Development of Synthetic Glycan Tools for Investigating Plant Cell Wall Pectins

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Summary

Plants are the most important natural resources. They can serve as energy source or provide raw materials for many industrial applications. The plant materials used mostly consist of plant cell walls. The cell walls are very rigid and heterogeneous networks of biopolymers such as proteins, lignin and most importantly polysaccharides which encapsulate every plant cell. These structures account for physical strength and defense mechanisms of the plant and play an important role in plant development. Pectins are very complex and highly branched cell wall polysaccharides. However, their distribution, function and biosynthesis have not yet been fully understood. In order to improve the economic usability of the cell wall, a better understanding of the composition, functions and biosynthesis of cell wall polysaccharides is essential. Libraries of chemically synthesized well-defined oligosaccharides and functionalized glycosyl donors can serve as important tools to advance existing experimental methods for investigating structure and biosynthesis of plant polysaccharides.

In chapter 2.1, the automated solid-phase synthesis of 23 arabinogalactan (AG) oligosaccharide fragments of pectin side chains is described. Automated glycan assembly (AGA) allowed the assembly of a library of structurally related type-I and type-II AGs on a linker-functionalized solid support from a set of differently protected galactose, arabinose and glucuronic acid monosaccharide building blocks (BBs). The synthetic glycan structures obtained were used to assign the exact binding epitopes of cell wall glycan-directed mAbs using glycan microarrays. Furthermore, the synthetic type-I AGs have been used to determine the substrate specificities of three *endo*-galactanases which are cell wall-degrading enzymes that are used to deconstruct pectin polymers for structural analysis. A detailed knowledge of the substrate specificities of these cell wall-degrading enzymes is required to deduce the composition of the original polysaccharide.

In chapter 2.2, solid-phase and solution-phase syntheses of apiose-substituted α(1,4)-homogalacturonan backbone structures, as found in rhamnoglacturonan-II (RG-II) were initiated. A set of three galactose BBs and one apiose BB was applied in AGA. However, the automated solid-phase synthesis of the backbone scaffold suffered from limited diastereoselective control during the first glycosylation reaction with the linker and the low nucleophilicity of the C4-hydroxyl group of galactose. In a solution-phase test series the diastereoselectivity during the glycosylation reaction with the linker could be improved using a fluorinated linker, but satisfactorily results were not obtained. Subsequently, two galacturonic acid lactone BBs were tested in solution-phase to assemble a (1,4)-linked galacturonic acid backbone consisting exclusively of α-linkages.

However, the galacturonic acid lactone BBs were also not applicable to assemble oligogalacturonates beyond disaccharides.

In chapter 2.3, four differently functionalized UDP-galactopyranoses (UDP-Gal*p*) and one functionalized UDP-arabinofuranose (UDP-Ara*f*) were chemically synthesized. The azido- or amino-functionalized sugar-nucleotides served as enzyme substrates in first proof-of-principle studies towards the development of a new high-throughput assay for the characterization of putative plant glycosyltransferases on glycan microarrays. It was shown that the tested transferases tolerate these modifications of the glycosyl donor substrates. The azido- or amino-functionalized residues were incorporated into acceptor oligosaccharides on microarray surfaces by the glycosyltransferases and the enzymatic reaction products were subsequently visualized by selective reaction of fluorescent probes with the azido- or the amino-groups.

Zusammenfassung

Pflanzliche Rohstoffe sind von enormer wirtschaftlicher Bedeutung. Sie sind sowohl als Energieträger als auch als Ausgangsstoff vieler industrieller Prozesse. Der Großteil dieser Rohstoffe wird aus den Pflanzenzellwänden gewonnen. Pflanzenzellwände sind hochkomplexe und sehr stabile Netzwerke aus diversen Biopolymeren wie Proteinen, Lignin und Polysacchariden. Die Zellwände tragen sowohl zur physikalischen Stabilität als auch zu Abwehrfunktionen der Pflanzenzelle bei und spielen zudem eine wichtige Rolle in der Pflanzentwicklung. Pektine sind eine Klasse von Polysacchariden in der Pflanzenzellwand und bestehen aus komplexen, hoch verzweigten Polysacchariden, deren Vorkommen, Funktion und Biosynthese allerdings noch immer nicht vollständig verstanden sind. Um die ökonomische Nutzbarkeit dieses Zellwandmaterials zu verbessern, ist ein vertieftes Verständnis der Zusammensetzung und der Funktion von Zellwandpolysacchariden wesentlich. Bibliotheken synthetischer, strukturell definierter Oligosaccharide und funktionalisierter Glycosyldonoren können wertvolle Werkzeuge sein, um die experimentellen Methoden zur Untersuchung der Struktur und Biosynthese von pflanzlichen Polysacchariden zu verbessern.

In Kapitel 2.1 wird die automatisierte Festphasensynthese von 23 Arabinogalactan-Oligosacchariden, die als verzweigende Seitenketten in Pektinen vorkommen, beschrieben. Mit der automatisierten Festphasensynthese konnte eine Bibliothek von strukturell verwandten Typ-I und Typ-II Arabinogalactanen an einem Linker-funktionalisiertem Harz mit Hilfe verschiedener Galactose-, Arabinose- und Glucuronsäure-Monosaccharid-Bausteine hergestellt werden. Die erhaltenen synthetischen Glykane wurden anschließend benutzt, um die exakten Bindungsepitope monoklonaler Antikörper, die Zellwandstrukturen binden, mit Hilfe von Glykan Microarrays zu bestimmen. Außerdem wurden die synthetischen Typ-I Arabinogalactane benutzt, um die Substratspezifitäten von drei *endo*-Galactanasen, die Pektine in Zellwänden zersetzen, zu bestimmen. Die Bestimmung der Enzymsubstratspezifitäten von Pektin-zersetzenden Enzymen ermöglicht es, über Verdauungsexperimente genauere Aussagen über die Struktur der Polysaccharide zu machen.

In Kapitel 2.2 werden sowohl Festphasensynthesen als auch Synthesen in Lösung von Apiose-substituierten α(1,4)-Homogalacturonan-Strukturen, die in pektinischem Rhamnoglacturonan-II vorkommen, untersucht. Für die automatisierte Festphasensynthese wurden drei Galactose und ein Apiose Baustein entwickelt. Während der Glykosylierung des Festphasen-Linkers mit dem ersten Galactose-Baustein wurde eine unzureichende Diastereoselektivität beobachtet. Die geringe Nucleophilie der Galactose C4-Hydroxygruppen führte zusätzlich zu niedrigen Ausbeuten in der

Festphasensynthese von Apiose substituierten α(1,4)-Homogalacturonanen. In Lösung konnte die Diastereoselektivität der ersten Glycosylierung mit Hilfe eines fluorierten Linkers verbessert werden. Es wurden desweiteren mehrere Galacturonsäurelacton-basierte Monosaccharid-Bausteine in Lösung als alternative α(1-4)-selektive Glycosyldonoren getestet. Dabei stellte sich heraus, dass auch diese Bausteine zu unreaktiv sind, um größere Oligogalacturonan Strukturen zu synthetisieren.

In Kapitel 2.3 wird die chemische Synthese von vier funktionalisierten UDP-Galactopyranosen (UDP-Gal*p*) als auch die Synthese von funktionalisierter UDP-Arabinofuranose (UDP-Ara*f*) beschrieben. Die Azid- oder Amin-funktionalisierten Zucker-Nukleotide dienten als Enzymsubstrate in vorläufigen Studien zur Entwicklung neuer Hochdurchsatzmethoden für die Charakterisierung von Glycosyltransferasen auf Glykan-Microarrays. Es konnte gezeigt werden, dass die untersuchten Glycosyltransferasen die Modifizierung der Glycosyldonorsubstrate tolerieren. Die Azid- oder Amin-funktionalisierten Bausteine wurden von den getesteten Transferasen auf Oligosaccharidakzeptoren auf der Microarrayoberfläche übertragen und die enzymatischen Reaktionsprodukte konnten auf der Oberfläche durch selektive Reaktion der eingeführten Azid- oder Amingruppen mit Fluoreszenzfarbstoffen visualisiert werden.

List of Publications

Publications:

M. P. Bartetzko, F. Schuhmacher, H. S. Hahm, P. H. Seeberger, F. Pfrengle, *Org. Lett.*, **2015**, *17*, 4344-4347.

M. Wilsdorf, D. Schmidt, M. P. Bartetzko, P. Dallabernardina, F. Schuhmacher, P. Seeberger, F. Pfrengle, *Chem. Comm.*, **2016,** *52*, 10187-10189.

M. P. Bartetzko, F. Schuhmacher, P. H. Seeberger, F. Pfengle, *J. Org. Chem.*, **2017**, *82*, 1842-1850.

C. Ruprecht, M. P. Bartetzko, D. Senf, P. Dallabernardina, I. Boos, M. C. F. Andersen, T. Kotake, J. P. Knox, M. G. Hahn, M.H. Clausen, F. Pfrengle, *Plant Physiol.*, **2017**, *175*, 1094-1104.

Conference Presentations:

M. P. Bartetzko, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated Glycan Assembly of Plant Cell Wall derived Arabinogalactan Oligosaccharides. Poster presentation delivered at the JCF-Frühjahrssymposium, Kiel, Germany, March 2016.

M. P. Bartetzko, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated Glycan Assembly of Plant Cell Wall derived Arabinogalactan Oligosaccharides. Poster presentation delivered at the International Carbohydrate Symposium 2016, New Orleans, USA, July 2016.

M. P. Bartetzko, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated Glycan Assembly of Plant Cell Wall derived Arabinogalactan Oligosaccharides. Poster presentation delivered at the 1st Biomolecular Systems Conference, Berlin, Germany, November 2016.

M. P. Bartetzko, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated Glycan Assembly of Pectin Oligosaccharides from the Plant Cell Wall. Oral presentation delivered at the Berliner Chemie Symposium, Berlin, Germany, April 2017.

M. P. Bartetzko, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated glycan assembly of pectin oligosaccharides provides biochemical tools for the epitope mapping of plant cell wall glycan-directed antibodies and characterization of glycosyl hydrolases. Poster presentation delivered at the 19th Tetraheadron Symposium, Riva del Garda, Italy, June 2018.

List of Abbreviations

Ac	acetyl
AG	arabinogalactan
AGA	automated glycan assembly
AGP	arabinogalactan protein
Api*f*	apiofuranose
Ar	aryl
Ara*f*	arabinofuranose
Azmb	2-(azidomethyl)benzoyl
aq.	aqueous
BAIB	bis(acetoxy)iodobenzene
BB	building block
Bn	benzyl
Bu	butane
Bz	benzoyl
c	concentration
CAZy	carbohydrate-active enzyme database
Cbz	carboxylbenzyl
CESA	cellulose synthase
COSY	correlation spectroscopy
CSA	camphorsulfonic acid
*cyclo*Sal	*cyclo*-saligenyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-en
DCE	dichloroethane
DCM	dichloromethane
DEAD	diethyl azocarboxylate
DIC	*N,N'*-diisopropylcarbodiimide
DMAP	dimethylaminopyridine
DMF	dimethylformamide
Dha	3-deoxy-D-lyxo-heptulosaric acid
ELISA	enzyme-linked immunosorbent assay
ELSD	evaporative light scattering detector
ESI	electrospray ionization
Et	ethyl
EtOAc	ethyl acetate
FEP	fluorinated ethylene propylene
Fmoc	fluorenylmethyloxycarbonyl
FTIR	fourier transform infrared
GalA	galacturonic acid
Gal*p*	galactopyranose
GC	gas chromatography
GH	glycosyl hydrolase
GT	glycosyltransferase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hex	hexane
HG	homogalacturonan
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence spectroscopy
IE	ion exchange
IR	infra red
Kdo	3-deoxy-D-manno-oct-2-ulsonic acid

LC	liquid chromatography
Lev	levulinoyl
LG	leaving group
mAb	monoclonal antibody
Me	methyl
MeCN	acetonitrile
MPIKG	Max Planck Institute of Colloids and Interfaces
MS	mass spectrometry
Nap	(2-methyl)naphthyl
NDP	nucleotide diphosphate
NHS	*N*-hydroxysuccinimide
NIS	*N*-iodosuccinimide
NMP	nucleotide monophosphate
NMR	nuclear magnetic resonance
NP	normal phase
o.d.	outer diameter
PG	protecting group
Ph	phenyl
Piv	pivaloyl
PMB	*p*-methoxybenzyl
QTOF	quadrupole time of flight
RG	rhamnogalacturonan
Rha*p*	rhamnopyranose
RP	reversed phase
rt	room temperature
sat.	saturated
TBS	*tert*-butyldimethylsilyl
*t*BuOOH	*tert*-butyl hydroperoxide
TCA	trichloroacetyl
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl radical
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofurane
TfOH	trifluoroacetic acid
THPTA	tris-hydroxypropyltriazolylmethylamine
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TLC	thin layer chromatography
TMSONa	sodium trimethylsilanolate
TOF	time of flight
Ts	*p*-toluenesulfonyl
T*t*BPy	2,4,6-tri-*tert*-butylpyridine
UDP	uridine diphosphate
UMP	uridine monophosphate
UV	ultraviolet

Symbols


D-Galactose


D-Glucose


D-Mannose


L-Arabinose

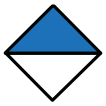

D-Xylose


L-Fucose


L-Rhamnose


D-Apiose


D-Galacturonic acid


D-Glucuronic acid


3-Deoxy-D-heptulosaric acid


3-Deoxy-D-manno-oct-2-ulosonic acid

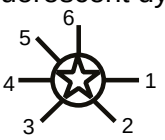
Aceric acid


Fluorescent dye


Monoclonal antibody


Solid support

Glycosyltransferase



Linkage

1 Introduction

1.1 Biomass as Resource

The global energy consumption is constantly growing on average at 2% per year due to population- and economic growth.[1] Atmospheric carbon dioxide levels, the major cause of global warming, are rising accordingly.[2] Nowadays huge efforts are made to establish alternative, renewable energy sources that have a lower environmental impact. In 2016, the world energy council estimated the contribution of renewable energies to the global energy consumption at 18% in which the energy derived from biological raw materials (biomass) accounted approximately 14%.[3] A big part of the biomass used, is transformed into biofuels which are one option for reducing the carbon emissions in the transport sector. Biofuels are defined as 1st-, 2nd- and 3rd-generation biofuels. Until today 1st-generation biofuels which are produced from food crops with high content of starch or oil contribute the major part to biofuel production. As the production of 1st-generation biofuels directly competes for land and water needed for food production, current research efforts focus on the further development of 2nd- and 3rd-generation biofuels. 2nd-Generation biofuels are produced from non-food biomass by utilization of cellulose, hemicellulose and lignin (lignocellulosic biomass).[4] 3rd-Generation biofuels are derived from algae. Energy crops grown for the production of 2nd-generation biofuels include for example simple plants such as switchgrass which tolerate cold temperature, have low water and nutrition requirements and can grow on a broad range of land types not suited for food production.[5]

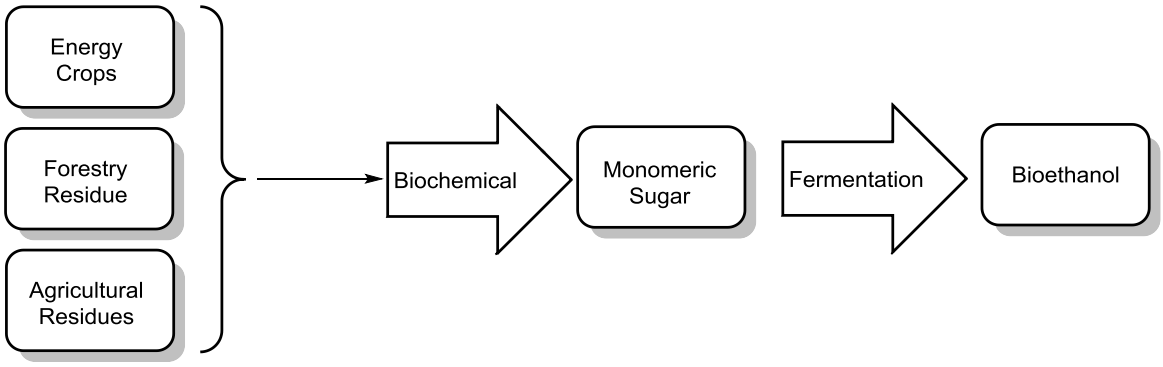


Figure 1: Conversion pathway from lignocellulosic biomass to second generation biofuels.

Exploitation of lignocellulosic biomass is hampered by some technical and economic challenges. To make the desired polysaccharides available, recalcitrance caused by lignin has to be overcome before the polysaccharides can be hydrolyzed. Hydrolysis is achieved chemically through the addition of sulphuric acid or biochemically through

enzymatic hydrolysis which is highly favorable over acidic hydrolysis due to higher yields, higher selectivity, less environmental impact and production of less or no by-products.[6] After the hydrolysis, the monomeric sugars are subjected to fermentation processes, yielding bioethanol which can be used as biofuel (fig. 1). Enzyme costs are a major drawback[7] of the utilization of lignocellulose polysaccharides and lowering the costs by improved biomass processing is required. Facilitation of biomass processing could be achieved by alteration of the lignocellulose composition in bioenergy plants[8] or by exploration of existing plants with higher amounts of easily accessible and digestible polysaccharides[9]. In order to do so the structure and biosynthesis of plant cell wall polysaccharides have to be understood in detail.

1.2 The Plant Cell Wall

1.2.1 Form and Function

On top of the cell membrane plant cells are coated with a cell wall that provides protection and mechanical support to the plant cell.[10] The cell wall is formed by a highly complex network of biopolymers that also plays an important role in the intercellular communication and the plant's defense responses against potential pathogens.[11] Despite the high variability in the cell wall composition of different plant species and cell types, cell wall structures can be categorized into primary and secondary cell walls. The primary cell wall is primarily found on growing cells and is a flexible network of polysaccharides capable of expansion. The secondary cell wall on the other hand is a rigid structure which also contains the phenolic polymer lignin and is found on cells that have ceased growth.[12]

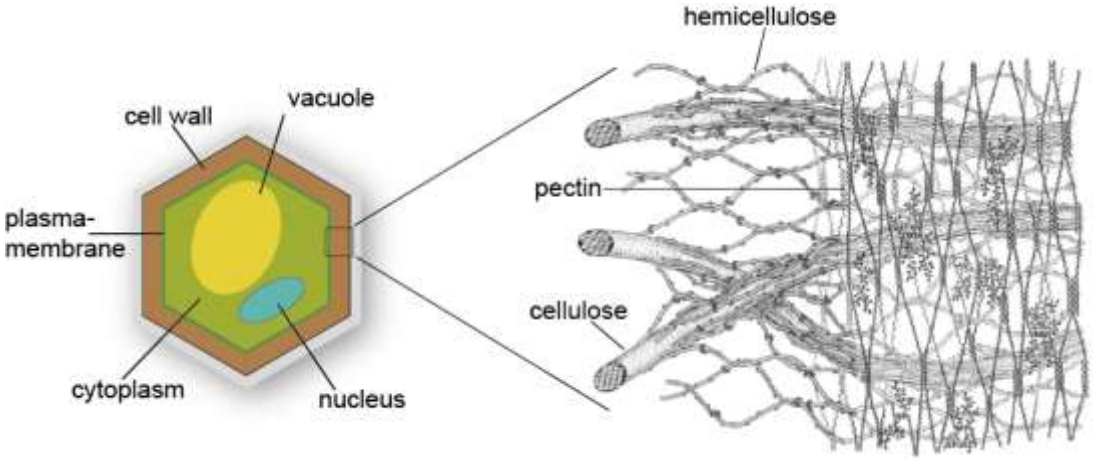


Figure 2: Schematic representation of a plant cell. Cellulose microfibrils, represented as thick rods, are embedded into a matrix of hemicellulose and pectin polysaccharides (modified from Carpita *et al*[13]).

Polysaccharides found in plant cell walls include cellulose, hemicellulose and pectin (fig. 2). Each class of cell wall polysaccharides has characteristic structural features that lead to different mechanical properties and functions of the polysaccharides.

1.2.2 Cellulose

Cellulose is the major component of the plant cell wall and therefore a highly abundant material on earth, with a wide range of traditional applications, e.g. as cotton in textile industry, pulp material in paper products or as additive in cosmetics.[14] In recent years cellulose has also gained attention as a raw material for the production of more advanced materials like cellulose nanomaterials.[15] Cellulose forms a network structure on the outside of the plasmamembrane of plant cells and thereby provides mechanical strength to plants that allows them to grow as tall as 100 m. The rigid network of cellulose in the plant cell wall is build up from water-insoluble string-like highly ordered crystalline microfibrils which consist of approximately 36 linear β(1,4)-linked D-glucopyranose chains (fig. 3).[16] Each chain can contain up to 25,000 glucose units organized in a pattern of cellobiose basic repeating units, accounting for a twofold screw-axis symmetry.[17] Intra-molecular hydrogen bonds between the C3-hydroxyl group and the ring oxygen of the adjacent glucose unit fixate this spatial orientation of the cellobiose units (fig. 3). This high symmetry of the glucan chains allows the single polymeric molecule to align with other β(1,4)-glucan chains through intermolecular hydrogen bonds, thereby forming very rigid microfibrils.

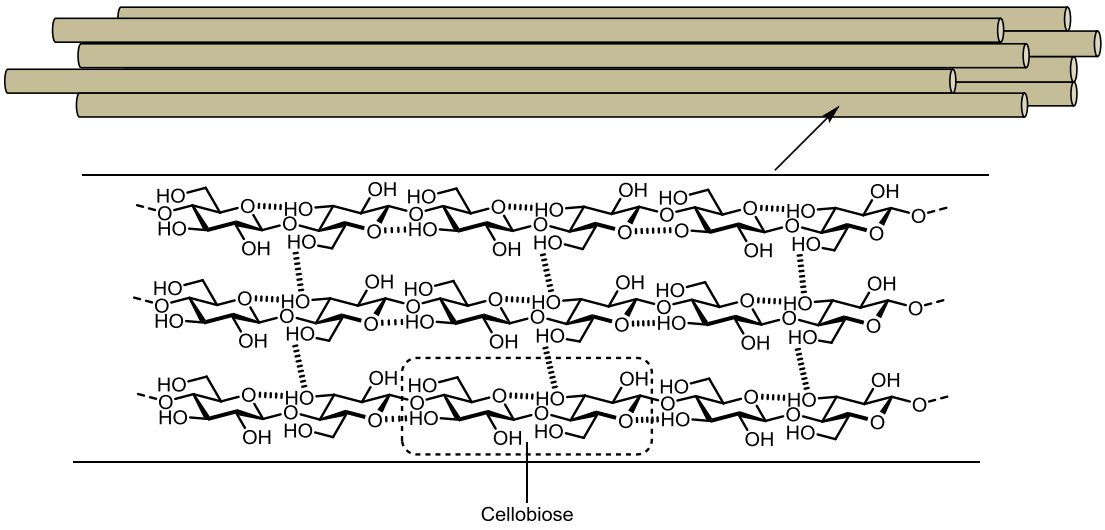


Figure 3: Schematic depiction of cellulose. Aggregates of microfibrils form a string-like network. Each microfibril consists of several β(1,4)-glucan polymers that are highly ordered and fixed by intra- and inter-molecular hydrogen bonds (dotted lines).

1.2.3 Hemicellulose

In contrast to cellulose, hemicelluloses cover a much more heterogeneous class of polysaccharides. Hemicelluloses are non-crystalline cell wall polysaccharides that have β(1,4)-linked backbones of D-pyranose moieties, mainly glucose, xylose and mannose.[18] The major classes of hemicelluloses are xyloglucans, mannans and xylans (fig. 4). Xyloglucans are ubiquitous plant polysaccharides that consist of a β(1,4)-D-glucan backbone decorated with α(1,6)-linked xylose substituents that can be further elongated with other glycosyl or nonglycosyl residues.[19] Mannans are polysaccharides composed of a β(1,4)-D-mannose backbone substituted with α(1,6)-galactose monosaccharides. They are found in all cell walls but the molar ration of mannose to galactose varies with plant species between 1:1 to 4:1.[20] Xylan polysaccharides of land plants are composed of a β(1,4)-D-xylose backbone that, depending on the plant species, are highly decorated with various monosaccharide moieties.[21] Depending on the type of substituents, xylans can be subdivided into homoxylan, arabinoxylan, glucuronoxylan and arabinoglucuronoxylan. A forth class of important hemicelluloses are mixed-linkage glucans. Mixed-linkage glucans are only prevalent in grasses and cereals[22] and therefore often not considered as a major hemicellulose. The structure of mixed-linkage glucans is similar to cellulose, as it is build up from unbranched, linear β(1,4)-linked glucose residues, but have an alternative linkage (β(1,3)-linkage) before every third or fourth glucose residue.[23,24]

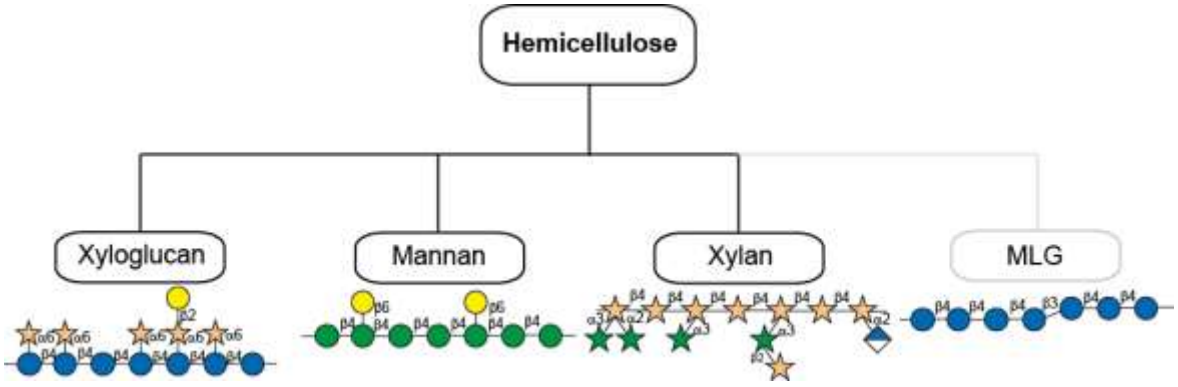


Figure 4: Classes of hemicellulosic polysaccharides and schematic representation of their structures.

Hemicelluloses are generally considered to be tethering glycans that bind to cellulose microfibrils through non-covalent interactions and thereby play an important role in cell wall growth and stability.[25] Recently, it has also been shown that hemicellulosic mannan structures form covalent linkages to lignin and thereby “glue” the polysaccharide component of the secondary cell wall to the lignin matrix.[26] Additionally, hemicelluloses are considered to act as signaling molecules, as it has for example been shown that xyloglucan breakdown products can induce cell responses.[27]

1.2.4 Pectin

Pectin polysaccharides are very complex and heterogeneous matrix polymers, mainly found in the primary cell wall of all land plants, and are almost absent in the secondary cell wall.[28] Primary cell wall polysaccharides consist of approximately 35% pectin in dry weight.[29] From the primary cell wall the pectin structures are easily extracted using acidified hot water. Due to their high solubility in aqueous solutions, pectin polysaccharides are long known and are broadly used in food production as gelling, stabilizing or thickening agents.[30] In the plant cell walls, pectin structures fulfill a variety of tasks. Pectins are involved in intercellular adhesion, control the porosity of the cell wall on a growing plant cell, activate plant defense responses and provide a gel-like matrix for the deposition of cellulose microfibrils.[31] As part of the daily human diet, pectins also fulfill a nutritional function and many studies have shown beneficial health effects of pectin polysaccharides due to their prebiotic character in the intestine.[32,33] To fully understand the functions of pectin in plant growth and development and to verify the beneficial health effects of nutritional pectins, the chemical structure of pectin has been investigated intensively. Though the composition of pectin polysaccharides varies with the source, generally five polysaccharides domains are found in pectin polysaccharides: The major domain, homogalacturonan (HG), that comprises 60-70% of the pectin structure, rhamnogalacturonan I (RG-I) that makes up 20-35% of pectin, rhamnoglacturonan II (RG-II) that can represent up to 10% of pectin and the less abundant apiogalacturonan and xylogalacturonan. It is believed that these pectin domains are covalently linked via their backbones. However, the exact structure and connectivity is still under debate.[34]

1.2.4.1 Homogalacturonan

Homogalacturonan (HG) makes up the smooth region of pectin as it is a linear homopolymer of α(1,4)-linked galacturonic acids that can be partially methylesterified at the C6 carboxylic acid or *O*-acetylated at the C2- or C3-position.[35] HG domains in pectin polysaccharides contain 80-120 galacturonic acid residues.[36] The extend of methylesterification is highly regulated by the cell and plays an important role in plant development.[37] The gelating properties of HG can be controlled by the degree of methylesterification. Unmethylated C6 carboxylic acids are negatively charged and HG regions with more than ten free carboxylic acids in line can coordinate to Ca^{2+} ions which may allow the coordination of a second HG molecule.[38] The Ca^{2+}-induced dimerization cross-links pectin polymers and is responsible for gelation.

1.2.4.2 Rhamnogalacturonan I

Rhamnogalacturonan-I (RG-I) is usually extracted together with HG, but is a much more complex, branched pectin domain. The backbone of RG-I has the disaccharide repeating unit α(1,4)-D-galacturonic acid-α(1,2)-L-rhamnopyranose.[37] Depending on the plant source, this backbone can be highly branched at the C4-position of the rhamnose residues with neutral arabinan, galactan or arabinogalactan side chains. These side-chains can be very heterogeneous in structure and composition. Arabinan structures from RG-I are often found to be α(1,5)-linked with terminal α(1,3)-linked arabinose or galactose residues. The galactan side chains usually contain β-(1,4)-linked galactose residues, with a length of up to 43-47 residues.[39] Even more complex are arabinogalactan (AG) side chains which include type-I AGs and type-II AGs. Type-I AGs are the most prevalent side-chain structures found in RG-I polymers. The precise composition of type-I AG side chains differs between plant species and tissues, but many general structural elements are conserved. All type-I AGs are composed of a Gal-β(1,4)-Gal backbone that may be substituted with α(1,3)-linked single arabinofuranoses, short α(1,3)- or α(1,5)-linked arabinan oligosaccharides, or with β(1,6)-linked galactose residues (fig. 5).[34] Type-II AGs are build up by β(1,3)-linked galactan backbones that are highly decorated with β(1,6)-galactan chains that might be further substituted with α(1,3)-L-arabinofuranoses.[13,29,37] Type-II AGs are not only found as side-chains in RG-I, but are also associated with arabinogalactan-proteins (AGPs) which consist of AG polysaccharides attached to a hydoxyproline-rich peptide backbone. AGPs often co-extract with pectin structures. Direct proof of their functions in the plant cell wall remains elusive.[40] The type-II AGs can also be further modified by the attachment of single glucuronic acid residues at the non-reducing ends of the β(1-6)- or the β(1,3)-galactan chains.[41] The described complexity and diversity of RG-I polysaccharides clearly implies important biological functions. However, a detailed understanding of the structure-function correlation has not yet been achieved.

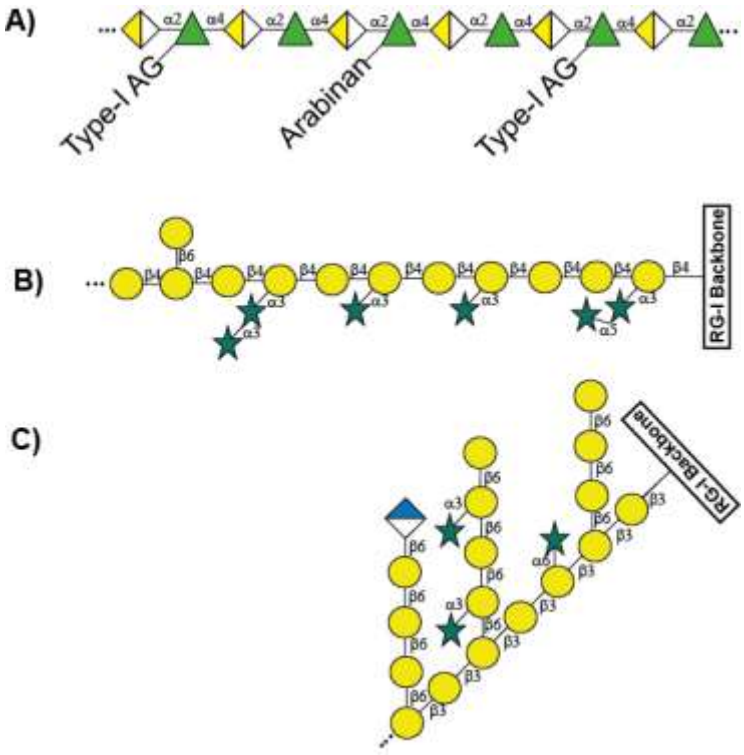


Figure 5. Schematic representation of RG-I polysaccharides. A) RG-I backbone substituted with different side-chain polysaccharides at the C4-position of certain rhamnose residues. B) Type-I AG consists of a β(1,4)-galactan backbone decorated with various galactose and arabinose substitutions. C) Type-II AG consists of a β(1,3)-linked galactan backbone highly branched with side-chains consisting of β(1,6)-linked galactans that may be further substituted with α(1,3)-linked arabinose residues.

1.2.4.3 Rhamnogalacturonan II

Among the four major domains that constitute the pectin polymers, rhamnogalacturonan-II is the structurally most complex polysaccharide. It comprises 13 different monosaccharide sugars in 21 different glycosidic linkages, among them the rare monosaccharides apiose, aceric acid, Kdo and Dha.[42] Structural elucidation of RG-II has made vast progress in the last 20 years and has led to constant refinements. RG-II has a HG backbone carrying six distinct side chains. Side chains A and B are octa- and heptasaccharides respectively, with a D-apiose residue β(1,2)-linked to the backbone as the first monosaccharide unit. Side chains C and D are disaccharides linked to the C2-position of a backbone galacturonic acid residue. Side chains E and F are L-arabinofuranose monosaccharides α(1,3)-linked to the HG backbone. The RG-II structure is remarkably preserved in higher plant species,[43] and often only minor modifications are found in the side-chains,[44] suggesting an important role of RG-II in plant development. It has been found that the RG-II polysaccharides dimerize through formation of a borate

ester between the apiosyl residues of the side chain A of neighboring moleculs, organizing the pectin structure and influencing plant growth (fig. 6)[45].

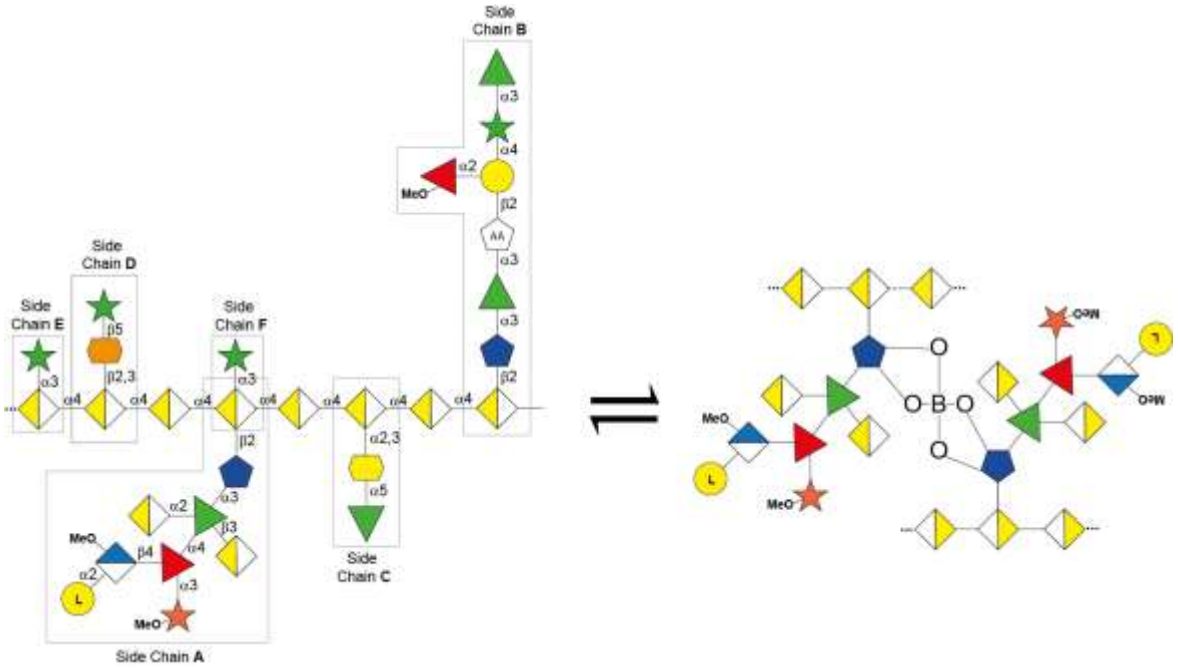


Figure 6: Structure of RG-II. Oligosaccharide side chains A-F are connected to a homogalacturonan backbone (distances between side chains are not representative). The side chains A of two RG-II monomers form borate ester between the apiosyl residues.

1.2.5 Biosynthesis of Plant Cell Wall Glycans

Plant cells deposit large amounts of differentiated polysaccharides in the cell wall during plant growth and development. In the course of building up the cell wall structures, a plant cell can modify the composition of the polysaccharides with regard to the required properties of the cell wall structures by the enhanced production of certain polysaccharides. For the assembly of this variety of polysaccharides in the plant cell wall, a complicated biological machinery is needed and it is obvious that the investigation of cell wall biosynthesis is of major interest in order to advance the basic understanding of plant development and to optimize cell wall utilization. Plenty genes encoding glycosyltransferases, which transfer an activated sugar to an acceptor substrate, have been identified by genetic studies. The gene products (glycosyltransferases) are mainly characterized by the description of phenotypical changes of transgenic knock-out plants but the respective enzymes often remain structurally and mechanistically uncharacterized.[46]

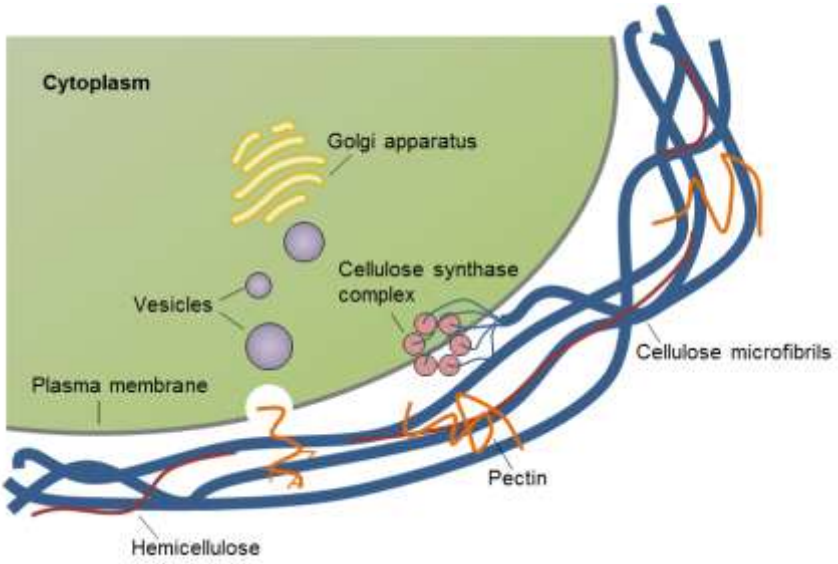


Figure 7: Schematic representation of primary cell wall synthesis. Cellulose is synthesized by large cellulose synthase complexes on the plasma membrane. Hemicellulose and pectin are synthesized in the Golgi apparatus and transported to the wall surface in vesicles. Adapted from *Cosgrove*.[12]

Today the biosynthesis of the major cell wall component cellulose is relatively well understood. Cellulose is synthesized at the plasma membrane by membrane-integrated cellulose synthase complexes and the nascent cellulose microfibrils are directly secreted into the cell wall (fig. 7). The microfibrils are assembled at rosette-like structures consisting of six hexagonally arranged subunits which itself consist of multiple cellulose synthase proteins (CESA). Each CESA is a glycosyltransferase (GT) and synthesizes linear β(1,4)-glucan chains from cytosolic UDP-activated glucose. During the synthesis of the β(1,4)-glucan chains the activated glucose monosaccharides are attached to the C4-hydroxyl group at the non-reducing end of the growing polymer which remains attached to the synthase. Despite the good understanding of the cellulose polymerization process, the initiation of cellulose synthesis is still under debate and has to be further investigated.[47,48]

In contrast to cellulose, the cell wall matrix polysaccharides (hemicellulose and pectin) are synthesized from activated monosaccharide donors (sugar nucleotides) exclusively in the Golgi apparatus and only then transported to the cell surface in Golgi-derived vesicles (fig. 7).[49] The assembly of complex branched hemicelluloses or pectins in the Golgi-apparatus involves a set of glycosyltransferases, sugar-nucleotide transporters and sugar-nucleotide-interconversion enzymes that have to act concertedly.

The β(1,4)-backbone structures of hemicellulosic xyloglucans, mannans and mixed-linked glucans are synthesized by glycosyltransferases from the cellulose synthase like family (CAZy family: GT2).[50] Xylan backbones are believed to be synthesized by GTs from other GT families which have only partly been described. Some enzymes responsible for the attachment of side-chains to the hemicellulose backbones have been identified, but many

more remain unknown. The mechanisms controlling the patterning of side chain substitutions of xyloglucans, mannans and xylans have not yet been elucidated.

The synthesis of pectin polysaccharides in the Golgi-apparatus requires a large number of GTs due to their complex structure. It is hypothesized that at least 67 different transferases are needed.[34] Until today only few of these transferases have been identified and even fewer have been characterized regarding their substrate specificity *in vitro* through heterologous expression.[51] Moreover, it is not yet understood if pectin polysaccharides are synthesized in a consecutive way by successive additions of sugar monosaccharides to the non-reducing end of the growing polymer or if pectin subdomains are synthesized individually first before they are stitched together.

The backbone structure of homogalacturonan is synthesized by galacturonosyltransferase complexes that transfer D-GalA residues in an α(1,4)-linkage from UDP-D-GalA onto the non-reducing end of HG acceptors.[52,53] The biosynthesis of rhamnogalacturonan-I backbones requires a galacturonosyltransferase and a rhamnosyltransferase, but none of the involved enzymes have been identified yet.[51] So far, only few transferases involved in the synthesis of RG-I arabinan[54,55] or galactan[56] side chains have been identified and mechanistically characterized by *in vitro* incubation of the enzymes with acceptor substrates and analysis of the enzymatic reaction products. The biosynthesis of RG-II requires at least 21 glycosyltransferases, assuming that one enzyme can form one glycosidic linkage.[57] Hitherto, little progress by forward or reverse genetics has been made in identifying the actual glycosyltransferases involved. Due to redundancy or lethality of mutations in transgenic knock-out plants, phenotypical changes of the mutated plants could not be investigated.[58]

To further answer the questions why plants developed such complex pectin structures and how modifications of these structures may influence plant development, a more detailed understanding of pectin biosynthesis is urgently required. Research towards understanding the biosynthesis of RG-I or RG-II is hampered by a lack of corresponding well-defined RG-II oligosaccharide fragments and of suited assays to characterize a large number of putative glycosyltransferases. Access to comprehensive libraries of pectin oligosaccharides and the development of novel high-throughput assays to screen glycosyltransferases would strongly accelerate research towards a better understanding of cell wall biosynthesis.[34]

1.2.6 Plant Cell Wall Analysis

1.2.6.1 Plant Cell Wall Monosaccharide Composition Analysis

To be able to obtain detailed information on structures of plant cell wall polysaccharides, such as the ones described above, the monosaccharide composition and the linkage-types can be investigated. Typically, the monosaccharide composition of cell wall polysaccharides is analyzed by gas chromatography-mass spectrometry (GC-MS) based methods.[59] Therefore, the polysaccharides are extracted from the plant material, hydrolyzed under acidic conditions and the resulting monosaccharides are derivatized into aldol acetates after “hydride”-mediated reduction.[60] Formation of the acetylated alditols facilitates the separation of the acid hydrolyzates of polysaccharides by gas chromatography, and the monosaccharides can be analyzed by their retention time relative to internal standards. However, identification of peracetylated alditol acetate derivatives does not allow for drawing any conclusions on the structure of the parent polysaccharide as no information about the linkage types are generated. Information about the linkage types can be obtained by a methylation analysis. In a methylation analysis, hydroxyl groups not involved in glycosidic linkages in the parent polysaccharide are methylated before hydrolysis of the polysaccharide. Methylation of the polysaccharides is achieved by reaction with methyl iodide[61]. After hydrolysis of the polysaccharide the partially methyl protected monosaccharides are reduced and derivatized to the partially methylated alditol acetates in which only the positions that were involved in glycosidic linkages carry an acetyl moiety.[59] The partially methylated alditol acetates are subsequently separated by GC and the peaks are analyzed by MS. Unfortunately, information about the anomeric configuration of the monosaccharides is lost during derivatization and has to be analyzed by other means such as NMR spectroscopy of intact polysaccharides or oligosaccharide fragments thereof.[62]

1.2.6.2 Plant Cell Wall Glycan-Directed Monoclonal Antibodies

The cell wall polysaccharides form a very complex network structure that differs between plant species and tissues with regard to cell biological events by the deposition of different types of polysaccharides. *In muro* localization of specific polymers in the plant tissue is necessary to investigate the contribution of individual polysaccharides to cell wall property and function. Nowadays, immunohistochemical methods are broadly applied to

visualize polymer deposition in plant tissues and to trace polysaccharide modifications during cell wall differentiation (fig. 8).[63] For the detection of specific substructures (epitopes) of the polymers, plant cell wall glycan-directed monoclonal antibodies (mAbs) have emerged as most powerful tool.[64] After the mAbs bind to specific epitopes in the cell wall, they can be detected with labeled secondary antibodies which are visualized by light or electron microscopy.[65] Nowadays, a set of more than 180 plant cell wall glycan-directed mAbs[66] has been generated by hybridoma techniques.[67]

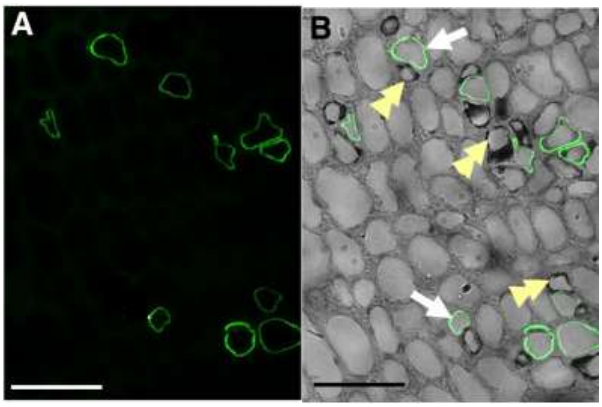


Figure 8: *In muro* analysis of cell wall glycans using mAbs in fluorescence microscopy experiments. A) Immunofluorescence of the type-I AG directed mAb LM26 binding to isolated cells in a transverse section of sugar beet storage root. B) Same section with overlaid bright field image.[68] (White arrows indicate immunolabeled cells, yellow arrows indicate specialized cells next to the labeled cells)

The available mAbs are routinely used to identify polysaccharides in plant tissues, but the information gained is often limited as the exact binding epitopes of the antibodies mostly remain unknown. Commonly, mAbs are generated by a hybridoma technique, where myeloma cells are fused with spleen cells from a mouse which was immunized with extracted oligo- or polysaccharides beforehands.[67] Usually, the generated mAbs are purified and probed with either extracted polysaccharides or purified monosaccharides to determine their binding specificities in enzyme-linked immunosorbent assays (ELISA)[66] or in carbohydrate microarrays[69]. However, extracted polysaccharides contain many possible binding epitopes, hence determination of the exact binding epitope of the mAb is not possible. And isolated oligosaccharides may contain impurities of unknown glycans, even after extensive purification, and thereby may create false positive results. An alternative approach using synthetic oligosaccharides enables the determination of the exact binding epitopes and circumvents issues with impurities. Microarrays equipped with synthetic oligosaccharides have been shown to be versatile tools to identify protein-glycan interactions.[70] Synthetic glycans can be immobilized on microarray surfaces in very small quantities by different covalent or non-covalent methods.[71] Incubation of the immobilized oligosaccharides with an mAb of interest followed by incubation with a fluorescently-

labelled secondary antibody then enables the characterization of the mAb with regard to its binding specificity (fig 9).[69]

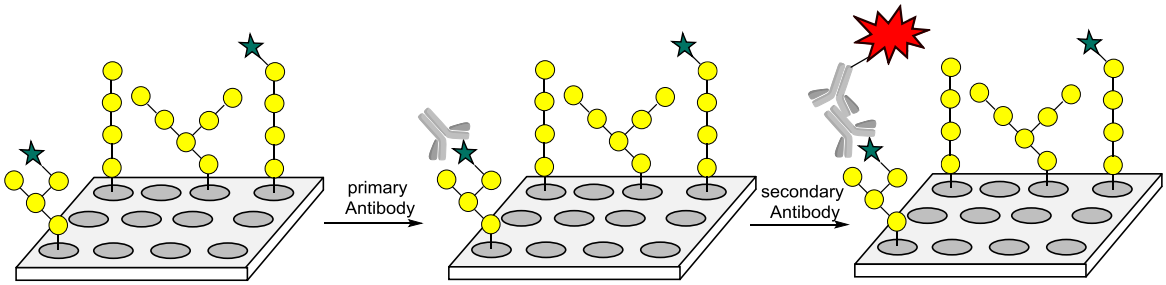


Figure 9: Schematic representation of a carbohydrate microarray assay for determining the binding specificity of an antibody. Oligosaccharides are covalently linked to a microarray surface and probed with mAbs. Antibodies that bind to specific structures are subsequently labeled with a secondary antibody carrying a fluorescent label.

1.2.6.3 Enzymatic Cell Wall Degradation

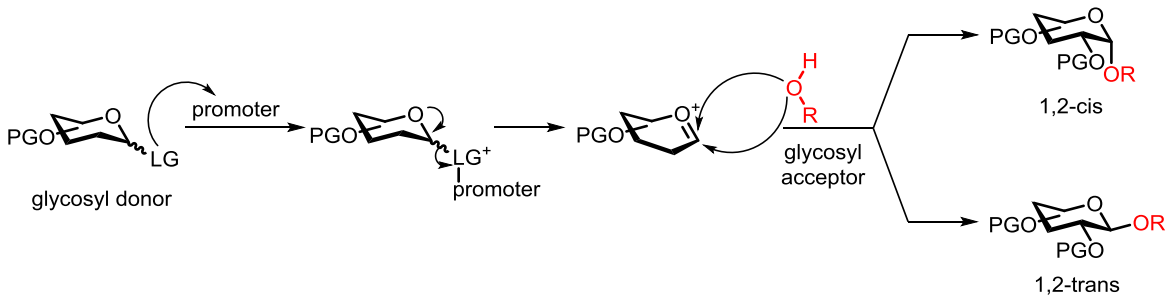
Knowledge about the occurrence and structure of plant cell wall polysaccharides is important for their application as raw material, e.g. in biofuel production. The monosaccharide composition of cell wall extracted polysaccharides can be analyzed as described in chapter 1.2.5.1 and polysaccharide motifs can be localized using immunohistochemical methods (chapter 1.2.5.2). However, degradation of extracted polysaccharides into smaller oligosaccharides which can be further analyzed e.g. by NMR spectroscopy[72] is a commonly applied[73-76] alternative analytical method that allows a rapid analysis of cell wall composition. The polysaccharides can be depolymerized by physicochemical or enzymatic methods in which enzymatic degradation is highly favorable due to higher selectivity under milder conditions.[77] Glycosyl hydrolases (GHs), capable of hydrolyzing polysaccharides, are classified into 153 families depending on their amino acid sequence.[78] Among them, a wide range of pectin degrading enzymes is found which are further categorized into two groups. The first group acts on the HG backbone while the second group acts on the highly branched RG regions of pectin, releasing shorter fragments of the side chains.[77] While pectin-modifying enzymes of the first group are routinely applied in the food industry, publications on the second group are very limited due to the limited access to well-defined substrates for biochemical characterization of the enzymes.[79] Availability of libraries of well-defined pectin-related oligosaccharides would therefore be of great value to characterize pectin-degrading enzymes. Only if the substrate specificities of these enzymes are known the detailed structure of degraded polysaccharides can be determined.

1.3 Synthetic Glycans

The chemical synthesis of plant-derived oligosaccharides offers a great opportunity to obtain well-defined model compounds as analytical tools. Well-defined and pure synthetic oligosaccharides can facilitate the characterization of the substrate specificities of plant cell wall glycan-directed mAbs, glycosyl hydrolases and glycosyltransferases. Although vast progress in the production of oligosaccharides as probes for biological research or as lead compounds for drug and vaccine discovery has been made in the last decades,[80] the chemical synthesis of oligosaccharides remains challenging. The synthesis of complex branched carbohydrate structures requires sophisticated protecting group strategies to enable differentiation of the reactive sites, and elaborated reaction conditions to maintain stereocontrol during the glycosylation reactions. However, current examples of syntheses of large carbohydrate structures such as the biggest synthetic glycan to date, consisting of 92 monosaccharide units[81], show the potential of chemical glycan synthesis.

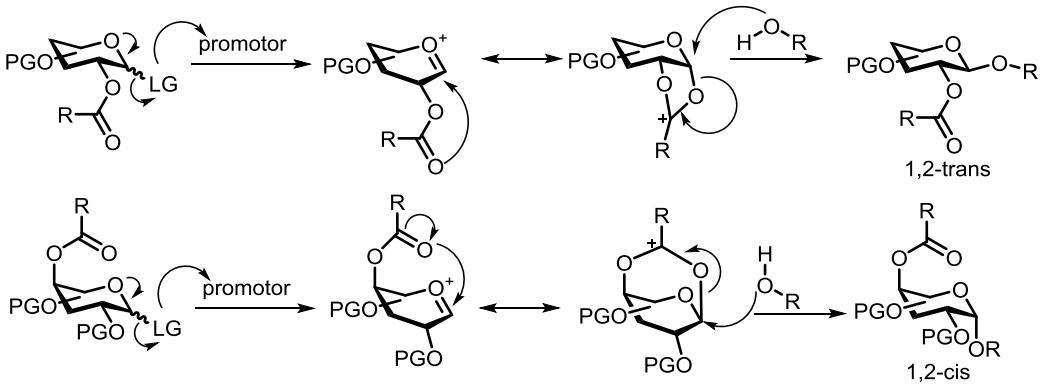
1.3.1 The Glycosylation Reaction

The fundamental reaction in carbohydrate chemistry is the glycosylation reaction in which a hemiacetal glycosyl donor is reacted with a glycosyl acceptor in the presence of a promotor to form a glycosidic linkage. 130 years ago, *Fischer* reported the glycosylation reaction of an unprotected monosaccharide with various alcohols using hydrochloric acid as the promotor.[82] Nowadays, many variations of this basic reaction principle are applied in carbohydrate synthesis. Typically, the monosaccharide glycosyl donor is activated through the installation of an anomeric leaving group which reacts with an electrophilic promotor to yield a reactive intermediate that can further react with a nucleophilc acceptor (scheme 1). The most commonly used glycosyl donors are glycosyl halides[83], glycosyl imidates[84], thioglycosides[85] and glycosyl phosphates,[86] but many more have been described.[87]



Scheme 1: Basic mechanism of a glycosylation reaction.

The common mechanistic depiction of the glycosylation reaction (scheme 1) describes the reaction mechanism of the glycosylation reaction as an S_N1 mechanism, with the formation of an oxocarbenium ion as the rate determining step.[88] The reactive oxocarbenium ion is attacked by a nucleophilic acceptor to form a glycosidic bond. The reaction of the nucleophile with the oxocarbenium ion leads to the formation of a 1,2-*cis*- or a 1,2-*trans* isomer and often gives mixtures of both isomers. Despite the progress being made in the field of glycan synthesis, controlling the stereoselectivity during the glycosylation reaction remains a major challenge, as many factors which have not yet been fully understood influence the reactivity of the glycosyl donor and acceptor and the stereoselectivity in the glycosidic bond formation.[88] Often, the stereoselectivity is controlled through participating protecting groups at the C2-position of a glycosyl donor or at more remote positions. Typically, 1,2-*trans* isomers are obtained selectively when the carbonyl group of a participating ester protecting group at the C2-position can attack the oxocarbenium ion to form an acyloxonium ion. The cyclic acyloxonium ion shields one of the diastereotropic faces, allowing the nucleophilic attack only to occur from the opposite face (scheme 2). Remote protecting groups located at the “upper” side can also be used to exploit anchimeric assistance from the other diastereotropic side of the oxocarbenium ion, leading to the selective formation of 1,2-*cis* glycosidic linkages (scheme 2).[89,90]



Scheme 2: Basic mechanisms of glycosylation reactions with anchimeric assistance of participating protecting groups at C2- or C4-position of the glycosyl donor.

Further, the stereoselectivity in a glycosylation reaction can be controlled by the reaction temperature, as the anomeric effect[91] favors the formation of the axially linked product in a thermodynamically controlled reaction, whereas formation of the equatorially linked product is kinetically favored. In addition, solvent effects can have a significant impact on the stereoselectivity of a glycosylation reaction. Using acetonitrile as the solvent for the glycosylation reaction enhances the formation of the equatorially linked product due to an axial coordination of acetonitrile to the anomeric carbon of the oxocarbenium ion. In contrast, the usage of ether solvents can enhance the formation of the axially linked product due to equatorial coordination of the ether to the anomeric carbon of the

oxocarbenium ion. However, the solvent effect is still not fully understood and the exact mechanisms remain to be clarified.[92] In summary, each glycosylation is a unique reaction that has to be carefully designed to avoid common side reactions such as hydrolysis of the glycosyl donor, eliminations of the leaving group, orthoester-formation or aglycon transfer (transfer of non-glycosyl residues such as released leaving groups to the oxocarbeniumion) and to provide high stereoselectivity.[93]

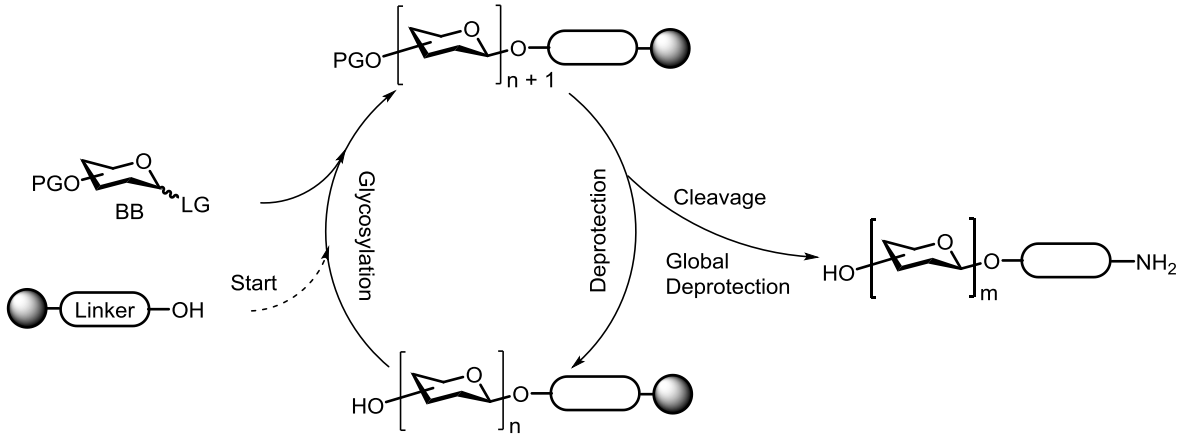
1.3.2 Synthesis of Plant Glycans

The chemical synthesis of plant glycans can provide essential molecular tools for studying plant cell wall structure and function. However, the chemical synthesis of these glycans has only received limited attention in comparison to the synthesis of mammalian or bacterial glycans.[94] Due to the high complexity of plant glycan structures, the reported syntheses focus on the assembly of shorter oligosaccharide fragments of the polysaccharide structures. A comprehensive overview over plant glycans synthesized so far has been given by *Kinnaert et al.* recently.[95] The majority of the reported syntheses relied on cumbersome series of solution-phase glycosylation-, deprotection- and purification steps, employing protected monosaccharide or disaccharide glycosyl donors. It is evident that a systematic synthetic approach to comprehensive libraries of closely related fragments of cell wall glycans would have an important impact on the investigation of plant cell wall structure and function.

1.3.3 Automated Glycan Assembly

Chemical syntheses of highly repetitive natural macromolecules such as peptides[96] and oligonucelotides[97] are nowadays routinely performed on solid-phase in an automated fashion. The assembly of structurally complex molecules on a solid support has many advantages over the assembly in solution-phase. Linking the growing molecule to a solid support allows the application of high excesses of reagents and simplifies the purification of intermediates or final products, as reagents can easily be washed off the solid-phase. The automated solid-phase synthesis of oligosaccharides (automated glycan assembly / AGA) has been introduced in 2001[98] and is not yet as routinely used as automated peptide- or oligonucleotide synthesis. In contrast to the synthesis of peptides or oligonucleotides, the synthesis of oligosaccharides requires a more sophisticated protecting group strategy as well as very elaborate activation and deprotection procedures.[99-101] In AGA, the target glycan structures are assembled on a solid support

resin, which is equipped with a cleavable linker that has to be inert to all reaction conditions applied during the automated synthesis. After attachment of the first monosaccharide to the linker through a glycosylation reaction, a temporary protecting group of the monosaccharide is removed selectively, providing a free hydroxyl group which can then act as a glycosyl acceptor. Subsequently, cycles of alternating glycosylation and deprotection reactions are applied until the desired oligosaccharide is assembled (scheme 3). Acetyl capping can be applied after each glycosylation step to block all unreacted nucleophiles, minimizing the accumulation of deletion sequences.[102] In the end, the oligosaccharide is cleaved from the solid support and the permanent protecting groups are removed by methanolysis and hydrogenolysis.

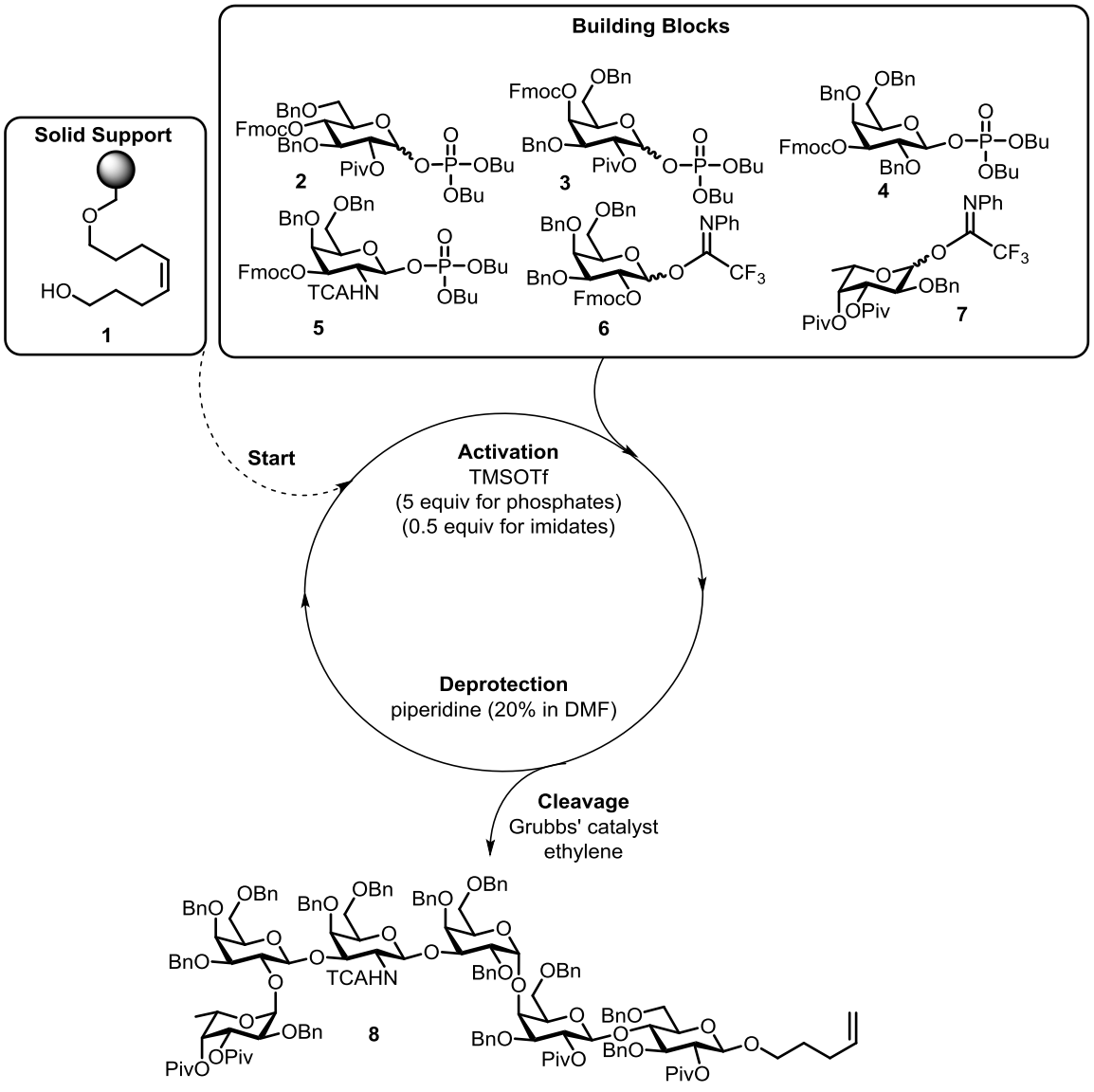


Scheme 3: Schematic representation of an automated glycan assembly procedure.

To ensure a successful synthesis of the oligosaccharide structures, the monosaccharide building blocks (BBs) used must be carefully designed. Permanent protecting groups (PGs) mask all hydroxyl groups that are not manipulated during the oligosaccharide synthesis. Temporary PGs can be selectively cleaved off on the solid support and allow elongation of the structure at specific positions. Commonly, benzyl ethers (Bn), benzoyl esters (Bz), azides and trichloroacetyl (TCA) groups are used as permanent PGs.[101] Fluorenylmethoxycarboyl (Fmoc)[101], levulinoyl (Lev)[101], (2-methyl)naphthyl (Nap)[103] and 2-(azidomethyl)benzoyl (Azmb)[104] routinely serve as orthogonal temporary PGs.

To show how AGA can be applied to obtain complex biological carbohydrate probes for example for vaccine development, the automated solid-phase synthesis of the tumor vaccine candidate Globo-H[105] is exemplary shown in scheme 4. The reported synthesis of Globo-H[105] demonstrated how complex glycan probes containing multiple 1,2-*trans* and 1,2-*cis* linkages can be assembled automatically. Six BBs equipped with phosphate or imidate leaving groups were applied. Five of the employed BBs contained the temporary protecting group Fmoc, which is completely stable under the acidic conditions required for

activation of the glycosyl donors. The oligosaccharide was assembled in an automated fashion by iterative glycosylation and deprotection steps on a solid support equipped with octenediol linker **1**, which was cleaved by olefin cross-metathesis, giving a pentenyl linker functionalized oligosaccharide. BBs with a participating PG (pivaloyl (Piv), TCA or Fmoc) in the C2-position enabled the stereoselective installation of 1,2-*trans* glycosidic linkages. The installation of the required 1,2-*cis* galactosidic linkage between the third (**3**) and the second BB (**4**) was achieved by using a BB without participating PG in the C2-position and activation of the BB at low temperature (-50 °C) in the presence of the α-directing solvent diethylether. Stereoselctive installation of the fucosyl BB (**7**) was supported by remote participation of Piv-ester groups. Once the hexasaccharide was assembled, the fully protected oligosaccharide **8** was cleaved from the solid support and isolated in 30% yield after column chromatography. Subsequently, the permanent PGs were removed using birch conditions.



Scheme 4: Automated Glycan Assembly of Globo-H.[105]

In the Max Planck Institute of Colloids and Interfaces (MPIKG) several fully automated oligosaccharide synthesizers[106] are available. Centerpiece of the synthesizers is a reaction vessel in which the linker-functionalized solid support (resin) is placed on top of a filter. Solvents are supplied from a solvent cabinet to the reaction vessel by applying argon pressure. The temperature inside the reaction vessel is controlled by a thermostat and can be regulated from -50° C to +50° C. Activator-, deprotection- and building block-solutions are delivered to the reaction vessel in high precision via syringe pumps. Mixing of the reaction mixture is ensured by bubbling argon through the vessel (fig. 10). After each step, excess reagents are drained through the filter by argon overpressure and the resin is washed with different solvents.

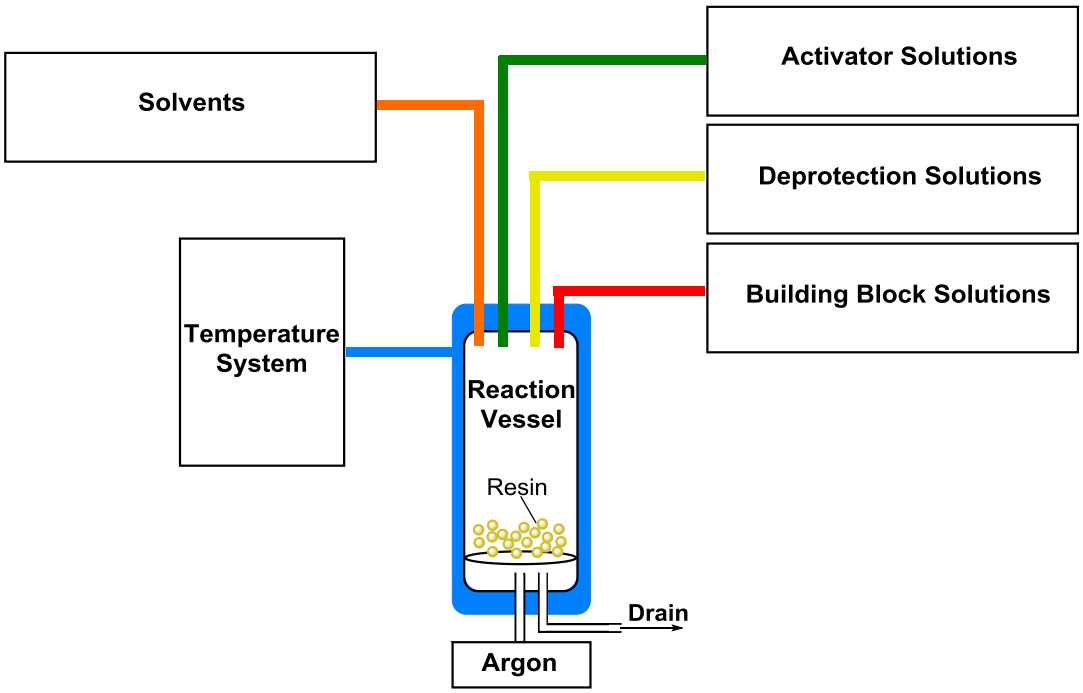
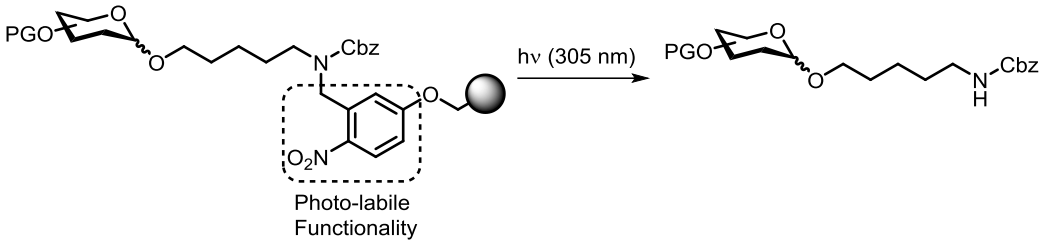


Figure 10: Schematic representation of the modules of an automated oligosaccharide synthesizer.

After a completed synthesis of an oligosaccharide the resin is taken out of the reaction vessel and the oligosaccharide is cleaved from the solid support. Today, the solid support is commonly furnished with a photo-labile linker that is stable towards the most frequently applied glycosylation and deprotection conditions.[107] The photolabile linker containing a *p*-nitrophenyl moiety (scheme 5) is cleaved from the solid support by UV light (305 nm) in a continuous flow reactor, yielding protected oligosaccharides equipped with a carboxybenzyl (Cbz) protected aminopentyl linker at the reducing end.

Since the first report of an automated solid-phase synthesis of an oligosaccharide in 2001, the technique has proven to be a powerful tool to access a wide range of oligosaccharides in appropriate times. AGA has been used to produce a variety of

biologically interesting probes such as potential vaccine candidates[108,109] or to synthesize long oligomers up to 30mers[110] and 50mers[102,111] which can be used to study material properties of carbohydrates.



Scheme 5: Cleavage of the most commonly used photo-labile linker for AGA.

1.4 Aims of this Thesis

The scientific motivation of this work was to provide a number of synthetic glycan tools for plant cell wall research. These glycan tools are plant cell wall-related probes that can be used to study for example the substrate specificities of cell wall-modifying enzymes or to assign the binding epitopes of mAbs used for high resolution imaging of cell wall polysaccharides. A better understanding of structure, function and biosynthesis of cell wall polysaccharides is crucial to enhance the usability of plant polysaccharides as resources for energy and materials. These polysaccharides include pectin polysaccharides which are in the context of biofuel production often neglected. Pectin can change the accessibility of hemicellulose or cellulose to enzymatic degradation[112] and pectin itself is a source of carbohydrates that can be further processed.[113]

This work aimed at the synthesis of well-defined pectin oligosaccharides as probes for the characterization of cell wall glycan-directed mAbs and of cell wall-degrading glycosylhydrolases. Additionally, the synthesis of functionalized sugar nucleotides as substrates for the development of novel high-throuput assays for the characterization of pectin-related glycosyltransferases was an important goal of this work.

The first aim was the synthesis of type-I and type-II AG oligosaccharides found in pectin. Automated glycan assembly was chosen to establish a library of structurally related oligosaccharides from galactose-, arabinose- and galacturonic acid monosaccharide BBs. The synthesis of type-I and type-II AGs required two sets of BBs with different protecting group patterns, allowing the installation of complex side chains to differently linked galactan backbones (chapter 2.1).

The second aim was the synthesis of a small library of RG-II backbone oligosaccharides substituted with the most internal monosaccharide (apiose) of side chains A or B (chapter 2.2). For the synthesis of RG-II oligosaccharides a number of

challenges had to be addressed, such as the installation of multiple α(1,4)-galacturonic acid linkages and the synthesis of the apiose BB. Hence, several synthetic approaches to assemble the oligosaccharide on solid-phase or in solution-phase were designed to accomplish this goal.

The third aim of this thesis was the chemical synthesis of azido- and amino-functionalized sugar nucleotides for the development of new high-throughput assays to screen putative plant glycosyltransferases for their substrate specificities. It was planned to incubate glycan microarrays equipped with synthetic plant oligosaccharides with the functionalized sugar nucleotides and to visualize the enzymatic reaction products through their selective reaction with fluorescent probes (chapter 2.3).

2 Results and Discussion

2.1 Automated Glycan Assembly of Arabinogalactans[1]

2.1.1 Synthesis of Type-I Arabinogalactans

2.1.1.1 Previous Chemical Syntheses of Type-I Arabinogalactans

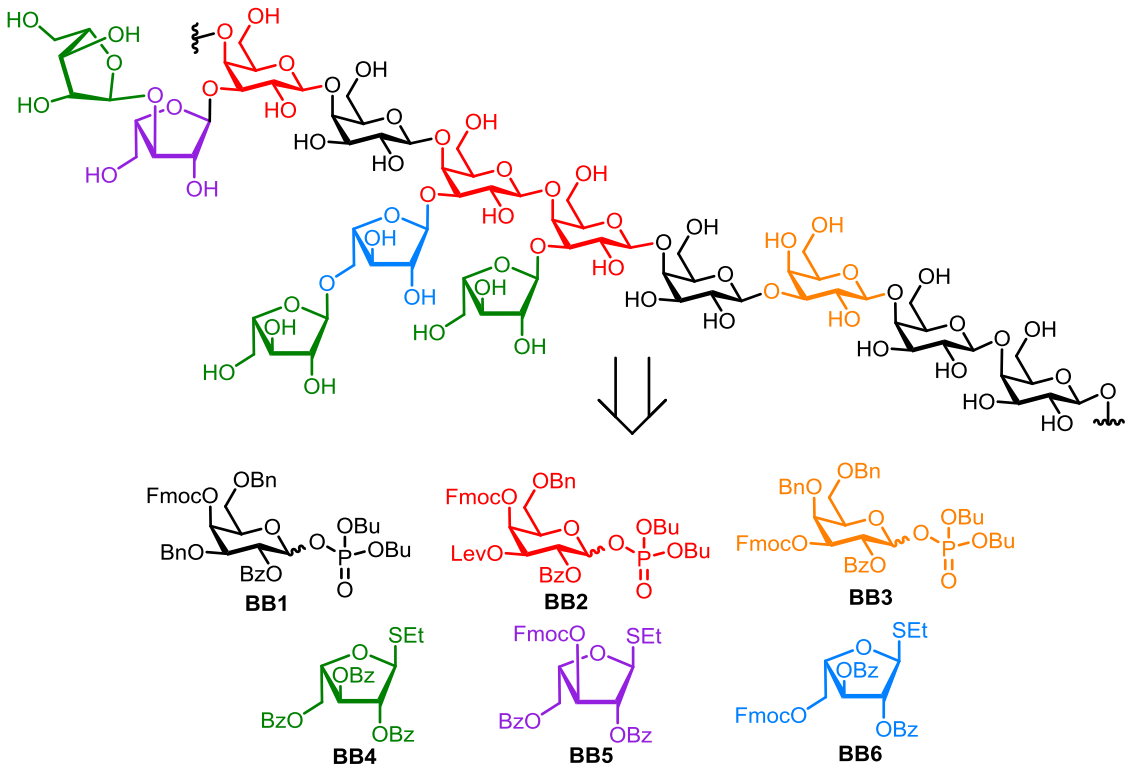
β(1,4)-Linked oligogalactans are challenging synthetic targets due to the low nucleophilicity of the axial C4-hydroxyl function of galactose which can be even lower when sterically demanding protecting groups at the C3- and C6-position are used. Only few syntheses of β(1,4)-D-galacto-oligosaccharides have been described in literature[114-119], some of which only reached the trisaccharide level[114,115]. The first chemical synthesis of a β(1,4)-D-galacto-trisaccharide was reported by *Schuerch et al.* who coupled glycosyl halide mono- or disaccharide donors to a galactose acceptor with a free hydroxyl group at the C4-position upon activation with silver triflate in a labor intensive ten step procedure.[114] Later, *Kováč et al.* reduced the number of synthetic steps for the synthesis of the β(1,4)-D-galacto-trisaccharide by using a different set of protecting groups. However, the use of electron-withdrawing ester protecting groups reduced the reactivity of the C4-hydroxyl function drastically and thereby reduced the yields immensly.[115] Significant progress towards the synthesis of longer β(1,4)-galactan structures could be made when *Lichtenthaler et al.* reported the synthesis of a β(1,4)-D-galacto-hexasaccharide in a 2+2+2 approach using disaccharide thioglycosides as glycosyl donors.[116] The most impressive examples for the solution-phase synthesis of type-I AGs have recently been reported by *Andersen et al*.[118] They described the synthesis of a small library of oligogalactans including structures with seven successive β(1,4)-linked galactose residues. The syntheses have been achieved by iterative glycosylations with *n*-pentenyl disaccharides followed by a late state glycosylation of a hexasaccharide core

[1] This chapter has been modified in parts from the following articles:
M. P. Bartetzko, F. Schuhmacher, H. S. Hahm, P. H. Seeberger, F. Pfrengle, *Org. Lett.*, **2015**, *17*, 4344-4347, Automated Glycan Assembly of Oligosaccharides Related to Arabinogalactan Proteins.
M. Wilsdorf, D. Schmidt, **M. P. Bartetzko,** P. Dallabernardina, F. Schuhmacher, P. Seeberger, F. Pfrengle, *Chem. Comm.*, **2016,** *52*, 10187-10189. A Traceless Photocleavable Linker for the Automated Glycan Assembly of Carbohydrates with Free Reducing Ends.
M. P. Bartetzko, F. Schuhmacher, P. H. Seeberger, F. Pfengle, *J. Org. Chem.*, **2017**, *82*, 1842-1850*,* Determining Substrate Specificities of β(1,4)-Endogalactanases Using Plant Arabinogalactan Oligosaccharides Synthesized by Automated Glycan Assembly
C. Ruprecht, **M. P. Bartetzko**, D. Senf, P. Dallabernardina, I. Boos, M. C. F. Andersen, T. Kotake, J. P. Knox, M. G. Hahn, M.H. Clausen, F. Pfrengle, *Plant Physiol.*, **2017**, *175*, 1094-1104. A Synthetic Glycan Microarray Enables Epitope Mapping of Plant Cell Wall Glycan-Directed Antibodies.

with single galactose or arabinose moieties or short galactan or arabinan disaccharides.[118] Unlike linear- and β(1,6)-branched β(1,4)-linked galactan oligosaccharides, α(1,3)-substituted type-I AG oligosaccharides have not been prepared so far.[116,118]

2.1.1.2 Automated Glycan Assembly of Type-I Arabinogalactans

Due to the highly repetitive structure of type-I AGs, automated glycan assembly is well suited to assemble libraries of type-I AGs from a small set of monosaccharide BBs (scheme 6). The BBs were designed for the synthesis of a comprehensive library of type-I AG oligosaccharides which contain characteristic structural features such as α(1,3)-substitution of the β(1,4)-galactan backbone with arabinose, substitution of the backbone with short α(1,3)- or α(1,5)-arabinans or single β(1,3)-linkages in the galactan backbone. β(1,3)-Galactan linkages have been found in type-I AG from potato.[120] Three differentially protected arabinose and three differentially protected galactose BBs were required (scheme 6).

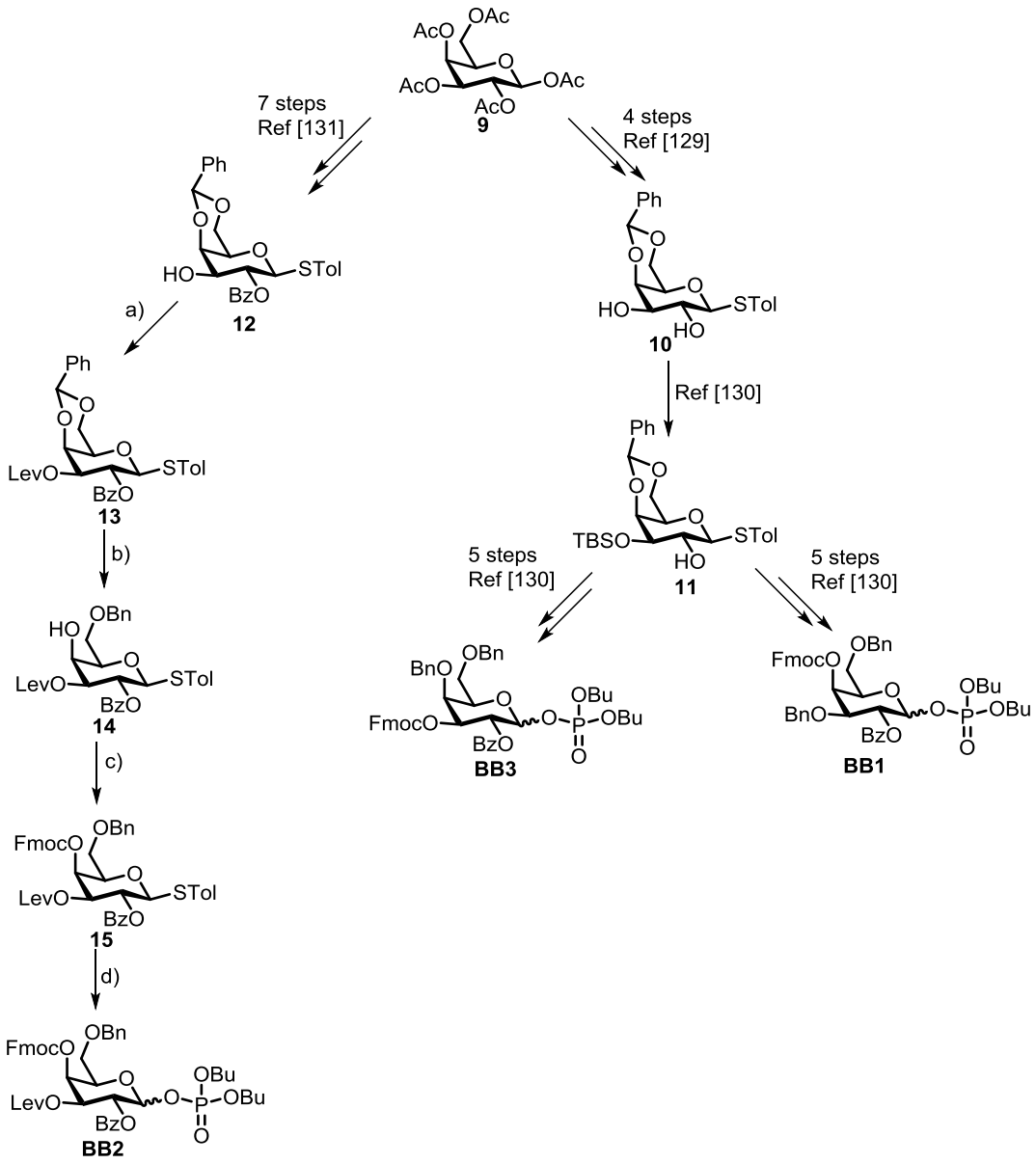


Scheme 6: Chemical structure of a type-I AG polysaccharide and the required building blocks for the automated glycan assembly of representative oligosaccharide fragments.

The two galactose BBs required for the synthesis of the β(1,4)-linked galactan backbone (**BB1**[121] and **BB2**) were equipped with a base labile Fmoc group in the C4-position for chain elongation. For the installation of side-chains in the C3-position, **BB2** was equipped with a temporary Lev protecting group that can be cleaved using hydrazine and is fully orthogonal to the Fmoc group.[101,122,123] The third galactose BB (**BB3**)[124], with an Fmoc protecting group in the C3-position, was used for elongation of the galactose solely in the C3-position. All remaining hydroxyl groups in **BB1**, **BB2** and **BB3** are not modified during the oligosaccharide assembly process and were permanently protected as benzyl ethers and benzoyl esters. The benzoyl protecting group in the C2-position provides the required stereocontrol for β-selective glycosylation. Additionally, by using dibutyl phosphate as the anomeric leaving group in the galactose building blocks, β-stereoselectivities during glycosylation reactions are maintained by avoiding α-directing ether solvents[105] which are commonly used during the activation of thioglycosides on solid support.[101] It has been observed before, that the activation of galactose thioglycoside BBs in the presence of ether solvents results in poor 1,2-*trans* stereoselectivities even in the presence of participating 2-*O*-protecting groups.[125-127]

Three different L-arabinofuranose BBs (**BB4**-**BB6**) were used for the installation of single arabinose substituents, α(1,3)-linked arabinan disaccharides, and an α(1,5)-linked arabinan trisaccharide. The arabinose building blocks were equipped with benzoyl esters as permanent protecting groups and a temporary Fmoc-protecting group at the respective position that is elongated during oligoarabinan synthesis. The arabinose BBs were used as thioglycoside donors as arabinose thioglycoside BBs provided good results in solution- or solid-phase syntheses previously.[103,128]

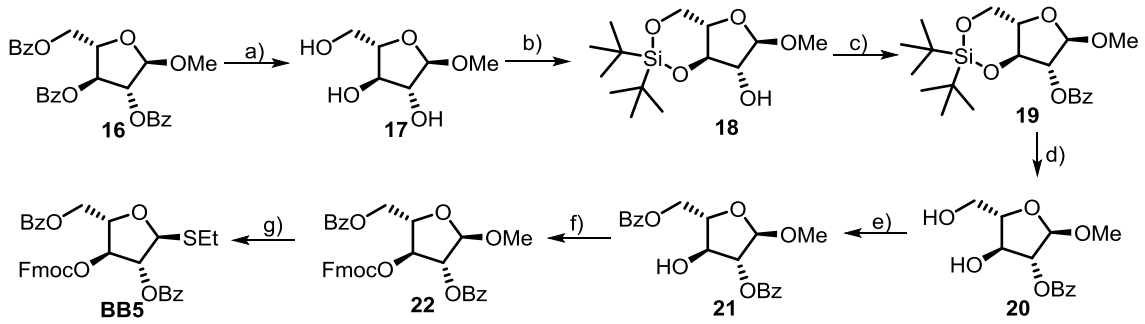
All three galactose BBs (**BB1**, **BB2** and **BB3**) were synthesized starting from peracetylated β-D-galactose **9** (scheme 7). The BBs **BB1** and **BB3** were obtained by a divergent synthesis starting from the reported benzylidene protected intermediate **10**[129] and have been previously used in the automated glycan assembly of few linear type-I and type-II arabinogalactan oligosaccharides (Max Bartetzko, MSc thesis, MPIKG).[130] **BB2** was synthesized starting from literature known intermediate **12**.[131] The levulinoyl group was introduced in good yields at the C3-position by DIC-promoted esterification giving **13**. Subsequent selective opening of the benzylidene acetal yielded intermediate **14** with a free hydroxyl group in the C4-position, which allowed the introduction of Fmoc as a second temporary protecting group at the C4-position. Exchange of the anomeric thioether leaving group for a phosphate provided the corresponding glycosyl donor **BB2** (scheme 7).



Scheme 7: Synthesis of galactose BBs **BB1**, **BB2** and **BB3**. Reagents and conditions: a) LevOH, DIC, DMAP, rt, DCM, 85%; b) Et_3SiH, TFAA, TFA, rt, DCM, quant.; c) FmocCl, pyridine, rt, DCM, 66%; d) $HOP(O)(OBu)_2$, NIS, TfOH, 0 °C, DCM, 69%.

The arabinose **BB6** was synthesized as previously described for the corresponding D-arabinofuranose[128] and **BB4**[132] was available in the laboratory and kindly provided by Deborah Senf. Arabinose **BB5** was available from 2,3,5-tri-*O*-benzoyl-1-methyl-α-L-arabinofuranose (**16**), which was also kindly provided by Deborah Senf. Methanolysis of the ester protecting groups of **16** gave **17**. Selective protection of the C4- and the C5-hydroxyl functions as cyclic silyl acetal yielded **18**, which could be selectively protected in the C2-position with a benzoyl ester, yielding **19**. Deprotection of the C3- and the C5-hydroxyl groups by removal of the silyl acetal with HF·pyridine and selective

protection of the primary hydroxyl function using benzoic acid in a Mitsunobu reaction gave access to the desired intermediate **21**. The free C3-hydroxyl group in **21** was then reacted with FmocCl to give **22** which was further reacted with ethanethiol to obtain the final arabinofuranose building block **BB5** (scheme 8).

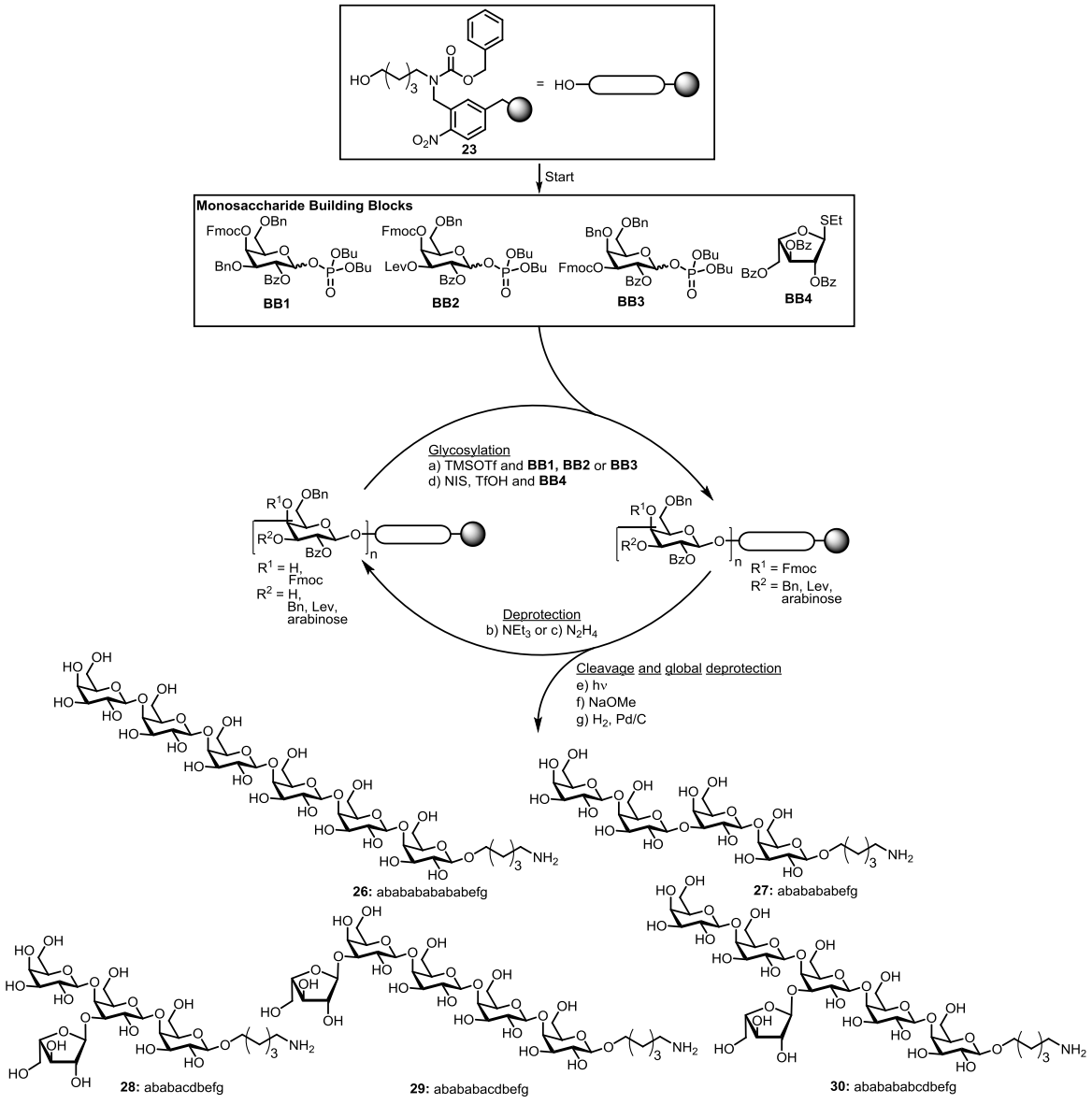


Scheme 8: Synthesis of arabinose **BB5**. Reagents and conditions: a) NaOMe, rt, MeOH/DCM, 99%; b) $(t\text{Bu})_2\text{Si}(\text{OTf})_2$, 2,6-lutidine, -5 °C, DMF, 58%; c) BzCl, rt, pyridine, 94%; d) HF·pyridine, 0 °C to rt, THF, 71%; e) BzOH, DEAD, Ph_3P, rt, THF, 77%; f) FmocCl, pyridine, rt ,DCM, 81%; g) EtSH, $\text{Et}_2\text{O}\cdot\text{BF}_3$, 0 °C, DCM, 76%.

After obtaining **BB1–BB6,** the automated glycan assembly of eight oligosaccharide fragments of pectic type-I AG polysaccharides was performed by alternating glycosylation and deprotection procedures on a solid support functionalized with a photo-labile linker (**23**) that yields oligosaccharides with an aminopentyl linker at the reducing end.[107] Glycosylations with phosphate building blocks **BB1**–**BB3** were generally performed using two times 3.8 equivalents building block and stoichiometric amounts of the activator TMSOTf. Thioglycoside building blocks **BB4**–**BB6** were used in two glycosylation cycles adding 3.8 equivalents building block each and activated with a slight excess of NIS[133] together with catalytic amounts of triflic acid. Deprotection of Fmoc was achieved with triethylamine (20% in DMF). Levulinoyl protecting groups were removed with a hydrazine acetate solution (0.15 M). Following assembly of the galactan backbone, capping with benzoic anhydride in the presence of dimethylaminopyridine (DMAP) allowed for temporary Fmoc protecting groups to be used for the selective elongation of arabinan side chains. The fully deprotected oligosaccharides were obtained following light-induced cleavage from the solid support, methanolysis of the ester protecting groups, and hydrogenolysis of the benzyl ethers and the Cbz-group at the linker.

The synthesis of a β(1,4)-linked galactan disaccharide (**24**) and a linear β(1,4)-linked galactan tetrasaccharide (**25**) on solid support was achieved in good yields previously (Max Bartetzko, MSc thesis, MPIKG).[130] Therefore the linear β(1,4)-linked galactan hexasaccharide **26** was prepared accordingly by alternating glycosylations with **BB1** and Fmoc deprotection reactions (scheme 9). The low reactivity of the axial C4-hydroxy nucleophile of galactose was likely responsible for the low yields that were

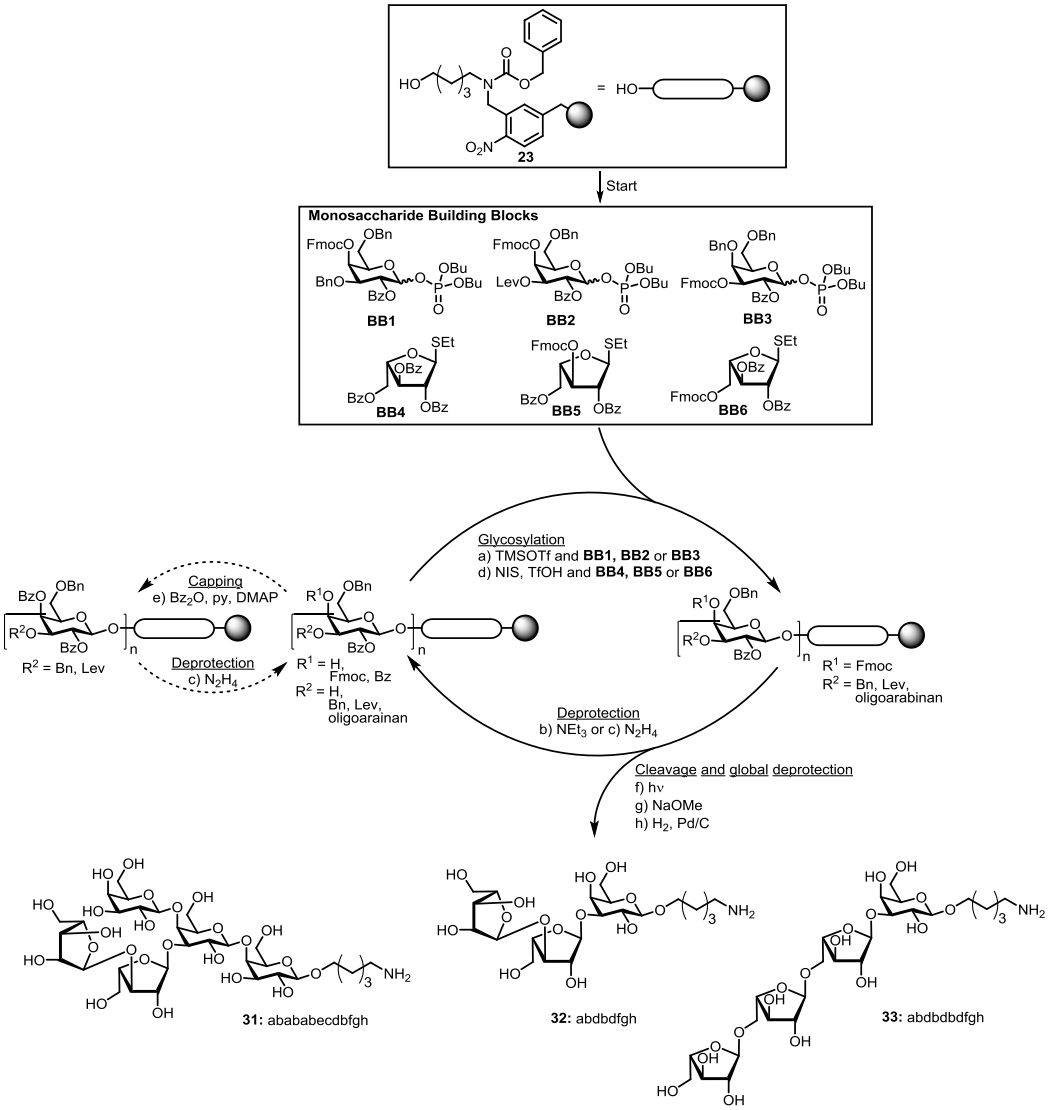
obtained after multiple formations of this challenging glycosidic linkage. β(1,4)-, β(1,3)-Mixed-linkage galactan tetrasaccharide **27**, a structural component in the backbone of type-I AGs in potato[120], was prepared in moderate yields by incorporation of **BB3** as the second BB in the AGA process (scheme 4). Arabinogalactan oligosaccharides **28**-**30** that contain α(1,3)-linked arabinose either at the central (**28** and **30**) or the terminal position of the backbone (**27**) were prepared by incorporation of the levulinoyl substituted BB (**BB2**) into the galactan backbone synthesis and selective removal of the levulinoyl protecting group after full assembly of the galactan backbone before final attachment of **BB4** (scheme 9).



Scheme 9: Automated glycan assembly of type-I arabinogalactan oligosaccharides **26**-**30**. Reagents and conditions: a) Twice 3.8 equiv of **BB1**, **BB2** or **BB3**, TMSOTf, DCM, -35 °C (5 min) → -20 °C (30 min) or -30 °C (5 min) → -10 °C (30 min); b) Three cycles of 20% NEt_3 in DMF, 25 °C (5 min); c) Three cycles of 0.15 M hydrazine in pyridine/AcOH/H_2O (4:1:0.25), 25 °C (30 min); d) Twice 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min); e) hv (305 nm); f) NaOMe, THF, 16 h; g) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16h. **26**: 2%, **27**: 16%, **28**:

6%, **29**: 7% **30**: 5% (yields are based on resin loading). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

Arabinose BBs **BB5** and **BB6** were required for the synthesis of short α(1,3)- and α(1,5)-linked arabinan oligomers. These arabinan oligosaccharides were either attached to a single galactose unit (**32** and **33**) or a galactan trisaccharide backbone (**29**). The oligosaccharides **30** and **31** were synthesized by attachment of 3-*O*-Fmoc protected galactose **BB3** to the solid support and iterative Fmoc removal and glycosylation reactions with **BB5**, **BB6** or **BB4** (scheme 10). The synthesis of the more complex type-I arabinogalactan oligosaccharide **31** required a capping step after assembly of the β(1,4) backbone to ensure exclusive attachment of the second arabinose moiety to the C3-position of the first side-chain arabinose moiety. For that purpose, the terminal Fmoc-group was removed after assembly of the β(1,4)-galactan backbone and the resulting free hydroxyl group was masked as an ester. It was found that the standard capping protocol previously applied in automated glycan assembly,[103] employing acetic anhydride in pyridine, was not suitable in this case – the resulting acetyl groups did not withstand the conditions required for the removal of Lev-groups. Thus, benzoyl esters were used instead of acetyl groups as capping moieties. These are more stable to the hydrazine used for Lev-deprotection.[134] Quantitative capping was achieved by incubating the resin-bound oligogalactan for 30 min at 40 °C with a solution of benzoic anhydride and DMAP in DCE (scheme 10). Using the benzoyl capping method, compound **31** was synthesized successfully. All fully de-protected type-I arabinogalactan oligosaccharides (**26**-**33**) were obtained after light-induced cleavage from the solid support, global deprotection by methanolysis and subsequent hydrogenolysis in yields of 4-38% after HPLC purification. The purity of all de-protected type-I AGs was ensured by analysis with HPLC, NMR-spectroscopy, and high-resolution mass spectrometry.



Scheme 10: Automated glycan assembly of type-I arabinogalactan oligosaccharides **29**-**31** including arabinan side-chains. a) Twice 3.8 equiv of **BB1**, **BB2** or **BB3**, TMSOTf, DCM, -35 °C (5 min) → -20 °C (30 min) or -30 °C (5 min) → -10 °C (30 min); b) Three cycles of 20% NEt_3 in DMF, 25 °C (5 min); c) Three cycles of 0.15 M hydrazine in pyridine/AcOH/H_2O (4:1:0.25), 25 °C (30 min); d) Twice 3.8 equiv **BB4**, **BB5** or **BB6** NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min); e) Three cycles of 0.5 M Bz_2O and 0.25 M DMAP in DCE, pyridine, 40 °C (30 min); f) hv (305 nm); g) NaOMe, THF, 16 h; h) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16h. **31**: 8%, **32**: 17%, **33**: 38% (yields are based on resin loading). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

It is noteworthy that a slow isomerization of hexasaccharide **30** was observed upon storing the oligosaccharide as a stock solution in water (2 mM). While the HPLC analysis of **30** revealed a single product peak immediately following the last deprotection reaction, an additional peak of identical mass appeared after storing **30** for several days in water at 4 °C. ^{1}H-NMR analysis revealed a shift of the signal for the anomeric arabinose proton from 5.48 ppm to 5.24 ppm (fig. 11). Apparently, the arabinofuranose isomerized into the corresponding β-arabinopyranose.

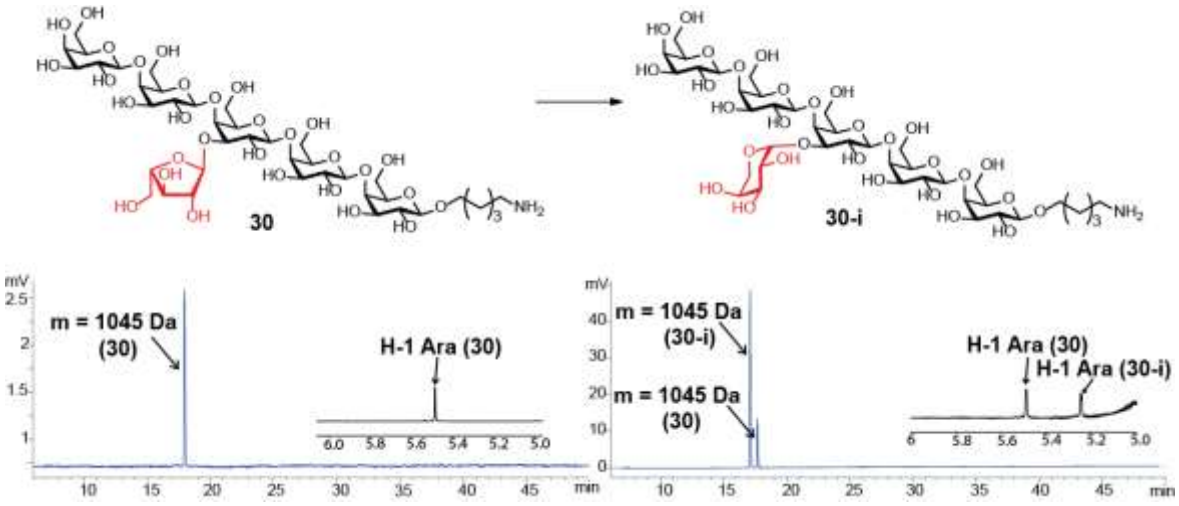


Figure 11: Isomerization of arabinogalactan **30.** Left: HPLC analysis (ELSD trace) and ^{1}H-NMR chemical shift of the anomeric arabinose proton of **30** directly after synthesis. Right: HPLC analysis and ^{1}H-NMR chemical shifts of the anomeric arabinose proton of **30** after storage in aqueous solution for several days.

This isomerization reaction may have been intramolecularly catalyzed by the aminoalkyl linker. Base-catalyzed isomerizations of methyl arabinofuranosides into arabinopyranosides in the presence of pyridine have been observed previously.[135] None of the other arabinogalactan oligosaccharides underwent isomerization.

2.1.2 Synthesis of Type-II Arabinogalactans

2.1.2.1 Previous Chemical Syntheses of Type-II Arabinogalactans

While the synthesis of type-I arabinogalactans (AG) is difficult due to the low reactivity of the axial C4-hydroxyl function, the synthesis of type-II arabinogalactans is comparably easier because of higher reactivities of the C3- and the C6-hydroxyl groups. Several approaches towards the synthesis of type-II AG fragments have been published. Most of these syntheses focus on β(1,6)-galactans partially substituted with L-arabinofuranoses. A comprehensive overview of the synthesized type-II AG structures with a β(1,6) backbone was published in a recent review.[95] One of the most impressive work found in literature is the assembly of a β(1,6)-galactan backbone consisting of up to 15 residues which are partially substituted at the C2- or C3-positions with arabinofuranosyl moieties.[136] Li and co-workers synthesized these arabinogalactans of varying length from five different monosaccharide building blocks in a block-wise approach in a series of tedious glycosylation-, deprotection- and purification steps.

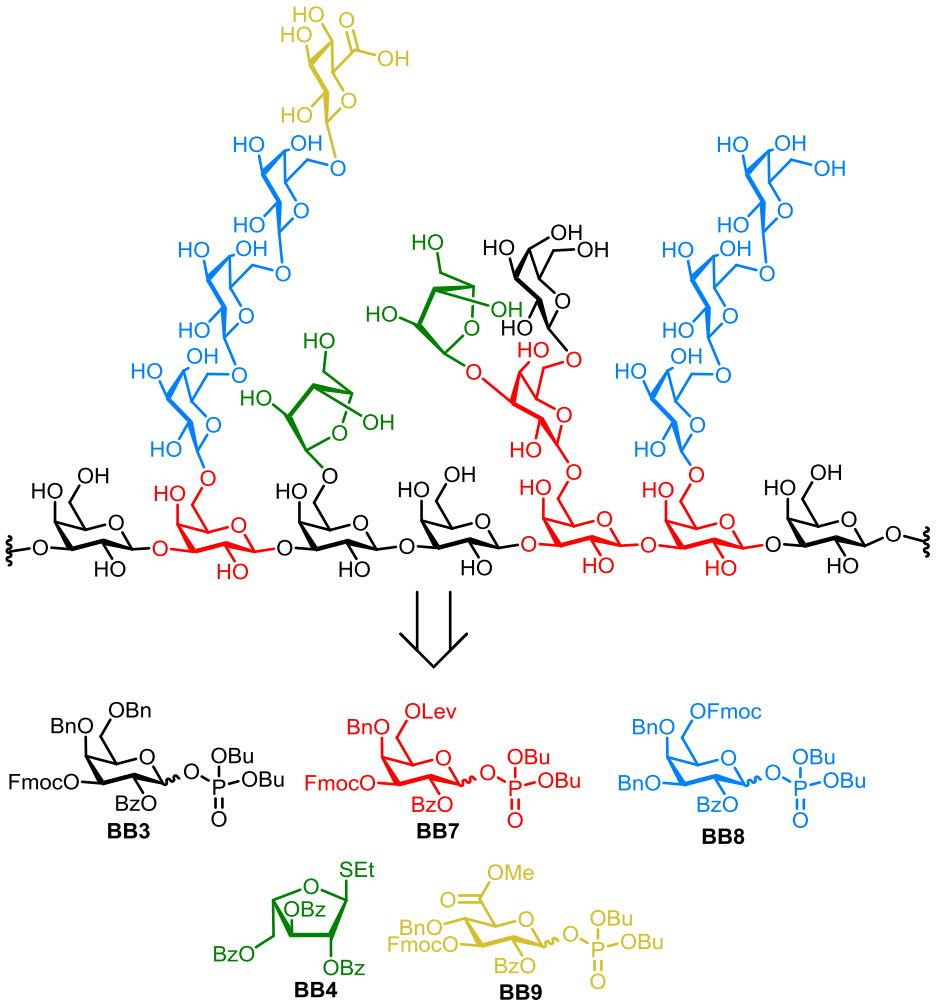
Only few syntheses of well-defined β(1,3)-linked galactans have been reported. Most of them were disaccharides and trisaccharides.[126,127,137-140] Longer β(1,3)-galactan structures

have been synthesized by Kováč and co-workers who used a single building block to assemble a series of linear β(1,3)-galactans up to a heptamer.[125] However, the synthesis suffered from relatively low yields and inconvenient purification procedures due to the formation of undesired side-products such as α-glycosides or orthoesters. Recently, another report describing the synthesis of linear and β(1,6)-branched β(1,3)-galactans by iterative couplings of monosaccharide and disaccharide thioglycoside donors has been published.[141] They achieved β(1,6)-branching of the β(1,3)-galactan backbone by incorporation of a monosaccharide building block with a temporary acetyl protecting group at the C6-position. Selective removal of the acetyl protecting group allowed the installation of single arabinofuranose or single galactose and β(1,6)- or β(1,3)-digalactan side chains at different positions of the β(1,3)-heptagalactan backbone after the full assembly of the backbone. Nevertheless, access to a more elaborated library of complex type-II AGs containing features of the β(1,3)-galactan backbone and/or the β(1,6)-galactan side chains that may be partially substituted with arabinofuranosyl or glucuronic acid moieties is still missing for comprehensive biological studies.

2.1.2.2 Automated Glycan Assembly of Type-II Arabinogalactans

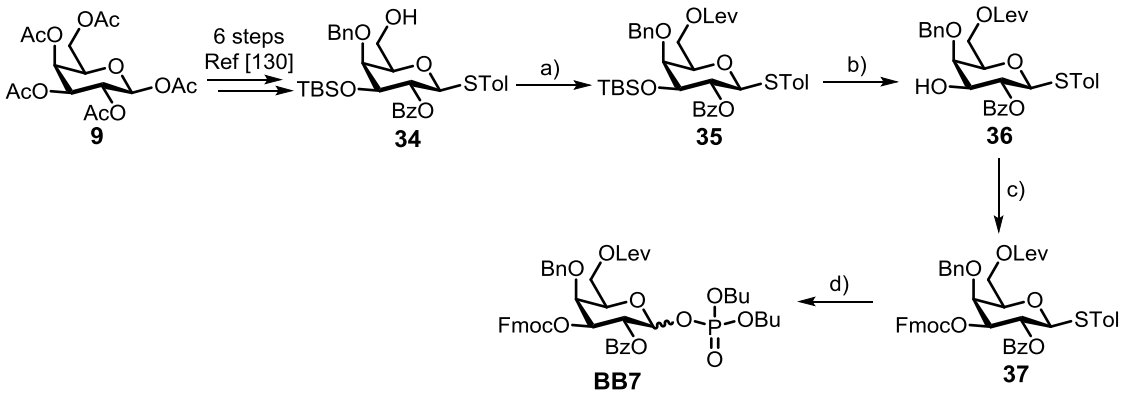
In order to produce a large number of structurally related type-II arabinogalactans by automated glycan assembly, a number of monosaccharide BBs had to be designed (scheme 11). Linear β(1,3)-galactan oligosaccharides and β(1,6)-galactan side-chains with partial decorations of arabinofuranosyl oligosaccharides or glucuronic acid residues can be assembled from a set of five BBs (**BB3**–**BB4** and **BB7**–**BB9**). Additionally to the two BBs, which were used in the synthesis of type-I AGs and described in chapter 2.1.1.2 (**BB3** and **BB4**), two additional galactose BBs were required. **BB7** was equipped with a temporary Fmoc protecting group in the C3-position for β(1,3)-galactan chain elongation and an orthogonal Lev protecting group in the C6-position, which allowed the installation of β(1,6)-linked side chains after removal of the Lev group on solid support. The galactose **BB8** had Fmoc as the single temporary protecting group in the C6-position. This BB can be used for elongation of β(1,6)-galactan chains. In the C2-position of both BBs, benzoyl esters (Bz) ensure selective formation of the β-anomers through neighboring group participation. All remaining positions were protected with benzyl ethers. Additionally, the glucuronic ester donor **BB9** was employed to terminate β(1,6)-galactans with a β(1,6)-linked glucuronic acid moiety. A precursor of glucuronic acid **BB9** equipped with a thiol leaving group was available in my laboratory[142] and was kindly provided by my colleague

Andrew Kononov. The thioglycoside leaving group of the precursor was exchanged for the phosphate leaving group using an established procedure.[142]



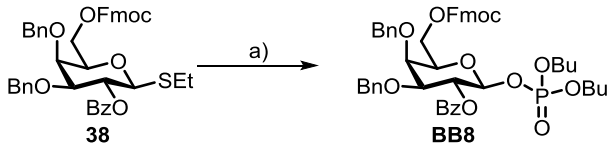
Scheme 11: Chemical structure of a type-II arabinogalactan polysaccharide and the required building blocks for the assembly of representative oligosaccharide fragments on solid support.

The synthesis of **BB7** with two orthogonal protecting groups at the C3- and C6-positions started from common intermediate **34** which was prepared from **9** in six steps following an established procedure (Max Bartetzko, MSc thesis, MPIKG).[130] The primary hydroxyl group of **32** was protected as a levulinoyl ester in high yields using levulinic acid under standard esterification conditions. Removal of the *tert*-butyldimethylsilyl protecting group at the C3-position using boron trifluoride etherate provided **36** in quantitative yield. Installation of the temporary Fmoc protecting group at the C3-position gave **35** which finally could be transformed into the desired **BB7** by exchange of the anomeric leaving group for a phosphate (scheme 12).



Scheme 12: Synthesis of galactose **BB7**. Reagents and conditons: a) LevOH, DIC, DMAP, rt, DCM 91%; b) $Et_2 \cdot BF_3$, 0 °C, MeCN, quant.; c) FmocCl, pyridine, rt, DCM, 75%; d) $HOP(O)(OBu)_2$, NIS, TfOH, 0 °C, DCM, 92%.

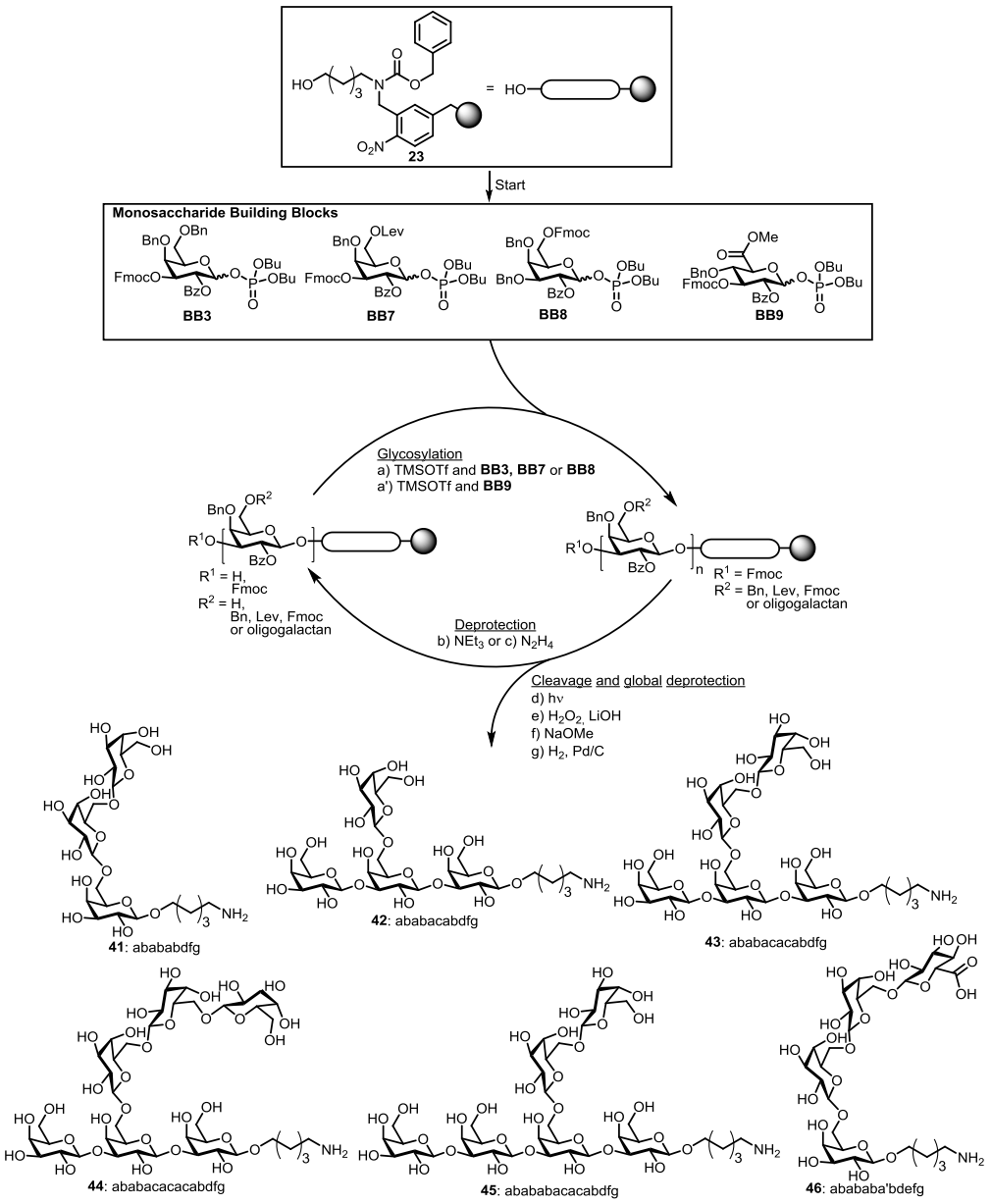
Glycosyl phosphate **BB8** was synthesized from **38**[143], which was provided by my colleague Heung Sik Hahm. The thioether leaving group was exchanged for the phosphate using NIS and catalytic amounts of TfOH (scheme 13).



Scheme 13: Synthesis of galactose **BB8**. Reagents and conditions: a) $HOP(O)(OBu)_2$, NIS, TfOH, 0 °C, DCM, 80%.

With the required building blocks in hand, further type-II AG fragments were synthesized on solid support in addition to previously synthesized linear β(1,3)-linked tri- (**39**) and tetragalactan (**40**) structures (Max Bartetzko, MSc thesis, MPIKG).[130] As expected, synthesis of a β(1,6)-linked trigalactoside proceeded in high yields and perfect stereoselectivity when using phosphate **BB8**. Hence, the stage was set to assemble linear and branched galactan oligosaccharides **41**-**46**. Glycosylations were performed in two cycles using 3.8 equivalents of galactose building blocks **BB3**, **BB7** and **BB8** and equimolar amounts of TMSOTf. Every glycosylation was followed either by an Fmoc- or Lev-deprotection step, depending on which position of the respective galactose unit was subsequently elongated. All branched structures were synthesized by assembling the linear β(1,3)-linked backbone first and subsequently attaching the β(1,6)-linked side chain. After completion of the automated syntheses, the protected oligosaccharides were cleaved from the solid support and treated with sodium methoxide in methanol. The resulting semi-protected products were subjected to hydrogenolysis and the fully deprotected oligogalactans **41**-**46** were obtained in 5-43% overall yield, based on the calculated loading of linker-functionalized resin **23** (scheme 14). In the case of glucuronic acid containing tetrasaccharide **46** the β(1,6)-galactan chain was assembled using only

one time five equivalents of **BB8** per glycosylation step, as high conversions in glycosylation reactions employing **BB8** were observed in the synthesis of oligosaccharide **41**. The terminal glucuronic acid residue on the other hand was coupled using two times five equivalents of **BB9** and stoichiometric amounts of the activator TMSOTf at elevated temperatures as has been used previously for this BB.[142]

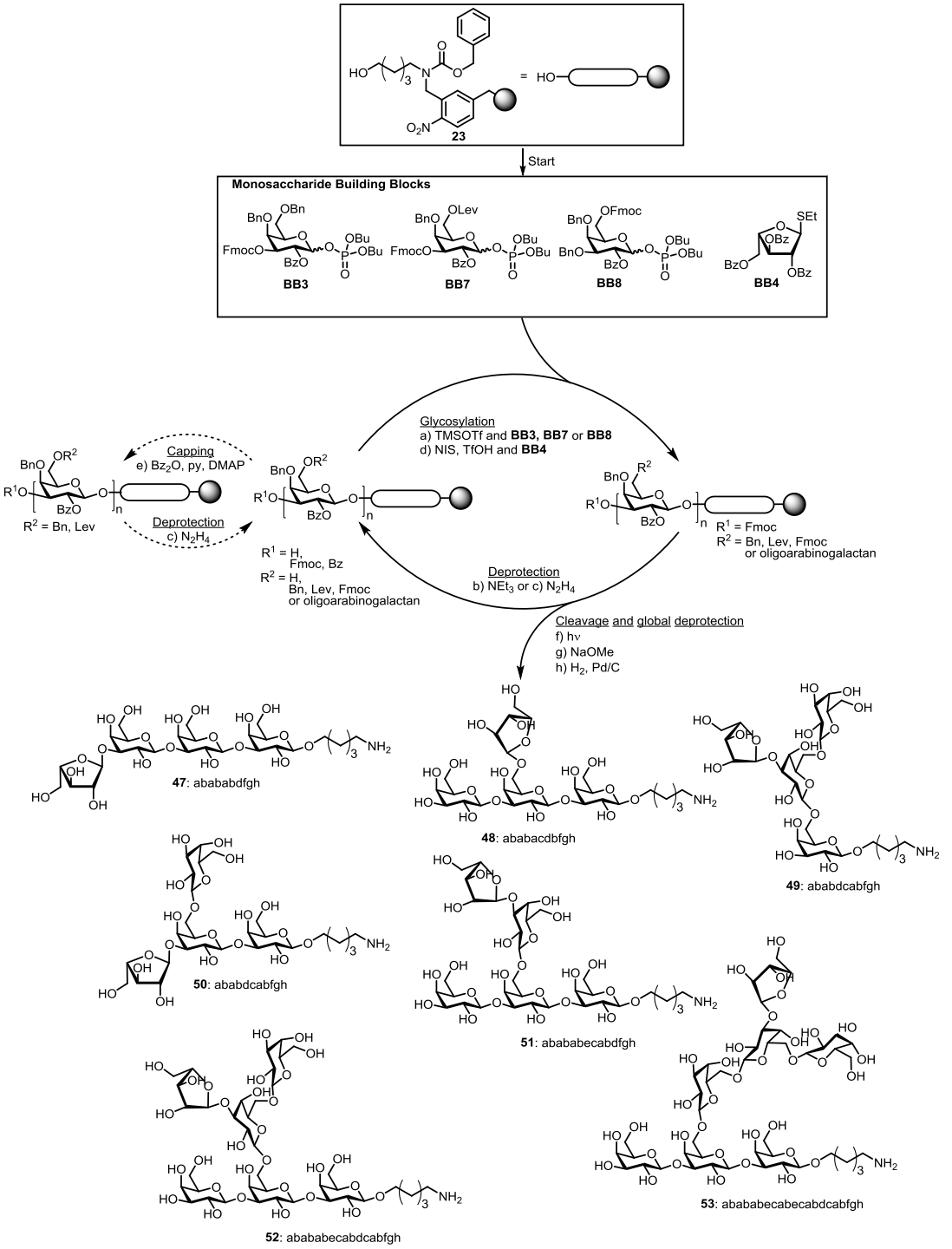


Scheme 14: Automated glycan assembly of type-II arabinogalactan oligosaccharides **41**-**46**. Reagents and conditions: a) Twice 3.8 equiv **BB3**, **BB7**, **BB8** or one time 5 equiv **BB8** for the synthesis of **46**, TMSOTf, DCM, -35 °C (5 min) → -20 °C (30 min); a') twice 5 equiv **BB9** TMSOTf, DCM, -15 °C (45 min) → 0 °C (15 min); b) Three cycles of 20% NEt_3 in DMF, 25 °C (5 min); c) Three cycles of 0.15 M hydrazine in pyridine/AcOH/H_2O (4:1:0.25), 25 °C (30 min); d) hv (305 nm); e) H_2O_2, H_2O, THF, -5 C to rt, 16 h; f) NaOMe, THF, 16 h; g) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16h. **41**: 39%, **42**: 15%, **43**: 10%, **44**: 7%, **45**: 5% **46**: 43% (yields are based on resin loading). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

After cleavage of the completely assembled oligosaccharide **46** from the solid support, the ester was saponificated using lithium hydroxide and hydrogen peroxide. The resulting tetrasaccharide containing the free carboxylic acid was subjected to methanolysis and subsequent hydrogenolysis. The fully deprotected tetrasaccharide **46** was obtained in 43% overall yield.

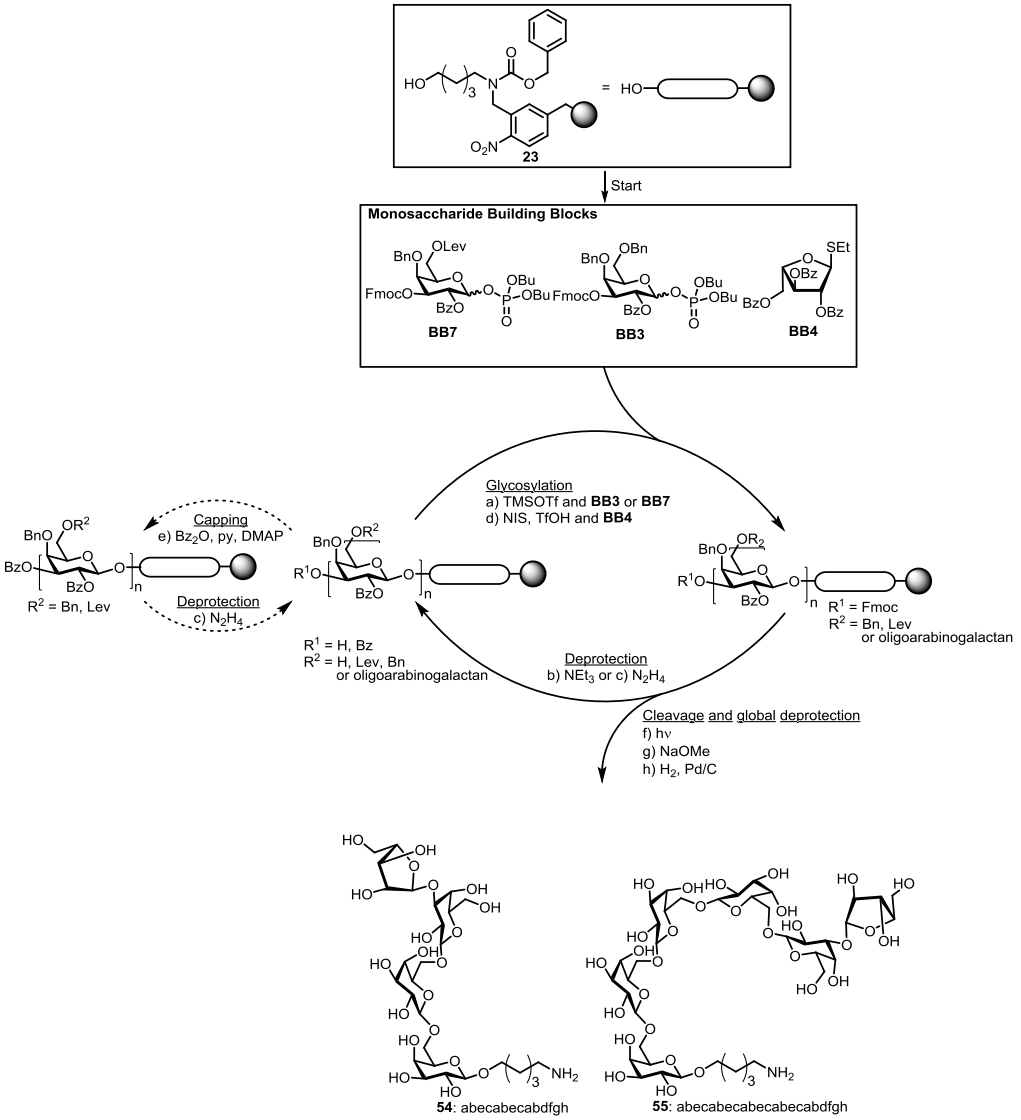
Addition of L-arabinose building block **BB4**[11] to the previously used set of galactose building blocks (**BB3**, **BB7**, **BB9**) enabled the production of seven additional oligosaccharides that represent substructures of type-II arabinogalactan (scheme 15). In the synthesized AG oligosaccharides, the arabinose unit is either attached to the β(1,3)-linked galactan backbone (**47**, **48**, **50**) or to the β(1,6)-linked galactan side chain (**49**, **51**-**54**). The syntheses of the more elaborate oligosaccharides **43**-**45** required capping steps during the automated synthesis to ensure the selective attachment of the arabinose to the desired galactose unit. Capping of the C3-position of the terminal galactose at the β(1,3)-galactan backbone was achieved as described above for the synthesis of type-I AG oligosaccharide **31** using benzoic anhydride and DMAP. After capping of the backbone, the side-chain was introduced by iterative Lev-deprotection and glycosylation reactions. The arabinose building block **BB4** was then selectively attached to the side chain after Fmoc-deprotection.

The same glycosylation protocols that were established for the synthesis of oligogalactans **41**-**46** were applied for AG oligosaccharides **47**-**53**. Thioglycoside **BB4** was activated with NIS and catalytic amounts of TfOH in DCM. After cleavage of the products from the solid support, global deprotection and purification, AG oligosaccharides **47**-**53** were obtained in yields between 5 and 39%, based on the calculated resin loading (scheme 15).



Scheme 15: Automated glycan assembly of arabinose containing type-II arabinogalactan oligosaccharides **47**-**53**. Reagents and conditions: a) Twice 3.8 equiv **BB3**, **BB7** or **BB8**, TMSOTf, DCM, -35 °C (5 min) → -20 °C (30 min); b) Three cycles of 20% NEt_3 in DMF, 25 °C (5 min); c) Three cycles of 0.15 M hydrazine in pyridine/AcOH/H_2O (4:1:0.25), 25 °C (30 min); d) Twice 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min); e) Three cycles of 0.5 M Bz_2O and 0.25 M DMAP in DCE, pyridine, 40 °C (30 min); f) hv (305 nm); g) NaOMe, THF, 16 h; h) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16h. **47**: 17%, **48**: 17%, **49**: 16%, **50**: 10%, **51**: 16%, **52**: 11%, **53**: 9% (yields are based on resin loading). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

Finally, **BB3**, **BB4** and **BB7** were used to assemble two type-II AG structures which resemble β(1,6)-galactose side chains that are capped with an arabinofuranosyl residue at the C3-position of the terminal galactose residue. Since **BB8** with a single temporary protecting group in the C6-position was not available in sufficient amounts at the time of the synthesis, the synthesis of oligosaccharides **54** and **55** was performed using the more complex **BB7**. Key step in the syntheses of **54** and **55** using **BB7** was the exchange of the Fmoc protecting group at the C3-position of **BB7** with a benzoyl ester on solid support after each glycosylation reaction with **BB7**. After every capping step removal of the levulinoyl protecting group at the C6-position allowed extension of the β(1,6)-galactan chain. **BB3** was finally used to terminate the β(1,6)-galactan chain, allowing the introduction of an α-linked arabinose at the C3-position on the terminal galactose after removal of the Fmoc group (scheme 16). Light-induced cleavage from the solid support, followed by global deprotection and purification steps, gave tetrasaccharide **54** in moderate yield (10%) and hexasaccharide **55** in low yield (1%). The difficult handling of the semi-protected oligosaccharide after methanolysis of the ester protecting groups lowered the overall yield of **55** drastically. The semi-protected version of **55** formed aggregates which could only partially be dissolved in methanol and methanol solvent mixtures that were used for hydrogenolysis. The purity of all deprotected type-II AGs was ensured by characterization with analytical HPLC, NMR-spectroscopy, and high-resolution mass spectrometry.



Scheme 16: Automated glycan assembly of type-II arabinogalactan oligosaccharides **54** and **55** using **BB7** for backbone elongation. Reagents and conditions: a) One time 5 equiv **BB3** or **BB7**, TMSOTf, DCM, -35 °C (5 min) → -20 °C (30 min); b) Three cycles of 20% NEt_3 in DMF, 25 °C (5 min); c) Three cycles of 0.15 M hydrazine in pyridine/AcOH/H_2O (4:1:0.25), 25 °C (30 min); d) Twice 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min); e) Three cycles of 0.5 M Bz_2O and 0.25 M DMAP in DCE, pyridine, 40 °C (30 min); f) hv (305 nm); g) NaOMe, THF, 16 h; h) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16h. **54**: 10%, **55**: 1% (yields are based on resin loading). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

2.1.2.3 Automated Glycan Assembly of Type-II Arabinogalactans with Free Reducing Ends Using a Traceless Linker

Having established reliable routes towards the assembly of type-II AGs by means of AGA, the synthesized BBs (**BB3**,**BB4**, **BB7** and **BB8**) were used to test a lately developed photo-labile traceless linker for the syntheses of complex and branched

glycans.[144] Free reducing end glycans produced by AGA represent valuable probes for biological assays that do not tolerate unnatural modifications or can serve as standards for LC/MS measurements[145]. Linker **56** (fig. 12) has been designed in analogy to the established photo-labile linker **23**[107] which provides oligosaccharides equipped with an alkyl linker after UV-light induced cleavage from the solid support. By introducing a benzyl spacer in the newly developed linker **56**, oligosaccharides equipped with a reductive-labile group at the reducing end are obtained. This benzylic group is removed upon a final hydrogenolysis reaction, releasing the free reducing end glycan after global deprotection.[146-148]

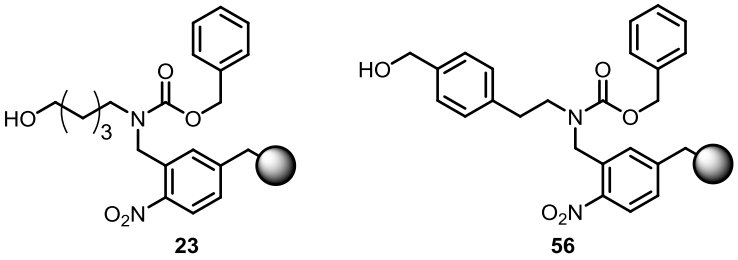
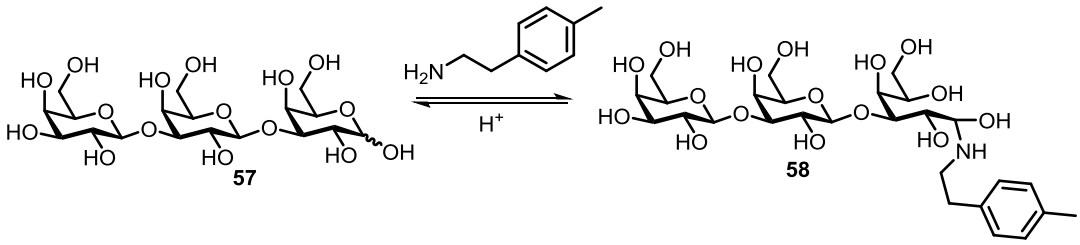


Figure 12: Photo-labile linkers for the solid phase synthesis of oligosaccharides. **23**: Linker commonly used in AGA for the synthesis of aminopentyl-functionalized oligosaccharides. **56**: Traceless linker for the solid phase synthesis of free-reducing end oligosaccharides.

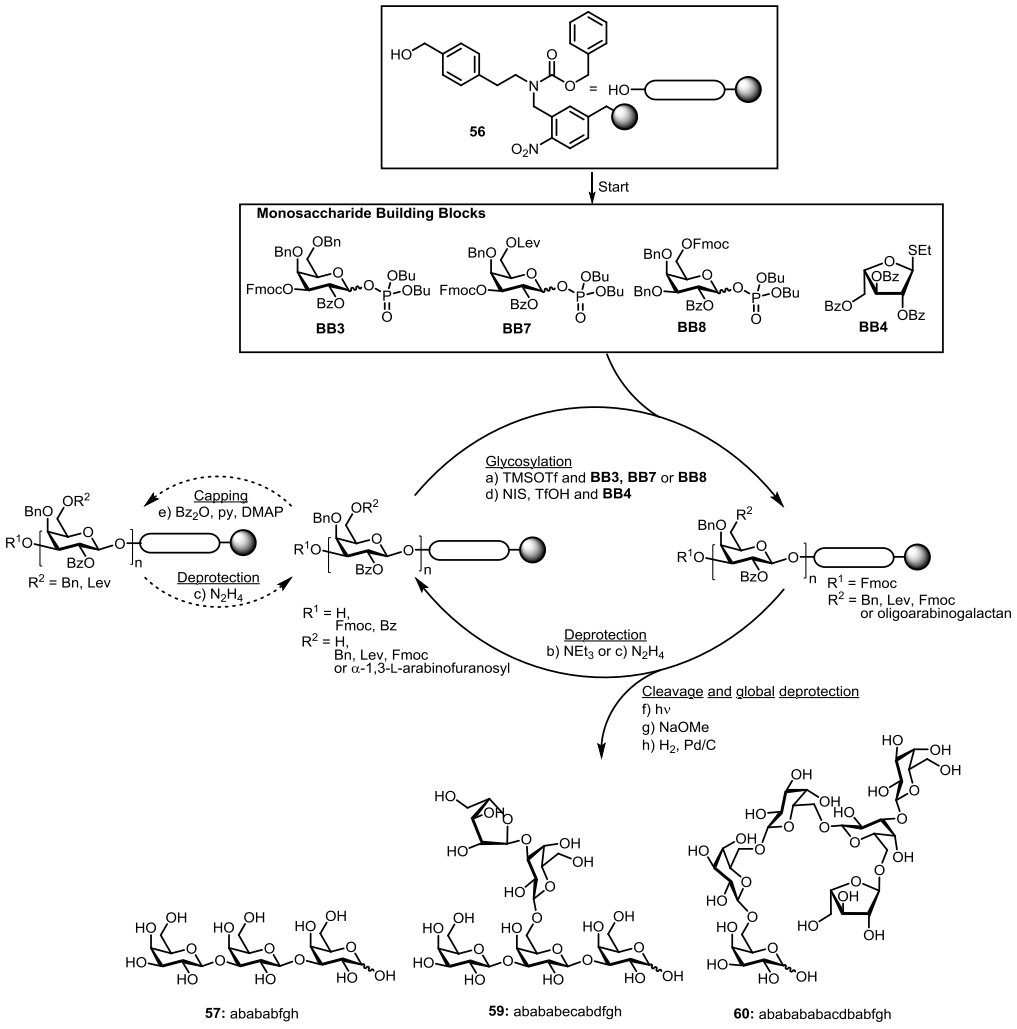
First, the applicability of the traceless photo-labile linker for automated glycan assembly was evaluated by synthesizing a simple linear target structure. To obtain a β(1,3)-linked galactan trisaccharide with free reducing end, **BB3** was coupled to a Merrifield resin equipped with linker **56** in three rounds of glycosylation and subsequent Fmoc-deprotection steps. Photo-induced cleavage of the trisaccharide from the solid support and purification using preparative normal phase HPLC gave the protected trisaccharide in good yield (49%). Global deprotection by methanolysis and subsequent hydrogenolysis gave the fully deprotected free reducing end trisaccharide **57** together with 2-(*p*-tolyl)ethane-1-amine as a side product.



Scheme 17: Formation of a heminaminal ether **58** from **57** and the amine released during the hydrogenolysis.

During concentration of the product under reduced pressure the amine partially reacted with the open chain form of the trisaccharide to form a hemiaminal ether[149] **57**

which could be converted back into the free reducing end trisaccharide **57** by acidic hydrolysis (scheme 17). More complex branched structures **59** and **60** could be synthesized from **BB3**, **BB7**, **BB8** and **BB4** when also Lev-deprotection and benzoyl capping procedures where included into the process (scheme 18). After global deprotection and subsequent acidic hydrolysis of the hemiaminal side products α/β-mixtures of type-II AG oligosaccharides **59** and **60** were obtained in yields (10% for **59** and 4% for **60**) comparable to the ones obtained in syntheses of similar target structures employing the standard aminopentyl linker **23**.



Scheme 18: Automated glycan assembly of free reducing end type-II arabinogalactan oligosaccharides **53**, **55** and **56**. Reagents and conditions: a) Twice 4 equiv **BB3**, **BB7** or twice 3 equiv **BB8**, TMSOTf, DCM, -35 °C (5 min) → -20 °C (30 min); b) Three cycles of 20% NEt_3 in DMF, 25 °C (5 min); c) Three cycles of 0.15 M hydrazine in pyridine/AcOH/H_2O (4:1:0.25), 25 °C (30 min); d) Twice 3 or 4 equiv **BB4**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min); e) Three cycles of 0.5 M Bz_2O and 0.25 M DMAP in DCE, pyridine, 40 °C (30 min); f) hv (305 nm); g) NaOMe, THF, 16 h; h) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16h. **57**: 8%, **59**: 10%, **60**: 4% (yields are based on resin loading). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

The successful syntheses of free reducing end AG oligosaccharides **57**, **59** and **60** proved that linker **56** is compatible with all common protocols for AGA, including glycosylations with glycosyl phosphates and thioglycosides, cleavage of temporary protecting groups such as Fmoc and Lev and capping of free hydroxyl functions as benzoyl esters.

2.1.3 Characterization of Arabinogalactan-Directed Monoclonal Antibodies

A great number of mAbs are commonly used to identify and localize pectin structures in plant cell walls.[150,151] The library of arabinogalactan oligosaccharides prepared by AGA provided an excellent toolbox to determine the exact binding epitopes of AG-directed mAbs. My colleague Dr. Ruprecht printed 26 synthetic type-I and type-II (arabino-)galactans on microarray slides to identify the binding epitopes of 43 AG directed mAbs.[152] It was found that the vast majority of the tested antibodies binds to β(1,6)-linked galactans with a slight variety towards the substitution pattern of the β(1,6) galactan chain. More differentiated binding patterns were observed in the case of seven particular AG-directed mAbs. In figure 13 the microarray scans after incubation of the array with antibodies JIM 14 and JIM 16 are exemplary shown.

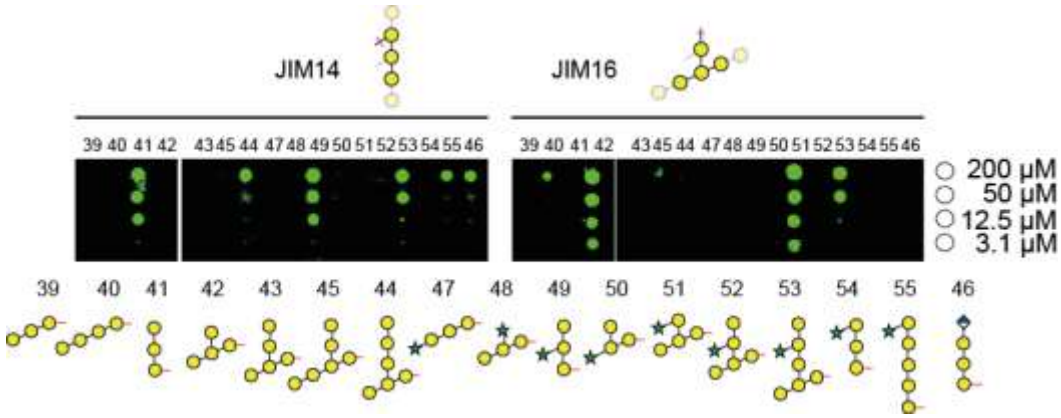


Figure 13: Identification of the binding epitopes of AG directed mAbs. Shown are the representative scans of microarrays incubated with JIM14 and JIM16. The compounds were printed in four different concentrations as indicated on the right. Light linkages and light monosaccharide symbols on the upper structures indicate positions that are allowed but not required for binding. Positions that must not be substituted are indicated by red bars.

JIM14 bound to five structures containing at least three consecutive β(1,6)-linked galactose moieties which may be arabinosylated at the central galactose residue. Interestingly, JIM14 differentiated between compounds **54** and **55** indicating that it binds to internal epitopes of β(1,6)-galactans but not to the non-reducing end of β(1,6)-

galactans. JIM 16 showed a completely different binding pattern as it only bound to compounds **42** and **51**, implying that it recognizes a β(1,3)-galactan backbone when substituted with a single β(1,6)-linked galactose moiety.

2.1.4 Identification of Substrate Specificities of β(1,4)-*endo*-Galactan Hydrolases

The type-I arabinogalactan oligosaccharides assembled by AGA (**26** to **33**) together with the previously synthesized β(1,4)-linked galactan disaccharide (**24**) and the previously synthesized β(1,4)-linked galactan tetrasaccharide (**25**) (Max Bartetzko, MSc thesis, MPIKG) represent useful probes to investigate the specificity of *endo*-galactanases. Hydrolases that degrade plant cell wall polysaccharides are important tools to analyze the structure of cell wall polysaccharides.[73,74] Short oligosaccharide fragments released by the enzymes can be analyzed by HPLC, mass spectrometry and NMR spectroscopy to draw conclusions on the structure of the original AG polysaccharides. However, information on the minimal oligomer length required for hydrolysis and the degree of arabinose substitution tolerated by the enzyme is essential. Three representative β(1,4)-*endo*-galactanases from glycosyl hydrolase (GH) family 53 were investigated: E-EGALN from *Aspergillus niger*, E-GALCJ from *Cellvibrio japonicus*, and E-GALCT from *Clostridium thermocellum*. Oligosaccharides (**24**-**33**) were incubated with the enzymes for 3 h at 40 °C before stopping the reaction by heat inactivation of the enzyme at 80 °C. The resulting digestion products were analyzed by HPLC coupled to an ELS-detector and a mass spectrometer. HPLC analysis of the digestion products revealed different minimal length requirements for hydrolysis by the galactanases (fig. 14). While E-GALCJ completely degraded tetragalactoside **25**, E-EGALN as well as E-GALCT only partially hydrolyzed **25**. Since neither the linker-functionalized mono- nor disaccharide was detected, E-GALCJ probably hydrolyzed the linker first and then cleaved the central bond of the resulting free reducing tetrasaccharide. Hexasaccharide **26**, in contrast to tetrasaccharide **25**, was hydrolyzed equally well by the two galactanases, although slightly different product patterns were observed. Mixed-linkage tetragalactoside **27** remained intact during incubation with the galactanases, proving the strict selectivity of the galactanases for β(1,4)-glycosidic linkages. Next, the effect of arabinose substitution on digestion efficiency was investigated. Arabinogalactan tetrasaccharide **28** was not hydrolyzed by any of the galactanases. Hexasaccharide **30**, bearing an arabinose residue in the central position, was cleaved by E-EGALN and E-GALCT but not E-GALCJ between the first two galactose units.

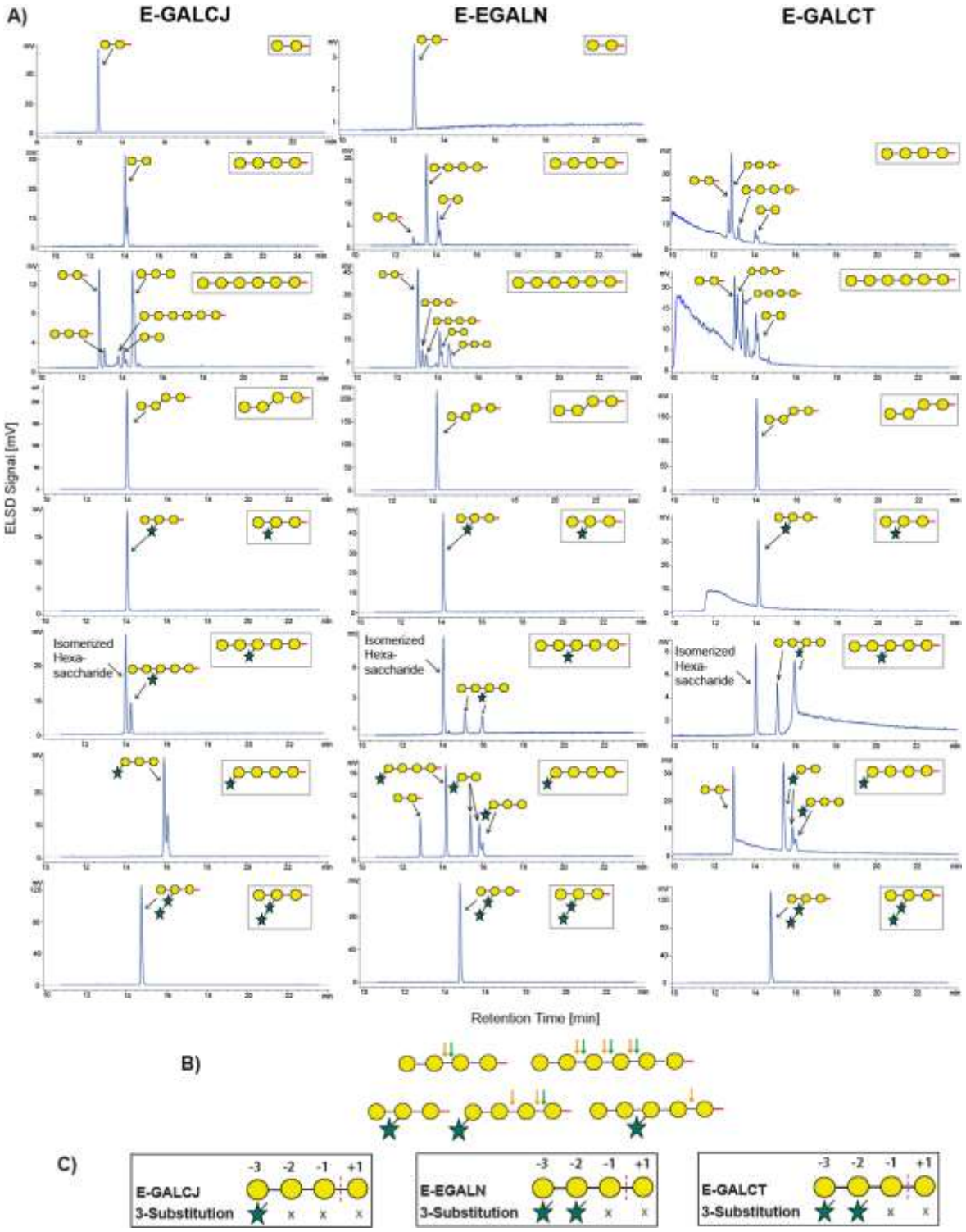


Figure 14: Digestion of synthetic arabinogalactan oligosaccharides with the β(1,4)-endo-galactanases E-GALCJ, E-EGALN and E-GALCT and analysis of the resulting hydrolysis products by HPLC-MS. (a) HPLC analysis of the products after incubation of the respective oligosaccharides (indicated by boxes) with the galactanases. Peaks are annotated with AG fragments either carrying an aminopentyl linker or with free reducing end (with or without red bar). Note that α- and β-anomers of the free reducing sugars elute as separate- or double-peaks. (b) The cutting sites derived from (a) are summarized and indicated by arrows. (c) General requirements for arabinose substitutions relative to the cutting site of E-GALCJ and E-EGALN galactanases. "X" denotes galactose residues that must not be substituted with arabinofuranose. The reducing end of the structures is located on the right.

Thus, E-EGALN and E-GALCT do tolerate arabinose substitution in the -2 subsite relative to the site of hydrolysis. The isomerized hexasaccharide **30-i** was not digested by E-EGALN or E-GALCT, suggesting a specific recognition of the arabinofuranose. Further information was provided by analysis of the hydrolysis products obtained after digestion of pentasaccharide **29** having the arabinose substitution in the terminal position. E-GALCJ tolerated arabinose substitution in the -3 subsite and cleaved the bond between the first two galactose residues. E-GALN and E-GALCT on the other hand additionally cleaved the bond between the second and third galactose unit, demonstrating their ability to accept arabinose substitution in both the -2 and -3 subsite. Neither galactanase tolerated substitution in direct proximity to the cleavage site as no corresponding hydrolysis fragments were detected. In summary, the results demonstrate that the substrate specificities of these GH53 β1,4-endo-galactanases differ in the number of subsites that are important for substrate binding and in their tolerance for arabinose substitution.

2.1.5 Conclusion and Outlook

In conclusion, a comprehensive library of pectic AG oligosaccharides has been synthesized from nine different monosaccharide BBs using AGA. The library contains eight type-I AG fragments with β(1,4)-galactan backbones of different length and varying arabinose or oligo-arabinan substitutions. The library further contains 15 type-II AG fragments resembling structural features such as the β(1,3)-galactan backbone, β(1,6)-galactan side chains, different arabinose substitutions or side-chains terminated with glucuronic acid. Furthermore, the same synthetic AGA protocols used for the assembly of type-I and type-II AG oligosaccharides were applied to test a newly developed photo-labile linker that releases free-reducing end oligosaccharides. The synthetic strategy applied in the solid-phase syntheses of the AG oligosaccharides relied on galactose BBs equipped with the orthogonal temporary protecting groups Fmoc and Lev at the respective positions used for chain elongation or branching. By establishing a benzoyl capping procedure that was performed during the assembly on solid support, the number of accessible structures was further increased while keeping the number of monosaccharide BBs limited. The structures were obtained in satisfying yields after cleavage from the solid support and global deprotection. All glycans equipped with an aminopentyl linker were printed together with previously synthesized shorter β(1,3)- and β(1,4)-galactans[130] as glycan microarrays. Binding studies using AG-directed mAbs on the microarrays enabled the determination of the exact binding epitopes for several anti-AG antibodies.[152] The newly obtained information will facilitate the identification of AG

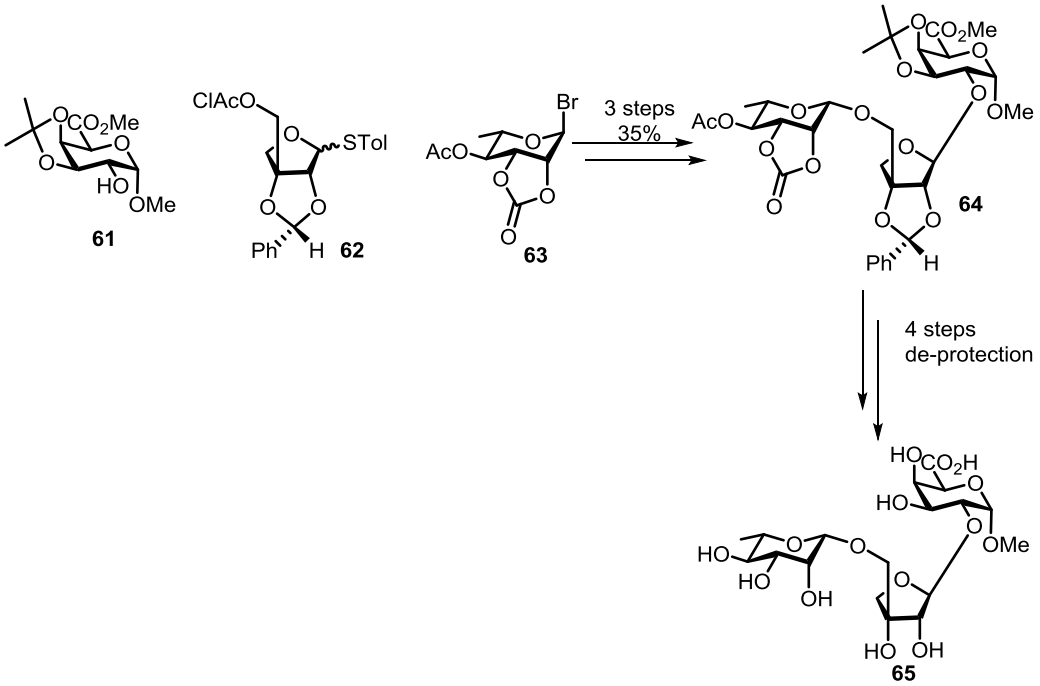
structures in plant cell walls by immunolabeling studies. The type-I oligosaccharides further served as valuable tool to determine the substrate specificities of β(1,4)-*endo*-galactanases. It was discovered that the galactanases recognize and hydrolyze arabinogalactan oligosaccharides of different lengths and arabinose substitution patterns. These findings have implications for future structural analyses of pectic polysaccharides. In summary, synthetic AG oligosaccharides have proved to be highly valuable tools to increase our understanding of plant cell wall biology. In the future, further extension of the library with longer and more elaborately substituted β(1,4)-galactans will allow even more detailed investigations into plant cell wall structures.

2.2 Synthesis of RG-II Fragments

2.2.1 Previous Chemical Synthesis of RG-II Fragments

Despite the great scientific need for well-defined fragments of RG-II, only a small number of syntheses have been published.[95] The assembly of unsubstituted HG oligosaccharides is rather straightforward and the syntheses of quite large oligosaccharides have been described in literature. *Ogawa et al.* reported the synthesis of a α(1,4)-dodecagalacturonic acid that was assembled from digalactoside building blocks equipped with benzyl-, benzylidene- and acetyl protecting groups. The disaccharide donors were used as glycosyl fluorides. After complete assembly of the dodecasaccharide the acetyl groups were removed and the hydroxyl groups were oxidized by a sequence of Swern oxidation and oxidation with $NaClO_2$ in water. Subsequently, the final α(1,4)-dodecagalacturonic acid was obtained by global deprotection using palladium on charcoal under a hydrogen atmosphere.[153] The synthesis of partially methylated tri- and hexasaccharide fragments of HG by post-glycosylation oxidation has been reported by *Clausen et al*. They introduced two orthogonal protecting groups at the C6-positions of the galactose building blocks to allow selective synthesis of the free acid or the methyl ester after assembly of the oligosaccharide.[154,155] Interestingly, both groups report high α-selectivity for the (1,4)-glycosylation reactions when employing 2,3-dibenzylated or 2,3,4-tribenzylated galactose building blocks. Although the 1,2-*cis*-configuration of the glycosidic linkages in the galacturonic acid backbone cannot be ensured via participating mechanism of neighboring protecting groups remote participation from C4 or C6 and solvent effects during the glycosylation reactions can promote the desired α-selectivity. So far only two groups have reported the synthesis of oligosaccharides containing both, an apiose and a galacturonic acid residue.[156,157] Reimer and co-workers synthesized a β-D-Api*f*-α(1,2)-D-Gal*p*A-OMe disaccharide by glycosylation of the C2 hydroxyl group of a protected galacturonate acceptor with a thioapiofuranose donor and subsequent global deprotection.[156]

Later, Field and co-workers published the synthesis of a β-Rha*p*-β(1,3)-Api*f*-α(1,2)-Gal*p*A-OMe trisaccharide starting from the same galacturonic acid acceptor **61** previously used by Reimer and co-workers. Successive glycosylations with 2,3-*O*-carbonate-protected rhamnopyranosyl bromide BB **63** and 2,3-*O*-benzylidene-protected apiofuranosyl thioglycoside BB **62** (scheme 19) provided fully protected trisaccharide fragment **64** which could be deprotected by sequential hydrogenolysis, acidic cleavage of the isopropylidene acetal, methanolysis and saponification of the ester to give **65**.



Scheme 19: Synthesis of apiose-containing trisaccharide fragment **65** of RG-II by Field and co-workers.[157] Note that the rhamnose moiety is β-linked to the apiose residue unlike in the natural structure.

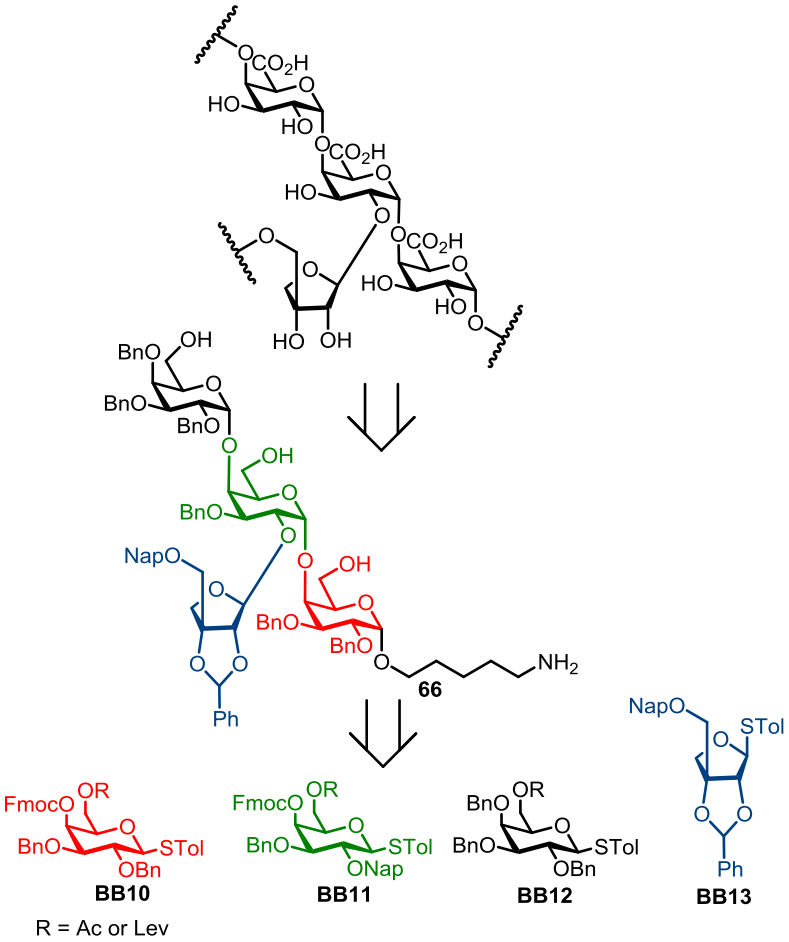
Syntheses of side chain A and B motifs from disaccharides[156-159] to hexasaccharides[160] were reported. Some of the resulting compounds have been used to confirm the molecular structure of RG-II. A fragment of side chain A with a central rhamnose moiety substituted with galacturonic acid at the C2- and C3-position and with fucose at the C4-position has been synthesized by *Chauvin et al.* in 2005. They glycosylated a rhamnose acceptor with a fucose glycosyl donor at the C4-position and galactose glycosyl donors at the C2- and the C3-position followed by a late stage oxidation of the primary hydroxyl functions of the galactose residues to the corresponding carboxylic acids using TEMPO.[159] Synthetic tetra- and hexasaccharide fragments of side chain B have been reported by *Rao et al.*[160,161]. In their synthesis of the side chain B hexasaccharide fragment a central arabinopyranose moiety was decorated with a β-linked rhamnopyranose at the C3-postion before it was reacted with a D-Gal*p*-α(1,2)-2-*O*-methyl-Fuc disaccharide acceptor upon activation of the anomeric thiol leaving group of the arabinopyranose. Finally, a β(1,2)-rhamnose-β(1,3)-arabinofuranosise side chain was installed at the C2-position of the arabinopyranose by sequential deprotection and glycosylation reactions. This synthesis impressively showed how also structurally complex and sterically very demanding side chain fragments of RGII can be assembled chemically. Unfortunately, due to the recent corrections of the stereochemistry of the Rha*p*-Api*f*-linkage in the side-chains A and B by *Gilbert et al.*[162], many of the side-chain oligosaccharide syntheses became obsolete, and oligosaccharide samples with correct stereochemistry are urgently needed.

So far, no syntheses of HG backbone structures substituted with parts of the RG-II side-chains have been reported. Libraries of HG backbone structures branched with truncated side-chains would be valuable tools for the production and characterization of RG-II specific antibodies and to characterize RG-II-modifying enzymes such as glycosyltransferases. HG backbone oligosaccharides of RG-II substituted with small side-chain structures are for example interesting synthetic tools for clarifying the question if during biosynthesis the RG-II side-chains are coupled *en block* to a preexisting HG backbone in the cell wall or if the side-chains are consecutively attached to the backbone while it is synthesized in the Golgi-apparatus.[163]

2.2.2 Automated Glycan Assembly of Substituted Homogalacturonan Fragments

Tetrasaccharide **66** (scheme 20) was identified as a central target structure for developing a general synthetic strategy towards a synthetic library of RG-II oligosaccharides that resembles branched HG backbone motifs of different lengths. The retrosynthetic analysis (scheme 20) of **66** reveals a number of chemical challenges associated with its synthesis. Foremost, oligosaccharides containing several uronic acids are challenging targets, as protected uronic acid glycosyl donors are of comparably low reactivity due to the electron-withdrawing ester function.[164] An elegant way to bypass this problem is the post-glycosylation oxidation strategy in which the oligosaccharide is fully constructed using glycosyl donors without the carboxylic acid function before the C6-hydroxyl groups are oxidized to the corresponding carboxylic acid. Another synthetic challenge is the inherent low nucleophilicity of the galactose C4-hydroxyl group[165] as described in chapter 2.1.1. The third synthetic challenge towards the preparation of structure **66** is the need for a suitable apiose building block. As D-apiose is a very rare sugar, the building block has to be synthesized from D-mannose[157], L-arabinose[157], D-xylose[166] or *de novo*[167].

Inspired by the above described syntheses of oligogalacturonans, a new synthetic approach towards the synthesis of RG-II fragments employing automated glycan assembly has been designed. The galacturonic acid backbone should be assembled from galactoside BBs equipped with a set of protecting groups that allows introduction of side chains at the C2-position of specific galacturonic acid moieties as well as a post-glycosylation oxidation after cleavage of the assembled oligosaccharide from the solid support.



Scheme 20: Retrosynthesis of a HG-trisaccharide substituted with apiose, the first monosaccharide of RG-II side chains A and B.

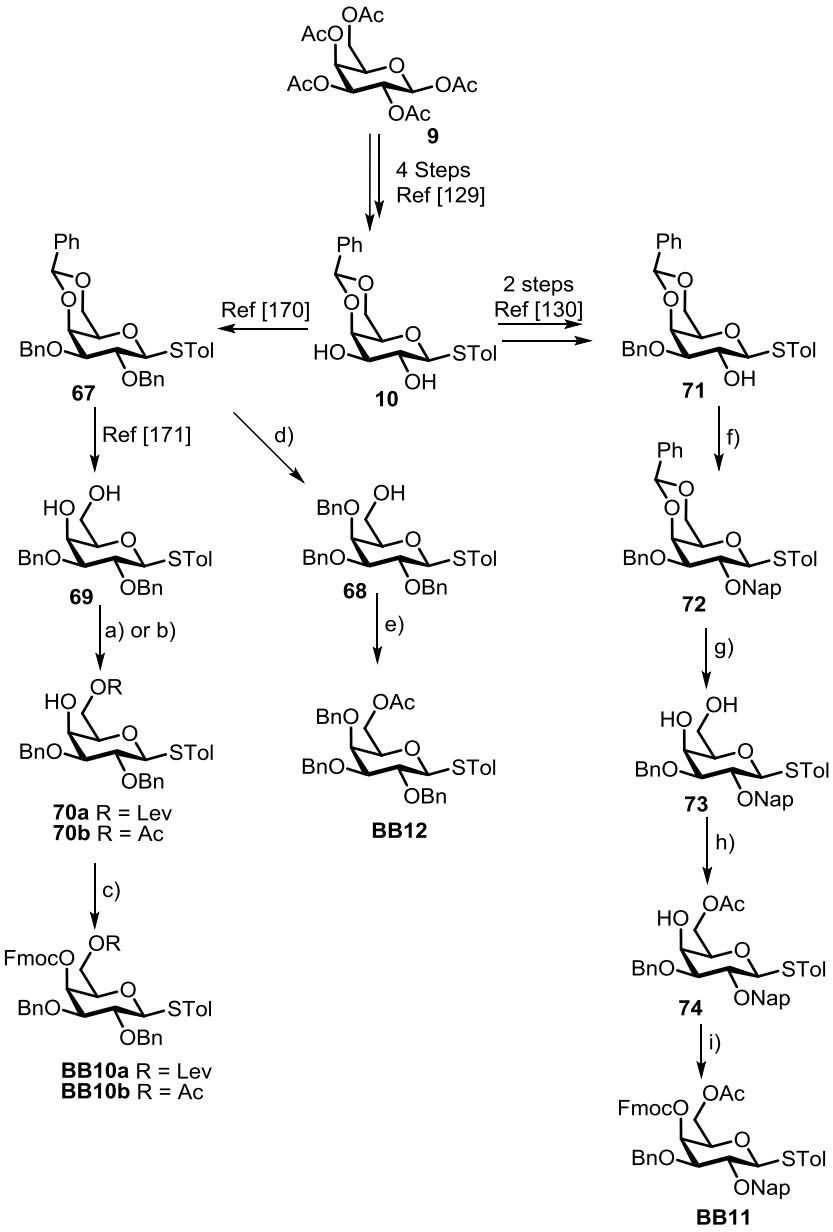
In order to enable a post-glycosylation oxidation, the three galactose building blocks (**BB10**-**BB12**) were equipped with an ester protecting group at C6 which can be selectively removed after the glycosylation reactions, making the primary hydroxy group available for oxidation to the acid. For a first synthetic test on the solid support, **BB10** was equipped with a levulinoyl ester at the C6-position, as this temporary protecting group is routinely used in automated glycan assembly and can be removed by applying routine protocols on solid support. Removal of the protecting groups at the C6-positions of the galactan backbone on solid support reduces the required number of chemical modifications of the oligosaccharide in solution. Alternatively, **BB10** was equipped with an acetyl ester at the C6-position that has to be removed after cleavage of the oligosaccharide from the solid support, as methanolysis of ester groups on the solid support is difficult. However, this BB might provide better glycosylation yields. **BB10** and **BB11** carry an Fmoc group at C4 as a temporary protecting group to allow elongation of the α(1,4)-linked backbone. Installation of ester and carbonate groups at the C6- (**BB12**) or C4- and C6- (**BB10** and **BB11**) position should promote the formation of α-glycosidic linkages due to remote participation of these protecting groups.[168] **BB11** was further

protected with an *O*-naphthyl ether in the C2-position. The naphthyl ether serves as a non-participating temporary protecting group that can be cleaved on solid support under oxidative conditions,[103,104] liberating the hydroxy function at C2 for further glycosylation with apiose **BB13**. The remaining hydroxy groups of the galactose building blocks were protected with benzyl ethers.

The apiose BB was equipped with a 2,3-*O*-benzylidene protecting group which renders the apiose glycosyl donor β-stereoselective and can be removed under the same conditions as the benzyl protecting groups.[157] At the C3'-position, the apiose BB carried a naphthyl ether which later may be removed during the automated glycan assembly process for further elongation of the side chain. All building blocks were used as thioglycosides and activated under standard conditions.[101]

BB10 – **BB12** were synthesized in a divergent manner starting from intermediate **10** which is available from peracetylated D-galactose **9** in four steps.[129] To synthesize **BB10** and **BB12**, the benzylidene-protected intermediate **10** was further protected in the C2- and C3-positions with benzyl groups, yielding intermediate **67**[169]. **BB12** for termination of the α(1-4)-backbone chain was obtained by regioselective reductive opening of the benzylidene acetal with BH_3, yielding quantitatively intermediate **68.** The resulting free primary hydroxyl group at the C6-position was then protected as an acetyl ester to yield **BB12**. To get access to **BB10**, which allows α(1,4)-chain elongation after removal of the temporary protecting group at the C4-position, the benzylidene acetal was fully removed under strongly acidic conditions using a TFA/H_2O-mixture. Subsequently, the primary hydroxyl function of **69**[170] was selectively protected either as a levulinoyl ester (**70a**) or as an acetyl ester (**70b**) by applying standard esterification methods. Finally, the Fmoc protecting group was installed as a temporary protecting group at the C4-position.

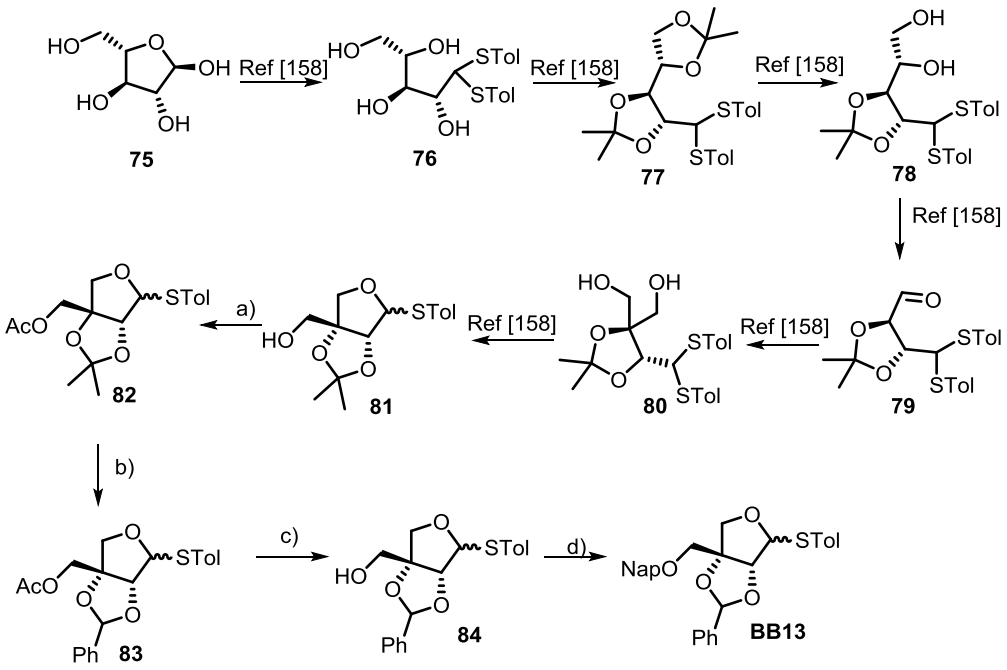
To obtain naphthyl protected **BB11**, the intermediate **10** was selectively protected with a benzyl group in the C3-position, following the same two step procedure[130] as described above for the synthesis of **BB1**. Installation of the naphthyl ether at the C2-position was performed with naphthyl bromide and **72** was obtained in good yield. Subsequent removal of the benzylidene acetal under acidic conditions, giving 4,6-dihydroxy intermediate **73**, was only achieved in moderate yields. The reaction could not be pushed to completion, as longer stirring under acidic conditions provoked cleavage of the naphthyl ether. The acetyl protecting group at the C6-position (**74**) and the Fmoc protecting group at the C4-position (**BB11**) were introduced as described for **BB10** in good yields.



Scheme 21: Synthesis of D-galactopyranose BBs **BB10**, **BB11** and **BB12**. Reagents and conditions: a) LevOH, DIC, DMAP DCM, -6 °C to rt, 81%; b) AcOH, DCC, DMAP DCM, 0 °C to rt, 82%; c) FmocCl, pyridine, DCM, rt, 57% for **BB10a**, 75% for **BB10b**; d) BH_3, TMSOTf, DCM, 0 °C to rt, quantitative; e) AcOH, DIC, DMAP DCM, 0 °C to rt, 58%; f) NapBr, NaH, DCM/DMF, 0 °C to rt, 89%; g) TFA/H_2O, DCM, rt, 51% h) AcOH, DIC, DMAP, DCM, 0 °C to rt, 66%; i) FmocCl, pyridine, DCM, rt, 67%.

Apiose **BB13** was synthesized from reported 2,3-*O*-isopropylidene-1-thio-α-D-erythrofuranoside (**81**) (scheme 22).[157] Intermediate **81** was synthesized following literature procedures[157] via the 2,3-*O*-isopropylidene protected L-arabinose dithioacetal **78**. Oxidative cleavage of the vicinal C4 and C5 hydroxyl groups of **78** yielded aldehyde **79**. This aldehyde was then reacted in an aldol-Cannizzaro reaction to give the acyclic version of apiose derivative **80** which could be cyclized using NIS as a promoter. It is noteworthy that the 2,3-*O*-isopropylidene acetal protecting group in apiose has been described as too stable to be removed from an oligosaccharide without decomposition

and therefore was exchanged for a 2,3-*O*-benzylidene protecting group which can be readily removed under hydrogenolytic conditions.[157] Attempts to exchange the acetal protecting group in presence of the naphthyl protecting group failed due to the lability of the Nap group to strong acids. The 3'-hydroxy group of intermediate **81** was thus temporarily protected as an acetyl ester. This allowed the exchange of the acetal protecting group to yield the 2,3-*O*-benzylidene acetal **83**. Subsequent deprotection of the 3'C-*OH* under basic conditions and installation of the naphthyl ether yielded the desired **BB13**.



Scheme 22: Synthesis of apiose **BB13**. Reagents and conditions: a) AcOH, DCC, DMAP DCM, 0 °C to rt, 97%; b) TFA/H_2O, DCM rt then $PhCH(OMe)_2$, CSA, rt, 35%, c) NaOMe, MeOH, rt, 96%, d) NapBr, NaH, 0 °C to rt, 86%.

With the four desired BBs (**BB10**–**BB13**) in hand, suitable reaction conditions for the assembly of target structure **66** by AGA were explored. In an initial test series the levulinoyl protected **BB10a** was compared to the acetyl protected **BB10b** regarding its efficiency in glycosylation reactions. Therefore **BB10a** was coupled to the photo-labile linker-functionalized resin **23** by glycosylation with NIS and catalytic amounts of TfOH using two cycles of 3.8 equiv BB at -10 °C. After Fmoc-deprotection, another glycosylation using two cycles with 3.8 equiv **BB10a** at -10 °, Fmoc-deprotection and removal of the Lev-groups were performed. HPLC analysis of the crude product after UV-light induced cleavage from the solid support revealed that mainly linker-bound monosaccharide was formed in the form of an α/β-mixture (fig. 15). This means that the glycosylation between the linker and the first monosaccharide did not proceed in a stereoselective manner, probably because the primary hydroxyl group of the linker was too nucleophilic for

significant stereochemical control in the reaction.[171] Next, acetyl protected **BB10b** was tested in AGA to see if a change in the protecting group at the C6-position improves glycosylation efficiency. The acetyl protected disaccharide **85b** was assembled on solid support by two rounds of glycosylation and deprotection. The glycosylations were again performed with NIS and catalytic amounts of TfOH using two cycles of 3.8 equiv of BB at -10 °C. HPLC analysis after cleavage of the product from the solid support showed that the desired disaccharide was obtained as the major product. The stereochemistry of this product was determined by 2D-NMR spectroscopy after HLPC purification (fig. 15). The second glycosylation reaction resulted in the exclusive formation of the desired α-(1,4)-glycosidic linkage between the two galactose moieties. Interestingly, the isolated disaccharide was found to be almost exclusively β-linked to the linker (fig. 15 B and C) suggesting that the β-linked monosaccharide is a better glycosyl acceptor than the α-linked monosaccharide.[165,172]

Any attempts to assemble longer α(1,4)-galactan oligosaccharides using the same glycosylation conditions as described above, gave unsatisfying results (fig. 16). The yields in the syntheses of trisaccharides **86** and **87** were low and purification of the desired compounds by HPLC proved to be very difficult, due to the formation of diastereomeric mixtures of the oligosaccharides that were linked either in an α- or β-configuration to the linker.

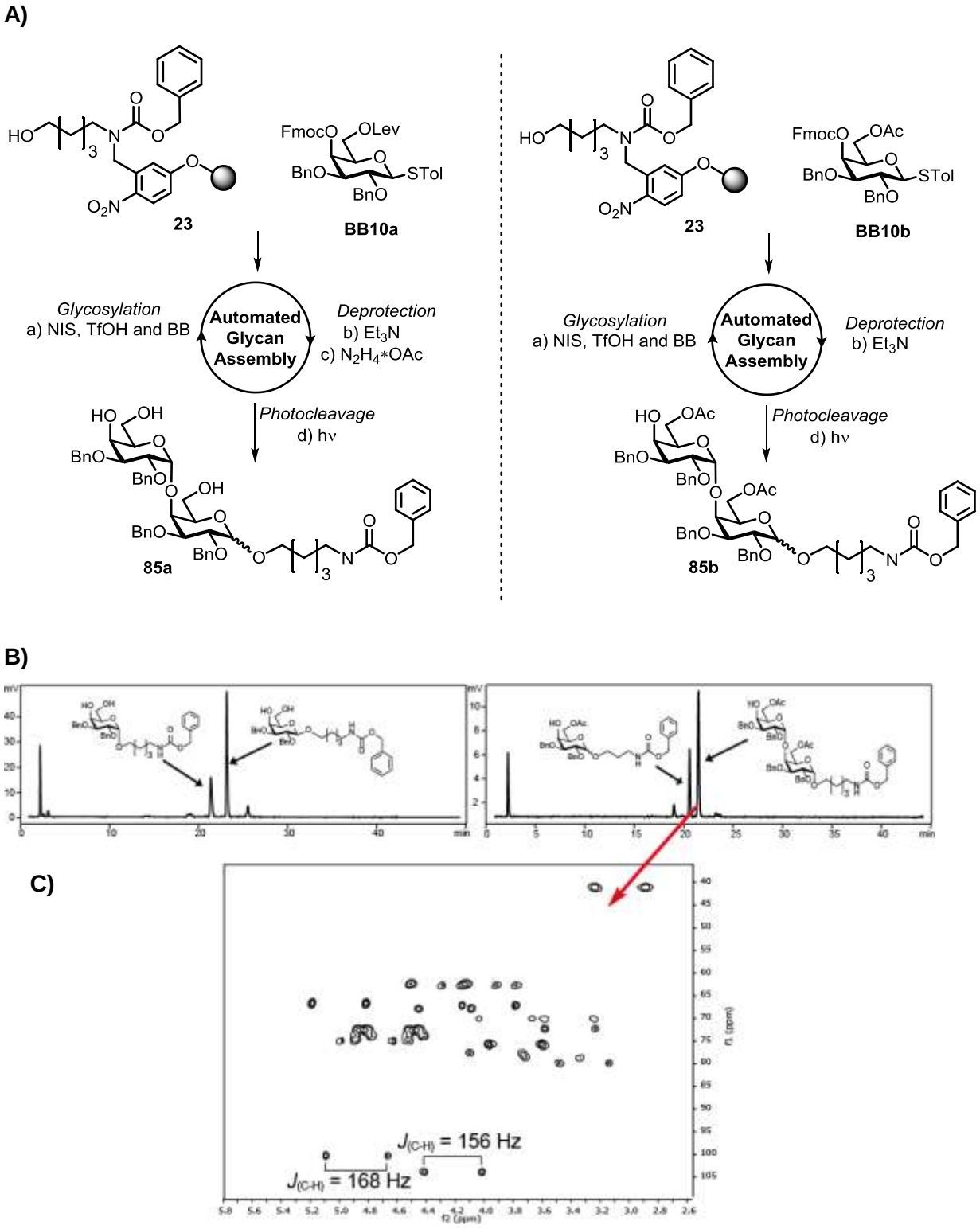


Figure 15: A) Comparison of galactosyl donors **BB10a** and **BB10b** in the automated glycan assembly of disaccharides **85a** and **85b**. Reagents and conditions: 2 x 3.8 equiv of **BB10a** or **BB10b**, NIS, TfOH, DCM/dioxane, -30 °C (5 min) → -10 °C (30 min); b) 3 cycles of 20% NEt_3 in DMF, 25 °C, c) (5 min), N_2H_4·HOAc (155 mM) in pyridine/AcOH/H_2O 4:1:0.25, 25 °C (30 min); d) hν (305 nm). B) Crude NP HPLC chromatograms of **85a** and **85b** (ELSD trace). **85a** was only obtained in traces. C) C-H coupled HSQC-NMR of product **85b:** coupling constant (C1'-H1') of 168 Hz indicates an α-linkage between the galactose moieties and coupling constant (C1-H1) of 156 Hz indicates a β-linkage between the first galactose moiety and the linker.[62]

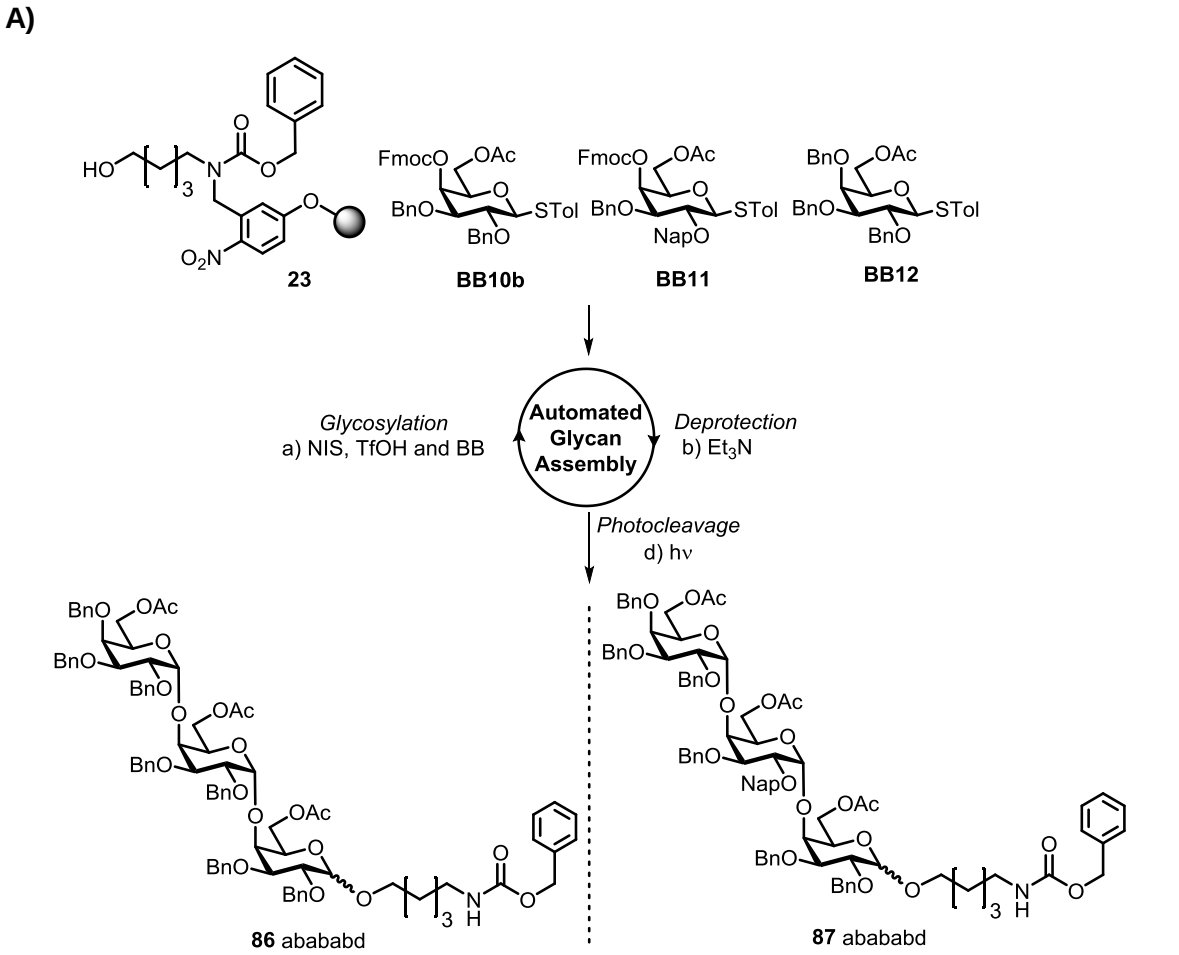


Figure 16: A) Automated glycan assembly of trisaccharides **86** and **87**. Reagents and conditions: 2 x 3.8 equiv of **BB1b, BB2** or **BB3** NIS, TfOH, DCM/dioxane, -30 °C (5 min) → -10 °C (30 min); b) 3 cycles of 20% NEt_3 in DMF, 25 °C (5 min), c) hν (305 nm). B) HPLC-chromatograms of the crude products (ELSD trace). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

2.2.3 Solution-Phase Synthesis of the RG-II Galacturonan Backbone

2.2.3.1 Solution-Phase Synthesis of the RG-II Galacturonan Backbone using a Fluorinated Linker

To see if the stereo-control over the first glycosylation reaction can be improved, a series of test glycosylations was run in solution phase to exploit the use of a different linker. Recently, it was shown that the use of a modified linker which carries two fluorine atoms in close proximity to the nucleophilic hydroxyl group can improve the stereoselectivity in the glycosylation of the linker. The fluorine atoms decrease the oxygen nucleophilicity and thereby switch the stereochemical outcome of the reaction to preferentially form the α-glycosides.[173] Small amounts of the fluorinated linker were kindly provided by my colleague Dacheng Shen.

Reacting **BB10b** with 2,2-difluoro-3-amino-1-propanol-linker **88** after activation of the thioglycoside donor with NIS and catalytic amounts of TfOH at 0 °C followed by removal of the Fmoc-protecting group gave linker-functionalized monosaccharide **89** in good yield (70%) (fig. 4). Compound **89** was exclusively obtained as the α-anomer which was confirmed by 2D-NMR spectroscopy analysis (fig. 17). **89** was subsequently reacted with thioglycoside donor **BB11**. After removal of the temporary Fmoc-protecting group, linker-functionalized disaccharide **90** was obtained and the α-selectivity of the glycosylation reaction was again confirmed by 2D-NMR spectroscopy analysis (fig. 17). Anyhow, due to poor yields (28% for glycosylation and subsequent Fmoc removal) in the second glycosylation reaction only small quantities of disaccharide (**90**) were obtained. Attempts to further use disaccharide **90** as an acceptor substrate in a glycosylation reaction with donor **BB12** were not successful. Several factors may have contributed to the failure of this glycosylation reaction: i) The low nucleophilicity of a galactose C4-hydroxyl group is even lower when the acceptor galactose is α-configured. Theoretical calculations showed that the C4-OH of an α-linked galactose cannot form a strong hydrogen bond with the pyranosyl oxygen atom and is therefore less nucleophilic.[174] ii) The bulky naphthyl group at the C2-position might have increased the steric bulk around the axial C4-hydroxyl group, as the naphthyl moiety is most likely pushed above the ring plane of the acceptor galactose as the space below the ring plane is occupied by the first galactose moiety bound via the 1,2-*cis*-glycosidic linkage. Hence, it was concluded that the assembly of longer α(1,4)-galactan structures in reasonable amounts is not possible with this set of BBs.

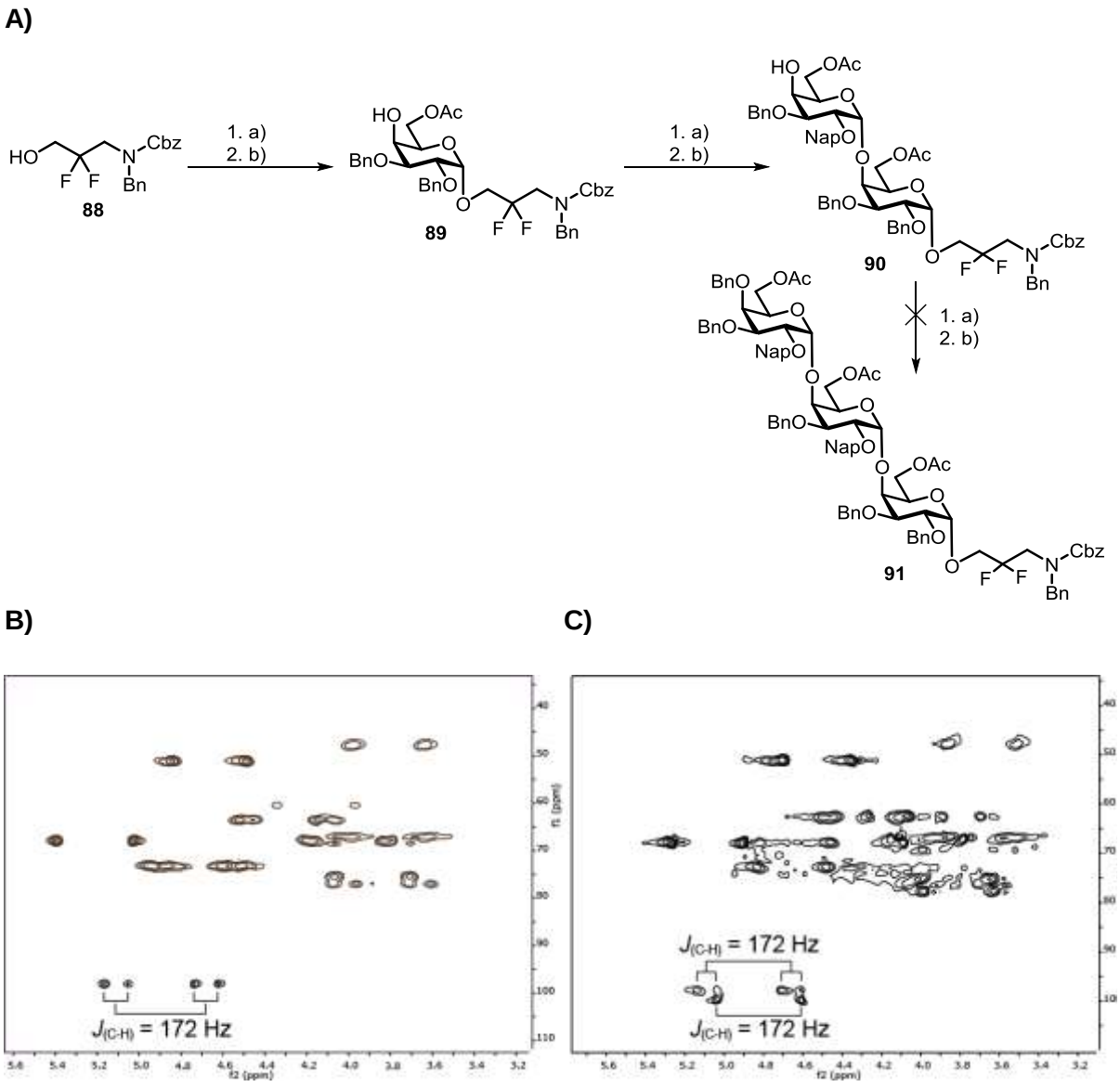
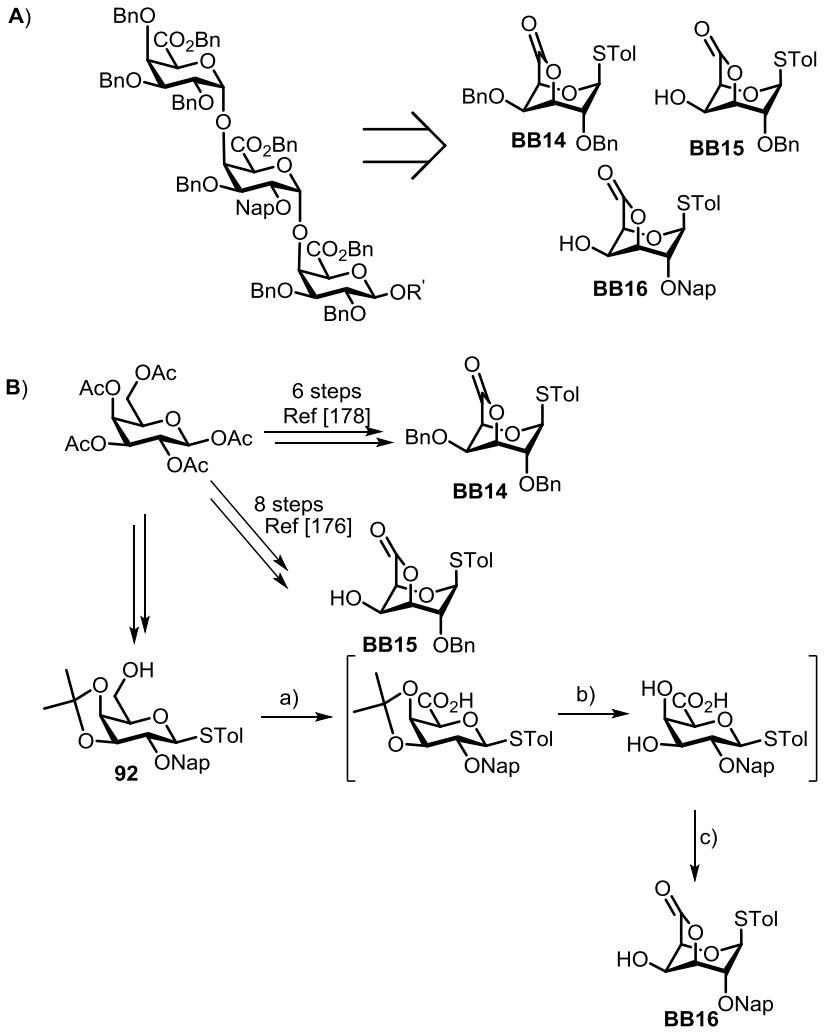


Figure 17: A) Solution-phase synthesis of α(1,4)–linked galactan oligosaccharides using a difluorinated linker. Reagents and conditions: a) NIS, TfOH 0 °C to rt then b) NEt_3 rt; Yields: **89**: 70% two steps, **90** 28% two steps B) Coupled HSQC-NMR of **89** showing a coupling constant of 172 Hz for C1-H1 (peak appears as duplet of a duplet due to fluorine coupling) indicating a 1,2-*cis*-linkage. C) Coupled HSQC-NMR of **90** reveal coupling constants of 172 Hz for C1-H1 and for C1'-H1', indicating the presence of two 1,2-*cis*-linkages.

2.2.3.2 Solution-Phase Synthesis of the RG-II Galacturonan Backbone Using Galacturonic Acid Lactone Buidling Blocks

Although the stereoselectivity for the attachment of the first galactose moiety to the linker could be improved significantly by the usage of the fluorinated linker **88**, the results in the syntheses of longer α(1,4)-galactans employing BBs **BB10b** to **BB12** remained unsatisfying. Therefore, it was decided to change the synthetic strategy and BB design to overcome the inherent low reactivity of the C4-hydroxyl group of α-linked galactose residues. A very attractive strategy to incorporate α-galacturonic acid moieties into oligosaccharide structures has been published by *Codée et al.* They employed galacturonic acid 3,6-lactones in the synthesis of trisaccharide repeating units of the capsular polysaccharide of *Streptococcus pneumonia*, Sp1. The employed galacturonic acid lactone BBs (**BB14** and **BB15**) are conformationally locked and thus more reactive and selective in glycosylations, providing α-linked galacturonides in good yields.[175] The activation of the galacturonic acid lactone BBs by a preactivation-based strategy with diphenyl sulfoxide and triflic anhydride at -45 °C gave rise to α-selective glycosylation, presumably through the intermediacy of an axial β-triflate that is replaced by the nucleophile in an S_N2 type reaction.[176] It was shown that the C4 hydroxyl groups of these BBs were good nucleophiles and can act as glycosyl acceptors.[175] By locking the sugar in the $^1C_4$-chair conformation, the C4-hydroxyl group is forced into an equatorial position which increases its nucleophilicity. Based on these results, it was anticipated that the RG-II galacturonic acid backbone could be synthesized by consecutive coupling of lactone building blocks **BB14**, **BB15** and **BB16** (scheme 22). The galacturonic acid lactone **BB14** was synthesized following literature procedures[175,177] by chemo- and regioselective oxidation of the primary hydroxy group of 2,4-*O*-dibenzyl-3,6-dihydroxy-1-thio galactopyranoside and concurrent intramolecular lactonization with the C3 hydroxyl function. **BB15** was prepared following an eight step literature synthesis.[175] 2-*O*-naphthyl-protected **BB16** was analogously synthesized from **92**. The naphthyl protecting group remained stable during the TEMPO and BAIB mediated oxidation of the primary hydroxyl function (scheme 22).

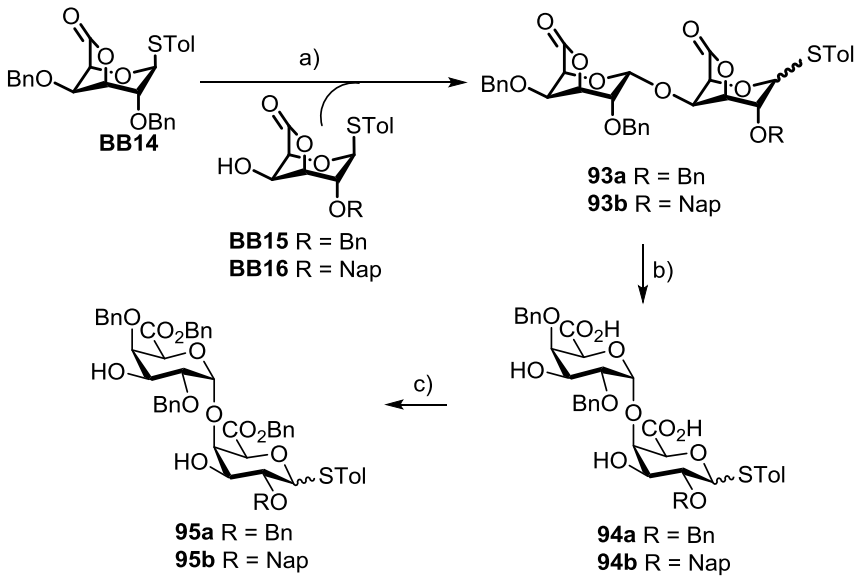


Scheme 22: A) Retrosynthetic approach towards a protected α(1,4)-galacturonic acid oligosaccharide with a 2-*O*-naphthyl protecting group at the central GalA unit group for later installation of a RG-II side chain. B) Galacturonic acid lactone BB synthesis starting from peracetylated β-D-galactose. **BB14** and **BB15** were synthesized following literature procedures. **BB16** was synthesized analogously to **BB15**. Reagents and conditions: a) TEMPO, BAIB, DCM, rt; b) AcOH/H_2O 65 °C; ethyl chloroformate, DIPEA, rt, 17% over three steps.

With the galacturonic acid lactone BBs in hand, the assembly of galacturonic acid oligosaccharides was tested. The desired oligosaccharides were assembled from the non-reducing end to the reducing end by iterative preactivation of the glycosyl donor followed by addition of the acceptor and purification of the resulting oligosaccharide. Preactivation of donor **BB14** by the addition of diphenyl sulfoxide and triflic anhydride in the presence of tri-*tert*-butyl pyridine as buffer at -45 °C led to formation of a reactive β-triflate intermediate which was reacted with acceptor **BB15** or **BB16** at -60 °C. Unfortunately, the desired disaccharides **93a** and **93b** were only obtained in moderate yields (28%-35%). The disaccharide **93a** was obtained as an anomeric mixture of the α- and β-thioglycosides (fig. 18), indicating that an aglycon transfer occurred which is a

commonly observed side reaction when a glycosyl acceptor possessing a thioglycoside aglycon is reacted with an activated glycosyl donor.[131]

A)



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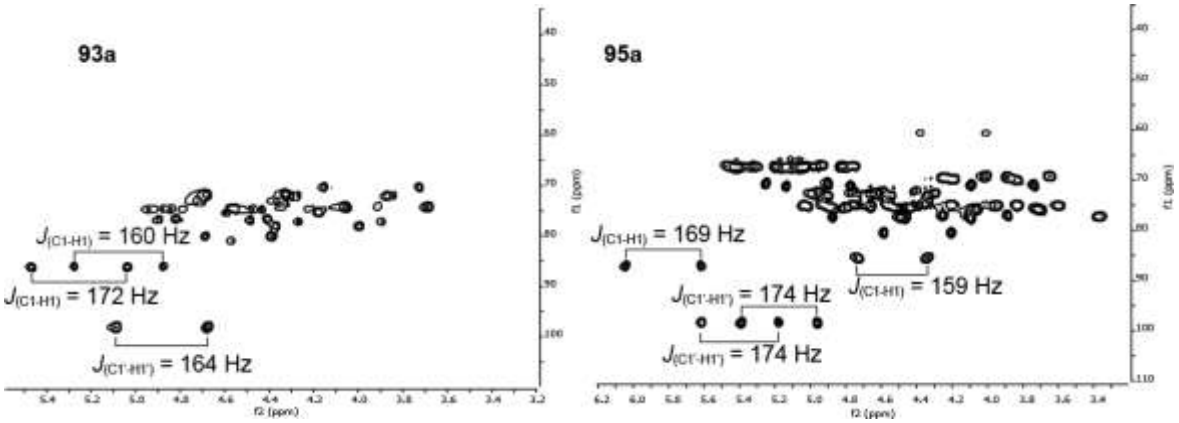
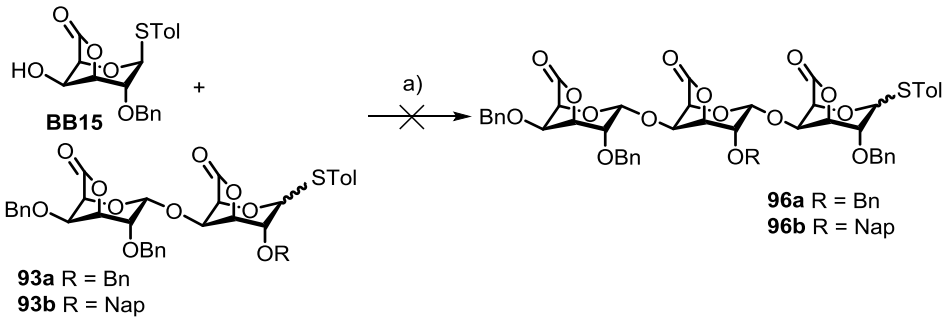


Figure 18: A) Assembly of two galacturonic acid lactone building blocks followed by lactone opening and subsequent protection of the carboxylic acid function. Reagents and conditions: a) Ph_2SO, T*t*BPy, Tf_2O -60 °C to -45 °C then to -60 °C and addition of acceptor **BB15** or **BB16**; b) TMSONa, DCM, rt; c) Cs_2CO_3, BnBr, DMF, rt. B) Coupled HSQC-NMR of **93a** shows two different coupling constants for C1-H1 (JC-H = 160 Hz and J_{C-H} = 172 Hz) as result of an aglycon transfer side reaction. Coupled HSQC-NMR of the diastereomeric mixture of **95a**.

The lactone rings of disaccharides **93a** and **93b** were hydrolyzed applying TMSONa as a nucleophile and the resulting carboxylic acids were subsequently esterified by treatment with BnBr and Cs_2CO_3 (fig 18). 2D NMR-spectroscopic analysis confirmed that only the α-linked disaccharides **94a** and **94b** were obtained (fig. 18). The syntheses of longer galacturonic acid lactone oligosaccharides were planned to be performed by preactivation of lactone disaccharides **93a** and **93b** and addition of further lactone BB

acceptor (**BB15**). The reaction was monitored by TLC and MS, but no reaction progress was observed. Even when the temperature was slowly increased to -10 °C after preactivation with Ph_2O and Tf_2O, no product formation was observed by MS or TLC. Also exchanging the activators to the strongly electrophilic reagents dimethyl(methylthio)sulfonium triflate (DMTST)[178] at temperatures from -78 °C to rt or dimethyldisulfide and Tf_2O[179] at -78 °C to rt did not activate the lactone BBs, but only led to decomposition of the glycoside donor at higher temperatures.

These results confirmed previous findings by *van der Marel et al.* who could only activate galacturonic acid lactone monosaccharides by preactivation with diphenylsulfoxide and triflic anhydride or *in situ* by the extremely electrophilic *p*-nitrophenylsulfenyl triflate promoter system but not with a NIS/triflic acid promoter system. As all attempts to activate **93a** or **93b** failed, the successive usage of galacturonic acid lactone BBs for the assembly of longer structures was considered a synthetic dead end (scheme 23).



Scheme 23: Activation of donors **93a** and **93b** for glycosylation reaction with acceptor **BB15** could not be realized. Conditions and reagents: a) Ph_2SO, T*t*BPy, Tf_2O -60 °C to -45 °C then to -60 °C and addition of acceptor **BB6** followed by slow warming to room temperature while tight monitoring of the reactionby TLC and MS.

2.2. 4 Conclusion and Outlook

In conclusion, the assembly of a RG-II fragment library using different types of BBs either using AGA or solution-phase synthesis was attempted. To assemble an apiose substituted homogalacturonan backbone on solid support, three galactose building blocks suited for a post-glycosylation oxidation strategy and one apiose BB have been synthesized. Though the protected α(1,4)-galactan trisaccharides (**86** and **87**) could be synthesized on solid phase using AGA, the yields remained unsatisfactorily low due to the low nucleophilicity of the C4-hydroxyl group of galactose. It was found that the low reactivity of the C4 nucleophile is especially problematic when the acceptor galactose moiety is equipped with a naphthyl protecting group at the C2-position. Furthermore, the

observed limited diastereoselective control over the first glycosylation reaction with the linker made the isolation of sufficient amounts of material for post-assembly modifications of the oligosaccharide impossible. In a solution phase test series the diastereoselectivity during the glycosylation reaction with the linker could be improved using a fluorinated linker giving the α-linked glycan exlusively. Unfortunately the following glycosylation reactions in solution phase still remained low yielding due to the inherent low nucleophilicity of the C4-*O*H of the galactose BBs.

To overcome the impeding low nucleophilicity of the C4-*O*H of galactose, a series of galacturonic acid lactone BBs was synthesized and evaluated for their potential to assemble α(1,4)-linked galacturonic acid oligosaccharides. By locking the galacturonic acid as lactone in the $^4C_1$-conformation and forcing the C4-*O*H into an equatorial position the reactivity of the C4-*O*H should be increased. It was shown that two galacturonic acid lactone BBs can be reacted α-selectively by preactivation of the donor substrate with diphenyl sulfoxide and triflic anhydride. Unfortunately, larger galacturonic acid oligosaccharides could not be synthesized as the disaccharide donors **93a** and **93b** could not be activated for further glycosylation.Libraries of RG-II backbone structures of different length carrying different side-chain fragments remain challenging but highly interesting synthetic targets. To achieve the assembly of a comprehensive library of RG-II fragments in high yields by AGA, new monosaccharide building blocks will have to be designed and evaluated. One useful modification of the BBs could be the exchange of the 2-*O*-naphthyl protecting group of **BB11** to a less sterically demanding temporary protecting group such as PMB. The results of the preliminary studies presented in this chapter imply that a solid support equipped with a fluorinated linker[173] may be used in AGA to overcome problems in the stereocontrol during the first glycosylation reaction that attaches the galactose BB to the linker. Furthermore, the yields in AGA might be improved applying capping steps after each glycosylation reaction as recent studies have demonstrated.[102]

2.3 Chemical Synthesis of Functionalized Sugar Nucleotides for Glycan Microarray Screenings of Plant Glycosyltransferases

2.3.1 High-throughput Characterization of Plant Glycosyltransferases

During the last decade, many enzymes that are involved in cell wall polysaccharide biosynthesis (mainly glycosyltransferases) have been identified by genetic screens.[180] Unfortunately, biochemical characterization of these enzymes lags behind, partly due to a lack of suitable assays which allow a high-throughput characterization of the substrate specificities of glycosyltransferases. Novel approaches towards quantitative high-throughput analyses of plant glycosyltransferase activities that are applicable with a wide range of enzymes, are operationally simple and give reproducible results, are highly needed to push forward the biochemical characterization of plant glycosyltransferases.The major obstacle in the development of high-throughput methods so far has been the lack of well-defined oligosaccharide acceptor substrates. Due to our development of glycan microarrays that are equipped with plant related oligosaccharides obtained by AGA, further progress towards high-throughput methods may be realized. A possibility to determine the substrate specificities of glycosyltransferases is to incubate such a microarray with the respective enzyme and to determine which oligosaccharide served as a substrate. However, detection of glycosyl transferase binding is less straightforward than in the case of mAbs or lectins, as the glycosyl transferases do not bind permanently to the array. Therefore, specific detection of the products of the enzymatic reactions is required. In the literature several methods to detect the products of enzymatic reactions on microarrays have been described. A very versatile approach is to use mass spectrometry for the detection of the glycans immobilized as self-assembled monolayers on gold[181] or on a lipid bilayer conjugated to an indium-tin oxide surface,[182] but so far these specialized chips are not commercially available and the number of glycans that can be immobilized is significantly lower than for standard arrays. Other very sensitive assays use ^{14}C-labled sugar donors[69] or ^{3}H-labled sugar donors[183], which allow the radiometric detection of incorporated monosaccharides to specific glycans on the array. Though this technique is very sensitive, handling of the radioactive probes needs specialized equipment, trained personal and produces radioactive waste that has to be costly disposed and therefore is unsuited for the development of high-throughput assays. A third method to detect the enzymatic reaction products on the microarray is the use of lectins or antibodies as molecular probes.[184,185] However, specific molecular probes for the reaction products often are not available. The fourth method of detection makes use

of chemically functionalized sugar nucleotide donors. When chemically modified, the donor substrate can be detected on the microarray after the enzymatic reaction by introduction of a label at the functionalized groups.[186] Though chemical modifications of the sugar nucleotide donors may not be accepted by all classes of glycosyltransferases, small modifications of the sugar nucleotide donors such as the introduction of azide- or alkyne-functionalities are generally well tolerated by glycosyltransferases, as reported in metabolic glycan engineering studies.[187-191] All the methods summarized above are valuable tools for the detection of the reaction products on glycan microarrays after incubation with glycosyltransferases and sugar nucleotides.

In this chapter, the development of a new high-throughput method for the characterization of plant glycosyltransferases using carbohydrate microarrays is described. In this assay, microarrays equipped with plant-derived oligosaccharides are incubated with putative glycosyltransferases and functionalized UDP-sugar donors (fig. 19). After incubation, the enzymes and the side products are washed off the microarray surface and a fluorescent label is attached to the functionalized monosaccharide units that have been incorporated into the oligosaccharide acceptors immobilized on the surface (fig. 19). Read-out of the fluorescent signals on the microarray surface then allows to determine the acceptor substrate specificities of the glycosyltransferases (fig. 19).

It was envisioned that UDP-sugar donors functionalized with azido- or amino-groups would serve best for this purpose as azido- or amino-functionalities are small and easily reacted with a fluorescent dye by copper catalyzed “click”-chemistry[192] or NHS-ester coupling. Two functionalized UDP-Gal*p*s (**97** and **99**) were chosen as initial donors for realizing proof of principle experiments. Galactose is ubiquitous in pectin polysaccharides and in hemicelluloses. Still, many galactosyltransferases involved in the biosynthesis of galactose containing polysaccharides have not been biochemically characterized yet. The sugar donor for the synthesis of these galactose containing polysaccharides is UDP-α-D-Gal.[193] Natural UDP-Gal*p* is commercially available and can serve as positive control to evaluate the tolerance of the putative galactosyltransferases towards modifications of the UDP-Gal*p* substrates in a HPLC assay. The use of azido-functionalized UDP-donors enables the use of a reliable labeling strategy based on copper catalyzed “click”-chemistry for the attachment of an alkyne-linked fluorophore to the products of the enzymatic reactions on the array surface. The azido functionalized UDP-Gal*p*s (**97** and **99**) may later be reduced to the corresponding amino-functionalized UDP-Gal*p*s (**98** and **100**). Besides functionalized UDP-Gal*p*, functionalized UDP-Ara*f* **101** was chosen to be synthesized (fig. 19). L-Ara*f* is a common component of plant cell wall polysaccharides. The biosynthesis of branched pectic arabinan structures for example

requires up to 18 different arabinosyltransferases[194] which use UDP-β-L-Ara*f* as donor substrates.[195] However, until today very few arabinofuranosyltransferases have been biochemically characterized. The characterization of arabinosyltransferases was mainly hampered by the limited availability of UDP-L-Ara*f*.[194] So far, only the characterization of one arabinosyltransferase using exogenous acceptor substrates and UDP-β-L-Ara*f* has been reported.[196] To establish a high-throughput assay for arabinosyltransferases as described above, access to sufficient amounts of functionalized UDP-Ara*f* as well as non-functionalized UDP-Ara*f* to confirm potential hits in a conventional HPLC-assay were required.

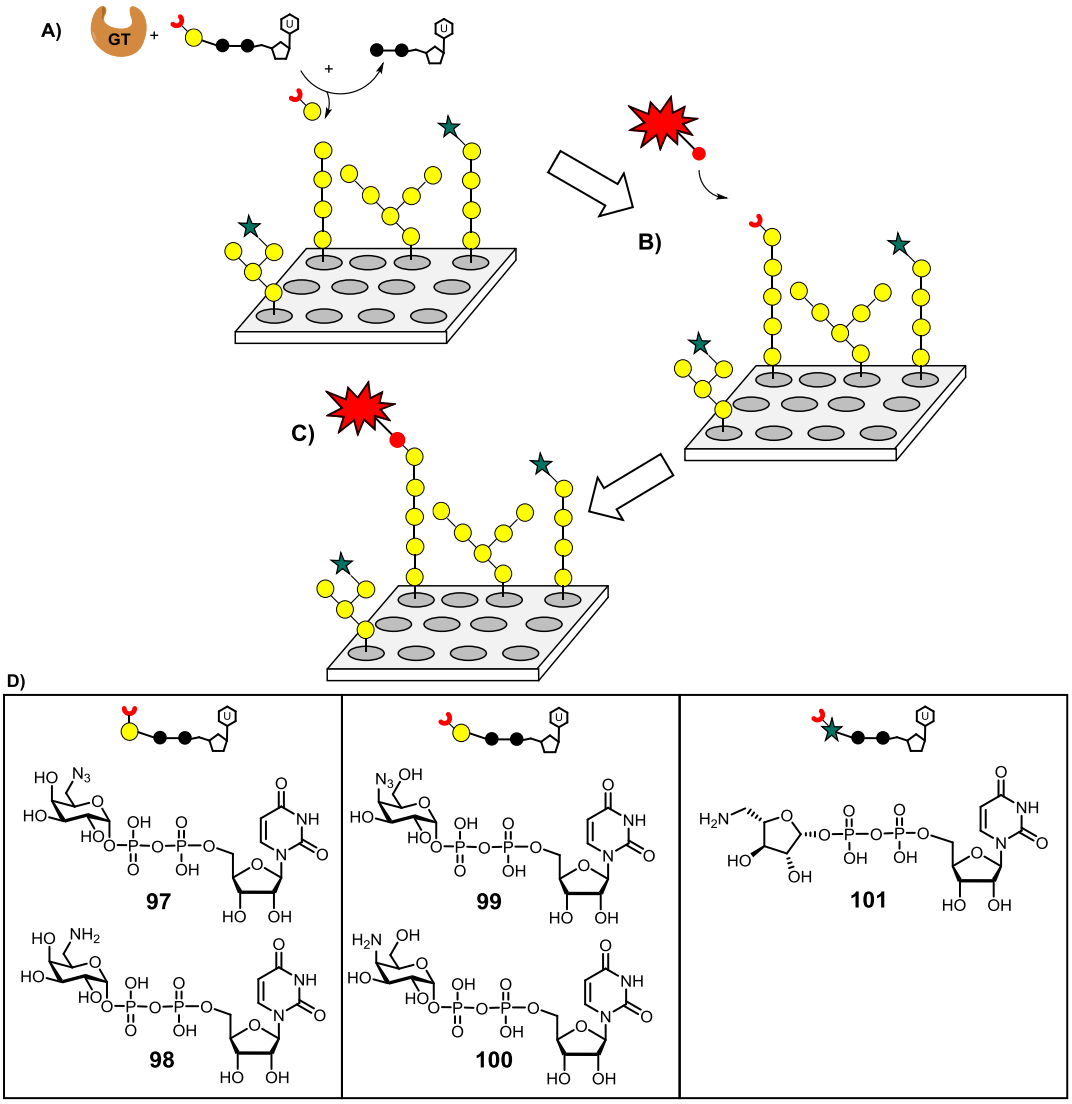


Figure 19: Microarray assay for the high-throughput characterization of plant glycosyltransferases employing functionalized sugar nucleotides. A) The microarray that is equipped with synthetic oligosaccharides of defined structures is incubated with a putative glycosyltransferase and a functionalized sugar-nucleotide. B) After the enzymatic reaction the microarray is washed, and the functionalized monosaccharides incorporated into surface bound glycans are labeled by reaction with a fluorescent probe. C) Read-out of the fluorescent signals. D) Functionalized sugar-nucleotides to be synthesized.

2.3.2 General Considerations for the Chemical Synthesis of Sugar-Nucleotides

Glycosyltransferases assemble monosaccharide precursors into larger glycan structures by successively adding sugar-nucleotides to oligosaccharide acceptors. Sugar-nucleotides are activated forms of monosaccharides which are composed of a sugar derivative and a nucleoside diphosphate (NDP-sugars) or a nucleoside monophosphate (NMP-sugars).[197] The majority of monosaccharide donors for the biosynthesis of plant glycans are NDP-sugars.[193] Chemical synthesis that makes naturally occurring sugar-nucleotides or modified analogues available in large quantities as substrates for the study of glycan biosynthesis, as enzyme inhibitors or as tools for assay development is of great importance. The chemical synthesis of sugar nucleotides is non-trivial and needs sophisticated synthetic approaches: Low solubility in organic solvents and susceptibility of the glycosidic and pyrophosphate bonds to hydrolytic cleavage complicate the handling and purification of the compounds.[197] NDP-sugars are usually synthesized from an activated nucleoside monophosphate and a glycosyl monophosphate, which are coupled to form the pyrophosphate (fig. 20). The advantage of this synthetic approach is the predetermined anomeric configuration of the sugar monophosphates which prevents the formation of diastereomeric mixtures of the final compound.

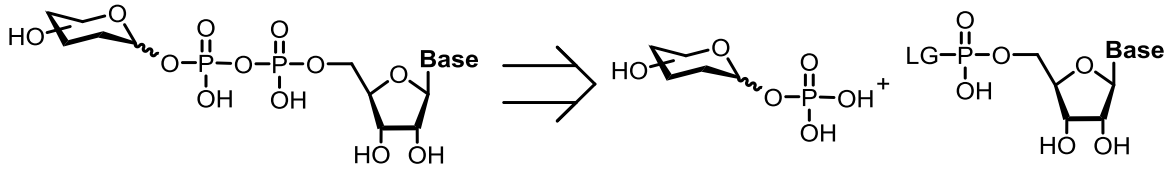
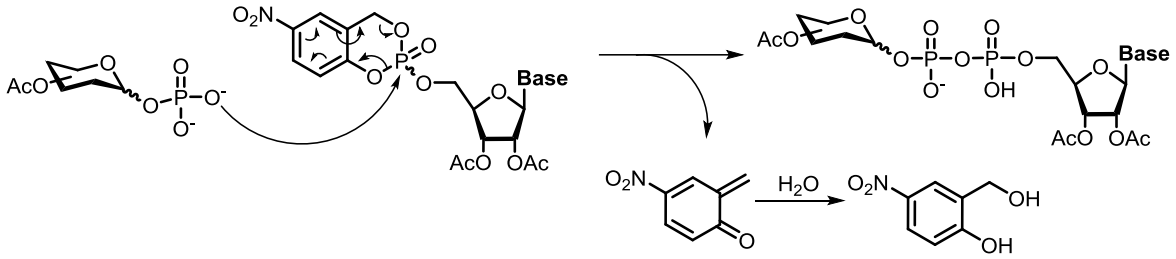


Figure 20: General synthetic strategy for the synthesis of NDP-sugars. The pyrophosphate bond is formed by coupling of a glycosyl phosphate and an activated nucleoside monophosphate. The nucleoside monophosphate is activated by installation of a suitable leaving group (LG).

The most commonly used nucleoside monophosphate derivatives for the synthesis of sugar-nucleotides are phosphoric acid amides, usually nucleoside phosphoromorpholidates.[198,199] Generally, condensation reactions between a sugar 1-phosphate and a nucleoside monophosphate morpholidate are slow and the chemical yields are low. The reaction can be accelerated by the addition of catalytic amounts of 1*H*-tetrazole which acts as both an acidic and a nucleophilic catalyst.[200] *Meier et al.* reported a conceptually new approach to sugar nucleotides that employs pyranose-1-phosphates and *cyclo*-saligenyl (*cyclo*Sal) nucleosyl phosphate triesters as active esters (scheme 24).[201] They showed that the *cyclo*Sal method can be applied to couple various protected sugar phosphates with the nucleosides of thymine, cytosine, uracil, guanine and adenine.

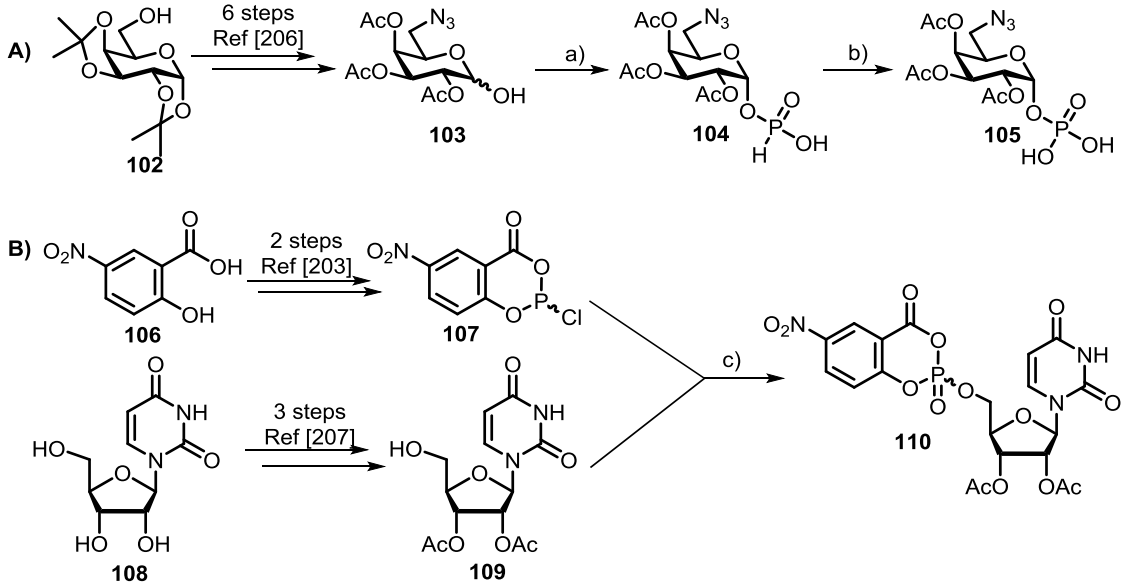
Coupling is achieved efficiently using mild conditions and DMF as the solvent in good overall yields (22-88%).



Scheme 24: Formation of the pyrophosphate bond using *cyclo*Sal-phosphate triesters and pyranose-1-phosphates.[202]

2.3.3 Chemical Synthesis of Functionalized UDP-Galactopyranose

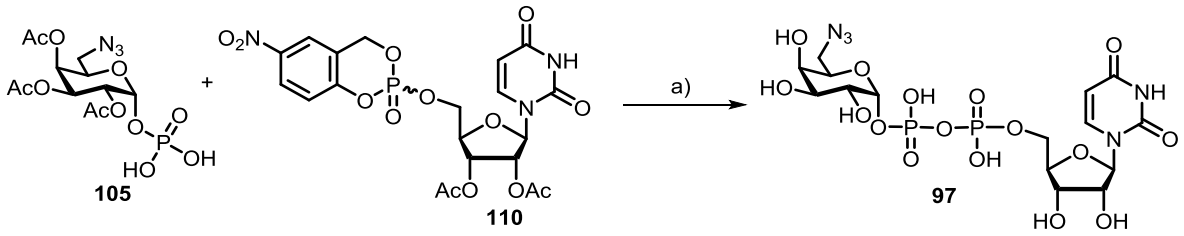
The synthesis of compound **97** was realized by applying the *cyclo*Sal method established by *Meier et al*.[201,203] for coupling 6-azido galactose phosphate **105** with activated uridine monophosphate **110**. Functionalization of the C6-position of galactose was performed first as it requires few synthetic steps and the synthesis of 6-azido-UDP-Gal*p* was already reported in literature using UMP-morpholidate chemistry.[204] However, it was decided to synthesize the functionalized UDP-galactoses using the *cyclo*Sal strategy, as higher yields and shorter reaction times were anticipated.



Scheme 25: Synthesis of the acetyl protected intermediates **105** and **110** A) Synthesis of 2,3,4-tri-*O*-6-azido-6-deoxy-α-D-galactopyranosyl 1-phosphate (**105**) from 1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**102**).[204] Reagents and conditions: a) 2-Chloro-4*H*-benzodioxaphosphorin-4-one, NEt_3, dioxane, rt, then H_2O, rt, then H_3PO_3, MeCN, rt, 67% b) *t*BuOOH, I_2, THF, rt, 30%. B) Synthesis of 5-chloro-*cyclo*Sal-2',3'-*O*-diacetyluridine monophosphate (**110**) by coupling 2,3-di-*O*-acetyl uridine (**109**) with 5-nitro-*cyclo*Sal phosphorochloridite (**107**). Reagents and conditions: c) NEt_3, MeCN, 0°C to rt then oxone, water, rt, 24% over two steps.

The synthesis of key intermediate **103** was performed according to literature procedures[205] starting from protected galactose **102**. With **103** in hand, the anomeric phosphate group was installed via the galactopyranosyl phosphite intermediate **104**. Phosphitylation of **103** with 2-chloro-4*H*-benzodioxaphosphorin-4-one gave a reactive cyclic phosphite which was converted into **104** by hydrolysis, yielding an anomeric mixture. The anomeric mixture was dissolved again in MeCN and equilibrated into the thermodynamically favored α-anomer **104** using phosphorous acid as reported in literature.[204] Subsequently, the anomeric phosphite group of **104** was oxidized to give the corresponding phosphate **105**.

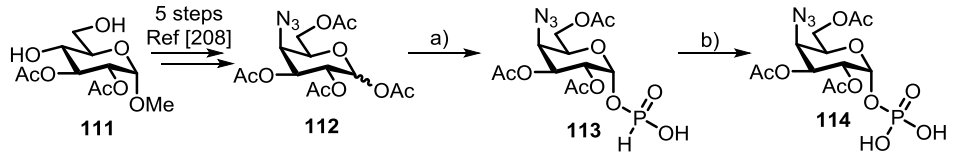
In order to obtain *cyclo*Sal-activated UMP **110**, compound **107**[201], which was obtained from 5-nitrosalicylic acid by reduction of the acid with borane and further reaction with phosphorous trichloride, was reacted with partially protected uridine **109**[206]. Formation of the pyrophosphate bond in **97** was achieved by coupling acetyl protected galactose 1-phosphate **105** with the active ester **110**. The two components were stirred at rt in DMF under argon atmosphere in the presence of molecular sieves. Removal of the acetyl protecting groups upon stirring with triethylamine in a mixture of water and methanol gave then the desired uridine 5'-(6-azido-6-deoxy-α-D-galactopyranosyl) diphosphate (**97**) which could be isolated in 25% yield after ion exchange chromatography and subsequent solid phase extraction using a Carbograph column (scheme 26).



Scheme 26: Synthesis of UDP-6-azido-Gal*p* (**97**) starting from acetyl protected uridine *cyclo*Sal phosphate triester **110** and the acetyl protected galactosyl 1-phosphate **105**. Reagents and conditions: Molecular sieves (4 Å), DMF, rt; then MeOH/H_2O/NEt_3 (7:3:1), rt, 25% over two steps.

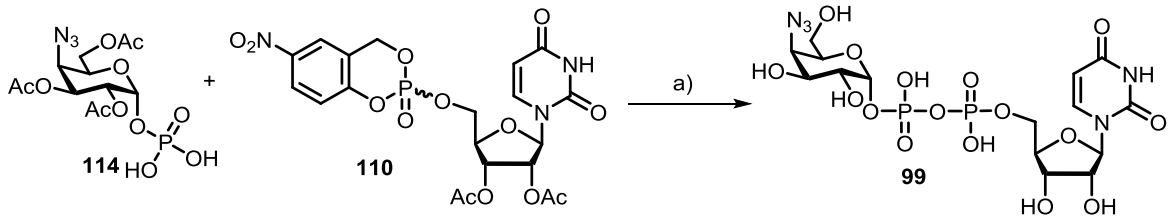
To obtain 4-azido functionalized galactose phosphate **99**, a synthesis starting from the commercially available compound **111** was designed. Literature known intermediate **112**[207] was obtained by acetylation of the primary hydroxyl group in **111**, subsequent mesylation of the C4-hydroxyl group and displacement of the mesyloxy function at the C4-position by an azide in an S_N2 reaction under inversion of the C4 stereocenter. Exchange of the anomeric methoxy group by an acetyl group then gave the desired intermediate **112** as reported in literature.[207] Selective de-*O*-acetylation at C1 using hydrazinium

acetate in DMF[205] allowed the installation of the anomeric phosphate as described above for the synthesis of **104** (scheme 27).



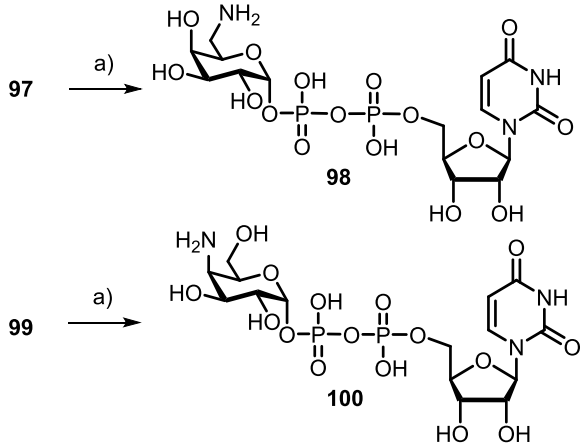
Scheme 27: Synthesis of 4-azido-galactosyl 1-phosphate **114** from advanced glucose intermediate **111**. Reagents and conditions: a) N_2H_4•AcOH, DMF, 50 °C then 2-chloro-4*H*-benzodioxaphosphorin-4-one; NEt_3, dioxane, rt; then H_2O, rt; then H_3PO_3, MeCN, rt, 64% b) *t*BuOOH, I_2, THF, rt, 33%.

Coupling of **114** to active ester **110**, as described above for the synthesis of compound **97** and subsequent removal of the acetyl protecting groups by methanolysis gave the functionalized galactosyl nucleotide donor **99** (scheme 28).



Scheme 28: Synthesis of UDP-4-azido-Gal*p* **99** starting from the acetyl protected uridine *cyclo*Sal phosphate trimester **110** and the acetyl protected 4-azido-4-deoxy-galactosyl 1-phosphate **114**. Reagents and conditions: a) Molecular sieves (4 Å), DMF, rt, then MeOH/H_2O/NEt_3 (7:3:1), rt, 15% over two steps.

Synthesis of amino-functionalized UDP-Gal*p* **98** and **100** was achieved by stirring the azide in water together with palladium on activated carbon under a hydrogen atmosphere yielding UDP-6-amino-Gal*p* **98**. Though the reduction of compound **99** turned out to be rather slow and was thus more prone to side reactions such as the reduction of the uracil double bound, the reduction to 4-amino-UDP-Gal*p* **100** could be achieved under the same reaction conditions as for the synthesis of **98** (scheme 29).



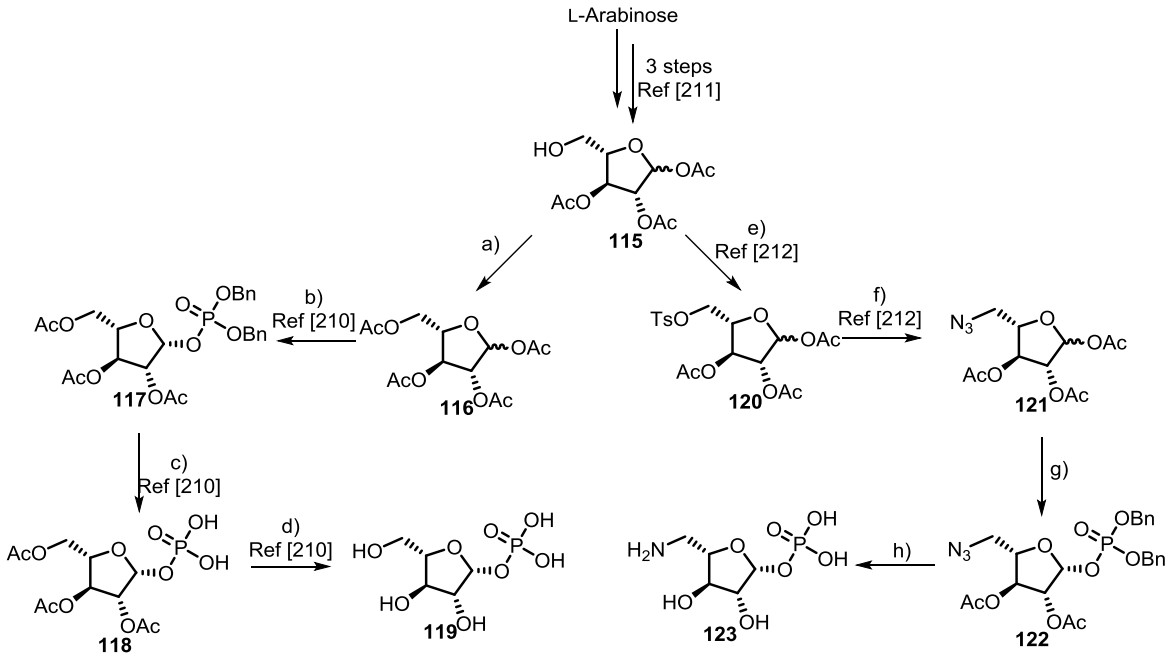
Scheme 29: Synthesis of UDP-6-amino-Gal*p* **98** and UDP-4-amino-Gal*p* **100**. Reagents and conditions: a) 10 wt % Pd/C, H_2, H_2O, rt, 84% for **98** and quant. for **100**.

2.3.4 Chemical Synthesis of UDP-Arabinofuranose and Functionalized UDP-Arabinofuranose

Unfortunately, natural UDP-β-L-Ara*f* is expensive and fairly unstable.[208] There is only one chemical synthesis of UDP-Ara*f* reported.[209] It is based on UMP-morpholidate chemistry, using β-L- Ara*f* 1-phosphate which is coupled to morpholidate-activated UMP. However, it was decided to synthesize the substrates **101** and **124** applying the *cyclo*Sal strategy employing **110** to establish a synthetic method for the large-scale synthesis of natural and unnatural UDP-Ara*f* donor substrates. The required β-L-Ara*f* 1-phosphate **118** and 5-amino-5-deoxy-β-L-Ara*f* 1-phosphate **122** were synthesized in a divergent manner starting from L-arabinose. The key intermediate **115** was synthesized following a reported procedure.[210] L-Arabinose was forced into the furanose conformation by protecting the C5-hydroxyl group as a trityl-ether and the remaining hydroxyl groups as acetyl esters. Removal of the trityl group under acidic conditions gave then **115**. **115** was either further acetylated with acetic anhydride in pyridine to give peracetylated Ara*f* derivative **116** or equipped with an azide group at the C5-position. Installation of the azide functionality was achieved via tosylation of the C5-hydroxyl group (**120**) and subsequent S_N2 reaction with sodium azide, analogously to a reported procedure[211] for the synthesis of the respective D-isomer.

To obtain the 1,2-*cis*-configured β-L-Ara*f* 1-phosphate **117**, peracetylated Ara*f* intermediate **116** was reacted with trimethylsilyl bromide and dibenzyl phosphate following a reported procedure[209]. The β-anomer of phosphate **117** was obtained exclusively, albeit in low yields. Removal of the benzyl protecting groups by catalytic hydrogenation over 10% palladium on charcoal in the presence of trimethylamine afforded the acetyl protected phosphate **118**[209] which was further deprotected by stirring in a methanol-water mixture at high pH, yielding the fully deprotected *β*-Ara*f* 1-phosphate **119**.[209]

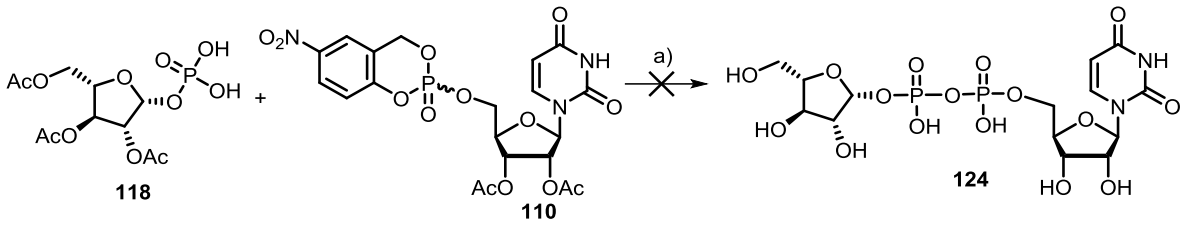
Azido functionalized arabinofuranose **121** was converted into 5-azido-5-deoxy-β-Ara*f* 1-phosphate **122** following the same synthetic strategy. Reaction of the anomeric mixture of **121** with trimethylsilyl bromide resulted in the formation of the α-bromide, which was to unstable to be isolated, but could directly be reacted with dibenzyl phosphate, giving **122** as the 1,2-*cis*-isomer exclusively. Assignment of the anomeric proton of **122** with a characteristic doublet of a doublet at 6.01 ppm and coupling constants $J_{1,P}$ and $J_{1,2}$ of 5.5 Hz and 4.0 Hz confirmed the 1,2-*cis*-configuration.[212]



Scheme 30: Synthesis of *β*-L-Ara*f* 1-phosphate **119** and 5-amino *β*-L-Ara*f* 1-phosphate **123**. Reagents and conditions: a) Ac_2O, pyridine, rt, 95%; b) TMSBr, DCM, 0 °C to rt then dibenzyl phosphate, NEt_3, 0 °C to rt, 21%; c) Pd/C (10 wt%), NEt_3, rt; d) NEt_3, MeOH/H_2O (3:2), rt, 94% over two steps; e) TsCl, pyridine, DCM, rt, 70%; f) NaN_3, DMF, 70 °C, 79%; g) TMSBr, DCM, 0 °C to rt then dibenzyl phosphate, NEt_3, 0 °C to rt, 9%; h) Pd/C (10 wt%), NEt_3, rt then NEt_3 MeOH/H_2O (3:2), rt, quant.

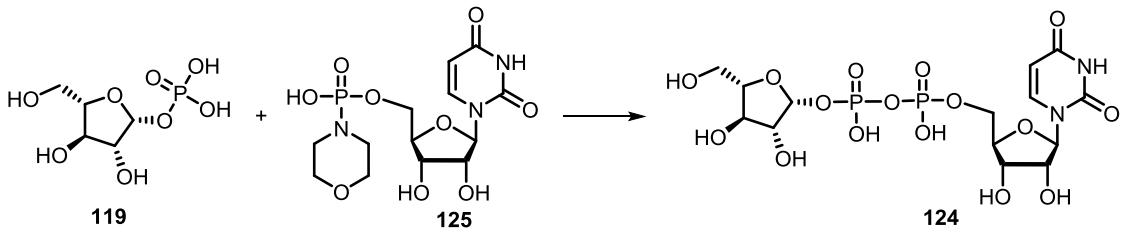
Unfortunately, the synthesis of the desired intermediate **122** was low yielding (10% maximum yield obtained). Similar observations were reported in the literature for the synthesis of 5-*N*-acetyl arabinofuranosyl 1-phosphate, concluding that arabinofuranosyl 1-phophate derivatives which do not have a strongly electron withdrawing group at the C5-position are too unstable to be efficiently handled or purified.[211] Thus, **122** was quickly converted into arabinofuranosyl 1-phosphate **123** by reduction of the azide and simultaneous removal of the benzyl protecting-groups under hydrogenolytic conditions, and subsequent cleavage of the acetyl protecting groups under basic conditions (scheme 30).

With β-L-Ara*f* phosphates **118** and the amino functionalized β-L-Ara*f* phosphate **123** in hand, coupling reactions with activated UMP were tested. In an initial test reaction the tri-*O*-acetyl protected arabinofuranosyl phosphate **118** was coupled to **110** by stirring the reactants in DMF at rt under argon atmosphere with activated molecular sieves. Formation of the acetyl protected UDP-*β*-L-arabinofuranose was detected by MS, but unfortunately subsequent removal of the acetyl protecting groups by methanolysis resulted in decomposition of the pyrophosphate bond (scheme 31).



Scheme 31: Synthesis of UDP-β-L-Ara*f* using the *cyclo*Sal active ester of UMP. Removal of the acetyl protecting groups under basic conditions has led to decomposition of the pyrophosphate bond. Reagents and conditions: a) molecular sieves (4 Å), DMF, rt, then MeOH/H_2O/NEt_3 (7:3:1), rt.

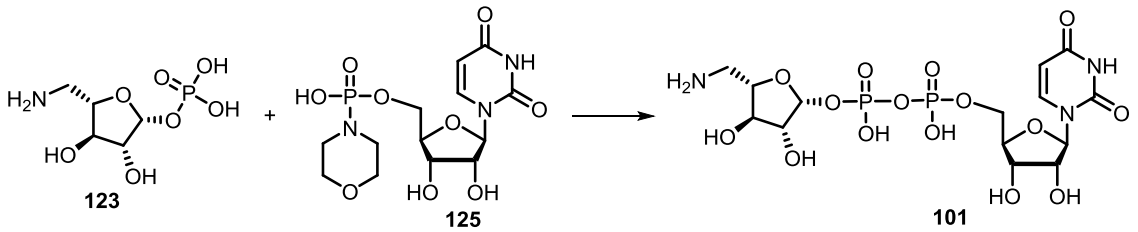
To avoid the necessity to deprotect the final UDP-Ara*f*, next the traditional method using deprotected sugar-1-phoshates and UMP-morpholidate was tested by stirring 1-phosphate **119** together with UMP-morpholidate in anhydrous pyridine and 1*H*-tetrazole as activator (scheme 32) for three days at rt.[209] The desired UDP-β-L-Ara*f* was isolated in pure form, but in very low yield (1%) after several HPLC and ion exchange chromatography purification steps. Apparently, fully deprotected arabinofuranosyl 1-phosphates are relatively unstable[211] which might have caused slow degradation of the starting material **119** during the coupling reaction with **125** (scheme 32). Furthermore, partial isomerization of UDP-Ara*f* into the UDP-Ara*p* form has been observed upon storing the crude reaction mixture at -20 °C before HPLC purification.



Scheme 32: Synthesis of UDP-β-L-Ara*f* using the UMP-morpholidate method. Reagents and conditions: a) 1*H*-tetrazole, pyridine, rt, 1%.

The amino functionalized UDP-β-L-Ara*f* **101** was synthesized analogously to **124** (scheme 33). Couplings of amino functionalized sugar phosphates to morpholidate activated nucleoside monophosphates have been reported in literature.[213] Purification by semi-preparative HPLC using a Hypercarb column and subsequent ion exchange chromatography gave small amounts (100 µg) of the desired UDP 5-amino-5-deoxy-β-L-arabinofuranose (**101**). Analysis by NMR spectroscopy (^{1}H and ^{31}P) confirmed the structure of the desired compound. The anomeric signal of arabinose appeared as a doublet of doublet at 5.65 ppm (J = 5.9, 3.2 Hz), indicating a 1,2-*cis* configuration, as for **124**. The ^{31}P NMR-spectrum showed two doublets of the same intensity, indicating the presence of a diphosphate linkage. Though only small quantities of the product were

isolated, the availability of compound **101** will allow the evaluation of putative plant arabinosyltransferases on the microarray.



Scheme 33: Synthesis of amino-functionalized UDP-*β*-L-Ara*f* using UMP-morpholidate method. Reagents and conditions: a) 1*H*-tetrazole, pyridine, rt, 1%.

2.3.5 Characterization of Plant Galactosyltransferases on Glycan Microarrays Using Functionalized UDP-Galactopyranose.

Preliminary studies establishing the new high-throughput screening method for glycosyltransferases on glycan microarrays described above were performed using the functionalized UDP-Gal*p* sugar donors (**97** and **98**) and the well-known galactosyltransferase GALACTAN SYNTHASE 1 (GALS1). In the literature, it was reported that heterologously expressed and affinity-purified GALS1 from glycosyltransferase family GT92 successively transfers galactose residues from UDP-Gal onto β(1,4)-galactopentaose and thereby forms new β(1,4)-galactosyl linkages.[56] The soluble catalytic domain of GALS1 and GALS2 were obtained from our collaboration partner Dr. Breeanna Urbanowicz (CCRC, Athens, GA, USA) who expressed the enzymes by transient transfection of suspension culture HEK293 cells as previously reported for the expression of AtXXT2.[214]

First, enzyme activity was confirmed by my colleague Dr. Ruprecht by incubation of GALS1 with the linear β(1,4)-linked tetragalactoside **25** and natural UDP-Gal followed by HPLC-MS analysis of the reaction products. HPLC analysis confirmed the previously described activity of the enzyme with natural UDP-Gal. Penta-, hexa-, hepta- and octasaccharides could be detected (fig. 21). Next, tolerance of GALS1 towards azido- and amino-functionalization of the sugar donor was investigated by incubating GALS1 with the linear β(1,4)-linked tetragalactoside **25** and azido- and amino-functionalized UDP-Gal derivatives **97** and **98** followed by HPLC-MS analysis of the reaction products. It was observed that GALS1 tolerates modifications at the Gal-C6-position of the donor substrates, as azido-functionalized penta- hexa- and heptasaccharides were formed (fig. 21). The 6-amino-Gal-UDP donor substrate **98** was well tolerated by GALS1 as well, but only the formation of the amino-functionalized pentasaccharide was detected (fig. 21),

implying that the amino-functionalized pentasaccharide did not serve as an acceptor substrate for further elongation with 6-amino-Gal residues.

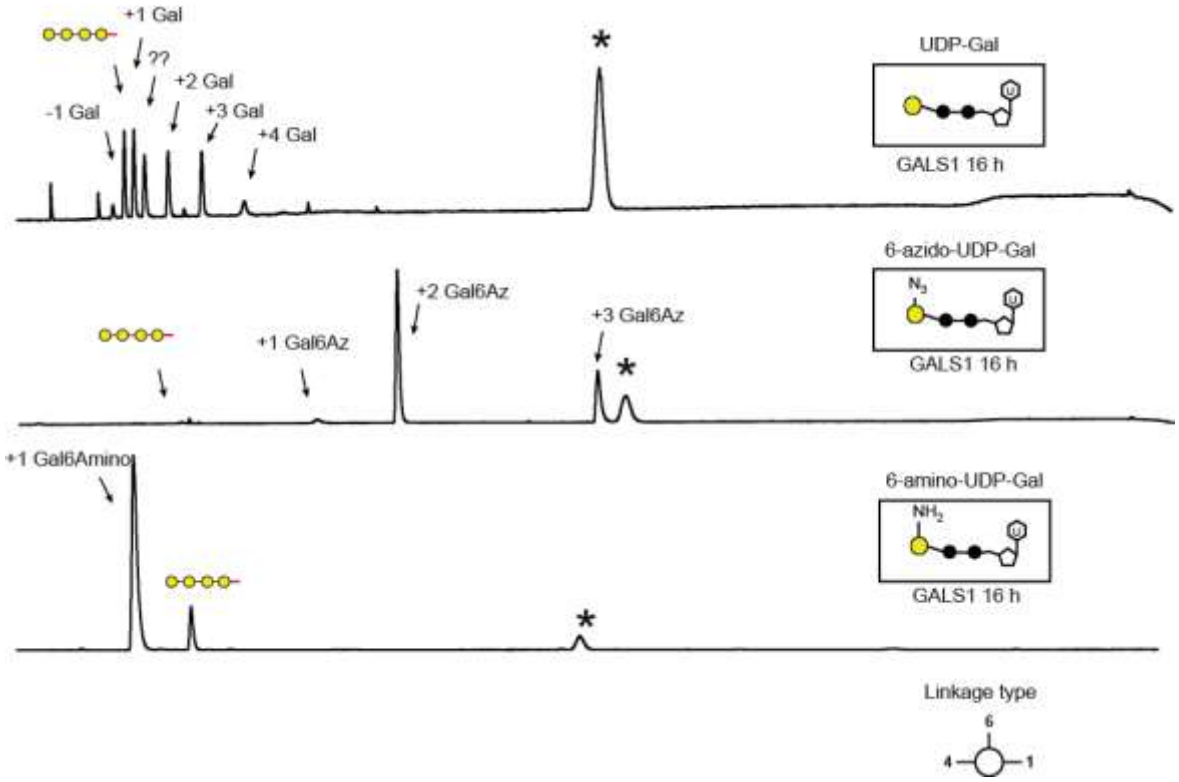


Figure 21: HPLC analysis (ELSD traces) of GALS1-catalyzed reactions of tetragalactoside acceptor **25** with natural UDP-Gal (top chromatogram), 6-azido-UDP-Gal (middle chromatogram) and 6-amino-UDP-Gal (lower chromatogram). Peaks are annotated with galactan oligosaccharides. The enzymes were incubated together with the acceptor and the donor substrate in HEPES buffer (50 mM, pH 6.8) for 16 h. The enzymatic reactions were stopped by heating to 80 °C before the crude reaction mixture was subjected to analytical HPLC-analysis. Star symbol marks UDP side-product.

After having confirmed the tolerance of GALS1 towards different modifications of the UDP-donor substrates, first experiments to test the microarray assay were performed by my colleague Dr. Colin Ruprecht.

The microarrays were equipped with a set of 44 glycans of which each was printed in two different concentrations. Additionally, 11-azido-3,6,9-trioxaundecan-1-amine was printed on the microarray surface as a positive control (fig. 22). The glycans were obtained by AGA as described in chapter 2 (compound 1 to 24 and 26, fig. 22), provided by my colleagues Dr. Pietro Dallabernardina[215,216] (compound 38 to 41, fig. 22) and Deborah Senf[103] (compound 37, fig. 22), by collaboration partners[118,141,217] (compounds 25, 28 to 36 and 42, fig. 22), or purchased as polysaccharides from Megazyme (compound 43 and 44 fig. 22).

The microarray was incubated with GALS1 and 6-azido-UDP-Gal (**97**) (0.01 – 0.5 mM) for 16 h in a Mn^{2+}-containing HEPES buffer (50 mM, pH 6.8) solution.

Subsequently, the reaction mixture was washed off the microarray surface and the azido groups incorporated into surface attached glycans were allowed to react with the fluorescent dye Sulfo-Cy5-alkyne in the presence of $CuSO_4$, a THPTA ligand and sodium ascorbate. After incubation for 1 h, the slides were washed, and the products were detected by fluorescence read-out at 635 nm. A fluorescent signal was only detected at positions that were equipped with β(1,4)-galactans consisting of at least four galactose moieties (fig. 22, **A**, compound 2,3 and 6), confirming the previously reported acceptor-substrate specificity of GALS1[56]. Concurring results were obtained for the incubation of a microarray slide with GALS1 and 4-azido-UDP-Gal **99** (fig. 22, **C**). Detection of incorporated amino functionalities after incubation of the glycan microarray with GALS1 and 6-amino-UDP Gal was also successful (fig. 22, **B**). Coupling of the fluorescent dye was achieved by reaction of the amino groups with a crosslinker containing an NHS ester and an azide before application of the alkyne-functionalized fluorescent dye for the "click-chemistry" reaction. The pattern of fluorescent signals for the incorporated amino functions was equal to the pattern of the incorporated azido functions (fig. 22, **B** compare to **A**), demonstrating that both methods of visualization perform equally well.

Interestingly, a similar but not identical pattern of fluorescent signals was detected when the microarray was incubated with GALS2 and 6-azido UDP Gal. GALS2 is another galactosyltransferase from glycosyltransferase family GT92 in *Arabidopsis* which was also obtained from our collaboration partner Dr. Breeanna Urbanowicz. GALS2 catalyzed the attachement of 6-azido-functionalized galactose from donor **97** into different β(1,4)-linked galactan oligosaccharides (compounds 1 to 3 and 6 and 7 fig. 22, **D**). However, these were very preliminary results, and enzyme and donor concentrations during incubation on the microarray probably have to be carefully adjusted for each enzyme to enable a reliable comparison between different enzymes.

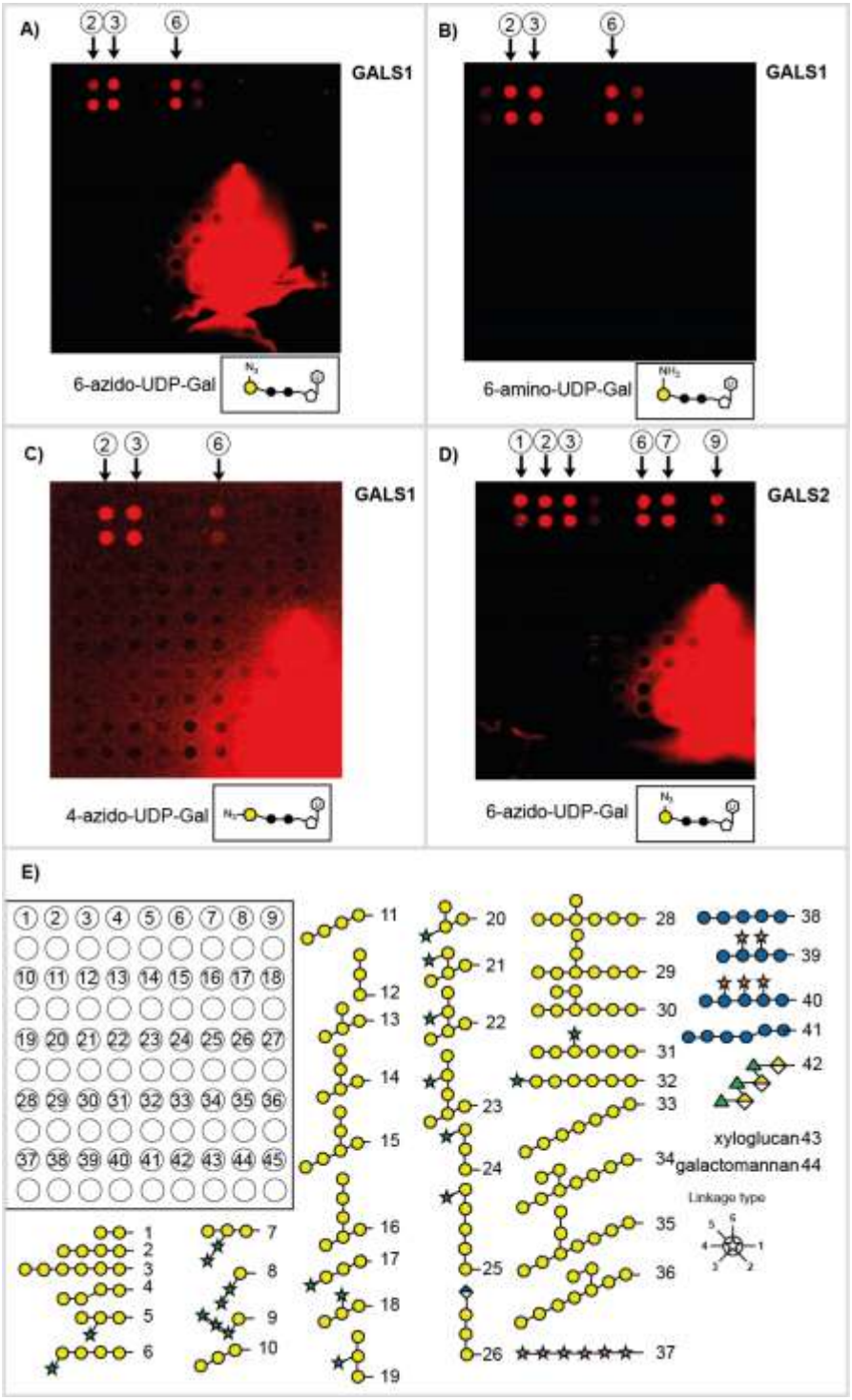


Figure 22: Microarray assays to study the substrate specificities of galactosyltransferases GALS1 and GALS2. **A)** Fluorescent signal after incubation with GALS1 and 6-azido-UDP-Gal and subsequent reaction with Sulfo Cy5-alkyne. **B)** Fluorescent signal after incubation with GALS1 and 6-amino-UDP-Gal and subsequent reaction with a NHS-ester-linked azide and Sulfo-Cy5-alkyne. **C)** Fluorescent signal after incubation with GALS1 and 4-azido-UDP-Gal. **D)** Fluorescent signal on the microarray after incubation with GALS2 and 6-azido-UDP-Gal. **E)** Printing pattern of glycans on the microarray. The absolute intensities of the signals in A)-D) cannot be directly compared, as the pictures were taken with different gain. Bright signals in the bottom right result from the positive control spots.

2.3.6 Conclusion and Outlook

In summary, a set of five functionalized and one natural UDP-sugar has been synthesized as substrates for the glycan microarray-based characterization of glycosyltransferases. Functionalized galactosyl 1-phosphates equipped with an azido-group (**97** and **99**) were coupled with activated UMP using the *cyclo*Sal activation method in good yields. The azido-functionalized UDP-galactoses **97** and **99** were further transformed into the respective amino-functionalized UDP-galactoses **98** and **100** by reduction of the azido groups. However, the *cyclo*Sal method failed for coupling arabinofuranosyl 1-phosphate **124** to UMP, due to degradation of the product upon subsequent removal of the acetal protecting groups. Instead, natural UDP-Ara*f* **124** as well as amino-functionalized UDP-Ara*f* **101** could be synthesized in low quantities by coupling the unprotected Ara*f* 1-phosphates **119** and **123** to morpholidate activated UMP. The functionalized galactose-UDP substrates **97**, **98**, **99** and **100** were used for preliminary proof-of-principle studies to investigate the substrate specificities of glycosyltransferases on a microarray equipped with synthetic oligosaccharides.

The enzymatic reaction products after incubation of the glycan microarrays with GALS1 or GALS2 and the functionalized donors (**97** to **100**) were visualized by reaction of the array-bound azido- or to the amino- functionalities with fluorescent probes. Analysis of the fluorescent signals on the microarray confirmed the expected substrate specificities of GALS1. Detection of the fluorescent signal after incubation with the genetically-related GALS2 revealed that GALS2 has a similar substrate specificity to GALS1.

Hence, it was shown that the newly developed method employing functionalized UDP-sugars on glycan microarray surfaces allows a high-throughput analysis of the substrate specificities of plant glycosyltransferases. Further functionalized sugar-nucleotides will be prepared in future to enable the characterization of a large number of putative glycosyltransferases.

3 Experimental Part

3.1 General Information

The automated syntheses were performed on a self-build synthesizer developed in the Max Planck Institute of Colloids and Interfaces. Galactan-degrading enzymes were obtained from Megazyme International Ireland. Linker-functionalized resins **23**[107] and **56**[144] were synthesized according to literature procedures. Resin loading was determined by performing one glycosylation (Module **A**) with large excess of **BB3** followed by DBU promoted Fmoc-cleavage and determination of dibenzofulvene production by measuring its UV absorbance.[218] Advanced intermediates for the synthesis of the BBs for AG oligosaccharides *p*-tolyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**12**)[131], *p*-tolyl 2-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilane-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**11**)[130], 2,3,5-tri-*O*-benzoyl-1-methyl-α-L-arabinofuranoside (**16**)[128], ethyl 2-*O*-benzoyl-3,4-*O*-dibenzyl-6-*O*-fluorenylcarbonylmethoxy-1-thio-D-galactopyranoside (**38**)[143], and the building blocks dibutoxyphosphoryloxy 2-*O*-benzoyl-3,6-dibenzyl-4-*O*-fluorenylcarbonylmethoxy-D-galactopyranoside (**BB1**)[130], dibutoxyphosphoryloxy 2-*O*-benzoyl-3-*O*-fluorenylcarbonylmethoxy-4,6-*O*-dibenzyl-D-galactopyranoside (**BB3**)[130], ethyl 2,3-di-*O*-benzoyl-5-*O*-fluorenylcarbonylmethoxy-1-thio-α-L-arabinofuranoside (**BB6**)[128], ethyl 2,3,5-tri-*O*-benzoyl-1-thio-α-L-arabinofuranoside (**BB4**)[128], methyl-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-di-*O*-butylphosphatidyl-D-glucopyranosyluronate (**BB9**)[142] were synthesized as described before. Phosphate BBs (**BB1**-**BB3** and **BB7**-**BB9**) were used in the automated synthesis as mixtures of α/β-anomers. Advanced intermediates for the synthesis of BBs for RG-II fragments *p*-tolyl 2,3-*O*-dibenzyl-1-thio-β-D-galactopyranoside (**69**)[170,171], *p*-tolyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**71**)[130], *p*-toyl 3-*C*-hydroxymethyl-2,3-*O*-isopropylidene-1-thio-β-D-erythrofuranoside (**81**)[157] and *N*-benzyl-*N*-benzyloxycarbonyl-2,2-difluoro-3-amino-1-propanol (**88**)[173] were synthesized as reported. Lactone BBs *p*-tolyl 2,4-dibenzyl-1-thio-β-D-galactopyranosidurono-3,6-lactone (**BB14**)[177], *p*-tolyl 2-*O*-benzyl-1thio-β-D-galactopyranosidurono-3,6-lactone (**BB15**)[175] and intermediate *p*-tolyl-3,4-*O*-isopropylidene-6-*O*-(1-methoxy-1-methylethyl)-1-thio-β-D-galactopyranoside[175,219] were synthesized analogously to the reported *S*-phenyl derivatives. Advanced intermediates for the synthesis of sugar nucleotides 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-D-galactopyranose (**103**)[205], 1,2,3,4-tertra-*O*-acetyl-4-azido-4-deoxy-D-galactopyranose (**112**)[207], 2,3-di-*O*-acetyl-urindine (**109**)[206], 5-nitro-*cyclo*Sal phosphorochloridite (**107**)[201] and 1,2,3-tri-*O*-acetyl-L-arabinofuranose (**115**)[210] were

synthesized as reported in the literature. Uridine 5'-monophosphomorpholidate 4-morpholine-N,N'-dicyclohexylcarboxamidine salt (**125**) was purchased from Sigma-Aldrich and used without further purifications. Solvents and reagents were used as supplied without any further purification. Anhydrous solvents were taken from a dry solvent system (JC-Meyer Solvent Systems). Column chromatography was carried out using Fluka Kieselgel 60 (230-400 mesh). NMR spectra were recorded on a Varian 400-MR (400 MHz), a Varian 600- (600 MHz), a Bruker AVIII 400 (400MHz) or a Bruker AVIII 700 (700 MHz) spectrometer using solutions of the respective compound in $CDCl_3$, D_2O and MeOD. NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. Spectra recorded in $CDCl_3$ or MeOD used the solvent residual peak chemical shift as internal standard ($CDCl_3$: 7.26 ppm ^{1}H, 77.1 ppm ^{13}C; MeOD: 3.31 ppm ^{1}H, 49.0 ppm ^{13}C). Spectra recorded in D_2O used either residual acetic (D_2O: 2.08 ppm ^{1}H) acid or formic acid (D_2O: 8.26 ppm ^{1}H) as internal standards in ^{1}H NMR and an acetic acid (D_2O: 21.03 ppm ^{13}C) or formic acid (D_2O: 166.31 ppm ^{13}C) spike as internal standard in ^{13}C NMR. The chemical shifts in ^{31}P NMR of compound **97**-**100** were referenced to the reported shifts of **97**[204]. The chemical shifts in ^{31}P NMR in compounds **124** and **101** were referenced to the reported shifts of **124**[209]. NMR peaks of BBs and intermediates were assigned by COSY and HSQC NMR experiments. Yields of final deprotected oligosaccharides were determined after removal of residual acetic acid. Optical rotations were measured using a UniPol L1000 polarimeter (Schmidt&Haensch) with concentrations expressed as g/100 mL. IR spectra were recorded on a Spectrum 100 FTIR spectrophotometer (Perkin-Elmer). High resolution mass spectra were obtained using a 6210 ESI-TOF (Agilent) or a Xevo J2-XGQTOF (Waters) mass spectrometer. Analytical HPLC was performed on an Agilent 1200 series coupled to a quadrupole ESI LC/MS 6130 using a YMC-Pack DIOL-300-NP column (150 x 4.6 mm), a Phenomenex Luna C5 column (250 x 4.6 mm), a Thermo Scientific Hypercarb column (150 x 4.6 mm), or a Phenomenex Synergy Hydro RP18 (250 x 4.6 mm). Preparative HPLC was performed on an Agilent 1200 series using a preparative YMC-Pack-DIOL-300-NP (150 x 20 mm), a semi-preparative Phenomenex Luna C5 column (250 x 10 mm), a semi-preparative Thermo Scientific Hypercarb column (150 x 4.6 mm) or a semi-preparative Phenomenex Hydro RP18 column (250 x 10 mm). Ion exchange chromatography was performed on a Knauer Azura system using a GE HiTrap Q XL 5 mL column with a linear gradient of 100% aq. 0.05 M NH_4OAc (pH 6.8) buffer to 100% aq 0.5 M NH_4OAc (pH 6.8) buffer.

3.1 Synthesizer Modules and Conditions

After placement of the linker-functionalized resin **23** or **56** (12.9-22.1 µmol of hydroxyl groups) in the reaction vessel the resin was washed with DMF, THF, and DCM. Subsequently the glycosylation (Module **A** and **D**), deprotection (Module **B** and **C**) and capping (Module **E**) steps were performed. Mixing of the components was accomplished by bubbling Argon through the reaction mixture.

Module A: Glycosylation with Glycosyl Phosphates. The resin (12.9-22.1 µmol of hydroxyl groups) was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to -30 °C. Prior to the glycosylation reaction the resin was washed with TMSOTf in DCM and DCM. For the glycosylation reaction the DCM was drained and a solution of phosphate BB (3-5 equiv in 1 mL DCM) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by the addition of TMSOTf in DCM (3-5 equiv in 1 mL DCM). The glycosylation was performed for 5 min at -35 °C (**BB1**, **BB3**, **BB7** and **BB8**) or -30 °C (**BB2**) and for 30 min at -20 °C (**BB1**, **BB3**, **BB7** and **BB8**) or -10 °C (**BB2**) or for 15 min at -15 °C and for 45 min at 0 °C (**BB9**). Subsequently the solution was drained, and the resin was washed twice with DCM. The whole procedure was repeated once to enhance conversion of the acceptor sites.

Module B: Fmoc Deprotection. The resin was washed with DMF, swollen in 2 mL DMF and the temperature of the reaction vessel was adjusted to 25 °C. Prior to the deprotection step the DMF was drained and the resin was washed with DMF three times. For Fmoc deprotection 2 mL of a solution of 20% Et_3N in DMF was delivered to the reaction vessel. After 5 min the solution was drained and the whole procedure was repeated another two times. After Fmoc deprotection was complete the resin was washed with DMF, THF and DCM.

Module C: Lev Deprotection. The resin was washed with DCM three times and the temperature of the reaction vessel was adjusted to 25 °C. For Lev deprotection 1.3 mL DCM remained in the reaction vessel and 0.8 mL of a solution of 0.15 M hydrazine acetate in pyridine/AcOH/H_2O (4:1:0.25) was delivered to the reaction vessel. After 30 min the reaction solution was drained and the whole procedure was repeated another two times. After Lev deprotection was complete the resin was washed with DMF, THF and DCM.

Module D: Glycosylation with Thioglycosides. The resin (16.9-22.1 µmol of hydroxyl groups) was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to -30 °C. Prior to the glycosylation reaction the resin was washed with TMSOTf in DCM and DCM. For the glycosylation reaction the DCM was drained and a solution of thioglycoside BB (3 to 5 equiv. in 1 mL DCM) was delivered to the reaction

vessel. After the set temperature was reached, the reaction was started by the addition of NIS (3.4-4.4 equiv.) and TfOH (0.4 equiv.) in DCM/dioxane (3:1). The glycosylation was performed for 5 min at -40 °C and for 40 min at -20 °C. Subsequently the solution was drained, and the resin was washed with DCM. The whole procedure was repeated once to enhance conversion of the acceptor sites. Afterwards the resin was washed three times with DCM at 25 °C.

Module E: Benzoylation. The temperature was adjusted to 25 °C and the resin washed with pyridine (2 mL) three times. For benzoylation temperature was set to 40 °C and 2 mL pyridine and 1 mL of a solution containing 0.5 M benzoic anhydride and 0.25 M DMAP in DCE were delivered to the reaction vessel. After 30 min the reaction solution was drained and the whole procedure was repeated another two times. After capping was complete the resin was washed with DCM.

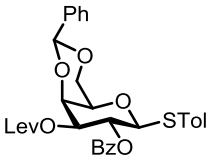
Cleavage from the Solid Support. After assembly of the oligosaccharides, cleavage from the solid support was accomplished by modification of a previously published protocol,[107] using the Vapourtec E-Series UV-150 photoreactor Flow Chemistry System. The medium pressure metal halide lamp is filtered using the commercially available red filter. The resin, suspended in DCM, was loaded into a plastic syringe. The suspension was then pumped using a syringe pump (PHD2000, Harvard Aparatus) at 1 mL/min through a 10 mL reactor, constructed of 1/8 inch o.d. FEP tubing. The total volume within the photoreactor was 9 mL. The temperature of the photoreactor was maintained at 20 °C and the lamp power was 80%. The exiting flow was deposited in a 10 mL syringe containing a filter, with a collection flask beneath the syringe.

3.3 Automated Glycan Assembly of Pectin Arabinogalactans

3.3.1 Building Block Synthesis

3.3.1.1 Synthesis of Galactose Building Blocks

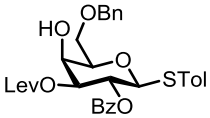
_p_-Tolyl 2-_O_-benzoyl-3-_O_-levulinoyl-4,6-_O_-benzylidene-1-thio-β-D-galactopyranoside (13)



A solution of galactose derivative **12**[131] (4.20 g, 8.75 mmol) in DCM (50 mL) at 0 °C was treated with DMAP (0.64 g, 5.25 mmol, 0.6 equiv), DIC (2.05 mL, 13.1 mmol, 1.5 equiv) and levulinic acid (1.43 mL, 14.0 mmol, 1.6 equiv). The solution was stirred for 3 h at rt. A white precipitate slowly formed and the solution turned slightly pink. After complete conversion of the starting material, the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hex = 2:3) to give galactose derivative **13** (4.30 g, 7.46 mmol, 85% yield).

$[\alpha]_D^{25}$ = -14.7 (c 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.03 (d, *J* = 7.6 Hz, 2H, Ar), 7.59 (t, *J* = 7.4 Hz, 1H,. Ar), 7.50 – 7.33 (m, 9H, Ar), 7.06 (d, *J* = 7.5 Hz, 2H, Ar), 5.53 (t, *J* = 9.8 Hz, 1H, H-2), 5.48 (s, 1H, PhC*H*), 5.17 (d, *J* = 9.9 Hz, 1H, H-3), 4.81 (d, *J* = 9.7 Hz, 1H, H-1), 4.40 (m, *J* = 12.6 Hz, 2H, H-4, H-6), 4.04 (d, *J* = 12.3 Hz, 1H, H-6) 3.64 (s, 1H, H-5), 2.67 – 2.37 (m, 4H, Lev), 2.34 (s, 3H, SPhCH_3), 1.86 (s, 3H, Lev(CH_3)) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 206.2 (C=O), 172.1 (C=O), 165.0 (C=O), 138.5, 137.7, 134.6, 133.3, 129.9, 129.8, 129.6, 129.2, 128.5, 128.2, 127.2, 126.7 (18 C, Ar), 101.2 (Ph*C*H), 85.4 (C-1), 73.67 (C-4), 73.4 (C-3), 69.9 (C-5), 69.2 (C-6), 67.4 (C-2), 37.9 (Lev), 29.5 (Lev(*C*H_3)), 28.3 (Lev), 21.4 (SPh*C*H_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{32}H_{32}NaO_8S$: 599.1715, found: 599.1687. IR (neat) ν_{max} = 1711, 1252, 997, 717 cm^{-1}.

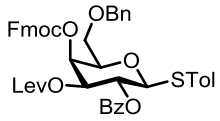
_p_-Tolyl 2-_O_-benzoyl-3-_O_-levulinoyl-6-_O_-benzyl-1-thio-β-D-galactopyranoside (14)



Galactose derivative **13** (4.30 g, 7.46 mmol) was dissolved in anhydrous DCM (50 mL) under nitrogen atmosphere and triethylsilane (7.16 mL, 44.7 mmol, 6 equiv) and TFAA (1.00 mL, 7.46 mmol, 1 equiv) were added. The solution was stirred at rt for 15 min before TFA (3.42 mL, 44.7 mmoL, 6 equiv) was added dropwise. The reaction was stirred at rt for additional 2 h before it was quenched by the addition of sat. aq. $NaHCO_3$ solution

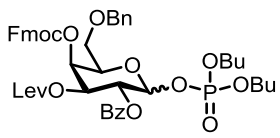
and washed with brine. The organic phase was separated, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. Compound **14** (approx. 4.32 g, 7.46 mmol) was directly used in the next step without further purification.

p-Tolyl 2-*O*-benzoyl-3-*O*-levulinoyl-4-*O*-fluorenylcarbonylmethoxy-6-*O*-benzyl-1-thio-β-D-galactopyranoside (15)



14 (3.00 g, 5.18 mmol) was dissolved in anhydrous DCM (50 mL) and anhydrous pyridine (2.09 mL, 25.9 mmol, 5 equiv) was added. The solution was stirred at rt for 15 min before it was cooled down to 0 °C and 3.99 g (15.4 mmol, 3 equiv) FmocCl were added. The reaction was allowed to slowly warm up to rt and stirred for 6 h. The solvent was removed under vacuum and the residue co-evaporated with toluene twice before compound **15** was recrystallized from boiling ethanol in 66% yield (2.75 g, 3.43 mmol).
$[\alpha]_D^{25}$ = +1.0 (c 0.4, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.02 (d, *J* = 7.3 Hz, 2H, Ar), 7.76 (d, *J* = 6.4 Hz, 2H, Ar), 7.62 (d, *J* = 6.9 Hz, 3H, Ar), 7.48 – 7.21 (m, 12H, Ar), 7.03 (d, *J* = 7.8 Hz, 2H, Ar), 5.59 (t, *J* = 10.0 Hz, 1H, H-2), 5.37 (s, 1H, H-4), 5.22 (d, *J* = 9.9 Hz, 1H, H-3), 4.81 (d, *J* = 10.0 Hz, 1H, H-1), 4.56 – 4.18 (m, 6H, C*H*$_2$Ph, Fmoc), 3.94 (t, *J* = 6.0 Hz, 1H, H-5), 3.66 (dt, *J* = 16.2, 9.4 Hz, 2H, H-6), 2.48 – 2.21 (m, 7H, Lev, SPhC*H*$_3$), 1.87 (s, 3H, LevC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 205.8 (C=O), 165.2 (C=O), 154.9, 143.4, 143.3, 143.2, 141.4, 141.3, 138.4, 137.7, 133.5, 133.0, 130.0, 129.8, 129.5, 129.0, 128.6, 128.5, 128.1, 128.0, 127.9, 127.5, 127.4, 127.3, 125.5, 125.3, 120.2, 120.1 (30 C, Ar), 87.4 (C-1), 76.1 (C-5), 73.7 (*C*H$_2$Ph), 72.7 (C-3), 72.0 (C-4), 70.4 (Fmoc), 67.9 (C-2), 67.9 (C-6), 46.6 (Fmoc), 37.7 (Lev), 29.5 (Lev), 28.0 (Lev), 21.3 (SPh*C*H$_3$) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{47}H_{44}NaO_{10}S$: 823.2552, found: 823.2570. IR (neat) ν_{max} = 1722, 1246, 1094, 707 cm^{-1}.

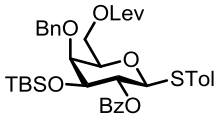
Dibutoxyphosphoryloxy 2-*O*-benzoyl-3-*O*-levulinoyl-4-*O*-fluorenylcarbonylmethoxy-6-*O*-benzyl-α/β-D-galactopyranoside (BB2)



To a suspension of activated molecular sieve (4 Å, 1.50 g) in anhydrous DCM (20 mL) was added dibutyl phosphate (1.40 mL, 6.86 mmol, 2 equiv) and the mixture was stirred for 30 min. Subsequently, the molecular sieve was allowed to settle for 30 min and the supernatant was added to a solution of **15** (2.75 g, 3.43 mmol) in anhydrous DCM (30 mL) and cooled to 0 °C under argon atmosphere. NIS (1.00 g, 4.46 mmol, 1.3 equiv)

and TfOH (0.06 mL, 0.69 mmol, 0.2 equiv) were added and the resulting purple reaction mixture was stirred for 1 h. The reaction was quenched by the addition of sat. aq. $NaHCO_3$-solution and washed with sat. aq. $Na_2S_2O_3$-solution until the color of the organic layer changed from purple to colorless. The layers were separated, the organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The product was purified by silica gel column chromatograph (EtOAc/hex = 1:4 to 1:1) to give phosphate **BB2** (2.08 g, 2.38 mmol, 69% yield) as a mixture of *α/β*-anomers as a highly viscous and sticky oil.
Analytical data for *β*-anomer: $[\alpha]_D^{25}$ = -4.9 (c 2.3, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.08 – 8.03 (m, 2H, Ar), 7.78 (dd, *J* = 7.0, 4.4 Hz, 2H, Ar), 7.67 (dd, *J* = 11.2, 7.4 Hz, 2H, Ar), 7.61 – 7.56 (m, 1H, Ar), 7.50 – 7.34 (m, 6H, Ar), 7.33 – 7.21 (m, 5H, Ar), 5.72 (dd, *J* = 10.5, 8.0 Hz, 1H, H-2), 5.48 (t, *J* = 7.7 Hz, 1H, H-1), 5.44 (d, *J* = 2.8 Hz, 1H, H-4), 5.24 (dd, *J* = 10.5, 3.3 Hz, 1H, H-3), 4.55 – 4.43 (m, 3H, C*H*$_2$Ph, Fmoc), 4.38 – 4.28 (m, 2H, C*H*$_2$Ph, Fmoc), 4.10 – 3.98 (m, 3H , H-5, H-6), 3.81 – 3.61 (m, 4H, OBu), 2.58 – 2.23 (m, 4H, Lev), 1.92 (s, 3H Lev(C*H*$_3$)), 1.65 – 1.55 (m, 2H, OBu), 1.41 – 1.28 (m, 4H, OBu), 1.02 (dq, *J* = 14.7, 7.4 Hz, 2H, OBu), 0.89 (t, *J* = 7.4 Hz, 3H, OBu(C*H*$_3$)), 0.69 (t, *J* = 7.4 Hz, 3H, OBu(C*H*$_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 205.8 (C=O), 165.2 (C=O), 154.9 (C=O), 143.3, 143.2, 141.4, 141.3, 137.5, 133.6, 130.0, 129.1, 128.6, 128.5, 128.1, 128.0, 127.8, 127.5, 127.4, 125.4, 120.2, 120.2 (Ar 24 C) , 96.9 (d, *J* = 4.7 Hz, C-1), 73.7 (*C*H$_2$Ph), 72.8 (C-5), 71.4 (C-4), 71.3 (C-3), 70.6 (Fmoc), 69.25 (d, *J* = 9.1 Hz, C-2), 68.0 (d, *J* = 6.3 Hz, OBu), 67.9 (d, *J* = 6.3 Hz, OBu), 67.1 (C-6), 46.7 (Fmoc), 37.7 (Lev), 31.9 (d, *J* = 7.4 Hz, OBu), 31.7 (d, *J* = 7.3 Hz, OBu), 29.5 (Lev), 27.8 (Lev), 18.7 (OBu), 18.4 (OBu), 13.7 (OBu), 13.5 (OBu) ppm. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{47}H_{45}O_{10}S$: 887.3408, found: 887.3461. IR (neat) ν_{max} =1249, 1025, 740, 711 cm^{-1}.

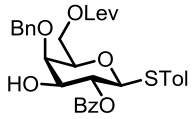
_p_-Tolyl 2-_O_-benzoyl-3-_O_-_tert_-butyldimethylsilyl-4-_O_-benzyl-6-_O_-levulinoyl-1-thio-β-D-galactopyranoside (35)



A solution of **34**[130] (6.07 g, 10.2 mmol) in DCM (100 mL) at 0 °C was treated with DMAP (0.75 g 6.13 mmol, 0.6 equiv), DIC (1.93 g, 15.3 mmol, 1.5 equiv) and levulinic acid (1.7 mL, 16.4 mmol, 1.6 equiv) was added. The solution was stirred for 3 h at rt. A white precipitate slowly formed and the solution became pink. After complete conversion, the solvent was removed *in vacuo* and the product was purified by silica gel column chromatography (hex/EtOAc = 3:2) to give **35** as a colorless foam in 91% yield (6.42 g, 9.27 mmol)
$[\alpha]_D^{25}$ = +23.1 (c 1.4, $CHCl_3$). ^{1}H NMR (400 MHz,$CDCl_3$) δ = 8.06 – 8.02 (m, 2H, Ar), 7.58 (t, *J* = 7.4 Hz, 1H, Ar), 7.45 (t, *J* = 7.7 Hz, 2H, Ar), 7.38 – 7.26 (m, 7H, Ar), 7.01 (d, *J* = 7.9

Hz, 2H, Ar), 5.64 – 5.59 (m, 1H, H-2), 5.10 (d, *J* = 11.4 Hz, 1H, PhC*H*$_2$), 4.71 (d, *J* = 9.6 Hz, 1H, H-1), 4.56 (d, *J* = 11.4 Hz, 1H, PhC*H*$_2$), 4.32 (dd, *J* = 11.2, 6.9 Hz, 1H, H-6), 4.21 (dd, *J* = 11.2, 5.5 Hz, 1H, H-6), 3.97 (d, *J* = 8.8 Hz, 1H, H-3), 3.81 – 3.73 (m, 2H, H-4, H-5), 2.74 (t, *J* = 6.5 Hz, 2H, Lev(C*H*$_2$)), 2.53 (t, *J* = 6.6 Hz, 2H, Lev(C*H*$_2$)), 2.29 (s, 3H, SPhC*H*$_3$), 2.19 (s, 3H, LevC*H*$_3$), 0.77 (s, 9H, TBS), 0.10 (d, *J* = 3.5 Hz, 3H, TBS), -0.10 (s, 3H, TBS) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 206.6, 172.6, 165.3 (3 C, C=O), 138.5, 137.7, 133.1, 132.6, 130.4, 129.9, 129.5, 128.4, 128.3, 127.9, 127.6 (18 C, Ar), 87.2 (C-1), 76.9, 76.1, 75.7 (C-3), 75.0 (Ph*C*H$_2$), 71.0 (C-2), 63.6 (C-6), 38.0 (Lev), 29.9 (Lev), 27.9 (Lev), 25.6 (TBS), 21.2 (SPh*C*H$_3$), 17.9 (TBS), -3.92 (TBS), -4.98 (TBS) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{38}H_{48}NaO_8SSi$: 715.2736, found: 715.2652 IR (neat) ν_{max} = 1719, 1260, 1088, 1069 cm^{-1}.

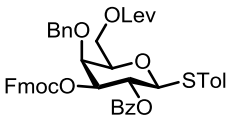
p-Tolyl 2-*O*-benzoyl-4-*O*-benzyl-6-*O*-levulinoyl-1-thio-β-D-galactopyranoside (36)



35 (6.37 g, 9.27 mmol) was dissolved in MeCN (80 mL), the solution was cooled to 0 °C and boron trifluoride etherate (1.3 mL, 10.1 mmol, 1.1 equiv) was added. The solution was stirred at 0 °C for ten min and subsequently quenched by the addition of sat. aq. $NaHCO_3$-solution. The product was extracted from the aq. solution with DCM and the organic layer was washed with water twice. The organic layer was dried over $MgSO_4$. The product **36** was obtained in quantitative yields (5.32 g, 9.27 mmol) as a transparent oil after removal of the solvent *in vacuo*.

$[\alpha]_D^{25}$ = +4.7 (c 1.1, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.05 (d, *J* = 8.1 Hz, 2H, Ar), 7.57 (dd, *J* = 10.6, 4.2 Hz, 1H, Ar), 7.45 (t, *J* = 7.7 Hz, 2H, Ar), 7.37 – 7.28 (m, 7H, Ar), 7.04 (d, *J* = 7.9 Hz, 2H, Ar), 5.23 (t, *J* = 9.7 Hz, 1H, H-2), 4.82 (d, *J* = 11.6 Hz, 1H, PhC*H*$_2$), 4.76 – 4.66 (m, 2H, PhC*H*$_2$, H-1), 4.35 (dd, *J* = 11.1, 6.9 Hz, 1H, H-6), 4.17 (dd, *J* = 11.2, 6.0 Hz, 1H, H-6), 3.88 (d, *J* = 2.3 Hz, 1H, H-3), 3.83 (dd, *J* = 9.5, 3.2 Hz, 1H, H-4), 3.75 (t, *J* = 6.4 Hz, 1H, H-5), 2.74 (t, *J* = 6.5 Hz, 2H, Lev(C*H*$_2$)), 2.54 (t, *J* = 6.4 Hz, 2H, Lev(C*H*$_2$)), 2.30 (s, 3H, SPhC*H*$_3$), 2.17 (s, 3H, LevC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 206.9, 172.5, 166.8 (3 C, C=O), 138.1, 137.8, 133.4, 133.1, 130.0, 129.6, 129.6, 128.8, 128.5, 128.5, 127.9, 127.9 (18 C, Ar), 86.3 (C-1), 76.5 (C-3), 76.1 (C-5), 75.5 (Ph*C*H$_2$), 74.4(C-4), 72.1 (C-2), 63.0 (C-6), 37.9 (Lev), 29.9 (Lev), 27.8(Lev), 21.2 (SPh*C*H$_3$) ppm. $[M+Na]^+$ calcd. for $C_{32}H_{34}NaO_8S$: 601.1872; found 601.1911. IR (neat) ν_{max} =1723, 1713 1271, 1250 cm^{-1}.

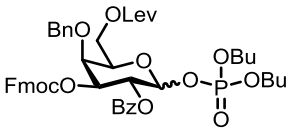
_p_-Tolyl 2-_O_-benzoyl-3-_O_-fluorenylcarbonylmethoxy-4-_O_-dibenzyl-6-_O_-levulinoyl-1-thio-β-D-galactopyranoside (37)



To a solution of **36** (5.32 g, 9.27 mmol) in DCM (100 mL) and pyridine (2.5 mL, 30.9 mmol, 3.3 equiv) Fmoc-Cl (4.83 g, 18.6 mmol, 2 equiv) was added and the solution was stirred at rt for 6 h. Subsequently the mixture was evaporated *in vacuo* and the resulting oil re-dissolved in DCM (50 mL). The organic layer was washed with brine three times, dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. Recrystallization from boiling ethanol gave the pure product **37** in 75% yield (5.60 g, 6.99 mmol) as a colorless solid.

$[\alpha]_D^{25}$ = +35.7 (c 1.1, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.08 – 8.03 (m, 2H, Ar), 7.69 (dd, *J* = 7.5, 4.2 Hz, 2H, Ar), 7.56 (t, *J* = 7.4 Hz, 1H, Ar), 7.49 – 7.29 (m, 13H, Ar), 7.20 – 7.03 (m, 4H, Ar), 5.72 (t, *J* = 10.0 Hz, 1H, H-2), 5.07 (dd, *J* = 10.0, 2.8 Hz, 1H, H-3), 4.83 (d, *J* = 11.5 Hz, 1H, PhC*H*$_2$), 4.80 (d, *J* = 10.0 Hz, 1H, H-1), 4.53 (d, *J* = 11.4 Hz, 1H, PhC*H*$_2$), 4.37 – 4.29 (m, 2H, H-6, Fmoc), 4.27 – 4.14 (m, 2H, H-6, Fmoc), 4.07 (t, *J* = 7.5 Hz, 1H, Fmoc), 4.04 (d, *J* = 2.4 Hz, 1H, H-4), 3.81 (t, *J* = 6.4 Hz, 1H, H-5), 2.74 (t, *J* = 6.1 Hz, 2H, Lev(C*H*$_2$)), 2.53 (t, *J* = 6.5 Hz, 2H, Lev(C*H*$_2$)), 2.31 (s, 3H, SPhC*H*$_3$), 2.18 (s, 3H, Lev(C*H*$_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 206.5, 172.4, 165.1, 154.5 (4 C, C=O), 143.3, 142.8, 141.3, 141.2, 138.2, 137.5, 133.4, 133.0, 130.0, 129.6, 129.6, 129.1, 129.1, 128.5, 128.5, 128.4, 128.0, 127.9, 127.2, 127.2, 125.2, 125.0, 120.1 (26 C, Ar), 87.1 (C-1), 79.1 (C-3), 75.9 (C-5), 75.0 (Ph*C*H$_3$), 73.5 (C-4), 70.3 (Fmoc), 68.6 (C-2), 62.7 (C-6), 46.5 (Fmoc), 37.9 (Lev), 29.9 (Lev), 27.8 (Lev), 21.3 (SPh*C*H$_3$) ppm. $[M+Na]^+$ calcd. for $C_{47}H_{44}NaO_{10}S$: 823.2552, found: 823.2558. IR (neat) ν_{max} = 1742, 1723, 1273, 1249 cm^{-1}.

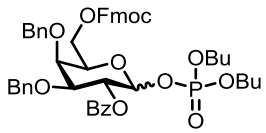
Dibutoxyphosphoryloxy 2-_O_-benzoyl-3-_O_-fluorenylcarbonylmethoxy-4-_O_-benzyl-6-_O_-levulinoyl-α/β-D-galactopyranoside (BB7)



To a suspension of activated powdered molecular sieves (4 Å, 1 g) in anhydrous DCM (20 mL) was added dibutyl phosphate (1.0 mL, 5.01 mmol, 2 equiv) and the mixture was stirred for 1.5 h. Subsequently the molecular sieves were allowed to settle for 30 min, the supernatant was added to a solution of **37** (1.85 g, 2.50 mmol) in anhydrous DCM (50 mL) and cooled to 0 °C under argon atmosphere. NIS (0.73 g, 3.26 mmol, 1.3 equiv) and TfOH (0.07 mL, 0.79 mmol, 0.3 equiv) were added and the purple reaction mixture was stirred for 1 h. The reaction was quenched by addition of sat. aq. $NaHCO_3$-solution

and washed with sat. aq. $Na_2S_2O_3$-solution until the color of the organic layer changed from purple to colorless. The layers were separated, the organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo*. The product was purified by silica gel column chromatograph (EtOAc/hex = 1:8 to 1:1) to give the phosphate **BB7** (2.04 g, 2.30 mmol, 92% yield) as a mixture of α/β-anomers (α/β~1:2) as a highly viscous and sticky oil.
Analytical data for β-anomer: $[\alpha]_D^{25}$ = +28.8 (c 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, $CCDl_3$) δ = 8.10 – 8.02 (m, 2H, Ar), 7.70 – 7.65 (m, 2H, Ar), 7.55 (t, *J* = 7.4 Hz, 1H, Ar), 7.48 (d, *J* = 7.5 Hz, 1H, Ar), 7.45 – 7.40 (m, 1H, Ar), 7.39 – 7.28 (m, 6H, Ar), 7.16 – 7.06 (m, 2H, Ar), 5.81 (dd, *J* = 10.5, 8.0 Hz, 1H, H-2), 5.42 (t, *J* = 7.7 Hz, 1H, H-1), 5.02 (dd, *J* = 10.5, 2.9 Hz, 1H, H-3), 4.80 (d, *J* = 11.2 Hz, 1H, PhCH_2), 4.52 (d, *J* = 11.2 Hz, 1H, PhCH_2), 4.40 – 4.18 (m, 4H, H-6, Fmoc), 4.15 – 3.97 (m, 5H, H-4, Fmoc, OBu), 3.91 (t, *J* = 6.4 Hz, 1H, H-5), 3.71 (ddq, *J* = 27.5, 9.8, 6.7 Hz, 2H, OBu), 2.75 (t, *J* = 6.5 Hz, 2H, Lev), 2.52 (t, *J* = 6.5 Hz, 2H, Lev), 2.19 (s, 3H, CH_3(Lev)), 1.65 – 1.57 (m, 2H, OBu), 1.42 – 1.21 (m, 4H, OBu), 1.02 (dq, *J* = 14.7, 7.4 Hz, 2H, OBu), 0.90 (t, *J* = 7.4 Hz, 3H, CH_3(OBu)), 0.68 (t, *J* = 7.4 Hz, 3H, CH_3(OBu)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.4, 172.3, 165.1, 154.4 (4C, C=O), 143.2, 142.8, 141.3, 141.2, 137.2, 133.5, 130.0, 129.2, 128.6, 128.6, 128.2, 127.9, 127.2, 127.1, 125.2, 125.0, 120.1 (18 C, Ar), 96.8 (d, *J* = 5.0 Hz), 77.4 (C-3), 75.4 (PhCH_2), 73.1 (C-4), 72.9 (C-5), 70.3 (Fmoc), 69.8 (C-2), 68.1 (d, *J* = 6.3 Hz, 1C OBu), 68.0 (d, *J* = 6.3 Hz, OBu), 62.2 (C-6), 46.5 (Fmoc), 37.9 (Lev), 32.1 (d, *J* = 7.5 Hz, 1C, OBu), 31.8 (d, *J* = 7.4 Hz, 1C, OBu) 29.9 (Lev), 27.8 (Lev), 18.6, 18.3, 13.6, 13.4 (4C, OBu) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{48}H_{55}O_{14}NaP$: 909.3227, found: 909.3265. IR (neat) ν_{max} = 1738, 1272, 1250, 1028 cm^{-1}.

Dibutoxyphosphoryloxy 2-*O*-benzoyl-3,4-*O*-dibenzyl-6-*O*-fluorenylcarbonylmethoxy-α/β-D-galactopyranoside (BB8)



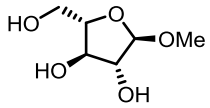
To a suspension of activated powdered molecular sieves (4 Å, 1g) in anhydrous DCM (50 mL) was added dibutyl phosphate (0.6 mL, 3.0 mmol, 2.2 equiv) and the mixture was stirred for 1.5 h. Subsequently the molecular sieves were allowed to settle for 30 min, the supernatant was added to a solution of ethyl 2-*O*-benzoyl-3,4-*O*-dibenzyl-6-*O*-fluorenylcarbonylmethoxy-1-thio-β-D-galactopyranoside (**38**)[143] (0.98g, 1.38 mmol) in anhydrous DCM (100 mL) and cooled to 0 °C under argon atmosphere. NIS (0.41 g, 1.80 mmol, 1.3 equiv) and TfOH (0.04 mL, 0.45 mmol, 0.3 equiv) were added and the purple reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched by addition of sat. aq. $NaHCO_3$-solution and washed with sat. aq. $Na_2S_2O_3$-solution until the color of

the organic layer changed from purple to colorless. The layers were separated, the organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo*. The product was purified by silica gel column chromatography (EtOAc/hex = 1:8 to 1:1) to give the phosphate **BB8** (0.98 g, 1.11 mmol, 80% yield) as a mixture of α/β anomers (α/β~2:1) as a highly viscous and sticky oil.

Characterization for α-anomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.05 (d, *J* = 7.5 Hz, 2H, Ar), 7.78 (d, *J* = 7.5 Hz, 2H, Ar), 7.63 – 7.55 (m, 3H, Ar), 7.43 (dt, *J* = 10.8, 7.6 Hz, 4H, Ar), 7.37 – 7.23 (m, 12H, Ar), 5.94 (dd, *J* = 6.7, 3.5 Hz, 1H, H-1), 5.66 (dt, *J* = 10.4, 3.2 Hz, 1H, H-2), 5.02 (d, *J* = 11.4 Hz, 1H, PhCH_2), 4.74 (s, 2H, PhCH_2), 4.64 (d, *J* = 11.4 Hz, 1H, PhCH_2), 4.41 (m, 2H, Fmoc, H-5), 4.33 – 4.26 (m, 2H, Fmoc, H-6), 4.25 – 4.19 (m, 2H, H-6, Fmoc), 4.16 (dd, *J* = 10.4, 2.4 Hz, 1H, H-3), 4.06 – 3.95 (m, 3H, H-4, OBu), 3.94 – 3.75 (m, 2H, OBu), 1.58 – 1.48 (m, 2H, OBu), 1.38 – 1.23 (m, 4H, OBu), 1.14 (dq, *J* = 15.1, 7.4 Hz, 2H, OBu), 0.84 (t, *J* = 7.4 Hz, 3H, OBu), 0.74 (t, *J* = 7.4 Hz, 3H, OBu) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.6, 154.7 (2 C, C=O), 143.1, 141.2, 137.6, 137.6, 133.2, 129.8, 129.5, 128.4, 128.4, 128.3, 128.3, 127.9, 127.8, 127.6, 127.1, 125.0, 120.0 (26 C, Ar), 94.8 (d, *J* = 6.4 Hz, C-1), 75.7 (C-5), 74.8 (Ph*C*H_3), 73.6 (C-4), 72.9 (Ph*C*H_2), 70.3 (Fmoc), 70.3 (C-2), 69.9 (C-5), 67.75 (d, *J* = 5.6 Hz, OBu), 67.69 (d, *J* = 5.7 Hz, OBu), 66.4 (C-6), 46.6 (Fmoc), 32.07 (d, *J* = 7.3 Hz, OBu), 31.89 (d, *J* = 6.8 Hz, OBu), 18.5 (OBu), 18.3 (OBu), 13.5 (OBu), 13.4 (OBu) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{50}H_{55}NaO_{12}P$: 901.3328, found: 901.3199. IR (neat) ν_{max} = 1743, 1724, 1251, 1027 cm^{-1}.

3.3.1.2 Synthesis of Arabinose Building Blocks

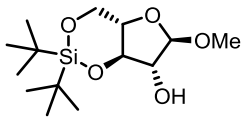
Methyl-1-α-L-arabinofuranoside (17)



Benzoyl protected arabinofuranoside **16**[128] (5.00 g, 10.5 mmol) was dissolved in a mixture of MeOH and DCM (2:1) and sodium methoxide (2.60 g, 47.2 mmol, 4.5 equiv) was added. The solution was stirred overnight at rt before it was neutralized by the addition of H^+-Amberlite resin. After filtration through a plug of cotton the solvents were removed *in vacuo* and the residue was purified through a short pad of silica gel (DCM/MeOH = 1:0 to 9:1) to give **17** (1.71 g, 10.4 mmol, 99%).

^{1}H NMR (400 MHz, CD_3OD) δ = 4.80 (s, 1H), 3.99 – 3.92 (m, 2H), 3.89 – 3.85 (m, 1H), 3.79 (dd, *J* = 11.9, 3.2 Hz, 1H), 3.68 (dd, *J* = 11.8, 5.3 Hz, 1H), 3.41 (s, 3H) ppm. The analytical data is in agreement with literature data.[220]

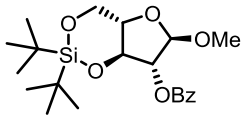
3,5-*O*-(Di-*tert*-butylsilanediyl)-1-methyl-α-L-arabinofuranoside (18)



Methyl-1-α-L-arabinofuranoside (1.71 g, 10.4 mmol) was dissolved in anhydrous DMF (15 mL) at -5 °C and di-*tert*-butylsilanediyl bis(trifluoromethanesulfonate) (5.24 mL, 15.6 mmol, 1.4 equiv) was added. After stirring the resulting solution for 5 min, 2,6-dimethylpyridine (4.24 mL, 36.4 mmol, 3.5 equiv) was added dropwise and the reaction was stirred at -5 °C for ten more minutes before it was quenched by the addition of MeOH. The reaction mixture was sequentially washed with 1 M HCl, sat. aq. $NaHCO_3$ solution and brine. Purification by silica gel chromatography (EtOAc/hex = 1:9) gave 1.83 g (6.00 mmol, 58%) of arabinofuranoside **18** as a colorless solid.

$[\alpha]_D^{25}$ = -51.1 (c 1.4, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.81 (d, *J* = 3.3 Hz, 1H, H-1), 4.38 – 4.29 (m, 1H, H-4), 4.12 (dt, *J* = 7.4, 3.8 Hz, 1H, H-2), 3.99 – 3.88 (m, 3H, H-3, H-5), 3.42 (s, 3H, OMe), 1.06 (s, 9H, *t*Bu), 0.99 (s, 9H, *t*Bu) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 109.0 (C-1), 81.8 (C-2), 81.7 (C-3), 73.8 (C-5), 67.6 (C-4), 56.2 (O*C*H_3), 27.5 (*t*Bu), 27.2 (*t*Bu), 22.8 (*C*$(CH_3)_3$), 20.2 (*C*$(CH_3)_3$) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{14}H_{28}NaO_5Si$: 327.1603, found: 327.1611. IR (neat) ν_{max} = 1086, 1026, 929, 805 cm^{-1}.

2-*O*-Benzoyl-3,5-*O*-(di-*tert*-butylsilanediyl)-1-methyl-α-L-arabinofuranoside (19)

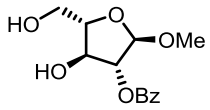


Silane-acetal protected arabinofuranoside **18** (1.83 g, 6.00 mmol) was dissolved in anhydrous pyridine (40 mL) and the resulting solution was stirred at 0 °C for ten minutes before benzoyl chloride (2.79 mL, 24.0 mmol, 4 equiv) was added. After the addition was complete the cooling bath was removed and the reaction mixture was stirred overnight. The reaction mixture was diluted with DCM, poured into ice-cold water and stirred for 20 min. The organic layer was separated and washed with sat. aq $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (EtOAc/hex 1:9) gave arabinofuranoside **19** as colorless solid (2.31 g, 5.66 mmol, 94%).

$[\alpha]_D^{25}$ = 14.7 (c 0.1, $CHCl_3$)^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.10 – 8.05 (m, 2H, Ar), 7.62 – 7.57 (m, 1H, Ar), 7.47 (dd, *J* = 10.6, 4.7 Hz, 2H, Ar), 5.32 (dd, *J* = 7.0, 2.3 Hz, 1H, H-2), 4.89 (d, *J* = 2.3 Hz, 1H, H-1), 4.43 (dd, *J* = 8.8, 4.7 Hz, 1H, H-5), 4.29 (dd, *J* = 9.4, 7.0 Hz, 1H, H-3), 4.12 – 3.96 (m, 2H, H-4 H-5), 3.43 (s, 3H, OMe), 1.06 (s, 9H, C(C*H*$_3$)), 1.01 (s, 9H C(C*H*$_3$)) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.0 (C=O), 133.4, 129.9, 128.6 (6 C, Ar), 107.7 (C-1), 83.3 (C-3), 80.2 (C-2), 73.5 (C-5), 67.6 (C-4), 55.9 (O*C*H_3), 27.5 (C(*C*H_3),

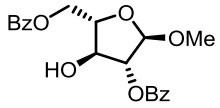
27.2 (C(*C*H_3), 22.8 (*C*(CH_3), 20.28 (*C*(CH_3). ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{21}H_{32}NaO_6Si$: 431.1860, found: 431.1934. IR (neat) ν_{max} = 1732, 1267, 1093, 717 cm^{-1}.

2-*O*-Benzoyl-1-methyl-α-L-arabinofuranoside (20)



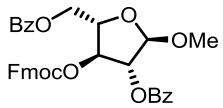
Arabinofuranoside **19** (2.31 g, 5.66 mmol) was stirred in a Sarstedt 50 mL plastic tube in a mixture of THF (30.0 mL) and py (5.00 mL) at 0 °C and a solution of HF·pyridine (0.80 mL, 6.23 mmol, 1.1 equiv) was added dropwise to the reaction mixture. The reaction was warmed up to rt and stirred for additional 3 h before it was poured into ethyl acetate and the resulting mixture was neutralized with sat. aq. $NaHCO_3$ solution. The organic phase was separated, dried over $MgSO_4$, and concentrated. Product **20** (1.08 g, 4.03 mmol, 71%) was directly used for subsequent reactions without further purification.
^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.04 – 8.00 (m, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 5.16 (s, 1H), 5.07 (s, 1H), 4.19 (b, 2H), 3.94 (d, *J* = 11.4 Hz, 1H), 3.80 (d, *J* = 11.7 Hz, 1H), 3.46 (s, 3H) ppm. NMR data is in agreement with literature data.[221]

2,6-*O*-Dibenzoyl-1-methyl-α-L-arabinofuranoside (21)



A solution of DEAD (0.99 mL, 6.04 mmol, 1.5 equiv) and benzoic acid (0.74 g, 6.04 mmol, 1 equiv) in anhydrous THF was added dropwise to a solution of **20** (1.08 g, 4.03 mmol) and triphenylphosphine (1.58 g, 6.04 mmol, 1 equiv) in anhydrous THF. The yellow color of the solution of DEAD and BzOH in THF disappears slowly upon addition. The reaction mixture was stirred at rt for 2 h. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography (EtOAc/hex = 1:3 to 1:1) to give **21** in 77% (1.15 g, 3.10 mmol) yield.
^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.05 – 8.00 (m, 4H), 7.63 – 7.58 (m, 1H), 7.55 – 7.50 (m, 1H), 7.47 – 7.42 (m, 2H), 7.35 (t, *J* = 7.8 Hz, 2H), 5.20 (s, 1H), 5.13 – 5.12 (m, 1H), 4.64 (dd, *J* = 11.9, 3.9 Hz, 1H), 4.53 (dd, *J* = 11.9, 5.5 Hz, 1H), 4.43 (td, *J* = 5.6, 4.0 Hz, 1H), 4.24 (dd, *J* = 5.1, 2.5 Hz, 1H), 3.48 (s, 3H) ppm. The analytical data is in agreement with literature data.[221]

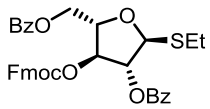
2,6-*O*-Dibenzoyl-3-*O*-fluorenylcarbonylmethoxy-1-methyl-α-L-arabinofuranoside (22)



21 (1.15 g, 2.95 mmol) was dissolved in anhydrous DCM (20 mL) under nitrogen atmosphere and anhydrous pyridine (2.38 mL, 29.5 mmol, 10 equiv) was added. The solution was stirred at rt for 10 min before FmocCl (1.53 g, 5.91 mmol, 2 equiv) was added. The reaction mixture was stirred at rt for 6 h and the solvent was evaporated. The residue was purified by silica gel chromatography (EtOAc/hex = 1:7 to 1:3) to give the Fmoc-protected product **22** (1.43 g, 2.40 mmol, 81%) as colorless solid.

$[\alpha]_D^{25}$ = +2.6 (c 1.0, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.02 (dd, *J* = 21.9, 7.9 Hz, 4H, Ar), 7.77 (d, *J* = 7.5 Hz, 2H, Ar), 7.64 – 7.54 (m, 3H, Ar), 7.51 (t, *J* = 7.5 Hz, 1H, Ar), 7.40 (q, *J* = 7.6 Hz, 4H, Ar), 7.31 (q, *J* = 7.4 Hz, 4H, Ar), 5.45 (s, 1H, H-2), 5.29 (d, *J* = 5.2 Hz, 1H, H-3), 5.14 (s, 1H, H-1), 4.78 (dd, *J* = 11.6, 3.1 Hz, 1H, H-5), 4.61 (dd, *J* = 12.0, 4.6 Hz, 1H. H-5), 4.56 – 4.51 (m, 1H, H-4), 4.50 – 4.37 (m, 2H, Fmoc), 4.26 (t, *J* = 7.4 Hz, 1H, Fmoc), 3.50 (s, 3H, OMe) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.3 (C=O), 154.6(C=O), 143.5, 143.3, 143.2, 141.4, 141.4, 133.6, 133.2, 130.0, 129.9, 128.6, 128.5, 128.1, 128.0, 127.4, 127.3, 127.3, 125.3, 125.3, 120.2, 120.2, 120.2 (22C, Ar), 106.8 (C-1), 82.1 (C-2), 80.8 (C-3), 80.3 (C-4), 70.6 (Fmoc), 63.6 (C-5), 55.1 (OMe), 46.8 (Fmoc) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{35}H_{30}NaO_9$: 617.1787, found: 617.1793. IR (neat) ν_{max} = 1725, 1254, 727, 709 cm^{-1}.

Ethyl-2,6-*O*-dibenzoyl-3-*O*-fluorenylcarbonylmethoxy-1-thio-α-L-arabinofuranoside (BB5)



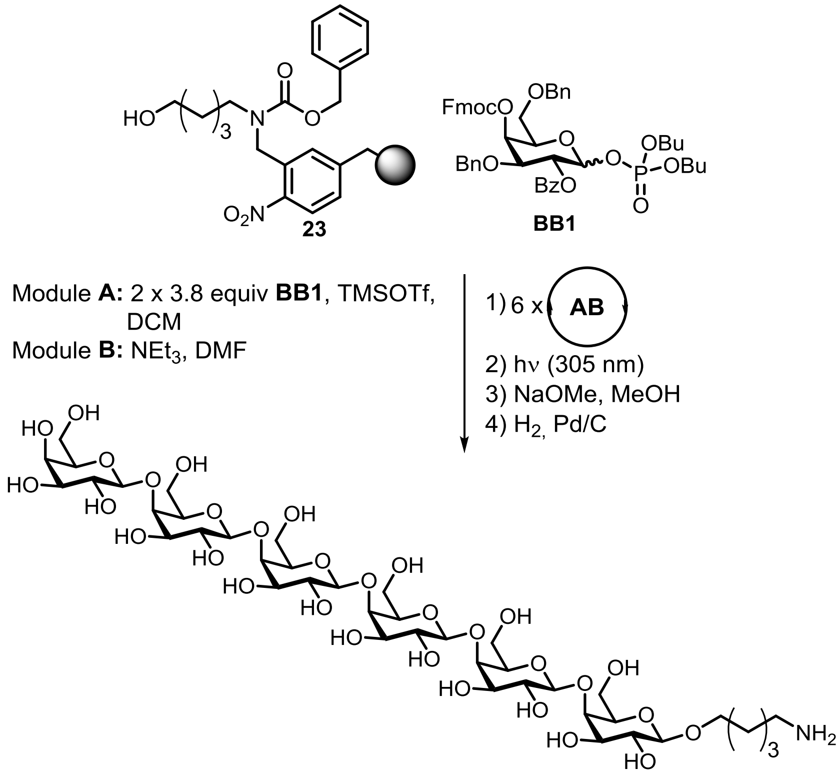
Arabinose derivative **22** (1.43 g, 2.40 mmol) was dissolved in anhydrous DCM (20 mL) and ethanethiol (0.25 mL, 3.36 mmol, 1.4 equiv) was added. The solution was cooled to 0 °C and boron trifluoride etherate (0.91 mL, 7.20 mmol, 3 eq.) was added dropwise. The reaction mixture was stirred for 5 h at 0°C, subsequently diluted with DCM, and washed with sat. aq. $NaHCO_3$ solution. The crude product was purified by silica column chromatography (hex/EtOAc 8:1) to give thioglycoside **BB5** as colorless solid in 76% yield (1.14 g, 1.83 mmol).

$[\alpha]_D^{25}$ = -37.1 (c 0.5, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.03 (dd, *J* = 16.0, 7.5 Hz, 4H, Ar), 7.77 (d, *J* = 7.5 Hz, 2H, Ar), 7.65 – 7.55 (m, 3H, Ar), 7.52 (t, *J* = 7.5 Hz, 1H, Ar), 7.40 (dd, *J* = 10.1, 5.4 Hz, 4H, Ar), 7.35 – 7.28 (m, 4H, Ar), 5.57 (s, 1H, H-1), 5.48 (s, 1H, H-2), 5.32 (d, *J* = 5.5 Hz, 1H, H-3), 4.79 – 4.62 (m, 2H, H-5), 4.49 – 4.36 (m, 3H, H-4, Fmoc),

4.27 (t, *J* = 7.6 Hz, 1H, Fmoc), 2.87 – 2.67 (m, 2H, SC*H*$_2$CH$_3$), 1.36 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.2 (C=O), 165.5 (C=O), 154.5 (C=O), 143.3, 143.1, 141.4,133.7, 133.2, 130.0, 129.8, 128.6, 128.5, 128.1, 127.4, 127.3, 125.3, 125.3, 120.2, 120.2 (22C, Ar), 88.04 (C-1), 82.9 (C-2), 81.1 (C-3), 80.3 (C-4), 70.7 (Fmoc), 63.3 (C-5), 46.8 (Fmoc), 25.4 S*C*H$_2$CH$_3$, 14.9 (SCH$_2$*C*H$_3$) ppm. m/z $[M+Na]^+$ calcd. for $C_{35}H_{30}NaO_9$: 635.1715, found: 617.1723. IR (neat) ν_{max} = 1721, 1244, 1094, 707 cm^{-1}.

3.3.2 Automated Glycan Assembly of Type-I Arabinogalactans

Aminopentyl-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (26)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

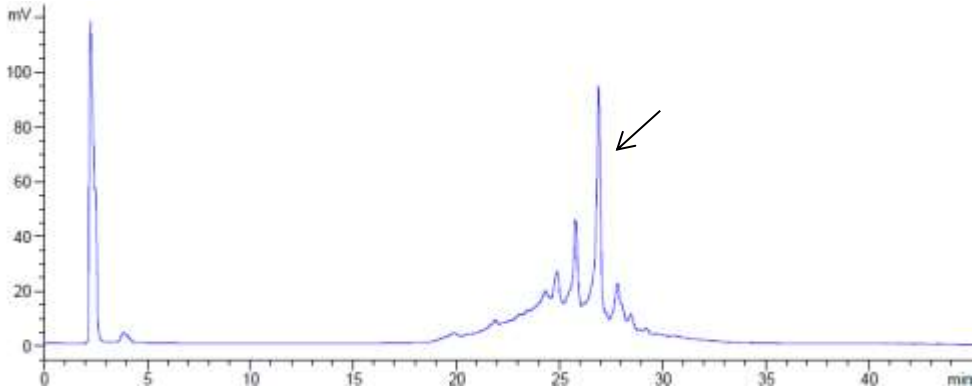
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected hexasaccharide **26**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

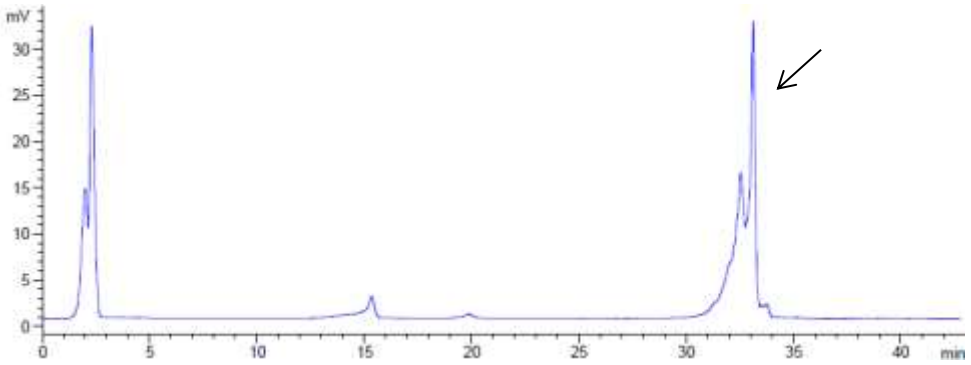
Crude NP-HPLC of protected hexasaccharide **26** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 30% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected hexasaccharide **26** was dissolved in THF (3 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The product was not soluble in MeCN/water and therefore not purified by reversed phase HPLC but instead used in the final deprotection step without purification.

Crude RP-HPLC of semi-protected hexasaccharide **26** (ELSD trace):



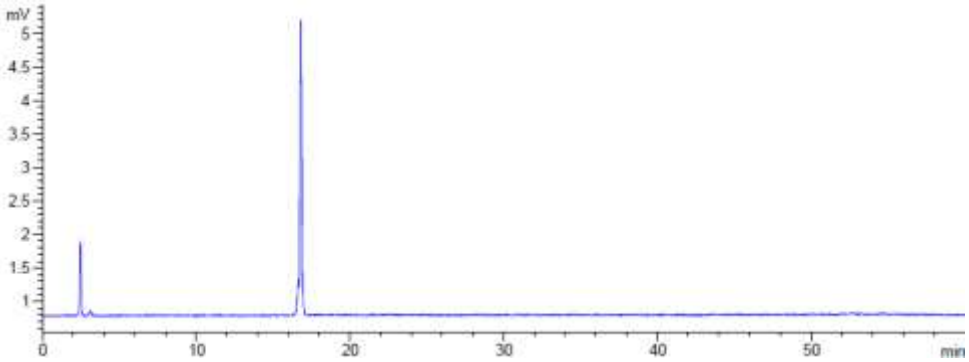
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 5 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight at rt. After filtration of the reaction mixture through a syringe filter the solvents were evaporated and the fully deprotected

hexasaccharide **26** was purified by reversed phase HPLC using a semi-preparative Hypercarb column (0.4 mg, 0.36 µmol, 2% over 15 steps, based on resin loading).

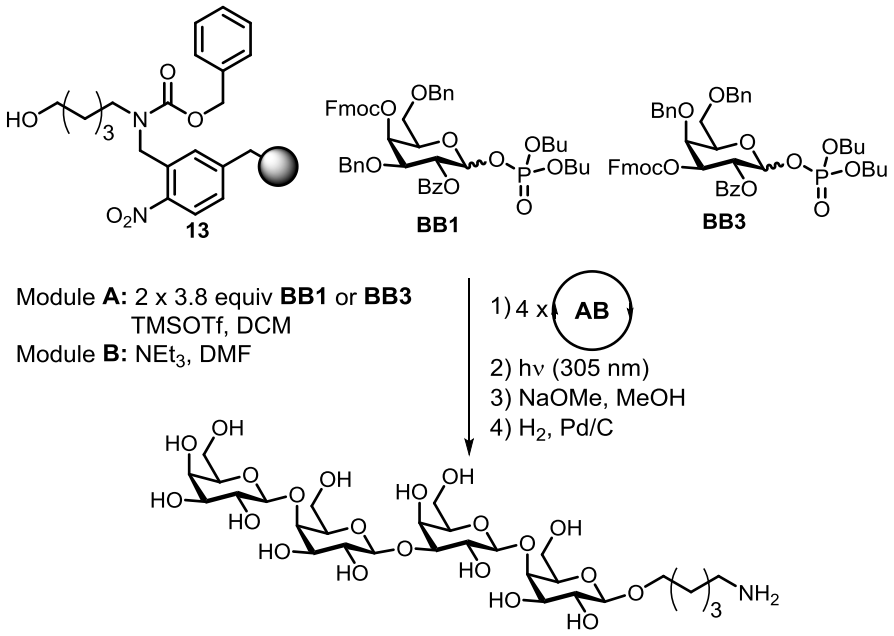
^{1}H NMR (600 MHz, D_2O) δ = 4.59 – 4.55 (m, 4H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.35 (d, *J* = 8.0 Hz, 1H), 4.10 (d, *J* = 3.2 Hz, 4H), 3.88 – 3.82 (m, 3H), 3.80 – 3.57 (m, 30H), 3.55 – 3.48 (m, 1H), 2.94 (t, *J* = 7.5 Hz, 2H), 1.61 (tt, *J* = 14.1, 7.2 Hz, 4H), 1.42 – 1.36 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O) δ = 104.3, 104.2, 102.6, 77.6, 77.5, 77.1, 75.1, 74.5, 74.4, 74.28, 74.22, 73.2, 72.7, 71.87, 71.80, 71.3, 71.2, 70.0, 68.6, 60.9, 60.7, 60.6, 60.5, 39.3, 28.1, 26.3, 22.0 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{41}H_{73}NNaO_{31}$: 1098.4064; found 1098.4043.

RP-HPLC of deprotected hexasaccharide **26** (ELSD trace):



RP-HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (27)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

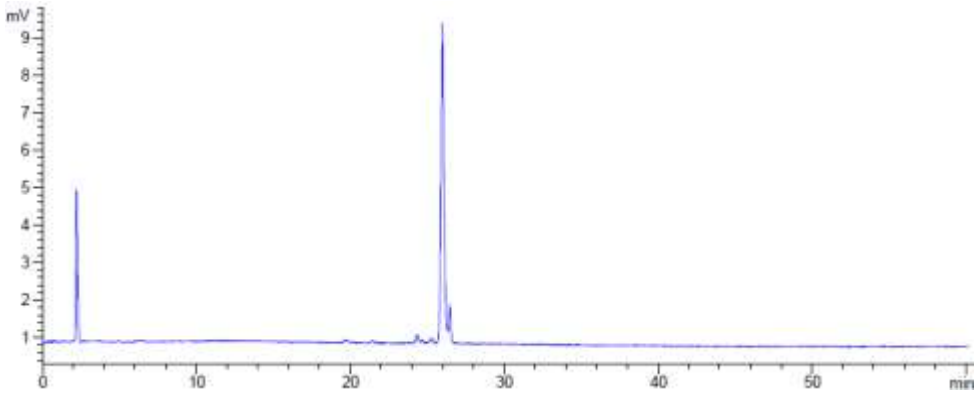
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected tetrasaccharide **27**.

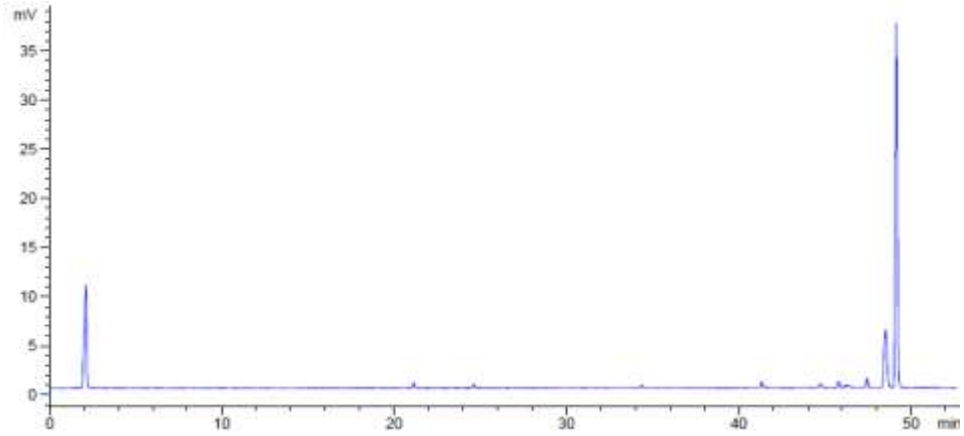
Crude NP-HPLC of protected terasaccharide **27** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The crude protected tetrasaccharide **27** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.13 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected tetrasaccharide **27**.

Crude RP-HPLC of semi-protected tetrasaccharide **27** (ELSD trace):

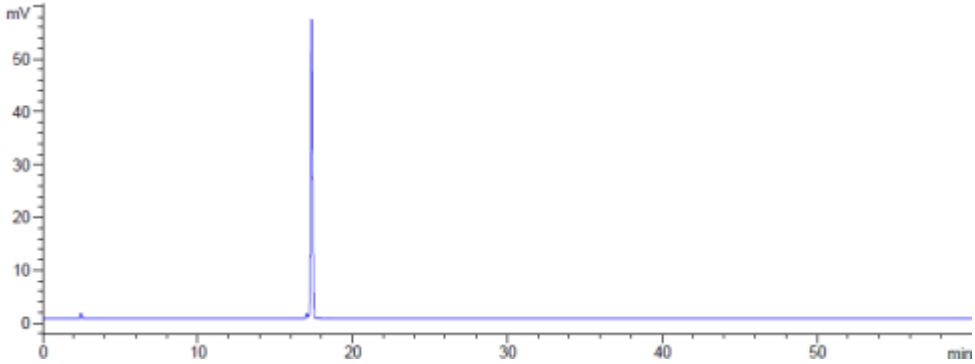


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight at rt. After filtration of the reaction mixture through a syringe filter the solvents were evaporated and the fully deprotected tetrasaccharide **27** was purified by reversed phase HPLC using a semi-preparative Hypercarb column (2.0 mg, 2.66 µmol, 16% over 11 steps, based on resin loading).

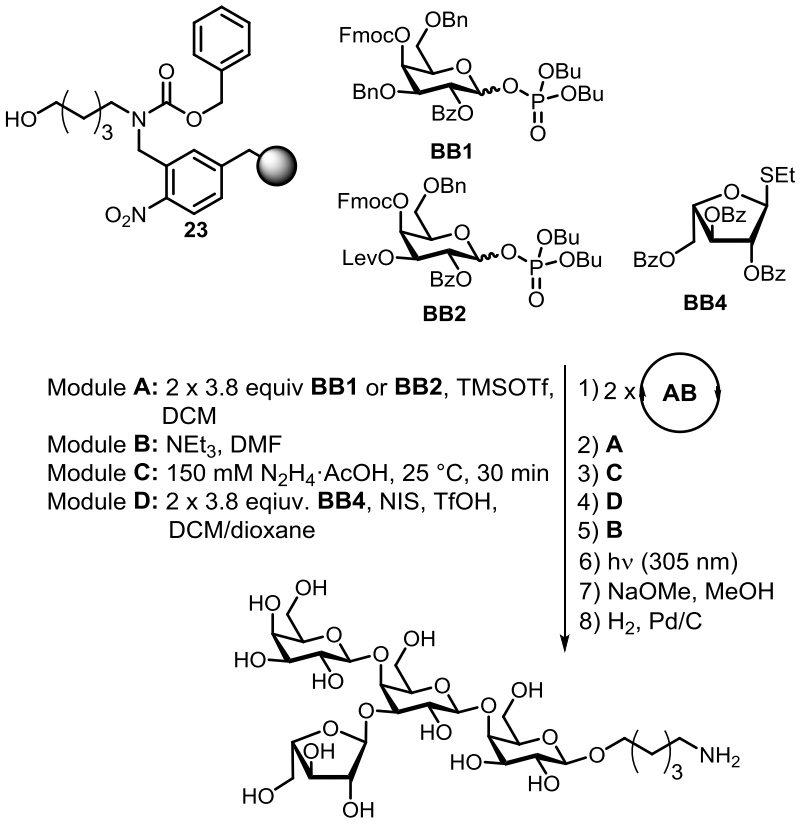
^{1}H NMR (600 MHz, D_2O) δ = 4.48 – 4.44 (m, 2H), 4.41 (d, *J* = 7.8 Hz, 1H), 4.23 (d, *J* = 7.9 Hz, 1H), 3.99 (t, *J* = 3.6 Hz, 2H), 3.96 (d, *J* = 3.2 Hz, 1H), 3.76 – 3.71 (m, 2H), 3.68 – 3.63 (m, 4H), 3.61 – 3.46 (m, 16H), 3.39 (ddd, *J* = 9.9, 7.9, 3.7 Hz, 1H), 2.84 – 2.80 (m, 2H), 1.54 – 1.45 (m, 4H), 1.30 – 1.24 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 99.5, 99.3, 97.9, 77.4, 72.7, 72.4, 70.4, 70.0, 69.4, 68.6, 68.2, 68.0, 66.7, 66.6, 65.9, 65.2, 63.8, 63.7, 56.2, 56.2, 55.7, 34.6, 23.4, 21.6, 17.3 ppm. ESI-HRMS**:** m/z $[M+Na]^+$ calcd. for $C_{29}H_{53}NNaO_{21}$: 774.3007; found: 774.2991.

RP-HPLC of deprotected tetrasaccharide **27** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl β-D-galactopyranosyl-(1→4)-3-*O*-[α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (28)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB2**, TMSOTf, DCM, 2 x 35 min, -30 °C to -10 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

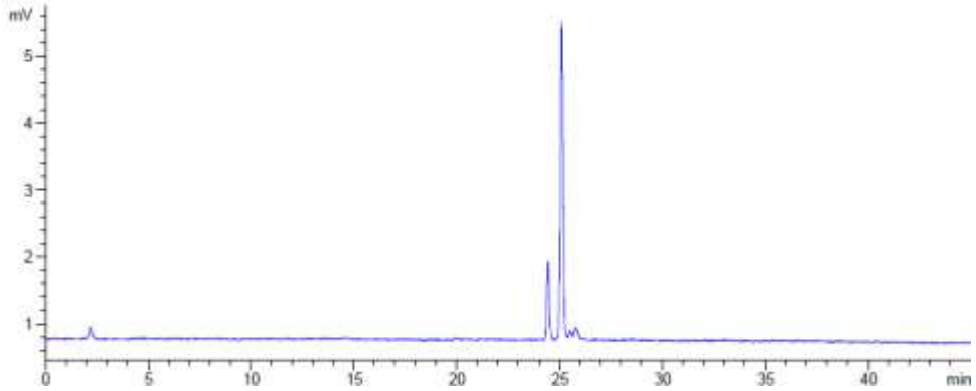
Module **C** (150 mM N_2H_4·AcOH, 3 x 30 min, 25 °C)

Module **D** (2 x 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected tetrasaccharide **28**.

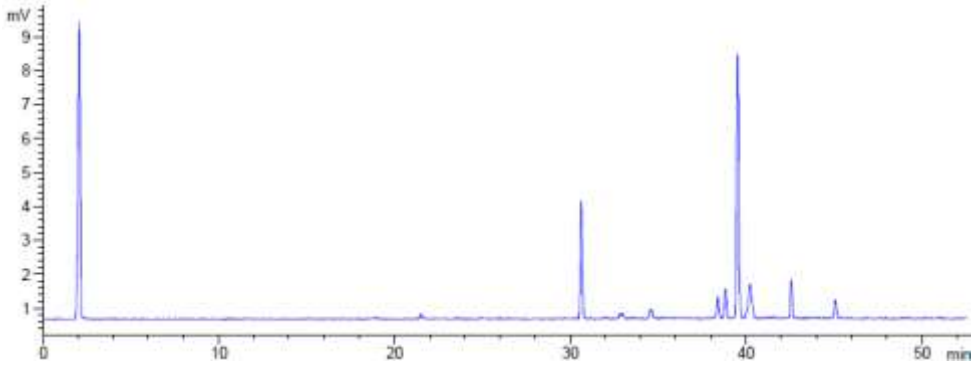
Crude NP-HPLC of protected terasaccharide **28** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected tetrasaccharide **28** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The semi-protected tetrasaccharide was purified by semi-preparative RP HPLC (C5 column).

Crude RP-HPLC of semi-protected tetrasaccharide **28** (ELSD trace):

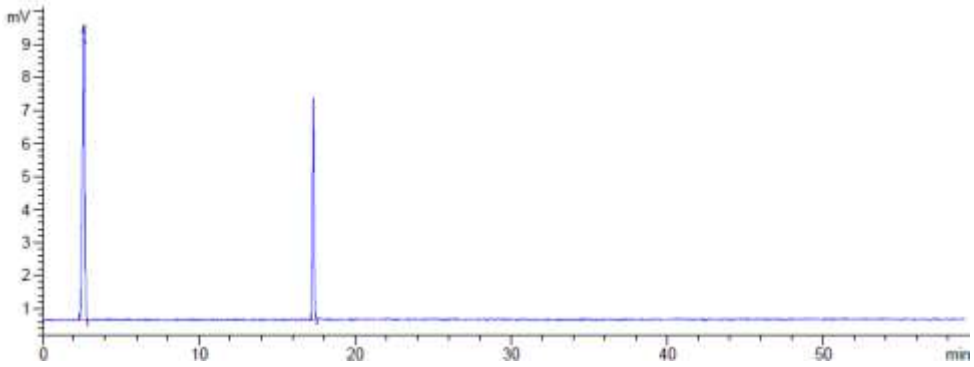


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide. The compound was purified once more after hydrogenation by filtration through a short C18 SepPak column (100% H_2O) to yield the deprotected tetrasaccharide **28** (0.8 mg, 1.04 µmol, 6%).

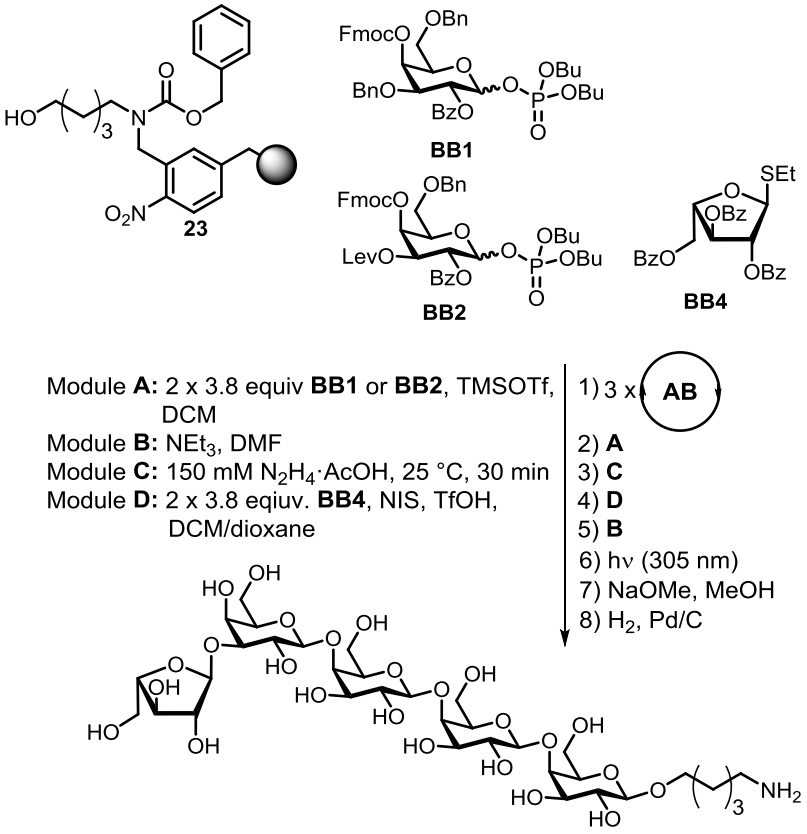
^{1}H NMR (600 MHz, D_2O) δ = 5.41 (s, 1H), 4.87 (s, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.49 (s, 1H), 4.39 – 4.30 (m, 3H), 4.15 – 4.05 (m, 3H), 4.03 – 3.78 (m, 17H), 3.77 – 3.69 (m, 1H), 3.15 (t, *J* = 7.4 Hz, 2H), 1.88 – 1.80 (m, 4H), 1.66 – 1.58 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 107.1, 102.1, 100.7, 100.3, 82.0, 78.9, 78.0, 73.9, 72.8, 72.2, 72.0, 71.1, 70.5, 68.9, 67.8, 66.4, 59.0, 58.8, 58.3, 46.8, 37.2, 25.9, 19.8 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{28}H_{51}NNaO_{20}$: 744.2902; found 744.2856.

RP-HPLC of the deprotected tetrasaccharide **28** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-3-*O*-[α-L-arabinofuranosyl]-β-D-galactopyranoside (29)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

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Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB2**, TMSOTf, DCM, 2 x 35 min, -30 °C to -10 °C)

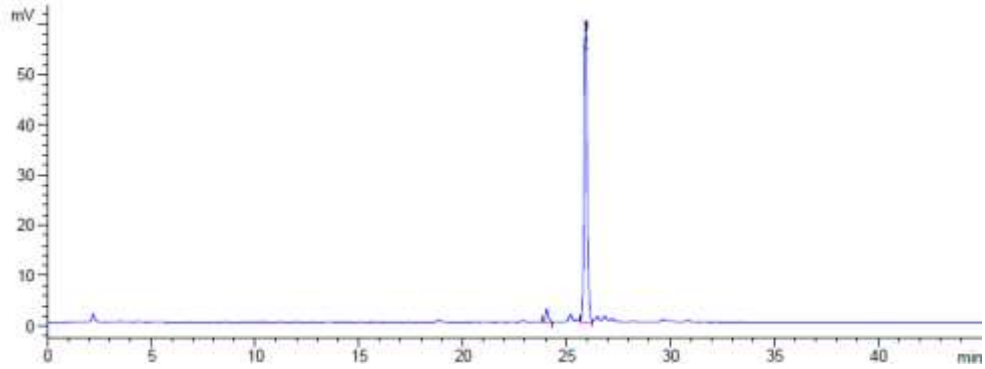
Module **C** (150 mM N_2H_4·AcOH, 3 x 30 min, 25 °C)

Module **D** (2 x 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected pentasaccharide **29**.

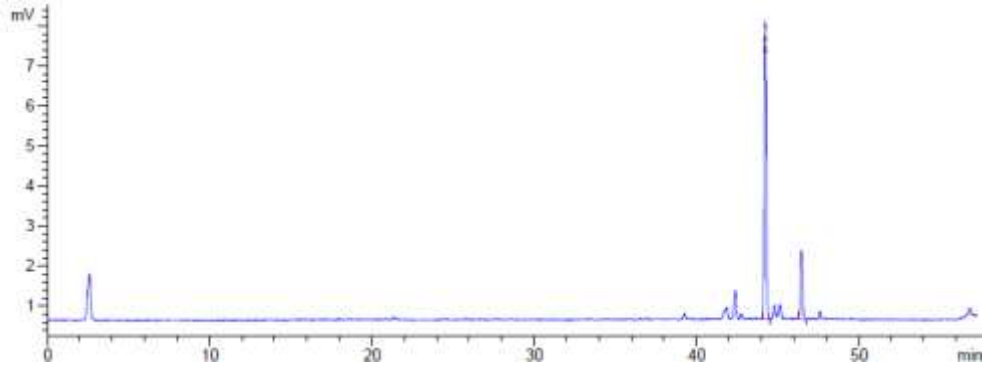
Crude NP-HPLC of protected pentasaccharide **29** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected pentasaccharide **29** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The semi-protected pentasaccharide was purified by semi-preparative RP HPLC (C5 column).

Crude RP-HPLC of semi-protected pentasaccharide **29** (ELSD trace):

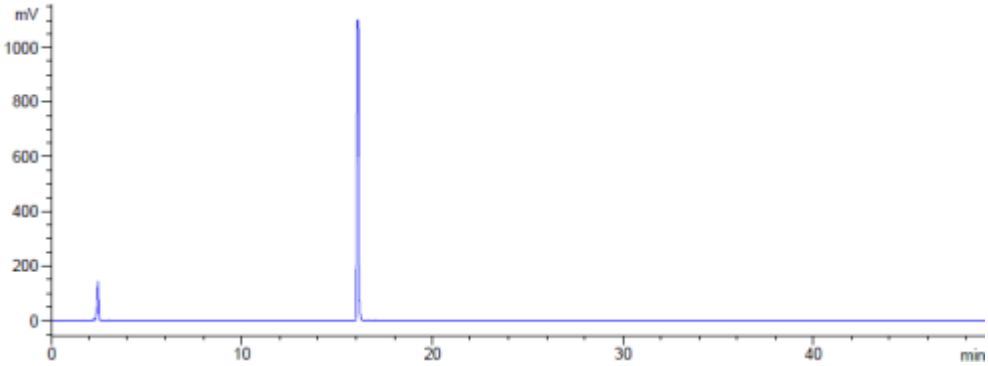


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide. The compound was purified once more after hydrogenation by filtration through a C18 SepPak column (100% water) to yield the deprotected pentasaccharide **29** (1.1 mg, 1.23 µmol, 7%).

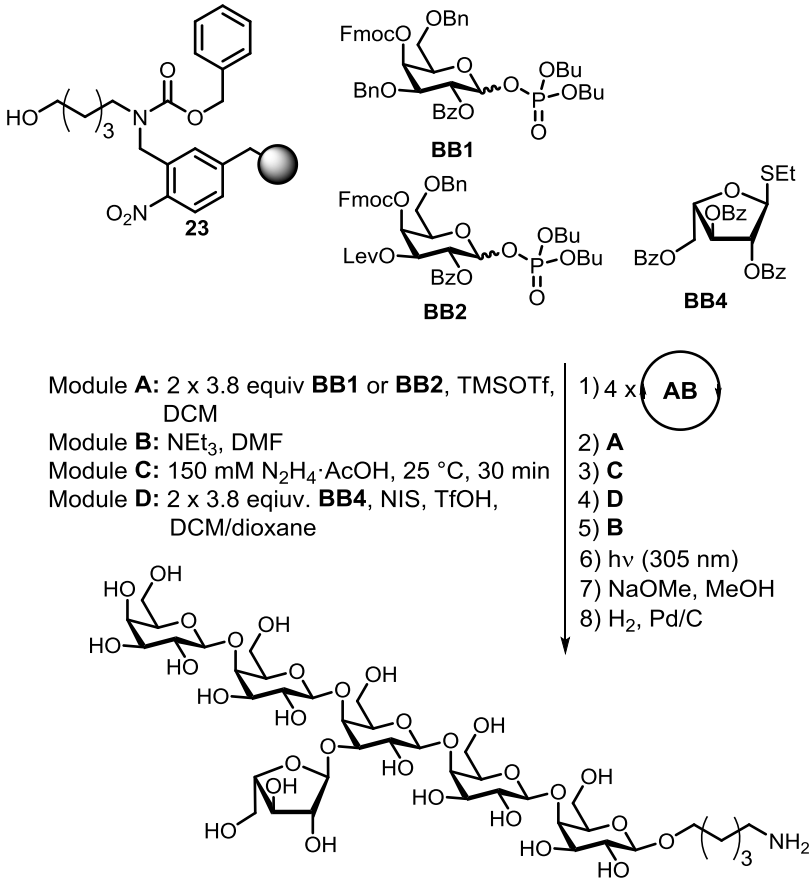
^{1}H NMR (600 MHz, D_2O) δ = 5.41 (s, 2H), 4.85 – 4.78 (m, 3H), 4.59 (dd, *J* = 7.9, 1.2 Hz, 1H), 4.38 (dt, *J* = 3.0, 1.5 Hz, 1H), 4.37 – 4.32 (m, 3H), 4.32 – 4.28 (m, 1H), 4.26 (s, 1H), 4.13 – 4.07 (m, 2H), 4.04 – 3.81 (m, 21H), 3.77 – 3.72 (m, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 1.89 – 1.81 (m, 4H), 1.67 – 1.59 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 106.9, 102.1, 102.0, 101.9, 100.4, 81.6, 79.0, 77.9, 75.5, 75.3, 75.1, 74.3, 72.7, 72.3, 72.2, 72.0, 71.0, 70.9, 69.6, 69.0, 68.3, 67.7, 66.2, 58.9, 58.6, 58.5, 58.4, 58.3, 37.1, 25.9, 24.1, 19.8 ppm. ESI-HRMS**:** m/z $[M+Na]^+$ calcd. for $C_{34}H_{61}NNaO_{25}$: 906,3425; found 906.3387.

RP-HPLC of deprotected tetrasaccharide **29** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-3-*O*-[α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (30)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB2**, TMSOTf, DCM, 2 x 35 min, -30 °C to -10 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

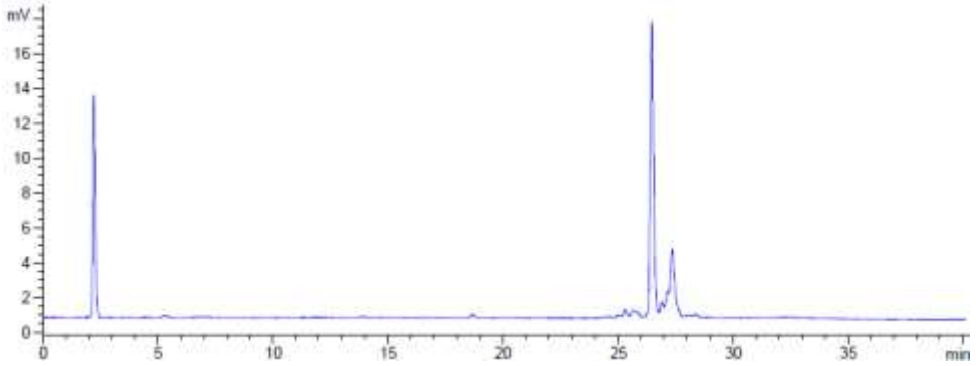
Module **C** (150 mM N_2H_4·AcOH, 3 x 30 min, 25 °C)

Module **D** (2 x 3.8 equiv **BB4** NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected hexasaccharide **30**.

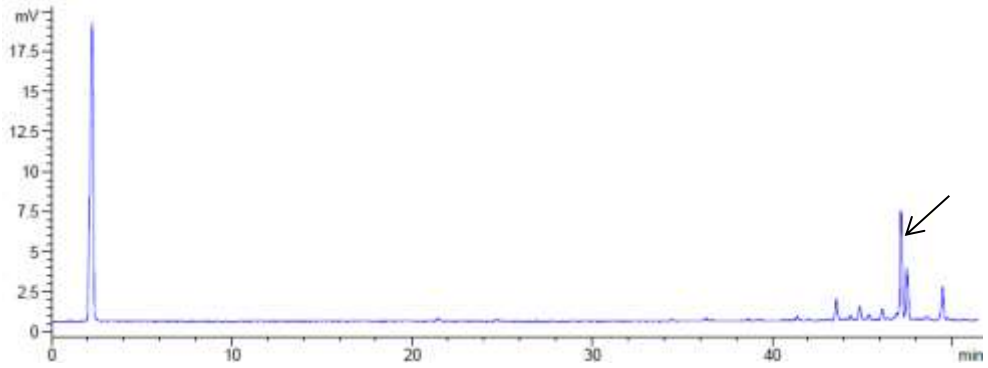
Crude NP-HPLC of protected hexasaccharide **30** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected hexasaccharide **30** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The semi-protected hexasaccharide was purified by semi-preparative RP HPLC (C5 column).

Crude RP-HPLC of semi-protected hexasaccharide **30** (ELSD trace):



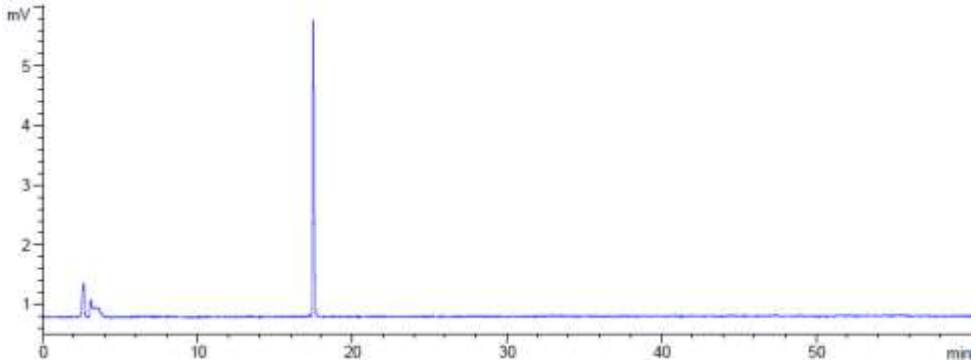
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide. The compound was purified once more after hydrogenation by RP HPLC using a semi-preparative Hypercarb column to yield the deprotected hexasaccharide **30** (0.8 mg, 0.79 µmol, 5%).

^{1}H NMR (700 MHz, D_2O) δ = 5.48 (d, *J* = 1.4 Hz, 1H), 4.70 (d, *J* = 8.0 Hz, 1H), 4.68 (d, *J* = 7.8 Hz, 1H), 4.64 (d, *J* = 7.9 Hz, 1H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H),

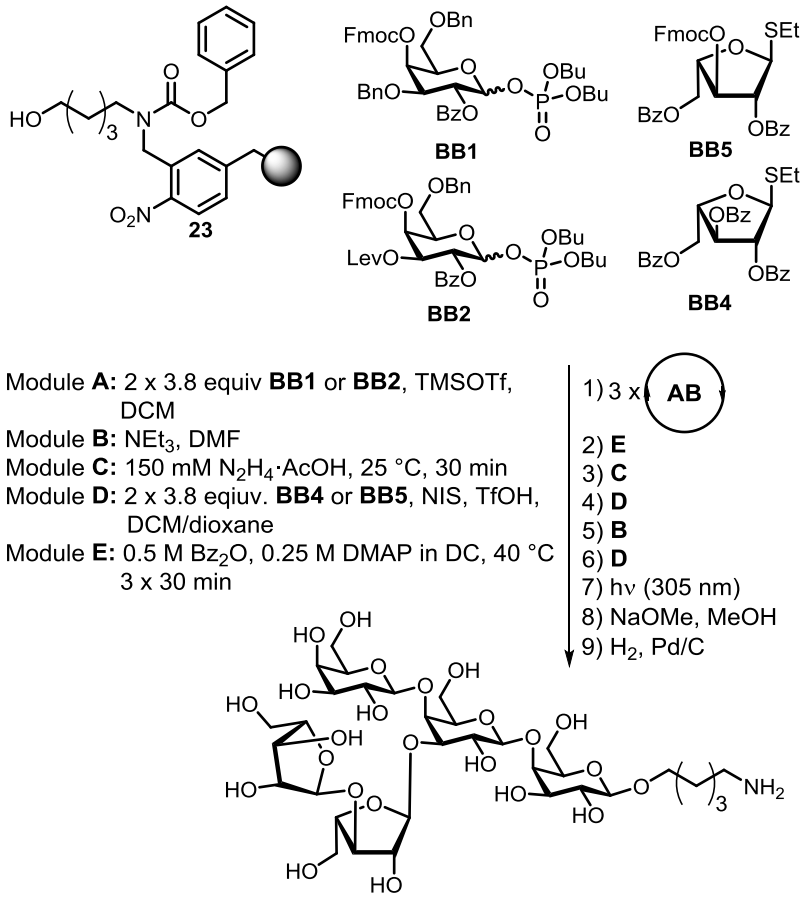
4.22 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.21 – 4.16 (m, 4H), 4.08 (td, *J* = 6.1, 3.4 Hz, 1H), 3.97 – 3.89 (m, 4H), 3.86 – 3.64 (m, 25H), 3.60 – 3.56 (m, 2H), 3.01 (t, *J* = 7.5 Hz, 2H), 1.73 – 1.65 (m, 4H), 1.49 – 1.43 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 107.2, 102.1, 102.0, 100.7, 100.4, 82.1, 78.9, 78.1, 75.7, 75.5, 74.9, 74.0, 72.9, 72.3, 72.2, 72.1, 72.0, 71.0, 70.9, 70.5, 69.7, 69.3, 69.1, 69.0, 68.8, 67.7, 66.4, 59.1, 58.7, 58.5, 58.4, 58.4, 58.3, 37.1, 25.9, 24.2, 19.8 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{40}H_{72}NNaO_{30}$: 1046.4139; found 1046.4105.

RP-HPLC of deprotected hexasaccharide **30** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl β-D-galactopyranosyl-(1→4)-3-*O*-[3-*O*-[α-L-arabinofuranosyl]-α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (31)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB2**, TMSOTf, DCM, 2 x 35 min, -30 °C to -10 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv **BB1**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (0.5 M Bz_2O, 0.25 M DMAP in DCE, 3 x 30 min, 40 °C)

Module **C** (150 mM N_2H_4·AcOH, 3 x 30 min, 25 °C)

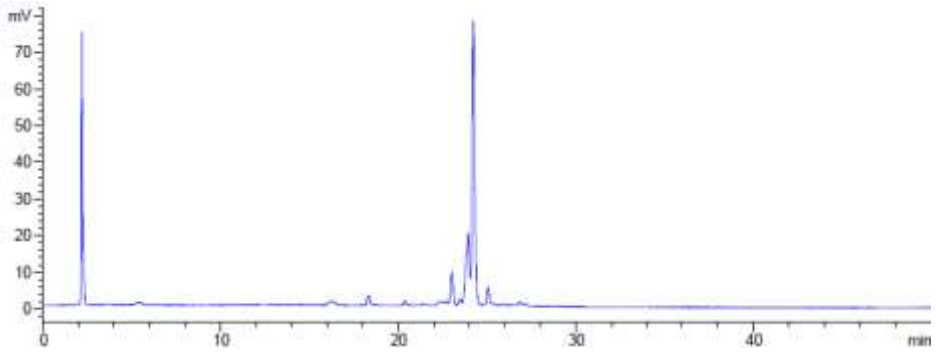
Module **D** (2 x 3.8 equiv **BB5**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected pentasaccharide **31**.

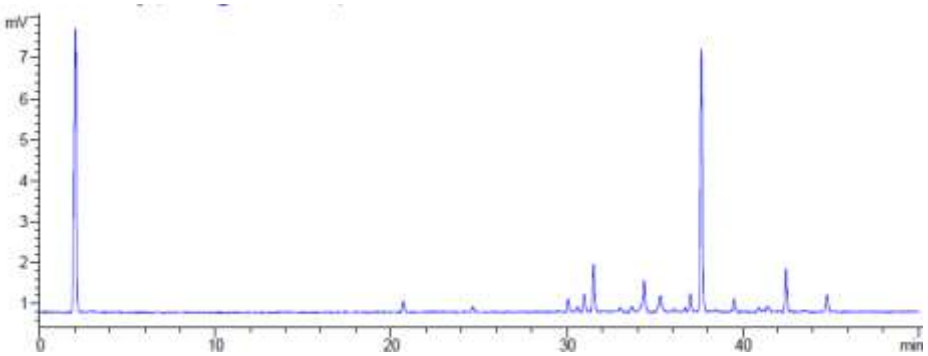
Crude NP-HPLC of protected pentasaccharide **31** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected pentasaccharide **31** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.6 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The semi-protected pentasaccharide was purified by semi-preparative RP HPLC (C5 column).

Crude RP-HPLC of semi-protected pentasaccharide **31** (ELSD trace):



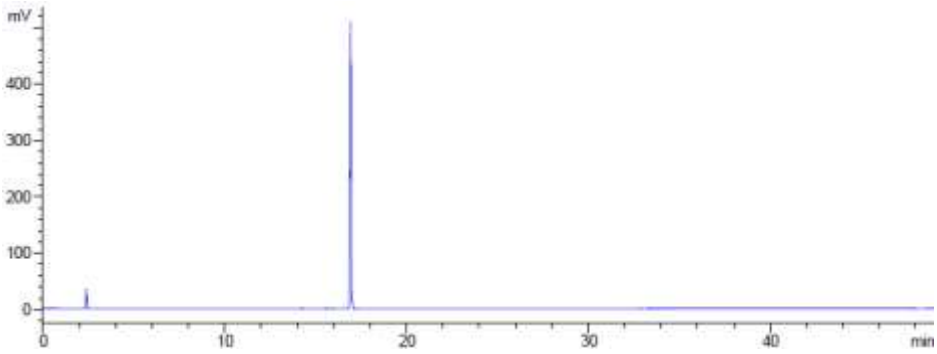
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected pentasaccharide. The compound was purified once more after hydrogenation by filtration through a short C18 SepPak column (100% H_2O) to yield the deprotected pentasaccharide **31** (1.1 mg, 1.31 µmol, 8%).

^{1}H NMR (600 MHz, D_2O) δ = 5.42 (s, 1H), 5.36 (s, 1H), 4.89 – 4.86 (m, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.55 (dd, *J* = 2.9, 1.4 Hz, 1H), 4.52 (s, 1H), 4.39 – 4.36 (m, 1H), 4.34 (d, *J* = 3.0 Hz, 1H), 4.28 (dd, *J* = 3.3, 1.5 Hz, 1H), 4.26 (dd, *J* = 6.6, 3.2 Hz, 1H), 4.19 (td, *J* = 6.1,

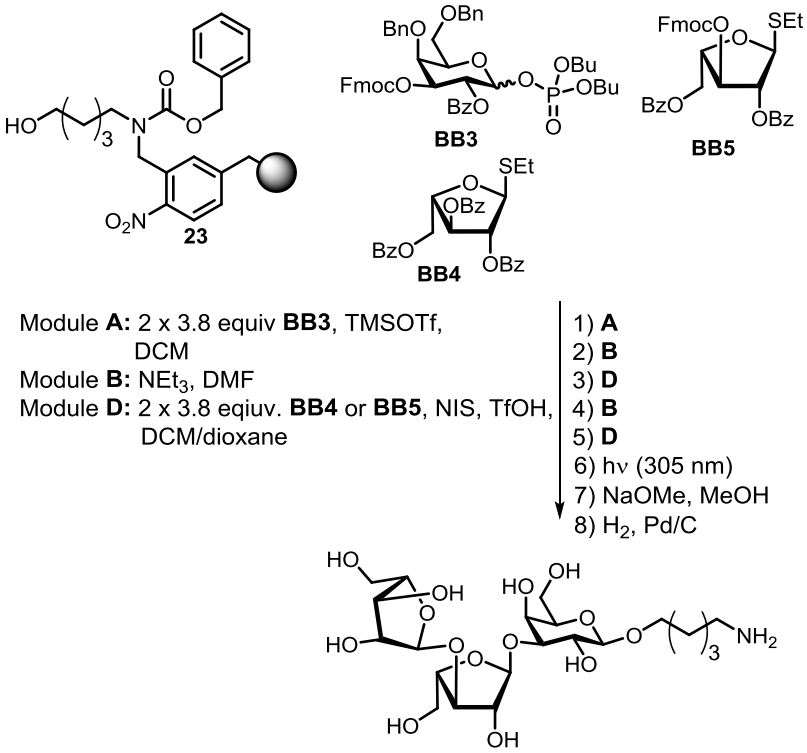
3.1 Hz, 1H), 4.14 – 4.07 (m, 2H), 4.05 (dd, *J* = 9.3, 3.2 Hz, 1H), 4.03 – 3.81 (m, 19H), 3.73 (ddd, *J* = 17.6, 9.8, 7.9 Hz, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 1.90 – 1.81 (m, 4H), 1.66 – 1.59 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 107.4, 104.5, 102.2, 100.3, 100.1, 81.5, 80.3, 79.3, 79.2, 77.9, 77.7, 75.8, 74.2, 72.9, 72.4, 72.0, 71.1, 70.4, 69.0, 68.8, 67.7, 66.4, 58.8, 58.4, 58.3, 37.1, 25.9, 24.2, 19.8 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{33}H_{59}NNaO_{24}$: 876.3324; found 876.3301.

RP-HPLC of deprotected pentasaccharide **31** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl α-L-arabinofuranosyl-(1→3)-α-L-arabinofuranosyl-(1→3)-β-D-galactopyranoside (32)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

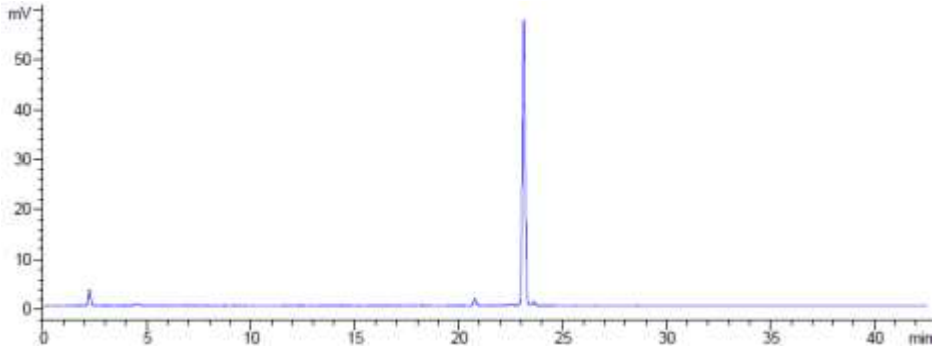
Module **D** (2 x 3.8 equiv **BB5**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected trisaccharide **32**.

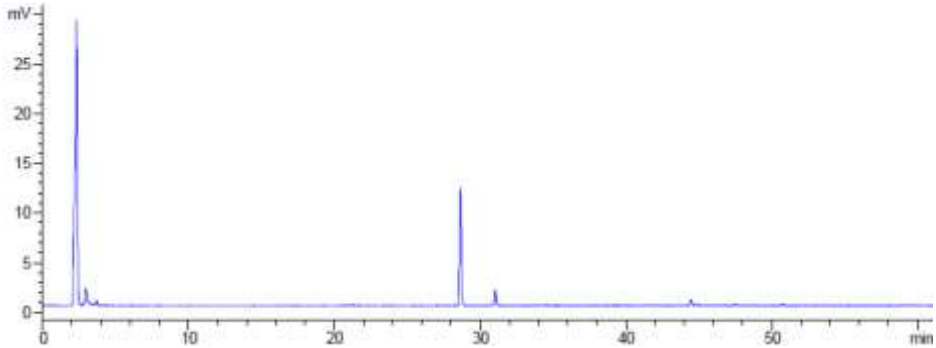
Crude NP-HPLC of protected trisaccharide **32** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected trisaccharide **32** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The semi-protected trisaccharide was purified by semi-preparative RP HPLC (C5 column).

Crude RP-HPLC of semi-protected trisaccharide **32** (ELSD trace):

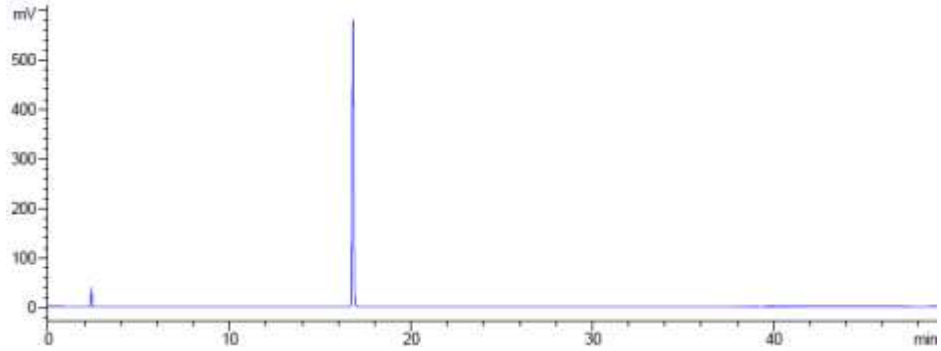


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected trisaccharide. The compound was purified once more after hydrogenation by RP HPLC using a semi-preparative Hypercarb column yielding the deprotected trisaccharide **32** (1.5 mg, 2.83 µmol, 17%).

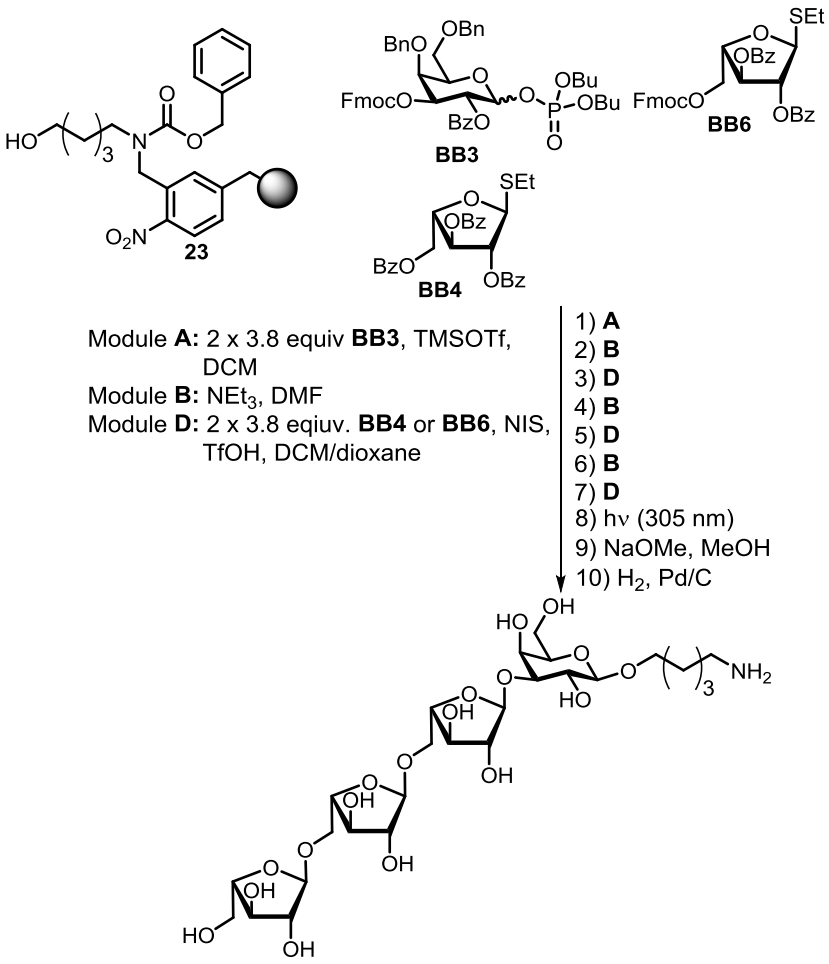
^{1}H NMR (600 MHz, D_2O) δ = 5.26 (s, 1H), 5.19 (s, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.37 (d, *J* = 1.3 Hz, 1H), 4.23 (td, *J* = 5.8, 3.1 Hz, 1H), 4.14 – 4.12 (m, 1H), 4.10 (d, *J* = 3.2 Hz, 1H), 4.05 (dd, *J* = 5.9, 2.6 Hz, 2H), 3.98 – 3.93 (m, 2H), 3.86 (ddd, *J* = 25.8, 12.3, 3.1 Hz, 2H), 3.80 – 3.68 (m, 7H), 3.62 (dd, *J* = 9.7, 8.1 Hz, 1H), 3.02 (t, *J* = 7.5 Hz, 2H), 1.75 – 1.65 (m, 4H), 1.51 – 1.44 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 111.8, 109.7, 105.0, 86.7, 85.5, 84.7, 83.6, 82.6, 82.3, 79.0, 77.56, 72.53, 72.4, 71.0, 63.7, 63.6, 63.4, 41.9, 30.7, 28.9, 24.6 ppm. ESI-HRMS**:** m/z $[M+Na]^+$ calcd. for $C21H_{39}NNaO_{14}$: 552.2268; found 552.2240.

RP-HPLC of deprotected trisaccharide **32** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→3)-β-D-galactopyranoside (33)



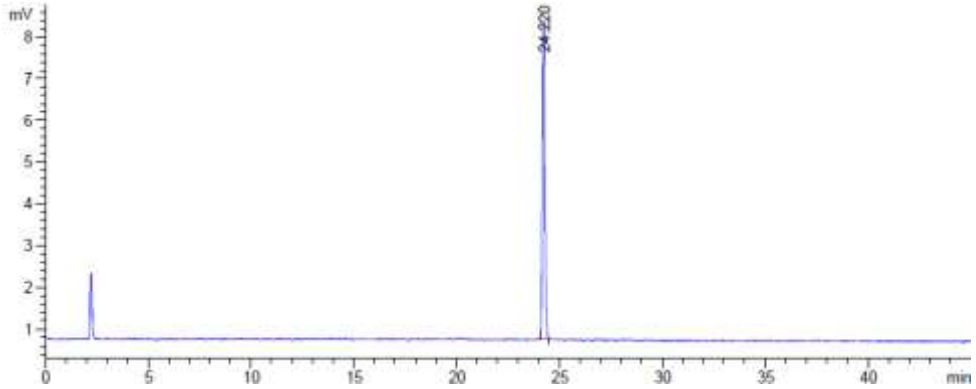
Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 3.8 equiv **BB6**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **D** (2 x 3.8 equiv **BB6**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **D** (2 x 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane, 2 x 45 min, -40 °C to -20 °C)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded protected tetrasaccharide **33**.

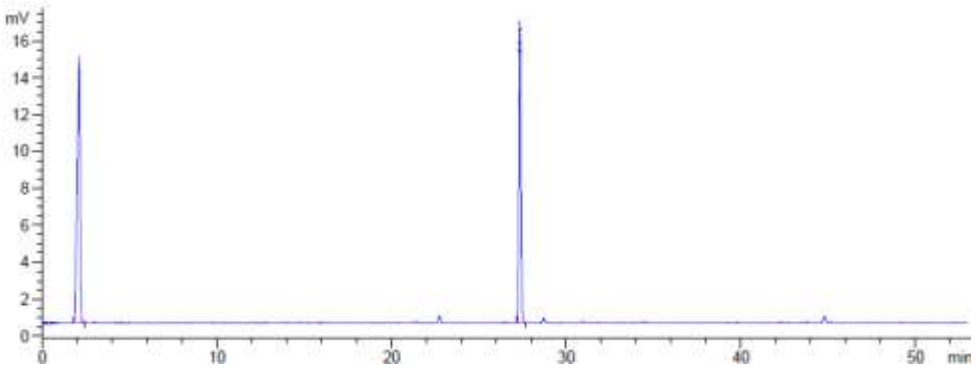
Crude NP-HPLC of protected tetrasaccharide **33** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected tetrasaccharide **33** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.54 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The semi-protected tetrasaccharide was purified by semi-preparative RP HPLC (C5 column).

Crude RP-HPLC of semi-protected tetrasaccharide **33** (ELSD trace):

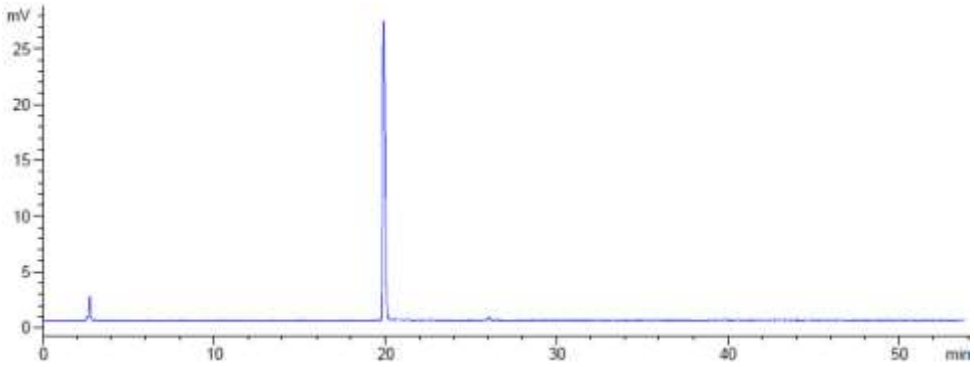


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide **33** in 38% yield (4.2 mg, 6.35 µmol).

^{1}H NMR (600 MHz, D_2O) δ = 5.40 (s, 1H), 5.24 (s, 2H), 4.61 (d, *J* = 7.9 Hz, 1H), 4.43 – 4.38 (m, 1H), 4.38 – 4.35 (m, 2H), 4.28 (s, 2H), 4.26 – 4.23 (m, 2H), 4.18 – 4.15 (m, 2H), 4.13 – 4.08 (m, 2H), 4.06 – 4.02 (m, 2H), 4.00 – 3.83 (m, 9H), 3.77 (t, *J* = 8.9 Hz, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 1.91 – 1.78 (m, 4H), 1.67 – 1.58 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 107.2, 105.4, 105.3, 100.4, 81.9, 80.2, 80.1, 79.1, 78.8, 78.7, 78.3, 74.6, 74.6, 74.4, 72.9, 67.8, 67.8, 66.4, 64.8, 64.7, 59.1, 58.8, 37.3, 26.0, 24.3, 20.0 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{26}H_{47}NNaO_{18}$: 684.2690; found 684.2670.

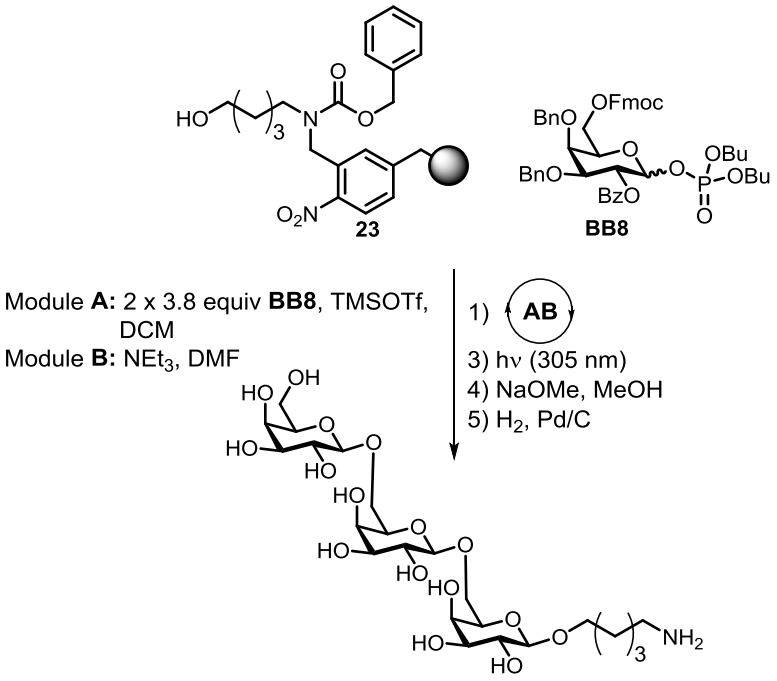
RP-HPLC of deprotected tetrasaccharide **33** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

3.3.3 Automated Glycan Assembly of Type-II Arabinogalactans

Aminopentyl-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (41)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

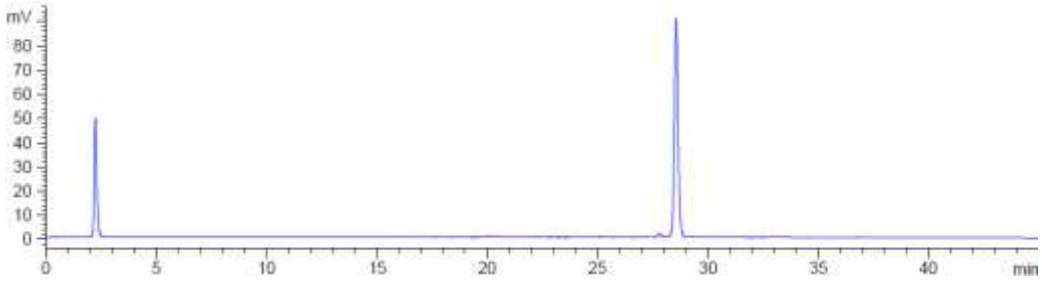
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected trisaccharide **41**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

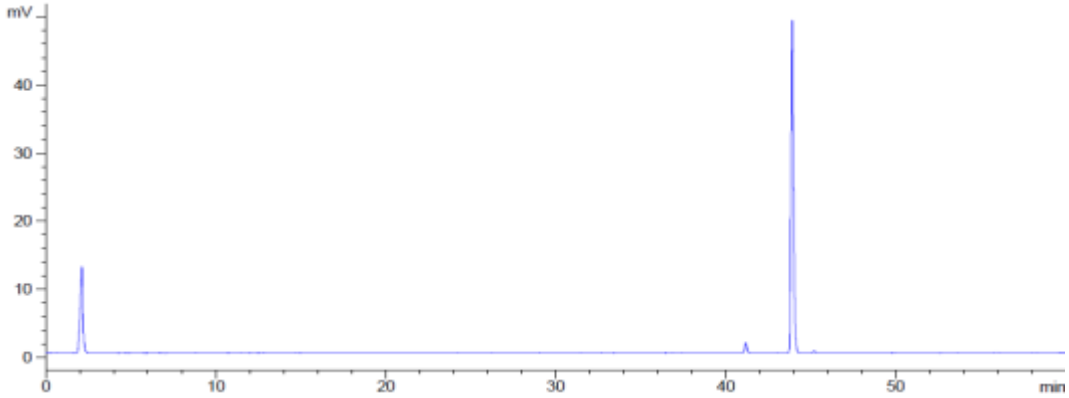
Crude NP-HPLC of protected trisaccharide **41** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected trisaccharide **41** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected trisaccharide.

Crude RP-HPLC of semi-protected trisaccharide **41** (ELSD trace):



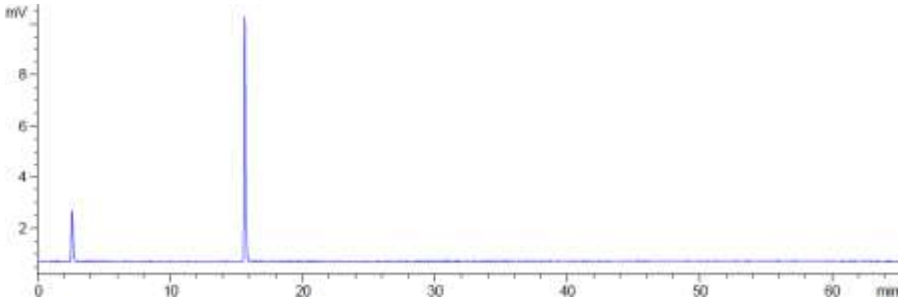
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 8 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected trisaccharide **41** (4.0 mg, 6.72 µmol, 39% over 9 steps, based on resin loading).

^{1}H NMR (700 MHz, D_2O) δ = 4.64 (d, *J* = 7.9 Hz, 1H), 4.62 (d, *J* = 7.8 Hz, 1H), 4.58 (d, *J* = 7.9 Hz, 1H), 4.23 – 4.21 (m, 2H), 4.15 – 4.05 (m, 8H), 3.98 – 3.91 (m, 2H), 3.88 – 3.81 (m, 5H), 3.72 – 3.66 (m, 3H), 3.18 (t, *J* = 7.3 Hz, 2H), 1.89 – 1.82 (m, 4H), 1.65 – 1.60 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O) δ = 101.1, 101.1, 100.5, 72.9, 71.5, 70.5, 70.4, 70.4,

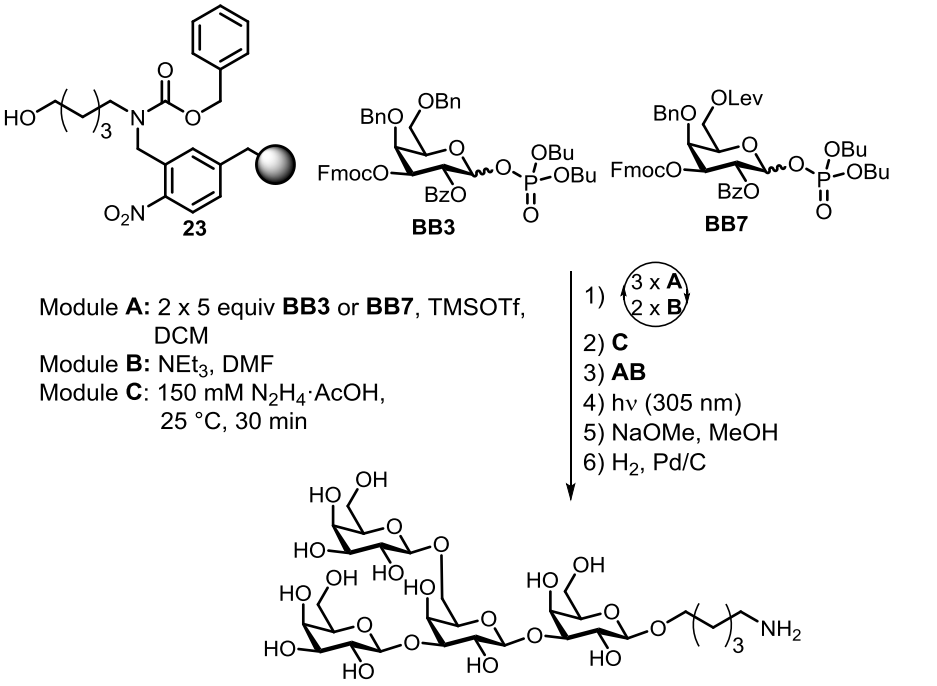
68.5, 68.5, 67.9, 67.0, 66.9, 66.4, 66.4, 58.8, 37.1, 25.9, 24.2, 19.8 ppm. HRMS: m/z $[M+H]^+$ calcd. for $C_{23}H_{43}NO_{16}$: 590.2660, found: 590.2710

RP-HPLC of deprotected trigalactoside **41** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (42)



Linker functionalized resin **23** (43 mg, 13.7 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 5 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 5 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 5 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

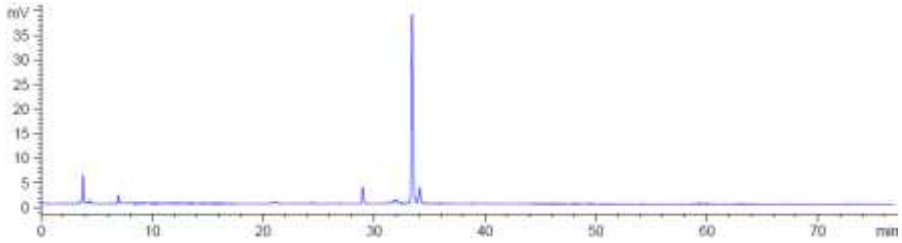
Module **C** (3 cycles)

Module **A** (2 x 5 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **42**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

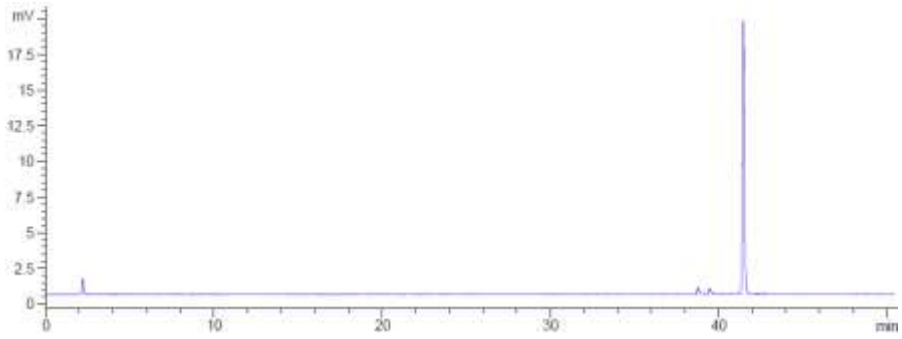
Crude NP-HPLC of protected tetrasaccharide **42** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected, branched tetrasaccharide **42** was dissolved in THF (5 mL) and NaOMe (0.5 mL 0.5 M in MeOH) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of semi-protected trisaccharide **42** (ELSD trace):

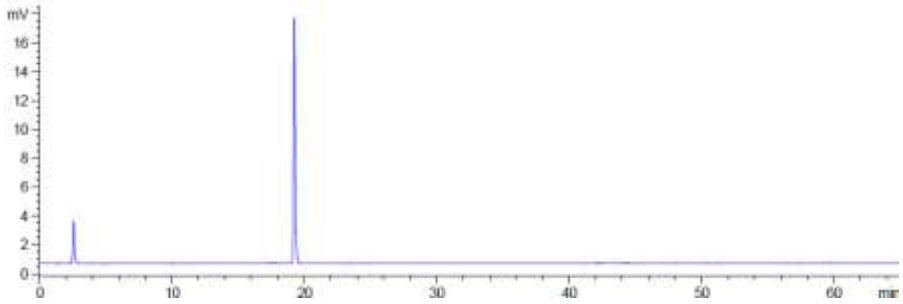


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 8 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide **42** (1.5 mg, 2.00 µmol, 15% over 11 steps, based on resin loading).

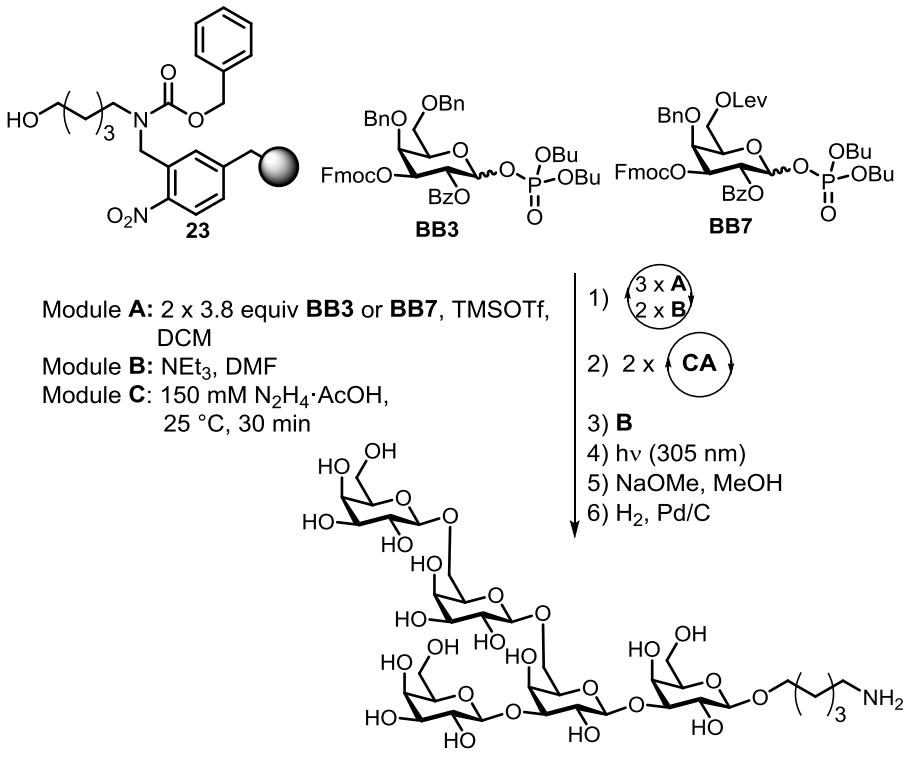
^{1}H NMR (600 MHz, D_2O) δ = 4.79 (d, *J* = 7.8 Hz, 1H), 4.72 (d, *J* = 7.6 Hz, 1H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.54 (d, *J* = 7.9 Hz, 1H), 4.34 (dd, *J* = 7.2, 3.2 Hz, 2H), 4.18 – 4.12 (m, 1H), 4.09 – 3.98 (m, 5H), 3.97 – 3.92 (m, 2H), 3.92 – 3.70 (m, 15H), 3.62 (dd, *J* = 9.8, 8.0 Hz, 1H), 3.12 (t, *J* = 7.5 Hz, 2H), 1.80 (tt, *J* = 14.0, 7.2 Hz, 4H), 1.61 – 1.55 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 102.9, 102.5, 102.1, 101.0, 81.0, 80.6, 73.7, 73.7, 73.4, 72.0, 71.3, 71.1, 69.6, 69.4, 68.8, 68.5, 68.5, 67.8, 67.2, 67.2, 67.1, 67.0, 59.6, 59.6, 59.5, 38.0, 26.8, 25.0, 20.7 ppm. HRMS: m/z $[M+Na]^+$ calcd. for $C_{29}H_{53}NNaO_{21}$: 774.3007, found 774. 3030

RP-HPLC of deprotected tetrasaccharide **42** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[6-*O*-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (43)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.75 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

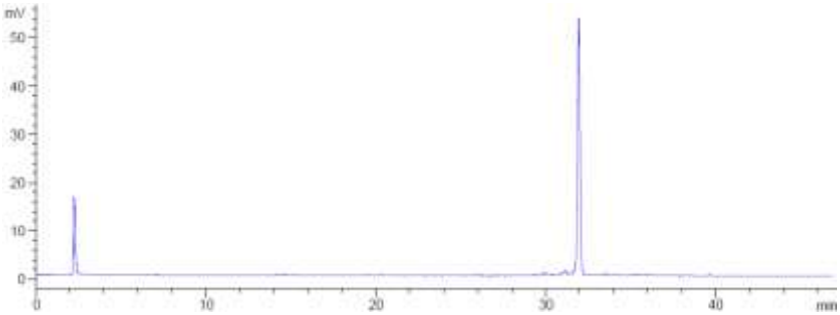
Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide **43**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

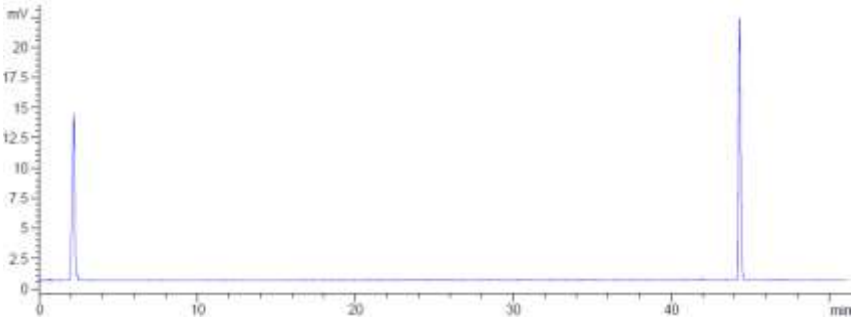
Crude NP-HC of protected pentasaccharide **43** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min)

The protected, branched pentasaccharide **43** was dissolved in THF (5 mL) and NaOMe (1 mL 0.5 M in MeOH) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

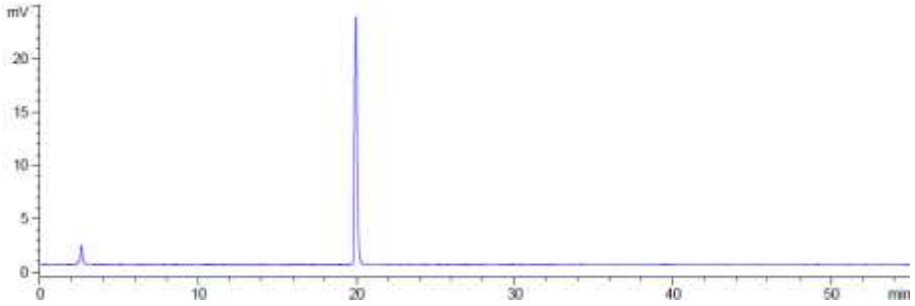
Crude RP-HPLC of semi-protected pentasaccharide **43** (ELSD trace):



HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

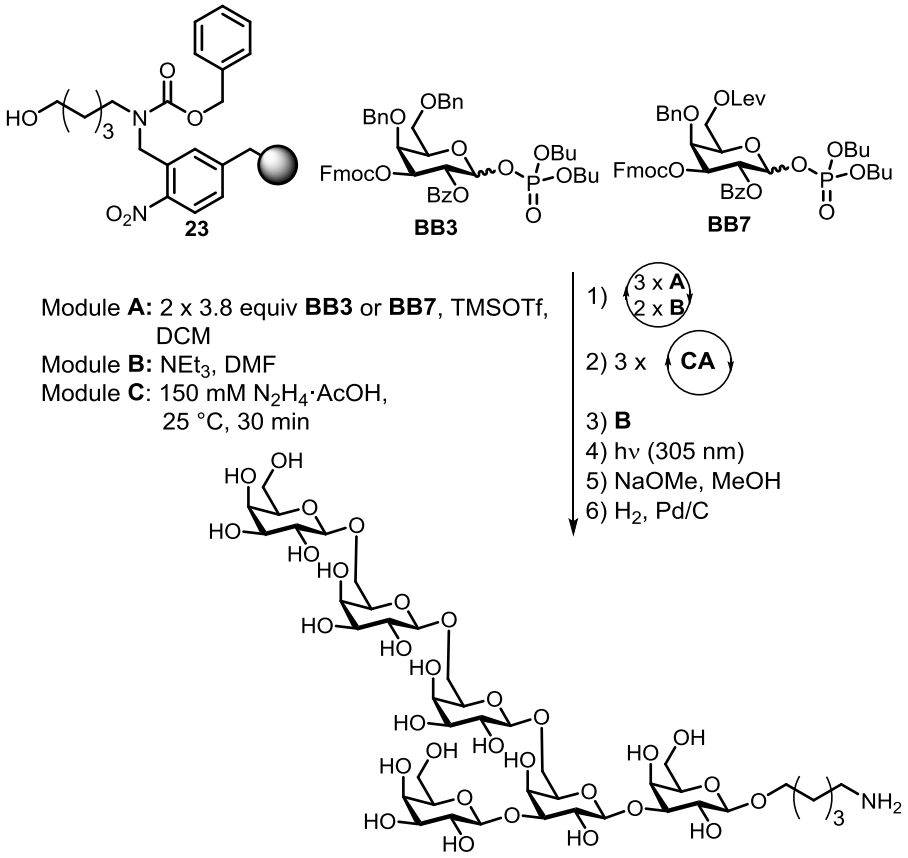
The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 8 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected pentasaccharide **43** (1.6 mg, 1.73 µmol, 10% over 13 steps, based on resin loading).
^{1}H NMR (700 MHz, D_2O) δ = 4.82 (d, *J* = 7.8 Hz, 1H), 4.75 (d, *J* = 7.7 Hz, 1H), 4.61 – 4.56 (m, 3H), 4.37 (dd, *J* = 8.1, 2.9 Hz, 2H), 4.19 – 4.14 (m, 2H), 4.11 – 3.98 (m, 9H), 3.97 – 3.73 (m, 17H), 3.69 – 3.63 (m, 2H), 3.14 (t, *J* = 7.5 Hz, 2H), 1.82 (tt, *J* = 14.3, 7.4 Hz, 4H), 1.63 – 1.56 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O) δ = 102.0, 101.5, 101.2, 101.1, 100.0, 80.1, 79.7, 72.8, 72.8, 72.5, 71.4, 70.9, 70.4, 70.3, 70.2, 68.7, 68.4, 68.4, 67.9, 67.6, 67.5, 67.1, 67.0, 66.3, 66.1, 66.0, 58.7, 58.7, 58.6, 37.0, 25.8, 24.1, 19.8 ppm. HRMS: m/z $[M+H]^+$ calcd. for $C_{35}H_{64}NO_{26}$: 914.3716, found: 914.3783

RP-HPLC of the deprotected pentasaccharide **43** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[6-*O*-[6-*O*-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (44)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

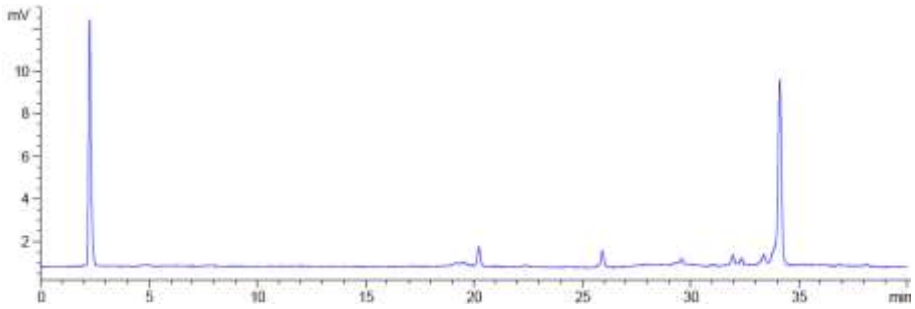
Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected hexasaccharide **44**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

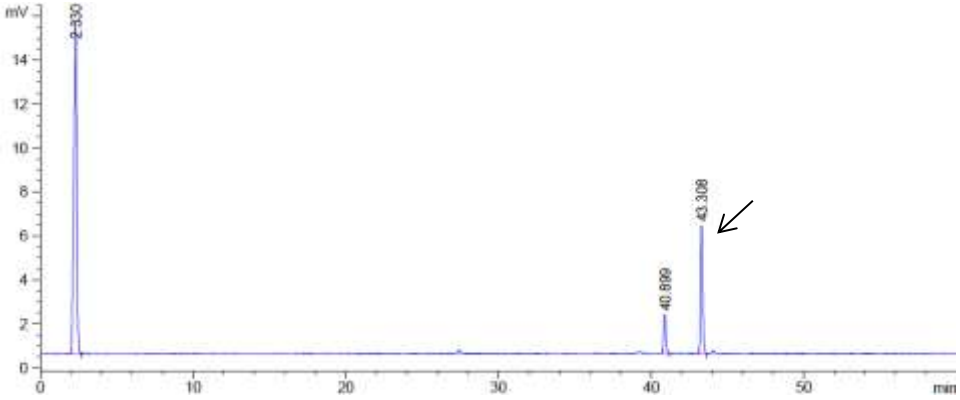
Crude NP-HPLC of protected hexasaccharide **44** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min)

The protected, branched hexasaccharide **44** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of the semi-protected hexasaccharide **44** (ELSD trace):



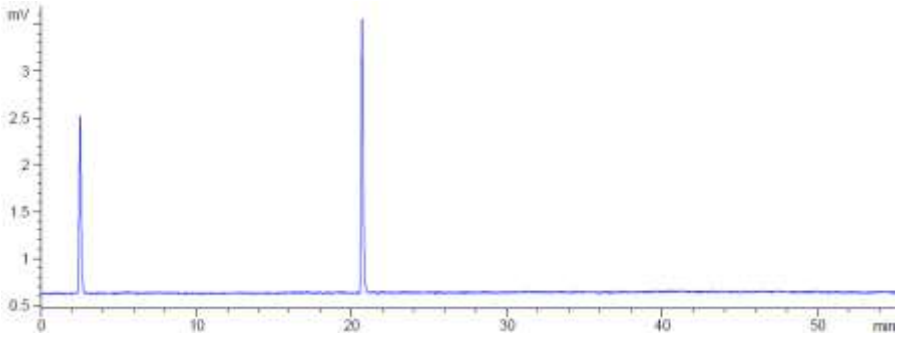
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 8 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide **44** (0.9 mg, 0.84 µmol, 7% over 15 steps, based on resin loading).

^{1}H NMR (700 MHz, D_2O) δ = 4.86 (d, *J* = 7.9 Hz, 1H), 4.79 (d, *J* = 7.7 Hz, 1H), 4.66 – 4.60 (m, 4H), 4.43 – 4.39 (m, 2H), 4.24 – 4.18 (m, 3H), 4.15 – 4.06 (m, 11H), 4.04 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.01 – 3.81 (m, 17H), 3.78 (dd, *J* = 9.8, 7.7 Hz, 1H), 3.73 – 3.67 (m, 3H), 3.19 – 3.16 (m, 2H), 1.90 – 1.83 (m, 4H), 1.66 – 1.61 (m, 2H) ppm. ^{13}C NMR (176 MHz,

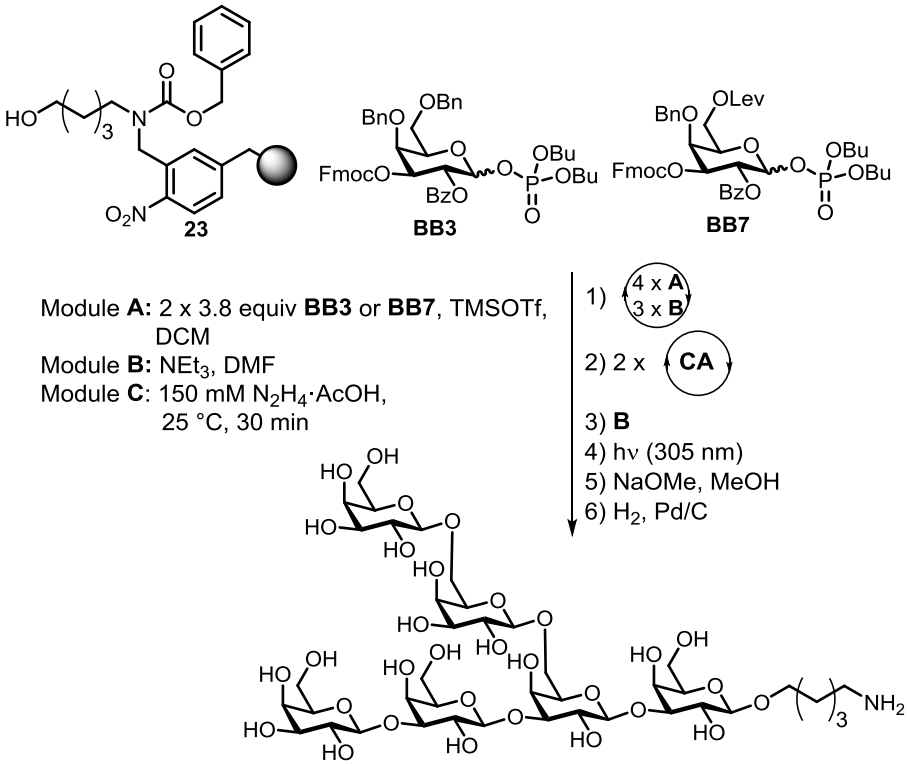
D_2O) δ = 102.1, 101.6, 101.2 (3 C), 100.1, 80.2, 79.7, 72.9, 72.8, 72.5, 71.5, 71.4, 71.0, 70.5, 70.3, 70.3, 68.8, 68.5, 67.9, 67.7, 67.6, 67.2, 67.1, 67.0, 66.4, 66.2, 66.1, 58.8, 58.7, 37.1, 25.9, 24.2, 19.8 ppm. HRMS: m/z $[M+Na]^+$ calcd. for $C_{41}H_{73}NNaO_{31}$: 1098.4064 found: 1098.4163

RP-HPLC of deprotected hexasaccharide **44** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[6-*O*-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (45)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

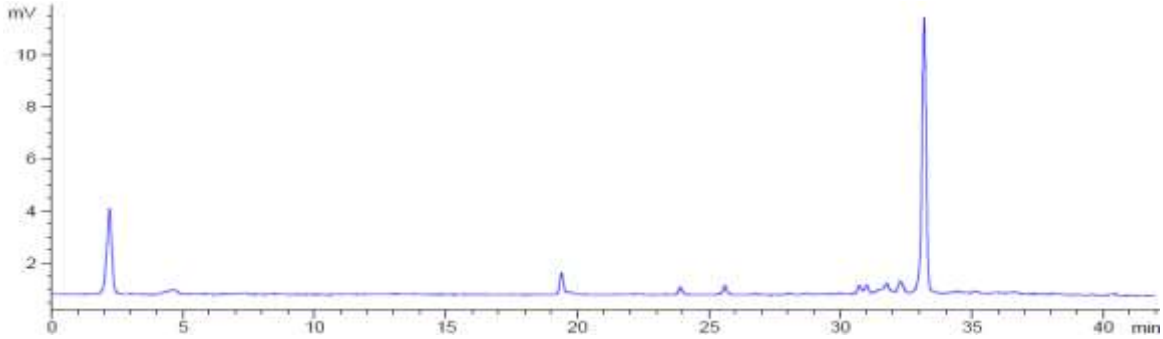
Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected hexasaccharide **45**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

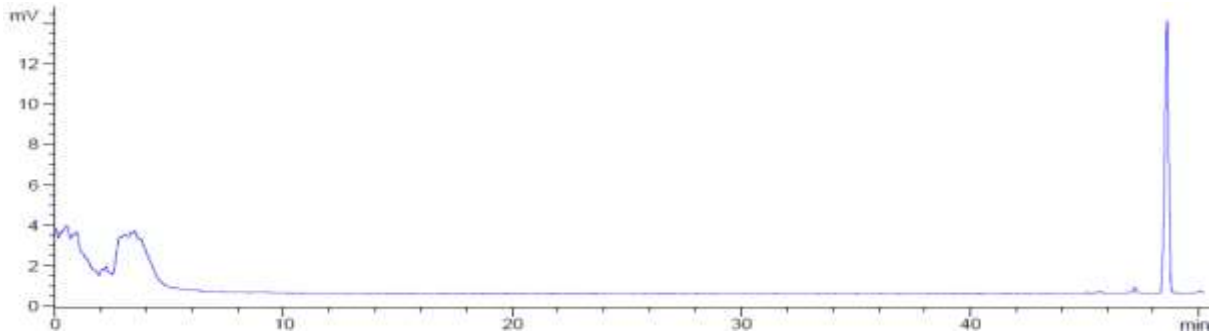
Crude NP-HPLC of protected hexasaccharide **45** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min)

The protected branched hexasaccharide **45** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of the semi-protected hexasaccharide **45** (ELSD trace):



HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

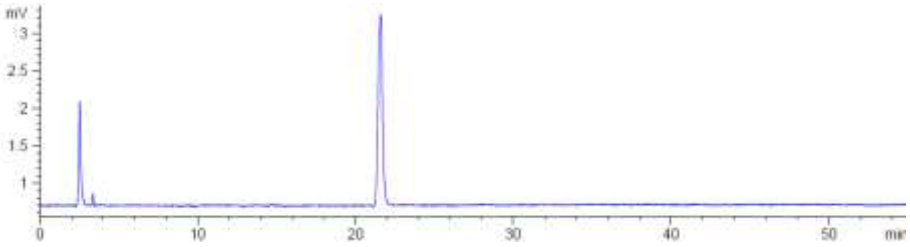
The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide **45** (0.9 mg, 0.84 µmol, 5% over 15 steps, based on resin loading).

^{1}H NMR (600 MHz, D_2O) δ = 4.87 – 4.84 (m, 2H), 4.79 (d, *J* = 7.6 Hz, 1H), 4.63 – 4.60 (m, 3H), 4.42 – 4.35 (m, 3H), 4.23 – 4.18 (m, 2H), 4.13 – 4.03 (m, 8H), 4.02 – 3.76 (m, 23H), 3.73 – 3.66 (m, 2H), 3.20 – 3.16 (m, 2H), 1.90 – 1.82 (m, 4H), 1.67 – 1.61 (m, 2H) ppm.

^{13}C NMR (151 MHz, D_2O) δ = 102.0, 101.7, 101.6, 101.2, 101.2, 100.1, 80.2, 79.8, 79.7,

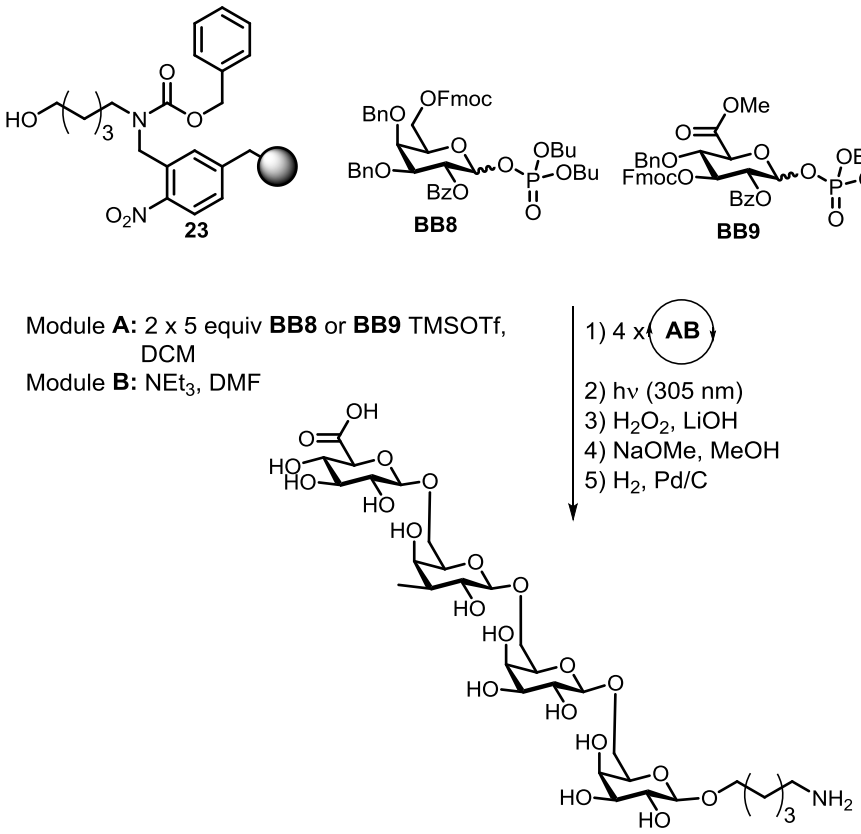
72.9, 72.8, 72.5, 72.4, 71.4, 71.0, 70.5, 70.4, 70.3, 68.8, 68.5, 68.5, 67.9, 67.7, 67.6, 67.2, 67.0, 66.4, 66.3, 66.2, 66.1, 66.1, 58.8, 58.7, 58.7, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z $[M+Na]^+$ calcd. for $C_{41}H_{73}NNaO_{31}$: 1098. 4064, found: 1098.4173

RP-HPLC of the deprotected hexasaccharide **45** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, 0.7 mL/min).

Aminopentyl β-D-glucopyranosyluronate-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (46)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

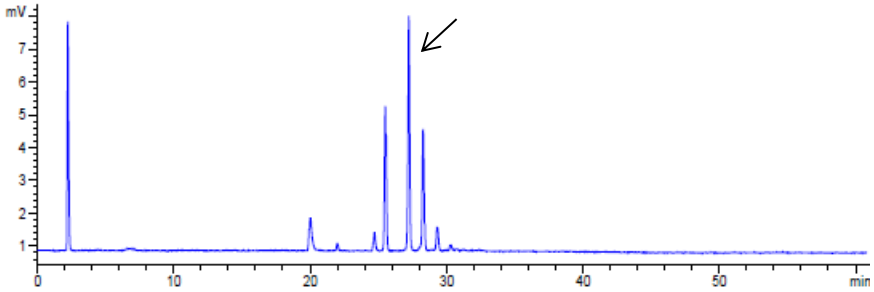
Module **A** (1 x 5 equiv. **BB8**, TMSOTf, DCM, 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (1 x 5 equiv. **BB8**, TMSOTf, DCM, 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (1 x 5 equiv. **BB8**, TMSOTf, DCM, 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 5 equiv. **BB9**, TMSOTf, DCM, 60 min, -15 °C to 0 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **46**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

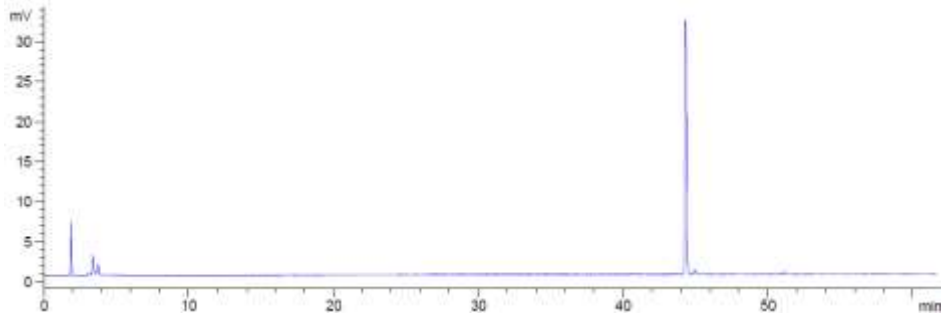
Crude NP-HPLC of protected tetrasaccharide **46** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected tetrasaccharide **46** (6 mg, 3.06 µmol) was dissolved in THF (1 mL) and cooled to -5 °C. Hydrogen peroxide (1 µL of a 30% solution in water, 0.01 µmol, 3.5 equiv) was added under stirring followed by the addition of LiOH (0.02 mL of a 1M solution in water, 0.02 mmol, 7 equiv). The solution was stirred for 2 hours at lowered temperature (-5 °C to 0 °C) and then overnight at rt. After stirring overnight the reaction mixture was again cooled to 0 °C, NaOH (0.05 mL of a 1 M solution in water, 0.05 mmol, 15 equiv) and MeOH (0.15 mL) were added dropwise. The mixture was allowed to warm to rt and stirred over the weekend followed by neutralization by Amberlite H^+ resin. The resin was filtered of and the solvents were removed *in vacuo*. The crude semi-protected intermediate was dissolved in H_2O/MeCN (1:1, 4 mL) and purified over semi-preparative C5 HPLC yielding 2 mg (1.31 µmol, 43%) of the semi-protected intermediate.

Crude RP-HPLC of semi-protected tetrasaccharide **46** (ELSD trace):

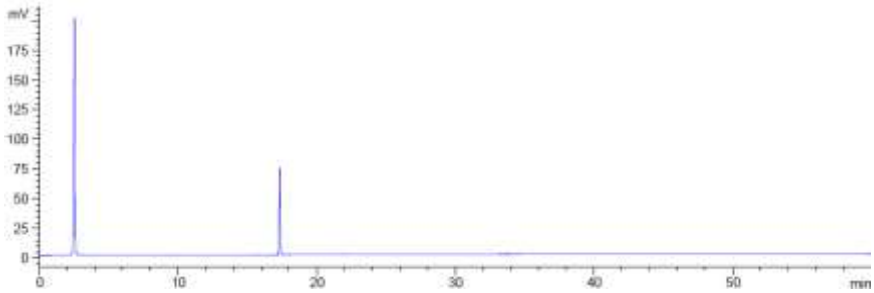


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product (2 mg, 1.3 µmol) was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated and the fully de-protected tetrasaccharide **46** (1.1 mg, 1.3 µmol, 100%) was obatained after purification by elution form a C18 SepPak column (50 mg).

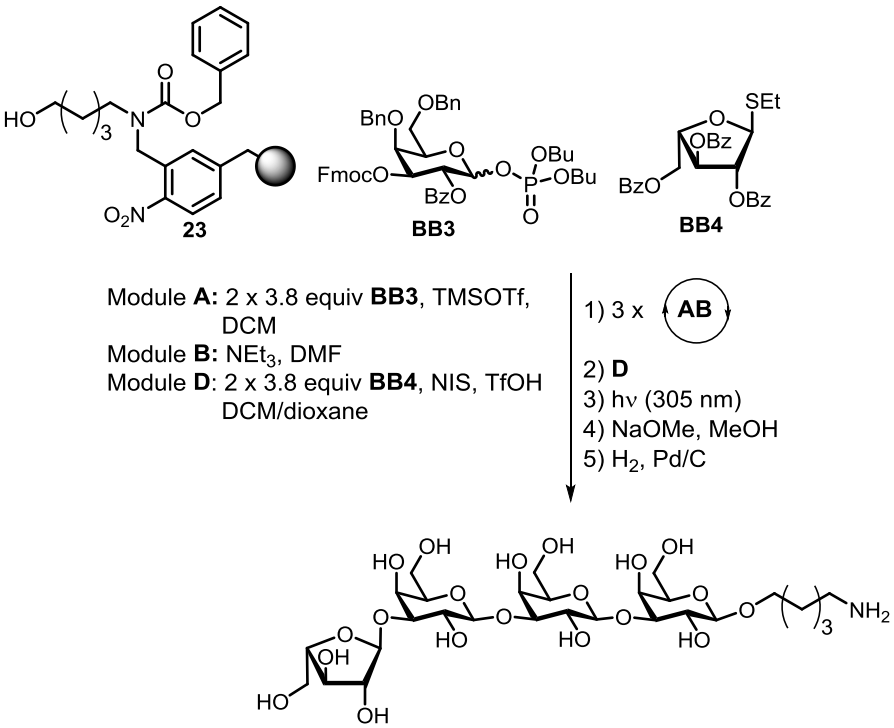
^{1}H NMR (600 MHz, D_2O) δ = 4.69 (d, *J* = 7.7 Hz, 1H), 4.66 (d, *J* = 7.7 Hz, 1H), 4.62 (d, *J* = 7.7 Hz, 1H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.24 – 4.18 (m, 4H), 4.15 – 4.05 (m, 11H), 3.90 – 3.81 (m, 5H), 3.72 – 3.66 (m, 3H), 3.51 (t, *J* = 8.1 Hz, 1H), 3.18 (t, *J* = 7.3 Hz, 2H), 1.86 (dp, *J* = 13.7, 7.2, 6.7 Hz, 4H), 1.63 (p, *J* = 7.9 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 101.2, 101.0, 100.5, 100.5, 73.8, 73.3, 71.5, 70.7, 70.44, 70.36, 69.5, 68.48, 68.46, 67.9, 66.4, 66.3, 37.1, 25.9, 24.2 19.8. HRMS: m/z $[M+H]^+$ calcd. for $C_{29}H_{51}NO_{22}$: 766.2981, found: 766.2990

RP-HPLC of deprotected tetrasaccharide **46** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, 0.7 mL/min).

Aminopentyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-α-L-arabinofuranoside (47)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

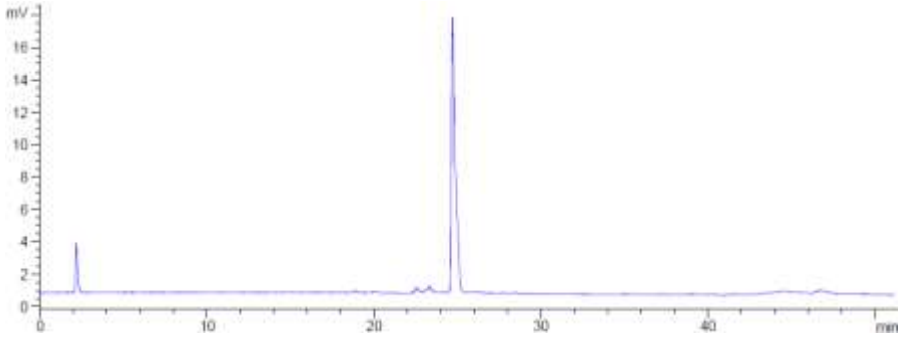
Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **47**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

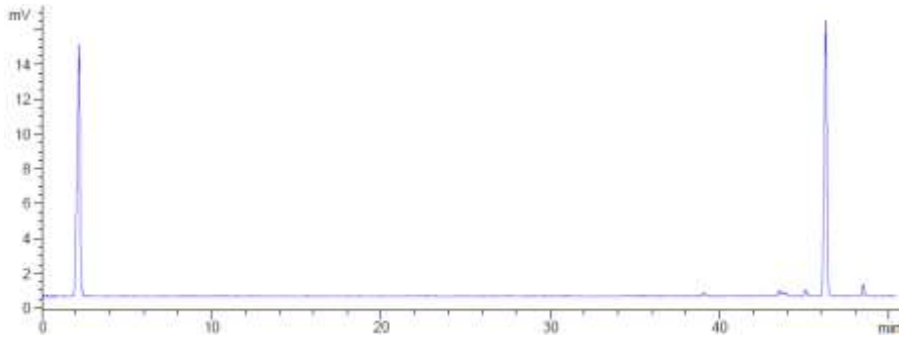
Crude NP-HPLC of protected tetrasaccharide **47** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min)

The protected tetrasaccharide **47** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of the semi-protected tetrasaccharide **47** (ELSD trace):

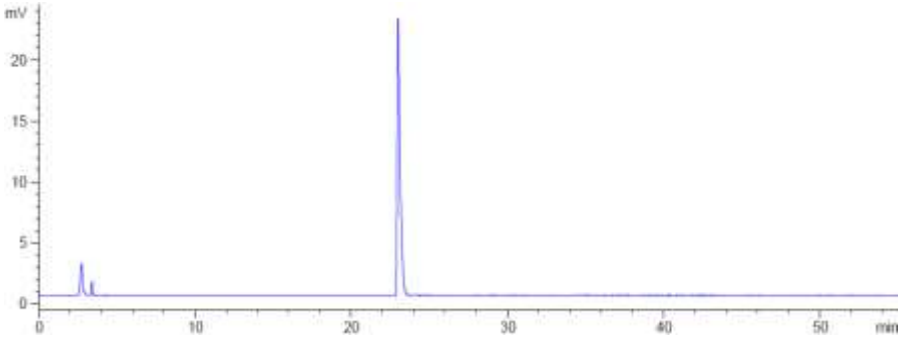


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide **47** (2.0 mg, 2.81 µmol, 17% over 10 steps, based on resin loading).
^{1}H NMR (700 MHz, D_2O) δ = 6.13 (s, 1H), 5.59 – 5.54 (m, 2H), 5.34 (d, *J* = 8.0 Hz, 1H), 5.11 – 5.08 (m, 3H), 5.03 – 4.99 (m, 2H), 4.86 – 4.82 (m, 2H), 4.75 – 4.70 (m, 3H), 4.69 – 4.55 (m, 15H), 3.90 (t, *J* = 7.5 Hz, 2H), 2.62 – 2.54 (m, 4H), 2.38 – 2.33 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O) δ = 107.0, 101.8, 101.8, 100.1, 81.6, 80.2, 79.8, 79.0, 77.8, 74.3,

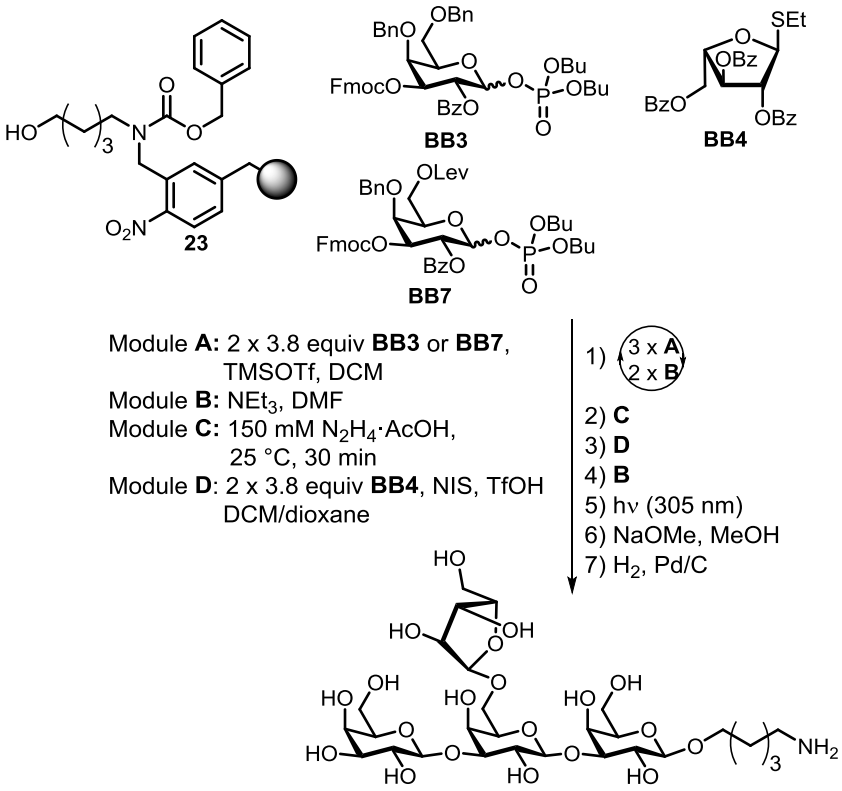
72.7, 72.5, 72.5, 68.0, 67.7, 67.6, 66.2, 66.1, 59.0, 58.6, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z $[M+H]^+$ calcd. for $C_{28}H_{51}NO_{20}$: 722.3082, found: 722.3036.

RP-HPLC of the deprotected tetrasaccharide **47** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl β-D-galactopyranosyl-(1→3)-6-*O*-[α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (48)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

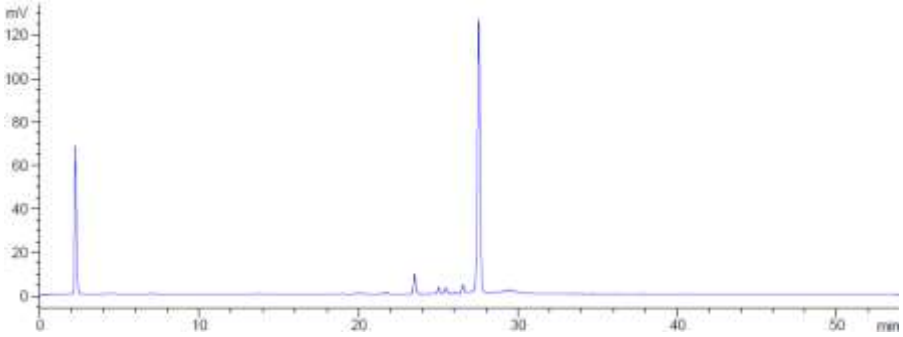
Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **C** (3 cycles)
Module **D** (2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **48**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

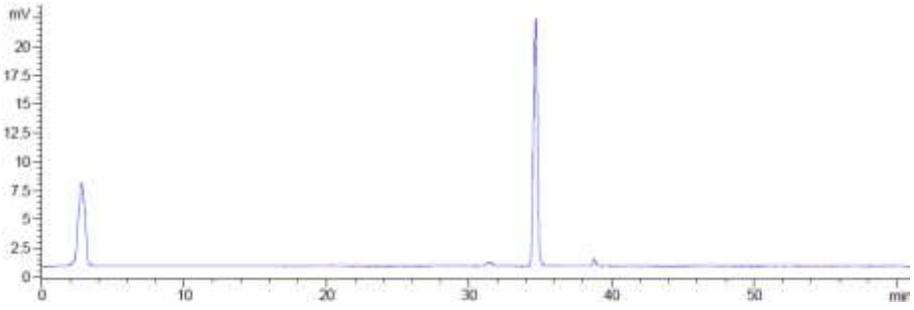
Crude NP-HPLC of protected tetrasaccharide **48** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min)

The protected tetrasaccharide **48** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH 1 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

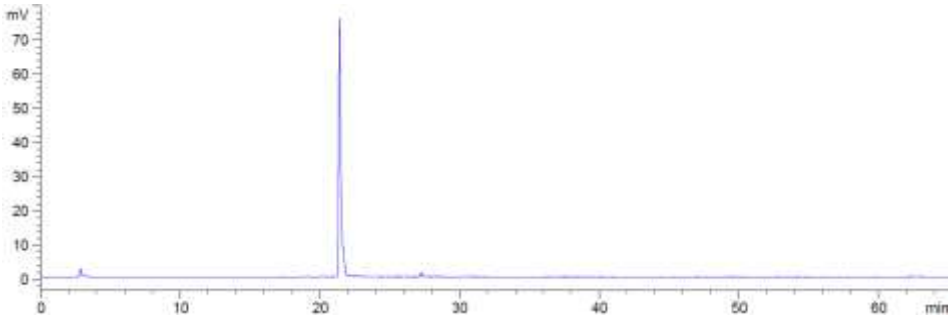
Crude RP-HPLC of semi-protected terasaccharide **48** (ELSD trace):



HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

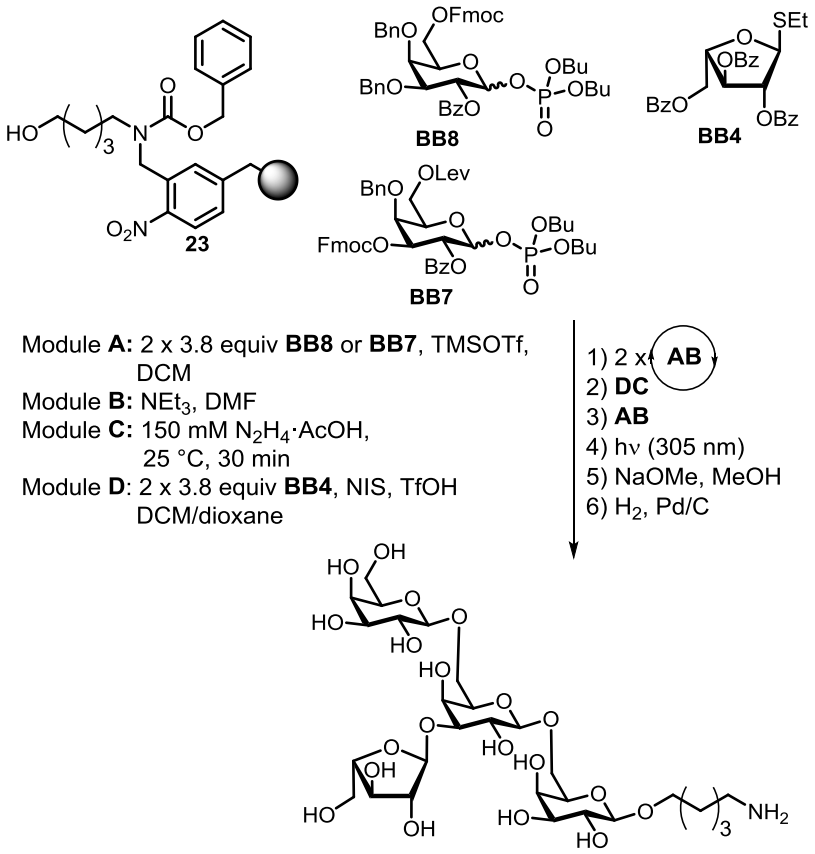
The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide **48** (2.0 mg, 2.72 µmol, 17% over 11 steps, based on resin loading).
^{1}H NMR (600 MHz, D_2O) δ = 5.15 (d, *J* = 1.4 Hz, 1H), 4.77 (d, *J* = 7.7 Hz, 1H), 4.70 (d, *J* = 7.6 Hz, 1H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.29 (dd, *J* = 12.5, 3.2 Hz, 2H), 4.16 (dd, *J* = 3.4, 1.7 Hz, 1H), 4.13 (td, *J* = 6.0, 3.3 Hz, 1H), 4.07 – 4.00 (m, 3H), 3.99 – 3.95 (m, 2H), 3.94 – 3.83 (m, 9H), 3.82 – 3.73 (m, 6H), 3.70 (dd, *J* = 9.9, 7.7 Hz, 1H), 3.13 – 3.07 (m, 2H), 1.82 – 1.73 (m, 4H), 1.55 (dt, *J* = 15.4, 7.6 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 106.9, 103.3, 102.9, 101.4, 82.9, 81.6, 81.0, 80.1, 75.5, 74.1, 73.8, 72.3, 71.5, 70.0, 69.1, 68.9, 68.8, 67.6, 67.5, 67.5, 66.4, 60.2, 60.0, 60.0, 38.4, 27.2, 25.4, 21.1 ppm. HRMS: m/z $[M+H]^+$ calcd. for $C_{28}H_{51}NO_{20}$: 722.3082, found: 722.3042.

RP-HPLC of the deprotected tetrasaccharide **48** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1 % of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-3-*O*-[α-L-arabinofuranosyl]-(1→6)-β-D-galactopyranoside (49)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

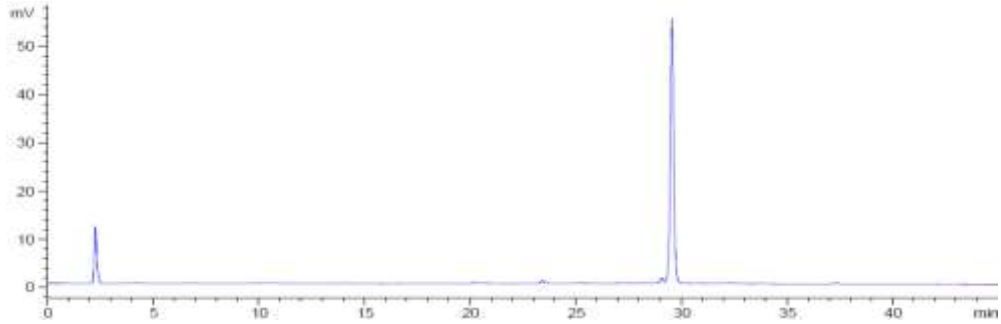
Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **49**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

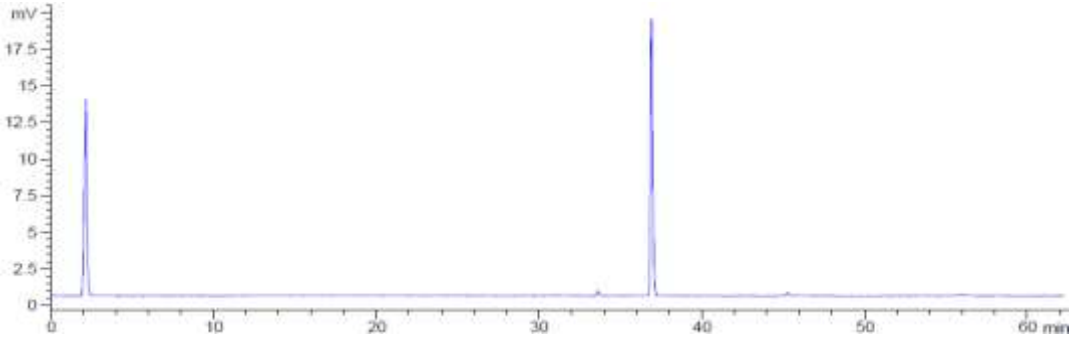
Crude NP-HPLC of protected tetrasaccharide **49** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min)

The protected tetrasaccharide **49** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH 1 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of semi-protected tetrasaccharide **49** (ELSD trace):

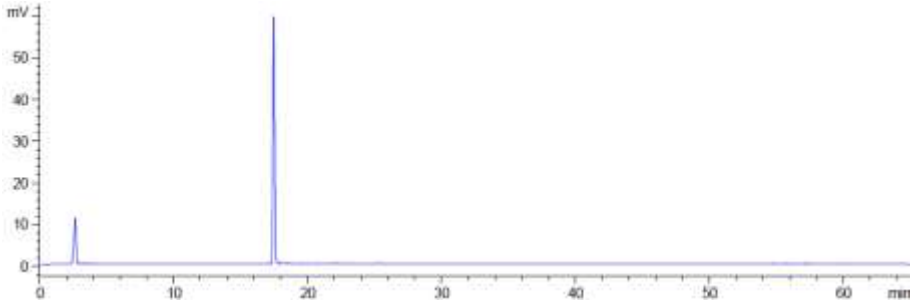


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide **49** (2.0 mg, 2.77 µmol, 16% over 11 steps, based on resin loading).
^{1}H NMR (700 MHz, D_2O) δ = 5.39 (s, 1H), 4.70 (d, *J* = 7.8 Hz, 1H), 4.62 (d, *J* = 7.9 Hz, 1H), 4.59 (d, *J* = 7.8 Hz, 1H), 4.38 – 4.37 (m, 1H), 4.32 (d, *J* = 2.3 Hz, 1H), 4.31 – 4.28 (m, 1H), 4.24 – 4.19 (m, 2H), 4.15 – 4.05 (m, 8H), 4.00 (d, *J* = 12.5 Hz, 1H), 3.98 – 3.80 (m, 9H), 3.72 – 3.66 (m, 2H), 3.18 (t, *J* = 7.4 Hz, 2H), 1.90 – 1.82 (m, 4H), 1.66 – 1.60 (m,

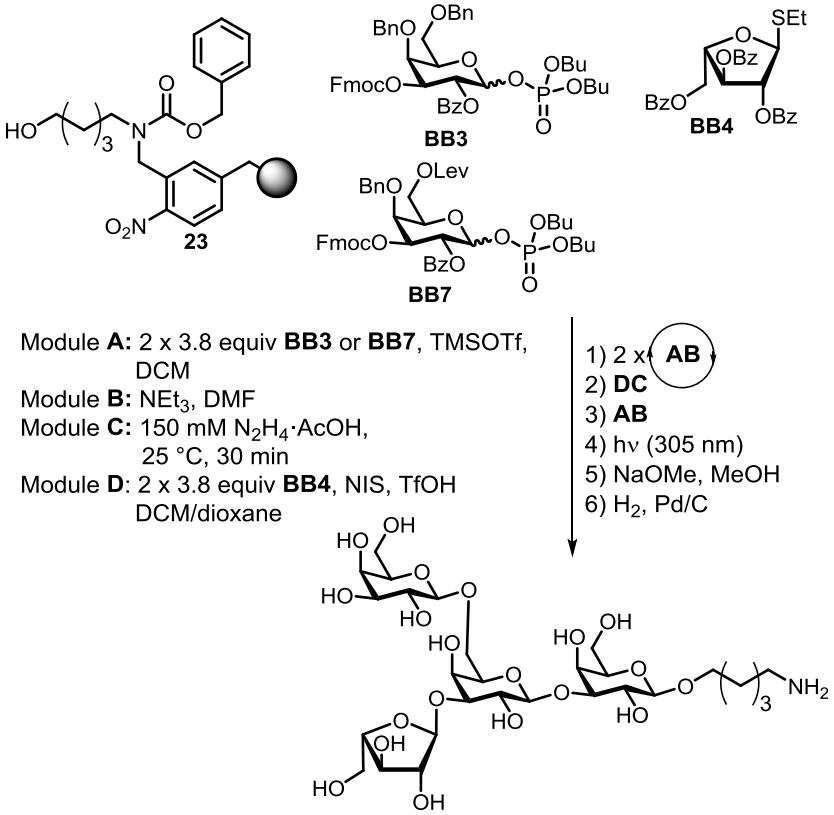
2H) ppm. ^{13}C NMR (176 MHz, D_2O) δ = 107.0, 101.1, 100.8, 100.4, 81.6, 79.0, 77.9, 74.3, 72.9, 71.5, 71.3, 70.4, 70.4, 68.5, 68.4, 67.9, 67.5, 67.0, 66.8, 66.4, 66.4, 66.2, 58.9, 58.7, 37.1, 25.9, 24.2, 19.8 ppm. HRMS: m/z $[M+Na]^+$ calcd. for $C_{28}H_{51}NO_{20}$: 744.2902, found: 744.2903.

RP-HPLC of deprotected tetrasaccharide **49** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-α-L-arabinofuranoside (50)

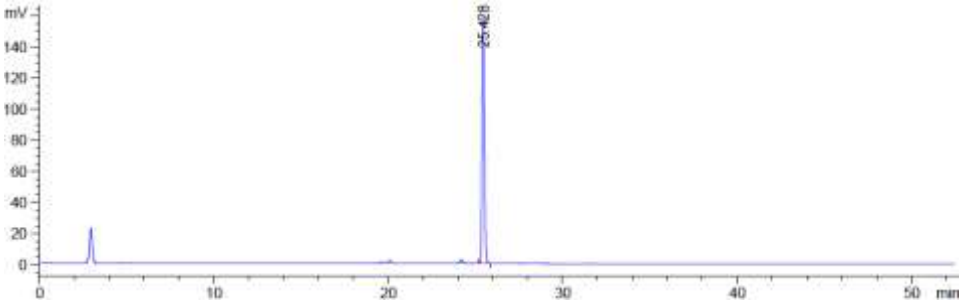


Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **D** (2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
Module **C** (3 cycles)
Module **A** (2 x 3.75 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **50**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

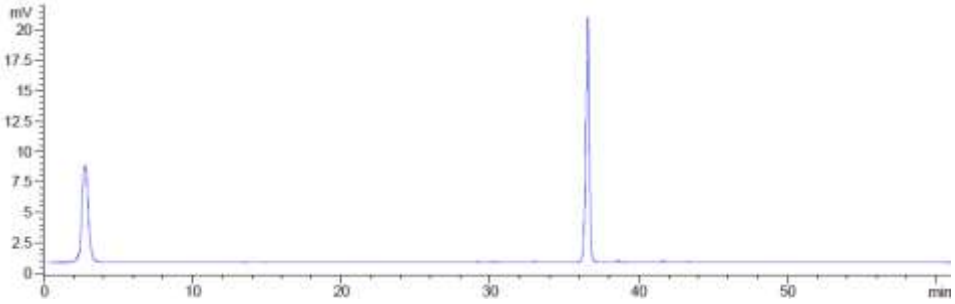
Crude NP-HPLC of protected tetrasaccharide **50** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The Tetrasaccharide **50** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

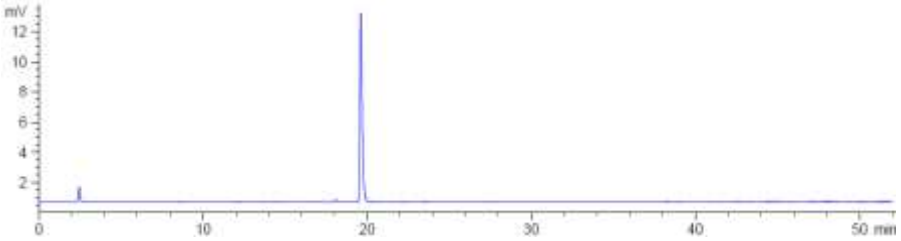
Crude RP-HPLC of semi-protected terasaccharide **50** (ELSD trace):



HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

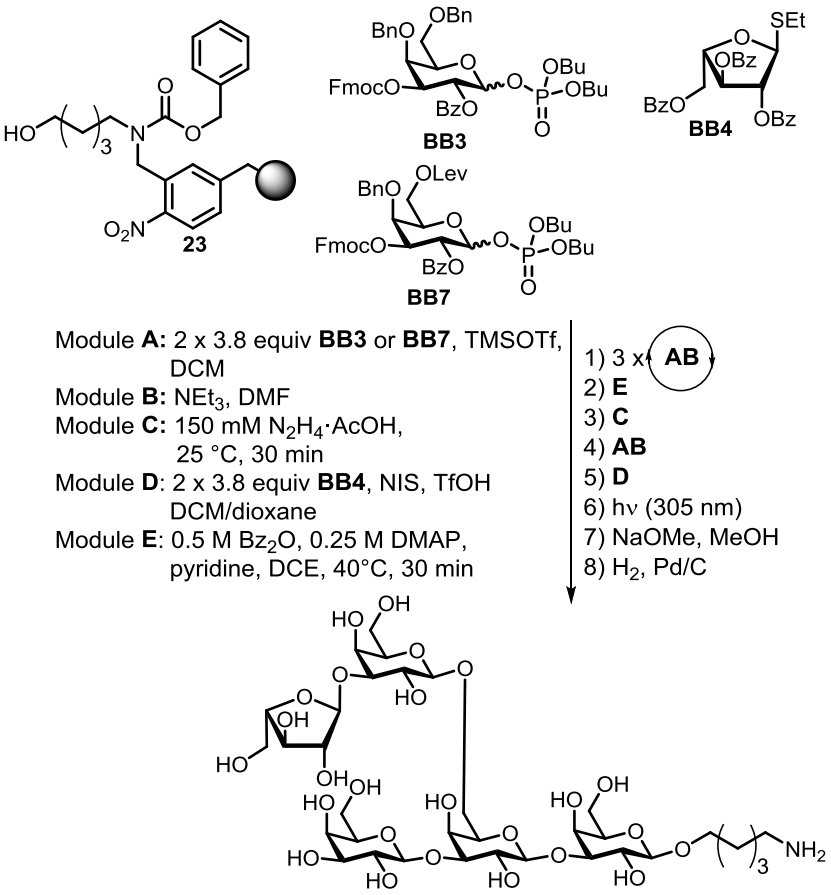
The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected tetrasaccharide **50** (1.2 mg, 1.66 µmol, 10% over 11 steps, based on resin loading).
^{1}H NMR (600 MHz, D_2O) δ = 5.40 (d, *J* = 1.3 Hz, 1H), 4.62 (d, *J* = 8.0 Hz, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.40 – 4.37 (m, 2H), 4.30 (ddd, *J* = 12.3, 6.1, 3.0 Hz, 2H), 4.20 (dd, *J* = 10.3, 3.0 Hz, 1H), 4.15 – 4.03 (m, 5H), 4.02 – 3.97 (m, 3H), 3.96 – 3.83 (m, 11H), 3.83 – 3.79 (m, 1H), 3.69 (dd, *J* = 9.9, 7.9 Hz, 1H), 3.20 – 3.16 (m, 2H), 1.86 (tt, *J* = 14.2, 7.1 Hz, 4H), 1.67 – 1.60 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 107.0, 101.7, 101.2, 100.1, 81.6, 80.1, 79.0, 77.6, 74.3, 72.9, 72.5, 71.2, 70.5, 68.5, 67.9, 67.7, 67.6, 66.9, 66.3, 66.2, 66.2, 58.9, 58.7, 58.7, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z $[M+H]^+$ calcd. for $C_{28}H_{51}NO_{20}$: 722.3082, found: 722.3125.

RP-HPLC of deprotected tetrasaccharide **50** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[3-*O*-[α-L-arabinofuranosyl]-β-D-galactopyranosyl] β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (51)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)

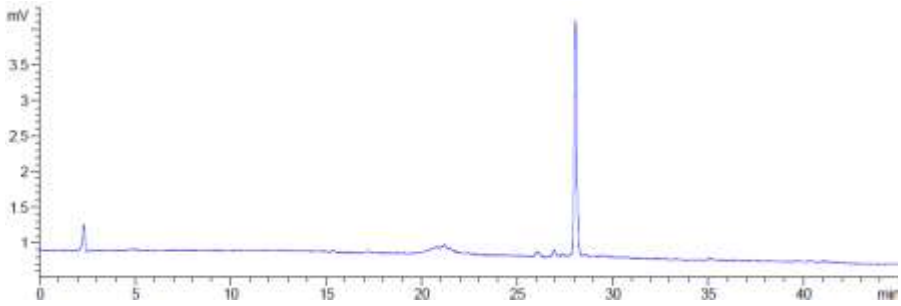
Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide **51**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

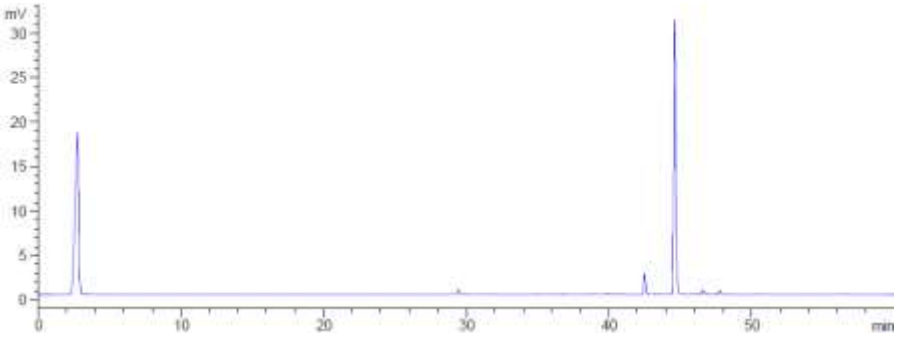
Crude NP-HPLC of protected pentasaccharide **51** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected branched pentasaccharide **51** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of semi-protected pentasaccharide **51** (ELSD trace):

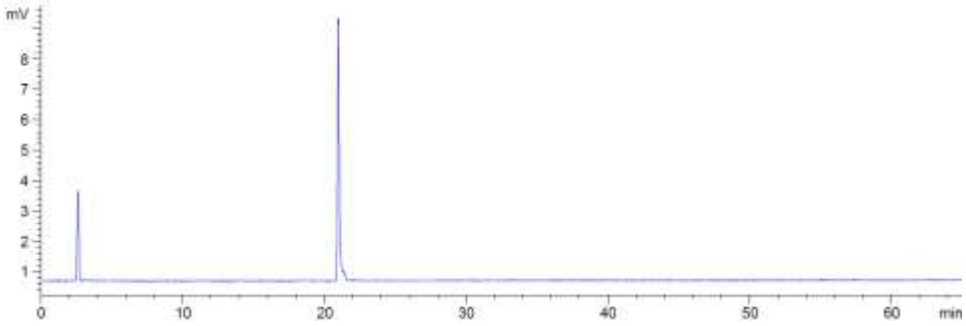


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected pentasaccharide **51** (2.7 mg, 3.05 µmol, 16% over 14 steps, based on resin loading).
^{1}H NMR (600 MHz, D_2O) δ = 5.40 (s, 1H), 4.85 (d, *J* = 7.7 Hz, 1H), 4.78 (d, *J* = 7.6 Hz, 1H), 4.65 (d, *J* = 7.9 Hz, 1H), 4.62 (d, *J* = 8.0 Hz, 1H), 4.42 – 4.37 (m, 3H), 4.31 – 4.25 (m, 2H), 4.21 (q, *J* = 8.7 Hz, 1H), 4.14 – 4.04 (m, 6H), 4.03 – 3.76 (m, 24H), 3.18 (t, *J* = 7.4 Hz, 2H), 1.90 – 1.81 (m, 4H), 1.63 (dt, *J* = 15.0, 7.7 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 106.9, 102.1, 101.6, 101.0, 100.1, 81.6, 80.1, 79.7, 79.0, 78.0, 74.2, 72.8, 72.7,

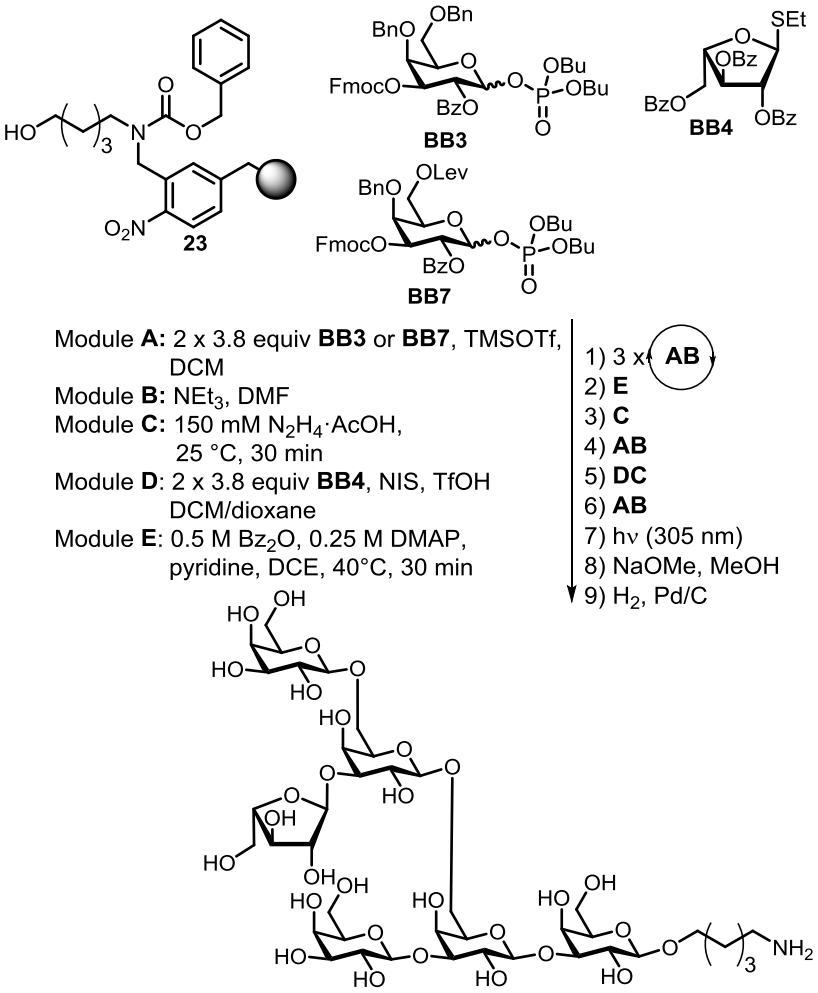
72.5, 71.1, 70.3, 68.7, 67.9, 67.7, 67.6, 67.0, 66.3, 66.2, 66.2, 66.1, 58.9, 58.7, 58.7, 37.1, 25.9, 24.1, 19.8 ppm. HRMS: m/z $[M+H]^+$ calcd. for $C_{34}H_{61}NO_{25}$: 884.3610, found: 884.3550.

RP-HPLC of deprotected pentasaccharide **51** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[3-*O*-[α-L-arabinofuranosyl]-6-*O*-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (52)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

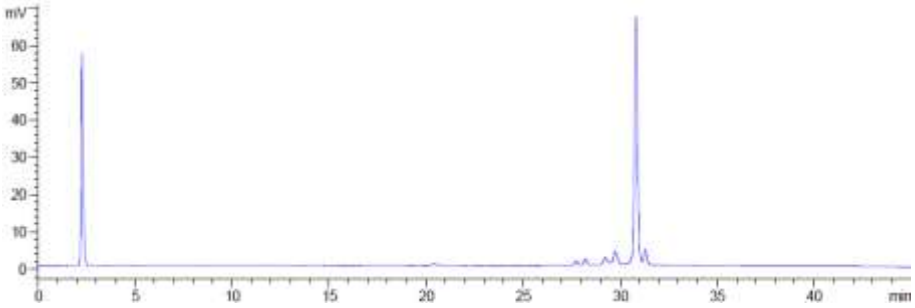
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Module **C** (3 cycles)

Module **A** (2 x 3.75 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected hexasaccharide **52**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

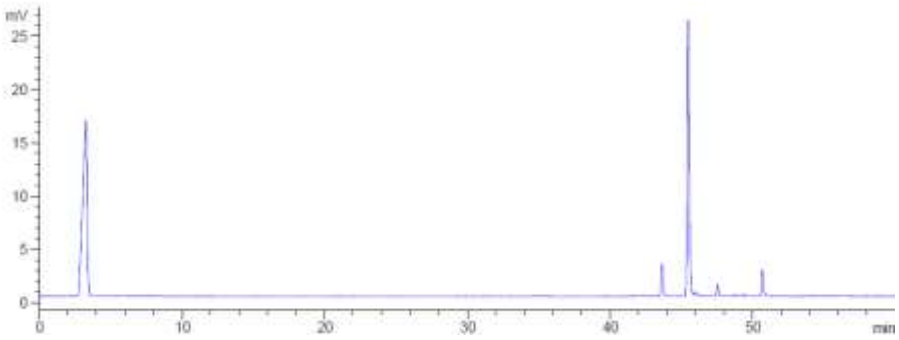
Crude NP-HPLC of protected hexasaccharide **52** (ELSD trace):



HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected hexasaccharide **52** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH 0.5 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

Crude RP-HPLC of semi-protected hexasaccharide **52** (ELSD trace):

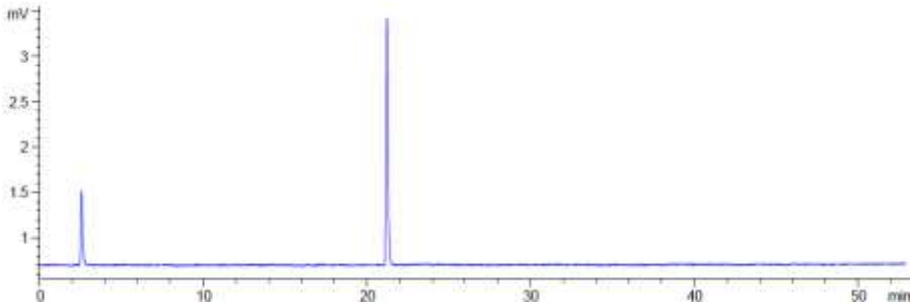


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture

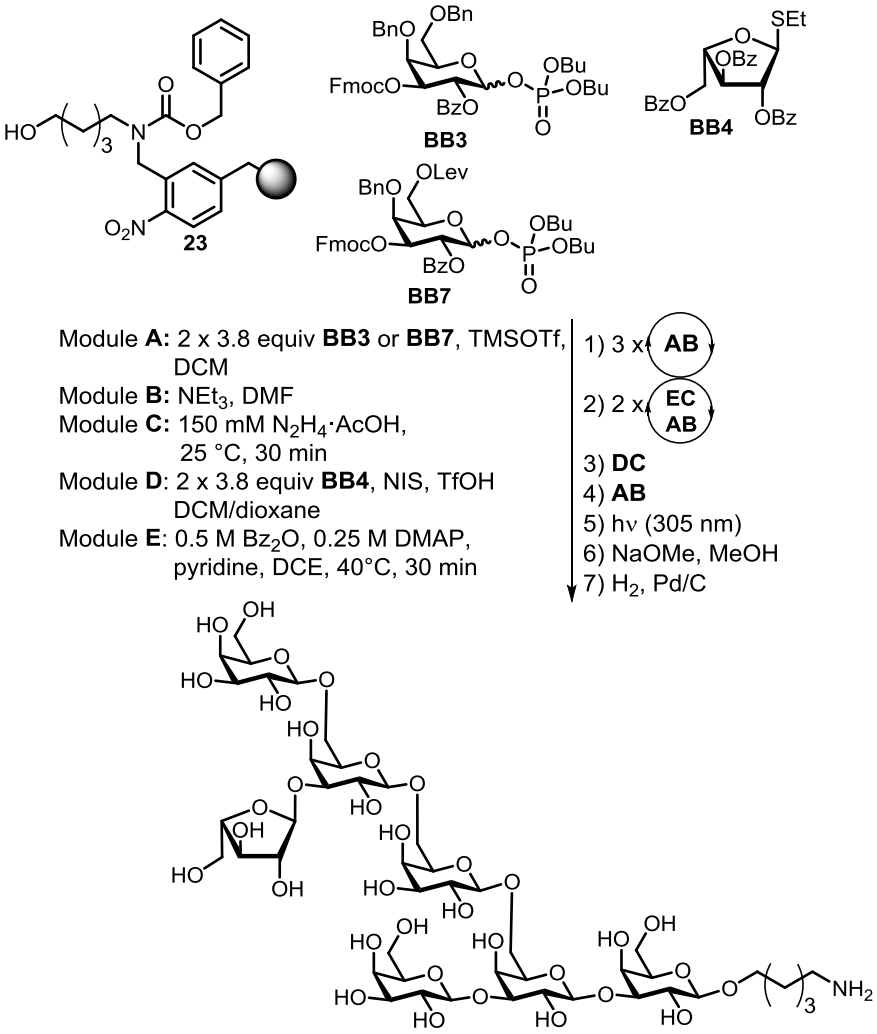
through a syringe filter the solvents were evaporated to provide the fully deprotected hexasaccharide **52** (1.9 mg, 1.82 µmol, 11% over 17 steps, based on resin loading).
^{1}H NMR (600 MHz, D_2O) δ = 5.33 (s, 1H), 4.79 (d, *J* = 7.8 Hz, 1H), 4.72 (d, *J* = 7.6 Hz, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.56 – 4.54 (m, 2H), 4.35 – 4.30 (m, 3H), 4.24 – 4.21 (m, 2H), 4.15 – 4.09 (m, 2H), 4.07 – 3.99 (m, 8H), 3.96 (dd, *J* = 9.9, 3.1 Hz, 1H), 3.94 – 3.90 (m, 2H), 3.90 – 3.84 (m, 7H), 3.84 – 3.69 (m, 11H), 3.63 (dd, *J* = 9.7, 8.0 Hz, 1H), 3.11 (t, *J* = 7.4 Hz, 2H), 1.83 – 1.75 (m, 4H), 1.59 – 1.53 (m, 2H) ppm.^{13}C NMR (151 MHz, D_2O) δ = 108.0, 103.1, 102.7, 102.3, 102.1, 101.2, 82.7, 81.3, 80.8, 80.1, 79.0, 75.3, 74.0, 73.9, 73.6, 72.3, 72.0, 71.6, 71.4, 69.8, 69.6, 69.0, 68.8, 68.7, 68.7, 68.3, 68.0, 67.4, 67.4, 67.3, 67.3, 67.2, 60.0, 59.9, 59.8, 59.8, 38.2, 27.0, 25.2, 20.9 ppm. HRMS: m/z $[M+Na]^+$ calcd. for $C_{40}H_{71}NO_{30}$: 1068.3958, found: 1068.4056.

RP-HPLC of deprotected hexaasaccharide **52** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min)

Aminopentyl-β-D-galactopyranosyl-(1→3)-6-*O*-[6-*O*-[3-*O*-[α-L-arabinofuranosyl]-6-*O*-[β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (53)



Linker functionalized resin **23**(53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)

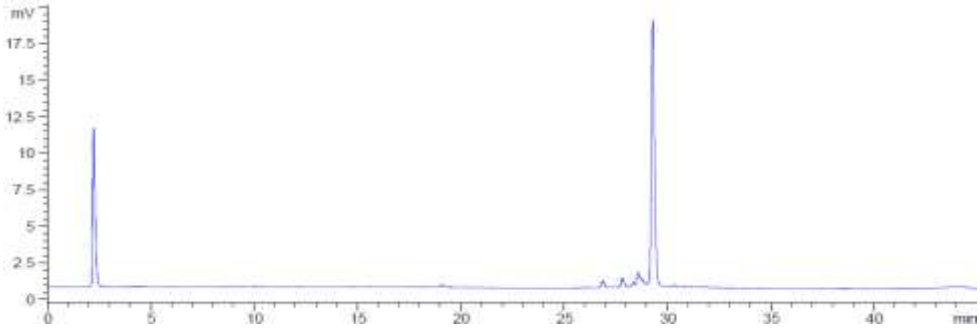
Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)
Module **A** (2 x 3.8 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **D** (2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
Module **C** (3 cycles)
Module **A** (2 x 3.8 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected heptasaccharide **53**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column.

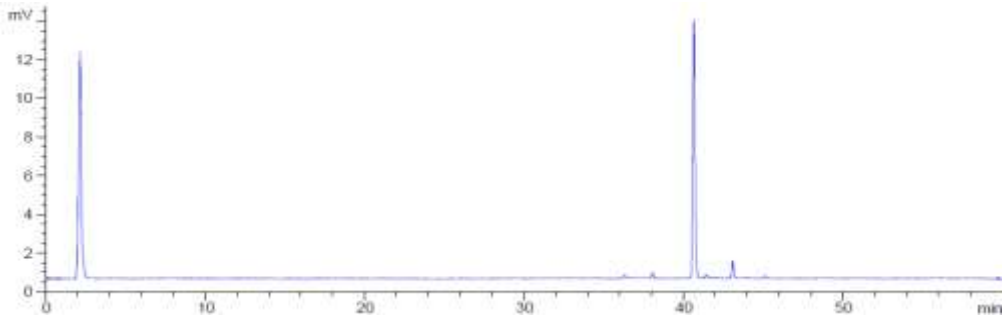
Crude NP-HPLC of protected heptasaccharide **53** (ELSD trace):



HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The protected heptasaccharide **53** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The solution was stirred overnight at rt. The reaction mixture was neutralized using prewashed Amberlite IR-120 resin, the resin was filtered off and the solvent was removed *in vacuo*. The semi-protected product was purified by semi-preparative RP-HPLC (C5 column).

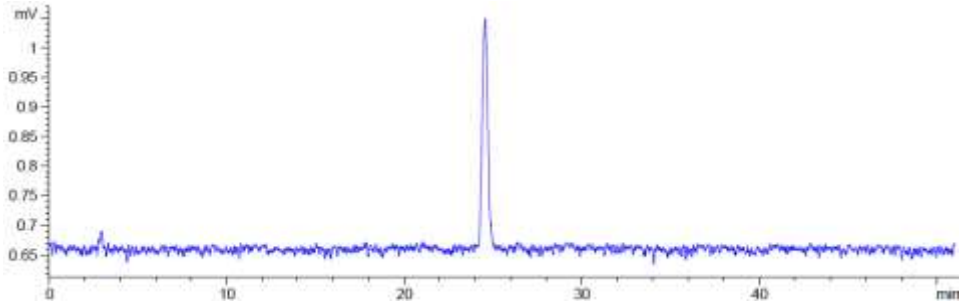
Crude RP-HPLC of semi-protected heptasaccharide **53** (ELSD trace):



HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

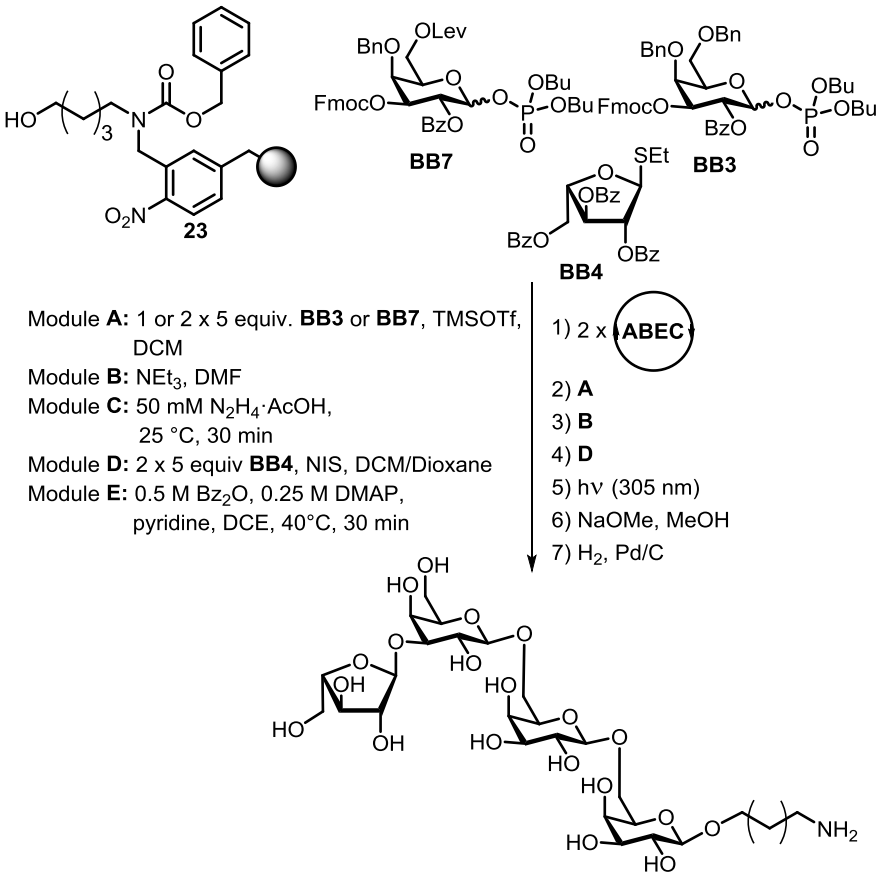
The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected heptasaccharide **53** (1.9 mg, 1.55 µmol, 9% over 21 steps, based on resin loading).
^{1}H NMR (600 MHz, D_2O) δ = 5.40 (d, *J* = 0.8 Hz, 1H), 4.86 (d, *J* = 7.7 Hz, 1H), 4.78 (d, *J* = 7.6 Hz, 1H), 4.69 (d, *J* = 7.9 Hz, 1H), 4.62 (dd, *J* = 7.9, 4.6 Hz, 3H), 4.41 (dd, *J* = 8.1, 3.2 Hz, 2H), 4.38 (dd, *J* = 3.4, 1.5 Hz, 1H), 4.32 – 4.28 (m, 2H), 4.23 – 4.18 (m, 3H), 4.14 – 4.05 (m, 11H), 4.03 (dd, *J* = 9.9, 3.2 Hz, 1H), 4.01 – 3.97 (m, 2H), 3.96 – 3.90 (m, 7H), 3.90 – 3.80 (m, 11H), 3.78 (dd, *J* = 9.8, 7.7 Hz, 1H), 3.72 – 3.66 (m, 2H), 3.20 – 3.16 (m, 2H), 1.90 – 1.82 (m, 4H), 1.63 (dt, *J* = 15.3, 7.7 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 107.0, 102.1, 101.6, 101.2, 101.2, 100.9, 100.1, 81.6, 80.3, 79.7, 79.1, 77.9, 74.4, 72.9, 72.9, 72.6, 71.4, 71.4, 71.0, 70.5, 70.4, 70.3, 68.8, 68.5, 68.5, 68.0, 67.7, 67.6, 67.3, 67.2, 67.2, 66.9, 66.4, 66.4, 66.2, 66.2, 66.1, 59.0, 58.8, 58.8, 58.7, 37.1, 25.9, 24.2, 19.9 ppm. HRMS: m/z $[M+Na]^+$ calcd. for $C_{46}H_{81}NNaO_{35}$: 1230.4486, found: 1230.4396.

RP-HPLC of deprotected heptasaccharide **53** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min).

Aminopentyl α-L-arabinofuranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (54)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 5 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)

Module **A** (1 x 5 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)

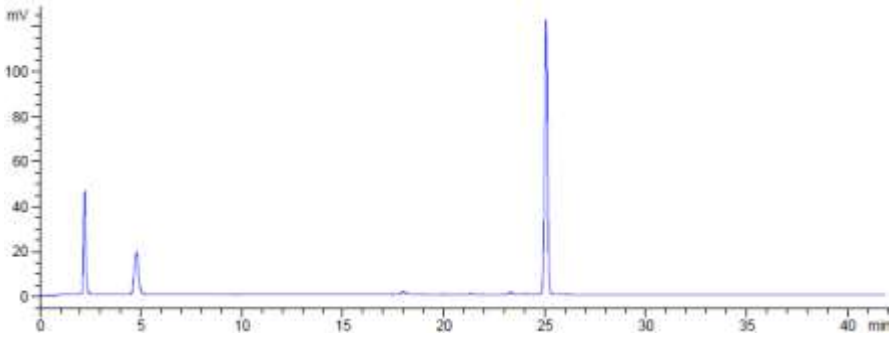
Module **A** (1 x 5 equiv. **BB3** TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 5 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected tetrasaccharide **54**.

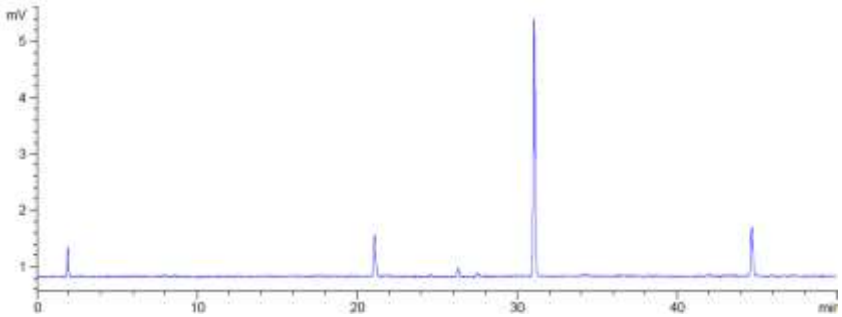
Crude NP-HPLC of ptotected tetrasaccharide **54** (ELSD trace):



HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected tetrasaccharide **54** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.5 mL) was added. The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The semi-protected trisaccharide was purified by semi-preparative RP HPLC (C5 column).

Crude RP-HPLC of the semi-protected tetrasaccharide **54** (ELSD trace):



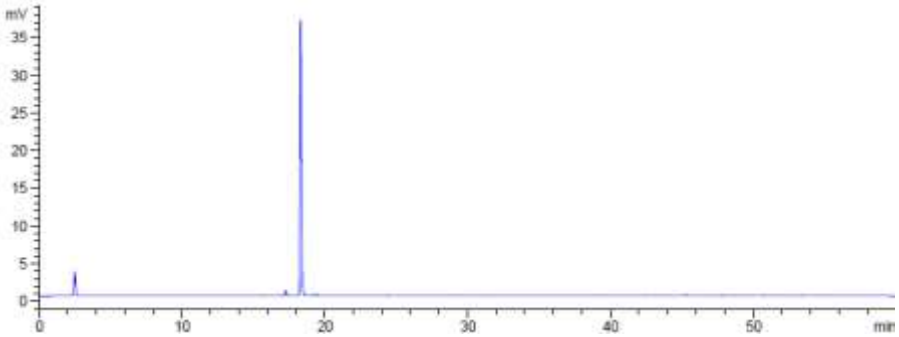
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/H_2O/AcOH (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 5 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated. The fully deprotected tetrasaccharide **54** was re-dissolved in water and purified by filtration through a SepPak C18 column to give the tetrasaccharide **54** (1.2 mg, 1.66 µmol, 8% over 14 steps, based on resin loading).

^{1}H NMR (600 MHz, D_2O) δ = 5.41 (d, *J* = 1.2 Hz, 1H), 4.68 (d, *J* = 7.9 Hz, 1H), 4.65 (d, *J* = 7.9 Hz, 1H), 4.59 (d, *J* = 8.0 Hz, 1H), 4.38 (dd, *J* = 3.5, 1.7 Hz, 1H), 4.32 – 4.26 (m, 2H),

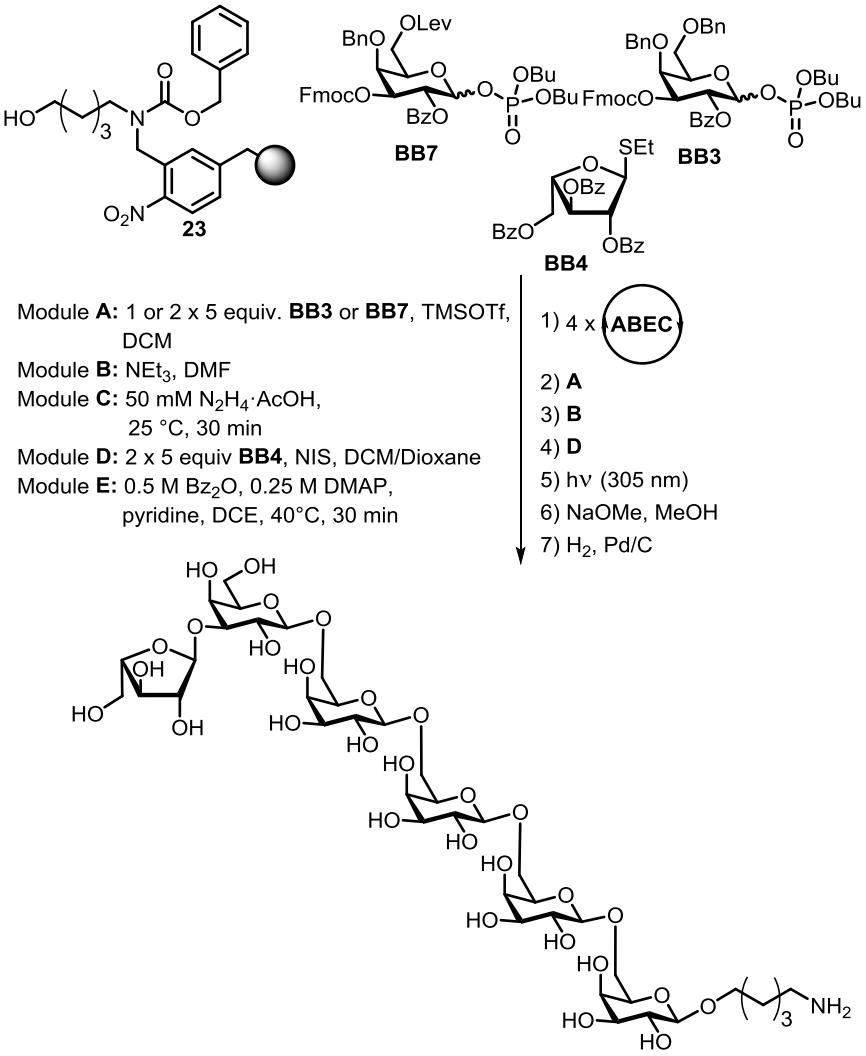
4.23 (ddd, *J* = 10.0, 4.7, 3.2 Hz, 2H), 4.17 – 4.04 (m, 8H), 4.02 – 3.80 (m, 11H), 3.69 (ddd, *J* = 17.8, 9.8, 8.0 Hz, 1H), 3.19 – 3.15 (m, 2H), 1.91 – 1.82 (m, 4H), 1.64 (q, *J* = 8.0 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 107.0, 101.1, 100.9, 100.5, 81.6, 79.1, 78.0, 74.3, 72.8, 71.6, 71.5, 70.4, 70.4, 68.5, 67.9, 67.7, 67.1, 66.5, 66.4, 66.2, 59.0, 58.7, 37.2, 26.0, 24.3, 19.9 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{28}H_{51}NNaO_{20}$: 744.2897, found: 744.2928.

RP-HPLC of the deprotected tetrasaccharide **54** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

Aminopentyl α-L-arabinofuranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (55)



Linker functionalized resin **23** (53 mg, 16.9 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (1 x 5 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)

Module **A** (1 x 5 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)

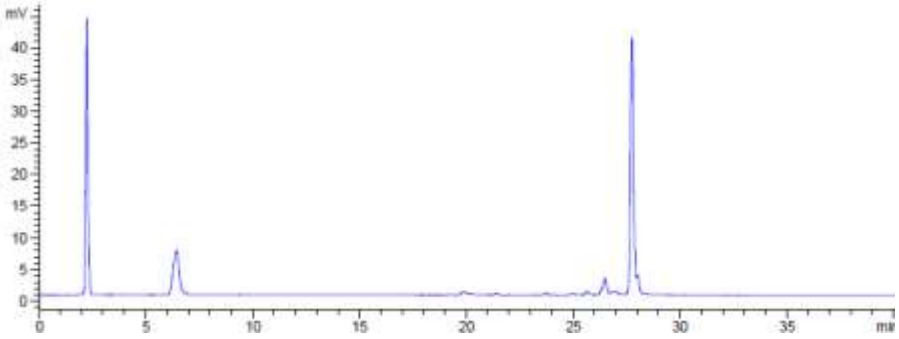
Module **A** (1 x 5 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (3 cycles)

Module **C** (3 cycles)
Module **A** (1 x 5 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **E** (3 cycles)
Module **C** (3 cycles)
Module **A** (1 x 5 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Module **D** (2 x 5 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide **55**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column (18 mg, 6.06 µmol, 36%).

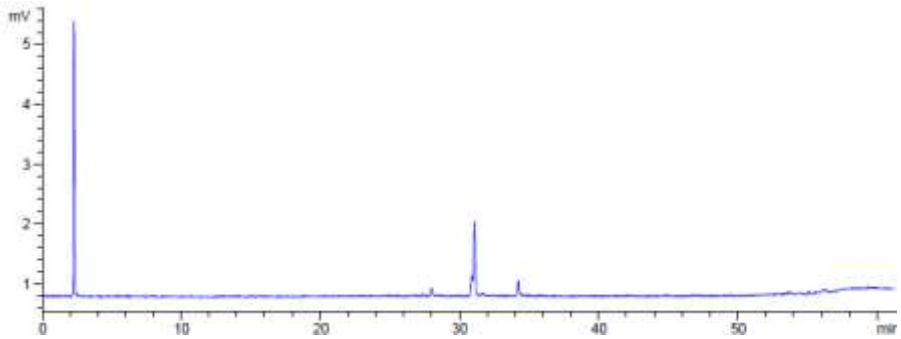
Crude NP-HPLC of ptotected pentasaccharide **55** (ELSD trace):



HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected pentasaccharide **55** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 0.3 mL) was added. Upon addition of the base a precipitate formed which could be dissolved again by addition of MeOH (1 mL). The reaction mixture was stirred overnight at rt and subsequently neutralized by the addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The product was not soluble in MeCN/water and therefore not purified by reversed phase HPLC but instead used in the final deprotection step without purification.

Crude RP-HPLC of the semi-protected tetrasaccharide **55** (ELSD trace):



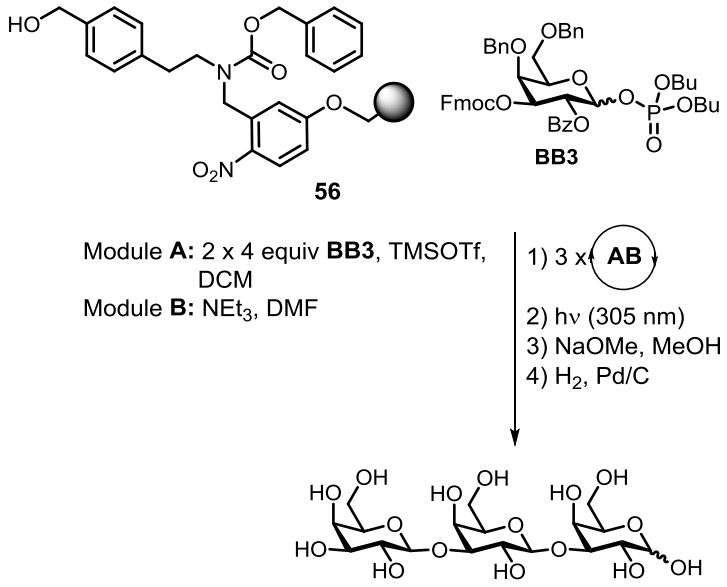
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The crude semi-protected pentasaccharide **55** was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min, and stirred under a H_2-atmosphere overnight at rt. Due to incomplete benzyl cleavage after stirring overnight the hydrogenation procedure was repeated and the reaction was left stirring at rt overnight again. After filtration of the reaction mixture through a syringe filter the solvents were evaporated and the fully deprotected hexasaccharide **55** was purified by reversed phase HPLC using a semi-preparative Hypercarb column (0.2 mg, 0.14 µmol, 1% over 18 steps, based on resin loading).

^{1}H NMR (700 MHz, D_2O) δ = 5.41 (d, *J* = 1.6 Hz, 1H), 4.68 (d, *J* = 8.0 Hz, 1H), 4.67 – 4.63 (m, 3H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.38 (dd, *J* = 3.5, 1.8 Hz, 1H), 4.30 (td, *J* = 6.2, 3.3 Hz, 1H), 4.28 (d, *J* = 3.3 Hz, 1H), 4.22 (ddd, *J* = 8.3, 6.2, 2.7 Hz, 4H), 4.15 – 4.05 (m, 14H), 4.00 (dd, *J* = 12.2, 3.3 Hz, 1H), 3.97 – 3.92 (m, 2H), 3.91 – 3.81 (m, 9H), 3.73 – 3.66 (m, 4H), 3.20 – 3.17 (m, 2H), 1.86 (dp, *J* = 14.4, 7.3 Hz, 4H), 1.63 (p, *J* = 7.8 Hz, 2H) ppm. ESI-HRMS: m/z $[M+H]^+$ calcd. For $C_{40}H_{72}NO_{30}$: 1046,4139, found: 1046.4185. The product **55** was not obtained in sufficient amounts to measure an adequate ^{13}C NMR spectrum.

3.3.4 Automated Glycan Assembly of Arabinogalactans with Free Reducing Ends

β-D-Galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-D-galactopyranose (57)



Linker functionalized resin **56** (60 mg, 15.1 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 4 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **C** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 4 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

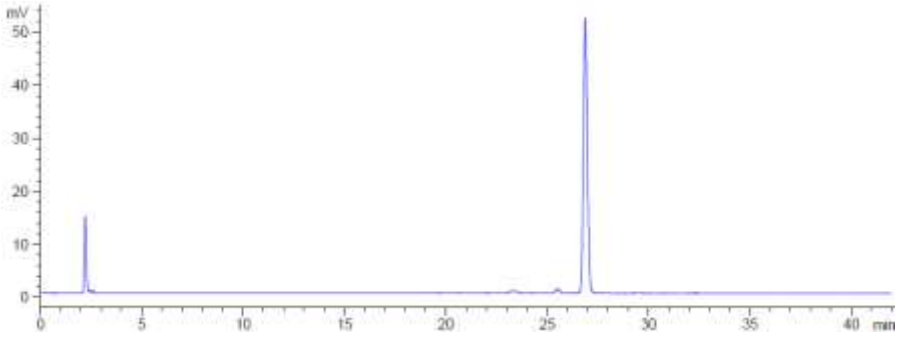
Module **C** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 4 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **C** (20% NEt_3 in DMF, 3 x 5 min, rt)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected trisaccharide **57**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column (12 mg, 7.39 µmol, 49%).

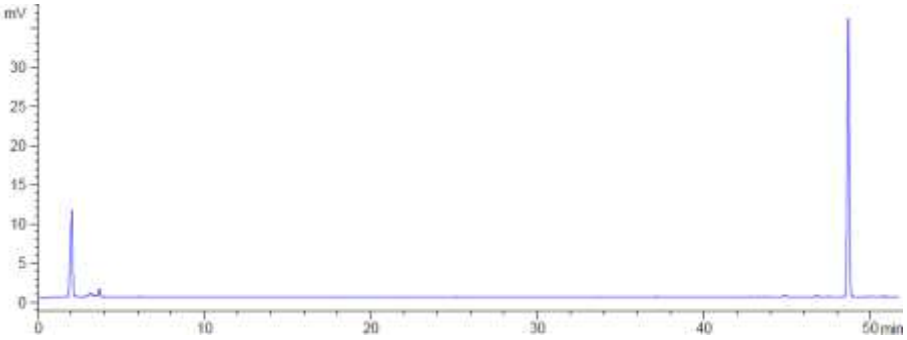
Crude NP-HPLC of protected trisaccharide **57** (ELSD trace):



HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected trisaccharide **57** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected trisaccharide.

Crude RP-HPLC of the semi-protected trisaccharide **57** (ELSD trace):



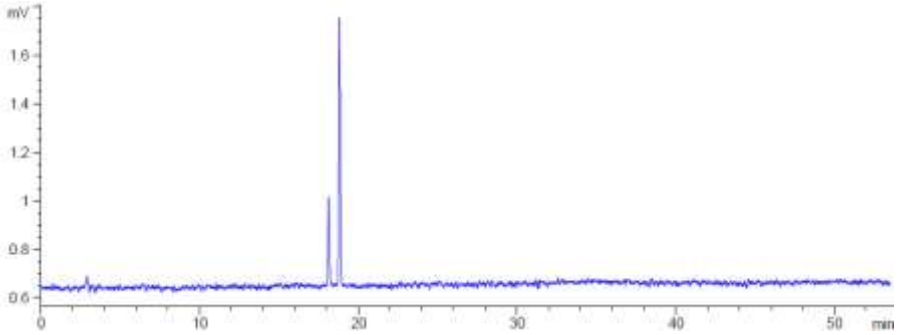
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/H_2O/AcOH (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 5 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated. The fully deprotected trisaccharide **57** was re-dissolved in 0.5 mL water and stirred for one hour with a spatula tip of H^+-Amberlite resin to hydrolyze a side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully de-protected trisaccharide **57** was directly purified by reversed phase HPLC using a semi-

preparative Hypercarb column to give an α/β–mixture of the trisaccharide **57** (0.6 mg, 1.19 µmol, 8% over 9 steps, based on resin loading).

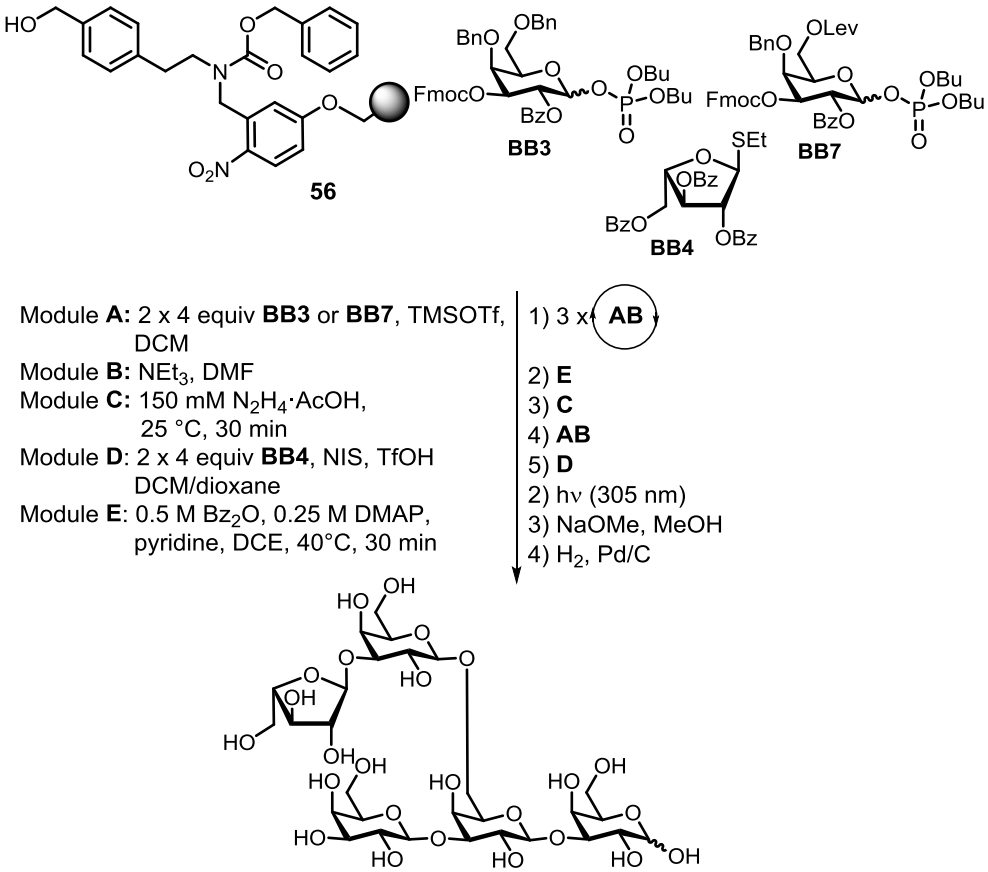
^{1}H NMR (600 MHz, D_2O) δ = 5.30 (d, *J* = 3.4 Hz, 1H), 4.70 (t, *J* = 7.4 Hz, 1H), 4.65 (t, *J* = 8.1 Hz, 2H), 4.28 (d, *J* = 2.0 Hz, 1H), 4.22 (b, 2H), 4.14 (t, *J* = 6.1 Hz, 1H), 4.01 (qd, *J* = 10.3, 3.2 Hz, 1H), 3.94 (d, *J* = 3.2 Hz, 1H), 3.88 – 3.82 (m, 2H), 3.81 – 3.61 (m, 13H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 106.6, 98.7, 85.0, 84.6, 81.9, 77.3, 77.2, 75.1, 73.6, 73.5, 72.8, 71.1, 70.9, 63.7, 63.5, 63.5, 63.4, 63.4 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{18}H_{32}NaO_{16}$: 527.1588, found: 527.1534

RP-HPLC of the deprotected trisaccharide **57** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

β-D-Galactopyranosyl-(1→3)-6-*O*-[3-*O*-[α-L-arabinofuranosyl]-β-D-galactopyranosyl]-β-D-galactopyranosyl-(1→3)-D-galactopyranose (59)



Linker functionalized resin **56** (60 mg, 15.1 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 4 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 4 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 4 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **E** (0.5 M Bz_2O, 0.25 M DMAP, Py, DCE, 3 x 30 min, 40 °C)

Module **C** (0.15 M N_2H_4 in Py/AcOH/H_2O (4:1:0.25), 3 x 30 min, 25 °C)

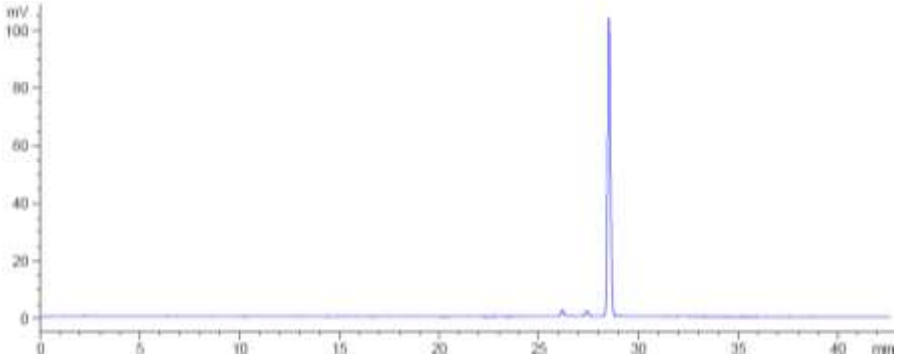
Module **A** (2 x 4 equiv. **BB3**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **D** (2 x 4 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected pentasaccharide **58**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column (17 mg, 6.72 µmol, 45%).

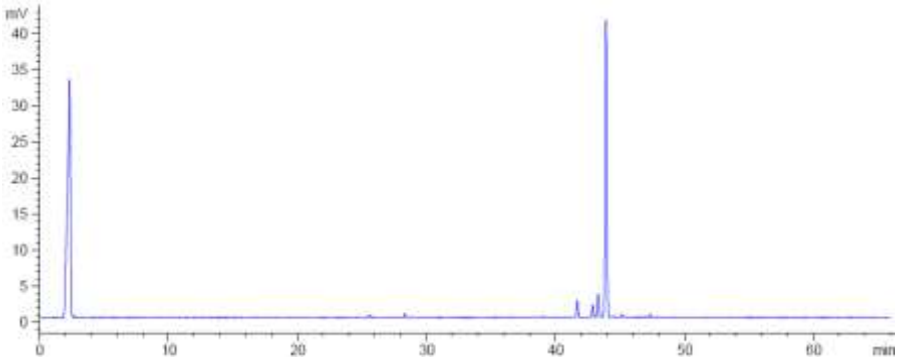
Crude NP-HPLC of protected pentasaccharide **59** (ELSD trace):



HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected pentasaccharide **59** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected hexasaccharide.

Crude RP-HPLC of the semi-protected pentasaccharide (ELSD trace):

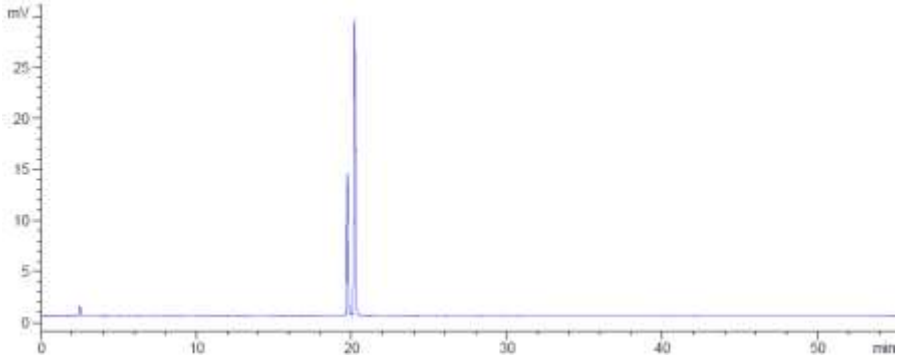


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/H_2O/AcOH (4:2:2:1, 3 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated until approx. 1 mL of the solvent mixture remained and the solvents were removed at a lyophilizer. The fully deprotected pentasaccharide **59** was re-dissolved in 0.5 mL water and purified by reversed phase

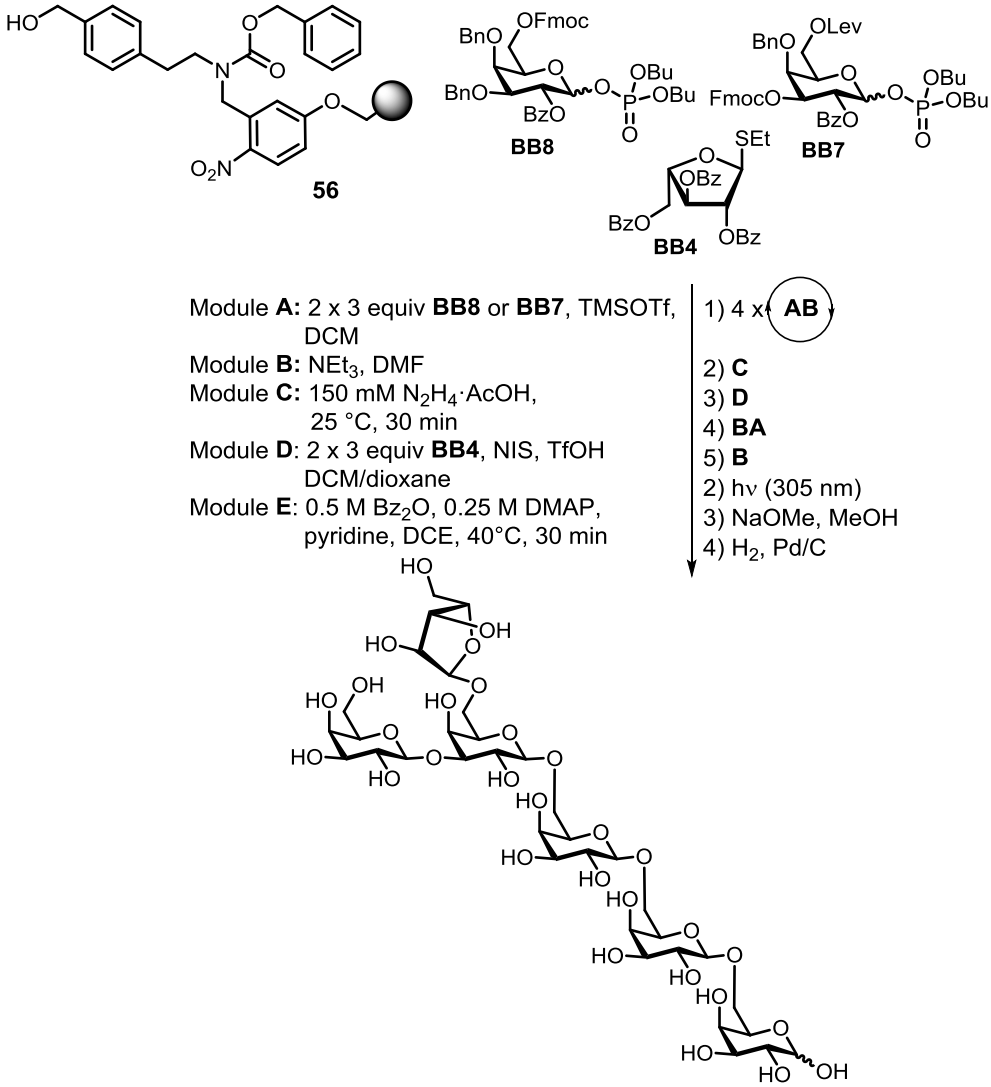
HPLC using a semi-preparative Hypercarb column to give an α/β-mixture of the pentasaccharide **59** (1.2 mg, 1.48 µmol, 10% over 14 steps, based on resin loading).
^{1}H NMR (600 MHz, D_2O): δ = 5.15 (s, 1H), 5.10 (s, 1H), 4.55 (t, *J* = 8.0 Hz, 1H), 4.48 (t, *J* = 8.6 Hz, 1H), 4.34 (d, *J* = 6.2 Hz, 1H), 4.16 (s, 1H), 4.08 (d, *J* = 15.4 Hz, 3H), 3.97 (d, *J* = 15.5 Hz, 2H), 3.93 – 3.74 (m, 7H), 3.73 – 3.45 (m, 17H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 111.8, 111.8, 106.9, 106.4, 106.4, 105.8, 98.7, 98.7, 94.7, 86.4, 84.9, 84.9, 84.5, 83.8, 83.8, 82.8, 82.8, 81.9, 79.0, 79.0, 77.6, 77.5, 77.3, 75.8, 75.8, 75.1, 73.6, 73.5, 72.7, 72.6, 72.5, 71.8, 71.7, 71.7, 71.7, 71.1, 71.0, 71.0, 71.0, 70.9, 69.9, 63.8, 63.7, 63.5 ppm. $[M+Na]^+$ calcd. for $C_{29}H_{50}NaO_{25}$: 821.2538, found: 821.2538

RP-HPLC of the deprotected pentasaccharide **59** (ELSD trace):



HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

β-D-Galactopyranosyl-(1→3)-[6-*O*-[α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-D-galactopyranose (60)



Linker functionalized resin **56** (85 mg, 22.1 µmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A** (2 x 3 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3 equiv. **BB7**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)

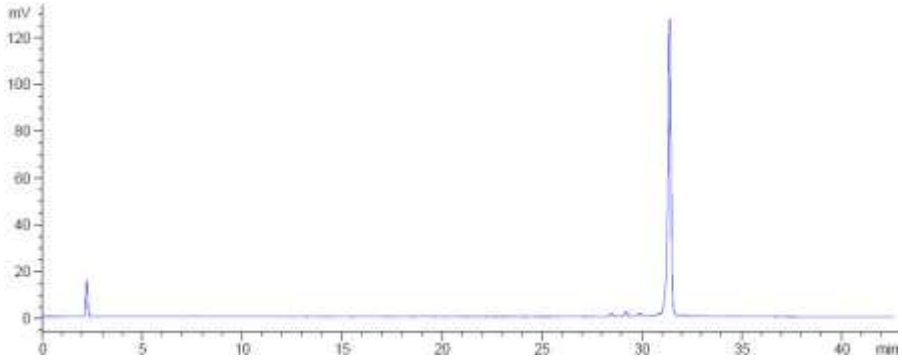
Module **C** (0.15 M N_2H_4 in Py/AcOH/H_2O (4:1:0.25), 3 x 30 min, 25 °C)

Module **D** (2 x 3 equiv. **BB4**, NIS, TfOH, DCM/Dioxane, 2 x 45 min, -40 °C to -20 °C)

Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)

Module **A** (2 x 3 equiv. **BB8**, TMSOTf, DCM, 2 x 35 min, -35 °C to -20 °C)
Module **B** (20% NEt_3 in DMF, 3 x 5 min, rt)
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the protected hexasaccharide **60**. The crude product was purified by normal phase HPLC using a preparative YMC Diol column (23 mg, 8.01 µmol, 36%).

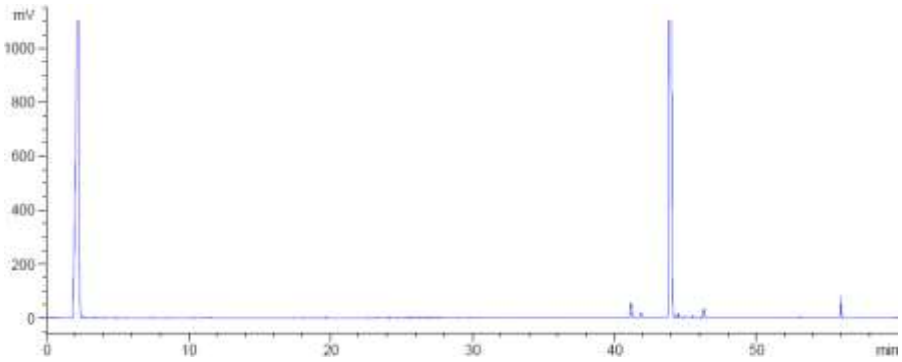
Crude NP-HPLC of protected hexasaccharide **60** (ELSD trace):



HPLC was performed using a YMC Diol column and a linear gradient from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

The protected hexasaccharide **60** was dissolved in THF (5 mL) and NaOMe (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo* to afford the semi-protected hexasaccharid.

Crude RP-HPLC of the semi-protected hexasaccharide **60** (ELSD trace):



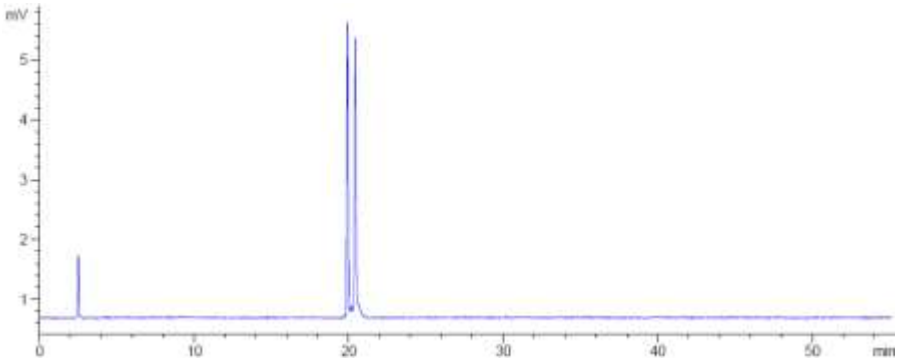
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H_2O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

The product was dissolved in a mixture of EtOAc/MeOH/H_2O/AcOH (4:2:2:1, 5 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10 mg). The suspension was flushed with argon for 10 min, then saturated with H_2 for 10 min and stirred under a H_2-atmosphere overnight. The hydrogenation procedure was repeated two times until all protecting groups were removed. After filtration of the reaction

mixture through a syringe filter the solvents were evaporated. The fully deprotected hexasaccharide **60** was re-dissolved in 0.5 mL water and stirred for one hour with a spatula tip of H^+-Amberlite resin to hydrolyze a side product that has been formed by condensation of the oligosaccharide with the cleaved linker. The fully deprotected hexasaccharide **60** was purified by reversed phase HPLC using a semi-preparative Hypercarb column to give an α/β-mixture of the hexasaccharide **60** (0.8 mg, 0.85 µmol, 4% over 15 steps, based on resin loading).

^{1}H NMR (700 MHz, D_2O) δ = 5.29 (d, *J* = 3.8 Hz, 1H), 5.11 (s, 1H), 4.65 – 4.61 (m, 2H), 4.55 (d, *J* = 7.9 Hz, 1H), 4.52 – 4.47 (m, 2H), 4.31 (dd, *J* = 7.8, 4.0 Hz, 1H), 4.23 (d, *J* = 3.0 Hz, 1H), 4.14 – 4.13 (m, 1H), 4.10 – 4.05 (m, 5H), 4.00 – 3.67 (m, 24H), 3.65 – 3.62 (m, 1H), 3.58 – 3.55 (m, 2H), 3.52 (dd, *J* = 9.8, 8.1 Hz, 1H) ppm. ^{13}C NMR (176 MHz, D_2O) δ = 107.8, 104.3, 103.4, 103.2, 103.1, 96.4, 92.3, 83.8, 82.2, 81.0, 76.4, 75.0, 73.7, 73.5, 72.6, 72.5, 71.7, 71.0, 70.7, 70.6, 69.7, 69.5, 69.4, 69.3, 69.2, 68.9, 68.7, 68.6, 68.5, 68.2, 67.3, 61.1, 60.9 ppm. $[M+Na]^+$ calcd. for $C_{35}H_{60}NaO_{30}$: 983.3067, found: 983.3007

RP-HPLC of the deprotected hexasaccharide **60** (ELSD trace):

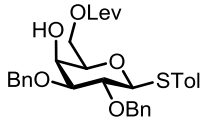


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H_2O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H_2O in MeCN (10 min, flow rate 0.7 mL/min).

3.4 Synthesis of RG-II Fragments

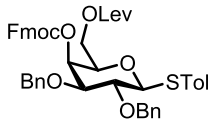
3.4.1 Synthesis of Building Blocks for the Automated Glycan Assembly of Substituted Homogalacturonan Fragments

_p_-Tolyl 2,3-_O_-dibenzyl-6-_O_-levulinoyl-1-thio-β-D-galactopyranoside (70a)



To a solution of dihydroxy galactose **69**[170] (3.00 g, 6.43 mmol) in anhydrous DCM (150 mL) at -6 °C (salt-water-ice bath) were subsequently added DMAP (78.1 mg, 0.643 mmol, 0.1 equiv) and DIC (1.00 mL, 6.43 mmol, 1 equiv). Levulinic acid (0.622 mL, 6.11 mmol) dissolved in anhydrous DCM (10 mL) was added dropwise. The solution was stirred at 0 °C for 1 h, slowly allowed to warm to rt and kept stirring at rt overnight. The reaction was quenched by the addition of water (10 mL) and the reaction mixture was washed with brine. The organic phase was separated, dried over $MgSO_4$, the solvent was removed *in vacuo* and the residue was subjected to silica column chromatography (hex/EtOAc = 3:2), yielding the levulinoyl protected intermediate **70a** (2.93 g, 5.19 mmol, 81%) as a colorless foam. $[\alpha]_D^{25}$ = +15.9 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49 – 7.44 (m, 2H, Ar), 7.44 – 7.39 (m, 2H, Ar), 7.38 – 7.28 (m, 8H, Ar), 7.11 – 7.07 (m, 2H, Ar), 4.83 (d, *J* = 10.3 Hz, 1H, OC*H*$_2$Ph), 4.74 (d, *J* = 10.3 Hz, 1H, OC*H*$_2$Ph), 4.71 (d, *J* = 1.8 Hz, 2H, OC*H*$_2$Ph), 4.55 (d, *J* = 9.7 Hz, 1H, H-1), 4.40 – 4.29 (m, 2H, H-6), 4.03 – 3.99 (m, 1H, H-4), 3.69 (dd, *J* = 9.7, 9.0 Hz, 1H, H-2), 3.63 – 3.55 (m, 2H, H-3, H-5), 2.78 – 2.73 (m, 2H, OLev), 2.60 – 2.55 (m, 2H, OLev), 2.40 (dd, *J* = 2.8, 1.1 Hz, 1H, 4-O*H*), 2.32 (s, 3H, SPhC*H*$_3$), 2.18 (s, 3H, OLev) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 206.7 (C=O), 172.8 (C=O), 138.3, 137.8, 137.6, 132.7, 129.9, 129.8, 128.7, 128.5, 128.4, 128.2, 128.1, 128.0 (18 C, Ar), 88.0 (C-1), 82.4 (C-3), 76.9 (C-2), 75.9 (O*C*H$_2$Ph), 75.6 (C-5), 72.4 (O*C*H$_2$Ph), 66.5 (C-4), 63.4 (C-6), 38.1 (OLev), 30.0 (OLev), 28.0 (OLev), 21.3 (SPh*C*H$_3$) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{32}H_{36}NaO_7S$: 587.2074, found: 587.2087. IR (neat) ν_{max}: 1704, 1088 cm^{-1}.

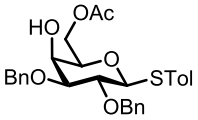
_p_-Tolyl 2,3-_O_-dibenzyl-4-_O_-fluorenylcarbonylmethoxy-6-_O_-levulinoyl-1-thio-β-D-galactopyranoside (BB10a)



Intermediate **70a** (0.699 g, 1.24 mmol) was dissolved in anhydrous DCM (50 mL) under argon atmosphere. Anhydrous pyridine (0.400 mL, 4.96 mmol) was added at rt, the solution was stirred at rt for 5 min and FmocCl (1.28 g, 4.96 mmol, 4 equiv) was added.

The reaction was left stirring at rt under argon atmosphere overnight. As after stirring overnight still starting material was detected by TLC, another 1.00 g (3.88 mmol, 3.1 equiv) FmocCl was added and the reaction was left stirring at rt for additional three hours. Recrystallization from boiling EtOH gave the desired product (0.554 g, 0.704 mmol, 57%) as a colorless solid. $[\alpha]_D^{25}$ = -1.4 (*c* 1.0, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.79 (d, *J* = 7.6 Hz, 2H, Ar), 7.63 (dd, *J* = 19.2, 7.4 Hz, 2H, Ar), 7.51 (d, *J* = 7.9 Hz, 2H, Ar), 7.47 – 7.38 (m, 4H, Ar), 7.33 (m, 7H, Ar), 7.22 – 7.15 (m, 3H, Ar), 7.11 (d, *J* = 7.8 Hz, 2H, Ar), 5.41 (d, *J* = 3.1 Hz, 1H, H-4), 4.84 – 4.75 (m, 3H, OC*H*$_2$Ph), 4.64 (d, *J* = 9.6 Hz, 1H, H-1), 4.57 (d, *J* = 11.3 Hz, 1H, OC*H*$_2$Ph), 4.47 – 4.30 (m, 3H, H-6', Fmoc), 4.27 – 4.19 (m, 2H,H-6´´, Fmoc), 3.82 (t, *J* = 6.9 Hz, 1H, H-5), 3.78 (t, *J* = 9.5 Hz, 1H, H-2), 3.69 (dd, *J* = 9.2, 3.1 Hz, 1H, H-3), 2.75 (t, *J* = 6.5 Hz, 2H, *O*Lev), 2.60 – 2.56 (m, 2H, OLev), 2.31 (s, 3H, SPh*C*H$_3$), 2.17 (s, 3H, OLev) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 206.6 (C=O), 172.4 C=O), 155.1, 143.7, 143.3, 141.4, 141.4, 138.3, 137.9, 137.6, 132.7, 129.8, 129.7, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 127.9, 127.3, 127.3, 125.5, 125.3, 120.1, 120.1 (30 C, Ar), 88.2 (C-1), 81.0 (C-3), 76.7 (C-2), 76.0 (O*C*H$_2$Ph), 74.1 (C-5), 72.2 (O*C*H$_2$Ph), 70.9 (C-4), 70.3 (OFmoc), 62.3 (C-6), 46.7 (OFmoc), 38.1 (OLev), 29.9 (OLev), 28.0 (OLev), 21.3 (SPh*C*H$_3$) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{47}H_{46}NaO_9S$: 809.2755, found 809.2771. IR (neat) ν_{max}: 1745, 1707, 1260, 1235 cm^{-1}.

_p_-Tolyl 2,3-_O_-dibenzyl-6-_O_-acetyl-1-thio-β-D-galactopyranoside (70b)

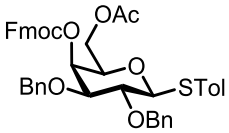


To a solution of **69**[170] (1.68 g, 3.61 mmol) in DCM (50 mL) at 0 °C were subsequently added DMAP (44.0 mg, 0.361 mmol, 0.1 equiv), DCC (0.745 g, 3.61 mmol, 1 equiv) and acetic acid (0.227 mL, 3.97 mmol, 1.1 equiv) dissolved in DCM (5.00 mL). The solution was stirred at rt for 2 h. A white precipitate slowly formed. After complete conversion, the solvent was removed under reduced pressure and purification by column chromatography (hex/EtOAc = 3:1 to 3:2) yielded **70b** (1.51 g, 2.97 mmol, 82% yield) as a colorless solid.

$[\alpha]_D^{25}$ = +8.8 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.50 – 7.46 (m, 2H, Ar), 7.44 – 7.41 (m, 2H, Ar), 7.39 – 7.29 (m, 8H, Ar), 7.12 – 7.07 (m, 2H, Ar), 4.85 (d, *J* = 10.3 Hz, 1H, OC*H*$_2$Ph), 4.74 (d, *J* = 10.3 Hz, 1H, OC*H*$_2$Ph), 4.71 (s, 2H, OC*H*$_2$Ph), 4.55 (d, *J* = 9.7 Hz, 1H, H-1), 4.39 – 4.28 (m, 2H, H-6), 3.99 – 3.97 (m, 1H, H-4), 3.70 (t, *J* = 9.3 Hz, 1H, H-2), 3.64 – 3.55 (m, 2H, H-3, H-5), 2.40 (dd, J = 2.7, 1.2 Hz, 1H, 4-O*H*) 2.33 (s, 3H, SPhC*H*$_3$), 2.07 (s, 3H, OAc) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.9 (C=O), 138.2, 137.8, 137.6, 132.7, 129.9, 129.7, 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 128.0 (18 C, Ar), 88.0 (C-1), 82.4 (C-3), 77.0 (C-2), 75.9 (O*C*H$_2$Ph), 75.7 (C-5), 72.5 (O*C*H$_2$Ph), 66.8

(C-4), 63.4 (C-6), 21.2 (SPh*C*H_3), 21.0 (OAc) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{29}H_{32}NaO_6S$: 531.1812, found: 531.1812. IR (neat) ν_{max}: 1718, 1270, 1081, 1046 cm^{-1}.

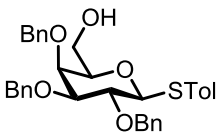
_p_-Tolyl phenyl 2,3-_O_-dibenzyl-4-_O_- fluorenylcarbonylmethoxy -6-_O_-acetyl-1-thio-β-D-galactopyranoside (BB10b)



70b (1.43 g, 2.81 mmol) was dissolved in anhydrous DCM (50 mL) under argon atmosphere and pyridine (0.453 mL, 5.62 mmol, 2 equiv) was added at rt. FmocCl (1.46 g, 5.62 mmol, 2 equiv) was added and the reaction was left stirring under argon atmosphere overnight. The solvent was removed *in vacuo* and the residue was subjected to silica gel column chromatography (hex/EtOAc = 4:1) to obtain product **BB10b** (1.54 g, 2.10 mmol, 75%) as a colorless solid.

$[\alpha]_D^{25}$ = -2.7 (*c* 0.2, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.82 – 7.75 (m, 2H, Ar), 7.67 – 7.58 (m, 2H, Ar), 7.53 – 7.49 (m, 2H, Ar), 7.46 – 7.39 (m, 4H, Ar), 7.38 – 7.26 (m, 7H, Ar), 7.21 – 7.16 (m, 3H, Ar), 7.13 – 7.09 (m, 2H, Ar), 5.37 (dd, *J* = 3.3, 1.0 Hz, 1H, H-4), 4.84 – 4.74 (m, 3H, OC*H*$_2$Ph), 4.62 (d, *J* = 9.7 Hz, 1H, H-1), 4.56 (d, *J* = 11.2 Hz, 1H, OC*H*$_2$Ph), 4.48 – 4.20 (m, 5H, H-6, Fmoc), 3.84 – 3.72 (m, 2H, H-2, H-5), 3.68 (dd, *J* = 9.1, 3.2 Hz, 1H, H-3), 2.31 (s, 3H, SPh*C*H_3), 2.08 (s, 3H, OAc) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7 (C=O), 155.1 (C=O), 143.6, 143.2, 141.4, 141.3, 138.2, 138.0, 137.5, 132.7, 129.8, 129.7, 128.5, 128.4, 128.4, 128.0, 128.0, 127.9, 127.9, 127.3, 127.2, 125.5, 125.3, 120.2, 120.1, 120.1 (30 , Ar), 88.2 (C-1), 81.0 (C-3), 76.7 (C-2), 76.0 (O*C*H$_2$Ph), 74.3 (C-5), 72.2 (O*C*H$_2$Ph), 71.0 (C-4), 70.3 (Fmoc), 62.5 (C-6), 46.7 (Fmoc), 21.3 (SPh*C*H_3), 20.9 (OAc) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{44}H_{42}NaO_8S$: 753.2503, found: 753.2493. IR (neat) ν_{max}: 1743, 1258, 1225, 1034 cm^{-1}.

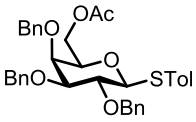
_p_-Tolyl 2,3,4-tri-_O_-benzyl-1-thio-β-D-galactopyranoside (68)



To solution of 4-methyl phenyl 2,3-*O*-dibenzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (67)[222] (6.99 g, 12.6 mmol) in anhydrous DCM (50 mL) under argon atmosphere at 0 °C were sequentially added borane (25.2 mL of a 1 M solution in THF, 25.2 mmol, 2 equiv) and TMSOTf (1.14 mL, 6.30 mmol, 0.5 equiv). The reaction was stirred at 0°C for 90 min before it was allowed to warm to rt. The reaction was left stirring at rt for one hour. After completion, the reaction was quenched by the addition of NEt_3 (1.00 mL) and MeOH (10.0 mL) and the resulting mixture was concentrated *in vacuo*,

yielding intermediate 68 (7.00 g, 12.6 mmol) in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.48 – 7.44 (m, 2H), 7.42 – 7.29 (m, 15H), 7.04 (d, *J* = 7.9 Hz, 2H), 4.98 (d, *J* = 11.6 Hz, 1H), 4.83 (d, *J* = 10.2 Hz, 1H), 4.79 – 4.74 (m, 3H), 4.67 – 4.56 (m, 3H), 3.92 (t, *J* = 9.4 Hz, 1H), 3.87 – 3.80 (m, 1H), 3.61 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.52 (dd, *J* = 11.3, 5.1 Hz, 1H), 3.45 – 3.40 (m, 1H), 2.31 (s, 3H) ppm. The analytical data is in agreement with literature data.[223]

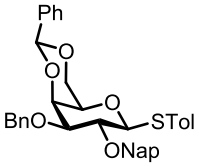
_p_-Tolyl 2,3,4-tri-_O_-benzyl-6-_O_-acetyl-1-thio-β-D-galactopyranoside (BB12)



To a solution of **68** (7.00 g, 12.6 mmol) in DCM (200 mL) at 0 °C were added DMAP (0.154 g, 1.26 mmol, 0.1 equiv), DIC (1.96 mL, 12.6 mmol, 1 equiv), and acetic acid (0.792 mL, 13.8 mmol, 1.1 equiv) dissolved DCM (5.00 mL). A white precipitate slowly formed when the solution was stirred for 3 h at rt. The solvent was removed *in vacuo* and purification over silica column chromatography (hex/EtOAc = 3:2) yielded **BB12** (4.36 g, 7.28 mmol, 58% yield) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52 (d, *J* = 7.6 Hz, 2H), 7.46 – 7.31 (m, 15H), 7.07 (d, *J* = 7.6 Hz, 2H), 5.03 (d, *J* = 11.4 Hz, 1H), 4.87 (d, *J* = 10.0 Hz, 1H), 4.81 – 4.75 (m, 3H), 4.68 (d, *J* = 11.8 Hz, 1H), 4.61 (d, *J* = 9.4 Hz, 1H), 4.30 (dd, *J* = 11.2, 6.7 Hz, 1H), 4.15 (dd *J* = 11.3, 5.5 Hz, 1H), 3.96 (t, *J* = 9.3 Hz, 1H), 3.90 – 3.86 (m, 1H), 3.63 (dt, *J* = 12.6, 4.6 Hz, 2H), 2.34 (s, 3H), 2.03 (s, 3H) ppm.The analytical data is in agreement with literature data.[224]

_p_-Tolyl 2-_O_-naphtyl-3-_O_-benzyl-4,6-_O_-benzylidene-1-thio-β-D-galactopyranoside (72)

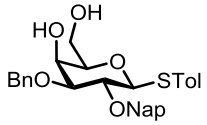


The benzylidene intermediate **71**[225] (3.98 g, 8.57 mmol) was dissolved in a mixture of anhydrous DCM and anhydrous DMF (1:1, 100 mL) under argon atmosphere. To the solution were added sodium hydride (1.03 g, 26.0 mmol, 60% dispension in mineral oil, 3 equiv) and BnBr (2.32 mL, 3.34 g, 19.5 mmol, 2.3 equiv) at 0° C. The ice-bath was removed and the mixture was stirred at rt for 3 h. After quenching of the excess sodium hydride by careful addition of methanol, the reaction was worked up with sat. aq. NH_4Cl-solution and brine. The organic phase was dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The residue was subjected to silica gel column chromatography

(hex/EtOAc = 10:1), yielding **72** (4.62 g, 7.64 mmol, 89% yield over two steps) as a colorless solid.

$[\alpha]_D^{25}$ = -32.6 (*c* 2.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86 – 7.79 (m, 4H, Ar), 7.65 – 7.57 (m, 3H, Ar), 7.56 – 7.45 (m, 4H, Ar), 7.42 – 7.38 (m, 3H, Ar), 7.36 – 7.32 (m, 2H, Ar), 7.30 – 7.25 (m, 3H, Ar), 7.03 – 6.98 (m, 2H,Ar), 5.49 (s, 1H, PhC*H*), 4.88 (s, 2H, ONapC*H*$_2$), 4.73 (s, 2H, OC*H*$_2$Ph), 4.61 (d, *J* = 9.5 Hz, 1H, H-1), 4.39 (dd, *J* = 12.3, 1.6 Hz, 1H, H-6'), 4.17 (d, *J* = 3.0 Hz, 1H, H-4), 4.00 (dd, *J* = 12.3, 1.7 Hz, 1H, H-6''), 3.90 (t, *J* = 9.4 Hz, 1H, H-2), 3.66 (dd, *J* = 9.2, 3.4 Hz, 1H, H-3), 3.43 (b, 1H, H-5), 2.31 (s, 3H SPhC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.2, 138.0, 137.8, 136.2, 133.6, 133.4, 133.1, 129.8, 129.2, 128.7, 128.5, 128.2, 128.17, 128.1, 127.98, 127.96, 127.81, 126.84, 126.4, 126.3, 126.0, 125.9 (22 C, Ar), 101.5 (Ph*C*H), 86.7 (C-1), 81.5 (C-3), 75.6 (ONap*C*H$_2$), 75.5 (C-2), 73.7 (C-4), 71.9 (O*C*H$_2$Ph), 69.9 (C-5), 69.6 (C-6), 21.3 (SPh*C*H$_3$) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{38}H_{36}NaO_5S$: 627.2176, found: 627.2187. IR (neat) ν_{max}: 1100, 1090, 1057 cm^{-1}.

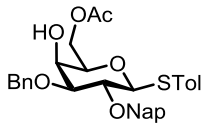
p-Tolyl 2-*O*-naphtyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (73)



To a solution of **72** (4.60 g, 7.61 mmol) in DCM (200 mL) a solution of TFA (5.00 ml, 64.9 mmol, 8.5 equiv) in water (20.0 mL) was added. The reaction was stirred at rt for 1 h before it was quenched by the addition of sat. aq. $NaHCO_3$-solution after one hour, although TLC did not indicate full conversion of the educt but also the formation of the naphthyl de-protected side-product. The separated organic phase was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (hex/EtOAc = 1:1) yielding **73** (1.99 g, 3.85 mmol, 51% yield).

$[\alpha]_D^{25}$ = +12.1 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.87 – 7.79 (m, 4H, Ar), 7.57 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.51 – 7.45 (m, 4H, Ar), 7.37 – 7.28 (m, 5H, Ar), 7.14 – 7.08 (m, 2H, Ar), 5.01 (d, *J* = 10.5 Hz, 1H, ONapC*H*$_2$), 4.91 (d, *J* = 10.6 Hz, 1H, ONapC*H*$_2$), 4.72 (d, *J* = 1.9 Hz, 2H, OC*H*$_2$Ph), 4.63 (d, *J* = 9.8 Hz, 1H, H-1, H-1), 4.09 – 4.06 (m, 1H, H-4), 4.02 – 3.94 (m, 1H, H-6'), 3.84 – 3.75 (m, 2H, H-2, H-6''), 3.61 (dd, *J* = 8.9, 3.3 Hz, 1H, H-3), 3.52 – 3.46 (m, 1H, H-5), 2.63 (dd, *J* = 2.1, 1.1 Hz, 1H, 4-O*H*)2.33 (s, 3H, SPhC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.0, 137.6, 135.8, 133.4, 133.2, 132.7, 129.9, 129.8, 128.7, 128.2, 128.1, 128.0, 127.8, 127.0, 126.4, 126.2, 126.0 (22 C, Ar), 88.0 (C-1), 82.6 (C-3), 78.1 (C-5), 76.9 (C-2), 75.9 (ONap*C*H$_2$), 72.4 (O*C*H$_2$Ph), 67.5 (C-4), 62.9 (C-6), 21.3 (SPh*C*H$_3$) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{31}H_{32}NaO_5S$: 539.1863, found: 539.1861. IR (neat) ν_{max}: 1085, 1056, 808 cm^{-1}.

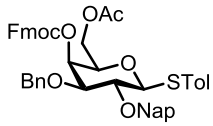
p-Tolyl 2-*O*-naphtyl-3-*O*-benzyl-6-*O*-acetyl-1-thio-β-D-galactopyranoside (74)



To a solution of **73** (1.96 g, 3.79 mmol) in anhydrous DCM (100 mL) at 0 °C were added DMAP (46.0 mg, 0.379 mmol, 0.1 equiv), DIC (0.621 mL, 3.98 mmol, 1.05 equiv), and acetic acid (0.239 mL, 4.17 mmol, 1.1 equiv) dissolved in anhydrous DCM (5.00 mL). The solution was stirred at rt overnight. A white precipitate slowly formed. After complete conversion, the solvent was removed *in vacuo* and the residue was subjected to purification by silica gel column chromatography (hex/EtOAc = 3:2), yielding **74** (1.41 g, 2.52 mmol, 66% yield) as a colorless solid.

$[\alpha]_D^{25}$ = +19.9 (*c* 2.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.87 – 7.79 (m, 4H, Ar), 7.57 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.51 – 7.46 (m, 4H, Ar), 7.36 – 7.28 (m, 5H, Ar), 7.14 – 7.07 (m, 2H, Ar), 5.01 (d, *J* = 10.6 Hz, 1H, ONapC*H*$_2$), 4.90 (d, *J* = 10.5 Hz, 1H, ONapC*H*$_2$), 4.72 (s, 2H, OC*H*$_2$Ph), 4.59 (d, *J* = 9.8 Hz, 1H, H-1), 4.40 – 4.31 (m, 2H, H-6',H-6''), 4.00 (d, *J* = 2.6 Hz, 1H, H-4), 3.76 (t, *J* = 9.3 Hz, 1H, H-2), 3.65 – 3.58 (m, 2H, H-3, H-5), 2.33 (s, 3H, SPhC*H*$_3$), 2.08 (s, 3H, OAc) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.0 (C=O), 137.9, 137.6, 135.7, 133.4, 133.3, 133.2, 132.7, 129.9, 129.8, 128.7, 128.2, 128.1, 128.0, 127.8, 127.0, 126.4, 126.2, 126.0 (22 C, Ar), 88.1 (C-1), 82.4 (C-3), 77.1 (C-2), 75.9 (ONap*C*H$_2$), 75.7 (C-5), 72.5 (O*C*H$_2$Ph), 66.8 (C-4), 63.5 (C-6), 21.3 (SPh*C*H$_3$), 21.0 (OAc) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{33}H_{34}NaO_6S$: 581.1968, found: 581.1981. IR (neat) ν_{max}: 1717, 1272, 815 cm^{-1}.

p-Tolyl 2-*O*-naphtyl-3-*O*-benzyl-4-*O*-fluorenylcarbonylmethoxy-6-*O*-acetyl-1-thio-β-D-galactopyranoside (BB11)

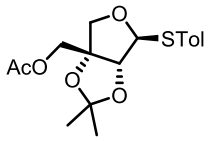


74 (1.20 g, 2.15 mmol) was dissolved in anhydrous DCM (100 mL) under argon atmosphere and pyridine (0.347 mL, 4.30 mmol, 2 equiv) was added. The solution was stirred at rt for 5 min and FmocCl (1.11 g, 4.30 mmo, 2 equiv) was added. The reaction was stirred for 6 h at rt, the solvent was removed *in vacuo* and the product **BB11** was recrystallized as a colorless solid from boiling ethanol (1.13 g, 1.45 mmol, 67% yield).

$[\alpha]_D^{25}$ = +6.9 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86 – 7.74 (m, 6H, Ar), 7.72 – 7.39 (m, 10H, Ar), 7.38 – 7.27 (m, 4H, Ar), 7.17 (dd, *J* = 5.2, 1.9 Hz, 2H, Ar), 7.14 – 7.09 (m, 2H, Ar), 5.39 (dd, *J* = 3.2, 1.0 Hz, 1H, H-4), 5.00 – 4.90 (m, 2H, OC*H*$_2$Ph), 4.81 (d, *J* = 11.3 Hz, 1H, ONapC*H*$_2$), 4.66 (d, *J* = 9.7 Hz, 1H, H-1), 4.58 (d, *J* = 11.3 Hz, 1H,

ONapC*H*$_2$), 4.47 – 4.32 (m, 2H, H-6', H-6''), 4.32 – 4.22 (m, 3H, C-5, OFmoc), 3.88 – 3.81 (m, 2H, H-2, OFmoc), 3.71 (dd, *J* = 9.1, 3.2 Hz, 1H, H-3), 2.31 (s, 3H, SPhC*H*$_3$), 2.08 (s, 3H, OAc) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7 (C=O), 155.2 (C=O), 144.2, 143.7, 143.3, 141.5, 141.4, 138.0, 137.6, 135.7, 133.4, 133.2, 132.8, 129.8, 129.8, 128.4, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.3, 127.3, 127.3, 127.0, 126.4, 126.1, 126.0, 125.5, 125.3, 125.1, 120.2, 120.2, 120.2 (34 C, Ar), 88.3 (C-1), 81.1 (C-3), 77.4 (C-2), 76.1 (O*C*H$_2$Ph), 74.4 (Fmoc), 72.3 (ONap*C*H$_2$), 71.1 (C-4), 70.4 (Fmoc), 62.5 (C-6), 46.7 (C-5), 21.3 (SPh*C*H$_3$), 21.0 (OAc) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{48}H_{44}NaO_8S$: 803.2649, found: 803.2678. IR (neat) ν_{max}: 1744, 1259, 1226 cm^{-1}.

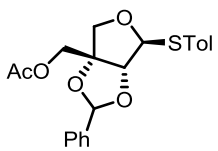
_p_-Tolyl 3-_C_-acetoxymethyl-2,3-_O_-isopropylidene-1-thio-β-D-erythrofuranoside (82)



To a solution of the β-anomer of **81**[157] (0.208 g, 0.702 mmol) in anhydrous DCM (20 mL) at 0 °C was subsequently added DMAP (85.7 mg, 0.0702 mmol, 0.1 equiv), DIC (0.120 mL, 0.772 mmol, 1.1 equiv) and acetic acid (48.2 µL, 0.842 mmol, 1.2 equiv). The solution was stirred at rt overnight. A white precipitate slowly formed. After complete consumption of the educt, the solvents were removed *in vacuo* and the residue was subjected to silica gel column chromatography (hex/EtOAc = 3:2) to obtain **82** (0.230 g, 0.680 mmol, 97% yield) as a colorless solid.

$[\alpha]_D^{25}$ = -230.9 (c 0.5, $CHCl_3$).^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.31 (m, 2H, Ar), 7.20 – 7.09 (m, 2H, Ar), 5.60 (s, 1H, H-1), 4.54 (s, 1H, H-2), 4.41 (d, *J* = 11.7 Hz, 1H, H-3'a), 4.29 (d, *J* = 11.7 Hz, 1H, H-3'a), 4.17 (d, *J* = 10.5 Hz, 1H, H-4a), 4.04 (d, *J* = 10.5 Hz, 1H, H-4b), 2.33 (s, 3H, SPhC*H*$_3$), 2.15 (s, 3H, COC*H*$_3$), 1.48 (s, 3H, C(C*H*$_3$)$_2$), 1.41 (s, 3H, C(C*H*$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8 (C=O), 138.2, 133.0, 130.0, 129.4 (Ar, 6*C*), 114.4 (*C*(CH_3), 93.5 (*C*-1), 90.6 (*C*-3), 87.8 (*C*-2), 73.8 (*C*-4), 65.4 (*C*-3'), 27.8 (C(*C*H$_3$)$_2$, 27.6 (C(*C*H$_3$)$_2$, 21.3 (SPh*C*H$_3$), 21.0 (CO*C*H$_3$). ESI-HRMS $[M+Na]^+$ calcd. for $C_{17}H_{22}NaO_5S$: 361.1080, found: 361.1068. IR (neat) ν_{max} =1748, 1222, 1075, 1037 cm^{-1}.

_p_-Tolyl 3-_C_-acetoxymethyl-2,3-_O_-benzylidene-1-thio-β-D-erythrofuranoside (83)

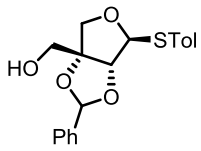


The acetyl protected intermediate **82** (0.230 g, 0.680 mmol) was dissolved in 90% aq. TFA (2.00 mL) and stirred at rt for 90 min before the solvent was removed *in vacuo*. The residue was co-evaporated with toluene (three times 10 mL). The crude intermediate

was dissolved in anhydrous toluene (5 mL) and (dimethoxymethyl)benzene (0.508 mL, 3.39 mmol, 5 equiv) and a catalytic amount of CSA were added. The reaction was stirred at rt overnight. The reaction was quenched by the addition of excess NEt_3, the solvents were removed *in vacuo* and the residue was subjected to silica gel column chromatography (hex/EtOAc = 9:1) yielding **83** (90.0 mg, 0.233 mmol, 35% yield over two steps).

$[\alpha]_D^{25}$ = -189.9 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.54 – 7.49 (m, 2H, Ar), 7.43 – 7.34 (m, 5H, Ar), 7.17 – 7.10 (m, 2H, Ar), 5.93 (s, 1H, PhC*H*), 5.77 (s, 1H, H-1), 4.61 (s, 1H, H-2), 4.51 (d, *J* = 11.9 Hz, 1H, H-3'a), 4.42 (d, *J* = 11.9 Hz, 1H, H-3'b), 4.29 – 4.19 (m, 2H, H-4), 2.34 (s, 3H, SPhC*H*$_3$), 2.18 (s, 3H, COC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7 (C=O), 138.3, 135.8, 133.0, 130.3, 130.1, 129.1, 128.6, 127.3 (Ar, 12C), 106.6 (Ph*C*H), 92.8 (C-1), 90.6 (C-3), 87.8 (C-2), 72.9 (C-4), 64.1 (C-3'), 21.3 (SPh*C*H$_3$), 21.0 (COC*H*$_3$) ppm. ESI-HRMS $[M+Na]^+$ calcd. for $C_{21}H_{22}NaO_5S$: 409.1080, found: 409.1080. IR (neat) ν_{max} = 1746, 1222, 1061, 1041 cm^{-1}.

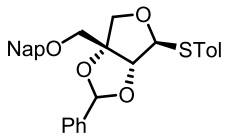
_p_-Tolyl 3-_C_-hydroxy-2,3-_O_-benzylidene-1-thio-β-D-erythrofuranoside (84)



Intermediate **83** (90.0 mg, 0.233 mmol) was dissolved in MeOH (5 mL) at rt and sodium methoxide (62.6 mg, 1.16 mmol, 5 equiv) was added. The reaction was stirred at rt overnight and quenched by the addition of H^+-Amberlite resin until neutral pH. The resin was filtered off and the solvent was removed under reduced pressure to give product **84** in 96% yield (0.08 g, 0.22 mmol).

$[\alpha]_D^{25}$ = -239.2 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.55 – 7.48 (m, 2H, Ar), 7.43 – 7.33 (m, 5H, Ar), 7.17 – 7.11 (m, 2H, Ar), 5.96 (s, 1H, PhC*H*), 5.79 (s, 1H, H-1), 4.62 (s, 1H, H-2) 4.26 – 4.14 (m, 2H, H-4), 4.04 (d, *J* = 12.0 Hz, 1H, H-3'a), 3.97 (d, *J* = 11.9 Hz, 1H, H-3'b), 2.34 (s, 3H, SPhC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 133.0, 130.2, 130.1, 129.1, 128.6, 127.2 (12 C, Ar), 106.7 (Ph*C*H), 93.0 (C-1), 92.6 (C-3), 87.4 (C-2), 73.1 (C-4), 63.8 (C'-3), 21.3 (SPh*C*H$_3$) ppm. ESI-HRMS $[M+Na]^+$ calcd. for $C_{19}H_{20}NaO_4S$: 347.0975, found: 367.0981. IR (neat) ν_{max} =2926, 2855, 1060, 1041 cm^{-1}.

_p_-Tolyl 3-_C_-naphthyloxymethyl-2,3-_O_-benzylidene-1-thio-β-D-erythrofuranoside (BB13)

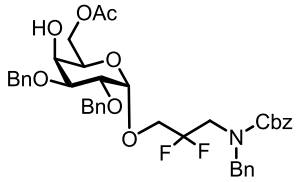


Intermediate **84** (77.0 mg, 0.224 mmol) was dissolved in a mixture of anhydrous DCM and anhydrous DMF (1:1, 10 mL) under argon atmosphere. The solution was cooled to 0 °C and sodium hydride (16.1 mg, 0.671 mmol, 3 equiv) and naphthyl bromide (99.0 mg, 0.447 mmol, 2 equiv) were added. The reaction was stirred under argon atmosphere at rt overnight. The reaction was quenched by the addition of sat. aq. NH_4Cl-solution until no further gas evolved from the mixture. The organic phase was separated, washed with brine, dried over $MgSO_4$ and filtered. The solvent was removed *in vacuo* and the residue was subjected to silica gel column chromatography. The product **BB13**eluted with hex/EtOAc (9:1) (93.0 mg, 0.192 mmol, 86% yield).

$[\alpha]_D^{25}$ = -149.6 (c 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.92 – 7.79 (m, 4H, Ar), 7.56 – 7.45 (m, 5H, Ar), 7.42 – 7.31 (m, 5H, Ar), 7.16 – 7.05 (m, 2H, Ar), 5.98 (s, 1H, PhC*H*), 5.76 (s, 1H, H-1), 4.85 (s, 2H, OC*H*$_2$Nap), 4.69 (s, 1H, H-2), 4.26 – 4.15 (m, 2H, H-4), 3.88 (s, 2H, H-3'), 2.33 (s, 3H, SPhC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.0, 136.3, 135.3, 133.4, 133.2, 133.2, 132.8, 130.1, 130.0, 129.6, 128.5, 128.5, 128.0, 127.9, 127.3, 126.6, 126.4, 126.2, 125.6 (Ar, 22 C), 106.7 (Ph*C*H), 93.0 (C-1), 92.1 (C-3), 87.7 (C-2), 74.0 (O*C*H$_2$Nap), 73.0 (C-4), 70.7 (C-3), 21.3 (SPh*C*H$_3$) ppm. ESI-HRMS $[M+Na]^+$ calcd. for $C_{30}H_{28}NaO_4S$: 507.1601, found: 507.1604. IR (neat) ν_{max} =1056, 809, 731, 697 cm^{-1}.

3.4.2 Solution-Phase Synthesis of the RG-II Galacturonan Backbone using a Fluorinated Linker

(3-_N_-Benzyl-_N_-benzyloxycarbonylamino)-2,2-difluoro-1-propyl (2,3-di-_O_-benzyl-6-_O_-acetyl)-α-D-galactopyranoside (89)

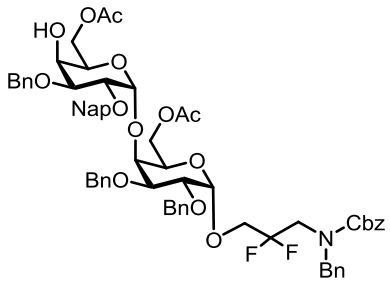


BB10b (0.200 g, 0.274 mmol, 2.7 equiv) and difluoro-linker **88**[173] (34.0 mg, 0.101 mmol) were dissolved in a mixture of anhydrous DCM (2 mL) and anhydrous dioxane (0.2 mL). The mixture was cooled to 0 °C and NIS (67.8 mg, 0.303 mmol,

3.0 equiv) and TfOH (one drop) were added. The reaction was slowly allowed to warm to rt and stirred until TLC confirmed full consumption of the acceptor. The reaction was quenched by the addition of sat. aq. $NaHCO_3$-solution and sat. aq. $Na_2S_2O_3$-solution. The organic phase was separated, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was taken up in DCM (9 mL) and NEt_3 (3.00 mL) was added. The reaction was stirred overnight at ambient temperature. The reaction mixture was washed with sat. aq. NH_4Cl-solution, the organic phase was separated, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was subjected to silica gel column chromatography (hex/EtOAc = 3:1 to 3:2) yielding product **89** (50.0 mg, 0.0695 mmol, 70% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44 – 7.08 (m, 20H, Ar), 5.19 (s, 2H), 5.02 – 4.53 (m, 7H), 4.37 – 4.19 (m, 2H), 4.02 – 3.56 (m, 8H), 2.05 (s, 3H, OAc) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.9, 137.0, 128.7, 128.6, 128.5, 128.3, 128.2, 128.04, 128.01, 127.9, 127.6, 127.5, 98.1, 75.5, 73.1, 68.2, 67.9, 67.7, 63.6, 60.5, 51.3, 20.9 ppm. ESI-MS: m/z $[M+Na]^+$ calcd. for $C_{40}H_{43}F_2NNaO_9$: 742.2, found: 742.2.

(3-*N*-Benzyl-*N*-benzyloxycarbonylamino)-2,2-difluoro-1-propyl (2,3-di-*O*-benzyl-6-*O*-acetyl)-α-D-galactopyranosyl-(1,4)-(2,3-di-*O*-benzyl-6-*O*-acetyl)-α-D-galactopyranoside (90)



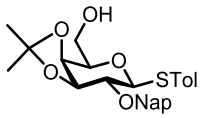
Donor **BB11** (47.7 mg, 0.0610 mmol, 2 equiv) and acceptor **89** (22.0 g, 0.0306 mmol) were dissolved in a mixture of anhydrous DCM (2 mL) and anhydrous dioxane (0.2 mL), and molecular sieves (4 Å, powdered, 0.100 g) was added. The mixture was stirred for 10 min before it was cooled to 0 °C and treated with NIS (15.1 mg, 0.0672 mmol, 2.2 equiv) and TfOH (one drop). The reaction mixture was stirred at 0°C, slowly allowed to warm to rt and stirred for further two hours. The reaction was stopped by the addition of sat. aq. $NaHCO_3$-solution and sat. aq. Na_2SO_3-solution. The organic phase was separated, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude Fmoc protected intermediate was dissolved in DCM (1 mL) and NEt_3 (0.100 mL) was added. The reaction was stirred at rt overnight before it was worked up with sat. aq. NH_4Cl-solution. The organic phase was separated, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification over a silica gel column (hex/EtOAc = 3:1 to 3:2) yielded **90** (10.0 mg, 0.00866 mmol, 28% yield over two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86 – 7.71 (m, 4H), 7.52 – 7.42 (m, 3H), 7.39 – 7.22 (m, 25H), 5.15 (s, 2H), 5.02 – 4.59 (m, 10H), 4.42 – 4.26 (m, 2H), 4.22 – 4.10 (m, 1H), 4.07 – 4.01 (m, 1H), 3.98 – 3.84 (m, 4H), 3.83 – 3.57 (m, 4H), 1.92 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.61 170.60, 138.1, 137.1, 135.6, 133.4, 133.1, 128.7, 128.68, 128.63, 128.4, 128.3, 128.2, 128.1, 128.08, 128.03, 127.97, 127.92, 127.8, 127.6, 127.4, 126.5, 126.2, 126.1, 100.0, 98.0, 77.9, 75.5, 75.2, 73.0, 72.4, 69.4, 67.9, 67.8, 67.0, 62.8, 62.6, 21.0, 20.9 ppm. ESI-MS: m/z $[M+Na]^+$ calcd. for $C_{62}H_{67}F_2NNaO_{15}$: 1176.4, found: 1176.2.

3.4.3 Solution-Phase Synthesis of the RG-II Galacturonan Backbone using Galacturonic Acid Lactone Building Blocks

3.4.3.1 Synthesis of Galacturonic Acid Lactone Building Blocks

_p_-Tolyl 2-_O_-naphtyl-3,4-_O_-isopropylidene-1-thio-β-D-galactopyranoside (92)

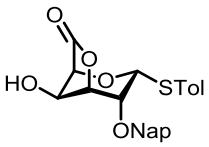


To a solution of *p*-tolyl-3,4-*O*-isopropylidene-6-*O*-(1-methoxy-1-methylethyl)-1-thio-β-D-galactopyranoside [219] (6.29 g, 15.8 mmol) in anhydrous THF (50 mL) was added NaH (1.26 g, 31.6 mmol, 2 equiv of a 60% dispersion in mineral oil) at 0°C. The mixture was stirred at 0 °C for 15 min before naphthyl bromide (3.84 g, 17.4 mmol, 1.1 equiv) was added. After removal of the ice-bath the reaction was allowed to warm to rt and stirred overnight. When full consumption of the starting material was detected by TLC, the reaction was quenched by the addition of MeOH and water and the aqueous phase was extracted with DCM (three times 100 mL). The organic phase was separated, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was dissolved in MeOH (20 mL) which resulted in a cloudy solution. CSA (36.7 mg, 0.158mmol, 0.01 equiv) was added and the solution was stirred at rt for 15 min until the solution became clear. The solvent was evaporated *in vacuo* and the residue was subjected to silica gel column chromatography (hex/EtOAc = 4:1 to 1:1). The product was isolated in 42% yield over three steps (3.10 g, 6.64 mmol) as a colorless solid.

^{1}H NMR (400 MHz $CDCl_3$) δ = 7.88 – 7.76 (m, 4H, Ar), 7.58 (dd, *J* = 8.3, 1.7 Hz, 1H, Ar), 7.53 – 7.39 (m, 4H,Ar), 7.08 (d, *J* = 7.9 Hz, 2H, Ar), 4.99 (d, *J* = 11.5 Hz, 1H, OC*H*$_2$Nap), 4.85 (d, *J* = 11.5 Hz, 1H, OC*H*$_2$Nap), 4.61 (d, *J* = 9.6 Hz, 1H, H-1), 4.31 (t, *J* = 6.0 Hz, 1H, H-3), 4.18 (dd, *J* = 5.8, 1.9 Hz, 1H, H-4), 3.94 (dd, *J* = 10.8, 6.6 Hz, 1H, H-6'), 3.82 – 3.73

(m, 2H, H-5, H-6''), 3.56 (dd, *J* = 9.6, 6.4 Hz, 1H, H-2), 2.31 (s, 3H, SPhCH_3), 1.37 (s, 3H, CH_3), 1.35 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.0, 135.3, 133.3, 133.2, 132.8, 129.8, 129.5, 128.2, 128.0, 127.8, 127.3, 126.5, 126.1, 126.0 (16 C, Ar), 110.5 ($(O)_2C(CH_3)$), 86.4 (C-1), 80.0 (C-3), 78.3 (C-2), 76.8 (C-5) 74.1 (C-4), 73.6 (OCH_2Nap), 62.8 (C-6), 27.9 (CH_3), 26.5 (CH_3), 21.3 (SPhCH_3) ppm. ESI-MS: m/z $[M+Na]^+$ calcd. for $C_{27}H_{30}NaO_5S$: 489.2, found: 489.2.

_p_-Tolyl 2-_O_-naphthtyl-1-thio-β-D-galactopyranosidurono-3,6-lactone (BB16)

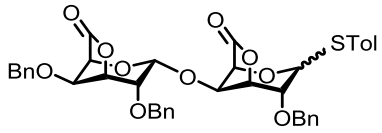


Intermediate **92** (3.00 g, 6.43 mmol) was dissolved in DCM (50 mL), and H_2O (20 mL), TEMPO (0.201 g, 1.29 mmol, 0.2 equiv) and BAIB (5.19 g, 16.1 mmol, 2.5 equiv) were added. After stirring the solution for 2 h at rt, sat. aq. $Na_2S_2O_3$-solution was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The crude acid (theo. amount: 9.13 mmol) was stirred at 65 °C in AcOH/H_2O (4:1, 50 mL) until TLC showed consumption of the starting material. The mixture was concentrated *in vacuo* and co-evaporated with toluene (three times 50 mL). The crude product was dissolved in anhydrous DCM (60 mL) followed by the addition of DIPEA (1.34 mL, 7.72mmol, 1.2 equiv) of ethyl chloroformate (0.673 mL, 7.07 mmol, 1.1 equiv). After stirring at rt for 3 h, the mixture was evaporated *in vacuo* and the residue was subjected to silica gel column chromatography (hex/EtOAc = 4:1 to 3:2) to afford lactone **BB16** (0.459 g, 1.09 mmol, 17% yield).

$[\alpha]_D^{25}$ = -122.5 (c 0.5, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.88 – 7.83 (m, 3H, Ar), 7.79 – 7.75 (m, 1H, Ar), 7.53 (dt, *J* = 6.2, 3.4 Hz, 2H, Ar), 7.44 (dd, *J* = 8.5, 1.7 Hz, 1H, Ar), 7.26 – 7.22 (m, 2H, Ar), 7.08 – 7.03 (m, 2H, Ar), 5.32 (s, 1H, H-1), 4.85 – 4.73 (m, 3H, H-3, OCH_2Nap), 4.70 – 4.66 (m, 1H; H-4), 4.33 (d, *J* = 4.7 Hz, 1H, H-2), 4.00 (t, *J* = 1.4 Hz, 1H, H-5), 2.31 (s, 3H, SPhCH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.1 (C=O), 138.7, 133.9, 133.6, 133.4, 133.3, 129.9, 129.7, 128.9, 128.1, 127.9, 127.5, 126.7, 126.6, 125.8 (16 C, Ar), 86.0 (C-1), 81.3 (C-3), 78.6 (C-2), 73.6 ,(OCH_2Nap) 72.5 (C-5), 70.1 (C-4), 21.3 (SPhCH_3) ppm. $[M+Na]^+$ calcd. for $C_{24}H_{22}NaO_5S$: 445.1080, found: 445.1078. IR (neat) ν_{max} =3433, 1765, 1071, 811 cm^{-1}.

3.4.3.2 Synthesis of RG-II Galacturonan Backbione Disaccharides using Galacturonic Acid Lactone Building Blocks

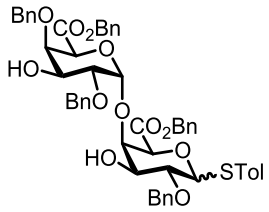
2,4-Dibenzyl-*O*-α-D-galactopyranosiduronyl-3,6-lactone-(1→4)-4-methyl phenyl 2-*O*-benzyl-1-thio-α/β-D-galactopyranosidurono-3,6-lactone (93a)



A solution of donor **BB14** (0.100 g, 0.216 mmol), diphenyl sulfoxide (48.1 mg, 0.238 mmol, 1.1 equiv) and tri-*tert*-butylpyridine (80.2 mg, 0.324 mmol, 1.5 equiv) in DCM (5 mL) was stirred over activated powdered molecular sieves (4 Å). The mixture was cooled to -60 °C before triflic acid anhydride (40.0 µL, 0.238 mmol, 1.1 equiv) was added. The mixture was allowed to warm to -45 °C before it was re-cooled to -60 °C and a mixture of acceptor **BB15** (0.121 g, 0.324 mmol, 1.5 equiv) over activated powdered molecular sieves (4 Å) in a small amount of DCM was added. The reaction was stirred under argon atmosphere and the reaction mixture was allowed to warm to -10 °C. The reaction was quenched by the addition of NEt_3 (0.15 mL, 1.10 mmol, 5 equiv), diluted with DCM and washed with sat. aq. $NaHCO_3$-solution. The aqueous phase was extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (hex/EtOAc = 3:1) to afford product **93a** (0.0540 mg, 0.076 mmol, 35% yield) as a diasteromeric mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.28 – 7.14 (m, 30H), 7.08 (ddd, *J* = 12.1, 7.4, 1.6 Hz, 4H), 6.98 (dd, *J* = 7.9, 5.5 Hz, 4H), 5.19 (s, 1H, H-1β), 5.02 (d, *J* = 2.8 Hz, 1H, H-1α), 4.86 – 4.81 (m, 3H), 4.73 (dd, *J* = 5.1, 1.7 Hz, 1H), 4.70 (d, *J* = 11.6 Hz, 1H), 4.65 – 4.58 (m, 2H), 4.57 – 4.51 (m, 5H), 4.50 – 4.40 (m, 6H), 4.32 (ddd, *J* = 9.5, 6.8, 5.5 Hz, 4H), 4.12 (d, *J* = 4.8 Hz, 1H), 4.07 (d, *J* = 1.6 Hz, 1H), 4.04 – 3.98 (m, 3H), 3.88 (d, *J* = 1.5 Hz, 1H), 3.81 (dt, *J* = 5.2, 2.7 Hz, 2H), 2.20 (s, 3H), 2.19 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.4 (C=O), 171.2 (C=O), 171.0 (C=O), 170.8 (C=O), 138.7, 138.2, 137.0, 136.9, 136.7, 136.6, 136.5, 136.4, 133.4, 131.6, 130.1, 130.0, 129.8, 129.6, 128.89, 128.85, 128.8, 128.7, 128.64, 128.63, 128.5, 128.45, 128.41, 128.3, 128.2, 128.1, 128.0, 127.9, 98.5 (C-1'), 98.0 (C-1'), 86.3 (C-1β), 86.1 (C-1α), 81.0, 80.2, 80.19, 80.15, 78.1, 77.3, 77.1, 76.8, 75.3, 74.8, 74.7, 74.6, 74.3, 74.2,5 74.23, 73.8, 73.1, 72.19, 72.17, 71.8, 70.5, 21.3, 21.2 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{24}H_{22}NaO_5S$: 445.1080, found: 445.1078.

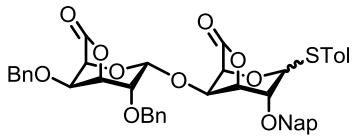
Benzyl 2,4-dibenzyl-*O*-α-D-galactopyranosiduronate-(1→4)-benzyl 4-methyl phenyl 2-*O*-benzyl-1-thio-α/β-D-galactopyranosiduronate (95a)



To a solution of **93a** (42.0 mg, 0.0715 mmol) in DCM (5 mL) at rt was added 75.1 µL (0.0751 mmol, 1.05 equiv) of 1 M TMSONa in DCM. After 15 min full conversion of the educt was detected by TLC and the reaction was neutralized with a drop of acetic acid. The solvent was removed by co-evaporation with toluene (two times 10 mL). The remaining yellow solid was re-dissolved in anhydrous DMF (5 mL) under argon atmosphere and Cs_2CO_3 (51.3 mg, 0.157 mmol 2.2 equiv) and BnBr (25.5 µL, 0.215 mmol, 3 equiv) were added at rt. The reaction was monitored by TLC (hex/EtOAc = 1:1) until full conversion of the educt was detected after 90 min stirring at rt. The reaction mixture was diluted with EtOAc and the organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (hex/EtOAc = 2:1) gave **95a** (0.0200 g, 0.0216 mmol, 30% yield) as a diasteromeric mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.56 – 7.51 (m, 2H), 7.47 – 7.23 (m, 50H), 7.21 – 7.17 (m, 2H), 7.06 (d, *J* = 8.0 Hz, 2H), 6.83 (d, *J* = 7.9 Hz, 2H), 5.81 (t, *J* = 2.4 Hz, 1H), 5.38 (d, *J* = 3.4 Hz, 1H, H-1α), 5.28 – 5.17 (m, 3H), 5.16 – 5.14 (m, 2H), 5.12 (s, 1H, H-1β), 5.09 – 5.04 (m, 2H), 5.02 – 4.90 (m, 4H), 4.85 – 4.76 (m, 2H), 4.75 – 4.68 (m, 3H), 4.66 (d, *J* = 1.9 Hz, 2H), 4.63 – 4.43 (m, 8H), 4.37 (dd, *J* = 2.6, 1.2 Hz, 1H), 4.28 – 4.24 (m, 2H), 4.06 – 4.01 (m, 2H), 4.01 – 3.97 (m, 2H), 3.91 – 3.78 (m, 4H), 2.36 (s, 3H), 2.30 (s, 3H) ppm.
^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.8, 168.0, 167.8, 167.6, 138.4, 138.3, 138.2, 138.0, 137.9, 137.7, 137.5, 137.1, 135.4, 135.3, 135.2, 135.0, 134.9, 131.8, 130.1, 129.8, 129.5, 128.81, 128.80, 128.69, 128.66, 128.62, 128.59, 128.56, 128.54, 128.51, 128.46, 128.40, 128.39, 128.32, 128.28, 128.23, 128.1, 128.0, 127.85, 127.81, 127.7, 127.6, 127.3, 98.5, 98.4, 87.2, 85.4, 80.4, 77.6, 77.2, 77.0, 76.9, 76.0, 75.7, 75.5, 75.1, 75.0, 74.9, 74.8, 72.9, 72.6, 72.1, 71.2, 71.0, 70.6, 69.9, 69.5, 69.2, 67.4, 67.2, 67.1, 67.0, 21.3, 21.2, 21.1 ppm.
ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{54}H_{54}NaO_{12}S$: 949.3228, found: 949.3242.

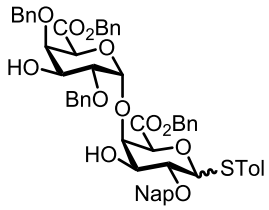
2,4-Dibenzyl-*O*-α-D-galactopyranosiduronyl-3,6-lactone-(1→4)-4-methyl phenyl 2-*O*-naphthyl-1-thio-α/β-D-galactopyranosidurono-3,6-lactone (93b)



A solution of donor **BB14** (50.0 mg, 0.108 mmol), diphenyl sulfoxide (24.0 mg, 0.119 mmol, 1.1 equiv) and tri-*tert*-butylpyridine (40.1 mg, 0.162 mmol, 1.5 equiv) in DCM (5 mL) was stirred over activated powdered molecular sieves (4 Å). The mixture was cooled to -60 °C before triflic acid anhydride (0.0200 mL, 0.119 mmol, 1.1 equiv) was added. The mixture was allowed to warm to -45 °C before it was re-cooled to -60 °C and a mixture of acceptor **BB16** (68.4 mg, 0.162 mmol, 1.5 equiv) over activated molecular sieves (4 Å) in a small amount of DCM was added. The reaction was stirred under argon atmosphere and the reaction mixture was allowed to warm to -10 °C. The reaction was quenched with NEt_3 (0.0753 mL, 0.540 mmol, 5 equiv.), diluted with DCM and washed with sat. aq. $NaHCO_3$-solution. The aqueous phase was extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (hex/EtOAc = 9:1 to 7:1) to afford product **93b** in 35% yield (29.0 mg, 0.0381 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93 – 7.83 (m, 4H Ar), 7.78 (d, *J* = 1.6 Hz, 1H), 7.57 – 7.50 (m, 2H), 7.47 – 7.43 (m, 1H), 7.39 – 7.28 (m, 8H), 7.25 – 7.20 (m, 3H), 7.07 – 7.03 (m, 2H), 5.28 (s, 1H, H-1), 5.02 (dd, *J* = 4.9, 1.4 Hz, 1H), 4.98 (d, *J* = 2.5 Hz, 1H, H-1'), 4.78 (s, 2H), 4.76 – 4.72 (m, 2H), 4.68 (td, *J* = 4.5, 1.5 Hz, 1H), 4.59 (d, *J* = 2.9 Hz, 2H), 4.51 – 4.45 (m, 2H), 4.33 (d, *J* = 4.8 Hz, 1H), 4.18 (d, *J* = 1.5 Hz, 1H), 4.03 (d, *J* = 1.4 Hz, 1H), 3.95 (dd, *J* = 5.0, 2.5 Hz, 1H), 2.32 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.4, 171.3, 138.7, 137.0, 136.9, 136.7, 133.8, 133.4, 133.3, 133.25, 133.22, 131.7, 130.1, 129.9, 129.6, 128.9, 128.8, 128.7, 128.5, 128.4, 128.2, 128.0, 127.9, 127.6, 126.6, 126.5, 98.1, 80.2, 78.3, 75.3, 74.7, 74.3, 73.5, 72.2, 71.8, 71.7, 70.5, 21.3 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{44}H_{40}NaO_{10}S$: 783.2234, found: 783.2245.

Benzyl 2,4-dibenzyl-*O*-α-D-galactopyranosiduronat-(1→4)-benzyl 4-methyl phenyl 2-*O*-naphthyl-1-thio-α/β-D-galactopyranosiduronate (95b)



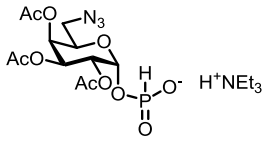
To a solution of **93b** (10.0 mg, 0.0132 mmol) in DCM (1 mL) at rt was added 13.8 µL (0.0138 mmol, 1.05 equiv) of 1 M TMSONa in DCM. After 15 min full conversion of the educt was detected by TLC and the reaction was neutralized with a drop of acetic acid. The solvent was removed by co-evaporation with toluene (two times 10 mL). The remaining yellow solid was re-dissolved in anhydrous DMF (5 mL) under argon atmosphere and Cs_2CO_3 (9.46 mg, 0.0290 mmol 2.2 equiv) and BnBr (4.70 µL, 0.0396 mmol, 3 equiv) were added at rt. The reaction was monitored by TLC until full conversion of the educt was detected after 90 min stirring at rt. The reaction mixture was diluted with EtOAc and the organic phase washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (hex/EtOAc = 2:1) gave product **95b** in 20% yield (3.00 mg, 0.00259 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86 – 7.68 (m, 8H), 7.59 – 7.10 (m, 50H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.83 (d, *J* = 7.8 Hz, 2H), 5.78 (d, *J* = 4.6 Hz, 1H, H-1α), 5.28 (d, *J* = 2.6 Hz, 1H), 5.26 – 4.98 (m, 7H), 4.93 (dd, *J* = 12.2, 4.0 Hz, 2H), 4.84 (d, *J* = 11.8 Hz, 2H), 4.80 – 4.58 (m, 9H), 4.51 (d, *J* = 9.1 Hz, 1H, H-1β), 4.47 – 4.32 (m, 5H), 4.23 (s, 1H), 4.12 (d, *J* = 7.2 Hz, 1H), 4.01 – 3.92 (m, 2H), 3.89 (t, *J* = 2.0 Hz, 1H), 3.82 – 3.58 (m, 10H), 2.30 (s, 4H), 2.29 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.4, 170.3, 170.1, 169.5, 168.7, 167.9, 167.8, 167.5, 138.3, 138.2, 137.8, 137.6, 137.4, 135.3, 135.2, 135.1, 134.9, 134.8, 134.4, 133.1, 133.09, 133.06, 133.00, 131.7, 130.0, 129.7, 129.6, 129.5, 128.8, 128.7, 128.65, 128.63, 128.62, 128.60, 128.56, 128.53, 128.48, 128.45, 128.43, 128.3, 128.24, 128.22, 128.18, 128.15, 128.0, 127.99, 127.95, 127.80, 127.78, 127.73, 127.72, 127.66, 127.55, 127.50, 126.4, 126.24, 126.21, 126.1, 126.0, 98.2 (C-1'), 98.1 (C-1'), 87.2 (C-1α), 87.0, 85.3 (C-1β), 79.6, 75.9, 75.5, 75.2, 74.9, 74.8, 74.7, 74.3, 72.8, 72.5, 72.4, 72.0, 71.1, 70.8, 70.6, 69.8, 69.4, 69.1, 67.3, 67.2, 67.1, 66.9, 29.7, 21.3, 21.1 ppm. ESI-MS: m/z $[M+Na]^+$ calcd. for $C_{58}H_{23}NaO_{12}S$: 999.3, found: 999.2.

3.5 Synthesis of Functionalized Sugar Nucleotides

3.5.1 Synthesis of Functionalized UDP-Gal*p*

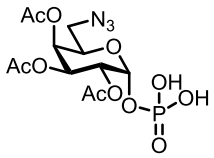
2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl phosphite mono trimethylamine salt (104)



To a solution of an anomeric mixture of **103**[205] (0.600 g, 1.81 mmol) in anhydrous dioxane (2 mL) containing NEt_3 (0.0510 mL, 3.62 mmol, 2 equiv), 2-chloro-4*H*-benzodioxaphosphorin-4-one (0.439 g 2.17 mmol,1.2 equiv) was added and the mixture was stirred at rt for 30 min, before water (0.100 mL) was added. The solution was stirred for additional 5 min before it was concentrated *in vacuo*. The residue was dissolved in THF (2 mL). A colorless precipitate formed within 2 min. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The crude residue was subjected to silica gel column chromatography (DCM/MeOH 20:1 to 9:1) to afford 0.418 g (0.842 mmol) of the anomeric mixture of the galactopyranosyl phosphite **104**. Compound **104** was dissolved in anhydrous MeCN and phosphorous acid (0.414 g, 5.05 mmol, 6 equiv) was added. The mixture was stirred at rt under argon atmosphere overnight before the reaction mixture was neutralized by the addition of NEt_3 (0.819 mL, 5.89 mmol, 7 equiv) and concentrated *in vacuo*. Filtration of the crude residue through a short plug of silica (DCM/MeOH 8.5:1.5) yielded exclusively the α-anomer of the triethylamine salt **104** (0.418 g, 0.842 mmol, 47%) as a sticky oil.

^{1}H NMR (400 MHz, MeOD) δ = 5.79 (dd, *J* = 8.7, 3.5 Hz, 1H), 5.46 (dd, *J* = 3.4, 1.3 Hz, 1H), 5.38 (dd, *J* = 10.8, 3.4 Hz, 1H), 5.17 – 5.12 (m, 1H), 4.41 (ddd, *J* = 7.2, 5.4, 1.4 Hz, 1H), 3.48 (dd, *J* = 12.7, 7.5 Hz, 1H), 3.28 (dd, *J* = 12.7, 7.4 Hz, 1H), 3.21 (q, *J* = 7.3 Hz, 6H), 2.16 (s, 3H), 2.07 (s, 3H), 1.96 (s, 3H), 1.31 (t, *J* = 7.3 Hz, 9H) ppm. ^{13}C NMR (101 MHz, MeOD) δ = 171.9, 171.8, 171.5, 93.2 (d, $J_{C\text{-}P}$ = 4.6 Hz), 70.2, 70.0, 68.95, 68.90 (d, $J_{C\text{-}P}$ = 5.9 Hz), 51.5, 47.8, 20.7, 20.6, 20.5, 9.2 ppm. The analytical data is in agreement with literature data.[204]

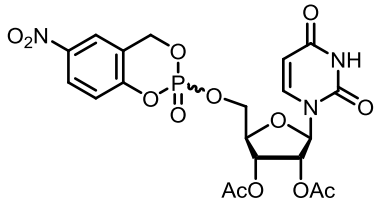
2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl phosphate (105)



104 (0.100 g, 0.202 mmol) was dissolved in THF (5 mL) and passed through a 5 mL syringe filled with H^+-ion exchange resin (prewashed Amberlite). The column was flushed with additional THF (10 mL) and the eluent was concentrated to approx. 5 mL. *t*BuOOH (48.6 µL of 5 M in decan, 0.243 mmol, 1.2 equiv) and a spatula tip of iodine were added. The solution turned brownish and was stirred at rt under argon atmosphere overnight. The reaction was neutralized by the addition of NEt_3 (34.0 µL, 0.243 mmol) and concentrated *in vacuo*. The product was purified by reverse-phase HPLC using a semi-preparative Synergy-C18 column (H_2O + 0.1% formic acid in MeCN, the product eluted with 30% MeCN) yielding the galactose-1-phosphate **105** (20.0 mg, 0.05 mmol, 25%).

^{1}H NMR (400 MHz, MeOD) δ = 5.81 (dd, *J* = 7.4, 3.4 Hz, 1H,), 5.49 (dd, *J* = 3.4, 1.3 Hz, 1H), 5.37 (dd, *J* = 10.9, 3.4 Hz, 1H), 5.13 – 5.07 (m, 1H), 4.42 – 4.35 (m, 1H 4.39 (t, *J* = 6.3 Hz, 2H), 3.49 (dd, *J* = 12.7, 6.9 Hz, 1H), 3.34 – 3.31 (m, 1H), 2.16 (s, 3H), 2.07 (s, 3H), 1.98 (s, 3H) ppm. ^{13}C NMR (101 MHz, MeOD) δ = 172.0, 171.9, 171.5, 94.29 (d, J_{C-P} = 4.9 Hz), 70.2, 69.7, 68.9 (d, J_{C-P} = 7.3 Hz), 68.6, 51.3, 20.6, 20.6, 20.5 ppm. The analytical data is in agreement with literature data.[204]

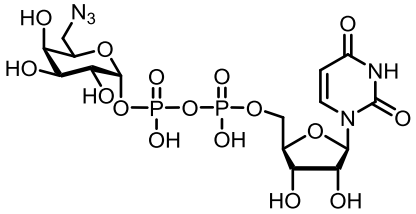
5-Nitro-*cyclo*Sal-2',3'-*O*-diacetyluridine monophosphate (110)



To a solution of acetyl protected uracil **109**[206] (0.100 g, 0.305 mmol) in MeCN (2 mL) was added NEt_3 (0.118 mL, 0.854 mmol, 2.8 equiv) at 0 °C. Subsequently, 5-nitro-*cyclo*-Sal-chlorophosphite (**107**)[203] (0.610 mmol. 2 equiv) dissolved in anhydrous MeCN (5 mL) was added. The solution turned bright yellow immediately upon addition of the phosphite. The solution was allowed to warm to rt and stirred until full consumption of the uracil starting material was detected by TLC. To the stirred solution at rt was added 0.464 g (3.05 mmol, 10 equiv) oxone dissolved in water (5 mL). After stirring for 30 min at rt, DCM was added and the organic phase washed with water three times. The organic phase was separated, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude product was subjected to silica gel column chromatography (hex/EtOAc/AcOH = 1:4:0.1), yielding a sticky oil that was dissolved in anhydrous MeCN and freeze-dried to give a diastereomeric mixture of **110** (65.0 mg, 0.120 mmol, 24%) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.16 (d, *J* = 7.4 Hz, 1H, Ar), 8.27 – 8.19 (m, 1H, Ar), 8.09 (td, *J* = 2.7, 1.3 Hz, 1H, Ar), 7.36 (dd, *J* = 8.2, 7.4 Hz, 1H, Ar), 7.23 (dd, *J* = 9.0, 4.8 Hz, 1H, Ar), 5.90 (dd, *J* = 7.8, 5.3 Hz, 1H), 5.58 – 5.45 (m, 2H), 5.43 – 5.27 (m, 2H), 4.64 – 4.54 (m, 1H), 4.52 – 4.41 (m, 1H), 4.35 – 4.28 (m, 1H), 2.12 (s, Hz, 1.5H), 2.10 (s, 1.5 H), 2.09 (s, 1.5 H) 2.09 (s, 1.5 H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.9, 169.8, 162.94, 162.90, 154.5, 154.43, 154.42, 154.3, 150.1, 150.0, 144.22, 144.19, 140.4, 140.3, 125.93, 125.91, 125.89, 122.0, 121.7, 121.65, 121.64, 121.5, 120.0, 119.9, 119.8, 119.7, 103.5, 103.4, 88.9, 88.6, 80.5, 80.4, 77.5, 77.2, 76.8, 72.8, 72.7, 69.7, 68.4, 68.3, 68.1, 68.0, 67.7, 67.6, 20.7, 20.6, 20.56, 20.53 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{20}H_{20}N_3NaO_{13}P$: 564.0626, found: 564.0624

Uridine 5'-(6-azido-6-deoxy-α-D-galactopyranosyl) diphosphate (97)

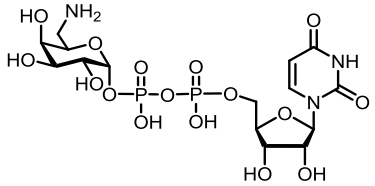


The galactosyl-1-phosphate **105** (0.0200 g, 0.0486 mmol, 1.0 equiv) was dissolved under argon atmosphere in anhydrous DMF (3 mL) and 3 spatula tips of activated powdered molecular sieves (4 Å) were added. After 1 h storage over the activated molecular sieves, phosphate ester **110** (34.0 mg, 0.0628 mmol, 1.3 equiv) was added and the reaction mixture was stirred at rt overnight. The reaction mixture was subsequently filtered through a short bed of celite and the solvent was removed *in vacuo*. The residue was dissolved in water and washed with EtOAc. The aqueous phase was concentrated *in vacuo* yielding the crude intermediate (41.0 mg). The crude intermediate was dissolved in MeOH/H_2O/NEt_3 (7:3:1; 5.5 mL) and stirred at rt overnight. The solvent was removed *in vacuo*. The crude product was taken up in water (4.50 mL) and subjected to ion exchange chromatography, in five portions of 900 µL each. Fractions that still contained impurities after ion exchange chromatography were subsequently subjected to a Carbograph C18 column from which the product eluted with 10% MeCN in water to obtain the azido-functionalized UDP-galactose **97** in 25% yield (6.75 g, 0.0114 mmol).

^{1}H NMR (600 MHz, D_2O) δ = 7.98 (d, *J* = 8.1 Hz, 1H), 6.05 – 5.96 (m, 2H), 5.64 (dd, *J* = 7.1, 3.6 Hz, 1H), 4.40 – 4.38 (m, 2H), 4.33 – 4.19 (m, 4H), 4.02 (d, *J* = 3.3 Hz, 1H), 3.94 (dd, *J* = 10.3, 3.3 Hz, 1H), 3.81 (dt, *J* = 10.1 Hz, 1H), 3.59 (dd, *J* = 12.7, 7.2 Hz, 1H), 3.48 (dd, *J* = 12.8, 6.0 Hz, 1H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 168.9, 154.5, 144.3, 105.3, 98.45 (d, *J* = 6.5 Hz), 91.1, 85.95 (d, *J* = 9.2 Hz), 76.5, 72.8, 72.3, 71.89 (d, *J* = 2.6 Hz), 71.01 (d, *J* = 8.1 Hz), 67.6, 53.0 ppm. ^{31}P NMR (243 MHz, D_2O) δ = -11.2 (d, *J* = 20.8 Hz),

-12.9 (d, *J* = 20.8 Hz) ppm. ESI-HRMS: m/z [M-H]$^-$ calcd for $C_{15}H_{23}N_5O_{16}P_2$: 590.0542, found: 590.0550. The analytical data is in agreement with literature data.[204]

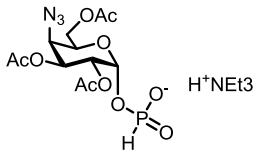
Uridine 5'-(6-amino-6-deoxy-α-D-galactopyranosyl) diphosphate (98)



The azido functionalized UDP-galactose **97** (0.500 mg, 0.846 µmol) was dissolved in H_2O (1 mL) and Pd/C (10 wt% on activated carbon, 2.00 mg) was added. The solution was stirred under H_2 atmosphere for 30 min at rt before the catalyst was filtered off through a RC-syringe filter. The solvent was removed *in vacuo* yielding 0.400 mg (0.708 µmol, 84%) of **98**.

^{1}H NMR (600 MHz, D_2O) δ = 7.95 (d, *J* = 8.2 Hz, 1H), 6.02 – 5.97 (m, 2H), 5.67 (dd, *J* = 6.5, 3.9 Hz, 1H), 4.42 – 4.36 (m, 3H), 4.33 – 4.26 (m, 2H), 4.25 – 4.18 (m, 1H), 4.05 (d, *J* = 3.2 Hz, 1H), 3.96 (dd, *J* = 10.3, 3.3 Hz, 1H), 3.87 – 3.83 (m, 1H), 3.32 – 3.24 (m, 2H) ppm. ESI-HRMS: m/z [M-H]$^-$ calcd for $C_{15}H_{24}N_3O_{16}P_2$: 564.0637, found: 564.0652. The analytical data is in agreement with literature data.[204]

2,3,6-Tri-*O*-acetyl-4-azido-4-deoxy-α-D-galactopyranosyl phosphite (113)

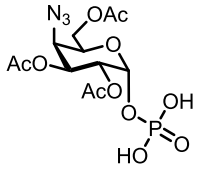


To a solution of 1,2,3,6-tetra-*O*-acetyl-4-azido-4-deoxy-α-D-galactopyranose[207] (**112**) (150 mg, 0.402 mmol) in DMF (4 mL), hydrazine acetate (41.0 mg 0.446 mmol, 1.1 equiv) was added and the solution was stirred for 90 min at 50 °C. The reaction was subsequently diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo* to give 2,3,6-tri-*O*-acetyl-4azido-4-deoxy-α/β-D-galactopyranose (133 mg, 0.402 mmol) as a colorless oil. Without further purification, parts of the anomeric mixture (120 mg, 0.362 mmol, 1 equiv) were dissolved in anhydrous dioxane (5 mL) containing NEt_3 (120 µL, 0.861 mmol, 2.4 equiv) under argon atmosphere, and 2-chloro-4*H*-benzodioxaphosphorin-4-one (89.0 mg, 0.435 mmol, 1.2 equiv) was added to the mixture. The reaction mixture was stirred at rt for 30 min before water (0.200 mL) was added, and the reaction was stirred for further 5 min. After concentration of the reaction mixture *in vacuo,* the residue was dissolved in THF (2 mL). Upon solvation in THF a colorless precipitate formed within 2 min. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (DCM/MeOH = 20:1 to 9:1 to DCM/MeOH/AcOH = 9:1:0.1) yielded

131 mg (0.265 mmol, 66%) of **113** as an anomeric mixture of the monotriethylamine salt. The anomeric mixture was dissolved in anhydrous MeCN (4 mL) and phosphorous acid (162 mg, 1.98 mmol, 6 equiv) was added. The mixture was stirred under argon atmosphere at rt overnight. The reaction mixture was neutralized by the addition of NEt_3 (275 µL, 1.98 mmol) and the solvent was removed *in vacuo*. The product was filtered through a short plug of silica (DCM/MEOH = 9:1), yielding 4-azido-α-galactosyl phosphite **113** (91.0 mg, 0.184 mmol, 64%) as slightly brown sticky oil.

$[\alpha]_D^{25}$ = +42.9 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, MeOD) δ = 5.68 (dd, *J* = 8.6, 3.5 Hz, 1H, H-1), 5.53 (dd, *J* = 10.6, 3.7 Hz, 1H, H-3), 5.16 (ddd, *J* = 10.8, 3.7, 1.6 Hz, 1H, H-2), 4.42 (td, *J* = 6.3, 1.7 Hz, 1H, H-5), 4.30 (dd, *J* = 3.8, 1.7 Hz, 1H, H-4), 4.19 (dd, *J* = 6.2, 4.6 Hz, 2H, H-6), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc) ppm. ^{13}C NMR (101 MHz, MeOD) δ = 172.2 (C=O), 171.8 (C=O), 171.2 (C=O), 93.2 (d, J_{C-P} = 4.7 Hz, C-1), 70.7 (C-3), 69.2 (d, J_{C-P} = 5.8 Hz, C-2), 68.8 (C-5), 64.1 (C-6), 62.4 (C-4) 20.6 (OAc), 20.6 (OAc), 20.4 (OAc) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{12}H_{18}N_3NaO_{10}P$: 418.0622, found: 418.0620. IR (neat) ν_{max}: 2115, 1744, 1214, 918 cm^{-1}.

2,3,6-Tri-*O*-acetyl-4-azido-4-deoxy-α-D-galactopyranosyl phosphate (114)

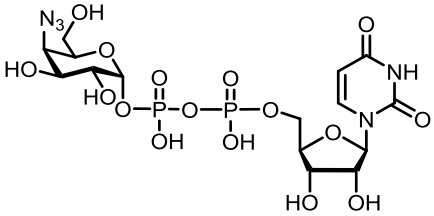


The mono-triethylammonium salt of phosphite **113** (46.0 mg, 0.0929 mmol) was dissolved in THF (5 mL) and passed through a 5 mL syringe filled with H^+-ion exchange resin (prewashed Amberlite). The column was flushed with additional THF (10 mL) and the eluent was concentrated *in vacuo* to approx 5 mL. *t*BuOOH (22.3 µL, 5 M in decane, 0.111 mmol, 1.1 equiv) and a spatula tip of iodine were added. The resulting brown solution was stirred under Ar atmosphere at rt overnight. The reaction was neutralized by the addition of NEt_3 (16.0 µL 0.111 mmol) and concentrated *in vacuo* yielding **114**. The crude product was dissolved in water and purified by HPLC using a semipreparative Synergy C18 column. The product was eluted from the column with 31% MeCN in H_2O containing 0.1% formic acid in 33% yield (12.0 mg, 0.0292 mmol).

$[\alpha]_D^{25}$ = +64.9 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, MeOD) δ = 5.73 (dd, *J* = 7.2, 3.5 Hz, 1H, H-1), 5.52 (dd, *J* = 10.7, 3.7 Hz, 1H, H-3), 5.13 (dt, *J* = 10.8, 2.6 Hz, 1H, H-2), 4.40 (t, *J* = 6.0 Hz, 1H, H-5), 4.33 (dd, *J* = 3.8, 1.5 Hz, 1H, H-4), 4.21 (d, *J* = 6.2 Hz, 2H, H-6), 2.12 (s, 3H, OAc), 2.07 (d, *J* = 2.1 Hz, 6H, OAc). ^{13}C NMR (101 MHz, MeOD) δ = 172.3 (C=O), 171.9 (C=O), 171.2 (C=O), 94.26 (d, *J* = 4.9 Hz, C-1), 70.3 (C-3), 69.12 (d, *J* = 7.5 Hz, C-2), 69.0 (C-5), 63.8 (C-6), 62.2 (C-4), 20.6 (OAc), 20.6 (OAc), 20.4 (OAc). ESI-HRMS:

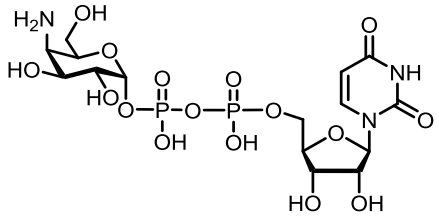
m/z $[M+Na]^+$ calcd for $C_{12}H_{18}N_3NaO_{11}P$: 434.0571, found: 434.0579. IR (neat) ν_{max}: 2115, 1743, 1218, 943 cm^{-1}.

Uridine 5'-(4-azido-4-deoxy-α-D-galactopyranosyl) diphosphate (99)



114 (10.0 mg, 0.0243 mmol) was dissolved in anhydrous DMF (2 mL) and three spatula tips of activated powdered molecular sieves (4 Å) were added. After storage over activated molecular sieves for 1 h, phosphate ester **110** (17.1 mg, 0.0316 mmol, 1.3 equiv) was added and the reaction mixture was stirred at rt overnight. The reaction mixture was subsequently filtered through a short bed of celite and the solvent was removed *in vacuo*. The residue were dissolved in water and washed with EtOAc. The aqueous phase was concentrated *in vacuo*, yielding the crude intermediate. The crude intermediate was dissolved in $MeOH/H_2O/NEt_3$ (7:3:1, 5.50 mL) and stirred at rt overnight. The solvents were removed *in vacuo* and the residue was taken up in 1.80 mL water. The resulting solution was subjected to ion exchange chromatography in two portions of 0.9 mL each. Fractions that still contained impurities after ion exchange chromatography were subsequently subjected to a Carbograph C18 column, from which the product eluted with 10% MeCN in water. In total 2.10 mg (0.00355 mmol, 15%) of **99** were obtained.
^{1}H NMR (700 MHz, D_2O) δ 7.96 (d, *J* = 8.1 Hz, 1H, uracil), 6.02 – 5.96 (m, 2H, uracil, H-1 Rib), 5.60 (dd, *J* = 6.8, 4.0 Hz, 1H, H-1 Gal), 4.41 – 4.36 (m, 2H, H-2 Rib, H-3 Rib), 4.30 – 4.23 (m, 3H, H-4 Rib, H-5 Gal, H-5a Rib), 4.23 – 4.17 (m, 2H, H-3 Gal, H-5b Rib), 4.13 (d, *J* = 2.3 Hz, 1H, H-4 Gal), 3.81 (d, *J* = 10.4 Hz, 1H, H-2 Gal), 3.77 (dd, *J* = 11.8, 6.9 Hz, 1H, H-6a Gal), 3.72 (dd, *J* = 11.6, 5.5 Hz, 1H, H-6b Gal). ^{13}C NMR (176 MHz, D_2O) δ = 168.9 (C=O), 154.5 (C=O), 144.3 (uracil), 105.3 (uracil), 98.2 (d, *J* = 6.3 Hz, C-1 Gal) , 91.0 (C-1 Rib), 85.87 (d, *J* = 9.1 Hz, C-4 Rib), 76.4 (C-3 Rib), 72.9 (C-5 Gal), 72.4 (C-3 Gal), 72.3 (C-2 Rib), 71.14 (d, *J* = 8.7 Hz, C-2 Gal), 67.58 (d, *J* = 5.5 Hz, C-5 Rib), 65.9 (C-4 Gal), 63.5 (C-6 Gal) ppm. ^{31}P NMR (243 MHz, D_2O) δ = -11.3 (d, *J* = 20.7 Hz), -13.0 (d, *J* = 20.7 Hz) ppm. ESI-HRMS: m/z $[M-H]^-$ calcd for $C_{15}H_{23}N_5O_{16}P_2$: 590.0542, found: 590.0552.

Uridine 5'-(4-amino-4-deoxy-α-D-galactopyranosyl) diphosphate (100)

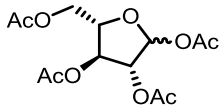


The azido functionalized UDP-galactose **99** (0.380 mg, 0.643 µmol) was dissolved in 1.5 mL H_2O and Pd/C (10 wt% on activated carbon, 2.00 mg) was added. The solution was stirred under H_2 atmosphere for 30 min at rt before the catalyst was filtered off through a syringe filter. The solvent was removed using a lyophilizer, yielding 0.363 mg (0.643 µmol, quant.) of **100**.

^{1}H NMR (600 MHz, D_2O) δ = 7.95 (d, *J* = 8.0 Hz, 1H, uracil), 5.99 – 5.93 (m, 2H, uracil, H-1 Rib), 5.68 – 5.64 (m, 1H, H-1 Gal), 4.35 (d, *J* = 8.5 Hz, 2H), 4.28 – 4.06 (m, 5H), 3.80 (d, *J* = 5.2 Hz, 2H), 3.74 – 3.62 (m, 2H, H-6 Gal) ppm. ESI-HRMS: m/z $[M-H]^-$ calcd for $C_{15}H_{24}N_3O_{16}P_2$: 564.0637, found: 564.0640.

3.5.2 Synthesis of Natural and Functionalized UDP-Ara*f*

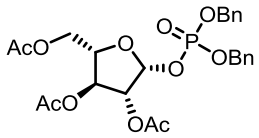
1,2,3,5-Tetra-*O*-acetyl-L-arabinofuranose (116)



To a solution of 1,2,3–tri-*O*-acetyl-L-arabinofuranose (**115**)[210] (0.100 g, 0.362 mmol) in anhydrous pyridine (10 mL) under argon atmosphere, acetic anhydride (68.4 µL, 0.724 mmol, 2 equiv) was added. The reaction was stirred at rt until TLC indicated consumption of the starting material. The reaction was quenched by the addition of few a drops water and the solvent was removed *in vacuo*. The residue was subjected to silica gel column chromatography (hex/EtOAc = 2:1) to give **116** as an anomeric mixture (α/β 1:1) in the form of a colorless oil (0.109 g, 0.343 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.38 (d, *J* = 3.9 Hz, 1H), 6.19 (s, 1H), 5.37 – 5.34 (m, 3H), 5.21 (d, *J* = 1.7 Hz, 1H), 5.07 – 5.03 (m, 1H), 4.43 – 4.33 (m, 2H), 4.29 – 4.18 (m, 3H), 2.14 – 2.08 (7 s, 21H), 2.03 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8, 170.7, 170.4, 170.1, 169.9, 169.6, 169.4, 99.4, 93.8, 82.5, 80.6, 79.8, 75.3, 74.7, 64.7, 63.2, 21.2, 21.2, 20.9, 20.9, 20.9, 20.9, 20.6, ppm. The analytical data is in agreement with literature data.[226]

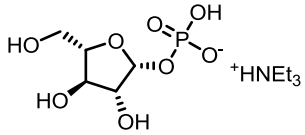
Dibenzyl 2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl phosphate (117)[209]



To a solution of **116** (0.733 g, 2.31 mmol) in anhydrous DCM (10 mL) under argon atmosphere at 0 °C was added bromotrimethylsilane (6.07 mL, 4.60 mmol, 20 equiv) dropwise. After addition the solution was allowed to warm to rt and the reaction was stirred at rt overnight before the solvent and excess bromotrimethylsilane were removed *in vacuo*. The crude residue was taken up in anhydrous toluene (5 mL) under argon atmosphere and a solution of dibutyl phosphate (0.834 g 3.00 mmol, 1.3 equiv) and anhydrous triethylamine (0.964 mL, 6.93 mmol, 3 equiv) in anhydrous toluene (10 mL) was added dropwise at 0 °C. The solution was allowed to warm to rt and the reaction was stirred overnight at rt. The solvent was removed *in vacuo* and the crude residue was subjected to silica gel column chromatography (hex/EtOAc = 3:2 to 1:1) to afford compound **117** (0.260 g, 0.484 mmol, 21%) as the β-anomer exclusively.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40 – 7.30 (m, 10H Ar), 6.02 (dd, *J* = 5.5, 4.3 Hz, 1H), 5.44 – 5.37 (m, 1H), 5.24 (ddd, *J* = 7.4, 4.3, 2.0 Hz, 1H, H-2), 5.07 – 5.03 (m, 4H), 4.48 – 4.40 (m, 1H), 4.21 (ddd, *J* = 6.8, 5.7, 3.7 Hz, 1H), 4.17 – 4.10 (m, 1H), 2.10 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.3, 170.2, 128.7, 128.0, 97.88 (d, *J* = 5.1 Hz), 80.0, 75.98 (d, *J* = 7.0 Hz), 74.0, 69.6, 69.5, 69.5, 64.6, 20.9, 20.8, 20.5 ppm. The analytical data is in agreement with literature data.[209]

β-L-arabinofuranosyl 1-phosphate (119)[209]

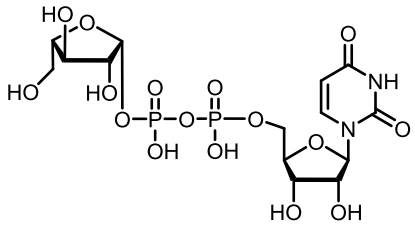


To a solution of **117** (0.110 g, 0.205 mmol) in NEt_3 (10 mL) was added Pd/C (10 wt% on activated carbon, 20.0 mg) and the mixture was stirred under hydrogen atmosphere for 2 h at rt. The catalyst was filtered off through a syringe filter and the solvent was removed *in vacuo*. The crude residue was dissolved in a mixture of MeOH/H_2O (3:2, 3.50 mL) and NEt_3 (0.500 mL) was added. The reaction was stirred at rt overnight. The solvents were removed *in vacuo*, yielding **119** as the monotriethylammonium salt in the form of a sticky oil (64.0 mg, 0.193 mmol, 94%).

^{1}H NMR (400 MHz, D_2O) δ = 5.41 (dd, *J* = 5.3, 3.7 Hz, 1H), 4.07 – 4.02 (m, 2H), 3.85 – 3.73 (m, 1H), 3.70 (dd, *J* = 12.6, 3.0 Hz, 1H), 3.60 – 3.55 (m, 1H), 3.10 (q, *J* = 7.3 Hz, 9H), 1.17 (t, *J* = 7.3 Hz, 12H) ppm. ^{13}C NMR (101 MHz, D_2O) δ = 97.66 (d, *J* = 5.8 Hz),

83.1, 77.44 (d, *J* = 7.7 Hz), 74.0, 63.0, 47.4, 9.1 ppm. The analytical data is in agreement with literature data.[209]

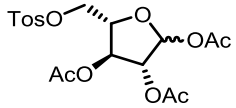
Uridine 5'-(β-L-arabinofuranosyl) diphosphate (124)



The triethylammonium salt of β-L-arabinofuranosyl phosphate (**119**)[209] (64.0 mg, 0.193 mmol) was dried by co-evaporation with anhydrous pyridine (3 x 2 mL) before commercially available uridine 5′-monophosphomorpholidate 4-morpholine-N,N′-dicyclohexylcarboxamidine salt (**125**) (0.305 g, 0.444 mmol, 2.3 equiv) was added and the mixture was again dried by co-evaporation with anhydrous pyridine (2 x 2 mL). The residue was left under high vacuum for 90 min before it was dissolved in anhydrous pyridine (2 mL) under argon atmosphere at rt. To the mixture was added a solution of 1*H*-tetrazole in MeCN (1.33 mL of a 0.45 M solution, 0.598 mmol. 3.1 equiv) and the reaction was allowed to stir at rt for three days before the solvent was removed *in vacuo*. The crude product was taken up in water and subjected to HPLC purification using a semi-preparative reversed-phase Hypercarb column. The product was eluted with 25% MeCN in aqueous ammonium acetate (0.05 M, pH 6.9), after running a gradient of aqueous ammonium acetate/MeCN. As the product still contained UMP as an impurity after semi-preparative HPLC, the product was subjected to ion exchange chromatography, yielding 0.730 mg (0.00136 mmol, 1%) of pure UDP-β-L-arabinofuranose.

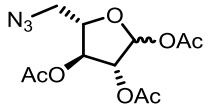
^{1}H NMR (600 MHz, D_2O) δ = 7.97 (d, *J* = 8.2 Hz, 1H, Uracil), 6.00 (d, *J* = 4.1 Hz, 1H, H-1 Rib), 5.99 (d, *J* = 8.1 Hz, 1H, Uracil), 5.65 (dd, *J* = 5.6, 3.6 Hz, 1H, H-1 Ara), 4.40 – 4.38 (m, 2H, H-2 Rib, H-3 Rib), 4.28 – 4.19 (m, 3H, H-4 Rib, H-5a Rib, H-5b Rib), 4.19 – 4.13 (m, 2H, H-2 Ara, H-3 Ara), 3.96 – 3.89 (m, 1H, H-4 Ara), 3.81 (dd, *J* = 12.6, 3.1 Hz, 1H, H-5a Ara), 3.71 (dd, *J* = 12.7, 6.1 Hz, 1H, H-5b Ara) ppm. ^{31}P NMR (243 MHz, D_2O) δ = -11.5 (d, *J* = 21.1 Hz), -12.9 (d, *J* = 21.1 Hz). ESI-HRMS: m/z $[M-H]^-$ calcd for $C_{14}H_{21}N_2O_{16}P_2$: 535.0372, found: 535.0383. The analytical data is in agreement with literature data.[209]

1,2,3-Tri-*O*-acetyl-5-tosyl-L-arabinofuranose (120)



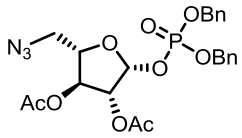
To a solution of 1,2,3–tri-*O*-acetyl-L-arabinofuranose (**115**)[210] (0.584 g, 2.11 mmol) in anhydrous DCM under argon atmosphere were added anhydrous pyridine (1.50 mL, 18.6 mmol 9 equiv) and tosyl chloride (0.605 g, 3.17 mmol, 1.5 equiv) and the reaction was stirred at rt overnight. The reaction was quenched by the addition of sat. aq. $NaHCO_3$-solution and the resulting mixture was extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude product was subjected to silica gel column chromatography (hex/EA 3:1 to 1:1) to give an anomeric mixture (α/β = 1:1) of **120** as a colorless oil (0.636 g, 1.48 mmol, 70% yield).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.83 – 7.76 (m, 2H, Ar), 7.39 – 7.30 (m, 2H, Ar), 6.33 (d, *J* = 3.9 Hz, 1H, H-1(β)), 6.11 (s, 1H, H-1(α)), 5.34 – 5.28 (m, 2H, H-2(β), H-3(β)), 5.15 (d, *J* = 1.5 Hz, 1H, H-2(α)), 4.99 (dt, *J* = 4.3, 1.0 Hz, 1H, H-3(α)), 4.34 – 4.08 (m, 6H, H-4(α), H-4(β), H-5(α), H-5(β), H-5a(α), H-5a(β)), 2.45 (d, *J* = 1.2 Hz, 6H, CH_3(α), CH_3(β)), 2.10 (s, 3H), 2.10 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.05 (s, 3H, OAc). The analytical data is in agreement with literature data for an anomeric mixture of the D-isomer.[211]

1,2,3-Tri-*O*-acetyl-5-azido-5-deoxy-L-arabinofuranose (121)



To a solution of **120** (0.444 g, 1.03 mmol) in anhydrous DMF under argon atmosphere was added sodium azide (0.267 g, 4.12 mmol, 4 equiv). The reaction was stirred at 70 °C overnight. The reaction was quenched by the addition of an excess of water and the reaction mixture was extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude product was subjected to silica gel column chromatography (hex/EtOAc = 2:1) to give an anomeric mixture of **121** as a colorless oil (0.245 g, 0.813 mmol, 79% yield).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.40 (d, *J* = 4.1 Hz, 1H, H-1 (β)), 6.22 (s, 1H, H-1 (α)), 5.41 – 5.33 (m, 2H, H-2 (β),H-3 (β)), 5.22 (dd, *J* = 1.4, 0.5 Hz, 1H, H-2 (α)), 5.05 (ddd, *J* = 4.6, 1.4, 0.8 Hz, 1H, H-3 (α)), 4.29 (td, *J* = 4.6, 3.1 Hz, 1H, H-4 (α)),4.15 – 4.11 (m, 1H, H-4 (β)), 3.69 (dd, *J* = 13.3, 3.2 Hz, 1H, H-5a (α)), 3.61 (dd, *J* = 13.3, 3.6 Hz, 1H, H-5a (β)), 3.45 (ddd, *J* = 13.4, 5.5, 1.9 Hz, 2H, H-5b (α/β)), 2.14, 2.13, 2.12 (s each, 3H each OAc (α)), 2.12, 2.10, 2.09 (s each, 3H each, OAc (β)) ppm. The analytical data is in agreement with literature data for an anomeric mixture of the D-isomer.[211]

Dibenzyl 2,3-di-*O*-acetyl-5-azido-5-deoxy-β-L-arabinofuranosyl phosphate (122)

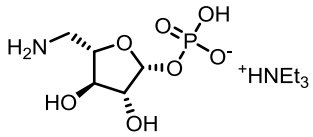


To a solution of **121** (0.670 g, 2.22 mmol) in anhydrous DCM (10 mL) under argon atmosphere at 0 °C was added bromotrimethylsilane (5.90 mL, 44.5 mmol, 20 equiv) dropwise. After addition the solution was allowed to warm to rt and the reaction was stirred at rt overnight before the solvent and excess bromotrimethylsilane were removed *in vacuo*. The crude residue was taken up in anhydrous toluene (10 mL) under argon atmosphere and a solution of dibutyl phosphate (0.678 g 2.44 mmol, 1.1 equiv) and anhydrous triethylamine (0.927 mL, 6.66 mmol, 3 equiv) in anhydrous toluene (2 mL) was added dropwise at 0 °C. The solution was allowed to warm up to rt and the reaction was stirred overnight at rt. The solvent was removed *in vacuo* and the crude residue was subjected to silica gel column chromatography (hex/EtOAc = 3:2 to 1:1) to exclusively afford the β-isomer of compound **122** (0.107 g, 0.206 mmol, 9%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40 – 7.30 (m, 10H, Ar), 6.01 (dd, *J* = 5.5, 4.0 Hz, 1H, H-1), 5.30 – 5.20 (m, 2H, H-2, H-3), 5.07 (d, *J* = 4.5 Hz, 2H, C*H*$_2$Ph), 5.05 (d, *J* = 4.3 Hz, 2H, C*H*$_2$Ph), 4.11 – 4.07 (m, 1H, H-4), 3.53 – 3.49 (m, 2H, H-5), 2.10 (s, 3H, OAc), 1.94 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.5 (C=O), 170.1 (C=O), 135.57 (d, $J_{C\text{-}P}$ = 7.2 Hz), 128.8, 128.8, 128.8, 128.7, 128.1 (12 C, Ar), 97.9 (d, $J_{C\text{-}P}$ = 5.2 Hz, C-1), 81.4 (C-4), 75.8 (d, $J_{C\text{-}P'}$ = 6.6 Hz, C-2), 74.9 (C-3), 69.67 (t, $J_{C\text{-}P}$ = 5.4 Hz, *C*H$_2$Ph) 53.8 (C-5), 20.9 (OAc), 20.5 (OAc) ppm. ESI-MS: m/z $[M\text{-}Na]^+$ calcd for $C_{23}H_{26}N_3O_9P$: 542.1, found: 542.8

Due to the low stability of **122** IR data and optical rotation could not be measured.

5-Amino-5-deoxy-β-L-arabinofuranosyl 1-phosphate (123)

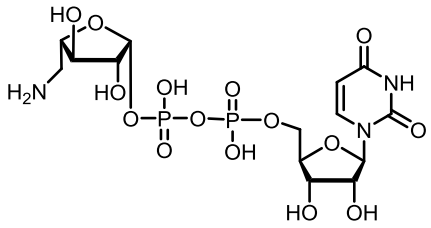


To a solution of **122** (0.100 g, 0.193 mmol) in a mixture of MeOH/NEt_3 (1:1, 4 mL) was added Pd/C (10 wt% on activated carbon, 50.0 mg) and the mixture was stirred under hydrogen atmosphere for 30 min at rt. The catalyst was filtered off through a syringe filter and the solvent was removed *in vacuo*. The crude residue was dissolved in a mixture of MeOH/H_2O (3:2, 3.50 mL) and NEt_3 (1.00 mL) was added. The reaction was stirred at rt overnight. The solvents were removed *in vacuo*, yielding the monotriethylammonium salt of **123** as a brownish sticky oil (0.0637 g, 0.193 mmol, quant).

^{1}H NMR (400 MHz, D_2O) δ = 5.44 (dd, *J* = 5.2, 4.0 Hz, 1H), 4.11 – 3.98 (m, 3H, H-2, H-3, H-4), 3.23 – 3.20 (m, 1H, H-5^a), 3.16 (d, *J* = 7.3 Hz, 1H, H-5^b), 3.11 (q, *J* = 7.3 Hz, 6H,

NEt_3), 1.18 (t, *J* = 7.3 Hz, 9H, NEt_3) ppm. ^{13}C NMR (101 MHz, D_2O/MeOD) δ = 98.5 (d, *J* = 5.7 Hz, C-1), 79.7 (C-4), 78.6 (d, *J* = 7.8 Hz, C-.2), 76.8 (C-3), 48.1 (NEt_3), 43.2 (C-5), 9.7 (NEt_3) ppm. ESI-HRMS: m/z $[M-H]^-$ calcd for $C_5H_{12}NO_7P$: 228.0279, found: 228.0289. Due to the low stability of **123** IR data and optical rotation could not be measured.

Uridine 5'-(5-amino-5-deoxy-β-L-arabinofuranosyl) diphosphate (101)



The triethylammonium salt of 5-amino-5-deoxy-β-L-arabinofuranosyl phosphate (**123**) (19.0 mg, 0.0576 mmol) was dried by co-evaporation with anhydrous pyridine (3 x 2 mL) before commercially available uridine 5′-monophosphomorpholidate 4-morpholine-N,N′-dicyclohexylcarboxamidine salt (**125**) (90.7 mg, 0.132 mmol, 2.3 equiv) was added and the mixture was dried by co-evaporation with anhydrous pyridine (2 x 2 mL). The residue was left under high vacuum for 90 min before it was dissolved in anhydrous pyridine (2 mL) under argon atmosphere at rt. To the mixture was added a solution of 1*H*-tetrazole in MeCN (0.397 mL of a 0.45 M solution, 0.179 mmol. 3.1 equiv) and the reaction was allowed to stir at rt for three days before the solvent was removed *in vacuo*. The crude product was taken up in water and subjected to HPLC-purification using a semi-preparative reversed-phase Hypercarb column. The product was eluted with 25% MeCN in aqueous ammonium acetate (0.05 M, pH 6.8) running a gradient of aqueous ammonium acetate/MeCN. As the product was still contaminated with UMP after semi-preparative HPLC, the product was subjected to IE chromatography (GE HiTrap Q XL 5 mL column, from 100% 0.05 M NH_4OAc buffer (pH 6.8) to 100% 0.5 M NH_4OAc buffer (pH 6.8)) yielding 0.120 mg (0.00024 mmol, 0.4%) of **101**.

^1H NMR (600 MHz, D_2O) δ = 7.94 (d, *J* = 8.1 Hz, 1H, Uracil), 5.96 – 5.93 (m, 2H, H-1 Rib, Uracil), 5.65 (dd, *J* = 5.9, 3.2 Hz, 1H, H-1 Ara), 4.32 – 4.27 (m, 2H, H-2 Rib, H-3 Rib), 4.23 – 4.17 (m, 2H,), 4.14 (d, *J* = 4.9 Hz, 2H), 4.10 – 4.08 (m, 1H), 4.04 (dd, *J* = 5.2, 2.7 Hz, 1H), 3.37 – 3.24 (m, 2H, H-5a Ara, H-5b Ara) ppm. ^{31}P NMR (243 MHz, D_2O) δ = -10.02 (d, *J* = 21.5 Hz), -11.40 (d, *J* = 21.2 Hz) ppm. ESI-HRMS: m/z $[M-H]^-$ calcd for $C_{14}H_{23}N_3O_{15}P_2$: 534.0532, found: 534.0542.

4 Literature

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