Synthesis of new enzyme stabilisers inspired by compatible solutes of hyperthermophilic microorganisms

Eva Correia Lourenço

Supervisor: Dr. Maria Rita Ventura
Co-supervisor: Professor Helena Santos

Dissertation presented to obtain the Ph.D in Biotechnology

Instituto de Tecnologia Química e Biológica António Xavier

Universidade Nova de Lisboa

Oeiras, November, 2013

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

ITQB INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA ANTÓNIO XAVIER /UNL
Knowledge Creation

UNIVERSIDADE NOVA DE LISBOA

The research work described in this thesis was performed at the Bioorganic Chemistry and Cell Physiology & NMR laboratories, Instituto de Tecnologia Química e Biológica António Xavier - Universidade Nova de Lisboa, Oeiras, Portugal. This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through a PhD grant to Eva Lourenço (SFRH/47702/2008) and the grant # PEst-OE/EQB/LA0004/2011.

Acknowledgments

To Doctor Maria Rita Ventura for believing in my scientific skills and giving me the opportunity to develop my scientific career. Her support, guidance and availability during these four years made this work possible.

To Professor Helena Santos for the important scientific contribution given throughout the PhD, assertiveness and critical analysis of my work, and for the prompt response to my questions.

To Professor Christopher Maycock for the enthusiasm in discussing science, and for always encouraging me to go forward.

To Dr. Sofia Miguel for all the discussions about our work, the friendship and support which greatly contributed to overcome less positive days.

To my colleagues from the Bioorganic Chemistry Lab and Organic Synthesis Lab, Paula Rodrigues, Vanessa Miranda, Saul Silva and Osvaldo Ascenso for the friendly and supportive environment.

To my colleagues in the Cell Physiology & NMR lab, especially Ana Mingote, Ana Esteves and Marta Rodrigues for always making me feel welcome and their patience for teaching me new techniques.

To the CERMAX for the use of the NMR facilities, especially to Helena Matias for her efforts to carry out the HR-MAS work.

To Fundação para a Ciência e a Tecnologia for the financial support provided by the Ph.D. grant, and Instituto de Tecnologia Química e Biológica António Xavier, for providing the conditions for conducting quality scientific work.

To the family I have chosen, my friends.
To Aurélien and Vasco.
And at last, to my mother and father.

Table of Contents

List of Figures:

List of Schemes:

List of Tables:

Abbreviations

Ac	Acetate
Ac_2O	Acetic anhydride
AcOH	Acetic acid
BnBr	Benzyl bromide
CPME	Cyclopentyl methyl ether
DIC	N,N'-diisopropylcarbodiimide
DIP	di-*myo*-inositol phosphate
DGP	di-Glycerol phosphate
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DSF	Differential Scanning Fluorimetry
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
GG	α-D-Glucosyl-D-glycerate
GGG	α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2)-D-glycerate
GL	α-D-Glucosyl-*S*-lactate
Hex	Hexane
HOBt	Hydroxybenzotriazole
HR-MS	High Resolution Mass Spectrometry
HR-MAS NMR	High Resolution Magic Angle Spining NMR
HSQC	Heteronuclear Single Quantum Correlation NMR
iPr_2NEt	*N*,*N*-Diisopropylethylamine
MDH	Malate dehydrogenase
Me	Methyl
MeOH	Methanol
MG	α-D-Mannosyl-D-glycerate
MGA	α-D-Mannosyl-D-glyceramide
MGG	α-D-Mannopyranosyl-(1→2)-α-D-glucopyranosyl-(1→2)-D-glycerate
MGly	α-D-Mannosyl-glycolate
MGGly	(2*R*)-2-(1-*O*-α-D-mannopyranosyl)-3-(1-*O*-α-D-

	glucopyranosyl)-glycerate
ML	α-D-Mannosyl-*S*-lactate
NaOMe	Sodium methoxide
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
Ph	Phenyl
TLC	Thin layer chromatography
SNase	Staphylococcal nuclease
TBAF	Tetra-*n*-butylammonium fluoride
TBDPSCl	*tert*-Butylchlorodiphenylsilane
TBDMS	*tert*-Butyldimethylsilane
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Tr	Trityl or triphenylmethyl

Abstract

In response to osmotic or heat stress, marine hyperthermophiles (thriving optimally at or above 80ºC) accumulate ionic compatible solutes such as α-D-mannosyl-D-glycerate (MG). It has been proposed that these hypersolutes stabilise intracellular components, such as proteins and enzymes, allowing them to withstand high growth temperatures. Actually, the efficacy of these solutes in the stabilisation of a number of model proteins has been demonstrated in vitro. Since several human pathologies such as Alzheimer's, Creutzfeldt-Jacob's, cystic fibrosis and Parkinson's diseases have been associated with the structural instability of proteins, and consequent protein aggregation, the development of reliable strategies to improve protein stability is of great importance and could lead to several pharmaceutical and biotechnological applications.

The main goals of this doctoral work were the development of strategies for the synthesis of newly isolated natural solutes, such as α-D-mannopyranosyl-(1→2)- α-D-glucopyranosyl-(1→2)-D-glycerate (MGG) and (2R)-2-(1- α-D-mannopyranosyl)-3-(1-O-α-D-glucopyranosyl)-glycerate (MGlyG), and the construction of a solute library inspired by the structure of natural hypersolutes, mainly MG.

Hypersolutes are present in limited quantities in their natural producers and chemical synthesis was considered a convenient method to obtain, study and confirm the structure of the isolated compounds. The most challenging feature in carbohydrate chemistry is the stereoselective introduction of the glycosidic bond, hence, to enhance the anomeric selectivity of the glycosylation reaction for the preparation of these compounds new synthetic methodologies for the synthesis of 1,2-cis glycosides were developed. In this work, several galactose, glucose and glucosamine thioglycosides were prepared and used as donors. The influence of the nature of the protective groups on different positions of the donor, solvent and temperature on the stereochemical outcome of these glycosylation reactions was studied using a wide range of acceptors, ranging from unhindered linear primary alcohols to other sugars, using NIS/TfOH as activator. The results showed that for the studied hexoses, the presence of a strong electron withdrawing group at the C-6 position of the donors had an influence in the anomeric selectivity, favouring the formation of

1,2-cis glycosides. In the case of galactose this effect could be enhanced by the presence of an additional ester group at the C-4 position of the donor.

In the present work, the natural MGG was efficiently synthesised in solution and on solid support in a stereocontrolled manner. Different strategies using two different thioglucosides bearing a free C-2 hydroxyl group were designed and tested in solution. These thioglucosides were used as glycosyl acceptors in the first glycosylation reaction with the tetraacetylmannosyl trichloroacetimidate, and as donors in the second glycosylation reaction with the glycerate acceptor. The glycosylation reactions with both thioglucosides were highly stereoselective affording exclusively the α-anomer. For the solid supported synthesis, a thioglucoside was efficiently immobilized on the solid support (Tentagel MB-NH2) by the C-6 hydroxyl group using a succinate linker, and successfully used in the synthesis of MGG. The stereoselectivity of the glycosylation reactions was reproducible on solid support, affording the desired α-product. Characterisation of the products while attached to the solid support was performed using HR-MAS NMR.

A synthetic strategy for the synthesis of the recently isolated MGlyG was successfully developed based on the glycosylation reaction of ethyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio-α/β-D-glucopyranoside donor with a partially protected mannosyl glycerate acceptor. Optimisation studies were performed changing the solvent and temperature for these reaction in order to improve the 1,2-*cis* stereoselectivity. The effectiveness of MGlyG for the protection of model enzymes against heat induced inactivation was evaluated, using differential scanning fluorimetry (DSF). For comparison, the protection induced by natural compatible solutes, like MG, GG or GGG, was assessed by the same method. The results demonstrated that for the studied enzymes (malate dehydrogenase, staphylococcal nuclease and lysozyme) MGlyG was the best stabiliser, and that the extent of protein stabilisation rendered by the solute depends on the specific solute/enzyme examined.

The mechanisms that govern protein stabilisation by hypersolutes are still unclear and a subject of interest for the literature. To understand what are the structural features of these compounds that are determinant for stabilisation, a solute library was developed in this work. Twenty-one new synthetic analogues derived from different hexoses, such as glucose, galactose,

mannose and glucosamine have been synthesised and their thermostabilisation properties assessed using DSF. The comparative analysis of the results obtained reinforces the idea of the importance of charge for the stabilisation effect, and that the extent of stabilisation is determined by specific interactions between solutes and proteins. Furthermore, the importance of the nature of the sugar and the non-glycosidic moiety of the molecule for the stabilisation effect were studied, and results suggested that the structure of the non-glycosidic moiety had more influence on the stabilisation effect than the nature of the hexose.

Resumo

Hipertermófilos marinhos capazes de proliferar optimamente acima dos 80ºC acumulam solutos compatíveis iónicos (hipersolutos), tais como o α-D-manosil-D-glycerato (MG), como resposta a condições de stress osmótico e de temperatura. Dada a capacidade destes hipersolutos de estabilizarem componentes intracelulares, nomeadamente proteínas, foi proposta a acumulação destes compostos intracelularmente como estratégia destes organismos para suportar temperaturas elevadas. Para além disso, testes in vitro demonstraram a capacidade estabilizadora destes solutos numa variedade de proteínas modelo. Uma vez que a instabilidade estrutural das proteínas e a sua consequente agregação tem sido associada a uma série de doenças humanas, tais como a Alzheimer, a doença Creutzfeldt-Jacob, a fibrose quística e a doença de Parkinson, o desenvolvimento de estratégias que visam o aumento da estabilidade das proteínas é de grande interesse e poderá ter inúmeras aplicações farmacêuticas e biotecnológicas.

Os principais objectivos desta tese de doutoramento foram o desenvolvimento de estratégias para a síntese de compostos naturais recentemente isolados, tais como o α-D-manosil-(1→2)-α-D-glucosil-(1→2)-D-glicerato (MGG) e (*2R*)-2-(1-*O*-α-D-manosil)-3-(1-*O*-α-D-glucosil)-glicerato (MGlyG), e a construção de uma biblioteca de compostos baseada na estrutura de solutos produzidos naturalmente pelos hipertermófilos, nomeadamente o MG.

Uma vez que estes hipersolutos ocorrem em baixas concentrações nos produtores naturais , o método mais conveniente para os obter, estudar e confirmar a sua estrutura é a síntese química. Um dos maiores desafios da síntese de açúcares é a introdução estereosselectiva da ligação glicosídica; por isso, para melhorar a selectividade anomérica da reacção de glicosilação na síntese destes compostos foram desenvolvidas novas metodologias para a obtenção de 1,2-*cis* glicósidos. Neste trabalho foram sintetizados e utilizados vários tioglucósidos derivados da glucose, galactose e glucosamina. Utilizando diversos aceitadores glicosídicos a influência da natureza dos grupos protectores em diferentes posições do dador, bem como o solvente e a temperatura, na selectividade da reacção de glicosilação foi avaliada utilizando o sistema NIS/TfOH como activador. Os resultados obtidos para as hexoses estudadas demonstram que grupos electroatractores

na posição C-6 dos dadores têm uma influência directa na selectividade anomérica da reacção, favorecendo a formação de 1,2-*cis* glicósidos. No caso da galactose, este efeito pode ser acentuado pela presença adicional de um grupo éster na posição C-4 do dador glicosídico.

No presente trabalho, o soluto natural MGG foi eficientemente e esterosselectivamente sintetizado em solução e em suporte sólido. Foram desenvolvidas e testadas duas vias de síntese em solução utilizando dois dadores tioglucosídicos contendo o grupo hidroxilo do C-2 livre. Estes tioglucósidos foram utilizados como aceitadores numa primeira glicosilação com o dador tetraacetilmanosil tricloroacetamidato, e posteriormente como dadores na reacção de glicosilação com o glicerato como aceitador. As reacções de glicosilação com ambos os tioglucósidos foram estereosselectivas, tendo-se formado exclusivamente o anómero α. A síntese em suporte sólido foi conseguida com sucesso através da imobilização do tioglucósido na resina Tentagel MB-NH_2 através do C-6 e utilizando como "linker" o succinato. A estereosselectividade das reacções em solução foi reproductível em suporte sólido, tendo-se obtido exclusivamente o produto α pretendido. A caracterização dos compostos em suporte sólido foi realizada através de RMN HR-MAS.

Para a síntese do recentemente isolado MGlyG foi desenvolvida uma estratégia tendo como base a reacção de glicosilação entre o dador etil 6-*O*-acetil-2,3,4-tri-*O*-benzil-1-tio- α/β-D-glucopiranósido e o manosil glicerato parcialmente protegido como aceitador. Uma vez que a reacção de glicosilação não foi estereosselectiva, foi necessário proceder-se à realização de um estudo para optimização das condições de reacção (solvente e temperatura). A capacidade termoestabilizadora do MGlyG foi avaliada através de fluorimetria diferencial de varrimento (DSF) em três enzimas modelo (malato desidrogenase, staphylococcal nuclease e lisozima). Como termo de comparação foi também avaliada pelo mesmo método a capacidade termoestabilisadora de alguns solutos compatíveis naturais, tais como o MG, o GG ou GGG. Os resultados obtidos para as três enzimas em estudo demonstraram que o MGlyG é o melhor termoestabilisador, e que o grau de estabilização conferido pelos solutos depende de interacções específicas entre o par soluto/enzima em questão.

Uma vez que os mecanismos que controlam a estabilização de proteínas pelos hipersolutos são ainda desconhecidos , para compreender quais os determinantes estruturais destes compostos para a estabilização foi desenvolvida uma biblioteca de solutos análogos aos produzidos na natureza pelos hipertermófilos. Para a construção da biblioteca foram sintetizados 21 novos análogos derivados de diferentes hexoses, tais como a glucose, galactose, manose e glucosamina, e as suas propriedades termoestabilisadoras avaliadas em proteínas modelo. A análise comparativa dos resultados obtidos reforça o conceito da importância da carga eléctrica dos compostos para o efeito de estabilização, e de que o grau de estabilização conferido é determinado por interacções específicas entre os solutos e as proteínas. Neste trabalho foi ainda estudado o modo como a estrutura da hexose, bem como da parte não glicosídica da molécula influenciam o efeito de estabilização, sendo que os resultados sugerem que a estrutura da parte não glicosídica tem maior relevância do que a estrutura da hexose.

Chapter 1

Introduction

Introduction

Hypersolutes

Halotolerant and moderately halophilic microorganisms accumulate compatible solutes in the cytoplasm to face fluctuations in the osmotic pressure of their environment.[1] Compatible solutes are low molecular weight and highly soluble organic compounds, namely amino acids and derivatives, sugars and polyols that are able to protect cell structures under stress conditions and do not interfere with normal cell function.

In response to osmotic or heat stress, marine hyperthermophiles (microorganisms thriving optimally at or above 80ºC) use the same strategy and accumulate ionic compatible solutes also known as hypersolutes.[1] In contrast with neutral or zwitterionic compounds, more commonly found in mesophiles, hypersolutes are in general negatively charged and most fall into two categories: hexose derivatives, like α-D-mannosyl-D-glycerate (MG) and polyol-phosphodiesters such as di-*myo*-inositol phosphate (DIP) (Figure 1).[1] Although the preference for the accumulation of negatively charged compounds is still unclear, in vitro studies have proved not only the efficacy of these solutes in the stabilisation of a number of model proteins, but also that charged compounds rendered better protection against aggregation, unfolding and inactivation of proteins than neutral compounds.[2-5] The increase in the concentration of these solutes not only with the increase of the osmotic pressure of the medium but also with the growth temperature led to the proposal that they would play a role in thermoadaptation by stabilising intracellular components, such as proteins and enzymes, allowing them to withstand high growth temperatures.[1, 6]

MG is one of the most widespread[6] solutes in hyper/thermophilic organisms and the efficacy of this natural solute in the protection of protein structures has been amply illustrated[2, 4]. Due to the small amounts of these hypersolutes available from their natural sources, chemical synthesis was considered the best route to obtain, study and confirm the structure of the isolated compounds. More recently, other isolated natural solutes, like α-D-glucosyl-D-glycerate (GG) and α-D-glucopyranosyl-(1→6)-α-D-

glucopyranosyl-(1→2)-D-glucopyranosyl-(1→2)-D-glycerate (GGG) (Figure 1) with a more complex structure, have been synthesised.[7]

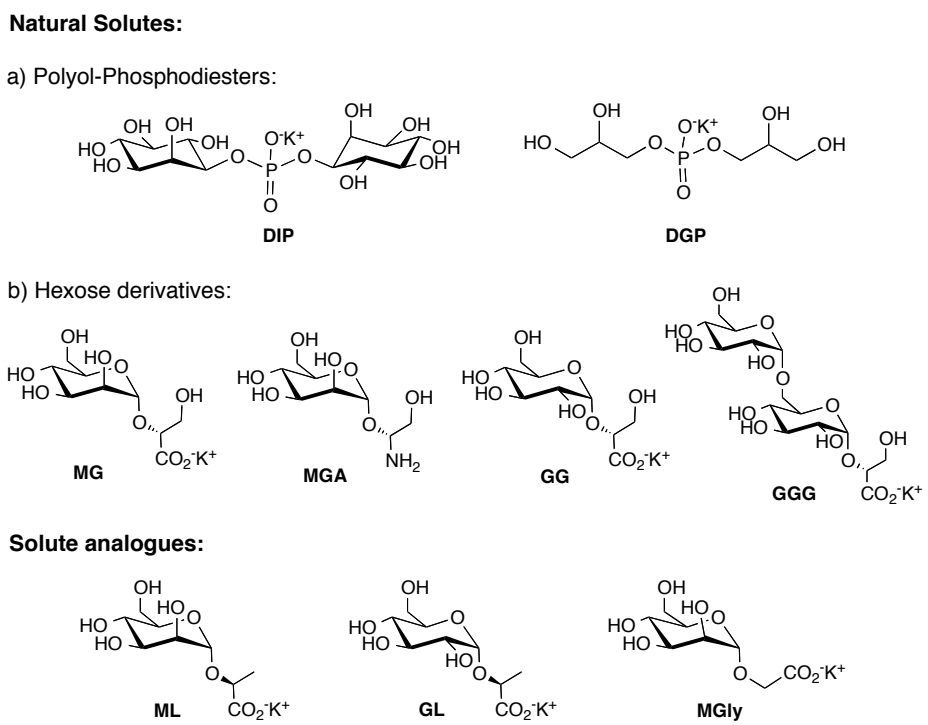


Figure 1. Examples of natural compatible solutes and analogues.

Protein stability can be defined in a very simplistic way as the capacity of the polypeptide chain to maintain a specific three-dimensional conformation that allows it to perform a given function, and results of the equilibrium between folding and unfolding forces, which is highly influenced on the interactions between the protein and the solvent. Although the principles underlying the stabilisation effect of compatible solutes are still unknown, it is accepted to be a consequence of the alteration of the environment that surrounds the protein. More specifically, the solvent (in this case water) physicochemical properties.[8] In the presence of the solute, the hydrated tertiary native structure of the protein in aqueous solution suffers a structural compaction and rigidification.[9] This concept is supported by the study of the effect of MG in the internal motions of the hyperstable staphylococcal nuclease (SNase), where it was

shown that, in the presence of the solute, the protein backbone motions were restricted in a concentration-dependent manner.[10] Also, the fact that different solutes stabilise proteins to different extents suggests the presence of weak interactions between the solutes and the protein surface[9], without interfering with the biological function of the protein.

The success of many industrial and pharmaceutical processes is highly dependent on the preservation of the native structure and activity of enzymes and other proteins. Moreover, since several afflicting human pathologies have been associated with structural instability of proteins, and consequent protein aggregation, like Alzheimer's, Creutzfeldt-Jacob's, Huntington's, cystic fibrosis and Parkinson's, the development of reliable strategies to improve protein stability is of great importance.

Some classical compatible solutes from mesophiles, like trehalose[11], that are able to sustain and preserve a wide array of biological molecules have been used as stabilisers in several commercially available therapeutic products, food and cosmetic products. Based on the proposed role of hypersolutes as thermoprotectors and on their superior stabilisation properties against denaturation, aggregation and inactivation effects several biotechnological applications can be considered. Furthermore, since enhanced stability of proteins to high temperature usually implies improved resistance to other types of stress conditions, such as pH, freezing, radiation, dehydration, oxidation and other chemical modifications, suggests that the use of hypersolutes can be considered for the global enhancement of protein stability.[8, 12] In fact, MG is one of the most studied hypersolutes with respect to its protein stabilising properties and has been successfully used with this purpose in different types of applications, for example: in the quality improvement of DNA microarrays[13]; in the inhibition of aggregation of proteins involved in Alzheimer's[14], Parkinson's[15] and Huntington's[16] disease; and in the stability improvement of the retro and adenoviral vectors[17] allowing long-term storage (half-life >1 year). In addition, its high compatibility and lack of toxicity has made it a very promising candidate for several industrial

applications as stabilising agent of biomaterials, which have been disclosed in a number of patent applications[18-21].

Recently in another study[3], several molecules chemically related to MG were synthesised and in some cases (like α-D-mannosyl-(*S*)-lactate (ML), Figure 1) showed a superior ability to protect model enzymes against heat-induced denaturation, aggregation and inactivation. This finding showed that an apparently small structural change, the glycerate moiety being replaced by a lactate, had positive effect in the protein thermostabilisation and has stimulated the production of new synthetic analogues. The possibilities of solute engineering having MG as the reference compound are very large, so in this work a solute library was constructed, and the diversity of these analogue structures was introduced in three levels: the nature of the sugar (mannose, glucose, galactose and glucosamine), both anomers for each sugar and the glycosyl acceptor (like, for example methyl (*2R*)-D-glycerate, methyl (*S*)-lactate, glycerol, methyl glycolate, methyl butyrate or dimethyl malate) used in the glycosylation reaction. The solute library provided a wide range of structurally different sugar derivatives that could give insight into the key features necessary for protein stabilization, and synthetic analogues with improved stabilisation properties.

Carbohydrates Synthesis

Carbohydrates play an important role in many biological processes and can be found in nature in various forms, from simple monosaccharides to more complex polysaccharides, glycoconjugates or glycosides in which a monosaccharide is joined by glycosidic bonds to other monosaccharides or other functional groups. From the three major classes of biopolymers - oligonucleotides, oligopeptides and oligosaccharides (Figure 2) - the synthesis of oligosaccharides is the most challenging and has been less explored than that of other biomolecules. Compared with other biopolymers such as nucleic acids, proteins and peptides, in which their biological activity depends on the sequence of nucleotides or amino acids, in the case of oligosaccharides, the situation is more complex. For oligosaccharides,

besides the sequence of the monomeric structures, other aspects such as the functional groups and their stereochemistry, the conformation of the sugars and the stereoselective formation of glycosidic linkages must be considered.

In order to perform biophysical and biochemical studies of biologically active carbohydrates to understand their biological function, sufficient quantities of defined oligosaccharides are required and which are difficult to obtain from their natural sources due to their microheterogeneity and low concentrations and low biomass yields. The chemical synthesis of oligosaccharides for biological studies is a powerful tool to provide pure, structurally defined and homogeneous samples in substantial quantity, and simultaneously allows for synthesising several structures including non-natural ones.

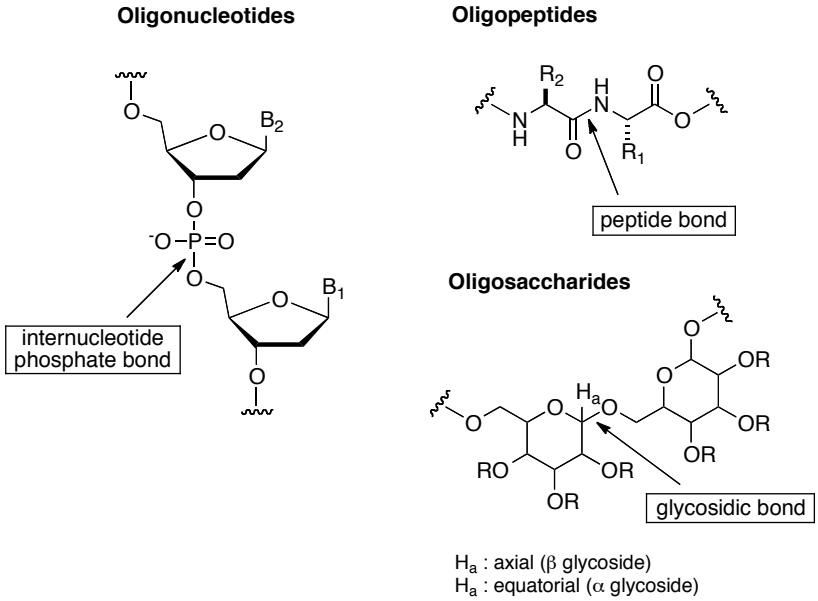
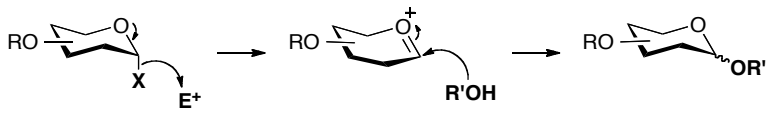


Figure 2. The three major classes of biooligomers.

The structural complexity of carbohydrates can be translated in two major chemical challenges: the multitude of functional groups with similar reactivity that need to be distinguished, and the creation of a new stereogenic center when the glycosidic bond is formed.

The development of stereoselective methods for the introduction of the glycosidic bond between the monosaccharide units during the glycosylation

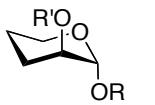
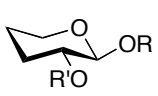
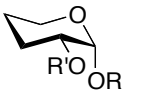
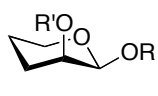
reaction plays a central role in carbohydrates chemistry. The glycosidic bond is formed by a nucleophilic attack by an alcohol (R'OH), or by the hydroxyl group of a partially protected sugar moiety – the glycosyl acceptor - to the anomeric center of the glycosyl donor often through a unimolecular S_N1 mechanism. The reaction is generally performed in the presence of an activator called promoter (E) that assists the departure of the leaving group (X) and in some cases, other additives such as molecular sieves or any base that may act as acid scavenger are used (Scheme 1).[22]



Scheme 1. Glycosylation reaction.

Despite the glycosylation reaction being known for centuries and the existence of many glycosylation methods available for glycosidic bond formation, none of them is general, as there are many factors to take in consideration. Although 1,2-*trans* glycosides (Table 1) can be stereoselectively prepared in the classical manner by using the C-2 acyl neighbouring group participation, the stereoselective formation of 1,2-*cis* glycosides (Table 1), like α-glucopyranosides, α-galactopyranosides and β-mannopyranosides, still represents a challenge and proceeds with poorer stereocontrol, which results in mixtures of diastereomers.

Table 1. Stereochemistry of the anomeric bond.

	α	β
1,2-*trans*		
1,2-*cis*		

For example, this can be easily illustrated by comparing the selectivities obtained for the synthesis of the 1,2-*cis* glucosides, GG (>10:1 1,2-*cis*/1,2-*trans* isomers) and GL (4:1 1,2-*cis*/1,2-*trans* isomers), which are difficult to attain in a stereocontrolled manner, with the selectivities obtained for the synthesis of the 1,2-*trans* mannosides, MG and ML (1:0 1,2-*trans*/1,2-*cis* isomers).[3, 7]

Many factors, such as the nature of the leaving and protective groups on the donor, the steric hindrance of the glycosyl acceptor, the reaction solvent, temperature, and the promoting system can influence the outcome of the reaction and should be taken into account.[22] So improved and more general methods for the control of the 1,2-*cis* stereoselectivity of the glycosylation reaction are needed.

When planning a synthetic strategy for carbohydrates synthesis another crucial task is the preparation of properly functionalized building blocks and deprotection strategies. This involves the use of multistep transformations for differentiation of functional groups (amino and hydroxyl) of similar reactivity and, in most cases, purification by chromatography is needed after each step. In order to simplify the labour-intensive, time consuming and expensive solution phase synthesis of these compounds, many research efforts have been directed towards the development of efficient strategies and methodologies for solid phase synthesis.

Although the solid phase synthesis was initially almost exclusively dedicated to oligopeptides and provided the basis for automated peptide synthesis, the use of functionalised solid supports has been extended to the synthesis of oligonucleotides and oligosaccharides, proving to be a powerful tool for combinatorial chemistry in the preparation of compound libraries. However, the solid phase synthesis of oligosaccharides and oligosaccharide libraries, for the reasons mentioned above, is much more demanding than the synthesis of peptides or nucleic acids and therefore less developed and automatised than the other two. The automation of solid phase oligosaccharide synthesis holds great promise for the future, and is expected to simplify the synthesis of biologically relevant sugar structures.

Using solid supports for the synthesis of compatible solutes will allow a more efficient preparation of these compounds in a relatively short time, essentially by using well established solution phase reactions and taking advantage of the main feature of solid supports - the purification step, which can be accomplished by a simple filtration, allowing the use of excess reagents to drive reactions to completion.

The development of improved and more efficient chemical synthesis of these compounds is crucial for the expansion of the industrial applications of hypersolutes.

References

1. Costa, M. S.; Santos, H.; Galinski, E. A., An overview of the role and diversity of compatible solutes in Bacteria and Archaea. In *Biotechnology of Extremophiles*, Antranikian, G., Ed. Springer Berlin Heidelberg: **1998**; Vol. 61, pp 117-153.

2. Ramos, A.; Raven, N. D. H.; Sharp, R. J.; Bartolucci, S.; Rossi, M.; Cannio, R.; Lebbink, J.; vanderOost, J.; deVos, W. M.; Santos, H., Stabilization of enzymes against thermal stress and freeze-drying by mannosylglycerate. *Applied and Environmental Microbiology* **1997,** 63, 4020-4025.

3. Faria, T. Q.; Mingote, A.; Siopa, F.; Ventura, R.; Maycock, C.; Santos, H., Design of new enzyme stabilizers inspired by glycosides of hyperthermophilic microorganisms. *Carbohydrate Research* **2008,** 343, 3025-3033.

4. Borges, N.; Ramos, A.; Raven, N. D. H.; Sharp, R. J.; Santos, H., Comparative study of the thermostabilizing properties of mannosylglycerate and other compatible solutes on model enzymes. *Extremophiles* **2002,** 6, 209-216.

5. Lamosa, P.; Burke, A.; Peist, R.; Huber, R.; Liu, M. Y.; Silva, G.; Rodrigues-Pousada, C.; LeGall, J.; Maycock, C.; Santos, H., Thermostabilization of proteins by diglycerol phosphate, a new compatible

solute from the hyperthermophile Archaeoglobus fulgidus. *Applied and Environmental Microbiology* **2000,** 66, 1974-1979.

6. Santos, H.; da Costa, M. S., Compatible solutes of organisms that live in hot saline environments. *Environmental Microbiology* **2002,** 4, 501-509.

7. Lourenco, E. C.; Maycock, C. D.; Ventura, M. R., Synthesis of potassium (*2R*)-2-*O*-alpha-D-glucopyranosyl-(1 -> 6)-alpha-D-glucopyranosyl-2,3-dihydroxypropanoate a natural compatible solute. *Carbohydrate Research* **2009,** 344, 2073-2078.

8. Lentzen, G.; Schwarz, T., Extremolytes: natural compounds from extremophiles for versatile applications. *Applied Microbiology and Biotechnology* **2006,** 72, 623-634.

9. Pais, T. M.; Lamosa, P.; dos Santos, W.; LeGall, J.; Turner, D. L.; Santos, H., Structural determinants of protein stabilization by solutes - The importance of the hairpin loop in rubredoxins. *Febs Journal* **2005,** 272, 999-1011.

10. Pais, T. M.; Lamosa, P.; Garcia-Moreno, B.; Turner, D. L.; Santos, H., Relationship between protein stabilization and protein rigidification induced by mannosylglycerate. *Journal of Molecular Biology* **2009,** 394, 237-250.

11. Ohtake, S.; Wang, Y. J., Trehalose: Current use and future applications. *Journal of Pharmaceutical Sciences* **2011,** 100, 2020-2053.

12. Luley-Goedl, C.; Nidetzky, B., Glycosides as compatible solutes: biosynthesis and applications. *Natural Product Reports* **2011,** 28, 875-896.

13. Mascellani, N.; Liu, X. P.; Rossi, S.; Marchesini, J.; Valentini, D.; Arcelli, D.; Taccioli, C.; Citterich, M. H.; Liu, C. G.; Evangelisti, R.; Russo, G.; Santos, J. M.; Croce, C. M.; Volinia, S., Compatible solutes from hyperthermophiles improve the quality of DNA microarrays. *Bmc Biotechnology* **2007,** 7.

14. Ryu, J.; Kanapathipillai, M.; Lentzen, G.; Park, C. B., Inhibition of beta-amyloid peptide aggregation and neurotoxicity by alpha-D-mannosylglycerate, a natural extremolyte. *Peptides* **2008,** 29, 578-584.

15. Faria, C.; Jorge, C. D.; Borges, N.; Tenreiro, S.; Outeiro, T. F.; Santos, H., Inhibition of formation of α-synuclein inclusions by mannosylglycerate in a yeast model of Parkinson's disease. *Biochimica et Biophysica Acta (BBA) - General Subjects* **2013,** 1830, 4065-4072.

16. Jorge, C. D.; Ventura, R.; Maycock, C.; Outeiro, T. F.; Santos, H.; Costa, J., Assessment of the efficacy of solutes from extremophiles on protein aggregation in cell models of Huntington's and Parkinson's diseases. *Neurochemical Research* **2011,** 36, 1005-1011.

17. Cruz, P. E.; Silva, A. C.; Roldao, A.; Carmo, M.; Carrondo, M. J. T.; Alves, P. M., Screening of novel excipients for improving the stability of retroviral and adenoviral vectors. *Biotechnology Progress* **2006,** 22, 568-576.

18. Santos, H. R., A.; da Costa, M. S. Utilização de manosilglicerato na termoestabilização, osmoprotecção e protecção contra a desidratação de componentes celulares e células. INPI (Instituto Nacional de Propriedade Industrial) - Invention patent, submited in June 1996.

19. Santos, H. R., A.; da Costa, M. S. Thermostabilization, osmoprotection, and protection against desiccation of enzymes, cell components, and cells by mannosylglycerate. European patent submitted in 14/03/97 with nº 97670002.1 and published in 07/01/98 Bulletin 1998/02.

20. Lamosa, P. F., T. Q.; Ventura, M. R.; Maycock, C. D.; Santos, H. Derivados sintéticos de manosil-glicerato para a estabilização e/ou preservação de biomateriais. Patent: PAT 103442 P; 24/02/2006.

21. Santos, H. d. C., M. S.; Empadinhas, N.; Albuquerque, L. Identification of a bifunctional gene for mannosylglycerate synthesis and development of a high-scale-production heterologous system based on Saccharomyces cerevisiae. International patent submitted in 17/10/2003, 2003 (process nº 3398007Y).

22. Demchenko, A. V., *Handbook of Chemical Glycosylation*. Wiley: 2008.

Chapter 2

Stereoselective 1,2-*cis* Glycosylations - Towards the Synthesis of Protein Stabilisers

This chapter contains data published in:

Eva C. Lourenço, M. Rita Ventura; The synthesis of compatible solute analogues - solvent effects on selective glycosylation. *Carbohydrates Research* **2011**, 346, 163-168.

Eva C. Lourenço, M. Rita Ventura; The effect of electron withdrawing protecting groups at positions 4 and 6 on 1,2-*cis* galactosylation. *Tetrahedron* **2013**, 69, 7090-7097.

The author contributed fully to this chapter, namely in the planning of the experimental work, performing the experiments and writing of the manuscript.

Stereoselective 1,2-*cis* Glycosylations - Towards the Synthesis of Protein Stabilisers

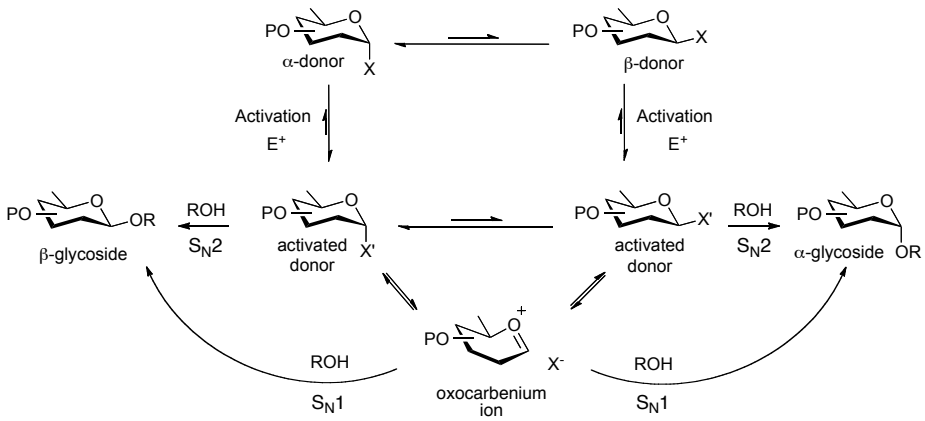
Abstract

Thioglycosides are extremely versatile and have been widely employed in the synthesis of complex carbohydrates and carbohydrate libraries. Since obtaining high stereoselectivity in 1,2-*cis* glycosylation reactions is challenging in this chapter the use of thioglycosides as donors in this reaction was explored. In order to improve the α anomeric selectivity of the glycosylation reaction different thioglucosides, thiogalactosides and 2-azido-2-deoxythioglucosides were prepared as donors and the NIS/TfOH system used as the promoter. The influence of the nature of the protective groups on different positions of the donor, solvent and temperature in this reaction was studied using a wide range of acceptors (from unhindered primary hydroxyl groups to other sugars). Studies showed that the α-stereoselectivity of thioglucosides and 2-azido-2-deoxythioglucosides could be improved by the use of an electron-withdrawing group (acetyl or acyl) at the C-6 position of these donors, and in the case of thiogalactosides by the use of an acyl group both at the C-4 and C-6 position. In general, it was observed that donors possessing stronger electron-withdrawing groups at these positions have a higher α-directing effect. Glycosylations of the different thioglycosides with several acceptors showed that careful optimisation of the solvent system and temperature is a powerful way to obtain high yields and α-stereoselectivity. These glycosides are key intermediates for the synthesis of new analogues of compatible solutes.

Introduction

The key step in the synthesis of carbohydrates is the stereoselective introduction of the glycosidic bond between the monosaccharide units during the glycosylation reaction. The glycosylation is a complex phenomenon which detailed mechanism is still unknown and subject of several studies[1-3]. It is commonly accepted that involves the nucleophilic attack by an alcohol (ROH) – the glycosyl acceptor - to the anomeric center of the glycosyl donor in the presence of the promoter (E). Regarding the mechanism, the reaction cannot

be considered simply in terms of pure unimolecular (S_N1) or pure bimolecular mechanism (S_N2), and was described by Crich[2] as "continuum of mechanisms" ranging between the pure S_N2 mechanism at the one extreme and the S_N1 mechanism with free oxocarbenium ions at the other (Scheme 2). Where according to a given donor-acceptor pair, the reaction conditions may favour either a bimolecular or a monomolecular rate-determining step (Scheme 2).[2]



Scheme 2. Full spectrum of mechanisms from S_N2 all the way to pure S_N1.[2]

When considering that the new glycosidic linkage creates a chirality center, many factors influence the stereochemical outcome of this reaction, like:

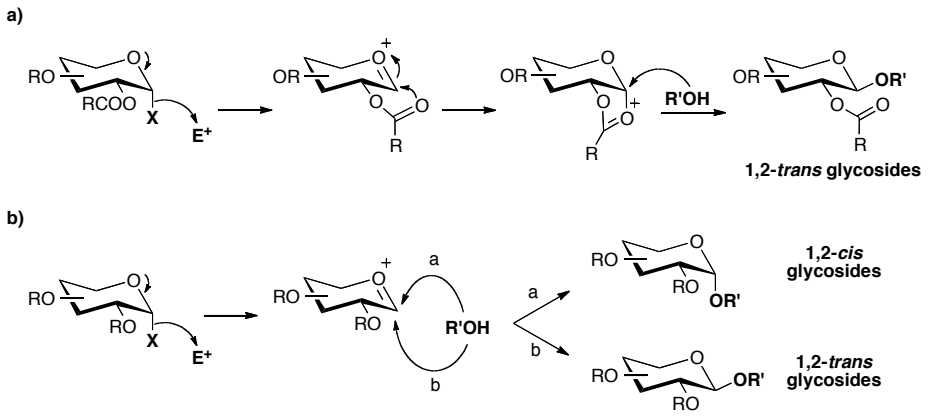
- the nature of the protecting groups, specially the group bound to the C-2 of the sugar ring;
- the reactivity of the anomeric centre of the glycosyl donor, the acceptor and the promoter;
- the solvent;
- the steric hindrance of the acceptor;
- the temperature at which the reaction takes place;

need to be taken into account when designing the glycosidic donors to accomplish the desired product.

The choice of the proper protecting groups has great influence in the reactivity of the donor - they affect the nucleophilicity of the anomeric function

and thereby the rate of attack of the anomeric group on the electrophilic promoter species. And they determine the stability of the partial positive charge on the anomeric center, which develops upon expulsion of the anomeric leaving group. A glycoside donor is deactivated by electron-withdrawing protecting groups (such as acyl functions) and termed "disarmed", since they decrease the electron density, therefor, the nucleophilicity of the anomeric function and destabilise the oxocarbenium ion transition state.[3] Whereas electron releasing protective groups (such as aryl or alkyl functions) are more reactive and described "armed".

The protecting group on C-2, adjacent to the anomeric center, has the highest impact in the outcome of the glycosylation reaction as it can participate by formation of an intermediate if it is an ester (like acetyl, benzoyl, 2-phthalimido, Scheme 3. a), or not if it is an ether (Scheme 3. b).[1]



R: alkyl, aryl. X: Leaving Group. E^+: Promoter.

Scheme 3. Glycosylation Reaction: a) C-2 Group Participation – Disarmed donor; b) C-2 Non-participative Group - Armed donor.

The most commonly applied nonparticipating groups are benzyl for neutral sugars and azide for 2-amino-2-deoxy sugars, but other protective groups have also been used. In this case, since the nucleophilic attack would be almost equally possible from either the upper (β product) or the bottom face (α product) of the sugar ring and although the α-product is thermodynamically favoured by the anomeric effect, the kinetic β-product is often obtained

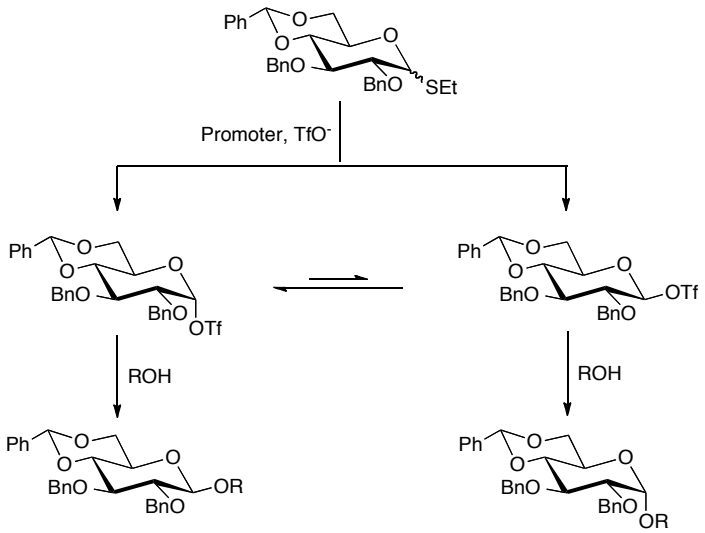
(Scheme 3. b)). This is why the formation of 1,2-*trans* glycosides can easily be accomplished by neighbouring group participation of the 2-acyl group, while 1,2-*cis* glycosides it is far more complicated.

Even though the effect of substituents remote to the anomeric center is less important for the stereochemical outcome of the glycosylation reaction than those at C-2, their significant contribution needs to be addressed.[1] Several reports have been speculating about remote group participation by esters from C-3[4-7], C-4[5, 6, 8-11] (axial and equatorial) and C-6[4, 9, 10] positions. These assumptions are mostly based on the observation of changes in stereoselectivity when esters replace ethers at those positions. Distinguish between the electron-withdrawing effect from the effect of remote participation of the protecting groups on the outcome of the reaction is a controversial and a non-unanimous subject.[2, 4, 11, 12]

In a recent study, several molecules chemically related to MG were synthesised and in some cases showed a superior ability to protect model enzymes against heat-induced denaturation, aggregation and inactivation.[13] This finding has stimulated the production of new synthetic analogues from different hexoses, such as mannose, glucose, galactose and glucosamine. In this work, although it would be interesting to test both anomers of each solute analogue, since in nature these compounds are present in the α-form the main challenge would be the stereoselective synthesis of the α-glucosides, the α-galactosides and the α-glucosamine analogues.

Despite the several methods available for the formation of 1,2-*cis* glycosides none of them is general and when applied to different acceptors the stereoselectivity is not predictable in many cases. Lemieux[14] and Crich[15] have both developed methods using “armed” donors - benzyl ethers - bearing non-participative groups on C-2 and obtained preferentially the 1,2-*cis* isomers. Crich’s method[15] is based on the use of thioglucosides that upon treatment and activation with triflic anhydride lead to the formation of triflate intermediates (Scheme 4). He proposed the existence of a system where the α– and β-triflates are in dynamic equilibrium and since the β-triflate is less stable, more reactive, reacts faster with the glycosyl acceptor by nucleophilic

substitution. This leads to the formation of the α-glucoside and directs the system equilibrium in that direction. Crich also found that size and steric hindrance of the acceptor had a great influence in this reaction. With smaller and less hindered glycosyl acceptors, like methanol, a α/β mixture is obtained, which can be explained by the fast direct displacement of the α-triflate (more stable) that directs the system equilibrium in that direction.

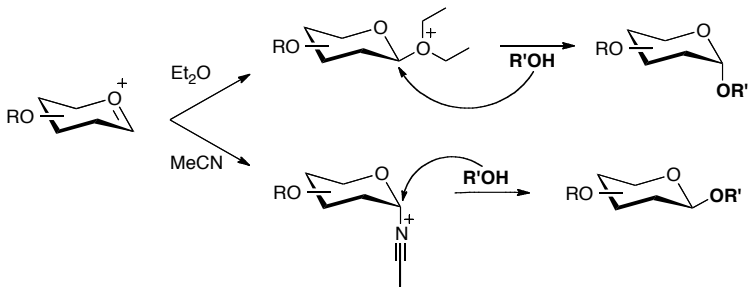


Scheme 4. Glycosylation reaction scheme according to Crich.[15]

More recently, in the synthesis of GG and GGG[16], thioglucoside **1** (Scheme 6) was used as the glucosidic donor and in both glycosylation reactions the α-anomer was the major product (α/β >10:1), using the NIS/TfOH system as the promoter. The presence of an acetate group at C-6 position of the thioglucoside **1** should favor the formation of the α-product because of the disarming effect of this electron-withdrawing group, lowering the reactivity of the donor when compared with the tetrabenzylated thioglucoside.[17] In addition, bulky or strong electron-withdrawing substituents at the C-6 position can influence the outcome of the reaction by electronically shielding the upper face of sugar ring, favoring the nucleophilic attack from the opposite side and therefore the formation of 1,2-*cis* glycosides.[18] However, when the same

thioglucoside **1** was used for the synthesis of the GG analogue, GL, under the same conditions with a less bulky, more reactive acceptor, like (*S*)-lactate, a α/β ratio of 4:1 was obtained.[13] Reactive alcohols are stronger nucleophiles and react faster, which turns difficult to control the selectivity and tend to give lower 1,2-*cis* stereoselectivity.[1]

The importance of solvents for the stereochemical outcome of the glycosylation reaction is well known in literature.[1] In general, polar solvents have a tendency to direct the glycosylation in an β-selective way while non-polar solvents, like dichloromethane or toluene, increase the proportion of α-glycoside. Moreover there are other contributions that can be manipulated in order to favour the synthesis of the desired products, like the participating properties of some ethereal and nitrile solvents. In the case of diethyl ether, a β–diethyl oxonium-ion intermediate may be formed due to steric reasons, which after nucleophilic displacement with inversion of configuration would give α-product (Scheme 5).[1] When using nitrile solvents, on contrary, the nitrilium cation formed in situ adopts axial orientation, leading towards the formation of the β-glycosides (Scheme 5).[1]



Scheme 5. Solvent coordination to the glycosyl donor.

Boons and co-workers[19] studied the α-directing effect of ethers as solvents in the glycosylation of a armed tetrabenzylated β-thioglucoside, and found that by performing the reaction in a mixture of toluene:1,4-dioxane obtained higher α-selectivities (α/β 15.3:1). Ethyl ether was tested in mixtures with dichloromethane and toluene, but even though the results were better in

toluene than in dichloromethane, were worst than with 1,4-dioxane. Dichloromethane was the worst solvent affording more β-anomer (α/β 0.7:1) and THF was not used in this study.

Bonnaffé and co-workers[20] performed an optimisation study of the solvent system for the α-selective glycosylation reaction of a 2-azido trichloroacetimidate disaccharide derived from glucose. Ethyl ether and dichloromethane showed the worst results both in terms of diastereoselectivity (α/β 70:30) and yield (<74%). They found that THF enhanced the α-stereoselectivity (α/β 93:7) and lowered the yield (66%) of the reaction, but when used as mixture THF/Et_2O (9:1) they obtained the best results (α/β 92:8, 90%).

In another study, Ito and co-workers[21] described the optimisation of the solvent system during the construction of three sequential α-glucosidic bonds using 4,6-*O*-cyclohexylidene-protected thioglucoside donors. It was shown that the best solvent systems were 1:1 mixtures of $CHCl_3$/Et_2O and $CHCl_3$/CPME.

A common point in these studies is that all of the acceptors used were other glycosides, mono and higher saccharides, and no smaller or more reactive alcohols were studied.

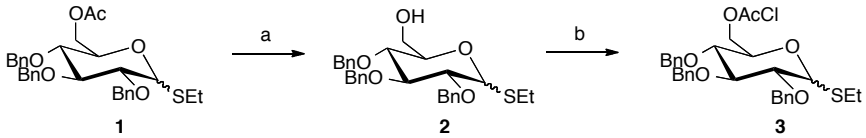
In order to improve the α-selectivity of the glycosylation reaction for the synthesis of new synthetic 1,2-*cis* analogues from glucose, galactose and glucosamine, several thioglycosides were prepared as donors. Using the NIS/TfOH[16] system as the promoter, the influence of the nature of the protective groups on different positions of the donor, as well as the solvent and the temperature on these glycosylations, were studied using a wide range of acceptors.

Results and Discussion

1,2-*cis* Stereoselective Glucosylations

Following the same line of research used for the synthesis of the natural compatible solutes GG and GGG[16], thioglucoside **1** (Scheme 6) was prepared

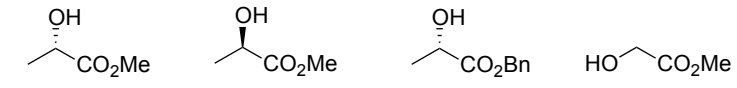
according to literature in 3 steps from methyl glucose and used to study the influence of the solvent and temperature on the glycosylation reaction. To further investigate the influence of protecting groups thioglucoside donor **3** was synthesised with a more electron-withdrawing group at the 6-position (chloroacetyl) from donor **1** (Scheme 6).



1 **2** **3**

a) NaOMe, MeOH, 0ºC, 96%. b) Chloroacetyl chloride, Py, 0ºC, 86%.

Scheme 6. Synthesis of thioglucosyl donor **3.**



4 Methyl (*S*)-lactate

5 Methyl (*R*)-lactate

6 Benzyl (*S*)-lactate

7 Methyl glycolate

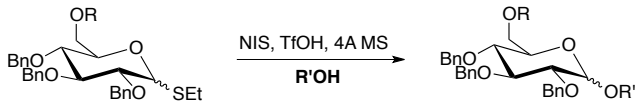
8 Methyl (*2R*)-*O*-*tert*-butyldimethylsilyl-2,3-dihydroxipropanoate

9 Methyl (*2R*)-3-*O*-*tert*-butyldiphenylsilyl-2,3-hidroxypropanoate

Figure 3. Glycosyl acceptors.

Based on the work of Boons[19], Bonnaffée[20] and Ito[21], a series of experiments changing the solvent system and the temperature was conducted. The results of the glycosylation reaction of donor **1** and **3** with a series of small and reactive glycosyl acceptors (Figure 3) using NIS/TfOH[16] system in the presence of 4Å MS are described in Table 2.

Table 2. Effect of solvent and temperature on the stereoselectivity of the glycosylation of donors **1** and **3**.



1: R=Ac
3: R=AcCl

Entry	R'OH	T (ºC)	Solvent	Donor 1			Donor 3		
				Product (Yield,%)	t (mn)	α/β	Product (Yield,%)	t (mn)	α/β
1	**4**	0	CH_2Cl_2	**10** (91)	10	4:1	**16** (90)	5	5.5:1
2			CH_2Cl_2 (THF, 2 eq)	**10** (83)	10	4:1			
3			Tol/Dioxane (1:3)	**10** (43) [a]	80	6:1			
4			Tol/Dioxane (1:3)[b]	**10** (78)	20	6.3:1	**16** (85)	5	7.8:1
5			THF/Et_2O (1:9)	**10** (87)	10	6:1			
6			THF/Et_2O (9:1)	**10** (60)	10	5.8:1			
7			Et_2O	**10** (85)	10	7.5:1	**16** (90)	5	9.3:1
8			THF	**10** (72)	45	6.5:1	**16** (85)	5	6.8:1
9		-60	CH_2Cl_2	**10** (83)	10	7:1	**16** (94)	20	8.5:1
10			Et_2O	**10** (81)	45	9:1	**16** (90)	25	14.2:1
11	**5**	0	CH_2Cl_2	**11** (85)	10	4:1			
12			Et_2O	**11** (76)	15	7.9:1			
13		-60	CH_2Cl_2	**11** (82)	10	6.5:1			
14			Et_2O	**11** (83)	45	8.3:1			
15	**6**	0	CH_2Cl_2	**12** (93)	10	4:1			
16			Et_2O	**12** (82)	15	8.3:1			
17		-60	CH_2Cl_2	**12** (97)	10	6.1:1			

18			Et_2O	**12** (81)	45	10:1	**17** (85)	20	11:1
7	**7**	0	CH_2Cl_2	**13** (93)	10	4.2:1			
20			Et_2O	**13** (85)	45	4.4:1			
21		-60	CH_2Cl_2	**13** (96)	10	4.2:1			
22			Et_2O	**13** (88)	45	13:1	**18** (70)	20	11:1
23	**8**	-60	Et_2O	**14** (30) [b,c]	45	1:0			
24	**9**	0	CH_2Cl_2	**15** (96)	30	>10:1			
25		0	Et_2O	**15** (94)	45	1:0			

[a] Hydrolysis product also recovered. [b] TMSTOf was used instead of TfOH.

[c] Initial donor was recovered.

As expected, ethereal solvents like ethyl ether, 1,4-dioxane and THF, gave higher α-selectivity than dichloromethane that is a halogenated solvent. This study showed that by using the standard glycosylation conditions - dichloromethane at 0ºC - the worst results were obtained in terms of selectivity for the two donors with all of the acceptors.

Glycosylation of donor **1** with methyl (*S*)-lactate **4** in the toluene/dioxane (1:3) mixture used by Boons[19] improved the α/β ratio (6:1), however the reaction was slower (80 min), we obtained only 43% yield and recovered the corresponding reducing monosaccharide as by-product (Table 2, entry 3). Changing the promoter to NIS/TMSOTf increased the yield (78%), but the α/β ratio remained almost the same (α/β 6.3:1, Table 2, entry 4).

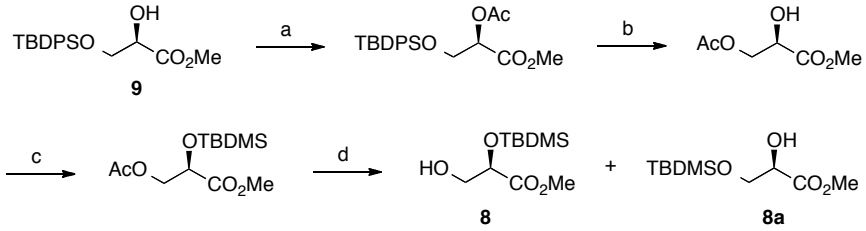
The THF/Et_2O (9:1) mixture employed by Bonnaffé[20] was not beneficial in this case and resulted in a 5.8:1 α/β ratio and only 60% yield (Table 2, entry 6). By reversing the proportions of both solvents in the mixture, THF/Et_2O (1:9), the yield was enhance to 87% still the α/β ratio remained almost unchanged (α/β 6:1, Table 2, entry 5). Performing the reaction in ethyl ether a higher anomeric ratio of 7.5:1 α/β and 85% yield was obtained (Table 2, entry

7). However, when using only THF both selectivity and yield were lower, and the reaction was slower (Table 2, entry 8).

Concerning the study of the effect of temperature, conducting the reaction in dichloromethane and ethyl ether at -60ºC afforded in both cases an enhance in the α-anomeric selectivity (Table 2, entries 9 and 10). In the case of dichloromethane (α/β 7:1) the yield was slightly lower (83%) but the reaction time remained the same (10 min) (Table 2, entry 9). With ethyl ether the donor was consumed more slowly (45 min) but this was compensated by improvement of the α/β ratio 9:1 and the good yield (81%, Table 2, entry 10). Regarding the glycosylation with methyl (*R*)-lactate **5** in dichloromethane and ethyl ether both at 0 and 60ºC (Table 2, entries 11-14) the results were very similar to those obtained for methyl (*S*)-lactate **4**. With the bulkier benzyl (*S*)-lactate **6** the highest α-anomeric selectivity was achieved in ethyl ether at -60ºC (α/β 10:1, Table 2, entry 18). When comparing the α/β ratio obtained for the glycosylation reaction in dichloromethane at 0ºC using methyl (*S*)-lactate **4,** methyl (*R*)-lactate **5** and benzyl (*S*)-lactate **6** as acceptors the results were the same (α/β 4:1, Table 2, entry 1, 11 and 15), meaning that under this conditions neither the (*R*),(*S*)-stereochemistry and bulkiness of the ester moiety of the lactate acceptor was relevant for the anomeric selectivity.

Glycosylation with the primary hydroxyl group of the small methyl glycolate **7** acceptor in ethyl ether at -60ºC afforded excellent results both in terms of α/β ratio (13:1) and yield (88%, Table 2, entry 22). Reaction with primary hydroxyl group of methyl glycerate **8** was attempted and even though only the α-anomer was isolated the yield was low and initial donor **1** was recovered (Table 2, entry 23). On contrary, glycosylation with the sterically hindered secondary hydroxyl group of methyl glycerate **9** afforded a good α/β ratio (>10:1) and yield both in dichloromethane and ethyl ether at 0ºC (Table 2, entries 24 and 25). Methyl glycerate acceptor **9** was prepared according to literature[16, 22], and acceptor **8** prepared from **9** using standard protection/deprotection reactions (Scheme 7). Although the synthesis of methyl glycerate acceptor **8** seems straightforward, the low yield (16%) is due to the migration of the protecting group on the secondary hydroxyl group after deprotection of the primary hydroxyl. When using acetate to protect the

secondary hydroxyl group total migration occurs, and the same phenomenon was observed when using a benzoyl group at this position.



a) Ac_2O, Py, DMAP, 0ºC-r.t., 94%. b) TBAF, THF, 0ºC-r.t., 75%. c) TBDMSCl, imidazole, DMF, 0ºC-r.t., 74%. d) NaOMe, MeOH, 0ºC, 16% **8**, 76% **8a**.

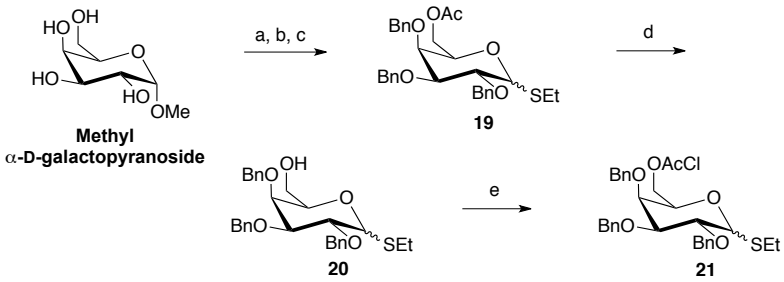
Scheme 7. Synthesis of methyl (2*R*)-*O*-*tert*-butyldimethylsilyl-2,3-dihydroxipropanoate **8**.

Concerning the influence of protecting groups at the 6-position for most of the cases donor **3** showed a slightly higher α-selectivity than donor **1,** which is an evidence of the importance for the anomeric selectivity of a more electron-withdrawing group at this position.

1,2-*cis* Stereoselective Galactosylations

Aiming to determine the importance of the sugar structure for the protein stabilisation effect, the synthesis of analogues from galactose was attempted. In general, glycosylations of thiogalactosides afford lower 1,2-*cis* selectivities and are less studied. This lack of selectivity can be explained by a higher reactivity due to unfavorable steric effects of the axially oriented substituent at C-4.

Using the same methodology used for glucose, thiogalactosides **19** and **21** (Scheme 8) were synthesised from methyl D-galactopyranoside, and conducted a series of experiments changing the solvent system and the temperature.



a) BnBr, NaH, DMF, 0ºC-r.t., 90%. b) Ac_2O/AcOH, H_2SO_4, 0ºC, 75%, α/β=3.7:1. c) EtSH, $BF_3.Et_2O$, CH_2Cl_2, 0ºC, 91%, α/β=2.4:1. d) NaOMe, MeOH, 0ºC, 81%. e) Chloroacetyl anhydride, Py, 0ºC, 90%.

Scheme 8. Synthesis of thiogalactosyl donors **19** and **21.**

In this study not only small and reactive alcohols (Figure 3) were chosen as acceptors but also other molecules with a more complex structure, like lipids, aminoacids, steroids, other sugars and bulkier alcohols (Figure 4).

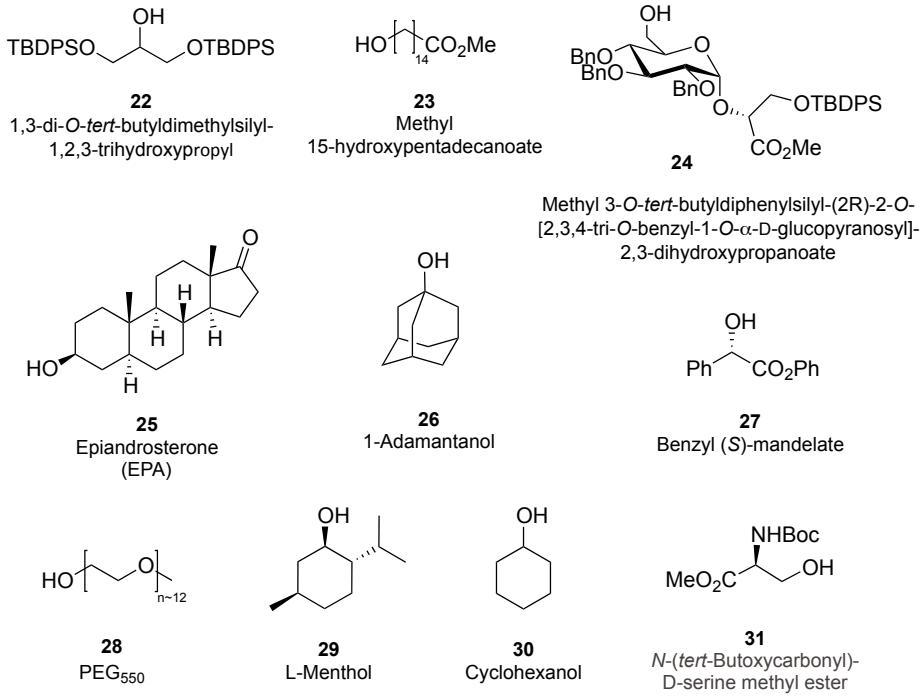
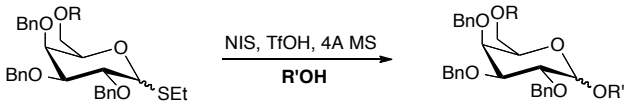


Figure 4. Glycosyl acceptors.

The results of the glycosylation reaction of donor **19** and **21** using NIS/TfOH system in the presence of 4Å MS are described in Table 3.

Table 3. Effect of solvent and temperature on the stereoselectivity of the glycosylation of donor **19** and **21**.



19: R=Ac
21: R=AcCl

Entry	R'OH	T (°C)	Solvent	Donor 19			Donor 21		
				Product (Yield,)	t (mn)	α/β	Product (Yield,%)	t (mn)	α/β
1	**4**	r.t.	CH_2Cl_2				**36** (76)	5	3.8:1
2		0	CH_2Cl_2	**32** (87)	5	3:1	**36** (95)	5	3.4:1
3			CH_2Cl_2	**32** (68)	30	2.6:1			
4			THF	**32** (15) [a]	60	5:1			
5			Et_2O	**32** (70)	40	3:1			
6			Tol:Dioxane (1:3)	**32** (54) [b]	10	2.5:1			
7		-60	CH_2Cl_2	**32** (83)	5	1.5:1	**36** (81)	15	1.8:1
8			Et_2O	**32** (40)	86	2.2:1			
9	**6**	0	CH_2Cl_2	**33** (92)	5	2.8:1			
10			Et_2O	**33** (84)	40	2.7:1			
11		-60	CH_2Cl_2	**33** (82)	10	1.7:1			
12			Et_2O	**33** (86)	40	4:1			
13	**7**	0	CH_2Cl_2	**34** (87)	5	3:1	**37** (80) [c]	5	3:1
14			Et_2O	**34** (92)	15	2.2:1			
15		-60	CH_2Cl_2	**34** (96)	5	1.2:1			
16			Et_2O	**34** (95)	15	1.4:1			

17	**9**	0	CH_2Cl_2	**35** (70)	5	2.4:1	**38** (86)	5	2.6:1
18	**22**						**39** (68)	5	2.4:1
19	**23**						**40** (63)	5	1:2.3
20	**24**						**41** (70)	5	1.4:1
21	**25**						**42** (57)	5	1:1.7
22	**26**						**43** (82)	5	1:1.3
23	**27**						**44** (70)	5	1:0
24	**28**						**45** (97) [c]	30	1:1
25	**29**						**46** (85)	5	1:1.5
26	**30**						**47** (93)	5	1:1.2
27	**31**						**48** (91)	5	2:1

[a] 43% of starting material and 38% of the hydrolysis product were recovered. [b] 18% of the hydrolysis product was recovered. [c] More equivalents of TfOH were added during the reaction.

Contrary to what happened with the glucose donors, glycosylation with methyl (*S*)-lactate using dichloromethane (Table 3, entry 2) as solvent gave better results than ethereal solvents. Also when using dichloromethane the reaction times were shorter and by leaving the reaction longer, the anomeric selectivity and the yield were decreased (Table 3, entry 3). Galactosylation in THF afforded the highest selectivity (α/β 5:1, Table 3, entry 4) but unfortunately the reaction time was longer, the yield was only 15% and we recovered 43% of starting material and 38% of the hydrolysis product.

The toluene/dioxane (1:3) mixture, employed by Boons and co-workers[19], afforded not only the lowest selectivity but also low yield (α/β 2.5:1, 54%, Table 3, entry 6). When using diethyl ether the same α/β ratio 3:1 was obtained as in dichloromethane but the reaction time was longer and the yield was lower (Table 3, entry 5).

When comparing the results obtained for the galactosylation reaction at different temperatures, the temperature dependence is obvious and that the

higher α-selectivites were obtained at higher reaction temperatures (Table 3, entries 1-2 and 7). This effect has been attributed to the fact that the addition to the donor at low temperatures (-60ºC) is subjected to kinetic control, and when the temperature is increased the effect of the remote neighbouring group at C-6 is enhanced.[9]

Experiments with the bulkier benzyl (*S*)-lactate in dichloromethane did not improve the anomeric selectivity (Table 3, entries 9 and 11), but when using ethyl ether as solvent at -60ºC the α/β ratio increased to 4:1 (Table 3, entry 12). Reaction with the primary hydroxyl group of methyl glycolate in dichloromethane at 0ºC afforded a α/β ratio of 3:1 (Table 3, entry 13) and worst results were obtained when other conditions were tested (Table 3, entries 14-16).

Having found that the best solvent for the glycosylation reaction of thiogalactoside donor **19** was dichloromethane, a series of experiments were performed with thiogalactoside donor **21** in order to assess if a more electron-withdrawing group at the C-6 position of the donor would have a dramatic effect on the selectivity of the reaction.

The results of the glycosylation reaction of donor **21** with methyl (*S*)-lactate in dichloromethane at different temperatures (Table 3, entries 1-2 and 6), confirmed the temperature dependence. The lower the reaction temperature the lower the anomeric selectivity, and the higher the reaction temperature the lower the yield of the reaction.

When comparing the results obtained for the two donors (**19** and **21**), we found that using the chloroacetyl thiogalactoside **21** with the small and reactive acceptors did not improve the anomeric selectivity and the reaction time remained the same (Table 3, entries 2, 7, 13 and 17). However, in the case of benzyl (*S*)-mandelate it was obtained exclusively the α-anomer (Table 3, entry 23).

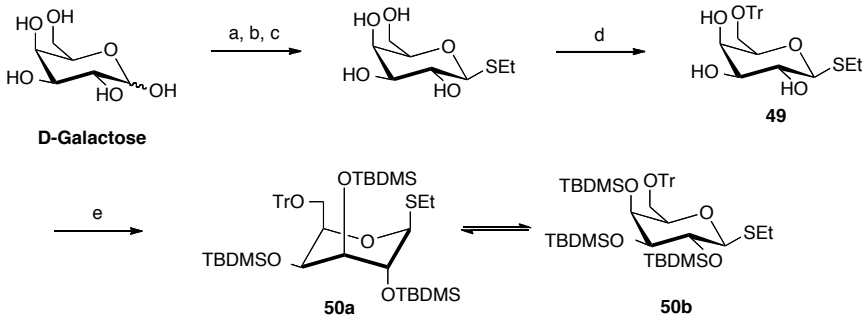
Contrary to what was expected, glycosylation of donor **21** with bulkier and less reactive acceptors (Table 3, entries 18-22 and 24-25) afforded lower anomeric selectivity, with more β anomer being obtained.

As predictable, thiogalactoside donors were more reactive and less selective than the corresponding thioglucosides. These results were in accordance with

the work of Wong and co-workers[23] who studied the structural effect of monosaccharides on the reactivity of the glycosylation reaction by comparing reaction rates of several thioglycoside donors with MeOH using NIS/TfOH system. In this study, for example, the perbenzylated tolylthiogalactoside reacts 6.4 times faster than the corresponding thioglucoside, which was explained by the stereo and inductive effects and possible involvement of the axial 4-*O* lone-pair electrons in the stabilization of the oxocarbenium ion intermediate.

The concept of an axial polar substituent stabilizing a positive charge to a higher degree than the corresponding equatorial equivalent explains these findings, and is the concept behind the idea of very reactive super-armed glycosyl donors developed by Bols and co-workers[24]. Super-armed donors are synthesised by forcing conformational changes, in this case forcing the oxygen substituents into an axial position by choosing bulky silyl protecting groups.

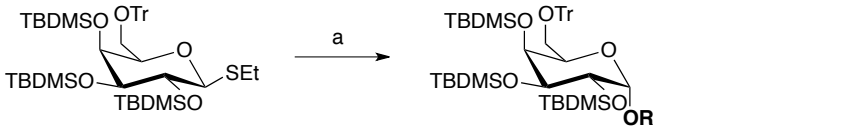
Since changing the solvent system and temperature were unable to improve the selectivity of the glycosylation reaction for the synthesis of galactosyl derivatives, based on the work of Bols[24] a super-armed thiogalactosyl donor **50** (Scheme 9) was designed, synthesised and studied. Super armed donor **50** was synthesised in five steps from D-galactose, which after selective protection of the primary hydroxyl group at C-6 position of the ethyl β-D-thiogalactoside in the form of trityl ether[25] **49** was silylated with TBDMSOTf according to literature[24]. When protected with bulky silyl groups, donor **50** (Scheme 9) would suffer a conformational change to a more “axial-rich” conformation that would enhance their reactivity.



a) Ac_2O, Py, DMAP, 0ºC-r.t., quant. b) $ZnCl_2$, EtSH, 0ºC-r.t., 65%. c) NaOMe, MeOH, r.t., 86%.
d) TrCl, Py, DMAP, 60ºC, 81%. e) TBDMSOTf, Py, DMAP, 60ºC, 83%.

Scheme 9. Synthesis of super armed thiogalactosyl donor **50**.

Since in solution donor **50** is in slow equilibrium between several conformers the peaks on ^{1}H NMR spectra were broad and complex. The quality of the spectra could be improved by cooling the samples in $CDCl_3$ to -30°C where the equilibration was slow and the different conformers gave sharper signals. To test the donor, the glycosylation reaction was performed using the NIS/TfOH system as the promoter in dichloromethane at -78ºC, and as acceptors methyl (*S*)-lactate **4** and methyl glycerate **9** (Scheme 10).



51, R: (*S*)-CH(CH_3)CO_2Me , 48%, α/β=1:0.
52, R: (*R*)-CH(CH_2OTBDPS)CO_2Me, 22%, α/β=1:0.

a) ROH: **4** or **9**, NIS, TfOH, 4A MS, CH_2Cl_2, -78ºC.

Scheme 10. Glycosylation reaction of super armed thiogalactosyl donor **50**.

Despite the fact of the good 1,2-*cis* selectivities achieved for both acceptors, the yields obtained were disappointing (Scheme 10) due to degradation of the initial donor. Examples in literature[24] show higher yields when other sugars are used as acceptors, which are not comparable to our relatively small and reactive alcohols with few functional groups. Possibly with less reactive acceptors this would be an efficient approach. Moreover, the super armed

thiogalactosyl donor used in literature bears a benzyl group at the C-6 position, the acid lability of the trityl group of donor **50** can also explain the differences in the yields obtained.

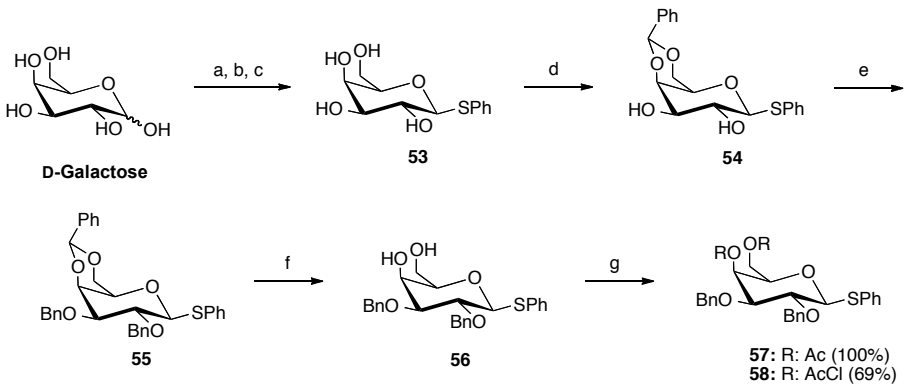
Aiming for a selective and efficient method to obtain the α-galactosyl derivatives, based on works reported on literature[5, 6, 8-10], the influence of remote neighbouring group at C-4 position of the donor was studied.

Boons and co-workers[8] observed the effect of having a participating neighboring group at C-4 position of thiogalactosyl donors for the stereochemical outcome of glycosylations. They tested several tribenzylated thiogalactosides bearing different groups at C-4 position, and obtained high α-anomeric selectivity when using electron-donating groups (like esters) in a 1,4-dioxane/toluene solvent system. Kalikanda et al.[5] followed the same rationale and tested a dibenzylated thiogalactoside donor bearing acetate groups at C-3 and C-4 positions using the NIS/TfOH promoter system in dichloromethane. Excellent α-selectivity was observed in all test reactions using other glycosides as glycosyl acceptors and a superior stereoselectivity was observed when compared with tetrabenzyl protected galactose donor.

This C-4 participating neighboring group effect was also found in studies with other types of donors, for example Lin and co-workers[9] reported that galactosyl phosphite donors bearing acyl remote participating groups (benzoyl) at C-4 and C-6 positions promoted high α-anomeric selectivities in glycosylations with primary and secondary hydroxyl acceptors. In a more recent study, Matta and coworkers[10] compared the use of dibenzoyl esters to benzylidene as protective groups at C-4 and C-6 positions of trichloroacetimidate donors with 2-naphtylmethyl (NAP) non-participating group at C-2, and the higher α-selectivities in the glycosylations were obtained with the dibenzoyl trichloroacetimidate donor.

Based on these studies, thiogalactoside donors **57** and **58** were synthesised with a further acetate or chloroacetyl group respectively at C-4 position. The diacetate thiogalactosyl donor **57** was prepared in seven steps according to the literature[26,27,28] from D-galactose (Scheme 11) using standard protection and deprotection steps. The dichloroacetyl thiogalactosyl donor **58** was

prepared in the same manner from diol **56** after esterification with chloroacetic anhydride (Scheme 11).



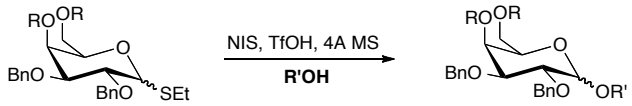
a) Ac_2O, DMAP, Py, 0ºC-r.t. b) PhSH, $BF_3.Et_2O$, CH_2Cl_2, reflux, over-night. (90% in two steps.) c) NaOMe, MeOH, 0ºC-r.t., 98%. d) $PhCH(OMe)_2$, CSA, THF, reflux, 90%. e) BnBr, NaH, DMF, 0ºC, 86%. f) MeOH: CH_2Cl_2 (2:1), *p*-TSOH (cat.), r.t., 88%. g) Ac_2O or Chloroacetic anhydride, Py, 0ºC.

Scheme 11. Synthesis of thiogalactosyl donors **57** and **58**.

Donors **57** and **58** were used in the glycosylation study with several acceptors (Figure 3, Figure 4) at different temperatures using the NIS/TfOH system in dichloromethane, the solvent in which previously we obtained the best results for thiogalactoside donors **19** and **21**. The results are presented in Table 4.

When comparing the results obtained with the thiogalactosyl donors **57** and **58** (Table 4) to the ones obtained previously with donor **21** (Table 3) the di-acetyl donors gave, in general, significant higher α-anomeric selectivities for almost all of the acceptors. As expected, dichloroacetyl donor **58** provided higher α selectivity than the diacetate donor **57**, which can be explained by having stronger electron-withdrawing esters. Also diethyl ether proved to be, once more, a worst solvent than dichloromethane for the glycosylations with galactose.

Table 4. Effect of solvent and temperature on the stereoselectivity of the glycosylation of donor **57** and **58**.



57: R=Ac
58: R=AcCl

Entry	R'OH	T (ºC)	Solvent	Donor 57			Donor 58		
				Product (Yield,%)	t (mn)	α/β	Product (Yield,%)	t (mn)	α/β
1	**4**	r.t.	CH_2Cl_2	**59** (96)	5	10:1	**72** (67)	5	1:0
2		0	CH_2Cl_2	**59** (80)	5	10:1	**72** (74)	5	1:0
3			Et_2O	**59** (56) [a, b]	110	6.7:1	**72** (20) [a, c]	110	1:0
4		-60	CH_2Cl_2	**59** (91)	15	8.6:1	**72** (63) [d]	15	1:0
5	**7**	0	CH_2Cl_2	**60** (84) [a]	15	6.3:1	**73** (63) [a]	5	7.5:1
6	**9**			**61** (90)	5	1:0	**74** (86)	5	1:0
7	**22**			**62** (86)	5	1:0	**75** (69)	5	1:0
8	**23**			**63** (77)	5	1.2:0	**76** (63)	5	1.5:1
9	**24**			**64** (69)	15	1:0	**77** (89)	15	1:0
10	**25**			**65** (91)	5	2.2:1	**78** (41)	15	3.8:1
11	**26**			**66** (91)	5	6.5:1	**79** (89)	5	6.2:1
12	**27**			**67** (68)	5	1:0	**80** (79)	5	1:0
13	**28**			**68** (99) [a]	40	1.9:1	**81** (99) [a]	60	1.5:1
14	**29**			**69** (85)	5	2.7:1	**82** (94)	5	3.4:1
15	**30**			**70** (78) [a]	50	1.1:1	**83** (87)	5	2.5:1
16	**31**			**71** (69) [e]	5	2:1	**84** (73) [f]	5	2:1

Caption of Table 4: [a] More equivalents of TfOH were added during the reaction. [a] 15% of starting material was recovered. [b] 50% of starting material was recovered. [d] 19% of the hydrolysis product was recovered. [e] 21% of starting material was recovered. [f] 20% of starting material was recovered.

Galactosylation with more hindered small acceptors afforded higher anomeric selectivities (Table 4, entries 1-7 and 12). When methyl (*R*)-glycerate **9** and the glycerol **22** (Table 4, entries 6 and 7) were used as acceptors both donors **57** and **58** provided only the α-anomer. In the case of the small and reactive methyl (*S*)-lactate **4**, the α-anomer was exclusively obtained when using thiogalactosyl donor **58** (Table 4, entries 1-4). Glycosylation with benzyl (*S*)-mandelate, as it was previously seen with donor **21**, also afforded only the α-galactoside.

In most of the studies found in the literature that showed good α-selectivities the acceptors used were other glycosides, mono or higher saccarides, and the same happened when another sugar was used as the acceptor, the α-anomer was formed exclusively (Table 4, entry 9).

In some cases, like when using EPA **25**, adamantanol **26** and menthol **29** as acceptors, even though the improvement was not as significant as in other cases, the selectivity of the reaction was inversed (Table 4, entries 10-11 and 14) when compared with the results obtained with donor **21** (Table 3, entries 21-22 and 25). In the case of the reaction with the EPA acceptor (Table 4, entry 10) a superior stereoselectivity was obtained (donor **57** α/β 2.2:1, donor **58** α/β 3.8:1) when compared with the results obtained in literature[29] for the tetrabenzyl protected galactose donor with cholestanol a very similar molecule to EPA (α/β 1:1).

The lipophilic primary alcohols, **23** and **28** (Table 4, entries 8 and 13), with both donors gave the worst α/β ratios showing no selectivity and no tendency for improvement. This lack of stereocontrol can be explained by the tendency of lipids to aggregate and hence change reactivity.[30]

In the case of *N*-Boc serine methyl ester **31** there was no improvement (Table 4, entry 16), the α/β ratio (2:1) was the same as with donor **21** (Table 3, entry 27). A low α-selectivity for the glycosylation of *N*-acylated β-hydroxy

amino acids have been explained in the literature[31] by a decrease in the nucleophilicity of the glycosyl acceptor due to hydrogen-bonding between the OH and NH groups of the acceptor. Also, the use of Fmoc as the amine protecting group instead of Boc, and the use of a benzyl ester instead of methyl ester as the carboxyl protecting group have both proven to improve the α-selectivity of the reaction[31,32]. However, this was not attempted in this study.

The results obtained undoubtedly confirm the importance of the ester functionality at the C-4 position of the thiogalactosyl donor for the stereoselectivity of the glycosylation reaction. Although the 4-*O*-chloroacetyl group could disarm the system slightly, the higher α-stereoselectivity can be attributed to a remote stereocontrol and electronic effect of the C-4 ester in the galactoside donor. Boons[8], Kalikanda[5], Lin[9] and Matta[10] have suggested the possibility of remote group participation by axial carboxylated esters on C-4 position leading to improved synthesis of α-galactosides. This hypothesis was studied by Crich and co-workers[2, 12] who found no experimental evidence in support of neighbouring group participation at this position and at the C-6 position. Crich defends that no single effect is responsible for these results and the mechanism for this remote stereocontrol is still unknown. The remarkable α-anomeric selectivities obtained with donors **57** and **58** cannot be explained by neighbouring group participation of the esters at C-4 and C-6, but to stereoelectronic and conformational influences. The preferential formation of the α-anomer can be explained based on the system proposed by Crich[15] (Scheme 4) and on the reactivity of the respective triflate intermediates, where the β-triflate, being unstable and more reactive shifts the equilibrium towards the formation of the α-galactoside by nucleophilic substitution by the alcohol in an S_N2 manner.

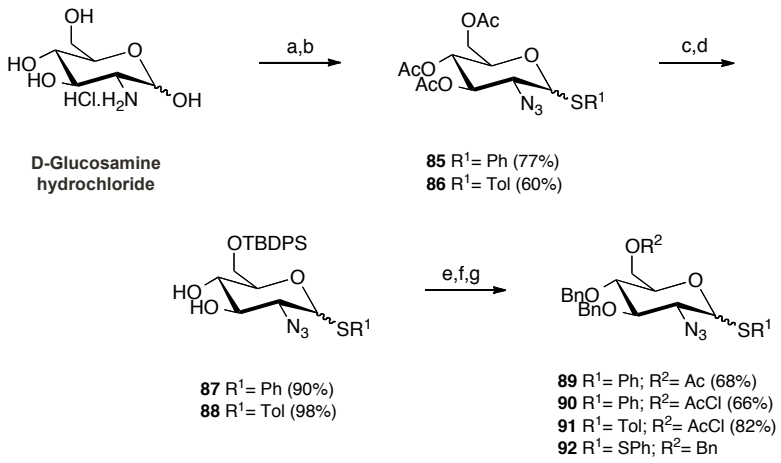
1,2-*cis* Stereoselective 2-Azido-2-DeoxyGlucosylations

Glucosamine is one of the most abundant monosaccharides and an important building block present in many natural molecules involved in a number of biological processes. Although structurally similar to glucose it

possesses an amine group in the C-2 position that in solution and according to pH can be charged. In order to study the effect of an amine group, glucosamine analogues were synthesised. Synthesis of 1,2-*trans* 2-amino-2-deoxy-glycosides can be achieved by applying the C-2 neighbouring group participation methodology, however 1,2-*cis* 2-amino-2-deoxy-glycosides face the same problems that we presented for 1,2-*cis* glucosides and galactosides.

For the synthesis of the α-glucosamine analogues, the most common way is to protect the amino functionality by masking it in the form of an azide at C-2 position of the donor. The use of 2-azido-2-deoxyglucosyl the trichloroacetimidate donors for the synthesis of α-glucosamine compounds have been widely reported, due to the non-participating nature of the azide group and subsequent convenient conversion to the amine. Similar glycosylations using thioglycosides have been less studied.

Several 2-azido-2-deoxythioglucosides were prepared from the readily available glucosamine hydrochloride salt that after treatment with imidazole-1-sulfonyl azide hydrochloride salt, followed by acetylation, produced per-*O*-acetyl 2-azido-2-deoxyglucosyl acetate (Scheme 12).[33] Thioglucoside formation with the respective thiol in the presence of boron trifluoride–diethyl ether was sluggish and 20-30% of the initial acetate was recovered. In the future the solution to this problem could be the use of sonication[34, 35], however we did not test this method. Thioglycosides **85** and **86** were deacetylated by the *Zemplé* method and the primary hydroxyl group selectively silylated before benzylation of the secondary hydroxyl groups. The 2-azido*per*-*O*-benzyl-2-deoxythioglucoside donor **92** (Scheme 12) was obtained as a by-product of the benzylation reaction of compound **87** in 7% yield. After deprotection of the silyl group, esterification with acetic or chloroacetic anhydride gave donors **89**-**91**.



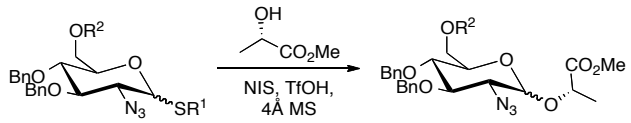
a) (i) K_2CO_3, $CuSO_4.5H_2O$, imidazole-1-sulfonyl azide hydrochloride, MeOH, r.t. (ii) Ac_2O, Py, DMAP, r.t., 82% (over two steps). b) PhSH or TolSH, $BF_3.OEt_2$, CH_2Cl_2, 0ºC-r.t. c) NaOMe, MeOH, 0ºC-r.t. d) TBDPSCl, Py, DMAP, r.t. e) NaH, BnBr, DMF, 0ºC-r.t. f) TBAF, THF, r.t. g) Ac_2O or $ClAc_2O$, Py, DMAP, r.t.

Scheme 12. Synthesis of 2-azido-2-deoxythioglucoside donors **89-92.**

Following our line of work and aiming to determine the best conditions to obtain the new synthetic 1,2-*cis* glucosamine analogues, the influence of the different protecting groups at the 6-position of the donor, as well as the solvent and the temperature on these glycosylations, were studied. Using the NIS/TfOH system as the promoter and methyl (*S*)-lactate **4** as the acceptor. These results are presented in Table 5.

Since most common solvents found in literature for these donors are dichloromethane and diethyl ether, initial studies of the reactivity of the donors were done in dichloromethane. The selectivities were generally lower than those for the corresponding thioglucosides donors and lowering the temperature showed no great impact on the selectivity, however the reaction time increased considerably (Table 5, entries **7** and **12**). Contrary to what happened with thioglucoside donors, when using ethyl ether as solvent 2-azido-2-deoxythioglucosides donors did not react and the initial donor was recovered (Table 5, entries **5** and **10**).

Table 5. Effect of solvent, temperature and protecting groups on the stereoselectivity of the glycosylation of donors **89**-**92**.



89-92

Entry	R^1	R^2	T (ºC)	Solvent	Product (Yield,%)	t (mn)	α/β
1	Ph	Bn	-10	CH_2Cl_2	**93** (73)	10	1:1
2				CH_2Cl_2:Et_2O (1:1.8) [a]	**93** (78)	120	2.6:1
3		Ac	r.t.	CH_2Cl_2	**94** (87)	5	3:1
4			-10	CH_2Cl_2	**94** (90)	10	3.5:1
5				Et_2O	**94** (0) [b]	90	-
6				CH_2Cl_2:Et_2O (1:1.8) [a]	**94** (96)	120	7.3:1
7			-78	CH_2Cl_2	**94** (98)	120	2.5:1
8		AcCl	r.t.	CH_2Cl_2	**95** (96)	5	3.6:1
9			-10	CH_2Cl_2	**95** (85)	10	4.2:1
10				Et_2O	**95** (0) [b]	90	-
11				CH_2Cl_2:Et_2O (1:2) [a]	**95** (84)	120	8.7:1
12			-78	CH_2Cl_2	**95** (87)	120	4.2:1
13	Tol	AcCl	-10	CH_2Cl_2	**95** (93)	10	4:1
14				CH_2Cl_2:Et_2O (1:2) [a]	**95** (99)	120	10.3:1
15			-78	CH_2Cl_2:Et_2O (1:2) [a]	**95** (99)	120	8:1

[a] Adition of NIS(2 eq.)/TfOH system in solution.

[b] Recovery of the initial donor.

Wong and co-workers[36] reported the use of a solvent mixture of CH_2Cl_2/Et_2O (1:4) with the NIS/TfOH system for obtaining α-2-azido-2-deoxyglucosides

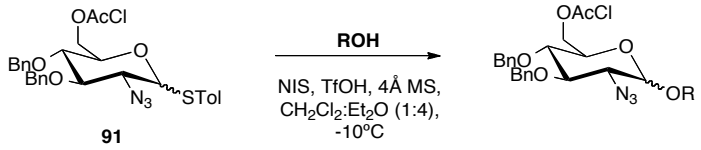
from a tribenzylated 2-azido-2-deoxythioglucoside **92**. The selectivity obtained for the glycosylation using 2-deoxystreptamine as acceptor was in the range of 10:1 α/β ratio and 58% yield. The use of diethyl ether as cosolvent was fundamental to this selectivity because when the reaction was performed in dichloromethane at -78°C the α/β ratio was only 2.5:1. Using the CH_2Cl_2/Et_2O (1:4) solvent combination employed by Wong[36] and direct addition of the NIS/TfOH system to the reaction mixture, did not afford any improvement and we recovered the initial donor. However, when using this solvent combination and adding the NIS/TfOH system in solution to the reaction mixture there was a remarkable improvement of the α-selectivity for almost all of the donors (Table 5, entries 6, 11 and 14). Performing the reaction at a lower temperature lowered the selectivity from 10.3:1 α/β ratio at 0ºC to 8:1 at -78ºC (Table 5, entries 14 and15).

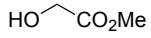
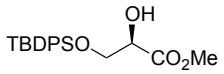
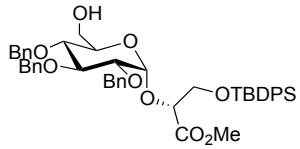
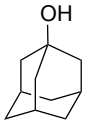
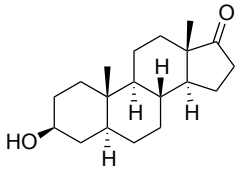
Other important aspect is that when using these conditions but only one equivalent of NIS, the reaction was incomplete and the initial donor was recovered. This problem was overcome by the addition of two equivalents of NIS. Although the influence of the anomeric thio-group for the stereoselectivity of the glycosylation has been reported in literature[37], in this case this effect is less obvious since phenyl and tolyl are both bulky thio-groups with similar properties.

Comparing the results obtained with different protecting groups at the 6-position of the donors it is evident that the presence of an ester-protecting group at this position influences the stereoselectivity of the reaction. Donors **89**, **90** and **91** showed higher α-selectivity than **92**.

Since the best results for the glycosylation reaction with methyl (*S*)-lactate were obtained using a mixture of CH_2Cl_2/Et_2O (1:4) at -10ºC with donor **91** (Table 5, entry 14), these conditions were selected to study the selectivity of the reaction using a wide range of acceptors (Figure 3, Figure 4) under these conditions. The results are presented on Table 6.

Table 6. Glycosylation of donor **91** with different acceptors.



Entry	R^3OH	Product (Yield,%)	t (mn)	α/β
1	 **7**	**96** (76)	180	12:1
2	 **9**	**97** (84)	120	>10:1
3	 **24**	**98** (42)[(a)]	120	5:1
4	 **26**	**99** (50)	120	6.2:1
5	 **25**	**100** (46)[(a)]	120	3.2:1

[a] Recovery of the initial donor.

Glycosylation with small and reactive donors, such as methyl glycolate **7** and methyl glycerate **9**, gave better α-selectivities and better yields (Table 6, entry 1 and 2). When the acceptor was bulkier the stereoselectivity of the reaction was lower, so were the yields and non-reacted initial donor was recovered (Table 6, entry 3-5).

Conclusion

The determinant step during the synthesis of any oligosaccharide, glycoconjugates or glycosides is the control of the anomeric stereochemistry during the course of the glycosylation reaction.

In this study, several thioglycosides derived from glucose, galactose and glucosamine were synthesised in order to determine the best reaction conditions to obtain stereoselectively 1,2-*cis* glycosides. Since the stereoselectivity of the glycosylation reaction is dependent of many factors, the influence of solvent, temperature and reactivity of its components (like nature and position of protecting groups, and different acceptors) were studied. All synthesised thioglycoside donors were stable to many different chemical manipulations, and under the presence of soft electrophilic reagents, such as the NIS/TfOH promoter system, afforded clean glycosylations.

As expected, the best results for the synthesis of 1,2-*cis* glucosides with the thioglucosides **1** and **3** were obtained when using ethyl ether as the solvent for all the small and reactive acceptors tested. Also, the α-selectivity could be further improved by lowering the reaction temperature to -60ºC. The presence of the ester group at the C-6 position proved to be important for the stereoselectivity and this effect can be enhanced by the presence of a stronger electron-withdrawing group at this position.

Using the same conditions for the synthesis of 1,2-*cis* galactosides, glycosylations with the thiogalactosides **19** and **21** produced lower stereoselectivities, which could not be improved by changing the solvent or the temperature. These findings could be explained by the higher reactivity and shorter reaction times of thiogalactoside donors when compared to the corresponding thioglucosides. Nevertheless the conditions that showed best α-selectivities were dichloromethane at -10ºC. Trying to develop an efficient method for the synthesis of 1,2-*cis* galactosides, a super-armed thiogalactosyl donor **50** was synthesised and tested. Although glycosylation with the small and reactive acceptors gave only the desired α-products, the yields were poor.

The influence of the nature of the protecting group at the C-4 position of galactosyl donors on the stereochemical outcome of the glycosylation reaction

was studied. Thiogalactosyl donors bearing acetyl and chloroacetyl groups both on C-4 and C-6 positions were synthesised, and higher α-selectivities were obtained with almost all the acceptors tested in this reaction, proving the α-directing effect of ester functionalities at the C-4 position of galactosyl donors. In addition, comparing the results obtain for the two donors (**57** and **58**) led to the conclusion that stronger electron-withdrawing groups gave better results, in our case the dichloroacetyl thiogalactosyl donor **58**.

For the synthesis of the α-glucosamine analogues, several 2-azido-2-deoxythioglucoside donors were synthesised, with the amine group conveniently protected in the form of a non-participating azide, which afterwards could be converted to the amine. Performing the glycosylation reaction at -10 ºC and the use of a mixture of CH_2Cl_2:Et_2O (1:4) as solvent improved the anomeric selectivity favoring the α product. Glycosylation with small and reactive acceptors gave better results than the ones obtained with bulkier and less reactive alcohols.

Regarding the results obtained for each hexose, reactivity is dependent on their structure and of the protecting groups used. Each hexose needs to be considered independently and the conditions of the reaction optimized according to it.

In these studies with the different hexoses, when comparing different acceptors it is clear that the reactivity and structure of the acceptors plays an important role on the stereoselectivity of the glycosylation reaction. In most of the studies described in the literature the acceptors are other sugars or bulkier molecules that can interact in several ways with the donor, so the results cannot be compared to the small reactive acceptors that we used. Moreover, alcohol reactivity is typically known to be inversely correlated with the 1,2-*cis* stereoselectivity meaning that, most reactive hydroxyls are stronger nucleophiles, giving faster reactions which being difficult to control produce lower α-selectivities. Acknowledging this, the good results obtained in some cases with the small reactive acceptors were highly rewarding.

In summary, although many factors that influence stereoselectivity have been object of study for many years, it is still difficult to find a general method to obtain 1,2-*cis* glycosides or predict the α stereoselectivity when using

different acceptors. Furthermore, full understanding of the mechanisms involved in the glycosylation reaction would provide insight for the development of methodologies to control anomeric stereochemistry. In this study, different methods were developed to enhance the α-selectivity for the synthesis of 1,2-*cis* glucosides, galactosides and glucosamines that are intermediates in the synthesis of several analogues of hypersolutes. The methods developed can be very useful for the efficient synthesis of higher saccharides.

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through grant PEst-OE/EQB/LA0004/2011. E.L. acknowledges FCT for a PhD grant SFRH/47702/2008. We thank CERMAX for the use of the NMR spectrometers, which were purchased within the framework of the National Programme for Scientific Re-equipment, contract REDE/1517/RMN/2005, with funds from POCI 2010 (FEDER) and FCT.

References

1. Demchenko, A. V., *Handbook of Chemical Glycosylation*. Wiley: 2008.

2. Bohe, L.; Crich, D., A propos of glycosyl cations and the mechanism of chemical glycosylation. *Comptes Rendus Chimie* **2011,** 14, 3-16.

3. Mydock, L. K.; Demchenko, A. V., Mechanism of chemical *O*-glycosylation: from early studies to recent discoveries. *Organic & Biomolecular Chemistry* **2010,** 8, 497-510.

4. Baek, J. Y.; Lee, B. Y.; Jo, M. G.; Kim, K. S., beta-Directing effect of electron-withdrawing groups at O-3, O-4, and O-6 positions and alpha-directing effect by remote participation of 3- *O* -acyl and 6-*O*-acetyl groups of donors in mannopyranosylations. *Journal of the American Chemical Society* **2009,** 131, 17705-17713.

5. Li, Z. T.; Zhu, L. S.; Kalikanda, J., Development of a highly alpha-selective galactopyranosyl donor based on a rational design. *Tetrahedron Letters* **2011,** 52, 5629-5632.

6. Kalikanda, J.; Li, Z. T., Study of the stereoselectivity of 2-azido-2-deoxygalactosyl donors: Remote protecting group effects and temperature dependency. *Journal of Organic Chemistry* **2011,** 76, 5207-5218.

7. Ustyuzhanina, N.; Komarova, B.; Zlotina, N.; Krylov, V.; Gerbst, A.; Tsvetkov, Y.; Nifantiev, N., Stereoselective alpha-glycosylation with 3-*O*-acetylated D-gluco donors. *Synlett* **2006**, 921-923.

8. Demchenko, A. V.; Rousson, E.; Boons, G. J., Stereoselective 1,2-cis-galactosylation assisted by remote neighboring group participation and solvent effects. *Tetrahedron Letters* **1999,** 40, 6523-6526.

9. Cheng, Y. P.; Chen, H. T.; Lin, C. C., A convenient and highly stereoselective approach for alpha-galactosylation performed by galactopyranosyl dibenzyl phosphite with remote participating groups. *Tetrahedron Letters* **2002,** 43, 7721-7723.

10. Khaja, S. D.; Kumar, V.; Ahmad, M.; Xue, J.; Matta, K. L., Novel galactosyl donor with 2-naphthylmethyl (NAP) as the non-participating group at C-2 position: efficient synthesis of alpha-galactosyl ceramide. *Tetrahedron Letters* **2010,** 51, 4411-4414.

11. Ma, Y. Y.; Lian, G. Y.; Li, Y.; Yu, B. A., Identification of 3,6-di-*O*-acetyl-1,2,4-*O*-orthoacetyl-alpha-D-glucopyranose as a direct evidence for the 4-*O*-acyl group participation in glycosylation. *Chemical Communications* **2011,** 47, 7515-7517.

12. Crich, D.; Hu, T.; Cai, F., Does neighboring group participation by non-vicinal esters play a role in glycosylation reactions? Effective probes for the detection of bridging intermediates. *The Journal of Organic Chemistry* **2008,** 73, 8942-8953.

13. Faria, T. Q.; Mingote, A.; Siopa, F.; Ventura, R.; Maycock, C.; Santos, H., Design of new enzyme stabilizers inspired by glycosides of hyperthermophilic microorganisms. *Carbohydrate Research* **2008,** 343, 3025-3033.

14. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K., Halide Ion Catalyzed glycosidation reactions syntheses of alpha-linked disaccharides. *Journal of the American Chemical Society* **1975,** 97, 4056-4062.

15. Crich, D.; Cai, W. L., Chemistry of 4,6-*O*-benzylidene-D-glycopyranosyl triflates: Contrasting behavior between the gluco and manno series. *Journal of Organic Chemistry* **1999,** 64, 4926-4930.

16. Lourenco, E. C.; Maycock, C. D.; Ventura, M. R., Synthesis of potassium (*2R*)-2-*O*-alpha-D-glucopyranosyl-(1 -> 6)-alpha-D-glucopyranosyl-2,3-dihydroxypropanoate a natural compatible solute. *Carbohydrate Research* **2009,** 344, 2073-2078.

17. Paulsen, H., Advances in selective chemical syntheses of complex oligosaccharides. *Angewandte Chemie-International Edition in English* **1982,** 21, 155-173.

18. Frechet, J. M.; Schuerch, C., Solid-Phase synthesis of oligosaccharides. Steric control by C-6 substituents in glucoside syntheses. *Journal of the American Chemical Society* **1972,** 94, 604-&.

19. Demchenko, A.; Stauch, T.; Boons, G. J., Solvent and other effects on the stereoselectivity of thioglycoside glycosidations. *Synlett* **1997**, 818-&.

20. Lucas, R.; Hamza, D.; Lubineau, A.; Bonnaffe, D., Synthesis of glycosaminoglycan oligosaccharides - An unexpected inhibitory effect of a remote *N*-acetyl group upon trichloroacetimidate-mediated couplings. *European Journal of Organic Chemistry* **2004**, 2107-2117.

21. Ishiwata, A.; Munemura, Y.; Ito, Y., Synergistic solvent effect in 1,2-*cis*-glycoside formation. *Tetrahedron* **2008,** 64, 92-102.

22. Lok, C. M.; Ward, J. P.; Vandorp, D. A., Synthesis of chiral glycerides starting from D-serine and L-serine. *Chemistry and Physics of Lipids* **1976,** 16, 115-122.

23. Zhang, Z. Y.; Ollmann, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C. H., Programmable one-pot oligosaccharide synthesis. *Journal of the American Chemical Society* **1999,** 121, 734-753.

24. Pedersen, C. M.; Nordstrom, L. U.; Bols, M., "Super armed" glycosyl donors: Conformational arming of thioglycosides by silylation. *Journal of the American Chemical Society* **2007,** 129, 9222-9235.

25. France, R. R.; Compton, R. G.; Davis, B. G.; Fairbanks, A. J.; Rees, N. V.; Wadhawan, J. D., Selective electrochemical glycosylation by reactivity tuning. *Organic & Biomolecular Chemistry* **2004,** 2, 2195-2202.

26. Sugimura, H.; Watanabe, K., Formal synthesis of cytosamine - A component of nucleoside antibiotics, the amicetin family. *Synthetic Communications* **2001,** 31, 2313-2321.

27. Li, Z. T.; Gildersleeve, J. C., Mechanistic studies and methods to prevent aglycon transfer of thioglycosides. *Journal of the American Chemical Society* **2006,** 128, 11612-11619.

28. Crich, D.; de la Mora, M.; Vinod, A. U., Influence of the 4,6-*O*-benzylidene, 4,6-*O*-phenylboronate, and 4,6-*O*-polystyrylboronate protecting groups on the stereochemical outcome of thioglycoside-based glycosylations mediated by 1-benzenesulfinyl piperidine/triflic anhydride and N-iodosuccinimide/trimethylsilyl triflate. *Journal of Organic Chemistry* **2003,** 68, 8142-8148.

29. Crich, D.; Smith, M., 1-Benzenesulfinyl piperidine/trifluoromethanesulfonic anhydride: A potent combination of shelf-stable reagents for the low-temperature conversion of thioglycosides to glycosyl triflates and for the formation of diverse glycosidic linkages. *Journal of the American Chemical Society* **2001,** 123, 9015-9020.

30. Kononov, L. O.; Malysheva, N. N.; Orlova, A. V.; Zinin, A. I.; Laptinskaya, T. V.; Kononova, E. G.; Kolotyrkina, N. G., Concentration dependence of glycosylation outcome: A clue to reproducibility and understanding the reasons behind. *European Journal of Organic Chemistry* **2012**, 1926-1934.

31. Lin, C. C.; Shimazaki, M.; Heck, M. P.; Aoki, S.; Wang, R.; Kimura, T.; Ritzen, H.; Takayama, S.; Wu, S. H.; WeitzSchmidt, G.; Wong, C. H., Synthesis of sialyl Lewis X mimetics and related structures using the glycosyl phosphite methodology and evaluation of E-selectin inhibition. *Journal of the American Chemical Society* **1996,** 118, 6826-6840.

32. Polt, R.; Szabo, L.; Treiberg, J.; Li, Y. S.; Hruby, V. J., General-Methods for alpha-*O*-Ser/Thr or beta-*O*-Ser/Thr glycosides and glycopeptides - Solid-phase synthesis of *O*-glycosyl cyclic enkephalin analogs. *Journal of the American Chemical Society* **1992,** 114, 10249-10258.

33. Goddard-Borger, E. D.; Stick, R. V., An efficient, inexpensive, and shelf-stable diazotransfer reagent: Imidazole-1-sulfonyl azide hydrochloride. *Organic Letters* **2007,** 9, 3797-3800.

34. Deng, S. L.; Gangadharmath, U.; Chang, C. W. T., Sonochemistry: A powerful way of enhancing the efficiency of carbohydrate synthesis. *Journal of Organic Chemistry* **2006,** 71, 5179-5185.

35. Mong, K. K. T.; Yen, Y. F.; Hung, W. C.; Lai, Y. H.; Chen, J. H., Application of 2-azido-2-deoxythioglycosides for beta-glycoside formation and oligosaccharide synthesis. *European Journal of Organic Chemistry* **2012**, 3009-3017.

36. Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S. C.; Wong, C. H., Design and synthesis of new aminoglycoside antibiotics containing neamine as an optimal core structure: Correlation of antibiotic activity with in vitro inhibition of translation. *Journal of the American Chemical Society* **1999,** 121, 6527-6541.

37. Boons, G. J.; Stauch, T., Stereoselectivity in glycosidic bond formation: Studies on the anomerisation of thioglycosides. *Synlett* **1996**, 906-+.

Chapter 3

Synthesis of MGG: A Natural Compatible Solute

This chapter contains data published in:

Eva C. Lourenço, M. Rita Ventura; Synthesis of potassium (2*R*)-2-*O*-α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoate: a natural compatible solute. *Eur. J. Org. Chem*. **2011**, 6698–6703.

The author contributed fully to this chapter, namely in the planning of the experimental work, performing the experiments and writing of the manuscript.

Synthesis of MGG: A Natural Compatible Solute

Abstract

An efficient and stereocontrolled synthesis of the recently isolated and rare natural solute α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-(1→2)-D-glycerate potassium salt (MGG) was achieved in solution and on solid support. Different strategies using two thioglucosides bearing a free C-2 hydroxyl group were tested in solution. These thioglucosides were used as glycosyl acceptors in the first glycosylation reaction with the tetraacetylmannosyl trichloroacetimidate, and as donors in the second glycosylation reaction with the glycerate acceptor. The glycosylation reactions with both thioglucosides were highly stereoselective affording exclusively the α-anomer. For the solid supported synthesis the thioglucoside donor/acceptor was efficiently immobilized on the solid support (Tentagel MB-NH_2) by the C-6 hydroxyl group using a succinate linker, and successfully used in the synthesis of MGG.

Introduction

The study of the intracellular accumulation of organic solutes in *Petrotoga miotherma*[1] led to the identification of a new solute, α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-(1→2)-D-glycerate (MGG, Figure 5), which more recently was also isolated from *Petrotoga mobilis*[2]. While in *Petrotoga miotherma*[1], MGG is accumulated during low-level of osmotic adaptation and it is not used against oxidative or heat stress, in *Petrotoga mobilis*[2] it is the major compatible solute under osmotic and thermal stress. The ability of MGG to protect malate dehydrogenase (pig heart MDH), a model enzyme, against heat inactivation and freeze-drying led to a patent for the stabilization and preservation of biomaterials.[1] Until the present date, these are the only two organisms known to accumulate MGG.

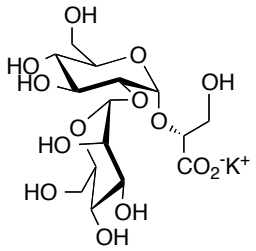


Figure 5. MGG, **101**.

The structure of the novel MGG which was established to be (2*R*)-2-*O*-α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoate, is related to the compatible solutes α-D-mannosyl-D-glycerate (MG), α-D-glucosyl-D-glycerate (GG) and α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2)-D-glycerate (GGG) (Figure 1), where a hexose moiety is α-lynked to the C-2 of a glyceric unit. Since this hypersolute is available in very small amounts from its natural sources, chemical synthesis was considered the best route to obtain, study and confirm the structure of the isolated compound.

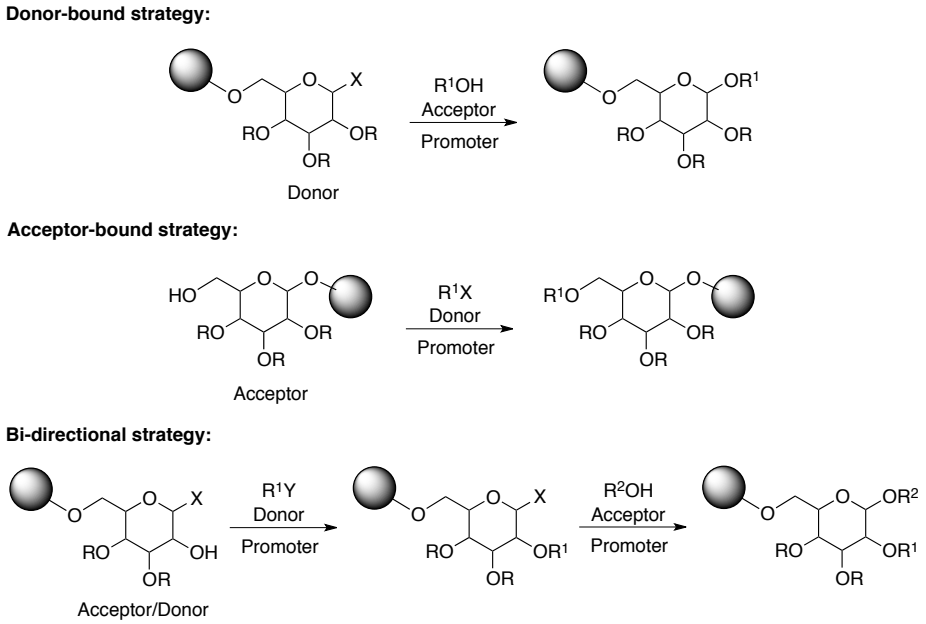
Chemical synthesis of carbohydrates normally includes time-consuming protecting group manipulations to differentiate several hydroxyl groups of similar reactivity, complicated stereoselective glycosylations and long overall routes. Impelled by the automated solid phase oligopeptide and oligonucleotide synthesis different strategies for the assembly of oligosaccharides on solid supports have been developed to allow a more efficient preparation of these compounds in a relatively short time.[3, 4]

The use of polymer bound scaffolds has attractive advantages, like: better yields due to the use of excess reagents; simpler, easier and faster purification steps, since excess reagents and non-bound by-products are removed by washing the solid support; and it is a chemically/environmentally/economically sustainable process, because even though excess reagents are used there is less use of solvents.

When planning the synthesis of oligosaccharides on solid support some considerations need to be taken, such as: the design of an overall synthetic strategy (protective groups, experimental conditions and the stereospecificity

of the glycosylation reaction); the selection of a suitable solid support and linker; and how the reactions are monitored.

In general, there are two main strategies for the solid supported synthesis of these molecules: attachment of the glycosyl donor to the polymeric support via an anchoring group (donor-bound strategy, Scheme 13), or attachment of the glycosyl acceptor by fixing the anomeric position to the support (acceptor-bound strategy, Scheme 13). Alternatively, mixed strategies of these two have been explored leading to a bidirectional synthesis approach, where a glycosyl donor/acceptor is immobilised to the solid support bearing at the same time a suitably differentiated acceptor site and an anomeric donor function for chain elongation in two directions (Scheme 13).



Scheme 13. Solid support synthetic strategies (X, Y: leaving group).

Selection of the solid support is determinant for the synthesis design since it limits the use of reagents and reaction conditions. In this case, a commercially available copolymer of polyethylene glycol (PEG) and polysterene (PS), known as Tentagel-NH_2, was used as solid support. Even though, these copolymers have amphiphilic properties the presence of 50-70% of PEG

chains determines the properties they exhibit. The possibility of using a wide range of solvents, from protic to aprotic, is one of the biggest advantages of this type of resins. The localisation of the reactive site at the end of the long PEG flexible spacer well separated from the PS backbone, allows the high mobility of these chains and the immobilised molecules to exhibit a "solution-like" behaviour[5, 6], which is the reason for the successful use of Tentagel resins in oligosaccharide synthesis[7, 8]. Flexibility, mobility, accessibility and solvation properties allow the reaction to be conducted almost as in a homogenous solution. However, when compared with other resins, Tentagel resins are less stable, more expensive and have lower loading capacity.

Other determinant step when planning the synthesis is the choice of the appropriate linker between the solid support and first monosaccharide, since it will determine all other protecting-group and coupling manipulations. The linker can be considered as a protecting group that is attached to the solid support, but is essential that the linker forms stable linkages with both the resin and the donor, to be inert to all reaction conditions and easily cleaved at the end of the synthesis.

Although, solid supported synthesis has been successfully used in oligopeptide and oligonucleotide synthesis, oligosaccharide synthesis often affords lower overall yields, since efficiency of glycosylations on solid support are significantly lower (50-90%) than that of peptide bond formation (>95%)[4]. This can be explained by the existence of highly reactive and unstable intermediary species giving rise to different by-products.

The use of solid supported synthesis is also limited due to: the need for well established reaction conditions; the reactivity of the molecules attached to the solid support may be different than in solution; the high cost of some solid supports; and difficulty in monitoring the reaction and characterising products or intermediates during the course of the synthesis.

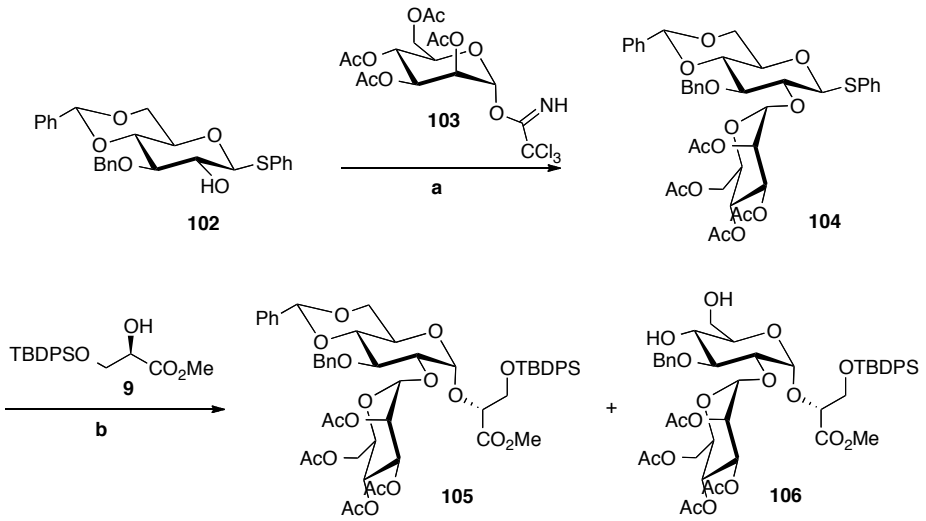
The present work describes the synthesis of the MGG using two different strategies, one in solution and other using a solid support.

Results and Discussion

Solution-phase Synthesis

The main challenges in the synthesis of MGG **101** were the need for the C-2 hydroxyl group of glucose to be free in order to form a glycosidic bond with mannose, and the stereoselective formation of the 1,2-*cis* glucosidic bond with glycerate.

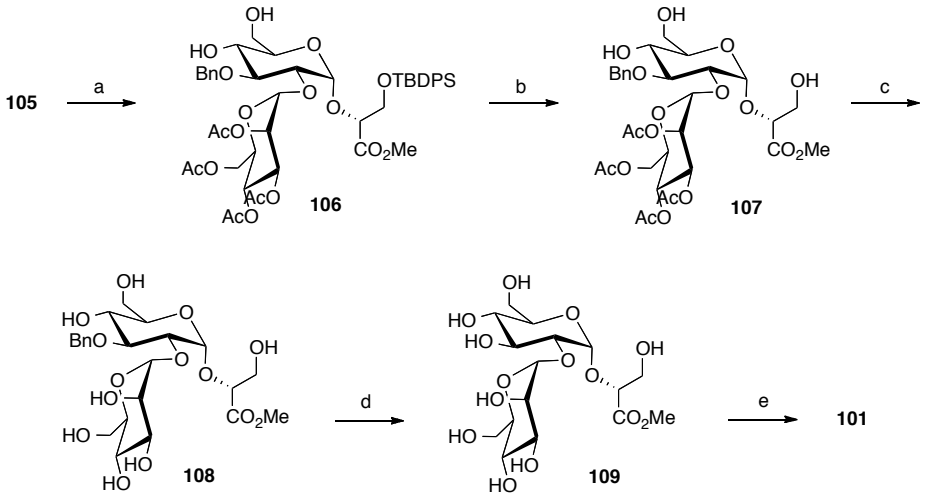
For the synthesis of MGG in solution, a strategy was developed based on thioglycoside **102**[9] bearing a free hydroxyl group at C-2, and at the same time similar to the donor developed by Crich et al.[10, 11] which have proved to provide α-glucosides with high stereoselectivity. Thioglucoside **102** would act first as a glycosyl acceptor in the reaction with the mannosyl trichloroacetimidate donor **103,** and afterwards could be activated as a donor in the glycosylation reaction with the glycerate derivative **9**[12] (Scheme 14).



a) TMSOTf, CH_2Cl_2, -20ºC, 74%. b) NIS, TMSOTf, CH_2Cl_2, 4Å MS, 0ºC, **105**: 40%, **106**: 23%.

Scheme 14. Glycosylation reactions for the solution phase synthesis of MGG, **105**.

The first glycosylation reaction between mannose trichloroacetimidate donor **103**[13] and the thioglucoside **102** as the acceptor, using TMSOTf as the promoter, afforded as expected by the use of C-2 acyl neighboring group participation strategy exclusively the α-mannoside **104** in 74% yield. Glycosylation between thioglucoside **104** and methyl 3-*O*-*tert*-butyldiphenylsilylglycerate acceptor **9**, using the NIS/TMSOTf system as the promoter, gave the desired product **105** and no β-anomer was detected by NMR (Scheme 14). Although, the second glycosylation was highly selective the yield was only 40% due to the lability of the benzylidene-protecting group. The use of TMSOTf instead of TfOH was unable overcome this issue and 23% of the deprotected product **106** was recovered. However, since the next step would be the deprotection of the benzylidene this was not a major problem.



a) p-TsOH, CH_2Cl_2, r.t., 91% b) TBAF, THF, r.t., 80%. c) NaOMe, MeOH, 0ºC, quant. d) H_2 (g), Pd/C, EtOH, 50 psi, r.t., quant. e) KOH, H_2O, r.t., quant.

Scheme 15. Deprotection strategy to obtain MGG, **101**.

Hydrolysis of the benzylidene-protecting group of fully protected MGG **105** furnished the diol **106** (91%, Scheme 15). Selective fluorolysis of the silyl ether with tetrabutylammonium fluoride (TBAF, 80%), followed by deacetylation by *Zemplé* method and hydrogenolysis of the benzyl group

afforded the fully unprotected MGG **109** (Scheme 15). After the final hydrolysis of the methyl ester, MGG **101** (Scheme 15) was obtained in the form of the potassium salt with 34% overall yield (calculated from the first glycosylation reaction). The NMR data (Table 7) of the synthetic product were identical to those described in literature[1] for the natural product.

Table 7. Comparison of ^{13}C NMR chemical shifts for the synthetic potassium salt of MGG **101** with data from the natural product[1].

^{13}C, δ (ppm)	α (1→2) mannosyl moiety					
	C-1	C-2	C-3	C-4	C-5	C-6
Natural	99.90	72.53	72.88	69.33	75.47	63.50
Synthetic	100.1	72.7	73.1	70.6	75.7	63.9

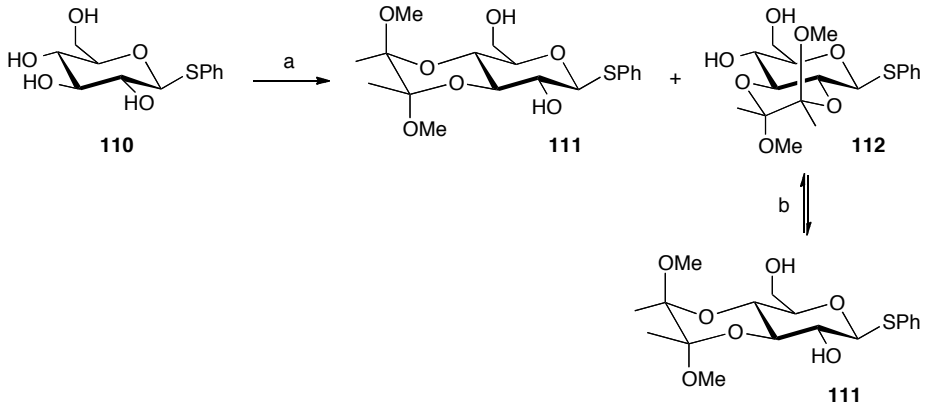
^{13}C, δ (ppm)	α (1→2) glucosyl moiety					
	C-1	C-2	C-3	C-4	C-5	C-6
Natural	96.60	76.76	73.85	72.24	74.62	63.30
Synthetic	96.8	76.9	74.1	72.5	74.8	63.5

^{13}C, δ (ppm)	Gliceryl moiety		
	C-1	C-2	C-3
Natural	179.2	81.0	65.7
Synthetic	179.5	81.3	65.9

Solid-supported Synthesis

The use of a bidirectional approach (Scheme 13) for the solid-supported synthesis of MGG **101** required the development of a new synthetic strategy, where the donor with the C-2 free hydroxyl group would be linked to the resin through the C-6 position. A new thioglycoside **111** was designed bearing the C-2 and C-6 hydroxyl groups free for manipulation (Scheme 16). Butane-2,3-bis-acetals have been widely used in carbohydrates synthesis for the selective protection of vicinal diequatorial diols[14, 15]. Treatment of phenyl

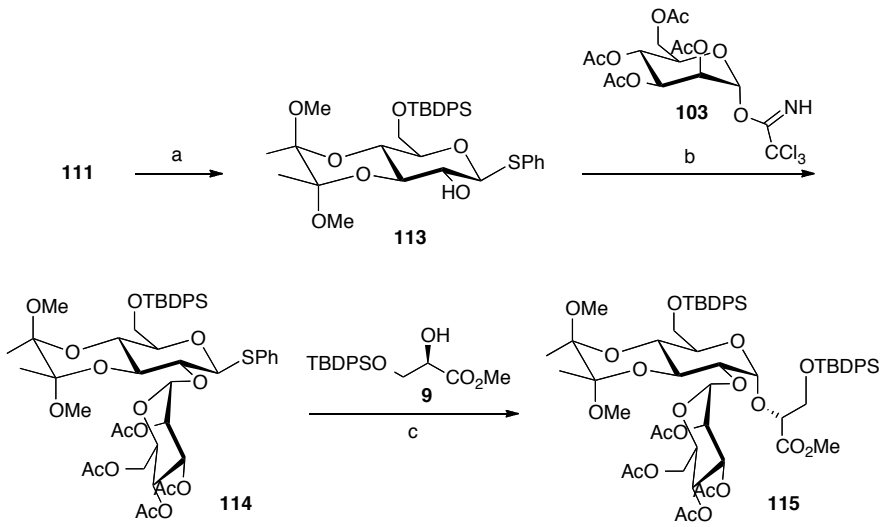
thioglucopyranoside **110**[10] with 2,2,3,3-tetramethoxybutane afforded a separable mixture of 1:1 of the bisacetals **111** and **112** (Scheme 16)[16, 17]. The undesired isomer **112** could be recycled afterwards by overnight reflux in methanol with a catalytic amount of CSA, affording once again a 1:1 mixture of isomers that increased the yield of the desired isomer **111** to 70%.



a) 2,2,3,3-Tetramethoxybutane, $CH(OCH_3)_3$, CSA, MeOH, Δ, 96%. b) Camphorsulfonic acid (CSA), MeOH, Δ, 91%.

Scheme 16. Synthesis of thioglucoside **111**.

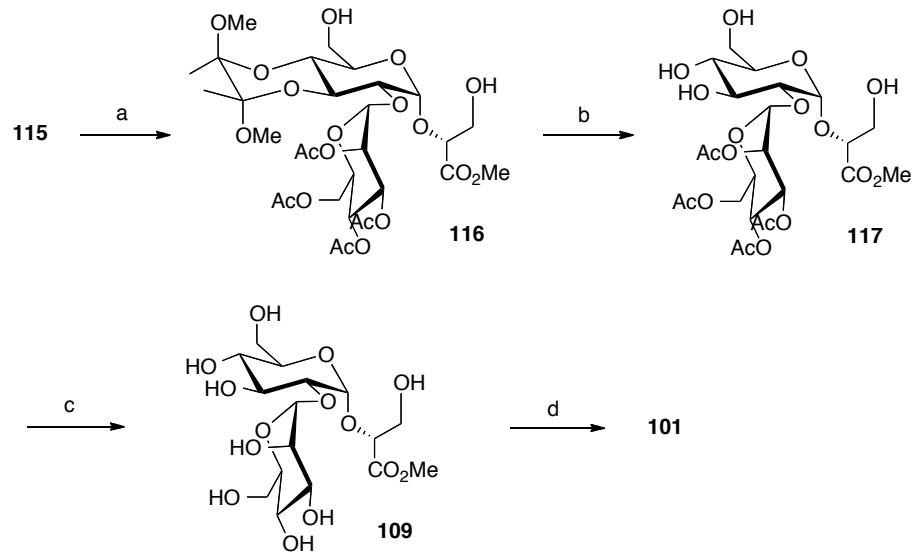
The tuning of the reaction conditions for maximum yields and anomeric selectivity for the synthesis of MGG **101** was first carried out using solution phase reactions (Scheme 17). In order to block the C-6 position selective silylation of the primary hydroxyl group of **111** was accomplished using *tert*-butyldiphenylsilyl chloride and imidazole with 89% yield.



a) TBDPSCl, imidazole, DMF, r.t., 89%. b) TMSOTf, CH_2Cl_2, -20ºC, 58%. c) NIS, TMSOTf, 4Å MS, CH_2Cl_2, 0ºC, 60%.

Scheme 17. Optimisation for the solid supported synthesis of MGG.

Glycosylation of the mannose donor **103** and thioglucoside **113** as the acceptor, using TMSOTf as the promoter, afforded the α-product **114** in 58% yield. The second glycosylation reaction between disaccharide donor **114** and methyl 3-*O*-*tert*-butyldiphenylsilylglycerate **9**, in the presence of the promoter NIS/TMSOTf, afforded exclusively the desired α-glucoside **115** (Scheme 17). The use of 6-*O*-bulky protecting groups on the donor, like TBDPS, for long-range stereocontrol of 1,2-*cis* glucosylations has been reported in the literature[18]. Although both glycosylation reactions were highly selective the reason for the moderate yields was the partial hydrolysis of the acetal under the glycosylation conditions.



a) TBAF, THF, r.t., 71%. b) TFA/CH_2Cl_2/H_2O, r.t., 60%. c) NaOMe, MeOH, r.t., quant. d) KOH, H_2O, r.t., quant.

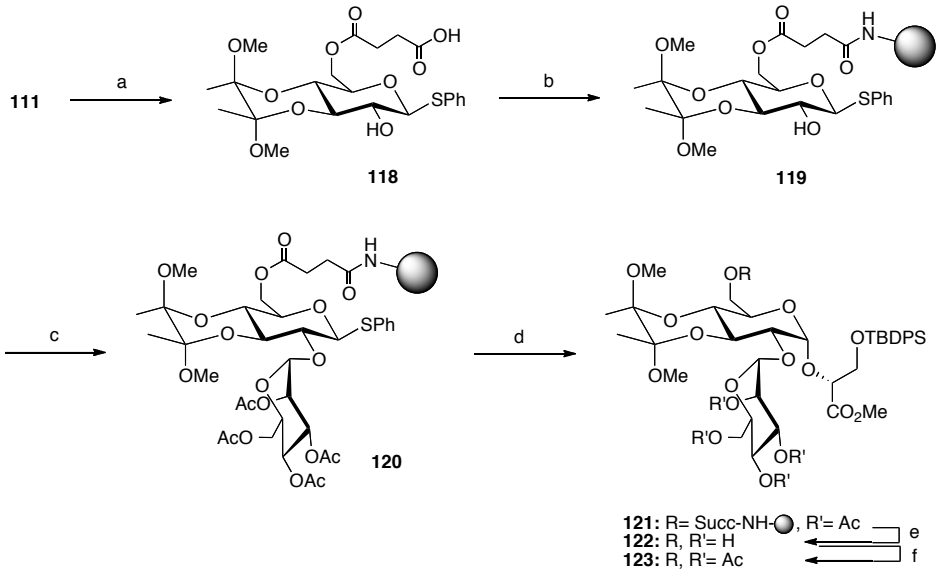
Scheme 18. Deprotection strategy for the synthesis of MGG, **101**.

Removal of the silyl ethers of **115** with TBAF (71%), followed by hydrolysis of the acetal with TFA in dichloromethane/water (60%), deacetylation by the *Zemplé* method of the acetates, and final hydrolysis of the methyl ester of the glycerate afforded the final product MGG **101** in the form of the potassium salt (Scheme 18). With the strategy tested and optimized in solution we were ready to proceed for the synthesis on solid support.

Several stereoselective glycosylation reactions on solid support have been reported in the literature[3, 19], but the exact degree of stereoselectivity is not predictable and there is no guarantee that the stereochemical outcome observed in solution-phase chemistry can be reproduced in solid-phase synthesis.

In our synthetic strategy for the solid supported synthesis of MGG **101**, immobilization of the thioglycoside **111** on the Tentagel MB-NH_2 resin was accomplished through a succinate bridge, involving the C-6 hydroxyl group of the donor and the native amino function of the Tentagel resin (Scheme 19).[7, 8, 20, 21] The succinate ester was chosen as a linker to exert the same

orienting effect that is assigned in literature to the acetate group in the C-6 position of the thioglucoside donor[12, 22].



a) Succinic anhydride, iPr_2NEt, DMAP, CH_2Cl_2, r.t., 90%. b) Tentagel MB-NH_2, diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), CH_2Cl_2, r.t. c) **104**, TMSOTf, CH_2Cl_2, -20ºC. d) **9**, NIS, TMSOTf, 4Å MS, CH_2Cl_2, 0ºC. e) NaOMe, MeOH, r.t. f) Ac_2O, pyridine, DMAP, r.t., quant.

Scheme 19. Solid supported synthesis of MGG.

Selective formation of the hemisuccinic acid moiety on the primary C-6 hydroxyl group of diol **111** was achieved under basic conditions using succinic anhydride in good yield (90%), leaving the C-2 hydroxyl group free (Scheme 19). Thioglucoside donor **118** was successfully anchored to the solid support through the formation of an amide linkage between the carboxylic group of the succinic acid moiety and the amine active sites of the Tentagel resin, using standard carbodiimide coupling. Immobilisation was confirmed by HR-MAS NMR spectroscopy experiments (^1H, ^{13}C and HSQC) and an initial loading of 0.32 $mmol.g^{-1}$ of resin was estimated from the dry weight difference.

Using a bi-directional strategy the resin-bound thioglucoside **119** first acted as a glycosyl acceptor in the glycosylation reaction with mannosyltrichloroacetimidate donor **103** using TMSOTf[7, 8] as in solution.

Afterwards, the resulting α-disaccharide **120** was used as a donor upon reaction with the glycerate acceptor **9** in the presence of the NIS/TMSOTf promoter system[20], and HR-MAS NMR experiments confirmed formation of the 1,2-*cis* glucoside **121**. This technique has proven to be an excellent tool for monitoring the reaction progress and quickly estimated the specificity of the coupling step without the need to cleave the product from the resin. The glycosylation reactions were as selective on solid support as in solution. The major advantage of performing the glycosylation reactions on the solid support was the purification step, where the excess of reagents was simply washed away by filtering the resin beads.

Treatment of the fully protected MGG **121** attached to the solid support with sodium methoxide in methanol, cleaved the product from the resin and hydrolysed the acetate groups from the mannose moiety (Scheme 19). Acetylation of the free hydroxyl groups was performed in order to better purify and characterize the crude obtained from the resin to confirm the success of the polymer-bound glycosylations.

Overall yield of the synthesis on solid support was disappointing, 18% based on calculated immobilised **118**. Contrary to most procedures found in literature that remark the use of longer glycosylation times[7, 8, 20] and the need to cap the unreacted sites with acetate[7, 8] after each reaction on solid support, in the present case these strategies did not make any difference to the overall efficiency of the synthesis.

Conclusion

In this work, the natural compatible solute MGG was synthesised in solution and on a solid support. The synthetic challenges of MGG were the formation of the 1,2-*cis* glycosidic bond between glucose and the secondary hydroxyl group of glycerate, and the design of a thioglucoside donor bearing the C-2 hydroxyl group free for coupling with the mannose donor.

Solution-phase synthesis using the benzylidene protected thioglucoside acceptor/donor **102** afforded the final product with the overall yield of 34% (calculated from the first glycosylation reaction).

Thioglucoside donor **111** was designed to be used in the solid-phase synthesis in a bidirectional approach, bearing the C-6 hydroxyl group differentiated to be immobilised to the resin and the C-2 position free to react as an acceptor with mannose. In order to test and optimise the reaction conditions for the solid-phase synthesis, the synthesis of MGG **101** from thioglucoside donor **111** was first carried out in solution with an overall yield of 15% (calculated from the first glycosylation reaction). Solution-phase glycosylations using both thioglucoside donors **102** and **113** were stereoselective affording exclusively the α-product.

A similar synthesis was carried out successfully with the thioglucoside donor **111** immobilised on a solid support (Tentagel MB-NH_2 resin) by a succinate linker, affording a partially deprotected product in 18% overall yield. The stereoselectivity of the glycosylation reactions was reproducible on solid support, affording at each step of the synthesis only the α-product.

Although solid-phase synthesis seems very promising in the construction of carbohydrate libraries and several advances have been made in this field for the synthesis of complex carbohydrates there are still obstacles to overcome, such as the need for reactions with high degree of efficiency. Reactions with low degree of efficiency after several couplings lead to low quality products and difficulties in their isolation due to simpler purification steps. Other major downsize is still the high price of these resins. HR-MAS NMR proved to be a powerful analytical tool for the characterisation of the products while attached to the solid support.

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through grant PEst-OE/EQB/LA0004/2011. E.L. acknowledges FCT for a PhD grant SFRH/47702/2008. We thank CERMAX for the use of the NMR spectrometers, which were purchased within the framework of the National Programme for Scientific Re-equipment, contract REDE/1517/RMN/2005, with funds from POCI 2010 (FEDER) and FCT. Doctor Pedro Lamosa and Doctor Helena Matias for the support with the HR-MAS NMR.

References

1. Jorge, C. D.; Lamosa, P.; Santos, H., α-D-Mannopyranosyl-(1→2)-α-D-glucopyranosyl-(1→2)-glycerate in the thermophilic bacterium Petrotoga miotherma − structure, cellular content and function. *FEBS Journal* **2007,** 274, 3120-3127.

2. Fernandes, C.; Mendes, V.; Costa, J.; Empadinhas, N.; Jorge, C.; Lamosa, P.; Santos, H.; da Costa, M. S., Two alternative pathways for the synthesis of the rare compatible solute mannosylglucosylglycerate in Petrotoga mobilis. *Journal of Bacteriology* **2010,** 192, 1624-1633.

3. Seeberger, P. H.; Haase, W. C., Solid-phase oligosaccharide synthesis and combinatorial carbohydrate libraries. *Chemical Reviews* **2000,** 100, 4349.

4. Ito, Y.; Manabe, S., Solid-phase oligosaccharide synthesis and related technologies. *Current Opinion in Chemical Biology* **1998,** 2, 701-708.

5. Gerritz, S. W.; Trump, R. P.; Zuercher, W. J., Probing the reactivity of solid supports via Hammett relationships. *Journal of the American Chemical Society* **2000,** 122, 6357-6363.

6. Li, W. B.; Yan, B., Effects of polymer supports on the kinetics of solid-phase organic reactions: A comparison of polystyrene- and Tentagel-based resins. *Journal of Organic Chemistry* **1998,** 63, 4092-4097.

7. Adinolfi, M.; Barone, G.; DeNapoli, L.; Iadonisi, A.; Piccialli, G., Solid phase synthesis of oligosaccharides. *Tetrahedron Letters* **1996,** 37, 5007-5010.

8. Adinolfi, M.; Barone, G.; De Napoli, L.; Iadonisi, A.; Piccialli, G., Use of controlled pore glass in solid phase oligosaccharide synthesis. Application to the semiautomated synthesis of a glyconucleotide conjugate. *Tetrahedron Letters* **1998,** 39, 1953-1956.

9. Koketsu, M.; Kuwahara, M.; Sakurai, H.; Ishihara, H., First synthesis of a trisaccharide of glycosylkaemferide: A resistance factor in carnations. *Synthetic Communications* **2004,** 34, 239-245.

10. Crich, D.; Cai, W. L., Chemistry of 4,6-*O*-benzylidene-D-glycopyranosyl triflates: Contrasting behavior between the gluco and manno series. *Journal of Organic Chemistry* **1999,** 64, 4926-4930.

11. Crich, D.; Smith, M., 1-Benzenesulfinyl piperidine/trifluoromethanesulfonic anhydride: A potent combination of shelf-stable reagents for the low-temperature conversion of thioglycosides to glycosyl triflates and for the formation of diverse glycosidic linkages. *Journal of the American Chemical Society* **2001,** 123, 9015-9020.

12. Lourenco, E. C.; Maycock, C. D.; Ventura, M. R., Synthesis of potassium (*2R*)-2-*O*-alpha-D-glucopyranosyl-(1 -> 6)-alpha-D-glucopyranosyl-2,3-dihydroxypropanoate a natural compatible solute. *Carbohydrate Research* **2009,** 344, 2073-2078.

13. Schmidt, R. R.; Michel, J., Facile synthesis of alpha-*O*-glycosyl and beta-*O*-glycosyl imidates - Preparation of glycosides and disaccharides. *Angewandte Chemie-International Edition in English* **1980,** 19, 731-732.

14. Montchamp, J. L.; Tian, F.; Hart, M. E.; Frost, J. W., Butane 2,3-bisacetal protection of vicinal diequatorial diols. *Journal of Organic Chemistry* **1996,** 61, 3897-3899.

15. Ley, S. V.; Baeschlin, D. K.; Dixon, D. J.; Foster, A. C.; Ince, S. J.; Priepke, H. W. M.; Reynolds, D. J., 1,2-diacetals: A new opportunity for organic synthesis. *Chemical Reviews* **2001,** 101, 53-80.

16. de Kort, M.; Valentijn, A. R. P. M.; van der Marel, G. A.; van Boom, J. H., Synthesis of clustered disaccharide polyphosphate analogues of adenophostin A. *Tetrahedron Letters* **1997,** 38, 7629-7632.

17. Crich, D.; Jayalath, P., Stereocontrolled formation of beta-glucosides and related linkages in the absence of neighboring group participation: Influence of a trans-fused 2,3-*O*-carbonate group. *Journal of Organic Chemistry* **2005,** 70, 7252-7259.

18. Tokimoto, H.; Fujimoto, Y.; Fukase, K.; Kusumotot, S., Stereoselective glycosylation using the long-range effect of a [2-(4-phenylbenzyl)oxycarbonyl]benzoyl group. *Tetrahedron-Asymmetry* **2005,** 16, 441-447.

19. Dreef-Tromp, C. M.; Willems, H. A. M.; Westerduin, P.; van Veelen, P.; van Boeckel, C. A. A., Polymer-supported solution synthesis of heparan sulphate-like oligomers. *Bioorganic & Medicinal Chemistry Letters* **1997,** 7, 1175-1180.

20. Zhu, T.; Boons, G. J., A two-directional approach for the solid-phase synthesis of trisaccharide libraries. *Angewandte Chemie-International Edition* **1998,** 37, 1898-1900.

21. Hunt, D. K.; Seeberger, P. H., Linker influence on the stereochemical outcome of glycosylations utilizing solid support-bound glycosyl phosphates. *Organic Letters* **2002,** 4, 2751-2754.

22. Lourenço, E. C.; Ventura, M. R., Synthesis of Potassium (*2R*)-2-*O*-α-D-Mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoate: A Naturally Compatible Solute. *European Journal of Organic Chemistry* **2011,** 2011, 6698-6703.

Chapter 4

Synthesis of MGlyG: A Newly Isolated Compatible Solute

The author contributed fully to this chapter, namely in the planning of the experimental work, performing the experiments and writing of the manuscript.

Synthesis of MGlyG: A Newly Isolated Compatible Solute

Abstract

An efficient synthesis of the newly isolated and rare compatible solute (2*R*)-2-(1-*O*-α-D-mannopyranosyl)-3-(1-*O*-α-D-glucopyranosyl)-glycerate (MGlyG) was accomplished. A tetraacetylmannosyl trichloroacetimidate was used first as a donor in the glycosylation reaction with the glycerate derivative and, after deprotection of the primary hydroxyl group of glycerate as the acceptor in the glycosylation reaction with the ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α/β-D-glucopyranoside. As expected the α-anomer was the only product of the first glycosylation reaction. Although the formation of the 1,2-*cis* glucoside was more challenging, after optimisation studies the α-anomer was obtained as the major product.

The effectiveness of MGlyG for the protection of model enzymes against heat induced inactivation was evaluated, using differential scanning fluorimetry (DSF). For comparison, the protection induced by natural compatible solutes, like MG, GG or GGG, was assessed by the same method. The results obtained demonstrated that for the studied enzymes (malate dehydrogenase, staphylococcal nuclease and lysozyme) MGlyG was the best stabiliser, and that the extent of protein stabilisation rendered by the solute depends on the specific solute/enzyme examined.

Introduction

The new compatible solute (2*R*)-2-(1-*O*-α-D-mannopyranosyl)-3-(1-*O*-α-D-glucopyranosyl)-glycerate (MGlyG, Figure 6) has been recently isolated and characterised.[1]

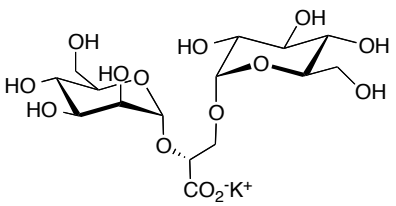


Figure 6. MGlyG, **124.**

Although, the position of the glyceric unit in bridge between the two hexoses has never been detected before, the newly isolated MGlyG is structurally related to the compatible solutes α-D-mannosyl-D-glycerate (MG, Figure 1) where a mannose moiety is α-lynked to the C-2 of a glyceric unit, and to the disaccharide α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-(1→2)-glycerate (MGG, Figure 5) which is composed by the same structural units – mannose, glucose and glyceric acid – lynked in a different manner.

To confirm the structure of the isolated MGlyG and study his biochemical properties it is required sufficient amounts of the pure compound, which is not possible from its natural source where it is available in low concentrations. Hence it was highly desirable to establish a methodology for the chemical synthesis of MGlyG.

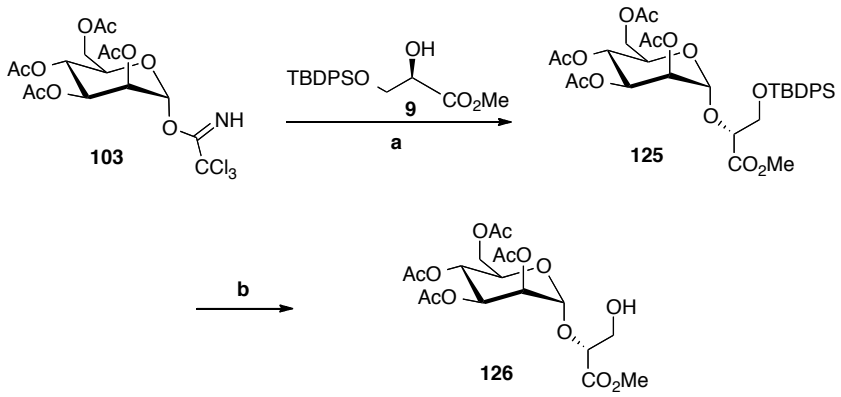
In the present work the chemical synthesis of MGlyG was established and the ability of MGlyG to protect three model proteins - malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme - against heat denaturation was assessed using differential scanning fluorimetry (DSF). Differential scanning fluorimetry (DSF) also known as thermofluor, is a high-throughput technique for monitoring thermal stability changes in proteins developed by Pantoliano et al.[2] Initially developed as a high-throughput screen for buffer optimization and ligand-induced stabilisation in proteins, DSF has been successfully used in screening for ligands and small molecules. This technique determines the protein melting temperature (T_M) by monitoring protein unfolding using a fluorescent reporter dye, which interacts with the hydrophobic regions of the protein that are exposed upon protein thermal denaturation. Although there are other methods available to determine the melting temperature of proteins, like differential scanning calorimetry (DSC) or circular dichroism (CD), the DSF is the most suitable for this work due to the need for only small amounts of protein (micrograms) and solutes (milligrams), and the ability to screen simultaneously different conditions.

Results and Discussion

Chemical Synthesis

The key step in the synthesis of MGlyG **124** is the selective formation of the 1,2-*cis* glucosidic bond between glucose and the primary hydroxyl group of glycerate for which there are no general methods. The synthetic strategy was developed starting from the synthesis of α-mannosyl glycerate **126**, that afterwards would act as acceptor in the glycosylation reaction with the thioglucoside **1.**

For the synthesis of α-mannosyl glycerate acceptor **126**, the α-mannose trichloroacetimidate derivative **103**[3] was used as donor in the glycosylation reaction with the methyl 3-*O*-*tert*-butyldiphenylsilylglycerate acceptor **9** (Scheme 20). Methyl glycerate acceptor **9** was prepared according to experimental procedures described in literature[4, 5]. 1,2-*Trans* mannoside **125** was exclusively obtained in 85% yield applying the C-2 acyl neighbouring group participation methodology, using acetates as protecting groups and under $BF_3.OEt_2$ activation. Removal of the silyl ether from the primary hydroxyl group of glycerate afforded the α-mannosyl glycerate **126** in 89% yield, and ready to be used as the acceptor in the next glycosylation step.

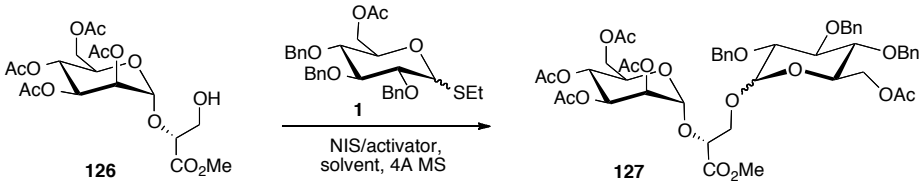


a) $BF_3.OEt_2$, CH_2Cl_2, 0ºC-r.t., 85%. b) TBAF, THF, r.t., 89%.

Scheme 20. Synthesis of partially protected MG, **126**.

Based on previous glycosylation studies (Chapter 2) thioglucoside **1**, which is readily obtained in three steps from methyl glucose[5], was chosen as donor for the second glycosylation reaction. In order to obtain high 1,2-*cis* stereoselectivity in this glycosylation reaction, a series of experiments changing the solvent, temperature and the activator were conducted. The results of the glycosylation reaction of donor **1** using NIS in the presence of 4Å MS are described in
Table 8.

Table 8. Optimisation of the glycosylation reaction of acceptor **126**.



Entry	Solvent	Activator	T (ºC)	t (mn)	Yield, (%)	α/β
1	CH_2Cl_2	TfOH	-20	15	84	1.7:1
2		TMSOTf		15	73	1.7:1
3			-60	30	90	2.1:1
4	Et_2O	TfOH	-20	15	62	4:1
5			-60	60	65	4.6:1
6		TMSOTf		90[a]	47[b]	4.7:1

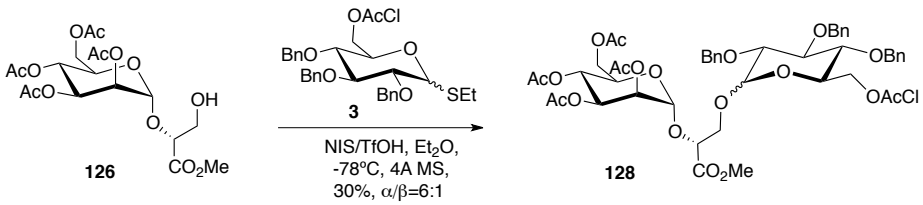
[a] More equivalents of TMSOTf were added during the reaction. [b] Initial donor was recovered.

As expected from previous results (Chapter 2, Table 2), using an ethereal solvent like ethyl ether, gave higher α-selectivity than dichloromethane that is a halogenated solvent. Using dichloromethane gave the worst results in terms of selectivity, even at lower temperatures. The use of ethyl ether enhanced the α-selectivity, although in general, lowered the reaction yields.

Concerning the use of different activators, comparing the results obtained with TfOH and TMSOTf using dichloromethane (Table 8, entries 1 and 2) or ethyl ether (Table 8, entries 5 and 6) as solvent, it is clear that the activator has no influence on the selectivity of the glycosylation reaction.

Conducting the reaction at lower temperatures (-60ºC, Table 8, entries 3 and 5-6) afforded only a minor improvement in the α-anomeric selectivity. When using ethyl ether at -60ºC the donor was consumed much slower (Table 8, entries 5 and 6), and when using TMSOTf the lowest reaction yield was obtained (43%, Table 8, entry 6).

Glycosylation with the thioglucoside donor **3** bearing a more electrophilic group at the C-6 position (chloroacetyl) used in Chapter 2 was also attempted to improve the α-selectivity (Scheme 21). The reaction conditions chosen were the ones that gave the best results with thioglucoside donor **1** - TfOH in Et_2O at -60ºC (Table 8, entry 5). Although the α/ proportion have improved to 6:1 the reaction yield dropped to 30%, with 60% recovery of the donor **3** and 10% of the hydrolysed donor in the anomeric position.

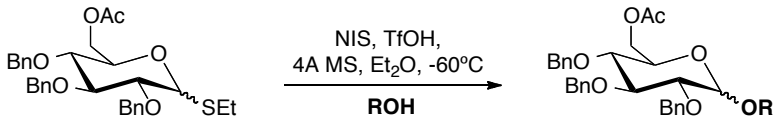


Scheme 21. Glycosylation of acceptor **126** with thioglucoside donor **3**.

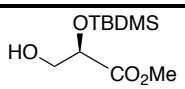
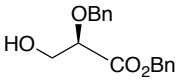
In light of the results obtained and aiming to develop the best route to efficiently obtain the MGlyG **124**, changing the strategy to alter the glycosylations order was considered. The possibility of performing first the glycosylation of thioglucoside donor **1** with glycerate, followed by the glycosylation with the mannose donor **103** was studied. Glycosylation of thioglucoside donor **1** with different acceptors chemically related to methyl glycerate **9**, but bearing the first hydroxyl group free and the secondary one protected was tested (Table 9). Acceptor **8** (Table 9, entry 1) was obtained as described in Chapter 2, and acceptor **129** (Table 9, entry 2) was obtained after benzylation of the methyl glycerate **9** with BnBr/NaH followed by

deprotection of the primary hydroxyl group with TBAF. Since the best results for the glycosylation reaction with methyl glycerate **9** were obtained with TfOH in Et_2O at -60ºC (Table 8, entry 5) these conditions were selected to study the selectivity of the reaction using acceptors **8** and **129**. The results obtained are summarised in Table 9.

Table 9. Glycosylation of donor **1** with different acceptors.



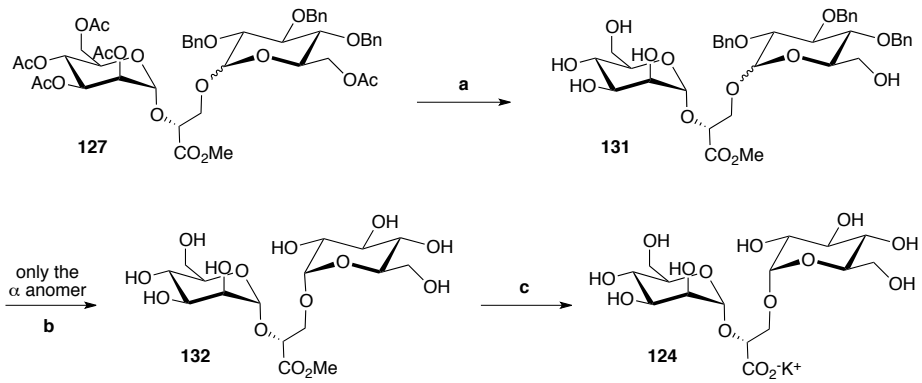
1

Entry	ROH	Product	t (mn)	Yield (%)	α/β
1	 **8**	**14**	45	30[a]	1:0
2	 **129**	**130**	60	54	2.8:1

[a] 64% of the initial donor was recovered.

Even though glycosylation with the primary hydroxyl group of methyl glycerate **8** afforded only the α-anomer, the yield was low (30%) and initial donor **1** was recovered (Table 9, entry 1). Glycosylation with the primary hydroxyl group of benzyl glycerate **129** gave worst results in terms of α/β ratio (2.8:1), but better yield (54%, Table 9, entry 2). These results can be explained by the difference in the steric hindrance of the protecting groups on the secondary hydroxyl group of glycerate. Benzyl ether being a smaller group, less sterically hindred than the *tert*-butyldimethylsilyl ether afforded worst results. Attempts were made with other protecting groups on the secondary hydroxyl group of methyl glycerate, such as THP but the reaction yields were lower than 12%.

Since the results obtained with this strategy were disappointing, the MGlyG synthesis was developed based on the first strategy and proceeded from the protected MGlyG **127** (Scheme 22).



a) NaOMe, MeOH, r.t., 90%. b) H_2(g), Pd/C,AcOEt, r.t., 50 psi, quant. c) KOH, H_2O, r.t., quant.

Scheme 22. Deprotection scheme for the synthesis of MGlyG, **124**.

After methanolysis of the acetates from the fully protected MGlyG **127** (90%) followed by flash column chromatography, the mixture of the α/β anomers could be separated and the synthesis was carried on with only the α anomer (Scheme 22). Hydrogenolysis of the benzyl groups and finally hydrolysis of the methyl ester afforded the potassium salt of MGlyG **124** (Scheme 22). The synthesis overall yield calculated from the first glycosylation step was 36%.

Performance of MGlyG as stabilizer for model enzymes

The stabilising effect of MGlyG **124** was assessed for three model enzymes - MDH, SNase and lysozyme – using DSF. The stabilising effect of MGlyG was compared with other natural compatible solutes that are structurally related to MGlyG, like MG, GG and GGG (Figure 1, Chapter 1).

The DSF based stability assays were performed at each protein working pH and the melting temperatures (T_M) values determined in the absence (control experiments) and in the presence of solutes, and at different solute concentrations.

An example of the typical denaturation curve of SNase obtained from the fluorescence data is presented on Figure 7. The stabilisation effect of different concentrations of MGlyG (0.1 and 0.25 M) on SNase is described by the

positive shift of the protein-unfold transition slop relatively to the control experiment.

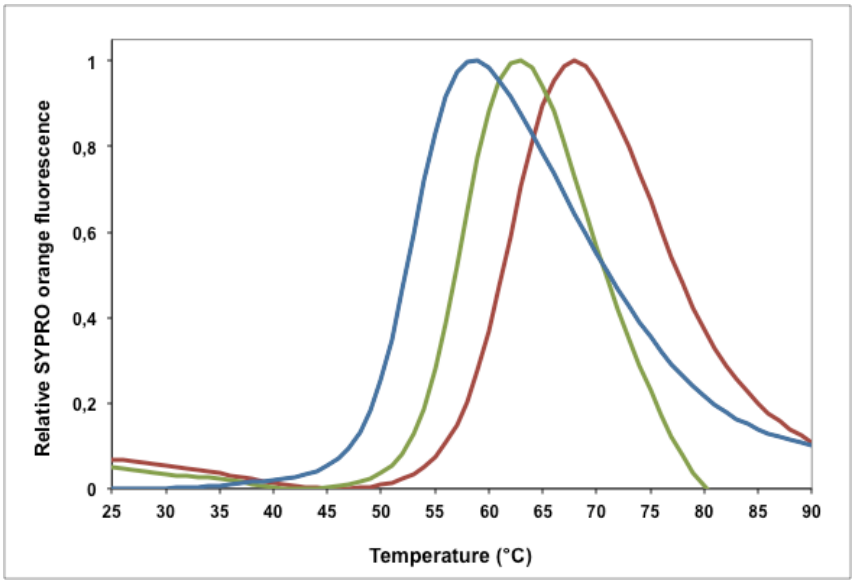


Figure 7. Curves obtained for the fluorescence data of SNase, comparing the stabilisation effect of different concentrations of MGlyG - 0.1M (green) and 0.25 M (red) - with the control experiment (absence of solute (blue)).

The denaturation curves for each assay were analysed, and the melting temperatures determined by the calculation of the first derivative, which corresponds to the midpoint temperature of the protein-unfolding transition. In the absence of solutes, malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme have melting temperatures (T_M) of 50, 52 and 71ºC respectively. The unfolding temperature shifts (ΔT_M) were calculated by comparing the T_M values obtained in the presence of solutes with the T_M values of the control experiments (absence of solutes). Positive ΔT_M values correspond to an increase in the T_M meaning that the protein is more stable and more energy (heat) is needed to unfold it. Negative ΔT_M values correspond to a decrease in the T_M meaning that the protein is less stable. The increment in the melting temperature (ΔT_M) induced by the presence of MGlyG and the other natural structurally related solutes is depicted in Figure 8.

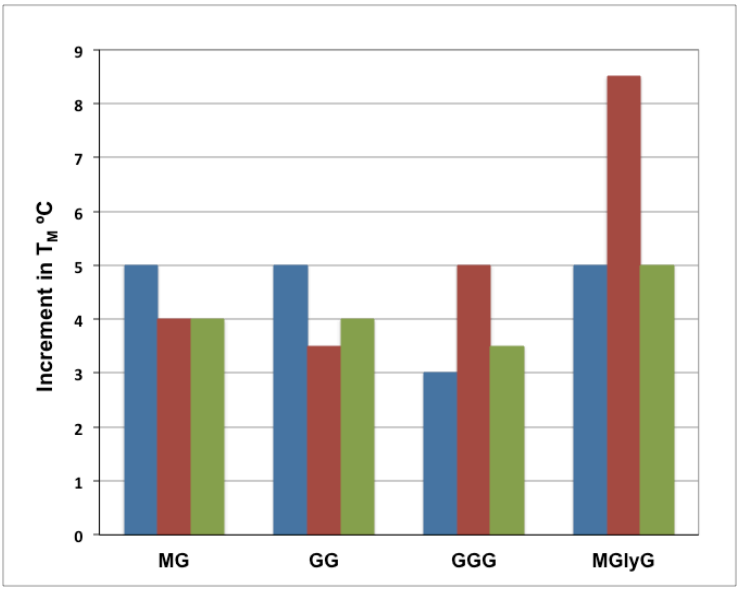


Figure 8. Increment in the melting temperature (T_M) of malate dehydrogenase (MDH, blue bars), staphylococcal nuclease (SNase, red bars) and lysozyme (green bars) in the presence of 0.25 M of different solutes. The melting temperatures (T_M) in the absence of solutes were 50 ºC for MDH, 52 ºC for SNase and 71ºC for lysozyme.

As expected from previous results obtained using DSC for MG, GG[6] and GGG[7] all compounds had a stabilising effect on the tested proteins. However, this effect was not general, the degree of stabilisation of each solute, in most cases, was different for each protein. Meaning that the degree of stabilisation rendered by each solute is dependent on the specific protein/solute system studied. In fact, these findings support the hypothesis of the existence of specific weak interactions, or loci for preferential binding sites on the protein surface.[8]

The results obtained demonstrated that at 0.25 M, MGlyG was the best stabiliser for SNase and lysozyme. Comparing the results obtained with the disaccharides - GGG and MGlyG – with the monosaccharides - GG and MG – the fact that these compounds have an extra glycosyl unit brings no clear benefit for stabilisation. Except in the case of SNase, where MGlyG was clearly the best stabiliser. Also, the fact that MGlyG gave the best results and it is the only solute in which the primary hydroxyl group of the glyceric unit is

not free can probably indicate that the carboxylic acid group is more important than the alcohol for the stabilisation effect.

The dependence of the increment of T_M (stabilisation) on the concentration of MGlyG was studied using MDH, SNase and Lysozyme (Figure 9), and also by comparing it with the effect of the other solutes on SNase (Figure 10).

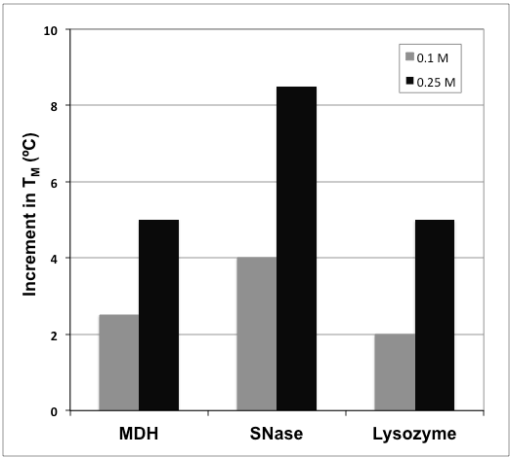


Figure 9. Dependece of MDH, SNase and lysozyme melting temperatures on the concentration of MGlyG.

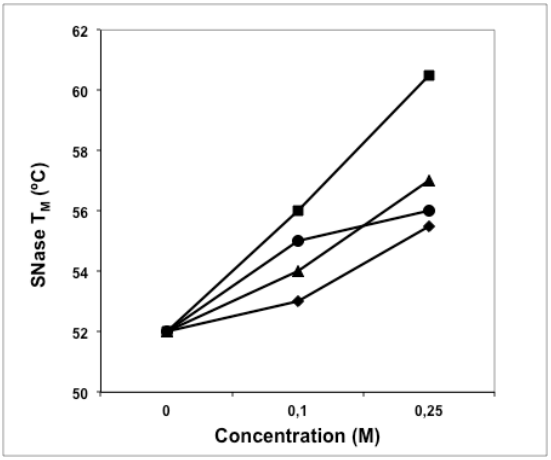


Figure 10. Dependece of SNase melting temperature on the concentration of MGlyG (squares), GGG (triangles), MG (circles) and GG (diamonds).

Although only two concentrations were tested, the stabilisation effect was directly proportional to the concentration of solute as expected, and MGlyG

was the best stabiliser for SNase. The concentration of MGlyG required to increase the T_M of SNase by 4ºC was 0.1 M while for the others solutes the same increment was produced only at 0.25 M.

Conclusion

The efficient synthesis of the natural solute MGlyG has been achieved with 36% overall yield (calculated from the first glycosylation step). The main challenge of the synthesis of MGlyG was to form the α-glycosidic bond between glucose and the primary hydroxyl group of glycerate, for which there are good procedures but still no general method. A synthetic strategy was designed starting with the synthesis of the partially protected MG acceptor **126** to be used in the glycosylation reaction with the thiogluocoside donor. In order to obtain high 1,2-*cis* stereoselectivity in the glycosylation reaction 6-acetoxy perbenzylated thioglucoside donor was used to optimise the reaction conditions. Although the reaction was not selective, ethyl ether at -60ºC and TfOH were found to be best conditions with α/β proportion of 4.6:1. Other synthetic strategies, like changing the order of glycosylations, using a different thioglucoside donor or different glycerate derivatives, were tried without much success. Although, the product was obtained as a mixture of anomers, after deprotection of the acetate groups the anomers could be separated and the desired final product obtained.

The effectiveness of the newly synthesised natural solute MGlyG for the protection of model enzymes against heat-induced denaturation was evaluated using DSF. For comparison, the protection induced by other natural compatible solutes, like MG, GG and GGG was assessed. The results obtained demonstrated that for SNase and lysozyme MGlyG was the best stabiliser and that the extent of protein stabilisation rendered by each solute depends on the specific solute/enzyme examined.

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through grant PEst-OE/EQB/LA0004/2011. E.L. acknowledges FCT for a PhD grant SFRH/47702/2008. We thank CERMAX for the use of the NMR spectrometers, which were purchased within the framework of the National Programme for Scientific Re-equipment; contract REDE/1517/RMN/2005, with funds from POCI 2010 (FEDER) and FCT. Doctor Tiago Bandeiras for the support in the DSF experiments.

References

1. Lamosa, P. E., N.; Costa , M.; Santos, H., *Unpublished results.*

2. Pantoliano, M. W.; Petrella, E. C.; Kwasnoski, J. D.; Lobanov, V. S.; Myslik, J.; Graf, E.; Carver, T.; Asel, E.; Springer, B. A.; Lane, P.; Salemme, F. R., High-density miniaturized thermal shift assays as a general strategy for drug discovery. *Journal of Biomolecular Screening* **2001,** 6, 429-440.

3. Schmidt, R. R.; Michel, J., Facile synthesis of Alpha-*O*-glycosyl and Beta-*O*-glycosyl imidates - Preparation of glycosides and disaccharides. *Angewandte Chemie-International Edition in English* **1980,** 19, 731-732.

4. Lok, C. M.; Ward, J. P.; Vandorp, D. A., Synthesis of chiral glycerides starting from D-serine and L-serine. *Chemistry and Physics of Lipids* **1976,** 16, 115-122.

5. Lourenco, E. C.; Maycock, C. D.; Ventura, M. R., Synthesis of potassium (*2R*)-2-*O*-alpha-D-glucopyranosyl-(1 -> 6)-alpha-D-glucopyranosyl-2,3-dihydroxypropanoate a natural compatible solute. *Carbohydrate Research* **2009,** 344, 2073-2078.

6. Faria, T. Q.; Mingote, A.; Siopa, F.; Ventura, R.; Maycock, C.; Santos, H., Design of new enzyme stabilizers inspired by glycosides of hyperthermophilic microorganisms. *Carbohydrate Research* **2008,** 343, 3025-3033.

7. Lamosa, P.; Rodrigues, M. V.; Goncalves, L. G.; Carr, J.; Ventura, R.; Maycock, C.; Raven, N. D.; Santos, H., Organic solutes in the deepest phylogenetic branches of the Bacteria: identification of alpha(1-6)glucosyl-

alpha(1-2)glucosylglycerate in Persephonella marina. *Extremophiles* **2013,** 17, 137-146.

8. Pais, T. M.; Lamosa, P.; dos Santos, W.; LeGall, J.; Turner, D. L.; Santos, H., Structural determinants of protein stabilization by solutes - The importance of the hairpin loop in rubredoxins. *Febs Journal* **2005,** 272, 999-1011.

Chapter 5

Synthesis of a Solute Library and Assessment as Protein Thermostabilisers

The author contributed fully to this chapter, namely in the planning of the experimental work, performing the experiments and writing of the manuscript.

Synthesis of a Solute Library and Assessment as Protein Thermostabilisers

Abstract

Mannosylglycerate (MG) is one of the most widespread solutes in hyper/thermophilic organisms, and its efficacy in the protection of protein structures has been amply illustrated. In the present work, several molecules inspired by the structure of natural compatible solutes, mainly mannosylglycerate, were synthesised. The diversity of the chemical library of new synthetic solutes was introduced in three levels: the nature of the sugar (glucose, galactose and mannose), both anomers for each sugar and the glycosyl acceptor (methyl (*2R*)-L-glycerate, methyl (*2R*)-D-glycerate, ethyl 3-hydroxybutyrate, dimethyl (*S*)-malate, methyl (*S*)-lactate and methyl glycolate) used in the glycosylation reaction. The effectiveness of the 21 newly synthesised compounds as protein thermostabilisers was assessed using differential scanning fluorimetry (DSF). The importance of the nature of the sugar and the non-glucosidic moiety for the stabilisation effect was studied.

Introduction

In a recent study, several molecules chemically related to mannosylglycerate were synthesized and their effectiveness for the protection of model enzymes against heat-induced denaturation, aggregation and inactivation was evaluated.[1] In this study, α-mannosyllactate (ML) (Figure 1, Chapter 1), a non-natural solute, proved to be superior to MG in the stabilisation rendered to model enzymes. The results showed that an apparently small structural change, the glycerate moiety being replaced by a lactate, which lacks the primary hydroxyl group and has the opposite configuration, had positive effect in the protein thermostabilisation. Thus, the possibilities of solute engineering having MG as the reference compound are very large.

Since several human pathologies such as Alzheimer's, Creutzfeldt-Jacob's, cystic fibrosis and Parkinson's diseases, have been associated with the structural instability of proteins, and consequent protein aggregation, the development of reliable strategies to improve protein stability is of great

importance and could have several pharmaceutical and biotechnological applications.

With the objective of synthesising new organic compounds with increased protein stabilisation properties inspired by the structure of natural compatible solutes, a chemical library based on sugar derivatives was prepared. The diversity of the analogue structures was introduced by using different hexoses, such as glucose, galactose, mannose and glucosamine, and by using different glycosyl acceptors during the glycosylation reaction.

Although most common solutes derived from hexoses are mannosides and glucosides, galactose and glucosamine analogues were also synthesised in this work to assess the contribution of the sugar structure for the stabilisation effect. To our knowledge, only one galactose containing compatible solute has been isolated from hyperthermophiles, the β-galactopyranosyl-5-hydroxylysine (GalHI) from *Thermococcus litoralis* (Figure 11).

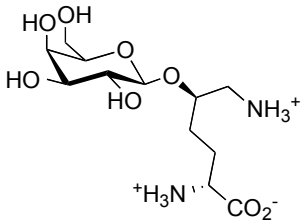


Figure 11. β-Galactopyranosyl-5-hydroxylysine (GalHI).

Several amino acids, like glutamate, proline, and glutamine, can function as compatible solutes in many mesophilic organisms and both α- and β-amino acids are used for osmoadaptation.[2] Amino acids are common compatible solutes, chargeable according to media pH, and it is known that charge is important for the stabilisation effect of hypersolutes. In order to determine if the amino group or the extra charge would enhance the stabilisation effect it is necessary to synthesise glucosamine derivatives. Glucosamine is an important building block present in many natural molecules involved in a number of biological processes. To our knowledge, only one glucosamine containing compatible solute has been isolated from hyperthermophiles, the di-*N*-acetyl-glucosamine phosphate (DAGAP) from *Rubrobacter*

xylanophilus (Figure 12).[3] DAGAP is structurally similar to the phosphodiester compatible solutes found in hyperthermophiles, like DIP or DGP, however, the role as a compatible solute has been refuted due to the concentrations that are too low to contribute to the cell's osmotic balance.

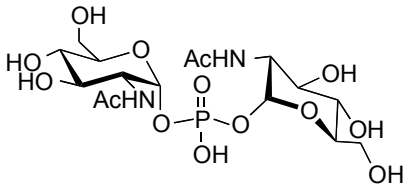
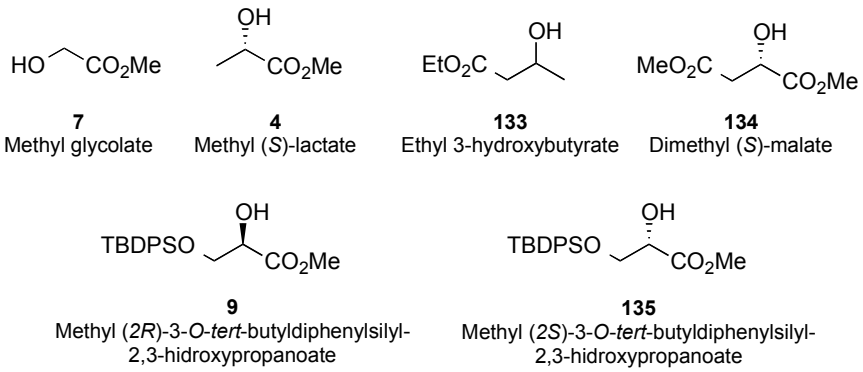


Figure 12. Di-*N*-acetyl-glucosamine phosphate (DAGAP).

Since previous studies[1] have proven the superiority of charged organic solutes as enzymes thermostabilisers, all of the acceptors chosen are charged and structurally related to glycerate with point modifications (Figure 13), such as more or less carbon atoms, loss of a hydroxyl group, an additional carboxylic group and the configuration at the asymmetric center, when present.



7
Methyl glycolate

4
Methyl (*S*)-lactate

133
Ethyl 3-hydroxybutyrate

134
Dimethyl (*S*)-malate

9
Methyl (*2R*)-3-*O*-*tert*-butyldiphenylsilyl-2,3-hidroxypropanoate

135
Methyl (*2S*)-3-*O*-*tert*-butyldiphenylsilyl-2,3-hidroxypropanoate

Figure 13. Glycosyl acceptors.

The solute library provided a wide range of structurally different sugar derivatives that could give insight into the key features necessary for protein stabilisation.

The ability of the newly synthesised solutes to protect three model proteins - MDH, SNase and lysozyme - against heat denaturation was assessed using DSF.

Results and Discussion

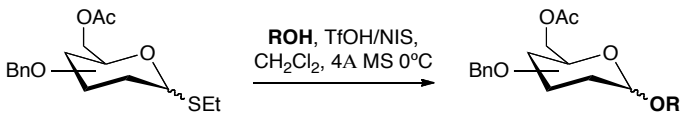
Synthesis of the New Analogues of Compatible Solutes

The design of the properly functionalized building blocks is a crucial step for the stereoselective introduction of the glycosidic linkage during the glycosylation reaction.

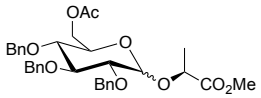
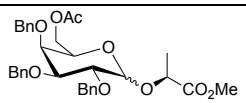
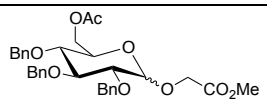
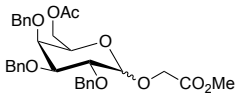
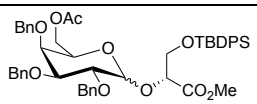
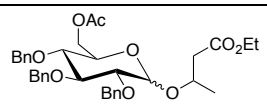
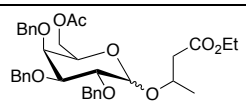
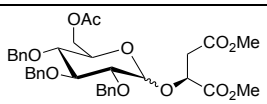
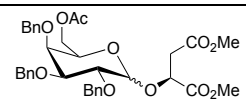
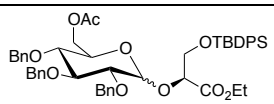
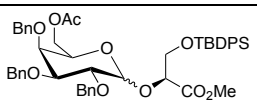
For the synthesis of the glucose and galactose derivatives thioglycoside donors were synthesised, and the method of choice for the formation of the thioglycosides **1** and **19** was oriented according to previous optimisation studies (Chapter 2), so that the configuration of the glycosidic linkage was preferentially α. The results obtained for the glycosylation reaction of donors **1** and **19** with the glycosyl acceptors (Figure 13) using NIS/TfOH[4] system in dichloromethane are described in Table 10. All acceptors were commercially available with exception of the methyl glycerate derivatives **9** and **135**, which were synthesised according to the experimental procedures reported for D-serine[4, 5].

Although for most of the glycosyl acceptors the reaction gave a mixture of anomers, this was not a problem, since it would be interesting to test α and β anomers separately to determine the importance of the stereochemistry of the anomeric position for the stabilization effect. This was the case for the D/L-glycerate and malate galactosyl derivatives.

Table 10. Results obtained for the glycosylation reaction with the thioglycoside donors **1** and **19**.



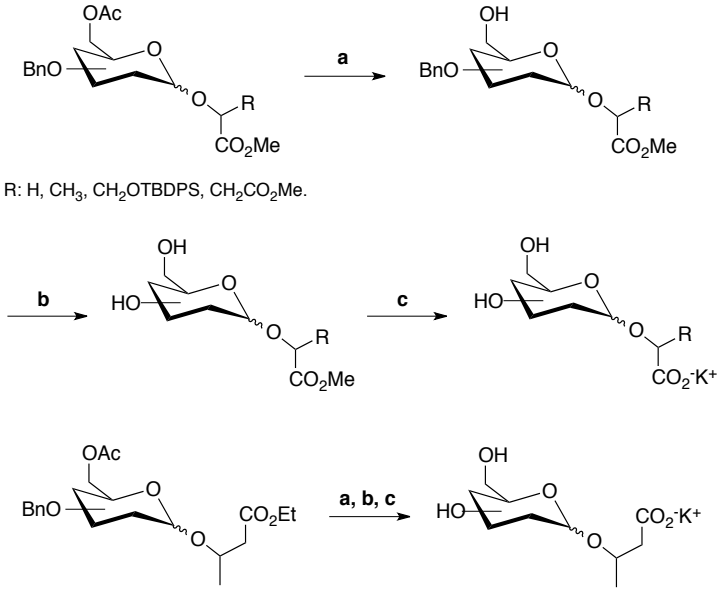
1: Glucose
19: Galactose

ROH	Donor	Product		Yield (%)	α/β
4	1		**10**	91	4:1
	19		**32**	87	3:1
7	1		**13**	93	4:1
	19		**34**	95	3:1
9	19		**35**	88	2:1
133	1		**136**	84	9:1[a]
	19		**137**	95	3:1[a]
134	1		**138**	94	7:1
	19		**139**	87	5:1
135	1		**140**	98	>10:1
	19		**141**	88	2.6:1

[a] Calculated after deprotection of the acetate group.

After the successive removal of the protective groups using common organic reactions, such as methanolysis of the acetates, fluorolysis of the silyl ether in the case of the glycerate analogues (compounds **35**, **140** and **141**) and

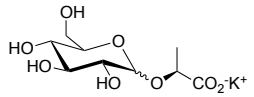
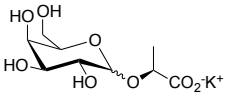
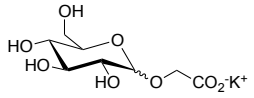
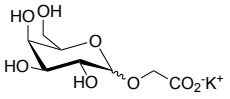
hydrolysis of the ester group (Scheme 23), the desired products were obtained (Table 11).

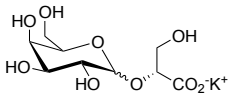
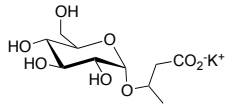
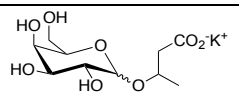
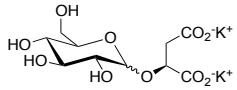
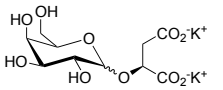
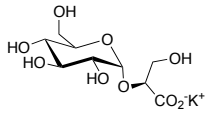
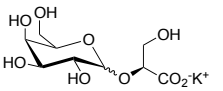


a) NaOMe, MeOH, 0ºC-r.t. b) H_2(g), Pd/C, 50 psi, AcOEt, r.t. c) KOH/H_2O, r.t.

Scheme 23. General deprotection scheme for the glucose and galactose analogues.

Table 11. Final products and overall yields[a] for glucose and galactose derivatives.

Compound number	Final Product		Yield (%)[a]
10		**142**	78
32		**143**	83
13		**144**	73
34		**145**	77

35		**146 α** **147 β**	61 21
136		**148**	68
137		**149**	86
138		**150**	42
139		**151 α** **152 β**	57 16
140		**153**	60
141		**154 α** **155 β**	43 14

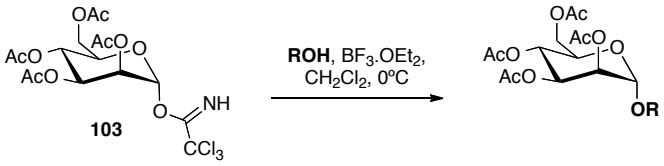
[a] Calculated from the glycosylation reaction.

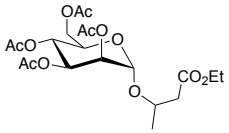
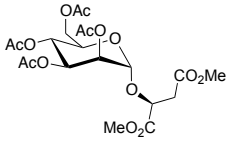
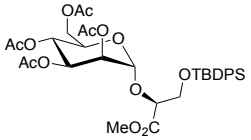
Overall the products were successfully obtained in good yields and the α anomer was the major product. Although in some cases a mixture of α and β anomers was obtained (enriched on the first), they were used for a preliminary screening where the most promising compounds would then be tested separately. The lowest yields were obtained for the derivatives of the dimethyl (*S*)-malate (**150**, **151** and **152**, Table 11) due to the use of base in the removal of the acetates and in the hydrolysis of the methyl ester, which promoted the hydrolysis of the anomeric position, by elimination of the malate moiety. This problem has been reported in the literature in the synthesis of bacillithiol (BSH).[6] To minimize the elimination of the malate moiety, careful ester hydrolysis left traces of the mono-ester in the final product.

For the synthesis of the 1,2-*trans* mannosides, the C-2 acyl neighboring group participation strategy was applied using acetates as protecting groups,

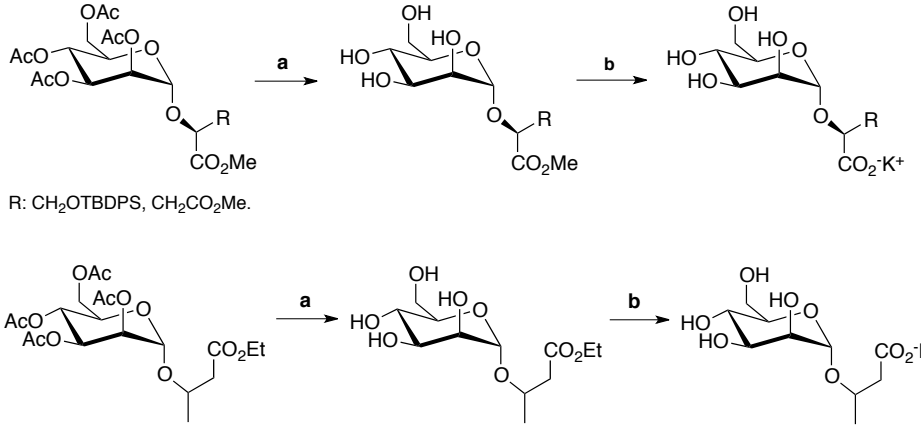
and trichloroacetamidates as glycosyl donors, which are relatively fast to prepare and unexpensive. Glycosylation reaction between the mannose trichloroacetimidate donor **103** and the glycosyl acceptors (Figure 13), using $BF_3.OEt_2$ as the promoter, afforded as expected exclusively the α-products. The results obtained for the glycosylation reaction with the mannose donor **103** are presented on Table 12.

Table 12. Results obtained for the glycosylation reaction with the mannose trichloroacetimidate donor **104**.



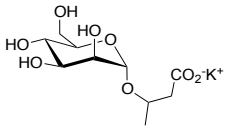
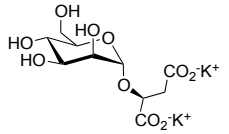
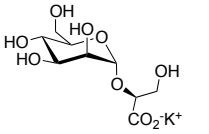
ROH	Product		Yield (%)
133		**156**	91
134		**157**	88
135		**158**	73

Successive removal of the protective groups using common organic reactions (Scheme 24), afforded the desired products (Table 13) in good overall yields.



Scheme 24. General deprotection scheme for the mannose analogues.

Table 13. Final products and overall yields[a] for mannose derivatives.

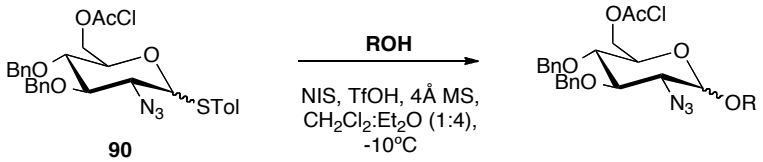
Compound number	Final Product		Overall Yield (%)[a]
156		**159**	73
157		**160**	69
158		**161**	50

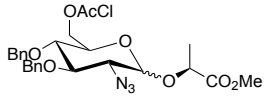
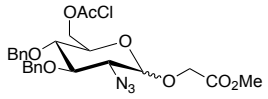
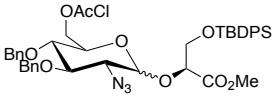
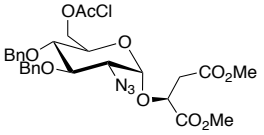
[a] Calculated from the glycosylation reaction.

Hydrolysis of the acetate groups and of the methyl ester of mannosyl dimethyl (*S*)-malate derivative presented the same problem described above for the glucose and galactose derivatives.

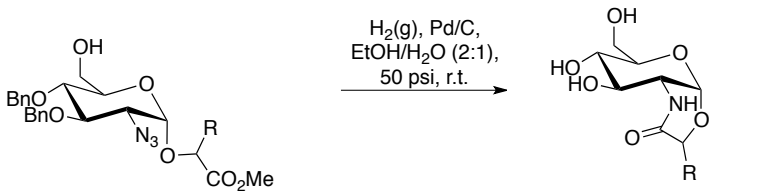
The 1,2-*cis* glucosamine derivatives were synthesised from the 2-azido-2-deoxythioglucoside donor **90** synthesised in Chapter 2, and the glycosylation reaction with the glycosyl acceptors from Figure 13 conducted in a mixture of CH_2Cl_2/Et_2O (4:1) at -10ºC and using NIS/TfOH as promotor. The results are presented on Table 14.

Table 14. Results obtained for the glycosylation reaction with the 2-azido-2-deoxythioglucoside donor **90**.



ROH	Product		Yield (%)	α/β
4		**95**	86	8.7:1
7		**96**	76	12:1
9		**97**	84	>10:1
134		**162**	86	1:0

As expected from previous studies (Chapter 2) the α anomer was the major product for all of the acceptors used. After methanolysis of the acetate group and in the case of the glycerate derivative fluorolysis of the silyl ether, catalytic hydrogenation of the benzyl group with simultaneous reduction of the azide promoted the formation of an undesired cyclic amide (Scheme 25). This effect was only observed for the α anomers, and led to uncharged derivatives.

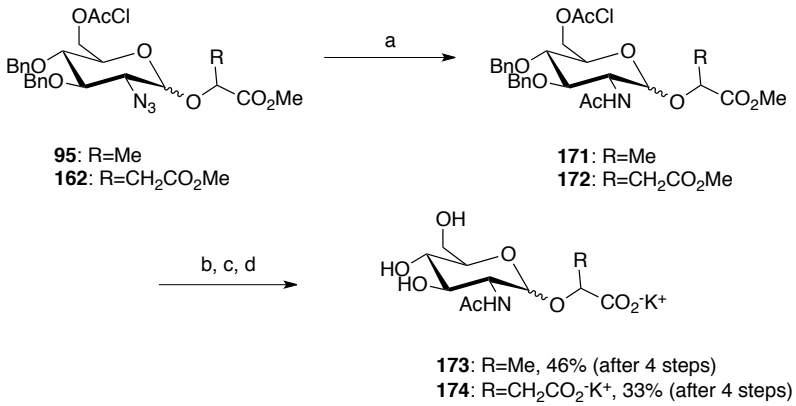


163: R=Me
164: R=H
165: R=CH_2OH
166: R=CH_2CO_2Me

167: R=Me, 84%
168: R=H, 81%
169: R=CH_2OH, 80%
170: R=CH_2CO_2H, 87%

Scheme 25. Hydrogenation of the 2-azido-2-deoxyglucoside derivatives.

Since it is known that charge is important for the stabilisation effect, to overcome this problem previous reduction of the azide using a Staudinger reaction, followed by protection of the resulting amine group with acetate (Scheme 26) blocked the amine and avoided cyclisation. After removal of the protecting groups and hydrolysis of the methyl ester the final *N*-acetyl glucosamine derivatives were obtained.



95: R=Me
162: R=CH_2CO_2Me

171: R=Me
172: R=CH_2CO_2Me

173: R=Me, 46% (after 4 steps)
174: R=$CH_2CO_2^-K^+$, 33% (after 4 steps)

a) (i) Ph_3P, THF, AcOH, 0ºC-r.t. (ii) Ac_2O, 0ºC. b) NaOMe, MeOH, 0ºC.
c) H_2 (g), Pd/C, AcOEt, 50 psi, r.t. d) KOH/H_2O, r.t.

Scheme 26. Synthesis of the *N*-acetyl glucosamine derivatives.

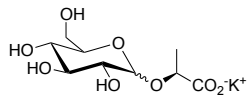
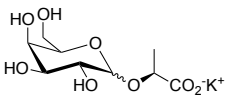
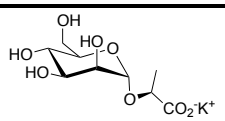
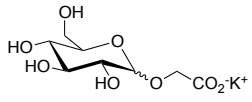
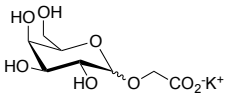
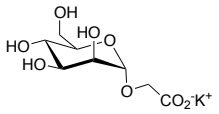
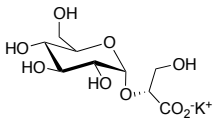
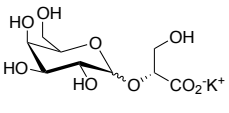
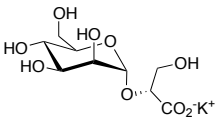
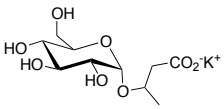
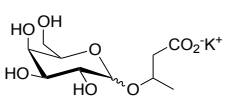
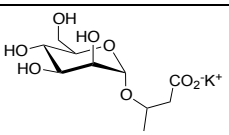
Assessment of the ability of the new analogues as thermostabilisers

The ability of the new synthetic analogues to stabilise three model proteins against thermal stress was assessed using DSF. Previous studies involved the use of differential scanning calorimetry (DSC)[1] or the determination of

enzymatic activities[7], however these methods are time consuming and large quantities of solutes and proteins are needed. DSF is a high-throughput method that allows the simultaneous screening of different conditions and requires low quantities of both solute and protein, which means fast access to information about the stabilising properties of the solutes. The careful analysis of the results can lead to the design of new structural modifications that will improve the properties of the compounds in order to produce better stabilisers.

In this study, MDH, SNase and lysozyme were used as model proteins, and the stabilising effect of the synthetic compounds was compared with the effect of natural solutes, like MG and GG as well as potassium chloride, and other previously synthesised non-natural solutes, like MGlyc and ML. All compounds tested are presented in Table 15 and Table 16.

Table 15. Chemical structures of the natural and synthetic glucose, galactose and mannose derivatives tested in this study.

Glucose derivatives	Galactose derivatives	Mannose derivatives
 GL, **142**	 GaL, **143**	 ML, **175**
 GGlyc, **144**	 GaGlyc, **145**	 MGlyc, **176**
 GG (D), **177**	 GaG (α, D), **146** GaG (β, D), **147**	 MG (D), **178**
 GBut, **148**	 GaBut, **149**	 MBut, **159**

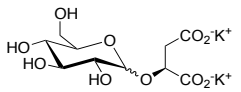
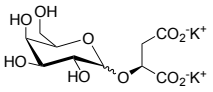
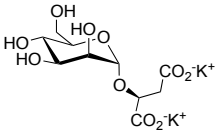
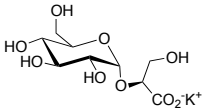
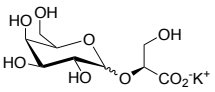
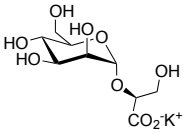
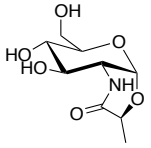
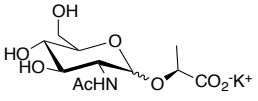
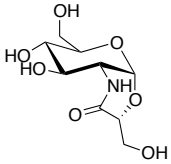
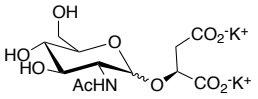
 GMal, **150**	 GaMal (α), **151** GaMal (β), **152**	 MMal, **160**
 GG (L), **153**	 GaG (α, L), **154** GaG (β, L), **155**	 MG (L), **161**

Table 16. Chemical structures of the synthetic glucosamine and *N*-acetyl glucosamine derivatives tested in this study.

Glucosamine derivatives	***N*-Acetyl Glucosamine derivatives**
 GNHL, **167**	 NAcGL, **173**
 GNHG, **169**	 NAcGM, **174**

The DSF based stability assays were performed at each protein working pH and the melting temperatures (T_M) values determined in the absence (control experiments) and in the presence of solutes, at different solute concentrations. The denaturation curves for each assay were analysed, and the melting temperatures determined by the calculation of the first derivative, which corresponds to the midpoint temperature of the protein-unfolding transition. In the absence of solutes, MDH, SNase and lysozyme have melting temperatures (T_M) of 50, 52 and 71ºC respectively. The unfolding temperature shifts (ΔT_M) were calculated by comparing the T_M values obtained in the

presence of solutes with the T_M values of the control experiments (absence of solutes). Positive ΔT_M values correspond to an increase in the T_M meaning that the protein is more stable and more energy (heat) is needed to unfold it. Negative ΔT_M values correspond to a decrease in the T_M meaning that the protein is less stable.

The increment in the melting temperature (ΔT_M) of the three enzymes induced by the presence of the synthetic and the natural solutes (Table 15, Table 16) is depicted in Figure 14.

From the analysis of the large data set presented in Figure 14 it is clear that the degree of stabilisation rendered by each compound is different for each protein. This reinforces the concept that the extent of thermoprotection provided by organic solutes depends on specific protein/solute interactions. This specificity has been reported in literature[8], where the study of minimal alterations on the protein sequence produced considerable differences in the extent of stabilisation rendered by a given solute.

Also, the fact that GNHL **167** and GNHG **169** (cyclic glucosamine derivatives) were poor stabilisers was expected, since reported studies[1] have demonstrated the superior thermostabilisation properties of charged solutes. When plotting the increment of the melting temperature of MDH versus those of SNase and lysozyme, a general view of the results arises. Although the results are quite disperse, it is clear that the non-charged solutes **167** and **169**, in opposition to the charged ones, are clustered in the lower-left corner of the graphic (Figure 15). However, charge alone cannot be responsible for the stabilisation, since KCl is a charged inorganic salt and its stabilisation effect is similar to the non-charged solutes, showing that the major contribution arises from the organic moiety of the solutes.

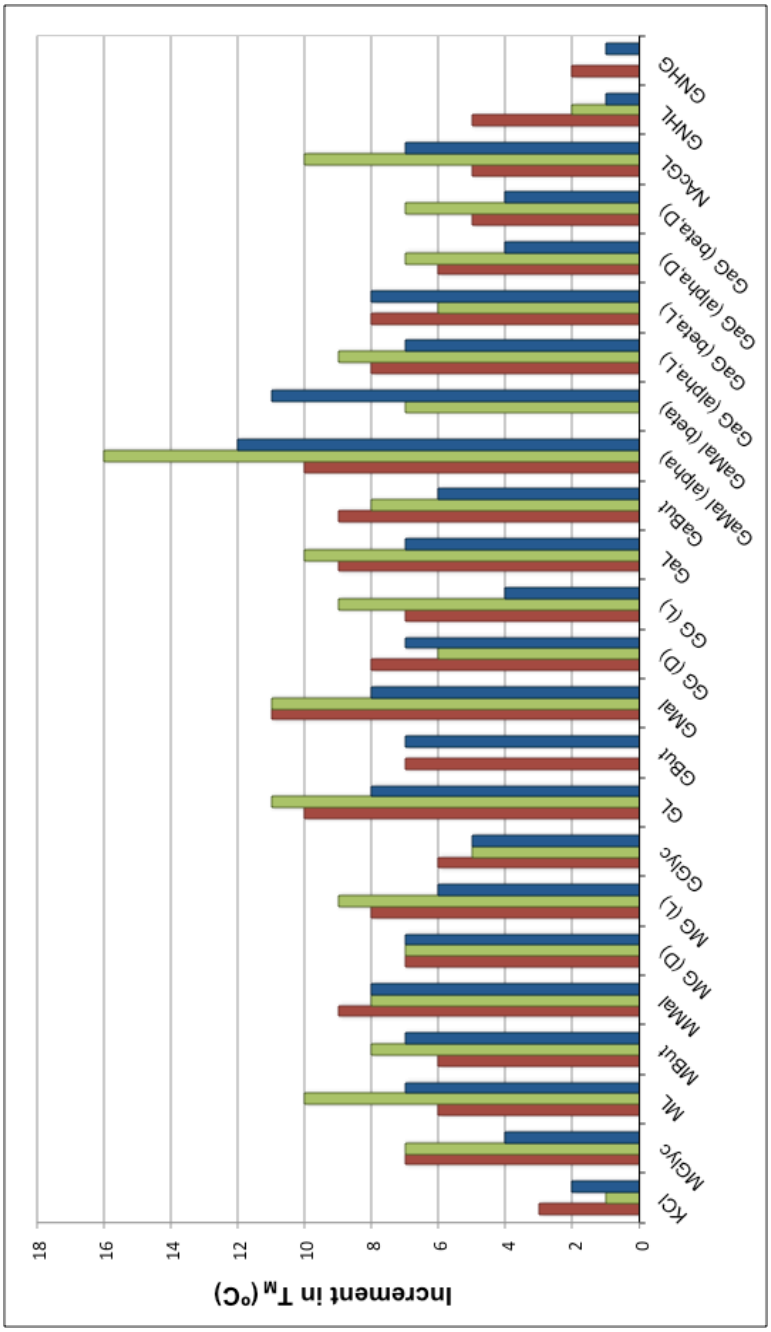


Figure 14. Increment in the melting temperature (T_M) of malate dehydrogenase (MDH, red bars), staphylococcal nuclease (SNase, green bars) and lysozyme (blue bars) in the presence of 0.5 M of different solutes. The melting temperatures (T_M) in the absence of solutes were 50 ºC for MDH, 52 ºC for SNase and 71ºC for lysozyme.

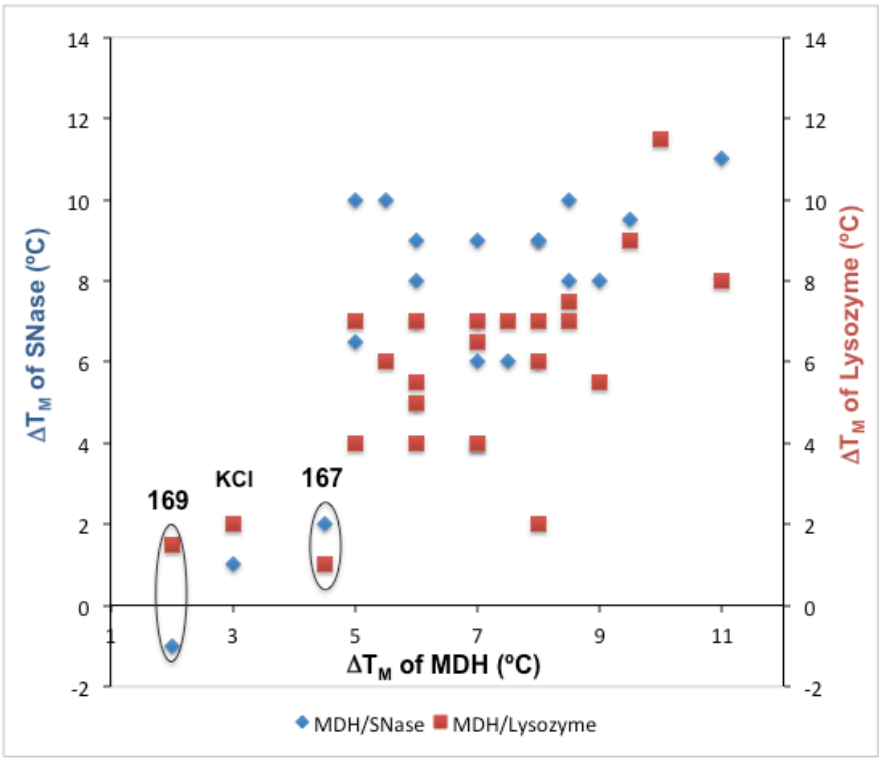


Figure 15. Stabilising effect of different compounds against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinate axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

In order to determine which are the structural features important for the stabilisation effect, different types of analysis were done based on the results obtained. However, since the degree of stabilisation depends on the protein studied, the rationalisation of the data becomes difficult.

Analysis of different glucose (Figure 17) and galactose (Figure 18) derivatives showed the importance of the non-glycosidic group attached to the hexose. By changing the nature of the group attached to the anomeric center of the hexose, a significant difference in the melting temperature of the proteins was observed. For both hexoses, malate derivatives were the best stabilisers, which can be explained by the presence of the extra charge in these molecules and reinforces the importance of charge for the stabilisation effect. These findings are in agreement with a study found in literature[9] where

the stabilising effect of several carboxylic acids on four proteins increased with the number of carboxylic groups in the salt. Lactate derivatives were also good stabilisers, although when compared with the natural glycerate (*R*), lactate lacks the primary hydroxyl group and has the opposite configuration (*S*). In the case of glucose, GGlyc that lacks the CH_2OH group and has lost the asymmetric center was the poorest stabiliser tested. This results and the fact that butyrate and the natural glycerate had a similar behaviour lead to the hypothesis that the primary hydroxyl group present in glycerate is not essential for the stabilising effect. Although the order of efficiency depends on the enzyme studied, for most cases, the following series was found: glycolate<glycerate<butyrate<lactate<malate.

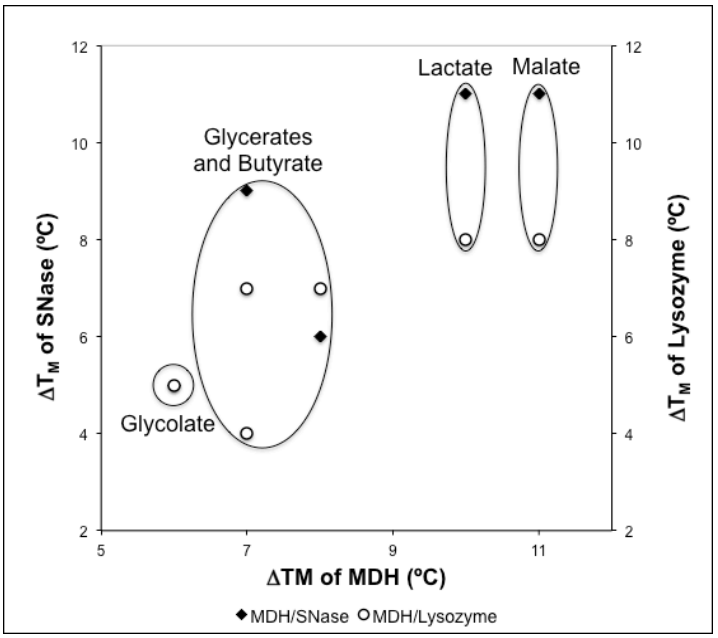


Figure 16. Stabilising effect of different glucose derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinates axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

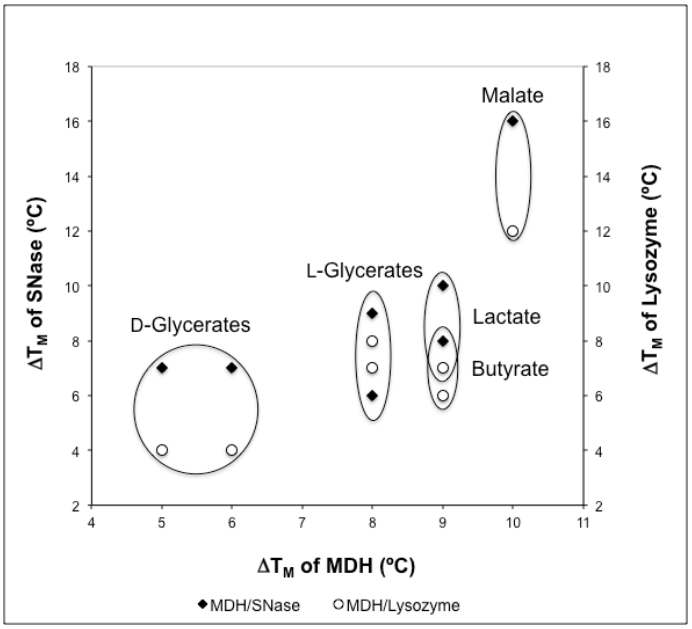


Figure 17. Stabilising effect of different galactose derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinates axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

Concerning the importance of the sugar structure different lactate (Figure 18) and malate derivatives (Figure 19) were analysed. In the case of lactate derivatives (Figure 18), a small change in the stabilisation effect of the different hexoses was observed, with exception of MDH, where changing the structure of the hexose had a larger impact in the degree of stabilisation. In this case, *N*-acetyl glucosamine derivative was the poorest stabiliser, and glucose and galactose were better stabilisers than mannose. For malate derivatives (Figure 19) the sugar structure had a bigger impact in the stabilisation effect of the three proteins. α-Galactosyl malate was the best stabiliser, and mannosyl malate gave the lowest stabilisation effect. Although, the effect can not be generalised, from the results obtained glucose and galactose derivatives were better stabilisers than mannose derivatives.

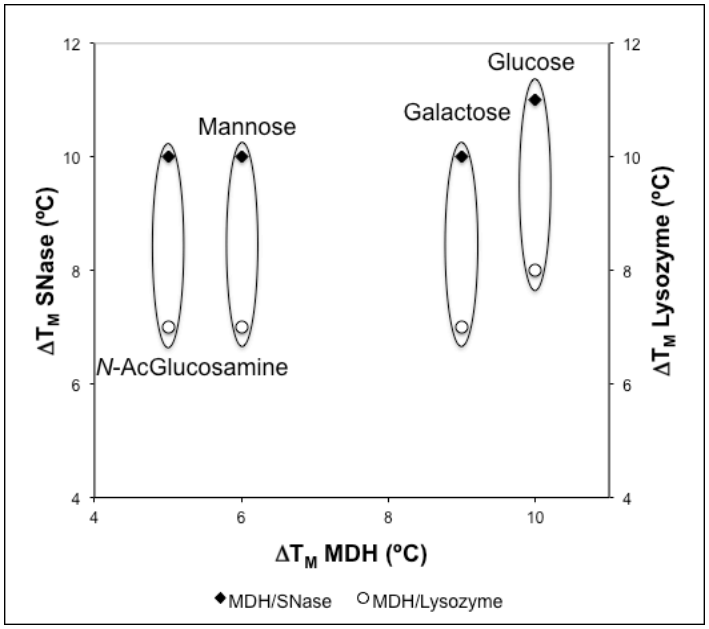


Figure 18. Stabilising effect of different lactate derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinate axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

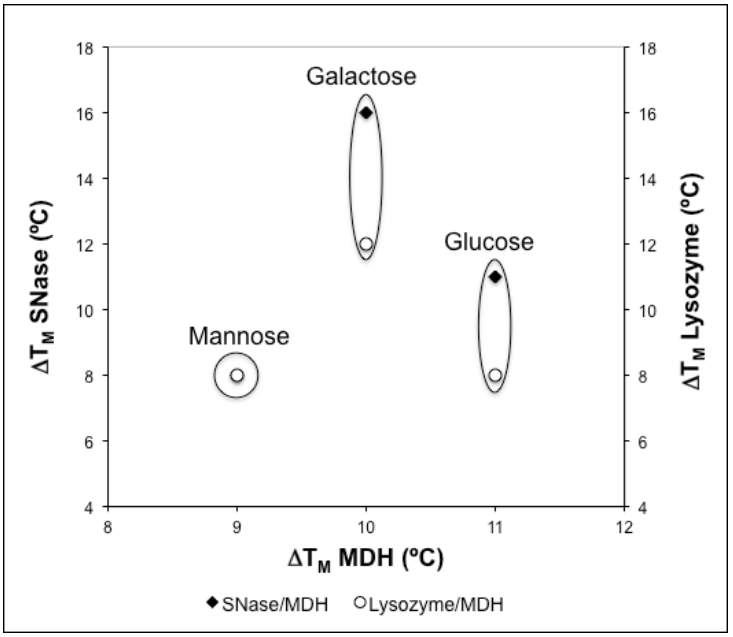


Figure 19 Stabilising effect of different malate derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In

the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinate axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

In nature hypersolutes are found in the α-anomeric configuration, to determine the importance of the glycosidic linkage of the sugar for the stabilisation effect, different α and β anomers of D and L-galactosyl glycerates were studied (Figure 20). Results obtained for the three enzymes showed that L-glycerates were better stabilisers than the natural D-glycerate derivatives, and that the α-anomers were better stabilisers than those with the β configuration.

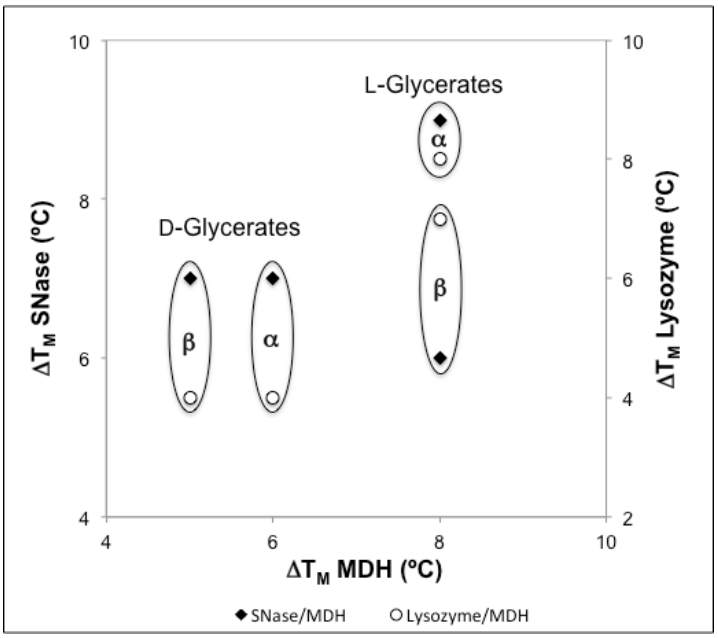


Figure 20. Stabilising effect of different galactosyl glycerate derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinate axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

In order to study the dependence of the increment of the melting temperature on the concentration of the solutes, the proteins were tested at different solute concentrations – 0.1, 0.25 and 0.5 M (Figure 21, Figure 22

and Figure 23). For the three proteins, results showed that independent of the degree of stabilisation, the stabilisation effect was directly proportional to the concentration of the solute. Although the results obtained seem to follow a general trend, when taking a closer look in the case of SNase (Figure 21) and lysozyme (Figure 23) α-galactosyl malate is clearly the best stabiliser. In the case of MDH the results show that glucosyl malate was the best stabiliser for this enzyme.

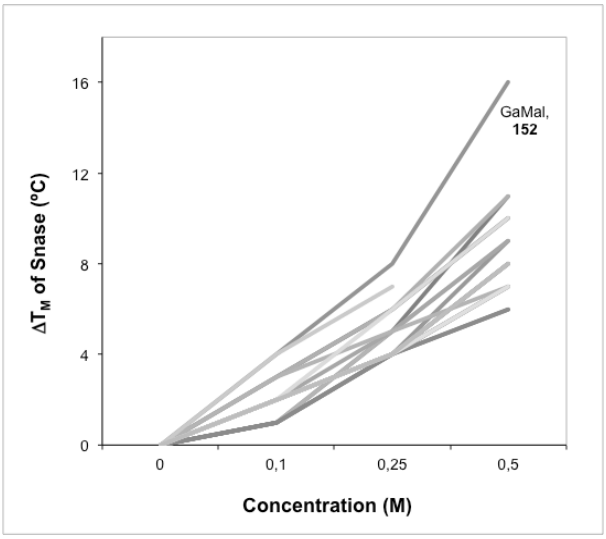


Figure 21. Dependence of SNase melting temperature on the concentration of solutes.

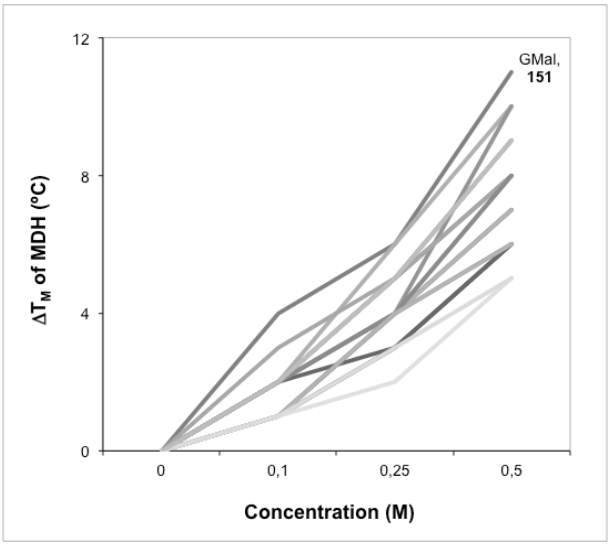


Figure 22. Dependence of MDH melting temperature on the concentration of solutes.

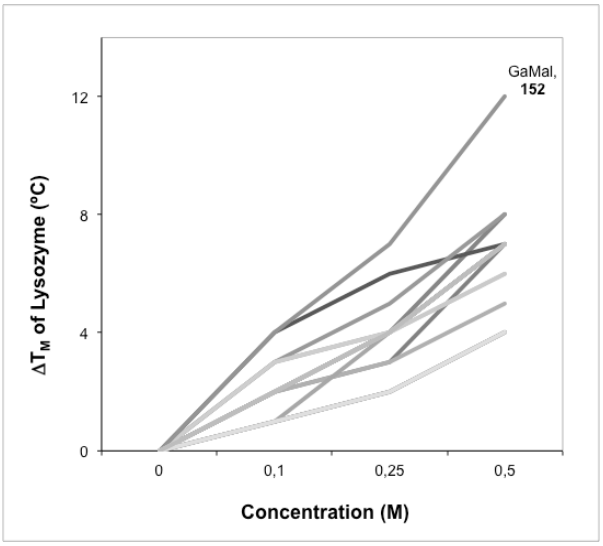


Figure 23. Dependence of lysozyme melting temperature on the concentration of solutes.

Conclusion

In this work, 21 new analogues of hypersolutes were efficiently synthesised based on glucose, galactose, mannose and glucosamine. α-Manosides were selectively obtained from the tetraacetylmannosyl trichloroacetimidate donor, and glucosides and galactosides from the respective thioglycoside donors with high α-anomeric selectivity. After cleavage of the protecting groups the final products were obtained with good overall yields. α–Glucosamine analogues were prepared from the 2-azido-2-deoxythioglucoside donors and the α-product was obtained as the major product. Since the catalytic hydrogenation of the azide group promoted the formation of a cyclic amide between the amine and the carboxylic acid from non-sugar moiety, to overcome this problem *N*-acetyl glucosamine analogues were prepared.

The effectiveness of the new compounds in the protection of model proteins against heat-induced inactivation was assessed using DSF, and compared with the effect of natural solutes, like MG and GG as well as potassium chloride, and other previously synthesised non-natural solutes, like MGlyc and

ML. DSF proved to be an excellent high-throughput method to obtain rapid information about the stabilising properties of the molecules. However, in order to obtain more accurate results further improvements have to be made related to the quantification of these compounds and the presence of inorganic KCl salt that can interfere with the results.

Analysis of the results obtained showed that the stabilisation effect is not general, and strongly depends on specific protein-solute interactions. Although some solutes showed superior thermostabilisation properties, the degree of stabilisation is different for each protein.

The presence of charge is one the most important features for the stabilisation effect. Uncharged solutes, like the glucosamine cyclic derivatives, gave the lowest stabilisation, and malate derivatives, bearing a double charge, were the best stabilisers.

Concerning the use of different hexoses, glucose and galactose derivatives were better stabilisers than the respective mannose and *N*-acetylglucosamine derivatives. However, the results showed that the group attached to the sugar had more influence for the stabilisation effect than the nature of the sugar.

The results obtained with the α and β anomers of galactosyl glycerates, showed that the α derivatives were better stabilisers. To confirm this trend future tests should be made with other β derivatives.

In the future, it would be interesting to test the stabilization effect of these compounds in a wider range of proteins with biotechnological relevance, like biopharmaceuticals. Although, the possibilities for solute engineering are large, the results obtained in the current study have presented some guidelines for the design of new analogues. Since the negative charge is determinant for the stabilization effect, it would be interesting to add an additional negative charge to the sugar moiety, such as a phosphate, a sulphate or a carboxylic acid (uronic acid) groups. Also, using other alcohols as glycosyl acceptors, or the introduction of other modifications on the hexose or in the aglycon moiety, such as replacing the hydroxyl groups for fluorine, thiol or amine groups.

In conclusion, this study provides structural insight for future modifications, as well as confirmation about the structural features that might play a role in the stabilisation effect rendered by hypersolutes.

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through grant PEst-OE/EQB/LA0004/2011. E.L. acknowledges FCT for a PhD grant SFRH/47702/2008. We thank CERMAX for the use of the NMR spectrometers, which were purchased within the framework of the National Programme for Scientific Re-equipment; contract REDE/1517/RMN/2005, with funds from POCI 2010 (FEDER) and FCT. Doctor Tiago Bandeiras and Ana Mingote for the support in the DSF experiments.

References

1. Faria, T. Q.; Mingote, A.; Siopa, F.; Ventura, R.; Maycock, C.; Santos, H., Design of new enzyme stabilizers inspired by glycosides of hyperthermophilic microorganisms. *Carbohydrate Research* **2008,** 343, 3025-3033.

2. Costa, M. S.; Santos, H.; Galinski, E. A., An overview of the role and diversity of compatible solutes in Bacteria and Archaea. In *Biotechnology of Extremophiles*, Antranikian, G., Ed. Springer Berlin Heidelberg: 1998; Vol. 61, pp 117-153.

3. Empadinhas, N.; Mendes, V.; Simoes, C.; Santos, M. S.; Mingote, A.; Lamosa, P.; Santos, H.; da Costa, M. S., Organic solutes in Rubrobacter xylanophilus: the first example of di-myo-inositol-phosphate in a thermophile. *Extremophiles* **2007,** 11, 667-673.

4. Lourenco, E. C.; Maycock, C. D.; Ventura, M. R., Synthesis of potassium (*2R*)-2-*O*-alpha-D-glucopyranosyl-(1 -> 6)-alpha-D-glucopyranosyl-2,3-dihydroxypropanoate a natural compatible solute. *Carbohydrate Research* **2009,** 344, 2073-2078.

5. Lok, C. M.; Ward, J. P.; Vandorp, D. A., Synthesis of Chiral Glycerides Starting from D-Serine and L-Serine. *Chemistry and Physics of Lipids* **1976,** 16, 115-122.

6. Sharma, S. V.; Jothivasan, V. K.; Newton, G. L.; Upton, H.; Wakabayashi, J. I.; Kane, M. G.; Roberts, A. A.; Rawat, M.; La Clair, J. J.; Hamilton, C. J., Chemical and chemoenzymatic syntheses of Bacillithiol: A unique low-molecular-weight thiol amongst low G+C gram-positive bacteria. *Angewandte Chemie-International Edition* **2011,** 50, 7101-7104.

7. Jorge, C. D.; Lamosa, P.; Santos, H., α-D-Mannopyranosyl-(1→2)-α-D-glucopyranosyl-(1→2)-glycerate in the thermophilic bacterium Petrotoga miotherma − structure, cellular content and function. *FEBS Journal* **2007,** 274, 3120-3127.

8. Pais, T. M.; Lamosa, P.; dos Santos, W.; LeGall, J.; Turner, D. L.; Santos, H., Structural determinants of protein stabilization by solutes - The importance of the hairpin loop in rubredoxins. *Febs Journal* **2005,** 272, 999-1011.

9. Kaushik, J. K.; Bhat, R., A mechanistic analysis of the increase in the thermal stability of proteins in aqueous carboxylic acid salt solutions. *Protein Science* **1999,** 8, 222-233.

Chapter 6

Conclusion

Compatible solutes isolated from hyperthermophiles have shown the ability to preserve cell components against heat induced inactivation, making them promising candidates for several biotechnological applications. Although these compounds have been known for a long time, the mechanism responsible for stabilisation is still unclear. To understand the molecular mechanism for protein stabilisation by hypersolutes, it is crucial to understand what are the structural features of these compounds that are important for this effect.

The main goals of this work were the synthesis and study of new isolated hypersolutes with challenging structures - MGG **101** and MGlyG **124** - and the development of a solute library inspired by the structure of natural hypersolutes, mainly MG.

Since the 1,2-*cis* equatorial relationship of the glycosidic bond found in the natural solutes is difficult to obtain in a stereocontrolled manner, the most challenging step for the synthesis of the new natural hypersolutes and the synthetic analogues is the glycosylation reaction. In this work, new methodologies were developed in order to efficiently obtain these compounds with high stereoselectivity. Several thioglycoside donors derived from glucose, galactose and glucosamine were synthesised and tested in the glycosylation reaction with a wide range of acceptors using NIS/TfOH system as the promoter. The reported studies showed that, although many factors influence stereoselectivity of the glycosylation reaction, careful design of the donors, optimisation of the solvent system and temperature are powerful tools to obtain high α-stereoselectivities and good yields. However, when applied to different acceptors the α stereoselectivity is not predictable in many cases. The influence of the nature of the protecting groups was found to be determinant for the stereoselectivity of the glycosylation reaction, such as the α-directing effect of ester functionalities at the C-6 position of the thioglycoside donors, and in the case of the thiogalactosides an additional ester group at the C-4 position had also a very important role. Particular relevance was given to the development of these donors that can be used as versatile building blocks for the efficient synthesis of higher saccharides.

The newly isolated hypersolutes of more complex structure, MGG and MGlyG, were efficiently synthesised with high stereoselectivity. The challenging 1,2-*cis* linkages present in these molecules were introduced in a stereocontrolled manner applying the developed glycosylation methods for glucose. In this work, MGG was also synthesised on solid support using a bidirectional approach. Although, the solid supported synthesis of peptides is well establish and fully automated, the synthesis of carbohydrates is more complex due to the low efficiency of coupling reactions. In the solid supported synthesis of MGG, coupling to the solid support and the successive glycosylation reactions were successfully achieved with the desired stereoselectivity, however the overall yield was lower than in solution. The chemical synthesis has proven to be the method of choice to obtain and study the natural isolated compounds.

In search for better protein stabilisers, a chemical library inspired by the structure of natural compatible solutes was created. 21 new synthetic analogues derived from glucose, galactose, mannose and glucosamine using several glycosyl acceptors structurally related to the natural glycerate were synthesised with good overall yields. DSF has proved to be an excellent method for the fast screening of the newly synthesised compounds as protein thermostabilisers. Analysis of the results reinforced the concept that the degree of stabilisation rendered by each solute is not general, being dependent of the existence of specific solute/protein interactions, since the same solute can stabilise different proteins to very different extents. Additionally, the structural diversity of the solute library has provided insight about the influence of the structure of these molecules for the stabilisation effect. The careful analysis of the data set has provided two major conclusions about the structural features of these molecules: charge has proven to be one of the most important structural features for the stabilisation effect, malate glycosides bearing two negative charges being the best stabilisers; and that the structure of the glycosyl acceptor had more influence on the stabilisation effect than the nature of the hexose.

Protein stability is a subject of great importance for many biotechnological, industrial and pharmaceutical areas, where compounds with increased protein stabilisation properties would have several applications. In this work, new

methods were developed for the efficient synthesis and screening of natural and synthetical hypersolutes as protein thermostabilisers. These contributions can be a platform for the synthesis of other molecules of biological interest.

Chapter 7

Experimental Procedures

The author contributed fully to this chapter, namely in the planning of the experimental work, performing the experiments and writing of the manuscript.

Chemical Synthesis

Materials and Analysis

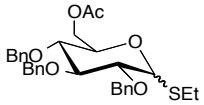
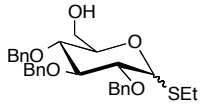
All the reactions were carried out under an inert atmosphere (argon), except when the solvents were not dried. Air sensitive materials were handed in a Braun MB 150-GI glove box. The synthesized compounds were purified by **f**lash chromatography using Silica Gel Merck 60 (0.040-0.063 mm), and preparative TLC using Merck 60 F254 silica gel. Analytical TLC was performed on aluminum-backed Merck 60 F254 silica gel plates. ^{1}H NMR spectra were obtained at 400 MHz in $CDCl_3$ or D_2O with chemical shift values (δ) in ppm downfield from tetramethylsilane in the case of $CDCl_3$, and ^{13}C NMR spectra were obtained at 100.61 MHz in $CDCl_3$ or D_2O. Assignments are supported by 2D correlation NMR studies and the peaks assigned when possible. Specific rotations ($[\alpha]^{20}_{D}$) were measured by using a Perkin–Elmer D241 automatic polarimeter.

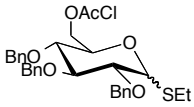
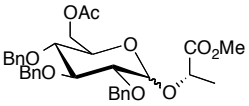
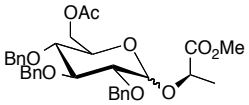
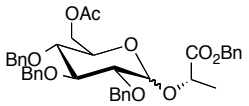
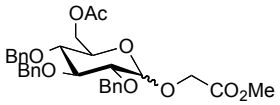
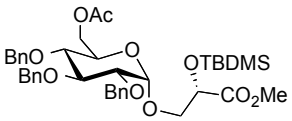
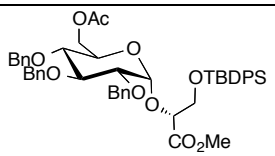
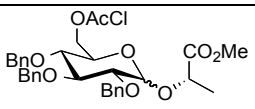
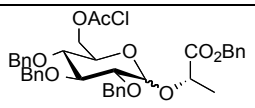
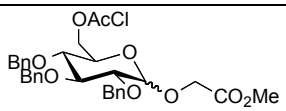
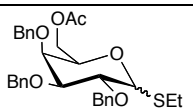
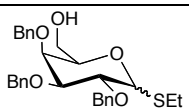
Solvent and Reagent Purification

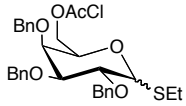
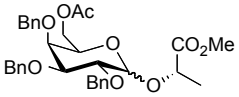
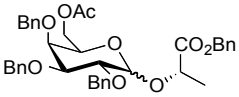
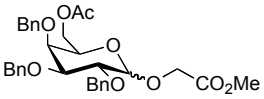
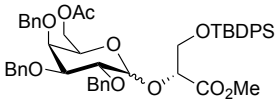
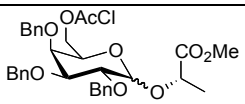
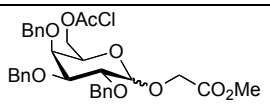
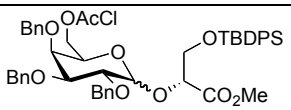
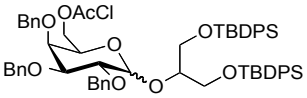
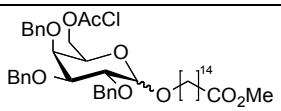
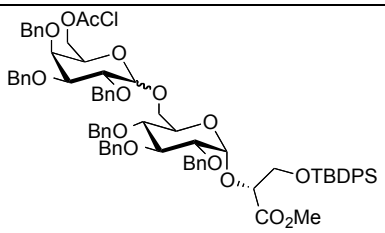
Reagents and solvents were purified and dried according to Ref.[1].

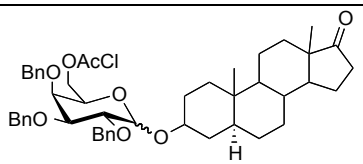
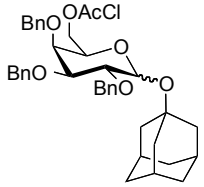
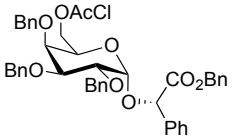
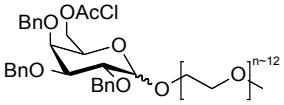
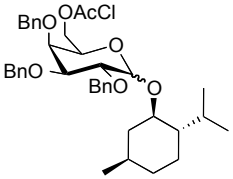
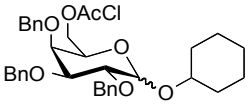
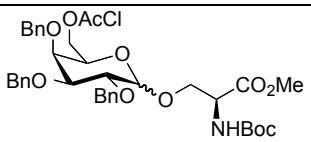
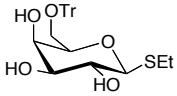
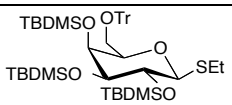
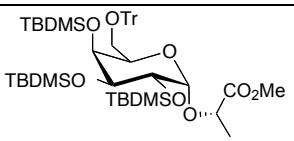
Graphical Index of Compounds and Experiments

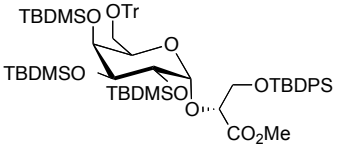
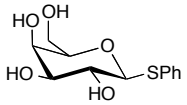
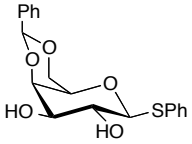
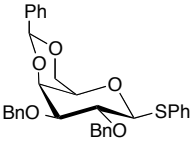
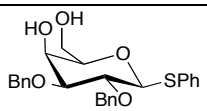
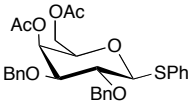
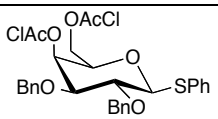
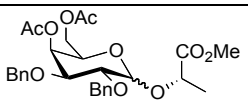
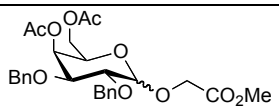
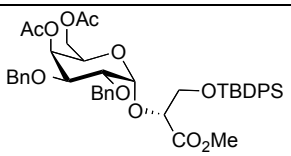
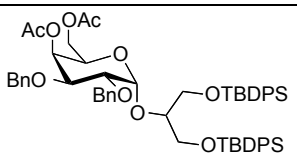
Table 17. Graphical Index of Compounds and Experiments.

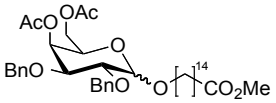
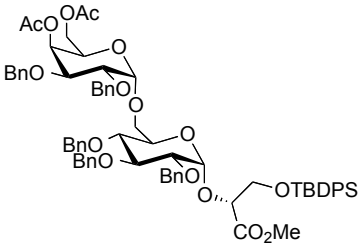
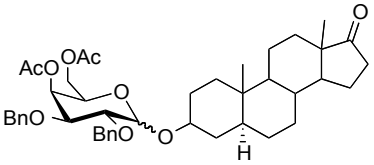
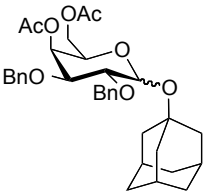
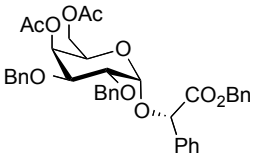
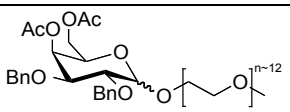
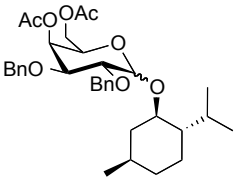
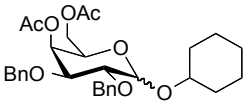
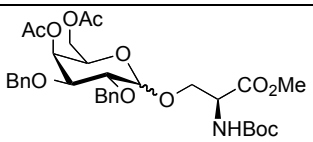
Compound number	Compound	Experiment	Page
1		Experiment 1	150
2		Experiment 2	150

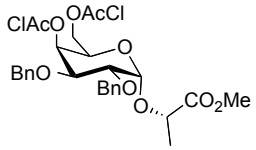
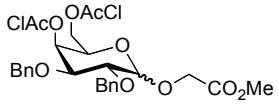
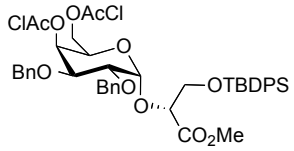
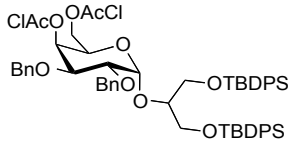
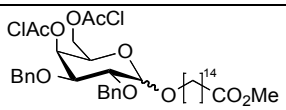
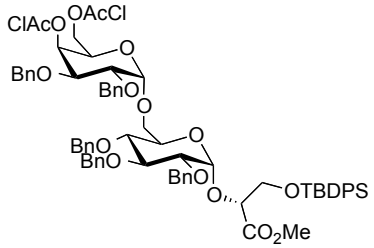
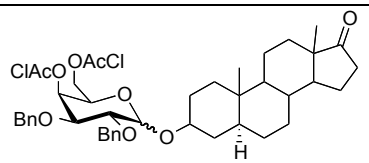
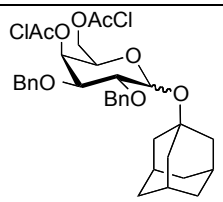
3		Experiment 3	151
10		Experiment 5	152
11		Experiment 6	**153**
12		Experiment 7	**153**
13		Experiment 8	**154**
14		Experiment 9	**154**
15		Experiment 10	**155**
16		Experiment 11	**156**
17		Experiment 12	**156**
18		Experiment 13	**157**
19		Experiment 14	**157**
20		Experiment 15	**159**

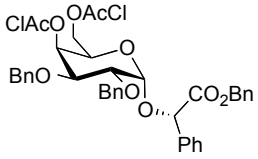
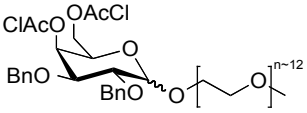
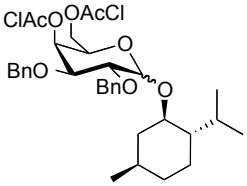
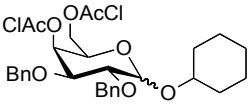
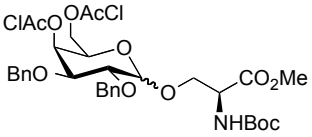
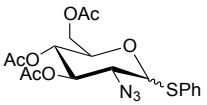
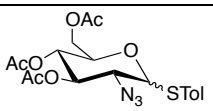
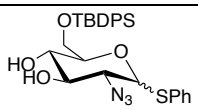
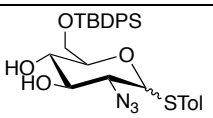
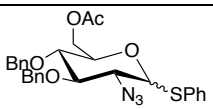
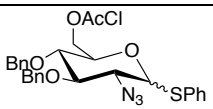
21		Experiment 16	**160**
32		Experiment 17	**161**
33		Experiment 18	**161**
34		Experiment 19	**162**
35		Experiment 20	**162**
36		Experiment 21	**163**
37		Experiment 22	**163**
38		Experiment 23	**164**
39		Experiment 24	**165**
40		Experiment 25	**165**
41		Experiment 26	**166**

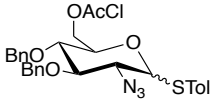
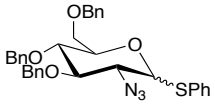
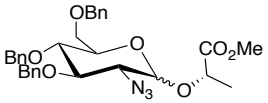
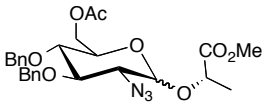
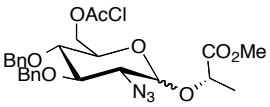
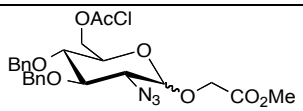
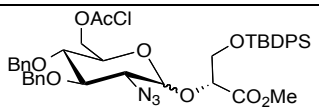
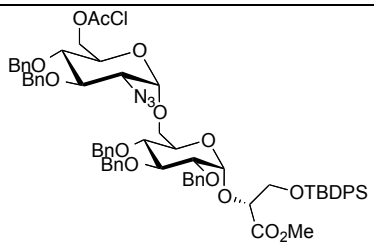
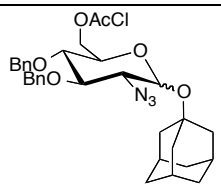
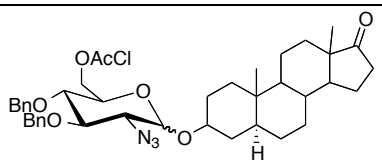
42		Experiment 27	**166**
43		Experiment 28	**167**
44		Experiment 29	**168**
45		Experiment 30	**168**
46		Experiment 31	**169**
47		Experiment 32	**170**
48		Experiment 33	**170**
49		Experiment 34	**171**
50		Experiment 35	**172**
51		Experiment 36	**173**

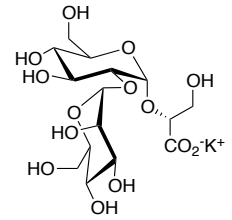
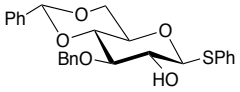
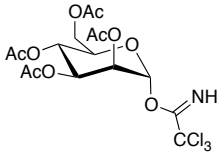
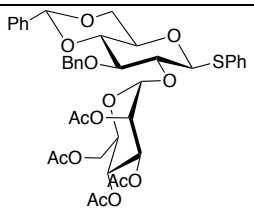
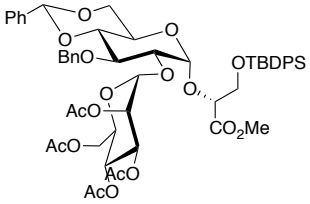
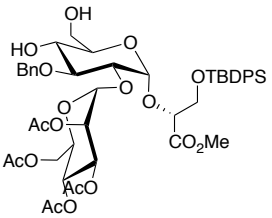
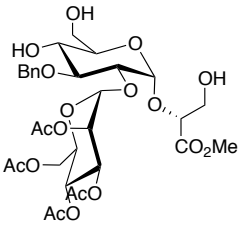
52		Experiment 37	**173**
53		Experiment 38	**174**
54		Experiment 39	**174**
55		Experiment 40	**174**
56		Experiment 41	**174**
57		Experiment 42	**175**
58		Experiment 43	**175**
59		Experiment 44	**176**
60		Experiment 45	**177**
61		Experiment 46	**177**
62		Experiment 47	**178**

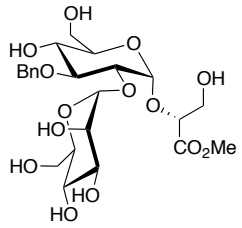
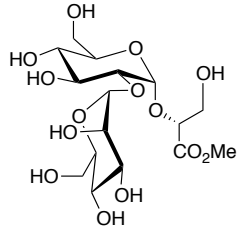
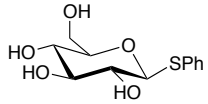
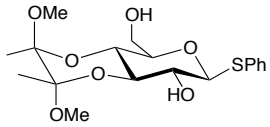
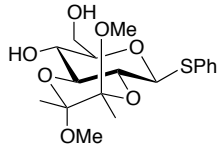
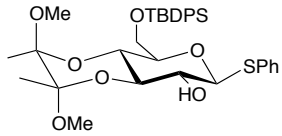
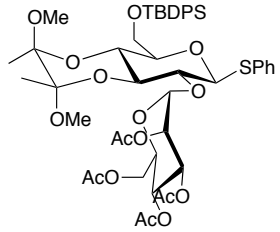
63		Experiment 48	**178**
64		Experiment 49	**179**
65		Experiment 50	**180**
66		Experiment 51	**181**
67		Experiment 52	**181**
68		Experiment 53	**182**
69		Experiment 54	**182**
70		Experiment 55	**183**
71		Experiment 56	**184**

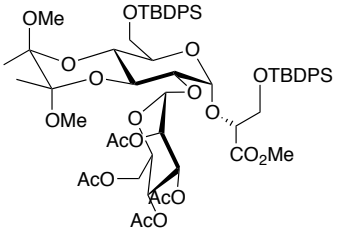
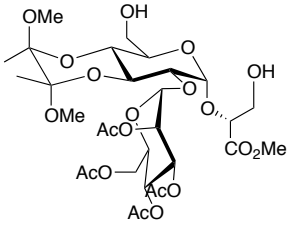
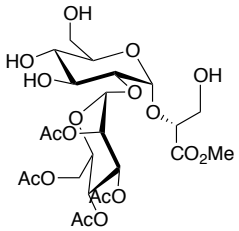
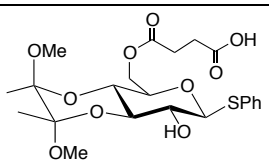
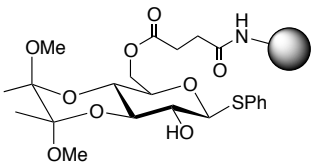
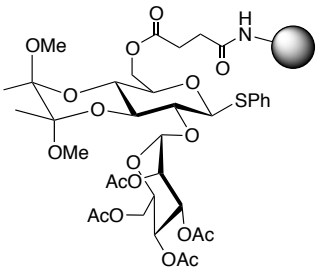
72		Experiment 57	**185**
73		Experiment 58	**185**
74		Experiment 59	**186**
75		Experiment 60	**187**
76		Experiment 61	**187**
77		Experiment 62	**188**
78		Experiment 63	**189**
79		Experiment 64	**189**

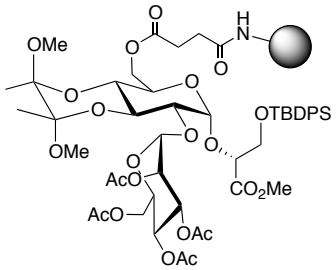
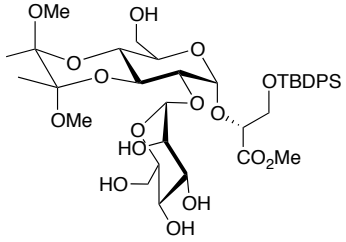
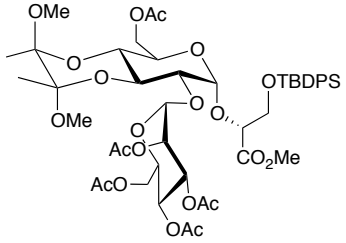
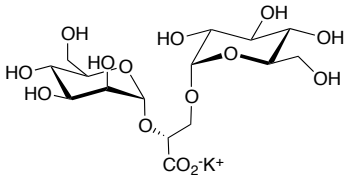
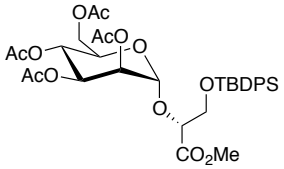
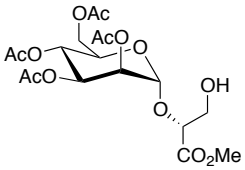
80		Experiment 65	**190**
81		Experiment 66	**191**
82		Experiment 67	**191**
83		Experiment 68	**193**
84		Experiment 69	**194**
85		Experiment 70	**194**
86		Experiment 71	**195**
87		Experiment 72	**196**
88		Experiment 73	**197**
89		Experiment 74	**197**
90		Experiment 75	**199**

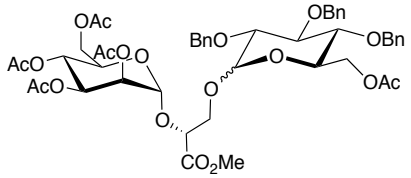
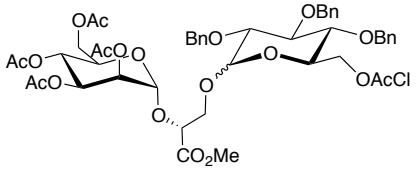
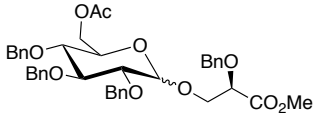
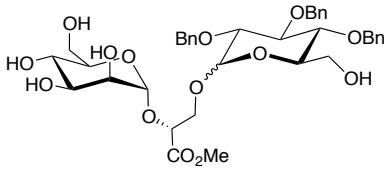
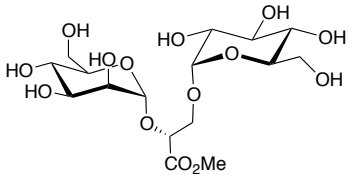
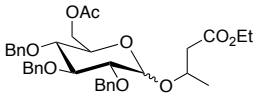
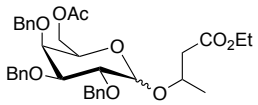
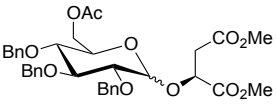
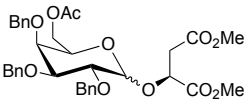
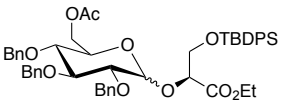
91		Experiment 76	**199**
92		Experiment 74	**197**
93		Experiment 77	**199**
94		Experiment 78	**200**
95		Experiment 79	**200**
96		Experiment 80	**201**
97		Experiment 81	**202**
98		Experiment 82	**202**
99		Experiment 83	**203**
100		Experiment 84	**203**

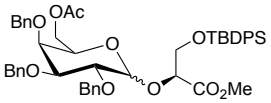
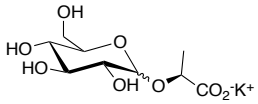
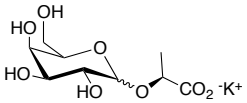
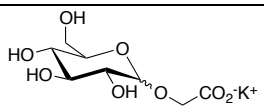
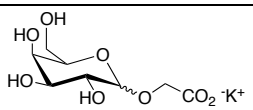
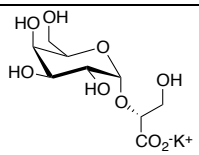
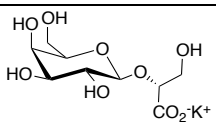
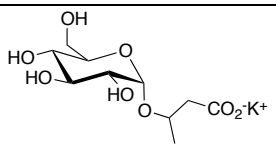
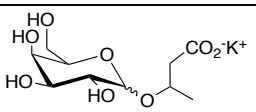
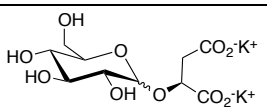
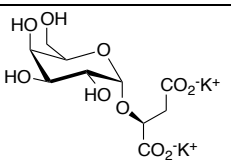
101		Experiment 93	**209**
102		Experiment 85	**204**
103		Experiment 86	**204**
104		Experiment 87	**204**
105		Experiment 88	**205**
106		Experiment 89	**206**
107		Experiment 90	**207**

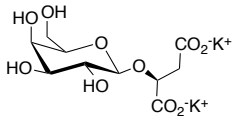
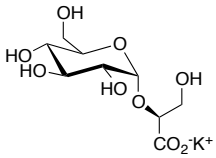
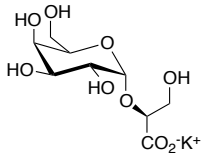
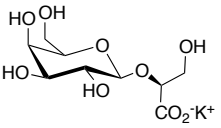
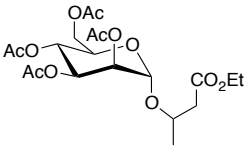
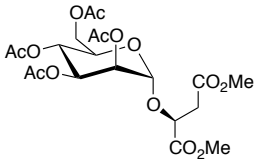
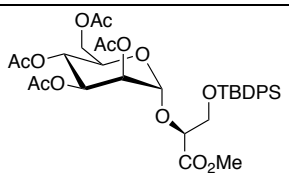
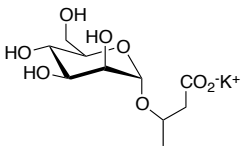
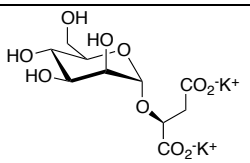
108		Experiment 91	**207**
109		Experiment 92	**208**
110		Experiment 94	**209**
111		Experiment 95	**209**
112		Experiment 95	**209**
113		Experiment 96	**209**
114		Experiment 97	**210**

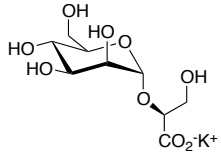
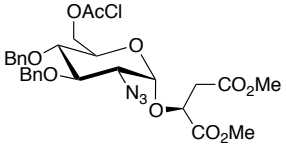
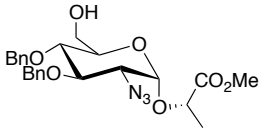
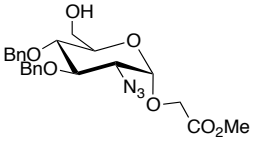
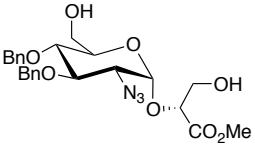
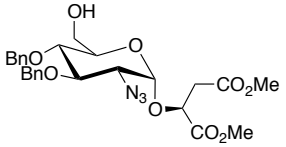
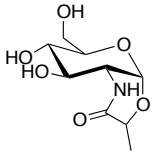
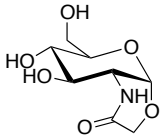
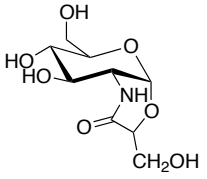
115		Experiment 98	**211**
116		Experiment 99	212
117		Experiment 100	**213**
118		Experiment 101	**213**
119		Experiment 102	**214**
120		Experiment 103	**214**

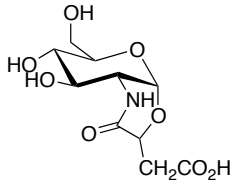
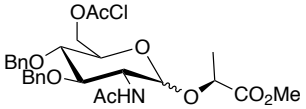
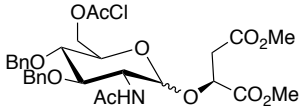
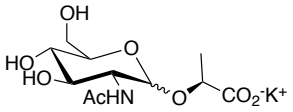
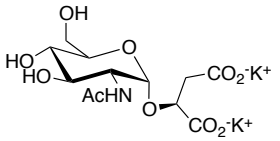
121		Experiment 104	**215**
122		Experiment 105	**215**
123		Experiment 106	**215**
124		**Experiment 114**	221
125		Experiment 107	**216**
126		Experiment 108	**217**

127		Experiment 109	**218**
128		Experiment 110	**219**
130		Experiment 111	**219**
131		Experiment 112	**220**
132		**Experiment 113**	**221**
136		Experiment 115	**222**
137		Experiment 116	**223**
138		Experiment 117	**223**
139		Experiment 118	**224**
140		Experiment 119	**225**

141		Experiment 120	**226**
142		Experiment 121	**226**
143		Experiment 122	**227**
144		Experiment 123	**227**
145		Experiment 124	**228**
146		Experiment 125	**228**
147		Experiment 125	**228**
148		Experiment 126	**229**
149		Experiment 127	**230**
150		Experiment 128	**231**
151		Experiment 129	**231**

152		Experiment 129	**231**
153		Experiment 130	**232**
154		Experiment 131	**233**
155		Experiment 132	**233**
156		Experiment 133	**234**
157		Experiment 134	**235**
158		Experiment 135	**235**
159		Experiment 136	**236**
160		Experiment 137	**236**

161		Experiment 138	**237**
162		Experiment 139	**237**
163		Experiment 140	**238**
164		Experiment 141	**239**
165		Experiment 142	**234**
166		Experiment 143	**240**
167			
168			
169			

170	
171	
172	
173	
174	

Experimental Procedures

Experiment 1. Synthesis of Ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α/β-D-glucopyranoside (1)

The synthesis of compound **1** was carried out according to the procedure described in the literature[2].

Experiment 2. Synthesis of Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α/β-D-glucopyranoside (2)

A solution of NaOMe 1N (1.12 mL, 1.12 mmol) in MeOH was added to a stirred solution of **1** (1.0 g, 1.86 mmol) in MeOH (5 mL) at 0 ºC. After 1 hour the reaction mixture was neutralized with saturated aqueous NH_4Cl. The aqueous phase was extracted with EtOAc (3x10 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed.

The crude product was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) to afford the product **2** (0.90 g, 96%, α:β =2.6:1) as a white solid. **FT-IR** (KBr disk) $\upsilon_{máx.}$: 3370-3515 (O-H) cm^{-1}. **^{1}H RMN** ($CDCl_3$): δ 7.40-7.25 (m, Ph), 5.35 (d, *J* = 5.4 Hz, H-1 (α)), 4.97-4.84 (m, C$\underline{H}_2$Ph), 4.80-4.71 (m, C$\underline{H}_2$Ph), 4.66-4.62 (m, C$\underline{H}_2$Ph), 4.50 (d, *J* = 9.8 Hz, H-1 (β)), 4.10-4.09 (m), 3.88 (dt, *J* = 3.3 Hz, *J* = 10.0 Hz, H-5 (α)), 3.88 (t, *J* = 9.1 Hz), 3.79-3.66 (m), 3.60-3.50 (m), 3.43-3.35 (m, H-2 (β), H-5 (β)), 2.82-2.62 (m, SC$\underline{H}_2$CH$_3$ (β)), 2.60-2.45 (m, SC$\underline{H}_2$CH$_3$ (α)), 1.33-1.25 (m, SCH$_2$C$\underline{H}_3$) ppm. **^{13}C RMN** ($CDCl_3$): δ 138.7, 138.2, 137.9, 137.8, 128.6-127.6, 86.5, 85.3 (C-1 (β)), 83.0 (C-1 (α)), 82.4, 81.8 (C-2 (β)), 79.7 (C-2 (α)), 79.3 (C-5 (β)), 77.7, 77.2, 75.7 (C$\underline{H}_2$Ph), 75.5 (C$\underline{H}_2$Ph (β)), 75.1 (C$\underline{H}_2$Ph (β)), 75.0 (C$\underline{H}_2$Ph (α)), 72.4 (C$\underline{H}_2$Ph (α)), 71.1 (C-5 (α)), 62.2 (C-6 (β)), 62.0 (C-6 (α)), 25.2 (SC$\underline{H}_2$CH$_3$ (β)), 23.7 (SC$\underline{H}_2$CH$_3$ (α)), 15.1 (SCH$_2$C$\underline{H}_3$ (β)), 14.6 (SCH$_2$C$\underline{H}_3$ (α)) ppm.

Experiment 3. Synthesis of Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-1-thio-α/β-D-glucopyranoside (3)

To a stirred solution of **2** (1.05 g, 2.12 mmol) in pyridine (5 mL) at 0ºC was added chloroacetyl chloride (0.186 mL, 2.33 mmol), after 1 hour more chloroacetyl chloride (0.169 mL, 2.12 mmol) was added at 0ºC. After complete conversion of the starting material water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to furnish a yellow viscous residue. Purification by flash column chromatography on silica gel (10:90, EtOAc/Hex) afforded the product **3** as viscous colourless foam (1.04 g, 86%). **FT-IR** (film) $\upsilon_{máx.}$: 1762, 1743 (C=O) cm^{-1}. **^{1}H RMN** ($CDCl_3$): δ 7.39-7.25 (m, Ph), 5.35 (d, *J* = 5.4 Hz, H-1 (α)), 4.98-4.94 (m, C$\underline{H}_2$Ph), 4.91-4.83 (m, C$\underline{H}_2$Ph), 4.78-4.71 (m, C$\underline{H}_2$Ph), 4.64 (d, *J* = 11.7 Hz, C$\underline{H}_2$Ph (α)), 4.60-4.56 (m, C$\underline{H}_2$Ph), 4.47 (d, *J* = 9.8 Hz, H-1 (β)), 4.44-4.41 (m, H-6 (β)), 4.34-4.22 (m), 4.03-3.95 (m, AcCl), 3.91-3.86 (m, H-3 (α)), 3.79 (dd, *J* = 5.4 Hz, *J* = 9.5 Hz, H-2 (α)), 3.70 (t, *J* = 8.7 Hz, H-3 (β)), 3.53-3.52 (m, H-4 (β)), 3.48-3.40 (m, H-4 (α), H-2 (β)), 2.73-2.66 (m, SC$\underline{H}_2$CH$_3$ (β)), 2.61-2.45 (m, SC$\underline{H}_2$CH$_3$ (α)), 1.33-1.25 (m, SCH$_2$C$\underline{H}_3$) ppm. **^{13}C RMN** ($CDCl_3$): δ 166.97 (C$\underline{O}$CH$_2$Cl (β)), 166.95 (C$\underline{O}$CH$_2$Cl (β)), 138.4, 138.2, 137.7, 137.6, 137.5,

128.5-127.7, 86.6 (C-3 (β)), 85.2 (C-1 (β)), 82.9 (C-1 (α)), 82.4 (C-3 (α)), 81.6 (C-2 (β)), 79.4 (C-2 (α)), 76.5 (C-4), 75.8 ($\underline{C}H_2Ph$ (β)), 75.7 ($\underline{C}H_2Ph$ (α)), 75.5 ($\underline{C}H_2Ph$ (β)), 75.0 ($\underline{C}H_2Ph$ (β)), 74.8 ($\underline{C}H_2Ph$ (α)), 72.3 ($\underline{C}H_2Ph$ (α)), 72.4 ($\underline{C}H_2Ph$ (α)), 68.6 (C-5), 64.8 (C-6 (β)), 64.7 (C-6 (α)), 40.68 ($CO\underline{C}H_2Cl$ (β)), 40.62 ($CO\underline{C}H_2Cl$ (α)), 25.1 ($S\underline{C}H_2CH_3$ (β)), 23.7 ($S\underline{C}H_2CH_3$ (α)), 15.1 ($SCH_2\underline{C}H_3$ (β)), 14.7 ($SCH_2\underline{C}H_3$ (α)) ppm.

Experiment 4. General glycosylation procedure

A suspension of thioglycoside donor (0.15 mmol), acceptor (0.15 mmol) and 4Å MS in the solvent/mixture of solvents indicated (1 mL) was stirred for 1 h at room temperature then cooled to 0°C. *N*-Iodosuccinimide (0.19 mmol) and TfOH (0.9 μL) were added at 0°C and when the reaction was complete (TLC), 10% $Na_2S_2O_3$ aqueous solution (2 mL) and saturated aqueous $NaHCO_3$ (1 mL) were added and the mixture was extracted with CH_2Cl_2 (3x5 mL); the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed under vacuum. The crude product was purified by preparative TLC (3:7, EtOAc/Hex). The α/β ratio of the isolated product was measured by ^{1}H NMR (400 MHz, $CDCl_3$) spectra.

Experiment 5. Synthesis of Methyl (*2S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)propanoate (10)

The glycosylation reaction of donor **1** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1743 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.42-7.24 (m, Ph), 5.14 (d, *J* = 8.6 Hz, $C\underline{H}_2Ph$), 5.01 (d, *J* = 8.6 Hz, $C\underline{H}_2Ph$), 4.96 (d, *J* = 8.6 Hz, $C\underline{H}_2Ph$), 4.89-4.70 (m, $C\underline{H}_2Ph$), 4.75 (d, *J* = 3.0 Hz, H-1 (α)), 4.63 (d, *J* = 12.0 Hz, $C\underline{H}_2Ph$), 4.57 (d, *J* = 10.9 Hz, $C\underline{H}_2Ph$), 4.50 (d, *J* = 6.2 Hz, H-1 (β)), 4.32 (dd, *J* = 12.2 Hz, *J* = 3.9 Hz, H-6), 4.15-4.02 (m, H-3, H-5, H-6, $C\underline{H}CH_3$), 3.71 (s, OMe (β)), 3.70 (s, OMe (α)), 3.55-3.48 (m, H-2, H-4), 2.03 (s, Ac (β)), 2.00 (s, Ac (α)) 1.50 (d, *J* = 5.5 Hz, $CH(C\underline{H}_3)$ (β)), 1.44 (d, *J* = 5.4 Hz, $CHC\underline{H}_3$ (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.9, 172.7, 138.6, 138.5, 138.0, 135.6, 135.5, 132.8, 129.9, 129.8, 128.5–127.4, 102.4

(C-1 (β)), 97.8 (C-1 (α)), 84.3, 82.0, 81.6, 80.1, 75.7, 75.6, 75.1, 75.0, 74.6, 74.4, 73.4, 72.8, 71.5, 62.0, 61.8, 52.1, 52.0, 19.1, 18.0 ppm.

Experiment 6. Synthesis of Methyl (*2R*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)propanoate (11)

The glycosylation reaction of donor **1** with acceptor **5** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1.**FT-IR** (film) $\upsilon_{máx.}$: 1742 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.43–7.25 (m, Ph), 5.10 (d, *J* = 3.4 Hz, H-1(α)), 5.06 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.89 (d, *J* = 11.9 Hz, 1H, C$\underline{H}_2$Ph), 4.88 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph), 4.81 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.72 (d, *J* = 11.9 Hz, C$\underline{H}_2$Ph), 4.55 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph), 4.48 (d, *J* = 7.7 Hz, H-1(β)), 4.37 (q, *J* = 7.0 Hz, C$\underline{H}$CH$_3$), 4.26 (d, *J* = 3.4 Hz, H-6), 4.08 (t, *J* = 9.2Hz, H-3), 3.83 (ddd, *J* = 3.2Hz, J=3.2Hz, *J* = 10.0Hz, H-5), 3.76 (s, OMe (α)), 3.72 (s, OMe (β)), 3.58 (dd, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.48 (t, *J* = 9.7 Hz, H-4), 2.04 (s, Ac (β)), 2.02 (s, Ac (α)), 1.50 (d, *J* = 7.0 Hz, CHC$\underline{H}_3$ (α)), 1.46 (d, *J* = 6.8 Hz, CHC$\underline{H}_3$ (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.9 (β), 172.5 (α), 170.7 (β), 170.6 (α), 138.6 (α), 138.4 (β), 138.2 (β), 137.9 (α), 137.6 (α), 137.5 (β), 128.5-127.6, 103.4 (C-1 (β)), 95.3 (C-1 (α)), 84.5 (β), 81.8 (β), 81.5 (α), 78.9 (α), 77.1 (α), 75.7 (α), 75.6 (β), 75.5 (β), 75.2 (α), 75.0 (β), 74.9 (β), 72.9 (β), 72.3 (α), 69.7 (α), 69.3 (α), 62.9 (α), 62.8 (β), 51.9 (α), 20.7 (α), 18.4 (α), 18.2 (β) ppm.

Experiment 7. Synthesis of Benzyl (*2S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)propanoate (12)

The glycosylation reaction of donor **1** with acceptor **6** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$:1739 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.34–7.24 (m, Ph), 5.18–5.09 (m, $CO_2C\underline{H}_2$Ph), 4.99 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph), 4.86 (d, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.82 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph), 4.79 (d, *J* = 12.1 Hz, C$\underline{H}_2$Ph), 4.77 (d, *J* = 3.1 Hz, H-1 (α)), 4.63 (d, *J* = 12.0 Hz, C$\underline{H}_2$Ph), 4.54 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph), 4.51 (d, *J* = 7.8 Hz, H-1 (β)), 4.20-4.09(m, H-5,

H-6, C<u>H</u>CH$_3$), 4.06-3.99 (m, H-3, H-6), 3.54-3.47 (m, H-2, H-4), 2.01 (s, Ac (β)), 1.97 (s, Ac (α)), 1.51 (d, *J* = 6.8 Hz, CHC<u>H</u>$_3$ (β)), 1.45 (d, *J* = 6.8 Hz, CHC<u>H</u>$_3$ (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.1 (β), 171.9 (α), 170.7 (β), 170.6 (α), 138.6 (α), 138.5 (β), 138.4 (β), 138.1 (α), 138.0 (α), 137.7 (β), 135.4 (α), 128.6–127.6, 102.4 (C-1 (β)), 97.3 (C-1 (α)), 84.4 (β), 81.8 (C-3 (α)), 79.9 (C-2 (α)), 77.1 (β), 77.0 (C-4 (α)), 75.7 (α), 75.0 (β), 74.8 (α), 74.5 (β), 73.8 (<u>C</u>HCO$_2$CH$_3$ (α)), 73.3 (α), 72.9 (β), 72.8 (β), 69.1 (C-5 (α)), 66.7 (α), 63.0 (β), 62.7 (C-6 (α)), 20.8 (OCO<u>C</u>H$_3$), 19.1 (CO$_2$<u>C</u>H$_3$ (β)), 17.8 (CO$_2$<u>C</u>H$_3$ (α)) ppm.

Experiment 8. Synthesis of Methyl 2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)acetate (13)

The glycosylation reaction of donor **1** with acceptor **7** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$:1742 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.32–7.25 (m, Ph), 5.05 (d, *J* = 4.0 Hz, H-1(α)), 5.03 (d, *J* = 11.2Hz, C<u>H</u>$_2$Ph), 4.88 (d, *J* = 10.8Hz, C<u>H</u>$_2$Ph), 4.86 (d, *J* = 11.9 Hz, C<u>H</u>$_2$Ph), 4.81 (d, *J* = 10.8 Hz, C<u>H</u>$_2$Ph), 4.77 (d, *J* = 11.9 Hz, C<u>H</u>$_2$Ph), 4.55 (d, *J* = 10.9 Hz, C<u>H</u>$_2$Ph), 4.51 (d, *J* = 7.7 Hz, H-1 (β)), 4.27 (d, *J* = 16.6 Hz, C<u>H</u>$_2$CO$_2$Me), 4.25-4.24 (m, H-6), 4.15 (d, *J* = 16.6 Hz, C<u>H</u>$_2$CO$_2$Me), 4.06 (t, *J* = 9.2 Hz, H-3), 3.91 (dt, *J* = 3.1 Hz, *J* = 10.1 Hz, H-5), 3.76 (s, CO$_2$Me (α)), 3.75 (s, CO$_2$Me (β)), 3.59 (dd, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2 (α)), 3.54-3.50 (m, H-2 (β)), 3.48 (dd, *J* = 9.2 Hz, *J* = 9.7 Hz, H-4), 2.03 (s, Ac (β)), 2.02 (s, Ac (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.0, 138.6, 137.9, 137.8, 128.5, 128.47, 128.45, 128.3, 128.1, 128.0, 127.95, 127.92, 127.7, 103.2 (C-1 (β)), 96.3 (C-1 (α)), 81.6, 79.3, 77.0, 75.8, 75.0, 72.7, 69.3, 63.3, 62.9, 51.9, 20.8 ppm. **Anal. Calcd for** $C_{32}H_{36}O_9$: C, 68.07; H, 6.43. Found: C, 68.60, H, 6.54.

Experiment 9. Synthesis of Methyl (*2R*)-*tert*-butyldimethylsilyl-3-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2,3-dihydroxypropanoate (14)

The glycosylation reaction of donor **1** with acceptor **8** was performed according to the procedure described in experiment 4. The results are

presented in Table 2, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.38–7.25 (m, 15H, Ph), 4.95 (d, 1H, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.88 (d, 1H, *J* = 11.1 Hz, C$\underline{H}_2$Ph), 4.87 (d, 1H, *J* = 3.5 Hz, H-1), 4.77 (d, 1H, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.72-4.66 (m, 2H, C$\underline{H}_2$Ph), 4.56 (d, 1H, *J* = 11.1 Hz, C$\underline{H}_2$Ph), 4.45 (dd, 1H, *J* = 4.4 Hz, *J* = 6.2 Hz, $CH_2C\underline{H}$(OTBDMS)CO_2Me), 4.24-4.22 (m, 2H, H-6), 4.00 (t, 1H, *J* = 9.2 Hz, H-3), 3.88 (dt, 1H, *J* = 3.0 Hz, *J* = 10.0 Hz, H-5), 3.81-3.75 (m, 1H, C$\underline{H}_2$CH(OTBDMS)CO_2Me), 3.72-3.67 (m, 1H, C$\underline{H}_2$CH(OTBDMS)CO_2Me), 3.70 (s, 3H, OMe), 3.54-3.44 (m, 2H, H-2, H-4), 2.01 (s, 3H, Ac), 0.91 (s, 9H, *t*-But), 0.13 (s, 6H, $SiMe_2$) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.2, 170.7, 138.6, 138.3, 138.1, 128.6-127.6, 97.1 (C-1), 81.7 (C-3), 79.2 (C-2), 77.1 (C-4), 75.7 ($\underline{C}H_2$Ph), 74.8 ($\underline{C}H_2$Ph), 72.6 ($\underline{C}H_2$Ph), 72.0 ($CH_2\underline{C}$H(OTBDMS)CO_2Me)), 68.8 ($\underline{C}H_2$CH(OTBDMS)CO_2Me), 65.0 (C-5), 63.1 (C-6), 52.0 ($CO_2\underline{Me}$), 25.7 (SiC($\underline{C}H_3$)$_3$), 20.8 (CO$\underline{C}H_3$), 18.3 (Si$\underline{C}$(CH_3)$_3$), 4.9 (Si($\underline{C}H_3$)$_2$) ppm.

Experiment 10. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(*2R*)-2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2,3-dihydroxypropanoate (15)

The glycosylation reaction of donor **1** with acceptor **9** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1. **Alpha anomer: $[\alpha]_D^{20}$** +45.2 (c 1.45, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.38-7.37 (5H, m), 7.37-7.22 (25H, m), 5.16 (1H, d, *J* = 3.6 Hz, H-1), 5.02 (1H, d, *J* = 10.6 Hz), 4.89 (2H, dd, *J* = 11.9 Hz, *J* = 3.1 Hz), 4.76 (1H, d, *J* = 10.5Hz), 4.70 (1H, d, *J* = 11.8Hz), 4.54 (1H, d, *J* = 11.2 Hz), 4.49 (1H, dd, *J* = 6.3 Hz, J = 4.3 Hz), 4.15-3.94 (6H, m), 3.73 (3H, s), 3.60 (1H, dd, *J* = 9.7 Hz, *J* = 3.5 Hz), 3.48 (1H, t, *J* = 9.5 Hz), 1.99 (3H, s), 1.03 (9H, s) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.6, 170.2, 138.7, 138.1, 138.0, 135.6, 135.5, 133.0, 132.8, 129.8, 129.8, 128.4, 128.3, 128.2, 128.2, 128.1, 127.8, 127.7, 127.6, 94.8 (C-1), 81.6, 75.8, 74.8, 72.0, 71.9, 69.0, 65.8, 64.6, 62.9, 51.9, 26.7, 20.8, 19.1 ppm. **HR-MS:** calcd for $C_{49}H_{56}O_{10}Si$ $[M]^+$: 559.2130; found: 559.2125.

Experiment 11. Synthesis of Methyl (*2S*)-2-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-glucopyranosyl)propanoate (16)

The glycosylation reaction of donor **3** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1751 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.37-7.24 (m, Ph), 5.13 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph (β)), 5.00 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (α)), 4.96 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph (β)), 4.90-4.77 (m, C$\underline{H}_2$Ph), 4.72 (d, *J* = 3.8 Hz, H-1 (α)), 4.68-4.55 (m, C$\underline{H}_2$Ph), 4.50 (d, *J* = 7.6 Hz, H-1 (β)), 4.31-4.24 (m), 4.16-4.11 (m), 4.06-3.90 (m), 3.71 (s, OMe (β)), 3.70 (s, OMe (α)), 3.55-3.45 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.6 ($\underline{C}O_2$Me), 166.9 ($\underline{C}OCH_2Cl$), 138.6, 138.1, 137.8, 128.5-128.0, 127.9, 127.7, 102.3 (C-1 (β)), 97.5 (C-1 (α)), 84.4 (β), 81.3 (C-3 (α)), 80.0 (C-2 (α)), 75.7 (C-4 (α)), 74.8 ($\underline{C}H_2$Ph), 74.6 ($\underline{C}H_2$Ph), 74.1 ($\underline{C}HCH_3$), 73.4 ($\underline{C}H_2$Ph), 72.8 (β), 72.5 (β), 69.0 (C-5 (α)), 64.3 (C-6 (α)), 52.1 (OMe), 40.7 ($CO\underline{C}H_2Cl$), 17.9 ($CH\underline{C}H_3$) ppm.

Experiment 12. Synthesis of Benzyl (*2S*)-2-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-glucopyranosyl)propanoate (17)

The glycosylation reaction of donor **3** with acceptor **6** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1745 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.34-7.27 (m, Ph), 5.13 (dd, *J* = 12.2 Hz, *J* = 23.8 Hz, $CO_2C\underline{H}_2$Ph (α)), 5.00 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (α)), 4.99 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (α)), 4.87 (d, *J* = 11.1 Hz, C$\underline{H}_2$Ph (α)), 4.82 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph (α)), 4.78 (d, *J* = 12.1 Hz, C$\underline{H}_2$Ph (α)), 4.73 (d, *J* = 3.7 Hz, H-1 (α)), 4.61 (d, *J* = 12.0Hz, C$\underline{H}_2$Ph (α)), 4.57 (d, *J* = 11.2 Hz, C$\underline{H}_2$Ph (α)), 4.51 (d, *J* = 7.6 Hz, H-1 (β)), 4.19-4.06 (m), 4.02 (t, *J* = 9.2 Hz, H-3 (α)), 3.90 (dd, *J* = 14.8 Hz, *J* = 21.1 Hz, AcCl), 3.52-3.44 (m, H-2 (α), H-4 (α)), 1.45 (d, *J* = 6.7 Hz, CHC$\underline{H}_3$ (α)), 1.50 (d, *J* = 6.9 Hz, CHC$\underline{H}_3$ (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 171.9 ($\underline{C}O_2$Bn), 166.9 ($\underline{C}OCH_2Cl$), 138.6, 138.1, 138.0, 135.3, 128.9-127.7, 102.3 (C-1 (β)), 97.4 (C-1 (α)), 81.8 (C-3 (α)), 79.9 (C-2 (α)), 76.6 (C-4 (α)), 75.8 ($\underline{C}H_2$Ph), 74.7

($\underline{C}H_2Ph$), 74.0 ($\underline{C}HCH_3$), 73.4 ($\underline{C}H_2Ph$), 69.0 (C-5 (α)), 66.8 ($CO_2\underline{C}H_2Ph$), 64.2 (C-6 (α)), 40.6 ($CO\underline{C}H_2Cl$), 17.9 ($CH\underline{C}H_3$) ppm.

Experiment 13. Synthesis of Methyl 2-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-glucopyranosyl)acetate (18)

The glycosylation reaction of donor **3** with acceptor **7** was performed according to the procedure described in experiment 4. The results are presented in Table 2, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1757 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.42-7.24 (m, Ph), 5.07 (d, *J* = 11,0 Hz, $C\underline{H}_2Ph$ (β)), 5.03 (d, *J* = 10.4 Hz, $C\underline{H}_2Ph$ (α)), 5.02 (s, H-1 (α)), 4.97 (d, *J* = 10.9 Hz, $C\underline{H}_2Ph$ (β)), 4.90-4.71 (m, $C\underline{H}_2Ph$), 4.58 (d, *J* = 11.0 Hz, $C\underline{H}_2Ph$ (α)), 4.51 (d, *J* = 7.6 Hz, H-1 (β)), 4.38-4.23 (m), 4.16-4.00 (m), 3.98-3.92 (m), 3.76 (s, OMe), 3.67 (t, *J* = 8.3 Hz, H-3 (β)), 3.57 (dd, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2 (α)), 3.47 (t, *J* = 9.4 Hz, H-4 (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.0 ($\underline{C}O_2Me$), 166.9 ($\underline{C}OCH_2Cl$), 138.5, 137.8, 137.7, 128.5-127.7, 96.3 (C-1 (α)), 81.6 (C-3 (α)), 79.3 (C-2 (α)), 76.5 (C-4 (α)), 75.8 ($\underline{C}H_2Ph$), 74.9 ($\underline{C}H_2Ph$), 72.8 ($\underline{C}H_2Ph$), 69.1 (C-5 (α)), 64.4 (C-6 (α)), 63.4 (OMe), 40.6 ($CO\underline{C}H_2Cl$) ppm.

Experiment 14. Synthesis of Ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α/β-D-galactopyranoside (19)

To a stirred solution of methyl α–D-galactopyranoside (2.0 g, 10.3 mmol) in DMF (20 mL) was added benzyl bromide (6.3 mL, 51.5 mmol). The mixture was cooled to 0ºC and sodium hydride (1.48 g, 61.8 mmol) was added portion-wise. The reaction was kept over-night at r.t. and after complete conversion of the starting material MeOH was added at 0ºC. The mixture was extracted with Et_2O and the combined organic phases dried, filtered and concentrate. Purification by flash column chromatography on silica gel (10:90, EtOAc/Hex) afforded the product as a viscous colourless foam (5.14 g, 90%).

Concentrated sulphuric acid (1.0 mL) was added dropwise to a stirred solution of the methyl tetra-*O*-benzylgalactopyranoside (5.72 g, 10.7 mmol) in acetic acid/acetic anhydride (1:1, 50 mL) at 0ºC. After complete conversion of the starting material the reaction mixture was quenched with saturated

$NaHCO_3$ solution and ice-cold distilled water until pH 7. The mixture was extracted with EtOAc (3x70 mL) and the combined organic dried, filtered and concentrated in vacuum. The residue was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) to give the diacetate (4.29 g, 75%, α:β = 3.7:1) as a viscous colourless foam. **FT-IR** (film) $\upsilon_{máx.}$: 1745 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.26 (m, Ph), 6.39 (d, *J* = 2.5 Hz, H-1 (α)), 5.58 (d, *J* = 5.0 Hz, H-1 (β)), 4.99-4.95 (m), 4.89-4.60 (m), 4.20-3.95 (m), 3.92-3.87 (m), 3.83 (d, *J* = 2.0 Hz), 3.68 (t, *J* = 6.0 Hz), 3.62 (dd, *J* = 2.8 s Hz, *J* = 10.0 Hz), 2.11 (s, Ac (α)), 2.04 (s, Ac (β)), 1.98 (s, Ac (α)), 1.96 (s, Ac (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 138.5, 138.0, 137.9, 128.5-127.4, 94.1 (C-1 (β)), 90.7 (C-1 (α)), 82.4 (β), 78.6 (α), 78.1 (β), 75.4 (α), 75.3 ($\underline{C}H_2Ph$ (β)), 74.7 ($\underline{C}H_2Ph$ (α)), 74.4 ($\underline{C}H_2Ph$ (β)), 74.3 (α), 73.44 ($\underline{C}H_2Ph$ (α)), 73.40 ($\underline{C}H_2Ph$ (α)), 73.1 ($\underline{C}H_2Ph$ (β)), 73.2 (β), 72.9 (β), 70.8 (α), 63.1 (C-6 (α)), 62.9 (C-6 (β)), 21.1 ($CO\underline{C}H_3$ (α)), 21.0 ($CO\underline{C}H_3$ (β)), 20.8 ($CO\underline{C}H_3$ (α)), 20.7 ($CO\underline{C}H_3$ (β)) ppm. **HR-MS:** calcd for $C_{31}H_{34}O_8Na^+$ $[M+Na]^+$: 557.21459; found: 557.21447.

Ethanethiol (1.56 mL, 20.7 mmol) was added to a stirred solution of diacetate (3.69 g, 6.9 mmol) in DCM (30 ml). The reaction mixture was cooled to 0ºC and boron trifluoride diethyl etherate (1.31 mL, 10.35 mmol) added dropwise. After complete conversion of starting material the reaction mixture was diluted with CH_2Cl_2 (40 mL) and quenched with saturated $NaHCO_3$ solution until pH 7. The aqueous phase was extracted with CH_2Cl_2 (2$\times$40 mL) and the combined organic extracts dried, filtered and concentrated in vacuum. The residue was purified by flash column chromatography (20:80, EtOAc/Hex) to give the thiogalactoside **19** (3.39 g, 91%, α/β=2.4:1) as a viscous colourless foam. **Alpha product: $[\alpha]_D^{20}$** +113.8 (c 2.32, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1743 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.27 (m, 15H, Ph), 5.49 (d, 1H, *J* = 3.5 Hz, H-1), 4.95 (d, 1H, *J* = 7.0 Hz, $C\underline{H}_2Ph$), 4.86 (d, 1H, *J* = 7.5 Hz, $C\underline{H}_2Ph$), 4.76-4.66 (m, 3H, $C\underline{H}_2Ph$), 4.61 (d, 1H, *J* = 7.0 Hz, $C\underline{H}_2Ph$), 4.31-4.25 (m, 2H, H-2, H-5), 4.18 (dd, 1H, *J* = 2.8 Hz, *J* = 4.5 Hz, H-6), 4.05 (dd, 1H, *J* = 3.3Hz, *J* = 3.8Hz, H-6), 3.85-3.84 (m, 1H, H-4), 3.79 (dd, 1H, *J* = 1.8 Hz, J=4.5 Hz, H-3), 2.62-2.44 (m, 2H, $SC\underline{H}_2CH_3$), 1.97 (s, 3H, OAc), 1.27 (t, 3H, *J* = 4.5 Hz, $SCH_2C\underline{H}_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.4 ($\underline{C}OCH_3$), 138.7,

138.2, 138.1, 128.5-127.5, 83.2 (C-1), 79.4, 76.4, 74.7, 74.5 ($\underline{C}H_2Ph$), 73.7 ($\underline{C}H_2Ph$), 72.5 ($\underline{C}H_2Ph$), 68.9, 63.4 (C-6), 23.5 ($S\underline{C}H_2CH_3$), 20.8 ($CO\underline{C}H_3$), 14.7 ($SCH_2\underline{C}H_3$) ppm. **HR-MS:** calcd for $C_{31}H_{36}O_6SH^+$ $[M+H]^+$: 537.23054; found: 537.23155. **Beta product:** $\mathbf{[\alpha]_D^{20}}$ +17.1 (c 1.18, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1742 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.39-7.26 (m, 15H, Ph), 4.98 (d, 1H, *J* = 7.3 Hz, $C\underline{H}_2Ph$), 4.89 (d, 1H, *J* = 6.3 Hz, $C\underline{H}_2Ph$), 4.83-4.74 (m, 3H, $C\underline{H}_2Ph$), 4.65 (d, 1H, *J* = 7.5Hz, $C\underline{H}_2Ph$), 4.41 (d, 1H, *J* = 6.0Hz, H-1), 4.22 (dd, 1H, *J* = 2.8Hz, *J* = 4.3 Hz, H-6), 4.05 (dd, 1H, *J* = 3.8 Hz, J=3.3 Hz, H-6), 3.87-3.82 (m, 2H, H-2, H-4), 3.58-3.53 (m, 2H, H-3, H-5), 2.80-2.64 (m, 2H, $SC\underline{H}_2CH_3$), 1.96 (s, 3H, OAc), 1.29 (t, 3H, *J* = 4.5 Hz, $SCH_2\underline{C}H_3$) ppm. **^{13}C NMR ($CDCl_3$):** δ 170.5 ($\underline{C}OCH_3$), 138.28, 138.25, 138.22, 128.5-127.6, 85.4 (C-1), 84.1, 78.4, 75.9, 75.8 ($\underline{C}H_2Ph$), 74.3 ($\underline{C}H_2Ph$), 73.3, 73.1 ($\underline{C}H_2Ph$), 63.3 (C-6), 24.9 ($S\underline{C}H_2CH_3$), 20.8 ($CO\underline{C}H_3$), 15.1 ($SCH_2\underline{C}H_3$) ppm. **HR-MS:** calcd for $C_{31}H_{36}O_6SH^+$ $[M+H]^+$: 537.23054; found: 537.23002.

Experiment 15. Synthesis of Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α/β-D-galactopyranoside (20)

The procedure of Experiment 2 was applied to compound **19**, affording the product **20** (0.856 g, 81%, α:β=2.6:1) as a white solid. **Alpha product: f.p.** 84.0-85.1 ºC. $\mathbf{[\alpha]_D^{20}}$ +145.2 (c 1.03, CH_2Cl_2). **FT-IR** (KBr disk) $\upsilon_{máx.}$: 3422-3508 (O-H) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.28 (m, 15H, Ph), 5.51 (d, 1H, *J* = 3.5 Hz, H-1), 4.97 (d, 1H, *J* = 7.3 Hz, $C\underline{H}_2Ph$), 4.87 (d, 1H, *J* = 7.5 Hz, $C\underline{H}_2Ph$), 4.72 (q, 2H, *J* = 7.3 Hz, *J* = 9.3 Hz), 4.64 (d, 1H, *J* = 7.3Hz), 4.30 (dd, 1H, *J* = 3.3 Hz, *J* = 3.5 Hz), 4.13-4.10 (m, 1H), 3.88-3.87 (m, 1H), 3.73 (dd, 1H, *J* = 3.3 Hz, *J* = 4.0 Hz), 3.53 (dd, 1H, *J* = 3.3 Hz, *J* = 3.8 Hz), 2.61-2.45 (m, 2H, $SCH_2C\underline{H}_3$), 1.27 (t, 3H, *J* = 4.5 Hz, $SCH_2C\underline{H}_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 138.6, 138.2, 138.1, 128.5-127.6, 83.4 (C-1), 79.5, 76.2, 75.2, 74.5 ($\underline{C}H_2Ph$), 73.7 ($\underline{C}H_2Ph$), 72.8 ($\underline{C}H_2Ph$), 70.5, 62.5 (C-6), 23.5 ($S\underline{C}H_2CH_3$), 14.6 ($SCH_2\underline{C}H_3$) ppm. **Anal. Calcd for $C_{29}H_{34}O_5S$:** C, 70.42; H, 6.93; S, 6.48. **Found:** C, 69.93; H, 6.86; S, 6.18. **Beta anomer: f.p.** 100.3-101.4 ºC. $\mathbf{[\alpha]_D^{20}}$ +6.6 (c 0.89, CH_2Cl_2). **FT-IR** (KBr disk) $\upsilon_{máx.}$: 3420-3510 (O-H) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.28 (m, 15H, Ph), 4.97 (d, 1H, *J* = 7.3 Hz), 4.90 (d, 1H, *J* =

6.3 Hz), 4.81 (d, 1H, *J* = 6.3 Hz), 4.80-4.74 (m, 2H), 4.43 (d, 1H, *J* = 6.3 Hz, H-1), 3.87-3.76 (m, 3H), 3.58 (dd, 1H, *J* = 1.8 Hz, *J* = 4.0 Hz), 3.47 (dd, 1H, *J* = 3.3 Hz, *J* = 3.8 Hz), 3.42-3.39 (m, 1H), 2.82-2.67 (m, 2H, $SC\underline{H}_2CH_3$), 1.30 (t, 3H, *J* = 4.5 Hz, $SCH_2C\underline{H}_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 138.3, 138.2, 138.19, 128.5-127.6, 85.4 (C-1), 84.1, 78.6, 78.5, 75.8 ($\underline{C}H_2Ph$), 74.1 ($\underline{C}H_2Ph$), 73.1 ($\underline{C}H_2Ph$), 73.0, 62.2 (C-6), 24.9 ($S\underline{C}H_2CH_3$), 15.1 ($SCH_2\underline{C}H_3$) ppm. **Anal. Calcd for $C_{29}H_{34}O_5S$:** C, 70.42; H, 6.93; S, 6.48. **Found:** C, 70.17; H, 6.97; S, 6.32.

Experiment 16. Synthesis of Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-1-thio-α/β-D-galactopyranoside (21)

To a stirred solution of **20** (0.845 g, 1.70 mmol) in pyridine (5 mL) at 0ºC was added chloroacetic anhydride (0.320 g, 1.87 mmol). After complete conversion of the starting material water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to furnish a yellow viscous residue. Purification by flash column chromatography on silica gel (20:80, EtOAc/hexane) afforded the product **21** as a viscous colourless foam (0.878 g, 90%). **HRMS:** calcd for $C_{31}H_{35}ClO_6SNa^+$ $[M+Na]^+$: 593.1740; found 593.1735. **Alpha product: $[\alpha]_D^{20}$** +104.00 (c 1.60, CH_2Cl_2). **FT-IR (film) $\upsilon_{máx.}$:** 1745 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.28 (m, 15H, Ph), 5.47 (d, 1H, *J* = 5.4 Hz, H-1), 4.96 (d, 1H, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.87 (d, 1H, *J* = 11.8 Hz, $C\underline{H}_2Ph$), 4.76-4.67 (m, 3H, $C\underline{H}_2Ph$), 4.62 (d, 1H, *J* = 11.6Hz, $C\underline{H}_2Ph$), 4.32-4.25 (m, 3H, H-2, H-4, H-6), 4.15-4.06 (m, 1H, H-6), 3.96-3.88 (m, 2H, AcCl), 3.85-3.83 (m, 1H, H-5), 3.79 (dd, 1H, *J* = 2.8 Hz, *J* = 9.7 Hz, H-3), 2.62-2.35 (m, 2H, $SC\underline{H}_2CH_3$), 1.28-1.24 (m, 3H, $SCH_2C\underline{H}_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.8 ($\underline{C}OCH_2Cl$), 138.5, 138.1, 138.0, 128.5-127.6, 83.1 (C-1), 79.2 (C-3), 76.1 (C-2), 74.4 ($\underline{C}H_2Ph$), 74.3 (C-5), 73.8 ($\underline{C}H_2Ph$), 72.5 ($\underline{C}H_2Ph$), 68.5 (C-4), 64.9 (C-6), 40.5 ($CO\underline{C}H_2Cl$), 23.5 ($S\underline{C}H_2CH_3$), 14.7 ($SCH_2\underline{C}H_3$) ppm. **Beta product: $[\alpha]_D^{20}$** +2.03 (c 1.02, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1747 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.28 (m, 15H, Ph), 4.99 (d, 1H, J=11.8 Hz, $C\underline{H}_2Ph$), 4.89 (d, 1H, *J* = 10.2 Hz, $C\underline{H}_2Ph$), 4.83-4.75 (m, 3H, $C\underline{H}_2Ph$), 4.65 (d, 1H, *J* = 11.8 Hz, $C\underline{H}_2Ph$), 4.42 (d, 1H, *J* = 9.6 Hz, H-1), 4.31 (dd, 1H, *J* = 6.8 Hz, *J* = 11.1 Hz, H-6), 4.10 (dd, 1H, *J* = 5.9, Hz, *J* = 11.1 Hz, H-6), 3.91 (dd,

2H, *J* = 15.0, Hz, v19.0 Hz, AcCl), 3.87-3.80 (m, 2H, H-2, H-4), 3.59-3.55 (m, 2H, H-3, H-5), 2.81-2.65 (m, 2H, $SC\underline{H}_2CH_3$), 1.30 (t, 3H, *J* = 7.4 Hz, $SCH_2C\underline{H}_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.8 ($\underline{C}OCH_2Cl$), 138.1, 128.5-127.6, 85.4 (C-1), 84.0 (C-3), 78.3 (C-2), 75.8 ($\underline{C}H_2Ph$), 75.5 (C-5), 74.1 ($\underline{C}H_2Ph$), 73.3 ($\underline{C}H_2Ph$), 72.9 (C-4), 64.8 (C-6), 40.6 ($CO\underline{C}H_2Cl$), 24.9 ($S\underline{C}H_2CH_3$), 15.1 ($SCH_2\underline{C}H_3$) ppm.

Experiment 17. Synthesis of Methyl (*2S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)propanoate (32)

The glycosylation reaction of donor **19** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1743 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.45-7.25 (m, Ph), 5.12 (d, *J* = 10.8 Hz, $C\underline{H}_2Ph$), 4.98-4.58 (m, $C\underline{H}_2Ph$), 4.85 (s, H-1 (α)), 4.51-4.44 (m, $C\underline{H}CH_3$ (β)), 4.45 (d, *J* = 7.6 Hz, H-1 (β)), 4.21-3.95 (m, H-6 (α), H-6 (β), H-5(α), $C\underline{H}CH_3$ (α)), H-2 (α), H-6 (α), H-6 (β), H-3 (α)), 3.93 (sl, 1H, H-4 (α)), 3.86 (dd, *J* = 7.6Hz, *J* = 9.6Hz, H-2 (β)), 3.75 (d, *J* = 2.2 Hz, H-4 (β)), 3.69 (s, OMe (β)), 3.67 (s, OMe (α)), 3.58-3.44 (m, H-3 (β), H-5 (β)), 1.96 (s, Ac (β)), 1.95 (s, Ac (α)), 1.49 (d, *J* = 6.9 Hz, $CHC\underline{H}_3$ (β)), 1.43 (d, *J* = 6.8 Hz, $CHC\underline{H}_3$ (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 173.0, 172.9, 170.5, 138.8, 138.7, 138.5, 138.3, 138.1, 128.6, 128.4, 128.37, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.57, 127.53, 127.45, 127.43, 102.6 (C-1 (α)), 98.0 (C-1 (β)), 81.9 (C-3 (β)), 79.1 (C-2 (β)), 78.9 (C-3 (α)), 76.3 (C-2 (α)), 74.9, 74.57, 74.53, 74.2, 73.7, 73.4, 73.2, 72.8, 72.5, 72.1 (C-5 (β)), 68.9 (C-5 (α)), 63.1 (C-6 (α)), 62.9 (C-6 (β)), 51.98, 51.94, 20.7, 19.1, 17.8 ppm.

Experiment 18. 4.2.9. Synthesis of Benzyl (*2S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D- galactopyranosyl)propanoate (33)

The glycosylation reaction of donor **19** with acceptor **6** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.37–7.23 (m, Ph), 5.15–5.07 (m, $CO_2C\underline{H}_2Ph$), 4.97-4.89 (m,

C$\underline{H}_2$Ph), 4.86-4.84 (m, H-1 (α)), 4.82-4.49 (m, C$\underline{H}_2$Ph), 4.46 (d, *J* = 7.6 Hz, H-1 (β)), 4.19-4.12 (m, H-5, C$\underline{H}$CH$_3$), 4.10-3.96 (m, H-2, H-3, H-6), 3.87 (bs, H-4), 1.93 (s, Ac (β)), 1.92 (s, Ac (α)), 1.50 (d, *J* = 6.9 Hz, CHC$\underline{H}_3$ (β)), 1.44 (d, *J* = 6.8 Hz, CHC$\underline{H}_3$ (α)) ppm. **^{13}C NMR** (CDCl$_3$): δ 172.2, 170.4, 138.7, 138.5, 138.2, 128.2-127.4, 102.6 (C-1(β)), 98.0 (C-1 (α)), 81.9 (β), 79.1 (β), 78.9 (α), 76.3 (α), 74.9 (β), 74.6 (α), 74.5 (α), 74.2 (β), 73.7 (β), 73.5 (α), 73.2, 72.9 (β), 72.4 (β), 72.2 (β), 68.9, 66.6 (α), 66.5 (β), 63.0 (α), 62.9 (β), 20.8, 17.8 ppm.

Experiment 19. Synthesis of Methyl 2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)acetate (34)

The glycosylation reaction of donor **19** with acceptor **7** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1743 (C=O) cm^{-1}. **^{1}H NMR** (CDCl$_3$): δ 7.43-7.26 (m, Ph), 5.08 (d, *J* = 10.0 Hz, C$\underline{H}_2$Ph), 5.05 (d, *J* = 3.6 Hz, H-1 (α)) 4.99-4.91 (m, C$\underline{H}_2$Ph), 4.85-4.72 (m, C$\underline{H}_2$Ph), 4.67-4.60 (m, C$\underline{H}_2$Ph), 4.48 (d, *J* = 7.6 Hz, H-1 (β)), 4.39-4.26 (m, C$\underline{H}_2$CO$_2$Me), 4.22-3.98 (m, H-6 (α), H-6 (β), C$\underline{H}_2$CO$_2$Me, H-2 (α), H-6 (β), H-6 (α), H-5 (α), H-3 (α)), 3.93–3.88 (m, H-2 (β), H-4 (α)), 3.77 (bs, H-4 (β), OMe (β)), 3.75 (bs, OMe (α)), 3.56–3.48 (m, H-3 (β), H-5 (β)), 1.98 (s, Ac (α)), 1.97 (s, Ac (β)) ppm. **^{13}C NMR** (CDCl$_3$): δ 170.5, 170.2, 138.7, 138.3, 138.1, 138.0, 128.5–128.1, 103.3 (C-1 (β)), 97.3 (C-1 (α)), 81.8 (β), 79.0 (β), 78.6 (α), 76.0 (α), 75.0 (β), 74.7 (α), 74.6 (α), 74.3 (β), 73.7 (α), 73.6 (β), 73.1 (α), 72.9 (β), 72.2 (β), 69.1 (α), 65.5 (β), 63.6 (α), 63.4 (α), 63.0 (β), 51.9, 20.8 ppm.

Experiment 20. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(*2R*)-2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)-2,3-dihydroxypropanoate (35)

The glycosylation reaction of donor **19** with acceptor **9** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **^{1}H NMR** (CDCl$_3$): δ 7.65-7.61 (m), 7.45-7.24 (m), 5.23 (d, *J* = 3.4 Hz, H-1 (α)), 4.97-4.58 (m), 4.51 (dd, *J* = 6.8 Hz, *J* = 4.8

Hz), 4.43 (d, *J* = 7.7 Hz, H-1 (β), 4.28-4.23 (m), 4.14-3.86 (m), 3.79 (s), 3.71 (s), 1.97 (s), 1.86 (s), 1.03 (s), 1.01 (s) ppm. **^{13}C NMR** (CDCl3): δ 170.8, 170.6, 138.5, 138.3, 138.2, 135.6, 135.5, 129.8, 129.7, 128.5-127.4, 103.9 (C-1 (β)), 82.0 (C-1 (α)), 79.7, 79.0, 75.1, 74.3, 73.4, 73.1, 72.4, 63.6, 63.1, 51.9, 26.6, 20.8, 19.1 ppm.

Experiment 21. Synthesis of Methyl (*2S*)-2-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranosyl)propanoate (36)

The glycosylation reaction of donor **21** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1751 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.44-7.25 (m, Ph), 5.12 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph (β)), 4.98-4.94 (m, C$\underline{H}_2$Ph), 4.91-4.82 (m, C$\underline{H}_2$Ph), 4.82 (d, *J* = 3.9 Hz, H-1 (α)), 4.79-4.70 (m, C$\underline{H}_2$Ph), 4.68-4.59 (m, C$\underline{H}_2$Ph), 4.45 (d, *J* = 7.6 Hz, H-1 (β)), 4.27 (dd, *J* = 6.6 Hz, *J* = 11.0 Hz, H-6 (β)), 4.20 (t, *J* = 6.2 Hz), 4.13-3.96 (m), 3.93-3.84 (m), 3.74-3.69 (m), 3.70 (s, OMe (β)), 3.69 (s, OMe (α)), 3.53-3.49 (m), 1.48 (d, *J* = 7.0 Hz, CHC$\underline{H}_3$ (β)), 1.42 (d, *J* = 6.8 Hz, CHC$\underline{H}_3$ (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.9 (C$\underline{O}_2$Me), 166.9 (C$\underline{O}$CH$_2$Cl (α)), 166.8 (C$\underline{O}$CH$_2$Cl (β)), 138.7, 138.6, 138.5, 138.4, 138.1, 138.0, 128.8-127.5, 102.6 (C-1 (β)), 98.1 (C-1 (α)), 81.8, 79.0, 78.8, 78.5, 76.3, 75.3, 74.9 (C$\underline{H}_2$Ph(β)), 74.4 (C$\underline{H}_2$Ph (α)), 74.3, 74.1 (C$\underline{H}_2$Ph (β)), 73.8 (C$\underline{H}_2$Ph (β)), 73.6 (C$\underline{H}_2$Ph (α)), 73.54 (C$\underline{H}_2$Ph (α)), 73.52, 72.55, 72.52, 71.8, 70.4, 68.3, 64.7 (C-6 (α)), 64.3 (C-6 (β)), 52.1 (OMe (α)), 52.0 (OMe (β)), 40.7 (CO$\underline{C}$H$_2$Cl (α)), 40.5 (CO$\underline{C}$H$_2$Cl (β)), 19.0 (CH$\underline{C}$H$_3$ (β)), 17.8 (CH$\underline{C}$H$_3$ (α)) ppm. **HR-MS:** calcd for $C_{33}H_{37}ClO_9Na^+$ [M+Na]$^+$: 635.2018; found: 635.2018.

Experiment 22. Synthesis of Methyl 2-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranosyl)acetate (37)

The glycosylation reaction of donor **21** with acceptor **7** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1755 (C=O) cm^{-1}. **^{1}H NMR**

($CDCl_3$): δ 7.42-7.25 (m, Ph), 5.07 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (β)), 5.02 (d, *J* = 3.3 Hz, H-1 (α)), 4.99-4.91 (m, C$\underline{H}_2$Ph), 4.86-4.72 (m, C$\underline{H}_2$Ph), 4.66-4.60 (m, C$\underline{H}_2$Ph), 4.48 (d, *J* = 7.6 Hz, H-1 (β)), 4.39-4.18 (m), 4.11-3.97 (m), 3.95-3.87 (m), 3.74 (s, OMe), 3.55-3.52 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.2 ($\underline{C}O_2$Me (α)), 169.9 ($\underline{C}O_2$Me (β)), 166.85 ($\underline{C}OCH_2Cl$ (α)), 166.83 ($\underline{C}OCH_2Cl$ (β)), 138.7, 138.6, 138.3, 138.2, 138.1, 138.0, 128.6-127.5, 103.3 (C-1 (β)), 97.5 (C-1 (α)), 81.7, 79.0, 78.5, 75.9, 75.0 ($\underline{C}H_2$Ph (β)), 74.5 ($\underline{C}H_2$Ph (α)), 74.4, 74.2 ($\underline{C}H_2$Ph (β)), 73.8 ($\underline{C}H_2$Ph (α)), 73.7 ($\underline{C}H_2$Ph (β)), 73.2 ($\underline{C}H_2$Ph (α)), 72.6, 71.9, 68.8, 65.5 ($\underline{C}H_2CO_2$Me (β)), 64.9 (C-6 (α)), 64.4 (C-6 (β)), 63.9 ($\underline{C}H_2CO_2$Me (α)), 51.9 (OMe), 40.6 ($CO\underline{C}H_2Cl$ (α)), 40.5 ($CO\underline{C}H_2Cl$ (β)) ppm. **HR-MS:** calcd for $C_{32}H_{35}ClO_9Na^+$ $[M+Na]^+$: 621.1862; found: 621.1852.

Experiment 23. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(2*R*)-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranosyl)-2,3-dihydroxypropanoate (38)

The glycosylation reaction of donor **21** with acceptor **9** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1743, 1749 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.66-7.61 (m, Ph), 7.45-7.25 (m, Ph), 5.22 (d, *J* = 3.5 Hz, H-1 (α)), 4.96-4.81 (m, C$\underline{H}_2$Ph), 4.78-4.66 (m, C$\underline{H}_2$Ph), 4.61-4.56 (m, C$\underline{H}_2$Ph), 4.50 (dd, *J* = 3.8 Hz, *J* = 7.0 Hz, C$\underline{H}CO_2$Me), 4.41 (d, *J* = 7.7 Hz, H-1 (β)), 4.26-4.20 (m), 4.17-4.09 (m), 4.06-3.85 (m), 3.79-3.68 (m), 3.79 (s, OMe (β)), 3.72 (s, OMe (α)), 3.49-3.44 (m), 1.03 (s, *t*-Bu (α)), 1.00 (s, *t*-Bu (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.2 ($\underline{C}O_2$Me), 166.6 ($\underline{C}OCH_2Cl$), 138.8, 138.5, 138.1, 135.6-135.5, 133.1, 132.9, 132.8, 129.8, 128.5-127.5, 104.0 (C-1 (β)), 95.5 (C-1 (α)), 81.8, 79.8, 78.9, 78.4, 75.9, 75.1 ($\underline{C}H_2$Ph (β)), 74.6 ($\underline{C}HCO_2$Me), 74.4 ($\underline{C}H_2$Ph (α)), 74.3, 74.2 ($\underline{C}H_2$Ph (β)), 73.8 ($\underline{C}H_2$Ph (α)), 73.5 ($\underline{C}H_2$Ph (β)), 72.8, 72.2 ($\underline{C}H_2$Ph (α)), 72.0, 68.3, 64.7, 64.6, 64.5, 63.6, 52.0 (OMe (α)), 51.9 (OMe (β)), 40.6 ($CO\underline{C}H_2Cl$ (β)), 40.4 ($CO\underline{C}H_2Cl$ (α)), 26.7 (*t*-Bu), 19.2 ($Si\underline{C}(CH_3)_3$) ppm. **HR-MS:** calcd for $C_{49}H_{55}ClO_{10}SiNa^+$ $[M+Na]^+$: 889.3145; found: 889.3158.

Experiment 24. Synthesis of Methyl 1,3-di-*O*-*tert*-butyldimethylsilyl-(2*R*)-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranosyl)-1,2,3-trihydroxypropyl (39)

The glycosylation reaction of donor **21** with acceptor **22** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1746, 1763 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.66-7.57 (m), 7.40-7.11 (m), 5.11 (d, *J* = 3.4 Hz, H-1 (α)), 4.96-4.79 (m, C$\underline{H}_2$Ph), 4.73-4.56 (m, C$\underline{H}_2$Ph), 4.45 (d, *J* = 7.7 Hz, H-1 (β)), 4.08-3.72 (m), 3.69 (bs, H-4 (β)), 3.60 (dd, *J* = 15.0 Hz, *J* = 30.0 Hz, AcCl), 3.42 (dd, *J* = 2.8 Hz, *J* = 9.7Hz, H-3 (β)), 3.34 (dd, J=6.3 Hz, J=6.7 Hz, H-5 (β)), 1.03 (s, *t*-Bu), 1.02 (s, *t*-Bu), 1.00 (s, *t*-Bu), 0.98 (s, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.5 ($\underline{C}OCH_2Cl$), 138.8, 138.46, 138.41, 138.2, 138.1, 135.6-135.5, 133.7-133.0, 129.8-129.5, 128.6-127.5, 103.2 (C-1 (β)), 96.2 (C-1 (α)), 81.9, 80.7, 79.4, 78.7, 77.2, 76.4, 74.9 (C$\underline{H}_2$Ph (β)), 74.3 (C$\underline{H}_2$Ph (α)), 74.2, 73.7 (C$\underline{H}_2$Ph (α)), 73.6 (C$\underline{H}_2$Ph (β)), 72.9 (C$\underline{H}_2$Ph (α)), 72.8, 71.4, 67.7, 64.6, 64.2, 63.9, 63.7, 63.2, 62.9, 40.5 (CO$\underline{C}H_2$Cl (β)), 40.4 (CO$\underline{C}H_2$Cl (α)), 26.9 (*t*-Bu), 26.84 (*t*-Bu), 26.82 (*t*-Bu), 26.7 (*t*-Bu), 19.3 (Si$\underline{C}(CH_3)_3$), 19.2 (Si$\underline{C}(CH_3)_3$), 19.1 (Si$\underline{C}(CH_3)_3$) ppm.

Experiment 25. Synthesis of Methyl 15-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranosyl)decapentanoate (40)

The glycosylation reaction of donor **21** with acceptor **23** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1736 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.26 (m, Ph), 4.98-4.89 (m, C$\underline{H}_2$Ph), 4.85-4.80 (m, C$\underline{H}_2$Ph), 4.80 (d, *J* = 3.2 Hz, H-1 (α)), 4.77-4.73 (m, C$\underline{H}_2$Ph), 4.67-4.60 (m, C$\underline{H}_2$Ph), 4.33 (d, *J* = 7.6 Hz, H-1 (β)), 4.29 (dd, *J* = 6.4 Hz, *J* = 11.0 Hz, H-6 (β)), 4.22 (dd, *J* = 7.1 Hz, *J* = 11.1 Hz, H-6 (α)), 4.10 (dd, *J* = 6.4 Hz, *J* = 11.0 Hz, H-6 (β)), 4.07-4.01 (m, H-6 (α), H-2 (α)), 3.96-3.81 (m), 3.75 (d, *J* = 2.1 Hz, H-4 (β)), 3.66 (s, OMe), 3.62-3.40 (m), 2.29 (t, *J* = 7.5 Hz), 1.67-1.55 (m), 1.39-1.24 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 174.5 ($\underline{C}O_2$Me (α)), 174.3 ($\underline{C}O_2$Me (β)),

166.82 (COCH$_2$Cl (α)), 166.80 (COCH$_2$Cl (β)), 138.7, 138.6, 138.5, 138.4, 138.1, 128.6-127.5, 103.9 (C-1(β)), 97.4 (C-1(α)), 82.1, 79.4, 78.9, 76.6, 75.2 (CH$_2$Ph (β)), 74.4 (CH$_2$Ph (α)), 74.3, 74.1 (CH$_2$Ph (β)), 73.6 (CH$_2$Ph (α)), 73.5(CH$_2$Ph (β)), 73.3 (CH$_2$Ph (α)), 72.5, 71.5, 70.1, 68.4, 67.9, 65.1 (C-6 (α)), 64.4 (C-6 (β)), 51.4 (OMe), 40.6 (COCH$_2$Cl), 34.1, 29.7, 29.64, 29.61, 29.49, 29.46, 29.40, 29.2, 29.1, 26.2, 26.1, 24.9 ppm.

Experiment 26. Synthesis of Methyl 3-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (41)

The glycosylation reaction of donor **21** with acceptor **24** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1751 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.70-7.66 (m, Ph), 7.43-7.08 (m, Ph), 5.23 (d, *J* = 3.4 Hz, H-1 Glu (β,α)), 5.15 (d, *J* = 3.5 Hz, H-1 Glu (α,α)), 4.99-4.89 (m, CH$_2$Ph), 4.93 (d, *J* = 5.7 Hz, H-1 Gal (α,α)), 4.86-4.80 (m, CH$_2$Ph), 4.78-4.46 (m, CH$_2$Ph), 4.26-4.19 (m), 4.20 (d, *J* = 7.8 Hz, H-1 Gal (β,α)), 4.15-3.42 (m), 3.72 (s, OMe (α,α)), 3.57 (s, OMe (β,α)), 1.02 (s, *t*-Bu (α,α)), 1.01 (s, *t*-Bu (β,α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.3 (CO$_2$Me), 170.2 (CO$_2$Me), 166.8 (COCH$_2$Cl), 166.7 (COCH$_2$Cl), 139.0-138.1, 135.7, 135.6, 133.1-132.9, 129.8, 129.7, 128.5-127.2, 104.0 (C-1 Gal (β)), 97.9 (C-1 Gal (α)), 94.5 (C-1 Glu (β,α)), 94.4 (C-1 Glu (α,α)), 82.1, 81.7, 81.6, 79.6, 79.2, 79.0, 78.3, 77.6, 77.5, 76.5, 75.7 (CH$_2$Ph), 75.6 (CH$_2$Ph), 75.3 (CH$_2$Ph), 74.7 (CH$_2$Ph), 74.6 (CH$_2$Ph), 74.5, 74.47, 74.43 (CH$_2$Ph), 74.40, 74.2 (CH$_2$Ph), 73.5 (CH$_2$Ph), 73.4 (CH$_2$Ph), 72.9, 72.5 (CH$_2$Ph), 71.9 (CH$_2$Ph), 71.8 (CH$_2$Ph), 71.7, 70.8, 70.1, 68.3, 68.2, 66.2, 65.1, 64.8, 64.4, 52.0 (OMe (α,α)), 51.8 (OMe (β,α)), 40.6 (COCH$_2$Cl), 40.5 (COCH$_2$Cl), 26.7 (*t*-Bu), 19.2 (SiC(CH$_3$)$_3$) ppm.

Experiment 27. Synthesis of 3-*O*-(2,3,4-tri-O-benzyl-6-O-chloroacetyl-α/β-D-galactopyranosyl)-epiandrosterone (42)

The glycosylation reaction of donor **21** with acceptor **25** was performed according to the procedure described in experiment 4. The results are

presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1737 (C=O st) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.27 (m, Ph), 4.98-4.92 (m, C$\underline{H}_2$Ph), 4.89 (d, *J* = 3.7 Hz, H-1 (α)), 4.89-4.72 (m, C$\underline{H}_2$Ph), 4.68-4.60 (m, C$\underline{H}_2$Ph), 4.45 (d, *J* = 7.7 Hz, H-1 (β)), 4.29 (dd, *J* = 6.6 Hz, *J* = 11.0 Hz, H-6 (β)), 4.23-4.18 (m, H-6 (α)), 4.11-4.00 (m), 3.96-3.95 (m), 3.92 (d, *J* = 5.3 Hz, AcCl (α)), 3.88 (d, *J* = 5.1 Hz, AcCl (β)), 3.86-3.80 (m, H-2 (β), H-4 (α)), 3.74 (d, *J* = 2.3 Hz, H-4 (β)), 3.65-3.57 (m), 3.53-3.47 (m), 2.43 (dd, *J* = 8.7 Hz, *J* = 19.1 Hz), 2.13-2.01 (m), 1.95-1.88 (m), 1.84-1.19 (m), 1.14-1.03 (m), 1.01-0.90 (m), 0.84 (d, *J* = 9.7 Hz), 0.67 (td, *J* = 3.4 Hz, *J* = 11.4 Hz) ppm. **^{13}C NMR** ($CDCl_3$): δ 165.8 (C$\underline{O}$OCH$_2$Cl), 137.7, 137.5, 137.1, 127.5-126.4, 94.5 (C-1 (α)), 78.0, 75.4, 75.3, 72.6 (C$\underline{H}_2$Ph (α)), 72.2 (C$\underline{H}_2$Ph (α)), 66.9, 64.3 (C-6 (α)), 53.4, 50.3, 46.7, 44.2, 39.5 (CO$\underline{C}$H$_2$Cl (α), 35.8, 34.8, 34.7, 34.0, 30.5, 29.8, 27.4, 26.3, 20.7, 19.4, 12.7, 11.3 ppm.

Experiment 28. Synthesis of 1-*O*-Adamantanyl-2,3,4-tri-O-benzyl-6-O-chloroacetyl-α/β-D-galactopyranoside (43)

The glycosylation reaction of donor **21** with acceptor **26** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1741, 1743 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.25 (m, Ph), 5.31 (d, *J* = 3.4 Hz, H-1 (α)), 4.99-4.94 (m, C$\underline{H}_2$Ph), 4.88 (d, *J* = 11.6 Hz, C$\underline{H}_2$Ph (α)), 4.82 (d, *J* = 11.7 Hz, C$\underline{H}_2$Ph (β)), 4.76-4.70 (m, C$\underline{H}_2$Ph), 4.67-4.59 (m, C$\underline{H}_2$Ph), 4.60 (d, *J* = 7.6 Hz, H-1 (β)), 4.28 (dd, *J* = 7.1 Hz, *J* = 11.0 Hz, H-6 (β)), 4.28 (dd, *J* = 7.1 Hz, *J* = 10.5 Hz, H-6 (α)), 4.15 (t, *J* = 5.3 Hz ou 6.96, H-5 (α)), 4.07-3.95 (m, H-6 (β), H-6 (α), H-2 (α), H-3 (α)), 3.92-3.87 (m, AcCl (α), AcCl (β), H-4 (α)), 3.81 (t, *J* = 9.6 ou 7.9 Hz, H-2 (β)), 3.72 (d, *J* = 2.4 Hz, H-4 (β)), 3.53-3.49 (m, H-5 (β), H-3 (α)), 2.12 (bs), 1.91-1.76 (m), 1.65-1.57 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 165.8 (C$\underline{O}$OCH$_2$Cl), 137.8, 137.7, 137.5, 137.4, 137.3, 137.1, 127.7-127.2, 126.9-126.4, 95.5 (C-1 (β)), 89.5 (C-1 (α)), 81.5 (C-3 (β)), 78.4 (C-2 (β)), 78.0 (C-3 (α)), 75.4 (C-2 (α)), 74.2 (C$\underline{H}_2$Ph (β)), 74.1, 73.5 (C-4 (α)), 73.4, 73.3 (C$\underline{H}_2$Ph (α)), 73.0 (C$\underline{H}_2$Ph (β)), 72.6 (C$\underline{H}_2$Ph (β)), 72.3 (C$\underline{H}_2$Ph (α)), 72.0 (C$\underline{H}_2$Ph (α)),

71.6 (C-4 (β)), 70.3 (C-5 (β)), 66.5 (C-5 (α)), 64.2 (C-6 (α)), 63.7 (C-6 (β)), 41.6, 41.4, 39.6, 39.5, 35.2, 35.2 ($CO\underline{C}H_2Cl$ (β)), 29.6 ($\underline{C}H(CH_2)_3$) ppm.

Experiment 29. Synthesis of Benzyl (2*S*)-2-(2,3,4-tri-O-benzyl-6-O-chloroacetyl-α-D-galactopyranosyl)-2-phenylacetate (44)

The glycosylation reaction of donor **21** with acceptor **27** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. $[\alpha]_D^{20}$ + 72.9 (c 0.82, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1748 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.52-7.49 (m, 2H, Ph), 7.43-7.18 (m, 23H, Ph), 5.15 (s, 1H, $C\underline{H}CO_2Bn$), 5.12 (dd, 1H, *J* = 6.5 Hz, *J* = 16.5 Hz, $CO_2C\underline{H}_2Ph$), 4.96 (d, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.92 (d, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.87 (d, *J* = 3.6 Hz, H-1(α)), 4.78 (d, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.66 (d, *J* = 11.7 Hz, $C\underline{H}_2Ph$), 4.60 (d, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.48 (d, *J* = 11.7 Hz, $C\underline{H}_2Ph$), 4.18-4.10 (m, 3H, H-3, H-5, H-6), 4.05-3.99 (m, 2H, H-2, H-6), 3.91-3.82 (m, 3H, H-4, AcCl) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.9 ($\underline{C}O_2Bn$), 166.8 ($\underline{C}OCH_2Cl$), 138.6, 138.2, 138.1, 135.5, 135.4, 128.8-128.2, 127.9-127.5, 96.4 (C-1(α)), 78.7 (C-3), 77.4 ($\underline{C}HCO_2Bn$), 76.2 (C-2), 74.4 ($\underline{C}H_2Ph$), 74.3 (C-4), 73.6 ($\underline{C}H_2Ph$), 73.1 ($\underline{C}H_2Ph$), 68.9 (C-5), 66.8 ($CO_2\underline{C}H_2Ph$), 64.8 (C-6), 40.6 ($CO\underline{C}H_2Cl$) ppm. **HR-MS:** calcd for $C_{44}H_{43}ClO_9Na^+$ $[M+Na]^+$: 773.2493; found: 773.2485.

Experiment 30. Synthesis of 1-ω-Methoxy-PEG$_{550}$yl-2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranoside (45)

The glycosylation reaction of donor **21** with acceptor **28** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1715, 1757 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.26 (m, Ph), 4.98-4.94 (m, $C\underline{H}_2Ph$), 4.91-4.88 (m, $C\underline{H}_2Ph$), 4.90 (d, *J* = 3.5 Hz, H-1 (α)), 4.84–4.60 (m, $C\underline{H}_2Ph$), 4.40 (d, *J* = 7.6 Hz, H-1 (β)), 4.28 (dd, *J* = 6.6 Hz, *J* = 11.0 Hz), 4.21 (dd, *J* = 7.0 Hz, *J* = 11.0 Hz), 4.12-3.82 (m), 3.77-3.50 (m), 3.37 (s, OMe) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.88 ($\underline{C}OCH_2Cl$), 166.81 ($\underline{C}OCH_2Cl$), 138.7, 138.5, 138.3, 138.1, 138.0, 128.6-128.1, 127.9-127.5, 104.0 (C-1 (β)), 97.6 (C-1 (α)), 81.9, 79.2, 78.8, 76.4, 75.0, 74.4, 74.3, 73.6, 73.5, 73.2, 72.6, 71.9, 71.6, 70.5, 70.4, 70.3,

70.2, 68.9, 68.0, 66.9, 64.9, 64.4, 59.0 (OMe), 40.7 ($CO\underline{C}H_2Cl$), 40.6 ($CO\underline{C}H_2Cl$) ppm. **HR-MS:** calcd for $C_{54}H_{81}ClO_{19}Na^+$ $[M+Na]^+$: 1091.4958; found: 1091.4934.

Experiment 31. Synthesis of *O*-1-$_L$-Menthyl-2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranoside (46)

The glycosylation reaction of donor **21** with acceptor **29** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1762 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.27 (m, Ph), 5.01 (d, *J* = 3.5 Hz, H-1 (α)), 4.97 (d, *J* = 11.8 Hz, $C\underline{H}_2Ph$ (β)), 4.94 (d, *J* = 10.8 Hz, $C\underline{H}_2Ph$ (α)), 4.88-4.64 (m, $C\underline{H}_2Ph$), 4.61 (d, *J* = 11.6 Hz, $C\underline{H}_2Ph$ (α)), 4.37 (d, *J* = 7.7 Hz, H-1 (β)), 4.27-4.22 (m, H-6 (α), H-6 (β)), 4.10-4.01 (m), 3.98-3.91 (m), 3.87 (dd, *J* = 14.8 Hz, *J* = 18.4 Hz, AcCl), 3.77 (dd, *J* = 7.8 Hz, *J* = 11.0 Hz, H-2 (β)), 3.72 (d, *J* = 2.3 Hz, H-4 (β)), 3.53-3.48 (m), 3.38 (td, *J* = 4.2 Hz, *J* = 10.6 Hz, $C\underline{H}O$ menthol (β)), 3.32 (td, *J* = 4.3 Hz, *J* = 10.6 Hz, $C\underline{H}O$ menthol (α)), 2.43-2.32 (m, $C\underline{H}(CH_3)_2$ menthol), 2.12-2.09 (m, $CHC\underline{H}_2CH$ menthol (β)), 2.05-2.02 (m, $CHC\underline{H}_2CH$ menthol (α)), 1.67-1.56 (m, $C\underline{H}_2C\underline{H}_2$ menthol), 1.41-1.21 (m, $C\underline{H}CH_3$, $C\underline{H}CH(CH_3)_2$ menthol), 1.03-1.75 (m), 0.83 (d, *J* = 7.1 Hz), 0.72 (d, *J* = 6.8 Hz), 0.68 (d, *J* = 6.9 Hz) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.9 ($\underline{C}OCH_2Cl$), 166.8 ($\underline{C}OCH_2Cl$), 138.7, 138.68, 138.61, 138.5, 138.25, 138.23, 128.6-128.2, 127.8-127.4, 101.9 (C-1 (β)), 99.3 (C-1 (α)), 82.5, 80.6, 79.3, 78.96, 78.92, 76.8, 75.2, 74.5, 74.4, 74.1, 73.7, 73.6, 73.1, 73.0, 71.5, 68.2, 65.4, 64.8, 48.8 ($\underline{C}HCH(CH_3)_2$ menthol (α)), 47.9 ($\underline{C}HCH(CH_3)_2$ menthol (β)), 42.9 ($CHC\underline{H}_2CH$ menthol (α)), 41.2 ($CHC\underline{H}_2CH$ menthol (β)), 40.63 ($CO\underline{C}H_2Cl$ (α)), 40.62 ($CO\underline{C}H_2Cl$ (β)), 34.4, 34.2, 31.7 ($\underline{C}HCH_3$ menthol (α)), 31.5 ($\underline{C}HCH_3$ menthol (β)), 24.8 ($\underline{C}H(CH_3)_2$ menthol (β)), 24.5 ($\underline{C}H(CH_3)_2$ menthol (α)), 23.0, 22.9, 22.4, 22.2, 21.1, 16.0, 15.5 ppm.

Experiment 32. Synthesis of *O*-1-Cyclohexyl-2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranoside (47)

The glycosylation reaction of donor **21** with acceptor **30** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1759, 1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.21 (m, Ph), 4.98 (s, H-1 (α)), 4.98-4.73 (m, C$\underline{H}_2$Ph), 4.68-4.60 (m, C$\underline{H}_2$Ph), 4.44 (d, *J* = 7.6 Hz, H-1 (β)), 4.32-4.27 (m, H-6 (β)), 4.23-4.19 (m, H-6 (β)), 4.09-4.01 (m), 3.96-3.80 (m), 3.73 (s, H-4 (β)), 3.70-3.62 (m), 3.52-3.50 (m), 1.95-1.89 (m), 1.82-1.73 (m), 1.54-1.12 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.8 ($\underline{C}OCH_2Cl$), 166.7 ($\underline{C}OCH_2Cl$), 138.8, 138.7, 138.6, 138.4, 138.2, 138.1, 128.7-128.2, 127.9-127.5, 102.2 (C-1 (β)), 95.6 (C-1 (α)), 82.3 (C-3 (β)), 79.4 (C-2 (β)), 79.0 (C-3 (α)), 77.8, 76.5 (C-2 (α)), 75.8, 75.2 ($\underline{C}H_2$Ph), 74.4 (C-4 (α)), 74.1 ($\underline{C}H_2$Ph), 73.6 ($\underline{C}H_2$Ph), 73.1 ($\underline{C}H_2$Ph), 72.5 (C-4 (β)), 71.5 (C-5 (β)), 67.9 (C-5 (α)), 65.3 (C-6 (α)), 64.5 (C-6 (β)), 40.6 (CO$\underline{C}H_2$Cl), 33.6, 33.3, 31.9, 31.6, 25.6, 25.5, 24.5, 24.2, 24.0, 23.9 ppm.

Experiment 33. Synthesis of 3-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-galactopyranosyl)-*N*-Boc-L-ser-methyl ester (48)

The glycosylation reaction of donor **21** with acceptor **31** was performed according to the procedure described in experiment 4. The results are presented in Table 3, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1714, 1747 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.25 (m, Ph), 5.73 (d, *J* = 8.6 Hz, N$\underline{H}$Boc (α)), 5.46 (d, *J* = 7.8 Hz, N$\underline{H}$Boc (β)), 4.96 (d, *J* = 11.5 Hz, C$\underline{H}_2$Ph), 4.87-4.83 (m, C$\underline{H}_2$Ph), 4.80-4.72 (m, C$\underline{H}_2$Ph), 4.76 (s, H-1 (α)), 4.65-4.59 (m, C$\underline{H}_2$Ph), 4.45-4.43 (m, C$\underline{H}$NHBoc), 4.32 (d, *J* = 7.6 Hz, H-1 (β)), 4.29-4.19 (m), 4.15-3.96 (m), 3.92-3.73 (m), 3.74 (s, OMe (β)), 3.66 (s, OMe (α)), 3.53-3.50 (m), 1.43 (s, *t*-Bu (α)), 1.42 (s, *t*-Bu (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.8 ($\underline{C}O_2$Me (α)), 170.7 ($\underline{C}O_2$Me (β)), 167.0 ($\underline{C}OCH_2Cl$ (α)), 166.8 ($\underline{C}OCH_2Cl$ (β)), 155.5 (NH$\underline{C}O_2$*t*-But), 138.4, 138.37, 138.30, 138.1, 137.9, 128.6-128.2, 127.9-127.5, 104.3 (C-1 (β)), 99.7 (C-1 (α)), 81.9, 78.9, 78.5, 76.3, 75.4, 74.5, 74.3, 74.2, 73.5, 73.4, 72.5, 71.8, 71.1, 69.0, 65.0 (C-6 (α)), 64.3 (C-6 (β)), 54.2 ($\underline{C}$HNHBoc

(α)), 53.9 (CHNHBoc (β)), 52.6 (OMe (β)), 52.4 (OMe (α)), 40.7 ($CO\underline{C}H_2Cl$ (α)), 40.6 ($CO\underline{C}H_2Cl$ (β)), 28.3 (*t*-Bu) ppm.

Experiment 34. Synthesis of Ethyl 6-*O*-trityl-1-thio-β-D-glucopyranoside (49)

To a suspension of D-galactose (2.0 g, 11.1 mmol) in pyridine (10 mL) at 0ºC was added acetic anhydride (10.6 mL, 0.11 mol) and a catalytic amount of DMAP. The reaction mixture was stirred at r.t. overnight, then poured into saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (3x20 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under vacuum to afford the pentaacetate (4.29 g, 99%) as a very viscous gum.

To a stirred solution of galactose pentaacetate (2.5 g, 6.39 mmol) in ethanethiol (12.5 mL, 0.166 mol) was added zinc chloride (1.22 g, 8.95 mmol) at 0ºC. The reaction mixture was kept at 0ºC overnight, and then diluted with CH_2Cl_2 (10 mL) and saturated aqueous $NaHCO_3$ solution was added. After extraction with CH_2Cl_2 (2X40 mL) the combined organic extracts were dried ($MgSO_4$), filtered and concentrated in vacuum. The residue was purified by flash column chromatography (30:70, EtOAc/Hex) to give the β-thiogalactoside (1.2 g, 48%) as a viscous colourless foam, and the initial pentaacetate (1.6 g, 64%).

A solution of NaOMe 1N (1.94 mL, 1.94 mmol) in MeOH was added to a stirred solution of the β-thiogalactoside (1.13 g, 2.89 mmol) in MeOH (8.7 mL) at 0ºC. After 1 hour the reaction mixture was neutralized with Dowex-H^+ resin. Filtration and evaporation of solvents afforded the product as viscous colourless foam (0.65 g, 100%).

The tetrol (0.2 g, 0.89 mol) and trityl chloride (0.31 g, 1.11 mmol) were dissolved in pyridine (3 mL) and stirred under argon. After 24 hours DMAP (13.0 mg, 0.11 mmol) and trityl chloride (0.25 g, 0.89 mmol) were added and the mixture stirred for a further 12 hours. The reaction mixture was concentrated in vacuum and the residue dissolved in dichloromethane (5 mL), washed with a saturated aqueous NH_4Cl solution (2x3 mL) and a saturated aqueous $NaHCO_3$ solution (2x3 mL). The organic layer was then dried ($MgSO_4$) and concentrated under vacuum to afford the triol **49** (0.34 g, 81%).

as a viscous colourless foam. **^{1}H NMR** (DMSO): δ 7.44-7.42 (m, 6H, Ph), 7.34-7.30 (m, 6H, Ph), 7.26-7.23 (m, 3H, Ph), 4.99-4.98 (m, 1H), 4.87-4.86 (m, 1H), 4.47-4.46 (m, 1H), 4.34-4.32 (m, 1H), 3.65-3.3.63 (m, 1H), 3.28-3.24 (m, 1H, H-6), 3.01-3.00 (m, 1H, H'-6), 2.78-2.71 (m, 1H, $SC\underline{H}_2CH_3$), 2.67-2.60 (m, 1H, $SC\underline{H}_2CH_3$), 1.26 (t, 3H, *J* = 7.6 Hz, $SCH_2C\underline{H}_3$). **^{13}C NMR** (DMSO): δ 144.4, 128.7, 128.3, 127.4, 86.2 ($\underline{C}Ph_3$), 85.5 (C-1), 78.0, 75.0, 70.1, 69.6, 64.3 (C-6), 23.5 ($S\underline{C}H_2CH_3$), 15.7 ($SCH_2\underline{C}H_3$) ppm.

Experiment 35. Synthesis of Ethyl 2,3,4-tetra-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-1-thio-β-D-galactopyranoside (50)

Thiogalactoside **49** (0.16 g, 0.35 mmol) and a catalytic amount of DMAP were dissolved in pyridine (4 mL), and the solution was cooled at 0ºC. Then *tert*-butyldimethyl silyl triflate (0.49 mL, 2.14 mmol) was added, and the solution stirred for 30 minutes at 0ºC and then for 24 hours at 60ºC. MeOH (1.5 mL) was added, the reaction mixture was diluted with EtOAc (4 mL). After washing the reaction mixture with an aqueous HCl 10% solution, the aqueous phase was extracted with EtOAc. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under vacuum. The crude product was purified by preparative TLC (10:90, EtOAc/Hex) to give the fully protected compound **50** (0.24 g, 83%) as a very viscous gum. **^{1}H NMR** ($CDCl_3$, 400 MHz, 243K): δ 7.76-7.67 (m, 6H, Ph), 7.59-7.50 (m, 9H, Ph), 4.53 (d, 1H *J* = 8.8 Hz, H-1), 4.41-4.36 (m, 1H), 4.13 (d, 1H, *J* = 8.8 Hz), 3.83 (d, *J* = 8.9 Hz, 1H), 3.74-3.71 (m, 2H), 3.34-3.29 (m, 1H, H-6), 3.03-2.96 (m, 1H, $SC\underline{H}_2CH_3$), 2.89-2.82 (m, 1H, $SC\underline{H}_2CH_3$), 1.52 (m, 3H, $SCH_2C\underline{H}_3$), 1.26 (s, 9H, *t*-Bu), 1.18 (s, 9H, *t*-Bu), 0.98 (s, 9H, *t*-Bu), 0.44 (s, 6H, $SiMe_2$), 0.41 (s, 6H, $SiMe_2$), 0.34 (s, 6H, $SiMe_2$) ppm. **^{13}C NMR** ($CDCl_3$, 100 MHz, 243K): δ 143.8, 128.7, 128.0, 127.2, 86.63 (CPh_3), 86.52 (C-1), 77.93, 77.79, 72.3, 70.4, 62.3 (C-6), 27.1 ($C(\underline{C}H_3)_3$), 26.7 ($C(\underline{C}H_3)_3$), 26.0 ($C(\underline{C}H_3)_3$), 24.0 ($S\underline{C}H_2CH_3$), 19.7 (C-Si), 18.6 (C-Si), 18.4 (C-Si), 14.8 ($SCH_2\underline{C}H_3$), -1.5 (Si-$\underline{C}H_3$), -3.3 (Si-$\underline{C}H_3$), -3.7 (Si-$\underline{C}H_3$), -4.0 (Si-$\underline{C}H_3$), -4.2 (Si-$\underline{C}H_3$), -4.6 (Si-$\underline{C}H_3$) ppm.

Experiment 36. Synthesis of Methyl (*2S*)-2-(2,3,4-tetra-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-α-D-galactopyranosyl)propanoate (51)

A suspension of thioglycoside donor **50** (68.0 mg, 0.084 mmol), methyl (*S*)-lactate (8 μL, 0.084 mmol) and 4Å MS in CH_2Cl_2 (1 mL) was stirred for 1 hour at room temperature then cooled to -78°C. *N*-Iodosuccinimide (20.8 mg, 0.092 mmol) and TfOH (0.37 μL, 4.20 μmol) were added at -78°C. After 15 minutes the reaction was completed (TLC) and the temperature was allowed to rise. The reaction mixture was diluted with CH_2Cl_2 (1 mL), and 10% $Na_2S_2O_3$ aqueous solution (1 mL) and saturated aqueous $NaHCO_3$ solution (1 mL) were added. The mixture was extracted with CH_2Cl_2 (3x2 mL) and the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed under vacuum. The crude product was purified by preparative TLC (10:90, EtOAc/Hex) to give the product **51** (34.0 mg, 48%) as a viscous residue. **^{1}H NMR** ($CDCl_3$): δ 7.71-7.68 (m, 6H, Ph), 7.57-7.46 (m, 9H, Ph), 5.07 (d, 1H, *J* = 2.6 Hz, H-1), 4.55-4.50 (m, 2H), 4.42-4.29 (m, 3H), 3.86 (s, 3H, CO_2Me), 3.63-3.60 (m, 1H), 3.35-3.31 (m, 1H), 1.69 (d, *J* = 6.8 Hz, 3H, $CHC\underline{H}_3$), 1.18 (s, 18H, *t*-Bu), 0.96 (s, 9H, *t*-Bu), 0.36-0.27 (m, 18H, $SiMe_2$).

Experiment 37. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(*2R*)-2-*O*-(2,3,4-tetra-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-α-D-galactopyranosyl)-2,3-dihydroxypropanoate (52)

The glycosylation reaction of donor **50** (46.0 mg, 0.057 mmol) with acceptor **9** (20.0 mg, 0.057 mmol) was performed according to the procedure described in experiment 36. The results are presented in Table 3, Chapter 1. Product **52** was obtained with 22% yield as a viscous residue. **^{1}H NMR** ($CDCl_3$): δ 7.72-7.66 (m, 4H, Ph), 7.45-7.33 (m, 12H, Ph), 7.25-7.15 (m, 9H, Ph), 4.96 (bs, 1H, H-1), 4.60-4.58 (m, 1H, $C\underline{H}CH_2OTBDPS$), 4.07-3.84 (m, 6H), 3.68 (s, 3H, CO_2Me), 3.56-3.49 (m, 1H), 3.09-3.06 (m, 1H), 1.01 (s, 9H, *t*-Bu), 0.92 (s, 9H, *t*-Bu), 0.79 (s, 9H, *t*-Bu), 0.73 (s, 9H, *t*-Bu), 0.12 (s, 3H, $SiMe_2$), 0.09 (s, 3H, $SiMe_2$), 0.03-0.01 (m, 9H, $SiMe_2$), -0.16 (s, 3H, $SiMe_2$).

Experiment 38. Synthesis of Phenyl 1-thio-β-D-galactopyranoside (53)

The synthesis of compound **53** was carried out according to the procedure described in the literature[3] with 98% yield.

Experiment 39. Synthesis of Phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (54)

To a stirred solution of **53** (0.834 g, 3.0 mmol) in THF (5 mL) at r.t. was added benzaldehyde dimethylacetal (0.920 mL, 6.12 mmol) and a catalytic amount of camphorsulfonic acid. The reaction mixture was refluxed and after 3 hours it was quenched with saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc, dried ($MgSO_4$), filtred and concentrated in vacuum. Purification by flash chromatography on silica gel (70:30, EtOAc/Hex) afforded the product **54** as a viscous colorless foam (0.878 g, 90%). Characterisation data of compound **54** identical to the literature[4].

Experiment 40. Synthesis of Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (55)

The synthesis of compound **55** was carried out according to the procedure described in the literature[5] with 86% yield.

Experiment 41. Synthesis of Phenyl 2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside (56)

To a stirred solution of **55** (1.10 g, 2.03 mmol) in dry CH_2Cl_2 (3 mL) was added MeOH (6 mL) and a catalytic amount of *p*-toluenesulfonic acid at r.t. After 12h the reaction mixture was quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, dried ($MgSO_4$) and concentrated in vacuum. Purification by flash chromatography on silica gel (50:50, EtOAc/Hex) afforded the product **56** as a viscous colourless foam (0.808 g, 88%), and recovered **55** (0.121, 11%).

Experiment 42. Synthesis of Phenyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside (57)

To a stirred solution of **56** (0.810 g, 1.78 mmol) in pyridine (5 mL) at 0ºC was added acetic anhydride (0.507 mL, 5.36 mmol) and a catalytic amount of DMAP. After complete conversion of the starting material water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to furnish a viscous residue. Purification by flash column chromatography on silica gel (70:30, EtOAc/Hex) afforded the product **57** as a viscous colourless foam (0.960 g, 100%). **$[\alpha]^{20}_D$:** +29.5 (c 0.50, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1741 (C=O st) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.59-7.56 (m, 2H, Ph), 7.41-7.26 (m, 13H, Ph), 5.54 (d, 1H, *J* = 1.5 Hz, H-4), 4.79-4.72 (m, 3H, C$\underline{H}_2$Ph), 4.67-4.65 (m, 1H, H-1), 4.50 (d, 1H, *J* = 11.0 Hz, C$\underline{H}_2$Ph), 4.17 (d, 2H, *J* = 6.4 Hz, H-6, H-6), 3.80 (dd, 1H, *J* = 6.0Hz, *J* = 7.0Hz, H-5), 3.69-3.63 (m, 2H, H-2, H-3), 2.14 (s, 3H, Ac), 2.06 (s, 3H, Ac) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 138.1, 137.4, 133.4, 132.2, 128.7-127.6, 87.7 (C-1), 80.9 (C-2), 75.8 ($\underline{C}H_2$Ph), 74.4 (C-5), 72.0 ($\underline{C}H_2$Ph), 66.5 (C-4), 62.3 (C-6), 20.8 (CO$\underline{C}H_3$), 20.7 (CO$\underline{C}H_3$) ppm. **HR-MS:** calcd for $C_{30}H_{32}O_7SNa^+$ $[M+Na]^+$: 559.1766; found: 559.1766.

Experiment 43. Synthesis of Phenyl 2,3-di-*O*-benzyl-4,6-di-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (58)

To a stirred solution of **56** (0.530 g, 1.17 mmol) in pyridine (3 mL) at 0ºC was added chloroacetic anhydride (0.440 g, 2.57 mmol). After complete conversion of the starting material water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to furnish a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20 EtOAc/hexane) afforded the product **58** as a viscous colourless foam (0.488 g, 69%). **$[\alpha]^{20}_D$:** +23.6 (c 0.59, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1747, 1762 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.57-7.54 (m, 2H, Ph), 7.40-7.28 (m, 13H, Ph), 5.56 (d, 1H, *J* = 2.9 Hz, H-4), 4.79-4.71 (m, 3H, C$\underline{H}_2$Ph), 4.66 (d, 1H, *J* = 9.3 Hz, H-1), 4.52 (d, 1H, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.36-4.24 (m, 2H, H-6, H-6), 4.18-4.09 (m, 2H, AcCl), 4.05 (s, 2H, AcCl), 3.87 (dd, 1H, *J* = 6.7 Hz, *J* = 6.4

Hz, H-5), 3.70-3.61 (m, 2H, H-2, H-3) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.0 ($\underline{C}OCH_2Cl$), 166.9 ($\underline{C}OCH_2Cl$), 137.9, 137.0, 132.9, 132.5, 128.9, 128.5-127.9 (m), 87.7 (C-1), 80.6 (C-3), 76.5 (C-2), 75.9 ($\underline{C}H_2Ph$), 73.9 (C-5), 72.5 ($\underline{C}H_2Ph$), 68.7 (C-4), 63.5 (C-6), 40.7 ($CO\underline{C}H_2Cl$), 40.5 ($CO\underline{C}H_2Cl$) ppm. **HR-MS:** calcd for $C_{30}H_{30}Cl_2O_7SNa^+$ $[M+Na]^+$: 627.0987; found: 627.0968.

Experiment 44. Synthesis of Methyl (*2S*)-2-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosyl)propanoate (59)

The glycosylation reaction of donor **57** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1736 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.35-7.26 (m, Ph), 5.60 (d, *J* = 2.9 Hz, H-4 (α)), 5.47 (d, *J* = 3.2 Hz, H-4 (β)), 5.06 (d, *J* = 10.7 Hz, $C\underline{H}_2Ph$ (β)), 4.85 (d, *J* = 3.9 Hz, H-1 (α)), 4.84 (d, *J* = 12.0 Hz, $C\underline{H}_2Ph$ (α)), 4.75 (d, *J* = 10.8 Hz, $C\underline{H}_2Ph$ (α)), 4.72-4.65 (m), 4.60 (d, *J* = 12.0 Hz, $C\underline{H}_2Ph$ (α)), 4.56 (d, *J* = 10.8 Hz, $C\underline{H}_2Ph$ (α)), 4.51-4.47 (m), 4.49 (d, *J* = 7.5 Hz, H-1 (β)), 4.42 (t, *J* = 6.5 Hz, H-5 (α)), 4.22 (t, *J* = 7.0 Hz, H-5 (β)), 4.18-4.07 (m), 4.03 (dd, *J* = 3.3 Hz, *J* = 10.1 Hz, H-3 (α)), 3.94 (dd, *J* = 6.7 Hz, *J* = 11.2 Hz, H-6 (α)), 3.78 (dd, *J* = 3.8 Hz, *J* = 10.1 Hz, H-2 (α)), 3.72 (s, CO_2Me (α)), 3.70 (s, CO_2Me (β)), 3.63-3.53 (m), 2.14 (s, Ac (β)), 2.10 (s, Ac (α)), 2.07 (s, Ac (β)), 2.05 (s, Ac (α)), 1.45 (d, *J* = 6.8 Hz, $CHC\underline{H}_3$ (β)), 1.51 (d, *J* = 6.8 Hz, $CHC\underline{H}_3$ (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.8, 170.5, 170.4, 170.3, 138.5, 138.0, 128.3-127.5, 102.5 (C-1 (β)), 98.5 (C-1 (α)), 78.8 (β), 78.4 (β), 75.9 (C-3 (α)), 75.6 (β), 75.3 (C-2 (α)), 75.1 ($\underline{C}H_2Ph$ (β)), 74.3 (β), 74.0 ($\underline{C}HCH_3$ (α)), 73.5 ($\underline{C}H_2Ph$ (α)), 73.0 (β), 72.4 ($\underline{C}H_2Ph$ (β)), 72.2 ($\underline{C}H_2Ph$ (α)), 70.8 (β), 67.7 (C-4 (α)), 67.2 (C-5 (α)), 66.5 (β), 62.1 (C-6 (β)), 61.9 (C-6 (β)), 52.1 ($CO_2\underline{Me}$), 20.9 ($CO\underline{C}H_3$ (β)), 20.89 ($CO\underline{C}H_3$ (α)), 20.83 ($CO\underline{C}H_3$ (β)), 20.7 ($CO\underline{C}H_3$ (α)), 19.0 ($CH\underline{C}H_3$ (β)), 18.0 ($CH\underline{C}H_3$ (α)) ppm. **HR-MS:** calcd for $C_{28}H_{34}O_{10}Na^+$ $[M+Na]^+$: 553.2044; found: 553.2035.

Experiment 45. Synthesis of Methyl 2-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosyl)acetate (60)

The glycosylation reaction of donor **57** with acceptor **7** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1742 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.26 (m, Ph), 5.51 (d, *J* = 2.4 Hz, H-4 (α)), 5.48 (d, *J* = 2.6 Hz, H-4 (β)), 5.04 (d, *J* = 3.7 Hz, H-1 (α)), 4.81 (d, *J* = 11.8 Hz, C$\underline{H}_2$Ph (α)), 4.76 (d, *J* = 11.8 Hz, C$\underline{H}_2$Ph (α)), 4.74 (d, *J* = 11.1 Hz, C$\underline{H}_2$Ph (α)), 4.58 (d, *J* = 11.1 Hz, C$\underline{H}_2$Ph (α)), 4.53 (d, *J* = 11.3 Hz, C$\underline{H}_2$Ph (β)), 4.50 (d, *J* = 7.6 Hz, H-1 (β)), 4.41 (d, *J* = 16.0 Hz, C$\underline{H}_2CO_2Me$ (β)), 4.30 (d, *J* = 16.0 Hz, C$\underline{H}_2CO_2Me$ (β)), 4.29 (d, *J* = 16.4 Hz, C$\underline{H}_2CO_2Me$ (α)), 4.27-4.23 (m), 4.14 (d, *J* = 16.4 Hz, C$\underline{H}_2CO_2Me$ (α)), 4.15-4.00 (m), 3.82 (dd, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2 (α)), 3.76 (s, OMe), 3.66 (dd, *J* = 7.6 Hz, *J* = 9.6 Hz, H-2 (β)), 3.57 (dd, *J* = 3.5 Hz, *J* = 9.6 Hz, H-3 (β)), 2.14 (s, Ac (β)), 2.11 (s, Ac (α)), 2.07 (s, Ac (β)), 2.06 (s, Ac (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5, 170.4, 170.3, 170.0, 138.5, 138.3, 138.0, 128.3-127.6, 103.3 (C-1 (β)), 97.6 (C-1 (α)), 78.7 (C-3 (β)), 78.4 (C-2 (β)), 75.5 (C-3 (α)), 75.2 r (β)), 75.0 (C-2 (α)), 73.3 (C$\underline{H}_2$Ph (α)), 72.4 (C$\underline{H}_2$Ph (α)), 72.3 (C$\underline{H}_2$Ph (β)), 70.9 (C-4 (β)), 67.8 (C-4 (α)), 67.3 (C-5 (α)), 66.4 (C-5 (β)), 66.0 (C$\underline{H}_2CO_2Me$ (β)), 64.1 (C$\underline{H}_2CO_2Me$ (α)), 62.3 (C-6 (α)), 61.9 (C-6 (β)), 51.9 ($CO_2\underline{Me}$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$) ppm.

Experiment 46. Synthesis of Methyl 3-*O*-*tert*-butyldiphenylsilyl-(2*R*)-2-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-2,3-dihydroxypropanoate (61)

The glycosylation reaction of donor **57** with acceptor **9** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +95.88 (c 1.12, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1745 (C=O) cm^{-1}. **^{1}H NMR (**$CDCl_3$): δ 7.68-7.65 (m, 4H, Ph), 7.43-7.26 (m, 16H, Ph), 5.44 (d, 1H, *J* = 2.3 Hz, H-4), 5.23 (d, 1H, *J* = 3.6 Hz, H-1 (α)), 4.85 (d, 1H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.75 (d, 1H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.69 (d, 1H, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.54 (d, 1H, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.51 (dd, 1H, *J* =

3.9 Hz, *J* = 7.2 Hz, C<u>H</u>CO$_2$Me), 4.27 (t, 1H, *J* = 6.5 Hz, H-5), 4.06-3.96 (m, 5H, H-3, H-6, H-6, C<u>H$_2$</u>OTBDPS), 3.85 (dd, 1H, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2), 3.73 (s, 3H, CO$_2$Me), 2.09 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.02 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** (CDCl$_3$): δ 170.4, 170.3, 170.0, 138.6, 138.2, 135.6, 135.5, 132.9, 132.8, 129.9, 128.3-127.5, 95.6 (C-1 (α)), 75.4 (C-3), 75.0, 74.9, 72.4 (<u>C</u>H$_2$Ph), 67.8 (C-4), 66.9 (C-5), 64.6 (<u>C</u>H$_2$OTBDPS), 62.3 (C-6), 52.1 (CO$_2$<u>Me</u>), 26.7 (*t*-Bu), 20.9 (CO<u>C</u>H$_3$), 20.7 (CO<u>C</u>H$_3$), 19.2 (Si<u>C</u>(CH$_3$)$_3$) ppm. **HR-MS:** calcd for $C_{44}H_{52}O_{11}SiNa^+$ $[M+Na]^+$: 807.3171; found: 807.3165.

Experiment 47. Synthesis of 1,3-di-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-1,2,3-trihydroxypropyl (62)

The glycosylation reaction of donor **57** with acceptor **22** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +62.45 (c 2.63, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1744 (C=O) cm^{-1}. **^{1}H NMR** (CDCl$_3$): δ 7.67-7.61 (m, 8H, Ph), 7.41-7.14 (m, 22H, Ph), 5.43 (d, 1H, *J* = 2.9 Hz, H-4), 5.10 (d, 1H, *J* = 3.5 Hz, H-1 (α)), 4.68 (d, 1H, *J* = 10.9 Hz, C<u>H$_2$</u>Ph), 4.67 (d, 1H, *J* = 11.9 Hz, C<u>H$_2$</u>Ph), 4.55 (d, 1H, *J* = 11.9 Hz, C<u>H$_2$</u>Ph), 4.49 (d, 1H, *J* = 11.0 Hz, C<u>H$_2$</u>Ph), 4.23 (t, 1H, *J* = 6.7 Hz, H-5), 3.99-3.75 (m, 8H), 3.72 (dd, 1H, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2), 2.08 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.00 (s, 18H, *t*-Bu) ppm. **^{13}C NMR** (CDCl$_3$): δ 170.4 (CO<u>C</u>H$_3$), 170.3 (CO<u>C</u>H$_3$), 138.4, 138.1, 135.6, 135.4, 133.4, 133.3, 133.2, 133.1, 129.7, 128.3-127.5, 96.2 (C-1 (α)), 77.3, 75.7 (C-3), 75.4 (C-2), 73.0 (<u>C</u>H$_2$Ph), 72.3 (<u>C</u>H$_2$Ph), 67.8 (C-4), 66.4 (C-5), 64.0 (<u>C</u>H$_2$OTBDPS), 62.8 (<u>C</u>H$_2$OTBDPS), 62.1 (C-6), 26.84 (*t*-Bu), 26.82 (*t*-Bu), 20.9 (CO<u>C</u>H$_3$), 20.6 (CO<u>C</u>H$_3$), 19.2 (Si<u>C</u>(CH$_3$)$_3$), 19.1 (Si<u>C</u>(CH$_3$)$_3$) ppm. **HR-MS:** calcd for $C_{59}H_{70}O_{10}Si_2Na^+$ $[M+Na]^+$: 1017.4400; found: 1017.4394.

Experiment 48. Synthesis of Methyl 15-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosyl)decapentanoate (63)

The glycosylation reaction of donor **57** with acceptor **23** was performed according to the procedure described in experiment 4. The results are

presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1740 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.33-7.25 (m, Ph), 5.55 (d, *J* = 3.1 Hz, H-4 (α)), 5.49 (d, *J* = 2.3 Hz, H-4 (β)), 4.88 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.82 (d, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.81 (d, *J* = 4.4 Hz, H-1 (α)), 4.76-4.70 (m, C$\underline{H}_2$Ph), 4.62 (d, *J* = 12.0 Hz, C$\underline{H}_2$Ph), 4.56 (d, *J* = 11.1 Hz, C$\underline{H}_2$Ph), 4.53 (d, *J* = 11.4 Hz, C$\underline{H}_2$Ph), 4.38 (d, *J* = 6.9 Hz, H-1 (β)), 4.16-4.09 (m), 4.07-4.04 (m), 3.99-3.91 (m), 3.78-3.73 (m), 3.66 (s, CO_2Me), 3.65-3.42 (m), 2.29 (t, *J* = 7.5 Hz), 2.14 (s, Ac), 2.11 (s, Ac), 2.07 (s, Ac), 2.05 (s, Ac), 1.70-1.54 (m), 1.41-1.25 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 174.3 ($\underline{C}O_2Me$), 170.57 ($\underline{C}OCH_3$), 170.53 ($\underline{C}OCH_3$), 170.48 ($\underline{C}OCH_3$), 170.40 ($\underline{C}OCH_3$), 138.6, 138.5, 138.1, 137.7, 128.3-127.6, 103.8 (C-1 (β)), 97.6 (C-1 (α)), 79.0, 78.8, 76.0, 75.6, 75.3 ($\underline{C}H_2$Ph), 73.4 ($\underline{C}H_2$Ph), 72.3 ($\underline{C}H_2$Ph), 72.2 ($\underline{C}H_2$Ph), 70.6, 70.5, 68.6, 67.9, 66.6, 66.5, 62.6 (C-6), 62.0 (C-6), 51.4, 34.1, 29.7-29.1, 26.2, 26.1, 24.9, 20.93 (CO$\underline{C}H_3$), 20.90 (CO$\underline{C}H_3$), 20.7 (CO$\underline{C}H_3$) ppm.

Experiment 49. Synthesis of Methyl 3-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-[4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (64)

The glycosylation reaction of donor **57** with acceptor **24** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +112.06 (c 0.88, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$:1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.70-7.67 (m, 4H, Ph), 7.39-7.17 (m, 31H, Ph), 5.46 (d, 1H, *J* = 3.0 Hz, H-4 Gal), 5.14 (d, 1H, *J* = 3.5 Hz, H-1 Glu (α)), 4.98 (d, 1H, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.97 (d, 1H, *J* = 1.8 Hz, H-1 Gal (α), 4.87 (d, 1H, *J* = 11.5 Hz, C$\underline{H}_2$Ph), 4.83 (d, 1H, *J* = 11.6 Hz, C$\underline{H}_2$Ph), 4.74 (d, 1H, *J* = 10.7 Hz, C$\underline{H}_2$Ph), 4.70 (d, 1H, *J* = 11.3 Hz, C$\underline{H}_2$Ph), 4.69 (d, 1H, *J* = 12.1 Hz, C$\underline{H}_2$Ph), 4.60 (d, 1H, *J* = 12.1 Hz, C$\underline{H}_2$Ph), 4.59 (d, 1H, *J* = 11.6 Hz, C$\underline{H}_2$Ph), 4.53 (d, 1H, *J* = 11.5 Hz, C$\underline{H}_2$Ph), 4.52 (d, 1H, *J* = 11.3 Hz, C$\underline{H}_2$Ph), 4.48 (dd, 1H, *J* = 4.0 Hz, *J* = 5.9 Hz, C$\underline{H}CO_2Me$), 4.09-3.86 (m, 8H), 3.77-3.60 (m, 4H), 3.75 (s, 3H, CO_2Me), 3.48 (dd, 1H, *J* = 3.6 Hz, *J* = 9.5 Hz, H-2 Glu), 2.09 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.02 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5, 170.4, 170.2, 138.9, 138.7, 138.6, 138.3, 138.0, 135.7,

135.6, 133.1, 132.8, 129.7, 128.3-127.3, 98.0 (C-1 Gal (α)), 94.5 (C-1 Glu (α)), 81.7 (C-3 Glu), 79.5 (C-2 Glu), 75.7 ($\underline{C}H_2Ph$), 75.5, 75.3, 74.7 ($\underline{C}H_2Ph$), 74.5, 72.7 ($\underline{C}H_2Ph$), 72.0 ($\underline{C}H_2Ph$), 71.9 ($\underline{C}H_2Ph$), 70.8, 67.9, 66.7 (C-4 Gal), 66.1 (C-6 Gal), 64.7, 62.5 (C-6 Glu), 52.0 (CO_2Me), 26.7 (*t*-Bu), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 19.2 ($Si\underline{C}(CH_3)_3$) ppm. **HR-MS:** calcd for $C_{71}H_{80}O_{16}SiNa^+$ $[M+Na]^+$: 1239.5008; found: 1239.5109.

Experiment 50. Synthesis of 3-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosyl)-epiandrosterone (65)

The glycosylation reaction of donor **57** with acceptor **25** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1741 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.36-7.27 (m, Ph), 5.55 (d, *J* = 3.0 Hz, H-4 (α)), 5.47 (d, *J* = 2.8 Hz, H-4 (β)), 4.97 (d, *J* = 3.6 Hz, H-1 (α)), 4.88 (d, *J* = 10.9 Hz, $C\underline{H}_2Ph$ (β)), 4.80 (d, *J* = 12.0 Hz, $C\underline{H}_2Ph$ (α)), 4.75-4.71 (m, $C\underline{H}_2Ph$), 4.62 (d, *J* = 12.0 Hz, $C\underline{H}_2Ph$ (α)), 4.55 (d, *J* = 11.3 Hz, $C\underline{H}_2Ph$), 4.50 (d, *J* = Hz, $C\underline{H}_2Ph$), 4.48 (d, *J* = 7.2 Hz, H-1 (β)), 4.23 (t, *J* = 6.4 Hz, H-5 (α)), 4.19-4.10 (m, H-6 (α), H-6 (β), H-6 (β)), 4.03 (dd, *J* = 7.3 Hz, *J* = 11.1 Hz, H-6 (α)), 3.95 (dd, *J* = 3.3 Hz, *J* = 10.0 Hz, H-3 (α)), 3.77-3.73 (m, H-2 (α), H-5 (β)), 3.64-3.48 (m), 2.43 (dd, *J* = 8.9 Hz, *J* = 19.2 Hz), 2.13 (s, Ac (β)), 2.10 (s, Ac (α)), 2.06 (s, Ac (β)), 2.05 (s, Ac (α)), 2.11-2.01 (m), 1.95-1.43 (m), 1.40-1.20 (m), 1.15-1.07 (m), 1.01-0.91 (m), 0.68 (td, *J* = 3.3 Hz, *J* = 11.5 Hz) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 138.6, 138.5, 138.1, 137.8, 128.3-127.6, 102.3 (C-1 (β)), 96.2 (C-1 (α)), 79.7 (C-2 (β)), 79.2 (C-3 (β)), 78.9, 76.1 (C-3 (α)), 75.5 (C-2 (α)), 75.4 ($\underline{C}H_2Ph$ (β)), 73.3 ($\underline{C}H_2Ph$ (α)), 72.3 ($\underline{C}H_2Ph$ (α)), 72.2 ($\underline{C}H_2Ph$ (β)), 70.5 (C-5 (β)), 67.9 (C-4 (α)), 66.6 (C-5 (α)), 66.5 (C-4 (β)), 62.7 (C-6 (α)), 62.0 (C-6 (β)), 54.4, 51.4, 47.8, 45.2, 44.8, 36.9, 35.85, 35.8, 35.0, 34.7, 31.5, 30.9, 29.5, 28.5, 28.4, 27.5, 21.7, 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$), 13.8, 12.3, 12.2 ppm.

Experiment 51. Synthesis of 1-*O*-Adamantanyl-4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranoside (66)

The glycosylation reaction of donor **57** with acceptor **26** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **Alpha product: $[\alpha]_D^{20}$** +108.3 (c 0.94, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1745 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.36-7.25 (m, 10H, Ph), 5.56 (d, 1H, *J* = 2.9 Hz, H-4), 5.32 (d, 1H, *J* = 3.8 Hz, H-1 (α)), 4.76 (d, 1H, *J* = 11.8 Hz, C$\underline{H}_2$Ph), 4.73 (d, 1H, *J* = 10.6Hz, C$\underline{H}_2$Ph), 4.64 (d, 1H, *J* = 11.9 Hz, C$\underline{H}_2$Ph), 4.55 (d, 1H, *J* = 10.7 Hz, C$\underline{H}_2$Ph), 4.35 (t, 1H, *J* = 6.6 Hz, H-5), 4.12-4.03 (m, 2H, H-6, H-6), 3.99 (dd, 1H, *J* = 3.4 Hz, *J* = 10.0 Hz, H-3), 3.75 (dd, 1H, *J* = 3.8 Hz, *J* = 10.0 Hz, H-2), 2.15 (bs, 3H, C$\underline{H}(CH_2)_3$), 2.10 (s, Ac), 2.04 (s, Ac), 1.93-1.78 (m), 1.69-1.59 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.69 ($\underline{C}OCH_3$), 170.62 ($\underline{C}OCH_3$), 138.7, 138.4, 128.4-127.6, 90.8 (C-1 (α)), 76.2 (C-3), 75.7 (C-2), 74.8 (O$\underline{C}(CH_2)_3$), 73.3 ($\underline{C}H_2$Ph), 72.3 ($\underline{C}H_2$Ph), 68.2 (C-4), 66.3 (C-5), 62.7 (C-6), 42.6, 36.3, 30.8 ($\underline{C}H(CH_2)_3$), 21.0 (CO$\underline{C}H_3$), 20.8 (CO$\underline{C}H_3$) ppm. **Beta product: $[\alpha]_D^{20}$** +41.7 (c 0.17, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.36-7.25 (m, 10H, Ph), 5.46 (d, 1H, *J* = 2.4 Hz, H-4 (β)), 4.92 (d, 1H, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.67-4.56 (m, 3H, C$\underline{H}_2$Ph, H-1 (β)), 4.44 (d, 1H, *J* = 10.9 Hz,C$\underline{H}_2$Ph), 4.14-4.03 (m, 2H, H-6), 3.72-3.67 (m, 1H, H-5), 3.55-3.48 (m, 2H, H-2, H-3), 2.15 (bs), 2.13 (s), 2.10 (s), 2.04 (s), 1.93-1.78 (m), 1.69-1.59 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.76 ($\underline{C}OCH_3$), 170.72 ($\underline{C}OCH_3$), 138.8, 138.0, 128.4-127.6, 96.5 (C-1 (β)), 79.6 (C-3), 78.9 (C-2), 75.6 (O$\underline{C}(CH_2)_3$), 75.5 ($\underline{C}H_2$Ph), 72.5 ($\underline{C}H_2$Ph), 70.6 (C-5), 66.8 (C-4) 62.3 (C-6), 42.7, 42.5, 36.4, 30.8 ($\underline{C}H(CH_2)_3$), 21.1 (CO$\underline{C}H_3$), 20.9 (CO$\underline{C}H_3$) ppm.

Experiment 52. Synthesis of Benzyl (*2S*)-2-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-2-phenylacetate (67)

The glycosylation reaction of donor **57** with acceptor **27** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +101.8 (c 1.57, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1743 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.44-7.15 (m, 20H, Ph), 5.57 (d,

1H, *J* = 3.0 Hz, H-4), 5.19-5.09 (m, 3H, CHPh, CO_2CH_2Ph), 4.88 (d, 1H, *J* = 3.6 Hz, H-1 (α)), 4.75 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.69 (d, 1H, *J* = 11.8 Hz, CH_2Ph), 4.58 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.45 (d, 1H, *J* = 11.8 Hz, CH_2Ph), 4.37 (t, 1H, *J* = 6.4 Hz, H-5), 4.13 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H-3), 4.08 (dd, 1H, *J* = 6.0 Hz, *J* = 11.2 Hz, H-6), 3.96 (dd, 1H, *J* = 6.9 Hz, *J* = 11.1 Hz, H-6), 3.77 (dd, 1H, *J* = 3.6 Hz, *J* = 9.9 Hz, H-2), 2.09 (s, 3H, Ac), 2.03 (s, 3H, Ac) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5 ($COCH_3$), 170.3 ($COCH_3$), 169.7 (CO_2Bn), 138.3, 138.0, 135.39, 135.34, 128.8-128.0, 127.9-127.3, 96.7 (C-1 (α)), 77.8 (CHPh), 75.9 (C-3), 75.3 (C-2), 73.3 (CH_2Ph), 72.2 (CH_2Ph), 67.7 (C-4), 67.4 (C-5), 66.9 (CO_2CH_2Ph), 62.2 (C-6), 20.8 ($COCH_3$), 20.7 ($COCH_3$) ppm. **HR-MS:** calcd for $C_{39}H_{40}O_{10}Na^+$ $[M+Na]^+$: 691.2514; found: 691.2504.

Experiment 53. Synthesis of 1-ω-Methoxy-PEG$_{550}$yl-4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosy (68)

The glycosylation reaction of donor **57** with acceptor **28** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1716, 1742 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.37-7.26 (m, Ph), 5.55 (d, *J* = 2.6 Hz, H-4 (α)), 5.48 (d, *J* = 2.4 Hz, H-4 (β)), 4.91 (d, *J* = 3.6 Hz, H-1 (α)), 4.90 (d, *J* = 10.8 Hz, CH_2Ph), 4.80 (d, *J* = 12.0 Hz, CH_2Ph), 4.74 (d, *J* = 11.1 Hz, CH_2Ph), 4.71 (d, *J* = 12.3 Hz, CH_2Ph), 4.64 (d, *J* = 12.0 Hz, CH_2Ph), 4.56 (d, *J* = 11.0 Hz, CH_2Ph), 4.52 (d, *J* = 11.4 Hz, CH_2Ph), 4.44 (d, *J* = 7.2 Hz, H-1 (β)), 4.21-4.18 (m), 4.15-4.10 (m), 4.06-4.01 (m), 3.98 (dd, *J* = 3.4 Hz, *J* = 9.9 Hz, H-3 (α)), 3.81-3.53 (m), 3.37 (s, CO_2Me), 2.13 (s, Ac), 2.11 (s, Ac), 2.07 (s, Ac), 2.06 (s, Ac) ppm. **HR-MS:** calcd for $C_{49}H_{79}O_{20}$ $[M+H]^+$: 987.5159; found: 987.5157.

Experiment 54. Synthesis of O-1-L-Menthyl-4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-1-*O*-Menthyl–α/β-D-galactopyranoside (69)

The glycosylation reaction of donor **57** with acceptor **29** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1746 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.36-7.25 (m, Ph), 5.56 (d, *J* = 2.6 Hz, H-4 (α)), 5.46 (s, H-4 (β)),

5.02 (d, *J* = 3.5 Hz, H-1 (α)), 4.87 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (β)), 4.80 (d, *J* = 11.6 Hz, C$\underline{H}_2$Ph (α)), 4.73 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph (α)), 4.68 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (β)), 4.62 (d, *J* = 11.6 Hz, C$\underline{H}_2$Ph (α)), 4.56-4.50 (m, C$\underline{H}_2$Ph), 4.42 (d, *J* = 6.2 Hz, H-1 (β)), 4.29 (t, *J* = 6.2 Hz, H-5 (α)), 4.14-4.04 (m, H-6 (α), H-6 (α), H-6 (β), H-6 (β)), 3.99 (dd, *J* = 3.0 Hz, *J* = 10.0 Hz, H-3 (α)), 3.77 (dd, *J* = 3.5 Hz, *J* = 10.0 Hz, H-2 (α)), 3.72 (t, *J* = 6.6 Hz, H-5 (β)), 3.57-3.50 (m, H-2 (β), H-3 (β)), 3.41 (td, *J* = 4.1 Hz, *J* = 10.6 Hz, C$\underline{H}$-O menthol (β)), 3.34 (td, *J* = 4.2 Hz, *J* = 10.6 Hz, C$\underline{H}$-O menthol (α)), 2.44-2.31 (m, C$\underline{H}$(CH$_3$)$_2$), 2.14-2.05 (m, CHC$\underline{H}_2$CH), 2.14 (s, Ac (β)), 2.11 (s, Ac (α)), 2.07 (s, Ac (α)), 2.05 (s, Ac (β)), 1.67-1.60 (m, C$\underline{H}_2$C$\underline{H}_2$), 1.42-1.25 (m, C$\underline{H}$CH$_3$, C$\underline{H}$CH(CH$_3$)$_2$), 1.08-0.80 (m), 0.75 (d, *J* = 6.7 Hz), 0.68 (d, *J* = 6.8 Hz) ppm. **^{13}C NMR** (CDCl$_3$): δ 170.6 (C$\underline{O}$CH$_3$), 170.59 (C$\underline{O}$CH$_3$), 170.58 (C$\underline{O}$CH$_3$), 170.4 (C$\underline{O}$CH$_3$), 138.66, 138.62, 138.1, 137.9, 128.3-128.0, 127.9-127.4, 101.7 (C-1 (β)), 99.5 (C-1 (α)), 81.2 (CH-O menthol (α)), 79.5 (CH-O menthol (β)), 79.3, 78.7, 76.1 (C-3 (α)), 76.0 (C-2 (α)), 75.3 (C$\underline{H}_2$Ph (β)), 73.8 (C$\underline{H}_2$Ph (α)), 72.3 (C$\underline{H}_2$Ph (β)), 71.8 (C$\underline{H}_2$Ph (α)), 70.4 (C-5 (β)), 68.0 (C-4 (α)), 66.8 (C-5 (α)), 66.7 (C-4 (β)), 63.0 (C-6 (α)), 62.3 (C-6 (β)), 48.7 (C$\underline{H}$CH(CH$_3$)$_2$ (α)), 47.9 (C$\underline{H}$CH(CH$_3$)$_2$ (β)), 42.9 (CH$\underline{C}$H$_2$CH (α)), 41.3 (CH$\underline{C}$H$_2$CH (β)), 34.3, 34.2, 31.7 (C$\underline{H}$CH$_3$ (α)), 31.5 (C$\underline{H}$CH$_3$ (β)), 25.0 (C$\underline{H}$(CH$_3$)$_2$ (β)), 24.4 (C$\underline{H}$(CH$_3$)$_2$ (α)), 23.0, 22.9, 22.4, 22.2, 21.0, 20.98 (CO$\underline{C}$H$_3$), 20.92 (CO$\underline{C}$H$_3$), 20.8 (CO$\underline{C}$H$_3$), 20.7 (CO$\underline{C}$H$_3$), 16.0, 15.5 ppm.

Experiment 55. Synthesis of 1-*O*-Cyclohexyl-4,6-di-*O*-acetyl-2,3-di-*O*-benzyl- -α/β-D-galactopyranoside (70)

The glycosylation reaction of donor **57** with acceptor **30** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1744 (C=O) cm^{-1}. **^{1}H NMR** (CDCl$_3$): δ 7.36-7.25 (m, Ph), 5.56 (d, *J* = 2.8 Hz, H-4 (α)), 5.48 (d, *J* = 2.3 Hz, H-4 (β)), 4.99 (d, *J* = 3.6 Hz, H-1 (α)), 4.91 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.79 (d, *J* = 12.0 Hz, C$\underline{H}_2$Ph), 4.75-4.69 (m, C$\underline{H}_2$Ph), 4.62 (d, *J* = 12.0 Hz, C$\underline{H}_2$Ph), 4.57-4.49 (m, C$\underline{H}_2$Ph), 4.48 (d, J=7.2 Hz, H-1 (β)), 4.23 (t, *J* = 6.2 Hz, H-5 (α)), 4.20-4.09 (m, H-6 (α), H-6 (β), H-6 (β)), 4.03 (dd, *J* = 7.4 Hz, *J* = 11.2 Hz, H-6

(α)), 3.97 (dd, *J* = 3.3 Hz, *J* = 10.0 Hz, H-3 (α)), 3.78-3.73 (m, H-2 (α), H-5 (α)), 3.70-3.63 (m), 3.61-3.49 (m), 2.13 (s, Ac), 2.10 (s, Ac), 2.06 (s, Ac), 2.05 (s, Ac), 1.99-1.84 (m), 1.77-1.75 (m), 1.62-1.17 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.58 ($\underline{C}OCH_3$), 170.56 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 138.6, 138.1, 137.8, 128.3-127.5, 102.1 (C-1 (β)), 95.9 (C-1 (α)), 79.4, 78.5 (C<u>H</u>-O cyclohexanol), 76.3 (C<u>H</u>-O cyclohexanol), 76.1 (C-3 (α)), 75.6 (C-2 (α)), 75.3 ($\underline{C}H_2Ph$), 73.2 ($\underline{C}H_2Ph$), 72.2 ($\underline{C}H_2Ph$), 70.5 (C-5 (β)), 68.0 (C-4 (α)), 66.6 (C-4 (β)), C-5 (α)), 62.6 (C-6 (α)), 62.0 (C-6 (β)), 33.7, 33.3, 32.0, 31.6, 25.58, 25.55, 24.5, 24.2, 24.1, 24.0, 20.97 ($CO\underline{C}H_3$), 20.92 ($CO\underline{C}H_3$), 20.77 ($CO\underline{C}H_3$), 20.75 ($CO\underline{C}H_3$) ppm.

Experiment 56. Synthesis of 3-*O*-(4,6-Di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosyl)-*N*-Boc-L-ser-methyl ester (71)

The glycosylation reaction of donor **57** with acceptor **31** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **Alpha product: $[\alpha]_D^{20}$** +99.0 (c 1.25, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1715, 1745 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.32-7.26 (m, 10H, Ph), 5.62 (d, 1H, *J* = 8.2 Hz, N<u>H</u>Boc), 5.55 (s, 1H, H-4), 4.80 (s, 1H, H-1 (α)), 4.77 (d, 1H, *J* = 12.0 Hz, $C\underline{H}_2Ph$), 4.73 (d, 1H, *J* = 11.1 Hz, $C\underline{H}_2Ph$), 4.58 (d, 1H, *J* = 11.9 Hz, $C\underline{H}_2Ph$), 4.53 (d, 1H, *J* = 11.0 Hz, $C\underline{H}_2Ph$), 4.47-4.45 (m, 1H, C<u>H</u> serine), 4.17-4.02 (m, 4H, H-5, H-6, H-6, $C\underline{H}_2$ serine), 3.90-3.83 (m, 2H, H-3, $C\underline{H}_2$ serine), 3.77-3.74 (m, 1H, H-2), 3.66 (s, 3H, CO_2Me), 2.10 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.45 (s, 3H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.5, 170.2, 155.4, 138.4, 137.9, 128.36, 128.34, 127.9, 127.7, 127.6, 99.2 (C-1 (α)), 75.6 (C-3), 75.4 (C-2), 73.4 ($\underline{C}H_2Ph$), 72.1 ($\underline{C}H_2Ph$), 70.2 ($\underline{C}H_2$ serine), 67.5 (C-4), 67.3 (C-5), 62.3 (C-6), 54.1 (<u>C</u>H serine), 52.5 ($CO_2\underline{C}H_3$), 28.3 (*t*-Bu), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$) ppm. **Beta product: $[\alpha]_D^{20}$** +38.8 (c 0.57, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1715, 1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.39-7.28 (m, 10H, Ph), 5.48 (s, 2H, N<u>H</u>Boc, H-4), 4.80 (d, 1H, *J* = 10.7 Hz, $C\underline{H}_2Ph$), 4.75-4.69 (m, 2H, $C\underline{H}_2Ph$), 4.50 (d, 1H, *J* = 11.3 Hz, $C\underline{H}_2Ph$), 4.49-4.46 (m, 1H, C<u>H</u> serine), 4.36 (d, 1H, *J* = 7.1 Hz, H-1 (β)), 4.35-4.31 (m, 1H, $C\underline{H}_2$ serine), 4.13 (d, 2H, *J* = 6.5 Hz, H-6, H-6), 3.83

(dd, 1H, *J* = 3.4 Hz, *J* = 10.4 Hz, C<u>H</u>$_2$ serine), 3.75 (s, 3H, CO_2Me), 3.75-3.72 (m, 1H, H-5), 3.58-3.52 (m, 2H, H-2, H-3), 2.14 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.42 (s, 3H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.5, 170.3, 155.4, 138.2, 137.5, 128.3, 128.1, 128.0, 127.8, 127.7, 104.1 (C-1 (β)), 79.0, 78.3, 75.4 (<u>C</u>H_2Ph), 72.1 (<u>C</u>H_2Ph), 70.8 (C-5), 70.3 (<u>C</u>H_2 serine), 66.3 (C-4), 61.9 (C-6), 54.0 (<u>C</u>H serine), 52.6 (CO_2<u>C</u>H_3), 28.3 (*t*-Bu), 20.8 (CO<u>C</u>H_3), 20.7 (CO<u>C</u>H_3) ppm.

Experiment 57. Synthesis of Methyl (*2S*)-2-(2,3-di-*O*-benzyl-4,6-di-*O*-chloroacetyl-α-D-galactopyranosyl)propanoate (72)

The glycosylation reaction of donor **58** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +79.1 (c 0.63, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1747 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.33-7.27 (m, 10H, Ph), 5.63 (d, 1H, *J* = 2.9 Hz, H-4), 4.83 (d, 1H, *J* = 3.8 Hz, H-1 (α)), 4.82 (d, 1H, *J* = 12.0 Hz, C<u>H</u>$_2$Ph), 4.73 (d, 1H, *J* = 10.8 Hz, C<u>H</u>$_2$Ph), 4.59 (d, 1H, *J* = 12.0 Hz, C<u>H</u>$_2$Ph), 4.58 (d, 1H, *J* = 10.8 Hz, C<u>H</u>$_2$Ph), 4.53 (t, 1H, *J* = 6.5 Hz, H-5), 4.21 (dd, 1H, *J* = 6.2 Hz, *J* = 11.2 Hz, H-6), 4.11-4.03 (m, 7H), 3.75 (dd, 1H, *J* = 3.8 Hz, *J* = 10.1 Hz, H-2), 3.72 (s, 3H, CO_2Me), 1.45 (d, 3H, *J* = 6.8 Hz, CHC<u>H</u>$_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.8 (<u>C</u>O_2Me), 166.9 (<u>C</u>OCH_2Cl), 138.3, 137.7, 128.4-127.7, 98.5 (C-1 (α)), 75.6 (C-2), 75.1 (C-3), 74.3 (<u>C</u>HCH_3), 73.6 (<u>C</u>H_2Ph), 72.6 (<u>C</u>H_2Ph), 69.9 (C-4), 66.7 (C-5), 63.4 (C-6), 52.1 (CO_2<u>C</u>H_3), 40.7 (CO<u>C</u>H_2Cl), 40.6 (CO<u>C</u>H_2Cl), 18.0 (CH<u>C</u>H_3) ppm. **HR-MS:** calcd for $C_{28}H_{32}O_{10}Cl_2Na^+$ $[M^+ + Na]^+$: 621.1265; found: 621.1257.

Experiment 58. Methyl 2-(2,3-di-*O*-benzyl-4,6-di-*O*-chloroacetyl-α/β-D-galactopyranosyl)acetate (73)

The glycosylation reaction of donor **58** with acceptor **7** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1751 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.39-7.27 (m, Ph), 5.59 (d, *J* = 2.4 Hz, H-4 (α)), 5.50 (d, *J* = 2.2 Hz, H-4 (β)), 5.00 (d, *J* = 3.6 Hz, H-1 (α)), 4.80 (d, *J* = 11.9 Hz, C<u>H</u>$_2$Ph (α)), 4.74

(d, *J* = 11.8 Hz, C$\underline{H}_2$Ph (α)), 4.73 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph (α)), 4.60 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph (α)), 4.55 (d, *J* = 11.2 Hz, C$\underline{H}_2$Ph (β)), 4.52 (d, *J* = 7.1 Hz, H-1 (β)), 4.40-4.14 (m), 4.11-4.03 (m), 3.79 (dd, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2 (α)), 3.76 (s, CO_2Me), 3.66-3.55 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.9 ($\underline{C}O_2Me$ (α)), 169.5 ($\underline{C}O_2Me$ (β)), 167.0 ($\underline{C}OCH_2Cl$ (β)), 166.9 ($\underline{C}OCH_2Cl$ (α)), 166.8 ($\underline{C}OCH_2Cl$ (α)), 138.0, 137.6, 128.4-127.6, 103.2 (C-1 (β)), 97.9 (C-1 (α)), 78.3 (C-3 (β)), 78.1 (C-2 (β)), 75.2 (C-3 (α)), 74.8 (C-2 (α)), 73.4 ($\underline{C}H_2Ph$), 72.7 ($\underline{C}H_2Ph$), 70.3 (β), 70.0 (C-4 (α)), 68.5 (β), 66.8 (C-5 (α)), 66.0 ($C\underline{H}_2CO_2Me$ (β)), 64.5 ($C\underline{H}_2CO_2Me$ (α)), 63.5 (C-6 (α)), 63.1 (C-6 (β)), 52.0 ($CO_2\underline{C}H_3$), 40.7 ($CO\underline{C}H_2Cl$), 40.5 ($CO\underline{C}H_2Cl$) ppm.

Experiment 59. Methyl 3-*O*-*tert*-butyldiphenylsilyl-(2*R*)-2-*O*-(4,6-di-*O*-chloroacetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-2,3-dihydroxypropanoate (74)

The glycosylation reaction of donor **58** with acceptor **9** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +69.4 (c 2.02, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1750 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.67-7.64 (m, 4H, Ph), 7.42-7.26 (m, 16H, Ph), 5.40 (d, 1H, *J* = 2.8 Hz, H-4), 5.23 (d, 1H, *J* = 3.5 Hz, H-1 (α)), 4.85 (d, 1H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.74 (d, 1H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.66 (d, 1H, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.55 (d, 1H, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.51 (dd, 1H, *J* = 3.8 Hz, *J* = 7.2 Hz, C$\underline{H}CO_2Me$), 4.32 (t, 1H, *J* = 6.5 Hz, H-5), 4.15-4.00 (m, 7H), 3.91 (s, 2H, AcCl), 3.81 (dd, 1H, *J* = 3.5 Hz, *J* = 9.9 Hz, H-2), 3.73 (s, 3H, CO_2Me), 1.03 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.8 ($\underline{C}O_2Me$), 166.9 ($\underline{C}OCH_2Cl$), 166.6 ($\underline{C}OCH_2Cl$), 138.3, 137.8, 135.5, 135.4, 132.9, 132.8, 129.9, 128.3, 128.2, 127.9-127.6, 95.5 (C-1), 75.1 (C-3), 75.0 ($\underline{C}HCO_2Me$), 74.7 (C-2), 72.8 ($\underline{C}H_2Ph$), 72.4 ($\underline{C}H_2Ph$), 70.0 (C-4), 66.3 (C-5), 64.5 ($\underline{C}H_2OTBDPS$), 63.3 (C-6), 52.1 ($CO_2\underline{C}H_3$), 40.7 ($CO\underline{C}H_2Cl$), 40.4 ($CO\underline{C}H_2Cl$), 26.7 (*t*-Bu), 19.2 ($Si\underline{C}(CH_3)_3$) ppm. **HR-MS:** calcd for $C_{44}H_{50}O_{11}Cl_2SiNa^+$ $[M+Na]^+$: 875.2392; found: 875.2369.

Experiment 60. 1,3-di-*O*-*tert*-butyldiphenylsilyl-(2*R*)-2-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-1,2,3-trihydroxypropyl (75)

The glycosylation reaction of donor **58** with acceptor **22** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. $[\alpha]_D^{20}$ +66.9 (c 12.15, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1747, 1766 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.63-7.61 (m, 8H, Ph), 7.43-7.27 (m, 18H, Ph), 7.18-7.14 (m, 4H, Ph), 5.39 (d, 1H, *J* = 2.9 Hz, H-4), 5.06 (d, 1H, *J* = 3.5 Hz, H-1 (α)), 4.64 (d, 2H, *J* = 11.4 Hz, C$\underline{H}_2$Ph), 4.54 (d, 1H, *J* = 11.9 Hz, C$\underline{H}_2$Ph), 4.50 (d, 1H, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.26 (t, 1H, *J* = 6.6 Hz, H-5), 4.14-3.91 (m, 6H), 3.88-3.72 (m, 6H), 3.67 (dd, 1H, *J* = 3.4 Hz, *J* = 10.0 Hz, H-2), 1.01 (s, 18H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.0 ($\underline{C}OCH_2Cl$), 166.6 ($\underline{C}OCH_2Cl$), 138.1, 137.8, 135.6-135.5, 133.3, 133.2, 133.0, 129.83, 129.80, 128.3, 128.2, 128.0, 127.8-127.6, 96.2 (C-1 (α)), 77.6 (O$\underline{C}$H($CH_2OTBDPS)_2$), 75.4 (C-3), 75.1 (C-2), 73.1 ($\underline{C}H_2$Ph), 72.6 ($\underline{C}H_2$Ph), 70.0 (C-4), 65.8 (C-5), 64.1 (OCH($\underline{C}H_2OTBDPS)_2$), 63.2 (C-6), 62.8 (OCH($\underline{C}H_2OTBDPS)_2$), 40.7 (CO$\underline{C}H_2$Cl), 40.4 (CO$\underline{C}H_2$Cl), 26.9 (*t*-Bu), 26.8 (*t*-Bu), 19.3 (Si$\underline{C}(CH_3)_3$), 19.1 (Si$\underline{C}(CH_3)_3$) ppm. **HR-MS:** calcd for $C_{59}H_{68}O_{10}Cl_2Si_2Na^+$ $[M+Na]^+$: 1085.3620; found: 1085.3622.

Experiment 61. Synthesis of Methyl 15-*O*-(2,3-di-*O*-benzyl-4,6-di-*O*-chloroacetyl-α/β-D-galactopyranosyl)decapentanoate (76)

The glycosylation reaction of donor **58** with acceptor **23** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1736, 1764 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.35-7.27 (m, Ph), 5.57 (d, *J* = 3.3 Hz, H-4 (α)), 5.51 (d, *J* = 2.1 Hz, H-4 (β)), 4.87 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.807 (d, *J* = 3.6 Hz, H-1 (α)), 4.805 (d, *J* = 12.0 Hz, C$\underline{H}_2$Ph), 4.73 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph), 4.72 (d, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.70 (d, *J* = 10.4 Hz, C$\underline{H}_2$Ph), 4.61 (d, *J* = 12.2 Hz, C$\underline{H}_2$Ph), 4.58 (d, *J* = 11.0 Hz, C$\underline{H}_2$Ph), 4.54 (d, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.39 (d, *J* = 7.1 Hz, H-1 (β)), 4.32 (dd, *J* = 6.8 Hz, *J* = 11.2 Hz, H-6 (β)), 4.25 (dd, *J* = 6.8 Hz, *J* = 11.2 Hz, H-6 (β)), 4.21-4.19 (m, H-6 (α),H-6 (α), H-5 (α)), 4.15 (d, *J* = 2.2 Hz, AcCl (β)), 4.10 (d, *J* = 2.8 Hz, AcCl (α)), 4.08 (s, AcCl (β)), 4.06 (s, AcCl (α)),

4.00 (dd, *J* = 3.4 Hz, *J* = 10.0 Hz, H-3 (α)), 3.95-3.90 (m), 3.83 (t, *J* = 7.0 Hz, H-5 (β)), 3.73 (dd, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2 (α)), 3.66 (s, CO_2Me), 3.63-3.43 (m), 2.29 (t, *J* = 7.5 Hz), 1.67-1.57 (m), 1.42-1.24 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 174.3 ($\underline{C}O_2Me$), 167.1 ($\underline{C}OCH_2Cl$), 167.0 ($\underline{C}OCH_2Cl$), 166.9 ($\underline{C}OCH_2Cl$), 166.8 ($\underline{C}OCH_2Cl$), 138.3, 137.7, 137.4, 128.4-127.7, 103.7 (C-1 (β)), 97.6 (C-1 (α)), 78.6, 78.5, 75.7 (C-3 (α)), 75.4 ($\underline{C}H_2Ph$ (β)), 75.3 (C-2 (α)), 73.4 ($\underline{C}H_2Ph$ (α)), 72.7 ($\underline{C}H_2Ph$ (α)), 72.6 ($\underline{C}H_2Ph$ (β)), 70.6 (β), 70.0 (C-4 (α)), 68.7, 68.6 (C-4 (β), C-5 (β)), 66.0 (C-5 (α)), 63.7 (C-6 (α)), 63.1 (C-6 (β)), 51.4 ($CO_2\underline{C}H_3$), 40.8 ($CO\underline{C}H_2Cl$), 40.7 ($CO\underline{C}H_2Cl$), 40.6 ($CO\underline{C}H_2Cl$), 40.5 ($CO\underline{C}H_2Cl$), 34.1, 29.6-29.1, 26.2, 26.1, 24.9 ppm. **HR-MS:** calcd for $C_{40}H_{56}Cl_2O_{10}Na^+$ $[M+Na]^+$: 789.3143; found: 789.3119.

Experiment 62. Methyl 3-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-[2,3-di-*O*-benzyl-4,6-di-*O*-chloroacetyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (77)

The glycosylation reaction of donor **58** with acceptor **24** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +98.5 (c 0.76, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1751 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.69-7.68 (m, 4H, Ph), 7.40-7.17 (m, 31H, Ph), 5.48 (d, 1H, *J* = 2.9 Hz, H-4 Gal), 5.15 (d, 1H, *J* = 3.3 Hz, H-1 (α) Glu), 4.98 (d, 1H, *J* = 10.6 Hz, $C\underline{H}_2Ph$), 4.92 (d, 1H, *J* = 3.2 Hz, H-1 (α) Gal), 4.88 (d, 1H, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.84 (d, 1H, *J* = 11.5 Hz, $C\underline{H}_2Ph$), 4.73 (d, 1H, *J* = 10.6 Hz, $C\underline{H}_2Ph$), 4.67 (d, 2H, *J* = 11.8 Hz, $C\underline{H}_2Ph$), 4.60 (d, 1H, *J* = 11.4 Hz, $C\underline{H}_2Ph$), 4.58 (d, 1H, *J* = 12.0 Hz, $C\underline{H}_2Ph$), 4.53 (d, 1H, *J* = 11.2 Hz, $C\underline{H}_2Ph$), 4.52 (d, 1H, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.47 (t, 1H, *J* = 4.64 ou 5.08 Hz, $\underline{C}HCO_2Me$), 4.19-3.88 (m, 13H), 3.76 (s, 3H, CO_2Me), 3.74-3.58 (m, 4H), 3.51 (dd, 1H, *J* = 3.4 Hz, *J* = 9.4 Hz, H-2), 1.03 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.2 ($\underline{C}O_2Me$), 167.0 ($\underline{C}OCH_2Cl$), 166.7 ($\underline{C}OCH_2Cl$), 138.8, 138.6, 138.4, 138.2, 137.7, 135.6, 135.5, 133.0, 132.8, 129.7, 128.3-127.3, 97.8 (C-1 (α) Gal), 94.4 (C-1 (α) Glu), 81.7 (C-3 Glu), 79.5 (C-2 Glu), 77.4 (C-4 Glu), 75.7 ($\underline{C}H_2Ph$), 75.3 (C-2 Gal), 75.0, 74.7 ($\underline{C}H_2Ph$), 74.5 ($\underline{C}HCO_2Me$), 72.8 ($\underline{C}H_2Ph$), 72.4 ($\underline{C}H_2Ph$), 71.9 ($\underline{C}H_2Ph$), 70.8, 70.1, 66.3 (C-6 Glu), 66.2, 64.7

($\underline{C}H_2$OTBDPS), 63.7 (C-6 Gal), 52.0 ($CO_2\underline{C}H_3$), 40.7 ($CO\underline{C}H_2Cl$), 40.5 ($CO\underline{C}H_2Cl$), 26.7 (*t*-Bu), 19.2 ($Si\underline{C}(CH_3)_3$) ppm. **HR-MS:** calcd for $C_{71}H_{78}O_{16}Cl_2SiNa^+$ [M^+ + Na]: 1307.4328; found: 1307.4334.

Experiment 63. Synthesis of 3-*O*-(2,3-Di-*O*-benzyl-4,6-di-*O*-chloroacetyl-α/β-D-galactopyranosyl)-epiandrosterone (78)

The glycosylation reaction of donor **58** with acceptor **25** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1737, 1764 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.35-7.27 (m, Ph), 5.58 (d, *J* = 2.7 Hz, H-4 (α)), 5.50 (d, *J* = 1.9 Hz, H-4 (β)), 4.97 (d, *J* = 3.7 Hz, H-1 (α)), 4.88 (d, *J* = 10.9 Hz, $C\underline{H}_2Ph$ (β)), 4.79 (d, *J* = 12.0 Hz, $C\underline{H}_2Ph$ (α)), 4.73-4.69 (m, $C\underline{H}_2Ph$), 4.63-4.56 (m, $C\underline{H}_2Ph$), 4.54 (d, *J* = 11.3 Hz, $C\underline{H}_2Ph$ (β)), 4.50 (d, *J* = 7.4 Hz, H-1 (β)), 4.34-4.30 (m), 4.25-4.20 (m), 4.15-4.04 (m), 3.99 (dd, *J* = 10.0 Hz, *J* = 3.4 Hz, H-3 (α)), 3.83 (t, *J* = 7.0 Hz, H-5 (β)), 3.73 (dd, *J* = 10.0 Hz, *J* = 3.7 Hz, H-2 (α)), 3.57-3.48 (m), 2.44 (dd, *J* = 19.2 Hz, *J* = 8.5 Hz), 2.11-2.01 (m), 1.96-1.63 (m), 1.59-1.43 (m), 1.39-1.20 (m), 1.15.1.07 (m), 1.02-0.91 (m), 0.68 (td, *J* = 0.63 Hz, *J* = 11.4 Hz) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.2 ($\underline{C}OCH_2Cl$ (β)), 167.0 ($\underline{C}OCH_2Cl$), 166.9 ($\underline{C}OCH_2Cl$), 138.4, 138.3, 137.8, 128.4-127.7, 102.2 (C-1 (β)), 95.8 (C-1 (α)), 79.7, 78.8, 77.1, 75.7 (C-3 (α)), 75.4 ($\underline{C}H_2Ph$ (β)), 75.2 (C-2 (α)), 73.4 ($\underline{C}H_2Ph$), 72.6 ($\underline{C}H_2Ph$), 70.1 (C-4 (α)), 70.0 (C-5 (β)), 68.7 (C-4 (β)), 66.0 (C-5 (α)), 63.9 (C-6 (α)), 63.2 (C-6 (β)), 54.4, 51.4, 47.8, 45.2, 44.7, 40.8 ($CO\underline{C}H_2Cl$ (β)), 40.7 ($CO\underline{C}H_2Cl$), 40.5 ($CO\underline{C}H_2Cl$), 36.8, 35.8, 35.7, 35.0, 31.5, 30.8, 29.5, 28.4, 27.4, 21.7, 20.5, 14.2, 13.8, 12.9, 12.3 ppm. **HR-MS:** calcd for $C_{43}H_{54}Cl_2O_9Na^+$ $[M+Na]^+$: 807.3043; found: 807.3018.

Experiment 64. Synthesis of 1-*O*-Adamantanyl-2,3-di-*O*-benzyl-4,6-di-*O*-chloroacetyl-α/β-D-galactopyranoside (79)

The glycosylation reaction of donor **58** with acceptor **26** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **HR-MS:** calcd for $C_{34}H_{40}Cl_2O_8Na^+$ $[M+Na]^+$: 669.1998; found: 669.1986. **Alpha product: $[\alpha]_D^{20}$** +89.8 (c 0.85, CH_2Cl_2).

FT-IR (film) $\upsilon_{máx.}$: 1743, 1763 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.29-7.19 (m, 10H, Ph), 5.52 (d, 1H, *J* = 2.4 Hz, H-4), 5.23 (d, 1H, *J* = 3.7 Hz, H-1 (α)), 4.68-4.62 (m, 2H, C$\underline{H}_2$Ph), 4.63 (d, 1H, *J* = 11.9 Hz, C$\underline{H}_2$Ph), 4.57 (d, 1H, *J* = 10.6 Hz, C$\underline{H}_2$Ph), 4.35 (t, 1H, *J* = 6.5 Hz, H-5), 4.17-4.07 (m, 2H, H-6, H-6), 4.02 (d, 2H, *J* = 3.1 Hz, AcCl), 3.98 (s, 2H, AcCl), 3.95 (dd, 1H, *J* = 3.4 Hz, J=10.0 Hz, H-3), 3.65 (dd, 1H, *J* = 3.7 Hz, *J* = 10.0 Hz, H-2), 2.13 (bs, 3H, C$\underline{H}(CH_2)_3$), 1.79-1.76 (m, 3H), 1.72-1.69 (m, 3H), 1.60-1.51 (m, 6H) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.2 ($\underline{C}OCH_2Cl$), 167.0 ($\underline{C}OCH_2Cl$), 138.5, 138.0, 128.4-127.8, 90.6 (C-1 (α)), 75.3 (C-3), 75.4 (C-2), 75.1, 73.3 ($\underline{C}H_2Ph$), 72.6 ($\underline{C}H_2Ph$), 70.4 (C-4), 65.8 (C-5), 64.0 (C-6), 42.5, 40.9 ($CO\underline{C}H_2Cl$), 40.7 ($CO\underline{C}H_2Cl$), 36.3, 30.7 ($\underline{C}H(CH_2)_3$) ppm. **Beta product: $[\alpha]_D^{20}$** +38.0 (c 0.35, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1747, 1762 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.35-7.28 (m, 10H, Ph), 5.49 (d, 1H, *J* = 2.4 Hz, H-4), 4.91 (d, 1H, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.72-4.66 (m, 2H, C$\underline{H}_2$Ph), 4.53 (d, 1H, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.30 (dd, 1H, *J* = 7.4 Hz, *J* = 11.2 Hz, H-6), 4.22 (dd, 1H, *J* = 5.9 Hz, *J* = 11.2 Hz, H-6), 4.14 (d, 2H, *J* = 2.3 Hz, AcCl), 4.05 (s, 2H, AcCl), 3.83 (t, 1H, *J* = 6.5 Hz, H-5), 3.60-3.53 (m, 2H, H-2, H-3), 2.16 (bs, 3H, C$\underline{H}(CH_2)_3$), 1.91-1.88 (m, 3H), 1.79-1.77 (m, 3H), 1.67-1.58 (m, 6H) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.4 ($\underline{C}OCH_2Cl$), 167.0 ($\underline{C}OCH_2Cl$), 138.5, 137.6, 128.6-127.7, 96.4 (C-1 (β)), 79.2, 78.6, 75.8, 75.5 ($\underline{C}H_2Ph$), 72.8 ($\underline{C}H_2Ph$), 70.0 (C-5), 69.0 (C-4), 63.6 (C-6), 42.7, 40.9 ($CO\underline{C}H_2Cl$), 40.6 ($CO\underline{C}H_2Cl$), 36.3, 30.8 ($\underline{C}H(CH_2)_3$) ppm.

Experiment 65. Synthesis of Benzyl (*2S*)-2-*O*-(4,6-di-*O*-chloroacetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-2-phenylacetate (80)

The glycosylation reaction of donor **58** with acceptor **27** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **$[\alpha]_D^{20}$** +80.5 (c 2.10, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1747 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.49-7.13 (m, 20H, Ph), 5.58 (d, 1H, *J* = 3.0 Hz, H-4), 5.18-5.11 (m, 3H, C$\underline{H}$Ph, $CO_2C\underline{H}_2Ph$), 4.87 (d, 1H, *J* = 3.6 Hz, H-1 (α)), 4.73 (d, 1H, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.66 (d, 1H, *J* = 11.8 Hz, C$\underline{H}_2$Ph), 4.63 (d, 1H, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.45-4.42 (m, 2H, H-5, C$\underline{H}_2$Ph), 4.18-4.04 (m, 3H, H-3, H-6, H-6), 4.08 (s, 2H, AcCl), 4.02 (s, 2H, AcCl), 3.73

(dd, 1H, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.7 ($\underline{C}O_2Me$), 166.9 ($\underline{C}OCH_2Cl$), 166.8 ($\underline{C}OCH_2Cl$), 138.1, 137.7, 135.3, 135.1, 128.9-128.0, 127.9-127.4, 96.7 (C-1 (α)), 78.0 ($\underline{C}HPh$), 75.5 (C-3), 75.1 (C-2), 73.3 ($\underline{C}H_2Ph$), 72.6 ($\underline{C}H_2Ph$), 69.9 (C-4), 67.0 ($CO_2\underline{C}H_2Ph$), 66.9 (C-5), 63.4 (C-6), 40.7 ($CO\underline{C}H_2Cl$), 40.5 ($CO\underline{C}H_2Cl$) ppm. **HR-MS:** calcd for $C_{39}H_{40}Cl_2O_{10}Na^+$ $[M+Na]^+$: 759.1734; found: 759.1726.

Experiment 66. Synthesis of 1-ω-Methoxy-PEG$_{550}$yl-4,6-di-*O*-chloroacetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranoside (81)

The glycosylation reaction of donor **58** with acceptor **28** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1716, 1760 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.46-7.25 (m, Ph), 5.58 (d, *J* = 2.7 Hz, H-4 (α)), 5.50 (bs, H-4 (β)), 4.90 (d, *J* = 10.5 Hz, $C\underline{H}_2Ph$), 4.89 (d, *J* = 3.6 Hz, H-1 (α)), 4.83-4.53 (m, $C\underline{H}_2Ph$), 4.47 (d, *J* = 6.7 Hz, H-1 (β)), 4.32–4.19 (m), 4.16–4.06 (m), 4.04–3.97 (m), 3.90–3.53 (m), 3.37 (s, OMe) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.1 ($\underline{C}OCH_2Cl$), 167.0 ($\underline{C}OCH_2Cl$), 166.9 ($\underline{C}OCH_2Cl$), 138.4, 138.2, 137.7, 137.3, 128.4-128.0, 127.9-127.6, 103.8 (C-1 (β)), 97.7 (C-1 (α)), 78.5, 78.4, 75.5 (C-3 (α)), 75.1, 73.4, 72.6, 72.5, 71.9, 71.8, 70.4, 70.3, 70.1, 70.0 (C-4 (α)), 69.3, 68.6 (C-4 (β)), 67.3, 66.0 (C-5 (α)), 63.5 (C-6 (α), 63.1 (C-6 (β), 59.0 (OMe), 40.77 ($CO\underline{C}H_2Cl$), 40.75 ($CO\underline{C}H_2Cl$), 40.6 ($CO\underline{C}H_2Cl$), 40.5 ($CO\underline{C}H_2Cl$) ppm.

Experiment 67. Sinthesis of *O*-1-L-Menthyl-4,6-di-*O*-chloroacetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranoside (82)

The glycosylation reaction of donor **58** with acceptor **29** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **HR-MS:** calcd for $C_{34}H_{44}Cl_2O_8Na^+$ $[M+Na]^+$: 673.2305; found: 673.2294. **Alpha product: $[\alpha]_D^{20}$** +52.8 (c 2.48, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1748, 1765 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.32-7.26 (m, 10H, Ph), 5.58 (s, 1H, H-4), 5.00 (d, 1H, *J* = 3.0 Hz, H-1 (α)), 4.77 (d, 1H, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.71 (d, 1H, *J* = 10.9 Hz, $C\underline{H}_2Ph$), 4.62 (d, 1H, *J* = 11.6 Hz,

CH_2Ph), 4.57 (d, 1H, *J* = 10.9 Hz, CH_2Ph), 4.36 (t, 1H, *J* = 6.4 Hz, H-5), 4.25-4.15 (m, 2H, H-6, H-6), 4.11 (s, 2H, AcCl), 4.07 (s, 2H, AcCl), 4.02 (dd, 1H, *J* = 2.0 Hz, *J* = 10.0, H-3), 3.73 (dd, 1H, *J* = 2.9 Hz, *J* = 10.0 Hz, H-2), 3.34 (td, 1H, *J* = 4.0 Hz, *J* = 10.5 Hz, CH-O menthol), 2.41-2.35 (m, 1H, CHCH(CH_3)$_2$), 2.06-2.03 (m, 1H, CHCH_2CH), 1.64-1.57 (m, 3H, CHCH_2CH_2CH), 1.43-1.35 (m, 1H, CHCH_3), 1.30-1.25 (m, 1H, CHCH(CH_3)$_2$), 1.07-0.77 (m, 2H, CHCH_2CH, CHCH_2CH_2CH), 0.91 (d, 3H, *J* = 6.5 Hz, CHCH_3), 0.83 (d, 3H, *J* = 6.6 Hz, CH(CH_3)$_2$), 0.69 (d, 3H, *J* = 6.7 Hz, CH(CH_3)$_2$) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.0 (C=O), 166.9 (C=O), 138.3, 137.7, 128.3, 128.2, 128.0, 127.7, 127.6, 127.5, 99.4 (C-1 (α)), 81.3 (CH-O menthol), 75.78, 75.75, 73.8 (CH_2Ph), 72.2 (CH_2Ph), 70.1 (C-4), 66.2 (C-5), 63.9 (C-6), 48.7 (CHCH(CH_3)$_2$), 42.8 (CHCH_2CH), 40.7 (COCH_2Cl), 40.5 (COCH_2Cl), 34.2 (CHCH_2CH_2CH), 31.7 (CHCH_3), 24.5 (CHCH(CH_3)$_2$), 22.9 (CHCH_2CH_2CH), 22.4 (CHCH_3), 21.0 (CH(CH_3)$_2$), 16.0 (CH(CH_3)$_2$) ppm. **Beta product: $[\alpha]_D^{20}$** - 0.75 (c 0.53, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1763 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.36-7.27 (m, 10H, Ph), 5.49 (d, 1H, *J* = 3.2 Hz, H-4), 4.87 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.71 (d, 1H, *J* = 11.2 Hz, CH_2Ph), 4.67 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.54 (d, 1H, *J* = 11.2 Hz, CH_2Ph), 4.44 (d, 1H, *J* = 7.6 Hz, H-1 (β)), 4.28-4.12 (m, 4H, AcCl, H-6, H-6), 4.05 (s, 2H, AcCl), 3.80 (t, 1H, *J* = 6.4 Hz, H-5), 3.57 (dd, 1H, *J* = 3.4 Hz, *J* = 9.6 Hz, H-3), 3.50 (dd, 1H, *J* = 7.6 Hz, *J* = 9.5 Hz, H-2), 3.42 (td, 1H, *J* = 4.2 Hz, *J* = 10.7 Hz, CH-O menthol), 2.34-2.26 (m, 1H, CHCH(CH_3)$_2$), 2.11-2.08 (m, 1H, CHCH_2CH), 1.67-1.64 (m, 2H, CHCH_2CH_2CH), 1.39-1.24 (m, 2H, CHCH_3, CHCH(CH_3)$_2$), 1.02-0.80 (m, 9H), 0.74 (d, 3H, *J* = 6.8 Hz, CH(CH_3)$_2$) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.2 (C=O), 166.9 (C=O), 138.4, 137.5, 128.4, 128.2, 128.17, 128.14, 127.8, 127.6, 101.4 (C-1 (β)), 79.1 (CH-O menthol), 79.0 (C-3), 78.5 (C-2), 75.3 (CH_2Ph), 72.7 (CH_2Ph), 69.2 (C-5), 68.9 (C-4), 63.4 (C-6), 47.9 (CHCH(CH_3)$_2$), 41.1 (CHCH_2CH), 40.8 (COCH_2Cl), 40.5 (COCH_2Cl), 34.3 (CHCH_2CH_2CH), 31.5 (CHCH_3), 25.1 (CHCH(CH_3)$_2$), 23.0 (CHCH_2CH_2CH), 22.1 (CHCH_3), 21.0 (CH(CH_3)$_2$), 15.6 (CH(CH_3)$_2$) ppm.

Experiment 68. Synthesis of *O*-1-Cyclohexyl-4,6-di-*O*-chloroacetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranoside (83)

The glycosylation reaction of donor **58** with acceptor **30** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **HR-MS:** calcd for $C_{30}H_{36}Cl_2O_8Na^+$ $[M+Na]^+$: 617.1679; found: 617.1675. **Alpha product: $[\alpha]_D^{20}$** +94.0 (c 1.06, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1744, 1762 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.32-7.28 (m, 10H, Ph), 5.58 (d, 1H, *J* = 2.9 Hz, H-4), 4.98 (d, 1H, *J* = 3.6 Hz, H-1 (α)), 4.77 (d, 1H, *J* = 11.9 Hz, C$\underline{H}_2$Ph), 4.71 (d, 1H, *J* = 10.7 Hz, C$\underline{H}_2$Ph), 4.63-4.57 (m, 2H, C$\underline{H}_2$Ph), 4.31 (t, 1H, *J* = 6.4 Hz, H-5), 4.24-4.17 (m, 2H, H-6, H-6), 4.14–4.06 (m, 4H, AcCl), 4.00 (dd, 1H, *J* = 3.3 Hz, *J* = 10.0 Hz, H-3), 3.73 (dd, 1H, *J* = 3.3 Hz, *J* = 10.0 Hz, H-2), 3.56-3.49 (m, 1H, C$\underline{H}$-O cyclohexanol), 1.92-1.74 (m, 4H), 1.58-1.53 (2H, m), 1.47-1.14 (m, 4H) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.0 (C$\underline{O}$OCH$_2$Cl), 166.9 (C$\underline{O}$OCH$_2$Cl), 138.3, 137.8, 128.3, 128.0, 127.8, 127.7, 95.8 (C-1 (α)), 76.4 (C$\underline{H}$-O cyclohexanol), 75.7 (C-3), 75.3 (C-2), 73.3 (C$\underline{H}_2$Ph), 72.6 (C$\underline{H}_2$Ph), 70.1 (C-4), 66.0 (C-5), 63.8 (C-6), 40.7 (CO$\underline{C}$H$_2$Cl), 40.5 (CO$\underline{C}$H$_2$Cl), 33.3, 31.5, 25.5, 24.4, 24.1 ppm. **Beta product: $[\alpha]_D^{20}$** +22.7 (c 0.65, CH_2Cl_2). **FT-IR** (film) $\upsilon_{máx.}$: 1748, 1760 (C=O) cm^{-1}. **^{1}H NMR ($CDCl_3$)**: δ 7.35-7.27 (m, 10H, Ph), 5.50 (d, 1H, *J* = 1.8 Hz, H-4), 4.90 (d, 1H, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.70 (t, 2H, *J* = 11.4 Hz, C$\underline{H}_2$Ph), 4.54 (d, 1H, *J* = 11.2 Hz, C$\underline{H}_2$Ph), 4.49 (d, 1H, *J* = 7.4 Hz, H-1 (β)), 4.32 (dd, 1H, *J* = 6.9 Hz, *J* = 11.2 Hz, H-6), 4.23 (dd, 1H, *J* = 6.5 Hz, *J* = 11.2 Hz, H-6), 4.15 (dd, 2H, *J* = 15.1 Hz, *J* = 18.1 Hz, AcCl), 4.07 (s, 2H, AcCl), 3.82 (t, 1H, *J* = 6.7 Hz, H-5), 3.70-3.63 (m, 1H, C$\underline{H}$-O cyclohexanol), 3.60-3.53 (m, 2H, H-2, H-3), 1.98-1.91 (m, 2H), 1.79-1.74 (m, 2H), 1.57-1.50 (m, 2H), 1.47-1.37 (m, 2H), 1.34-1.17 (m, 2H) ppm. **^{13}C NMR** ($CDCl_3$): δ 167.2 (C$\underline{O}$OCH$_2$Cl), 166.9 (C$\underline{O}$OCH$_2$Cl), 138.3, 137.4, 128.4, 128.3, 128.15, 128.11, 127.8, 127.7, 102.1 (C-1 (β)), 78.8, 78.6 (C$\underline{H}$-O cyclohexanol), 78.5, 75.3 (C$\underline{H}_2$Ph), 72.6 (C$\underline{H}_2$Ph), 70.0 (C-5), 68.7 (C-4), 63.2 (C-6), 40.8 (CO$\underline{C}$H$_2$Cl), 40.5 (CO$\underline{C}$H$_2$Cl), 33.6, 31.9, 25.5, 24.0, 23.9 ppm.

Experiment 69. Synthesis of 3-*O*-(4,6-Di-*O*-chloroacetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosyl)-*N*-Boc-L-ser-methyl ester (84)

The glycosylation reaction of donor **58** with acceptor **31** was performed according to the procedure described in experiment 4. The results are presented in Table 4, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1714, 1715, 1747 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.37-7.27 (m, Ph), 5.58 (d, 2H, *J* = 8.2 Hz, NHBoc, H-4 (α)), 5.49 (d, *J* = 2.7 Hz, H-4 (β)), 5.44 (d, *J* = 7.9 Hz, NHBoc (β)), 4.80 (d, *J* = 10.7 Hz, CH_2Ph (β)), 4.79 (s, H-1 (α)), 4.77-67 (m), 4.59-4.51 (m), 4.48-4.45 (m, CH serine), 4.37 (d, *J* = 7.6 Hz, H-1 (β)), 4.31-4.16 (m), 4.15-4.06 (m), 3.92 (dd, *J* = 3.3 Hz, *J* = 10.0 Hz, H-3 (α)), 3.86-3.80 (m), 3.78-3.71 (m, H-2 (α)), 3.76 (s, CO_2Me (β)), 3.67 (s, CO_2Me (α)), 3.59 (dd, *J* = 3.0 Hz, *J* = 9.6 Hz, H-3 (β)), 3.53 (dd, *J* = 9.3 Hz, *J* = 17.0 Hz, H-2 (β)), 1.45 (s, *t*-Bu (α)), 1.42 (s, *t*-Bu (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7 (CO_2Me), 167.0 ($COCH_2Cl$), 166.99 ($COCH_2Cl$), 166.94 ($COCH_2Cl$), 138.1, 137.5, 137.1, 128.4-127.7, 104.0 (C-1 (β)), 99.3 (C-1 (α)), 78.6 (β), 78.0 (β), 75.5 (β), 75.3 (C-3 (α)), 75.1 (C-2 (α)), 73.5 (α), 72.59 (β), 72.52 (α), 70.6 (CH_2 serine (α)), 70.4 (CH_2 serine (β)), 70.2 (C-5 (β)), 69.7 (C-4 (α)), 68.4 (C-4 (b)), 66.8 (C-5 (a)), 63.5 (C-6 (a)), 63.0 (C-6 (b)), 54.1 (CH serine (α)), 54.0 (CH serine (β)),52.6 (OMe (β)), 52.5 (OMe (α)), 40.7 ($COCH_2Cl$ (β)), 40.67 ($COCH_2Cl$ (α)), 40.61 ($COCH_2Cl$ (α)), 40.5 ($COCH_2Cl$ (β)), 28.3 (*t*-Bu) ppm. **HR-MS:** calcd for $C_{33}H_{41}Cl_2O_{12}NNa^+$ $[M+Na]^+$: 736.1904; found: 736.1889.

Experiment 70. Synthesis of Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-D-glucopyranoside (85)

To a solution of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranose[6] (6.42 g, 17.2 mmol) and thiophenol (3.55 mL, 34.4 mmol) in CH_2Cl_2 (60 mL) at 0ºC was added and $BF_3.OEt_2$ (9.81 mL, 77.4 mmol). The reaction mixture was stirred at r.t. for 48 hours, then diluted with CH_2Cl_2 and washed with $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were dried with $MgSO_4$, filtered and concentrated under vacuum. The crude was purified by flash column chromatography on

silica gel (30:70, EtOAc/hexane) to afford **85** (5.40 g, 74%, α/β=2.5:1) as a colourless viscous foam, and to recover the initial tetraacetate (1.30 g, 20%). **FT-IR** (film) $\upsilon_{máx.}$: 1740 (C=O), 2107 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.60-7.58 (m, Ph), 7.51-7.48 (m, Ph), 7.38-7.28 (m, Ph), 5.64 (d, *J* = 5.6 Hz, H-1 (α)), 5.34 (t, *J* = 10.0, H-3 (α)), 5.10-5.02 (m, H-3 (β), H-4 (α)), 4.92 (t, *J* = 10.0 Hz, H-4 (β)), 4.62-4.57 (m, H-5 (α)), 4.49 (d, *J* = 10.0, H-1 (β)), 4.29 (dd, *J* = 5.2 Hz, *J* = 12.4 Hz, H-6 (α)), 4.23-4.18 (m, H-6 (β)), 4.16-4.01 (m, H-2 (α), H'-6 (α)), 3.71-3.67 (m, H-5 (β)), 3.41 (t, *J* = 10.0, H-2 (β)), 2.105 (Ac (α)), 2.06 (Ac (β)), 2.05 (Ac (α)), 2.04 (Ac (β)), 2.03 (Ac (α)), 2.00 (Ac (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.4, 169.7, 134.1, 132.24, 132.21, 130.1, 129.2, 129.1, 128.0, 86.5 (C-1 (α)), 85.3 (C-1 (β)), 75.7 (β), 74.4 (β), 72.0 (α), 68.7 (α), 68.5 (α), 68.0 (β), 62.6 (β), 62.0 (C-6 (β), 61.9 (C-6(α)), 61.6 (α), 20.7 (Ac (β), 20.66 (Ac (α)), 20.64 (Ac (β)), 20.61 (Ac (α)), 20.5 (Ac (β)) ppm.

Experiment 71. Synthesis of *p*-Tolyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-D-glucopyranoside (86)

To a solution of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranose[6] (3.87 g, 10.4 mmol) and p-toluenethiol (2.57 g, 20.7 mmol) in CH_2Cl_2 (60 mL) at 0ºC was added and $BF_3.OEt_2$ (6.57 mL, 51.8 mmol). The reaction mixture was stirred at r.t. for 60 hours, then diluted with CH_2Cl_2 and washed with $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were dried with $MgSO_4$, filtered and concentrated under vacuum. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/hexane) to afford **85** (2.26 g, 50%, α/β=1.8:1) as a colourless viscous foam, and to recover the initial tetraacetate (1.69 g, 44%). **FT-IR** (film) $\upsilon_{máx.}$: 1747 (C=O), 2109 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.48 (d, *J* = 8.0 Hz, Ph), 7.38 (d, *J* = 7.6 Hz, Ph), 7.14 (t, *J* = 10.8 Hz, Ph), 5.56 (d, *J* = 5.6 Hz, H-1 (α)), 5.33 (t, *J* = 9.6 Hz, H-3 (α)), 5.9-5.01 (m, H-3 (β), H-4 (α)), 4.90 (t, *J* = 10.0 Hz, H-4 (β)), 4.63-4.59 (m, H-5 (α)), 4.42 (d, *J* = 10.0 Hz, H-1 (β)), 4.29 (dd, *J* = 5.2 Hz, *J* = 12.4 Hz, H-6 (α)), 4.22 (dd, *J* = 4.4 Hz, *J* = 12.0 Hz, H-6 (β)), 4.16 (dd, *J* = 2.0 Hz, *J* = 12.4 Hz, H'-6 (β)), 4.08-4.01 (m, H-2 (α), H'-6 (α)), 3.69-3.65 (m, H-5 (β)), 3.36 (t, *J* = 10.0 Hz, H-2 (β)), 2.37 (Me (β)), 2.33

(Me (α)), 2.09 (Ac (β)), 2.05 (Ac (α)), 2.04 (Ac (α)), 2.00 (Ac (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5, 170.4, 169.8, 169.7, 169.6, 139.3, 138.4, 134.6, 132.8, 130.0, 129.8, 128.5, 126.1, 86.8 (C-1 (α)), 85.7 (C-1 (β)), 75,7 (C-5 (β)), 74.4 (C-3 (β)), 72.0 (C-3 (α)), 68.7 (C-4 (α)), 68.3 (C-5 (α)), 68.0 (C-4 (β)), 62.4 (C-2 (β)), 62.0 (C-6 (β)), 61.9 (C-6 (α)), 61.6 (C-2 (α)), 21.2 (Me), 21.1 (Me), 20.7 (Ac), 20.66 (Ac), 20.61 (Ac), 20.5 (Ac).

Experiment 72. Synthesis of Phenyl 2-azido-6-*O-tert*-butyldiphenylsilyl-2-deoxy-1-thio-α/β-D-glucopyranoside (87)

A solution of NaOMe 1N (6.73 mL, 6.73 mmol) in MeOH was added to a stirred solution of **85** (4.75 g, 11.21 mmol) in MeOH (20 mL) at 0 ºC. After 3 hours the starting material had been consumed. The reaction mixture was diluted with MeOH and Dowex-H^+ resin was added until neutral pH. Filtration and evaporation of the solvents afforded the triol (3.23 g, 97%) as a viscous gum. To a solution of triol (2.46 g, 8.27 mmol) in pyridine (20 mL) at rt was added TBDPSCl (2.36 mL, 9.10 mmol), followed by a catalytic amount of DMAP. The mixture was stired overnight, then quenched with H_2O (20 mL), extracted with CH_2Cl_2 (3x20 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated. Purification by flash column chromatography (30:70 AcOEt/hexane) afforded the product **87** (4.12 g, 93%, α/β=1.8:1) as a white solid. **Alpha product: FT-IR** (film) $\upsilon_{máx.}$: 2108 (N_3), 3406 (OH) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.76-7.72 (m, 4H, Ph), 7.50-7.39 (m, 8H, Ph), 7.28-7.24 (m, 3H, Ph), 5.55 (d, 1H, *J* = 4.9 Hz, H-1), 4.29-4.24 (m, 1H, H-5), 3.96-3.81 (m, 4H, H-2, H-6, H'-6, H-3), 3.73 (t, 1H, *J* = 8.9 Hz, H-4), 2.67 (bs, 2H, OH), 1.00 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 135.6, 135.5, 133.5, 132.7, 132.6, 132.1, 129.99, 129.94, 129.0, 127.88, 127.81, 127.6, 87.2 (C-1), 73.4, 72.9, 71.1, 64.5 (C-6), 63.4, 26.8 ($C(\underline{C}H_3)_3$), 19.2 (<u>C</u>-Si) ppm. **Beta product: FT-IR** (film) $\upsilon_{máx.}$: 2111 (N_3), 3387 (OH) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.72-7.68 (m, 4H, Ph), 7.46-7.35 (m, 8H, Ph), 7.29-7.23 (m, 3H, Ph), 4.45 (d, 1H, *J* = 10.0 Hz, H-1), 3.98-3.91 (m, 2H, H-6), 3.62 (t, 1H, *J* = 9.1 Hz, H-4), 3.51 (t, 1H, *J* = 9.1 Hz, H-3), 3.40-3.35 (m, 1H, H-5), 3.27 (t, 1H, *J* = 9.4 Hz, H-2), 2.87 (bs, 2H, OH), 1.06 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 135.6, 135.5, 133.2, 132.8,

132.6, 131.4, 129.95, 129.92, 128.9, 128.2, 127.86, 127.84, 86.2 (C-1), 78.7, 77.1, 71.3, 64.5, 64.2 (C-6), 26.8 (C($\underline{C}H_3)_3$), 19.2 ($\underline{C}$-Si) ppm.

Experiment 73. Synthesis of *p*-Tolyl 2-azido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-α/β-D-glucopyranoside (88)

The procedure of experiment 72 was applied to compound **86** affording compound **88** as a colourless viscous gum in 98% yield (α/β=1.8:1) over the two steps. **FT-IR** (film) $\upsilon_{máx.}$: 2108 (N_3), 3385 (OH) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.74-7.65 (m, Ph), 7.47-7.26 (m, Ph), 7.07-7.01 (m, Ph), 5.44 (d, *J* = 5.1 Hz, H-1 (α)), 4.39 (d, *J* = 10.0 Hz, H-1 (β)), 4.25 (dt, *J* = 4.7 Hz, *J* = 9.4 Hz, H-5 (α)), 3.95-3.83 (m, H-3 (α), H-6 (α), H-6 (β)), 3.78 (dd, *J* = 5.2 Hz, *J* = 10.3 Hz, H-2 (α)), 3.68 (t, *J* = 9.0 Hz, H-4 (α)), 3.61 (t, *J* = 9.1 Hz, H-4 (β)), 3.50 (t, *J* = 9.1 Hz, H-3 (β)), 3.37 (dt, *J* = 4.5 Hz, *J* = 9.1 Hz, H-5 (β)), 3.24 (t, *J* = 9.6 Hz, H-2 (β)), 2.84 (bs, OH), 3.32 (s, Me (β)), 2.29 (s, Me (α)), 1.07 (s, *t*-Bu (β)), 1.06 (s, *t*-Bu (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 138.5, 137.9, 136.7, 135.6-135.5, 133.8, 132.9-132.6, 129.9-129.7, 127.8-127.7, 87.6 (C-1 (α)), 86.1 (C-1 (β)), 78.7 (β), 77.3 (β), 73.4 (α), 72.8 (α), 71.3 (α), 71.2 (β), 64.4 (C-6 (α)), 63.3 (β), 64.1 (C-6 (β)), 63.5 (α), 26.88 (C($\underline{C}H_3)_3$ (α)), 26.82 (C($\underline{C}H_3)_3$ (β)), 21.17 (Me (β)), 21.12 (Me (α)), 19.23 ($\underline{C}$-Si) ppm.

Experiment 74. Synthesis of Phenyl 6-*O*-acetyl-2-azido-3,4,di-*O*-benzyl-2-deoxy-1-thio-α/β-D-glucopyranoside (89)

To a stirred solution of **87** (3.91 g, 7.30 mmol) and benzyl bromide (1.97 mL, 22.6 mmol) in DMF (15 mL) at 0ºC was added portion-wise sodium hydride (0.45 g, 18.6 mmol). After 2 hours, MeOH was added at 0ºC and the reaction mixture was quenched with a saturated aqueous solution and extracted with Et_2O. The combined organic phases were dried with $MgSO_4$, filtered and evaporated in vacuum. Purification by flash column chromatography (10:90 AcOEt/hexane) afforded the dibenzylated product (4.45 g, 85%) as a whyte solid, and the tribenzylated product **92** (0.31 g, 7%) as a viscous gum.

Compound 92: FT-IR (film) $\upsilon_{máx.}$: 2105 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.61-7.59 (m), 7.52-7.48 (m), 7.41-7.16 (m), 5.61 (d, *J* = 5.4 Hz, H-1 (α)), 4.93-4.77 (m), 4.63-4.52 (m), 4.44 (d, *J* = 12.0 Hz), 4.41 (d, *J* = 10.0 Hz, H-1 (β)), 4.38-4.35 (m), 3.95 (dd, *J* = 10.0, 5.4 Hz), 3.85-3.72 (m), 3.65-3.58 (m), 3.53-3.47 (m), 3.37-3.32 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 137.8, 137.7, 137.6, 133.6, 133.4, 132.0, 129.0, 128.9, 128.5-127.5, 87.2 (C-1 (α)), 85.9 (C-1 (β)), 85.0 (β), 81.8 (α), 79.3 (β), 78.2 (α), 77.5 (β), 75.8 (β), 75.1 (α), 75.0 (β), 73.45 (α), 73.43 (β), 71.8 (α), 68.7 (C-6 (β)), 68.2 (C-6 (α)), 65.0 (β), 64.1 (α).

To a solution of the dibenzylated product (2.42 g, 3.38 mmol) in THF (10 mL) at r.t. was added TBAF (1.15 g, 4.39 mmol). The reaction mixture was stirred for 3 hours and then water was added. The mixture was extracted with AcOEt (3x20 mL), dried (MgSO4) and concentrated to furnish a yellow viscous residue. Purification by flash column chromatography (30:70, AcOEt/hexane) afforded the alcohol (1.43 g, 88%) as a viscous gum.

To a stirred solution of the alcohol (1.396 g, 2.92 mmol) in pyridine (5 mL) at 0ºC was added acetic anhydride (0.55 mL, 5.85 mmol) and a catalytic amount of DMAP. After complete conversion of the starting material water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to furnish a viscous residue. Filtration through celite with a mixture of EtOAc/hexane (10/90) afforded the product **89** as a viscous colourless gum (1.38 g, 91%, α/β=1.4:1). **^{1}H NMR** ($CDCl_3$): δ 7.64-7.57 (m), 7.57-7.50 (m), 7.47-7.28 (m), 5.62 (d, *J* = 5.3 Hz, H-1 (α)), 5.00-4.86 (m), 4.62 (t, *J* = 10.9 Hz), 4.50-4.42 (m), 4.45 (d, *J* = 10.1 Hz, H-1 (β)), 4.33-4.27 (m), 4.25-4.19 (m), 3.98 (dd, *J* = 10.3 Hz, *J* = 5.3 Hz), 3.89 (dd, *J* = 10.2 Hz, *J* = 8.7 Hz), 3.63-3.47 (m), 3.37 (t, *J* = 9.7 Hz), 2.08 (s), 2.02 (s). **^{13}C NMR** ($CDCl_3$): δ 170.56, 170.54, 137.46, 137.40, 137.35, 133.8, 133.0, 132.3, 131.0, 129.12, 128.94, 128.58, 128.52, 128.22, 128.18, 128.15, 128.13, 128.09, 128.01, 127.98, 127.87, 86.9 (C-1 (α)), 85.9 (C-1 (β)), 85.1, 81.8, 78.0, 77.34, 77.24, 77.12, 77.02, 75.97, 75.86, 75.19, 75.13, 70.1, 65.1, 64.1, 62.87, 62.79, 20.83, 20.78 ppm.

Experiment 75. Synthesis of Phenyl 2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-1-thio-α/β-D-glucopyranoside (90)

The procedure of experiment 74 was applied to compound **87** using chloroacetic anhydride and affording compound **90** as a colourless viscous gum in 66% (α/β=1.6:1) yield over the three steps. Characterisation data of compound **90** identical to the literature[7].

Experiment 76. Synthesis of *p*-Tolyl 2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-1-thio-α/β-D-glucopyranoside (91)

The procedure of experiment 74 was applied to compound **88** using chloroacetic anhydride and affording compound **91** as a colourless viscous gum in 82% yield (α/β=1:1) over the three steps. **FT-IR** (film) $\upsilon_{máx.}$: 1739, 1765 (C=O), 2107 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.45 (d, *J* = 8.1 Hz, Ph), 7.41-7.24 (m, Ph), 7.13-7.11 (m, Ph), 5.50 (d, *J* = 5.1 Hz, H-1 (α)), 4.95 (d, *J* = 10.5 Hz, C$\underline{H}_2$Ph (α)), 4.91-4.81 (m), 4.61-4.55 (m), 4.50-4.43 (m), 4.35-4.30 (m), 4.33 (d, *J* = 10.1 Hz, H-1 (β)), 4.24 (dd, *J* = 11.9 Hz, *J* = 4.6 Hz), 3.98 (s), 3.94-3.83 (m), 3.57-3.41 (m), 3.28 (t, *J* = 9.6 Hz, H-2 (β)), 2.35 (s, PhC$\underline{H}_3$), 2.33 (s, PhC$\underline{H}_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.85, 166.80, 139.1, 138.2, 137.42, 137.32, 134.5, 132.7, 129.97, 129.79, 129.0, 128.62, 128.60, 128.30, 128.22, 128.21, 128.19, 128.13, 128.11, 128.07, 126.6, 87.1 (C-1 (α)), 85.9 (C-1 (β)), 85.2, 81.8, 77.7, 77.4, 77.1, 76.76, 76.74, 76.72, 76.02, 75.88, 75.11, 75.04, 69.8, 64.9, 64.35, 64.22, 64.05, 40.64, 40.57, 21.22, 21.15 ppm.

Experiment 77. Synthesis of Methyl (*2S*)-2-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α/β-D- glucopyranosyl)propanoate (93)

The glycosylation reaction of donor **92** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 5, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1752 (C=O), 2108 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.34-7.24 (m, Ph), 7.17-7.14 (m, Ph), 4.98 (d, *J* = 3.6 Hz, H-1 (α)), 4.90 (d, *J* = 10.6 Hz, C$\underline{H}_2$Ph), 4.87 (s), 4.81-4.76 (m, C$\underline{H}_2$Ph), 4.59 (d, *J* = 12.0 Hz, C$\underline{H}_2$Ph), 4.54-4.42 (m), 4.43 (d, *J* = 7.3 Hz, H-1 (β)), 4.10

(dt, *J* = 2.4 Hz, J = 10.1 Hz), 4.07-4.02 (m), 3.78-3.72 (m), 3.67 (s, CO_2Me), 3.60 (t, *J* = 9.1 Hz, H-3 (β)), 3.52 (dd, *J* = 1.9 Hz, J =10.8 Hz), 3.43 (t, *J* = 7.9 Hz, H-2 (β)), 3.34 (dd, *J* = 3.6 Hz, *J* = 10.3 Hz, H-2 (α)), 1.50 (d, *J* = 6.9 Hz, CHCH_3 (β)), 1.46 (d, *J* = 6.7 Hz, CHCH_3 (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.6, 138.0-137.8, 128.5-128.3, 128.0-127.6, 100.6 (C-1 (β)), 98.8 (C-1 (α)), 83.0 (β), 79.9 (α), 78.1 (α), 77.5 (β), 75.5 (β), 75.3 (α), 75.0 (β), 74.9 (α), 74.6 (α), 73.4, 72.2 (β), 71.3 (α), 68.5 (β), 68.0 (α), 66.0 (β), 63.1 (α), 52.1 (CO_2Me (β)), 52.0 (CO_2Me (α)), 18.8 (CHCH_3 (β)), 18.1 (CHCH_3 (α)) ppm.

Experiment 78. Synthesis of Methyl (*2S*)-2-(6-*O*-acetyl-2-azido-3,4,di-*O*-benzyl-2-deoxy-α/β-D- glucopyranosyl)propanoate (94)

The glycosylation reaction of donor **89** with acceptor **4** was performed according to the procedure described in experiment 4. The results are presented in Table 5, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1741 (C=O), 2107 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.39-7.23 (m), 4.95 (d, *J* = 3.7 Hz, H-1 (α)), 4.93-4.83 (m), 4.78 (d, *J* = 10.7 Hz, CH_2Ph (β)), 4.59-4.54 (m), 4.48-4.43 (m), 4.44 (d, *J* = 7.8 Hz, H-1 (β)), 4.33-4.14 (m), 4.07 (dd, *J* = 8.8 Hz, *J* = 10.3 Hz, H-3 (α)), 3.73 (s, CO_2Me (α)), 3.78 (s, CO_2Me (β)), 3.57 (dd, *J* = 8.9 Hz, *J* = 9.8 Hz, H-4 (α)), 3.45-3.41 (m), 3.32 (dd, *J* = 3.7 Hz, *J* = 10.3 Hz, H-2 (α)), 2.03 (Ac (β)), 2.02 (Ac (α)), 1.48 (d, *J* = 6.9 Hz, CHCH_3 (β)), 1.47 (d, *J* = 6.8 Hz, CHCH_3 (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.4, 170.6, 137.8 (β), 137.7 (α), 137.6 (α), 137.4 (β), 128.54, 128.51, 128.0, 127.9, 100.6 (C-1 (β)), 98.7 (C-1 (α)), 82.9 (β), 79.8 (α), 77.8 (α), 77.1 (β), 75.6 (CH_2Ph (β)), 75.4 (CH_2Ph (α)), 75.09 (CH_2Ph (β)), 75.02 (CH_2Ph (α)), 74.9 (α), 73.0 (β), 72.4 (β), 69.7 (a), 66.0 (β), 63.1 (α), 62.7 (C-6 (β)), 62.5 (C-6 (α)), 52.1 (CO_2Me), 20.8 (Ac), 18.7 (CHCH_3 (β)), 18.2 (CHCH_3 (α)) ppm.

Experiment 79. Synthesis of Methyl (*2S*)-2-(2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-α/β-D- glucopyranosyl)propanoate (95)

The glycosylation reactions of donor **90** and **91** with acceptor **4** were performed according to the procedure described in experiment 4. The results

are presented in Table 5, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1742 (C=O), 2106 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.23 (m, Ph), 4.96-4.84 (m), 4.93 (d, *J* = 3.4 Hz, H-1 (α)), 4.79 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (β)), 4.62-4.56 (m), 4.46-4.39 (m), 4.45 (d, *J* = 7.9 Hz, H-1 (β)), 4.34-4.21 (m), 4.07 (dd, *J* = 8.8 Hz, *J* = 10.3 Hz), 4.03-3.93 (m), 3.78 (s, CO_2Me (β)), 3.73 (s, CO_2Me (α)), 3.56 (dd, *J* = 8.9 Hz, *J* = 9.9 Hz), 3.48-3.41 (m), 3.31 (dd, *J* = 3.7 Hz, *J* = 10.3 Hz), 1.47 (d, *J* = 6.8 Hz, CHC$\underline{H}_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.4, 166.8, 137.6, 137.5, 128.5, 128.1-128.0, 100.5 (C-1 (β)), 98.7 (C-1 (α)), 82.9 (β), 80.0 (α), 77.3 (α), 76.6 (β), 75.6 (β), 75.5 (α), 75.0 (α), 74.9, 72.8 (β), 72.4 (β), 69.5 (α), 65.9 (β), 64.1 (C-6 (β)), 64.0 (C-6 (α)), 63.1 (α), 52.1 (CO_2Me), 40.67 (CO$\underline{C}H_2$Cl (α)), 40.65 (CO$\underline{C}H_2$Cl (β)), 18.7 (CH$\underline{C}H_3$ (β)), 18.2 (CH$\underline{C}H_3$ (β)) ppm.

Experiment 80. Synthesis of Methyl 2-(2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-α/β-D-glucopyranosyl)acetate (96)

The glycosylation reaction of donor **91** with acceptor **7** was performed according to the procedure described in experiment 4. The results are presented in Table 6, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1759 (C=O), 2110 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.40-7.26 (m, Ph), 5.02 (d, *J* = 3.5 Hz, H-1 (α)), 4.96-4.84 (m), 4.81 (d, *J* = 10.6 Hz, C$\underline{H}_2$Ph (β)), 4.60 (d, *J* = 11.1 Hz, C$\underline{H}_2$Ph (a)), 4.55 (d, *J* = 12.0 Hz, C$\underline{H}_2$Ph (β)), 4.45 (d, *J* = 6.5 Hz, H-1 (β)), 4.38 (dd, *J* = 3.6 Hz, *J* = 10.2 Hz, H-6 (α)), 4.31-4.25 (m), 4.18 (d, *J* = 16.6 Hz), 4.07-3.94 (m), 3.78 (s, CO_2Me (β)), 3.77 (s, CO_2Me (α)), 3.55 (dd, *J* = 8.8 Hz, *J* = 10.0 Hz, H-4 (α)), 3.44 (dd, *J* = 3.6 Hz, *J* = 10.2 Hz, H-2 (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.5, 166.8, 137.5, 137.3, 128.58, 128.55, 128.16, 128.13, 128.0, 101.2 (C-1 (β)), 97.8 (C-1 (α)), 80.2 (α), 77.2 (α), 75.6 ($\underline{C}H_2$Ph (α)), 75.0 ($\underline{C}H_2$Ph (α)), 72.8 (β), 69.6 (α), 65.9 (β), 64.3 (α), 64.0 (α), 63.3 (C-2 (α)), 52.1 ($CO_2\underline{C}H_3$ (α)), 40.6 (CO$\underline{C}H_2$Cl (α)) ppm.

Experiment 81. Synthesis of Methyl (*2R*)-*tert*-butyldimethylsilyl-3-(2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-α/β-D- glucopyranosyl)-2,3-dihydroxyropanoate (97)

The glycosylation reaction of donor **91** with acceptor **9** was performed according to the procedure described in experiment 4. The results are presented in Table 6, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1743 (C=O), 2107 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.69-7.26 (m, Ph), 7.69-7.61 (m, Ph), 7.45-7.28 (m, Ph), 7.22-7.19 (m, Ph), 5.10 (d, *J* = 3.5 Hz, H-1 (α)), 4.93 (d, *J* = 10.4 Hz, C$\underline{H}_2$Ph (α)), 4.86 (d, *J* = 11.3 Hz, C$\underline{H}_2$Ph (α)), 4.83 (d, *J* = 10.4 Hz, C$\underline{H}_2$Ph (α)), 4.57 (d, *J* = 11.3 Hz, C$\underline{H}_2$Ph (α)), 4.46 (dd, *J* = 3.8 Hz, *J* = 6.6 Hz, C$\underline{H}$CH$_2$OTBDPS), 4.40 (d, *J* = 8.1 Hz, H-1 (β)), 4.28-4.23 (m), 4.14.4.15 (m), 4.02-3.90 (m), 3.75 (s, CO_2Me (α)), 3.56-3.51 (m), 3.38 (dd, *J* = 3.5 Hz, *J* = 10.3 Hz, H-2 (α)), 1.04 (s, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.6, 166.8, 137.65, 137.63, 135.6-135.5, 132.8, 132.7, 129.9, 129.8, 128.6, 128.5, 128.4, 128.1, 128.0, 127.88, 127.85, 127.78, 127.75, 102.2 (C-1 (β)), 96.3 (C-1 (α)), 83.1 (β), 80.08 (α), 80.02 (β), 75.63 (α), 75.60 (C$\underline{H}_2$Ph (α)), 75.5 (C$\underline{H}_2$Ph (β)), 75.0 (C$\underline{H}_2$Ph (β)), 74.8 (C$\underline{H}_2$Ph (α)), 72.9 (β), 71.9 (α), 69.2, 65.86 (β), 65.81, 64.4 (α), 64.0 (α), 63.9 (β), 63.07 (α), 63.04 (β), 52.3 ($CO_2\underline{C}H_3$ (α)), 52.1 ($CO_2\underline{C}H_3$ (β)), 40.6 (CO$\underline{C}H_2$Cl (β)), 40.5 (CO$\underline{C}H_2$Cl (α)), 26.7 (*t*-Bu (α)), 26.6 (*t*-Bu (β)), 19.2 (C-Si (β)), 19.1 (C-Si (α)).

Experiment 82. Synthesis of Methyl 3-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-[2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-α/β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (98)

The glycosylation reaction of donor **91** with acceptor **24** was performed according to the procedure described in experiment 4. The results are presented in Table 6, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1752 (C=O), 2108 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.71-7.69 (m, Ph), 7.46-7.19 (m, Ph), 5.23 (d, J = 3.5 Hz, H-1 (α)), 5.02-4.81 (m), 4.79-4.68 (m), 4.63 (d, *J* = 11.2 Hz, C$\underline{H}_2$Ph (β)), 4.58-4.52 (m), 4.39 (dd, *J* = 1.8 Hz, *J* = 12.0 Hz, β), 4.22 (dd, J=1.8 Hz,

J=11.9 Hz, α), 4.13-3.83 (m), 3.73-3.46 (m), 3.72 (s, CO_2Me (β)), 3.70 (s, CO_2Me (α)), 3.41 (dd, *J* = 2.5 Hz, *J* = 5.8 Hz), 3.28 (dd, *J* = 3.4 Hz, *J* = 10.2 Hz), 1.04 (s, t-Bu (α)), 1.03 (t-Bu (β)) ppm.

Experiment 83. Synthesis of 1-*O*-Adamantanyl-2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-α/β-D-glucopyranoside (99)

The glycosylation reaction of donor **91** with acceptor **26** was performed according to the procedure described in experiment 4. The results are presented in Table 6, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1763, 1743 (C=O), 2104 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.23 (m, Ph), 5.29 (d, *J* = 3.7 Hz, H-1 (α)), 4.93-4.84 (m), 4.77 (d, *J* = 10.8 Hz, CH_2Ph (β)), 4.59 (d, *J* = 6.4 Hz, H-1 (β)), 4.58 (d, *J* = 10.8 Hz, CH_2Ph (α)), 4.57 (d, *J* = 11.0 Hz, CH_2Ph (β)), 4.38-4.29 (m), 4.20 (dd, *J* = 5.3 Hz, J=11.6 Hz, β), 4.13 (ddd, J=2.4 Hz, J=4.3Hz, J=10.0 Hz, α), 4.11-3.92 (m), 3.51 (dd, *J* = 8.8 Hz, *J* = 9.9 Hz, H-4 (α)), 3.17 (dd, *J* = 3.6 Hz, *J* = 10.3 Hz, H-2 (α)), 2.16 (bs), 1.88-1.76 (m), 1.67-1.57 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.9, 137.8 (β), 137.5 (α), 128.6-128.5, 128.13, 128.10, 128.0, 127.9, 95.1 (C-1 (β)), 95.0 (C-1 (α)), 83.3 (β), 79.9 (α), 77.9 (α), 77.1 (β), 75.7, 75.5 ($\underline{C}H_2Ph$ (β)), 75.2 ($\underline{C}H_2Ph$ (α)), 75.1 ($\underline{C}H_2Ph$ (α)), 74.9 ($\underline{C}H_2Ph$ (β)), 72.4 (β), 68.4 (α), 66.4 (β), 64.7 (C-6 (β)), 64.6 (C-6 (α)), 63.1 (α), 42.36 ($CO\underline{C}H_2Cl$ (β)), 42.30 ($CO\underline{C}H_2Cl$ (α)), 40.6, 46.1, 30.68 (β), 30.62 (α).

Experiment 84. Synthesis of 3-*O*-(2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-α/β-D-glucopyranosyl)-epiandrosterone (100)

The glycosylation reaction of donor **91** with acceptor **25** was performed according to the procedure described in experiment 4. The results are presented in Table 6, Chapter 1. **FT-IR** (film) $\upsilon_{máx.}$: 1742, 1762 (C=O), 2107 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.46-7.43 (m, Ph), 7.41-7.24 (m, Ph), 7.13 (d, *J* = 7.9 Hz, Ph), 5.30 (d, *J* = 3.6 Hz, H-1 (α)), 4.94-4.82 (m), 4.78 (d, *J* = 10.8 Hz, $C\underline{H}_2Ph$ (β)), 4.61-4.55 (m), 4.48 (dd, *J* = 11.9, *J* = 1.9 Hz), 4.39-4.29 (m), 4.24 (dd, *J* = 11.9, *J* = 4.6 Hz), 4.22-4.18 (m), 4.14 (ddd, *J* = 10.1 Hz, *J* = 4.5 Hz, *J* = 2.5 Hz), 4.08 (dd, *J* = 10.3 Hz, *J* = 8.7 Hz, H-3 (α)), 4.03-3.89 (m),

3.54-3.39 (m), 3.28 (dd, *J* = 10.0, *J* = 9.3 Hz, H-5 (α)), 3.17 (dd, *J* = 10.3, *J* = 3.6 Hz, H-2 (α)), 2.35 (s), 2.19-2.13 (m), 1.92-1.75 (m), 1.67-1.57 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 166.99, 166.79, 139.1, 137.9, 137.51, 137.48, 137.32, 137.31, 134.5, 129.8, 128.66, 128.60, 128.56, 128.52, 128.51, 128.29, 128.19, 128.18, 128.14, 128.10, 128.06, 128.02, 127.94, 126.6, 95.1 (C-1 (β)), 91.1 (C-1 (α)), 85.9 (α), 85.1 (α), 83.3 (β), 80.0 (α), 78.0 (α), 77.1 (β), 76.75, 76.71, 76.2, 76.0, 75.8, 75.5, 75.25, 75.12, 75.04, 74.95, 72.4 (β), 68.5 (α), 66.4 (β), 64.85 (α), 64.74 (β), 64.67, 64.2, 63.1 (α), 42.36, 42.31, 40.6, 36.16, 36.11, 30.68, 30.62, 21.2

Experiment 85. Synthesis of Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranose (102)

The synthesis of compound **102** was carried out according to the procedure described in the literature[8].

Experiment 86. Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (103)

The synthesis of compound **103** was carried out according to the procedure described in the literature[9].

Experiment 87. Synthesis of Phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranose (104)

Acceptor **102** (0.140 g, 0.31 mmol) was added to a solution of trichloroacetamidate **103** (0.344 g, 0.46 mmol) in dry CH_2Cl_2 (3 mL). The solution was cooled to -20°C and TMSOTf (56.5 µL, 0.31 mmol) was slowly added. When the reaction was completed, a saturated aqueous solution of $NaHCO_3$ (2 mL) was added, followed by extractions with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (50:50, EtOAc/hexane) to afford **104** as a white foam (0.180 g, 74%). **FTIR** (film) υ

$_{máx}$: 1749 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.55-7.52 (m, 2H, Ph), 7.45-7.42 (m, 2H, Ph), 7.38-7.28 (m, 11H, Ph), 5.58 (s, 1H, C<u>H</u>Ph), 5.56 (d, *J* = 3.1 Hz, 1H, H-1 Man), 5.40 (dd, *J* = 3.1 Hz, *J* = 1.8 Hz, 1H), 5.35-5.26 (m, 2H), 5.06 (d, *J* = 10.9 Hz, 1H, C<u>H</u>$_2$Ph), 4.69 (d, *J* = 9.3 Hz, 1H, H-1 Glu), 4.66 (d, *J* = 10.9 Hz, 1H, C<u>H</u>$_2$Ph), 4.39 (dd, *J* = 10.5 Hz, *J* = 5.0 Hz, 1H), 4.28 (dt, *J* = 9.4 Hz, *J* = 2.5 Hz, 1H), 3.83-3.71 (m, 5H), 3.60 (dd, *J* = 12.6 Hz, *J* = 2.2 Hz, 1H), 3.53-3.48 (m, 1H), 2.17 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.00 (s, 3H, Ac) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.6, 169.99, 169.85, 169.4, 137.7, 137.0, 132.64, 132.58, 129.17, 129.06, 128.40, 128.32, 128.28, 128.24, 128.0, 126.0, 101.3 (C-1 (α) Man), 97.9 (C-1 (β) Glu), 89.1, 81.9, 80.8, 77.4, 77.1, 76.8, 76.6, 75.1 (<u>C</u>H$_2$Ph), 70.1, 69.31, 69.15, 68.76, 68.58, 65.6, 61.4, 20.9 (CO<u>C</u>H$_3$), 20.7 (CO<u>C</u>H$_3$) ppm.

Experiment 88. Synthesis of Methyl 3-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl]-2,3-di-hydroxypropanoate (105)

A suspension of thioglycoside **104** (0.370 g, 0.47 mmol), glycerate **9** (0.204 g, 0.57 mmol) and 4 Å molecular sieves in CH_2Cl_2 (3 mL) was stirred at r.t. for 1 hour. The solution was cooled to 0°C and N-iodosuccinimide (0.135 g, 0.60 mmol) and TMSOTf (42 µL, 0.24 mmol) were added. After all of the starting material had been consumed, a 10% aqueous solution of $Na_2S_2O_3$ (2 mL) and a saturated aqueous solution of $NaHCO_3$ (1 mL) were added to the mixture. After extraction with CH_2Cl_2, the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed in vacuum. The crude product was purified by flash column chromatography (30:70, EtOAc/hexane) to afford the glycoside **105** as a colourless viscous foam (0.190 g, 40%), and the product of the deprotection of the benzylidene acetal **106** (0.100 g, 23%). **$[\alpha]^{20}_D$** = +51.9 (*c* = 2.67, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 1752 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.72-7.70 (m, 4H, Ph), 7.45-7.25 (m, 16H, Ph), 5.56 (s, 1H, C<u>H</u>Ph), 5.49-5.43 (m, 2H), 5.46 (s, 1H, H-1 Man), 5.31-5.20 (m, 2H), 5.26 (bs, 1H, H-1 Glu), 4.92 (d, *J* = 11.0 Hz, 1H, C<u>H</u>$_2$Ph), 4.79 (d, *J* = 10.9 Hz, 1H, C<u>H</u>$_2$Ph), 4.44 (dd, *J* = 6.4, 3.4 Hz, 1H, C<u>H</u>CH$_2$OTBDPS), 4.28-4.24 (m, 1H), 4.21-3.92 (m, 8H), 3.89 (dd, *J* = 9.5, 3.7 Hz, 1H), 3.72-3.63 (m, 2H), 3.66 (s, 3H, CO_2Me),

2.16 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.05 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.6, 169.86, 169.66, 169.63, 169.48, 138.2, 137.3, 135.64, 135.61, 135.54, 135.51, 133.05, 132.86, 129.88, 129.81, 128.9, 128.6, 128.31, 128.26, 128.12, 127.90, 127.81, 127.78, 127.66, 126.1, 101.5 101.3 (C-1 (α) Man), 94.8 (C-1 (β) Glu), 94.3, 82.3, 76.5, 75.5 ($\underline{C}H_2Ph$), 75.1, 73.5, 69.36, 69.20, 68.8, 68.4, 65.9, 64.5, 62.7, 61.9, 52.0 ($CO_2\underline{C}H_3$), 26.7 ($C(\underline{C}H_3)_3$) 20.87 ($CO\underline{C}H_3$), 20.76 ($CO\underline{C}H_3$), 20.73 ($CO\underline{C}H_3$), 20.68 ($CO\underline{C}H_3$), 19.1 (C-Si) ppm.

Experiment 89. Synthesis of Methyl 3-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-α-D-glucopyranosyl]-2,3-di-hydroxypropanoate (106)

To a solution of glycoside **105** (0.190 g, 0.185 mmol) in CH_2Cl_2 (2 mL) was added a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture was stirred until all of the starting material was consumed as confirmed by TLC. After adition of saturated aqueous solution of $NaHCO_3$ (1 mL) and extraction with CH_2Cl_2, the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed in vacuum. The crude product was purified by preparative TLC (50:50, EtOAc/hexane) to afford **106** as a colourless viscous foam (0.158 g, 91%). **$[\alpha]^{20}_D$** = +69.3 (*c* = 1.21, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 3483 (OH), 1751 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.71-7.68 (m, 4H, Ph), 7.45-7.26 (m, 11H, Ph), 5.48-5.45 (m, 2H, H-2 Man, H-3 Man), 5.31-5.27 (m, 1H, H-4 Man), 5.31 (s, 1H, H-1 Man), 5.21 (d, *J* = 3.3 Hz, 1H, H-1 Glu), 5.02 (d, *J* = 11.6 Hz, 1H, $C\underline{H}_2Ph$), 4.77 (d, *J* = 11.6 Hz, 1H, $C\underline{H}_2Ph$), 4.44 (dd, *J* = 6.5 Hz, *J* = 3.4 Hz, 1H, $C\underline{H}CH_2OTBDPS$), 4.18-4.11 (m, 2H, H-5 Man, H-6 Man), 4.09-4.02 (m, 2H, H'-6 Man, $CHC\underline{H}_2OTBDPS$), 3.95 (dd, *J* = 10.8 Hz, *J* = 3.4 Hz, 1H, $CHC\underline{H}_2OTBDPS$), 3.90-3.75 (m, 3H, H-2 Glu, H-3 Glu, H-5 Glu), 3.70-3.68 (m, 2H, H-6 Glu, H'-6 Glu), 3.66 (s, 3H, CO_2Me), 3.59 (dd, *J* = 9.6 Hz, *J* = 8.8 Hz, 1H, H-2 Glu), 2.17 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.06 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.6, 169.93, 169.74, 169.69, 169.54, 138.3, 135.60, 135.51, 133.1, 132.9, 129.87, 129.85, 128.6, 127.89, 127.85, 127.82, 127.79, 94.5 (C-1 Man), 93.6 (C-1 Glu), 80.0, 75.6 ($\underline{C}H_2Ph$), 75.0 ($\underline{C}HCH_2OTBDPS$), 73.7, 71.3, 70.3, 69.23, 69.21, 68.7,

65.8, 64.5 (CH<u>C</u>H$_2$OTBDPS), 62.17 (C-6 Man), 62.01 (C-6 Glu), 52.0 (CO_2<u>C</u>H$_3$), 26.7 (C(<u>C</u>H$_3$)$_3$), 20.86 (CO<u>C</u>H$_3$), 20.76 (CO<u>C</u>H$_3$), 20.66 (CO<u>C</u>H$_3$), 19.2 (C-Si) ppm.

Experiment 90. Synthesis of Methyl (*2R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-α-D-glucopyranosyl]-2,3-di-hydroxypropanoate (107)

TBAF (1M in THF; 0.14 mL, 0.14 mmol) was added to a solution of **106** (0.110 g, 0.117 mmol) in THF (2 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by preparative TLC (80:20, EtOAc/hexane) afforded product **107** as a viscous colourless foam (0.050 g, 73%). **$[\alpha]^{20}_D$** = +84.1 (*c* = 1.00, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 3441 (O–H), 1747 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.42-7.26 (m, 5H, Ph), 5.43 (dd, *J* = 3.2 Hz, *J* = 1.7 Hz, 1H, H-2 Man), 5.36-5.25 (m, 2H, H-3 Man, H-4 Man), 5.23 (d, *J* = 1.4 Hz, 1H, H-1 Man), 5.20 (d, *J* = 3.4 Hz, 1H, H-1 Glu), 4.94 (d, *J* = 11.4 Hz, 1H, C<u>H</u>$_2$Ph), 4.86 (d, *J* = 11.5 Hz, 1H, C<u>H</u>$_2$Ph), 4.39 (dd, *J* = 6.1 Hz, *J* = 3.2 Hz, 1H, C<u>H</u>CH$_2$OH), 4.15-3.74 (m, 10H), 3.70 (s, 3H, CO_2Me), 3.58 (t, *J* = 3.5 Hz, 1H), 3.10 (bs, 3H), 2.16 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.95 (s, 3H, Ac) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.2, 169.91, 169.77, 169.60, 138.3, 128.55, 128.49, 127.81, 127.72, 94.6 (C-1 Man), 93.9 (C-1 Glu), 79.7, 75.6 (<u>C</u>H$_2$Ph), 75.3 (<u>C</u>HCH$_2$OH), 73.8, 71.9, 71.0, 69.4, 69.1, 68.7, 65.5, 63.1, 62.3 (C-6 Man), 62.0 (C-6 Glu), 52.2 (CO_2<u>C</u>H$_3$), 20.84 (CO<u>C</u>H$_3$), 20.73 (CO<u>C</u>H$_3$), 20.70 (CO<u>C</u>H$_3$), 20.64 (CO<u>C</u>H$_3$) ppm.

Experiment 91. Synthesis of Methyl (*2R*)-2-*O*-[α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-α-D-glucopyranosyl]-2,3-di-hydroxypropanoate (108)

A 1N solution of NaOMe (57 µL, 0.057 mmol) in MeOH was added to a stirred solution of **107** (0.058 g, 0.082 mmol) in MeOH (1 mL) at 0 °C. After complete conversion of the starting material, previously activated Dowex-H^+ resin was added until neutral pH. After filtration with MeOH and water, the

solvent was removed in vacuum to yield **108** as a viscous colourless foam (0.026 g, quantitative). **^{1}H NMR** (D_2O): δ 7.50-7.40 (m, 5H), 5.33 (d, *J* = 2.7 Hz, 1H, H-1 Glu), 5.16 (d, *J* = 1.5 Hz, 1H, H-1 Man), 4.85 (s, 2H, C$\underline{H}_2$Ph) 4.57 (t, *J* = 4.0 Hz, 1H, C$\underline{H}$CH$_2$OH), 4.09 (dd, *J* = 3.3 Hz, *J* = 1.7 Hz, 1H, H-2 Man), 4.00-3.94 (m, 2H, CHC$\underline{H}_2$OH), 3.89-3.61 (m, 10H), 3.81 (s, 3H, CO_2Me), 3.56 (t, *J* = 9.2 Hz, 1H) ppm. **^{13}C NMR** (D_2O): δ 172.0 ($\underline{C}O_2$Me), 137.1, 129.0, 128.7, 128.5, 97.0 (C-1 Man), 94.0 (C-1 Glu), 79.5, 75.9 ($\underline{C}H_2$Ph), 75.2 ($\underline{C}$HCH$_2$OH), 73.4, 72.7, 72.3, 70.5, 69.8, 69.4, 66.2, 62.4 (CH$\underline{C}$H$_2$OH), 60.4, 60.2, 52.7 ($CO_2\underline{C}H_3$) ppm.

Experiment 92. Synthesis of Methyl (2*R*)-2-*O*-[α-D-mannopyranosyl-(1→2)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (109)

From compound **108**: Benzyl ether **108** (0.036 g, 0.067 mmol) in EtOH (1 mL) was hydrogenated at 50 psi in the presence of Pd/C 10% (0.25 equiv). After 3 hours, the reaction mixture was filtered and the solvent was evaporated to afford ester **109** as a very viscous colourless foam (0.640 g, quantitative).

From compound **117**: A 1 N solution of NaOMe (34 µL, 0.03 mmol) in MeOH was added to a stirred solution of **117** (0.036 g, 0.06 mmol) in MeOH (1 mL) at 0 °C. After 1 hour, previously activated Dowex-H$^+$ resin was added until neutral pH. After filtration with MeOH and water, the solvent was removed in vacuum to yield **118** as a viscous colourless foam (0.026 g, quantitative).

$[\alpha]^{20}_D$ = +135.6 (*c* = 2.03, H_2O). **^{1}H NMR** (D_2O): δ 5.26 (d, *J* = 3.4 Hz, 1H, H-1 Glu), 5.09 (d, *J* = 1.3 Hz, 1H, H-1 Man), 4.52 (t, *J* = 3.8 Hz, 1H, C$\underline{H}$CH$_2$OH), 4.03 (dd, *J* = 1.6 Hz, *J* = 3.6 Hz, 1H, H-2 Man), 3.91-3.90 (m, 2H, CHC$\underline{H}_2$OH), 3.87-3.63 (m, 10H), 3.77 (s, 3H, CO_2Me), 3.45 (t, *J* = 9.0 Hz, 1H) ppm. **^{13}C NMR** (D_2O): δ 174.6 ($\underline{C}O_2$Me), 99.6 (C-1 Man), 96.8 (C-1 Glu), 77.9 ($\underline{C}$HCH$_2$OH), 76.3, 75.2, 74.7, 73.5, 72.8, 72.4, 71.8, 69.1, 64.9 (CH$\underline{C}$H$_2$OH), 63.4, 62.9, 55.3 ($CO_2\underline{Me}$) ppm. **HRMS**: calcd. for $C_{16}H_{28}O_{14}Na^+$ [M^+ + Na] 467.1371; found 467.1372.

Experiment 93. Synthesis of Potassium (2*R*)-2-*O*-α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoate (101)

A solution of 2 M KOH (320 µL) was added to a stirred solution of ester **118** (0.026 g, 0.06 mmol) in H_2O (1 mL). After all of the starting material had been consumed, the pH was adjusted to 7 with 10% HCl and the solvent was evaporated to afford **1** as a viscous colourless foam (0.027 g, quantitative). The NMR spectroscopic data were identical to those of a natural sample. **$[\alpha]^{20}_D$** = +67.8 (*c* = 0.85, H_2O). **FTIR** (film) $\upsilon_{máx.}$: 3315 (OH), 1655 (C=O) cm^{-1}. **^{1}H NMR** (D_2O): δ 5.12 (d, *J* = 3.4 Hz, 1H, H-1 Glu), 5.06 (d, *J* = 1.12 Hz, 1H, H-1 Man), 4.13 (dd, *J* = 3.2 Hz, *J* = 7.0 Hz, 1H, CHC<u>H</u>$_2$OH), 4.04-4.02 (dd, *J* = 1.6 Hz, *J* = 3.2 Hz 1H, H-2 Man), 3.85-3.58 (m, 12H), 3.39 (t, *J* = 9.3 Hz, 1H) ppm. **^{13}C NMR** (D_2O): δ 176.5 (CO_2^-), 97.1 (C-1 Man), 93.8 (C-1 Glu), 78.3 (<u>C</u>HCH$_2$OH), 73.9, 72.7, 71.8, 71.1, 70.1, 69.7, 69.5, 66.6, 62.9, 60.9, 60.5 ppm. **HRMS**: calcd. for $C_{15}H_{26}O_{14}K^+$ [M^+ + H] 469.09541; found 469.0954.

Experiment 94. Synthesis of Phenyl 1-thio-β-D-glucopyranoside (110)

The synthesis of compound **110** was carried out according to the procedure described in the literature[10].

Experiment 95. Synthesis of Phenyl 3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranoside (111) and Phenyl 2,3-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranoside (112)

The synthesis of compound **110** was carried out according to the procedure described in the literature[11].

Experiment 96. Synthesis of Phenyl 6-*O*-tert-butyldiphenylsilyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranoside (113)

TBDPSCl (1.28 mL, 4.92 mmol) was added to a solution of **111** (0.634 g, 1.64 mmol) in dry DMF (5 mL) at r.t., followed by imidazole (0.391 g, 5.74 mmol). After 12 h at room temperature, the reaction was quenched with H_2O

(5 mL), extracted with CH_2Cl_2 (3x5 mL), and the combined organic phases were dried (MgSO4) and concentrated. Purification by flash column chromatography on silica gel (30:70 EtOAc/hexane) afforded the product **113** as a white solid (0.908 g, 89%). **$[\alpha]^{20}_D$** = +34.1 (*c* = 0.17, CH_2Cl_2). **m.p.** 69.2–71.4 °C. **FTIR** (film) $\upsilon_{máx}$: 3496 (O–H) cm^{-1}. **^{1}H NMR ($CDCl_3$):** δ 7.67-7.72 (m, 4H, Ph), 7.57-7.55 (m, 2H, Ph), 7.42-7.31 (m, 6H, Ph), 7.23-7.19 (m, 3H, Ph), 4.57 (d, *J* = 9.3 Hz, 1H, H-1), 3.99-3.90 (m, 2H, H-6, H'-6), 3.82-3.73 (m, 2H, H-3, H-4), 3.59-3.52 (m, 2H, H-2, H-5), 3.31 (s, 3H, OMe), 3.19 (s, 3H, OMe), 1.33 (s, 3H, Me), 1.28 (s, 3H, Me), 1.06 (s, 9H, *t*-Bu) ppm. **^{13}C NMR ($CDCl_3$):** δ 135.8, 135.5, 133.7, 133.1, 132.8, 131.9, 129.6, 129.5, 129.0, 127.9, 127.66, 127.63, 99.7 ($CH_3\underline{C}OCH_3$), 99.4 ($CH_3\underline{C}OCH_3$), 88.5 (C-1), 78.9 (C-5), 73.7, 69.1 (C-2), 64.9, 61.9 (C-6), 48.2 ($O\underline{C}H_3$), 48.0 ($O\underline{C}H_3$), 26.9 ($C(\underline{C}H_3)_3$), 19.3 (C-Si), 17.7 ($C\underline{C}H_3$), 17.6 ($C\underline{C}H_3$) ppm. **Anal. Calcd for $C_{34}H_{44}O_7SSi$** (624.86): calcd. C 65.35, H 7.10, S 5.13; found C 65.00, H 6.86, S 5.01.

Experiment 97. Synthesis of Phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-6-*O*-*tert*-butyldiphenylsilyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranoside (114)

Acceptor **113** (0.254 g, 0.41 mmol) was added to a solution of trichloroacetamidate **103** (0.250 g, 0.51 mmol) in dry CH_2Cl_2 (4 mL). The solution was cooled to -20°C and TMSOTf (92.30 µL, 0.51 mmol) was slowly added. When the reaction was completed, a saturated aqueous solution of $NaHCO_3$ (2 mL) was added, followed by extractions with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (20:80 EtOAc/hexane) to afford **114** as a white foam (0.237 g, 58%). **$[\alpha]^{20}_D$** = +54.1 (*c* = 1.30, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$:.1752 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.73-7.70 (m, 4H, Ph), 7.55-7.53 (m, 2H, Ph), 7.43-7.19 (m, 9H, Ph), 5.51 (s, 1H, H-1 Man), 5.35-5.33 (m, 3H, H-2 Man, H-3 Man, H-4 Man), 4.73-4.72 (m, 1H, H-5 Man), 4.65 (d, *J* = 8.7 Hz, 1H, H-1 Glu), 4.29 (dd, *J* = 3.6 Hz, *J* = 12.0 Hz, 1H, H-6 Man), 4.18-4.15 (m, 1H, H'-6 Man), 3.95-3.88 (m, 2H, H-6 Glu, H'-6 Glu), 3.79–3.76 (m, 3H, H-2 Glu, H-3 Glu, H-4 Glu), 3.55 (m, 1H, H-5 Glu), 3.32 (s, 3H, OMe), 3.17 (s, 3H, OMe), 2.20 (s, 3H, Ac), 2.12 (s, 3H, Ac),

2.01 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.26 (s, 3H, Me), 1.25 (s, 3H, Me), 1.06 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.8, 169.9, 169.8, 169.6, 135.8, 135.5, 133.9, 133.6, 133.0, 131.9, 131.5, 129.6, 129.5, 129.0, 127.6, 127.5, 99.9 ($CH_3\underline{C}OCH_3$), 99.3 ($CH_3\underline{C}OCH_3$), 97.1 (C-1 Man), 88.7 (C-1 Glu), 78.5 (C-5 Glu), 73.4, 72.8, 69.2, 69.1, 68.6 (C-5 Man), 66.0 (C-5 Man), 65.1 (C-2 Glu), 62.0 (C-6 Glu, C-6 Man), 48.0 ($CH_3CO\underline{C}H_3$), 47.9 ($CH_3CO\underline{C}H_3$), 26.8 ($C(\underline{C}H_3)_3$), 20.9 ($CO\underline{C}H_3$), 20.71 ($CO\underline{C}H_3$), 20.70 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 19.3 (C-Si), 17.6 ($\underline{C}H_3COCH_3$), 17.3 ($\underline{C}H_3COCH_3$) ppm. **Anal. Calcd for $C_{48}H_{62}O_{16}SSi$** (955.15): calcd. C 60.36, H 6.54; found C 60.50, H 6.40.

Experiment 98. Synthesis of Methyl 3-*O*-*tert*-butyldiphenylsilyl-(*2R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-6-*O*-*tert*-butyldiphenylsilyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-di-hydroxypropanoate (115)

A suspension of thioglycoside **114** (0.387 g,0.40 mmol), glycerate **9** (0.143 g, 0.40 mmol) and 4 Å molecular sieves in CH_2Cl_2 (3 mL) was stirred at r.t. for 1 h. The solution was cooled to 0°C and N-iodosuccinimide (0.099 g, 0.44 mmol) and TMSOTf (36 µL, 0.20 mmol) were added. After all of the starting material had been consumed, a 10% aqueous solution of $Na_2S_2O_3$ (2 mL) and a saturated aqueous solution of $NaHCO_3$ (1 mL) were added and the mixture was extracted with CH_2Cl_2; the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed in vacuum. The crude product was purified by flash column chromatography (30:70 EtOAc/hexane) to afford glycoside **115** as a colourless viscous foam (0.286 g, 60%). **$[\alpha]^{20}_D$** = +83.3 (*c* = 1.00, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 1753, 1641 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.68-7.62 (m, 8H, Ph), 7.42-7.30 (m, 12H, Ph), 5.46 (dd, *J* = 3.4 Hz, *J* = 9.9 Hz, 1H, H-3 Man), 5.40-5.39 (m, 1H, H-2 Man), 5.29 (t, *J* = 10.0 Hz, 1H, H-4 Man), 5.20 (d, *J* = 1.4 Hz, 1H, H-1 Man), 5.13 (d, *J* = 3.8 Hz, 1H, H-1 Glu), 4.52-4.48 (m, 1H), 4.37 (dd, *J* = 5.2 Hz, *J* = 3.8 Hz, 1H, $C\underline{H}CH_2$OTBDPS), 4.29-4.18 (m, 3H, H-6 Man, H'-6 Man, H-3 Glu), 3.97 (dd, *J* = 5.4 Hz, *J* = 10.5 Hz, 1H, H-6 Glu), 3.91-3.83 (m, 2H, H'-6 Glu, H-2 Glu), 3.80-3.71 (m, 4H, H-4 Glu, H-5 Glu, $CHC\underline{H}_2$OTBDPS), 3.72 (s, 3H, CO_2Me), 3.28 (s, 3H, OMe), 3.17 (s, 3H, OMe), 2.17 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.97 (s, 3H,

Ac), 1.27 (s, 6H, Me), 1.01 (s, 9H, *t*-Bu), 1.00 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.3, 169.8, 169.6, 169.5, 135.8, 135.6, 135.5, 135.4, 133.7, 133.2, 132.7, 129.8, 129.7, 129.6, 129.5, 127.8, 127.7, 127.5, 127.4, 99.6 ($CH_3\underline{C}OCH_3$), 99.5 ($CH_3\underline{C}OCH_3$), 94.6 (C-1 Man), 93.9 (C-1 Glu), 74.6 ($\underline{C}HCH_2OTBDPS$), 70.9 (C-2 Glu), 70.4 (C-5 Glu), 69.6 (C-2 Man), 69.1 (C-3 Man), 68.5 (C-5 Man), 67.8 (C-3 Glu), 66.3 (C-4 Man), 65.8 (C-4 Glu), 64.4 (C-6 Man), 62.3 (C-6 Man), 61.7 ($CH\underline{C}H_2OTBDPS$), 51.9 ($CO_2\underline{C}H_3$), 47.9 ($CH_3CO\underline{C}H_3$), 47.8 ($CH_3CO\underline{C}H_3$), 26.7 ($C(\underline{C}H_3)_3$), 26.5 ($C(\underline{C}H_3)_3$), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.69 ($CO\underline{C}H_3$), 20.67 ($CO\underline{C}H_3$), 19.3 (C-Si), 19.1 (C-Si), 17.6 ($\underline{C}H_3COCH_3$), 17.6 ($\underline{C}H_3COCH_3$) ppm. **Anal. Calcd for $C_{62}H_{82}O_{20}Si_2$** (1203.49): calcd. C 61.88, H 6.87; found C 61.90, H 6.75.

Experiment 99. Synthesis of Methyl (*2R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (116)

TBAF (1M in THF; 0.22 mL, 0.22 mmol) was added to a solution of **115** (0.246 g, 0.20 mmol) in THF (3 mL) at room temperature. The reaction mixture was stirred for 3 h and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by column chromatography (EtOAc) afforded product **116** as a viscous colourless foam (0.103 g, 71 %). **$[\alpha]^{20}_D$** = +155.2 (*c* = 1.82, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 3463 (O–H), 1747, 1636 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 5.39-5.37 (m, 1H, H-2 Man), 5.35-5.28 (m, 2H, H-3 Man, H-4 Man), 5.19 (d, *J* = 3.8 Hz, 1H, H-1 Glu), 5.13 (s, 1H, H-1 Man), 4.50-4.48 (m, 1H, H-5 Man), 4.39-4.36 (m, 1H, $C\underline{H}CH_2OH$), 4.26-4.17 (m, 2H, H-6 Man, H'-6 Man), 4.10 (t, *J* = 10.0 Hz, 1H, H-3 Glu), 3.95-3.81 (m, 5H), 3.75-3.67 (m, 2H), 3.73 (s, 3H, OMe), 3.34 (s, 3H, CO_2Me), 3.24 (s, 3H, OMe), 2.17 (s, 3H, OMe), 2.13 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.30 (s, 3H, Me), 1.26 (s, 3 H, Me) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.1, 169.9, 169.74, 169.70, 99.8 ($CH_3\underline{C}OCH_3$), 99.7 ($CH_3\underline{C}OCH_3$), 94.9 (C-1 Man), 94.6 (C-1 Glu), 75.4 ($\underline{C}HCH_2OH$), 71.4 (C-2 Glu), 70.0 (C-5 Glu), 69.3 (C-3 Man), 69.2 (C-2 Man), 68.7 (C-5 Man), 67.4 (C-3 Glu), 66.0 (C-4 Glu), 65.8 (C-4 Man), 63.3 ($CH\underline{C}H_2OH$), 62.1 (C-6 Man), 60.9 (C-6 Glu), 52.2 ($CO_2\underline{C}H_3$), 47.9

($CH_3CO\underline{C}H_3$), 47.8 ($CH_3CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 17.6 ($\underline{C}H_3COCH_3$), 17.5 ($\underline{C}H_3COCH_3$) ppm. **HRMS:** calcd. for $C_{30}H_{46}O_{20}Na^+$ [M^+ + Na] 749.2475; found 749.2451.

Experiment 100. Synthesis of Methyl (*2R*)-2-*O*-[2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (117)

A mixture of TFA/H_2O (5:1) (0.8 mL) was added to a solution of **116** (0.093 g, 0.13 mmol) in CH_2Cl_2 (1 mL) at r.t. The reaction mixture was stirred until all of the starting material had been consumed and then it was concentrated to give a viscous residue. Purification by flash column chromatography (1:4, MeOH/CH_2Cl_2) afforded product **117** as a viscous colourless foam (0.055g, 70%). $[\alpha]^{20}_D$ = +72.2 (*c* = 0.46, EtOH). **FTIR** (film) $\upsilon_{máx}$: 3460 (O–H), 1748 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 5.43-5.42 (m, 1H, H-2 Man), 5.38 (dd, *J* = 3.2 Hz, *J* = 10.0 Hz, 1H, H-3 Man), 5.26 (t, *J* = 10.0 Hz, 1H, H-4 Man), 5.20 (d, *J* = 3.6 Hz, 1H, H-1 Glu), 5.17 (d, *J* = 1.4 Hz, 1H, H-1 Man), 4.48 (t, *J* = 4.2 Hz, 1H, $C\underline{H}CH_2OH$), 4.46-4.39 (m, 2H, H-5 Man, H-6 Man), 4.15-4.12 (m, 1H, H'-6 Man), 3.88-3.68 (m, 7H), 3.69 (s, 3H, CO_2Me), 3.43 (t, *J* = 9.4 Hz, 1H, H-4 Glu), 2.18 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.98 (s, 3H, Ac) ppm. **^{13}C NMR** ($CDCl_3$): δ 173.7, 173.1, 172.8, 172.7, 172.0, 95.4 (C-1 Man), 94.8 (C-1 Glu), 75.9 ($\underline{C}HCH_2OH$), 75.4 (C-2 Glu), 72.2 (C-5 Glu), 70.9 (C-3 Glu), 69.7 (C-3 Man), 69.3, 68.4 (C-5 Man), 65.5 (C-4 Man), 62.3 ($CH\underline{C}H_2OH$), 61.8 (C-6 Man), 60.3 (C-6 Glu), 52.7 ($CO_2\underline{C}H_3$), 20.1 ($CO\underline{C}H_3$), 20.0 ($CO\underline{C}H_3$) ppm. **HRMS**: calcd. for $C_{24}H_{36}O_{18}Na^+$ [M^+ + Na] 635.1794; found 635.2451.

Experiment 101. Synthesis of 6-*O*-[Phenyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranosyl] Succinate (118)

Succinic anhydride (0.041 g, 0.41 mmol), followed by diisopropylethylamine (171 µL, 0.98 mmol) and a catalytic amount of DMAP were added to a solution of **111** (0.160 g, 0.41 mmol) in CH_2Cl_2 (2 mL) at 0°C. The reaction mixture was stirred as the temperature was allowed to rise to r.t. After 3 hours, the reaction was quenched and washed with H_2O. The aqueous phase

was extracted with AcOEt (3x 5 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated. Purification by flash column chromatography (80:20 EtOAc/hexane) afforded product **118** as a viscous colourless oil (0.180 g, 90 %). $[\alpha]^{20}_D$ = +110.5 (*c* = 0.19, CH_2Cl_2). **FTIR** (film) $\nu_{máx}$: 3488 (O–H), 1736 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.56–7.53 (m, 2H, Ph), 7.33–7.29 (m, 3H, Ph), 4.52 (d, *J* = 9.3 Hz, 1H, H-1), 4.48 (dd, *J* = 1.9 Hz, *J* = 11.8 Hz, 1H), 4.21 (dd, *J* = 5.4, *J* = 11.9 Hz, 1H), 3.77–3.71 (m, 2H), 3.59 (t, *J* = 9.8Hz, 1H), 3.48 (t, *J* = 9.32 Hz, 1H), 3.30 (s, 3H), 3.21 (s, 3H), 2.72–2.63 (m, 4H), 1.32 (s, 3H), 1.28 (s, 3H) ppm. **^{13}C NMR** ($CDCl_3$): δ 176.6, 171.8, 133.5, 130.9, 128.9, 128.5, 99.8, 99.7, 88.0 (C-1), 75.7, 73.3, 69.0, 65.6, 62.8, 48.05, 48.02, 28.7, 28.6, 17.6, 17.5 ppm. **HRMS**: calcd. for $C_{22}H_{30}O_{10}SNa^+$ [M^+ + Na] 509.1452; found 509.1454.

Experiment 102. Synthesis of Resin-Bound 119

Thioglucoside **118** (0.130 g, 0.27 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0°C. HOBt (0.036 g, 0.27 mmol) was added, followed by DIC (0.041 mL, 0.37 mmol), and the solution was stirred at 0 °C for 10 min. The reaction mixture was transferred by cannula to Tentagel MB-NH_2 resin (0.601 g, 0.24 mmol) swelled in CH_2Cl_2 (2 mL), and the reaction was shaken at room temperature for 6 hours. The resin was filtered, and rinsed three times with DMF, MeOH and CH_2Cl_2, and dried in vacuum over phosphorus pentoxide. **HR-MAS ^{1}H NMR** spectroscopy indicated successful coupling, with a theoretical resin loading of 0.32 mmol.g-^{1}, as calculated from the resin weight gain.

Experiment 103. Synthesis of Resin-Bound 120

Resin-bound thioglucoside acceptor **119** (0.711 g, 0.23 mmol) was swelled in CH_2Cl_2 (2 mL) with 4 Å molecular sieves. Donor **103** (0.340 g, 0.69 mmol) was dissolved in CH_2Cl_2, transferred by cannula to the reaction flask and shaken for 1 hour. The reaction was cooled to -20°C and TMSOTf (0.049 mL, 0.27 mmol) was added. The solution phase was filtered after 15 min and the resin washed three times with DMF, MeOH, Et_2O and CH_2Cl_2. The resin was dried in vacuum over phosphorus pentoxide. **HR-MAS ^{1}H NMR** spectroscopy

indicated successful coupling, with a theoretical resin loading of 0.28 mmol.g^{-1}, as calculated from the resin weight gain.

Experiment 104. Synthesis of Resin-Bound 121

Resin-bound donor **120** (0.350 g, 0.10 mmol) was swelled in CH_2Cl_2 (2 mL) with 4 Å molecular sieves. Acceptor **9** (0.280 g, 0.78 mmol) was dissolved in CH_2Cl_2, transferred by cannula to the reaction flask and the reaction mixture was shaken at r.t. for 1 h. The reaction was cooled to -20°C, NIS (0.037 g, 0.12 mmol) and TMSOTf (0.013 mL, 0.07 mmol) were added, and the reaction shaken at -20°C for min. The solution phase was filtered out and the resin was washed three times with DMF, MeOH, Et_2O and CH_2Cl_2. The resin was dried in vacuum over phosphorus pentoxide. **HR-MAS ^{1}H NMR** spectroscopy indicated successful coupling with a theoretical resin loading of 0.186mmol.g^{-1}, as calculated from resin weight gain.

Experiment 105. Cleavage from the Resin: Methyl 3-*O*-*tert*-butyldiphenylsilyl-(2*R*)-2-*O*-[α-D-mannopyranosyl-(1→2)-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (122)

Resin **121** (0.430 g) was swelled in CH_2Cl_2 for 30 min and then suspended in MeOH. A 1 N solution of NaOMe (0.6 equiv.) was added and the reaction was stirred for 5 h at r.t. The resin was filtered and washed six times with CH_2Cl_2 and MeOH. The solution phase was treated with previously activated Dowex-H^+, filtered with MeOH and concentrated under reduced pressure. Purification was accomplished by preparative TLC (1:9, MeOH/ CH_2Cl_2) to give the desired product **122** (2 mg, 18%). This product was very polar and for further characterization it was acetylated to afford **123**. Compound **122**: ^{1}H NMR ($CDCl_3$): δ 7.68-7.64 (m, 4H), 7.42-7.39 (m, 6H), 5.16-5.12 (m, 2H, H-1 Man, H-1 Glu), 4.41-4.39 (m, 1H), 4.15-3.58 (m, 14H), 3.75 (s, 3H), 3.21 (s, 3H), 3.17 (s, 3H), 1.26 (s, 3H), 1.22 (s, 3H), 1.02 (s, 9H) ppm.

Experiment 106. Synthesis of Methyl 3-*O*-*tert*-Butyldiphenylsilyl-(2*R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-6-*O*-acetyl-3,4-di-*O*-

(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (123)

Acetic anhydride (5 µL, $5.3x10^{-2}$ mmol) and a catalytic amount of DMAP were added to a solution of **122** (2 mg, $2.5x10^{-3}$ mmol) in pyridine (0.5 mL) at r.t. After 12 hours, the reaction was quenched with H_2O (1 mL), extracted with EtOAc (3x2 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated. Purification by flash column chromatography on silica gel (30:70, EtOAc/hexane) afforded product **123** as a viscous colourless oil (3 mg, 100 %). **$[\alpha]^{20}_D$** = +101.5 (*c* = 1.99, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 1747 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.70-7.66 (m, 4H, Ph), 7.43-7.42 (m, 6H, Ph), 5.45 (dd, *J* = 3.4 Hz, *J* = 9.9 Hz, 1H), 5.39–5.37 (m, 1H), 5.30 (t, *J* = 10.0 Hz, 1H), 5.18 (d, *J* = 3.7 Hz, 1H, C-1 Glu), 5.16 (d, *J* = 0.96 Hz, 1 H, C-1 Man), 4.50-4.46 (m, 1H), 4.44 (dd, *J* = 6.0 Hz, *J* = 3.6 Hz, 1H), 4.23-4.10 (m, 5H), 4.07-4.00 (m, 2H), 3.93-3.88 (m, 2H), 3.71-3.64 (m, 1H), 3.68 (s, 3H), 3.29 (s, 3H), 3.19 (s, 3H), 2.17 (s, 3H),2.13 (s, 3H),2.04 (s, 3H), 2.03 (s, 3H),1.97 (s, 3H), 1.27 (s, 3H), 1.26 (s, 3H), 1.04 (s, 9H) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.6, 170.0, 169.8, 169.7, 169.6, 135.6, 135.5, 133.1, 132.8, 129.8, 129.7, 127.9, 127.86, 127.84, 99.8 (C-1 Man), 99.7 (C-1 Glu), 94.7, 94.2, 75.0, 70.8, 69.4, 69.1, 68.5, 67.5, 67.4, 66.2, 66.0, 64.3, 62.1, 62.0, 60.3, 52.0, 47.8, 47.7, 26.6, 20.9, 20.76, 20.71, 20.70, 20.6, 19.1, 17.6, 17.5, 14.1 ppm.

Experiment 107. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(*2R*)-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2,3-dihydroxypropanoate (125)

Acceptor **9** (0.546 g, 1.52 mmol) was added to a solution of trichloroacetamidate **103** (0.750 g, 1.52 mmol) in dry CH_2Cl_2 (5 mL). The solution was cooled to 0°C and $BF_3.OEt_2$ (0.193 mL, 1.52 mmol) was slowly added. When the reaction was completed, a saturated aqueous solution of $NaHCO_3$ was added, followed by extractions with CH_2Cl_2 (3x10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (30:70, EtOAc/hexane) to afford **125** as a white foam (0.888 g, 85%). **^{1}H NMR**

($CDCl_3$): δ 7.71-7.67 (m, 4H, Ph), 7.45-7.41 (m, 6H, Ph), 5.49-5.45 (m, 2H, H-2, H-3), 5.33-5.27 (t, *J* = 9.9 Hz, 1H, H-4), 5.04 (d, *J* = 1.3 Hz, 1H, H-1), 4.49 (dd, *J* = 6.9 Hz, *J* = 3.4 Hz, 1H, C<u>H</u>CH_2OTBDPS), 4.23-4.17 (m, 2H), 4.03-3.99 (m, 1H), 3.97-3.90 (m, 2H), 3.72 (s, 3H, CO_2Me), 2.16 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.05 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.6, 169.81, 169.68, 169.62, 169.52, 135.57, 135.47, 132.70, 132.56, 129.94, 129.89, 127.92, 127.90, 96.3 (C-1), 75.6, 69.2, 69.0, 68.7, 65.9, 64.4, 62.2, 52.3 (CO_2<u>C</u>H_3), 26.7 (C(<u>C</u>H_3)$_3$), 20.88 (CO<u>C</u>H_3), 20.71 (CO<u>C</u>H_3), 20.69 (CO<u>C</u>H_3), 20.63 (CO<u>C</u>H_3), 19.1 (C-Si) ppm.

Experiment 108. Synthesis of Methyl (*2R*)-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2,3-dihydroxypropanoate (126)

TBAF (1M in THF; 1.28 mL, 1.28 mmol) was added to a solution of **125** (0.880 g, 1.28 mmol) in THF (6 mL) at room temperature. The reaction mixture was stirred for 3 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded product **126** as a viscous colourless foam (0.511 g, 89 %). **$[\alpha]^{20}_D$** = +73.2 (*c* = 4.27, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 1751 (C=O), 3493 (O–H) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 5.43-5.37 (m, 2H, H-2, H-3), 5.28 (t, *J* = 9.9 Hz, 1H, H-4), 5.06 (d, *J* = 1.6 Hz, 1H, H-1), 4.38 (dd, *J* = 5.8 Hz, *J* = 3.4 Hz, 1H, C<u>H</u>CH_2OH), 4.25 (dd, *J* = 12.3 Hz, *J* = 5.6 Hz, 1H, H-6), 4.15-4.09 (m, 2H, H-5, H'-6), 3.98 (dd, *J* = 12.1 Hz, *J* = 3.4 Hz, 1H, CHC<u>H</u>$_2$OH), 3.91 (dd, *J* = 12.1 Hz, *J* = 5.9 Hz, 1H, CHC<u>H</u>$_2$OH), 3.78 (s, 3H, CO_2Me), 2.16 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.00 (s, 3H, Ac) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.5, 169.68, 169.64, 169.55, 96.89, 96.85 (C-1), 76.0, 69.28, 69.22, 68.8, 66.26, 66.21, 62.5, 52.4 (CO_2<u>C</u>H_3), 20.75 (CO<u>C</u>H_3), 20.61 (CO<u>C</u>H_3), 20.58 (CO<u>C</u>H_3), 20.54 (CO<u>C</u>H_3) ppm.

Experiment 109. Synthesis of Methyl (*2R*)-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-3-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-2,3-dihydroxypropanoate (127)

A suspension of thioglucoside donor **1** (0.128 g, 0.24 mmol), acceptor **126** (0.107 g, 0.24 mmol) and 4Å MS in diethyl ether (4 mL) was stirred for 1 h at room temperature then cooled to -60°C. *N*-Iodosuccinimide (0.069 g, 0.30 mmol) and TfOH (1.42 μL) were added at -60°C and when the reaction was complete, 10% $Na_2S_2O_3$ aqueous solution (2 mL) and saturated aqueous $NaHCO_3$ solution (1 mL) were added. The mixture was extracted with CH_2Cl_2 (3x6 mL), the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed under vacuum. The crude product was purified by preparative TLC (50:50, EtOAc/Hex) to afforded product **126** as a viscous colourless foam (0.143 g, 65 %, α/β=4.6:1). **FTIR** (film) $\upsilon_{máx}$: 1745 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.38-7.25 (m, Ph), 5.45-5.27 (m), 5.06 (d, *J* = 1.7 Hz, H-1 Man (α, α)), 5.05 (d, *J* = 1.7 Hz, H-1 Man (α, β)), 4.99 (d, *J* = 10.7 Hz, C$\underline{H}_2$Ph (α, α)), 4.92 (d, *J* = 10.9, C$\underline{H}_2$Ph (α, β)), 4.91 (d, *J* = 10.9, C$\underline{H}_2$Ph (α, β)), 4.87-4.73 (m), 4.87 (d, *J* = 3.9 Hz, H-1 Glu (α, α)), 4.69-4.65 (m), 4.61-4.57 (m), 4.54 (dd, *J* = 7.0 Hz, *J* = 3.2 Hz), 4.45 (d, *J* = 7.8 Hz, H-1 Glu (α, β)), 4.35-4.31 (m), 4.29-4.17 (m), 4.16-4.09 (m), 4.02-3.93 (m), 3.90-3.83 (m), 3.76-3.72 (m), 3.74 (s, CO_2Me (α, α)), 3.70 (s, CO_2Me (α, β)), 3.67-363 (m), 3.55 (dd, *J* = 9.5 Hz, *J* = 3.6 Hz, H-2 Glu (α,α)),3.52-3.48 (m), 3.44 (dd, *J* = 9.1 Hz, *J* = 7.9 Hz, H-2 Glu (α,β)), 2.154 (s, Ac (α, α)), 2.151 (s, Ac (α, β)), 2.09 (s, Ac (α, β)), 2.07 (s, Ac (α, α)), 2.04 (s, Ac (α, β)), 2.03 (s, Ac (α, α)), 1.97 (s, Ac (α, β)), 1.96 (s, Ac (α, α)), 1.82 (s, Ac (α, β)), 1.79 (s, Ac (α, α)) ppm.**^{13}C NMR** ($CDCl_3$): δ 170.7-169.2, 138.5, 138.3, 138.2, 138.1, 138.0, 137.6, 128.4-127.6, 104.2 (C-1 Glu (α, β)), 97.5 (C-1 Glu (α, α)), 96.6 (C-1 Man), 84.5 (α, β), 82.1 (α, β), 82.0 (α, α), 80.0 (α, α), 77.3 (α, β), 75.72 (α, β), 75.70 (α, α), 75.0 (α, α), 74.9 (α, β), 74.7 (α, β), 73.9 (α, α), 73.0 (α, β), 72.9 (α, α), 69.6 (α, β), 69.25 (α, α), 69.20, 69.15 (α, α), 68.95 (α, α), 68.8 (α, β), 68.3 (α, β), 67.8 (α, α), 66.0 (α, β), 65.6 (α, α), 62.95 (α, β), 62.85 (α, β), 62.3 (α, β), 62.1 (α, α), 52.5 ($CO_2\underline{C}H_3$ (α, α)), 52.4 ($CO_2\underline{C}H_3$ (α, β)), 20.87 (α, α), 20.85 (α, α), 20.84 (α, β), 20.76 (α, β), 20.73 (α, β), 20.70 (α, α), 20.62 (α, α),

20.60 (α, β), 20.4 (α, α), 20.3 (α, β) ppm. **HRMS**: calcd. for $C_{47}H_{56}O_{19}Na^+$ [M^+ + Na] 947.3313; found 947.3303.

Experiment 110. Synthesis of Methyl (*2R*)-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α/β-D-glucopyranosyl)-2,3-dihydroxypropanoate (128)

The glycosylation reaction of thioglucoside donor **3** (0.178 g, 0.313 mmol) and acceptor **126** (0.140 g, 0.313 mmol) was performed according to the procedure described in experiment 106. The crude was purified by preparative TLC (40:60, EtOAc/Hex) affording the product **128** as a viscous colourless foam (0.090 g, 30 %, α/β=6:1), and recovery of initial donor **3** (0.085 g, 60 %) and of the hydrolysed donor in the anomeric position (0.017 g, 10 %). **FTIR** (film) $\upsilon_{máx}$: 1753 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.37-7.24 (m), 5.47-5.38 (m), 5.34-5.29 (m), 5.06 (d, *J* = 1.7 Hz, H-1 Man (α, α)), 5.05 (d, *J* = 1.6 Hz, H-1 Man (α, β)), 4.99 (d, *J* = 10.7 Hz), 5.06-5.04 (m), 4.99 (d, *J* = 10.7 Hz), 4.86 (d, *J* = 11.2 Hz), 4.81-4.73 (m), 4.64 (dd, *J* = 15.8, 11.6 Hz), 4.48-4.25 (m), 4.18-4.09 (m), 4.06-3.93 (m), 3.90-3.84 (m), 3.76-3.67 (m), 3.76 (s, CO_2Me (α, α)), 3.74 (s, CO_2Me (α, β)), 3.63-3.46 (m), 2.17 (s), 2.16 (s), 2.12 (s), 2.11 (s), 2.04 (s), 2.03 (s), 1.99 (s), 1.98 (s) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.69, 170.58, 169.85, 169.82, 169.78, 169.72, 169.69, 169.58, 169.1, 167.0, 138.4, 138.2, 137.9, 128.53, 128.46, 128.39, 128.07, 127.90, 127.88, 127.87, 127.82, 127.71, 97.4, 96.6, 95.99, 95.93, 82.1, 80.0, 77.29, 77.28, 75.7, 74.9, 73.8, 73.55, 73.49, 73.0, 69.31, 69.27, 69.22, 69.15, 69.02, 68.94, 68.93, 68.6, 67.7, 66.05, 65.99, 65.6, 64.5, 62.35, 62.32, 62.22, 62.14, 52.56, 52.41, 40.7, 37.89, 37.76, 23.96, 23.88, 23.83, 22.2, 22.0, 20.89, 20.86, 20.81, 20.71, 20.69, 20.63, 20.4 ppm.

Experiment 111. Synthesis of Methyl (*2R*)-2-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-acetyl-α/β-D-glucopyranosyl)-2,3-dihydroxypropanoate (130)

The glycosylation reaction of thioglucoside donor **3** (0.009 g, 0.017 mmol) and acceptor **129** (0.005 g, 0.017 mmol) was performed according to the procedure described in experiment 106. The crude was purified by

preparative TLC (30:70, EtOAc/Hex) affording the product **130** as a viscous colourless gum (0.007 g, 54 %, α/β=2.8:1). **FTIR** (film) $\upsilon_{máx}$: 1741 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.37-7.26 (m, Ph), 5.20 (d, *J* = 3.5 Hz, H-1 (α)), 4.98-4.94 (m), 4.89-4.84 (m, 3H), 4.81-4.77 (m), 4.74 (d, *J* = 7.7 Hz, H-1 (β)), 4.70 (d, *J* = 11.8 Hz, C$\underline{H}_2$Ph (α)), 4.57 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph (α)), 4.37-4.17 (m), 4.10 (dt, *J* = 10.0, 3.2 Hz), 4.00 (t, *J* = 9.2 Hz), 3.72-3.67 (m), 3.59-3.48 (m, 3H), 3.41 (dd, *J* = 9.1 Hz, *J* = 7.7 Hz), 2.04 (s, Ac (α)), 2.03 (s, Ac (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.8, 138.5, 137.77, 137.69, 128.52, 128.50, 128.45, 128.16, 128.10, 128.08, 128.02, 127.87, 127.83, 127.76, 97.4 (C-1 (β)), 91.2 (C-1 (α)), 84.5, 83.0, 81.6, 80.0, 77.26, 77.20, 75.78, 75.74, 75.06, 75.04, 74.8, 73.3, 73.1, 68.9, 63.15, 63.08, 20.9 ppm.

Experiment 112. Synthesis of Methyl (*2R*)-2-*O*-(α-D-mannopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-2,3-dihydroxypropanoate (131)

A 1N solution of NaOMe (0.17 mL, 0.17 mmol) in MeOH was added to a stirred solution of **127** (0.264 g, 0.28 mmol) in MeOH (1 mL) at 0 °C. After complete conversion of the starting material, previously activated Dowex-H^+ resin was added until neutral pH. After filtration with MeOH, the solvent was removed in vacuum. Purification by flash column chromatography on silica gel (40:10, $CHCl_3$/MeOH) afforded the α (0.156 g, 76 %) and β (0.028 g, 14 %) products as viscous colourless gums. **Alpha product: $[\alpha]^{20}_D$** = +83.9 (*c* = 0.72, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 1747 (C=O), 3384 (OH) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.34-7.24 (m, 15H, Ph), 5.00 (s, 1H, H-1 Man), 4.91 (d, *J* = 10.9 Hz, 1H, C$\underline{H}_2$Ph), 4.88 (d, *J* = 3.0 Hz, 1H, H-1 Glu), 4.81 (d, *J* = 11.2 Hz, 1H, C$\underline{H}_2$Ph), 4.76 (d, *J* = 11.0 Hz, 1H, C$\underline{H}_2$Ph), 4.68-4.57 (m, 3H), 4.52-4.50 (m, 1H, C$\underline{H}$$CO_2$Me), 4.08 (bs, 1H, H-2 Man), 3.94-3.85 (m, 4H), 3.79-3.70 (m, 5H), 3.68-3.54 (m, 2H), 3.64 (s, 3H, CO_2Me), 3.47 (dd, *J* = 9.1, 3.6 Hz, 1H, H-2 Glu), 3.41 (t, *J* = 9.3 Hz, 1H, H-4 Glu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.3 ($\underline{C}O_2$Me), 138.7, 138.1, 137.9, 128.47, 128.43, 128.35, 128.17, 128.03, 127.94, 127.90, 127.85, 127.57, 98.9 (C-1 Man), 96.8 (C-1 Glu), 81.6, 80.1, 77.4, 75.6, 75.0, 73.8 ($\underline{C}$HCO_2Me), 73.1, 72.8, 71.5, 71.2, 70.4, 67.27

($\underline{C}H_2CHCO_2Me$), 61.7, 61.1, 52.4 ($CO_2\underline{C}H_3$) ppm. **HRMS**: calcd. for $C_{37}H_{46}O_{14}Na^+$ [M^+ + Na] 737.2785; found 737.2775. **Beta product: $[\alpha]^{20}_D$** = +42.6 (*c* = 2.51, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 1747 (C=O), 3371 (OH) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.34-7.20 (m, 15H, Ph), 5.02 (bs, 1H, H-1 Man), 4.86-4.73 (m, 4H), 4.63-4.57 (m, 2H), 4.44 (bs, 1H, $C\underline{H}CO_2Me$), 4.37 (d, *J* = 7.7 Hz, 1H, H-1 Glu), 4.19 (bs, 1H, H-2 Man), 4.11-3.46 (m, 11H), 3.54 (s, 3H, CO_2Me), 3.38 (t, *J* = 8.4 Hz, 1H, H-2 Glu), 3.33-3.21 (m, 1H, H-5 Glu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.3 ($\underline{C}O_2Me$), 138.8, 138.2, 138.0, 128.1, 127.77, 127.57, 127.52, 103.9 (C-1 Glu), 99.8 (C-1 Man), 84.4, 82.0, 77.46, 75.6, 75.5, 74.6, 74.3 ($\underline{C}HCO_2Me$), 73.1, 71.6, 71.0, 70.6 ($\underline{C}H_2CHCO_2Me$), 70.4, 66.3, 61.3, 60.8, 52.4 ($CO_2\underline{C}H_3$) ppm. **HRMS**: calcd. for $C_{37}H_{46}O_{14}Na^+$ [M^+ + Na] 737.2785; found 737.2777.

Experiment 113. Synthesis of Methyl (*2R*)-2-*O*-(α-D-mannopyranosyl)-3-*O*-(α-D-glucopyranosyl)-2,3-dihydroxypropanoate (132)

Benzyl ether **131** (α anomer) (0.225 g, 0.31 mmol) in EtOH/EtOAc (0.1:2.9, 3 mL) was hydrogenated at 50 psi in the presence of Pd/C 10% (0.25 equiv). After 8 hours, the reaction mixture was filtered and the solvent was evaporated to afford ester **132** as a viscous colourless foam (0.140 g, quantitative). **^{1}H NMR** (D_2O): δ 4.93 (d, *J* = 1.2 Hz, 1H, H-1 Man), 4.88 (d, *J* = 3.7 Hz, 1H, H-1 Glu), 4.70-4.67 (m, 1H, $C\underline{H}CO_2Me$), 4.08 (dd, *J* = 11.0 Hz, *J* = 4.2 Hz, 1H, $C\underline{H}H'CHCO_2Me$), 4.01-4.00 (m, 1H, H-2 Man), 3.84-3.74 (m, 4H), 3.75 (s, 3H, CO_2Me), 3.71-3.52 (m, 5H), 3.48-3.40 (m, 2H), 3.32 (m, 1H, H-4 Glu) ppm. **^{13}C NMR** (D_2O): δ 172.0 ($\underline{C}O_2CH_3$), 99.3 (C-1 Man), 98.1 (C-1 Glu), 73.7 ($\underline{C}HCO_2Me$), 73.4, 73.0, 72.1, 71.3, 70.2, 69.7, 69.3, 67.6 ($\underline{C}H_2CHCO_2Me$), 66.6, 60.9, 60.3, 53.0 ($CO_2\underline{C}H_3$) ppm.

Experiment 114. Synthesis of Potassium (*2R*)-2-*O*-(α-D-mannopyranosyl)-3-*O*-(α-D-glucopyranosyl)-2,3-dihydroxypropanoate (124)

A solution of 2 M KOH (0.29 mL) was added to a stirred solution of ester **132** (0.130 g, 0.29 mmol) in H_2O (2 mL). After all of the starting material had been

consumed, the pH was adjusted to 7 with 10% HCl and the solvent was evaporated to afford **124** as a viscous colorless foam (0.137 g, quantitative). **$[\alpha]^{20}_D$** = +89.7 (*c* = 0.33, H_2O). **^{1}H NMR** (D_2O): δ 4.85 (d, *J* = 3.7 Hz, 1H, H-1 Glu), 4.84 (d, *J* = 1.5 Hz, 1H, H-1 Man), 4.29 (dd, *J* = 5.1 Hz, *J* = 2.2 Hz, 1H, C<u>H</u>CO_2^-), 4.01 (dd, *J* = 3.3 Hz, *J* = 1.6 Hz, 1H, H-2 Man), 3.96 (dd, *J* = 10.6 Hz, *J* = 5.2 Hz, 1H, C<u>H</u>H'$CHCO_2^-$), 3.85-3.76 (m, 3H), 3.71-3.56 (m, 7H), 3.44 (dd, *J* = 9.8 Hz, *J* = 3.7 Hz, 1H, H-2 Glu), 3.31 (t, *J* = 9.6 Hz, 1H, H-4 Glu) ppm. **^{13}C NMR** (D_2O): δ 176.1 (<u>C</u>O_2^-), 98.8 (C-1 Man), 97.7 (C-1 Glu), 75.7 (<u>C</u>HCO_2^-), 73.16, 73.04, 71.71, 71.53, 70.3, 69.9, 69.6, 68.3 (<u>C</u>$H_2CHCO_2^-$), 66.9, 61.0, 60.4 ppm. **HRMS**: calcd. for $C_{15}H_{26}O_{14}K$ [M^+ + H] 469.0960; found 469.0940.

Experiment 115. Synthesis of Ethyl 3-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-3-hydroxybutyrate (136)

A suspension of thioglucoside donor **1** (0.815 g, 1.52 mmol), ethyl 3-hydroxybutyrate **133** (0.197 mL, 1.52 mmol) and 4Å MS in CH_2Cl_2 (6 mL) was stirred for 1 h at room temperature then cooled to 0°C. *N*-Iodosuccinimide (0.434 g, 1.93 mmol) and TfOH (0.112 mL) were added at 0°C and when the reaction was complete, 10% $Na_2S_2O_3$ aqueous solution (6 mL) and saturated aqueous $NaHCO_3$ solution (3 mL) were added. The mixture was extracted with CH_2Cl_2 (3x6 mL), the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed under vacuum. The crude product was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) to afforded product **136** as a viscous colourless foam (0.771 g, 84 %). **FTIR** (film) $\upsilon_{máx}$: 1736 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.36-7.26 (m), 5.03-4.96 (m), 4.95-4.74 (m), 4.71-4.62 (m), 4.58-4.54 (m), 4.50 (d, *J* = 8.0 Hz, H-1 (β)), 4.31-3.92 (m), 3.89-3.85 (m), 3.67-3.62 (m), 3.54-3.44 (m), 3.43-3.37 (m), 2.86-2.80 (m), 2.74-2.68 (m), 2.68-2.62 (m), 2.48-2.35 (m), 2.02 (s), 1.33-1.16 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 171.2, 170.77, 170.75, 138.67, 138.66, 138.18, 138.03, 137.95, 137.81, 128.41, 128.40, 128.36, 128.30, 128.17, 128.13, 128.11, 128.10, 128.03, 127.99, 127.95, 127.92, 127.87, 127.85, 127.77, 127.67, 127.64, 103.5 (C-1 (β)), 102.4 (C-1 (β)), 96.8 (C-1 (α)), 94.4 (C-1 (α)), 84.7, 81.95, 81.79, 79.90, 79.80, 77.5, 77.3, 75.73, 75.72, 75.68, 75.1, 74.9,

73.3, 72.8, 72.1, 69.6, 68.92, 68.87, 63.16, 63.01, 60.55, 60.46, 42.1, 41.8, 21.5, 20.8, 19.1, 14.20, 14.17, 14.14 ppm.

Experiment 116. Synthesis of Ethyl 3-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)-3-hydroxybutyrate (137)

The glycosylation reaction of thiogalactoside donor **19** (0.638 g, 1.19 mmol) and ethyl 3-hydroxybutyrate **133** (0.170 mL, 1.31 mmol) was performed according to the procedure described in experiment 115. The crude was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) affording the product **137** as a viscous colourless gum (0.685 g, 95 %). **FTIR** (film) $\upsilon_{máx}$: 1736 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.39-7.26 (m), 5.08 (d, *J* = 3.7 Hz, H-1 (α)), 4.98-4.93 (m), 4.90-4.86 (m), 4.83-4.64 (m), 4.61 (d, *J* = 11.5 Hz), 4.46 (d, *J* = 7.7 Hz, H-1 (β)), 4.45 (d, *J* = 7.6 Hz, H-1 (β)), 4.27-3.95 (m), 3.93-3.88 (m), 3.82-3.78 (m), 3.76-3.75 (m), 3.54-3.48 (m), 2.89 (dd, *J* = 15.5 Hz, *J* = 5.2 Hz), 2.72-2.62 (m), 2.48-2.37 (m), 1.99 (s), 1.98 (s), 1.96 (s), 1.95 (s), 1.35-1.17 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 171.28, 171.18, 170.7, 138.76, 138.64, 138.59, 138.48, 138.43, 138.27, 138.24, 138.19, 128.61, 128.56, 128.41, 128.32, 128.31, 128.20, 128.15, 128.04, 127.99, 127.97, 127.81, 127.74, 127.73, 127.66, 127.62, 127.60, 127.55, 127.54, 127.51, 127.44, 127.42, 103.8 (C-1 (β)), 102.9 (C-1 (β)), 97.6 (C-1 (α)), 95.3 (C-1 (α)), 82.33, 82.28, 79.4, 78.98, 78.95, 76.38, 76.32, 75.2, 75.0, 74.77, 74.70, 74.51, 74.27, 74.22, 73.56, 73.47, 73.45, 73.42, 73.04, 72.98, 72.85, 72.00, 71.92, 71.75, 69.4, 68.72, 68.56, 63.69, 63.60, 63.1, 62.9, 60.48, 60.41, 60.36, 60.34, 42.7, 42.16, 42.05, 41.87, 30.9, 21.9, 21.4, 20.79, 20.77, 20.75, 20.4, 19.1, 14.21, 14.18, 14.16, 14.13 ppm.

Experiment 117. Synthesis of Dimethyl (*2S*)-2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-2-hydroxysuccinate (138)

The glycosylation reaction of thiogalactoside donor **1** (0.850 g, 1.58 mmol) and dimethyl (*S*)-malate **134** (0.208 mL, 1.58 mmol) was performed according to the procedure described in experiment 115. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the

product **138** as a viscous colourless gum (0.949 g, 94 %, α/β=7:1). **FTIR** (film) $\upsilon_{máx}$: 1739 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.36-7.26 (m, Ph), 5.05 (d, *J* = 3.7 Hz, H-1 (α)), 4.99-4.95 (m), 4.87 (d, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.81 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 5.06-4.50 (m), 4.77 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph), 4.72-4.62 (m), 4.56 (d, *J* = 10.9 Hz, C$\underline{H}_2$Ph), 4.52 (t, *J* = 6.5 Hz, CHC$\underline{H}_2CO_2Me$ (α)), 4.30-4.26 (m), 4.16-4.13 (m), 4.00 (t, *J* = 9.3 Hz, H-3 (α)), 3.74 (s, CO_2Me (α)), 3.74 (s, CO_2Me (β)), 3.67 (s, CO_2Me (β)), 3.58 (s, CO_2Me (α)), 3.55-3.48 (m), 2.94-2.93 (m, 1H), 2.93 (d, *J* = 5.9 Hz, CHC$\underline{H}_2CO_2Me$ (β)), 2.83 (d, *J* = 6.5 Hz, CHC$\underline{H}_2CO_2Me$ (α)), 2.04 (s, Ac (β)), 2.01 (s, Ac (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 171.2, 170.7, 170.4, 138.6, 138.1, 137.9, 128.49, 128.44, 128.31, 128.12, 128.08, 127.95, 127.87, 127.74, 127.72, 127.63, 102.8 (C-1 (β)), 98.5 (C-1 (α)), 81.6, 79.7, 75.7 ($\underline{C}H_2$Ph), 75.0 ($\underline{C}H_2$Ph), 74.6 ($\underline{C}HCH_2CO_2Me$ (α)), 72.7 ($\underline{C}H_2$Ph), 69.5, 62.8 (C-6 (α)), 52.4 ($CO_2\underline{C}H_3$), 51.9 ($CO_2\underline{C}H_3$), 37.4 (CH$\underline{C}H_2CO_2Me$), 20.85 (Ac (α)), 20.83 (Ac (β)) ppm.

Experiment 118. Synthesis of Dimethyl (*2S*)-2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)-2-hydroxysuccinate (139)

The glycosylation reaction of thiogalactoside donor **19** (1.20 g, 2.23 mmol) and dimethyl (*S*)-malate **134** (0.294 mL, 2.23 mmol) was performed according to the procedure described in experiment 115. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the product **139** as a viscous colourless gum (1.235 g, 87 %, α/β=5:1). **FTIR** (film) $\upsilon_{máx}$: 1740 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.25 (m, 1H, Ph), 5.10 (d, *J* = 3.7 Hz, H-1 (α)), 5.01 (d, *J* = 10.8 Hz), 4.97-4.94 (m), 4.88-4.83 (m), 4.77-4.57 (m), 4.56-4.53 (m), 4.22-4.16 (m), 4.12 (d, *J* = 7.1 Hz, H-1 (β)), 4.09-3.96 (m), 3.93 (t, *J* = 1.2 Hz), 3.83 (dd, *J* = 9.7, 7.7 Hz), 3.75-3.71 (m), 3.72 (s, CO_2Me), 3.56 (s, CO_2Me), 3.54-3.48 (m), 2.95 (d, *J* = 6.0 Hz, CHC$\underline{H}_2CO_2Me$ (α)), 2.82 (d, *J* = 5.5 Hz, CHC$\underline{H}_2CO_2Me$ (β)), 1.98 (s, Ac), 1.96 (s, Ac) ppm.

Experiment 119. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(*2S*)-2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-2,3-dihydroxypropanoate (140)

The glycosylation reaction of thiogalactoside donor **1** (0.300 g, 0.56 mmol) and acceptor **135** (0.200 g, 0.56 mmol) was performed according to the procedure described in experiment 115. The crude was purified by flash column chromatography on silica gel (10:90, EtOAc/Hex) affording the product **140** as a viscous colourless gum (0.463 g, 98 %, α/β>10:1). **FTIR** (film) $\upsilon_{máx}$: 1743 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.68-7.66 (m, Ph), 7.40-7.27 (m, 3H), 5.22 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph (β)), 4.96 (d, *J* = 10.8 Hz, C$\underline{H}_2$Ph (α)), 4.87-4.78 (m), 4.80 (d, *J* = 3.99 Hz, H-1 (α)), 4.67 (d, *J* = 12.2 Hz, C$\underline{H}_2$Ph (α)), 4.58-4.53 (m), 4.29-4.25 (m), 4.15-4.06 (m), 4.02-3.92 (m), 3.75 (s, CO_2Me (β)), 3.72 (s, CO_2Me (α)), 3.51-3.44 (m), 2.00 (s, Ac (α)), 1.98 (s, Ac (β)), 1.03 (s, *t*-Bu (α)), 1.01 (s, *t*-Bu (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.9, 170.7, 138.7, 138.05, 137.97, 135.63, 135.61, 135.58, 133.2, 132.8, 129.8, 128.55, 128.49, 128.25, 128.13, 128.05, 127.97, 127.94, 127.88, 127.87, 127.84, 127.79, 127.76, 127.71, 127.65, 98.7 (C-1 (α)), 81.7 (α), 79.9 (α), 79.3 (α), 77.3 (α), 75.7 (α), 74.9 (α), 73.0 (α), 69.4 (α), 64.3 (α), 62.8 (α), 52.0 ($CO_2\underline{C}H_3$), 26.70 ($C(\underline{C}H_3)_3$ (α)),, 26.62 ($C(\underline{C}H_3)_3$ (β)), 20.9 (Ac), 19.2 (C-Si) ppm.

Experiment 120. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(*2S*)-2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)-2,3-dihydroxypropanoate (141)

The glycosylation reaction of thiogalactoside donor **1** (0.300 g, 0.56 mmol) and acceptor **135** (0.200 g, 0.56 mmol) was performed according to the procedure described in experiment 115. The crude was purified by flash column chromatography on silica gel (10:90, EtOAc/Hex) affording the product **140** as a viscous colourless gum (0.463 g, 98 %, α/β>10:1). **Alpha product: FTIR** (film) $\upsilon_{máx}$: 1746 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.67 (d, *J* = 6.8 Hz, 4H, Ph), 7.43-7.17 (m, 21H, Ph), 4.96-4.94 (m, 2H), 4.95 (d, *J* = 11.6 Hz, 1H, C$\underline{H}_2$Ph), 4.95 (d, *J* = 3.6 Hz, 1H, H-1), 4.86 (d, *J* = 11.6 Hz, 1H, C$\underline{H}_2$Ph), 4.75-4.70 (m, 2H), 4.62-4.58 (m, 2H), 4.15-4.09 (m, 3H), 4.06-3.91

(m, 7H), 3.70 (s, 3H, CO_2Me), 1.97 (s, 3H, Ac), 1.03 (s, 9H, *t*-Bu). **^{13}C NMR** ($CDCl_3$): δ 171.1, 170.5, 138.8, 138.44, 138.28, 135.65, 135.57, 133.1, 132.9, 129.8, 128.39, 128.37, 128.31, 128.27, 127.84, 127.72, 127.59, 127.51, 127.42, 99.3 (C-1), 78.8, 78.4, 76.3, 74.62, 74.56, 73.5, 73.2, 69.1, 64.6, 63.0, 51.9, 26.7, 20.8, 19.2 ppm. **Beta product: FTIR** (film) $\upsilon_{máx}$: 1744 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.71-7.66 (m, 4H, Ph), 7.44-7.28 (m, 21H, Ph), 5.23 (d, *J* = 10.8 Hz, 1H, C$\underline{H}_2$Ph), 5.00 (d, *J* = 11.6 Hz, 1H, C$\underline{H}_2$Ph), 4.88 (d, *J* = 11.7 Hz, 1H, C$\underline{H}_2$Ph), 4.75 (m, 2H), 4.68 (d, *J* = 11.6 Hz, 1H, C$\underline{H}_2$Ph), 4.58-4.54 (m, 1H), 4.75 (d, *J* = 7.6 Hz, 1H, H-1), 4.16-4.11 (m, 2H), 4.08-4.07 (m, 2H), 4.02 (dd, *J* = 11.2 Hz, *J* = 6.2 Hz, 1H), 4.00 (dd, *J* = 11.2 Hz, *J* = 6.2 Hz, 1H), 3.76 (s, 3H, CO_2Me), 3.55 (dd, *J* = 9.7 Hz, *J* = 2.7 Hz, 1H), 3.51-3.48 (m, 1H), 1.94 (s, 3H, Ac), 1.03 (s, 9H, *t*-Bu) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.7, 170.5, 138.9, 138.6, 138.2, 135.6, 133.23, 133.09, 129.70, 129.66, 128.60, 128.50, 128.39, 128.30, 128.14, 127.76, 127.67, 127.60, 127.4, 102.7 (C-1), 81.9, 79.2, 76.9, 74.9, 74.3, 73.8, 73.1, 72.1, 65.0, 62.9, 51.9, 26.65, 26.63, 20.8, 19.2 ppm.

Experiment 121. Synthesis of Potassium (*2S*)-2-(α-D-glucopyranosyl)propanoate (142)

A solution of NaOMe 1N (0.443 mL, 0.443 mmol) in MeOH was added to a stirred solution of **10** (0.427 g, 0.74 mmol) in MeOH (4 mL) at 0 ºC. After 1 h the reaction mixture was neutralized with saturated aqueous NH_4Cl. The aqueous phase was extracted with EtOAc and the combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed. The crude product was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) to afford the α (0.310 g, 78%) and β-alcohol (0.027 g, 7%) as viscous colourless gums.

A solution of the α-alcohol (0.300 g, 0.56 mmol) in EtOAc was hydrogenated at 50 psi in the presence of Pd/C 10% (0.25 equiv). After 5 hours, the reaction mixture was filtered and the solvent was evaporated to afford the ester as a very viscous colourless foam (0.149 g, quantitative). A solution of 2 M KOH (0.28 mL) was added to a stirred solution of the ester (0.149 g, 0.56 mmol) in H_2O (2 mL). After all of the starting material had been consumed, the pH was adjusted to 7 with 10% HCl and the solvent was evaporated to afford **142** as a

viscous colorless foam (0.162 g, quantitative). **$[\alpha]^{20}_{D}$** = +107.2 (*c* = 0.60, H_2O). **^{1}H NMR** (D_2O): δ 4.93 (d, *J* = 3.9 Hz, 1H, H-1), 3.96 (q, *J* = 6.8 Hz, 1H, C<u>H</u>CH_3), 3.75-3.68 (m, 5H), 3.44 (dd, *J* = 9.9 Hz, *J* = 4.0 Hz, 1H, H-2), 3.35 (t, *J* = 9.3 Hz, 1H, H-4), 1.28 (d, *J* = 6.8 Hz, 3H, CHC<u>H</u>$_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 181.0 ($CHCO_2^-$), 97.3 (C-1), 75.5 (<u>C</u>HCH_3), 73.1 (C-3), 71.9 (C-5), 71.5 (C-2), 69.4 (C-4), 60.1 (C-6), 17.5 (CH<u>C</u>H_3) ppm.

Experiment 122. Synthesis of Potassium (*2S*)-2-(α/β-D-galactopyranosyl)propanoate (143)

The methanolysis of the acetate group of the galactoside **32** (1.720 g, 2.63 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (1.350 g, 96 %, α/β=3:1). After catalytic hydrogenation of the benzyl ethers (1.323 g, 2.46 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 118, the compound **143** was obtained as a viscous colorless foam (0.715 g, quantitative, α/β=3:1). **FTIR** (film) $\upsilon_{máx}$: 1635 (C=O), 3332 (O-H) cm^{-1}. **^{1}H NMR** (D_2O): δ 4.97 (d, *J* = 3.9 Hz, H-1 (α)), 4.58 (q, *J* = 7.0 Hz, C<u>H</u>CH_3 (β)), 4.37 (d, *J* = 7.7 Hz, H-1 (β)), 4.25 (q, *J* = 6.9 Hz, C<u>H</u>CH_3 (α)), 3.93-3.88 (m), 3.84-3.79 (m), 3.76-3.64 (m), 3.63-3.52 (m), 3.47 (dd, *J* = 9.9, 7.7 Hz,), 1.38 (d, *J* = 7.0 Hz, CHC<u>H</u>$_3$ (β)), 1.35 (d, *J* = 6.8 Hz, CHC<u>H</u>$_3$ (α)) ppm. **^{13}C NMR** ($CDCl_3$): δ 187.3 ($CHCO_2^-$), 101.8 (C-1 (β)), 98.9 (C-1 (α)), 75.2, 73.7, 73.1, 72.6, 71.4, 70.7, 69.21, 69.07, 68.5, 68.0, 60.84, 60.80, 52.68, 52.62, 17.1 (CH<u>C</u>H_3) ppm.

Experiment 123. Synthesis of Potassium 2-(α/β-D-glucopyranosyl)acetate (144)

The methanolysis of the acetate group of the glucoside **13** (0.940 g, 1.66 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (0.765 g, 88 %, α/β=11:1). After catalytic hydrogenation of the benzyl ethers (0.715

g, 1.37 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **144** was obtained as a viscous colorless foam (0.378 g, quantitative, α/β=10:1). **^{1}H NMR** (D_2O): δ 4.88 (d, *J* = 3.8 Hz, H-1 (α)), 4.41 (d, *J* = 7.9 Hz, H-1 (β)), 4.22 (d, *J* = 15.6 Hz, C<u>H</u>H'CO_2^- (β)), 4.06 (d, *J* = 15.5 Hz, C<u>H</u>H'CO_2^- (α)), 4.03 (d, *J* = 15.8 Hz, CH<u>H</u>'CO_2^- (α)), 3.88 (d, *J* = 15.5 Hz, CH<u>H</u>'CO_2^- (β)), 3.79-3.61 (m), 3.46 (dd, *J* = 9.8, 3.8 Hz), 3.34 (t, *J* = 9.5 Hz) ppm. **^{13}C NMR** ($CDCl_3$): δ 177.4, 102.3 (C-1 (β)), 98.3 (C-1 (α)), 75.9 (β), 75.5 (β), 73.1, 71.9, 71.5, 69.5, 68.5 (<u>C</u>$H_2CO_2^-$ (β)), 66.8 (<u>C</u>$H_2CO_2^-$ (α)), 60.61 (C-6 (β)), 60.43 (C-6 (α)) ppm.

Experiment 124. Synthesis of Potassium 2-(α/β-D-galactopyranosyl)acetate (145)

The methanolysis of the acetate group of the galactoside **34** (1.079 g, 1.91 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (0.800 g, 81 %, α/β=2:1). After catalytic hydrogenation of the benzyl ethers (0.787 g, 1.50 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **145** was obtained as a viscous colorless foam (0.416 g, quantitative, α/β=2:1). **^{1}H NMR** (D_2O): δ 4.91 (d, *J* = 3.9 Hz, H-1 (α)), 4.34 (d, *J* = 7.7 Hz, H-1 (β)), 4.24 (d, *J* = 15.6 Hz, C<u>H</u>H'CO_2^- (β)), 4.06 (d, *J* = 15.6 Hz, C<u>H</u>H'CO_2^- (α)), 4.03 (d, *J* = 15.6 Hz, CH<u>H</u>'CO_2^- (β)), 3.92-3.84 (m), (d, *J* = 15.6 Hz, CH<u>H</u>'CO_2^- (α)), 3.75-3.71 (m), 3.69-3.58 (m), 3.52 (dd, *J* = 10.0 Hz, *J* = 7.6 Hz, H-2 (β)) ppm. **^{13}C NMR** ($CDCl_3$): δ 177.5, 102.9 (C-1 (β)), 98.3 (C-1 (α)), 75.2 (β), 72.6 (β), 71.1 (α), 70.8 (β), 69.5 (α), 69.2 (α), 68.6 (β), 68.5 (<u>C</u>$H_2CO_2^-$ (β)), 68.4 (α), 66.8 (<u>C</u>$H_2CO_2^-$ (α)), 61.15 (C-6 (α)), 60.95 (C-6 (β)) ppm.

Experiment 125. Synthesis of Potassium (*2R*)-2-*O*-(α/β-D-galactopyranosyl)-2,3-dihydroxypropanoate (146, 147)

The methanolysis of the acetate group of the galactoside **35** (1.078 g, 1.29 mmol) was performed according to the procedure described in experiment

121. The crude was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) affording the α (0.671 g, 66 %) and the β-alcohol (0.286 g, 28 %) as viscous colourless gums.

TBAF (1M in THF; 0.83 mL, 0.83 mmol) was added to a solution of the α–galactoside (0.655 g, 0.83 mmol) in THF (4 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded the α-diol as a viscous colourless gum (0.457 g, 92%). After catalytic hydrogenation of the benzyl ethers from the α-diol (0.140 g, 1.50 mmol) and hydrolisis of the methyl ester according to the procedure described in experiment 121, the compound **146** was obtained as a viscous colorless foam (0.416 g, quantitative). **Alpha product 146: $[\alpha]^{20}_D$** = +127.6 (*c* = 0.62, H_2O). **^{1}H NMR** (D_2O): δ 4.97 (d, *J* = 3.9 Hz, 1H, H-1), 4.13 (dt, *J* = 4.7 Hz, *J* = 2.3 Hz, 1H, C<u>H</u>CH_2OH), 3.99 (t, *J* = 6.2 Hz, 1H,), 3.93-3.87 (m, 2H), 3.81 (dd, *J* = 12.1, 3.2 Hz, 1H), 3.77-3.70 (m, 2H), 3.69-3.64 (m, 2H, H-6, H'-6) ppm. **^{13}C NMR** (D_2O): δ 177.1 (CH<u>C</u>O_2^-), 97.6 (C-1), 79.2 (<u>C</u>HCH_2OH), 71.3, 69.6, 69.3, 68.5, 63.1 (CH<u>C</u>H_2OH), 61.2 (C-6) ppm.

The same strategy was applied for the deprotection of the β-galactoside (0.266 g, 0.34 mmol). After fluorolysis (0.140 g, 76%), catalytic hydrogenation of the benzyl ethers from the β-diol (0.340 g, 0.615 mmol) and hydrolisis of the methyl ester, the compound **147** was obtained as a viscous colorless foam (0.188 g, quantitative). **Beta product 147:^{1}H NMR** (D_2O): δ 4.42 (d, *J* = 7.5 Hz, 1H, H-1), 4.11 (dd, *J* = 6.5 Hz, *J* = 3.2 Hz, 1H, C<u>H</u>CH_2OH), 3.83-3.77 (m, 2H), 3.73-3.67 (m, 2H), 3.64-3.53 (m, 4H) ppm. **^{13}C NMR** (D_2O): δ 177.9 (CH<u>C</u>O_2^-), 102.6 (C-1), 81.3 (<u>C</u>HCH_2OH), 75.1, 72.6, 70.9, 68.7, 62.6 (CH<u>C</u>H_2OH), 60.9 (C-6) ppm.

Experiment 126. Synthesis of Potassium 3-*O*-(α-D-glucopyranosyl)-3-hydroxybutyrate (148)

The methanolysis of the acetate group of the glucoside **136** (0.823 g, 1.36 mmol) was performed according to the procedure described in experiment

121. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the α (0.594 g, 82 %) and the β-alcohol (0.066 g, 9 %) as viscous colourless gums.

After catalytic hydrogenation of the benzyl ethers of the α-alcohol (0.516 g, 0.94 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **148** was obtained as a viscous colorless foam (0.285 g, quantitative). **^{1}H NMR** (D_2O): δ 4.98 (d, *J* = 4.0 Hz, H-1), 4.97 (d, *J* = 4.2 Hz, H-1), 4.14-4.04 (m, C<u>H</u>CH_3), 3.80-3.59 (m), 3.45-3.39 (m, H-2), 3.34-3.28 (m, H-4), 2.47 (dd, *J* = 14.2 Hz, *J* = 6.9 Hz, CHC<u>H</u>$_2CO_2^-$), 2.40-2.22 (m, CHC<u>H</u>$_2CO_2^-$), 1.21 (d, *J* = 6.1 Hz, CHC<u>H</u>$_3$), 1.14 (d, *J* = 5.9 Hz, CHC<u>H</u>$_3$) ppm. **^{13}C NMR** (D_2O): δ 180.19, 180.16, 97.6 (C-1), 94.9 (C-1), 73.4 (<u>C</u>HCH$_3$), 73.15 (<u>C</u>HCH$_3$), 73.06, 71.9, 71.51, 71.48, 71.32, 70.5, 69.62, 69.56, 60.6 (C-6), 60.3 (C-6), 45.6 (CH<u>C</u>H$_2CO_2^-$), 44.5 (CH<u>C</u>H$_2CO_2^-$), 20.6 (CH<u>C</u>H$_3$), 18.0 (CH<u>C</u>H$_3$) ppm.

Experiment 127. Synthesis of Potassium 3-*O*-(α/β-D-galactopyranosyl)-3-hydroxybutyrate (149)

The methanolysis of the acetate group of the galactoside **137** (0.709 g, 1.17 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (0.584 g, 88 %, α/β=3:1). After catalytic hydrogenation of the benzyl ethers (0.573 g, 1.01 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **145** was obtained as a viscous colorless foam (0.309 g, quantitative, α/β=3:1). **^{1}H NMR** (D_2O): δ 5.01 (d, *J* = 3.9 Hz, H-1 (α)), 4.99 (d, *J* = 3.8 Hz, H-1 (α)), 4.42 (d, *J* = 8.4 Hz, H-1 (β)), 4.40 (d, *J* = 8.1 Hz, H-1 (β)), 4.23-4.04 (m), 3.97-3.89 (m), 3.84 (t, *J* = 3.9 Hz), 3.78-3.54 (m), 3.40 (dd, *J* = 9.6, 8.2 Hz), 2.54-2.44 (m), 2.40-2.22 (m), 1.22-1.13 (m) ppm. **^{13}C NMR** (D_2O): δ 180.3, 179.9, 101.9 (C-1 (β)), 101.1 (C-1 (β)), 97.9 (C-1 (α)), 95.1 (C-1 (α)), 75.14, 75.10, 74.99, 74.0, 73.4, 72.79, 72.65, 71.02, 71.01, 70.8, 70.52, 70.44, 69.58, 69.46, 69.30, 69.1, 68.72, 68.56, 68.46, 68.2, 61.23, 61.09, 60.9, 45.75 (CH<u>C</u>H$_2CO_2^-$), 45.57

($CH\underline{C}H_2CO_2^-$), 44.5 ($CH\underline{C}H_2CO_2^-$), 20.6 ($CH\underline{C}H_3$), 19.3 ($CH\underline{C}H_3$), 18.0 ($CH\underline{C}H_3$) ppm.

Experiment 128. Synthesis of Potassium (*2S*)-2-*O*-(α/β-D-glucopyranosyl)-2-hydroxysuccinate (150)

The methanolysis of the acetate group of the glucoside **138** (0.263 g, 0.41 mmol) was performed according to the procedure described in experiment 121. The crude was purified by preparative TLC (50:50, EtOAc/Hex) affording the desired alcohol (0.117 g, 48 %) as a viscous colourless gum, and recovery of the initial **138** (0.068 g, 26 %) and the product of the hydrolysis at the anomeric position (0.046 g, 25 %,). After catalytic hydrogenation of the benzyl ethers (0.565 g, 0.95 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **145** was obtained as a viscous colorless foam (0.290 g, 94 %). **^{1}H NMR** (D_2O): δ 4.94 (d, *J* = 3.9 Hz, H-1 (α)), 4.39 (d, *J* = 7.9 Hz, H-1 (β)), 4.19 (dd, *J* = 10.4, *J* = 3.1 Hz, 1H, $CO_2^-C\underline{H}CH_2CO_2^-$), 3.80-3.63 (m), 3.45-3.28 (m), 3.21-3.15 (m), 2.59 (dd, *J* = 15.1 Hz, *J* = 2.9 Hz, $CHC\underline{H}_2CO_2^-$ (β)), 2.52 (dd, *J* = 15.2 Hz, *J* = 3.2 Hz, $CHC\underline{H}_2CO_2^-$ (α)), 2.42 (dd, *J* = 15.2 Hz, *J* = 10.4 Hz, $CHC\underline{H}_2CO_2^-$ (α)) ppm. **^{13}C NMR** (D_2O): δ 179.5, 179.1, 135.3 (C-1 (β)), 99.7 (C-1 (α)), 95.9, 79.0, 75.92, 75.72, 74.1, 73.1, 72.2, 71.8, 71.4, 69.6, 69.2, 60.7, 60.0, 41.5 ppm.

Experiment 129. Synthesis of Potassium (*2S*)-2-*O*-(α/β-D-galactopyranosyl)-2-hydroxysuccinate (151, 152)

The methanolysis of the acetate group of the galactoside **139** (1.215 g, 1.91 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (50:50, EtOAc/Hex) affording the α (0.642 g, 57 %) and β-alcohol (0.176 g, 16 %) as viscous colourless gums, and recovery of the starting material **139** (0.020 g, 16 %) and the product of the hydrolysis at the anomeric position (0.067 g, 8 %). After catalytic hydrogenation of the benzyl ethers from the α (0.640 g, 1.07 mmol) and β-alcohol (0.159 g, 0.27 mmol) and hydrolysis of the

methyl ester according to the procedure described in experiment 121, the compounds **151** (0.401 g, quantitative) and **152** (0.100 g, quantitative) were obtained as viscous colorless foams. **Alpha product 151: FTIR** (film) $\upsilon_{máx}$: 1634 (C=O), 3332 (O-H) cm^{-1}. **^{1}H NMR** (D_2O): δ 4.96 (d, *J* = 4.0 Hz, 1H, H-1) 4.20 (dd, *J* = 10.3, 3.1 Hz, $CO_2^-C\underline{H}CH_2CO_2^-$), 4.05-4.02 (m), 3.94 (d, *J* = 2.8 Hz), 3.86 (dd, *J* = 10.4 Hz, *J* = 3.3 Hz), 3.70-3.54 (m), 2.54 (dd, *J* = 15.2 Hz, 3.2 Hz, 1H, $CHC\underline{H}_2CO_2^-$), 2.43 2.42 (dd, *J* = 15.2 Hz, *J* = 10.3 Hz, 1H, $CHC\underline{H}_2CO_2^-$) ppm. **Beta product 152: FTIR** (film) $\upsilon_{máx}$: 1736 (C=O), 3410 (O-H) cm^{-1}. **^{1}H NMR** (D_2O): δ 4.52 (dd, *J* = 10.0 Hz, *J* = 3.1 Hz, 1H, $CO_2^-\underline{C}HCH_2CO_2^-$), 4.33 (d, *J* = 7.5 Hz, 1H, H-1), 3.83-3.82 (m, 1H), 3.77-3.50 (m, 5H), 2.62 (dd, *J* = 15.2, 3.1 Hz, 1H, $CH\underline{C}H_2CO_2^-$), 2.44 (dd, *J* = 15.2, 10.0 Hz, 1H, $CH\underline{C}H_2CO_2^-$). **^{13}C NMR** (D_2O): δ 179.4 (CO_2^-), 179.0 (CO_2^-), 102.0 (C-1), 77.6 ($\underline{C}HCH_2CO_2^-$), 75.4, 72.8, 70.9, 68.8, 61.3 (C-6), 41.9 ($CH\underline{C}H_2CO_2^-$) ppm.

Experiment 130. Synthesis of Potassium (*2S*)-2-*O*-(α-D-glucopyranosyl)-2,3-dihydroxypropanoate (153)

The methanolysis of the acetate group of the glucoside **140** (0.450 g, 0.54 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) affording the α (0.299 g, 70 %) and the β-alcohol (0.030 g, 7 %) as viscous colourless gums.

TBAF (1M in THF; 0.37 mL, 0.37 mmol) was added to a solution of the α-glucoside (0.290 g, 0.37 mmol) in THF (2 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded the α-diol as a viscous colourless gum (0.162 g, 80%). After catalytic hydrogenation of the benzyl ethers from the α-diol (0.150 g, 0.27 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **153** was obtained as a viscous colorless foam (0.077 g, quantitative). **^{1}H NMR** (D_2O): δ 4.96 (d, *J* = 3.9 Hz, 1H, H-1), 3.96 (dd, *J* = 3.3 Hz, *J* = 6.5 Hz, 1H, $C\underline{H}CH_2OH$), 3.79- 3.75 (m, 3H), 3.72-3.63 (m, 3H), 3.48 (dd, *J* = 3.9 Hz, *J* = 9.9 Hz, 1H, H-2), 3.37 (t, *J* =

9.6 Hz, 1H, H-4) ppm. **^{13}C NMR** (D_2O): δ 177.4 (CO_2^-), 99.2 (C-1), 81.4 ($\underline{C}HCH_2OH$), 72.9, 72.2, 71.7 (C-2), 69.3 (C-4), 62.6 ($CH\underline{C}H_2OH$), 60.0 (C-6) ppm.

Experiment 131. Synthesis of Potassium (*2S*)-2-*O*-(α-D-galactopyranosyl)-2,3-dihydroxypropanoate (154)

The methanolysis of the acetate group of the α-galactoside **141** (0.640 g, 0.77 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the alcohol (0.572 g, 94 %) as a viscous colourless residue.

TBAF (1M in THF; 0.83 mL, 0.88 mmol) was added to a solution of the α–galactoside (0.695 g, 0.88 mmol) in THF (5 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded the diol as a viscous colourless gum (0.343 g, 71%). After catalytic hydrogenation of the benzyl ethers from the diol (0.310 g, 0.56 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **154** was obtained as a viscous colorless foam (0.172 g, quantitative). **^{1}H NMR** (D_2O): δ 5.01 (d, *J* = 3.9 Hz, 1H, H-1), 4.28 (t, *J* = 4.2 Hz, 1H, $C\underline{H}CH_2OH$), 4.18-3.92 (m, 4H), 3.85-3.58 (m, 4H) ppm.

Experiment 132. Synthesis of Potassium (*2S*)-2-*O*-(β-D-galactopyranosyl)-2,3-dihydroxypropanoate (155)

The methanolysis of the acetate group of the β-galactoside **141** (0.275 g, 0.33 mmol) was performed according to the procedure described in experiment 121. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol (0.243 g, 93 %) as a viscous colourless residue.

TBAF (1M in THF; 0.83 mL, 0.44 mmol) was added to a solution of the β–galactoside (0.350 g, 0.44 mmol) in THF (3 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded the diol as a viscous colourless gum (0.151 g, 62 %). After catalytic hydrogenation of the benzyl ethers from the diol (0.140 g, 0.25 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 121, the compound **146** was obtained as a viscous colorless foam (0.078 g, quantitative). **^{1}H NMR** (D_2O): δ 4.38 (d, *J* = 7.4 Hz, 1H, H-1), 4.28 (dd, *J* = 6.2 Hz, *J* = 2.9 Hz, 1H, C<u>H</u>CH_2OH), 3.84 (dt, *J* = 7.2, 3.8 Hz, 2H), 3.76-3.68 (m, 3H), 3.66-3.53 (m, 4H) ppm. **^{13}C NMR** (D_2O): δ 177.1 (CO_2^-), 102.5 (C-1), 81.4 (<u>C</u>HCH_2OH), 75.3, 72.8, 71.0, 68.6, 63.2 (CH<u>C</u>H_2OH), 61.0 (C-6) ppm.

Experiment 133. Synthesis of Ethyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-3-hydroxybutyrate (156)

Ethyl 3-dihydroxybutyrate (0.348 mL, 2.68 mmol) was added to a solution of trichloroacetamidate **103** (1.100 g, 2.23 mmol) in dry CH_2Cl_2 (6 mL). The solution was cooled to 0°C and $BF_3.OEt_2$ (0.0.282 mL, 2.23 mmol) was slowly added. When the reaction was completed, a saturated aqueous solution of $NaHCO_3$ was added, followed by extractions with CH_2Cl_2 (3x15 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (40:60, EtOAc/hexane) to afford **156** (0.943 g, 91 %) as a viscous colourless residue. **FTIR** (film) $\upsilon_{máx}$: 1743 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 5.33-5.22 (m), 5.17 (dd, *J* = 2.8 Hz, *J* = 1.8 Hz,), 5.14 (dd, *J* = 3.3 Hz, *J* = 1.8 Hz), 4.99 (d, *J* = 1.6 Hz, H-1), 4.96 (d, *J* = 1.6 Hz, H-1), 4.30-4.05 (m), 2.67-2.57 (m), 2.52-2.38 (m), 2.16 (s), 2.15 (s), 2.11 (s), 2.09 (s), 2.04 (s), 2.03 (s), 1.99 (s), 1.98 (s), 1.32-1.22 (m) ppm. **^{13}C NMR** ($CDCl_3$): δ 171.0, 170.79, 170.75, 170.63, 170.1, 169.93, 169.88, 169.76, 169.74, 98.2 (C-1), 94.8 (C-1), 73.5, 70.1, 69.78, 69.69, 69.09, 69.00, 68.7, 66.4, 66.0, 64.2, 62.6, 62.3, 60.75, 60.67, 42.7, 41.9, 22.4, 21.3, 20.93, 20.89, 20.75, 20.70, 18.7, 14.17, 14.14 ppm.

Experiment 134. Synthesis of Dimethyl (*2S*)-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-hydroxysuccinate (157)

The glycosylation reaction of trichloroacetamidate donor **103** (1.540 g, 3.12 mmol) and ethyl dimethyl dimethyl (*S*)-malate **134** (0.494 mL, 3.75 mmol) was performed according to the procedure described in experiment 133. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the product **157** as a viscous colourless gum (1.359 g, 88 %). **$[\alpha]^{20}_D$** = +15.29 (*c* = 1.19, CH_2Cl_2). **FTIR** (film) $\upsilon_{máx}$: 1745 (C=O) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 5.39-5.29 (m, 2H, H-3, H-4), 5.23 (dd, *J* = 3.1, 1.8 Hz, 1H, H-2), 5.01 (d, *J* = 1.6 Hz, 1H, H-1), 4.58-4.52 (m, 1H, C<u>H</u>CH_2CO_2Me), 4.39-4.35 (m, 1H, H-5), 4.28 (dd, *J* = 12.3, 4.6 Hz, 1H, H-6), 4.05 (dd, *J* = 12.4, 2.3 Hz, 1H, H'-6), 3.84 (s, 3H, CO_2Me), 3.74 (s, 3H, CO_2Me), 2.92-2.79 (m, 4H, CHC<u>H_2</u>CO_2Me), 2.19 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.01 (s, 3H, Ac). **^{13}C NMR** ($CDCl_3$): δ 170.77, 170.72, 170.1, 169.89, 169.87, 169.76, 99.5 (C-1), 75.0, 69.4, 69.0, 67.3, 65.8, 62.2, 52.5, 52.3, 38.5, 20.89, 20.78, 20.74, 20.69.

Experiment 135. Synthesis of Methyl 3-*O*-*tert*-butyldimethylsilyl-(*2S*)-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2,3-dihydroxypropanoate (158)

The glycosylation reaction of trichloroacetamidate donor **103** (1.30 g, 2.64 mmol) and aceptor **135** (0.946 g, 2.64 mmol) was performed according to the procedure described in experiment 133. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the product **158** as a viscous colourless gum (1.33 g, 73 %). **^{1}H NMR** ($CDCl_3$): δ 7.67-7.65 (m, 4H, Ph), 7.47-7.39 (m, 6H, Ph), 5.43-5.31 (m, 3H, H-2, H-3, H-4), 5.01 (d, *J* = 1.4 Hz, 1H, H-1), 4.36 (ddd, *J* = 9.9 Hz, *J* = 4.3 Hz, *J* = 2.2 Hz, 1H, H-5), 4.31-4.25 (m, 2H), 4.03 (dd, *J* = 12.3, 2.2 Hz, 1H), 3.94-3.93 (m, 2H), 3.74 (s, 3H, CO_2Me), 2.15 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.04 (s, 9H, *t*-Bu) ppm.

Experiment 136. Synthesis of Potassium 3-*O*-(α-D-mannopyranosyl)-3-hydroxybutyrate (159)

A solution of NaOMe 1N (0.36 mL, 0.36 mmol) in MeOH was added to a stirred solution of **156** (0.276 g, 0.60 mmol) in MeOH (3 mL) at 0 ºC. After complete conversion of the starting material, previously activated Dowex-H^+ resin was added until neutral pH. After filtration with MeOH and water, the solvent was removed in vacuum to yield the deprotected mannoside as a viscous colourless gum (0.157 g, 90 %).

A solution of 2 M KOH (0.78 mL) was added to a stirred solution of the previously mannoside (0.431 g, 1.56 mmol) in H_2O (4 mL). After all of the starting material had been consumed, the pH was adjusted to 7 with 10% HCl and the solvent was evaporated to afford **159** as a viscous colorless foam (0.446 g, quantitative). **^{1}H NMR** (D_2O): δ 4.91 (d, *J* = 7.5 Hz), 4.18-4.09 (m, (C<u>H</u>CH_3), 3.84-3.77 (m), 3.74-3.63 (m), 3.57 (t, *J* = 8.9 Hz), 2.43-2.21 (m, (CHC<u>H</u>$_2CO_2^-$)), 1.20 (d, *J* = 6.1 Hz, CHC<u>H</u>$_2CO_2^-$), 1.14 (d, *J* = 5.6 Hz, CHC<u>H</u>$_2CO_2^-$) ppm. **^{13}C NMR** ($CDCl_3$): δ 179.9 (CH_2<u>C</u>O_2^-), 179.8 (CH_2<u>C</u>O_2^-), 99.7 (C-1), 96.5 (C-1), 73.4, 72.9, 72.5, 70.57, 70.52, 70.41, 70.29, 70.24, 66.84, 66.72, 61.0 (C-6), 60.7 (C-6), 45.5 (CH<u>C</u>$H_2CO_2^-$), 44.8 (CH<u>C</u>$H_2CO_2^-$), 20.8 (CH<u>C</u>H_3) ppm.

Experiment 137. Synthesis of Potassium (*2S*)-2-*O*-(α-D-mannopyranosyl)-2-hydroxysuccinate (160)

The methanolysis of the acetate groups of the mannoside **157** (1.343 g, 2.72 mmol) was performed according to the procedure described in experiment 136. The crude was purified by column chromatography on silica gel (20:80, MeOH/CH_2Cl_2) affording the desired deprotected mannoside (0.685 g, 78 %) as a viscous colourless gum, and the product of the hydrolysis at the anomeric position, the D-mannopyranoside (0.100 g, 20 %). After hydrolysis of the methyl ester according to the procedure described in experiment 133 compound **160** was obtained as a viscous colorless foam (0.0.786 g, quantitative). **^{1}H NMR** (D_2O): δ 4.85 (t, *J* = 15.6 Hz, 1H), 4.85 (s, 1H, H-1),

4.24 (dd, *J* = 8.2 Hz, *J* = 4.7 Hz, 1H), 3.90-3.58 (m, 5 H), 2.79-2.64 (m, 2H) ppm.

Experiment 138. Synthesis of Potassium (*2S*)-2-*O*-(D-mannopyranosyl)-2,3-dihydroxypropanoate (161)

TBAF (1M in THF; 0.38 mL, 0.38 mmol) was added to a solution of the **158** (0.220 g, 0.32 mmol) in THF (3 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by preparative TLC (60:40, EtOAc/hexane) afforded the alcohol as a viscous colourless gum (0.103 g, 72 %). The methanolysis of the acetate groups of the alcohol (0.518 g, 1.15 mmol) was performed according to the procedure described in experiment 136. After complete conversion of the starting material, previously activated Dowex-H^+ resin was added until neutral pH. After filtration with MeOH and water, the solvent was removed in vacuum to yield the deprotected mannoside as a viscous colourless gum (0.312 g, 96 %). Hydrolysis of the methyl ester according to the procedure described in experiment 136, the compound **161** was obtained as a viscous colorless foam (0.300 g, quantitative). **^{1}H NMR** (D_2O): δ 4.89 (d, *J* = 1.4 Hz, 1H, H-1), 4.02 (dd, *J* = 7.1, 3.2 Hz, 1H, C<u>H</u>CO_2^-), 3.99 (dd, *J* = 3.4, 1.6 Hz, 1H, H-), 3.89 (dd, *J* = 9.5, 3.4 Hz, 1H), 3.79 (dd, *J* = 12.2, 3.1 Hz, 1H, H-), 3.74-3.61 (m, 5H) ppm. **^{13}C NMR** ($CDCl_3$): δ 100.8, 80.6 (C-1), 73.2, 70.5, 70.1, 66.5, 62.5, 60.5 ppm.

Experiment 139. Synthesis of Dimethyl (*2S*)-2-*O*-(2-azido-3,4,di-*O*-benzyl-6-*O*-chloroacetyl-2-deoxy-α-D-glucopyranosyl)-2-hydroxysuccinate (162)

A suspension of thioglucoside donor **91** (0.750 g, 1.32 mmol), methyl (*S*)-malate **134** (0.197 mL, 1.52 mmol) and 4Å MS in CH_2Cl_2:Et_2O (1:4, 20 mL) was stirred for 1 h at room temperature then cooled to 0°C. A solution of *N*-iodosuccinimide (0.0.594 g, 2.64 mmol) and TfOH (0.027 mL) in CH_2Cl_2:Et_2O (1:1, 20 mL) was added at 0°C. After complete conversion of the starting material, 10% $Na_2S_2O_3$ aqueous solution (20 mL) and saturated aqueous

$NaHCO_3$ solution (10 mL) were added. The mixture was extracted with CH_2Cl_2 (3x20 mL), the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed under vacuum. The crude product was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) to afforded product **162** as a viscous colourless foam (0.672 g, 84 %).

^{1}H NMR ($CDCl_3$): δ 7.39-7.26 (m, 10H, Ph), 5.05 (d, *J* = 3.8 Hz, 1H, H-1), 4.91-4.85 (m, 3H), 4.60 (d, *J* = 11.2 Hz, 1H), 4.54 (t, *J* = 6.5 Hz, 1H, C<u>H</u>CH_2CO_2Me), 4.35-4.24 (m, 3H), 4.04-3.93 (m, 3H), 3.75 (s, 3H, CO_2Me), 3.73 (s, 3H, CO_2Me), 3.55 (t, *J* = 9.2 Hz, 1H, H-4), 3.30 (dd, *J* = 10.4, 3.8 Hz, 1H, H-2), 2.85 (d, *J* = 6.6 Hz, 2H, CHC<u>H</u>$_2CO_2Me$) ppm. **^{13}C NMR** ($CDCl_3$): δ 170.8, 170.2, 166.9, 137.66, 137.51, 128.55, 128.53, 128.06, 128.04, 128.00, 100.0 (C-1), 79.9, 77.3, 77.0, 76.7, 75.47, 75.33, 74.9, 69.7, 64.0, 63.2, 52.5, 52.2, 40.7, 37.6 ppm.

Experiment 140. Synthesis of Methyl (*2S*)-2-(2-azido-3,4,di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)propanoate (163)

A solution of NaOMe 1N (0.46 mL, 0.46 mmol) in MeOH was added to a stirred solution of **95** (0.470 g, 0.77 mmol) in MeOH (5 mL) at 0 ºC. After 1 hour the reaction mixture was neutralized with saturated aqueous NH_4Cl. The aqueous phase was extracted with EtOAc and the combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed. The crude product was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) to afford 163 (0.355 g, 98%) as a viscous colourless gum. **FTIR** (film) $\upsilon_{máx}$: 1753 (C=O), 2108 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.39-7.27 (m, 10H, Ph), 4.96 (d, *J* = 3.7 Hz, 1H, H-1), 4.90 (s, 2H, C<u>H</u>$_2$Ph), 4.87 (d, *J* = 11.1 Hz, 1H, C<u>H</u>$_2$Ph), 4.67 (d, *J* = 11.1 Hz, 1H, C<u>H</u>$_2$Ph), 4.18-4.02 (m, 3H), 3.74-3.73 (m, 3H, CO_2<u>Me</u>), 3.72-3.71 (m, 2H, H-6, H'-6), 3.59 (t, *J* = 9.5 Hz, 1H, H-4), 3.29 (dd, *J* = 10.4 Hz, *J* = 3.7 Hz, 1H, H-2), 1.48 (d, *J* = 6.8 Hz, 3H, CHC<u>H</u>$_3$) ppm. **^{13}C NMR** ($CDCl_3$): δ 172.7 (<u>C</u>O_2Me), 137.9, 128.52, 128.48, 128.04, 127.96, 127.88, 99.0 (C-1), 79.8, 78.0 (C-4), 75.42 (<u>C</u>H_2Ph), 75.23, 75.03 (<u>C</u>H_2Ph), 72.1, 63.2 (C-2), 61.5 (C-6), 52.2 (<u>C</u>O_2Me), 18.3 (CH<u>C</u>H_3) ppm.

Experiment 141. Synthesis of Methyl 2-(2-azido-3,4,di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)acetate (164)

The procedure of experiment 140 was applied to compound **96** (0.500 g, 0.94 mmol) affording compound **164** as a viscous colourless gum (0.393 g, 92 %). **FTIR** (film) $\upsilon_{máx}$: 1757 (C=O), 2110 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.39-7.28 (m, 10H, Ph), 5.04 (d, *J* = 3.5 Hz, 1H, H-1), 4.93-4.84 (m, 3H, C$\underline{H}_2$Ph), 4.67 (d, *J* = 11.1 Hz, 1H, C$\underline{H}_2$Ph), 4.27 (d, *J* = 16.3 Hz, 1H, C$\underline{H}$H'CO_2Me), 4.18 (d, *J* = 16.4 Hz, 1H, CH$\underline{H}$'CO_2Me), 4.05 (t, *J* = 9.6 Hz, 1H, H-3), 3.85-3.70 (m, 3H), 3.77 (s, 3H, $CO_2\underline{Me}$), 3.62 (t, *J* = 9.4 Hz, 1H, H-4), 3.42 (dd, *J* = 10.3, 3.6 Hz, 1H, H-2) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.7 ($\underline{C}O_2$Me), 137.8, 128.56, 128.50, 128.12, 128.09, 128.07, 128.02, 127.93, 97.9 (C-1), 80.0 (C-3), 77.6 (C-4), 75.6 ($\underline{C}H_2$Ph), 75.1 ($\underline{C}H_2$Ph), 72.1 (C-5), 64.3 ($\underline{C}H_2CO_2$Me), 63.4 (C-2), 61.5 (C-6), 52.1 ($\underline{C}O_2$Me) ppm.

Experiment 142. Synthesis of Methyl (*2R*)-*tert*-butyldimethylsilyl-3-(2-azido-3,4,di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3-dihydroxyropanoate (165)

TBAF (1M in THF; 1.14 mL, 1.14 mmol) was added to a solution of **97** (0.830 g, 1.03 mmol) in THF (7 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (50:50, EtOAc/hexane) afforded the alcohol as a viscous colourless gum (0.401 g, 72%). The procedure of experiment 140 was applied to the alcohol (0.296 g, 0.55 mmol) affording **165** as a viscous colourless gum (0.261 g, 92 %). **FTIR** (film) $\upsilon_{máx}$: 1739 (C=O), 2108 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.43-7.29 (m, 12H, Ph), 5.20 (d, *J* = 3.6 Hz, 1H, H-1), 4.97-4.88 (m, 3H, C$\underline{H}_2$Ph), 4.69 (d, *J* = 11.0 Hz, 1H, C$\underline{H}_2$Ph), 4.40 (dd, *J* = 5.3 Hz, *J* = 3.6 Hz, 1H, C$\underline{H}CH_2OH$), 4.08 (dd, *J* = 10.2 Hz, *J* = 9.0 Hz, 1H, H-3), 4.00-3.92 (m, 2H, CHC$\underline{H}_2$OH), 3.88-3.77 (m, 2H), 3.83 (s, 3H, $CO_2\underline{Me}$), 3.72 (dd, *J* = 12.1 Hz, *J* = 4.6 Hz, 1H, H-6), 3.63 (t, *J* = 9.5 Hz, 1H, H-4), 3.43 (dd, *J* = 10.3 Hz, *J* = 3.7 Hz, 1H, H-2) ppm. **^{13}C NMR** ($CDCl_3$): δ 169.8 ($\underline{C}O_2$Me), 137.76, 137.59, 128.59, 128.56, 128.52, 128.12, 128.06,

128.04, 127.97, 96.9 (C-1), 79.8 (C-3), 77.7 (C-4), 75.67 (CHCH$_2$OH), 75.52 (CH$_2$Ph), 75.3 (CH$_2$Ph), 72.2, 63.34 (C-2), 63.32 (CHCH$_2$OH), 61.5 (C-6), 52.5 (CO$_2$Me) ppm.

Experiment 143. Synthesis of Dimethyl (*2S*)-2-*O*-(2-azido-3,4,di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-hydroxysuccinate (166)

The procedure of experiment 140 was applied to compound **162** (0.670 g, 1.10 mmol) affording compound **166** as a viscous colourless gum (0.429 g, 73 %). **FTIR** (film) $\upsilon_{máx}$: 1739 (C=O), 2107 (N_3) cm^{-1}. **^{1}H NMR** ($CDCl_3$): δ 7.41-7.29 (m, 10H, Ph), 5.11 (d, *J* = 3.8 Hz, 1H, H-1), 4.94-4.88 (m, 3H, CH$_2$Ph), 4.71 (d, *J* = 11.1 Hz, 1H, CH$_2$Ph), 4.58 (dd, *J* = 7.2 Hz, *J* = 5.9 Hz, 1H, CHCH$_2$CO$_2$Me), 4.10-4.02 (m, 2H, H-3, H-5), 3.78 (s, 3H, CO$_2$Me), 3.78 (s, 3H, CO$_2$Me), 3.75-3.74 (m, 2H, H-6, H'-6), 3.62 (dd, *J* = 9.8 Hz, *J* = 9.2 Hz, 1H, H-4), 3.32 (dd, *J* = 10.4 Hz, *J* = 3.8 Hz, 1H, H-2), 2.91-2.89 (m, 2H, CHCH$_2$CO$_2$Me) ppm. **^{13}C NMR** ($CDCl_3$): δ 171.0 (CO$_2$Me), 170.2 (CO$_2$Me), 137.8, 128.52, 128.47, 128.02, 127.96, 127.88, 100.2 (C-1), 79.6 (C-3), 77.9 (C-4), 75.49 (CHCH$_2$CO$_2$Me), 75.39 (CH$_2$Ph), 75.0 (CH$_2$Ph), 72.3 (C-5), 63.3 (C-2), 61.4 (C-6), 52.5 (CO$_2$Me), 52.2 (CO$_2$Me), 37.6 (CHCH$_2$CO$_2$Me) ppm.

Differential scanning fluorimetry

Materials

Mannosylglycerate (MG), glucosylglycerate (GG), glucosylglucosylglycerate (GGG), mannosyl glycolate (MGly) and mannosyl lactate (ML) were obtained by chemical synthesis as described in literature[2, 12]. New synthetic compounds were obtained by chemical synthesis as described in Chapter 7. The desired compounds were purified by size exclusion chromatography on a Sephadex G-10 column eluted with water. The fractions containing the pure compounds were pooled, lyophilized. Purity and concentration of the compounds was assessed by ^{1}H NMR spectra obtained at 500 MHz spectrometer in D_2O. For quantification purposes, spectra were acquired with

a repetition delay of 60 s with formate as concentration standard. Only samples with purity higher than 98% were used. Mitochondrial malate dehydrogenase from pig heart (MDH) was purchased from Roche, and hen egg white lysozyme from Sigma-Aldrich. These enzymes were used without further purification. Recombinant staphylococcal nuclease A (SNase) was produced and purified from Escherichia coli cells as described by Faria et al.[13] Protein concentration was determined from UV absorbance at 280 nm, using 0.28 $(mg/mL)^{-1}cm^{-1}$ for the extinction coefficient of MDH[14], 2.58 $(mg/mL)^{-1}cm^{-1}$ for lysozyme[15] and 0.93 $(mg/mL)^{-1}cm^{-1}$ for SNase[16].

DSF assay

The protein melting temperature (T_M) determination was performed by monitoring protein unfolding with the fluoroprobe SYPRO Orange dye (Molecular Probes), which although completely quenched in aqueous environment, emits fluorescence upon binding to protein hydrophobic patches. Such increase in fluorescence can be measured as a function of temperature. The thermal shift assay was performed on an iCycle iQ5 Real Time PCR Detection System (Bio-Rad), equipped with a charge-coupled device (CCD) camera and a Cy3 filter with excitation and emission wavelengths of 490 and 575 nm, respectively. This equipment can simultaneously detect the fluorescence changes in 96-well plates (low profile plate, Bio-Rad) and thus can be used for parallel thermal stability assays. The 96-well plates are sealed with optical quality sealing tape (Bio-Rad) and centrifuged at 2500 g for 2 minutes immediately before the assay to remove possible air bubbles. The plates are subsequently heated from 20 to 90 ºC with stepwise increments of 1 ºC with a 60 seconds equilibration time, followed by the fluorescence read out. In a typical assay with a total volume of 20 µL, a protein concentration from 0.14 to 0.21 mg/mL, and a dye concentration of 5 fold were used to guarantee the best signal to noise ratio. Protein stock solutions of SNase or MDH were prepared in phosphate buffer (20 mM of sodium phosphate, pH 7.6), and lysozyme was prepared in citrate

buffer (40 mM sodium citrate, 110 mM NaCl, pH 6.0). These stock solutions were extensively dialyzed against the same buffer before the assays. Protein concentrations approximately 1.9 μM were used for MDH, 12.4 μM for SNase and 13 μM for lysozyme. Solute solutions were prepared in water with the respective concentrations. The assay was prepared by adding 2 μL of protein to 8 μL of dye buffer solution, and 10 μL of solute solution, all prepared in the protein purification buffer except for the solutes solutions. Fluorescence intensities versus temperature are used to calculate the protein melting temperature (T_M) by determining the first derivative (d(Rfu)/dT) to extract the exact transition inflection point.

References

1. Armarego, W. L. F.; Chai, C., *Purification of Laboratory Chemicals*. Elsevier Science: 2012.

2. Lourenco, E. C.; Maycock, C. D.; Ventura, M. R., Synthesis of potassium (*2R*)-2-*O*-alpha-D-glucopyranosyl-(1 -> 6)-alpha-D-glucopyranosyl-2,3-dihydroxypropanoate a natural compatible solute. *Carbohydrate Research* **2009,** 344, 2073-2078.

3. Sugimura, H.; Watanabe, K., Formal synthesis of cytosamine - A component of nucleoside antibiotics, the amicetin family. *Synthetic Communications* **2001,** 31, 2313-2321.

4. Ellervik, U.; Magnusson, G., A high yielding chemical synthesis of sialyl Lewis x tetrasaccharide and Lewis x trisaccharide; Examples of regio- and stereodifferentiated glycosylations. *Journal of Organic Chemistry* **1998,** 63, 9314-9322.

5. Li, Z. T.; Gildersleeve, J. C., Mechanistic studies and methods to prevent aglycon transfer of thioglycosides. *Journal of the American Chemical Society* **2006,** 128, 11612-11619.

6. Goddard-Borger, E. D.; Stick, R. V., An efficient, inexpensive, and shelf-stable diazotransfer reagent: Imidazole-1-sulfonyl azide hydrochloride. *Organic Letters* **2007,** 9, 3797-3800.

7. Csiki, Z.; Fugedi, P., Synthesis of glycosaminoglycan oligosaccharides. Part 4: Synthesis of aza-L-iduronic acid-containing analogs of heparan sulfate oligosaccharides as heparanase inhibitors. *Tetrahedron* **2010,** 66, 7821-7837.

8. Koketsu, M.; Kuwahara, M.; Sakurai, H.; Ishihara, H., First synthesis of a trisaccharide of glycosylkaemferide: A resistance factor in carnations. *Synthetic Communications* **2004,** 34, 239-245.

9. Hanessian, S., *Preparative Carbohydrate Chemistry*. Taylor & Francis: 1997.

10. Crich, D.; Cai, W. L., Chemistry of 4,6-O-benzylidene-D-glycopyranosyl triflates: Contrasting behavior between the gluco and manno series. *Journal of Organic Chemistry* **1999,** 64, 4926-4930.

11. Crich, D.; Jayalath, P., Stereocontrolled formation of beta-glucosides and related linkages in the absence of neighboring group participation: Influence of a trans-fused 2,3-O-carbonate group. *Journal of Organic Chemistry* **2005,** 70, 7252-7259.

12. Faria, T. Q.; Mingote, A.; Siopa, F.; Ventura, R.; Maycock, C.; Santos, H., Design of new enzyme stabilizers inspired by glycosides of hyperthermophilic microorganisms. *Carbohydrate Research* **2008,** 343, 3025-3033.

13. Faria, T. Q.; Lima, J. C.; Bastos, M.; Macanita, A. L.; Santos, H., Protein stabilization by osmolytes from hyperthermophiles - Effect of mannosylglycerate on the thermal unfolding of recombinant nuclease a from Staphylococcus aureus studied by picosecond time-resolved fluorescence and calorimetry. *Journal of Biological Chemistry* **2004,** 279, 48680-48691.

14. Thorne, C. J. R., Properties of Mitochondrial Malate Dehydrogenases. *Biochimica Et Biophysica Acta* **1962,** 59, 624-&.

15. Aune, K. C.; Tanford, C., Thermodynamics of denaturation of lysozyme by guanidine hydrochloride .I. Dependence on Ph at 25 degrees. *Biochemistry* **1969,** 8, 4579.

16. Fuchs, S.; Cuatreca.P; Anfinsen, C. B., An improved method for purification of Staphylococcal Nuclease. *Journal of Biological Chemistry* **1967,** 242, 4768-&.