Bacterial glycomimetics: synthesis and applications

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Synthesis and biological evaluation of oligomers mimicking *Enterococcus faecalis* Diheteroglycan capsular polysaccharide

Introduction

Enterococcal bacteria are a major cause of hospital-acquired infections,[1] and enterococci are considered a major health threat because of their growing resistance against antibiotics such as vancomycin, linezolid, tigecycline and daptomycin.[2,3] Together with the slow development of new antibiotics this has led to the need for alternative treatments to fight bacterial infections.[4,5] Passive or active vaccination presents a potential solution to this issue. To this end glycoconjugate vaccines may be developed which can be based on the conjugation of bacterial polysaccharides such as teichoic acids, capsular polysaccharides (CPS), exopolysaccharides or lipopolysaccharides to a carrier protein.[6,7] *E. faecalis* is a commensal Gram-positive bacterium that inhabits the gastrointestinal tract of humans and other mammals. Because of the limited number of serotypes known for this bacterium, the development of a glycoconjugate vaccine is an attractive goal.[8,9,10] As an opportunistic pathogen it is generally poorly virulent, but it can cause severe infections and *E. faecalis* is one of the most important sources of nosocomial infections, causing septic shock, urinary tract infections, peritonitis and endocarditis.[11] Four different serotypes have been discovered (CPS A-D), based on immunoreactivity of antisera raised against prototype strains.[12, 13, 14] It has been observed that antibodies raised against serotypes A and B are not able to induce opsonization of serotypes C and D. These antibodies are directed towards lipoteichoic acid (LTA) cell-wall components, which are masked by a CPS in serotypes C and D.

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The type C and D CPS are constituted by two different polysaccharides named tetraheteroglycan (THG) and diheteroglycan (DHG). THG is composed of a mixture of rhamnose, glucose, glucosamine, galactosamine, ribitol and phosphate, but its exact structure has not been elucidated. DHG is constituted by glucose and galactose with acetyl and lactic acid appendages.[15, 16] Recently Theilacker *et al.* elucidated the DHG structure, showing the repeating unit to be →6)-β-Galf-(1→3)-β-D-Glcp-(1→ with partial *O*-acetylation at position 5 and lactic acid substitution at position 3 of the Galf residue (Figure 1). The lactic acid appendage was shown to have the *R* configuration by Krylov *et al*. DHG was proposed as a good target for the development of a glycoconjugate vaccine, since rabbit antiserum against this oligosaccharide showed protective properties against *E. faecalis* serotypes C and D.[10, 17]

However, extraction and purification of the saccharide from biological sources leads to microheterogeneous mixtures making it difficult to study the structure-immunogenicity relationships. A synthetic approach can deliver well-defined structures, of a pre-determined length, which can be used to generate homogeneous glycoconjugate vaccines. This chapter deals with the solution phase synthesis and the biological evaluation of a set of oligosaccharides based on the backbone of the DHG polysaccharide of *E. faecalis.* Besides the synthetic approach was adapted for the development of fully automated solid phase synthesis.

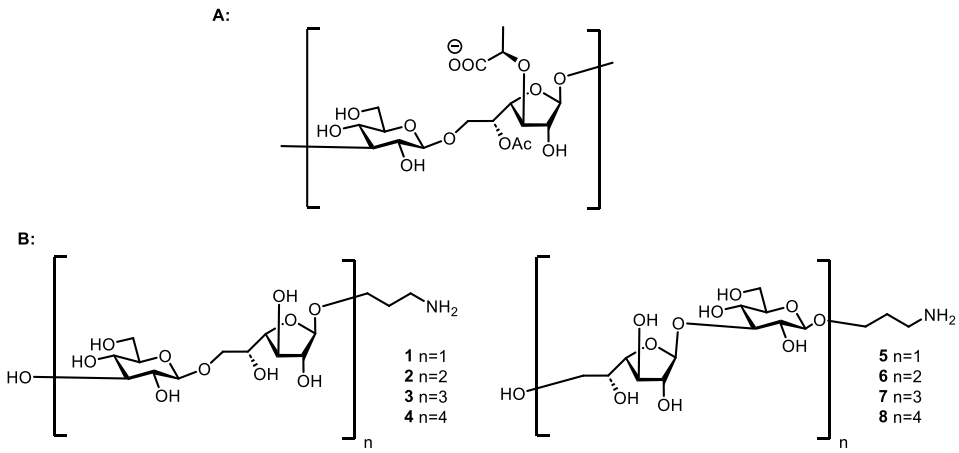


Figure 1. ***A:*** *Diheteroglycan repeating unit of E. faecalis;* ***B:*** *Synthetic oligomers.*

Results and discussion

As the DHG is built up from disaccharide repeats two types of oligosaccharides may be generated, based on [Glc*p*-Gal*f*] or [Gal*f*-Glc*p*] dimer repeating unit, respectively (Figure 1). In collaboration with the group of Nifantiev the compounds shown in Figure 1 were

targeted and this Chapter describes the synthesis of DHG oligosaccharides **1**-**4**, varying in length from one to four disaccharide residues and equipped with a propylamine spacer for conjugation purposes.[18]

It was projected to assemble target oligomers **1**-**4** by iterative [2+2] couplings, starting from dimer **9** having an azidopropanol spacer at the reducing end and key dimer **10** (Figure 2). In these dimers the benzoyl esters (Bz) and silylidene ketals function as permanent protecting groups while the levulinic (Lev) ester is used to temporary mask the C-3-OH of the glucosyl moiety. Key dimer **10** can be obtained from monosaccharide building blocks **11** and **12**.

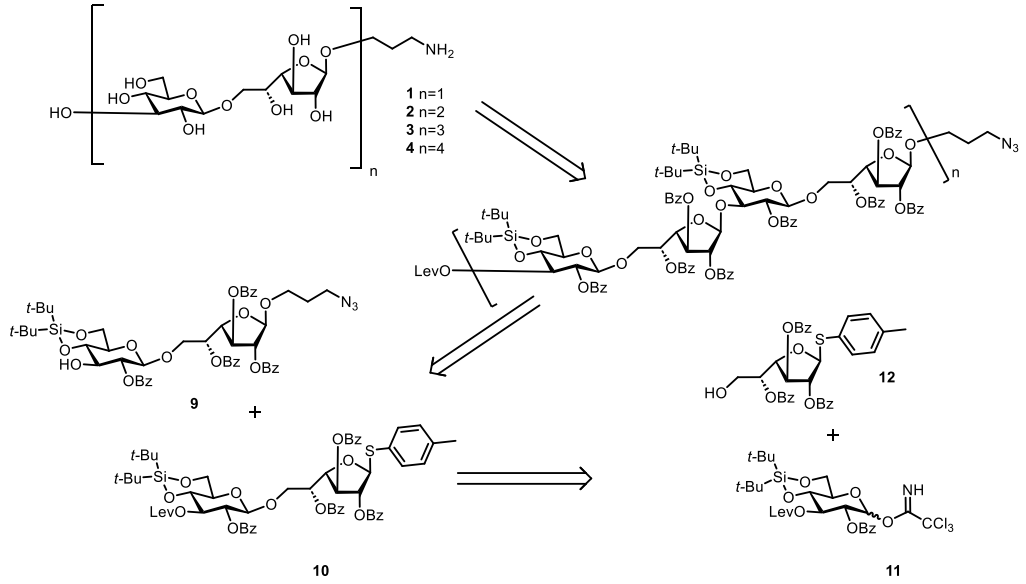
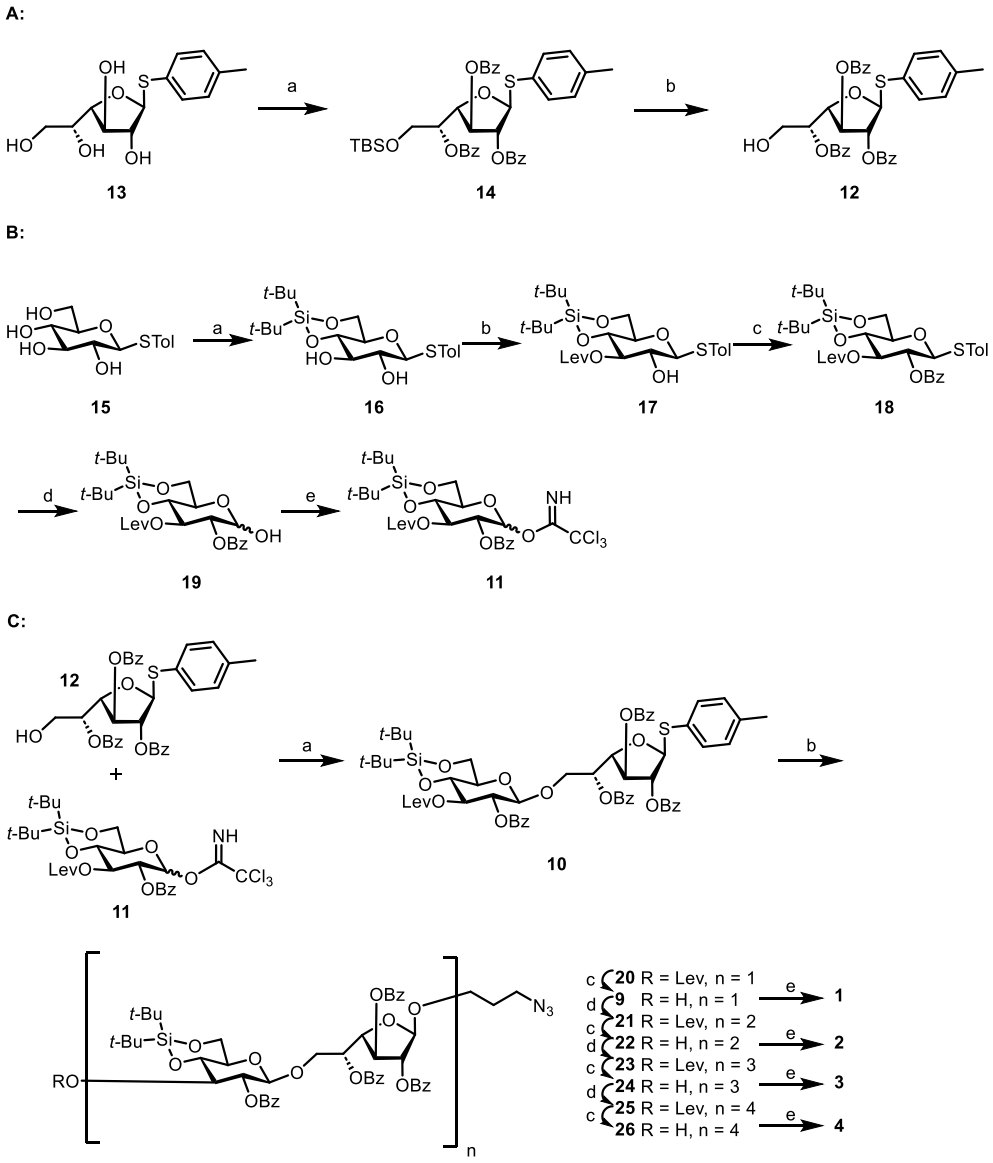


*Figure 2. Retrosynthetic analysis of target oligosaccharides **1**-**4**.*

For the synthesis of Galf building block **12** a known procedure, starting from commercially available D-(+)-galactose was employed to achieve thioglycoside **13** (Scheme 1).[19, 20] A one-pot two-step procedure, comprising the regioselective protection of the C-6-OH with a *tert*-butyldimethylsilyl ether (TBS) using TBSCl and catalytic amount of DMAP in a mixture of Py/DMF at 0°C, followed by the addition of BzCl to mask the remaining hydroxyls, provided fully protected **14** in 74% yield. In order to remove the TBS group, **14** was dissolved in 80% AcOH /ACN, heated to 40°C and stirred overnight giving building block **12** in 80% yield. This method to remove the silyl group was preferred over the more often used conditions using a fluoride source to avoid any migration of the C-5-benzoate to the liberated primary alcohol.

The synthesis of glucose building block **11** started with compound **15**, which was treated with di-*tert*-butylsilyl-*bis*-triflate in DMF at -40°C to regioselectively protect both the C-



Scheme 1. ***A:*** *Synthesis of Galf building block* ***12*** *a) (i) TBSCl, DMAP, DMF/Pyr (1:1), 0°C, then add BzCl, 0°C to r.t., 74%; b) AcOH (80%), ACN, 40°C, 80%.* ***B:*** *Synthesis of Glucose building block* ***11.*** *a) di-tert-silanedyl-bis-triflate, DMF, -40°C, 93%; b) (i) di-butyltin-oxide, toluene, 110°C, (ii) Lev$_2$O (0.5 M in DCM), 85%; c) BzCl, DCM/Pyr (1:1), 0°C to r.t., 85%; d) TFA, NIS, DCM, 0°C to r.t., 87% e) 2-2-2-trichloroacetonitrile, K$_2$CO$_3$, DCM, 0°C to r.t.; 80%.* ***C:*** *Synthesis di-, tetra-, hexa- and octamer (****1-4****). a) TMSOTf, MS3Å, DCM, 0°C, 80%; b) HO(CH$_2$)$_3$N$_3$, TMSOTf, NIS, MS3Å, DCM, -30°C, 70%; c) hydrazine acetate, Pyr/AcOH (4:1), 0°C, 92% (****9****), 86% (****22****), 84% (****24****), 87% (****26****); d) TMSOTf, NIS, MS3Å, DCM, -30°C, 77% (****21****), 83% (****23****), 86% (****25****); e) (i) Et$_3$N•3HF (0.1 M in THF), (ii) NaOMe (0.54 M in MeOH), MeOH, (iii) Pd black, H$_2$, MilliQ H$_2$O, AcOH, 80% (****1****), 82% (****2****), 71% (****3****), 63% (****4****) overall yields.*

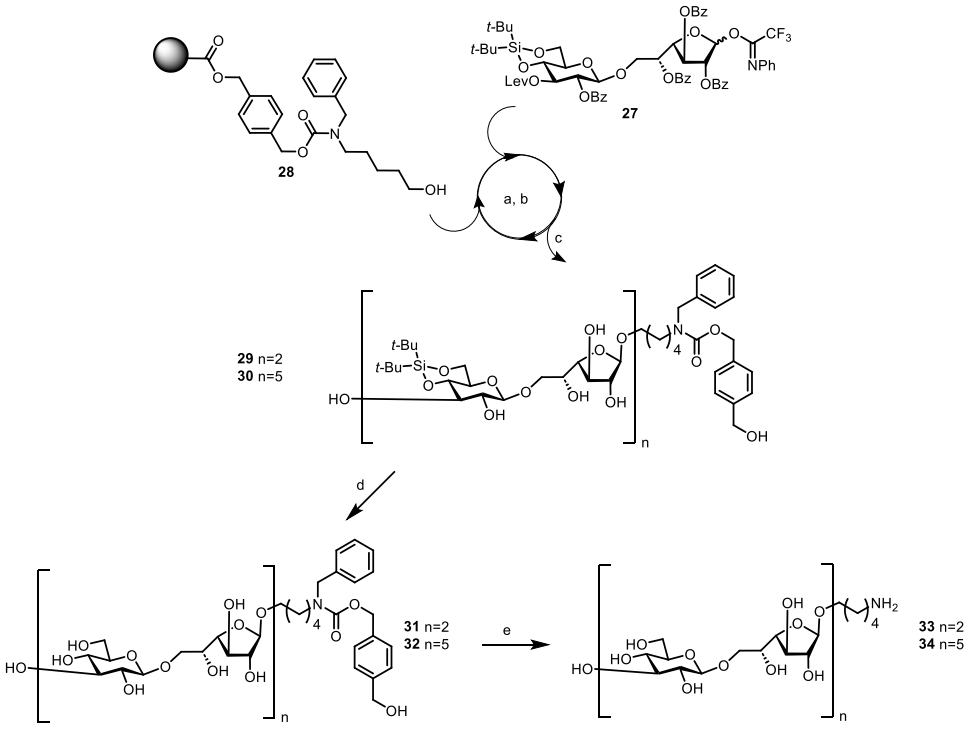
4- and C-6-OH to give **16** in 93% yield (Scheme 1B). The silylidene group was chosen because it is very stable under acidic glycosylation conditions and because it endows the neighbouring C-3-OH with excellent reactivity and increases the solubility of the product as compared to its benzylidene protected counterpart.[21, 22] In order to selectively introduce the Lev ester on the C-3-OH, compound **16** was refluxed in toluene together with di-butyltin-oxide and then treated with levulinic anhydride (Lev_2O) yielding **17** in 85%. The remaining C-2-OH was protected efficiently as a benzoate ester to ensure neighbouring group participation during the glycosylation reaction. To allow coupling with **12**, thioglycoside **18** was hydrolysed using stochiometric amounts of *N*-iodosuccinimide (NIS) and trifluoroacetic acid (TFA) at 0°C to deliver **19** as an α/β mixture in 87% yield. Finally, trichloroacetimidate donor **11** was generated in 80% yield by reaction of **19** with potassium carbonate (K_2CO_3) and 2-2-2-trichloroacetonitrile.

With monosaccharide building blocks **11** and **12** in hand, key disaccharide **10** was produced in 80% yield by TMSOTf catalysed glycosylation reaction in DCM at 0° C (Scheme 1C). Next, to acquire longer oligosaccharides, a [2+2] coupling approach was undertaken in which thioglycoside dimer **10** functions as a suitable donor and the orthogonal Lev ester at the C-3-OH of the glucose moiety allows elongation. At first a C3 spacer was installed on a small portion of dimer **10** using NIS/TfOH as activating agent and azido-propanol as acceptor giving protected target dimer **20** in 70%. Elongation of dimer **20** to tetramer **21** comprised two steps. First the Lev ester was cleaved using hydrazine acetate in DCM followed by NIS mediated coupling of the generated acceptor **9** with donor **10**, furnishing protected target tetramer **21** in 77%. Repetition of this reaction sequence using tetramer **21** gave fully protected target hexamer **24** in similar yield. Analogously, the synthesis of octamer **26** proceeded uneventful, indicating the effectiveness and the reproducibility of the applied procedure even for longer oligomers.

The deprotection of the four fragments **9, 22, 24** and **26** was carried out by cleavage of the silyl groups using a solution of $Et_3N{\cdot}3HF$ in THF and purification by flash chromatography. Subsequent removal of the benzoyl esters under Zemplén conditions, reduction of the azide functionality of the obtained crude product through a hydrogenolysis and finally purification by size-exclusion chromatography providing target oligosaccharides **1**-**4** all in excellent yields.

The synthetic route described above towards the desired DHG fragments has proven to be reliable and consistent, as also evidenced by a multi-milligram scale synthesis that delivered up to 40 mg of octasaccharide **4**. On the other hand, the procedure, comprising the iterative [2+2] couplings, ensuing deprotections of the Lev group, the accompanying purifications and characterizations, was time consuming. A potential acceleration of the overall process is represented by the development of a solid phase approach, using the fully automated oligosaccharide Glyconeer® synthesizer. Although only the introduction of trans glycosidic bonds is required, in the elongation protocol of the solid phase

synthesis repeated dimer couplings were preferred over alternating monomer couplings to avoid any benzoyl migration taking place from the Galf C-5 to the neighbouring C-6 position during the intermediate deprotections. It was decided to change the coupling method by converting thioglycoside **10** into the corresponding *N*-phenyl trifluoroacetimidate donor. Although thio donors have been applied in an automated synthesizer, imidate donors are used more often and allow to recycle the excess hydrolysed donor after each coupling. Therefore, dimer **10** was hydrolysed using NBS in wet acetone in quantitative yield and directly transformed into the trifluoroimidate donor **27**, which was preferred over the trichloroimidate for its higher stability.[23] The carboxylic acid-functionalized polystyrene resin **28**, functionalized with the base labile linker of the group of Reichardt was selected as a suitable solid support.[24] To find the



*Scheme 2. Solid-phase synthesis of tetramer **33** and decamer **34**. a) Coupling, 3 x (3 eq **27**, 0.2 eq TfOH, DCM, -5°C to r.t.; b) Deblock, H_2NNH_2 acetate, Pyr/AcOH (4:1), 40°C; c) Cleavage, THF/MeOH (3:1), NaOMe 0.01M in MeOH; d) HF•Pyr, Pyr, 32% (**31**), 8% (**32**); e) Pd black, H_2, H_2O, AcOH, 95% (**33**), 70% (**34**).*

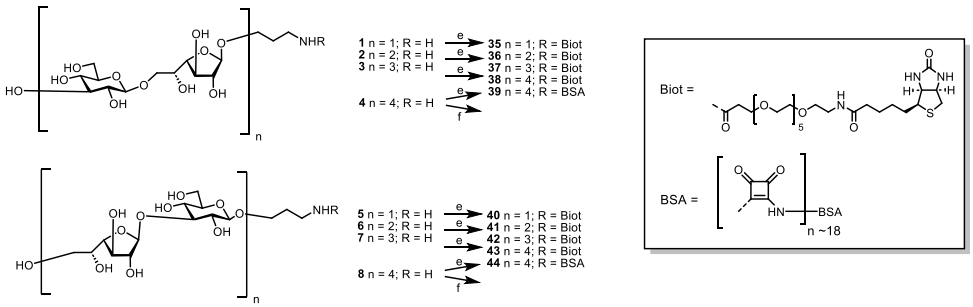
best coupling conditions resin **28** was incubated three consecutive times with 3 equivalents of donor **27** and a catalytic amount of TfOH as activator at -30°C, followed by treatment with a solution of hydrazine acetate at 40°C to cleave the levulinoyl ester. After cleavage from the resin using a sodium methoxide solution, no dimer was detected and only unreacted spacer was obtained. To explain this result different coupling conditions in solution were explored, where the temperature and amounts of activator

were varied. Surprisingly, it was observed that donor **27** required a relatively high temperature (>0°C) to react, showing a substantial difference in reactivity compared to its thio counterpart. With the so-optimized coupling conditions, the automated syntheses of a tetra- and decasaccharide (Scheme 2) were achieved, yielding compound **29** in 32% and **30** in 8% after cleavage from the solid support and silyl deprotection. The target tetra- and decamer (**33** and **34**) were obtained by hydrogenolysis of **31** and **32** in 95% and 70% yield, respectively.

The automated approach proved to be an efficient way to streamline synthetic efforts, especially when stereoselective glycosylations are viable and building blocks donors are readiliy obtained through a scalable and effective synthetic route.

Biological results

The immunogenicity of the DGH oligomers **1**-**4** was evaluated alongside the set of DHG oligosaccharides with the alternate [Galf-Glcp] frameshift **5-8** (Scheme 3). Both sets were functionalized with a biotin moiety, by treatment of the aminopropyl glycosides with a biotin reagent. The hexaethylenglycol spacer was chosen to allow efficient spatial presentation of the oligosaccharides in the glycoarray for recognition after the immobilization on streptavidin coated ELISA plates.



Scheme 3. Biotinylation of DHG oligosaccharides and conjugation of the octamers to BSA: e) C_6F_5O-Biot, Et_3N, DMF, roomtemperature (70–80%); (f) first, diethyl squarate, Et_3N, EtOH, H_2O; second, BSA, borate buffer (pH 9.0).

Once immobilized on the ELISA plates, the fragments were evaluated for their ability to bind to antibodies in serum raised against native DHG. As shown in Figure 3, the synthetic DHG oligosaccharides were recognized in a length-depended manner, with the longest fragments binding best to the antibodies. Binding, however, was significantly less than binding of the native DHG structure, probably due to the lack of decorations (acetyl and lactic acid substituents) on the synthetic fragments and their limited length as compared to the native oligosaccharide.

With the objective to establish whether the generated fragments can function as possible antigen for vaccine development, two glycoconjugates were generated.

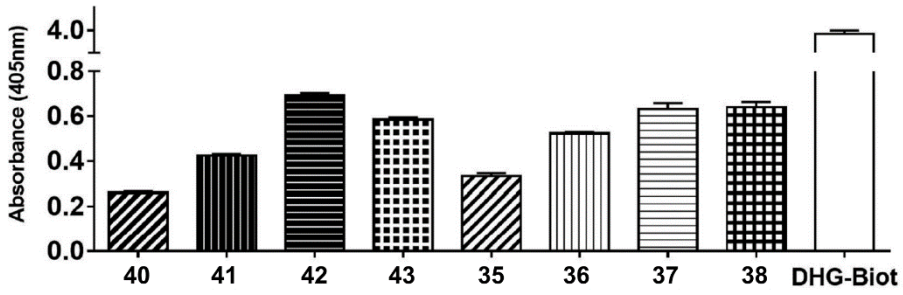


Figure 3. Antibodies raised against native DHG from E. faecalis Type 2 were evaluated for their ability to bind specifically the different synthetic DHG resembles of native DHG.

To this end, octamers **4** and **8** were treated with diethyl squarate yielding the conjugation-ready squarate DHG-oligomers and subsequently coupled to the free amino groups of BSA at pH 9 (Scheme 3). The newly formed BSA conjugates **39** and **44** were analyzed by MALDI-TOF showing an average loading of 18 octasaccharides per protein.

Next, two New Zeeland rabbits were immunized with BSA conjugates **39** and **44**, and different bleeds were taken during the 90 days immunization period and checked for their immunoreactivity against BSA, native DHG and octamers **44** and **39** respectively (Figure 4 A-D). No activity was observed against the BSA carrier alone, indicating that no significant levels of antibodies were produced against the carrier protein. Low amounts

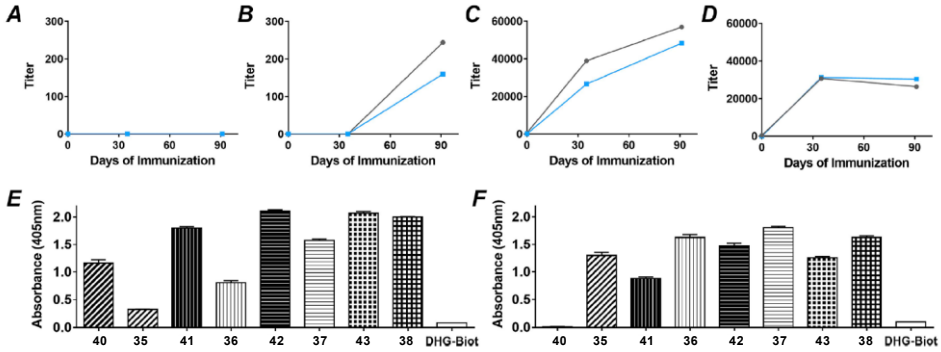


*Figure 4. Specificity of sera raised against synthetic **39** and **44** conjugates. Rabbit sera obtained during immunization schedule were examined for specificity toward the different antigens. Streptavidin coated plates were coated with 1 µg per well of (A) carrier protein BSA, (B) the native DHG, and synthetic (C) **44** and (D) **39** conjugates. Rabbit sera anti-**44** (black) and anti-**39** (blue) were plated in 2-fold serial dilutions, starting with a dilution of 50 µg IgG/mL for each serum tested. (E) Anti-**44** and (F) anti-**39** at 1.25 and 2.4 µg/mL, respectively, were also examined for their immunoreactivity toward synthetic DHG saccharides, dimers **40** and **35**, tetramers **41** and **36**, hexamers **42** and **37**, and octamers **43** and **38**. Biotinylated DHG (DHG-Biot) was used as the control to assess the preferred ability of the anti-DHG serum for the antigenic structures tested.*

of antibodies were detected against native DHG, which can be explained by the structural differences between the synthetic and native structures. On the other hand, as expected, high amounts of antibodies were detected against the synthetic antigens, with the serum raised against conjugate **44** having a higher antibody titer. Because of the different antibody titers, the sera raised against conjugate **39** were used with a 1.87 times higher concentration in subsequent experiments. The sera were also tested for their ability to recognize the other shorter and frameshifted synthetic fragments. As shown in Figure 4 (E and F) both sera were responsive towards oligosaccharides **35**-**38** and **40**-**43** but not against native DHG. For the shorter fragments (especially the dimers and tetramers) the nature of the dimer repeating played an important role in recognition.

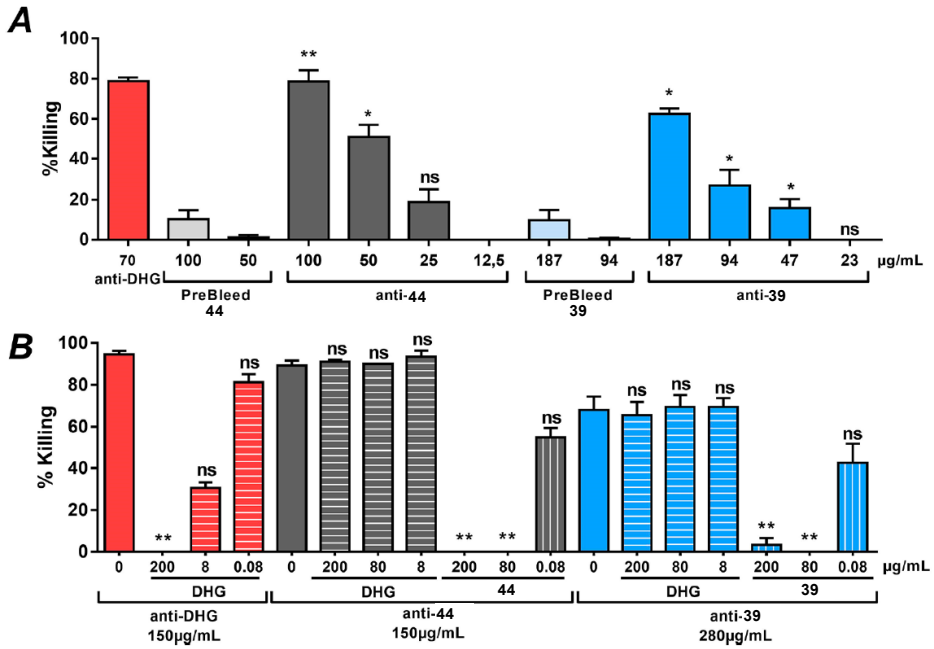


*Figure 5. Analysis of opsonophagocytic killing activity of the antibodies. (A) Sera raised against **39** and **44** conjugates were evaluated in an opsonophagocytic assay to determine the opsonophagocytic killing activity of the sera against E. faecalis Type 2. Anti-**44** (gray) and anti-**39** (blue) sera were used at different concentrations, as shown in the x-axis. Serum raised against the native DHG polysaccharide was used as a positive control (red). (B) Specificity of the sera against the antigens was confirmed by inhibiting the opsonophagocytic killing activity with different amounts of the different antigens. Purified antibodies raised against synthetic **44** (gray) and **39** (blue) conjugates were used at a dilution yielding between 60 and 90% of killing and absorbed out with different amounts of native DHG (horizontal stripes) or synthetic DHG-conjugate (vertical stripes) as inhibitors. Serum raised against the native DHG polysaccharide was absorbed out with native DHG as a positive control. Sera without inhibitors were used as a positive control for opsonophagocytic killing. Effective opsonophagocytic (or inhibition of) killing in the anti-conjugate sera (anti-**44** and anti-**39**) was compared to pre-immune rabbit sera (PreBleed, in lighter color) by nonparametric Kruskal–Wallis test, followed by multiple comparisons using Dunn's post-test. Bars represent the mean of data, and the error bars represent the standard error of the mean (ns, nonsignificant, *P < 0.05, and **P < 0.01).*

The opsonophagocytic killing activity (OPA) of the sera raised against the BSA conjugates **39** and **44** was evaluated against the *E. faecalis* type 2 strain, carrying the DHG polysaccharide. The pre-bleeds were used to see whether any opsonic antibodies against the target bacterium were present before the immunization with the conjugates. Both sera showed good killing activity, comparable to the activity of serum raised against native DHG (Figure 5A).[25]

Opsonophagocytic inhibition assay (OPIA) experiments confirmed the specificity of the antibodies toward the target antigen, showing complete inhibition of killing when the conjugates **44** and **39** were used at 80 and 200 ug/mL to block antibody binding (Figure 5B).

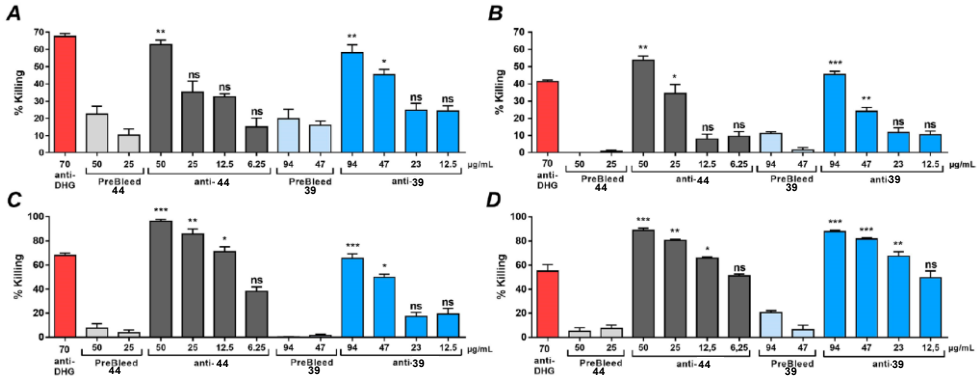
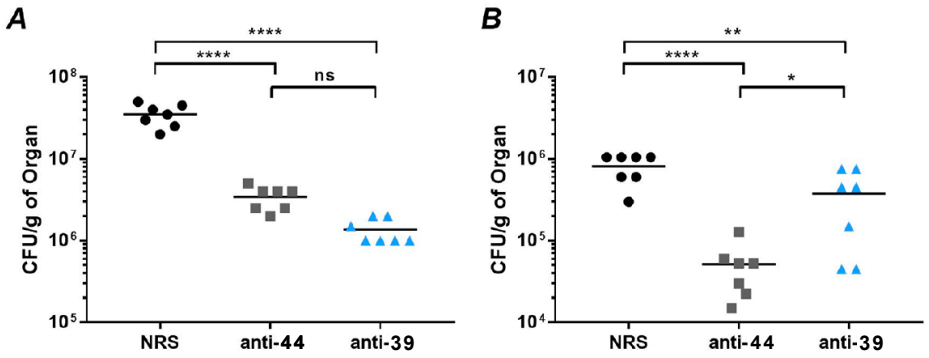


*Figure 6. Opsonophagocytic killing activity of anti-**44** and anti-**39** sera against E. faecalis encapsulated bacterial strains. The antibodies raised against synthetic **44** (gray) and **39** (blue) conjugates were used at different concentrations. Evaluated E. faecalis CPS-C strains were (A) E. faecalis V583 and (B) E. faecalis FA2-2 and E. faecalis CPS-D strains were (C) E. faecalis Type 5 and (D) E. faecalis Type 18. Effective opsonophagocytic killing in the anti-conjugate sera (anti-4c and anti-8c) was compared to pre-immune rabbit sera (PreBleed, in lighter color) by a nonparametric Kruskal–Wallis test, followed by multiple comparisons using Dunn's post-test. Bars represent the mean of data, and the error bars represent the standard error of the mean (ns, nonsignificant, *P < 0.05, **P < 0.01, ***P < 0.001).*

Interestingly the addition of native DHG did not lead to any inhibition, even at the highest concentrations, indicating poor interaction of the native polysaccharide with the antibodies in the serum. This data can be interpreted to be a result of the lack of substituents on the synthetic fragments used. The sera raised against **39** and **44** were also evaluated in OPA experiments against different *E. faecalis* CPS-C (V583, FA2-2) and *E. faecalis* CPS-D strains (Type 5, Type 18). Figure 6 shows a concentration dependent killing of these strains by both sera, indicating good cross-reactivity towards these encapsulated strains.

Finally, the protective potential of the sera raised against the synthetic fragments was evaluated in a mouse sepsis model, in which mice were passively immunized with the two sera or normal rabbit serum (NRS). Subsequently, mice were challenged with *E.*

faecalis type 2 for two days and then sacrificed to check the bacterial counts in their livers and kidneys. The results displayed in Figure 7 clearly show the ability of the sera to reduce



the colony counts in comparison to the NRS control.

*Figure 7. Mouse sepsis model. Mice were passively immunized with the sera raised against **44** (gray squares) and **39** (blue triangles) conjugates and challenged with E. faecalis Type 2. After 48 h of challenge, mice were sacrificed and their livers and kidneys were removed to assess viable counts. Panels (A) and (B) show the resulting viable counts in mice livers and kidneys challenged with E. faecalis Type 2, respectively. Each point represents the bacterial counts from a single mouse. Bars indicate the median CFU/g of organ for the group. Statistical analysis was done by one-way analysis of variance (ANOVA) with a Dunnett's correction post-test comparing between the animals immunized with the antibodies raised against the DHG-protein conjugates and control animals immunized with normal rabbit serum (NRS, black circles). Horizontal bars represent geometric means (ns, nonsignificant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).*

Conclusion

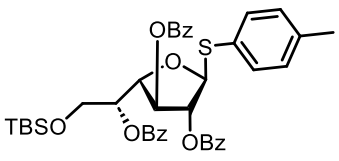
This Chapter describes the first synthesis of oligosaccharides, mimicking the native DHG structure of *E. faecalis*, ranging in length from dimers to octamers. The prepared oligomers consist of [Glcp-Galf] dimer repeats and their capacity to serve as synthetic antigen was assessed together with oligomers of equal length but built up with [Galf-Glcp] dimer repeats. Serum, raised against native DHG, was shown to bind to the biotin functionalized oligosaccharides in a length dependent manner but to a significantly lesser extent than to the native structure. Octasaccharides **4** and **8** have been used to generate BSA glycoconjugates for immunization of rabbits and the resulting sera have been analyzed in OPA, OPIA and *in vivo* experiments demonstrating good recognition and killing activity towards encapsulated *E. faecalis* strains. Even though the structures lack the lactic acid and acetyl decorations, the synthetic DHG-glycoconjugates could be used to raise antibodies with adequate opsonophagocytic activity. The sera raised with neo-glycoconjugates recognized different encapsulated *E. faecalis* strains and could be used to reduce the bacterial burden in a mouse sepsis model by passive immunization. These findings represent a concrete step towards the development of a vaccine able to combat encapsulated *E. faecalis*. The solid phase approach that has been developed to streamline the synthesis of these fragments represents an easy and fast way to

streamline the production of synthetic DHG oligomers. With the availability of building blocks that incorporate the different lactic acid and acetylation decoration patterns, a library of synthetic DHG fragments can be assembled to establish structure-immunogenicity relationships.

Experimental part

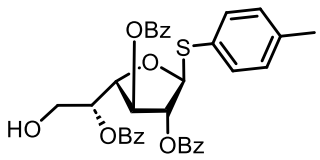
General procedures and materials: All chemicals (Acros, Biosolve, Sigma-Aldrich, TCI, etc) were used as received and all reactions were effectuated under an argon atmosphere, at ambient temperature (22°C), unless stated otherwise. For the TLC analysis were used aluminium sheets (Merck , TLC silica gel 60 F_{254}), sprayed with a solution of H_2SO_4 (20%) in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}\bullet 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\bullet 2H_2O$ (10g/L) in 10% aqueous H_2SO_4 or with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in H_2O and then heated at ≈ 140°C. For the column chromatography was used 40-63 µm 60Å silica gel (SD Screening Devices). NMR spectra (^1H, ^{13}C and ^{31}P) were recorded with a Bruker AV-400liq or a Bruker DMX-400solid or a Bruker AV-500 or a Bruker AV-600. High resolution mass spectra were recorded by direct injection on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (soure voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R= 60000 at m/z 400 (mass range m/z= 150-2000) and dioctylphthalate (m/z= 391.28428)as a lock mass.

***p*-Tolyl 2,3,5-tri-*O*-benzoyl-6-*O*-*tert*-butyldimethylsislyl-1-thio-β-D-galactofuranoside 14**



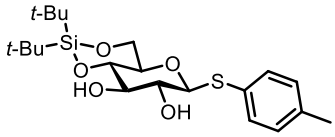
p-Tolyl 1-thio-β-galactofuranoside13 (20.0 mmol, 5.73 g) was dissolved in a mixture of DMF/Pyr (100 mL, 1:1 ratio) and cooled to 0°C. To the mixture was added DMAP (10.0 mmol, 1.22 g) and TBSCl (26.0 mmol, 3.32 g). After the starting material was fully converted, to the mixture was added BzCl (90.0 mmol, 10.45 mL). The reaction mixture was stirred overnight. The mixture was diluted with CH_2Cl_2, and H_2O was added. The solution was washed 3 times with a 1M HCl solution, once with H_2O, 3 times with $CuSO_4$ sat. solution, once with H_2O, once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **14** (14.8 mmol, 10.55 g) in 74% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ=8.09 – 7.06 (m, 19H, CH_{arom}), 5.75 – 5.70 (m, 1H, H-1), 5.68 – 5.60 (m, 3H, H-2, H-3, H-5), 4.98 (dd, *J* = 5.0, 3.9 Hz, 1H, H-4), 4.04 – 3.89 (m, 2H, H-6), 2.32 (s, 3H, CH_3*STol*), 0.83 (s, 9H, *t*-Bu), 0.05 (s, 6H, 2xCH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 165.9 – 165.5(C_q), 138.2 – 128.6 (CH_{arom}), 91.4 (C-1) , 82.6 (C-3), 80.7 (C-4), 78.0 (C-5), 73.2 (C-2), 61.3 (C-6), 25.8(*t*-Bu), 21.3 (CH_3*STol*), 18.3 (C_q) -5.32 (2xCH_3). **HRMS** *m/z*: $[M+Na]^+$ Calcd 735.2418; found 735.2432

***p*-Tolyl 2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside 12**



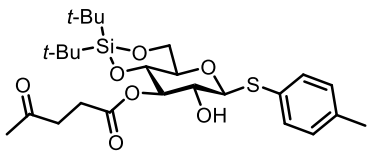
Compound **14** (14.8 mmol, 10.55 g) was dissolved in ACN (100 mL) and a 80% solution AcOH (150mL) was added. The reaction mixture was heated up to 40°C and stirred overnight. The mixture was concentrated in *vacuo* and purified by flash chromatography (EtOAc/Pentane), providing **12** (11.8 mmol, 7.15 g) in 80% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 8.15 – 7.09 (m, 19H, CH_{arom}), 5.76 (m, 1H, H-1), 5.70 – 5.62 (m, 3H, H-2, H-3 and H-5), 4.92 (m, 1H, H-4), 4.11 – 4.00 (m, 2H, H-6), 2.33 (s, 3H, CH_3*STol*). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 138.5 (C_q), 133.8 – 128.6 (CH_{arom}), 91.5 (C-1), 82.3 (C-3, C-4), 78.2 (C-5), 73.3 (C-2), 62.7 (C-6). **HRMS** *m/z*: $[M+Na]^+$ Calcd 621.1554; found 621.1561

***p*-Tolyl 4,6-*O*-di-tert-butylsilylene-1-thio-β-D-glucopyranoside 16**



Compound **15** (24.45 mmol, 7.0 g) was co-evaporated twice with pyridine and dissolved in DMF (245 mL). The solution was cooled down to -40°C and di-*tert*-silanedyl-*bis*-triflate (23.22 mmol, 7.6 mL) was added dropwise. After 3 h Pyridine (73.3 mmol, 5.9 mL) was added to the mixture. The reaction mixture was diluted with Et_2O (500 mL) and washed 3 times with H_2O. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **16** (22.73 mmol, 9.70 g) in 93% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ= 7.41 – 7.30 (m, 2H, CH_{arom}), 7.19 – 7.10 (m, 2H, CH_{arom}), 4.64 (dd, *J* = 9.7, 1.8 Hz, 1H, H-1), 4.10 (m, 1H, H-6), 3.80 (m, 1H, H-6), 3.69 – 3.39 (m, 3H, H-4, H-5, H-3), 3.20 (t, *J* = 9.2 Hz, 1H, H-2), 2.31 (s, 3H, CH_3*STol*), 2.22 (s, 2H, OH), 1.03 (s,3H, *t*-Bu), 0.98 (s, 9H, *t*-Bu). **^{13}C NMR** (101 MHz, CD_3CN) δ= 138.6 (C_q), 132.8 (CH_{arom}), 130.6 (CH_{arom}), 130.4 (C_q), 88.8 (C-1), 78.5 (C-3), 77.6 (C-4), 75.1 (C-5), 73.2 (C-2), 67.0 (C-6), 27.8 (*t*-Bu), 27.5 (*t*-Bu), 20.4 (CH_3*STol*). **HRMS** *m/z*: $[M+Na]^+$ Calcd 449.1788; found 449.1795

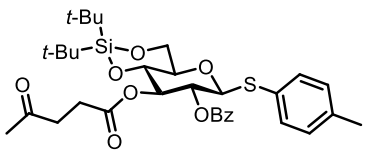
***p*-Tolyl 3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-1-thio-β-D-glucopyranoside 17**



Compound **16** (12.31 mmol, 5.25 g) was co-evaporated 3 times with toluene and dissolved in Toluene (120 mL). To the solution was added di-butyltin-oxide (12.92 mmol, 3.22 g). The reaction mixture was heated up to 110°C and stirred for 4 h. The reaction was cooled down to rt. and a 0.5 M solution in CH_2Cl_2 of Lev_2O (22.15 mmol, 44.3 mL) was added. The mixture was stirred overnight and MeOH was added. The reaction mixture was concentrated in *vacuo*, dissolved in EtOAc (120 mL) and washed 3 times with a 10% w/v solution of KF, once with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **17** (10.46 mmol, 5.49 g) in 85%

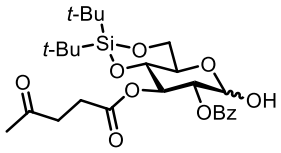
yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 7.47 – 7.39 (m, 2H), 7.17 – 7.09 (m, 2H), 5.05 (t, *J* = 9.2 Hz, 1H), 4.59 (d, *J* = 9.7 Hz, 1H), 4.21 (dd, *J* = 10.2, 5.0 Hz, 1H), 3.89 (t, *J* = 10.2 Hz, 1H), 3.76 (t, *J* = 9.4 Hz, 1H), 3.56 – 3.41 (m, 2H), 3.16 (d, *J* = 3.4 Hz, 1H), 2.78 (td, *J* = 6.6, 6.2, 4.5 Hz, 2H), 2.70 – 2.55 (m, 2H), 2.34 (s, 3H), 2.16 (s, 3H), 1.02 (s, 9H), 0.95 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ=^{13}C NMR (101 MHz, $CDCl_3$) δ 206.9, 172.6, 138.6, 133.7, 129.8, 127.7, 89.1, 78.0, 77.5, 77.2, 76.8, 74.9, 74.5, 70.7, 66.1, 38.3, 29.8, 28.2, 27.4, 26.9, 22.6, 21.2, 19.9. **HRMS** *m/z*: $[M+Na]^+$ Calcd 547.2156; found 547.2157

***p*-Tolyl 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-1-thio-β-D-glucopyranoside 18**



Compound **17** (10.46 mmol, 5.49 g) was co-evaporated 3 times with toluene and dissolved in a mixture of CH_2Cl_2/Pyridine (100 mL, 1:1 ratio). The solution was cooled down to 0°C and BzCl (35 mmol, 4.26 mL) was added. The reaction mixture was allowed to reach rt and was stirred overnight. The mixture was diluted with CH_2Cl_2 (50 mL) and H_2O was added. The mixture was washed 3 times with a 1.0 M solution of HCl, once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **18** (8.85 mmol, 5.57 g) in 85% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 8.22 – 7.09 (m, 9H, CH_{arom}), 5.31 (t, *J* = 9.3 Hz, 1H, H-3), 5.17 (t, *J* = 9.7 Hz, 1H, H-2), 4.82 (d, *J* = 9.9 Hz, 1H, H-1), 4.25 (m, 1H, H-6), 3.93 (m, 2H, H-4, H-6), 3.56 (m, 1H, H-5), 2.66 – 2.41 (m, 4H, 2x CH_2-Lev), 2.33 (s, 3H, CH_3*STol*), 2.03 (s, 3H, CH_3 Lev), 1.04 (s, 9H, *t*-Bu), 0.96 (s, 9H, *t*-Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 133.8 (C_q), 133.5 – 128.6 (CH_{arom}), 87.3 (C-1), 75.9 (C-3), 75.1 (C-2), 74.7 (C-4), 70.6 (C-5), 66.18 (C-6), 38.19 (CH_2 Lev), 28.2 , 27.4(*t*-Bu), 26.9 (CH_3 Lev). **HRMS** *m/z*: $[M+Na]^+$ Calcd 651.2418; found 651.2433

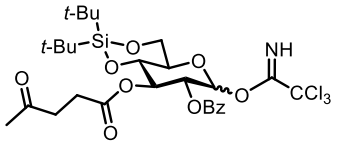
2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-α/β-D-glucopyranoside 19



Compound **18** (1.32 mmol, 0.89 g) was co-evaporated 3 times with toluene and dissolved in dry CH_2Cl_2 (13 mL). The solution was cooled down to 0°C, NIS (1.45 mmol, 0.327 g) and TFA (1.45 mmol, 0.11 mL) were added. The ice bath was removed and the solution was stirred for 2 h. The reaction mixture was cooled down to 0°C and piperidine (3.96 mmol, 0.392 mL) was added. The solution was stirred for 45 min and a sat. saolution of $Na_2S_2O_3$ was added. The mixture was washed 2 times with a sat. solution of $Na_2S_2O_3$, once with 1.0 M solution of HCl, once with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **19** (1.14 mmol, 0.597 g) in 87% yield. **^{1}H NMR** (400 MHz,

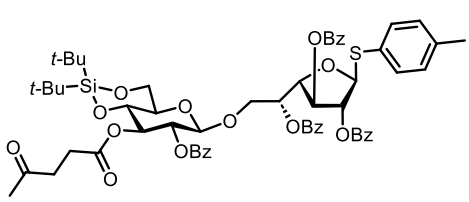
$CDCl_3$) δ= α anomer 8.03 – 7.14 (m, 5H, , CH_{arom}), 5.65 (t, *J* = 9.8 Hz, 1H, H-3), 5.56 (t, *J* = 3.7 Hz, 1H, H-1), 5.08 – 4.96 (m, 1H, H-2), 4.16 (m, 1H, H-5), 4.02 – 3.87 (m, 3H, H-4, H-6), 2.80 (s, 1H, OH), 2.66 – 2.49 (m, 4H, 2x CH_2-Lev), 2.05 (s, 3H, CH_3 Lev), 1.05 (s, 9H, *t*-Bu), 1.00 (s, 9H, *t*-Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 133.6(C_q), 130.1 - 128.6 (CH_{arom}),90.9 (C-1), 75.4 (C-4), 72.0 (C-2), 71.6 (C-3), 71.6(C-5) 66.6 (C-6), 38.3, 28.2, 27.5, 27.0, 22.8, 20.1. **HRMS** *m/z*: $[M+Na]^+$ Calcd 545.2177; found 545.2183

2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-α/β-D-glucopyranosyl N-phenyl-trichloroacetimidate 11



Compound **19** (1.14 mmol, 0.597 g) was co-evaporated 3 times with toluene and dissolved in dry CH_2Cl_2 (11 mL). The solution was cooled down to 0°C, K_2CO_3 (4.56 mmol, 0.632 g) and 2-2-2-trichloroacetonitrile (6.84 mmol, 0.687 mL) were added. The reaction mixture was heated up to rt and stirred overnight. The reaction mixture was filtrate over celite and concentrated in *vacuo*. The crude was was purified by flash chromatography (EtOAc/Pentane), providing **11** (0.91 mmol, 0.608 g) in 80% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ= 8.98 (s, 1H), 7.98 – 7.88 (m, 3H), 7.66 – 7.59 (m, 2H), 7.52 – 7.43 (m, 3H), 6.56 (d, *J* = 3.6 Hz, 1H), 5.65 – 5.59 (m, 1H), 5.40 (dd, *J* = 9.8, 8.2 Hz, 1H), 5.29 (dd, *J* = 10.2, 3.6 Hz, 1H), 4.20 (dd, *J* = 9.9, 5.0 Hz, 1H), 4.17 – 4.09 (m, 4H), 3.99 – 3.90 (m, 2H), 3.87 (dd, *J* = 9.7, 4.9 Hz, 1H), 2.67 – 2.59 (m, 3H), 2.51 – 2.41 (m, 3H), 2.16 (s, 8H), 1.05 (s, 16H), 0.98 (d, *J* = 15.2 Hz, 16H). **^{13}C NMR** (126 MHz, CD_3CN) δ= 172.9, 166.2, 160.9, 160.6, 134.6, 130.6, 129.6, 96.8, 94.1, 75.2, 74.7, 72.3, 71.9, 71.2, 70.0, 66.8, 66.7, 38.4, 38.4, 29.7, 28.8, 27.6, 27.1, 23.0, 20.5.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside 10**



Compound **12** (0.362 mmol, 0.217 g) and compound **11** (0.449 mmol, 0.300 g) were co-evaporated 3 times with toluene and dissolved in CH_2Cl_2 (1.7 mL) with freshly activated MS3Å. The solution was cooled down to 0°C and stirred for 15 min. To the reaction mixture was added TMSOTf (0.036 mmol, 6.4 µL). After 2.5 h to the reaction mixture was added TEA. The solution was washed once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **10** (0.289 mmol, 0.319 g) in 80% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 8.05 – 7.99 (m, 2H), 7.91 – 7.86 (m, 2H), 7.86 – 7.80 (m, 2H), 7.79 – 7.73 (m, 2H), 7.63 – 7.56 (m, 1H), 7.52 – 7.36 (m, 8H), 7.30 – 7.16 (m, 8H), 7.08 (dd, *J* = 7.2, 1.4 Hz, 2H), 5.76 (ddd, *J* = 6.8, 5.3, 3.8 Hz, 1H), 5.63 (d, *J* = 1.6 Hz, 1H), 5.55 – 5.49 (m, 2H), 5.30 – 5.17 (m, 2H), 4.80 (dd, *J* = 5.0, 3.9

Hz, 1H), 4.74 (d, *J* = 7.4 Hz, 1H), 4.17 (td, *J* = 10.4, 5.2 Hz, 2H), 3.98 – 3.80 (m, 3H), 3.50 (td, *J* = 9.9, 5.0 Hz, 1H), 2.61 – 2.39 (m, 4H), 2.32 (s, 3H), 2.00 (s, 3H), 1.03 (s, 9H), 0.97 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 205.8, 171.9, 165.6, 165.5, 165.4, 165.1, 138.3, 133.6, 133.6, 133.6, 133.5, 133.1, 133.0, 130.1, 129.9, 129.8, 129.8, 129.7, 129.5, 129.3, 129.2, 129.1, 128.9, 128.6, 128.5, 128.4, 101.3, 91.4, 82.4, 80.9, 77.9, 77.5, 77.2, 76.8, 74.8, 74.5, 71.6, 71.0, 70.9, 67.4, 66.1, 38.1, 28.2, 27.4, 27.0, 22.7, 21.3, 20.0. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1125.3733; found 1125.3766

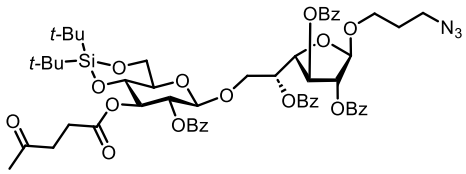
General procedure A:

Starting acceptor (1 eq.) and donor (1.4 eq.) were co-evaporated 3 times with toluene and dissolved in dry CH_2Cl_2 (0.2 M) with freshly activated MS3Å. To the solution was added NIS (1.3 eq.). The reaction mixture was cooled down to -30°C and TMSOTf (0.2-0.3 eq.) was added. The reaction was stirred until the total conversion of the acceptor (≈3 h). To the reaction mixture were added TEA (3-4 eq.) and a sat. solution of $Na_2S_2O_3$. The solution was washed once with sat. solution of $Na_2S_2O_3$, once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane).

General procedure B:

Starting compound was co-evaporated 3 times with toluene and was dissolved in a mixture of Pyr/AcOH (0.1 M, 4:1 ratio) and cooled to 0°C. To the solution was added hydrazine acetate (2 eq.) The reaction was stirred until full conversion of the starting material (≈2 h). The reaction mixture was diluted with EtOAc and was added H_2O. The mixture was washed once with H_2O, once with a 1.0 M solution of HCl, once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane).

3-azidopropyl 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 20

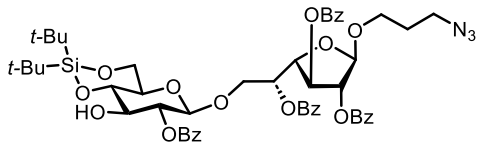


Acceptor $HO(CH_2)_3N_3$ (1.10 mmol, 0.111 g) was coupled to donor **10** (1.54 mmol, 1.544 g) using general procedure **A** providing **20** (0.77 mmol, 0.832 g) in 70% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.99 – 7.94 (m, 2H), 7.93 – 7.88 (m, 2H), 7.88 – 7.83 (m, 2H), 7.80 – 7.75 (m, 2H), 7.60 – 7.55 (m, 1H), 7.51 (tt, *J* = 7.3, 1.3 Hz, 1H), 7.48 – 7.39 (m, 4H), 7.30 – 7.18 (m, 7H), 5.70 (ddd, *J* = 6.9, 5.7, 3.5 Hz, 1H), 5.45 (dd, *J* = 5.4, 1.4 Hz, 1H), 5.35 (d, *J* = 1.3 Hz, 1H), 5.25 (t, *J* = 9.4 Hz, 1H), 5.23 – 5.15 (m, 2H), 4.74 (d, *J* = 7.7 Hz, 1H), 4.51 (dd, *J* = 5.4, 3.4 Hz, 1H), 4.17 (ddd,

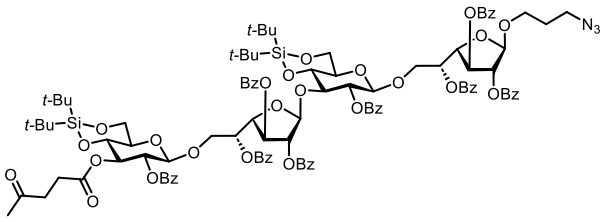
J = 15.8, 10.4, 5.3 Hz, 2H), 3.96 (dd, *J* = 10.5, 7.0 Hz, 1H), 3.91 – 3.78 (m, 3H), 3.59 – 3.48 (m, 2H), 3.43 (t, *J* = 6.7 Hz, 2H), 2.61 – 2.51 (m, 2H), 2.51 – 2.39 (m, 2H), 2.00 (s, 3H), 1.89 (dqd, *J* = 9.3, 6.9, 5.0 Hz, 2H), 1.03 (s, 9H), 0.97 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ= 205.8, 171.9, 165.6, 165.6, 165.1, 133.6, 133.5, 133.1, 133.1, 130.0, 129.9, 129.8, 129.5, 129.3, 129.2, 129.1, 128.6, 128.5, 128.4, 128.4, 105.6, 101.3, 82.3, 80.9, 77.5, 77.4, 77.2, 76.9, 74.9, 74.5, 71.6, 70.9, 70.9, 67.3, 66.1, 64.2, 48.5, 38.2, 29.7, 29.1, 28.2, 27.4, 27.0, 22.8, 20.0. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1102.3975; found 1102.3994

3-azidopropyl 2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 9



Compound **20** (0.650 mmol, 0.702 g) was deprotected using general procedure **B** providing **9** (0.603 mmol, 0.592 g) in 92% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ= 7.95 (td, *J* = 8.4, 1.4 Hz, 4H), 7.86 (ddd, *J* = 8.4, 4.7, 1.3 Hz, 4H), 7.59 – 7.54 (m, 1H), 7.50 (tt, *J* = 7.5, 1.4 Hz, 1H), 7.46 (tt, *J* = 7.4, 1.3 Hz, 1H), 7.41 (td, *J* = 7.6, 2.1 Hz, 3H), 7.29 – 7.14 (m, 9H), 5.71 (td, *J* = 6.3, 3.3 Hz, 1H), 5.45 (dd, *J* = 5.5, 1.3 Hz, 1H), 5.36 (d, *J* = 1.3 Hz, 1H), 5.21 (s, 1H), 5.15 (dd, *J* = 9.1, 7.9 Hz, 1H), 4.74 (d, *J* = 7.9 Hz, 1H), 4.52 (dd, *J* = 5.4, 3.4 Hz, 1H), 4.17 (ddd, *J* = 15.8, 10.4, 5.5 Hz, 2H), 3.99 (dd, *J* = 10.5, 6.7 Hz, 1H), 3.86 (t, *J* = 10.2 Hz, 1H), 3.84 – 3.72 (m, 3H), 3.55 (ddd, *J* = 10.0, 6.5, 5.2 Hz, 1H), 3.49 – 3.39 (m, 3H), 2.52 (s, 1H), 1.87 (dqd, *J* = 8.9, 6.9, 5.1 Hz, 2H), 1.04 (s, 10H), 0.99 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ= 165.7, 165.6, 165.6, 165.5, 133.5, 133.4, 133.1, 133.0, 130.0, 129.9, 129.8, 129.7, 129.6, 129.6, 129.2, 129.1, 129.0, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 125.4, 105.5, 101.1, 82.3, 80.8, 77.5, 77.4, 77.2, 77.1, 76.9, 75.3, 73.4, 70.9, 70.6, 66.9, 66.1, 64.2, 48.4, 29.0, 27.5, 27.0, 22.8, 20.0. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1004.3608; found 1004.3638

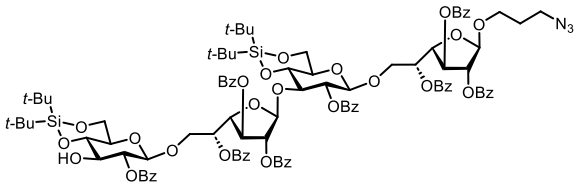
3-azidopropyl 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 21



Acceptor **9** (0.553 mmol, 0.543 g) was coupled to donor **10** (0.774 mmol, 0.854 g) using general procedure **A** providing **21** (0.425 mmol, 0.834 g) in 77% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ= 7.97 – 7.93 (m, 2H), 7.90 (ddd, *J* = 8.3, 2.6, 1.3 Hz, 4H), 7.88 – 7.83 (m, 2H), 7.75 – 7.71 (m, 2H), 7.71 – 7.68 (m, 2H), 7.68 – 7.64 (m, 2H), 7.61 – 7.29 (m, 15H), 7.26 (s, 5H), 7.20 (td, *J* = 7.6, 5.4 Hz, 5H), 7.16 – 7.12 (m, 1H), 7.06

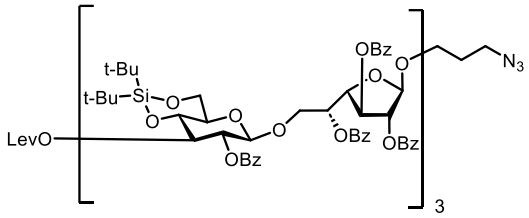
(dt, *J* = 15.4, 7.8 Hz, 4H), 6.98 (t, *J* = 7.8 Hz, 2H), 5.82 (dt, *J* = 8.7, 3.3 Hz, 1H), 5.66 (td, *J* = 6.3, 3.3 Hz, 1H), 5.42 (dd, *J* = 5.4, 1.3 Hz, 1H), 5.39 – 5.28 (m, 4H), 5.24 – 5.11 (m, 4H), 4.66 (dd, *J* = 13.4, 7.9 Hz, 2H), 4.60 (t, *J* = 4.3 Hz, 1H), 4.49 (dd, *J* = 5.5, 3.3 Hz, 1H), 4.17 (ddt, *J* = 11.5, 7.9, 3.1 Hz, 3H), 4.09 (dd, *J* = 10.2, 5.0 Hz, 1H), 4.00 – 3.77 (m, 9H), 3.55 (ddd, *J* = 10.0, 6.5, 5.2 Hz, 1H), 3.51 – 3.40 (m, 4H), 2.58 – 2.36 (m, 4H), 1.98 (s, 3H), 1.93 – 1.83 (m, 2H), 1.02 (s, 9H), 0.97 (d, *J* = 2.8 Hz, 18H), 0.88 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ= 205.7, 171.9, 165.6, 165.5, 165.4, 165.3, 164.8, 164.7, 133.8, 133.5, 133.4, 133.3, 133.1, 133.0, 132.9, 132.7, 132.6, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7, 129.7, 129.6, 129.5, 129.5, 129.5, 129.3, 129.2, 129.1, 129.0, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 106.3, 105.5, 101.5, 101.0, 82.4, 82.3, 82.0, 80.8, 78.6, 77.5, 75.6, 74.8, 74.5, 73.4, 71.4, 71.3, 71.0, 70.8, 70.8, 68.9, 66.7, 66.4, 66.1, 64.1, 48.4, 38.1, 29.6, 29.0, 28.7, 28.1, 27.4, 27.3, 27.2, 26.9, 22.7, 22.6, 20.0. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1982.7102; found 1982.7068

3-azidopropyl 2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 22



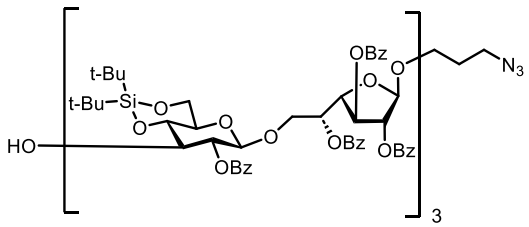
Compound **21** (0.408 mmol, 0.800 g) was deprotected using general procedure **B** providing **22** (0.351 mmol, 0.654 g) in 86% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 7.99 – 7.93 (m, 2H), 7.90 (ddd, *J* = 8.3, 3.0, 1.3 Hz, 4H), 7.87 – 7.83 (m, 2H), 7.80 – 7.70 (m, 6H), 7.60 – 7.46 (m, 4H), 7.46 – 7.31 (m, 11H), 7.30 – 7.02 (m, 14H), 6.98 (t, *J* = 7.8 Hz, 2H), 5.79 (dt, *J* = 8.4, 3.4 Hz, 1H), 5.66 (td, *J* = 6.3, 3.3 Hz, 1H), 5.44 – 5.37 (m, 2H), 5.37 – 5.27 (m, 3H), 5.21 – 5.15 (m, 2H), 5.07 (dd, *J* = 9.0, 7.9 Hz, 1H), 4.69 – 4.60 (m, 3H), 4.49 (dd, *J* = 5.4, 3.3 Hz, 1H), 4.16 (tt, *J* = 9.9, 3.9 Hz, 3H), 4.07 (dd, *J* = 10.2, 5.0 Hz, 1H), 4.00 – 3.85 (m, 5H), 3.85 – 3.75 (m, 3H), 3.75 – 3.65 (m, 2H), 3.54 (ddd, *J* = 10.0, 6.6, 5.4 Hz, 1H), 3.50 – 3.35 (m, 4H), 2.60 (d, *J* = 2.3 Hz, 1H), 1.94 – 1.81 (m, 2H), 1.04 (s, 11H), 0.97 (d, *J* = 5.2 Hz, 20H), 0.87 (s, 10H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 165.5, 165.6, 165.5, 165.4, 165.3, 164.9, 164.7, 133.5, 133.4, 133.4, 133.1, 133.0, 132.8, 132.7, 132.6, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.6, 129.5, 129.5, 129.2, 129.1, 129.1, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 128.1, 106.4, 105.5, 101.3, 101.0, 82.4, 82.3, 82.0, 80.8, 78.6, 77.5, 77.1, 75.7, 75.4, 73.4, 73.3, 71.5, 71.1, 70.8, 70.5, 68.6, 66.7, 66.5, 66.2, 64.2, 48.5, 29.0, 27.5, 27.3, 27.3, 27.1, 22.8, 22.6, 20.1, 20.0. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1884.6734; found 1884.6691

3-azidopropyl 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 23



Acceptor **22** (0.291 mmol, 0.542 g) was coupled to donor **10** (0.422 mmol, 0.466 g) using general procedure **A** providing **23** (0.241 mmol, 0.686 g) in 83% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 7.98 – 7.92 (m, 2H), 7.92 – 7.80 (m, 9H), 7.75 – 7.59 (m, 10H), 7.59 – 7.29 (m, 22H), 7.29 – 7.11 (m, 13H), 7.11 – 6.94 (m, 12H), 5.78 (td, *J* = 8.8, 4.5 Hz, 2H), 5.65 (dt, *J* = 6.3, 3.2 Hz, 1H), 5.41 (dd, *J* = 5.4, 1.4 Hz, 1H), 5.37 – 5.09 (m, 12H), 4.68 – 4.53 (m, 5H), 4.48 (dd, *J* = 5.5, 3.3 Hz, 1H), 4.21 – 4.11 (m, 4H), 4.07 (ddd, *J* = 10.1, 7.3, 5.0 Hz, 2H), 3.98 – 3.74 (m, 13H), 3.58 – 3.50 (m, 1H), 3.49 – 3.34 (m, 5H), 2.56 – 2.36 (m, 5H), 1.98 (s, 3H), 1.94 – 1.81 (m, 2H), 1.07 – 0.98 (m, 13H), 0.99 – 0.93 (m, 26H), 0.86 (d, *J* = 9.7 Hz, 18H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 205.8, 171.9, 165.6, 165.6, 165.5, 165.3, 165.3, 164.9, 164.8, 164.7, 164.44, 133.5, 133.4, 133.3, 133.1, 133.0, 132.9, 132.7, 132.6, 132.4, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 106.4, 106.3, 105.5, 101.5, 101.2, 101.0, 82.5, 82.3, 82.0, 81.9, 80.8, 78.7, 78.6, 77.5, 75.7, 75.6, 74.8, 74.5, 73.3, 73.2, 71.4, 71.4, 71.3, 71.0, 71.0, 70.9, 70.8, 69.0, 68.9, 68.5, 68.4, 66.7, 66.5, 66.4, 66.1, 64.2, 48.5, 38.1, 29.7, 29.0, 28.2, 27.4, 27.3, 27.3, 27.2, 27.0, 22.7, 22.6, 22.5, 20.1, 20.0. **HRMS** *m/z*: [M] Calcd 2840.0336; found 2840.075

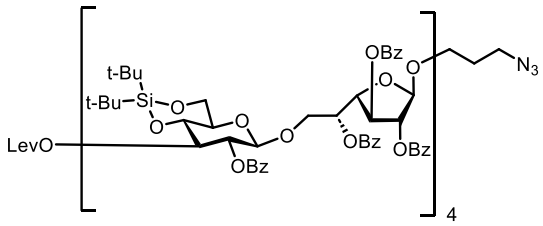
3-azidopropyl 2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 24



Compound **23** (0.220 mmol, 0.625 g) was deprotected using general procedure **B** providing **24** (0.184 mmol, 0.507 g) in 84% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 7.98 – 7.92 (m, 2H), 7.92 – 7.81 (m, 9H), 7.80 – 7.65 (m, 8H), 7.64 – 7.59 (m, 2H), 7.59 – 7.30 (m, 22H), 7.30 – 7.02 (m, 19H), 6.97 (td, *J* = 7.8, 2.7 Hz, 4H), 5.78 (dd, *J* = 8.4, 4.1 Hz, 2H), 5.65 (td, *J* = 6.3, 3.3 Hz, 1H), 5.41 (d, *J* = 5.4 Hz, 1H), 5.39 – 5.28 (m, 6H), 5.28 – 5.17 (m, 2H), 5.13 (d, *J* = 8.7 Hz, 2H), 5.06 (dd, *J* = 9.0, 7.9 Hz, 1H), 4.69 – 4.51 (m, 5H), 4.48

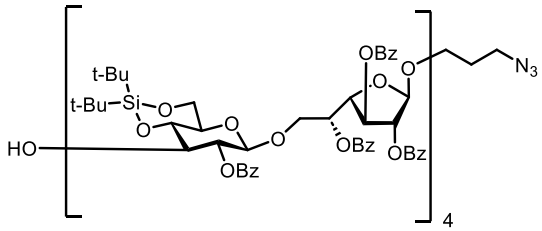
(dd, *J* = 5.4, 3.3 Hz, 1H), 4.20 – 4.11 (m, 4H), 4.06 (dd, *J* = 10.2, 5.0 Hz, 2H), 3.98 – 3.64 (m, 14H), 3.54 (dt, *J* = 10.0, 5.7 Hz, 1H), 3.50 – 3.32 (m, 5H), 2.59 (s, 1H), 1.87 (dtd, *J* = 11.7, 6.8, 4.9 Hz, 2H), 1.03 (s, 11H), 1.01 – 0.92 (m, 28H), 0.86 (d, *J* = 10.1 Hz, 20H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 165.7, 165.6, 165.5, 165.5, 165.4, 165.3, 165.3, 164.9, 164.8, 164.7, 164.5, 133.5, 133.4, 133.4, 133.1, 132.9, 132.8, 132.7, 132.6, 132.4, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.5, 129.2, 129.1, 129.1, 128.8, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 128.1, 106.4, 106.3, 105.5, 101.2, 101.2, 101.0, 82.4, 82.4, 82.0, 81.9, 80.8, 78.8, 78.7, 77.4, 77.1, 76.8, 75.7, 75.6, 75.4, 73.4, 73.3, 73.2, 71.5, 71.4, 71.1, 71.0, 70.8, 70.5, 68.6, 68.5, 66.7, 66.5, 66.5, 66.2, 64.2, 48.5, 29.0, 27.5, 27.3, 27.1, 22.8, 22.6, 22.5, 20.1, 20.0. **HRMS** *m/z*: [M] Calcd 2743.0002; found 2743.0066

3-azidopropyl 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 25



Acceptor **24** (0.153 mmol, 0.419 g) was coupled to donor **10** (0.214 mmol, 0.236 g) using general procedure **A** providing **25** (0.132 mmol, 0.493 g) in 86% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 7.98 – 7.92 (m, 2H), 7.92 – 7.81 (m, 10H), 7.75 – 7.57 (m, 14H), 7.57 – 7.11 (m, 41H), 7.11 – 6.92 (m, 18H), 5.78 (ddq, *J* = 15.2, 8.4, 4.4, 3.8 Hz, 3H), 5.65 (td, *J* = 6.3, 3.3 Hz, 1H), 5.41 (d, *J* = 5.5 Hz, 1H), 5.37 – 5.26 (m, 8H), 5.26 – 5.16 (m, 4H), 5.16 – 5.07 (m, 4H), 4.68 – 4.58 (m, 3H), 4.55 (dt, *J* = 8.1, 3.3 Hz, 4H), 4.48 (dd, *J* = 5.4, 3.3 Hz, 1H), 4.11 (dtd, *J* = 29.8, 10.0, 5.6 Hz, 8H), 3.98 – 3.72 (m, 17H), 3.59 – 3.50 (m, 1H), 3.50 – 3.33 (m, 6H), 2.56 – 2.33 (m, 4H), 1.98 (s, 3H), 1.87 (dtd, *J* = 11.6, 6.7, 4.9 Hz, 2H), 1.02 (s, 11H), 0.95 (dd, *J* = 6.7, 4.9 Hz, 37H), 0.86 (d, *J* = 10.1 Hz, 28H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 205.8, 171.9, 165.7, 165.6, 165.5, 165.3, 165.3, 165.2, 164.9, 164.8, 164.7, 164.5, 133.5, 133.4, 133.3, 133.1, 133.0, 132.9, 132.7, 132.6, 132.4, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 106.4, 106.3, 105.5, 101.2, 82.5, 82.4, 82.0, 81.9, 80.8, 78.8, 78.7, 78.7, 77.4, 75.7, 75.6, 74.8, 74.6, 73.4, 73.2, 73.1, 71.4, 71.4, 71.3, 71.1, 71.0, 70.9, 70.8, 69.0, 68.5, 66.7, 66.5, 66.2, 64.2, 48.5, 38.1, 29.7, 29.0, 28.2, 27.4, 27.3, 27.2, 27.0, 22.7, 22.6, 22.5, 20.1, 20.0. **HRMS** *m/z*: [M] Calcd 3722.3529; found 3722.9320

3-azidopropyl 2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-di-tert-butylsilylene-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 26



Compound **25** (0.119 mmol, 0.443 g) was deprotected using general procedure **B** providing **26** (0.104 mmol, 0.377 g) in 87% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 7.99 – 7.93 (m, 2H), 7.92 – 7.82 (m, 10H), 7.80 – 7.64 (m, 10H), 7.64 – 7.29 (m, 33H), 7.29 – 7.01 (m, 26H), 6.97 (ddt, *J* = 10.0, 4.6, 2.2 Hz, 6H), 5.77 (td, *J* = 6.7, 5.4, 2.8 Hz, 3H), 5.65 (td, *J* = 6.3, 3.3 Hz, 1H), 5.41 (d, *J* = 5.5 Hz, 1H), 5.39 – 5.26 (m, 8H), 5.26 – 5.17 (m, 3H), 5.16 – 5.02 (m, 4H), 4.64 (t, *J* = 7.7 Hz, 2H), 4.57 (ddt, *J* = 11.5, 7.9, 3.5 Hz, 5H), 4.48 (dd, *J* = 5.4, 3.3 Hz, 1H), 4.14 (ddt, *J* = 11.5, 6.9, 4.4 Hz, 5H), 4.06 (ddd, *J* = 10.7, 5.3, 2.7 Hz, 3H), 3.97 – 3.65 (m, 18H), 3.54 (dt, *J* = 9.9, 5.8 Hz, 1H), 3.50 – 3.32 (m, 6H), 2.60 (d, *J* = 2.3 Hz, 1H), 1.87 (dtd, *J* = 11.8, 6.8, 4.9 Hz, 2H), 1.04 (s, 11H), 1.01 – 0.90 (m, 38H), 0.90 – 0.80 (m, 29H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 165.6, 165.5, 165.4, 165.3, 165.2, 164.9, 164.8, 164.7, 164.6, 164.5, 133.5, 133.4, 133.3, 133.1, 133.0, 132.8, 132.7, 132.6, 132.4, 132.4, 130.1, 130.0, 129.9, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.2, 129.1, 129.0, 128.8, 128.7, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 106.4, 106.4, 106.3, 105.5, 101.2, 101.2, 101.1, 101.0, 82.4, 82.4, 82.0, 81.9, 80.8, 78.8, 78.7, 78.6, 77.5, 77.1, 75.7, 75.6, 75.6, 75.4, 73.4, 73.3, 73.2, 73.1, 71.5, 71.4, 71.0, 70.9, 70.8, 70.5, 68.6, 68.5, 66.7, 66.5, 66.2, 64.2, 48.5, 29.0, 27.5, 27.3, 27.3, 27.1, 22.8, 22.6, 22.5, 20.1, 20.00. **HRMS** *m/z*: [M] Calcd 3623.3128; found 3623.8100

General procedure C:

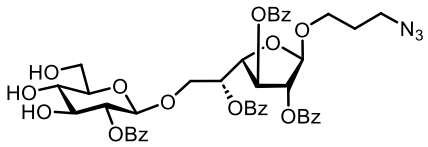
Starting compound was co-evaporated with toluene, cooled down to 0°C and a 0.1 M solution in THF of $Et_3N•3HF$ (2 eq. per silyl group) was added. The reaction mixture was stirred until full conversion of the starting material (≈3 h). The solution was diluted with CH_2Cl_2 and washed once with a sat. solution of $NaHCO_3$ and once with H_2O. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (MeOH/ CH_2Cl_2).

General procedure D:

Starting compound was dissolved in MeOH (0.01-0.02 M) and a 0.54 M solution in MeOH of NaOMe (0.5 eq.) was added. The reaction mixture was stirred until full conversion of the starting material (≈24 h). To the mixture was added AcOH glacial until pH 7 is reached. The solution was concentrated in *vacuo*. The crude was dissolved in MilliQ H_2O (2.0 mL).

To the reaction mixture was added 4-5 drops of glacial AcOH. The mixture was purged with Ar. To the solution was added a scup of Pd black. The reaction mixture was purged with H_2 for a few seconds and stirred under H_2 atmosphere for ≈1 h. The solution was concentrated in *vacuo*. The crude was purified by size-exclusion chromatography (Sephadex LH-20, MeOH/MilliQ H_2O 9:1 ratio).

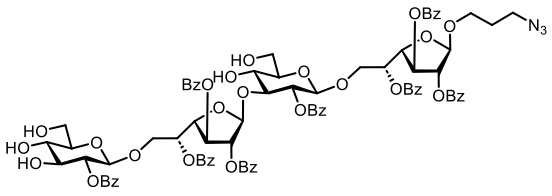
3-azidopropyl 2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 1a



Compound **9** (0.030 mmol, 0.029 g) was deprotected using general procedure **C** providing **1a** (0.026 mmol, 0.022 g) in 87% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.03 – 7.94 (m, 4H), 7.85 (ddt, *J* = 17.3, 6.7, 1.4 Hz, 4H), 7.61 – 7.55 (m, 1H), 7.52 – 7.40 (m, 5H), 7.31 – 7.20 (m, 8H), 5.89 (ddd, *J* = 7.2, 5.5, 3.4 Hz, 1H), 5.49 (dt, *J* = 5.1, 1.0 Hz, 1H), 5.39 (d, *J* = 1.3 Hz, 1H), 5.24 (s, 1H), 4.97 (dd, *J* = 9.2, 7.8 Hz, 1H), 4.73 (d, *J* = 7.8 Hz, 1H), 4.51 (dd, *J* = 5.1, 3.4 Hz, 1H), 4.19 – 4.05 (m, 2H), 3.92 (d, *J* = 12.1 Hz, 1H), 3.83 (ddd, *J* = 9.9, 7.1, 5.2 Hz, 2H), 3.70 (p, *J* = 9.0 Hz, 2H), 3.58 (ddd, *J* = 9.9, 6.4, 5.2 Hz, 1H), 3.43 (q, *J* = 6.7 Hz, 3H), 3.03 (s, 2H), 2.86 (d, *J* = 7.5 Hz, 1H), 1.88 (dq, *J* = 12.0, 6.9 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 166.7, 166.2, 165.9, 165.7, 133.7, 133.5, 133.4, 133.3, 130.1, 130.0, 129.9, 129.6, 129.3, 129.1, 129.0, 128.7, 128.5, 128.5, 128.4, 105.7, 101.1, 82.2, 81.5, 77.8, 75.9, 75.8, 74.6, 71.6, 71.0, 67.8, 64.3, 62.1, 48.4, 29.1. **HRMS** *m/z*: $[M+Na]^+$ Calcd 864.2586; found 864.2621

3-azidopropyl 2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 2a

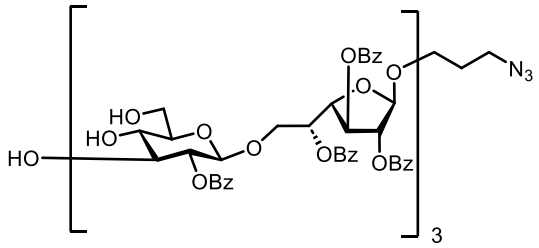


Compound **22** (0.040 mmol, 0.075 g) was deprotected using general procedure **C** providing **2a** (0.036 mmol, 0.058 g) in 91% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.96 (ddd, *J* = 9.9, 8.0, 5.9 Hz, 8H), 7.90 – 7.78 (m, 6H), 7.60 – 7.37 (m, 13H), 7.29 – 7.07 (m, 15H), 5.82 (d, *J* = 8.0 Hz, 1H), 5.58 (ddd, *J* = 8.6, 5.9, 2.4 Hz, 1H), 5.46 (d, *J* = 4.8 Hz, 1H), 5.41 – 5.34 (m, 3H), 5.31 (t, *J* = 8.4 Hz, 1H), 5.24 – 5.19 (m, 2H), 5.08 (t, *J* = 8.4 Hz, 1H), 4.73 (dt, *J* = 7.7, 3.8 Hz, 2H), 4.61 (d, *J* = 7.7 Hz, 1H), 4.52 (t, *J* = 4.2 Hz, 1H), 4.10 (d, *J* = 19.7 Hz, 4H), 4.00 (d, *J* = 12.3 Hz, 1H), 3.96 – 3.67 (m, 11H), 3.55 (dt, *J* = 10.4, 5.8 Hz, 1H), 3.41 (t, *J* = 6.7 Hz, 6H), 2.11 (d, *J* = 45.0 Hz, 1H), 1.88 (hept, *J* = 7.0 Hz, 2H), 1.26 (s, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 166.4, 166.1, 165.9, 165.8, 165.7, 165.1, 164.9, 133.6, 133.5, 133.4, 133.3, 133.2, 132.7, 130.3, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5,

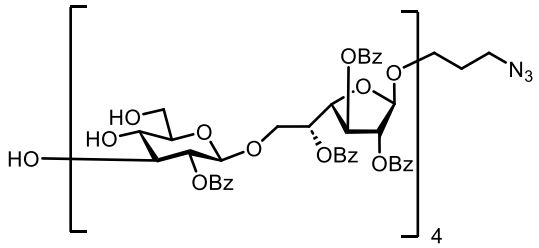
129.3, 129.2, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 107.6, 105.6, 101.0, 100.4, 85.5, 82.7, 82.2, 81.3, 81.1, 77.7, 77.2, 76.8, 76.6, 76.0, 75.8, 75.3, 74.4, 72.2, 71.3, 70.1, 70.0, 69.4, 67.1, 65.5, 64.2, 62.1, 61.4, 48.5, 46.5, 29.0. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1604.4691; found 1604.4727

3-azidopropyl 2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 3a



Compound **24** (0.025 mmol, 0.069 g) was deprotected using general procedure **C** providing **3a** (0.021 mmol, 0.048 g) in 82% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ= 8.02 – 7.84 (m, 17H), 7.79 (dd, *J* = 17.1, 7.7 Hz, 4H), 7.63 – 7.51 (m, 5H), 7.51 – 7.34 (m, 16H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.17 (ddq, *J* = 22.9, 15.4, 7.6 Hz, 20H), 7.03 (t, *J* = 7.6 Hz, 2H), 5.84 – 5.76 (m, 1H), 5.63 – 5.53 (m, 2H), 5.46 – 5.18 (m, 12H), 5.13 (t, *J* = 8.3 Hz, 1H), 4.80 (ddd, *J* = 11.7, 5.7, 2.2 Hz, 2H), 4.67 (t, *J* = 8.8 Hz, 2H), 4.61 (d, *J* = 7.6 Hz, 1H), 4.49 (dd, *J* = 5.1, 3.0 Hz, 1H), 4.34 (s, 2H), 4.21 – 3.94 (m, 12H), 3.94 – 3.64 (m, 11H), 3.50 (dt, *J* = 23.1, 7.9 Hz, 5H), 3.40 (t, *J* = 6.7 Hz, 3H), 1.92 – 1.78 (m, *J* = 7.5 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ= 166.3, 166.0, 165.8, 165.7, 165.6, 165.5, 165.4, 165.00, 164.9, 164.7, 133.5, 133.4, 133.3, 133.2, 133.1, 133.0, 132.9, 132.6, 130.2, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 107.6, 105.5, 101.0, 100.6, 100.4, 85.5, 85.0, 82.9, 82.6, 82.3, 81.2, 81.1, 80.9, 77.6, 77.0, 76.4, 75.8, 75.7, 75.1, 74.4, 72.4, 71.2, 70.3, 70.1, 69.9, 69.8, 67.1, 65.6, 65.5, 64.2, 62.5, 61.4, 48.74, 29.0. **HRMS** *m/z*: $[M+Na]^{2+}$ Calcd 1184.3361; found 1184.3396

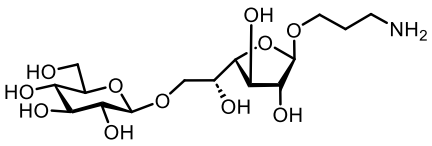
3-azidopropyl 2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 4a



Compound **26** (0.047 mmol, 0.171 g) was deprotected using general procedure **C** providing **4a** (0.036 mmol, 0.110 g) in 76% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ= 8.05 – 7.86 (m, 22H), 7.86 – 7.74 (m, 7H), 7.62 – 7.50 (m, 7H), 7.50 – 7.33 (m, 23H), 7.29 (t,

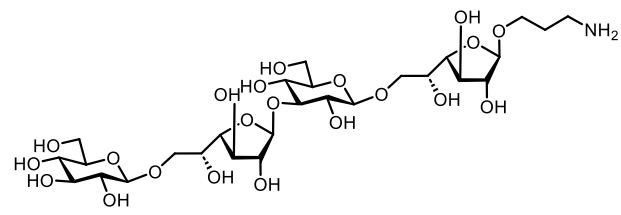
J = 7.5 Hz, 1H), 7.27 – 7.00 (m, 30H), 5.80 (td, *J* = 6.4, 3.2 Hz, 1H), 5.58 (dtd, *J* = 12.6, 6.8, 6.2, 3.0 Hz, 3H), 5.46 (d, *J* = 5.2 Hz, 1H), 5.44 – 5.20 (m, 15H), 5.12 (t, *J* = 8.6 Hz, 1H), 4.87 (dd, *J* = 5.5, 2.2 Hz, 1H), 4.82 (dd, *J* = 5.8, 2.2 Hz, 1H), 4.77 (dd, *J* = 5.9, 2.7 Hz, 1H), 4.74 – 4.63 (m, 3H), 4.60 (d, *J* = 7.7 Hz, 1H), 4.52 (dd, *J* = 5.1, 3.3 Hz, 1H), 4.23 – 3.68 (m, 34H), 3.51 (dddt, *J* = 18.1, 15.0, 9.0, 4.4 Hz, 5H), 3.39 (q, *J* = 6.3, 5.6 Hz, 4H), 1.86 (dt, *J* = 12.7, 6.3 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ= 166.3, 166.0, 165.8, 165.7, 165.6, 165.5, 165.3, 165.2, 165.0, 164.9, 164.8, 164.7, 133.5, 133.5, 133.4, 133.3, 133.2, 133.1, 133.0, 132.8, 132.7, 132.6, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.0, 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 108.0, 107.6, 107.5, 105.5, 100.9, 100.7, 100.6, 100.5, 85.2, 84.4, 84.3, 82.8, 82.5, 82.4, 82.2, 81.4, 81.2, 80.9, 80.8, 77.7, 77.1, 76.9, 76.5, 76.3, 76.2, 75.9, 75.8, 75.1, 74.4, 74.0, 72.7, 72.5, 72.4, 71.3, 70.7, 70.5, 70.1, 69.9, 69.5, 69.4, 69.1, 66.8, 66.4, 65.9, 65.6, 64.2, 62.4, 62.0, 61.6, 61.5, 48.4, 29.0. **HRMS** *m/z*: $[M+Na]^{2+}$ Calcd 1554.4414; found 1554.4414

3-azidopropyl β-D-glucopyranosyl-(1→6)-β-D-galactofuranoside 1



Compound **1a** (0.017 mmol, 0.014 g) was deprotected using general procedure **D** providing **1** (0.015 mmol, 0.006 g) in 92% yield. **^{1}H NMR** (500 MHz, D_2O) δ= 5.01 (d, *J* = 1.9 Hz, 1H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.09 (dd, *J* = 5.3, 3.3 Hz, 1H), 4.08 – 3.97 (m, 4H), 3.91 (dd, *J* = 12.3, 2.3 Hz, 1H), 3.89 – 3.83 (m, 1H), 3.78 – 3.63 (m, 3H), 3.52 – 3.43 (m, 2H), 3.38 (dd, *J* = 9.8, 9.0 Hz, 1H), 3.31 (dd, *J* = 9.4, 7.9 Hz, 1H), 3.15 – 3.10 (m, 2H), 1.98 (ddt, *J* = 12.5, 7.6, 3.8 Hz, 2H), 1.90 (s, 3H). **^{13}C NMR** (126 MHz, D_2O) δ= 107.2, 102.8, 83.4, 80.7, 76.5, 76.0, 75.7, 73.2, 71.1, 69.6, 65.8, 60.8, 37.9, 26.6. **HRMS** *m/z*: $[M+Na]^{+}$ Calcd 422.1633; found 422.1635

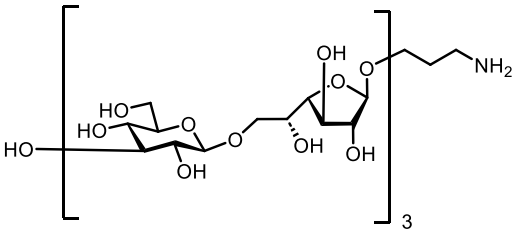
3-azidopropyl β-D-glucopyranosyl-(1→6)-β-D-galactofuranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-galactofuranoside 2



Compound **2a** (0.024 mmol, 0.038 g) was deprotected using general procedure **D** providing **2** (0.022 mmol, 0.016 g) in 90% yield. **^{1}H NMR** (400 MHz, D_2O) δ= 5.30 (d, *J* = 1.8 Hz, 1H), 5.01 (d, *J* = 1.8 Hz, 1H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.50 (dd, *J* = 8.0, 1.1 Hz, 1H), 4.16 (dt, *J* = 3.3, 1.4 Hz, 1H), 4.14 – 3.98 (m, 10H), 3.95 – 3.83 (m, 3H), 3.80 – 3.62 (m, 7H), 3.53 – 3.42 (m, 6H), 3.38 (ddd, *J* = 9.8, 8.9, 1.2 Hz, 1H), 3.31 (ddd, *J* = 9.3, 7.8, 1.2 Hz, 1H), 3.13 (t, *J* = 6.9 Hz, 2H), 2.03 – 1.94 (m, 2H), 1.90 (s, 3H). **^{13}C NMR** (126 MHz, D_2O) δ= 108.2, 107.2, 102.8, 102.6,

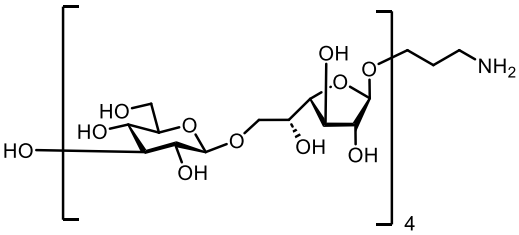
83.4, 83.1, 82.1, 81.3, 80.7, 76.6, 76.5, 76.0, 75.8, 75.7, 73.3, 73.2, 71.4, 71.2, 69.7, 69.3, 68.2, 65.8, 60.8, 37.9, 26.6. **HRMS** *m/z*: $[M+Na]^+$ Calcd 746.2689; found 746.2702

3-azidopropyl β-D-glucopyranosyl-(1→6)-β-D-galactofuranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-galactofuranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-galactofuranoside 3



Compound **3a** (0.014 mmol, 0.033 g) was deprotected using general procedure **D** providing **3** (0.012 mmol, 0.013 g) in 87% yield. **^{1}H NMR** (500 MHz, D_2O) δ= 5.30 (d, *J* = 1.8 Hz, 2H), 5.01 (d, *J* = 1.9 Hz, 1H), 4.55 – 4.48 (m, 3H), 4.16 (dd, *J* = 3.4, 1.8 Hz, 2H), 4.14 – 4.08 (m, 6H), 4.08 – 3.96 (m, 9H), 3.95 – 3.83 (m, 5H), 3.74 (dddd, *J* = 18.5, 12.4, 8.2, 5.3 Hz, 7H), 3.69 – 3.62 (m, 3H), 3.53 – 3.42 (m, 9H), 3.41 – 3.36 (m, 1H), 3.31 (dd, *J* = 9.4, 7.9 Hz, 1H), 3.13 (t, *J* = 6.9 Hz, 2H), 1.98 (p, *J* = 6.6 Hz, 2H), 1.90 (s, 3H). **^{13}C NMR** (126 MHz, D_2O) δ= 108.2, 107.2, 102.8, 102.6, 83.4, 83.0, 82.1, 82.0, 81.2, 80.7, 76.6, 76.6, 76.5, 76.0, 75.8, 75.7, 75.7, 73.3, 73.2, 71.4, 71.4, 71.2, 69.7, 69.6, 69.3, 69.2, 68.2, 65.8, 60.8, 37.9, 26.6. **HRMS** *m/z*: $[M+H]^+$ Calcd 1048.3926; found 1048.3948

3-azidopropyl β-D-glucopyranosyl-(1→6)-β-D-galactofuranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-galactofuranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-galactofuranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-galactofuranoside 4



Compound **4a** (0.018 mmol, 0.055 g) was deprotected using general procedure **D** providing **4** (0.015 mmol, 0.020 g) in 83% yield. **^{1}H NMR** (500 MHz, D_2O) δ= 5.29 (d, *J* = 1.8 Hz, 3H), 5.00 (d, *J* = 1.8 Hz, 1H), 4.55 – 4.47 (m, 4H), 4.15 (dd, *J* = 3.4, 1.8 Hz, 3H), 4.14 – 3.98 (m, 17H), 3.95 – 3.82 (m, 5H), 3.78 – 3.61 (m, 12H), 3.52 – 3.41 (m, 11H), 3.41 – 3.35 (m, 1H), 3.30 (dd, *J* = 9.4, 7.9 Hz, 1H), 3.14 – 3.08 (m, 2H), 2.02 – 1.92 (m, 2H), 1.89 (s, 3H). **^{13}C NMR** (126 MHz, D_2O) δ= 108.2, 107.2, 102.8, 102.6, 83.4, 83.0, 82.1, 81.2, 80.7, 76.6, 76.0, 75.7, 75.6, 73.3, 73.2, 71.4, 71.2, 69.7, 69.6, 69.2, 68.2, 65.8, 60.8, 37.9, 26.7. **HRMS** *m/z*: $[M+H]^+$ Calcd 1372.4983; found 1372.5004

Automated synthesis methods:

All solvents used in the machine are pre-dried 24h before use on a 4Å ms and are HPLC grade. The activator and the deblock solutions are always freshly prepared before use and are made using pre-dried solvents.

Method A: agitation of the resin during couplings and washes

After addition of the appropriate solvent, an Ar flow was applied from the bottom of the reaction vessel, suspending the resin in solution. The flow duration was 15s for the washing steps while for the coupling steps was of 10s every 30s.

Method B: swelling of the resin

Dry resin was inserted in the reaction vessel and washed with DCM (3x) and alternating THF (3x) and DCM(3x).

Method C: coupling cycle

The resin was suspended in DCM, the reaction vessel was emptied and the building block solution added (1.0 mL) followed by flushing the building block line with DCM (0.5 mL) while being agitated. The temperature was set to -5°C while employing method A. A 10 min pause was started, after which the activator solution (0.1 M TfOH solution in DCE, 300 µL) was added, keeping the temperature below -5°C. The delivery line was flushed with an additional DCM (0.5 mL) to the reaction vessel. Temperature was set to 20°C and method A was applied for 1h, after which the reaction vessel was emptied and the mixture collected in the fraction collector. The resin was washed with DCM (3x 2.0 mL) and the washes were eluted to the fraction collector.

Method E: deblock cycle

The resin was washed with DMF (4x 3.0 mL). The deblock solution added (0.08 M hydrazine acetate solution in pyridine/AcOH 4:1, 3.0 mL) and the temperature was set to 40°C, followed by a 5-minute incubation applying method A. The temperature was kept at 40°C, after which the solid support was incubated 10 minutes applying protocol A. Then the reaction vessel was emptied and the resin washed with DMF (3x 3.0 mL) running method A.

Method F: washing after coupling

The temperature was set to 20°C. The resin washed with MeOH (3x 2.0 mL), alternating THF (6x 2.0 mL) and DCM (5x 3.0 mL) while applying method A.

Method G: washing after deblock

The temperature was set to 20°C. The resin washed with DMF (4x 3.0 mL), DCM (4x 3.0 mL), alternating THF (6x 3.0 mL), 0.01M AcOH in THF (6x 3.0 mL) and DCM (8x 5 mL).

Method H: resin isolation

To the dry resin was added DCM or MeOH (first 2 times DCM and last 2 times MeOH), after which the resin was agitated for 15s. The suspended resin was removed with a glass pipet.

Method I: cleavage from the resin

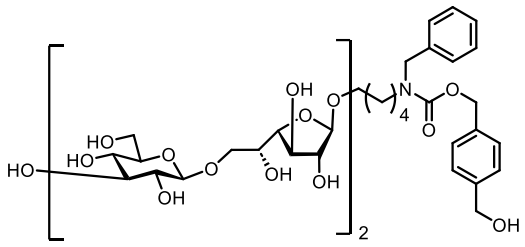
The isolated resin from the synthesizer was charged in a 10 mL microwave glass vial and a mixture of THF/MeOH (3:1, 4.0 mL) was added together with a solution of NaOMe 0.01M in MeOH (0.2 eq). The mixture was agitated overnight at r.t. The organic solution containing the crude was concentrated *in vacuo* and dissolved in pyridine (0.01M) and HF•Pyr (2eqx Sylil group) was added and stirred overnight. The reaction mixture was concentrated *in vacuo* and the remaining crude purified by flash chromatography.

Method	# Cycles	Description	Time	Temperature	
B	1	Swelling of the resin	30 min	20°C	
C	3	Coupling: 3eq donor, 1eq TfOH	60 min	-5→20°C	**Elongation block**
F	3	Wash after coupling	10 min	20°C	
E	3	Deblock: 9eq H2NNH2 AcOH	15 min	40°C	
G	3	Wash after deblock	10 min	20°C	
H	1	Resin isolation	5 min	r.t.	
I	1	Cleavage from the resin	12h	r.t.	

General procedure E: Automated synthesis of Diheteroglycan fragments

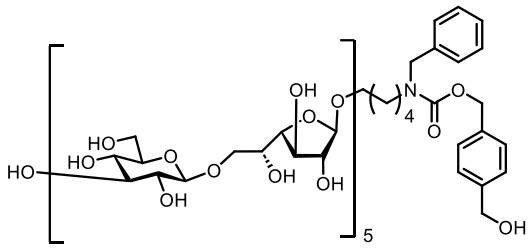
Functionalized resin **28** (0.03 mmol, 0.1 g) was introduced in the reaction vessel followed by method B. The elongation block was repeated ''n'' times where ''n'' is the number of repeating units to instal. Method H was used to isolate the resin and method I to cleave the fragments from the solid support.

Tetramer 31



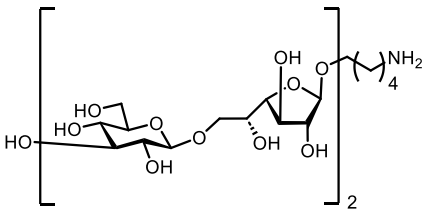
Functionalized resin **28** was treated using general procedure **E** providing compound **31** (0.022 mmol, 0.029 g) in 32% yield.**^{1}H NMR** (400 MHz, MeOD) δ = 7.47 – 7.13 (m, 8H), 5.29 (d, *J* = 1.8 Hz, 1H), 5.16 (d, *J* = 14.2 Hz, 2H), 4.82 (d, *J* = 8.0 Hz, 1H), 4.61 (d, *J* = 9.9 Hz, 2H), 4.51 (s, 2H), 4.32 (dd, *J* = 10.5, 7.5 Hz, 2H), 4.15 – 3.97 (m, 6H), 3.97 – 3.81 (m, 6H), 3.75 – 3.50 (m, 6H), 3.44 – 3.17 (m, 18H), 1.64 – 1.43 (m, 4H), 1.29 (s, 3H). **^{13}C NMR** (101 MHz, MeOD) δ = 128.2, 127.8, 127.3, 126.8, 108.5, 108.0, 103.2, 83.9, 82.9, 82.1, 81.2, 77.3, 77.1, 76.6, 76.4, 76.3, 73.7, 71.5, 71.1, 70.1, 69.8, 69.5, 68.6, 63.5, 61.2. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1028.39452; found 1028.39463.

Decamer 32



Functionalized resin **28** was treated using general procedure **E** providing compound **32** (0.0031 mmol, 0.010 g) in 8% yield. **^{1}H NMR** (500 MHz, D_2O) δ = 7.47 – 7.01 (m, 10H), 5.37 – 5.23 (m, 4H), 5.10 (d, *J* = 29.8 Hz, 2H), 4.92 (d, *J* = 16.1 Hz, 1H), 4.67 – 4.54 (m, 2H), 4.54 – 4.38 (m, 7H), 4.22 – 3.80 (m, 32H), 3.80 – 3.53 (m, 16H), 3.53 – 3.14 (m, 20H), 1.45 (s, 5H), 1.22 (d, *J* = 44.2 Hz, 4H), 0.99 (t, *J* = 11.2 Hz, 1H). **^{13}C NMR** (126 MHz, D_2O) δ = 186.4, 128.8, 128.2, 128.0, 127.6, 108.2, 107.2, 102.8, 102.6, 83.0, 82.5, 82.1, 82.1, 82.0, 81.2, 81.1, 76.6, 76.6, 76.4, 76.0, 75.7, 75.6, 73.3, 73.3, 73.2, 71.4, 69.7, 69.3, 69.2, 68.2, 63.6, 60.8. **HRMS** *m/z*: $[M+Na]^{2+}$ Calcd 1000.85937; found 1000.85937.

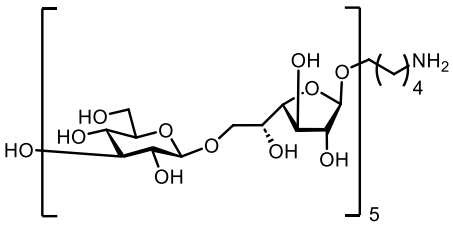
Tetramer 33



Compound **31** (0.0079 mmol, 0.008 g) was deprotected using general procedure **D** providing **33** (0.0075 mmol, 0.005 g) in 95% yield. **^{1}H NMR** (500 MHz, D_2O) δ = 5.29 (d, *J* = 1.8 Hz, 1H), 4.98 (d, *J* = 2.3 Hz, 1H), 4.50 (dd, *J* = 16.6, 8.0 Hz, 2H), 4.17 – 3.86 (m, 12H), 3.78 – 3.26 (m, 15H), 3.07 – 2.91 (m, 2H), 1.65 (dp, *J* = 21.1, 7.1, 6.6 Hz, 4H), 1.43 (q, *J* = 8.3 Hz, 2H). **^{13}C NMR** (126 MHz, D_2O) δ = 108.2, 107.1, 102.8, 102.6, 83.0, 82.6, 82.0, 81.2, 80.9, 76.6, 76.4, 76.0, 75.8, 75.6, 73.3, 73.2,

71.3, 71.2, 69.7, 69.5, 69.3, 68.2, 68.1, 60.8, 39.4, 28.1, 26.5, 22.3. **HRMS** *m/z*: $[M+H]^+$ Calcd 752.31828; found 752.31828.

Decamer 34



Compound **32** (0.0031 mmol, 0.010 g) was deprotected using general procedure **D** providing **34** (0.0022 mmol, 0.005 g) in 70% yield. **^{1}H NMR** (500 MHz, D_2O) δ = 5.30 (d, *J* = 1.8 Hz, 4H), 5.00 (d, *J* = 2.2 Hz, 1H), 4.57 – 4.47 (m, 5H), 4.17 (dd, *J* = 3.4, 1.8 Hz, 4H), 4.11 (pd, *J* = 6.6, 3.6 Hz, 9H), 4.07 – 3.96 (m, 12H), 3.92 (dq, *J* = 12.3, 2.5 Hz, 5H), 3.75 (dtd, *J* = 19.0, 12.2, 10.6, 6.7 Hz, 11H), 3.66 (t, *J* = 8.9 Hz, 4H), 3.58 (d, *J* = 10.0 Hz, 1H), 3.54 – 3.43 (m, 14H), 3.42 – 3.27 (m, 3H), 3.03 – 2.97 (m, 2H), 1.67 (dt, *J* = 21.5, 7.7 Hz, 5H), 1.44 (t, *J* = 7.8 Hz, 2H). **^{13}C NMR** (126 MHz, D_2O) δ = 108.2, 107.1, 102.8, 102.6, 83.1, 82.7, 82.1, 81.3, 81.0, 76.6, 76.5, 76.0, 75.8, 75.7, 73.3, 73.2, 71.4, 71.2, 69.7, 69.5, 69.3, 69.3, 68.2, 68.1, 60.8, 39.4, 28.2, 26.5, 23.3, 22.3. **HRMS** *m/z*: $[M+H]^{2+}$ Calcd 862.82125; found 862.82131.

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