



An efficient synthesis of linear -(16)-galactan oligosaccharides related to plant cell wall glycans

Andersen, Mathias Christian Franch; Arentoft, Camilla Anna Søholt; Boos, Irene; Kinnaert, Christine; Awan, Shahid Iqbal; Clausen, Mads Hartvig

Published in:
Trends in Carbohydrate Research

Publication date:
2017

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Andersen, M. C. F., Arentoft, C. A. S., Boos, I., Kinnaert, C., Awan, S. I., & Clausen, M. H. (2017). An efficient synthesis of linear -(16)-galactan oligosaccharides related to plant cell wall glycans. *Trends in Carbohydrate Research*, 9(2), 27-37.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

An efficient synthesis of linear β -(1 \rightarrow 6)-galactan oligosaccharides related to plant cell wall glycans

Mathias C. F. Andersen, Camilla A. S. Arentoft, Irene Boos, Christine Kinnaert, Shahid I. Awan, Mads H. Clausen*

Center for Nanomedicine and Theranostics, Department of Chemistry, Technical University of Denmark, Kemitorvet, Building 207, 2800 Kgs. Lyngby, Denmark.

Abstract:

Galactans are linear structures mainly found in arabinogalactan glycans and RG-I side chains. As a follow-up to our work on both β -(1 \rightarrow 3)-linked and β -(1 \rightarrow 4)-linked galactans, we herein report a convergent synthesis of β -(1 \rightarrow 6)-galactan using our previously synthesized 4,6-benzylidene protected disaccharide as a key building block. However, the regioselective reductive opening of the 4,6-benzylidene protected disaccharide turned out to become more challenging as the length of the oligosaccharide increased and a second differential protected disaccharide building block carrying a chloroacetyl group on the 6-position was used to elongate the chain in a more efficient way.

Keywords:

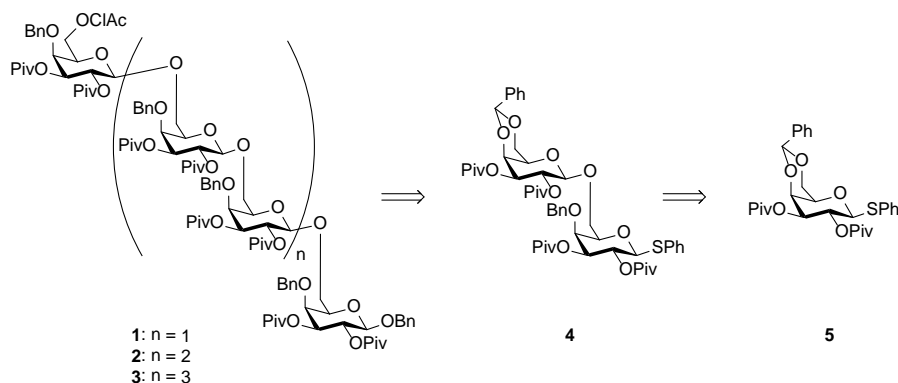
Plant cell wall oligosaccharides, Arabinogalactans, RG-I, β -(1 \rightarrow 6)-D-galactans.

Introduction:

β -(1 \rightarrow 6)-D-galactans are linear structures mainly found in arabinogalactan (AG) glycans. The latter are cell wall polysaccharides widely distributed in the plant kingdom and contain high proportions of

* Corresponding author: Prof. Mads H. Clausen
E-mail: mhc@kemi.dtu.dk, Tel.: +45 4525 2131

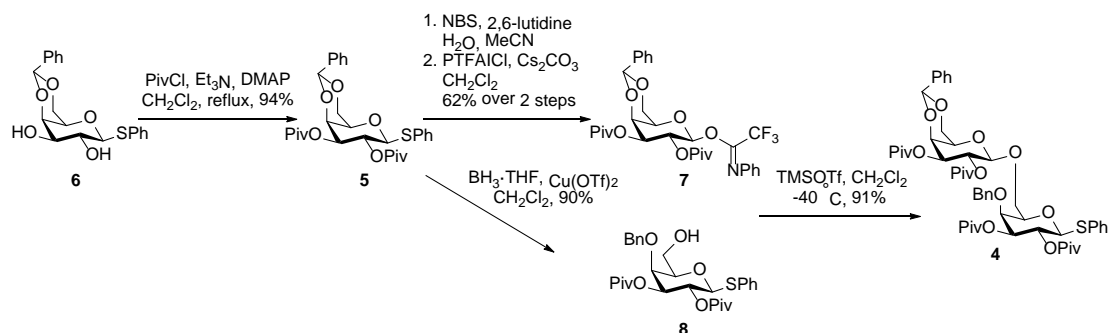
galactose and arabinose. They are present in many plants both as hemicellulose and arabinogalactans proteins.¹ Due to their structural complexity and developmental origin, they are usually divided into two main groups: type I and type II.² Type I AGs are characterized by a linear β -(1 \rightarrow 4)-linked D-Galp chain, which may be substituted with single L-Araf, short arabinan oligosaccharide, or with β -(1 \rightarrow 6)-linked Galp residues. Type II AGs are highly branched polysaccharides composed of a β -(1 \rightarrow 3) or (1 \rightarrow 6)-linked-D-galactan backbone often substituted at C-3 and/or C-6 with β -galactan or arabinogalactan chains.³ Type II AGs make up the carbohydrate part of arabinogalactan proteins. Unlike the other β -galactans, linear and branched (1 \rightarrow 6)-linked galactans have been prepared via a wide range of strategies. The first synthesis of a tetrameric arabinogalactan with a β -(1 \rightarrow 6)-linked backbone was achieved in 1998 by van Boom and co-workers, based on the use of 1,2-anhydrosugar building blocks.⁴ Kong and colleagues later reported several syntheses of well-defined arabinogalactans carrying different lengths of arabinan side chain.⁵⁻¹⁰ The strategy was based on block synthesis, which allowed for the consecutive couplings of two tetrasaccharide building blocks. Later, the groups of Yang and Zhang developed one-pot glycosylation strategies for the synthesis of 3,6-branched tetra- and hexaarabinogalactans.^{11,12} Following our previous work on galactans on β -(1 \rightarrow 4)-linked and β -(1 \rightarrow 3)-linked D-Galp,¹³⁻¹⁵ we herein report the synthesis of β -(1 \rightarrow 6)-linked D-Galp using the same disaccharide **4** as a central building block.



Scheme 1: Retrosynthesis of β -(1 \rightarrow 6)-linked linear galactans.

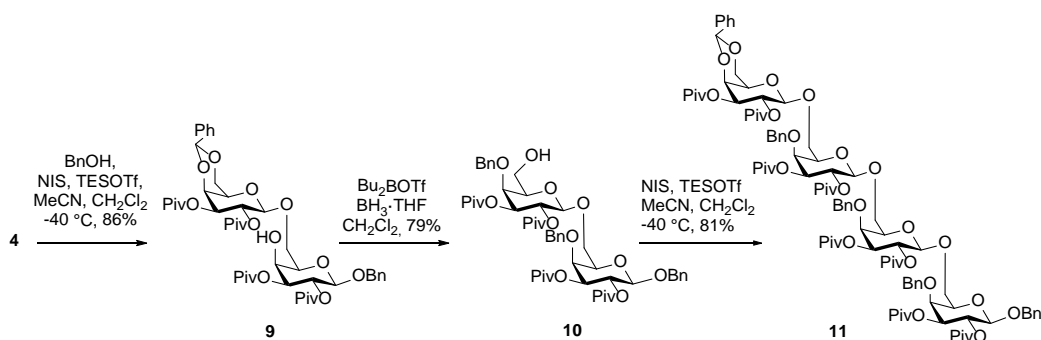
Results and Discussion:

We envisioned to prepare three linear β -(1 \rightarrow 6)-linked galactans **1-3** from the central disaccharide building block **4** as block strategies, which usually enables the synthesis of longer chains in a better overall yield by decreasing the number of critical glycosylations steps (see Scheme 1).



Scheme 2: Synthesis of mono- and disaccharide building blocks.

An esterification of the diol **6**¹³ with pivaloyl chloride in the presence of 0.5 equiv. of DMAP afforded the fully protected galactoside **5** (Scheme 2). As previously reported by Jiang and co-workers,¹⁶ only the 3-position was protected if the reaction was carried out at 22 °C and with 0.1 equiv. of DMAP. The obtained thioglycoside **5** was then hydrolyzed using NBS/H₂O/2,6-lutidine and further converted into the corresponding trifluoroacetimidate donor **7** by treatment with PTFAICl and Cs₂CO₃.¹⁷ Regioselective reductive opening of thioglycoside **5** with BH₃·THF and Cu(OTf)₂ afforded the desired 6-OH acceptor in 90% yield.¹⁸ TMSOTf-promoted glycosylation between trifluoroacetimidate donor **7** and acceptor **8** gave building block disaccharide **4** in 91% yield. It was crucial to perform the reaction at -40 °C to achieve good stereoselectivity.

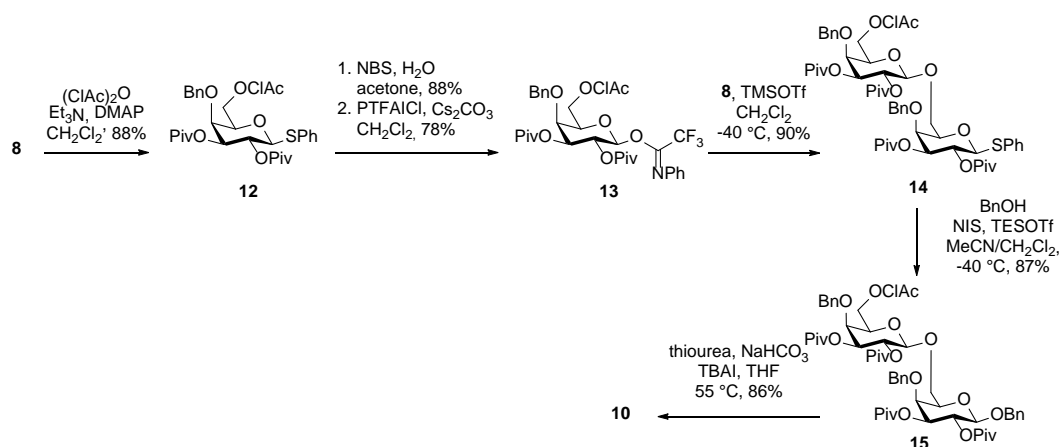


Scheme 3: First attempt to synthesize β -(1 \rightarrow 6)-linked tetrasaccharide.

The next step was to convert thioglycoside **4** to a reducing end 6-*OH* acceptor (Scheme 3). Therefore, an NIS-TESOTf promoted glycosylation of benzyl alcohol with **4** was performed to afford reducing end disaccharide **9** in 86% yield. However, the following regioselective opening of the benzylidene acetal turned out to be more challenging than expected. Mostly hydrolysis of the glycosidic linkage was observed when using the same conditions as previously (Cu(OTf)₂/BH₃·THF), and the desired alcohol **10** was isolated in only 35% yield. Similar results were obtained after treatment with PhBCl₂/Et₃SiH at -78 °C.¹⁹ Finally, treatment with Bu₂BOTf/BH₃·THF succeeded in affording disaccharide acceptor **10** in 79% yield.^{20,21} However, the reaction still gave rise to hydrolysis. It was believed that it would become harder to avoid this side-reaction when performing the same step on longer galactan chains as the number of glycosidic linkages that could potentially be hydrolyzed would increase with the length of the galactan. Therefore, it was decided to change the strategy.

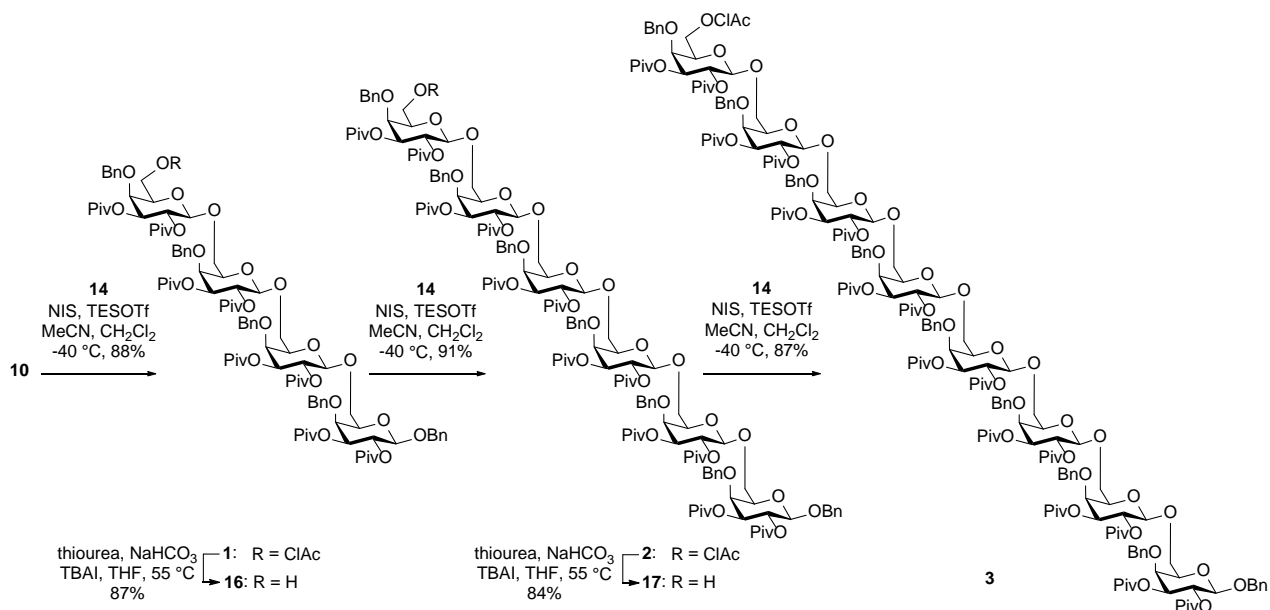
The C6-*OH* of thioglycoside **8** was protected with a chloroacetyl group to give thioglycoside **12** (Scheme 4). Hydrolysis with NBS in wet acetone afforded the hemiacetal, which was converted to the trifluoroacetimidate donor **13** and coupled to thioglycoside **8** in a TMSOTf-promoted glycosylation. The reaction was slow compared to the glycosylation with benzylidene acetal-protected trifluoroacetimidate donor **7**; nevertheless, disaccharide **14** was isolated in 90% yield as a single stereoisomer. The reducing end acceptor **10** could now be prepared without problems by NIS/TESOTf-mediated glycosylation of benzyl alcohol and disaccharide **14** affording fully protected

disaccharide **15**. Further, deprotection of the chloroacetyl group with thiourea, NaHCO₃, and TBAI furnished the disaccharide acceptor **10** in 86% yield.



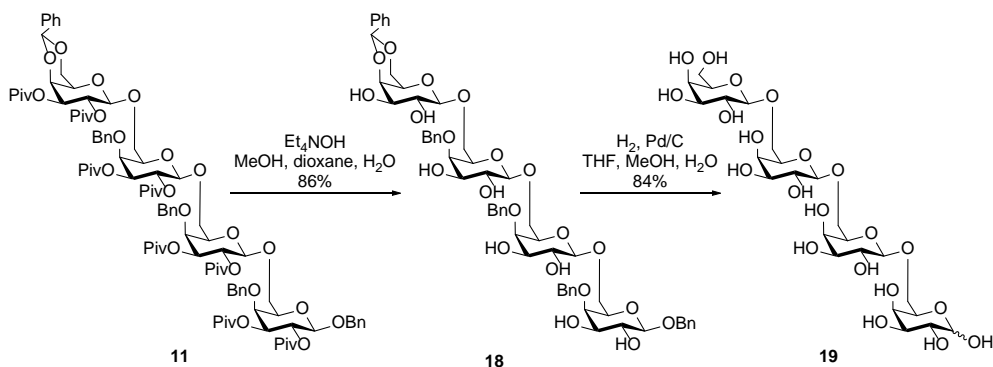
Scheme 4: Synthesis of chloroacetyl-protected building blocks.

NIS/TESOTf-promoted glycosylation of disaccharide acceptor **10** with disaccharide donor **14** gave the tetrasaccharide **1** in 87% yield (Scheme 5). The chloroacetyl group was then selectively removed to give acceptor **16**. The following NIS/TESOTf-promoted glycosylation of **16** with donor **14** gave hexasaccharide **2** in 91% yield. Repeating the two last steps once again afforded the fully protected octasaccharide **3** in 73% yield over two steps. The yield and reaction time were very similar in the three glycosylations.



Scheme 5: Synthesis of linear β -(1 \rightarrow 6)-galactans.

Global deprotection of tetrasaccharide **11** was achieved in two steps (Scheme 6). First, the pivaloyl groups were removed with Et_4NOH , then hydrogenolysis over Pd/C gave the fully unprotected tetrasaccharide **19** in 72% overall yield.



Scheme 6: Deprotection of tetrasaccharide 11.

Conclusion:

A convergent synthetic strategy for β -(1 \rightarrow 6)-D-galactans was developed using a key building block **14**. Linear galactans up to an octasaccharide were prepared, and it is likely that much longer oligosaccharides can be reached with this protecting group pattern since the reactivity of the

consecutive glycosylations was similar. These well-defined glycans together with our previously synthesized β -(1 \rightarrow 4)- and β -(1 \rightarrow 3)-D-galactans can help to elucidate the structure and function of arabinogalactan proteins.

Experimental:

General Information:

Starting material, reagents and solvents were purchased from commercial suppliers and were used without further purification. All solvents are HPLC-grade. Anhydrous solvents were obtained from Innovative Technology PS-MD-7 Pure-solv solvent purification system. All of the reactions were carried out in flame-dried glassware under an inert atmosphere. Thin-layer chromatography (TLC) was performed on Merck aluminium sheets pre-coated with silica, C-60 F254 plates. Compounds were visualized by charring after dipping in CAM stain ($\text{Ce}(\text{SO}_4)_2$ (1.6 g) and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (4 g) in 10 % sulfuric acid (200 mL). Eluent systems are specified for each R_f value, and ratios are given as volume ratios.

Evaporation of solvents was performed with a VWR International Laborota 400 under reduced pressure (*in vacuo*) at temperatures ranging between 35 - 55 °C. Traces of solvent were removed under reduced pressure using an oil pump. Flash chromatography was performed using Matrex 60 Å silica gel (35-70 μm) as the stationary phase by the general procedure developed by Still *et al.* The eluent system is specified in the protocol reported for each synthesis. Eluent ratios are given as volume ratios.

NMR-spectra were recorded on a Bruker Ascend 400, Bruker Avance 800 MHz, Varian Mercury 300 B, Bruker DQX 400 and Bruker AC 500 spectrometers. Chemical shifts (δ) are reported in ppm downfield from TMS ($\delta = 0$) using solvent resonance as the internal standard. The spectra were recorded in CDCl_3 , CD_3OD , D_2O or DMSO-d_6 .

IR analyses were performed on a Bruker Alpha-P FT-IR instrument where solid compounds are applied directly to the instrument. Optical rotations were measured on a Perkin-Elmer Model 241 Polarimeter. The solvents used were either CHCl₃ or H₂O.

Melting points were measured on a Stuart melting point SMP30 and reported in °C uncorrected.

UPLC/MS analysis was performed on a Waters AQUITY UPLC system equipped with PDA and SQD MS detector. Column: AQUITY UPLC BEH C18 1.7µm, 2.1 x 50mm. Column temp: 65 °C. Flow rate: 0.6 ml/min. Solvent A: 0.1% formic acid in water, Solvent B: 0.1% formic in MeCN. Gradient: 5% B to 100% B in 2.4 min, hold 0.1 min, total run time 2.6 min. High-resolution LC-DAD-MS was performed on an Agilent 1100 system equipped with a photodiode array detector (DAD) and coupled to an LCT orthogonal time-of-flight mass spectrometer (Waters-Micromass, Manchester, UK) with Z-spray electrospray ionization (ESI) source and a LockSpray probe and controlled MassLynx 4.0 software. LC-MS calibration from m/z 100-900 was done with a PEG mixture. Standard separation involved a LUNA 2 column with a MeCN (50 ppm TFA) in water gradient starting from 15% to 100% over 25 minutes with a flow rate of 0.3 mL/min.

Spectroscopic data is not given for *N*-phenyl trifluoroacetimidates due to their low stability.

Experimental Procedures:

Phenyl 4,6-*O*-benzylidene-2,3-di-*O*-pivaloyl-1-thio-β-D-galactopyranoside (5)

Compound **6** (10 g; 27.74 mmol) was dissolved in CH₂Cl₂ (250 mL). Et₃N (15.5 mL; 110.98 mmol), DMAP (1.70 g; 13.87 mmol) and pivaloyl chloride (9.4 mL; 110.98 mmol) were added to the solution and the reaction mixture was heated to 45 °C for 4 h. The reaction mixture was cooled to 0 °C, quenched with MeOH (20 mL), washed with water (2x200 mL), dried over MgSO₄ and concentrated. The product was purified by flash chromatography (toluene/EtOAc 19:1) to afford **5** as a white amorphous powder. R_f 0.58 (9:1 toluene/EtOAc). Yield 13.79 g (94%). [α]_D²⁰ = 18.6° (c 1.0, CDCl₃).

IR (neat, cm^{-1}): 3062, 2972, 2933, 2906, 2872, 1732, 1584, 1479, 1457, 1397, 1367, 1281, 1157, 1139, 1092, 1045, 1026. **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 7.52 – 7.47 (m, 2H, Ar-H), 7.31 (m, 8H, Ar-H), 5.42 (s, 1H, $-\text{CH}^{\text{benzylidene}}$), 5.36 (t, $J_{1,2} = J_{2,3} = 9.9$ Hz, 1H, H-2), 4.88 (dd, $J_{2,3} = 9.9$, $J_{3,4} = 3.5$ Hz, 1H, H-3), 4.66 (d, $J_{1,2} = 9.9$ Hz, 1H, H-1), 4.33 (d, $J_{3,4} = 3.5$ Hz, 1H, H-4), 4.31 (dd, $J_{6a,6b} = 12.4$, $J_{5,6z} = 1.1$ Hz, 1H, H-6a), 3.95 (dd, $J_{6a,6b} = 12.4$, $J_{5,6b} = 1.7$ Hz, 1H, H-6b), 3.52 (m, 1H, H-5), 1.14 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$), 1.04 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$). **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 178.2, 176.2, 137.7, 133.1 (2C), 131.9, 129.0, 128.8 (2C), 128.1 (2C), 128.0, 126.2 (2C), 100.6, 85.7, 73.3, 73.1, 69.7, 69.2, 65.9, 39.0, 38.8, 27.2 (3C), 27.0 (3C). HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{29}\text{H}_{36}\text{NaO}_7\text{S}$ 551.2079; Found 551.2071.

4,6-*O*-Benzylidene-2,3-di-*O*-pivaloyl- β -D-galactopyranose *N*-phenyl trifluoroacetimidate (7)

Compound **5** (10.0 g; 18.91 mmol) was dissolved in MeCN (250 mL) and water (25 mL). NBS (13.5 g; 75.66 mmol) was added, and the reaction was stirred at 50 °C for 4 h. The solution was diluted with EtOAc (500 mL) and washed with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ (200 mL) and sat. aq. NaHCO_3 (200 mL). The organic phase was dried over MgSO_4 , filtered and concentrated. The residue was purified by flash chromatography (4:1 toluene/EtOAc) to afford the corresponding hemiacetal. R_f 0.17 (4:1 toluene/EtOAc). Further, this hemiacetal was dissolved in CH_2Cl_2 (200 mL) and cooled to 0 °C. Cs_2CO_3 (11.9 g; 36.65 mmol) was added followed by *N*-phenyl trifluoroacetimidoyl chloride (7.6 g; 36.65 mmol). The ice bath was removed, and the reaction mixture was stirred for 12 h. It was then filtered, through a plug of Celite, concentrated and purified by flash chromatography (9:1 toluene/EtOAc) to give **352** as a white amorphous product. Yield: 6.9 g (62% over two steps).

Phenyl 4-*O*-benzyl-2,3-di-*O*-pivaloyl-1-thio- β -D-galactopyranoside (8)

A 1 M solution of BH_3 ·THF complex in THF (189.2 mL) was added to a solution of **5** (20 g; 37.8 mmol) in DCM (100 mL) at 0 °C. The mixture was stirred for 10 min, and freshly dried $\text{Cu}(\text{OTf})_2$ (2.1 g, 5.67 mmol) was added to the solution. After stirring for a 5 h, the mixture was cooled to 0 °C,

and the reaction was quenched by addition of triethylamine (5.3 mL, 37.8 mmol) and methanol (69 mL, caution: hydrogen gas was evolved). The resultant mixture was concentrated at reduced pressure followed by coevaporation with methanol. The residue was purified by flash column chromatography (9:1 toluene/EtOAc) to afford **8** as a slightly yellow amorphous material.

R_f 0.21 (9:1 toluene/EtOAc). Yield: 18.1 g (90%). $[\alpha]_D^{20} = 2.2^\circ$ (c 1.0, CDCl₃). **IR** (neat, cm⁻¹): 3522, 3062, 3032, 2970, 2934, 2906, 2872, 1733, 1479, 1457, 1397, 1365, 1279, 1162, 1138, 1082, 1041.

¹H NMR (400 MHz, CDCl₃) δ 7.42 – 7.35 (m, 2H, Ar-H), 7.32 – 7.14 (m, 8H, Ar-H), 5.42 (t, $J_{1,2} = J_{2,3} = 9.9$ Hz, 1H, H-2), 5.01 (dd, $J_{2,3} = 9.9$, $J_{3,4} = 2.9$ Hz, 1H, H-3), 4.74 (d, $J_{CH_2} = 11.3$ Hz, 1H, 0.5xCH₂^{Bn}), 4.64 (d, $J_{1,2} = 9.9$ Hz, 1H, H-1), 4.39 (d, $J_{CH_2} = 11.3$ Hz, 1H, 0.5xCH₂^{Bn}), 3.89 (d, $J_{3,4} = 2.9$ Hz, 1H, H-4), 3.76 (dd, $J_{6a,6b} = 11.2$, $J_{5,6a} = 6.9$ Hz, 1H, H-6a), 3.61 – 3.53 (dd, $J_{5,6a} = 6.9$, $J_{5,6b} = 5.2$ Hz, 1H), 3.47 (dd, $J_{6a,6b} = 11.2$, $J_{5,6b} = 5.2$ Hz, 1H, H-6b), 1.14 (s, 9H, 3xCH₃^{Piv}), 1.11 (s, 9H, 3xCH₃^{Piv}). **¹³C NMR** (101 MHz, CDCl₃) δ 177.7, 176.5, 137.5, 132.9, 131.9 (2C), 128.9 (2C), 128.5 (2C), 128.0, 127.8 (2C), 127.7, 86.7, 78.8, 75.1, 74.8, 74.2, 67.4, 61.8, 38.9, 38.7, 27.2 (3C), 27.2 (3C). **HRMS** (ESI-TOF) m/z: [M + Na]⁺ Calcd for C₂₉H₃₈NaO₇S 553.2236; Found 553.2231.

Phenyl 4,6-O-benzylidene-2,3-di-O-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-O-benzyl-2,3-di-O-pivaloyl- β -D-1-thio-galactopyranoside (4)

To a 250 mL flame-dried flask was added **8** (4.7 g, 8.76 mmol) and the **7** (6.4 g, 10.50 mmol). The mixture was co-evaporated with toluene (2x100 mL) and subjected to vacuum overnight. The mixture dissolved in CH₂Cl₂ (100 mL) and cooled to -40 °C. TMSOTf (0.20 mL; 0.88 mmol) was added and the reaction mixture was stirred at -40 °C for 1.5h. Et₃N (1 mL) was added and the reaction mixture was concentrated. The crude compound was purified by flash chromatography (19:1 toluene/EtOAc) to afford **4**. R_f 0.52 (9:1 toluene/EtOAc). Yield: 7.6 g (91%). $[\alpha]_D^{20} = 11.4^\circ$ (c 1.0, CDCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.44 – 7.34 (m, 4H, H^{SPH}), 7.29 – 7.13 (m, 11H, H^{SPH}. Ar-H), 5.43 (s, 1H, -CH^{benzylidene}), 5.38 (t, $J_{1,2} = J_{2,3} = 9.8$ Hz, 1H, H-2), 5.33 (dd, $J_{2,3} = 9.7$, $J_{1,2} = 8.0$ Hz, 1H, H-2'),

5.02 (dd, $J_{2,3} = 9.8$, $J_{3,4} = 2.9$ Hz, 1H, H-3), 4.79 (dd, $J_{2,3} = 9.7$, $J_{3,4} = 3.7$ Hz, 1H, H-3'), 4.68 (d, $J_{\text{CH}_2} = 11.2$ Hz, 1H, $-\text{CH}_2^{\text{Bn}}$), 4.59 (d, $J = 9.8$ Hz, 1H, H-1), 4.55 (d, $J_{\text{CH}_2} = 11.2$ Hz, 1H, $-\text{CH}_2^{\text{Bn}}$), 4.45 (d, $J = 8.0$ Hz, 1H, H-1'), 4.33 – 4.28 (m, 1H, H-4'), 4.22 – 4.15 (m, 1H, H-6a'), 3.95 (dd, $J = 12.5$, 1.9 Hz, 1H, H-6b'), 3.86 (m, 2H, H-4, H-6a), 3.75 – 3.63 (m, 2H, H-6b, H-5), 3.39 – 3.35 (m, 1H, H-5'), 1.13 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$), 1.09 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$), 1.07 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$), 1.07 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$). ^{13}C NMR (101 MHz, CDCl_3) δ 178.1, 177.4, 176.5, 176.4, 138.1, 137.5, 132.8, 132.1 (2C), 128.9 (2C), 128.8, 128.2 (2C), 128.0 (2C), 127.7, 127.5 (2C), 127.4, 125.9 (2C), 100.7, 100.5, 86.4, 77.6, 74.9, 74.6, 74.5, 73.0, 71.8, 68.7, 68.1, 67.3, 66.9, 66.4, 38.9, 38.8, 38.7, 27.2, 27.2 (3C), 27.1 (3C), 27.1 (3C), 27.0 (3C). HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{52}\text{H}_{58}\text{NaO}_{14}\text{S}$: 971.4228; Found 971.4229.

Benzyl 4,6-*O*-benzylidene-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranoside (9)

Compound **4** (2.0 g; 2.11 mmol) was dried azeotropically with toluene (2x30 mL) and left under vacuum over night. Freshly distilled benzyl alcohol (0.26 mL; 2.52 mmol) was added and the mixture was dissolved in dry CH_2Cl_2 (30 mL) and cooled to -40 °C. Then NIS (497 mg; 2.21 mmol) and TESOTf (45 μL ; 0.21 mmol) were added and the reaction mixture was stirred at -40 °C until TLC revealed full conversion of the donor (4 h). The solution was diluted with CH_2Cl_2 (100 mL) and washed with sat. aq. Na_2SO_3 (50 mL) and sat. aq. NaHCO_3 (50 mL), dried over MgSO_4 and concentrated. The product was purified by flash chromatography (4:1 Heptan/EtOAc) to yield a white amorphous solid. R_f 0.35 (9:1 toluene/EtOAc). Yield: 1.7 g (86 %). $[\alpha]_D^{20} = 24.6^\circ$ (c 1.0, CDCl_3). ^1H NMR (400 MHz, CDCl_3) δ 7.41 (m, 2H, H^{Bn}), 7.35 – 7.13 (m, 13H, H^{Bn} , Ar-H), 5.45 (s, 1H, $-\text{CH}^{\text{benzylidene}}$), 5.42 (dd, $J_{2,3} = 10.6$, $J_{1,2} = 8.1$ Hz, 1H, H-2), 5.37 (dd, $J_{2,3} = 10.4$, $J_{1,2} = 7.9$ Hz, 1H, H-2'), 4.96 (dd, $J_{2,3} = 10.6$, $J_{3,4} = 3.1$ Hz, 1H, H-3), 4.82 (dd, $J_{2,3} = 10.4$, $J_{3,4} = 3.8$ Hz, 1H, H-3'), 4.77 (d, $J_{\text{CH}_2} = 12.0$ Hz, 1H, CH_2^{a}), 4.70 (d, $J_{\text{CH}_2} = 11.2$ Hz, 1H, CH_2^{b}), 4.55 (d, $J_{\text{CH}_2} = 11.2$ Hz, 1H, CH_2^{a}),

4.49 (d, $J_{\text{CH}_2} = 12.0$ Hz, 1H, CH_2^a), 4.48 (d, $J = 7.9$ Hz, 1H, H-1'), 4.42 (d, $J = 7.9$ Hz, 1H, H-1), 4.32 (dd, $J_{3,4} = 3.8$, $J_{4,5} = 1.0$ Hz, 1H, H-4'), 4.20 (dd, $J_{6a,6b} = 12.5$, $J_{5,6a} = 1.6$ Hz, 1H, H-6a'), 3.97 (dd, $J_{6a,6b} = 12.5$, $J_{5,6b} = 1.8$ Hz, 1H, H-6b'), 3.89 (dd, $J = 9.8$, 5.9 Hz, 1H, H-6a), 3.85 (d, $J_{3,4} = 3.0$ Hz, 1H, H-4), 3.73 – 3.62 (m, 1H, H-5, H-6b), 3.41 – 3.37 (m, 1H, H-5'), 1.11 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$), 1.09 (s, 18H, $6 \times \text{CH}_3^{\text{Piv}}$), 1.04 (s, 9H, $3 \times \text{CH}_3^{\text{Piv}}$). **^{13}C NMR** (101 MHz, CDCl_3) δ 178.1, 177.6, 176.7, 176.5, 138.0, 137.5, 137.0, 128.8, 128.2 (4C), 128.0 (2C), 127.8 (2C), 127.7 (2C), 127.6, 127.5, 125.9 (2C), 100.8, 100.5, 100.1, 77.2, 75.1, 74.7, 73.8, 73.5, 73.0, 71.9, 70.3, 69.3, 68.7, 68.1, 66.9, 66.4, 38.9, 38.9, 38.7, 38.7, 27.2 (3C), 27.1 (3C), 27.1 (3C), 27.0 (3C). **HRMS** (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{53}\text{H}_{70}\text{NaO}_{15}$ 969.4612; Found 969.4614.

Benzyl 4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranoside (10)

A solution of 1 M BH_3 in THF (10 mL) was added to a 50 ml dry flask containing 1 mmol of compound **9** at 0 °C, and the solution was stirred for 5 minutes. A solution of 1 M Bu_2BOTf in CH_2Cl_2 (1 mL) was then added to the clear solution slowly. After 1 hour at 0 °C, TLC showed that the starting material had disappeared. Triethylamine (0.5 mL) was added followed by careful addition of methanol until the evolution of H_2 had ceased. The reaction mixture was co-distilled with methanol three times after which the product was purified by flash chromatography (9:1 toluene/EtOAc) to afford **10** as a colorless amorphous material. Yield: 749 mg (79%).

Benzyl 4,6-*O*-benzylidene-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranoside (11)

To a 25 mL flame-dried flask was added **10** (500 mg, 0.53 mmol) and **4** (658 mg, 2.05 mmol). The mixture was dried azeotropically with toluene (2x10 mL) and subjected to vacuum overnight. It was then dissolved in dry CH_2Cl_2 (5 mL) and dry MeCN (5 mL), cooled to -30 °C, followed by addition

of NIS (157 mg; 0.70 mmol) and TESOTf (14 mg; 0.05 mmol). The reaction mixture was stirred at -30 °C until TLC revealed full conversion of the donor (1.5h). The solution was diluted with CH₂Cl₂ (100 mL) and washed with sat. aq. Na₂S₂O₃ (100 mL) and sat. aq. NaHCO₃ (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated. The product was purified by flash chromatography (9:1 toluene/EtOAc) to afford **11** as a white amorphous material. R_f 0.45 (9:1 toluene/EtOAc). Yield: 792 mg (81%). ¹H NMR (400 MHz, CDCl₃) δ 7.52 – 7.01 (m, 35H), 5.44 (s, 1H, CH^{benzylidene}), 5.42 (dd, J_{2,3} = 10.1, J_{1,2} = 8.7 Hz, 1H, H-2), 5.37 (dd, J_{2,3} = 10.2, J_{1,2} = 8.0 Hz, 1H, H-2), 5.30 (dd, J_{2,3} = 10.4, J_{1,2} = 7.9 Hz, 1H, H-2), 5.29 (dd, J_{2,3} = 10.7, J_{1,2} = 8.0 Hz, 1H, H-2), 4.99 (dd, J_{2,3} = 10.6, J_{3,4} = 2.8 Hz, 1H, H-3), 4.96 (dd, J_{2,3} = 10.3, J_{3,4} = 2.7 Hz, 1H, H-3), 4.94 (dd, J_{2,3} = 10.3, J_{3,4} = 2.9 Hz, 1H, H-3), 4.82 (dd, J_{2,3} = 10.3, J_{3,4} = 3.7 Hz, 1H, H-3), 4.76 (d, J_{CH2} = 12.0 Hz, 1H, 0.5xCH₂^{Bn}), 4.70 – 4.45 (m, 7H, 3.5xCH₂^{Bn}), 4.41 (d, J_{1,2} = 7.9 Hz, 1H, H-1), 4.36 (d, J_{1,2} = 8.0 Hz, 1H, H-1), 4.36 (d, J_{1,2} = 7.9 Hz, 1H, H-1), 4.32 (d, J_{3,4} = 3.1 Hz, 1H), 4.29 (d, J_{1,2} = 7.9 Hz, 1H, H-1), 4.14 (d, J = 11.8 Hz, 1H, 0.5xH-6), 4.01 – 3.77 (m, 8H, 3xH-4, 2.5xH-6), 3.72 – 3.32 (m, 5H, 4xH-5, H-6), 1.11 – 1.02 (m, 72H, 24xCH₃^{Piv}). ¹³C NMR (101 MHz, CDCl₃) δ 178.2, 177.6, 177.5, 177.5, 176.8 (2C), 176.8, 176.6, 138.5, 138.4, 138.2, 137.7, 137.0, 128.9-126.0 (35C), 101.2, 101.1, 100.9, 100.6, 100.0, 75.4, 75.2, 75.1, 74.7, 74.5, 74.4, 73.9, 73.7, 73.6, 73.5, 73.1, 73.0, 72.0, 71.9, 70.4, 70.3, 69.7, 69.4, 68.8, 68.7, 68.2, 67.0, 67.0, 66.5, 66.5, 66.1, 66.0, 39.0 (2C), 38.9 (2C), 38.8 (2C), 38.8 (2C), 27.3 (3C), 27.3 (6C), 27.3 (3C), 27.2 (3C), 27.2 (6C), 27.1 (3C).

Phenyl 4-O-benzyl-6-O-chloroacetyl-2,3-di-O-pivaloyl-1-thio-β-D-galactopyranoside (12)

Compound **8** (30.0 g; 56.53 mmol) was dissolved in CH₂Cl₂ (560 mL) and cooled to 0 °C. (ClAc)₂O (15.0 g; 87.62 mmol), Et₃N (12.7 mL; 90.45 mmol) and DMAP (138 mg; 1.13 mmol) was added and the reaction was stirred at 0 °C for 1 h. The reaction mixture was concentrated and purified by flash chromatography (20:1 toluene/EtOAc) to afford **12** as a yellowish amorphous powder. R_f 0.57 (9:1 toluene/EtOAc). Yield 30.2 g (88%). [α]_D²⁰ = -6.7° (c 1.0, CDCl₃). ¹H NMR (400 MHz, CDCl₃) δ

7.45 – 7.33 (m, 2H, H^{SPh}), 7.31 – 7.11 (m, 8H, H^{SPh}, H^{Bn}), 5.41 (t, $J_{1,2} = J_{2,3} = 9.9$ Hz, 1H, H-2), 5.02 (dd, $J_{2,3} = 9.9$, $J_{3,4} = 2.9$ Hz, 1H, H-3), 4.78 (d, $J_{CH2} = 11.4$ Hz, 1H, -CH₂^{Bn}), 4.62 (d, $J_{1,2} = 9.9$ Hz, 1H, H-1), 4.38 (d, $J_{CH2} = 11.4$ Hz, 1H, -CH₂^{Bn}), 4.29 (dd, $J_{6a,6b} = 11.2$, $J_{5,6a} = 6.9$ Hz, 1H, H-6a), 4.04 (dd, $J_{6a,6b} = 11.2$, $J_{5,6b} = 5.8$ Hz, 1H, H-6b), 3.98 – 3.80 (m, 3H, -CH₂^{AcCl}, H-4), 3.73 (ddd, $J_{5,6a} = 6.9$, $J_{5,6b} = 5.8$, $J_{4,5} = 1.2$ Hz, 1H, H-5), 1.15 (s, 9H, 3xCH₃^{Piv}), 1.13 (s, 9H, 3xCH₃^{Piv}). ¹³C NMR (101 MHz, CDCl₃) δ 177.8, 176.6, 166.8, 137.4, 133.0, 132.4 (2C), 128.9 (2C), 128.6 (2C), 128.1, 128.0 (2C), 128.0, 87.0, 75.6, 74.9, 74.9, 73.9, 67.3, 64.3, 40.6, 39.1, 38.9, 27.3 (3C), 27.3 (3C). HRMS (ESI-TOF) m/z: [M + Na]⁺ Calcd for C₃₁H₄₀ClNaO₈S 630.2030; Found 630.2030.

4-O-Benzyl-6-O-chloroacetyl-2,3-di-O-pivaloyl-β-D-galactopyranose N-phenyl trifluoroacetimidate (13)

Compound **12** (20.0 g; 32.94 mmol) was dissolved in acetone (330 mL) and water (30 mL). NBS (14.8 g; 65.88 mmol) was added, and the reaction was stirred at 22 °C for 3 h. The solution was diluted with EtOAc (500 mL) and washed with sat. aq. Na₂S₂O₃ (200 mL) and sat. aq. NaHCO₃ (200 mL). The organic phase was dried over MgSO₄, filtered and concentrated. The residue was purified by flash chromatography (4:1 toluene/EtOAc) to afford **13**. R_f 0.19 (4:1 toluene/EtOAc) Yield: 14.9 g (88%). Hemiacetal (14.9 g; 28.88 mmol) was dissolved in CH₂Cl₂ (300 mL) and cooled to 0 °C. Cs₂CO₃ (18.8 g; 57.75 mmol) was added followed by *N*-phenyl trifluoroacetimidoyl chloride (12.0 g; 57.75 mmol). The ice bath was removed, and the reaction mixture was stirred for 12 h. It was then filtered, through a plug of Celite, concentrated and purified by flash chromatography (15:1 toluene/EtOAc) to give **13** as a white amorphous product. Yield: 15.5 g (78%).

Phenyl 4-O-benzyl-6-O-chloroacetyl-2,3-di-O-pivaloyl-β-D-galactopyranosyl-(1→6)-4-O-benzyl-2,3-di-O-pivaloyl-1-thio-β-D-galactopyranoside (14)

To a 500 mL flame-dried flask was added **8** (7.2 g, 13.56 mmol) and **13** (11.2 g, 16.28 mmol). The mixture was co-evaporated with toluene (2x200 mL) and subjected to vacuum overnight. The mixture

dissolved in CH₂Cl₂ (200 mL) and cooled to -40 °C. TMSOTf (0.23 mL; 1.35 mmol) was added, and the reaction mixture was stirred at -40 °C for 2h. Et₃N (1 mL) was added, and the reaction mixture was concentrated. The crude compound was purified by flash chromatography (9:1 toluene/EtOAc) affording **14**. R_f 0.5 (4:1 toluene/EtOAc). Yield: 12.5 g (90%).

Benzyl 4-O-benzyl-6-O-chloroacetyl-2,3-di-O-pivaloyl-β-D-galactopyranosyl-(1→6)-4-O-benzyl-2,3-di-O-pivaloyl-β-D-galactopyranoside (15)

Compound **14** (3.0 g; 2.92 mmol) was dried azeotropically with toluene (2x50 mL) and subjected to vacuum overnight. Benzyl alcohol (378 mg; 3.50 mmol) was added. The mixture was dissolved in dry CH₂Cl₂ (50 mL) and cooled to -40 °C. NIS (689 mg; 3.06 mmol) and TESOTf (77 mg; 0.29 mmol) was added and the reaction mixture was stirred at -40 °C until TLC revealed full conversion of the donor (4 h). The solution was diluted with CH₂Cl₂ (100 mL) and washed with sat. aq. Na₂S₂O₃ (100 mL) and sat. aq. NaHCO₃ (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated. The product was purified by flash chromatography (19:1 toluene/EtOAc) to afford **15** as a white amorphous material. R_f 0.49 (9:1 toluene/EtOAc). Yield: 2.60 g (87%). $[\alpha]_D^{20} = -14.3^\circ$ (c 1.0, CDCl₃). **IR** (neat, cm⁻¹): 3064, 3031, 2971, 2935, 2906, 2872, 1734, 1496, 1479, 1456, 1397, 1366, 1278, 1141, 1073, 1041. **¹H NMR** (400 MHz, CDCl₃) δ 7.48 – 6.88 (m, 15H, Ar-H), 5.40 (dd, $J_{2,3} = 10.4$, $J_{1,2} = 7.9$ Hz, 1H, H-2), 5.35 (dd, $J_{2,3} = 10.4$, $J_{1,2} = 7.8$ Hz, 1H, H-2'), 4.95 (dd, $J_{2,3} = 10.4$, $J_{3,4} = 3.0$ Hz, 1H, H-3'), 4.94 (dd, $J_{2,3} = 10.4$, $J_{3,4} = 3.1$ Hz, 1H, H-3), 4.80 (d, $J_{CH_2} = 11.8$ Hz, 1H, 0.5xCH₂^{Bn}), 4.77 (d, $J_{CH_2} = 11.6$ Hz, 1H, 0.5xCH₂^{Bn}), 4.70 (d, $J_{CH_2} = 11.8$ Hz, 1H, 0.5xCH₂^{Bn}), 4.48 (d, $J_{CH_2} = 11.6$ Hz, 1H, 0.5xCH₂^{Bn}), 4.42 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.38 (d, $J_{CH_2} = 11.4$ Hz, 1H, 0.5xCH₂^{Bn}), 4.39 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1'), 4.15 (dd, $J_{6a,6b} = 11.1$, $J_{5,6a} = 6.4$ Hz, 1H, H-6a'), 4.02 (dd, $J_{6a,6b} = 11.1$, $J_{5,6b} = 6.5$ Hz, 1H, H-6b'), 3.88 – 3.78 (m, 5H, H-4, H-4', H-6a, CH₂^{AcCl}), 3.68 – 3.54 (m, 3H, H-5, H-5', H-6b), 1.15 (s, 9H, 3xCH₃^{Piv}), 1.11 (s, 9H, 3xCH₃^{Piv}), 1.09 (s, 9H, 3xCH₃^{Piv}), 1.04 (s, 9H, 3xCH₃^{Piv}). **¹³C NMR** (101 MHz, CDCl₃) δ 177.9, 177.7, 176.8, 176.7, 166.8, 138.0,

137.3, 137.0, 128.6 (2C), 128.4 (2C), 128.3 (2C), 128.2 (2C), 128.2, 127.9 (2C), 127.8 (2C), 127.8 (2C), 101.3, 100.1, 75.2, 75.1, 74.7, 73.8, 73.7 (2C), 73.5, 71.9, 70.3, 69.3, 69.3, 67.5, 63.8, 40.6, 39.1, 39.0, 38.9, 38.8, 27.3 (3C), 27.3 (3C), 27.3 (3C), 27.2 (3C). **HRMS** (ESI-TOF) m/z : $[M + Na]^+$ Calcd for $C_{55}H_{73}ClNaO_{16}$ 1047.4485; Found 1047.4484.

Benzyl 4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranoside (10)

Compound **9** (2.9 g; 2.83 mmol) was dissolved in dry THF (50 mL). Thiourea (645 mg; 8.48 mmol), Bu_4NI (209 mg; 0.57 mmol) and $NaHCO_3$ (784 mg; 9.33 mmol) were added and the reaction mixture was heated to 55 °C for 6 h. The mixture was filtered, concentrated and purified by flash chromatography (9:1 toluene/EtOAc). R_f 0.22 (9:1 toluene/EtOAc). Yield: 2.39 g (89%). $[\alpha]_D^{20} = -8.0^\circ$ (c 1.0, $CDCl_3$). **1H NMR** (400 MHz, $CDCl_3$) δ 7.44 – 7.00 (m, 15H, Ar-H), 5.40 (dd, $J_{2,3} = 10.4$, $J_{1,2} = 7.9$ Hz, 1H, H-2), 5.34 (dd, $J_{2,3} = 10.4$, $J_{1,2} = 7.8$ Hz, 1H, H-2'), 4.93 (dd, $J_{2,3} = 10.4$, $J_{3,4} = 2.8$ Hz, 1H, H-3), 4.93 (dd, $J_{2,3} = 10.4$, $J_{3,4} = 2.8$ Hz, 1H, H-3'), 4.80 – 4.74 (m, 2H, CH_2^{Bn}), 4.72 (d, $J_{CH_2} = 11.4$ Hz, 1H, $0.5 \times CH_2^{Bn}$), 4.50 (d, $J = 11.3$ Hz, 1H, $0.5 \times CH_2^{Bn}$), 4.49 (d, $J_{CH_2} = 12.0$ Hz, 1H, $0.5 \times CH_2^{Bn}$), 4.41 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.37 (d, $J_{CH_2} = 11.4$ Hz, 1H, $0.5 \times CH_2^{Bn}$), 4.33 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1'), 3.87 (d, $J_{3,4} = 2.8$ Hz, 1H, H-4'), 3.85 (d, $J_{3,4} = 2.8$ Hz, 1H, H-4), 3.83 – 3.77 (m, 1H, H-6a), 3.67 – 3.56 (m, 3H, H-6b, H-6a', H-5), 3.50 – 3.43 (m, 1H, H-5'), 3.43 – 3.34 (m, 1H, H-6b'), 1.14 (s, 9H, $3 \times CH_3^{Piv}$), 1.11 (s, 9H, $3 \times CH_3^{Piv}$), 1.10 (s, 9H, $3 \times CH_3^{Piv}$), 1.03 (s, 9H, $3 \times CH_3^{Piv}$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.7, 176.7, 175.7, 175.6, 137.0, 136.5, 135.8, 127.5 (2C), 127.2 (2C), 127.2 (2C), 127.1 (2C), 126.8 (2C), 126.6 (2C), 126.6 (2C), 100.3, 98.9, 73.9, 73.9, 73.4, 73.0, 72.8, 72.6, 72.5, 69.2, 68.3, 68.2, 66.4, 60.6, 37.9, 37.9, 37.7, 37.7, 26.2 (3C), 26.2 (3C), 26.1 (3C), 26.1 (3C). **HRMS** (ESI-TOF) m/z : $[M + Na]^+$ Calcd for $C_{53}H_{72}NaO_{15}$ 971.4769; Found 971.4774.

Benzyl 4-O-benzyl-6-O-chloroacetyl-2,3-di-O-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-O-benzyl-2,3-di-O-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-O-benzyl-2,3-di-O-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-O-benzyl-2,3-di-O-pivaloyl- β -D-galactopyranoside (1)

To a 100 mL flame-dried flask was added **10** (1.5 g, 1.58 mmol) and the **14** (2.11 g, 2.05 mmol). The mixture was dried azeotropically with toluene (2x50 mL) and subjected to vacuum overnight. It was then dissolved in dry CH₂Cl₂ (25 mL) and dry MeCN (25 mL), cooled to -30 °C, followed by addition of NIS (472 mg; 2.10 mmol) and TESOTf (42 mg; 0.16 mmol). The reaction mixture was stirred at -30 °C until TLC revealed full conversion of the donor (1.5 h). The solution was diluted with CH₂Cl₂ (150 mL) and washed with sat. aq. Na₂S₂O₃ (100 mL) and sat. aq. NaHCO₃ (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated. The product was purified by flash chromatography (20:1 toluene/EtOAc) to afford a white amorphous material. R_f 0.43 (9:1 toluene/EtOAc). Yield: 2.6 g (88%). [α]_D²⁰ = -18.8° (c 1.0, CDCl₃) **IR** (neat, cm⁻¹): 2971, 2935, 2907, 2872, 1733, 1479, 1456, 1397, 1365, 1278, 1132, 1070, 1042. **¹H NMR** (400 MHz, CDCl₃) δ 7.47 – 6.98 (m, 25H, Ar-H), 5.39 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 7.9 Hz, 1H, H-2), 5.33 (dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 5.29 (dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 5.28 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 7.8 Hz, 1H, H-2), 5.01 – 4.90 (m, 4H, H-3¹-H-3⁴), 4.79 (d, J_{CH2} = 11.4 Hz, 1H, 0.5xCH₂^{Bn}), 4.76 (d, J_{CH2} = 12.0 Hz, 1H, 0.5xCH₂^{Bn}), 4.65 (m, 3H, 1.5xCH₂^{Bn}), 4.54 (d, J_{CH2} = 11.0 Hz, 1H, 0.5xCH₂^{Bn}), 4.53 (d, J_{CH2} = 11.1 Hz, 1H, 0.5xCH₂^{Bn}), 4.49 (d, J_{CH2} = 11.3 Hz, 1H, 0.5xCH₂^{Bn}), 4.48 (d, J_{CH2} = 12.0 Hz, 1H, 0.5xCH₂^{Bn}), 4.40 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.37 (d, J_{CH2} = 11.4 Hz, 1H, 0.5xCH₂^{Bn}), 4.36 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.29 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.29 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.09 (dd, $J_{6a,6b}$ = 11.0, $J_{5,6a}$ = 6.2 Hz, 1H, H-6a⁴), 4.00 (dd, $J_{6a,6b}$ = 11.0, $J_{5,6b}$ = 6.6 Hz, 1H, H-6b⁴), 3.95 – 3.76 (m, 7H, H-4¹-H-4⁴, 1.5xH-6), 3.73 (d, J = 1.5 Hz, 1H, H-5⁴), 3.67 – 3.48 (m, 4H, H-5¹-H-5³, 0.5xH-6), 3.42 – 3.31 (m, 2H, H-6), 1.15 (s, 9H, 3xCH₃^{Piv}), 1.10 (s, 9H, 3xCH₃^{Piv}), 1.09 (s, 9H, 3xCH₃^{Piv}), 1.09 (s, 9H, 3xCH₃^{Piv}), 1.08 (s, 9H, 3xCH₃^{Piv}), 1.08 (s, 18H, 6xCH₃^{Piv}), 1.03 (s, 9H, 3xCH₃^{Piv}). **¹³C NMR**

(101 MHz, CDCl₃) δ 177.9, 177.6, 177.5, 177.5, 176.8, 176.8, 176.8, 176.8, 166.8, 138.4, 138.3, 138.2, 137.4, 137.0, 128.6, 128.3, 128.3, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 101.3, 101.2, 101.1, 100.0, 77.3, 75.3, 75.2, 75.1, 75.1, 74.8, 74.5, 74.4, 73.7, 73.6, 73.5, 73.1, 73.0, 73.0, 71.9, 70.3, 69.7, 69.7, 69.4, 69.3, 67.0, 66.4, 66.2, 63.8, 40.5, 39.1, 39.0, 38.9, 38.9, 38.9, 38.8, 38.8, 38.8, 27.3, 27.3, 27.3, 27.3, 27.3, 27.2, 27.2, 27.2.

Benzyl 4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranoside (16)

Compound **1** (3.1 g; 1.66 mmol) was dissolved in dry THF (50 mL). Thiourea (380 mg; 4.98 mmol), Bu₄NI (123 mg; 0.33 mmol) and NaHCO₃ (460 mg; 5.48 mmol) was added and the reaction mixture was heated to 55 °C for 8 h. The mixture was filtered, concentrated and purified by flash chromatography (9:1 toluene/EtOAc) to give **16**. R_f 0.17 (9:1 toluene/ EtOAc). Yield: 2.59 g (87%).

$[\alpha]_D^{20} = -14.8^\circ$ (c 1.0, CDCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.40 – 7.00 (m, 25H), 5.47 – 5.20 (m, 4H, H-2¹-H-2⁴), 5.03 – 4.86 (m, 4H, H-3¹-H-3⁴), 4.81 – 4.44 (m, 9H, 4.5xCH₂^{Bn}), 4.40 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1^{1/2/3/4}), 4.35 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1^{1/2/3/4}), 4.28 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1^{1/2/3/4}), 4.24 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1^{1/2/3/4}), 3.94 – 3.74 (m, 8H, H-4¹-H-4⁴, 2xH-6), 3.64 – 3.25 (m, 8H, H-5¹-H-5⁴, 2xH-6), 1.13 (s, 9H, 3xCH₃^{Piv}), 1.12 – 1.06 (m, 54H, 18xCH₃^{Piv}), 1.03 (s, 9H, 3xCH₃^{Piv}). **¹³C NMR** (101 MHz, CDCl₃) δ 177.7, 177.6, 177.5, 177.4, 176.7, 176.7 (2C), 176.7, 138.3, 138.3, 138.1, 137.5, 136.9, 128.5, 128.2, 128.1, 128.1, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.5, 127.5, 127.4, 127.4, 101.4, 101.0, 101.0, 99.9, 77.2, 75.1, 75.0, 75.0, 75.0, 74.9, 74.6, 74.1, 73.8, 73.5, 73.4, 73.1, 73.0, 73.0, 73.0, 70.1, 69.6, 69.5, 69.4, 69.3, 66.9, 66.4, 66.2, 61.6, 38.9, 38.9, 38.8 (2C), 38.8, 38.7 (2C), 38.7, 27.2-27.1 (24C).

Benzyl 4-*O*-benzyl-6-*O*-chloroacetyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-

galactopyranosyl-(1→6)-4-O-benzyl-2,3-di-O-pivaloyl-β-D-galactopyranosyl-(1→6)-4-O-benzyl-2,3-di-O-pivaloyl-β-D-galactopyranosyl-(1→6)-4-O-benzyl-2,3-O-dipivaloyl-β-D-galactopyranoside (2)

To a 100 mL flame-dried flask was added **16** (2.15 g, 1.20 mmol) and the **14** (1.60 g, 1.56 mmol). The mixture was dried azeotropically with toluene (2x50 mL) and subjected to vacuum overnight. It was then dissolved in dry CH₂Cl₂ (20 mL) and dry MeCN (20 mL), cooled to -30 °C, followed by addition of NIS (359 mg; 1.60 mmol) and TESOTf (32 mg; 0.12 mmol). The reaction mixture was stirred at -30 °C until TLC revealed a full conversion of the donor (1 h). The solution was diluted with CH₂Cl₂ (200 mL) and washed with sat. aq. Na₂S₂O₃ (100 mL) and sat. aq. NaHCO₃ (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated. The product was purified by flash chromatography (20:1 toluene/EtOAc) to afford **2** as a white amorphous material. R_f 0.45 (9:1 toluene/EtOAc). Yield: 2.95 g (91%). $[\alpha]_D^{20} = -11.6^\circ$ (c 1.0, CDCl₃). **IR**(neat, cm⁻¹): 3065, 3031, 2971, 2935, 2906, 2872, 1733, 1479, 1457, 1397, 1365, 1278, 1132, 1070. **¹H NMR** (400 MHz, CDCl₃) δ 7.40 – 7.00 (m, 35H), 5.48 – 5.14 (m, 6H, H-2¹-H-2⁶), 5.05 – 4.88 (m, 6H, H-3¹-H-3⁶), 4.79 (d, $J_{CH2} = 11.3$ Hz, 1H, 0.5xCH₂^{Bn}), 4.76 (d, $J_{CH2} = 12.0$ Hz, 1H, 0.5xCH₂^{Bn}), 4.69 – 4.44 (m, 11H, 5.5xCH₂^{Bn}), 4.40 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1^{1/2/3/4/5/6}), 4.375 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1^{1/2/3/4/5/6}), 4.37 (d, $J_{CH2} = 11.4$ Hz, 1H, 0.5xCH₂^{Bn}), 4.35 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1^{1/2/3/4/5/6}), 4.30 – 4.21 (m, 3H, H-1^{1/2/3/4/5/6}, H-1^{1/2/3/4/5/6}, H-1^{1/2/3/4/5/6}), 4.07 (dd, $J_{6a,6b} = 11.1$, $J_{5,6a} = 6.2$ Hz, 1H, H-6a⁶), 3.99 (dd, $J_{6a,6b} = 11.1$, $J_{5,6b} = 6.6$ Hz, 1H, H-6b⁶), 3.94 – 3.75 (m, 11H, H-4¹-H-4⁶, 2.5xH-6), 3.71 (d, $J_{CH2} = 2.1$ Hz, 2H, -CH₂^{AcCl}), 3.68 – 3.44 (m, 8H, H-5¹-H-5⁶, H-6), 3.36 (dd, $J_{6a,6b} = 9.3$, $J_{5,6a} = 5.3$ Hz, 1H, 0.5xH-6), 3.27 (m, 2H, H-6), 1.15 (s, 9H, 3xCH₃^{Piv}), 1.11 – 1.05 (m, 90H, 30xCH₃^{Piv}), 1.03 (s, 9H). **¹³C NMR** (101 MHz, CDCl₃) δ 177.7, 177.5, 177.4, 177.3 (2C), 177.3, 176.7 (2C), 176.7 (2C), 176.6, 166.6, 138.5 (2C), 138.3, 138.2, 138.1, 137.2, 136.9, 128.5-127.1 (35C), 101.2, 101.1 (2C), 99.9, 77.2, 75.2, 75.1, 75.0,

74.6, 74.4, 74.2, 73.6, 73.5, 73.4, 73.0, 72.9, 72.8, 71.8, 70.1, 69.6, 69.6, 69.3, 69.1, 66.9, 65.8, 63.6, 40.4-38.7 (12C), 27.2-27.1 (36C).

Benzyl 4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)- 4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranoside (17)

Compound **2** (2.15 g; 0.79 mmol) was dissolved in dry THF (50 mL). Thiourea (241 mg; 3.18 mmol), Bu₄NI (59 mg; 0.16 mmol) and NaHCO₃ (293 mg; 3.49 mmol) was added and the reaction mixture was heated to 55 °C for 8 h. The mixture was filtered, concentrated and purified by flash chromatography (9:1 toluene/EtOAc) to afford **17** as a slightly yellow amorphous material. R_f 0.22 (9:1 toluene/EtOAc). Yield: 1.75 g (84%). [α]_D²⁰ = -22.5° (c 1.0, CDCl₃) **IR** (neat, cm⁻¹): 2971, 2935, 2872, 1734, 1496, 1479, 1278, 1133, 1070, 1045. **¹H NMR** (400 MHz, CDCl₃) δ 7.36 – 7.08 (m, 35H), 5.39 (dd, $J_{2,3} = 10.4$, $J_{1,2} = 7.9$ Hz, 1H, H-2), 5.29 (m, 5H, 5xH-2), 5.04 – 4.89 (m, 6H, 6xH-3), 4.76 (d, $J_{CH2} = 11.8$ Hz, 1H, 0.5xCH₂^{Bn}), 4.72 – 4.45 (m, 12H, 6xCH₂^{Bn}), 4.43 – 4.19 (m, 7H, 0.5xCH₂^{Bn}, 6xH-1), 3.96 – 3.77 (m, 11H, 6xH-4, 2.5xH-6), 3.64 – 3.18 (m, 13H, 6xH-5, 3.5xH-6), 1.15 – 1.02 (m, 108H, 36xCH₃^{Piv}). **¹³C NMR** (101 MHz, CDCl₃) δ 177.9-176.8 (12C), 138.6-137.0 (7C), 128.6-127.4 (35C), 101.5-100.1 (6C), 75.2, 75.1, 75.09, 74.8, 74.5, 74.4, 74.2, 73.9, 73.7, 73.5, 73.1, 73.0, 70.3, 69.7, 69.5, 69.4, 67.2, 67.0, 66.3, 61.7, 39.0-38.8 (12C), 27.3-27.2 (36C).

Benzyl 4-*O*-benzyl-6-*O*-chloroacetyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)- 4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranosyl-(1 \rightarrow 6)-4-*O*-benzyl-2,3-di-*O*-pivaloyl- β -D-galactopyranoside (3)

To a 50 mL flame-dried flask was added **17** (1.2 g, 0.46 mmol) and the **14** (609 mg, 0.59 mmol). The mixture was dried azeotropically with toluene (2x25 mL) and subjected to vacuum overnight. It was then dissolved in dry CH₂Cl₂ (20 mL) and dry MeCN (20 mL), cooled to -30 °C, followed by addition of NIS (136 mg; 0.61 mmol) and TESOTf (12 mg; 0.05 mmol). The reaction mixture was stirred at -30 °C until TLC revealed full conversion of the donor (1 h). The solution was diluted with CH₂Cl₂ (200 mL) and washed with sat. aq. Na₂S₂O₃ (100 mL) and sat. aq. NaHCO₃ (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated. The product was purified by flash chromatography (15:1 toluene/EtOAc) to afford **3** as a white amorphous material. R_f 0.37 (9:1 toluene/EtOAc). Yield: 1.4 g (87%). $[\alpha]_D^{20} = -15.7^\circ$ (c 1.0, CDCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.39 – 6.97 (m, 45H), 5.42 – 5.20 (m, 8H, H-2¹-H-2⁸), 5.02 – 4.90 (m, 8H, H-3¹-H-3⁸), 4.79 (d, $J_{CH_2} = 11.4$ Hz, 1H, 0.5xCH₂^{Bn}), 4.76 (d, $J_{CH_2} = 12.1$ Hz, 1H, 0.5xCH₂^{Bn}), 4.72 – 4.44 (m, 15H, 7.5xCH₂^{Bn}), 4.40 (d, $J = 7.9$ Hz, 1H, H-1^{1/2/3/4/5/6}), 4.37 (d, $J_{CH_2} = 11.4$ Hz, 1H, 0.5xCH₂^{Bn}), 4.35 (d, $J = 7.8$ Hz, 1H, H-1^{1/2/3/4/5/6}), 4.30 – 4.19 (m, 6H, 6xH-1), 4.06 (dd, $J_{6a,6b} = 11.1$, $J_{5,6a} = 6.2$ Hz, 1H, H-6a⁸), 3.99 (dd, $J_{6a,6b} = 11.0$, $J_{5,6a} = 6.6$ Hz, 1H, H-6b⁸), 3.95 – 3.73 (m, 17H, H-4¹-H-4⁸, 4.5xH-6), 3.70 (d, $J_{CH_2} = 2.3$ Hz, 2H, CH₂^{AcCl}), 3.67 – 3.44 (m, 10H, H-5¹-H-5⁸, H-6), 3.36 (dd, $J = 9.3$, 5.2 Hz, 1H, 0.5xH-6), 3.32 – 3.14 (m, 4H, 2xH-6), 1.15 (s, 9H, 3xCH₃^{Bn}), 1.08 (dd, 126H, 42xCH₃), 1.03 (s, 9H, 3xCH₃^{Bn}). **¹³C NMR** (101 MHz, CDCl₃) δ 177.7-176.6 (16C), 166.6, 138.5, 138.5, 138.5, 138.5, 138.3, 138.2, 138.1, 137.2, 136.9, 128.5-127.1 (45C), 101.2-101.1 (7C), 99.9, 77.2, 75.2, 75.1, 75.0, 74.6, 74.3, 74.2, 73.6, 73.5, 73.5, 73.4, 73.0, 72.9, 72.8, 72.8, 72.7, 72.7, 71.8, 70.1, 69.7, 69.6, 69.3, 69.1, 40.4, 39.0-38.7 (16C), 27.2-27.0 (48C).

Benzyl 4,6-O-benzylidene-β-D-galactopyranosyl-(1→6)-4-O-benzyl-β-D-galactopyranosyl-(1→6)-4-O-benzyl-β-D-galactopyranosyl-(1→6)-4-O-benzyl-β-D-galactopyranoside (18)

To a 50 mL flask was added **11** (500 mg, 0.45 mmol), dioxane (10 mL), and water (10 mL). Afterwards, 1 M Bu₄NOH in MeOH (4.5 mL, 4.5 mmol) was added and the reaction mixture was

stirred at 55 °C, overnight. After completion of the reactions, it was neutralized with amberlite IR-120 H⁺ resin. Furthermore it was filtered and concentrated. The product was purified by flash chromatography (7% MeOH/DCM) to afford **18** as a white amorphous material. R_f 0.30 (9:1 DCM/MeOH). Yield: 268 mg (86%). $[\alpha]_D^{20} = -35.86^\circ$ (c 1.1, MeOH). **IR** (neat, cm⁻¹): 3550, 3100, 3062, 3029, 2873, 1473, 1364, 1153, 1043. **¹H NMR** (300 MHz, CD₃OD) δ 7.55 – 7.11 (m, 25H), 5.54 (s, 1H), 4.94-4.88 (m, 1H), 4.77-4.58 (m, 6H), 4.35-3.15 (m, 29H). **¹³C NMR** (75 MHz, CD₃OD) δ 139.6, 139.6, 139.4, 139.0, 138.3, 129.1, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.2, 127.9, 127.8, 127.6, 126.9, 104.5, 104.3, 103.3, 103.9, 103.1, 101.6, 77.2, 77.1, 76.6, 75.5, 75.9, 74.8, 75.0, 73.9, 72.0, 72.1, 71.3, 71.1, 69.3, 68.9, 68.3, 68.0, 67.2.

β-D-Galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-D-galactopyranose (19)

To a 100 mL flame-dried flask under N₂-atmosphere was added **18** (222 mg, 0.20 mmol), THF (10 mL), and MeOH (30 mL). Further, it was followed by the addition of 10% Pd/C (250 mg), and the reaction mixture was stirred for 3 h under H₂-atmosphere. Furthermore, water (10 mL) was added, and resulting mixture was stirred for 48 h under the same conditions. After the completion of reactions, the reaction mixture was filtered through a plug of Celite and concentrated. The product was purified by reverse phase chromatography to afford **19** as a pale yellow oil. Yield: 111 mg (84%). $[\alpha]_D^{20} = 8.81^\circ$ (c 1.1, H₂O). **IR** (neat, cm⁻¹): 3500, 3100, 2895, 2484, 1587, 1383, 1132, 1025. **¹H NMR** (500 MHz, D₂O) δ 5.25 (d, *J* = 3.8 Hz, 1H), 4.58 (d, *J* = 7.9 Hz, 1H), 4.49-4.40 (m, 4H), 4.26 (dd, *J* = 8.0, 4.3 Hz, 1H), 4.09-3.41 (m, 34H). **¹³C NMR** (50 MHz, D₂O) δ 106.0, 105.9, 105.8, 99.1, 77.8, 76.4, 75.4, 75.2, 74.5, 73.4, 71.9, 71.6, 71.4, 71.3, 70.9, 63.7.

Acknowledgement:

We acknowledge financial support from the Danish Council for Independent Research “A biology-driven approach for understanding enzymatic degradation of complex polysaccharide systems”

(Grant Case no.: 107279), the Carlsberg Foundation, the Danish Strategic Research Council (GlycAct and SET4Future projects), the Villum Foundation (PLANET project) and the Novo Nordisk Foundation (Biotechnology-based Synthesis and Production Research).

References:

- (1) Heredia, A.; Jiménez, A.; Guillén, R. *Z. Lebensm. Unters. Forsch.* **1995**, *200*, 24–31.
- (2) Clarke, A. E.; Anderson, R. L.; Stone, B. A. *Phytochemistry* **1979**, *18* (4), 521–540.
- (3) Mohnen, D. *Curr. Opin. Plant Biol.* **2008**, *11*, 266–277.
- (4) Timmers, C. M.; Wigchert, S. C. M.; Leeuwenburgh, M. A.; van der Marel, G. A.; van Boom, J. H. *European J. Org. Chem.* **1998**, *1*, 91–97.
- (5) Gu, G.; Yang, F.; Du, Y.; Kong, F. *Carbohydr. Res.* **2001**, *336* (2), 99–106.
- (6) Zeng, Y.; Li, A.; Kong, F. *Tetrahedron Lett.* **2003**, *44* (45), 8325–8329.
- (7) Li, A.; Zeng, Y.; Kong, F. *Carbohydr. Res.* **2004**, *339* (3), 673–681.
- (8) Li, A.; Kong, F. *Carbohydr. Res.* **2004**, *339* (11), 1847–1856.
- (9) Li, A.; Kong, F. *Bioorg. Med. Chem.* **2005**, *13* (3), 839–853.
- (10) Du, Y.; Pan, Q.; Kong, F. *Carbohydr. Res.* **2000**, *323* (1–4), 28–35.
- (11) Huang, H.; Han, L.; Lan, Y.-M.; Zhang, L.-L. *J. Asian Nat. Prod. Res.* **2014**, *16* (6), 640–647.
- (12) Liang, X.-Y.; Liu, Q.-W.; Bin, H.-C.; Yang, J.-S. *Org. Biomol. Chem.* **2013**, *11*, 3903–3917.
- (13) Andersen, M. C. F.; Kračun, S. K.; Rydahl, M. G.; Willats, W. G. T.; Clausen, M. H. *Chem. - Eur. J.* **2016**, *22*, 11543–11548.
- (14) Andersen, M. C. F.; Boos, I.; Marcus, S. E.; Kračun, S. K.; Rydahl, M. G.; Willats, W. G. T.;

Knox, J. P.; Clausen, M. H. *Carbohydr. Res.* **2016**, *436*, 36–40.

- (15) Andersen, M. C. F. *Synthesis and Application of Plant Cell Wall Oligogalactans*, Technical University of Denmark, 2014.
- (16) Jiang, L.; Chan*, T.-H. *J. Org. Chem.* **1998**, *63*, 6035–6038.
- (17) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42* (12), 2405–2407.
- (18) Shie, C.-R.; Tzeng, Z.-H.; Kulkarni, S. S.; Uang, B.-J.; Hsu, C.-Y.; Hung, S.-C. *Angew. Chemie Int. Ed.* **2005**, *44* (11), 1665–1668.
- (19) Sakagami, M.; Hamana, H. *Tetrahedron Lett.* **2000**, *41* (29), 5547–5551.
- (20) Hernández-Torres, J. M.; Achkar, J.; Wei, A. *J. Org. Chem.* **2004**, *69* (21), 7206–7211.
- (21) Jiang, L.; Chan, T.-H. *Tetrahedron Lett.* **1998**, *39* (5–6), 355–358.