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Abstract

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Determining Substrate Specificities of β1,4-Endo-Galactanases Using Plant Arabinogalactan Oligosaccharides Synthesized by Automated Glycan Assembly

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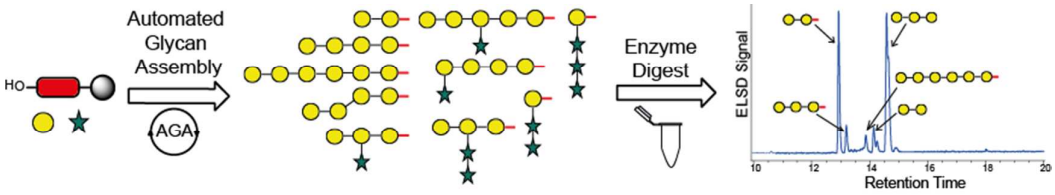


ABSTRACT: Pectin is a structurally complex plant polysaccharide with many industrial applications in food products. The structural elucidation of pectin is aided by digestion assays with glycosyl hydrolases. We report the automated glycan assembly of oligosaccharides related to the arabinogalactan side chains of pectin as novel biochemical tools to determine the substrate specificities of endo-galactanases. Analysis of the digestion products revealed different requirements for the lengths and arabinose substitution pattern of the oligosaccharides to be recognized and hydrolyzed by the galactanases.

Pectin is a highly complex polysaccharide found in the cell walls of all land plants, assuming multiple functions in plant growth and development.[1] Industrially, pectic polysaccharides are used as gelling agents and stabilizers in food production.[2] Pectin also receives growing attention from the pharmaceutical industry as it is beneficial for human health, e.g. by reducing cholesterol[3] and serum glucose levels.[4] There are three major classes of pectic polysaccharides: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). The backbone of HG and RG-II is a homopolymer of α1,4-

linked D-galacturonic acid. RG-I is composed of the disaccharide repeating unit [→2)-α-L-Rhap-(1→4)-α-D-GalpA-(1→] that is highly decorated with arabinans, galactans, and type-I arabinogalactans (AGs). The composition of type-I AG side chains differs between plant species and tissues, but many structural features are conserved. All type-I AGs are composed of a Gal-β1,4-Gal backbone that may be substituted with α1,3-linked single arabinofuranoses, short α1,3- or α1,5-linked arabinan oligosaccharides, or with β1,6-linked galactose residues (Fig. 1).[5]

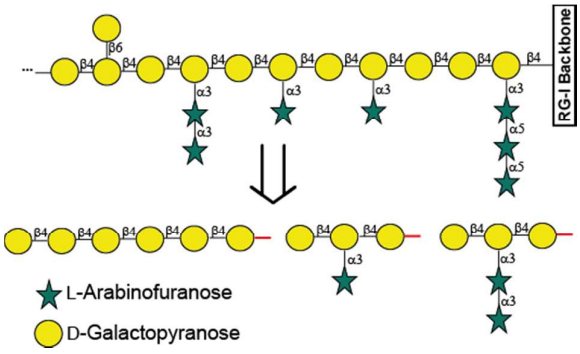


Figure 1. Schematic representation of type-I AG polysaccharides and different oligosaccharide substructures as potential synthetic targets.

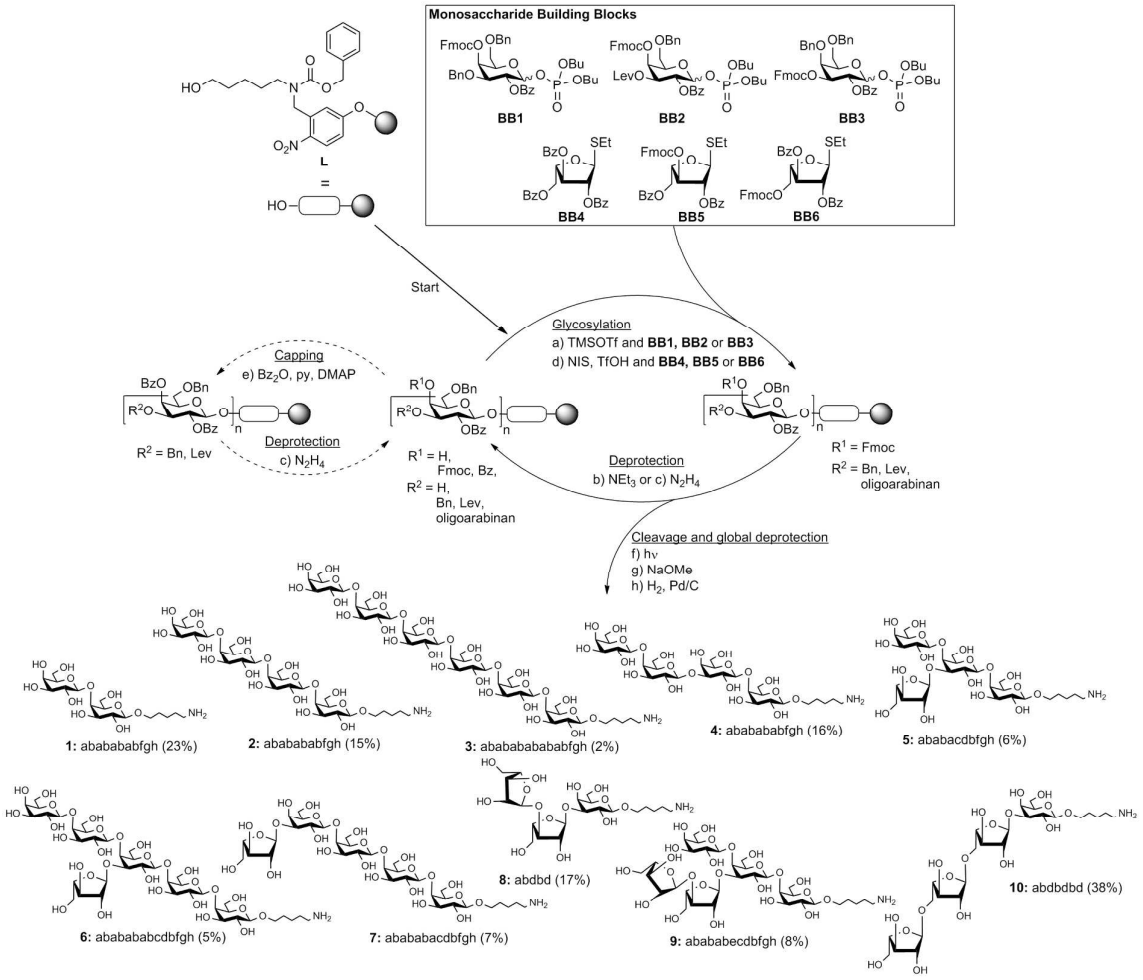
Glycosyl hydrolases acting on pectin are powerful tools to investigate its molecular structure.[6] Short oligosaccharide fragments released by the enzymes can be analyzed by HPLC, mass spectrometry, and NMR-spectroscopy. However, missing information concerning the substrate specificities of these pectinases often prevents comprehensive conclusions about the structure of the digested polysaccharides. Determining the substrate specificities of β1,4-endo-galactanases that cleave the galactan backbone of type-I AGs suffers from a low availablity of pure AG samples.[7] Hence, the structure of type-I AGs is poorly characterized across plant species.

Synthetic oligosaccharides are ideal substrates for investigating glycosyl hydrolases as they are well-defined and provide digestion products that are readily analyzed.[8] Unlike linear- and

β1,6-branched β1,4-linked galactan oligosaccharides, α1,3-substituted type-I AG oligosaccharides have not been prepared previously.[9] Automated glycan assembly[10] served us well to rapidly procure collections of plant cell wall oligosaccharides as valuable tools for the characterization of cell wall glycan-directed antibodies.[11] Here, we describe the use of type-I AG oligosaccharides containing α1,3-linked arabinofuranosides prepared by automated glycan assembly to determine the substrate specificities of three β1,4-endo-galactanases (Fig. 1).

The AG oligosaccharides were prepared from six monosaccharide building blocks (BBs) (Scheme 1). Two galactose building blocks were required for the synthesis of the β1,4-linked galactan backbone. Both BBs (**BB1**[12] and **BB2**) rely on a base labile fluorenylmethoxycarbonyl (Fmoc) group in the C-4 position for chain elongation. For the installation of substituents in the C-3 position, one of the BBs (**BB2**) was equipped with a temporary levulinoyl (Lev) protecting group that can be cleaved using hydrazine and is fully orthogonal to the Fmoc group.[10a, 13] A third galactose building block (**BB3**)[11a] with an Fmoc protecting group in the C-3 position was used for elongation of the galactose solely in the C-3 position. All remaining hydroxyl groups in **BB1**, **BB2** and **BB3** are not modified during the oligosaccharide assembly process and were permanently protected as benzyl ethers and benzoyl esters. Inspired by good results in the synthesis of type-II AG oligosaccharides, the galactose building blocks were equipped with phosphate leaving groups.[11a] Three different L-arabinofuranose BBs (**BB4-BB6**) were used for the installation of single arabinose residues, α1,3-linked arabinan disaccharides, and an α1,5-linked arabinan trisaccharide. The arabinose building blocks were equipped with benzoyl esters as permanent protecting groups and temporary Fmoc-protecting groups at the respective position that is elongated during oligoarabinan syntheses. Arabinose thioglycoside BBs showed good results previously.[11a, 11c]

Scheme 1. Automated glycan assembly of type-I arabinogalactan oligosaccharides.



Reagents and conditions: a) Twice 3.8 or 5 equiv of **BB1**, **BB2** or **BB3**, TMSOTf, DCM, -35°C (5 min) → -20 °C (30 min) or -30 °C (5 min) → -10 °C (30 min); b) Three cycles of 20% NEt_3 in DMF, 25 °C (5 min); c) Three cycles of 0.15 M hydrazine in py/AcOH/H_2O (4:1:0.25), 25 °C (30 min); d) Twice 3.8 equiv **BB4**, **BB5** or **BB6** NIS, TfOH, DCM/dioxane, -40 °C (5 min) → -20 °C (40 min); e) Three cycles of 0.5 M Bz_2O and 0.25 M DMAP in DCE, py, 40 °C (30 min); f) hν (305 nm); g) NaOMe, THF, 16 h; h) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16h. **1**: 23%, **2**: 15%, **3**: 2%, **4**: 16%, **5**: 8%, **6**: 5%, **7**: 7%, **8**: 17%, **9**: 8%, **10**: 38% (yields are based on resin loading). The letter code below the structures represents the synthesizer modules and deprotection steps used for the syntheses.

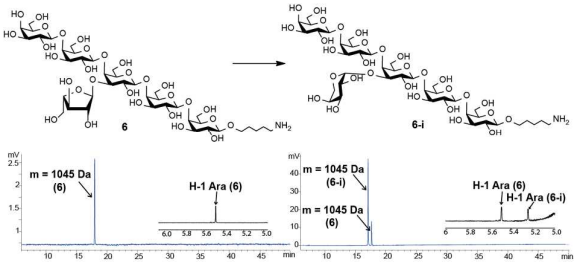
The automated glycan assembly of ten oligosaccharide fragments of pectic type-I AG polysaccharides was based on building blocks **BB1-BB6**. Alternating glycosylation and deprotection procedures were performed on a solid support functionalized with a photolabile linker that yields oligosaccharides with an aminopentyl linker at the reducing end.[14] Glycosylations with phosphate building blocks **BB1-BB3** were generally performed using two times 3.8 equivalents building block[15] and stochiometric amounts of the activator trimethylsilyl trifluoromethanesulfonate (TMSOTf). Thioglycoside building blocks **BB4-BB6** were used in two glycosylation cycles adding 3.8 equivalents building block and activated with a slight excess of *N*-iodosuccinimide (NIS)[16] together with catalytic amounts of triflic acid. Deprotection of Fmoc was achieved with triethylamine (NEt_3) (20% in dimethylformamide (DMF)). Levulinoyl protecting groups were removed with a hydrazine acetate solution (0.15 M). Following assembly of the galactan backbone, capping with benzoic anhydride in the presence of dimethylaminopyridine (DMAP) allowed for temporary Fmoc protecting groups to be used for the selective elongation of arabinan side chains. The fully deprotected oligosaccharides were obtained following light-induced cleavage from the solid support, methanolysis of the ester protecting groups, and hydrogenolysis of the benzyl ethers and the caboxybenzyl (Cbz) group that remains from the linker.

Initially, the linear β1,4-linked galactan oligosaccharides **1-3** were prepared. The low reactivity of the axial C4-hydroxy nucleophile on galactose is responsible for the decreased yields as the length of the galactan backbone increased. Still, linear β1,4-linked oligogalactans as long as hexasaccharides were prepared, albeit in low yields (**3**). Arabinogalactan oligosaccharides **5-7** that contain α1,3-linked arabinose either at the central (**5** and **6**) or the terminal position of the backbone (**7**) were prepared by incorporation of the levulinoyl substituted BB (**BB2**) into the galactan backbone. Arabinose BBs **BB5** and **BB6** were key to the synthesis of short α1,3- and α1,5-linked arabinan oligomers. These arabinan oligosaccharides were either attached to a single galactose unit (**8** and **10**) or a galactan

trisaccharide backbone (**9**). Finally, β1,4-, β1,3-mixed linkage galactan tetrasaccharide **4**, a structural component in the backbone of type-I AGs in potato, was prepared.[17]

When preparing stock solutions of the oligosaccharides in water (2 mM), we observed a slow isomerization of hexasaccharide **6**. While the HPLC analysis of **6** revealed a single product peak immediately following the last deprotection reaction, an additional peak of identical mass appeared after storing **6** for several days in water at 4 °C. ^{1}H-NMR analysis revealed a shift of the signal for the anomeric arabinose proton from 5.48 ppm to 5.24 ppm (Scheme 2). We hypothesize that the arabinofuranose isomerized into the corresponding β-arabinopyranose. This isomerization reaction may have been intramolecularly catalyzed by the aminoalkyl linker. Base-catalyzed isomerizations of methyl arabinofuranosides into arabinopyranosides in the presence of pyridine have been observed previously.[18] None of the other arabinogalactan oligosaccharides underwent isomerization.

Scheme 2. Isomerization of arabinogalactan **6**



Left: HPLC analysis (ELSD trace) and ^{1}H-NMR chemical shift of the anomeric arabinose proton of **6** directly after synthesis. Right: HPLC analysis and ^{1}H-NMR chemical shifts of the anomeric arabinose proton of **6** after storage in aqueous solution for several days.

The arabinogalactan oligosaccharides are ideal probes to investigate the specificity of β1,4-endo-galactanases. The minimal oligomer length required for galactan backbone hydrolysis and the degree of arabinose substitution tolerated by the respective enzymes are of particular interest. This information is essential to draw conclusions on the molecular structure of AG

polysaccharides after digestion. Three representative β1,4-endo-galactanases from the glycosyl hydrolase (GH) family 53 were investigated: E-EGALN from *Aspergillus niger*, E-GALCJ from *Cellvibrio japonicus*, and E-GALCT from *Clostridium thermocellum*. Oligosaccharides were incubated with the enzymes for 3 h at 40 °C before stopping the reaction by heat inactivation of the enzyme at 80 °C. The resulting digestion products were analyzed by HPLC coupled to an ELS-detector and a mass spectrometer. HPLC analysis of the digestion products revealed different minimal length requirements for hydrolysis by the galactanases (Figure 2). While E-GALCJ completely degraded tetragalactoside **2**, E-EGALN only partially hydrolyzed **2**. Since neither the linker-functionalized mono- nor disaccharide was detected, we assume that E-GALCJ hydrolyzed the linker first and then cleaved the central bond of the resulting free reducing tetrasaccharide. Hexasaccharide **3**, in contrast to tetrasaccharide **2**, was hydrolyzed equally well by the two galactanases, although a slightly different product pattern was observed. Mixed-linkage tetragalactoside **4** remained intact during incubation with the galactanases, proving their strict selectivity for β1,4-glycosidic linkages.

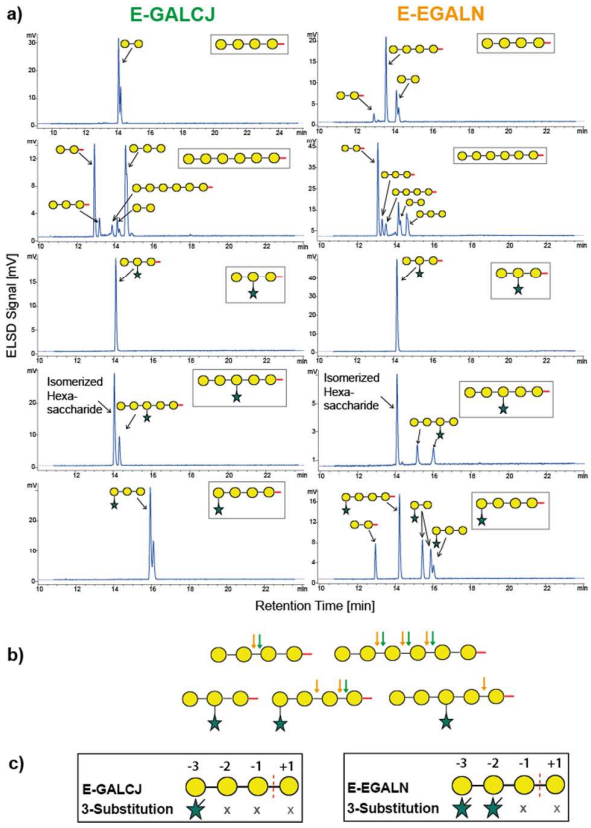


Figure 2. Digestion of synthetic arabinogalactan oligosaccharides with the β1,4-endo-galactanases E-GALCJ and E-EGALN and analysis of the resulting hydrolysis products by HPLC-MS. (a) HPLC analysis of the products after incubation of the respective oligosaccharides (indicated by boxes) with the galactanases. Peaks are annotated with AG fragments either carrying an aminopentyl linker or with free reducing end (with or without red bar). Note that α- and β-anomers of the free reducing sugars elute as separate- or double-peaks. (b) The cutting sites derived from (a) are summarized and indicated by arrows. (c) General requirements for arabinose substitutions relative to the cutting site of E-GALCJ and E-EGALN galactanases. "X" denotes galactose residues that must not be substituted with arabinofuranose. The reducing end of the structures is located on the right.

Next, we investigated the effect of arabinose substitution on digestion efficiency. Arabinogalactan tetrasaccharide **4** was not hydrolyzed by any of the galactanases. Hexasaccharide **6**, bearing an arabinose residue in the central position, was cleaved by

E-EGALN but not E-GALCJ between the first two galactose units. Thus, E-EGALN does tolerate arabinose substitution in the -2 subsite relative to the site of hydrolysis. The isomerized hexasaccharide **6-i** was not digested by E-EGALN, suggesting a specific recognition of the arabinofuranose. Further information was provided by analysis of the hydrolysis products obtained after digestion of pentasaccharide **7** having the arabinose substitution in the terminal position. E-GALCJ tolerated arabinose substitution in the -3 subsite and cleaved the bond between the first two galactose residues. E-GALN on the other hand additionally cleaved the bond between the second and third galactose unit, demonstrating its ability to accept arabinose substitution in both the -2 and -3 subsite. Neither galactanase tolerated substitution in direct proximity to the cleavage site as no corresponding hydrolysis fragments were detected. The third investigated galactanase E-GALCT gave results similar to E-GALN. In summary, our results demonstrate that the substrate specificities of these GH53 β1,4-endo-galactanases differ in the number of subsites that are important for substrate binding and in their tolerance for arabinose substitution.

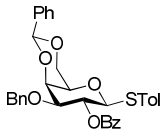
In conclusion, we discovered that β1,4-endo-galactanases recognize and hydrolize arabinogalactan oligosaccharides of different lengths and arabinose substitution patterns. These findings have implications for future structural analyses of pectic polysaccharides. Key to these studies was a collection of synthetic type-I AG oligosaccharides assembled by automated glycan assembly. Despite the inherent challenges associated with C-4 glycosylation of galactose, we were able to synthesize AG hexasaccharides. The oligosaccharide tools are currently applied to the characterization of biosynthetic enzymes and arabinogalactan- and arabinan-directed antibodies.

Experimental Section

The automated syntheses were performed on a self-built synthesizer developed in the Max Planck Institute of Colloids and Interfaces. Linker-functionalized resin **L** was synthesized according to literature procedures.[14] Resin loading was determined by performing one glycosylation (Module **A**) with large excess of **BB3** followed by 1,8-diazabicyclo[5.4.0]undec-7-en promoted Fmoc-cleavage and determination of dibenzofulvene production by measuring its UV absorbance.[19] Advanced intermediates *p*-tolyl 3-*O*-*tert*-butyldimethylsilane-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[11a], *p*-tolyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[20], 2,3,5-tri-*O*-benzoyl-1-methyl-α-L-arabinofuranoside[21], and building blocks ethyl 2,3-di-*O*-benzoyl-5-*O*-fluorenylcarbonylmethoxy-1-thio-α-L-arabinofuranoside (**BB6**)[21], ethyl 2,3,5-tri-*O*-benzoyl-1-thio-α-L-arabinofuranoside (**BB4**)[21] were synthesized according to literature procedures for D-isomers. **BB1** was synthesized in close analogy to a previously published building block.[12]Phosphate BBs (**BB1** to **BB3**) were used in the automated synthesis as mixtures of α/β-anomers. Solvents and reagents were used as supplied without any further purification. Anhydrous solvents were taken from a dry solvent system. Column chromatography was carried out using Fluka Kieselgel 60 (230-400 mesh). NMR spectra were recorded 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 $CDCl_3$ used the solvent residual peak chemical shift as internal standard ($CDCl_3$: 7.26 ppm ^1H, 77.16 ppm ^{13}C). Spectra recorded in D_2O used either residual acetic acid (AcOH) (D_2O: 2.08 ppm ^1H) or formic acid (D_2O: 8.26 ppm ^1H) (compound **4**) as internal standards in ^1H NMR and an acetic acid (D_2O: 21.03 ppm ^{13}C) or a formic acid (D_2O: 166.31 ppm ^{13}C) (compound **4**) spike as internal standard in ^{13}C NMR. NMR peaks of BBs and BB intermediates were assigned by COSY and HSQC NMR experiments. Yields of final deprotected oligosaccharides were determined after removal of residual acetic acid. Optical rotations were measured in concentrations expressed

as g/100 mL. IR spectra were recorded on a FTIR spectrophotometer. High resolution mass spectra were obtained using a ESI-TOF mass spectrometer. Analytical HPLC was performed using a YMC-Pack DIOL-300-NP column (150 x 4.6 mm), a Phenomenex Luna C5 column (250 x 4.6 mm), or a Thermo Scientific Hypercarb column (150 x 4.6 mm). Preparative HPLC was performed on an Agilent 1200 series using a preparative YMC-Pack-DIOL-300-NP (150 x 20 mm), a semi-preparative Phenomenex Luna C5 column (250 x 10 mm) or a semi-preparative Thermo Scientific Hypercarb column (150 x 4.6 mm).

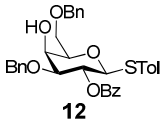
Building Block Synthesis



11

2-O-Benzoyl-3-O-benzyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside ***(11)***. 3-*O-Tert*-butyldimethylsilyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[11a] (3.58 g, 7.32 mmol) was dissolved in 20 mL of anhydrous DMF at -10 °C under Ar, 0.702 g (17.6 mmol) of NaH (60% dispersion in mineral oil) were added. The mixture was stirred for 10 min before 2.61 ml (22.0 mmol) of benzyl bromide were added. The solution was allowed to stir for another 4 h at -10 °C under Ar. The reaction was quenched by the addition of aq. sat. NH_4Cl solution. The reaction mixture was diluted with dichloromethane (DCM) and the organic layer was separated, washed with brine and dried over Na_2SO_4. The solvent was removed *in vacuo*.The residue was taken up in 40 mL tetrahydrofurane (THF), and 15.0 mL (15.0 mmol) of a 1 M tetrabutylamonium fluoride (TBAF) solution in THF was added. The solution was stirred overnight at rt. The solvent was evaporated and the crude product was taken up in DCM and washed with aq. sat. NH_4Cl solution and brine. The solvent was evaporated and the residue together with 4.97 g (22.0 mmol) benzoic anhydride and 0.447 g (3.66 mmol) DMAP was dissolved up in 25 mL anhydrous DCM under Ar at 0 °C. Triethylamine (4.08 mL, 29.3 mmol) was added and the solution was allowed to stir overnight at rt. The solution was diluted

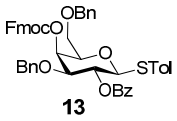
with DCM and washed with aq. sat. $NaHCO_3$ solution and brine. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The product was subjected to silica gel column chromatography (ethyl acetate (EtOAc)/hexane (hex)/DCM = 1.5:8:0.5 to 8:2:0.5) and the **11** was obtained in 34% yield (1.39 g, 2.45 mmol). $[\alpha]_D^{25}$ = +25.3 (c 1.07, $CHCl_3$). IR (neat). ν_{max} 1718, 1260, 1086, 1057 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 – 8.02 (m, 2H, Ar), 7.63 – 7.57 (m, 1H, Ar), 7.48 (d, *J* = 8.0, 4H, Ar), 7.46 – 7.42 (m, 2H, Ar), 7.38 – 7.33 (m, 3H, Ar), 7.22 – 7.11 (m, 5H, Ar), 7.04 (d, *J* = 7.9, 2H, Ar), 5.50 (t, *J* = 9.7, 1H, H-2), 5.47 (s, 1H, PhC*H*O_2), 4.76 (d, *J* = 9.8, 1H, H-1), 4.63 (d, *J* = 12.8, 1H, PhCH_2), 4.55 (d, *J* = 12.8, 1H, PhCH_2), 4.38 (dd, *J* = 12.3, 1.4, 1H, H-6), 4.23 (d, *J* = 3.0, 1H, H-4), 4.02 (dd, *J* = 12.3, 1.5, 1H, H-6), 3.76 (dd, *J* = 9.6, 3.3, 1H, H-3), 3.48 (d, *J* = 0.8, 1H, H-5), 2.32 (s, 3H, SPhCH_3). ^{13}C NMR{^1H} (101 MHz, $CDCl_3$): δ 164.9 (C=O), 138.2, 137.7, 137.6, 134.4, 133.0, 130.2, 129.9, 129.5, 129.0, 128.3, 128.2, 128.1, 127.69, 127.67, 127.5, 126.7 (24C, Ar), 101.3 (Ph*C*HO_2), 85.4 (C-1), 78.2, 73.1, 71.0, 70.0, 69.3, 69.1, 21.3 (SPh*C*H_3). ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{34}H_{32}NaO_6S$: 591.1817; found 591.1833.



12

2-O-Benzoyl-3,6-O-dibenzyl-1-thio-β-D-galactopyranoside ***(12)*** To 1.37 g (2.41 mmol) of **11** in 50 mL anhydrous DCM under Ar atmosphere at 0 °C were added 2.31 mL (14.5 mmol) of triethylsilane and 0.340 mL (2.41 mmol) of trifluoroacetic acid anhydride (TFAA). The solution was stirred at 0 °C for 15 min and 1.11 mL (14.5 mmol) trifluoroacetic acid (TFA) were added slowly. The ice bath was removed and the reaction was allowed to stir for additional 3 h. The reaction mixture was washed with aq. sat. $NaHCO_3$ solution and brine. The organic layer was separated, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield 1.10 g (1.92 mmol, 80%) of **12**. $[\alpha]_D^{25}$ = +30.7 (c 0.10, $CHCl_3$). IR (neat). ν_{max} 1732, 1266, 1084, 1047 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 – 8.01 (m, 2H,

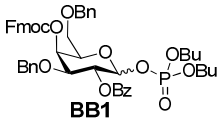
Ar), 7.64 – 7.58 (m, 1H, Ar), 7.47 (t, *J* = 7.7, 2H, Ar), 7.39 – 7.28 (m, 7H, Ar), 7.21 – 7.10 (m, 5H, Ar), 7.02 (d, *J* = 7.9, 2H, Ar), 5.47 (t, *J* = 9.7, 1H, H-2), 4.71 – 4.64 (m, 2H, H-1, PhCH_2), 4.59 (s, 2H, PhCH_2), 4.51 (d, *J* = 12.3, 1H, PhCH_2), 4.16 (d, *J* = 2.3, 1H, H-4), 3.88 – 3.79 (m, 2H, H-6), 3.69 (t, *J* = 6.0, 1H, H-5), 3.65 (dd, *J* = 9.3, 3.2, 1H, H-3), 2.29 (s, 3H, SPhCH_3). ^{13}C NMR{^{1}H} (101 MHz, $CDCl_3$): δ 165.4 (C=O), 138.1, 138.0, 137.1, 133.2, 133.0 , 130.1, 130.0, 129.6, 129.3, 128.57, 128.54, 128.4, 128.3, 128.0, 127.99, 127.97, 127.90,(24C, Ar), 87.0 (C-1), 79.4 (C-3), 77.5 (C-5), 73.8 (Ph*C*H_2), 71.4 (Ph*C*H_2), 69.8 (C-2), 69.3 (C-6), 66.4 (C-4), 21.2 (SPh*C*H_3). ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{34}H_{34}NaO_6S$: 593.1973; found 593.1986.



13

2-O-Benzoyl-3,6-O-dibenzyl-4-O-fluorenylcarbonylmethoxy-1-thio-β-D-galactopyranoside ***(13)***. Pyridine (0.306 mL, 3.80 mmol) was added to a solution of 1.08 g (1.90 mmol) **12** in 10 mL anhydrous DCM. The mixture was stirred for 15 min and 0.982 g (3.80 mmol) FmocCl were added. After stirring the reaction mixture for 18 h at rt the solvent was removed under reduced pressure and the residue was co-evaporated with toluene twice. The remaining oil was taken up in DCM, washed with brine twice, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was recrystallized from ethanol (EtOH) to give fully protected thioglycoside **13** as colorless crystals in 88% yield (1.32 g, 1.66 mmol). $[\alpha]_D^{25}$ = +38.9 (c 0.10, $CHCl_3$). IR (neat) ν_{max} 1747, 1720, 1252, 1234 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.99 (m, 2H, Ar), 7.78 (dd, *J* = 7.3, 3.4, 2H, Ar), 7.70 (d, *J* = 7.4, 1H, Ar), 7.66 – 7.57 (m, 2H, Ar), 7.51 – 7.29 (m, 13H, Ar), 7.15 – 7.08 (m, 3H, Ar), 6.98 – 7.06 (m, 4H, Ar), 5.57 (t, *J* = 9.8, 1H, H-2), 5.53 (d, *J* = 2.8, 1H, H-4), 4.75 (d, *J* = 10.1, 1H, H-1), 4.68 (d, *J* = 12.6, 1H, PhCH_2), 4.57 – 4.43 (m, 4H, PhCH_2, Fmoc), 4.29 – 4.22 (m, 2H, Fmoc), 3.85 (t, *J* = 6.4, 1H, H-5), 3.78 – 3.65 (m, 3H, H-3, H-6), 2.28 (s, 3H, SPhCH_3). ^{13}C NMR{^{1}H} $CDCl_3$ (101 MHz, $CDCl_3$): δ 165.2, 155.1 (2C C=O), 143.8, 143.2, 141.4, 141.3, 138.1, 137.6, 137.2, 133.2,

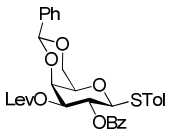
132.8, 130.09, 130.06, 129.7, 129.5, 128.5, 128.4, 128.3, 128.06, 128.02, 127.97, 127.93, 127.7, 127.45, 127.41, 125.8, 125.4, 120.07, 120.04 (36C, Ar), 87.6 (C-1), 77.5 (C-3), 76.1 (C-5), 73.9 (Ph*C*H_2), 71.1 (Ph*C*H_2), 70.6 (C-4), 70.3 (Fmoc), 69.6 (C-2), 68.2 (C-6), 46.6 (Fmoc), 21.2 (SPh*C*H_3). ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{49}H_{44}NaO_8S$: 815.2654; found 815.2658.



BB1

*Dibutoxyphosphoryloxy 2-O-benzoyl-3,6-O-dibenzyl-4-O-fluorenylcarbonylmethoxy-β-D-galactopyranoside (**BB1**)*. Powdered 4 A molecular sieve (5.00 g) was heated and dried under vacuum for 30 min before 25 mL anhydrous DCM and dibutyl phosphate (0.659 mL, 3.32 mmol) were added. The mixture was stirred for 1 h. After stirring, the molecular sieve was allowed to settle for 30 min and the supernatant was added to a solution of **13** (1.32 g, 1.66 mmol) in 10 mL anhydrous DCM, cooled to 0 °C under Ar and NIS (0.486 g, 2.16 mmol) and TfOH (0.044 mL, 0.499 mmol) were added. The purple reaction mixture was stirred for 1 h. The reaction was quenched with aq. sat. $NaHCO_3$ solution and washed with aq. sat. $Na_2S_2O_3$ solution until the color of the organic layer changed from purple to colorless. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash chromatography over silica gel (EtOAc/hex = 1:8 to 2:3) to give phosphate **BB1** as a mixture of α/β anomers (1.01 g, 1.15 mmol, 69% yield) as a highly viscous and sticky oil (analytical data for β-anomer). $[\alpha]_D^{25}$ = +38.0 (c 0.20, $CHCl_3$). IR (neat). ν_{max} 1733, 1250, 196, 1026 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 – 8.00 (m, 2H), 7.78 (dd, *J* = 7.3, 3.1, 2H), 7.71 (d, *J* = 7.4, 1H), 7.66 – 7.57 (m, 2H), 7.48 – 7.28 (m, 10H), 7.25 – 7.22 (m, 1H), 7.15 – 7.07 (m, 3H), 7.05 – 6.99 (m, 2H), 5.66 (dd, *J* = 10.0, 8.2, 1H, H-2), 5.54 (d, *J* = 2.6, 1H, H-4), 5.36 (t, *J* = 7.8, 1H, H-1), 4.69 (d, *J* = 12.7, 1H, PhCH_2), 4.57 – 4.49 (m, 3H, Fmoc, PhCH_2), 4.46 (d, *J* = 12.6, 1H, PhCH_2), 4.29 – 4.22 (m, 2H, Fmoc), 4.08 – 3.99 (m, 2H

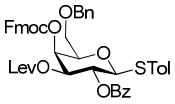
OBu), 3.96 (t, *J* = 6.5, 1H, H-5), 3.78 – 3.62 (m, 5H, H-3, H-6, OBu), 1.65 – 1.56 (m, 2H, OBu), 1.35 (dq, *J* = 14.7, 7.4, 2H, OBu), 1.30 – 1.22 (m, 2H, OBu), 1.00 (dq, *J* = 14.6, 7.4, 2H, OBu), 0.89 (t, *J* = 7.4, 3H, C*H*$_3$), 0.66 (t, *J* = 7.4, 3H, C*H*$_3$). ^{13}C NMR{^{1}H} (101 MHz, $CDCl_3$): δ 165.1, 155.0 (2C, C=O), 143.7, 143.2, 141.4, 141.3, 137.4, 137.0, 133.4, 130.1, 129.6, 128.6, 128.5, 128.3, 128.1, 128.08, 128.03, 127.98, 127.94, 127.91, 127.4, 125.8, 125.4, 120.09, 120.06 (30C, Ar), 97.0 (d, J = 4.8, C-1), 76.0, 73.9, 72.8 (C-5), 71.2 (Ph*C*H$_2$), 70.7 (d, J = 8.7, C-2), 70.4 (Fmoc), 70.0, 68.1 (d, J = 6.4), 67.9 (d, J = 6.4), 67.3, 46.6, 32.1 (d, J = 7.4), 31.8 (d, J = 7.3), 18.6, 18.3, 13.7, 13.5. ESI-HRMS: m/z $[M+K]^+$ calcd for $C_{50}H_{55}KO_{12}P$: 917.3063; found 917.3093.



14

p-Tolyl 2-O-benzoyl-3-O-levulinoyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside ***(14)***. A solution of p-tolyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[20] (4.20 g, 8.75 mmol) in DCM (Volume: 50 mL) at 0 °C was treated with DMAP (0.641 g, 5.25 mmol), N,N´-diiopropylcarbodiimind (2.05 mL, 13.1 mmol) and levulinic acid (1.43 mL, 14.0 mmol). The solution was stirred for 3 h at rt. A white precipitate slowly formed and the solution turned slightly pink. After complete conversion of the starting material, the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hex = 2:3) to give galactose derivative **14** (4.30 g, 7.46 mmol, 85% yield). $[\alpha]_D^{25}$ = -14.7 (c 1.1, $CHCl_3$). IR (neat) ν_{max} 1711, 1252, 997, 717 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.6 Hz, 2H, Ar), 7.59 (t, *J* = 7.4 Hz, 1H,. Ar), 7.50 – 7.33 (m, 9H, Ar), 7.06 (d, *J* = 7.5 Hz, 2H, Ar), 5.53 (t, *J* = 9.8 Hz, 1H, H-2), 5.48 (s, 1H, PhC*H*), 5.17 (d, *J* = 9.9 Hz, 1H, H-3), 4.81 (d, *J* = 9.7 Hz, 1H, H-1), 4.40 (m, *J* = 12.6 Hz, 2H, H-4, H-6), 4.04 (d, *J* = 12.3 Hz, 1H, H-6) 3.64 (s, 1H, H-5), 2.67 – 2.37 (m, 4H, Lev), 2.34 (s, 3H, SPhC*H*$_3$), 1.86 (s, 3H, Lev(C*H*$_3$)). ^{13}C NMR{^{1}H} (101 MHz, $CDCl_3$) δ 206.2 (C=O), 172.1 (C=O), 165.0 (C=O),

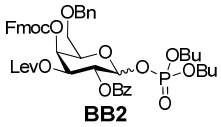
138.5, 137.7, 134.6, 133.3, 129.9, 129.8, 129.6, 129.2, 128.5, 128.2, 127.2, 126.7 (18 C, Ar), 101.2 (Ph*C*H), 85.4 (C-1), 73.6 (C-4), 73.4 (C-3), 69.9 (C-5), 69.2 (C-6), 67.4 (C-2), 37.9 (Lev), 29.5 (Lev(*C*H_3)), 28.3 (Lev), 21.4 (SPh*C*H_3).ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{32}H_{32}NaO_8S$: 599.1715, found: 599.1687.



15

*p-Tolyl 2-O-benzoyl-3-O-levulinoyl-4-O-fluorenylcarbonylmethoxy-6-O-benzyl-1-thio-β-D-galactopyranoside **(15)**.* Galactose derivative **14** (4.30 g, 7.46 mmol) was dissolved in 50 mL anhydrous DCM under nitrogen atmosphere and 7.16 mL (44.7 mmol) triethylsilane and 1.00 mL (7.46 mmol) TFAA were added. The solution was stirred at rt for 15 min before 3.42 mL (44.7 mmoL) TFA were added dropwise. The reaction was stirred at rt for additional 2 h before it was quenched by the addition of aq. sat. $NaHCO_3$ solution and washed with brine. The organic phase was separated, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was dissolved in anhydrous DCM (50 mL) and 2.09 mL (25.9 mmol) anhydrous pyridine was added. The solution was stirred at rt for 15 min before it was cooled down to 0 °C and 3.99 g (15.4 mmol) FmocCl were added. The reaction was allowed to slowly warm up to rt and stirred for 6 h. The solvent was removed under vacuum and the residue co-evapoated with toluene twice before compound **15** was recrystallized from hot ethanol in 66% yield (2.75 g, 3.43 mmol). $[\alpha]_D^{25}$ = +1.0 (c 0.4, $CHCl_3$). IR (neat) ν_{max} 1722, 1246, 1094, 707 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 7.3 Hz, 2H, Ar), 7.76 (d, *J* = 6.4 Hz, 2H, Ar), 7.62 (d, *J* = 6.9 Hz, 3H, Ar), 7.48 – 7.21 (m, 12H, Ar), 7.03 (d, *J* = 7.8 Hz, 2H, Ar), 5.59 (t, *J* = 10.0 Hz, 1H, H-2), 5.37 (s, 1H, H-4), 5.22 (d, *J* = 9.9 Hz, 1H, H-3), 4.81 (d, *J* = 10.0 Hz, 1H, H-1), 4.56 – 4.18 (m, 6H, CH_2Ph, Fmoc), 3.94 (t, *J* = 6.0 Hz, 1H, H-5), 3.66 (dt, *J* = 16.2, 9.4 Hz, 2H, H-6), 2.48 – 2.21 (m, 7H, Lev, SPhCH_3), 1.87 (s, 3H, LevCH_3). ^{13}C NMR{^1H} (101 MHz, $CDCl_3$) δ 205.8 (C=O), 165.2

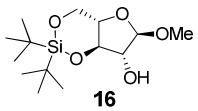
(C=O), 154.9, 143.4, 143.3, 143.2, 141.4, 141.3, 138.4, 137.7, 133.5, 133.0, 130.0, 129.8, 129.5, 129.0, 128.6, 128.5, 128.1, 128.0, 127.9, 127.5, 127.4, 127.3, 125.5, 125.3, 120.2, 120.1 (30 C, Ar), 87.4 (C-1), 76.1 (C-5), 73.7 (CH_2Ph), 72.7 (C-3), 72.0 (C-4), 70.4 (Fmoc), 67.99 (C-2), 67.95 (C-6), 46.6 (Fmoc), 37.7 (Lev), 29.5 (Lev), 28.0 (Lev), 21.3 (SPhCH_3). ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{47}H_{44}NaO_{10}S$: 823.2552, found: 823.2570.



BB2

Dibutoxyphosphoryloxy 2-O-benzoyl-3-O-levulinoyl-4-O-fluorenylcarbonylmethoxy-6-O-benzyl-α/ß-D-galactopyranoside ***(BB2)***. To a suspension of 1.50 g dried 4 A molecular sieve in 20 mL anhydrous DCM was added dibutyl phosphate (1.40 mL, 6.86 mmol) and the mixture was stirred for 30 min. Subsequently, the molecular sieve was allowed to settle for 30 min and the supernatant was added to a solution of **15** (2.75 g, 3.43 mmol) in 30 mL anhydrous DCM and cooled to 0 °C under Ar atmosphere. NIS (1.00 g, 4.46 mmol) and TfOH (0.060 mL, 0.686 mmol) were added and the resulting purple reaction mixture was stirred for 1 h. The reaction was quenched by the addition of aq. sat. $NaHCO_3$-solution and washed with aq. sat. $Na_2S_2O_3$-solution until the color of the organic layer changed from purple to colorless. The layers were separated, the organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo*. The product was purified by silica gel column chromatograph (EtOAc/hex = 1:4 to 1:1) to give phosphate **BB2** (2.08 g, 2.38 mmol, 69% yield) as a mixture of α/β anomers as a highly viscous and sticky oil (analytical data for β-anomer). $[\alpha]_D^{25}$ = -4.9 (c 2.3, $CHCl_3$). IR (neat) ν_{max} 1249, 1025, 740, 711 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.03 (m, 2H, Ar), 7.78 (dd, *J* = 7.0, 4.4 Hz, 2H, Ar), 7.67 (dd, *J* = 11.2, 7.4 Hz, 2H, Ar), 7.61 – 7.56 (m, 1H, Ar), 7.50 – 7.34 (m, 6H, Ar), 7.33 – 7.21 (m, 5H, Ar), 5.72 (dd, *J* = 10.5, 8.0 Hz, 1H, H-2), 5.48 (t, *J* = 7.7 Hz, 1H, H-1), 5.44 (d, *J* = 2.8 Hz, 1H, H-4), 5.24 (dd, *J* = 10.5, 3.3 Hz, 1H, H-3), 4.55 – 4.43 (m, 3H, CH_2Ph, Fmoc), 4.38 – 4.28 (m, 2H, CH_2Ph, Fmoc), 4.10 – 3.98 (m,

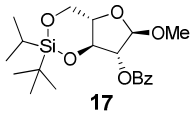
3H , H-5, H-6), 3.81 – 3.61 (m, 4H, OBu), 2.58 – 2.23 (m, 4H, Lev), 1.92 (s, 3H Lev(CH_3)), 1.65 – 1.55 (m, 2H, OBu), 1.41 – 1.28 (m, 4H, OBu), 1.02 (dq, J = 14.7, 7.4 Hz, 2H, OBu), 0.89 (t, J = 7.4 Hz, 3H, OBu(CH_3)), 0.69 (t, J = 7.4 Hz, 3H, OBu(CH_3)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.8 (C=O), 171.7 (C=O), 165.2 (C=O), 154.9, 143.4, 143.2, 141.4, 141.3, 137.5, 133.7, 130.0, 129.2, 128.7, 128.6, 128.1, 128.0, 127.9, 127.6, 127.5, 125.5, 120.3, 120.2 (24 C Ar), 96.91 (d, J = 4.7 Hz, C-1), 73.8, 72.8, 71.4, 71.4, 71.3, 70.6, 69.45, 69.36, 68.23, 68.17, 68.1, 68.0, 67.1, 46.7, 37.7, 32.2, 32.1, 32.0, 31.9, 29.6, 28.0, 18.0, 18.4, 14.4, 13.7, 13.5. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{48}H_{55}NaO_{14}P$: 909.3222, found: 909.3235.



16

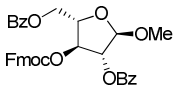
3,5-O-(Di-tert-butylsilanediyl)-1-methyl-α-L-arabinofuranoside ***(16)***. 2,3,5-*O*-tri-benzoyl-1-methyl-α-L-arabinofuranoside[21] (5.00 g, 10.5 mmol) was dissolved in a mixture of methanol (MeOH) and DCM (2:1) and sodium methoxide (NaOMe) (2.60 g, 47.2 mmol) was added. The solution was stirred overnight at rt before it was neutralized by the addition of H^+-Amberlite resin. After filtration through a plug of cotton the solvents were removed *in vaccuo* and the residue was purified through a short pad of silica gel (DCM/MeOH = 1:0 to 9:1) to give 1-methyl-α-L-arabinofuranoside[22] (1.71 g, 10.4 mmol, 99%). Methyl-1-α-L-arabinofuranoside (1.71 g, 10.4 mmol) was dissolved in 15 mL anhydrous DMF at -5 °C and di-*tert*-butylsilanediyl bis(trifluoromethanesulfonate) (5.24 mL, 15.6 mmol) was added. After stirring the resulting solution for 5 min, 2,6-dimethylpyridine (4.24 mL, 36.4 mmol) was added dropwise and the reaction was stirred at -5 °C for ten more minutes before it was quenched by the addition of MeOH. The reaction mixture was sequentially washed with 1 M HCl, aq. sat. $NaHCO_3$ solution and brine. Purification by silica gel chromatography (EtOAc/hex = 1:9) gave 1.83 g (6.00 mmol, 58%) of arabinofuranoside **16** as a colorless solid. $[\alpha]_D^{25}$ = -51.1 (c 1.4, $CHCl_3$). IR (neat) ν_{max} 1086, 1026, 929, 805 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.81 (d, J = 3.3 Hz, 1H, H-1), 4.38 – 4.29 (m, 1H, H-4), 4.12 (dt, J = 7.4, 3.8

Hz, 1H, H-2), 3.99 – 3.88 (m, 3H, H-3, H-5), 3.42 (s, 3H, OMe), 1.06 (s, 9H, *t*Bu), 0.99 (s, 9H, *t*Bu). ^{13}C NMR{^{1}H} (101 MHz, $CDCl_3$) δ 109.0 (C-1), 81.8 (C-2), 81.7 (C-3), 73.8 (C-5), 67.6 (C-4), 56.2 (OCH_3), 27.5 (*t*Bu), 27.2 (*t*Bu), 22.8 ($C(CH_3)_3$), 20.2 ($C(CH_3)_3$). ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{14}H_{28}NaO_5Si$: 327.1603, found: 327.1611.



17

2-O-Benzoyl-3,5-O-(di-tert-butylsilanediyl)-1-methyl-α-L-arabinofuranoside ***(17)***. Arabinofuranoside **16** (1.83 g, 6.00 mmol) was dissolved in 40 mL anhydrous pyridine and the resulting solution was stirred at 0 °C for ten minutes before benzoyl chloride (2.79 mL, 24.0 mmol) was added. After the addition was complete the cooling bath was removed and the reaction mixture was stirred overnight. The reaction mixture was diluted with DCM, poured into ice-cold water and stirred for 20 min. The organic layer was separated and washed with aq. sat. $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. Purification by silica gel chromatography (EtOAc/hex 1:9) gave arabinofuranoside **17** as colorless solid (2.31 g, 5.66 mmol, 94%). $[\alpha]_D^{25}$ = +15.7 (c 0.1, $CHCl_3$). IR (neat) ν_{max} = 1732, 1267, 1093, 717 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 – 8.05 (m, 2H, Ar), 7.62 – 7.57 (m, 1H, Ar), 7.47 (dd, *J* = 10.6, 4.7 Hz, 2H, Ar), 5.32 (dd, *J* = 7.0, 2.3 Hz, 1H, H-2), 4.89 (d, *J* = 2.3 Hz, 1H, H-1), 4.43 (dd, *J* = 8.8, 4.7 Hz, 1H, H-5), 4.29 (dd, *J* = 9.4, 7.0 Hz, 1H, H-3), 4.12 – 3.96 (m, 2H, H-4 H-5), 3.43 (s, 3H, OMe), 1.06 (s, 9H, C(CH_3)), 1.01 (s, 9H C(CH_3)). ^{13}C NMR{^{1}H} (101 MHz, $CDCl_3$) δ 166.0 (C=O), 133.4, 129.9, 128.6 (6 C, Ar), 107.7 (C-1), 83.3 (C-3), 80.2 (C-2), 73.5 (C-5), 67.6 (C-4), 55.9 (OCH_3), 27.5 (C(CH_3), 27.2 (C(CH_3), 22.8 ($C(CH_3)$, 20.28 ($C(CH_3)$. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{21}H_{32}NaO_6Si$: 431.1860, found: 431.1851.

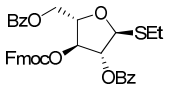


18

2,5-O-Dibenzoyl-3-O-fluorenylcarbonylmethoxy-1-methyl-α-L-arabinofuranoside ***(18)***.

Arabinofuranoside **17** (2.31 g, 5.66 mmol) was stirred in a Sarstedt 50 mL plastic tube in a mixture of THF (30.0 mL) and pyridine (py) (5.00 mL) at 0 °C and a solution of HF*py (0.802 mL, 6.23 mmol) was added dropwise to the reaction mixture. The reaction was warmed up to rt and stirred for additional 3 h before it was poured into ethyl acetate and the resulting mixture was neutralized with aq. sat. $NaHCO_3$ solution. The organic phase was separated, dried over $MgSO_4$, and concentrated. The residue was dissolved together with triphenylphosphine (1.58 g, 6.04 mmol) in 20 mL anhydrous THF. A solution of diethyl azodicarboxylate (DEAD) (0.986 mL, 6.04 mmol) and benzoic acid (0.737 g, 6.04 mmol) in 20 mL anhydrous THF was added dropwise to the solution. The reaction mixture was stirred at rt for 2 h. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography (EtOAc/hex = 1:3 to 1:1) to give 2,5-*O*-dibenzoyl-1-methyl-α-L-arabinofuranoside[23] in 77% (1.15 g, 3.10 mmol) yield. 2,5-*O*-dibenzoyl-1-methyl-α-L-arabinofuranoside (1.10 g, 2.95 mmol) was dissolved in 20 mL anhydrous DCM under nitrogen atmosphere and 2.38 mL of anhydrous pyridine (29.5 mmol) were added. The solution was stirred at rt for 10 min before FmocCl (1.53 g, 5.91 mmol) was added. The reaction mixture was stirred at rt for 6 h and the solvent was evaporated. The residue was purified by silica gel chromatography (EtOAc/hex = 1:7 to 1:3) to give the Fmoc-protected product **18** (1.43 g, 2.40 mmol, 81%) as colorless solid. $[\alpha]_D^{25}$ = +2.6 (c 1.0, $CHCl_3$). IR (neat) ν_{max} 1725, 1254, 727, 709 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.0 Hz, 2H, Ar), 7.99 (d, *J* = 7.8 Hz, 2H, Ar), 7.77 (d, *J* = 7.5 Hz, 2H, Ar), 7.64 – 7.54 (m, 3H, Ar), 7.51 (t, *J* = 7.5 Hz, 1H, Ar), 7.40 (q, *J* = 7.6 Hz, 4H, Ar), 7.31 (q, *J* = 7.4 Hz, 4H, Ar), 5.45 (s, 1H, H-2), 5.29 (d, *J* = 5.2 Hz, 1H, H-3), 5.14 (s, 1H, H-1), 4.78 (dd, *J* = 11.6, 3.1 Hz, 1H, H-5), 4.61 (dd, *J* = 12.0, 4.6 Hz, 1H. H-5), 4.56 – 4.51 (m, 1H, H-4), 4.50 – 4.37 (m, 2H, Fmoc), 4.26 (t, *J* = 7.4 Hz, 1H, Fmoc), 3.50 (s, 3H, OMe). ^{13}C NMR{^1H} (101 MHz, $CDCl_3$) δ 166.3 (C=O), 154.6(C=O), 143.5, 143.3, 143.2, 141.48, 141.44, 133.6, 133.2, 130.0, 129.9, 128.6, 128.5,

128.1, 128.0, 127.4, 127.36, 127.33, 125.3, 120.2 (22C, Ar), 106.8 (C-1), 82.1 (C-2), 80.8 (C-3), 80.3 (C-4), 70.6 (Fmoc), 63.6 (C-5), 55.1 (OMe), 46.8 (Fmoc). ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{35}H_{30}NaO_9$: 617.1787, found: 617.1793.



BB5

Ethyl-2,5-O-dibenzoyl-3-O-fluorenylcarbonylmethoxy-1-thio-α-L-arabinofuranoside ***(BB5)***. Arabinose derivative **18** (1.43 g, 2.40 mmol) was dissolved in 20 mL anhydrous DCM and ethanethiol (0.249 mL, 3.36 mmol) was added. The solution was cooled to 0 °C and boron trifluoride etherate (0.913 mL, 7.20 mmol, 3 eq.) was added dropwise. The reaction mixture was stirred for 5 h at 0°C, subsequently diluted with DCM, and washed with aq. sat. $NaHCO_3$ solution. The crude product was purified by silica column chromatography (hex/EtOAc 8:1) to give thioglycoside **BB5** as colorless solid in 76% yield (1.14 g, 1.83 mmol). $[\alpha]_D^{25}$ = -37.1 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.03 (dd, *J* = 16.0, 7.5 Hz, 4H, Ar), 7.77 (d, *J* = 7.5 Hz, 2H, Ar), 7.65 – 7.55 (m, 3H, Ar), 7.52 (t, *J* = 7.5 Hz, 1H, Ar), 7.40 (dd, *J* = 10.1, 5.4 Hz, 4H, Ar), 7.35 – 7.28 (m, 4H, Ar), 5.57 (s, 1H, H-1), 5.48 (s, 1H, H-2), 5.32 (d, *J* = 5.5 Hz, 1H, H-3), 4.79 – 4.62 (m, 2H, H-5), 4.49 – 4.36 (m, 3H, H-4, Fmoc), 4.27 (t, *J* = 7.6 Hz, 1H, Fmoc), 2.87 – 2.67 (m, 2H, SCH_2CH_3), 1.36 (t, *J* = 7.4 Hz, 3H, SCH_2CH_3) ppm. ^{13}C NMR{^{1}H} (101 MHz, $CDCl_3$) δ = 166.2 (C=O), 165.5 (C=O), 154.5 (C=O), 143.3, 143.1, 141.4,133.7, 133.2, 130.0, 129.8, 128.6, 128.5, 128.1, 127.4, 127.3, 125.39, 125.36, 120.2, 120.2 (22C, Ar), 88.0 (C-1), 82.9 (C-2), 81.1 (C-3), 79.7 (C-4), 70.7 (Fmoc), 63.3 (C-5), 46.8 (Fmoc), 25.4 SCH_2CH_3, 14.9 (SCH_2CH_3). m/z $[M+Na]^+$ calcd. for $C_{36}H_{32}NaO_8S$: 647.1710, found: 647.1723. IR (neat) ν_{max} = 1721, 1244, 1094, 707 cm^{-1}.

Automated Glycan Assembly *Synthesizer Modules and Conditions*

Linker-functionalized resin **L** (12.9-16.9 μmol of hydroxyl groups) was placed in the reaction vessel of the automated oligosaccharide synthesizer and swollen for 30 min in DCM. Before the synthesis the resin was washed with DMF, THF and dichloromethane (DCM).

Subsequently the glycosylation (Module A and Module D), deprotection (Module B and Module C) and capping (Module E) steps were performed. Mixing of the components was accomplished by bubbling argon through the reaction mixture.

Module A: Glycosylation with Glycosyl Phosphates

The resin (12.9-16.9 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 (3.8 or 5 equiv in 1 mL DCM) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by the addition of TMSOTf in DCM (3.8 or 5 equiv in 1 mL DCM). The glycosylation was performed for 5 min at -35 °C (**BB1** and **BB3**) or -30 °C (**BB2**) and then at -20 °C (**BB1** and **BB3**) or -10 °C (**BB2**) for 30 min. Subsequently the solution was drained and the resin was washed three times with DCM. The whole procedure was repeated once to enhance conversion of the acceptor sites.

Module B: Fmoc Deprotection.

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

Module C: Lev Deprotection

The resin was washed with DCM three times and the temperature of the reaction vessel was adjusted to 25 °C. For Lev deprotection 1.3 mL DCM remained in the reaction vessel and

0.8 mL of a solution of 0.15 M hydrazine acetate in Py/AcOH/H_2O (4:1:0.25) was delivered to the reaction vessel. After 30 min the reaction solution was drained and the whole procedure was repeated another two times. After Lev deprotection was complete the resin was washed with DMF, THF and DCM.

Module D: Glycosylation with Thioglycosides

The resin (16.9 µmol of hydroxyl groups) was swollen in DCM (2 mL) and the temperature of the reaction vessel was adjusted to -30 °C. Prior to the glycosylation reaction the resin was washed with TMSOTf in DCM and DCM. For the glycosylation reaction the DCM was drained and a solution of thioglycoside BB (3.8 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 (4.4 equiv) and TfOH (0.4 equiv) in DCM/dioxane (3:1). The glycosylation was performed for 5 min at -40 °C and for 40 min at -20 °C. Subsequently the solution was drained and the resin was washed with DCM. The whole procedure was repeated once to ensure full conversion of all acceptor sites. Afterwards the resin was washed three times with DCM at 25 °C.

Module E: Benzoyl Capping

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 1,2-dichloroethane (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 following the previously published protocol.[11a]

Global Deprotection

The protected oligosaccharides were dissolved in THF (3 mL) and NaOMe (0.5 M in MeOH, 0.5-1 mL) was added. The reaction mixture was stirred overnight and subsequently neutralized by addition of prewashed Amberlite IR-120 resin. The resin was filtered off and the solvents were removed *in vacuo*. The crude product was purified by preparative HPLC, 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, 10-20 mg). The suspension was saturated with H_2 for 30 min and stirred under an H_2-atmosphere overnight. After filtration of the reaction mixture through a syringe filter the solvents were evaporated to provide the fully deprotected oligosaccharides.

Aminopentyl-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside ***(1)*** The synthesizer modules were applied as follows: A(**BB1**)-B-A(**BB1**)-B. The resulting disaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and subjected to hydrogenolysis, providing **1** (1.3 mg) in 23% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 4.73 (d, *J* = 7.8 Hz, 1H), 4.56 (d, *J* = 7.9 Hz, 1H), 4.31 (d, *J* = 3.2 Hz, 1H), 4.09 – 4.03 (m, 2H), 3.99 (dd, *J* = 11.7, 5.8 Hz, 1H), 3.94 – 3.79 (m, 9H), 3.72 (dd, *J* = 10.0, 7.8 Hz, 2H), 3.15 (t, *J* = 7.5 Hz, 2H), 1.82 (tdd, *J* = 12.7, 8.3, 6.4 Hz, 4H), 1.60 (tt, *J* = 8.7, 6.5 Hz, 2H); ^{13}C NMR{^{1}H} (151 MHz, D_2O) δ = 106.9, 105.2, 79.8, 77.7, 76.8, 75.9, 75.4, 74.0, 73.9, 72.6, 71.2, 63.6, 63.1, 42.0, 30.8, 29.0, 24.7; ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{17}H_{34}NO_{11}$: 428.2127; found 428.2141.

Aminopentyl-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside ***(2)*** The synthesizer modules were applied as follows: A(**BB1**)-B-A(**BB1**)-B-A(**BB1**)-B-A(**BB1**)-B. The resulting tetrasaccharide was

purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and subjected to hydrogenolysis, providing **2** (1.5 mg) in 15% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 4.72 – 4.69 (m, 2H), 4.66 (d, *J* = 7.8 Hz, 1H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.25 – 4.22 (m, 3H), 4.01 – 3.96 (m, 2H), 3.94 – 3.70 (m, 19H), 3.68 – 3.62 (m, 2H), 3.07 (t, *J* = 7.5 Hz, 2H), 1.75 (tt, *J* = 14.2, 7.1 Hz, 4H), 1.52 (dt, *J* = 15.4, 7.7 Hz, 2H); ^{13}C NMR{^{1}H} (151 MHz, D_2O) δ 103.84, 103.81, 103.7, 102.1, 77.2, 77.0, 76.6, 74.6, 73.99, 73.93, 73.7, 72.8, 72.6, 72.2, 71.3, 71.2, 70.8, 70.7, 69.4, 68.0, 60.4, 60.2, 60.1, 60.0, 38.8, 27.6, 25.8, 21.5; ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{29}H_{53}NNaO_{21}$: 774.3007; found 774.3005.

*Aminopentyl-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**3**)* The synthesizer modules were applied as follows: A(BB**1**)-B-A(BB**1**)-B-A(**BB1**)-B-A(**BB1**)-B-A(**BB1**)-B-A(**BB1**)-B. The resulting hexasaccharide was purified after global deprotection by reversed phase HPLC using a Hypercarb column, providing **3** (0.4 mg) in 2% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 4.59 – 4.55 (m, 4H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.35 (d, *J* = 8.0 Hz, 1H), 4.10 (d, *J* = 3.2 Hz, 4H), 3.88 – 3.82 (m, 3H), 3.80 – 3.57 (m, 30H), 3.55 – 3.48 (m, 1H), 2.94 (t, *J* = 7.5 Hz, 2H), 1.61 (tt, *J* = 14.1, 7.2 Hz, 4H), 1.42 – 1.36 (m, 2H); ^{13}C NMR{^{1}H} (176 MHz, D_2O) δ 104.3, 104.2, 102.6, 77.6, 77.5, 77.1, 75.1, 74.5, 74.4, 74.28, 74.22, 73.2, 72.7, 71.87, 71.80, 71.3, 71.2, 70.0, 68.6, 60.9, 60.7, 60.6, 60.5, 39.3, 28.1, 26.3, 22.0; ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{41}H_{73}NNaO_{31}$: 1098.4064; found 1098.4043.

*Aminopentyl-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**4**)* The synthesizer modules were applied as follows: A(**BB1**)-B-A(**BB3**)-B-A(**BB1**)-B-A(**BB1**)-B. The resulting tetraasaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and subjected to hydrogenolysis, providing **4** (2.0 mg) in 16% yield based on resin loading.

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

*Aminopentyl β-D-galactopyranosyl-(1→4)-3-O-[α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**5**)* The synthesizer modules were applied as follows: A(**BB1**)-B-A(**BB2**)-B-A(**BB1**)-C-D(**BB4**)-B. The resulting tetrasaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and subjected to hydrogenolysis, resulting **5** (0.8 mg), in 6% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 5.41 (s, 1H), 4.87 (s, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.49 (s, 1H), 4.39 – 4.30 (m, 3H), 4.15 – 4.05 (m, 3H), 4.03 – 3.78 (m, 17H), 3.77 – 3.69 (m, 1H), 3.15 (t, *J* = 7.4 Hz, 2H), 1.88 – 1.80 (m, 4H), 1.66 – 1.58 (m, 2H); ^{13}C NMR{^{1}H} (151 MHz, D_2O) δ 107.1, 102.1, 100.7, 100.3, 82.0, 78.9, 78.0, 73.9, 72.8, 72.2, 72.0, 71.1, 70.5, 68.9, 67.8, 66.4, 59.0, 58.8, 58.3, 46.8, 37.2, 25.9, 19.8 ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{28}H_{51}NNaO_{20}$: 744.2902; found 744.2932.

*Aminopentyl β-D-galactopyranosyl-(1→4)- β-D-galactopyranosyl-(1→4)-3-O-[α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**6**)* The synthesizer modules were applied as follows: A(**BB1**)-B-A(**BB1**)-B-A(**BB2**)-B-A(**BB1**)-B-A(**BB1**)-C-D(**BB4**)-B. The resulting hexasaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and purified again after hydrogenolysis using reversed phase HPLC (Hypercarb column), providing **6** (0.8 mg) in 5% yield based on resin loading. ^{1}H NMR (700 MHz, D_2O) δ 5.48 (d, *J* = 1.4 Hz,

1H), 4.70 (d, *J* = 8.0 Hz, 1H), 4.68 (d, *J* = 7.8 Hz, 1H), 4.64 (d, *J* = 7.9 Hz, 1H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.22 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.21 – 4.16 (m, 4H), 4.08 (td, *J* = 6.1, 3.4 Hz, 1H), 3.97 – 3.89 (m, 4H), 3.86 – 3.64 (m, 25H), 3.60 – 3.56 (m, 2H), 3.01 (t, *J* = 7.5 Hz, 2H), 1.73 – 1.65 (m, 4H), 1.49 – 1.43 (m, 2H); ^{13}C NMR{^{1}H} (151 MHz, D_2O) δ 107.2, 102.1, 102.0, 100.7, 100.4, 82.1, 78.9, 78.1, 75.7, 75.5, 74.9, 74.0, 72.9, 72.3, 72.2, 72.1, 72.0, 71.0, 70.9, 70.5, 69.7, 69.3, 69.1, 69.0, 68.8, 67.7, 66.4, 59.1, 58.7, 58.5, 58.4, 58.4, 58.3, 37.1, 25.9, 24.2, 19.8; ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{40}H_{72}NO_{30}$: 1046.4139; found 1046.4105.

Aminopentyl β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-3-O-[α-L-arabinofuranosyl]-β-D-galactopyranoside (7) The synthesizer modules were applied as follows: A(**BB1**)-B-A(**BB1**)-B-A(**BB1**)-B-A(**BB2**)-C-D(**BB4**)-B. The resulting pentasaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and subjected to hydrogenolysis, providing **7** (1.1 mg) in 7% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 5.41 (s, 2H), 4.85 – 4.78 (m, 3H), 4.59 (dd, *J* = 7.9, 1.2 Hz, 1H), 4.38 (dt, *J* = 3.0, 1.5 Hz, 1H), 4.37 – 4.32 (m, 3H), 4.32 – 4.28 (m, 1H), 4.26 (s, 1H), 4.13 – 4.07 (m, 2H), 4.04 – 3.81 (m, 21H), 3.77 – 3.72 (m, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 1.89 – 1.81 (m, 4H), 1.67 – 1.59 (m, 2H); ^{13}C NMR{^{1}H} (151 MHz, D_2O) δ 106.9, 102.1, 102.0, 101.9, 100.4, 81.6, 79.0, 77.9, 75.5, 75.3, 75.1, 74.3, 72.7, 72.3, 72.2, 72.0, 71.0, 70.9, 69.6, 69.0, 68.3, 67.7, 66.2, 58.9, 58.6, 58.5, 58.4, 58.3, 37.1, 25.9, 24.1, 19.8. ESI-HRMS**:** m/z $[M+H]^+$ calcd for $C_{34}H_{62}NO_{25}$: 884.3610; found 884.3636.

*Aminopentyl α-L-arabinofuranosyl-(1→3)-α-L-arabinofuranosyl-(1→3)-β-D-galactopyranoside (**8**)* The synthesizer modules were applied as follows: A(**BB3**)-B-D(**BB5**)-B-D(**BB4**). The resulting trisaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and subjected to hydrogenolysis, providing **8** (1.5 mg) in 17% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 5.26 (s, 1H), 5.19

(s, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.37 (d, *J* = 1.3 Hz, 1H), 4.23 (td, *J* = 5.8, 3.1 Hz, 1H), 4.14 – 4.12 (m, 1H), 4.10 (d, *J* = 3.2 Hz, 1H), 4.05 (dd, *J* = 5.9, 2.6 Hz, 2H), 3.98 – 3.93 (m, 2H), 3.88 (dd, *J* = 12.3, 3.0 Hz, 1H), 3.84 (dd, *J* = 12.3, 3.2 Hz, 1H), 3.80 – 3.68 (m, 7H), 3.62 (dd, *J* = 9.7, 8.1 Hz, 1H), 3.02 (t, *J* = 7.5 Hz, 2H), 1.75 – 1.65 (m, 4H), 1.51 – 1.44 (m, 2H);^{13}C NMR{^{1}H} (151 MHz, D_2O) δ 111.8, 109.7, 105.0, 86.7, 85.5, 84.7, 83.6, 82.6, 82.3, 79.0, 77.56, 72.53, 72.4, 71.0, 63.7, 63.6, 63.4, 41.9, 30.7, 28.9, 24.6; ESI-HRMS**:** m/z $[M+Na]^+$ calcd for $C21H_{39}NNaO_{14}$: 552.2268; found 552.2240.

*Aminopentyl β-D-galactopyranosyl-(1→4)-3-O-[3-O-[α-L-arabinofuranosyl]-α-L-arabinofuranosyl]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**9**)* The synthesizer modules were applied as follows: A(**BB1**)-B-A(**BB2**)-B-A(**BB1**)-B-E-C-D(**BB5**)-B-D(**BB4**). The resulting pentasaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and subjected to hydrogenolysis, providing **9** (1.1 mg) in 8% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 5.42 (s, 1H), 5.36 (s, 1H), 4.89 – 4.86 (m, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.55 (dd, *J* = 2.9, 1.4 Hz, 1H), 4.52 (s, 1H), 4.39 – 4.36 (m, 1H), 4.34 (d, *J* = 3.0 Hz, 1H), 4.28 (dd, *J* = 3.3, 1.5 Hz, 1H), 4.26 (dd, *J* = 6.6, 3.2 Hz, 1H), 4.19 (td, *J* = 6.1, 3.1 Hz, 1H), 4.14 – 4.07 (m, 2H), 4.05 (dd, *J* = 9.3, 3.2 Hz, 1H), 4.03 – 3.81 (m, 19H), 3.73 (ddd, *J* = 17.6, 9.8, 7.9 Hz, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 1.90 – 1.81 (m, 4H), 1.66 – 1.59 (m, 2H); ^{13}C NMR{^{1}H} (151 MHz, D_2O) δ 107.4, 104.5, 102.2, 100.3, 100.1, 81.5, 80.3, 79.3, 79.2, 77.9, 77.7, 75.8, 74.2, 72.9, 72.4, 72.0, 71.1, 70.4, 69.0, 68.8, 67.7, 66.4, 58.8, 58.4, 58.3, 37.1, 25.9, 24.2, 19.8; ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{33}H_{59}NNaO_{24}$: 876.3324; found 876.3301.

*Aminopentyl α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→3)-β-D-galactopyranoside (**10**)* The synthesizer modules were applied as follows: A(**BB3**)-B-D(**BB6**)-B-D(**BB6**)-B-D(**BB4**). The resulting tetrasaccharide was purified after methanolysis of the benzoyl esters using reversed phase HPLC (C5 column) and

subjected to hydrogenolysis, providing **10** (1.1 mg) in 38% yield based on resin loading. ^{1}H NMR (600 MHz, D_2O) δ 5.40 (s, 1H), 5.24 (s, 2H), 4.61 (d, *J* = 7.9 Hz, 1H), 4.43 – 4.38 (m, 1H), 4.38 – 4.35 (m, 2H), 4.28 (s, 2H), 4.26 – 4.23 (m, 2H), 4.18 – 4.15 (m, 2H), 4.13 – 4.08 (m, 2H), 4.06 – 4.02 (m, 2H), 4.00 – 3.83 (m, 9H), 3.77 (t, *J* = 8.9 Hz, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 1.91 – 1.78 (m, 4H), 1.67 – 1.58 (m, 2H); ^{13}C NMR{^{1}H} (151 MHz, D_2O) δ 107.2, 105.4, 105.3, 100.4, 81.9, 80.2, 80.1, 79.1, 78.8, 78.7, 78.3, 74.6, 74.6, 74.4, 72.9, 67.8, 67.8, 66.4, 64.8, 64.7, 59.1, 58.8, 37.3, 26.0, 24.3, 20.0; ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{26}H_{47}NNaO_{18}$: 684.2690; found 684.2670.

Analysis of Glycosyl Hydrolase Substrate Specificities

The β1,4-endo-galactanases were purchased from Megazyme (Bray, Ireland) and used in the following buffers that were suggested by the manufacturer: 200 mM sodium acetate (NaOAc) (pH 4) for E-EGALN, 100 mM NaOAc (pH 4.5) for E-GALCT and 100 mM $NaPO_4$ (pH 8) for E-GALCJ. The enzyme was used at a concentration of 1 U/ml. The oligosaccharides were used at a concentration of 1 mM. All reactions were carried out at 40 °C and terminated by incubation at 80 °C for 5 min. The reactions were analyzed on an Agilent 1200 Series HPLC equipped with an Agilent 6130 quadrupole mass spectrometer (MS) and an Agilent 1200 Evaporative Light Scattering Detector (ELSD). The oligosaccharides were separated on a Hypercarb column (150 x 4.6 mm, Thermo Scientific) using a water (including 0.1% formic acid) - acetonitrile (MeCN) gradient at a flow-rate of 0.7 ml/min starting at 2.5% MeCN for 5 min, ramping up to 15% MeCN at 8 min, followed by a slow increase of MeCN to 30% at 40 min, a steep ramp to 100% MeCN at 43.5 min, a decline back to 2.5% MeCN from 46 min to 47 min, and equilibration until 55 min at 2.5% MeCN. The peaks in the ELSD traces were assigned based on their retention time and the corresponding masses in the MS.

Associated Content

Supporting Information available free of charge: Supporting Information includes reaction schemes of building block synthesis, ^{1}H, ^{13}C NMR spectra of novel building blocks and novel building block intermediates, reaction schemes of oligosaccharide synthesis, ^{1}H, ^{13}C and 2D NMR spectra, HPLC traces of protected and unprotected oligosaccharides and HPLC traces of enzyme oligosaccharide digests.

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