Chemical synthesis of fragments of streptococcal cell wall polysaccharides

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Chapter 5

Summary and Future Prospects

This thesis describes the design and synthesis of fragments of various cell wall carbohydrates of the *Streptococcus* species, including the branched Group B-specific antigen (GBC) of Group B *Streptococcus*, the recently discovered glycerol phosphate (GroP) modified group A carbohydrate and the *O*-acetylated type 1 capsular polysaccharide of *Streptococcus pneumoniae*. All the synthesized fragments were equipped with a spacer at the reducing end for further conjugation with proteins or active small molecules to explore the mechanisms of carbohydrate-based vaccines in immune responses and to develop novel vaccines. To investigate structure-activity relationship, several fragments of each polysaccharide were assembled varying in length.

Chapter 1 introduces the diversity and different recognition functions of carbohydrates and provides an overview of the developments and state of the art of isolated and synthetic carbohydrate-based vaccines. The mechanisms by which the immune system reacts to carbohydrate-based vaccines, being either pure oligosaccharides, glycoconjugates or zwitterionic polysaccharides, are summarized. Some examples of synthetic oligosaccharide vaccines for *Streptococcus* that offer protection against, amongst others, Group A *Streptococcus*, Group B *Streptococcus* and *Streptococcus pneumoniae*, are described.

Chapter 2 describes the chemical synthesis of representative fragments of the Group B-specific antigen using highly convergent pathways, including a [3 + 5] glycosylation and [5 + 8] phosphoramidite coupling. Three branched oligosaccharides were obtained in multi-milligram quantities, including a pentasaccharide **1**, an octasaccharide **2** and a tridecasaccharide **3**, all of which contain an amino spacer for conjugation purposes (Figure 1). To evaluate the biological activities of these oligosaccharides, the bioconjugation with two different carrier proteins, non-toxic mutant of diphtheria toxin CRM197 and human serum albumin HSA, was accomplished by Jacopo Enotarpi of Leiden University (Figure 2 and 3). Conjugation of three synthesized oligosaccharides with an excess of reactive di-(*N*-succinimidyl)-glutarate (DSG), then covalent coupling to carrier protein yielded the CRM197 and HSA conjugates with an average of 29 pentasaccharide, 25 octasaccharide, 18 tridecasaccharide molecules per CRM197; 32 pentasaccharide, 30 octasaccharide, 20 tridecasaccharide molecules per HSA (Figure 3, Table 1). These conjugates were purified by filtration against sodium phosphate buffer and characterized by SDS-PAGE and capillary electrophoresis–mass spectrometry (CE-MS) analysis (Figure 3) to estimate the carbohydrate/protein molar ratio. The conjugates will be probed for binding to serum and (monoclonal) antibodies directed at *Group B Streptococcus* polysaccharides and immunization studies in mice.

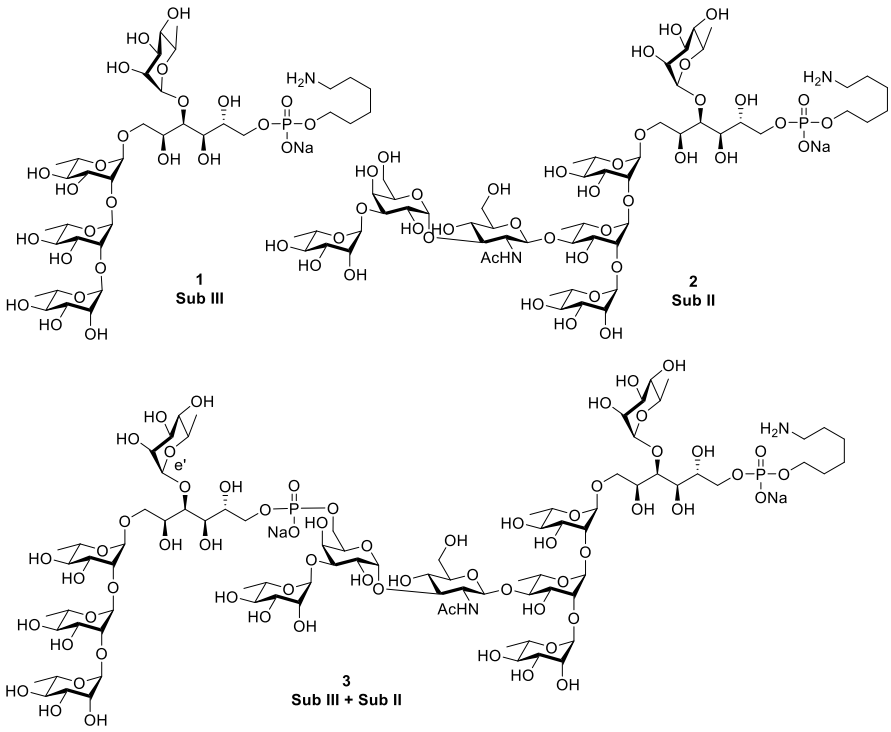
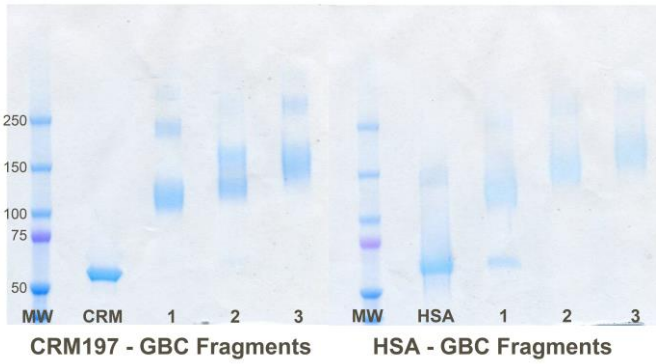


Figure 1. Three conjugation-ready GBC fragments.



Conjugate	Protein mg available
CRM197 - 1	*2,3*
CRM197 - 2	*2,6*
CRM197 - 3	*1,1*
HSA - 1	*1,8*
HSA - 2	*2,6*
HSA - 3	*1,6*

Figure 2. Conjugation of synthesized oligosaccharides with carrier proteins CRM197 and HSA.

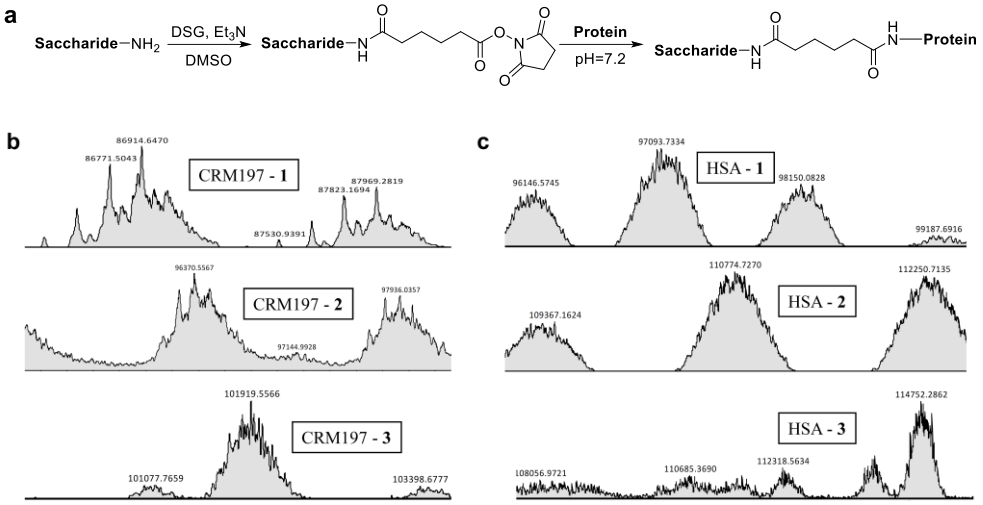


Figure 3. Conjugation of oligosaccharides with CRM197 and HSA. **a**, Oligosaccharides **1** - **3** were conjugated with CRM197 and HSA proteins using the DSG derivatization method; **b**, CE-MS analysis of CRM197 conjugates measure the average molecular weight to estimate the carbohydrate/protein molar ratio; **c**, CE-MS analysis of HSA conjugates measure the average molecular weight to estimate the carbohydrate/protein molar ratio. Mass of CRM197 is 58.4 kDa and HSA is 66.5 kDa.

Table 1. The carbohydrate loadings of glycoconjugates CRM197 and HSA conjugates.

Conjugates	CRM197 - **1**	CRM197 - **2**	CRM197 - **3**	HSA - **1**	HSA - **2**	HSA - **3**
Carbohydrate/protein	29	25	18	32	30	20

Chapter 3 describes the first total synthesis of fragments of the recently discovered glycerol phosphate (GroP) modified group A carbohydrate (GAC), termed GroP GAC. The corresponding fragments of the GAC-fragments, lacking the glycerol phosphate appendages, were also synthesized, and all six synthesized oligosaccharides **4** – **9** contain a spacer terminated with a free amine for the further modification (Figure 4). A properly protected trisaccharide was adopted as the repeating unit building block to assemble the desired six targets, including two tri-, two hexa- and two nonasaccharides, employing [3 + 3] and [3 + 6] glycosylations. The fragments will be coupled to a carrier protein to provide the corresponding glycoconjugates for further vaccine development. Based on the NMR analysis of the isolated GroP GAC, approximately 25% of the GAC sidechain GlcNAc carries a GroP at its O-6 position (GroP : GlcNAc = 1 : 3). In the generated fragments, all the GlcNAc sidechains were modified with a GroP moiety (GroP : GlcNAc = 1 : 1). To build a more

complete fragment library of GAC, to prepare more glycoconjugate vaccine candidates and to investigate the significance of the GroP, other fragments would be synthesized in the future, with varying GroP : GlcNAc ratio and different substitution patterns.

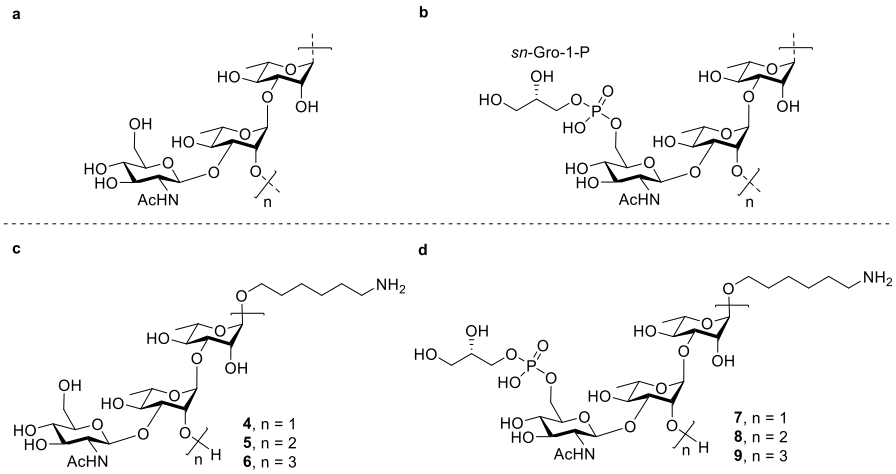


Figure 4. **a**, The structure of GAC. **b**, the structure of glycerol phosphate modified GAC. **c**, the designed fragments of GAC **4** - **6**. **d**, the designed fragments of GroP GAC **7** - **9**.

Chapter 4 describes the first synthesis of fragments of the *O*-acetylated type 1 capsular polysaccharide of *Streptococcus pneumoniae*, termed *O*-Ac Sp1, including a tri-, a hexa- and a nonasaccharide (Figure 5). Sp1 is one of the zwitterionic polysaccharides (ZPSs), a rare class of immunomodulatory agents, that can provoke a T-cell mediated immune responses after being processed by the antigen-presenting cells (APC) and binding to the major histocompatibility complex class II (MHC II). Considering future conjugation and the presence of free amines in the *O*-Ac Sp1, a vicinal diol spacer was attached at the reducing end. A post-oxidation glycosylation strategy was first evaluated to introduce the carboxylates at an early stage of the synthesis, but unfortunately this strategy had to be abandoned because of the poor stereoselectivity of a model [2 + 2] glycosylation. Therefore, the previously developed strategy for the synthesis of the non-acetylated Sp1 oligosaccharides was adopted. The regioselective oxidation of multiple primary alcohols in the complex oligosaccharide was accomplished using a modified TEMPO-BAIB oxidation protocol. It was observed that over-oxidation could take place leading to cleavage of the glycosidic bond, of the galactose moiety that was to be oxidized. The generated trisaccharide was used to probe the stability of the C-3-*O*-acetyl group, which was shown to be labile under neutral and slightly basic conditions. At slightly acidic pH, the acetyl group is stable, without migration and cleavage taking place. The structure of the *O*-Ac Sp1 oligosaccharide targets will be investigated

employing molecular dynamics simulations and NMR spectroscopy to evaluate the role of the acetyl on the 3D-structure of these oligosaccharides. Binding studies using enzyme-linked immunosorbent assay (ELISA) and saturation transfer difference (STD) NMR experiments will reveal the role of the acetyl groups in the interaction with anti-Sp1 antibodies.

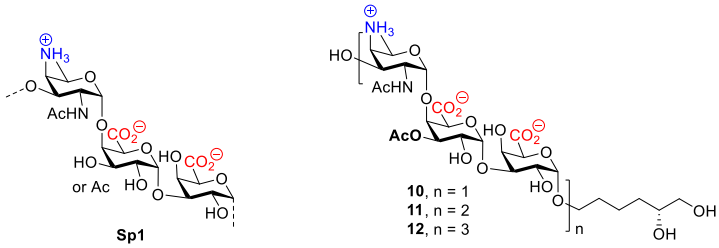


Figure 5. The structure of Sp1 and designed fragments of *O*-Ac-Sp1 **10** – **12**.

Synthesis of PS A1 Repeating Unit

To further probe structure-activity relationship studies for ZPSs, the synthesis of another zwitterionic polysaccharide, PS A1, was explored. PS A1 is isolated from *Bacteroides fragilis*, which is a gram-negative and generally commensal bacterium, colonizing the human colon. The structure of PS A1 is made up of tetrasaccharide repeating units, containing the rare 2-acetamido-4-amino-2,4,6-trideoxygalactose (D-AAT) and a pyruvate substituted galactose residue (Figure 6A).[1] Encouraged by the specific immunomodulating properties of zwitterionic polysaccharide (outlined in Chapter 1), the synthesis of PS A1 fragments has been reported by several groups, including the groups of Van der Marel,[2] Seeberger[3] and Andreana[4]. Although some solutions have been offered to overcome the synthetic challenges of this complex structure, to date, only the assembly of a tetrasaccharide, *i. e.* one repeating unit was accomplished.

Previous reports have reported on the poor nucleophilicity of the galactosamine C-4-OH in glycosylations of the D-AAT donor and trisaccharide *DB(A)* or disaccharide *BA*, due to the steric crowding between the pyruvalated galactose and the C-6 benzyl ether of the galactosamine residue.[2-3] To accomplish the total synthesis of one repeating unit, a coupling between a *C(D)B* trisaccharide donor and pyruvalated galactose (*A*) acceptor was performed. However, to obtain the longer repeating unit **13** – **15** and analogues **16** – **18**, lacking the galactofuranose residues (Figure 6A), to establish structure-activity relationship, a more effective and convergent strategy is necessary. Considering the reactivity of the building blocks, a new retrosynthetic analysis pathway was designed using a [1 + [2 + 1]] strategy: [*D* + [*CB* + *A*]] (Figure 6B). Because of the free amine in the repeating unit, the propargyl group

was selected as the terminus of spacer for the further conjugation. Accordingly, to circumvent hydrogenation conditions for global deprotection, acyl and carbamate groups were chosen as the protecting groups, including levulinoyl (Lev), and acetyl (Ac) esters and a phenoxyacetyl (Pac) to mask the D-AAT amine, which all can be removed with mild basic condition. The PS A1 fragments **13** – **15** can be obtained from the corresponding protected oligosaccharides **19** – **21**, which in turn can be generated by glycosylation of the linear oligosaccharide acceptors **22** – **24** with an appropriate galactofuranose donor. The linear fragments **16** – **18** were planned to be derived from **22** – **24**. These linear oligosaccharides can be obtained from key trisaccharide **25** and **26**, which can be synthesized from the four monosaccharides **27**, **28**, **30** and **31**.

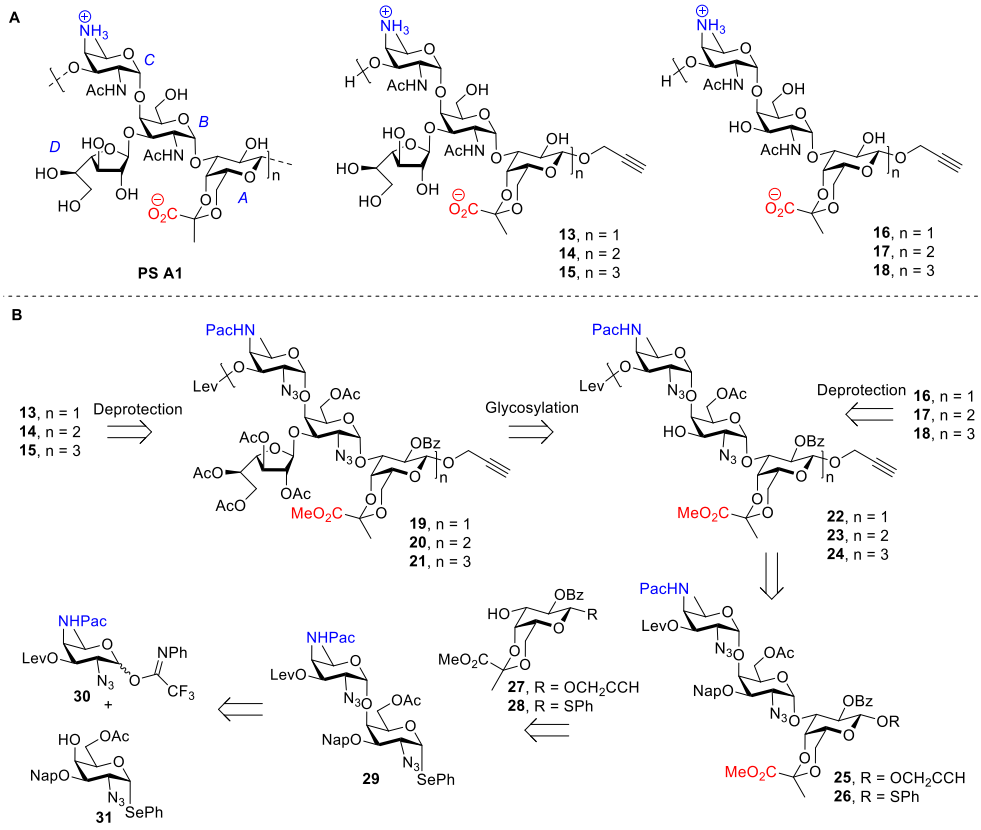
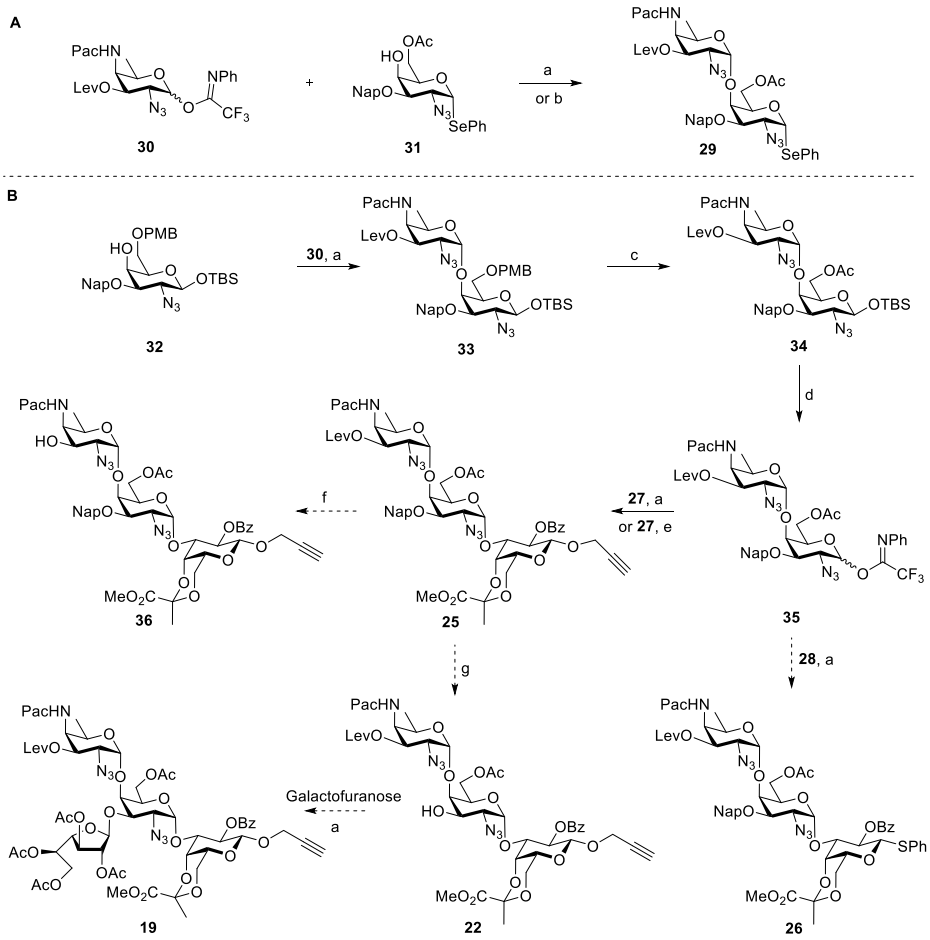


Figure 6. A) Structure of PS A1 repeating unit and the designed fragments. B) the first retrosynthetic analysis of the fragments **13** – **18**.

According to (modified) previously reported procedures, the four designed monosaccharides were readily prepared (See the Experiment Section). The synthesis of the fragments commenced with the glycosylation between D-AAT donor **30** and galactose donor

31. Unexpectedly, the yield of disaccharide **29** is very low (Scheme 1A). This outcome may be attributed to the poor reactivity of the C-4-OH in the galactosamine building block. To improve the yield of this glycosylation, another galactose building block **32** was explored as acceptor, bearing an electron-donating *p*-methoxybenzyl (PMB) protective group at the C-6 hydroxyl. The glycosylation between **32** and **30** generated the disaccharide **33** under the promotion of TBSOTf in 68% yield. Considering the lability of the pyruvyl acetal group under acidic conditions, the PMB ether was transformed to acetyl ester in 96% yield over two steps to provide the disaccharide **34**. To simplify the next glycosylation, imidate donor **35** was generated from silyl ether **34**. The ensuing [2 + 1] glycosylation between donor **35** and acceptor **27** was carried out in the presence of TBSOTf to construct the trisaccharide **25** in 62% yield. Unfortunately, the α/β selectivity of this glycosylation was very poor (1:1). Although the α/β-ratio could be increased to more than 10:1 with the use of DMF as an additive,[5] the yield of this glycosylation was rather poor (32%). Optimization of this glycosylation is required to effectively generate the trisaccharide. To this end other additives, such as methyl(phenyl)formamide may be explored in combination with slightly elevated temperatures.[6]



Scheme 1. Attempted synthesis pathway of the fragments of PS A1.

Reagents and conditions: a) TBSOTf, 4Å MS, DCM, 0 °C, **29**, 23%; **33**, 68%; **25**, 62% (α/β = 1 : 1). b) TfOH, 4Å MS, DCM, 0 °C, 25%. c) i, HCl/HFIP, DCM, HFIP, triethylsilane; ii, Ac_2O, pyridine, 96% (over two steps). d) i, HF/Py, THF, pyridine, 77%; ii, *N*-phenyltrifluoroacetimidoyl chloride, Cs_2CO_3, acetone, 98%. e) TBSOTf, 4Å MS, DMF, DCM, 0 °C, 32% (α/β > 10 : 1).

Inspired by the higher reactivity of 3,6-tethered glycosyl donors,[7] a second generation retrosynthetic analysis was designed (Figure 7), in which the key linear oligosaccharides **40** – **42** were planned to be synthesized from the corresponding oligosaccharides **43** – **45** after ring opening and selective benzoylation. The oligosaccharides **43** – **45** can be obtained from glycosylations using disaccharide acceptor **46** – **47** and D-AAT donor **30**. The two different disaccharides **46** – **47** could be synthesized by glycosylation of tethered galactose donor **40** and acceptor **27** or **28**. In this pathway, the stereoselectivity in the formation of **46** and **47** is

expected to be controlled by the bulky 3,6-silylidene group.

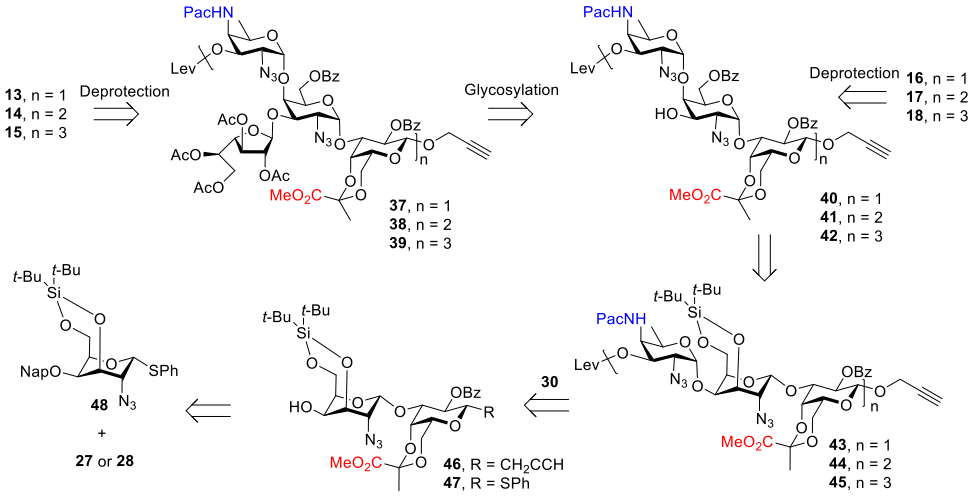


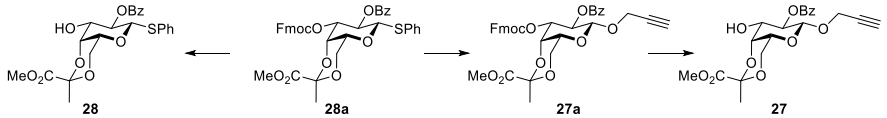
Figure 7. The second generation retrosynthetic analysis of the fragments **13** – **18**.

Experimental section

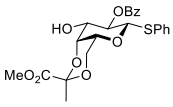
General experimental procedures

All reagents were of commercial grade and used as received. All moisture sensitive reactions were performed under an argon atmosphere. DCM used in the glycosylation reactions was dried with flamed 4Å molecular sieves before being used. Reactions were monitored by TLC analysis with detection by UV (254 nm) and where applicable by spraying with 20% sulfuric acid in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulfuric acid (aq.) followed by charring at ~150 °C. Flash column chromatography was performed on silica gel (40-63µm). ^1H and ^{13}C spectra were recorded on a Bruker AV 400 or Bruker AV 500 or Bruker AV 600 and Bruker AV 850 in $CDCl_3$ or D_2O. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard (^1H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hz. All ^{13}C spectra are proton decoupled. NMR peak assignments were made using COSY and HSQC experiments, where applicable Clean TOCSY, HMBC and GATED experiments were used to further elucidate the structure. The anomeric product ratios were analyzed through integration of proton NMR signals.

Experimental Procedures and Characterization Data of Products

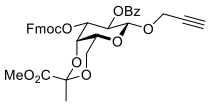


Phenyl 2-*O*-benzoyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-thio-β-D-galactopyranoside (28)



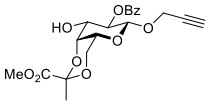
Phenyl 2-*O*-benzoyl-3-*O*-fluorenylmethyloxycarbonyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-thio-β-D-galactopyranoside **28a**[3] (4.93 g, 7,23 mmol, 1 eq) was dissolved in DCM and triethyl amine (60 mL, 434 mmol, 60 eq) is added and the solution stirred for four hours and thirty minutes. The solution is co-evaporated with toluene and concentrated *in vacuo*. The compound is purified by flash chromatography (PE/EA 3:1 - 1:1) to yield compound **28** (2.82 g, 6.03 mmol, 83%). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.11 – 8.05 (m, 2H, arom), 7.64 – 7.42 (m, 5H, arom), 7.32 – 7.23 (m, 3H, arom), 5.24 (t, *J* = 9.7 Hz, 1H, H-2), 4.78 (d, *J* = 9.8 Hz, 1H, H-1), 4.23 – 4.20 (m, 1H, H-4), 4.17 (dt, 1H, H-6), 4.04 – 3.97 (dt, 1H, H-6), 3.86 – 3.77 (m, 4H, OMe, H-3), 3.54 – 3.48 (m, 1H, H-5), 2.66 (d, *J* = 10.7 Hz, 1H, OH), 1.57 (s, 3H, Me,). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.08 (CO_2Me), 166.00 (Bz), 133.63, 133.23, 131.54, 129.94, 129.83, 128.74, 128.39, 128.25 (C_{arom}), 98.62 (C_{quat}), 85.23 (C-1),72.67 (C-3), 71.33 (C-4), 70.46 (C-2), 69.11 (C-5), 65.29 (C-6), 52.78 (C_{OMe}), 25.71 (C_{Me}).

Propynyl 2-*O*-benzoyl-3-*O*-fluorenylmethyloxycarbonyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-β-D-galactopyranoside (27a)

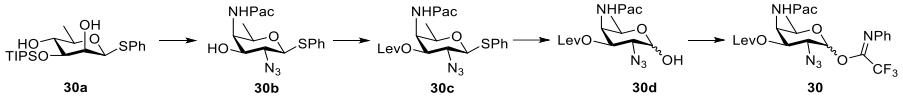


Compound **28a** (1.08 g, 1.59 mmol, 1 eq), diphenyl sulfoxide (0.41 g, 2.02 mmol, 1.27 eq) and 2,4,6-Tri-*tert*-butylpyrimidine (TTBP) (0.99 g, 3.99 mmol, 2.5 eq) were added to a flask and co-evaporated with toluene (3×) under an argon atmosphere. Dry DCM (3×) (36 mL) and molecular sieves (3Å) were added and the solution reduced to -60 °C. Triflic anhydride (Tf_2O) (0. 35 mL, 2.06 mmol, 1.30 eq) was added and the solution stirred for thirty minutes. Propynyl alcohol (0.27 mL, 4.76 mmol, 3 eq) was added and the solution allowed to warm to -40 °C. and stirred overnight. The reaction was quenched with sodium bicarbonate, diluted with ethyl acetate, and washed with water (1×) and brine (3×). The compound was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The column was purified by flash chromatography (PE/EA 5:1 - 1:1) to yield compound **27a** (0.80 g, 1.27 mmol, 80%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.11 – 8.04 (m, 2H), 7.85 – 7.79 (m, 2H), 7.71 – 7.45 (m, 5H), 7.42 – 7.33 (m, 2H), 7.25 (td, *J* = 7.5, 1.1 Hz, 1H), 7.15 (td, *J* = 7.5, 1.1 Hz, 1H), 5.60 (dd, *J* = 10.3, 8.0 Hz, 1H, H-2), 5.13 (dd, *J* = 10.4, 3.7 Hz, 1H, H-3), 5.07 (d, *J* = 8.0 Hz, 1H), 4.55 (dd, *J* = 3.8, 1.1 Hz, 1H, H-4), 4.46 – 4.34 (m, 3H, alkyn CH_2, Fmoc CH_2), 4.34 – 4.21 (m, 2H, Fmoc CH_2, H-5), 4.12 (dd, *J* = 12.9, 1.9 Hz, 1H, H-6), 4.03 (dd, *J* = 12.9, 1.7 Hz, 1H, H-6), 3.87 – 3.81 (m, 1H), 3.65 (s, 3H, OMe), 2.96 (t, *J* = 2.4 Hz, 1H), 1.53 (s, 3H). ^{13}C NMR (101 MHz, Acetone) δ 168.81, 163.84, 153.00, 140.14, 132.34, 128.65, 127.57, 126.81, 126.78, 126.18, 126.11, 124.15, 124.07, 118.99 (C_{arom}), 97.31 (C-1), 74.36 (C-3), 68.82 (Fmoc CH_2), 68.00 (C-4), 64.41, 63.81 (C-6), 54.30, 50.82 (OCH_3), 45.37, 24.22 (CH_3).

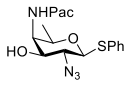
Propynyl 2-*O*-benzoyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-β-D-galactopyranoside (27)



compound **27a** (0.797 g, 1.27 mmol, 1 eq) was dissolved in DCM (12 mL) and triethyl amine (9 mL, 69 mmol, 55 eq) added and the solution stirred overnight. The reaction was co-evaporated with toluene and concentrated *in vacuo*. The compound was purified by column chromatography (PE/EA 3:1 - 1.5:1) to yield compound **27** (0.385 g, 0.94 mmol, 75%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.13 – 8.03 (m, 2H), 7.69 – 7.57 (m, 1H), 7.57 – 7.44 (m, 2H), 5.34 (dd, *J* = 9.9, 8.1 Hz, 1H, H-2), 4.88 (d, *J* = 8.0 Hz, 1H, H-1), 4.37 (d, *J* = 2.4 Hz, 2H, CH_2), 4.26 (dd, *J* = 3.7, 1.2 Hz, 1H, H-4), 4.18 (d, *J* = 8.4 Hz, 1H, 3-OH), 4.09 (dd, *J* = 12.9, 1.9 Hz, 1H, H-6), 4.05 – 3.94 (m, 2H, H-3, H-6), 3.78 (s, 3H, OMe), 3.68 (t, *J* = 1.6 Hz, 1H, H-5), 2.93 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, Acetone) 170.64 (CO_2Me), 165.84 (Bz), 133.55, 131.02, 130.03, 128.96, 99.07 (C-1), 98.96, 79.58, 76.14, 72.80 (C-2), 72.16 (C-4), 71.19 (C-3), 66.37 (C-5), 65.46 (C-6), 55.65, 52.49, 25.89.

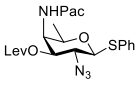


Phenyl 2-azido-6-deoxy-4-*N*-phenoxyacetimide-1-thio-β-D-galactopyranoside (30b)



Phenyl 6-deoxy-3-*O*-triisopropylsilyl-1-thio-β-D-mannopyranoside **30a** (7.9 g, 19.2 mmol, 1.0 eq) was dissolved in DCM (170 ml) with pyridine (20 mL, 250 mmol, 13.0 eq), then Tf_2O (19.3 mL, 115.2 mmol, 6.0 eq) was added to the reaction mixture at -10 ℃, and slowly warm up to 10 ℃ in 2 h. After TLC showed complete consumption of the starting material, the reaction mixture was diluted with DCM and washed with 1M HCl solution and saturated aqueous sodium bicarbonate. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in dry CH_3CN (250 mL), $TBAN_3$ (5.56 g, 19.6 mmol, 1.02 eq) solution in CH_3CN (25 mL) was slowly added to the reaction mixture at -30 ℃ and stirred one day. The reaction was warmed slowly to -20℃ and stir for additional 2 days. After TLC showed complete consumption of the starting material, 7N NH_3 in methanol (40 mL) was added in -20℃. The reaction was slowly warmed to 5 ℃ and stirred 3 days. After TLC showed complete consumption of the starting material, the mixture was concentrated *in vacuo*. The residue was dissolved in THF (190 mL) and water (95 mL), and then sodium bicarbonate (6.5 g, 76.8 mmol, 4.0 eq) was added and cooled to 0 ℃. After phenoxyacetyl chloride (PacCl) (5.3 mL, 38.4 mmol, 2.0 eq). the mixture was stirred for overnight at room temperature. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with EtOAc. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EA (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was dissolved in THF (190 mL) and AcOH (2.2 mL, 38.4 mmol, 2 eq). Then 1M TBAF in THF (39 mL, 39 mmol, 2 eq) was added in 0 ℃. The reaction mixture was stirred for overnight. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous ammonium chloride and diluted with EA. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EA (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 5:1:1 - 2:1:1) to yield compound **30b** (4.2 g, 10.2 mmol, 53%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.62 – 7.52 (m, 2H), 7.41 – 7.27 (m, 5H), 7.12 – 7.05 (m, 1H), 6.99 – 6.92 (m, 2H), 6.70 (d, *J* = 8.7 Hz, 1H, NH), 4.64 – 4.49 (m, 2H), 4.38 (d, *J* = 10.2 Hz, 1H, H-1), 4.32 – 4.24 (m, 1H, H-4), 3.84 – 3.72 (m, 2H, H-5, H-3), 3.02 (t, *J* = 9.9 Hz, 1H, H-2), 1.16 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.29 (Pac), 157.03 (Pac), 133.60, 131.23, 130.09, 129.27, 128.72, 122.73, 115.04, 86.48 (C-1), 75.19 (C-3), 73.61 (C-5), 67.43 (Pac), 62.65 (C-2), 53.41 (C-4), 17.17 (C-6).

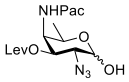
Phenyl 2-azido-6-deoxy-3-*O*-levulinoyl-4-*N*-phenoxyacetimide-1-thio-β-D-galactopyranoside (30c)



Compound **30b** (4.22 g, 10.18 mmol, 1.0 eq) was co-evaporated with anhydrous toluene three times under nitrogen and dissolved in DCM (100 mL). Reduced to 0 ℃, levulinic acid (3.3 g, 28.4 mmol, 2.8 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (3.15 g, 20.4 mmol, 2.0 eq) and 4-dimethylaminopyridine (DMAP) (250 mg, 2 mmol, 0.2 eq) were added. The reaction was stirred for overnight. The reaction was diluted with DCM and washed with saturated aqueous sodium bicarbonate and brine. The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 7:1:1 – 2:1:1) to yield compound **30c** (5.2 g, 10.2 mmol, 100%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.61 – 7.52 (m, 2H), 7.42 – 7.29 (m, 5H), 7.12 – 7.05 (m, 1H), 6.99 – 6.93 (m, 2H), 6.56 (d, *J* =

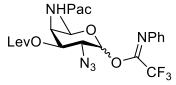
9.5 Hz, 1H, NH), 4.78 (dd, *J* = 10.2, 3.9 Hz, 1H, H-3), 4.64 – 4.48 (m, 2H), 4.47 – 4.37 (m, 2H, H-4, H-1), 3.86 – 3.76 (m, 1H, H-5), 3.04 (t, *J* = 10.2 Hz, 1H, H-2), 2.90 – 2.45 (m, 4H, Lev), 2.19 (s, 3H, Lev), 1.15 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.60 (Lev), 172.07 (Lev), 169.48 (Pac), 157.06 (Pac), 133.57, 131.10, 130.08, 129.35, 128.85, 122.71, 115.02, 86.73 (C-1), 75.00 (C-3), 73.68 (C-5), 67.47 (Pac), 59.65 (C-2), 49.99 (C-4), 37.91 (Lev), 29.92 (Lev), 27.94 (Lev), 16.95 (C-6). HR-MS: Calculated for $C_{25}H_{28}N_4O_6S$ [M+Na$^+$]: 535.1622, found: 535.1635. TLC: Rf = 0.5 (PE/EA = 1/1, v/v).

2-*N*-azido-6-deoxy-3-*O*-levulinoyl-4-*N*-phenoxyacetimide-α/β-D-galactopyranoside (30d)

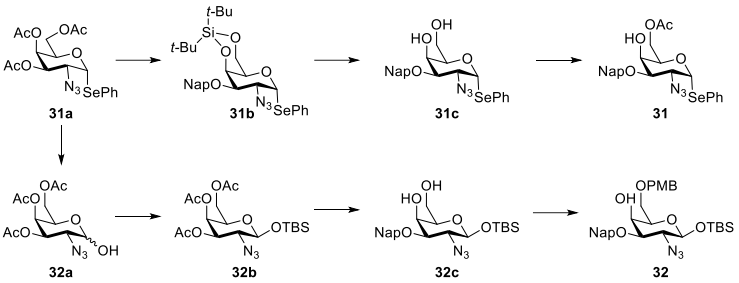


Compound **30c** (0.949 g, 1.85 mmol, 1 eq) was dissolved in DCM (10 mL) and reduced to 0 °C. NIS (0.626 g, 2.78 mmol, 1.5 eq) and TFA (0.17 mL, 2.22 mmol, 1.2 eq) were added and the solution stirred for 1 hour. NIS (0.2 g, 0.925 mmol, 0.5 eq) and TFA (0.01 mL, 1.3 mmol, 0.7 eq) were added and the solution stirred for a further 1 hour. The reaction was quenched with triethyl amine and sodium thiosulphate. The solution was diluted with DCM and washed with brine (3×). The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/DCM/EA 3:1:1 - 1:1:1) to yield the titled compound **30d** (0.61 g, 1.46 mmol, 79%). NMR assignment for the major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.45 – 7.22 (m, 2H, arom), 7.09 – 6.89 (m, 3H, arom), 6.84 (d, *J* = 9.5 Hz, 1H, NH), 6.34 (d, *J* = 4.9 Hz, 1H, OH), 4.76 (dd, *J* = 10.9, 4.0 Hz, 1H, H-3), 4.68 – 4.54 (m, 3H, H-1, Pac), 4.45 – 4.38 (m, 1H, H-4), 3.90 – 3.71 (m, 1H, H-5), 2.92 – 2.43 (m, 4H, Lev) , 2.17 (d, *J* = 1.5 Hz, 3H, Lev), 1.09 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.19 (Lev), 172.01 (Lev), 169.89 (Pac), 156.84, 129.89, 122.45, 114.86, 96.33 (C-1), 73.04 (C-3), 68.88 (C-5), 67.22 (Pac), 62.27 (C-2), 50.13 (C-4), 37.84 (Lev), 29.80 (Lev), 27.92 (Lev) , 16.55 (C-6).

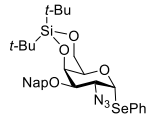
***N*-phenyl-trifluoroacetimidoyl 2-*N*-azido-3-*O*-levulinoyl-5-methyl-4-*N*-phenoxyacetimide-β-D-galactopyranoside (30)**



Compound hemiacetal **30d** (0.568 g, 1.35 mmol, 1.0 eq) was dissolved in acetone (13.5 mL) and reduced to 0 °C. *N*-phenyl trifluoroacetimidoyl chloride (0.41 g, 1.98 mmol, 1.47 eq) and cesium carbonate (0.527 g, 1.62 mmol, 1.2 eq) were added. The solution was allowed to warm to RT and stirred for overnight. The reaction was quenched with triethyl amine and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 5:1 - 1:1) to yield compound **30** (0.646 g, 1.09 mmol, 81%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.42 – 7.30 (m, 4H), 7.28 – 7.12 (m, 2H), 7.06 – 6.98 (m, 3H), 6.95 – 6.87 (m, 2H), 5.03 – 4.86 (m), 4.76 – 4.60 (m), 4.52 – 4.39 (m), 4.22 – 3.96 (m), 3.87 – 3.71 (m), 2.91 – 2.39 (m, 4H), 2.12 (s, 3H), 1.17 – 1.09 (m, 3H). ^{13}C NMR (126 MHz, Acetone) δ 204.33 (Lev), 171.90 (Lev), 169.56 (Pac), 158.47 (Pac), 143.84, 130.21, 130.19, 129.49, 125.15, 122.24, 122.21, 119.70, 115.38, 115.32, 96.67, 73.06, 71.06, 67.57, 60.86, 50.14, 37.88, 29.31, 28.34, 16.22 (C-6).

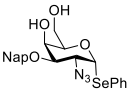


Phenyl 2-*N*-azido-4,6-*O*-di-*tert*-butylsilylidene-3-*O*-(2-methylnaphthyl)-1-seleno-β-D-galactopyranoside (31b)



Phenyl 3,4,6-*O*-acetyl-2-*N*-azido-1-seleno-β-D-galactopyranoside (**31a**) (9.4 g, 20 mmol, 1.0 eq) was dissolved in MeOH (40 mL). Sodium methoxide (25 wt. % in methanol) (0.5 mL, 2.2 mmol, 0.11 eq) was added at 0 ℃. The reaction was allowed to warm to RT and stirred for overnight. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with amberlite H^+, filtered and concentrated. The crude was dissolved in pyridine (100 mL) and then cooled to -20 ℃. Di-tert-butylsilyl ditriflate (6.5 mL, 20 mmol, 1.0 eq) was added. The mixture was warmed to RT slowly. After TLC showed complete consumption of the starting material, the reaction was quenched with MeOH and diluted with ethyl acetate. The solution was washed with 1M HCl, water and brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was dissolved in dry DMF (100 mL) and cooled to 0 °C. Sodium hydride (1.2 g, 30 mmol, 1.5 eq) and NapBr (8.8 g, 40 mmol, 2.0 eq) were added. After TLC showed complete consumption of the starting material, the reaction was quenched with MeOH and diluted with ethyl acetate. The mixture was washed with water and brine, then dried with $MgSO_4$, filtered, and evaporated to dryness. The compound was purified by flash chromatography (PE/EA 100:1 - 50:1) to yield compound **31b** (9.99 g, 16 mmol, 80%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 – 7.80 (m, 4H), 7.61 – 7.43 (m, 5H), 7.31 – 7.20 (m, 3H), 5.95 (d, *J* = 5.3 Hz, 1H, H-1), 4.95 – 4.82 (m, 2H, Nap), 4.59 (dd, *J* = 3.0, 1.1 Hz, 1H, H-4), 4.35 (dd, *J* = 10.2, 5.3 Hz, 1H, H-2), 4.21 (dd, *J* = 12.5, 2.1 Hz, 1H, H-6), 4.05 – 3.95 (m, 2H, H-5, H-6), 3.68 (dd, *J* = 10.3, 2.9 Hz, 1H, H-3), 1.06 (s, 9H, *t*-Bu), 1.04 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.25, 134.59, 133.38, 133.25, 129.25, 128.58, 128.52, 128.08, 128.07, 127.95, 127.86, 126.72, 126.31, 126.15, 125.91, 85.99 (C-1), 78.87 (C-3), 70.87 (Nap), 70.10 (C-5), 69.46 (C-4), 67.09 (C-6), 59.83 (C-2), 27.74, 27.43, 23.52, 20.87.

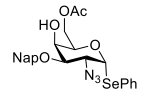
Phenyl 2-*N*-azido-3-*O*-(2-methylnaphthyl)-1-seleno-β-D-galactopyranoside (31c)



Compound **31b** (2.5 g, 4 mmol, 1.0 eq) was dissolved in anhydrous THF (20 mL). Then 1M TBAF in THF (8 mL, 8 mmol, 2.0 eq) was added in 0 ℃. The reaction mixture was stirred at RT. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous ammonium chloride and diluted with EA. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EA (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 3:1 - 2:1) to yield compound **31c** (1.75 g, 3.91 mmol, 98%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.98 – 7.85 (m, 4H), 7.68 – 7.59 (m, 3H), 7.55 – 7.45 (m, 2H), 7.34 – 7.25 (m, 3H), 6.04 (d, *J* = 5.3 Hz, 1H, H-1), 5.01 (d, *J* = 11.7 Hz, 1H, Nap), 4.86 – 4.79 (m, 1H, Nap), 4.53 – 4.46 (m,

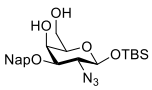
1H, H-4), 4.45 – 4.36 (m, 2H, 4-OH, H-2), 4.29 – 4.19 (m, 1H, H-5), 3.93 – 3.77 (m, 3H, 6-OH, H-6, H-3), 3.71 (dd, *J* = 10.7, 5.9 Hz, 1H, H-6). ^{13}C NMR (101 MHz, Acetone) δ 136.66, 135.40, 134.20, 133.90, 129.85, 129.82, 128.73, 128.66, 128.49, 128.39, 127.18, 126.95, 126.74, 126.73, 86.69 (C-1), 80.02 (C-3), 74.54 (C-5), 71.20 (Nap), 65.87 (C-4), 61.92 (C-6), 61.48 (C-2).

Phenyl 6-*O*-acetyl-2-*N*-azido-3-*O*-(2-methylnaphthyl)-1-seleno-β-D-galactopyranoside (31)



Compound **31c** (1.75 g, 3.95 mmol, 1 eq) was dissolved in CH_3CN (20 mL) and cooled to 0 °C. 2-Aminoethyl diphenylborinate (188 mg, 0.8 mmol, 0.2 eq), DIPEA (1.36 mL, 7.82 mmol, 2.0 eq) and acetyl chloride (0.4 mL, 5.48 mmol, 1.4 eq) were added. The reaction was stirred and warmed slowly to RT. After analysis by TLC showed complete consumption of the starting material, diluted with EtOAc, and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 4:1 – 3/1) to yield compound **31** (1.71 g, 3.5 mmol, 89%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.98 – 7.94 (m, 1H), 7.94 – 7.86 (m, 3H), 7.68 – 7.59 (m, 3H), 7.55 – 7.47 (m, 2H), 7.35 – 7.29 (m, 3H), 6.08 (d, *J* = 5.3 Hz, 1H, H-1), 5.05 – 4.98 (m, 1H, Nap), 4.88 – 4.80 (m, 1H, Nap), 4.55 – 4.36 (m, 4H, H4-OH, H-5, H-4, H-2), 4.32 – 4.22 (m, 2H, H-6), 3.83 (dd, *J* = 10.4, 2.9 Hz, 1H, H-3), 1.93 (s, 3H, OAc). ^{13}C NMR (101 MHz, Acetone) δ 170.80 (OAc), 136.61, 135.36, 134.23, 133.95, 129.86, 129.59, 128.77, 128.69, 128.52, 128.48, 127.28, 127.00, 126.78, 86.02 (C-1), 79.76 (C-3), 72.04 (C-5), 71.47 (Nap), 66.07 (C-4), 64.30 (C-6), 61.27 (C-2), 20.76 (OAc).

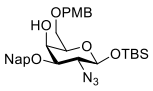
***Tert*-butyldiphenylsilyl 2-*N*-azido-3-*O*-(2-methylnaphthyl)-β-D-galactopyranoside (32c)**



Tert-butyldimethylsilyl 3,4,6-*O*-acetyl-2-*N*-azido-β-D-galactopyranoside (32b)[8] (6.9 g 15.5 mmol, 1.0 eq) was dissolved in methanol (130 mL) and DCM (10 mL) and cooled to 0 °C. Sodium methoxide (0.1 mL, 0.03 eq) was added and the solution allowed to warm to RT and stirred for 3 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched by amberlite H^+, filtered, and evaporated directly. The crude was dissolved in ACN (62 mL) and anisaldehyde dimethyl acetal ($PMPCH(OMe)_2$) (3.8 mL, 22.2 mmol, 1.4 eq) and TsOH (0.26 g, 1.51 mmol, 0.1 eq) were added. The reaction stirred at 30 °C, 130 mbar for 1 hour with excess ACN was added once solvent had evaporated after 20 minutes. This resulted in a purple solution. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethylamine, co-evaporated with toluene and concentrated *in vacuo*. The crude was dissolved in DMF (23 mL) and reduced to 0 °C. 2-(bromomethyl)naphthalene (NapBr) (6.85 g, 31 mmol, 2eq) and sodium hydride (1.24 g, 31 mmol, 2.0 eq) was slowly added, and the solution stirred overnight. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched by slowly adding ethyl acetate, methanol and finally water. The reaction was diluted with ethyl acetate and washed with brine (2×). The solution was dried with $MgSO_4$, filtered, co-evaporated with toluene and concentrate *in vacuo*. The crude was dissolved in acetic acid in water solution (450 mL, 73%) and stirred overnight at 40 °C. After analysis by TLC showed complete consumption of the starting material, the reaction was extracted into ethyl acetate (2×), washed with water and brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The column was purified by

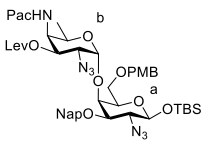
flash chromatography (PE/DCM/EA 10:10:1 - 2:1:1) to yield compound **32c** (1.21 g, 3.1 mmol, 20% over 4 steps,). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 – 7.73 (m, 4H), 7.60 – 7.40 (m, 3H), 4.86 (s, 2H, Nap), 4.45 (d, *J* = 7.7 Hz, 1H, H-1), 4.03 – 3.86 (m, 2H, H-4, H-6), 3.83 – 3.73 (m, 1H, H-6), 3.60 (dd, *J* = 10.1, 7.7 Hz, 1H, H-2), 3.46 – 3.35 (m, 1H, H-5), 3.29 (dd, *J* = 10.2, 3.3 Hz, 1H, H-3), 0.94 (s, 9H, TBS), 0.15 (s, 6H, TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.79, 133.41, 133.39, 128.81, 128.16, 128.00, 127.22, 126.60, 126.49, 125.95, 97.54 (C-1), 78.74 (C-3), 74.51 (C-5), 72.54 (Nap), 66.46 (C-4), 65.41 (C-2), 62.55 (C-6), 25.85 (TBS), -3.93 (TBS), -4.88 (TBS).

***Tert*-butyldiphenylsilyl 2-*N*-azido-6-*p*-methoxybenzyl-3-*O*-(2-methylnaphthyl)-β-D-galactopyranoside (32)**



Compound **32c** (295 mg, 0.64 mmol, 1 eq) was dissolved in ACN (4.0 mL) forming a cloudy mixture. Potassium iodide (KI) (107 mg, 0.64 mmol, 1 eq), potassium carbonate (K_2CO_3) (97 mg, 0.704 mmol, 1.1 eq), 2-aminoethyl diphenylborinate (30 mg, 0.13 mmol, 0.2 eq) and 4-methoxybenzyl chloride (0.13 mL, 0.96 mmol, 1.5 eq) were added. The reaction was heated to 60 °C and stirred for 24 hours. The reaction turns yellow/orange after 3-4 hours but remains cloudy. After 24 hours the solution had a red/brown color, and everything had dissolved. A yellow solid was stuck to the side of the flask. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with water, which dissolved the yellow solid. The reaction was diluted with ethyl acetate and washed with brine (3×). The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 4:1) to yield compound **32** (330 mg, 0.57 mmol, 89%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.95 – 7.83 (m, 4H, Nap), 7.59 (dd, *J* = 8.4, 1.7 Hz, 1H, Nap), 7.54 – 7.46 (m, 2H Nap), 7.31 – 7.24 (m, 2H, PMB), 6.92 – 6.83 (m, 2H, PMB), 4.94 (d, *J* = 12.1, 0.8 Hz, 1H, Nap), 4.78 (d, *J* = 12.1, 0.9 Hz, 1H, Nap), 4.60 (d, *J* = 7.6 Hz, 1H, H-1), 4.48 (s, 2H, PMB), 4.24 – 4.15 (m, 1H, H-4), 4.11 (d, *J* = 4.0, 0.9 Hz, 1H, 4-OH), 3.78 (s, 3H, PMB), 3.77 – 3.68 (m, 2H, H-5, H-6), 3.68 – 3.57 (m, 2H, H-2, H-6), 3.49 (dd, *J* = 10.3, 3.1 Hz, 1H, H-3), 0.95 (s, 9H, TBS), 0.17 (s, 6H, TBS). ^{13}C NMR (101 MHz, Acetone) δ 206.20, 160.14 (PMB), 136.98 (Nap), 134.23 (Nap), 133.94 (Nap),, 131.57 (PMB), 130.64 (PMB), 129.93, 128.74, 128.67, 128.51, 127.13, 126.96, 126.76, 126.71, 114.40, 97.79 (C-1), 80.22 (C-3), 74.53 (C-5), 73.38 (PMB), 71.67 (Nap), 70.01 (C-6), 66.68 (C-2), 65.84 (C-4), 55.48 (PMB), 26.06 (TBS), -3.87 (TBS), -4.94 (TBS).

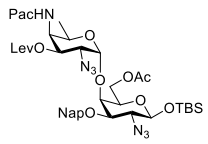
***Tert*-butyldiphenylsilyl 4-*O*-(2-*N*-azido-3-*O*-levulinoyl-5-methyl-4-*N*-phenoxyacetimide-α-D-galactopyranoside) 2-*N*-azido-6-*O*-*p*-methoxybenzyl-3-*O*-(2-methylnaphthyl)-β-D-galactopyranoside (33)**



Donor **30** (0.489 g, 0.84 mmol, 1.22 eq) and acceptor **32** (0.408 g, 0.69 mmol, 1 eq) were co-evaporated with toluene (3×) and placed under an argon atmosphere. Dry DCM (18 mL) and molecular sieves (4Å) were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and a solution of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (0.0365 g, 0.138 mmol, 0.2 eq) in dry DCM (0.1 mL), dried with molecular sieves (4Å), was added. The solution was stirred for 24 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with $NaHCO_3$ and diluted with DCM. The solution was washed with water (2×) and brine (3×). The aqueous layer was extracted with DCM (3×), dried with $MgSO_4$, filtered, and

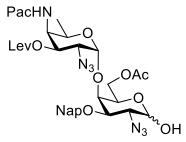
concentrated *in vacuo*. The compound was purified first by size exclusion (DCM/MeOH 1:1) and then flash chromatography (PE/EA 8:1 - 2:1 - PE/DEA 2:1:1) to yield compound **33** (0.460 g, 0.469 mmol, 68%).[1]H NMR (400 MHz, Acetone-d6) δ 7.98 – 7.94 (m, 1H, Nap), 7.93 – 7.84 (m, 3H), 7.63 (dd, *J* = 8.5, 1.6 Hz, 1H, Nap), 7.54 – 7.45 (m, 2H), 7.36 – 7.28 (m, 4H), 7.07 – 6.97 (m, 4H, Pac), 6.97 – 6.91 (m, 2H, PMB), 5.35 (dd, *J* = 11.4, 3.9 Hz, 1H, H-3b), 5.10 (d, *J* = 3.9 Hz, 1H, H-1b), 5.02 (d, *J* = 12.9 Hz, 1H, Nap), 4.91 – 4.81 (m, 2H, H-5b, Nap), 4.69 – 4.57 (m, 3H, H-1a, Pac), 4.56 -4.44 (m, 3H, H-4b, PMB) 4.29 (d, *J* = 2.9 Hz, 1H, H-4a), 3.94 (t, *J* = 8.7 Hz, 1H, H-6a), 3.81 – 3.78 (m, 4H, H-2a, PMB), 3.77 – 3.70 (m, 1H, H-5a), 3.70 – 3.60 (m, 2H, H-2b, H-6a), 3.50 (dd, *J* = 10.7, 2.9 Hz, 1H, H-3a), 2.88 – 2.42 (m, 4H, Lev), 2.12 (s, 3H, Lev), 0.96 (s, 9H, TBS), 0.79 (d, *J* = 6.4 Hz, 3H, C-6b), 0.18 (s, 6H, TBS). [13]C NMR (101 MHz, Acetone) δ 206.10 (Lev), 172.25 (Lev), 169.48 (Pac), 160.15 (PMB), 158.43 (Pac), 136.59, 134.03, 133.74, 130.82, 130.34, 130.17, 128.68, 128.54, 128.38, 126.85, 126.79, 126.59, 126.24, 122.41, 115.45, 114.38, 99.46 (C-1b), 97.88 (C-1a), 78.99 (C-3a), 73.41 (C-5a), 73.31 (C-4a), 73.26 (PMB), 72.70 (Nap), 70.54 (C-3b), 67.81 (Pac), 67.67 (C-6b), 66.74 (PMB), 65.30 (C-2a), 58.73 (C-5b), 55.43 (C-2b), 51.24 (C-4b), 38.09 (Lev), 29.56 (Lev), 28.62 (Lev), 25.98 (TBS), 16.59 (C-6b), -3.95 (TBS), -4.90 (TBS).

***Tert*-butyldiphenylsilyl 4-*O*-(2-*N*-azido-3-*O*-levulinoyl-5-methyl-4-*N*-phenoxyacetimide-α-D-galactopyranoside) 6-*O*-acetyl-2-*N*-azido-3-*O*-(2-methylnaphthyl)-β-D-galactopyranoside (34)**



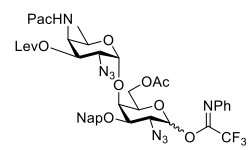
Compound **33** (115 mg, 0.12 mmol, 1.0 eq) and triethylsilane were dissolved in DCM (1.2 mL) and hexafluoro-*iso*-propanol (HFIP) (1.2 mL). Then 0.1M HCl in HFIP (0.12 mL, 0.012 mmol, 0.1 eq) was added to the mixture. The reaction was stirred at rt for 30 mins. After analysis by TLC showed complete consumption of the starting material, then the mixture was diluted with DCM and the reaction quenched with saturated Na_2HCO_3. The organic phase was washed with water and brine, dried with MgSO4, filtered, and concentrated *in vacuo*. The crude was dissolved in the pyridine (1.0 mL) and put in the ice bath. DMAP (1.5 mg, 0.012 mmol, 0.1 eq) and Ac_2O (0.2 mL) were added. The reaction was stirred for overnight. After analysis by TLC showed complete consumption of the starting material, the reaction was concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 3:1 - 2:1) to yield compound **34** (101 mg, 0.115 mmol, 96%). [1]H NMR (400 MHz, Acetone-d_6) δ 7.99 – 7.93 (m, 1H), 7.93 – 7.84 (m, 3H), 7.62 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.36 – 7.27 (m, 2H), 7.06 (d, *J* = 9.7 Hz, 1H), 7.02 – 6.94 (m, 3H), 5.30 (dd, *J* = 11.4, 4.0 Hz, 1H), 5.09 (d, *J* = 3.9 Hz, 1H), 5.06 – 4.99 (m, 1H), 4.88 (dd, *J* = 12.7, 0.9 Hz, 1H), 4.84 – 4.76 (m, 1H), 4.69 – 4.54 (m, 3H), 4.53 – 4.45 (m, 2H), 4.38 – 4.28 (m, 2H), 3.83 – 3.74 (m, 3H), 3.55 (dd, *J* = 10.7, 2.9 Hz, 1H), 2.86 – 2.40 (m, 4H), 2.12 (s, 3H), 2.01 (s, 3H), 0.94 (s, 9H), 0.81 (d, *J* = 6.4 Hz, 3H), 0.19 – 0.14 (m, 6H). [13]C NMR (101 MHz, Acetone) δ 205.18, 171.34, 169.51, 168.62, 157.58, 135.66, 133.14, 132.88, 129.41, 127.77, 127.64, 127.47, 126.01, 125.94, 125.69, 125.45, 121.48, 114.56, 99.18, 96.94, 78.12, 73.92, 72.31, 71.75, 69.94, 66.92, 65.57, 64.71, 62.19, 58.26, 50.32, 37.17, 28.59, 27.73, 25.02, 19.73, 15.60, -5.02, -5.94.

4-*O*-(2-*N*-azido-3-*O*-levulinoyl-5-methyl-4-*N*-phenoxyacetimide-α-D-galactopyranoside) 6-*O*-acetyl-2-*N*-azido-3-*O*-(2-methylnaphthyl)-α/β-D-galactopyranoside (35a)



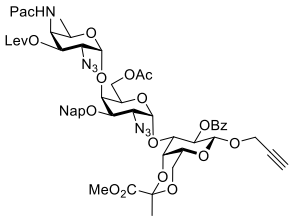
Compound **34** (100 mg, 0.111 mmol, 1.0 eq) was dissolved in THF (1.0 mL) and pyridine (2.0 mL), then cooled to 0 °C. Hydrogen fluoride (HF)/pyridine (70%) (0.2 mL) was added dropwise. The solution was stirred for overnight. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate slowly and diluted with EtOAc. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EtOAc (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 3:2 - 1:1) to yield compound **35a** (67 mg, 0.085 mmol, 77%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.96 (d, *J* = 6.2 Hz, 1H), 7.93 – 7.83 (m, 3H), 7.65 – 7.58 (m, 1H), 7.54 – 7.44 (m, 2H), 7.36 – 7.27 (m, 2H), 7.11 – 7.04 (m, 1H), 7.03 – 6.95 (m, 3H), 6.43 – 6.00 (m, 1H), 5.41 – 5.33 (m, 0.6H), 5.30 – 5.21 (m, 1H), 5.16 – 5.01 (m, 2H), 4.91 – 4.82 (m, 1H), 4.78 – 4.55 (m, 3.4H), 4.52 – 4.43 (m, 1.6H), 4.43 – 4.33 (m, 2H), 4.31 – 4.23 (m, 1H), 4.13 (dd, *J* = 10.9, 2.7 Hz, 0.6H), 3.91 – 3.72 (m, 2.4H), 3.58 (dd, *J* = 10.7, 2.8 Hz, 0.4H), 2.87 – 2.75 (m, 1H), 2.75 – 2.64 (m, 1H), 2.63 – 2.50 (m, 1H), 2.50 – 2.39 (m, 1H), 2.14 – 2.10 (m, 3H), 2.03 – 1.98 (m, 3H), 0.84 – 0.72 (m, 3H). ^{13}C NMR (126 MHz, Acetone) δ 206.35, 172.42, 172.39, 170.66, 170.63, 169.76, 158.59, 136.70, 136.66, 134.19, 133.88, 130.43, 130.42, 128.82, 128.76, 128.65, 128.47, 126.99, 126.94, 126.87, 126.68, 126.45, 126.37, 122.50, 122.49, 115.57, 100.14, 100.07, 97.27, 93.00, 79.74, 76.09, 75.78, 75.18, 72.98, 72.72, 72.25, 71.41, 71.01, 68.94, 67.91, 65.92, 65.73, 63.11, 61.05, 59.63, 59.45, 51.36, 38.15, 29.59, 28.72, 28.71, 20.75, 20.72, 16.57.

***N*-phenyl-trifluoroacetimidoyl 4-*O*-(2-*N*-azido-3-*O*-levulinoyl-5-methyl-4-*N*-phenoxyacetimide-α-D-galactopyranoside) 6-*O*-acetyl-2-*N*-azido-3-*O*-(2-methylnaphthyl)-α/β-D-galactopyranoside (35)**



Compound hemiacetal **35a** (65 mg, 0.0823 mmol, 1.0 eq) was dissolved in acetone (2.0 mL) and reduced to 0 °C. *N*-phenyl trifluoroacetimidoyl chloride (36 mg, 0.17 mmol, 2.0 eq) and cesium carbonate (28 mg, 0.085 mmol, 1.0 eq) were added. The solution was allowed to warm to RT and stirred for overnight. The reaction was quenched with triethyl amine, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 1:1 - 1:1) to yield compound **35** (78 mg, 0.081 mmol, 99%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.02 – 7.95 (m, 1H), 7.94 – 7.83 (m, 3H), 7.69 – 7.58 (m, 1H), 7.55 – 7.46 (m, 2H), 7.41 – 7.24 (m, 4H), 7.17 – 7.09 (m, 1H), 7.08 – 6.95 (m, 3H), 6.95 – 6.85 (m, 2H), 5.31 (dd, *J* = 11.4, 4.0 Hz), 5.24 (dd, *J* = 11.3, 4.0 Hz), 5.20 – 5.05 (m), 4.96 – 4.80 (m), 4.78 – 4.68 (m), 4.69 – 4.54 (m), 4.53 – 4.38 (m), 4.33 – 4.12 (m), 3.90 – 3.75 (m), 2.87 – 2.64 (m, 2H), 2.61 – 2.39 (m, 2H), 2.16 – 2.10 (m), 2.10 – 2.07 (m), 2.01 – 1.97 (m), 0.85 – 0.74 (m, 3H). ^{13}C NMR (126 MHz, Acetone) δ 206.20, 172.36, 170.54, 170.52, 169.68, 169.66, 158.64, 158.62, 136.34, 134.22, 134.19, 133.96, 133.93, 130.46, 130.44, 129.73, 129.69, 128.86, 128.69, 128.66, 128.50, 127.24, 127.00, 126.91, 126.78, 126.76, 126.50, 126.28, 125.24, 122.51, 122.47, 119.92, 115.61, 115.60, 115.57, 100.38, 100.30, 79.60, 76.97, 75.20, 74.61, 74.23, 72.80, 72.43, 72.36, 71.30, 71.24, 71.17, 67.98, 67.95, 67.82, 65.94, 65.90, 62.91, 62.75, 62.65, 59.80, 59.57, 59.50, 55.43, 51.38, 51.31, 38.20, 38.15, 29.84, 29.59, 28.77, 28.73, 20.70, 20.66, 16.61, 16.54.

Propynyl 3-*O*-(6-*O*-acetyl-2-azido-4-*O*-(2-azido-3-*O*-levulinoyl-6-deoxy-4-*N*-phenoxyacetimide-α-D-galactopyranoside)-3-*O*-napthylmethyl-β-D-galactopyranoside)-2-*O*-benzoyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-β-D-galactopyranoside (25)



Donor **35** (92 mg, 0.098 mmol, 1 eq) and acceptor **27** (81 mg, 0.195 mmol, 2.0 eq) were co-evaporated with toluene (3×) and placed under an argon atmosphere. Dry DCM (2 mL) and 4Å molecular sieves were added and the solution stirred for 30 minutes before being reduced to - 70 °C. DMF (121 μL, 1.57 mmol, 16 eq) and TBSOTf (11.3 μL, 0.049 mmol, 0.5 eq) were added to the reaction. The solution was warmed to rt and stirred for overnight. The reaction was quenched with $NaHCO_3$, washed with water (2×) and brine (3×). The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 2:1 - 1:1) to yield compound **25** (37 mg, 0.031 mmol, 32%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.99 – 7.84 (m, 6H), 7.66 – 7.58 (m, 1H), 7.55 – 7.38 (m, 5H), 7.35 – 7.24 (m, 2H), 7.05 – 6.91 (m, 4H), 5.54 – 5.46 (m, 1H), 5.39 (d, *J* = 2.8 Hz, 1H), 5.19 (dd, *J* = 11.3, 3.9 Hz, 1H), 4.99 – 4.80 (m, 3H), 4.67 – 4.49 (m, 5H), 4.43 – 4.32 (m, 4H), 4.22 – 4.08 (m, 4H), 4.06 – 3.98 (m, 1H), 3.98 – 3.90 (m, 1H), 3.87 – 3.74 (m, 6H), 3.73 – 3.63 (m, 2H), 2.81 – 2.62 (m, 2H), 2.57 – 2.32 (m, 2H), 2.11 (s, 3H), 1.94 (s, 3H), 1.54 (s, 3H), 0.68 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (126 MHz, Acetone) δ 172.35, 171.06, 170.23, 169.64, 165.50, 158.63, 136.48, 134.26, 134.03, 133.97, 130.86, 130.46, 130.44, 130.38, 130.30, 130.25, 129.39, 128.85, 128.81, 128.71, 128.69, 128.51, 126.97, 126.93, 126.90, 126.73, 126.32, 122.51, 115.63, 115.60, 100.02, 99.44, 99.36, 94.77, 76.47, 76.20, 75.60, 74.13, 73.00, 70.84, 70.81, 69.75, 67.95, 67.57, 66.60, 65.84, 65.76, 62.28, 59.75, 59.32, 56.02, 52.86, 51.30, 38.14, 28.70, 26.03, 20.79, 16.50.

Reference

[1] H. Baumann, A. O. Tzianabos, J. R. Brisson, D. L. Kasper and H. J. Jennings, *Biochemistry* **1992**, *31*, 4081-4089.

[2] L. J. van den Bos, T. J. Boltje, T. Provoost, J. Mazurek, H. S. Overkleeft and G. A. van der Marel, *Tetrahedron Lett.* **2007**, *48*, 2697-2700.

[3] R. Pragani and P. H. Seeberger, *J. Am. Chem. Soc.* **2011**, *133*, 102-107.

[4] P. Eradi, S. Ghosh and P. R. Andreana, *Org. Lett.* **2018**, *20*, 4526-4530.

[5] L. Wang, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codée, *J. Am. Chem. Soc.* **2018**, *140*, 4632-4638.

[6] a) L. Wang, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codée, *Eur. J. Org. Chem.* **2019**, *2019*, 1994-2003; b) L. Wang, F. Berni, J. Enotarpi, H. S. Overkleeft, G. van der Marel and J. D. C. Codée, *Org. Biomol. Chem.* **2020**, *18*, 2038-2050; c) L. Wang in *Reagent Controlled Synthesis of 1,2-cis-Oligosaccharides*, *Vol. PhD* Leiden University, **2020**.

[7] M. Heuckendorff, C. M. Pedersen and M. Bols, *J. Org. Chem.* **2013**, *78*, 7234-7248.

[8] R. Pragani, P. Stallforth and P. H. Seeberger, *Org. Lett.* **2010**, *12*, 1624-1627.