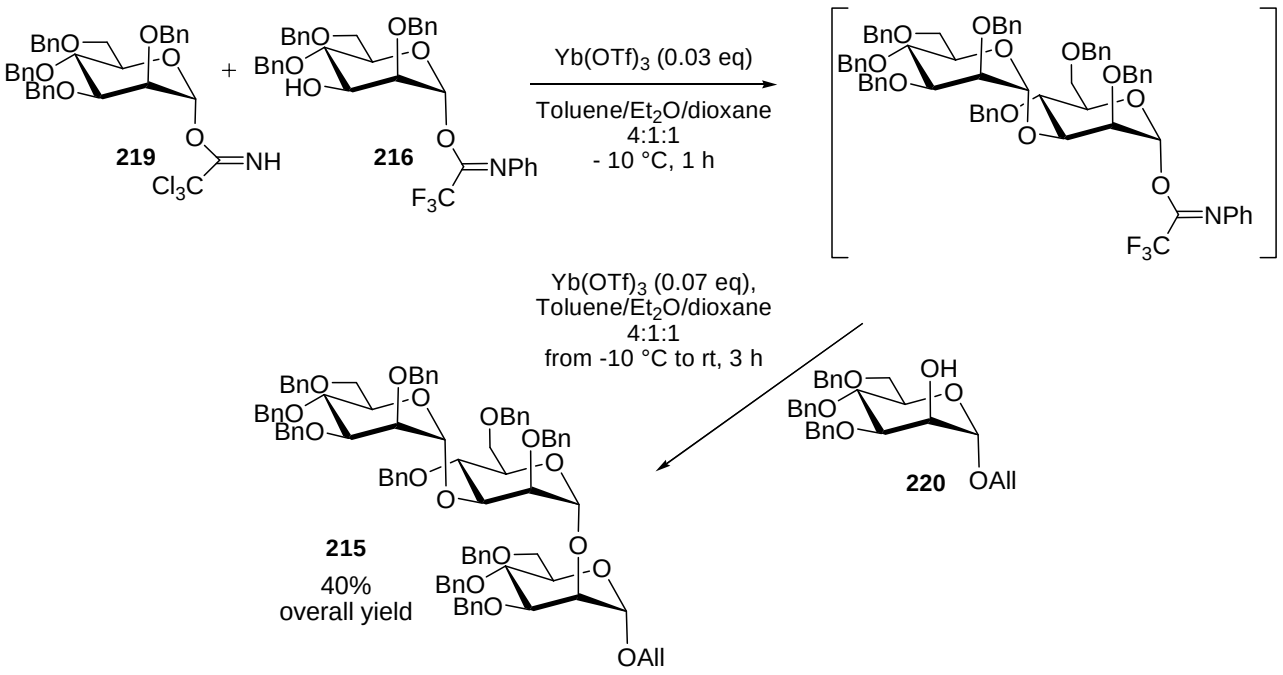
[20] a) Young, M.; Haavik, S.; Paulsen, B. S.; Broker, M.; Barnes, R. M. R. *Carbohydr. Polym.* **1996**, *30*, 243; b) Young, M.; Davies, M. J.; Bailey, D.; Gradwell, M. J.; Paulsen, B. S.; Wold, J. K.; Broker, M.; Barnes, R. M. R.; Hounsell, E. F. *Glycoconjugate J.* **1998**, *15*, 815.

O-benzyl-α-mannopyranoside[21] was submitted to a sequence of double deallylation and regioselective anomeric installation of the trifluoroacetimidate group that afforded the desired building-block **216**.



SCHEME **7.6** Synthesis of 3-OH Mannosyl Donor **216**.

In this case the one-pot synthesis was performed in a solvent mixture containing dioxane and diethyl ether to maximize the α-selectivity of the $Yb(OTf)_3$ promoted glycosidation steps as suggested by our previous observations.[16] The initial coupling between **216** (1 eq) and **219** (1.4 eq) was performed at -10 °C under the agency of catalytic $Yb(OTf)_3$ (0.03 eq) (*Scheme 7.7*). After one hour acceptor **220** (1.4 eq) was added to the mixture together with an additional amount of promoter (0.07 eq) and the temperature was allowed to raise. Chromatographic purification of the mixture afforded **215** as the only detectable trisaccharide in a good 40% overall yield.



SCHEME **7.5** One-Pot Synthesis of Trisaccharide **215** *via* Sequential Activation of Donors **219** and **216**.

It should be noted that this result is comparable with the overall glycosidation yields reported in a recent synthesis of the analogous sequence by a conventional stepwise

[21] Ogawa, T.; Yamamoto, H. *Carbohydr. Res.* **1985**, *137*, 79.

approach,[22] and with the results obtained in the one-pot synthesis of similar mannan sequences.[5d] On the other hand, to the best of our knowledge the here reported syntheses are representing the first examples of one-pot preparation of trisaccharides under catalytic activation (an overall 10% amount of promoter is sufficient for both glycosidation steps).

Conclusion

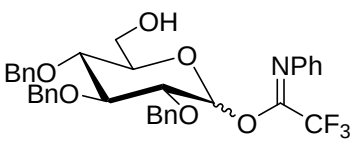
In conclusion, we have reported that the different reactivity of glycosyl trichloro- and (*N*-phenyl)trifluoroacetimidates can be suitably exploited for the one-pot assembly of trisaccharides without using the stoichiometric activation of the donors. In addition, a good stereo control was achieved without resorting to donors equipped with "disarming" participating groups. In perspective, the inclusion of electronically disarmed building blocks in the proposed approach would offer a further element of flexibility which may useful for the one-pot assemblage of even longer sequences.

Experimental Section

General Methods and Material

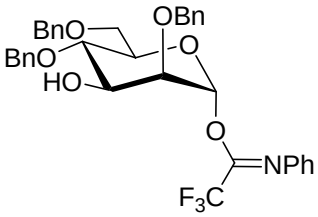
Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian spectrometers at 200 or 300 MHz and 50 or 75 MHz, respectively; or on a Bruker Avance 500 spectrometer at 500 MHz. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Analytical thin layer chromatography (TLC) was performed on E. Merck pre-coated (25 nm) silica gel 60F-254 plates. Column chromatography was preformed using Carlo Erba 0.06-0.20 silica gel. Acid washed molecular sieves and $Yb(OTf)_3$ were dried by overnight heating at 200 °C under vacuum before their use in glycosidations. Anhydrous solvents stored over molecular sieves (purchased from Aldrich) were used for performing the reactions.

Procedure for the synthesis of glycosyl (*N*-phenyl) trifluoroacetimidates from diols: (*N*-Phenyl) trifluoroacetimidoyl chloride (55 μL, 0.45 mmol) was added at room temperature to a mixture of 2,3,4-tri-*O*-benzyl glucopyranose (100 mg, 0.22 mmol) and K_2CO_3 (37 mg, 0.26 mmol) in acetone (2 mL). After ca. two hours a few drops of pyridine were added and the mixture was filtered on a short pad of neutral alumine (eluent dichloromethane). The residue was chromatographed on neutral aluminum oxide I (eluent: petroleum ether/ ethyl acetate from 85:15 to 7:3) to yield **213** (91 mg, yield 66 %) as an oil. Analogous procedure was adopted for the synthesis of **216** (38 % over two steps).

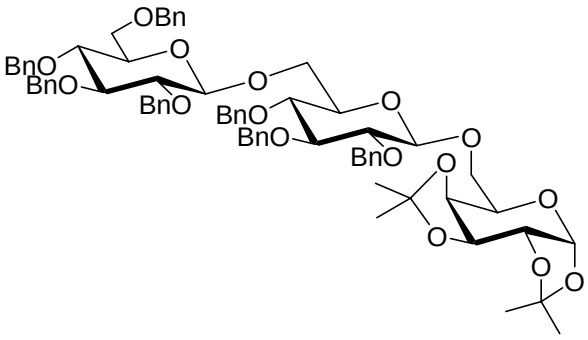


213. (β-anomer): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60-6.80 (aromatic protons), 5.75 (1H, bs, H-1), 5.00-4.40 (benzyl CH_2), 4.00-3.20 (6H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.3, 138.3, 137.8, 137.6, 129.3-127.8, 126.2, 124.4, 120.6, 119.3, 97.0 (C-1), 84.3, 81.0, 76.7, 76.0, 75.6, 75.2, 75.1, 61.4.

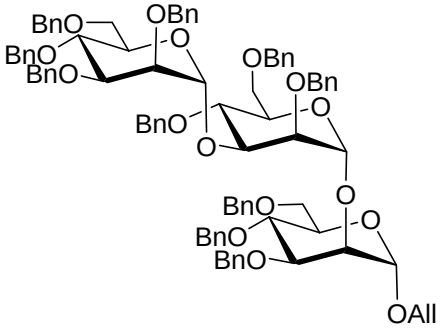
[22] Carpenter, C.; Nepogodiev, S. A. *Eur. J. Org. Chem.* **2005**, 3286.



216. (α-anomer): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50-6.80 (aromatic protons), 6.42 (1H, bs, H-1), 4.95-4.55 (benzyl CH_2), 4.08 (1H, td, $J_{2,3}$ = 3.3 Hz, $J_{3,OH}$ = $J_{3,4}$ = 9.3 Hz, H-3), 4.00-3.70 (5H), 2.45 (d, 3-OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.4, 138.1, 138.0, 137.1, 128.7-127.5, 124.4, 120.6, 119.4, 94.7 (C-1), 76.0, 75.6, 75.1, 73.8, 73.4, 72.8, 71.3, 68.6.



214. Trichloroacetimidate **152** (38 mg, 56 μmol) and trifluoroacetimidate **213** (25 mg, 40 μmol) were coevaporated three times in anhydrous toluene and then, after the addition of freshly activated acid washed molecular sieves, dissolved in acetonitrile (0.5 mL). The mixture was cooled at -30°C and then a solution of $Yb(OTf)_3$ (0.7 mg, 1.2 μmol) in pivalonitrile (30 μL) was added. After consumption of the trichloroacetimidate donor (1 h), a solution of acceptor **21** (13 mg, 56 μmol) in acetonitrile (0.9 mL) and a further aliquot of $Yb(OTf)_3$ (1.6 mg, 2.8 μmol) in pivalonitrile (70 μL) were added and the mixture was allowed to warm spontaneously to room temperature. A few drops of pyridine were added and the mixture was filtered on a short pad of silica gel. The residue was chromatographed on a silica gel column eluted with ether petroleum: ethyl acetate mixtures to yield trisaccharide **214** (27 mg, 55 % yield) slightly contaminated by minor amounts of anomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.22 (aromatic protons), 5.75 (1H, d, $J_{1,2}$ = 4.8 Hz, H-1 Gal), 5.10-4.40 (16 H), 4.43 and 4.41 (2H, 2xd, $J_{1,2}$ = 7.2 Hz, 2x H-1 Glc), 4.28 (1H, dd, $J_{2,3}$ = 2.4 Hz, H-2 Gal), 4.25-3.40 (15 H), 1.50, 1.38, 1.30, 1.22 (12H, 4xs, acetonides CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 138.7, 138.6, 138.5, 138.2, 128.3-127-7, 109.3, 108.5, 104.4, 104.0, 96.3, 84.8, 84.5, 81.8, 81.5, 78.0, 77.8, 77.3, 77.1, 76.5, 75.7, 75.6, 75.0, 74.8, 74.7, 74.6, 74.2, 73.5, 71.3, 70.7, 70.5, 70.0, 68.9, 68.6, 67.4, 26.1, 25.9, 25.0, and 24.4.



215. Trichloroacetimidate **219** (58 mg, 85 μmol) and trifluoroacetimidate **216** (37 mg, 60 μmol) were coevaporated three times in anhydrous toluene and then, after the addition of freshly activated acid washed molecular sieves, dissolved in 4:1 toluene/diethyl ether (0.5 mL). The mixture was cooled at -10°C and then a solution of $Yb(OTf)_3$ (1.2 mg, 1.7 μmol) in dioxane (100 μL) was added. After consumption of the trichloroacetimidate donor (ca 30 min), a solution of acceptor **220** (41 mg, 84 μmol) in 4:1 toluene/diethyl ether (1.2 mL) and a further aliquot of $Yb(OTf)_3$ (2.8 mg, 4.0 μmol) in dioxane (230 μL) were added and the mixture was allowed to warm spontaneously to room temperature. After ca 3 hours a few drops of pyridine were added and the mixture was filtered on a short pad of silica gel. The residue was chromatographed on a silica gel column eluted with ether petroleum: ethyl acetate mixtures to yield trisaccharide **215** (34 mg, 40 % yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-6.90 (aromatic protons), 5.83 (1H, m, -$CH_2C\underline{H}=CH_2$), 5.25-5.22 (2H, H-1 and -$CH_2CH=C\underline{H}_{trans}$), 5.20 (1H, d, $J_{1,2}$ = 1.2 Hz, H-1), 5.13 (1H, bd, $J_{1,2}$ = 10.4 Hz, -$CH_2CH=C\underline{H}_{cis}$), 4.97 (1H, d, $J_{1,2}$ = 1.2 Hz, H-1), 4.90-4.30 (20 H), 4.21

(1H, dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.15-3.55 (19 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 138.9, 138.6, 138.5, 138.4, 138.3, 13.9, 128.3-127.0, 117.1, 99.5, 99.4, 98.2, 80.1, 79.9, 75.5, 75.3, 75.2, 75.0, 74.8, 73.3, 72.6, 72.3, 72.1, 71.8, 69.4, 68.9, 67.8.

Chapter 8

New Routes to Glycosyl Phosphates and Their Application to *in Situ* Glycosylation Reactions

Introduction

The chemical synthesis of oligosaccharides is of utmost importance to procure tools for glycobiology in sufficient amounts.[1] A plethora of glycosylating agents carrying a variety of anomeric leaving groups have been developed to construct glycosidic linkages.[2] Glycosyl chlorides, bromides, iodides, trichloroacetimidates, fluorides, *n*-pentenyl glycosides, anhydro sugars, as well as anomeric aryl sulfoxides, and thioglycosides have been applied to the construction of complex oligosaccharides and glycosylated natural products.[3] Despite the ever increasing number of glycosylating agents available, one of the most limiting factors for the advancement of glycochemistry is surely the lack of a generally applicable glycosylation method.

Glycosyl phosphate triesters, the nature's glycosyl donors, proved to be effective glycosylating agents for the chemical synthesis of carbohydrates.[4,5] However, difficulties associated with the synthesis of anomeric phosphates diminished the synthetic utility of this class of glycosylating agents. Glycosyl phosphates can be synthesized from anomeric lactols either by treatment with a suitable chlorophosphonate in the presence of a base,[6] or *via* a dehydrative mechanism that takes advantage of the formation of an anomeric oxosulfonium species (*Scheme 8.1*).[7]

---

[1] a) Garegg, P. *J. Med. Res. Rev*. **1996**, *16*, 345; b) Seeberger, P. H. *Chem. Commun*. **2003**, 1115.

[2] Toshima, K.; Tatsuta, K. *Chem. Rev*. **1993**, *93*, 1503.

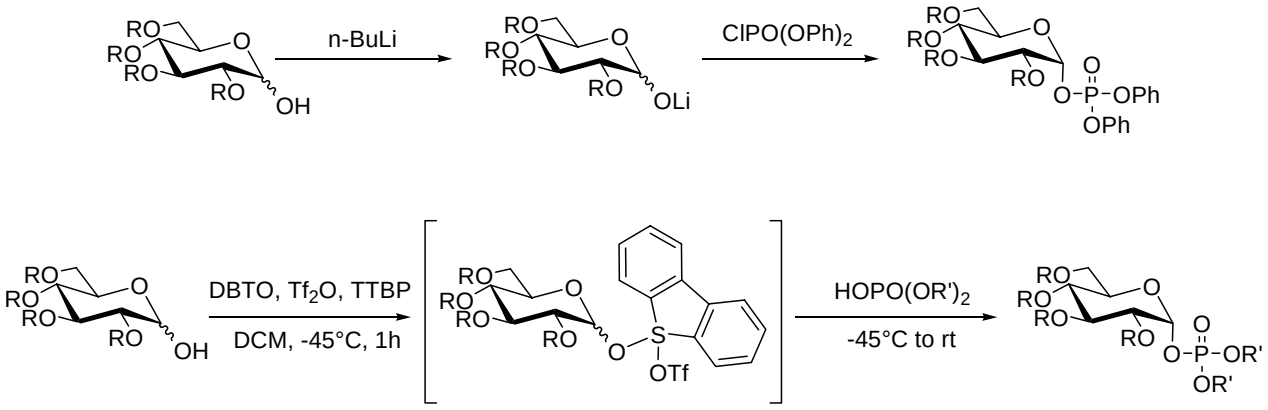
[3] a) Koenigs, W.; Knorr, E. *Chem. Ber*. **1901**, *34*, 957; b) Lemieux, R. U.; Hayami, J. *Can. J. Chem*. **1965**, *43*, 2162; c) Paulsen, H.; Lockhoff, O. *Chem. Ber.* **1981**, *114*, 3102; d) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem*. **1994**, *50*, 21; e) Mukaiama, T.; Murai, Y.; Shoda, S. *Chem. Lett*. **1981**, 431; f) Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. *J. Chem. Soc., Chem. Commun*. **1988**, 823; g) Seeberger, P. H.; Bilodeau, M. T.; Danishefsky, S. J. *Aldrichim. Acta* **1997**, *30*, 75; h) Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881; i) Garegg, P. J. *Adv. Carbohydr. Chem. Biochem*. **1997**, *52*, 179.

[4] See *Chapter 1*, pp. ff-gg.

[5] Hashimoto, S.; Honda, T.; Ikegami, S. *J. Chem. Soc. Chem. Comm.* **1989**, 685.

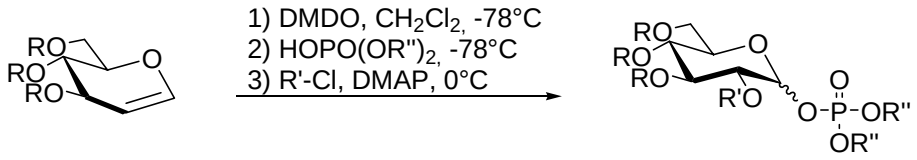
[6] Sabesan, S.; Neira, S. *Carbohydr. Res.* **1992**, *223*, 169.

[7] Garcia, B. A.; Gin, D. Y. *Org. Lett.* **2000**, *2*, 2135.



SCHEME 8.1 Glycosyl Phosphate Syntheses from Anomeric Lactols.

Alternative approaches involve the conversion of parent glycosylating agents such as glycosyl trichloroacetimidates, halides, or *n*-pentenyl- and thioglycosides into the corresponding phosphates.[8] Introduction of a one-pot protocol[9] gave ready access to glycosyl phosphate building blocks that now serve as key monomers for the synthesis of complex oligosaccharides in solution and by automated solid-phase synthesis (*Scheme 8.2*).[10]



SCHEME 8.2 Glycosyl Phosphate Synthesis *via* One-Pot Conversion of 1,2-Glycals.

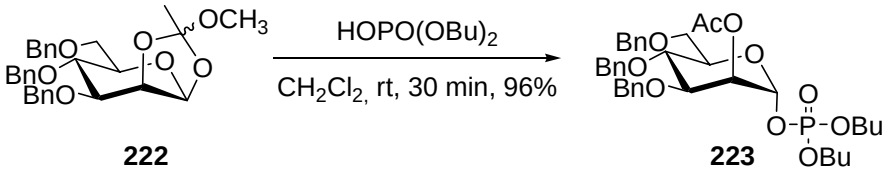
Although extremely efficient, the main drawback of this protocol entails the use of dimethyldioxirane (DMDO). DMDO has to be freshly prepared, can be dangerous to handle and makes reaction scale-up difficult.[11] Furthermore, this procedure doesn't allow the generation of C-2 epimers of glucose, such as mannosyl phosphates. That's due to the peculiar reaction mechanism that proceeds through the formation of an 1,2-anhydro sugar intermediate that, right after, undergoes the phosphate diester nucloephilic attack. As a matter of fact, the epoxidation step turned to be very stereo-selective, leading to the exclusive equatorial orientation of the epoxide oxygen at C-2 position, thus enabling only the synthesis of gluco-type anhydro sugars.
The need to access a host of glycosyl phosphate monomers for use in automated oligosaccharide synthesis prompted the pursuit of efficient synthetic means to generate glycosyl phosphates.

[8] a) Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1984**, 680; b) Adelhorst, K.; Whitesides, G. M. *Carbohydr. Res.* **1993**, *242*, 69; c) Boons, G-J; Burton, A.; Wyatt, P. *Synlett* **1996**, 310; d) Pale, P.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 4547; e) Timmers, C. M.; van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1998**, *17*, 471.
[9] a) Plante, O. J.; Andrade, R. B.; Seeberger, P. H. *Org. Lett.* **1999**, *2*, 211; b) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2001**, *123*, 9545.
[10] Plante, O. J.; Palmacci. E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523.
[11] Love, K. R; Seeberger, P. H. *Org. Synth.* **2005**, *81*, 225.

General applicability and ready scale-up of such a transformation are essential. 1,2-Glycosyl orthoesters are valuable synthetic intermediates in the preparation of carbohydrate building blocks.[12] Like 1,2-glycals, 1,2-glycosyl orthoesters possess only three hydroxyl groups to be differentiated. Treatment of 1,2-glycosyl orthoesters with excess alcohol nucleophiles under Lewis acid activation generates 2-*O*-acyl glycosides with 1,2-*trans* configuration.[13] In the 1970s, per-acetylated 1,2-orthoesters served as precursors to glycosyl phosphate monoesters and nucleotide 5'-diphospho sugars albeit in highly variable yields.[14]
Here, we describe the stereoselective conversion of 1,2-orthoesters to glycosyl 1-phosphate triesters by employing phosphate diesters both as nucleophile and acidic activator.

Results and Discussion

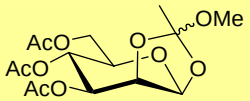
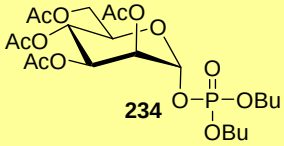
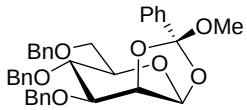
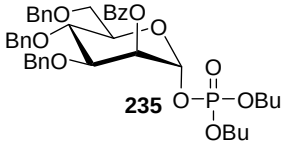
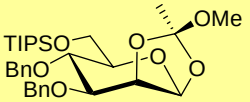
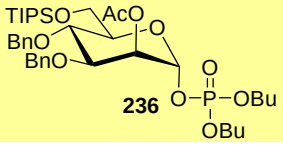
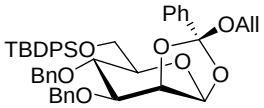
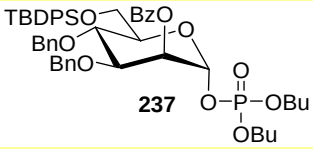
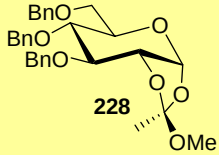
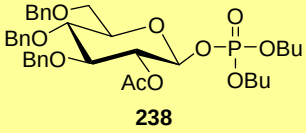
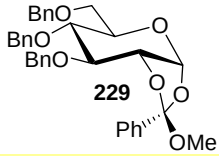
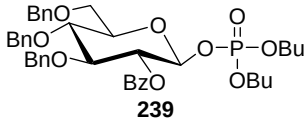
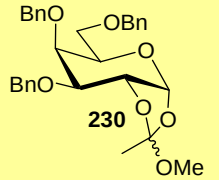
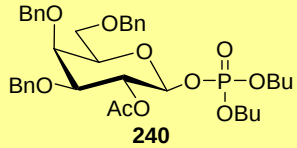
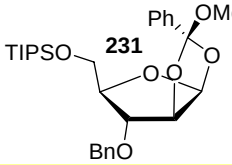
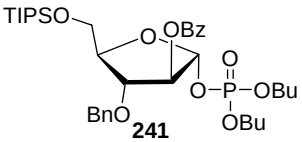
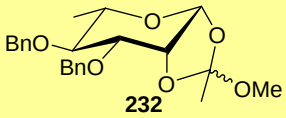
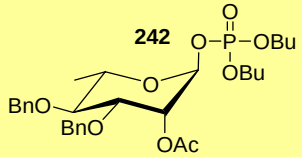
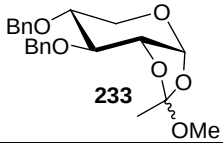
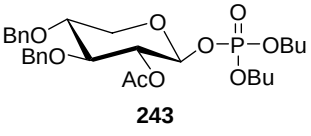
Tribenzylmannosyl 1,2-orthoacetate **222** served as test substrate *en route* to finding the optimal reaction conditions. Slow addition of a solution of **222** in dichloromethane to a solution of dibutyl phosphate in the presence of molecular sieves (MS) yielded the desired glycosyl phosphate **223**. In order to minimize the excess of dibutyl phosphate and to simplify the work-up procedures, different bases to quench the acidic dibutyl phosphate were tested. Just three equivalents of dibutyl phosphate are sufficient to convert **222** into mannosyl phosphate **223** in 30 min at room temperature (*Scheme 8.3*). Activated 4Å MS function as drying agent[15] while excess dibutyl phosphate was quenched by the addition of triethylamine. Filtration through a pad of Et_3N-deactivated silica gel, followed by column chromatography afforded **223** in 96% yield.



SCHEME 8.3 Conversion of 1,2-Orthoester **222** to Mannosyl Phosphate **223**.

The protocol can be further simplified by adding dibutyl phosphate dropwise to a solution of 1,2-orthobenzoate **225** in CH_2Cl_2. The corresponding mannosyl phosphate **235** was isolated in 97% yield. This addition sequence can also be employed when handling orthoacetates such as **222**, although longer reaction times are required to achieve full conversion. With a suitable protocol in hand, the synthetic scope of this transformation was explored using a range of mannose **224**-**227**, glucose **228**,**229**, galactose **230**, arabinofuranose **231**, rhamnose **232** and xylose **233** 1,2-orthoesters (*Table 8.1*).

[12] Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2199.
[13] a) Ogawa, T.; Beppu, K.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, C6; b) Leroux, J.; Perlin, A. S. *Carbohydr. Res.* **1981**, *94*, 108.
[14] a) Volkova, L. V.; Danilov, L. L.; Evstigneeva, R. P. *Carbohydr. Res.* **1974**, *32*, 165; b) Tsai, J.-H; Behrman E. J. *Carbohydr. Res*. **1978**, *64*, 297; c) Salam, M. A.; Behrman E. J. *Carbohydr. Res*. **1981**, 90, 83; *Carbohydr. Res*. **1982**, *101*, 339; Behrman E. J. *Carbohydr. Res*. **1982**, *102*, 139.
[15] AW 300 molecular sieves were also examined, but their acidity resulted in significant formation (5-20%) of the corresponding *O*-methyl-glycoside due to the rearrangement of the 1,2-orthoester.

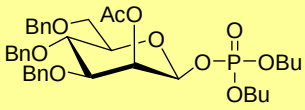
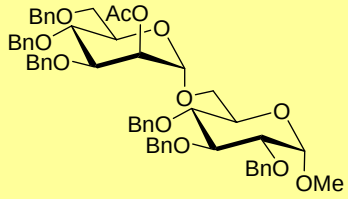
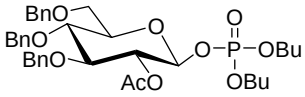
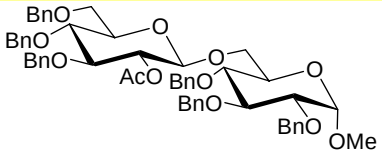
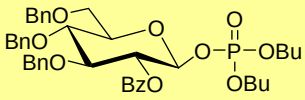
Entry[a]	1,2-Orthoester	Product	Yield (%)
1	 **224**	 **234**	86[c]
2	 **225**	 **235**	97 86[b]
3	 **226**	 **236**	95[c]
4	 **227**	 **237**	89
5	 **228**	 **238**	83 89[c]
6	 **229**	 **239**	quant
7	 **230**	 **240**	92 90[c]
8	 **231**	 **241**	quant[c]
9	 **232**	 **242**	92
10	 **233**	 **243**	90

[a]Conditions: 3 equiv of dibutyl phosphate were added by syringe to a CH_2Cl_2 solution of sugar at rt; [b]Multigram scale; [c]Addition of the sugar as solution to dibutyl phosphate (3 equiv) in CH_2Cl_2 at rt.

TABLE 8.1 Preparation of Various Glycosyl Phosphates from 1,2-Orthoesters.

The corresponding glycosyl phosphates were obtained in high yield. 1,2-Orthobenzoates (**225**, **227**, **229**, **231**) reacted faster with dibutyl phosphate (30 min) than 1,2-orthoacetates

(**222**, **224**, **226**, **228**, **230**, **232**, **233**) that required reaction times of 2-3 h. These differences in reactivity can be rationalized when considering that the phenyl moiety at the orthoester helps to stabilize the carboxonium intermediate during orthoester ring opening.
Since the procurement of large amounts of monosaccharide building blocks is required to supply the starting materials for automated oligosaccharide synthesis, process scalability is important. The proof-of-principle reactions were carried out on 0.2–0.5 mmol scale, but the process can be readily scaled up, as demonstrated for the preparation of several grams of **235**.
The work-up and purification procedure called for filtration through a plug of silica followed by column chromatography. NMR spectra of the crude glycosyl phosphates obtained after simple silica gel filtration indicated very high purity of the desired products. Therefore, we began to examine the efficiency of crude glycosyl phosphates as glycosylating agents.
The crude mannosyl and glucosyl phosphates **223, 238**, **239** derived from the corresponding orthoesters (1.2 eq.) were coupled to methyl glucoside **244** (1.0 eq.) at -30 °C by TMSOTf activation (*Table 8.2*). The desired 1,2-*trans*-linked disaccharides **245–247** were obtained in excellent yield and complete stereoselectivity.[5] The formation of disaccharide orthoester by-products was not observed.

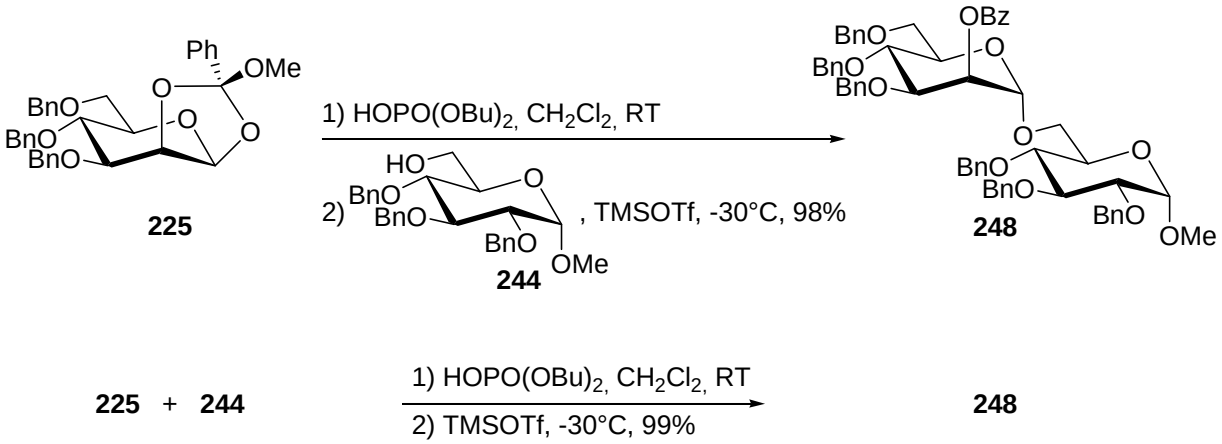
Entry[a]	*Donor*	*Acceptor*	*Product*	*Yield (%)*
1	 **223**	 **244**	 **245**	98
2	 **238**	**244**	 **246**	97
3	 **239**	**244**	 **247**	91

[a]Glycosidations were carried out with 1.2 equiv of donor, 1.0 equiv of acceptor and 1.2 equiv of TMSOTf at -30 °C in CH_2Cl_2 , within 1 h.

TABLE 8.2 Glycosidations with Crude Glycosyl Phosphates.

The carbohydrate impurities generated during glycosyl phosphate synthesis did not interfere with the glycosylations. Thus, a one-pot coupling following the *in situ* generation of glycosyl phosphates should be possible since excess acidic dibutyl phosphate was not expected to negatively affect the glycosylation.
To evaluate the *in situ* route, mannosyl phosphate **235** was generated from orthoester **225** (1.2 eq.) as described above. Then, a solution of glucoside **244** (1.0 eq.) in CH_2Cl_2 was added. After cooling the reaction mixture to -30 °C three equivalents of TMSOTf were

required to activate the glycosyl phosphate. Partial quenching of TMSOTf by some remaining phosphoric acid necessitated this increased amount of activator. Disaccharide **248** was isolated in 98% yield (*Scheme 8.4*). The formation of *O*-methyl glycoside was not observed, probably because the molecular sieves that are present in the reaction mixture act as methanol scavenger. A further simplification of the reaction protocol was achieved when orthoester **225** was treated with dibutyl phosphate in the presence of the coupling partner, nucleophile **244**. The *in-situ* generated glycosyl phosphate was activated by simple addition of TMSOTf to furnish disaccharide **248** in excellent yield.



SCHEME 8.4 Glycosidations Using *in situ* Generated Glycosyl Phosphates

1,2-Glycosyl orthoesters had been used previously as glycosylating agents.[16] Direct coupling generally resulted in poor yield and excess orthoester was required to improve coupling yields. Only a limited range of substrates such as 1,2-pentenol and –thio orthoesters were effective in the assembly of complex carbohydrates.[17] Based on the new procedure reported here, 1,2-orthoesters may be viewed as latent glycosylating agents similar to 1,2-glycals whereby the *in-situ* conversion to glycosyl phosphates is employed for efficient couplings.

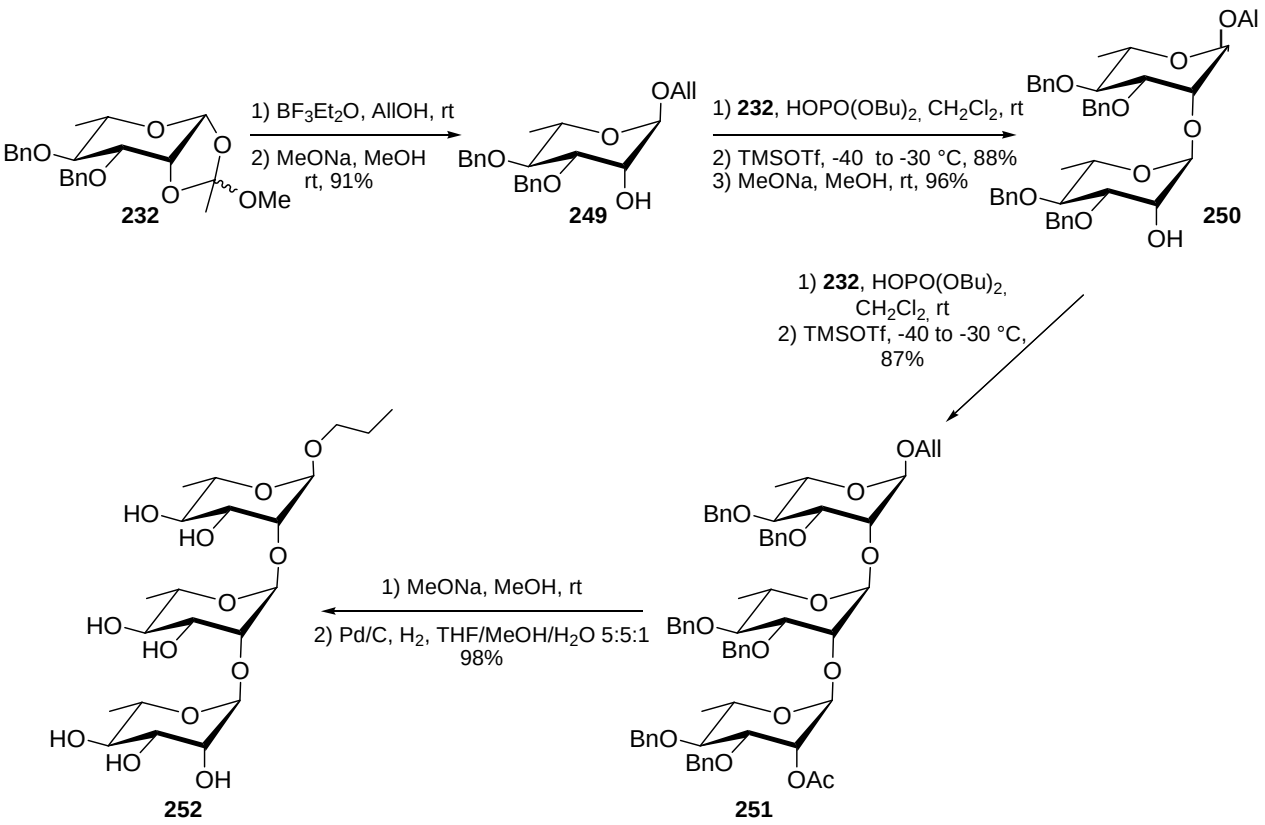
The synthetic utility of the new approach was further demonstrated by the rapid assembly of a group B *Streptococcus* trirhamnoside epitope.[18] Rhamnose orthoester **232** served as the only building block for both glycosylating agent **242** and nucleophile **249** *via* chain elongation with *in-situ* generation of glycosyl phosphates. Allyl rhamnoside **249** was obtained in 91% yield by selective opening of **232** under acidic conditions with an excess of allyl alcohol and subsequent cleavage of the acetate. Treatment of orthoester **232** with dibutyl phosphate in the presence of **249**, followed by the activation with TMSOTf, gave dirhamnoside in 88% yield. Removal of the acetate furnished **250** that served as

[16] a) Kochetkov, N. K.; Khorlin, A. J.; Bochkov, A. F. *Tetrahedron* **1967**, *23*, 693; b) Kochetkov, N. K.; Bochkov, A. F.; Sokolovskaya, T. A.; Snyatkova, V. J. *Carbohydr. Res.* **1971**, *16*, 17; c) Wang, W.; Kong, F. *J Org. Chem.*. **1998**, *63*, 5744.

[17] a) Allen, J. G.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1999**, *121*, 468; b) Jayapraskash, K. N.; Radhakrishnan, K. V.; Fraser-Reid. B. *Tetrahedron Lett.* **2002**, *43*, 6953; c) Kochetkov, N. K.; Backinowsky, L. V.; Tsvetkov, Y. E. *Tetrahedron Lett.* **1977**, *18*, 3681; d) Backinowsky, L. V.; Tsvetkov, Y. E.; Balan, N. F.; Byramova, N. E.; Kochetkov, N. K. *Carbohydr. Res.* **1980**, *85*, 209; e) Wang, W.; Kong, F. *Angew. Chem. Int. Ed.* **1999**, *38*, 1247.

[18] a) Michon, F.; Katzenellenbogen, E.; Kasper, D. L.; Jennings, H. J. *Biochemistry* **1987**, *26*, 476; b) Michon, F.; Brisson, J.-R.; Dell, A.; Kasper, D. L.; Jennings, H. J. *Biochemistry* **1988**, *27*, 5341; c) Michon, F.; Chalifur, R.; Feldman, R.; Wessels, M.; Kasper, D. L.; Gamian, A.; Pozsgay, V.; Jennings, H. J. *Infect. Immun.* **1991**, *59*, 1690.

nucleophile in the coupling with the *in-situ* generated rhamnosyl phosphate to yield 87% of trirhamnoside **251**. Removal of all protective groups furnished trirhamnoside **252** in 98% yield (*Scheme 8.5*).



SCHEME 8.5 Synthesis of Rhamnose Trisaccharide **252**.

On the basis of the excellent results obtained with glycosyl 1,2-orthoesters, we extended our protocol for the synthesis of glycosyl phosphates to less usual building blocks such as 1,2-oxazolines and 1,2,6-mannosyl orthoester.[19]

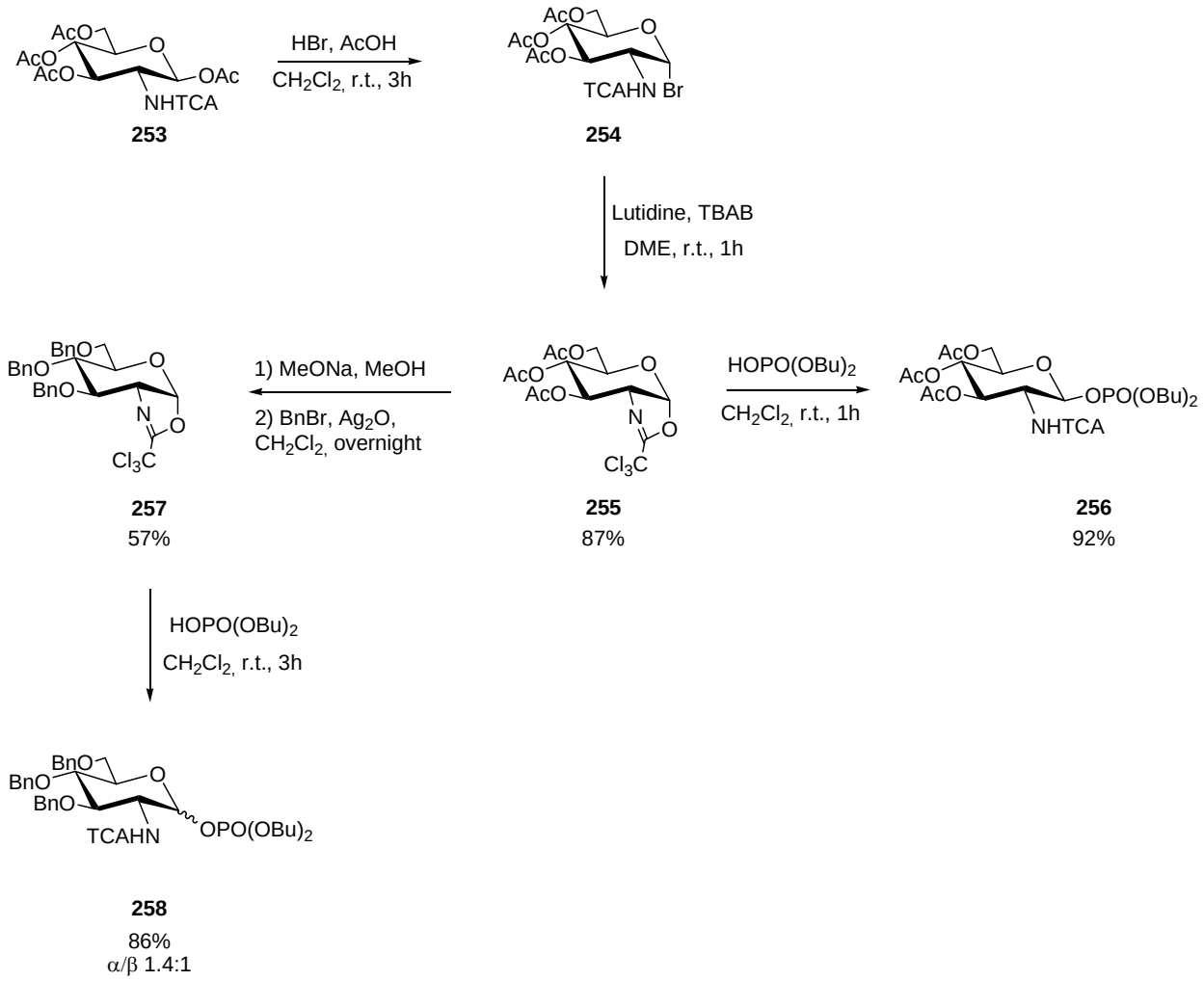
1,2-Oxazolines can be considered as C-2 nitrogenated analogues of 1,2-orthoesters, in fact, as these latter, they are easily prepared from the corresponding anomeric bromides by basic treatment and undergo nucleophilic attack in acidic conditions. Although glycosamine phosphates proved to be very effective glycosylating agents,[20] their use has been limited by their difficult synthesis, often achieved by converting parent glycosyl donors or using labile chlorophosphates. Despite the presence in literature of some glucosamine phosphates preparations *via* oxazoline opening, their application was limited to the obtainment of unprotected derivatives to be used as probes for biological tests.[21] We intended to develop a feasible protocol to easily access synthetically useful glucosamine donors.

[19] Ravidà, A; Liu, X; Seeberger, P. H. unpublished results.

[20] Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Org. Lett.* **2000**, *24*, 3841.

[21] a) Khorlin, A. Y.; Zurabyan, S. E.; Antonenko, T. S. *Tetrahedron Lett.* **1970**, *55*, 4803; b) Busca, P.; Martin, O. R. *Tetrahedron Lett.* **1998**, *39*, 8101.

Glucosamine phosphates **256** and **258** were synthesized from the corresponding trichloro-oxazoline **255**[22] and **257** in excellent to good yield, using the above described protocol (*Scheme 8.6*). The use of trichloroacetamide protection at the C-2 position is due to the better coupling yields registered when such functionalized glucosamine are used as donors, if compared with acetamide protection.

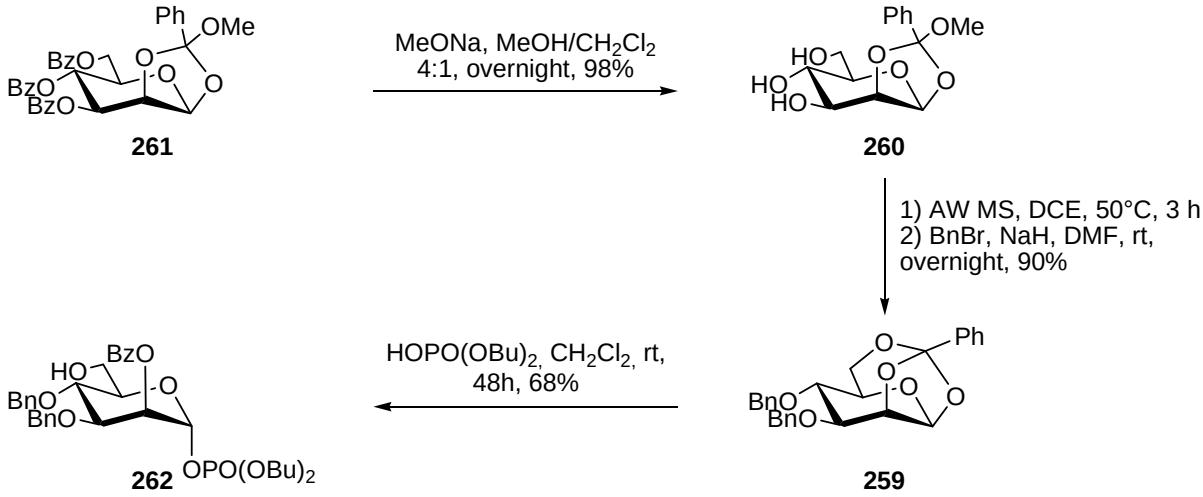


SCHEME 8.6 Synthesis of Glucosamine Phosphates **256** and **258**.

Noteworthy, the armed glucosamine phosphate **258** was isolated as an α/β mixture. The major reactivity of this derivative, if compared with the disarmed **256**, allowed the more reactive β-anomer to isomerize into the more stable α-phosphate. This behaviour had reported to occur in the one-pot procedure from glycals as well, and can be taken as starting point for further studies, as the different reactivities exhibited by α- and β-phosphates as donors found interesting application in sequential one-pot glycosidations.[9]
As previously anticipated, the protocol was also applied to the unusual 1,2,6-mannosyl orthoester **259**,[23] whose synthesis was achieved developing a new mild procedure (*Scheme 8.7*). Triol **260** has been treated with acid washed molecular sieves (AW 300 MS) in

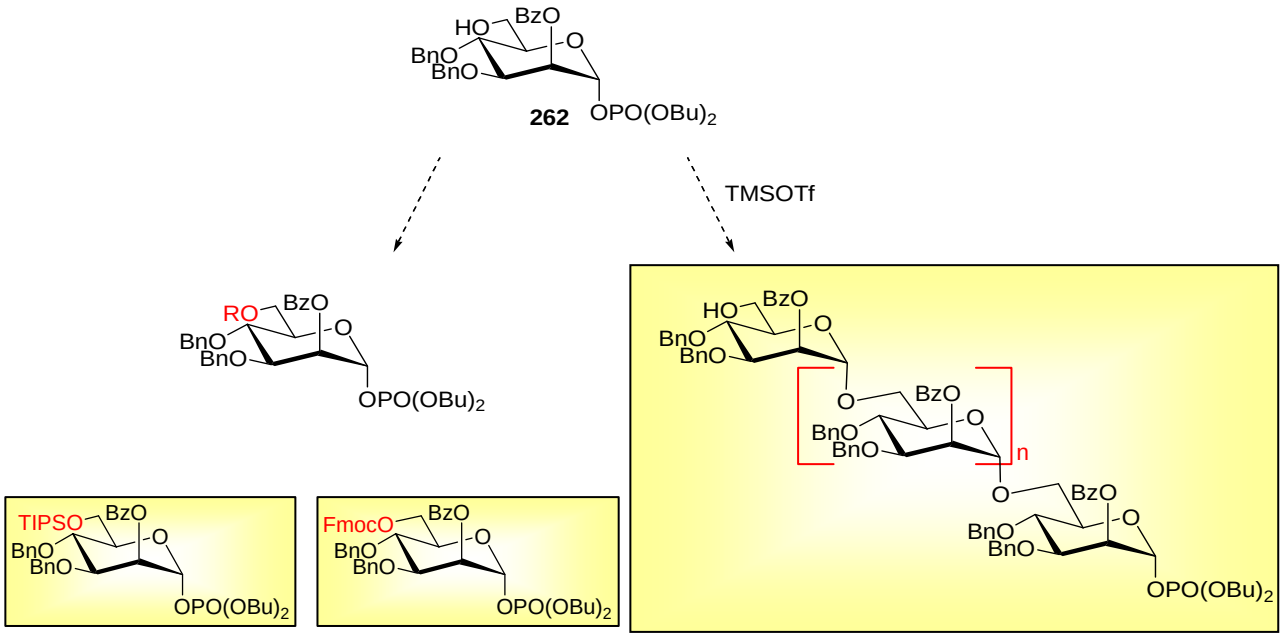
[22] a) Wolfrom, M. L.; Bhat, H. B. *J. Org. Chem.* **1967**, *32*, 1821 ; b) Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189.
[23] Hiranuma, S.; Kanie, O.; Wong, C.-H. *Tetrahedron Lett.* **1999**, *40*, 6423.

dichloroethane in order to promote the conversion into the tricyclic orthoester, whose *in situ* benzylation afforded **259** in 90% yield over two steps. Derivative **259** slugghishly reacted with 5 equiv. of dibutyl phosphate furnishing the partially protected mannosyl phosphate **262** in 68% yield.



SCHEME 8.7 Synthesis of 6-OH Mannosyl Phosphate **262**.

The thus obtained phosphate **262** is a very interesting building block, since the free hydroxy group at the C-6 can be further orthogonally protected or directly involved in one-pot synthetic strategies (*Scheme 8.8*).



SCHEME 8.8 Feasible Applications of Mannosyl Phosphate **262**.

Conclusion

In summary, we developed a highly effective protocol to convert 1,2-glycosyl orthoesters to C2-acyl glycosyl phosphates. The synthetic scope was established by preparing a series of mannosyl, glucosyl, galactosyl, rhamnosyl, xylosyl and arabinofuranosyl phosphates in high yield. Pure glycosyl phosphates are obtained following column chromatography. *In situ* generation of glycosyl phosphates from 1,2-orthoesters allows for simple and efficient glycoside formation as shown for a trirhamnoside antigen. Straightforward access to glycosyl phosphate building blocks will facilitate the synthesis of complex carbohydrates in solution and by automated solid phase synthesis. Furthermore, the effective protocol thus developed was extended to the syntheses of useful glucosamine phosphates from 1,2-oxazolines. A novel mild procedure has been developed for the synthesis of the unusual 1,2,6 mannosyl orthoester that has been then subjected to dibutyl phosphate treatment to yield the very versatile of 6-OH mannosyl phosphate, whose applications will be reported in due course.

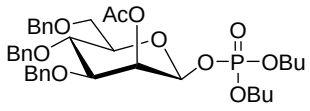
Experimental Section

General Methods and Material

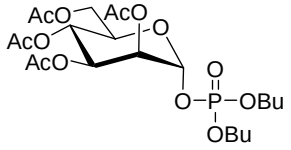
All chemicals used were reagent grade and used as supplied except where noted. All reactions were performed in oven-dried glassware under an inert atmosphere (nitrogen or argon) unless noted otherwise. Reagent grade dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), diethyl ether (Et_2O) and toluene (PhMe) were passed through activated neutral alumina column prior to use. Reagent grade N,N-dimethylformamide (DMF) and methanol (MeOH) were dried over activated molecular sieves prior to use. Pyridine, triethylamine and acetonitrile were distilled over CaH_2 prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates (0.25mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution or phosphomolybdic acid (PMA) or sulforic acid ethanol solution, or spraying with Bial's reagent (orcinol in acidic ethanol). Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh).
^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Varian Mercury 300 (300 MHz), Varian Gemini 300 (300 MHz), Bruker DRX400 (400 MHz), Bruker DRX500 (500 MHz), in $CDCl_3$ with chemical shifts referenced to internal standards $CDCl_3$ (7.26 ppm ^1H, 77.0 ppm ^{13}C) unless otherwise stated. ^{31}P spectra are reported in δ value relative to H_3PO_4 (0.0 ppm) as an external reference. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for ^1H NMR data. Signals were assigned by means of DEPT, ^1H-^1H COSY and ^1H-^{13}C HSQC spectra. High-resolution mass spectral (HRMS) analyses were performed by the MS-service at the Laboratorium für Organische Chemie (LOC) at ETH Zürich. High-resolution MALDI and ESI mass spectra were run on an IonSpec Ultra and a Bruker BioAPEXII instruments respectively. In case of MALDI-MS, 2,5-dihydroxybenzoic acid (DHB) or 3-hydroxypyridine 2-carboxylic acid (3-HPA) or 2-(4-hydroxyphenylazo) benzoic acid (HABA) served as the matrix. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin-Elmer 241

polarimeter. Elemental analyses were performed by the Microanalytical Laboratory of the LOC, ETH Zürich.

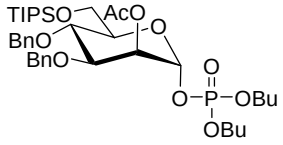
Synthesis of Glycosyl Phosphates: General Procedure A.
Suitably protected 1,2-orthoester (1.0 equiv) was azeotropically dryed with toluene (3 x 2 mL) followed by 1 h under vacuum. Activated Molecular sieves under flux of argon were added and the sugar was dissolved in CH_2Cl_2 (1 mL/0.10 mmol 1,2-orthoester). After 15 min stirring at room temperature, the mixture was added dropwise (within 30 min) to a 3 M solution of dibutyl phosphate in CH_2Cl_2 (3 equiv) in the presence of molecular sieves. After completion of the reaction (TLC analysis), the reaction was cooled to 0°C and triethylamine (4 equiv) was added. The solution was warmed to room temperature and filtered off through a pad of Et_3N-deactivated silica gel. The resulting mixture was purified by flash silica column chromatography.



223. General procedure A with orthoester **222**[24] (253 mg, 0.50 mmol), dibutyl phosphate (300 µL, 1.5 mmol), 4Å MS (250 mg), CH_2Cl_2 (5 mL), room temperature, 30 min gave **223** (328 mg, 96%). The spectral data were in agreement with those in the literature.[25]

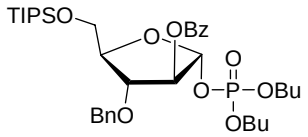


234. General procedure A with orthoester **224**[25] (72 mg, 0.20 mmol), dibutyl phosphate (120 µL, 0.6 mmol), 4Å MS (80 mg), CH_2Cl_2 (2 mL), room temperature, 3 h gave **234** (93 mg, 86%). The spectral data were in agreement with those in the literature.[26]



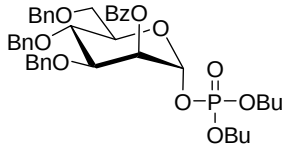
236. General procedure A with orthoester **226**[26] (115 mg, 0.20 mmol), dibutyl phosphate (120 µL, 0.60 mmol), 4Å MS (100 mg), CH_2Cl_2 (2 mL), room temperature, 40 min gave **236** (143 mg, 95%). R_f 0.62 (Hexanes/EtOAc = 6 : 4); $[\alpha]_D^{r.t.}$ = +21.2 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.37-7.27 (m, 10 H, arom.), 5.62 (dd, *J* = 6.3, 2.1 Hz, 1 H, H-C(1)), 5.40 (t, *J* = 2.4 Hz, 1 H, H-C(2)), 4.92 (AB, *J* = 10.5 Hz, 1 H, OC*H*HPh), 4.73 (AB, *J* = 9.0 Hz, 1 H, OC*H*HPh), 4.70 (AB, *J* = 9.0 Hz, 1 H, OCH*H*Ph), 4.57 (AB, *J* = 11.4 Hz, 1 H, OCH*H*Ph), 4.19-3.81 (m, 9 H, H-C(3), H-C(4), H-C(5), Ha-C(6), HbC(6), 2 x OC*H*$_2$CH$_2$CH$_2$CH$_3$), 2.12 (s, 3 H, COC*H*$_3$), 1.70-1.60 (m, 4 H, 2 x OCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.47-1.33 (m, 4 H, 2 x OCH$_2$CH$_2$C*H*$_2$CH$_3$), 1.11-1.07 (m, 21 H, 3 x C*H*(C*H*$_3$)$_3$), 0.94 (t, *J* = 7.5 Hz, 3 H, OCH$_2$CH$_2$CH$_2$C*H*$_3$), 0.94 (t, *J* = 7.5 Hz, 3 H, OCH$_2$CH$_2$CH$_2$C*H*$_3$); ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.36; IR (film): 3005, 2962, 2867, 1744, 1492, 1464, 1374, 1262, 1172, 1105, 1029, 960, 882, 652, 626 cm^{-1}. Anal. Calcd for $C_{39}H_{63}O_{10}SiP$; C, 62.38; H, 8.46; P, 4.12. Found: C, 62.63; H, 8.59; P, 4.07; HRMS-ESI Calcd for $C_{39}H_{63}O_{10}SiPNa$ 773.3820 (M+Na): Found: 773.3835.

[24] Ziegler, T.; Lemanski, G. *Eur. J. Org. Chem.* **1998**, 163.
[25] Soldaini, G.; Cardona, F.; Goti, A. *Org. Lett.* **2005**, *7*, 725.
[26] Seeberger, P. H.; Hewitt, M. C.; Snyder, D. PCT Int. Appl. 2004005532, 2004, 69.



241. General procedure A with orthoester **231**[27] (77 mg, 0.15 mmol), dibutyl phosphate (150 µL, 0.45 mmol), 4Å MS (100 mg), CH_2Cl_2 (1.5 mL), room temperature, 1 h gave **241** (104 mg, quant.). R_f 0.30 (Hexanes/EtOAc = 6 : 4); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.00 (dd, *J* = 8.7, 0.9 Hz, 2 H, H_o Bz), 7.59 (tt, *J* = 7.2, 1.8 Hz, 1 H, H_p Bz), 7.44 (tt, *J* = 7.5, 1.5 Hz, 2 H, H_m Bz), 7.36-7.22 (m, 5 H, arom.), 5.97 (d, *J* = 4.8 Hz, 1 H, H-C(1)), 5.52 (d, *J* = 1.2 Hz, 1 H, H-C(2)), 4.82 (AB, *J* = 11.7 Hz, 1 H, OC*H*HPh), 4.65 (AB, *J* = 12.0 Hz, 1 H, OC*H*HPh), 4.41 (q, *J* = 4.5 Hz, 1 H), 4.20 (d, *J* = 5.1 Hz, 1 H), 4.08 (dq, *J* = 6.6, 0.9 Hz, 4 H, $OCH_2CH_2CH_2CH_3$), 3.89 (dd, *J* = 11.1, 4.5 Hz, 1 H, Ha-C(5)), 3.82 (dd, *J* = 11.1, 5.1 Hz, 1 H, Hb-C(5)), 1.70-1.61 (m, 4 H, 2 x $OCH_2CH_2CH_2CH_3$), 1.46-1.33 (m, 4 H, 2 x $OCH_2CH_2CH_2CH_3$), 1.00 (s, 18 H, 3 x $CH(CH_3)_3$), 0.99 (q, *J* = 4.0 Hz, 3 H, 3 x C*H* $(CH_3)_3$), 0.91 (t, *J* = 7.2 Hz, 6 H, 2 x $OCH_2CH_2CH_2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 165.0, 137.5, 133.4, 129.7-127.7 (10 C arom.), 103.4 (d, $^2J_{C,P}$ = 5.5 Hz, 1 C, C(1)), 85.9, 82.4, 82.2, 82.1, 72.3, 67.7, 62.5, 32.3 (d, $^3J_{C,P}$ = 7.2 Hz), 18.8, 18.0, 13.7, 12.0; ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.75.

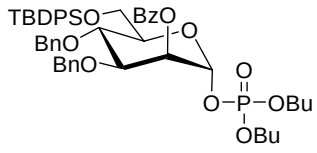
Synthesis of Glycosyl Phosphates: General Procedure B.
Suitably protected 1,2-orthoester (1.0 equiv) and activated molecular sieves were mixed under argon. CH_2Cl_2 (1 mL/0.10 mmol 1,2-orthoester) was added, and the mixture was stirred at room temperature for 15 min. Dibutyl phosphate (3 equiv) was added dropwise within 30 min. After completion of the reaction (TLC analysis), the reaction was cooled to 0°C and triethylamine (4 equiv) was added. The solution was warmed to room temperature and filtered off through a pad of Et_3N-deactivated silica gel. The resulting mixture was purified by flash silica column chromatography.



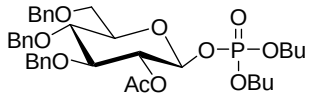
235. General procedure B with orthoester **225**[28] (200 mg, 0.35 mmol), dibutyl phosphate (210 µL, 1.05 mmol), 4Å MS (200 mg), CH_2Cl_2 (3.5 mL), room temperature, 30 min gave **235** (253 mg, 97%).
General procedure B with orthoester **225** (2.60 g, 4.57 mmol), dibutyl phosphate (2.72 mL, 13.72 mmol), 4Å MS (1.5 g), CH_2Cl_2 (10 mL), room temperature, 50 min gave **235** (2.93 g, 86%). R_f 0.61 (Hexanes/EtOAc = 1 : 1); $[\alpha]_D^{r.t.}$ = -3.8 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.09 (d, *J* = 7.2 Hz, 2 H arom.), 7.57 (t, *J* = 7.2 Hz, 1 H arom.), 7.41-7.22 (m, 17 H arom.), 5.83 (dd, *J* = 6.3, 2.1 Hz, 1 H, H-C(1)), 5.71 (d, *J* = 2.4 Hz, 1 H, H-C(2)), 4.91 (AB, *J* = 10.8 Hz, 1 H, C*H*HPh), 4.83 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh), 4.76 (AB, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.62 (AB, *J* = 11.4 Hz, 1 H, C*H*HPh), 4.60 (AB, *J* = 11.4 Hz, 1 H, C*H*HPh), 4.56 (AB, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.23-4.03 (m, 7 H, H-C(3), H-C(4), H-C(5), 2x $CH_2CH_2CH_2CH_3$), 3.95 (dd, *J* = 10.8, 3.3 Hz, 1 H, Ha-C(6)), 3.79 (dd, *J* = 11.1, 1.5 Hz, 1 H, Hb-C(6)), 1.68 (m, 4 H, $CH_2CH_2CH_2CH_3$), 1.42 (m, 4H, $CH_2CH_2CH_2CH_3$), 0.96 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$), 0.95 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 165.5, 138.5, 138.4, 137.9, 133.6, 130.2-127.8 (20 C ar.), 95.9 (d, $^2J_{C,P}$ = 5.5 Hz, 1 C, C(1)), 77.5, 75.6, 74.0, 73.8, 73.7, 72.0, 68.9, 68.7, 68.2 (d, $^2J_{C,P}$ = 4.3 Hz, 2 C, $CH_2CH_2CH_2CH_3$), 32.4 (d, $^3J_{C,P}$ = 6.1 Hz, 2 C, $CH_2CH_2CH_2CH_3$), 18.9, 13.8; ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.25; IR (film): 3007, 2963, 2874, 1724, 1603, 1496, 1453, 1362, 1268, 1167, 1095, 1028, 961, 913 cm^{-1}. Anal. Calcd for $C_{42}H_{51}O_{10}P$; C, 67.55; H, 6.88; P, 4.15.

[27] Hölemann, A.; Seeberger P. H. unpublished results.
[28] Prepared in a similar fashion as the *n*-pentenyl-analogue in Fraser-Reid, B.; Udodung, U. E.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S:; Roberts, C.; Madsen, R. *Synlett* **1992**, 927.

Found: C, 67.50; H, 6.94; P, 4.21; HRMS-ESI Calcd for $C_{42}H_{51}O_{10}PNa$ 769.3112 (M+Na): Found: 769.3098.

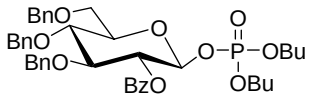


237. General procedure B with orthoester **227**[29] (150 mg, 0.20 mmol), dibutyl phosphate (120 µL, 0.60 mmol), 4Å MS (200 mg), CH_2Cl_2 (2 mL), room temperature, 40 min gave **237** (159 mg, 89%). R_f 0.59 (Hexanes/EtOAc = 6 : 4); $[\alpha]_D^{r.t.}$ = -15.4 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.19 (dd, *J* = 7.2, 1.2 Hz, 2 H, H_o Bz), 7.78 (dd, *J* = 6.0, 1.8 Hz, 2 H, H_oa TBDPS), 7.75 (dd, *J* = 6.9, 1.2 Hz, 2 H, H_ob TBDPS), 7.59 (tt, *J* = 7.5, 1.2 Hz, 1 H, H_p Bz), 7.45-7.22 (m, 18 H), 5.89 (d, *J* = 6.0, 2.1 Hz, 1 H, H-C(1)), 5.78 (t, *J* = 2.1 Hz, 1 H, H-C(2)), 5.03 (AB, *J* = 10.8 Hz, 1 H, C*H*HPh), 4.87 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh), 4.76 (AB, *J* = 10.5 Hz, 1 H, CH*H*Ph), 4.66 (AB, *J* = 11.4 Hz, 1 H, CH*H*Ph), 4.43 (t, *J* = 9.6 Hz, 1 H, H-C(4)), 4.21-3.90 (m, 8 H, H-C(3), H-C(5), Ha-C(6), Hb-C(6), 2 x $OCH_2CH_2CH_2CH_3$), 1.75-1.56 (m, 4 H, $OCH_2CH_2CH_2CH_3$), 1.50-1.28 (m, 4 H, $OCH_2CH_2CH_2CH_3$), 1.16 (s, 9 H, *t*-Bu), 0.95 (t, *J* = 7.2 Hz, 3 H, $OCH_2CH_2CH_2CH_3$), 0.88 (t, *J* = 7.2 Hz, 3 H, $OCH_2CH_2CH_2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 165.3, 138.3, 137.7, 135.8, 135.5, 133.4, 133.3, 132.7, 130.0, 129.7, 129.6, 129.5, 128.4, 128.3, 128.1, 127.8, 127.6, 96.7 (d, $^2J_{C,P}$ = 5.3 Hz, 1 C, C(1)), 75.6, 74.2, 73.4, 72.0, 68.9, 68.8, 67.9 (d, $^2J_{C,P}$ = 5.5 Hz, 2 C, $CH_2CH_2CH_2CH_3$), 62.1, 32.3 (d, $^3J_{C,P}$ = 6.7 Hz, 2 C, $CH_2CH_2CH_2CH_3$), 27.0, 19.6, 18.9, 13.7; ^{31}P-NMR (121MHz, $CDCl_3$): δ (ppm) -2.38; IR (film): 3008, 2962, 2872, 1724, 1492, 1453, 1428, 1362, 1268, 1169, 1113, 1027, 959, 910, 823, 614 cm^{-1}; HRMS-ESI Calcd for $C_{51}H_{63}O_{10}PSiNa$ 917.3820 (M+Na): Found: 917.3803.



238. General procedure B with orthoester **228**[30] (150 mg, 0.3 mmol), dibutyl phosphate (180 µL, 0.9 mmol), 4Å MS (150 mg), CH_2Cl_2 (3 mL), room temperature, 1.5 h gave **238** (170 mg, 83%).

General procedure A with orthoester **228** (75 mg, 0.15 mmol), dibutyl phosphate (90 µL, 0.45 mmol), 4Å MS (80 mg), CH_2Cl_2 (1.5 mL), room temperature, 30 min gave **238** (91 mg, 89%). R_f 0.54 (Hexanes/EtOAc = 1 : 1); $[\alpha]_D^{r.t.}$ = 16.7 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.34-7.18 (m, 15 H arom.), 5.20 (t, *J* = 7.5 Hz, 1 H), 5.10 (t, *J* = 8.1 Hz, 1 H), 4.82 (AB, *J* = 11.7 Hz, 1 H, C*H*HPh), 4.80 (AB, *J* = 10.8 Hz, 1 H, C*H*HPh), 4.68 (AB, *J* = 11.7 Hz, 1 H, CH*H*Ph), 4.61 (AB, *J* = 11.7 Hz, 1 H, C*H*HPh), 4.59 (AB, *J* = 10.8 Hz, 1 H, CH*H*Ph), 4.51 (AB, *J* = 12.0 Hz, 1 H, CH*H*Ph), 4.11-3.95 (m, 4 H), 3.85-3.67 (m, 4 H), 3.62-3.57 (m, 1 H), 1.97 (s, 3 H), 1.68-1.58 (m, 4 H, $CH_2CH_2CH_2CH_3$), 1.42-1.33 (m, 4H, $CH_2CH_2CH_2CH_3$), 0.93 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$), 0.90 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 169.6, 138.2, 138.1, 137.9, 128.7-127.9 (15 C ar.), 96.7 (d, $^2J_{C,P}$ = 4.8 Hz, 1 C, C(1)), 82.6, 77.5, 75.8, 75.4, 75.3, 73.7, 73.3, 68.4, 68.1 (d, $^2J_{C,P}$ = 6.0 Hz, 2 C, $CH_2CH_2CH_2CH_3$), 32.2 (d, $^3J_{C,P}$ = 6.1 Hz, 2 C, $CH_2CH_2CH_2CH_3$), 21.0, 18.8 13.8; ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.13; IR (film): 3005, 2964, 2872, 1749, 1492, 1451, 1364, 1267, 1082, 1046, 903, 631 cm^{-1}.



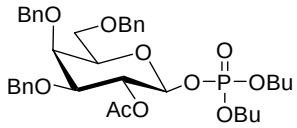
239. General procedure B with orthoester **229**[31] (114 mg, 0.2 mmol), dibutyl phosphate (120 µL, 0.6 mmol), 4Å MS (100 mg), CH_2Cl_2 (2

[29] Prepared in a similar fashion as the *n*-pentenyl-analogue in Udodung, U. E.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1993**, *115*, 7886.

[30] Boren, H. B.; Ekborg, G.; Eklind, K.; Garegg, P. J.; Pilotti, A.; Swahn, C. –J. *Acta Chem. Scand.* **1973**, *27*, 2639.

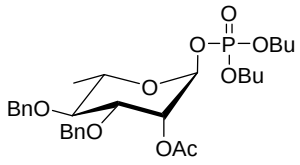
[31] Ekborg, G.; Glaudemans, C. P.J. *Carbohydr. Res.* **1984**, *129*, 287.

mL), room temperature, 30 min gave **239** (149 mg, quant). R_f 0.64 (Hexanes/EtOAc = 1 : 1); $[\alpha]_D^{r.t.}$ = 26.9 (*c* = 2.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.05 (d, *J* = 8.0 Hz, 2 H arom.), 7.58 (t, *J* = 7.8 Hz, 1 H arom.), 7.44 (t, *J* = 7.8 Hz, 2 H arom.), 7.36-7.20 (m, 15 H arom.), 5.44-5.37 (m, 2 H), 4.85 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh), 4.78 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh), 4.68 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh), 4.64 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh), 4.63 (AB, *J* = 10.5 Hz, 1 H, C*H*HPh), 4.54 (AB, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.12-4.01 (m, 4 H), 3.96-3.64 (m, 5 H), 1.73-1.57 (m, 2 H), 1.48-1.24 (m, 4H), 1.09-0.94 (m, 2 H), 0.90 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$), 0.69 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 165.0, 137.8, 137.7, 137.5, 133.3, 129.8-127.7 (20 C ar.), 96.7, 82.1, 77.5, 75.8, 75.2, 73.6, 73.4, 68.3, 68.0, 67.8, 32.2, 31.9, 18.7, 18.4, 13.8, 13.5; ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.25; IR (film): 3007, 2963, 2875, 1230, 1602, 1496, 1453, 1361, 1266, 1094, 1070, 1029, 908, 647 cm^{-1}.

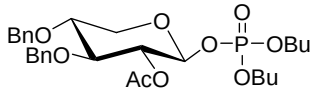


240. General procedure B with orthoester **230**[32] (100 mg, 0.2 mmol), dibutyl phosphate (120 µL, 0.6 mmol), 4Å MS (250 mg), CH_2Cl_2 (2 mL), room temperature, 1 h gave **240** (126 mg, 92%).

General procedure A with orthoester **230** (76 mg, 0.15 mmol), dibutyl phosphate (90 µL, 0.45 mmol), 4Å MS (80 mg), CH_2Cl_2 (1.5 mL), room temperature, 30 min gave **240** (92 mg, 90%). R_f 0.47 (Hexanes/EtOAc = 1 : 1); $[\alpha]_D^{r.t.}$ = 10.0 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.37-7.24 (m, 15 H arom.), 567 (AB, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.59 (AB, *J* = 11.4 Hz, 1 H, CH*H*Ph), 4.51 (AB, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.43 (AB, *J* = 12.0 Hz, 2 H, CH*H*Ph), 4.06-3.92 (m, 5 H), 3.72-3.52 (m, 4 H), 2.03 (s, 3 H), 1.65-1.54 (m, 4 H, $CH_2CH_2CH_2CH_3$), 1.43-1.26 (m, 4H, $CH_2CH_2CH_2CH_3$), 0.91 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$), 0.87 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_2CH_2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 169.3, 138.2, 137.6, 128.4-127.5 (15 C ar.), 97.0, 79.7, 77.3, 74.7, 74.2, 73.6, 72.3, 72.2, 71.3, 68.0, 32.2, 21.1, 18.7 13.7; ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.42. IR (film): 3008, 2961, 2874, 1750, 1496, 1454, 1368, 1265, 1103, 1059, 1028, 909, 866, 600 cm^{-1}.



242. General procedure B with orthoester **232**[33] (100 mg, 0.25 mmol), dibutyl phosphate (150 µL, 0.75 mmol), 4Å MS (100 mg), CH_2Cl_2 (3 mL), room temperature, 3 h, gave **242** (132 mg, 92%). R_f 0.22 (Hexanes/EtOAc = 3 : 1); $[\alpha]_D^{r.t.}$ = -18.1 (*c* = 3.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, *J* = 7.5 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H), 1.33 (d, *J* = 6.3 Hz, 3H), 1.33-1.47 (m,4H), 1.66 (tt, *J* = 6.6, 6.6 Hz, 4H), 3.47 (t, *J* = 9.6 Hz, 1H), 3.93-4.09 (m, 7H), 4.53 (d, *J* = 11.1 Hz, 1H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.71 (d, *J* = 11.1 Hz, 1H), 4.92 (d, *J* = 11.5 Hz, 1H), 5.43 (dd, *J* = 3.0, 2.1 Hz, 1H), 5.56 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.25-7.38 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 18.0, 18.8, 21.1, 32.5, 67.8, 68.5, 69.7, 71.9, 75.5, 77.1, 79.3, 95.5, 127.7-128.3, 137.5, 138.1, 169.7; ^{31}P NMR (121 MHz, $CDCl_3$) δ –2.22; IR (film): 2961, 1749, 1456, 1372, 1279, 1231, 1167, 1110, 1063, 1025, 958, 736 cm^{-1}. Anal. Calcd for $C_{30}H_{43}O_9P$; C, 62.27; H, 7.49; P, 5.35. Found: C, 62.25; H, 7.47; P, 5.39.

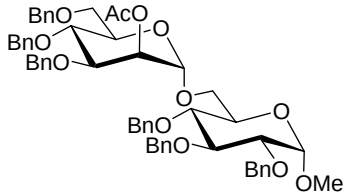


243. General procedure B with orthoester **233**[34] (103 mg, 0.27 mmol), dibutyl phosphate (150 µL, 0.75 mmol), 4Å MS (100 mg), CH_2Cl_2 (3 mL), room temperature, 1 h, gave **243** (131 mg, 90%) . R_f

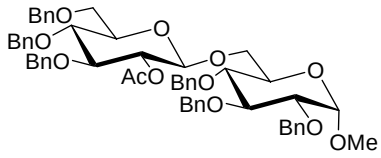
[32] Asai, N.; Fusetani, N.; Matsunaga, S. *J. Nat. Prod.* **2001**, *64*, 1210.
[33] Demetzos, C.; Skaltsounis, A. L.; Razanamahefa, B.; Tillequin, F. *J. Nat. Prod.* **1994**, *57,* 1234.
[34] Lichtenthaler, F. W.; Metz, T. *Eur. J. Org. Chem.* **2003**, 3081-3093.

0.26 (Hexanes/EtOAc = 1 : 1); $[\alpha]_D^{r.t.}$ = +2.4 (*c* = 1.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.2 Hz, 6H), 1.31-1.42 (m, 4H), 1.56-1.68 (m, 4H), 1.97 (s, 3H), 3.41 (dd, *J* = 12.0, 8.4 Hz, 1H), 3.62 (t, *J* = 7.8 Hz, 1H), 3.60-3.70 (m, 1H), 3.92-4.07 (m, 1H+4H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.80 (d, *J* = 12.0 Hz, 1H), 4.99 (dd, *J* = 7.8, 7.8 Hz, 1H), 5.20 (dd, *J* = 6.6 Hz, 1H), 7.25-7.39 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 18.8, 21.0, 32.3, 93.9, 68.1, 72.0, 73.3, 74.7, 76.8, 79.8, 97.1, 127.9-128.7, 137.9, 138.2, 169.6; ^{31}P NMR (121 MHz, $CDCl_3$) δ –2.15; IR (film): 2961, 1749, 1496, 1454, 1372, 1281, 1231, 1028, 911 cm^{-1} Anal. Calcd for $C_{29}H_{41}O_9P$; C, 61.69; H, 7.32; P, 5.49. Found: C, 61.43; H, 7.29; P, 5.67.

Crude Glycosyl Phosphate Couplings: General Procedure C.
Crude glycosyl phosphate (1.2 equiv) and acceptor (1.0 equiv) were combined and azeotropically dried with toluene (3 x 5 mL) followed by 1 h under vacuum. The mixture was dissolved in CH_2Cl_2 (2 mL/0.10 mmol acceptor) and cooled to -30°C for 15 min before trimetylsilyltriflate (1.2 equiv) was added dropwise. After completion of the reaction (TLC analysis) triethylamine (2 equiv) was added. The solution was warmed to room temperature, the solvent was removed and the resulting mixture was purified by flash silica gel chromatography.

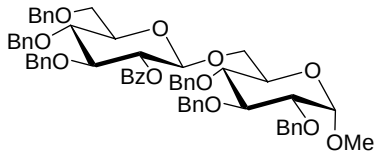


245. General procedure C with donor **223** (205 mg, 0.30 mmol), acceptor **244** (116 mg, 0.25 mmol), and TMSOTf (58 µL, 0.30 mmol), 30 min afforded **245**[35] (230 mg, 98%) as a colorless oil after flash silica gel column chromatography (25% EtOAc/Hexane) R_f 0.64.; $[\alpha]_D^{r.t.}$ = 38.5 (*c* = 1.0, $CHCl_3$); ^1H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.40-7.13 (m, 30 H arom.), 5.40 (s, 1 H), 5.01 (AB, *J* = 10.8 Hz, 1 H, C*H*HPh), 4.91-4.40 (m, 5 H), 4.60 (d, *J* = 3.3 Hz, 1 H), 4.00 (t, *J* = 9.6 Hz, 1 H), 3.96-3.53 (m, 10 H), 3.45 (t, *J* = 9.3 Hz, 1 H), 3.33 (s, 3 H), 2.15 (s, 3 H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 170.2, 138.6, 138.4, 138.1, 138.0, 137.7, 128.4-127.5 (30 C ar.), 98.0, 97.8, 82.1, 80.1, 77.6, 75.8, 75.1, 75.0, 74.2, 73.4, 71.6, 71.5, 69.7, 68.7, 68.5, 66.1, 55.2, 21.3. IR (film): 3067, 3008, 2927, 2862, 1743, 1600, 1496, 1454, 1363, 1248, 1139, 1089, 1046, 1028, 911, 600 cm^{-1}.



246. General procedure C with building block **238** (205 mg, 0.30 mmol), acceptor **244** (116 mg, 0.25 mmol), and TMSOTf (58 µL, 0.30 mmol), 30 min afforded of **246**[36] (228 mg, 97%) as a colorless oil after flash silica gel column chromatography (45% EtOAc/Hexane) R_f 0.76. $[\alpha]_D^{r.t.}$ = 9.7 (*c* = 1.0, $CHCl_3$); ^1H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.38-7.18 (m, 30 H arom.), 5.08 (t, *J* = 8.1 Hz, 1 H), 4.99 (AB, *J* = 10.8 Hz, 1 H, CH*H*Ph), 4.87-4.78 (m, 4 H), 4.83 (d, *J* = 3.6 Hz, 1 H), 4.67 (AB, *J* = 11.4 Hz, 1 H, CH*H*Ph), 4.66 (AB, *J* = 11.5 Hz, 1 H, CH*H*Ph), 4.62-4.52 (m, 5 H), 4.42 (d, *J* = 7.8 Hz, 1 H), 4.14-4.10 (m, 1 H), 3.99 (t, *J* = 9.3 Hz, 1 H), 3.80-3.65 (m, 6 H), 3.57-3.44 (m, 3 H), 3.37 (s, 3 H), 1.90 (s, 3 H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 169.1, 138.8, 138.2, 138.1, 137.7, 128.4-127.5 (30 C ar.), 100.9, 98.0, 83.1, 82.1, 79.8, 78.1, 77.8, 75.7, 75.4, 75.1, 74.9, 73.5, 73.0, 69.7, 68.8, 68.0, 55.2, 21.1. IR (film): 3067, 3008, 2932, 2862, 1748, 1496, 1454, 1362, 1154, 1066, 1028, 913, 600 cm^{-1}.

[35] Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447.
[36] Shi, L.; Kim, Y.-L.; Gin, D. Y. *J. Am. Chem. Soc.* **2001**, *123*, 6940.



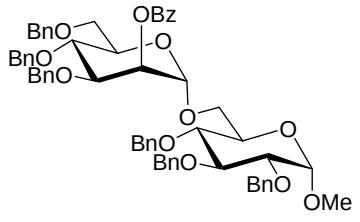
247. General procedure C with donor **239** (150 mg, 0.20 mmol), acceptor **244** (77 mg, 0.17 mmol), and TMSOTf (39 µL, 0.20 mmol), 40 min afforded **247** (155 mg, 91%) as a colorless oil after flash silica gel column chromatography (45% EtOAc/Hexane) R_f 0.77; $[\alpha]_D^{r.t.}$ = 22.6 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.95 (d, *J* = 7.8 Hz, 2 H), 7.46 (t, *J* = 6.9 Hz, 1 H), 7.34-7.02 (m, 32 H arom.), 5.37 (t, *J* = 8.4 Hz, 1 H), 4.89 (AB, *J* = 10.8 Hz, 1 H, CH*H*Ph), 4.83 (AB, *J* = 10.8 Hz, 1 H, C*H*HPh), 4.77-4.54 (m, 8 H), 4.60 (d, *J* = 3.6 Hz, 1 H), 4.49 (d, *J* = 3.3 Hz, 1 H), 4.45 (AB, *J* = 11.1 Hz, 1 H, CH*H*Ph), 4.29 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh), 4.17-4.13 (m, 1 H), 3.92-3.65 (m, 7 H), 3.60-3.56 (m, 1 H), 3.50-3.36 (m, 2 H), 3.21 (s, 3 H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 164.9, 138.8, 138.2, 138.1, 137.8, 137.7, 132.9, 129.8, 129.7, 128.3-127.3 (34 C ar.), 101.2, 97.9, 82.7, 81.8, 79.6, 78.0, 75.0, 74.6, 73.6, 73.4, 73.3, 69.4, 68.8, 68.0, 54.9. IR (film): 3067, 3008, 2932, 2928, 2872, 1729, 1600, 1496, 1453, 1360, 1267, 1154, 1092, 1070, 1028, 913 cm^{-1}.

One-pot Glycosylation: General Procedure D.

Suitably protected 1,2-orthoester (1.2 equiv) and activated molecular sieves were mixed under argon. CH_2Cl_2 (1 mL/0.10 mmol 1,2-orthoester) was added, and the mixture was stirred at room temperature for 15 min. Dibutyl phosphate (3.6 equiv) was added dropwise within 30 min. After completion of the reaction (TLC analysis), a solution of the acceptor (1.0 equiv) in CH_2Cl_2 (1 mL/0.01 mmol acceptor) was added dropwise and the mixture was cooled to -30°C. After the mixture was stirred for 15 min, TMSOTf (3.6 equiv) was added. After completion of the reaction (TLC analysis), triethylamine (4 equiv) was added. The solution was warmed to room temperature and filtered off through a pad of Et_3N-deactivated silica gel. The resulting mixture was purified by flash silica column chromatography.

One-pot Glycosylation: General Procedure E.

1,2-Orthoester (1.2 equiv) and acceptor (1.0 equiv) were combined and azeotropically dried with toluene (3 x 5 mL) followed by 1 h under vacuum. Activated molecular sieves were mixed under argon, the mixture was dissolved in CH_2Cl_2 (2 mL/0.10 mmol acceptor) at room temperature. After the mixture was stirred for 15 min, dibutyl phosphate (3.6 equiv) was added dropwise within 30 min. After the formation of the desired glycosyl phosphate (TLC analysis), the reaction mixture was cooled to -30°C and TMSOTf (3.6 equiv) was added dropwise. After completion of the reaction (TLC analysis), triethylamine (4 equiv) was added. The solution was warmed to room temperature and filtered off through a pad of Et_3N-deactivated silica gel. The resulting mixture was purified by flash silica column chromatography.

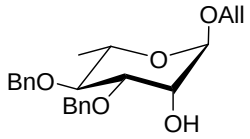


248. General Procedure D with 1,2-orthoester **225** (170 mg, 0.30 mmol), dibutyl phosphate (178 µL, 0.90 mmol), acceptor **244** (116 mg, 0.25 mmol), and TMSOTf (174 µL, 0.90 mmol) afforded 245 mg (98%) of **248**[37] as colorless oil after flash silica gel chromatography (30% EtOAc/Hexane) R_f 0.71.

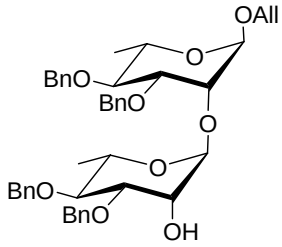
General Procedure E with 1,2-orthoester **225** (142 mg, 0.25

[37] Mathew, F.; Mach, M.; Hazen, K.C; Fraser-Reid, B. *Tetrahedron Lett.* **2003**, *44*, 9051.

mmol), acceptor **244** (97 mg, 0.21 mmol), dibutylphosphate (149 µL, 0.75 mmol), and TMSOTf (145 µL, 0.75 mmol) afforded 208 mg (99%) of **248** as colorless oil after flash silica gel chromatography.

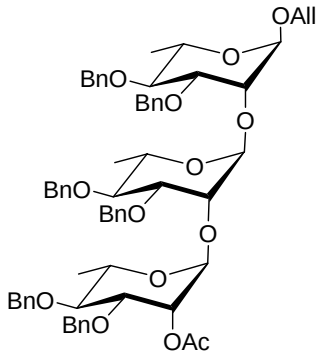


249. To a solution of rhamnose orthoester **232** (0.91 g, 2.27 mmol), allyl alcohol (2.3 mL, 33.9 mmol) in dry CH_2Cl_2 (9 mL) was added $BF_3 \cdot Et_2O$ (114 µL, 0.90 mmol) at room temperature in the presence of activated powdered 4Å molecular sieves (1 g). After stirring for 3 h, the mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was then washed with saturated aqueous $NaHCO_3$ solution, water and dried over Na_2SO_4. The solvents were removed in *vacuo* and the residue was dissolved in a solution of NaOMe (11.8 mg, 0.2 mmol) in MeOH (6 mL). The mixture was stirred then at room temperature for ca. 12 h. The solvents were then removed in *vacuo* and the residue was purified by silica gel column chromatography to give **245**[38] as a syrup (793 mg, 91% 2 steps). R_f 0.22 (Hexanes/EtOAc = 4 : 1).$[\alpha]_D^{rt}$ = -32.1 (*c* = 1.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, *J* = 6.3 Hz, 3H), 2.55 (d, *J* = 2.1 Hz, 1H), 3.48 (t, *J* = 9.3 Hz, 1H), 3.72-3.82 (m, 1H), 3.89 (dd, *J* = 9.3, 6.3 Hz, 1H), 3.94-4.01 (m, 1H), 4.06-4.09 (m, 1H), 4.14-4.20 (m, 1H), 4.65 (d, *J* = 10.8 Hz, 1H), 4.71 (s, 2H), 4.87 (d, *J* = 1.5 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 5.18-5.32 (m, 2H), 5.83-5.96 (m, 1H), 7.26-7.39 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.1, 67.6, 68.0, 68.7, 72.2, 75.6, 80.2, 80.3, 98.3, 117.6, 127.9-128.7 (CH-Aryl), 134.0, 138.1, 138.6; IR (film): 3459, 3063, 2910, 1497, 1364, 1211, 1061, 922, 737 cm^{-1}. Anal. Calcd for $C_{23}H_{28}O_5$; C, 71.85; H, 7.34. Found: C, 71.81; H, 7.53.

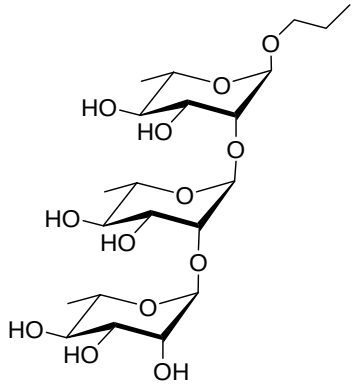


250. A solution of rhamnoside **249** (226 mg, 0.59 mmol) and rhamnose orthoester **232** (260 mg, 0.65 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min in the presence of activated 4Å MS (300 mg) and was then added dibutyl phosphate (385 µL, 1.94 mmol) dropwise. After stirring for 3 h, the mixture was cooled to -40 °C and TMSOTf (354 µL, 1.94 mmol) added. The glycosylation went to completion in 30 min and Et_3N (1 mL) was added to quench the excess acid. The mixture was filtered through a pad of Celite and the filtrate was concentrated in *vacuo*. The residue was then purified by silica gel column chromatography to give allyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (384 mg, 88%). The dirhamnoside (384 mg, 0.51 mmol) was then dissolved in a solution of NaOMe in MeOH (0.01 M, 3 mL) at room temperature. After stirring for 12 h, the solvent was removed in *vacuo* and the residue was purified by silica gel column chromatography to give **250**[39] (340 mg, 96%) as a white solid. R_f 0.30 (Hexanes/EtOAc = 3 : 1). $[\alpha]_D^{rt}$ = -35.9 (*c* = 0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (d, *J* = 6.3 Hz, 6H), 2.43 (d, *J* = 1.8 Hz, 1H), 3.39 (t, *J* = 9.3 Hz, 1H), 3.47 (t, *J* = 9.3 Hz, 1H), 3.64-3.96 (m, 5H), 4.03-4.15 (m, 3H), 4.58-4.72 (m, 6H), 4.76 (d, *J* = 1.8 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 5.08 (d, *J* = 1.5 Hz, 1H), 5.14-5.25 (m, 2H), 5.79-5.92 (m, 1H), 7.25-7.39 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.1, 18.3, 67.9, 68.2, 68.9, 72.4, 72.5, 74.8, 75.6, 75.7, 79.8, 80.1, 80.3, 80.6, 98.2, 101.0, 117.5, 127.9-128.9 (CH-Aryl), 134.1, 138.2, 138.5, 138.6, 138.8; IR (film): 3477, 3005, 2930, 1497, 1389, 1078, 985, 912 cm^{-1}. HRMS-MALDI (*m/z*): $[M + Na]^+$ Calcd for $C_{43}H_{50}O_9$, 710.3455; Found: 710.3453.

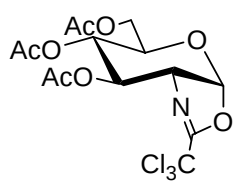
[38] Bousqueta, E.; Khitri, M.; Lay, L.; Nicotra, L.; Panza, L.; Russo, R. *Carbohydr. Res.* **1998**, *311*, 171-181.
[39] Bélot, F.; Wright, K.; Costachel, C.; Phalipon, A.; Mulard, L. A. *J. Org. Chem.* **2004**, *69*, 1060-1074.



251. A solution of dirhamnoside **250** (192 mg, 0.27 mmol) and rhamnose orthoester **232** (120 mg, 0.30 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 30 min in the presence of activated 4Å MS (200 mg) and was then added dibutyl phosphate (178 μL, 0.90 mmol) dropwise. After stirring for 3 h, the mixture was cooled to -40 °C and TMSOTf (164 μL, 0.90 mmol) was added. The glycosylation was completed in 30 min and Et_3N (0.5 mL) was added to quench the excess acid. The mixture was filtered through a pad of Celite and the filtrate was concentrated in *vacuo*. The residue was then purified by silica gel column chromatography to give **251** (261mg, 87%) as a syrup. R_f 0.55 (Hexanes/EtOAc = 3 : 1); $[\alpha]_D^{r.t.}$ = -26.1 (*c* = 2.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (d, *J* = 6.3 Hz, 3H), 1.26 (d, *J* = 6.3 Hz, 6H), 2.13 (s, 3H), 3.35 (t, *J* = 9.2 Hz, 1H), 3.40 (t, *J* = 9.3 Hz, 1H), 3.44 (t, *J* = 9.3 Hz, 1H), 3.61-3.98 (m, 8H), 4.06-4.11 (m, 2H), 4.52-4.75 (m, 10H), 4.84-4.91 (m, 2H), 4.99 (d, *J* = 1.7 Hz, 1H), 5.07 (d, *J* = 1.8 Hz, 1H), 5.16 (ddt, *J* = 10.4, 2.9, 1.3 Hz, 1H), 5.22 (ddt, *J* = 17.0, 2.9, 1.3 Hz, 1H), 5.54 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.78-5.88 (m, 1H), 6.98-7.37 (m, 30H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.9, 18.0, 21.1, 67.6, 68.0, 68.3, 68.5, 69.0, 71.8, 72.1, 72.2, 74.5, 74.7, 75.2, 75.3, 75.4, 77.2, 77.8, 79.1, 79.6, 80.1, 80.2, 80.3, 97.9, 99.1, 100.4, 117.2, 127.4-128.4, 133.8, 138.1, 138.3, 138.5, 138.5, 138.5, 138.6, 170.0; IR (film): 3025, 2913, 1744, 1492, 1451, 1364, 1077, 918 cm^{-1}. HRMS-MALDI Calcd for $C_{65}H_{74}O_{14}Na$ 1101.4971 (M+Na): Found: 1101.495.



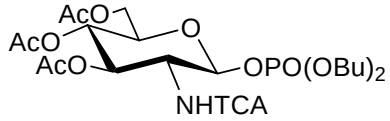
252. Tri-rhamnoside **251** (23 mg, 21.3 μmol) was dissolved in a solution of NaOMe in MeOH (0.01 M, 1 mL) and stirred at room temperature for 10 h. Afterwards, the solvent was removed in *vacuo* and the residue was passed through a pad of silica gel to give allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (21 mg). The tri-rhamnoside was then dissolved in a mixture of THF/MeOH/H_2O (5:5:1, 5 mL) and stirred in presence of Pd/C (30 mg) under H_2 atmosphere for 15 h. The mixture was then filtered through a pad of Celite and the solvents were evaporated in *vacuo*. Subsequent lyophilization gave **252** (11 mg, 98% 2 steps) as a white solid, ^{1}H NMR (500 MHz, CD_3OD) δ 0.88 (t, *J* = 7.5 Hz, 3H), 1.17 (d, *J* = 6.5 Hz, 3H), 1.18 (d, *J* = 6.5 Hz, 3H+3H), 1.50-1.58 (m, 2H), 3.24-3.33 (m, 3H), 3.47-3.73 (m, 9H), 3.91 (dd, *J* = 3.5, 2.0 Hz, 1H), 3.94 (dd, *J* = 3.5, 2.0 Hz, 1H), 4.71 (d, *J* < 1Hz, 1H), 4.86 (d, *J* = 1.5 Hz, 1H), 5.01 (d, *J* = 1.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 9.83, 16.7, 16.9, 16.9, 22.6, 68.7, 69.1, 69.1, 70.7, 70.8, 70.9, 71.1, 72.8, 73.0, 73.1, 78.7, 79.2, 99.1, 101.5, 102.7; HRMS-ESI Calcd for $C_{21}H_{38}O_{13}Na$ 521.22046 (M+Na): Found: 521.22043.



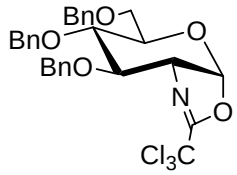
255. 1.872 g (3.80 mmol) of glucosamine derivative **253**[40] were dissolved into 2.5 mL of CH_2Cl_2 and to this solution 1.7 mL of HBr (33% solution in AcOH) were admixed at room temperature. After three hours stirring, the solvent was removed, the residual syrup was dissolved in ethyl ether and the solution was evaporated again. Glucosamine

[40] a) Silva, D. J.; Wang, H.; Allanson, N. M.; Jain, R. K.; Sofia, M. J. *J. Org. Chem.* **1999**, *64*, 5926; b) Dempsey, A. M.; Hough, L. *Carbohydr. Res.* **1975**, *41*, 63.

bromide **254** was directly used in the following step. Crude **254** together with 1,220 g (3.80 mmol) of TBAB were coevaporated in dry toluene. The mixture was dissolved in 1.5 mL of dry CH_2Cl_2 and then 663 μL (5.70 mmol) of lutidine were added at room temperature. After reaction completion (TLC analysis), the reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic phase was concentrated and purified on silica gel by column chromatography furnishing oxazoline **255** (1.430 g, 3.31 mmol) in 87% yield over two steps. $[\alpha]_D^{r.t.}$ = 21.4 (*c* = 2.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 6.29 (t, *J* = 7.5, 1 H, H-C(1)), 5.34 (t, *J* = 2.4 Hz, 1 H, H-C(3)), 4.89 (dt, *J* = 8.1, 1.8 Hz, 1 H, H-C(4)), 4.43 (dq, *J* = 7.5, 1.2 Hz, 1 H, H-C(2)), 4.23 (dd, *J* = 12.0, 3.0 Hz, 1 H, Ha-C(6)), 4.13 (dd, *J* = 12.0, 5.7 Hz, 1 H, Hb-C(6)), 3.75-3.69 (m, 1 H, H-C(5)), 2.09 (s, 3 H, CH_3 Ac), 2.04 (s, 3 H, CH_3 Ac), 2.03 (s, 3 H, CH_3 Ac); ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 170.6, 169.6, 169.1, 162.9, 103.3, 77.5, 69.1, 68.9, 67.8, 64.8, 63.6, 21.7, 21.1, 20.9; IR (film): 3032, 1747, 1660, 1370, 1248, 1139, 1040, 970, 909, 830, 600 cm^{-1}. HRMS-ESI Calcd for $C_{14}H_{16}NO_8Cl_3Na$ 455.6336 (M+Na): Found: 455.9801.

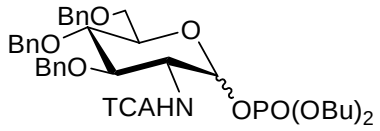


256. General procedure B with oxazoline **255** (189 mg, 0.44 mmol), dibutyl phosphate (240 μL, 1.3 mmol), 4Å MS (150 mg), CH_2Cl_2 (3 mL), room temperature, 1 h gave **256** (258 mg, 92%). $[\alpha]_D^{r.t.}$ = 5.48 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.26 (d, *J* = 9.6 Hz 1 H, NHTCA.), 5.51 (t, *J* = 8.1, 1 H, H-C(1)), 5.41 (t, *J* = 9.6 Hz, 1 H, H-C(3)), 5.04 (t, *J* = 9.6 Hz, 1 H, H-C(4)), 4.28-3.90 (m, 7 H, H-C(2), Ha-C(6), Hb-C(6) and 2 x $OCH_2CH_2CH_2CH_3$), 3.79-3.74 (m, 1 H, H-C(5)), 2.04 (s, 3 H, CH_3 Ac), 1.99 (s, 3 H, CH_3 Ac), 1.97 (s, 3 H, CH_3 Ac), 1.62-1.52 (m, 4 H, 2 x $OCH_2CH_2CH_2CH_3$), 1.38-1.28 (m, 4 H, 2 x $OCH_2CH_2CH_2CH_3$), 0.86 (t, *J* = 7.5 Hz, 3 H, $OCH_2CH_2CH_2CH_3$), 0.85 (t, *J* = 7.5 Hz, 3 H, $OCH_2CH_2CH_2CH_3$); ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.95; IR (film): 3401, 2964, 1749, 1713, 1523, 1368, 1248, 1181, 1033, 908, 842, 600 cm^{-1}. Anal. Calcd for $C_{22}H_{35}NO_{12}PCl_3$; C, 41.10; H, 5.49; N, 2.18; P, 4.82. Found: C, 41.20; H, 5.78; N, 2.19; P, 4.67; HRMS-ESI Calcd for $C_{22}H_{35}NO_{12}PCl_3Na$ 664.08547 (M+Na): Found: 664.0854.

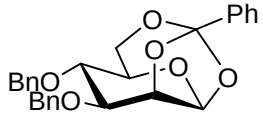


257. Oxazoline **255** (248 mg, 0.57 mmol) was dissolved into 3 mL of CH_2Cl_2/MeOH 3:1 mixture, then 12 μL of MeONa (0.5 M solution in MeOH, 5.70 μmol) were added at room temperature. After stirring overnight, the solvent was removed under reduced pressure and the resulting white solid was directly used in the following step. The triol and 200 mg of freshly activated molecular sieves were fluxed under argon atmosphere then 2 mL of dry CH_2Cl_2 were added. After q0 minutes stirring at room temperature, 68 μL (5.13 mmol) of BnBr and 1.46 g (6.84 mmol) of freshly prepared Ag_2O were added at room temperature. After reaction completion (TLC analysis), the reaction mixture was filtered through a pad of celite and the solvent was evaporated under reduced pressure. The crude was purified on silica gel by column chromatography furnishing **257** (187 mg, 0.32 mmol) in 57% yield over two steps. $[\alpha]_D^{r.t.}$ = 45.3 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.38-7.26 (m, 13 H, aromatic), 7.21-7.18 (m, 2 H, aromatic), 6.38 (t, *J* = 7.5, 1 H, H-C(1)), 4.72 (AB, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.64 (AB, *J* = 12.3 Hz, 1 H, C*H*HPh), 4.58 (AB, *J* = 12.0 Hz, 1 H, CH*H*Ph), 4.56 (AB, *J* = 11.7 Hz, 1 H, C*H*HPh), 4.50 (AB, *J* = 12.0 Hz, 1 H, CH*H*Ph), 4.49 (ddd, *J* = 0.9, 3.0, 8.0 Hz, 1 H, H-C(2)), 4.32 (AB, *J* = 12.0 Hz, 1 H, CH*H*Ph), 4.12 (t, *J* = 3.0 Hz, 1 H, H-C(4)), 3.75 (ddd, *J* = 1.2, 2.7, 8.7 Hz, 1 H, Ha-C(3)), 3.69

(m, 1 H, H-C(5)), 3.67 (m, 2 H, H-C(6a) and H-C(6b)); ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 162.5 (C oxazoline), 137.8, 137.6, 137.4 (C aromatic), 128.5, 128.3, 128.2, 128.0, 127.9, 127.7, 127.7, 127.6 (CH aromatic), 77.1 (*C*Cl_3), 75.7, 74.2, 73.2, 71.8, 71.6 (C carbinolic), 71.3, 69.2, 65.9 (benzyl); IR (film): 3008, 2923, 2867, 1657, 1496, 1454, 1363, 1318, 1138, 1096, 1072, 1028, 882, 820, 600 cm^{-1}. Anal. Calcd for $C_{29}H_{28}NO_5Cl_3$; C, 60.38; H, 4.89; N, 2.43. Found: C, 60.94; H, 5.21; N, 2.27; HRMS-ESI Calcd for $C_{29}H_{28}NO_5Cl_3Na$ 598.0925 (M+Na): Found: 598.0923.

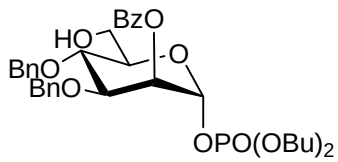


258. General procedure B with oxazoline **257** (104 mg, 0.18 mmol), dibutyl phosphate (110 μL, 0.54 mmol), 4Å MS (150 mg), CH_2Cl_2 (3 mL), room temperature, 3 h gave **257** as α/β mixture 1.4:1.(122 mg, 86%). ^{1}H-NMR (300 MHz, $CDCl_3$) significative signals: δ (ppm) 8.34 (d, *J* = 9.6 Hz 1 H, NHTCA.), 7.37-7.01 (m, 15 H aromatic), 5.75 (dd, *J* = 5.8, 3.3, 1 H, H-C(1)-α anomer), 5.51 (t, *J* = 7.7, 1 H, H-C(1)-β anomer), 4.85 (AB, *J* = 11.1 Hz, 1 H, C*H*HPh β-anomer), 4.80 (t, *J* = 10.8 Hz, 2 H, CH_2Ph α-anomer), 4.75 (AB, *J* = 11.0 Hz, 1 H, CH*H*Ph β-anomer), 4.64-4.44 (overlapped AB systems, 4 H α-anomer and 4 H β-anomer), 4.32 (tt, *J* = 10.0, 3.3 Hz, 1 H, H-C(2) α-anomer), 4.21 (t, *J* = 7.8 Hz, 1 H, H-C(2) β-anomer), 4.10-4.01 (m, 12 H, overlapped signals 2 x OCH_2CH$_2$CH$_2$CH$_3$, H-C(3) and H-C(4) α- and β- anomers), 4.00-4.39 (m, 2 H, Ha-C(6) and Hb-C(6) α anomer), 3.83-3.75 (m, 2 H, Ha-C(6) and Hb-C(6) β anomer), 3.69-3.64 (m, 2 H , H-C(5) α and β anomers), 1.71-1.55 (m, 8 H, 2 x OCH$_2$CH_2CH$_2$CH$_3$ α- and β anomers), 1.45-1.28 (m, 8 H, 2 x OCH$_2$CH$_2$CH_2CH$_3$ α- and β anomers), 0.98-0.90 (m, 12 H, 2 x OCH$_2$CH$_2$CH$_2$CH_3 α- and β-anomers); ^{13}C-NMR major anomer (125 MHz, $CDCl_3$): δ (ppm) 162.2, 138.1, 137.9 137.8, (C aromatic), 128.8, 128.6, 128.6, 128.5, 128.1, 128.1, 127.9, 127.8 (CH aromatic), 96.9 (C-1 β-anomer), 96.3(C-1 α-anomer), 77.9 (*C*Cl_3), 75.7, 75.4, 74.1, 73.2, 68.4, 68.1, 67.7, 32.4, 32.3, 18.8, 13.8; ^{31}P-NMR (121 MHz, $CDCl_3$): δ (ppm) -2.05 (α-anomer) and -3.02 (β-anomer); IR (film): 3415, 3008, 2962, 2875, 1719, 1600, 1515, 1454, 1361, 1265, 1149, 1028, 949, 822, 600 cm^{-1}. Anal. Calcd for $C_{37}H_{47}NO_9PCl_3$; C, 56.46; H, 6.02; N, 1.78; P, 3.94. Found: C, 56.74; H, 6.12; N, 1.83; P, 3.84; HRMS-ESI Calcd for $C_{37}H_{47}NO_9PCl_3Na$ 808.1946 (M+Na): Found: 808.1952.



259. 137 mg (0.46 mmol) of derivative **260** together with 120 mg of AW 300 MS were fluxed under argon atmosphere and then 2 mL of dry dichloroethane were added. After completion (8 hours at 50 °C), the solvent was evaporated and the resulting crude was dissolved in 2 mL of DMF. To this solution 64 mg of NaH and 190 μL of BnBr were admixedand the mixture was kept reacting at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl ether, the organic phase was evaporated and the residue was purified by column chromatography furnishing **259** (185 mg, 0.41 mmol) in 90% yield over two steps. $[\alpha]_D^{r.t.}$ = 2.39 (*c* = 2.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 7.71-7.68 (m, 2 H, aromatic), 7.49-7.35 (m, 13 H, aromatic), 5.89 (d, *J* = 5.7, 1 H, H-C(1)), 4.89 (AB, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.85 (AB, *J* = 12.3 Hz, 1 H, C*H*HPh), 4.84 (AB, *J* = 11.7 Hz, 1 H, CH*H*Ph), 4.69 (AB, *J* = 11.3 Hz, 1 H, C*H*HPh), 4.68 (dd, *J* = 2.4, 6.0 Hz, 1 H, H-C(2)), 4.29-4.22 (m, 3 H), 3.83-3.78 (m, 2 H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 138.3, 137.4, 129.6 (C aromatic), 128.8-128.1 (14 CH aromatic), 126.2, 122.1, 99.9, 79.5, 77.6, 74.6, 73.4, 72.6, 70.8; IR (film):

3067, 3008, 2932, 2868, 1496, 1453, 1368, 1324, 1291, 1162, 1133, 1102, 1077, 1049, 1025, 1006, 960, 917, 841, 600 cm^{-1}. Anal. Calcd for $C_{27}H_{26}O_6$; C, 72.63; H, 5.87. Found: C, 72.50; H, 5.91.



258. General procedure B with **259** (100 mg, 0.22 mmol), dibutyl phosphate (133 µL, 0.67 mmol), 4Å MS (150 mg), CH_2Cl_2 (3 mL), room temperature, 48 h gave **258** (100 mg, 68%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.09-8.05 (m, 2 H), 7.64-7.58 (m, 1 H), 7.50-7.45 (m, 2 H), 7.37-7.24 (m, 10 H), 5.76 (dd, *J* = 6.0, 2.1 Hz, 1 H, H-C(1)), 5.65 (t, *J* = 2.7 Hz, 1 H, H-C(2)), 4.92 (AB, *J* = 10.8 Hz, 1 H, C*H*HPh), 4.79 (AB, *J* = 11.4 Hz, 1 H, C*H*HPh), 4.65 (AB, *J* = 10.8 Hz, 1 H, CH*H*Ph), 4.59 (AB, *J* = 11.4 Hz, 1 H, CH*H*Ph), 4.14-3.83 (m, 5 H), 1.17-1.62 (m, 4 H, 2 x $OCH_2CH_2CH_2CH_3$), 1.47-1.35 (m, 4 H, 2 x $OCH_2CH_2CH_2CH_3$), 0.98-0.91 (m, 6 H, 2 x $OCH_2CH_2CH_2CH_3$) ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 165.1, 137.9, 137.5, 133.4, 129.8-127.7 (15 C aromatic), 95.6, 77.3, 75.4, 73.8, 73.3, 71.8, 68.6, 68.1, 67.4, 61.7, 32.4, 32.3, 18.8, 13.7; ^{31}P-NMR (121MHz, $CDCl_3$): δ (ppm) -2.45.

Conclusion

In this work several synthetic aspects of carbohydrate chemistry have been explored.
The main focus has been addressed to the development and implementation of mild glycosidation procedures. Despite the ever increasing number of glycosylating agents available, to date one of the most limiting factors for the advancement of glycochemistry is surely the lack of a generally applicable method. Furthermore, the most versatile glycosylation methods, thioglicosydes, glycosyl trihaloacetimidates and phosphate triesters, although very efficient, suffer from the use of strong Lewis and Brønsted acids as promoters (such as $BF_3 \cdot Et_2O$, TMSOTf, NIS and TfOH). These reagents not only complicate the experimental procedures because of their corrosiveness, moisture sensitivity and difficulty to be handled, but, because of their strong acidity, they also limit the pattern of protecting groups to be used. Therefore, the search of new mild glycosylation promoters, able to overcome these experimental problems, is continuously pursued by several research groups. Recently, in our laboratory some novel protocols for the mild activation of trihaloacetimidate donors have been developed. Particularly, $Yb(OTf)_3$, commercially available acid washed molecular sieves (AW 300 and 500 MS) and $Bi(OTf)_3$ proved to effectively promote the activation of variously protected glycosyl trihaloacetimidates, becoming a valid alternative to the harsh promoters classically employed. In this thesis a particular emphasis is put on the use of $Yb(OTf)_3$ and AW MS. The experimental advantages connected with the use of these cheap and easy to handle reagents had been evidenced by previous work. $Yb(OTf)_3$ was reported to be compatible with a wide range of very acid-labile protecting groups, as demonstrated by the unprecedented withstanding of the dimethoxytrityl moiety in the glycosylation medium. Whereas the insolubility of AW MS guarantees an extreme simplification of the experimental procedure, the promoters being easily removed from the reaction mixture by simple filtration.
Interestingly, the two systems exhibited different behaviours towards glycosylation stereoselectivity. $Yb(OTf)_3$ proved to be extremely versatile, being compatible with the mostly used methodologies to direct the stereochemical outcome of glycosylations, while the control of the stereochemistry in AW MS-promoted reaction could be effectively performed only exerting the anchimeric assistance of acyl protecting groups, thus limiting the application of this latter promoting system to the generation of peculiar types of glycosidic bonds.

In order to test the effectiveness of $Yb(OTf)_3$ and AW 300 MS promoting systems and contextually expand the scope of these approaches, we investigate their application to the synthesis of biologically relevant oligosaccharide antigens such as Lewis^x trisaccharide and Globo-H tetrasaccharide reducing end (*Chapter 3*). The synthesis of these oligosaccharides posed several synthetic challenges due to their structural complexity, among the others, the construction of non trivial α-L-fucosidic linkages. A preliminary investigation on the feasible use $Yb(OTf)_3$ and AW 300 MS in α-fucosylations promotion preceded the assembly of the target molecules. Once settled the suitable conditions for performing α-fucosylations, Lewis^x and Globo-H tetrasaccharide were synthesized exploiting in all glycosidation steps exclusively the mild activating systems developed in our laboratory. Moreover, the planned synthetic strategies relied on the use of building blocks conveniently prepared from cheap precursors often following unprecedented synthetic pathways. The isolation in good overall yield of both oligosaccharide targets witnessed the versatility of the proposed glycosylation protocols, evidencing , at the same time, the feasibility of the proposed strategy as a valid synthetic alternative for the obtainment of these compounds of undoubted pharmacological interest. Noteworthy, in the course of Lewis^x and Globo-H assembly we faced the need of effectively accessing to a galactosyl 1,2-orthoester as key precursor for the obtainment of a crucial building block. This experimental necessity prompted us to investigate a feasible protocol for the convenient one-pot synthesis of such a derivative. In the course of our survey a novel procedure for glycosyl iodides synthesis has been developed. Peracetylated sugars were effectively converted into the corresponding anomeric iodides by treatment with the I_2/Et_3SiH combined system, effectively used as source of HI. The iodination protocol was then applied to the one-pot synthesis of the glycosyl orthoester of our interest, that could effectively be isolated in good overall yield and with a clear simplification of the experimental procedure. The protocol was also extended to the syntheses of synthetically useful 1,2-ethylidene and 1,2-glycal derivatives (*Chapter 2*).

A further study was conducted in order to improve the control of the stereochemical outcome of $Yb(OTf)_3$-promoted α-glycosylation reactions (*Chapter 4*). Particularly, the use of dimethoxyethane (DME) as a novel α-stereodirecting solvent was proposed. Considerable improvements of α-selectivities were registered using DME as cosolvent, achieving good results even with primary acceptors as well as with amino acidic residues. Furthermore, the beneficial effect of DME as α-stereodirecting solvent was effectively proved also in TMSOTf-promoted glycosylations, thus evidencing its general applicability.

Our experience in carbohydrate elaboration exploiting mild procedures prompted the implementation of two different projects with the aim of synthesizing novel classes of glycoconjugated with pharmacological activity.
In the first project, in collaboration with dr. Pezzella of University "Federico II" of Napoli, the regioselective glycosylation of 17β-estradiol and derivatives thereof was performed (*Chapter 5*). The scope of the work was the improvement of pharmacokinetic and pharmacodinamic properties of the drugs, exerting a minimal number of chemical operation on the active molecules. Glycosylations either at the phenol or carbinol site of the unprotected hormone were carried out taking advantage of the different nature, and therefore reactivity, of the two hydroxyl groups. Phenol hydroxyl was glycosylated by glycosyl iodides (synthesized with the I_2/Et_3SiH-based protocol) in a double phase

reaction, while the carbinol hydroxyl was chemoselectively glycosylated in acidic conditions exploiting the AW 300 MS activation of glycosyl trichloroacetimidate donors. The glycoconjugated derivatives thus generated will be subjected to biological tests to evaluate the effect of the glycoconjugation on their pharmacological activity.
The aim of the second project, supported by Ministero dell'Istruzione, dell'Università e della Ricerca as a "Research Programme of Prominent National Interest", was the design, synthesis and screening of β-lactam-glycoconjugates (*Chapter 6*). Two class of novel β-lactam-glycoconjugates were synthesized. The extreme sensitivity of the chosen β-lactam structure called for the exploitation of very mild protocols for performing glycosylation and deprotection reactions. In the first class of glycoconjugates, the generation of a direct linkage between the β-lactam and the saccharidic moieties was performed. The glycosylation of the sensitive β-lactam acceptor exploited the mild activation of *N*-phenyl trifluoroacetimidate donors with ytterbium(III) triflate. In the second approach a succinyl spacer was interposed between the two cyclic molecules. The glycoconjugates thus obtained underwent hydrogenolytic deprotection of the saccharidic portions without the β-lactam moiety being affected. Evaluation of these unprecedented derivatives for antibiotic activity against resistant bacteria furnished promising results for the second type of glycoconjugates. Further studies are in progress. New β-lactam structures have been screened and will be conjugated to suitable saccharidic structures generating a second generation of antibiotic β-lactam-glycoconjugates.

From the experience matured in activating glycosyl trihalo-acetimidates donors with $Yb(OTf)_3$, a novel one-pot approach for the synthesis of oligosaccharides was developed exerting the different reactivity exhibited by glycosyl trichloro- and *N*-phenyl trifluoro-acetimidates.(*Chapter 7*). Trisaccharide structures were effectively synthesized by sequentially activating glycosyl trichloroacetimidates and the fluorinated analogues by adjusting the glycosylation conditions in due course. To the best of our knowledge, this represents the first case of trisaccharides one-pot synthesis carried out using exclusively trihaloacetimidates as donors and, furthermore, using just catalytic amounts of promoter in every glycosylation step.

In the last project a highly effective protocol to convert 1,2-glycosyl orthoesters to C2-acyl glycosyl phosphates was developed (*Chapter 8*). The synthetic scope was established by preparing a series of mannosyl, glucosyl, galactosyl, rhamnosyl, xylosyl and arabinofuranosyl phosphates in high yield. *In situ* generation of glycosyl phosphates from 1,2-orthoesters allowed simple and efficient trirhamnoside antigen assembly. Furthermore, the effective protocol thus developed was extended to the syntheses of useful glucosamine phosphates from 1,2-oxazolines and mannosyl 1,2,6 orthoester. A novel mild procedure has been developed for the synthesis of the unusual 1,2,6 mannosyl orthoester that has been then subjected to dibutyl phosphate treatment to yield the very versatile of 6-OH mannosyl phosphate, whose applications will be reported in due course. Straightforward access to glycosyl phosphate building blocks will facilitate the synthesis of complex carbohydrates in solution and by automated solid phase synthesis.