Synthesis of Arabinoxylan Oligo- and Polysaccharides from the Plant Cell Wall

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Content

Summary

Every plant cell is enclosed by a cell wall that controls its volume and shape. It provides structural support, tissue elasticity, and protection against pathogenic organisms. The sophisticated network of biomolecules that form the plant cell wall consists of proteins, lignin, and primarily of polysaccharides. The polysaccharides are structurally highly diverse, which makes the analysis of individual functions and interactions challenging. Well-defined probes of oligo- or polysaccharides would greatly facilitate these analyses but they are difficult to obtain from natural sources. Chemical synthesis is a powerful alternative to prepare these probes.

One of the main components of plant cell wall polysaccharides is the hemicellulose xylan. Xylans possess a common backbone consisting of β-1,4-linked xylopyranoses which may be partially acetylated and substituted with arabinofuranosyl or (4-*O*-methyl)glucuronosyl residues. In chapter 2.1 a collection of oligosaccharides related to plant arabino- and glucuronoxylans was synthesized by automated glycan assembly, which is a powerful tool for the synthesis of oligosaccharide libraries. By iterative addition of different monosaccharide building blocks (BB) to a linker-functionalized resin, xylan oligosaccharides of different length and complexity have been obtained. Among these were α-1,2- and α-1,3-substituted arabino- and glucuronoxylan oligosaccharides. To enable selective substitution of the xylan backbone with arabinose and glucuronic acid units the xylose BBs were equipped with 2-(methyl)naphthyl (Nap) and 2-(azidomethyl)benzoyl (AZMB) groups that were used for the first time as protecting groups in automated glycan assembly. The glucuronoxylan oligosaccharides were obtained by using a glucose BB during oligosaccharide assembly that was afterwards converted in a two-step-oxidation procedure into the corresponding glucuronic acid. The synthetic oligosaccharides served as excellent tools for the characterization of binding epitopes of plant cell wall glycan-directed monoclonal antibodies and for determining the substrate specificities of cell wall-degrading enzymes. Monoclonal antibodies are used for high resolution imaging of plant cell walls, providing important information on the structure and function of cell wall polysaccharides. Using glycan microarray technology many binding epitopes were determined that were previously unknown with the help of the synthetic oligosaccharides. Cell wall-degrading enzymes are crucial for the deconstruction of lignocellulosic biomass, making the hydrolysis products available for various industrial applications. The substrate specificities of xylanases and arabinofuranosidases were determined by analyzing the digestion products after incubation of the synthetic oligosaccharides with these enzymes.

In chapter 2.2 the synthesis of artificial arabinoxylan polysaccharides with defined substitution patterns is described. Polysaccharides from plant biomass are explored extensively as renewable resources for the production of materials and fuels. However, the heterogeneous nature of non-cellulosic polysaccharides such as arabinoxylan makes it difficult to correlate molecular structure with macroscopic properties. To study the impact of specific structural features of the polysaccharides on e.g. crystallinity or affinity to other cell wall components, collections of polysaccharides with defined repeating units are required. A collection of artificial arabinoxylan polysaccharides with systematically altered branching patterns was obtained by glycosynthase-catalyzed polymerization of glycosyl fluorides derived from arabinoxylan oligosaccharides that were procured either chemically, chemo-enzymatically, or from a commercial source. These artificial arabinoxylan polysaccharides, that are not accessible by other means, represent ideal probes for structure-property relationship studies. The crystallinity of the polysaccharides was qualitatively determined by powder X-ray diffraction, revealing that the specific substitution pattern has a higher impact on crystallinity of xylans than the degree of substitution. Experiments using quartz crystal microbalance with dissipation (QCMD) on a cellulosic surface indicated that polysaccharides carrying arabinose substituents, which are evenly spaced, adsorb more strongly to cellulose than arabinoxylans with other substitution patterns.

Zusammenfassung

Jede Pflanzenzelle ist von einer Zellwand umschlossen, die ihr Volumen und ihre Form bestimmt. Sie sorgt für strukturelle Stabilität, Elastizität des Gewebes und Schutz vor Krankheitserregern. Das hochentwickelte System aus Biomolekülen, das die Pflanzenzelle bildet, besteht aus Proteinen, Lignin und zum größten Teil aus Polysacchariden. Die Polysaccharide sind strukturell sehr vielfältig, was die Untersuchung ihrer individuellen Funktionen und Interaktionen erschwert. Strukturell klar definierte Oligo- und Polysaccharide würden diese Analysen stark vereinfachen, aber sie sind nur schwer aus natürlichen Quellen zu gewinnen. Die chemische Synthese ist daher eine vielversprechende Alternative, um diese herzustellen.

Eine der Hauptkomponenten der pflanzlichen Polysaccharide in der Zellwand ist die Hemizellulose Xylan. Xylane besitzen ein Rückgrat, das aus β-1,4-verknüpften Xylopyranosen besteht, welche partiell acetyliert und mit Arabinofuranose oder (4-*O*-Methyl)glucuronsäure substituiert sein können. In Kapitel 2.1 wurde eine Bibliothek von Oligosacchariden, die strukturell mit pflanzlichen Arabino- und Glucuronoxylanen verwandt sind, mittels automatisierter Oligosaccharidsynthese hergestellt, welche eine leistungsstarke Technik für die Synthese von Oligosaccharid-Bibliotheken ist. Durch die iterative Verknüpfung von Monosaccharid-Bausteinen an ein Harz, das mit einem Linker funktionalisiert wurde, konnten Xylanoligosaccharide mit verschiedener Länge und Komplexität erhalten werden. Darunter waren α-1,2- und α-1,3-substituierte Arabino- und Glucuronoxylanoligosaccharide. Um die selektive Substitution des Xylan-Rückgrats mit Arabinose- und Glucuronsäure-Substituenten zu ermöglichen, wurden die Xylose-Bausteine mit 2-(Methyl)naphthyl- (Nap) und 2-(Azidomethyl)benzoyl- (AZMB) Gruppen ausgestattet, die das erste Mal als Schutzgruppen in der automatisierten Oligosaccharidsynthese benutzt wurden. Die Glucuronoxylanoligosaccharide wurden durch die Verwendung eines Glucose-Bausteins in der Oligosaccharid-Synthese erhalten. Die Glucose-Einheit wurde dann in einem zweistufigen Prozess in die entsprechende Glucuronsäure überführt. Die synthetischen Oligosaccharide fanden Anwendung in der Charakterisierung von monoklonalen Antikörpern, die Zellwandpolysaccharide erkennen und bei der Bestimmung von Substratspezifitäten von Enzymen, die Pflanzenzellwände zersetzen. Monoklonale Antikörper werden in hochauflösenden bildgebenden Verfahren eingesetzt, um wichtige Informationen über die Struktur und Funktion von pflanzlichen Zellwandpolysacchariden zu erhalten. Durch die Verwendung der Glykan-Microarray-Technologie wurden mit Hilfe der synthetischen Oligosaccharide zahlreiche Bindungsepitope von monoklonalen Antikörpern bestimmt, die bisher unbekannt waren. Zellwand-zersetzende Enzyme sind für den Abbau von lignozellulosischer Biomasse von

Bedeutung, der wichtige Hydrolyseprodukte für viele industrielle Anwendungen zugänglich macht. Die Substratspezifitäten von Xylanasen und Arabinofuranosidasen wurden bestimmt, indem nach der Inkubation der Oligosaccharide mit den jeweiligen Enzymen die Abbauprodukte analysiert wurden.

In Kapitel 2.2 wird die Synthese von artifiziellen Arabinoyxlanpolysacchariden mit definiertem Substitutionsmuster beschrieben. Polysaccharide aus pflanzlicher Biomasse werden intensiv als erneuerbare Ressourcen für die Produktion von Materialien und Kraftstoffen genutzt. Die heterogene Beschaffenheit nicht-zellulosischer Polysaccharide wie Arabinoxylanen erschwert es jedoch, die molekulare Struktur mit makroskopischen Eigenschaften zu korrelieren. Um den Einfluss von speziellen strukturellen Merkmalen der Polysaccharide auf z.B. Kristallinität oder die Affinität zu anderen Zellwandkomponenten zu untersuchen, wird eine Bibliothek von Polysacchariden mit definierten Wiederholungseinheiten benötigt. Eine solche Bibliothek von artifiziellen Polysacchariden mit systematisch verändertem Substitutionsmuster wurde in dieser Arbeit durch Glykosynthase-katalysierte Polymerisation von Glykosylfluoriden erzeugt. Diese wurden aus Arabinoxylanoligosacchariden hergestellt, die wiederum entweder chemisch, chemo-enzymatisch oder auf kommerziellem Weg erhalten wurden. Diese artifiziellen Arabinoxylanpolysaccharide, die nicht auf anderem Weg erhältlich sind, stellen ideale Werkzeuge für die Untersuchung von Struktur-Eigenschafts-Beziehungen dar. Die Kristallinität der Polysaccharide wurde qualitativ mittels Pulver-Röntgendiffraktometrie bestimmt, was ergab, dass das spezifische Substitutionsmuster einen größeren Einfluss auf die Kristallinität von Xylanen hat als der Substitutionsgrad. Experimente mittels Quarzkristall-Mikrowaage mit Dissipation (QCMD) auf einer zellulosischen Oberfläche weisen zudem darauf hin, dass Polysaccharide, die Arabinose-Substituenten in geraden Abständen tragen, stärker an Zellulose binden, als Arabinoxylane mit anderen Substitutionsmustern.

List of Publications

Publications:

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. DOI: https://10.1104/pp.17.00737. Copyright by the American Society of Plant Biologists.

D. Senf, C. Ruprecht, G. H. M. deKruijff, S. O. Simonetti, F. Schuhmacher, P. H. Seeberger, F. Pfrengle, *Chem. Eur. J.* **2017**, *23*, 3197-3205. DOI: https:// 10.1002/chem.201605902.

M. Wilsdorf, D. Schmidt, M. P. Bartetzko, P. Dallabernardina, F. Schuhmacher, P. H. Seeberger, F. Pfrengle, *Chem. Comm.* **2016**, *52*, 10187-10189. DOI: https://10.1039/C6CC04954K.

D. Schmidt, F. Schuhmacher, A. Geissner, P. H. Seeberger, F. Pfrengle, *Chem. Eur. J.* **2015**, *21*, 5709-5713. DOI: https://10.1002/chem.201500065.

Conference Presentations:

D. Senf, G. H. M. deKruijff, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated Glycan Assembly of Xylan Oligosaccharide Fragments and Their Biological Evaluation. Poster presentation delivered at the 1st Biomolecular Systems Conference, Berlin, Germany, November 2016.

D. Schmidt, G. H. M. deKruijff, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated Glycan Assembly of Arabinoxylan Fragments and Their Biological Evaluation. Poster presentation delivered at the International Carbohydrate Symposium 2016, New Orleans, USA, July 2016.

D. Schmidt, G. H. M. deKruijff, F. Schuhmacher, P. H. Seeberger, F. Pfrengle. Automated Glycan Assembly of Arabinoxylan Fragments and Their Biological Evaluation. Poster presentation delivered at the Riken Symposium, Berlin, April 2016.

Abbreviation Index

Ac	acetyl
ACN	acetonitrile
AGX	arabino(glucurono)xylan
Ar	aryl
AX	arabinoxylan
aq.	aqueous
Azmb	2-(azidomethyl)benzoyl
BAIB	bis(acetoxy)iodobenzene
BB	building block
Bn	benzyl
br	broad
Bz	benzoyl
Cbz	carboxylbenzyl
DAST	diethylaminosulfurtrifluorid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DCM	dichloromethane
DDQ	dichlorodicyanobenzoquinone
DMAP	*N,N*-dimethylaminopyridine
DMF	dimethylformamid
DMP	Dess-Martin-periodinane
ELSD	evaporative light scattering detector
ESI	electrospray ionization
Et	ethyl
EtOAc	ethyl acetate
FA	ferulic acid
Fmoc	fluorenylmethoxycarbonyl
FTIR	fourier transform infrared
GC	gas chromatography
GH	glycosyl hydrolase
GT	glycosyltransferase
GX	glucuronoxylan
Hex	hexane
HG	homogalacturonan

HPAEC	high-pH anion-exchange chromatography
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence spectroscopy
Lev	levulinoyl
LevOH	levulinic acid
LG	leaving group
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
Me	methyl
MS	mass spectrometry
Nap	(2-methyl)naphtyl
NDP	nucleotide diphosphate
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
NP	normal phase
PG	protecting group
Ph	phenyl
*p*TsOH	*p*-toluenesulfonic acid
QCMD	quartz crystal microbalance with dissipation
RG	rhamnogalacturonan
RP	reversed phase
rt	room temperature
sat.	saturated
TBDMS	*tert*-butyl-di-methyl-silyl
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl radical
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofurane
Tol	toluene
UDP	uridine diphosphate
UV	ultraviolett

Symbols

D-Xylose
(Xyl)

L-Arabinose
(Ara)

D-Glucuronic acid
(GlcA)

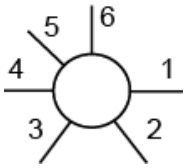
D-Glucose
(Glc)

D-Mannose
(Man)

D-Galactose
(Gal)

D-Fucose
(Fuc)

Bonds



Solid support

Monoclonal antibody

1 Introduction

1.1 Plant Cell Wall

1.1.1 Architecture

The plant cell wall is a highly organized and sophisticated network of different polysaccharides, proteins and aromatic compounds.[1,2] It encloses every cell of the plant and therefore controls its volume and shape. Furthermore, the cell wall provides structural support for the plant, tissue elasticity, and protection against pathogenic organisms.[3] Growing cells are surrounded by a thin, flexible, and expandable cell wall, which is called primary cell wall. Mature cells deposit additional layers of polysaccharides and lignin inside the old one, thereby forming a more rigid secondary cell wall that can differ in its composition significantly from the primary cell wall.[2,4] The main component of cell walls are the polysaccharides, which can be categorized into the three classes cellulose, hemicellulose, and pectin (Figure 1).[5,6] Apart from their structural role they are involved in signaling and the immune response to pathogens.[7,8]

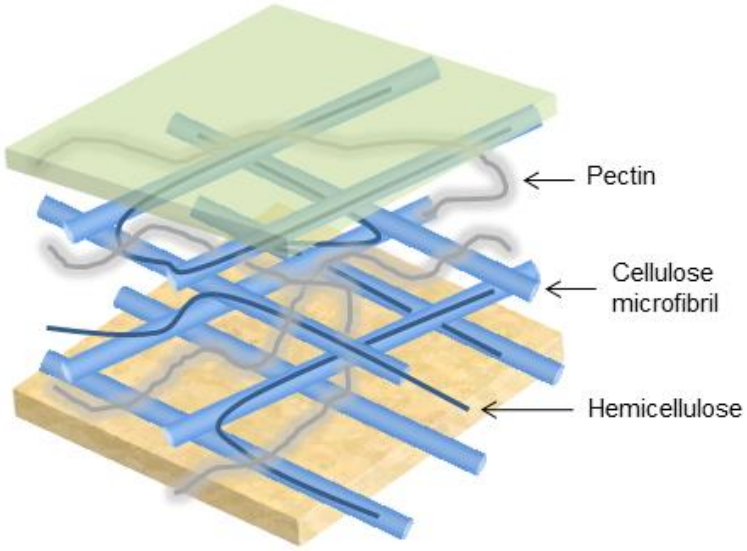


Figure 1. Schematic representation of the plant cell wall and its components.[5]

Plant cell wall glycans are the largest source of biomass on earth, making them an attractive target for industrial utilization, such as in the paper and pulp industry, or as a renewable resource for the production of second-generation biofuels and various materials.[9,10] Moreover, a considerable amount of our diet contains plant cell wall polysaccharides in the form of dietary fibers in fruits, vegetables, and cereals. Their regular uptake benefits our health by reducing the risk of diseases, such as high blood

pressure, heart diseases, diabetes, and obesity.[11,12] In the pharmaceutical industry immunostimulatory[13-15] and anti-tumor[14,16,17] effects of plant glycans are explored.

1.1.2 Cellulose

Cellulose is the most abundant macromolecule on earth, which makes up to 1.5 x 10^{12} tons of the total annual biomass production.[18] Already thousands of years ago cellulose fibers were used as material for clothing or in the Egyptian papyri. Today, this biopolymer is still used for the production of textile fibers, paper products and biofuels, but also in cosmetics, coatings, laminates, and optical films.[18-20]

Cellulose is the main component of plant cell walls. This homopolymer consists of linear chains of β-1,4-linked D-glucose dimers (Figure 2), whose adjacent glucose residues adapt an alternating spatial conformation, due to strong hydrogen bonds between the hydrogen atom of O3 and the ring oxygen atom of the next residue. Cellulose microfibrils are formed through further intermolecular hydrogen bonds connecting 20-40 glucan chains. Each glucan molecule can reach a length of 8000-15000 monosaccharide units, depending on the plant source.[2] These strong inter- and intramolecular hydrogen bonds make the microfibril a rigid and water-insoluble polymer which is mainly responsible for the structural support of the cell wall.

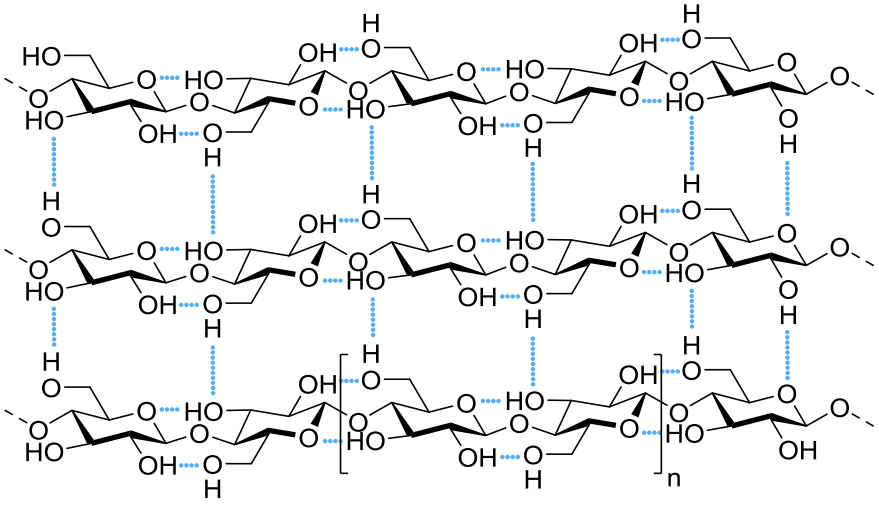


Figure 2. Structural model of a cellulose microfibril. The repeating unit of a single glucose chain is cellobiose (shown in brackets). Intra- and intermolecular hydrogen bonds are indicated by blue, dotted lines.

In plants, cellulose is synthesized in a multi-step process by a membrane-bound enzyme complex with six subunits, which are arranged in a rosette shape. Each of them contains multiple catalytic sites, the cellulose synthase proteins (CESA) that synthesize a

single cellulose chain from UDP-glucose. The parallel chains produced by every subunit then self-assemble into a microfibril.[21-23]

1.1.3 Hemicelluloses

1.1.3.1 Occurrence, Structure and Biosynthesis

The term hemicellulose originated from the early belief that the water-extractable polysaccharides in the plant cell wall were precursors for cellulose. Even though this is now known to be false, the term is still commonly used. Hemicelluloses are a heterogeneous class of low-weight polysaccharides with mostly β-1,4-linked backbones (Figure 3). They are often referred to as matrix polysaccharides as they form a tight network with cellulose microfibrils to prevent their aggregation.[24,25]

Depending on the plant species, the types of hemicellulosic polysaccharides that are present differ. In all seed plants, except for grasses, xyloglucans are the main hemicellulose (Figure 3a). They consist of repetitive units of a β-1,4-linked D-glucose tetrasaccharide, of which three consecutive glucose residues are decorated with side chains. These always consist of xylosyl residues, which may be further extended by galactosyl and fucosyl residues.[26] Mannans (Figure 3b) are found in variable amounts in all cell walls and their backbone can be substituted with additional galactose residues.[27] Mixed-linkage glucans (Figure 3c) possess a β-1,4-linked D-glucose backbone, which is

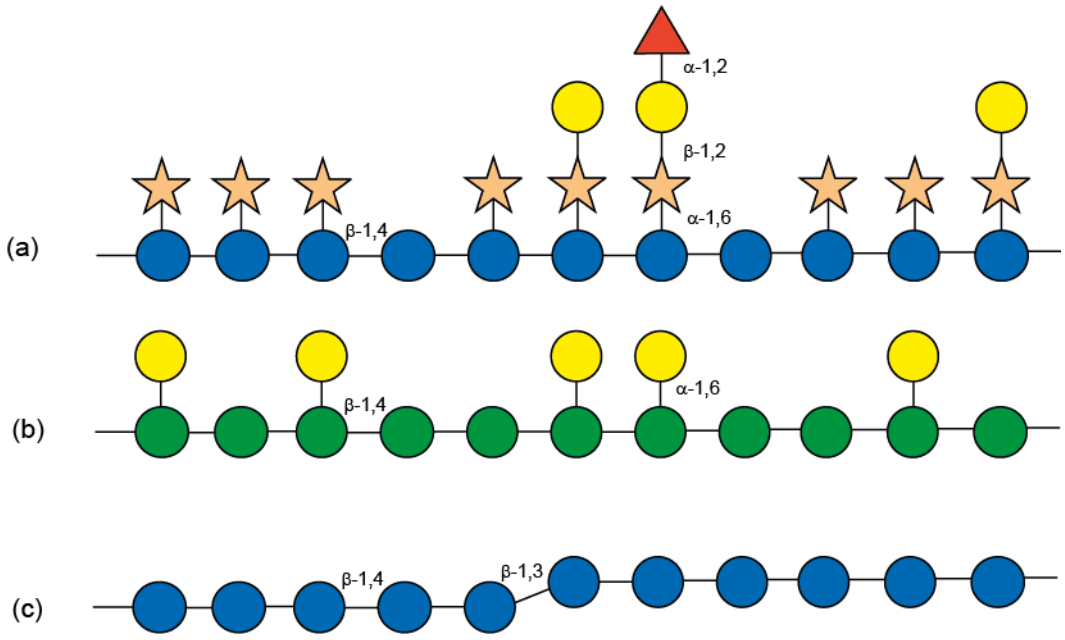


Figure 3. Main polysaccharide classes of hemicelluloses besides xylans: (a) xyloglucans, (b) mannans, and (c) mixed-linkage glucans.

occasionally disrupted by a single β-1,3-linkage. They are exclusively present in the primary cell wall of grasses and play an important role in cell growth.[28] Another class of hemicelluloses arexylans (not shown here), which consist of a β-(1→4)-linked D-xylose backbone. They are mostly present in grasses and cereals and will be discussed in the next chapter.

Industrial utilization of hemicelluloses has become increasingly interesting since up to 50% of the biomass dry weight are hemicellulosic polysaccharides. They are mostly used as raw material for products with industrial significance, such as ethanol, xylitol, and 2,3-butandiol[29] or as novel materials.[24]

Hemicellulosic polysaccharides are synthesized in the Golgi apparatus of the cell a variety of glycosyltransferases. The β-1,4-linked backbones of xyloglucans,[30] mannans,[31,32] and mixed-linkage glucans[33] are synthesized by glycosyltransferases that belong to the cellulose synthase-like (CSL) gene family. The xylan backbone is synthesized by glycosyltransferases of other families and even though many of the involved enzymes have been identified, their exact role remains unclear.[34] After backbone synthesis, the substituents are attached by further glycosyltransferases[35] and the final polysaccharide is transported to the cell wall in vesicles.

1.1.3.2 Xylans

Xylans are the most abundant non-cellulosic polysaccharides in the plant kingdom.[25,34] The xylan backbone consists of β-(1→4)-linked D-xylopyranosides decorated with variable substitution patterns that result in an immense structural diversity across plant species (Figure 4). Arabinoxylans (AX) are an important xylan subclass in which the backbone is substituted with L-arabinofuranosyl residues in the C2- and/or C3-position (Figure 4).[36] They are the most abundant type of xylans in cereals.[37] In woody tissues, glucuronoxylans (GX) are the main class of xylans. Their backbone is substituted with D-glucuronic acid or the 4-*O*-methylated D-glucuronic acid derivative exclusively in the C2-position of the xylose residues. Glucurono(arabino)xylans (GAX), that are mostly found in grasses, carry both types of substituents.[38] The backbone of GXs and GAXs may be partially acetylated and the arabinose in AXs and GAXs may be esterified with ferulic acid (FA), which forms dimers in the plant cell wall, linking two xylan polymers together.[39,40]

The molecular structure of xylans is known to have a strong impact on the macroscopic properties. A decrease in arabinose-substitution is accompanied by a decrease in solubility due to increased interchain interactions which result in aggregation.[41] Crystallinity is similarly affected by substitution. In the crystalline state (and in solution) xylan adopts a left-handed triple helical conformation of antiparallel chains.[42] Substiuents

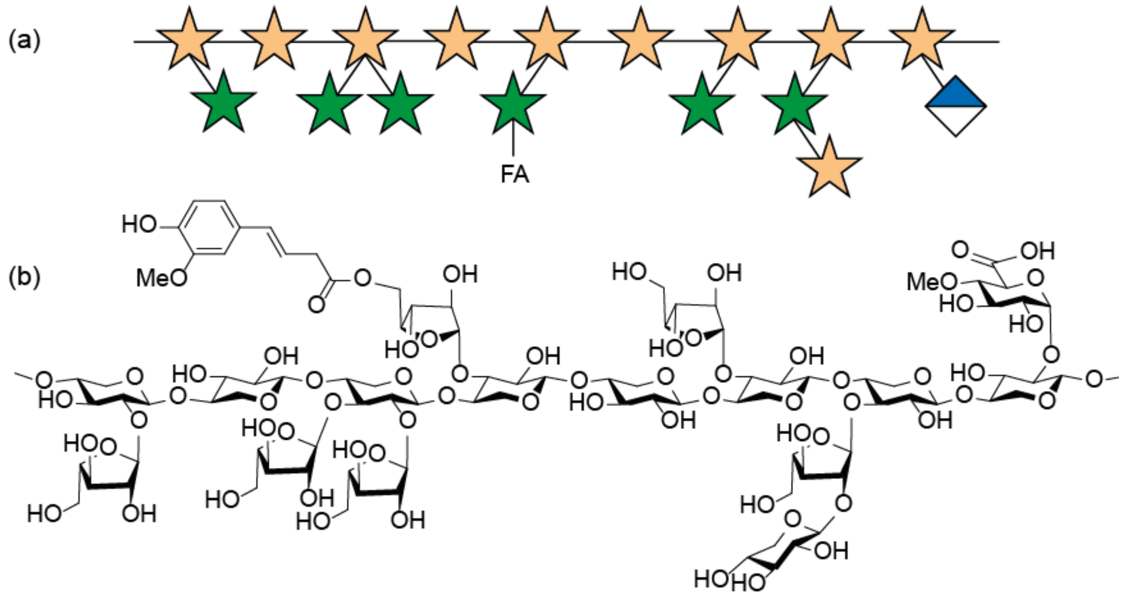


Figure 4. Schematic representation of a GAX structure in CFG nomenclature[43] (a) and as chemical structure (b).

disrupt the interactions between xylan chains and therefore decrease crystallinity significantly. Furthermore, adsorption to cellulose usually increases with a lower degree of substitution leaving the backbone free to hydrogen-bond to the cellulose microfibril.[41,44-46] However, recently it was shown[47,48] that in glucuronoxylans the discrete molecular structure of the substitution pattern affects binding to cellulose more significantly. Upon interaction, xylan adopts a flattened two-fold helical screw conformation[49,50] in contrast to the three-fold helical screw conformation in solution.[42] The former conformation can only be adopted by domains of the polymer that carry evenly spaced substituents.[51,52] This shows that there is an important relationship between structure and function in xylans.

The high abundance of xylans makes them an interesting natural source for various applications, e.g. in the food industry as a stabilizer and additive. Because of the beneficial health effects of dietary fibers xylans are utilized as ingredients in functional foods.[11,17,53,54] Moreover, their non-food applications include the production of second generation biofuels from lignocellulosic biomass,[55,56] oxygen barrier films for packaging,[57,58] and as a strengthener in paper production.[59]

1.1.4 Pectin

The most heterogeneous and complex class of plant polysaccharides is pectin. Their ability to form a gel-like matrix in the cell wall is taken advantage of in their main industrial applications as gelling and stabilizing agents in food products and as a fat substitute in

baked goods.[60,61] In the plant cell wall, pectins are involved in cell-cell-adhesion and have numerous functions during cell expansion where they promote cell wall flexibility.[4,62-65]

The chemical structure of pectins is rich in negatively charged carboxylic acids and can be divided into five domains, namely homogalacturonan (HG), xylogalacturonan (XGA), apiogalacturonan (AGA), rhamnogalacturonan II (RG-II), and rhamnogalacturonan I (RG-I). All five domains are believed to be covalently linked.[63] The major constituent of all pectin is HG. Its backbone consists of α-1,4-linked D-galacturonic acid residues[66] to which D-xylopyranosyl (XGA) and D-apiofuranosyl residues (AGA) or complex side chains consisting of up to 12 different monosaccharides (RG-II) may be attached.[63,67,68] RG-I is constructed from an [α-1,4-D-GlcA-α-1,2-L-Rha] disaccharide repeating unit that is highly substituted with galactan, arabinan, and arabinogalactan oligosaccharide side chains.[63,64]

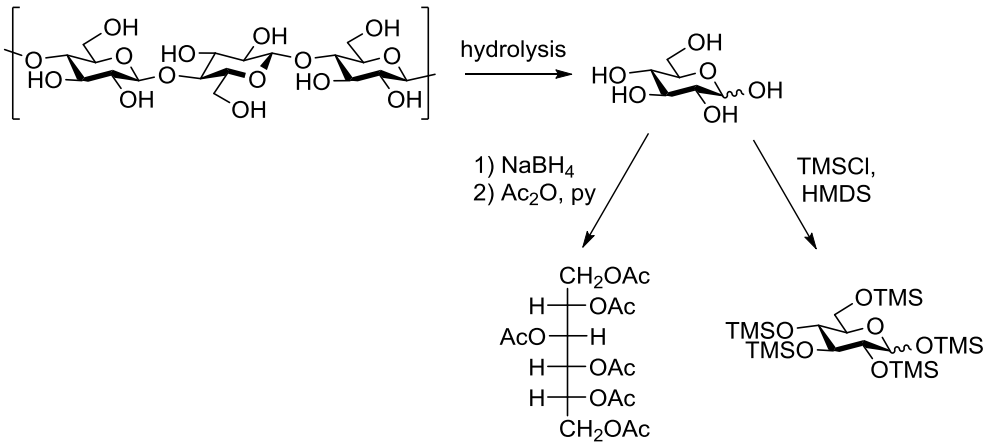
The biosynthesis of pectin occurs presumably in the Golgi apparatus and, considering its structural diversity, it involves a plethora of glycosyltransferases of which only few have been identified to date.[63,69,70]

1.1.5 Plant Cell Wall Analysis

1.1.5.1 Monosaccharide Composition Analysis

The fine structures and relative quantities of plant cell wall polysaccharides vary significantly not only between plant species, but also between tissues of the same plant. Therefore, a detailed knowledge about their composition is necessary to understand their functions in the cell wall. To analyze the occurrence and structure of plant glycans, the monosaccharide composition of the polysaccharides can be determined by chromatographic and spectroscopic methods. To do so, the polysaccharides are first isolated by extraction from plants. After acidic or enzymatic hydrolysis, the monosaccharides are released and analyzed qualitatively and quantitatively by gas chromatography (GC). For their identification, they are compared to authentic standards,[71] often in combination with mass spectrometry (MS). In order to achieve the requisite volatility, chemical modifications to the carbohydrates prior to analysis are necessary.[72,73] Often the “peracetylated alditol acetate” derivatization[74] or protection of the hydroxyl groups with trimethylsilyl groups[75] are performed (Scheme 1). A more recent method for composition analysis utilizes high-pH anion-exchange chromatography (HPAEC), which does not require derivatization of the monosaccharides.[76] However, none of the above described methods provides information about the type of linkages between the monosaccharides. The most common method to analyze the linkages is methylation of the hydroxyl groups of the polysaccharide before hydrolysis. The resulting monosaccharides

carry unprotected hydroxyl groups in positions that were previously bound in a glycosidic linkage. Analysis of GC retention times by using the peracetylated alditol acetate method and MS-fragmentation patterns allow the identification of the linkages.[72] Their stereochemistry has to be determined by NMR spectroscopy of the polysaccharides as the anomeric configuration is lost during the derivatization.[77]



Scheme 1. Hydrolysis of polysaccharides to monosaccharides and their derivatization for GC-MS analysis for quantitative determination of the monosaccharide composition. Left: peracetylated alditol acetate, right: trimethylsilylated monosaccharide.

1.1.5.2 Cell Wall Glycan-Directed Monoclonal Antibodies

The complexity and heterogeneity of plant cell wall glycans is still not well understood. However, it has become apparent that the plant cell wall is a highly sophisticated network, which is able react to environmental changes e.g. by compensatory deposition of one polymer for another or release of certain oligosaccharides to trigger an immune response.[8,78] Previously, studies of the molecular structure of polysaccharides in the context of an intact cell wall were limited to lectins, which significantly lack diversity and specificity.[79] The use of monoclonal antibodies that were raised against plant polysaccharides has greatly improved the number and versatility of probes for high resolution cell wall imaging in plant research (Figure 5). Antibodies are proteins with specific recognition capacities that bind to only one single epitope.[80,81] Differences in the composition of the plant cell wall between cell types and even within the wall surrounding of an individual cell can be detected using monoclonal antibodies. Also, it was shown with antibodies that the occurrence of epitopes changes during plant growth.[82-85] To date more than 200 monoclonal antibodies have been raised against most classes of plant cell wall polysaccharides by different laboratories. The monoclonal antibodies were screened for their specificity with libraries of polysaccharides in enzyme-linked immunosorbent assays

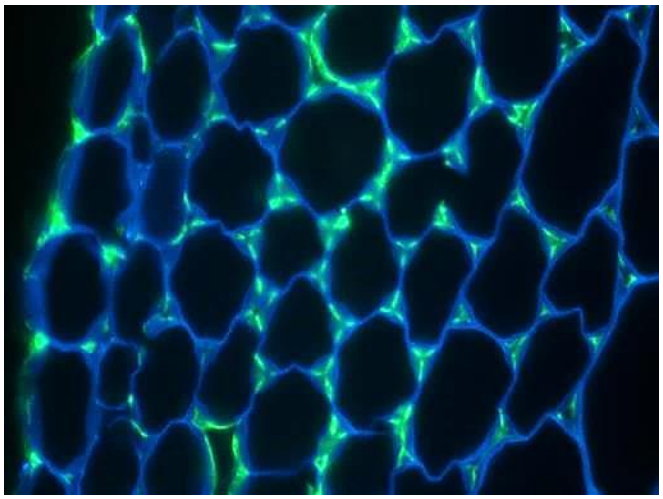


Figure 5. Micrograph showing the epidermis and outer cortical tissue of a tobacco stem section. The cell walls were stained with Calcufluor (blue). Fluorescence (green) indicates binding of xylans by antibody LM10.[85]

(ELISA) or in high-throughput analyses by using the carbohydrate microarray technology.[86] However, the specific binding epitopes of the antibodies are often not known in detail, which limits the applicability of the antibodies. For their characterization so far isolated polysaccharides have largely been used, which do not allow for the determination of the exact chemical entity bound by the monoclonal antibody.[87,88] A detailed characterization requires access to pure and well-defined oligosaccharides, which can only be obtained comprehensively by labor-intensive chemical syntheses.

1.1.6 Cell Wall-Degrading Enzymes

The hydrolysis of complex carbohydrates by glycosyl hydrolases (GHs) is vital to the natural degradation of plant cell walls to ensure recycling of carbon and its release into the carbon cycle. Also, many industrial applications such as bread making, animal feed industry,[89-91] paper manufacturing,[90] biofuel production,[9,55,92] and the production of high quality brews[93] depend on the use of GHs. To improve all of these processes, a detailed understanding of the cell wall degradation at the molecular level is necessary.

The most fundamental enzymatic activity for the hydrolysis of cell wall glycans is hydrolysis of the backbone and its side chains. Xylan-degrading enzymes for example include xylanases, β-D-xylosidases, and arabinofuranosidases. These enzymes for instance hydrolyze arabinoxylans to arabinoxylan oligosaccharides, which are used as prebiotics in the food industry.[11,12] The GHs have been classified into families (CAZY database)[94] based on the amino acid sequence in the catalytic domain. Xylanases have been classified into glycosyl hydrolase (GH) families 5, 8, 10 and 11. Most xylanases belong to

the families GH10 and GH11.[95] To efficiently degrade the polysaccharides into short oligosaccharides or its monosaccharide components numerous GHs act cooperatively. The substrate specificities of several GH families have been determined by analysis of the hydrolysis products after incubation with natural polysaccharides.[96,97] To identify the individual oligosaccharides in complex hydrolysis mixtures, sophisticated analytical techniques are used.[98,99] These experiments are aided by crystal structures of ligand-enzyme complexes and mutational analysis of potential binding sites.[100-104]

1.2 Synthesis of Carbohydrates

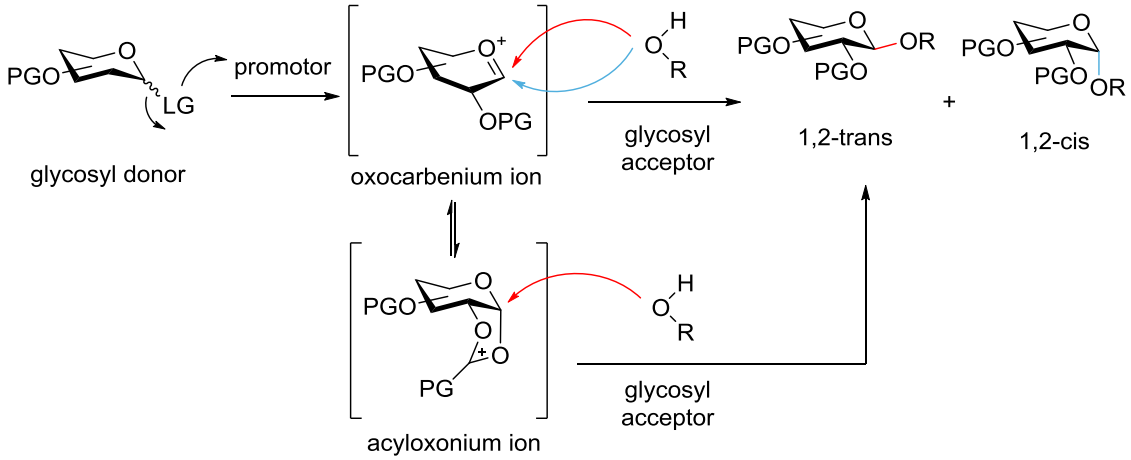
The analysis of individual functions and interactions of plant cell wall polysaccharides is challenging due to their molecular heterogeneity and diversity. Synthetic oligosaccharides can reduce this complexity as they represent model compounds for the larger polysaccharides.[5,6] A library of tailored oligosaccharides may be particularly useful in the characterization of substrate specificities of glycosyl hydrolases or the epitopes of cell wall glycan-directed antibodies.[105-107] These oligosaccharides may also serve as glycosyl acceptors for the characterization of glycosyl transferases to elucidate biosynthetic pathways. Artificial polysaccharides with defined substitution patterns may be helpful for the correlation of structural aspects and macroscopic properties.

Over the past 100 years many chemical and enzymatic methods have been developed for the efficient synthesis of oligo- and polysaccharides. These methods will be briefly discussed in the following chapter.

1.2.1 The Chemical Glycosylation Reaction

In carbohydrate synthesis, the most important reaction is the glycosylation reaction between an activated glycosyl donor and a nucleophile, the glycosyl acceptor.[108,109] The first glycosylation reaction was discovered in the late 19th century by Michael[110] who obtained a protected phenyl glucoside by nucleophilic attack of phenolate to peracetylated glucosyl chloride. Few years later, Fischer[111] synthesized a methyl glucoside from glucose in methanol and HCl. A more controlled glycosylation reaction that uses glycosyl halides as donors was reported by Koenigs and Knorr.[112] This method is still applied today. Numerous glycosyl donors including thioglycosides, imidates, and phosphates have been developed in the meantime. Even though many more protocols for the formation of glycosidic bonds have been described, the synthesis of carbohydrates remains more challenging than that of other biopolymers, since oligosaccharides are often highly branched molecules where each glycosidic linkage can exist in two different

stereoisomeric forms. Furthermore, the mechanism of the glycosylation reaction is still not fully understood. It is believed that the glycosyl donor forms an oxocarbenium ion upon activation with an electrophilic promotor, which leads to dissociation of the leaving group (Scheme 2).[113] Most glycosylation reactions proceed through a contact ion pair rather than a "naked" oxocarbenium ion.[114] The contact ion pair can be attacked by a free hydroxyl group of the glycosyl acceptor to form a new bond. The attack can take place either from the upper (red arrow) or the lower face (blue arrow), which gives the 1,2-*trans* (often β-anomer) or the 1,2-*cis* glycoside (often α-anomer), respectively. Often a mixture of both isomers is obtained. The complexity of the glycosylation reaction is further increased by several alternative reaction pathways that may occur. The glycosyl donor can undergo side reactions, such as hydrolysis, elimination, rearrangements, or orthoester formation.[115]



Scheme 2. Mechanism of the glycosylation reaction with and without a participating group in the C2-position of the glycosyl donor.

The stereoselectivity of the reaction is influenced by many parameters and it is desirable to gain control over the outcome. In general, 1,2-*trans* products are more easily accessible in high selectivity due to the possibility to make use of the neighboring group participation effect (Scheme 2, lower pathway). When an ester group is installed as a participating protecting group in the neighboring C2 position, its acyl moiety can form an acyloxonium ion complex. This complex blocks the α-face and leaves only the β-face free for the subsequent attack of the glycosyl acceptor. Hence, the 1,2-*trans* product is formed with high selectivity.

Without neighboring group participation, the stereoselectivity can be controlled through temperature and other reaction parameters. The α-anomer is the thermodynamically favored product due to the anomeric effect, which stabilizes an axial configuration at the anomeric center. It can be preferably obtained by increasing the

reaction temperature, whereas the β-product is formed in larger amounts at low temperatures. The reaction outcome can also be controlled via the choice of solvent, an effect which is known as the "solvent effect". While the use of acetonitrile favors the formation of the β-product, the use of diethylether, THF, and 1,4-dioxane predominantly give the α-product.[114,116-118] The protecting groups of the glycosyl donor may also assist in the selective formation of 1,2-*cis* glycosidic bonds when remote participating protecting groups are used.[119] These are often ester protecting groups at the C6-position of the glycosyl donor that shield the β-face either sterically or by electronic interactions and therefore favor the attack from the α-face. Also, many other sophisticated protecting groups that influence the stereochemical outcome of the reaction through specific reaction mechanisms have been developed.[119-123]

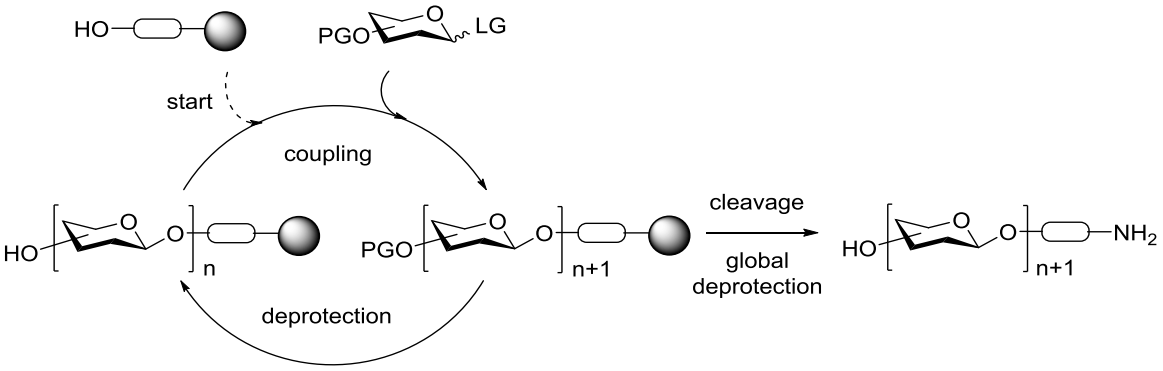
Despite the challenges associated with the formation of glycosidic bonds, many complex oligosaccharides have been successfully synthesized. Recently, even the synthesis of an arabinogalactan containing 92 monosaccharide units was reported.[124] Also the synthesis of plant glycans has gained some attention, and among many others,[5,6] a pectic homogalacturonan dodecasaccharide was synthesized.[125] Still, the chemical synthesis of carbohydrates remains labor-intensive, particularly due to the many purification steps that are required.

1.2.2 Automated Glycan Assembly

Automated solid-phase synthesis provides fast access to a large number of structurally complex compounds. Solid-phase synthesis was introduced by Merrifield,[126] for which he received the Nobel prize in 1984.[127] This technique allows for the use of excess reagents, enabling high conversion, and facilitates purification of the intermediates, as all reagents can be removed by simple filtration. Automated solid-phase synthesis of peptides[128] and oligonucleotides[129] has been well established for decades. Although solid supports have been used also in carbohydrate synthesis for over 40 years,[130] the first automated solid-phase oligosaccharide synthesis (automated glycan assembly) was only reported in 2001.[131]

In solid-phase synthesis of oligosaccharides the first monosaccharide is attached to a linker-functionalized resin in a glycosylation reaction and one or more protecting groups are removed (Scheme 3). Through alternating cycles of coupling and deprotection steps the oligosaccharide chain grows until the desired length is reached. Then, the oligosaccharide is cleaved from the resin and global deprotection using methanolysis and hydrogenolysis is performed in solution to give the final product. The monosaccharide

building blocks (BBs) must be suitably protected with permanent protecting groups (PGs) that are not affected during the assembly of the oligosaccharide chain, and temporary PGs that can be selectively removed on the solid phase. Moieties such as benzyl ethers (Bn), benzoyl esters (Bz), silyl ethers, azides, and trichloroacetyl groups (TCA) have been used as permanent PGs, whereas fluorenylmethoxycarbonyl (Fmoc), levulinoyl (Lev), (2-methyl)naphtyl (Nap),[132] and 2-(azidomethyl)benzoyl (Azmb)[133] have been routinely used as temporary PGs. For elongation of the backbone, Fmoc is most commonly used as it can be quantitatively removed using mild bases.



Scheme 3. Schematic representation of the automated glycan assembly process.

The first automated synthesizer was built based on a modified peptide synthesizer.[131] In 2012, a new synthesizer was reported that can be considered to be the "first fully automated oligosaccharide synthesizer",[134,135] which is currently used in an improved version of automated glycan assembly at the Max Planck Institute of Colloids and Interfaces (MPIKG, Figure 6). The synthesizer is placed in a fume hood and contains a reaction vessel (Figure 6 yellow box), in which the resin is placed. The temperature in the vessel is controlled by an external cooling/heating device which can adjust the temperature from -50 °C to +50 °C. Solvents such as DCM, DMF, and THF can be delivered to the vessel from bottles in a safety cabinet (Figure 6 blue box). The solvents are used to wash the resin thoroughly after each reaction step. In most reactions DCM is used as the solvent as the resin swells well in it, making the oligosaccharides better accessible to reagents and building blocks. Reagents are introduced into the vessel from bottles containing stock solutions in DCM or DMF. These can be BBs (Figure 6 yellow box), activation reagents (Figure 6 red box), and deprotection reagents (Figure 6 green box). The modular setup of the synthesizer allows for the use of all common glycosyl donors including thioglycosides,[134] phosphates, and imidates.[131] Bubbling of Argon through a filter on the bottom of the vessel ensures constant mixing during the reactions.

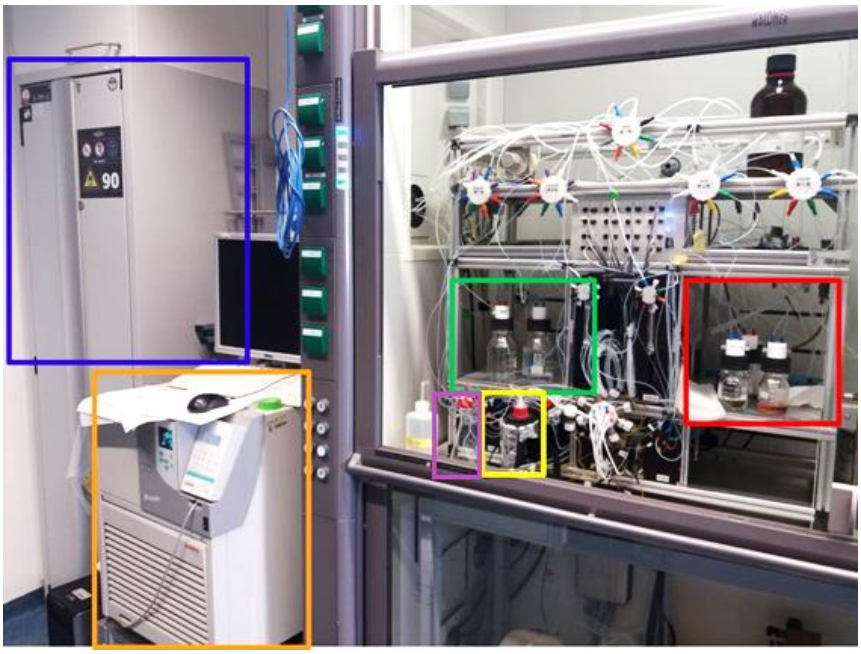
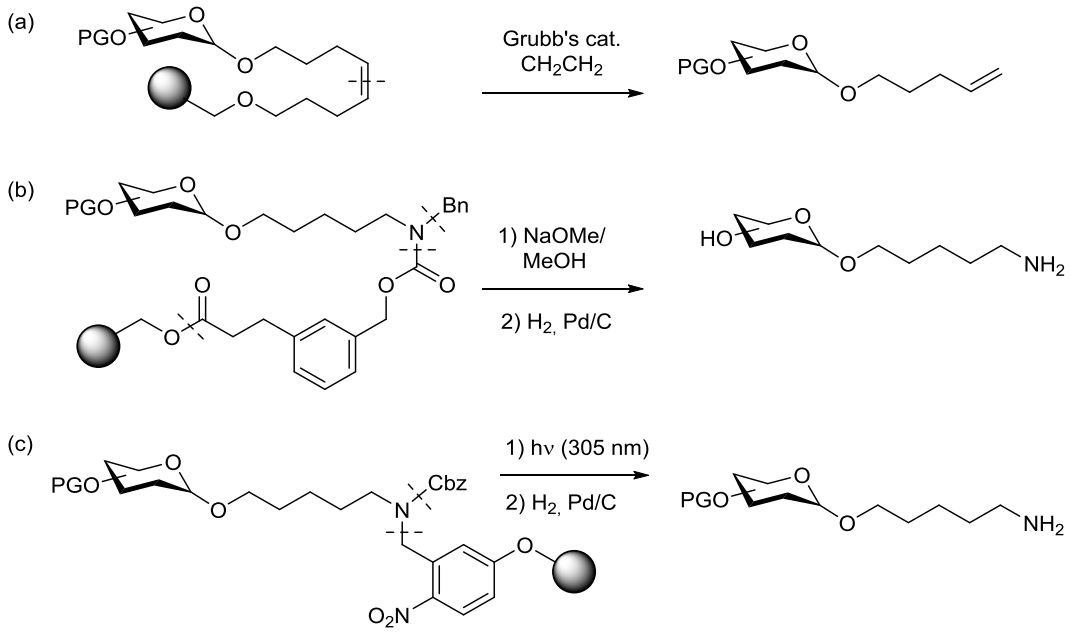


Figure 6. Home built-synthesizer at the MPIKG. Blue box: solvent cabinet; orange box: cryostat; green box: deprotection solutions; red box: activation solutions; purple box: building blocks; yellow box: reaction vessel.

When a reaction step is finished, the solution containing all excess reagents and soluble byproducts is drained through the filter by applying Argon pressure from the top.

After the synthesis the oligosaccharide is cleaved from the resin. This cleavage process requires some particular attention. The linker that is attached to the resin has to meet several requirements. It needs to be stable under the activation and deprotection conditions used over a wide range of temperatures. It is also desirable that the linker provides the oligosaccharides after cleavage in a form that allows for further functionalization. This was initially accomplished by using an octenediol linker (Scheme 4a), which after cleavage by using Grubb's catalyst gives oligosaccharides functionalized with a pentenyl linker.[131,136,137] Later, a carbamate-based linker that provides the oligosaccharide with an aminopentyl linker by methanolysis and hydrogenolysis was developed (Scheme 4b).[134,138] However, the double bond in the octenediol linker excluded the usage of thioglycosides, and the carbamate-based linker was not stable towards strongly acidic conditions as are required for the coupling of phosphate donors. These restrictions have led to the development of a third linker that contains a *p*-nitrophenol moiety and can be cleaved using light at a certain wavelength (Scheme 4c).[139] This linker is used in most modern automated glycan assembly syntheses as it can withstand the widest range of reaction conditions. After cleavage the oligosaccharides are released with a Cbz-protected aminopentyl linker at the reducing end. Subsequent hydrogenolysis provides a terminal amine that can be exploited for coupling to glass surfaces or proteins.



Scheme 4. Commonly used linkers for automated glycan assembly and the conditions for their cleavage.

The photocleavage is performed in a continuous-flow photoreactor. A suspension of the resin in DCM is pumped through tubing (Figure 7, green box) that is wrapped around a mercury lamp (305 nm) (Figure 7, red box). This setup circumvents the problems that are usually encountered with photochemistry in batch reactors, due to the exponential decrease of light intensity with increasing distance from the source, and allows for efficient

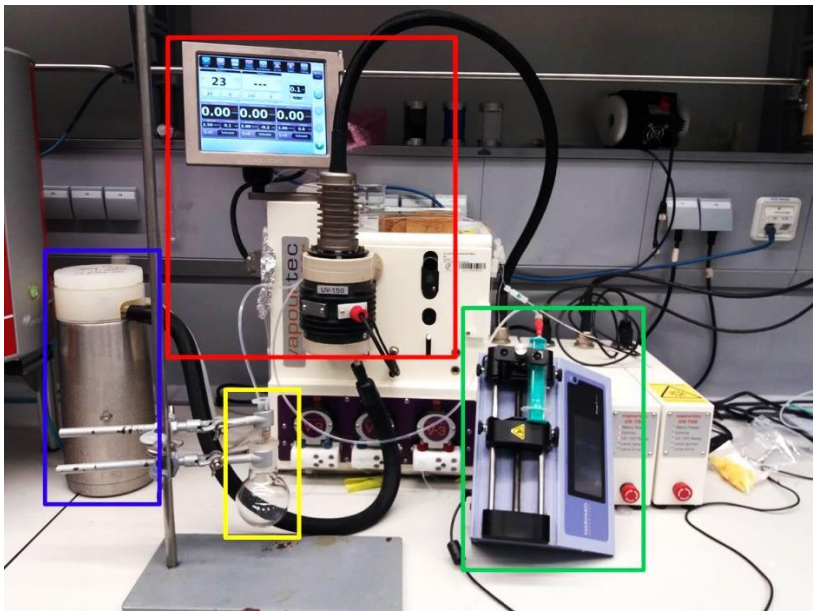


Figure 7: Continuous flow reactor. Red: photolamp, blue: cooling device, yellow: solution containing cleaved oligosaccharides, green: syringe pump with syringe containing the resin.

irradiation of the resin with light. As the UV lamp produces heat it must be constantly cooled (Figure 7, blue box). At the outlet of the tubing the resin is filtered off and the solution containing the oligosaccharide is collected (Figure 7, yellow box).

Automated glycan assembly has enabled the synthesis of many biologically relevant molecules,[140,141] such as chondroitin sulfate glycosaminoglycans[139] that play important roles in infectious diseases and the tumor-associated Globo-H antigen.[142] The synthesis of oligosaccharides as potential vaccine candidates was also reported.[143,144] The synthesis of both, 1,2-*trans*- and 1,2-*cis*-linkages in automated glycan assembly is possible, as demonstrated by the successful syntheses of oligosaccharides containing a β-mannose[145,146] or even multiple *cis*-linkages.[147] The limit of accessible glycan length is constantly extended, with the assembly of a 30-mer[148] and a 50-mer mannoside reported recently.[149] Current limitations of automated glycan assembly are that the oligosaccharides are only produced in small amounts and the instrumental setup is expensive.

1.2.3 Enzymatic Synthesis

Another approach for the preparation of carbohydrates is enzymatic synthesis. Enzymes have high substrate specificities and are highly stereoselective so that no protecting group manipulations are necessary. Two different classes of enzymes can be explored for the transfer of glycosyl donors to an acceptor: glycosyltransferases (GT) and glycosyl hydrolases (GH). GTs are responsible for the majority of glycosylations in nature and can transfer activated glycosyl moieties to a hydroxyl group of a saccharide chain. Lipids, aryl moieties, and heteroatoms of amino acids, such as nitrogen or sulfur, can also act as the acceptor to give glycoconjugates. The donor is typically a nucleotide diphosphate (NDP) sugar, e.g. uridine diphosphate (UDP)-xylose (Figure 8). However, since the required NDP sugars are highly expensive and the expression of many enzymes is still challenging, the synthetic utility of GTs for the synthesis of oligosaccharides on larger scale is somewhat limited.

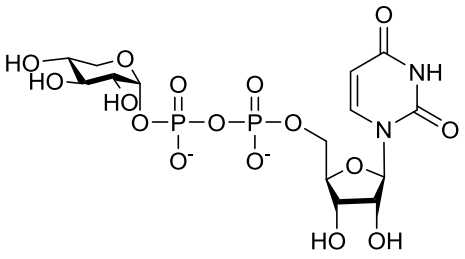
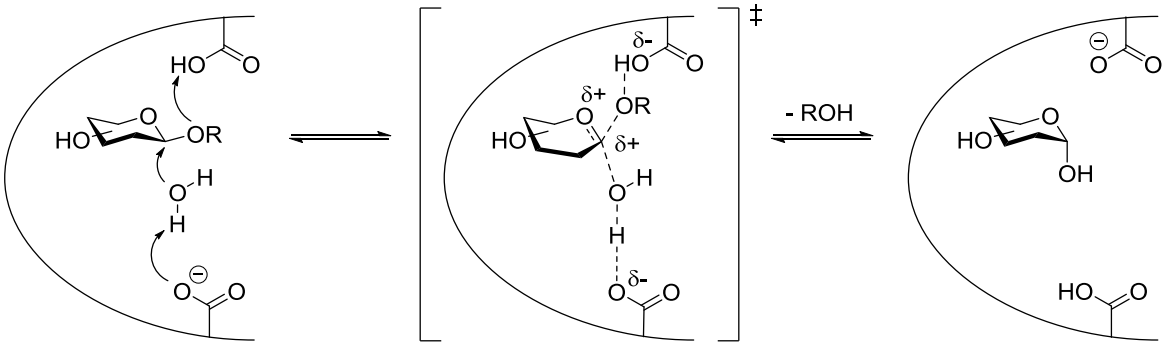


Figure 8. UDP-xylose as an example of a NDP sugar.

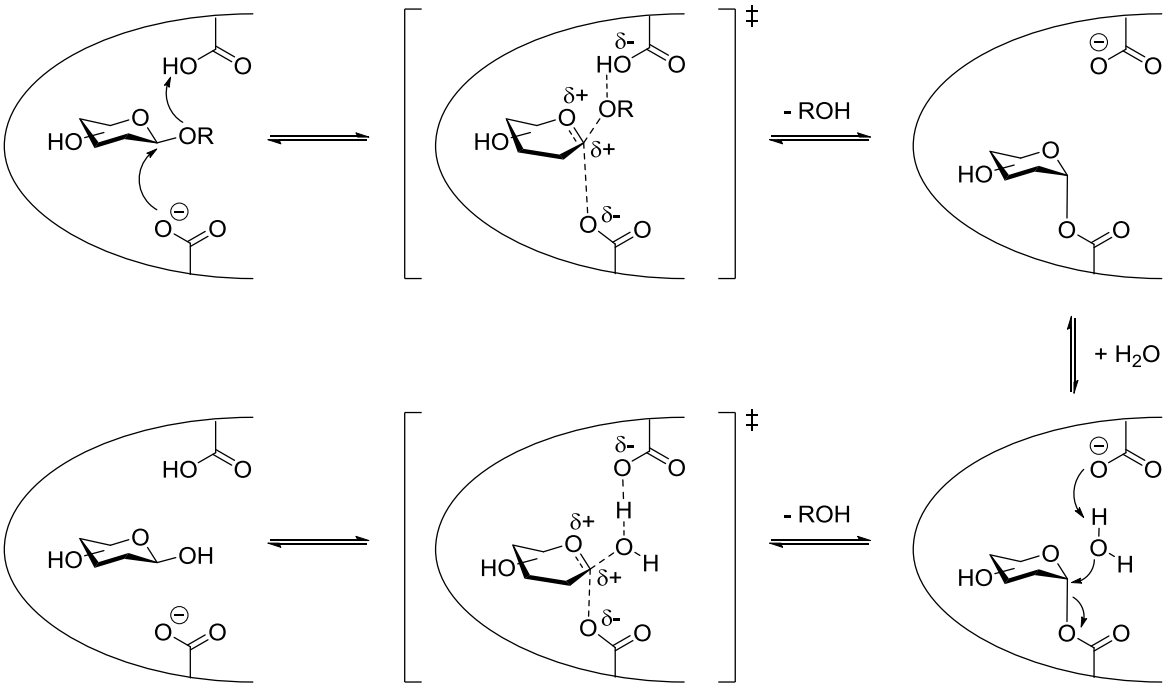
GHs act mainly as glycan-degrading enzymes in nature. In this case, water is the glycosyl acceptor and the glycosidic bond is hydrolyzed. However, the reaction is reversible, and GHs can also catalyze the formation of glycosidic bonds, which will be described in detail later in this chapter. There are two groups of GHs: retaining and inverting GHs. The active sites of both types of enzymes contain two carboxylic acid residues, usually aspartic acid and glutamic acid.[151-153] Inverting GHs use a direct displacement mechanism (Scheme 5), in which the substrate and water must be present simultaneously. The carboxylic acids, which are ~10 Å apart, provide assistance in the form of a general acid-base-catalysis to facilitate the nucleophilic attack of water.[154] The anomeric carbon in the transition state has substantial sp^2-character and can be considered oxocarbenium-like.[150] The resulting product has an anomeric configuration opposite to the one of the starting material. Hence, the reaction proceeds under inversion of configuration.



Scheme 5. Direct displacement mechanism in inverting GHs.[150]

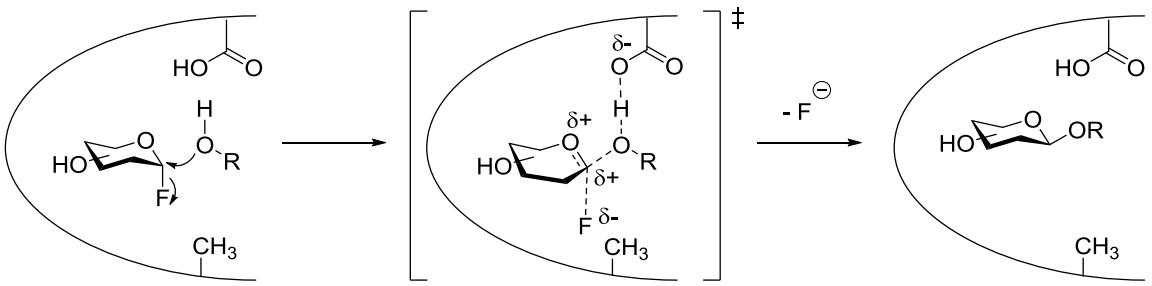
Retaining GHs follow a double-displacement mechanism (Scheme 6). Here, the carboxylic acids are only ~5 Å apart. This enables one of the carboxylic acids to function as a nucleophile. The carboxylate attacks at the anomeric center and forms a covalent substrate-enzyme-intermediate. This reaction is facilitated by the other carboxylic acid that protonates the leaving group. The corresponding carboxylate anion acts as a catalytic base in the second step, by deprotonating water to facilitate its subsequent nucleophilic attack at the anomeric center.[154] Both reactions proceed via oxocarbenium-like transition states. The product is obtained in the same configuration as the starting material with an overall retention of configuration.

GHs can also catalyze the reverse reaction, the formation of a glycosidic bond, which was exploited in the “thermodynamic approach”. High concentrations of two sugars were treated with a GH to shift the equilibrium towards the condensation (glycosylation reaction) in order to produce polysaccharides. In the “kinetic approach” an already



Scheme 6. Double-displacement mechanism in retaining GHs.[150]

activated sugar donor was used, which gives high steady state concentrations of the covalent-enzyme-intermediate that can then be trapped with a suitable glycosyl acceptor. However, yields are generally low, as the product is always also a substrate for hydrolysis.[155,156] In order to efficiently utilize GHs to build up oligosaccharides, their hydrolytic activity had to be eliminated. This was accomplished first in 1998 by Withers *et al.*[157] who genetically replaced the nucleophilic amino acid in the active site of β-retaining GHs with an alanine. These mutants were termed glycosynthases[150,157,158] (Scheme 7). In glycosynthase-catalyzed reactions an activated glycosyl donor such as a glycosyl fluoride with the opposite anomeric configuration of the natural substrate is attacked by the glycosyl acceptor, which is activated by acid catalysis of the non-mutated carboxylic acid in the active site. This S_N2-type reaction inverts the configuration at the anomeric position. Therefore, the use of α-fluorides results in the formation of β-products when retaining



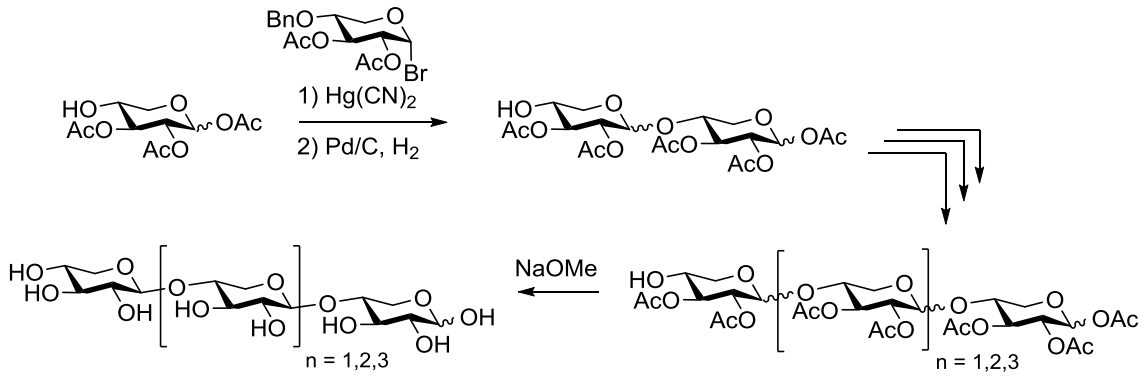
Scheme 7. Mechanism of a glycosylation reaction catalyzed by a retaining glycosynthase.[157]

glycosynthases are used. Now, the scope of this technology has been expanded to produce α-configurated products as well.[158] The use of leaving groups other than fluoride is limited because of the restricted space in the catalytic domain of the enzyme and only few alternatives such as the use of β-glycosyl azides have been reported.[159]

Self-condensation of the donor is possible when glycosynthases are used that are derived from *endo*-glycosidases.[160-162] In this way, polysaccharides of different classes including xylans,[163-165] xyloglucans[166-169] and mixed-linked glucans[169] with molar masses up to 60 kDa have been obtained. By introduction of substituents at the oligosaccharide monomer, the global substitution pattern of the polysaccharide can be tailored. This strategy was applied to the synthesis of functionalized cellulose from cellobiosyl fluoride donors that carry amino-, bromo-, and even sulfur substitution at the 6′ position.[170,171]

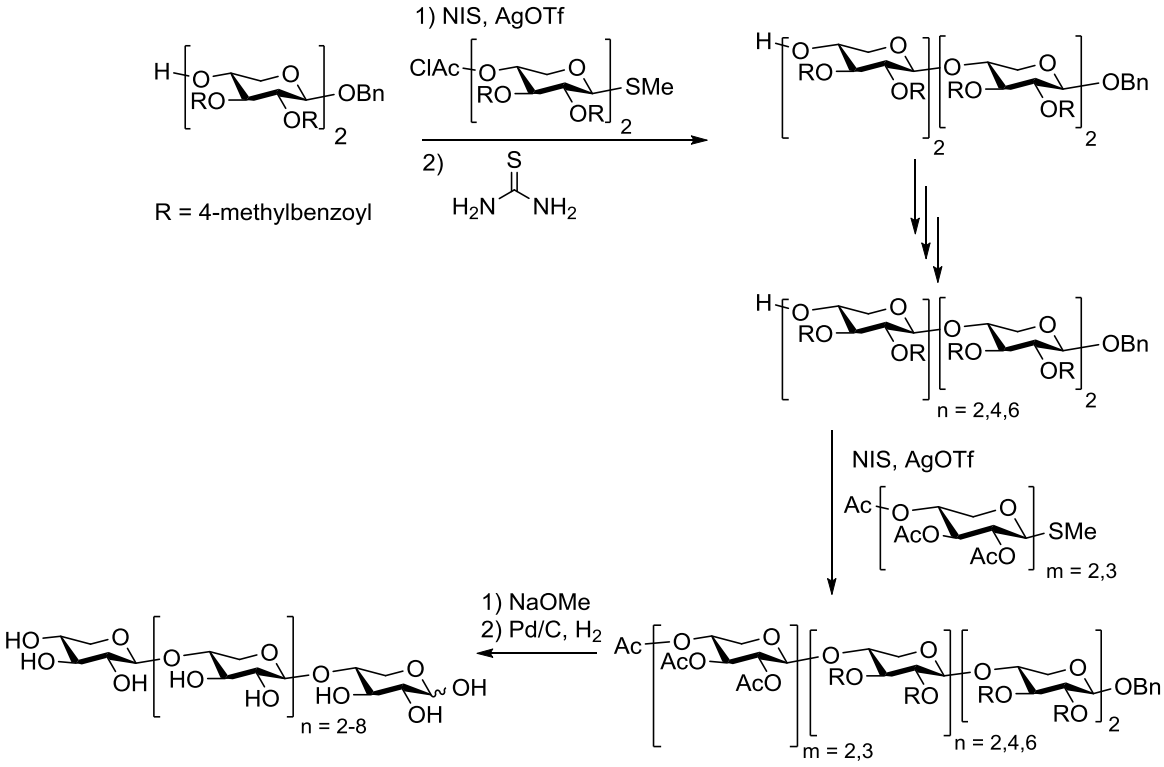
1.2.4 Chemical Synthesis of Xylan Oligosaccharides

Chemical syntheses for many classes of complex plant oligosaccharides have been described.[5,6] For xylans mostly syntheses of linear structures up to decaoligosaccharides[172-175] have been reported. The first synthesis of linear xylans was described by Hirsch and Kováč in 1981 (Scheme 8).[172,173] In their synthetic strategy a peracetylated xylosyl bromide donor was reacted with an acetyl protected xyloside acceptor in a mercury cyanide-promoted glycosylation reaction. A benzyl protecting group at the C4'-position of the resulting disaccharide was subsequently removed by hydrogenolysis. Iteration of the two steps, followed by a final methanolysis, provided a collection of tri- to pentaxylosides. However, the glycosylation reactions proceeded with poor stereoselectivity (α/β 1:1.5) and low yields (<25%).



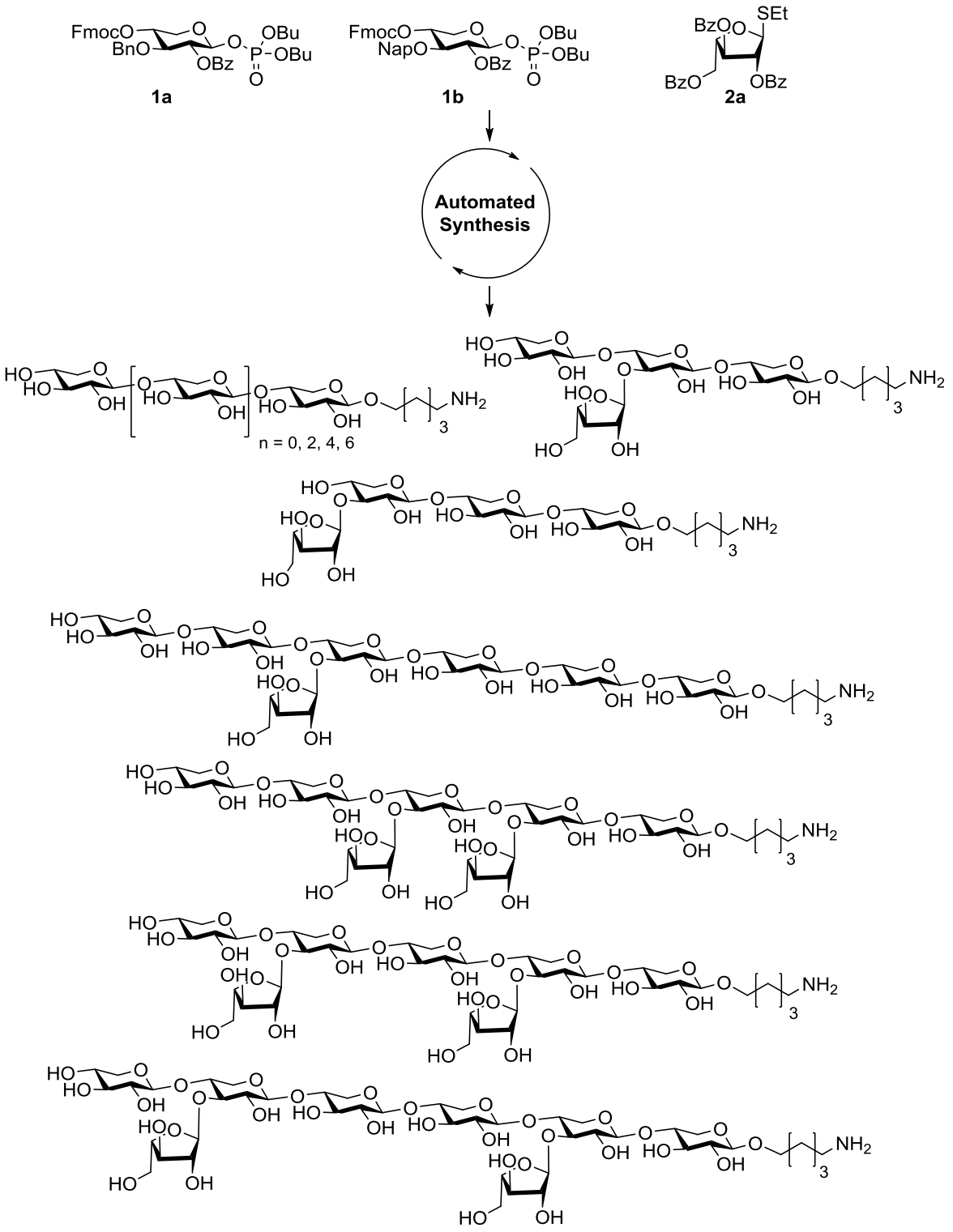
Scheme 8. Stepwise synthesis of xylooligosaccharides from tri- to pentaxylosides by Hirsch and Kováč.[172,173]

By using a blockwise approach of suitably protected thioglycosides, Takeo *et. al.* (Scheme 9)[175] achieved better coupling yields (~70%). They first synthesized tetra- to octaxylosides by using disaccharide BBs. The disaccharide BB at the reducing end was protected with a permanent benzyl ether at the anomeric center. The free hydroxyl group in the C4-position was reacted in an NIS/AgOTf-promoted glycosylation reaction with a thioglycoside disaccharide BB and a chloroacetyl protecting group in the C4-position of this BB was removed by treatment with thiourea. By repeating these steps, oligosaccharides up to an octasaccharide were obtained. These oligosaccharides were then reacted with peracetylated di- or trisaccharide thioglycoside donors to give a collection of oligosaccharides, containing tetra- to decaxylosides after global deprotection.



Scheme 9. Block-synthesis of xylooligosaccharides up to decaxylosides by Takeo *et al*.[175].

Until recently the only syntheses of substituted xylans were reported for a D-glucuronic acid-substituted tri-[176] and pentasaccharide.[177] As arabinoxylan oligosaccharides are of great economic interest due to their beneficial prebiotic effects[11,54] and antioxidative activities,[17] a collection of α-1,3-arabinofuranose substituted xylan oligosaccharides has been recently synthesized by automated glycan assembly (Deborah Senf, MSc thesis, MPIKG, Scheme 10).[132] Three monosaccharide BBs including two xylose and one arabinose BB were used for the automated glycan assembly. The xylose BBs were equipped with an Fmoc-group in the C4-position, which was selectively



Scheme 10. Automated glycan assembly of α-1,3-arabinofuranose substituted xylan oligosaccharides from three monosaccharide BBs.[132]

deprotected to enable chain elongation. Linear xylosides were produced by alternating coupling cycles with xylose BB **1a** and Fmoc-deprotection cycles. Branching in the C3-position was enabled by introducing xylose BB **1b** into the backbone. The xylose residue

was protected at the C3-position with a (2-methyl)naphtyl (Nap) protecting group that can be selectively removed using DDQ.[178-180] The resulting free hydroxyl group was then glycosylated with arabinose BB **2a**. After cleavage from the resin and global deprotection the final oligosaccharides were obtained.

1.3 Aims of this Thesis

The general aim of this thesis was to contribute to plant biological research by providing synthetic glycans as tools for investigating the plant cell wall.

The first aim was to characterize plant glycan-directed monoclonal antibodies, which are probes for high resolution imaging of cell wall polysaccharides and xylan-deconstructing enzymes, which are key to the utilization of plant biomass (chapter 2.1). For this purpose, a collection of arabino- and glucuronoxylan oligosaccharides from the plant cell wall was required. Automated glycan assembly was envisioned to be a powerful technology to gain fast access to these oligosaccharides containing many repetitive structural motifs. While the assembly of the xylan backbone is straightforward, attachment of arabinofuranosyl- and glucuronosyl residues to the backbone requires the design of BBs with sophisticated protecting group patterns and an efficient synthetic strategy towards a stereoselective formation of *cis*-glucosidic bonds.

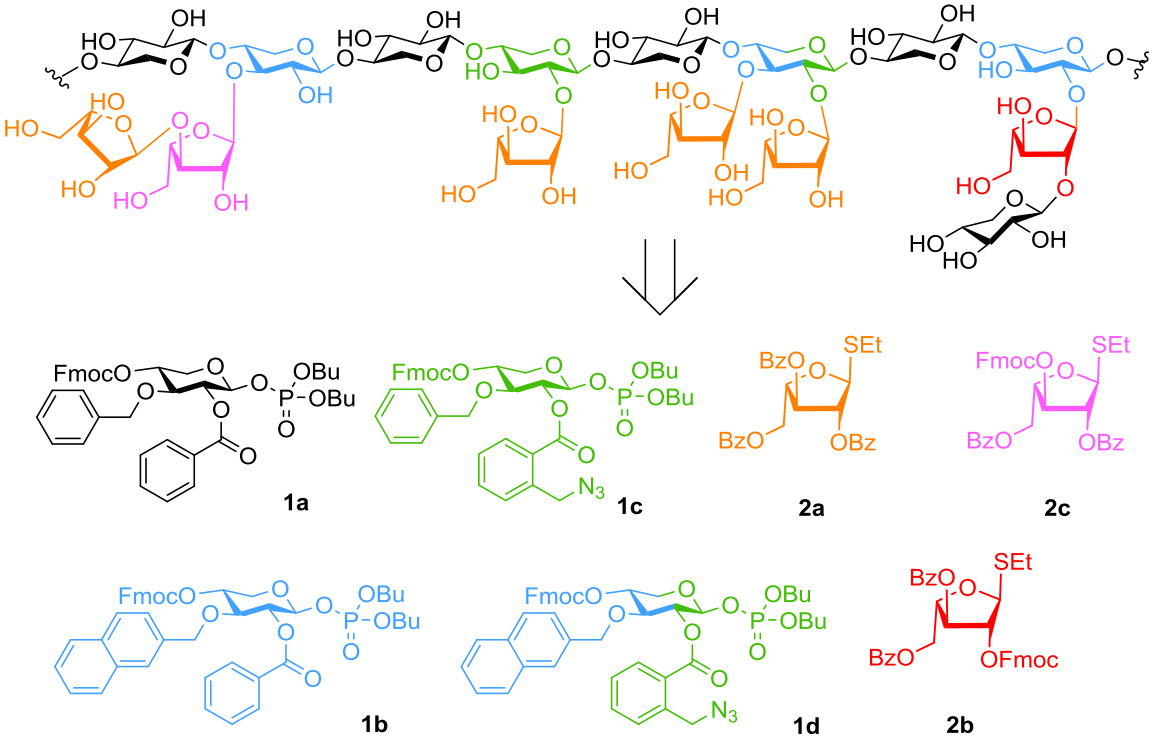
The second aim of the thesis was to provide synthetic tools for studying the structure-function-relationship of arabinoxylans that are important for the plant cell wall architecture. The structural inhomogeneity of many cell wall polysaccharides such as arabinoxylan makes the correlations of the molecular structure and macroscopic properties difficult. To study the impact of specific structural features on physico-chemical properties, such as crystallinity and the affinity to other cell wall components, collections of synthetic polysaccharides with defined repeating units were required. Therefore, a library of artificial arabinoxylan polysaccharides with defined substitution patterns was planned to be synthesized from oligosaccharide monomers by glycosynthase-mediated polymerization (chapter 2.2). The required oligosaccharide monomers can be obtained by chemical and chemo-enzymatic synthesis, or procured commercially.

2 Results and Discussion

2.1 Automated Glycan Assembly of Arabino- and Glucuronoxylan Oligosaccharides[1]

2.1.1 Automated Glycan Assembly of Arabinoxylan Oligosaccharides

The efficient automated glycan assembly of selected oligosaccharide fragments of arabinoxylan requires differentially protected BBs that are readily synthesized and can be employed in a modular fashion. Four BBs were designed for construction of the xylan backbone (Scheme 11). BB **1a** was used for linear chain elongation. It was equipped with a base-labile Fmoc-protecting group in the C4-position and, for permanent protection of the C2- and C3-positions, a benzoyl ester and a benzyl ether, respectively. In order to enable substitution of the backbone, temporary protecting groups were selected for the

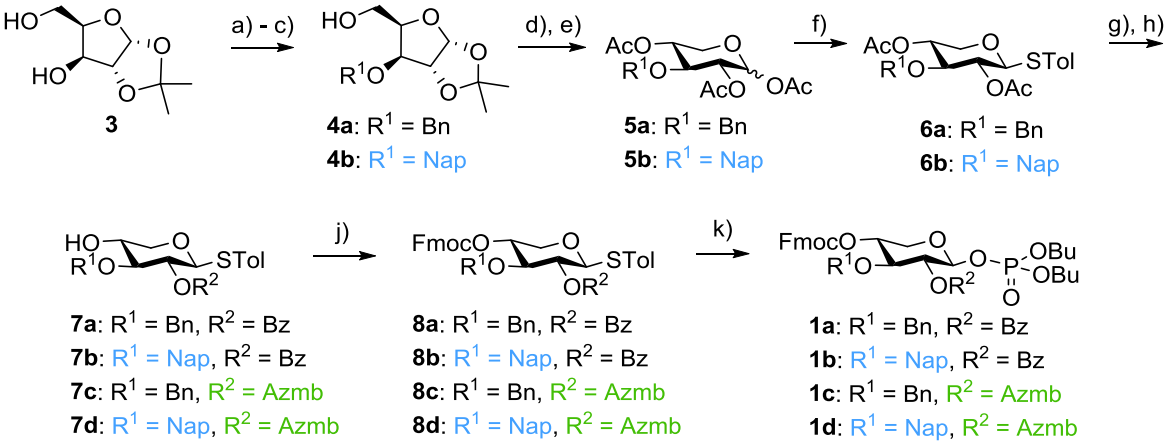


Scheme 11. Chemical structure of an arabinoxylan polysaccharide and the building blocks required for the assembly of representative oligosaccharide fragments.

[1] This chapter has been modified in part from the following articles: Schmidt, D.; Schuhmacher, F.; Geissner, A.; Seeberger, P. H.; Pfrengle, F., *Chem. Eur. J.* **2015,** *21* (15), 5709-5713. DOI: https://10.1002/chem.201500065. Senf, D.; Ruprecht, C.; deKruijff, G. H. M.; Simonetti, S. O.; Schuhmacher, F.; Seeberger, P. H.; Pfrengle, F., *Chem. Eur. J.* **2017,** *23* (13), 3197-3205. DOI: https:// 10.1002/chem.201605902.

hydroxyls at C2 and C3 that ensure similar reactivity of BBs **1b**-**d** compared to BB **1a**. Exchange of the benzyl for a (2-methyl)naphtyl (Nap) ether in **1b** allows for installation of arabinose substituents in the C3-position. Nap ethers are readily cleaved under oxidative conditions[178-180] and have been previously used for the synthesis of a small library of α-1,3-substituted arabinoxylan oligosaccharides (Deborah Senf, MSc thesis, MPIKG).[132] For temporary protection of the C2-hydroxyl the use of a 2-(azidomethyl)benzoyl (Azmb) ester in BB **1c** is particularly attractive. The Azmb-group provides both the required participating effect for selective β-(1→4)-glycosylations and the potential for chemoselective removal using alkyl phosphines. Azmb had already proven to be a powerful protecting group in the solution-phase synthesis of different complex oligosaccharides[181-183] and was recently added to the toolbox of temporary protecting groups for automated glycan assembly (Goswin deKruijff, MSc thesis, MPIKG).[133] Combination of the Nap and Azmb protecting groups allows for the introduction of doubly substituted xylose units when using BB **1d**. All xylose BBs were equipped with phosphate leaving groups that gave the best results in the synthesis of plant cell wall oligosaccharides previously.[106,184] Arabinofuranose BBs **2a**-**c** enable substitution of the backbone either with single arabinose units or elongated oligosaccharide side chains.

The design of the protecting group pattern in BBs **1a**-**d** allowed for their synthesis to diverge at a late stage (Scheme 12). Starting from 1,2-*O*-isopropylidene-D-xyloside **3** a simple three step transformation gave the 3-*O*-Bn and 3-*O*-Nap protected derivatives **4a** and **4b** in good yields.[132,185] After acidic cleavage of the acetonide with concomitant equilibration of the furanose to the pyranose form, peracetylated **5a**,**b** were obtained after



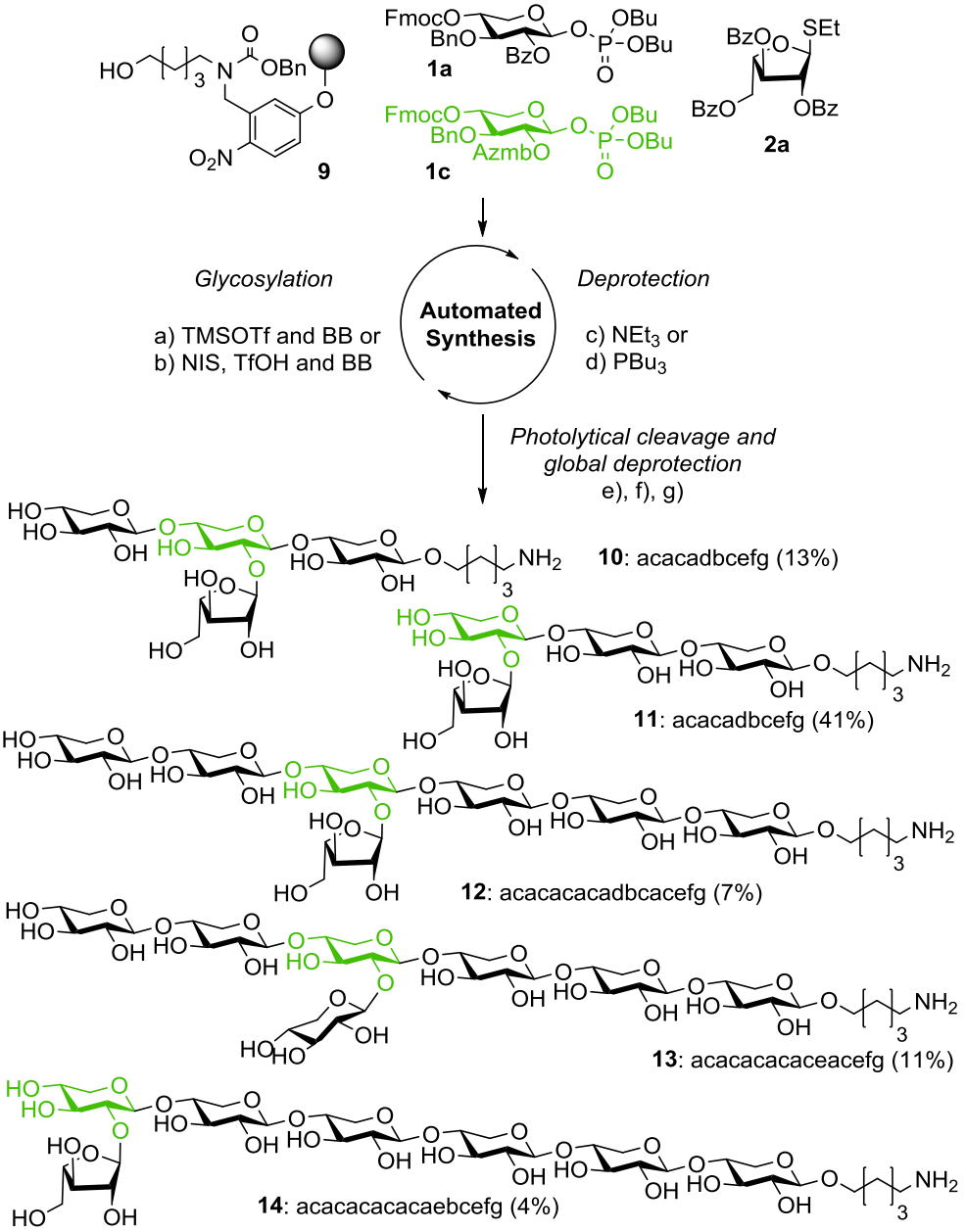
Scheme 12. Synthesis of xylose building blocks **8a**-**d**. Reagents and conditions: a) TrCl, DMAP, NEt_3, DMF; b) R^1Br, NaH, TBAI; c) TsOH, MeOH/Et_2O, H_2O (100:10:1), **4a**: 72%, **4b**: 62% (3 steps); d) H_2O/AcOH, reflux; e) Ac_2O, DMAP; f) HSTol, $BF_3{\cdot}OEt_2$, 0 °C, **6a**: 62%, **6b**: 48% (3 steps); g) NaOMe, MeOH, CH_2Cl_2; h) $R^2{}_2O$, 5-10 mol% $Yb(OTf)_3$, dioxane, **7a**: 71%, **7b**: 61%, **7c**: 64%, **7d**: 44% (2 steps); j) FmocCl, pyridine, CH_2Cl_2, **8a**: 79%, **8b**: 76%, **8c**: 87%, **8d**: 68%; k) $HOP(O)(OBu)_2$, *N*-iodosuccinimide, triflic acid, **1a**: 87%, **1b**: 95%, **1c**: 60%, **1d**: 63%.

treatment with acetic anhydride in pyridine. Subsequently, **5a** and **5b** were converted into the corresponding thioglycosides **6a** and **6b** and the acetyl groups were cleaved off by methanolysis. An ytterbium triflate catalyzed selective protection of the C2-position[186] using either Bz_2O or $Azmb_2O$ gave the Bz esters **7a,b** and Azmb esters **7c,d**, respectively. The glycosyl donors **1a**-**d** were finally obtained by protection of the free hydroxyls at C4 with FmocCl (**8a**-**d**) and introduction of a phosphate ester as the anomeric leaving group. The arabinose BBs **2a** and **2b** were synthesized as reported previously.[132] Arabinose BB **2c**[187] was available in our group and kindly provided by Max Bartetzko.

After their successful synthesis, BBs **1a**-**d** were added to a Merrifield resin equipped with a photocleavable linker **9**[139] (Scheme 13) via alternating cycles of glycosylation and deprotection reactions. Glycosylations with phosphate BBs **1a**-**d** were originally performed with two times 5 equiv of glycosyl donor and stoichiometric amounts of TMSOTf as an activator (module A).[132] However, two cycles using 1.8 equiv of donor each were also found to be sufficient to obtain full conversion in the construction of the xylan backbone. Thioglycoside BBs **2a**-**c** were also reacted using as little as two times 1.8 equiv BB upon activation with stoichiometric NIS and catalytic amounts of TfOH (module B). The temporary protecting groups were removed according to standard deprotection modules that are routinely used in automated glycan assembly. Fmoc was readily removed using triethylamine (module C), while deprotection of the hydroxyl group at 3-OH was accomplished best by treating the resin with a DDQ solution in DCE/MeOH/H_2O (64:16:1) at 40 °C in seven incubation cycles of 20 min (module D).[132] For deprotection of the Azmb ester, incubation of the resin with a solution of PBu_3 in THF/H_2O (5:1) at 45 °C in 6 cycles of 30 min each (module E) was performed. Key in this reaction was the use of the right amount of water to hydrolyze iminophosphoranes formed during the course of the reaction.[133,183]

Having established reliable glycosylation and deprotection conditions for all BBs and protecting groups, a number of xylan oligosaccharides decorated with α-1,2-linked arabinofuranoses (**10**-**14**) were prepared (Scheme 13). For the two tetrasaccharides **10** and **11** the full xylan backbone was synthesized first using xylose BBs **1a** and **1c**, and then the arabinose substituent was added using BB **2a** after selective deprotection of the Azmb group. For the longer xylan structures, however, the product yields in the synthesis of 2-substituted compounds decreased with the number of glycosylations performed after introduction of the Azmb-protected BB **1c**. It was advantageous to remove the Azmb-group at an earlier stage of the assembly and continue with elongation of the backbone after introduction of the substituent. Using this strategy, the two heptasaccharides **12** and **13** were prepared. The latter, a branched oligosaccharide comprised only of

xylopyranoses, resembles branched xylans that are present in the mucilage surrounding plant seeds.[188]



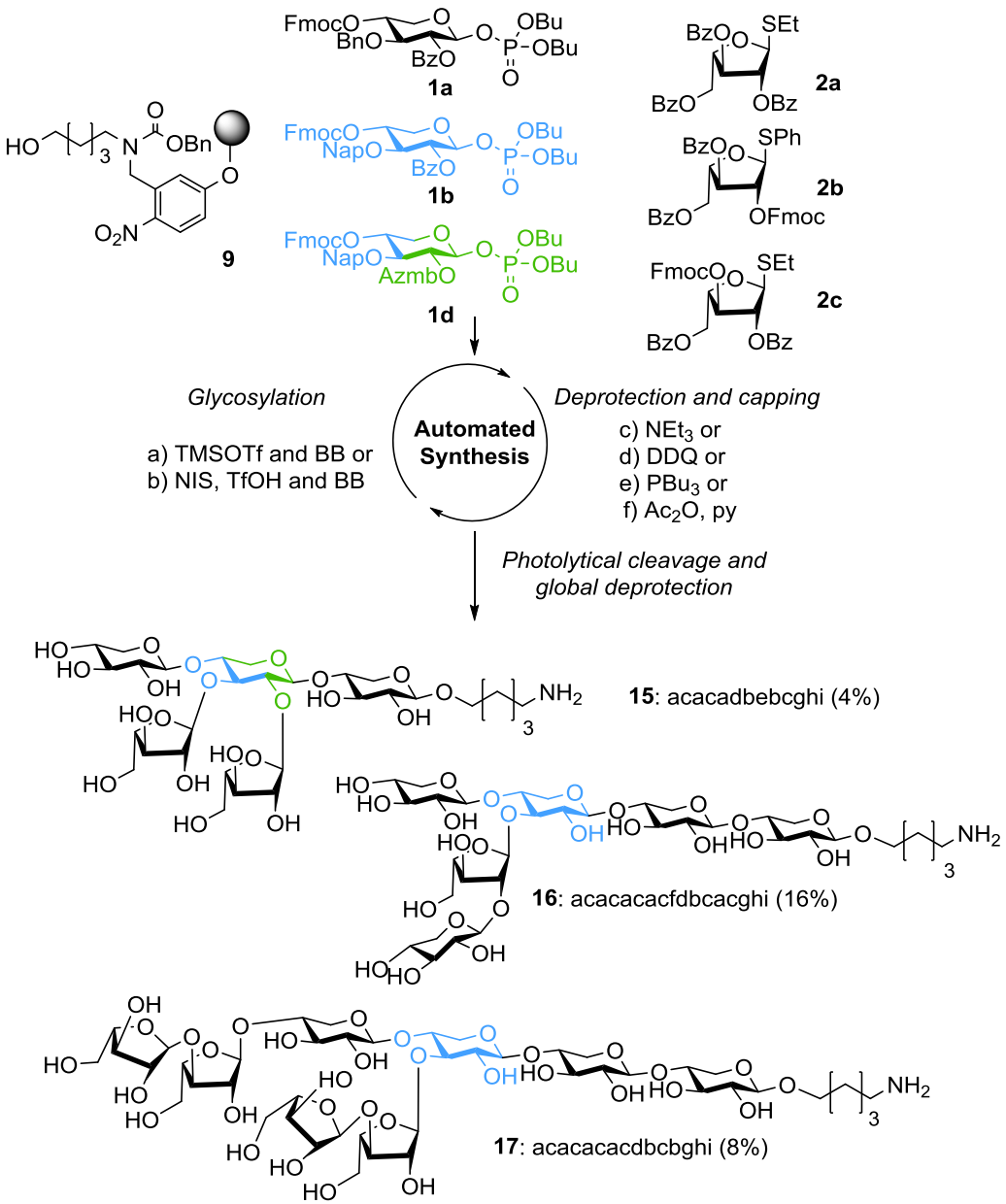
Scheme 13. Automated glycan assembly of α-1,2-substituted arabinoxylan oligosaccharides **10**–**14**. Reagents and conditions: a) 2 x 5 equiv, 2 x 3.7 equiv or 2 x 1.8 equiv of BB **1a** or **1c**, TMSOTf, DCM, -35 °C (5 min) → -15 °C (30 min) (Module A); b) 2 x 5 equiv or 2 x 1.8 equiv of BB **2a**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min) (Module B); c) 3 cycles of 20% NEt_3 in DMF, 25 °C (5 min) (Module C); d) 6 cycles of PBu_3, THF/H_2O (5:1), 45 °C, 30 min (Module E); e) hν (305 nm); f) NaOMe, DCM/MeOH, 12 h; g) H_2, Pd/C, EtOAc/MeOH/H_2O/HOAc, 12 h (yields are based on resin loading). The letter code below the structures represents the reaction sequence applied in the respective synthesis

For the terminally substituted heptasaccharide **14**, the backbone was assembled by introducing BB **1c** as the last xylose unit. After deprotection of the Azmb-group, arabinose BB **2a** was attached to the resulting free hydroxyl group. Due to the low yields obtained in the syntheses of large structures the synthesis of oligosaccharides carrying two α-1,2-linked arabinosyl substituents was not attempted. The fully deprotected oligosaccharides **10**-**14** were obtained after light-induced cleavage from the resin and global deprotection consisting of methanolysis and subsequent hydrogenolysis in yields of 4-41%.

Using a combination of the two orthogonal Nap and Azmb protecting groups in a single BB (**1d**) the synthesis of pentasaccharide **15** having two substituents linked to same xylose unit was achieved (Scheme 14). In order to attach these two arabinofuranose residues, first the Nap ether was deprotected and the resulting free hydroxyl was then glycosylated with **2a** before deprotection of the Azmb ester allowed for another glycosylation with **2a**. After light-induced cleavage from the resin and deprotection of the benzoyl protecting groups by methanolysis, hydrogenolysis was performed as with the previous compounds by using a solvent mixture of MeOH, EtOAc, H_2O and AcOH. AcOH is used to avoid adsorption of the amino-group to the palladium catalyst. However, in this case the acidic conditions caused partial cleavage of one of the arabinose substituents, which resulted in a low isolated yield (4%) for pentasaccharide **15**.

Next, two α-1,3-substituted arabinoxylan oligosaccharides containing disaccharide side chains were synthesized to expand the small library of oligoarabinoxylosides that was previously synthesized (Deborah Senf, MSc thesis, MPIKG).[132] Both side chains are frequently found in grasses and often esterified with ferulic acid.[189] Their automated glycan assembly was performed by following the well-established protocol developed previously (Deborah Senf, MSc thesis, MPIKG).[132] For the synthesis of both oligosaccharides the xylose backbone was first fully assembled using BBs **1a** and **1b**. The synthesis of hexasaccharide **16**, containing a β-1,2-D-xylopyranosyl-α-1,3-L-arabinofuranosyl disaccharide side chain required capping of the terminal xylose residue after backbone assembly using acetic anhydride and pyridine (module F). Only then, the Nap group was cleaved to enable glycosylation with arabinose BB **2b** that contains an Fmoc protecting group for elongation of the side chain. Capping of the backbone was thus needed for the subsequent selective glycosylation of the arabinose with xylose BB **1a**. Instead of octasaccharide **17** we originally intended the synthesis of a hexasaccharide containing only one arabinan side chain connected to the C3-position of the penultimate xylose residue. The synthesis of this hexasaccharide would have required capping of the terminal xylose residue in the backbone, similar to the synthesis of **16**. Due to technical difficulties the capping module was not performed during the automated synthesis and the

hydroxyl group at the C4-position remained unprotected. After deprotection of the Nap group and glycosylation reaction with arabinose BB **2c**, the highly reactive arabinosyl donor has glycosylated both free hydroxyl groups simultaneously. The subsequent Fmoc deprotection and glycosylation with arabinose BB

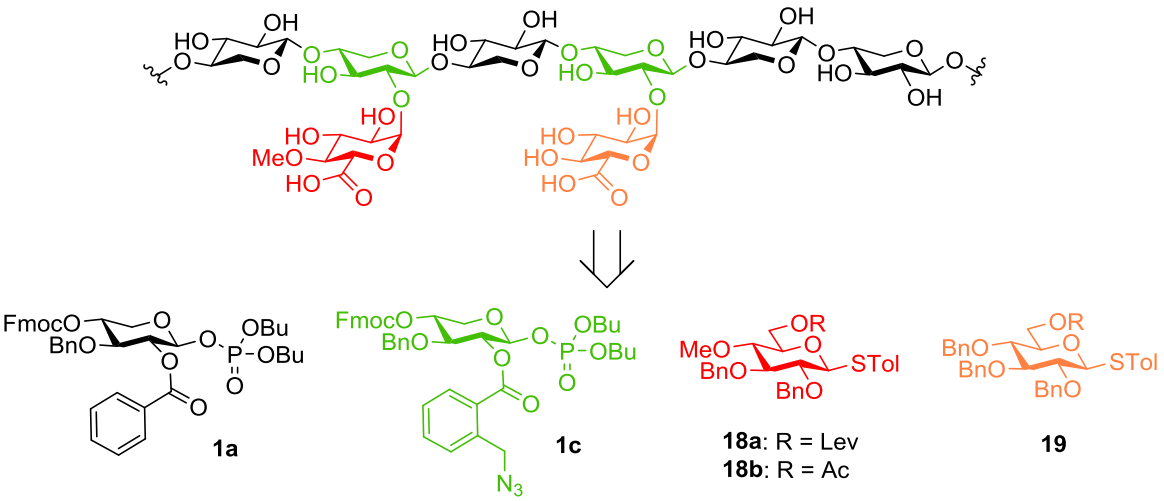


Scheme 14. Automated glycan assembly of α-1,2-α-1,3-disubstituted and α-1,3-substituted arabinoxylan oligosaccharides **15**–**17**. Reagents and conditions: a) 2 x 5 equiv, 2 x 3.7 equiv or 2 x 1.8 equiv of BB **1a**, **1b** or **1d**, TMSOTf, DCM, -35 °C (5 min) → -15 °C (30 min) (Module A); b) 2 x 5 equiv or 2 x 1.8 equiv of BB **2a**, **2b** or **2c**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min) (Module B); c) 3 cycles of 20% NEt_3 in DMF, 25 °C (5 min) (Module C); d) 7 cycles of 0.1 M DDQ in DCE/MeOH/H_2O (64:16:1), 40 °C, 20 min (Module D); e) 6 cycles of PBu_3, THF/H_2O (5:1), 45 °C, 30 min (Module E); f) 3 cycles of Ac_2O, pyridine, 25 °C, 30 min (Module F); g) hν (305 nm); h) NaOMe, DCM/MeOH, 12 h; i) H_2, Pd/C, EtOAc/MeOH/H_2O/HOAc, 12 h (yields are based on resin loading). The letter code below the structures represents the reaction sequence applied in the respective synthesis

2a were also performed twice resulting in the formation of the protected version of octasaccharide **17**. The fully deprotected oligosaccharides **16** and **17** were obtained after light-induced cleavage from the resin and global deprotection consisting of methanolysis and subsequent hydrogenolysis in yields of 16 and 8%.

2.1.2 Automated Glycan Assembly of Glucuronoxylan Oligosaccharides

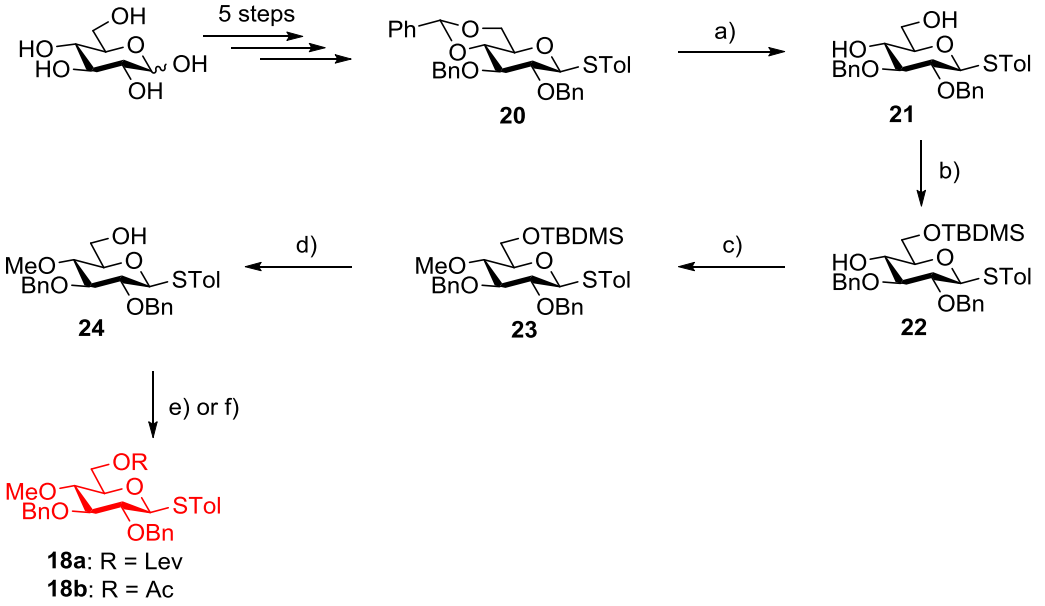
The automated glycan assembly of glucuronic acid substituted oligoxylosides required only two xylose BBs for the assembly of the backbone (Scheme 15) as substitution only occurs at the C2-position in natural glucuronoxylans. A glucose BB was utilized for the automated glycan assembly in combination with a post-assembly oxidation strategy because glucuronic acid BBs are disarmed donors and generally exhibit low reactivity.[190] To favor the formation of an α-glucosidic bond, as opposed to the β-anomer, the C6-position was protected with an ester-group that enables remote participation.[147] Two types of glucose BBs were designed to introduce either glucuronic acid (**19**) or its 4-*O*-methylated derivative (**18a** and **18b**) as substituents of the xylose backbone. BB **18** was synthesized with both a Lev- and an Ac-protecting group in the C6-position in order to investigate their ability to favor the formation of the α-glucosidic bond by remote participation.



Scheme 15. Chemical structure of a glucuronoxylan polysaccharide and the building blocks required for the assembly of representative oligosaccharide fragments.

The two 4-*O*-methylated BBs were synthesized starting from D-glucose which was transformed in five steps to acetal **20**.[191] Complete removal of the acetal by TFA gave dialcohol **21** that was selectively protected at the C6-position as a sterically demanding

TBDMS ether. The free hydroxyl group in **22** was then deprotonated with NaH and reacted with MeI to introduce the 4-*O*-methyl group (**23**). The final BBs were obtained after removal of the silyl ether (**24**) and subsequent esterification of the C6-position. The Lev-group was introduced by DCC-promoted esterification of the free hydroxyl group with LevOH to give **18a**. For **18b**, the free hydroxyl group was protected using Ac_2O in pyridine.



Scheme 16. Synthesis of glucose BBs **18a** and **18b**. Reagents and conditions: a) TFA/H_2O, DCM, quant., b) TBDMSCl, DMF, 96%, c) NaH, MeI, DMF, 93%, d) TBAF, THF, 95%, e) LevOH, DCC, **18a**: 60%; f) Ac_2O, py, **18b**: 98%.

With the 4-*O*-methylated glucose BBs in hand, the glycosylation conditions for efficient automated glycan assembly were investigated (Figure 9a). For this purpose, model syntheses of disaccharides **25a** and **25b** consisting of one xylosyl and one glucosyl residue were chosen. The 2-*O*-Azmb protected xylose BB **1c** was coupled to linker-functionalized resin **9** (module A) and the C2-position was deprotected (module E). Subsequently, glycosylations with glucose BB **18a** and **18b** were performed. In order to promote the desired α-selectivity relatively high temperatures, compared to the conditions used with other thioglycoside BBs, were chosen for the glycosylation reaction (module B: 2 x 1.8 equiv of BB **1a** or **1b**, NIS, TfOH, DCM/dioxane, -20 °C (5 min) → -5 °C (40 min)). The formation of the 1,2-*cis* product was further promoted by the solvent effect exhibited by dioxane, which is commonly used instead of DCM in solid-phase glycosylations with thioglycosides in order to help the activator NIS to dissolve. After cleavage from the solid support, MALDI and NP-HPLC analysis confirmed the formation of the 1,2-*cis*-glucosylated products **25a** and **25b** with high selectivity for both donors (α/β ~ 7:1, Figure

9b). The stereochemistry of the main product was determined by 2D-NMR spectroscopy (Figure 9c) after HPLC purification. The Lev-protected BB **18a** was less reactive in the glycosylation reaction and a significant amount of monosaccharide **26** was detected. The following syntheses were thus performed with 6-*O*-Ac-protected BB **18b**.

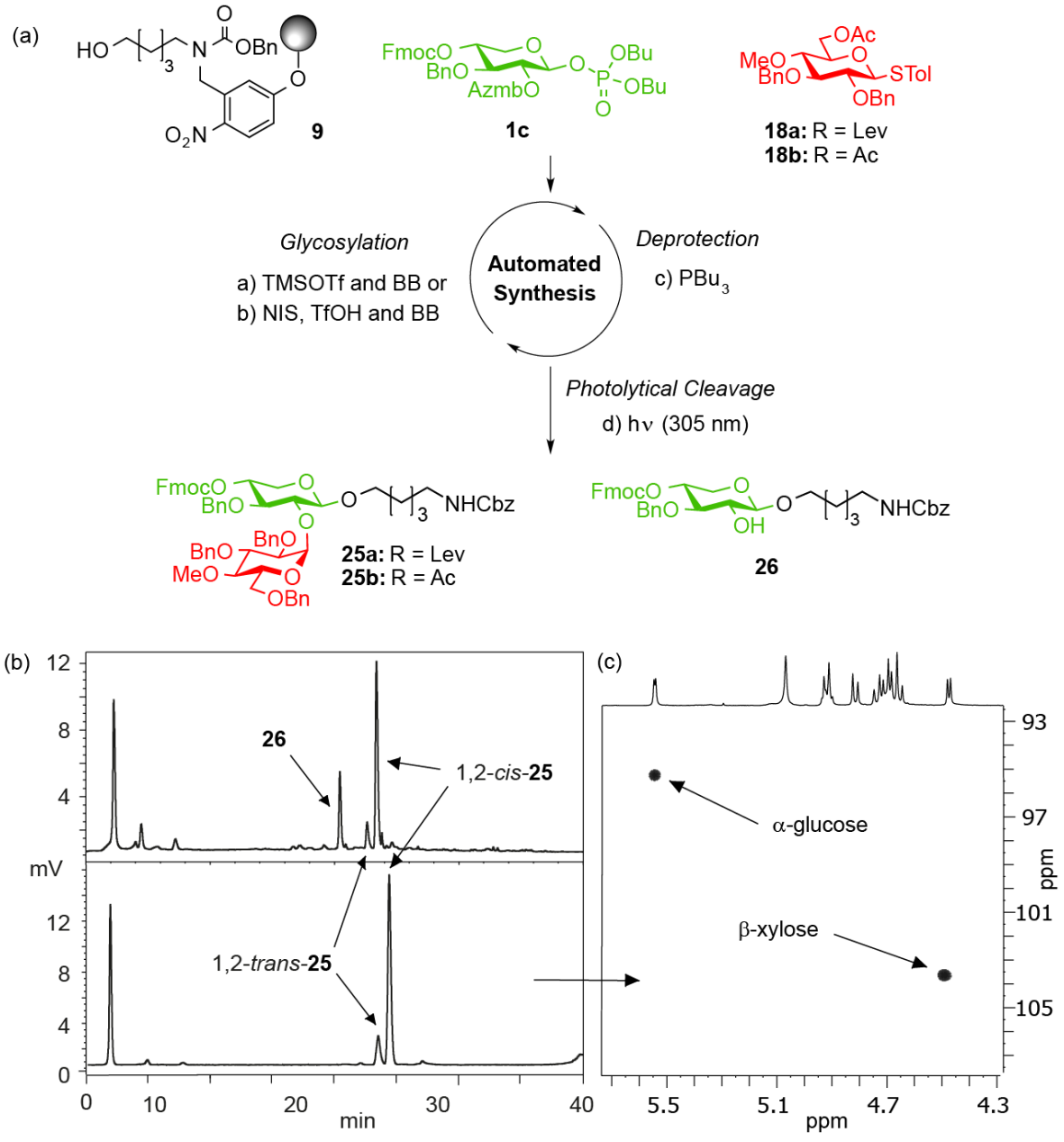
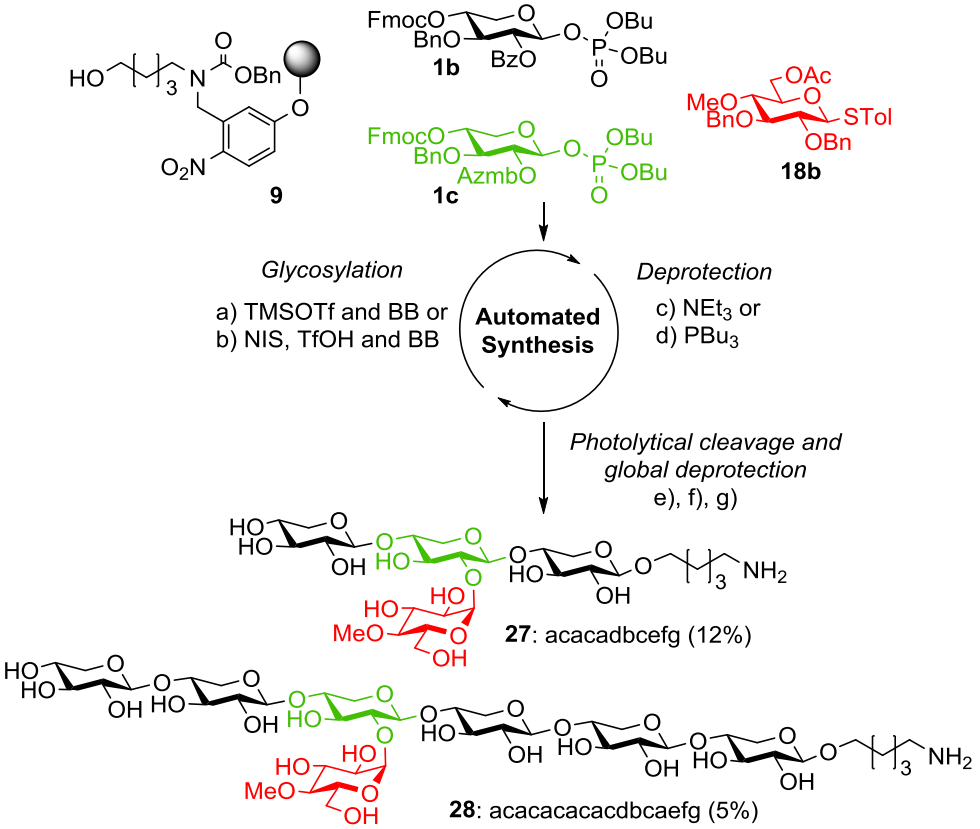


Figure 9. (a) Comparison of glucosyl donors **18a** and **18b** by automated glycan assembly of disaccharides **25a** and **25b**. Side product **26** is only obtained when BB **18a** is used. Reagents and conditions: a) 2 x 1.8 equiv of BB **1c**, TMSOTf, DCM, -35 °C (5 min) → -15 °C (30 min) (Module A); b) 2 x 1.8 equiv of BB **18a** or **18b**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min) (Module B); c) 6 cycles of PBu_3, THF/H_2O (5:1), 45 °C, 30 min (Module E); d) hν (305 nm). (b) Crude NP HPLC chromatograms of **25a** and **25b** (trace of evaporative light scattering detector (ELSD)). Both syntheses provided the product with good α–selectivity (α/β~7:1). (c) Anomeric region of HSQC-NMR spectrum of disaccharide **25b** after HPLC purification. The two anomeric peaks belong to the β-xylosyl and α-glucosyl residues.

After having successfully established efficient glycosylation conditions, 4-*O*-methylated glucose-substituted xylan oligosaccharides **27** and **28** (Scheme 17) were synthesized by automated glycan assembly using BBs **1a**, **1c**, and glucose BB **18b**.



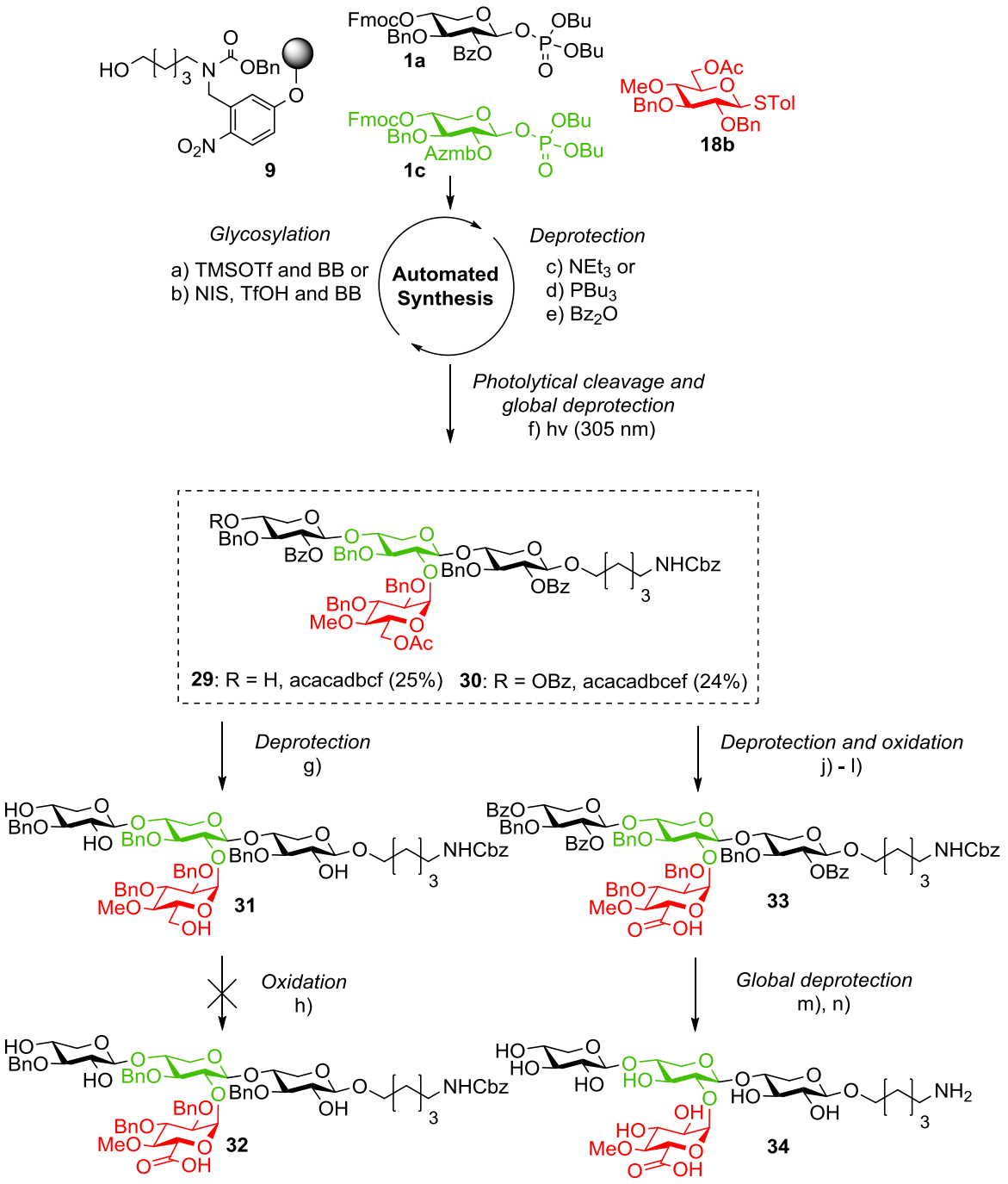
Scheme 17. Automated glycan assembly of glucose-substituted xylan oligosaccharides **27** and **28**. Reagents and conditions: a) 2 x 1.8 equiv of BB **1a** or **1c**, TMSOTf, DCM, -35 °C (5 min) → -15 °C (30 min) (Module A); b) 2 x 1.8 equiv of BB **18b** or **18**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min) (Module B); c) 3 cycles of 20% NEt_3 in DMF, 25 °C (5 min) (Module C); d) 6 cycles of PBu_3, THF/H_2O (5:1), 45 °C, 30 min (Module E); e) hν (305 nm); f) NaOMe, DCM/MeOH, 12 h; g) H_2, Pd/C, EtOAc/MeOH/H_2O/HOAc, 12 h, (yields are based on resin loading). The letter code below the structures represents the reaction sequence applied in the respective synthesis.

Analogous to the syntheses of α-1,2-linked arabinoxylan oligosaccharides, the xylose backbone of tetrasaccharide **27** was fully assembled before a final glycosylation with glucose BB **18b** was performed. For the synthesis of heptasaccharide **28**, the assembly of the backbone was interrupted after the xylosyl residue to which xylose BB **1c** was added and continued after introduction of the glucosyl substituent. This allowed the removal of the Azmb-group at an earlier stage of the assembly, preventing partial decomposition of this group during the further automated glycan assembly process. After photolytical cleavage from the resin, the fully protected versions of tetra- and heptasaccharides **27** and **28** were obtained. HPLC analysis of the crude reaction mixtures confirmed high α-selectivity for both syntheses. The respective 1,2-*trans* compounds could not be identified

in the mixture of side products. After global deprotection, the fully deprotected 4-*O*-Me-glucose-substituted xylan oligosaccharides **27** and **28** were obtained. In order to synthesize the desired glucuronoxylan oligosaccharides a suitable post-assembly oxidation procedure had to be developed. After automated glycan assembly of protected glucose-substituted xylan tetrasaccharide **29**, its further transformation to the respective glucuronic acid was envisioned by selective oxidation of the primary hydroxyl group in the C6-position after methanolysis of all protective esters (Scheme 18 left). The primary oxidant (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and the secondary oxidant bis(acetoxy)iodobenzene (BAIB) were added to the semi-protected tetrasaccharide **31** in a biphasic DCM/H_2O (1:1, v/v) solvent system (Table 1, Entry 1).[192-194] The reaction was monitored by ESI-MS. Either no reaction was observed or when longer reaction times were used only decomposition of the starting material was observed. Therefore, also other protocols for TEMPO-mediated oxidations were tested. The addition of the phase-transfer-catalyst Bu_4NCl (Entry 2)[195] gave the same reaction outcome. Next, the solvents were changed to the monophasic mixture of ACN/H_2O (1:1, v/v).[196] The use of ACN resulted in an increase of solubility of tetrasaccharide **31** compared to DCM. However, performing the oxidation reaction in ACN/H_2O at room temperature (Entry 3) or at 0 °C (Entry 4) also led to decomposition of the starting material. It has been reported that increasing the pH of the reaction by the addition of $NaHCO_3$ can accelerate the reaction (Entry 5).[197] Still, only decomposition of the starting material was observed. When a protocol using NaOCl as the secondary oxidant (Entry 6) in a bicarbonate-buffered ACN/H_2O mixture was employed,[198] no progression of the reaction was observed. Finally, the reaction was performed in the presence of KBr in the biphasic system DCM/H_2O (Entry 7).[194] It was reported that KBr reacts with NaOCl to form HOBr *in situ*, which is a much more potent secondary oxidant.[196] However, again only decomposition of the starting material was observed.

Table 1. Different reaction conditions tested for the TEMPO-mediated oxidations of **31** to **32**.

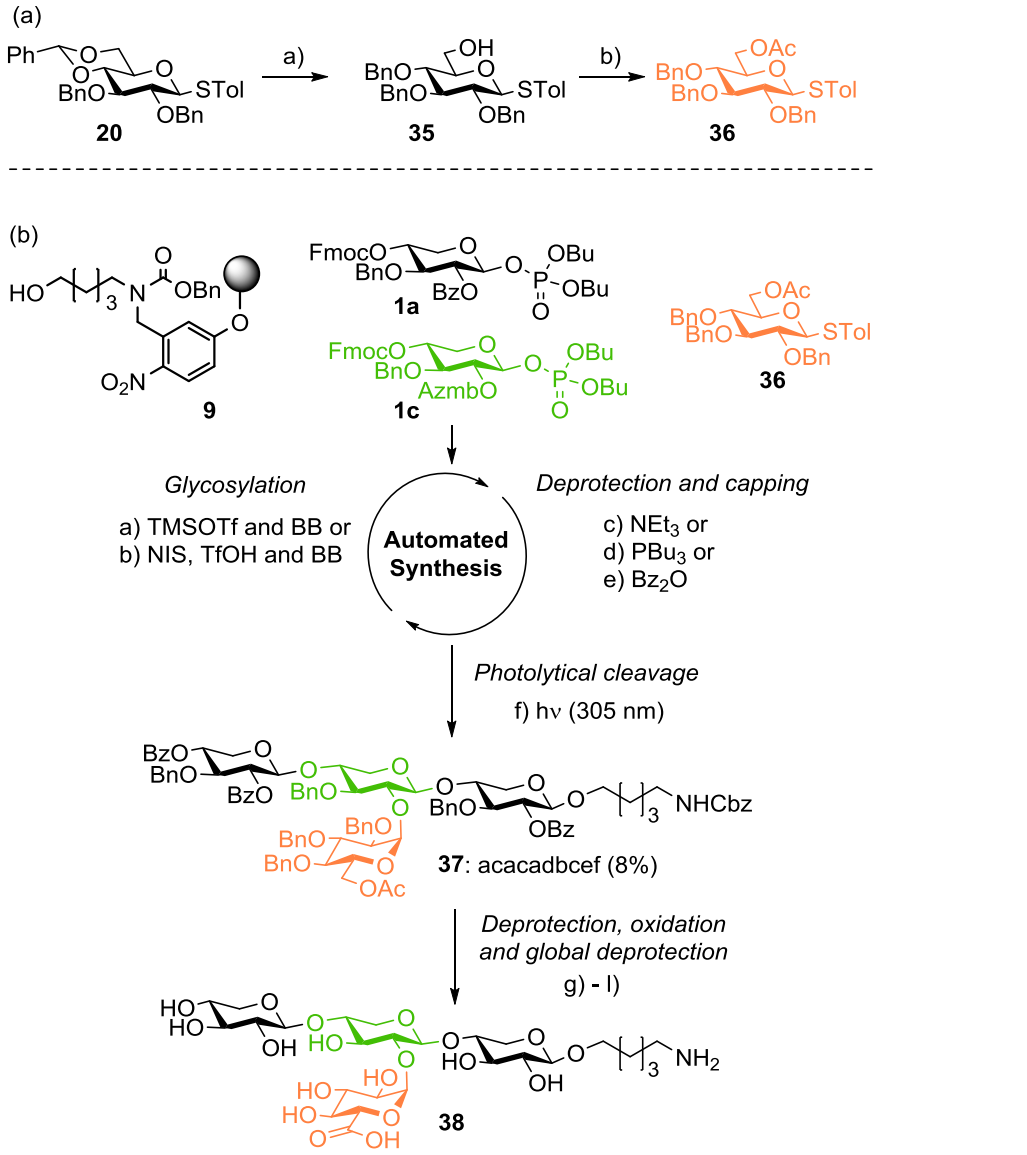
Entry	TEMPO + Additives	Solvents (1:1, v/v)	T	Outcome
1	BAIB	DCM/H_2O	r.t.	decomposition
2	BAIB/Bu_4NCl	DCM/H_2O	r.t.	decomposition
3	BAIB	ACN/H_2O	r.t.	decomposition
4	BAIB	ACN/H_2O	0 °C	decomposition
5	BAIB/$NaHCO_3$	ACN/H_2O	r.t.	decomposition
6	NaOCl/$NaHCO_3$	ACN/H_2O	r.t.	no reaction
7	NaOCl/KBr/$NaHCO_3$	DCM/H_2O	r.t.	decomposition



Scheme 18. Automated glycan assembly of oligosaccharides **29** and **30** and different post-assembly oxidation strategies towards glucuronoxylan oligosaccharides **32** and **34**. Reagents and conditions: a) 2 x 1.8 equiv of BB **1a** or **1c**, TMSOTf, DCM, -35 °C (5 min) → -15 °C (30 min) (Module A); b) 2 x 1.8 equiv of BB **18b**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min) (Module B); c) 3 cycles of 20% NEt_3 in DMF, 25 °C (5 min) (Module C); d) 6 cycles of PBu_3, THF/H_2O (5:1), 45 °C, 30 min (Module E); e) Bz_2O, DMAP (Module G); f) hν (305 nm), yields are based on resin loading; the letter codes below the structure represents the reaction sequence applied in the respective synthesis; g) NaOMe, MeOH/DCM, 16 h, h) TEMPO, BAIB; j) *p*TsOH, DCM/MeOH (9:1), 40 °C, 16 h; k) DMP, DCM, H_2O, 2 d; l) $NaOCl_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH/THF/H_2O (2:1:1), 2 h **33**: 55% over 3 steps; m) NaOMe, THF, 16h; n) H_2, Pd/C, EtOAc/MeOH/H_2O/HOAc, 12 h, **34**: 21% over 2 steps.

Based on these results a different oxidation strategy had to be applied. A two-step oxidation approach via the respective aldehyde was pursued that had previously been used for example in the synthesis of pectic homogalacturonan hexasaccharides.[199] Unlike the previously described TEMPO-mediated oxidation reactions, this reaction is not selective towards primary alcohols. The oxidation had to be performed at the fully protected stage where only the hydroxyl group at the C6-position of the glucose residue is deprotected. Thus, fully protected tetrasaccharide **30** was prepared by automated glycan assembly (Scheme 18 right). The synthesis of tetrasaccharide **30** was performed in the same way as of tetrasaccharide **29**. However, the hydroxyl group at the terminal C4-position was capped with a benzoyl protecting group in a final esterification step. After photolytical cleavage, the acetyl group at the C6-position was selectively removed using *p*TsOH[200] and the resulting free hydroxyl group was oxidized by performing the following two reactions. First, treatment with Dess-Martin periodinane (DMP) gave the respective aldehyde and then Pinnick oxidation with $NaOCl_2$ provided the fully protected 4-*O*-methylated glucuronic acid-substituted oligoxyloside **33** in 55% yield. Subsequent removal of the benzoyl esters by methanolysis and of the benzyl ethers by hydrogenolysis afforded the fully deprotected glucuronoxylan oligosaccharide **34**.

The synthesis of the analogous non-methylated glucuronoxylan oligosaccharide **38** required the synthesis of glucose BB **36** (Scheme 19). As the previous comparison of glucose BBs **18a** and **18b** had shown that acetyl-substituted BB **18b** gives better conversion in the glycosylation reaction, glucose BB **36** was only synthesized as the respective 6-*O*-Ac-substituted compound (Scheme 19a). The synthesis started from acetal **20**, which had also served as the starting material for the synthesis of BB. Opening of the benzylidene acetal with BH_3·THF and TMSOTf gave alcohol **35** and the free hydroxyl at the C6-position was subsequently protected with an acetate-group to give glucose BB **36**. Glucose BB **36** was used in the automated glycan assembly of the fully protected glucose-substituted xylan oligosaccharide **37** (Scheme 19b). After selective removal of the acetate group at the glucose residue using *p*TsOH, the above-described two-step oxidation was applied. The respective fully protected non-methylated glucuronic acid-substituted oligosaccharide was obtained in 28% yield. Subsequent removal of the benzoyl esters by methanolysis and of the benzyl ethers by hydrogenolysis afforded the glucuronoxylan oligosaccharide **38** in 49% yield. Due to the many post-assembly transformations required and the low yield achieved in the synthesis of glucose-substituted xylan heptasaccharide **28**, the synthesis of larger glucuronoxylan oligosaccharides was not attempted.



Scheme 19. (a) Synthesis of glucose BB **36**. Reagents and conditions: a) THF·BH_3, TMSOTf, 51%, b) Ac_2O, py, 83%, (b) Synthesis of glucuronoxylan oligosaccharide **38**. Reagents and conditions: a) 2 x 1.8 equiv of BB **1a** or **1c**, TMSOTf, DCM, -35 °C (5 min) → -15 °C (30 min) (Module A); b) 2 x 1.8 equiv of BB **36**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min) (Module B); c) 3 cycles of 20% NEt_3 in DMF, 25 °C (5 min) (Module C); d) 6 cycles of PBu_3, THF/H_2O (5:1), 45 °C, 30 min (Module E); e) Bz_2O, DMAP (Module G); f) hν (305 nm), the yield is based on resin loading; the letter code below the structure represents the reaction sequence applied in the respective synthesis; g) *p*TsOH, DCM/MeOH (9:1), 40 °C, 16 h; h) DMP, DCM, H_2O, 2 d; j) $NaOCl_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH/THF/H_2O (2:1:1), 2 h, 28% over 3 steps; k) NaOMe, THF, 16h; l) H_2, Pd/C, EtOAc/MeOH/H_2O/HOAc, 12 h. **38**: 49% over 2 steps.

2.1.3 Characterization of Xylan-Directed Monoclonal Antibodies

Monoclonal antibodies that recognize plant cell wall glycans provide important information on the structure and localization of cell wall polysaccharides. The prepared arabino- and glucuronoxylan oligosaccharides represent ideal probes to determine previously unknown epitopes of xylan-directed monoclonal antibodies. The oligosaccharides were printed together with the previously synthesized (Deborah Senf, MSc thesis, MPIKG)[132] collection of four linear oligoxylans and eight α-1,3-substituted oligoarabinoxylans as a glycan microarray and probed with 209 plant cell wall glycan-directed monoclonal antibodies in collaboration with my colleague, Dr. Ruprecht. Binding of the monoclonal antibodies to the synthetic oligosaccharides was identified by incubation of the array with the primary monoclonal antibody, which is then bound by a fluorescently-labelled secondary antibody (Figure 10). The positions on the microarray slide that were recognized by a mAb were detected by using a microarray scanner.

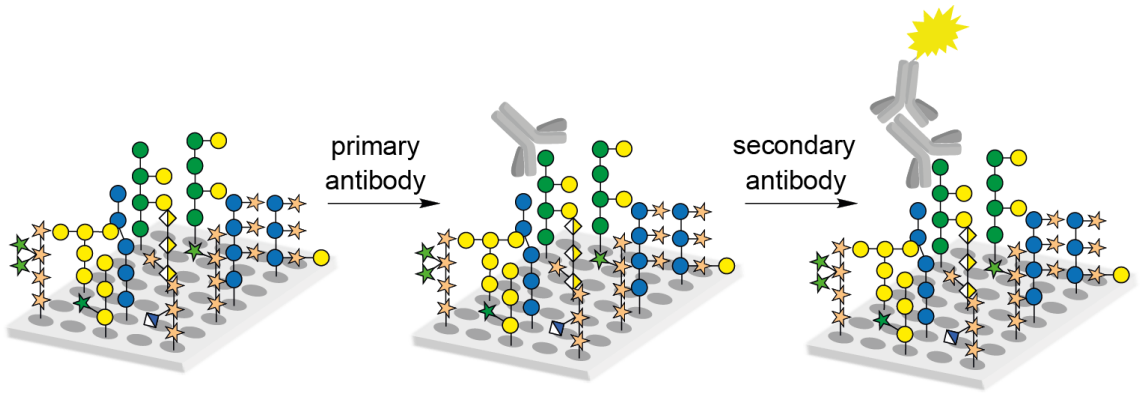


Figure 10. Schematic representation of a glycan microarray assay for characterizing the binding epitopes of cell wall glycan-directed monoclonal antibodies.

We identified 30 monoclonal antibodies that bound to the synthetic xylan oligosaccharides. Microarray scans of a selection of these antibodies are shown in Figure 11. Monoclonal antibody LM11 for example recognized the β-1,4-linked xylose backbone with a high tolerance for backbone substitution. In some cases the binding epitopes were more precisely characterized. As expected all linear oligoxylans were detected by monoclonal antibody LM10 that was originally raised against a synthetic pentaxyloside-BSA conjugate. Previously, LM10 was thought to bind only to low- or unsubstituted xylans.[201] However, in our assay LM10 bound to all arabinose substituted xylan structures except for the oligosaccharides that carry an arabinose substituent at the non-reducing xylan residue (**X**, **11**, **14**, **17**). For the three oligosaccharides **X**, **11** and **17**, the absence of binding can be explained by the high degree of substitution next to the non-reducing xylose residue.

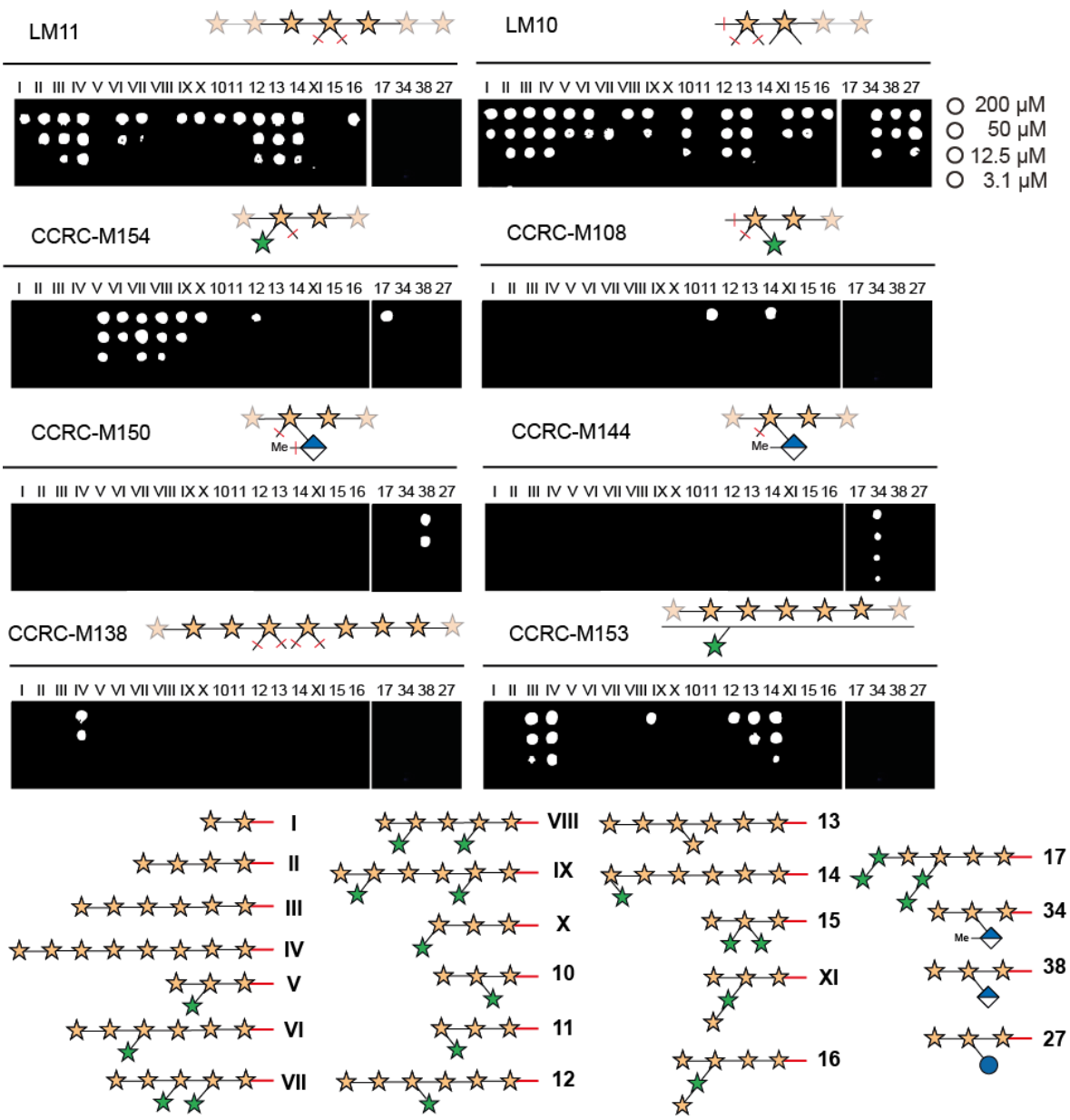


Figure 11. Detection of arabino- and glucuronoxylan oligosaccharides by xylan-directed monoclonal antibodies. Representative microarray scans of selected examples are shown. Each compound was printed in four concentrations as indicated on the right. Light linkages and light monosaccharide symbols indicate positions for substitutions that are allowed but not required for binding. Red bars represent positions, which must not be substituted.

To confirm that LM10 exclusively binds to the non-reducing end even when longer unsubstituted xylan stretches are present, the lowly substituted heptasaccharide **14**, with only one arabinosyl substituent at the non-reducing end, was prepared. No binding to heptasaccharide **14** was detected.

Other monoclonal antibodies recognized completely different epitopes. CCRC-M154 bound exclusively to oligoxylans substituted with α-1,3-linked arabinose (**V**-**X**) residues and CCRC-M108, CCRC-M109, and CCRC-M110 to 2-substituted arabinoxylans.

Glucuronoxylan oligosaccharide **38** was selectively recognized by CCRC-M150 and its 4-*O*-methylated glucuronic acid derivative **34** by CCRC-M144, CCRC-M145, CCRC-M146, and CCRC-M155. It was furthermore confirmed that LM28 binds xylans that are substituted with either of the glucuronic acid derivatives.[202] None of the monoclonal antibodies bound to glucose-substituted xylan **27**, which indicates that the carboxylic acid moiety is essential for binding.

The antibodies used in this study were previously grouped into four clades based on their binding properties towards natural xylan polysaccharides (Xylan group 1-4).[88] In general, monoclonal antibodies from Xylan group 1 (e.g. CCRC-M138, CCRC-M153) were found to bind the β-1,4-linked backbone of low-substituted xylans. Interestingly, different binding patterns towards the synthetic oligoarabinoxylosides were observed not only for antibodies from the same clade but also for antibodies raised against the same polysaccharide antigen. For example, while CCRC-M138 detected only the linear octaxyloside, CCRC-M153 also bound to arabinose substituted xylosides. Both antibodies were originally raised against the same natural oat xylan and bind the same group of natural xylans.

None of the antibodies showed either strong or selective binding to penta- and hexasaccharides **XI** and **16** containing the β-1,2-D-xylopyranosyl-α-1,3-L-arabinofuranosyl substituent. Similarly, the α-1,3-L-arabinofuranosyl-α-1,3-L-arabinofuranosyl side chain was not recognized, making these antigens promising candidates for immunizations and production of monoclonal antibodies towards these otherwise undetectable epitopes. The results demonstrate that libraries of synthetic plant oligosaccharides can be used to determine the binding specificities of cell wall glycan-directed antibodies with high precision. With knowledge of the exact epitopes of these monoclonal antibodies, they can be now used as selective markers for xylans that carry specific substituents.

2.1.4 Active Site-Mapping of Xylan-Degrading Enzymes

Xylan-degrading enzymes are important tools for many industrial applications such as the production of biofuel.[9,55,92] A detailed understanding of the enzymatic degradation of xylan at the molecular level is beneficial for its efficient utilization.[55,203] The prepared collection of arabinoxylan oligosaccharides represents suitable substrates for the characterization of the substrate specificities of xylan-degrading enzymes. My colleague, Dr. Ruprecht, investigated a number of commercially available endo-xylanases and arabinofuranosidases, i.e. a GH family 10 xylanase from *Cellvibrio japonicas* (XYNACJ),[204] a GH11 xylanase from rumen microorganism (XYRU6),[205] and two GH43

arabinofuranosidases from *Bacteroides ovatus* (ABFBO17 and ABFBO25) using the synthetic xylan oligosaccharides.[204,206] Simple end-point measurements were performed by incubating the enzymes with the respective oligosaccharide for 3 h at 40 °C and then terminating the reaction by heat inactivation. The resulting fragments were analyzed by HPLC coupled to a mass spectrometer and an ELSD. Exemplary results for the GH10 xylanase XYNACJ are shown in Figure 12a. As expected, linear octasaccharide **IV** was readily degraded by the xylanase, whereas the arabinose substituents in **VI** and **12** directed the cleavage to the glycosidic bond. Depending on whether the arabinose was α-1,2- or α-1,3-linked, the backbone was further hydrolyzed either one or two units before the substituent. Based on the identified fragments, the cutting sites for each of the oligosaccharides and for both types of endo-xylanases investigated were determined (Figure 12b). Summarizing these results, general requirements for arabinose substitutions that are tolerated by the GH10 xylanase were deduced (Figure 12c). These results are

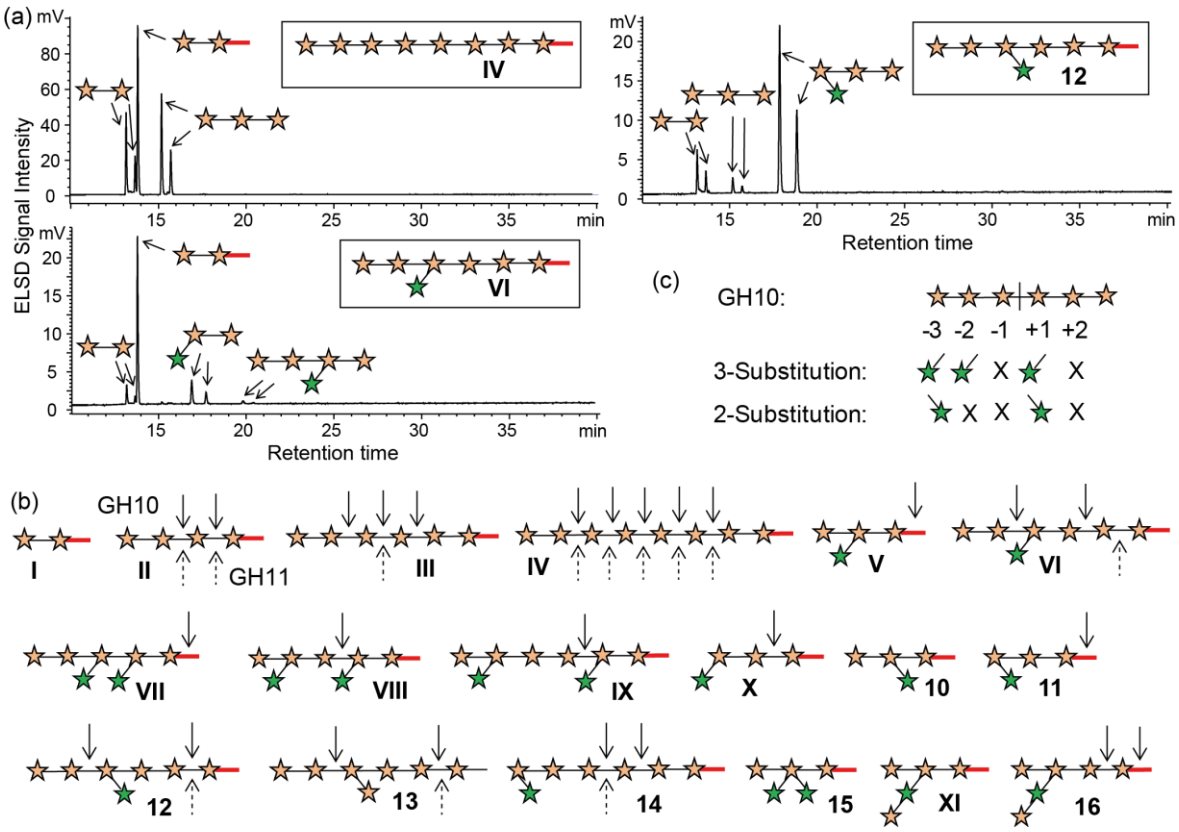


Figure 12. Investigation of the substrate specificities of GH10 and GH11 endo-xylanases using synthetic xylan oligosaccharides. (a) HPLC analyses of reactions of a GH10 xylanase with different substrates (indicated by boxes). Peaks are annotated with xylan fragments with aminopentyl linker or with free reducing end (with or without red bar). Note that the α- and the β-form of the fragments with free reducing end elute separately. (b) Cutting sites of GH10 and GH11 xylanases in all prepared arabinoxylan oligosaccharides. Thin arrows indicate minor activity at the respective site. (c) General requirements for arabinose substitutions relative to the cutting site of the GH10 xylanase derived from the results obtained in (b). The "X" denotes xylose residue positions that must not be substituted. The reducing end of the structures is located on the right.

consistent with previously published data on the active site of GH10 xylanases which is conserved between most, but not all, family members.[95] Due to their larger xylan recognition site and the limited size of the prepared oligosaccharides a similar generalization was not possible for the GH11 xylanases.

Another important class of enzymes in the degradation of arabinoxylans are arabinofuranosidases that can act on mono-[207,208] or double-substituted[209-211] xylose residues. To investigate the substrate specificities of these enzymes, experiments with two different GH 43 arabinofuranosidases were performed (Figure 13). The results indicated a strong preference of ABFBO25 for cleavage of α-1,2- compared to α-1,3-linked arabinoses (Figure 13a) as confirmed by time-course experiments using compounds **V**, **X**, **10**, and **11** (Figure 13b). Compound **15** with two arabinose substitutions at the same xylose residue was exclusively not digested by ABFBO25, but represented the only substrate for ABFBO17 (Figure 13c). In agreement with previous studies,[203,204,206] ABFBO17 selectively removed the α-1,3-linked arabinose, leaving the α-1,2-substituted arabinose compound

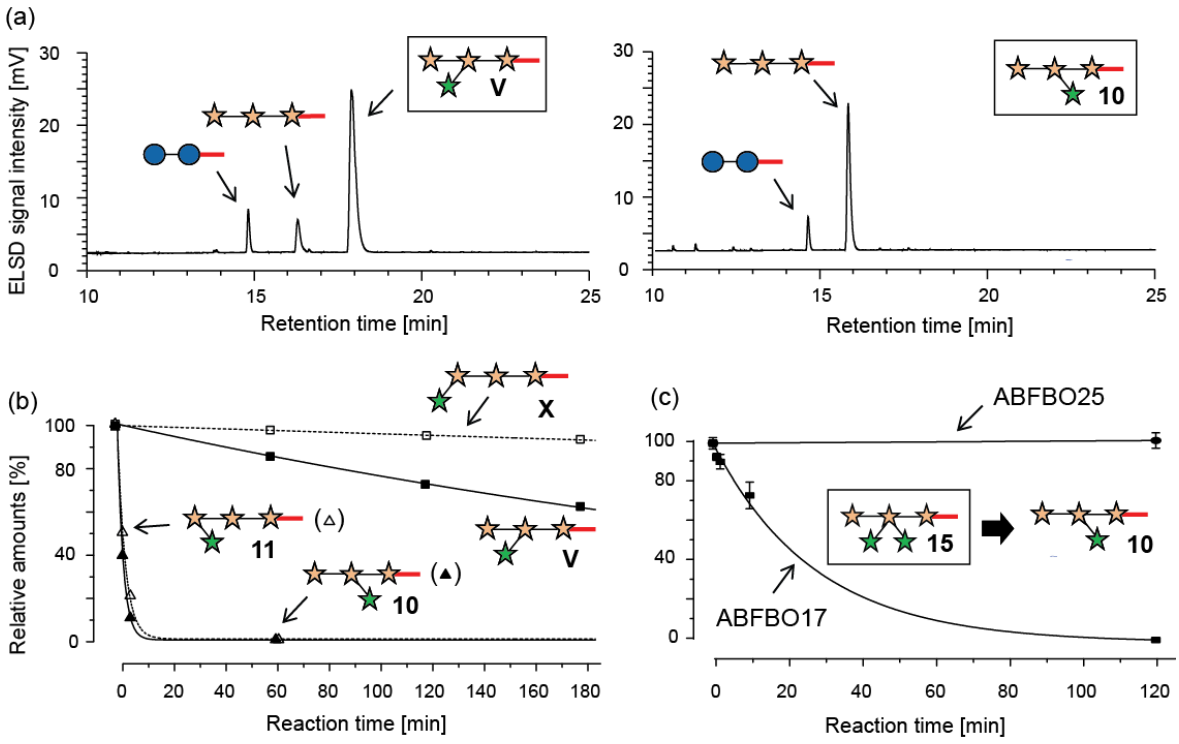


Figure 13. Arabinofuranosidase activities on different arabinoxylan oligosaccharide substrates. (a) HPLC analysis of a reaction of a GH43 arabinofuranosidase from *Bacteroides ovatus* (ABFBO25) with xylotriose substituted with arabinose at the C3- (**V**) or the C2- (**X**) position after 60 min incubation time. The structure of the substrates is indicated by boxes. The glucose disaccharide was used as internal standard for quantification. (b) Time-course experiment using four different tetrasaccharides as substrates for ABFBO25. (c) Time-course experiment for ABFBO17, another GH43 arabinofuranosidase from *Bacteroides ovatus*, and ABFBO25 using a C2-, C3-disubstituted arabinoxylan oligosaccharide as substrate (indicated by a box). Note that only ABFBO17 specifically removes C3-substituted arabinose from C2-, C3-disubstituted xyloses. These results were obtained at an enzyme concentration of 0.1 U/mL.

10 as the product. The exclusive selectivity of ABFBO17 and ABFBO25, which belong to the same GH family, for either mono- or disubstituted arabinoxylosides indicate distinct properties in the catalytic domain of these enzymes.

2.1.5 Conclusion and Outlook

In conclusion, a set of 12 xylan oligosaccharides with arabinofuranose and glucuronic acid substituents attached to the C2- and/or C3-position of the backbone xylose units was synthesized by automated glycan assembly. Elongation of the xylan backbone was accomplished by iterative additions of C4-fluorenylmethoxylcarbonyl (Fmoc) protected xylose building blocks to a linker-functionalized resin. Substituents were selective attached to the backbone using fully orthogonal 2-(methyl)naphthyl (Nap) and 2-(azidomethyl)benzoyl (Azmb) protecting groups at the C2- and C3-hydroxyls of the xylose building blocks. The attachment of glucuronic acid substituents required the use of glucose BBs that were oxidized to the corresponding glucuronic acid after the full oligosaccharide has been assembled to overcome low reactivity of glucuronic acid donors. High 1,2-*cis* stereoselectivity in the glycosylation reactions was obtained by using an ester protecting group in the C6-position of glucose that exhibited remote participation. Different post-assembly oxidation strategies had to be examined. Firstly, a TEMPO-based strategy was tested, which did not lead to the desired product under several different reaction conditions. Next, a two-step oxidation procedure via the respective aldehyde as an intermediate was pursued that yielded the desired glucuronic acid oligosaccharides.

The library of synthetic xylan oligosaccharides was printed as glycan microarray and probed with anti-xylan antibodies. The binding specificities of several antibodies were precisely determined, providing the required information for the specific detection of many complex molecular structures of xylan polysaccharides in the plant cell wall. The absence of binding towards the oligosaccharides containing disaccharide side chains makes these antigens promising candidates for immunizations and production of monoclonal antibodies, as these epitopes remain undetectable otherwise. This requires the production of larger amounts of the respective oligosaccharides, which can be achieved by multiple rounds of automated glycan assembly followed by combined global deprotection in solution. Future efforts will aim at the synthesis of esterified oligoxylosides, as xylans are often substituted with acetyl or ferulate esters. The current synthetic strategy does not allow for their synthesis as the ester groups would be cleaved during methanolysis of the benzoates and new BBs will have to be designed.

The collection of arabinoxylan oligosaccharides was further utilized for determining the substrate specificities of xylan-degrading enzymes. The synthetic arabinoxylan

oligosaccharides were incubated with different glycosyl hydrolases followed by HPLC-MS analysis of the digestion products. Simple end-point measurements revealed the substrate specificities of one GH10 xylanase and two arabinofuranosidases. More detailed information on the preferences of the enzymes for specific oligosaccharides was elucidated through time-course experiments. The developed HPLC-based assay using synthetic arabinoxylan oligosaccharides rather than natural polysaccharide substrates shortens analysis time to a single day and provides a powerful tool for screening xylan-degrading enzymes. Future syntheses of even larger xylan oligosaccharides will enable the active site-mapping of xylanases belonging to the other GH families (5, 8, and 11). These xylanase families are less studied than the GH10 xylanases and insights into substrate binding properties are urgently needed.

2.2 Artificial Polysaccharides with Well-Defined Branching Patterns[2]

Cellulose and xylan are the major polysaccharides in lignocellulosic biomass and thus promising renewable resources for the production of materials and fuels.[212] While cellulose is regularly utilized for these purposes, the exploration of xylan is lagging behind due to its structural complexity.[25,34] Arabinoxylans, as a subclass of xylans, form long polymeric chains that are randomly substituted with arabinofuranosyl residues (Figure 14a). The molecular composition of xylans has a strong impact on their macroscopic properties such as solubility[41] and crystallinity as the substituents disrupt the interactions between individual xylan chains.[213] Adsorption of xylan to cellulose is also strongly affected by its discrete substitution pattern as demonstrated recently by Dupree *et al*. for glucuronoxylans.[41,44-48]

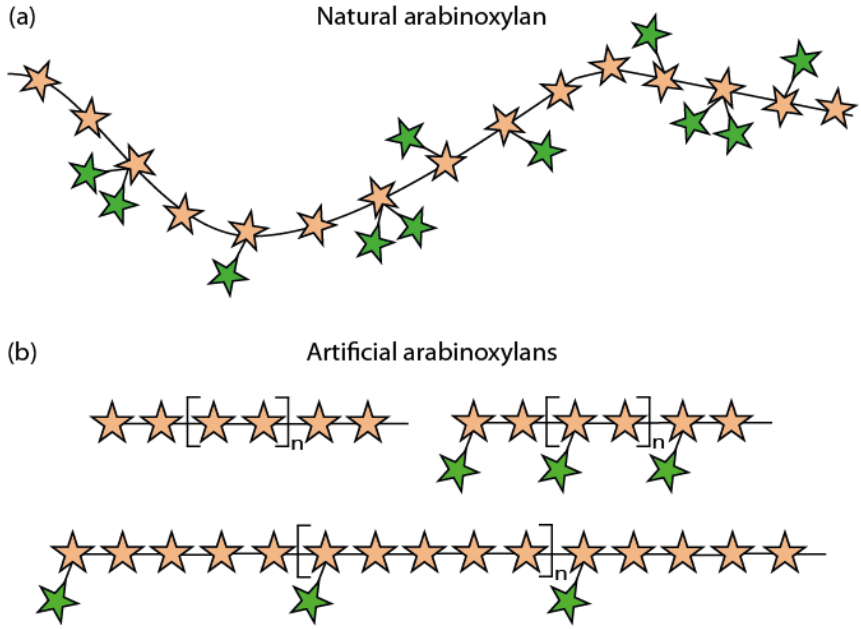


Figure 14. Schematic representation of (a) a natural xylan with random branching pattern and (b) artificial arabinoxylans with defined branching patterns.

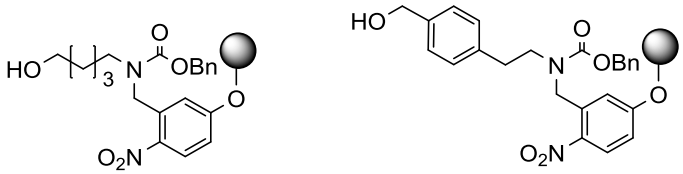
Detailed investigations into structure-property relationships of complex polysaccharides are hampered by their inhomogeneity as they are extracted from natural sources. Polysaccharides with defined branching patterns can only be obtained synthetically using a bottom-up approach. Enzymatic polymerizations using

[2] This chapter has been modified in part from the following articles: Senf, D.; Ruprecht, C.; Matic, A.; Pfrengle, F., *Angew. Chem*., **2018**, *submitted*.

glycosynthases[157] have proven useful for the synthesis of artificial polysaccharides of many different classes, such as xyloglucans and homopolymers of xylans.[155,162-170] However, substituted xylan polysaccharides have not been synthetically prepared. In this chapter, the synthesis of artificial arabinoxylan polysaccharides with defined branching patterns (Figure 14b) by glycosynthase-catalyzed polymerization of a number of different oligosaccharide fluorides is described. To catalyze the polymerization of the arabinoxylan oligosaccharide fluorides, the glycosynthase XynAE265G was chosen as it is able to produce xylan homopolymers containing more than 100 monosaccharide units.[163]

2.2.1 Proof-of-Principle using Automated Glycan Assembly

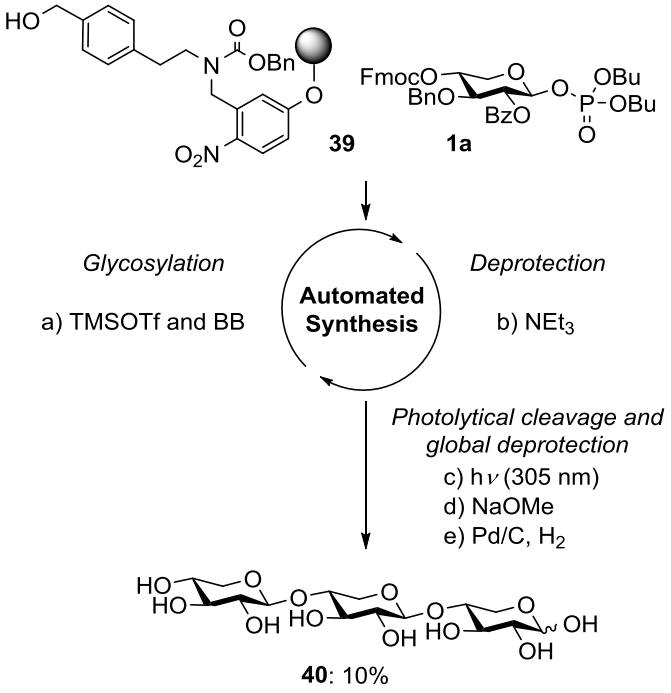
Arabinoxylan oligosaccharide fluorides can be easily obtained from the corresponding oligosaccharides with free reducing ends. Automated glycan assembly offers a fast synthetic route to these oligosaccharides (chapter 2.1). Recently, a new photo-labile linker (**39**, Figure 15 right) was developed,[214] that is structurally analogous to the established linker **9**[139] that is routinely used to produce linker-functionalized glycans. This new linker releases oligosaccharides with free reducing ends after light-induced cleavage and deprotection. The benzyl moiety that is incorporated in the linker releases the anomeric hydroxyl group after a final hydrogenolysis.[215-217]



9 **39**

Figure 15. Resin-bound photo-labile linkers for automated glycan assembly. Left: established linker **9**;[139] right: modified traceless linker **39**.

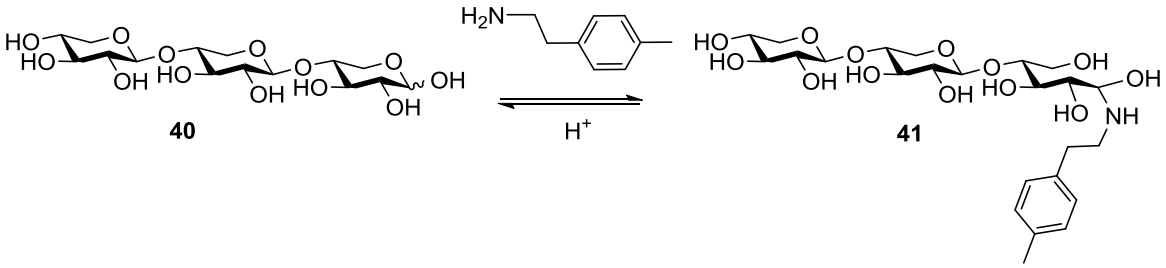
For the automated glycan assembly of arabinoxylan oligosaccharides with free reducing ends, linear trisaccharide **40** served as an initial target structure to investigate the efficiency of the glycan assembly using the new linker (Scheme 20). Xylose BB **1a** was iteratively added to a Merrifield resin equipped with linker **39** via alternating cycles of glycosylation and deprotection reactions. The fully protected trisaccharide was obtained after photolytical cleavage in high purity. Sequential deprotection of the permanent protecting groups by methanolysis and hydrogenolysis gave trisaccharide **40** accompanied by a primary amine, which was formed as a side product during hydrogenolysis. Upon removal of the solvents, the open chain form of **40** reacted partially



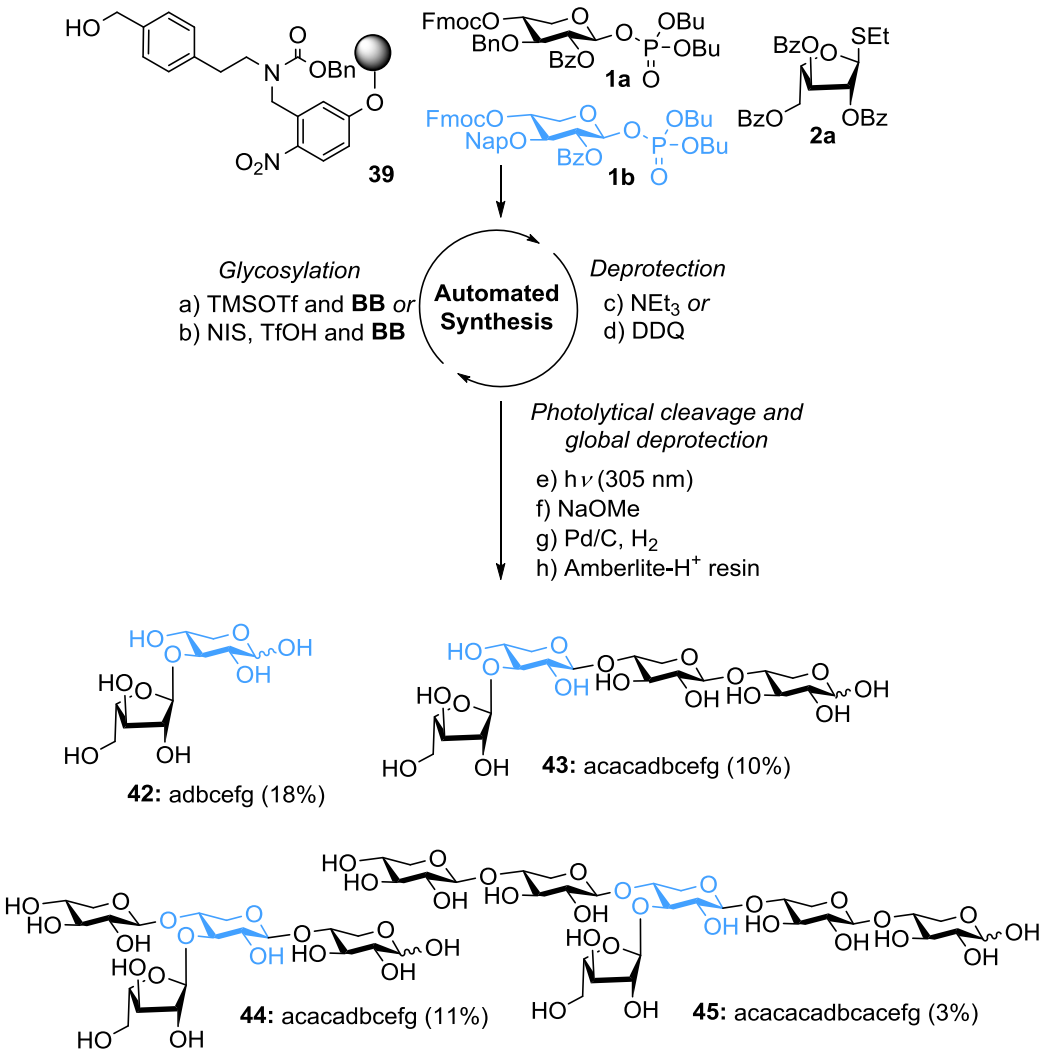
Scheme 20. Automated glycan assembly of trisaccharide **40** using traceless linker **39**.

with the primary amine of the cleaved-off linker to form a hemiaminal ether (Scheme 21). This mixture was converted to the desired product **40** under acidic conditions before it was subjected to final a HPLC purification.

In order to proceed with the synthesis of α-1,3-substituted arabinoxylan oligosaccharides, the stability of linker **39** during DDQ-mediated removal of the Nap-protecting group of xylose BB **1b** on the solid-phase had to be ensured. Thus, disaccharide **42** was synthesized by automated glycan assembly using BBs **1b** and **2a** (Scheme 22). After photo-induced cleavage of the oligosaccharide from the solid support, no side products were observed by HPLC confirming the stability of linker **39** during Nap-deprotection.



Scheme 21. Formation of hemiaminal ether **41** from trisaccharide **40** and a primary amine, which was formed as a side product during hydrogenolysis.



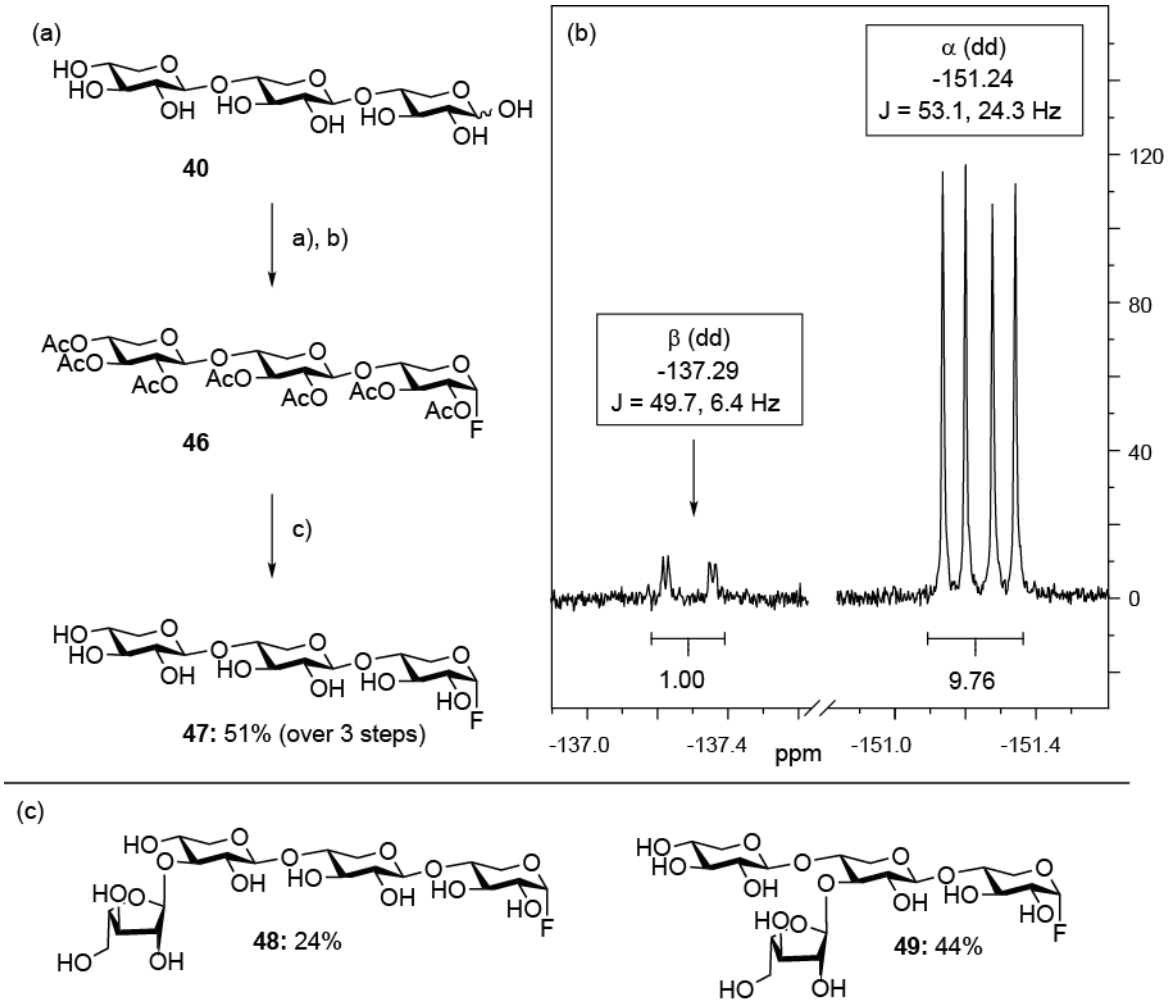
Scheme 22. Automated glycan assembly of arabinoxylan oligosaccharides with free reducing end (**42**-**45**). Reagents and conditions: a) 2 x 1.8 equiv of BB **1a** or **1b**, TMSOTf, DCM, -35 °C (5 min) → -15 °C (30 min) (Module A); b) 2 x 1.8 equiv of BB **2a**, NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min) (Module B); c) 3 cycles of 20% NEt_3 in DMF, 25 °C (5 min) (Module C); d) 7 cycles of 0.1 M DDQ in DCE/MeOH/H_2O (64:16:1), 40 °C, 20 min (Module D); e) hν (305 nm); f) NaOMe, DCM/MeOH, 16h; g) H_2, Pd/C, EtOAc/MeOH/H_2O/HOAc, 12 h. The yields are based on resin loading. The letter code below the structures represents the reaction sequence applied in the respective synthesis.

Next, three target molecules (**43**-**45**) were selected for the automated glycan assembly of α-1,3-substituted arabinoxylan oligosaccharides to evaluate their potential to serve as substrates in the glycosynthase-catalyzed polymerization. Tetrasaccharide **43**, with an arabinose substituent attached to the non-reducing end xylose unit, was expected to be a good substrate in the polymerization reaction, as glycosynthase XynAE265G[163] is derived from a xylanase belonging to the GH family 10, and GH10 xylanases only accommodate arabinose substitution in certain positions alongside the xylan backbone. Tetrasaccharide **44** with arabinose substitution at the penultimate xylose residue is less likely to be an acceptor. As a third substrate, hexasaccharide **45** will be prepared.

For the automated glycan assembly of tetrasaccharides **43** and **44**, the full xylan backbone was synthesized before the Nap ether was deprotected using DDQ to allow for the final glycosylation with arabinose BB **2a**. For the synthesis of hexasaccharide **45**, the assembly of the backbone was interrupted after the xylosyl residue to which xylose BB **1b** was added. After subsequent deprotection of the Nap ether and attachment of the arabinose side chain, the backbone synthesis was completed. The fully deprotected arabinoxylan oligosaccharides **42**-**45** were obtained after global deprotection, acidic hydrolysis of the hemiaminal ether and final HPLC-purification.

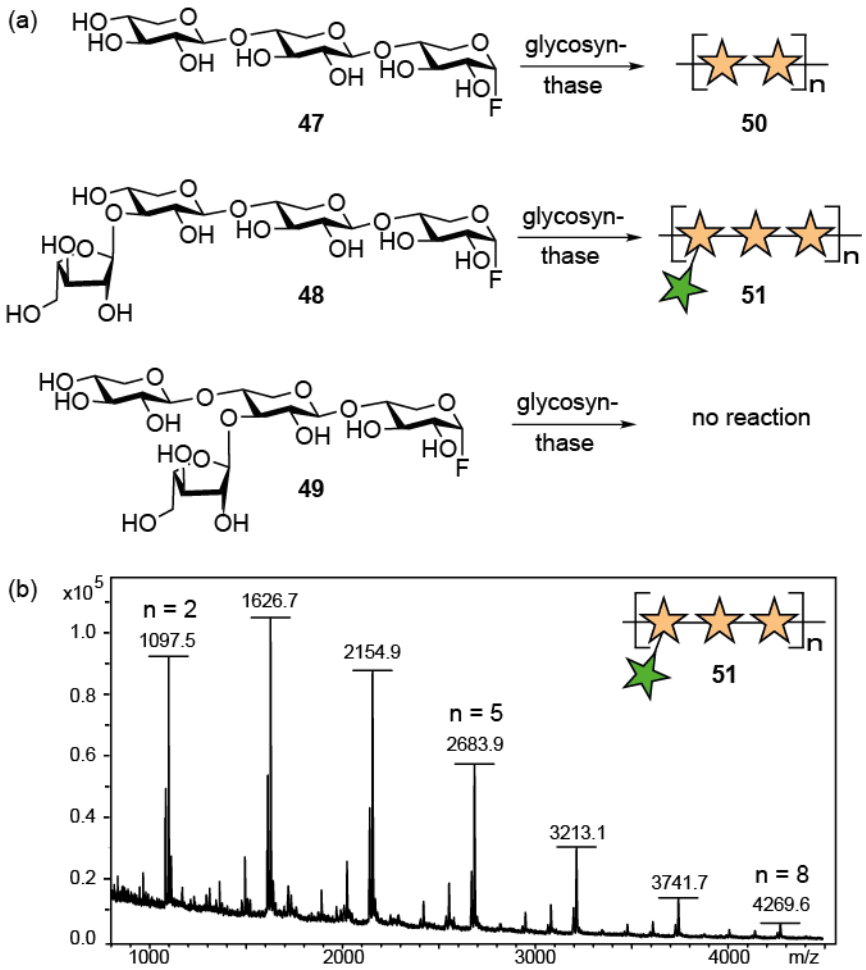
Transformation of the deprotected oligosaccharides into glycosyl fluorides was performed in a three-step procedure consisting of peracetylation, fluorination, and deacetylation. The synthesis is shown exemplary for trisaccharide **40** in Scheme 23a. The fluorination of the peracetylated trisaccharide was performed using HF/pyridine at low temperature,[218-222] providing α-fluoride **46** with high selectivity (α/β ~ 10:1). The configuration of the anomeric center was confirmed by the characteristic geminal coupling constant (J = 53 Hz for α-fluoride) between the anomeric H- and F-atoms in ^{1}H and ^{19}F NMR spectroscopy (Scheme 23b).[223] Removal of the acetate groups by treatment with 1 equiv NaOMe at 0 °C finally afforded deprotected trisaccharide fluoride **47**. The fluorination of arabinose-substituted oligosaccharides **43**, **44** and **45** was performed analogously. However, the strongly acidic conditions of the fluorination reaction provoked partial cleavage of the arabinofuranose in some cases, which explains the low yields obtained for **48** and **49** (Scheme 23c). The respective glycosyl fluoride of hexasaccharide **45** could not be isolated after treatment with HF/pyridine, probably due to difficulties resulting from the very small scale of this reaction.

With the desired glycosyl fluorides **47**-**49** in hand, enzymatic polymerizations were performed by overnight incubation of the respective glycosyl fluoride with glycosynthase XynAE265G (Scheme 24a). The formation of polysaccharides was investigated by MALDI-TOF. The peaks in the MALDI spectrum confirmed that the polysaccharides were formed from the starting oligosaccharides and that these polysaccharides represent oligomers of the oligosaccharide fluorides. In the polymerization reaction of trisaccharide donor **47** and tetrasaccharide donor **48** polysaccharides containing up to 33 and 32 monosaccharide units, respectively, were formed (Scheme 24b). Conversely, tetrasaccharide **49** did not undergo polymerization. Substitution with arabinose in the position next the non-reducing end (+2 position) was not accommodated by the glycosynthase, which is in line with the substrate specificities of GH10 xylanases (see Chapter 2.1.4). Polysaccharide **51**, which was formed by polymerization of **48**, is the first



Scheme 23. (I)Transformation of deprotected trisaccharide **40** to the respective glycosyl fluoride **47**. Reagents and conditions: a) Ac_2O, DMAP, py; b) HF/py, 5 h, -10 °C; c) NaOMe, MeOH, 0 °C. (II) α- and β-anomeric fluoride peaks in the ^{19}F NMR spectrum of crude trisaccharide fluoride **46**. (III) Tetrasaccharide fluorides **48** and **49** that were synthesized analogously to **47**.

example of an artificial arabinoxylan polysaccharide with a defined substitution pattern. These results show that enzymatic polymerization using the glycosynthase technology is a fast and convenient synthetic route towards regularly substituted polysaccharides. However, the scale of these polymerization reactions was limited to less than 1 mg due to the limited scale of the automated glycan assembly.



Scheme 24. (a) Glycosynthase-catalyzed polymerization of glycosyl fluorides **47**, **48** and **49**. (b) MALDI-MS spectrum of polysaccharide **51**, showing the formation of chain lengths up to n = 8 (32 monosaccharides).

2.2.2 Chemical Solution-Phase Synthesis of Arabinoxylan Oligosaccharides

The polymerization of arabinoxylan oligosaccharides obtained from automated glycan assembly provided a proof-of-principle for the approach of synthesizing artificial arabinoxylan polysaccharides with well-defined substitution patterns by glycosynthase-catalyzed polymerization of arabinoxylan oligosaccharides. However, the scale of these reactions was very small. Therefore, three oligosaccharides (**43**, **52** and **53**) were produced in larger quantities by chemical synthesis to serve as monomers in the enzymatic polymerization reactions (Figure 16). For the synthesis of linear xylan polysaccharides, xylobiose **52** was chosen as a substrate instead of trisaccharide donor **40** due to its simpler synthesis. The arabinose-substituted structures **53** and **43** carry α-1,3-linked arabinofuranosyl substituents at the non-reducing end xylose units to ensure accommodation of the substituents by glycosynthase XynAE265G.[163]

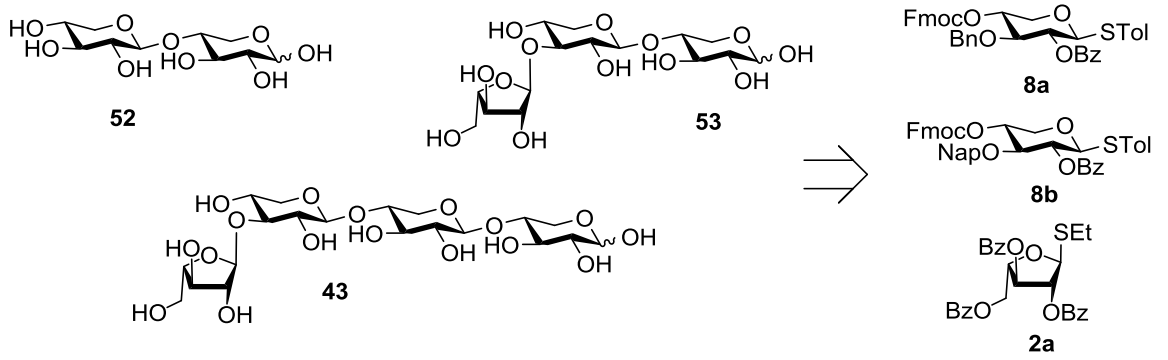
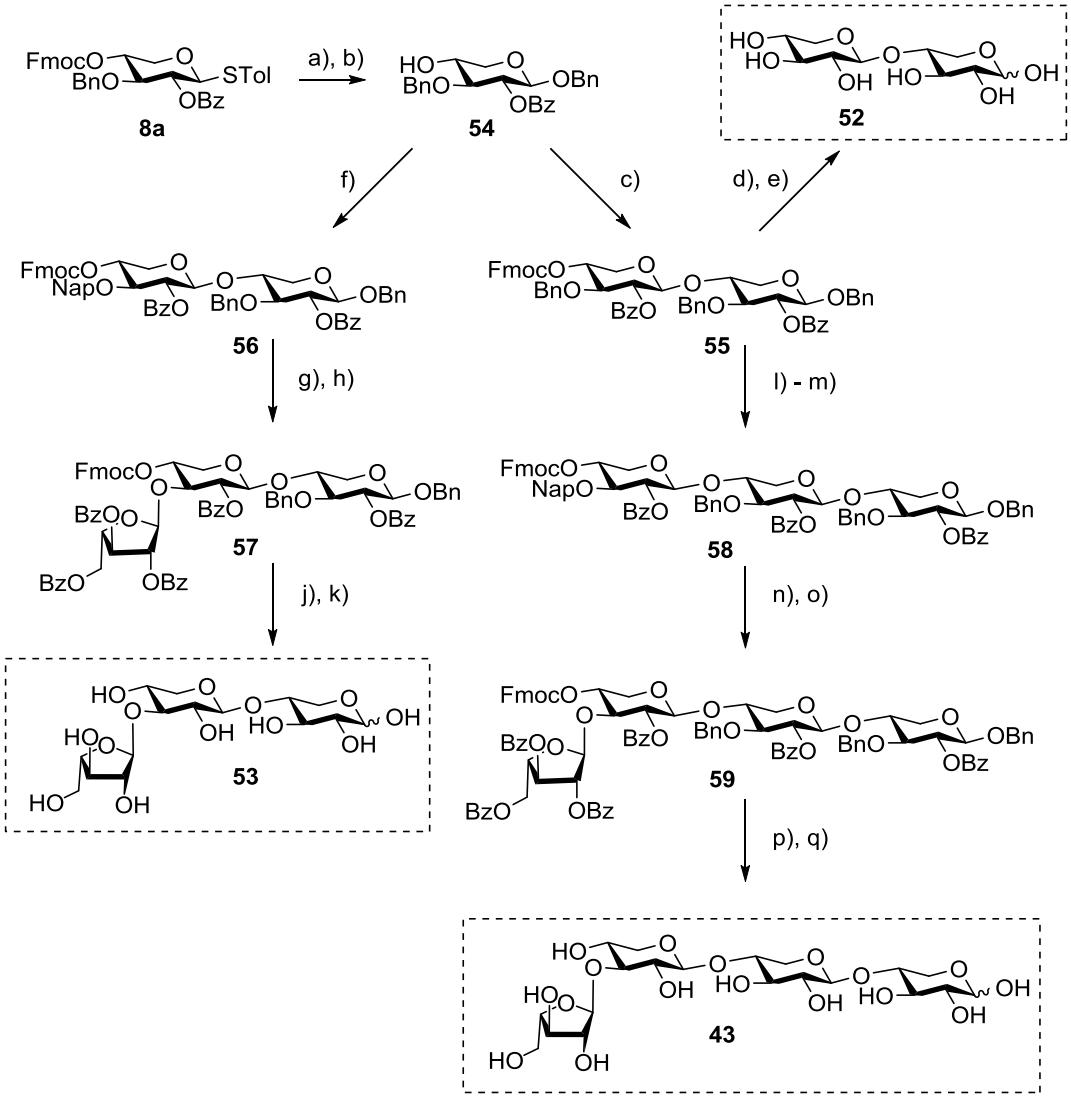


Figure 16. (Arabino-)xylan oligosaccharides **43**, **52** and **53** and the monosaccharide BBs for their solution-phase synthesis.

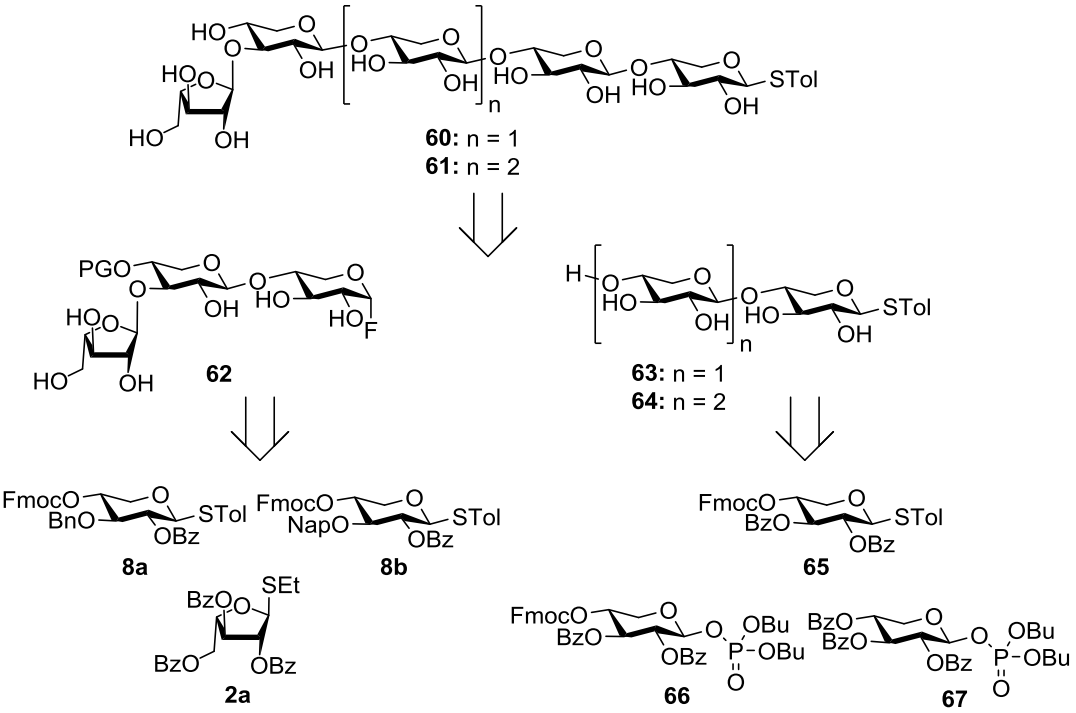
For the chemical synthesis of oligosaccharides **43**, **52** and **53**, the same protecting group strategy was used as for the automated glycan assembly of the α-1,3-substituted arabinoxylan oligosaccharides described in the previous chapter (Scheme 25). The synthesis started from thioglycoside BB **8a**, which was reacted with BnOH and NIS/TfOH. After the C4-position was deprotected by using NEt_3, chain elongation with thioglycoside **8a** was performed to give fully protected disaccharide **55**. Subsequently, global deprotection afforded fully deprotected disaccharide **52**. For the synthesis of trisaccharide **53**, glycosylation of the free hydroxyl group of xylose acceptor **54** was performed with Nap-protected BB **8b** to give disaccharide **56**. Deprotection of the hydroxyl group at the C3-position with DDQ was followed by a glycosylation reaction with arabinose BB **2a**. After methanolysis and hydrogenolysis of **57**, fully deprotected trisaccharide **53** was obtained. Finally, the synthesis of tetrasaccharide **43** was performed starting from fully protected disaccharide **55**. After Fmoc-deprotection using NEt_3, chain elongation at the C4-position was performed with Nap-protected BB **8b** to afford trisaccharide **58**. Subsequent removal of the Nap group in the C3-position using DDQ was followed by the attachment of the arabinose side chain by glycosylation of the free hydroxyl group with BB **2a**, which gave fully protected tetrasaccharide **59**. Methanolysis and hydrogenolysis afforded the fully deprotected tetrasaccharide **43**. In this case, it was crucial to add triethylamine (0.1 equiv) directly after the hydrogenolysis in order to prevent acid-induced cleavage of the arabinose side chain during removal of the solvents. Traces of HCl are believed to be formed during hydrogenolysis from residual $PdCl_2$ in the Pd-catalyst.[224,225]



Scheme 25. Chemical synthesis of di-, tri- and tetrasaccharides **43**, **52** and **53**. Reagents and conditions: a) NIS, TfOH, BnOH, -10 °C, 30 min; b) NEt_3, DCM, 2 h, 93% over 2 steps; c) NIS, TfOH, DCM, BB **8a**, -15 °C, 30 min, 96%; d) NaOMe, MeOH/DCM, 71%, e) H_2, Pd/C, MeOH, EtOAc, H_2O, 78%; f) NIS, TfOH, DCM, BB **8b**, -15 °C, 30 min, 84%; g) DDQ, MeOH/DCM 79% h) NIS, TfOH, BB **2a**, -30 °C, 30 min, 93%; j) NaOMe, MeOH/DCM, 73%, k) H_2, Pd/C, MeOH, EtOAc, H_2O, 99%; l) NEt_3, DCM, 2 h, 90%; m) NIS, TfOH, DCM, BB **8b**, -15 °C, 30 min; n) DDQ, MeOH/DCM 75% over 2 steps; o) NIS, TfOH, BB **2a**, -30 °C, 30 min, 79%; p) NaOMe, MeOH/DCM, 65%; q) H_2, Pd/C, MeOH, EtOAc, H_2O, NEt_3; the yield was calculated over 2 steps after the next transformation.

2.2.3 Chemo-Enzymatic Synthesis of Arabinoxylan Oligosaccharides

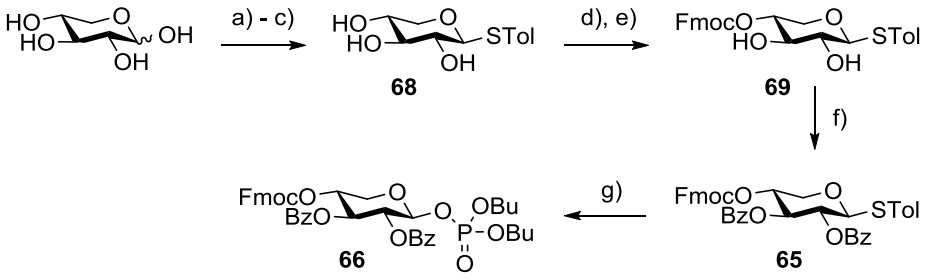
Larger α-1,3-arabinofuranosyl-substituted xylan oligosaccharides **60** and **61** were obtained by a chemo-enzymatic approach (Scheme 26), in which arabinoxylan trisaccharide donor **62** was ligated in a controlled manner to di- and trixyloside acceptors **63** and **64** using glycosynthase XynAE265G.[163]



Scheme 26. Retrosynthetic analysis of penta- and hexasaccharides **60** and **61**, PG = protecting group.

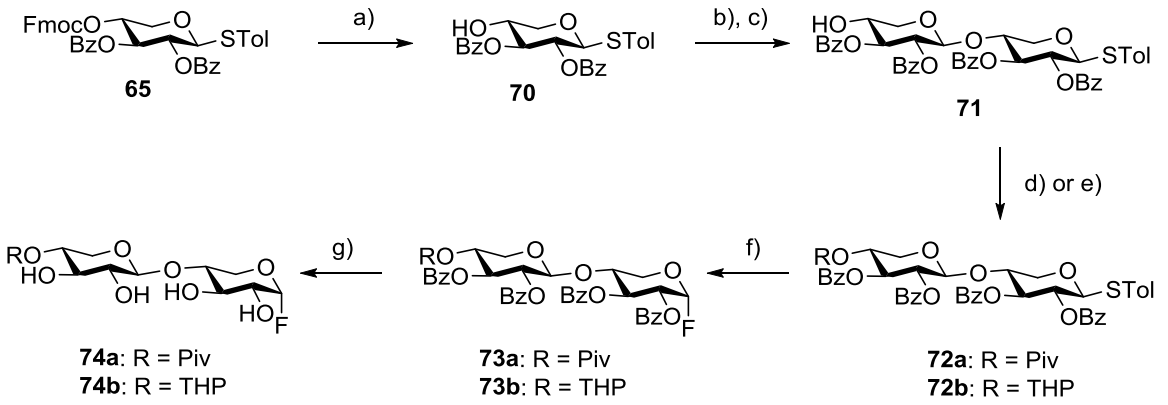
To prevent enzyme-catalyzed self-condensation of trisaccharide donor **62**, it had to be equipped with a suitable protecting group (PG) at the C4-position of the non-reducing xylose residue. For this purpose, the tetrahydropyranosyl (THP)- and pivaloyl (Piv)-groups were evaluated. The THP-group had already successfully been used in the step-wise enzymatic synthesis of cellodextrins[226] and xyloglucan oligosaccharides.[227] It is cleavable under relatively mild acidic conditions in aqueous solution.[228,229] However, THP-cleavage also occurs during the acidic conditions in the fluorination reaction to prepare the glycosyl fluoride and THP has thus to be reintroduced in an additional synthetic step. It was therefore desirable to find a different protecting group that is stable towards HF/pyridine. The Piv-group was envisioned to be a suitable alternative as it is stable to acidic, oxidative and reductive conditions, and can be removed with strong bases.[230,231] However, if the

Piv-group is accommodated by the glycosynthase during the enzymatic ligation and if the Piv-protected glycosyl fluoride can be selectively deacetylated in the last step remained elusive. In order to investigate the suitability of both protecting groups, model disaccharide fluorides **74a** and **74b** equipped with either the Piv- or THP-group at the C4'-position were synthesized (Scheme 28). The model disaccharides were synthesized from xylose donors **65** and **66** as their synthesis requires fewer synthetic steps and they are also required for the synthesis of di- and trisaccharide acceptors **63** and **64**. The synthesis of **65** and **66** was performed starting from D-xylose according to a previously established reaction sequence in our group (Michael Wilsdorf, unpublished results, Scheme 27). Peracetylation of D-xylose using Ac_2O in pyridine, followed by $BF_3{\cdot}OEt_2$-promoted thioglycosylation with TolSH[232] and the removal of the acetate groups using NaOMe, gave fully deprotected thioglycoside **68**. Subsequently, selective protection of the hydroxyl group in the C4-position with Fmoc was accomplished by first refluxing thioglycoside **69** in presence of Bu_2SnO in 1,4-dioxane[232] and then reacting the resulting tin acetal with FmocCl. Upon aqueous workup the tin acetal was cleaved, and Fmoc-protected compound **69** was obtained. Protection of the remaining hydroxyl groups with benzoyl chloride gave thioglycoside **65**. In the last step the thiol leaving group was exchanged by a phosphate leaving group and glycosyl phosphate **66** was obtained.



Scheme 27. Synthesis of xylose donors **65** and **66**. Reagents and conditions: a) Ac_2O, pyridine, DMAP; b) HSTol, $BF_3{\cdot}OEt_2$, 55%; c) NaOMe, MeOH/DCM, 65%; d) Bu_2SnO, dioxane, reflux e) FmocCl, DCM, 72%; f) BzCl, pyridine, DMF, 64%; g) $HOP(O)(OBu)_2$, NIS, TfOH, DCM, 0 °C, 84%.

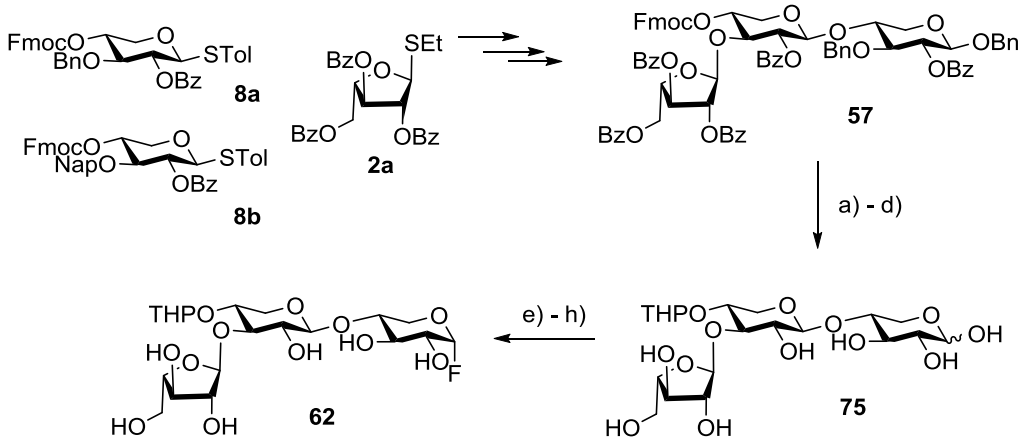
For the synthesis of disaccharides **74a** and **74b** (Scheme 28), thioglycoside **65** was deprotected using NEt_3 and the resulting free hydroxyl group in **70** was reacted with phosphate donor **66** to give disaccharide **71**. Introduction of the Piv-group was performed using PivCl and pyridine[233] and introduction of the THP-group using DHP and catalytic amounts of *p*TsOH.[226] Transformation of the resulting protected disaccharides **72a** and **72b** into the respective fluorides **73a** and **73b** was accomplished using HF/pyridine and NIS, which is used to activate the thioether.[234] In the case of glycosyl fluoride **73b** the THP-group was lost and had to be reintroduced in the C4'-position. Subsequently, the



Scheme 28. Synthesis of 4'-O-protected disaccharides **74a** and **74b** for the investigation of suitable PGs in the chemo-enzymatic synthesis of penta- and hexasaccharides **60** and **61**. Reagents and conditions: a) NEt_3, DCM, 3 h, 80%; b) TMSOTf, DCM, **66**, 66%; c) NEt_3, DCM, 3 h, 59% over 2 steps; d) PivCl, py, DCM, 0 °C to r.t., 82%; e) DHP, CSA, DCM, 80%; f) (I) NIS, HF, py, -10 °C, 5 h, **73a**: 42%; (II) for **73b** only: DHP, CSA, DCM, 82% over 2 steps; g) NaOMe, MeOH, 0 °C, **74b**: 89%.

benzoyl groups were removed. Methanolysis using catalytic amounts of NaOMe at 0 °C afforded the 4'-*O*-THP-protected disaccharide fluoride **74b**. The synthesis of **74a** failed because the Piv ester was partially cleaved under these conditions. Using NEt_3 in methanol at 0 °C for removal of the benzoyl esters[231] did not improve the results. Thus, the Piv-group was not suitable for protection of the glycosyl fluorides and trisaccharide donor **62** was synthesized equipped with a THP-group at the C4'-position.

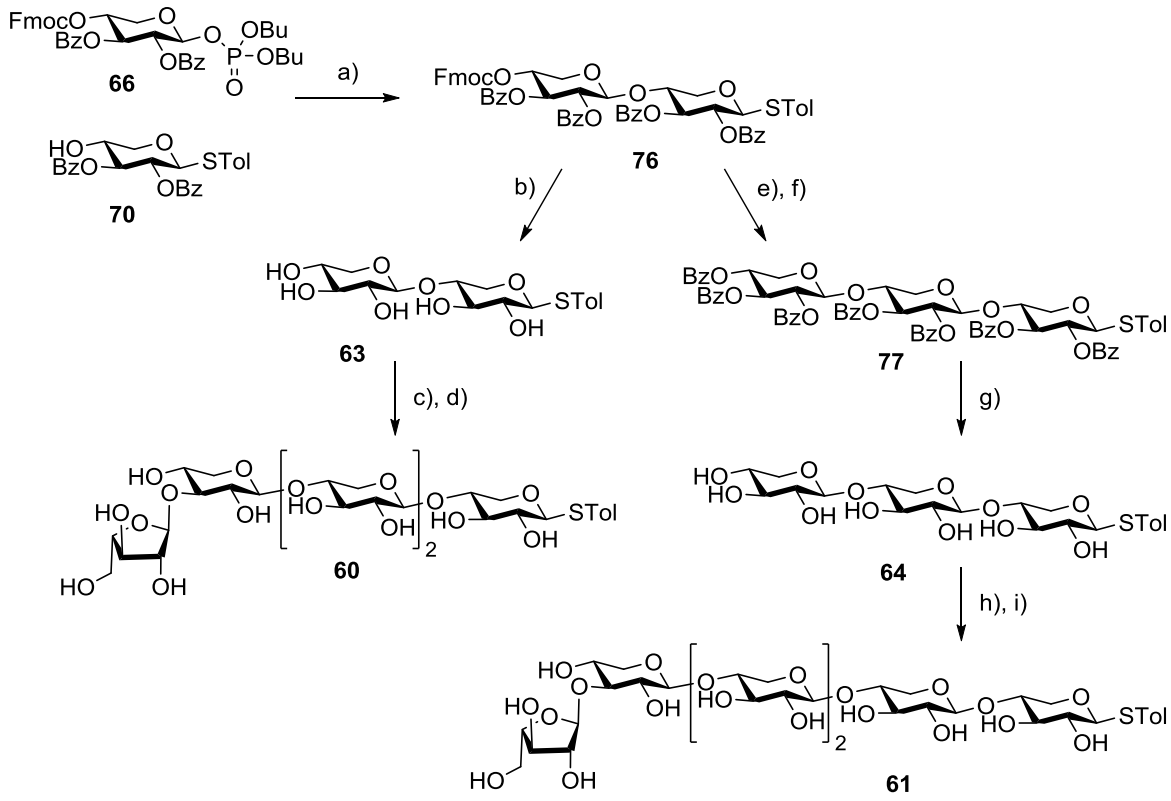
The synthesis of protected glycosyl fluoride **62** was performed starting from trisaccharide **57** that had already been assembled from BBs **8a**,**b** and **2a** for the chemical solution-phase synthesis of trisaccharide **53** in the previous chapter (Scheme 29). The



Scheme 29. Synthesis of 4'-*O*-THP-protected trisaccharide fluoride **62**. Reagents and conditions: a) NEt_3, DCM, 2 h, rt, 90%; b) DHP, *p*-TsOH, DCM, 4 h, rt; c) NaOMe, MeOH/DCM, 16 h, rt, 66% over 2 steps; d) H_2, Pd/C, MeOH/EtOAc/$Na_xH_xPO_4$ buffer, rt, 24 h; e) Ac_2O, py, 4 h, rt; f) HF/py, DCM, -30 °C → -20 °C, 2 h, 60% over 3 steps; g) DHP, *p*-TsOH, DCM, 4 h, rt, 91%; h) NaOMe, MeOH, 0 °C, 96%.

Fmoc-protecting group in the C4-position was exchanged by a THP-group in two steps,and subsequent methanolysis of the benzoyl esters and hydrogenolysis of the benzyl ethers provided 4'-*O*-THP-protected trisaccharide **75**. Due to the acid-sensitivity of the THP-protecting group, it was necessary to perform the hydrogenolysis in a solvent mixture containing sodium phosphate buffer to prevent acid-promoted THP-cleavage.[224,225] Peracetylation of **75** was then followed by fluorination with HF/pyridine at low temperature to provide the corresponding glycosyl fluoride.[218-222] Reaction temperature and time were carefully adjusted to ensure formation of the thermodynamically favored α-fluoride without cleavage of the acid-labile arabinofuranose substituent. The THP-group did not stay intact under these reaction conditions and was subsequently reintroduced at the C4'-position using DHP and catalytic amounts of *p*TsOH. Removal of the acetate groups by treatment with catalytic amounts of NaOMe (1 equiv) at 0 °C finally afforded THP-protected glycosyl fluoride **62**.

Next, acceptors **63** and **64** for the enzymatic ligation reaction were synthesized (Scheme 30). Glycosylation of the previously synthesized phosphate donor **66** with



Scheme 30. Synthesis of di- and trisaccharide acceptors **63** and **64** and enzymatic ligations providing penta- and hexasaccharides **60** and **61**. Reagents and conditions: a) TMSOTf, DCM, 66%; b) NaOMe, MeOH/DCM, 2 h, 94%; c) **62**, XynAE265G, rt, $Na_xH_xPO_4$ buffer, 16 h; d) 1 M HCl, rt, 30 min, 78% over 2 steps; e) NEt_3, DCM, 1 h, 72%; f) TMSOTf, DCM, BB **67**, 72% g) NaOMe, MeOH/DCM, 16 h, 90%; h) **62**, XynAE265G, rt, $Na_xH_xPO_4$ buffer, 16 h; i) 1 M HCl, rt, 30 min, 76% over 2 steps.

thioglycoside **70** afforded disaccharide **76**. Subsequent treatment of **76** with NaOMe to remove the ester protecting groups gave disaccharide acceptor **63**. Overnight-incubation of glycosyl fluoride **62** and acceptor **63** with the glycosynthase XynAE265G followed by acid-promoted removal of the THP protecting group gave the desired pentasaccharide **60** in good yield. Trisaccharide acceptor **64** was obtained after glycosylation of disaccharide **76** with perbenzoylated phosphate donor **67** and removal of the benzoyl esters in the resulting trisaccharide **77** using NaOMe. Enzymatic ligation of glycosyl fluoride **62** and acceptor **64** with the glycosynthase XynAE265G was performed as with acceptor **63**. The thioether moiety in **60** and **61** provided a good hydrophobic handle for purification and permitted their later conversion into the corresponding glycosyl fluorides.

2.2.4 Synthesis of Glycosyl Fluorides and Enzymatic Polymerization

In addition to the synthesized oligosaccharides **43**, **52**, **53** and **61**-**62**, α-1,2-substituted and α-1,2-α-1,3-disubstituted arabinoxylan tetra- and pentasaccharide (**78** and **79**) were procured commercially in limited amounts to serve as monomers in the enzymatic polymerization reaction (Figure 17). All of these oligosaccharides were converted into the corresponding glycosyl fluorides.

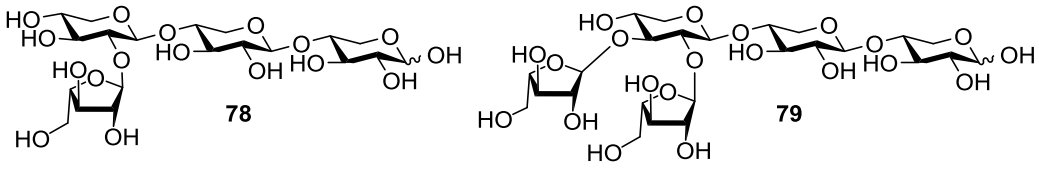
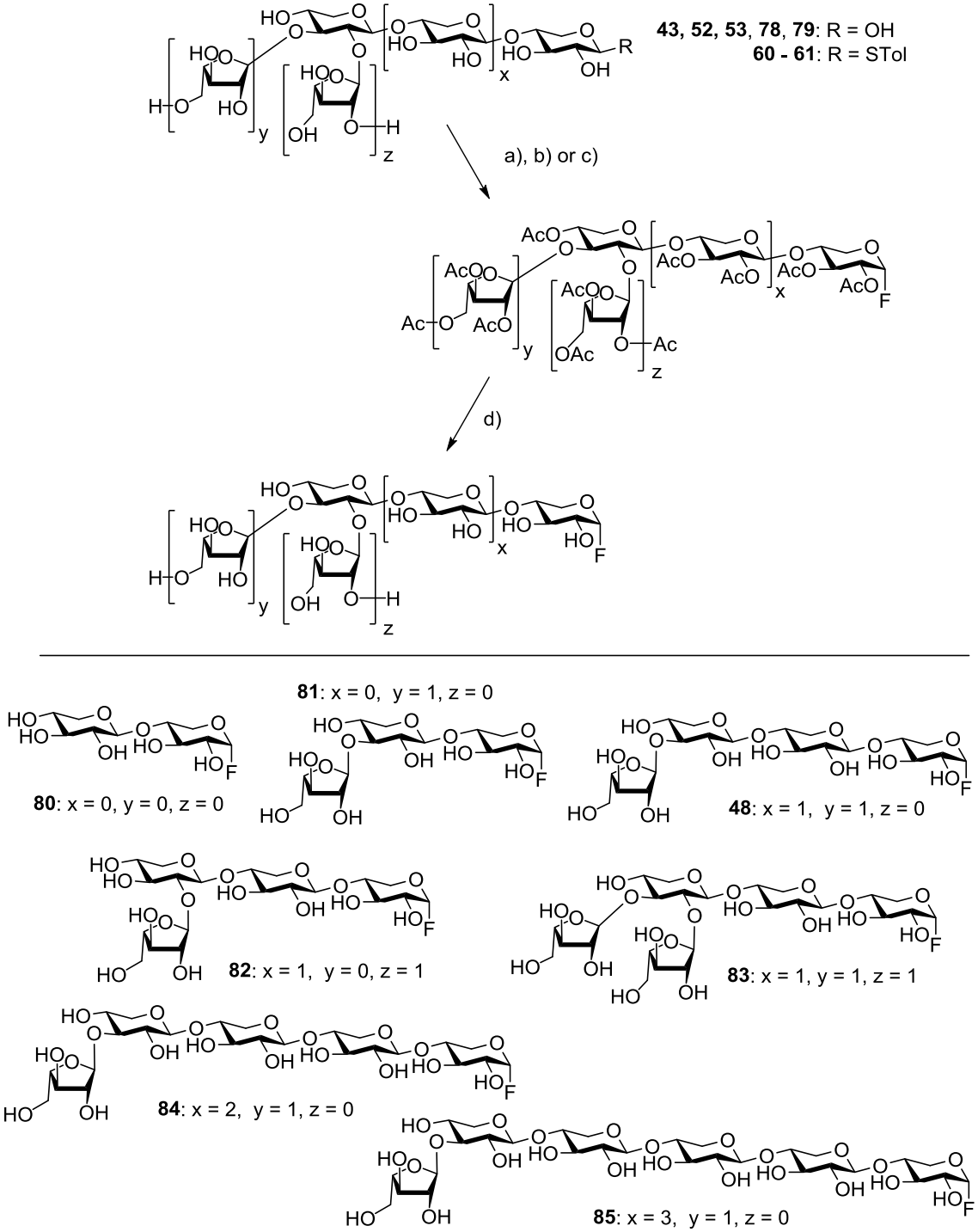


Figure 17. Commercially available α-1,2-substituted and α-1,2-α-1,3-disubstituted arabinoxylan oligosaccharides **78** and **79**.

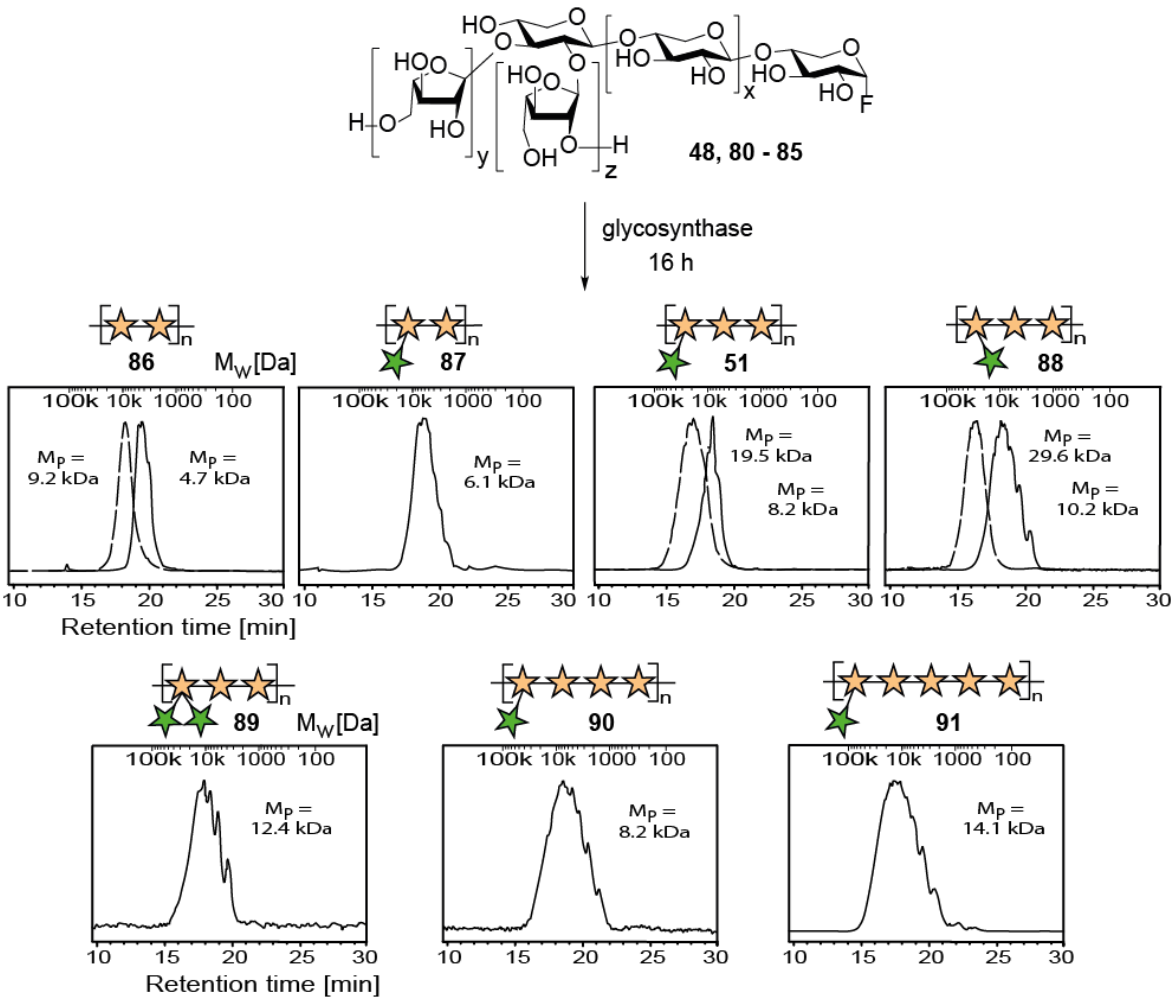
Transformation into the glycosyl fluorides was accomplished by the previously described three step procedure consisting of peracetylation, fluorination, and deacetylation (Scheme 31).[218-222] In the case of the peracetylated derivates of **52**, **53** and **78** fluorination was performed using HF/pyridine at low temperature. Activation of the peracetylated oligosaccharides derived from thioglycosides **60** and **61** required the admixture of NIS to activate the thioether leaving group.[234] A milder procedure was applied to oligosaccharides **43** and **79**, as the usage of HF/pyridine provoked cleavage of the arabinofuranose. In this procedure, the hydroxyl group at the anomeric center was selectively deprotected using hydrazine acetate first, and the resulting hydroxyl group converted then with *N*,*N*'-diethylaminosulfur trifluoride (DAST) into the corresponding β-

fluoride. The β-fluorides were finally equilibrated into the desired α-fluoride-derivates of **43** and **79** using a small amount of HF/pyridine.[235]



Scheme 31. Synthesis of glycosyl fluorides **48** and **80**–**85**. Reagents and conditions: a) Ac_2O, py, **A**: 42%, **B**: 78%; **C**: 90% over 2 steps, **D**: 94%, **E**: 96%; b) only **C** and **E**: I) N_2H_4·AcOH, DMF, 0 °C, 5 h, II) DAST, DCM, 0 °C, 1 h, c) NIS (for **F** and **G** only), HF/py, DCM, -30 °C → -10 °C, 2-5 h, **A**: 79%, **B**: 89%; **C**: 77% over 3 steps, **D**: 43%, **E**: 44% over 3 steps, **F**: 53% over 2 steps, **G**: 52% over 2 steps; h) NaOMe, MeOH, 0 °C, **80**: quant., **81**: quant.; **48**: quant., **82**: quant., **83**: 96%, **84**: 99%, **85**: quant.

With the desired arabinoxylan glycosyl fluorides in hand, enzymatic polymerizations were performed by overnight incubation of the respective glycosyl fluoride with XynAE265G, and arabinoxylan polysaccharides in amounts up to 55 mg (**87**) were obtained in one batch (Scheme 32). In some cases (**86**, **51**, and **88**), precipitates of water-insoluble polysaccharides were formed during the reactions while in the other cases the reaction products remained fully soluble. When precipitated polysaccharides were formed, they were collected by centrifugation and the remaining solution was passed through a short C18 cartridge to isolate the soluble polysaccharide fraction. Water-soluble polysaccharides were directly subjected to C18 reversed-phase chromatography. MALDI mass spectrometry was used to confirm the formation of polymers of the starting oligosaccharides. The molecular mass distribution of the products was derived from high-



Scheme 32. Synthesis of artificial arabinoxylan polysaccharides by enzymatic polymerization of arabinoxylan oligosaccharide fluorides. The molecular mass distribution of the resulting polysaccharides was analyzed by HPSEC chromatography using a TSK gel 3000 column and an ELSD detector. Chromatograms with two ELSD traces were obtained when a water-insoluble polysaccharide fraction (dashed line) and a water-soluble fraction (solid line) was obtained. These fractions were analyzed separately.

performance size-exclusion chromatography (HPSEC) calibrated with pullulan standards (Scheme 32) since it cannot be deduced from mass spectrometry experiments due to the size dependent molecule desorption from the MALDI matrix.[236,237] The molecular weight at the peak maximum (M_P) of the different polysaccharide products was found to range from 4.7 kDa for the water-soluble fraction of linear xylan **86** to 29.6 kDa for the water-insoluble fraction of arabinoxylan **88**. These values correspond to 36 (n = 18) and 224 (n = 56) monosaccharides, respectively. The observed differences in the degree of polymerization (DP) of the artificial polysaccharides are most likely a result of the substrate specificity of the enzyme that transfers some of the arabinoxylan oligosaccharides more efficiently than others. There is an inherent limitation for the maximum achievable lengths of xylan polysaccharides that is determined by their solubility. Thus, the M_p of the linear xylan polysaccharide for example did not exceed 9.2 kDa.

In addition to the linear xylan **86**, water-insoluble polysaccharide fractions were obtained for arabinoxylans **51** and **88**, which carry the arabinofuranosyl substituents at the 2- and 3-position of every third xylose residue, respectively. In line with this, arabinoxylans **51** and **88** showed significant crystallinity whereas **87**, **90**, and **91**, having differently spaced substituents, were completely amorphous, as qualitatively determined by powder X-ray diffraction (Figure 18). The specific substitution pattern of arabinoxylans **51** and **88** might favor a stable threefold helical screw conformation, with the substituents all pointing in the same direction.[238] Such a conformation would enable interactions between the xylan backbones of individual molecules, explaining the low solubility and high crystallinity of **51** and **88**. This contradicts the commonly accepted notion that solubility and crystallinity of xylans directly correlate with the degree of substitution.[41,44]

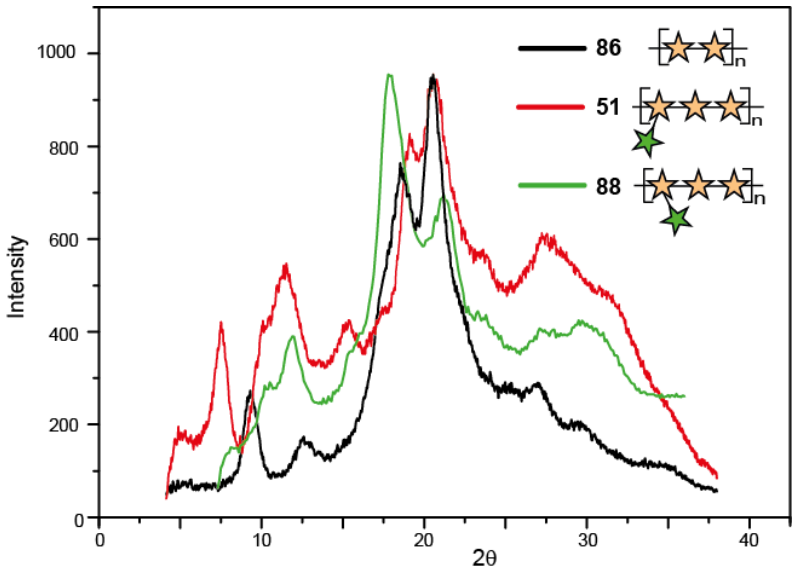


Figure 18. X-Ray diffractogram of the polysaccharide **86**, **51** and **88** that show crystallinity.

2.2.5 Adsorption of Arabinoxylan Polysaccharides to a Cellulosic Surface

The ability of selected polysaccharides to bind to cellulose was investigated as Dupree *et al.* have found that only glucuronoxylans having evenly spaced substituents are able to bind to the cellulose microfibril due to the conformational change from a two-fold to a three-fold helical screw.[47,51,239] The prepared collection of synthetic polysaccharides represents ideal substrates to investigate this effect for arabinose-substituted xylans. No or evenly spaced arabinose substituents at the xylose backbone should enable binding to cellulose, whereas unevenly spaced arabinose-substitution is expected to reduce the ability of the respective polysaccharide to bind. The experiments were performed using a quartz crystal microbalance with dissipation (QCMD) by Saina Kishani and Guillermo Toriz Gonzalez at the Chalmers University of Technology in Göteborg (Sweden). QCMD is a highly sensitive mass measuring system based on the principles of piezoelectricity. The quartz crystal is brought into oscillation and upon deposition of a substrate the frequency of the oscillation changes. The frequency is monitored and can be converted to units of adsorbed mass by using the Sauerbrey equation.[240,241] The experiments were performed by slowly flowing a solution containing one of the polysaccharides over a spin-coated cellulose model surface (Figure 19a). Rinsing before and after polysaccharide deposition was performed to remove loosely attached material. The mass of the adsorbed polysaccharides was measured over time. As substrates for these experiments the three polysaccharides **86**, **87** and **51** were chosen for the investigation of their ability to bind to cellulose as relatively large amounts of sample were available. In order to reduce effects resulting from the overall length of the polysaccharides, the soluble fractions of **86**, **87** and **51** having similar M_P values were compared.

For all three polysaccharides, a layer of material was deposited when the cellulosic surface was exposed to the polysaccharide-containing solution (Figure 19b, 5-25 min). As expected, the deposited amount of linear xylan polysaccharide **86** was the largest, since it has no substituents and the xylan backbone can freely interact with the cellulosic surface. Evenly substituted arabinoxylan **87** was adsorbed in higher amounts than unevenly substituted arabinoxylan **51**. This difference became even more apparent after the final rinsing step (at 25 min) was performed. The deposited layer of **51** is almost completely removed, while for **86** and **87** only a small decrease in the adsorbed mass was observed.

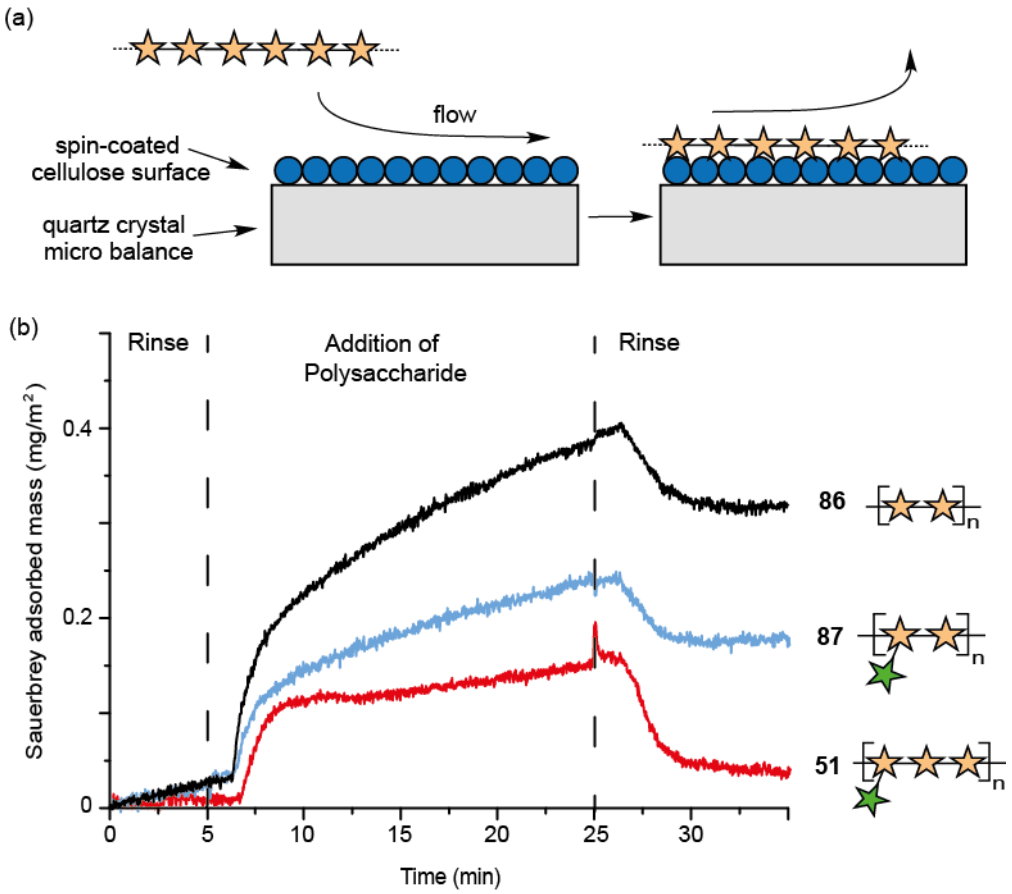


Figure 19. Adsorption of synthetic polysaccharides **86**, **87** and **51** to a surface spin-coated with cellulose. (a) Schematic representation of the experiment. A solution of the polysaccharide flows over the cellulosic surface. (b) Adsorbed mass of the polysaccharides on a cellulosic surface calculated by the Sauerbrey equation.

These results indicate that also for arabinoxylans evenly spaced substituents enable stronger binding to cellulose, which could be explained by a conformational change from a three-fold to a two-fold helical screw. The availability of artificial polysaccharides with a defined substitution pattern, which are not accessible by other means, allowed us to directly investigate this effect outside of the plant cell wall.

2.2.6 Conclusion and Outlook

In conclusion, the glycosynthase technology was explored for the polymerization of synthetic arabinoxylan oligosaccharides into artificial polysaccharides with well-defined branching patterns. The oligosaccharides that served as substrates for the polymerization reactions were initially obtained in small amounts by automated glycan assembly using a photolabile, traceless linker as a proof-of-principle for the glycosynthase-catalyzed polymerization and to investigate the substrate specificity of the glycosynthase. Thereafter, a set of seven oligosaccharides was produced by chemical and chemo-

enzymatic synthesis, and successfully used in enzymatic polymerization reactions. The obtained polysaccharides had molecular masses up to 80 kDa (606 monosaccharides), which are, to the best of our knowledge, the largest polysaccharides produced by glycosynthase technology to date. Due to the defined nature of the polysaccharide branching patterns, specific properties were observed for particular members of the prepared arabinoxylan collection rather than the simple linear correlation between crystallinity and degree of substitution reported previously.[44]

The ability of three polysaccharides (**86**, **87** and **51**), having either no, evenly spaced or unevenly spaced substituents, to adsorb to a spin coated cellulosic surface were compared using QCMD.[41,44,50] The results indicate that evenly spaced arabinose substituents enable stronger binding of the xylan polysaccharides to cellulose than unevenly spaced substituents. Currently, this investigation is being extended to the remaining four polysaccharides. Also, further physico-chemical properties of the synthetic polysaccharides, such as their ability to absorb water, their immunomodulatory properties,[242] as well as their potential to serve as substrates in assays to determine the specificities of xylan-degrading enzymes are being investigated.[91,95]

3 Experimental Part

3.1 General Information

The automated syntheses were performed on a self-built synthesizer developed in the Max Planck Institute of Colloids and Interfaces. Xylan-degrading enzymes were obtained from Megazyme International Ireland. Linker-functionalized resin **9**[139] and **39**[214] were prepared as reported in the literature. The resin loading was determined as described previously.[106] Xylose BBs **1a**-**c**,[132,133] arabinose BBs **2a**, **2b**[132] and **2c**,[187] 4-methylphenyl 3-*O*-(2-methyl)naphtyl-1-thio-β-D-xylopyranoside,[132] 44-Methylphenyl 4,6-di-*O*-benzylidene-1-thio-β-D-glucopyranoside,[243] and 2-(azidomethyl)benzoic anhydride[244,245] were prepared as reported in the literature. Oligosaccharides **78** (A^2XX) and **79** ($A^{2,3}XX$) were obtained from Megazyme International Ireland. 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) or a Bruker AVIII 700 (700 MHz) spectrometer using solutions of the respective compound in $CDCl_3$ or D_2O. NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. Spectra recorded in $CDCl_3$ and D_2O used the solvent residual peak chemical shifts as internal standards ($CDCl_3$: 7.26 ppm ^1H, 77.0 ppm ^{13}C; D_2O: 4.80 ppm ^1H). Yields of the final deprotected oligosaccharides obtained by automated glycan assembly 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 mass spectrometer (Agilent). Analytical HPLC was performed on an Agilent 1200 series coupled to a quadrupole ESI LC/MS 6130 using a), a YMC-Diol-300 column (150 x 4.6 mm) with 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), a Phenomenex Luna C5 column (250 x 4.6 mm) with linear gradients from 80% to 0% water in ACN (50 min, flow rate 1 mL/min), or a Thermo Scientific Hypercarb column (150 x 4.6 mm) with linear gradients from 97.5% to 0% water in ACN (45 min, flow rate 0.7 mL/min) Preparative HPLC was performed on an Agilent 1200 series using a preparative a YMC-Diol-300 column (150 x 20 mm), a semi-preparative Phenomenex Luna C5 column (250 x 10 mm) or a semi-preparative Thermo Scientific Hypercarb column (150 x 10 mm). Analytical HPSEC was performed on an Agilent 1200 series using a TSKgel G3000 PW column at 0.4 mL/min

flowrate. For filtration syringe filters (RC, 0.45 μm) from Roth were used. Glycosynthase XynAE265G[163] was provided by my colleague Dr. Ruprecht. X-ray powder diffraction patterns were recorded using a Nanostar with an X-ray beam (68 μm in diameter; λ =1.542 Å). All samples were placed in sealed capillary at a relative humidity of 53% and if possible at 90%. The calibration of sample to detector was performed using quartz as standard with the software DPDAK and all patterns were corrected for empty beam background.

3.2 Synthesizer Modules and Conditions

Linker-functionalized resin **9** or **39** (12.5-33.8 μmol or 44.2 μmol of hydroxyl groups) were placed in the reaction vessel of the automated oligosaccharide synthesizer and swollen for at least 30 min in DCM. Before every synthesis the resin was washed with DMF, THF and DCM. Subsequently the glycosylation (Module **A** and **B**) and deprotection (Module **D** and **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 (12.5-44.2 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 then DCM only. For the glycosylation reaction the DCM was drained and a solution of phosphate BB (1.8 equiv in 1 mL DCM) was delivered to the reaction vessel at -35 °C. The reaction was started by the addition of TMSOTf in DCM (1.8 equiv in 1 mL DCM). The glycosylation was performed for 5 min at -35 °C and then for 30 minutes at -15 °C. Subsequently the solution was drained and the resin washed three times with DCM. The whole procedure was repeated once to increase conversion of the acceptor sites. Afterwards the resin was washed three times with DCM at 25 °C.

Module B: Glycosylation with Thioglycosides. The resin (12.5-44.2 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 then DCM only. For the glycosylation reaction the DCM was drained and a solution of thioglycoside BB (1.8-5 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 (4.44 equiv or 8.88 equiv) and TfOH (0.44 equiv or 0.88 equiv) in DCM/dioxane (2:1). The glycosylation was performed for 5 min at -40 °C or -20 °C and then for 40 min at -20 °C or -5 °C. Subsequently the solution was drained and the resin was washed with DCM. The whole procedure was repeated once to ensure full conversion of all acceptor sites. Afterwards the resin was washed three times with DCM at 25 °C.

Module C: 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 reaction 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 D: Nap Deprotection. The resin was washed with DCM three times and the temperature of the reaction vessel was adjusted to 40 °C. For Nap deprotection the DCM was drained and 1.5 mL of a 0.1 M DDQ solution in DCE/MeOH/H_2O (64:16:1) was delivered to the reaction vessel. After 20 min the reaction solution was drained and the whole procedure was repeated another six times. After Nap deprotection was complete the resin was washed with DMF, THF and DCM.

Module E: Azmb Deprotection. The resin was washed with THF, swollen in 2 mL THF and the temperature of the reaction vessel was adjusted to 25 °C. Prior to the deprotection step the THF was drained and the resin was washed with THF three times. For Azmb deprotection, 2 mL of a solution of PBu_3 (7.5 or 15 equiv.) in THF containing 5% H_2O was delivered to the reaction vessel at 45 °C. After 30 min the solution was drained and the whole procedure was repeated another five times. After Azmb deprotection was complete the resin was washed with THF and DCM.

Module F: Acetylation. The temperature of the reaction vessel was adjusted to 25 °C and the resin washed with pyridine (2 mL) three times. For capping, 1 mL pyridine and 1 mL acetic anhydride 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 pyridine and DCM.

Module G: 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.

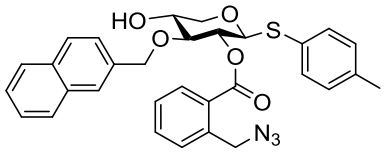
Cleavage from the solid support. After assembly of the oligosaccharides, cleavage from the solid support was accomplished by modification of a previously published protocol,[106] using the Vapourtec E-Series UV-150 photoreactor Flow Chemistry System. The medium pressure metal halide lamp is filtered 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 photo-reactor 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 Arabino- and Glucuronoxylan Oligosaccharides

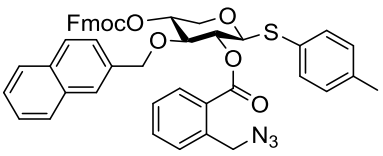
3.3.1 Synthesis of Xylose Building Blocks

4-Methylphenyl 2-O-(azidomethyl)benzoyl-3-O-(2-methyl)naphtyl-1-thio-β-D-xylo-pyranoside (7d)



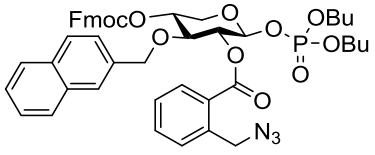
4-Methylphenyl 3-*O*-(2-methyl)naphtyl-1-thio-β-D-xylopyranoside **7b** (1.50 g, 3.78 mmol), $Yb(OTf)_3$ (470 mg, 760 μmol) and 2-(azidomethyl)benzoic anhydride (1.78 g, 5.30 mmol) were dissolved in dioxane (30 mL) and stirred for 2 d. The reaction was quenched with MeOH (10 mL) and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc/hex = 9:1 to 6:1) to give 4-methylphenyl 2-*O*-(azidomethyl)benzoyl-3-*O*-(2-methyl)naphtyl-4-*O*-fluorenylcarbox-ymethyl-1-thio-β-D-xylopyranoside **7d** (933 mg, 1.68 mmol, 44%) as a colorless solid. $[\alpha]_{25}^{D}$ = -7.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06-7.91 (m, 1H, Ar*H*), 7.82-7.70 (m, 4H, Ar*H*), 7.62-7.53 (m, 1H, Ar*H*), 7.53-7.32 (m, 7H, Ar*H*), 7.16-7.06 (m, 2H, Ar*H*), 5.35 (t, *J* = 6.6 Hz, 1H, H-2), 5.00 (d, *J* = 6.6 Hz, 1H, H-1), 4.95 (d, *J* = 11.9 Hz, 1H, CH_2N_3), 4.83 (d, *J* = 11.9 Hz, 1H, CH_2N_3), 4.79 (d, *J* = 14.7 Hz, 1H, CH_2Ar), 4.70 (d, *J* = 14.6 Hz, 1H, CH_2Ar), 4.41 (dd, *J* = 11.8, 4.0 Hz, 1H, H-5a), 3.85 (td, *J* = 6.9, 3.9 Hz, 1H, H-4), 3.74 (t, *J* = 6.6 Hz, 1H, H-3), 3.49 (dd, *J* = 11.8, 7.1 Hz, 1H, H-6b), 2.33 (d, *J* = 3.0 Hz, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.0 (C=O), 138.1, 137.6, 134.9, 133.1, 133.0, 132.7, 131.0, 129.8, 129.7, 128.4, 128.2, 128.1, 127.9, 127.7, 126.8, 126.2, 126.0, 125.6 (15 C, Ar), 87.1 (C-1), 80.0 (C-3), 73.9 (CH_2N_3), 71.4 (C-2), 68.7 (C-4), 66.4 (C-5), 52.9 (CH_2Ar), 21.1 (CH_3) ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{31}H_{29}N_3NaO_5S$: 578.1726; found: 578.1724. IR (neat): ν_{max} = 2103, 1724, 1252, 1070 cm^{-1}.

4-Methylphenyl 2-*O*-(azidomethyl)benzoyl-3-*O*-(2-methyl)naphtyl-4-*O*-fluorenylcarboxy-methyl-1-thio-β-D-xylopyranoside (8d)



4-Methylphenyl 2-O-(azidomethyl)benzoyl-3-O-(2-methyl)naphtyl-4-O-fluorenylcarboxyme-thyl-1-thio-β-D-xylopyranoside **7d** (902 mg, 1.62 mmol) was dissolved in DCM (15 mL) and pyridine (15 mL) and FmocCl (504 mg, 1.94 mmol) were added. After stirring the reaction mixture for 2 h water was added and the phases were separated. The organic phase was washed with 10% HCl (50 mL) and brine (50 mL), and the combined organic phases were dried with Na_2SO_4. Column chromatography (SiO_2, EtOAc/hex = 1:8 to 1:5) gave 4-methylphenyl 2-*O*-(azidomethyl)benzoyl-3-*O*-(2-methyl)naphtyl-4-*O*-fluorenylcarboxymethyl-1-thio-β-D-xylopyranoside **8d** (862 mg, 1.11 mmol, 68%) as a colorless solid. $[\alpha]_{25}^{D}$ = -19.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09-8.05 (m, 1H, Ar*H*), 7.76-7.71 (m, 6H, Ar*H*), 7.52-7.34 (m, 6H, Ar*H*), 7.29-7.19 (m, 4H, Ar*H*), 7.11 (m, 3H, Ar Ar*H* 5.37 (t, *J* = 5.6, 1H, H-2), 5.12 (d, *J* = 5.5 Hz, 1H, H-1), 4.91-4.82 (m, 3H, CH_2-Ar, H-4), 4.75 (s, 2H, CH_2-Ar), 4.56 (dd, *J* = 12.4, 3.7 Hz, 1H, H-5a), 4.38 (dd, *J* = 7.1, 2.5 Hz, 2H, Fmoc-CH_2), 4.15 (t, *J* = 7.1 Hz, 1H, Fmoc-C*H*), 3.93 (t, *J* = 5.9 Hz, 1H, H-3), 3.67 (dd, *J* = 12.4, 5.8 Hz, 1H, H-5b), 2.32 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.0 (C=O), 154.3 (C=O), 143.1, 143.0, 141.2, 138.1, 137.8, 134.6, 133.1, 133.0, 132.6, 131.5, 129.7, 129.5, 128.2, 128.0, 127.9, 127.6, 127.1, 126.8, 126.1, 126.0, 125.7, 125.0, 125.0, 120.1 (Ar), 86.3 (C-1), 75.6 (C-4), 73.5 (*C*H_2-Ar), 73.0 (C-4), 70.2 (C-2), 70.0 (Fmoc-*C*H_2), 62.4 (C-5), 52.9 (*C*H_2-Ar), 46.7 (Fmoc-*C*H), 21.1 (CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{46}H_{39}N_3NaO_7S$: 800.2406; found: 800.2347. IR (neat): ν_{max} = 2103, 1748, 1247, 1074 cm^{-1}.

Dibutoxyphosphoryloxy 2-*O*-(azidomethyl)benzoyl-3-*O*-(2-methyl)naphtyl-4-*O*-fluorenylcarboxymethyl-β-D-xylopyranoside (1d)

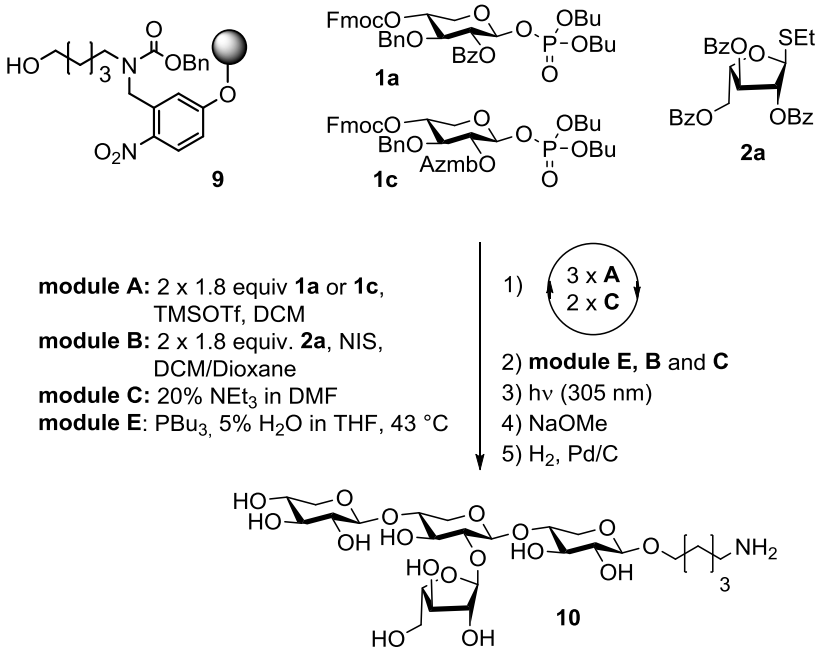


To a suspension of 1 g dried and powdered molecular sieves in DCM (10 mL) was added dibutyl phosphate (0.320 mL, 1.61 mmol) and the mixture was stirred for 30 min. The supernatant of this mixture was added to a solution of 4-methylphenyl 2-*O*-(azidomethyl)benzoyl-3-*O*-(2-methyl)naphtyl-4-*O*-fluorenylcarboxy-methyl-1-thio-β-D-xylopyranoside **8d** (500 mg, 0.640 mmol) in DCM (10 mL) and cooled to 0 °C. 1-

Iodopyrrolidine-2,5-dione (174 mg, 0.770 mmol) and TfOH (20.0 μL, 0.190 mmol) were added and the mixture was stirred for 90 min. The mixture was quenched with aq. sat. $NaHCO_3$ solution (20 mL) and washed with aq. sat. $Na_2S_2O_3$ solution (20 mL). The organic phase was separated and dried with Na_2SO_4. The residue was subjected to column chromatography (SiO_2, EtOAc/hex=1:8) and dibutoxyphosphoryloxy 2-*O*-(azidomethyl)benzoyl-3-*O*-(2-methyl)naphtyl-4-*O*-fluorenylcarboxymethyl-β-D-xylopyranoside **1d** (350 mg, 410 μmol, 63%, α/β ~ 1:13) was obtained as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, β-anomer): δ = 8.05-8.02, 7.77-7.67, 7.66-7.61, 7.57-7.19 (m, 19H, Ar*H*), 5.54 (dd, *J* = 6.9, 4.8 Hz, 1H, H-1), 5.33 (dd, *J* = 6.1, 4.8 Hz, 1H, H-2), 4.95-4.76 (m, 3H, H-4, CH_2Ar), 4.76-4.68 (m, 2H, CH_2N_3), 4.41-4.32 (m, 3H, H-5a, Fmoc-CH_2), 4.16 (t, *J* = 7.1 Hz, 1H, Fmoc-C*H*), 4.08-4.00 (m, 2H, OBu), 3.93 (d, *J* = 12.7 Hz, 1H, H-3), 3.92-3.75 (m, 2H, OBu), 3.71 (dd, *J* = 12.5, 6.0 Hz, 1H, H-5b), 1.65-1.58 (m, 2H, Bu), 1.45-1.30 (m, 4H, Bu), 1.15 (h, *J* = 7.5 Hz, 2H, Bu), 0.89 (t, *J* = 7.4 Hz, 3H, CH_3), 0.74 (t, *J* = 7.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, β-anomer): δ = 164.7 (C=O), 154.2 (C=O), 143.1, 143.0, 141.3, 138.0, 134.6, 133.3, 133.1, 133.0, 131.5, 129.5, 128.2, 128.1, 127.9, 127.9, 127.6, 127.3, 127.2, 127.2, 126.8, 126.1, 126.0, 125.6, 125.0, 125.0, 120.1 (28 C, Ar), 95.9 (C-1), 74.9 (C3-), 73.4 (CH_2-Nap), 72.7 (C-4), 70.1 (Fmoc), 69.5 (C-2), 67.9 (2C, OBu), 61.1 (C-5), 52.9 (CH_2-N_3), 46.6 (Fmoc), 32.0, 31.9, 18.6, 18.4 (4C, Bu), 13.5, 13.4 (2C, CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{47}H_{50}N_3NaO_{11}P$: 886.3081; found: 886.3072. IR (neat): ν_{max} = 2103, 1735, 1270, 1070 cm^{-1}

3.3.2 Automated Glycan Assembly of Arabinoxylan Oligosaccharides

Aminopentyl β-D-xylopyranosyl-(1→4)-2-*O*-[α-L-arabinofuranosyl]-β-D-xylo-pyranosyl-(1→4)-β-D-xylopyranoside (10)



Linker-functionalized resin **9** (52.0 mg, 16.9 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

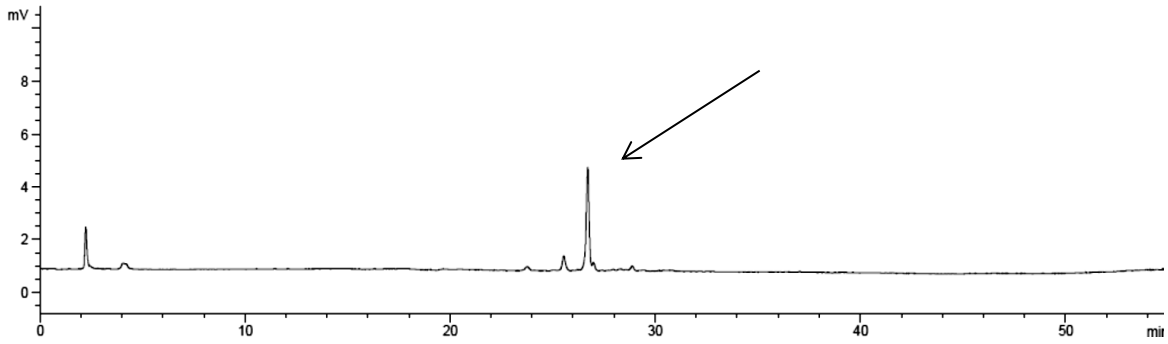
Module **E**: 15 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

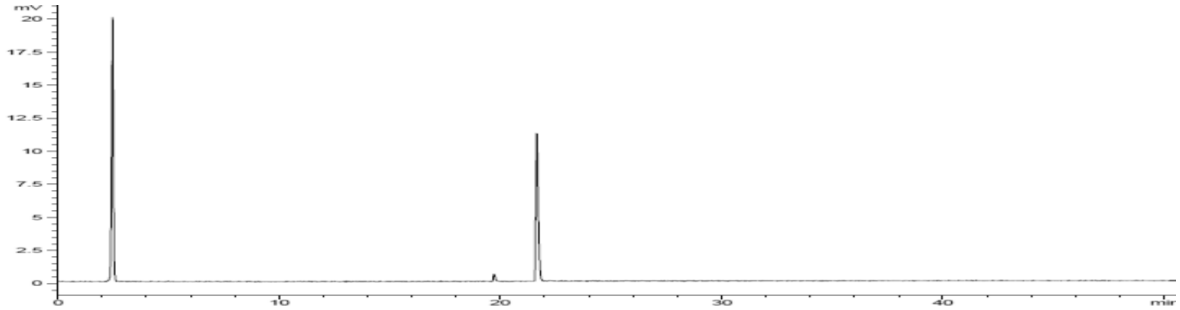
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected tetrasaccharide.

Crude NP-HPLC (ELSD trace):

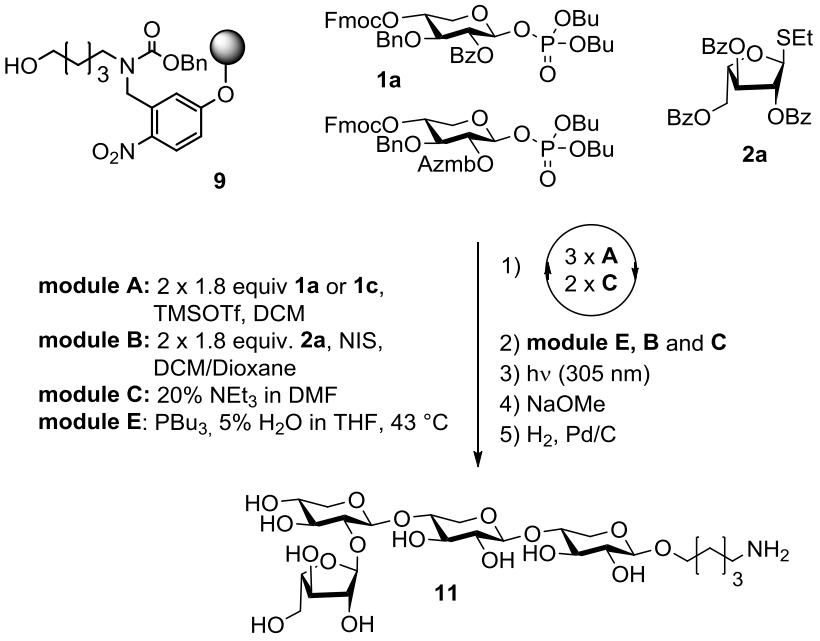


The protected tetrasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected tetrasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected tetrasaccharide **10** (1.4 mg, 2.19 μmol, 13% over 11 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 5.21 (s, 1H), 4.51 (d, *J* = 7.4 Hz, 1H), 4.39 (d, *J* = 7.8 Hz, 1H), 4.34 (d, *J* = 7.9 Hz, 1H), 4.11-4.08 (m, 1H), 4.08-4.01 (m, 3H), 3.92-3.86 (m, 2H), 3.82-3.78 (m, 1H), 3.77-3.73 (m, 2H), 3.72-3.67 (m, 1H), 3.66-3.58 (m, 3H), 3.57-3.53 (m, 1H), 3.49 (t, *J* = 9.2 Hz, 1H), 3.39-3.31 (m, 4H), 3.25-3.17 (m, 3H), 2.93 (t, *J* = 7.5 Hz, 2H), 1.65-1.55 (m, 4H), 1.40-1.34 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 108.5, 102.8, 101.9, 100.1, 84.5, 80.9, 79.0, 76.6, 76.4, 76.0, 75.6, 73.9, 73.7, 72.9, 72.8, 70.1, 69.2, 65.2, 62.9, 62.8, 61.3, 39.3, 28.2, 26.4, 22.1 ppm. ESI-HRMS: m/z = $[M+H]^+$ calcd. for $C_{25}H_{46}NO_{17}^+$: 632.2760, found 632.2804.

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



Aminopentyl 2-*O*-[α-L-arabinofuranosyl]-β-D-xylopyranosyl-(1→4)-β-D-xylo-pyranosyl-(1→4)-β-D-xylopyranoside (11)



Linker-functionalized resin **9** (52.0 mg, 16.9 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

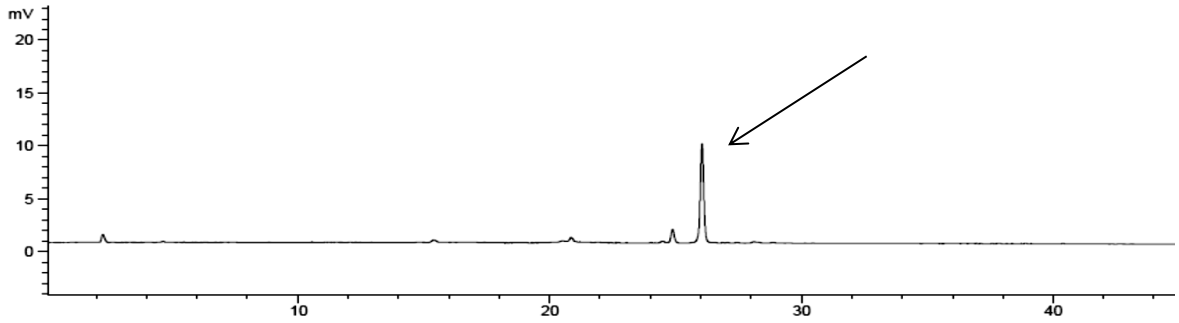
Module **E**: 15 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

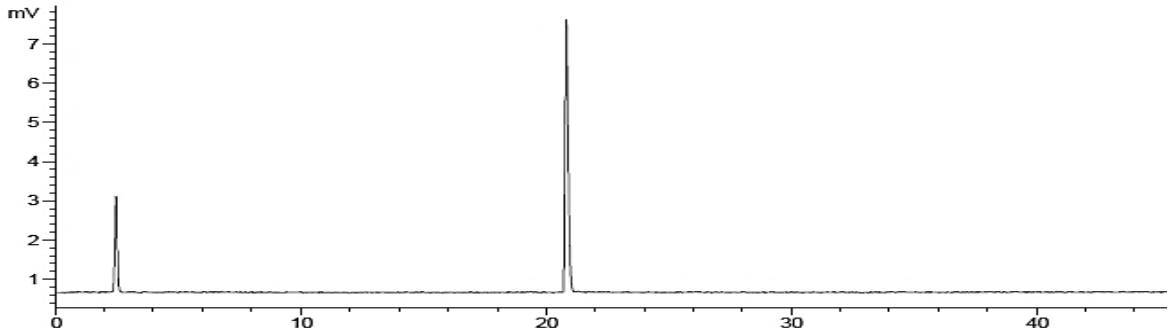
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected tetrasaccharide.

Crude NP-HPLC (ELSD trace):

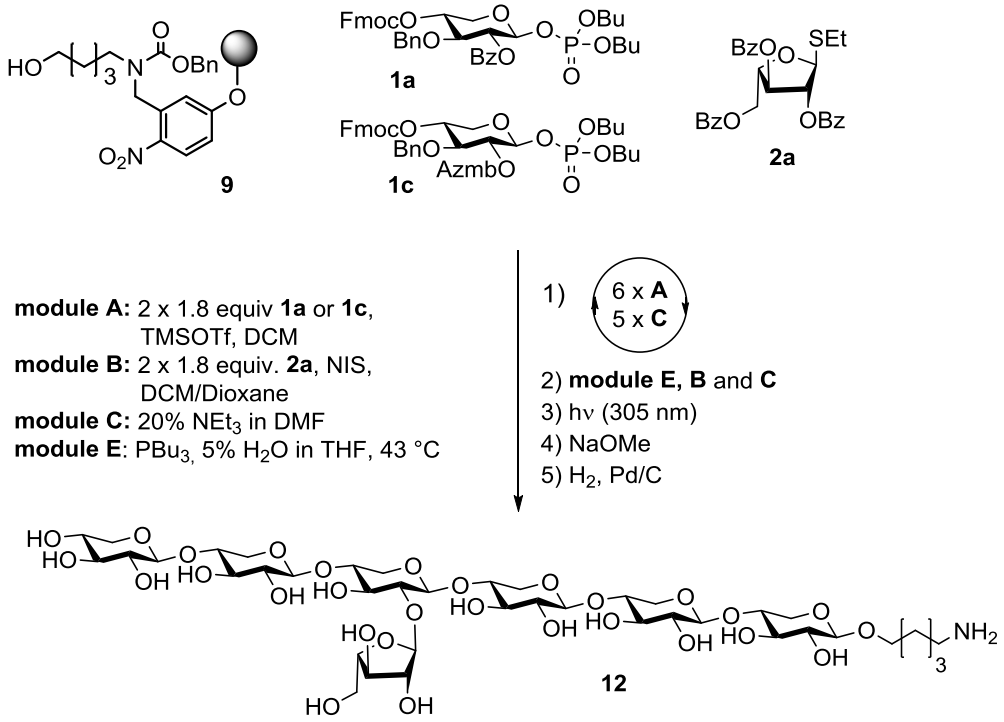


The protected tetrasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected tetrasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected tetrasaccharide **11** (4.4 mg, 6.92 μmol, 41% over 11 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 5.29 (s, 1H), 4.57 (d, *J* = 7.5 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.21-4.04 (m, 4H), 4.02-3.51 (m, 12H), 3.46-3.36 (m, 3H), 3.35-3.25 (m, 3H), 3.02 (s, 2H), 1.76-1.62 (m, 4H), 1.52-1.41 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 111.1, 105.4, 104.3, 102.9, 87.2, 83.6, 80.7, 79.3, 79.0, 78.7, 78.3, 76.5, 76.3, 75.5, 75.3, 72.7, 71.7, 67.6, 65.6, 65.5, 63.9, 42.0, 30.8, 29.0, 24.7 ppm. ESI-HRMS: m/z = $[M+H]^+$ calcd. for $C_{25}H_{46}NO_{17}$: 632.2766; found: 632.2770.

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



Aminopentyl β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-2-*O*-[α-L-arabino-furanosyl]-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (12)



Linker-functionalized resin **9** (104 mg, 33.8 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **E**: 15 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

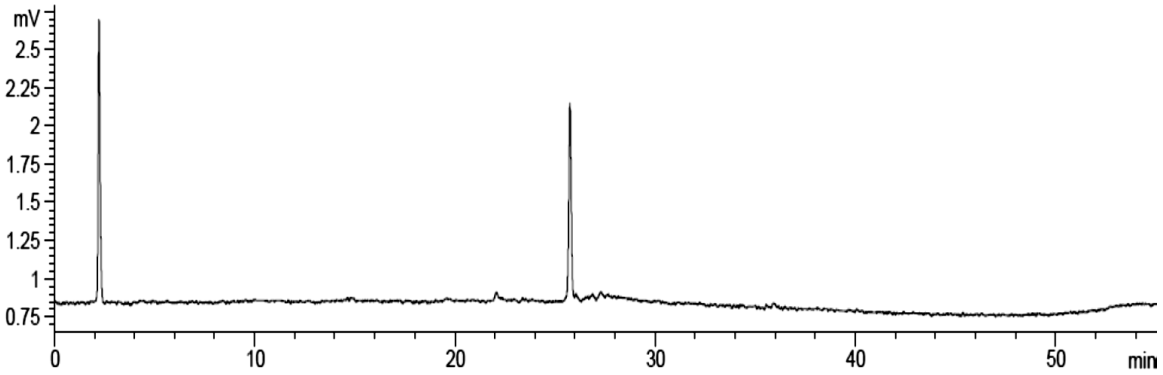
Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

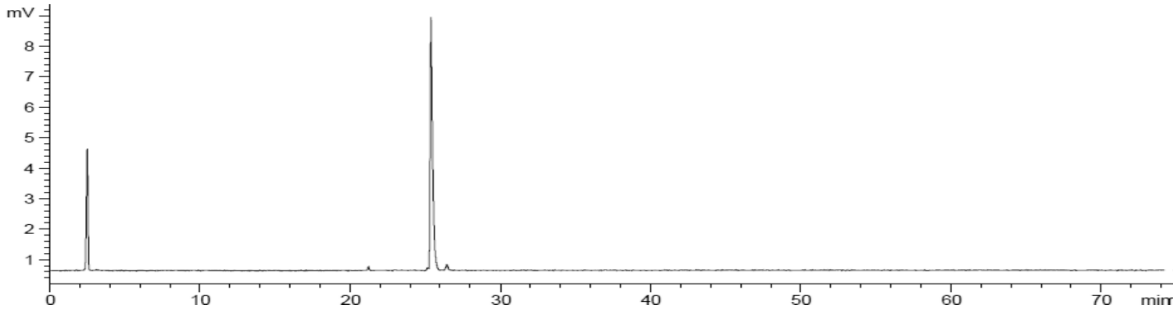
Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a

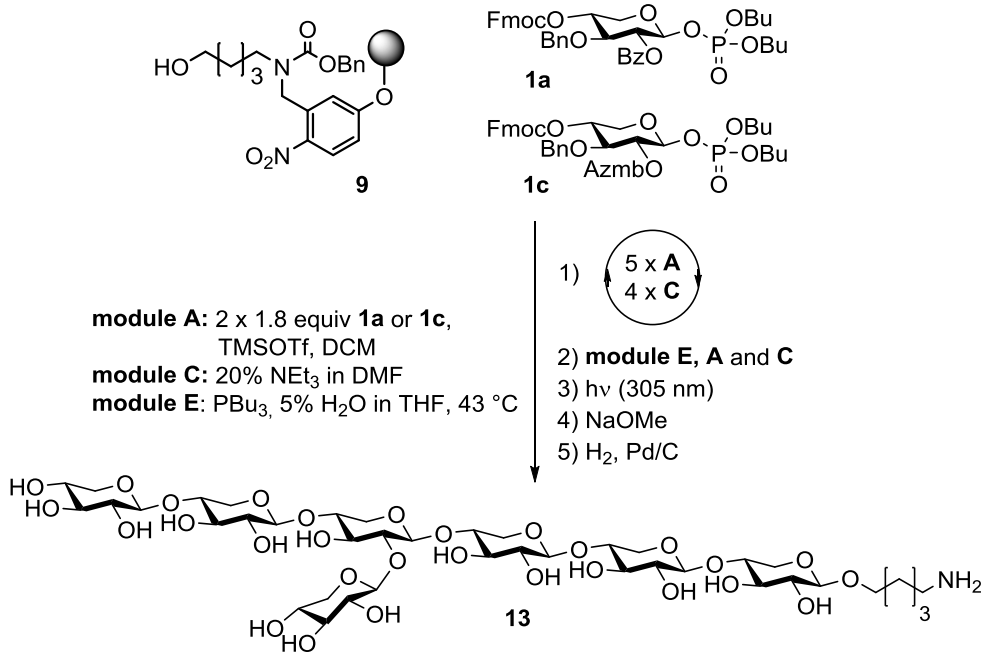
preparative YMC diol column gave the protected heptasaccharide.
Crude NP-HPLC (ELSD trace):



The protected heptasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) 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 heptasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected heptasaccharide **12** (2.6 mg, 2.48 μmol, 7% over 17 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 5.30 (t, *J* = 0.9 Hz, 1H), 4.60 (d, *J* = 7.4 Hz, 1H), 4.52-4.46 (m, 4H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.18 (dd, *J* = 2.9, 1.3 Hz, 1H), 4.17-4.07 (m, 7H), 4.01-3.95 (m, 2H), 3.92-3.61 (m, 13H), 3.60-3.54 (m, 4H), 3.49-3.36 (m, 7H), 3.35-3.25 (m, 6H), 3.01 (t, *J* = 7.6 Hz, 2H), 1.73-1.64 (m, 4H), 1.46 (p, *J* = 7.8 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 111.0, 105.4, 104.4, 104.3, 104.3, 104.2, 102.6, 87.2, 83.6, 80.5, 79.3, 79.0 (2C), 78.9 (2C), 78.6, 78.2, 76.4, 76.3, 76.2 (2C), 75.6, 75.4, 75.3 (2C), 72.8, 71.8, 71.1, 67.8, 65.6, 65.5 (2C), 65.4, 63.9, 42.0, 30.8, 29.1, 25.9, 24.7, 22.6 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{40}H_{70}NO_{29}$: 1028.4034; found 1028.4050.
RP-HPLC of the deprotected heptasaccharide (ELSD trace):



Aminopentyl β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-2-*O*-[β-D-xylopyranosyl]-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (13)



Linker-functionalized resin **9** (104 mg, 33.8 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

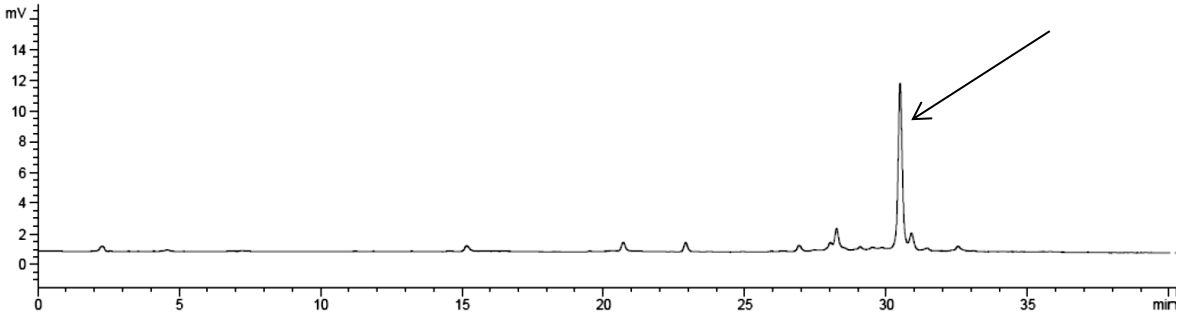
Module **E**: 15 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

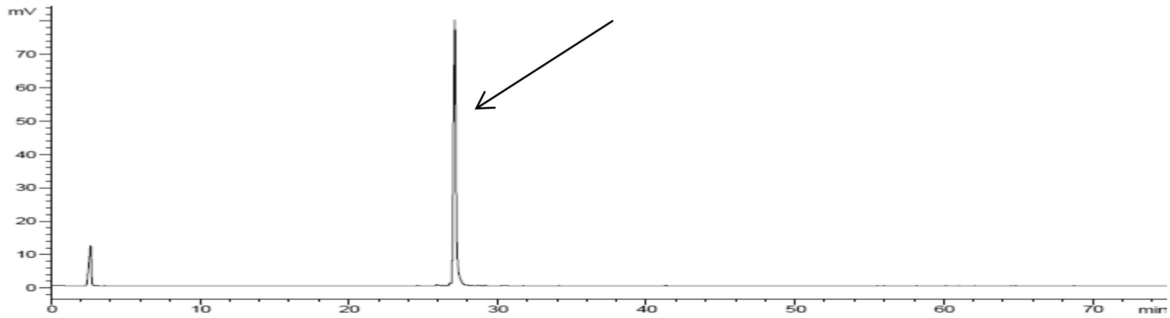
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected heptasaccharide.

Crude NP-HPLC (ELSD trace):

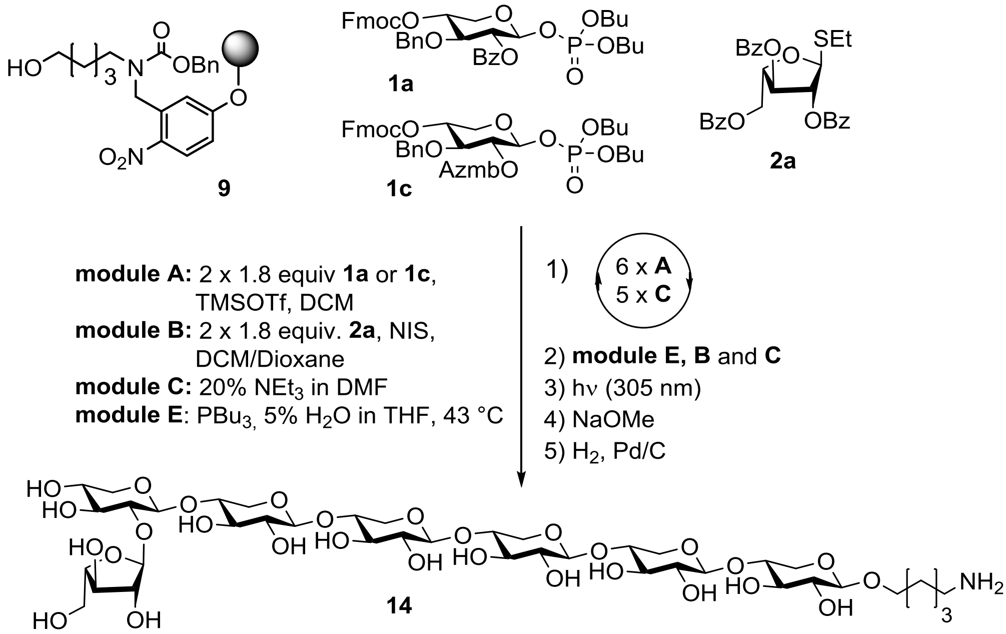


The protected heptasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected heptasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected heptasaccharide **13** (3.8 mg, 3.69 μmol, 11% over 16 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 4.68-4.62 (m, 2H), 4.52-4.46 (m, 4H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.17-4.06 (m, 5H), 4.01-3.95 (m, 2H), 3.92-3.74 (m, 7H), 3.72-3.51 (m, 8H), 3.50-3.24 (m, 15H), 3.05-2.98 (m, 2H), 1.73-1.64 (m, 4H), 1.50-1.43 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 106.3, 105.4, 104.4, 104.3 (2C), 104.2, 102.7, 82.9, 78.9 (3C), 78.8, 78.2, 78.0, 76.4, 76.3, 76.2 (3C), 75.7, 75.6, 75.4, 75.3 (2C), 72.7, 71.8, 67.8, 67.8, 65.6, 65.5 (2C), 65.0, 41.9, 30.8, 29.0, 24.7 ppm. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{40}H_{70}NO_{29}$: 1028.4034; found: 1028.4048.

RP-HPLC of the deprotected heptasaccharide (ELSD trace):



Aminopentyl α-L-arabinofuranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (14)



Linker-functionalized resin **9** (104 mg, 33.8 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

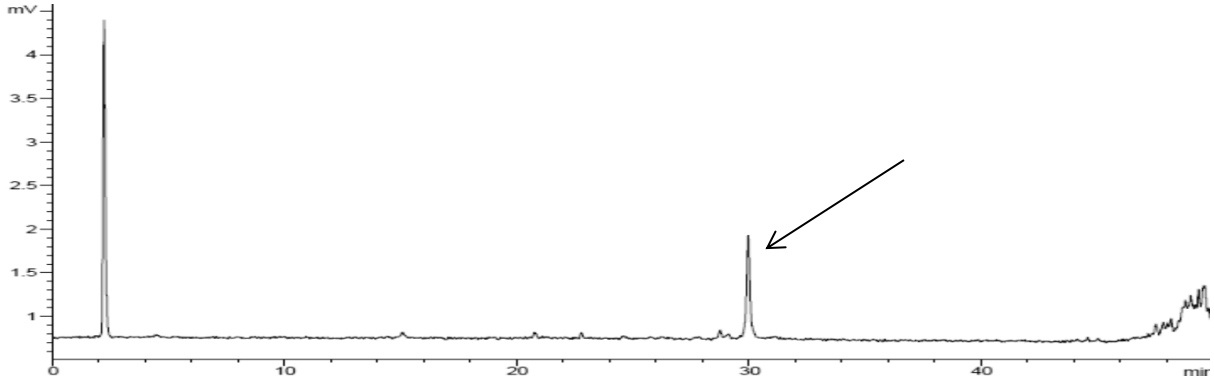
Module **E**: 15 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

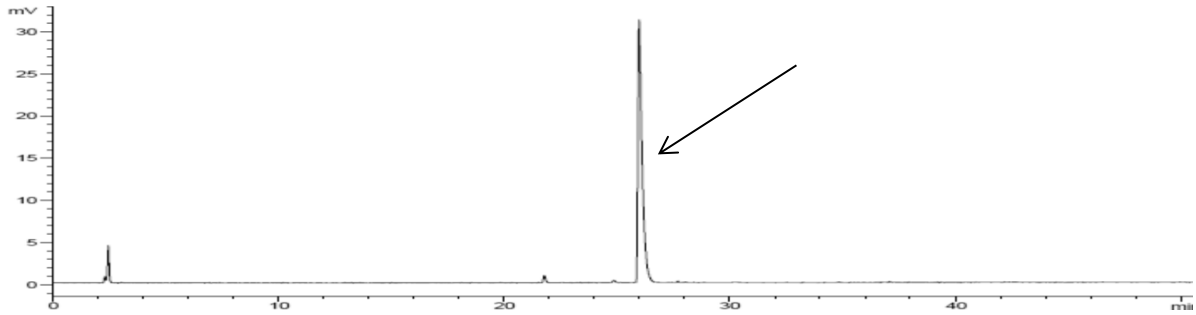
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected heptasaccharide.

Crude NP-HPLC (ELSD trace):

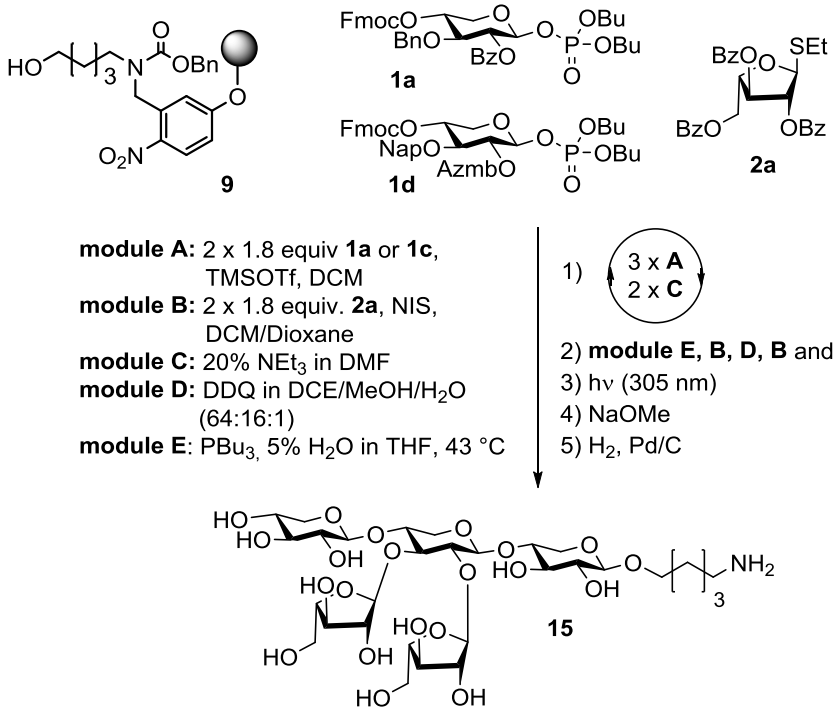


The protected heptasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected heptasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected heptasaccharide **14** (1.65 mg, 1.61 μmol, 5% over 17 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): 5.22 (s, 1H), 4.49 (d, *J* = 7.6 Hz, 1H), 4.42 (d, *J* = 7.7 Hz, 4H), 4.35 (d, *J* = 7.8 Hz, 1H), 4.12 – 3.99 (m, 6H), 3.92 (dd, *J* = 11.7, 5.4 Hz, 1H), 3.88 (dd, *J* = 5.8, 2.9 Hz, 1H), 3.84 – 3.79 (m, 1H), 3.78 – 3.68 (m, 6H), 3.67 – 3.57 (m, 4H), 3.53 – 3.45 (m, 6H), 3.38 – 3.29 (m, 6H), 3.27 – 3.19 (m, 6H), 2.94 (t, *J* = 7.6 Hz, 2H), 1.61 (dp, *J* = 21.2, 7.2, 6.8 Hz, 4H), 1.38 (p, *J* = 7.9 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 108.4, 102.7, 101.6, 101.6, 100.2, 84.5, 80.9, 78.1, 76.6, 76.3, 76.2, 76.0, 75.6, 73.8, 73.7, 73.6, 72.9, 72.6, 70.1, 69.0, 64.9, 63.0, 62.9, 62.8, 61.2, 39.3, 28.1, 26.3 ppm. ESI-HRMS: m/z [M+H]+ calcd. for $C_{40}H_{70}NO_{29}$: 1028.4034; found: 1028.4022.

RP-HPLC of the deprotected heptasaccharide (ELSD trace):



Aminopentyl β-D-xylopyranosyl-(1→4)-2-O-[α-L-arabinofuranosyl]-3-O-[α-L-arabinofuranosyl] β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (15)



Linker-functionalized resin **9** (104 mg, 33.8 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1d**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **E**: 15 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

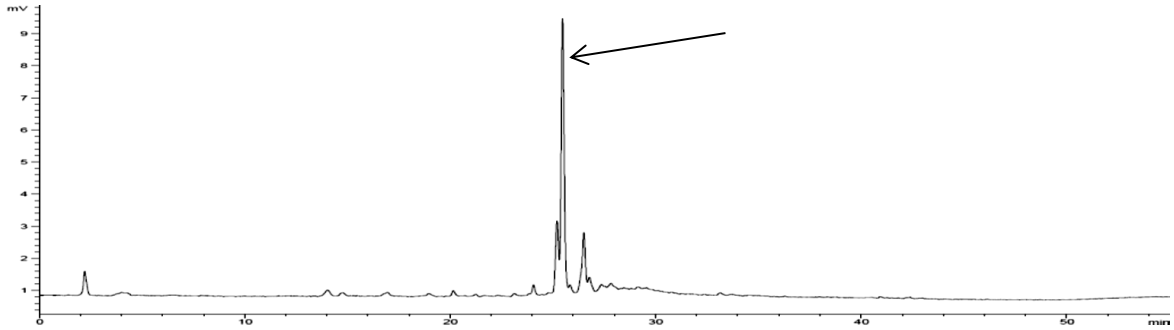
Module **D**: DDQ, DCE/MeOH/H2O (64:16:1), 7 x 30 min, 40 °C

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

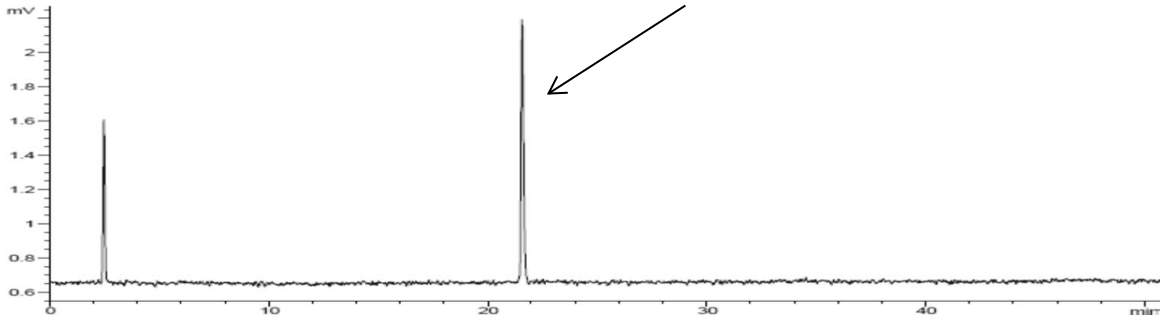
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected pentasaccharide.

Crude NP-HPLC (ELSD trace):

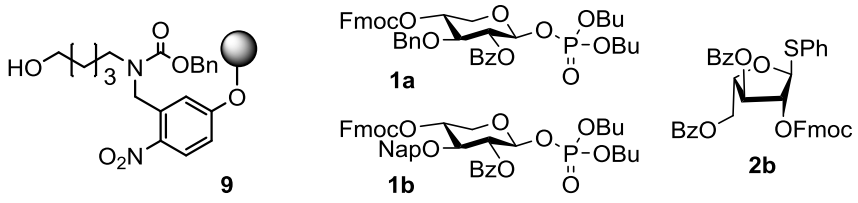


The protected pentasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 1.00 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected pentasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated. After purification by preparative HPLC using a Hypercarb column fully deprotected pentasaccharide **15** (1.1 mg, 1.45 μmol, 4% over 13 steps, based on resin loading) was obtained. ^{1}H NMR (700 MHz, D_2O): δ = 5.14 (s, 1H), 5.09 (s, 1H), 4.50 (d, *J* = 7.2 Hz, 1H), 4.29 (dd, *J* = 17.5, 7.9 Hz, 2H), 4.18 (d, *J* = 4.7 Hz, 1H), 4.06-3.95 (m, 5H), 3.86-3.39 (m, 16H), 3.29 (t, *J* = 9.5 Hz, 3H), 3.13 (q, *J* = 11.0, 9.4 Hz, 3H), 2.84 (q, *J* = 7.1 Hz, 1H), 1.58-1.46 (m, 4H), 1.37-1.26 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): δ = 111.3, 110.7, 105.4, 104.0, 103.9, 102.5, 87.0, 83.8, 83.6, 83.3, 81.2, 80.1, 79.8, 79.2, 78.4, 78.2, 76.5, 76.3, 75.6, 72.7, 71.8, 67.7, 65.5, 65.1, 63.8, 63.7, 41.9, 30.8, 29.0, 24.7 ppm. ESI-HRMS: m/z = $[M+H]^+$ calcd. for $C_{30}H_{54}NO_{21}^+$: 764.3188; found 764.3169.

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

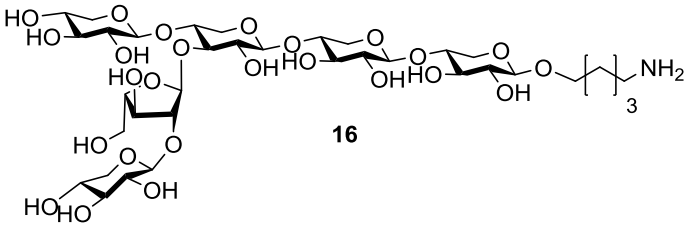


Aminopentyl β-D-xylopyranosyl-(1→4)-3-*O*-[2-*O*-[β-D-xylopyranosyl]-α-L-arabino-furanosyl]-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (16)



module A: 2 x 1.8 equiv **1a** or **1c**, TMSOTf, DCM
module B: 2 x 1.8 equiv. **2b**, NIS, DCM/Dioxane
module C: 20% NEt_3 in DMF
module D: DDQ in DCE/MeOH/H_2O (64:16:1)
module F: Ac_2O, pyridine

1) 4 x **A**, 4 x **C**
2) **module F, D, B, C, A** and **C**
3) hν (305 nm)
4) NaOMe
5) H_2, Pd/C



Linker-functionalized resin **9** (104 mg, 33.8 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1b**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **F**: Ac_2O, pyridine, 30 min

Module **D**: DDQ, DCE/MeOH/H2O (64:16:1), 7 x 30 min, 40 °C

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

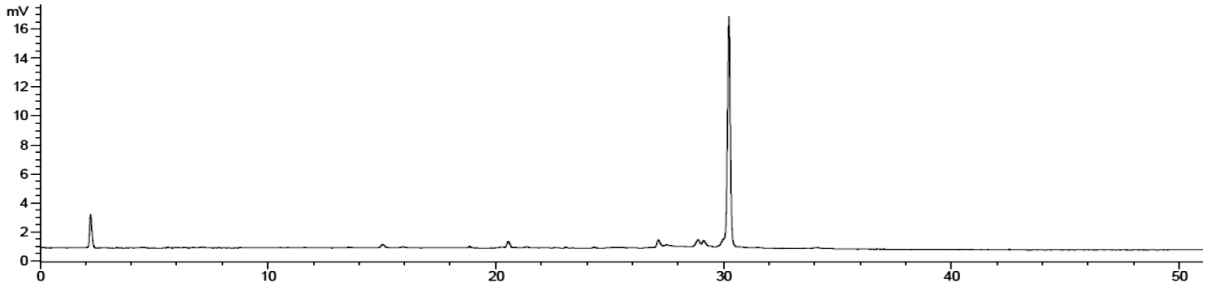
Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

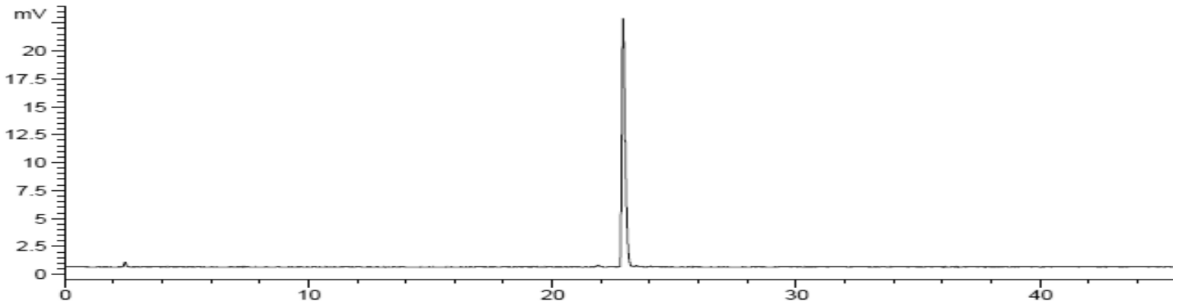
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected hexasaccharide.

Crude NP-HPLC (ELSD trace):

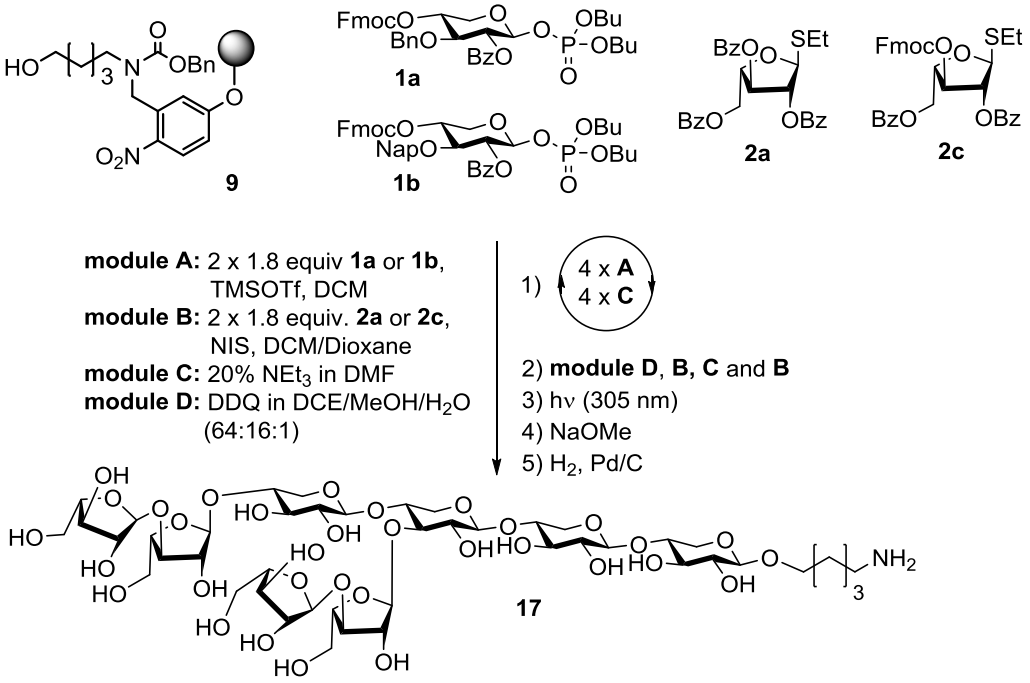


The protected Hexasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 1.00 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected hexasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected hexasaccharide **16** (15.5 mg, 17.3 μmol, 17% over 17 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 5.56 (s, 1H), 4.57 (d, *J* = 7.9 Hz, 1H), 4.52 (d, *J* = 7.7 Hz, 1H), 4.49 (d, *J* = 7.7 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.31 (td, *J* = 5.5, 3.6 Hz, 1H), 4.29 – 4.26 (m, 1H), 4.16-4.05 (m, 4H), 3.98 (dd, *J* = 11.6, 5.5 Hz, 1H), 3.95-3.53 (m, 13H), 3.50-3.21 (m, 12H), 3.02 (t, *J* = 7.6 Hz, 2H), 1.75-1.62 (m, 4H), 1.50-1.40 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 109.0, 105.4, 105.2, 104.3, 104.2, 104.0, 91.3, 86.6, 80.1, 79.0, 78.9, 78.3, 78.2, 78.1, 76.5, 76.2, 76.2, 75.8, 75.6, 75.6, 75.4, 75.3, 72.7, 71.8, 71.7, 67.9, 67.7, 65.6, 65.5, 65.4, 63.5, 41.9, 30.8, 29.0, 24.7 ppm. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{35}H_{62}NO_{25}$: 896.3611; found: 896.3641.

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



Aminopentyl α-L-arabinofuranosyl-(1→3)-α-L-arabinofuranosyl-(1→4)-β-D-xylo-pyranosyl-(1→4)-3-*O*-[3-*O*-[a-L-arabinofuranosyl]-α-L-arabinofuranosyl]-β-D-xylo-pyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (17)



Linker-functionalized resin **9** (104 mg, 33.8 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1b**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **D**: DDQ, DCE/MeOH/H2O (64:16:1), 7 x 30 min, 40 °C

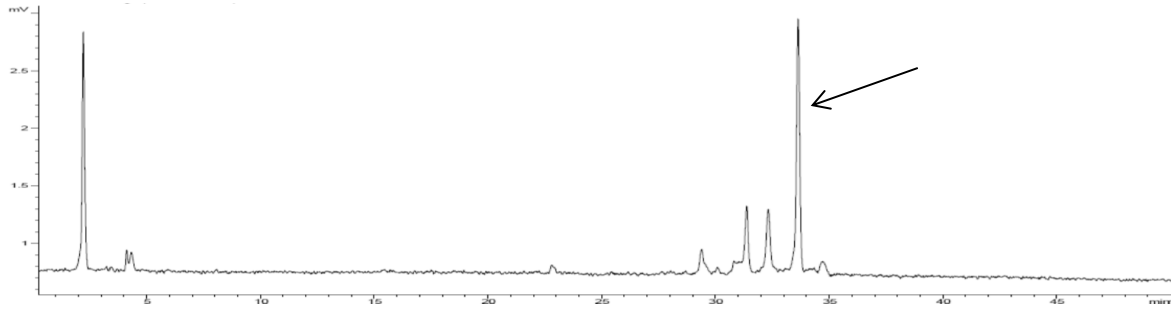
Module **B**: 2 x 1.8 equiv **2c**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **B**: 2 x 1.8 equiv **2a**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

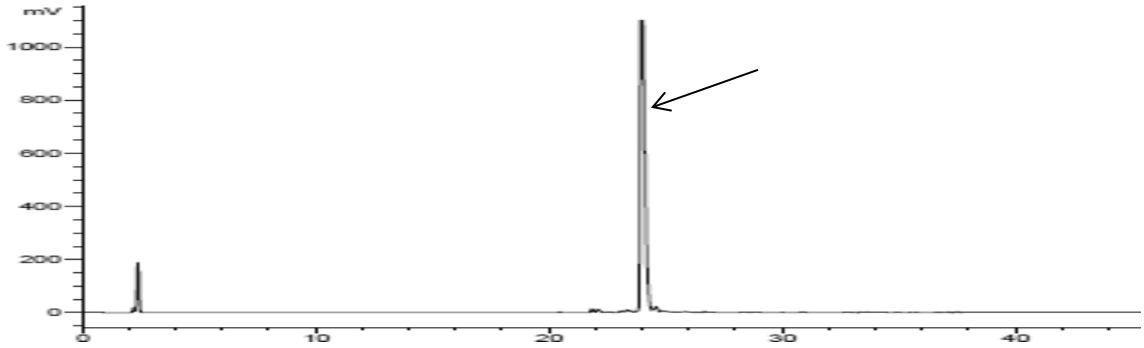
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected octasaccharide.

Crude NP-HPLC (ELSD trace):



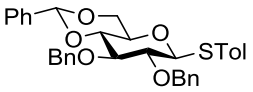
The protected octasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 1.00 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected octasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected octasaccharide **17** (2.4 mg, 2.07 μmol, 8% over 15 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 5.17 (s, 1H), 5.02 (s, 2H), 5.01 (s, 1H), 4.99 (s, 1H), 4.38 (d, *J* = 7.7 Hz, 1H), 4.34 (d, *J* = 7.7 Hz, 1H), 4.31 (d, *J* = 7.7 Hz, 1H), 4.28 (d, *J* = 7.2 Hz, 4H), 4.20 – 4.18 (m, 1H), 4.13 – 4.08 (m, 2H), 4.03 – 3.92 (m, 7H), 3.91 – 3.87 (m, 4H), 3.82 – 3.79 (m, 2H), 3.76 – 3.52 (m, 20H), 3.42 (t, *J* = 9.2 Hz, 2H), 3.38 (t, *J* = 9.2 Hz, 1H), 3.32 – 3.21 (m, 5H), 3.20 – 3.10 (m, 5H), 2.87 (t, *J* = 7.6 Hz, 2H), 1.58 – 1.49 (m, 4H), 1.31 (p, *J* = 7.8 Hz, 2H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 110.8, 109.8, 109.6, 108.3, 105.4, 104.2, 104.2, 103.7, 86.7, 86.3, 85.6, 85.2, 84.9, 84.7, 84.0, 83.7, 82.7, 82.2, 81.2, 79.1, 78.9, 76.6, 76.4, 76.3, 76.2, 75.8, 75.5, 75.3, 72.7, 65.5, 65.5, 65.2, 63.8, 63.7, 63.5, 63.1, 41.9, 30.8, 29.0, 24.7 ppm. ESI-HRMS: m/z [M+H]+ calcd. for $C_{45}H_{78}NO_{33}^+$: 1160.4462; found: 1160.4506.

RP-HPLC (ELSD trace) of the deprotected octasaccharide:



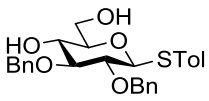
3.3.3 Synthesis of Glucose Building Blocks 18a and 18b

4-Methylphenyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-1-thio-β-D-glucopyranoside (20)



4-Methylphenyl 4,6-di-*O*-benzylidene-1-thio-β-D-glucopyranoside (6.03 g, 16.1 mmol) was dissolved in DMF (25 mL) and cooled to 0 °C. Sodium hydride (1.55 g, 64.4 mmol, 60% in mineral oil) was added slowly. After the mixture was stirred for 10 min, benzyl bromide (11.0 g, 64.4 mmoL) was added dropwise. The reaction mixture was stirred for 2 h at 0 °C until the starting material was fully consumed. Then, aq. sat. NH_4Cl solution was added until pH 7 was reached. The aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with aq. sat. NH_4Cl solution (40 mL), H_2O (40 mL) and brine (40 mL). The organic phase was dried with Na_2SO_4 and the residue was submitted to column chromatography (SiO_2, EtOAc/hex = 1:4) to afford 4-methylphenyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-1-thio-β-D-glucopyranoside **20** (6.65 g, 12.0 mmol, 74%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50-7.23 (m, 17 H, Ar), 7.13-7.09 (m, 2H, Ar), 5.58 (s, 1H, PhC*H*) 4.76-4.95 (m, 4H, CH_2Ar), 4.69 (d, *J* = 9.7, 1H, H-1), 4.38 (dd, *J* = 4.9, 10.4 Hz, 1H, H-6a), 3.86-3.76 (m, 2H, H-3, H-6b), 3.68 (t, *J* = 9.1 Hz, 1H, H-4), 3.53-3.39 (m, 2H, H-2, H-5), 2.34 (s, 3H, CH_3) ppm. The analytical data is in agreement with literature data.[243]

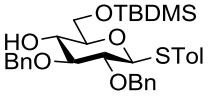
4-Methylphenyl 2,3-di-*O*-benzyl-1-thio-β-D-glucopyranoside (21)



To a solution of 4-methylphenyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-1-thio-β-D-glucopyranoside **20** (6.65 g, 12.0 mmol) in DCM (25 mL) was added an aqueous solution of TFA (80% in water (v/v), 5 mL). The solution was stirred at r.t. for 3 h, quenched with

aq. sat. $NaHCO_3$ solution (50 mL) and extracted with DCM (2 x 50 mL). The organic layer was washed with brine (25 ml) and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure to afford 4-methylphenyl 2,3-di-*O*-benzyl-1-thio-β-D-glucopyranoside **21** (5.58 g, 12.0 mmol, quant.) as a colorless solid. $[\alpha]_{25}^{D}$ = +2.4 (*c* 0.7, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44-7.29 (m, 12H, Ar*H*), 7.15-7.09 (m, 2H, Ar*H*), 4.97 (dd, *J* = 10.9, 3.6 Hz, 2H, CH_2Ar), 4.75 (d, *J* = 10.2 Hz, 1H, CH_2Ar), 4.70 (d, *J* = 11.5 Hz, 1H, CH_2Ar), 4.66 (d, *J* = 9.2 Hz, 1H, H-1), 3.87 (dd, *J* = 11.8, 3.5 Hz, 1H, H-6a), 3.74 (dd, *J* = 11.8, 5.4 Hz, 1H, H-6b), 3.54 (t, *J* = 8.8 Hz, 1H, H-3), 3.49 (t, *J* = 8.3 Hz, 1H, H-4), 3.44 (t, *J* = 8.5 Hz, 1H, H-2), 3.43-3.30 (m, 1H, H-5), 2.32 (s, 3H, CH_3), 2.00 (br, 2H, O*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.2, 138.0, 137.8, 132.5, 129.8, 129.4, 128.7, 128.5, 128.3, 128.1, 128.0, 127.9 (18C, Ar-C), 88.0 (C-1), 86.0 (C-4), 80.8 (C-2), 79,0 (C-5), 75.4 (*C*H_2Ar), 70.4 (C-3), 62.7 (C-6), 21.1 (*C*H_3) ppm. ESI-HRMS: m/z [M+Na]+ calcd. for $C_{27}H_{30}O_5SNa$: 489.1712; found: 489.1711. IR (neat): ν_{max} = 3408, 2871, 1454, 1355, 1029, 697 cm^{-1}.

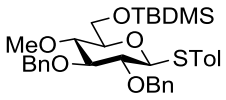
4-Methylphenyl 2,3-di-*O*-benzyl-6-*O*-tertbutyldimethylsilyl-1-thio-β-D-glucopyranoside (22)



A solution of 4-methylphenyl 2,3-di-*O*-benzyl-1-thio-β-D-glucopyranoside **21** (5.58 g, 12.0 mmol) and TBDMSCl (1.80 g, 12.0 mmol) in DMF (20 mL) was cooled to 0 °C and imidazole (1.58 g, 23.2 mmol) was added. The reaction mixture was stirred at r.t. for 1 h. The reaction was quenched by the addition of water (25 mL) and the mixture was extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with 1 M HCl (25 mL), water (25 mL) and brine (25 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated to give 4-methylphenyl 2,3-di-*O*-benzyl-6-*O*-tertbutyldimethylsilyl-1-thio-β-D-glucopyranoside **22** (6.64 g, 11.4 mmol, 96%) as a colorless solid. $[\alpha]_{25}^{D}$ = +1.6 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41-7.09 (m, 14H, Ar), 4.94 (dd, *J* = 10.8, 3.3 Hz, 2H, CH_2Ar), 4.72 (t, *J* = 10.9 Hz, 2H, CH_2Ar), 4.64 (d, *J* = 9.4, 1H, H-1), 3.85 (dd, *J* = 11.3, 3.4 Hz, 1H, H-6a), 3.73 (dd, *J* = 11.9, 5.3 Hz, 1H, H-6b), 3.56 (t, *J*= 9.1 Hz, 1H, H-3), 3.50 (t, *J*= 8.4 Hz, 1H, H-3), 3.46 (t, *J* = 9.3 Hz, 1H, H-4), 3.34 (dt, *J* = 9.8, 5.0 Hz, 1H, H-5), 2.32 (s, 3H, CH_3), 0.90 (s, 9H, Si(CH_3)$_3$), 0.08 (s, *J* = 2.3, 6H, SiCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.3, 138.0, 137.9, 132.5, 129.8, 129.5, 128.7, 128.5, 128.3, 128.1, 128.0, 127.9 (18 C, Ar), 88.0 (C-1), 86.0 (C-3), 80.8 (C-4), 79,0 (C-5), 75.4 (C-2), 75.4 (C-6), 70.4 (*C*H_2Ar), 62.7 (*C*H_2Ar), 25.6 (Si(*C*H_3)$_3$), 21.1 (*C*H_3), -3.6 (Si*C*H_3) ppm. ESI-HRMS: m/z [M+Na]+ calcd. for $C_{33}H_{44}O_5SNa$: 603.2576;

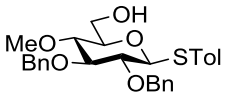
found: 603.2526. IR (neat): ν_{max} = 3401, 2922, 1494, 1455, 1058, 698 cm^{-1}.

4-Methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (23)



To a solution of 4-methylphenyl 2,3-di-*O*-benzyl-6-*O*-tertbutyldimethylsilyl-1-thio-β-D-glucopyranoside **22** (6.64 g, 11.4 mmol) in DMF (25 mL) was added sodium hydride (550 mg, 22.8 mmol, 60% in mineral oil). The mixture was stirred for 10 min at 0 °C and CH_3I (1.40 mL, 22.9 mmol) was added dropwise. After stirring the reaction mixture for 2 h at 0 °C aq. sat. $NaHCO_3$ solution (50 mL) was added. The mixture was extracted with EtOAc (2 x 50 mL). The organic layer was washed with brine (50 mL) and dried over Na_2SO_4. The solution was filtered, and the solvent was evaporated. The crude product was submitted to column chromatography (SiO_2, hex/EtOAc = 4:1) to afford 4-methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside **23** (6.33 g, 10.6 mmol, 93%) as a colorless solid. $[\alpha]_{25}^{D}$ = +27.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz. $CDCl_3$): δ = 7.51-7.47 (m, 2H, Ar), 7.30-7.43 (m, 10H, Ar), 7.10-7.07 (m, 2H, Ar), 4.92-4.74 (m, 3H, CH_2Ar), 4.69 (d, *J* = 10.3 Hz, 1H, CH_2Ar), 4.55 (d, *J* = 9.7, 1H, H-1), 3.93-3.80 (m, 2H, H-6), 3.58 (m, 4H, H-2, O-CH_3), 3.41 (d, *J* = 9.2 Hz, 1H, H-3), 3.35 (d, *J* = 9.4 Hz, 1H, H-4), 3.23-3.17 (m, 1H, H-5), 2.33 (s, 3H, CH_3), 0.94 (s, 9H, C(CH_3)$_3$), 0.14 (s, 3H, Si-CH_3), 0.10 (s, 3H, Si-CH_3) ppm. ^{13}C NMR (100 MHz. $CDCl_3$): δ = 138.4, 138.3, 137.6, 132.7, 129.7, 129.6, 128.4, 128.4, 128.1, 128.1, 127.8, 127.8 (18C, Ar), 87.4 (H-1), 86.8 (H-2), 80.3 (H-3), 80.0 (H-5), 79.0 (H-4), 75.9 (*C*H_2Ar), 75.3 (*C*H_2Ar), 62.0 (C-6), 60.6 (O-*C*H_3), 25.9 (C(*C*H_3)$_3$), 21.1 (*C*H_3), 18.3 (*C*(*C*H_3)$_3$), -5.1 (Si-*C*H_3i), -5.4 (Si-*C*H_3) ppm. ESI-HRMS: *m/z* [M+Na]+ calcd. for $C_{34}H_{46}O_5SSiNa$: 617.2733; found: 617.2704. IR (neat): ν_{max} = 2928, 1494, 1455, 1253, 1151 cm^{-1}.

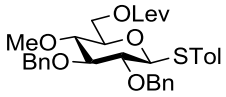
4-Methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-1-thio-β-D-glucopyranoside (24)



To a solution of 4-methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-tert-butyldimethylsilyl-1-thio-β-D-glucopyranoside **23** (6.33 g, 10.6 mmol) in THF (20 mL) was added TBAF (4.48 g, 17.2 mmol) and the mixture was stirred at r.t. for 3 h. Then the reaction was quenched by the addition of brine (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL) and dried over Na_2SO_4. After filtration the mixture was concentrated, and the resulting residue purified by column

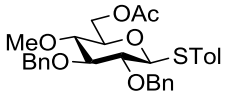
chromatography (SiO_2, hex/EtOAc = 4:1) to give 4-methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-1-thio-β-D-glucopyranoside **24** (4.85 g, 10.1 mmol, 95%) as a colorless solid. $[\boldsymbol{\alpha}]_{\mathbf{25}}^{\boldsymbol{D}}$ = +18.8 (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45-7.28 (12H, Ar), 7.13-7.10 (m, 2H, Ar), 4.91-4.81 (m, 3H, C*H*$_2$Ar), 4.75 (d, *J* = 10.2 Hz, 1H, C*H*$_2$Ar), 4.60 (d, *J* = 9.8 Hz, 1H, H-1), 3.87 (dd, *J* = 12.6, 3.9 Hz 1H, H-6a), 3.72 (dd, *J* = 3.7, 11.7 Hz, 1H, H-6b), 3.59 (t, *J* = 8.6 Hz, 1H, H-3), 3.53 (s, 3H, O-C*H*$_3$), 3.39 (t, *J* = 9.1, 1H, H-2), 3.30-3.22 (m, 2H, H-4, H-5), 2.32 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.3, 138.0, 137.9, 132.7, 129.8, 129.3, 128.4, 128.4, 128.2, 127.9, 127.9, 127.7 (18C, Ar), 87.7 (C-1), 86.4 (C-3), 80.8 (C-2), 79.7 (C-5), 79.2 (C-4), 75.7 (*C*H$_2$Ar), 75.5 (*C*H$_2$Ar), 62.1 (C-6), 60.8 (O-*C*H$_3$), 21.1 (*C*H$_3$) ppm. ESI-HRMS: *m/z* [M+Na]+ calcd. for $C_{28}H_{32}O_5SNa$: 503.1868; found: 503.2269. IR (neat): ν_{max} = 3330, 2907, 1496, 1132 cm^{-1}.

4-Methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-O-levulinoyl-1-thio-β-D-xylopyranoside (18a)



To a solution of compound **24** (595 mg, 1.24 mmol) in DCM (2.00 mL) was added levulinic acid (432 mg, 372 mmol) at 0 °C. DCC (511 mg, 2.48 mmol) and DMAP (160 mg, 1.24 mmol) were added. The resulting mixture was stirred at r.t. overnight. The reaction mixture was filtered through a plug of silica in EtOAc and the solvents were evaporated. Column chromatography (SiO_2, hex/EtOAc = 6:1) afforded 4-methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-O-levulinoyl-1-thio-β-D-xylopyranoside **18a** as a colorless solid (356 mg, 0.611 mmol, 50%). $[\boldsymbol{\alpha}]_{\mathbf{25}}^{\boldsymbol{D}}$ = +2.44 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.44 (d, *J*= 7.4 Hz, 2H, ArH), 7.29-7.42 (m, 10H, ArH), 7.10 (d, *J*= 7.4 Hz, 2H, ArH), 4.72 - 4,81 (m, 4H, C*H*$_2$Ar), 4.57 (d, *J* = 4,6, 1H, H-1), 4.38 (dd, *J* = 2.0 and 11.8 Hz, 1H, H-6), 3.60 (t, *J*= 3.6 Hz 1H, H-3), 3.53 (s, 3H, OC*H*$_3$), 3.44 (q, *J*= 3,4 Hz, 1H, H-5), 3.42 (t, *J* = 3.4 Hz, 1H, H-4), 2.77 (m, *2*H, *H*$_2$C-C=O), 2.64 (t, *J* = 2.64 Hz, 1H, CH_2-C(O)=O), 2.34 (s, 3H, C*H*$_3$) and 2.20 (s, 3H, *H*$_3$C-C=O) ppm. ^{13}C NMR (100 MHz): 206.4 (C=O), 172.5 (CO=O), 138.2, 136.0, 132.7, 129.6, 128.4, 128.4, 128.2, 127.9, 127.9, 127.8 (18C, Ar), 87.7 (C-1), 86.4 (C-3), 80.5 (C-2), 79.8 (C-4), 76.9 (C-5), 75.8 (*C*H$_2$Ar), 75.4 (*C*H$_2$Ar), 63.5 (C-6), 60.9 (O*C*H$_3$), 37,9 (H$_2$*C*-C=O), 29.9 (H$_3$*C*-C=O), 27.9 (H$_2$*C*-C(O)=O) and 21.1 (*C*H$_3$) ppm. ESI-HRMS: m/z [M+Na]+ calcd. for $C_{33}H_{38}O_7SNa^+$: 601.2236; found 601.2216. IR (neat) νmax = 1738, 1719, 1495, 1455, 1152, 1068 cm^{-1}.

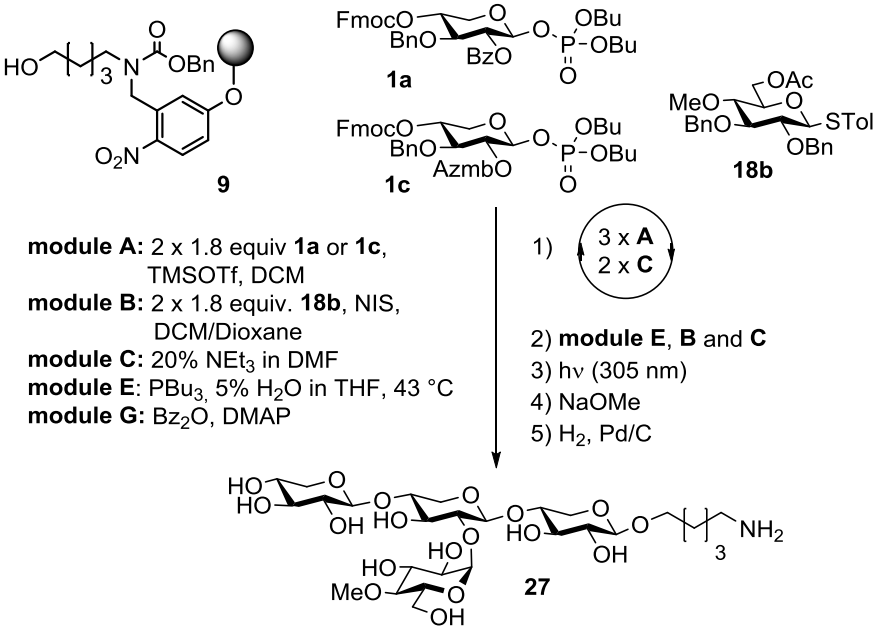
4-Methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-O-acetyl-1-thio-β-D-xylopyranoside (18b)



4-Methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-1-thio-β-D-xylopyranoside **24** (1.00 g, 2.08 mmol) was dissolved in pyridine (10 mL). DMAP (30.0 mg, 210 μmol) and acetic anhydride (400 μl, 4.16 mmol) were added and the reaction mixtures was stirred at r.t. for 2 h. The reaction mixture was diluted with EtOAc (10 mL) and washed with 1 M HCl (10 mL) and brine (10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (SiO_2, EtOAc/hex = 1:15 to 1:10) gave 4-methylphenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-acetyl-1-thio-β-D-xylopyranoside **18b** (898 mg, 1.71 mmol, 83%) as a colorless solid. $[\alpha]_{25}^{D}$ = +13.6 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42-7.32 (m, 4H, Ar), 7.31-7.17 (m, 8H, Ar), 7.03 (m, 2H, Ar), 4.87-4.68 (m, 3H, C*H*$_2$Ar), 4.66 (d, *J* = 10.3 Hz, 1H, C*H*$_2$Ar), 4.50 (d, *J* = 9.4 Hz, 1H, H-1), 4.32 (d, *J* = 11.6 Hz, 1H, H-6a), 4.16 (dd, *J* = 11.6, 5.8 Hz, 1H, H-6b), 3.53 (t, *J* = 8.9 Hz, 1H), 3.45 (s, 3H, O=CC*H*$_3$), 3.40-3.32 (m, 2H, H-2, H-6), 3.14 (t, *J* = 9.4 Hz, 1H), 2.26 (s, 3H, C*H*$_3$), 2.04 (s, 3H, OCH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.9 (C=O), 138.3, 138.1, 138.0, 132.8, 129.7, 129.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9 (12 Ar), 87.8 (C-1), 86.6 (C-3), 80.7 (C-2), 80.0 (C-4), 77.0 (C-5), 75.9 (*C*H_2Ar), 75.6 (*C*H_2Ar), 63.5 (C-6), 61.0 (O=C*C*H_3), 21.2 (*C*H_3), 21.0 (O-*C*H_3) ppm. ESI-HRMS: *m/z* [M+Na]+ calcd. for $C_{30}H_{34}NaO_6S$: 545.1974; found: 545.1988. IR (neat): ν_{max} = 1744, 1236, 1100, 698 cm^{-1}.

3.3.4 Automated Glycan Assembly of 4-*O*-Methyl Glucuronoxylan Oligosaccharides

Aminopentyl β-D-xylopyranosyl-(1→4)-2-*O*-[4-*O*-methyl-α-D-glucopyranosyl]- β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (27)



Linker-functionalized resin **9** (104 mg, 66.7 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

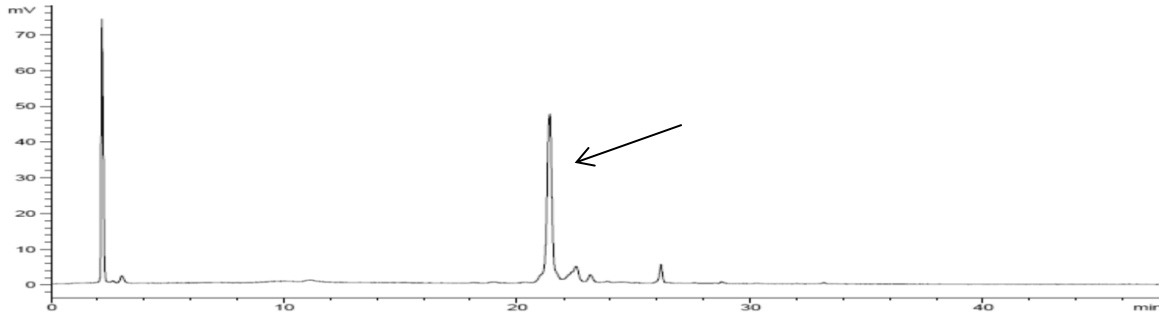
Module **E**: 40 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **18b**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

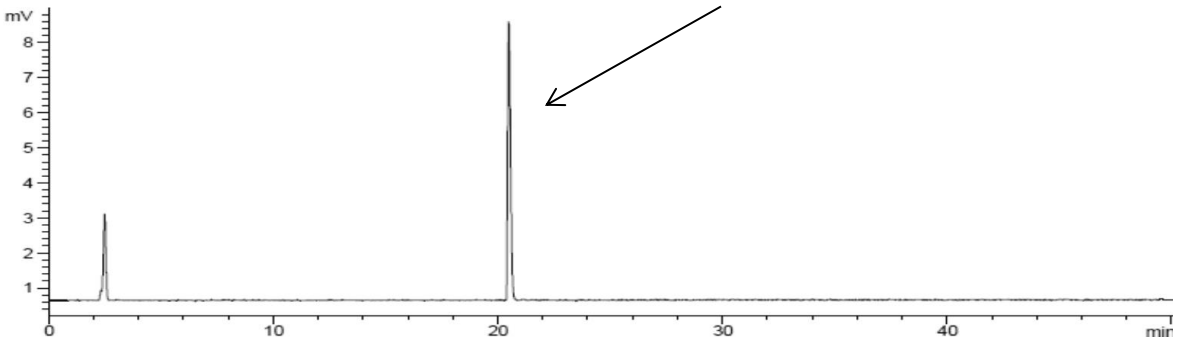
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected tetrasaccharide (26.0 mg, 16.7 μmol, 25%) as a single stereoisomer.

Crude NP-HPLC (ELSD trace):

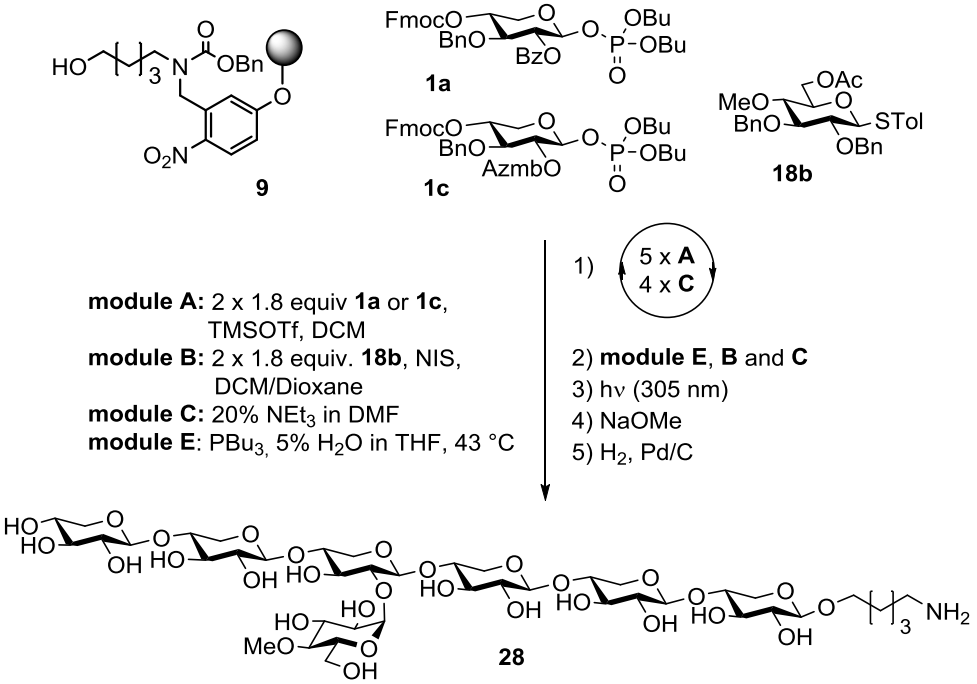


The protected tetrasaccharide (26.0 mg, 16.7 µmol) was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.600 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by the addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the 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. 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, 10.0 mg). The suspension was saturated with H_2 for 30 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 **27** (1.0 mg, 1.51 µmol, 9% over 9 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 5.13 (d, *J* = 3.8 Hz, 1H), 4.51 (d, *J* = 7.3 Hz, 1H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.28 (dd, *J* = 7.9, 1.5 Hz, 1H), 4.02 – 3.92 (m, 3H), 3.84 (dd, *J* = 11.7, 5.4 Hz, 1H), 3.78 – 3.72 (m, 1H), 3.71 – 3.61 (m, 5H), 3.58 – 3.41 (m, 7H), 3.39 (dd, *J* = 9.9, 3.8 Hz, 1H), 3.34 – 3.22 (m, 4H), 3.20 – 3.08 (m, 4H), 3.03 (t, *J* = 5.8 Hz, 1H), 2.90 – 2.85 (m, 1H), 1.59 – 1.50 (m, 4H), 1.32 (p, *J* = 7.7 Hz, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): δ = 105.4, 104.6, 100.3, 81.6, 79.4, 79.1, 78.7, 78.2, 76.5, 75.6, 75.4, 75.2, 75.1, 74.0, 73.2, 72.8, 71.8, 67.8, 65.4, 62.6, 62.5, 47.2, 42.0, 30.8, 29.0, 24.8, 24.1 ppm. ESI-HRMS: m/z [M+H]+ calcd. for $C_{27}H_{50}NO_{18}^+$: 676.3028; found: 676.2969.

RP-HPLC (ELSD trace):



Aminopentyl β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-2-*O*-[4-*O*-methyl-α-D-glucopyranosyl]-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4) (28)



Linker-functionalized resin **9** (104 mg, 33.8 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

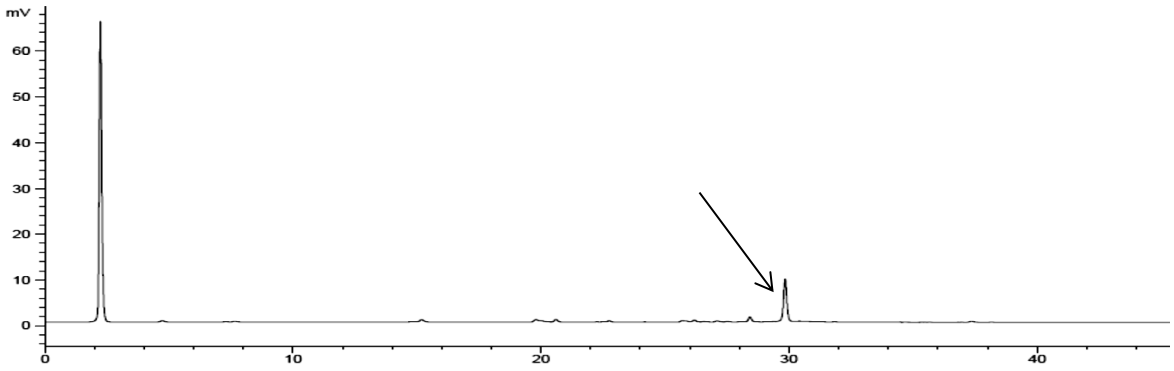
Module **E**: 40 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **18b**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

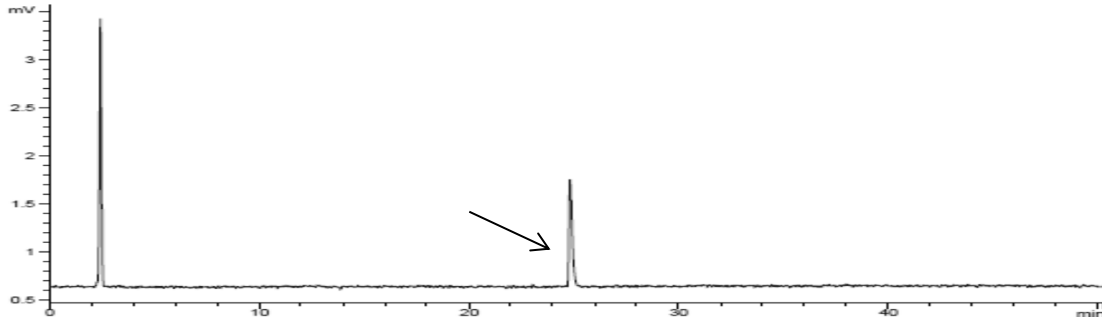
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photo-reactor afforded the crude product. Purification by normal phase HPLC using a pre-parative YMC diol column gave the protected heptasaccharide (12.6 mg, 5.06 μmol, 15%) as a single stereoisomer.

Crude NP-HPLC (ELSD trace):

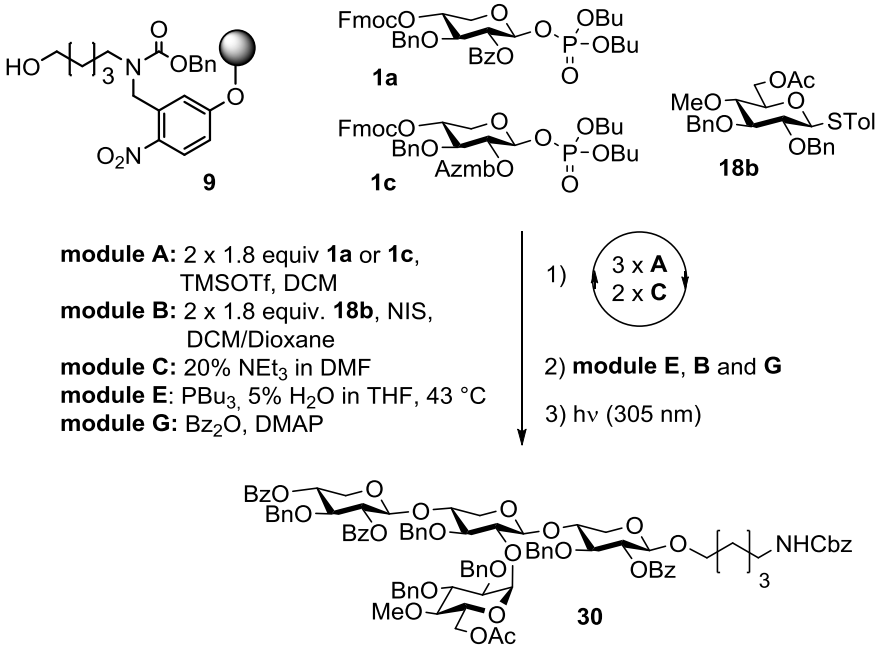


The protected heptasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 1.00 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected heptasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 min and stirred under a H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter (RC 0.45 μm) the solvents were evaporated to provide the fully deprotected heptasaccharide **28** (1.82 mg, 1.70 μmol, 5% over 15 steps, based on resin loading). ^{1}H NMR (600 MHz, D_2O): δ = 5.15 (d, *J* = 3.8 Hz, 1H), 4.52 (d, *J* = 7.5 Hz, 1H), 4.41 – 4.26 (m, 5H), 4.06 – 3.91 (m, 4H), 3.85 (dd, *J* = 11.6, 5.4 Hz, 1H), 3.80 – 3.37 (m, 19H), 3.35 – 3.07 (m, 13H), 2.88 (t, *J* = 7.6 Hz, 2H), 1.62 – 1.51 (m, 4H), 1.38 – 1.26 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): δ = 105.4, 104.4, 104.2, 103.9, 100.2, 81.6, 79.3, 79.1, 79.0, 78.2, 76.2, 75.5, 75.3, 75.2, 75.1, 75.0, 73.9, 73.2, 72.7, 71.7, 68.6, 67.8, 65.5, 62.4, 42.0, 30.8, 29.2, 24.7, 16.7 ppm. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{42}H_{74}NO_{30}^+$: 1072.4296; found: 1072.4302.

RP-HPLC (ELSD trace):



Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-[2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-acetyl-α-D-glucopyranosyl]-3-*O*-benzyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (30)



Linker-functionalized resin **9** (104 mg, 66.7 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

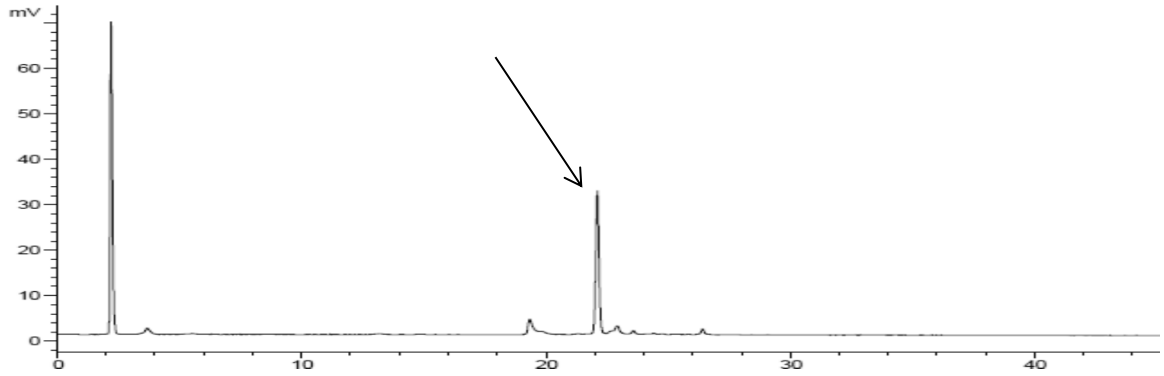
Module **E**: 40 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **18b**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

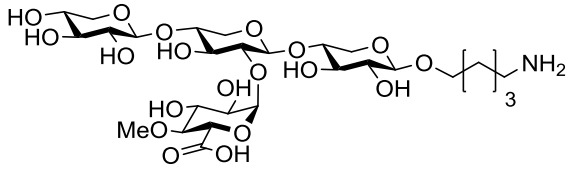
Module **G**: 0.5 M Bz_2O and 0.25 M DMAP in DCE

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave protected tetrasaccharide **30** (26.5 mg, 16.4 μmol, 24%) as a single stereoisomer.

Crude NP-HPLC (ELSD trace):

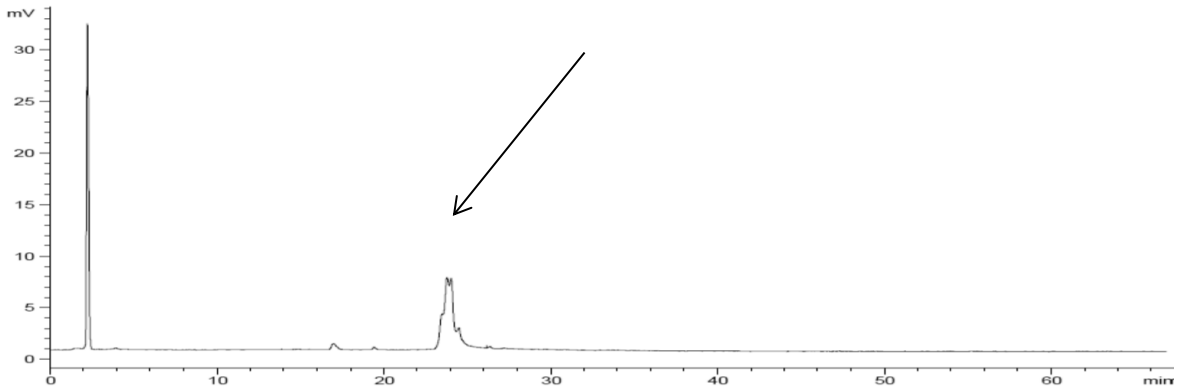


Aminopentyl β-D-xylopyranosyl-(1→4)-2-*O*-[4-*O*-methyl-α-D-glucopyranosyluronic acid]-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (34)



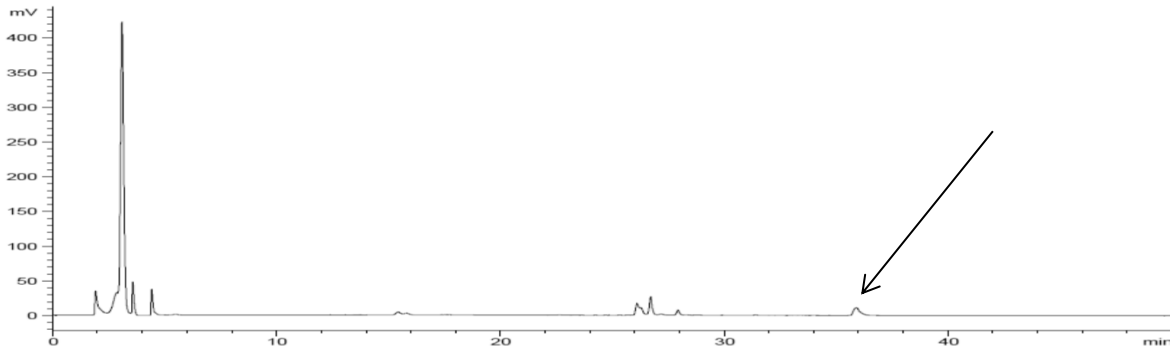
Fully protected tetrasaccharide **30** was dissolved in DCM (1.8 mL) and MeOH (0.2 mL) and *p*TsOH (3.12 mg, 16.4 μmol) was added. The mixture was refluxed overnight and then washed with aq. sat. $NaHCO_3$ solution. The organic phase was dried and concentrated and the crude tetrasaccharide was used in the next step without further purification. The crude tetrasaccharide was dissolved in DCM (10 mL) and Dess-Martin-Periodinate (10.4 mg, 24.6 μmol) and water (1.00 μL) were added. The reaction mixture was stirred at r.t. for 2 d. DCM (5 mL) was added and the reaction was quenched by the addition of aq. sat. $Na_2S_2O_3$ solution (5 mL) and aq. sat. $NaHCO_3$ solution (5 mL). The organic phase was separated, and the aqueous phase was extracted twice with DCM (10 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed *in vacuo*.

Crude NP-HPLC (ELSD trace):



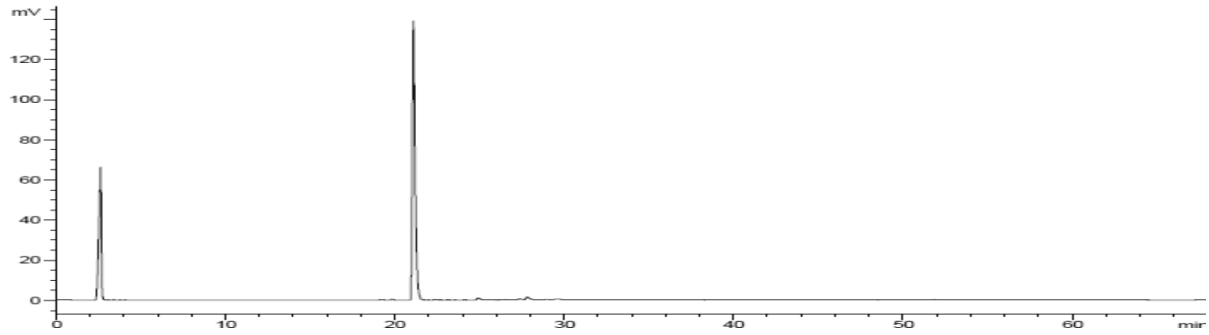
The crude aldehyde was taken up in THF (5 mL) and *t*BuOH (10 mL) and 2-methyl-but-2-ene (90.0 μL, 820 μmol) were added. $NaClO_2$ (14.8 mg, 164 μmol) and NaH_2PO4 (14.8 mg, 123 μmol) in water (5 mL) were added and the reaction mixture was stirred at r.t. for 2 h. The reaction was quenched by the addition of 1 M HCl solution (10 mL) and the aqueous phase was extracted with DCM (3 x 5 mL). The organic phase was dried, and the solvents were evaporated. Purification by normal phase HPLC using a preparative YMC diol column gave the protected glucuronic acid tetrasaccharide (14.2 mg, 8.95 μmol, 54% over 3 steps).

Crude NP-HPLC (ELSD trace):



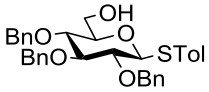
The protected glucuronic acid tetrasaccharide was dissolved in in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.6 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by the addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected tetrasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 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 **34** (1.3 mg, 1.89 μmol, 3% over 12 steps, based on resin loading). ^{1}H NMR (400 MHz, D_2O): δ = 5.27 (d, *J* = 3.8 Hz, 1H), 4.61 (d, *J* = 7.5 Hz, 1H), 4.44 (d, *J* = 7.8 Hz, 1H), 4.39 (d, *J* = 7.9 Hz, 1H), 4.31 (d, *J* = 10.1 Hz, 1H), 4.09 (dt, *J* = 11.8, 5.8 Hz, 2H), 3.96 (dd, *J* = 11.6, 5.4 Hz, 1H), 3.89-3.49 (m, 9H), 3.46-3.14 (m, 11H), 2.98 (t, *J* = 7.6 Hz, 2H), 1.73-1.57 (m, 4H), 1.49-1.38 (m, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): δ = 110.0, 102.7, 101.9, 101.3, 97.3, 82.4, 76.7, 76.3, 75.9, 75.5, 73.8, 72.8, 72.7, 72.3, 72.1, 71.2, 70.0, 69.1, 65.1, 62.8, 62.7, 59.8, 39.3, 28.1, 26.3, 22.0 ppm. ESI-HRMS: m/z [M+H]+ calcd. for $C_{27}H_{48}NO_{19}$: 690.2821; found: 676.2815.

RP-HPLC (ELSD trace):



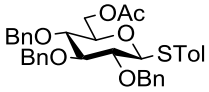
3.3.5 Synthesis of Glucose Building Block 36

4-Methylphenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (35)



A 1 M solution of borane tetrahydrofurane complex (15.1 mL, 15.1 mmol) and TMSOTf (0.34 mL, 1.89 mmol) were sequentially added to a solution of 4-methylphenyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-1-thio-β-D-glucopyranoside **20** (2.10 g, 3.79 mmol) in DCM (25 mL) at 0 °C. After stirring the reaction mixture for 4 h, triethylamine (10 mL) and methanol (50 mL) were added. The resulting mixture was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, EtOAc/hex = 1:4 to 2:3) to afford 4-methylphenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside **35** (1.03 g, 1.85 mmol, 49%) as a colorless solid. 1H NMR (400 MHz, $CDCl_3$): δ = 7.44 - 7.27 (m, 17H, Ar*H*), 7.12 (d, *J* = 7.9 Hz, 2H, Ar*H*), 4.94 - 4.82 (m, 4H, ArCH_2), 4.76 (d, *J* = 10.2 Hz, 1H, ArCH_2), 4.64 (d, *J* = 10.3 Hz, 2H, ArCH_2, H-1), 3.87 (dd, *J* = 12.0, 2.6 Hz, 1H, H-6a), 3.76 - 3.65 (m, 2H, H-4, H-6b), 3.55 (t, *J* = 9.4 Hz, 1H, H-3), 3.46 (dd, *J* = 9.7, 8.8 Hz, 1H, H-2), 3.36 (ddd, *J* = 9.7, 4.9, 2.7 Hz, 1H, H-5), 2.34 (s, 3H, CH_3) ppm. The analytical data is in agreement with literature data.[246]

4-Methylphenyl 2,3,4-tri-*O*-benzyl-6-*O*-acetyl-1-thio-β-D-glucopyranoside (36)

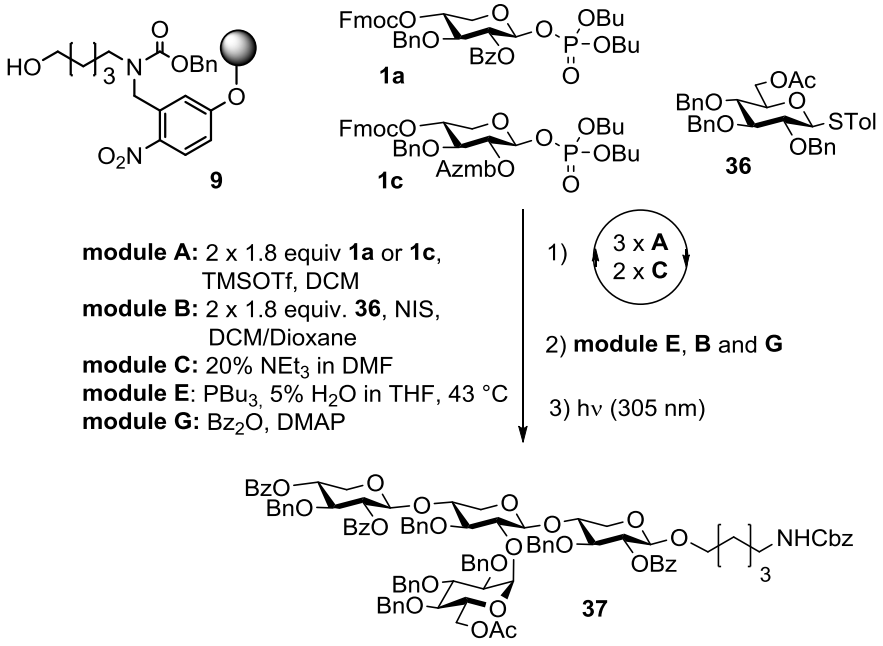


4-Methylphenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside **35** (1.03 g, 1.85 mmol) was dissolved in anhydrous DCM (50 mL) and acetic anhydride (5.25 mL, 55.5 mmol) and DMAP (23.0 mg, 0.185 mmol) were added. The reaction mixture was stirred at r.t. for 2 h. The reaction mixture was quenched by the addition of 1 M HCl solution (20 mL) and the phases were separated. The organic phase was washed with brine (20 mL) and was dried

with Na_2SO_4. The crude product was purified by column chromatography (SiO_2, EtOAc/hex = 1:6) to afford 4-methylphenyl 2,3,4-tri-*O*-benzyl-6-*O*-acetyl-1-thio-β-D-glucopyranoside **36** (1.03 g, 1.85 mmol, 89%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50 – 7.43 (m, 2H, Ar*H*), 7.42 – 7.38 (m, 2H, Ar*H*), 7.36 – 7.27 (m, 13H, Ar*H*), 7.13 – 7.06 (m, 2H, Ar*H*), 4.93 (dd, *J* = 10.5, 3.1 Hz, 2H, ArCH_2), 4.88 – 4.81 (m, 2H, ArCH_2), 4.74 (d, *J* = 10.3 Hz, 1H, ArCH_2), 4.61 – 4.54 (m, 2H, H-1, ArCH_2), 4.36 (d, *J* = 11.8 Hz, 1H, H-6a), 4.20 (dd, *J* = 11.5, 3.6 Hz, 1H, H-6b), 3.77 – 3.67 (m, 1H, H-5), 3.60 – 3.37 (m, 3H, H-2, H-3, H-4), 2.34 (s, 3H, CH_3), 2.05 (s, 3H, CH_3) ppm. The analytical data is in agreement with literature data.[247]

3.3.6 Automated Glycan Assembly of Glucuronoxylan Oligosaccharide

Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-acetyl-α-D-glucopyranosyl]-3-*O*-benzyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (37)



Linker-functionalized resin **9** (104 mg, 64.7 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1c**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

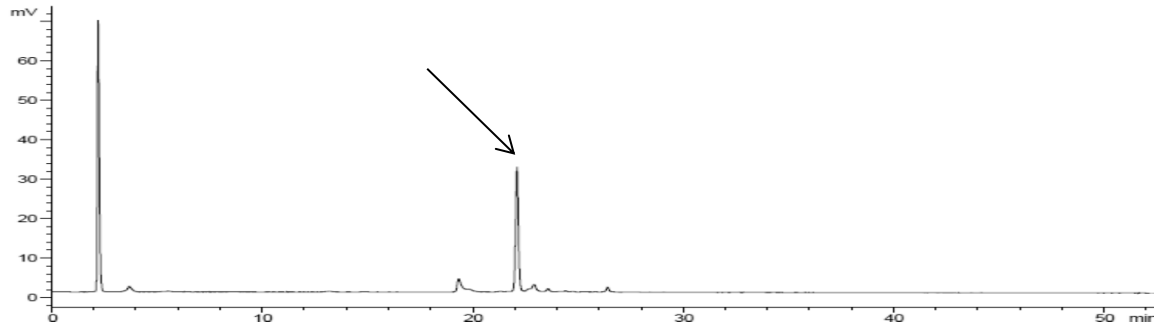
Module **E**: 40 equiv. PBu_3, 5% H_2O in THF, 6 x 30 min, 45 °C

Module **B**: 2 x 1.8 equiv **36**, NIS, DCM/dioxane, 2 x 45 min, -20 to -5 °C

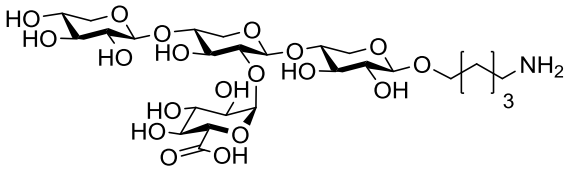
Module **G**: 0.5 M Bz_2O and 0.25 M DMAP in DCE

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave protected tetrasaccharide **38** (9.00 mg, 5.32 µmol, 8%) as a single stereoisomer.

Crude NP-HPLC (ELSD trace):



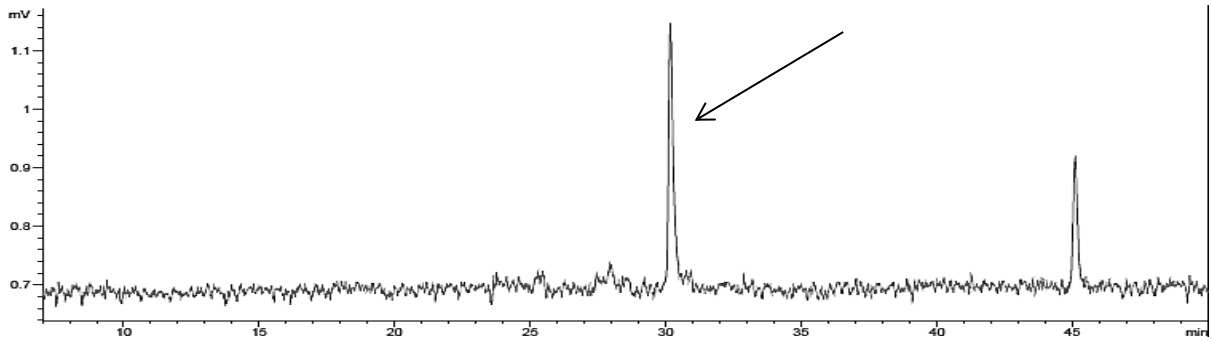
Aminopentyl β-D-xylopyranosyl-(1→4)-2-*O*-[α-D-glucopyranosyluronic acid]-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (38)



Fully protected tetrasaccharide **37** (9.00 mg, 5.32 µmol) was dissolved in DCM (1.8 mL) and MeOH (0.2 mL) and *p*TsOH (1.13 mg, 5.91 µmol) was added. The mixture was refluxed overnight and then washed with aq. sat. $NaHCO_3$ solution (1 mL). The organic phase was dried and concentrated and the crude tetrasaccharide was used in the next step without further purification. The crude tetrasaccharide was dissolved in DCM (5 mL) and Dess-Martin-Periodinate (3.47 mg, 8.19 µmol) and water (0.1 µL) were added. The reaction mixture was stirred at r.t. for 2 d. DCM (5 mL) was added and the reaction was quenched by the addition of aq. sat. $Na_2S_2O_3$ solution (5 mL) and then aq. sat. $NaHCO_3$ solution (5 mL). The organic phase was separated, and the aqueous phase was extracted twice with DCM (10 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude aldehyde was taken up in THF (2 mL) and *t*BuOH (4 mL) and 2-methylbut-2-ene (29.0 µL, 273 µmol) were added. $NaClO_2$ (4.94 mg, 54.6 µmol) and NaH_2PO4 (4.91 mg, 41.0 µmol) in water (2 mL) were added and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was quenched by the

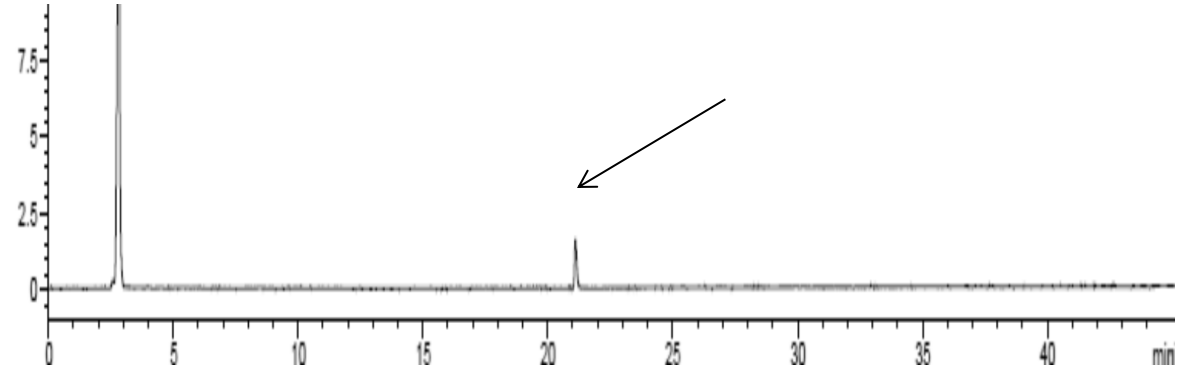
addition of 1 M HCl solution (5 mL) and the aqueous phase was extracted with DCM (3 x 5 mL). The organic phase was dried, and the solvents were evaporated. Purification by normal phase HPLC using a preparative YMC diol column gave the protected glucuronic acid tetrasaccharide (2.50 mg, 1.50 µmol, 28% over 3 steps).

Crude NP-HPLC (ELSD trace):



The protected glucuronic acid tetrasaccharide was dissolved in THF (1 mL) and NaOMe (0.5 M in MeOH, 0.100 mL) was added. The reaction mixture was stirred overnight and was subsequently neutralized by the addition of prewashed Amberlite IR-120 (H^+) 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. The product was dissolved in a mixture of EtOAc/MeOH/AcOH/H_2O (4:2:2:1, 1.00 mL) and the resulting solution was added to a round-bottom flask containing Pd/C (10% Pd, 10.0 mg). The suspension was saturated with H_2 for 30 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 **38** (0.8 mg, 0.592 µmol, 0.9% over 12 steps, based on resin loading). ^{1}H NMR (400 MHz, D_2O): δ = 5.18 (d, *J* = 3.8 Hz, 1H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.31 – 4.21 (m, 2H), 3.99 (dt, *J* = 12.3, 6.2 Hz, 2H), 3.84 (dd, *J* = 11.6, 5.4 Hz, 1H), 3.80 – 3.08 (m, 18H), 2.87 (t, *J* = 7.5 Hz, 2H), 1.61 – 1.45 (m, 4H), 1.40 – 1.25 (m, 2H) ppm. ESI-HRMS: m/z [M+H]+ calcd. for $C_{26}H_{45}NNaO_{19}^+$: 698.2483; found: 698.2497.

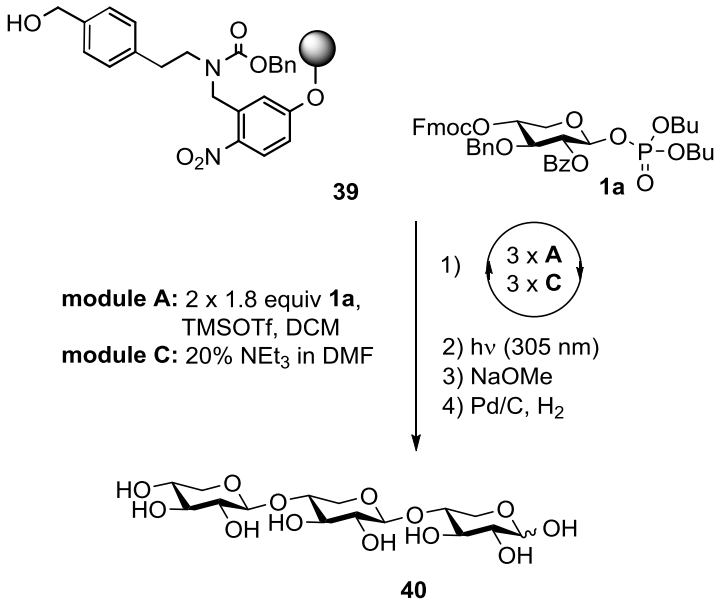
RP-HPLC (ELSD trace):



3.4 Artificial Polysaccharides with Well-Defined Branching Patterns

3.4.1 Automated Glycan Assembly of Arabinoxylan Oligosaccharides with Free Reducing Ends

β-D-Xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-D-xylopyranose (40)



Linker-functionalized resin **39** (170 mg, 44.2 μmol) was placed in the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

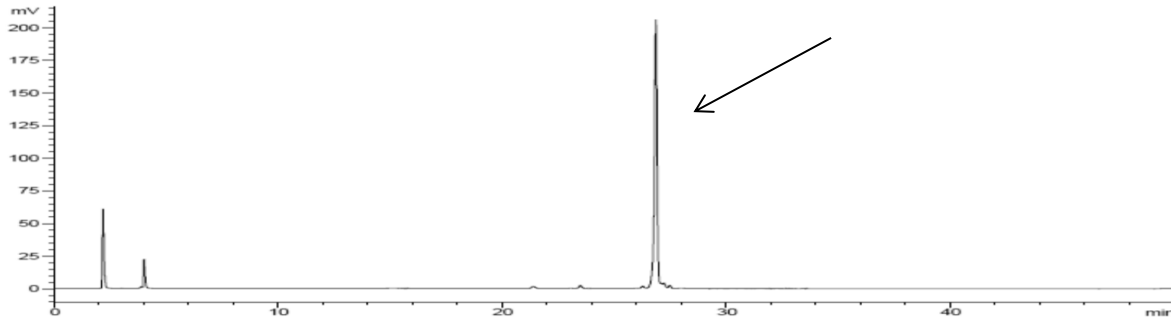
Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

Module **A**: 2 x 1.8 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 4 x 5 min, r.t.

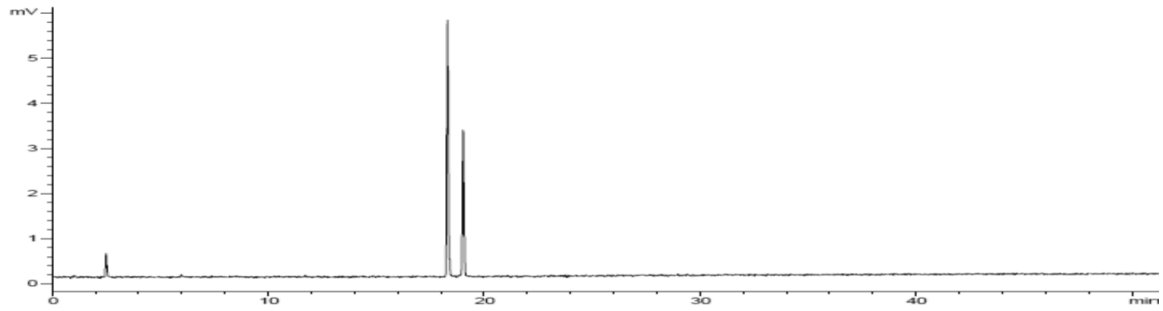
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected tetrasaccharide (30.1 mg, 23.8 μmol, 54%).

Crude NP-HPLC (ELSD trace):

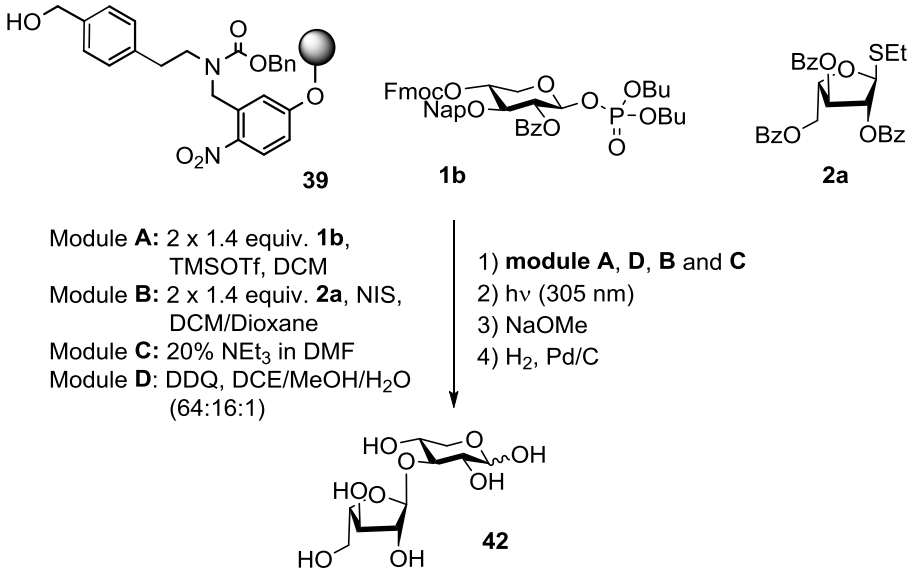


The protected trisaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected trisaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 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 was left. The fully deprotected trisaccharide **40** was stirred with a spatula tip of H^+-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected trisaccharide **40** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an α/β-mixture of the trisaccharide **40** (1.9 mg, 4.64 µmol, 10% over 9 steps, based on resin loading). ^{1}H NMR (400 MHz, D_2O): δ = 5.04 (d, *J* = 3.7 Hz, α-1H), 4.44 (d, *J* = 7.9 Hz, β-1H), 4.32 (t, *J* = 7.2 Hz, 5H), 3.96 (dd, *J* = 11.8, 5.3 Hz, 2H), 3.95 – 3.86 (m, 2H), 3.82 (dd, *J* = 11.6, 5.5 Hz, 2H), 3.70 – 3.56 (m, 9H), 3.52 – 3.36 (m, 9H), 3.31 – 3.19 (m, 6H), 3.19 – 3.07 (m, 9H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 101.8, 101.6, 96.4, 91.9, 76.5, 76.3, 75.5, 73.9, 73.8, 73.6, 72.7, 72.6, 71.3, 70.9, 69.1, 65.1, 62.9 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{15}H_{26}NaO_{13}^+$: 437.1271; found 437.1254.

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



2-*O*-[α-L-Arabinofuranosyl]-D-xylopyranose (42)



Linker-functionalized resin **39** (170 mg, 44.2 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.4 equiv **1b**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

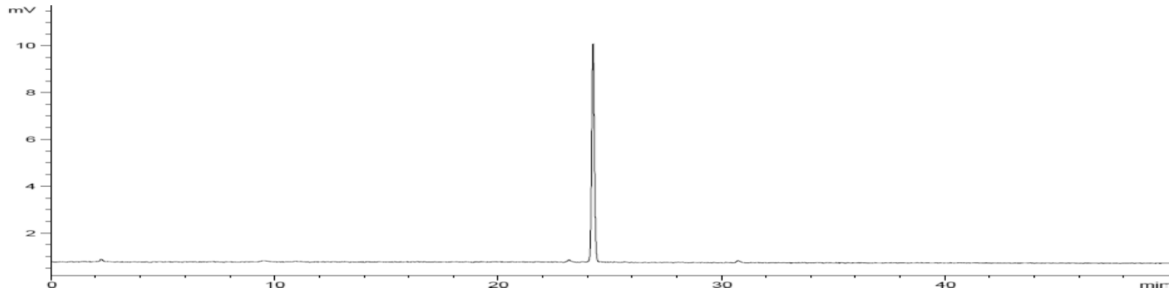
Module **D**: 0.1 M DDQ in DCE/MeOH/H_2O (64:16:1)

Module **B**: 2 x 1.4 equiv **2a**, NIS, 2 x 35 min, -40 °C to -20 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected disaccharide (10.2 mg, 10.6 μmol, 24%)

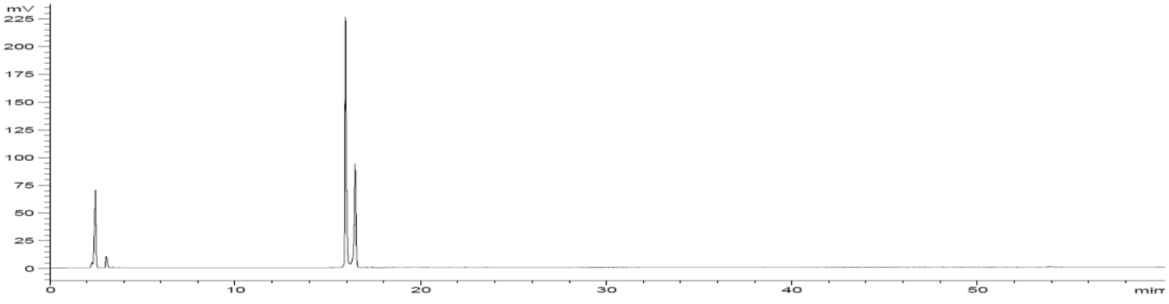
Crude NP-HPLC (ELSD trace):



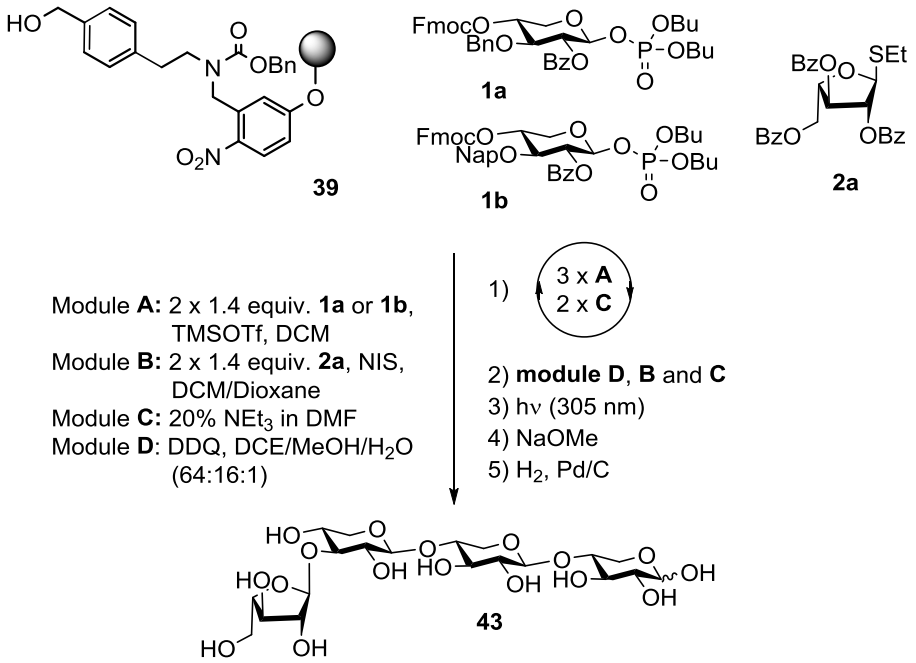
The protected disaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected disaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 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 was left. The fully deprotected disaccharide **42** was stirred with a spatula tip of H^+-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected disaccharide **42** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an α/β-mixture of the disaccharide **42** (2.2 mg, 7.72 µmol, 18% over 7 steps, based on resin loading). ^{1}H NMR (400 MHz, D_2O): δ = 5.13 (d, *J* = 13.8 Hz, 1H), 5.00 (d, *J* = 3.4 Hz, 1H, α-1H), 4.42 (d, *J* = 8.0 Hz, 1H, β-1H), 4.08 – 3.95 (m, 2H), 3.85 – 3.70 (m, 2H), 3.68 – 3.36 (m, 6H), 3.25 – 3.10 (m, 1H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 108.1, 108.0, 96.3, 92.1, 83.8, 83.7, 81.8, 81.0, 79.2, 76.3, 73.8, 71.1, 67.8, 67.7, 64.8, 61.0, 60.8 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{10}H_{18}NaO_9^+$: 305.0849; found 305.0848.

RP-HPLC (ELSD trace):



α-L-Arabinofuranosyl-(1→3)-β-D-Xylopyranosyl-β-D-xylopyranosyl-D-xylopyranose (43)



Linker-functionalized resin **39** (340 mg, 88.4 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1b**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

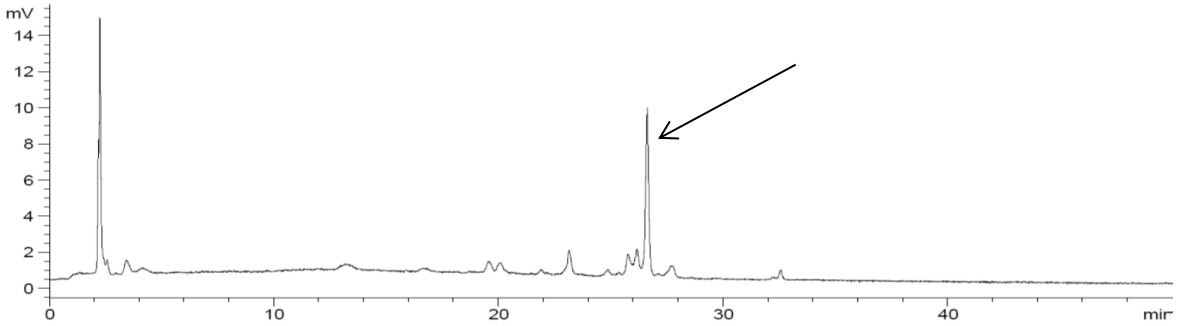
Module **D:** 0.1 M DDQ in DCE/MeOH/H_2O (64:16:1)

Module **B**: 2 x 1.4 equiv **2a**, NIS, 2 x 35 min, -40 °C to -20 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

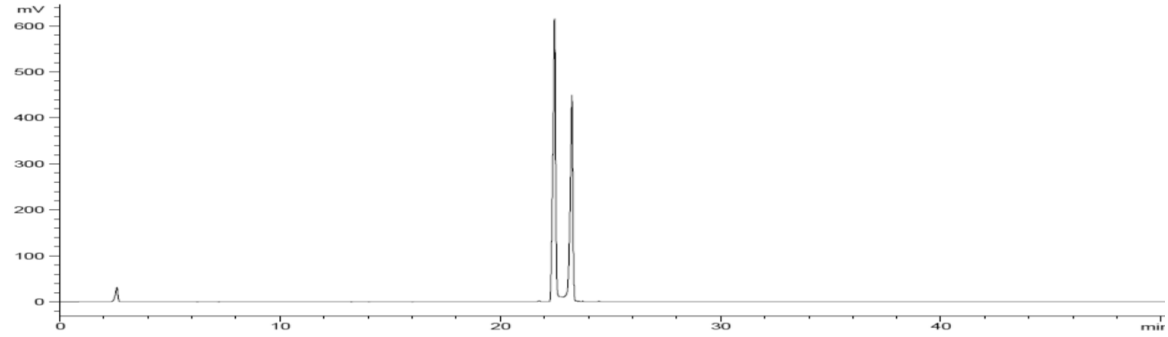
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photo-reactor afforded the crude product. Purification by normal phase HPLC using a pre-parative YMC diol column gave the protected tetrasaccharide (30.0 mg, 18.5 μmol, 21%).

Crude NP-HPLC (ELSD trace):

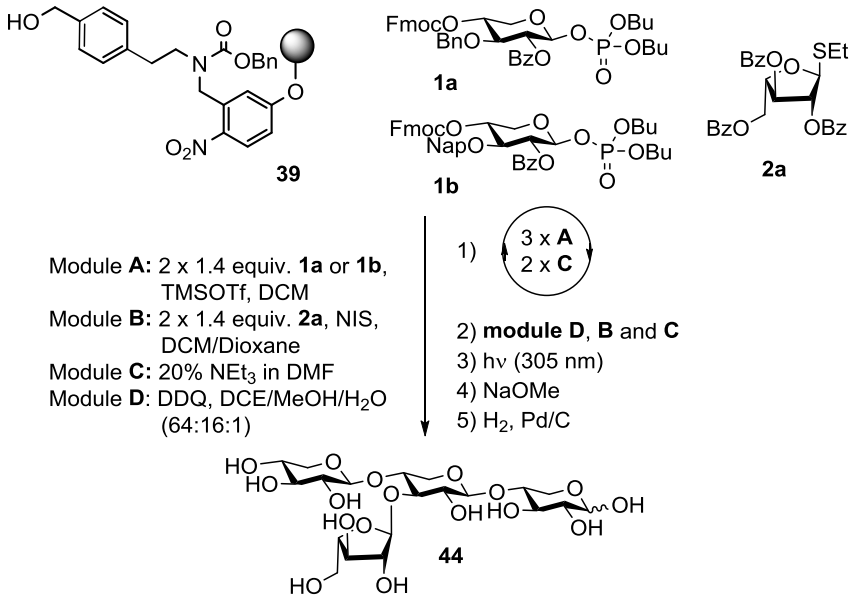


The protected tetrasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected tetrasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 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 was left. The fully deprotected tetrasaccharide **43** was stirred with a spatula tip of H^+-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected tetrasaccharide **43** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an α/β-mixture of the tetrasaccharide **43** (0.75 mg, 1.37 µmol, 2% over 11 steps, based on resin loading). For characterization see chemical solution-phase synthesis of **43** (chapter 3.4.3).

RP-HPLC (ELSD trace):



β-D-Xylopyranosyl-2-*O*-[α-L-arabinofuranosyl]-β-D-xylopyranosyl-D-xylopyranose (44)



Linker-functionalized resin **39** (170 mg, 44.2 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1b**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

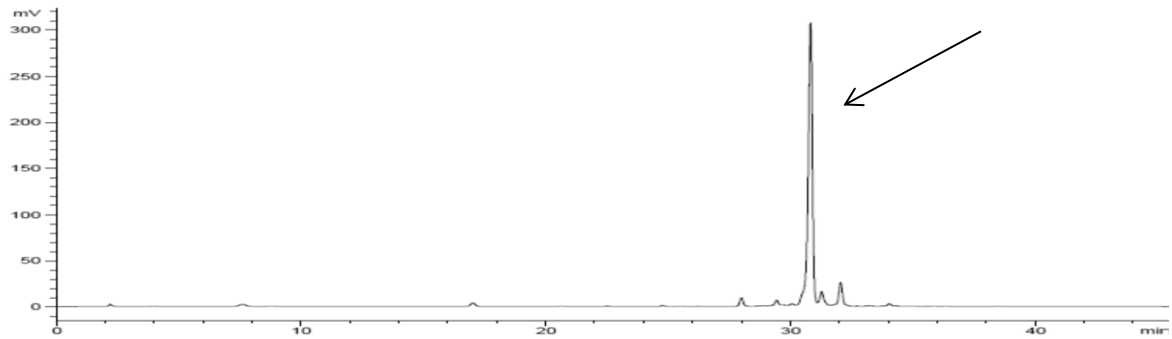
Module **D:** 0.1 M DDQ in DCE/MeOH/H_2O (64:16:1)

Module **B**: 2 x 1.4 equiv **2a**, NIS, 2 x 35 min, -40 °C to -20 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

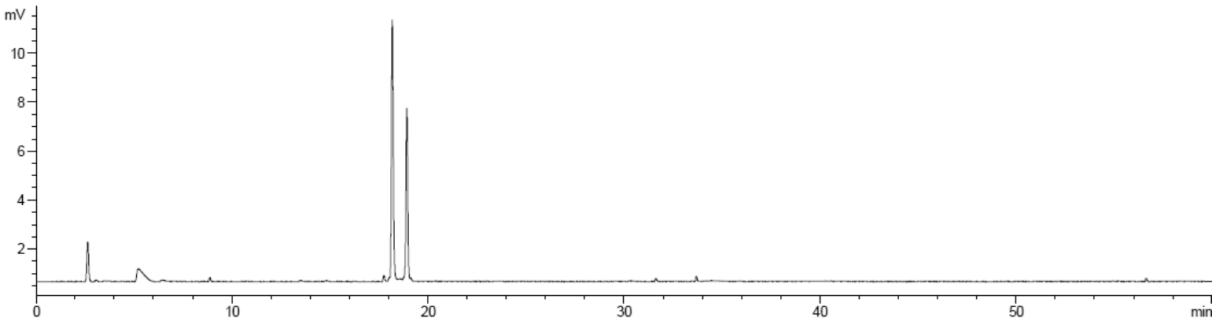
Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photoreactor afforded the crude product. Purification by normal phase HPLC using a preparative YMC diol column gave the protected tetrasaccharide (28.1 mg, 17.4 μmol, 40%).

Crude NP-HPLC (ELSD trace):

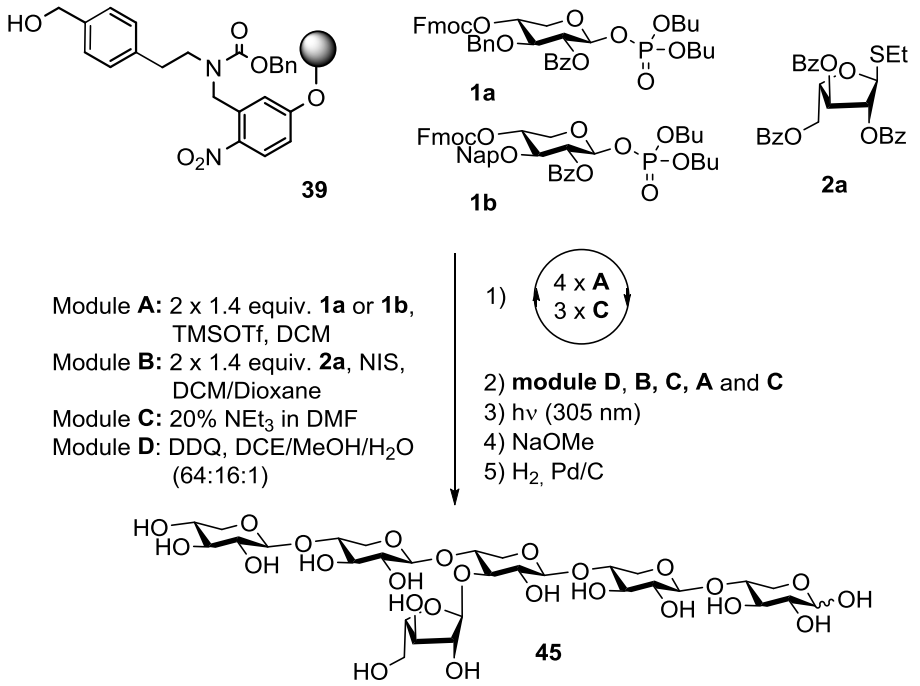


The protected tetrasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected tetrasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 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 was left. The fully deprotected tetrasaccharide **44** was stirred with a spatula tip of H^+-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected trisaccharide **44** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an α/β-mixture of the tetrasaccharide **44** (2.6 mg, 4.68 µmol, 11% over 11 steps, based on resin loading). ^{1}H NMR (400 MHz, D_2O): δ = 5.37 (s, 1H), 5.16 (s, 1H, α-1H), 4.56 (d, *J* = 7.9 Hz, 1H, β-1H), 4.48 (d, *J* = 7.6 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.29 – 4.21 (m, 1H), 4.16 – 4.00 (m, 3H), 3.95 – 3.64 (m, 9H), 3.61 – 3.47 (m, 3H), 3.45 – 3.31 (m, 4H), 3.30 – 3.16 (m, 3H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 107.5, 101.6, 101.4, 96.4, 92.0, 84.7, 80.6, 77.2, 77.1, 76.6, 76.4, 75.5, 73.9, 73.8, 73.5, 73.2, 72.9, 71.3, 69.1, 65.0, 62.9, 62.7, 61.2, 58.7 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{20}H_{34}NaO_{17}^+$: 569.1694; found 569.1697.

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



β-D-Xylopyranosyl-2-*O*-[α-L-arabinofuranosyl]-β-D-xylopyranosyl-D-xylopyranose (45)



Linker-functionalized resin **39** (170 mg, 44.2 μmol) was placed in the reaction vessel of the synthesizer and synthesizer modules were applied as follows:

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1b**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **E:** 0.1 M DDQ in DCE/MeOH/H_2O (64:16:1)

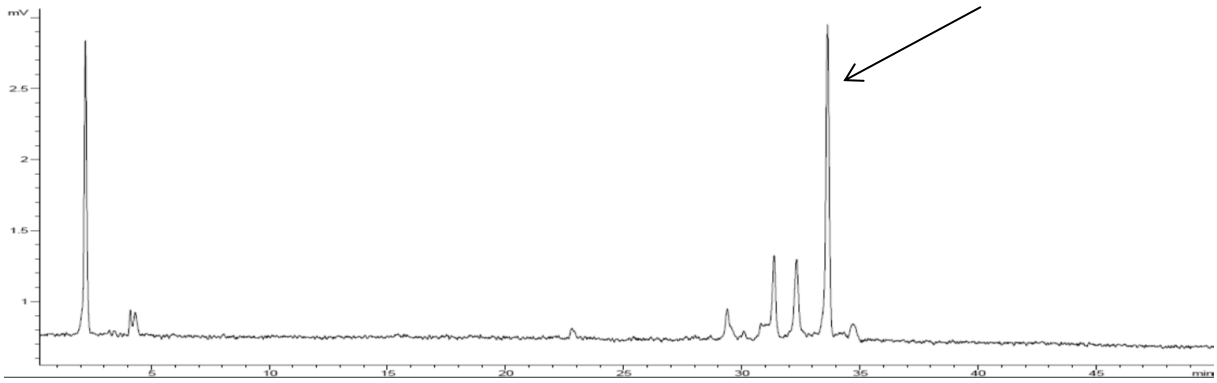
Module **B**: 2 x 1.4 equiv **2a**, NIS, 2 x 35 min, -40 °C to -20 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

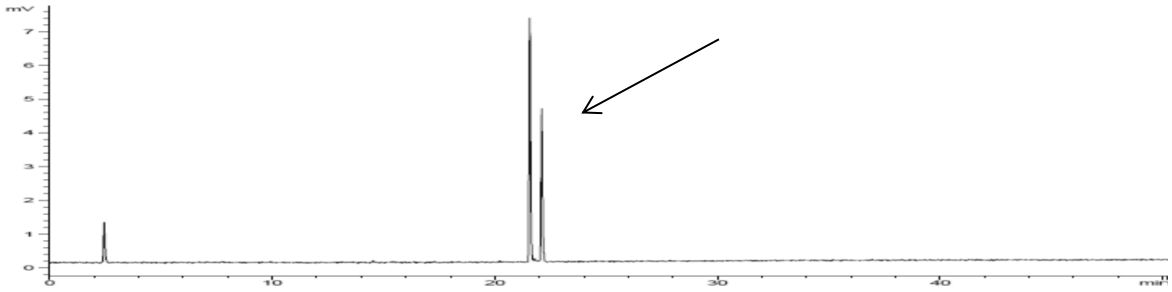
Module **A**: 2 x 1.4 equiv **1a**, TMSOTf, DCM, 2 x 35 min, -35 °C to -15 °C

Module **C**: 20% NEt_3 in DMF, 3 x 5 min, r.t.

Cleavage from the resin using UV irradiation at 305 nm in a continuous flow photo-reactor afforded the crude product. Purification by normal phase HPLC using a pre-parative YMC diol column gave the protected hexasaccharide (22.1 mg, 9.73 μmol, 22%). Crude NP-HPLC (ELSD trace):

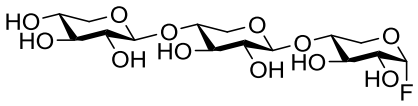


The protected hexasaccharide was dissolved in DCM/MeOH (2:1, 3 mL) and NaOMe (0.5 M in MeOH, 0.500 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were removed *in vacuo.* The crude product was purified by reversed phase HPLC using a semi-preparative C5 column affording the semi-protected hexasaccharide. 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, 10.0 mg). The suspension was saturated with H_2 for 30 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 was left. The fully deprotected hexasaccharide **45** was stirred with a spatula tip of H^+-Amberlite resin to hydrolyze the side product that has formed by condensation of the oligosaccharide with the cleaved linker. Without removal of the water the fully deprotected trisaccharide **45** was directly purified by reversed phase HPLC using a semi-preparative hypercarb column to give an α/β-mixture of the hexasaccharide **45** (1.0 mg, 1.25 µmol, 3% over 15 steps, based on resin loading). ^{1}H NMR (400 MHz, D_2O): δ = 5.20 (s, 1H), 4.99 (d, *J* = 3.6 Hz, 1H, α-1H), 4.39 (dd, *J* = 7.7, 1.7 Hz, 1H), 4.34 – 4.22 (m, 4H), 4.09 (q, *J* = 5.1 Hz, 1H), 3.98 – 3.83 (m, 4H), 3.80 – 3.69 (m, 3H), 3.68 – 3.30 (m, 15H), 3.29 – 3.02 (m, 10H) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 110.2, 110.0, 104.9, 104.4, 104.2, 103.9, 87.3, 83.3, 82.6, 82.5, 79.9, 79.8, 79.1, 79.0, 78.9, 78.2, 76.2, 76.2, 75.9, 75.6, 75.5, 75.3, 75.3, 74.0, 72.5, 71.8, 67.8, 65.5, 65.5, 65.4, 65.2, 63.9, 63.9, 63.2 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{20}H_{34}NaO_{17}^+$: 833.2539; found 833.2526.
RP-HPLC (ELSD trace):



3.4.2 Synthesis of Glycosyl Fluorides and Glycosynthase-catalyzed Polymerization

β-D-Xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (47)

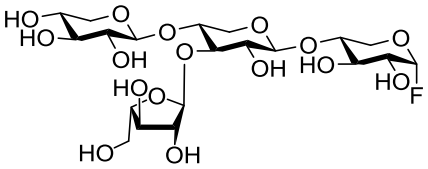


β-D-Xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-D-xylopyranose **40** (1.90 mg, 4.64 μmol) was dissolved in acetic anhydride (1.50 mL) and pyridine (1.50 mL) and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was diluted with DCM (5 mL) and washed with 1 M HCl solution (2 mL). The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated to give the crude product (2.51 mg). The protected trisaccharide was dissolved in anhydrous DCM (0.5 mL) in a plastic vessel. The solution was cooled to -25 °C and HF/pyridine (0.5 mL) was added. The biphasic reaction mixture was rigorously stirred at -10 °C for 5 h. The reaction mixture was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 2 mL). The phases were separated and the organic phase was washed with aq. sat. $NaHCO_3$ solution (2 mL). The aqueous phase was reextracted with DCM (2 x 2 mL) and the combined organic phases were dried with Na_2SO_4. After evaporation of the solvent the crude product was purified by NP HPLC using a Luna Silica Prep Column to give trisaccharide fluoride **46** (1.41 mg, 1.98 μmol, 51%) as a colorless solid. Disaccharide fluoride **46** (1.41 mg, 1.98 μmol,) was dissolved in anhydrous MeOH (1.00 mL) at 0 °C. NaOMe (0.5 M in MeOH, 4.00 μL, 1.98 μmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated to give the fully unprotected fluoride **47** (0.830 mg, 1.99 μmol, 100%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 5.46 (d, *J* = 53.3 Hz, 1H), 4.29 (t, *J* = 8.5 Hz, 3H), 3.93 (dd, *J* = 12.0, 5.3 Hz, 1H), 3.80 (td, *J* = 11.1, 5.2 Hz, 2H), 3.72 – 3.33 (m, 9H), 3.29 – 3.03 (m, 5H) ppm.

α-L-Arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (48)

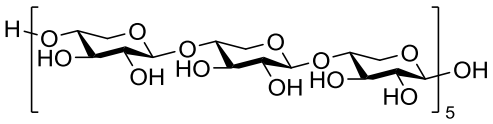
For synthesis of glyocsyl fluoride **48** see chapter 3.4.5.1.

β-D-Xylopyranosyl-(1→4)-3-*O*-[α-L-arabinofuranosyl]-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (49)

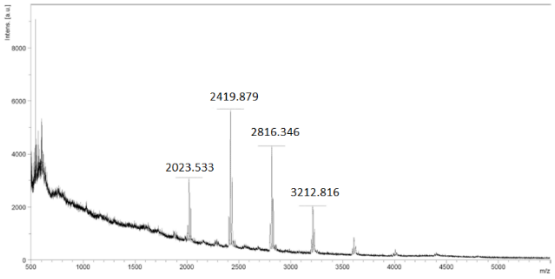


Tetrasaccharide **43** (2.28 mg, 4.17 μmol) was dissolved in acetic anhydride (1.50 mL) and pyridine (1.50 mL) and the reaction mixture was stirred at r.t. for 3 h. The reaction mixture was diluted with DCM (5 mL) and washed with 1 M HCl (2 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated to give the crude product (2.52 mg). The protected trisaccharide was dissolved in anhydrous DCM (3.00 mL) in a plastic vessel. The solution was cooled to -25 °C and HF/pyridine (3.00 mL) was added. The biphasic system was stirred rigorously for 4 h at -10 °C. The reaction mixture was diluted with DCM (2 mL) and was quenched by dropping the mixture carefully into aq. ammonia solution (25%, 2 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (2 mL) and reextracted with DCM (2 x 20 mL). The combined organic phases were dried with Na_2SO_4 and the solvents were evaporated. The crude product was purified using a Luna Silica Prep Column to give the protected tetrasaccharide fluoride (1.72 mg, 1.85 μmol) as a colorless solid. The tetrasaccharide fluoride (1.72 mg, 1.85 μmol) was dissolved in anhydrous MeOH (1.00 mL) at 0 °C. NaOMe (0.5 M in MeOH, 3.70 μL, 1.85 μmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated to give fully deprotected tetrasaccharide fluoride **49** (88.0 mg, 0.211 mmol, 100%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 5.46 (dd, *J* = 53.4, 2.8 Hz, 1H), 5.21 (s, 1H), 4.33 (d, *J* = 7.7 Hz, 1H), 4.26 (d, *J* = 7.6 Hz, 1H), 4.10 – 4.06 (m, 1H), 3.99 – 3.89 (m, 2H), 3.85 – 3.33 (m, 12H), 3.28 – 3.18 (m, 3H), 3.12 – 3.01 (m, 2H) ppm.

(1→4)-β-D-Xylan $(XX)_n$ (50)



An aqueous solution of fluoride donor **47** (10 mM, 99.0 μL) and a phosphate buffered solution of XynAE265G (9.30 mg/mL, 25.0 μL) were added to sodium phosphate buffer (100 mM, pH 7, $V_{Fluoride}/V_{buffer}$ = 1:5). The reaction was shaken at r.t. overnight. A white precipitate was formed. It was centrifuged and washed three times with MilliQ water and analyzed by MALDI MS.

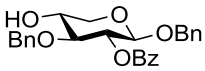


α-L-Arabinofuranosyl-(1→3)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylan $(A^3XX)_n$ (51)

For synthesis of polysaccharide from glycosyl fluoride **48** see chapter 3.4.5.2.

3.4.3 Chemical Solution-Phase Synthesis of Arabinoxylan Oligosaccharides

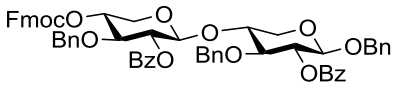
Benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (54)



A mixture of xylose BB **8a** (5.00 g, 7.43 mmol), benzyl alcohol (3.09 mL, 29.7 mmol) and molecular sieves (2.00 g) in anhydrous DCM (250 mL) was stirred under an atmosphere of Argon for 30 min. The mixture was cooled to -20 °C and NIS (2.51 g, 11.2 mmol) and TfOH (0.131 mL, 1.49 mmol) were added. After stirring for 40 min at -20 °C NEt_3 (10 mL) was added and the reaction mixture was stirred for another 30 min. The mixture was filtered and the filtrate was washed with 1 M HCl solution (100 mL) and brine (100 mL). The aqueous layers were extracted with DCM (2 x 100 mL). The

combined organic phases were dried with Na_2SO_4 and the solvent was evaporated. The pale yellow oil was purified by column chromatography (SiO_2, EtOAc/Hex = 1:6) to give **54** (3.01 g, 6.93 mmol, 93%) as a colorless solid. $[\alpha]_D^{25}$ = -18.4 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 − 8.04 (m, 2H, Ar*H*), 7.67 − 7.62 (m, 1H, Ar*H*), 7.53 − 7.48 (m, 6H, Ar*H*), 7.41 − 7.38 (m, 1H, Ar*H*), 7.30 − 7.21 (m, 9H, Ar*H*), 5.36 (dd, *J* = 8.0 Hz, *J* = 6.4 Hz, 1H, H-2), 4.90 (d, *J* = 12.5 Hz, 1H, C*H*$_2$Ph), 4.79 (d, *J* = 11.5 Hz, 1H, C*H*$_2$Ph), 4.67 (d, *J* = 11.5 Hz, 1H, C*H*$_2$Ph), 4.65 (d, *J* = 12.5 Hz, 1H, C*H*$_2$Ph), 4.65 (d, *J* = 6.4 Hz, 1H, H-1), 4.16 (dd, *J* = 11.6 Hz, 4.6 Hz, 1H, H-5a), 3.90 (td, *J* = 8.1 Hz, *J* = 4.6 Hz, 1H, H-4), 3.66 (t, *J* = 7.7 Hz, 1H, H-3), 3.39 (dd, *J* = 11.7 Hz, 8.6 Hz, 1H, H-5b), 2.69 − 2.40 (br, 1H, O*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.3, 137.9, 137.1, 133.4, 129.9, 129.8, 128.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.1 (19C, Ar), 99.4 (C-1), 80.8 (C-3), 73.9 (*C*H_2Ph), 72.4 (C-2), 70.0 (*C*H_2Ph), 69.2 (C-4), 64.5 (C-5) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{26}H_{26}O_6Na^+$: 457.1622; found: 457.1665. IR (neat): ν_{max} = 1727, 1454, 1269, 1071 cm^{-1}.

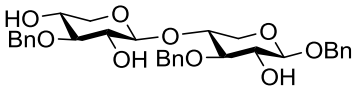
Benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-fluorenylcarboxymethyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (55)



A mixture of compound **54** (6.04 g, 8.98 mmol), xylose BB **8a** (3.00 g, 6.90 mmol) and activated molecular sieves (4 Å, 2.00 g) in anhydrous DCM (45 mL) was stirred at r.t. for 30 min under an Argon atmosphere. After the mixture was cooled to −30 °C, NIS (6.21 g, 27.6 mmol) and TfOH (0.121 mL, 1.38 mmol) were added. After the reaction mixture was stirred for 30 min at −30 °C, the reaction mixture was diluted with DCM (50 mL) and quenched by the addition of aq. sat. $NaHCO_3$ solution (50 mL). The phases were separated and the organic phase was washed with aq. sat. $Na_2S_2O_3$ solution (1 x 50 mL) and H_2O (50 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography (EtOAc/Hex = 1:6) to give **55** (6.50 g, 6.61 mmol, 96 %) as a colorless solid. $[\alpha]_D^{25}$ = -18.4 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 - 8.08 (m, 2H, Ar*H*), 8.01 - 7.93 (m, 2H, Ar*H*), 7.83 - 7.72 (m, 2H, Ar*H*), 7.68 - 7.54 (m, 4H, Ar*H*), 7.52 - 7.39 (m, 6H, Ar*H*), 7.36 - 7.06 (m, 17H, Ar*H*), 5.35 - 5.26 (m, 2H, H-2, H-2'), 4.91 - 4.78 (m, 4H, H-1', H-4', C*H*$_2$Ph), 4.76 - 4.67 (m, 2H, C*H*$_2$Ph), 4.65 (d, *J* = 11.4 Hz, 1H, C*H*$_2$Ar), 4.58 (d, *J* = 12.6 Hz, 1H, C*H*$_2$Ph), 4.50 (d, *J* = 7.2 Hz, 1H, H-1), 4.45 (dd, *J* = 7.3, 3.3 Hz, 2H, H-5'a, Fmoc-C*H*$_2$), 4.34 - 4.22 (m, 2H, H-5'a, Fmoc-C*H*), 4.12 - 4.02 (m, 2H, H-4, H-5a), 3.91 (t, *J* = 6.4 Hz, 1H, H-3'), 3.69 (t, *J* = 8.3 Hz, 1H, H-3), 3.52 (dd, *J* = 12.4, 6.5 Hz, 1H, H-5'b), 3.30 - 3.19 (m, 1H, H-5b) ppm.

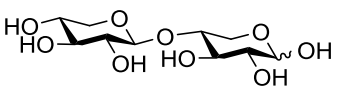
^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.2, 165.2, 154.4 (3C, C=O), 143.2, 143.2, 141.3, 141.3, 137.9, 137.3, 137.0, 133.5, 133.1, 129.9, 129.9, 129.5, 128.6, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.7, 127.6, 127.5, 127.3, 127.2, 125.1, 120.2 (41C, Ar), 99.8 (C-1), 98.5 (C-1'), 79.2 (C-3), 75.8 (C-3'), 75.6 (C-4), 74.5 (*C*H_2Ph), 73.4 (*C*H_2Ph), 73.3 (C-4'), 72.6 (C-2), 70.6 (C-2'), 70.1 (Fmoc-*C*H_2), 70.0 (*C*H_2Ph), 62.6 (C-5), 60.6 (C-5'), 46.8 (Fmoc-*C*H) ppm. ESI-HRMS: m/z = $[M+H]^+$ calcd. for $C_{60}H_{54}NaO_{13}^+$: 1005.3462; found 1005.3480. IR(neat): ν_{max} = 1728, 1452, 1225, 1097 cm^{-1}.

Benzyl 3-*O*-benzyl-β-D-xylopyranosyl-(1→4)-3-*O*-benzyl-β-D-xylopyranoside (SI2)



The protected disaccharide **55** (3.00 g, 3.05 mmol) was dissolved in DCM (10 mL) and MeOH (20 mL). NaOMe (0.5 M in MeOH, 10.4 mL, 5.19 mmol) was added and the reaction mixture was stirred at r.t. for 18 h. The reaction mixture was neutralized by the addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated. The crude product was filtered through a plug of silica gel (SiO_2, EtOAc/Hex = 1:1) to give **SI2** (1.20 g, 2.17 mmol, 71%) as a yellow oil. $[\alpha]_D^{25}$ = -4.5 (*c* 0.68, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 – 7.13 (m, 15H, Ar*H*), 4.83 (d, *J* = 11.5 Hz, 1H, C*H*$_2$Ar), 4.78 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ar), 4.74 (d, *J* = 11.4 Hz, 1H, C*H*$_2$Ar), 4.67 (d, *J* = 11.3 Hz, 1H, C*H*$_2$Ar), 4.59 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ar), 4.59 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ar), 4.54 – 4.45 (m, 1H, C*H*$_2$Ar), 4.41 (d, *J* = 5.5 Hz, 1H, H-1), 4.31 (d, *J* = 7.0 Hz, 1H, H-1'), 4.03 (dd, *J* = 12.0, 4.1 Hz, 1H, H-5a), 3.92 (dd, *J* = 11.6, 5.0 Hz, 1H, H-5a'), 3.82 – 3.76 (m, 1H, H-4), 3.64 – 3.53 (m, 2H, H-2, H-4'), 3.52 – 3.45 (m, 2H, H-3, H-2'), 3.32 – 3.24 (m, 2H, H-3', H-5b), 3.14 (dd, *J* = 11.6, 9.3 Hz, 1H, H-5b'), 2.78 (s, 1H, OH), 2.64 (s, 1H, OH), 2.13 (s, 1H, OH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.4, 138.1, 137.3, 128.8, 128.6, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0 (18C, Ar), 102.0 (C-1'), 101.6 (C-1), 82.7 (C-3'), 79.1 (C-3), 74.5 (*C*H_2Ar), 73.9 (*C*H_2Ar), 73.8 (C-4), 72.5 (C-2'), 72.0 (C-2), 70.6 (*C*H_2Ar), 69.2 (C-4'), 65.2 (C-5'), 62.2 (C-5) ppm. ESI-HRMS: m/z = $[M+H]^+$ calcd. for $C_{31}H_{36}NaO_9^+$:575.2257; found 575.2260. IR(neat): ν_{max} = 1269, 1064, 1028, 699 cm^{-1}.

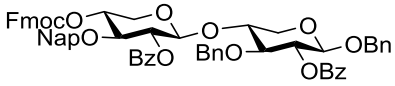
β-D-Xylopyranosyl-(1→4)-D-xylopyranose (52)



The semi-protected disaccharide **SI2** (1.20 g, 2.17 mmol) was dissolved in MeOH (15 mL) and Pd/C (300 mg, 10% Pd) was added. Hydrogen gas was bubbled through the reaction mixture for 10 min and the reaction mixture was stirred under a hydrogen

atmosphere for 2 d. The reaction mixture was diluted with H_2O (5 mL) and the catalyst was filtered off by using a syringe filter (4.5 μm). After evaporation of the solvents fully deprotected disaccharide **52** (480 mg, 1.70 mmol, 78%, α/β ~ 1:1) was obtained as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 5.16 (d, *J* = 3.6 Hz, 0.5H, H-1α), 4.56 (d, *J* = 7.8 Hz, 0.5H, H-1β), 4.43 (d, *J* = 7.8 Hz, 1H, H-1'), 4.03 (dd, *J* = 11.7, 5.3 Hz, 0.5H), 3.94 (dd, *J* = 11.6, 5.4 Hz, 1H), 3.84 – 3.69 (m, 2.5H), 3.64 – 3.48 (m, 2.5H), 3.44 – 3.37 (m, 1H), 3.37 – 3.18 (m, 2.5H) ppm. The analytical data matches the one reported in literature.[248]

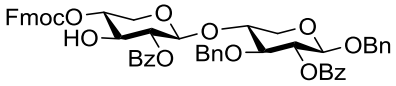
Benzyl 2-*O*-benzoyl-3-*O*-(2-methyl)naphthyl-4-*O*-fluorenylmethoxycarbonyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (56)



A mixture of compound **54** (10.4 g, 14.4 mmol), BB **8b** (5.00 g, 11.5 mmol) and activated molecular sieves (4 Å, 2.00 g) in anhydrous DCM (50 mL) was stirred at r.t. for 30 min under an Argon atmosphere. After the mixture was cooled to −30 °C, NIS (10.4 g, 46.0 mmol) and TfOH (0.417 mL, 2.30 mmol) were added and the reaction mixture was stirred at −30 °C for 30 min. The reaction mixture was diluted with DCM (50 mL) and quenched by the addition of aq. sat. $NaHCO_3$ solution (50 mL). The phases were separated and the organic phase was washed with aq. sat. $Na_2S_2O_3$ solution (50 mL) and H_2O (50 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography (EtOAc/Hex = 1:6) to give **56** (10.0 g, 9.71 mmol, 84%) as a colorless solid. $[a]_D^{25}$ = -21.5 (*c* 0.9, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.16 − 8.12 (m, 1H, Ar*H*), 8.04 − 7.99 (m, 2H, Ar*H*), 7.84 − 7.60 (m, 8H, Ar*H*), 7.52 − 7.13 (m, 12H, Ar*H*), 7.28 − 7.11 (m, 12H, Ar*H*), 5.40 (dd, *J* = 6.7, 5.2 Hz, 1H, H-2), 5.35 (dd, *J* = 8.8, 7.2 Hz, 1H, H-2'), 4.99 − 4.95 (m, 3H, H-4, C*H*$_2$Ph), 4.93 − 4.83 (m, 3H, H-1, C*H*$_2$Ph), 4.67 (d, *J* = 11.4 Hz, 1H, C*H*$_2$Ph), 4.61 (d, *J* = 12.6 Hz, 1H, C*H*$_2$Ph), 4.54 − 4.48 (m, 3H, H-1', Fmoc-C*H*$_2$), 4.34 (dd, *J* = 12.3, 4.0 Hz, 1H, H-5a), 4.29 − 4.24 (m, 1H, Fmoc-C*H*), 4.13 − 4.05 (m, 2H, H-4', H-5a'), 3.99 (t, *J* = 6.6 Hz, 1H, H-3), 3.72 (t, *J* = 8.2 Hz, 1H, H-3'), 3.54 (dd, *J* = 12.3, 6.8 Hz, 1H, H-5b), 3.27 (td, *J* = 10.9, 4.9 Hz, 1H, H-5b') ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 162.6, 162.5 (3C, C=O), 151.8, 140.7, 140.6, 138.8, 135.4, 135.3, 134.5, 132.3, 130.9, 130.6, 130.5, 130.5, 127.4, 127.4, 127.3, 126.9, 126.5, 126.0, 125.7, 125.7, 125.7, 125.6, 125.6, 125.6, 125.5, 125.4, 125.2, 125.1, 125.1, 124.9, 124.7, 124.7, 124.5, 123.6, 123.4, 122.8, 122.6, 117.6 (46C, Ar), 97.2 (C-1'), 96.2 (C-1), 76.7 (C-3'), 73.5 (C-3), 73.2 (C-4'), 71.8 (*C*H$_2$Ph), 71.0 (C-4), 71.0 (*C*H$_2$Ph), 70.0 (C-2'), 68.2 (C-2), 67.5 (*C*H$_2$Ph), 67.5 (Fmoc-*C*H$_2$), 60.1 (C-5'), 58.2 (C-5), 44.3 (Fmoc-

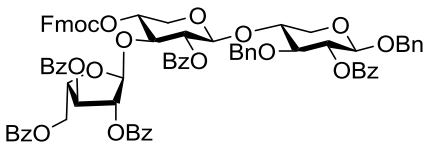
*C*H) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{64}H_{56}NaO_{13}^+$: 1055.3619; found: 1055.3632. IR (neat): ν_{max} = 1729, 1452, 1225, 1070 cm^{-1}.

Benzyl 2-O-benzoyl-4-O-fluorenylmethoxycarbonyl-β-d-xylopyranosyl-(1→4)-2-O-benzoyl-3-O-benzyl-β-D-xylopyranoside (SI3)



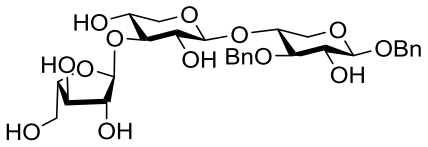
The protected disaccharide **56** (10.0 g, 9.71 mmol) was dissolved in a mixture of DCM (50 mL), MeOH (12.5 mL) and H_2O (0.5 mL), and DDQ (6.61 g, 29.1 mmol) was added. The reaction mixture was stirred at r.t. for 4 h. The reaction mixture was diluted with DCM (30 mL) and washed with aq. sat. $NaHCO_3$ solution (30 mL) and H_2O (30 mL). The organic layers were dried with Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 4:1) to give **SI3** (6.89 g, 7.72 mmol, 79%) as a colorless oil. $[\alpha]_D^{25}$ = -30.9 (*c* 0.75, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.01 − 7.96 (m, 2H, Ar*H*), 7.86 − 7.80 (m, 2H, Ar*H*), 7.62 − 7.53 (m, 2H, Ar*H*), 7.48 − 7.36 (m, 4H, Ar*H*), 7.32 − 7.20 (m, 6H, Ar*H*), 7.17 − 6.96 (m, 12H, Ar*H*), 5.16 (dd, *J* = 8.5, 7.0 Hz, 1H, H-2), 4.98 (dd, *J* = 6.7, 5.1 Hz, 1H, H-2'), 4.69 (d, *J* = 5.1 Hz, 1H, H-1'), 4.65 (d, *J* = 12.6 Hz, 1H, C*H*$_2$Ph), 4.60 (d, *J* = 11.2 Hz, 1H, C*H*$_2$Ph), 4.53 (d, *J* = 11.2 Hz, 1H, C*H*$_2$Ph), 4.40 (d, *J* = 12.6 Hz, 1H, C*H*$_2$Ph), 4.35 (d, *J* = 7.0 Hz, 1H, H-1), 4.28 − 4.20 (m, 2H, Fmoc-C*H*$_2$), 4.10 − 4.00 (m, 2H, H-5a', Fmoc-C*H*), 3.95 − 3.85 (m, 3H, H-4, H-5a, H-3'), 3.55 (t, *J* = 8.2 Hz, 1H, H-3), 3.32 (dd, *J* = 12.5, 6.5 Hz, 1H, H-5b'), 3.14 − 3.06 (m, 1H, H-5b) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 165.8, 165.2, 154.7 (3C, C=O), 143.3, 143.1, 141.3, 137.6, 137.0, 133.6, 133.2, 130.0, 129.9, 129.8, 129.3, 128.6, 128.5, 128.4, 128.3, 128.3, 128.1, 128.0, 127.7, 127.3, 125.2, 125.1, 120.1 (36C, Ar), 99.6 (C-1), 98.5 (C-1'), 79.0 (C-3), 75.4 (C-3'), 74.4 (*C*H_2Ph), 74.0 (C-4'), 72.5 (C-2), 71.9 (C-2'), 70.2 (Fmoc-*C*H_2), 70.0 (*C*H_2Ph), 69.8 (C-4), 62.3 (C-5), 60.5 (C-5'), 46.7 (Fmoc-*C*H) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{53}H_{48}O_{13}Na^+$: 915.2987; found: 915.2817. IR (neat): ν_{max} = 1727, 1258, 1097, 1069 cm^{-1}.

Benzyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-2-*O*-benzoyl-4-*O*-fluorenylmethoxycarbonyl-β-D-xylopyranosyl-(1→4)-3-*O*-benzyl-2-*O*-benzoyl-β-D-xylopyranoside (57)



A mixture of **SI3** (6.95 g, 7.78 mmol), arabinose BB **2a** (5.13 g, 10.1 mmol) and activated molecular sieves (4 Å, 1.00 g) in anhydrous DCM (50 mL) was stirred at r.t. for 30 min under an Argon atmosphere. After the mixture was cooled to −40 °C, NIS (7.00 g, 31.1 mmol) and TfOH (0.137 mL, 1.56 mmol) were added. The reaction mixture was allowed to warm to −20 °C for 30 min. The reaction mixture was diluted with DCM (50 mL) and quenched by the addition of aq. sat. $NaHCO_3$ solution (50 mL). The phases were separated and the organic phase was washed with aq. sat. $Na_2S_2O_3$ solution (50 mL) and H_2O (50 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated. The crude product was purified via column chromatography (SiO_2, EtOAc/Hex = 1:4) to give fully protected trisaccharide **57** (9.64 g, 7.21 mmol, 93%) as a colorless solid. $[\alpha]_D^{25}$ = -26.3 (*c* 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.10 – 7.88 (m, 8H, Ar*H*), 7.71 – 7.27 (m, 22H, Ar*H*), 7.21 – 7.06 (m, 13H, Ar*H*), 5.57 – 5.49 (m, 1H, H-4‘‘), 5.46 (d, *J* = 6.8 Hz, 1H, H-1‘‘), 5.44 – 5.38 (m, 1H, H-2‘), 5.37 (s, 1H, H-2‘‘), 5.22 (t, *J* = 8.7 Hz, 1H, H-2), 4.98 – 4.84 (m, 1H, H-4‘), 4.84 (d, *J* = 11.4 Hz, 1H, C*H*$_2$Ph), 4.82 – 4.72 (m, 2H, H-5a‘‘, H-1‘), 4.71 – 4.54 (m, 4H, H-3‘‘, C*H*$_2$Ph, C*H*$_2$Ph), 4.50 (d, *J* = 13.2 Hz, 1H, H-5b‘‘), 4.40 (d, *J* = 7.3 Hz, 1H, H-1), 4.35 – 4.26 (m, 1H, H-3‘), 4.26 – 4.19 (m, 1H, H-5a‘), 4.15 – 4.08 (m, 1H, Fmoc-C*H*$_2$), 4.05 – 3.92 (m, 4H, H-4, H-5a, Fmoc-C*H*, Fmoc-C*H*$_2$), 3.67 (t, *J* = 8.4 Hz, 1H, H-3), 3.52 – 3.40 (m, 1H, H-5b‘), 3.12 (t, *J* = 10.3 Hz, 1H, H-5b) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 166.2, 165.6, 165.2, 165.1, 165.0, 164.9 (6C, C=O), 154.2, 154.1, 145.5, 145.3, 144.5, 144.0, 143.1, 142.2, 140.9, 140.8, 140.3, 140.1, 140.0, 139.8, 137.9, 137.1, 137.0, 136.9, 134.3, 134.2, 133.9, 133.8, 133.7, 133.4, 133.3, 133.0, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5, 125.0, 124.9, 121.8, 121.7, 120.2 (54C, Ar), 105.7 (C-1‘‘), 100.4 (C-1‘), 99.7 (C-1), 82.1 (2C, C-3‘‘, C-2‘‘), 78.9 (C-3), 77.5 (C-4‘‘), 77.3 (C-4), 74.5 (*C*H_2Ph), 74.3 (C-3’), 73.2 (C-4’), 72.4 (C-2), 70.0 (C-5‘‘), 69.6 (Fmoc-*C*H_2), 63.8 (*C*H_2Ph), 62.8 (C-5), 62.3 (C-5‘), 46.3 (Fmoc-*C*H) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{79}H_{68}O_{20}Na^+$:1359.4202; found: 1359.4196. IR (neat): ν_{max} = 1725, 1256, 1107, 1069, cm^{-1}.

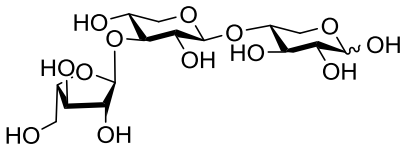
Benzyl α-L-arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-3-*O*-benzyl-β-D-xylopyranoside (SI4)



The protected trisaccharide **57** (2.50 g, 1.87 mmol) was dissolved in DCM (6 mL) and MeOH (12 mL). NaOMe (0.5 M in MeOH, 10.5 mL, 5.23 mmol) was added and the

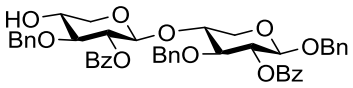
reaction mixture was stirred at r.t. for 18 h. The reaction was neutralized by the addition of Amberlite IR-120 (H^+) resin until the pH was 7. The resin was filtered off and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc to EtOAc/MeOH =20:1) to give semi-protected trisaccharide **SI4** (813 mg, 1.37 mmol, 73%) as a yellow oil. $[\alpha]_D^{25}$ = -8.7 (*c* 0.71, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 - 7.21 (m,8H, Ar*H*), 5.30 (d, J = 1.7 Hz, 1H, H-1''), 4.79 (d, J = 11.9 Hz, 1H, C*H*$_2$Ph), 4.74 - 4.60 (m, 3H, H-1', C*H*$_2$Ph), 4.48 (d, J = 11.9 Hz, 1H, C*H*$_2$Ph), 4.35 (d, J = 6.3 Hz, 1H, H-1), 4.21 (s, 1H, H-2''), 4.18 - 4.06 (m, 3H, H-3, H-4, H-5a), 4.01 - 3.90 (m, 1H, H-5a''), 3.88 - 3.77 (m, 2H, H-3', H5a'), 3.76 - 3.60 (m, 4H, H-2', H-4', H-5b' H-4''), 3.50 (d, J = 7.2 Hz, 3H, H-2, H5b, H-3''), 3.19 (t, J = 10.3 Hz, 1H, H-5b'') ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.1, 137.5, 128.5, 128.5, 128.1, 127.9, 127.9, 127.8 (8C, Ar), 108.5 (C-1''), 101.8 (C-1'), 100.7 (C-1), 84.3, 82.9, 81.4, 77.4, 76.4, 73.9, 72.7, 70.1, 68.9, 68.6, 65.5, 61.4, 59.3 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{29}H_{38}NaO_{13}^+$: 617.2210; found 617.2214. IR (neat): ν_{max} = 3392, 1062, 1029, 699 cm^{-1}.

α-L-Arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-D-xylopyranose (53)



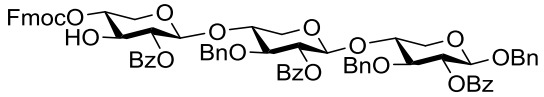
The semi-protected trisaccharide **SI4** (813 mg, 1.37 mmol) was dissolved in MeOH (8 mL) and Pd/C (300 mg, 10% Pd) was added. Hydrogen gas was bubbled through the reaction mixture for 10 min and the reaction mixture was stirred under a hydrogen atmosphere for 2 d. The reaction mixture was diluted with H_2O (5 mL) and the catalyst was filtered off by using a syringe filter (4.5 μm). After evaporation of the solvents fully deprotected trisaccharide **53** (565 mg, 1.36 mmol, 99%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 5.16 (d, *J* = 1.8 Hz, 1H, H-1''), 5.02 (d, *J* = 3.6 Hz, 0.5H, H-1α), 4.41 (d, *J* = 7.9 Hz, 0.5H, H-1β), 4.31 (d, *J* = 7.8 Hz, 1H; H-1'), 4.00 (dd, *J* = 5.5, 3.6 Hz, 2H), 3.91 – 3.76 (m, 3H), 3.68 – 3.47 (m, 6H), 3.42 (t, *J* = 8.1 Hz, 1H), 3.37 (t, *J* = 8.0 Hz, 1H), 3.28 – 3.09 (m, 3H), 3.08 (dd, *J* = 9.4, 7.8 Hz, 1H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 107.9 (C-1''), 101.6 (C-1'), 101.6 (C-1β), 96.3 (C-1α), 91.8, 83.8, 81.2, 81.2, 81.0, 76.5, 76.4, 76.3, 73.8, 73.7, 72.7, 72.7, 71.2, 70.8, 67.6, 64.9, 62.8, 61.1, 58.7 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{15}H_{26}NaO_{13}^+$: 437.1271; found 437.1342. IR (neat): ν_{max} = 1352, 1277, 1021, 899 cm^{-1}.

Benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (SI5)



The protected disaccharide **55** (6.05 g, 6.11 mmol) was dissolved in DCM (50 mL). NEt_3 (8.45 mL, 61.1 mmol) was added and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was quenched by the addition of 1 M HCl solution (50 mL). The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 2:1) to give compound **SI5** (4.17 g, 5.49 mmol, 90%) as a colorless solid. $[\alpha]_D^{25}$ = -33.0 (*c* 0.74, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 – 8.03 (m, 2H, Ar*H*), 7.95 – 7.90 (m, 2H, Ar*H*), 7.65 – 7.54 (m, 2H, Ar*H*), 7.52 – 7.39 (m, 4H, Ar*H*), 7.25 – 7.05 (m, 15H, Ar*H*), 5.25 – 5.19 (m, 2H, H-2, H-2'), 4.79 – 4.71 (m, 3H, CH_2Ar), 4.70 (d, *J* = 5.9 Hz, 1H, H-1), 4.61 (dd, *J* = 11.5, 3.1 Hz, 2H, CH_2Ar), 4.52 (d, *J* = 12.6 Hz, 1H, CH_2Ar), 4.43 (d, *J* = 7.2 Hz, 1H, H-1'), 4.09 (dd, *J* = 11.8, 4.4 Hz, 1H, H-5a'), 4.03 – 3.94 (m, 2H, H-5a, H-4'), 3.83 – 3.74 (m, 1H, H-4), 3.67 – 3.60 (m, 2H, H-3, H-3'), 3.31 (dd, *J* = 11.9, 8.0 Hz, 1H, H-5b'), 3.20 – 3.12 (m, 1H, H-5b) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.1, 165.0 (2C, C=O), 137.9, 137.6, 137.0, 133.4, 133.0, 129.9, 129.8, 129.7, 129.5, 128.6, 128.5, 128.2, 128.1, 128.0, 127.6, 127.4 (30C, Ar), 99.8 (C-1), 99.6 (C-1'), 80.2 (C-3), 79.0 (C-3'), 76.1 (C-4'), 74.2 (*C*H_2Ar), 73.8 (*C*H_2Ar), 72.4 (C-2), 72.2 (C-2'), 69.9 (*C*H_2Ar), 68.7 (C-4), 64.1 (C-5'), 62.6 (C-5) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{45}H_{44}NaO_{11}^+$: 783.2781; found: 783.2797. IR (neat): ν_{max} = 1731, 1211, 1095, 1070 cm^{-1}.

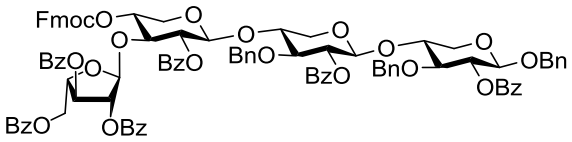
Benzyl 2-*O*-benzoyl-4-*O*-fluorenylcarboxymethyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (SI6)



Disaccharide **SI5** (3.70 g, 4.87 mmol), xylose BB **8b** (4.22 g, 5.84 mmol) and powdered molecular sieves (4 Å, 500 mg) in anhydrous DCM (27.0 mL) were cooled to -30 °C. NIS (4.38 g, 19.4 mmol) and TfOH (1.06 mL, 5.84 mmol) were added and the reaction mixture was stirred at −30 °C. After 30 min, the reaction mixture was diluted with DCM (25 mL) and quenched by the addition of aq. sat. $NaHCO_3$ solution (50 mL). The phases were separated and the organic phase was washed with aq. sat. $Na_2S_2O_3$ solution (50 mL) and H_2O (50 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated. Flash column chromatography (SiO_2, EtOAc/Hex = 1:4) afforded the crude

trisaccharide **58** (7.00 g). The crude trisaccharide **58** (3.40 g) was dissolved in a mixture of DCM (20.0 mL), MeOH (5.00 mL) and H_2O (0.100 mL) and DDQ (1.69 g, 7.51 mmol) was added. The reaction mixture was stirred at r.t. for 4 h. The reaction mixture was diluted with DCM (20 mL) and washed with aq. sat. $NaHCO_3$ solution (30 mL) and H_2O (30 mL). The organic layer was dried with Na_2SO_4 and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 4:1) to give compound **SI6** (2.28 g, 1.87 mmol, 75% over 2 steps). $[\alpha]_D^{25}$ = -36.8 (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 – 8.09 (m, 2H, Ar*H*), 8.02 – 7.96 (m, 2H, Ar*H*), 7.92 – 7.87 (m, 2H, Ar*H*), 7.80 – 7.75 (m, 2H, Ar*H*), 7.64 – 7.38 (m, 14H, Ar*H*), 7.32 – 7.27 (m, 2H, Ar*H*), 7.22 – 7.03 (m, 14H, Ar*H*), 5.23 – 5.15 (m, 2H, H-2, H-2'), 5.07 (dd, *J* = 7.0, 5.3 Hz, 1H, H-2''), 4.80 – 4.55 (m, 8H, H-1', H-1'', H-4'', C*H*$_2$Ar), 4.48 (d, *J* = 12.6 Hz, 1H,C*H*$_2$Ar), 4.42 (dd, *J* = 7.4, 1.8 Hz, 2H, Fmoc-C*H*$_2$), 4.37 (d, *J* = 7.3 Hz, 1H, H-1), 4.23 (t, *J* = 7.3 Hz, 1H, Fmoc-C*H*), 4.18 (dd, *J* = 12.6, 4.3 Hz, 1H, H-5a''), 4.05 – 3.86 (m, 5H, H-4, H-5a, H-5a', H-4', H-3''), 3.72 (t, *J* = 7.9 Hz, 1H, H-3'), 3.63 – 3.54 (m, 1H, H-3), 3.45 (dd, *J* = 12.5, 6.9 Hz, 1H, H-5b''), 3.22 (dd, *J* = 11.6, 8.3 Hz, 1H, H-5b'), 3.12 – 3.01 (m, 1H, H-5b) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.9, 165.2, 165.1, 154.6 (4C, C=O), 143.2, 143.0, 141.3, 138.0, 137.5, 136.9, 133.8, 133.4, 133.0, 129.9, 129.8, 129.5, 129.2, 128.7, 128.6, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.6, 127.4, 127.2, 125.2, 125.1, 120.1 (48C, Ar), 100.1 (C-1''), 99.6 (C-1), 98.5 (C-1'), 78.8 (C-3), 78.6 (C-3'), 76.5 (C-4), 75.1 (C-4'), 74.3 (*C*H$_2$Ar), 74.2 (*C*H$_2$Ar), 74.1 (C-4''), 72.5, 72.3 (2C, C-2, C-2'), 72.0 (C-1''), 70.3 (C-3''), 70.2 (Fmoc-*C*H$_2$), 70.0 (2C, *C*H$_2$Ar), 62.6 (C-5), 62.0 (C-5'), 60.6 (C-5''), 46.6 (Fmoc-*C*H) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{72}H_{66}NaO_{18}^+$: 1241.4147; found: 1241.4208. IR (neat): ν_{max} = 1728, 1261, 1097, 1069 cm^{-1}.

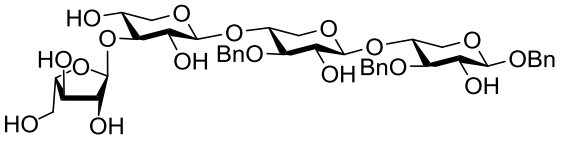
Benzyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-2-*O*-benzoyl-4-*O*-fluorenyl-carboxymethyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (59)



Trisaccharide **SI6** (2.07 g, 1.70 mmol), arabinose BB **2a** (1.12 g, 2.21 mmol) and powdered molecular sieves (4 Å, 250 mg) in anhydrous DCM (15.0 mL) were cooled to -40 °C. NIS (1.52 g, 6.80 mmol) and TfOH (30.0 μL, 0.340 mmol) were added and the reaction mixture was stirred at −20 °C. After 30 min, the reaction mixture was diluted with DCM (20 mL) and quenched by the addition of aq. sat. $NaHCO_3$ solution (25 mL). The phases were separated and the organic phase was washed with aq. sat. $Na_2S_2O_3$ solution

(25 mL) and H_2O (25 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 4:1) to give tetrasaccharide **59** (2.26 g, 1.36 mmol, 79%) as a colorless solid. $[\alpha]_D^{25}$ = -41.2 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ =8.00 – 7.75 (m, 10H, Ar*H*), 7.68 (s, 1H, Ar*H*), 7.61 – 6.88 (m, 42H, Ar*H*), 5.45 – 5.40 (m, 1H, H-4'''), 5.38 – 5.30 (m, 2H, H-2'', H-1'''), 5.28 (s, 1H, H-2'''), 5.08 – 5.00 (m, 2H, H-2, H-2'), 4.87 – 4.79 (m, 1H, H-4''), 4.75 (d, *J* = 11.5 Hz, 1H, C*H*$_2$Ar), 4.70 – 4.44 (m, 8H, H-1'', H-3''', H-5a''', C*H*$_2$Ar), 4.40 – 4.33 (m, 2H, H-1', H-5a'''), 4.27 – 4.17 (m, 2H, H-1, H-3''), 4.13 (dd, *J* = 11.8, 4.9 Hz, 1H, H-5a''), 4.03 – 3.69 (m, 7H, H-4, H-4', Fmoc-C*H*$_2$, Fmoc-C*H*, H-5a, H-5a'), 3.58 (t, *J* = 8.3 Hz, 1H, H-3'), 3.45 (t, *J* = 8.2 Hz, 1H, H-3), 3.41 – 3.29 (m, 1H, H-5b'''), 3.00 – 2.86 (m, 2H, H-5b, H-5b') ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 166.1, 166.1, 165.7, 165.6, 165.2, 165.0, 165.0, 164.9, 154.2, 154.1 (10C, C=O), 145.3, 144.0, 140.0, 139.8, 138.0, 137.8, 137.2, 137.1, 137.0, 134.3, 134.2, 133.9, 133.8, 133.7, 133.5, 133.4, 133.2, 133.0, 130.0, 129.9, 129.8, 129.7, 129.5, 129.3, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 127.8, 127.6, 127.6, 127.4, 121.8, 120.2 (66C, Ar), 106.0 (C-1'''), 100.5 (2C, C-1', C-1''), 99.6 (C-1), 82.2 (C-3'''), 82.1 (C-2'''), 78.7, 78.5 (2C, C-3, C-3'), 77.4 (2C, C-4, C-4'), 67.8 (C-4'''), 74.9 (C-3'''), 74.2 (2C, *C*H$_2$Ar), 73.4 (C-4''), 72.5, 72.3 (2C, C-2, C-2'), 70.0 (C-5'''), 69.1 (Fmoc-*C*H$_2$), 63.8 (*C*H$_2$Ar), 62.7, 62.5 (3C, C-5, C-5', C-5''), 46.2 (Fmoc-*C*H) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{98}H_{86}NaO_{25}^+$: 1685.5356; found: 1685.5345. IR (neat): ν_{max} = 1723, 1253, 1096, 1068, 709 cm^{-1}.

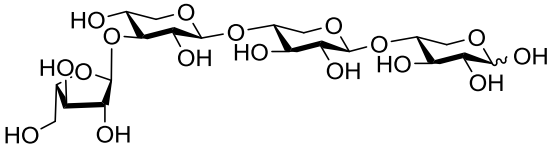
Benzyl α-L-arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-3-*O*-benzyl-β-D-xylopyranosyl-(1→4)-3-*O*-benzyl-β-D-xylopyranoside (SI7)



The protected tetrasaccharide **59** (1.90 g, 1.14 mmol) was dissolved in DCM (4.00 mL) and MeOH (8.00 mL). NaOMe (0.5 M in MeOH, 185 mg, 3.42 mmol) was added and the reaction mixture was stirred at r.t. for 18 h. The reaction mixture was neutralized by the addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc to EtOAc/MeOH = 20:1) to give semi-protected tetrasaccharide **SI7** (607 mg, 744 mmol, 65%) as a yellow oil. $[\alpha]_D^{25}$ = -99.2 (*c* 0.48, $CHCl_3$). ^{1}H NMR (400 MHz , $CDCl_3$): δ = 7.41 – 7.27 (m, 15H, Ar*H*), 5.28 (s, 1H, H-1'''), 4.85 (d, *J* = 11.8 Hz, 1H), 4.73 – 4.62 (m, 6H), 4.59 – 4.50 (m, 2H, H-1'), 4.27 (d, *J* = 6.2 Hz, 1H, H-1), 4.22 – 4.00 (m, 6H), 3.95 – 3.72 (m, 5H), 3.70 – 3.42 (m, 12H), 3.39 – 3.30 (m, 2H), 3.21 – 3.11 (m, 1H) ppm. ^{13}C

NMR (101 MHz, $CDCl_3$): δ = 138.2, 137.7, 137.2, 128.4, 128.3, 128.1, 127.9, 127.8, 127.6, 108.4, 101.4 (2C), 99.2, 84.1, 82.7, 81.1, 78.9, 76.4, 76.2, 73.6, 73.4, 73.1, 72.8, 72.5, 70.8, 70.3, 68.6 (2C), 65.3, 61.2, 60.8, 59.2 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{41}H_{52}NaO_{17}^+$: 839.3102; found: 839.3132. IR (neat): ν_{max} = 1256, 1061, 1028, 752 cm^{-1}.

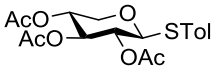
α-L-Arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-D-xylopyranose (54)



The semi protected tetrasaccharide **SI7** (550 mg, 0.674 mmol) was dissolved in MeOH (10.0 mL) and EtOAc (5.00 mL) and Pd/C (250 mg, 10% Pd) was added. Hydrogen gas was bubbled through the reaction mixture for 10 min and the reaction mixture was stirred under a hydrogen atmosphere for 2 d. The reaction mixture was diluted with H_2O (5.00 mL) and NEt_3 (0.1 mL) was added. The catalyst was filtered off by using a syringe filter (4.5 μm) and the solvents were evaporated to give crude compound **43** (294 mg). The compound was used in the next step without further purification.

3.4.4 Chemo-Enzymatic Synthesis of Arabinoxylan Oligosaccharides

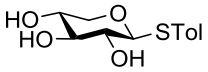
4-Methylphenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside (SI8)[232]



D-Xylose (15.0 g, 100 mmol) was suspended in Et_2O (100 mL) and acetic anhydride (57.0 mL, 600 mmol), pyridine (48.0 mL, 600 mmol) and DMAP (100 mg, 0.800 mmol) were added. The mixture was stirred at r.t. for 20 h. The homogeneous reaction mixture was washed with 2 M HCl solution (3 x 150 mL) and the organic phase was dried over Na_2SO_4. After filtration, evaporation of the solvent and co-evaporation with toluene, the crude acetyl 2,3,4-tri-*O*-acetyl-1-D-xylopyranoside (32.5 g) was obtained as a colorless oil. The crude compound (32.5 g) was dissolved in anhydrous DCM (200 mL) and TolSH (13.7 g, 110 mmol) was added. After the reaction mixture was cooled to 0 °C, $BF_3{\cdot}Et_2O$ (26.6 mL, 210 mmol) was added dropwise under Argon atmosphere. The reaction mixture was warmed up to r.t. and stirred overnight. The deep red mixture was quenched with aq. sat. $NaHCO_3$ solution (200 mL). The layers were separated, and the organic layer was washed with aq. sat. $NaHCO_3$ solution (200 mL). The organic phase was dried over

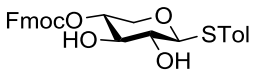
Na_2SO_4, filtered and concentrated to yield the crude product as a brown oil. The mixture was recrystallized from Hex/EtOAc to obtain 4-methylphenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside **SI8** (20.7 g, 55%) as colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 – 7.33 (m, 2H, Ar*H*), 7.17 – 7.09 (m, 2H, Ar*H*), 5.17 (t, *J* = 8.3 Hz, 1H, H-3), 4.99 – 4.83 (m, 1H, H-4), 4.71 (d, *J* = 8.5 Hz, 1H, H-1), 4.25 (dd, *J* = 11.7, 5.0 Hz, 1H, H-5a), 3.39 (dd, *J* = 11.7, 9.0 Hz, 1H, H-5b), 2.34 (s, 3H, C*H*$_3$), 2.10 (s, 3H, C*H*$_3$), 2.04 (s, 6H, C*H*$_3$) ppm. The analytical data matches the one reported in literature.[232]

4-Methylphenyl 1-thio-β-D-xylopyranoside (68)[232]



4-Methylphenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside **SI8** (16.0 g, 41.8 mmol) was dissolved in DCM (35.0 mL) and MeOH (35.0 mL). To this mixture NaOMe (2.30 g, 41.8 mmol) was added and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin. After filtration the solvent was evaporated. The residue was dissolved in the smallest amount of MeOH possible (about 5 mL). After the addition of a mixture of Et_2O and Hex (35 mL, 1:1 v/v) a fine solid precipitated. The solid was filtered off and washed with Et_2O to give 4-methylphenyl 1-thio-β-D-xylopyranoside **68** (7.01 g, 27.3 mmol, 65%) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.44 – 7.34 (m, 2H, Ar*H*), 7.18 – 7.05 (m, 2H, Ar*H*), 4.41 (d, *J* = 8.4 Hz, 1H, H-1), 3.88 (dd, *J* = 11.2, 5.2 Hz, 1H, H-3), 3.47 – 3.35 (m, 1H, H-4), 3.32 – 3.24 (m, 1H, H-5a), 3.20 – 3.08 (m, 2H, H-2, H-5b), 2.28 (s, 3H, C*H*$_3$) ppm. The analytical data matches the one reported in literature.[232]

4-Methylphenyl 4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-xylopyranoside (69)[232]



4-Methylphenyl 1-thio-β-D-xylopyranoside **68** (7.01 g, 27.3 mmol) was dissolved in anhydrous 1,4-dioxane (120 mL). Dibutyltin oxide (8.20 g, 32.8 mmol) was added. The suspension was refluxed for 2 h. After the solvent was evaporated a brown foam was obtained. The residue was taken up in anhydrous DCM (100 mL), cooled to 0 °C and FmocCl (7.10 g, 27.3 mmol) and pyridine (8.80 mL, 109 mmol) were added. The mixture was stirred at r.t. for 2 h. The reaction mixture was quenched by the addition of aq. sat. $NaHCO_3$ solution (50 mL) and a colorless solid precipitated. The mixture was filtered through a plug of celite and the phases were separated. The organic layer was washed with aq. sat. $NaHCO_3$ solution (2 x 50 mL). The aq. phase was reextracted with DCM (30 mL) and the combined organic phases were dried over Na_2SO_4. After filtration the

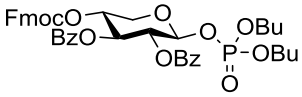
mixture was concentrated and the crude product was recrystallized from EtOAc/Hex to give 4-methylphenyl 4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-xylopyranoside **69** as a colorless solid (9.42 g, 19.6 mmol, 72%). $[a]_D^{25}$ = -43.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 – 7.67 (m, 2H, Ar*H*), 7.63 – 7.53 (m, 2H, Ar*H*), 7.45 – 7.36 (m, 4H, Ar*H*), 7.35 – 7.26 (m, 2H, Ar*H*), 7.18 – 7.11 (m, 2H, Ar*H*), 4.70 – 4.61 (m, 1H, H-4), 4.51 – 4.35 (m, 3H, Fmoc-CH_2, H-1), 4.23 (t, ^3J=7.2 Hz, 1H, Fmoc-C*H*), 4.18 (dd, *J* = 11.4, 5.4 Hz 1H, H-5a), 3.76 (t, *J* = 8.9 Hz, 1H, H-3), 3.40 – 3.29 (m, 2H, H-5b, H-2), 2.36 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 154.6, 143.2, 143.0, 141.3, 138.9, 133.8, 129.9, 129.9, 127.2, 127.9, 125.0, 125.1, 120.1 (18C, Ar), 88.4 (1C, C-1), 75.1 (1C, C-3), 74.2 (1C, C-4), 71.7 (1C, C-2), 70.2 (1C, Fmoc-CH_2), 66.4 (1C, C-5), 46.6 (1C, Fmoc-*C*H), 21.2 (1C, CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{27}H_{26}NaO_6S^+$: 501.1348; found: 501.1335. IR (neat): ν_{max} = 1754, 1261, 1089, 1069, 1037, 971, 758, 737, 710 cm^{-1}.

4-Methylphenyl 2,3-di-*O*-benzoyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-xylopyranoside (65)

4-Methylphenyl 4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-xylopyranoside **69** (9.00 g, 18.8 mmol) was dissolved in anhydrous DCM (100 mL) and cooled to 0 °C. Pyridine (9.20 mL, 113 mmol) and benzoyl chloride (8.73 mL, 75.0 mmol) were added. The mixture was stirred at r.t. for 4 h. To quench the reaction 1 M HCl solution (50 mL) was added. After separation of the phases the organic layer was washed with aq. sat. $NaHCO_3$ solution (3 x 30 mL), dried over Na_2SO_4 and concentrated. The crude product was recrystallized from EtOAc/Hex to give 4-methylphenyl 2,3-di-*O*-benzoyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-xylopyranoside **65** (8.32, 12.1 mmol, 64%) as colorless crystals. $[a]_D^{25}$ = -7.60 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 – 7.74 (m, 4H, Ar*H*), 7.48 – 7.42 (m, 2H, Ar*H*), 7.28 – 7.17 (m, 4H, Ar*H*), 7.14 – 7.03 (m, 8H, Ar*H*), 6.98 – 6.92 (m, 1H, Ar*H*), 6.91 – 6.80 (m, 3H, Ar*H*), 5.37 (t, *J* = 6.7 Hz, 1H, H-3), 5.13 (t, *J* = 6.4 Hz, 1H, H-2), 4.88 (d, *J* = 6.2 Hz, 1H, H-1), 4.75 – 4.65 (m, 1H, H-4), 4.29 (dd, *J* = 12.3, 3.8 Hz, 1H, H-5a), 4.13 – 3.97 (m, 2H, Fmoc-CH_2), 3.86 (t, *J* = 7.6 Hz, 1H, Fmoc-C*H*), 3.51 (dd, *J* = 12.3, 6.7 Hz, 1H, H-5b), 2.04 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, CDCl3): δ = 165.2, 165.2, 154.3, 143.3, 143.1, 141.3, 141.2, 138.6, 134.7, 133.7, 133.5, 133.3, 130.7, 130.2, 130.1, 129.9, 129.3, 129.2 129.0, 128.9, 128.6, 128.5, 128.0, 127.3, 125.3, 125.2, 120.1 (30C, Ar), 86.6 (1C, C-1), 71.5 (1C, C-4), 70.6 (1C, Fmoc-CH_2), 70.5 (1C, C-3), 69.8 (1C, C-2), 63.4 (1C, C-5), 46.6 (1C, Fmoc-*C*H), 21.3 (1C, CH_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{41}H_{34}NaO_8S^+$: 709.1872; found: 709.1856. IR

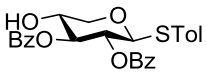
(neat): ν_{max} = 1727, 1451, 1276, 1244, 1092 cm^{-1}.

Dibutoxyphosphoryloxy 2,3-di-*O*-benzoyl-4-*O*-fluorneylmethoxycarbonyl-1-thio-β-D-xylopyranoside (66)



4-Methylphenyl 2,3-di-*O*-benzoyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-xylopyranoside **65** (5.12 g, 7.43 mmol) was dissolved in anhydrous DCM (150 mL) and cooled to 0 °C under Argon atmosphere. Dibutyl hydrogen phosphate (3.68 mL, 18.6 mmol), NIS (2.01 g, 8.99 mmol) and TfOH (0.20 mL, 2.23 mmol) were added. After stirring for 1 h at 0 °C the reaction was quenched with aq. sat. $NaHCO_3$ solution (100 mL) and washed with aq. sat. $Na_2S_2O_3$ solution (50 mL). The organic phase was dried over Na_2SO_4, filtered and after the removal of the solvent the crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 4:1) to give phosphate donor **66** as a colorless oil (4.83 g, 6.21 mmol, 84%, α/β = 1:10). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12 – 7.99 (m, 4H, Ar*H*), 7.75 – 7-70 (m, 2H, Ar*H*), 7.59 - 7.46 (m, 4H, Ar*H*), 7.42 – 7.32 (m, 6H, Ar*H*), 7.29 – 7.14 (m, 2H, Ar*H*), 5.69 – 5.60 (m, 2H, H-1, H-3), 5.42 (dd, *J* = 6.4, 4.8 Hz, 1H, H-2), 5.02 (td, *J* = 6.2, 3.7 Hz, 1H, H-4), 4.45 (dd, *J* = 12.6, 3.8 Hz, 1H, H-5a), 4.37 – 4.30 (m, 2H, Fmoc-CH_2), 4.16 (t, *J* = 7.6 Hz, 1H, Fmoc-C*H*), 4.12 – 4.03 (m, 2H, OBu), 3.95 – 3.83 (m, 3H, OBu, H-5b), 1.70 – 1.59 (m, 2H, Bu), 1.48 – 1.34 (m, 4H, Bu), 1.23 – 1.10 (m, 2H, Bu), 0.90 (t, *J* = 7.4 Hz, 3H, OBu), 0.76 (t, *J* = 7.4 Hz, 3H, OBu) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.0, 164.9, 154.2 (3C, C=O), 143.1, 142.9, 141.2, 133.6, 130.0, 129.9, 128.8, 128.5, 127.9, 127.2, 125.1, 120.0 (24C, Ar), 95.8 (1C, C-1), 70.9 (1C, C-4), 70.5 (1C, Fmoc-*C*H_2), 69.0 (1C, C-3), 68.9 (1C, C-2), 68.1, 68.0 (2C, OBu), 61.3 (1C, C-5), 46.5 (Fmoc-*C*H), 32.2, 32.0 (2C, Bu), 18.6, 18.4 (2C, Bu), 13.6, 13.7 (2C, OBu) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{42}H_{45}NaO_{12}P^+$: 795.2456; found: 795.2550. IR (neat): ν_{max} = 1729, 1246, 1026, 712 cm^{-1}.

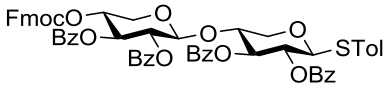
4-Methylphenyl 2,3-di-*O*-benzoyl-1-thio-β-D-xylopyranoside (70)



4-Methylphenyl 2,3-di-*O*-benzoyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-xylopyranoside **65** (3.44 g, 5.22 mmol) was dissolved in DCM (50 mL). To the mixture was added NEt_3 (2.90 mL, 20.1 mmol). After the reaction mixture was stirred for 10 h at r.t. the reaction was quenched by the addition of 1 M HCl solution (30 mL). The layers were separated and the aqueous layer was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by

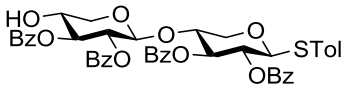
column chromatography (SiO_2, Hex/EtOAc = 3:1 to 2:1) to give 4-methylphenyl 2,3-di-*O*-benzoyl-1-thio-β-D-xylopyranoside **70** (1.89 g, 4.09 mmol, 80%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 – 7.96 (m, 4H, Ar*H*), 7.59 – 7.49 (m, 2H, Ar*H*), 7.45 – 7.36 (m, 6H, Ar*H*), 7.17 – 7.09 (m, 2H, Ar*H*), 5.47 – 5.24 (m, 2H, H-2, H-3), 5.01 (d, *J* = 7.5 Hz, 1H, H-1), 4.41 (dd, *J* = 11.9, 4.4 Hz, 1H, H-5a), 4.12 – 3.90 (m, 1H, H-4), 3.58 (dd, *J* = 11.8, 8.2 Hz, 1H, H-5b), 3.17 (s, 1H, OH), 2.34 (s, 3H, C*H*$_3$) ppm. The analytical data matches the one reported in literature.[249]

4-Methylphenyl 2,3-di-*O*-benzoyl-4-*O*-fluoromethoxycarbonyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-benzoyl-1-thio-β-D-xylopyranoside (SI9)



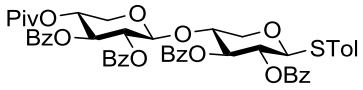
4-Methylphenyl 2,3-di-*O*-benzoyl-1-thio-β-D-xylopyranoside **70** (0.970 g, 2.09 mmol) and phosphate donor **66** (1.93 g, 2.51 mmol) were dissolved in anhydrous DCM (65.0 mL) and cooled to -78 °C under Argon atmosphere. After TMSOTf (0.378 mL, 2.09 mmol) was added, the mixture was slowly warmed up to 0 °C. After 4 h the reaction was quenched with aq. sat. $NaHCO_3$ solution (80 mL). The layers were separated and the aqueous layer was extracted with DCM (2 x 50 mL). The combined organic phases were dried over Na_2SO_4 and filtered. After the solvent was evaporated the crude product was purified by silica gel flash column chromatography (SiO_2, Hex/EtOAc = 4:1 to 2:1) to give **SI19** as a colorless solid (1.42 g, 1.38 mmol, 66%). $[a]_D^{25}$ = 7.45 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 7.91 (m, 8H, Ar*H*), 7.75 – 7.69 (m, 2H, Ar*H*), 7.57 – 7.33 (m, 17H, Ar*H*), 7.26 – 7.13 (m, 3H, Ar*H*), 7.11 – 7.06 (m, 2H, Ar*H*), 5.64 (t, *J* = 8.0 Hz, 1H, H-3), 5.52 (t, *J* = 6.6 Hz, 1H, H-3‘), 5.30 (t, *J* = 8.2 Hz, 1H, H-2), 5.25 – 5.15 (m, 1H, H-2‘), 4.94 – 4.88 (m, 2H, H-1, H-1‘), 4.77 – 4.70 (m, 1H, H-4‘), 4.38 – 4.17 (m, 3H, Fmoc-C*H*$_2$, H-5a), 4.15 – 4.02 (m, 2H, Fmoc-C*H*, H-4), 3.91 (dd, *J* = 12.5, 3.7 Hz, 1H, H-5a’), 3.50 (dd, *J* = 12.0 Hz, *J* = 8.8 Hz, 1H, H-5b), 3.42 (dd, *J* = 12.5 Hz, 6.2 Hz, 1H, H’-5b), 2.31 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.4, 165.2, 165.1, 164.9, 154.1 (5C, C=O), 143.1, 142.9, 141.2, 141.2, 138.4, 133.5, 133.3, 133.3, 130.0, 129.9, 129.8, 129.7, 129.4, 129.3, 129.0, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 127.9, 127.2, 125.1, 125.0, 120.0 (42C, Ar), 99.5 (C-1‘), 86.9 (C-1), 75.2 (C-4), 73.1 (C-3), 71.2 (C-4‘), 70.4 (C-2), 70.3 (Fmoc-*C*H_2), 69.7 (C-2‘), 69.4 (C-3‘), 65.6 (C-5‘), 60.4 (C-5), 46.5 (Fmoc-*C*H), 21.2 (*C*H_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{60}H_{50}NaO_{15}S^+$: 1049.2819; found: 1049.2802. IR (neat): v_{max} = 1731, 1211, 1095, 1070 cm^{-1}.

4-Methylphenyl 2,3-di-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-benzoyl-1-thio-β-D-xylopyranoside (71)



The protected disaccharide **SI9** (1.35 g, 1.74 mmol) was dissolved in anhydrous DCM (18.0 mL) and NEt_3 (23.4 mL, 174 mmol) was added. The reaction mixture was stirred for 1 h and was quenched by the addition of 1 M HCl solution (25 mL). The phases were separated and the aqueous phase was extracted with DCM (20 mL). The combined organic phases were dried with Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 3:2) to give **71** (1.01 g, 1.25 mmol, 72%) as a colorless solid. $[a]_D^{25}$ = 30.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 – 7.91 (m, 8H, ArH), 7.58 – 7.48 (m, 4H, ArH), 7.45 – 7.30 (m, 10H, ArH), 7.12 – 7.00 (m, 2H, ArH), 5.64 (t, *J* = 8.2 Hz, H-3), 5.40 – 5.13 (m, 3H, H-2, H-2', H-3'), 4.90 (d, *J* = 8.4 Hz, H-1), 4.78 (d, *J* = 6.0 Hz, H-1'), 4.15 (dd, *J* = 12.0, 4.9 Hz, H-5a), 4.08 – 3.98 (m, 1H, H-4), 3.78 – 3.64 (m, 2H, H-4', H-5a'), 3.46 (dd, *J* = 12.0, 9.0 Hz, H-5b), 3.18 (dd, *J* = 11.8, 7.7 Hz, H-5b'), 2.31 (s, 1H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 167.1, 165.6, 165.3, 165.1 (4C, C=O), 138.5, 133.7, 133.6, 133.3, 133.3, 130.1, 130.0, 130.0, 129.9, 129.8, 129.8, 129.8, 129.6, 129.4, 129.1, 128.8, 128.7, 128.6, 128.5, 128.5 (30C, Ar), 101.1 (C-1), 87.0 (C-1'), 76.1 (C-4), 75.5 (C-3'), 73.4 (C-3), 70.7, 70.5 (2C, C-2, C-2'), 68.4 (C-4'), 66.2 (C-5), 64.5 (C-5'), 21.3 (*C*H_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{45}H_{40}NaO_{12}S^+$: 827.2138; found: 827.2112. IR (neat): ν_{max} = 1730, 1311, 1094, 1070, 709 cm^{-1}.

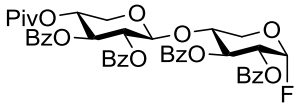
4-Methylphenyl 4-*O*-pivaloyl-2,3-di-*O*-benzoyl-β-D-(1→4)-xylopyranosyl-(1→4)-2,3-di-*O*-benzoyl-1-thio-β-D-xylopyranoside (72a)



Disaccharide **71** (0.690 g, 0.860 mmol) was dissolved in anhydrous DCM (1 mL) and pyridine (1 mL). PivCl (0.160 mL, 1.29 mmol) was added at 0 °C and the reaction mixture was stirred at r.t. for 24 h. Then, the reaction was stopped by the addition of sat. aq. $NaHCO_3$-Lösung (10 mL). The aqueous phase was extracted with DCM (2 x 5 mL) and the combined organic phases were dried with Na_2SO_4. The solvent was removed and the crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 6:1) to give **72a** (0.634 g, 0.713 mmol, 83%) as a colorless solid. $[\alpha]_D^{25}$ = 16,8 (*c* 0,6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (ddd, *J* = 8.5, 3.5, 1.3 Hz, 4H, Ar), 7.96 – 7.91 (m, 2H, Ar), 7.91 – 7.85 (m, 2H, Ar), 7.58 – 7.46 (m, 4H, Ar), 7.46 – 7.34 (m, 9H, Ar), 7.34 – 7.28 (m,

2H, Ar), 7.08 (d, *J* = 7.9 Hz, 2H, Ar), 5.62 (t, *J* = 7.9 Hz, 1H, H-3‘), 5.53 (t, *J* = 8.4 Hz, 1H, H-2), 5.29 (t, *J* = 8.1 Hz, 1H, H-2‘), 5.23 (dd, *J* = 8.6, 6.5 Hz, 1H, H-3), 4.94 – 4.85 (m, 2H, H-4, H-1), 4.79 (d, *J* = 6.5 Hz, 1H, H-1‘), 4.15 (dd, *J* = 12.0, 4.8 Hz, 1H, H-5a‘), 4.05 – 3.95 (m, 1H, H-4‘), 3.73 (dd, *J* = 11.9, 5.0 Hz, 1H, H-5a), 3.46 (dd, *J* = 12.1, 8.6 Hz, 1H, H-5b‘), 3.17 (dd, *J* = 12.0, 8.4 Hz, 1H, H-5b), 2.31 (s, 3H, SPhC*H*$_3$), 1.00 (s, 9H, OC(C*H*$_3$)$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 177.0, 165.2, 164.8, (4C, C=O) 138.2, 133.3, 133.2, 133.1, 133.0, 129.9, 129.7, 129.7, 129.6, 129.5, 129.3, 129.1, 129.0, 128.9, 128.8, 128.4, 128.6, 128.3, 126.2 (20C, Ar), 101.15 (C-1'), 86.8 (C-1), 75.8 (C-4‘), 80.0 (C-3‘), 71.3 (C-2), 71.1 (C-2‘), 70.4 (C-3), 68.2 (C-4), 65.7 (C-5), 61.8 (C-5‘), 28.6 (3C, OC(*C*H$_3$)$_3$), 20.8 (SPhCH$_3$) ppm. IR: ν_{max} = 1734, 1453, 1268, 1095, 1070 cm^{-1}. ESI-HRMS: m/z = [m+Na]$^+$ calcd. for $C_{50}H_{48}NaO_{13}S$: 911.2713; found: 911.2845

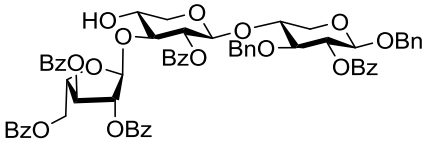
4-Methylphenyl 4-*O*-pivaloyl-2,3-di-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-D-xylopyranosyl fluoride (73a)



Disaccharide **72a** (598 mg, 0.678 mmol) was dissolved in anhydrous DCM (15 mL) and cooled to –50 °C. NIS (227 mg, 1.01 mmol) and HF/pyridin (85.0 μL, 1.04 mmol) were added and the reaction mixture was stirred for 30 min at -50 °C. Then, HF/pyridin (6.00 mL) was added dropwise and the reaction mixture was stirred at –10 °C for 5 h. When the reaction was finished, the reaction mixture was diluted with DCM (10 mL) and the solution was carefully dropped into an aq. NH_3 solution (25%, 30 mL). The phases were separated, and the organic phase was washed with sat. aq. $NaHCO_3$ solution (30 mL). The organic phase was dried with $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 4:1) to give glycosyl fluoride **73a** (0.216 g, 0.275 mmol, 41%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 – 7.94 (m, 6H, Ar), 7.93 – 7.86 (m, 2H, Ar), 7.58 – 7.47 (m, 4H, Ar), 7.44 – 7.33 (m, 9H, Ar), 5.95 (t, *J* = 9.8 Hz, 1H, H-3), 5.83 (dd, *J* = 53.1, 2.7 Hz, 1H, H-1), 5.59 – 5.51 (m, 1H, H-3‘), 5.29 – 5.20 (m, 1H, H-2‘), 5.20 – 5.08 (m, 1H, H-2), 4.94 (td, *J* = 8.0, 4.7 Hz, 1H, H-4‘), 4.84 (d, *J* = 6.1 Hz, 1H, H-1‘), 4.18 (td, *J* = 9.7, 6.6 Hz, 1H, H-4), 3.85 (q, *J* = 5.0, 4.5 Hz, 2H, H-5), 3.79 – 3.70 (m, 1H, H-5a‘), 3.19 (dd, *J* = 12.0, 7.9 Hz, 1H, H-5b’), 1.03 (s, 9H, OC(C*H*$_3$)$_3$) ppm. ^{13}C NMR (151 MHz, $CDCl_3$,): δ = 177.1, 165.7, 165.3, 164.8, (5C, C=O), 133.5, 133.4, 133.3, 133.0, 129.9, 129.7, 129.6, 128.9, 128.8, 128.5, 128.4, 128.3 (16C, Ar) 103.9 (d, *J* = 229.6 Hz, C-1), 100.9 (C-1‘) 75.6 (C-4), 71.5 (C-2‘), 71.4 (C-2), 71.0 (C-3‘), 70.0 (C-3), 68.1 (C-4‘), 61.7 (C-5‘), 61.3 (C-5), 26.7 (3C, OC(*C*H$_3$)$_3$) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = -151.07 (dd, *J* = 53.1, 24.1 Hz) ppm.

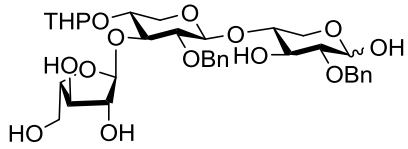
ESI-HRMS: m/z = $[m+Na]^+$ calcd. for $C_{43}H_{41}FNaO_{13}$: 807.2429; found: 807.2450

Benzyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-2-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-xylopyranoside (SI10)



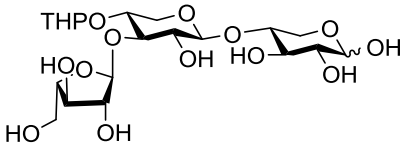
The fully protected trisaccharide **57** (6.75 g, 5.05 mmol) was dissolved in DCM (50.0 mL). NEt_3 (7.03 mL, 50.5 mmol) was added and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was quenched by the addition of 2 M HCl solution (50 mL) and the phases were separated. The organic phase was washed with brine (50 mL) and dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (EtOAc/Hex = 1:2) to give compound **SI10** (5.08 g, 4.56 mmol, 90%) as a yellow oil. $[a]_D^{25}$ = -7.4 (*c* 0.76, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 – 8.00 (m, 6H, Ar), 7.95 – 7.90 (m, 2H, Ar*H*), 7.73 – 7.67 (m, 2H, Ar*H*), 7.64 – 7.28 (m, 15H, Ar*H*), 7.21 – 7.07 (m, 10H, Ar*H*), 5.53 (dd, *J* = 4.9, 2.1 Hz, 1H, H-4''), 5.41 (d, *J* = 2.1 Hz, 1H, H-3''), 5.39 (s, 1H, H-1''), 5.29 (dd, *J* = 8.9, 7.5 Hz, 1H, H-2'), 5.21 (dd, *J* = 8.7, 7.2 Hz, 1H, H-2), 4.82 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.79 – 4.71 (m, 3H, H-2'', H-5a'', CH_2Ph), 4.67 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.65 – 4.58 (m, 2H, H-1', CH_2Ph), 4.49 (d, *J* = 12.6 Hz, 1H, H-5b''), 4.39 (d, *J* = 7.2 Hz, 1H, H-1), 4.10 (dd, *J* = 12.0, 5.2 Hz, 1H, H-5a'), 4.01 – 3.89 (m, 2H, H-4, H-5a), 3.89 – 3.82 (m, 1H, H-4'), 3.78 (t, *J* = 8.7 Hz, 1H, H-3'), 3.65 (dd, *J* = 8.7, 7.8 Hz, 1H, H-3), 3.28 (dd, *J* = 12.0, 9.5 Hz, 1H, H-5b'), 3.10 (dd, *J* = 11.5, 9.0 Hz, 1H, H-5b) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 166.2, 165.8, 165.3, 165.2, 165.0 (5C, C=O), 138.1, 137.1, 133.9, 133.6, 133.3, 133.2, 133.1, 130.2, 130.0, 129.9, 129.9, 129.8, 129.8, 129.6, 128.9, 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 127.7, 127.7, 127.6 (42 C, Ar), 107.5 (C-1''), 101.4 (C-1'), 99.7 (C-1), 84.3 (C-3'), 81.9 (C-2''), 81.6 (C-3''), 78.9 (C-3), 77.4 (C-4), 77.2 (C-4''), 74.3 (*C*H_2Ph), 72.4 (C-2), 72.3 (C-2'), 70.1 (C-5''), 69.1 (C-4'), 65.4 (C-5'), 63.9 (*C*H_2Ph), 62.9 (C-5) ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{64}H_{58}NaO_{18}^+$: 1137.3521; found 1137.3535. IR (neat): ν_{max} = 1070, 1097, 1267, 1726 cm^{-1}.

Benzyl α-L-arabinofuranosyl-(1→3)-4-*O*-tetrahydropyranosyl-β-D-xylopyranosyl-(1→4)-3-*O*-benzyl-β-D-xylopyranoside (SI11)



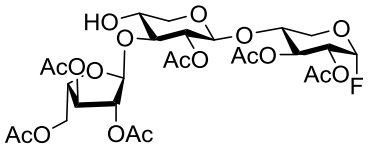
A solution of trisaccharide **SI10** (5.03 g, 4.51 mmol), DHP (1.02 mL, 11.3 mmol) and *p*TsOH monohydrate (17.0 mg, 90.0 μmol) in anhydrous DCM (75.0 mL) was stirred at r.t. for 4 h. The reaction mixture was diluted with DCM (100 mL) and washed with aq. $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated to obtain the crude trisaccharide (5.60 g). The crude compound (5.60 g) was dissolved in anhydrous DCM (14 mL) and MeOH (28.0 mL). NaOMe (0.5 M in MeOH, 16.3 mL, 8.17 mmol) was added and the reaction mixture was stirred at r.t. overnight. The reaction mixture was neutralized by the addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc) to give semi-protected trisaccharide **SI11** (2.01 g, 2.96 mmol, 66% over 2 steps) as a mixture of two diastereomeres. $[\alpha]_D^{25}$ = -91.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 – 7.19 (m, 10H), 5.47 (s, 1H, H-1‘‘), 4.81 (d, *J* = 11.9 Hz, 1H), 4.78 – 4.65 (m, 3H), 4.64 – 4.46 (m, 3H), 4.41 – 4.29 (m, 2H), 4.20 – 4.05 (m, 4H), 4.04 – 3.38 (m, 15H), 3.33 – 3.22 (m, 1H), 1.79 – 1.67 (m, 2H), 1.54 – 1.42 (m, 4H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.1, 137.9, 137.4, 129.1, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 125.3, 108.2, 107.6, 101.9, 101.5, 101.1, 101.0, 100.9, 99.7, 87.1, 86.6, 79.5, 78.3, 78.2, 77.6, 77.4, 77.1, 76.8, 75.7, 74.7, 74.5, 74.2, 73.9, 73.1, 72.9, 72.3, 70.5, 70.3, 70.2, 69.9, 65.7, 64.9, 63.3, 62.0, 61.6, 60.8, 60.3, 31.5, 30.7, 25.2, 24.9, 21.5, 19.9 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{34}H_{46}NaO_{14}^+$:701.2785; found: 701.2804. IR (neat): ν_{max} = 699, 752, 1027, 1064 cm^{-1}.

α-L-Arabinofuranosyl-(1→3)-4-O-tetrahydropyranosyl-β-D-xylopyranosyl-(1→4)-D-xylopyranose (75)



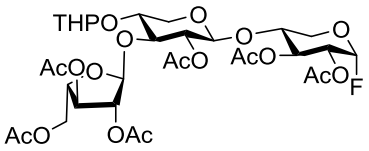
The semi-protected trisaccharide **SI11** (700 mg, 1.03 mmol) was dissolved in MeOH (15 mL), EtOAc (3.5 mL) and sodium phosphate buffer (3.5 mL, 100 mM, pH 7), and Pd/C (700 mg, 10% Pd) was added. Hydrogen gas was bubbled through the reaction mixture for 10 min and was stirred under a hydrogen atmosphere for 1 d. The reaction mixture was diluted with H_2O (5 mL) and the solution was filtered over a short celite column. After evaporation of the solvents the crude trisaccharide **75** (411 mg) was obtained as a mixture of four diastereomers, which was used in the next step without further purification.

2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI12)



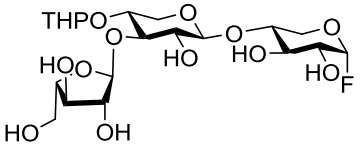
The crude trisaccharide **75** (411 mg) and DMAP (16.7 mg, 0.137 mmol) were dissolved in pyridine (25 mL). Acetic anhydride (412 μL, 412 mmol) was slowly added and the reaction mixture was stirred at r.t. for 4 h. The reaction mixture was diluted with DCM (20 mL) and washed with 1 M HCl (20 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated, and the crude product was filtered over a short silica column (SiO_2, Hex/EtOAc = 2:1 to 1:1) to give the crude peracetylated trisaccharide (801 mg) as a mixture of four diastereomers. The crude trisaccharide (260 mg) was dissolved in anhydrous DCM (3.00 mL) in a plastic vessel. The solution was cooled to -30 °C and HF/pyridine (2.00 mL) was added. The biphasic system was stirred rigorously for 2 h at -20 °C. The reaction mixture was diluted with DCM (5 mL) and was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 10 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (20 mL) and reextracted with DCM (2 x 20 mL). The solvents were evaporated and the crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 2:1) to give **SI12** (293 mg, 0.440 mmol, 60% over 3 steps, α/β = 8:1) as a colorless solid. $[a]_D^{25}$ = -22.6 (*c* 1.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, α-anomer): δ = 5.62 (dd, *J* = 53.0, 2.8 Hz, 1H, H-1), 5.38 (t, *J* = 9.5 Hz, 1H, H-3), 5.04 – 4.91 (m, 3H, H-1‘‘, H-2‘‘, H-4‘‘), 4.86 – 4.74 (m, 2H, H-2, H-2‘), 4.40 – 4.32 (m, 3H, H-1‘, H-3‘‘, H-5a‘‘), 4.25 – 4.16 (m, 1H, H-5b‘‘), 4.01 (dd, *J* = 11.9, 5.3 Hz, 1H, H-5a‘), 3.86 – 3.64 (m, 5H, H-4, H-4‘, H-5, OH), 3.42 (t, *J* = 8.7 Hz, 1H, H-3‘), 3.23 (dd, *J* = 11.9, 9.5 Hz, 1H, H-5b‘), 2.13 – 2.00 (m, 18H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, α-anomer): δ = 170.5, 170.3, 170.2, 169.8, 169.7, 169.6, 169.2 (6C, C=O), 107.4 (C-1‘‘), 103.95 (d, *J* = 229.2 Hz, C-1), 101.5 (C-1‘), 84.8 (C-3‘), 81.4 (C-4‘‘), 80.7 (C-3‘‘), 76.3 (C-2‘‘),75.4 (C-4‘), 71.0 (C-3‘), 70.54 (d, *J* = 24.9 Hz, C-2), 69.5 (C-3), 68.7 (C-4), 65.2 (C-5‘), 63.1 (C-5‘‘), 61.18 (d, *J* = 4.8 Hz, C-5), 20.8, 20.8, 20.7, 20.7, 20.7, 20.6 (6C, *C*H_3) ppm. ^{19}F NMR (376 MHz, $CDCl_3$, α-anomer): δ = -151.00 (dd, *J* = 53.1, 24.3 Hz). ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{27}H_{37}FNaO_{18}^+$: 691.1862; found: 691.1878. IR (neat): ν_{max} = 1746, 1227, 1372, 1041 cm^{-1}.

2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2-*O*-acetyl-4-*O*-tetrahydropyranosyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI13)



A solution of **SI12** (190 mg, 0.284 mmol), DHP (64.0 μL, 0.711 mmol) and *p*TsOH monohydrate (0.910 mg, 4.79 μmol) in anhydrous DCM (1.50 mL) was stirred at r.t. for 4 h. The reaction mixture was diluted with DCM (10 mL) and washed with aq. sat. $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 1:1) to give fully protected fluoride **SI13** (197 mg, 0.261 mmol, 91%) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.62 (dd, *J* = 53.1, 2.8 Hz, 1H, H-1), 5.43 – 5.34 (m, 1H, H-3), 5.17 (0.5H, H-1''), 5.14 (0.5H, H-1''), 5.01 – 4.89 (m, 2H, H-2'', H-4''), 4.87 – 4.74 (m, 3H, H-2, H-2', H-4'), 4.70 – 4.63 (m, 1H, C*H*), 4.51 – 4.15 (m, 4H, H-1', H-3'', H-5''), 4.14 – 4.00 (m, 1H, CH_2), 3.88 – 3.64 (m, 5H, CH_2, H-4, H-3'), 3.53 – 3.43 (m, 1H, CH_2), 3.34 – 3.24 (m, 1H, CH_2), 2.13 – 2.01 (m, 18H, CH_3), 1.85 – 1.66 (m, 2H, CH_2), 1.61 – 1.43 (m, 4H, CH_2) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.6, 170.3, 169.6, 169.2, 169.0, 106.5, 105.8, 103.97 (d, *J* = 228.9 Hz), 101.3, 100.5, 96.6, 81.2, 80.8, 80.6, 78.4, 75.7, 75.6, 75.3, 74.7, 72.3, 71.3, 70.7, 70.5, 69.6, 69.6, 65.0, 63.3, 63.2, 62.9, 61.9, 61.2, 30.6, 30.5, 25.2, 20.9, 20.8, 20.7, 20.6, 20.0, 19.6 ppm. ^{19}F NMR (376 MHz, $CDCl_3$): d = -150.96 (dd, *J* = 53.1, 24.4 Hz) ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{32}H_{45}FNaO_{19}^+$: 775.2437; found: 775.2466. IR (neat): ν_{max} = 1226, 1372, 1041, 706 cm^{-1}.

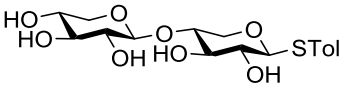
α-L-Arabinofuranosyl-(1→3)-4-*O*-tetrahydropyranosyl-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (62)



Glycosyl fluoride **SI13** (87 mg, 0.116 mmol) was dissolved in anhydrous MeOH (2.50 mL) and anhydrous DCM (0.500 mL) at 0 °C. NaOMe (0.5 M in MeOH, 0.450 mL, 0.260 mmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated to give 4-*O*-THP protected fluoride **62** (55.5 mg, 0.111 mmol, 96%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ =

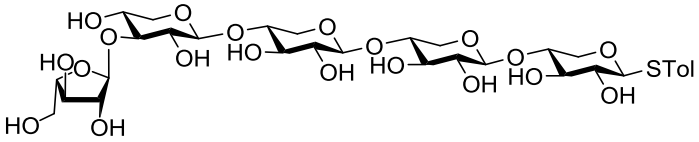
5.60 (dd, *J* = 53.3, 2.8 Hz, 1H, H-1), 5.40 (s, 0.5H, H-1''), 5.32 (s, 0.5H, H-1''), 4.74 – 4.64 (m, 1H, C*H*), 4.48 – 4.40 (m, 1H, H-1'), 4.21 – 3.48 (m, 15H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4', H-5, H-5', H-5'', CH_2), 3.44 – 3.24 (m, 2H, CH_2, H-2), 1.83 – 1.68 (m, 2H, CH_2), 1.57 – 1.39 (m, 4H, CH_2) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 108.4, 108.3, 107.7, 106.0, 101.7, 101.6, 101.5, 97.9, 84.5, 84.3, 81.1, 80.8, 80.4, 77.5, 76.7, 76.2, 75.5, 74.8, 73.5, 73.2, 71.4, 71.1, 70.9, 70.8, 64.3, 63.9, 63.8, 62.7, 61.1, 61.0, 61.0, 60.9, 30.2, 24.4, 24.4, 19.5, 19.3 ppm. ^{19}F NMR (376 MHz, D_2O): δ = -122.34, -152.77 (dd, *J* = 53.3, 26.3 Hz) ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{20}H_{33}FNaO_{13}^+$: 523.1803; found: 523.1830.

4-Methylphenyl β-D-xylopyranosyl-(1→4)-1-thio-β-D-xylopyranoside (63)



The protected disaccharide **76** (730 mg, 0.907 mmol) was dissolved in MeOH (10.0 mL) and DCM (5.00 mL) and NaOMe (0.5 M in MeOH, 907 μL, 0.453 mmol) was added. The mixture was stirred at r.t. for 2 h. After the reaction was finished the mixture was neutralized with Amberlite IR-120 (H^+) resin, fitered, and the solvents were evaporated. The crude product was purified by using a Sep-Pak C18-cartridge (waters, 3cc, H_2O/MeOH = 9:1 to 4:1 to 1:1) to give acceptor **63** (332 mg, 0.885 mmol, 94%) as a colorless solid. $[\alpha]_D^{25}$ = -76.8 (*c* 0.54, $CHCl_3$). ^{1}H NMR (400 MHz, D_2O): δ = 7.48 – 7.40 (m, 2H, ArH), 7.27 – 7.22 (m, 2H, ArH), 4.65 (d, *J* = 9.7 Hz, 1H, H-1), 4.41 (d, *J* = 4.41, 1H, H-2'), 4.08 (dd, *J* = 11.6, 5.3 Hz, 1H, H-5a), 3.93 (dd, *J* = 11.6, 5.4 Hz, 1H, H-5a'), 3.72 (ddd, *J* = 10.3 Hz, 9.1 Hz, 5.2 Hz, 1H, H-4), 3.64 – 3.52 (m, 2H, H-4', H-3), 3.46 – 3.17 (m, 5H, H-2, H-5b, H-2', H-3', H-5b'), 2.32 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 132.8, 132.6, 130.0, 130.3 (6C, Ar), 101.7 (C-1'), 88.1 (C-1), 76.0 (C-4), 75.5 (C-3'), 75.1 (C-4'), 72.7 (C-2), 71.5 (C-2'), 69.1 (C-3), 66.5 (C-5), 65.1 (C-5') ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{17}H_{24}NaO_8S^+$: 411.1090; found: 411.1126. IR (neat): ν_{max} = 3347, 1035, 980, 806 cm^{-1}.

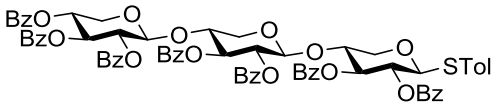
4-Methylphenyl α-L-arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-1-thio-β-D-xylopyranoside (60)



A 300 mM aqueous solution of 4-*O*-THP protected fluoride **62** (166 μL, 50.0 μmol), a 300 mM aqueous solution of acceptor **63** (200 μL, 60.0 μmol) and a solution of XynAE265G (620 μL, 7.70 mg/mL in $Na_xH_xPO_4$ buffer) was added to $Na_xH_xPO_4$ buffer

(616 μL, 100 mM). The reaction mixture was shaken at r.t. overnight. The reaction was stopped by heating the solution to 80 °C for 15 min. The reaction mixture was acidified by the addition of 1 M HCl solution (100 μL, 1.00 mmol) and it was shaken at r.t. for 30 min. The reaction mixture was neutralized by the dropwise addition of 1 M NaOH solution. The reaction mixture was centrifuged and the supernatant was subjected to a Sep-Pak C18-cartridge (waters, 6cc, H_2O/MeOH: 1:0 to 1:1) to give compound **60** as a colorless solid (30.5 mg, 38.8 µmol, 78%). $[a]_D^{25}$ = -97.8 (*c* 0.44, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 7.41 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H), 5.29 (d, *J* = 1.7 Hz, 1H), 4.62 (d, *J* = 9.7 Hz, 1H), 4.47 – 4.36 (m, 3H), 4.15 – 4.10 (m, 2H), 4.09 – 4.00 (m, 3H), 3.98 – 3.88 (m, 2H), 3.80 – 3.45 (m, 8H), 3.39 – 3.19 (m, 7H), 2.28 (s, 3H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 139.2, 132.8, 130.0, 127.3, 108.0, 101.6, 101.5, 101.5, 88.1, 83.9, 81.3, 81.1, 76.4, 76.3, 76.2, 75.9, 75.1, 73.5, 72.8, 72.6, 72.5, 71.5, 67.7, 66.4, 64.9, 62.9, 61.1, 20.1 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{32}H_{48}NaO_{20}S^+$: 807.2357; found: 807.2372. IR (neat): ν_{max} = 3327, 1042, 897, 806 cm^{-1}.

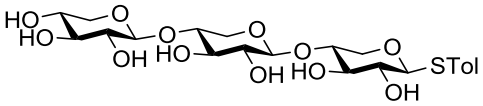
4-Methylphenyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-benzoyl-1-thio-β-D-xylopyranoside (77)



Disaccharide **71** (100 mg, 0.124 mmol) and phosphate donor **68** (98.0 mg, 0.149 mmol) were dissolved in anhydrous DCM (2.50 mL) and cooled to -50 °C. TMSOTf (27.0 µL, 0.149 mmol) was added and the reaction mixture was allowed to slowly warm up to -10 °C. After 2 h the reaction was quenched with aq. sat. $NaHCO_3$ solution (5 mL). The layers were separated and the aqueous layer was extracted with DCM (2 x 5 mL). The combined organic phases were dried over Na_2SO_4 and filtered. After the solvent was evaporated the crude product was purified by silica gel flash column chromatography (SiO_2, Hex/EtOAc = 4:1) to give protected trisaccharide **77** (113 mg, 90.5 μmol, 72%) as a colorless solid. $[a]_D^{25}$ = -4.1 (*c* 0.91, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): d = 8.00 – 7.86 (m, 14H, Ar*H*), 7.59 – 7.47 (m, 7H, Ar*H*), 7.42 – 7.27 (m, 17H, Ar*H*), 7.09 – 7.05 (m, 2H, Ar*H*), 5.62 – 5.55 (m, 2H, H-3, H-3''), 5.51 (t, *J* = 8.1 Hz, 1H, H-3'), 5.26 (t, *J* = 8.3 Hz, 1H, H-2), 5.18 – 5.12 (m, 2H, H-2', H-2''), 5.00 (td, *J* = 6.4, 4.0 Hz, 1H, H-4''), 4.87 (d, *J* = 8.4 Hz, 1H, H-1), 4.70 (dd, *J* = 5.6, 3.3 Hz, 2H, H-1', H-1''), 4.10 (dd, *J* = 12.0, 4.9 Hz, 1H, H-5a), 3.99 – 3.91 (m, 2H, H-4, H-5a''), 3.82 (td, *J* = 8.2, 4.8 Hz, 1H, H-4'), 3.52 (dd, *J* = 12.3, 4.8 Hz, 1H, H-5a'), 3.42 (dd, *J* = 12.0, 8.9 Hz, 1H, H-5b), 3.34 (dd, *J* = 12.3, 6.4 Hz, 1H, H-5b''), 3.13 (dd, *J* = 12.3, 8.6 Hz, 1H, H-5b'), 2.31 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (101

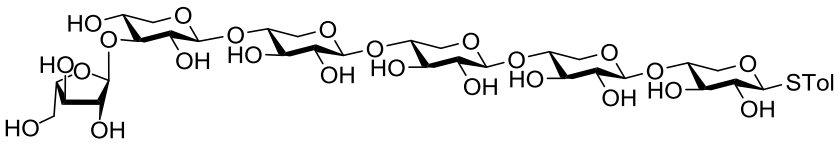
MHz, $CDCl_3$): δ = 165.4, 165.4, 165.3, 165.2, 165.2, 165.0, 164.9 (6C, C=O), 138.4, 133.4, 133.4, 133.3, 133.2, 133.1, 130.0, 129.9, 129.8, 129.7, 129.5, 129.4, 129.1, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3 (48C, Ar*C*), 101.0, 99.4 (2C, C-1', C-1''), 86.9 (C-1), 75.8 (C-4), 74.8 (C-4'), 73.3 (C-3), 71.9 (C-3'), 71.4 (C-2'), 70.4 (C-2), 70.0 (C-2''), 69.5 (C-3''), 68.5 (C-4''), 66.0 (C-5), 62.1 (C-5'), 60.8 (C-5''), 21.2 (*C*H_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{71}H_{60}NaO_{19}S^+$: 1271.3347; found: 1271.3386. IR (neat): ν_{max} = 1687, 1327, 1292, 707 cm^{-1}.

4-Methylphenyl β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-1-thio-β-D-xylopyranoside (64)



The protected trisaccharide **77** (105 mg, 84.1 μmol) was dissolved in anhydrous MeOH (5.00 mL) and anhydrous DCM (2.50 mL). NaOMe (0.5 M in MeOH, 84.2 μL, 42.1 μmol) was added. The mixture was stirred at r.t. overnight. After the reaction was finished the mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and the solvents were evaporated. The crude product was purified by using a Sep-Pak C18-cartridge (waters, 3cc, H_2O/MeOH = 9:1 to 4:1 to 1:1) to give acceptor **64** (39.4 mg, 75.7 μmol, 90%) as a colorless solid. $[\alpha]_D^{25}$ = -90.2 (*c* 0.53, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 7.34 – 7.24 (m, 2H), 7.09 – 7.02 (m, 2H), 4.51 (d, *J* = 9.7 Hz, 1H), 4.31 (d, *J* = 7.8 Hz, 1H), 4.28 (d, *J* = 7.8 Hz, 1H), 4.02 – 3.90 (m, 2H), 3.84 (dd, *J* = 11.5, 5.4 Hz, 1H), 3.69 – 3.35 (m, 5H), 3.30 (t, *J* = 9.2 Hz, 1H), 3.26 – 3.09 (m, 6H), 2.16 (s, 3H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 139.0, 132.8, 129.9, 127.3, 101.7, 101.4, 88.0, 76.2, 75.9, 75.5, 75.1, 73.5, 72.6, 72.5, 71.5, 69.1, 66.4, 65.1, 62.9, 20.2 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{22}H_{32}NaO_{12}S^+$: 543.1512; found: 543.1509. IR (neat): ν_{max} = 3378, 1164, 1043, 984 cm^{-1}.

4-Methylphenyl α-L-arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-1-thio-β-D-xylopyranoside (61)



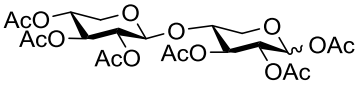
A 300 mM aqueous solution of 4-*O*-THP protected fluoride **62** (248 μL, 74.4 μmol), a 300 mM aqueous solution of acceptor **64** (207 μL, 62.0 μmol) and a solution of

XynAE265G (690 µL, 9.3 mg/mL in $Na_xH_xPO_4$ buffer) was added to $Na_xH_xPO_4$ buffer (750 µL, 100 mM). The reaction mixture was shaken at r.t. overnight. The mixture was stopped by heating the solution to 80 °C for 15 min. The reaction mixture was acidified by the addition of 1 M HCl solution (125 µL, 1.25 mmol) and it was shaken at r.t. for 30 min The reaction mixture was neutralized by the dropwise addition of 1 M NaOH solution. The reaction mixture was centrifuged and the supernatant was subjected to a Sep-Pak C18-cartridge (waters, 3cc, H_2O/MeOH: 1:0 to 1:1) to give compound **61** as a colorless solid (43.5 mg, 47.5 µmol, 76%). $[\alpha]_D^{25}$ = -111.8 (*c* 0.21, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 7.32 (d, *J* = 7.8 Hz, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 5.19 (s, 1H), 4.54 (d, *J* = 9.7 Hz, 1H), 4.40 – 4.29 (m, 4H), 4.06 – 3.91 (m, 6H), 3.88 – 3.79 (m, 2H), 3.70 – 3.51 (m, 7H), 3.49 – 3.35 (m, 5H), 3.29 – 3.09 (m, 10H), 2.20 (s, 3H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 139.2, 132.8, 129.9, 127.3, 108.0, 101.6, 101.5, 101.5, 88.1, 83.9, 81.3, 81.1, 76.4, 76.3, 76.2, 76.2, 75.9, 75.1, 73.5, 72.8, 72.6, 72.5, 71.5, 67.7, 66.4, 64.9, 62.8, 61.1, 20.1 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{37}H_{56}NaO_{24}S^+$: 939.2780; found: 939.2818. IR (neat): ν_{max} = 3371, 1044, 988 cm^{-1}.

3.4.5 Synthesis of Glycosyl Fluorides and Enyzmatic Polymerization

3.4.5.1 Synthesis of Glycosyl Fluorides

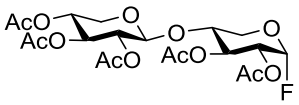
Acetyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-D-xylopyranoside (SI14)



The crude β-D-xylopyranosyl-(1→4)-D-xylopyranose **52** (480 mg) was dissolved in acetic anhydride (4.81 mL, 51.0 mmol) and pyridine (15 mL). DMAP (20.8 mg, 0.170 mmol) was added and the reaction mixture was stirred at r.t. for 4 h. The reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl solution (10 mL). The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (Hex/EtOAc = 2:1 → 1:1) to give compound **SI14** (690 mg, 1.29 mmol, 42% over 3 steps, α/β = 48/52) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.19 (t, *J* = 2.9 Hz, 0.5H, C-1α), 5.62 (d, *J* = 7.4 Hz, 0.5H, C-1β), 5.37 (t, *J* = 9.7 Hz, 0.5H, C-3α), 5.14 (t, *J* = 8.6 Hz, 0.5H, C-3β), 5.08 (t, *J* = 7.8 Hz, 1H, C-3'), 4.98 – 4.91 (m, 1H, C-2), 4.92 – 4.81 (m, 1H, C-4'), 4.78

(t, *J* = 7.0 Hz, 1H, C-2'), 4.57 – 4.50 (m, 1H, C-2), 4.08 (dd, *J* = 12.5, 5.1 Hz, 1H, H-5a'), 3.99 (ddd, *J* = 12.0, 5.1, 1.7 Hz, 0.5H, H-5aβ), 3.89 – 3.80 (m, 1H, H-4), 3.80 – 3.74 (m, 0.5H, H-5aα), 3.65 (t, *J* = 11.0 Hz, 0.5H, H-5bα), 3.45 (dd, *J* = 11.8, 9.5 Hz, 0.5H H-5bβ), 3.37 (ddd, *J* = 11.8, 7.6, 2.8 Hz, 1H, H-5b'), 2.09 – 1.98 (m, 18H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.1, 170.0, 169.9, 169.9, 169.9, 169.8, 169.7, 169.3, 169.2, 169.1 (10C, C=O), 100.0 (C-1β'), 99.7 (C-1α'), 92.3 (C-1β), 89.3 (C-1α), 75.2 (C-4α/β), 74.3 (C-4α/β), 72.1 (C-3β), 70.6 (2C, C-3α/β'), 70.5 (C-2α/β'), 70.4 (C-2α/β'), 69.9 (C-3α), 69.5 (C-2), 68.4 (C-4'), 68.4 (C-4'), 63.5 (C-5β), 61.7 (C-5'), 61.3 (C-5α), 21.0, 21.0, 20.9, 20.9, 20.9, 20.9, 20.8, 20.7, 20.7, 20.6 (10C, *C*H_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{22}H_{30}NaO_{15}^+$: 557.1482; found: 557.1484. IR (neat): ν_{max} = 1041, 1213, 1718, 1747, 2925 cm^{-1}.

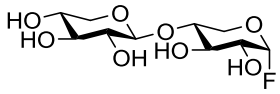
2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI15)



Disaccharide **SI14** (560 mg, 1.048 mmol) was dissolved in anhydrous DCM (3.00 mL) in a plastic vessel. The solution was cooled to -25 °C and HF/pyridine (3.00 mL) was added. The biphasic reaction mixture was rigorously stirred at -10 °C for 5 h. The reaction mixture was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 10 mL). The phases were separated and the organic phase was washed with aq. sat. $NaHCO_3$ solution (20 mL). The aqueous phase was reextracted with DCM (2 x 20 mL) and the combined organic phases were dried with Na_2SO_4. After evaporation of the solvent the crude product was subjected to silica column chromatography (EtOAc/Hex = 1:3) to give disaccharide fluoride **SI15** (408 mg, 0.825 mmol, 79%, α/β =12:1) as a colorless solid. $[a]_D^{25}$ = -40.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, α-anomer): δ = 5.63 (dd, *J* = 53.0, 2.7 Hz, 1H, H-1), 5.40 (t, *J* = 9.8 Hz, 1H, H-3), 5.08 (t, *J* = 7.5 Hz, 1H, H-3'), 4.91 – 4.73 (m, 3H, H-2, H-2', H-4'), 4.57 (d, *J* = 5.8 Hz, 1H, H-1'), 4.10 (dd, *J* = 12.0, 4.5 Hz, 1H, H-5a'), 3.94 – 3.81 (m, 2H, H-4, H-5a), 3.79 – 3.67 (m, 1H, H-5b), 3.40 (dd, *J* = 12.1, 7.4 Hz, 1H, H-5b'), 2.08 (s, 3H, C*H*$_3$), 2.06 – 2.01 (m, 12H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, α-anomer): δ = 170.3, 170.1, 170.0, 169.7, 169.2 (5C, C=O), 104.08 (d, *J* = 229.2 Hz, C-1), 99.5 (C-1'), 74.3 (C-4), 70.8 (C-2), 70.6 (C-2'), 70.3 (C-3'), 69.6 (C-3), 68.3 (C-4'), 61.5 (C-5'), 61.0 (d, *J* = 4.9 Hz, C-5), 21.0, 20.9, 20.8, 20.8, 20.7 (5C, *C*H_3) ppm. ^{19}F NMR (376 MHz, $CDCl_3$, α-anomer): δ = -151.01 (dd, *J* = 53.1, 24.3 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{20}H_{27}FNaO_{13}^+$: 517.1333; found: 517.1326.

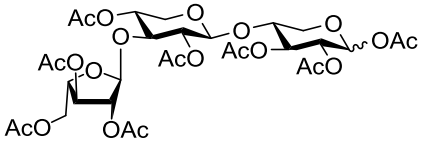
IR(neat): ν_{max} = 1746, 1371, 1235, 1047 cm^{-1}.

β-D-Xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (80)



Disaccharide fluoride **SI15** (170 mg, 0.211 mmol) was dissolved in anhydrous MeOH (10.0 mL) and anhydrous DCM (1.00 mL) at 0 °C. NaOMe (0.5 M in MeOH, 1.00 mL, 0.500 mmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated to give β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride **80** (60.0 mg, 0.211 mmol, 100%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 5.63 (dd, *J* = 53.3, 2.8 Hz, 1H, H-1), 4.45 (d, *J* = 7.8 Hz, 1H, H-1‘), 4.02 – 3.92 (m, 2H, H-5a‘, H-5a), 3.91 – 3.51 (m, 5H, H-4‘, H-5b, H-2, H-3, H-4), 3.42 (t, *J* = 9,2 Hz, 1H, H-3‘), 3.35 – 3.20 (m, 2H, H-2‘, H-5b‘) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 107.1 (d, *J* = 223.7 Hz, C-1), 101.7 (C-1‘), 75.5 (C-3‘), 72.6 (C-2‘), 71.1, 70.8, 70.7, 69.0, (4C, C-2, C-3, C-4, C-4‘) 65.1 (C-5), 61.0 (C-5‘) ppm. ^{19}F NMR (376 MHz, D_2O): δ = –152.8 (dd, *J* = 53.3, 26.4 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{10}H_{17}FNaO_8^+$: 307.0805; found: 307.0800.

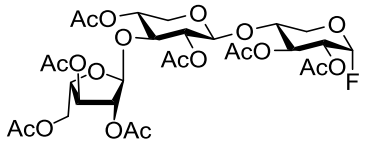
Acetyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-D-xylopyranoside (SI16)



Trisaccharide **53** (567 mg, 1.37 mmol) was dissolved in acetic anhydride (3.87 mL, 41.1 mmol) and pyridine (5 mL). DMAP (16.7 mg, 0.137 mmol) was added and the reaction mixture was stirred at r.t. for 4 h. The reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl (10 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (Hex/EtOAc = 2:1 to 1:1) to give fully protected trisaccharide **SI16** (800 mg, 1.06 mmol, 78%, α/β = 47/53) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.19 (d, *J* = 3.7 Hz, 0.5H, H-1α), 5.63 (d, *J* = 7.4 Hz, 0.5H, H-1β), 5.38 (dd, *J* = 10.2, 8.6 Hz, 0.5H, H-3α), 5.15 (t, *J* = 8.6 Hz, 0.5H, H-3β), 5.06 (s, 1H, C-1‘‘), 4.99 – 4.84 (m, 5H, H-2, H-2‘, H-4‘, C-4‘‘), 4.48 – 4.41 (m, 1H, H-1‘), 4.41 – 4.32 (m, 1H, H-5a‘‘), 4.23 – 4.13 (m, 2H, H-5b‘‘), 4.06 – 3.95 (m, 1.5H, H-5a‘, H-5aβ), 3.86 – 3.72 (m, 2.5H, H-4, H-3‘, H-5aα), 3.71 – 3.63 (m, 0.5H, H-5bα), 3.47 (dd, *J* = 12.0, 9.3 Hz, 0.5H, H-

5bβ), 3.30 (dd, *J* = 12.1, 7.8 Hz, 1H, H-5b‘), 2.18 – 1.97 (m, 18H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7, 170.5, 170.1, 170.0, 170.0, 169.8, 169.8, 169.7, 169.7, 169.4, 169.3, 169.2, 169.2 (13C, C=O), 106.6 (C-1β‘‘), 106.5 (C-1α‘‘), 101.0 (C-1β‘), 100.4 (C-1α'), 92.3 (C-1β), 89.3 (C-1α), 81.4 (C-4’’), 81.1 (C-3’’), 77.3 (C-3’), 76.7 (C-2’’), 75.8 (C-4β), 74.6 (C-4α), 72.1 (C-3β), 71.4 (C-2β'), 71.1 (C-2α’), 70.0 (2C, C2α/β), 69.7 (C-2α), 69.6 (C-4β'), 69.5 (C-4α'), 63.6 (C-5β), 63.4 (C-5’’), 62.3 (C-5β'), 62.2 (C-5α'), 61.5 (C-5α), 21.6, 21.1, 21.0, 21.0, 20.9, 20.9, 20.9, 20.8, 20.8, 20.7 (10C, *C*H_3) ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{31}H_{42}NaO_{21}^+$:773.2116; found: 773.2144. IR (neat): ν_{max} = 1041, 1070, 1220, 1746 cm^{-1}.

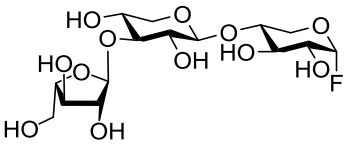
2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI17)



The protected trisaccharide **SI16** (550 mg, 0.733 mmol) was dissolved in anhydrous DCM (3.00 mL) in a plastic vessel. The solution was cooled to -25 °C and HF/pyridine (3.00 mL) was added. The biphasic system was stirred rigorously for 5 h at -10 °C. The reaction mixture was diluted with DCM (5 mL) and was quenched by dropping the mixture carefully into aq. ammonia solution (25%, 10 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (20 mL) and reextracted with DCM (2 x 20 mL). The combined organic phases were dried with Na_2SO_4 and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 1:2) to give trisaccharide fluoride **SI17** (463 mg, 0.652 mmol, 89%) as a colorless solid. $[\alpha]_D^{25}$ = -57.0 (*c* 0.92, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.63 (dd, *J* = 53.1 Hz, 2.8 Hz, 1H, H-1), 5.40 (dd, *J* = 10.2, 8.5 Hz, 1H, H-3), 5.07 (s, 1H, H-1’’), 4.94 − 4.76 (m, 5H, H-2, H-2’, H-4’, H-3’’, H-4’’), 4.47 (d, *J* = 6.11 Hz, 1H, H-1’), 4.37 (dd, *J* = 10.8, 2.5 Hz, 1H, H-5a’’), 4.24 − 4.14 (m, 2H, H-3’’, H-5b’’), 4.04 (dd, *J* = 12.1, 4.8 Hz, 1H, H-5a’), 3.88 − 3.73 (m, 4H, H-4, H-5a, H-5b, H-3’), 3.32 (dd, *J* = 12.1, 7.5 Hz, 1H, H-5b’), 2.12 − 2.02 (m, 21H, C*H*$_3$) ppm.^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.5, 170.4, 170.2, 169.9, 169.5, 169.1 (7C, C=O), 106.4 (C-1’’), 103.94 (d, *J* = 229.0 Hz, C-1), 100.3 (C-1’), 81.2 (C-4’’), 81.0 (C-3’’), 76.8 (C-3’), 76.6 (C-2’’), 74.6 (C-3), 71.0 (C-2’), 70.5 (d, *J* = 24.9 Hz, C-2), 69.4 (2, C-3, C-4’), 63.2 (C-5’’), 61.9 (C-5’), 61.0 (d, *J* = 4.7 Hz, C-5), 20.9, 20.8, 20.7, 20.6 (7C, *C*H_3) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): d = -151.06 (dd, *J* = 53.2, 24.0 Hz) ppm. ^{19}F-NMR (400 MHz, $CDCl_3$): d = -150.92 (dd, *J* = 53.1, 24.3 Hz) ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{29}H_{39}FNaO_{19}^+$: 733.1967; found: 733.1978. IR (neat): ν_{max} = 1048, 1223, 1371,

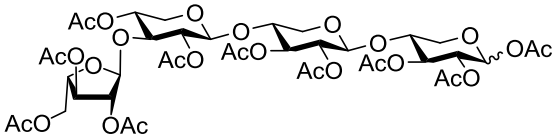
1746 cm^{-1}.

α-L-Arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (821)



Trisaccharide fluoride **SI17** (150 mg, 0.211 mmol) was dissolved in anhydrous MeOH (9.00 mL) and anhydrous DCM (1.00 mL) at 0 °C. NaOMe (0.5 M in MeOH, 1.00 mL, 0.500 mmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated to give fully deprotected trisaccharide fluoride **81** (88.0 mg, 0.211 mmol, 100%) as a colorless solid. ^{1}H-NMR (400 MHz, D_2O): δ = 5.60 (dd, *J* = 53.3, 2.8 Hz, 1H), 5.30 (d, *J* = 1.5 Hz, 1H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.23 – 4.06 (m, 2H), 4.04 – 3.88 (m, 3H), 3.85 – 3.61 (m, 6H), 3.61 – 3.48 (m, 2H), 3.43 – 3.22 (m, 3H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 108.0, 107.2 (d, *J* = 223.7 Hz), 101.7, 83.9, 81.4, 81.1, 76.4, 75.5, 72.7, 71.01 (d, *J* = 24.9 Hz), 70.8, 67.7, 64.9, 61.2 ppm. ESI-HRMS: m/z = $[M+Na]^+$ calcd. for $C_{15}H_{25}FNaO_{12}^+$: 439.1228; found: 439.1230.

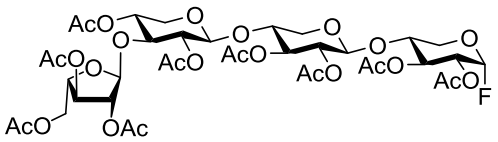
Acetyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-D-xylopyranoside (SI18)



Tetrasaccharide **43** (200 mg) and DMAP (4.47 mg, 36.6 μmol) were dissolved in pyridine (5 mL). Acetic anhydride (692 μL, 7.32 mmol) was added and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl (10 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (SiO_2, Hex/EtOAc = 2:1→1:1) to give protected trisaccharide **SI18** (318 mg, 329 μmol, 90%, α/β = 1:1.5) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.13 (d, *J* = 3.7 Hz, 0.5H, H-1α), 5.58 (d, *J* = 7.2 Hz, 0.5H, H-1β), 5.30 (dd, *J* = 10.2, 8.7 Hz, 0.5H, H-3α), 5.07 (t, *J* = 8.4 Hz, 0.5H, H-3α), 5.03 – 4.96 (m, 2H, H-3', H-1'''), 4.91 – 4.76 (m, 5H, H-2, H-2'', H-2''', H-4'', H-4'''), 4.68 (dd, *J* = 8.7, 6.9 Hz, 1H, H-2'), 4.45 – 4.38 (m, 2H, H-1', H-1''), 4.34 – 4.29 (m, 1H, H-5a'''), 4.17 – 4.07 (m,

2H, H-3''', H-5b'''), 3.99 – 3.90 (m, 1.5H, H-5aβ, H-5a''), 3.86 (dd, *J* = 11.9, 5.0 Hz, 1H, H-5a'), 3.81 – 3.66 (m, 3.5H, H-4, H-5aα, H-4', H-3''), 3.58 (t, *J* = 10.6 Hz, 0.5H, H-5bα), 3.40 (dd, *J* = 12.1, 9.1 Hz, 0.5H, H-5bβ), 3.33 – 3.19 (m, 2H, H-5b', H-5b''), 2.13 – 1.90 (m, 30H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.5, 170.4, 169.9, 169.8, 169.8, 169.8, 169.6, 169.6, 169.5, 169.4, 169.4, 169.2, 169.1, 169.0 (10C, C=O), 106.3 (C-1'''), 100.8, 100.4, 100.2, 100.2 (2C, C-1α/β', C-1α/β''), 92.1 (C-1β), 89.1 (C-1α), 81.2 (C-4'''), 80.9 (C-3'''), 76.6 (2C, C-2''', C-3''), 75.8 (C-4β), 74.7 (C-4α), 74.6 (C-4'), 72.0 (C3β), 71.9. 71.8 (C-3'), 71.0 (C-2''), 70.9 (C-2'), 69.9 (C-3α), 69.6 (C-2β), 69.4 (2C, C-2α, C-4''), 63.3 (C-5β), 63.2 (C-5'''), 62.7, 62.7 (C-5'), 61.9, 61.9 (C-5''), 61.3 (C-5α), 20.9, 20.8, 20.8, 20.7, 20.6, 20.5 (20C, *C*H$_3$) ppm. ESI-HRMS: m/z [M+Na]$^+$ calcd. for $C_{40}H_{54}NaO_{27}^+$: 989.2750; found: 989.2785. IR (neat): ν_{max} = 1748, 1373, 1221, 1046 cm^{-1}.

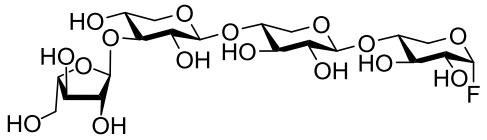
2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-d-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI19)



Tetrasaccharide **SI18** (150 mg, 0.155 mmol) was dissolved in anhydrous DMF (6 mL) at 0 °C. Hydrazine acetate (14.2 mg, 0.155 mmol) was added and the reaction mixture was stirred at 0 °C for 5 h. The reaction mixture was quenched by the addition of 1 M HCl solution (10 mL). The phases were separated and the organic phase was washed with aq. sat. $NaHCO_3$ solution (10 mL). The organic phase was dried with Na_2SO_4 and the solvent was evaporated to give crude anomeric alcohol (145 mg). The compound (145 mg) was dissolved in anhydrous DCM (5 mL). The solution was cooled to 0 °C and Argon gas was bubbled through the solution for 5 min. DAST (1.55 M in DCM, 100 μL, 0.155 mmol) was added and the reaction mixture was stirred at 0 °C. After 1 h the reaction mixture was diluted with DCM (5 mL) and the reaction mixture was washed with H_2O (20 mL). The organic phase was dried with Na_2SO_4 and the solvent was evaporated to give crude β-fluoride (135 mg). The compound (135 mg) was dissolved in anhydrous DCM (1.00 mL) in a plastic vessel. The solution was cooled to -30 °C and HF/pyridine (0.200 mL) was added. The biphasic system was stirred rigorously for 2 h at -10 °C. The reaction mixture was diluted with DCM (5 mL) and the reaction mixture was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 3 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (10 mL) and reextracted with DCM (2 x 10 mL). The combined organic phases were dried over Na_2SO_4 and the solvents were evaporated.

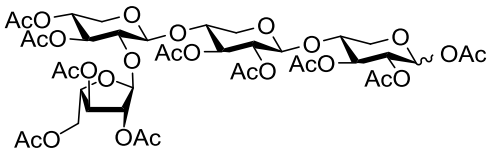
The crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 2:3 to 1:1) to give protected tetrasaccharide fluoride **SI19** (110 mg, 0.119 mmol, 77% over 3 steps) as a colorless solid. $[\alpha]_D^{25}$ = -19.5 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ =5.62 (dd, *J* = 53.1, 2.8 Hz, 1H, H-1), 5.37 (t, *J* = 9.6 Hz, 1H, H-3), 5.06 (s, 1H, H-1'''), 5.04 (t, *J* = 8.2 Hz, 1H, H-3'), 4.92 – 4.90 (m, 1H, H-2'''), 4.89 – 4.84 (m, 3H, H-2'', H-4'', H-4'''), 4.80 (ddd, *J* = 24.3, 10.2, 2.8 Hz, 1H, H-2), 4.70 (dd, *J* = 8.4, 6.7 Hz, 1H, H-2'), 4.47 (d, *J* = 6.7 Hz, 1H, H-1'), 4.45 (d, *J* = 6.3 Hz, 1H, H-1''), 4.36 (dd, *J* = 11.5, 3.2 Hz, 1H, H-5a'''), 4.20 – 4.18 (m, 1H, H-3'''), 4.16 (dd, *J* = 11.5, 5.9 Hz, 1H, H-5b'''), 4.01 (dd, *J* = 12.1, 4.8 Hz, 1H, H-5a''), 3.91 (dd, *J* = 12.0, 4.9 Hz, 1H, H-5a'), 3.86 – 3.81 (m, 2H, H-4, H-5a), 3.79 – 3.70 (m, 3H, H-5b, H-4', H-3''), 3.33 (dd, *J* = 12.0, 8.8 Hz, 1H, H-5b'), 3.29 (dd, *J* = 12.1, 7.6 Hz, 1H, H-5b''), 2.10 – 2.06 (m, 18H, C*H*$_3$), 2.03 (s, 3H, C*H*$_3$), 2.01 (s, 3H, C*H*$_3$), 2.00 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.5, 170.4, 170.2, 169.8, 169.5, 169.4, 169.1 (10C, C=O), 106.3 (C-1'''), 103.94 (d, *J* = 229.3 Hz, C-1), 100.4 (C-1'), 100.2 (C-1''), 81.3 (C-4'''), 81.0 (C-3'''), 76.7 (2C, C-3'', C-2'''), 74.9 (C-4), 74.5 (C-4'), 71.8 (C-3'), 71.1 (C-2''), 70.9 (C-2''), 70.57 (d, *J* = 24.8 Hz, C-2), 69.6 (C-3), 69.5 (C-4''), 63.3 (C-5'''), 62.6 (C-5'), 62.0 (C-5''), 61.1, 61.05 (d, *J* = 4.6 Hz, C-5'''), 20.9, 20.8, 20.7, 20.7, 20.7, 20.6, 20.6 (9C, *C*H_3) ppm. ^{19}F NMR (564 MHz, $CDCl_3$): δ = -151.10 (dd, *J* = 53.1, 24.3 Hz). ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{38}H_{51}FNaO_{25}^+$: 949.2601; found: 949.2641. IR (neat): ν_{max} = 1748, 1373, 1223, 1050 cm^{-1}.

α-L-Arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (48)



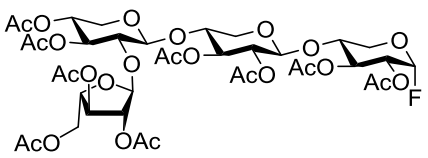
The protected tetrasaccharide fluoride **SI19** (70 mg, 0.0756 mmol) was dissolved in anhydrous MeOH (1.50 mL) and anhydrous DCM (0.400 mL) at 0 °C. NaOMe (0.5 M in MeOH, 90.0 μL, 0.0756 mmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated to give fully deprotected tetrasaccharide fluoride **48** (42.0 mg, 0.0756 mmol, 100%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ =5.50 (dd, *J* = 53.3, 2.9 Hz, 1H), 5.19 (s, 1H), 4.38 – 4.31 (m, 2H), 4.07 – 3.35 (m, 17H), 3.30 – 3.11 (m, 5H) ppm.^{13}C NMR (101 MHz, D_2O): δ = 108.0, 107.11 (d, *J* = 223.8 Hz), 101.6, 83.9, 81.3, 81.1, 76.4, 76.3, 75.4, 73.5, 72.7, 72.5, 71.1, 70.8, 70.7, 67.7, 64.9, 62.9, 61.1, 60.95 (d, *J* = 4.1 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{20}H_{33}FNaO_{16}^+$: 571.1650; found: 571.1664.

Acetyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl-(1→2)-3,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-D-xylopyranoside (SI20)



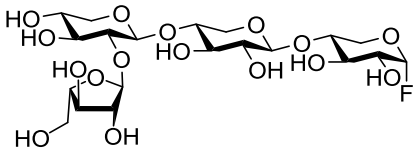
The unprotected tetrasaccharide **78** (30.0 mg, 54.9 µmol) was dissolved in pyridine (5 mL) and acetic anhydride (5.19 mL, 54.9 mmol) and DMAP (0.670 mg, 5.49 µmol) were added. The reaction mixture was diluted with DCM (5 mL) and washed with 1 M HCl (5 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated to give protected tetrasaccharide **SI20** (50.1 mg, 51.7 µmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.18 (d, *J* = 3.7 Hz, 0.5H, H-1α), 5.63 (d, *J* = 7.1 Hz, 0.5H, H-1β), 5.35 (t, *J* = 9.6 Hz, 0.5H, H-3α), 5.16 – 5.02 (m, 3.5H, H-3β,H-3', H-3''), 4.96 – 4.89 (m, 3H, H-2α/β, H-2''', H-4'''), 4.88 – 4.77 (m, 1H, H-4''), 4.75 (dd, *J* = 8.7, 6.8 Hz, 1H, H-2'), 4.52 – 4.46 (m, 1H, H-1'α/β), 4.42 (d, *J* = 6.4 Hz, 1H, H-1''), 4.40 – 4.30 (m, 2H, H-3''', H-5a'''), 4.17 (dd, *J* = 11.5, 5.6 Hz, 1H, H-5b'''), 4.07 – 3.96 (m, 2.5H, H-5aβ, H-5a', H-5a''), 3.86 – 3.73 (m, 2.5H, H-5aα, H-4, H-4'), 3.68 – 3.59 (m, 0.5H, H-5bα), 3.54 (dd, *J* = 8.8, 6.4 Hz, 1H, H-2''), 3.49 – 3.36 (m, 1.5H, H-5bβ, H-5b'), 3.31 (dd, *J* = 11.9, 8.3 Hz, 1H, H-5b''), 2.18 – 1.92 (m, 30H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7, 170.7, 170.2, 170.2, 170.1, 170.0, 170.0, 169.9, 169.7, 169.7, 169.6, 169.6, 169.4, 169.3, 169.2, 169.1, 106.5 (C-1'''), 101.3 (C-1''), 100.7 (C-1'b), 100.2 (C-1'α), 92.1 (C-1β), 89.2 (C-1α), 81.2 (C-4'''), 80.8 (C-3'''), 76.9 (C-2'''), 75.8 (C-2''), 75.6 (C-4β), 74.4 (C-4α), 74.2 (C-4'), 71.9, 71.8, 71.7 (3C, C-3β, C-3', C-3''), 71.0 (C-2'β), 70.8 (C-2'α), 69.9 (C-3a), 69.6 (C-2β), 69.4 (C-2α), 69.2 (C-4''), 63.3 (2C, C-5β, C-5'''), 62.6 (C-5'), 62.1 (C-5α), 61.3 (C-5''), 21.0, 20.9, 20.8, 20.7, 20.6 (10C, *C*H_3) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{40}H_{54}NaO_{27}^+$: 989.2750; found: 989.2781. IR (neat): ν_{max} = 1750, 1371, 1236, 1050 cm^{-1}.

2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→2)-3,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI21)



The protected tetrasaccharide **SI20** (50.1 mg) was dissolved in anhydrous DCM (2.00 mL) in a plastic vessel. The solution was cooled to -30 °C and HF/pyridine (1.50 mL) was added. The biphasic system was stirred rigorously for 2 h at -20 °C. The reaction mixture was diluted with DCM (10 mL) and was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 10 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (10 mL) and reextracted with DCM (2 x 10 mL). The combined organic phases were dried over Na_2SO_4 and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 2:1) to give protected tetrasaccharide fluoride **SI21** (20.5 mg, 22.1 μmol, 43%) as a colorless solid. $[a]_D^{25}$ = -19.5 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.62 (dd, *J* = 53.0, 2.8 Hz, 1H, H-1), 5.37 (t, *J* = 9.4 Hz, 1H, H-3), 5.13 – 5.07 (m, 2H, H-1’’’, H-3’’), 5.05 (t, *J* = 8.2 Hz, 1H, H-3’), 4.97 – 4.88 (m, 2H, H-2’’’, H-4’’’), 4.88 – 4.76 (m, 2H, H-2, H-4’’), 4.72 (dd, *J* = 8.4, 6.5 Hz, 1H, H-2’), 4.51 (d, *J* = 6.5 Hz, 1H, H-1’), 4.43 (d, *J* = 6.4 Hz, 1H, H-1’’), 4.38 (dd, *J* = 11.8, 3.9 Hz, 1H, H-5a’’’), 4.35 – 4.30 (m, 1H, H-3’’’), 4.17 (dd, *J* = 11.8, 5.7 Hz, 1H, H-5b’’’), 4.03 (dd, *J* = 12.0, 4.9 Hz, 1H, H-5a’), 3.99 (dd, *J* = 11.9, 5.0 Hz, 1H, H-5a’’), 3.89 – 3.76 (m, 3H, H-4, H-4’, H-5a), 3.75 – 3.67 (m, 1H, H-5b), 3.54 (dd, *J* = 8.8, 6.5 Hz, 1H, H-2’’), 3.42 (dd, *J* = 12.1, 8.7 Hz, 1H, H-5b’), 3.31 (dd, *J* = 12.1, 8.3 Hz, 1H, H-5b’’), 2.13 – 1.87 (m, 27H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.6, 170.1, 170.0, 169.9, 169.5, 169.4, 169.2 (9C, C=O), 106.5 (C-1’’’), 103.9 (d, *J* = 229.1 Hz, C-1), 101.3 (C-1’’), 100.2 (C-1’), 81.2 (C-4’’’), 80.8 (C-3’’’), 76.8 (C-2’’’), 75.9 (C-2’’), 74.6 (C-4), 74.1 (C-4’), 71.9 (C-3’’), 71.6 (C-3), 70.8 (C-2’), 70.5 (d, *J* = 24.7 Hz, C-2), 69.5 (C-3), 69.2 (C-4’’), 63.2 (C-5’’’), 62.5 (C-5’), 62.1 (C-5’’), 61.0 (d, *J* = 4.7 Hz, C-5), 20.8, 20.7, 20.6, 20.5 (9C, CH_3) ppm. ^{19}F-NMR (400 MHz, $CDCl_3$): δ = -152.79 (dd, *J* = 53.3, 26.4 Hz). ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{38}H_{51}FNaO_{25}^+$:949.2601; found: 949.2597. IR (neat): ν_{max} = 1748, 1373, 1228, 1050 cm^{-1}.

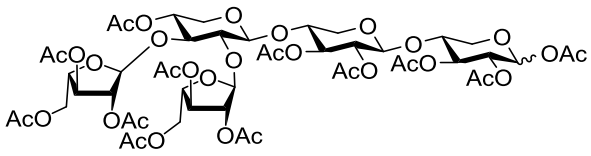
α-L-Arabinofuranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (82)



The protected tetrasaccharide fluoride **SI21** (20.5 mg, 22.1 μmol) was dissolved in anhydrous MeOH (2.00 mL) at 0 °C. NaOMe (0.5 M in MeOH, 40.0 μL, 22.1 μmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvent was evaporated to give fully deprotected tetrasaccharide fluoride **82**

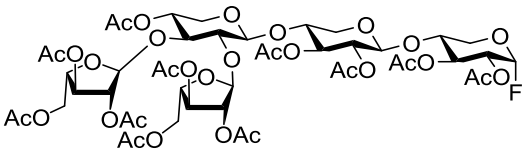
(12.1 mg, 22.1 μmol, 100%) as a colorless solid. ^{1}H-NMR (400 MHz, D_2O): δ = 5.91 – 5.44 (m, 1H), 5.26 (s, 1H), 4.54 (d, *J* = 7.5 Hz, 1H), 4.45 (d, *J* = 7.7 Hz, 1H), 4.27 – 4.07 (m, 3H), 4.02 – 3.49 (m, 13H), 3.43 – 3.23 (m, 4H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 108.4, 107.1 (d, *J* = 223.6 Hz), 101.6, 100.1, 84.5, 80.9, 78.0, 76.6, 76.0, 75.6, 75.4, 73.6, 72.6, 70.99 (d, *J* = 24.9 Hz), 70.7, 69.0, 64.9, 63.0, 61.2, 60.96 (d, *J* = 3.2 Hz) ppm. ^{19}F-NMR (400 MHz, D_2O): δ = -152.79 (dd, *J* = 53.3, 26.4 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{20}H_{33}NaO_{16}F^+$: 571.1650; found: 571.1657.

Acetyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2-*O*-[2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl]-4-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-D-xylopyranoside (SI22)



Pentasaccharide **79** (50.0 mg, 73.7 μmol) was dissolved in pyridine (8 mL) and acetic anhydride (6.96 mL, 73.7 mmol) and DMAP (0.899 mg, 7.37 μmol) were added. The reaction mixture was stirred at r.t. for 2 h. The reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl (10 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 1:1) to give fully protected pentasaccharide **SI22** (84.0 mg, 71.1 μmol, 96%, α/β = 2:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.15 (d, *J* = 3.7 Hz, 0.5H), 5.60 (dd, *J* = 7.1, 1.7 Hz, 0.5H), 5.34 – 5.28 (m, 0.5H), 5.23 (d, *J* = 10.6 Hz, 2H), 5.08 (td, *J* = 8.2, 1.7 Hz, 0.5H), 5.01 (t, *J* = 8.3 Hz, 1H), 4.98 – 4.93 (m, 2H), 4.92 – 4.85 (m, 2H), 4.84 – 4.80 (m, 1H), 4.78 (q, *J* = 7.1 Hz, 1H), 4.72 – 4.67 (m, 1H), 4.51 – 4.45 (m, 1H), 4.41 – 4.37 (m, 1H), 4.37 – 4.27 (m, 3H), 4.18 – 4.07 (m, 3H), 4.01 – 3.94 (m, 1.5H), 3.90 (dd, *J* = 10.9, 4.4 Hz, 1H), 3.84 – 3.71 (m, 3.5H), 3.63 – 3.55 (m, 1.5H), 3.46 – 3.33 (m, 1.5H), 3.19 (dd, *J* = 12.3, 7.4 Hz, 1H), 2.18 – 1.84 (m, 36H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7, 170.7, 170.4, 170.1, 169.9, 169.8, 169.8, 169.7, 169.5, 169.5, 169.5, 169.4, 169.3, 169.2, 169.2, 169.1, 168.9, 106.3, 106.1, 101.2, 101.2, 100.4, 100.0, 92.0, 89.1, 81.0, 80.8, 80.6, 80.5, 77.8, 77.8, 77.5, 77.2, 75.2, 74.1, 73.6, 73.6, 71.7, 71.6, 70.9, 70.8, 70.8, 69.9, 69.6, 69.4, 64.2, 63.4, 63.3, 63.3, 63.1, 62.7, 62.4, 62.4, 61.3, 20.9, 20.8, 20.7, 20.6, 20.5, 20.4 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{49}H_{66}NaO_{33}^+$:1205.3384; found: 1205.3425. IR (neat): ν_{max} = 1746, 1371, 1223, 1046 cm^{-1}.

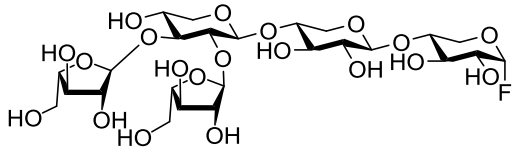
2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2-*O*-[2,3,5-tri-*O*-acetyl-α-L-arabino-furanosyl]-4-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI23)



The fully protected pentasaccharide **SI22** (73.0 mg, 61.7 μmol) was dissolved in anhydrous DMF (1.50 mL) at 0 °C. Hydrazine acetate (5.70 mg, 61.7 μmol) was added and the reaction mixture was stirred at 0 °C for 5 h. The reaction mixture was quenched by the addition of 1 M HCl solution (10 mL). The phases were separated and the organic phase was washed with aq. sat. $NaHCO_3$ solution (10 mL). The organic phase was dried with Na_2SO_4 and the solvent was evaporated to give crude anomeric alcohol (70.3 mg). The crude compound (70.3 mg) was dissolved in anhydrous DCM (0.500 mL). The solution was cooled to 0 °C and Argon was bubbled through the solution for 5 min. DAST (8.10 μL, 61.7 μmol) was added and the reaction mixture was stirred at 0 °C. After 1 h the reaction mixture was diluted with DCM (5 mL) and the reaction mixture was washed with H_2O (5 mL). The organic phase was dried with Na_2SO_4 and the solvent was evaporated to give crude β-fluoride (71.0 mg). The crude compound (71.0 mg) was dissolved in anhydrous DCM (2.00 mL) in a plastic vessel. The solution was cooled to -30 °C and HF/pyridine (0.200 mL) was added. The biphasic system was stirred rigorously for 2 h at -15 °C. The reaction mixture was diluted with DCM (5 mL) and was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 3 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (5 mL) and reextracted with DCM (2 x 5 mL). The combined organic phases were dried over Na_2SO_4 and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 1:1) to give protected pentasaccharide fluoride **SI23** (31.2 mg, 27.3 μmol, 44% over 3 steps) as a colorless solid. $[\alpha]_D^{25}$ = -17.4 (*c* 0.73, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.61 (dd, *J* = 53.1, 2.8 Hz, 1H), 5.37 (t, *J* = 9.4 Hz, 1H), 5.29 (s, 1H), 5.27 (s, 1H), 5.08 – 4.97 (m, 3H), 4.92 (d, *J* = 4.8 Hz, 1H), 4.88 – 4.75 (m, 3H), 4.71 (dd, *J* = 8.3, 6.5 Hz, 1H), 4.52 (d, *J* = 6.5 Hz, 1H), 4.47 – 4.29 (m, 4H), 4.21 – 4.14 (m, 3H), 4.00 (dd, *J* = 12.0, 4.7 Hz, 1H), 3.93 (dd, *J* = 12.2, 5.2 Hz, 1H), 3.85 (t, *J* = 8.5 Hz, 2H), 3.80 – 3.66 (m, 1H), 3.62 (dd, *J* = 9.4, 6.6 Hz, 1H), 3.43 (dd, *J* = 12.0, 8.5 Hz, 1H), 3.22 (dd, *J* = 12.2, 7.4 Hz, 1H), 2.26 – 1.88 (m, 33H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.9, 170.6, 170.3, 170.1, 170.0, 169.7, 169.6, 169.4, 106.5, 106.3, 104.1 (d, *J* = 229.0 Hz), 101.5, 100.1, 81.2, 81.0, 80.9, 80.7, 78.0, 77.8, 77.4, 77.3, 74.5, 73.8, 71.8, 71.0, 70.7 (d, *J* = 24.7 Hz), 70.6, 69.7, 63.6,

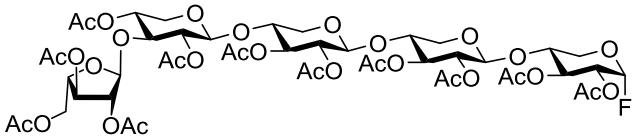
63.5, 62.9, 62.5, 61.2 (d, *J* = 4.6 Hz), 21.1, 21.0, 20.9, 20.8, 20.7 ppm.^{19}F-NMR (400 MHz, $CDCl_3$): δ = -150.99 (dd, *J* = 53.1, 24.1 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{47}H_{63}FNaO_{31}^+$: 1165.3235; found: 1165.3219. IR (neat): ν_{max} = 1740, 1370, 1216, 1023 cm^{-1}.

α-L-Arabinofuranosyl-(1→3)-2-*O*-[α-L-arabinofuranosyl]-β-D-xylopyranosyl-(1→4)- β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (83)



The protected pentasaccharide fluoride **SI23** (20.0 mg, 17.5 μmol) was dissolved in anhydrous MeOH (4.00 mL) at 0 °C. NaOMe (0.5 M in MeOH, 35.0 μL, 17.5 μmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvent was evaporated to give fully deprotected pentasaccharide fluoride **83** (11.4 mg, 16.8 μmol, 96%) as a colorless solid. ^{1}H-NMR (400 MHz, D_2O): δ = 5.63 (dd, *J* = 53.3, 2.8 Hz, 1H), 5.12 (s, 1H), 5.12 (s, 1H), 4.58 (d, *J* = 7.4 Hz, 1H), 4.46 (d, *J* = 7.7 Hz, 1H), 4.36 – 4.07 (m, 5H), 4.05 – 3.88 (m, 4H), 3.89 – 3.62 (m, 13H), 3.37 – 3.19 (m, 3H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 108.6, 108.4, 107.1 (d, *J* = 224.5 Hz), 101.6, 99.9, 84.3, 83.9, 82.0, 81.1, 81.0, 78.0, 76.6, 76.4, 75.8, 75.4, 73.6, 72.6, 71.0 (d, *J* = 24.8 Hz), 70.7, 67.9, 64.7, 62.9, 61.1 (d, *J* = 7.3 Hz) ppm. ^{19}F-NMR (400 MHz, D_2O): δ = -152.79 (dd, *J* = 53.5, 26.2 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{25}H_{41}FNaO_{20}^+$: 703.2073; found: 703.2083.

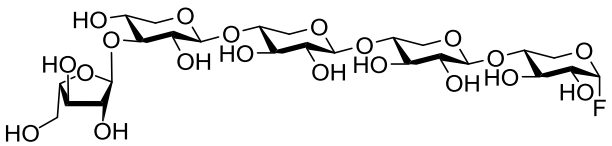
2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI24)



Pentasaccharide **60** (30.5 mg, 38.8 μmol) was dissolved in pyridine (5.00 mL) and acetic anhydride (3.76 mL, 38.9 mmol) and DMAP (0.474 mg, 3.88 μmol) were added. The reaction mixture was stirred at r.t. for 3 h. The reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl (10 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated to give crude

protected thioglycoside (59.0 mg). The crude compound (40.1 mg) was dissolved in anhydrous DCM (1.50 mL) in a plastic vessel. The solution was cooled to -50 °C and NIS (8.78 mg, 39.0 μmol) and HF/pyridine (50.0 μL) were added. The biphasic system was stirred rigorously for 30 min, then more HF/pyridine (1.00 mL) was added and the reaction mixture was allowed to warm up to -15 °C. After 2 h the reaction mixture was diluted with DCM (10 mL) and was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 5 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (10 mL) and reextracted with DCM (2 x 10 mL). The combined organic phases were dried over Na_2SO_4 and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 1:1 → 3:2) to give protected pentasaccharide fluoride **SI24** (16.0 mg, 14.0 μmol, 53% over 2 steps) as a colorless solid. $[\alpha]_D^{25}$ = -56.8 (*c* 0.93, H_2O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.62 (dd, *J* = 53.0, 2.8 Hz, 1H, H-1), 5.38 (t, *J* = 9.6 Hz, 1H, H-3), 5.10 – 4.99 (m, 3H, H-1’’’’, H-3’, H-3’’), 4.93 – 4.76 (m, 5H, H-2, H-2’’’, H-4’’’, H-2’’’’, H-3’’’’), 4.74 – 4.64 (m, 2H, H-2’, H-2’’), 4.49 – 4.42 (m, 3H, H-1’, H-1’’, H-1’’’), 4.36 (dd, *J* = 11.3, 3.0 Hz, 1H, H-5a’’’’), 4.24 – 4.14 (m, 2H, H-4’’’’, H-5b’’’’), 4.01 (dd, *J* = 12.1, 4.9 Hz, 1H, H-5a’’’), 3.94 – 3.87 (m, 2H, H-5a’, H-5a’’), 3.87 – 3.79 (m, 2H, H-4, H-5a), 3.79 – 3.68 (m, 4H, H-5b, H-4’, H-4’’, H-3’’’), 3.42 – 3.21 (m, 3H, H-5b’, H-5b’’, H-5b’’’), 2.18 – 1.96 (m, 33H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.6, 170.5, 170.3, 169.9, 169.9, 169.6, 169.5, 169.4, 169.2 (11C, C=O), 106.5 (C-1’’’’), 104.06 (d, *J* = 229.5 Hz, C-1), 100.3 (3C, C-1’, C-1’’, C-1’’’), 81.4 (C-4’’’’), 81.1 (C-3’’’’), 76.8 (2C, C-3’’’, C-2’’’’), 74.9 (C-4), 74.7 (2C, C-4’, C-4’’), 72.0, 71.8 (2C, C-3’, C-3’’), 71.2 (C-2’’’), 71.0, 70.8 (2C, C-2’, C-2’’), 70.7 (d, *J* = 24.7 Hz, C-2), 69.6 (2C, C-3, C-4’’’), 63.4 (C-5’’’’), 62.7, 62.5 (2C, C-5’, C-5’’), 62.1 (C-5’’’), 61.1 (C-5), 21.1, 21.0, 20.9, 20.8, 20.7 (11C, *C*H_3) ppm. ^{19}F NMR (564 MHz, $CDCl_3$): δ = -151.07 (dd, *J* = 53.0, 24.3 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{47}H_{63}FNaO_{31}^+$: 1165.3235; found: 1165.3230. IR (neat): ν_{max} = 1748, 1372, 1221, 1050 cm^{-1}.

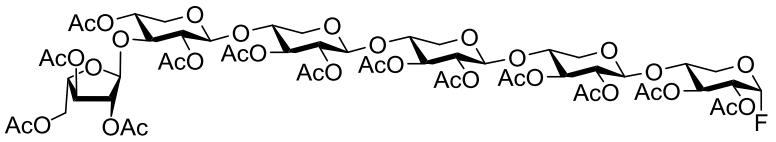
α-L-Arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (84)



Pentasaccharide fluoride **SI24** (21.0 mg, 18.3 μmol) was dissolved in anhydrous MeOH (2.00 mL) and anhydrous DCM (0.200 mL) at 0 °C. NaOMe (0.5 M in MeOH, 36.6 μL, 18.3 μmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin.

The resin was filtered off and the solvents were evaporated to give fully deprotected pentasaccharide fluoride **84** (12.4 mg, 18.2 μmol, 99%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 5.57 (dd, *J* = 53.3, 2.8 Hz, 1H), 5.26 (d, *J* = 1.6 Hz, 1H), 4.45 – 4.36 (m, 3H), 4.13 – 4.09 (m, 2H), 4.05 – 4.01 (m, 2H), 3.94 – 3.86 (m, 3H), 3.81 – 3.76 (m, 1H), 3.76 – 3.68 (m, 4H), 3.66 – 3.56 (m, 3H), 3.56 – 3.44 (m, 3H), 3.35 – 3.19 (m, 7H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 108.1, 107.24 (d, *J* = 223.7 Hz), 101.7, 101.7, 101.6, 84.0, 81.5, 81.2, 76.5, 76.4, 76.3, 75.5, 73.7, 73.6, 72.8, 72.7, 72.7, 71.08 (d, *J* = 25.0 Hz), 70.8, 67.8, 65.0, 63.0, 63.0, 61.2, 61.1 (d, *J* = 4.2 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{25}H_{41}FNaO_{20}^+$: 703.2073; found: 703.2077.

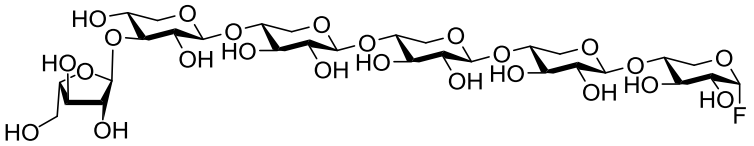
2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-D-xylopyranosyl fluoride (SI25)



The unprotected hexasaccharide **79** (51.8 mg, 56.6 μmol) was dissolved in pyridine (10 mL) and acetic anhydride (5.34 mL, 56.6 mmol) and DMAP (0.689 mg, 5.66 μmol) were added. The reaction mixture was stirred at r.t. for 3 h. The reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl (10 mL) solution. The phases were separated and the organic phase was dried with Na_2SO_4. The solvent was evaporated to give crude protected thioglycoside (85.2 mg). The crude compound (39.9 mg) was dissolved in anhydrous DCM (1.50 mL) in a plastic vessel. The solution was cooled to -50 °C and NIS (7.59 mg, 33.7 μmol) and HF/pyridine (0.05 mL) were added. The biphasic system was stirred rigorously for 30 min, then more HF/pyridine (1.00 mL) was added and the reaction mixture was allowed to warm up to -15 °C. After 3 h the reaction mixture was diluted with DCM (10 mL) and was quenched by dropping the mixture carefully into an aq. ammonia solution (25%, 10 mL). The organic phase was washed with aq. sat. $NaHCO_3$ solution (10 mL) and reextracted with DCM (2 x 10 mL). The combined organic phases were dried over Na_2SO_4 and the solvents were evaporated. The crude product was purified by column chromatography (SiO_2, EtOAc/Hex = 1:1 to 3:2) to give protected hexasaccharide fluoride **SI25** (18.9 mg, 13.9 μmol, 52% over 2 steps) as a colorless solid. $[\alpha]_D^{25}$ = -68.2 (*c* 0.67, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.63 (dd, *J* = 53.1, 2.8 Hz, 1H, H-1), 5.38 (dd, *J* = 10.2, 8.6 Hz, 1H, H-3), 5.12 – 4.99 (m, 4H, H-1‘‘‘‘, H-3‘, H-3‘‘, H-3‘‘‘), 4.94 – 4.75 (m, 5H, H-2, H-2‘‘‘‘, H-4‘‘‘‘, H-2‘‘‘‘‘, H-3‘‘‘‘‘), 4.75 – 4.66 (m, 3H, H-2‘, H-2‘‘,

H-2‘‘‘), 4.51 – 4.42 (m, 4H, H-1‘, H-1‘‘, H-1‘‘‘, H-1‘‘‘‘), 4.39 – 4.33 (m, 1H, H-5a‘‘‘‘‘), 4.22 – 4.13 (m, 2H, H-4‘‘‘‘, H-5b‘‘‘‘‘), 4.01 (dd, *J* = 12.1, 4.8 Hz, 1H, H-5a‘‘‘‘), 3.94 – 3.87 (m, 2H, H-5a‘, H-5a‘‘, H-5a‘‘‘), 3.86 – 3.67 (m, 6H, H-4, H-5, H-4‘, H-4‘‘, H-4‘‘‘, H-3‘‘‘‘), 3.35 – 3.25 (m, 4H, H-5b‘, H-5b‘‘, H-5b‘‘‘), 2.14 – 1.94 (m, 39H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.6, 170.4, 170.3, 169.9, 169.8, 169.8, 169.6, 169.4, 169.4, 169.3, 169.2 (13C, C=O), 106.3 (C-1‘‘‘‘), 103.93 (d, *J* = 229.1 Hz, C-1), 100.3, 100.3, 100.2, 100.0 (4C, C-1‘, C-1‘‘, C-1‘‘‘, C-1‘‘‘‘), 81.3 (C-4‘‘‘‘), 81.0 (C-3‘‘‘‘),76.6 (2C, C-3‘‘‘, C-2‘‘‘‘), 74.9, 74.6, 74.4 (4C, C-4, C-4‘, C-4‘‘, C-4‘‘‘), 71.9, 71.7 (3C, C-3‘, C-3‘‘, C-3‘‘‘), 70.9, 70.7 (4C, C-2‘, C-2‘‘, C-2‘‘‘, C-2‘‘‘‘), 70.54 (d, *J* = 24.9 Hz, C-2), 69.5 (2C, C-3, C-4‘‘‘‘), 63.3 (C-5‘‘‘‘), 62.6, 62.4, 62.4 (3C, C-5‘, C-5‘‘, C-5‘‘‘), 61.9 (C-5‘‘‘‘), 61.02 (d, *J* = 4.8 Hz, C-5), 21.0, 20.9, 20.8, 20.7, 20.6 (13C, *C*H$_3$) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = -151.05 (dd, *J* = 53.0, 24.1 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{56}H_{75}FNaO_{37}^+$: 1381.3869; found: 1381.3931. IR (neat): ν_{max} = 1745, 1372, 1219, 1048 cm^{-1}.

α-L-Arabinofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-D-xylopyranosyl fluoride (85)

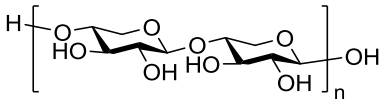


The protected hexasaccharide fluoride **SI25** (18.9 mg, 13.9 μmol) was dissolved in anhydrous MeOH (1.00 mL) and anhydrous DCM (0.200 mL) at 0 °C. NaOMe (0.5 M in MeOH, 30.0 μL, 13.9 μmol) was added dropwise and the solution was stirred at 0 °C for 1 h. The reaction mixture was neutralized by the careful addition of Amberlite IR-120 (H^+) resin. The resin was filtered off and the solvents were evaporated to give fully deprotected hexasaccharide fluoride **85** (11.3 mg, 13.9 μmol, 100%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ = 5.51 (dd, *J* = 53.3, 2.8 Hz, 1H), 5.19 (s, 1H), 4.40 – 4.26 (m, 4H), 4.10 – 3.90 (m, 5H), 3.88 – 3.80 (m, 3H), 3.77 – 3.36 (m, 13H), 3.28 – 3.09 (m, 9H) ppm. ^{13}C NMR (101 MHz, D_2O): δ = 108.0, 107.17 (d, *J* = 223.4 Hz), 101.6, 101.6, 101.6, 83.9, 81.4, 81.1, 76.4, 76.3, 76.3, 75.4, 73.6, 73.6, 72.7, 72.6, 72.6, 71.1, 70.9, 70.8, 67.7, 64.9, 62.9, 61.2, 61.0 ppm. ^{19}F NMR (564 MHz, D_2O): δ = -152.67 (dd, *J* = 53.3, 26.5 Hz) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{30}H_{49}FNaO_{24}^+$: 835.2496; found: 835.2507.

3.4.5.2 Glycosynthase-Catalyzed Enzymatic Polymerizations

General procedure: An aqueous solution of the fluoride donor (600 mM) and a phosphate buffered solution of XynAE265G (7.70 mg/mL, $V_{Fluoride}/V_{XT6E265G}$ = 1:3.6) were added to $Na_xH_xPO_4$ buffer (100 mM, $V_{Fluoride}/V_{buffer}$ = 1:5). The reaction was shaken at r.t. overnight. If a white precipitate was formed, it was centrifuged and washed three times with MilliQ water to give a water-insoluble fraction of polysaccharides. The aqueous phase was removed with a pipette, heated to 80 °C for 15 min to inactivate the glycosynthase and the precipitate was centrifuged off. The remaining solution was passed through a Sep-Pak C18-cartridge (Waters, H_2O/MeOH: 1:0 to 1:1) to give a water-soluble fraction of polysaccharides. If no precipitate was formed the solution was heated to 80 °C for 15 min to inactivate the glycosynthase and the precipitate was centrifuged off. The remaining solution was passed through a Sep-Pak C18-cartridge (Waters, H_2O/MeOH: 1:0 to 1:1) to a give water-soluble fraction of polysaccharides.

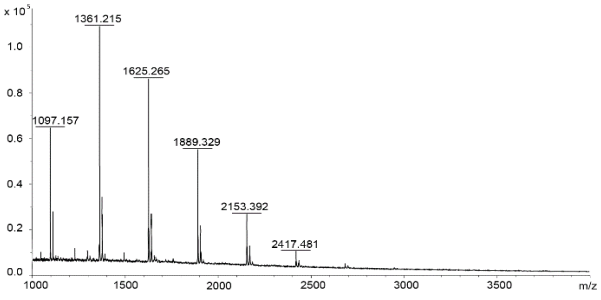
(1→4)-β-D-Xylan $(XX)_n$ (86)



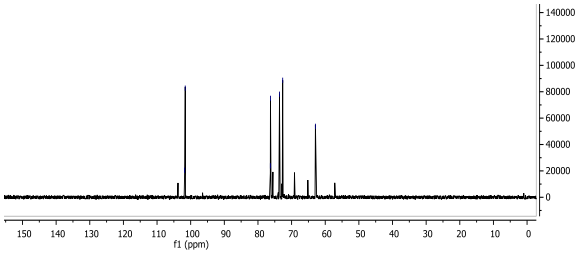
Fluoride donor: 34.6 mg

Water-soluble fraction: 0.98 mg (3%)

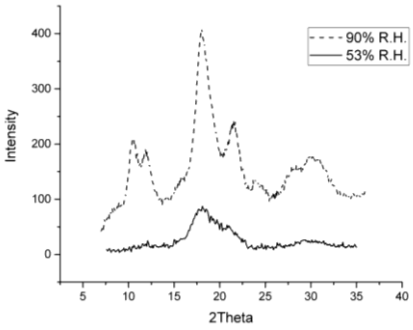
MALDI-TOF-MS:



^{13}C NMR (D_2O, 161 MHz):

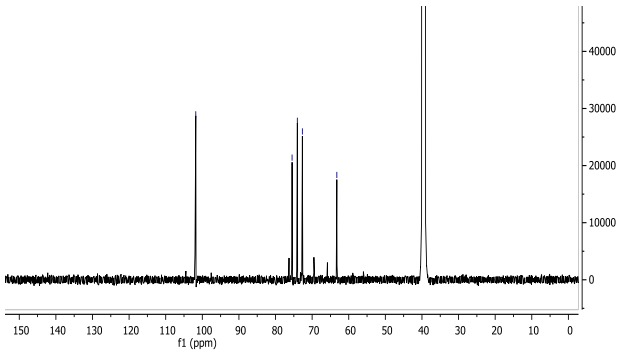


X-Ray diffractorgramm:

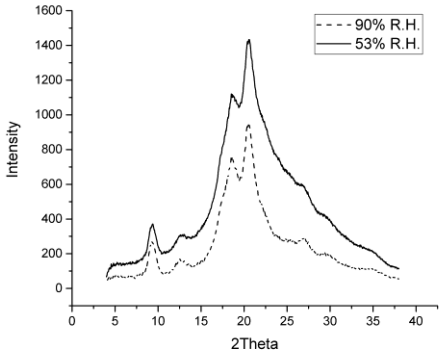


Water-insoluble fraction: 13.9 mg (43%)

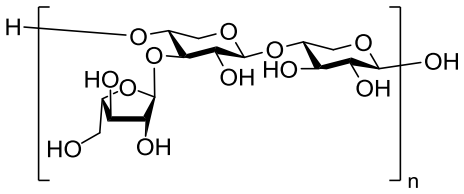
^{13}C NMR (DMSO-d_6, 161 MHz):



X-Ray diffractorgramm:

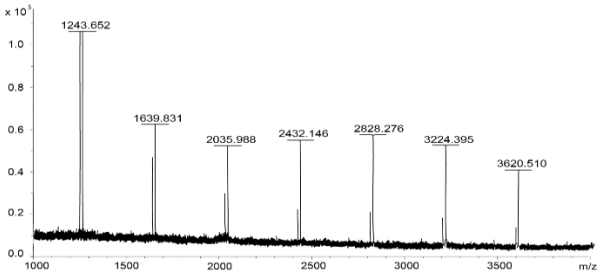


α-L-Arabinofuranosyl-(1→3)-β-D-xylosyl-(1→4)-β-D-xylan ($A^3X)_n$ (87)

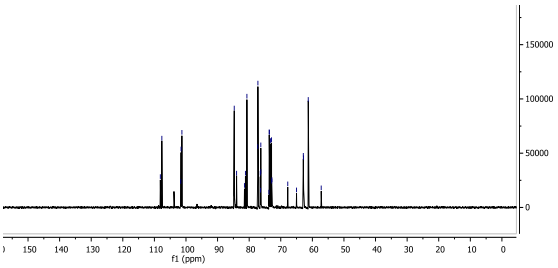


Fluoride donor: 103 mg; Yield: 55.0 mg (51%)

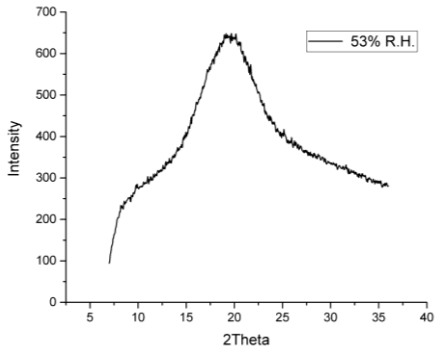
MALDI-TOF-MS:



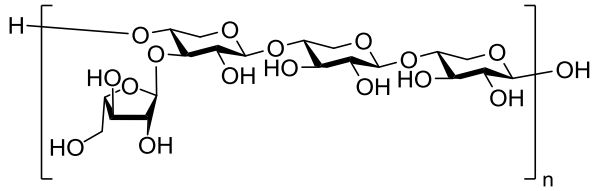
^{13}C NMR (D_2O, 161 MHz):



X-Ray diffractorgramm:



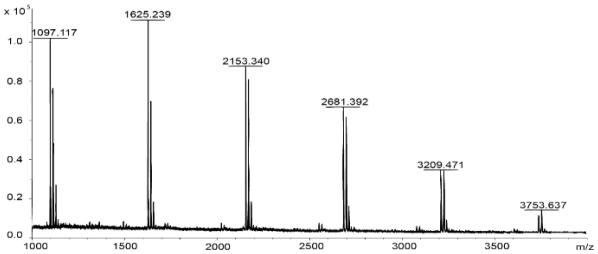
α-L-Arabinofuranosyl-(1→3)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylan ($A^3XX)_n$ (51)



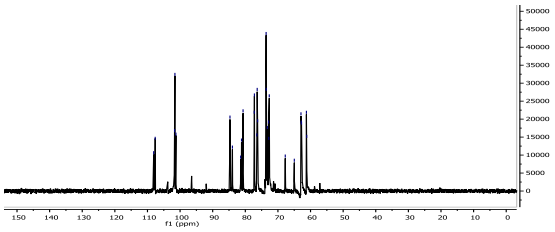
Fluoride donor: 45.4 mg

Water-soluble fraction: 5.87 mg, (13%)

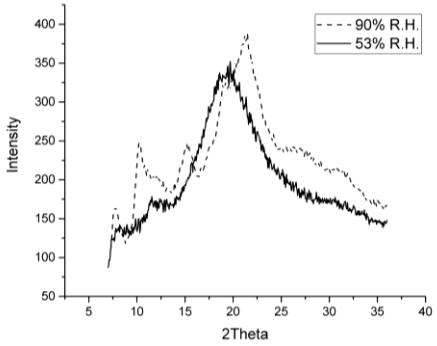
MALDI-TOF-MS:



^{13}C NMR (D_2O, 161 MHz):

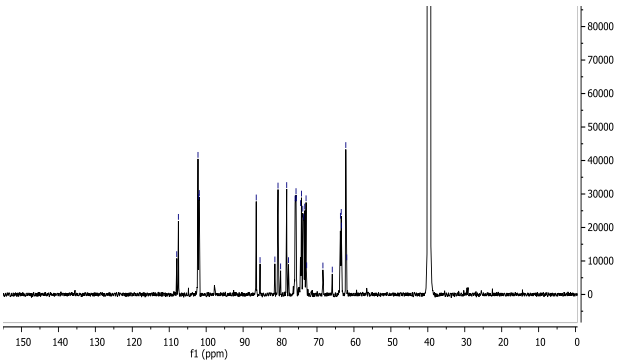


X-Ray diffractorgramm:

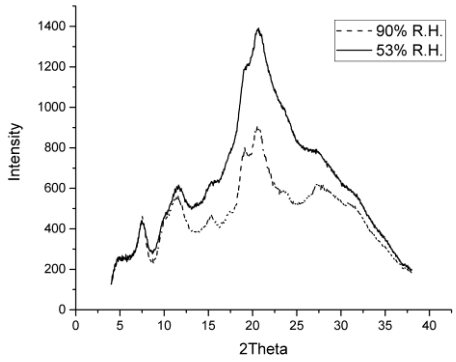


Water-insoluble fraction: 24.6 mg (56%)

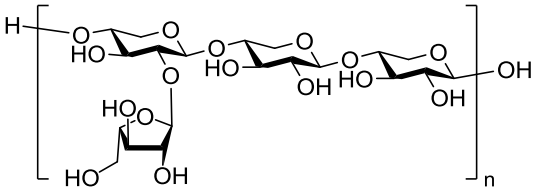
^{13}C NMR (DMSO-d_6, 161 MHz):



X-Ray diffractorgramm:



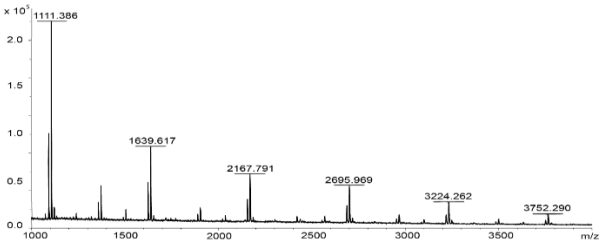
α-L-Arabinofuranosyl-(1→2)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylan $(A^2XX)_n$ (88)



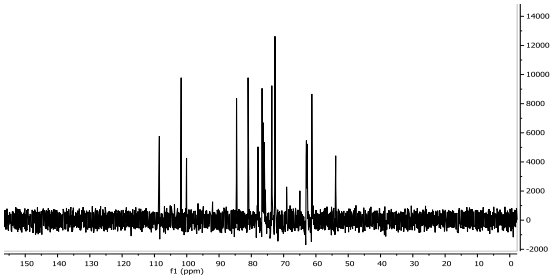
Fluoride donor: 4.18 mg

Water-soluble fraction: 0.42 mg (10%).

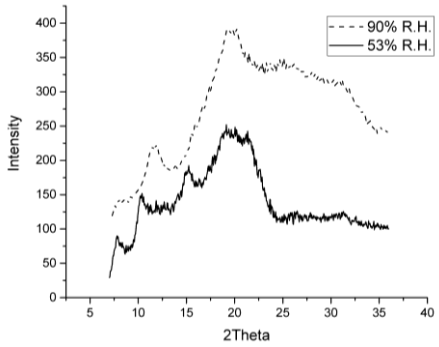
MALDI-TOF-MS:



^{13}C NMR (D_2O, 161 MHz):

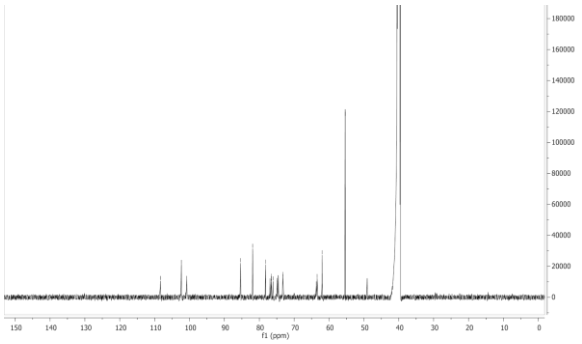


X-Ray diffractorgramm:

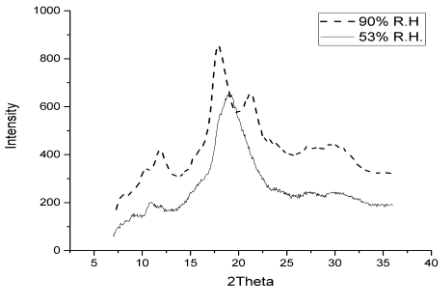


Water-insoluble fraction: 2.31 mg (57%)

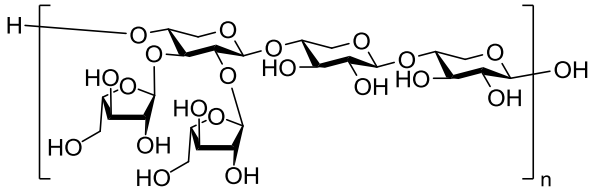
^{13}C NMR (DMSO-d_6, 161 MHz):



X-Ray diffractorgramm:

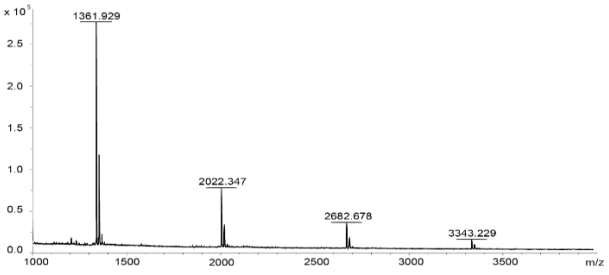


α-L-Arabinofuranosyl-(1→3)-2-*O*-[α-L-arabinofuranosyl]-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylan ($A^{2,3}XX)_n$ (89)

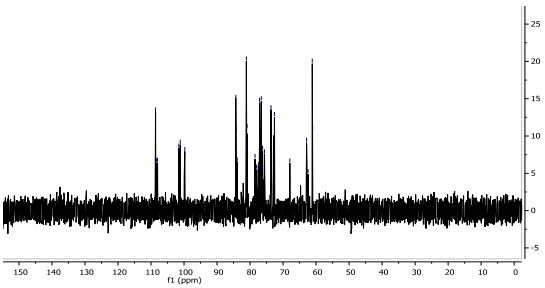


Fluoride donor: 6.00 mg; Yield: 1.83 mg (31%)

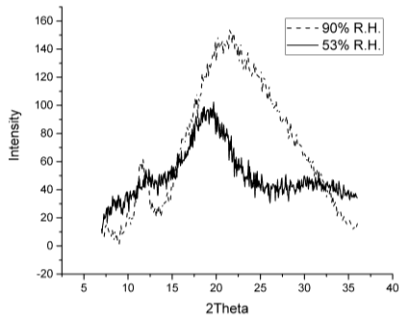
MALDI-TOF-MS:



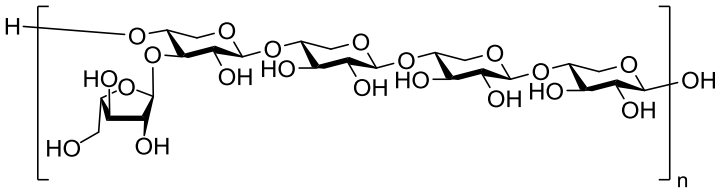
^{13}C NMR (D_2O, 161 MHz):



X-Ray diffractorgramm:

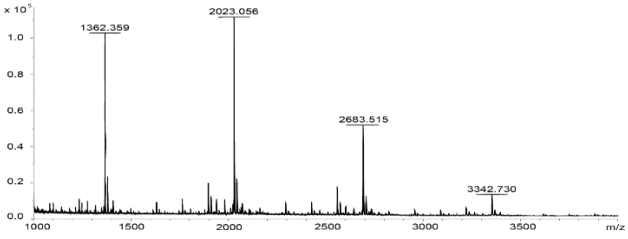


α-L-Arabinofuranosyl-(1→3)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylan ($A^3XXX)_n$ (90)

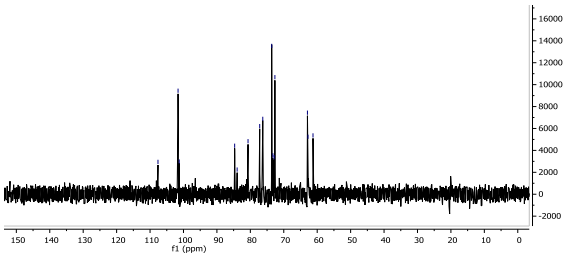


Fluoride donor: 4.49 mg; Yield: 1.24 mg (28%)

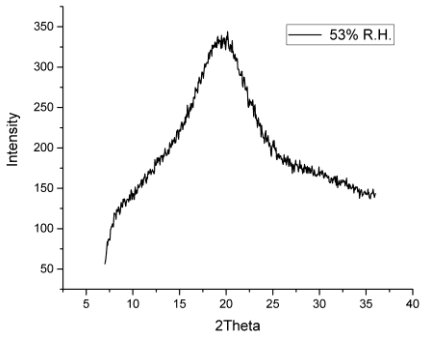
MALDI-TOF-MS:



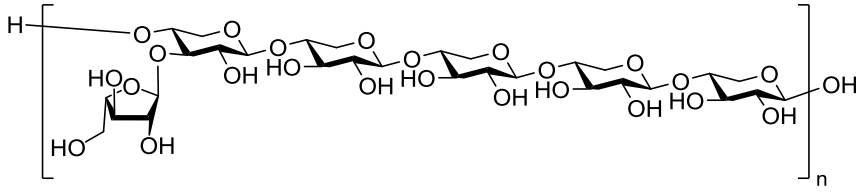
^{13}C NMR (D_2O, 161 MHz):



X-Ray diffractorgramm:

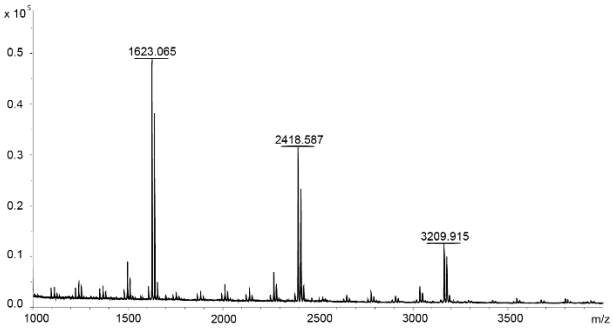


α-L-Arabinofuranosyl-(1→3)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylosyl-(1→4)-β-D-xylan ($A^3XXXX)_n$ (91)

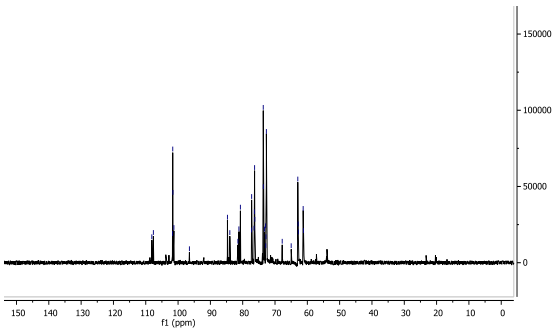


Fluoride donor: 3.65 mg; Yield: 0.98 mg (27%)

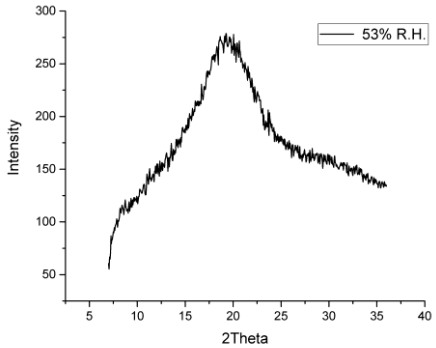
MALDI-TOF-MS:



^{13}C NMR (D_2O, 161 MHz):



X-Ray diffractorgramm:



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