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Zakharova, Alexandra

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Synthesis of Oligosaccharide Fragments of the Pectic Polysaccharide Rhamnogalacturonan I

PhD Thesis

Alexandra Zakharova

Department of Chemistry
Technical University of Denmark

March 2013
Synthesis of Oligosaccharide Fragments of the Pectic Polysaccharide Rhamnogalacturonan I

Alexandra Zakharova
Preface

The work described in this thesis was 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 was a part of the EU Marie Curie research network LeanGreenFood.

Chapter 1 discusses the general aspects of oligosaccharide synthesis and includes a literature review of 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 the synthesis of the branched oligosaccharide fragments of rhamnogalacturonan I and tells about the synthesis of two tetrasaccharide intermediates with diarabinan and digalactan side chains.

Chapter 4 contains the experimental procedures and compound characterization data.
Acknowledgements

I would like to express my sincere gratitude to the following people:

My advisors Associate Professor Mads H. Clausen and Professor Robert Madsen for taking me into the project and guiding during the three years, for helping to keep on track and always being a source of new research ideas. Mads – for your help with writing up my results, for the great times at the two carbohydrate conferences and your support during the preparation for my talk at one of them, for the unforgettable group dinners, for your optimism and sense of humor. Robert – for the valuable advice when the important decisions had to be made, for sharing your opinions and experiences.

Mathias Andersen – for being the best labmate and for your friendship. For your famous “I can’t see what can go wrong in this reaction...”. For teaching me some Danish and changing for the better my Russian mentality. For our many chemistry discussions and your thoughtful and detailed comments about the thesis. Hope you remember our deal you have to take me to Noma if you become a professor.

Present and past members of the Clausen group. Personally: Martin Pedersen – for your help with the branched structures project, for the great atmosphere in the office when I was writing the thesis and for translating the abstract. Mathilde Daugaard – for sharing your experience on arabinose chemistry and for bringing structure to common work. Brian Dideriksen – for being a great source of practical help and synthesis of some starting materials. Beatrice Bonora – for the nice talks and for keeping the lab so tidy, it made my last working days easier.
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Anne Hector – for your help with NMR, Janne Borg Rasmussen and Brian Ekman-Gregersen – for keeping the chemicals in order, Tina Gustafsson – for assistance with purification of compounds and obtaining MS data, Paul-Eric Wibe – for taking care of our always broken oil pump and other equipment, Lars Bruhn for great small talks.

Associate Professor Charlotte Held Gotfredsen for inviting me to be a teaching assistant for the NMR course; I had a lot of fun teaching and also learnt a lot together with the students. And for caring.

Fellow PhD students for creating a good working and social atmosphere at the department. Personally: Agnese Maggi, Kennedy Taveras, Ragnhild Ohm – for the great times we had outside work. Stig Christensen and Jens Engel-Andreasen – for sharing the duties at the PhD Club. Claus Bang – for your sense of humor. All of you for the memorable New Year Parties and the trip to Moscow.

My dear friends Ilya Makarov and Vitaly Komnatnyy for sharing you lives with me during these years, for you friendship, humor and support.

Mum, dad and my sister – for your love, compliments and criticism. My boyfriend – for your support, understanding my willingness to go abroad and trust in me.
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 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 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 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 tilhørende pektiner, sammen med homogalacturonan og rhamnogalacturonan II. Den kemiske struktur af rhamnogalacturonan I er kompleks med et skelet bestående skiftevis af α-forbundne L-rhamnose og D-galacturonsyre-enheder med mange forgreninger af arabinaner, galactaner eller arabinogalactaner placeret på C-4 i rhamnosrester.

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 den kemiske syntese af pektin oligosakkarider.

I denne afhandling præsenteres strategier for kemisk syntese af lineære og forgrenede oligosakkaridfragmente af rhamnogalacturonan.

Den første vellykkede syntese af et fuldt ubeskyttet lineært hexasakkaridfragment af rhamnogalacturonan I er opnået. Strategien implementerer en høj modulær tilgang, der drager fordel af armed-disarmed effekten til chemoselektivt at generere en n-pentenyl disakkarid nøgledonor.

To beskyttede n-pentenyl tetrasakkarid mellemprodukter, 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 konstrueret for at muliggøre kobling af større forgrenede rhamnogalacturonan I fragmenter.
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>All</td>
<td>Allyl, prop-2-ene-1-yl</td>
</tr>
<tr>
<td>Api</td>
<td>Apiose</td>
</tr>
<tr>
<td>BDA</td>
<td>Butane diacetal</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>BSP</td>
<td>1-Benzenesulfinyl piperidine</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>Benzoyl</td>
</tr>
<tr>
<td>CAN</td>
<td>Ammonium cerium(IV) nitrate</td>
</tr>
<tr>
<td>ClAc</td>
<td>Chloroacetyl</td>
</tr>
<tr>
<td>CSA</td>
<td>Camphor-10-sulfonic acid</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
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<tr>
<td>DABCO</td>
<td>1,4-Diazabicyclo[2.2.2]octane</td>
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<tr>
<td>DAST</td>
<td>(Diethylamino)sulfur trifluoride</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
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<td>DDQ</td>
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<td>Dha</td>
<td>3-Deoxy-D-lyxo-2-heptulosaric acid</td>
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<td>DMAP</td>
<td>4-(Dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
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<td>Dess-Martin periodinane</td>
</tr>
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<td>DMTST</td>
<td>Dimethylthiomethylsulfonium triflate</td>
</tr>
<tr>
<td>DQF-COSY</td>
<td>Double quantum filtered correlation spectroscopy</td>
</tr>
<tr>
<td>EDG</td>
<td>Electron-donating group</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>EWG</td>
<td>Electron-withdrawing group</td>
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<td>f</td>
<td>Furanose</td>
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<td>Abbreviation</td>
<td>Full Form</td>
</tr>
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<td>-----------</td>
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<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>Fuc</td>
<td>Fucose</td>
</tr>
<tr>
<td>Gal</td>
<td>Galactose</td>
</tr>
<tr>
<td>GalA</td>
<td>Galacturonic acid</td>
</tr>
<tr>
<td>HG</td>
<td>Homogalacturonan</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple bond correlation spectroscopy</td>
</tr>
<tr>
<td>HRMS</td>
<td>High-resolution mass spectrometry</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single quantum coherence</td>
</tr>
<tr>
<td>IDCP</td>
<td>Iodonium di-<em>sym</em>-collidine perchlorate</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared spectroscopy</td>
</tr>
<tr>
<td>Kdo</td>
<td>2-Keto-3-deoxy-D-manno-octulosonic acid</td>
</tr>
<tr>
<td>LG</td>
<td>Leaving group</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix assisted laser desorption ionization time of flight</td>
</tr>
<tr>
<td>MCPBA</td>
<td><em>m</em>-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MS</td>
<td>Molecular Sieves, Mass spectrometry</td>
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<tr>
<td>NAP</td>
<td>2-Naphthylmethyl</td>
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<tr>
<td>NBS</td>
<td><em>N</em>-Bromosuccinimide</td>
</tr>
<tr>
<td>NIS</td>
<td><em>N</em>-Iodosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>p</td>
<td>Pyranose</td>
</tr>
<tr>
<td>PFBz</td>
<td>Pentafluorobenzoyl</td>
</tr>
<tr>
<td>PG</td>
<td>Protective group</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>PMB</td>
<td><em>p</em>-Methoxybenzyl</td>
</tr>
<tr>
<td>R</td>
<td>Radical</td>
</tr>
<tr>
<td>RG</td>
<td>Rhamnogalacturonan</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Rha</td>
<td>Rhamnose</td>
</tr>
<tr>
<td>RRV</td>
<td>Relative reactivity values</td>
</tr>
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<td>t</td>
<td>Tert</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>TBA</td>
<td>Tetrabutylammonium</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-Butyldimethylsilyl</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-Butyldiphenylsilyl</td>
</tr>
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<td>TEMPO</td>
<td>2,2,6,6-Tetramethylpiperidine-1-oxyl</td>
</tr>
<tr>
<td>TES</td>
<td>Triethylsilyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>Tol</td>
<td>Tolyl, p-Methylphenyl</td>
</tr>
<tr>
<td>Tr</td>
<td>Trityl, triphenylmethyl</td>
</tr>
<tr>
<td>Ts</td>
<td>Tosyl, p-toluenesulfonyl</td>
</tr>
</tbody>
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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.\textsuperscript{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.\textsuperscript{2} It is also used in the production of biodegradable films, surface modifiers for medical devices, materials for biomedical implantation, and for drug delivery.\textsuperscript{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).\textsuperscript{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.\textsuperscript{5}

HG, the most abundant component of pectin, is a homopolymer of $\alpha$-(1$\rightarrow$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.\textsuperscript{6}

The chemical structure of RG I, the second most abundant class of pectic polysaccharides, is complex, having a backbone of alternating $\alpha$-(1$\rightarrow$4)-linked L-rhamnose and $\alpha$-(1$\rightarrow$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.

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 12 different sugars linked with 20 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

![Diagram of L-rhamnose and D-galacturonic acid]

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 plant cell wall acting enzymes. 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. 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, glycosydic 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. All the considerations given herein are based on the simplified and commonly used glycosylation mechanism. 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-\textit{cis} product is thermodynamically favored due to the anomeric effect,\textsuperscript{17} in many cases substantial amount of the kinetic 1,2-\textit{trans} product can be formed and the $\alpha/\beta$-mixtures can be obtained by reason of the irreversible nature of glycosylation reactions. Galactosyl and mannosyl donors tend to form $\alpha$-products, while $\alpha/\beta$-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 stereoselective formation of the 1,2-\textit{trans} glycosidic linkage is achieved.

\begin{scheme}
\begin{center}
\includegraphics{Scheme1.png}
\end{center}
\end{scheme}

\textbf{Scheme 1} Stereoselectivity in glycosylation reaction (for carbohydrates with the gluco-configuration). LG – leaving group, PG – protective group.
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). An impressive number of different protective groups has been developed, and the optimal conditions for their introduction and removal have been established. Preparation of monosaccharide building blocks with various protective group patterns has been described. 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. 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, glycosyl trichloroacetimidates and recently introduced N-phenyl trifluoroacetimidates, glycosyl halides, glycosyl sulfoxides, glycalcs, n-pentenyl glycosides, glycosyl thioimidates, glycosyl phosphates, etc. Various conditions are available for activation of each type of glycosyl donor. Thioglycosides, pentenyl glycosides and glycosyl imidates were employed in this work; thus their properties will be discussed in details.

Thioglycosides

Thioglycosides are 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. 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), iodonium di-sym-collidine perchlorate (IDCP), methyl trifluoromethanesulfonate (MeOTf), phenylselenyl triflate (PhSeOTf), dimethylthiomethylsulfonium triflate (DMTST), and the recently introduced sulfonium triflate activators 1-benzenesulfinyl piperidine/triflic anhydride (BSP/Tf$_2$O), and diphenyl sulfoxide/Tf$_2$O (Ph$_2$SO/Tf$_2$O) (Figure 2).

\[ O \quad N-I \quad \cdot \quad TFOH \quad \rightleftharpoons \quad O \quad N-H \quad I^{\ominus} \quad \cdot \quad OTf \]

\[ \text{MeOTf} \]

\[ \text{PhSeCl} + \text{AgOTf} \quad \rightarrow \quad \text{PhSe}^{\cdot} \cdot \text{OTf} \quad + \quad \text{AgCl} \]

\[ \text{MeS-SMe} \quad + \quad \text{MeOTf} \quad \rightarrow \quad \text{MeS-S-S-Me} \quad \cdot \quad \text{OTf} \quad \text{(DMTST)} \]

\[ \text{R} \quad \text{S}^{\cdot} \quad \text{OTf} \quad + \quad \text{TF}_{2}O \quad \rightleftharpoons \quad \text{R} \quad \text{S}^{\cdot} \quad \text{OTf} \quad \text{(BSP)} \]

\[ \text{R} = \text{N} \quad \text{(BSP)} \quad \text{R} = \text{Ph} \quad \text{(Ph}_{2}\text{SO)} \]

**Figure 2** Electrophilic reagents used for activation of thioglycosyl donors
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. 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. 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). Treatment of a thioglycoside with oxidants, such as m-chloroperoxybenzoic acid (MCPBA), affords a glycosyl sulfoxide. This makes thioglycosides particularly useful building blocks in chemoselective glycosylation strategies (vide infra).

![Figure 3](image-url) Transformation of thioglycosides into other glycosyl donors

Although thioglycosides are potent and widely employed glycosyl donors, possible aglycon transfer make 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.
**n-Pentenyl glycosides**

*n*-Pentenyl glycosides as glycosyl donors were introduced by Fraser-Reid and co-workers. 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 conditions. Pentenyl glycosides can be activated with NIS/TfOH and NIS/triethylsilyl trifluoromethanesulfonate (TESOTf) or with the less potent promoter IDCP. Alike the thio functionality, the *n*-pentenyl 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. Reaction of a pentenyl glycoside with NBS/water liberates a free hydroxyl group at the anomeric position.

**Trichloroacetimidates and N-phenyl trifluoroacetimidates**

Glycosyl imidate donors, developed by Schmidt, are probably the most commonly used nowadays owing to their ability to perform as very powerful glycosyl donors under mildly acidic conditions. Apart from application in classic oligosaccharide synthesis, trichloroacetimidates have also been used for solid-supported oligosaccharide assembly. Glycosyl trichloroacetimidates can be prepared from the corresponding anomic 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, K₂CO₃, Cs₂CO₃, can be employed. Trichloroacetiminate donors are activated with catalytic amounts of Lewis acid, typically TMSOTf or boron trifluoride diethyl etherate (BF₃·Et₂O). 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 a corresponding glycosyl acetamide is occasionally observed (Scheme 2). These obstacles can often be overcome by using $N$-phenyl trifluoroacetimidates that are considerably less reactive$^{55}$ presumably due to the lower basicity of the substituted nitrogen atom and do not undergo the corresponding rearrangement.

![Scheme 2 Rearrangement of glycosyl trichloroacetimidate](image)

In certain cases, the so-called “inverse” protocol, where the glycosyl acceptor and a catalytic amount 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 its 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:

- Reactivities of pyranosides differ as a function of sugar. Reactivity decreases in the order fucose > galactose > mannose > glucose > sialic acid.
- Protecting 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 > OBl > 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.
- Reactivity depends on the nature of leaving groups. Bulky leaving groups at the anomeric position decrease reactivity. \( \text{Para-substituents in the phenyl ring influence reactivity in the order } \text{OMe} > \text{H} > \text{NO}_2 \).
- 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\( \_2 \text{O} \). The less reactive donors can subsequently be activated when CH\( \_2 \text{Cl}_2 \) is used as a solvent.

### 1.2.3 Synthetic strategies for oligosaccharide assembly

#### Linear vs. convergent approach

Fundamentally, there are two distinct approaches to the oligosaccharide assembly: linear and convergent. In a linear approach, the carbohydrate chain is extended by one monosaccharide unit at a time (Scheme 3). 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 can be employed (Scheme 4). In this strategy, smaller oligosaccharide building blocks are synthesized separately and subsequently used for the assembly of a larger oligosaccharide.
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 leaving 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. 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. 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). 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.

![Scheme 5 Orthogonal approach in oligosaccharide synthesis](image)

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 IDCP-catalyzed glycosylations over pentenyl glycosides protected with electron-withdrawing ester protective groups (“disarmed”).

![Scheme 6 Armed-disarmed approach in oligosaccharide synthesis](image)

This difference in reactivity can be explained as follows: 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](image)

**Scheme 7** Activation of pentenyl glycosides in glycosylation reaction

The armed-disarmed approach has been applied to glycosylations with other classes of glycosyl donors, including thioglycosides,39 glycals30 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](image)

**Scheme 8** Disarming of the 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 the wide range of its practical applications and desire to understand its structure and functions in details have inspired many researches to pursue chemical syntheses of pectic oligosaccharides. A number of strategies towards the synthesis of oligosaccharide fragments of HG, RG I and RG II have been reported in the literature. 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.\textsuperscript{68} 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 should be noted that the non-oxidized carbohydrates are generally more reactive glycosyl donors than their oxidized counterparts,\textsuperscript{69-71} where reactivity is decreased by the presence of the electron-withdrawing carboxyl groups. 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

<table>
<thead>
<tr>
<th>Synthetic oligosaccharide fragment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homogalacturonan fragments</strong></td>
<td></td>
</tr>
<tr>
<td>(\alpha-D-GalpA-((1\rightarrow4))-D-GalpA)</td>
<td>Magaud et al.72</td>
</tr>
<tr>
<td>Two monomethyl esterified isomers</td>
<td></td>
</tr>
<tr>
<td>Protected mono- and dimethyl- esterified methyl (\alpha)- and (\beta)-glycosides</td>
<td>Magaud et al.73</td>
</tr>
<tr>
<td>Protected dimethyl esterified allyl (\beta)-glycoside</td>
<td>Kramer et al.74</td>
</tr>
<tr>
<td>Protected dimethyl esterified allyl (\alpha)-glycoside</td>
<td>Vogel et al.75</td>
</tr>
<tr>
<td>(\alpha-D-GalpA-((1\rightarrow4))-(\alpha-D-GalpA-((1\rightarrow4))-D-GalpA)</td>
<td>Clausen et al.76</td>
</tr>
<tr>
<td>Three monomethyl esterified isomers</td>
<td></td>
</tr>
<tr>
<td>Protected fully methyl esterified allyl (\beta)-glycoside</td>
<td>Kramer et al.74</td>
</tr>
<tr>
<td>(\alpha-D-GalpA-((1\rightarrow4))-((\alpha-D-GalpA-((1\rightarrow4)))-D-GalpA)</td>
<td>Clausen &amp; Madsen67</td>
</tr>
<tr>
<td>Five partially methyl esterified compounds</td>
<td></td>
</tr>
<tr>
<td>(\alpha-D-GalpA-((1\rightarrow4))-((\alpha-D-GalpA-((1\rightarrow4)))-\beta-D-GalpA-OPr)</td>
<td>Nakahara &amp; Ogawa77</td>
</tr>
<tr>
<td>(\alpha-D-GalpA-((1\rightarrow4))-((\alpha-D-GalpA-((1\rightarrow4)))-\beta-D-GalpA-\beta-L-Rhap-OMe)</td>
<td>Nakahara &amp; Ogawa78</td>
</tr>
<tr>
<td><strong>Rhamnogalacturonan II fragments</strong></td>
<td></td>
</tr>
<tr>
<td>(\beta-L-Rhap-((1\rightarrow3'))-(\beta-D-ApiJ-OMe)</td>
<td>Buffet et al.79</td>
</tr>
<tr>
<td>(\beta-L-Rhap-((1\rightarrow3'))-(\beta-D-ApiJ-OMe)</td>
<td>Nepogodiev et al.80</td>
</tr>
<tr>
<td>(\beta-L-Rhap-((1\rightarrow3'))-(\beta-D-ApiJ-((1\rightarrow2))-(\alpha-D-GalpA-OMe)</td>
<td>Chauvin et al.81</td>
</tr>
<tr>
<td>(\beta-L-Rhap-((1\rightarrow3'))-(\beta-D-ApiJ-((1\rightarrow2))-(\alpha-D-GalpA-OMe)</td>
<td>Nepogodiev et al.82</td>
</tr>
<tr>
<td>(\alpha-L-Fucp-((1\rightarrow4))-(\alpha-L-Rhap) (free disaccharide and methyl (\alpha)- and (\beta)-glycosides)</td>
<td>Egelund et al.83</td>
</tr>
<tr>
<td>(\beta-D-GalpA-((1\rightarrow3))-(\alpha-L-Rhap-OMe)</td>
<td>Chauvin et al.84</td>
</tr>
<tr>
<td>(\beta-D-GalpA-((1\rightarrow3))-(\alpha-D-GalpA-((1\rightarrow2))-(\alpha-L-Rhap-OMe)</td>
<td>Chauvin et al.84</td>
</tr>
<tr>
<td>Synthetic oligosaccharide fragment</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>α-L-Fucp-(1→4)-[β-D-GalpA-(1→3)]-[α-D-GalpA-(1→2)]-α-L-Rhap-OMe</td>
<td>Chauvin et al.$^{84}$</td>
</tr>
<tr>
<td>Acef</td>
<td>Jones et al.$^{85}$</td>
</tr>
<tr>
<td>β-L-Acef-(1→3)-α-L-Rhap-OMe (partially protected)</td>
<td>Nepogodiev et al.$^{82}$</td>
</tr>
<tr>
<td>α-L-Rhap-(1→3)-α-L-Arap-(1→4)-[2-O-β-L-MeFucp-(1→2)]-β-D-Galp-O(CH$_3$)$_2$NH$_2$</td>
<td>Timmer et al.$^{86}$</td>
</tr>
<tr>
<td>β-L-Araf-(1→3)-α-L-Rhap-(1→2)-[α-L-Rhap-(1→3)]-[α-L-Arap-(1→4)-2-OMe-β-L-Fucp-(1→2)]-β-D-Galp-O(CH$_3$)$_2$NH$_2$</td>
<td>de Oliveira et al.$^{87}$</td>
</tr>
<tr>
<td>β-L-Araf-(1→3)-α-L-Rhap-(1→2)-[α-L-Rhap-(1→3)]-[α-L-Arap-(1→4)-O(CH$_3$)$_2$NH$_2$</td>
<td>Rao &amp; Boons$^{88}$</td>
</tr>
</tbody>
</table>

**Rhamnogalacturonan I fragments**

<table>
<thead>
<tr>
<th>Synthetic oligosaccharide fragment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-D-GalpA-(1→2)-α-L-Rhap-(1→4)-D-GalpA (dimethyl esterified and partially protected)</td>
<td>Nolting et al.$^{90}$</td>
</tr>
<tr>
<td>α-L-Rhap-(1→4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→4)-β-D-GalpA-OPr</td>
<td>Maruyama et al.$^{91}$</td>
</tr>
<tr>
<td>α-L-Rhap-(1→4)-α-D-GalpA-(1→2)-2-α-L-Rhap-(1→4)-α-D-GalpA-OMe (with free and dimethyl esterified GalpA residues)</td>
<td>Nemati et al.$^{92}$</td>
</tr>
<tr>
<td>α-L-Rhap-(1→4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→4)-D-GalpA (with free and monomethyl esterified GalpA residues)</td>
<td>Reiffarth &amp; Reimer$^{93}$</td>
</tr>
<tr>
<td>α-L-Rhap-(1→4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→4)-D-GalpA</td>
<td>Scanlan et al.$^{94}$</td>
</tr>
</tbody>
</table>

### 1.3.1 Synthetic studies of RG I oligosaccharides

RG I polysaccharides have a common backbone with repeating disaccharide unit α-D-GalpA-(1→2)-α-L-Rhap-(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 \(\beta-(1\rightarrow4)\)-linked D-galactose residues. Arabinans are chains of \(\alpha-(1\rightarrow5)\)-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 \(\beta-(1\rightarrow4)\)-galactan with arabinan branches; highly branched arabinogalactan II with \(\beta-(1\rightarrow3)\)-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.\(^{47}\)

![Image of RG I chemical structure]

**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**.
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). 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 the RG I backbone**

In 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). 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.
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\textsuperscript{(5)} 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 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, 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. 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 the 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). The strategy employed trityl-cyanoethylidene condensation and thioglycoside methodology. Galacturonic acid was used as a starting material.
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). 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.
Scheme 12 Modular design approach for synthesis of RG I fragments by Vogel and co-workers
Rhamnosyl donor 21 and galactosyluronic acceptor 22 were coupled in the TMSOTf-catalyzed glycosylation reaction to produce the 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.90 Contrary to the observations of Reimer and co-workers,93 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 the fully unprotected propyl glycoside of RG I tetrasaccharide

Takeda and co-workers\(^9\) 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 the 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 (BnBr) 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 TEMPO, KBr and NaClO in aqueous NaHCO\(_3\), which provided the target tetrasaccharide 51 in 37% yield over two steps.
Scheme 13 Synthesis of the 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).$^{94}$ 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 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/NaClO₂ 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/H₂O 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 chapter, synthesis of the fully unprotected linear fragment of the RG I backbone is described. Its structure is depicted in Figure 6.

Figure 6 Target hexasaccharide fragment of the RG I backbone
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 more reactive glycosyl donors than corresponding uronic acids, where the reactivity is decreased by the presence of the electron-withdrawing carboxyl groups. 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-alturonic 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.

![Figure 7 Retrosynthesis of the linear hexasaccharide fragment of the RG I backbone](image-url)

66 67 68 69 70
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 anomic positions were chosen due to their ability to function both as leaving groups and as temporary protective groups and perform well in armed-disarmed couplings98 (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 can 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 conditions97 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. 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). 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 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.
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 BF₃·OEt₂-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 Ley¹⁰⁰,¹⁰¹ 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 TBAI. The BDA protective group was then hydrolyzed under acidic conditions to afford diol 76. The reaction had to be performed carefully as 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 (BnBr) 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.

The BF₃·OEt₂-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 BnBr 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-toluenesulfonic 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 the possibilities of their chemoselective coupling (Table 2).

Table 2 Attempts to synthesize the thiophenyl disaccharide donor 83

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>D:A</th>
<th>Activator</th>
<th>Solvent</th>
<th>T, °C</th>
<th>Yield, %</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>1.2</td>
<td>NIS/TESOTf²</td>
<td>Et₂O</td>
<td>–20</td>
<td>51</td>
<td>83+84 mixt.</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>1.2</td>
<td>NIS/TESOTf</td>
<td>CH₂Cl₂</td>
<td>–20</td>
<td>50</td>
<td>83+84 mixt.</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>1.2</td>
<td>NIS/TESOTf</td>
<td>1:1 CH₂Cl₂/Et₂O</td>
<td>–20</td>
<td>45</td>
<td>83+84 mixt.</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>1.2</td>
<td>NIS/TESOTf</td>
<td>Et₂O</td>
<td>–40</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>1.2</td>
<td>NIS/TESOTf</td>
<td>Et₂O</td>
<td>0</td>
<td>35</td>
<td>83+84 mixt.</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>1.8</td>
<td>NIS/TESOTf</td>
<td>Et₂O</td>
<td>–20</td>
<td>48</td>
<td>83+84 mixt.</td>
</tr>
<tr>
<td>7</td>
<td>69</td>
<td>1.2</td>
<td>I₂³</td>
<td>CH₂Cl₂</td>
<td>20</td>
<td>&lt;20</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>69</td>
<td>1.2</td>
<td>I₂</td>
<td>CH₂Cl₂</td>
<td>20</td>
<td>&lt;15</td>
<td>K₂CO₃ added</td>
</tr>
</tbody>
</table>
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 83 but only as approximately an 1.5:1 mixture with the trisaccharide by-product 84 in a total yield of 51% (entry 1). The trisaccharide by-product 84 presumably arose from glycosylation of acceptor 70 with the disaccharide donor 83 formed in the course of the reaction. The mixture of 83 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 donors58 and, in addition, the disaccharide donor 83 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$_2$Cl$_2$ (entry 2) or 1:1 ether/CH$_2$Cl$_2$ 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).

Subjecting the mixture of disaccharide 83 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 83 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$_2$Cl$_2$ at 20 °C in the presence of 4 Å molecular sieves with or without additives such as potassium carbonate and tetrabutylammonium iodide (TBAI) (entries 7,8 and
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. Interestingly, in order to enable the formation of the 1,2-\textit{trans}-diequatorial junction in the bicyclic product 85 the sugar ring underwent a conformational change from \( \text{aC}^1 \) to \( \text{cC}^1 \), as evident from the NMR spectra.

![Scheme 18 Iodine-promoted formation of C-glycoside](image)

Given the lack of success in synthesizing the disaccharide 83 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\(_2\)Cl\(_2\) 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\(_2\)Cl\(_2\) 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 \textit{in situ} anomerisation conditions (vide infra) did not afford the target disaccharide 83 presumably due to the insufficient nucleophilicity of the C-4 hydroxyl group in the galactosyl acceptor 70.
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 results are summarized in Error! Reference source not found.).

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 was 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 can be applied. However, unlike the 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 building block 92

2.2.4 Synthesis of the pentenyl disaccharide donor

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** Glycosylation conditions for synthesis of the pentenyl disaccharide 83

<table>
<thead>
<tr>
<th>Entry</th>
<th>D:A&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Time</th>
<th>Solvent</th>
<th>T, °C</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>1.5 h</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>–20</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>40 min</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>–20</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>3 h</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>–40</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>20 min</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>30 min</td>
<td>1:1 CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;/Et&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>–20</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>1.2</td>
<td>15 min</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>–20</td>
<td>45</td>
</tr>
</tbody>
</table>

<sup>1</sup>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 temperature (–40 °C) the coupling was less efficient (entry 3); at higher temperature (0 °C) more decomposition products were observed (entry 4). Performing the reaction in a 1:1 ether/CH<sub>2</sub>Cl<sub>2</sub> mixture (entry 5) instead of pure ether did not change the glycosylation yield,
while using pure CH₂Cl₂ (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 essential 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 galactose acceptor 93 bearing an acetyl group](image)

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](image)

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 assemble the target hexasaccharide 66. It was planned to be obtained from 83 (Figure 8).

![Figure 8 Disaccharide acceptor 95](image)

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 $\alpha/\beta$-mixture (as judged by NMR). Such a low stereoselectivity was observed presumably due to the high reactivity of benzyl alcohol.

![Scheme 23 Glycosylation of benzyl alcohol with disaccharide donor 83](image)

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 $\alpha/\beta$-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. 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 the α-anomer in 90% yield (Scheme 25).

Scheme 25 Synthesis of benzyl disaccharide 98

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, but other oxidizing agents, such as ammonium cerium(IV) nitrate (CAN), can be used. For acidic hydrolysis, TFA or, as recently reported by Liu and co-workers, HF/pyridine can be employed. Examples of selective hydrogenolysis of the NAP-ether in the presence of benzyl ethers are also known.

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 a NAP-group

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>Solvent</th>
<th>T, °C</th>
<th>Time, h</th>
<th>Yield, %</th>
<th>Work-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DDQ</td>
<td>CH₂Cl₂/MeOH/H₂O</td>
<td>20</td>
<td>3</td>
<td>75</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>DDQ</td>
<td>CH₂Cl₂/H₂O</td>
<td>20</td>
<td>2</td>
<td>67</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>DDQ</td>
<td>CH₂Cl₂/MeOH/H₂O</td>
<td>20</td>
<td>3</td>
<td>42</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>DDQ</td>
<td>CH₂Cl₂/phosphate buffer pH 7.2</td>
<td>20</td>
<td>12</td>
<td>38</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>DDQ</td>
<td>CH₂Cl₂/MeOH/H₂O</td>
<td>0</td>
<td>24</td>
<td>70</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>HF/Py</td>
<td>toluene</td>
<td>20</td>
<td>2</td>
<td>30</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>TFA</td>
<td>toluene</td>
<td>20</td>
<td>2</td>
<td>65</td>
<td>B</td>
</tr>
<tr>
<td>8</td>
<td>TFA</td>
<td>toluene</td>
<td>0</td>
<td>24</td>
<td>65</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>TFA</td>
<td>toluene</td>
<td>20</td>
<td>2</td>
<td>40</td>
<td>A</td>
</tr>
</tbody>
</table>

¹Isolated yields after flash chromatography
²A – direct evaporation, B – work-up with saturated aqueous NaHCO₃

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 CH₂Cl₂/MeOH (entry 1) was found to be preferable to using CH₂Cl₂ 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 NaHCO₃, 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 8). Work-up with a saturated aqueous solution of NaHCO₃ 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 CH₂Cl₂/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).
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 temperature (0 °C) was 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.
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\textsuperscript{111} to aldehydes and then with sodium chlorite\textsuperscript{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.\textsuperscript{113} The protected hexasaccharide 102 was obtained in 60% yield over 3 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 the global deprotection
2.3 NMR assignment of the target hexasaccharide

The structure of the 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 \(\alpha\)- and the \(\beta\)-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: \(\alpha\)- and \(\beta\)-linkages for 1Gal (1\(\alpha\)H\(1 \delta_H 5.32, J = 5.7\) Hz, 1\(\beta\)H\(1 \delta_H 4.60, J = 7.4\) Hz), \(\alpha\)-linkage for 2+4Rha and 6Rha (2+4H\(1 \delta_H 5.29, J = 4.9\) Hz, 6H\(1 \delta_H 5.25, J = 4.2\) Hz), \(\alpha\)-linkage for 3+5Gal (3+5H\(1 \delta_H 5.05, J = 5.2\) Hz). Some of the anomeric configurations could be confirmed by measuring the one-bond C-H coupling constants from the HMBC spectrum. The \(\text{J}_{C,H}\) values determined were 169.6 Hz for 2+4Rha and 173.5 Hz for 6Rha indicating the \(\alpha\)-linkages and 160.3 Hz for 1\(\beta\)Gal indicating the \(\beta\)-linkage.

The HMBC spectrum was used to locate 1\(\alpha\)C\(6\), 1\(\beta\)C\(6\) and 3+5C\(6\) carboxylic acid resonances (strong signals for 1\(\beta\)C\(6\) and 3+5C\(6\), weak signal for 1\(\alpha\)C\(6\)). The \(^{13}\)C resonances of 1\(\alpha\)C\(4\), 1\(\beta\)C\(4\), 2+4C\(2\) and 3+5C\(4\) 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+4H\(1\) and 1\(\alpha\)C\(4\), 3+5H\(1\) and 2+4C\(2\), 6H\(1\) and 3+5C\(4\) in the HMBC spectrum.
Table 5 \(^1^H\) and \(^{13}^C\) resonance assignments for the target hexasaccharide 66

<table>
<thead>
<tr>
<th>Residue</th>
<th>Position in the sugar ring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1α-GalA</td>
<td>5.32</td>
</tr>
<tr>
<td></td>
<td>93.2</td>
</tr>
<tr>
<td>1β-GalA</td>
<td>4.60</td>
</tr>
<tr>
<td></td>
<td>97.1</td>
</tr>
<tr>
<td>2+4Rha</td>
<td>5.29</td>
</tr>
<tr>
<td></td>
<td>99.4</td>
</tr>
<tr>
<td>3+5GalA</td>
<td>5.05</td>
</tr>
<tr>
<td></td>
<td>98.4</td>
</tr>
<tr>
<td>6Rha</td>
<td>5.25</td>
</tr>
<tr>
<td></td>
<td>101.6</td>
</tr>
</tbody>
</table>
**Figure 9** $^1$H NMR of hexasaccharide 66

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

In summary, we have presented the first successful synthesis of a fully unprotected hexasaccharide fragment of the RG I backbone, employing a highly modular synthesis that takes advantage of the armed-disarmed effect to generate the key disaccharide donor in a chemoselective fashion. We envisioned that this strategy 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 the 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 the 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,92 the branched RG I fragments have not been previously 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 (see Chapter 4).

Here, 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.
3.1 Retrosynthetic analysis

Thinking about 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 13 Error! Reference source not found.).
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. To make the whole synthesis logical and 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 glycosydic linkages in the diarabinan and digalactan side chain fragments required using the 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. Obviously, 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.

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

Figure 13 Retrosynthetic analysis of the target tetrasaccharide 105

Figure 14 Monosaccharide building blocks required for the 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 4 steps; its synthesis is shown in Scheme 29.

First, the non-protected monosaccharide was transformed into the methyl glycoside 114 in two straightforward steps: a Fischer glycosylation\textsuperscript{115} of methanol under kinetic control (to insure the formation of the furanose form) followed by benzylation with benzoyl chloride (BzCl) in pyridine.\textsuperscript{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)\textsuperscript{117} to give hemiacetal 115 in 70% yield. Subsequent reaction with N-phenyl trifluoroacetimidoyl chloride\textsuperscript{26} in the presence of cesium carbonate in CH$_2$Cl$_2$ 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.

N-phenyl trifluoroacetimidate donor 112 was prepared from commercially available D-galactose in 4 steps; its synthesis is shown in Scheme 30. The nonprotected monosaccharide was converted into the tetrabenoate 116 in 87% yield by treatment with benzoyl chloride (BzCl) in pyridine.\textsuperscript{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\textsuperscript{119} to afford hemiacetal 118 in 70% yield over 2 steps. Reaction of 118 with N-phenyl trifluoroacetimidoyl chloride\textsuperscript{26} in the presence of cesium carbonate in CH$_2$Cl$_2$ afforded donor 112 as a $\alpha/\beta$-mixture in 85% yield.

![Scheme 30 Synthesis of the galactose imidate donor](image)

Acceptor 113 was synthesized in 2 steps from diol 89 (Scheme 31), which was employed in our synthesis of the linear hexasaccharide 66.
First, the C-2 and C-3 hydroxyl groups were protected with benzoyl groups by the reaction with benzoyl chloride (BzCl) 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 of the C-6 monobenzylated products. The regioselectivity of this process depends on the reagents used. For instance, employing LiAlH₄–AlCl₃ generally gives the C-4 monobenzylated products, while using NaCNBH₃–HCl provides the C-6 isomer. A number of other reagents are also available. The reductive opening of the benzylidene acetal in 119 with NaCNBH₃–HCl in tetrahydrofuran gave acceptor 113 in 82% yield.

**Synthesis of the rhamnose acceptor**

Rhamnose thioglycoside acceptor 108 was designed to bear a temporary 2-naphthylmethyl (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), copper(II), mercury(II), and nickel(II) and boron-containing compounds. The most widely used of these methods are tin-mediated alkylations. Cyclic dibutylstannylene derivatives of carbohydrates can be prepared by reaction with dibutyltin(IV) oxide (Bu₂SnO) or dibutyldimethoxytin (Bu₂Sn(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.

In the fully unprotected rhamnosyl glycosides, tin chemistry offers a method for selective protection of the C-3 hydroxyl group. Rhamnose triol 73 was selectively benzylated at the C-3 position, in 55% yield, by reaction with Bu₂SnO followed by treatment with benzyl bromide (BnBr) in the presence of tetrabutylammonium iodide (TBAI) in refluxing toluene (Scheme 32).

![Scheme 32 Regioselective benzylation of triol 73](image)

In general, because of its higher acidity the C-2 hydroxyl displays the highest reactivity among all secondary hydroxyl groups in carbohydrates. Therefore, we expected that it would be possible to selectively protect the C-2 position in diol 120 with a NAP-group. In 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. When 120 was subjected to the reaction with 2-(bromomethyl)naphthalene (NAPBr) in the mixture of CH₂Cl₂ and aqueous sodium hydroxide in the presence of the phase-transfer catalyst tetrabutylammonium hydrogen sulfate (TBAHSO₄), 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 sufficient amounts of the unreacted
starting material left after the 48 hour reaction. Interestingly, the reaction of 120 with NAPBr in the presence of sodium hydride and tetrabutylammonium iodide (TBAI) in DMF (entry 2) in our hands gave higher yields than the protection under the phase-transfer conditions. This reaction produced the desired 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 and potassium iodide in CH₂Cl₂ gave 108 in less than 30% 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 of diol 114 with trichloroacetimidate 121 in ether (entry 4) procured mainly the undesired regioisomer presumably due to the less steric hindrance of the equatorial C-4 hydroxyl group.

Table 6 Selective protection of the C-2 hydroxyl group in rhamnose derivative 114

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction conditions</th>
<th>T, °C</th>
<th>Time, h</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NAPBr, TBAHSO₄, aq. NaOH, CH₂Cl₂</td>
<td>40</td>
<td>48</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>NAPBr, NaH, TBAI, DMF</td>
<td>0 to 20</td>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>NAPBr, Ag₂O, KI, CH₂Cl₂</td>
<td>20</td>
<td>48</td>
<td>&lt;40</td>
</tr>
<tr>
<td>4</td>
<td>121, TMSOTf, Et₂O</td>
<td>0 to 20</td>
<td>12</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

121 was prepared from 1-naphthalenemethanol by treatment with trichloroacetonitrile in the presence of cesium carbonate in CH₂Cl₂.
3.2.2 Synthesis of the disaccharide side chains

Synthesis of diarabinan donor

Synthesis of the diarabinan N-phenyl trifluoroacetimidate donor 109 was performed in 6 steps starting from the arabinose donor 110 (Scheme 33).

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 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 CH₂Cl₂ 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

Scheme 33 Synthesis of the diarabinan N-phenyl trifluoroacetimidate donor 109
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)-glycosydic 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 diarabinan](image)

Triol 123 was transformed into the fully protected arabinose derivative 126 through 2 steps performed one-pot. First, the primary hydroxyl group in 123 was selectively protected with the tert-butylidiphenylsilyl (TBDPS) group by treatment with tert-butylidiphenylchlorosilane (TBDPSCI) in pyridine at 0 °C. This was followed by the esterification of the remaining free hydroxyls with benzoyl esters in 82% yield over 2 steps. The TBDPS-group is 126 was then selectively cleaved in 75% yield by treatment with a 1M solution of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran 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 126 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 1H NMR spectra of the main three fractions obtained after the 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 benzoyl protective groups.

Finally, hemiacetal 126 was transformed to the target disaccharide donor 109 in 87% yield by the reaction with N-phenyl trifluoroacetimidoyl chloride\textsuperscript{26} in the presence of cesium carbonate in CH\textsubscript{2}Cl\textsubscript{2}.

**Synthesis of digalactan**

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\textsubscript{2}Cl\textsubscript{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-glycosydic 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)\textsuperscript{33} in the mixture of acetone and water resulted in multiple products. Alternatively, this transformation could be performed in 2 steps. First, the pentenyl disaccharide 129 was titrated with a solution of bromine in CH\textsubscript{2}Cl\textsubscript{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\textsuperscript{119}. This approach afforded hemiacetal 131 in 69% yield over 2 steps. Reaction of 131 with N-phenyl trifluoroacetimidoyl chloride\textsuperscript{26} in the presence of cesium carbonate in CH\textsubscript{2}Cl\textsubscript{2} gave the target digalactan donor 132 in 85% yield.

\begin{center}
\textbf{Scheme 35} Synthesis of the digalactan N-phenyl trifluoroacetimidate donor 130
\end{center}
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 X.

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-glycosydic linkage. The benzoyl esters in 107 were exchanged for the permanent benzyl protective groups in 2 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 (BnBr) 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](image-url)
The digalactan-containing trisaccharide 106 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 (BnBr) 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](image)

**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 CH₂Cl₂ in the presence of TMSOTf. The reaction proceeded smoothly according to TLC and the disaccharide product 137 was subjected directly to the Zemplén conditions. Triol 138 was isolated in 70% yield over 2 steps. The TMSOTf-mediated glycosylation of the primary C-5 hydroxyl group in 138 with
the same donor 109 in CH₂Cl₂ 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 deacylation conditions (MeONa, MeOH) followed by the protection of the free hydroxyls with the benzyl groups (treatment with benzyl bromide (BnBr) in the presence of NaH and catalytic amounts of TBAI in DMF). The target trisaccharide donor 106 was obtained in XX% yield.

Scheme 38 Synthesis of trisaccharide 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 5 steps instead of 9 starting from the same monosaccharide building blocks 108 and 110.
Synthesis of tetrasaccharides

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 Et₂O 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](image)

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 the chemoselective glycosylations. The methods were tested on the coupling of two monosaccharides 69 and 92 (Table 7).
Table 7 Screening of the glycosylation conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acceptor</th>
<th>Activator</th>
<th>Solvent</th>
<th>T, °C</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>NIS/Yb(OTf)_3</td>
<td>CH₂Cl₂</td>
<td>−20</td>
<td>n.d.</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
<td>NIS/Yb(OTf)_3</td>
<td>CH₂Cl₂</td>
<td>0</td>
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</tr>
<tr>
<td>3</td>
<td>92</td>
<td>MeOTf</td>
<td>CH₂Cl₂</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>NIS/Yb(OTf)_3</td>
<td>CH₂Cl₂</td>
<td>0</td>
<td>&lt;20²</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>MeOTf</td>
<td>CH₂Cl₂</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>93</td>
<td>Ph₂SO/Tf₂O</td>
<td>CH₂Cl₂</td>
<td>−60</td>
<td>n.d.</td>
</tr>
<tr>
<td>7</td>
<td>93</td>
<td>DMTST</td>
<td>CH₂Cl₂</td>
<td>−40</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>93</td>
<td>Me₂S₂/Tf₂O</td>
<td>CH₂Cl₂</td>
<td>−40</td>
<td>68</td>
</tr>
<tr>
<td>9</td>
<td>93</td>
<td>Me₂S₂/Tf₂O</td>
<td>Et₂O</td>
<td>−40</td>
<td>38</td>
</tr>
</tbody>
</table>

¹n.d. – almost no disaccharide product was observed

²based on TLC analysis

Fraser-Reid and co-workers have demonstrated 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 could be selectively activated over the disarmed pentenyl glycosides by NIS/Yb(OTf)_3. When a mixture of 69 and 92 in CH₂Cl₂ 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 process was discussed in Chapter 2) through an intramolecular cyclization. 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\textsuperscript{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\textsubscript{2}Cl\textsubscript{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)\textsubscript{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.\textsuperscript{48} One of these promoters is a combination of diphenyl sulfoxide and triflic anhydride (Ph\textsubscript{2}SO/Tf\textsubscript{2}O) recently introduced by van der Marel and co-workers.\textsuperscript{143} It was shown to be capable of activating various thioglycosides and promoting high yielding glycosylations. When donor 69 was
treated with Ph₂SO/Tf₂O at –60 °C in CH₂Cl₂ 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 Ph₂SO/Tf₂O under the same conditions without adding acceptor 93. After 5 minutes the reaction was stopped by addition of a saturated aqueous NaHCO₃. 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 Fugedi⁴³ and the dimethyl disulfide-triflic anhydride (Me₂S₂/Tf₂O) system developed later by Fugedi and co-workers.¹⁴⁴ The DMTST-promoted glycosylation of acceptor 93 with donor 69 at –40 °C in CH₂Cl₂ (entry 7) resulted in 40% yield of disaccharide 94. The same reaction mediated by Me₂S₂/Tf₂O (entry 8) furnished the target disaccharide 94 in 68% yield. Changing the solvent from CH₂Cl₂ 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 Me₂S₂/Tf₂O was used as a promoter and the glycosylation was performed in CH₂Cl₂. 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 the found glycosylation conditions to the coupling of the trisaccharide donor 106 with acceptor 93. Regrettably, treatment of 106 and 93 with Me₂S₂/Tf₂O at –40 °C in CH₂Cl₂ resulted mainly in the undesired formation of the C-glycoside. The target tetrasaccharide 140 was obtained in only 20% yield (Scheme 40).
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 be used instead of the NAP-group as it could be selectively removed in the presence of the C-6 acetyl group by treatment with thiourea.

The monosaccharide donor 141 bearing the ClAc-group was prepared (Scheme 41) and the possibility of its coupling with acceptor 93 was studied (Table 8).

The exchange of the NAP-group for the ClAc was performed in 2 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 CH₂Cl₂ and methanol. The released hydroxyl group was then esterified by the reaction with trichloroacetic anhydride (ClAc₂O) in the presence of triethylamine in CH₂Cl₂ at 0 °C. Donor 141 was obtained in 92% yield.

We tested the promoter system that performed best in the previous experiments (Me₂S₂/Tf₂O) and the two systems where the side reactions were
caused by the cyclization of the donor (NIS/Yb(OTf)₃ and Ph₂SO/Tf₂O). The Me₂S₂/Tf₂O-mediated glycosylation of acceptor 94 with donor 141 performed at −40 °C in CH₂Cl₂ (entry 1) resulted in the formation of the disaccharide product 142 in 60% yield. Substitution of the NAP-group for the ClAc-group did not significantly change the yield of the NIS/Yb(OTf)₃-promoted coupling (entry 2). Even though the formation of the cyclic C-glycoside was not observed, significant decomposition of the acceptor took place under the reaction conditions leading to the low yield. However, the outcome of the Ph₂SO/Tf₂O-mediated glycosylation (entry 3) was improved and disaccharide 142 was obtained in 45% yield.

**Table 8** Coupling of ClAc donor 141

<table>
<thead>
<tr>
<th>Entry</th>
<th>Activator</th>
<th>Solvent</th>
<th>T, °C</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Me₂S₂/Tf₂O</td>
<td>CH₂Cl₂</td>
<td>−40</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>NIS/Yb(OTf)₃</td>
<td>CH₂Cl₂</td>
<td>0</td>
<td>&lt;10</td>
</tr>
<tr>
<td>3</td>
<td>Ph₂SO/Tf₂O</td>
<td>CH₂Cl₂</td>
<td>−60</td>
<td>45</td>
</tr>
</tbody>
</table>

To conclude, exchanging the NAP-group for the ClAc allowed for avoiding the undesired cyclization reaction. Me₂S₂/Tf₂O and Ph₂SO/Tf₂O were found to be the most promising promoter systems and were subsequently applied in the glycosylation with the trisaccharide donors.

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

![Chemical structure](image)

Scheme 42 Introducing the ClAc group into the trisaccharide donor

Trisaccharide 111 subjected to treatment with DDQ in the presence of water in the mixture of CH₂Cl₂ and methanol resulting in the formation of 143 in 73% yield. The hydroxyl group in 143 was protected with the ClAc-ester in 94% yield by reaction with trichloroacetic anhydride (ClAc₂O) in the presence of triethylamine in CH₂Cl₂ at 0 °C.

Glycosylation of acceptor 93 with the prepared trisaccharide donor 144 was studied. When Me₂S₂/Tf₂O was applied as a promoting system and the reaction was performed in CH₂Cl₂ at −40 °C, the tetrasaccharide product 145 was isolated in 20% yield. Using Ph₂SO/Tf₂O as a promoter and performing the glycosylation in in CH₂Cl₂ at −60 °C allowed for obtaining 145 in 45% yield.
Table 4 Synthesis of the diarabinan-containing tetrasaccharide 145

<table>
<thead>
<tr>
<th>Entry</th>
<th>Activator</th>
<th>Solvent</th>
<th>T, °C</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Me₂S₂/Tf₂O</td>
<td>CH₂Cl₂</td>
<td>–40</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Ph₂SO/Tf₂O</td>
<td>CH₂Cl₂</td>
<td>–60</td>
<td>40</td>
</tr>
</tbody>
</table>

We believe that further optimization of the reaction conditions is possible leading to higher yields of the target tetrasaccharide 145, which will be the focus of future efforts.
4 Experimental

General information

All reagents and solvents were purchased from Sigma-Aldrich and used without further purification, except for diethyl ether that was distilled over sodium/benzophenone prior to use in glycosylation reactions. 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(SO₄)₂ (2.5 g) and (NH₄)₆Mo₇O₂₄ (6.25 g) in 10% aqueous H₂SO₄ (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. Chemical shifts δ are reported in ppm using the solvent resonance as the internal standard (CDCl₃: ¹H 7.27 ppm, ¹³C 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.

General Procedure I for glycosylation reactions

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 disaccharide 3b) or to 0 °C (for synthesis of the tetrasaccharide 9 and the hexasaccharide 11), 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 triethylamine (0.1 ml), diluted with CH₂Cl₂ (50 ml) and washed with 10% aq. Na₂S₂O₃ (2 x 20 ml). The combined aqueous phases were extracted with CH₂Cl₂ (20 ml). The combined organic phases were dried with MgSO₄, 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 CH₂Cl₂/MeOH 4:1 (15 ml) and water (0.5 ml) was added followed by addition of DDQ (480 mg, 2.1 mmol). The reaction mixture was stirred at room temperature until TLC (toluene/EtOAc 10:1) showed completion of the reaction (2 – 5 h). The reaction mixture was diluted with CH₂Cl₂ (100 ml) and washed with sat. aq. NaHCO₃ (2 x 50 ml). The combined aqueous phases were extracted with CH₂Cl₂ (20 ml). The combined organic phases were dried with MgSO₄, filtered, concentrated and purified by flash chromatography (toluene/EtOAc 15:1).

**Synthesis of monosaccharide building block**

\[
\text{AcO} \quad \text{OAc} \\
\text{AcO} \quad \text{OAc}
\]

L-Rhamnose monohydrate (20 g, 0.11 mol) was suspended in CH₂Cl₂ (100 ml), cooled in ice bath (0 °C) then Et₃N (120 ml, 0.878 mol) and DMAP (1.34 g, 0.011 mol) were added. Ac₂O (62 ml, 0.66 mol) was added dropwise (during the addition the temperature should be kept below 5°C). The reaction was allowed to stay at room temperature. When the reaction completed (checked by TLC, eluent: heptan/EtOAc: 1:1) MeOH (2.5 ml) was added. Resulting mixture was diluted with DCM (100 ml) and washed with 1 M HCl (2 x 50 ml) and water (50 ml). Organic phase was dried over MgSO₄ and concentrated on rotovap. Yield 95% (yellow oil, α/β-mixture).
Rhamnose tetraacetate (35 g, 0.105 mol) was dissolved in DCM (300 ml) under N$_2$-atm. and cooled in ice bath (0 °C). Boron trifluoride etherate (15 ml, 0.116 ml) was added and the mixture was allowed to stay for 15-20 min then thiophenol (12 ml, 0.116mol) was added, ice bath was removed and the mixture was stirred at room temperature. Note: If the reaction is not completed after 5 hours (TLC analysis, eluent: heptan/EtOAc: 3:1) additional amounts of thiophenol (5 ml) and boron trifluoride etherate (6 ml) are required. When TLC showed completion the reaction mixture was quenched with MeOH (10 ml) and diluted with DCM (200 ml). The organic phase was washed with sat. NaHCO$_3$ (3 x 100 ml), dried over MgSO$_4$ and concentrated on rotovap to give yellow crystals. The crystals were washed with heptane on a glass filter. Yield (85 %, white solid).
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-α-D-galactopyranoside

Prepared from 4 and 5b according to the General Procedure I. Colorless foam, 78% yield. Rf 0.47 (toluene/EtOAc 10:1).

$^{1}$H NMR (500 MHz, CDCl$_3$) $\delta$ 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$_3$) $\delta$ 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; [α]$_{D}^{2}$ +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$_5$O$_{11}$Na [M+Na]+: 1111.40; Found: 1111.44.

Benzy 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

Pentenyl glycoside 3b (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 CH₂Cl₂ (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 Br₂ in CH₂Cl₂ 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).

¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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; [α]D²² +37.6 (c 1.4, CHCl₃); IR (neat) 1740 cm⁻¹ (C=O). m/z (MALDI-TOF MS) Calcd. for C₆₅H₸₇F₁₁O₁₁Na[M+Na]+: 1111.39; Found: 1111.39.

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

Prepared from 7 according to the General Procedure II. White foam, 74% yield. Rf 0.21 (toluene/EtOAc 10:1).

¹H NMR (500 MHz, CDCl₃) 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\(_3\)) \( \delta \) 158.4, 147.1, 143.7, 138.2, 138.1, 137.9, 136.8, 128.8, 128.3, 128.2, 128.2, 128.1, 128.0, 127.8, 127.7, 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\(_5\)O\(_{11}\)Na \([M+Na]^+\): 993.3249; Found: 993.3249.

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

Prepared from 3b and 8 according to the General Procedure I. White foam, 71% yield. Rf 0.47 (toluene/EtOAc 10:1).

\( ^1 \)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 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 (d, \( J = 10.8 \) Hz, 1H), 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\(_3\)) \( \delta \) 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, 127.3, 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-1-rhamnopyranosyl-(1→4)-2,3-di-O-benzyl-6-O-pentafluorobenzyol-α-D-galactopyranosyl-(1→2)-2,3-di-O-benzyl-α-1-rhamnopyranosyl-(1→4)-2,3-di-O-benzyl-6-O-pentafluorobenzyol-α-D-galactopyranoside

Prepared from 9 according to the General Procedure II. White foam, 76% yield. Rf 0.20 (toluene/EtOAc 10:1).

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 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$_3$) $\delta$ 158.3, 157.9, 147.2, 144.8, 143.7, 141.4, 139.1, 138.6, 138.3, 138.2, 138.1, 138.0, 137.9, 137.7, 137.59, 136.8, 135.7, 128.8, 128.4, 128.3, 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.
Benzy1 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

3b and 10 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).

1H NMR (500 MHz, CDCl3) 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); 13C NMR (75 MHz, CDCl3) δ 138.7, 138.6, 138.5, 138.4, 138.4, 138.3, 138.3, 138.1, 138.0, 137.1, 135.8, 133.1, 132.8, 128.4, 128.4, 128.3, 128.3, 128.3, 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; [α]D22 +95.9 (c 0.9, CHCl3). m/z (MALDI-TOF MS) Calcd. for C138H148O28Na[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)

To a suspension of the Dess-Martin periodinane (210 mg, 0.49 mmol) in anhydrous CH₂Cl₂ (5 mL) was added a solution of 2 (250 mg, 0.11 mmol) in CH₂Cl₂ (7 mL). The reaction was stirred for 1 h, then diluted with Et₂O (25 mL), quenched with 10% aq. Na₂S₂O₅ (25 mL), and stirred for 30 min. The organic phase was separated and washed with sat. aq. NaHCO₃ (20 ml). The combined aqueous phases were extracted with Et₂O (2 × 20 ml), dried (MgSO₄) and concentrated. The crude aldehyde was dissolved in THF (2.5 mL) followed by addition of iBuOH (5 mL), 2-methyl-but-2-ene (1.6 ml, 15 mmol), and a solution of NaClO₂ (270 mg, 3.0 mmol) and NaH₂PO₄·H₂O (310 mg, 2.25 mmol) in H₂O (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 Et₂O (3 × 30 ml). The combined organic phases were dried with MgSO₄, filtered and concentrated to afford the crude acid. Rf 0.41 (CH₂Cl₂/MeOH 95:5). The crude acid was dissolved in EtOAc (6 mL) and titrated with PhCHN₂113 (0.5 M sol. in Et₂O) until TLC (toluene/EtOAc 10:1) showed full conversion (2 h). *Note: PhCHN₂ 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).

¹H NMR (300 MHz, CDCl₃) 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, 128.4, 128.4, 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$_{140}$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**

11 (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$_2$O (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 C$_{18}$ silica column (eluent H$_2$O) and lyophilized to furnish 1 as white foam (54 mg, 95%).

$[\alpha]_D^{22}$ +33.2 (c 0.4, H$_2$O). 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.
Screening of the conditions for removing the NAP-group

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

All reactions were monitored by TLC (heptane/EtOAc 1:1), Rf of X 0.55. The reactions were worked up according to either Procedure A or Procedure B. Product X was isolated by flash chromatography in 5:1 heptane/EtOAc.

**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$_2$Cl$_2$ (20 ml) and washed with sat. aq. NaHCO$_3$ (2 × 10 ml). The combined aqueous phases were extracted with CH$_2$Cl$_2$ (2 × 20 ml). The combined organic phases were dried (MgSO$_4$), filtered, concentrated and purified by flash chromatography.

Product X was obtained as a colorless foam. The analytical data of X matched with previously reported.$^{110}$

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.48 – 7.52 (m, 2H), 7.25 – 7.46 (m, 13H), 5.59 (d, $J = 1.5$ Hz, 1H), 4.96 (d, $J = 11.0$ Hz, 1H), 4.76 (s, 2H), 4.71 (d, $J = 11.0$ Hz, 1H), 4.23 – 4.32 (m, 2H), 3.92 (dd, $J = 9.2, 3.2$ Hz, 1H), 3.61 (t, $J = 9.2$ Hz, 1H), 2.93 (d, $J = 1.8$ Hz, 1H), 1.38 (d, $J = 6.2$ Hz, 3H).

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 138.4, 137.8, 134.3, 131.4, 129.1, 128.7, 128.6, 128.2, 128.1, 127.9, 127.4, 87.2, 80.3, 80.2, 75.6, 72.3, 70.2, 68.9, 18.0.

**DDQ**

**Entry 1.** To a solution of X (300 mg, 0.5 mmol) in CH$_2$Cl$_2$/MeOH 4:1 (5 ml) and water (0.2 ml) was added DDQ (160 mg, 0.7 mmol, 1.4 eq.). 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. 42% yield.
**Entry 2.** Same as Entry 1, but the reaction was worked up according to the Procedure B. 75% yield.

**Entry 3.** Same as Entry 2, but the reaction was performed at 0 °C for 24 h. 70% yield.

**Entry 4.** Same as Entry 2, but the reaction was performed in CH₂Cl₂ (5 ml). 67% yield.

**Entry 5.** Same as Entry 2, but K₂HPO₄/KH₂PO₄ buffer (1M, pH 7.2, 1 ml) was added instead of H₂O. 38% yield.

**HF/Pyridine**

**Entry 6.** To a solution of X (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. 30% yield.

**TFA**

**Entry 7.** To a solution of X (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. 40% yield.

**Entry 8.** Same as Entry 7, but the reaction was worked up according to the Procedure B. 65% yield.

**Entry 9.** Same as Entry 8, but the reaction was performed at 0 °C for 24 h. 65% yield.

**NMR analysis of hexasaccharide**

Hexasaccharide 1 (50 mg) was dissolved in 2 ml D₂O and freeze dried, this was repeated twice and then 1 was dissolved in 99.9% D₂O 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 (δH 4.79 ppm) and the CH₃-groups in rhamnose (δC 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. The 1D $^{13}$C spectrum wasn’t available and $^{13}$C chemical shift values were obtained from the HSQC and the HSQC-TOCSY spectra.
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