Synthesis of Oligosaccharide Fragments of the Pectic Polysaccharide Rhamnogalacturonan I

PhD Thesis

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Preface

The work described in this PhD thesis has been carried out at the Department of Chemistry, Technical University of Denmark from March 2010 until March 2013 under the supervision of Associate Professor Mads H. Clausen and Professor Robert Madsen. The project has been a part of the EU Marie Curie research network LeanGreenFood.

Chapter 1 discusses the general aspects of oligosaccharide synthesis and includes a literature review on the chemical syntheses of rhamnogalacturonan I oligosaccharides.

Chapter 2 describes the synthesis of a fully unprotected linear hexasaccharide fragment of the rhamnogalacturonan I backbone.

Chapter 3 presents the strategy for synthesis of branched oligosaccharide fragments of rhamnogalacturonan I and tells about the synthesis of two tetrasaccharide intermediates with diarabinan and digalactan side chains.

Chapter 4 contains experimental procedures and compound characterization data.

Acknowledgements

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Anne Hector for help with obtaining numerous NMRs. Janne B. Rasmussen and Brian Ekman-Gregersen for keeping the chemicals in order. Tina Gustafsson for assistance with purification of compounds and obtaining MS data. Paul Erik Wibe for taking care of our always broken oil pump and other equipment. Lars E. Bruhn for knowing what is where and for the interesting small talks.

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Mum, dad and my sister Eugenia for your love, support, compliments and criticism. My fiancé Pavel for your love, understanding and belief in me and for reminding me that there are other things in life except for work.

I like to think that I have done it all on my own but I know the truth: this work would not be possible without all your help. Thank you so much!

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Abstract

Pectin is a highly heterogeneous polysaccharide of plant origin. It is found in the primary cell wall and contributes to various cell functions, including support, defense, signaling, and cell adhesion. Pectin also plays important role as a food additive, serving as stabilizing and thickening agent in products such as jams, yoghurts and jellies.

Rhamnogalacturonan I is one of the structural classes of pectic polysaccharides, along with homogalacturonan and rhamnogalacturonan II. The chemical structure of rhamnogalacturonan I is complex having a backbone consisting of alternating α-linked L-rhamnose and D-galacturonic acid units with numerous branches of arabinans, galactans and arabinogalactans positioned at C-4 of the rhamnose residues.

The structural complexity of pectin together with a wide range of its practical applications and a desire to understand its structure and functions in details have inspired many researches to pursuit chemical syntheses of pectic oligosaccharides.

Herein, the strategies for chemical synthesis of linear and branched oligosaccharide fragments of rhamnogalacturonan I are presented. The first successful synthesis of a fully unprotected linear hexasaccharide fragment of the rhamnogalacturonan I backbone has been accomplished. The strategy employs a highly modular approach that takes advantage of the armed-disarmed effect to generate the key *n*-pentenyl disaccharide glycosyl donor in a chemoselective fashion. Two protected *n*-pentenyl tetrasaccharide intermediates bearing the digalactan and the diarabinan side-chains have been synthesized. The suitably protected mono- and disaccharide donors have been utilized in the chemoselective glycosylations. The protective group pattern is designed to allow the assembly of larger branched rhamnogalacturonan I fragments.

Dansk Resumé

Pektin er et meget heterogent polysakkarid af vegetabilsk oprindelse. Det findes i den primære cellevæg og bidrager til forskellige cellefunktioner inklusiv støtte, forsvar, signalering og celleadhæsion. Pektin er et vigtigt tilsætningsstof i fødevarer, hvor det fungerer som stabilisator og fortykningsmiddel i fødevarer såsom marmelade, yoghurt og geléer.

Rhamnogalacturonan I er en af de strukturelle polysakkaridgrupper i pektiner, sammen med homogalacturonan og rhamnogalacturonan II. Den kemiske struktur af rhamnogalacturonan I er kompleks med et skelet bestående skiftevis af α-bundne L-rhamnose og D-galacturonsyre-enheder med mange forgreninger af arabinaner, galactaner og arabinogalactaner placeret på C-4 i rhamnosesukrene.

Den strukturelle kompleksitet af pektin sammen med den brede vifte af praktiske anvendelsesmuligheder samt et ønske om at forstå dets struktur og funktion i detaljer har inspireret mange forskere til at forfølge kemisk syntese af pektin oligosakkarider.

I denne afhandling præsenteres strategier for kemisk syntese af lineære og forgrenede oligosakkaridfragmenter af rhamnogalacturonan I. Den første vellykkede syntese af et fuldt ubeskyttet lineært hexasakkaridfragment af rhamnogalacturonan I er opnået. Strategien implementerer en modulær tilgang, der drager fordel af armed-disarmed effekten til chemoselektivt at generere en vigtig *n*-pentenyl disakkarid donor. To beskyttede *n*-pentenyl tetrasakkaridmellemprodukter, forsynet med digalactan og diarabinan sidekæder, er blevet syntetiseret. Mono- og disakkarid donorer er blevet anvendt i chemoselektive glycosyleringer med egnede beskyttelsesgrupper. Mønsteret af beskyttelsesgrupperne er designet til at muliggøre kobling af større forgrenede rhamnogalacturonan I fragmenter.

List of Abbreviations

Ac	Acetyl
All	Allyl, prop-2-en-1-yl
Api	Apiose
BDA	Butane diacetal
Bn	Benzyl
BSP	1-Benzenesulfinyl piperidine
Bu	Butyl
Bz	Benzoyl
CAN	Ammonium cerium(IV) nitrate
ClAc	Chloroacetyl
CSA	Camphor-10-sulfonic acid
d	Doublet
DABCO	1,4-Diazabicyclo[2.2.2]octane
DAST	(Diethylamino)sulfur trifluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
DMAP	4-(Dimethylamino)pyridine
DMF	*N*,*N*-Dimethylformamide
DMP	Dess-Martin periodinane
DMTST	Dimethylthiomethylsulfonium triflate
DQF-COSY	Double quantum filtered correlation spectroscopy
EDG	Electron-donating group
Et	Ethyl
EWG	Electron-withdrawing group
f	Furanose
FT-IR	Fourier transform infrared spectroscopy

Fuc	Fucose
Gal	Galactose
GalA	Galacturonic acid
HG	Homogalacturonan
HMBC	Heteronuclear multiple bond correlation spectroscopy
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IDCP	Iodonium di-*sym*-collidine perchlorate
IR	Infrared spectroscopy
LG	Leaving group
m	Multiplet
MALDI-TOF	Matrix assisted laser desorption ionization time of flight
MCPBA	*m*-Chloroperoxybenzoic acid
Me	Methyl
MS	Molecular sieves; mass spectrometry
NAP	2-Naphthylmethyl
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
p	Pyranose
PFBz	Pentafluorobenzoyl
PG	Protective group
Ph	Phenyl
PMB	*p*-Methoxybenzyl
R	Radical
RG	Rhamnogalacturonan
Rha	Rhamnose
RRV	Relative reactivity values

s	Singlet
t	*Tert*
t	Triplet
TBA	Tetrabutylammonium
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TES	Triethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Tol	Tolyl, *p*-methylphenyl
Tr	Trityl, triphenylmethyl
Ts	Tosyl, *p*-toluenesulfonyl
TTBP	2,4,6-Tri-*tert*-butylpyrimidine

Contents

1 Introduction

1.1 Pectin

"Pectin" is to some extent a deceptive term as it does not mean one type of molecule. In fact, pectin is a common name for the most structurally complex and diverse family of plant polysaccharides. It is a major component of the primary cell wall of all land plants and contributes to various cell functions, including support, defense, signaling and cell adhesion.[1] Pectin plays important role as a functional food ingredient, serving as stabilizing and thickening agent in the production of jams, jellies, yoghurts, fruit juice and confectionary products.[2] It is also used in the production of biodegradable films, surface modifiers for medical devices, materials for biomedical implantation, and for drug delivery.[3]

The properties of pectin have been known for many years, but recently a lot of knowledge about the fine structure of pectic polysaccharides has been gained. All pectic polysaccharides contain D-galacturonic acid (GalA) to a greater or lesser extent. Among them, three major classes have been identified: homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II).[4] It is believed that these polymers are covalently linked to each other, but a clear picture of how they are connected has not been obtained and several models exist.[5]

HG, the most abundant component of pectin, is a homopolymer of α-(1→4)-linked D-galacturonic acid (Figure 1). Its polysaccharide chain can be acetylated at C-2, C-3 or both and the carboxylic acid functionalities are often methyl esterified. These substituents are important structural modifications, as they can significantly influence the physical and chemical properties of polysaccharides.[6]

The chemical structure of RG I, the second most abundant class of pectic polysaccharides, is complex, having a backbone of alternating α-(1→4)-linked L-rhamnose and α-(1→2)-linked D-galacturonic acid units (Figure 1) with numerous branches of arabinans, galactans or arabinogalactans positioned at C-4 of the rhamnose residues, with substantial structural variation within these branches.

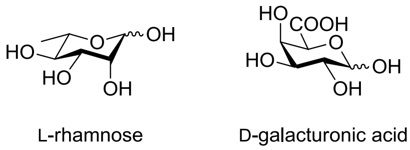


Figure 1 Chemical structures of L-rhamnose and D-galacturonic acid

RG II is the third major and the most structurally complex component of pectin. It has an HG backbone with various side chains consisting of twelve different monosaccharides linked with twenty different linkages. RG II contains monosaccharide units which are uncommon for other plant polysaccharides, such as D-apiose, 3-*C*-carboxy 5-deoxy-L-xylose (L-aceric acid), 2-*O*-methyl L-fucose, 2-*O*-methyl D-xylose, L-galactose, 3-deoxy-D-lyxo-2-heptulosaric acid (Dha) and 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo).[7]

Understanding pectin structure, function and biosynthesis is essential for understanding, and potentially modifying, cell wall structure.[1] This can lead to production of new "designer" pectin with improved properties.[2] Structurally defined oligosaccharide fragments of pectin can find a wide application for studying plant cell wall structure and function as well as enzymes acting on the plant cell wall. Pectic oligosaccharides can be obtained either by controlled chemical or enzymatic degradation of pectin followed by fractionation or by chemical synthesis. Although a number of studies of selective degradation of pectic polysaccharides have been published, the scope of the structures available by this method is still limited and the obtained oligosaccharides require extensive chromatographic purification.[8] Chemical synthesis, on the other hand, is capable of producing structurally diverse oligosaccharides of excellent purity and in sufficient amount. General aspects of oligosaccharide synthesis are discussed below.

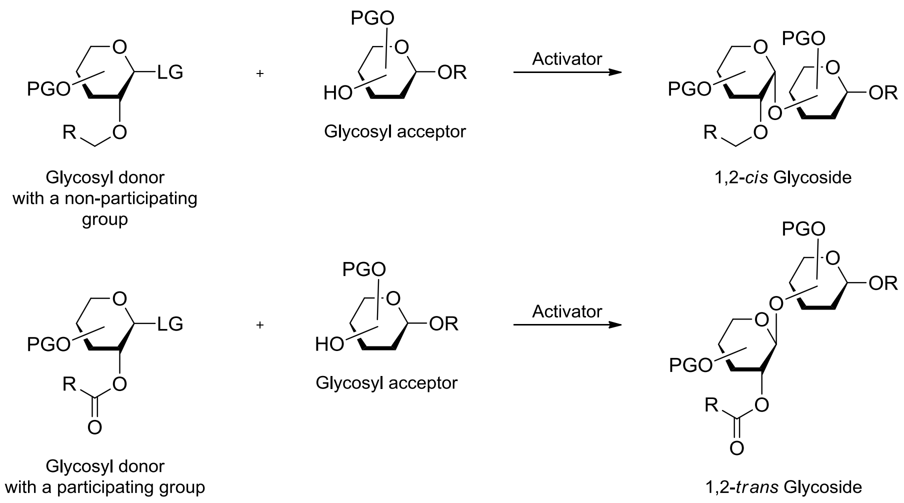
1.2 Oligosaccharide Synthesis – General Aspects

The importance of carbohydrate molecules has encouraged chemists to develop methods for creating glycosidic linkages and perform chemical syntheses of various oligosaccharides. The first glycosylation reactions were reported already in the end of the 19th century. Since then, a lot of knowledge has been accumulated and systematized. Many excellent books and reviews covering different aspects of oligosaccharide synthesis have been published.[9–14] It is not the aim of this short chapter to give a comprehensive overview of oligosaccharide synthesis. Instead, a brief introduction to the field will be given and the concepts closely related to the work described in the thesis will be discussed in more details. Additionally, the existing literature on synthesis of pectic oligosaccharides will be reviewed with specific attention paid to the syntheses of rhamnogalacturonan I fragments.

1.2.1 Glycosylation Reaction. Stereo- and Regioselectivity in the Formation of Glycosidic Linkage.

In oligosaccharide synthesis, glycosidic linkages between monosaccharide residues are created in glycosylation reactions. A glycosylation reaction is based on a nucleophilic displacement of a leaving group from a glycosyl donor by a free hydroxyl group of a glycosyl acceptor. The remaining hydroxyl groups of both the donor and the acceptor are usually protected with the suitable protective groups. Glycosylation reactions are performed in a stepwise and selective fashion to build up larger oligosaccharides with the desired chemical structure.

Despite glycosylation being a central reaction in carbohydrate chemistry, its mechanism has not been fully understood.[15,16] All the considerations given herein are based on the simplified and commonly used glycosylation mechanism.[12] As outlined in Scheme 1, a glycosylation reaction commences with an activator-assisted departure of a leaving group of a glycosyl donor, which results in a formation of an oxocarbenium ion, followed by a nucleophilic attack by the hydroxyl group of the glycosyl acceptor. The nature of the protective group installed at the C-2 position of the donor has a major impact on the stereoselectivity of glycosylation. In case the protective group at C-2 is non-participating (*i.e.* not capable of providing an anchimeric assistance), such as a benzyl ether, the nucleophilic attack on the oxocarbenium ion is possible from both the top and the bottom face of the sugar ring. Even though the 1,2-*cis* product is thermodynamically favored due to the anomeric effect,[17] in many cases substantial amounts of the kinetic 1,2-*trans* product are formed and the α/β-mixtures are obtained because of the irreversible nature of glycosylation reactions. Galactosyl and mannosyl donors tend to form α-products, while α/β-mixtures are usually obtained from glucosyl donors. Various factors including choice of protective groups, activator, reaction conditions (temperature, solvent) can affect the glycosylation outcome. When a participating protective group, such as an acetyl or a benzoyl ester, is installed at the C-2 position of a glycosyl donor, the glycosylation proceeds through an acyloxonium intermediate. In this case, the nucleophilic attack takes place preferentially from the top face of the sugar ring and a stereoselective formation of the 1,2-*trans* glycosidic linkage is achieved.



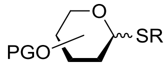
Scheme 1 Stereoselectivity in glycosylation reactions (for carbohydrates with the gluco-configuration). LG – leaving group, PG – protective group. Adapted from Nepogodiev *et al.*[8]

The regioselectivity in glycosylation reactions is usually secured by the suitable protection of the glycosyl acceptor, ensuring that only the hydroxyl group that needs to be glycosylated is left unprotected. The choice of protecting groups is dictated by their compatibility (in protection/deprotection and lability to other transformations), selectivity (in protection) and sequence (order of deprotection when other protective groups are employed).[18,19] An impressive number of different protective groups has been developed, and the optimal conditions for their introduction and removal have been established.[20] Preparation of monosaccharide building blocks with various protective group patterns has been described.[21] In certain cases difference in the reactivity of the hydroxyl groups in the partially protected acceptor can be exploited, meaning that a selective glycosylation of a more reactive hydroxyl group in the presence of a less reactive one can be achieved.[22] Typically, nucleophilicity of the hydroxyl groups is decreasing in the order primary hydroxyl > equatorial secondary hydroxyl > axial secondary hydroxyl.

1.2.2 Glycosyl Donors

A large number of potent glycosyl donors has been developed, most commonly used being thio/selenoglycosides,[23,24] glycosyl trichloroacetimidates[25] and recently introduced *N*-phenyl trifluoroacetimidates,[26] glycosyl halides,[27,28] glycosyl sulfoxides,[29] glycals,[30,31] *n*-pentenyl glycosides,[32] glycosyl thioimidates,[33,34] glycosyl phosphates,[35] etc. Various conditions are available for activation of each type of glycosyl donor.[14] Thioglycosides, pentenyl glycosides and glycosyl imidates were employed in this work; thus their properties will be discussed in details.

Thioglycosides



Thioglycoside

Thioglycosides, for the first time used as glycosyl donors by Ferrier and co-workers,[23] are nowadays one of the most widely used classes of glycosyl donors. This originates from their stability under a variety of reaction conditions, which allows for extensive protective group manipulations in the presence of the thio functionality. Thioglycosides are commonly prepared from the fully acetylated monosaccharides by Lewis acid catalyzed reactions with thiols.[36] Thioglycosides can be activated with a variety of electrophilic reagents. In the activation step, a lone pair of the sulfur atom of the glycosyl donor reacts with an electrophilic species, resulting in the formation of a sulfonium intermediate. This intermediate is a good leaving group and can be displaced by a hydroxyl group of the glycosyl acceptor. The most commonly employed thioglycoside activators are *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) or trimethylsilyl trifluoromethanesulfonate (TMSOTf),[37,38] iodonium di-*sym*-collidine perchlorate (IDCP),[39] methyl trifluoromethanesulfonate (MeOTf),[40] phenylselenyl triflate (PhSeOTf),[41,42] dimethylthiomethylsulfonium triflate (DMTST),[43] and the recently introduced sulfonium triflate activators 1-benzenesulfinyl piperidine/triflic anhydride (BSP/Tf_2O),[44] and diphenyl sulfoxide/Tf_2O (Ph_2SO/Tf_2O)[45] (Figure 2).

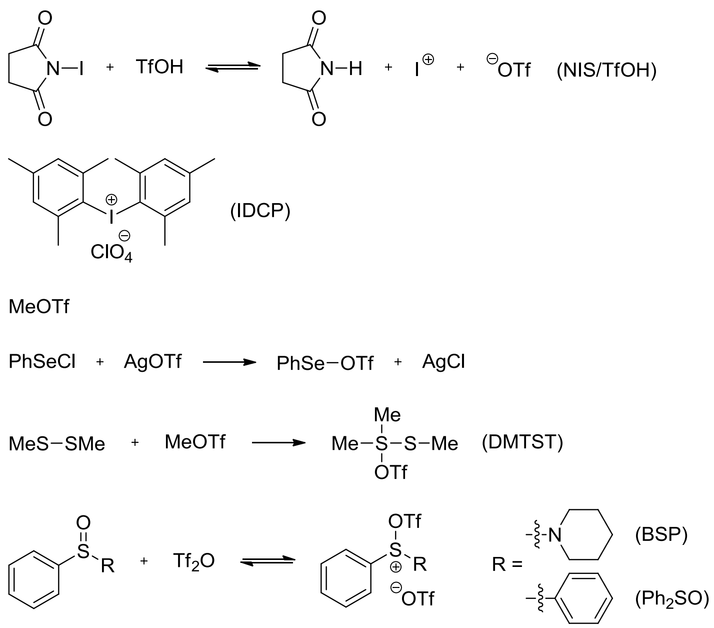


Figure 2 Electrophilic reagents for activation of thioglycosyl donors. Adapted from Codée *et al.*[46]

The thio functionality can serve not only as a leaving group, but also as a convenient temporary protective group for the anomeric position. Thioglycosides can be converted into a variety of glycosyl donors (Figure 3). For example, treatment of a thioglycoside with bromine provides a glycosyl bromide.[36] The resulting glycosyl bromide can be used in glycosylation reaction directly or after a purification step. The hemiacetal functionality can be accessed using *N*-bromosuccinimide (NBS) in wet acetone.[47] The obtained hemiacetal can be further transformed into a trichloroacetimidate glycosyl donor (*vide infra*). A glycosyl fluoride can be obtained when a thioglycoside is treated with *N*-bromosuccinimide/(diethylamino)sulfur trifluoride (NBS/DAST).[48] Treatment of a thioglycoside with oxidants, such as *m*-chloroperoxybenzoic acid (MCPBA),[29] affords a glycosyl sulfoxide. This makes thioglycosides particularly useful building blocks in chemoselective glycosylation strategies (*vide infra*).

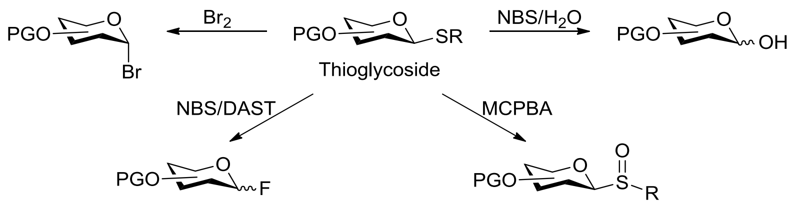
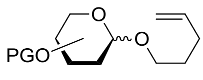


Figure 3 Transformation of thioglycosides into other types of glycosyl donors

Although thioglycosides are potent and widely employed glycosyl donors, possible aglycon transfer makes them less practical when acceptors of low nucleophilicity (e.g. due to steric hindrance) are used. The aglycon transfer can be rationalized as follows: the oxonium ion formed after the activation of the glycosyl donor is attacked by the sulfur atom of the thioglycoside instead of the hydroxyl group due to the low reactivity of this hydroxyl group. It was demonstrated that in some cases the aglycon transfer can be suppressed by employing less reactive thio glycosides with sterically demanding aglycones.[46]

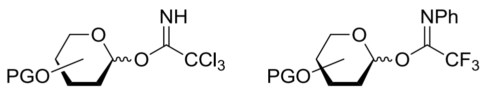
n-Pentenyl Glycosides



n-Pentenyl glycoside

n-Pentenyl glycosides as glycosyl donors were introduced by Fraser-Reid and co-workers.[32] They can be prepared according to standard procedures for making alkyl glycosides. The Fisher glycosylation provides a direct access to pentenyl glycosides from the non-protected monosaccharides. Alternatively, pentenyl glycosides can be obtained by a glycosylation of *n*-pentenyl alcohol with glycosyl acetates or under Koenings-Knorr[27] conditions. Pentenyl glycosides can be activated with NIS/TfOH and NIS/triethylsilyl trifluoromethanesulfonate (TESOTf)[49] or with the less potent promoter IDCP.[50] Alike the thio functionality, the *n*-pentenyloxy group is stable under the majority of protective group manipulation conditions, except those of catalytic hydrogenation, and therefore can serve as a temporary protective group for the anomeric position. By treatment with bromine, pentenyl glycosides can be transformed into glycosyl bromides.[51] Reaction of a pentenyl glycoside with NBS/water liberates a free hydroxyl group at the anomeric position.[33]

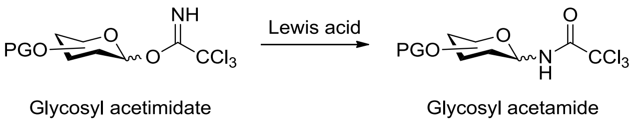
Trichloroacetimidates and *N*-Phenyl Trifluoroacetimidates



Glycosyl imidates

Glycosyl imidate donors, developed by Schmidt and co-workers,[25] are probably the most commonly used nowadays owing to their ability to perform as very powerful glycosyl donors under mildly acidic conditions.[52] Apart from application in classic oligosaccharide synthesis, trichloroacetimidates have also been used for solid-supported oligosaccharide assembly.[53]

Glycosyl trichloroacetimidates can be prepared from the corresponding anomeric hemiacetals by treatment with trichloroacetonitrile under basic conditions. Organic or inorganic bases, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), NaH, K_2CO_3, Cs_2CO_3, can be employed. Trichloroacetimidate donors are activated with catalytic amounts of Lewis acid, typically TMSOTf or boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$).[54] When glycosyl acceptors of low nucleophilicity are used, the high reactivity of trichloroacetimidate donors can become a disadvantage and lead to significant amounts of undesired side-products. A rearrangement of a glycosyl acetimidate into the corresponding glycosyl acetamide is occasionally observed (Scheme 2). These obstacles can often be overcome by using *N*-phenyl trifluoroacetimidates that do not undergo the corresponding rearrangement and are considerably less reactive[55] presumably due to the lower basicity of the substituted nitrogen atom and.



Scheme 2 Rearrangement of a glycosyl trichloroacetimidate

In certain cases, the so-called "inverse" protocol, where the glycosyl acceptor and a catalytic amounts of TMSOTf are premixed before the addition of the trichloroacetimidate donor, is advantageous as it diminishes decomposition of the glycosyl donor by the acid.[56]

Reactivity of Glycosyl Donors

It has long been known that electronic effects of the substituents in carbohydrates (both in the carbohydrate and the aglycon parts) have remarkable effects on their reactivity. Already in 1982 in Paulsen's classic review,[9] it was stated that "benzyl compounds are always more reactive than the acetylated or benzoylated derivatives". Ley and co-workers conducted the first systematic study to quantify the influence of protective groups on reactivity of glycosyl donors.[57] Later, Wong and co-workers performed a comprehensive examination of reactivity of a large number of differently protected *p*-methylphenyl thioglycosides (STol).[58] This was done in order to quantify the reactivity of glycosyl donors in terms of relative reactivity values (RRVs). RRVs were defined as the ratio of products derived from two glycosyl donors competing for one glycosyl acceptor. This quantification of reactivity led to several general observations[59]:

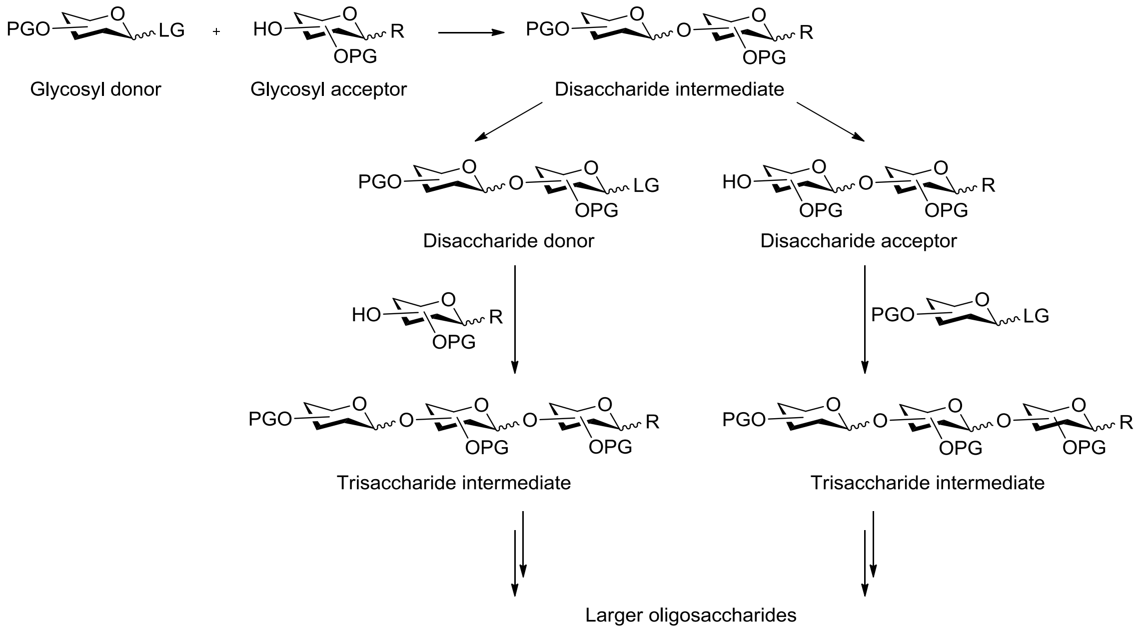
- Reactivities of pyranosides differ as a function of sugar. Reactivity decreases in the order fucose > galactose > mannose > glucose > sialic acid.
- Protective groups affect reactivity of glycosyl donors. The electron-withdrawing protective groups decrease reactivity by lowering the nucleophilicity of the anomeric thio functionality. This effect is decreased in the order OClAc > OBz > OAc > OBn > OH > OSilyl > H.
- The effect of a substituent is dependent on its position in the sugar ring. However, the position that affects the reactivity most is not the same for all sugars.
- Conformational effects play a role. Axial substituents increase reactivity.[60]
- Reactivity depends on the nature of leaving groups. Bulky leaving groups at the anomeric position decrease reactivity.[61] *Para*-substituents in the phenyl ring influence reactivity in the order OMe > H > NO_2.
- Reactivity can be tuned by using different solvents. More reactive glycosyl donors can be selectively activated over the less reactive ones when glycosylation is performed in Et_2O. The less reactive donors can subsequently be activated when CH_2Cl_2 is used as a solvent.[62]

To conclude, the reactivity of glycosyl donors is influenced by a variety of factors such as the nature of protective groups and the reaction conditions.

1.2.3 Synthetic Strategies for Oligosaccharide Assembly

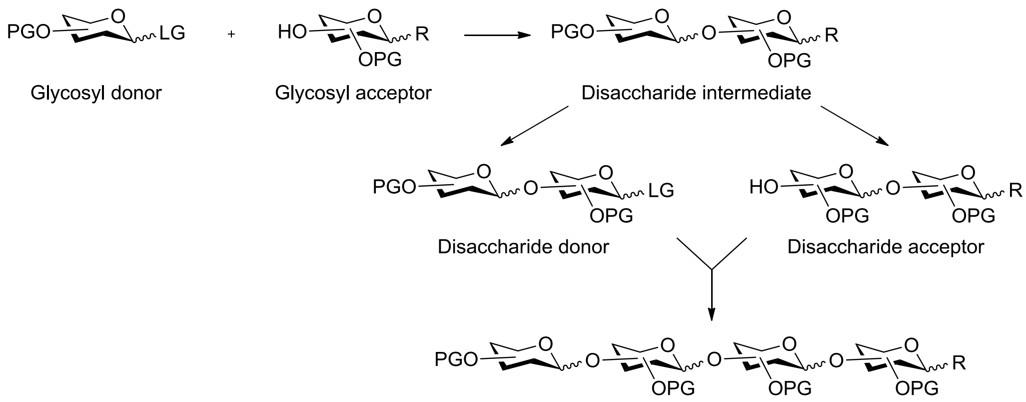
Linear vs. Convergent Approach

Fundamentally, there are two distinct approaches to oligosaccharide assembly: linear and convergent.[12] In a linear approach (Scheme 3), the carbohydrate chain is extended by one monosaccharide unit at a time. The oligosaccharide can be build starting from either the non-reducing or the reducing end. After coupling of two monosaccharide building blocks, the resulting disaccharide is converted either into a new glycosyl donor (by removing an anomeric protective group and installing a new leaving group) or into a new glycosyl acceptor (by removing the temporary protective group). This disaccharide is then coupled with a monosaccharide building block to provide a trisaccharide. The process is reiterated until an oligosaccharide of the desired length is obtained.



Scheme 3 Linear strategy in oligosaccharide synthesis

Alternatively, the convergent approach (Scheme 4) can be employed. In this strategy, smaller oligosaccharide building blocks are synthesized separately and subsequently used for the assembly of larger oligosaccharides.



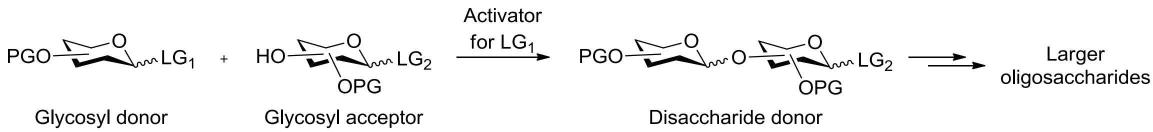
Scheme 4 Convergent strategy in oligosaccharide synthesis

A major advantage of the convergent approach over the linear synthesis is that it requires less protective group manipulations, which in general makes the synthesis shorter and increases its overall efficiency. Another benefit of the convergent strategy is the possibility to conduct "difficult" glycosylations at an earlier stage of the synthesis leaving "easy" coupling steps for the end.

Strategies for Chemoselective Glycosylations

In a selective glycosylation, two saccharides both bearing leavings groups at the anomeric position are coupled. Choice of the reaction conditions allows for the selective activation of one reaction partner over the other. This approach minimizes the number of synthetic steps, as no conversion of an anomeric protective group into a leaving group is required after the glycosylation step, and the obtained product can be taken directly into the next glycosylation. Various approaches to selective glycosylations have been developed.[12] Some of them are based on using different types of leaving groups at the anomeric position (the orthogonal strategy), while the others take advantage of the distinct reactivity of the building blocks caused by electronic or steric effects of the protective groups in their structure (the armed-disarmed strategy).

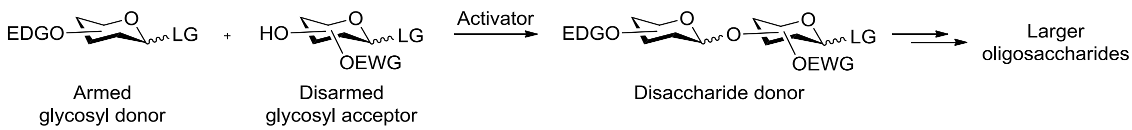
In the orthogonal strategy, two reaction partners bearing different leaving groups are employed.[63] These two leaving groups require two mutually distinct promoter systems. Thus, the selectivity of glycosylation reaction can be controlled by choosing a suitable activator (Scheme 5).



Scheme 5 Orthogonal approach in oligosaccharide synthesis

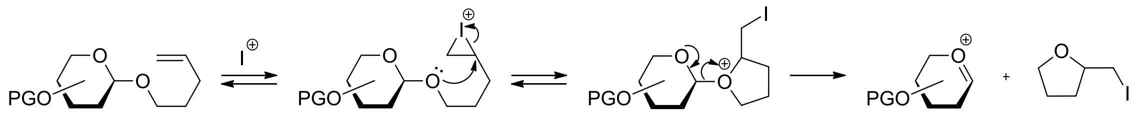
The advantage of the orthogonal strategy is that selectivity of the couplings does not depend on the relative reactivity of the building blocks allowing for more flexible choice of protective groups.

In contrast to the orthogonal strategy, the armed-disarmed approach employs the same type of the leaving group in both the donor and the acceptor. In this case, the selectivity of glycosylation is dictated by the different reactivity of the reaction partners (Scheme 6). The armed-disarmed approach was introduced by Fraser-Reid and co-workers, who discovered that pentenyl glycosides protected with electron-donating ether protective groups ("armed") could be selectively activated in the IDCP-catalyzed glycosylations over pentenyl glycosides protected with electron-withdrawing ester protective groups ("disarmed").[50]



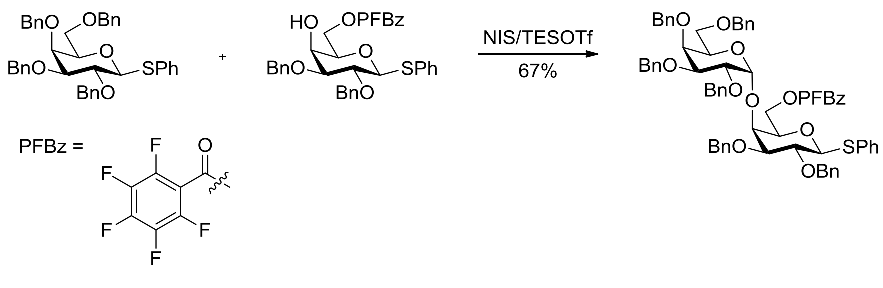
Scheme 6 Armed-disarmed approach in oligosaccharide synthesis; EDG – electron-donating group, EWG – electron-withdrawing group

This difference in reactivity can be explained as follows[64]: upon a reversible addition of the iodonium ion to the double bond, a cyclic iodonium ion is formed; it is then attacked by the lone pair of the oxygen atom of the *n*-pentenyloxy group to give the cyclic intermediate, which then collapses into the oxocarbenium ion and a molecule of 2-iodomethyltetrahydrofuran (Scheme 7). If the pentenyl glycoside is protected with electron-withdrawing groups, the nucleophilicity on the exocyclic oxygen is decreased and thus it becomes less reactive.



Scheme 7 Activation of a pentenyl glycoside in glycosylation reaction

The armed-disarmed approach has been applied to glycosylations with other classes of glycosyl donors, including thioglycosides,[39] glycals[30] and thioimidates.[65] Madsen and co-workers further expanded the scope of the armed-disarmed glycosylations by demonstrating that a glycosyl acceptor could be significantly "disarmed" by introducing a single strongly electron-withdrawing group at the C-6 position of the sugar ring.[66,67] The best results in glycosylations were obtained when a pentafluorobenzoyl (PFBz) group was used (Scheme 8). It is important that this strategy allows for the formation of the 1,2-*cis* glycosidic linkage in the subsequent glycosylation, while previously in the armed-disarmed couplings the C-2 position of the acceptor always contained an ester protective group dictating the formation of the 1,2-*trans* linkage.



Scheme 8 Disarming of a glycosyl acceptor by a remote pentafluorobenzoyl group

1.2.4 Concluding Remarks

Although modern carbohydrate chemistry has an extensive arsenal of methods to assemble virtually any oligosaccharide molecule, each case remains to be an individual and often laborious task. Unlike in peptide and nucleic acid chemistry, in carbohydrate synthesis there is yet no universal approach that would allow building any type of oligosaccharide. Owing to the complexity of the glycosylation reactions and a large number of factors to be carefully considered (including the nature of the protective groups, choice of a leaving group and reaction conditions), achieving high yields and good stereocontrol in many glycosylations remains a challenge.

1.3 Chemical Synthesis of Pectic Oligosaccharides

The structural complexity of pectin together with a wide range of its practical applications and a desire to understand its structure and functions in details have inspired many researches to pursuit chemical syntheses of pectic oligosaccharides. A number of strategies for the synthesis of oligosaccharide fragments of HG, RG I and RG II have been reported in the literature. Table 1 summarizes the published work on synthesis of oligosaccharide fragments of pectin.

Table 1 Oligosaccharide fragments of pectin which have been chemically synthesized. Adapted from Nepogodiev *et al.*[8]

Synthetic oligosaccharide fragment	Reference
Homogalacturonan fragments	
α-D-Gal*p*A-(1→4)-D-Gal*p*A	
Two monomethyl esterified isomers	Magaud *et al.*[68]
Protected mono- and dimethyl- esterified methyl α- and β-glycosides	Magaud *et al.*[69]

Synthetic oligosaccharide fragment	**Reference**
Protected dimethyl esterified allyl β-glycoside	Kramer *et al.*[70]
Protected dimethyl esterified allyl α-glycoside	Vogel *et al.*[71]
α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-D-Gal*p*A	
Three monomethyl esterified isomers	Clausen *et al.*[72]
Protected fully methyl esterified allyl β-glycoside	Kramer *et al.*[70]
α-D-Gal*p*A-(1→4)-{(α-D-Gal*p*A-(1→4)}$_4$-D-Gal*p*A	
Five partially methyl esterified compounds	Clausen & Madsen[67]
α-D-Gal*p*A-(1→4)-{(α-D-Gal*p*A-(1→4)}$_8$-D-Gal*p*A-β-D-Gal*p*A--OPr	Nakahara & Ogawa[73]
α-D-Gal*p*A-(1→4)-{(α-D-Gal*p*A-(1→4)}$_{10}$-D-Gal*p*A	Nakahara & Ogawa[74]
Rhamnogalacturonan II fragments	
β-D-Api*f*-(1→2)-α-D-Gal*p*A-OMe	Buffet *et al.*[75] Nepogodiev *et al.*[76]
β-L-Rha*p*-(1→3′)-β-D-Api*f*-OMe	Chauvin *et al.*[77]
β-L-Rha*p*-(1→3′)-β-D-Api*f*-(1→2)-α-D-Gal*p*A-OMe	Nepogodiev *et al.*[78]
α-L-Fuc*p*-(1→ 4)-L-Rha*p* (free disaccharide and methyl α-and β-glycosides)	Egelund *et al.*[79]
β-D-Gal*p*A-(1→3)-α-L-Rha*p*-OMe	Chauvin *et al.*[80]
β-D-Gal*p*A-(1→3)-[α-D-Gal*p*A-1→2]-α-L-Rha*p*-OMe	Chauvin *et al.*[80]
α-L-Fuc*p*-(1→4)-[β-D-Gal*p*A-(1→3)]-[α-D-Gal*p*A-(1→2)]-α-L--Rha*p*-OMe	Chauvin *et al.*[80]
Ace*f*	Jones *et al.*[81] Nepogodiev *et al.*[78] Timmer *et al.*[82]
β-L-Ace*f*-(1→3)-α-L-Rha*p*-OMe (partially protected)	de Oliveira *et al.*[83]

Synthetic oligosaccharide fragment	Reference
α-L-Rha*p*-(1→3)-α-L-Ara*p*-(1→4)-[2-*O*-β-L-MeFuc*p*-(1→2)]-β--D-Gal*p*-O$(CH_2)_3NH_2$	Rao & Boons[84]
β-L-Ara*f*-(1→3)-α-L-Rha*p*-(1→2)-[α-L-Rha*p*-(1→3)-]-α-L-Ara*p*--(1→4)-[2-OMe-β-L-Fuc*p*-(1→2)]-β-D-Gal*p*-O$(CH_2)_3NH_2$	Rao & Boons[84]
β-L-Ara*f*-(1→3)-α-L-Rha*p*-(1→2)-[α-L-Rha*p*-(1→3)-]-α-L-Ara*p*--O$(CH_2)_3NH_2$	Rao *et al.*[85]
Rhamnogalacturonan I fragments	
α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-D-Gal*p*A (dimethyl esterified and partially protected)	Nolting *et al.*[86]
α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-β-D--Gal*p*A-OPr	Maruyama *et al.*[87] Nemati *et al.*[88]
α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D--Gal*p*A-OMe (with free and dimethyl esterified Gal*p*A residues)	Reiffarth & Reimer[89]
α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-D-Gal*p*A (with free and monomethyl esterified Gal*p*A residues)	Scanlan *et al.*[90]

1.3.1 Synthetic Studies of RG I Oligosaccharides

RG I polysaccharides have a common backbone with repeating disaccharide unit α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4). The diversity of RG I structures is caused by the presence of various side chains of galactan, arabinan or arabinogalactan positioned at C-4 of the backbone rhamnose residues (Figure 4). RG I side chains are complex and variable. Galactans are mostly linear chains of β-(1→4)-linked D-galactose residues. Arabinans are chains of α-(1→5)-linked L-arabinofuranose residues that are frequently branched at C-3 and sometimes at C-2. Arabinogalactan side chains are mostly arabinogalactan I which is β-(1→4)-galactan with arabinan branches; highly branched arabinogalactan II with β-(1→3)-linked galactose residues that are more common in proteoglycans may also be part of RG I. Some of the galacturonic acid residues of RG I can be acetylated at C-2 and/or C-3.[47]

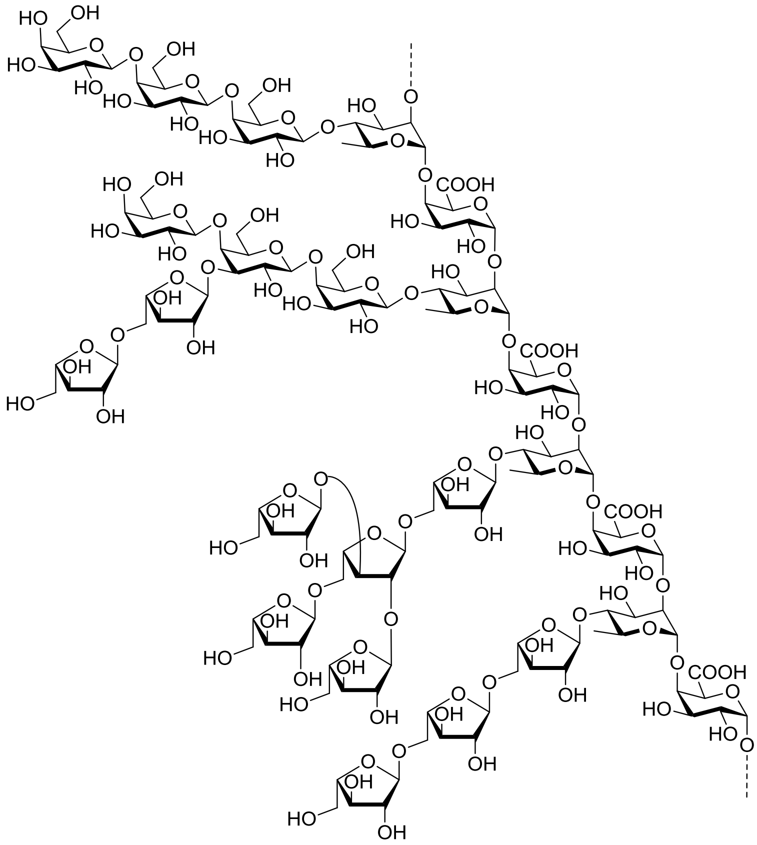


Figure 4 Representation of RG I chemical structure

Several chemical syntheses of fully and partially unprotected RG I oligosaccharide fragments have been performed, their structures are shown in Figure 5. Some of the strategies have used galacturonic acid as the starting material, while others have favored the oxidation of galactose to galacturonic acid at a late stage, i.e. pre- and postglycosylation-oxidation strategies, respectively. These two approaches are general for synthesis of oligosaccharides containing uronic acids.[91] In the preglycosylation-oxidation approach, suitably protected galacturonic acid derivatives are directly used in glycosylation reactions. In the postglycosylation-oxidation strategy, galactose derivatives are employed instead. When the desired oligosaccharide is assembled, temporary protective groups are removed to release the C-6 hydroxyl groups which are then oxidized to carboxylic acid functionalities. Although the postglycosylation-oxidation strategy requires additional protective group manipulations, it has been observed that the non-oxidized carbohydrates are generally more reactive glycosyl donors than their oxidized counterparts,[92–94] where reactivity is decreased by the presence of the electron-withdrawing carboxyl groups.

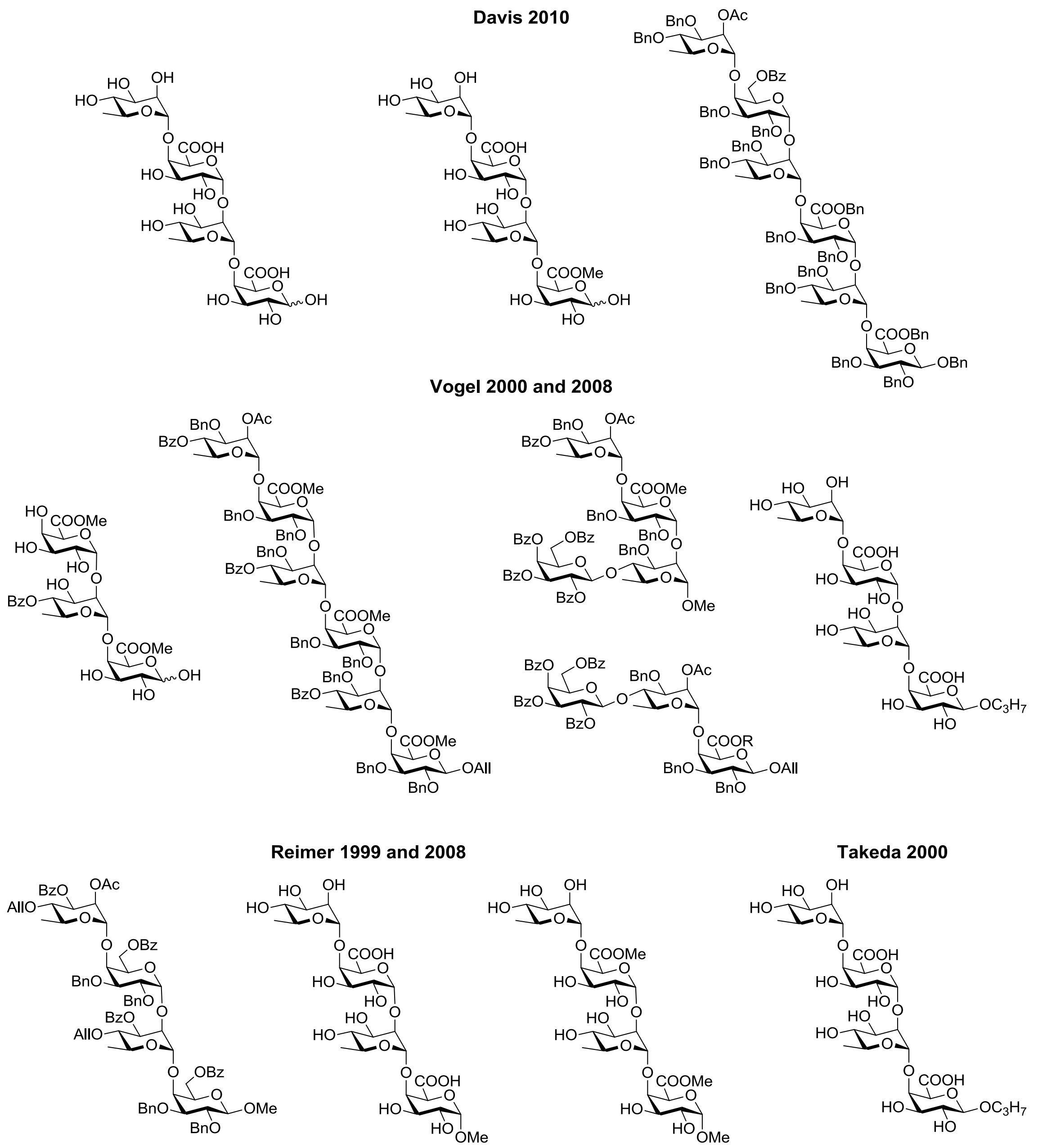
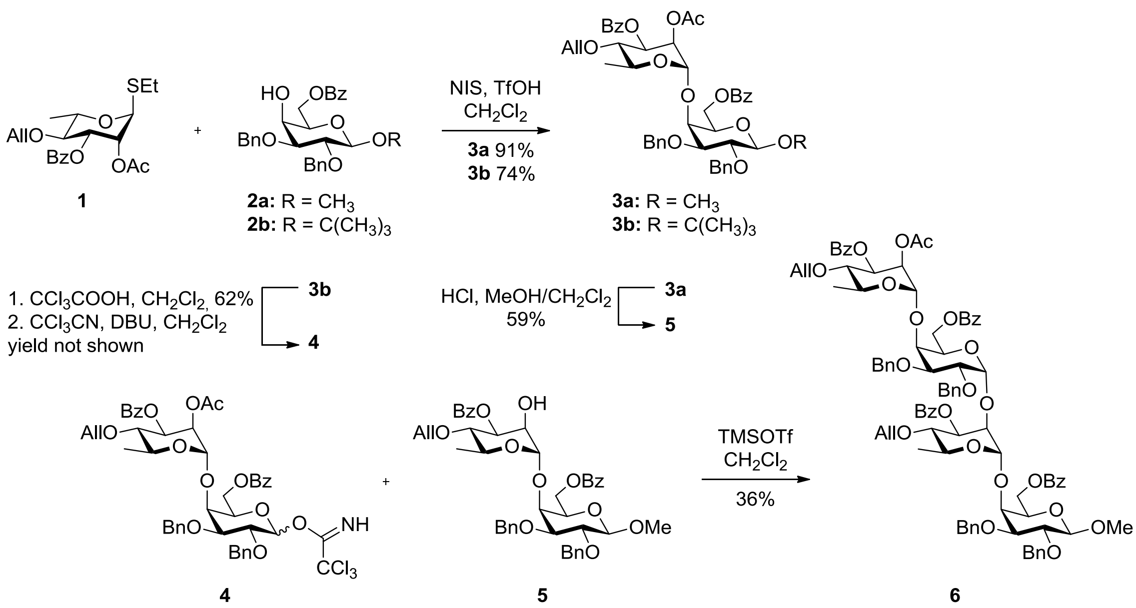


Figure 5 Published synthetic oligosaccharide fragments of RG I

Synthesis of a Protected Tetrasaccharide Intermediate

Reimer and co-workers reported the synthesis of the protected tetrasaccharide **6** containing galactose instead of galacturonic acid as an intermediate for the preparation of RG I fragments (Scheme 9).[95] Tetrasaccharide **6** was designed to be a key intermediate in overall synthetic strategy to synthesize RG I oligosaccharides. The C-2 acetyl protective group of the terminal rhamnosyl residue of **6** was envisioned to be selectively removed which would allow for further elongation of the main chain. Alternatively, removal of the C-4 allyl protective groups of the two rhamnosyl units would allow for introduction of side-chains. Finally, full deprotection and selective oxidation of the primary hydroxyl groups in the galactosyl residues would introduce the carboxylic acid functionalities found in the native RG I polysaccharide.

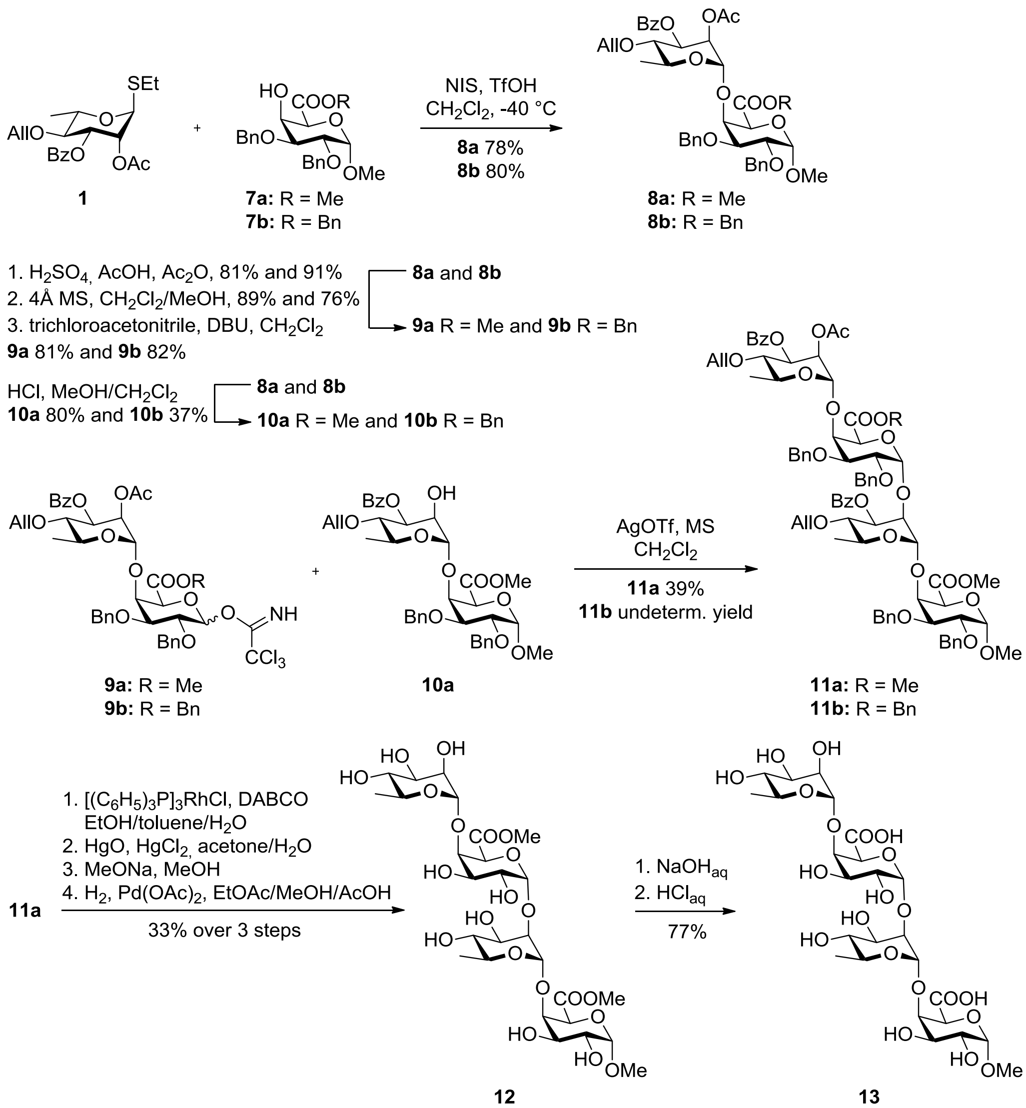


Scheme 9 Synthesis of a protected tetrasaccharide intermediate for the possible assembly of RG I oligosaccharides by Reimer and co-workers

In this synthesis, rhamnosyl thioglycoside donor **1** and galactosyl acceptor **2a** were coupled in a NIS/TfOH-catalyzed glycosylation reaction to give disaccharide **3a** in 91% yield. Similarly, reaction of the same glycosyl donor **1** with glycosyl acceptor **2b** afforded disaccharide **3b** in 74% yield. In a test reaction, it was demonstrated that selective removal of the C-4 allyl protective group in **3a** could be achieved, which indicated that selective deprotection of the C-4 positions of tetrasaccharide **1** and later introduction of the branching should be possible. Selective deprotection of the C-2 acetyl protective group in **3** was done by treatment with methanolic HCl and provided glycosyl acceptor **5**. Trichloroacetimidate **4** was obtained from **3b** by first treatment with trichloroacetic acid and then with trichloroacetonitrile and DBU. The TMSOTf-catalyzed coupling of disaccharides **4** and **5** afforded target tetrasaccharide **1** in 36% yield.

Synthesis of a Tetrasaccharide Fragment of RG I Backbone

In their later work Reimer and co-workers synthesized the fully unprotected methyl glycoside of the RG I tetrasaccharide, both in the methyl ester and the free carboxylic acid forms (Scheme 10).[89] A block synthesis approach was used, which allowed for the coupling of two disaccharide units derived from the same disaccharide intermediate to form the target tetrasaccharide. The C-4 positions of the rhamnosyl residues were orthogonally protected with allyl protective groups to allow for possible introduction of the side-chains. In this work, galacturonic acid was employed from the early stages. This lowered the overall number of synthetic steps by avoiding the late stage oxidation. Unfortunately, the key glycosylation reaction proved to be problematic and only low yields of the protected tetrasaccharide product could be obtained.

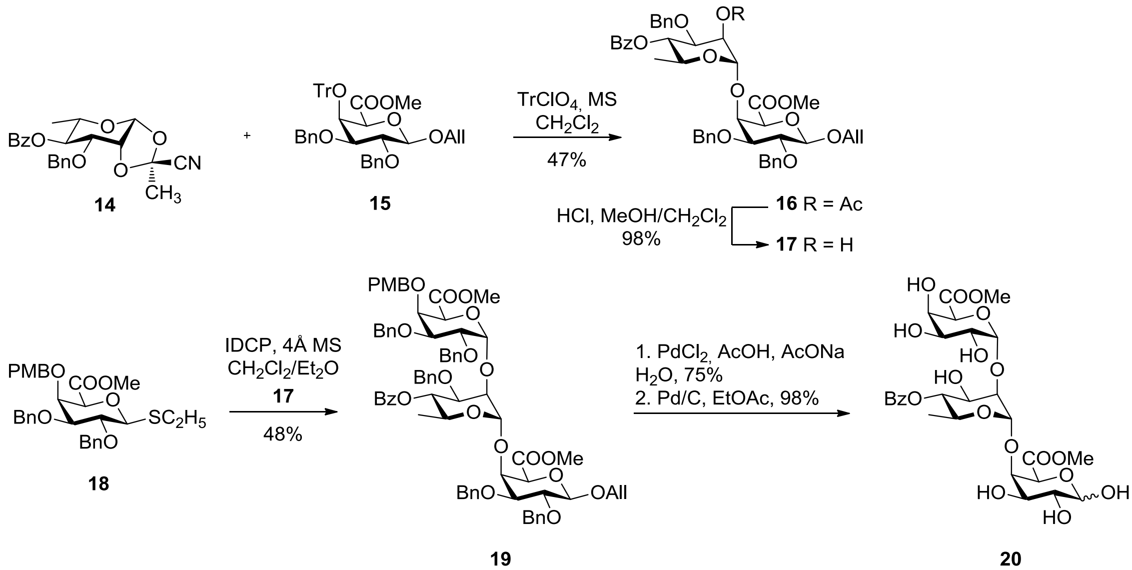


Scheme 10 Synthesis of a tetrasaccharide fragment of RG I backbone by Reimer and co-workers

This synthesis utilized two types of protected monosaccharide building blocks, rhamnosyl thioglycoside **1** (the same glycosyl donor was used in the previous work of the group[95]) and galacturonic acid derivatives **7a** and **7b**. The NIS/TfOH-catalyzed glycosylation reaction afforded disaccharides **8a** and **8b** in 78% and 80% yield, respectively. Both **8a** and **8b** were, in three steps, converted into trichloroacetimidate glycosyl donors **9a** and **9b**. Removal of the C-2 acetyl protective group of the rhamnose residue of **8a** and **8b** using methanolic HCl gave disaccharide acceptors **10a** and **10b** in 80% and 37% yield, respectively. The low yield of **10b** was caused by the transesterification of the benzyl ester as well as the loss of the C-2 acetyl. Disaccharide **10a** was used in further synthesis. Glycosylation of **10a** with glycosyl donors **9a** and **9b** turned out to be problematic. Only 39% yield of tetrasaccharide **11a** and an impure sample of tetrasaccharide **11b** were obtained when silver trifluoromethanesulfonate (AgOTf) was used as an activator. A number of methods were explored in an attempt to improve the outcome of the glycosylation reaction. Using TMSOTf or *t*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) as activators, as well as attempts to generate thioglycoside and bromide glycosyl donors, proved unsuccessful. The fully deprotected tetrasaccharide **12** in the methyl ester form was obtained from **11a** in three steps in 33% yield. The allyl protective groups were removed by treatment with Wilkinson's catalyst,[96] followed by a combination of mercury(II) oxide and mercury(II) chloride. Cleavage of the benzoyl and the acetyl protective groups was achieved under the Zemplén conditions.[97] The benzyl groups were removed by hydrogenolysis in presence of palladium(II) acetate catalyst. Treatment of **12** with aqueous NaOH, followed by acidification, afforded the fully unprotected tetrasaccharide **13** in the free carboxylic acid form in 77% yield.

Synthesis of a Partially Deprotected Trisaccharide Fragment of RG I Backbone

Vogel and co-workers prepared a partially deprotected RG I trisaccharide bearing a benzoyl group at C-4 of the rhamnose residue (Scheme 11).[86] The strategy employed trityl-cyanoethylidene condensation and thioglycoside methodology. Galacturonic acid was used as a starting material.

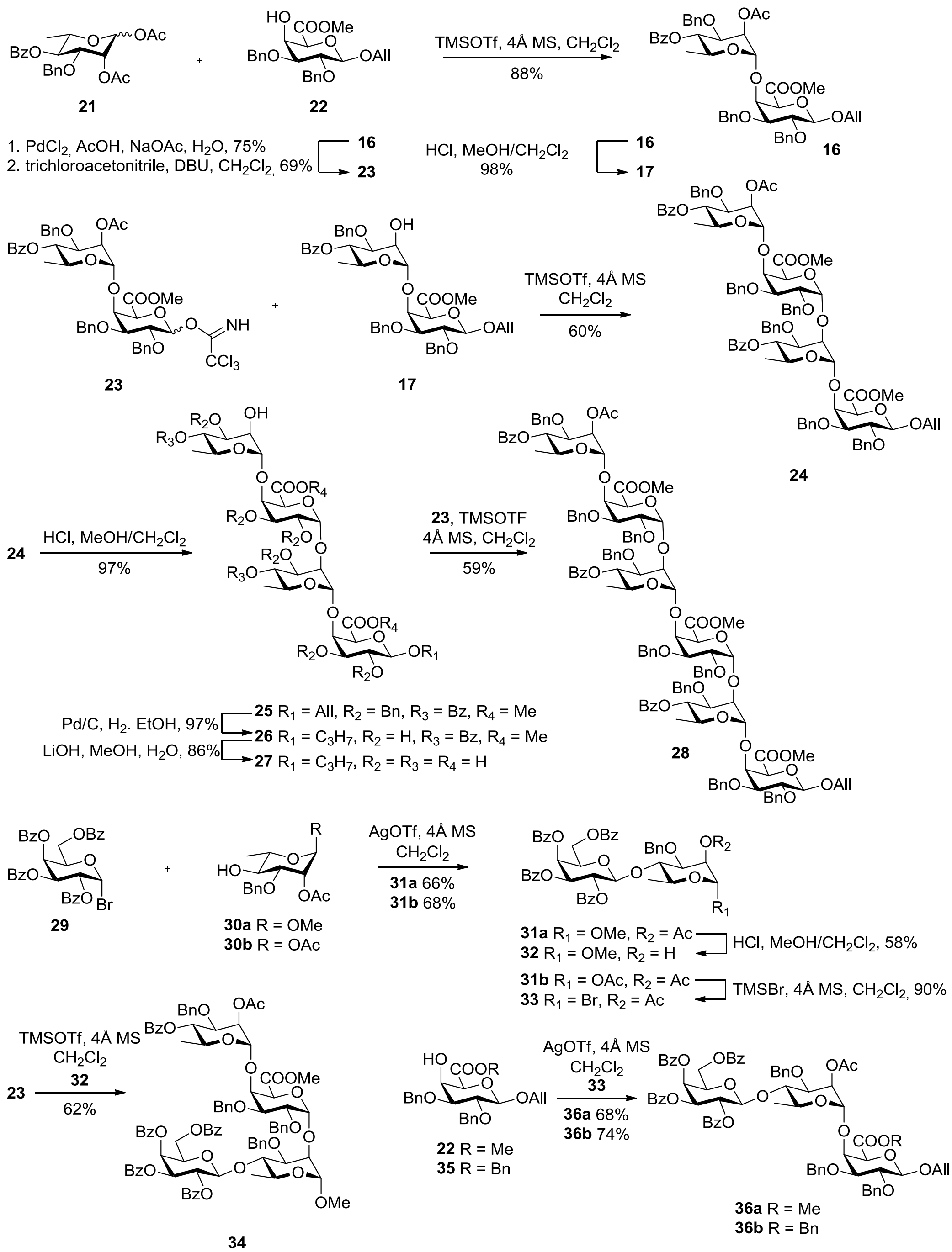


Scheme 11 Synthesis of a partially deprotected trisaccharide fragment of RG I backbone by Vogel and co-workers

Cyano-ethylidene rhamnosyl donor **14** was coupled with galactosyluronic acceptor **15** bearing a trityl protective group; disaccharide **16** was obtained in 47% yield. The C-2 acetyl group of the rhamnose residue of **16** was selectively removed by treatment with methanolic HCl resulting quantitatively in glycosyl acceptor **17**. The IDCP-catalyzed coupling of **17** with galactosyluronic thioglycoside donor **18** procured the trisaccharide product **19** in 48% yield. Finally, the allyl and benzyl protective groups were removed by palladium(II) chloride catalyzed deallylation, followed by hydrogenolysis over Pd/C to give the partially deprotected trisaccharide **20**.

Modular Design Approach for Synthesis of RG I Fragments

Later, Vogel and co-workers reported the synthesis of the fully unprotected propyl glycoside of the RG I tetrasaccharide (**27**), as well as synthesis of its protected hexasaccharide fragment (**28**) and the protected tri- (**36a** and **36b**) and tetrasaccharides (**34**) suitable for assembly of the branched RG I fragments (Scheme 12).[88]



Scheme 12 Modular design approach for synthesis of RG I fragments by Vogel and co-workers

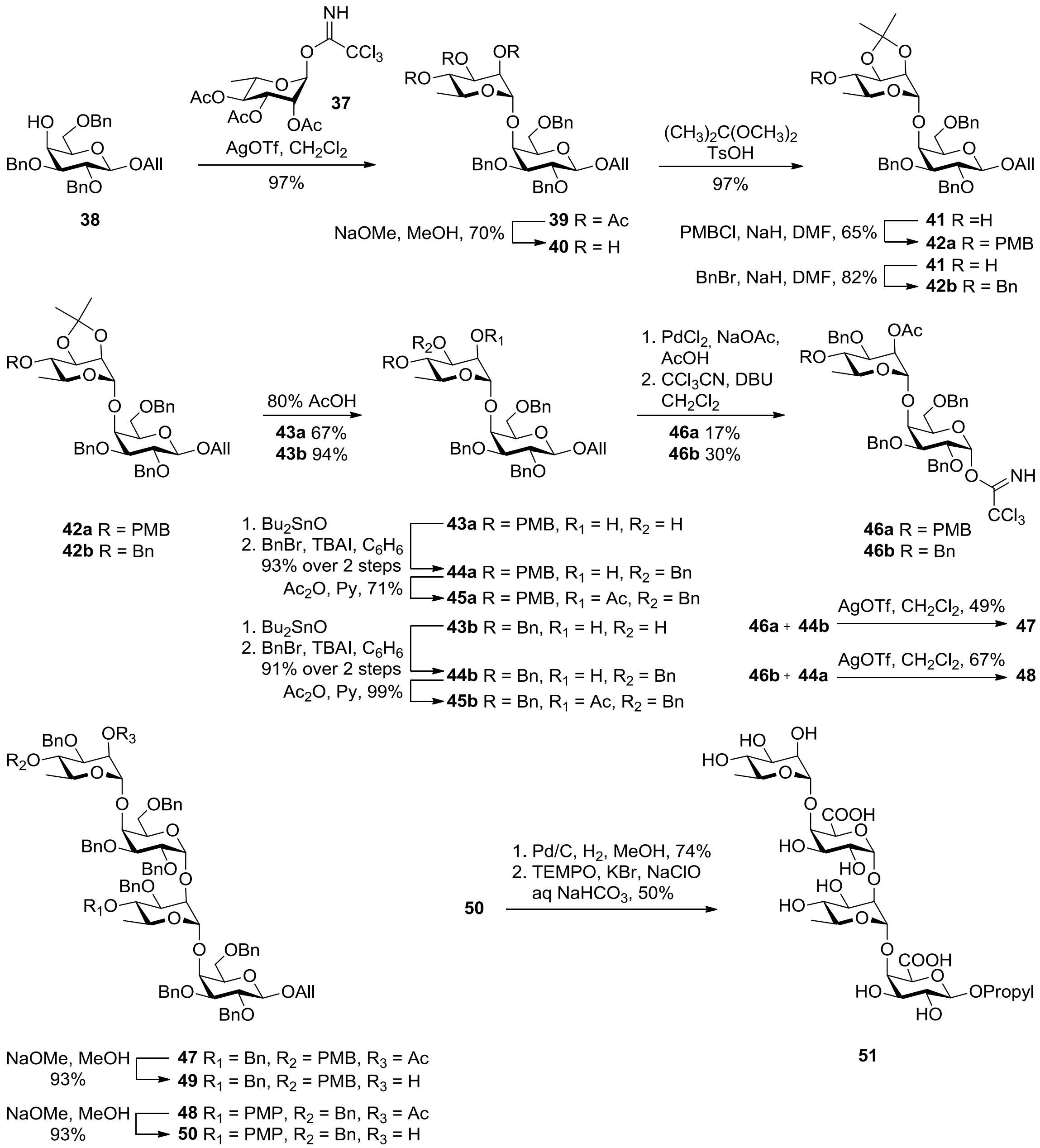
The synthesis was based on a modular principle and used galacturonic acid as the starting material. The oligosaccharides were designed to bear benzoyl protective groups at C-4 of the rhamnose residues to allow for possible attachment of branching.

Rhamnosyl donor **21** and galactosyluronic acceptor **22** were coupled in the TMSOTf-catalyzed glycosylation reaction to produce disaccharide **16** in 88% yield. Disaccharide **16** was then converted into a trichloroacetimidate donor **23** and glycosyl acceptor **17**. Donor **23** was obtained from **16** in two steps, first by palladium(II) chloride catalyzed deallylation and then by treatment with trichloroacetonitrile and DBU. Acceptor **17** was produced after selective deacetylation of **16** with methanolic HCl. The synthesis of **16** and its transformation into **17** were previously described by the same authors before.[86] Contrary to the observations of Reimer and co-workers,[89] the TMSOTf-catalyzed glycosylation of acceptor **17** with donor **23** provided the desired tetrasaccharide **24** in 60% yield. It was subjected to methanolic HCl to give tetrasaccharide **25**. The fully deprotected tetrasaccharide **27** was obtained from **25** in two steps, first by removal of the benzyl protective groups by hydrogenolysis over Pd/C and simultaneous reduction of the allyl group in the anomeric position to the propyl group, and then by the cleavage of the ester protective groups in methanol and water in the presence of lithium hydroxide. The potential application of the modular design approach to the synthesis of larger RG I fragments was demonstrated by preparation of the fully protected hexasaccharide **28** by the TMSOTf-catalyzed glycosylation of **27** with disaccharide donor **23** in 59% yield. In addition, smaller RG I fragments containing galactose monosaccharide branching were synthesized. The AgOTf-catalyzed coupling of the benzoylated galactosyl bromide **29** with either methyl rhamnoside **30a** or diacetate **30b** gave disaccharides **31a** and **31b** in 66% and 68% yield, respectively. Compound **33a** was converted into disaccharide glycosyl acceptor **34** by treatment with methanolic HCl. Acceptor **32** was then taken into the TMSOTf-catalyzed glycosylation with disaccharide donor **23** which provided the tetrasaccharide product **34** in 62% yield. Compound **33b** was transformed into glycosyl bromide **33** by treatment with bromotrimethylsilane (TMSBr) and coupled with galactosyluronate acceptors **22** and **35** to provide trisaccharides **36a** and **36b** in 68% and 74% yield, respectively.

Synthesis of a Fully Unprotected Propyl Glycoside of RG I Tetrasaccharide

Takeda and co-workers[87] prepared the unprotected propyl glycoside of RG I tetrasaccharide (**51**) employing trichloroacetimidate glycosyl donors and a late stage oxidation approach (Scheme 13). The rhamnose residues were bearing orthogonal *p*-methoxybenzyl (PMB) protective groups at C-4 allowing for possible introduction of side-chains.

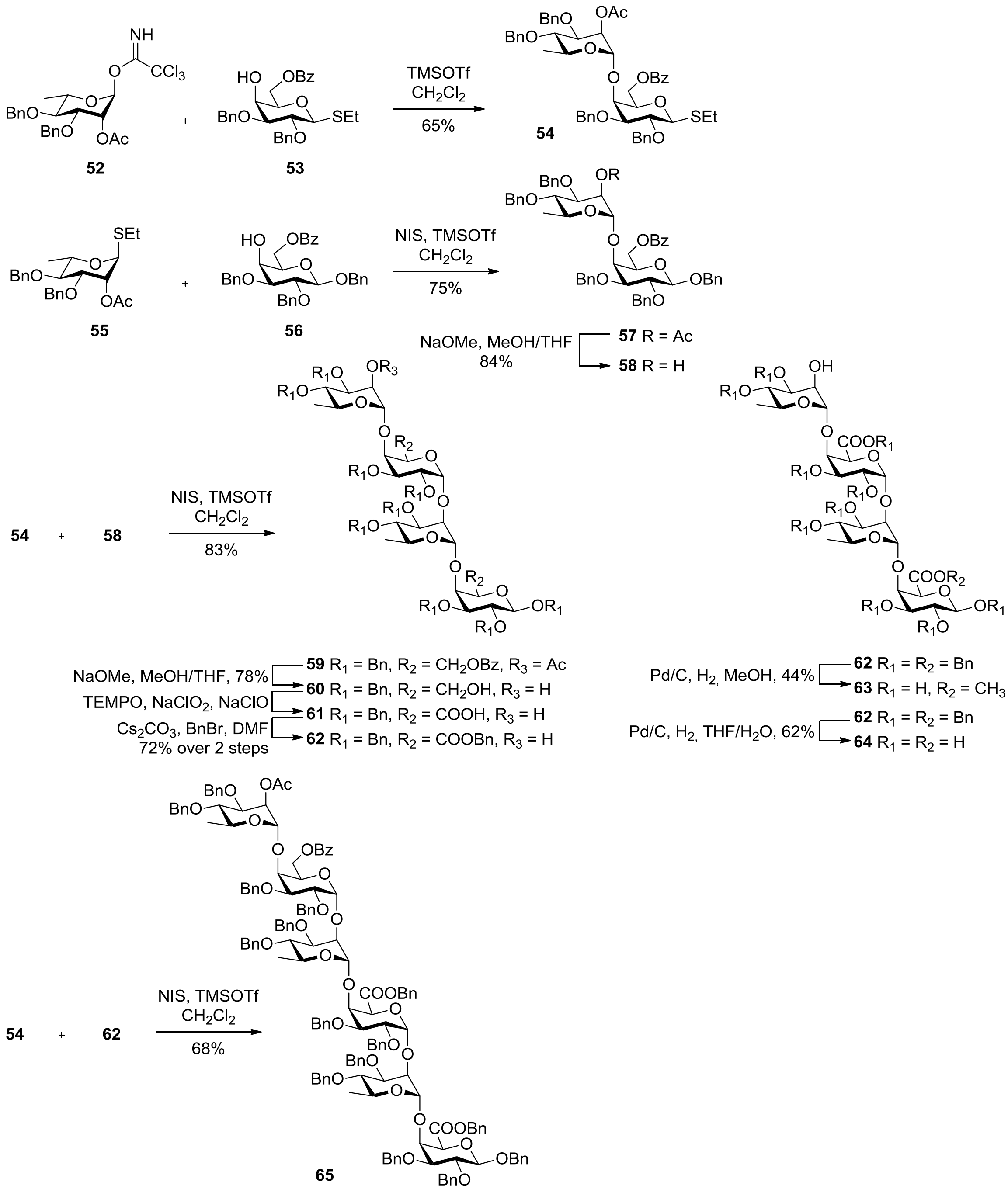
The trichloroacetimidate rhamnosyl donor **37** was coupled with galactose acceptor **28** in the AgOTf-catalyzed glycosylation reaction to give allyl disaccharide **39** in 97% yield. The acetyl protective groups of the rhamnose residue were removed by treatment with sodium methoxide in methanol. Isopropylidenation of the obtained partially protected disaccharide **40** followed by protection of the C-4 hydroxyl group of rhamnose with PMB and benzyl protective groups gave disaccharides **42a** and **42b**, respectively. Disaccharides **42a** and **42b** were then converted into acceptors **44a** and **44b** by acid-catalyzed hydrolysis of the acetonides followed by selective protection of C-3 in rhamnose with a benzyl group using dibutyltin(IV) oxide, benzyl bromide and tetrabutylammonium iodide (TBAI) in benzene. Disaccharides **44a** and **44b** were acetylated with acetic anhydride and then converted into glycosyl donors **46a** and **46b** in moderate yields by palladium (II) chloride catalyzed deallylation, followed by treatment of the resulting hemiacetal with trichloroacetonitrile and DBU. The AgOTf-catalyzed coupling of **46a** and **44b** gave tetrasaccharide **47** in 49% yield. Similarly, the AgOTf-catalyzed glycosylation of **46b** with **44a** furnished tetrasaccharide **48** in 67% yield. Both **47** and **48** were deacetylated by treatment with sodium methoxide in methanol to give tetrasaccharides **49** and **50**, respectively. Compound **50** was subjected to palladium-catalyzed hydrogenolysis followed by selective oxidation of the primary hydroxyl groups with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), KBr and NaClO in aqueous $NaHCO_3$, which provided tetrasaccharide **51** in 37% yield over two steps.



Scheme 13 Synthesis of a fully unprotected propyl glycoside of RG I tetrasaccharide by Takeda and co-workers

Synthesis of a Fully Unprotected RG I Tetrasaccharide, Its Methyl Ester and a Protected RG I Hexasaccharide Analog

In a recent report by Davis and co-workers an orthogonal approach was employed and combined with the late stage oxidation strategy to synthesize the fully unprotected RG I tetrasaccharide **64** and its methyl ester **63** (Scheme 14).[90] Interestingly, the initial attempt to couple a galactorhamnosyl disaccharide donor to the C-4 hydroxyl group of galactose of the disaccharide acceptor failed due to the lack of reactivity of the acceptor, forcing the authors to change the strategy and assemble this RG I tetrasaccharide through galactosylation instead of rhamnosylation. The potential of this methodology for iterative extension of the oligosaccharide chain was demonstrated by preparation of a fully protected analog of the native hexasaccharide **65**, containing both galactose and galacturonic acid residues.

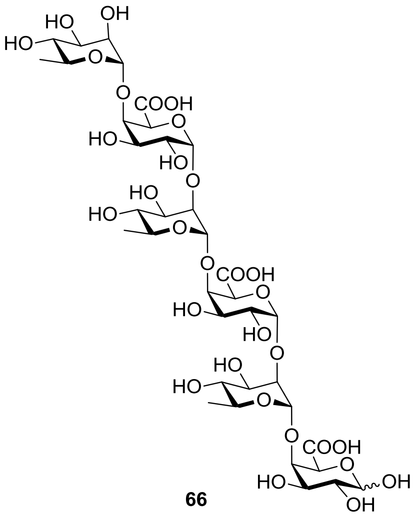


Scheme 14 Synthesis of a fully unprotected RG I tetrasaccharide, its methyl ester and a protected RG I hexasaccharide analog by Davis and co-workers

The TMSOTf-catalyzed coupling of the rhamnosyl trichloroacetimidate donor **52** with the galactosyl thioglycoside acceptor **53** gave disaccharide **54** in 65% yield. The obtained disaccharide donor **54** was used for assembly of tetrasaccharide **59** and the protected hexasaccharide **65**. Disaccharide acceptor **58** was prepared by the NIS/TMSOTf-catalyzed glycosylation of the galactosyl acceptor **56** with the rhamnosyl thioglycoside donor **52** in 75% yield, followed by selective deprotection of the C-2 acetyl group in the rhamnose residue. The key NIS/TMSOTf-catalyzed glycosylation of **58** with disaccharide donor **54** furnished the tetrasaccharide product **59** in 83% yield. Cleavage of the ester protective groups was achieved by treatment with sodium methoxide in methanol, giving tetrasaccharide **60**. Selective oxidation of the primary C-6 hydroxyl groups in **60** using sequential treatment with TEMPO/$NaClO_2$ and NaClO converted galactose residues into galacturonic acids, furnishing tetrasaccharide **61**. Carboxylic acid groups in **61** were benzylated to facilitate purification, and fully protected tetrasaccharide **62** was subjected to Pd/C-catalyzed hydrogenolysis. Careful control of the deprotection conditions allowed access to both monomethyl ester **63** (when MeOH was used as solvent) and carboxylic acid **64** (when THF/H_2O was employed). The potential of this strategy for elongation of RG I chain was shown by successful NIS/TMSOTf-catalyzed glycosylation of the tetrasaccharide acceptor **62** with the disaccharide donor **54**; the protected RG I hexasaccharide analog **65** was obtained in 68% yield.

2 Synthesis of a Linear Backbone Hexasaccharide Fragment

In this work, the target RG I oligosaccharide fragments were intended to be used for several biological applications, including studies of enzymes acting on RG I. Therefore, oligosaccharide fragments of sufficient length were desired. At first, a fully unprotected linear hexasaccharide fragment of the RG I backbone **66** was targeted; its structure is depicted in Figure 6.



66

Figure 6 Target hexasaccharide fragment of the RG I backbone

As discussed in Chapter 1, several oligosaccharide fragments of RG I have been prepared by chemical synthesis. Synthesis of the fully unprotected RG I hexasaccharide has not been previously reported. However, smaller fully and partially unprotected RG I oligosaccharides, as well as fully protected oligosaccharides up to hexamers have been prepared by different approaches.

2.1 Retrosynthetic Analysis

Retrosynthetic analysis of the target hexasaccharide **66** is shown in Figure 7. Choosing between the two possible approaches for synthesis of oligosaccharides containing uronic acids (that is, oxidation prior to or after glycosylation), we adopted the postglycosylation strategy. Although this approach requires additional synthetic steps to temporarily protect and subsequently oxidize the C-6 position in the galactose residues, it is known that the non-oxidized carbohydrates are generally more reactive glycosyl donors than corresponding uronic acids, where the reactivity is decreased by the presence of the electron-withdrawing carboxyl groups.[92] Moreover, introduction of the carboxylic acid functionalities at a late stage of the synthesis reduces the risk of possible side reactions, such as epimerization to L-altruronic acid and β-elimination leading to the formation of 4-deoxy-L-*threo*-hex-4-enopyranuronic acid. This postglycosylation-oxidation strategy proved to be successful in the synthesis of HG fragments previously performed in our group.[67,72]

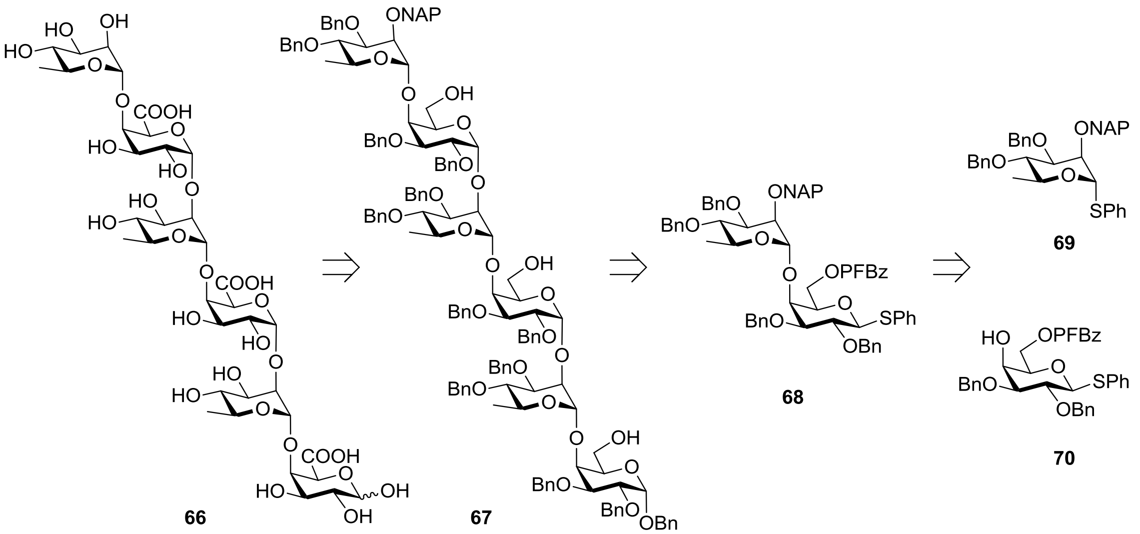


Figure 7 Retrosynthesis of the target linear hexasaccharide fragment of the RG I backbone

According to this reasoning, we envisioned that the target hexasaccharide **66** could be obtained from the partially deprotected hexasaccharide **67** by oxidation of the primary C-6 hydroxyl groups to the carboxylic acid functionalities, followed by a global deprotection. Hexasaccharide **67** was planned to be assembled by two iterative glycosylations using the disaccharide building block **68**. Employing the common disaccharide **68** in this convergent strategy would minimize the number of monosaccharide building blocks required for the synthesis. In fact, only the two monosaccharides **69** and **70** would be needed to complete the synthesis of hexasaccharide **66**. The common disaccharide donor **68** was designed to possess a nonparticipating benzyl (Bn) group at the C-2 position of the galactose residue, promoting the formation of the α-glycosidic linkage. Disaccharide **54** was intended to be produced through a chemoselective coupling between rhamnosyl donor **69** with a temporary blocked C-2 position and galactosyl acceptor **70** with a free hydroxyl group at the C-4 position and a temporary protective group at C-6. The thiophenyl functionalities in the anomeric positions were chosen due to their ability to function both as leaving groups and as temporary protective groups and perform well in armed-disarmed couplings[98] (for discussion of thiophenyl glycoside donor properties see Chapter 1).

2-Naphthylmethyl (NAP) group was chosen as a temporary protective group for the C-2 position in the rhamnosyl donor **69**. Since in rhamnose the formation of the α-glycosidic linkage is favored by the anomeric effect, a non-participating NAP-group at the C-2 position could be used. This group was chosen due to its arming nature, which was expected to be of advantage in the relation to our armed-disarmed approach. The NAP-ether is orthogonal to the groups used for the protection of the galactosyl acceptor **70**, therefore, at a later stage, it could be selectively removed by oxidative cleavage with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ)[20] to allow for elongation of the oligosaccharide chain at this position.

The C-6 position in the galactosyl acceptor **70** was capped with a pentafluorobenzoyl ester (PFBz) that later could be selectively removed under the Zemplén conditions[97] to release this position for oxidation. Apart from functioning as a temporary protective group, the PFBz-ester was also envisioned to tune the reactivity of thiophenyl glycoside **70**.[66] It is known that electron-withdrawing protective groups decrease the reactivity of glycosyl donors, and the donors protected with electron-donating (ether) groups can be selectively activated in a glycosylation reaction over the donors protected with electron-withdrawing (ester) groups. This phenomenon is known as the "armed-disarmed" effect (see Chapter 1 for more details)[50]. In the present strategy, the armed rhamnosyl thiophenyl donor **69** fully protected with ether groups was planned to be selectively activated over the disarmed galactosyl thiophenyl acceptor **70** bearing an electron-withdrawing PFBz-group. In addition to the electronic effects of the protective groups, rhamnose was expected to have a higher reactivity than galactose, because it is a deoxy sugar and lacks the electron-withdrawing hydroxyl group at the C-6 position.

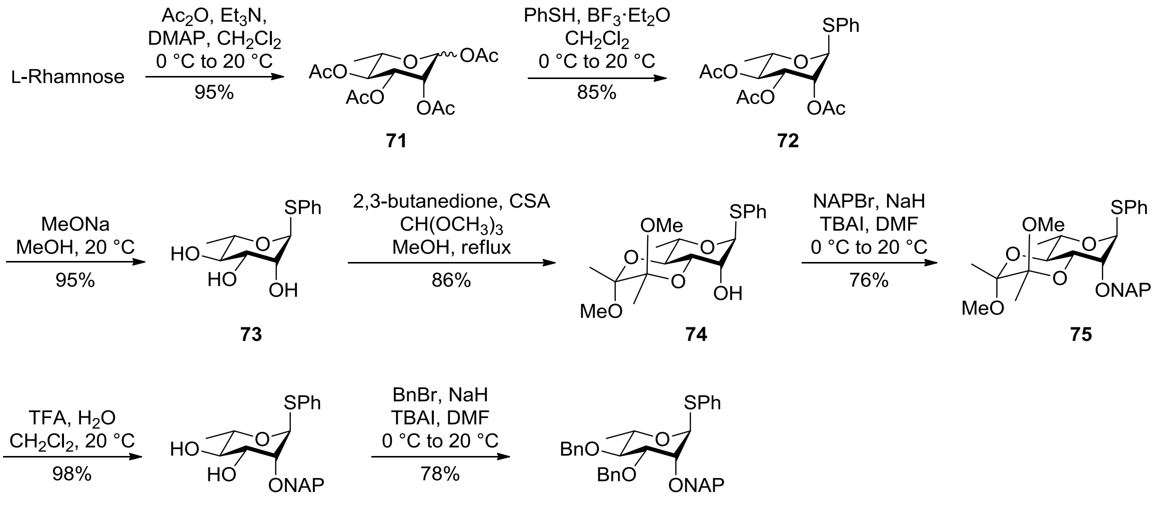
Benzyl groups were chosen for the permanent blocking of the rest of the hydroxyl groups in both the rhamnosyl donor **69** and the galactosyl acceptor **70**, as they are stable under most protective group manipulation conditions and can be removed under mild conditions such as palladium-catalyzed hydrogenolysis[99] at the end on the synthesis.

2.2 Synthesis of the Building Blocks and Assembly of the Target Hexasaccharide

2.2.1 Synthesis of the Thioglycoside Monosaccharide Building Blocks

As has been mentioned when discussing the retrosynthetic analysis of the target hexasaccharide **66**, only two monosaccharide building blocks **69** and **70** were required for its assembly.

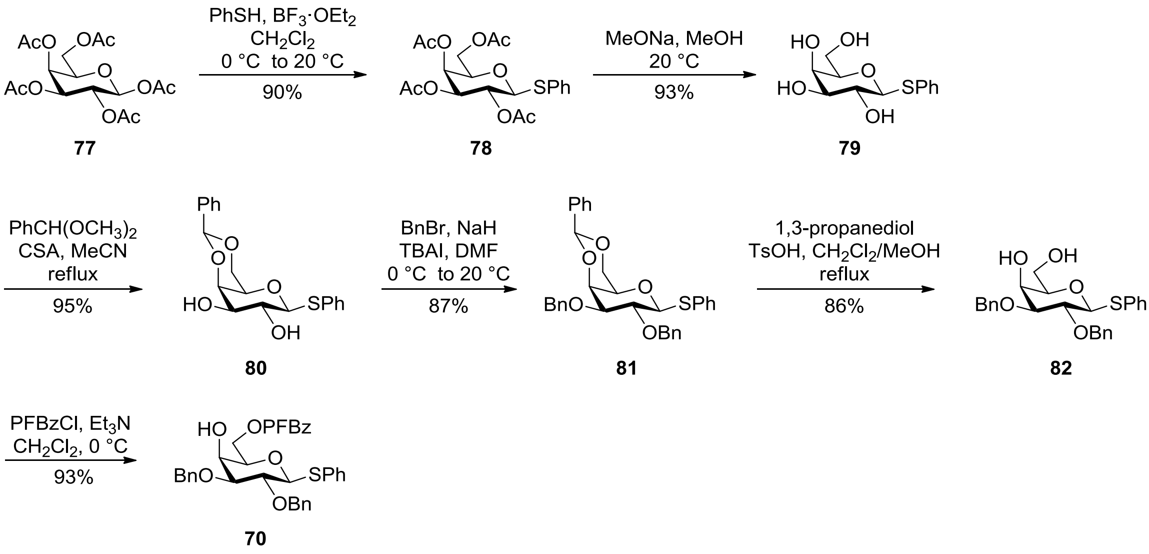
The rhamnose derivative **69** was obtained from commercially available L-rhamnose in seven steps; its synthesis is shown in Scheme 15.



Scheme 15 Synthesis of the rhamnosyl thioglycoside building block **69**

The nonprotected monosaccharide was converted into the tetraacetate **71** in 95% yield by treatment with acetic anhydride in the presence of triethylamine and 4-(dimethylamino)pyridine (DMAP). The $BF_3{\cdot}OEt_2$-mediated glycosylation of thiophenol with the obtained glycosyl acetate **71** provided rhamnosyl thiophenyl glycoside **72** in 85% yield. Subsequent deacetylation of **72** under the Zemplén conditions afforded triol **73** in 95% yield. The acid-catalyzed reaction of **73** with 2,3-butanedione allowed for selective protection of the *trans*-diequatorial C-2 and C-3 hydroxyl groups with a cyclic butane diacetal (BDA) protective group introduced by Ley[100,101] to give **74** in 86% yield. The free C-2 hydroxyl was then protected with a NAP-group in 76% yield by treatment with 2-(bromomethyl)naphthalene (NAPBr) in the presence of NaH and catalytic amounts of tetrabutylammonium iodide (TBAI). The BDA protective group was then hydrolyzed under acidic conditions to afford diol **76**. The reaction had to be performed carefully because prolonged treatment of **75** with acid resulted in partial cleavage of the NAP-group. The released hydroxyl groups were permanently protected with benzyl groups by treatment with benzyl bromide in the presence of NaH and catalytic amounts of TBAI to furnish the target rhamnose building block **69** in 78% yield.

The galactose derivative **70** was prepared from the commercially available β-D-galactose pentaacetate **77** in six steps; the synthesis is shown in Scheme 16.



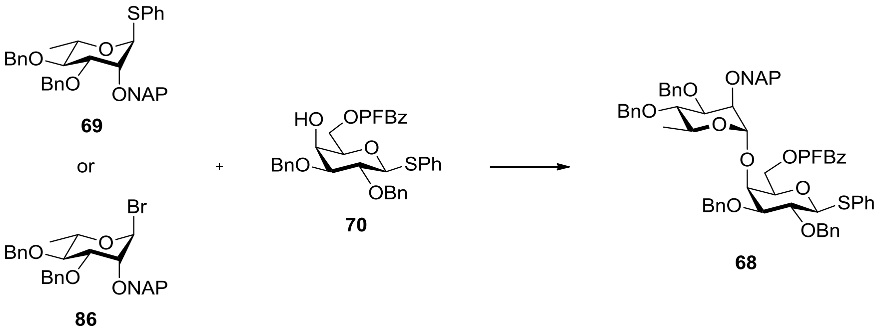
Scheme 16 Synthesis of the galactosyl thioglycoside building block **70**

The $BF_3 \cdot OEt_2$-catalyzed glycosylation of thiophenol with galactose tetraacetate **77** procured galactosyl thiophenyl glycoside **78** in 90% yield. Its treatment under the Zemplén conditions afforded tetraol **79** in 93% yield. The C-4 and C-6 hydroxyl groups in **79** were selectively protected with a benzylidene acetal by acid-catalyzed reaction with benzaldehyde dimethyl acetal to give diol **80** in 95% yield. The C-2 and C-3 hydroxyls of **80** were permanently protected with benzyl groups by treatment with benzyl bromide in the presence of NaH and catalytic amounts of TBAI to afford **81** in 87% yield. The benzylidene acetal protective group in **81** was cleaved by the reaction with *p-t*oluenesulfonic acid (TsOH) in the presence of 1,3-propanediol to give diol **82** in 86% yield. The primary C-6 hydroxyl was selectively protected with the pentafluorobenzoyl (PFBz) group by treatment with PFBzCl in the presence of triethylamine to provide the target galactose building block **70** in 93% yield.

2.2.2 Attempts to Synthesize the Thiophenyl Disaccharide Donor

Having synthesized the armed rhamnosyl donor **69** and the disarmed galactosyl acceptor **70**, we explored their chemoselective coupling (Table 2).

Table 2 Attempts to synthesize the thiophenyl disaccharide donor **68**



Entry	Donor	D:A[1]	Activator	Solvent	T, °C	Yield, %	Comments
1	**69**	1.2	NIS/TESOTf[2]	Et_2O	–20	50	**68**+**84** mixt.
2	**69**	1.2	NIS/TESOTf	CH_2Cl_2	–20	50	**68**+**84** mixt.
3	**69**	1.2	NIS/TESOTf	1:1 CH_2Cl_2/Et_2O	–20	45	**68**+**84** mixt.
4	**69**	1.2	NIS/TESOTf	Et_2O	–40	n.d.	
5	**69**	1.2	NIS/TESOTf	Et_2O	0	35	**68**+**84** mixt.
6	**69**	1.8	NIS/TESOTf	Et_2O	–20	48	**68**+**84** mixt.
7	**69**	1.2	I_2[3]	CH_2Cl_2	20	<20	
8	**69**	1.2	I_2	CH_2Cl_2	20	<15	K_2CO_3 added

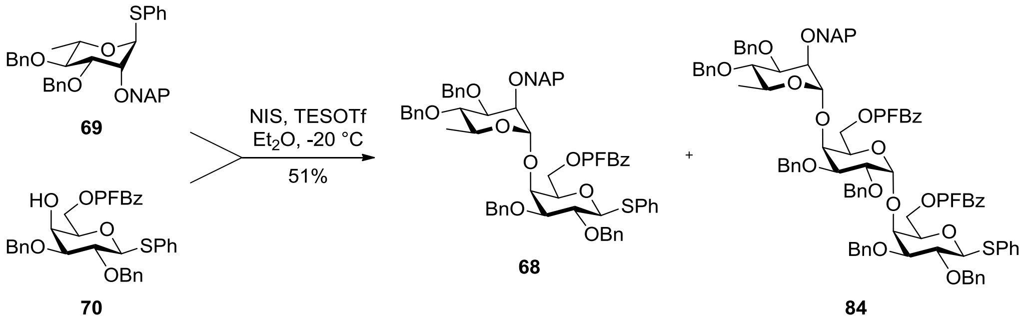
Entry	**Donor**	**D:A[1]**	**Activator**	**Solvent**	**T, °C**	**Yield, %**	**Comments**
9	**69**	1.2	I_2	CH_2Cl_2	20	<10	TBAI added
10	**86**	1.8	AgOTf[4]	CH_2Cl_2	–50	n.d.	
11	**86**	1.8	TBAI[5]	CH_2Cl_2	20	<10	

[1]D:A – donor/acceptor ratio. [2]1.1 equiv. of NIS relative to the donor and 0.15 equiv. of TESOTf relative to NIS. [3]All glycosylations with I_2 were performed in the presence of 4 Å MS; 1.2 equiv. of I_2 relative to the donor. [4]1.5 equiv. of AgOTf relative to the donor. [5]2 equiv. of TBAI relative to the donor.

When NIS/TESOTf was used as an activator and the glycosylation was performed in ether at –20 °C, the reaction (Scheme 17) procured the target disaccharide **68** but only as approximately an 1.5:1 mixture with the trisaccharide by-product **84** in a total yield of 50% (entry 1). The trisaccharide by-product **84** presumably arose from glycosylation of acceptor **70** with the disaccharide donor **68** formed in the course of the reaction. The mixture of **68** and **84** was essentially inseparable and could be partially separated only after several flash columns. The formation of trisaccharide under the chosen conditions was unexpected as, in general, disaccharide donors are considered to be less reactive than monosaccharide donors[58] and, in addition, the disaccharide donor **68** was believed to be disarmed by the presence on an electron-withdrawing PFBz-group.

In an attempt to optimize the glycosylation to avoid the undesired by-product formation, the solvent, reaction temperature and relative amounts of donor and acceptor were altered. Using CH_2Cl_2 (entry 2) or 1:1 ether/CH_2Cl_2 mixture (entry 3) instead of pure ether did not improve the reaction outcome. In both cases mixtures of the disaccharide and the trisaccharide products were obtained and the yields were comparable or even lower than those of glycosylations performed in ether. Lowering the temperature to –40 °C (entry 4) caused precipitation of the starting materials from the reaction mixture, while raising the temperature to 0 °C (entry 5) resulted in less clean glycosylations.

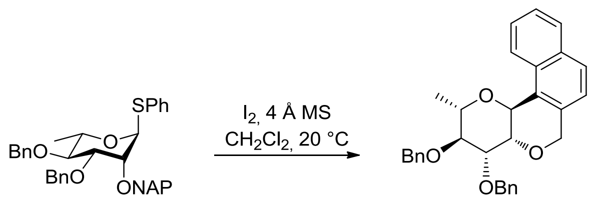
Using a larger excess of donor (1.8 equivalents compared to 1.2 equivalents used in the initial experiments) did not have a significant effect on the glycosylation result (entry 6).



Scheme 17 Formation of the trisaccharide by-product in the NIS/TESOTf-promoted glycosylation of **70** with **69**

Subjecting the mixture of disaccharide **68** and trisaccharide **84** to the NAP-group deprotection conditions (treatment with DDQ) allowed facile isolation of the deprotected disaccharide in the pure form. However, considering the overall yield, this result could not be evaluated as satisfactory.

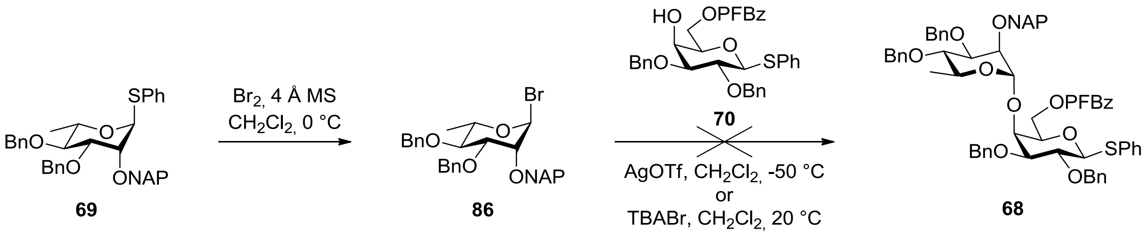
Trying to avoid the activation of the disaccharide donor **68** we examined the use of a mild activator for glycosylations. Molecular iodine was chosen for this purpose as it is known to be capable of activating armed thioglycoside donors under very mild conditions.[102,103] The glycosylations were performed in CH_2Cl_2 at 20 °C in the presence of 4 Å molecular sieves with or without additives such as potassium carbonate and TBAI (entries 7,8 and 9). The reactions were very slow (from 24 hours up to 5 days depending on the reaction conditions chosen) and resulted mainly in the formation of *C*-glycoside **85** through an intramolecular cyclization (Scheme 18). Similar electrophilic aromatic substitution on the NAP-group by an oxocarbenium ion was observed for mannose by Crich and co-workers.[104] Interestingly, in order to enable the formation of the 1,2-*trans*-diequatorial junction in the bicyclic product **85** the sugar ring underwent a conformational change from $_4C^1$ to $^4C_1$, as evident from the NMR spectra.



69 **85**

Scheme 18 Iodine-promoted formation of C-glycoside

Given the lack of success in synthesizing disaccharide **68** through the selective activation of the rhamnosyl donor **69** over the galactosyl acceptor **70**, we explored the opportunity of converting thioglycoside **69** into the corresponding glycosyl bromide and using the latter as a glycosyl donor (Scheme 19). Titrating **69** with a solution of bromine in CH_2Cl_2 in the presence of 4 Å molecular sieves at 0 °C afforded glycosyl bromide **86**, as judged by TLC. It was used directly, without purification, in the glycosylation with acceptor **70**. When AgOTf was used as an activator and the reaction was performed in CH_2Cl_2 at –50 °C, the decomposition of the acceptor was observed and the glycosylation resulted in a complex mixture of products. Notably, one of the by-products was found to be thioglycoside **69**, likely meaning that aglycon transfer of the thiophenyl group of the acceptor took place. Performing the reaction under the Lemieux *in situ* anomerisation conditions (vide infra) did not afford sufficient amounts of the target disaccharide **68** presumably due to the insufficient nucleophilicity of the *C*-4 hydroxyl group in the galactosyl acceptor **70**.



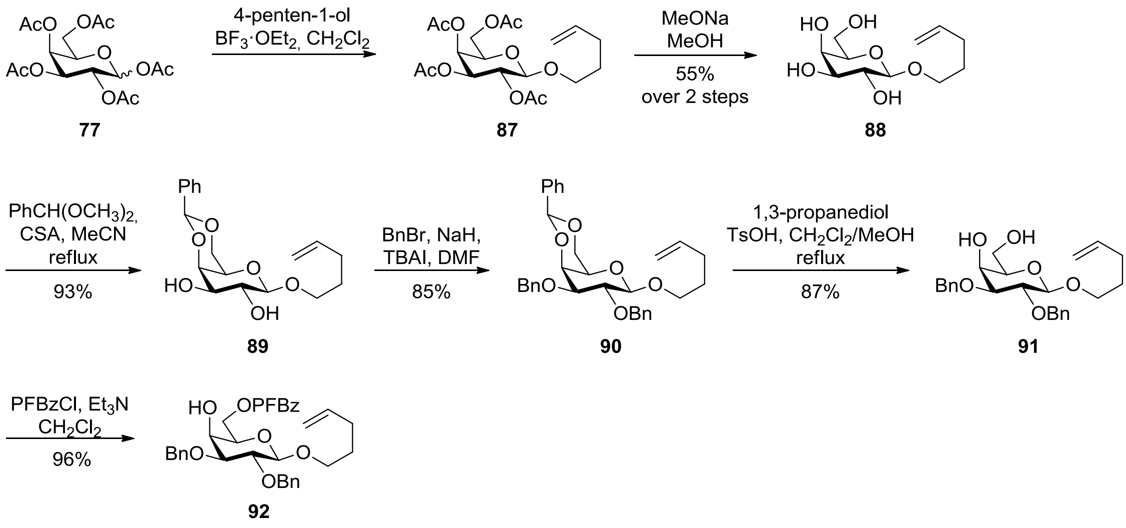
Scheme 19 Employing the glycosyl bromide donor **86** in synthesis of the thiophenyl disaccharide donor **68**

To conclude, the chemoselective activation of donor **69** over acceptor **70** proved to be unsuccessful and disaccharide **83** could not be obtained using this strategy in pure form and acceptable yield.

The major obstacles were observed to be the activation of the disaccharide product under the glycosylation conditions (leading to the formation of the trisaccharide by-product) and low nucleophilicity of the C-4 position in galactose (leading to side reactions or decomposition of the starting materials). In certain cases, nucleophilicity of the thiophenyl functionality was higher than nucleophilicity of the C-4 hydroxyl group, which led to the aglycon transfer. This had been previously observed in our laboratory for other similar systems and therefore seemed to be a general problem. We envisioned that substituting the thiophenyl functionality for the *n*-pentenyloxy group could be of advantage. Thioglycosides and pentenyl glycosides can be activated under essentially the same reaction conditions (see Chapter 1), meaning that the same armed-disarmed concept could be applied. However, unlike thioglycosides, pentenyl glycosides are not prone to aglycon transfer. According to this logic, we turned our attention to pentenyl glycosides as an alternative to thioglycosides.

2.2.3 Synthesis of the Pentenyl Monosaccharide Acceptor

Synthesis of the pentenyl galactose building block **92** was performed according to a route similar to the one employed for synthesis of the thiophenyl glycoside **70** (Scheme 20).

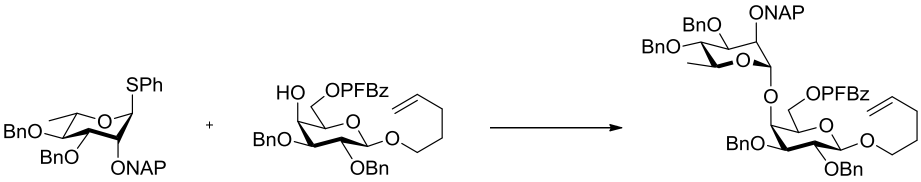


Scheme 20 Synthesis of the galactosyl pentenyl glycoside acceptor **92**

2.2.4 Synthesis of the Pentenyl Disaccharide Donor

Next we explored whether the armed-disarmed approach could be applied to glycosylation of the disarmed galactose pentenyl acceptor **92** with the armed rhamnose thioglycoside donor **69** (Table 3).

Table 3 Exploring the glycosylation conditions for synthesis of the pentenyl disaccharide **83**



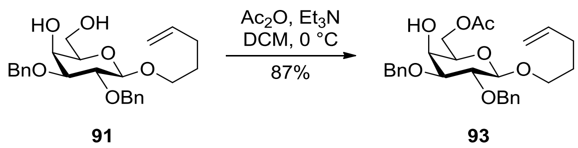
69 **92** **83**

Entry	D:A[1]	Time	Solvent	T, °C	Yield, %
1	1.1	1.5 h	Et_2O	–20	60
2	1.2	40 min	Et_2O	–20	78
3	1.2	3 h	Et_2O	–40	63
4	1.2	20 min	Et_2O	0	58
5	1.2	30 min	1:1 CH_2Cl_2/Et_2O	–20	75
6	1.2	15 min	CH_2Cl_2	–20	45

[1]D:A – donor/acceptor ratio. In all glycosylations 1.1 equiv. of NIS relative to the donor and 0.15 equiv. of TESOTf relative to NIS were used

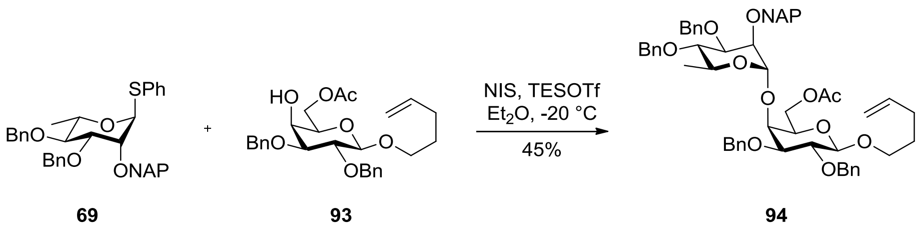
In the initial experiment, NIS/TESOTf was used as an activator and glycosylation reaction was performed in ether at –20 °C for 1.5 hours (entry 1). Under these reaction conditions, disaccharide product **83** could be obtained in 60% yield. Increasing the amount of donor from 1.1 to 1.2 equivalents relative to acceptor and performing the reaction for shorter time (40 minutes instead of 1.5 hours) resulted in 78% yield (entry 2). The reaction proceeded with very high α-selectivity; no β-product was isolated. Changing temperature did not improve the reaction outcome: at lower temperatures (–40 °C) the coupling was less efficient (entry 3); at higher temperatures (0 °C) more decomposition products were observed (entry 4). Performing the reaction in a 1:1 ether/CH_2Cl_2 mixture (entry 5) instead of pure ether did not change the glycosylation yield, while using pure CH_2Cl_2 (entry 6) decreased the yield significantly and disaccharide **83** was obtained in 45% yield.

It was interesting to find out whether the presence of the PFBz-group in the acceptor molecule was required for achieving selectivity in this glycosylation. In order to test this, galactose acceptor **93** bearing an acetyl group instead of a PFBz-group in the C-6 position was prepared from diol **91**. This was done by selective acetylation of the primary hydroxyl group by acetic anhydride in the presence of triethylamine at 0 °C (Scheme 21).



Scheme 21 Synthesis of the galactose acceptor **93** bearing an acetyl group

The synthesized acceptor **93** was glycosylated with donor **69** under identical reaction conditions (Scheme 22). The reaction resulted in a complex mixture of products, some of which were presumably formed due to decomposition of the acceptor. Disaccharide product **94** was obtained in 45% yield.

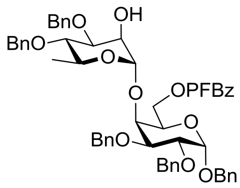


Scheme 22 Synthesis of disaccharide **94** bearing an acetyl group

Since the glycosylation with the acetylated acceptor proved to be less efficient than the one with the acceptor containing PFBz-group, the latter was used in the synthesis.

2.2.5 Synthesis of the Disaccharide Acceptor

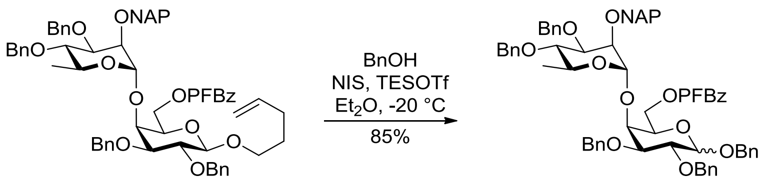
According to our synthetic planning, disaccharide acceptor **95** was required in order to assembly the target hexasaccharide **66**. It was planned to be obtained from **83** (Figure 8).



95

Figure 8 Disaccharide acceptor **95**

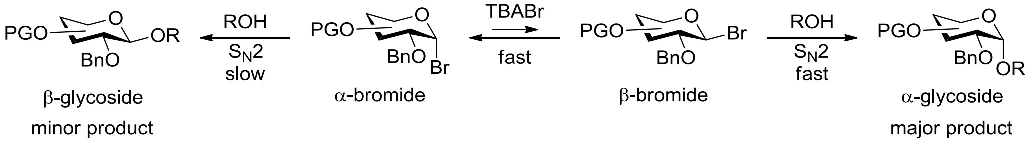
First, the anomeric position in disaccharide **83** had to be permanently protected. In order to do this, the *n*-pentenyloxy group had to be replaced by a benzyl ether. An initial attempt to glycosylate benzyl alcohol with donor **83** in the presence of NIS/TESOTf (Scheme 23) resulted in approximately 2:1 α/β-mixture (as judged by NMR). Such a low stereoselectivity was observed presumably due to the high reactivity of benzyl alcohol.



83 **96**

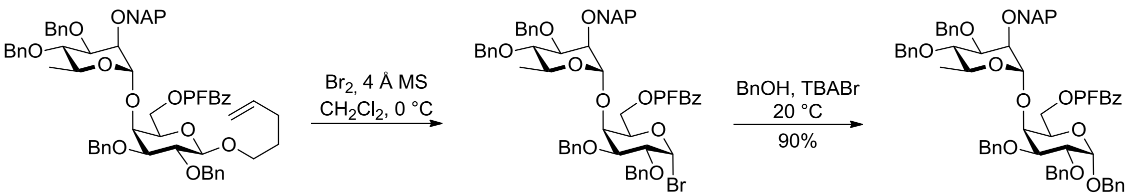
Scheme 23 Glycosylation of benzyl alcohol with the disaccharide donor **83**

This result was unsatisfactory for our purposes, as we intended to take disaccharide **96** into the following synthetic steps. A need to work with a α/β-mixture would significantly complicate the whole synthesis. In order to solve this issue, the glycosylation was performed again according to the Lemieux *in situ* anomerisation protocol.[105,106] This procedure employs glycosyl bromides as glycosyl donors. Lemieux and co-workers observed that equilibrium is achieved between the α- and the β-glycosyl bromides upon addition of tetrabutylammonium bromide (TBABr). The α-bromide is more stable due to the anomeric effect, while the β-bromide is more reactive towards a nucleophilic attack. For this reason, glycosylation preferentially occurs on the β-glycoside and due to its S_N2 fashion the α-product is formed. Under the conditions where the rate of equilibration between the α- and the β-bromides is much higher than the rate of the glycosylation reaction, a selective formation of the α-product can be achieved (Scheme 24).



Scheme 24 Glycosylation under the Lemieux conditions

To convert disaccharide **83** into glycosyl bromide **97**, it was titrated with a solution of bromine in CH_2Cl_2 in the presence of 4 Å molecular sieves at 0 °C. The resulting bromide **97** was taken directly, without purification, into the coupling with benzyl alcohol in the presence of TBABr at 20 °C. The reaction afforded benzyl glycoside **98** as a single α-anomer in 90% yield over two steps (Scheme 25).



83 **97** **98**

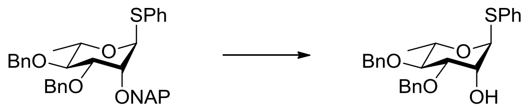
Scheme 25 Synthesis of the benzyl disaccharide **98** under the Lemieux conditions

To transform disaccharide **98** into the glycosyl acceptor **95**, the NAP-group had to be removed from the C-2 position in rhamnose. Selective deprotection of a

NAP-ether is usually achieved either by oxidative cleavage or by acidic hydrolysis. DDQ is commonly employed as an oxidant,[107] but other oxidizing agents, such as ammonium cerium(IV) nitrate (CAN)[20], can be used. For acidic hydrolysis trifluoroacetic acid (TFA)[20] or, as recently reported by Liu and co-workers, HF/pyridine[108] can be employed. Examples of selective hydrogenolysis of the NAP-ether in the presence of benzyl ethers are also known.[109]

In the synthesis of the target hexasaccharide **66**, removal of a NAP-group had to be performed several times. The optimal conditions for this transformation were obviously needed, and we therefore explored different methods available. The test reactions were carried out on a model system using monosaccharide **69** as a substrate. To assure that the outcome of the reaction did not significantly depend on the choice of monosaccharide as a substrate, selected conditions were repeated using disaccharide **83** as a starting material (see Chapter 4). The results of the screening are presented in Table 4.

Table 4 Screening of the reaction conditions for removal of the NAP-group in **69**



69 **99**

Entry	Reagent	Solvent	T, °C	Time, h	Yield,[1] %	Work-up[2]
1	DDQ	CH_2Cl_2/MeOH/H_2O	20	3	75	B
2	DDQ	CH_2Cl_2/H_2O	20	2	67	B
3	DDQ	CH_2Cl_2/MeOH/H_2O	20	3	42	A
4	DDQ	CH_2Cl_2/phosphate buffer pH 7.2	20	12	38	B
5	DDQ	CH_2Cl_2/MeOH/H_2O	0	24	70	B

Entry	Reagent	Solvent	T, °C	Time, h	Yield,[1] %	Work-up[2]
6	HF/Py	toluene	20	2	30	B
7	TFA	toluene	20	2	65	B
8	TFA	toluene	0	24	65	B
9	TFA	toluene	20	2	40	A

[1]Isolated yields after flash chromatography. [2]A – direct evaporation, B – work-up with saturated aqueous $NaHCO_3$

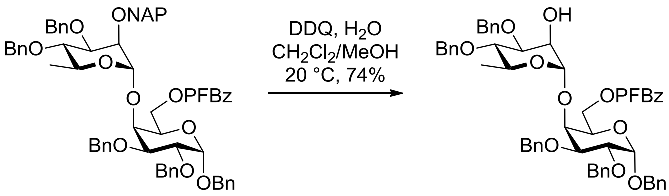
At first, the oxidative cleavage conditions were examined. DDQ was used as an oxidizing agent. The yields varied from 38 to 75% depending on the conditions chosen. Performing the reaction in CH_2Cl_2/MeOH (entry 1) was found to be preferable to using CH_2Cl_2 alone (entry 2). It turned out that the work-up conditions had an influence on the reaction outcome. Direct evaporation of the reaction mixture, followed by column chromatography purification (entry 3), gave lower yields than a work-up with saturated aqueous solution of $NaHCO_3$, followed by the same purification procedure (entry 1). Buffering the reaction mixture with pH 7.2 phosphate buffer (entry 4) did not lead to any improvement in terms of the yield; neither did lowering temperature of the reaction from 20 °C to 0 °C (entry 5).

When monosaccharide **69** was treated with HF/pyridine in toluene (entry 6), the benzyl ethers were cleaved as readily as the NAP-group, resulting in a formation of a complex mixture of compounds, from where the desired product could be isolated in only 30% yield. Discouraged by such a low selectivity, we did not try to optimize the method further.

An ability of TFA to cleave a NAP-group was observed in our synthesis of the rhamnose derivative **76**, where that process was an undesired side-reaction lowering the yield of the butane diacetal deprotection step. Here, we explored the possibility of using TFA to remove the NAP-group selectively. The reaction was carried out in toluene at 20 °C or 0 °C. The temperature difference did not have a significant influence on the reaction outcome. In both cases the product was obtained in 65% yield (entries 7 and 8h). Similarly to the DDQ-mediated deprotection work-up with a saturated aqueous solution of $NaHCO_3$ gave better results than direct evaporation of the reaction mixture (entry 9).

To summarize, in our hands the best results were obtained by treatment of **69** with DDQ in CH_2Cl_2/MeOH in the presence on small amounts of water at 20 °C for 3 hours, followed by a basic work-up. These conditions afforded alcohol **99** in 75% yield after flash chromatography. Prolonged reaction times as well as increasing the amount of DDQ resulted in partial cleavage of the benzyl ethers (results not shown in Table 4).[110]

Compound **98** was subjected to the aforementioned conditions to give disaccharide acceptor **95** in 74% yield (Scheme 26).

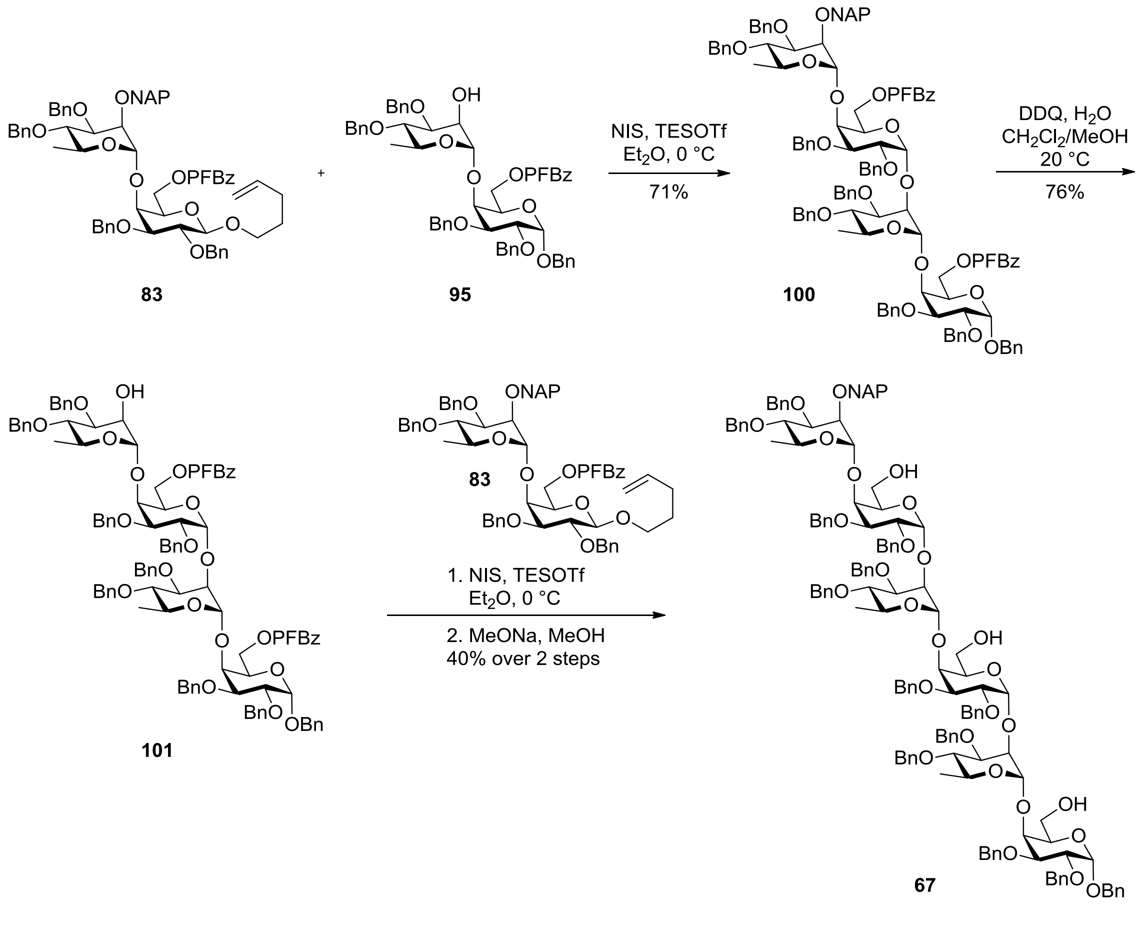


98 **95**

Scheme 26 Synthesis of the disaccharide acceptor **95**

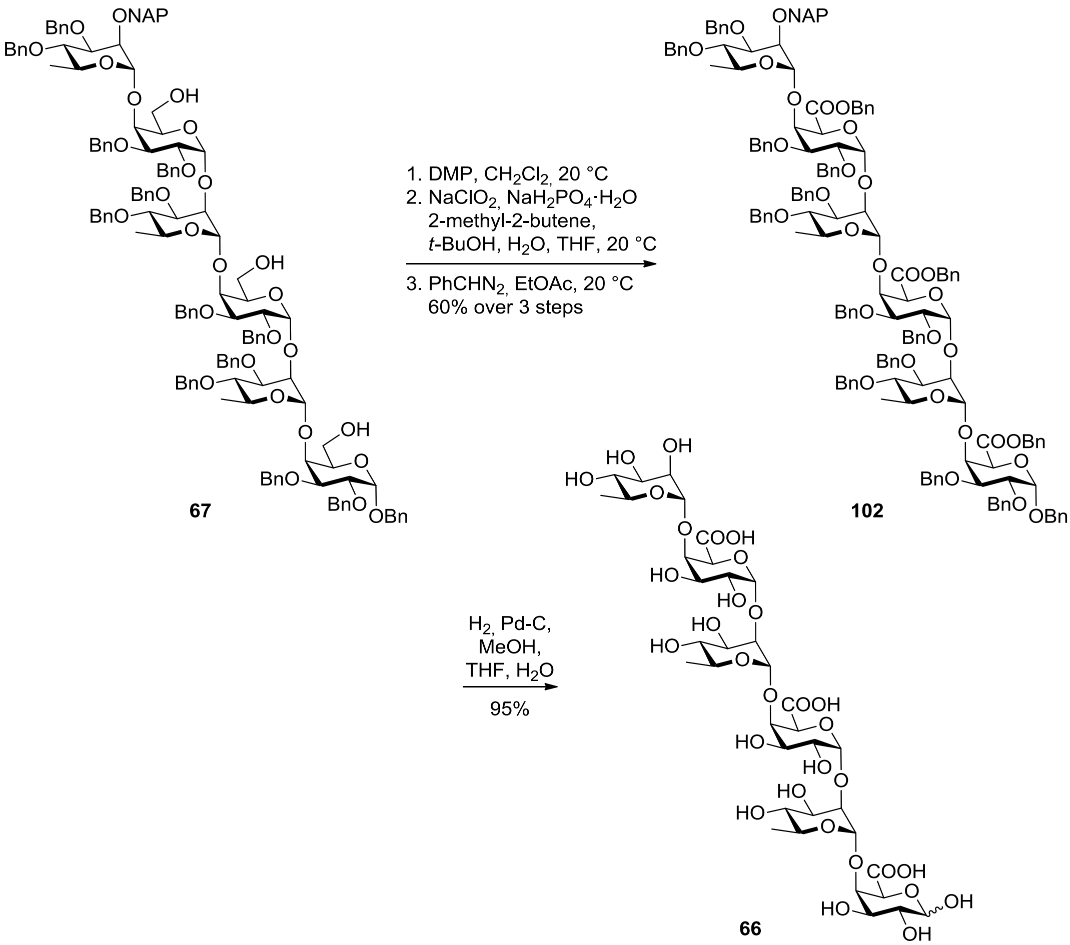
2.2.6 Assembly of the Target Hexasaccharide

Pentenyl disaccharide **83** was used as the key disaccharide donor in the further iterative assembly of the protected hexasaccharide **67** (Scheme 27). The NIS/TESOTf-catalyzed glycosylation of **95** with **83** led to the formation of tetrasaccharide **100** as a single α-isomer in 71% yield. Notably, in this case the reaction did not proceed at –20 °C (conditions used for the synthesis of disaccharide **83**) and higher temperatures (0 °C) were required. The obtained tetrasaccharide **100** was subjected to the same procedure for removal of the NAP-group with DDQ to furnish the tetrasaccharide **101** in 76% yield. Acceptor **101** was glycosylated again under the same conditions with the disaccharide donor **83**. The reaction resulted in an inseparable mixture of the hexasaccharide product with a by-product of an unidentified structure. After subjecting the mixture to the Zemplén deacylation conditions, the PFBz-groups at the C-6 position in galactose were selectively removed and triol **67** was successfully separated from the by-product and isolated in a pure form in 40% yield over two steps.



Scheme 27 Assembly of the partially protected hexasaccharide **67**

To obtain galacturonic acid residues, the liberated primary hydroxyl groups in **67** had to be oxidized into the carboxylic acid functionalities. This was done in two steps, first by oxidizing with Dess-Martin periodinane[111] to aldehydes and then with sodium chlorite[112] to carboxylic acids. The resulting carboxylic acid functionalities were protected as benzyl esters to facilitate purification. This was done by reaction with phenyldiazomethane that was formed prior to the reaction by vacuum pyrolysis of benzaldehyde tosylhydrazone sodium salt.[113] The protected hexasaccharide **102** was obtained in 60% yield over three steps. Finally, treatment of **102** under standard conditions for catalytic hydrogenolysis allowed removal of all the benzyl groups as well as the NAP-group furnishing, after a facile purification by reverse-phase column chromatography, the target fully unprotected hexasaccharide **66** in 95% yield.



Scheme 28 Oxidation of the C-6 positions in galactose and global deprotection

2.3 NMR Assignment of the Target Hexasaccharide

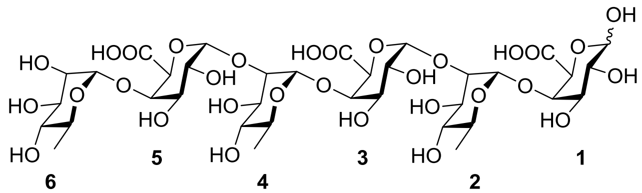
The structure of the synthesized fully unprotected hexasaccharide **66** was analyzed by 2D NMR spectroscopy; the full assignments of all ^{1}H and ^{13}C resonances are given in Table 5.

The obtained NMR data allowed us to differentiate and assign the resonances from the α- and the β-GalA at the reducing end. For the rest of the monosaccharide residues the effect of the anomeric configuration at the reducing end was not detectable under the chosen conditions. The internal residues 2Rha and 4Rha as well as 3GalA and 5GalA had the same resonances and the internal tetrasaccharide fragment appeared on the spectra as its repeating disaccharide unit.

The chemical shifts and the coupling constants (determined from the DQF-COSY spectrum) for the anomeric protons were as follows: α- and β-linkages for 1Gal (1αH1 δ_H 5.32, J = 5.7 Hz, 1βH1 δ_H 4.60, J = 7.4 Hz), α-linkage for 2+4Rha and 6Rha (2+4H1 δ_H 5.29, J = 4.9 Hz, 6H1 δ_H 5.25, J = 4.2 Hz), α-linkage for 3+5Gal (3+5H1 δ_H 5.05, J = 5.2 Hz). Some of the anomeric configurations could be confirmed by measuring the one-bond C-H coupling constants from the HMBC spectrum. The $^{1}J_{C,H}$ values determined were 169.6 Hz for 2+4Rha and 173.5 Hz for 6Rha indicating the α-linkages and 160.3 Hz for 1βGal indicating the β-linkage.[114]

The HMBC spectrum was used to locate 1αC6, 1βC6 and 3+5C6 carboxylic acid resonances (strong signals for 1βC6 and 3+5C6, weak signal for 1αC6). The ^{13}C resonances of 1αC4, 1βC4, 2+4C2 and 3+5C4 were shifted approximately 4-6 ppm downfield compared to the values for the unprotected monosaccharides, which indicated that those carbon atoms were engaged in the formation of the glycosidic linkages. This was also proven by the correlations between 2+4H1 and 1αC4, 3+5H1 and 2+4C2, 6H1 and 3+5C4 in the HMBC spectrum.

Table 5 ^{1}H and ^{13}C resonance assignments for the target hexasaccharide **66**



Residue	Position in the sugar ring					
	1	2	3	4	5	6
1α-GalA	5.32	3.93	4.09	4.43	4.45	
	93.2	70.6	75.5	78.1	71.6	175.7
1β-GalA	4.60	3.59	3.87	4.36	4.09	
	97.1	72.3	74.6	77.5	71.1	175.1
2+4Rha	5.29	4.15	3.93	3.44	3.80	1.27
	99.4	77.0	70.1	72.8	69.9	17.6
3+5GalA	5.05	3.94	4.14	4.44	4.70	
	98.4	68.8	71.3	77.3	72.2	175.9
6Rha	5.25	4.09	3.82	3.40	3.79	1.26
	101.6	71.1	71.0	73.0	69.7	17.6

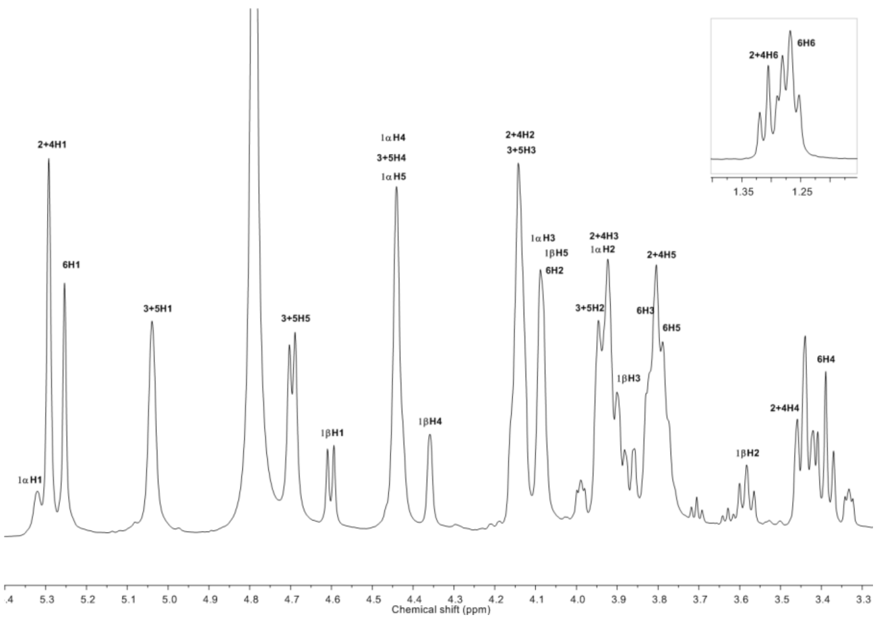


Figure 9 ^{1}H NMR of hexasaccharide **66**

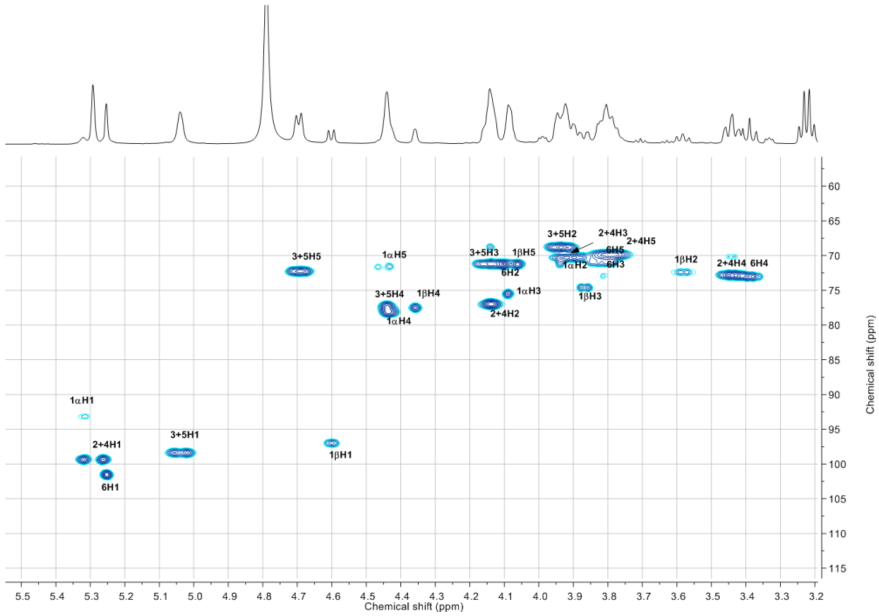


Figure 10 Fragment of HSQC spectra of hexasaccharide **66**

2.4 Conclusions

In summary, we have accomplished the first successful synthesis of the fully unprotected hexasaccharide fragment of the RG I backbone. The results of the work have been reported in Organic Letters; the paper is included in the Appendix.

The approach employed iterative glycosylations with a common disaccharide donor which was prepared by a chemoselective glycosylation of a disarmed pentenyl galactose glycosyl acceptor with an armed thiophenyl rhamnose glycosyl donor. The armed-disarmed effect was achieved by introducing an electron-withdrawing pentafluorobenzoyl group in the C-6 position of the acceptor.

The synthesis commenced with commercially available D-galactose pentaacetate and L-rhamnose. The optimal conditions for glycosylation steps and protection-deprotection manipulations were established. After twenty five overall synthetic steps, 50 mg of the target hexasaccharide was obtained.

The reactivity difference between the thiophenyl glycoside and the corresponding pentenyl glycosides observed in this work was somewhat surprising and we are currently investigating whether this is a general trend. The initial experiments (not described in this thesis) suggest that thioglycosides display higher reactivity than *n*-pentenyl glycosides in the NIS/TESOTf-promoted glycosylations. We are interested in seeing whether this difference is large enough to be practically used in chemoselective glycosylations.

We envisioned that this strategy developed for synthesis of the linear RG I hexasaccharide would allow for easy introduction of side-chains with galactan and arabinan, which was the focus of our next efforts summarized in Chapter 3.

3 Synthesis of RG I Oligosaccharides with Diarabinan and Digalactan Branching

In this chapter, our synthetic approach to the preparation of the branched RG I fragments is presented.

As discussed in Chapter 1, the RG I backbone is decorated with numerous side chains positioned at C-4 of the rhamnose residues, which causes the diversity of RG I structures. The RG I side chains are galactans, arabinans or arabinogalactans. Galactans are mostly linear chains of β-(1→4)-linked D-galactose residues. Arabinans are chains of α-(1→5)-linked L-arabinofuranose residues that are frequently branched at C-3 and sometimes at C-2. Arabinogalactan side chains are in most cases arabinogalactan I which is β-(1→4)-galactan with arabinan branches and less frequently arabinogalactan II with β-(1→3)-linked galactose residues.

To the best of our knowledge, except for the synthesis of the tri- and the tetrasaccharide intermediates containing a single galactose unit as a side chain by Vogel and co-workers,[88] the branched RG I fragments have not previously been prepared by chemical synthesis. Obtaining these structures is obviously of high interest because of a wide range of their potential applications for studying pectin and pectic enzymes.

Herein, we report the synthesis of two protected tetrasaccharides with diarabinan and digalactan branching (Figure 11, the protective groups used are discussed further) designed to be employed in the assembly of larger branched RG I oligosaccharides.

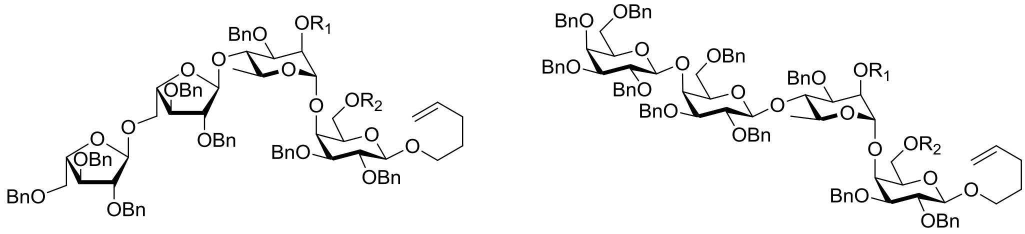


Figure 11 Structures of the target tetrasaccharides; R_1 and R_2 – temporary protective groups

3.1 Retrosynthetic Analysis

Considering the possible approaches to the synthesis of the branched RG I oligosaccharides, we wanted to base our strategy on the chemistry described in Chapter 2 that we had developed for the synthesis of the linear hexasaccharide. Here, the general synthetic approach is discussed using the branched RG I octasaccharide fragments **103** as an example (Figure 12).

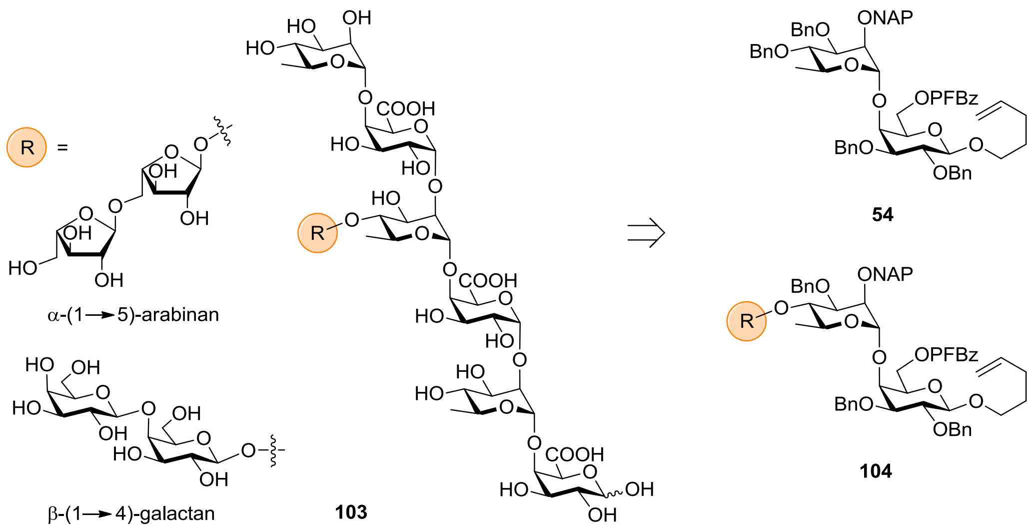


Figure 12 Retrosynthetic analysis of branched RG I oligosaccharides; R – disaccharide side-chains in the non-protected or protected form

It was envisioned that the backbone of **103** could be retrosynthetically disconnected into the "non-branched" disaccharide (**54**) and the "branched" tetrasaccharide (**104**) fragments. The "non-branched" disaccharide donor **54** was previously employed in our synthesis of the linear hexasaccharide **66**. In order to make the whole synthesis consistent, the same protective groups were chosen for the "branched" tetrasaccharide **104** as for the "non-branched" disaccharide **54**: the C-2 position in rhamnose was protected with a 2-naphthylmethyl (NAP) group, the C-6 position in galactose was protected with a pentafluorobenzoyl (PFBz) group and the remaining hydroxyls were permanently protected with benzyl groups. The structures of tetrasaccharides **105** and **111** are shown in Figure 13 and Figure 14.

The chosen protective group pattern dictated the approach to the synthesis of tetrasaccharides **105** and **111**. The 1,2-*trans* configuration of the glycosidic linkages in the diarabinan and digalactan side-chain fragments required using participating ester groups at the C-2 positions that later had to be exchanged for the permanent benzyl groups. At the same time, as has already been mentioned, the C-6 position in the backbone galactose residue was planned to be protected with the PFBz-group. However, the deprotection of the ester groups and the following protection of the released hydroxyls with benzyl groups could not be performed in the presence of the PFBz-group. This logic suggested that a corresponding trisaccharide fragment had to be prepared first, followed by the exchange of the protective groups and then by the coupling with the galactose acceptor **92**. This approach is illustrated in Figure 13 for the diarabinan-containing tetrasaccharide **105**.

The perbenzoylated trisaccharide **107** was planned to be prepared by glycosylating the rhamnose acceptor **108** with the diarabinan donor **109**. Disaccharide **109** could be obtained from the monosaccharide building block **110**.

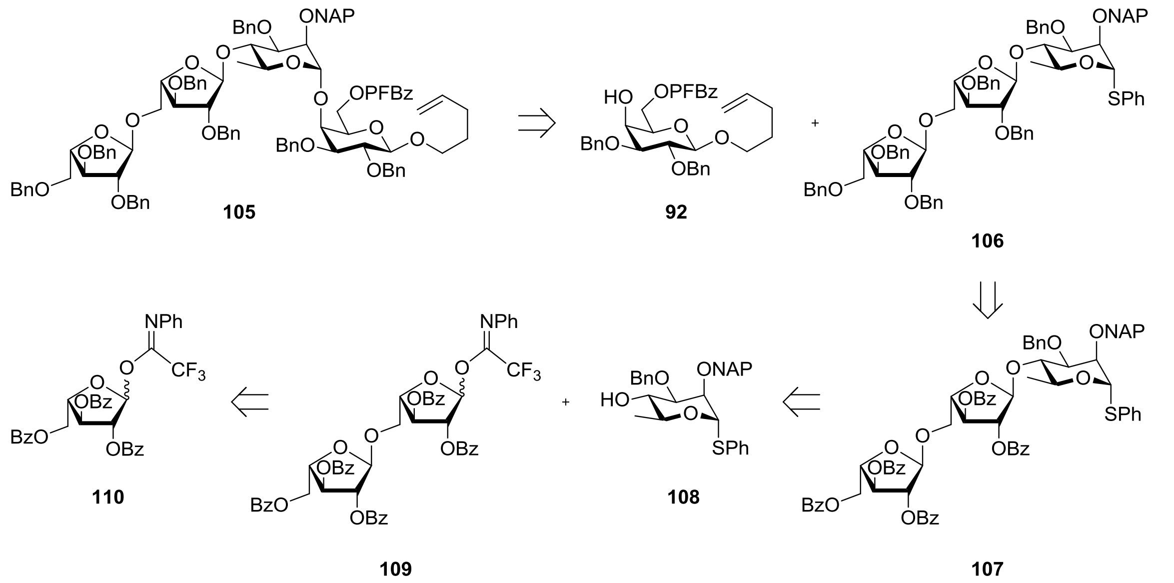


Figure 13 Retrosynthetic analysis of the target tetrasaccharide **105**

A similar approach was anticipated for the digalactan-containing tetrasaccharide **111**; the retrosynthetic breakdown of its structure into the monosaccharide building blocks is shown in Figure 14.

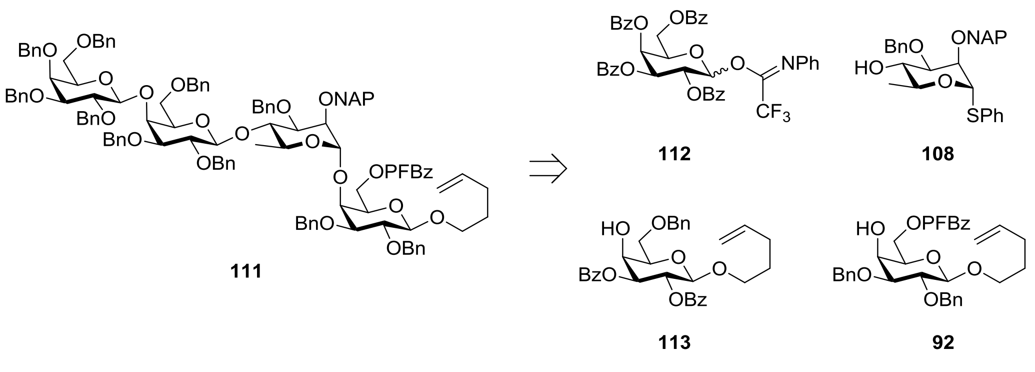


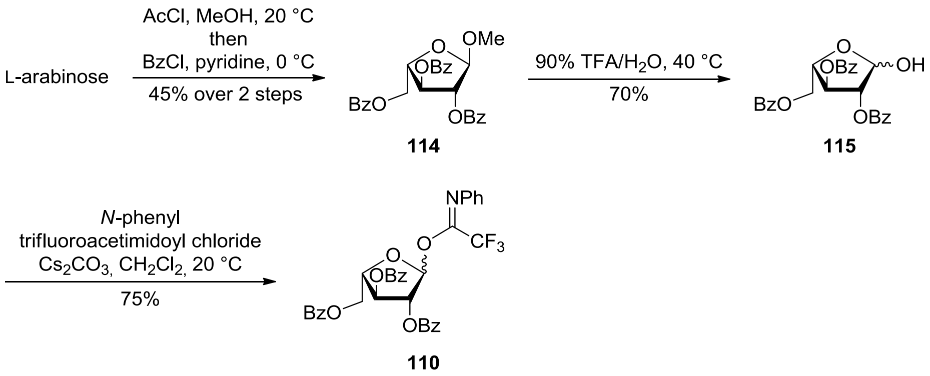
Figure 14 Monosaccharide building blocks required for synthesis of tetrasaccharide **111**

3.2 Synthesis of the Building Blocks and Assembly of the Target Tetrasaccharides

3.2.1 Synthesis of the Monosaccharide Building Blocks

Synthesis of the Arabinose *N*-Phenyl Trifluoroacetimidate Donor

The *N*-phenyl trifluoroacetimidate donor **110** was prepared from commercially available L-arabinose in four steps; its synthesis is shown in Scheme 29.



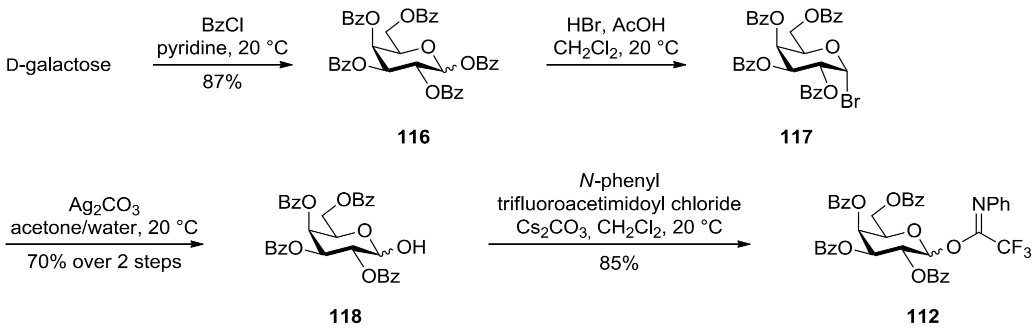
Scheme 29 Synthesis of the arabinose *N*-phenyl trifluoroacetimidate donor **110**

First, the non-protected monosaccharide was transformed into the methyl glycoside **114** in two straightforward steps: a Fischer glycosylation[115] of methanol under kinetic control (to insure the formation of the furanose form) followed by benzoylation with benzoyl chloride in pyridine.[116] Compound **114** was obtained as the α-isomer in 45% yield over two steps. The methyl group at the anomeric position of **114** was hydrolyzed by treatment with 90% aqueous trifluoroacetic acid (TFA)[117] to give hemiacetal **115** in 70% yield. Subsequent reaction with *N*-phenyl trifluoroacetimidoyl chloride[26] in the presence of cesium carbonate in CH_2Cl_2 afforded donor **110** as a α/β-mixture in 75% yield.

Synthesis of the Galactose Derivatives

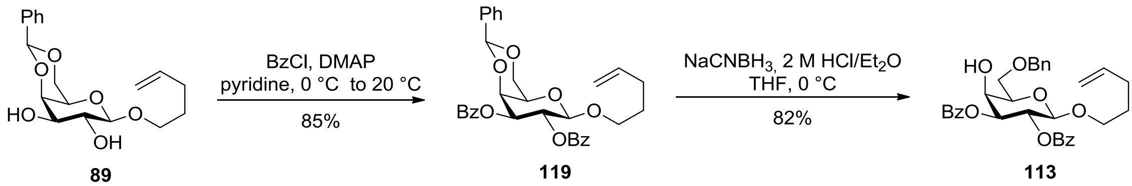
Galactose acceptor **92** was previously used in our synthesis of the linear hexasaccharide **66**; its synthesis is discussed in Chapter 2.

The *N*-phenyl trifluoroacetimidate donor **112** was prepared from commercially available D-galactose in four steps; its synthesis is shown in Scheme 30. The nonprotected monosaccharide was converted into the tetrabenzoate **116** in 87% yield by treatment with benzoyl chloride in pyridine.[118] Compound **116** was subjected to sequential anomeric bromination by the reaction with HBr in acetic acid. The resulting bromide **117** was taken directly, without purification, into the reaction with silver(I) carbonate in the mixture of acetone and water[119] to afford hemiacetal **118** in 70% yield over two steps. Reaction of **118** with *N*-phenyl trifluoroacetimidoyl chloride[26] in the presence of cesium carbonate in CH_2Cl_2 afforded donor **112** as a α/β-mixture in 85% yield.



Scheme 30 Synthesis of the galactose *N*-phenyl trifluoroacetimidate imidate donor **112**

Acceptor **113** was synthesized in two steps form diol **89** (Scheme 31), which was employed in our synthesis of the linear hexasaccharide **66**.



Scheme 31 Synthesis of the galactose pentenyl acceptor **113**

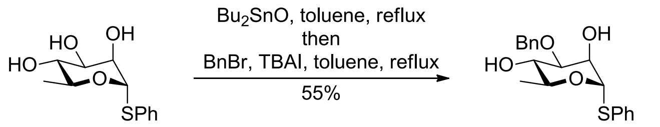
First, the C-2 and C-3 hydroxyl groups were protected with benzoyl groups by the reaction with benzoyl chloride in the presence of 4-(dimethylamino)pyridine (DMAP) in pyridine to afford **119** in 85% yield. The 4,6-benzylidene acetals can be regioselectively opened to give either the C-4 or the C-6 monobenzylated products.[120] The regioselectivity of this process depends on the reagents used. For instance, employing $LiAlH_4$–$AlCl_3$ generally gives the C-4 monobenzylated products,[121] while using $NaCNBH_3$–HCl provides the C-6 isomer.[122] A number of other reagents are also available.[123,124] The reductive opening of the benzylidene acetal in **119** with $NaCNBH_3$–HCl in tetrahydrofuran gave acceptor **113** in 82% yield.

Synthesis of the Rhamnose Acceptor

Rhamnose thioglycoside acceptor **108** was designed to bear a temporary NAP protective group in the C-2 position. The C-3 position had to be permanently blocked with a benzyl group. The C-4 hydroxyl group had to be left unprotected to allow for the future glycosylations at this position. The synthesis of **108** commenced with a triol **73** which was previously prepared in our synthesis of the linear hexasaccharide **66**. Two of the three hydroxyl groups in **73** had to be selectively alkylated.

Reagents capable of promoting regioselective alkylations of sugar hydroxyl groups have been developed, including tin(IV),[125] copper(II),[126,127] mercury(II),[126] nickel(II)[128] and boron[129]-containing compounds. The most widely used of these methods are tin-mediated alkylations.[125,130] Cyclic dibutylstannylene derivatives of carbohydrates can be prepared by reaction with dibutyltin(IV) oxide (Bu_2SnO) or dibutyldimethoxytin ($Bu_2Sn(OMe)_2$) with removal of water or methanol, respectively. These stannylene derivatives can subsequently be alkylated in benzene, toluene or DMF in the presence of added nucleophiles such as tetrabutylammonium halides or cesium fluoride to give the corresponding monosubstituted products in good yields. The stannylation of a diol enhances the nucleophilicity of one of the hydroxyl groups. In general, dibutyltin acetals derived from mixed primary and secondary diols are alkylated at the primary positions, while acetals derived from secondary diols are alkylated at the equatorial positions.[131]

In the fully unprotected rhamnosyl glycosides, tin chemistry offers a method for selective protection of the C-3 hydroxyl group.[132,133] Rhamnose triol **73** was selectively benzylated at the C-3 position, in 55% yield, by reaction with Bu_2SnO followed by treatment with benzyl bromide in the presence of tetrabutylammonium iodide (TBAI) in refluxing toluene (Scheme 32).

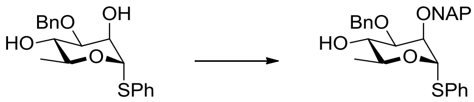


73 **120**

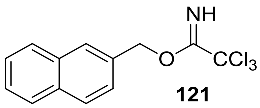
Scheme 32 Regioselective benzylation of triol **73**

In general, because of its higher acidity the C-2 hydroxyl displays the highest reactivity among all secondary hydroxyl groups in carbohydrates.[134] Therefore, we expected that it would be possible to selectively protect the C-2 position in diol **120** with a NAP-group. In the literature, there is an example of the selective benzylation of this position in a similar rhamnose derivative under the phase-transfer conditions in 52% yield.[135] When **120** was subjected to the reaction with 2-(bromomethyl)naphthalene (NAPBr) in the mixture of CH_2Cl_2 and aqueous sodium hydroxide in the presence of the phase-transfer catalyst tetrabutylammonium hydrogen sulfate ($TBAHSO_4$), product **108** was obtained in 42% yield (Table 6, entry 1). The relatively low yield in this transformation was caused by the formation of another regioisomer (where the protection occurred at the C-4 position) along with the significant amounts of the unreacted starting material left after 48 hours of reaction.

Table 6 Regioselective protection of the C-2 hydroxyl group in rhamnose derivative **120**



120 **108**

Entry	Reaction conditions	T, °C	Time, h	Yield, %
1	NAPBr, TBAHSO$_4$, aq. NaOH, CH_2Cl_2	40	48	42
2	NAPBr, NaH, TBAI, DMF	0 to 20	12	65
3	NAPBr, Ag_2O, KI, CH_2Cl_2	20	48	40
4	 **121**, TMSOTf, Et_2O[1]	0 to 20	12	25

[1]**121** was prepared from 1-naphthalenemethanol by treatment with trichloroacetonitrile in the presence of cesium carbonate in CH_2Cl_2

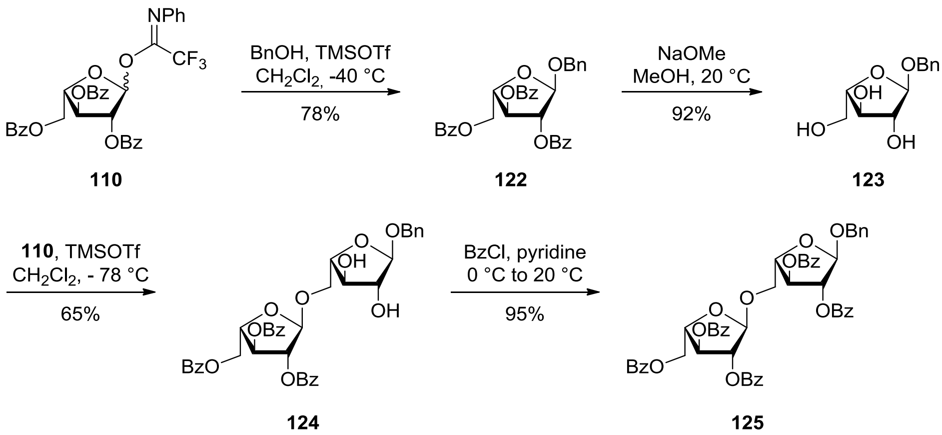
Interestingly, the reaction of **120** with NAPBr in the presence of sodium hydride and TBAI in DMF (entry 2) in our hands gave higher yields than the protection under the phase-transfer conditions. This reaction produced the desired NAP-protected sugar **108** in 65% yield. We also explored other methods available for introducing a NAP-group. Treatment of **120** with NAPBr in the presence of silver(I) oxide[136] and potassium iodide in CH_2Cl_2 gave **108** in 40% yield (entry 3). Together with the desired product **108**, another regioisomer and the dialkylated derivative were formed and some of the starting material remained unreacted even after 48 hours. The acid-catalyzed reaction[137] of diol

114 with trichloroacetimidate **121** in ether (entry 4) procured mainly the undesired regioisomer presumably because of the less steric hindrance of the equatorial C-4 hydroxyl group.

3.2.2 Synthesis of the Disaccharide Side Chains

Synthesis of the Diarabinan Donor

Synthesis of the diarabinan *N*-phenyl trifluoroacetimidate donor **109** was performed in six steps starting from the arabinose donor **110** (Scheme 33).



Scheme 33 Synthesis of the diarabinan *N*-phenyl benzyl glycoside **125** via a chemoselective glycosylation

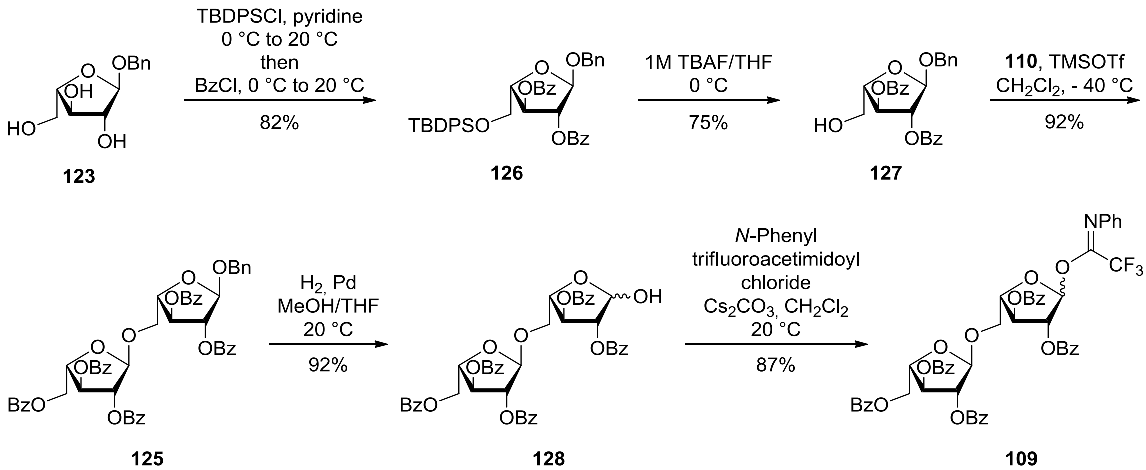
The TMSOTf-promoted glycosylation of benzyl alcohol with donor **110** provided benzyl glycoside **122** as the α-anomer in 78% yield. Subsequent treatment of **122** under the Zemplén deacylation conditions[97] afforded the nonprotected benzyl glycoside **123** in 92% yield.

The more reactive primary C-5 hydroxyl group of triol **123** was selectively glycosylated with a small excess (1.1 equivalents) of the same donor **110** activated with TMSOTf. When the reaction was performed in CH_2Cl_2 at –40 °C, the partially protected disaccharide **124** was obtained as the α-anomer in

55% yield. Lowering the temperature to –78 °C improved the glycosylation outcome and resulted in 65% yield. The selective glycosylation of the primary hydroxyl group in the presence of the secondary hydroxyls in arabinose was previously reported on a similar system by Kong and co-workers.[117,138]

Subsequent protection of the C-2 and the C-3 hydroxyls of **124** with the benzoate groups, conducted by treatment with benzoyl chloride in pyridine, furnished the fully protected disaccharide **125** in 95% yield. In ^{1}H NMR spectrum of **125**, the chemical shifts of the H-2 and H-3 signals moved downfield proving the formation of the (1→5)-glycosidic linkage.

Given the relatively low yields in the chemoselective coupling of **110** and **123**, we also explored an alternative route towards the synthesis of disaccharide **125** (Scheme 34).



Scheme 34 Synthesis of the diarabinan *N*-phenyl trifluoroacetimidate donor **109** via protective group manipulations

Triol **123** was transformed into the fully protected arabinose derivative **126** through two steps performed one-pot. First, the primary hydroxyl group in **123** was selectively protected with the *tert*-butyldiphenylsilyl (TBDPS) group by treatment with TBDPSCl in pyridine at 0 °C. This was followed by the esterification of the remaining free hydroxyls with benzoyl esters in 82% yield over two steps.[139] The TBDPS-group is **126** was then selectively cleaved in 75% yield by treatment with a 1M solution of tetrabutylammonium fluoride (TBAF) in THF at 0 °C. The resulting alcohol **127** was taken to the TMSOTf-promoted glycosylation with the same donor **110** to give the perbenzoylated benzyl glycoside **125** in 92% yield. This strategy allowed obtaining high yields in the glycosylation reaction. However, it included protection and deprotection of the C-5 hydroxyl and therefore contained more steps than the chemoselective glycosylation strategy. On the other hand, all the reactions were straightforward and the yields were generally high leading to the conclusion that in terms of the overall yield of disaccharide **125** starting from triol **123** these two methods were equally efficient.

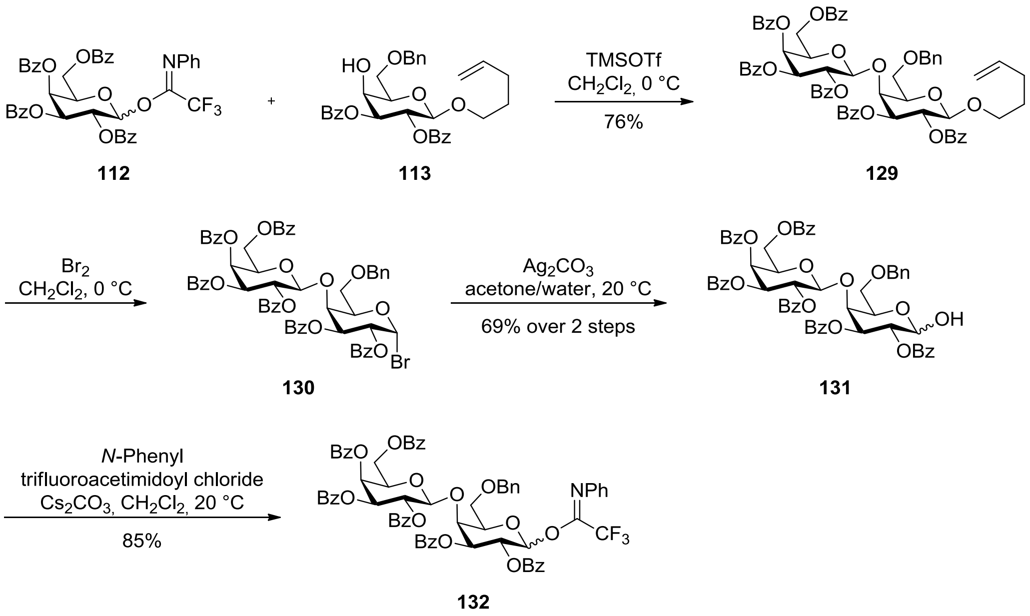
The benzyl group was used for temporary protection of the anomeric position in **125**. Its catalytic hydrogenolysis provided hemiacetal **128** in 92% yield (Scheme 34). The hydrogenolysis, although clean and high yielding, was very time consuming (the reaction took 5 days). Trying to speed it up, we performed the reaction under 10 bar pressure of hydrogen at 40 °C overnight. These conditions, unfortunately, resulted in a complex mixture of products that could be partially separated. In the ^{1}H NMR spectra of the main three fractions obtained after the purification flash column chromatography, the broad signals in the aliphatic region (1.0 – 2.0 ppm) were observed, which could indicate that the partial reduction of the benzoyl groups in **126** to cyclohexyls occurred under the reaction conditions. This hypothesis was proven by the fact that when these three products were taken separately into the next synthetic steps (discussed below), they all resulted in the same trisaccharide **133** after the removal of the ester protective groups.

Finally, hemiacetal **128** was transformed to the target disaccharide donor **109** in 87% yield by the reaction with *N*-phenyl trifluoroacetimidoyl chloride[26] in the presence of cesium carbonate in CH_2Cl_2.

Synthesis of the Digalactan Donor

The synthesis of the digalactan *N*-phenyl trifluoroacetimidate donor **132** (Scheme 35) commenced with the TMSOTf-promoted glycosylation of acceptor **113** with the perbenzoylated *N*-phenyl trifluoroacetimidate donor **112**. Initially, the reaction was performed in CH_2Cl_2 at –40 °C. Presumably due to the low nucleophilicity of the C-4 hydroxyl group in galactose, at this temperature the glycosylation was slow, and even after 2 hours almost no conversion to the disaccharide product **129** was observed. When the reactants were mixed at −40 °C and then warmed up immediately to 0 °C, and subsequently stirred at this temperature for 3 hours, disaccharide **129** could be obtained in 76% yield. The participating benzoyl group at the C-2 position of the donor **112** favored the formation of the β-glycosidic linkage.

The *n*-pentenyloxy group in **129** had to be hydrolyzed to the hemiacetal functionality. The initial attempt to perform this reaction by treatment with *N*-bromosuccinimide (NBS)[33] in the mixture of acetone and water resulted in multiple products. Alternatively, this transformation could be performed in two steps. First, the pentenyl disaccharide **129** was titrated with a solution of bromine in CH_2Cl_2 at 0 °C. Then the resulting bromide **130** was taken directly, without purification, into the reaction with silver(I) carbonate in the mixture of acetone and water.[119] This approach afforded hemiacetal **131** in 69% yield over two steps. Reaction of **131** with *N*-phenyl trifluoroacetimidoyl chloride[26] in the presence of cesium carbonate in CH_2Cl_2 gave the target digalactan donor **132** in 85% yield.



Scheme 35 Synthesis of the digalactan *N*-phenyl trifluoroacetimidate donor **132**

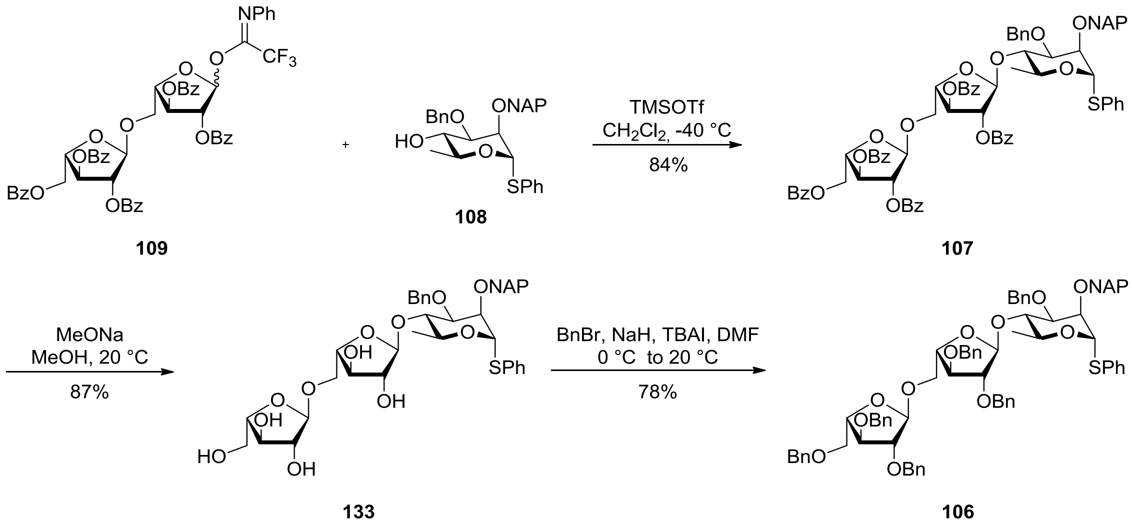
3.2.3 Assembly of the Target Tetrasaccharides

Synthesis of the Trisaccharide Donors

The prepared disaccharide donors **109** and **132** were used to construct trisaccharides **106** and **136**.

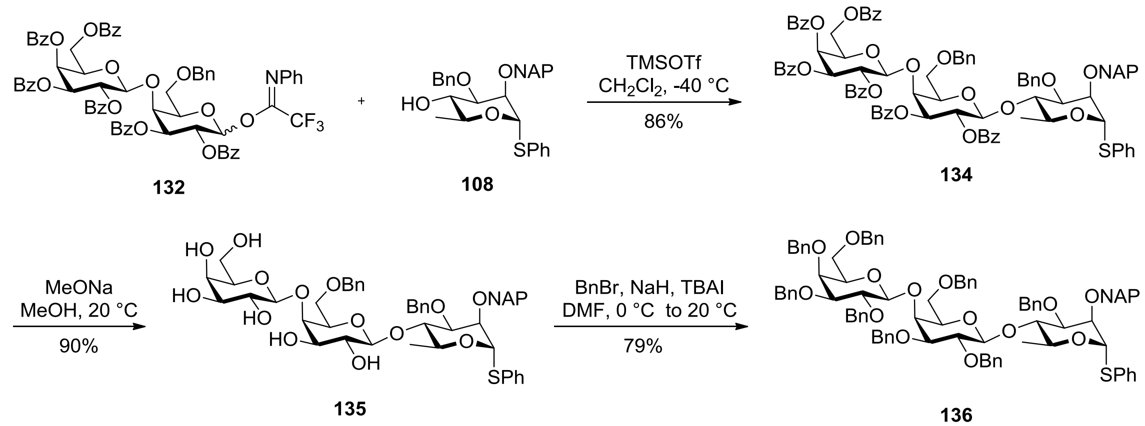
The synthesis of the diarabinan-containing trisaccharide **106** is shown in Scheme **36**. The TMSOTf-mediated coupling of the *N*-phenyl trifluoroacetimidate donor **109** with the rhamnose acceptor **108** afforded trisaccharide **107** in 84% yield. The presence on the participating benzoyl group at the C-2 position of the donor **109** ensured the formation of the α-glycosidic linkage. The benzoyl esters in **107** were exchanged for the permanent benzyl protective groups in two steps. First, treatment of **107** under the Zemplén deacylation conditions provided the partially protected trisaccharide **133** in 87% yield. Following reaction of **133** with benzyl bromide in the presence of

NaH and catalytic amounts of TBAI in DMF furnished the target trisaccharide donor **106** in 78% yield.



Scheme 36. Synthesis of the diarabinan-containing trisaccharide donor **106**

The digalactan-containing trisaccharide **136** was obtained by the similar route. Its synthesis commenced with the TMSOTf-promoted glycosylation of the same rhamnose acceptor **108** with the disaccharide donor **132**. The trisaccharide product **134** was obtained as the β-isomer in 86% yield. The deprotection of the benzoyl groups in **134** gave the partially protected trisaccharide **135** in 90% yield. The benzylation of the free hydroxyl groups in **135** with benzyl bromide in the presence of NaH and catalytic amounts of TBAI in DMF afforded the target trisaccharide donor in 79% yield.

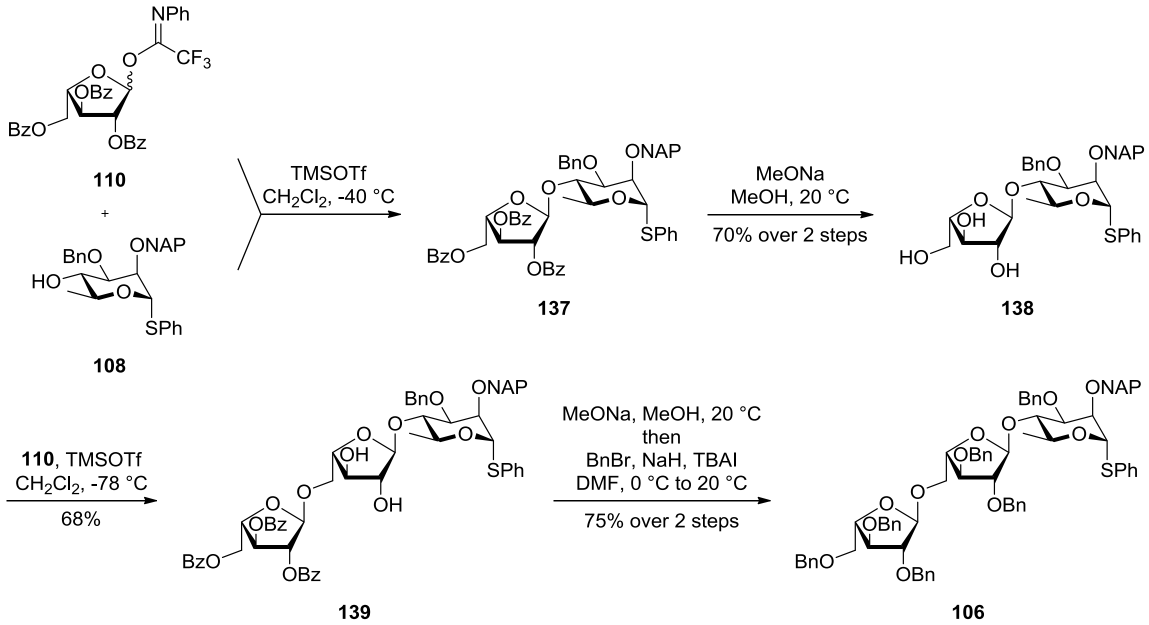


Scheme 37 Synthesis of digalactan-containing trisaccharide donor **136**

Alternative Approach to the Synthesis of the Diarabinan-Containing Trisaccharide

For the synthesis of the diarabinan-containing trisaccharide donor **106**, an alternative approach to the one described above was suggested. It was envisioned that the synthesis of **106** could be significantly simplified, as shown in Scheme 38.

The rhamnose acceptor **108** was glycosylated with the arabinose donor **109** in CH_2Cl_2 in the presence of TMSOTf. The reaction proceeded smoothly according to TLC and the disaccharide product **137** was subjected directly to the Zemplén conditions.[97] Triol **138** was isolated in 70% yield over two steps. The TMSOTf-mediated glycosylation of the primary C-5 hydroxyl group in **138** with the same donor **109** in CH_2Cl_2 furnished partially protected trisaccharide **139** in 68% yield. Similar to the glycosylations discussed previously, the participating benzoyl group at the C-2 position of the donor favored the formation of the 1,2-*trans* glycosidic linkages. Trisaccharide **139** was subjected to the Zemplén deacylation conditions followed by the protection of the free hydroxyls with the benzyl groups (treatment with benzyl bromide in the presence of NaH and catalytic amounts of TBAI in DMF). The target trisaccharide donor **106** was obtained in 75% yield over two steps.

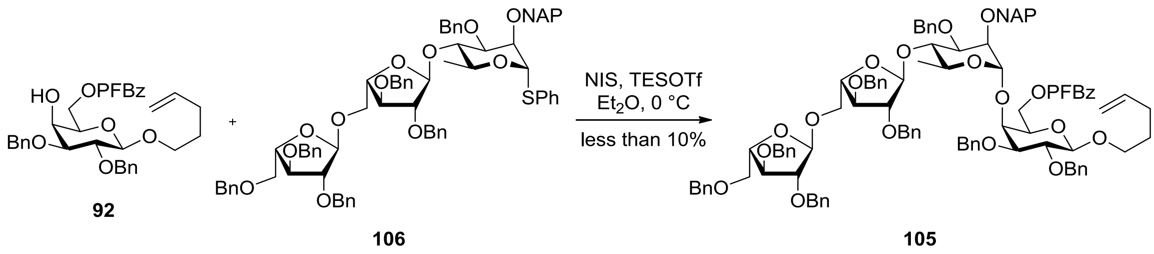


Scheme 38 Synthesis of trisaccharide **106** by the alternative approach

According to this strategy, the temporary protection of the anomeric position in arabinose was not required and only one arabinose monosaccharide building block **110** was used. This allowed synthesizing the target trisaccharide **106** in five steps instead of 9 starting from the same monosaccharide building blocks **108** and **110**.

Synthesis of the Target Tetrasaccharide Intermediates

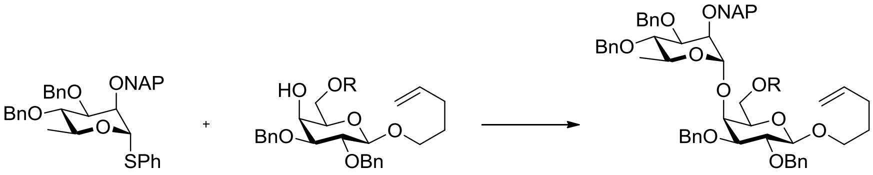
Having prepared the trisaccharide thiophenyl glycosyl donors **106** and **136**, we investigated the approaches for their coupling with the galactose acceptor **92**. At first, we examined the glycosylation of **92** with the diarabinan-containing donor **106** under the armed-disarmed conditions that were developed for the synthesis of the linear hexasaccharide and described in Chapter 2. The NIS/TESOTf-promoted glycosylation of **92** with **106** (Scheme 39) performed in Et_2O at 0 °C resulted in the formation of multiple products in essentially equal amounts. The yield of the desired tetrasaccharide was less than 10%, as judged by the TLC analysis.



Scheme 39 Armed-disarmed glycosylation of **92** with **106**

Because the application of NIS/TESOTf as a promoter did not result in an efficient glycosylation, we turned our attention to other methods available for activation of thioglycosides in chemoselective glycosylations.[46] The methods were tested on the coupling of two monosaccharides **69** and **92** (Table 7).

Table 7 Screening of the conditions for glycosylation of **92** and **93** with **69**

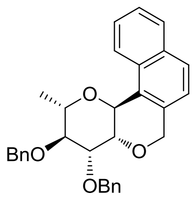


69 — **92** R = PFBz, **93** R = Ac — **83** R = PFBz, **94** R = Ac

Entry	Acceptor	Activator	Solvent	T, °C	Yield, %
1	**92**	NIS/$Yb(OTf)_3$	CH_2Cl_2	–20	n.d.[1]
2	**92**	NIS/$Yb(OTf)_3$	CH_2Cl_2	0	<10[2]
3	**92**	MeOTf	CH_2Cl_2	0	20
4	**93**	NIS/$Yb(OTf)_3$	CH_2Cl_2	0	<20[2]
5	**93**	MeOTf	CH_2Cl_2	0	25
6	**93**	Ph_2SO/Tf_2O	CH_2Cl_2	–60	n.d.
7	**93**	DMTST	CH_2Cl_2	–40	40
8	**93**	Me_2S_2/Tf_2O	CH_2Cl_2	–40	68
9	**93**	Me_2S_2/Tf_2O	Et_2O	–40	38

[1]N.d. – almost no disaccharide product was observed. [2]based on TLC analysis

Fraser-Reid and co-workers have demonstrated[140] that a mixture of NIS and lanthanide triflates can be successfully used as a very mild promoter in the chemoselective glycosylations. They have shown that thioglycosides can be selectively activated over disarmed pentenyl glycosides by NIS/$Yb(OTf)_3$.[141] When a mixture of **69** and **92** in CH_2Cl_2 was treated with NIS in the presence of $Yb(OTf)_3$ at –20 °C (entry 1), no formation of the disaccharide product **83** was observed. Instead, donor **69** was converted into the *C*-glycoside **85** (this side-reaction was discussed in Chapter 2) through an intramolecular cyclization.



85

When the reaction was performed at the higher temperature (0 °C, entry 2), a small amount (less than 10%, judged by TLC) of disaccharide **83** was formed, while **85** was still the major product. Further increase of the temperature did not improve the reaction outcome (results not shown in the table).

Demchenko and co-workers have reported[142] the use of methyl triflate (MeOTf) to selectively activate thioglycosides over pentenyl glycosides. When **69** and **92** were subjected to the treatment with MeOTf in CH_2Cl_2 at 0 °C (entry 3), disaccharide **83** was isolated in 20% yield. A substantial amount of the *C*-glycoside **85** was formed along with several other by-products.

Unfortunately for our synthesis, the aromatic system of the C-2 NAP-group exhibited a higher nucleophilicity than the C-4 hydroxyl group of acceptor **92**, which led to the formation of the cyclization by-product. We envisioned that the exchange of the PFBz-group at the C-6 position of acceptor **92** to a less electron-withdrawing and sterically demanding acetyl group could possibly increase the nucleophilicity of the C-4 hydroxyl group. The galactose acceptor **93** bearing the C-6 acetyl group was prepared from diol **91** as shown in Chapter 1.

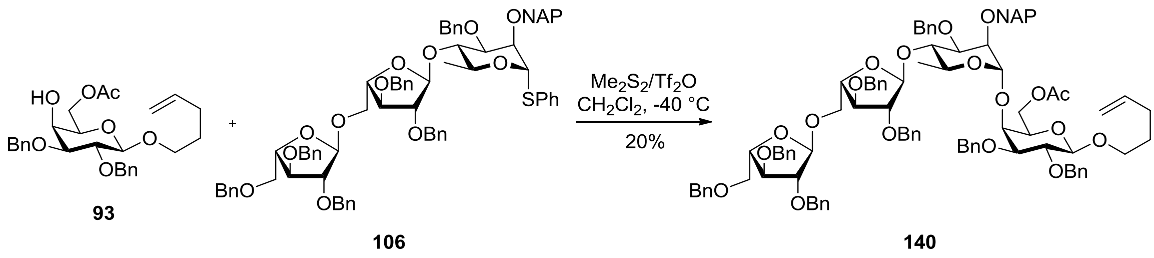
Acceptor **93** bearing the C-6 acetyl group was coupled with the same donor **69** in the NIS/$Yb(OTf)_3$- and MeOTf-promoted glycosylations (entries 4 and 5). In general, slightly higher yields of the disaccharide product were observed in these reactions compared to the ones performed with the PFBz-protected acceptor **92**.

Several sulfonium-based activator systems are available for the "preactivation" of thioglycosides with the *in situ* formation of the reactive glycosyl triflate intermediates that can be successfully coupled to a variety of glycosyl acceptors.[46] One of these promoters is a combination of diphenyl sulfoxide and triflic anhydride (Ph_2SO/Tf_2O) recently introduced by van der Marel and co-workers.[143] It was shown to be capable of activating various thioglycosides and promoting high yielding glycosylations. When donor **69** was treated with Ph_2SO/Tf_2O at –60 °C in CH_2Cl_2 for 5 minutes followed by addition of acceptor **93**, the formation of the cyclization product **85** was observed exclusively. In a separate experiment, **69** was treated with Ph_2SO/Tf_2O under the same conditions without adding acceptor **93.** After 5 minutes the reaction was stopped by addition of a saturated aqueous $NaHCO_3$. This led to the quantitative formation of **85** meaning that the donor was already converted into the *C*-glycoside before the acceptor was added.

As the next opportunity, we explored the use of thiophilic promoters such as dimethylthiomethylsulfonium triflate (DMTST) introduced by Garegg and Fugedi[43] and the dimethyl disulfide-triflic anhydride (Me_2S_2/Tf_2O) system developed later by Fugedi and co-workers.[144] The DMTST-promoted glycosylation of acceptor **93** with donor **69** at –40 °C in CH_2Cl_2 (entry 7) resulted in 40% yield of disaccharide **94**. The same reaction mediated by Me_2S_2/Tf_2O (entry 8) furnished the target disaccharide **94** in 68% yield. Changing the solvent from CH_2Cl_2 to ether (entry 9) resulted in the decrease of the yield to 38%.

In conclusion, the best results in the coupling of donor **69** with the acceptor **93** were obtained when Me_2S_2/Tf_2O was used as a promoter and the glycosylation was performed in CH_2Cl_2. These conditions gave the disaccharide product **94** in 68% yield. The efficacy of this reaction was comparable with the one performed under the armed-disarmed conditions.

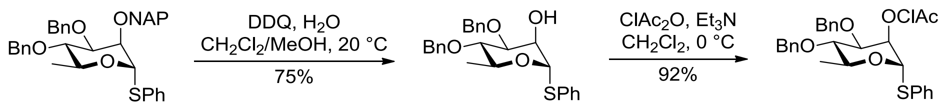
Inspired by this result, we applied these glycosylation conditions to the coupling of the trisaccharide donor **106** with acceptor **93**. Regrettably, treatment of **106** and **93** with Me_2S_2/Tf_2O at –40 °C in CH_2Cl_2 resulted mainly in the undesired formation of *C*-glycoside. The target tetrasaccharide **140** was obtained in only 20% yield (Scheme 40).



Scheme 40 Synthesis of tetrasaccharide **140**

All these observations led us to the conclusion that the presence of the C-2 NAP-group was the major obstacle for the successful glycosylations. Clearly, the NAP-group had to be replaced in order to avoid the formation of the cyclization by-product. The chloroacetyl (ClAc) ester was chosen to replace the NAP-group as it could be selectively removed in the presence of the C-6 acetyl group by treatment with thiourea.[20]

The monosaccharide donor **141** bearing the chloroacetyl group was prepared (Scheme 41) and coupling with acceptor **93** was studied (Table 8).



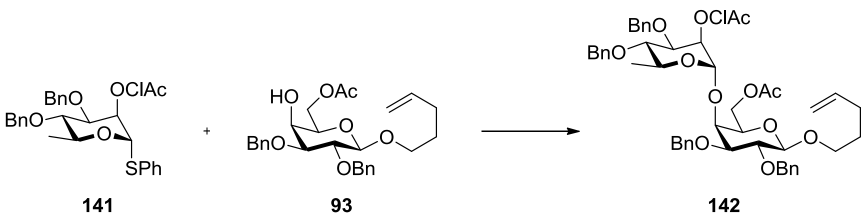
69 99 141

Scheme 41 Exchange of the NAP-group for the ClAc-group in the rhamnose donor **141**

The exchange of the NAP-group for the chloroacetyl was performed in two steps starting from donor **69**. The NAP-ether was cleaved in 75% yield by treatment with DDQ in the presence of water in the mixture of CH_2Cl_2 and methanol. The released hydroxyl group was then esterified by the reaction with trichloroacetic anhydride in the presence of triethylamine in CH_2Cl_2 at 0 °C. Donor **141** was obtained in 92% yield.

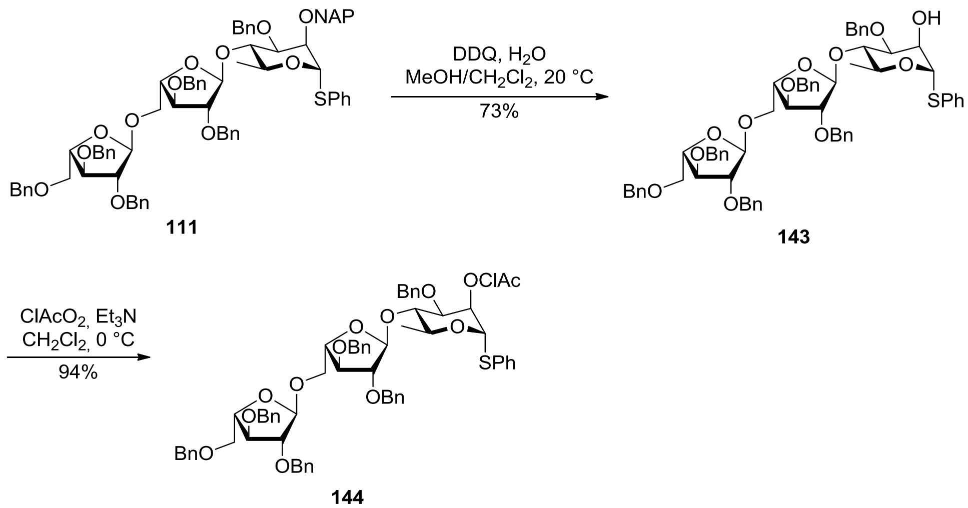
We tested the promoter system that performed best in the previous experiments (Me_2S_2/Tf_2O) and the two systems where the side reactions were caused by the cyclization of the donor (NIS/Yb(OTf)$_3$ and Ph_2SO/Tf_2O). The Me_2S_2/Tf_2O-mediated glycosylation of acceptor **94** with donor **141** performed at –40 °C in CH_2Cl_2 (entry 1) resulted in the formation of the disaccharide product **142** in 60% yield. Substitution of the NAP-group for the chloroacetyl roup did not significantly change the yield of the NIS/Yb(OTf)$_3$-promoted coupling (entry 2). Significant decomposition of the acceptor took place under the reaction conditions leading to the low yield. However, the outcome of the Ph_2SO/Tf_2O-mediated glycosylation (entry 3) was improved and disaccharide **142** was obtained in 40% yield.

Table 8 Coupling of **93** with donor **141** bearing a chloroacetyl group



Entry	Activator	Solvent	T, °C	Yield, %
1	Me_2S_2/Tf_2O	CH_2Cl_2	–40	60
2	NIS/$Yb(OTf)_3$	CH_2Cl_2	0	<10
3	Ph_2SO/Tf_2O	CH_2Cl_2	–60	45

To conclude, exchanging the NAP-group for the chloroacetyl allowed for avoiding the undesired cyclization reaction. Me_2S_2/Tf_2O and Ph_2SO/Tf_2O were found to be the most promising promoter systems and were subsequently applied in the glycosylation with the trisaccharide donors.



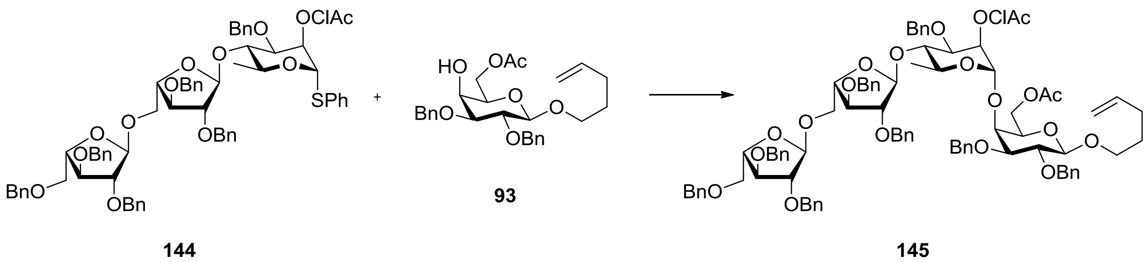
Scheme 42 Introducing the ClAc-group into the trisaccharide donor **144**

In the trisaccharides, the chloroacetyl group could not be introduced on an early stage because it would not be compatible with the conditions of cleavage of the benzoyl groups followed by introducing the benzyl groups. Thus, the chloroacetyl group had to replace the temporary NAP-group at a late stage. For synthesis of the trisaccharide donors bearing the chloroacetyl group a reaction sequence similar to the one performed for synthesis of monosaccharide donor **141** was used (Scheme 42).

Trisaccharide **111** was treated with DDQ in the presence of water in a mixture of CH_2Cl_2 and methanol resulting in the formation of **143** in 73% yield. The hydroxyl group in **143** was protected with the chloroacetyl ester in 94% yield by reaction with trichloroacetic anhydride in the presence of triethylamine in CH_2Cl_2 at 0 °C.

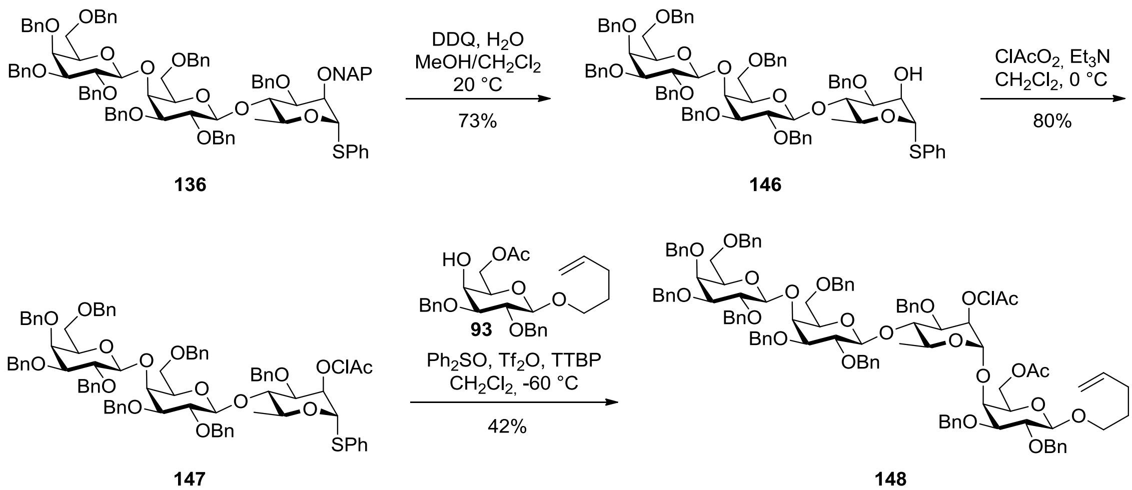
Glycosylation of acceptor **93** with the prepared trisaccharide donor **144** was studied (Table 9). When Me_2S_2/Tf_2O was applied as a promoting system and the reaction was performed in CH_2Cl_2 at –40 °C, the tetrasaccharide product **145** was isolated in 20% yield. Using Ph_2SO/Tf_2O as a promoter and performing the glycosylation in CH_2Cl_2 at –60 °C led to the isolation of **145** in 45% yield.

Table 9 Synthesis of the diarabinan-containing tetrasaccharide **145**



Entry	Activator	Solvent	T, °C	Yield, %
1	Me_2S_2/Tf_2O	CH_2Cl_2	–40	20
2	Ph_2SO/Tf_2O	CH_2Cl_2	–60	40

A similar synthetic sequence was performed on the digalactan-containing trisaccharide **136** (Scheme 43). Treatment with DDQ in the presence of water in a mixture of CH_2Cl_2 and methanol afforded **146** in 73% yield. The hydroxyl group in **143** was protected with the chloroacetyl ester in 80% yield by reaction with trichloroacetic anhydride in the presence of triethylamine in CH_2Cl_2 at 0 °C. The resulting trisaccharide donor **147** was taken into glycosylation with the galactose acceptor **93**. The glycosylation was carried out under the same conditions as for the diarabinan-containing trisaccharide donor **144**: Ph_2SO/Tf_2O was used as a promoter and the reaction was performed at –60 °C. The target tetrasaccharide **148** was obtained in 42% yield.



Scheme 43 Synthesis of tetrasaccharide **148** bearing the ClAc-group

3.3 Conclusions

To conclude, syntheses of two protected tetrasaccharide intermediates with diarabinan- and digalactan side-chains designed for assembly of larger RG I oligosaccharides have been performed.

In synthesis of the target tetrasaccharides, the side-chain diarabinan and digalactan were prepared first in the form of the *N*-phenyl trifluoroacetimidate donors. The TMSOTf-promoted coupling of these donors with the rhamnose acceptor allowed for obtaining the trisaccharide thioglycoside donors. Regrettably, the armed-disarmed approach which we developed for synthesis of the linear hexasaccharide was not efficient when applied for coupling of these trisaccharide donors with the galactose acceptor, neither were all other attempts to perform chemoselective glycosylations on these systems.

The major challenge we were facing was the low reactivity of the axial C-4 hydroxyl group in galactose. This problem was already seen in couplings between two monosaccharides (see Chapter 1) when it resulted in moderate yields. In certain cases the aromatic system of the NAP-group was observed to be more nucleophilic than the C-4 hydroxyl group of the acceptor resulting in the formation of *C*-glycoside as a by-product. When larger glycosyl donors (trisaccharides) were used this side-process became a major reaction taking place; almost no target tetrasaccharide products were obtained in glycosylations. This forced us to exchange the NAP-group in the trisaccharide donors for the chloroacetyl group in order to avoid the undesired cyclization. This approach proved to be successful and when the preactivation glycosylation protocol was employed, the target tetrasaccharides could be obtained in acceptable yields.

We envision that the prepared tetrasaccharides could be versatile building blocks in synthesis of larger branched fragments of RG I. The *n*-pentenyloxy group at the anomeric position allows for using them directly as glycosyl donors. The chloroacetyl group at the C-2 position of the rhamnose residue can be selectively removed converting the tetrasaccharides into the corresponding glycosyl acceptors. We are currently working on demonstrating that the tetrasaccharides can be efficiently used in further glycosylations to extend the oligosaccharide chain from both the reducing and the non-reducing ends.

4 Experimental

General Information

All reagents and solvents were purchased from Sigma-Aldrich and used without further purification, except for dry Et_2O and CH_2Cl_2 which were obtained from Innovative Technology PS-MD-7 Pure-solv solvent purification system. Tri-*tert*-butylpyrimidine (TTBP) was synthesized as described by Crich et al.[145] All reactions requiring anhydrous conditions were carried out in flame-dried glassware under inert atmosphere. Solvents were removed under reduced pressure (in vacuo) at temperature below 40 °C. All reactions were monitored by thin-layer chromatography (TLC) that was performed on Merck aluminum plates precoated with silica gel 60 F254. Compounds were visualized by heating after dipping in a solution of $Ce(SO_4)_2$ (2.5 g) and $(NH_4)_6Mo_7O_{24}$ (6.25 g) in 10% aqueous H_2SO_4 (250 mL). Column chromatography was performed using Geduran silica gel 60 with specified solvents. NMR spectra were recorded on a Varian Unity Inova 500 or a Varian Mercury 300 spectrometer or a Bruker Ascend 400. Chemical shifts δ are reported in ppm using the solvent resonance as the internal standart ($CDCl_3$: ^1H 7.27 ppm, ^{13}C 77.0 ppm). Coupling constants are reported in Hz, and the field is indicated in each case. Multiplicities are recorded as singlet (s), doublet (d), triplet (t) and multiplet (m). IR spectra were recorded neat on a Bruker Alpha FT-IR spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}). Optical rotations were measured with a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations of the solutions are given in 10^{-2} g ml^{-1}. MALDI-TOF mass spectra were obtained at Novozymes, Denmark or University of Southern Denmark using a Perseptive Biosystems Voyager-De instrument in positive-ion mode with 3,4-dihydroxybenzoic acid as the matrix, or using a Applied Biosystems MDS SCIEX 4800 Plus instrument in positive-ion mode with α-cyano-4-hydroxycinnamic acid as the matrix, respectively.

NMR Analysis of Hexasaccharide 66

Hexasaccharide **66** (50 mg) was dissolved in 2 ml D_2O and freeze dried, this was repeated twice and then **66** was dissolved in 99.9% D_2O and the solution was transferred to an NMR tube. All NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer at 20 °C. Chemical shifts were referenced to water (δ_H 4.79 ppm) and the CH_3-groups in rhamnose (δ_C 17.6 ppm). All spectra were processed in MNova 6.2.1 with zero filling in both dimensions. Two-dimensional spectra were processed with 90 (DQF-COSY, HSQC) or 60 (HMBC, HSQC-TOCSY) degree sine square functions in both dimensions. At the time of assigning the spectra, 1D ^{13}C spectrum was not available and ^{13}C chemical shift values were obtained from the HSQC and the HSQC-TOCSY spectra.

Reaction Conditions and Compound Characterization

In most cases general procedures are given. Syntheses of all new compounds are described in details. Some of the compounds were prepared according to the literature procedures. In these cases the procedures are not described and references are given instead.

General Procedure I for Glycosylation with Pentenyl Glycoside Donors

A mixture of the donor (1.2 mmol) and the acceptor (1.0 mmol) was co-evaporated with toluene (2 × 20 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous diethyl ether (15 mL) and cooled to –20 °C (for synthesis of the disaccharides **83** and **94**) or to 0 °C (for synthesis of the tetrasaccharide **100** and the hexasaccharide **67**), NIS (450 mg, 2.0 mmol) was added followed by addition of TESOTf (0.06 mL, 0.25 mmol). The reaction mixture was stirred at –20 °C or 0 °C until TLC (toluene/EtOAc 10:1) showed completion of the reaction (40 min – 1.5 h). The reaction mixture was quenched with Et_3N (0.1 ml), diluted with CH_2Cl_2 (50 ml) and washed with 10% aq. $Na_2S_2O_3$ (2 × 20 ml). The combined aqueous phases were extracted with CH_2Cl_2 (20 ml). The combined organic phases were dried with Na_2SO_4, filtered, concentrated and purified by flash chromatography (toluene/EtOAc 40:1).

General Procedure II for Removal of the 2-Naphthylmethyl (NAP) Group

The protected saccharide (1.5 mmol) was dissolved in a mixture of CH_2Cl_2 (12 ml) and MeOH (3 ml). Water (0.5 ml) was added followed by addition of DDQ (480 mg, 2.1 mmol). The reaction mixture was stirred at 20 °C until TLC (toluene/EtOAc 10:1) showed completion of the reaction (2–5 h). The reaction mixture was diluted with CH_2Cl_2 (100 ml) and washed with sat. $NaHCO_3$ (2 × 50 ml). The combined aqueous phases were extracted with CH_2Cl_2 (20 ml). The combined organic phases were dried with Na_2SO_4, filtered, concentrated and purified by flash chromatography (toluene/EtOAc 15:1).

General Procedure III for Removal of Acyl Protective Groups (Zemplén Conditions)

The protected saccharide (1 g) was dissolved in MeOH (10 ml) or, if it was not soluble in MeOH, in a mixture of MeOH (5 ml) and THF (5 ml) and 0.5 ml of freshly prepared 1M NaOMe solution in MeOH was added. The reaction mixture was stirred at 20 °C until TLC (heptane/EtOAc 1:1 and CH_2Cl_2/MeOH 10:1) showed the full conversion (1–24 h). The reaction mixture was then quenched by addition of Amberlite IR-120 (H^+) (10 ml) and stirred for additional 30 min. The resin was filtered off and the filtrate was concentrated and purified either by flash column chromatography in CH_2Cl_2/MeOH 10:1 or crystallization from EtOAc.

General Procedure IV for Benzylation of Hydroxyl Groups

To a solution of the starting saccharide (1 mmol, 3 mmol of OH-groups) in DMF (4 ml) BnBr (0.43 ml, 3.6 mmol) and TBAI (10 mg, 0.03 mmol) were added and the mixture was cooled in ice bath. NaH (145 mg, 3.6 mol, 60% in oil) was added and the mixture was stirred at 20 °C for 15 h and then quenched by addition of MeOH (0.2 ml). The reaction mixture was partially concentrated, diluted with EtOAc (20 ml) and washed with water (3 × 10 ml) and brine (10 ml). The organic phase was dried with Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (toluene/EtOAc 30:1).

General Procedure V for Preparation of the N-Phenyl Trifluoroacetimidate Glycosyl Donors

Hemiacetal (1.2 mmol) was dissolved in CH_2Cl_2 (12 mL) and 0.1 mL water. $ClC(=NPh)CF_3$ (0.50 g, 2,4 mmol) and Cs_2CO_3 (0.78 g, 2.4 mmol) were added and the reaction mixture was stirred at 20 °C until TLC (toluene/EtOAc 10:1) showed completion of the reaction (2–5 h). The reaction mixture was filtered through Celite, concentrated and the residue was purified by flash column chromatography (heptane/EtOAc 4:1).

General Procedure VI for Glycosylation with N-Phenyl Trifluoroacetimidate Glycosyl Donors

A mixture of the donor (1.0 mmol) and the acceptor (1.2 mmol) was co-evaporated with toluene (2 × 20 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (20 mL) and cooled to –40 °C. TMSOTf (0.018 ml, 0.1 mmol) was added and the reaction mixture was stirred at –40 °C until TLC (toluene/EtOAc 20:1) showed completion of the reaction (10–30 min). The reaction mixture was quenched by addition of Et_3N (0.1 ml), evaporated and purified by flash column chromatography (toluene/EtOAc 50:1).

General Procedure VII for the Regioselective Glycosylation with N-Phenyl Trifluoroacetimidate Glycosyl Donors

A mixture of the donor (1.1 mmol) and the acceptor (1.0 mmol) was co-evaporated with toluene (2 × 20 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (35 mL) and cooled to –78 °C. TMSOTf (0.018 ml, 0.1 mmol) was added and the reaction mixture was stirred at –78 °C until TLC (toluene/EtOAc 20:1) showed completion of the reaction (20 min – 1 h). The reaction mixture was quenched by addition of Et_3N (0.1 ml), evaporated and purified by flash chromatography (heptane/EtOAc 4:1).

General Procedure VIII for Introducing a Chloroacetyl (ClAc) Group

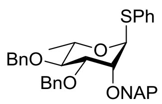
Diol **91** (2.0 g, 4.7 mmol) was dissolved in anhydrous CH_2Cl_2 (30 ml) and cooled in ice bath. Et_3N (1.05 ml, 7.5 mmol) was added followed by addition of $ClAc_2O$ (870 mg, 5.1 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed up to 20 °C, diluted with CH_2Cl_2 (50 ml) and washed with 0.1 M HCl

(2 × 15 ml). The organic phase was dried with Na_2SO_4, concentrated and purified by flash column chromatography (heptane/EtOAc 3:1 for partially protected saccharides or 50:1 toluene/EtOAc for fully protected saccharides).

General Procedure IX the Ph_2SO/Tf_2O-Promoted Glycosylations

A mixture of the donor (1.2 mmol), Ph_2SO (240 mg, 1.2 mmol) and TTBP (300 mg, 1.2 mmol) was co-evaporated with toluene (2 × 20 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (25 mL) and cooled to –60 °C. Tf_2O (0.22 ml, 1.32 mmol) was added and the reaction mixture was stirred for at –60 °C for 5 min, after which time a solution of the acceptor (1 mmol) in anhydrous CH_2Cl_2 (10 mL) was added (the acceptor was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h). The mixture was warmed to–40 °C over 2 h and Et_3N (0.5 ml) was added. The mixture was diluted with CH_2Cl_2 (50 ml) and washed with brine (2 × 15 ml), dried (Na_2SO_4), concentrated and purified by flash chromatography (toluene/EtOAc 50:1).

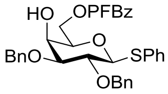
Phenyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 69



Prepared from phenyl 1-thio-α-L-rhamnopyranoside according to the synthetic sequence described in Chapter 2, which is similar to the one reported for methyl 1-thio-α-L-rhamnopyranoside.[146] The analytical data of **69** matched with previously reported.[147]

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.77 (m, 4H), 7.59 – 7.49 (m, 3H), 7.43 – 7.31 (m, 12H), 7.30 – 7.23 (m, 3H), 5.56 (d, *J* = 1.6 Hz, 1H), 5.05 (d, *J* = 10.8 Hz, 1H), 4.94 (d, *J* = 12.5 Hz, 1H), 4.86 (d, *J* = 12.5 Hz, 1H), 4.73 (d, *J* = 10.8 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.27 – 4.16 (m, 1H), 4.09 (dd, *J* = 3.0, 1.6 Hz, 1H), 3.91 (dd, *J* = 9.3, 3.0 Hz, 1H), 3.78 (t, *J* = 9.3 Hz, 1H), 1.43 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 138.2, 135.3, 134.5, 133.1, 133.00, 131.3, 128.9, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.2, 126.8, 126.1, 126.0, 125.9, 85.9, 80.5, 80.0, 76.4, 75.4, 72.2, 72.2, 69.4, 17.9.

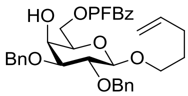
Phenyl 2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-1-thio-β-D-galactopyranoside 70



Prepared from commercially available D-galactose pentaacetate according to the literature procedure.[66] Its analytical data matched with those reported.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 – 7.52 (m, 2H), 7.47 – 7.29 (m, 10H), 7.27 – 7.21 (m, 3H), 4.88 (d, *J* = 10.3 Hz, 1H), 4.77 (d, *J* = 10.3 Hz, 1H), 4.75 – 4.66 (m, 3H), 4.66 (d, *J* = 9.7 Hz, 1H), 4.59 (dd, *J* = 11.6, 4.9 Hz, 1H), 4.04 (dd, *J* = 3.3, 1.0 Hz, 1H), 3.79 – 3.71 (m, 2H), 3.62 (dd, *J* = 8.9, 3.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.9, 137.4, 133.5, 131.9, 128.8, 128.6, 128.4, 128.2, 128.2, 127.9, 127.5, 87.9, 82.0, 76.8, 75.8, 75.3, 72.6, 66.7, 65.4.

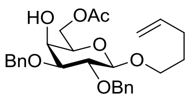
Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-1-thio-β-D-galactopyranoside 92



Prepared from commercially available D-galactose pentaacetate according to the literature procedure.[67] Its analytical data matched with those reported.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 – 7.28 (m, 10H), 5.91 – 5.75 (m, 1H), 5.07 – 4.92 (m, 3H), 4.82 – 4.72 (m, 1H), 4.69 (dd, *J* = 11.5, 7.3 Hz, 1H), 4.59 (dd, *J* = 11.5, 5.2 Hz, 1H), 4.38 (d, *J* = 7.7 Hz, 1H), 4.04 – 3.89 (m, 2H), 3.75 – 3.63 (m, 2H), 3.60 – 3.50 (m, 2H), 2.52 (dd, *J* = 2.2, 1.4 Hz, 1H), 2.24 – 2.12 (m, 2H), 1.85 – 1.70 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.7, 145.7, 143.3, 138.6, 138.1, 138.0, 137.8, 128.6, 128.4, 128.1, 128.0, 127.9, 127.7, 114.9, 108.0, 103.7, 80.3, 78.8, 75.3, 72.9, 71.7, 69.3, 66.9, 65.4, 30.3, 29.0.

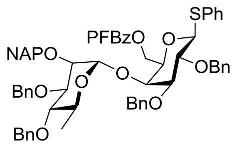
Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-acetyl-1-thio-β-D-galactopyranoside 93



Prepared from diol **91**[67] according to the literature procedure.[67] Its analytical data matched with those reported.

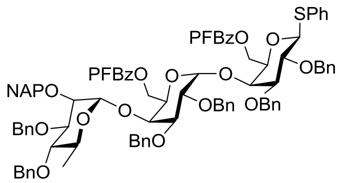
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42-7.28 (m, 10H), 6.82 (m, 1H), 5.03 – 4.92 (m, 3H), 4.76 (m, 1H), 4.75 (d, J = 11.1 Hz, 1H), 4.72 (d, J = 11.7 Hz, 1H), 4.38-4.30 (m, 3H), 3.96 (dt, J = 9.4, 6.4 Hz, 1H), 3.92 (bs, 1H), 3.65 (dd, J = 9.4, 7.7 Hz, 1H), 3.61-3.54 (m, 2H), 3.51 (dd, J = 9.4, 3.4 Hz, 1H), 2.49 (bs, 1H), 2.19 (m, 2H), 2.08 (s, 3H), 1.76 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 138.7, 138.2, 137.9, 128.6, 128.4 , 128.2, 128.1, 127.9, 127.8, 115.0, 103.8, 80.6, 78.9, 75.3, 72.8, 71.9, 69.5, 66.9, 63.2, 30.3, 29.1, 21.0.

Phenyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-1-thio-β-D-galactopyranoside 68



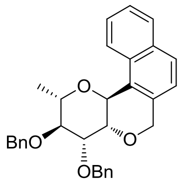
White foam, Rf 0.41 (toluene/EtOAc 25:1).
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 – 7.64 (m, 1H), 7.59 (m, 2H), 7.44 (dd, *J* = 20.5, 12.3 Hz, 5H), 7.35 – 7.09 (m, 23H), 7.05 (dd, *J* = 10.8, 3.9 Hz, 1H), 5.20 (d, *J* = 10.8 Hz, 1H), 4.82 (d, *J* = 10.9 Hz, 1H), 4.66 (d, *J* = 10.3 Hz, 1H), 4.61 – 4.42 (m, 10H), 4.34 (dd, *J* = 11.2, 5.1 Hz, 1H), 4.02 (s, 1H), 3.76 (qd, *J* = 12.0, 4.2 Hz, 3H), 3.64 (t, *J* = 6.1 Hz, 1H), 3.58 (t, *J* = 9.2 Hz, 1H), 3.51 – 3.37 (m, 2H), 1.23 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.6, 137.9, 137.8, 137.5, 137.2, 135.0, 133.2, 133.0, 132.9, 132.1, 128.8, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 126.7, 126.0, 125.9, 99.4, 88.0, 82.8, 80.0, 79.0, 77.2, 75.7, 75.5, 75.3, 73.8, 73.5, 72.7, 72.5, 69.4, 65.2, 17.6.

Trisaccharide by-product 84



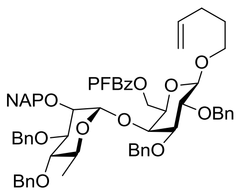
White foam, Rf 0.43 (toluene/EtOAc 25:1).
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 7.4 Hz, 1H), 7.61 – 7.52 (m, 2H), 7.48 – 7.43 (m, 3H), 7.40 – 7.05 (m, 36H), 5.27 (s, 1H), 5.19 (s, 1H), 4.84 – 4.78 (m, 2H), 4.75 – 4.68 (m, 4H), 4.67 – 4.59 (m, 4H), 4.58 – 4.51 (m, 2H), 4.50 – 4.38 (m, 6H), 4.30 (t, *J* = 6.6 Hz, 1H), 4.20 (dd, *J* = 10.7, 7.5 Hz, 1H), 4.15 – 4.09 (m, 2H), 3.95 (dd, *J* = 10.2, 2.3 Hz, 1H), 3.89 (d, *J* = 2.3 Hz, 1H), 3.85 (d, *J* = 1.9 Hz, 1H), 3.80 – 3.69 (m, 2H), 3.64 (dd, *J* = 12.2, 6.8 Hz, 1H), 3.59 (dd, *J* = 11.7, 7.4 Hz, 2H), 3.39 (dd, *J* = 9.4, 2.5 Hz, 1H), 1.17 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.8, 137.7, 137.5, 137.4, 137.2, 135.1, 133.2, 133.0, 132.9, 132.1, 128.8, 128.7, 128.4, 128.4, 128.3, 128.3, 128.3, 128.0, 127.9, 127.9, 127.7, 127.6, 127.6, 127.2, 126.5, 126.0, 125.8, 125.8, 99.9, 99.4, 88.1, 81.3, 79.9, 79.2, 77.9, 77.1, 75.9, 75.7, 75.5, 75.4, 75.0, 74.9, 74.3, 73.5, 72.8, 72.2, 69.4, 68.2, 64.4, 64.1, 17.5.

Cyclization by-product 85



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, *J* = 8.4 Hz, 1H), 7.73 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.50 – 7.37 (m, 2H), 7.37 – 7.20 (m, 10H), 7.01 (d, *J* = 8.5 Hz, 1H), 5.70 (d, *J* = 9.4 Hz, 1H), 5.10 (dd, *J* = 15.2, 1.6 Hz, 1H), 4.88 (d, *J* = 12.2 Hz, 2H), 4.64 (d, *J* = 12.2 Hz, 1H), 4.51 (d, *J* = 12.2 Hz, 1H), 4.43 (d, *J* = 12.2 Hz, 1H), 4.39 (bq, *J* = 7.2 Hz, 1H), 4.17 – 4.13 (m, 1H), 4.10 (dd, *J* = 9.4, 2.6 Hz, 1H), 3.53 (dd, *J* = 3.1, 0.5 Hz, 1H), 1.73 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 137.8, 133.8, 132.9, 131.9, 129.26, 128.3, 128.3, 128.2, 128.2, 127.6, 127.5, 127.5, 127.5, 126.0, 125.8, 125.2, 121.9, 78.2, 76.3, 75.9, 73.3, 73.0, 71.4, 69.8, 61.8, 16.4.

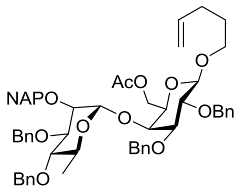
Pent-4-enyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-β-D-galactopyranoside 83



Prepared from **69** and **92** according to the General Procedure I. Colorless foam, 78% yield. Rf 0.47 (toluene/EtOAc 10:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.0 Hz, 1H), 7.67 (t, *J* = 8.0 Hz, 2H), 7.61 (bs, 1H), 7.45 – 7.38 (m, 3H), 7.39 – 7.22 (m, 20H), 5.86 – 5.77 (m, 1H), 5.32 (s, 1H), 5.01 (d, *J* = 17.1 Hz, 1H), 4.96 (d, *J* = 10.0 Hz, 1H), 4.92 (d, *J* = 11.0 Hz, 1H), 4.86 (d, *J* = 11.2 Hz, 1H), 4.74 – 4.53 (m, 8H), 4.52 – 4.45 (m, 1H), 4.34 (d, *J* = 7.5 Hz, 1H), 4.08 (d, *J* = 1.8 Hz, 1H), 3.96 – 3.87 (m, 3H), 3.82 – 3.75 (m, 1H), 3.69 (bt, *J* = 6.2 Hz, 1H), 3.63 (bt, *J* = 9.1 Hz, 1H), 3.56 – 3.48 (m, 3H), 2.19 – 2.12 (m, 2H), 1.84 – 1.68 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3, 146.9, 144.8, 143.6, 141.3, 139.1, 138.4, 138.4, 138.3, 137.8, 137.7, 135.8, 132.9, 132.7, 128.3, 128.1, 127.9, 127.8, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 126.2, 125.8, 125.7, 125.5, 114.7, 107.5, 103.7, 99.7, 81.1, 80.1, 79.2, 78.7, 75.3, 74.9, 74.7, 73.6, 73.5, 72.2, 72.1, 71.5, 69.2, 69.1, 65.2, 30.0, 28.7, 17.9; $[\alpha]_D^{22}$ +14.5 (c 1.3, $CHCl_3$); IR (neat) 1741 cm^{-1} (C=O). *m/z* (MALDI-TOF MS) Calcd for $C_{63}H_{61}F_5O_{11}Na$ $[M+Na]^+$: 1111.40; Found: 1111.44.

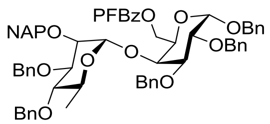
Pent-4-enyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside 94



Prepared from **69** and **93** according to the General Procedure I. Colorless foam, 45% yield. Rf 0.29 (toluene/EtOAc 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 – 7.46 (m, 4H), 7.33 – 7.10 (m, 23H), 5.79 – 5.63 (m, 1H), 5.27 (s, 1H), 4.97 – 4.92 (m, 1H), 4.91 – 4.86 (m, 1H), 4.83 (d, *J* = 11.1 Hz, 1H), 4.75 (d, *J* = 11.1 Hz, 1H), 4.64 – 4.38 (m, 8H), 4.22 (d, *J* = 7.2 Hz, 1H), 4.18 (d, *J* = 6.6 Hz, 1H), 4.09 (dd, *J* = 11.1, 6.2 Hz, 1H), 3.99 (d, *J* = 1.6 Hz, 1H), 3.78 (s, 2H), 3.59 – 3.33 (m, 6H), 2.12 – 2.01 (m, 2H), 1.96 (s, 3H), 1.71 – 1.60 (m, 2H), 1.25 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 138.5, 138.3, 137.8, 137.7, 135.8, 133.0, 132.7, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 127.4, 127.4, 127.3, 126.3, 125.8, 125.7, 125.5, 114.8, 103.8, 99.2, 81.5, 80.1, 79.3, 78.8, 75.2, 74.9, 74.8, 73.7, 72.5, 72.1, 71.6, 69.4, 68.8, 30.0, 28.7, 20.7, 17.9.

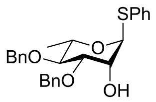
Benzyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranoside 98



Pentenyl glycoside **83** (4.01 g, 3.68 mmol) was co-evaporated with toluene (2 × 30 ml) and subjected to high vacuum for 2 h. The compound was dissolved in anhydrous CH_2Cl_2 (30 mL), preactivated 4 Å MS (2 g) were added and the mixture was stirred at room temperature for 20 min, cooled to 0 °C, and titrated with a 1 M solution of Br_2 in CH_2Cl_2 until a faint yellow color persisted. The solution was warmed to room temperature, followed by addition of BnOH (0.76 ml, 7.36 mmol) and TBABr (5.93 g, 18.4 mmol). The mixture was stirred for 24 h, filtered through Celite, concentrated and purified by flash chromatography (toluene/EtOAc 40:1) to furnish **7** as white foam (3.21 g, 90 %). Rf 0.46 (toluene/EtOAc 10:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.3 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 2H), 7.55 (s, 1H), 7.43 – 7.22 (m, 24H), 7.15 (d, *J* = 1.9 Hz, 4H), 5.32 (d, *J* = 1.5 Hz, 1H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.86 – 4.82 (m, 2H), 4.74 – 4.68 (m, 3H), 4.66 – 4.59 (m, 3H), 4.58 – 4.52 (m, 3H), 4.51 – 4.42 (m, 4H), 4.19 (s, 1H), 4.14 (t, *J* = 6.1 Hz, 1H), 4.00 (dd, *J* = 10.0, 2.7 Hz, 1H), 3.89 – 3.85 (m, 1H), 3.81 – 3.71 (m, 3H), 3.62 (t, *J* = 9.2 Hz, 1H), 1.32 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.4, 147.1, 143.7, 138.5, 138.4, 138.0, 137.7, 136.8, 135.8, 133.0, 132.8, 128.9, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.5, 127.5, 127.4, 127.3, 126.2, 125.8, 125.7, 125.6, 125.2, 99.7, 95.5, 80.0, 79.4, 78.1, 75.6, 75.1, 73.9, 72.7, 71.9, 71.8, 69.2, 68.8, 68.2, 65.8, 18.0; $[\alpha]_D^{22}$ +37.6 (c 1.4, $CHCl_3$); IR (neat) 1740 cm^{-1} (C=O). *m*/*z* (MALDI-TOF MS) Calcd for $C_{65}H_{59}F_5O_{11}Na$ [M+Na]$^+$: 1133.39; Found: 1111.39.

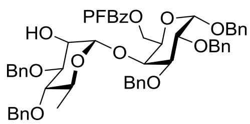
Phenyl 3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside 99



Prepared from **69** according to the General Procedure II. White foam, 75% yield. Its analytical data matched with trhose reported.[110]

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 – 7.50 (m, 2H), 7.25 – 7.45 (m, 13H), 5.60 (d, J = 1.5 Hz, 1H), 4.97 (d, J = 11.0 Hz, 1H), 4.76 (s, 2H), 4.70 (d, J = 10.8 Hz, 1H), 4.23 – 4.32 (m, 2H), 3.92 (dd, J = 9.1, 3.2 Hz, 1H), 3.61 (t, J = 9.3 Hz, 1H), 2.93 (d, J = 1.8 Hz, 1H), 1.38 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 137.9, 134.3, 131.4, 129.1, 128.8, 128.6, 128.2, 128.0, 127.9, 127.5, 87.2, 80.5, 80.2, 75.5, 72.3, 70.3, 69.0, 17.9.

Benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranoside 95

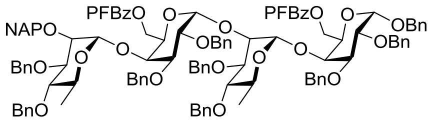


Prepared from **98** according to the General Procedure II. White foam, 74% yield. Rf 0.21 (toluene/EtOAc 10:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 25H), 5.15 (d, *J* = 1.7 Hz, 1H), 4.90 (d, *J* = 3.2 Hz, 1H), 4.86 (d, *J* = 11.5 Hz, 1H), 4.81 (d, *J* = 11.5 Hz, 1H), 4.73 (d, *J* = 11.5 Hz, 1H), 4.72 (d, *J* = 12.2 Hz, 1H), 4.66 (d, *J* = 12.2 Hz, 1H), 4.61 – 4.53 (m, 5H), 4.44 (d, *J* = 6.3 Hz, 2H), 4.18 – 4.16 (m, 1H), 4.13 (t, *J* = 6.2 Hz, 1H), 4.10 (s, 1H), 3.99 (dd, *J* = 10.0, 2.8 Hz, 1H), 3.83 – 3.75 (m, 3H), 3.44 (t, *J* = 9.0 Hz, 1H), 1.29 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.4, 147.1, 143.7, 138.2, 138.1, 137.9, 136.8, 128.8, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 125.1, 101.7, 95.5, 79.7, 79.3, 77.6, 76.0, 75.7, 74.8, 73.5, 72.8, 71.9, 68.8, 68.6, 68.5, 67.9, 65.7, 17.7; $[\alpha]_D^{22}$ +28.1 (c 1.1, $CHCl_3$); IR (neat)

1739 cm^{-1} (C=O). *m/z* (HRMS) Calcd for $C_{54}H_{51}F_5O_{11}Na$ [M+Na]$^+$: 993.3249; Found: 993.3249.

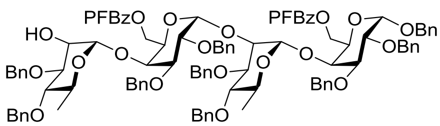
Benzyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-O-pentafluorobenzoyl-α-D-galactopyranosyl-(1→2)-2,3-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranoside 100



Prepared from **83** and **95** according to the General Procedure I. White foam, 71% yield. Rf 0.47 (toluene/EtOAc 10:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.6 Hz, 1H), 7.72 – 7.66 (m, 2H), 7.62 (s, 1H), 7.47 – 7.00 (m, 48H), 5.24 (s, 1H), 5.14 (s, 1H), 4.98 – 4.85 (m, 3H), 4.80 (d, *J* = 10.8 Hz, 1H), 4.74 – 4.26 (m, 22H), 4.23 – 4.17 (m, 2H), 4.14 – 4.09 (m, 1H), 4.08 (s, 2H), 4.01 (s, 1H), 3.98 – 3.92 (m, 2H), 3.91 – 3.86 (m, 2H), 3.85 – 3.73 (m, 3H), 3.65 (t, *J* = 9.3 Hz, 1H), 3.55 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.49 (t, *J* = 9.4 Hz, 1H), 1.32 (d, *J* = 6.1 Hz, 3H), 1.29 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3, 157.9, 147.2, 143.8, 138.6, 138.5, 138.4, 138.3, 138.2, 138.09, 138.1, 138.0, 136.8, 135.9, 133.0, 132.7, 128.8, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.4, 127.3, 127.1, 126.9, 126.5, 126.2, 125.8, 125.8, 125.6, 125.1, 100.1, 98.9, 95.4, 95.1, 80.2, 79.9, 79.7, 78.2, 77.8, 76.9, 76.3, 75.9, 75.7, 75.6, 75.2, 75.0, 73.9, 73.3, 72.8, 72.7, 72.0, 71.8, 71.5, 71.4, 69.2, 69.1, 68.8, 68.0, 67.4, 65.6, 64.7, 17.9; $[\alpha]_D^{22}$ +60.8 (c 1.1, $CHCl_3$); IR (neat) 1740 cm^{-1} (C=O). *m/z* (MALDI-TOF MS) Calcd for $C_{112}H_{102}F_{10}O_{21}Na$ [M+Na]$^+$: 1995.66; Found: 1996.58.

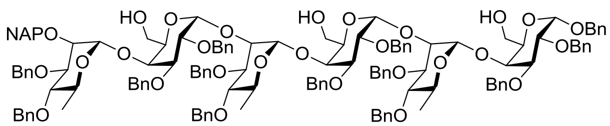
Benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranosyl-(1→2)-2,3-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranoside 101



Prepared from **100** according to the General Procedure II. White foam, 76% yield. Rf 0.20 (toluene/EtOAc 10:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.05 (m, 45H), 5.13 (s, 1H), 5.01 (d, *J* = 1.7 Hz, 1H), 4.90 – 4.83 (m, 2H), 4.83 – 4.76 (m, 2H), 4.71 – 4.48 (m, 18H), 4.44 – 4.29 (m, 2H), 4.21 (t, *J* = 9.0 Hz, 2H), 4.16 (s, 1H), 4.13 (dd, *J* = 10.7, 5.9 Hz, 1H), 4.10 – 4.06 (m, 1H), 4.04 (s, 1H), 3.99 (s, 1H), 3.95 – 3.86 (m, 2H), 3.83 – 3.71 (m, 4H), 3.55 (dd, *J* = 10.0, 3.5 Hz, 1H), 3.47 (t, *J* = 9.4 Hz, 1H), 3.40 (t, *J* = 9.0 Hz, 1H), 2.34 (s, 1H), 1.30 (d, *J* = 6.1 Hz, 3H), 1.21 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3, 157.9, 147.2, 144.8, 143.7, 141.4, 139.1, 138.6, 138.3, 138.2, 138.2, 138.1, 138.1, 138.0, 137.9, 137.7, 137.59, 136.8, 135.7, 128.8, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 127.0, 126.9, 126.4, 125.1, 107.3, 102.1, 99.0, 95.4, 95.1, 80.0, 79.9, 79.3, 78.2, 77.7, 76.5, 76.4, 76.2, 75.7, 75.0, 74.9, 73.8, 72.8, 72.7, 71.6, 71.5, 71.4, 69.2, 68.8, 68.5, 67.9, 67.1, 65.6, 64.5, 17.8, 17.7; $[\alpha]_D^{22}$ +55.4 (c 1.0, $CHCl_3$); IR (neat) 1740 cm^{-1} (C=O). *m*/*z* (MALDI-TOF MS) Calcd for $C_{101}H_{94}F_{10}O_{21}Na$ $[M+Na]^+$: 1855.60; Found: 1856.47.

Benzyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→2)-2,3-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→2)-2,3-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranoside 67

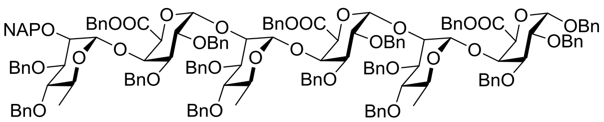


Compounds **83** and **101** were subjected to the glycosylation conditions according to the General Procedure I. The crude product was filtered through a plug of silica gel, the filtrate was evaporated and dissolved in MeOH/THF 2:1 (30 ml). Na (100 mg, 4.3 mmol) was added and the reaction mixture was stirred at room temperature until TLC revealed disappearance of the starting material (4 h). The reaction was quenched with Amberlite IR-120 H^+ (10 ml), the resin was filtered off, and the filtrate was concentrated and purified by flash chromatography (toluene/EtOAc 6:1) to furnish **2** as a white foam (580 mg, 40 % over 2 steps). Rf 0.57 (toluene/EtOAc 3:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.4 Hz, 2H), 7.56 (s, 1H), 7.43 – 7.07 (m, 68H), 5.24 (s, 1H), 5.06 (s, 1H), 5.02 (s, 1H), 4.91 (d, *J* = 10.9 Hz, 1H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.85 – 4.78 (m, 3H), 4.76 – 4.40 (m, 24H), 4.34

(d, J = 11.8 Hz, 1H), 4.33 (d, J = 11.6 Hz, 1H), 4.17 (s, 1H), 4.10 (s, 1H), 4.01 (d, J = 8.6 Hz, 3H), 3.97 – 3.93 (m, 1H), 3.93 – 3.71 (m, 14H), 3.67 – 3.38 (m, 10H), 1.32 (d, J = 6.1 Hz, 3H), 1.30 (d, J = 6.2 Hz, 3H), 1.27 (d, J = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.7, 138.6, 138.5, 138.4, 138.4, 138.4, 138.3, 138.2, 138.1, 138.0, 137.1, 135.8, 133.1, 132.8, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 127.9, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.3, 127.2, 127.1, 126.3, 125.9, 125.8, 125.6, 99.8, 99.5, 99.1, 95.8, 95.0, 94.8, 80.1, 79.8, 79.5, 78.7, 78.5, 77.8, 76.2, 76.1, 76.1, 75.5, 75.2, 75.2, 75.1, 74.9, 74.9, 73.8, 73.3, 73.0, 72.6, 72.5, 72.4, 71.9, 71.8, 71.7, 71.5, 70.5, 70.1, 69.6, 69.4, 69.2, 69.1, 61.8, 61.6, 61.5, 18.0; $[\alpha]_D^{22}$ +95.9 (c 0.9, $CHCl_3$). *m/z* (MALDI-TOF MS) Calcd for $C_{138}H_{148}O_{28}Na$ [M+Na]$^+$: 2276.01; Found: 2276.81.

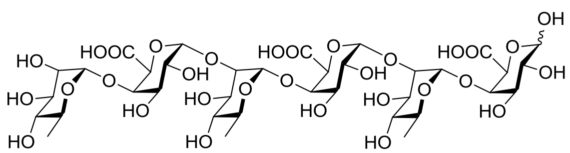
Benzyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→2)-2,3-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→2)-2,3-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosiduronate) 102



To a suspension of the Dess-Martin periodinane (210 mg, 0.49 mmol) in anhydrous CH_2Cl_2 (5 mL) was added a solution of **67** (250 mg, 0.11 mmol) in CH_2Cl_2 (7 mL). The reaction was stirred for 1 h, then diluted with Et_2O (25 mL), quenched with 10% aq. $Na_2S_2O_3$ (25 mL), and stirred for 30 min. The organic phase was separated and washed with sat. $NaHCO_3$ (20 ml). The combined aqueous phases were extracted with Et_2O (2 × 20 ml), dried (Na_2SO_4) and concentrated. The crude aldehyde was dissolved in THF (2.5 mL) followed by addition of *t*BuOH (5 mL), 2-methyl-but-2-ene (1.6 ml, 15 mmol), and a solution of $NaClO_2$ (270 mg, 3.0 mmol) and $NaH_2PO_4{\cdot}H_2O$ (310 mg, 2.25 mmol) in H_2O (2.5 mL). The reaction was stirred at room temperature until TLC (toluene/EtOAc 5:1) showed full conversion (2 h). The mixture was partially concentrated and acidified with 1 M aq. HCl. The aqueous phase was extracted with EtOAc (3 × 30 ml). The combined organic phases were dried with Na_2SO_4, filtered and concentrated to afford the crude acid. Rf 0.41 (CH_2Cl_2/MeOH 95:5). The crude acid was dissolved in EtOAc (6 mL) and titrated with $PhCHN_2$[113] (0.5 M sol. in Et_2O) until TLC (toluene/EtOAc 10:1) showed full conversion (2 h). *Note: $PhCHN_2$ is potentially explosive and may burn violently when exposed to air.* The reaction mixture was quenched with AcOH/EtOAc, concentrated and purified by flash chromatography (toluene/EtOAc 20:1) to furnish **11** as white foam (150 mg, 60 % over 3 steps). Rf 0.45 (toluene/EtOAc 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 – 7.71 (m, 1H), 7.65 – 7.58 (m, 2H), 7.54 (s, 1H), 7.34 – 7.03 (m, 83H), 5.33 (s, 2H), 5.29 (s, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 5.00 – 4.07 (m, 36H), 4.00 – 3.51 (m, 21H), 3.41 – 3.25 (m, 3H), 1.25 – 1.19 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 168.3, 168.1, 138.9, 138.7, 138.7, 138.5, 138.4, 138.3, 138.0, 137.9, 137.9, 137.8, 136.9, 135.9, 134.8, 133.1, 132.8, 128.9, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.2, 127.2, 127.1, 127.0, 126.9, 126.3, 125.9, 125.7, 125.2, 98.8, 97.9, 97.8, 96.7, 96.6, 96.1, 80.2, 79.8, 79.7, 78.9, 78.7, 77.8, 77.3, 77.2, 76.8, 75.4, 74.9, 74.9, 74.7, 74.7, 74.7, 74.5, 74.3, 74.0, 73.9, 73.6, 73.4, 73.2, 72.9, 72.6, 72.0, 72.0, 71.8, 71.6, 70.6, 70.3, 70.1, 68.6, 67.2, 67.1, 65.2, 18.2; $[\alpha]_D^{22}$ +53.3 (c 0.5, $CHCl_3$); IR (neat) 1732 cm^{-1} (C=O). *m/z* (MALDI-TOF MS) Calcd for $C_{159}H_{160}O_{31}Na$ $[M+Na]^+$: 2588.08; Found: 2590.04.

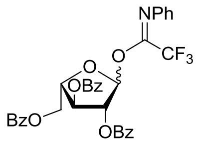
α-L-rhamnopyranosyl-(1→4)-(α-D-galactopyranosyluronic acid)-(1→2)-α-L-rhamnopyranosyl-(1→4)-(α-D-galactopyranosyluronic acid)-(1→2)-α-L-rhamnopyranosyl-(1→4)-D-galactopyranosiduronic acid 66



Compound **102** (150 mg, 0.058 mmol) was dissolved in MeOH/THF 3:1 (20 mL), 10% Pd/C (125 mg) was added, and stirred under an atmosphere of H_2 (1 atm) for 3 h, followed by addition of H_2O (5 mL). The reaction mixture was stirred at room temperature for 24 h, then another portion of 10% Pd/C (50 mg) was added, and the reaction mixture was stirred for additional 24 h, filtered through Celite and lyophilized yielding the

crude hexasaccharide **1**. The compound was purified on C18 silica column (eluent H_2O) and lyophilized to furnish **1** as white foam (54 mg, 95 %).
$[\alpha]_D^{22}$ +33.2 (c 0.4, H_2O). IR (neat) broad 3300 cm^{-1}, 1605 cm^{-1}. *m/z* (MALDI-TOF MS) Calcd for $C_{36}H_{56}O_{31}Na$ $[M+Na]^+$: 1007.27; Found: 1007.13.

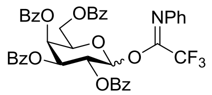
2,3,5-Tri-*O*-benzoyl-L-arabinofuranosyl *N*-phenyl trifluoroacetimidate 110



Prepared from hemiacetal **115**[117] according to the General Procedure V. White foam, 75% yield. Rf 0.29 (heptane/EtOAc 3:1).

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 165.9, 165.6, 165.4, 165.3, 164.9, 143.2, 143.1, 133.7, 133.7, 133.6, 133.0, 129.8, 129.8, 129.7, 129.6, 129.4, 129.4, 129.1, 128.6, 128.5, 128.4, 128.4, 128.2, 124.4, 124.1, 120.4, 119.4, 119.1, 102.1, 96.7, 84.1, 80.6, 80.4, 76.9, 76.0, 75.4, 64.7, 63.4.

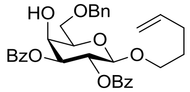
2,3,4,6-Tetra-*O*-benzoyl-D-galactopyranosyl *N*-phenyl trifluoroacetimidate 112



Prepared from hemiacetal **118**[119] according to the General Procedure V. White foam, 85% yield. Its analytical data matched with those reported.[148]

^1H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 7.3 Hz, 2 H), 8.01 (d,J = 7.1 Hz, 2 H), 8.02 (d, J = 7.3 Hz, 2 H), 7.78 (d, J = 7.3 Hz, 2 H),7.62–7.34 (m, 10 H), 7.26 (t, J = 7.7 Hz, 2 H), 7.10 (t, J = 7.1 Hz, 2H), 7.02 (t, J = 7.3 Hz, 1 H), 6.86 (bs, 1 H), 6.43 (d, J = 6.6 Hz, 2H), 6.17 (d, J = 2.2 Hz, 1 H), 6.04 (dd, J = 10.5, 3.0 Hz, 1 H), 5.92(dd, J = 10.5, 3.3 Hz, 1 H), 4.81 (m, 1 H), 4.63 (dd, J = 11.2, 6.9 Hz,1 H), 4.40 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 165.4, 165.5, 164.9, 142.94, 133.7, 133.5, 133.4, 133.2, 130.0, 129.9, 129.8, 129.7, 129.7, 129.2, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 124.5, 119.1, 95.1, 72.6, 71.3, 68.7, 67.8, 62.1.

Pent-4-enyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside 113

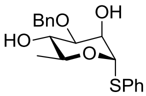


Prepared from **119** according to the literature procedure.[149] White foam, 82% yield. Rf 0.20 (toluene/EtOAc 10:1).

^1H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.83 (m, 4H), 7.39 – 7.29 (m, 2H), 7.27 – 7.13 (m, 9H), 5.70 (dd, *J* = 10.3, 7.9 Hz, 1H), 5.61 – 5.48 (m, 1H), 5.22 (dd, *J* = 10.3, 3.1 Hz, 1H), 4.73 (t, *J* = 1.3 Hz, 1H), 4.72 – 4.67 (m, 1H), 4.61 (d, *J* =

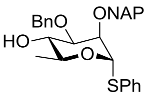
7.9 Hz, 1H), 4.48 (s, 2H), 4.25 (d, *J* = 3.0 Hz, 1H), 3.88 – 3.80 (m, 1H), 3.73 – 3.67 (m, 2H), 3.44 (dt, *J* = 9.7, 6.7 Hz, 1H), 2.85 (s, 1H), 1.97 – 1.77 (m, 2H), 1.62 – 1.40 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 165.2, 137.7, 137.5, 133.1, 132.8, 129.6, 129.5, 129.0, 128.3, 128.2, 128.1, 127.6, 127.6, 114.6, 101.3, 74.4, 73.5, 73.2, 69.6, 69.2, 68.9, 67.8, 29.6, 28.4.

Phenyl 3-*O*-benzyl-1-thio-α-L-rhamnopyranoside 120



Prepared from **73** according to the literature procedure.[150] White solid, 55% yield. Its analytical data matched with those reported.[110] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 – 7.50 (m, 2H), 7.22 – 7.43 (m, 8H), 5.53 (s, 1H), 4.72 (d, J = 11.6 Hz, 1H), 4.60 (d, J = 11.6 Hz, 1H), 4.23 – 4.26 (m, 1H), 4.12 – 4.20 (m, 1H), 3.62 – 3.69 (m, 2H), 2.68 (s, 1H), 2.36 (s, 1H), 1.32 (d, J = 6.2 Hz, 3H); ^{13}C NMR δ 137.3, 134.2, 131.3, 129.0, 128.8, 128.3, 128.2, 127.4, 87.5, 79.9, 72.0, 71.9, 69.7, 69.3, 17.8.

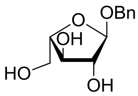
Phenyl 3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 108



Prepared from **120** according to the procedure described below (See Screening of the Reaction Conditions, Table 6, entry 2). Rf 0.22 (toluene/EtOAc 20:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (m, 4H), 7.59 – 7.26 (m, 11H), 5.64 (s, 1H), 4.90 (d, *J* = 12.4 Hz, 1H), 4.74 (d, *J* = 12.4 Hz, 1H), 4.59 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 11.7 Hz, 1H), 4.27 – 4.14 (m, 1H), 4.14 – 4.09 (m, 1H), 3.92 (t, *J* = 9.4 Hz, 1H), 3.72 (dd, *J* = 9.5, 3.0 Hz, 1H), 2.64 (s, 1H), 1.44 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.5, 135.0, 134.4, 133.0, 132.9, 131.1, 128.9, 128.4, 128.1, 127.8, 127.8, 127.5, 127.2, 126.7, 126.0, 125.8, 85.7, 79.5, 75.4, 71.9, 71.7, 71.4, 69.6, 17.6.

Benzyl α-L-arabinofuranoside 123

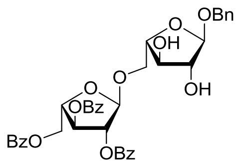


Prepared from **110** according to the literature procedure.[151] Its analytical data matched with those reported.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.38 – 7.27 (m, 5H), 5.34 (d, *J* = 5.3 Hz, 1H), 5.15 (d, *J* = 5.4 Hz, 1H), 4.82 (d, *J* = 1.9 Hz, 1H), 4.76 (t, *J* = 5.7 Hz, 1H), 4.65 (d, *J* = 12.1 Hz, 1H), 4.44 (d, *J* = 12.1 Hz, 1H), 3.88 – 3.83 (m, 1H), 3.82 – 3.76

(m, 1H), 3.70 – 3.55 (m, 2H), 3.49 – 3.40 (m, 1H), 3.37 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 138.1, 128.2, 127.7, 127.4, 107.2, 84.0, 82.2, 77.2, 68.2, 61.3.

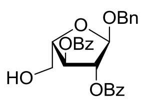
Benzyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranoside 124



Prepared from **110** and **123** according to the General Procedure VII. Its analytical data matched with those reported.[151]

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 – 8.04 (m, 2H), 8.00 – 7.87 (m, 6H), 7.56 – 7.16 (m, 14H), 5.54 (d, J = 4.9 Hz, 1H), 5.42 (d, J = 1.2 Hz, 1H), 5.31 (s, 1H), 5.06 (s, 1H), 4.79 (dd, J = 12.0, 3.3 Hz, 1H), 4.73 – 4.57 (m, 2H), 4.55 – 4.50 (m, 1H), 4.49 (s, 1H), 4.45 (s, 1H), 4.20 (m, 1H), 4.07 – 4.03 (m, 1H), 4.01 (s, 2H), 3.77 (dd, J = 11.0, 2.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 165.8, 165.3, 136.8, 133.6, 133.0, 130.1, 129.8, 129.7, 129.5, 128.6, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 107.0, 106.1, 85.8, 81.9, 79.2, 78.0, 77.4, 69.0, 66.9, 63.4.

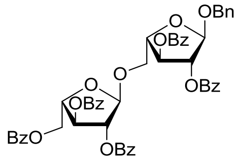
Benzyl 2,3-di-*O*-benzoyl α-L-arabinofuranoside 127



Prepared from **123** according to the literature procedure.[139] Rf 0.21 (toluene/EtOAc 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 – 7.87 (m, 4H), 7.60 – 7.46 (m, 2H), 7.45 – 7.01 (m, 7H), 5.53 (d, *J* = 1.4 Hz, 2H), 5.40 – 5.35 (m, 1H), 5.26 (s, 1H), 4.77 (d, *J* = 11.9 Hz, 1H), 4.54 (d, *J* = 11.9 Hz, 1H), 4.27 (m, 1H), 3.92 (t, *J* = 4.0 Hz, 2H), 2.15 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.14, 165.23, 137.23, 133.53, 133.50, 129.91, 129.80, 129.07, 129.00, 128.49, 128.43, 128.37, 127.72, 127.67, 104.67, 83.93, 81.68, 77.76, 68.65, 62.34.

Benzyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranoside 125

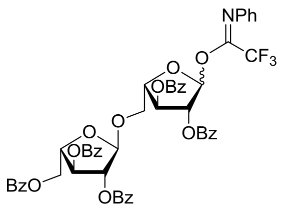


Prepared from **110** and **127** according to the General Procedure VI. Its analytical data matched with those reported.[151]

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.84 (m, 10H), 7.53 – 7.05 (m, 20H), 5.61 – 5.48 (m, 4H), 5.38 (s, 1H), 5.26 (s, 1H), 4.77 (dd, *J* = 11.8, 3.3

Hz, 2H), 4.71 – 4.65 (m, 1H), 4.59 (dd, *J* = 11.7, 4.6 Hz, 1H), 4.52 (d, *J* = 12.0 Hz, 1H), 4.46 – 4.39 (m, 1H), 4.17 (dd, *J* = 11.2, 4.7 Hz, 1H), 3.91 (dd, *J* = 11.2, 2.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 165.7, 165.6, 165.3, 165.2, 137.3, 133.4, 133.3, 133.2, 132.9, 129.8, 129.8, 129.7, 129.7, 129.7, 129.1, 129.0, 128.9, 128.8, 128.4, 128.3, 128.3, 128.2, 128.2, 127.6, 105.7, 104.7, 82.0, 81.8, 81.7, 81.2, 77.7, 77.2, 68.5, 66.1, 63.6.

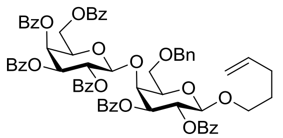
2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-L-arabinofuranosyl *N*-phenyl trifluoroacetimidate 109



Prepared from hemiacetal **128**[151] according to the General Procedure V. White foam, 87%. Rf 0.56 (toluene/EtOAc 10:1).

^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 165.6, 165.4, 165.3, 165.3, 165.2, 165.0, 165.0, 164.7, 143.0, 133.5, 133.4, 133.2, 133.1, 132.8, 129.7, 129.6, 129.5, 128.9, 128.8, 128.5, 128.4, 128.3, 128.3, 128.1, 128.0, 123.9, 119.0, 105.6, 96.6, 81.8, 81.2, 81.0, 77.6, 76.0, 74.7, 67.2, 63.4, 60.0.

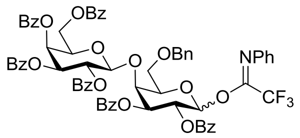
Pent-4-enyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranoside 129



Prepared from **112** and **113** according to the General Procedure VI. White foam, 76%. Rf 0.35 (toluene/EtOAc 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 – 7.93 (m, 4H), 7.91 – 7.69 (m, 8H), 7.49 – 7.12 (m, 23H), 5.79 (dd, J = 6.9, 3.1 Hz, 1H), 5.58 – 5.45 (m, 1H), 5.43 – 5.28 (m, 2H), 4.98 (d, J = 7.9 Hz, 1H), 4.74 (d, J = 1.1 Hz, 1H), 4.69 (dd, J = 9.2, 1.4 Hz, 1H), 4.58 (d, J = 7.5 Hz, 1H), 4.54 (d, J = 5.6 Hz, 1H), 4.47 (d, J = 2.8 Hz, 1H), 4.32 (dd, J = 11.2, 6.5 Hz, 1H), 4.22 (dd, J = 11.3, 6.6 Hz, 1H), 3.94 (t, J = 6.5 Hz, 1H), 3.88 – 3.74 (m, 3H), 3.47 – 3.34 (m, 1H), 1.89 – 1.78 (m, 1H), 1.56 – 1.40 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 166.6, 166.4, 166.2, 165.3, 138.8, 138.8, 134.4, 134.1, 133.6, 130.8, 130.7, 130.6, 130.5, 130.5, 130.5, 130.4, 130.3, 130.0, 129.8, 129.5, 129.4, 129.2, 129.1, 129.0, 129.0, 128.5, 128.5, 115.4, 102.0, 101.7, 74.9, 74.6, 74.5, 74.0, 72.6, 72.0, 70.9, 70.4, 70.3, 69.3, 68.9, 62.5, 30.6, 29.3.

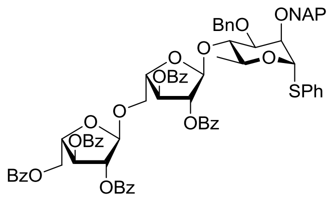
2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-D-galactopyranosyl *N*-phenyl trifluoroacetimidate 132



Hemiacetal **131** was prepared from pentenyl glycoside **129** as described in literature.[119] Compound **131** was converted into **132** according to the General Procedure V. White foam, 85%. Rf 0.47 (toluene/EtOAc 10:1).

^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 165.5, 165.4, 165.2, 164.0, 142.9, 137.7, 133.6, 133.5, 133.2, 133.1, 133.0, 132.8, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.0, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 128.1, 127.6, 127.5, 124.1, 119.0, 100.7, 95.0, 74.9, 73.5, 73.4, 72.5, 71.5, 71.2, 69.9, 69.0, 68.8, 67.9, 61.6.

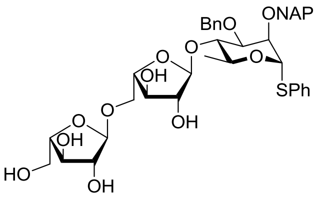
Phenyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 107



Prepared from **109** and **108** according to the General Procedure VI. White foam, 84%. Rf 0.40 (toluene/EtOAc 20:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 – 7.81 (m, 10H), 7.66 – 7.61 (m, 1H), 7.57 (m, 3H), 7.46 – 7.06 (m, 24H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.98 – 6.90 (m, 3H), 5.82 (s, 1H), 5.55 (bs, 1H), 5.50 (s, 1H), 5.47 (d, *J* = 4.7 Hz, 1H), 5.40 (bs, 1H), 5.35 (s, 1H), 4.75 – 4.57 (m, 4H), 4.56 – 4.36 (m, 4H), 4.15 – 3.97 (m, 3H), 3.92 – 3.76 (m, 3H), 1.29 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 165.6, 165.4, 165.0, 164.9, 137.5, 135.1, 134.5, 133.3, 133.2, 133.1, 132.9, 132.8, 131.0, 129.7, 129.6, 129.6, 129.5, 129.1, 128.9, 128.8, 128.7, 128.3, 128.2, 128.1, 127.7, 127.7, 127.5, 127.4, 127.1, 126.6, 125.9, 125.8, 125.8, 106.6, 105.9, 85.9, 82.5, 81.8, 81.5, 81.1, 80.2, 77.6, 77.6, 75.9, 75.5, 72.2, 71.8, 68.7, 66.4, 63.5, 18.0.

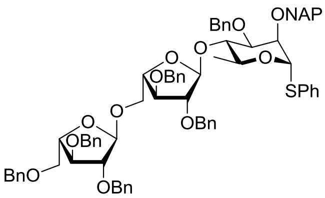
Phenyl α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 133



Prepared from **107** according to the General Procedure III. White foam, 87%. Rf 0.37 (CH_2Cl_2/MeOH 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 – 7.60 (m, 3H), 7.54 (bs, 1H), 7.39 – 7.30 (m, 4H), 7.26 – 7.13 (m, 7H), 7.12 – 7.07 (m, 2H), 5.38 (s, 1H), 5.32 (s, 1H), 4.93 (s, 1H), 4.66 (d, *J* = 12.4 Hz, 1H), 4.58 (d, *J* = 12.5 Hz, 1H), 4.36 (bs, 1H), 4.10 – 3.74 (m, 10H), 3.72 – 3.50 (m, 4H), 3.32 (s, 5H), 1.23 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.5, 134.8, 134.1, 133.0, 132.9, 131.4, 128.9, 128.5, 128.3, 128.1, 127.9, 127.8, 127.6, 127.3, 127.0, 126.1, 126.0, 125.9, 109.0, 107.9, 85.6, 85.3, 83.5, 81.6, 80.6, 79.3, 77.9, 76.8, 76.0, 75.8, 72.0, 71.9, 68.8, 66.3, 61.7, 17.9.

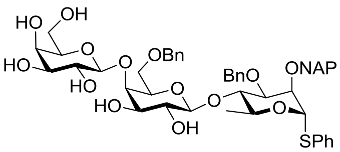
Phenyl 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzyl-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 106



Prepared from **133** according to the General Procedure IV. White foam, 78%. Rf 0.32 (toluene/EtOAc 20:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 – 7.64 (m, 1H), 7.63 – 7.56 (m, 3H), 7.39 – 7.34 (m, 3H), 7.31 – 7.25 (m, 4H), 7.24 – 7.11 (m, 29H), 7.03 – 6.95 (m, 2H), 5.55 (s, 1H), 5.45 (bs, 1H), 5.08 (s, 1H), 4.74 (d, *J* = 12.5 Hz, 1H), 4.63 (d, *J* = 12.5 Hz, 1H), 4.60 (s, 1H), 4.49 – 4.40 (m, 8H), 4.37 – 4.20 (m, 3H), 4.19 – 4.00 (m, 3H), 3.97 (dd, *J* = 5.0, 2.1 Hz, 5H), 3.87 – 3.73 (m, 3H), 3.64 – 3.45 (m, 3H), 1.30 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.0, 137.9, 137.8, 137.5, 137.5, 135.2, 134.5, 133.0, 132.9, 131.2, 128.9, 128.5, 128.3, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.2, 127.1, 126.9, 126.6, 126.0, 125.9, 125.8, 107.0, 106.3, 88.4, 88.0, 85.8, 83.4, 80.8, 80.6, 80.2, 76.1, 75.5, 73.3, 72.1, 72.0, 71.8, 71.5, 71.1, 69.5, 69.0, 66.0, 65.2, 17.9.

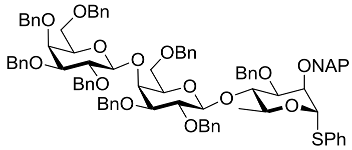
Phenyl β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 135



Compound **134** was prepared from **132** and **108** according to the General Procedure VI and taken without purification to the General Procedure III. White foam, 90%. Rf 0.25 (CH_2Cl_2/MeOH 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 – 7.52 (m, 4H), 7.42 – 7.26 (m, 3H), 7.27 – 7.01 (m, 15H), 5.34 (s, 1H), 4.66 – 4.24 (m, 14H), 3.97 (d, *J* = 6.5 Hz, 1H), 3.90 (s, 2H), 3.84 – 3.37 (m, 13H), 3.32 (d, *J* = 1.8 Hz, 1H), 1.24 (d, *J* = 5.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.0, 137.6, 135.0, 134.3, 133.0, 132.9, 131.1, 128.9, 128.5, 128.4, 128.3, 128.2, 127.8, 127.6, 127.4, 127.2, 126.8, 126.0, 125.9, 125.8, 105.7, 104.1, 85.5, 79.5, 79.2, 75.9, 74.5, 73.9, 73.6, 73.0, 72.6, 72.4, 72.2, 72.0, 68.9, 68.6, 68.3, 61.0, 17.8.

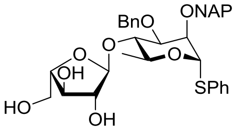
Phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 136



Prepared from **135** according to the General Procedure IV. White foam, 78%. Rf 0.27 (toluene/EtOAc 20:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.55 (m, 4H), 7.42 – 7.31 (m, 6H), 7.29 – 7.05 (m, 42H), 5.37 (d, *J* = 1.7 Hz, 1H), 5.02 (d, *J* = 11.0 Hz, 1H), 4.96 (d, *J* = 7.6 Hz, 1H), 4.89 (d, *J* = 11.6 Hz, 1H), 4.82 (d, *J* = 7.7 Hz, 1H), 4.76 – 4.59 (m, 7H), 4.54 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.46 (s, 1H), 4.34 – 4.25 (m, 4H), 4.16 (d, *J* = 11.2 Hz, 1H), 4.10 – 3.99 (m, 1H), 3.93 (t, *J* = 9.1 Hz, 1H), 3.83 – 3.76 (m, 2H), 3.74 – 3.63 (m, 4H), 3.57 – 3.50 (m, 2H), 3.50 – 3.44 (m, 2H), 3.43 – 3.32 (m, 5H), 1.27 (d, *J* = 6.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.2, 139.0, 138.9, 138.8, 138.5, 138.5, 138.3, 137.9, 135.2, 134.5, 133.1, 132.9, 131.4, 128.9, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.3, 127.2, 127.2, 127.1, 126.8, 126.0, 126.0, 125.8, 102.8, 102.2, 85.8, 82.4, 81.8, 80.4, 80.2, 79.7, 76.5, 76.1, 75.3, 74.6, 74.6, 74.3, 73.4, 73.4, 73.3, 73.1, 73.1, 72.3, 72.3, 72.0, 69.3, 69.1, 69.0, 68.6, 17.9.

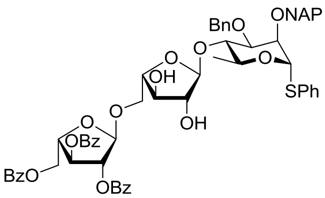
Phenyl α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 138



Acceptor **108** was glycosylated with donor **110** according to the General Procedure VI. The product was taken directly into the Zemplén deacylation according to General Procedure III. White foam, 70% over 2 steps. Rf 0.28 (CH_2Cl_2/MeOH 10:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.59 (m, 4H), 7.41 – 7.34 (m, 3H), 7.28 – 7.18 (m, 7H), 7.16 – 7.09 (m, 3H), 5.41 (s, 1H), 5.39 (d, *J* = 1.3 Hz, 1H), 4.72 (d, *J* = 12.4 Hz, 1H), 4.62 (d, *J* = 12.4 Hz, 1H), 4.39 (d, *J* = 1.3 Hz, 2H), 4.02 (dd, *J* = 4.8, 2.2 Hz, 1H), 3.97 – 3.88 (m, 4H), 3.83 (t, *J* = 9.4 Hz, 1H), 3.70 – 3.62 (m, 2H), 3.53 (dd, *J* = 11.8, 2.0 Hz, 1H), 3.46 (s, 3H), 1.26 (dd, *J* = 12.7, 6.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.4, 134.9, 134.1, 133.1, 133.0, 131.3, 128.9, 128.5, 128.2, 128.1, 127.9, 127.8, 127.6, 127.3, 126.9, 126.1, 126.0, 125.9, 109.5, 86.6, 85.6, 79.6, 79.3, 77.8, 76.8, 75.7, 72.0, 71.8, 68.6, 61.5, 17.9.

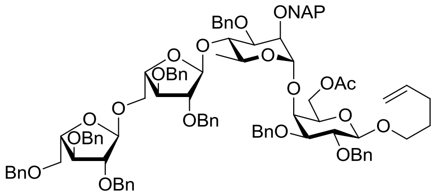
Phenyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside 139



Prepared from **110** and **138** according to the General Procedure VII. White foam, 68%. Rf 0.22 (toluene/EtOAc 4:1).

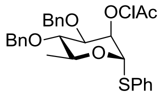
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 – 8.04 (m, 2H), 7.98 – 7.85 (m, 4H), 7.74 – 7.59 (m, 4H), 7.49 – 7.04 (m, 17H), 5.53 (d, *J* = 4.9 Hz, 1H), 5.50 (s, 1H), 5.42 (bs, 1H), 5.28 (s, 1H), 4.80 – 4.73 (m, 1H), 4.73 (s, 1H), 4.65 (d, *J* = 13.0 Hz, 1H), 4.60 (dd, *J* = 12.3, 5.1 Hz, 1H), 4.54 – 4.49 (m, 1H), 4.46 (d, *J* = 11.8 Hz, 1H), 4.38 (d, *J* = 11.8 Hz, 1H), 4.21 (d, *J* = 2.5 Hz, 1H), 4.03 – 3.91 (m, 5H), 3.87 (t, *J* = 9.3 Hz, 1H), 3.73 (dd, *J* = 10.9, 2.9 Hz, 1H), 3.66 (dd, *J* = 9.2, 3.0 Hz, 1H), 1.27 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 165.8, 165.3, 137.5, 135.1, 134.2, 133.5, 133.0, 133.0, 132.9, 131.1, 130.1, 129.8, 129.6, 129.5, 128.9, 128.6, 128.6, 128.4, 128.4, 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 127.6, 127.2, 126.6, 126.0, 125.9, 109.7, 106.0, 85.7, 85.5, 81.8, 81.8, 79.6, 79.3, 78.1, 77.4, 77.3, 75.5, 72.1, 71.5, 68.5, 67.1, 63.4, 18.1.

Pent-4-enyl 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzyl-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside 140



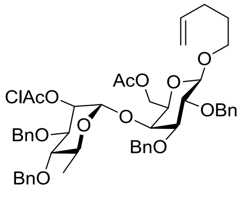
Colorless oil, Rf 0.45 (toluene/EtOAc 5:1). ^{13}C NMR (75 MHz, cdcl3) δ 170.4, 138.6, 138.5, 138.1, 138.0, 137.9, 137.9, 137.8, 137.5, 137.5, 136.1, 133.1, 132.8, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 127.0, 126.8, 125.4, 125.3, 114.8, 106.9, 106.4, 103.6, 101.8, 88.4, 87.9, 83.4, 83.4, 82.0, 80.7, 80.6, 79.4, 78.6, 77.2, 76.0, 75.2, 73.8, 73.3, 72.4, 72.1, 72.0, 71.8, 71.7, 71.4, 71.0, 70.2, 69.6, 69.4, 65.9, 62.3, 30.1, 28.9, 20.8, 18.0.

Phenyl 3,4-di-O-benzyl-2-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside 141



Prepared from **99** according to the General Procedure VIII. Colorless oil, 92%. Rf 0.54 (heptane/EtOAc 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.44 (m, 2H), 7.40 – 7.25 (m, 13H), 5.70 (dd, *J* = 3.1, 1.6 Hz, 1H), 5.47 (d, *J* = 1.4 Hz, 1H), 4.94 (d, *J* = 10.9 Hz, 1H), 4.73 (d, *J* = 11.2 Hz, 1H), 4.65 (d, *J* = 10.9 Hz, 1H), 4.57 (d, *J* = 11.2 Hz, 1H), 4.33 – 4.22 (m, 1H), 4.12 (s, 1H), 3.97 (dd, *J* = 9.3, 3.2 Hz, 1H), 3.52 (t, *J* = 9.4 Hz, 1H), 1.37 (d, *J* = 6.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 137.9, 137.2, 133.3, 131.6, 128.9, 128.2, 128.1, 128.0, 127.7, 127.7, 127.6, 127.5, 85.5, 79.6, 77.9, 75.2, 72.2, 71.8, 68.9, 40.6, 17.6.

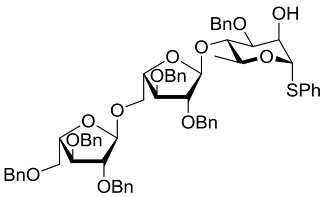
Pent-4-enyl 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside 142



Rf 0.30 (toluene/EtOAc 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 6.90 (m, 20H), 5.81 – 5.66 (m, 1H), 5.51 (s, 1H), 5.06 (s, 1H), 4.99 – 4.81 (m, 3H), 4.79 – 4.68 (m, 2H), 4.63 – 4.54 (m, 2H), 4.46 (d, *J* = 11.0 Hz, 1H), 4.37 (d, *J* = 10.9 Hz, 1H), 4.29 – 4.15 (m, 2H), 4.11 – 4.00 (m, 2H), 3.95 – 3.81 (m, 4H), 3.80 – 3.71 (m, 1H), 3.61 – 3.38 (m, 4H), 3.25 (t, *J* = 9.3

Hz, 1H), 2.15 – 2.00 (m, 2H), 1.95 (s, 3H), 1.74 – 1.60 (m, 2H), 1.20 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 166.0, 138.1, 137.8, 137.7, 137.6, 128.2, 128.2, 128.1, 128.1, 127.9, 127.7, 127.6, 127.5, 127.5, 127.4, 114.8, 103.7, 99.0, 81.0, 79.3, 78.5, 77.5, 74.9, 74.8, 73.6, 73.3, 71.8, 71.3, 70.6, 69.3, 68.5, 62.7, 40.7, 30.0, 28.7, 20.6, 17.7.

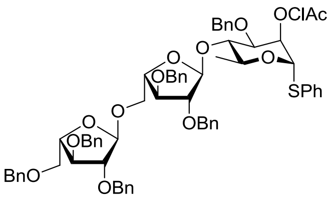
Phenyl 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzyl-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-1-thio-α-L-rhamnopyranoside 143



Prepared from **106** according to the General Procedure II. White foam, 73%. Rf 0.21 (toluene/EtOAc 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.34 (m, 2H), 7.26 – 7.12 (m, 31H), 7.07 (dd, *J* = 6.6, 3.0 Hz, 2H), 5.47 (s, 1H), 5.45 (d, *J* = 1.3 Hz, 1H), 5.06 (s, 1H), 4.59 (d, *J* = 11.5 Hz, 1H), 4.52 (d, *J* = 11.2 Hz, 1H), 4.48 – 4.24 (m, 10H), 4.20 – 4.07 (m, 4H), 4.01 – 3.92 (m, 3H), 3.86 – 3.73 (m, 4H), 3.62 – 3.43 (m, 3H), 1.24 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.0, 137.8, 137.8, 137.4, 137.4, 137.2, 134.0, 131.2, 128.9, 128.5, 128.3, 128.2, 128.2, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 106.9, 106.3, 88.3, 88.0, 86.9, 83.4, 83.3, 81.0, 80.6, 80.3, 75.4, 73.2, 72.1, 72.0, 71.8, 71.6, 71.3, 69.5, 69.3, 68.3, 66.1, 17.7.

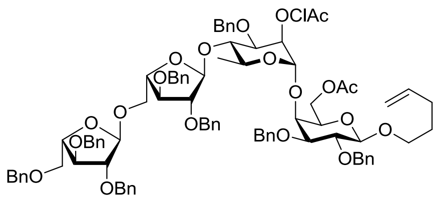
Phenyl 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzyl-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside 144



Prepared from **143** according to the General Procedure VIII. White foam, 94%. Rf 0.52 (toluene/EtOAc 10:1).

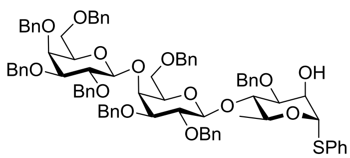
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.33 (m, 2H), 7.28 – 7.09 (m, 31H), 7.07 – 7.01 (m, 2H), 5.59 – 5.55 (m, 1H), 5.47 (s, 1H), 5.34 (d, *J* = 1.5 Hz, 1H), 5.07 (s, 1H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.50 – 4.38 (m, 7H), 4.38 – 4.20 (m, 4H), 4.20 – 4.09 (m, 3H), 3.99 (s, 2H), 3.97 – 3.93 (m, 3H), 3.88 – 3.80 (m, 3H), 3.75 (t, *J* = 10.8 Hz, 1H), 3.64 – 3.44 (m, 3H), 1.27 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 137.9, 137.7, 137.4, 137.3, 137.0, 133.4, 131.8, 129.0, 128.4, 128.3, 128.2, 128.2, 127.7, 127.6, 127.6, 127.5, 127.5, 106.9, 106.2, 88.1, 87.9, 85.7, 83.4, 83.3, 81.1, 80.6, 78.2, 75.4, 73.2, 72.1, 72.0, 71.7, 71.5, 71.3, 69.5, 68.7, 66.0, 40.7, 17.7.

Pent-4-enyl 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzyl-α-L-arabinofuranosyl-(1→4)-3-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside 145



Colorless oil, Rf 0.15 (toluene/EtOAc 10:1).
[1]H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.08 (m, 45H), 5.78 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.55 – 5.52 (m, 1H), 5.39 (d, *J* = 7.2 Hz, 1H), 5.11 (d, *J* = 1.6 Hz, 1H), 5.07 (s, 1H), 5.02 – 4.89 (m, 2H), 4.87 (d, *J* = 11.1 Hz, 1H), 4.78 – 4.70 (m, 3H), 4.62 (t, *J* = 10.6 Hz, 3H), 4.50 – 4.39 (m, 6H), 4.39 – 4.21 (m, 8H), 4.16 – 4.04 (m, 4H), 3.98 – 3.81 (m, 8H), 3.82 – 3.68 (m, 5H), 3.66 – 3.41 (m, 6H), 2.17 – 2.08 (m, 2H), 1.98 (s, 3H), 1.79 – 1.68 (m, 2H), 1.23 (d, *J* = 6.1 Hz, 3H).
[13]C NMR (75 MHz, $CDCl_3$) δ 170.4, 166.2, 138.1, 138.0, 137.8, 137.8, 137.7, 137.4, 137.4, 128.3, 128.3, 128.2, 128.2, 128.1, 127.8, 127.6, 127.6, 127.5, 114.9, 106.7, 106.2, 104.0, 99.0, 88.2, 87.9, 83.3, 81.1, 80.6, 80.6, 78.5, 77.8, 74.9, 73.5, 73.3, 73.2, 72.1, 72.0, 71.8, 71.6, 71.4, 71.2, 69.9, 69.7, 69.5, 68.3, 65.8, 62.6, 32.2, 30.1, 28.8, 20.7, 17.9.

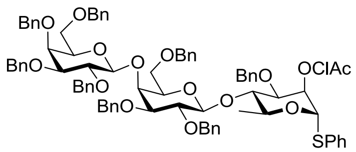
Phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-1-thio-α-L-rhamnopyranoside 146



Prepared from **136** according to the General Procedure II. White foam, 73%. Rf 0.23 (toluene/EtOAc 10:1).
[1]H NMR (400 MHz, CDCl3) δ 7.39 – 7.31 (m, 4H), 7.27 – 7.07 (m, 39H), 7.04 (d, J = 7.8 Hz, 2H), 5.39 (d, J = 1.3 Hz, 1H), 4.99 (d, J = 11.0 Hz, 1H), 4.92 (d, J = 7.6 Hz, 1H), 4.87 (d, J = 11.6 Hz, 1H), 4.74 – 4.66 (m, 3H), 4.65 – 4.52 (m, 5H), 4.46 (d, J = 11.6 Hz, 1H), 4.41 (s, 2H), 4.31 (d, J = 10.8 Hz, 1H), 4.28 – 4.18 (m, 4H), 4.12 – 4.02 (m, 1H), 4.01 – 3.98 (m, 1H), 3.77 – 3.71 (m, 2H),

3.71 – 3.61 (m, 4H), 3.53 – 3.41 (m, 2H), 3.40 – 3.29 (m, 5H), 1.20 (d, J = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 138.9, 138.7, 138.5, 138.4, 137.8, 137.6, 131.3, 128.9, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 127.8, 127.8, 127.7, 127.7, 127.7, 127.4, 127.3, 127.3, 127.3, 127.2, 125.2, 102.9, 102.2, 86.8, 82.3, 81.8, 80.5, 80.3, 79.7, 76.1, 75.4, 74.6, 74.6, 74.2, 73.4, 73.4, 73.3, 73.1, 72.3, 72.1, 69.7, 69.4, 69.1, 68.7, 68.3, 21.4, 17.7.

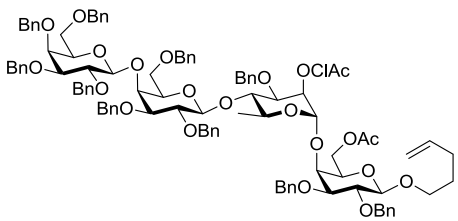
Phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside 147



Prepared from **146** according to the General Procedure VIII. White foam, 80%. Colorless oil, Rf 0.49 (toluene/EtOAc 10:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.31 (m, 4H), 7.27 – 7.04 (m, 39H), 7.02 (d, *J* = 7.4 Hz, 2H), 5.48 – 5.43 (m, 1H), 5.29 (d, *J* = 1.4 Hz, 1H), 5.01 (d, *J* = 11.0 Hz, 1H), 4.92 (d, *J* = 7.6 Hz, 1H), 4.87 (d, *J* = 11.6 Hz, 1H), 4.73 (d, *J* = 13.7 Hz, 1H), 4.70 (d, *J* = 17.3 Hz, 2H), 4.66 – 4.55 (m, 5H), 4.47 (d, *J* = 11.6 Hz, 1H), 4.42 (d, *J* = 2.4 Hz, 2H), 4.29 – 4.20 (m, 4H), 4.16 – 4.07 (m, 2H), 3.85 (d, *J* = 2.0 Hz, 2H), 3.75 (d, *J* = 2.9 Hz, 1H), 3.72 – 3.64 (m, 5H), 3.54 (dd, *J* = 10.1, 5.9 Hz, 1H), 3.49 – 3.41 (m, 1H), 3.42 – 3.30 (m, 5H), 1.25 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 139.0, 138.9, 138.8, 138.6, 138.4, 138.3, 137.8, 137.6, 137.3, 133.3, 131.8, 129.0, 128.9, 128.6, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 128.0, 127.8, 127.8, 127.7, 127.6, 127.3, 127.2, 125.1, 102.6, 102.2, 85.6, 82.1, 81.7, 80.3, 79.6, 78.4, 75.9, 75.5, 74.5, 74.2, 73.4, 73.3, 73.3, 73.1, 73.0, 72.2, 72.1, 72.0, 69.7, 69.4, 68.6, 68.6, 40.7, 17.7.

Pent-4-enyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside 148



Colorless oil, Rf 0.14 (toluene/EtOAc 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 6.6 Hz, 2H), 7.32 – 7.08 (m, 48H), 5.80 – 5.67

(m, 1H), 5.42 (dd, *J* = 3.0, 2.1 Hz, 1H), 5.11 (d, *J* = 1.6 Hz, 1H), 5.02 (d, *J* = 11.0 Hz, 1H), 4.94 (dd, *J* = 17.2, 1.7 Hz, 1H), 4.94 – 4.84 (m, 4H), 4.76 – 4.67 (m, 4H), 4.67 – 4.45 (m, 10H), 4.43 (d, *J* = 2.9 Hz, 2H), 4.35 – 4.24 (m, 4H), 4.19 (d, *J* = 2.2 Hz, 1H), 4.12 (dd, *J* = 11.1, 6.5 Hz, 1H), 4.04 (d, *J* = 9.6 Hz, 1H), 3.95 (d, *J* = 2.2 Hz, 1H), 3.93 – 3.88 (m, 1H), 3.85 – 3.58 (m, 8H), 3.58 – 3.29 (m, 10H), 2.16 – 2.05 (m, 2H), 1.92 (s, 3H), 1.77 – 1.64 (m, 2H), 1.21 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 166.1, 139.1, 139.0, 138.9, 138.7, 138.6, 138.5, 138.2, 137.9, 137.8, 137.6, 128.9, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 114.9, 104.0, 102.7, 102.4, 99.0, 82.1, 81.8, 81.3, 80.3, 79.6, 78.6, 77.8, 75.6, 75.5, 75.0, 74.6, 74.3, 73.4, 73.4, 73.4, 73.1, 73.1, 72.8, 72.4, 72.0, 71.4, 70.4, 69.8, 69.7, 68.7, 68.1, 62.6, 40.8, 30.1, 28.8, 20.7, 17.9.

Screening of the Reaction Conditions

Table 2: Synthesis of the Thiophenyl Disaccharide Donor 68

Glycosylations with Thiophenyl Glycosyl Donor 69

NIS/TESOTf-Promoted Glycosylations

In entries 1–6 the disaccharide product **68** was obtained in approx. 1:1 mixture with trisaccharide **84**. Yields are given for the mixture, % yields are calculated assuming that the product is disaccharide **68**.

Entry 1. A mixture of donor **69** (350 mg, 0.6 mmol) and acceptor **70** (320 mg, 0.5 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous diethyl ether (8 mL) and cooled to –20 °C. NIS (150 mg, 0.66 mmol) was added followed by addition of TESOTf (0.03 mL, 0.12 mmol). The reaction mixture was stirred at –20 °C until TLC (toluene/EtOAc 10:1) showed disappearance of the starting materials. The reaction mixture was quenched with Et_3N (0.1 ml), diluted with CH_2Cl_2 (25 ml) and washed with 10% aq. $Na_2S_2O_3$ (2 × 10 ml). The combined aqueous phases were extracted with CH_2Cl_2 (10 ml). The combined organic phases were dried with Na_2SO_4, filtered, concentrated and purified by flash chromatography (toluene/EtOAc 40:1). Yield 280 mg, 50%.

***Entry* 2.** Same as entry 1, but CH_2Cl_2 (8 ml) was used as solvent instead of diethyl ether. Yield 280 mg, 50%.

***Entry* 3.** Same as entry 1, but a mixture of CH_2Cl_2 (4 ml) and diethyl ether (4 ml) was used as solvent instead of pure diethyl ether. Yield 250 mg, 45%.

***Entry* 4.** Same as entry 1, but the glycosylation was performed at –40 °C instead of –20 °C. No product formation was seen, instead precipitation of the starting materials was observed.

***Entry* 5.** Same as entry 1, but the glycosylation was performed at 0 °C instead of –20 °C. Yield 195 mg, 35%.

***Entry* 6.** A mixture of donor **69** (520 mg, 0.9 mmol) and acceptor **70** (320 mg, 0.5 mmol) was taken into the glycosylation. Otherwise the same reaction conditions as in entry 1 were used. Yield 270 mg, 48%.

I_2-Promoted Glycosylations

In entries 7–9 and 10 product **68** was obtained in slightly impure form.

***Entry* 7.** A mixture of donor **69** (350 mg, 0.6 mmol) and acceptor **70** (320 mg, 0.5 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (20 mL), preactivated 4 Å MS (400 mg) was added and the mixture was stirred at 20 °C under N_2-atmosphere for 20 min. I_2 (180 mg, 0.72 mmol) was added and the reaction mixture was stirred at 20 °C until TLC (toluene/EtOAc 10:1) showed disappearance of the donor (24 h). The reaction mixture was filtered through a plug of Celite and washed with 10% aq. $Na_2S_2O_3$ (2 × 10 ml). The organic phase was dried with Na_2SO_4, filtered, concentrated and purified by flash chromatography (toluene/EtOAc 40:1). Yield 110 mg, 20%.

***Entry* 8.** Same as entry 7, but K_2CO_3 (83 mg, 0.6 mmol) was added before the addition of 4 Å MS. The reaction was staying for 5 days. Yield 85 mg, 15%.

***Entry* 9.** Same as entry 7, but TBAI (220 mg, 0.6 mmol) was added before the addition of 4 Å MS. The reaction was staying for 3 days Yield 55 mg, 10%.

Glycosylations with Glycosyl Bromide 86

Preparation of the glycosyl bromide **86**: Thiophenyl glycoside **69** (350 mg, 0.6 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. It was then dissolved in anhydrous CH_2Cl_2 (2 mL), cooled in ice bath and titrated with a 1M solution of Br_2 in anhydrous CH_2Cl_2 until a faint yellow color persisted. The prepared **86** was used in glycosylations without further purification.

AgOTf-Promoted Glycosylations

Entry 10. Acceptor **70** (210 mg, 0.33 mmol) was co-evaporated with toluene (2 × 10 ml), subjected to high vacuum for 2 h and dissolved in anhydrous CH_2Cl_2 (1 ml). Preactivated 4 Å MS (400 mg) and AgOTf (230 mg, 0.9 mmol) were added and the mixture was cooled to –50 °C. A solution of glycosyl bromide **86** was cannulated to the solution of acceptor. The reaction mixture was stirred at –50 °C for 2 h. Decomposition of the donor and the acceptor was observed. Yield of the product was not determined.

TBAI-Promoted Glycosylations

Entry 11. Acceptor **70** (210 mg, 0.33 mmol) was co-evaporated with toluene (2 × 10 ml), subjected to high vacuum for 2 h and dissolved in anhydrous CH_2Cl_2 (1 ml). Preactivated 4 Å MS (400 mg) and TBAI (385 mg, 1.2 mmol) were added and the reaction mixture was stirred at 20 °C for 15 h. Then it was diluted with CH_2Cl_2 (10 ml) and washed with sat. $NaHCO_3$ (2 × 10 ml). The organic phase was dried with Na_2SO_4, filtered, concentrated and purified by flash chromatography (toluene/EtOAc 40:1). Yield 55 mg (10%).

Table 3: Synthesis of the Pentenyl Disaccharide Donor 83

Entry 1. A mixture of donor **69** (315 mg, 0.55 mmol) and acceptor **92** (310 mg, 0.5 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous diethyl ether (8 mL) and cooled to –20 °C. NIS (135 mg, 0.6 mmol) was added followed by addition of TESOTf (0.025 mL, 0.1 mmol). The reaction mixture was stirred at –20 °C until TLC (toluene/EtOAc 10:1) showed completion of the reaction (1.5 h). The reaction mixture was quenched with Et_3N (0.1 ml), diluted with CH_2Cl_2 (25 ml) and washed with 10% aq. $Na_2S_2O_3$ (2 × 10 ml). The combined aqueous phases were extracted with CH_2Cl_2 (10 ml). The combined organic phases were dried with Na_2SO_4, filtered, concentrated and purified by flash chromatography (toluene/EtOAc 40:1). Yield 325 mg, 60%.

Entry 2. A mixture of donor **69** (345 mg, 0.6 mmol) and acceptor **92** (310 mg, 0.5 mmol) was taken into the glycosylation. Otherwise the same reaction conditions as in entry 1 were used. The reaction was done after 40 min (as shown by TLC). Yield 420 mg, 78%.

Entry 3. Same as entry 2, but the glycosylation was performed at –40 °C instead of –20 °C. The reaction took 3 h. Yield 340 mg, 63%.

Entry 4. Same as entry 2, but the glycosylation was performed at 0 °C instead of –20 °C. The reaction took 20 min. Yield 315 mg, 58%.

Entry 5. Same as entry 2, but a mixture of CH_2Cl_2 (4 ml) and diethyl ether (4 ml) was used as solvent instead of pure diethyl ether. The reaction took 30 min. Yield 405 mg, 75%.

Entry 6. Same as entry 2, but CH_2Cl_2 (8 ml) was used as solvent instead of diethyl ether. The reaction took less than 15 min. Yield 245 mg, 55%.

Table 4: Removing the NAP-Group in 69

All reactions were monitored by TLC (heptane/EtOAc 1:1). The reactions were worked up according to either Procedure A or Procedure B. Product **99** was isolated after flash chromatography (5:1 heptane/EtOAc) as white foam.

Work-up Procedure A. The reaction mixture was concentrated, co-evaporated with toluene (2 × 10 ml) and purified by flash chromatography.

Work-up Procedure B. The reaction mixture was diluted with CH_2Cl_2 (20 ml) and washed with sat. $NaHCO_3$ (2 × 10 ml). The combined aqueous phases were extracted with CH_2Cl_2 (2 × 20 ml). The combined organic phases were dried (Na_2SO_4), filtered, concentrated and purified by flash chromatography.

DDQ

Entry 1. To a solution of **69** (300 mg, 0.5 mmol) in a mixture of CH_2Cl_2 (4 ml), MeOH (1 ml) and water (0.2 ml) was added DDQ (160 mg, 0.7 mmol.). The reaction mixture was stirred at 20 °C until TLC showed completion of the reaction (3 h). The reaction was worked up according to the Procedure A. Yield 95 mg, 42%.

Entry 2. Same as entry 1, but the reaction was worked up according to the Procedure B. Yield 170 mg, 75%.

Entry 3. Same as entry 2, but the reaction was performed at 0 °C for 24 h. Yield 160 mg, 70%.

Entry 4. Same as entry 2, but the reaction was performed in CH_2Cl_2 (5 ml). Yield 150 mg, 67%.

Entry 5. Same as entry 2, but K_2HPO_4/KH_2PO_4 buffer (1M, pH 7.2, 1 ml) was added instead of H_2O. Yield 85 mg, 38%.

HF/Pyridine

Entry 6. To a solution of **69** (300 mg, 0.5 mmol) in toluene (1 mL) in a plastic centrifuge tube was added HF/pyridine (10.0 mmol, 0.25mL) with vigorous stirring. The reaction mixture was stirred at 20 °C until TLC showed completion of the reaction (2 h). The reaction was worked up according to the Procedure B. Yield 65 mg, 30%.

TFA

Entry 7. To a solution of **69** (300 mg, 0.5 mmol) in toluene (1 mL) was added TFA (9.3 ml). The reaction mixture was stirred at 20 °C until TLC showed completion of the reaction (2 h). The reaction was worked up according to the Procedure A. Yield 90 mg, 40%.

Entry 8. Same as entry 7, but the reaction was worked up according to the Procedure B. Yield 145 mg, 65%.

Entry 9. Same as entry 8, but the reaction was performed at 0 °C for 24 h. Yield 145 mg, 65%.

Table 6: Regioselective Protection of the C-2 Hydroxyl Group in Rhamnose Derivative 120

In all cases, the product was purified by flash column chromatography in heptane/EtOAc 4:1.

Entry 1. The solution of diol **120** (700 mg, 2.0 mmol) and NAPBr (490 mg, 2.2 mmol) in CH_2Cl_2 (20 ml) was mixed with 1M NaOH (8 ml) and $TBAHSO_4$ (135 mg, 0.4 mmol) was added. The mixture was heated under reflux for 48 h after which time it was diluted with CH_2Cl_2 (10 ml). The water phase was separated and washed with CH_2Cl_2 (2 × 10 ml). The combined organic phases were dried with Na_2SO_4, concentrated and purified. Yield 410 mg, 42%.

Entry 2. To a solution of diol **120** (700 mg, 2.0 mmol) in DMF (6 ml) NAPBr (490 mg, 2.2 mmol) and TBAI (75 mg, 0.2 mmol) were added and the mixture was cooled in ice bath. NaH (90 mg, 2.2 mmol, 60% in oil) was added and the mixture was stirred at 20 °C for 12 h and then quenched by addition of MeOH (0.2 ml). The reaction mixture was partially concentrated, diluted with EtOAc (20 ml) and washed with water (3 × 10 ml) and brine (10 ml). The combined water phase was washed with EtOAc (2 × 10 ml). The combined organic phase was dried with Na_2SO_4, concentrated and purified. Yield 640 mg, 65%.

Entry 3. To a solution of diol **120** (700 mg, 2.0 mmol), NAPBr (490 mg, 2.2 mmol) and TBAI (75 mg, 0.2 mmol) in DMF (6 ml) was added Ag_2O (700 mg, 3.0 mmol) and the mixture was stirred at 20 °C for 48 h after which time the reaction mixture was filtered through Celite, partially concentrated, diluted with EtOAc (20 ml) and washed with water (3 × 10 ml) and brine (10 ml). The combined water phase was washed with EtOAc (2 × 10 ml). The combined organic phase was dried with Na_2SO_4, concentrated and purified. Yield 390 mg, 40%.

Entry 4. A mixture of diol **120** (700 mg, 2.0 mmol) and **121** (660 mg, 2.2 mmol) was dissolved in Et_2O (20 ml) and the solution was cooled in ice bath. TMSOTf (0.035 ml, 0.2 mmol) was added and the mixure was allowed to gradually warm up to 20 °C and stirred at this temperature for 12 h after which time Et_3N (0.1 ml) was added. The mixture was concentrated and purified. Yield 250 mg, 25%.

Table 7: Glycosylation of 92 and 93 with 69

In all cases except for entry 6, prior to glycosylations a mixture of donor **69** (315 mg, 0.55 mmol) and acceptor **92** (310 mg, 0.5 mmol) or acceptor **93** (235 mg, 0.5 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. All glycosylations were monitored by TLC in toluene/EtOAc 10:1. The products were purified by flash column chromatography in toluene/EtOAc 40:1 (when **92** was used as an acceptor) or 20:1 (when **93** was used). The DMTST solution was prepared as follows: MeOTf (0.32 ml, 2.82 mmol) was added to a flame-dried flask containing Me_2S_2 (0.28 ml, 3.1 mmol). The mixture was stirred under inert atmosphere at 20 °C for 5 min, after which time CH_2Cl_2 (1 mL) was added. The prepared solution was used immediately in the glycosylation reactions. 1M solution of Me_2S_2/Tf_2O was prepared as follows: Me_2S_2 (0.10 ml, 1.1 mmol) was dissolved in CH_2Cl_2 (1 ml) and the solution was cooled to 0 °C. Tf_20 (0.17 ml, 1.0 mmol) was added and the mixture was stirred 0 °C for 20 min. The solution was used immediately in the glycosylation reactions.

Entry 1. A mixture of donor **69** and the acceptor **92** was dissolved in CH_2Cl_2 (20 ml), NIS (250 mg, 1.1 mmol) was added and the mixture was cooled to –20 °C. $Yb(OTf)_3$ (95 mg, 0.15 mmol) was added and the reaction mixture was stirred at –20 °C for 5 h, after which time the reaction was quenched by addition of Et_3N (0.3 ml). TLC control showed no product formation; only formation of *C*-glycoside **85** was observed.

Entry 2. Same as entry 1, but the reaction was performed at 0 °C instead of –20 °C. The yield of approx. 10% was judged by TLC; *C*-glycoside **85** was the major product.

Entry 3. A mixture of donor **69** and the acceptor **92** was dissolved in anhydrous CH_2Cl_2 (10 mL), 3 Å MS (500 mg) were added and the mixture was stirred at 20 °C under inert atmosphere for 30 min. The mixture was cooled to 0 °C, MeOTf (0.19 ml, 1.65 mmol) was added and the mixture was stirred at 0 °C for 2 h, after which time the reaction was quenched by addition of Et_3N (0.3 ml). The mixture was diluted with CH_2Cl_2 (30 ml), filtered through Celite, washed with water (10 ml) and brine (10 ml), concentrated and purified by flash column chromatography. Yield 110 mg, 20%.

Entry 4. Same as entry 2, but acceptor **93** was used. Yield approx. 20% (by TLC).

Entry 5. Same as entry 3, but acceptor **93** was used. Yield 120 mg, 25%.

Entry 6. A mixture of donor **69** (345 mg, 0.6 mmol), Ph_2SO (120 mg, 0.6 mmol) and TTBP (1500 mg, 0.6 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (12 mL) and cooled to –60 °C. Tf_2O (0.11 ml, 0.66 mmol) was added and the reaction mixture was stirred at –60 °C for 5 min, after which time a solution of acceptor **93** (235 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (5 mL) was added (the acceptor was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h). The mixture was stirred at –60 °C for 20 min. TLC control showed that *C*-glycoside **85** was formed exclusively.

Entry 7. A mixture of donor **69** and the acceptor **93** was dissolved in CH_2Cl_2 (8 ml), 4 Å MS (400 mg) and TTBP (135 mg, 0.55 mmol) were added and the mixture was cooled to –40 °C. DMTST solution (0.6 ml) was added and the reaction mixture was stirred at –40 °C for 20 min, after which time the reaction was quenched by addition of Et_3N (0.3 ml). The reaction mixture was diluted with CH_2Cl_2 (30 ml), filtered through Celite, washed with sat. $NaHCO_3$ (10 ml) and brine (10 ml), concentrated and purified by flash column chromatography. Yield 190 mg, 40%.

Entry 8. Same as entry 7, but 1M solution of Me_2S_2/Tf_2O in Et_2O (0.75 ml) was used instead of DMTST. Yield 320 mg, 68%.

Entry 9. Same as entry 8, but the reaction was performed in Et_2O (8 ml) instead of CH_2Cl_2. Yield 180 mg, 38%.

Table 8: Glycosylation of 93 with 141

A mixture of donor **141** (280 mg, 0.55 mmol) and acceptor **93** (235 mg, 0.5 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. All glycosylations were monitored by TLC in toluene/EtOAc 10:1. The product was purified by flash column chromatography in toluene/EtOAc 20:1.

Entry 1. Same procedure as in entry 8, table 7. Yield 290 mg, 60%.

Entry 2. Same procedure as in entry 2, table 7. Yield 50 mg, 10%, slightly impure product.

Entry 3. Same procedure as in entry 6, table 7. Yield 220 mg, 45%.

Table 9: Synthesis of Tetrasaccharide 145

A mixture of donor **144** (312 mg, 0.28 mmol) and acceptor **93** (120 mg, 0.25 mmol) was co-evaporated with toluene (2 × 10 ml) and subjected to high vacuum for 2 h. All glycosylations were monitored by TLC in toluene/EtOAc

10:1. The product was purified by flash column chromatography in toluene/EtOAc 10:1.

Entry 1. Same procedure as in entry 8, table 7. Yield 85 mg, 20%.

Entry 2. Same procedure as in entry 2, table 7. Yield mg 170 mg, 40%.

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Synthesis of a Backbone Hexasaccharide Fragment of the Pectic Polysaccharide Rhamnogalacturonan I

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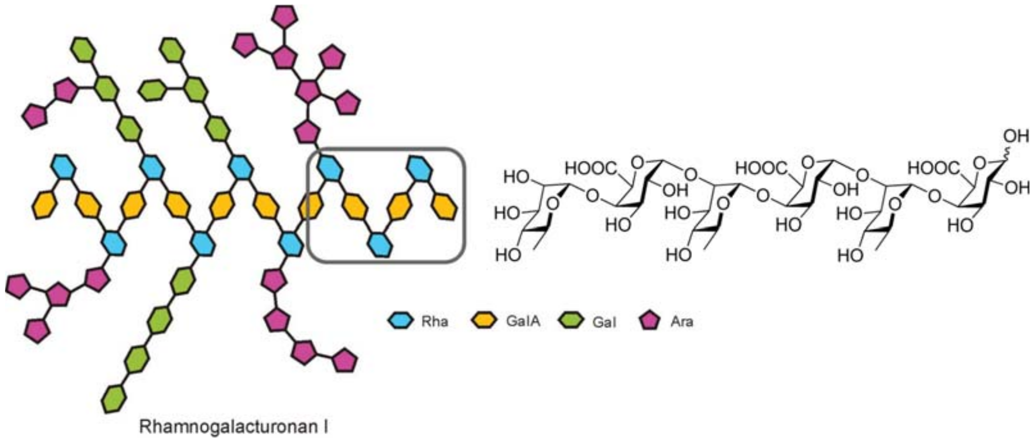
ABSTRACT



Synthesis of the fully unprotected hexasaccharide backbone of the pectic polysaccharide rhamnogalacturonan I is described. The strategy relies on iterative coupling of a common pentenyl disaccharide glycosyl donor followed by a late-stage oxidation of the C-6 positions of the galactose residues. The disaccharide donor is prepared by an efficient chemoselective armed-disarmed coupling of a thiophenyl rhamnoside donor with a pentenyl galactoside acceptor bearing the strongly electron-withdrawing pentafluorobenzoyl ester (PFBz) protective group.

Pectins are highly heterogeneous polysaccharides of plant origin. They are found in the primary cell wall and contribute to various cell functions, including support, defense, signaling, and cell adhesion.[1] Pectins also play important roles as food additives, serving as stabilizing and thickening agents in products such as jams, yogurts, and jellies.[2] Rhamnogalacturonan I (RG-I) is one of the structural classes of pectic polysaccharides, along with homogalacturonan, rhamnogalacturonan II, and xylogalacturonan.[3] The chemical structure of RG-I is complex having a backbone consisting of alternating α-linked L-rhamnose and D-galacturonic acid units with numerous branches of arabinans, galactans, or arabinogalactans positioned at C-4 of the rhamnose residues.

The structural complexity of pectin together with the wide range of its practical applications and desire to understand its structure and functions in details have inspired many researchers to pursue chemical syntheses of pectic oligosaccharides. Herein, we report the synthesis of a hexasaccharide fragment of the RG-I rhamnogalacturonan backbone (**1**, Figure 1).

Synthesis of the fully unprotected hexasaccharide fragment of RG-I has not been previously reported. However, smaller fully and partially unprotected RG-I oligosaccharides, as well as fully protected oligosaccharides up to

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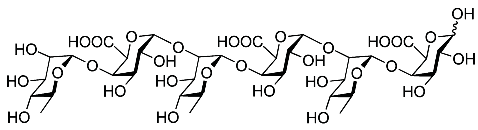

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Figure 1. Structure of the hexasaccharide fragment of RG-I.

hexamers, have been prepared by different approaches. Some of the strategies used galacturonic acid as the starting material, while others favored the oxidation of galactose to galacturonic acid at a late stage, i.e., pre- and postglycosylation–oxidation strategies, respectively. Reimer and co-workers[4] reported the synthesis of a protected tetrasaccharide containing galactose instead of galacturonic acid as an intermediate for the preparation of RG-I fragments. The protective group pattern was designed to allow for further chain elongation and introduction of branching. It was envisioned that the global deprotection and oxidation of the primary hydroxyl groups of the galactose units would furnish the native oligosaccharides. In later work, the group synthesized the fully unprotected methyl glycoside of an RG-I tetrasaccharide, both in the methyl ester and the free carboxylic acid forms.[5] In this case, a similar protective group pattern was used, but galacturonic acid was employed from the early stages. This lowered the overall number of synthetic steps by avoiding the late stage oxidation. Unfortunately, the key glycosylation reaction proved to be problematic and only low yields of the protected tetrasaccharide product could be obtained. Vogel and co-workers[6] prepared a partially deprotected RG-I trisaccharide bearing a benzoyl group at C-4 of the rhamnose residue where galacturonic acid was used as a starting material. Later, the same group reported the synthesis of the fully unprotected propyl glycoside of an RG-I tetrasaccharide, as well as synthesis of its protected hexasaccharide fragment and protected tri- and tetrasaccharides suitable for the assembly of the branched RG-I fragments.[7] The synthesis was based on a modular design principle and used galacturonic acid as the starting material. Takeda and co-workers prepared[8] the unprotected propyl glycoside of an RG-I tetrasaccharide using a late-stage oxidation approach. All the mentioned work employed the generation of glycosyl donors before each glycosylation step. In a recent report by Davis and co-workers,[9] a latent-active approach was utilized and combined with the late-stage oxidation strategy to synthesize the fully unprotected RG-I tetrasaccharide and its dimethyl ester. Interestingly, the initial attempt to couple a galactorhamnosyl disaccharide donor to the galactose of a disaccharide acceptor failed due to a lack of reactivity, forcing the authors to change the strategy and assemble the RG-I tetrasaccharide through galactosylation instead of rhamnosylation. The potential of this methodology for iterative elongation of the oligosaccharide chain was demonstrated by preparation of a fully protected analog of the native hexasaccharide, containing both galactose and galacturonic acid residues.

Retrosynthetic analysis of the target RG-I hexasaccharide **1** is depicted in Figure 2.

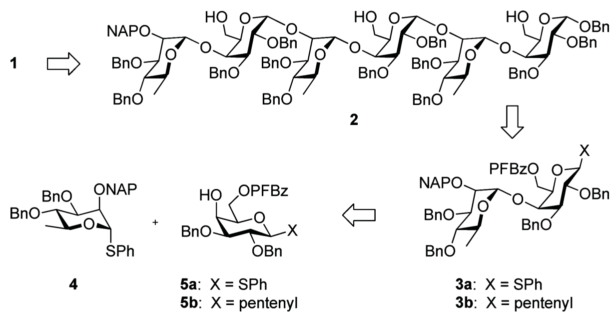

Figure 2. Retrosynthesis of the RG-I hexasaccharide **1**.

Choosing between the two possible approaches[10] for synthesis of oligosaccharides containing uronic acids (that is, oxidation prior to or after glycosylation), we adopted the postglycosylation strategy, which we had previously successfully employed[11] in the synthesis of homogalacturonans. Although it requires additional protective group manipulations, the nonoxidized carbohydrates are generally more reactive glycosyl donors than their oxidized counterparts,[12] where the reactivity is decreased by the presence of the electron-withdrawing carboxyl groups. Moreover, introduction of the carboxylic acid functionality at a late stage of the synthesis reduces the risk of possible side reactions, such as epimerization to L-altruronic acid and β-elimination leading to the formation of 4-deoxy-L-*threo*-hex-4-enopyranuronic acid. According to this reasoning, we envisioned that the target hexasaccharide **1** could be obtained from the partially deprotected hexasaccharide **2** by oxidation of the primary hydroxyl groups to the carboxylic acids followed by a global deprotection.

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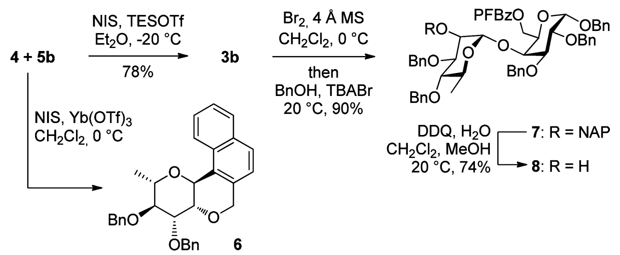
Hexasaccharide **2** in turn was planned to be assembled by two iterative glycosylations using disaccharide **3**. Employing the common disaccharide **3** would minimize the number of monosaccharide building blocks required for the synthesis. Donor **3** was designed to possess a nonparticipating benzyl (Bn) ether at C-2 promoting the formation of the α-glycosidic linkage and was intended to be produced through a chemoselective coupling between rhamnose donor **4** and galactose acceptor **5**. Donor **4** was designed to carry a nonparticipating 2-naphthylmethyl (NAP) ether at C-2 ensuring the formation of the α-glycosidic linkage and later on allowing for selective deprotection and elongation of the oligosaccharide chain at this position. The C-6 moiety in acceptor **5** was capped with a pentafluorobenzoyl ester (PFBz) that could be selectively removed to release this position for oxidation. Apart from functioning as a temporary protective group, the PFBz ester was also envisioned to tune the reactivity of **5**. It is known that electron-withdrawing protective groups decrease the reactivity of glycosyl donors,[13] and the donors protected with electron-donating (ether) groups can be selectively activated in a glycosylation reaction over the donors protected with electron-withdrawing (ester) groups. This phenomenon, first formulated by Fraser-Reid,[14] is known as the "armed-disarmed effect". In the present synthesis, the "armed" rhamnose donor **4** fully protected with ether groups was planned to be selectively activated over the "disarmed" galactose acceptor **5** bearing an electron-withdrawing PFBz group. In addition to the electronic effects of the protective groups, rhamnose was expected to have a higher reactivity, because it is a deoxy sugar and lacks the electron-withdrawing group at C-6 compared to galactose.[13]

Protected monosaccharide building blocks **4**,[15] **5a**,[16] and **5b**[11b] were synthesized from L-rhamnose and D-galactose (see the Supporting Information). Initially, the use of galactose thiophenyl acceptor **5a** in a chemoselective coupling with rhamnose thiophenyl donor **4** was explored. When *N*-iodosuccinimide (NIS) in the presence of a catalytic amount of triethylsilyl trifluoromethanesulfonate (TESOTf) was applied as the promoter it was possible to obtain the target disaccharide **3a** but, unfortunately, only as an inseparable mixture in almost equal amounts with a trisaccharide byproduct derived from a reaction of **3a** with **4**. Attempts to conduct this glycosylation under different reaction conditions (applying I_2 as the promoter, converting **4** into the corresponding glycosyl bromide and subsequent activation with silver triflate, or applying in situ anomerisation conditions) did not improve the reaction outcome. In some cases, most of donor **4** was converted into a *C*-glycoside through an intramolecular reaction (vide infra). Given the lack of success in synthesizing thiophenyl disaccharide **3a**, we turned to pentenyl glycosides as an alternative (Scheme 1). The NIS/TESOTf-mediated coupling of galactose pentenyl acceptor **5b** with the identical rhamnose donor **4** produced the desired disaccharide **3b** as the sole product, and we isolated the α-anomer in 78% yield after flash chromatography. As an alternative to the armed−disarmed approach that we describe here, we also explored selective activation of the thiophenyl glycoside with other promotors: MeOTf[17] resulted in a low yielding glycosylation with many byproducts while activation with NIS/$Yb(OTf)_3$[18] mainly led to the formation of the *C*-glycoside **6** (Scheme 1). Attempts to preactivate the glycosyl donor with diphenyl sulfoxide and triflic anhydride[19] also resulted in the formation of **6** as the major product. This could be circumvented by replacing the *O*-2 NAP protective group with chloroacetyl and with that thioglycoside donor the preactivation conditions gave a coupling yield of 45%. Nonetheless, the armed−disarmed coupling of **4** and **5b** resulted in the highest yield; the reactivity difference between the thiophenyl glycoside and the corresponding pentenyl glycosides was somewhat surprising, and we are currently investigating whether this is a general trend.

Scheme 1. Synthesis of the Disaccharide Building Blocks



To assemble the hexasaccharide from the disaccharide **3b**, it was first converted to the glycosyl bromide and then, by glycosylation of benzyl alcohol, to benzyl glycoside **7**. This two-step sequence ensured the formation of the α-glycoside, where direct activation with NIS/TESOTf resulted in an α/*β*-mixture. This was followed by removal of the NAP-group at C-2′ by oxidation with DDQ in CH_2Cl_2 in the presence of water furnishing disaccharide acceptor **8**. Pentenyl disaccharide **3b** was used as the key disaccharide donor in the further iterative assembly of hexasaccharide **2** (Scheme 2). Glycosylation of **8** with **3b** using the aforementioned conditions led to the formation of tetrasaccharide **9** as a single α-isomer in 71% yield. Tetrasaccharide **9** was subjected to the same procedure for removal of the NAP-group with DDQ to furnish tetrasaccharide acceptor **10**, which was coupled again with donor **3b** and the crude product was directly subjected to the Zemplén conditions, and after the selective removal of

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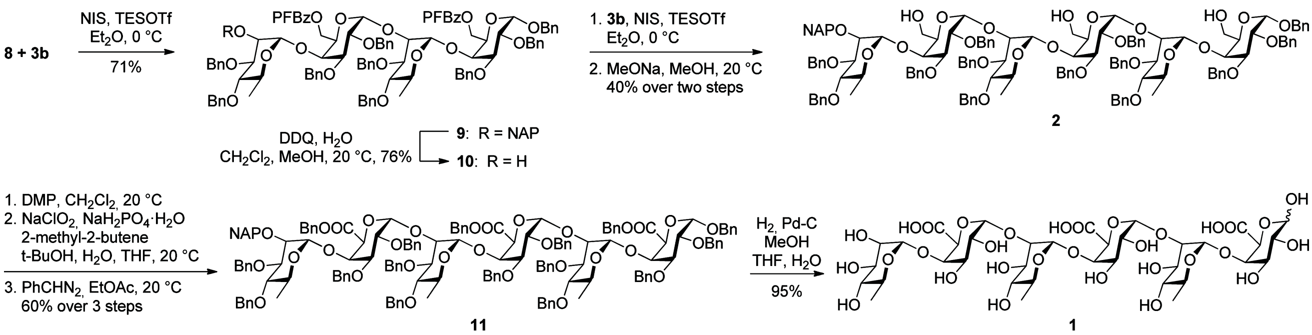
Scheme 2. Assembly of the Hexasaccharide



the PFBz-groups at C-6 of galactose, the hexasaccharide **2** was isolated in a pure form in 40% yield over two steps. The liberated primary alcohols were oxidized with Dess–Martin periodinane to aldehydes and then with $NaClO_2$ to carboxylic acids. The carboxylic acid functionalities were protected as benzyl esters by reaction with $PhCHN_2$ to facilitate purification. Finally, treatment of **11** under standard conditions for catalytic hydrogenolysis allowed removal of all the benzyl groups as well as the NAP group, furnishing the fully unprotected hexasaccharide **1**.

In summary, we have presented the first successful synthesis of a fully unprotected hexasaccharide RG-I fragment employing a highly modular synthesis that takes advantage of the armed–disarmed effect to generate the key disaccharide donor in a chemoselective fashion. We envision that this flexible strategy allows for easy introduction of side chains with galactan and arabinan, which will be the focus of future efforts, in addition to using hexasaccharide **1** in characterization of enzymes acting on RG-I.

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Supporting Information Available. Experimental procedures and analytical and spectral data, including copies of the NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.