Chemical synthesis of fragments of streptococcal cell wall polysaccharides

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Chemical Synthesis of Fragments of Streptococcal Cell Wall Polysaccharides

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To my family and my friends.

人生没有白走的路，

也没有白吃的苦，

当下跨出去的每一步，

都是未来的基石与铺垫。

Every experience in life is not rewardless,

Also, no pain is in vain,

Every step of the moment,

Be the cornerstones and foreshadowing of the future.

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List of abbreviations

Ac	acetyl
ACN	acetonitrile
All	allyl
aq.	aqueous
Arom	aromatic
atm	1 atmosphere = 10^5 Pa
BAIB	(diacetoxyiodo)benzene
Bn	benzyl
BOM	benzyloxymethyl
bs	broad singlet
BSP	1-benzenesulfinyl piperidine
Bu	butyl
t-Bu	*tert*-butyl
Bz	benzoyl
Cbz	benzyloxycarbonyl
ClAc	chloroacetyl
COD	1,5-cyclooctadiene
CSA	camphorsufonic acid
CSO	(1S)-(+)-(10-camphorsulfonyl)-oxaziridine
d	doublet
DCE	1,2-dichloroethane
DCM	dichloromethane
dd	doublet of doublets
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
$(DHQD)_2PHAL$	*bis*(dihydroquinidino)phthalazine
DIPEA	diisopropylethylamine
DMAP	*N,N*-4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DTBMP	2,6-*di*-*tert*-butyl-4-methylpyridine
DTBS	di-tert-butylsilylidene
EA/EtO Ac	ethyl acetate
EDCI	*N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride
eq	equivalents
Fmoc	9-fluorenylmethoxycarbonyl
Gal	galactose
Glc	glucose
GlcA	glucuronic acid
GlcN	glucosamine
$GlcN_3$	2-azido-2-deoxy glucose
GlcNAc	*N*-acetyl glucosamine
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HMBC	heteronuclear multiple-bond correlation
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence
IR	infrared
J	coupling constant
LAH	lithium aluminum hydride
LC-MS	liquid chromatography-mass spectrometry
Lev	levulinoyl
m	multiplet

MALDI	matrix-associated laser desorption ionization	t	triplet
Man	mannose	TBAB	tetra-*n*-butylammonium bromide
ManA	mannuronic acid	TBAF	tetra-*n*-butylammonium fluoride
Ms	methanesulfonyl	TBAI	tetra-*n*-butylammonium iodide
MS	mass spectrometry	TBDPS	*tert*-butyldiphenylsilyl
MS	molecular sieves	TBS	*tert*-butyldimethylsilyl
Nap	2-methylnaphthyl	TCA	trichloroacetyl
NBS	*N*-bromosuccinimide	TEA	triethylamine
NIS	*N*-iodosuccinimide	TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
NMR	nuclear magnetic resonance	Tf	trifluoromethanesulfonyl
NOESY	nuclear Overhauser effect spectroscopy	TfOH	triflic acid
Nu	nucleophile	Tf_2O	trifluoromethanesulfonic anhydride
NR	no reaction	TFA	trifluoroacetic acid
PE	petroleum ether	THF	tetrahydrofuran
Ph	phenyl	TIPS	triisopropylsilyl
Ph_2SO	diphenyl sulfoxide	TMS	trimethylsilyl
Phth	phthaloyl	TLC	thin layer chromatography
Piv	pivaloyl	TLR	toll like receptor
PMB	*para*-methoxybenzyl	Tol	*p*-tolyl
ppm	parts per million	Ts	*p*-toluenesulfonyl
Py	pyridine	TTBP	2,4,6-tri-*tert*-butylpyrimidine
q	quartet	*p*-TsOH	*p*-toluenesulfonic acid
RT	room temperature	UV	ultraviolet
Rf	retention factor		
s	singlet		
sat.	saturated		
SET	single electron transfer		

Chapter 1

General Introduction

1. Introduction

Carbohydrates, also named saccharides or glycans, are the most abundant of the four major classes of biomolecules (DNA, proteins, lipids and carbohydrates) in all living things.[1] Due to branched structures and various types of linkages, carbohydrates are structurally the most complex. They play many different roles in all forms of living organisms, as nutrients, in the storage of energy (e.g. starch and glycogen), as structural components (e.g. cellulose and chitin) and as signaling molecules and recognition elements, for example in the immune system and blood types. Besides, they are also important components of DNA, proteins, and

lipids. For example, the monosaccharide ribose (or deoxyribose) is a key constituent of coenzymes (e.g. ATP, FAD and NAD) and the backbone of the RNA (or DNA). A large part of all proteins is glycosylated, and glycans and lipids can be combined to provide glycolipids. As a result of the multiple asymmetric carbons in monosaccharides, varying stereoselectivity and regioselectivity of glycosidic linkages, different oligo/polysaccharide chain lengths, branching and additional functionalities such as carboxylate, sulfates and acetamides, an infinite number of saccharides exist in nature.

A small structural difference in a monosaccharide or glycosydic linkages can result in a significant change of the functions and properties of a glycan. For example, glucose and galactose only differ in one chiral center, but have a very different taste (Figure 1A). Difference of one hydroxyl group between ribose and deoxyribose defines DNA or RNA. Several structurally similar and important polysaccharides show in Figure 1B present how small structural changes lead to very different properties. Chitin and cellulose only differ between the acetylamino group and hydroxyl on position C-2. The difference between cellulose and amylose, which can be regarded as “unbranched starch”, is the configuration of the glycosydic bond (1,4-α vs 1,4-β). Glycogen contains a higher density of branches compared to amylopectin, a branched starch, on the same skeleton. The function and properties of these glycans are very different. Cellulose and chitin are both structural polysaccharides, and are components of plants and arthropods, respectively. Most non-ruminant animals cannot break down these polymers because they lack the cellulase enzyme. However, starch and glycogen are nutritional polysaccharides, which are easily broken down by these organisms. The more branched the polysaccharide is, the better its solubility in water and the more quickly it is digested.

A well-known example of cell-cell recognition that is determined by carbohydrates is the ABO blood group system.[2] Only one single terminal monosaccharide on the red blood cell surface determines blood types, establishing the rules for blood and organ transfusion.[3] The terminal carbohydrate structure of the A, B and H type II antigens, shown in Figure 1C, present on glycoproteins and glycolipids determines the blood group, and antigen(s) co-exists with antibodies directed at the missing antigen(s). For example, blood of group O only contains the H antigen (which is the precursor of A and B antigen), and has antibodies against both A and B.

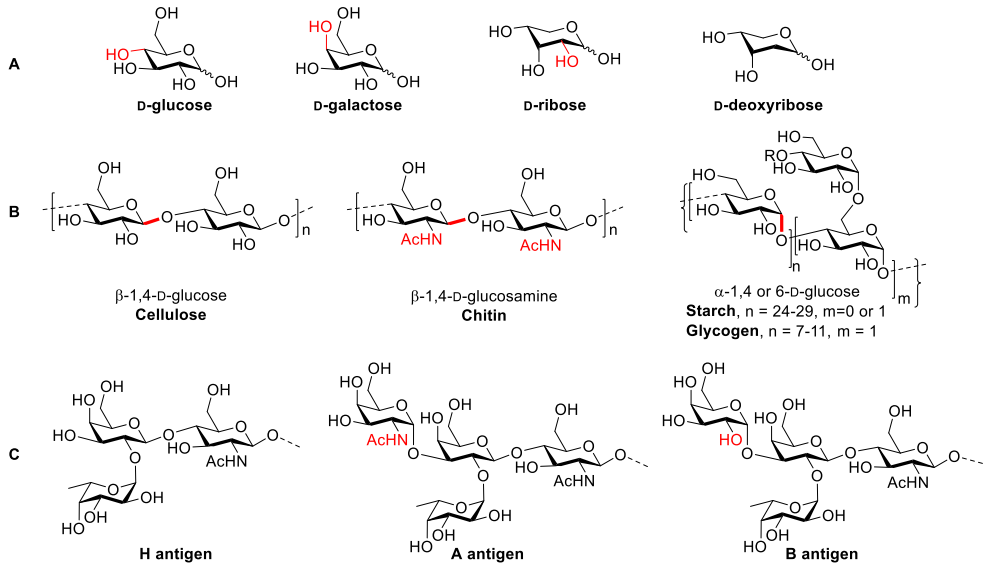


Figure 1. The structure of several mono-, oligo- and polysaccharides.

Specific recognition plays an all-important role in the immune system, which defends against infections by pathogens.[4] The cell surface of the most bacteria is surrounded by a cell wall polysaccharide (CWPS), which often is structurally unique and absent in host cells. Thus, CWPS can be regarded as pathogen signatures, and they can be used as a valuable epitopes (antigenic determinants) for the development of vaccines.[5] In general, carbohydrates are classified as T-cell-independent antigens, that can only induce specific short-lived IgM responses after being recognized by a B-cell receptor (BCR) with the aid of follicular dendritic cells (FDCs) (Fig. 2A). However, carbohydrates cannot be presented by major histocompatibility complex (MHC) proteins to activate T helper (T_h) cells, and therefore, they cannot induce immunological memory.[6] This deficiency can be made up by covalently conjugating the carbohydrate antigen with a carrier protein to provide a glycoconjugate vaccine (Fig. 2B).[7] The polysaccharide of this conjugate can react with the BCR to produce IgM response, while the peptide from the carrier protein can engage the T cell receptor (TCR) leading to the secretion of cytokines, inducing the switch from low affinity IgM to high affinity IgG antibodies and eliciting memory T (T_M) and memory B (B_M) cells for a long lasting immune response.

Some specific polysaccharides have been found that can activate the T cells, without conjugation to a protein (Fig. 2C). What these structures all have in common are negatively and positively charged groups, and therefore they are termed as zwitterionic polysaccharide (ZPS). They include a variety of structures, such as Sp1 from *Streptococcus pneumoniae*,

CP5 and CP8 from *Staphylococcus aureus*, and PS A1 from *Bacteroides fragilis* (see chapter 4).[8] ZPSs are regarded as T cell-dependent antigens, and they can be processed by antigen presenting cells (APCs) and presented by MHC-II proteins, leading to T helper cell activation.[9] Based on these unique properties, it has been suggested that ZPSs can be used to replace the carrier proteins to generate entirely carbohydrate-based vaccines.[10]

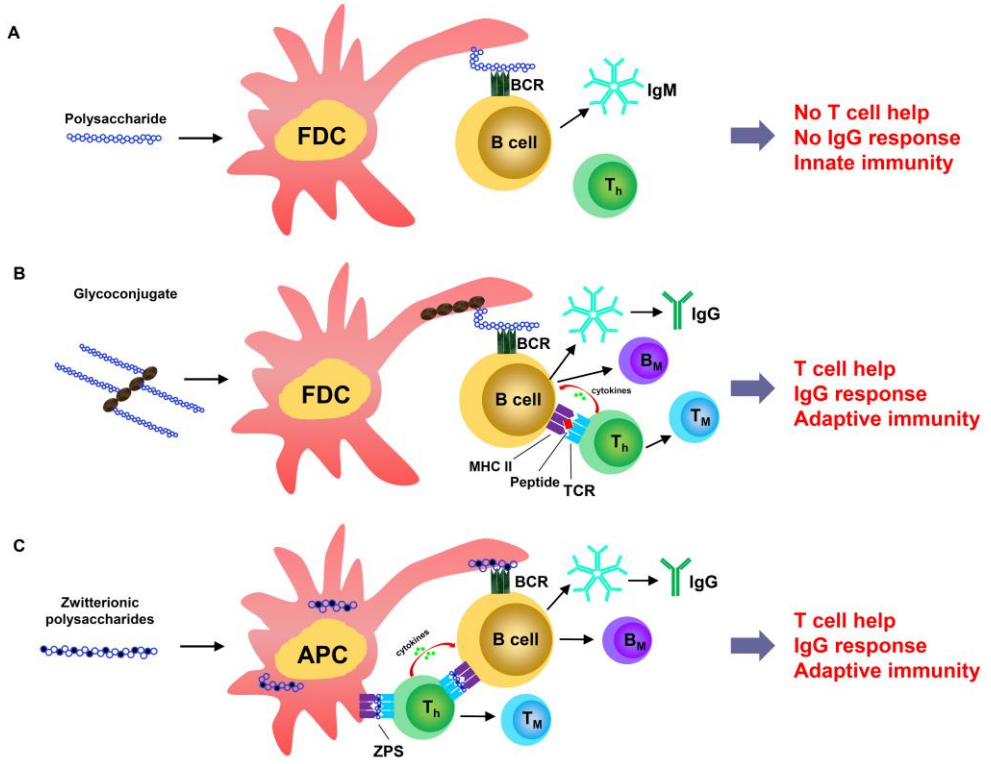


Figure 2. Interactions of polysaccharides and conjugate vaccines with the host immune system. A) Polysaccharides are recognized by a BCR leading to the production of IgM antibodies and no class switch to an IgG-responses or immunological memory. B) Glycoconjugates can not only interact with a BCR to produce the IgM, but also can recruit T cell help to provide the necessary co-stimulation to induce memory B cells and memory T cells to generate a long-lasting immune response. C) Zwitterionic polysaccharides can elicit a T helper cell response, upon presentation of APCs to T cells.

2. Carbohydrate-based Vaccines

Prevention is always better than treatment. Vaccination is a very powerful and selective weapon to combat pathogens. Compared to antibiotics or other medicine, vaccination can be

safer and cheaper. It has resulted in the eradication of smallpox, the near eradication of polio, and prevents numerous other infectious diseases. A vaccine trains the host immune system to recognize and combat pathogens. Carbohydrate-based vaccines aim to generate CWPS-specific antibodies which can provide protection against the targeted pathogen.[11] While glycoconjugate vaccines have provided a massive impact on public health, and every year billions of doses are used to vaccinate against for example *H. influenzae* type B (Hib), *Neisseria meningitidis* and pneumococcus, it is still not fully understood how the host immune system responds and processes these conjugates.

2.1 Carbohydrate-based vaccine development

CWPS often constitute the outermost layer of the cell surface, and it is the first moiety of a pathogen recognized by the host immune system. Therefore it is a natural antigen candidate.[12] The development of capsular polysaccharide (CPS)-based vaccines dates back to 1923 when the CPSs isolated from *Streptococcus pneumoniae* were shown to be immunoreactive.[9c, 13] It was shown in 1930s that carbohydrate-conjugates can induce the production of antibodies both in rabbits and humans.[14] The first CPS-based vaccine was approved by FDA in 1947 against *S. pneumoniae* using isolated pneumococcal polysaccharides. However, with the discovery of antibiotics, vaccine production was stalled in the 1950s. Due to the steady increase of antibiotic-resistant bacterial strains, CPS-based vaccines have received renewed interest. Because, CPSs elicit a poor immune response in children below two years old, vaccine development soon refocused on glycoconjugate vaccines,[14a] and the first CPS-conjugate vaccine was approved in the USA in 1987 for protection against Hib. Inspired by this success, various conjugate vaccines have been developed and licensed resulting in the remarkable reduction of Hib, *S. pneumoniae*, and *N. meningitidis* infections. Customarily, not all serogroups are covered in a vaccine. Among the 13 serogroups of *N. meningitidis*, at present, the inclusion of serotypes A, C, Y and W135 in the approved conjugate vaccines, like Menveo® (GSK), Menactra® (Sanofi Pasteur) and Nimenrix® (Pfizer) have effectively prevented most infections by this bacterium.

2.2 Novel synthetic glycoconjugate vaccine development

Although most of the above mentioned vaccines are based on isolated polysaccharides, vaccines based on synthetic oligosaccharides are being widely explored in recent years.[15] Synthetic oligosaccharide can be an attractive candidate because it guarantees minimal batch-to-batch variation, allows more controlled and precise conjugation chemistry and it can be used for detailed structure-activity relationships, for example, establishing minimal epitopes that can be used in vaccine optimization.

Taking advantage of ever more sophisticated strategies in carbohydrate synthesis, including one-pot sequential glycosylation reactions[16], (automated) solid-phase[17] and chemo-enzymatic syntheses[18], many oligosaccharides have been obtained to generate new vaccine candidates. The first licensed synthetic conjugate vaccine, Quimi-Hib® was developed in Cuba against the bacterium *Hemophilus influenza* type b (Hib). In this vaccine, the Hib ribose-ribitol phosphate oligomer is conjugated to the carrier protein tetanus toxin (TT) through a spacer attached at a pre-defined position of the oligosaccharide[19] (Fig. 3A). In 1974, Bundle and co-workers established that the CPS of the serotype A of *N. meningitidis* (MenA) as a structure composed of 1,6-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units (Fig. 3B).[20] Due to the instability of the anomeric phosphodiester, 1-C-phosphono analogues of MenA CPS were developed, which were conjugated to human serum albumin (HAS) to provide a vaccine entity, featuring a CPS mimic (as opposed to a close copy) (Fig. 3B). The immune response of the mimic showed promising responses in vivo.[21] A fully synthetic conjugate vaccine against *N. meningitidis* serotype C has also been developed. Guo and co-workers generated oligo-(2,8)-sialic acids, which were linked to monophosphoryl lipid A (MPLA) to provide a vaccine entity, capable of eliciting a robust immune responses (Fig. 3C).[22] Many CPSs of various bacteria have been synthesized over the years to develop synthetic conjugate vaccines, some of which are outlined in more detail below.

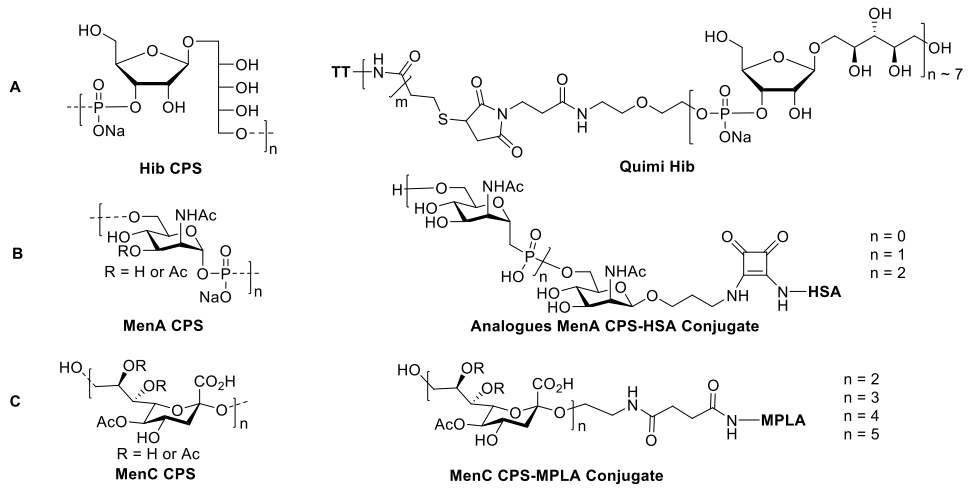


Figure 3. Structures of the repeating unit of several gram-negative bacteria CPS and their synthetic conjugate vaccines.

3. Examples of synthetic oligosaccharide vaccine for *Streptococcus*

Streptococci can cause a wide variety of fatal diseases, such as pneumonia, septicemia, meningitis, rheumatic fever and glomerulonephritis, and it tends to infect individuals with a weaker immune system, such as infants, young children, pregnant women, and the elderly.[23] Based on their hemolytic properties, they can be classified into three categories. The α-hemolytic species, such as *S. pneumoniae* and *S. viridans*i, show a greenish color on blood agar caused by the oxidization of iron in red blood cells; β-hemolytic species, such as Group A, B to V *Streptococci*, lead to a clear zone on blood agar due to complete rupture of red blood cells; γ-hemolytic species cause no hemolysis. Clinically, the most important bacteria are *S. pneumoniae*, *S. viridans*, Group A *Streptococcus* and Group B *Streptococcus*. In this chapter, examples of the CWPS syntheses of *S. pneumoniae*, GAS and GBS are provided.

3.1 Group A *Streptococcus* (GAS)

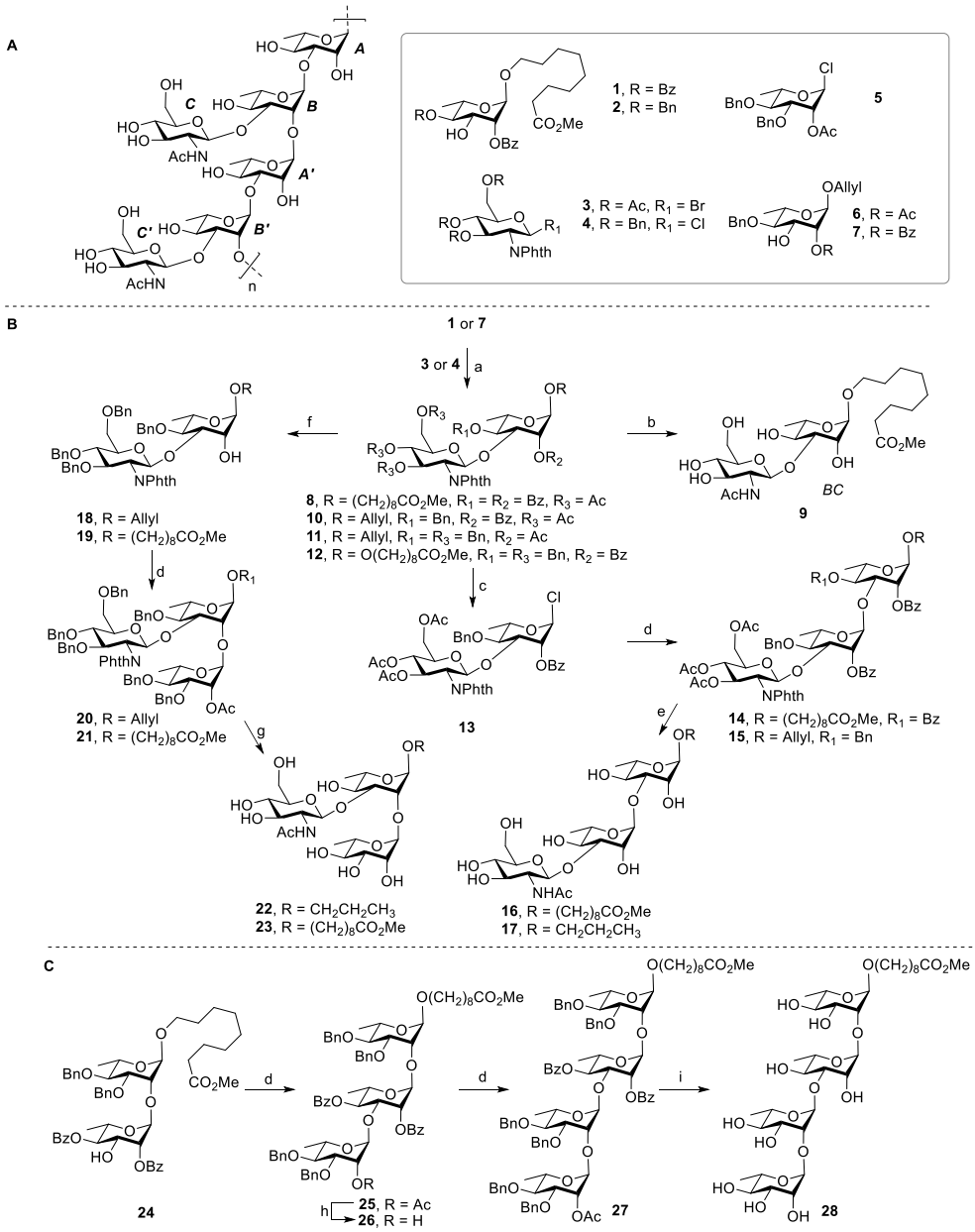
GAS also known as *Streptococcus pyogenes*, is an important human pathogen and ranks among the top ten causes of mortality and morbidity from an infectious disease. Although the GAS vaccines has been in development for almost a century, no vaccine has so far been licensed for use in humans.[24] The group A carbohydrate (GAC) was found to elicit immune responses in rabbits, mice and human,[25] and its structure, defined in 1970s, is composed of a polyrhamnose backbone containing immunodominant GlcNAc appendages[26] at the 3-position of the rhamnose residue (Scheme 1A).[27] So far, a variety of synthetic approaches including [3 + 1], [1 + 3], [3 + 2], [3 + 3], [3 + 6], [4 + 5] and [3 + 9] assembly strategies, have been developed to generate GAC fragments from di- up to dodecasaccharides.

The first synthesis of a GAC fragments was accomplished in 1981 by the Bundle group, who synthesized *BC* disaccharide **9** and tetrasaccharide *ABA′B′* **28**, representing a small rhamnose backbone (Scheme 1B and 1C).[28] In a Koenigs–Knorr reaction, rhamnose acceptor **1** and glucosyl bromide donor **3** were condensed using silver trifluoromethanesulfonate (AgOTf) and 2,4,6-trimethylpyridine (collidine) to give disaccharide **8** in 90% yield. A carboxylic ester terminated linker was used to allow for later modifications. Removal of the acetyl and benzoyl groups using NaOMe in MeOH, followed by deprotection of the phthaloyl group using hydrazine hydrate and acetylation of the released amine gave the deprotected disaccharide **9**. Meanwhile, a longer fragment of the rhamnose backbone was generated employing a Koenigs–Knorr glycosylation reaction between disaccharide acceptor **24** and rhamnose chloride donor **5** using AgOTf and tetramethylurea to provide trisaccharide **25** in 81% yield. The acetyl group was selectively removed by treatment with magnesium methoxide to generate trisaccharide acceptor **26**,

which could be glycosylated with donor **5** leading to tetrasaccharide **27** in 85% yield. The deprotected tetrasaccharide **28** was obtained after the global deprotection by subsequent basic hydrolysis and hydrogenation.

In the GAC structure, two trisaccharides can be recognized as repeating units, the linear *ABC* trimer or branched *B(C)A'* moiety. The linear *ABC* **16** and **17** were first constructed by the Pinto group via a [2 + 1] strategy (Scheme 1B).[29] Rhodium(I)-catalyzed isomerization and hydrolysis of the anomeric allyl group of disaccharide **10**, followed by a Vilsmeier-Haack reaction provided disaccharide chloride **13**. Then, glycosylation with rhamnose acceptors **1** or **7**, bearing different linkers, led to trisaccharides **14** and **15**. The deprotections were performed via methanolysis, hydrazinolysis, *N*-acetylation and hydrogenolysis to afford the trisaccharide as its 8-methoxycarbonyloctyl glycoside **16** for the preparation of conjugates and its propyl glycoside **17** for use as hapten for immunochemical studies. Besides, the branched *B(C)A'* **22** and **23** were prepared by the same group using a [1 + 2] pathway. Glycosylation of the monosaccharide donor **4** with rhamnose **6** or **2**, followed by acetyl removal gave disaccharide acceptors **18** and **19**. Treatment of these two acceptors with donor **5** under the silver triflate catalysis afforded the protected branched trisaccharides **20** and **21** in 81% and 62% yield, respectively. Deprotection of **20** and **21** was accomplished by methanolysis, hydrogenation, hydrazinolysis and *N*-acetylation to provide the trisaccharides **22** and **23**.

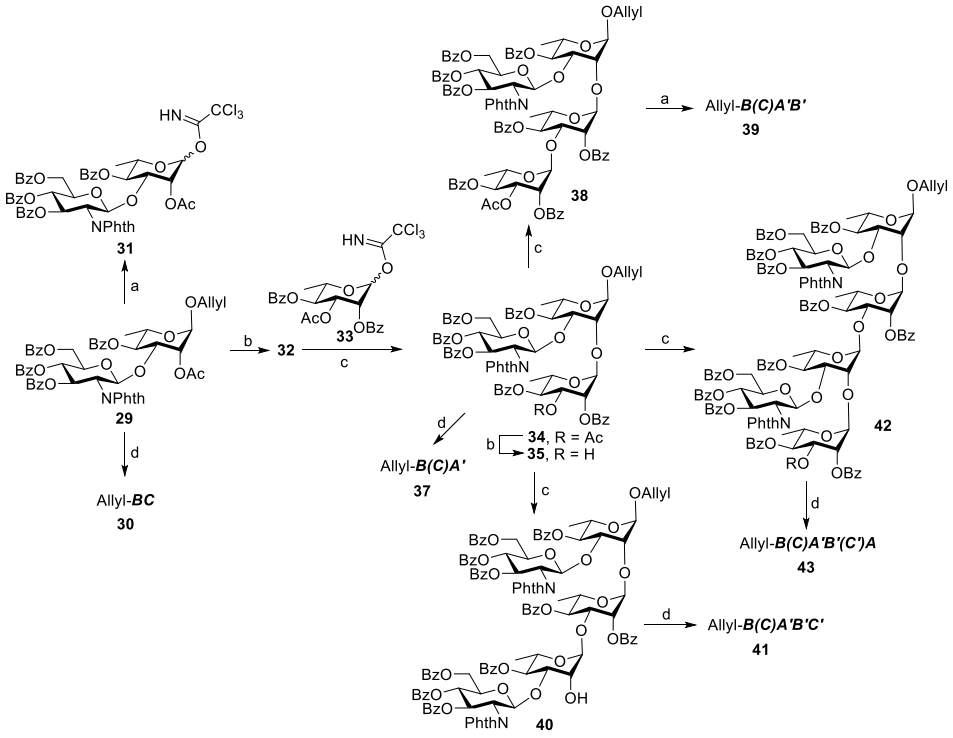
Next, pathways for the longer and modifiable GAC oligosaccharides were developed. Adopting the branched *B(C)A'* building block, several GAC fragments were generated via [1 + 3], [2 + 3] and [3 + 3] strategies (Scheme 2).[30] Trisaccharide **34** contains an allyl group on its anomeric position that could be used as a temporary protecting group for the assembly of longer oligosaccharides or as a permanent functionality that allows for conjugation of the synthetic antigens. Trisaccharide acceptor **35** could be obtained from **34** by removal of the acetyl group using HCl in MeOH. For ensuing further glycosylations, both the disaccharide **29** and trisaccharide **34** were converted to the corresponding imidate donors **31** and **36**, via the isomerization of the anomeric allyl group by Wilkinson's catalyst and hydrolysis of the so-formed enol ether, followed by the installment of trichloracetimidate moiety. Subsequently, the trisaccharide **35** was glycosylated with mono-, di- or trisaccharide imidates **31**, **33** and **36** under catalysis of TESOTf to construct tetra-, penta- and hexasaccharides **38**, **40** and **42**, respectively. Global deprotection and *N*-acetylated generated the allyl glycosides **30**, **37**, **39**, **41** and **43** in good yield.



Scheme 1. The structure of GAC and synthesis pathways of di-, tri, and tetrasaccharides

Reagents and conditions: a) AgOTf, collidine, 4Å MS, DCM, **8**, 90%; **10**, 90%; **11**, 61%; **12**, 57%. b) i, NaOMe, MeOH; ii, hydrazine hydrate, EtOH; iii, Ac_2O, MeOH, 80% (over three steps). c) i, $RhCl(PPh_3)_3$, EtOH, water, 86%; ii, HgO, $HgCl_2$, acetone, water, 93%; iii) Oxalyl chloride, *N*,*N*-dimethyl(chloromethyl-1-ene)ammonium chloride, DMF, DCM, 99%. d) AgOTf, tetramethylurea, DCM, -78 °C to rt, **14**, 53%; **15**, 76%; **20**, 81%; **21**, 62%; **25**, 81%; **27**, 85%. e) i, NaOMe, MeOH; ii, hydrazine hydrate, EtOH; iii, Ac_2O, MeOH; iv, Pd/C, H_2, AcOH, water, 75% (over

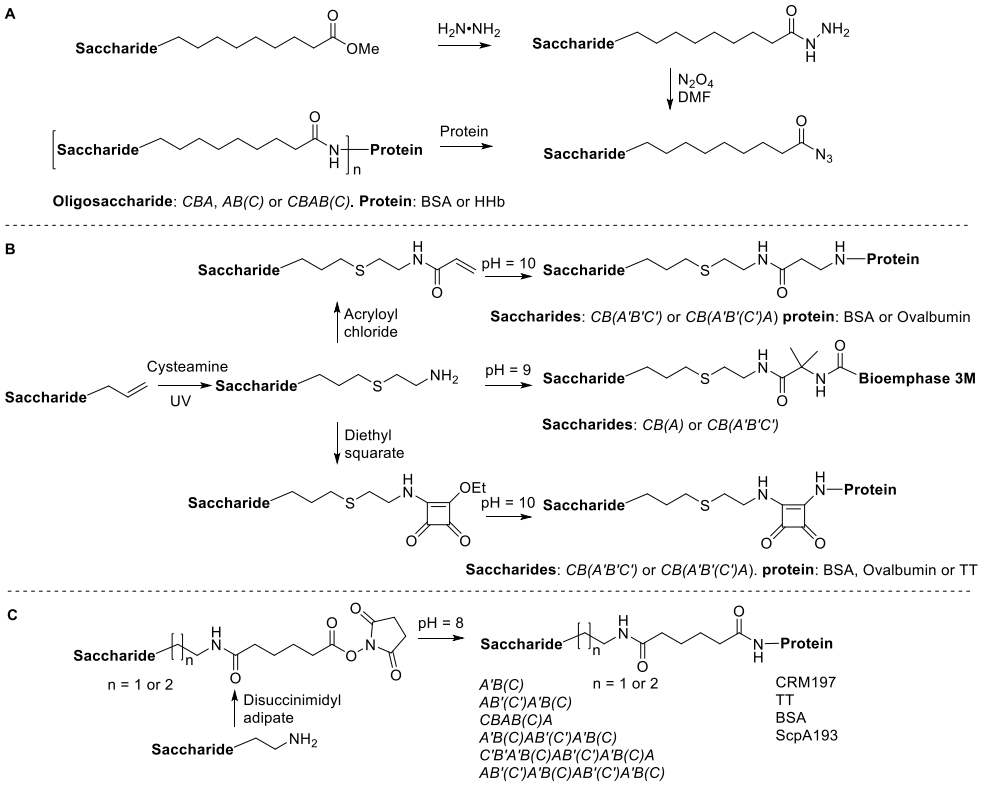
four steps). f) HCl in MeOH, **18**, 90%; NaOMe, NaOH, **19**, 58%. g) i, HCl in MeOH or NaOMe, MeOH; ii, Pd/C, H_2, AcOH, water; iii, N_2H_2•H_2O, EtOH; iv, Ac_2O, MeOH, **22**, 49%; **23**, 33% (over four steps). h) $Mg(OMe)_2$, MeOH, THF, 0 °C, 85%. i) i, Pd/C, AcOH; ii, NaOMe, MeOH, 63% (over 2 steps).



Scheme 2. The assembly of fragments of GAC using the branched *B(C)A'* as building block Reagents and conditions: a) i, $RhCl(PPh_3)_3$, 1,4-diazabicyclo[2.2.2]octane (DABCO), EtOH, water; ii, HgO, $HgCl_2$, acetone, water, 74%; iii, Cl_3CCN, K_2CO_3 or DBU, DCM, 76%. b) HCl in MeOH, **32**, 81%; **35**, 84%. c) TESOTf, DCM, -78 °C, 4Å MS, **34**, quant.; **38**, 80%, **40**, 76%; **42**, 43%. d) i, NaOMe, MeOH; ii, ethylenediamine, EtOH, reflux; iii, Ac_2O, MeOH, **30**, 68%; **37**, 70%; **39**, 88%; **41**, 86%; **43**, 84% (over four steps).

In comparing to the use of the branched *B(C)A'* trisaccharide repeating unit, the linear *CBA* trimer allows for effective neighboring group participation to control all the construction of the required 1,2-*trans* glycosidic bonds. The Pinto's group has assembled a variety of GAC fragments, such as *AB(C)A'*, *B(C)A'B'C'*, and *AB(C)A'B'C'* using the linear trisaccharide via [1 + 3],[31] [3 + 2][32] and [3 + 3][33] glycosylation strategies. So far, the largest GAC fragment was synthesized by Costantino and co-workers using the linear trisaccharide as repeating unit in a [3 + 3 + 3 + 3] glycosylation pathway to provide a dodecasaccharide.[34] Recently, Guo's group has also accomplished tri-, hexa- and nonasaccharides of GAC via a highly convergent strategy.[35]

To develop a GAS vaccine, many synthetic oligosaccharides were conjugated with different proteins. The carboxylic esters of the tri- and pentasaccharide, generated by the groups of Bundle and Pinto, were transformed via hydrazide derivatives to acyl azide intermediates[36] which were coupled to lysine residues of bovine serum albumin (BSA) and horse hemoglobin (HHb) (Scheme 3A).[37] For the allyl glycosides, different conjugation methods were developed to attach the oligosaccharides to both proteins or solid supports (Bioemphase 3M) to generate immunoaffinity columns (Scheme 3B).[38] Cysteamine hydrochloride was first added to the allyl glycosides, and then the adducts could be further functionalized via the introduced amine functionality. Different procedures were performed for conjugation of the penta- and hexasaccharides with proteins, such as BSA, ovalbumin and TT.[39] The ε-amino group of the protein lysines could be used for Michael-type addition[40] with the double bond of the *N*-acryloylated oligosaccharides, generated from the cysteamine adducts. Alternatively, the amines were linked with 3,4-diethoxy-3-cyclobutene-l,2-dione (diethyl squarate) and then coupled to the proteins. Use of the squarate adducts led to the incorporation of more glycans per protein. The immunogenicity of a hexasaccharide-TT glycoconjugate was confirmed in mice, inducing effective immunological memory. Disuccinimidyl adipate has also been used as a cross-linker to conjugate a hexa- and two dodecasaccharides with CRM197 (Scheme 3C).[34] Compared to conjugates of the bacterial polysaccharide, the oligosaccharide conjugates displayed similar immunogenicity and elicited comparable specific IgG titers in mice. Recently, branched tri-, hexa- and nonasaccharides, equipped with an amino spacer were conjugated with four different proteins, including CRM197, TT, BSA and Group A streptococcal C5a peptidase (ScpA193).[35] The latter protein was shown to be highly immunogenic and could be used to elicit specific antibodies that can inhibit streptococcal colonization (Scheme 3C). The ScpA193 was proved to be an effective carrier protein and its activity of boosting the immunogenicity was better than or at least comparable to CRM197 and TT. Because both the oligosaccharide and protein are derived from GAS, the ScpA193-conjugate could be functionalized as a promising bi-valent vaccine.



Scheme 3. Conjugation of the GAC fragments with various proteins or solid phase material

3.2 Group B *Streptococcus* (GBS)

Similar to GAS, infections of GBS, also known as *Streptococcus agalactiae*, represent a significant global public health problem, and a major cause of infections for pregnant women and newborns, but also immunocompromised people.[41] Intrapartum antibiotic prophylaxis (IAP) can substantially reduce early-onset infection of newborns, but do not present a solution for late onset infections (after 7 days of life) or later in life. So far, no vaccine is commercially available although much effort is being undertaken for this direction.[42] The capsular polysaccharides of GBS have been shown to be a major virulence factor, and it has been proposed that GBS uses its polysaccharide capsule to circumvent innate immune defenses of the host.[43] Based on the structure of the CPS (Figure 4A), currently, ten identified serotypes (Ia, Ib, and II to IX) have been described.[44] Serotype III is the most dominant serotype causing infection and colonizing 28% of mothers worldwide, with the serotypes Ia, Ib, II and V together responsible for more than 95% infections of GBS worldwide.[45]

The CPS of these serotypes represent promising vaccine candidates, as first demonstrated in 1930s by Rebecca Lancefield with CPS-specific protective rabbit sera.[46] Nowadays, a major focus is on the development of GAS glycoconjugate vaccines.[47] In 1996, the first glycoconjugate vaccine trial in humans was reported, conjugating type III CPS to a carrier protein TT (III-TT), which proved to be well-tolerated and immunogenic.[48] Afterwards, diverse monovalent and multivalent conjugate vaccines have been prepared and tested in preclinical or phase I/II clinical trials, including a CPS-CRM197 trivalent (serotypes Ia, IIb, III) and pentavalent (Ia, IIb, II, III, V) developed by Novartis/GSK.[49] These vaccines used CPSs, isolated from bacteria, but there is also significant interest in synthetic oligosaccharide conjugate vaccines. The structure of all CPS serotypes is made up of tetra- to heptasaccharide repeating units containing *N*-acetyl-D-neuraminic acid, L-rhamnose, D-galactose, D-glucose, *N*-acetyl-D-galactosamine and *N*-acetyl-D-glucosamine (Figure 4A). Besides the above CPSs, all GBS strains express the group B-specific antigen (GBC), first isolated by Jennings and co-workers (Figure 4B and Chapter 2).[50] This multiantennary structure is composed of L-rhamnose, D-galactose, D-*N*-acetylglucosamine and D-glucitol, with phosphate joints between different subunits. The synthesis of cell wall carbohydrate of GBS can be dated back to the 1980s, when several fragments of substructure III of GBC were accomplished with the assembly of a rhamnose trisaccharide and an oligosaccharide including the characteristic glucitol.[51]

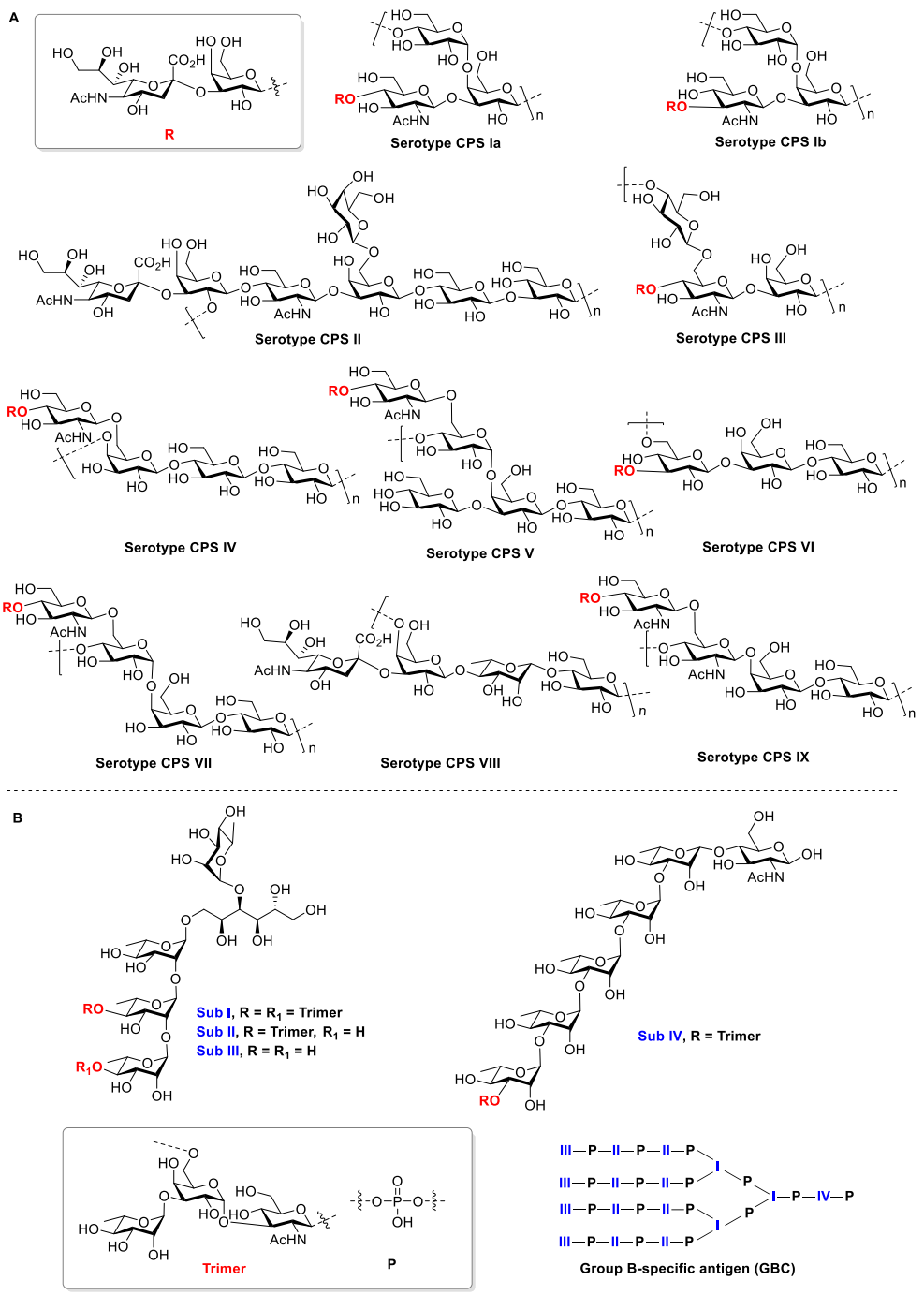
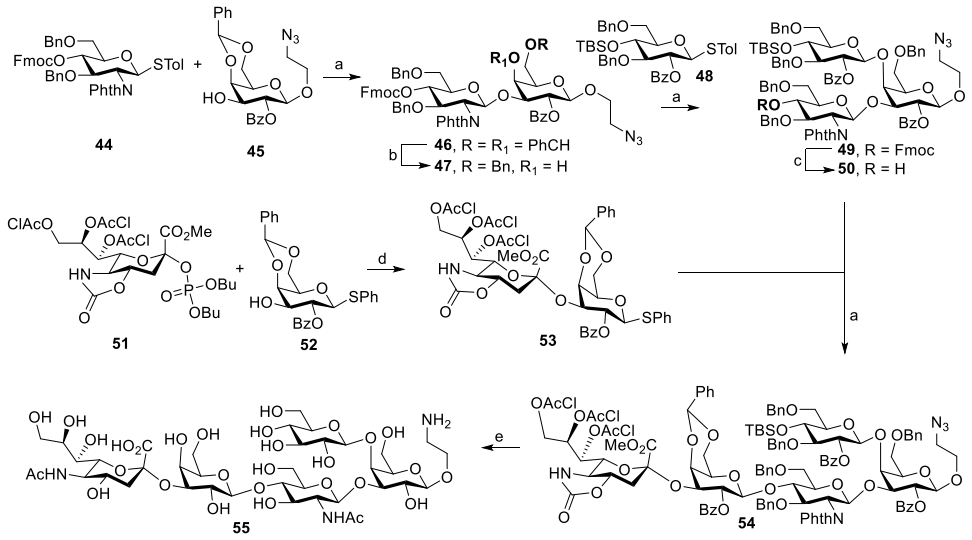


Figure 4. Structure of cell wall carbohydrate antigen of GBS

Serotype Ia and Ib

Even though the structure of the CPS serotypes were defined many years ago, and a chemoenzymatic synthesis strategy for the assembly of a type Ia CPS hexasaccharide was reported in 1996,[52] the first chemical synthesis of the type Ia repeating unit was not accomplished until 2015 when Guo's group assembled the pentasaccharide[53]. After probing various glycosylation strategies, the pentasaccharide repeating unit of serotype Ia CPS was obtained employing a convergent [2 + 3] pathway with a sialogalactoside disaccharide as the donor and a branched trisaccharide as the acceptor (Scheme 4). It is worth mentioning that the monosaccharide at the C-4-*O*-position of galactose had a big impact on the glycosylation of the 3-hydroxyl group, while the monosaccharide at the C-3 hydroxyl had little influence on the glycosylation of the C-4 alcohol. The synthesis commenced with the preparation of branched trisaccharide **50**. Glycosylation of **44** with **45** under the promotion of NIS/AgOTf provided **46** in 75% yield. Regioselective opening of the benzylidene ring, followed by glycosylation with **48** generated trisaccharide **49**. The branched acceptor **50** was generated by Fmoc-removal with triethylamine. The key [2 + 3] glycosylation was performed in the presence of NIS and AgOTf to yield **54** in 55% yield using acceptor **50** and sialogalactose disaccharide donor **53**, which was synthesized from **51** and **52**. Global deprotection of **54** was accomplished by desilylation, basic hydrolysis, acetylation and hydrogenation to provide the desired pentasaccharide **55**, which contained a free amino group at the reducing end for the further conjugation.



Scheme 4. Synthesis of the repeating unit of serotype **Ia** GBS CPS

Reagents and conditions: a) NIS, AgOTf, 4Å MS, DCM, **46**, 75%; **49**, 72%; **54**, 55%. b) $NaBH_3CN$, HCl, 4Å MS,

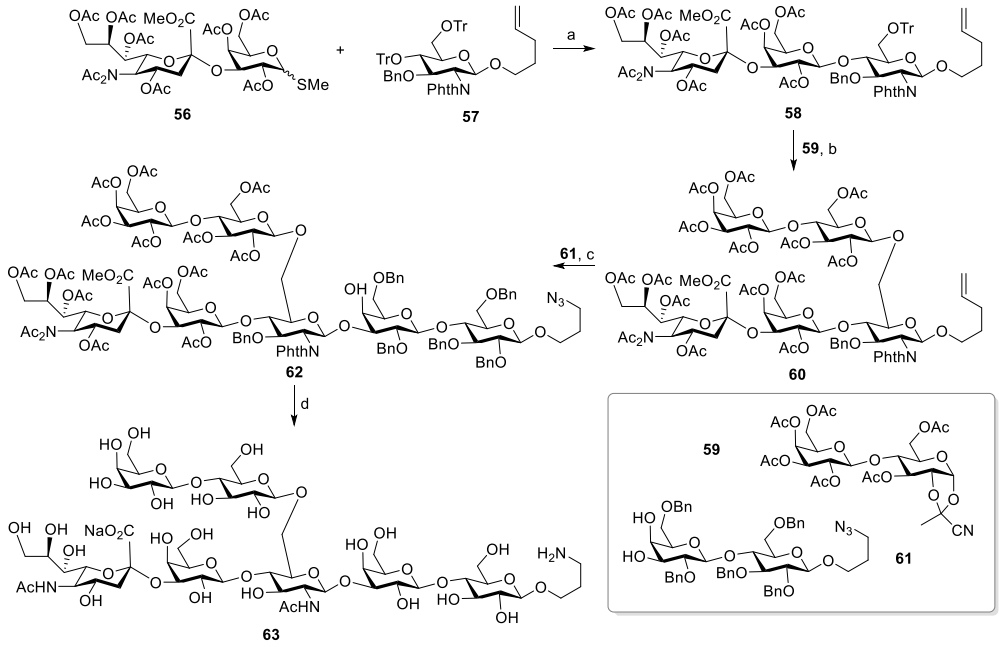
DCM, 95%. c) Et_3N, DCM, 100%. d) TMSOTf, DCM/MeCN, 4Å MS, 64%. e) i, Et_3N•3HF, THF; ii, LiOH, MeOH, H_2O, NH_2NH_2•H_2O; iii, Ac_2O, Pyridine; iv, NaOMe, MeOH; v, Pd/C, H_2, MeOH, 67% (over five steps).

Afterwards, more fragments of CPS type Ia were assembled including a decasaccharide composed of two repeating units,[54] a linear pentasaccharide repeating unit and a non-sialylated tetrasaccharide[55]. The synthesis of the decasaccharide was accomplished employing a convergent [2 * 2 + 6] dual glycosylation strategy using a double glycosylation with two copies of the sialogalactoside disaccharide donor and the branched hexasaccharide acceptor, which was generated using a [1 + 2 + 3] one-pot two-step synthesis pathway. Based on the successful [2 + 3] approach described above, the linear pentasaccharide repeating unit and the non-sialylated fragment were constructed by regioselective glycosylations using a trisaccharide diol containing a free Glc C3-OH and a free Gal C4-OH, exploiting the higher reactivity of the former alcohol over the latter one. The minimal structural difference between CPS Ia and Ib, (*i.e.* the 1-4 or 1-3 linkage of the sialogalactoside to the GlcNAc moiety), the synthesis of the repeating unit of CPS Ib, a branched and a linear pentasaccharide, containing a free amino group for future conjugation with carrier proteins, were accomplished employing similar strategies.[55]

Serotype III

On account of being the most prevalent and virulent, serotype III CPS has been the most studied CPS of the GBS polysaccharides. As early as 1990, desialylated tri- and tetrasaccharide fragments of the CPS were chemically synthesized by the group of Jennings.[56] Based on this success, one year later, the first complete repeating unit, a pentasaccharide, was achieved employing a combined chemical and enzymatic synthesis strategy, using a specific rat liver sialyltransferase to install the α-NeuNAc-moiety.[57] Taking advantage of this chemoenzymatic approach, two decasaccharides, representing two repeating units, were generated via enzymatic sialylation of the two terminal galactose residues of an octasaccharide.[58] A complete chemical synthesis of a heptasaccharide carrying an artificial spacer was accomplished in Boons group through a highly convergent strategy using a sialogalactosyl thioglycosyl donor and a *n*-pentenyl glucosamine building block (Scheme 5).[59] A chemoselective glycosylation of the thioglycoside donor **56** with di-*O*-tritylated acceptor **57** in the presence of MeOTf provided trisaccharide **58** with absolute regio- and stereoselectivity in excellent yield. The reactivity of C-4 hydroxyl in **57** is increased by tritylation due to the steric requirements of the C-4-*O*-trityl ether, resulting in elongation and polarization of the C-O bond of the secondary trityl ether. Next, the primary trityl ether was glycosylated with disaccharide donor **59** to assemble pentasaccharide **60** in

82% yield. Synthesis of the fully protected heptasaccharide **62** was accomplished employing the regioselective glycosylation of the *n*-pentenyl pentasaccharide donor **60** with disaccharide acceptor **61** under the promotion of NIS/TMSOTf. In the end, the target heptasaccharide **63** was obtained in 31% yield after a five-step deprotection, including removal of the esters, one sialyl *N*-acetyl group and the phthalimido group, *N*-acetylation of the glucosamine residue and hydrogenation of the benzyl ethers and azide. To detect antibodies in serum of pregnant women against the CPS type III by ELISA assay, the heptasaccharide was coupled to a poly(*N*-acryloyloxy)-succinimide polymer using the aminopropyl spacer.



Scheme 5. Synthesis of the repeating unit of serotype III GBS CPS

Reagents and conditions: a) MeOTf, 3Å MS, DCM, 96%. b) $TrClO_4$, DCM, 82%. c) NIS, TMSOTf, 3Å MS, 62%. d) i, LiI, pyridine; ii, ethylenediamine, *n*-butanol, H_2O; iii, Ac_2O, MeOH; iv, Pd/C, H_2, EtOH; v, 1N aq NaOH, 31%.

To explore the minimal structural requirements for antibody binding, various fragments of the type III CPS and mimics thereof were assembled, including a sialotrisaccharide, different pentasaccharides and a desialylated hexasaccharide.[60] Interestingly, the oligosaccharides of type III CPS could also be transformed to *S. pneumoniae* type 14 glycans using an enzymatic approach.[61] Recognition of the glycoconjugates with polyclonal CPS III specific serum indicated that the Glc β-(1→6) branch to GlcNAc is an important motif for antibody binding.[60b, 62]

3.3 *Streptococcus pneumoniae*

Streptococcus pneumoniae is a gram-positive bacterium causing fatal pneumonia, septicemia, otitis media and meningitis.[63] Pneumococcal infections lead to a very high morbidity and mortality rate worldwide, especially occurring in individuals with weaker immune systems, such as infants, young children and the elderly. The highly variable CPSs of *S. pneumoniae* have been identified to be one of the most important virulence determinants of pneumococci. The first-generation carbohydrate-based vaccine PPV23 which contains the 23 most prevalent serotypes, was developed by Merck and approved in the United States and in Europe in 1983.[64] However, PPSV23 cannot elicit a protective immune response in children, younger than 2 years old. To improve the immunogenicity, the second-generation pneumococcal conjugate vaccines (PCV7, PCV10 and PCV13, approved in 2010) were developed for use in children younger than 2 years of age. The vaccines PPSV23 (containing 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F) and PCV13 (containing 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) cover the most important, but not all of the 90 serotypes.[65]

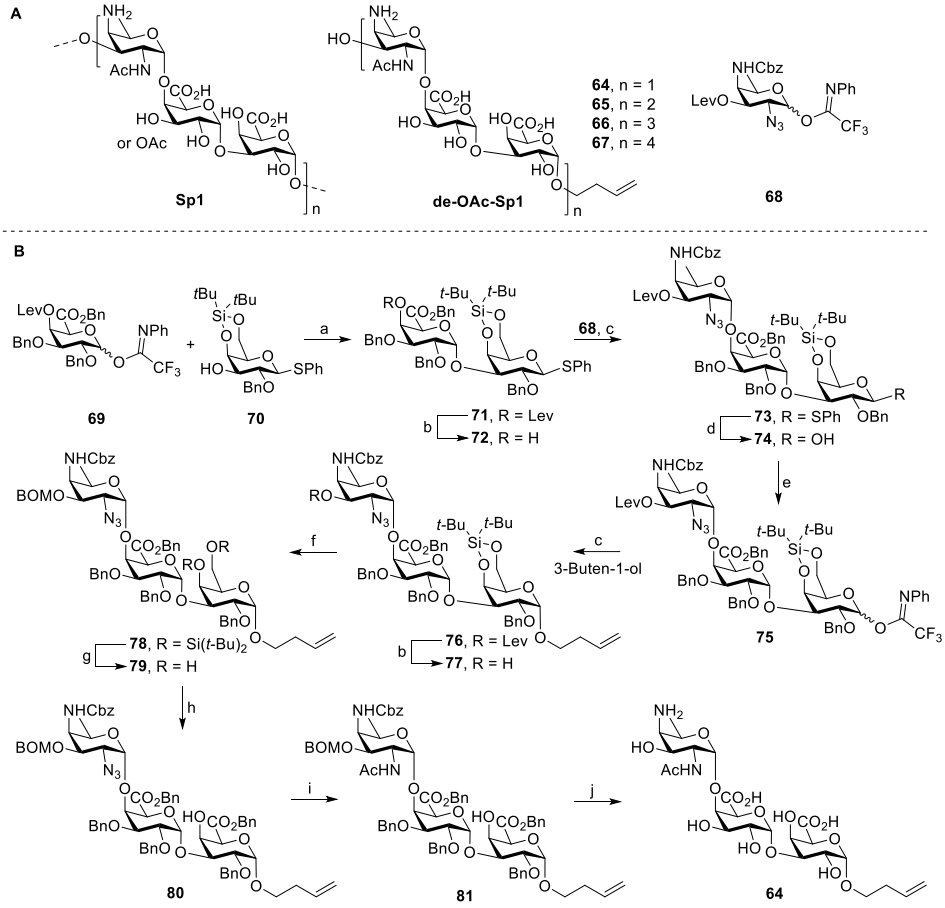
As described above most CPSs are T-cell-independent antigens which cannot lead to immunological memory without conjugating to a protein.[8a, 66] However, the serotype 1 CPS of *S. pneumoniae* (Sp1) is a zwitterionic polysaccharide (ZPS), which can provoke a T-cell dependent immune response as mentioned above (Figure 2). Sp1 is a linear polysaccharide consisting of a trisaccharide repeating unit, which contains two galacturonic acids and a rare 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (D-AAT) monosaccharide (Scheme 6A).[67] The structure presents three major challenges to a synthetic pathway, including stereoselective construction of the 1,2-*cis* glycosidic bonds, the introduction of the two uronic acids moieties and the rare AAT monosaccharide. Bundle and co-workers reported the first synthesis of Sp1-fragments with the assembly of oligosaccharides one and two repeating units.[68] The α-stereoselectivity of all glycosylations was achieved using remote participation effects. The uronic acids were introduced by the simultaneous oxidation of the two and four primary alcohols of trisaccharide and hexasaccharide, respectively, using a post glycosylation−oxidation strategy. Christina *et al.* reported the synthesis of all three possible frame-shifted Sp1 trisaccharides employing galacturonic acid-[3,6]-lactone as building blocks.[69] Longer oligomers could not be obtained using this approach because of poor selectivity in the crucial glycosylation reaction.

Recently, the assembly of larger fragments of Sp1, trisaccharide **64** up to dodecasaccharide **67**, were accomplished by Zhang *et al.* using a combination of a pre glycosylation−oxidation

with a post glycosylation−oxidation strategy to introduce the carboxylate groups (Scheme 6).[70] Crucial in the approach was the use of a trisaccharide featuring a silylidene protected galactose donor moiety to construct the 1,2-*cis* glycosydic linkages between the trimer repeating units. To generate the trimer repeating unit, first a glycosylation between galacturonic acid **69** and galactose acceptor **70** were performed to provide disaccharide **71** in 77% yield with 13:1 α/β stereoselectivity. Delevulinoylation and glycosylation with D-AAT donor **68** obtained the desired trisaccharide **73** in 85% yield with the same stereoselectivity. Next, this intermediate was transformed into trisaccharide imidate donor **75**. The spacer was stereoselectively glycosylated with donor **75** to furnish **76** as a single anomer. Removal of the levulinoyl group provided the trisaccharide acceptor **77**, which was used to generate the trisaccharide target, and for further elongated to acquire the longer oligosaccharides. To prevent the formation of the cyclic C-3-*O*-C-4-*N*-carbamate[69,71] during further modifications, a benzyloxymethyl group was installed on the free alcohol of **77**. Next, removal of the silylidene group, followed by regioselective oxidation of **79** using the TEMPO/BAIB reagent combination and benzylation gave trisaccharide **80** in good yield. The azide group of **80** was transformed into an acetamide using thioacetic acid. Finally, the global deprotection was accomplished by basic hydrolysis and Birch reduction to finish the trisaccharide **64**.

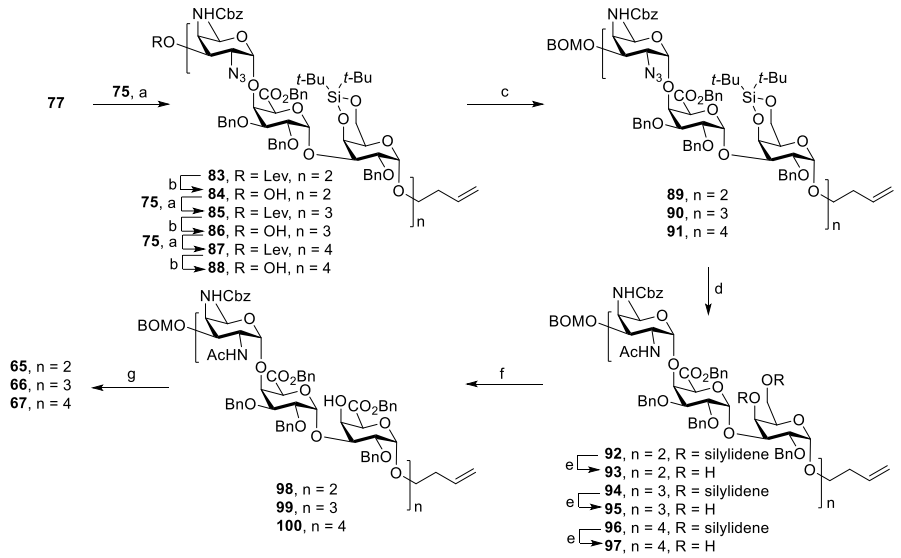
According to the successful synthesis of **64**, a next round of glycosylation with trisaccharide donor **75** and deprotection reactions provided the longer oligosaccharide hexasaccharide **83**, nonasaccharide **85** and dodecasaccharide **87** with good yield and excellent stereoselectivity (Scheme 7). However, to transform the generated oligomers into the target hexa-, nona- and dodecasaccharides, important improvements to the oxidation protocol had to be implemented as the TEMPO/BAIB oxidation conditions were not suitable to oxidize the multiple alcohols of the longer oligosaccharides. After a series of optimizations, the oxidation of the longer oligosaccharides was accomplished using a TEMPO/BAIB mediated oxidation under basic conditions, followed by benzylation using phenyldiazomethane, which provided a better yield than the use of benzyl bromide and K_2CO_3. Deprotection of the target oligosaccharides led to hexasaccharide **65** in 39% yield, nonasaccharide **66** in 55% yield and dodecasaccharide **67** in 47% yield. Structural studies and antibody recognition experiments with these oligosaccharides were conducted using NMR experiments, molecular dynamics (MD) calculations and ELISA experiments. It was revealed that the nona- and dodecasaccharides completed a full helical turn and showed better binding with both human and murine antibodies than the shorter oligosaccharides. The nonasaccharide may be an attractive candidate for the generation of an anti-Sp1 vaccine, representing the minimal epitope containing all structural features of the polysaccharide. To

this end, the alkene spacer can be used for conjugation purposes using a thiol-ene reaction.



Scheme 6. Synthesis of the monomeric repeating unit of Sp1 **64**

Reagents and conditions: a) TfOH, DCM, - 78 °C, 77%, α:β = 13:1. b) N_2H_4•H_2O, pyridine, AcOH, **72**, 89%; **77**, 98%. c) TBSOTf, DCM, 0 °C, **73**, 85%, α:β = 13:1; **76**, 82%, α only. d) NIS, TFA, DCM, 96%. e) *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone, 89%. f) BOMCl, DIPEA, TBAI, DCM, 89%. g) HF•Py, pyridine, THF, 94%. h) i. TEMPO, BAIB, DCM/tBuOH/H_2O, 4 °C; ii. Cs_2CO_3, BnBr, DMF, 84%. i) AcSH, pyridine, rt, 20 h, 66%. j) i, 1 M NaOH, THF, MeOH; ii, Na, NH_3 (liq.), THF, *t*-BuOH, allylcarbinol, 95%.



Scheme 7. Synthesis of the mono-, di-, tri- and tetrameric repeating unit of Sp1 **65**, **66**, **67**

Reagents and conditions: a) TBSOTf, DCM, **83**, 83%; **85**, 80%; **87**, 72%. b) N_2H_4•H_2O, pyridine, AcOH, **84**, 97%; **86**, 89%; **88**, 91%. c) BOMCl, DIPEA, TBAI, DCM, **89**, 81%; **90**, 89%; **91**, 84%. d) i, PPh_3, pyridine, H_2O, THF; ii. Ac_2O, pyridine, **92**, 93%; **94**, 88%; **96**, 99%. e) HF•Py, pyridine, THF, **93**, 91%; **95**, 88%; **97**, 91%. f) i. TEMPO, BAIB, $NaHCO_3$, EtOAc/*t*-BuOH/H_2O, 4 °C; ii. Cs_2CO_3, BnBr, DMF, or $PhCHN_2$, DCM, Et_2O, **98**, 45%; **99**, 51%; **100**, 49% (over two steps). g) i, 1 M NaOH, THF, MeOH; ii, Na, NH_3 (liq.), THF, *t*-BuOH, allylcarbinol, **65**, 39%; **66**, 55%; **67**, 47% (over two steps).

4. Aim and outline of this thesis

The aim of this thesis is to develop synthetic approaches to generate streptococcal oligosaccharides, which can be used to unravel structure-activity relationships and eventually to generate well-defined synthetic glycoconjugate vaccines. **Chapter 1** introduces the use of isolated and synthetic carbohydrates in vaccine development. Several syntheses of streptococcal CPSs are summarized to show the state-of-the-art in oligosaccharide synthesis and illustrate the use of synthetic oligosaccharides in the generation of anti-streptococcal conjugate vaccines. **Chapter 2** describes a chemical synthesis strategy to generate the terminal fragments of the Group B-specific antigen (GBC) of Group B *Streptococcus*. Highly convergent methods were employed to assemble GBC structures including a pentasaccharide, an octasaccharide through a [3 + 5] glycosylation strategy and a tridecasaccharide using a [5 + 8] phosphoramidite coupling. All structures were equipped with a spacer terminating in a free amine for the further conjugation with protein or other functional molecules. **Chapter 3** presents the first synthesis of fragments of the recently discovered glycerol phosphate (GroP)

modified group A carbohydrate (GAC), termed GroP GAC. Employing a linear trisaccharide as key repeating unit building block, tri-, hexa-, and nonasaccharides, with and without the GroP appendage, all bearing a free amine spacer at the reducing end were synthesized. **Chapter 4** describes the first assembly of the fragments of *O*-acetylated type 1 capsular polysaccharide of *Streptococcus pneumoniae* (Sp1), ranging from tri- to nonasaccharides. All fragments contain a diol terminated spacer that can be selectively oxidized in a Malaprade reaction to give an aldehyde for further modification. **Chapter 5** summarizes all the research presented in this thesis and provides some future prospect, including results of initial conjugation reactions and new synthesis pathways of fragments of another zwitterionic polysaccharide, PS A1, found in *Bacteroides fragilis*.

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Chapter 2

Chemical Synthesis of Fragments of the Multiantennary Group-Specific Polysaccharide of Group B *Streptococcus*

Introduction

July is international Group B *Strep* awareness month indicating that infections by Group B *Streptococcus* (GBS) remain a significant global public health problem.[1] GBS, also known as *Streptococcus agalactiae*, is a β-hemolytic gram-positive bacterium, and the most common cause of neonatal septicemia and meningitis, which are life-threatening for newborn babies.[2] A recent report estimates that 147 000 stillbirths and infant deaths annually are caused by GBS.[3] It was identified as a human pathogen in 1930s,[4] and is also a crucial cause of severe disease for susceptible individuals, such as pregnant women, immunocompromised patients and the elderly.

Intrapartum antibiotic strategies can lead to a significant decrease in early-onset disease (EOD, less than 7 days age), but have no effect on late-onset disease (LOD, 7 – 90 days of age). Until now, there is no vaccine commercially available to prevent GBS infections,[5] although clinical trials are ongoing. Novartis (now GSK) has developed and commenced a phase II clinical trial to evaluate the safety and immunogenicity in healthy pregnant women of a conjugate vaccine (NCT02046148), targeting GBS serotypes Ia, Ib and III. MinervaX has commenced phase I trials to investigate the safety and immunogenicity of a protein based vaccine in non-pregnant women among of 18 - 40 years old (NCT02459262).[5a, 6]

Bacterial cell-surface coated carbohydrates play a significant role in binding events and recognition by the immune system.[7] Bacterial cell wall polysaccharides (CWPS) are excellent targets to use in carbohydrate-based antibacterial vaccine.[8] As early as in 1938, Rebecca Lancefield demonstrated that the infection of mice by GBS could be prevented using CWPS-specific rabbit sera.[9] At least 10 different GBS serotypes can be distinguished on the basis of their capsular polysaccharide (CPS) structure, including type Ia, Ib, II, III, IV, V, VI, VII, VIII and IX.[10] Different CPSs have been explored in conjugate vaccines, but none have shown cross reactivity to other serotypes, even though the structure of some of the CPSs are highly similar.

The structure of the multiantennary group-specific polysaccharide (GBC, Fig. 1a) was first isolated from a type Ia GBS strain and reported by Jennings et al. in 1988.[11] The structure of GBC is assembled of numerous L-rhamnose and three other monosaccharides: D-galactose, D-*N*-acetylglucosamine and D-glucitol, with phosphate diesters joining the different subunits. Because of their immunological significance and due to the fact that bacterial polysaccharides are often not be obtained in sufficient purity and quantity, the chemical synthesis of bacterial oligosaccharides for vaccine purposes has drawn considerable attention in the fields of glycobiology.[8, 12] The chemical synthesis of the repeating units of serotypes Ia[13], Ib[13c], II[14], III[15] and V[16], have been published in the last 10 years by the groups of Adamo, Guo and Gao. Although the structure of GBC has been known for a long time, at present, only a trisaccharide[17] and tetrasaccharide[18] of the rhamnose moiety of the common antigen have been synthesized, and the role of this unique carbohydrate structure remains poorly understood. To make well-defined fragments of the GBC available for further studies and potential applications in novel conjugate vaccines, this Chapter describes the development of synthetic methodology to generate these fragments. As depicted in Fig. 1a, the GBC is built up from different substructures, and the target structures aimed at here representing the termini of the tetra-antennary structure. The boxed structure in Fig. 1a represents a tridecasaccharide, containing most components of the complete GBC and therefore

representing an attractive structure for immunological evaluation. It is built up from a pentasaccharide (Sub structure III) and an octasaccharide (Sub structure II), which are interconnected through a phosphate diester bridge. This Chapter describes the synthesis of conjugation-ready GBC-fragments **1** (the Sub III structure), **2** (the Sub II fragment) and **1** (the Sub II + Sub III oligomer). Because of the phosphate joints in the natural compound, a phosphate spacer was chosen to be coupled to the three different targets as shown in Fig. 1b.

Results and discussion

A retrosynthetic analysis towards the three targets is shown in Scheme 1. It was reasoned that the target tridecasaccharide **3** could be obtained from the protected tridecasaccharide **4** after a sequence of deprotection steps, including basic hydrolysis of the cyanoethyl, benzoyl groups and hydrogenation of the Bn, Nap and Cbz groups and transformation of the trichloroacetamide to the corresponding acetamide. Compound **4** could be constructed via a convergent [5 + 8] phosphate coupling strategy using pentasaccharide phosphoramidite **5** and the branched octasaccharide with a free galactosyl C-6-OH **6**. The key octasaccharide intermediate **6** was assembled by a [3 + 5] glycosylation strategy, which employed the trisaccharide **8** as donor and pentasaccharide **7** as acceptor. The latter pentasaccharide is also the precursor to the pentasaccharide phosphoramidite **5**. Both the tri- and pentasaccharides **7** and **8** were prepared via glycosylations using monosaccharide building blocks **A** to **F**.

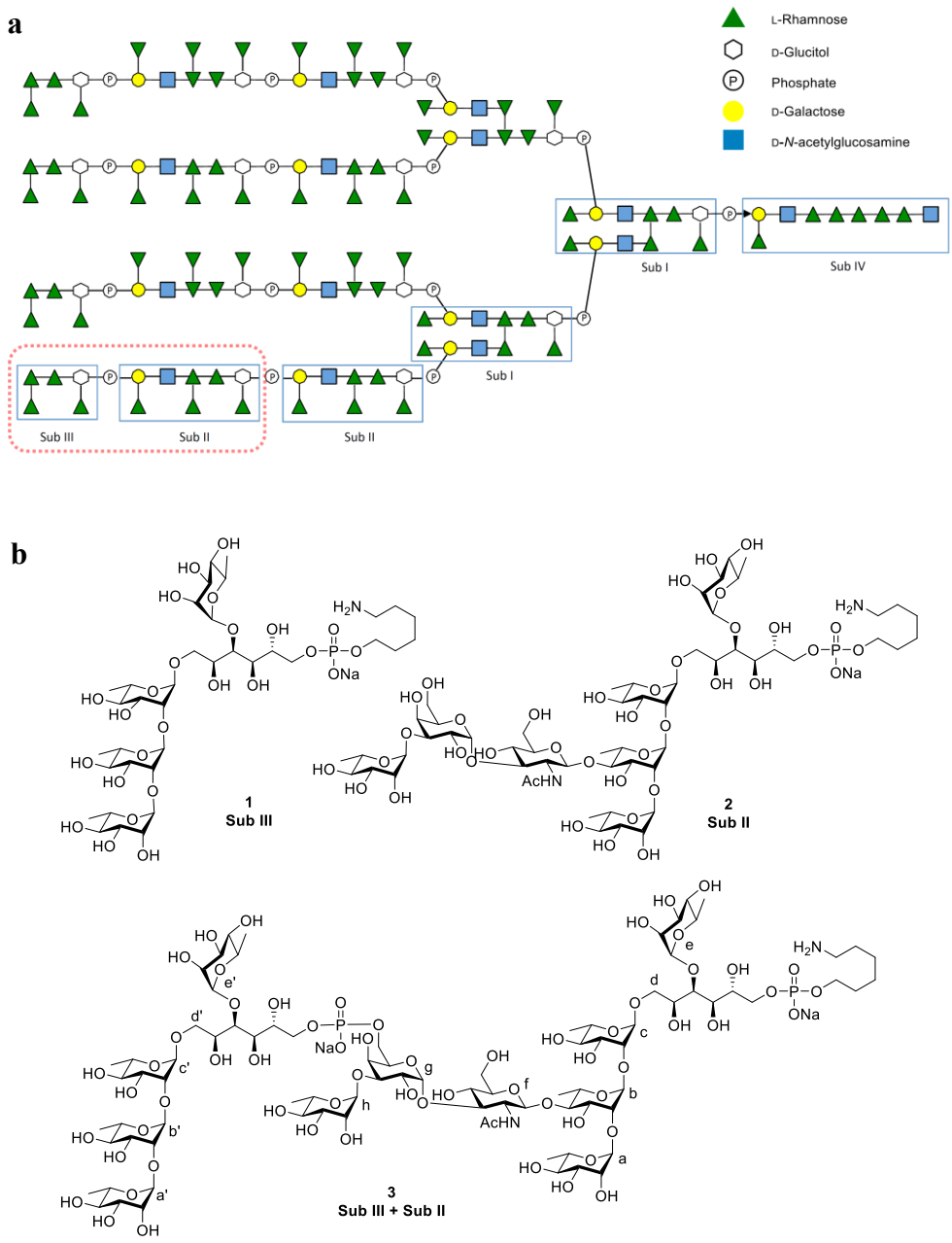
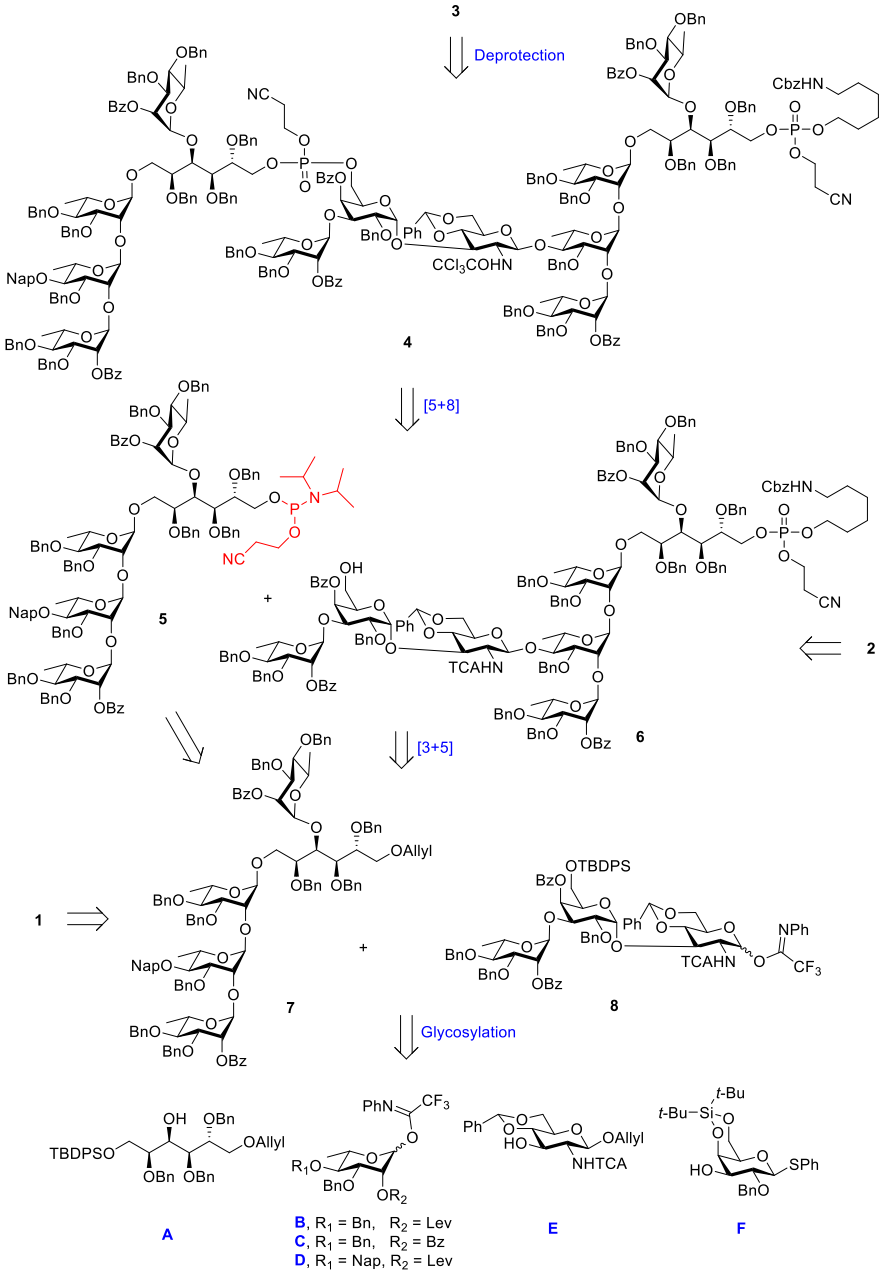
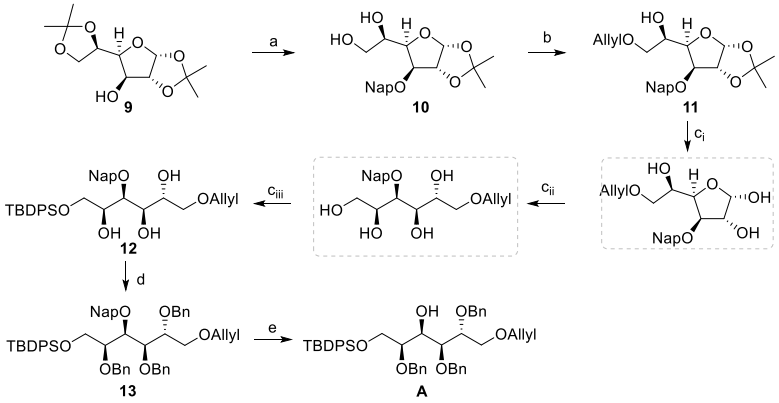


Figure 1. a) Structure of the GBC proposed by Jennings; b) Three conjugation-ready GBC fragments.



Scheme 1. Retrosynthetic analysis of the target oligosaccharides **1** – **3**.

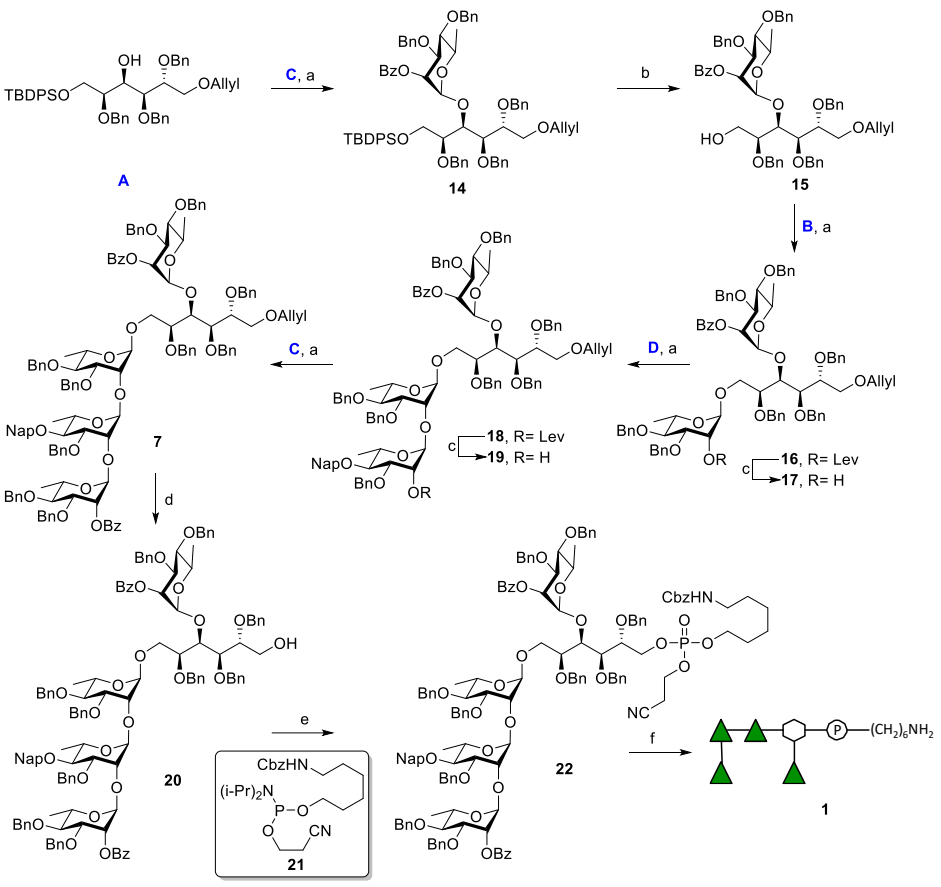
In the above described strategy, neighboring group participation is expected to control the anomeric selectivity in the glycosylations forming 1,2-*trans*-linkages. For the construction of the *cis*-galactosyl linkage, a silylidene group was to be used as stereochemistry controlling functionality.[19] Due to their high reactivity, convenient manipulation and facile purification, glycosyl *N*-phenyltrifluoroacetimidates were adopted as donors for all the glycosylation reactions. Of the listed building blocks (Scheme 1), rhamnosyl imidate donor **B**,[20] glucosamine acceptor **E**[21] and galactose acceptor **F**[12a] were prepared following reported procedures, while rhamnosyl imidate donors **C** and **D** were synthesized specifically for this study following adapted literature methods as described in the Experimental Section. A detailed description of glucitol acceptor **A** is shown in Scheme 2. 1,2-*O*-isopropylidene-3-*O*-napthylmethyl-α-D-glucofuranose **10**,[22] synthesized from diacetone-D-glucose **9**, was transformed into allyl-protected alcohol **11** in excellent yield *via* a borinic acid-catalyzed regioselective alkylation.[23] Subsequently, the ketal in **11** was removed in refluxing 80% acetic acid, which was followed by the sodium borohydride mediated reduction of the resulting hemiacetal and selective silylation using the bulky TBDPS group of the primary alcohol to afford triol **12** in 87% yield over three steps without purification of the intermediate products. Benzylation of the three hydroxyls was achieved in 94% yield. Detailed optimization of these benzylation conditions are showed in the Experimental Section, and several side products were identified, resulting from incomplete benzylation and silyl removal or migration. Finally, the D-glucitol building block **A** was obtained by removal of the Nap group in an oxidation with DDQ.



Scheme 2. Synthesis of the glucitol acceptor **A**.

Reagents and conditions: a) i, NapBr, NaH, DMF, 0 °C, 3 h; ii, 70% AcOH, rt, overnight, 92% (over 2 steps). b) 2-Aminoethyl diphenylborinate, KI, K_2CO_3, AllylBr, MeCN, 60 °C, 24 h, 91%. c) i, 80% AcOH, reflux, 2h; ii, $NaBH_4$, H_2O, EtOH-$CHCl_3$; iii, TBDPSCl, imidazole, DMF, 0 °C, 87% (over 3 steps). d) NaH, BnBr, TBAI, DMF, rt, 94%. e) DDQ, DCM/H_2O, 95%.

With all the six building blocks in hand, the assembly of the first target molecule **2** was undertaken as shown in Scheme 3. After an initial [3 + 2] model glycosylation showed that the stereochemistry of rhamnose-glucitol linkage was generated with poor selectivity,[24] the construction of the key pentasaccharide intermediate **7** was explored through a stepwise approach using monosaccharide building blocks. The first glycosylation between glucitol acceptor **A** and rhamnosyl donor **C** in the presence of TBSOTf as promotor gave disaccharide **14** in excellent yield. The selective deprotection of the TBDPS protecting group was performed utilizing TBAF in THF, and the subsequent glycosylation with donor building block **B** provided the trisaccharide **16** in 82% yield. Deprotection of the levulinoyl ester using $N_2H_4{\bullet}H_2O$ furnished trisaccharide acceptor **17** in 97% yield, which was glycosylated with building block **D** under the promotion of TBSOTf to furnish tetrasaccharide **18** in 71% yield. Selective removal of the levulinoyl group and subsequent glycosylation with building block **C** using the above-mentioned conditions provided the key intermediate pentasaccharide **7** in 87% yield. To complete the synthesis of target pentasaccharide **1**, de-allylation was performed using an isomerization reaction employing a catalytic amount of $Ir(COD)(Ph_2MeP)_2$, which was activated using H_2 in distilled THF. The resulting enol ether was cleaved by treatment with NIS and H_2O to provide the alcohol **20** in 90% yield.[25] Subsequently, the attachment of the spacer was achieved using phosphoramidite functionalized spacer **21** and dicyanoimidazole as an activator, followed by oxidation of the intermediate phosphite to the corresponding phosphate triester using CSO to give the fully protected pentasaccharide **22** in 90% yield over two steps. Finally, treatment of the pentasaccharide **22** with concentrated ammonia in dioxane led to removal of the cyanoethyl group, after which the compound was treated with NaOMe in MeOH/dioxane to remove the benzoyl esters. The subsequent palladium hydroxide mediated hydrogenation was performed in a mixture of water/*tert*-butanol under slightly acidic condition[26] at 1 atm for 3 days to give the target pentasaccharide **1** in 80% yield over the last 3 steps.

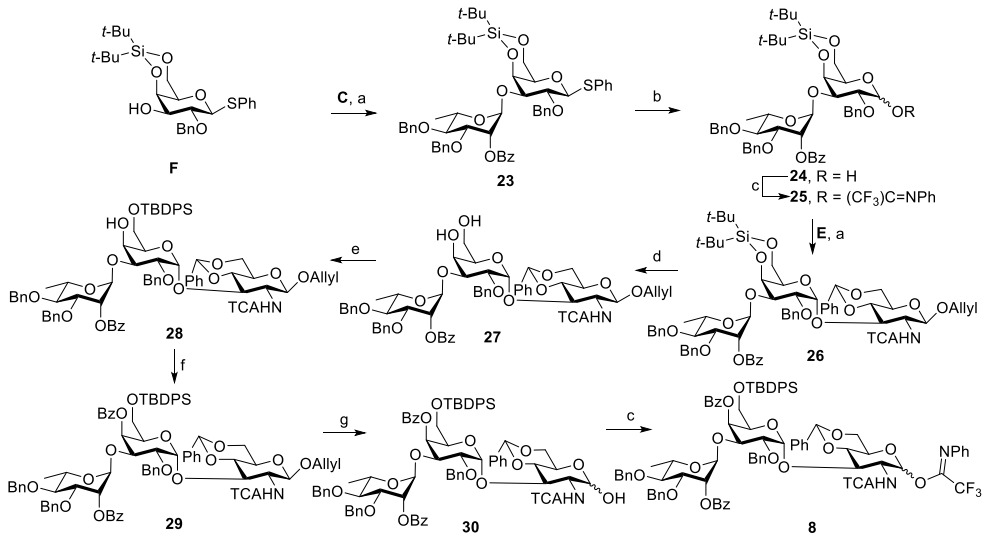


Scheme 3. Synthesis of the pentasaccharide **1**.

Reagents and conditions: a) TBSOTf, 4Å MS, DCM, 0 °C, **14**, 97%; **16**, 82%; **18**, 71%; **7**, 87%. b) TBAF, THF, 0 °C - RT, 93%. c) N_2H_4•H_2O, Py/AcOH, 0 °C - rt, **17**, 97%; **19**, 93%. d) $Ir(COD)(Ph_2MeP)_2$•PF_6, THF, H_2, 5s, then NIS and H_2O, 1h, 90%. e) **21**, DCI, MeCN, 3Å MS, 1h, then CSO, 15min, 90%. f) i, ammonium hydroxide, 1,4-dioxane; ii, NaOMe, MeOH/1,4-dioxane; iii, $Pd(OH)_2$/C, H_2, *t*-BuOH/H_2O, 3 days, 80%.

With the key pentasaccharide **7** in hand, and according to the retrosynthetic analysis, attention was turned to the trisaccharide donor **8** (Scheme 4). First, glycosylation of imidate donor **C** with acceptor **F** resulted in desired disaccharide **23** in 93% yield. Transformation of the thiophenyl donor into the corresponding imidate disaccharide donor gave **25** in excellent yield. Subsequently, the stereoselective formation of trisaccharide **26** was achieved through condensation **25** and acceptor **E** in 81% yield in the presence of catalytic amount of TBSOTf. To facilitate the phosphoramidite coupling to provide tridecasaccharide at a later stage, and prevent multiple protecting group manipulations on far-advanced intermediates, the

silylidene in **26** was removed using HF-Py in 87% yield, after which the primary C-6 alcohol of the galactoside moiety in **27** was selectively masked with a bulky TBDPS group to give **28**. The benzoylation of the remaining galactoside C-4 hydroxy was proved to be challenging, which can be attributed to the low reactivity of this alcohol. Several methods were tried, including the combination of BzCl and Et_3N. However, because of the acidic N-H of the glucosamine moiety, side-product was generated in which the amide was also benzoylated. The desired product **29** was finally obtained by stirring the substrate with BzCl in pyridine at RT for 3 days. Deprotection of the allyl group of the trisaccharide employing the iridium catalyst and subsequent NIS-mediated hydrolysis was followed by the installation of the imidate at the anomeric hydroxyl, to give the trisaccharide donor **8** in good yield.

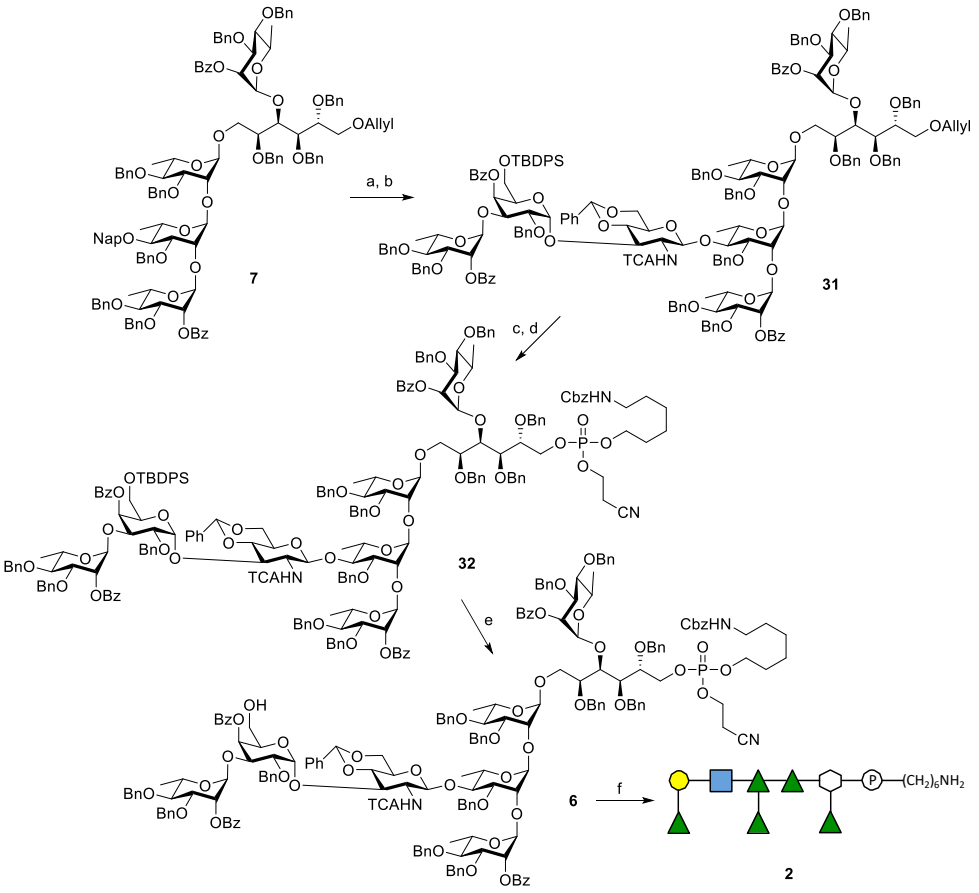


Scheme 4. Synthesis of the trisaccharide donor **8**.

Reagents and conditions: a) TBSOTf, DCM, 0 °C, 1h, 4Å MS, **23**, 93%; **26**, 81%. b) NIS, TFA, DCM, 0 °C, 0.5h, 94%. c) *N*-phenyltrifluoroacetimidoyl chloride, Cs_2CO_3, acetone, overnight, **25**, 93%; **8**, 81%. d) HF•Py, Py, THF, 87%. e) TBDPSCl, DMF, imidazole, 94%. f) BzCl, Py, DMAP, 3 days, 95%. g) $Ir(COD)(Ph_2MeP)_2•PF_6$, THF, H_2, 5s, then NIS and H_2O, 90%. TCA, trichloroacetyl.

To assemble the octasaccharide **2**, pentasaccharide **7** was first transformed into an acceptor by the selective removal of the Nap group using DDQ in DCM-H_2O (Scheme 5). Next, the [3 + 5] glycosylation with trisaccharide donor **8** using TBSOTf as a promotor gave octasaccharide **31** in 75% yield. As described for the synthesis of **22**, octasaccharide **32** was produced after de-allylation, and reaction with the phosphoramidite spacer and subsequent in situ oxidation, to give the fully protected product in high yield. Deprotection of the octasaccharide started with the removal of the TBDPS group using HF-Py in 93% yield to

provide **6**, having a free galactosyl C-6-OH. No migration of the neighboring benzoate was observed under this condition. Next, the same sequence of reactions was performed for the deprotection of pentasaccharide, to generate target octasaccharide **2**. Thus, first the cyanoethyl group and benzoate esters were removed, after which the reduction of all benzyl ethers, the benzylidene acetal and the benzyl carbamate and the concomitant transformation of the trichloroacetamide into the corresponding acetamide delivered GBC-octasaccharide **2**.

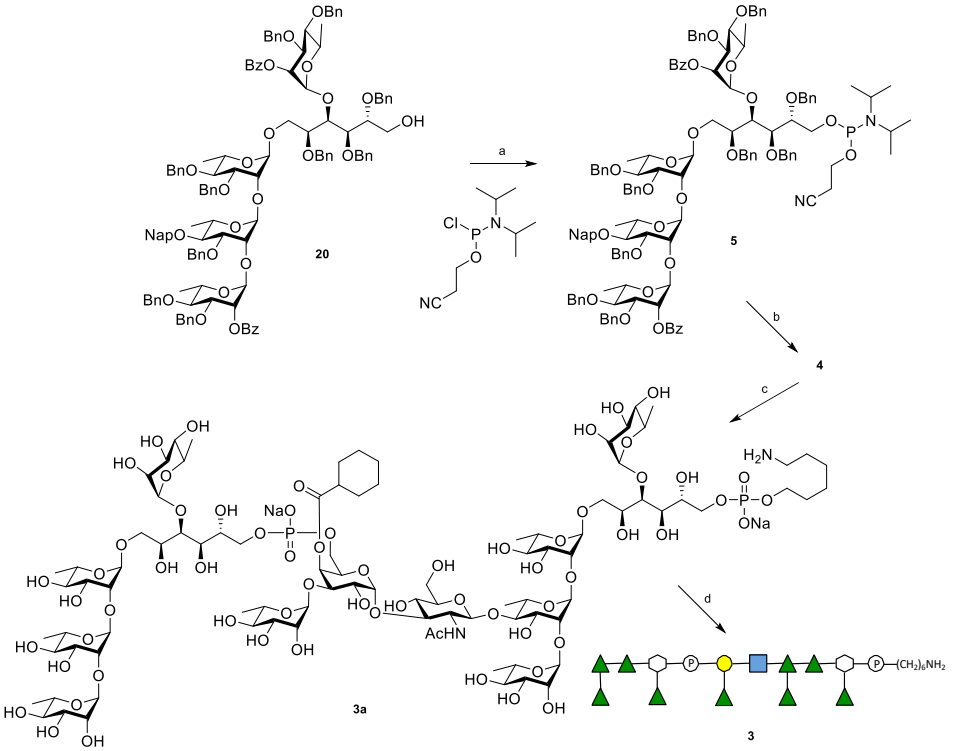


Scheme 5. The assembly of the octasaccharide **2**.

Reagents and conditions: a) DDQ, DCM/H_2O, 85%. b) **8**, TBSOTf, DCM, 4Å MS, 0 °C, 75%. c) $Ir(COD)(Ph_2MeP)_2$•PF_6, THF, H_2, then NIS and H_2O, 87%. d) **21**, DCI, ACN, 3Å MS, then, CSO, 92%. e) HF•Py, THF/Py, 93%. f) i, ammonium hydroxide, 1,4-dioxane; ii, NaOMe, MeOH/1,4-dioxane; iii, $Pd(OH)_2$/C, H_2, AcOH, *t*-BuOH/H_2O, 3 days, 70%

To be able to assemble the tridecasaccharide **3**, the pentasaccharide phosphoramidite **5** was synthesized from **20** using 2-cyanoethyl *N,N*-di-*iso*-propylchlorophosphoramidite and

DIPEA (Scheme 6). The key coupling of amidite **5** with octasaccharide **6** in the presence of DCI and in situ oxidation of the formed phosphite by CSO gave the fully protected tridecasaccharide **4** in 82% yield. When the global deprotection of this large oligosaccharide was performed using the procedures to generate **1** and **2**, undesired product **3a**, containing a cyclohexyl ester, was formed as the major product. This again underlines the low reactivity of the axial C-4 position of the galactose moiety. The cyclohexyl ester could be cleaved from **3a** by treatment with 1M NaOH for 24 hours, delivering the final compound **3**, after size exclusion chromatography.



Scheme 6. The assembly of the tridecasaccharide **3**.

Reagent and conditions: a) DIPEA, DCM, 3Å MS, 84%. b) **6**, DCI, ACN, 3Å MS, 2h, then, CSO, 15 min, 82%. c) i, ammonium hydroxide, 1,4-dioxane; ii, NaOMe, MeOH/1,4-diaxane; iii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days. d) 1M NaOH, H_2O, 52%.

The NMR data of compounds **1**, **2** and **3** closely matched the spectroscopic data reported for the isolated natural compounds, lacking the aminohexylphosphate spacer, as can be seen from Figure 2, Table 1 and 2.

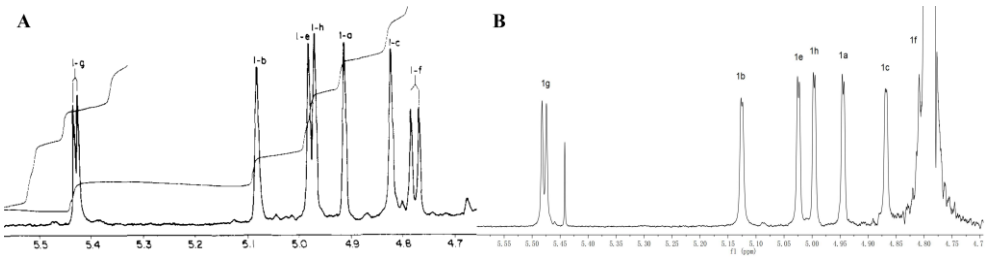


Figure 2. Partial ^{1}H NMR spectrum (anomeric proton region). A) isolated octasaccharide in D_2O. B) Synthesized target octasaccharide **2** in D_2O.

Table 1. ^{1}H NMR data (in D_2O) comparison of natural and synthetic fragments of GBC.

Anomeric Proton	Tridecasaccharide **3**		Octasaccharide **2**		Pentasaccharide **1**	
	Natural	Synthetic	Natural	Synthetic	Natural	Synthetic
1a	4.99	4.99	4.97	4.96		
1b	5.11	5.13	5.12	5.13		
1c	4.87	4.89	4.87	4.87		
1e	5.03	5.05	5.04	5.02		
1f	4.87	4.83	4.84	4.84		
1g	5.53	5.57	5.44	5.48		
1h	5.03	5.04	5.02	5.00		
1a’	4.98	4.97			4.97	4.99
1b’	5.12	5.14			5.10	5.14
1c’	4.89	4.90			4.87	4.90
1e’	5.03	5.02			5.03	5.05

Table 2. ^{13}C NMR data (in D_2O) comparison of natural and synthetic fragments of GBC.

Anomeric Carbon	Tridecasaccharide **3**		Octasaccharide **2**		Pentasaccharide **1**	
	Natural	Synthetic	Natural	Synthetic	Natural	Synthetic
1a	103.1	103.3	103.0	102.3		
1b	101.3	101.5	101.2	100.5		
1c	99.6	99.7	99.6	98.8		
1e	102.5	102.4	102.4	101.4		
1f	102.1	102.4	102.0	101.4		
1g	99.1	99.3	99.6	98.8		
1h	103.1	103.3	103.0	102.4		
1a’	103.0	103.2			102.9	102.3
1b’	101.7	101.8			101.6	100.9
1c’	99.6	99.7			99.6	98.8
1e’	102.1	102.3			102.4	101.4

Conclusion

The first chemical synthesis of fragments of the tetra-antennary group specific polysaccharide of GBS was achieved delivering the structures, equipped with a spacer for future conjugation purposes, in multi-milligrams quantities. The target structures represent the termini of the complex GBC and include branched oligosaccharides containing the pentasaccharide of GBC substructure III, the octasaccharide of substructure II and a tridecasaccharide, encompassing substructures II and III. A linear glycosylation strategy was used to construct the pentasaccharide **1**, a highly convergent [3 + 5] glycosylation approach delivered the octasaccharide **2** and a [5 + 8] strategy between a pentasaccharide phosphoramidite **5** and a branched octasaccharide with a free galactosyl C-6-OH **6** led to the challenging, complex tridecasaccharide target **3**. The spectroscopic data of the synthetic molecules matched well with those obtained for the isolated compounds. All the target fragments contain a free amino group at their downstream end for future regioselective modifications, such as conjugation with proteins, fluorophores or affinity tags, to provide compounds for various biological studies. Conjugation with a carrier protein such as CRM197, may deliver a conjugate vaccine that can be used to induce immunity against all GBS serotypes.

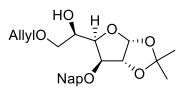
Experimental Section

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.

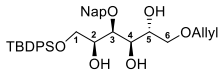
Experimental Procedures and Characterization Data of Products

6-Allyl-1,2-*O*-isopropylidene-3-*O*-(2-napthylmethyl)-α-D-glucofuranose (11)



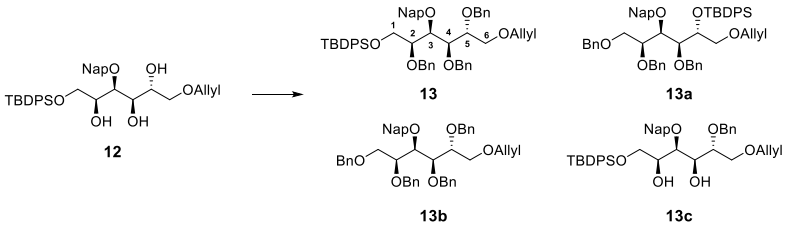
1,2-*O*-isopropylidene-3-*O*-(2-napthylmethyl)-α-D-glucofuranose[22] **10** (11.5 g, 32 mmol, 1 eq) was dissolved in CH_3CN (160 mL). 2-Aminoethyl diphenylborinate (2.1 g, 9.0 mmol, 0.28 eq), potassium iodide (7.4 g, 44.8 mmol, 1.4 eq) and potassium carbonate (6.64 g, 48 mmol, 1.5 eq), and allyl bromide (7 mL, 80 mmol, 2.5 eq) were added. The reaction was stirred at 60 °C for 24 hours. 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 10:1 – 3/1) to yield compound **11** (11.6 g, 29 mmol, 91%). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.87-7.74 (m, 4H, Nap), 7.54-7.40 (m, 3H, Nap), 5.95 (d, *J* = 4.0 Hz, 1H, H-1), 5.93-5.84 (m, 1H, $OCH_2C\mathit{H}CH_2$), 5.25 (dt, *J* = 17.2, 1.6 Hz, 1H, $OCH_2CHC\mathit{H}_2$), 5.16 (dt, *J* = 10.0, 1.6 Hz, 1H, OCH2CHC*H*2), 4.85 (d, *J* = 11.6 Hz, 1H, C*H*H), 4.76 (d, *J* = 12 Hz, 1H, CH*H*), 4.64 (d, *J* = 4.0 Hz, 1H, H-2). 4.18-4.14 (m, 3H, H-3, H-5, H-4), 4.01 (dt, 2H, *J* = 5.6, 1.2 Hz, $OC\mathit{H}_2CHCH_2$), 3.70 (dd, *J* = 3.2, 10 Hz, 1H, H-6), 3.55 (dd, *J* = 10, 6.0 Hz, 1H, H-6), 2.74 (d, *J* = 5.6 Hz, 1H, O*H*), 1.48 (s, 3H, CH_3), 1.31 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.86 (aromatic *C*), 134.56 ($OCH_2\mathit{C}HCH_2$), 133.28, 133.15 (aromatic *C*), 128.54, 128.02, 127.80, 126.83, 126.33, 126.18, 125.73 (aromatic *C*H), 117.37 ($OCH_2CH\mathit{C}H_2$), 111.85 (acetonide *C*), 105.26 (C-1), 82.44 (C-2), 82.00 (C-3), 79.96 (C-4), 72.54 (*C*H2), 72.41 ($O\mathit{C}H_2CHCH_2$), 72.00 (C-6), 68.02 (C-5), 26.87 ($\mathit{C}H_3$), 26.39 ($\mathit{C}H_3$). HR-MS: Calculated for $C_{23}H_{28}O_6$ $[M+Na]^+$: 423.1778, found: 423.1787. $[\alpha]^{20}_D$ = - 25.9° (c = 1, $CHCl_3$). TLC Rf = 0.40 (PE/EtOAc = 4/1, v/v).

6-Allyl-1-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2-napthylmethyl)-D-glucitol (12)



Compound **11** (11.6 g, 29 mmol, 1 eq) was dissolved in acetic acid (116 mL) and water (30 mL). The reaction was refluxed at 120 °C for 2 hours. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent, diluted in EtOAc and washed with saturated aqueous sodium bicarbonate, water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude was dissolved in ethanol (320 mL) and chloroform (160 mL). The reaction was cooled to 0 °C, sodium borohydride (6.6 g, 173.7 mmol, 6 eq) in water (160 mL) was added. It was slowly warmed to RT and stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid, and concentrated *in vacuo*. Diluted in EtOAc and washed with saturated aqueous sodium bicarbonate, water, and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was dissolved in DMF (58 mL) and cooled to 0 °C. *tert*-Butyl(chloro)diphenylsilane (TBDPSCl) (15 mL, 58 mmol, 2 eq) and imidazole (6 g, 88 mmol, 3 eq) were added at 0 °C. It was stirred at RT 4 hours and checked by TLC. After completed 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*. The compound was purified by flash chromatography (PE/EA 5:1 - 2:1) to yield compound **12** (15.16 g, 25.3 mmol, 87%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.79-7.71 (m, 4H, aromatic *H*), 7.65-7.62 (m, 4H, aromatic *H*), 7.46-7.30 (m, 9H, aromatic *H*), 5.92-5.82 (m, 1H, OCH_2CHCH_2), 5.24 (dt, *J* = 17.2, 1.6 Hz, 1H, OCH_2CHCH_2), 5.16 (dt, *J* = 10.4, 1.6 Hz, 1H, OCH_2CHCH_2), 4.89 (d, *J* = 11.4 Hz, 1H, CHH), 4.81 (d, *J* = 11.4 Hz, 1H, CHH), 4.09-4.06 (m, 1H, H-3), 4.00-3.97 (m, 3H, H-2, OCH_2CHCH_2), 3.85-3.67 (m, 5H, H-5, H-1, H-4, H-6), 3.56 (dd, *J* = 9.6, 6.0, Hz, 1H, H-6), 3.09-2.79 (m, 3H, 3 OH), 1.06 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.63 (aromatic CH), 135.52 (aromatic *C*), 134.42 (OCH_2CHCH_2), 133.24, 133.09, 133.07 (aromatic *C*), 129.91, 129.90, 128.29, 128.01, 127.86, 127.75, 126.99, 126.18, 126.06, 126.06 (aromatic CH), 117.48 (OCH_2CHCH_2), 76.75 (C-3), 74.98 (CH_2), 73.58 (C-2), 73.11 (C-4), 72.36 (OCH_2CHCH_2), 71.57 (C-6), 70.31 (C-5), 64.65 (C-1), 26.94 (3 CH_3), 19.26 ($C(CH_3)_3$). HR-MS: Calculated for $C_{36}H_{44}O_6Si$ $[M+Na]^+$: 623.2799, found : 623.2805. $[\alpha]^{20}_D$ = - 2.1° (c = 1, $CHCl_3$). TLC: Rf = 0.40 (PE/EtOAc = 7/3, v/v).

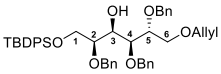
6-Allyl-2,4,5-tri-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2-naphthylmethyl)-D-glucitol (13)



Entry	Conditions	13	13a	13b	13c
1	DMF, NaH 6.0 eq, then BnBr 5.0 eq	51%	9%	19%	-
2	DMF, BnBr 4.0 eq, TBAI 0.3 eq, then NaH 4.0 eq	38% (>20:1)		9%	36%
3	DMF, BnBr 5.0 eq, TBAI 0.3 eq, then NaH 5.0 eq	52% (>20:1)		6%	41%
4	DMF, BnBr 10.0 eq, TBAI 3.0 eq, then NaH 6.0 eq	94% (>20:1)		trace	ND

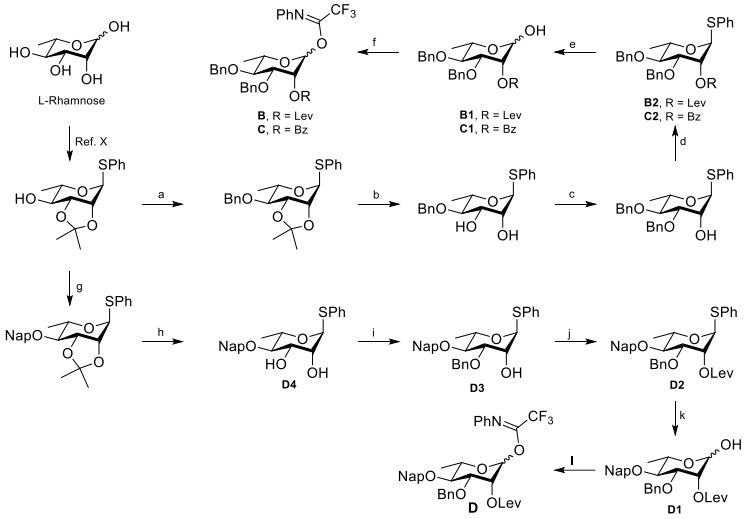
Triol **12** (1.62 g, 2.71 mmol, 1 eq) was dissolved in DMF (27 mL) and cooled to 0 °C. Benzyl bromide (3.2 mL, 27.1 mmol, 10 eq), tetrabutylammonium iodide (TBAI) (3 g, 8.12 mmol, 3 eq) were added. Then after sodium hydride (650 mg, 16.25 mmol, 6 eq) was added, the reaction was stirred at RT for 3 hours. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O, 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 50:1 – 20/1) to yield compound **13** (2.22 g, 2.55 mmol, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.79-7.75 (m, 1H, aromatic *H*), 7.71-7.60 (m, 7H, aromatic *H*), 7.44-7.30 (m, 8H, aromatic *H*), 7.26-7.19 (m, 16H, aromatic *H*), 5.93-5.83 (m, 1H, OCH_2CHCH_2), 5.25 (dt, *J* = 17.2, 2.0 Hz, 1H, OCH_2CHCH_2), 5.16 (dt, *J* = 10.4, 1.6 Hz, 1H, OCH_2CHCH_2), 4.82 (s, 2H, CH_2), 4.77 (d, *J* = 11.2 Hz, 1H, CH*H*), 4.66 (d, *J* = 12.0 Hz, 1H, C*H*H), 4.63 (d, *J* = 11.2 Hz, 1H, CH*H*), 4.58 (d, *J* = 11.6 Hz, 1H, CH*H*), 4.50 (d, *J* = 12.0 Hz, 1H, CH*H*), 4.38 (d, *J* = 12.0 Hz, 1H, CH*H*), 4.08-4.05 (m, 1H, H-5), 4.00-3.98 (m, 1H, H-2), 3.96-3.93 (m, 2H, OCH_2CHCH_2), 3.93-3.89 (m, 1H, H-1), 3.85-3.77 (m, 4H, H-6, H-4, H-1, H-3), 3.73-3.68 (m, 1H, H-6), 1.03 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.92, 138.89, 138.78, 136.31 (aromatic *C*), 135.80, 135.75 (aromatic *C*H), 135.00 (OCH_2CHCH_2), 133.49, 133.39, 133.34, 133.01 (aromatic *C*), 129.81, 129.76, 128.37, 128.33, 128.31, 128.11, 128.08, 128.00, 127.84, 127.80, 127.76, 127.57, 127.50, 127.46, 127.38, 126.89, 126.50, 125.97, 125.79 (aromatic *C*H), 116.86 (OCH_2CHCH_2), 80.42 (C-3), 79.63 (C-4), 79.50 (C-5), 78.95 (C-2), 75.06 (CH_2), 74.35 (CH_2), 73.07 (CH_2), 72.33 (OCH_2CHCH_2), 72.01 (CH_2), 70.05 (C-6), 63.63 (C-1), 26.99 (3 CH_3), 19.27 ($C(CH_3)_3$). HR-MS: Calculated for $C_{57}H_{62}O_6Si$ $[M+Na]^+$: 893.4208, found: 893.4217. $[\alpha]^{20}_D$ = - 1.4° (c = 1, $CHCl_3$). TLC: Rf = 0.50 (PE/EtOAc = 20/1, v/v).

6-Allyl-2,4,5-tri-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-D-glucitol (A)



Full protected **13** (2.37 g, 2.73 mmol, 1 eq) was dissolved in DCM (50 mL) and water (5 mL). After cooled to 0 °C, 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) (681 mg, 3 mmol, 1.1 eq) was added. The reaction was stirred at RT for 4 hours. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM, 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 30:1 – 15/1) to yield building block **A** (1.9 g, 2.6 mmol, 95%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.67-7.65 (m, 4H, aromatic *H*), 7.43-7.19 (m, 21H, aromatic *H*), 5.94-5.84 (m, 1H, OCH_2CHCH_2), 5.26 (dt, *J* = 17.2, 1.6 Hz, 1H, OCH_2CHCH_2), 5.16 (dt, *J* = 10.4, 1.6 Hz, 1H, OCH_2CHCH_2), 4.72-4.63 (m, 3H,), 4.54-4.47(m, 3H), 4.05 (bs, 1H, H-3), 3.97 (d, *J*

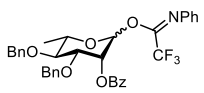
= 5.2 Hz, 2H, OC*H*$_2$CHCH$_2$),3.93-3.88 (m, 1H, H-1), 3.86-3.82 (m, 2H, H-5, H-4), 3.75-3.64 (m, 4H, H-6, H-1, H-2), 3.00 (s, 1H, O*H*), 1.04 (s, 9H, C(C*H*$_3$)$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.50, 138.21 (aromatic *C*), 135.73 (aromatic *C*H), 134.81 (OCH$_2$*C*HCH$_2$), 134.42, 133.24 (aromatic *C*), 129.77, 128.40, 128.38, 128.35, 128.05, 128.00, 127.80, 127.72, 127.67, 127.56 (aromatic *C*H), 117.01 (OCH$_2$CH*C*H$_2$), 80.43 (C-2), 79.44 (C-5), 77.80 (C-4), 73.61 (*C*H$_2$), 72.99 (*C*H$_2$), 72.58 (*C*H$_2$), 72.34 (O*C*H$_2$CHCH$_2$), 70.38 (C-3), 69.54 (C-6), 63.39 (C-1), 26.93 (3 *C*H$_3$), 19.24 (*C*(CH$_3$)$_3$). HR-MS: Calculated for $C_{46}H_{54}O_6Si$ [M+Na]$^+$: 753.3582, found: 753.3599. $[\alpha]^{20}_D$ = + 9.6° (c = 1, $CHCl_3$). TLC: Rf = 0.30 (PE/EtOAc = 10/1, v/v).



Scheme I. Synthesis of the rhamnoseyl donors **B**[20], **C**[27] and **D**[28]

Reagents ang conditions: a) BnBr, NaH, DMF, 4 h, 0 °C to rt, 96%. b) AcOH, 70 °C, 17 h, 95%. c) Bu_2SnO, toluene, 145 °C, 17 h, then BnBr, CsF, 120 °C, 17 h, 74%. d) Levulinic acid, EDCI, DMAP, DCM, 0 °C to rt, 19h, 96% (R = Lev); or BzCl, pyridine, DMAP, 0 °C to rt, 2h, 91% (R = Bz). e) NIS, TFA, DCM, 0 °C, 94% (R = Lev), 92% (R = Bz). f) $CF_3ClCNPh$, Cs_2CO_3, acetone, 0 °C - rt, 94% (R = Lev), quant. (R = Bz). g) NapBr, NaH, DMF, rt, 2 h. h) AcOH, 70 °C, overnight, 93% (over 2 steps). i) Bu_2SnO, toluene, 105 °C, 17h, then BnBr, DMF, rt, 5.5h, 72%. j) Levulinic acid, DIC, DMAP, DCM, 0 °C, 19h, 96%. k) NIS, TFA, DCM, 0 °C, 89%. l) $CF_3ClCNPh$, Cs_2CO_3, acetone, 0 °C - rt, 94%.

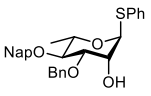
N-Phenyl-trifluoroacetimidate 2-*O*-benzoyl-3,4-di-*O*-benzyl-α/β-L-rhamnopyranoside (C)



Hemiacetal **C1** (11.8 g, 26.31 mmol, 1 eq) was dissolved in acetone (263 mL) and cooled to 0 °C. Cesium carbonate (9 g, 27.6 mmol, 1.05 eq) was added. After 15 min, *N*-phenyl trifluoroacetimidoyl chloride (6.6 g, 31.8 mmol, 1.2 eq) was added, and then it was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 100:1 – 20/1) to yield building block **C** (15.9 g, 25.6 mmol, 97%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 (d, *J*

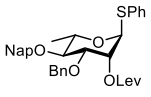
= 7.0 Hz, 2H, Bz), 7.61 – 7.51 (m, 1H, Bz), 7.51 – 7.40 (m, 2H, Bz), 7.39 – 7.18 (m, 12H), 7.13 – 7.01 (m, 1H), 6.88 – 6.78 (m, 2H), 6.31 (s, 1H, H-1), 5.75 (s, 1H, H-2), 4.93 (d, *J* = 10.8 Hz, 1H, CH_2), 4.82 (d, *J* = 11.2 Hz, 1H, CH_2), 4.67 – 4.61 (m, 2H, CH_2), 4.11 (dd, *J* = 9.4, 3.2 Hz, 1H, H-3), 4.05 – 3.91 (m, 1H, H-5), 3.64 (t, *J* = 9.5 Hz, 1H, H-4), 1.42 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.53 (Bz), 143.35, 142.83, 142.46, 142.11, 141.75, 138.11, 137.64, 133.53, 130.04, 129.59, 128.85, 128.59, 128.52, 128.49, 128.33, 127.99, 127.96, 124.53, 120.57, 120.32, 119.48, 117.47, 94.13 (C-1), 79.33 (C-4), 77.62 (C-3), 75.70 (*C*H_2), 72.12 (*C*H_2), 70.65 (C-5), 68.13 (C-2), 18.28 (C-6); HR-MS: Calculated for $C_{35}H_{32}F_3NO_6$ $[M+Na]^+$: 642.2074, found: 642.2070. TLC: Rf = 0.50 (PE/EtOAc = 20/1, v/v).

Phenyl 3-*O*-benzyl-4-*O*-(2-napthylmethyl)-1-thio-α-L-rhamnopyranoside (D3)



Phenyl 4-*O*-(2-napthylmethyl)-1-thio-α-L-rhamnopyranoside[28] **D4** (2.0 g, 5.04 mmol, 1 eq) was co-evaporated with anhydrous toluene three times under nitrogen and dissolved in anhydrous toluene (35 mL). Dibutyltin oxide (1.51 g, 1.2 eq) was added and the white suspension was heated to 145 °C. The reaction was stirred overnight after which the clear solution was cooled down. BnBr (785 µL, 1.3 eq) and CsF (1.53 g, 2 eq) were added and heated to 110 °C. After 6h, TLC showed complete consumption of the starting material, the reaction was diluted with EtOAc and washed with H_2O (2x), brine (2x). The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 8:1 - 5:1) to yield compound **D3** (1.8 g, 72%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 – 7.78 (m, 3H, Nap), 7.76 (s, 1H, Nap), 7.52 – 7.40 (m, 5H), 7.40 – 7.20 (m, 8H), 5.54 (s, 1H, H-1), 5.05 (d, *J* = 11.1 Hz, 1H, Nap), 4.82 (d, *J* = 11.1 Hz, 1H, Nap), 4.73 (s, 2H, Bn), 4.31 – 4.17 (m, 2H, H-2, H-5), 3.90 (dd, *J* = 9.0, 3.2 Hz, 1H, H-3), 3.59 (t, *J* = 9.3 Hz, 1H, H-4), 2.72 (s, 1H, H-2-OH), 1.33 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.72 (Bn), 135.86 (Nap), 135.86 (SPh), 134.23, 133.42, 131.52, 129.17, 128.77, 128.29, 128.25, 128.12, 128.05, 127.82, 127.48, 126.75, 126.23, 126.10, 126.05, 87.11 (C-1), 80.29 (C-4), 80.23 (C-3), 75.63 (Nap), 72.31 (Bn), 70.23 (C-2), 68.94 (C-5), 18.00 (C-6). HR-MS Calculated for $C_{30}H_{30}O_4S$ $[M+Na]^+$: 509.1757, found: 509.1769. $[\alpha]^{20}_D$ = - 213.9° (c = 1, $CHCl_3$). TLC Rf = 0.5 (PE/EtOAc = 4/1, v/v).

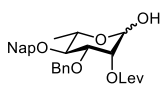
Phenyl 3-*O*-benzyl-2-*O*-levulinoyl-4-*O*-(2-napthylmethyl)-1-thio-α-L-rhamnopyranoside (D2)



The alcohol **D3** (1.8 g, 3.7 mmol, 1 eq) was co-evaporated with anhydrous toluene three times under nitrogen and dissolved in DCM (38 mL). Reduced to 0 °C, levulinic acid (1.2 g, 10.3 mmol, 2.8 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (1.2 g, 7.47 mmol, 2.0 eq) and 4-dimethylaminopyridine (DMAP) (46 mg, 0.38 mmol, 0.1 eq) were added. The reaction was stirred overnight and EDCI (1.2 g, 7.47 mmol, 2.0 eq) added, After TLC showed complete consumption of the starting material, the reaction was diluted with DCM and washed with H_2O (2x), brine. The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 9:1 - 4:1) to yield compound **D2** (2.15 g, 3.7 mmol, quantitive). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.94 – 7.84 (m, 3H), 7.82 (s, 1H), 7.59 – 7.46 (m, 5H), 7.46 – 7.30 (m, 8H), 5.73 – 5.65 (m, 1H), 5.49 (d, *J* = 1.7 Hz, 1H), 5.15 (d, *J* = 11.1 Hz, 1H), 4.87 (d, *J* = 11.1 Hz, 1H), 4.79 (d, *J* = 11.2 Hz, 1H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.39 – 4.26 (m, 1H),

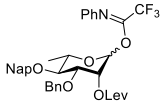
4.01 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.61 (t, *J* = 9.4 Hz, 1H), 2.89 – 2.65 (m, 4H), 2.20 (s, 3H), 1.43 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.29, 172.04, 137.81, 135.88, 133.92, 133.37, 133.07, 131.82, 129.17, 128.52, 128.28, 128.20, 128.00, 127.93, 127.78, 127.72, 126.76, 126.17, 126.13, 125.98, 86.06, 80.16, 78.35, 75.57, 71.77, 70.84, 69.10, 38.05, 29.90, 28.20, 18.05. HR-MS: Calculated for $C_{35}H_{36}O_6S$ $[M+Na]^+$: 607.2145, found: 607.2129. $[\alpha]^{20}_D$ = - 65.7° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EtOAc = 4/1, v/v).

3-*O*-benzyl-2-*O*-levulinoyl-4-*O*-(2-napthylmethyl)-α/β-L-rhamnopyranoside (D1)



The compound **D2** (7.58 g, 15.58 mmol, 1 eq) was dissolved in DCM (160 mL) and reduced to 0 °C. NIS (5.3 g, 1.5 eq) and TFA (1.8 mL, 1.5 eq) were added and the solution stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with $MgSO_4$, filtered, and concentrated in *vacuo*. The compound was purified by flash chromatography (PE/EA 4:1 - 1:1) to yield compound **D1** (7.52 g, 15.27 mmol, 98%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 – 7.75 (m, 3H, Nap), 7.74 (s, 1H, Nap), 7.52 – 7.38 (m, 3H, Nap), 7.37 – 7.21 (m, 5H, Bn), 5.43 – 5.36 (m, 1H, H-2), 5.16 – 5.10 (m, 1H, H-1), 5.06 (d, *J* = 11.1 Hz, 1H, CH_2), 4.82 – 4.66 (m, 2H, CH_2), 4.58 – 4.46 (m, 1H, CH_2), 4.08 – 3.95 (m, 2H, H-3, H-5), 3.53 – 3.36 (m, 1H, H-4), 2.97 – 2.56 (m, 4H, Lev), 2.15 (s, 3H, Lev), 1.32 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.63 (Lev), 172.19 (Lev), 138.05, 135.89, 133.28, 132.97, 128.36, 128.08, 128.07, 127.91, 127.69, 126.66, 126.08, 126.05, 125.86, 92.30 (C-1), 79.98 (C-4), 77.48 (C-3), 75.36, 71.60, 69.53 (C-2), 67.68 (C-5), 38.04 (Lev), 29.81 (Lev), 28.15 (Lev), 18.13 (C-6). HR-MS: Calculated for $C_{29}H_{32}O_7$ $[M+Na]^+$: 515.2040, found: 515.2047. TLC: Rf = 0.2 (PE/EtOAc = 2/1, v/v).

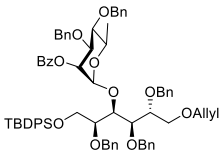
***N*-Phenyl-trifluoroacetimidate 3-*O*-benzyl-4-*O*-(2-napthylmethyl)-2-*O*-levulinoyl-α/β-L-rhamnopyranoside (D)**



Hemiacetal **D1** (720 mg, 1.46 mmol, 1.0 eq) was dissolved in Acetone (14 mL) and cooled to 0 °C. Cesium carbonate (476 mg, 1.5 mmol, 1.05 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (455 mg, 2.2 mmol, 1.5 eq) was added, and then it was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 10:1 - 5/1) to yield building block **D** (915 mg, 1.38 mmol, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 – 7.77 (m, 3H, Nap), 7.75 (d, *J* = 1.6 Hz, 1H, Nap), 7.53 – 7.40 (m, 3H, Nap), 7.40 – 7.26 (m, 7H), 7.16 – 7.06 (m, 1H), 6.86 – 6.76 (m, 2H), 6.15 (s, 1H, H-1), 5.49 (s, 1H, H-2), 5.08 (d, *J* = 11.0 Hz, 1H, CH_2), 4.88 – 4.70 (m, 2H, CH_2), 4.60 (d, *J* = 11.1 Hz, 1H, CH_2), 4.01 (dd, *J* = 9.4, 3.4 Hz, 1H, H-3), 3.97 – 3.84 (m, 1H, H-5), 3.54 (t, *J* = 9.5 Hz, 1H, H-4), 2.84 – 2.63 (m, 4H, Lev), 2.16 (s, 3H, Lev), 1.38 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.18 (Lev), 171.92 (Lev), 143.40, 137.73, 135.67, 133.41, 133.16, 128.87, 128.57, 128.40, 128.32, 128.05, 127.83, 126.97, 126.25, 126.21, 126.10, 124.55, 119.53, 94.15 (C-1), 79.30 (C-4), 77.50 (C-3), 75.76 (CH_2), 72.17 (CH_2), 70.57 (C-5), 67.90 (C-2), 38.07 (Lev), 29.93 (Lev), 28.13 (Lev), 18.20 (C-6). HR-MS: Calculated for $C_{37}H_{36}F_3NO_7$ $[M+Na]^+$:

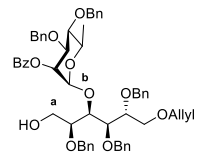
686.2336, found: 686.2377. TLC: Rf = 0.20 (PE/EtOAc = 9/1, v/v).

6-Allyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-D-glucitol (14)



Donor **C** (6.63 g, 10.7 mmol, 2 eq) and acceptor **A** (3.91 g, 5.35 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (54 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert* - butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (123 µL, 0.53 mmol, 0.1 eq) was added. The solution was stirred for 2 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 40:1 - 25:1) to yield compound **14** (6.0 g, 5.2 mmol, 97%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.08 – 7.99 (m, 2H, Bz), 7.67 – 7.59 (m, 4H), 7.59 – 7.53 (m, 1H, Bz), 7.44 (t, *J* = 7.8 Hz, 2H, Bz), 7.39 – 7.10 (m, 31H), 5.92 – 5.77 (m, 1H, OCH_2CHCH_2), 5.74 – 5.66 (m, 1H, H-2b), 5.27 – 5.17 (m, 2H, H-1b, OCH_2CHCH_2), 5.13 – 5.04 (m, 1H, OCH_2CHCH_2), 4.86 (d, *J* = 11.0 Hz, 1H, C*H*H), 4.75 – 4.49 (m, 7H, C*H*H), 4.46 (d, *J* = 11.5 Hz, 1H, C*H*H), 4.41 (d, *J* = 11.3 Hz, 1H, C*H*H), 4.26 – 4.17 (m, 1H, H-3a), 4.10 – 4.02 (m, 1H, H-5b), 4.02 – 3.86 (m, 5H, H-3b, OCH_2CHCH_2, 1a, 2a), 3.86 – 3.81 (m, 1H, H-5a), 3.81 – 3.74 (m, 2H, H-6a, 4a), 3.74 – 3.67 (m, 1H, H-1a), 3.67 – 3.60 (m, 1H, H-6a), 3.49 (t, *J* = 9.4 Hz, 1H, H-4b), 1.23 (d, *J* = 6.1 Hz, 3H, H-6b), 0.99 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.48 (Bz), 139.00, 138.87, 138.62, 138.52, 138.31, 135.82, 135.15 (OCH2*C*HCH2), 133.46, 133.20, 133.06, 130.36, 130.04, 129.77, 129.75, 128.41, 128.31, 128.30, 128.28, 128.27, 128.18, 128.08, 127.81, 127.77, 127.67, 127.64, 127.56, 127.40, 127.38, 127.34, 116.55, 99.07 (C-1b), 80.46 (C-4a), 80.14 (C-4b), 79.74 (C-5a), 78.37 (C-2a), 78.27 (C-3b), 77.50 (C-3a), 75.22, 73.70, 72.79, 72.29, 72.25, 71.50, 70.23 (C-6a), 69.77 (C-2b), 68.48 (C-5b), 63.31 (C-1a), 26.95 (TBDPS), 19.26 (TBDPS), 18.29 (C-6b). HR-MS: Calculated for $C_{73}H_{80}O_{11}Si[M+Na]^+$: 1183.5362, found: 1183.5402. $[\alpha]^{20}_D$ = + 11.9 (c = 1, $CHCl_3$). TLC: Rf = 0.40 (PE/EtOAc = 20/1, v/v).

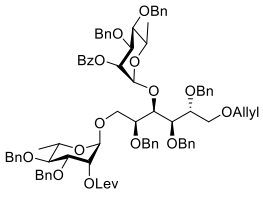
6-Allyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-D-glucitol (15)



Protected disaccharide **14** (6.14 g, 5.29 mmol, 1.0 eq) was dissolved in THF (110 mL) and cooled to 0 °C. Tetrabutylammonium fluoride hydrate (TBAF) (1.0 M in THF) (10 mL, 10 mmol, 2 eq) was added. The solution 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 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 10:1 - 4:1) to yield compound **15** (4.5 g, 4.9 mmol, 93%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.06 (d, *J* = 7.2 Hz, 2H, Bz), 7.60 – 7.52 (m, 1H, Bz), 7.44 (t, *J* = 7.7 Hz, 2H, Bz), 7.39 – 7.13 (m, 25H, Bn), 5.94 – 5.79 (m, 1H, OCH_2CHCH_2), 5.72 – 5.60 (m, 1H, H-2b), 5.24 (dd, *J* = 17.2, 1.8 Hz, 1H,

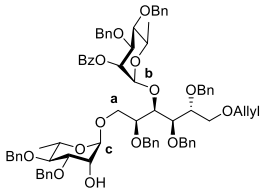
OCH_2CHCH_2), 5.18 – 5.07 (m, 2H, H-1b, OCH_2CHCH_2), 4.90 (d, *J* = 11.0 Hz, 1H, C*H*H), 4.79 – 4.65 (m, 4H, C*H*H), 4.65 – 4.56 (m, 3H, C*H*H), 4.56 – 4.45 (m, 2H, C*H*H), 4.11 (dd, *J* = 7.4, 3.3 Hz, 1H, H-3a), 4.08 – 3.72 (m, 9H), 3.72 – 3.64 (m, 2H, H-1a, 6a), 3.56 – 3.52 (m, 2H, H-1a, 4b) 1.27 (d, *J* = 6.2 Hz, 3H, H-6b). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.70 (Bz), 138.67, 138.64, 138.16, 138.13, 138.11, 134.90 (OCH2*C*HCH2), 133.18, 130.10, 129.97, 128.53, 128.42, 128.34, 128.32, 128.30, 128.08, 128.06, 128.03, 127.92, 127.81, 127.76, 127.72, 127.61, 127.53, 116.80 (OCH2CH*C*H2), 99.42 (C-1b), 80.07 (C-2a), 80.02 (C-4b), 79.28 (C-5a), 78.00 (C-4a), 77.96 (C-3b), 77.85 (C-3a), 75.25 (CH_2), 73.49 (Bn), 72.96 (CH_2), 72.46 (CH_2), 72.30 (O*C*H2CHCH2), 71.55 (CH_2), 69.88 (C-2b), 69.86 (C-6a), 68.58 (C-5b), 61.59 (C-1a), 18.22 (C-6b). HR-MS: Calculated for $C_{57}H_{62}O_{11}$ $[M+Na]^+$: 945.4184, found: 945.4222. $[\alpha]^{20}_D$ = + 3.3º (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/EtOAc = 4/1, v/v).

6-Allyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-(2-*O*-levulinoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-D-glucitol (16)



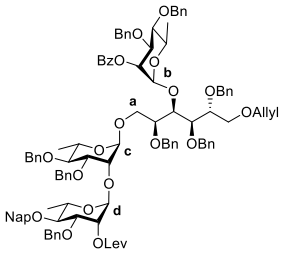
Donor **B** (5.36 g, 8.73 mmol, 1.8 eq) and acceptor **15** (4.38 g, 4.75 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (50 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 ºC and *tert* - butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (110 µL, 0.47 mmol, 0.1 eq) was added. The solution was stirred for 2 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 7:1 - 5:1) to yield compound **16** (5.0 g, 3.71 mmol, 79%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.07 – 8.01 (m, 2H, Bz), 7.59 – 7.52 (m, 1H, Bz), 7.43 (t, *J* = 7.8 Hz, 2H, Bz), 7.36 – 7.10 (m, 35H), 5.93 – 5.77 (m, 1H, OCH_2CHCH_2), 5.67 – 5.59 (m, 1H, H-2b), 5.38 – 5.30 (m, 1H, H-2c), 5.22 (dd, *J* = 17.2, 1.7 Hz, 1H, OCH_2CHCH_2), 5.14 – 5.06 (m, 2H, H-1b, OCH_2CHCH_2), 4.88 (d, *J* = 11.1 Hz, 2H, CH_2), 4.77 – 4.52 (m, 12H, H-1c, CH_2), 4.49 (d, *J* = 11.4 Hz, 1H, CH_2), 4.38 (d, *J* = 11.2 Hz, 1H, CH_2), 4.06 – 3.96 (m, 3H, H-5b, 3a, 3b), 3.96 – 3.88 (m, 2H, OCH_2CHCH_2), 3.88 – 3.55 (m, 9H,), 3.50 (t, *J* = 9.4 Hz, 1H, H-4b), 3.35 (t, *J* = 9.4 Hz, 1H, H-4c), 2.73 – 2.58 (m, 4H, Lev), 2.12 (s, 3H, Lev), 1.26 – 1.18 (m, 6H, H-6b, 6c). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.37 (Lev), 171.90 (Lev), 165.63 (Bz), 138.84, 138.76, 138.41, 138.24, 138.22, 138.20, 135.03 (OCH_2CHCH_2), 133.12, 130.26, 130.03, 128.45, 128.42, 128.37, 128.34, 128.33, 128.19, 128.17, 128.12, 128.08, 127.91, 127.71, 127.67, 127.65, 127.62, 127.59, 127.49, 116.76 (OCH_2CHCH_2), 98.96 (C-1b), 97.64 (C-1c), 80.07 (C-4b), 80.02 (C-4c), 79.40, 78.44, 78.06, 77.97, 77.93, 77.44 (C-3a), 75.28, 75.22, 73.57, 73.45, 72.37, 72.31 (OCH_2CHCH_2), 71.57, 69.86 (C-2b), 69.78 (C-6a), 69.04 (C-2c), 68.61 (C-5b), 67.91 (C-5c), 67.52 (C-1a), 38.19 (Lev), 29.93 (Lev), 28.27 (Lev), 18.26, 18.14. HR-MS: Calculated for $C_{82}H_{90}O_{17}$ $[M+Na]^+$: 1369.6070, found: 1369.6078. $[\alpha]^{20}_D$ = - 2.6º (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/EtOAc = 4/1, v/v).

6-Allyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-D-glucitol (17)



Trimer **16** (1.53 g, 1.14 mmol, 1.0 eq) was dissolved in pyridine (9 mL) and acetic acid (2.5 mL). After cooled to 0 °C, hydrazine hydrate (N_2H_4 50-60 %) (138 µL, 2.8 mmol, 2.5 eq) was added slowly. After stirred 20 min at RT, checked by TLC complete consumption of the starting material, quenched by acetone. 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 6:1 - 3:1) to yield compound **17** (1.38 g, 1.1 mmol, 97%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.09 – 8.01 (m, 2H, Bz), 7.58 – 7.49 (m, 1H, Bz), 7.42 (t, *J* = 7.8 Hz, 2H, Bz), 7.36 – 7.14 (m, 35H, Bn), 5.92 – 5.78 (m, 1H, OCH_2CHCH_2), 5.65 (dd, *J* = 3.2, 1.9 Hz, 1H, H-2b), 5.26 – 5.18 (m, 1H, OCH_2CHCH_2), 5.14 – 5.06 (m, 2H, H-1b, OCH_2CHCH_2), 4.93 – 4.83 (m, 2H, CH_2), 4.81 – 4.47 (m, 12H, CH_2, H-1c), 4.15 – 3.99 (m, 3H, H-3a, 3b, 5b), 3.99 – 3.95 (m, 1H, H-2c), 3.95 – 3.89 (m, 2H, OCH_2CHCH_2), 3.89 – 3.81 (m, 3H), 3.81 – 3.61 (m, 6H), 3.53 (t, *J* = 9.4 Hz, 1H, H-4b), 3.44 (t, *J* = 9.3 Hz, 1H, H-4c), 2.52 (s, 1H, 2c-OH), 1.32 – 1.19 (m, 6H, H-6b, 6c). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.72 (OBz), 138.79, 138.70, 138.65, 138.40, 138.14, 138.10, 138.02, 134.95 (OCH_2CHCH_2), 133.17, 130.08, 129.94, 128.48, 128.42, 128.39, 128.34, 128.31, 128.30, 128.27, 128.06, 128.05, 128.00, 127.89, 127.86, 127.84, 127.83, 127.63, 127.59, 127.58, 127.54, 127.41, 116.68 (OCH_2CHCH_2), 99.38 (C-1c), 98.88 (C-1b), 80.04 (C-4b), 79.93 (C-4c), 79.82 (C-3c), 79.35, 78.36, 77.96, 77.92, 77.44 (C-3a), 75.22, 75.18, 73.62, 73.08, 72.23, 72.22 (OCH_2CHCH_2), 71.85, 71.54, 69.91 (C-2b), 69.69 (C-6a), 68.55 (C-5b), 68.42 (C-2c), 67.65 (C-5c), 67.07 (C-1a), 18.23, 18.03. HR-MS: Calculated for $C_{77}H_{84}O_{15}$ $[M+Na]^+$: 1271.5702, found: 1271.5729. $[\alpha]^{20}_D$ = - 8.7 (c = 1, $CHCl_3$). TLC: Rf = 0.40 (Tol/EtOAc = 7/1, v/v).

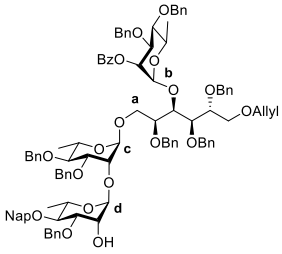
6-Allyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-2-*O*-(3-*O*-benzyl-2-*O*-levulinoyl-4-*O*-(2-napthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-D-glucitol (18)



Donor **D** (5.05 g, 7.6 mmol, 2.2 eq) and acceptor **17** (4.28 g, 3.43 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (34 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert* -butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (79 µL, 0.34 mmol, 0.1 eq) was added. The solution was stirred for 7 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 25:1 - 10:1) to yield compound **18** (4.23 g, 2.46 mmol, 71%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.08 – 7.99 (m, 2H, Bz), 7.85 – 7.75 (m, 3H, Nap), 7.73 (s, 1H, Nap), 7.57 – 7.50 (m, 1H, Bz), 7.48 – 7.09 (m, 45H), 5.90 – 5.78 (m, 1H, OCH_2CHCH_2), 5.67 (dd, *J* = 3.2, 1.9 Hz, 1H, H-2b), 5.54

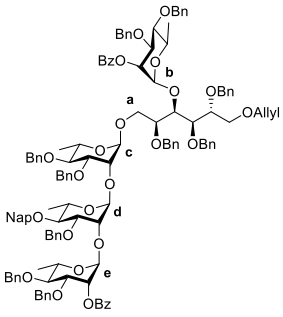
(dd, *J* = 3.3, 1.9 Hz, 1H, H-2d), 5.26 – 5.17 (m, 1H, OCH_2CHCH_2), 5.12 (d, *J* = 1.8 Hz, 1H, H-1b), 5.11 – 5.06 (m, 1H, OCH_2CHCH_2), 5.04 (d, *J* = 11.1 Hz, 1H, CH_2), 4.98 (d, *J* = 1.8 Hz, 1H, H-1d), 4.88 (d, *J* = 11.1 Hz, 2H, CH_2), 4.79 – 4.44 (m, 16H, CH_2, H-1c), 4.09 – 3.95 (m, 5H), 3.93 – 3.88 (m, 2H, OCH_2CHCH_2), 3.88 – 3.73 (m, 6H), 3.73 – 3.60 (m, 3H, H-1a, 5c, 6a), 3.56 (dd, *J* = 11.0, 3.0 Hz, 1H, H-1a), 3.51 (t, *J* = 9.4 Hz, 1H, H-4b), 3.46 – 3.33 (m, 2H, H-4d, 4c), 2.75 – 2.58 (m, 4H, Lev), 2.12 (s, 3H, Lev), 1.34 – 1.15 (m, 9H, 6b, 6c, 6d). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.26 (Lev), 171.80 (Lev), 165.53 (Bz), 138.86, 138.82, 138.70, 138.45, 138.36, 138.25, 138.18, 138.15, 136.05, 134.99, 133.38, 133.11, 133.05, 130.18, 129.99, 128.43, 128.40, 128.39, 128.32, 128.30, 128.26, 128.16, 128.12, 128.09, 128.07, 128.01, 127.97, 127.89, 127.75, 127.72, 127.68, 127.63, 127.60, 127.52, 127.44, 126.73, 126.20, 126.10, 125.91, 116.70 (OCH_2CHCH_2), 99.22 (C-1d), 99.00 (C-1b), 98.93 (C-1c), 80.08, 80.04, 79.60, 79.34, 78.49, 78.08, 78.04, 77.75, 77.44, 75.46, 75.27, 75.11, 74.77 (C-5d), 73.58, 73.33, 72.28, 72.25, 71.95, 71.68, 71.55, 69.77 (C-2b), 69.76 (C-6a), 69.23 (C-2d), 68.58, 68.26, 68.18, 67.15 (C-1a), 38.19 (Lev), 29.92 (Lev), 28.30 (Lev), 18.27, 18.16. HR-MS: Calculated for $C_{106}H_{114}O_{21}$ $[M+Na]^+$: 1745.7745, found: 1745.7706. $[\alpha]^{20}_D$ = - 0.6 (c = 1, $CHCl_3$). TLC: Rf = 0.30 (PE/EtOAc = 4/1, v/v).

6-Allyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-2-*O*-(3-*O*-benzyl-4-*O*-(2-napthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-D-glucitol (19)



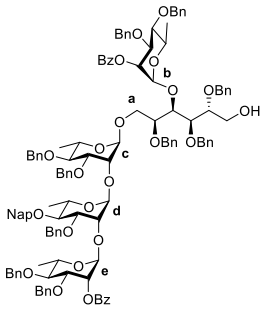
Tetramer **18** (1.22 g, 0.71 mmol, 1.0 eq) was dissolved in pyridine (6 mL) and acetic acid (1.5 mL). After cooled to 0 °C, hydrazine hydrate (N_2H_4 50-60 %) (70 µL, 1.4 mmol, 2.0 eq) was added slowly. After stirred 20 min at RT, checked by TLC complete consumption of the starting material, quenched by acetone. 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 5:1 - 3:1) to yield compound **19** (1.07 g, 0.66 mmol, 93%). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.04 (d, J = 7.7 Hz, 2H, Bz), 7.86 – 7.76 (m, 3H, Nap), 7.75 (s, 1H, Nap), 7.53 (t, J = 7.4 Hz, 1H, Bz), 7.49 – 7.08 (m, 45H), 5.90 – 5.76 (m, 1H, OCH_2CHCH_2), 5.67 (s, 1H, H-2b), 5.21 (d, J = 17.3 Hz, 1H, OCH_2CHCH_2), 5.15 – 5.04 (m, 3H, OCH_2CHCH_2, H-1b, 1d), 5.01 (d, J = 11.1 Hz, 1H, CH_2), 4.92 – 4.81 (m, 2H), 4.81 – 4.41 (m, 16H, CH_2, H-1c), 4.13 (s, 1H, H-2d), 4.09 – 3.95 (m, 4H), 3.95 – 3.73 (m, 9H), 3.73 – 3.60 (m, 3H), 3.56 (dd, J = 11.1, 3.0 Hz, 1H, H-1a), 3.50 (t, J = 9.4 Hz, 2H, H-4b, 4d), 3.36 (t, J = 9.4 Hz, 1H, H-4c), 2.44 (s, 1H, 2d-OH), 1.33 – 1.14 (m, 9H, H-6b, 6c, 6d). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.55 (Bz), 138.90, 138.83, 138.72, 138.38, 138.35, 138.19, 138.17, 138.14, 136.03, 135.00, 133.40, 133.12, 133.08, 130.19, 130.01, 128.62, 128.46, 128.45, 128.43, 128.41, 128.34, 128.32, 128.20, 128.18, 128.11, 128.09, 128.04, 128.01, 127.90, 127.85, 127.83, 127.77, 127.70, 127.65, 127.61, 127.54, 127.45, 126.67, 126.14, 126.12, 125.95, 116.73 (OCH2CHCH2), 100.82 (C-1d), 99.08 (C-1c), 99.06 (C-1b), 80.34, 80.26, 80.06, 79.69, 79.65, 79.35, 78.55, 78.09, 78.06, 77.49 (C-1a), 75.49, 75.29, 75.10, 74.77 (C-5d), 73.59, 73.37, 72.30, 72.26, 72.21, 72.14, 71.57, 69.78 (C-2b), 69.76 (C-6a), 68.85 (C-2d), 68.60 (C-2c), 68.18 (C-5c), 68.01 (C-5b), 67.20 (C-1a), 18.28, 18.21, 18.08. HR-MS: Calculated for $C_{101}H_{108}O_{19}$ $[M+Na]^+$: 1647.7377, found: 1647.7346. $[\alpha]^{20}_D$ = - 4.5 (c = 1, $CHCl_3$).TLC: Rf = 0.30 (PE/EtOAc = 4/1, v/v).

6-Allyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-2-*O*-(2-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-3-*O*-benzyl-4-*O*-(2-napthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-D-glucitol (7)



Donor **C** (1.3 g, 2.1 mmol, 3.0 eq) and acceptor **19** (1.14 g, 0.7 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (7 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and TBSOTf (19 µL, 0.07 mmol, 0.1 eq) was added. The solution was stirred for 2 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous $NaHCO_3$ and diluted with DCM. The solution was washed with water (2x), brine and extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 10:1 - 5:1) to yield compound **7** (1.25 g, 0.61 mmol, 87%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.13 (d, *J* = 8.0 Hz, 2H, Bz), 8.06 (d, *J* = 7.7 Hz, 2H, Bz), 7.86 – 7.76 (m, 4H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.53 – 7.05 (m, 57H), 7.01 (t, *J* = 7.5 Hz, 1H), 5.92 – 5.75 (m, 2H), 5.74 – 5.64 (m, 1H), 5.29 – 5.00 (m, 6H), 4.98 – 4.37 (m, 24H), 4.21 – 4.14 (m, 1H, H-4d), 4.14 – 4.08 (m, 1H), 4.08 – 3.99 (m, 4H), 3.99 – 3.88 (m, 4H), 3.88 – 3.73 (m, 7H), 3.73 – 3.61 (m, 3H), 3.61 – 3.46 (m, 4H), 3.40 – 3.30 (m, 1H), 1.35 – 1.15 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.61, 165.50, 138.84, 138.80, 138.70, 138.55, 138.49, 138.34, 138.23, 138.20, 138.16, 138.13, 136.10, 134.98, 133.40, 133.18, 133.08, 133.03, 130.26, 130.16, 130.01, 129.97, 128.47, 128.44, 128.40, 128.38, 128.36, 128.31, 128.28, 128.19, 128.17, 128.15, 128.12, 128.06, 127.97, 127.94, 127.91, 127.88, 127.84, 127.75, 127.71, 127.63, 127.59, 127.57, 127.49, 127.43, 126.82, 126.27, 126.05, 125.86, 116.67, 100.43, 99.25, 99.07, 99.01, 80.31, 80.26, 80.02, 79.44, 79.34, 78.54, 78.06, 77.94, 77.45, 75.45, 75.37, 75.24, 75.22, 75.09, 74.53, 73.56, 73.40, 72.29, 72.22, 72.05, 71.62, 71.54, 69.80, 69.74, 69.55, 68.55, 68.53, 68.39, 68.22, 67.15, 18.25, 18.23, 18.17. HR-MS Calculated for $C_{128}H_{134}O_{24}$ $[M+H]^+$: 2055.9338, found: 2055.9336. $[\alpha]^{20}_D$ = + 0.6 (c = 1, $CHCl_3$). TLC Rf = 0.50 (PE/EtOAc = 4/1, v/v).

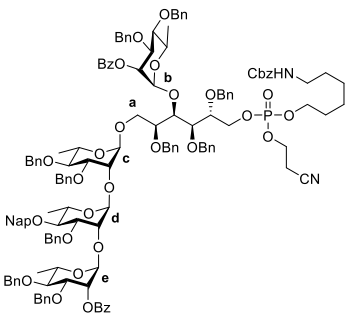
3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-2-*O*-(2-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-3-*O*-benzyl-4-*O*-(2-napthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-D-glucitol (20)



Allyl protected **7** (637 mg, 0.31 mmol, 1.0 eq) was dissolved in freshly distilled THF (6 ml). The mixture was degassed and placed under an argon atmosphere. (1,5-Cyclooctadiene) (pyridine)-(tricyclohexylphosphine)-iridium(I) hexafluorophosphate ($Ir(COD)(Ph_2MeP)_2 \cdot PF_6$) (17 mg, 0.02 mmol, 0.05 eq) was added and the reaction mixture was purged with H_2 for 5 seconds. The reaction mixture was stirred for 1 hour under an argon atmosphere. After analysis by TLC showed complete consumption of the starting material, diluted with THF (2 ml) and NIS (105 mg, 0.47 mmol, 1.5 eq), and water were added, and the solution stirred for 1 hours at room temperature. EtOAc was added and the organic layer was washed two times with saturated

aqueous sodium thiosulphate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (PE/EA 5:1 - 3:1) yielded **20** (563 mg, 0.28 mmol, 90%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.16 – 8.10 (m, 2H), 8.10 – 8.03 (m, 2H), 7.84 – 7.75 (m, 4H), 7.61 – 7.35 (m, 11H), 7.35 – 7.08 (m, 47H), 7.01 (t, *J* = 7.4 Hz, 1H), 5.84 – 5.76 (m, 1H), 5.74 – 5.67 (m, 1H), 5.19 – 5.09 (m, 3H), 5.06 (d, *J* = 11.1 Hz, 1H), 4.95 – 4.84 (m, 3H), 4.84 – 4.78 (m, 2H), 4.78 – 4.48 (m, 18H), 4.38 (d, *J* = 11.7 Hz, 1H), 4.20 – 3.99 (m, 7H), 3.99 – 3.88 (m, 2H), 3.87 – 3.77 (m, 6H), 3.77 – 3.62 (m, 5H), 3.60 – 3.47 (m, 4H), 3.36 (t, *J* = 9.4 Hz, 1H), 1.33 – 1.17 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.60, 165.55, 138.70, 138.56, 138.52, 138.45, 138.24, 138.19, 138.14, 138.10, 137.97, 136.04, 133.39, 133.17, 133.11, 133.03, 130.25, 130.14, 129.98, 129.95, 128.49, 128.46, 128.44, 128.41, 128.37, 128.35, 128.32, 128.31, 128.24, 128.20, 128.17, 128.15, 128.12, 128.07, 128.04, 127.93, 127.87, 127.82, 127.78, 127.76, 127.70, 127.69, 127.65, 127.64, 127.60, 127.55, 126.84, 126.27, 126.05, 125.86, 100.48 (C-1d), 99.24 (C-1b, 1e), 99.12 (C-1c), 80.32, 80.25, 80.23, 79.99, 79.66, 79.34, 79.11, 78.57, 77.90, 77.81, 77.38, 75.44, 75.37, 75.27, 75.24, 74.42, 73.89, 73.43, 72.19, 71.88, 71.84, 71.60, 71.56, 69.79 (C-2b), 69.56 (C-2e), 68.57, 68.53, 68.39, 68.16, 66.77 (C-1a), 60.39 (C-6a), 18.29, 18.22, 18.18. HR-MS: Calculated for $C_{125}H_{130}O_{24}$ $[M+H]^+$: 2015.9025, found: 2015.9010. $[\alpha]^{20}_D$ = + 3.8 (c = 1, $CHCl_3$). TLC: Rf = 0.40 (PE/EtOAc = 3/1, v/v).

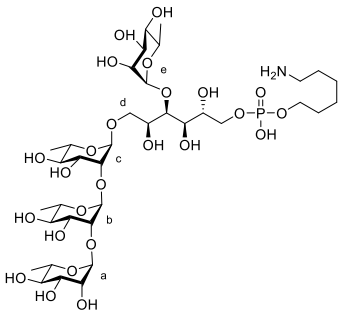
3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-2-*O*-(2-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-3-*O*-benzyl-4-*O*-(2-napthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-D-glucitol-*N*-benzyloxycarbonyl-6-aminohexanol-cyanoethyl phosphonate (22)



Alcohol **20** (238 mg, 0.12 mmol, 1.0 eq) was co-evaporated with dry acetonitrile 3 times. Dissolved in dry acetonitrile (4 ml), 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (0.94 mL, 0.24 mmol, 2.0 eq) and 3Å molecular sieves were added. The mixture was stirred for 15 mins under argon atmosphere. Benzyl 6-([*N*,*N*-diisopropylamino]-2-cyanoethyl-phosphite)-hexyl-1-carbamate (0.16M in acetonitrile) (1.5 mL, 0.24 mmol, 2.0 eq) was added. The reaction mixture was stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (0.71 mL, 0.36 mmol, 3.0 eq) was added. Stirred another 15 mins and diluted with EtOAc. The solution was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (DCM/Acetone 100:1 - 30:1) yielded **22** (252 mg, 0.11 mmol, 90%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.17 – 8.10 (m, 2H), 8.10 – 8.03 (m, 2H), 7.84 – 7.75 (m, 4H), 7.60 – 7.53 (m, 1H), 7.53 – 7.44 (m, 4H), 7.44 – 7.08 (m, 58H), 7.07 – 6.98 (m, 1H), 5.87 – 5.77 (m, 1H, H-2e), 5.70 – 5.60 (m, 1H, H-2b), 5.21 – 5.14 (m, 2H, H-1d, 1e), 5.14 – 5.02 (m, 4H, H-1b, CH_2), 4.95 – 4.84 (m, 4H, CH_2), 4.84 – 4.40 (m, 22H, H-1c, 6a, CH_2), 4.33 – 4.21 (m, 1H, H-6a), 4.21 – 4.15 (m, 1H, H-2d), 4.15 – 4.08 (m, 1H, H-3e), 4.08 – 3.77 (m, 16H), 3.77 – 3.48 (m, 6H, H-1a, 5c, 4b, 4d, 4e), 3.38 (t, *J* = 9.3 Hz, 1H, H-4c), 3.14 – 3.01 (m, 2H, CH_2NHCbz), 2.36 – 2.05 (m, 2H, OCH$_2$CH_2CN), 1.64 – 1.44 (m, 2H), 1.44 – 1.09 (m, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.51, 165.43, 156.35,

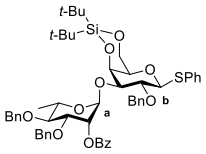
138.65, 138.44, 138.42, 138.40, 138.36, 138.09, 138.07, 137.96, 137.91, 137.87, 137.86, 137.81, 137.75, 137.73, 136.71, 135.96, 133.30, 133.10, 132.94, 130.15, 129.95, 129.89, 129.86, 128.45, 128.40, 128.38, 128.35, 128.32, 128.28, 128.25, 128.22, 128.16, 128.13, 128.10, 128.08, 128.06, 128.04, 128.00, 127.97, 127.95, 127.90, 127.83, 127.81, 127.76, 127.73, 127.70, 127.65, 127.61, 127.60, 127.55, 127.52, 127.49, 126.78, 126.21, 125.98, 125.79, 116.62, 116.56 (OCH_2CH_2CN), 100.36 (C-1d), 99.12 (C-1e), 98.97 (C-1c), 98.93, 98.89 (C-1b), 80.21, 80.17, 80.14, 79.88, 79.27, 79.07, 78.57, 78.52, 78.46, 78.41, 78.12, 78.06, 77.89, 77.82, 77.09, 77.01, 76.86, 75.34, 75.28, 75.11, 75.04 (C-2d), 74.41, 74.36, 73.71, 73.42, 73.37, 72.16, 72.12, 72.06, 71.89, 71.53, 71.50, 71.48, 69.64, 69.61, 69.45, 68.63, 68.47, 68.32 (C-5d), 68.18 (C-5c), 68.14, 68.12, 68.09, 66.80 (C-1a), 66.44 (Cbz), 66.34 (C-6a), 61.62, 61.58, 40.77 (CH_2Cbz), 29.97, 29.94, 29.91, 29.89, 29.65, 29.63, 29.25, 26.00, 24.89, 24.87, 19.21, 19.15, 19.09, 19.04, 18.19, 18.16, 18.14, 18.10. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.22, -0.66. HR-MS: Calculated for $C_{142}H_{153}N_2O_{29}P$ $[M+H]^+$: 2382.0369, found: 2382.0347. TLC: Rf = 0.50 (DCM/Acetone = 20/1, v/v).

3-*O*-(α-L-rhamnosyl)-1-*O*-(2-*O*-(2-*O*-(α-L-rhamnosyl)-α-L-rhamnosyl)-α-L-rhamnosyl)-D-glucitol-6-aminohexanol phosphate (1)



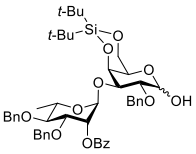
Full protected compound **22** (51.3 mg, 21.5 µmol, 1.0 eq) was dissolved in dioxane (6 mL) and ammonia solution (35%) (3 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The crude was dissolved in methanol (2 mL) and dioxane (1 mL). Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 20 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20 column) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (7 mL), water (4 mL) and 4 drops acetic acid. After $Pd(OH)_2$/C (51 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **2** (16.6 mg, 17.2 µmol, 80%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.141 (d, *J* = 1.8 Hz, 1H), 5.052 (d, *J* = 1.8 Hz, 1H), 4.987 (d, *J* = 1.8 Hz, 1H), 4.902 (d, *J* = 1.7 Hz, 1H), 4.167 – 4.067 (m, 5H), 4.067 – 3.970 (m, 3H), 3.957 – 3.635 (m, 15H), 3.515 – 3.440 (m, 4H), 3.027 – 2.982 (m, 2H), 1.745 – 1.609 (m, 4H), 1.492 – 1.382 (m, 4H), 1.358 – 1.237 (m, 12H). ^{13}C NMR (126 MHz, D_2O) δ 102.26, 101.44, 100.89, 98.84, 72.22, 72.16, 72.06, 71.96, 70.60, 70.48, 70.22, 70.13, 70.10, 70.00, 69.94, 69.87, 69.46, 69.35, 69.32, 69.21, 69.11, 68.85, 66.87, 66.83, 66.21, 66.17, 39.49, 29.53, 29.48, 26.75, 25.17, 24.47, 16.81, 16.77, 16.75, 16.69. ^{31}P NMR (202 MHz, D_2O) δ 1.96. HR-MS: Calculated for $C_{36}H_{68}NO_{25}P$ $[M+Na]^+$: 968.3710, found : 968.3719.

Phenyl 2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-1-thio-β-D-galactopyranoside (23)



Donor **C** (4.6 g, 7.5 mmol, 1.4 eq) and acceptor **F** (2.7 g, 5.37 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (54 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert* - butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (123 µL, 0.54 mmol, 0.1 eq) was added. The solution was stirred for 1 hour. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 10:1) to yield compound disaccharide **23** (4.65 g, 4.99 mmol, 93%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.09 – 8.07 (m, 2H, Bz), 7.58 – 7.48 (m, 5H), 7.43 (t, *J* = 7.8 Hz, 2H), 7.36 – 7.14 (m, 16H), 5.77 – 5.70 (m, 1H, H-2a), 5.15 (d, *J* = 1.8 Hz, 1H, H-1a), 4.95 (t, *J* = 11.2 Hz, 2H, C*H*$_2$), 4.81 (d, *J* = 10.0 Hz, 1H, C*H*$_2$), 4.74 (d, *J* = 11.5 Hz, 1H, C*H*$_2$), 4.70 – 4.62 (m, 2H, H-1b, C*H*$_2$), 4.56 (d, *J* = 11.5 Hz, 1H, C*H*$_2$), 4.43 (d, *J* = 3.1 Hz, 1H, H-4b), 4.24 – 4.08 (m, 4H, H-6b, 5a, 3a), 3.84 (t, *J* = 9.5 Hz, 1H, H-2b), 3.64 – 3.52 (m, 2H, H-3b, 4a), 3.30 (s, 1H, H-5b), 1.35 (d, *J* = 6.2 Hz, 3H, H-6a), 1.16 (s, 9H), 1.07 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.54 (Bz), 138.97, 138.02, 137.76, 134.83, 133.19, 132.08, 130.10, 129.95, 128.91, 128.88, 128.84, 128.51, 128.46, 128.40, 128.32, 128.20, 128.15, 128.00, 127.65, 127.45, 127.41, 127.37, 100.29 (C-1a), 88.95 (C-1b), 82.93 (C-3b), 80.03 (C-4a), 77.76 (C-3a), 77.03 (C-2b), 76.17 (*C*H$_2$), 74.73 (*C*H$_2$), 73.16 (C-4b), 71.76 (*C*H$_2$), 69.77 (C-2a), 68.52 (C-5a), 67.22 (C-6b), 27.94, 27.53, 23.52, 20.87, 18.49 (C-6a). HR-MS: Calculated for $C_{54}H_{64}O_{10}SSi$ $[M+Na]^+$: 955.3882, found: 955.3882. $[\alpha]^{20}_D$ = + 37.3 (c = 1, $CHCl_3$). TLC: Rf = 0.30 (PE/EA = 9/1, v/v).

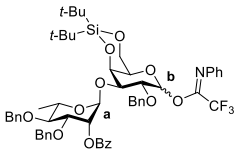
2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α/β-D-galactopyranoside (24)



Compound **23** (2.74 g, 2.94 mmol, 1.0 eq) was dissolved in DCM (30 mL) and reduced to 0 °C. NIS (727 mg, 3.23 mmol, 1.1 eq) and TFA (0.25 mL, 3.23 mmol, 1.1 eq) were added and the solution was stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with MgSO4, filtered and concentrated in vacuo. The compound was purified by flash chromatography (PE/EA 10:1 - 4:1) to yield compound **24** (2.37 g, 2.82 mmol, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.14 – 8.04 (m, 2H), 7.62 – 7.54 (m, 1H), 7.51 – 7.41 (m, 2H), 7.41 – 7.13 (m, 15H), 5.76 – 5.64 (m, 1H), 5.14-5.15 (m, 2H), 5.01 – 4.90 (m, 1H), 4.82 – 4.48 (m, 4H), 4.43 (d, *J* = 2.0 Hz, 1H), 4.25 – 4.16 (m, 1H), 4.16 – 4.00 (m, 2H), 3.96 – 3.81 (m, 2H), 3.61 – 3.48 (m, 1H), 3.42 – 3.31 (m, 1H), 3.11 (d, *J* = 2.0 Hz, 1H), 1.37 – 1.29 (m, 3H), 1.10 – 0.93 (m, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.78, 138.98, 138.11, 137.70, 133.26, 130.14, 130.02, 128.70, 128.67, 128.61, 128.52, 128.36, 128.33, 128.26, 128.23, 128.16, 128.12, 127.66, 127.52, 127.44, 127.43, 100.19, 91.92, 80.16, 77.78, 77.70, 74.80, 74.37, 73.75, 73.67, 71.66, 71.62, 69.78, 68.44, 67.47, 67.23,

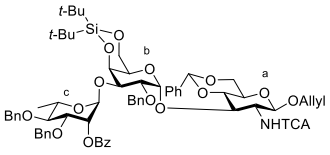
27.56, 27.54, 23.53, 20.84, 18.50. HR-MS: Calculated for $C_{48}H_{60}O_{11}Si$ $[M+K]^+$: 879.3536, found: 879.3521. TLC: Rf = 0.50 (PE/EA = 2/1, v/v).

N-Phenyl-trifluoroacetimidate 2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α/β-D-galactopyranoside (25)



Hemiacetal **24** (3.5 g, 4.16 mmol, 1.0 eq) was dissolved in Acetone (42 mL) and cooled to 0 ℃. Cesium carbonate (1.5 g, 4.6 mmol, 1.1 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (1.2 g, 5.78 mmol, 1.3 eq) was added, and then it was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 40:1 – 10/1) to yield compound **25** (4.0 g, 3.95 mmol, 95%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.13 – 8.00 (m, 2H, Bz), 7.61 – 7.49 (m, 1H), 7.48 – 7.41 (m, 2H), 7.41 – 7.10 (m, 18H), 7.09 – 7.00 (m, 1H), 6.75 (d, *J* = 7.7 Hz, 2H), 6.41 (s, 1H, H-1b), 5.75 – 5.63 (m, 1H, H-2a), 5.18 (d, *J* = 2.0 Hz, 1H, H-1a), 4.93 (d, *J* = 11.5 Hz, 1H, C*H*$_2$), 4.80 – 4.59 (m, 4H, C*H*$_2$), 4.58 – 4.45 (m, 2H, H-4b, C*H*$_2$), 4.28 – 4.12 (m, 2H, H-6b), 4.12 – 4.00 (m, 3H, H-2b, 3a, 5a), 3.96 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3b), 3.81 (s, 1H, H-5b), 3.57 (t, *J* = 9.3 Hz, 1H, H-4a), 1.34 (d, *J* = 6.3 Hz, 3H, H-5a), 1.04 (s, 9H), 0.98 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.78 (Bz), 143.97, 139.21, 138.35, 137.84, 133.16, 130.57, 130.09, 128.85, 128.69, 128.53, 128.39, 128.28, 128.19, 128.12, 127.68, 127.65, 127.44, 124.35, 119.70, 100.22 (C-1a), 94.89 (C-1b), 80.39 (C-4a), 78.00 (C-3a), 77.11 (C-3b), 74.86 , 73.71, 73.47 (C-2b, 4b), 71.79, 70.24 (C-5b), 69.93 (C-2a), 68.72 (C-5a), 66.92 (C-6b), 27.67, 27.55, 23.56, 20.94, 18.55 (C-6a). HR-MS: Calculated for $C_{56}H_{64}F_3NO_{11}Si$ $[M-[O(C=NPh)CF_3]+OH+Na]^+$: 863.3797, found: 863.3813. TLC: Rf = 0.80 (PE/EA = 4/1, v/v).

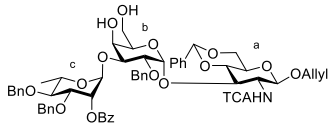
Allyl 4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-di-tert-butylsilylidene-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α-D-galactopyranosyl)-2-trichloroacetamido-2-deoxy-β-D-glucopyranoside (26)



Donor **25** (4.84 g, 4.79 mmol, 1.5 eq) and acceptor **E** (1.45 g, 3.2 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (32 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 ℃ and *tert* - butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (84 μL, 0.32 mmol, 0.1 eq) was added. The solution was stirred for 1.5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 10:1 - 5:1) to yield compound **26** (3.30 g, 2.59 mmol, 81%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 – 8.03 (m, 2H, Bz), 7.63 – 7.55 (m, 1H, Bz), 7.47 (t, *J* = 7.6 Hz, 2H, Bz), 7.42 – 7.22 (m, 12H), 7.22 – 7.16 (m, 3H), 7.16 – 7.03 (m, 5H), 7.00 (d, *J* = 7.5 Hz, 1H，N*H*TCA), 5.92 – 5.77 (m, 1H, OCH_2CHCH_2), 5.72 – 5.65 (m, 1H, H-2c), 5.54 (d, *J* = 3.5 Hz, 1H, H-1b), 5.47 (s, 1H, PhC*H*), 5.32 – 5.22 (m, 1H, OCH_2CHCH_2), 5.22 – 5.15 (m, 1H, OCH_2CHCH_2), 5.12 (s, 1H, H-1c), 5.07 (d, *J* =

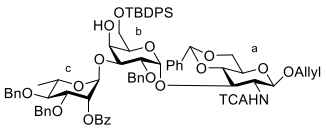
8.3 Hz, 1H, H-1a), 4.92 (d, *J* = 11.4 Hz, 1H, Bn), 4.73 (d, *J* = 11.5 Hz, 1H, Bn), 4.68 – 4.58 (m, 2H, Bn), 4.56 – 4.47 (m, 3H, Bn), 4.39 – 4.27 (m, 3H, H-5a, OCH_2CHCH$_2$), 4.15 – 3.95 (m, 5H, OCH_2CHCH$_2$, H-5c, H-3c), 3.94 – 3.70 (m, 4H, H-2b, H-4a), 3.64 (s, 1H), 3.59 – 3.47 (m, 3H, H-2a, H-4c), 1.29 (d, *J* = 6.3 Hz, 3H, H-6c), 0.99 (s, 9H, *t*-Bu), 0.89 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.69 (Bz), 161.69 (TCA), 139.00, 138.13, 137.61, 136.96, 133.31 (OCH$_2$*C*HCH$_2$), 133.22, 130.18, 130.02, 129.41, 128.52, 128.48, 128.35, 128.25, 128.21, 128.13, 127.86, 127.63, 127.53, 127.42, 126.31, 118.52 (OCH$_2$CH*C*H$_2$), 101.63 (Ph*C*H), 100.12 (C-1c), 98.62 (C-1a), 96.48 (C-1b), 92.50 (TCA), 82.75 (C-4a), 80.11 (C-4c), 77.82 (C-3c), 77.11, 74.79 (Bn), 73.77 (C-5a), 72.60 (C-2b), 72.54 (Bn), 71.71 (C-3a), 71.51 (Bn), 70.92 (O*C*H$_2$CHCH$_2$), 69.54, 68.83, 68.37 (C-2c), 67.67 (C-5c), 67.26, 65.94, 58.35 (C-2a), 27.56 (*t*-Bu), 27.38 (*t*-Bu), 23.55 (*t*-Bu), 20.77 (*t*-Bu), 18.48 (C-6c). HR-MS calculated for $C_{66}H_{78}Cl_3NO_{16}Si$ $[M+Na]^+$: 1296.4048, found: 1296.4050. $[\alpha]^{20}_D$ = + 43° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EtOAc = 4/1, v/v).

Allyl 4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α-D-galactopyranosyl)-2-trichloroacetamido-2-deoxy-β-D-glucopyranoside (27)



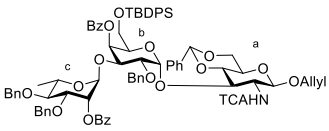
Compound **26** (222 mg, 0.174 mmol, 1.0 eq) was dissolved in THF (1 mL) and pyridine (1mL), then cooled to 0 °C and hydrogen fluoride (HF)/pyridine (70%) (0.1 mL) was added dropwise. The solution was stirred for overnight. After TLC showed complete consumption of the starting material, the reaction 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 (DCM/Acetone 20:1 - 7:1) to yield compound **27** (172 mg, 0.152 mmol, 87%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.48 (d, *J* = 9.4 Hz, 1H, NHTCA), 8.16 – 8.08 (m, 2H, Bz), 7.70 – 7.62 (m, 1H, Bz), 7.59 – 7.48 (m, 4H, Bz), 7.45 – 7.37 (m, 3H), 7.37 – 7.12 (m, 15H), 5.96 – 5.78 (m, 3H, OCH$_2$C*H*CH$_2$, H-1b, H-2c), 5.74 (s, 1H, PhC*H*), 5.36 (d, *J* = 1.8 Hz, 1H, H-1c), 5.29 (dq, *J* = 17.2, 1.8 Hz, 1H, OCH$_2$CHC*H*$_2$), 5.12 (dq, *J* = 10.6, 1.5 Hz, 1H, OCH$_2$CHC*H*$_2$), 4.96 – 4.78 (m, 3H, H-1a, Bn), 4.67 (dd, *J* = 11.6, 9.7 Hz, 2H, Bn), 4.59 – 4.43 (m, 3H, Bn, H-3a), 4.37 – 4.27 (m, 3H, OCH_2CHCH$_2$, H-6a), 4.25 – 4.04 (m, 6H, H-3c, H-2a), 4.04 – 3.71 (m, 7H, H-4a, H-5c, H-5a, H-6a, H-2b), 3.67 – 3.52 (m, 2H), 3.23 (s, 1H), 1.34 (d, *J* = 6.2 Hz, 3H, H-6c). ^{13}C NMR (101 MHz, Acetone) δ 165.96, 162.65, 139.64, 139.34, 139.24, 138.50, 134.87, 134.02, 130.93, 130.36, 129.77, 129.35, 128.98, 128.96, 128.87, 128.79, 128.76, 128.44, 128.34, 128.20, 128.12, 128.05, 127.10, 116.92 (OCH$_2$CH*C*H$_2$), 102.12 (Ph*C*H), 101.43 (C-1c), 99.80 (C-1a), 96.24 (C-1b), 93.60 (TCA), 83.98 (C-4a), 80.97, 79.16, 75.87, 75.65, 75.02, 72.81 (C-3a), 72.05, 71.46, 71.00, 70.59 (O*C*H$_2$CHCH$_2$), 70.47, 70.13 (C-2c), 69.08, 68.64 (C-5c), 66.47, 62.62, 57.37 (C-2a), 18.44 (C-6c). HR-MS: Calculated for $C_{58}H_{62}Cl_3NO_{16}$ $[M+NH_4]^+$: 1151.3472, found: 1151.3491. $[\alpha]^{20}_D$ = + 21.5° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (DCM/Acetone = 10/1, v/v).

Allyl 4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α-D-galactopyranosyl)-2-trichloroacetamido-2-deoxy-β-D-glucopyranoside (28)



Compound **27** (170 mg, 0.15 mmol, 1.0 eq) was dissolved in DMF (2 mL), then cooled to 0 °C and *tert*-butyl(chloro)diphenylsilane (TBDPSCl) (41.3 mg, 0.15 mmol, 1.0 eq) and imidazole (13 mg, 0.18 mmol, 1.2 eq) were added. The solution was stirred for overnight. After TLC showed complete consumption of the starting material, the reaction was 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 10:1 - 3:1) to yield compound **28** (193 mg, 0.14 mmol, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 – 8.02 (m, 2H, Bz), 7.71 – 7.63 (m, 4H), 7.61 – 7.54 (m, 1H, Bz), 7.50 – 7.43 (m, 2H, Bz), 7.43 – 7.07 (m, 26H), 6.85 (d, *J* = 8.2 Hz, 1H, NHTCA), 5.89 – 5.76 (m, 1H, OCH_2CHCH_2), 5.73 (dd, *J* = 3.3, 1.9 Hz, 1H, H-2c), 5.68 (d, *J* = 3.8 Hz, 1H, H-1b), 5.40 (s, 1H, PhC*H*), 5.30 – 5.21 (m, 2H, H-1c, OCH_2CHCH_2), 5.17 (dq, *J* = 10.4, 1.3 Hz, 1H, OCH_2CHCH_2), 4.90 (d, *J* = 10.9 Hz, 1H, Bn), 4.86 – 4.74 (m, 2H, H-1a, Bn), 4.62 (d, *J* = 10.9 Hz, 1H, Bn), 4.57 – 4.40 (m, 4H, Bn), 4.37 – 4.25 (m, 2H, H-6a, Bn), 4.08 – 3.95 (m, 4H, H-4a, OCH_2CHCH_2), 3.95 – 3.67 (m, 8H, H-6a, H-5c, H-2a), 3.58 – 3.42 (m, 2H), 2.51 (s, 1H), 1.32 (d, *J* = 6.2 Hz, 3H, H-6c), 1.07 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.57 (Bz), 161.75 (TCA), 138.44, 138.12, 137.90, 136.86, 135.86 (Bz), 135.78 (OCH_2CHCH_2), 133.29, 133.23, 132.99, 132.78, 130.06, 130.01, 129.98, 129.44, 128.53, 128.50, 128.47, 128.45, 128.38, 128.17, 128.12, 127.93, 127.81, 127.77, 127.70, 126.23, 118.29 (OCH_2CHCH_2), 101.69 (PhCH), 99.66 (C-1c), 99.30 (C-1a), 95.81 (C-1b), 92.41 (TCA), 82.84, 80.06, 78.03, 75.61, 75.50, 74.74, 72.23, 71.73, 71.62, 70.66 (OCH_2CHCH_2), 70.08, 69.83, 69.18, 68.81, 68.47, 65.89, 63.95, 57.54 (C-2a), 27.14 (TBDPS), 19.32 (TBDPS), 18.28 (C-6c). HR-MS: Calculated for $C_{74}H_{80}Cl_3NO_{16}Si$ $[M+NH_4]^+$: 1391.4647, found: 1391.4677. $[\alpha]^{20}_D$ = + 28.8° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 4/1, v/v).

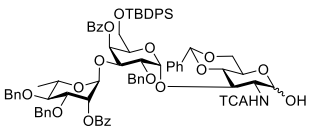
Allyl 4,6-*O*-benzylidene-3-*O*-(4-*O*-benzoyl-2-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α-D-galactopyranosyl)-2-trichloroacetamido-2-deoxy-β-D-glucopyranoside (29)



Compound **28** (2.47 g, 1.8 mmol, 1.0 eq) was dissolved in pyridine (18 mL), then cooled to 0 °C and 4-dimethylaminopyridine (DMAP) (110 mg, 0.9 mmol, 0.5 eq) were added, benzoyl chloride (BzCl) (630 µL, 5.4 mmol, 3 eq) was added dropwise. The solution was stirred for 3 days at RT. After TLC showed complete consumption of the starting material, the reaction was quenched by MeOH 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 10:1 - 5:1) to yield compound **29** (2.53 g, 1.71 mmol, 95%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.05 – 7.97 (m, 2H, Bz-2c), 7.86 – 7.77 (m, 2H, Bz-4b), 7.71 – 7.62 (m, 2H), 7.58 – 7.47 (m, 4H), 7.47 – 7.04 (m, 30H), 6.89 (d, *J* = 8.7 Hz, 1H, NHTCA), 5.91 – 5.72 (m, 2H, OCH_2CHCH_2, H-1b), 5.61 (d, *J* = 3.9 Hz, 1H, H-4b), 5.52 – 5.42 (m, 2H, H-2c, PhC*H*), 5.33 – 5.21 (m, 2H, OCH_2CHCH_2, H-1c), 5.21 – 5.12 (m, 1H, OCH_2CHCH_2), 4.83 (d, *J* = 11.6 Hz, 1H,

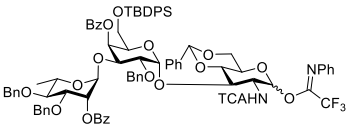
Bn), 4.70 (d, *J* = 8.3 Hz, 1H, H-1a), 4.66 – 4.24 (m, 9H, Bn, H-3a, H-3b, H-6a, OC*H*$_2$CHCH$_2$), 4.17 (d, *J* = 11.3 Hz, 1H, H-4a), 4.12 – 3.90 (m, 3H, H-5c, H-2a, OC*H*$_2$CHCH$_2$), 3.90 – 3.68 (m, 6H, H-2b, H-3c, H-6b, H-6a), 3.59 – 3.37 (m, 2H, H-4c, H-5a), 1.33 (d, *J* = 6.2 Hz, 3H, H-6c), 1.06 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.59 (Bz), 165.51 (Bz), 161.68 (TCA), 138.89, 138.15, 137.55, 136.95, 135.75, 135.53, 133.17 (OCH$_2$*C*HCH$_2$), 133.05, 132.99, 132.96, 132.73, 130.00, 129.91, 129.86, 129.81, 129.73, 129.41, 128.49, 128.36, 128.33, 128.31, 128.14, 128.11, 127.85, 127.78, 127.72, 127.67, 127.34, 127.32, 126.32, 118.11 (OCH$_2$CH*C*H$_2$), 101.84 (Ph*C*H), 99.71 (C-1a), 98.84 (C-1c), 95.40 (C-1b), 92.42 (TCA), 82.92, 79.35 (C-4c), 77.74, 75.38 (C-2b), 73.86 (Bn), 71.72, 71.65, 71.60, 71.46, 70.97 (C-4b), 70.35 (O*C*H$_2$CHCH$_2$), 70.17, 69.58 (C-2c), 68.72 (C-6a), 68.46 (C-5c), 65.87 (C-5a), 62.71 (C-6b), 56.95 (C-2a), 27.11 (TBDPS), 19.22 (TBDPS), 18.21 (C-6c). HR-MS: Calculated for $C_{81}H_{84}Cl_3NO_{17}Si$ $[M+NH_4]^+$: 1493.4912, found: 1493.4919. $[\alpha]^{20}_D$ = + 27.3° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 4/1, v/v).

4,6-*O*-benzylidene-3-*O*-(4-*O*-benzoyl-2-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α-D-galactopyranosyl)-2-trichloroacetamido-2-deoxy-α/β-D-glucopyranoside (30)



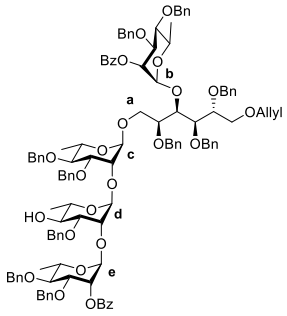
Allyl protected **29** (346 mg, 0.23 mmol, 1 eq) was dissolved in freshly distilled THF (3 ml). The mixture was degassed and placed under an argon atmosphere. (1,5-Cyclooctadiene)(pyridine)-(tricyclohexylphosphine)-iridium(I) hexafluorophosphate (Ir(COD)(Ph$_2$MeP)$_2$·PF$_6$) (10 mg, 0.01 mmol, 0.05 eq) was added and the reaction mixture was purged with H_2 for 5 seconds. The reaction mixture was stirred for 1 hour under an argon atmosphere. After analysis by TLC showed complete consumption of the starting material, diluted with THF (2 ml) and *N*-iodosuccinimide (NIS) (77.6 mg, 0.35 mmol, 1.5 eq), and water were added and the solution stirred for 2 hours at room temperature. EtOAc was added and the organic layer was washed two times with saturated aqueous sodium thiosulphate and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Column chromatography yielded **30** (300 mg, 0.21 mmol, 90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 – 7.95 (m, 2H), 7.77 – 7.71 (m, 2H), 7.69 – 7.59 (m, 2H), 7.56 – 7.21 (m, 24H), 7.21 – 7.02 (m, 16H), 5.80 (d, *J* = 3.5 Hz, 1H), 5.60 – 5.51 (m, 1H), 5.50 – 5.40 (m, 2H), 5.26 (s, 1H), 5.15 (t, *J* = 3.7 Hz, 1H), 4.80 (d, *J* = 11.6 Hz, 1H), 4.65 – 4.52 (m, 2H), 4.48 – 3.91 (m, 12H), 3.88 – 3.57 (m, 6H), 3.49 (t, *J* = 9.4 Hz, 1H), 1.34 (d, *J* = 6.1 Hz, 3H), 1.04 (s, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.71, 165.64, 161.80, 138.91, 138.16, 137.53, 137.13, 135.87, 135.79, 135.58, 133.13, 132.99, 130.02, 129.92, 129.82, 129.67, 129.55, 128.60, 128.45, 128.41, 128.37, 128.35, 128.22, 128.19, 128.17, 127.93, 127.89, 127.84, 127.79, 127.65, 127.41, 127.38, 126.50, 126.42, 102.14, 98.76, 95.68, 92.36, 91.74, 83.60, 79.47, 77.76, 77.36, 74.96, 73.79, 71.61, 71.21, 71.12, 71.09, 70.37, 70.19, 69.59, 69.04, 68.43, 62.87, 62.27, 54.44, 27.04, 19.18, 18.25. HR-MS: Calculated for $C_{78}H_{80}Cl_3NO_{17}Si$ $[M+H]^+$: 1436.4334, found: 1436.4340. TLC: Rf = 0.15 (PE/EA = 7/3, v/v).

_N_-Phenyl-trifluoroacetimidate 4,6-_O_-benzylidene-3-_O_-(4-_O_-benzoyl-2-_O_-benzyl-6-_O_-_tert_-butyldiphenylsilyl-3-_O_-(2-_O_-benzoyl-3,4-di-_O_-benzyl-α-L-rhamnopyranosyl)-α-D-galactopyranosyl)-2-trichloroacetamido-2-deoxy-α/β-D-glucopyranoside (8)



Hemiacetal **30** (282 mg, 0.2 mmol, 1 eq) was dissolved in acetone (3 mL) and cooled to 0 ℃. Cesium carbonate (66 mg, 0.2 mmol, 1.0 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (62 mg, 0.3 mmol, 1.5 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 10:1 – 4/1) to yield compound **8** (289 mg, 0.18 mmol, 91%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.10 – 7.91 (m, 3H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.72 – 7.60 (m, 2H), 7.60 – 7.00 (m, 38H), 6.95 – 6.71 (m, 2H), 5.79 (d, *J* = 3.6 Hz, 1H), 5.70 (t, *J* = 4.0 Hz, 1H), 5.61 – 5.36 (m, 2H), 5.36 – 5.17 (m, 1H), 4.98 – 4.75 (m, 1H), 4.75 – 3.44 (m, 18H), 1.48 – 1.31 (m, 3H), 1.15 – 0.95 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.55, 165.41, 161.93, 142.73, 139.01, 138.27, 137.48, 136.79, 136.69, 135.88, 135.80, 135.68, 135.63, 133.69, 133.08, 132.97, 132.92, 132.63, 130.49, 130.12, 130.05, 129.92, 129.85, 129.79, 129.68, 129.60, 128.98, 128.64, 128.50, 128.39, 128.21, 128.17, 128.04, 127.97, 127.90, 127.86, 127.78, 127.73, 127.66, 127.56, 127.39, 126.45, 126.33, 124.97, 119.26, 105.88, 102.32, 98.92, 96.23, 91.98, 82.61, 79.43, 79.32, 77.79, 77.68, 77.48, 77.16, 76.84, 75.70, 75.04, 73.87, 73.59, 72.18, 71.84, 71.67, 71.61, 70.95, 70.78, 70.61, 70.49, 70.34, 69.93, 69.68, 69.44, 68.58, 68.48, 68.22, 64.65, 63.37, 62.12, 61.41, 53.61, 27.03, 19.08, 18.21. HR-MS: Calculated for $C_{86}H_{84}Cl_3F_3N_2O_{17}Si$ $[M+H]^+$: 1607.4630, found: 1607.4565. TLC: Rf = 0.5 (PE/EA = 4/1, v/v).

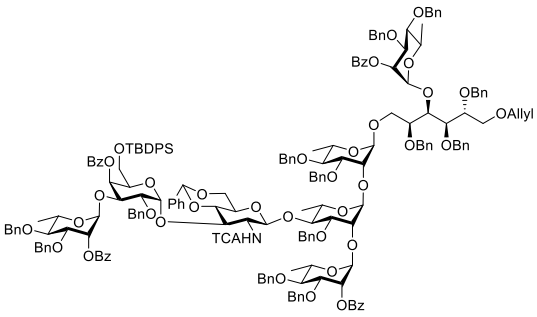
6-Allyl-3-_O_-(2-_O_-benzoyl-3,4-di-_O_-benzyl-α-L-rhamnopyranosyl)-2,4,5-tri-_O_-benzyl-1-_O_-(3,4-di-_O_-benzyl-2-_O_-(2-_O_-(2-_O_-benzoyl-3,4-di-_O_-benzyl-α-L-rhamnopyranosyl)-3-_O_-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-D-glucitol



Full protected **7** (903 mg, 0.44 mmol, 1 eq) was dissolved in DCM (5 mL) and water (0.5 mL). After cooled to 0 ℃, 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) (110 mg, 0.48 mmol, 1.1 eq) was added. The reaction was stirred at RT for 4 hours. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM 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 6:1 – 4/1) to yield title compound (716 mg, 0.37 mmol, 85%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.14 – 8.09 (m, 2H, Bz), 8.09 – 8.04 (m, 2H, Bz), 7.61 – 7.53 (m, 1H), 7.52 – 7.42 (m, 3H), 7.42 – 7.06 (m, 51H), 7.05 – 6.96 (m, 1H), 5.92 – 5.78 (m, 1H, OCH_2CHCH_2), 5.75 – 5.64 (m, 2H, H-2e, 2b), 5.27 – 5.17 (m, 2H, H-1d, OCH_2CHCH_2), 5.14 (d, *J* = 1.9 Hz, 1H, H-1b), 5.10 (dd, *J* = 10.5, 1.6 Hz, 1H, OCH_2CHCH_2), 5.00 (d, *J* = 1.9 Hz, 1H, H-1e), 4.94 – 4.85 (m, 3H, CH_2), 4.84 – 4.65 (m, 6H, H-1c, CH_2), 4.65 – 4.54 (m, 10H, CH_2), 4.54 – 4.43 (m, 2H, CH_2), 4.16 – 3.98 (m,

6H), 3.98 – 3.58 (m, 15H), 3.58 – 3.47 (m, 2H), 3.38 (t, *J* = 9.4 Hz, 1H), 2.43 (s, 1H), 1.33 – 1.22 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.51 (Bz), 138.75, 138.63, 138.49, 138.30, 138.16, 138.09, 137.91, 134.92 ($OCH_2\mathit{C}HCH_2$), 133.13, 133.05, 130.15, 130.09, 129.91, 128.55, 128.41, 128.36, 128.34, 128.32, 128.29, 128.26, 128.24, 128.22, 128.16, 128.12, 128.09, 128.07, 128.04, 128.01, 127.90, 127.84, 127.80, 127.74, 127.63, 127.58, 127.56, 127.54, 127.53, 127.48, 127.38, 116.61 ($OCH_2CH\mathit{C}H_2$), 100.52 (C-1d), 99.32 (C-1e), 98.97 (C-1b, 1c), 80.27, 80.07, 79.96, 79.38, 79.30, 78.89, 78.47, 78.00, 77.93, 77.43, 77.36, 75.35, 75.18, 74.99, 74.66, 74.63, 73.50, 73.33, 72.24, 72.16, 72.04, 71.80, 71.74, 71.60, 71.49, 69.74 (C-2b), 69.72 (C-6a), 69.52 (C-2e), 68.94, 68.52, 68.37, 68.16, 67.07 (C-1a), 18.20, 18.18, 18.15, 17.89. HR-MS: Calculated for $C_{117}H_{126}O_{24}$ $[M+H]^+$: 1915.8712, found: 1915.8734. $[\alpha]^{20}_D$ = + 0.5º (c = 1, $CHCl_3$). TLC: Rf = 0.30 (PE/EA = 4/1, v/v).

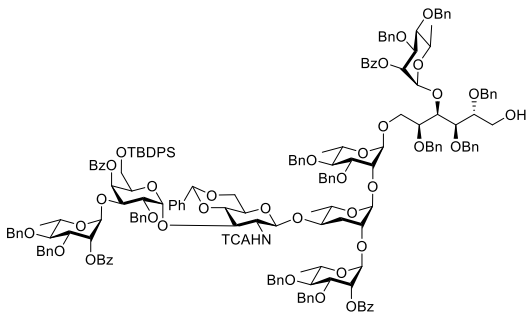
The synthesis of the octamer 31



Donor **8** (758 mg, 0.472 mmol, 2.0 eq) and acceptor **7d** (452 mg, 0.236 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (5 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 ℃ and *tert* - butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (6 µL, 0.023 mmol, 0.1 eq) was added. The solution was stirred for 4 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 10:1 - 4:1) to yield compound **31** (590 mg, 0.177 mmol, 75%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.13 – 8.08 (m, 2H), 8.07 – 8.03 (m, 2H), 8.03 – 7.98 (m, 2H), 7.83 – 7.77 (m, 2H), 7.62 – 7.55 (m, 3H), 7.55 – 7.04 (m, 86H), 7.04 – 6.94 (m, 3H), 6.69 (d, *J* = 8.9 Hz, 1H), 5.91 – 5.79 (m, 1H), 5.76 – 5.63 (m, 4H), 5.52 – 5.41 (m, 2H), 5.32 – 5.04 (m, 6H), 4.94 – 4.82 (m, 4H), 4.82 – 4.33 (m, 25H), 4.26 – 4.15 (m, 3H), 4.10 – 4.00 (m, 4H), 4.00 – 3.61 (m, 24H), 3.60 – 3.44 (m, 5H), 3.29 (t, *J* = 9.3 Hz, 1H), 1.37 (d, *J* = 6.2 Hz, 3H), 1.31 – 1.18 (m, 12H), 0.99 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.58, 165.55, 165.50, 165.37, 161.55, 139.13, 138.87, 138.83, 138.73, 138.37, 138.34, 138.32, 138.28, 138.26, 138.18, 138.15, 138.07, 137.70, 137.16, 135.85, 135.81, 135.65, 135.60, 135.02, 133.25, 133.07, 133.02, 132.99, 132.83, 132.81, 130.29, 130.20, 130.18, 129.97, 129.94, 129.87, 129.65, 129.56, 129.42, 128.74, 128.63, 128.51, 128.42, 128.40, 128.39, 128.36, 128.35, 128.32, 128.28, 128.27, 128.17, 128.14, 128.07, 127.98, 127.95, 127.90, 127.87, 127.85, 127.84, 127.78, 127.69, 127.65, 127.63, 127.61, 127.57, 127.50, 127.44, 127.38, 127.34, 127.30, 126.48, 126.32, 116.63, 101.96, 100.22, 99.97, 99.01, 98.96, 98.81, 95.64, 92.64, 83.14, 80.66, 80.14, 80.06, 79.50, 79.43, 79.32, 78.57, 78.16, 78.07, 77.94, 77.78, 77.46, 77.36, 75.55, 75.39, 75.28, 75.23, 75.11, 73.65, 73.37, 72.95, 72.32, 72.22, 72.01, 71.94, 71.67, 71.61, 71.56, 71.54, 71.02, 70.82, 69.95, 69.90, 69.78, 69.48, 68.85, 68.55, 68.48, 68.35, 68.10, 67.62, 67.15, 65.57, 61.97, 57.44, 27.06, 19.11, 18.35, 18.26, 18.21, 18.18,

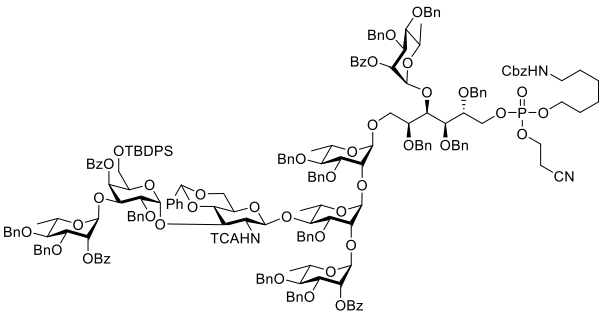
18.05. HR-MS: Calculated for $C_{195}H_{204}Cl_3NO_{40}Si$ $[M+H]^+$: 3333.2867, found: 3333.2771. $[\alpha]^{20}_D$ = + 28.6° (c = 1, $CHCl_3$). TLC: Rf = 0.30 (PE/EtOAc = 4/1, v/v).

The octasaccharide 31a



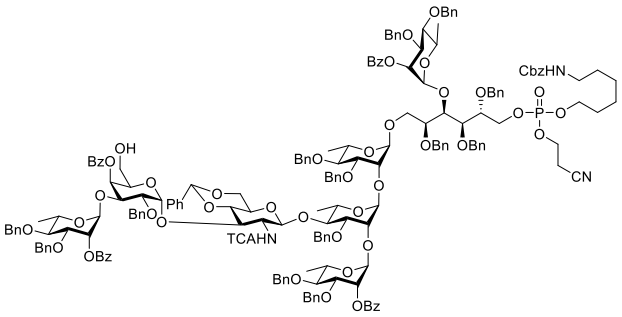
Allyl protected **31** (418 mg, 0.125 mmol, 1.0 eq) was dissolved in freshly distilled THF (4 ml). The mixture was degassed and placed under an argon atmosphere. (1,5-Cyclooctadiene) (pyridine)-(tricyclohexylphosphine)-iridium(I) hexafluorophosphate ($Ir(COD)(Ph_2MeP)_2{\cdot}PF_6$) (10 mg, 0.01 mmol, 0.1 eq) was added and the reaction mixture was purged with H_2 for 5 seconds. The reaction mixture was stirred for 1 hour under an argon atmosphere. After analysis by TLC showed complete consumption of the starting material, diluted with THF (2 ml) and *N*-iodosuccinimide (NIS) (42 mg, 0.19 mmol, 1.5 eq), and water were added, and the solution stirred for 2 hours at room temperature. EtOAc was added and the organic layer was washed two times with saturated aqueous sodium thiosulphate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (PE/EA 5:1 - 3:1) yielded **31a** (359 mg, 0.11 mmol, 87%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.13 – 8.05 (m, 4H), 8.04 – 7.98 (m, 2H), 7.84 – 7.77 (m, 2H), 7.62 – 7.55 (m, 3H), 7.55 – 7.03 (m, 86H), 7.03 – 6.94 (m, 3H), 6.71 (d, *J* = 9.0 Hz, 1H, N*H*TCA), 5.80 – 5.62 (m, 4H, H-1), 5.52 – 5.39 (m, 2H, PhC*H*), 5.28 (d, *J* = 1.7 Hz, 1H, H-1), 5.18 – 5.05 (m, 3H, H-1), 4.94 – 4.82 (m, 4H), 4.82 – 4.27 (m, 25H), 4.25 – 3.60 (m, 29H), 3.58 – 3.41 (m, 5H), 3.29 (t, *J* = 9.3 Hz, 1H), 2.32 (s, 1H), 1.37 (d, *J* = 6.1 Hz, 3H, H-6), 1.32 – 1.19 (m, 12H, H-6), 0.99 (s, 9H, *t*-Bu). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.57, 165.54, 165.35, 161.57, 139.11, 138.70, 138.59, 138.32, 138.29, 138.27, 138.23, 138.17, 138.12, 138.04, 137.97, 137.66, 137.13, 135.83, 135.63, 133.25, 133.11, 132.99, 132.83, 132.78, 130.25, 130.18, 130.16, 130.14, 129.96, 129.93, 129.86, 129.65, 129.56, 129.42, 129.08, 128.73, 128.63, 128.51, 128.46, 128.42, 128.39, 128.35, 128.34, 128.30, 128.28, 128.19, 128.16, 128.13, 128.12, 128.05, 127.99, 127.92, 127.89, 127.86, 127.84, 127.80, 127.78, 127.75, 127.71, 127.68, 127.65, 127.49, 127.47, 127.34, 127.30, 126.47, 125.36, 101.95, 100.18, 99.99, 99.30, 98.99, 98.80, 95.64, 92.60, 83.11, 80.66, 80.12, 80.03, 79.70, 79.47, 79.21, 78.65, 77.92, 77.90, 77.77, 77.36, 75.57, 75.34, 75.29, 74.89, 73.97, 73.65, 73.43, 72.93, 71.91, 71.76, 71.65, 71.60, 71.58, 71.52, 71.00, 70.78, 69.94, 69.81, 69.75, 69.45, 68.83, 68.53, 68.47, 68.33, 68.02, 67.61, 66.79, 65.56, 61.95, 60.40, 57.37, 29.75, 27.05, 19.10, 18.37, 18.30, 18.20, 18.15, 18.04. HR-MS: Calculated for $C_{185}H_{194}Cl_3NO_{39}Si$ $[M+H]^+$: 3293.2554, found: 3293.2729. $[\alpha]^{20}_D$ = + 26.8° (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/EtOAc = 3/1, v/v).

The octasaccharide 32



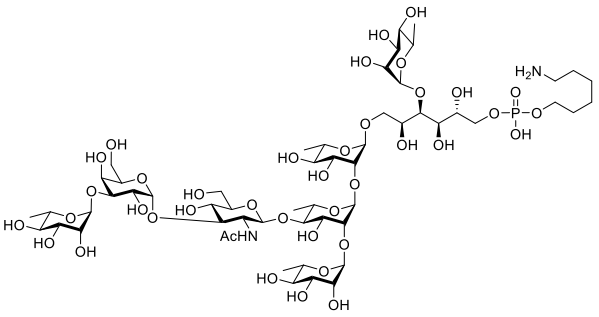
Alcohol **31a** (365 mg, 0.11 mmol, 1.0 eq) was co-evaporated with dry acetonitrile 3 times. Dissolved in dry acetonitrile (4 ml), 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (0.89 mL, 0.22 mmol, 2.0 eq) and 3Å molecular sieves were added. The mixture was stirred for 15 mins under argon atmosphere. Benzyl 6-([*N, N*-diisopropylamino]-2-cyanoethyl-phosphite)-hexyl-1-carbamate (0.16M in acetonitrile) (1.4 mL, 0.22 mmol, 2.0 eq) was added. The reaction mixture was stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (0.67 mL, 0.33 mmol, 3.0 eq) was added. Stirred another 15 mins and diluted with EtOAc. The solution was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (DCM/Acetone 100:1 - 10:1) yielded **32** (374 mg, 0.10 mmol, 92%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.14 – 7.98 (m, 6H, Bz), 7.81 (d, *J* = 7.6 Hz, 2H, Bz), 7.63 – 7.54 (m, 3H), 7.54 – 7.04 (m, 91H), 7.04 – 6.95 (m, 3H), 6.73 (d, *J* = 9.1 Hz, 1H), 5.82 – 5.63 (m, 4H), 5.54 – 5.41 (m, 2H), 5.28 (s, 1H), 5.19 – 5.02 (m, 5H), 4.92 – 4.34 (m, 30H), 4.33 – 4.11 (m, 5H), 4.09 – 3.44 (m, 32H), 3.31 (t, *J* = 9.3 Hz, 1H), 3.16 – 2.99 (m, 2H), 2.40 – 2.03 (m, 2H), 1.66 – 1.47 (m, 2H), 1.45 – 1.33 (m, 5H), 1.33 – 1.10 (m, 16H), 1.00 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.52, 165.47, 165.44, 165.30, 161.50, 156.37, 139.03, 138.68, 138.46, 138.43, 138.25, 138.23, 138.21, 138.11, 137.97, 137.93, 137.89, 137.87, 137.82, 137.75, 137.73, 137.57, 137.06, 136.72, 135.77, 135.56, 133.20, 133.12, 132.96, 132.91, 132.78, 132.70, 130.17, 130.08, 130.05, 129.98, 129.89, 129.85, 129.78, 129.59, 129.50, 129.37, 128.67, 128.49, 128.45, 128.41, 128.38, 128.32, 128.28, 128.20, 128.18, 128.15, 128.12, 128.10, 128.07, 128.04, 128.00, 127.93, 127.83, 127.81, 127.79, 127.76, 127.72, 127.68, 127.64, 127.60, 127.58, 127.53, 127.48, 127.43, 127.28, 127.24, 126.40, 116.73, 116.64, 101.89, 100.11, 99.94, 98.90, 98.85, 98.71, 95.56, 92.55, 83.04, 80.56, 80.03, 79.92, 79.39, 79.17, 78.66, 78.60, 78.53, 78.48, 78.16, 78.09, 77.92, 77.83, 77.68, 77.36, 77.00, 75.49, 75.36, 75.32, 75.25, 75.18, 74.94, 73.85, 73.56, 73.46, 73.39, 72.85, 72.22, 72.11, 71.86, 71.59, 71.57, 71.51, 71.44, 71.42, 70.92, 70.70, 69.86, 69.69, 69.66, 69.63, 69.35, 68.75, 68.62, 68.40, 68.28, 68.16, 68.12, 68.05, 67.57, 66.91, 66.47, 65.50, 61.88, 61.66, 61.62, 57.27, 40.80, 30.00, 29.98, 29.95, 29.92, 29.69, 26.99, 26.04, 24.93, 24.90, 19.25, 19.19, 19.16, 19.10, 19.03, 18.30, 18.21, 18.14, 18.11, 18.00. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.33, -0.80. MALDI-TOF: Calculated for $C_{209}H_{223}Cl_3N_3O_{45}PSi$ $[M+Na]^+$: 3681.372, found: 3680.636. TLC: Rf = 0.50 (DCM/Acetone = 20/1, v/v).

The octasacchride 6



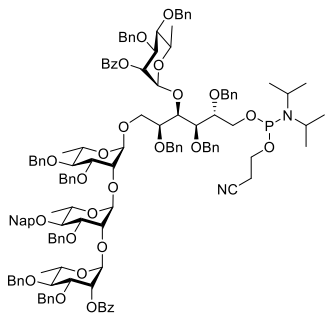
Compound **32** (355 mg, 0.097 mmol, 1.0 eq) was dissolved in THF (1 mL) and pyridine (1mL), then cooled to 0 °C and hydrogen fluoride (HF)/pyridine (70%) (0.1 mL) was added dropwise. The solution was stirred for 6 h. After TLC showed complete consumption of the starting material, the reaction was quenched 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 (DCM/Acetone 40:1 - 10:1) to yield compound **6** (308 mg, 0.09 mmol, 93%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.12 – 7.99 (m, 6H), 7.86 – 7.78 (m, 2H), 7.62 – 7.55 (m, 1H), 7.55 – 7.49 (m, 3H), 7.49 – 7.14 (m, 66H), 7.14 – 7.02 (m, 10H), 7.00 – 6.94 (m, 1H), 6.91 (d, *J* = 9.0 Hz, 1H), 5.70 (d, *J* = 3.5 Hz, 1H, H-1), 5.68 – 5.62 (m, 2H), 5.60 – 5.54 (m, 1H), 5.50 (s, 1H), 5.40 (d, *J* = 3.2 Hz, 1H), 5.27 (d, *J* = 1.8 Hz, 1H, H-1), 5.21 (s, 2H, H-1), 5.14 – 5.02 (m, 3H, H-1), 4.92 – 4.80 (m, 5H), 4.81 – 4.35 (m, 24H, H-1), 4.34 – 4.23 (m, 2H), 4.23 – 4.11 (m, 2H), 4.11 – 3.38 (m, 33H), 3.33 (t, *J* = 9.3 Hz, 1H), 3.16 – 3.00 (m, 2H, C*H*$_2$NHCbz), 2.48 – 2.37 (m, 1H), 2.37 – 2.06 (m, 2H, OCH$_2$*CH*$_2$CN), 1.65 – 1.45 (m, 2H), 1.45 – 1.33 (m, 2H), 1.33 – 1.09 (m, 19H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.84, 165.57, 165.45, 161.93, 156.38, 138.67, 138.64, 138.46, 138.44, 138.24, 138.16, 138.06, 137.99, 137.98, 137.96, 137.93, 137.89, 137.87, 137.81, 137.73, 137.34, 137.04, 136.72, 133.40, 133.23, 133.12, 133.05, 129.98, 129.90, 129.88, 129.86, 129.47, 129.30, 128.73, 128.66, 128.49, 128.45, 128.42, 128.39, 128.34, 128.31, 128.29, 128.27, 128.22, 128.18, 128.15, 128.12, 128.10, 128.04, 128.00, 127.96, 127.85, 127.83, 127.80, 127.77, 127.71, 127.68, 127.64, 127.62, 127.57, 127.54, 127.36, 127.32, 127.11, 126.50, 116.73, 116.64, 102.19, 100.35, 100.04, 99.04, 98.88, 98.83, 98.79, 95.94, 92.41, 83.00, 80.44, 80.04, 79.93, 79.43, 79.23, 78.64, 78.58, 78.51, 78.46, 78.16, 78.07, 77.92, 77.85, 77.72, 77.69, 77.36, 76.97, 75.50, 75.36, 75.32, 75.12, 74.96, 74.89, 74.47, 74.26, 74.08, 73.85, 73.42, 72.21, 72.00, 71.98, 71.73, 71.58, 71.42, 71.39, 71.09, 70.81, 69.68, 69.64, 69.57, 69.45, 69.43, 69.27, 68.81, 68.63, 68.54, 68.38, 68.18, 68.13, 68.06, 67.47, 66.97, 66.91, 66.49, 66.34, 65.71, 61.67, 61.63, 60.86, 57.17, 40.81, 30.01, 29.98, 29.95, 29.93, 29.69, 26.06, 26.04, 24.93, 24.91, 19.26, 19.20, 19.16, 19.11, 18.28, 18.21, 18.12, 18.11, 17.99. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.37, -0.85. HR-MS: Calculated for $C_{193}H_{205}Cl_3N_3O_{45}P$ $[M+NH_4]^+$: 3421.2721, found: 3421.2795. TLC: Rf = 0.3 (DCM/Acetone = 20/1, v/v).

The Octasaccharide 2



Full protected octamer **6** (64.5 mg, 18.8 µmol, 1.0 eq) was dissolved in dioxane (6 mL) and ammonia solution (35%) (3 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The crude was dissolved in methanol (3 mL) and dioxane (2 mL). Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 23 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (7 mL), water (3 mL) and 4 drops acetic acid. After $Pd(OH)_2/C$ (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **3** (19.2 mg, 13.2 µmol, 70%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.478 (d, *J* = 4.0 Hz, 1H), 5.125 (d, *J* = 1.8 Hz, 1H), 5.024 (d, *J* = 1.8 Hz, 1H), 4.996 (d, *J* = 1.7 Hz, 1H), 4.945 (d, *J* = 1.8 Hz, 1H), 4.868 (d, *J* = 1.7 Hz, 1H), 4.808 (d, *J* = 8.1 Hz, 1H), 4.148 – 3.944 (m, 11H), 3.940 – 3.858 (m, 6H), 3.858 – 3.674 (m, 19H), 3.674 – 3.596 (m, 2H), 3.491 – 3.377 (m, 5H), 2.977 – 2.900 (m, 2H), 2.061 (s, 3H), 1.639 (m, *J* = 6.9 Hz, 4H), 1.466 – 1.360 (m, 4H), 1.358 – 1.220 (m, 15H). ^{13}C NMR (126 MHz, D_2O) δ 174.45, 102.40, 102.32, 101.43, 101.40, 100.47, 98.81, 98.76, 80.06, 79.13, 78.70, 78.37, 77.83, 77.22, 75.44, 72.26, 72.05, 71.97, 71.02, 70.74, 70.60, 70.45, 70.22, 70.14, 70.12, 70.10, 70.02, 69.98, 69.46, 69.34, 69.24, 69.17, 69.11, 68.90, 68.84, 67.76, 67.65, 66.89, 66.24, 66.20, 60.51, 60.44, 54.42, 39.61, 29.56, 29.51, 27.32, 25.24, 24.52, 22.47, 17.21, 16.85, 16.74, 16.70, 16.68. ^{31}P NMR (202 MHz, D_2O) δ 1.96. HR-MS: Calculated for $C_{56}H_{101}N_2O_{39}P$ $[M+Na]^+$: 1457.5792, found : 1457.5803.

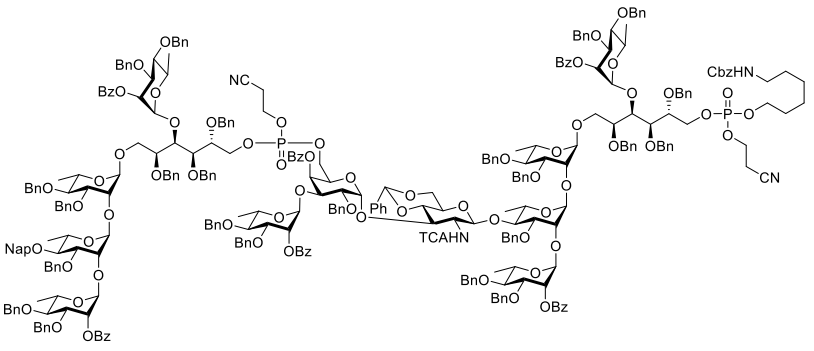
The pentasaccharide 5



Alcohol **20** (266 mg, 0.132 mmol, 1.0 eq) was co-evaporated with dry acetonitrile 3 times. Dissolved in dry DCM (5 ml), *N,N*-Diisopropylethylamine (DIPEA) (0.12 mL, 0.65 mmol, 5.0 eq) and 3Å molecular sieves were added. The mixture was stirred for 15 mins under argon atmosphere. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (90 µL, 0.39 mmol, 3.0 eq) was added. The reaction mixture was stirred for 1.5 hours. After analysis by TLC showed complete consumption of the starting material, DCM 5 mL was added. The solution was washed with

saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (PE/EA 8/1 – 3/1) yielded **5** (239 mg, 0.108 mmol, 84%). ^{1}H NMR (500 MHz, Acetonitrile-d_3) δ 8.15 – 8.07 (m, 2H), 8.07 – 7.99 (m, 2H), 7.88 – 7.78 (m, 4H), 7.66 (t, *J* = 7.5 Hz, 1H), 7.61 – 7.51 (m, 3H), 7.51 – 7.40 (m, 5H), 7.39 – 7.03 (m, 50H), 5.77 – 5.69 (m, 1H), 5.68 – 5.60 (m, 1H), 5.18 – 5.08 (m, 3H), 5.00 (d, *J* = 11.4 Hz, 1H), 4.89 – 4.39 (m, 21H), 4.22 – 3.47 (m, 24H), 3.46 – 3.36 (m, 1H), 2.57 – 2.30 (m, 2H), 1.32 – 1.04 (m, 28H). ^{13}C NMR (126 MHz, CD_3CN) δ 166.24, 166.18, 139.95, 139.84, 139.81, 139.78, 139.52, 139.49, 139.46, 139.41, 139.14, 139.11, 137.44, 134.44, 134.34, 134.20, 133.86, 130.93, 130.87, 130.56, 130.51, 129.69, 129.60, 129.30, 129.25, 129.20, 129.18, 129.16, 129.14, 129.11, 129.05, 128.96, 128.93, 128.87, 128.85, 128.82, 128.80, 128.71, 128.68, 128.59, 128.55, 128.53, 128.49, 128.45, 128.40, 128.33, 128.29, 127.51, 127.37, 127.10, 126.90, 118.26, 101.04, 99.70, 99.58, 81.08, 80.98, 80.86, 80.79, 80.24, 80.17, 80.04, 79.98, 79.72, 79.54, 79.46, 78.84, 78.82, 78.45, 78.16, 77.94, 77.80, 75.85, 75.73, 75.69, 75.65, 75.62, 75.51, 75.40, 75.36, 74.27, 73.75, 72.53, 72.44, 72.40, 72.37, 72.16, 72.12, 72.02, 70.60, 70.56, 70.17, 69.45, 69.43, 69.29, 69.26, 68.92, 67.72, 62.99, 62.89, 62.77, 59.49, 59.34, 59.31, 59.16, 43.91, 43.82, 25.20, 25.13, 25.07, 24.96, 24.90, 24.85, 21.01, 20.95, 20.89, 18.76, 18.65. ^{31}P NMR (202 MHz, CD_3CN) δ 149.50, 149.21. HR-MS: Calculated for $C_{134}H_{147}N_2O_{25}P$ $[M+H]^+$: 2216.0103, found: 2216.0112. TLC: Rf = 0.40 (PE/EA = 4/1, v/v).

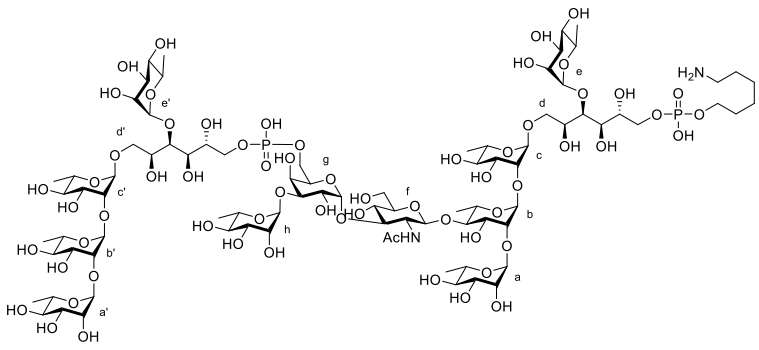
The Tridecasaccharide 4



Alcohol **6** (93 mg, 0.03 mmol, 1.0 eq) and phosphoramidite **5** (122 mg, 0.06 mmol, 2.0 eq) were co-evaporated with dry acetonitrile 3 times. Dissolved in dry acetonitrile (5 ml), and 3Å molecular sieves was added. After stirred 15 mins under argon atmosphere, 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (0.33 mL, 0.08 mmol, 3.0 eq) was added. The reaction mixture was stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (0.22 mL, 0.11 mmol, 4.0 eq) was added. Stirred another 15 mins and diluted with EtOAc. The solution was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (DCM/Acetone 60:1 - 10:1) yielded **4** (124 mg, 0.022 mmol, 82%). ^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.29 – 8.14 (m, 1H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.08 – 7.95 (m, 8H), 7.85 – 7.78 (m, 3H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.67 (d, *J* = 7.7 Hz, 1H), 7.64 – 7.55 (m, 2H), 7.55 – 6.88 (m, 143H), 5.93 – 5.81 (m, 1H), 5.80 – 5.74 (m, 1H), 5.70 – 5.62 (m, 2H), 5.60 – 5.51 (m, 1H), 5.51 – 5.41 (m, 3H), 5.24 (d, *J* = 4.8 Hz, 1H), 5.21 – 5.01 (m, 8H), 5.01 – 4.92 (m, 2H), 4.92 – 4.17 (m, 57H), 4.17 – 3.36 (m, 54H), 3.35 – 3.28 (m, 1H), 3.24 (t, *J* = 9.3 Hz, 1H), 3.17 – 3.03 (m, 2H), 2.38 – 1.87 (m,

2H), 1.83 – 1.68 (m, 2H), 1.59 – 1.51 (m, 2H), 1.43 – 1.35 (m, 2H), 1.33 – 1.05 (m, 31H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 165.96, 165.93, 165.65, 165.64, 165.56, 165.54, 165.51, 165.48, 162.29, 162.26, 156.47, 139.05, 138.96, 138.95, 138.88, 138.84, 138.79, 138.77, 138.62, 138.59, 138.56, 138.51, 138.47, 138.42, 138.41, 138.32, 138.31, 138.27, 138.23, 138.22, 138.19, 138.17, 138.13, 138.10, 138.04, 138.02, 138.00, 137.98, 137.96, 137.94, 137.93, 137.89, 137.81, 137.66, 137.53, 137.49, 137.11, 136.80, 136.09, 136.07, 133.43, 133.42, 133.25, 133.21, 133.18, 133.07, 130.27, 130.12, 130.06, 130.03, 130.01, 129.99, 128.68, 128.62, 128.56, 128.53, 128.50, 128.48, 128.47, 128.44, 128.42, 128.41, 128.39, 128.37, 128.36, 128.35, 128.34, 128.32, 128.31, 128.30, 128.29, 128.27, 128.25, 128.24, 128.23, 128.21, 128.20, 128.19, 128.18, 128.15, 128.11, 128.10, 128.06, 128.00, 127.96, 127.92, 127.90, 127.89, 127.87, 127.85, 127.81, 127.79, 127.78, 127.76, 127.75, 127.74, 127.69, 127.66, 126.35, 116.88, 116.81, 116.77, 102.02, 101.85, 100.58, 100.46, 100.40, 100.30, 100.28, 99.29, 99.18, 99.01, 98.99, 98.93, 98.87, 98.81, 98.75, 95.91, 95.53, 92.81, 83.49, 80.83, 80.81, 80.37, 80.34, 80.31, 80.29, 80.27, 80.19, 80.06, 80.04, 79.90, 79.88, 79.53, 79.38, 79.31, 79.23, 79.20, 78.67, 78.25, 78.15, 78.14, 78.09, 78.07, 78.06, 77.97, 77.92, 77.31, 77.23, 77.16, 77.09, 77.06, 77.01, 76.83, 76.77, 76.04, 75.58, 75.52, 75.50, 75.48, 75.43, 75.41, 75.37, 75.36, 75.33, 75.32, 75.27, 75.22, 75.20, 74.82, 74.59, 74.46, 74.39, 74.33, 74.27, 74.04, 74.03, 73.76, 73.69, 73.60, 73.57, 73.52, 73.51, 73.33, 73.23, 73.21, 72.95, 72.51, 72.36, 72.24, 72.23, 72.04, 72.01, 71.97, 71.79, 71.78, 71.68, 71.67, 71.65, 71.58, 71.55, 71.53, 71.46, 71.44, 71.29, 71.17, 70.55, 70.52, 69.72, 69.69, 69.66, 69.59, 69.54, 69.40, 69.38, 69.36, 69.34, 69.03, 68.82, 68.80, 68.67, 68.66, 68.63, 68.61, 68.56, 68.54, 68.42, 68.41, 68.25, 68.23, 68.20, 68.18, 68.16, 68.14, 67.79, 67.16, 66.99, 66.91, 66.75, 66.64, 66.55, 66.52, 65.49, 65.35, 62.37, 62.35, 62.01, 61.98, 61.74, 61.73, 61.72, 61.71, 56.83, 56.74, 40.96, 40.95, 30.11, 30.09, 30.06, 29.83, 29.81, 26.21, 26.19, 25.06, 25.04, 19.35, 19.31, 19.26, 19.23, 19.01, 18.97, 18.77, 18.74, 18.39, 18.31, 18.29, 18.27, 18.25, 18.21, 18.19, 18.13. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.48, -0.96, -2.57, -3.73. MALDI-FTICR: Calculated for $C_{321}H_{337}Cl_3N_4O_{71}P_2$ $[M+Na]^+$: 5573.1316, found: 5572.9005. TLC: Rf = 0.30 (DCM/Acetone = 20/1, v/v).

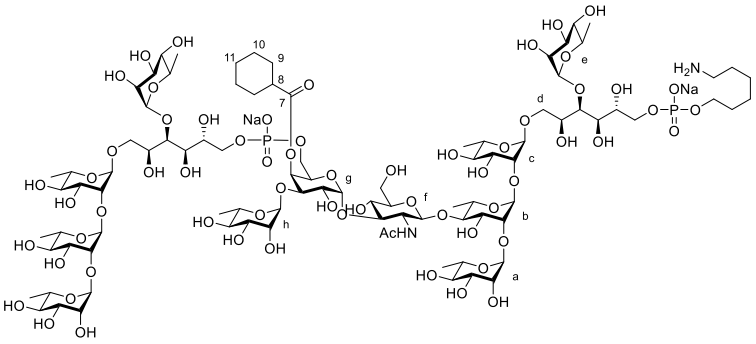
The Tridecasaccharide 3



Full protected tridecamer **4** (44 mg, 7.9 μmol, 1.0 eq) was dissolved in dioxane (5 mL) and ammonia solution (35%) (2 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The crude was dissolved in methanol (3 mL) and dioxane (2 mL). Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 56 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, the mixture was quenched with acetic acid and then quenched the excess acid using ammonia

solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The crude compound was dissolved in *tert*-butanol (7 mL), water (3 mL) and 2 drops acetic acid. After $Pd(OH)_2/C$ (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The crude was dissolved in sodium hydroxide (0.2 M, 2 mL), stirred overnight, quenched with acetic acid and then quenched the excess acid using ammonia solution. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **3a**, and the final compound **3** (9.4 mg, 4.1 µmol, 52%) was obtained after hydrolyzed by NaOH in water. ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.569 (d, *J* = 4.0 Hz, 1H, H-1g), 5.143 (d, *J* = 1.8 Hz, 1H, H-1b′), 5.133 (d, *J* = 1.8 Hz, 1H, H-1b), 5.052 (d, *J* = 1.8 Hz, 1H, H-1e), 5.044 (d, *J* = 1.8 Hz, 1H, H-1e′), 5.022 (d, *J* = 1.7 Hz, 1H, H-1h), 4.988 (d, *J* = 1.8 Hz, 1H, H-1a), 4.971 (d, *J* = 1.8 Hz, 1H, H-1a′), 4.903 (d, *J* = 1.7 Hz, 1H, H-1c′), 4.892 (d, *J* = 1.7 Hz, 1H, H-1c), 4.836 (dd, *J* = 7.2, 1.7 Hz, 1H, H-1f), 4.172 – 3.956 (m, 25H), 3.921 (m, *J* = 10.5, 7.3, 3.5 Hz, 8H), 3.887 – 3.703 (m, 28H), 3.705 – 3.638 (m, 3H), 3.522 – 3.424 (m, 10H), 2.995 – 2.933 (m, 2H), 2.117 (s, 3H), 1.703 – 1.632 (m, 4H), 1.478 – 1.391 (m, 4H), 1.375 – 1.248 (m, 27H). ^{13}C NMR (214 MHz, D_2O) δ 175.42 (Ac), 103.21 (C-1h, 1a′), 103.10 (C-1a), 102.31 (C-1f), 102.28 (C-1e), 102.20 (C-1e′), 101.72 (C-1b′), 101.37 (C-1b), 99.66 (C-1c′), 99.64 (C-1c), 99.21 (C-1g), 80.96, 79.60, 79.34, 79.32, 79.17, 79.03, 78.68, 78.50, 77.76, 76.28, 73.07, 73.01, 72.91, 72.84, 72.81, 71.85, 71.45, 71.44, 71.30, 71.27, 71.07, 71.06, 70.98, 70.96, 70.94, 70.92, 70.86, 70.83, 70.80, 70.77, 70.71, 70.30, 70.21, 70.19, 70.05, 70.02, 69.95, 69.66, 69.41, 68.60, 68.46, 67.88, 67.72, 67.06, 67.04, 64.93, 61.25, 55.25, 40.42, 30.38, 30.35, 28.02, 26.06, 25.35, 23.50, 18.08, 17.69, 17.68, 17.63, 17.62, 17.58, 17.53. ^{31}P NMR (202 MHz, D_2O) δ 1.95, 1.41. HR-MS: Calculated for $C_{86}H_{154}N_2O_{63}P_2$ $[M+H]^+$: 2285.8456, found : 2285.8435.

The tridecasaccharide 3a



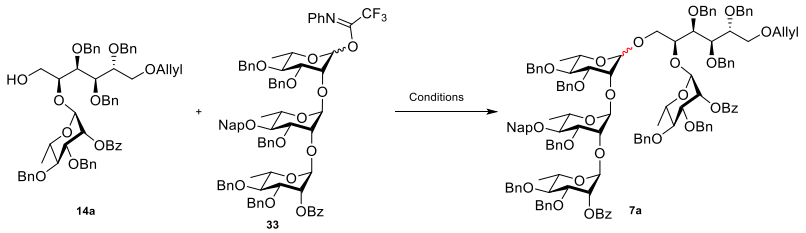
^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.57 (d, *J* = 3.5 Hz, 1H, H-1g), 5.41 – 5.36 (m, 1H, H-4g), 5.11 – 5.06 (m, 2H), 5.02 – 4.98 (m, 2H), 4.97 (d, *J* = 1.8 Hz, 1H), 4.94 – 4.89 (m, 2H), 4.86 – 4.83 (m, 2H), 4.16 (t, *J* = 6.4 Hz, 1H, H-5g), 4.11 – 3.91 (m, 28H), 3.90 – 3.54 (m, 50H), 3.47 – 3.33 (m, 13H), 2.96 (t, *J* = 7.5 Hz, 2H), 2.54 – 2.47 (m, 1H, H-8), 2.07 (s, 3H, NHAc), 1.93 – 1.81 (m, 2H, Cyclohexyl), 1.77 – 1.51 (m, 7H), 1.50 – 1.35 (m, 6H), 1.36 – 1.16 (m, 30H). ^{13}C NMR (214 MHz, D_2O) δ 178.68, 175.51 (NHAc), 103.26, 103.14, 102.74, 102.40, 102.28, 102.27, 102.21, 101.75, 101.38, 99.68, 99.64, 99.39, 80.97, 79.65, 79.37, 79.34, 79.30, 79.20, 78.67, 78.62, 78.49, 76.27, 75.04, 73.08, 73.06, 73.05, 73.00, 72.90, 72.83, 72.80, 72.77, 71.84, 71.76, 71.46, 71.43, 71.34, 71.30, 71.27, 71.06, 71.05, 71.00, 70.98, 70.96, 70.94, 70.92, 70.91, 70.86, 70.84, 70.81,

70.77, 70.74, 70.70, 70.32, 70.31, 70.28, 70.21, 70.13, 70.08, 70.04, 69.98, 69.96, 69.94, 69.72, 69.69, 69.47, 69.32, 68.63, 67.82, 67.80, 67.74, 67.72, 67.04, 67.02, 64.58 (C-6g), 61.19, 55.22, 43.79 (C-8), 40.30 (CH_2NH_2), 30.36, 30.33, 29.87, 29.39, 27.47, 26.15, 26.01, 25.80, 25.61, 25.33, 23.54, 18.09, 17.72, 17.69, 17.66, 17.64, 17.61, 17.59, 17.55, 17.54. ^{31}P NMR (202 MHz, D_2O) δ 1.93, 1.05.

Appendix

Due to the misinterpretation of GBC structure from the reported literature[11a], pentasaccharide **1a**, an regioisomeic analogue of the pentasaccharide **1** isolated from GBC, was synthesized. The synthesis of compound **1** was achieved using the optimized strategy of **1a**, which was described below.

Table I. The model reaction of convergent **[3+2]** glycosylation

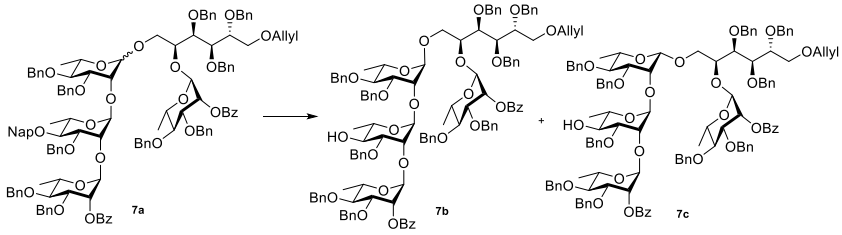


Entry	Reagents	solvent	Temp.	Yield	α/β
1	TBSOTf	DCM	0 ℃	55%	2.4 : 1
2	TBSOTf	DCM	-78 ℃	13%	1 : 2.1
3	TBSOTf, TMSI, Ph_3PO	DCM	RT	NR	-
4	TBSOTf	ACN	RT	65%	2.7 : 1
5	DMF, TfOH	DCM	-78 ℃ to 0 ℃	33%	1.9 : 1

For the reactions depicted in entry 1, 2 and 4, conditions were followed as described below. The reactions in 3 and 5, followed the published procedure.[29] Donor **33** (1.2 eq) and acceptor **14a** (1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM or ACN (2 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 ℃ or -78 ℃ and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) or another activator (0.1 eq) was added. The solution was stirred until TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 6:1 - 3:1) to yield a α/β mixture compound **7a**. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.12 (d, *J* = 7.7 Hz, 2H), 8.04 (d, *J* = 7.7 Hz, 2H), 7.86 – 7.72 (m, 4H), 7.65 – 7.40 (m, 10H), 7.40 – 7.06 (m, 54H), 6.99 (t, *J* = 7.5 Hz, 1H), 5.94 – 5.71 (m, 2H), 5.60 (s, 1H), 5.28 – 5.18 (m, 1H), 5.17 – 4.37 (m, 29H), 4.18 – 3.73 (m, 17H), 3.74 – 3.58 (m, 4H), 3.58 – 3.42 (m, 3H), 3.35 (t, *J* = 9.4 Hz, 1H), 1.38 – 1.14 (m, 19H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.65, 165.61, 138.81, 138.76, 138.58, 138.56, 138.51, 138.49, 138.43, 138.30, 138.24, 138.22,

136.12, 134.97, 133.45, 133.23, 133.18, 133.09, 130.31, 130.17, 130.06, 130.00, 128.58, 128.52, 128.49, 128.44, 128.41, 128.37, 128.33, 128.29, 128.21, 128.18, 128.11, 128.03, 127.96, 127.93, 127.84, 127.81, 127.76, 127.68, 127.65, 127.60, 127.57, 127.55, 127.49, 126.86, 126.31, 126.09, 125.90, 116.96, 100.45, 99.29, 99.12, 98.45, 80.33, 80.28, 79.97, 79.77, 79.17, 79.13, 78.67, 78.37, 78.13, 77.96, 77.73, 75.52, 75.43, 75.38, 75.31, 75.18, 74.92, 74.72, 74.16, 72.43, 72.34, 72.26, 72.16, 71.65, 71.61, 69.73, 69.60, 69.57, 68.60, 68.43, 68.38, 68.19, 66.57, 18.35, 18.28, 18.22, 18.19. HR-MS Calculated for $C_{128}H_{134}O_{24}$ $[M+H]^+$: 2055.9336, found: 2055.9309. $[\alpha]^{20}_D$ = - 1.8 (c = 1, $CHCl_3$) TLC: Rf = 0.20 (PE/EA = 9/1, v/v).

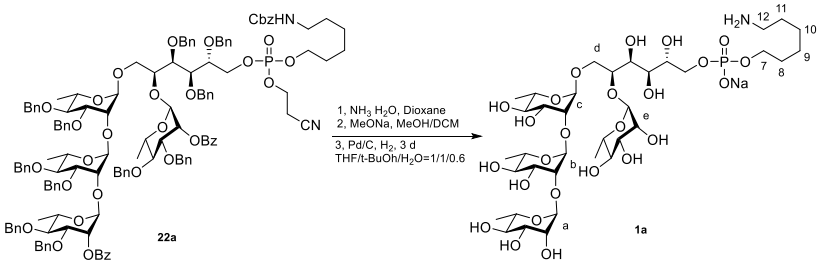
Scheme II. The Nap removal in pentasaccharide **7a** to give **7b** and **7c**



Fully protected **7a** (482 mg, 0.234 mmol, 1 eq) was dissolved in DCM (5 mL) and water (0.5 mL). After cooled to 0 ℃, 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) (59 mg, 0.26 mmol, 1.1 eq) was added. The reaction was stirred at RT for 4 hours. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM 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 6:1 – 3/1) to yield compound **7b** and **7c** (382 mg, 0.20 mmol, 85%). **α-isomer:** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.06 (m, 2H), 8.06 – 8.00 (m, 2H), 7.67 – 7.54 (m, 2H), 7.54 – 7.39 (m, 4H), 7.38 – 7.06 (m, 49H), 7.02 – 6.93 (m, 1H), 5.92 – 5.77 (m, 1H), 5.66 (t, *J* = 2.5 Hz, 1H), 5.60 (d, *J* = 2.8 Hz, 1H), 5.28 – 5.17 (m, 1H), 5.14 (d, *J* = 1.8 Hz, 1H), 5.12 – 5.05 (m, 1H), 5.00 (s, 1H), 4.96 – 4.72 (m, 6H), 4.71 – 4.37 (m, 16H), 4.10 – 3.74 (m, 14H), 3.74 – 3.45 (m, 9H), 3.35 (t, *J* = 9.4 Hz, 1H), 2.23 (s, 1H), 1.34 – 1.14 (m, 26H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.67, 165.64, 138.82, 138.81, 138.61, 138.52, 138.47, 138.30, 138.26, 138.22, 137.96, 135.01, 133.28, 133.21, 130.29, 130.21, 130.06, 130.03, 128.72, 128.55, 128.54, 128.50, 128.48, 128.46, 128.44, 128.41, 128.40, 128.38, 128.37, 128.34, 128.32, 128.30, 128.26, 128.22, 128.19, 128.10, 128.05, 127.98, 127.97, 127.93, 127.90, 127.79, 127.73, 127.72, 127.70, 127.69, 127.64, 127.58, 127.53, 116.98, 100.66, 99.43, 99.13, 98.51, 80.32, 80.19, 79.94, 79.80, 79.21, 78.92, 78.71, 78.41, 78.18, 78.03, 77.83, 75.52, 75.35, 75.15, 74.99, 74.95, 74.78, 74.16, 72.48, 72.38, 72.20, 71.88, 71.79, 71.75, 71.63, 69.76, 69.66, 69.63, 69.03, 68.49, 68.38, 68.23, 66.66, 18.36, 18.28, 18.21, 18.01. HR-MS: Calculated for $C_{117}H_{126}O_{24}$ $[M+H]^+$: 1915.8712, found: 1915.8657. $[\alpha]^{20}_D$ = - 2.7 (c = 1, $CHCl_3$). TLC: Rf = 0.20 (PE/EA = 4/1, v/v). **β-isomer**: ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.09 – 8.00 (m, 4H), 7.62 – 7.54 (m, 2H), 7.51 – 7.41 (m, 4H), 7.38 – 7.06 (m, 49H), 7.06 – 7.01 (m, 1H), 5.94 – 5.79 (m, 1H), 5.70 – 5.65 (m, 1H), 5.63 – 5.58 (m, 1H), 5.28 (d, *J* = 1.8 Hz, 1H), 5.23 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.10 (dd, *J* = 10.4, 1.6 Hz, 1H), 5.05 (d, *J* = 1.9 Hz, 1H), 4.92 (d, *J* = 1.9 Hz, 1H), 4.90 – 4.81 (m, 3H), 4.80 – 4.70 (m, 4H), 4.70 – 4.39 (m, 14H), 4.19 – 4.00 (m, 8H), 4.00 – 3.81 (m, 8H), 3.75 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.71 – 3.63 (m, 1H),

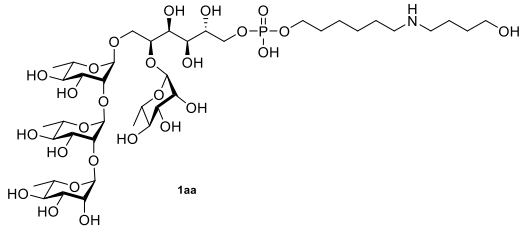
3.61 – 3.44 (m, 3H), 3.39 – 3.28 (m, 3H), 3.23 – 3.15 (m, 1H), 2.50 (s, 1H), 1.36 – 1.15 (m, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.60, 165.56, 138.78, 138.70, 138.57, 138.52, 138.47, 138.43, 138.29, 138.24, 138.22, 137.92, 135.01, 133.19, 133.17, 130.35, 130.25, 130.05, 128.75, 128.53, 128.52, 128.51, 128.49, 128.48, 128.45, 128.42, 128.40, 128.37, 128.35, 128.26, 128.25, 128.21, 128.18, 128.13, 127.93, 127.91, 127.88, 127.81, 127.75, 127.72, 127.69, 127.65, 127.64, 127.61, 127.59, 117.01, 100.72, 99.29, 99.10, 82.66, 80.33, 80.22, 79.64, 79.51, 79.47, 79.41, 78.95, 78.39, 78.20, 75.53, 75.46, 75.32, 75.08, 74.97, 73.64, 72.38, 72.27, 72.23, 72.09, 71.95, 71.92, 71.73, 71.45, 70.03, 69.89, 69.81, 69.56, 68.80, 68.40, 68.24, 18.53, 18.17. HR-MS: Calculated for $C_{117}H_{126}O_{24}$ $[M+Na]^+$: 1937.8531, found: 1937.8519. TLC: Rf = 0.40 (PE/EA = 4/1, v/v);

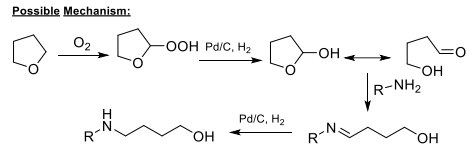
Scheme III. The optimization of the hydrogenation



Fully protected compound **22a** (10 mg, 4.3 μmol, 1.0 eq) was dissolved in dioxane (6 mL) and ammonia solution (35%) (3 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The crude was dissolved in methanol (2 mL) and DCM (1 mL). Sodium methoxide (25 wt. % in methanol) (0.05 mL, 0.22 mmol, 50 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20 column) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (2 mL), THF (2 mL), water (1.6 mL) and 4 drops acetic acid. After Pd/C (51 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The compound was firstly purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and then purified by HPLC (C30 column) to yield compound **1a** and **1aa**. ^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.10 (d, *J* = 1.8 Hz, 1H, H-1b), 4.99 (d, *J* = 1.8 Hz, 1H, H-1e), 4.96 (d, *J* = 1.8 Hz, 1H, H-1a), 4.89 (d, *J* = 1.7 Hz, 1H, H-1c), 4.13 – 4.03 (m, 4H), 4.03 – 4.00 (m, 1H), 4.00 – 3.93 (m, 3H), 3.93 – 3.81 (m, 7H), 3.81 – 3.62 (m, 7H), 3.51 – 3.38 (m, 4H), 3.00 – 2.92 (m, 2H, H-12), 1.71 – 1.59 (m, 4H), 1.46 – 1.36 (m, 4H), 1.35 – 1.22 (m, 12H). ^{13}C NMR (151 MHz, D_2O) δ 103.14 (C-1a), 102.37 (C-1e), 101.84 (C-1b), 99.88 (C-1c), 80.31, 79.21, 79.03, 73.08, 73.01, 72.92, 72.91, 71.11, 71.07, 70.94, 70.75, 70.72, 70.67, 70.43, 70.24, 70.11, 70.05, 69.93, 69.76, 68.13 (C-1d), 67.76 (d, *J* = 5.5 Hz, C-6d), 67.07 (d, *J* = 5.7 Hz, C-7), 67.03, 40.32 (C-12), 30.38, 30.34, 27.50, 26.04, 25.34, 17.73, 17.70, 17.66, 17.57. HR-MS: Calculated for $C_{36}H_{68}NO_{25}P$ $[M+Na]^+$: 968.3710, found : 968.3723. ^{31}P NMR (202 MHz, D_2O) δ 1.82.

Pentasaccharide 1aa





^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.15 – 5.12 (m, 1H), 5.02 (d, *J* = 1.8 Hz, 1H), 5.01 – 4.99 (m, 1H), 4.94 – 4.91 (m, 1H), 4.14 – 4.07 (m, 5H), 4.07 – 4.04 (m, 1H), 4.03 – 3.97 (m, 4H), 3.96 – 3.86 (m, 8H), 3.81 (dt, *J* = 9.8, 3.4 Hz, 2H), 3.79 – 3.67 (m, 6H), 3.65 (t, *J* = 6.3 Hz, 2H), 3.53 – 3.44 (m, 4H), 3.11 – 3.04 (m, 4H), 1.80 – 1.60 (m, 8H), 1.49 – 1.40 (m, 4H), 1.37 – 1.27 (m, 12H). ^{13}C NMR (151 MHz, D_2O) δ 103.19, 102.42, 101.90, 99.96, 80.35, 79.26, 79.10, 73.17, 73.10, 73.00, 71.19, 71.03, 70.84, 70.54, 70.30, 70.17, 70.12, 69.99, 69.85, 69.49, 68.20, 67.84 (d, *J* = 5.1 Hz), 67.11 (d, *J* = 5.7 Hz), 61.85, 48.45, 48.29, 30.43, 29.36, 26.38, 26.24, 25.43, 23.33, 21.04, 17.80, 17.78, 17.74, 17.64. ^{31}P NMR (202 MHz, D_2O) δ 1.95. HR-MS: Calculated for $C_{40}H_{76}NO_{26}P$ $[M+H]^+$: 1018.4466, found : 1018.4489.

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[24] Before the linear strategy was explored, a [3 + 2] model glycosylation between a rhamnosyl trisaccharide donor and a rhamnosylated glucitol disaccharide acceptor was performed. Unfortunately, the α:β ratio of this convergent glycosylation was poor (α:β = 2.4:1 at 0 °C; 1.2:1 at -78 °C, see Table I in the appendix of experimental part).

[25] Customarily, this type of enol ethers is cleaved by the combination of I_2 and $NaHCO_3$, however, these conditions proved unsuitable for this pentasaccharide, and the corresponding (*Z*) and (*E*) enol ethers were isolated. Fot the use of I_2 and $NaHCO_3$, see for example: D. van der Es, N. A. Groenia, D. Laverde, H. S. Overkleeft, J. Huebner, G. A. van der Marel and J. D. C. Codée, *Biorg. Med. Chem.* **2016**, *24*, 3893-3907.

[26] when THF was used in solution for the hydrogenation step to synthesize a pentasaccharide analogue, an unimaginable side product was speculated, the THF ring was opened and conjugated with the free amine of the spacer, and its structure was confirmed by HRMS and NMR data, and a possible mechanism was proposed in Scheme III of the appendix in experimental part. Additionally, when the THF was replaced by dioxane, the similar side-product was detected by LC-MS. In the end, water/tert-butanol system was selected as the optimum solvent for the hydrogenation step.

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Chapter 3

The First Total Synthesis of Repeating Units of Glycerol Phosphate Modified Capsular Polysaccharides from Group A *Streptococcus*

Introduction

Group A *Streptococcus* (GAS), also known as *Streptococcus pyogenes*, a β-hemolytic Gram-positive bacterium, has been recognized as a remarkable human pathogen, ranking among the top ten causes of infectious disease induced mortality and morbidity.[1] It is responsible for streptococcal pharyngitis, acute rheumatic fever (ARF) and infective endocarditis complicating rheumatic heart disease (RHD), inflicting school-age children and people with a weakened immune system, such as pregnant woman on a global scale, but especially in poor and overcrowded regions.[2]

Despite the use of effective antimicrobials, annually, at least 517,000 people die and 18.1 million suffer from GAS infected disease.[1b] To improve this situation, the development of a safe and effective vaccine is urgent. Bacterial cell-surface carbohydrates play a crucial role in host–bacteria interactions, including immunological recognition events.[3] In last century, a variety of GAS proteins and group A carbohydrate (GAC) molecules[4] have been evaluated as vaccine candidates. Because of the diversity of serotypes, it is difficult to design and synthesize a versatile oligosaccharide vaccine,[5] and until now, no safe and effective vaccine is available to prevent infections, even although human vaccination trial was launched 100 years ago.[6]

The GAC was first characterized by the group of Kindt in 1975, and is built up from a polyrhamnose backbone, containing alternating α-(1,2)- and α-(1,3)-linked residues, having an *N*-acetyl glucosamine (GlcNAc) attached to the rhamnosyl O-3 of the backbone (Fig. 1a).[7] This polysaccharide adopts a helical conformation with the polyrhamnose forming the core of the helix and the immunodominant *N*-acetylglucosamine residue displaying on the periphery.[4d, 8] Various GAC fragments, ranging from disaccharide to nonasaccharide structures, have been generated by different groups, including the groups of Bundle[4a], Pinto[9], costantino[4b] and Gu[4c]. Several conjugates were synthesized to explore carbohydrate-based GAS vaccines, which were successfully used for the induction of antibodies and raise of immune responses, showing protective efficacy comparable to the GAS polysaccharide conjugate.[4b, c] It has been speculated that the GlcNAc residues play an important role in evading the innate immune system, because strains which lack GlcNAc transferase, responsible for the decoration of the polyrhamnose backbone with these residues, show attenuated virulence.[8c]

Recently, a glycerol phosphate (GroP) modification on the GAC (GroP GAC) was discovered by Van Sorge and Korotkova and co-workers (Fig. 1b).[10] They revealed that the biosynthetic cluster, gacABCDEFGHIJKL, not only encodes for enzymes responsible for the synthesis of the polyrhamnose backbone and the installation of the GlcNAc sidechain, but also for a GroP transferase enzyme, that can transfer an *sn*-Gro-1-P moiety to the C-6 of the GAC-GlcNAc residues using phosphatidylglycerol as donor. Based on NMR analysis, approximately 25% of the GAC sidechain GlcNAcs are functionalized by GroP at its O-6 position.

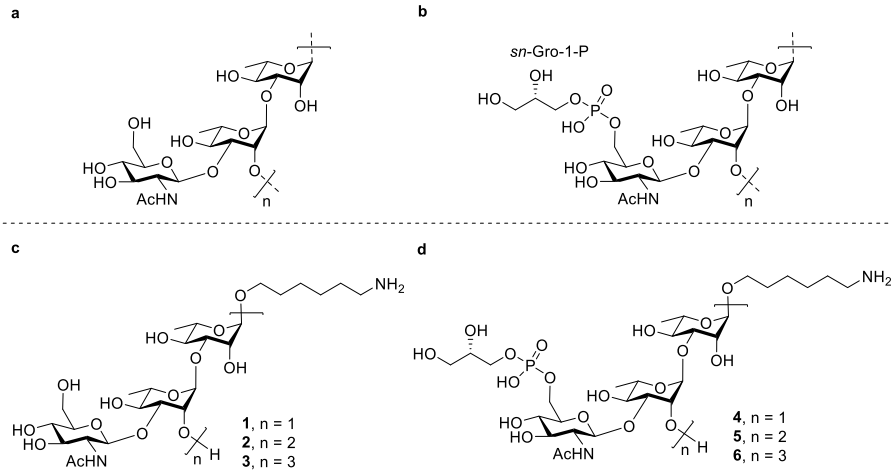


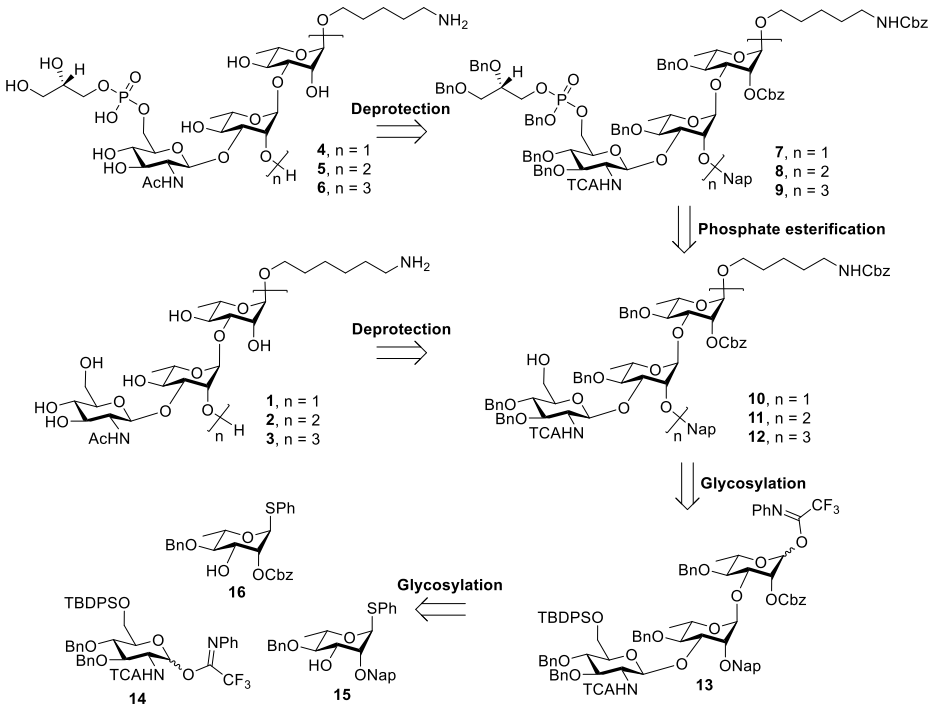
Figure 1. **a**, the structure of GAC. **b**, the structure of glycerol phosphate modified GAC. **c**, the designed fragments of GAC **1**-**3**. **d**, the designed fragments of GroP GAC **4**-**6**.

Fragments of the newly discovered *sn*-GroP modified GAC may be promising candidates to function as antigens in the development of a novel GAS vaccine. Therefore, this Chapter reports on the development of a synthetic methodology to generate GroP-GAC fragments of different length. Six fragments **1** - **6**, ranging from trisaccharides to nonasaccharides, with and without the *sn*-GroP-modification at each GlcNAc residue were designed as shown in Fig. 1c and 1d. For future conjugation purposes with proteins or other molecules, a spacer was required that can be chemoselectively addressed. To this and an aminohexanol spacer was included in the structures.

Results and discussion

Considering a late stage introduction of the glycerol phosphate groups and the required deprotection steps, the retrosynthetic analysis, shown in Scheme 1 was drafted. The spacer amine was protected with a benzyloxycarbonyl (Cbz) group. Commonly, primary amines are protected with both a Cbz and a benzyl group to prevent side reactions of the carbamate with electrophilic species generated during the glycosylation reactions. Removal of the benzyl group from the amine however can make the final deprotection step significantly more cumbersome. In addition, double protection of the amine leads to the formation of rotamers that complicate NMR analysis of the synthetic intermediates. Therefore, it was decided to solely protect the amine of the spacer with a Cbz group. To facilitate the global deprotection of the molecules, benzyl groups were planned to be used to mask the phosphotriesters and the C2-OH of the rhamnosides were blocked with benzyl carbonates. The latter groups can

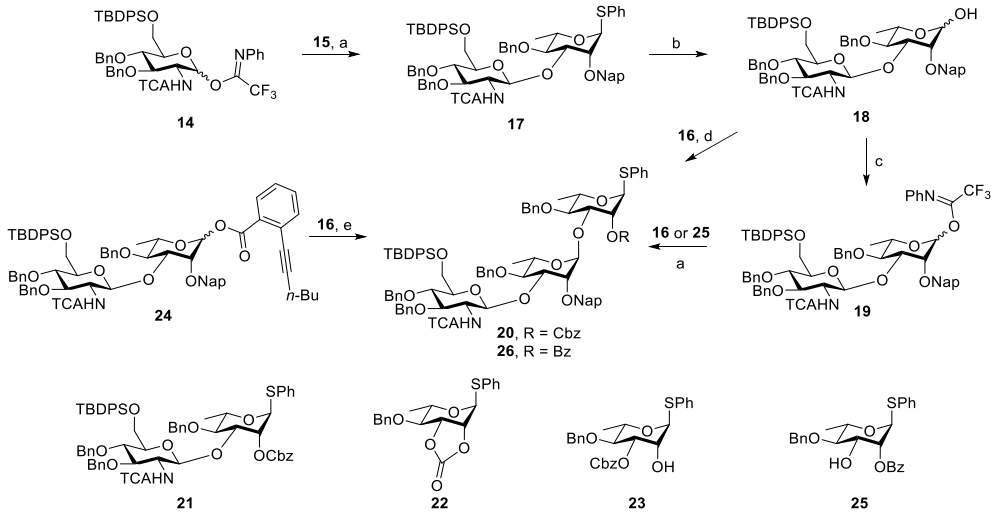
provide neighboring group participation during the construction of the desired α-rhamnosyl linkages. The 2-naphthylmethyl (Nap) group was chosen as temporary protecting group to mask the rhamnosyl C-2-OH to be elongated. This way, the most complex GroP modified nonasaccharide **6** could be obtained after only one hydrogenation step from the fully protected intermediate **9**, which in turn can be constructed by a coupling between a glycerol phosphoramidite and nonasaccharide **12**. The latter nonasaccharide, that can also be used to generate structures without a GroP appendage, could be obtained using three glycosylations with the key trisaccharide imidate donor **13**. It was envisioned that this key trisaccharide could be obtained from monosaccharides **14**-**16**.



Scheme 1. Retrosynthetic analysis of the GroP GAC-fragments. The orthogonal GlcNAc C-6-O-protecting group allows for the generation of fragments with and without a GroP groups.

Monosaccharide building blocks **14**-**16** were readily synthesized from D-glucosamine and L-rhamnose, and the detailed procedures are described in Scheme I – II in the Experiential Section. After all required building blocks were prepared, the glycosylation reactions outlined in Scheme 2 were undertaken. As expected, the TBSOTf mediated glycosylation between donor **14** and acceptor **15** produced disaccharide **17** in a good yield. To glycosylate the thioglycoside acceptor **16**, donor **17** was transformed into the corresponding imidate **19** in

two steps, including hydrolysis the thiophenyl acetal to the hemiacetal using NIS in a mixture of acetone/H_2O and installation of the imidate moiety. However, when the glycosylation conditions, used for the successful condensation of **14** and **15**, were employed to couple **19** and acceptor **16**, the desired trisaccharide **20** was obtained in a very poor yield (Table 1, entry 1). Three major side-products **21** - **23** were isolated and fully characterized with the aid of NMR-spectroscopy and HRMS-analysis. The last two byproducts were generated because of the acidic reaction conditions.[11] The transformation to provide disaccharide **21** would require cleavage of the glycosidic linkage in the donor disaccharide and subsequent addition of the acceptor. Why this has taken place is unclear at present but it is clear that the acid is infaust for the [2+1] glycosylation. To optimize the glycosylation, a dehydrative glycosylation was performed utilizing lactol **18** as donor. But even after significant optimization, the yield of **20** did not surpass 52% (Table 1, entry 2 and 3). Lastly, donor **24** was explored in combination with mild gold catalyzed activation conditions.[12] Unexpectedly, this glycosylation procedure produced an α/β-mixture in moderate yield (Table 1, entry 4 and 5). Based on these results, the acid labile Cbz group in **16** was replaced by a more stable benzoyl group and the use of monosaccharide **25** was explored. The glycosylation between imidate **19** and **25** using TBSOTf as promotor provided the key trisaccharide **26** in 73% as a single anomer (Table 1, entry 6).



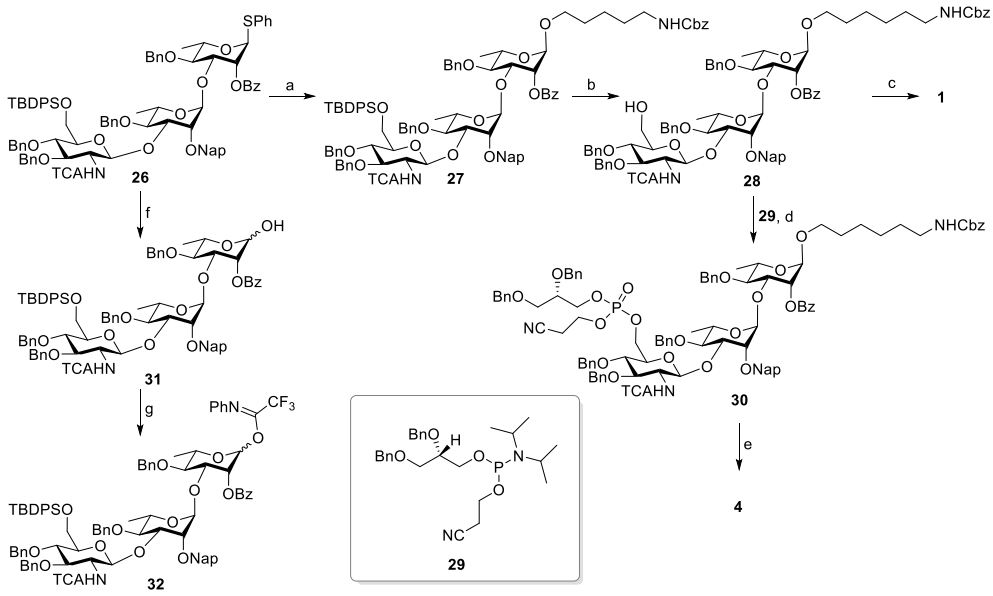
Scheme 2. The assembly of the key intermediate trisaccharide.

Reagents and conditions: a) TBSOTf, 4Å MS, DCM, 0 °C, **17**, 87%; **20**: 16%; **26**: 73%. b) NIS, acetone, water, 0 °C, 81%. c) Cs_2CO_3, acetone, *N*-phenyltrifluoroacetimidoyl chloride, 92%. d) Ph_2SO, DTBMP, DCM, 4Å MS, - 60 °C, then Tf_2O, 52%. e) PPh_3AuCl, $AgNTf_2$, DCM, 4Å MS, 0 °C, α/β mixture 55%.

Table 1. The optimization of [2+1] glycosylation

entry	donor	acceptor	condition	temperature	yield	α/β
1	**19**	**16**	TBSOTf	0 °C	16%	α only
2	**18**	**16**	Ph_2O, Tf_2O	- 40 °C to 0 °C	52%	α only
3	**18**	**16**	Ph_2O, Tf_2O	- 40 °C to -20 °C	28%	α only
4	**24**	**16**	PPh_3AuNTf_2	0 °C	55%	1.5/1
5	**24**	**16**	PPh_3AuNTf_2	- 78 °C	45%	1.1/1
6	**19**	**25**	TBSOTf	0 °C	73%	α only

With the key intermediate trisaccharide in hand, the synthesis of the first two target trisaccharides **1** and **4** were undertaken as depicted in Scheme 3. The glycosylation between the Cbz-protected aminohexanol spacer and thio-donor **26** was carried out under the promotion of NIS-TBSOTf in dry DCM to give the compound **27** in an excellent yield. Notably, treatment of **27** with HF/Pyridine or TBAF did not lead to the removal of the TBDPS group. The silyl ether could be selectively deprotected using TBAF and AcOH in THF at 50 °C for 5h.[13] The first target **1** was obtained from trisaccharide **28** after the removal of benzoyl group using NaOMe in methanol and global deprotection by hydrogenation, in 71% yield. The glycerol phosphate moiety was installed to the C-6 hydroxyl of the glucosamine residue utilizing glycerol phosphoramidite **29** that was activated by dicyanoimidazole, followed by in situ oxidation of the P(III) to P(V) using CSO. This way, phosphate **30** was assembled in 89% yield from **28**. In contrast to the described retrosynthetic pathway (Scheme 1), a cyanoethyl protecting group was used to mask the phosphate **30**, because the presence of the benzoate at the rhamnosyl C-2-hydroxyl required a deprotection step using basic conditions. The first GroP modified target trisaccharide **4** was obtained in 72% yield after a three step deprotection sequence, involving the subsequent removal the cyanoethyl and benzoyl groups using basic conditions and global hydrogenation using $Pd(OH)_2$ in *tert*-butanol/water.

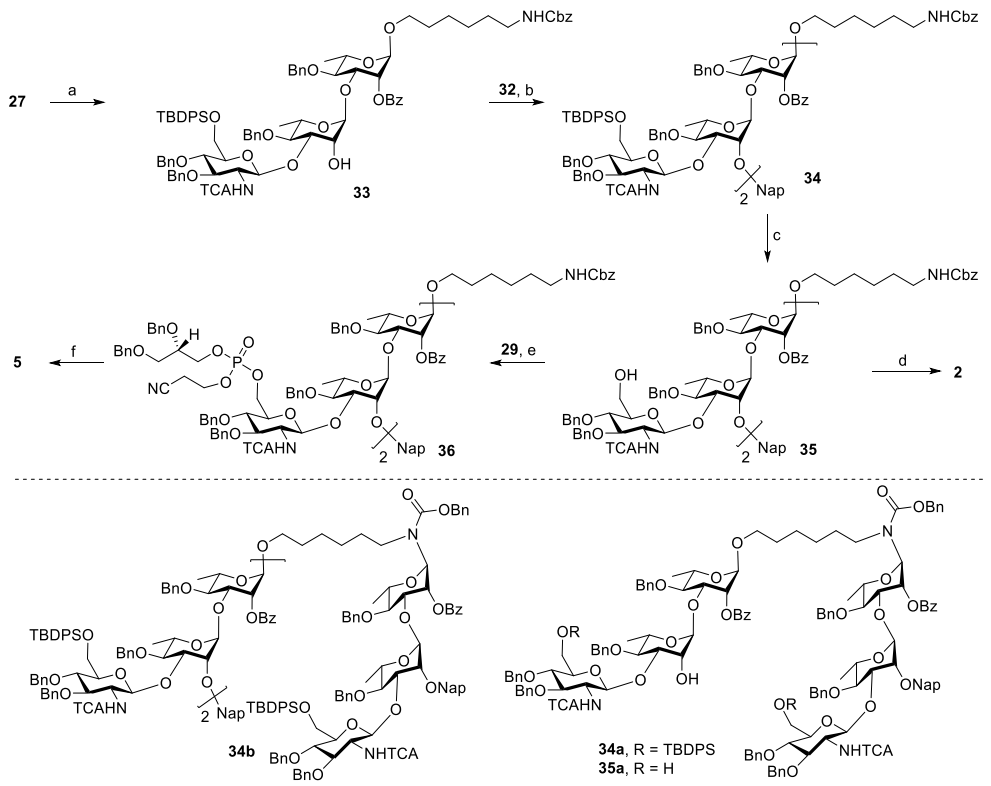


Scheme 3. The assembly of the target trisaccharides **1** and **4**.

Reagents and conditions: a) benzyl (6-hydroxyhexyl)carbamate, NIS, TBSOTf, 4Å MS, DCM, 0 °C, 95%. b) TBAF, AcOH, THF, 50 °C, 97%. c) i, NaOMe, MeOH/1,4-diaxane; ii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days, 71%. d) DCI, MeCN, 3Å MS, then CSO, 89%. e) i, ammonium hydroxide, 1,4-dioxane; ii, NaOMe, MeOH/1,4-diaxane; iii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days, 72%. f) NIS/TFA, DCM, 0 °C, 90%. g) *N*-phenyltrifluoroacetimidoyl chloride, Cs_2CO_3, acetone, 86%.

To assemble the hexa- and nonasaccharides, the trisaccharide donor **26** was transformed into imidate donor **32** using an NIS/TFA-mediated hydrolysis to provide the hemiacetal,[14] which was reacted with the *N*-phenyltrifluoroacetimidoyl chloride in the presence of Cs_2CO_3 to deliver **32** (Scheme 3). To elongate trisaccharide **27**, the Nap protecting group was removed oxidatively using DDQ in DCM and neutral water to provide the trisaccharide alcohol **33** in 91% yield as shown in Scheme 4. The [3 + 3] glycosylation represents a crucial but difficult step because of the low reactivity of the axial hydroxyl in L-rhamnose, the steric hindrance of the adjacent glucosamine residue and the possibility of *N*-glycosylation of the linker. The glycosylation between **33** and thio-donor **26** was tested employing NIS and TBSOTf as promotor to give the desired hexasaccharide **34** in 56% yield, alongside with 23% of the recovered acceptor **33** (Table 2, entry 1). Additionally, a significant amount of side-product nonasaccharide **34b** was isolated (21%), whose structure was verified by NMR. Another side product, **34a** was also obtained and its structure was determined after desilylation, giving product **35a**, by NMR and HRMS. The spacer *N*-glycosylation was not

observed in the generation of trisaccharide **27**, indicating that the lower reactivity of the rhamnosyl C-2-OH in **33**, opens up the way for this type of side reaction. To prevent the *N*-glycosylation, the imidate donor **32** was used and the reaction of this donor with **33** was optimized as shown in Table 2. It can be seen that a lower temperature was favorable for the construction of the desired product, and that the use of more donor leads to more side product **34b** (Table 2, entry 5). Finally, the use of 1.3 equivalents of the donor at – 20 °C was found to be optimal delivering the desired product in 78% yield (Table 2, entry 4). Subsequently, the two TBDPS ethers were removed by overnight treatment with TBAF/AcOH, to provide hexasaccharide **35** in 82% yield. To complete the synthesis of target hexasaccharide **2**, the compound **35** was deprotected by subsequent basic hydrolysis and hydrogenation using $Pd(OH)_2$ in *tert*-butanol and water to give compound **2** in 60% yield (5.5 mg). The conjugation of hexasaccharide **35** with phosphoramidite **29** using dicyanoimidazole as activator and in-situ oxidation by CSO proceeded smoothly to furnish the glycerol phosphate modified **36** in excellent yield. Hydrolysis of the cyanoethyl groups and subsequent removal of the benzoates was followed by global hydrogenation using $Pd(OH)_2$ in *tert*-butanol and water to afford the desired hexasaccharide **5**. Notably, the benzoyls of the two hexasaccharides were difficult to remove, and after the hydrogenation it appeared that they were in part still present. Therefore, the final product was treated with NaOH in water after the hydrogenation, to effectively remove the remaining benzoates and deliver the pure target compound, which was isolated in 65% yield (15 mg).



Scheme 4. The assembly of the target hexasaccharide of **2** and **5**

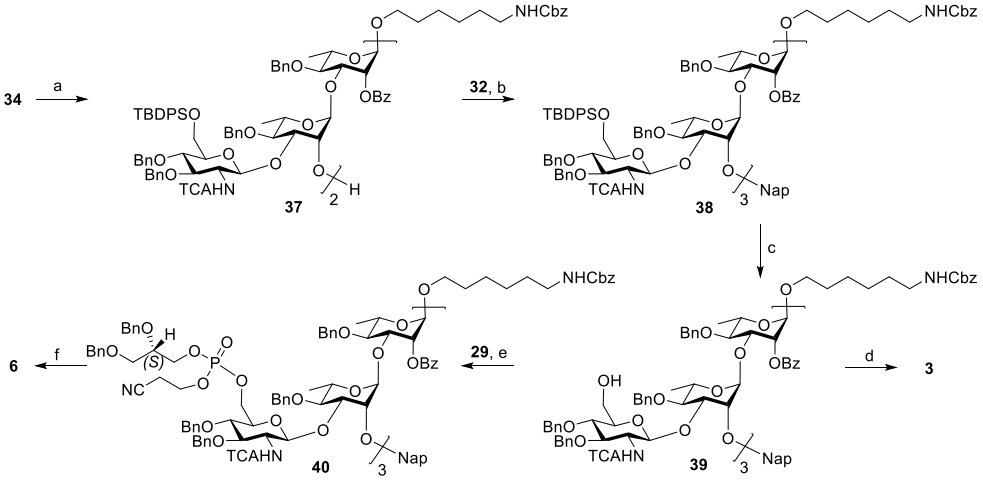
Reagents and conditions: a) DDQ, DCM, pH 7 phosphate buffer in water, 0 °C, 91%. b) TBSOTf, DCM, - 20 °C, 78%. c) TBAF, AcOH, THF, 50 °C, 82%. d) i, NaOMe, MeOH/1,4-diaxane; ii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days, 60% (over three steps). e) DCI, MeCN, 3Å MS, then CSO, 95%. f) i, ammonium hydroxide, 1,4-dioxane; ii, NaOMe, MeOH/1,4-diaxane; iii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days; iv, 1M NaOH, water, 65% (over four steps).

Table 2. Optimization of the **[3+3]** glycosylation

entry	donor	equivalent	temperature	**33**/%	**34**/%	**34b**/%
1	**26**	2.0	0 °C	23	56	21
2	**32**	1.2	0 °C	-	51	-
3	**32**	1.1	-20 °C	35	58	4
4	**32**	1.3	-20 °C	14	78	8
5	**32**	1.7	-20 °C	-	65	18

Based on the established procedure, described above, the two nonasaccharide targets **3** and **6** were synthesized via a [3 + 6] glycosylation as described in Scheme 5. First, and according

to the synthesis of **2** and **5**, removal of the Nap protecting group in hexasaccharide **34** through an oxidation by DDQ in a mixture of DCM and a neutral phosphate buffer, provided hexasaccharide **37** in 77%. Then, this hexasaccharide **37** was glycosylated with 1.3 equivalents of the trisaccharide imidate donor **32** under the promotion of TBSOTf at -20 °C to furnish the nonasaccharide **38** in 49% yield. The *N*-glycosylated side-product **38a** was formed in 21% under these conditions. Thereafter, the three TBDPS protecting groups were unmasked using TBAF/AcOH at 50 °C to generate the triol **39** in 65% yield. Because of the difficult deprotection of the benzoyl protecting groups, the sequence of the deprotection was reversed, performing the hydrogenation prior to the removal of the benzoyls. By doing so, the nonasaccharide **3** was generated in 54% yield (9.4 mg). The glycerol phosphate modified nonasaccharide was assembled from triol **39**. First the three protected glycerol phosphate triesters were installed using 9 equivalents of glycerol phosphoramidite **29** in combination with an excess DCI and oxidation of the so-formed phosphites by CSO. Then, the glycerol phosphate GAS nonasaccharide was deprotected by the removal of the cyanoethyl groups using ammonium hydroxide in dioxane, hydrogenation with $Pd(OH)_2$ and finally saponification of the benzoates to deliver the target nonasaccharide **6** in 65% yield (18 mg).



Scheme 5. The assembly of the two nonasaccharide targets **3** and **6**

Reagents and conditions: a) DDQ, DCM, pH 7 phosphate buffer in water, 0 °C, 77%. b) TBSOTf, DCM, - 20 °C, 49%. c) TBAF, AcOH, THF, 50 °C, 65%. d) i, $Pd(OH)_2$/C, H_2, AcOH, *t*-BuOH/H_2O, 3 days; ii, NaOH, water, 54%. e) DCI, MeCN, 3Å MS, then CSO, 91%. f) i, ammonium hydroxide, 1,4-dioxane; ii, $Pd(OH)_2$/C, H_2, AcOH, *t*-BuOH/H_2O, 3 days,; iii, NaOH, water, 65%.

Conclusion

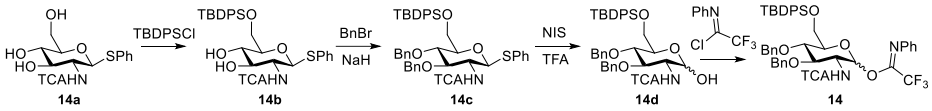
This Chapter describes the first total synthesis of glycerol phosphate modified fragments of the *Streptococcus* group A carbohydrate ranging in length from one to three repeating units. Also, the corresponding GAC-fragments lacking the glycerol phosphate appendages were synthesized. All six oligosaccharides were functionalized with a free amine terminated six-carbon spacer for further modification. A properly protected trisaccharide was used to assemble the two tri-, two hexa- and two nonasaccharides, employing a highly convergent strategy via [3 + 3] and [3 + 6] glycosylations. The glycosylation results showed that the reactivity of Cbz protected amines can lead to the formation of *N*-glycosylated side products, which become more prevalent upon decreasing reactivity of the acceptor alcohol. Nonetheless the desired tri-, hexa- and nonasaccharides were obtained in sufficient yields to complete the syntheses and deliver the target compounds in multi-milligram amounts. The set of compounds will be tested for their antigenic activity. The chemistry developed here can be readily adapted to generate GAC-fragments, that are substituted in a no-stoichiometric manner with glycerol phosphate groups. The generation of a set of substituted GAC-fragments will be valuable for more detailed structure-activity relationship studies.

Experimental section

General experimental procedures

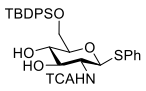
All reagents were of commercial grade and used as received. All moisture sensitive reactions were performed under an argon atmosphere. DCM used in the glycosylation reactions was dried with flamed 4Å molecular sieves before being used. Reactions were monitored by TLC analysis with detection by UV (254 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



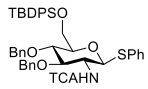
Scheme I. The synthesis of building block **14**.

Phenyl 2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-glucopyranoside (14b)



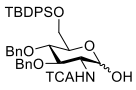
The known compound **14a**[15] (17.2 g, 41.2 mmol, 1.0 eq) was dissolved in DMF (140 mL) and cooled to 0 °C. *tert*-Butyl(chloro)diphenylsilane (TBDPSCl) (16 mL, 61.8 mmol, 1.5 eq) and imidazole (5.6 g, 82 mmol, 2 eq) were added at 0 °C. It was stirred at RT 4 hours and checked by TLC. After completed 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*. The compound was purified by flash chromatography (PE/EA/DCM 8:1:1 - 2:1:1) to yield compound **14b** (24.8 g, 38 mmol, 92%). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.77 – 7.64 (m, 4H), 7.54 – 7.45 (m, 2H), 7.45 – 7.30 (m, 6H), 7.27 – 7.17 (m, 3H), 7.13 (d, *J* = 7.8 Hz, 1H, NH), 4.93 (d, *J* = 10.2 Hz, 1H, H-1), 4.01 – 3.79 (m, 4H, H-6, H-3), 3.60 – 3.37 (m, 4H, H-2, H-4, H-5), 1.05 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.53 (NHTCA), 135.75, 135.71, 133.02, 132.92, 132.88, 132.05, 129.99, 129.16, 128.32, 127.94, 92.48 (TCA), 85.24 (C-1), 79.55 (C-5), 75.07 (C-3), 72.08 (C-4), 64.15 (C-6), 56.76 (C-2), 26.92 (*t*-Bu), 19.33 (*t*-Bu). HR-MS: Calculated for $C_{30}H_{34}Cl_3NO_5SSi$ $[M+Na]^+$: 676.08847, found: 676.08855. $[\alpha]^{25}_D$ = - 15.9° (c = 1, $CHCl_3$). TLC: Rf = 0.15 (PE/EA = 2/1, v/v).

Phenyl 3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-glucopyranoside (14c)



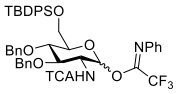
Diol **14b** (3.92 g, 6.0 mmol, 1 eq) was dissolved in DMF (3 mL) and THF (30 mL), then cooled to 0 °C. Sodium hydride (1.44 g, 36 mmol, 6 eq) was added, then after stirred 30 min, benzyl bromide (4.3 mL, 36 mmol, 6 eq) was added dropwise, the reaction was stirred for overnight. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O 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 14:1 – 12/1) to yield compound **14c** (3.85 g, 4.62 mmol, 77%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.82 – 7.75 (m, 2H), 7.73 – 7.67 (m, 2H), 7.60 – 7.55 (m, 2H), 7.44 – 7.38 (m, 2H), 7.38 – 7.18 (m, 15H), 7.18 – 7.12 (m, 2H), 6.88 (d, *J* = 8.0 Hz, 1H, NH), 5.15 (d, *J* = 10.1 Hz, 1H, H-1), 4.89 – 4.79 (m, 2H), 4.74 – 4.65 (m, 2H), 4.16 – 4.09 (m, 1H, H-3), 4.04 (dd, *J* = 11.4, 2.1 Hz, 1H, H-6), 3.96 (dd, *J* = 11.4, 3.5 Hz, 1H, H-6), 3.84 (t, *J* = 9.1 Hz, 1H, H-4), 3.67 – 3.57 (m, 1H, H-2), 3.57 – 3.48 (m, 1H, H-5), 1.09 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.55 (TCA), 137.97, 137.68, 135.98, 135.73, 133.47, 133.18, 132.91, 132.24, 129.87, 129.85, 129.20, 128.73, 128.61, 128.32, 128.22, 128.18, 127.97, 127.93, 127.86, 127.85, 92.65 (TCA), 84.61 (C-1), 81.65 (C-3), 80.34 (C-5), 78.17 (C-4), 75.72 (Bn), 75.02 (Bn), 62.60 (C-6), 56.98 (C-2), 26.97 (*t*-Bu), 19.42 (*t*-Bu). HR-MS: Calculated for $C_{44}H_{46}Cl_3NO_5SSi$ $[M+Na]^+$: 856.18237, found: 856.18220. $[\alpha]^{25}_D$ = - 11.3° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 10/1, v/v).

3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α/β-D-glucopyranoside (14d)

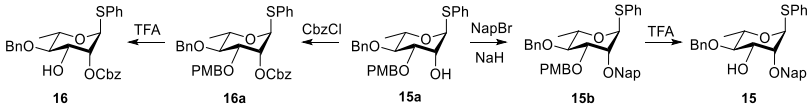


Compound **14c** (847 mg, 1.02 mmol, 1 eq) was dissolved in DCM (10 mL) and reduced to 0 °C. NIS (343 g, 1.52 mmol, 1.5 eq) and TFA (94 µL, 1.22 mmol, 1.2 eq) were added and the solution stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with $MgSO_4$, filtered and concentrated in *vacuo*. The compound was purified by flash chromatography (PE/EA 15:1 - 8:1) to yield compound **14d** (645 mg, 0.87 mmol, 85%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.75 – 7.62 (m, 4H), 7.46 – 7.22 (m, 14H), 7.21 – 7.13 (m, 2H), 6.91 (d, *J* = 9.2 Hz, 1H, NH), 5.27 (t, *J* = 3.4 Hz, 1H, H-1), 4.92 – 4.78 (m, 2H, Bn), 4.78 – 4.67 (m, 2H, Bn), 4.27 – 4.14 (m, 1H, H-2), 4.03 – 3.79 (m, 5H, H-6, H-5, H-4, H-3), 3.04 (d, *J* = 3.1 Hz, 1H, OH), 1.06 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.92 (TCA), 138.09, 137.86, 136.00, 135.72, 133.71, 133.14, 129.84, 128.67, 128.60, 128.17, 128.00, 127.95, 127.86, 127.73, 92.67 (TCA), 91.52 (C-1), 79.72 (C-3), 78.14 (C-4), 75.57 (Bn), 75.18 (Bn), 72.33 (C-5), 62.62 (C-6), 55.19 (C-2), 26.99 (*t*-Bu), 19.45 (*t*-Bu). HR-MS: Calculated for $C_{38}H_{42}Cl_3NO_6Si$ $[M+NH_4]^+$: 759.21852, found: 759.21809. TLC: Rf = 0.15 (PE/EA = 9/1, v/v).

_N_-Phenyl-trifluoroacetimidate 3,4-di-_O_-benzyl-2-trichloroacetamido-2-deoxy-6-_O-tert_-butyldiphenylsilyl-α/β-D-glucopyranoside (14)

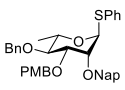


Hemiacetal **14d** (3.57 g, 4.80 mmol, 1.0 eq) was dissolved in acetone (50 mL) and cooled to 0 ℃. Cesium carbonate (1.9 g, 5.83 mmol, 1.2 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (1.5 g, 7.23 mmol, 1.5 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/Et_2O 10:1 – 7/1) to yield compound **14** (3.76 g, 4.11 mmol, 86%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.57 – 8.41 (m, 1H), 7.84 – 7.70 (m, 4H), 7.51 – 7.20 (m, 18H), 7.18 – 7.09 (m, 1H), 6.91 – 6.79 (m, 2H), 6.62 – 6.52 (m, 1H), 5.07 – 4.93 (m, 3H), 4.86 – 4.78 (m, 1H), 4.78 – 4.71 (m, 1H), 4.52 – 4.40 (m, 2H), 4.06 – 4.01 (m, 4H), 1.13 (s, 9H). ^{13}C NMR (126 MHz, Acetone) δ 162.95, 144.27, 139.40, 139.00, 136.45, 136.25, 134.11, 133.67, 130.58, 129.64, 129.09, 129.00, 128.59, 128.51, 128.49, 128.46, 128.41, 128.30, 125.24, 120.04, 105.51, 93.43, 78.77, 75.68, 75.43, 73.37, 71.78, 66.34, 63.27, 55.80, 27.19, 19.79. HR-MS: Calculated for $C_{46}H_{46}Cl_3F_3N_2O_6Si$ [M-[O(C=NPh)CF_3]+OH+NH_4]$^+$: 759.21852, found: 759.21811. TLC: Rf = 0.3 (PE/ Et_2O = 9/1, v/v).



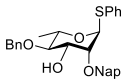
Scheme II. The synthesis of acceptors **15** and **16**

Phenyl 4-_O_-benzyl-3-_O-para_-methoxybenzyl-2-_O_-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside (15b)



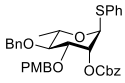
The known alcohol **15a**[11] (6.1 g, 13.07 mmol, 1 eq) was dissolved in DMF (40 mL), then cooled to 0 ℃. Sodium hydride (1.1 g, 26.2 mmol, 2 eq) was added, then 2-naphthylmethyl bromide (3.8 g, 17 mmol, 1.3 eq) was added, the reaction was stirred for 6h. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O 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 20:1 – 10/1) to yield compound **15b** (7.59 g, 12.5 mmol, 96%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.85 – 7.69 (m, 4H), 7.55 – 7.39 (m, 3H), 7.38 – 7.13 (m, 12H), 6.84 – 6.77 (m, 2H), 5.48 (d, *J* = 1.8 Hz, 1H, H-1), 4.98 (d, *J* = 10.8 Hz, 1H, Bn), 4.82 (q, *J* = 12.7 Hz, 2H, Nap), 4.65 (d, *J* = 10.9 Hz, 1H, Bn), 4.60 – 4.48 (m, 2H, PMB), 4.20 – 4.07 (m, 1H, H-5), 4.01 – 3.94 (m, 1H, H-2), 3.84 (dd, *J* = 9.4, 3.1 Hz, 1H, H-3), 3.76 (s, 3H, PMB), 3.70 (t, *J* = 9.4 Hz, 1H, H-4), 1.36 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.29 (PMB), 138.64, 135.43, 134.66, 133.25, 133.12, 131.45, 130.38, 129.55, 129.03, 128.45, 128.31, 128.06, 128.01, 127.77, 127.73, 127.32, 126.98, 126.18, 126.16, 126.04, 113.86, 86.06 (C-1), 80.56 (C-4), 79.73 (C-3), 76.63 (C-2), 75.49 (Bn), 72.37 (Nap), 72.02 (PMB), 69.54 (C-5), 55.29 (PMB), 18.03 (C-6). HR-MS: Calculated for $C_{38}H_{38}O_5S$ [M+Na]$^+$: 629.23322, found: 629.23345. $[\alpha]^{25}_D$ = - 45.9° (c = 1, $CHCl_3$). TLC: Rf = 0.5 (PE/EA = 9/1, v/v).

Phenyl 4-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside (15)



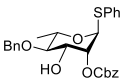
Compound **15b** (613 mg, 1.01 mmol, 1.0 eq) was dissolved in DCM (10 mL) and thiophenol (0.12 mL, 1.21 mmol, 1.2 eq), then TFA (1.0 mL) was added dropwise. The solution was stirred for 4 h at RT. After TLC showed complete consumption of the starting material, the reaction was quenched by saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 10:1 - 8:1) to yield compound **15** (456 mg, 0.94 mmol, 93%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.89 – 7.78 (m, 3H), 7.78 – 7.72 (m, 1H), 7.54 – 7.43 (m, 3H), 7.42 – 7.31 (m, 6H), 7.31 – 7.19 (m, 4H), 5.55 (d, *J* = 1.5 Hz, 1H, H-1), 4.90 (t, *J* = 11.5 Hz, 2H, CH_2), 4.68 (t, *J* = 11.2 Hz, 2H, CH_2), 4.23 – 4.10 (m, 1H, H-5), 4.08 – 4.02 (m, 1H, H-2), 4.01 – 3.92 (m, 1H, H-3), 3.43 (t, *J* = 9.2 Hz, 1H, H-4), 2.44 (s, 1H, 3-OH), 1.36 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.51, 134.83, 134.45, 133.30, 133.26, 131.67, 129.14, 128.68, 128.56, 128.08, 127.90, 127.87, 127.52, 127.24, 126.44, 126.33, 125.98, 85.31 (C-1), 82.52 (C-4), 80.09 (C-2), 75.28 (*C*H_2), 72.75 (*C*H_2), 72.19 (C-3), 68.77 (C-5), 18.06 (C-6). HR-MS: Calculated for $C_{30}H_{30}O_4S$ $[M+NH_4]^+$: 504.22031, found: 504.22046. $[\alpha]^{25}_D$ = - 86.8° (c = 1, $CHCl_3$). TLC: Rf = 0.6 (PE/EA = 3/1, v/v).

Phenyl 4-*O*-benzyl-2-*O*-benzyloxycarbonyl-3-*O*-*para*-methoxybenzyl-1-thio-α-L-rhamnopyranoside (16a)



Compound **15a** (4.04 g, 8.66 mmol, 1.0 eq) was dissolved in DCM (70 mL), then cooled to 0 °C and 4-dimethylaminopyridine (DMAP) (3.2 g, 26.19 mmol, 3 eq) were added, benzyloxycarbonyl chloride (CbzCl) (3.7 mL, 26.03 mmol, 3 eq) was added dropwise. The solution was stirred for overnight at RT. After TLC showed complete consumption of the starting material, the reaction was quenched by saturated aqueous sodium bicarbonate 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/Et_2O 20:1 - 10:1) to yield compound **16a** (4.6 g, 7.66 mmol, 88%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.41 (m, 2H), 7.40 – 7.21 (m, 15H), 6.87 – 6.79 (m, 2H), 5.50 (d, *J* = 1.6 Hz, 1H, H-1), 5.43 – 5.37 (m, 1H, H-2), 5.17 (s, 2H, Cbz), 4.90 (d, *J* = 10.8 Hz, 1H, Bn), 4.68 (d, *J* = 11.0 Hz, 1H, PMB), 4.59 (d, *J* = 10.9 Hz, 1H, Bn), 4.52 (d, *J* = 11.0 Hz, 1H, PMB), 4.25 – 4.14 (m, 1H, H-5), 3.89 (dd, *J* = 9.3, 3.1 Hz, 1H, H-3), 3.79 (s, 3H, PMB), 3.52 (t, *J* = 9.4 Hz, 1H, H-4), 1.32 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.43 (PMB), 154.82 (Cbz), 138.50, 135.11, 133.93, 131.86, 129.93, 129.86, 129.21, 128.70, 128.68, 128.51, 128.50, 128.09, 127.84, 127.78, 113.93, 85.95 (C-1), 80.04 (C-4), 77.97 (C-3), 75.66, 74.77 (C-2), 71.67, 70.09, 69.31 (C-5), 55.37 (PMB), 17.86 (C-6). HR-MS: Calculated for $C_{35}H_{36}O_7S$ $[M+NH_4]^+$: 618.25200, found: 618.25195. $[\alpha]^{25}_D$ = - 70.0° (c = 1, $CHCl_3$). TLC: Rf = 0.6 (PE/Et_2O = 9/1, v/v).

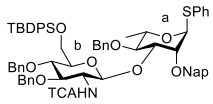
Phenyl 4-*O*-benzyl-2-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (16)



Compound **16a** (316 mg, 0.53 mmol, 1.0 eq) was dissolved in DCM (5 mL), and *p*-thiocresol (78 mg, 0.63 mmol, 1.2 eq) were added, then TFA (0.5 mL) was added dropwise. The solution was stirred for 4 h at RT. After TLC showed complete consumption of the starting material, the reaction

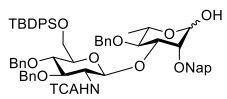
was quenched by saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 10:1 - 8:1) to yield compound **16** (217 mg, 0.45 mmol, 86%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.39 (m, 2H), 7.39 – 7.19 (m, 13H), 5.54 (d, *J* = 1.5 Hz, 1H, H-1), 5.25 – 5.19 (m, 1H, H-2), 5.14 (s, 2H, Cbz), 4.81 (d, *J* = 11.1 Hz, 1H, Bn), 4.66 (d, *J* = 11.1 Hz, 1H, Bn), 4.26 – 4.15 (m, 1H, H-5), 4.10 – 4.01 (m, 1H, H-3), 3.44 (t, *J* = 9.4 Hz, 1H, H-4), 2.58 (d, *J* = 5.8 Hz, 1H, 3-OH), 1.33 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.74 (Cbz), 138.16, 134.74, 133.79, 131.79, 129.13, 128.75, 128.68, 128.61, 128.59, 127.99, 127.91, 127.71, 85.56 (C-1), 81.78 (C-4), 78.15 (C-2), 75.39 (Bn), 70.77 (C-3), 70.27 (Cbz), 68.90 (C-5), 17.87 (C-6). HR-MS: Calculated for $C_{27}H_{28}O_6S$ $[M+NH_4]^+$: 498.19448, found: 498.19450. $[\alpha]^{25}_D$ = - 126.8° (c = 1, $CHCl_3$). TLC: Rf = 0.15 (PE/EA = 9/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside (17)



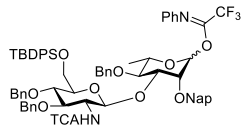
Donor **14** (7.6 g, 8.31 mmol, 2.0 eq) and acceptor **15** (2.03 g, 4.17 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (40 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (191 µL, 0.83 mmol, 0.2 eq) was added. The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 14:1) to yield compound **17** (4.4 g, 3.63 mmol, 87%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.83 – 7.74 (m, 4H), 7.74 – 7.65 (m, 4H), 7.59 – 7.53 (m, 1H), 7.44 – 7.37 (m, 2H), 7.37 – 7.12 (m, 24H), 7.10 – 7.02 (m, 2H), 6.72 (d, *J* = 8.9 Hz, 1H, NH), 5.50 (s, 1H, H-1a), 5.08 (d, *J* = 7.7 Hz, 1H, H-1b), 4.98 (d, *J* = 12.0 Hz, 1H, C*H*$_2$), 4.88 – 4.62 (m, 7H, C*H*$_2$), 4.54 (d, *J* = 10.8 Hz, 1H, C*H*$_2$), 4.28 – 4.13 (m, 3H, H-2a, H-3a, H-5a), 4.10 – 3.97 (m, 2H, H-2b, H-6b), 3.89 (dd, *J* = 11.2, 4.4 Hz, 1H, H-6b), 3.80 – 3.70 (m, 3H, H-4a, H-4b, H-3a), 3.62 – 3.52 (m, 1H, H-5b), 1.26 (d, *J* = 6.3 Hz, 3H, H-6a), 1.10 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.76 (TCA), 138.31, 137.67, 137.62, 136.02, 135.75, 135.63, 134.83, 133.36, 133.26, 133.09, 133.03, 130.94, 129.76, 128.92, 128.53, 128.50, 128.44, 128.06, 127.83, 127.80, 127.76, 127.68, 127.38, 127.04, 126.79, 126.43, 125.91, 125.72, 100.59 (C-1b), 92.52 (TCA), 86.65 (C-1a), 81.23 (C-4a), 80.43 (C-3b), 80.19 (C-2a), 78.01 (C-3a), 77.69 (C-4b), 76.41 (C-5b), 74.63 (*C*H$_2$), 74.53 (*C*H$_2$), 74.43 (*C*H$_2$), 73.76 (*C*H$_2$), 69.16 (C-5a), 63.04 (C-6b), 57.51 (C-2b), 27.07 (TBDPS), 19.39 (TBDPS), 17.97 (C-6a). HR-MS: Calculated for $C_{68}H_{70}Cl_3NO_9SSi$ $[M+NH_4]^+$: 1227.39444, found: 1227.39434. $[\alpha]^{25}_D$ = - 47.2° (c = 1, $CHCl_3$). TLC: Rf = 0.6 (PE/EA = 17/3, v/v).

4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α/β-L-rhamnopyranoside (18)



Compound **17** (4.40 g, 3.63 mmol, 1.0 eq) was dissolved in acetone (40 mL) and water (4 mL), then reduced to 0 °C. NIS (1.63 g, 7.24 mmol, 2.0 eq) was added and the solution stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The aqueous layer was extracted with DCM (3x), dried with MgSO4, filtered and concentrated in vacuo. The compound was purified by flash chromatography (PE/EA/DCM 8:1:1 – 6:1:1) to yield compound **18** (3.28 g, 2.93 mmol, 81%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.86 – 7.73 (m, 4H), 7.70 – 7.61 (m, 4H), 7.53 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.48 – 7.22 (m, 21H), 7.17 – 7.06 (m, 2H), 6.61 (d, *J* = 9.0 Hz, 0.6H), 6.54 (d, *J* = 9.1 Hz, 0.4H), 5.24 (d, *J* = 11.6 Hz, 0.4H), 5.16 – 5.09 (m, 0.6H), 5.07 – 4.96 (m, 1.6H), 4.85 – 4.70 (m, 3.5H), 4.70 – 4.54 (m, 3H), 4.33 (dd, *J* = 9.5, 3.1 Hz, 0.6H), 4.07 – 3.60 (m, 7.5H), 3.58 – 3.44 (m, 1.5H), 3.29 (dq, *J* = 9.2, 6.1 Hz, 0.4H), 2.60 (d, *J* = 3.4 Hz, 0.6H), 1.28 – 1.18 (m, 3H), 1.12 – 1.02 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.89, 161.86, 138.48, 138.24, 137.79, 137.77, 137.65, 137.61, 136.43, 135.86, 135.78, 135.75, 135.71, 135.65, 133.56, 133.43, 133.37, 133.29, 133.20, 133.16, 133.10, 133.04, 130.10, 129.99, 129.80, 128.69, 128.67, 128.65, 128.64, 128.62, 128.58, 128.46, 128.18, 128.13, 128.10, 128.08, 128.06, 127.99, 127.96, 127.94, 127.93, 127.86, 127.84, 127.82, 127.75, 127.54, 127.40, 127.36, 126.89, 126.55, 126.16, 126.03, 125.95, 125.74, 100.83, 100.69, 93.85, 93.28, 92.62, 92.55, 81.47, 80.77, 80.71, 80.66, 79.88, 79.85, 78.77, 78.17, 78.06, 76.39, 76.29, 75.74, 74.90, 74.84, 74.82, 74.75, 74.73, 74.57, 74.21, 71.54, 68.16, 62.77, 57.86, 57.75, 26.99, 19.48, 19.42, 18.20, 18.03. HR-MS: Calculated for $C_{62}H_{66}Cl_3NO_{10}Si$ $[M+NH_4]^+$: 1135.38598, found: 1135.38586. TLC: Rf = 0.20 (PE/Actone = 8/1, v/v).

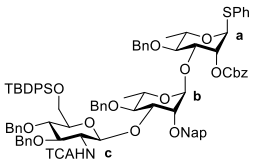
***N*-phenyl-trifluoroacetimidate 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α/β-L-rhamnopyranoside (19)**



The hemiacetal **18** (3.28 g, 2.93 mmol, 1.0 eq) was dissolved in acetone (30 mL) and cooled to 0 °C. Cesium carbonate (1.24 g, 3.81 mmol, 1.3 eq) was added. After 15 min, *N*-phenyl trifluoroacetimidoyl chloride (800 mg, 3.81 mmol, 1.3 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 10:1 – 5/1) to yield compound **19** (3.49 g, 2.7 mmol, 92%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.65 (d, *J* = 9.4 Hz, 1H), 8.07 (s, 1H), 8.03 – 7.96 (m, 2H), 7.96 – 7.88 (m, 1H), 7.86 – 7.79 (m, 1H), 7.76 – 7.65 (m, 4H), 7.53 – 7.21 (m, 24H), 7.14 – 7.03 (m, 3H), 6.75 – 6.67 (m, 1H), 6.00 – 5.65 (m, 1H), 5.35 – 5.17 (m, 2H), 5.17 – 5.00 (m, 2H), 4.94 – 4.79 (m, 3H), 4.79 – 4.69 (m, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.51 – 4.41 (m, 2H), 4.41 – 4.27 (m, 1H), 4.23 – 3.93 (m, 3H), 3.81 – 3.73 (m, 1H), 3.69 – 3.56 (m, 2H), 3.54 – 3.41 (m, 1H), 1.17 – 0.99 (m, 12H). ^{13}C NMR (126 MHz, Acetone) δ 162.46, 144.02, 139.29, 139.02, 138.63, 137.26, 135.94, 135.87, 133.92, 133.71, 133.69, 133.61, 130.36, 129.82, 129.17, 128.70, 128.64, 128.61, 128.53, 128.48, 128.37, 128.35, 128.28, 128.23, 128.16, 128.04, 127.95, 127.90, 127.88, 127.76, 127.73, 127.56, 126.36,

126.19, 124.72, 119.71, 119.38, 101.93, 93.52, 82.17, 81.19, 79.82, 79.10, 78.61, 76.84, 75.45, 75.29, 75.11, 74.94, 72.70, 63.75, 58.80, 26.89, 19.44, 17.73. HR-MS: Calculated for $C_{70}H_{70}Cl_3F_3N_2O_{10}Si$ [M-[O(C=NPh)CF_3]+OH+NH_4]$^+$: 1135.38598, found: 1135.38765. TLC: Rf = 0.15 (PE/ EA = 10/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-2-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (20)



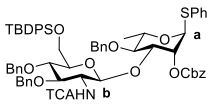
Imidate condition: Donor **19** (128.4 mg, 0.1 mmol, 1.0 eq) and acceptor **16** (66.0 mg, 0.14 mmol, 1.4 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (2 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (4.6 µL, 0.02 mmol, 0.2 eq) was added. The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 15:1:1 - 13:1:1) to yield compound **20** (25 mg, 16 µmol, 16%) and side product **21** (16.5 mg, 14 µmol, 14%).

Tf_2O/Ph_2SO mediated pre-activation condition: Donor **18** (52 mg, 46 µmol, 1.0 eq), Ph_2SO (21 mg, 104 µmol, 2.2 eq.) and DTBMP (24 mg, 117 µmol, 2.5 eq.) were co-evaporated with dry toluene three times under nitrogen. Then they were dissolved in DCM (2 mL) and activated 4Å molecular sieves and the reaction mixture stirred for 20 min at room temperature. The solution was cooled to -60°C and Tf_2O (8.5 µL, 50 µmol, 1.1 eq.) was slowly added. The reaction mixture was allowed to warm to -40°C in approximately 60 min, then added the acceptor **16** (45 mg, 94 µmol, 2 eq.) in DCM. The reaction mixture was allowed to warm slowly to 0 °C and stirred 5 h. After TLC showed complete consumption of the starting material, the reaction was quenched with Et_3N and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 15:1:1 - 13:1:1) to yield compound **20** (38 mg, 24 µmol, 52%).

Gold catalyzed condition: Donor **24** (42.2 mg, 32 µmol, 1.0 eq) synthesized following the reported procedure[12] and acceptor **16** (31.1 mg, 65 µmol, 2.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (2 mL) and 4Å molecular sieves were added and the solution stirred for 30 minutes at RT. The reaction was then cooled to 0 °C and a freshly prepared DCM solution of PPh_3AuNTf_2 (prepared by stirring 1:1 PPh_3AuCl (5.7 mg, 6.4 µmol, 0.2 eq) and $AgNTf_2$ (4.5 mg, 6.4 µmol, 0.2 eq) in DCM for 30 minutes). The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was filtered and concentrated *in vacuo*. The compound was firstly purified by size-exclusion chromatography (Sephadex LH-20, DCM/MeOH, 1:1 v/v) gave the α:β ratio, then purified by preparative TLC plates (Macherey-Nagel, pre-coated TLC plates SIL G-100 UV254) (PE/EA/DCM 8:1:1) to yield compound **20** (28 mg, 17.7 µmol, 55%) as a mixture of anomers.

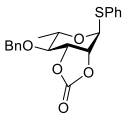
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.78 – 7.69 (m, 3H), 7.64 (s, 1H), 7.63 – 7.58 (m, 2H), 7.58 – 7.54 (m, 2H), 7.48 – 7.44 (m, 2H), 7.43 – 7.38 (m, 3H), 7.38 – 7.20 (m, 27H), 7.16 – 7.06 (m, 7H), 6.77 (d, *J* = 8.5 Hz, 1H, NHTCA), 5.54 (d, *J* = 1.7 Hz, 1H, H-1a), 5.25 (dd, *J* = 3.2, 1.8 Hz, 1H, H-2a), 5.11 – 5.02 (m, 4H, Cbz, H-1c, H-1b), 4.89 (d, *J* = 11.8 Hz, 1H, C*H*$_2$), 4.79 – 4.49 (m, 8H, C*H*$_2$), 4.35 – 4.25 (m, 2H, C*H*$_2$, H-3b), 4.20 – 4.03 (m, 2H, H-5a, H-3a), 3.94 – 3.62 (m, 8H, H-6c, H-2b, H-2c, H-5b, H-5c, H-4b), 3.53 – 3.41 (m, 2H, H-4a), 1.24 (d, *J* = 6.4 Hz, 3H, H-6a), 1.19 (d, *J* = 6.2 Hz, 3H, H-6b), 0.99 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.89 (NHTCA), 154.73 (Cbz), 138.45, 138.12, 137.91, 137.85, 136.08, 135.80, 135.58, 134.79, 133.91, 133.59, 133.34, 133.07, 133.00, 131.92, 129.97, 129.87, 129.21, 128.94, 128.70, 128.69, 128.66, 128.60, 128.54, 128.53, 128.48, 128.15, 128.05, 127.97, 127.94, 127.91, 127.80, 127.78, 127.76, 127.60, 127.57, 126.67, 126.32, 125.91, 125.72, 101.47 (C-1b), 99.81 (C-1c), 92.57 (NHTCA), 85.46 (C-1a), 81.35 (C-4b), 80.16, 80.08 (C-4a), 78.65, 77.84 (C-3a), 77.68 (C-2a), 76.52 (C-3b), 75.29, 74.50, 74.30, 74.17, 73.86, 70.17 (Cbz), 69.35 (C-5a), 68.76 (C-5b), 62.92 (C-6c), 57.96 (C-2c), 26.96 (TBDPS), 19.39, 18.11, 17.87. HR-MS: Calculated for $C_{89}H_{92}Cl_3NO_{15}SSi$ [M+NH_4^+]: 1597.53607, found: 1597.53592. $[\alpha]^{25}_D$ = - 37.1° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA/DCM = 8/1/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (21)



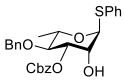
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.74 – 7.68 (m, 5H), 7.42 – 7.00 (m, 30H), 6.65 (d, *J* = 8.5 Hz, 1H), 5.50 (d, *J* = 1.6 Hz, 1H, H-1a), 5.38 (dd, *J* = 3.4, 1.6 Hz, 1H, H-2a), 5.14 (s, 2H, Cbz), 5.11 – 5.06 (m, 1H, H-1c), 4.82 – 4.51 (m, 6H, C*H*$_2$), 4.29 (dd, *J* = 9.5, 3.3 Hz, 1H, H-3a), 4.24 – 4.15 (m, 1H, H-5a), 4.03 (dd, *J* = 11.3, 2.8 Hz, 1H, H-6c), 3.95 (dd, *J* = 11.2, 4.4 Hz, 1H, H-6c), 3.90 – 3.72 (m, 3H, H-3c, H-4c, H-2c), 3.62 – 3.52 (m, 2H, H-4a, H-5c), 1.25 – 1.23 (m, 3H, H-6a), 1.13 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.69 (TCA), 154.44 (Cbz), 138.18, 137.93, 137.78, 135.97, 135.85, 135.34, 134.11, 133.62, 133.25, 131.63, 129.83, 129.81, 129.22, 129.14, 128.85, 128.70, 128.66, 128.61, 128.55, 128.53, 128.48, 128.16, 127.94, 127.92, 127.90, 127.88, 127.78, 127.70, 127.65, 127.58, 99.86 (C-1c), 92.60 (TCA), 85.62 (C-1a), 81.28 (C-4a), 80.01 (C-3c), 78.02 (C-2a), 77.86 (C-4c), 76.66 (C-5c), 75.01 (Bn), 74.77 (C-3a), 74.51 (Bn), 74.49 (Bn), 70.09 (Cbz), 69.07 (C-5a), 63.06 (C-6c), 57.91 (C-2c), 27.19 (TBDPS), 19.54 (TBDPS), 17.87 (C-6a). HR-MS: Calculated for $C_{65}H_{68}Cl_3NO_{11}SSi$ [M+NH_4]$^+$: 1221.36862, found: 1221.36830. $[\alpha]^{25}_D$ = - 44.1° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA/DCM = 8/1/1, v/v/v).

Phenyl 4-*O*-benzyl-2,3-*O*-carbonyl-1-thio-α-L-rhamnopyranoside (22)



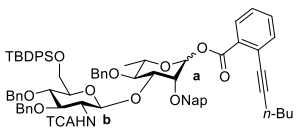
The analytical data were in full accord with the reported previously[16]. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.46 – 7.39 (m, 2H), 7.36 – 7.31 (m, 4H), 7.31 – 7.24 (m, 4H), 5.73 (d, *J* = 0.7 Hz, 1H, H-1), 4.85 – 4.74 (m, 3H, H-2, H-3, Bn), 4.57 (d, *J* = 11.3 Hz, 1H, Bn), 4.24 – 4.16 (m, 1H, H-5), 3.35 (dd, *J* = 9.7, 6.1 Hz, 1H, H-4), 1.23 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.14, 137.00, 132.47, 131.64, 129.24, 128.43, 128.30, 128.03, 82.05 (C-1), 80.14 (C-4), 78.93 (C-3), 77.47 (C-2), 73.38 (Bn), 65.49 (C-5), 17.53 (C-6). HR-MS: Calculated for $C_{20}H_{20}O_5S$ [M+NH_4]$^+$: 390.13697, found: 390.13702. TLC: Rf = 0.2 (PE/EA = 9/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (23)



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.39 (m, 2H), 7.39 – 7.15 (m, 13H), 5.44 (d, *J* = 1.8 Hz, 1H, H-1), 5.21 – 5.04 (m, 3H, H-3, Cbz), 4.67 (d, *J* = 11.0 Hz, 1H, Bn), 4.57 (d, *J* = 11.0 Hz, 1H, Bn), 4.36 (s, 1H, H-2), 4.31 – 4.20 (m, 1H, H-5), 3.66 (t, *J* = 9.4 Hz, 1H, H-4), 3.05 (s, 1H, 2-OH), 1.31 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.32, 137.83, 134.95, 133.97, 131.42, 129.06, 128.69, 128.67, 128.52, 128.48, 128.40, 127.92, 127.82, 127.42, 87.44 (C-1), 78.74 (C-4), 78.43 (C-3), 75.20 (Bn), 70.82 (C-2), 70.05 (Cbz), 69.06 (C-5), 17.78 (C-6). HR-MS: Calculated for $C_{27}H_{28}O_6S$ $[M+NH_4]^+$: 498.19448, found: 498.19438. $[\alpha]^{25}_D$ = - 77.2° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/EA = 9/1, v/v).

***ortho*-hexynylbenzoyl 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α/β-L-rhamnopyranoside (24)**

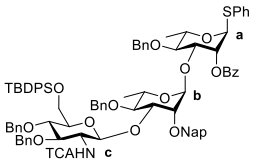


Hemiacetal **18** (258 mg, 213 µmol, 1.0 eq) was dissolved in DCM (3 mL). DMAP (52 mg, 426 µmol, 2.0 eq), DIPEA (148 µL, 852 µmol, 4.0 eq), EDCI·HCl (116 mg, 747 µmol, 3.5 eq) and freshly prepared *ortho*-hexynylbenzoic acid (129 mg, 639 µmol, 3 eq) were added and the mixture was stirred overnight. After analysis by TLC showed complete consumption of the starting material, diluted by DCM, the reaction was quenched with saturated aqueous sodium bicarbonate. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 7:1) to yield compound **24** (254 mg, 195 µmol, α:β 1:1.35, 92%). α-**24**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.86 – 7.76 (m, 4H), 7.73 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.59 – 7.45 (m, 6H), 7.45 – 7.36 (m, 3H), 7.36 – 7.04 (m, 22H), 6.82 (d, *J* = 8.7 Hz, 1H, NHTCA), 6.37 (d, *J* = 2.0 Hz, 1H, H-1a), 5.15 (d, *J* = 7.4 Hz, 1H, H-1b), 5.05 (d, *J* = 12.0 Hz, 1H, Nap), 4.89 – 4.59 (m, 7H, Bn, Nap), 4.36 (dd, *J* = 9.4, 3.1 Hz, 1H, H-3a), 4.04 – 3.81 (m, 7H, H-2a, H-2b, H-6b, H-5a, H-5b, H-3b), 3.77 (t, *J* = 9.5 Hz, 1H, H-4a), 3.61 – 3.53 (m, 1H, H-4b), 2.58 – 2.37 (m, 2H), 1.63 – 1.50 (m, 2H), 1.49 – 1.37 (m, 2H), 1.31 – 1.17 (m, 3H, H-6a), 0.97 – 0.85 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.19, 161.85 (NHTCA), 138.24, 137.76, 137.69, 136.07, 135.59, 135.53, 134.91, 133.38, 133.36, 133.11, 132.77, 131.97, 130.74, 130.71, 129.85, 129.83, 128.61, 128.57, 128.55, 128.12, 128.07, 127.99, 127.96, 127.90, 127.86, 127.80, 127.76, 127.73, 127.68, 127.65, 127.51, 127.12, 126.65, 126.23, 125.96, 125.76, 125.17, 100.61 (C-1b, J_{CH} = 163 Hz), 96.79, 92.87 (C-1a, J_{CH} = 176 Hz), 92.52 (NHTCA), 80.79 (C-4a), 79.92 (C-3b), 79.81, 77.96 (C-2a), 77.76 (C-3a), 77.46 (C-5b), 76.28 (C-4b), 75.04, 74.54, 74.44, 74.22, 70.77 (C-5a), 63.00 (C-6b), 57.37 (C-2b), 30.85, 26.87, 22.14, 19.69, 19.29, 18.13 (C-6a), 13.80. HR-MS: Calculated for $C_{75}H_{78}Cl_3NO_{11}Si$ $[M+Na]^+$: 1324.43019, found: 1324.43018.

β-**24**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.84 (d, *J* = 1.6 Hz, 1H), 7.81 – 7.73 (m, 2H), 7.73 – 7.67 (m, 1H), 7.67 – 7.57 (m, 5H), 7.53 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.46 – 7.37 (m, 3H), 7.36 – 7.22 (m, 18H), 7.21 (s, 1H), 7.16 (td, *J* = 7.7, 1.4 Hz, 1H), 7.13 – 7.05 (m, 2H), 6.64 (d, *J* = 8.8 Hz, 1H, NHTCA), 5.83 (d, *J* = 1.0 Hz, 1H, H-1a), 5.14 – 4.97 (m, 3H, CH_2, H-1b), 4.86 – 4.60 (m, 5H, CH_2), 4.52 (d, *J* = 10.8 Hz, 1H, CH_2), 4.16 (dd, *J* = 2.9, 1.1 Hz, 1H, H-2a), 4.01 – 3.82 (m, 3H, H-3a, H-6b, H-2b), 3.82 – 3.65 (m, 3H, H-6b, H-3b, H-4a), 3.61 – 3.43 (m, 3H, H-5b, H-5a, H-4b), 2.41 (t, *J* = 7.1 Hz, 2H), 1.63 – 1.52 (m, 2H), 1.49 – 1.38 (m, 2H), 1.28 (d,

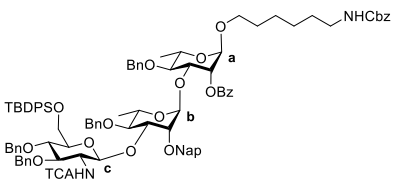
J = 6.1 Hz, 3H, H-6a), 1.02 (s, 9H, TBDPS), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.66, 161.83 (TCA), 138.16, 137.72, 137.62, 136.48, 135.67, 135.59, 134.50, 133.32, 133.23, 133.07, 132.94, 132.13, 130.74, 130.68, 129.97, 129.90, 128.63, 128.58, 128.56, 128.06, 128.01, 127.94, 127.91, 127.86, 127.80, 127.77, 127.63, 127.09, 127.05, 125.87, 125.71, 100.60 (C-1b, J_{CH} = 163 Hz), 97.01, 92.68 (C-1a, J_{CH} = 176 Hz), 92.54 (TCA), 80.45 (C-3b), 80.39 (C-4a), 79.99 (C-3a), 79.05, 78.44 (C-2a), 78.09 (C-5b), 76.37 (C-4b), 75.28, 74.97, 74.68, 74.65, 72.70 (C-5a), 62.88 (C-6b), 57.77 (C-2a), 30.73, 26.86, 22.16, 19.64, 19.30, 17.96 (C-6a), 13.77. TLC: Rf = 0.6-0.7 (PE/EA = 4/1, v/v).

Phenyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-1-thio-α-L-rhamnopyranoside (26)



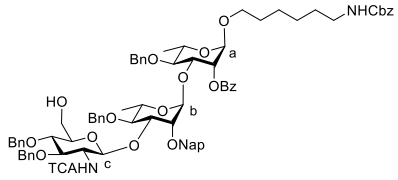
Donor **19** (703 mg, 0.54 mmol, 1.0 eq) and acceptor **25**[17] (497 mg, 1.1 mmol, 2 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (6 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (15 µL, 0.06 mmol, 0.1 eq) was added. The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 20:1:1 - 10:1:1) to yield compound **26** (610 mg, 0.39 mmol, 73%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 – 7.94 (m, 2H, Bz), 7.76 – 7.65 (m, 3H), 7.65 – 7.58 (m, 3H), 7.58 – 7.52 (m, 2H), 7.51 – 7.44 (m, 2H), 7.42 – 7.16 (m, 33H), 7.15 – 7.09 (m, 2H), 6.72 (d, *J* = 8.7 Hz, 1H, N*H*TCA), 5.73 – 5.65 (m, 1H, H-2a), 5.53 (d, *J* = 1.6 Hz, 1H, H-1a), 5.16 (d, *J* = 2.1 Hz, 1H, H-1b), 4.96 – 4.87 (m, 2H, CH_2, H-1c), 4.78 – 4.64 (m, 4H, CH_2), 4.64 – 4.49 (m, 5H, CH_2), 4.35 – 4.24 (m, 2H, H-5a, H-3a), 4.16 (dd, *J* = 9.0, 3.0 Hz, 1H, H-3b), 4.03 – 3.82 (m, 4H, H-2c, H-2b, H-5c, H-5b), 3.74 – 3.48 (m, 5H, H-3c, H-6c, H-4b, H-4a), 3.34 – 3.23 (m, 1H, H-4c), 1.35 (d, *J* = 6.2 Hz, 3H, H-6a), 1.20 (d, *J* = 6.2 Hz, 3H, H-6b), 1.00 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.71 (Bz), 161.89 (TCA), 138.41, 138.01, 137.94, 137.76, 135.99, 135.72, 135.51, 133.82, 133.54, 133.33, 133.27, 133.09, 133.00, 131.89, 129.78, 129.73, 129.71, 129.25, 129.17, 128.57, 128.54, 128.49, 128.10, 127.99, 127.92, 127.89, 127.84, 127.80, 127.75, 127.67, 127.65, 127.45, 127.22, 126.66, 126.31, 125.80, 125.63, 100.62 (C-1b, J_{CH} = 171 Hz), 100.46 (C-1c, J_{CH} = 162 Hz), 92.58 (TCA), 85.95 (C-1a, J_{CH} = 168 Hz), 80.92 (C-3c), 80.83 (C-4b), 80.78 (C-4a), 78.65 (C-2b), 77.43 (C-5c), 76.89 (C-3b), 76.34 (C-4c), 76.29 (C-3a), 74.95, 74.62, 74.36, 74.29 (C-2a), 74.07, 72.54, 69.25 (C-5a), 68.41 (C-5b), 62.67 (C-6c), 57.48 (C-2c), 26.98 (TBDPS), 19.43 (TBDPS), 18.10 (C-6a), 18.08 (C-6b). HR-MS: Calculated for $C_{88}H_{90}Cl_3NO_{14}SSi$ $[M+H]^+$: 1550.49896, found: 1550.49891. $[\alpha]^{25}_D$ = - 60.1° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 6/1, v/v).

_N_-benzyloxycarbonyl-6-aminohexanyl 2-_O_-benzoyl-4-_O_-benzyl-3-_O_-(4-_O_-benzyl-3-_O_-(3,4-di-_O_-benzyl-2-trichloroacetamido-2-deoxy-6-_O_-_tert_-butyldiphenylsilyl-β-D-glucopyranosyl)-2-_O_-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (27)



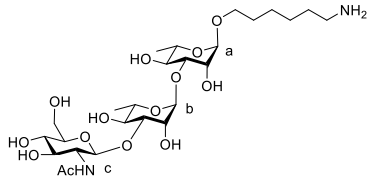
Donor **26** (201 mg, 0.13 mmol, 1.0 eq) and acceptor benzyl (6-hydroxyhexyl)carbamate (92 mg, 0.39 mmol, 3.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (4 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (6 µL, 0.026 mmol, 0.2 eq) and NIS (58 mg, 0.26 mmol, 2.0 eq) were added. The solution was stirred for 3 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 8:1 - 4:1) to yield compound **27** (208 mg, 0.123 mmol, 95%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.03 – 7.96 (m, 2H, Bz), 7.76 – 7.65 (m, 3H), 7.65 – 7.58 (m, 3H), 7.58 – 7.52 (m, 2H), 7.41 – 7.15 (m, 35H), 7.14 – 7.07 (m, 2H), 6.77 (d, *J* = 8.8 Hz, 1H, N*H*TCA), 5.44 – 5.38 (m, 1H, H-2a), 5.15 (d, *J* = 2.0 Hz, 1H, H-1b), 5.14 – 5.03 (m, 2H, Cbz), 4.93 – 4.78 (m, 4H, H-1c, H-1a, N*H*Cbz, CH_2), 4.76 – 4.62 (m, 4H, CH_2), 4.62 – 4.47 (m, 5H, CH_2), 4.30 (dd, *J* = 9.4, 3.4 Hz, 1H, H-3a), 4.16 (dd, *J* = 9.2, 3.0 Hz, 1H, H-3b), 4.02 – 3.76 (m, 5H, H-2c, H-2b, H-4c, H-5b, H-5a), 3.72 – 3.62 (m, 3H, H-4b, H-6c), 3.62 – 3.47 (m, 3H, H-6c, H-4a, H-3c), 3.44 – 3.34 (m, 1H), 3.30 – 3.22 (m, 1H, H-5c), 3.21 – 3.06 (m, 2H), 1.64 – 1.44 (m, 4H), 1.41 – 1.23 (m, 7H, H-6a), 1.18 (d, *J* = 6.1 Hz, 3H, H-6b), 0.99 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.91 (Bz), 161.85 (TCA), 156.45 (Cbz), 138.41, 138.02, 137.77, 136.74, 136.03, 135.69, 135.62, 135.47, 133.54, 133.24, 133.20, 133.08, 132.96, 132.12, 129.86, 129.74, 129.70, 129.68, 129.07, 128.63, 128.52, 128.48, 128.47, 128.44, 128.11, 128.06, 127.96, 127.91, 127.87, 127.82, 127.79, 127.77, 127.76, 127.69, 127.62, 127.60, 127.46, 127.20, 126.55, 126.26, 125.74, 125.55, 125.26, 100.61 (C-1b), 100.45 (C-1c), 97.29 (C-1a), 92.59 (TCA), 80.88 (C-4b, C-4a), 80.83 (C-3c), 78.79 (C-2b), 77.39 (C-4c), 76.73 (C-3b), 76.28 (C-5c), 76.17 (C-3a), 74.93, 74.56, 74.30, 73.99, 72.95 (C-2a), 72.25, 68.12 (C-5b), 67.83, 67.70 (C-5a), 66.55 (Cbz), 62.69 (C-6c), 57.45 (C-2c), 41.04, 29.92, 29.29, 26.95 (TBDPS), 26.51, 25.86, 19.38 (TBDPS), 18.20 (C-6a), 18.01 (C-6b). HR-MS: Calculated for $C_{96}H_{105}Cl_3N_2O_{17}Si$ $[M+Na]^+$: 1713.61403, found: 1713.61377. $[\alpha]^{20}_D$ = - 24.5º (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 3/1, v/v).

_N_-benzyloxycarbonyl-6-aminohexanyl 2-_O_-benzoyl-4-_O_-benzyl-3-_O_-(4-_O_-benzyl-3-_O_-(3,4-di-_O_-benzyl-2-trichloroacetamido-2-deoxy-β-D-glucopyranosyl)-2-_O_-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (28)



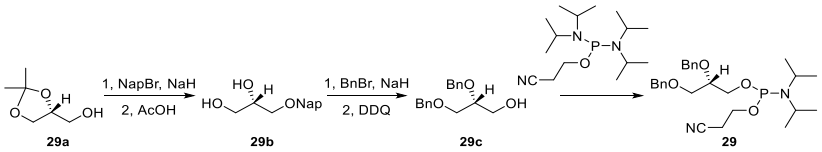
Protected trisaccharide **27** (91.8 mg, 54 µmol, 1.0 eq) was dissolved in anhydrous THF (3 mL) and AcOH (31 µL, 540 µmol, 10 eq). Then 1M TBAF in THF (540 µL, 540 µmol, 10 eq) was added in 0 °C. The reaction mixture was stirred at 50 °C for 5 h. After TLC showed complete consumption of the starting 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 - 4:1:1) to yield compound **28** (76.4 mg, 52.5 mmol, 97%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 – 8.04 (m, 2H, Bz), 7.82 – 7.72 (m, 3H), 7.64 (d, *J* = 1.6 Hz, 1H), 7.62 – 7.55 (m, 1H), 7.51 – 7.38 (m, 5H), 7.37 – 7.20 (m, 23H), 7.20 – 7.15 (m, 2H), 6.63 (d, *J* = 8.7 Hz, 1H), 5.40 – 5.33 (m, 1H, H-2a), 5.19 – 5.03 (m, 3H, H-1b, Cbz), 4.85 – 4.77 (m, 2H, H-1a), 4.77 – 4.56 (m, 9H, H-1c, CH_2), 4.52 (d, *J* = 12.1 Hz, 1H, CH_2), 4.46 (d, *J* = 11.1 Hz, 1H, CH_2), 4.26 (dd, *J* = 9.4, 3.3 Hz, 1H, H-3a), 3.97 (dd, *J* = 9.3, 2.9 Hz, 1H, H-3b), 3.84 – 3.71 (m, 4H, H-2b, H-5a, H-2c, H-5b), 3.68 – 3.48 (m, 4H, H-4b, H-3c, H-4a), 3.45 – 3.23 (m, 4H, H-6c, H-4c), 3.22 – 3.09 (m, 2H), 3.01 – 2.93 (m, 1H, H-5c), 1.64 – 1.45 (m, 4H), 1.42 – 1.22 (m, 7H, H-6a), 1.14 (d, *J* = 6.2 Hz, 3H, H-6b). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.95 (Bz), 161.97 (TCA), 156.51 (Cbz), 138.51, 138.23, 137.82, 137.76, 136.78, 135.87, 133.43, 133.23, 133.06, 130.03, 129.94, 128.69, 128.63, 128.61, 128.56, 128.48, 128.20, 128.15, 128.11, 128.08, 128.01, 127.93, 127.91, 127.85, 127.81, 127.67, 127.45, 127.31, 126.78, 126.42, 126.12, 125.88, 100.54 (C-1c), 100.25 (C-1b), 97.26 (C-1a), 92.47 (TCA), 80.84 (C-4a), 80.57 (C-4b), 80.29 (C-3c), 78.16 (C-2b), 77.98 (C-4c), 77.86 (C-3b), 76.72 (C-3a), 75.27 (C-5c), 75.05, 74.75, 74.69, 73.63, 73.15, 73.11 (C-2a), 68.96 (C-5b), 67.94, 67.82 (C-5a), 66.66 (Cbz), 61.70 (C-6c), 57.77 (C-2c), 41.13, 29.99, 29.35, 26.59, 25.93, 18.17 (C-6a), 18.02 (C-6b). HR-MS: Calculated for $C_{80}H_{87}Cl_3N_2O_{17}$ $[M+Na]^+$: 1470.54086, found: 1470.54103. $[\alpha]^{20}_D$ = - 26.0º (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/EA/DCM = 4/1/1, v/v/v).

6-aminohexanyl 3-_O_-(3-_O_-(2-acetamito-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (1)



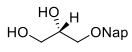
The protected trimer **28** (34.6 mg, 23.8 µmol, 1.0 eq) was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2$/C (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The crude was dissolved in sodium hydroxide (0.1 M, 5 mL), stirred overnight, quenched with acetic acid and then quenched the excess acid using ammonia solution. The compound was purified by gel filtration (HW-40, 0.15M, NH_4HCO_3 in H_2O) with a Shimadzu RID-10A refractive index detector and lyophilized to yield compound **1** (10.3 mg, 18.8 µmol, 71%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.98 (d, *J* =

1.8 Hz, 1H, H-1b), 4.72 (d, *J* = 1.8 Hz, 1H, H-1a), 4.67 (d, *J* = 8.5 Hz, 1H, H-1c), 4.24 (dd, *J* = 3.3, 1.8 Hz, 1H, H-2b), 3.96 (dd, *J* = 3.4, 1.8 Hz, 1H, H-2a), 3.91 – 3.85 (m, 2H, H-6c, H-3b), 3.81 – 3.64 (m, 6H, H-3a, H-5b, H-2c, H-6c, H-5a), 3.55 – 3.38 (m, 6H, H-3c, H-4a, H-4b, H-4a, H-5c), 2.95 (t, *J* = 7.6 Hz, 2H), 1.99 (s, 3H, NHAc), 1.68 – 1.53 (m, 4H), 1.44 – 1.32 (m, 4H), 1.28 – 1.20 (m, 6H, H-6a, H-6b). ^{13}C NMR (151 MHz, D_2O) δ 175.83 (NHAc), 103.60 (C-1c), 102.85 (C-1b), 100.53 (C-1a), 80.78 (C-3b), 79.06 (C-3a), 76.56 (C-5c), 74.57 (C-3c), 72.31 (C-4a), 71.79 (C-4b), 70.87 (C-2a), 70.75 (C-4c), 70.70 (C-3a), 70.19 (C-5b), 69.63 (C-5a), 68.68, 61.50 (C-6c), 56.65 (C-2c), 40.35, 29.19, 27.55, 26.28, 25.84, 23.09 (NHAc), 17.50, 17.48 (C-6a, C-6b). HR-MS: Calculated for $C_{26}H_{48}N_2O_{14}$ [M+H$^+$]: 613.31783, found: 613.31731.



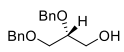
Scheme III. The synthesis of the space **29**.

1-*O*-(2-naphthylmethyl)-*sn*-glycerol (29b)



The commercially available reagent (R)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol **29a** (6.52 g, 49.3 mmol, 1.0 eq) was dissolved in DMF (150 mL), then cooled to 0 °C. Sodium hydride (3.94 g, 98.6 mmol, 2 eq) was added, then 2-naphthylmethyl bromide (16.4 g, 74.0 mmol, 1.5 eq) was added, the reaction was stirred for 7h. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude compound was dissolved in AcOH (200 mL) and water (200 mL). The mixture was warmed to 50 °C under 300 mbar in rotary evaporator for 4h. After analysis by TLC showed complete consumption of the starting material, concentrated *in vacuo*. The crude was dissolved in EA and washed with brine (3x). The aqueous layer was extracted with EA (3x), dried with MgSO4, filtered and concentrated in vacuo. The compound was purified by column chromatography (DCM/Acetone 9:1 – 4/1) to yield compound **29b** (10.07 g, 43.4 mmol, 88%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 – 7.77 (m, 3H, Nap), 7.77 – 7.70 (m, 1H, Nap), 7.53 – 7.36 (m, 3H, Nap), 4.67 (s, 2H, Nap), 3.96 – 3.81 (m, 1H, H-2), 3.67 (dd, *J* = 11.5, 3.8 Hz, 1H, H-3), 3.63 – 3.49 (m, 3H, H-3, H-1), 3.17 – 2.97 (m, 1H, 2-OH), 2.63 (s, 1H, 3-OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.29, 133.38, 133.21, 128.52, 128.05, 127.89, 126.85, 126.41, 126.22, 125.85, 73.83 (Nap), 71.90 (C-1), 70.90 (C-2), 64.21 (C-3). HR-MS: Calculated for $C_{14}H_{16}O_3$ [M+Na]$^+$: 255.09917, found: 255.09921. $[\alpha]^{25}_D$ = + 0.6° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (DCM/Acetone = 2/1, v/v).

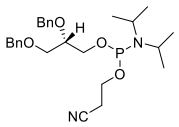
2,3-di-*O*-benzyl-*sn*-glycerol (29c)



Diol **29b** (10.0 g, 43.0 mmol, 1.0 eq) was dissolved in DMF (180 mL), then cooled to 0 °C. Benzyl bromide (16 mL, 129.0 mmol, 3.0 eq) was added and then sodium hydride (8.6 g, 215.0 mmol, 5 eq) was added slowly, the reaction was stirred for overnight. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O and washed with water and brine. The organic

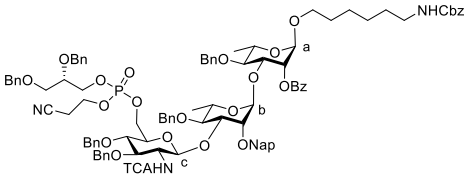
layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude compound was dissolved in DCM (500 mL) and water (50 mL). After cooled to 0 °C, 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) (12.0 g, 51.6 mmol, 1.2 eq) was added. The reaction was stirred at RT for 6 hours. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM 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 10:1 – 6/1) to yield compound **29c** (10.1 g, 37.1 mmol, 86%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.38 – 7.25 (m, 10H), 4.70 (d, *J* = 11.7 Hz, 1H, Bn), 4.61 (d, *J* = 11.8 Hz, 1H, Bn), 4.53 (d, *J* = 2.3 Hz, 2H, Bn), 3.80 – 3.56 (m, 5H, H-1, H-2, H-3), 2.24 – 2.17 (m, 1H, 1-OH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.35, 138.05, 128.56, 128.54, 127.91, 127.90, 127.84, 127.76, 78.16 (C-2), 73.62 (Bn), 72.25 (Bn), 70.27 (C-3), 62.93 (C-1). HR-MS: Calculated for $C_{17}H_{20}O_3$ $[M+NH_4]^+$: 290.17507, found: 290.17496. $[\alpha]^{25}_D$ = + 20.4° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/EA = 6/1, v/v).

2,3-di-*O*-benzyl-1-*O*-([*N*,*N*-diisopropylamino]-2-cyanoethylphosphite)-*sn*-glycerol (29)



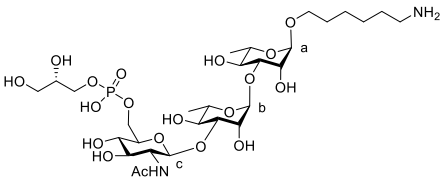
Alcohol **29c** (379 mg, 1.39 mmol, 1.0 eq) and diisopropylammonium tetrazolide (120 mg, 0.5 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (10 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. 0.3M Bis(diisopropylamino)(2-cyanoethoxy)phosphine in DCM (7.0 ml, 2.1 mmol, 1.5 eq) was added and the reaction mixture was stirred for 3 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with Et_3N and diluted with DCM. The solution was washed with saturated aqueous sodium bicarbonate and brine. The aqueous layer was extracted with DCM (1x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 6:1) to yield compound **29** (462 mg, 0.98 mmol, 70%). ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.42 – 7.24 (m, 10H, Bn), 4.71 – 4.59 (m, 2H, 2-OBn), 4.52 (s, 2H, 3-OBn), 3.86 – 3.53 (m, 9H), 2.69 – 2.53 (m, 2H, OCH_2CH_2CN), 1.22 – 1.11 (m, 12H, *i*-Pr). ^{13}C NMR (101 MHz, CD_3CN) δ 140.03, 139.69, 129.26, 129.20, 128.65, 128.63, 128.58, 128.44, 128.38, 119.53 (CN), 78.94, 78.92, 78.86, 78.84 (C-2), (C-3), 73.79 (OBn), 72.52, 72.50 (OBn), 70.79, 70.72 (C-3), 63.93, 63.91, 63.77, 63.75 (C-1), 59.47, 59.42, 59.29, 59.24 (OCH_2CH_2CN), 43.89, 43.87, 43.77, 43.75 ($CH(CH_3)_2$), 24.98, 24.91, 24.85 ($CH(CH_3)_2$), 21.03, 20.96 (OCH_2CH_2CN). ^{31}P NMR (162 MHz, CD_3CN) δ 149.47, 149.41. HR-MS: Calculated for $C_{26}H_{37}N_2O_4P$ $[M\text{-}N(i\text{-}Pr)_2+OH+NH_4]^+$: 407.17303, found: 407.17259. TLC: Rf = 0.5 (PE/EA = 6/1, v/v).

***N*-benzyloxycarbonyl-6-aminohexanyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-(2,3-di-*O*-benzyl-1-*O*-(2-cyanoethylphosphate)-*sn*-glycerol)-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (30)**



Alcohol **28** (57 mg, 39 µmol, 1.0 eq) and 0.1M phosphite **29** in ACN (1.2 mL, 118 µmol, 3.0 eq) were co-evaporated with dry acetonitrile 3 times under nitrogen. The mixture was dissolved in dry acetonitrile (4 ml) and 3Å molecular sieves was added. The mixture was stirred for 15 mins under argon atmosphere. 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (470 µL, 0.12 mmol, 3.0 eq) was added and the reaction mixture was stirred for 6 hours. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (314 µL, 0.16 mmol, 4.0 eq) was added. Stirred another 15 mins and diluted with EtOAc. The solution was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (PE/Acetone 5:1 - 3:1) yielded **30** (64 mg, 35 µmol, 89%). ^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 7.7 Hz, 2H, Bz), 7.83 – 7.72 (m, 3H), 7.72 – 7.68 (m, 1H), 7.64 – 7.58 (m, 1H), 7.53 – 7.46 (m, 2H), 7.46 – 7.40 (m, 4H), 7.39 – 7.19 (m, 32H), 7.16 – 7.10 (m, 2H), 6.79 (s, 1H), 5.44 – 5.37 (m, 1H), 5.18 – 5.04 (m, 3H), 4.92 – 4.33 (m, 14H), 4.27 – 4.19 (m, 1H), 4.16 – 3.96 (m, 5H), 3.93 – 3.85 (m, 1H), 3.84 – 3.50 (m, 11H), 3.48 – 3.35 (m, 3H), 3.23 – 3.10 (m, 3H), 2.23 – 1.91 (m, 2H), 1.62 – 1.45 (m, 4H), 1.41 – 1.17 (m, 7H), 1.14 – 1.04 (m, 3H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 166.05, 166.04, 162.11, 162.04, 156.52, 138.69, 138.64, 138.52, 138.06, 137.98, 137.93, 137.88, 137.81, 137.71, 137.70, 136.78, 136.20, 136.15, 133.48, 133.31, 133.28, 133.03, 132.99, 130.09, 130.08, 129.98, 128.72, 128.66, 128.64, 128.64, 128.59, 128.58, 128.56, 128.53, 128.52, 128.51, 128.25, 128.19, 128.15, 128.13, 128.10, 128.07, 128.06, 128.04, 128.00, 127.98, 127.94, 127.89, 127.88, 127.86, 127.85, 127.83, 127.80, 127.78, 127.77, 127.74, 127.73, 127.71, 127.67, 127.53, 127.41, 127.39, 126.48, 126.28, 126.25, 126.13, 126.08, 126.05, 125.89, 125.83, 116.70, 116.59, 100.44, 97.20, 92.37, 92.33, 80.41, 80.38, 80.22, 80.10, 78.72, 78.58, 77.76, 76.51, 76.49, 76.47, 76.44, 75.16, 75.10, 75.06, 75.02, 74.66, 74.56, 73.70, 73.67, 73.57, 73.49, 73.41, 73.25, 73.16, 72.22, 72.16, 68.78, 68.73, 68.59, 67.94, 67.93, 67.80, 67.71, 67.68, 67.50, 67.47, 66.68, 66.18, 66.11, 61.80, 61.78, 61.76, 58.33, 58.27, 41.15, 30.01, 29.84, 29.38, 26.63, 25.96, 19.19, 19.16, 19.11, 19.08, 18.18, 18.07. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.61, -0.82. HR-MS: Calculated for $C_{100}H_{109}Cl_3N_3O_{22}P$ $[M+NH_4]^+$: 1857.66442, found: 1857.66590. TLC: Rf = 0.35 (PE/Acetone = 3/1, v/v).

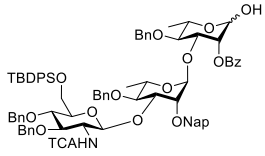
6-aminohexanyl 3-*O*-(3-*O*-(2-acetamito-2-deoxy-6-*O*-(1-*O*-phosphate-*sn*-glycerol)-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (4)



Full protected trimer **30** (10 mg, 5.4 µmol, 1.0 eq) was dissolved in dioxane (4 mL) and ammonia solution (35%) (2 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the

solvent. The crude was dissolved in methanol (2 mL) and dioxane (2 mL). Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 81 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (6 mL), water (3 mL) and 3 drops acetic acid. After $Pd(OH)_2/C$ (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **4** (3.0 mg, 3.9 µmol, 72%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.02 (d, *J* = 1.8 Hz, 1H, H-1b), 4.77 (d, *J* = 1.8 Hz, 1H, H-1c), 4.72 (d, *J* = 8.5 Hz, 1H, H-1c), 4.36 – 4.33 (m, 1H, H-2b), 4.22 – 4.16 (m, 1H, H-6c), 4.10 – 4.05 (m, 1H, H-6c), 4.03 – 3.99 (m, 1H, H-2a), 3.97 – 3.86 (m, 4H, H-3b), 3.86 – 3.81 (m, 1H, H-5b), 3.81 – 3.76 (m, 2H, H-3a, H-2c), 3.75 – 3.68 (m, 3H, H-5a), 3.63 (dd, *J* = 11.8, 6.1 Hz, 1H), 3.61 – 3.53 (m, 5H, H-5c, H-3c, H-4c, H-4a), 3.51 (t, *J* = 9.7 Hz, 1H, H-4b), 3.01 – 2.96 (m, 2H), 2.04 (s, 3H, NHAc), 1.70 – 1.58 (m, 4H), 1.48 – 1.38 (m, 4H), 1.31 (d, *J* = 6.3 Hz, 3H, H-6a), 1.29 (d, *J* = 6.3 Hz, 3H, H-6b). ^{13}C NMR (214 MHz, D_2O) δ 175.79 (NHAc), 103.76 (C-1c), 103.04 (C-1b), 100.52 (C-1a), 81.45 (C-3b), 79.32 (C-3a), 75.31, 75.27 (C-5c), 74.46 (C-3c), 72.12 (C-4a), 71.63 (C-4b), 71.59, 70.93 (C-2a), 70.63 (C-2b), 70.39 (C-4c), 70.11 (C-5b), 69.70 (C-5a), 68.64, 67.27, 67.25, 65.41, 65.39 (C-6c), 62.97, 56.62 (C-2c), 40.39, 29.19, 27.81, 26.29, 25.88, 23.08, 17.52, 17.49. ^{31}P NMR (162 MHz, D_2O) δ 1.44. HR-MS: Calculated for $C_{29}H_{55}N_2O_{19}P$ $[M+H]^+$: 767.32094, found: 767.32051.

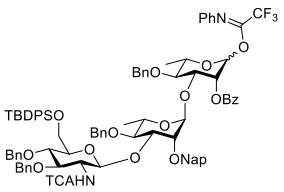
2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α/β-L-rhamnopyranoside (31)



The compound **26** (3.22 g, 2.07 mmol, 1 eq) was dissolved in DCM (20 mL) and reduced to 0 °C. NIS (934 mg, 4.15 mmol, 2.0 eq) and TFA (207 µL, 2.69 mmol, 1.3 eq) were added and the solution stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with $MgSO_4$, filtered and concentrated in *vacuo*. The compound was purified by flash chromatography (PE/Acetone 10:1 - 4:1) to yield compound **31** (2.72 g, 1.86 mmol, 90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 – 7.94 (m, 2H), 7.77 – 7.49 (m, 10H), 7.42 – 7.06 (m, 30H), 6.75 – 6.64 (m, 1H), 5.46 – 5.39 (m, 1H), 5.23 (d, *J* = 2.0 Hz, 1H), 5.13 (d, *J* = 2.0 Hz, 1H), 4.90 – 4.82 (m, 2H), 4.77 – 4.43 (m, 9H), 4.35 (dd, *J* = 9.4, 3.4 Hz, 1H), 4.13 (dd, *J* = 9.1, 3.1 Hz, 1H), 4.09 – 3.79 (m, 5H), 3.74 – 3.44 (m, 5H), 3.28 – 3.19 (m, 1H), 3.06 – 2.93 (m, 1H), 1.45 – 1.13 (m, 6H), 0.97 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.93, 161.90, 138.43, 138.05, 137.79, 136.01, 135.73, 135.66, 135.52, 135.47, 133.58, 133.28, 133.11, 133.00, 129.89, 129.81, 129.78, 129.76, 129.73, 128.68, 128.66, 128.63, 128.58, 128.53, 128.50, 128.47, 128.10, 128.07, 127.96, 127.93, 127.87, 127.82, 127.79, 127.74, 127.66, 127.49, 127.44, 127.41, 127.26, 127.24, 126.66, 126.32, 125.76, 125.59,

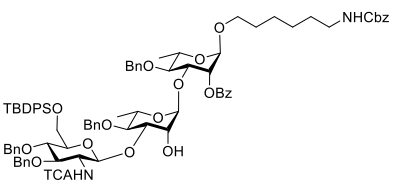
100.54, 92.58, 92.15, 80.88, 80.84, 78.74, 77.39, 76.30, 75.43, 74.78, 74.62, 74.35, 74.06, 73.10, 72.27, 68.14, 67.87, 62.68, 57.44, 29.80, 29.75, 26.96, 19.43, 18.24, 18.05. HR-MS: Calculated for $C_{82}H_{86}Cl_3NO_{15}Si$ [M+Na$^+$]: 1480.47245, found: 1480.47295. TLC: Rf = 0.6 (PE/Acetone = 3/1, v/v).

***N*-Phenyl-trifluoroacetimidate 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α/β-L-rhamnopyranoside (32)**



Hemiacetal **31** (3.57 g, 4.80 mmol, 1.0 eq) was dissolved in acetone (50 mL) and cooled to 0 °C. Cesium carbonate (1.9 g, 5.83 mmol, 1.2 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (1.5 g, 7.23 mmol, 1.5 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/Et_2O 10:1 – 7/1) to yield compound **32** (3.76 g, 4.11 mmol, 86%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.53 (d, *J* = 9.2 Hz, 1H), 8.18 – 8.07 (m, 2H), 7.99 – 7.85 (m, 4H), 7.76 – 7.69 (m, 2H), 7.68 – 7.60 (m, 3H), 7.58 – 7.22 (m, 31H), 7.20 – 7.09 (m, 3H), 6.97 (d, *J* = 7.8 Hz, 2H), 6.44 (s, 1H), 5.86 (s, 1H), 5.42 – 5.34 (m, 1H), 5.29 – 5.17 (m, 2H), 5.07 (d, *J* = 12.4 Hz, 1H), 4.96 (d, *J* = 11.4 Hz, 1H), 4.92 – 4.61 (m, 7H), 4.61 – 4.51 (m, 1H), 4.46 – 4.28 (m, 2H), 4.27 – 4.20 (m, 1H), 4.18 – 4.04 (m, 2H), 4.04 – 3.85 (m, 3H), 3.81 – 3.59 (m, 3H), 3.44 – 3.35 (m, 1H), 1.47 (d, *J* = 6.2 Hz, 3H), 1.08 (s, 12H). ^{13}C NMR (126 MHz, Acetone) δ 165.74, 162.63, 144.26, 139.77, 139.20, 138.94, 137.41, 136.31, 136.13, 134.27, 134.13, 133.81, 133.77, 130.54, 130.47, 130.29, 130.19, 129.56, 129.38, 129.10, 129.08, 128.90, 128.87, 128.83, 128.71, 128.63, 128.54, 128.48, 128.45, 128.38, 128.34, 128.30, 128.26, 128.24, 128.16, 128.13, 128.05, 127.94, 127.29, 127.18, 126.53, 126.34, 125.16, 120.04, 102.11, 100.76, 93.77, 82.26, 80.84, 80.47, 80.12, 78.58, 76.65, 75.39, 75.25, 75.20, 74.77, 74.48, 74.34, 71.51, 71.34, 69.39, 63.47, 58.87, 27.40, 19.84, 18.42, 18.27. HR-MS: Calculated for $C_{90}H_{90}Cl_3F_3N_2O_{15}Si$ [M-[O(C=NPh)CF_3]+OH+NH_4^+]: 1480.47245, found: 1480.47442. TLC: Rf = 0.6 (PE/Acetone = 4/1, v/v).

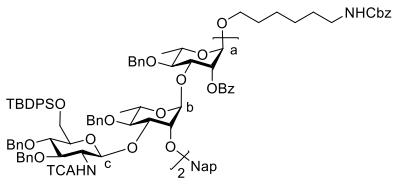
***N*-benzyloxycarbonyl-6-aminohexanyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (33)**



The full protected trisaccharide **27** (643 mg, 0.38 mmol, 1.0 eq) was dissolved in DCM (5 mL) and pH 7 water buffer (0.50 mL). After cooled to 0 °C, 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (172 mg, 0.76 mmol, 2.0 eq) was added. The reaction was stirred at RT for 6 hours. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM 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/DCM 7:1:1 – 4:1:1) to yield compound **33** (539 mg,

0.35 mmol, 91%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.07 – 8.01 (m, 2H), 7.67 – 7.59 (m, 4H), 7.56 – 7.49 (m, 1H), 7.44 – 7.36 (m, 6H), 7.36 – 7.15 (m, 23H), 7.15 – 7.10 (m, 2H), 7.09 – 7.02 (m, 2H), 6.87 (d, *J* = 7.8 Hz, 1H), 5.43 – 5.34 (m, 1H), 5.13 – 5.02 (m, 3H), 4.90 – 4.78 (m, 4H), 4.78 – 4.71 (m, 2H), 4.66 (q, *J* = 11.8, 11.0 Hz, 3H), 4.56 (d, *J* = 11.0 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.23 (dd, *J* = 9.4, 3.3 Hz, 1H), 4.06 – 4.00 (m, 1H), 3.95 (t, *J* = 9.2 Hz, 1H), 3.88 (dd, *J* = 8.6, 3.0 Hz, 1H), 3.85 – 3.76 (m, 1H), 3.76 – 3.53 (m, 6H), 3.47 (t, *J* = 9.0 Hz, 1H), 3.44 – 3.35 (m, 1H), 3.29 – 3.21 (m, 1H), 3.21 – 3.12 (m, 2H), 3.10 – 3.00 (m, 1H), 1.64 – 1.54 (m, 2H), 1.54 – 1.45 (m, 2H), 1.39 – 1.21 (m, 7H), 1.07 (d, *J* = 6.2 Hz, 3H), 1.05 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.98, 162.10, 156.47, 138.62, 138.28, 137.76, 137.66, 136.74, 135.76, 135.63, 133.25, 133.03, 132.68, 129.91, 129.88, 129.80, 129.10, 128.60, 128.56, 128.54, 128.53, 128.51, 128.46, 128.24, 128.13, 128.08, 128.04, 127.96, 127.91, 127.88, 127.84, 127.76, 127.68, 127.63, 127.61, 127.44, 127.38, 125.28, 101.49, 98.77, 97.06, 92.25, 81.13, 79.95, 79.81, 79.54, 78.54, 77.76, 76.08, 75.16, 75.13, 74.57, 73.85, 72.97, 69.81, 67.93, 67.88, 67.66, 66.57, 62.62, 58.47, 41.05, 29.92, 29.31, 26.92, 26.52, 25.88, 19.23, 18.16, 17.78. HR-MS: Calculated for $C_{85}H_{97}Cl_3N_2O_{17}Si$ $[M+NH_4]^+$: 1568.59603, found: 1568.59642. $[\alpha]^{20}_D$ = - 18.2° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA/DCM = 3/1/1, v/v/v).

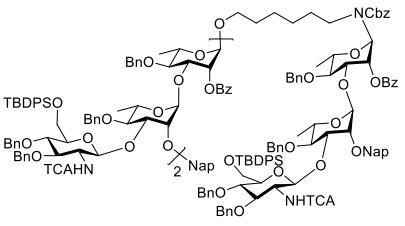
The hexasaccharide 34



Donor **32** (65 mg, 41.9 µmol, 1.3 eq) and acceptor **33** (50 mg, 32.2 µmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (2 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (1.5 µL, 8.4 µmol, 0.2 eq) was added. The solution was stirred for 3 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/Acetone 6:1 - 4:1) to yield compound **34** (75 mg, 25.0 µmol, 78%), recover acceptor **33** (7.8 mg, 5 µmol, 14%) and side product **34b** (12.3 mg, 2.8 µmol, 8%). ^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.61 (d, *J* = 6.9 Hz, 1H, NHTCA-c), 8.00 – 7.93 (m, 4H, Bz), 7.77 – 7.73 (m, 1H), 7.73 – 7.67 (m, 2H), 7.63 – 7.56 (m, 4H), 7.52 (ddd, *J* = 8.1, 5.1, 1.5 Hz, 5H), 7.49 – 7.46 (m, 2H), 7.46 – 7.43 (m, 1H), 7.42 – 7.11 (m, 59H), 7.11 – 7.07 (m, 2H), 6.94 – 6.90 (m, 2H), 6.75 (d, *J* = 8.4 Hz, 1H, NHTCA-c'), 5.50 (dd, *J* = 3.4, 1.8 Hz, 1H, H-2a'), 5.41 – 5.36 (m, 2H, H-1a', H-2a), 5.34 (d, *J* = 8.0 Hz, 1H, H-1c), 5.11 – 5.04 (m, 3H, Cbz, H-1b'), 5.00 (d, *J* = 1.6 Hz, 1H, H-1b), 4.94 (d, *J* = 7.7 Hz, 1H, H-1c'), 4.89 (d, *J* = 11.7 Hz, 1H, CH_2), 4.83 (d, *J* = 1.9 Hz, 1H, H-1a), 4.80 – 4.44 (m, 16H, CH_2), 4.44 – 4.34 (m, 3H, CH_2), 4.33 – 4.28 (m, 2H, H-3b, H-3a'), 4.22 (dd, *J* = 9.4, 3.5 Hz, 1H, H-3a), 4.15 (dd, *J* = 3.2, 1.7 Hz, 1H, H-2b), 4.10 (dd, *J* = 9.1, 2.9 Hz, 1H, H-3b'), 3.92 – 3.36 (m, 19H), 3.32 – 3.27 (m, 1H, H-5c'), 3.27 – 3.22 (m, 1H, H-5c), 3.22 – 3.15 (m, 2H), 3.15 – 3.08 (m, 1H, H-2c), 1.63 – 1.56 (m, 2H), 1.54 – 1.44 (m, 2H), 1.41 – 1.21 (m, 7H), 1.07 – 0.97 (m, 18H), 0.94 (s, 9H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 167.18, 166.22, 161.95, 161.88, 156.53 (Cbz), 138.92, 138.38, 138.36, 138.35, 138.32, 138.25, 138.04, 137.84, 136.84, 136.21, 135.83, 135.81, 135.65, 135.60, 133.63, 133.62, 133.47, 133.35, 133.32, 133.26, 133.15, 133.05, 129.95,

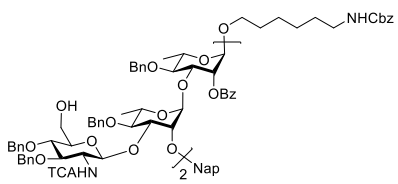
129.90, 129.80, 129.77, 129.69, 129.58, 128.64, 128.59, 128.55, 128.53, 128.52, 128.32, 128.31, 128.27, 128.12, 127.95, 127.94, 127.93, 127.88, 127.83, 127.75, 127.75, 127.66, 127.54, 127.48, 127.41, 127.38, 126.47, 126.21, 125.87, 125.66, 101.33 (C-1b, J_{CH} = 167 Hz), 100.46 (C-1b', J_{CH} = 172 Hz), 100.34 (C-1c', J_{CH} = 160 Hz), 98.38 (C-1a', J_{CH} = 175 Hz), 98.21 (C-1c, J_{CH} = 167 Hz), 97.16 (C-1a, J_{CH} = 166 Hz), 93.07 (TCA), 92.54 (TCA), 81.02, 80.82, 80.55, 80.47, 80.24 (C-3c'), 78.95 (C-4c), 78.76, 78.07 (C-3a, C-3b'), 77.67, 77.54, 77.31, 77.27 (C-2b), 76.98 (C-3b'), 76.41, 75.83 (C-5c), 75.44 (C-3b), 75.16, 74.98, 74.61, 74.48, 74.29, 74.25, 74.09, 73.99, 73.74, 73.46 (C-2d), 73.01 (C-2a), 68.83, 68.50, 68.37, 68.02, 67.55, 66.69 (Cbz), 62.94 (C-6c, C-6f), 61.17 (C-2c), 57.93 (C-2f), 41.16, 30.01, 29.84, 29.40, 27.12, 27.07, 26.61, 25.89, 19.45, 19.29, 18.29, 18.27, 18.12, 17.93. HR-MS: Calculated for $C_{167}H_{181}Cl_6N_3O_{31}Si_2$ $[(M+NH_4+NH_4)/2]^+$: 1513.05126, found: 1513.05116. $[\alpha]^{20}_D$ = - 31.5° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/Acetone = 4/1, v/v).

The side-product nonasaccharide 34b



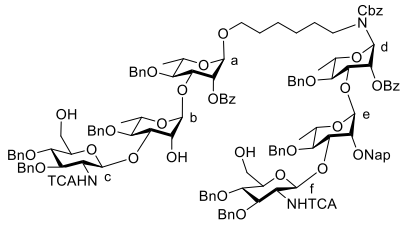
^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.59 (d, *J* = 6.8 Hz, 1H), 8.11 – 8.04 (m, 1H), 8.00 – 7.94 (m, 4H), 7.94 – 7.84 (m, 2H), 7.80 – 7.76 (m, 1H), 7.76 – 7.73 (m, 1H), 7.72 – 7.67 (m, 3H), 7.67 – 7.62 (m, 2H), 7.63 – 7.56 (m, 8H), 7.55 – 7.50 (m, 6H), 7.49 – 7.46 (m, 3H), 7.45 – 7.41 (m, 3H), 7.41 – 7.11 (m, 82H), 7.10 – 7.06 (m, 3H), 6.94 – 6.87 (m, 2H), 6.75 (d, *J* = 8.5 Hz, 1H), 5.96 (d, *J* = 9.5 Hz, 1H), 5.52 – 5.49 (m, 1H), 5.41 – 5.34 (m, 3H), 5.32 (d, *J* = 8.0 Hz, 1H), 5.12 – 5.04 (m, 2H), 5.03 – 4.95 (m, 4H), 4.93 (d, *J* = 7.6 Hz, 1H), 4.88 (d, *J* = 11.7 Hz, 1H), 4.79 – 4.54 (m, 17H), 4.53 – 4.46 (m, 5H), 4.44 – 4.35 (m, 7H), 4.32 – 4.27 (m, 2H), 4.27 – 4.16 (m, 3H), 4.16 – 4.13 (m, 1H), 4.11 – 4.01 (m, 3H), 3.98 – 3.91 (m, 1H), 3.91 – 3.33 (m, 25H), 3.31 – 3.15 (m, 6H), 3.14 – 3.07 (m, 2H), 2.82 – 2.74 (m, 1H), 1.61 – 1.54 (m, 2H), 1.53 – 1.44 (m, 2H), 1.42 – 1.09 (m, 13H), 1.05 – 1.02 (m, 15H), 1.01 – 0.99 (m, 12H), 0.94 (s, 9H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 167.16, 166.10, 165.44, 161.93, 161.89, 161.87, 156.53, 138.93, 138.51, 138.39, 138.38, 138.36, 138.33, 138.29, 138.05, 137.84, 137.82, 137.77, 137.76, 136.22, 136.04, 136.02, 136.00, 135.84, 135.83, 135.80, 135.70, 135.64, 135.60, 133.62, 133.46, 133.35, 133.28, 133.20, 133.13, 133.12, 133.04, 129.98, 129.97, 129.94, 129.89, 129.87, 129.79, 129.77, 129.67, 129.58, 128.67, 128.59, 128.56, 128.54, 128.53, 128.52, 128.50, 128.35, 128.31, 128.30, 128.26, 128.12, 128.00, 127.99, 127.98, 127.96, 127.94, 127.92, 127.88, 127.87, 127.80, 127.74, 127.69, 127.68, 127.65, 127.62, 127.52, 127.48, 127.47, 127.39, 127.32, 126.60, 126.46, 126.21, 126.14, 126.13, 125.94, 125.85, 125.65, 101.17, 100.42, 100.29, 98.41, 98.28, 98.15, 97.30, 97.16, 93.05, 92.54, 80.96, 80.85, 80.81, 80.63, 80.56, 80.51, 80.27, 78.90, 78.79, 78.21, 78.18, 78.15, 77.75, 77.66, 77.53, 77.37, 76.95, 76.87, 76.38, 75.80, 75.57, 75.01, 74.91, 74.73, 74.61, 74.49, 74.45, 74.27, 74.25, 74.04, 73.99, 73.94, 73.75, 73.70, 73.44, 72.95, 72.68, 71.38, 68.80, 68.46, 68.34, 68.28, 68.15, 67.96, 67.88, 67.81, 67.40, 66.70, 63.14, 62.90, 62.87, 61.84, 61.11, 57.89, 56.37, 41.17, 29.84, 29.29, 27.12, 27.10, 27.06, 27.04, 26.99, 26.81, 25.55, 19.45, 19.44, 19.28, 18.28, 18.26, 18.24, 18.17, 18.10, 17.94, 17.45, 16.58. MALDI-FTICR: Calculated for $C_{249}H_{265}Cl_9N_4O_{45}Si_3$ $[M+Na]^+$: 4452.4968, found: 4452.4100. $[\alpha]^{25}_D$ = - 43.8° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/Acetone = 4/1, v/v).

The hexasaccharide 35



Protected hexasaccharide **34** (54 mg, 18 µmol, 1.0 eq) was dissolved in anhydrous THF (4 mL) and AcOH (21 µL, 360 µmol, 20 eq). Then 1M TBAF in THF (360 µL, 360 µmol, 20 eq) was added in 0 °C. The reaction mixture was stirred at 50 °C 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/Acetone 4:1 - 2:1) to yield compound **35** (37.1 mg, 14.7 µmol, 82%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.15 (d, *J* = 6.8 Hz, 1H, NHTCA), 8.10 – 8.01 (m, 4H, Bz), 7.85 – 7.76 (m, 3H), 7.68 (s, 1H), 7.64 – 7.57 (m, 2H), 7.53 – 7.10 (m, 52H), 6.68 – 6.51 (m, 1H, NHTCA), 5.52 – 5.43 (m, 2H), 5.38 – 5.31 (m, 1H), 5.30 – 5.22 (m, 1H), 5.20 – 5.04 (m, 3H), 4.96 (d, *J* = 1.7 Hz, 1H), 4.91 – 4.37 (m, 21H), 4.31 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.21 – 4.14 (m, 2H), 4.10 (dd, *J* = 9.3, 3.0 Hz, 1H), 3.99 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.88 – 3.50 (m, 12H), 3.46 – 3.28 (m, 9H), 3.23 – 3.12 (m, 4H), 3.10 – 3.02 (m, 1H), 2.25 – 2.06 (m, 1H), 1.63 – 1.53 (m, 2H), 1.53 – 1.45 (m, 2H), 1.40 – 1.21 (m, 7H), 1.18 (d, *J* = 6.1 Hz, 3H), 1.06 (d, *J* = 6.1 Hz, 3H), 1.01 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.05, 166.09, 162.05, 161.97, 156.51, 138.65, 138.39, 138.22, 138.17, 138.11, 137.92, 137.79, 137.75, 136.80, 135.82, 133.81, 133.47, 133.27, 133.11, 130.03, 129.91, 129.56, 128.82, 128.80, 128.74, 128.68, 128.65, 128.63, 128.60, 128.51, 128.39, 128.31, 128.23, 128.18, 128.12, 128.09, 128.06, 127.99, 127.95, 127.88, 127.84, 127.74, 127.70, 127.62, 127.51, 127.48, 127.34, 126.79, 126.36, 126.18, 125.95, 101.12, 100.34, 98.79, 97.78, 96.98, 92.62, 92.43, 80.23, 79.85, 79.34, 78.77, 78.62, 78.05, 78.03, 77.65, 76.49, 75.35, 75.32, 75.13, 75.07, 74.79, 74.74, 74.67, 74.63, 74.19, 73.42, 73.26, 73.05, 69.20, 68.98, 68.55, 67.99, 67.67, 66.67, 61.74, 61.42, 60.93, 58.05, 41.13, 29.97, 29.82, 29.36, 26.58, 25.88, 18.14, 18.07, 17.83. HR-MS: Calculated for $C_{135}H_{145}Cl_6N_3O_{31}$ $[M+H]^+$: 2514.80660, found: 2514.81074. $[\alpha]^{20}_D$ = -57.4° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/Acetone = 3/1, v/v).

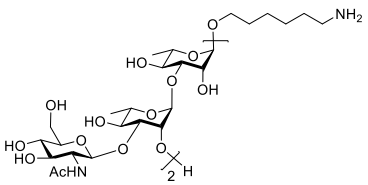
The side-product hexasaccharide 35a



^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.08 – 8.03 (m, 3H), 7.96 (d, *J* = 7.6 Hz, 2H), 7.82 – 7.76 (m, 3H), 7.76 – 7.71 (m, 2H), 7.63 – 7.58 (m, 1H), 7.52 – 7.43 (m, 7H), 7.41 – 7.18 (m, 39H), 7.15 – 7.11 (m, 5H), 6.75 – 6.67 (m, 2H, NHTCA), 6.01 (d, *J* = 9.6 Hz, 1H, H-1d), 5.39 (dd, *J* = 9.7, 3.4 Hz, 1H, H-2d), 5.33 (dd, *J* = 3.4, 1.9 Hz, 1H, H-2a), 5.10 – 5.05 (m, 3H, H-1b, Cbz), 4.95 – 4.88 (m, 2H, H-1f), 4.88 – 4.83 (m, 2H, H-1c), 4.82 – 4.57 (m, 15H, H-1a, C*H*$_2$), 4.52 (s, 1H, H-1e), 4.50 – 4.42 (m, 5H, H-3d), 4.36 (d, *J* = 12.1 Hz, 1H), 4.20 (dd, *J* = 9.4, 3.3 Hz, 1H, H-3a), 4.06 – 3.95 (m, 3H, H-5d, H-2f, H-2b), 3.87 (dd, *J* = 8.7, 3.0 Hz, 1H, H-3b), 3.85 – 3.80 (m, 1H, H-3c), 3.80 – 3.69 (m, 4H, H-2e, H-5a, H-5b), 3.69 – 3.51 (m, 9H), 3.50 – 3.45 (m, 2H), 3.41 (t, *J* = 9.1 Hz, 2H, H-4b), 3.26 – 3.19 (m, 3H, H-5c), 3.14 – 3.06 (m, 2H), 2.99 – 2.91 (m, 1H, H-4d), 1.52 – 1.45 (m, 2H), 1.41 – 1.34 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 3H, H-6b), 1.19 – 1.10 (m, 4H), 1.07

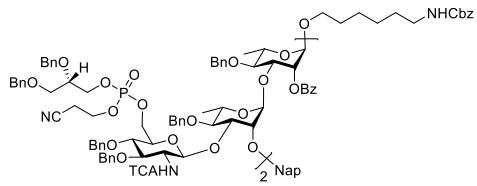
(d, *J* = 6.2 Hz, 3H, H-6a), 1.01 – 0.93 (m, 3H, H-6d), 0.67 (d, *J* = 6.0 Hz, 3H, H-6e). ^{13}C NMR (214 MHz, $CDCl_3$) δ 166.00, 165.38, 162.16 (NHTCA), 161.91 (NHTCA), 158.08 (Cbz), 138.57, 138.55, 138.31, 137.86, 137.80, 137.73, 137.72, 137.64, 135.84, 133.39, 133.37, 133.27, 133.17, 130.17, 129.97, 129.94, 129.35, 128.73, 128.72, 128.70, 128.69, 128.66, 128.65, 128.64, 128.62, 128.53, 128.42, 128.40, 128.32, 128.30, 128.21, 128.15, 128.13, 128.10, 128.06, 128.04, 128.03, 128.02, 127.96, 127.94, 127.89, 127.81, 127.75, 127.72, 127.69, 127.65, 127.52, 127.42, 126.74, 126.43, 126.22, 101.47 (C-1b), 100.73 (C-1f), 99.41 (C-1c), 97.18 (C-1a), 96.77 (C-1e), 92.63 (TCA), 92.28 (TCA), 80.57, 80.14, 80.03, 79.75 (C-3c), 78.15, 78.06, 77.79 (C-4c), 76.69 (C-2e), 75.90 (C-1d), 75.67, 75.47, 74.91, 74.71, 74.66, 74.55, 73.87, 73.15, 72.72, 71.47, 70.65, 68.82, 68.10, 68.08, 67.71 (C-2d), 67.70, 67.29 (Cbz), 61.80 (C-6c), 61.69 (C-6f), 58.07, 57.60, 45.99, 42.60, 29.85, 29.27, 26.84, 25.68, 18.20, 17.93, 17.29, 16.64. HR-MS: Calculated for $C_{135}H_{145}Cl_6N_3O_{31}$ $[M+H]^+$: 2514.80660, found: 2514.81265. $[\alpha]^{25}_D$ = - 67.0° (c = 0.2, $CHCl_3$). TLC: Rf = 0.1 (PE/Acetone = 3/1, v/v).

The target hexasaccharide 2



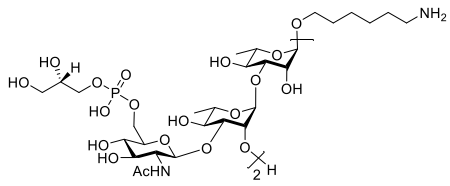
The hexamer **35** (20.6 mg, 8.2 µmol, 1.0 eq) was dissolved in methanol (3 mL) and dioxane (1 mL). Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 53 eq) was added. The reaction was stirred overnight. Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 53 eq) was added again. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The crude was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2/C$ (70 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered, using ammonia solution quenched the AcOH and concentrated *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector and lyophilized to yield compound **2** (5.5 mg, 5.0 µmol, 60%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.19 (d, *J* = 1.8 Hz, 1H), 5.09 (d, *J* = 1.8 Hz, 1H), 5.03 (d, *J* = 1.7 Hz, 1H), 4.76 (d, *J* = 1.7 Hz, 1H), 4.72 (d, *J* = 2.9 Hz, 1H), 4.71 (d, *J* = 3.0 Hz, 1H), 4.32 – 4.24 (m, 2H), 4.09 – 4.06 (m, 1H), 4.01 – 3.88 (m, 6H), 3.86 – 3.66 (m, 11H), 3.59 – 3.41 (m, 11H), 3.02 – 2.94 (m, 2H), 2.06 – 2.00 (m, 6H), 1.71 – 1.58 (m, 4H), 1.47 – 1.35 (m, 4H), 1.33 – 1.23 (m, 12H). ^{13}C NMR (126 MHz, D_2O) δ 174.97, 174.52, 102.79, 102.57, 101.76, 101.29, 101.02, 99.61, 79.91, 79.74, 77.46, 77.11, 76.00, 75.74, 75.66, 73.89, 73.69, 71.77, 71.51, 71.22, 70.92, 69.98, 69.89, 69.85, 69.79, 69.76, 69.41, 69.34, 68.70, 67.79, 60.84, 60.59, 55.89, 55.75, 39.46, 28.32, 26.68, 25.41, 24.96, 22.24, 22.20, 16.81, 16.66, 16.58, 16.50. HR-MS: Calculated for $C_{46}H_{81}N_3O_{27}$ $[M+2H^+]/2$: 554.76015, found: 554.75949.

The glycerol phosphate modified hexasaccharide 36



Alcohol **35** (37 mg, 14.7 µmol, 1.0 eq) and 0.1M phosphite **29** in ACN (588 µL, 58.8 µmol, 4.0 eq) were co-evaporated with dry acetonitrile 3 times under nitrogen. The mixture was dissolved in dry acetonitrile (2 ml) and 3Å molecular sieves was added. The mixture was stirred for 15 mins under argon atmosphere. 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (353 µL, 88 µmol, 6.0 eq) was added and the reaction mixture was stirred for 6 hours. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (180 µL, 88 µmol, 6.0 eq) was added. Stirred another 15 mins and diluted with EtOAc. The solution was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (PE/Acetone 3:1 - 2:1) yielded **36** (45.8 mg, 13.9 µmol, 95%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.80 – 8.62 (m, 1H), 8.16 – 7.99 (m, 4H), 7.86 – 7.67 (m, 4H), 7.66 – 7.02 (m, 64H), 6.80 (s, 1H), 5.55 – 5.42 (m, 1H), 5.38 – 5.24 (m, 3H), 5.19 – 5.04 (m, 3H), 5.04 – 3.24 (m, 80H), 3.23 – 2.99 (m, 4H), 2.26 – 1.94 (m, 4H), 1.64 – 1.44 (m, 4H), 1.40 – 1.30 (m, 4H), 1.29 – 0.93 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.27, 166.15, 166.08, 162.11, 162.05, 161.99, 156.50, 138.73, 138.70, 138.66, 138.61, 138.58, 138.53, 138.42, 138.37, 138.10, 138.08, 138.05, 138.00, 137.97, 137.93, 137.83, 137.78, 137.72, 136.80, 136.14, 133.88, 133.50, 133.43, 133.31, 133.28, 133.02, 132.99, 130.00, 129.95, 128.91, 128.68, 128.64, 128.60, 128.57, 128.54, 128.51, 128.49, 128.47, 128.46, 128.41, 128.38, 128.35, 128.31, 128.29, 128.22, 128.14, 128.11, 128.03, 127.99, 127.97, 127.96, 127.93, 127.88, 127.82, 127.79, 127.76, 127.75, 127.70, 127.68, 127.65, 127.61, 127.53, 127.47, 127.44, 127.35, 126.23, 126.18, 126.16, 126.09, 126.02, 125.91, 125.86, 116.75, 116.60, 116.54, 116.49, 101.46, 101.09, 100.41, 100.16, 99.53, 98.40, 98.31, 98.01, 97.02, 96.96, 92.90, 92.29, 92.25, 80.48, 80.22, 79.97, 79.76, 79.62, 79.51, 79.17, 78.42, 78.27, 78.18, 78.00, 77.64, 77.49, 77.29, 76.53, 76.51, 76.48, 76.45, 76.43, 76.40, 75.82, 75.49, 75.35, 75.13, 75.03, 74.99, 74.62, 74.59, 74.53, 74.47, 74.42, 74.24, 74.17, 74.09, 73.64, 73.57, 73.46, 73.42, 73.40, 73.34, 73.30, 73.14, 72.19, 72.15, 72.13, 69.03, 68.93, 68.84, 68.78, 68.71, 68.59, 68.53, 68.38, 68.36, 67.94, 67.90, 67.78, 67.73, 67.71, 67.68, 67.63, 67.49, 67.44, 67.28, 67.23, 66.63, 66.35, 66.21, 66.09, 61.83, 61.79, 61.75, 61.70, 61.66, 61.09, 60.87, 58.90, 58.78, 41.12, 29.96, 29.35, 26.58, 25.86, 19.21, 19.16, 19.11, 19.04, 18.98, 18.25, 18.15, 18.12, 18.10, 18.06, 17.83, 17.80. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.51, -0.56, -0.85, -1.01. HR-MS: Calculated for $C_{175}H_{189}Cl_6N_5O_{41}P_2$ $[(M+NH_4+NH_4)/2]^+$: 1662.05705, found: 1662.05788. TLC: Rf = 0.1 (PE/Acetone = 2.5/1, v/v).

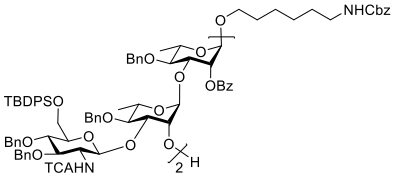
The glycerol phosphate modified hexasaccharide 5



Full protected trimer **36** (45 mg, 13.7 µmol, 1.0 eq) was dissolved in dioxane (6 mL) and ammonia solution (35%) (3 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The crude was dissolved in methanol (3 mL) and dioxane (3 mL). Sodium methoxide (25 wt. % in methanol)

(0.1 mL, 0.44 mmol, 32 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (7 mL), water (3 mL) and 2 drops acetic acid. After $Pd(OH)_2/C$ (45 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The crude was dissolved in water (5 mL) and 1M NaOH in water (0.5 mL). After stirred overnight, the reaction was quenched with acetic acid and then quenched the excess acid using ammonia solution. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **5** (12.5 mg, 8.8 µmol, 65%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.17 – 5.15 (m, 1H), 5.06 – 5.01 (m, 2H), 4.77 – 4.73 (m, 2H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.35 – 4.32 (m, 1H), 4.29 – 4.26 (m, 1H), 4.22 – 4.13 (m, 2H), 4.11 – 4.02 (m, 3H), 4.00 – 3.97 (m, 1H), 3.97 – 3.46 (m, 30H), 3.01 – 2.96 (m, 2H), 2.05 – 2.00 (m, 6H), 1.72 – 1.57 (m, 4H), 1.47 – 1.37 (m, 4H), 1.34 – 1.24 (m, 12H). ^{13}C NMR (151 MHz, D_2O) δ 175.86, 175.43, 104.00, 103.16, 102.88, 102.83, 101.85, 100.52, 81.87, 80.51, 78.65, 78.38, 78.26, 75.56, 75.51, 75.27, 75.22, 74.74, 74.50, 72.48, 72.37, 72.03, 71.66, 71.61, 70.93, 70.73, 70.66, 70.47, 70.26, 70.18, 70.14, 69.66, 68.65, 67.30, 65.58, 65.28, 62.99, 62.98, 56.77, 56.62, 40.36, 29.28, 27.62, 26.36, 25.97, 23.15, 23.11, 17.86, 17.62, 17.45. ^{31}P NMR (202 MHz, D_2O) δ 1.52, 1.47. HR-MS: Calculated for $C_{52}H_{95}N_3O_{37}P_2$ [M+Na^+]: 1438.50118, found: 1438.50211.

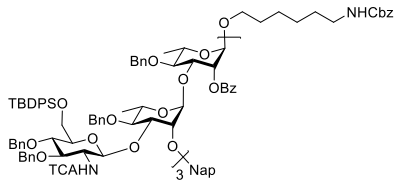
The hexasaccharide 37



The full protected hexamer **34** (597 mg, 0.2 mmol, 1.0 eq) was dissolved in DCM (3 mL) and pH 7 water buffer (0.3 mL). After cooled to 0 ℃, 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (91 mg, 0.4 mmol, 2.0 eq) was added. The reaction was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM 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 (DCM/EA 30:1 – 20:1) to yield compound **37** (437 mg, 0.15 mmol, 77%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.75 (d, *J* = 6.8 Hz, 1H), 8.09 – 7.94 (m, 4H), 7.69 – 7.56 (m, 6H), 7.56 – 7.15 (m, 58H), 7.14 – 7.04 (m, 5H), 6.97 – 6.89 (m, 2H), 6.76 (d, *J* = 7.7 Hz, 1H), 5.53 – 5.47 (m, 1H), 5.44 – 5.35 (m, 3H), 5.12 – 5.02 (m, 3H), 5.01 – 4.96 (m, 1H), 4.89 (d, *J* = 11.5 Hz, 1H), 4.86 – 4.82 (m, 1H), 4.81 – 4.53 (m, 17H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.45 – 4.32 (m, 4H), 4.26 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.20 (dd, *J* = 9.4, 3.5 Hz, 1H), 4.17 – 4.13 (m, 1H), 4.02 – 3.92 (m, 2H), 3.88 – 3.35 (m, 15H), 3.31 – 3.24 (m, 1H), 3.22 – 3.10 (m, 4H), 3.01 (d, *J* = 3.1 Hz, 1H), 1.62 – 1.54 (m, 2H), 1.53 – 1.44 (m, 2H), 1.40 – 1.17 (m, 7H), 1.05 (d, *J* = 6.2 Hz, 15H), 0.96 (d, *J* = 6.0 Hz, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.15, 166.26, 162.06, 162.03, 156.51, 138.94, 138.68, 138.65, 138.37, 138.32, 138.21, 137.83, 137.72, 135.86, 135.80, 135.72, 135.59, 133.70, 133.60, 133.34, 133.25, 133.09, 132.72, 130.11, 130.01, 129.99, 129.85, 129.79, 129.76, 129.68, 129.56, 128.78, 128.69, 128.65, 128.63, 128.58, 128.54, 128.50, 128.47, 128.42, 128.33, 128.29, 128.28, 128.25, 128.18, 128.13, 128.07, 128.03, 127.97,

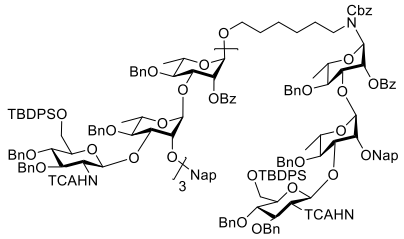
127.93, 127.89, 127.75, 127.71, 127.69, 127.64, 127.57, 127.50, 127.46, 127.42, 127.24, 101.48, 101.25, 98.67, 98.23, 97.98, 97.10, 93.09, 92.28, 81.97, 81.13, 80.47, 79.82, 79.67, 79.37, 79.03, 78.88, 78.44, 77.84, 77.62, 77.10, 76.10, 75.80, 75.30, 75.21, 75.09, 75.07, 74.58, 74.55, 74.53, 74.31, 74.16, 73.30, 73.04, 69.66, 68.52, 68.42, 68.30, 67.98, 67.51, 66.67, 63.02, 62.76, 61.33, 58.58, 41.14, 29.98, 29.84, 29.37, 27.09, 27.01, 26.59, 25.87, 19.34, 19.27, 18.27, 18.07, 18.00, 17.92. HR-MS: Calculated for $C_{156}H_{173}Cl_6N_3O_{31}Si_2$ [$(M+NH_4^+ + NH_4^+)/2$]: 1443.01997, found: 1443.01861. $[\alpha]^{20}_D$ = - 34.7° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (DCM/EA = 40/1, v/v).

The nonasaccharide 38



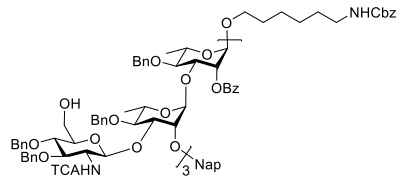
Donor **32** (47 mg, 29 µmol, 1.3 eq) and acceptor **37** (60 mg, 21 µmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (1 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was cooled to -20 °C and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (1.0 µL, 4.4 µmol, 0.2 eq) was added. The solution was stirred for 5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/Acetone 5:1 - 3:1) to yield compound **38** (44 mg, 10.2 µmol, 49%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.77 (d, *J* = 6.8 Hz, 1H), 8.62 (d, *J* = 6.9 Hz, 1H), 8.02 – 7.90 (m, 5H), 7.77 – 7.03 (m, 109H), 6.95 – 6.84 (m, 3H), 6.76 (d, *J* = 8.2 Hz, 1H), 5.55 – 5.42 (m, 3H), 5.41 – 5.31 (m, 4H), 5.12 – 5.06 (m, 2H), 5.00 – 4.94 (m, 2H), 4.90 – 4.82 (m, 2H), 4.82 – 4.52 (m, 20H), 4.52 – 4.47 (m, 2H), 4.45 – 4.28 (m, 8H), 4.27 – 4.08 (m, 5H), 3.93 – 3.83 (m, 3H), 3.83 – 3.24 (m, 28H), 3.20 – 3.04 (m, 4H), 1.61 – 1.56 (m, 2H), 1.53 – 1.45 (m, 2H), 1.34 – 1.27 (m, 7H), 1.15 (d, *J* = 6.2 Hz, 3H), 1.08 – 0.92 (m, 39H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.46, 167.18, 166.31, 162.08, 161.97, 161.85, 156.50, 138.91, 138.85, 138.42, 138.35, 138.32, 138.28, 138.24, 138.21, 138.19, 138.01, 137.81, 136.18, 135.82, 135.79, 135.72, 135.68, 135.62, 135.60, 133.64, 133.55, 133.40, 133.36, 133.34, 133.32, 133.26, 133.10, 133.02, 129.95, 129.88, 129.85, 129.77, 129.68, 129.56, 129.34, 128.77, 128.70, 128.68, 128.66, 128.62, 128.58, 128.54, 128.51, 128.49, 128.43, 128.35, 128.32, 128.27, 128.25, 128.23, 128.20, 128.17, 128.15, 128.05, 128.01, 127.99, 127.97, 127.93, 127.91, 127.87, 127.84, 127.81, 127.78, 127.75, 127.73, 127.70, 127.68, 127.65, 127.61, 127.58, 127.56, 127.50, 127.47, 127.43, 127.40, 126.45, 126.19, 125.84, 125.64, 101.59, 101.21, 100.46, 98.61, 98.20, 98.15, 97.92, 97.08, 93.10, 93.00, 92.52, 81.12, 81.03, 80.79, 80.47, 80.42, 80.21, 80.15, 79.13, 78.99, 78.85, 78.77, 78.54, 77.69, 77.63, 77.56, 77.50, 76.60, 76.40, 75.99, 75.83, 75.70, 75.40, 75.30, 75.08, 75.05, 74.57, 74.51, 74.29, 74.25, 74.22, 74.09, 73.96, 73.68, 73.48, 73.43, 73.07, 68.78, 68.72, 68.59, 68.42, 68.09, 67.98, 67.51, 66.67, 63.13, 62.93, 61.41, 61.17, 57.81, 41.13, 29.97, 29.83, 29.37, 27.15, 27.12, 27.05, 26.57, 25.83, 19.44, 19.25, 19.23, 18.28, 18.14, 18.08, 17.83. MALDI-FTICR: Calculated for $C_{238}H_{257}Cl_9N_4O_{45}Si_3$ $[M+Na]^+$: 4312.4342, found: 4312.3813. $[\alpha]^{20}_D$ = - 40.0° (c = 0.1, $CHCl_3$). TLC: Rf = 0.2 (PE/Acetone = 3/1, v/v).

The side-product dodecasaccharide 38a



^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.74 (d, *J* = 6.9 Hz, 1H), 8.61 (d, *J* = 6.8 Hz, 1H), 8.06 – 7.87 (m, 9H), 7.78 – 6.86 (m, 147H), 6.75 (d, *J* = 8.5 Hz, 1H), 5.97 (d, *J* = 9.5 Hz, 1H), 5.58 – 5.48 (m, 2H), 5.48 – 5.28 (m, 7H), 5.17 – 5.05 (m, 2H), 5.05 – 4.91 (m, 6H), 4.89 (d, *J* = 11.7 Hz, 1H), 4.83 – 4.02 (m, 53H), 3.99 – 3.35 (m, 36H), 3.35 – 3.06 (m, 9H), 2.79 (s, 1H), 1.53 – 1.38 (m, 4H), 1.31 – 0.73 (m, 57H), 0.55 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.42, 167.17, 166.17, 165.41, 162.05, 161.94, 161.83, 156.49, 156.10, 156.09, 138.91, 138.85, 138.50, 138.44, 138.35, 138.33, 138.30, 138.28, 138.24, 138.20, 138.19, 138.00, 137.80, 137.78, 137.75, 137.71, 136.18, 135.81, 135.79, 135.77, 135.71, 135.67, 135.59, 133.61, 133.56, 133.40, 133.34, 133.32, 133.17, 133.10, 133.01, 129.95, 129.91, 129.87, 129.84, 129.76, 129.72, 129.66, 129.58, 129.36, 128.73, 128.68, 128.64, 128.60, 128.56, 128.52, 128.50, 128.48, 128.46, 128.33, 128.30, 128.25, 128.24, 128.18, 128.15, 128.13, 128.11, 128.08, 128.03, 127.98, 127.96, 127.94, 127.91, 127.86, 127.82, 127.77, 127.73, 127.72, 127.70, 127.68, 127.66, 127.64, 127.62, 127.59, 127.55, 127.50, 127.48, 127.46, 127.44, 127.41, 127.38, 127.33, 127.29, 127.20, 126.57, 126.44, 126.18, 126.11, 125.92, 125.83, 125.62, 101.42, 101.12, 100.45, 98.62, 98.25, 98.21, 98.16, 97.99, 97.08, 93.09, 93.00, 92.53, 92.51, 81.08, 80.99, 80.79, 80.49, 80.21, 80.16, 79.08, 78.97, 78.79, 78.58, 78.41, 78.12, 77.74, 77.62, 77.56, 77.47, 77.37, 77.36, 77.02, 76.75, 76.56, 76.40, 75.98, 75.81, 75.24, 75.18, 75.04, 74.56, 74.53, 74.50, 74.44, 74.26, 74.22, 74.20, 74.04, 73.95, 73.91, 73.69, 73.64, 73.47, 73.37, 73.01, 72.97, 72.64, 71.36, 68.77, 68.70, 68.55, 68.41, 68.33, 68.27, 68.15, 68.05, 67.86, 67.39, 66.95, 66.64, 63.13, 63.07, 62.94, 61.35, 61.13, 57.77, 56.31, 42.62, 41.12, 29.25, 27.14, 27.12, 27.08, 27.04, 27.03, 26.75, 25.50, 19.42, 19.26, 19.24, 19.21, 18.31, 18.25, 18.13, 18.08, 17.85, 17.44, 16.55. MALDI-FTICR: Calculated for $C_{320}H_{341}C_{l12}N_5O_{59}Si_4$ $[M+Na]^+$: 5751.9068, found: 5751.7993. $[\alpha]^{20}_D$ = - 33.9° (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/Acetone = 3/1, v/v).

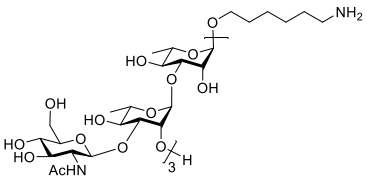
The nonasaccharide triol 39



Protected nonasaccharide **38** (10 mg, 2.3 µmol, 1.0 eq) was dissolved in anhydrous THF (1 mL) and AcOH (5.4 µL, 92 µmol, 40 eq). Then 1M TBAF in THF (92 µL, 92 µmol, 40 eq) was added in rt. The reaction mixture was stirred at 50 °C 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/Acetone/DCM 6:1:1 – 5:1:1) to yield compound **39** (5.4 mg, 1.5 µmol, 65%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.24 (d, *J* = 6.8 Hz, 1H), 8.15 – 8.00 (m, 7H), 7.82 – 7.73 (m, 3H), 7.67 (s, 1H), 7.64 – 7.56 (m, 3H), 7.53 – 7.07 (m, 74H), 6.64 – 6.54 (m, 1H), 5.57 (s, 1H), 5.53 – 5.41 (m, 3H), 5.36 – 5.24 (m, 3H), 5.18 (s, 1H), 5.08 (s, 2H), 5.00 – 4.36 (m, 32H), 4.33 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.28 – 4.07 (m, 6H), 4.01 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.94 – 3.49 (m, 14H), 3.49 – 3.25 (m, 13H),

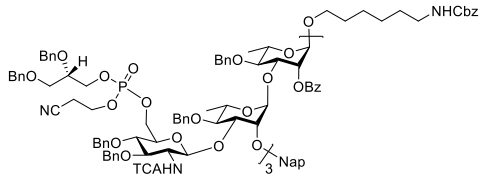
3.25 – 3.03 (m, 7H), 2.20 – 2.01 (m, 2H), 1.84 (s, 1H), 1.60 – 1.44 (m, 4H), 1.36 – 1.18 (m, 10H), 1.14 (d, *J* = 6.1 Hz, 3H), 1.05 (dd, *J* = 19.1, 6.2 Hz, 6H), 0.97 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.17, 167.02, 166.11, 162.09, 162.05, 161.93, 156.47, 138.68, 138.61, 138.36, 138.18, 138.12, 138.10, 137.94, 137.85, 137.75, 137.72, 136.77, 135.79, 133.81, 133.46, 133.23, 133.06, 130.03, 129.98, 129.53, 129.39, 128.85, 128.81, 128.78, 128.72, 128.68, 128.66, 128.63, 128.60, 128.58, 128.55, 128.50, 128.48, 128.43, 128.38, 128.35, 128.29, 128.26, 128.22, 128.19, 128.17, 128.13, 128.11, 128.09, 128.07, 128.02, 127.96, 127.94, 127.91, 127.85, 127.82, 127.80, 127.72, 127.67, 127.63, 127.61, 127.57, 127.49, 127.47, 127.41, 127.31, 126.74, 126.31, 126.15, 125.91, 101.26, 100.38, 98.75, 98.63, 97.85, 97.49, 96.90, 92.64, 92.58, 92.41, 80.27, 79.70, 79.53, 79.40, 79.26, 79.14, 78.83, 78.66, 78.56, 78.14, 78.00, 77.76, 77.59, 77.49, 77.10, 76.53, 75.93, 75.37, 75.32, 75.19, 75.06, 74.99, 74.80, 74.77, 74.73, 74.60, 74.16, 73.42, 73.37, 73.27, 73.04, 69.17, 69.10, 69.00, 68.62, 68.35, 67.93, 67.61, 66.64, 61.73, 61.41, 61.33, 61.06, 60.96, 58.01, 41.09, 29.93, 29.80, 29.33, 26.54, 25.82, 18.25, 18.16, 18.12, 18.04, 17.95, 17.76. HR-MS: Calculated for $C_{190}H_{203}Cl_9N_4O_{45}$ [M+2Na$^+$]/2: 1810.53502, found: 1810.55720. MALDI-FTICR: Calculated for $C_{190}H_{203}Cl_9N_4O_{45}$ [M+Na]$^+$: 3598.0808, found: 3597.9925. $[\alpha]^{20}_D$ = - 59.0 ° (c = 0.2, $CHCl_3$). TLC: Rf = 0.25 (PE/Acetone = 2/1, v/v).

The target nonasaccharide 3



The nonasaccharide **39** (38.8 mg, 10.8 µmol, 1.0 eq) was was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2$/C (80 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered, using ammonia solution quenched the AcOH and concentrated *in vacuo*. The crude was dissolved in water (5 mL). 1M Sodium hydroxide (0.5 mL) was added. The reaction was stirred overnight. The mixture was quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector and lyophilized to yield compound **3** (9.4 mg, 5.9 µmol, 54%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.17 – 5.13 (m, 2H, H-1), 5.07 – 5.04 (m, 2H, H-1), 5.01 – 4.98 (m, 1H, H-1), 4.72 (d, *J* = 1.7 Hz, 1H, H-1), 4.69 – 4.66 (m, 3H, H-1), 4.27 – 4.22 (m, 3H), 4.06 – 4.02 (m, 2H), 3.97 – 3.91 (m, 3H), 3.91 – 3.85 (m, 4H), 3.83 – 3.64 (m, 16H), 3.54 – 3.45 (m, 10H), 3.44 – 3.39 (m, 6H), 2.81 – 2.77 (m, 2H), 2.01 – 1.95 (m, 9H), 1.64 – 1.51 (m, 4H), 1.41 – 1.31 (m, 4H), 1.29 – 1.20 (m, 18H). ^{13}C NMR (214 MHz, D_2O) δ 175.78, 175.34, 175.31, 103.62 (C-1), 103.45 (C-1), 103.38 (C-1), 102.57 (C-1), 102.19 (C-1), 102.09 (C-1), 101.80 (C-1), 101.75 (C-1), 100.42 (C-1), 80.74, 80.64, 80.45, 78.22, 77.94, 77.50, 76.94, 76.87, 76.56, 76.54, 76.46, 74.73, 74.70, 74.50, 72.61, 72.58, 72.36, 72.12, 72.02, 71.72, 70.78, 70.69, 70.65, 70.63, 70.59, 70.24, 70.22, 70.21, 70.19, 70.17, 69.51, 68.66, 61.64, 61.61, 61.39, 56.69, 56.69, 56.56, 40.66, 29.34, 29.17, 26.39, 25.89, 23.05, 23.01, 17.66, 17.62, 17.49, 17.40, 17.32, 17.30. HR-MS: Calculated for $C_{66}H_{114}N_4O_{40}$ [M+2H$^+$]/2: 802.35774, found: 802.35720.

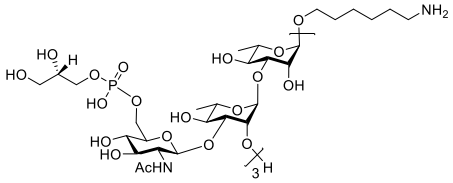
The glycerol phosphate modified nonasaccharide 40



Triol **39** (76 mg, 21.2 µmol, 1.0 eq) and 0.1M phosphite **29** in ACN (1.91 mL, 190.8 µmol, 9.0 eq) were co-evaporated with dry toluene 3 times under nitrogen. The mixture was dissolved in dry acetonitrile (4 ml) and 4Å molecular sieves was added. The mixture was stirred for 15 mins under argon atmosphere. 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (1.02 mL, 254.4 µmol, 12.0 eq) was added and the reaction mixture was stirred for 9 hours. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (510 µL, 254.4 µmol, 12.0 eq) was added. Stirred another 1hour and diluted with EtOAc. The solution was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Preparative TLC plate (Macherey-Nagel, pre-coated TLC plates SIL G-100 UV254) (DCM/Acetone 15:1) yielded compound **40** (91.3 mg, 19.2 µmol, 91%) as a mixture of anomers. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.84 – 8.63 (m, 2H), 8.14 – 8.00 (m, 5H), 7.83 – 7.67 (m, 4H), 7.66 – 6.96 (m, 109H), 6.83 – 6.71 (m, 1H), 5.53 – 5.45 (m, 1H), 5.44 – 5.25 (m, 5H), 5.18 – 5.03 (m, 3H), 5.01 – 3.28 (m, 102H), 3.28 – 3.03 (m, 5H), 2.24 – 1.95 (m, 6H), 1.58 – 1.44 (m, 4H), 1.43 – 0.80 (m, 22H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.47, 167.45, 167.43, 167.41, 167.28, 166.20, 166.11, 162.17, 162.13, 162.11, 162.08, 162.01, 161.96, 156.48, 138.65, 138.63, 138.58, 138.57, 138.53, 138.51, 138.41, 138.38, 138.37, 138.23, 138.20, 138.12, 138.09, 138.08, 138.05, 138.04, 138.03, 138.01, 137.99, 137.97, 137.95, 137.93, 137.86, 137.82, 137.77, 137.72, 136.82, 136.13, 133.96, 133.88, 133.47, 133.39, 133.30, 133.27, 133.01, 132.98, 130.11, 130.06, 130.01, 129.97, 129.93, 129.83, 129.51, 129.36, 129.34, 128.91, 128.86, 128.66, 128.65, 128.62, 128.61, 128.59, 128.58, 128.55, 128.51, 128.49, 128.47, 128.45, 128.43, 128.39, 128.38, 128.36, 128.34, 128.30, 128.27, 128.24, 128.18, 128.14, 128.12, 128.10, 128.09, 128.07, 128.05, 128.04, 128.01, 127.96, 127.94, 127.93, 127.90, 127.88, 127.86, 127.84, 127.83, 127.81, 127.80, 127.78, 127.75, 127.74, 127.69, 127.68, 127.66, 127.62, 127.59, 127.57, 127.55, 127.52, 127.50, 127.47, 127.45, 127.43, 127.35, 127.27, 126.40, 126.24, 126.22, 126.15, 126.12, 126.06, 126.00, 125.88, 125.83, 116.70, 116.56, 116.54, 116.51, 116.50, 116.45, 101.64, 101.59, 101.46, 101.19, 100.45, 100.22, 98.60, 98.54, 98.29, 98.21, 98.18, 98.13, 97.90, 97.85, 96.98, 96.91, 92.97, 92.95, 92.91, 92.88, 92.30, 92.26, 80.87, 80.67, 80.59, 80.40, 80.30, 80.08, 79.88, 79.65, 79.54, 78.94, 78.81, 78.55, 78.37, 78.26, 78.22, 78.14, 78.03, 77.83, 77.78, 77.66, 77.64, 77.61, 77.59, 77.29, 76.76, 76.55, 76.51, 76.47, 76.43, 76.42, 75.58, 75.39, 75.28, 75.23, 75.19, 75.09, 74.97, 74.94, 74.62, 74.59, 74.50, 74.48, 74.43, 74.39, 74.30, 74.27, 74.23, 74.19, 74.03, 73.79, 73.75, 73.68, 73.64, 73.57, 73.56, 73.53, 73.46, 73.44, 73.40, 73.33, 73.31, 73.28, 73.17, 72.32, 72.31, 72.19, 72.18, 72.13, 72.12, 68.99, 68.95, 68.94, 68.92, 68.88, 68.87, 68.81, 68.77, 68.72, 68.45, 68.41, 68.36, 68.29, 67.91, 67.86, 67.74, 67.71, 67.67, 67.65, 67.61, 67.56, 67.52, 67.49, 67.45, 67.36, 67.32, 67.28, 67.24, 66.59, 66.53, 66.50, 66.30, 66.21, 64.64, 61.85, 61.82, 61.80, 61.78, 61.74, 61.70, 61.66, 61.22, 61.15, 60.99, 60.93, 58.81, 58.72, 51.86, 41.10, 29.91, 29.78, 29.32, 26.53, 25.80, 19.42, 19.37, 19.22, 19.19, 19.14, 19.11, 19.09, 19.04, 18.99, 18.94,

18.94, 18.26, 18.17, 18.12, 18.08, 18.06, 18.06, 18.04, 17.98, 17.87, 17.83, 17.80, 17.77. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.54, -0.57, -0.86, -1.08, -1.10. HR-MS: Calculated for $C_{250}H_{269}Cl_9N_7O_{60}P_3$ [(M+NH_4^++NH_4^+)/2]: 2386.26497, found: 2386.23688. MALDI-FTICR: Calculated for $C_{250}H_{269}Cl_9N_7O_{60}P_3$ $[M+Na]^+$: 4759.4515, found: 4759.2796. TLC: Rf = 0.2 (DCM/Acetone = 14/1, v/v).

The target nonasaccharide 6



Full protected nonasaccharide **40** (51.6 mg, 10.9 μmol, 1.0 eq) was dissolved in dioxane (6 mL) and ammonia solution (35%) (3 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2/C$ (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The crude was dissolved in water (5 mL) and 1M NaOH in water (0.5 mL). After stirred overnight, the reaction was quenched with acetic acid and then quenched the excess acid using ammonia solution. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **6** (14.5 mg, 7.0 μmol, 65%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.21 – 5.18 (m, 1H, H-1), 5.15 (d, *J* = 1.8 Hz, 1H, H-1), 5.08 (d, *J* = 1.8 Hz, 1H, H-1), 5.05 (d, *J* = 1.9 Hz, 2H, H-1), 4.78 – 4.76 (m, 2H, H-1), 4.75 (d, *J* = 8.4 Hz, 1H, H-1), 4.72 (d, *J* = 8.5 Hz, 1H, H-1), 4.36 – 4.33 (m, 1H), 4.32 – 4.26 (m, 2H), 4.23 – 4.16 (m, 3H), 4.14 – 4.05 (m, 5H), 4.02 – 3.98 (m, 3H), 3.97 – 3.88 (m, 10H), 3.87 – 3.68 (m, 17H), 3.66 – 3.49 (m, 19H), 3.00 (t, *J* = 7.6 Hz, 2H), 2.07 – 2.01 (m, 8H), 1.74 – 1.59 (m, 4H), 1.49 – 1.39 (m, 4H), 1.36 – 1.25 (m, 18H). ^{13}C NMR (214 MHz, D_2O) δ 175.82, 175.44, 175.35, 103.94 (C-1), 103.30 (C-1), 103.06 (C-1), 102.95 (C-1), 102.80 (C-1), 102.61 (C-1), 101.90 (C-1), 101.64 (C-1), 100.49 (C-1), 81.85, 80.99, 80.15, 78.62, 78.46, 78.27, 77.90, 77.66, 75.54, 75.51, 75.45, 75.42, 75.25, 75.21, 74.76, 74.70, 74.49, 72.67, 72.45, 72.43, 72.26, 71.96, 71.68, 71.64, 71.63, 71.60, 71.59, 70.93, 70.72, 70.65, 70.47, 70.35, 70.23, 70.17, 70.16, 70.13, 70.06, 69.67, 68.62, 67.33, 67.32, 67.30, 67.27, 65.53, 65.43, 65.23, 65.21, 63.00, 62.98, 56.78, 56.73, 56.61, 40.35, 29.25, 27.57, 26.32, 25.93, 23.16, 23.15, 23.10, 17.90, 17.83, 17.60, 17.59, 17.52, 17.43. ^{31}P NMR (162 MHz, D_2O) δ 1.74, 1.69, 1.66. HR-MS: Calculated for $C_{75}H_{135}N_4O_{55}P_3$ [M+H^++Na^+]/2: 1044.35338, found: 1044.35484.

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Chapter 4

The First Total Synthesis of Acetylated Zwitterionic Polysaccharide Sp1 Fragments

Introduction

Bacterial cell-surface carbohydrates play a significant role in binding events with components from the host immune system.[1] Bacterial capsular polysaccharides (CPS) are excellent targets for designing carbohydrate-based antibacterial vaccines. Typically, bacterial capsular polysaccharides can only be recognized by B-cell receptors, thereby eliciting IgM responses, without inducing immunoglobulin class switching to form IgG isotypes.[2] They are considered to be "T-cell-independent antigens" which cannot be used as stand-alone vaccine entities, because they do not induce immunological memory.

Zwitterionic polysaccharides (ZPSs) are a rare class of immunomodulatory agents that can provoke a T-cell mediated immune responses. It has been shown that they can be processed by antigen-presenting cells and presented by major histocompatibility complex (MHC) class II-molecules to T helper cells.[3] Several ZPSs showed in Fig. 1 have been verified, such as Sp1 isolated from *Streptococcus pneumoniae*[4], CP5 and CP8 isolated from *Staphylococcus aureus*[5], PS A1, PS A2 and PS B isolated from *Bacteroides fragilis*[6]. Structurally, each of these molecules has both positively charged amino and negatively charged carboxylate or phosphate groups, which shape the unusual immunologic properties.[7] To explore structure–activity relationships and immunomodulatory mechanisms of ZPSs, pure and structurally well-defined oligosaccharides are required and therefore various chemical syntheses of these ZPSs have reported.[8]

The Sp1-polysaccharide is built up from trisaccharide repeating units, that in turn are composed of the rare α-2,4-di-amino-2,4,6-tri-deoxygalactose (D-AAT) and two α-D-galacturonic acid residues. The polysaccharide can carry *O*-acetyl groups at two thirds of the C-2 or C-3 positions of the 4-linked galacturonic acid residues. Recently, the assembly of zwitterionic, non-acetylated Sp1-oligosaccharides, up to the dodecasaccharide, has been achieved by combining pre-glycosylation oxidation and post-glycosylation oxidation chemistry.[8g] The 3D-structure of these Sp1 oligomers was analyzed via molecular dynamics simulations and NMR spectroscopy studies, and they were shown to adopt a right-handed helical structure with the nonasaccharide completing a full turn. It was found in ELISA and STD NMR[9] experiments that the longer oligosaccharides showed better binding to anti-Sp1 antibodies, but only a slight difference was shown between the nona- and dodecasaccharide, indicating that a single complete turn of the oligomers is sufficient for binding. This indicates that the Sp1-nonasaccharide can be a promising candidate for the development of a synthetic carbohydrate-based vaccine against *Streptococcus pneumoniae*. The role of the acetyl groups in the poly- and oligosaccharides however remain to be established.[4a, 10]

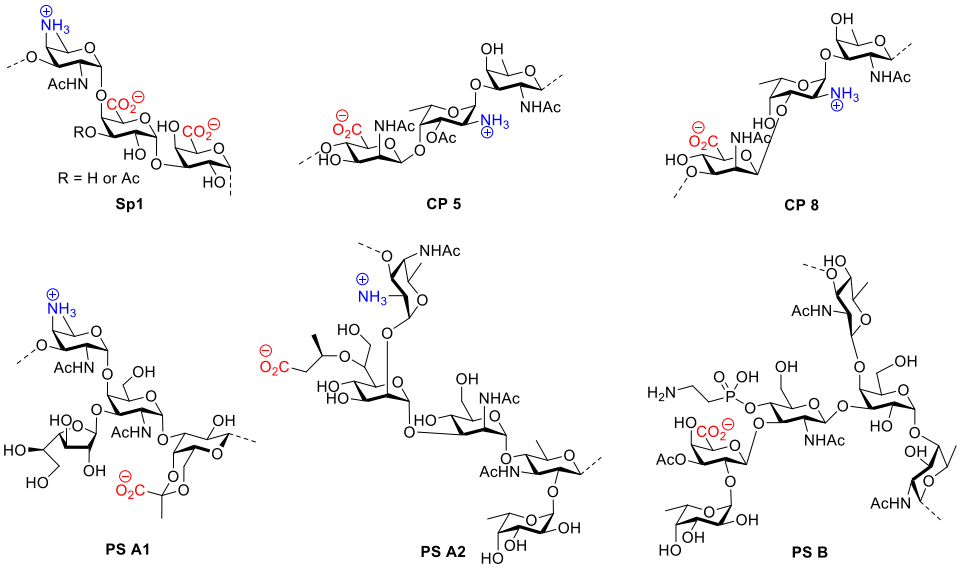


Figure 1. The chemical structures of naturally occurring ZPSs.

Streptococcus pneumoniae (or *pneumococcus*) is a dreaded alpha-hemolytic Gram-positive pathogen that can cause many types of fatal illnesses, including pneumonia, septicemia, meningitis, leading to high morbidity and mortality rates worldwide.[11] Pneumococcal infections can transmit via person-to-person contact, and mainly occur in individuals with weaker immune systems, such as infants, young children and the elderly, especially in developing countries, common during the winter and early spring months. Although antibiotic treatment for most pneumococcal infections is effective, it does induce the evolution of drug-resistant pneumococcal bacteria.

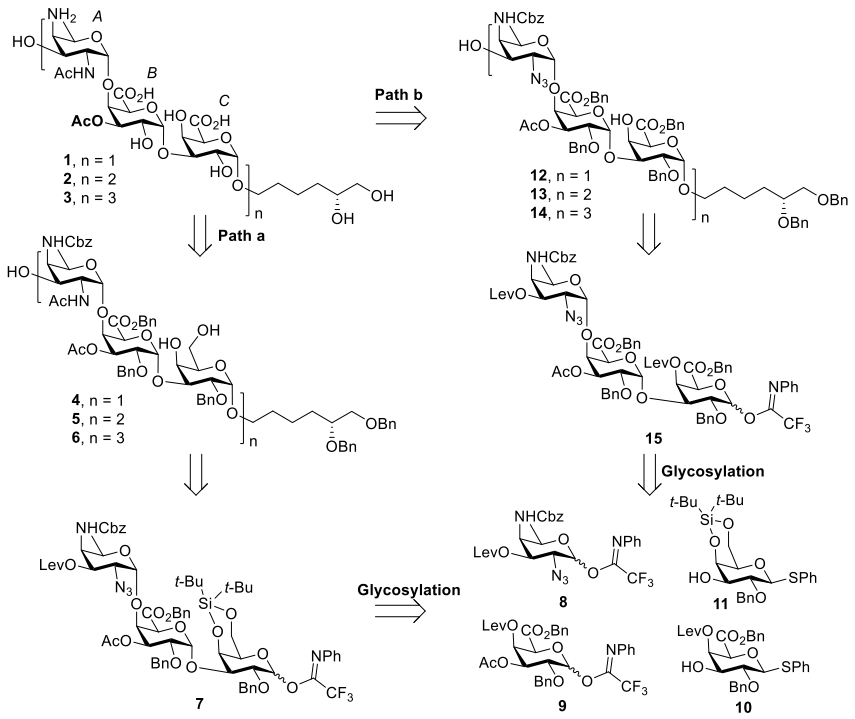
As early as 1946, the first pneumococcal polysaccharide vaccine was licensed.[12] Currently, two vaccines are available against the pneumococcal infections, including a 23-valent pneumococcal polysaccharide vaccine (PPSV23, Pneumovax, Merck) approved in 1983 and a 13-valent pneumococcal conjugate vaccine (PCV13, Prevnar, Pfizer) approved in 2010. Of the more than 90 serotypes of *pneumococcus*, only 25 serotypes were covered in these two vaccines.[13] Even though these vaccines have been very successful, the immunologic mechanism is not well understood and the large death tolls to date stimulate the development of novel well-defined vaccines.

To complement the series of de-acetylated Sp1 fragments described above, this chapter describes the development of a synthesis route to generate acetylated Sp1 fragments, which have been generated in multi-milligram amounts. The generated C-3-

O-acetyl Sp1 oligomers will be powerful tools to probe the effect of the acetyl groups on the structure of the oligosaccharides and binding to antibodies.

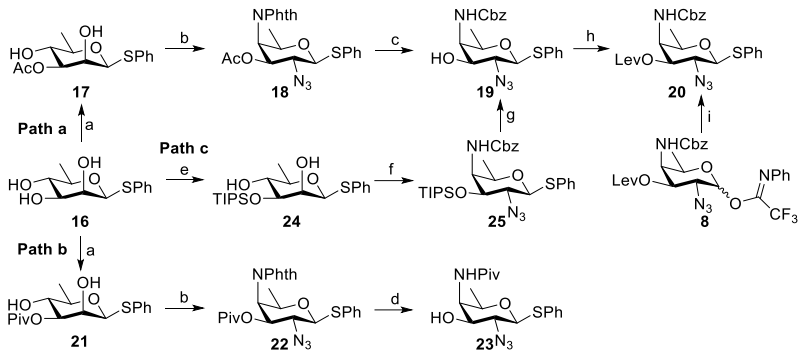
Result and discussion

The acetylated Sp1-fragments targeted in this chapter are shown in Scheme 1. The previously reported route towards the Sp1 oligosaccharides, combined a pre-glycosylation oxidation strategy with a post-glycosylation oxidation approach (path a in Scheme 1) to minimize the difficult oxidation events required on large oligosaccharides while enabling a robust and highly stereoselective glycosyl protocol. The de-OAc-Sp1 oligosaccharides were generated with a butenol spacer to enable thiol-ene conjugation chemistry. A Birch reduction was employed at the end of the syntheses to unmask all benzyl-type protecting groups. Taking the acetyl groups in the target compounds into consideration, a Birch reduction cannot be used for the deprotection and therefore a hydrogenation step will be required at the end of the synthesis. This thus precludes the use of an alkene-based linker and therefore a vicinal diol terminated spacer was chosen for the new targets. A selective oxidation by a Malaprade reaction can give the aldehyde for further modification. The oxidation and benzyl-ester formation steps in the syntheses of the long oligosaccharides proved to be extremely difficult. Therefore, another retrosynthetic plan built on a novel dual pre-glycosylation oxidation strategy (path b of Scheme 1), employing trisaccharide **15** as the pivotal building block. The required α-selectivity was expected to be controlled by the remote levulinoyl ester for the [3 + 3] and [3 + 6] glycosylations. It was planned to assemble the key trisaccharide **15** using glycosylation of the monosaccharide building blocks **8** – **10**.



Scheme 1. The designed fragments of *O*-Ac-Sp1 **1** – **3** and their retrosynthetic analysis.

The previously developed synthetic route (path a in Scheme 2) of the rare building block 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (D-AAT) **8**, which followed a modification of Kulkarni's protocol,[14] delivered **19** from triol **16** in only 19% yield.[8g] The most important side-products that were detected in this transformation were C-4-azido or C-4-hydroxyl substituted derivatives of the D-AAT building block. Therefore, different bulky protecting groups, such as Piv, TBS and TIPS groups, on the C-3 position of 6-deoxy-D-mannose **16** were probed to inhibit the formation of these side-products (routes b and c in Scheme 2). The three-step reaction sequence, involving triflation, azido substitution and phthalimide inversion delivered **22** from **21** in 37% yield. Unmasking the phthaloyl group however led to migration of the Piv group, generating the C-4-NHPiv compound **23**. In contrast, when the TIPS group was employed as the C-3-*O*-protecting group and ammonia as the nucleophile instead of potassium phthalimide, **16** was transformed into **19** in 50% yield over six steps (path c, Scheme 2). Following this route, the D-AAT sugar **8** can be rapidly obtained on multi-gram scale.

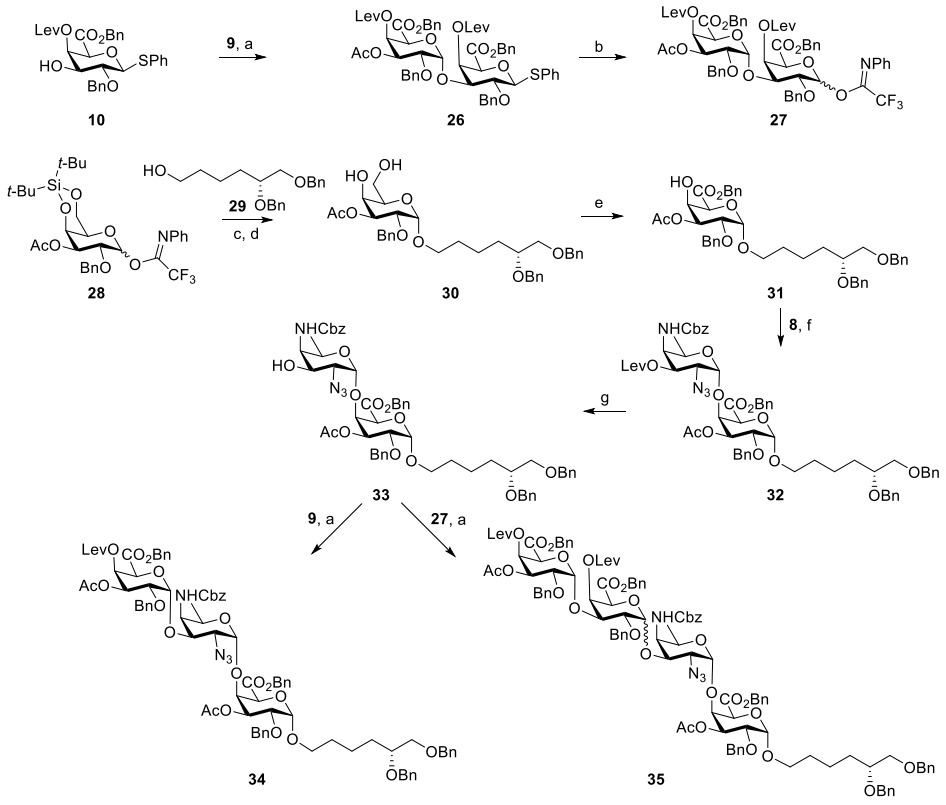


Scheme 2. The synthesis of building block D-AAT **8**.

Reagents and conditions: a) AcCl or pivaloyl chloride (PivCl), Me_2SnCl_2, DIPEA, THF, **17**, 90%; **21**, 89%. b) i, Tf_2O, pyridine, DCM; (ii) $TBAN_3$, CH_3CN, -30 °C; (iii) PhthNK, DMF, **18**, 30%; **22**, 37% (over three steps). c) i, Ethylenediamine, *n*-BuOH, reflux; (ii) CbzCl, $NaHCO_3$, THF/H_2O, 69%. d) i, Ethylenediamine, *n*-BuOH, reflux, 44%. e) TIPSCl, imidazole, DMF, 88%. f) i, Tf_2O, pyridine, DCM; (ii) $TBAN_3$, CH_3CN, -30 °C; (iii) NH_3 in MeOH; (iv) CbzCl, $NaHCO_3$, THF/H_2O, 62% (over four steps). g) TBAF, AcOH, THF, 91%. h) LevOH, EDCI, DIPEA, DMAP, DCM, 98%. i) i, NIS, TFA, DCM; ii, *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone; 91%.

The synthesis of the target oligosaccharides started with a feasibility study to see whether the designed strategy (path b in Scheme 1) could be used for the stereoselective fusion of the trisaccharides (*ABC*). It has been reported that the glycosylation of a C-4-*O*-Lev decorated D-galactopyranosyl uronate donor and silylidene galactose acceptor **11** can be achieved with effective stereoselectivity (α/β = 13 : 1).[8g] To further investigate the feasibility of the [*ABC* + *ABC* or *ABCABC*] glycosylation, two more model reactions were performed using [*C* + *AB*] and [*BC* + *AB*] glycosylations (Scheme 3). The detailed procedures for the synthesis of all required building blocks can be found in Experimental Section. Glycosylation between galactoside imidate donor **9** (*B*) and acceptor **10** (*C*) using TfOH as promotor provided a disaccharide **26** (*BC*) along with a side product resulting from an aglycon transfer of thioglycoside. Thereafter, disaccharide imidate donor **27** (*BC*) was generated by hydrolysis of thioglycoside **26** and installation of the imidate moiety in excellent yield. To accomplish the synthesis of disaccharide acceptor **33** (*AB*), spacer **29** (*vide infra*) was glycosylated with silylidene galactoside imidate donor **28** under the promotion of TBSOTf and ensuing desilylation delivered diol intermediate **30** in good yield. Then, selective oxidation of the diol **30** was performed under TEMPO-BAIB conditions, after benzylation of the carboxylate, to provide the C-4-OH galacturonic acid **31** in 94% yield. The desired disaccharide acceptor **33** (*AB*) was synthesized via glycosylation of **31** and D-AAT donor **8** (*A*) using TBSOTf as promotor,

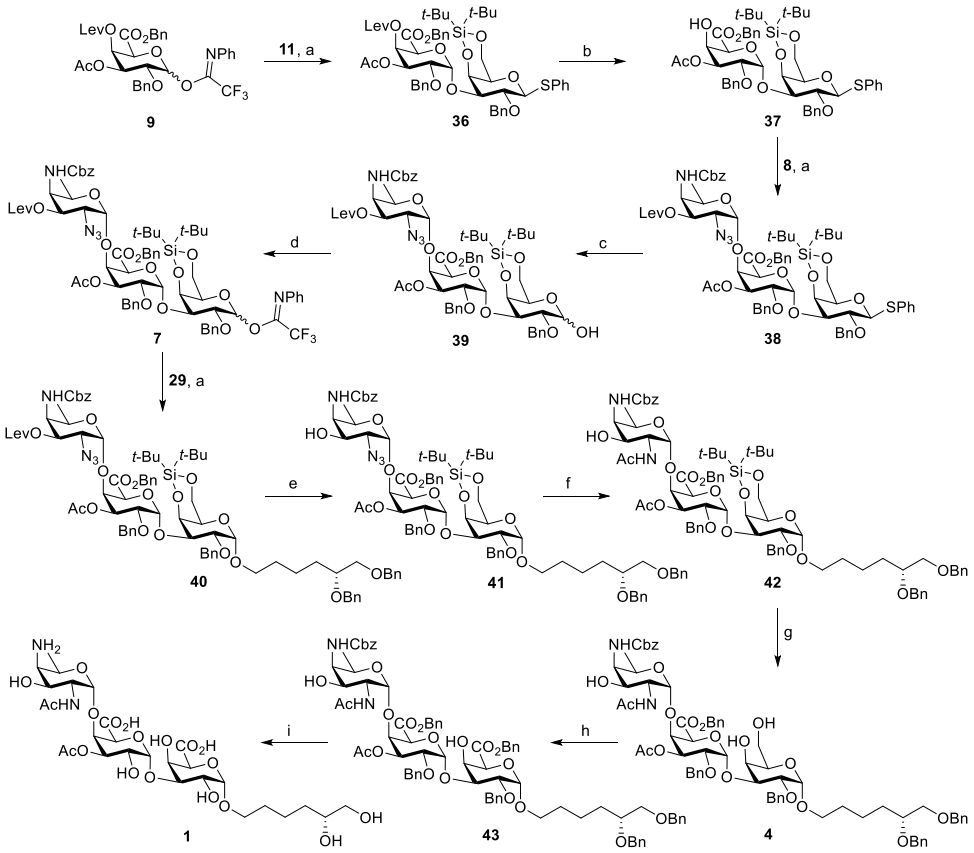
followed by delevulination by treatment with N_2H_4•H_2O in excellent yield. Next, the disaccharide acceptor **33** (*AB*) was investigated in the model glycosylations with the monosaccharide galactoside donor **9** (*B*) and a disaccharide donor **27** (*BC*). The stereoselectivity of [*B* + *AB*] glycosylation was excellent, and only α trisaccharide product **34** (*BAB*) was obtained. However, the model [*BC* + *AB*] glycosylation provided the tetrasaccharide **35** (*BCAB*) with very poor stereoselectivity (α/β = 2/1). Apparently, the functional group at the C-3-OH of the galacturonic acid donor plays a crucial role in determining the glycosylation stereoselectivity. The last model reaction clearly indicates a great risk to obtain the wanted stereoselectivity for the glycosylations to be used following route (b) for the synthesis of hexa- and nonasaccharides.



Scheme 3. The model glycosylation reactions.

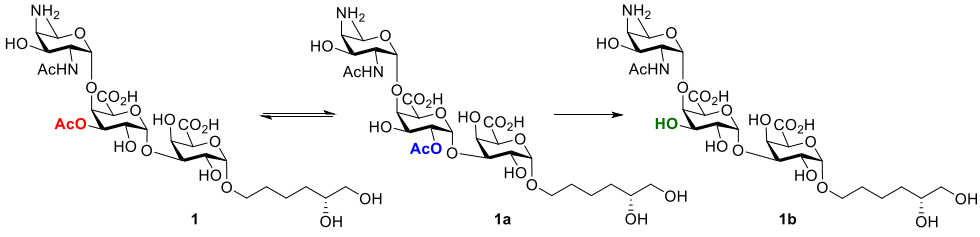
Reagents and conditions: a) TfOH, DCM, 5Å MS, 0 °C, **26**, 34%; **34**, 55% (α only); **35**, 52% (α/β = 2:1). b) i, NIS, TFA, DCM, 0 °C, 90%. ii, *N*-phenyltrifluoroacetimidoyl chloride, Cs_2CO_3, acetone, 91%. c) **29**, TBSOTf, DCM, 4Å MS, 0 °C, 94%. d) HF•Py, THF, pyridine, 0 °C, 85%. e) i, TEMPO, BAIB, *t*-BuOH, H_2O, DCM, 4 °C; ii, BnBr, Cs_2CO_3, DMF, 96% (over two steps). f) TBSOTf, DCM, 4Å MS, 0 °C, 83% (α/β = 9:1). g) N_2H_4•H_2O, pyridine, AcOH, 92%.

Because of the model experiments indicated route (b) to be challenging, attention was next focused on route (a), realizing that a difficult oxidation step was to be overcome. The synthesis commenced with the assembly of the trisaccharide target **1** (Scheme 4). First, the glycosylation between the imidate donor **9** and the acceptor **11** was performed in the presence of TBSOTf to provide the disaccharide **36** in 75% yield with excellent stereoselectivity. Subsequently, selective deprotection of the levulinoyl protecting group was affected by treatment with hydrazine acetate under acidic conditions to inhibit possible migration of the C-3-OAc, delivered the C4'-OH disaccharide **37** in 95% yield. Next, the glycosylation between acceptor **37** and the 6-deoxy-D-galactose analogue **8** was carried out under the promotion of TBSOTf to provide **38**/**38a** in 82% yield and a 5/1 α/β-ratio. The anomers could be separated at this stage and the synthesis was continued with the hydrolysis of the thioglycoside using the NIS-TFA system[15], to provide the corresponding hemiacetal **39**, which was followed by installation the imidate moiety in Cs_2CO_3 condition to provide the pivotal trisaccharide imidate donor **7**. As a spacer entity, (*R*)-5,6-bis(benzyloxy)hexan-1-ol **29** was selected, which was stereoselectively prepared according to literature procedures.[16] Taking advantage of the stereoselectivity controlled by the bulky silylidene, the glycosylation between the key trisaccharide donor **7** and this linker proceeded smoothly to furnish **40** in 85% yield, with complete stereoselectivity. Subsequently, hydrolysis of the levulinoyl group was performed using hydrazine monohydrate to provide the trisaccharide **41** in excellent yield. Reduction of the azido group using a Staudinger reaction, followed by selective *N*-acetylation provided the acetamide **42** in quantitative yield. Triol intermediate **4** could be prepared in 95% yield from **42** by treatment with hydrogen fluoride in pyridine. Regioselective oxidation to provide the carboxylic moiety was achieved using the TEMPO-BAIB oxidation system in a *tert*-butanol-DCM-water solution at 4 °C, which was followed by benzylation using BnBr in DMF to provide trisaccharide **43** in 81% yield. To complete the synthesis of the trisaccharide target **1**, all benzyl ethers, the benzyl esters and the benzyl carbamate were removed via a hydrogenation using $Pd(OH)_2$ as catalyst. After purification by gel filtration column, however, it was observed that the product was impure and NMR indicated the presence of side-products, in which the acetyl group had migrated (**1a**) and was hydrolyzed (**1b**) (see Scheme 5).[4c] Presumably, the acetyl group in the trisaccharide is very labile and cannot withstand the slightly basic conditions used for the gel filtration.[17] Therefore, the purity of the trisaccharide was checked, immediately after the hydrogenation reaction. After filtration of the catalyst and concentration, the trisaccharide proved to be pure and no sign of acetyl migration was detected by NMR spectroscopy.



Scheme 4. Assembly of the trisaccharide **1**.

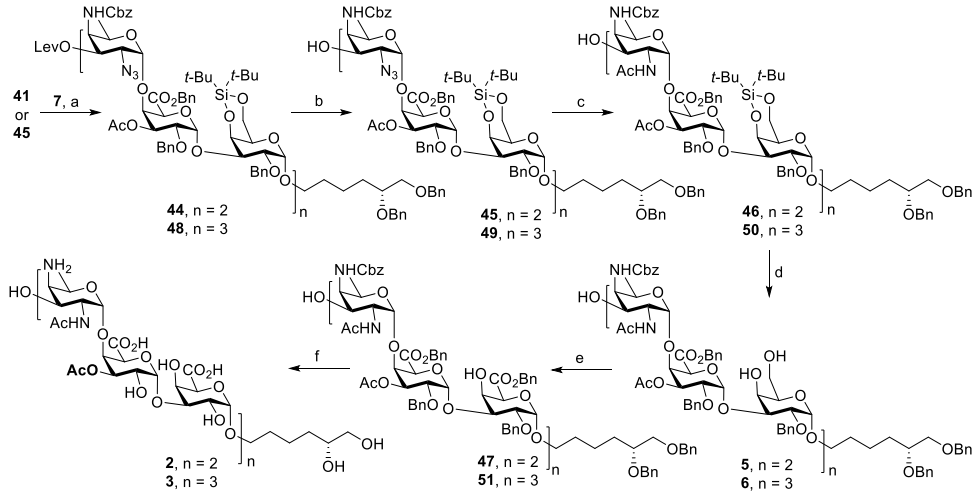
Reagents and conditions: a) TBSOTf, DCM, 5Å MS, 0 °C, **36**, 75%; **38**, 68% (β anomer **38a**, 14%); **40**, 85%. b) N_2H_4•AcOH, AcOH, THF, MeOH, 0 °C, 95%. c) NIS, TFA, DCM, 0 °C, 95%. d) *N*-phenyltrifluoroacetimidoyl chloride, Cs_2CO_3, acetone, 93%. e) N_2H_4•H_2O, pyridine, AcOH, 0 °C - RT, 95%. f) i, PPh_3, pyridine, H_2O, THF, 70 °C, 7 h; ii, Ac_2O, $NaHCO_3$, THF, H_2O, quantitative. g) HF•Py, THF, pyridine, 0 °C, 94%. h) i, TEMPO, BAIB, *t*-BuOH, H_2O, DCM, 4 °C; ii, BnBr, Cs_2CO_3, DMF, 81% (over two steps). i) $Pd(OH)_2$/C, H_2, *t*-BuOH, H_2O, 3 days, quantitative.



Scheme 5. Acetyl migration and hydrolysis in trisaccharide **1**.

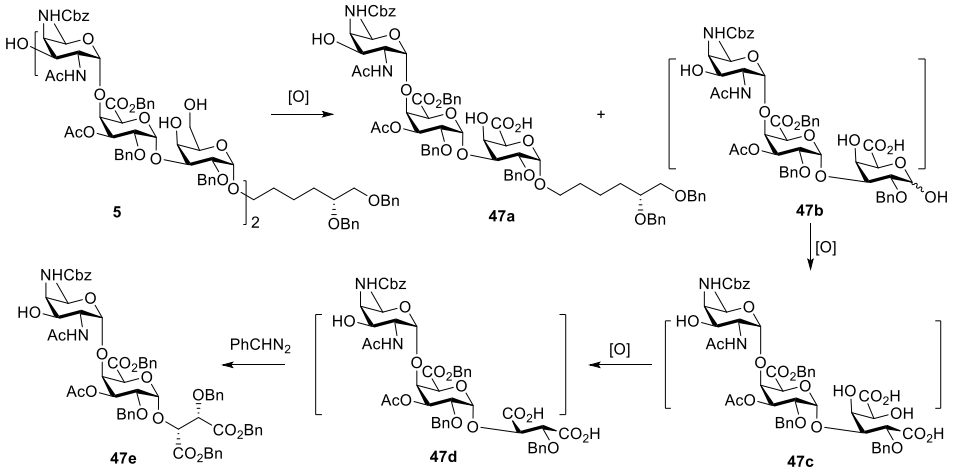
With the trisaccharide imidate donor **7** and acceptor **41** in hand, hexasaccharide **44** was synthesized via a [3 + 3] glycosylation mediated by TBSOTf in 83% yield as a single diastereoisomer (Scheme 6). Removal of the levulinoyl group using hydrazine monohydrate, as described for the synthesis of **41**, furnished hexasaccharide **45**. Subsequently, a [3 + 6] glycosylation and delevulination cycle was carried to provide the nonasaccharide **49**. Following the synthetic approach for trisaccharide target **1**, a similar functionalization and deprotection sequence were performed with hexasaccharide **45** and nonasaccharide **49** to provide the partially protected hexasaccharide **5** and nonasaccharide **6**, respectively. Previously it was shown that the regioselective oxidation of multiple primary alcohols in the longer oligosaccharide became increasingly difficult with increasing substrate length. The oxidation conditions, as previously optimized,[8g] were initially applied on hexasaccharide **5**, containing five free hydroxyls. Unfortunately, these conditions proved to be incompatible for the oxidation of **5**, and a major side-product was isolated. The structure of this compound was established using NMR and HRMS analysis and proved to be truncated disaccharide **47e**. A possible mechanism for the formation of **47e** is shown in Scheme 7. [18] Apparently, the oxidative conditions led to cleavage of the glycosydic bond at the junction of the trisaccharide repeating units. This would lead to two trisaccharide fragments **47a**, and **47b**. Subsequently, the hemiacetal **47b** can undergo a further oxidation to the di-acid intermediate **47c**, the diol of which can be oxidatively cleaved to provide, after another oxidation, di-acid **47d**, which upon benzylation provided **47e**. The formation of this side product indicated that the long time (3 days) used for the oxidation and the large excess of oxidants were too harsh for the substrate. Therefore, shorter reaction times were explored. The reaction was therefore monitored from 10 hours to 3 days, by thin-layer chromatography, indicating 24h to be optimal for the conversion of **5** into **47**. Thus, hexasaccharide **47** could be obtained in 58% yield after a reaction with the TEMPO/BAIB reagent combination for 24 hours at 4 ℃ in *t*-BuOH-water-EtOAc solution, followed by a benzylation using phenyldiazomethane. Similarly, the three primary alcohols of the nonasaccharide **6** were oxidized and benzylated to provide the corresponding nonasaccharide **51** in 66% yield over two steps. The cleavage of the nonasaccharide could not be completely prevented as LC-MS analysis of the reaction, which indicated the formation of trisaccharides and hexasaccharides, including **43** and **47**. The formation of these side products confirms the regioselectivity of the cleavage reactions, taking place at the anomeric center of the galactose residues that have to be oxidized. Unexpectedly, the deprotection procedure used for trisaccharide **1** was not suitable for the hexasaccharide, as products of acetyl migration and hydrolysis were detected by NMR and LC-MS. After various

optimizations of the solvent and reaction pH, the global deprotection was accomplished by performing the hydrogenation under mild acidic conditions to provide the target hexasaccharide **2** in 91% and nonasaccharide **3** in 90% yield (Figure 2).



Scheme 6. Assembly of the larger targets **2** and **3**.

Reagents and conditions: a) TBSOTf, DCM, 5Å MS, 0 °C, **44**, 83%; **48**, 85%. b) N_2H_4•H_2O, pyridine, AcOH, 0 °C - RT, **45**, 94%; **49**, 89%. c) i, PPh_3, pyridine, H_2O, THF, 70 °C, 7 h; ii, Ac_2O, $NaHCO_3$, THF, H_2O, **46**, 88%; **50**, 99%. d) HF•Py, THF, pyridine, 0 °C, **5**, 92%; **6**, 96%. e) i, TEMPO, BAIB, *t*-BuOH, H_2O, EtOAc or MeCN, 4 °C, 1d; ii, $PhCHN_2$, DCM, **47**, 58%; **51**, 66% (over two steps). f) $Pd(OH)_2$/C, H_2, *t*-BuOH, 0.1% AcOH in H_2O, 3 days, **2**, 91%; **3**, 90%.



Scheme 7. The possible mechanism for the formation of **47e**.

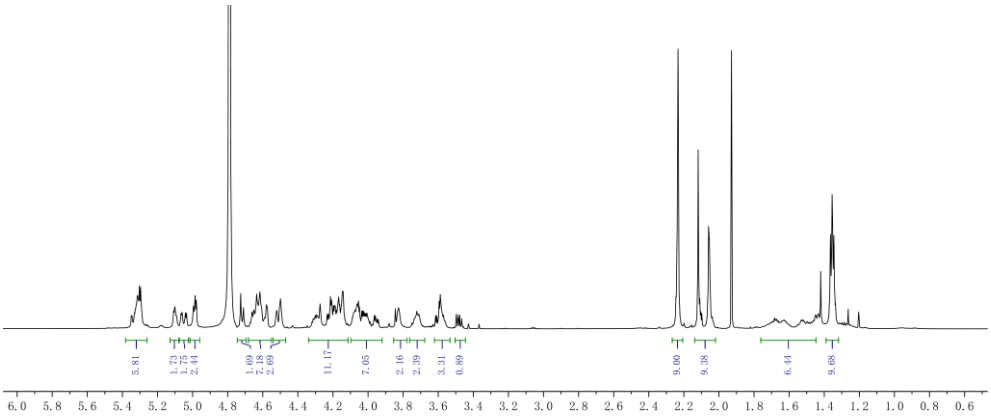


Figure 2. ^{1}H NMR spectrum of synthesized target nonasaccharide **3** in D_2O.

The syntheses of the acetylated oligomers clearly indicated the C-3-*O*-acetyl to be labile. To evaluate the stability of the acetyl group more accurately, a set of NMR experiments was set up. NMR analyses were performed on samples of **1** in D_2O phosphate buffer at different pD values (pD = pH + 0.4), ranging from pD 5.0 to pD 8.0. As shown in Figure 3 and 4, the acetyl group can migrate and hydrolyze from **1** to **1a** and **1b** under slightly basic conditions (pD = 8.0), and unexpectedly, migration also occurred, albeit sluggishly, at pD 7.0, which indicates that the acetyl may be labile also under very mild acidic conditions (pH = 6.6). At pD = 8.0 the 3-OAc ↔ 2-OAc migration was fast (Figure 4), with more than 50% of the 3-OAc migrating in the first 15 days. Recently a detailed acetyl migration study was performed on a β-mannosyl trisaccharide, carrying a C-3-*O*-acetyl. In this mannose system migration occurred much faster, because of the *cis*-relationship of the substituents at the C-2 and C-3 position of the mannose ring.[17] After 380 days the ratio of **1**, **1a** and **1b** was approximately 5:5:90. At lower pD (pD = 5.0 and 6.0) no migration or hydrolysis was observed over a period as long as 380 days. The stability studies underpinned the requirement for slightly acidic condition during the deprotection of the oligosaccharides. They also indicate that care should be taken when interaction studies are performed with these synthetic fragments or isolated Sp1-polysaccharides.

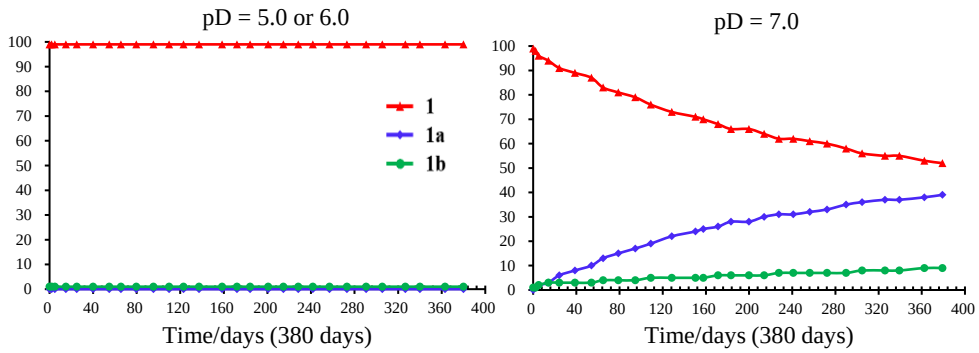


Figure 3. The *O*-Ac migration and hydrolysis of trisaccharide **1** in pD = 5, 6 and 7.

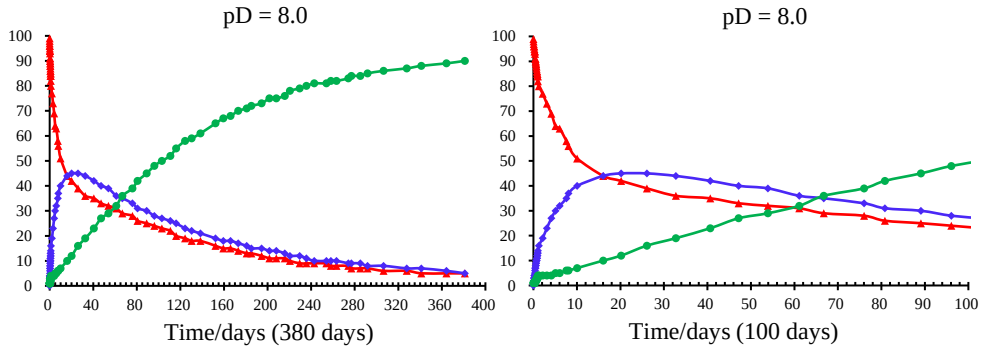


Figure 4. The *O*-Ac migration and hydrolysis of trisaccharide **1** in pD = 8.

Conclusion

In conclusion, three acetylated Sp1 fragments, a trisaccharide, hexasaccharide and nonasaccharide, were successfully assembled building on the previously developed synthesis approach, which strategically combined pre- and post-glycosylation oxidation events. The target molecules each contain a novel spacer, carrying a vicinal diol at its terminus, for future conjugation purposes. An alternative strategy that was devised to assemble the target oligosaccharides, hinging on the use of building blocks with the galacturonic acid moieties pre-installed (*i.e.* the use of a pre-glycosylation oxidation approach) was abandoned in an early stage as model experiments indicated that the glycosylation reactions proceeded with poor stereoselectivity. The regioselective oxidation of multiple primary alcohols in the complex oligosaccharide was accomplished using a modified TEMPO-BAIB oxidation. The formation of over-oxidized side products indicated the need to closely monitor the progress of the reactions. The pure 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 acetylated Sp1 fragments will be investigated employing molecular dynamics simulations and NMR spectroscopy to evaluate the role of the acetyl on the 3-D structure of these oligomers. Binding studies using the ELISA and STD NMR experiments will reveal the role of the acetyl groups in the interaction with anti-Sp1 antibodies.

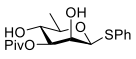
Experimental Section

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.

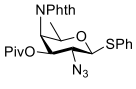
Experimental Procedures and Characterization Data of Products

Phenyl 3-*O*-pivaloyl-6-deoxy-1-thio-β-D-mannopyranoside (21)



Phenyl 6-deoxy-1-thio-β-D-mannopyranoside **16**[14b] (100 mg, 0.39 mmol, 1.0 eq) was dissolved in dry THF (2 mL). DIPEA (134 µL, 0.78 mmol, 2.0 eq), Me_2SnCl_2 (4.3 mg, 0.02 mmol, 0.05 eq) were added and stirred for 15 min. Then pivaloyl chloride (PivCl) (55 mg, 0.46 mmol, 1.1 eq) was added and the reaction was stirred at rt for 1 hour. After TLC showed complete consumption of the starting material, the reaction was quenched with 3% HCl solution and washed with H_2O (2x), brine. The organic phase was dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (DCM/Acetone 20:1 - 10:1) to yield compound **21** (118 mg, 0.35 mmol, 89%). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.49 – 7.42 (m, 2H), 7.31 – 7.21 (m, 3H), 4.91 (d, *J* = 1.1 Hz, 1H, H-1), 4.78 (dd, *J* = 9.8, 3.2 Hz, 1H, H-3), 4.30 – 4.24 (m, 1H, H-2), 3.71 (t, *J* = 9.5 Hz, 1H, H-4), 3.49 – 3.38 (m, 1H, H-5), 2.81 (s, 1H), 1.39 (d, *J* = 6.1 Hz, 3H, H-6), 1.23 (s, 9H, Piv). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.96 (Piv), 134.26, 131.17, 129.06, 127.53, 87.01 (C-1), 76.89 (C-5), 76.62 (C-3), 71.11 (C-2), 70.70 (C-4), 39.10, 27.14, 17.94 (C-6). HR-MS: Calculated for $C_{17}H_{24}O_5S$ $[M+Na]^+$: 363.12367, found: 363.12347. $[\alpha]^{20}_D$ = - 48.1° (c = 1, $CHCl_3$). TLC: Rf = 0.6 (DCM/Acetone = 10/1, v/v).

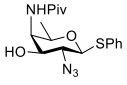
Phenyl 2-azido-3-*O*-pivaloyl-4-*N*-phthaloyl-6-deoxy-1-thio-β-D-galactopyranoside (22)



The compound **21** (115 mg, 0.338 mmol, 1.0 eq) was dissolved in DCM (6 ml) with pyridine (0.36 mL, 4.4 mmol, 13.0 eq), then Tf_2O (0.35 mL, 2.0 mmol, 6.0 eq) was added to the reaction mixture at -10 °C, and slowly warm up to 10 °C 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

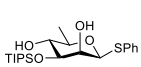
dissolved in dry CH_3CN (5 mL), $TBAN_3$ (96 mg, 0.338 mmol, 1.0 eq) solution in CH_3CN (1 mL) was slowly added to the reaction mixture at -30 ℃. The reaction was allowed to stir for 2 d at same temperature and then concentrated *in vacuo* under nitrogen. The residue was dissolved in DMF (2 mL), and then phthalimide potassium (135 mg, 0.73 mmol, 2.1 eq) was added to the reaction mixture and stirred for overnight at room temperature. The reaction mixture was diluted with EtOAc and washed with water and brine and then dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by column chromatography (PE/EA, 20:1 – 10:1) to yield compound **22** (61.4 mg, 0.124 mmol, 37%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.94 – 7.83 (m, 2H), 7.82 – 7.72 (m, 3H), 7.67 – 7.58 (m, 2H), 7.40 – 7.29 (m, 2H), 5.14 (dd, *J* = 9.2, 7.0 Hz, 1H, H-3), 4.88 (dd, *J* = 7.0, 2.9 Hz, 1H, H-4), 4.75 – 4.60 (m, 2H, H-2, H-1), 4.01 – 3.88 (m, 1H, H-5), 1.17 (d, *J* = 6.4 Hz, 3H, H-6), 0.98 (s, 9H, Piv). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.90 (Piv), 134.45, 133.63, 132.33, 129.09, 128.01, 123.73, 88.71 (C-1), 73.29 (C-5), 72.40 (C-3), 62.10 (C-2), 51.27 (C-4), 38.74, 26.82, 16.99 (C-6). HR-MS: Calculated for $C_{25}H_{26}N_4O_5S$ $[M+Na]^+$: 517.15161, found: 517.15180. $[\alpha]^{20}_D$ = + 62.9° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 4/1, v/v).

Phenyl 2-azido-4-*N*-pivaloyl-6-deoxy-1-thio-β-D-galactopyranoside (23)



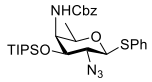
The compound **22** (31 mg, 0.06 mmol, 1.0 eq) was dissolved in butanol (3 mL) with ethylenediamine (0.3 mL), the reaction mixture was refluxed for 24 h. The reaction mixture concentrated *in vacuo*. The product was purified by column chromatography (DCM/MeOH 100:1 - 20:1) to yield product **23** (10 mg, 0.0274 mmol, 44%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 – 7.57 (m, 2H), 7.41 – 7.31 (m, 3H), 5.66 (d, *J* = 8.0 Hz, 1H, N*H*Piv), 4.37 (d, *J* = 10.2 Hz, 1H, H-1), 4.21 – 4.13 (m, 1H, H-4), 3.86 – 3.75 (m, 2H, H-5, H-3), 3.08 (t, *J* = 9.8 Hz, 1H, H-2), 2.36 (s, 1H), 1.24 (d, *J* = 6.7 Hz, 3H, H-6), 1.12 (s, 9H, Piv). ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.94 (Piv), 135.07, 129.29, 129.17, 129.15, 85.47 (C-1), 75.68 (C-3), 73.74 (C-5), 62.68 (C-2), 53.56 (C-4), 39.10, 27.59, 17.31 (C-6). $[\alpha]^{20}_D$ = - 34.0° (c = 0.1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 4/1, v/v).

Phenyl 6-deoxy-3-*O*-triisopropylsilyl-1-thio-β-D-mannopyranoside (24)

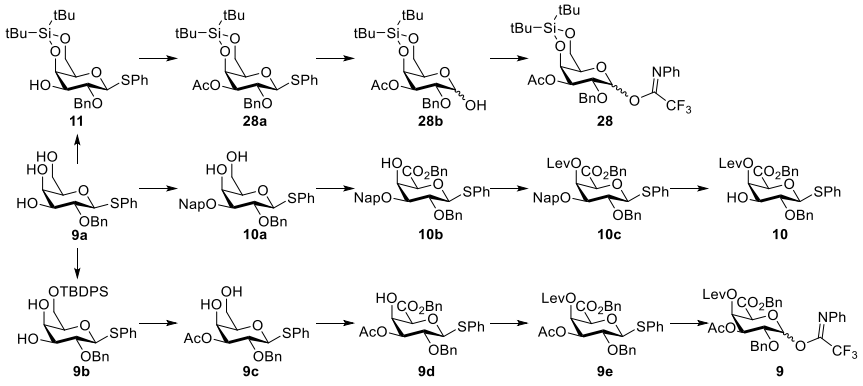


Compound phenyl 2-*O*-benzyl-1-thio-β-D-galactopyranoside **16**[14b] (19.7 g, 76.9 mmol, 1.0 eq) was dissolved in DMF (154 mL) and cooled to 0 ℃. Triisopropylsilyl chloride (TIPSCl) (33 mL, 153.8 mmol, 2.0 eq) and imidazole (31 g, 455 mmol, 6.0 eq) were added at 0 ℃. It was stirred at RT for 24 hours and checked by TLC. After completed 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*. The compound was purified by flash chromatography (PE/EA 20:1 - 8:1) to yield compound **24** (28 g, 67.9 mmol, 88%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.55 – 7.49 (m, 2H), 7.34 – 7.23 (m, 3H), 4.89 – 4.81 (m, 1H, H-1), 4.16 – 4.08 (m, 1H, H-2), 3.74 (dd, *J* = 8.8, 3.5 Hz, 1H, H-3), 3.63 – 3.54 (m, 1H, H-4), 3.41 – 3.29 (m, 1H, H-5), 2.66 (t, *J* = 1.8 Hz, 1H, 2-OH), 2.08 (d, *J* = 3.4 Hz, 1H, 4-OH), 1.41 (d, *J* = 6.1 Hz, 3H, H-6), 1.17 – 1.07 (m, 21H, TIPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 130.86, 129.01, 127.31, 86.39 (C-1), 76.62 (C-3), 75.94 (C-5), 73.34, 73.33 (C-2, C-4), 18.15, 18.14, 18.05 (C-6), 12.56. HR-MS: Calculated for $C_{21}H_{36}O_4SSi$ $[M+Na]^+$: 435.19958, found: 435.19957. $[\alpha]^{20}_D$ = - 40.0° (c = 1, $CHCl_3$). TLC: Rf = 0.5 (PE/EA = 4/1, v/v).

Phenyl 2-azido-4-*N*-benzyloxycarbonyl-6-deoxy-3-*O*-triisopropylsilyl-1-thio-β-D-galactopyranoside (25)

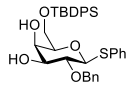


The compound **24** (1.57 g, 3.81 mmol, 1.0 eq) was dissolved in DCM (54 ml) with pyridine (4 mL, 50 mmol, 13.0 eq), then Tf_2O (3.8 mL, 22.9 mmol, 6.0 eq) was added to the reaction mixture at -10 ℃, and slowly warm up to 10 ℃ in 2 h. After TLC showed complete consumption of the starting material, the 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 (50 mL), $TBAN_3$ (1.11 g, 3.90 mmol, 1.02 eq) solution in CH_3CN (5 mL) was slowly added to the reaction mixture at -30 ℃ and stirred for 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 (10 mL) was added in -20℃. The reaction was slowly warmed to 5 ℃ and stirred for 3 days. After TLC showed complete consumption of the starting material, the mixture was concentrated *in vacuo*. The residue was dissolved in THF (28 mL) and water (19 mL), and then sodium bicarbonate (1.28 g, 15.2 mmol, 4.0 eq) was added and cooled to 0 ℃. After benzyl chloroformate (CbzCl) (1.1 mL, 7.6 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 compound was purified by flash chromatography (PE/EA 5:1 - 3:1) to yield compound **25** (3.0 mg, 3.0 mmol, 75%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 – 7.53 (m, 2H), 7.40 – 7.26 (m, 8H), 5.08 (q, *J* = 12.2 Hz, 2H, Cbz), 4.79 (d, *J* = 10.0 Hz, 1H, N*H*Cbz), 4.42 (d, *J* = 10.2 Hz, 1H, H-1), 4.01 – 3.93 (m, 1H, H-4), 3.77 (dd, *J* = 9.5, 4.4 Hz, 1H, H-3), 3.63 – 3.53 (m, 1H, H-5), 3.09 (t, *J* = 9.8 Hz, 1H, H-2), 1.24 (d, *J* = 6.3 Hz, 3H, H-6), 1.15 – 1.00 (m, 21H, TIPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.76 (Cbz), 133.08, 129.19, 128.57, 128.47, 128.21, 128.16, 87.37 (C-1), 74.64 (C-5), 73.86 (C-3), 67.01 (Cbz), 64.73 (C-2), 55.62 (C-4), 18.05, 18.02, 17.22 (C-6), 12.78. HR-MS: Calculated for $C_{29}H_{42}N_4O_4SSi$ [M+H$^+$]: 571.27688, found: 571.27703. $[\alpha]^{20}_D$ = + 0.6° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 20/1, v/v).



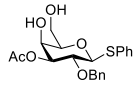
Scheme I. The synthesis of building blocks of galactose.

Phenyl 2-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (9b)



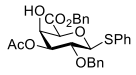
Compound phenyl 2-*O*-benzyl-1-thio-β-D-galactopyranoside **9a**[8g] (16.4 g, 45.3 mmol, 1.0 eq) was dissolved in DMF (91 mL) and cooled to 0 °C. *tert*-Butyl(chloro)diphenylsilane (TBDPSCl) (14.2 mL, 54.5 mmol, 1.2 eq) and imidazole (4.7 g, 69.0 mmol, 1.5 eq) were added at 0 °C. It was stirred at RT for 4 hours and checked by TLC. After completed 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*. The compound was purified by flash chromatography (PE/EA 6:1 - 3:1) to yield compound **9b** (26 g, 43.3 mmol, 96%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.76 – 7.64 (m, 4H), 7.59 – 7.51 (m, 2H), 7.46 – 7.16 (m, 14H), 4.89 (d, *J* = 10.9 Hz, 1H, C*H*$_2$), 4.69 (d, *J* = 10.9 Hz, 1H, C*H*$_2$), 4.64 – 4.55 (m, 1H, H-1), 4.06 – 4.01 (m, 1H, H-4), 3.98 – 3.87 (m, 2H, H-6), 3.66 – 3.57 (m, 2H, H-3, H-2), 3.49 – 3.42 (m, 1H, H-5), 2.74 (s, 1H), 1.06 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.16, 135.65, 135.57, 134.21, 132.84, 132.66, 131.33, 129.90, 128.92, 128.52, 128.25, 127.98, 127.83, 127.82, 127.22, 87.59 (C-1), 78.10 (C-3), 77.84 (C-5), 75.35, 75.23 (C-2), 69.60 (C-4), 63.88 (C-6), 26.80, 19.14. HR-MS: Calculated for $C_{35}H_{40}O_5SSi$ [M+Na$^+$]: 623.2258, found: 623.2256. $[\alpha]^{20}_D$ = + 6.8° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 3/1, v/v).

Phenyl 2-*O*-benzyl-3-*O*-acetyl-1-thio-β-D-galactopyranoside (9c)



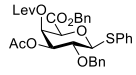
Compound **9b** (2.5 g, 4.24 mmol, 1.0 eq) was dissolved in dry THF (22 mL). DIPEA (1.5 mL, 8.5 mmol, 2.0 eq), Me_2SnCl_2 (50 mg, 0.23 mmol, 0.05 eq) were added and stirred for 15 min. Then acetyl chloride (362 µL, 5.07 mmol, 1.2 eq) was added and the reaction was stirred at rt for overnight. After TLC showed complete consumption of the starting material, the reaction was quenched with 3% HCl solution and washed with H_2O (2x), brine. The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in THF (30 mL) and pyridine (30 mL), then cooled to 0 °C and hydrogen fluoride (HF)/pyridine (70%) (3 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 (DCM/Acetone 20:1 - 5:1) to yield compound **9c** (1.5 g, 3.6 mmol, 85%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 – 7.52 (m, 2H), 7.37 – 7.23 (m, 8H), 4.92 (dd, *J* = 9.5, 3.1 Hz, 1H, H-3), 4.86 (d, *J* = 10.9 Hz, 1H, C*H*$_2$), 4.73 (d, *J* = 9.7 Hz, 1H, H-1), 4.58 (d, *J* = 11.0 Hz, 1H, C*H*$_2$), 4.19 (dd, *J* = 3.1, 1.0 Hz, 1H, H-4), 3.92 – 3.80 (m, 3H, H-2, H-6), 3.59 – 3.52 (m, 1H, H-5), 2.85 (s, 2H), 2.02 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.41 (OAc), 137.99, 133.49, 131.76, 129.19, 128.50, 128.03, 127.96, 127.77, 88.02 (C-1), 77.42 (C-5), 76.78 (C-3), 75.64 (*C*H$_2$), 75.42 (C-2), 68.77 (C-4), 62.98 (C-6), 21.10 (OAc). HR-MS: Calculated for $C_{21}H_{24}O_6S$ [M+Na$^+$]: 427.1186, found: 427.1185. $[\alpha]^{20}_D$ = +21.1° (c = 1, $CHCl_3$). TLC: Rf = 0.5 (DCM/Acetone = 4/1, v/v).

Benzyl phenyl 3-*O*-acetyl-2-*O*-benzyl-1-thio-β-D-galactopyranosyl uronate (9d)



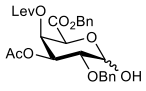
Compound **9c** (7.8 g, 19.3 mmol, 1.0 eq) was dissolved in DCM/*tert*-BuOH/H_2O (146 mL, 4/4/1, v/v/v). The mixture was cooled to 0 ℃ and treated with TEMPO (608 mg, 3.9 mmol, 0.2 eq) and BAIB (16 g, 48.2 mmol, 2.5 eq). After stirring for overnight at 4 ℃ and TLC showed complete consumption of the starting material, saturated aqueous sodium thiosulphate was added and diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DMF (77 mL), followed by addition of Cs_2CO_3 (6.4 g, 19.6 mmol, 1.0 eq) and BnBr (4.6 mL, 38.5 mmol, 2.0 eq) at 0ºC. The mixture was allowed to stir overnight at rt, and then diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (silica gel, pentane/DCM/EA, 7/2/1) yielded **9d** (7.6 g, 14.9 mmol, 77%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.68 – 7.60 (m, 2H), 7.41 – 7.21 (m, 13H), 5.28 – 5.19 (m, 2H, CH_2), 4.97 (dd, *J* = 9.6, 3.1 Hz, 1H, H-3), 4.88 (d, *J* = 11.0 Hz, 1H, CH_2), 4.67 (d, *J* = 9.7 Hz, 1H, H-1), 4.59 (d, *J* = 11.0 Hz, 1H, CH_2), 4.44 (dd, *J* = 3.1, 1.2 Hz, 1H, H-4), 4.19 (d, *J* = 1.2 Hz, 1H, H-5), 3.81 (t, *J* = 9.6 Hz, 1H, H-2), 2.23 (s, 1H), 2.01 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.10 (OAc), 167.21, 137.87, 135.06, 133.24, 132.63, 129.06, 128.72, 128.66, 128.64, 128.51, 128.44, 128.24, 128.04, 128.00, 87.99 (C-1), 76.84 (C-5), 75.82 (C-3), 75.67 (*C*H_2), 74.96 (C-2), 68.54 (C-4), 67.50 (*C*H_2), 21.00 (OAc). HR-MS: Calculated for $C_{28}H_{28}O_7S$ [M+Na^+]: 531.1448, found: 531.1448. $[\alpha]^{20}_D$ = + 4.5º (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/DCM/EA = 7/2/1, v/v/v).

Benzyl phenyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-1-thio-β-D-galactopyranosyl uronate (9e)



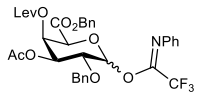
Compound **9d** (1.05 g, 2.1 mmol, 1.0 eq) was co-evaporated with anhydrous toluene three times under nitrogen and dissolved in DCM (20 mL). Reduced to 0 ℃, levulinic acid (668 mg, 5.8 mmol, 2.8 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (640 mg, 4.1 mmol, 2.0 eq) and 4-dimethylaminopyridine (DMAP) (50 mg, 0.41 mmol, 0.2 eq) were added. The reaction was stirred for 2 days. 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 (Tol/EA 20:1 - 10:1) to yield compound **9e** (1.21 g, 2.0 mmol, 99%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.72 – 7.62 (m, 2H), 7.41 – 7.23 (m, 13H), 5.76 – 5.71 (m, 1H, H-4), 5.23 (d, *J* = 11.9 Hz, 1H, CH_2), 5.12 (d, *J* = 11.9 Hz, 1H, CH_2), 5.02 (dd, *J* = 9.6, 3.4 Hz, 1H, H-3), 4.85 (d, *J* = 10.9 Hz, 1H, CH_2), 4.69 (d, *J* = 9.7 Hz, 1H, CH_2), 4.59 (d, *J* = 10.9 Hz, 1H, CH_2), 4.28 (d, *J* = 1.4 Hz, 1H, H-5), 3.70 (t, *J* = 9.7 Hz, 1H, H-2), 2.64 – 2.44 (m, 3H, Lev), 2.34 – 2.23 (m, 1H, Lev), 2.16 (s, 3H, Lev), 1.92 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.12 (Lev), 171.49 (Lev), 170.15 (OAc), 165.87 (CO_2Bn), 137.88, 135.14, 133.33, 132.78, 129.11, 129.04, 128.76, 128.74, 128.70, 128.52, 128.27, 128.22, 128.04, 128.03, 87.93 (C-1), 75.72, 75.37 (C-5), 74.84 (C-2), 73.85 (C-3), 68.95 (C-4), 67.60 (CO_2Bn), 37.72, 29.98 (Lev), 27.68, 20.69 (OAc). HR-MS: Calculated for $C_{33}H_{34}O_9S$ [M+Na^+]: 629.18157, found: 629.18103. $[\alpha]^{20}_D$ = + 4.6º (c = 1, $CHCl_3$). TLC: Rf = 0.2 (Tol/EA = 9/1, v/v).

Benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α/β-D-galactopyranosyl uronate (9f)



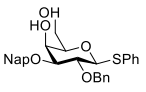
Compound **9e** (490 mg, 0.79 mmol, 1.0 eq) was dissolved in DCM (8 mL) and reduced to 0 °C. NIS (195 mg, 0.87 mmol, 1.1 eq) and TFA (67 µL, 0.87 mmol, 1.1 eq) were added and the solution stirred for 2 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). 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 the titled compound (390 mg, 0.76 mmol, 96%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.16 (m, 10H), 5.82 – 5.63 (m, 1H, H-4), 5.45 – 5.32 (m, 2H, H-1, H-3), 5.29 – 5.19 (m, 1H, CH_2), 5.13 – 5.00 (m, 1H, CH_2), 4.88 – 4.80 (m, 1H, H-5), 4.74 – 4.56 (m, 2H, CH_2), 3.93 – 3.65 (m, 2H, H-2), 2.66 – 2.35 (m, 3H, Lev), 2.31 – 2.09 (m, 4H, Lev), 2.01 – 1.89 (m, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.27, 206.19 (Lev), 171.52 (Lev), 170.35z, 170.33 (OAc), 167.42, 166.62 (CO_2Bn), 138.25, 137.69, 135.04, 134.92, 129.22, 128.73, 128.68, 128.65, 128.60, 128.40, 128.16, 128.01, 127.92, 127.82, 97.52, 91.95 (C-1), 77.01, 74.87, 73.51, 73.21 (C-2), 72.09, 71.92, 69.55 (C-4), 69.15 (C-3), 68.72, 68.53 (C-5), 67.78, 67.55 (C-6), 37.70, 37.68, 29.89, 27.58, 20.78 (OAc). HR-MS: Calculated for $C_{27}H_{30}O_{10}$ $[M+NH_4]^+$: 537.17312, found: 537.17302. TLC: Rf = 0.3 (PE/EA = 10/1, v/v).

Benzyl *N*-phenyl-trifluoroacetimidate 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α/β-D-galactopyranosyl uronate (9)



The corresponding hemiacetal (5.5 g, 10.7 mmol, 1.0 eq) was dissolved in acetone (110 mL) and cooled to 0 °C. Cesium carbonate (3.3 g, 12.9 mmol, 1.2 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (3.3 g, 15.9 mmol, 1.5 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by triethyl amine, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 5:1 – 2/1) to yield compound **9** (6.62 g, 9.66 mmol, 91%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.01 (m, 13H), 6.85 – 6.68 (m, 2H), 5.95 – 5.30 (m, 2H, H-4, H-3), 5.29 – 5.19 (m, 1H, CH_2), 5.15 – 4.88 (m, 2H, H-5, CH_2), 4.86 – 4.76 (m, 1H, CH_2), 4.73 – 4.64 (m, 1H, CH_2), 3.96 – 3.73 (m, 1H, H-2), 2.68 – 2.43 (m, 3H, Lev), 2.33 – 2.24 (m, 1H, Lev), 2.20 – 2.13 (m, 3H, Lev), 2.01 – 1.82 (m, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.07 (Lev), 171.39 (Lev), 170.11 (OAc), 165.26 (CO_2Bn), 137.44, 134.89, 129.31, 129.29, 128.86, 128.78, 128.74, 128.72, 128.70, 128.58, 128.56, 128.20, 128.12, 124.49, 119.36, 96.57 (C-1), 75.39, 74.91, 72.81, 71.89, 68.43, 67.79, 37.64, 29.91, 27.60, 20.61. HR-MS: Calculated for $C_{35}H_{34}F_3NO_{10}$ $[M+Na^+]$: 708.20270, found: 708.20297. TLC: Rf = 0.2 (PE/EA = 7/3, v/v).

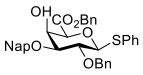
Phenyl 2-*O*-benzyl-3-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (10a)



The compound phenyl 2-*O*-benzyl-1-thio-β-D-galactopyranoside **9a**[8g] (4.47 g, 12.3 mmol, 1.0 eq) was dissolved in acetonitrile DCM (60 ml). Benzaldehyde dimethyl acetal (2.8 mL, 18.5 mmol, 1.5 eq) and *p*-Toluenesulfonic acid monohydrate (TsOH) (235 mg, 1.23 mmol, 0.1 eq) were added successively to the reaction mixture at RT. After stirred for overnight and TLC showed complete

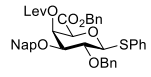
consumption of the starting material, the reaction mixture was quenched with triethyl amine and concentrated *in vacuo*. The intermediate was crystalized in ethanol and dissolved in dry DMF (35 mL). The reaction was cooled to 0 ℃, sodium hydride (1.0 g, 24.7 mmol, 2 eq) was added and stirred for 15 min. 2-(bromomethyl)naphthalene (4.1 g, 18.5 mmol, 1.5 eq) was added at 0 ℃. It was slowly wormed to RT and stirred for overnight. After analysis by TLC showed complete consumption of the starting material, quenched with MeOH and water. Diluted with EtOAc and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was dissolved in MeOH (150 mL) and DCM (150 mL). TsOH was added until the solution pH about 2. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched by triethylamine and concentrated *in vacuo*. The compound was purified by flash chromatography (DCM/Acetone 40:1 - 10:1) to yield compound **10a** (4.1, 8.2 mmol, 66%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.84 – 7.69 (m, 4H), 7.57 – 7.52 (m, 2H), 7.49 – 7.37 (m, 5H), 7.36 – 7.20 (m, 6H), 4.90 – 4.79 (m, 3H, CH_2), 4.76 (d, *J* = 10.3 Hz, 1H, CH_2), 4.64 (d, *J* = 9.8 Hz, 1H, H-1), 4.07 (d, *J* = 3.2 Hz, 1H, H-4), 3.94 (dd, *J* = 11.8, 6.6 Hz, 1H, H-6), 3.84 – 3.73 (m, 2H, H-6, H-2), 3.61 (dd, *J* = 8.9, 3.2 Hz, 1H, H-3), 3.49 – 3.41 (m, 1H, H-5), 2.80 (s, 1H, 4-OH), 2.37 (s, 1H, 6-OH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.21, 135.03, 133.79, 133.28, 133.18, 131.82, 129.09, 128.56, 128.52, 128.36, 128.02, 127.98, 127.83, 127.58, 126.91, 126.40, 126.27, 125.85, 87.72 (C-1), 82.31 (C-3), 78.07 (C-5), 77.10 (C-2), 75.89, 72.40, 67.50 (C-4), 62.80 (C-6). HR-MS: Calculated for $C_{30}H_{30}O_5S$ $[M+Na]^+$: 525.17062, found: 525.17051. $[\alpha]^{20}_D$ = + 5.3° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (DCM/Acetone = 10/1, v/v).

Benzyl phenyl 2-*O*-benzyl-3-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranosyl uronate (10b)



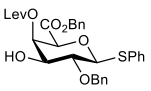
Compound **10a** (4.1 g, 8.2 mmol, 1.0 eq) was dissolved in DCM/*tert*-BuOH/H_2O (80 mL, 4/4/1, v/v/v). The mixture was cooled to 0 ℃ and treated with TEMPO (255 mg, 1.6 mmol, 0.2 eq) and BAIB (6.8 g, 20.4 mmol, 2.5 eq). After stirring for overnight at 4 ℃ and TLC showed complete consumption of the starting material, saturated aqueous sodium thiosulphate was added and diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DMF (32 mL), followed by addition of Cs_2CO_3 (4.0 g, 12.3 mmol, 1.5 eq) and BnBr (2.0 mL, 16.3 mmol, 2.0 eq) at 0 ℃. The mixture was allowed to stir overnight at rt, and then diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (silica gel, PE/EA, 5/1 – 3/1) yielded **10b** (2.9 g, 4.78 mmol, 59%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.86 – 7.76 (m, 2H), 7.76 – 7.69 (m, 2H), 7.68 – 7.61 (m, 2H), 7.51 – 7.30 (m, 13H), 7.28 – 7.19 (m, 3H), 5.27 (s, 2H, CO_2Bn), 4.93 – 4.74 (m, 4H, CH_2), 4.58 (d, *J* = 9.6 Hz, 1H, CH_2), 4.47 – 4.41 (m, 1H, H-4), 4.07 (d, *J* = 1.5 Hz, 1H, H-5), 3.77 (t, *J* = 9.3 Hz, 1H, H-2), 3.66 (dd, *J* = 8.9, 3.2 Hz, 1H, H-3), 2.50 (s, 1H, 4-OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.62 (CO_2Bn), 138.13, 135.38, 134.82, 133.36, 133.29, 133.23, 132.95, 129.00, 128.73, 128.60, 128.57, 128.44, 128.42, 128.10, 128.06, 128.04, 127.85, 127.00, 126.42, 126.30, 125.86, 87.73 (C-1), 81.70 (C-2), 76.96 (C-5), 76.54 (C-2), 75.96 (*C*H_2), 72.46 (Nap), 67.93 (C-4), 67.38 (CO_2Bn). HR-MS: Calculated for $C_{37}H_{34}O_6S$ $[M+Na]^+$: 629.19683, found: 629.19680. $[\alpha]^{20}_D$ = - 5.0° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/EA = 4/1, v/v).

Benzyl phenyl 2-*O*-benzyl-4-*O*-levulinoyl-3-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranosyl uronate (10c)



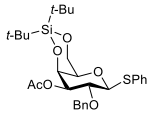
Compound **10b** (2.0 g, 3.3 mmol, 1.0 eq) was co-evaporated with anhydrous toluene three times under nitrogen and dissolved in DCM (33 mL). Reduced to 0 °C, levulinic acid (1.1 g, 9.5 mmol, 2.9 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (1.0 g, 6.4 mmol, 2.0 eq) and 4-dimethylaminopyridine (DMAP) (81 mg, 0.66 mmol, 0.2 eq) were added. The reaction was stirred for overnight. The reaction was diluted with DCM and washed with H_2O (2x), brine. The organic phase was dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (Tol/EA 50:1 - 30:1) to yield compound **10c** (2.1 g, 2.98 mmol, 90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.80 – 7.61 (m, 6H), 7.45 – 7.19 (m, 16H), 5.85 (dd, *J* = 3.1, 1.3 Hz, 1H, H-4), 5.19 (s, 2H, CH_2), 4.85 (d, *J* = 11.4 Hz, 1H, CH_2), 4.81 – 4.70 (m, 2H, CH_2), 4.63 – 4.55 (m, 2H, CH_2, H-1), 4.11 (d, *J* = 1.3 Hz, 1H, H-5), 3.73 – 3.57 (m, 2H, H-3, H-2), 2.65 – 2.43 (m, 4H, Lev), 2.08 (s, 3H, Lev). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.89 (Lev), 171.40 (Lev), 166.22 (CO_2Bn), 138.00, 135.01, 134.79, 133.14, 133.07, 132.88, 132.60, 128.85, 128.70, 128.49, 128.46, 128.26, 128.09, 128.04, 127.87, 127.81, 127.73, 127.53, 126.85, 125.99, 125.94, 125.85, 87.21 (C-1), 80.24 (C-3), 75.92 (C-2), 75.64, 75.40 (C-5), 71.78, 67.68 (C-4), 67.45 (C-6), 37.76, 29.65, 27.79 (Lev). HR-MS: Calculated for $C_{42}H_{40}O_8S$ [M+Na$^+$]: 727.23361, found: 727.23321. $[\alpha]^{20}_D$ = + 38.6° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (Tol/EA = 9/1, v/v).

Benzyl phenyl 2-*O*-benzyl-4-*O*-levulinoyl-3-*O*-acetyl-1-thio-β-D-galactopyranosyl uronate (10)



The compound **10c** (2.0 g, 2.98 mmol, 1.0 eq) was dissolved in DCM (60 mL) and water (6 mL). After cooled to 0 °C, 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (745 mg, 3.3 mmol, 1.1 eq) was added. The reaction was stirred at RT for 7 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched by saturated aqueous sodium thiosulphate, extracted with DCM, 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 5:1 – 2:1) to yield compound **10** (1.45 g, 2.6 mmol, 86%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.67 – 7.59 (m, 2H), 7.43 – 7.17 (m, 13H), 5.51 (dd, *J* = 3.4, 1.3 Hz, 1H, H-4), 5.12 (s, 2H, CO_2Bn), 4.80 (d, *J* = 10.6 Hz, 1H, CH_2), 4.71 (d, *J* = 10.6 Hz, 1H, CH_2), 4.53 (d, *J* = 9.6 Hz, 1H, H-1), 3.98 (d, *J* = 1.4 Hz, 1H, H-5), 3.75 (dd, *J* = 9.2, 3.4 Hz, 1H, H-3), 3.47 (t, *J* = 9.4 Hz, 1H, H-2), 3.27 (s, 1H), 2.61 – 2.52 (m, 2H, Lev), 2.49 – 2.34 (m, 2H, Lev), 2.08 (s, 3H, Lev). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.14 (Lev), 172.06 (Lev), 166.42 (*C*H_2), 138.11, 135.15, 133.00, 132.93, 128.88, 128.85, 128.57, 128.55, 128.44, 128.24, 127.93, 127.91, 87.13 (C-1), 77.35 (C-2), 75.48 (CO_2Bn), 75.41 (C-5), 73.29 (C-3), 71.35 (C-4), 67.42 (CO_2Bn), 38.02 (Lev), 29.76 (Lev), 27.96 (Lev). HR-MS: Calculated for $C_{31}H_{32}O_8S$ [M+Na$^+$]: 587.17101, found: 587.17111. $[\alpha]^{20}_D$ = -19.2 ° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/EA = 3/2, v/v).

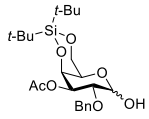
Phenyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-β-D-galactopyranoside (28a)



Compound **11** (1.76 g, 3.5 mmol, 1.0 eq) was dissolved in pyridine (35 mL). After reduced to 0 °C and 4- added dimethylaminopyridine (DMAP) (214 mg, 1.75 mmol, 0.5 eq), the acetyl chloride (AcCl) (275 µL, 3.9 mmol, 1.1 eq) was added dropwise. After stirred for overnight at RT and checked by TLC complete consumption of the starting material, the reaction was quenched by MeOH. The

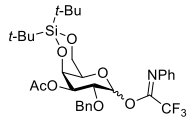
mixture was diluted with EtOAc and washed with H_2O (2x), brine. The organic phase was dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 30:1 - 15:1) to yield compound **28a** (1.42 g, 2.61 mmol, 75%). ^{1}H NMR (500 MHz, Chloroform-*d*)f δ 7.57 – 7.50 (m, 2H), 7.40 – 7.30 (m, 4H), 7.30 – 7.19 (m, 4H), 4.94 (d, *J* = 10.8 Hz, 1H, CH_2), 4.79 – 4.69 (m, 3H, H-3, H-1, CH_2), 4.68 – 4.63 (m, 1H, H-4), 4.23 – 4.12 (m, 2H, H-6), 3.90 (t, *J* = 9.6 Hz, 1H, H-2), 3.37 (d, *J* = 2.0 Hz, 1H, H-5), 2.06 (s, 3H, OAc), 1.12 (s, 9H), 1.01 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.57 (OAc), 138.13, 134.39, 132.20, 128.90, 128.41, 128.12, 127.86, 127.56, 88.59 (C-1, J_{CH} = 157.0 Hz), 77.33 (C-3), 75.79 (C-2), 75.74, 74.48 (C-5), 70.28 (C-4), 67.18 (C-6), 27.65, 27.60, 23.29, 21.00 (OAc), 20.76. HR-MS: Calculated for $C_{29}H_{40}O_6SSi$ [M+Na^+]: 567.22071, found: 567.22100. $[\alpha]^{20}_D$ = + 49.8º (c = 1, $CHCl_3$). TLC: Rf = 0.5 (PE/EtOAc = 9/1, v/v).

3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-α/β-D-galactopyranoside (28b)



Compound **28a** (1.18 g, 2.17 mmol, 1.0 eq) was dissolved in DCM (22 mL) and reduced to 0 °C. NIS (537 mg, 2.39 mmol, 1.1 eq) and TFA (184 µL, 2.39 mmol, 1.1 eq) were added and the solution stirred for 2 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 5:1 - 3:1) to yield compound **28b** (823 mg, 1.82 mmol, 84%). α anomer: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.27 (m, 5H), 5.22 (d, *J* = 3.6 Hz, 1H, H-1), 5.06 (dd, *J* = 10.2, 3.0 Hz, 1H, H-3), 4.79 – 4.64 (m, 3H, Bn, H-4), 4.27 – 4.17 (m, 1H, H-6), 4.12 (dd, *J* = 12.6, 1.7 Hz, 1H, H-6), 4.01 (dd, *J* = 10.2, 3.6 Hz, 1H, H-2), 3.97 (dd, *J* = 2.2, 1.2 Hz, 1H, H-5), 3.05 (s, 1H, 1-OH), 2.11 (s, 3H, OAc), 1.01 – 0.96 (m, 18H). α anomer: ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.88 (OAc), 137.77, 128.65, 128.49, 128.24, 128.22, 128.06, 127.86, 92.03 (C-1), 73.64 (Bn), 73.19 (C-2), 72.74 (C-3), 71.02 (C-4), 67.17 (C-6), 67.10 (C-5), 27.66, 27.35, 23.37, 21.17 (OAc), 20.76. HR-MS: Calculated for $C_{23}H_{36}O_7Si$ [M+Na^+]: 475.21225, found: 475.21208. TLC: Rf = 0.2 (PE/EA = 4/1, v/v).

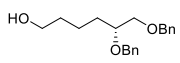
***N*-phenyl-trifluoroacetimidate 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-α/β-D-galactopyranoside (28)**



The hemiacetal **28b** (788 mg, 1.74 mmol, 1.0 eq) was dissolved in acetone (20 mL) and cooled to 0 °C. Cesium carbonate (688 mg, 2.11 mmol, 1.2 eq) was added. After 15 min, *N*-phenyl trifluoroacetimidoyl chloride (600 mg, 2.89 mmol, 1.7 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by triethyl amine, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 50:1 – 20:1) to yield compound **28** (1.05 g, 1.68 mmol, 97%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.78 – 7.67 (m, 1H), 7.47 – 7.27 (m, 5H), 7.27 – 7.19 (m, 1H), 7.13 (qt, *J* = 7.3, 1.2 Hz, 1H), 6.94 – 6.75 (m, 2H), 5.12 (dd, *J* = 10.4, 2.9 Hz, 1H, H-3), 4.89 – 4.60 (m, 3H, Bn, H-4), 4.47 – 3.99 (m, 4H, H-6, H-2, H-5), 2.10 (s, 3H, OAc), 1.10 – 0.95 (m, 18H). ^{13}C NMR (101 MHz, Acetone) δ 170.83, 170.67, 144.84, 139.31, 139.20, 130.00, 129.79, 129.32, 129.20, 128.87, 128.82, 128.74, 128.62, 126.69, 121.78, 120.23, 95.71 (C-

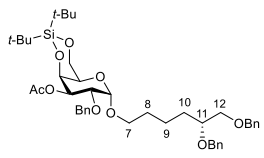
1), 76.08, 75.57, 73.97 (Bn), 72.95, 72.76, 72.66, 71.67, 70.63, 67.40 (C-6), 28.05, 28.02, 27.89, 27.76, 23.83, 21.34, 21.05, 20.98. HR-MS: Calculated for $C_{31}H_{40}F_3NO_7Si$ [M+Na$^+$]: 646.24183, found: 646.24202. TLC: Rf = 0.2/0.4 (α/β) (PE/EA = 20/1, v/v).

(*R*)-5,6-bis(benzyloxy)hexan-1-ol (29)



AD-mix-β (28.5 g) was dissolved in *tert*-BuOH/H_2O (192 mL, 1/1, v/v). The mixture was cooled to 0 °C and 2-((hex-5-en-1-yloxy)methyl)naphthalene[16b] (4.88 g, 20.3 mmol, 1.0 eq) was added and stirred for overnight. After TLC showed complete consumption of the starting material, solid sodium sulfite (25 g) was added slowly at 0 °C. The resultant suspension was allowed to warm to room temperature, stirred for an additional 1 h, and diluted with DCM. diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The aqueous phase was extracted with DCM, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated.[16a] The crude residue was dissolved in DMF (32 mL), sodium hydride (3.3 g, 81.2 mmol, 4.0 eq) was added and stirred for 15 min. Benzyl bromide (7.3 mL, 60.9 mmol, 3.0 eq) was added at 0 °C. It was slowly wormed to RT and stirred for overnight. After analysis by TLC showed complete consumption of the starting material, quenched with MeOH and water. Diluted with EtOAc and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was dissolved in DCM (400 mL) and water (40 mL). After cooled to 0 °C, 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (5.5 g, 24.2 mmol, 1.2 eq) was added. The reaction was stirred at RT for 7 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched by saturated aqueous sodium thiosulphate, extracted with DCM 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 10:1 – 4:1) to yield compound **29** (3.1 g, 9.9 mmol, 49%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 – 7.10 (m, 10H), 4.69 (d, *J* = 11.6 Hz, 1H, Bn), 4.59 – 4.52 (m, 3H, Bn), 3.65 – 3.47 (m, 5H), 1.64 – 1.31 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.94, 138.44, 128.47, 128.41, 127.93, 127.73, 127.69, 127.60, 78.10 (CH), 73.45, 72.85, 72.12, 62.81, 32.79, 31.80, 21.69. HR-MS: Calculated for $C_{20}H_{26}O_3$ [M+Na$^+$]: 337.1774, found: 337.1781. $[\alpha]^{20}_D$ = +13.7° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/EA = 4/1, v/v).

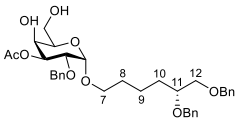
(*R*)-5,6-bis(benzyloxy)hexyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-α-D-galactopyranoside (30a)



Known compound donor **28** (1.06 g, 1.7 mmol, 1.0 eq) and the linker acceptor **29** (1.07 g, 3.4 mmol, 2.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (17 mL) and 4Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (80 µL, 0.34 mmol, 0.2 eq) was added. After stirred for 2 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 15:1 - 8:1) to yield compound **30a** (1.13 g, 1.59 mmol, 94%). ^{1}H

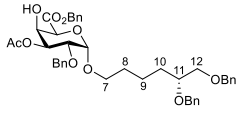
NMR (500 MHz, Chloroform-*d*) δ 7.37 – 7.22 (m, 15H), 5.05 (dd, *J* = 10.4, 3.1 Hz, 1H, H-3), 4.78 – 4.63 (m, 4H, H-1, H-4, CH_2), 4.59 (d, *J* = 12.1 Hz, 1H, CH_2), 4.56 – 4.50 (m, 3H, CH_2), 4.15 (dd, *J* = 12.5, 2.2 Hz, 1H, H-6), 4.05 (dd, *J* = 12.6, 1.8 Hz, 1H, H-6), 4.00 (dd, *J* = 10.4, 3.6 Hz, 1H, H-2), 3.73 – 3.65 (m, 1H, H-5), 3.63 – 3.46 (m, 4H, H-7, H-11, H-12), 3.43 – 3.31 (m, 1H, H-7), 2.09 (s, 3H, OAc), 1.64 – 1.29 (m, H-8, H-9, H-10), 0.98 (d, *J* = 4.4 Hz, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.77 (OAc), 139.01, 138.53, 138.38, 128.50, 128.48, 128.42, 128.22, 127.94, 127.92, 127.88, 127.72, 127.67, 127.60, 127.58, 97.84 (C-1), 78.17 (C-11), 73.45, 73.23, 72.94 (C-12), 72.91 (C-3), 72.72 (C-2), 72.18, 71.05 (C-4), 68.24 (C-7), 67.14 (C-6), 66.82 (C-5), 31.87, 29.57, 27.68, 27.35, 23.36, 22.15, 21.16 (OAc), 20.74. HR-MS: Calculated for $C_{43}H_{60}O_9Si$ [M+Na^+]: 771.3899, found: 771.3927. $[\alpha]^{20}_D$ = + 92.4º (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 9/1, v/v).

(*R*)-5,6-bis(benzyloxy)hexyl 3-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranoside (30)



Compound **30a** (166 mg, 0.235 mmol, 1.0 eq) was dissolved in THF (2 mL) and pyridine (2 mL), then cooled to 0 ℃ and 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 2:1 - 1:2) to yield compound **30** (120 mg, 0.2 mmol, 84%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.40 – 7.19 (m, 15H), 5.17 (dd, *J* = 10.4, 3.2 Hz, 1H, H-3), 4.79 (d, *J* = 3.7 Hz, 1H, H-1), 4.71 – 4.61 (m, 2H, CH_2), 4.60 – 4.45 (m, 4H, CH_2), 4.02 (d, *J* = 3.2 Hz, 1H, H-4), 3.92 (dd, *J* = 10.5, 3.7 Hz, 1H, H-2), 3.83 – 3.73 (m, 1H, H-5), 3.71 – 3.61 (m, 2H, H-6, H-7), 3.61 – 3.46 (m, 4H, H-11, H-6, H-12), 3.42 – 3.31 (m, 1H, H-7), 3.15 (s, 1H, 4-OH), 3.02 (s, 1H, 6-OH), 2.12 – 2.03 (m, 3H, OAc), 1.78 – 1.33 (m, 6H, H-8, H-9, H-10). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.34 (OAc), 138.54, 138.34, 138.27, 128.42, 128.39, 128.09, 127.79, 127.71, 127.69, 127.66, 127.63, 97.38 (C-1, J_{CH} = 167.0 Hz), 78.43 (C-11), 73.72 (C-2), 73.35, 72.91, 72.73 (C-12), 72.38, 72.29 (C-3), 69.23 (C-4), 69.05 (C-5), 67.65 (C-7), 62.75 (C-6), 31.46 (C-10), 29.21 (C-8), 21.94 (C-9), 21.12 (OAc). HR-MS: Calculated for $C_{35}H_{44}O_9$ [M+Na^+]: 631.2878, found: 631.2892. $[\alpha]^{20}_D$ = + 208.1º (c = 1, $CHCl_3$). TLC: Rf = 0.15 (PE/EA = 1/1, v/v).

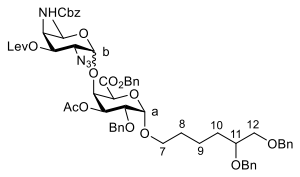
Benzyl (*R*)-5,6-bis(benzyloxy)hexyl 3-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl uronate (31)



Compound **30** (116 mg, 0.19 mmol, 1.0 eq) was dissolved in DCM/*tert*-BuOH/H_2O (4.5 mL, 4/4/1, v/v/v). The mixture was cooled to 0 ℃ and treated with TEMPO (6.0 mg, 0.04 mmol, 0.2 eq) and BAIB (158 mg, 0.48 mmol, 2.5 eq). After stirring for overnight at 4 ℃ and TLC showed complete consumption of the starting material, saturated aqueous sodium thiosulphate was added and diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DMF (4 mL), followed by addition of Cs_2CO_3 (62 g, 0.19 mmol, 1.0 eq) and BnBr (65 µL, 0.38 mmol, 2.0 eq) at 0℃. The mixture was allowed to stir overnight at rt, and then diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*.

Purification by column chromatography (PE/EA 7/3) yielded **31** (130 mg, 0.18 mmol, 96%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.20 (m, 20H), 5.31 – 5.14 (m, 3H, H-3, CO_2Bn), 4.89 (d, *J* = 3.6 Hz, 1H, H-1), 4.66 (dd, *J* = 12.0, 2.1 Hz, 2H, CH_2), 4.58 – 4.48 (m, 5H, CH_2, H-5), 4.48 – 4.41 (m, 1H, H-4), 3.92 (dd, *J* = 10.5, 3.5 Hz, 1H, H-2), 3.71 – 3.32 (m, 5H, H-7, H-11, H-12), 2.49 – 2.35 (m, 1H, 4-OH), 2.07 (s, 3H, OAc), 1.65 – 1.30 (m, 6H, H-8, H-9, H-10). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.13 (OAc), 168.39 (CO_2Bn), 138.87, 138.40, 138.10, 135.13, 128.63, 128.49, 128.47, 128.38, 128.32, 127.95, 127.85, 127.82, 127.63, 127.58, 127.49, 97.62 (C-1, J_{CH} = 170.0 Hz), 77.88 (C-11), 73.33, 73.09, 73.06 (C-2), 72.75 (C-12), 72.01, 71.38 (C-3), 69.80 (C-5), 69.04 (C-4), 68.96 (C-7), 67.22 (C-6), 31.72 (C-10), 29.37 (C-8), 21.92 (C-9), 21.05 (OAc). HR-MS: Calculated for $C_{42}H_{48}O_{10}$ [M+Na$^+$]: 735.3140, found: 735.3161. $[\alpha]^{20}_{D}$ = + 35.8° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/EA = 7/3, v/v).

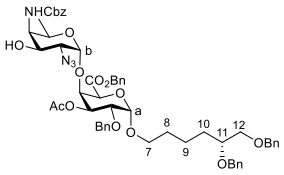
Benzyl ((*R*)-5,6-bis(benzyloxy)hexyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-3-*O*-levulinoyl-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl uronate) (32)



Known compound donor *N*-phenyl-trifluoroacetimidate 2-azido-3-*O*-levulinoyl-4-*N*-benzyloxycarbonyl-6-deoxy-α/β-D-galactopyranoside **8**[8g] (430 mg, 0.73 mmol, 2.5 eq) and the acceptor **31** (210 mg, 0.30 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (5 mL) and 4Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (7 µL, 0.03 mmol, 0.1 eq) was added. After stirred for 5 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 4:1 - 3:2) to yield 9:1 ratio of α/β mixed compound **32** (275.5 mg, 0.25 mmol, 83%). **α anomer 32**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.18 (m, 25H), 5.32 – 4.95 (m, 8H, H-1a, H-3a, H-3b, NHCbz, CH_2), 4.70 – 4.43 (m, 9H, H-1b, H-5b, H-4a, H-5a, CH_2), 4.23 – 4.07 (m, 2H, H-4b, H-5b), 3.91 (dd, *J* = 10.8, 3.5 Hz, 1H, H-2a), 3.69 – 3.36 (m, 5H, H-7, H-11, H-12), 3.20 (dd, *J* = 11.3, 3.9 Hz, 1H, H-2b), 2.84 – 2.36 (m, 4H, Lev), 2.14 (s, 3H, Lev), 2.00 (s, 3H, OAc), 1.62 – 1.28 (m, 6H), 1.05 (d, *J* = 6.3 Hz, 3H, H-6b). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.16 (Lev), 171.80 (Lev), 170.01 (OAc), 167.70 (CO_2Bn), 156.49 (Cbz), 138.75, 138.27, 137.60, 136.24, 134.67, 128.65, 128.55, 128.49, 128.44, 128.37, 128.29, 128.24, 128.19, 128.17, 127.92, 127.83, 127.71, 127.67, 127.48, 127.43, 127.34, 98.18 (C-1b, J_{CH} = 172.5 Hz), 97.03 (C-1a, J_{CH} = 170.0 Hz), 77.69 (C-11), 76.49 (C-4a), 73.15, 72.83, 72.60 (*C*H$_2$), 72.53 (C-2a), 71.84 (*C*H$_2$), 70.22 (C-3a), 70.07 (C-3b), 69.28 (C-5a), 68.76 (C-7), 67.27 (CO_2Bn), 66.92 (Cbz), 64.64 (C-5b), 57.45 (C-2b), 52.35 (C-4b), 37.79, 31.51, 29.64, 29.20, 27.84, 21.74, 21.16, 16.53 (C-6b). HR-MS: Calculated for $C_{61}H_{70}N_4O_{16}$ [M+Na$^+$]: 1137.4679, found: 1137.4712. $[\alpha]^{20}_{D}$ = + 116.0° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 3/2, v/v). **β anomer:** ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.42 – 7.19 (m, 25H), 5.32 (d, *J* = 12.6 Hz, 1H, CH_2), 5.22 (dd, *J* = 10.5, 3.1 Hz, 1H, H-3a), 5.15 (d, *J* = 12.3 Hz, 1H, CH_2), 5.09 – 4.93 (m, 3H, CH_2), 4.79 (d, *J* = 3.7 Hz, 1H, H-1a), 4.75 – 4.63 (m, 2H, CH_2), 4.60 – 4.47 (m, 7H, H-4a, H-3b, H-5a, CH_2), 4.19 (d, *J* = 8.0 Hz, 1H, H-1b), 4.11 (dd, *J* = 10.4, 3.7 Hz, 1H, H-2a), 4.02 (dd, *J* = 10.2, 4.0 Hz, 1H, H-4b), 3.63 – 3.45 (m, 6H, H-5b,

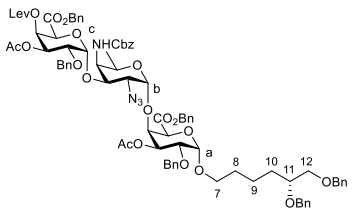
H-7, H-11, H-12, H-2b), 3.38 – 3.28 (m, 1H, H-7), 2.81 – 2.70 (m, 1H, Lev), 2.65 – 2.53 (m, 2H, Lev), 2.41 – 2.31 (m, 1H, Lev), 2.15 (d, *J* = 16.3 Hz, 6H, Lev), 1.62 – 1.25 (m, 6H, Lev, OAc), 1.10 (d, *J* = 6.3 Hz, 3H, H-6b). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.60 (Lev), 172.17 (Lev), 170.82 (OAc), 167.53 (CO_2Bn), 156.67 (Cbz), 138.96, 138.49, 138.37, 136.47, 135.07, 128.77, 128.67, 128.56, 128.54, 128.47, 128.40, 128.32, 128.04, 128.03, 127.97, 127.94, 127.91, 127.84, 127.73, 127.67, 127.58, 103.44 (C-1b, J_{CH} = 163.7 Hz), 97.93 (C-1a), 77.96 (C-11), 77.29 (C-4a), 73.67 (C-2a), 73.61, 73.43, 72.93 (C-3b), 72.85 (C-12), 72.12, 71.61 (C-3a), 69.58 (C-5b), 69.35 (C-5a), 69.25 (C-7), 67.15 (Bn), 67.12 (Bn), 61.40 (C-2b), 51.73 (C-4b), 37.90 (Lev), 31.84, 29.93 (Lev), 29.44, 27.92 (Lev), 21.97, 21.00 (OAc), 16.82 (C-6b). $[\alpha]^{20}_D$ = + 38.2º (c = 1, $CHCl_3$).

Benzyl ((*R*)-5,6-bis(benzyloxy)hexyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl uronate) (33)



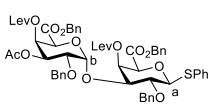
Compound **32** (234 mg, 0.21 mmol, 1.0 eq) was dissolved in pyridine (4 mL) and acetic acid (1 mL). After cooled to 0 °C, hydrazine hydrate (N_2H_4 • H_2O 50-60 %) (31 µL, 0.64 mmol, 3.0 eq) was added slowly. After stirred 20 min at RT, checked by TLC complete consumption of the starting material, quenched by acetone. 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 5:1 - 2:1) to yield compound **33** (196 mg, 0.19 mmol, 92%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.39 – 7.19 (m, 25H), 5.28 – 5.19 (m, 2H, H-3a, CH_2), 5.19 – 5.03 (m, 4H, CH_2), 5.00 (d, *J* = 3.7 Hz, 1H, H-1a), 4.70 – 4.44 (m, 9H, CH_2, H-1b, H-4a, H-5a), 4.16 – 4.03 (m, 2H, H-3b, H-5b), 4.01 – 3.92 (m, 1H, H-4b), 3.88 (dd, *J* = 10.7, 3.7 Hz, 1H, H-2a), 3.70 – 3.59 (m, 1H, H-7), 3.59 – 3.30 (m, 5H, H-7, H-11, H-12, 3b-OH), 3.02 (dd, *J* = 10.7, 3.9 Hz, 1H, H-2b), 2.03 (s, 3H, OAc), 1.63 – 1.28 (m, 6H, H-8, H-9, H-10), 1.08 (d, *J* = 6.4 Hz, 3H, H-6b). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.19 (OAc), 167.76 (CO_2Bn), 158.05 (Cbz), 138.86, 138.38, 137.80, 135.92, 134.81, 128.70, 128.63, 128.61, 128.52, 128.38, 128.36, 128.34, 128.30, 128.27, 128.15, 128.13, 127.99, 127.81, 127.77, 127.59, 127.53, 127.44, 98.75 (C-1b), 97.14 (C-1a), 77.84 (C-11), 76.89 (C-4a), 73.28, 72.73, 72.62 (C-2a), 71.96, 71.91, 70.61 (C-3a), 69.46 (C-5a), 68.90 (C-7), 68.36 (C-5b), 67.50 (Cbz), 67.42 (CO_2Bn), 65.19 (C-3b), 60.60 (C-2b), 55.78 (C-4b), 31.65, 29.33, 21.85, 21.27, 16.78 (C-6b). HR-MS: Calculated for $C_{56}H_{64}N_4O_{14}$ $[M+Na]^+$: 1039.4311, found: 1039.4344. $[\alpha]^{20}_D$ = + 98.8º (c = 1, $CHCl_3$). TLC: Rf = 0.2 (Tol/EA = 8/2, v/v).

Benzyl ((*R*)-5,6-bis(benzyloxy)hexyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-3-O-(Benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α-D-galactopyranosyl urinate)-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl uronate) (34)



Donor **9** (111.3 mg, 0.162 mmol, 3.0 eq) and acceptor **33** (55.8 mg, 55 µmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (1 mL) and 5Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and Trifluoromethanesulfonic acid (TfOH) (2.0 µL, 0.02 mmol, 0.4 eq) was added. After stirred for 5 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 3:1 – 1:1) to yield desired α anomer compound **34** (46 mg, 30.4 µmol, 55%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.41 – 7.17 (m, 35H), 5.83 – 5.77 (m, 1H, H-4c), 5.48 (d, *J* = 3.5 Hz, 1H, H-1c), 5.41 (dd, *J* = 10.7, 3.4 Hz, 1H, H-3c), 5.34 (d, *J* = 11.9 Hz, 1H, CO_2Bn), 5.28 – 5.19 (m, 2H, CH_2, H-3a), 5.12 – 5.03 (m, 2H, CH_2), 5.02 – 4.95 (m, 2H, CH_2), 4.92 (d, *J* = 3.6 Hz, 1H, H-1a), 4.79 (d, *J* = 1.8 Hz, 1H, H-5c), 4.75 – 4.44 (m, 12H, CH_2, H-1b, H-4a, H-5a), 4.31 – 4.20 (m, 1H, H-4b), 4.14 (dd, *J* = 10.5, 4.2 Hz, 1H, H-3b), 4.09 – 4.01 (m, 1H, H-5b), 3.90 – 3.81 (m, 2H, H-2a, H-2c), 3.77 – 3.43 (m, 4H, H-7, H-11, H-12), 3.43 – 3.32 (m, 1H, H-7), 3.12 (dd, *J* = 10.5, 3.9 Hz, 1H, H-2b), 2.60 – 2.29 (m, 4H, Lev), 2.12 (s, 3H, Lev), 2.04 (s, 3H, OAc), 1.85 (s, 3H, OAc), 1.62 – 1.28 (m, 6H, H-8, H-9, H-10), 1.05 (d, *J* = 6.5 Hz, 3H, H-6b). ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.99 (Lev), 171.26 (Lev), 170.32 (OAc), 169.97 (OAc), 167.76 (CO_2Bn), 166.88 (CO_2Bn), 156.87 (Cbz), 138.96, 138.49, 138.09, 137.98, 136.15, 135.08, 134.78, 129.30, 128.90, 128.83, 128.73, 128.67, 128.61, 128.58, 128.53, 128.46, 128.42, 128.40, 128.29, 128.26, 128.02, 127.93, 127.90, 127.81, 127.71, 127.66, 127.57, 97.61 (C-1b, J_{CH} = 172.0 Hz), 97.33 (C-1a, J_{CH} = 171.0 Hz), 93.98 (C-1c, J_{CH} = 171.0 Hz), 77.98 (C-11), 76.19 (C-4a), 73.42, 73.27 (C-2a), 73.21, 73.09, 72.87 (C-12), 72.11, 71.82 (C-2c), 70.47 (C-3a), 69.66 (C-4c), 69.37 (C-5a), 69.11 (C-5c), 69.01 (C-7), 68.83 (C-3c), 67.57 (6c-CO_2Bn), 67.48 (6a-CO_2Bn), 67.07 (Cbz), 65.82 (C-5b), 59.63 (C-2b), 50.56 (C-4b), 37.65 (Lev), 31.80, 29.89 (Lev), 29.46, 27.56 (Lev), 22.00, 21.36 (OAc), 20.68 (OAc), 16.57 (C-6b). HR-MS: Calculated for $C_{83}H_{92}N_4O_{23}$ [M+Na$^+$]: 1535.6045, found: 1535.6095. $[\alpha]^{20}_D$ = + 97.1° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 3/2, v/v).

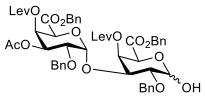
Benzyl (Phenyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α-D-galactopyranosyl urinate)-4-*O*-levulinoyl-1-thio-β-D-galactopyranosyl uronate) (26)



Donor **9** (572.3 mg, 0.84 mmol, 2.2 eq) and acceptor **10** (211 mg, 0.37 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (5 mL) and 5Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and Trifluoromethanesulfonic acid (TfOH) (13 µL, 0.15 mmol, 0.4 eq) was added. After stirred 2 hours and TLC showed complete consumption of the starting material, the reaction was

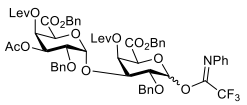
quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 4:1 - 3:2) to yield compound **26** (136 mg, 0.13 mmol, 34%) and SPh transferred byproduct **9e**[19] (140 mg, 0.23 mmol, 62%). **26**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.72 – 7.67 (m, 2H), 7.51 – 7.47 (m, 2H), 7.42 – 7.20 (m, 18H), 7.18 – 7.13 (m, 2H), 7.13 – 7.08 (m, 1H), 5.89 – 5.83 (m, 1H, H-4a), 5.49 – 5.44 (m, 1H, H-4b), 5.41 – 5.34 (m, 2H, H-3b, H-1b), 5.27 (d, *J* = 12.0 Hz, 1H, CH_2), 5.18 – 5.04 (m, 3H, CH_2), 4.75 – 4.66 (m, 2H, H-5b, CH_2), 4.65 – 4.59 (m, 2H, CH_2, H-1a), 4.57 – 4.48 (m, 2H, CH_2), 4.21 (d, *J* = 1.3 Hz, 1H, H-5a), 3.97 (dd, *J* = 9.5, 3.2 Hz, 1H, H-3a), 3.79 (dd, *J* = 10.5, 3.3 Hz, 1H, H-2b), 3.70 (t, *J* = 9.5 Hz, 1H, H-2a), 2.51 – 2.20 (m, 8H, Lev), 2.10 (s, 3H, Lev), 2.02 (s, 3H, Lev), 1.93 (s, 3H, OAc). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.04 (Lev), 205.95 (Lev), 171.42 (Lev), 171.28 (Lev), 170.20 (OAc), 166.76 (6b-CO_2Bn), 166.08 (6a-CO_2Bn), 138.02, 137.15, 135.19, 135.05, 133.18, 132.53, 129.02, 128.98, 128.81, 128.69, 128.64, 128.61, 128.58, 128.46, 128.40, 128.37, 128.23, 128.21, 127.81, 127.69, 93.14 (C-1b, J_{CH} = 174.0 Hz), 87.43 (C-1a, J_{CH} = 157.0 Hz), 76.32, 75.64 (C-5a), 75.26 (C-2a), 74.54 (C-3a), 73.65, 72.60 (C-2b), 69.41 (C-4b), 68.89 (C-3b), 68.39 (C-5b), 67.70, 67.09, 66.04 (C-4a), 37.76, 37.56, 29.83, 29.68, 27.90, 27.48, 20.73. HR-MS: Calculated for $C_{58}H_{60}O_{17}S$ [M+Na$^+$]: 1083.3443, found: 1083.3462. $[\alpha]^{20}_D$ = + 77.1° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 3/2, v/v).

Benzyl (2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α-D-galactopyranosyl urinate)-4-*O*-levulinoyl-α/β-D-galactopyranosyl uronate) (27a)



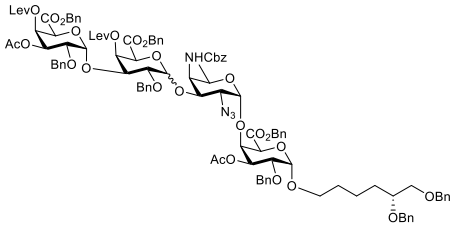
Compound **26** (112 mg, 0.11 mmol, 1.0 eq) was dissolved in DCM (4 mL) and reduced to 0 °C. NIS (26 mg, 0.12 mmol, 1.1 eq) and TFA (9.0 µL, 0.13 mmol, 1.1 eq) were added and the solution stirred for 2 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 3:1 - 3:2) to yield α/β mixed compound **27a** (92 mg, 0.095 mmol, 90%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.45 – 7.07 (m, 20H), 5.89 – 5.75 (m, 1H), 5.58 – 5.48 (m, 1H), 5.46 – 5.37 (m, 1H), 5.37 – 5.30 (m, 1H), 5.25 – 5.00 (m, 4H), 4.92 (dd, *J* = 44.2, 1.8 Hz, 1H), 4.78 – 4.45 (m, 6H), 4.30 (dd, *J* = 10.1, 3.5 Hz, 1H), 4.18 – 4.06 (m, 1H), 3.95 – 3.74 (m, 2H), 2.56 – 2.03 (m, 11H), 1.95 (dd, *J* = 24.8, 6.2 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.53, 206.26, 206.08, 206.05, 171.40, 171.38, 171.33, 171.29, 170.23, 170.15, 167.57, 166.98, 166.78, 138.19, 138.13, 137.53, 137.02, 128.95, 128.92, 128.89, 128.72, 128.65, 128.59, 128.56, 128.50, 128.45, 128.42, 128.40, 128.34, 128.31, 128.28, 127.69, 127.58, 127.56, 97.87, 93.59, 93.26, 91.06, 78.49, 75.90, 74.77, 73.58, 73.56, 73.13, 72.93, 72.86, 72.58, 72.29, 69.58, 69.50, 68.93, 68.82, 68.66, 68.51, 68.29, 67.81, 67.63, 67.07, 65.92, 37.74, 37.73, 37.55, 29.78, 29.77, 29.58, 29.52, 27.88, 27.49, 27.47, 20.69. HR-MS: Calculated for $C_{52}H_{56}O_{18}$ [M+Na$^+$]: 991.33589, found: 991.33578. TLC: Rf = 0.15 (PE/EA = 3/2, v/v).

Benzyl (*N*-phenyl-trifluoroacetimidate 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α-D-galactopyranosyl urinate)-4-*O*-levulinoyl-α/β-D-galactopyranosyl uronate) (27)



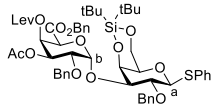
Hemiacetal **27a** (92 mg, 0.095 mmol, 1.0 eq) was dissolved in acetone (3 mL) and cooled to 0 ℃. Cesium carbonate (34 mg, 0.104 mmol, 1.1 eq) was added. After 15 min, *N*-phenyl trifluoroacetimidoyl chloride (30 mg, 0.14 mmol, 1.5 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by triethyl amine, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 5:1 – 2:1) to yield compound **27** (99 mg, 0.09 mmol, 91%). The crude compound was used for the further reaction without any purification. TLC: Rf = 0.5 (PE/EA = 3/2, v/v).

Benzyl ((*R*)-5,6-bis(benzyloxy)hexyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-3-O-(Benzyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α-D-galactopyranosyl urinate)-4-*O*-levulinoyl-α/β-D-galactopyranosyl urinate)-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl uronate) (35)



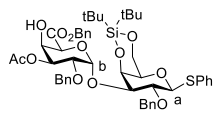
Donor **27** (60.0 mg, 52.6 µmol, 1.9 eq) and acceptor **33** (27.8 mg, 27.3 µmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (1 mL) and 5Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 ℃ and Trifluoromethanesulfonic acid (TfOH) (1.0 µL, 0.01 mmol, 0.4 eq) was added. After stirred 5 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 3:1 - 3:2) to yield 2:1 ratio of α/β mixed compound **35** (28 mg, 14.2 µmol, 52%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.15 (m, 45H), 5.95 – 5.78 (m, 1H), 5.68 – 5.50 (m, 1H), 5.45 – 4.41 (m, 23H), 4.39 – 3.96 (m, 5H), 3.94 – 3.44 (m, 12H), 3.44 – 3.32 (m, 1H), 3.17 – 2.98 (m, 1H), 2.54 – 2.16 (m, 8H), 2.10 (d, *J* = 6.2 Hz, 3H), 2.04 (d, *J* = 5.3 Hz, 3H), 1.98 – 1.86 (m, 6H), 1.63 – 1.31 (m, 6H), 1.09 – 1.00 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.20, 206.03, 205.99, 171.34, 171.32, 171.18, 170.39, 170.12, 170.03, 167.87, 167.75, 167.05, 167.02, 166.88, 165.92, 156.92, 156.43, 138.98, 138.51, 138.32, 138.06, 138.01, 137.39, 135.99, 135.50, 135.34, 135.25, 135.12, 134.91, 129.11, 129.04, 129.03, 128.93, 128.81, 128.76, 128.72, 128.71, 128.64, 128.59, 128.55, 128.49, 128.47, 128.45, 128.40, 128.38, 128.36, 128.25, 128.20, 128.16, 128.09, 128.03, 128.02, 127.97, 127.94, 127.72, 127.67, 127.59, 127.58, 102.08, 98.46, 97.90, 97.45, 97.31, 93.45, 93.32, 78.00, 76.66, 73.71, 73.52, 73.44, 73.38, 73.33, 73.19, 73.00, 72.90, 72.74, 72.61, 72.54, 72.12, 72.08, 71.39, 70.62, 70.59, 70.30, 69.70, 69.59, 69.50, 69.42, 69.37, 69.05, 68.82, 68.62, 67.83, 67.61, 67.52, 67.41, 67.24, 67.01, 66.96, 66.89, 61.99, 60.66, 59.94, 50.60, 37.81, 37.67, 37.64, 31.82, 31.77, 29.86, 29.61, 29.48, 27.98, 27.59, 27.55, 22.01, 21.39, 21.37, 20.79, 20.77, 19.37, 16.58, 14.02. HR-MS: Calculated for $C_{108}H_{118}N_4O_{31}$ [M+Na^+]: 1989.7672, found: 1989.7719. TLC: Rf = 0.2 (PE/EA = 3/2, v/v).

Phenyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-levulinoyl-α-D-galactopyranosyl urinate)-4,6-*O*-di-*tert*-butylsilylidene-1-thio-β-D-galactopyranoside (36)



The donor **9** (3.1 g, 4.52 mmol, 1.1 eq) and the acceptor **11** (2.0 g, 3.98 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (35 mL) and 5Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to -70 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (182 µL, 0.79 mmol, 0.2 eq) was added. After stirred overnight and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 5:1 - 3:1) to yield compound **36** (3.0 mg, 3.0 mmol, 75%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.59 – 7.48 (m, 4H), 7.35 – 7.23 (m, 13H), 7.22 – 7.17 (m, 2H), 7.17 – 7.10 (m, 1H), 5.63 – 5.58 (m, 1H, H-4b), 5.56 (dd, *J* = 10.5, 3.4 Hz, 1H, H-3b), 5.47 (d, *J* = 3.4 Hz, 1H, H-1b), 5.14 (d, *J* = 12.0 Hz, 1H, C*H*$_2$), 5.06 (d, *J* = 9.7 Hz, 1H, C*H*$_2$), 4.83 – 4.71 (m, 4H, H-5b, C*H*$_2$), 4.70 – 4.65 (m, 2H, H-1a, H-4a), 4.62 (d, *J* = 12.0 Hz, 1H, C*H*$_2$), 4.26 – 4.14 (m, 2H, H-6a), 3.96 (dd, *J* = 10.5, 3.3 Hz, 1H, H-2b), 3.89 (t, *J* = 9.5 Hz, 1H, H-2a), 3.71 (dd, *J* = 9.4, 2.9 Hz, 1H, H-3a), 3.32 (d, *J* = 2.3 Hz, 1H, H-5a), 2.55 – 2.32 (m, 3H, Lev), 2.16 – 2.07 (m, 4H, Lev), 1.94 (s, 3H, OAc), 1.09 (s, 9H), 1.02 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.87 (Lev), 171.29 (Lev), 170.06 (OAc), 166.91 (CO_2Bn), 137.83, 137.52, 134.99, 131.97, 128.97, 128.88, 128.73, 128.56, 128.45, 128.39, 128.32, 128.01, 127.73, 127.67, 127.45, 92.87 (C-1b, J_{CH} = 171.0 Hz), 88.89 (C-1a, J_{CH} = 157.2 Hz), 77.82 (C-3a), 76.39, 76.08 (C-2a), 74.54 (C-5a), 72.08, 71.90 (C-2b), 69.47 (C-4b), 69.03 (C-3b), 68.55 (C-5b), 68.18 (C-4a), 67.33, 67.18, 37.56, 29.78, 27.73, 27.69, 27.65, 27.49, 23.34, 20.69 (OAc). HR-MS: Calculated for $C_{54}H_{66}O_{14}SSi$ [M+Na$^+$]: 1021.3835, found: 1021.3843. $[\alpha]^{20}_D$ = + 94.3° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 7/3, v/v).

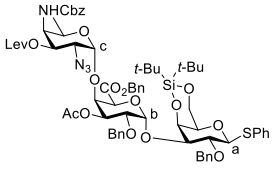
Phenyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl urinate)-4,6-*O*-di-*tert*-butylsilylidene-1-thio-β-D-galactopyranoside (37)



The compound **36** (3.0 g, 3.0 mmol, 1.0 eq) was dissolved in THF (30 mL), MeOH (3 mL) and acetic acid (3 mL). After cooled to 0 °C, hydrazine acetate (N_2H_4 • AcOH) (830 mg, 9.01 mmol, 3.0 eq) was added. After stirred 2 hours at RT, checked by TLC complete consumption of the starting material, the reaction was quenched by acetone. The solution was diluted by EtOAc and then 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/DCM 10:1:1 – 5:1:1) to yield compound **37** (2.6 g, 2.89 mmol, 95%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 – 7.52 (m, 2H), 7.52 – 7.46 (m, 2H), 7.36 – 7.15 (m, 16H), 5.54 (d, *J* = 3.5 Hz, 1H, H-1b), 5.49 (dd, *J* = 10.4, 3.2 Hz, 1H, H-3b), 5.18 (d, *J* = 12.4 Hz, 1H, C*H*$_2$), 5.03 (d, *J* = 9.9 Hz, 1H, C*H*$_2$), 4.95 (d, *J* = 12.3 Hz, 1H, C*H*$_2$), 4.87 – 4.79 (m, 2H, C*H*$_2$), 4.75 – 4.65 (m, 3H, H-1a, H-4a, H-5b), 4.56 (d, *J* = 12.1 Hz, 1H, C*H*$_2$), 4.32 – 4.26 (m, 1H, H-4b), 4.26 – 4.14 (m, 2H, H-6a), 4.06 (dd, *J* = 10.4, 3.4 Hz, 1H, H-2b), 3.89 (t, *J* = 9.5 Hz, 1H, H-2a), 3.73 (dd, *J* = 9.3, 2.9 Hz, 1H, H-3a), 3.32 (d, *J* = 2.2 Hz, 1H, H-5a), 2.06 (s, 3H, OAc), 1.08 (s, 9H), 1.02 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.98

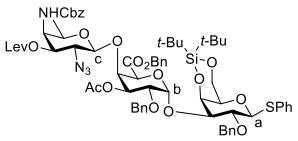
(OAc), 168.09 (CO_2Bn), 137.96, 137.79, 132.00, 129.08, 128.89, 128.62, 128.53, 128.46, 128.36, 128.27, 128.14, 127.87, 127.72, 127.49, 127.44, 125.35, 92.41 (C-1b, J_{CH} = 171.0 Hz), 88.83 (C-1a, J_{CH} = 157.0 Hz), 77.68 (C-3a), 76.28, 76.12 (C-2a), 74.50 (C-5a), 72.05 (C-2b), 71.72, 71.15 (C-3b), 69.90 (C-5b), 68.86 (C-4b), 68.21 (C-4a), 67.30 (C-6a), 67.00, 27.74, 27.65, 23.34, 21.03 (OAc), 20.71. HR-MS: Calculated for $C_{49}H_{60}O_{12}SSi$ [M+Na^+]: 923.3467, found: 923.3487. $[\alpha]^{20}_D$ = + 104.6º (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/DCM/EA = 3/1/1, v/v/v).

Phenyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-3-*O*-levulinoyl-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-4,6-*O*-di-*tert*-butylsilylidene-1-thio-β-D-galactopyranoside (38)



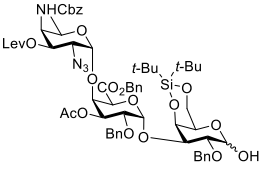
The donor **8**[8g] (1.6 g, 2.7 mmol, 1.5 eq) and the acceptor **37** (1.64 g, 1.82 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (18 mL) and 5Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 ºC and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (84 µL, 0.37 mmol, 0.2 eq) was added. After stirred 2 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 5:1:1 - 3:1:1) to yield desired α anomer compound **38** (1.6 g, 1.23 mmol, 68%) and byproduct β anomer **38a** (332 mg, 0.25 mmol, 14%). α anomer **38**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.57 – 7.53 (m, 2H), 7.51 – 7.46 (m, 2H), 7.41 – 7.20 (m, 21H), 5.57 (d, *J* = 3.6 Hz, 1H, H-1b), 5.45 (dd, *J* = 10.7, 2.9 Hz, 1H, H-3b), 5.24 – 5.12 (m, 2H, CH_2), 5.09 – 4.97 (m, 3H, H-3c, CH_2), 4.89 – 4.79 (m, 4H, CH_2), 4.73 (d, *J* = 3.0 Hz, 1H, H-4a), 4.66 (d, *J* = 9.8 Hz, 1H, H-1a), 4.61 (s, 1H, H-5b), 4.54 (d, *J* = 3.9 Hz, 1H, H-1c), 4.49 (d, *J* = 11.9 Hz, 1H, CH_2), 4.38 (d, *J* = 2.9 Hz, 1H, H-4b), 4.26 – 4.16 (m, 2H, H-6a), 4.16 – 4.10 (m, 1H, H-4c), 4.08 – 4.00 (m, 2H, H-2b, H-5c), 3.89 (t, *J* = 9.6 Hz, 1H, H-2a), 3.73 (dd, *J* = 9.4, 2.9 Hz, 1H, H-3a), 3.29 (d, *J* = 2.2 Hz, 1H, H-5a), 3.11 (dd, *J* = 11.3, 3.9 Hz, 1H, H-2c), 2.84 – 2.37 (m, 4H, Lev), 2.17 (s, 3H, Lev), 1.99 (s, 3H, OAc), 1.06 (d, *J* = 21.5 Hz, 21H, H-6c). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.37 (Lev), 171.97 (Lev), 170.24 (OAc), 167.20 (CO_2Bn), 156.61 (Cbz), 137.86, 132.03, 128.91, 128.70, 128.67, 128.55, 128.50, 128.45, 128.44, 128.27, 128.10, 127.93, 127.47, 98.50 (C-1c, J_{CH} = 170.8 Hz), 91.85 (C-1b, J_{CH} = 171.5 Hz), 88.89 (C-1a, J_{CH} = 158.0 Hz), 77.61 (C-3a), 76.76 (C-4b), 76.25, 76.04 (C-2a), 74.56 (C-5a), 71.93, 71.72 (C-2b), 70.31 (C-3b), 70.19 (C-3c), 69.55 (C-5b), 68.08 (C-4a), 67.38, 67.29, 67.21, 64.80 (C-5c), 57.64 (C-2c), 52.53 (C-4c), 38.00, 29.87, 28.01, 27.78, 27.66, 23.35, 21.39 (OAc), 20.74, 16.74 (C-6c). HR-MS: Calculated for $C_{68}H_{82}N_4O_{18}SSi$ [M+Na^+]: 1325.50063, found: 1325.50063. $[\alpha]^{20}_D$ = + 122.2º (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA/DCM = 3:1:1, v/v).

Phenyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-3-*O*-levulinoyl-4-*N*-benzyloxycarbonyl-6-deoxy-β-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-4,6-*O*-di-*tert*-butylsilylidene-1-thio-β-D-galactopyranoside (38a)



β anomer **38a**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 – 7.46 (m, 4H), 7.41 – 7.19 (m, 20H), 7.16 – 7.10 (m, 1H), 5.59 (d, *J* = 3.6 Hz, 1H, H-1b), 5.46 (dd, *J* = 10.3, 3.1 Hz, 1H, H-3b), 5.21 – 5.11 (m, 2H, C*H*$_2$), 5.11 – 4.99 (m, 2H, C*H*$_2$), 4.99 – 4.78 (m, 4H, C*H*$_2$), 4.78 – 4.48 (m, 6H, H-5b, H-4a, H-1a, H-3c, H-4b, C*H*$_2$), 4.28 (dd, *J* = 10.4, 3.5 Hz, 1H, H-2b), 4.25 – 4.13 (m, 3H, H-1c, H-6a), 4.09 – 3.98 (m, 1H, H-4c), 3.88 (t, *J* = 9.5 Hz, 1H, H-2a), 3.66 (dd, *J* = 9.3, 2.9 Hz, 1H, H-3a), 3.56 – 3.41 (m, 2H, H-5c, H-2c), 3.33 – 3.24 (m, 1H, H-5a), 2.84 – 2.28 (m, 4H), 2.15 (d, *J* = 2.0 Hz, 3H, Lev), 2.10 (s, 3H, OAc), 1.15 – 0.98 (m, 21H, H-6c). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.57 (Lev), 172.18 (Lev), 170.64 (OAc), 167.31 (CO_2Bn), 156.68 (Cbz), 138.14, 137.90, 135.04, 134.86, 132.14, 129.10, 128.94, 128.80, 128.75, 128.70, 128.66, 128.56, 128.54, 128.48, 128.43, 128.37, 128.06, 127.95, 127.78, 127.52, 127.47, 124.91, 103.15 (C-1c, J_{CH} = 163.8 Hz), 92.90 (C-1b, J_{CH} = 172.0 Hz), 88.84 (C-1a, J_{CH} = 157.0 Hz), 78.40 (C-3a), 76.86 (C-4b), 76.35, 76.16 (C-2a), 74.57 (C-5a), 73.10 (C-3c), 73.04 (C-2b), 72.27, 71.53 (C-3b), 69.90 (C-5b), 69.39 (C-5c), 68.54 (C-4a), 67.36, 67.16, 67.03, 61.43 (C-2c), 51.78 (C-4c), 37.92, 30.45, 29.83, 29.80, 27.84, 27.70, 23.45, 22.83, 21.00 (OAc), 20.78, 16.72 (C-6c). HR-MS: Calculated for $C_{68}H_{82}N_4O_{18}SSi$ [M+Na$^+$]: 1325.5006, found: 1325.5015. $[\alpha]^{20}_D$ = + 118.6º (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/EA/DCM = 3:1:1, v/v).

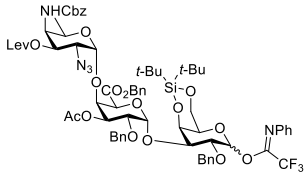
2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-3-*O*-levulinoyl-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-4,6-*O*-di-*tert*-butylsilylidene-α/β-D-galactopyranoside (39)



The compound **38** (1.56 g, 1.2 mmol, 1.0 eq) was dissolved in DCM (15 mL) and reduced to 0 °C. NIS (405 mg, 1.8 mmol, 1.5 eq) and TFA (111 µL, 1.44 mmol, 1.2 eq) were added and the solution stirred for 2 hours. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). 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 **39** (1.38 g, 1.14 mmol, 95%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.45 – 7.15 (m, 20H), 5.57 – 5.50 (m, 1H), 5.50 – 5.39 (m, 1H), 5.31 – 5.21 (m, 1H), 5.21 – 5.13 (m, 2H), 5.10 – 4.96 (m, 3H), 4.94 – 4.84 (m, 1H), 4.84 – 4.75 (m, 2H), 4.74 – 4.61 (m, 4H), 4.56 – 4.43 (m, 2H), 4.24 – 4.08 (m, 4H), 4.07 – 3.94 (m, 3H), 3.88 – 3.65 (m, 1H), 3.20 – 3.08 (m, 1H), 3.04 (s, 1H), 2.86 – 2.37 (m, 4H), 2.21 – 2.14 (m, 3H), 2.02 – 1.95 (m, 3H), 1.08 – 0.88 (m, 21H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.50, 172.04, 170.25, 167.55, 167.39, 156.64, 138.14, 137.80, 137.71, 137.55, 136.32, 134.86, 134.83, 128.83, 128.75, 128.74, 128.72, 128.65, 128.58, 128.44, 128.43, 128.20, 128.16, 128.12, 128.10, 128.03, 127.96, 127.90, 127.84, 98.49, 98.46, 97.91, 92.16, 91.77, 77.97, 76.79, 76.76, 75.77, 75.52, 73.69, 73.61, 72.71, 72.09, 71.90, 71.82, 71.36, 70.31, 70.28, 70.21, 70.18, 69.74, 69.48, 69.24, 68.21, 67.42, 67.28, 67.22, 67.18,

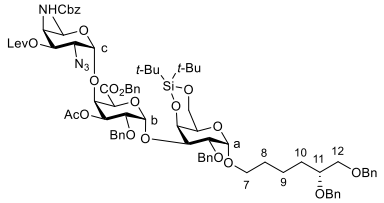
64.80, 57.66, 57.61, 52.54, 52.52, 38.01, 28.02, 27.75, 27.46, 27.29, 23.32, 21.38, 20.67, 16.74. HR-MS: Calculated for $C_{62}H_{78}N_4O_{19}Si$ [M+Na^+]: 1233.4922, found: 1233.4943. TLC: Rf = 0.2 (PE/EA/DCM = 1/1/1, v/v/v).

_N_-phenyl-trifluoroacetimidate 2-_O_-benzyl-3-_O_-(benzyl 3-_O_-acetyl-2-_O_-benzyl-4-_O_-(2-azido-3-_O_-levulinoyl-4-_N_-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-4,6-_O_-di-_tert_-butylsilylidene-α/β-D-galactopyranoside (7)



The hemiacetal **39** (1.53 g, 1.26 mmol, 1.0 eq) was dissolved in acetone (13 mL) and cooled to 0 ℃. Cesium carbonate (617 mg, 1.89 mmol, 1.5 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (524 mg, 2.5 mmol, 2.0 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by triethyl amine, filtered, and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 4:1 – 3/1) to yield compound **7** (1.64 mg, 1.19 mmol, 94%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.49 – 7.21 (m, 22H), 7.19 – 7.10 (m, 1H), 6.95 – 6.76 (m, 2H), 6.48 – 6.36 (m, 1H), 5.60 – 5.46 (m, 2H), 5.30 – 4.75 (m, 12H), 4.70 – 4.52 (m, 2H), 4.41 – 4.09 (m, 7H), 3.71 (dd, J = 11.5, 3.8 Hz, 1H), 2.87 – 2.33 (m, 4H), 2.16 – 2.12 (m, 3H), 2.09 (s, 3H), 1.20 – 1.13 (m, 3H), 1.12 – 0.92 (m, 18H). ^{13}C NMR (101 MHz, Acetone) δ 206.15, 206.04, 172.25, 170.63, 170.54, 168.33, 168.05, 157.75, 144.51, 144.30, 139.06, 139.01, 138.59, 138.57, 138.07, 136.18, 136.15, 129.53, 129.50, 129.35, 129.25, 129.18, 129.15, 129.11, 129.08, 129.03, 129.01, 128.94, 128.90, 128.86, 128.79, 128.74, 128.73, 128.67, 128.58, 128.46, 128.43, 128.31, 128.29, 125.06, 120.07, 119.93, 99.57, 99.49, 93.78, 93.68, 77.12, 76.84, 76.54, 75.86, 73.78, 73.33, 73.19, 73.03, 72.90, 72.63, 72.42, 72.40, 71.01, 70.97, 70.34, 70.23, 70.01, 69.97, 69.26, 67.57, 67.36, 67.22, 66.75, 65.78, 58.03, 57.99, 55.25, 53.49, 38.04, 29.78, 29.60, 28.58, 27.99, 27.79, 27.66, 23.64, 23.60, 21.36, 21.34, 21.14, 21.09, 17.01. HR-MS: Calculated for $C_{70}H_{82}F_3N_5O_{19}Si$ [M+Na^+]: 1404.5218, found: 1404.5259. TLC: Rf = 0.2 (PE/EA = 3/1, v/v).

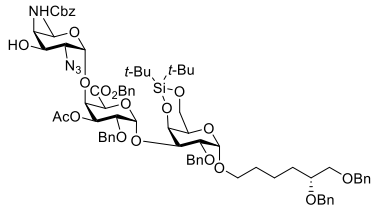
(_R_)-5,6-bis(benzyloxy)hexyl 2-_O_-benzyl-3-_O_-(benzyl 3-_O_-acetyl-2-_O_-benzyl-4-_O_-(2-azido-3-_O_-levulinoyl-4-_N_-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-4,6-_O_-di-_tert_-butylsilylidene-α-D-galactopyranoside (40)



Donor **7** (540 mg, 0.39 mmol, 1.0 eq) and the linker acceptor **29** (368 mg, 1.17 mmol, 3.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (5 mL) and 4Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 ℃ and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (18 μL, 0.08 mmol, 0.2 eq) was added. After stirred 2 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 8:1:1 - 4:1:1) to yield desired α anomer

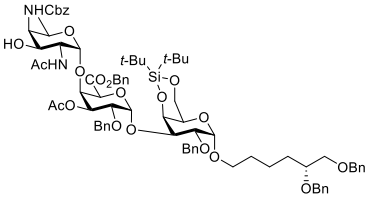
compound **40** (501 mg, 0.33 mmol, 85%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.45 – 7.17 (m, 30H), 5.59 (d, *J* = 3.5 Hz, 1H, H-1b), 5.51 (dd, *J* = 10.7, 2.9 Hz, 1H, H-3b), 5.31 – 5.23 (m, 1H, CH_2), 5.16 (d, *J* = 12.3 Hz, 1H, CH_2), 5.11 – 4.98 (m, 3H, H-3c, CH_2), 4.87 – 4.74 (m, 4H, CH_2, H-5b), 4.72 – 4.60 (m, 5H, CH_2, H-4a, H-1a, H-1c), 4.59 – 4.44 (m, 5H, CH_2, H-4b), 4.19 – 4.09 (m, 3H, CH_2, H-4c, H-5c), 4.09 – 3.99 (m, 3H, CH_2, H-3a, H-2b), 3.95 (dd, *J* = 10.2, 3.7 Hz, 1H, H-2a), 3.61 – 3.47 (m, 5H, H-5a, H-7, H-12, H-11), 3.43 – 3.35 (m, 1H, H-7), 3.16 – 3.10 (m, 1H, H-2c), 2.86 – 2.39 (m, 4H, Lev), 2.18 (s, 3H, Lev), 1.97 (s, 3H, OAc), 1.63 – 1.28 (m, 6H, H-8, H-9, H-10), 1.07 – 0.97 (m, 12H, H-6c), 0.87 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.48 (Lev), 172.09 (Lev), 170.21 (OAc), 167.62 (CO_2Bn), 156.67 (Cbz), 138.98, 138.50, 138.36, 138.06, 136.39, 134.94, 128.90, 128.75, 128.64, 128.59, 128.50, 128.48, 128.46, 128.42, 128.14, 127.92, 127.91, 127.88, 127.86, 127.84, 127.75, 127.72, 127.70, 127.64, 98.56 (C-1c, J_{CH} = 171.5 Hz), 97.65 (C-1a, J_{CH} = 167.6 Hz), 92.05 (C-1b, J_{CH} = 171.0 Hz), 78.23 (C-11), 77.04 (C-4b), 73.65, 73.45, 73.16 (C-2a), 73.00 (C-3a), 72.95 (C-12), 72.41 (C-2b), 72.24, 71.65, 70.26 (C-3c), 70.22 (C-3b), 69.97 (C-5b), 69.52 (C-4a), 68.11 (C-7), 67.45 (CO_2Bn), 67.25 (Cbz), 66.92 (C-5a), 64.80 (C-5c), 57.69 (C-2c), 52.61 (C-4c), 38.07 (Lev), 31.86, 29.94 (Lev), 29.46, 28.08 (Lev), 27.87, 27.31, 23.35, 22.02, 21.43 (OAc), 20.69, 16.79 (C-6c). HR-MS: Calculated for $C_{82}H_{102}N_4O_{21}Si$ [M+NH_4^+]: 1524.71440, found: 1524.71125. $[\alpha]^{20}_D$ = + 140.4° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/EA/DCM = 4:1:1, v/v).

(*R*)-5,6-bis(benzyloxy)hexyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-azido-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-4,6-*O*-di-*tert*-butylsilylidene-α-D-galactopyranoside (41)



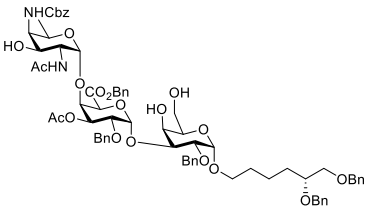
The compound **40** (356.6 mg, 0.237 mmol, 1.0 eq) was dissolved in pyridine (4 mL) and acetic acid (1 mL). After cooled to 0 °C, hydrazine hydrate (N_2H_4 • H_2O 50-60 %) (57 µL, 1.18 mmol, 5.0 eq) was added slowly. After stirred 20 min at RT, checked by TLC complete consumption of the starting material, quenched by acetone. 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/DCM 10:1:1 – 6:1:1) to yield compound **41** (317 mg, 0.225 mmol, 95%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.14 (m, 30H), 5.60 (d, *J* = 3.6 Hz, 1H, H-1b), 5.54 (dd, *J* = 10.7, 2.8 Hz, 1H), 5.30 – 5.19 (m, 1H), 5.18 – 4.98 (m, 4H), 4.87 – 4.74 (m, 3H), 4.73 – 4.57 (m, 5H, H-1a, H-1c), 4.57 – 4.41 (m, 5H), 4.19 – 3.85 (m, 8H), 3.64 – 3.33 (m, 7H), 2.95 (dd, *J* = 10.7, 3.8 Hz, 1H), 2.00 (s, 3H), 1.65 – 1.28 (m, 6H), 1.06 (d, *J* = 6.5 Hz, 3H), 1.01 (d, *J* = 5.7 Hz, 9H), 0.89 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.04, 167.54, 158.06, 138.83, 138.34, 138.19, 138.00, 135.88, 134.83, 128.64, 128.60, 128.54, 128.47, 128.37, 128.34, 128.33, 128.30, 128.27, 128.17, 128.15, 127.74, 127.69, 127.68, 127.63, 127.58, 127.55, 127.53, 127.47, 98.79 (C-1), 97.45 (C-1), 91.94 (C-1), 78.04, 76.93, 73.39, 73.26, 73.02, 72.85, 72.75, 72.13, 72.06, 71.21, 70.19, 69.91, 69.44, 68.18, 67.91, 67.49, 67.30, 67.11, 66.76, 65.02, 60.40, 55.79, 31.66, 29.29, 27.72, 27.18, 23.18, 21.86, 21.28, 20.55, 16.79. HR-MS: Calculated for $C_{77}H_{96}N_4O_{19}Si$ [M+NH_4^+]: 1426.67763, found: 1426.67712. $[\alpha]^{20}_D$ = + 120.5° (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/DCM/EA = 6/1/1, v/v/v).

(*R*)-5,6-bis(benzyloxy)hexyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-acetylamino-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-4,6-*O*-di-*tert*-butylsilylidene-α-D-galactopyranoside (42)



The compound **41** (97 mg, 0.07 mmol, 1.0 eq) was dissolved in THF (2 mL) and water (20 µL). Pyridine (88 µL, 1.1 mmol, 15 eq) and Ph_3P (72 mg, 0.27 mmol, 4.0 eq) were added and the reaction was allowed to stir for 7 h at 70 °C. After TLC showed complete consumption of the starting material, the reaction mixture was concentrated *in vacuo* and co-evaporated by toluene. The residue was dissolved in THF (2 ml) and water (0.5 mL), then sodium bicarbonate (24 mg, 0.29 mmol, 4.0 eq) and acetic anhydride (14 µL, 0.15 mmol, 2.0 eq) were added and stirred for overnight. After TLC showed complete consumption of the starting material, the reaction mixture was diluted with EtOAc and then washed with saturated aqueous sodium bicarbonate 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 (DCM/Acetone 10:1 – 5:1) to yield compound **42** (98 mg, 0.07 mmol, quantitative). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.41 – 7.19 (m, 30H), 5.98 (d, *J* = 8.6 Hz, 1H), 5.61 – 5.50 (m, 2H), 5.41 – 5.17 (m, 2H), 5.15 – 4.89 (m, 3H), 4.88 – 4.72 (m, 3H), 4.72 – 4.59 (m, 4H), 4.58 – 4.43 (m, 4H), 4.38 – 4.20 (m, 2H), 4.18 – 4.10 (m, 1H), 4.09 – 3.78 (m, 8H), 3.76 – 3.68 (m, 1H), 3.65 – 3.47 (m, 5H), 3.45 – 3.36 (m, 1H), 2.14 – 1.97 (m, 6H), 1.65 – 1.28 (m, 6H), 1.12 – 0.83 (m, 21H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.63, 170.07, 168.27, 157.62, 138.92, 138.39, 138.25, 137.84, 136.33, 134.04, 129.11, 129.00, 128.94, 128.60, 128.55, 128.47, 128.45, 128.41, 128.39, 128.33, 128.21, 128.16, 127.87, 127.83, 127.79, 127.76, 127.66, 127.60, 98.51, 97.57, 92.29, 78.13, 76.42, 73.64, 73.40, 73.28, 73.14, 72.86, 72.19, 72.12, 71.51, 70.11, 69.87, 69.49, 69.13, 68.13, 67.71, 67.20, 67.16, 66.77, 66.04, 55.37, 50.80, 31.81, 29.41, 27.85, 27.25, 23.43, 23.38, 21.98, 21.41, 20.65, 16.95. HR-MS: Calculated for $C_{79}H_{100}N_2O_{20}Si$ [M+H$^+$]: 1425.67115, found: 1425.67113. $[\alpha]^{20}_D$ = + 104° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (DCM/Acetone = 4/1, v/v).

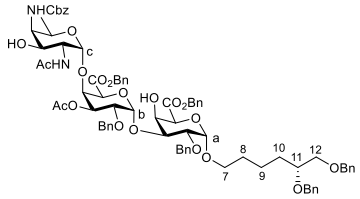
(*R*)-5,6-bis(benzyloxy)hexyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-acetylamino-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-α-D-galactopyranoside (4)



The compound **42** (94 mg, 0.066 mmol, 1.0 eq) was dissolved in THF (1 mL) and pyridine (1 mL), then cooled to 0 °C and hydrogen fluoride (HF)/pyridine (70%) (0.1 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 (DCM/Acetone 3:1 - 2:1) to yield compound **4** (80 mg, 0.062 mmol, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.09 (m, 30H), 5.97 (dd, *J* = 8.2, 4.1 Hz, 1H), 5.37 – 5.26 (m, 2H), 5.21 (d, *J* = 12.2 Hz, 1H), 5.10 – 4.97 (m, 3H), 4.90 – 4.78 (m, 2H), 4.76 –

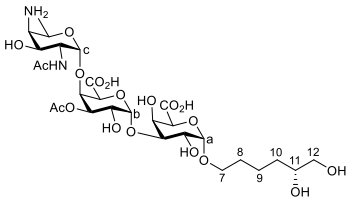
4.45 (m, 9H), 4.29 – 4.23 (m, 1H), 4.16 – 4.00 (m, 4H), 3.99 – 3.46 (m, 13H), 3.41 – 3.29 (m, 2H), 2.82 – 2.68 (m, 1H), 2.22 – 1.94 (m, 6H), 1.76 – 1.28 (m, 6H), 1.12 – 0.95 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.01, 170.37, 167.95, 157.77, 138.81, 138.37, 138.21, 136.70, 136.29, 134.03, 129.00, 128.90, 128.81, 128.73, 128.62, 128.50, 128.45, 128.42, 128.26, 128.15, 128.05, 127.84, 127.82, 127.77, 127.71, 127.68, 127.65, 98.31, 96.71, 94.18, 78.43, 76.11, 75.06, 74.56, 74.32, 73.41, 72.83, 72.75, 72.41, 72.34, 72.01, 70.46, 70.19, 69.15, 68.75, 67.64, 67.28, 67.12, 66.07, 62.85, 55.29, 50.72, 31.60, 29.31, 23.36, 21.99, 21.41, 16.99. HR-MS: Calculated for $C_{71}H_{84}N_2O_{20}$ [M+H$^+$]: 1285.56902, found: 1285.56928. $[\alpha]^{20}_D$ = + 105 ° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (DCM/Acetone = 2/1, v/v).

Benzyl ((*R*)-5,6-bis(benzyloxy)hexyl 2-*O*-benzyl-3-*O*-(benzyl 3-*O*-acetyl-2-*O*-benzyl-4-*O*-(2-acetylamino-4-*N*-benzyloxycarbonyl-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-α-D-galactopyranosyl uronate) (43)



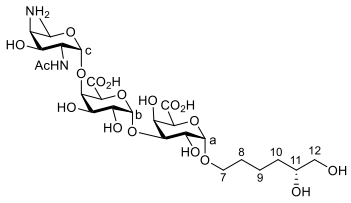
The compound **4** (62 mg, 0.05 mmol, 1.0 eq) was dissolved in DCM/*tert*-BuOH/H_2O (2.25 mL, 4/4/1, v/v/v). The mixture was cooled to 0 ℃ and treated with TEMPO (2.0 mg, 12.8 μmol, 0.25 eq) and BAIB (40.4 mg, 0.12 mmol, 2.5 eq). After stirring for overnight at 4 ℃ and TLC showed complete consumption of the starting material, saturated aqueous sodium thiosulphate was added and diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DMF (2 mL), followed by addition of Cs_2CO_3 (19 mg, 0.06 mmol, 1.2 eq) and BnBr (12 μL, 0.1 mmol, 2.0 eq) at 0℃. After the mixture was allowed to stir overnight at rt and TLC showed complete consumption of the starting material, the reaction was diluted with EtOAc and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (DCM/Acetone 10:1 – 5:1) yielded **43** (54.6 mg, 0.039 mmol, 81%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.42 – 7.08 (m, 35H), 5.90 (d, *J* = 7.7 Hz, 1H, N*H*Ac), 5.33 (d, *J* = 12.3 Hz, 1H, CH_2), 5.27 (dd, *J* = 10.7, 2.7 Hz, 1H, H-3b), 5.25 – 5.16 (m, 2H, CH_2), 5.12 (d, *J* = 9.8 Hz, 1H, CH_2), 5.07 (d, *J* = 12.2 Hz, 1H, CH_2), 5.01 (d, *J* = 11.8 Hz, 1H, CH_2), 4.93 (d, *J* = 3.6 Hz, 2H, H-1b, H-1a), 4.82 (d, *J* = 11.9 Hz, 1H, CH_2), 4.70 – 4.61 (m, 2H, CH_2, H-5b), 4.61 – 4.48 (m, 7H, CH_2), 4.40 (s, 1H, H-5a), 4.31 – 4.22 (m, 2H, H-4a, H-4b), 4.17 – 4.06 (m, 2H, H-3a, H-1c), 4.06 – 3.98 (m, 2H, H-4c, H-5c), 3.87 (dd, *J* = 9.8, 3.5 Hz, 1H, H-2a), 3.81 (dd, *J* = 10.6, 3.6 Hz, 1H, H-2b), 3.77 – 3.64 (m, 3H, H-3c, H-2c), 3.64 – 3.57 (m, 1H, H-7), 3.57 – 3.45 (m, 3H, H-11, H-12), 3.40 – 3.29 (m, 2H, H-7), 2.11 – 1.98 (m, 6H, NHAc, OAc), 1.61 – 1.49 (m, 4H), 1.49 – 1.38 (m, 1H), 1.38 – 1.28 (m, 1H), 1.06 (d, *J* = 6.3 Hz, 3H, H-6c). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.84 (NHAc), 170.04 (OAc), 168.44, 167.98 (CO_2Bn), 157.62 (Cbz), 138.98, 138.47, 138.02, 136.72, 136.32, 135.50, 134.07, 129.02, 128.99, 128.95, 128.86, 128.70, 128.67, 128.61, 128.59, 128.55, 128.50, 128.46, 128.43, 128.40, 128.31, 128.22, 127.99, 127.94, 127.88, 127.85, 127.81, 127.69, 127.67, 127.57, 98.36 (C-1c), 97.08 (C-1a), 94.54 (C-1b), 77.98 (C-11), 76.22 (C-4b), 74.79 (C-3a), 74.13, 74.07 (C-2a), 73.43, 72.85, 72.81, 72.73, 72.15 (C-2b), 72.09, 70.68 (C-3b), 70.13 (C-5b), 69.48, 69.43 (C-5a, H-5c), 68.82 (C-7), 67.68, 67.34 (Cbz), 67.20, 67.09 (C-4a), 66.12 (C-5c), 55.37 (C-4c), 50.89 (C-2c), 31.87 (C-10), 29.49 (C-8), 23.34 (NHAc), 21.97 (C-9), 21.42 (OAc), 17.05 (C-6c). HR-MS: Calculated for $C_{78}H_{88}N_2O_{21}$ [M+H$^+$]: 1389.59523, found: 1389.59579. $[\alpha]^{20}_D$ = + 90.2° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (DCM/Acetone = 4/1, v/v).

(*R*)-5,6-diol-hexyl 3-*O*-(2-*O*-acetyl-4-*O*-(2-acetylamino-4-amino-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-α-D-galactopyranosyl uronate (1)



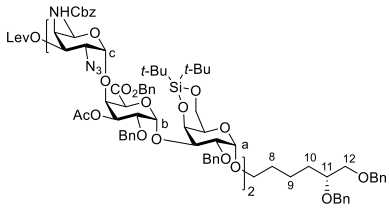
The protected trimer **43** (15 mg, 10.8 µmol, 1.0 eq) was dissolved in *tert*-butanol (7 mL) and water (3 mL). After $Pd(OH)_2/C$ (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo* to yield compound **1** (7.5 mg, 10.5 µmol, quantitative). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.33 (dd, *J* = 10.9, 2.9 Hz, 1H, H-3b), 5.30 (d, *J* = 3.9 Hz, 1H, H-1b), 5.02 – 4.96 (m, 2H, H-1c, H-1a), 4.84 (s, 1H, H-5b), 4.68 – 4.59 (m, 2H, H-5c, H-4b), 4.58 – 4.55 (m, 1H, H-4a), 4.53 (d, *J* = 1.4 Hz, 1H, H-5a), 4.23 (dd, *J* = 11.4, 4.4 Hz, 1H, H-3c), 4.18 – 4.12 (m, 1H, H-2b), 4.07 (dd, *J* = 10.3, 3.1 Hz, 1H, H-3a), 3.98 – 3.91 (m, 2H, H-2c, H-2a), 3.73 – 3.62 (m, 3H, H-7, H-11, H-4c), 3.58 – 3.50 (m, 2H, H-7, H-12), 3.46 – 3.40 (m, 1H, H-12), 2.19 (s, 3H, OAc), 2.03 (s, 3H, NHAc), 1.71 – 1.34 (m, 6H, H-8, H-9, H-10), 1.31 (d, *J* = 6.7 Hz, 3H, H-6c). ^{13}C NMR (101 MHz, D_2O) δ 174.90, 173.35, 172.69, 171.96, 98.46, 97.91 (C-1a, C-1c), 95.82 (C-1b), 76.30 (C-4b), 74.90 (C-3a), 71.63 (C-11), 71.24 (C-3b), 70.21 (C-5a), 70.07 (C-5b), 68.59 (C-7), 67.03 (C-4a), 66.13 (C-2b), 66.03 (C-2a), 65.38 (C-12), 63.73 (C-3c), 63.32 (C-5c), 55.16 (C-4c), 49.21 (C-2c), 31.90, 28.48, 22.23 (NHAc), 21.40, 20.80 (OAc), 16.03 (C-6c). HR-MS: Calculated for $C_{28}H_{46}N_2O_{19}$ [M+H$^+$]: 715.27675, found: 715.27682.

(*R*)-5,6-diol-hexyl 3-*O*-(4-*O*-(2-acetylamino-4-amino-6-deoxy-α-D-galactopyranosyl)-α-D-galactopyranosyl urinate)-α-D-galactopyranosyl uronate (1b)



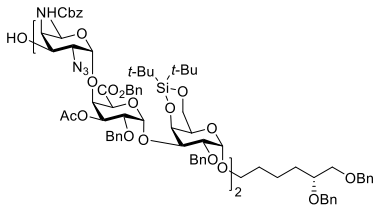
^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.22 (d, *J* = 3.9 Hz, 1H, H-1b), 4.98 – 4.93 (m, 2H, H-1a, H-1c), 4.78 – 4.71 (m, 1H, H-5c), 4.56 (d, *J* = 1.3 Hz, 1H, H-5b), 4.48 (dd, *J* = 3.3, 1.4 Hz, 1H, H-4a), 4.35 (dd, *J* = 3.2, 1.2 Hz, 1H, H-4b), 4.24 (d, *J* = 1.5 Hz, 1H, H-5a), 4.18 (dd, *J* = 11.3, 4.4 Hz, 1H, H-3c), 4.10 (dd, *J* = 10.6, 3.1 Hz, 1H, H-3b), 4.05 – 3.97 (m, 2H, H-3a, H-2c), 3.93 – 3.85 (m, 2H, H-2a, H-2b), 3.74 – 3.65 (m, 2H, H-7, H-11), 3.63 – 3.51 (m, 3H, H-4c, H-12, H-7), 3.49 – 3.41 (m, 1H, H-12), 2.09 (s, 3H, NHAc), 1.73 – 1.34 (m, 6H, H-8, H-9, H-10), 1.25 (d, *J* = 6.7 Hz, 3H, H-6c). ^{13}C NMR (214 MHz, D_2O) δ 175.96, 175.79, 175.14, 99.68 (C-1c, J_{CH} = 174.0 Hz), 99.27 (C-1a, J_{CH} = 172.0 Hz), 97.15 (C-1b, J_{CH} = 170.0 Hz), 80.89 (C-4b), 76.63 (C-3a), 72.56 (C-11), 72.01 (C-5b), 71.84 (C-5a), 69.41 (C-3b), 69.29 (C-7), 68.89 (C-2b), 68.57 (C-4a), 67.39 (C-2a), 66.31 (C-12), 65.51 (C-3c), 64.20 (C-5c), 56.21 (C-4c), 50.26 (C-2c), 32.83, 29.43, 23.24, 22.34, 16.35 (C-6c). HR-MS: Calculated for $C_{26}H_{44}N_2O_{18}$ [M+H$^+$]: 673.26619, found: 673.26633.

Hexamer of two repeating units 44



The donor **7** (194 mg, 0.14 mmol, 2.0 eq) and the acceptor **41** (99 mg, 0.07 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (3 mL) and 4Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and TBSOTf (3.2 µL, 13.9 µmol, 0.2 eq) was added. After stirred 2 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (Tol/EA 8:1 - 8:3) to yield desired α anomer compound **44** (151 mg, 58 µmol, 83%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.43 – 7.15 (m, 50H), 5.61 (d, *J* = 3.5 Hz, 1H, H-1), 5.56 (d, *J* = 3.5 Hz, 1H, H-1), 5.53 – 5.43 (m, 2H), 5.25 – 5.13 (m, 5H, H-1a$_1$), 5.12 – 4.90 (m, 6H), 4.88 – 4.62 (m, 15H, H-1a), 4.59 (dd, *J* = 11.5, 3.8 Hz, 2H, H-1c, H-1c$_1$), 4.56 – 4.40 (m, 8H), 4.29 – 4.09 (m, 7H), 4.09 – 3.97 (m, 6H), 3.97 – 3.86 (m, 2H), 3.71 (s, 1H), 3.62 – 3.44 (m, 5H), 3.43 – 3.35 (m, 1H, H-7), 3.14 (dd, *J* = 11.3, 4.0 Hz, 1H, H-2c$_1$), 3.07 (dd, *J* = 10.8, 3.8 Hz, 1H, H-2c), 2.86 – 2.36 (m, 4H), 2.17 (s, 3H), 2.01 (d, *J* = 8.7 Hz, 6H), 1.62 – 1.27 (m, 6H), 1.08 – 0.97 (m, 21H), 0.88 (s, 9H), 0.80 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.38, 172.00, 170.30, 170.03, 167.39, 167.33, 156.93, 156.57, 138.90, 138.41, 138.26, 138.01, 137.95, 137.92, 136.33, 136.13, 135.00, 134.88, 128.86, 128.68, 128.66, 128.57, 128.53, 128.50, 128.48, 128.42, 128.38, 128.33, 128.31, 128.12, 128.05, 127.83, 127.76, 127.70, 127.65, 127.63, 127.60, 127.56, 127.55, 98.24 (C-1c, C-1c$_1$), 97.58 (C-1a), 93.52 (C-1a$_1$), 92.00 (C-1b), 91.65 (C-1b$_1$), 78.09 (C-11), 77.07, 76.68, 73.50, 73.37, 73.01, 72.94, 72.91, 72.82, 72.58, 72.30, 72.13, 71.37, 71.31, 70.50, 70.33, 70.30, 70.15, 69.87, 69.47, 69.30, 68.08, 67.41, 67.25, 67.19, 67.15, 66.85, 65.92, 64.76, 59.80 (C-2c), 57.66 (C-2c$_1$), 52.51 (C-4c$_1$), 50.41 (C-4c), 37.98, 31.78, 29.85, 29.39, 28.00, 27.91, 27.82, 27.30, 27.24, 27.14, 23.30, 21.93, 21.37, 21.32 (OAc), 20.62, 20.54, 16.69, 16.57 (C-6c, C-6c$_1$). HR-MS: Calculated for $C_{139}H_{172}N_8O_{37}Si_2$ [M+ NH_4^++NH_4^+]/2: 1318.60192, found: 1318.60143. $[\alpha]^{20}_D$ = + 136.5 ° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (Tol/EA = 8:3, v/v).

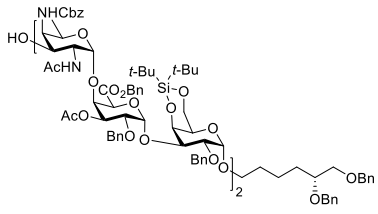
Hexamer of two repeating units 45



The compound **44** (585.1 mg, 0.225 mmol, 1.0 eq) was dissolved in pyridine (4 mL) and acetic acid (1 mL). After cooled to 0 °C, hydrazine hydrate (N_2H_4 • H_2O 50-60 %) (83 µL, 1.7 mmol, 7.5 eq) was added slowly. After stirred 20 min at RT, checked by TLC complete consumption of the starting material, quenched by acetone. 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 (DCM/Acetone 30:1 – 20:1) to yield compound **45** (530 mg, 0.212 mmol, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44 – 7.14 (m, 50H), 5.61 (d, *J* = 3.5 Hz, 1H), 5.55 (d, *J* = 3.5 Hz, 1H), 5.51 – 5.43 (m, 2H), 5.25 – 5.10 (m, 6H), 5.05 (d, *J* = 12.2 Hz, 1H), 5.03 – 4.87 (m, 3H), 4.87 – 4.39 (m, 23H), 4.30 –

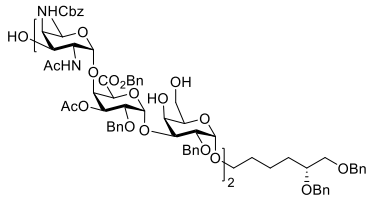
4.10 (m, 6H), 4.10 – 3.86 (m, 11H), 3.72 (s, 1H), 3.62 – 3.45 (m, 5H), 3.44 – 3.34 (m, 1H), 3.08 (dd, *J* = 10.8, 3.7 Hz, 1H), 2.97 (dd, *J* = 10.6, 3.8 Hz, 1H), 2.90 (s, 1H), 2.07 – 1.97 (m, 6H), 1.62 – 1.27 (m, 6H), 1.10 – 0.98 (m, 21H), 0.92 – 0.77 (m, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.36, 170.07, 167.43, 158.26, 156.99, 138.97, 138.47, 138.32, 138.10, 138.02, 136.19, 135.92, 135.07, 134.91, 128.89, 128.78, 128.75, 128.68, 128.62, 128.58, 128.57, 128.54, 128.48, 128.43, 128.38, 128.34, 128.32, 128.17, 127.89, 127.82, 127.71, 127.68, 127.62, 127.54, 98.64, 98.31, 97.64, 93.54, 92.07, 91.72, 78.16, 76.90, 73.56, 73.44, 73.05, 72.89, 72.40, 72.19, 71.43, 71.12, 70.63, 70.36, 70.01, 69.94, 69.53, 69.44, 68.95, 68.16, 67.74, 67.47, 67.38, 67.25, 66.91, 65.97, 65.11, 60.92, 59.89, 55.80, 50.48, 31.84, 29.45, 27.98, 27.88, 27.29, 27.19, 23.36, 21.98, 21.42, 21.38, 20.68, 20.60, 16.91, 16.64. HR-MS: Calculated for $C_{134}H_{166}N_8O_{35}Si_2$ [M+H$^+$]: 2504.10669, found: 2504.10992. $[\alpha]^{20}_D$ = + 140.9° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (DCM/Acetone = 20/1, v/v).

Hexamer of two repeating units 46



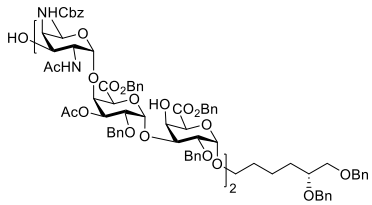
The compound **45** (85 mg, 34 μmol, 1.0 eq) was dissolved in THF (2 mL) and water (20 μL). Pyridine (42 μL, 0.5 mmol, 15 eq) and Ph_3P (37 mg, 0.14 mmol, 4.0 eq) were added and the reaction was allowed to stir for 7 h at 70 °C. After TLC showed complete consumption of the starting material, the reaction mixture was concentrated *in vacuo* and co-evaporated by toluene. The residue was dissolved in THF (1 ml) and water (0.5 mL), then sodium bicarbonate (12 mg, 0.14 mmol, 4.0 eq) and acetic anhydride (14 μL, 0.15 mmol, 4.0 eq) were added and stirred for overnight. After TLC showed complete consumption of the starting material, the reaction mixture was diluted with EtOAc and then washed with saturated aqueous sodium bicarbonate 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 (DCM/Acetone 5:1 – 4:1) to yield compound **46** (75.6 mg, 29.8 μmol, 88%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.51 – 6.96 (m, 50H), 6.17 – 6.05 (m, 1H), 5.63 – 5.46 (m, 5H), 5.34 – 4.88 (m, 10H), 4.88 – 4.59 (m, 13H), 4.59 – 4.41 (m, 5H), 4.40 – 4.29 (m, 2H), 4.29 – 3.81 (m, 16H), 3.78 – 3.31 (m, 11H), 2.61 (s, 1H), 2.16 – 1.92 (m, 12H), 1.65 – 1.29 (m, 6H), 1.13 – 0.74 (m, 42H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.88, 170.27, 170.16, 169.86, 168.35, 157.59, 156.96, 139.02, 138.46, 138.37, 138.28, 137.96, 137.91, 136.76, 136.42, 134.59, 134.08, 129.17, 128.94, 128.88, 128.82, 128.63, 128.50, 128.46, 128.40, 128.35, 128.25, 128.21, 128.04, 127.93, 127.86, 127.81, 127.68, 127.58, 99.10, 98.48, 97.54, 96.40, 92.84, 91.70, 78.15, 76.62, 73.46, 73.30, 73.19, 72.98, 72.91, 72.86, 72.43, 72.16, 72.05, 71.76, 71.59, 71.21, 70.21, 70.06, 69.96, 69.69, 69.64, 69.29, 68.31, 68.21, 67.80, 67.71, 67.64, 67.26, 67.23, 67.13, 66.93, 66.67, 66.61, 66.03, 55.47, 51.66, 51.09, 48.78, 31.87, 29.49, 27.91, 27.81, 27.30, 27.21, 23.46, 23.39, 23.31, 22.00, 21.42, 21.37, 20.68, 17.03, 16.94. HR-MS: Calculated for $C_{138}H_{174}N_4O_{37}Si_2$ [M+H$^+$+NH$_4^+$]/2: 1277.09032, found: 1277.09029. $[\alpha]^{20}_D$ = + 128.3° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (DCM/Acetone = 4/1, v/v).

Hexamer of two repeating units 5



The compound **46** (73 mg, 28.8 µmol, 1.0 eq) was dissolved in THF (1 mL) and pyridine (1 mL), then cooled to 0 °C and hydrogen fluoride (HF)/pyridine (70%) (0.1 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 (DCM/Acetone 4:1 - 1:1) to yield compound **5** (60 mg, 26.6 µmol, 92%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.41 – 7.09 (m, 50H), 6.09 – 5.92 (m, 2H), 5.40 – 4.86 (m, 13H), 4.86 – 4.41 (m, 17H), 4.38 (d, *J* = 3.7 Hz, 1H), 4.33 (d, *J* = 2.9 Hz, 1H), 4.25 (d, *J* = 10.8 Hz, 1H), 4.19 – 3.46 (m, 27H), 3.41 – 3.27 (m, 3H), 2.93 – 2.55 (m, 3H), 2.14 – 1.96 (m, 12H), 1.72 – 1.32 (m, 6H), 1.11 – 0.97 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.98, 170.85, 170.03, 169.87, 167.71, 157.55, 156.91, 138.79, 138.34, 138.25, 138.12, 137.03, 136.70, 136.45, 136.30, 134.20, 134.00, 129.06, 128.85, 128.83, 128.79, 128.78, 128.72, 128.66, 128.62, 128.59, 128.50, 128.43, 128.41, 128.15, 128.06, 127.87, 127.74, 127.72, 127.69, 127.66, 127.27, 98.61, 98.26, 96.72, 94.40, 94.25, 78.45, 78.02, 76.15, 75.54, 75.21, 74.55, 74.32, 74.22, 74.14, 73.99, 73.40, 72.87, 72.81, 72.54, 72.34, 72.09, 72.00, 70.71, 70.36, 70.23, 70.13, 69.28, 68.83, 67.62, 67.59, 67.22, 66.94, 66.81, 66.60, 66.03, 63.27, 62.87, 55.28, 50.95, 50.85, 48.50, 31.56, 29.73, 29.26, 23.56, 23.29, 21.96, 21.38, 21.35, 17.00, 16.98. HR-MS: Calculated for $C_{122}H_{142}N_4O_{37}$ $[M+2H^+]/2$: 1128.47492, found: 1128.47435. $[\alpha]^{20}_D$ = + 137.6 ° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (DCM/Acetone = 3/2, v/v).

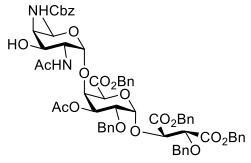
Hexamer of two repeating units 47



The compound **5** (11.6 mg, 5.14 µmol, 1.0 eq) was dissolved in EtOAc/*tert*-BuOH/H_2O (375 µL, 2/2/1, v/v/v). The mixture was cooled to 0 °C and treated with TEMPO (1.4 mg, 8.96 µmol, 1.7 eq), BAIB (14 mg, 42 mmol, 8 eq) and $NaHCO_3$ (4.5 mg, 53.6 µmol, 10 eq). After stirring for 24 hours at 4 °C and TLC showed complete consumption of the starting material, saturated aqueous sodium thiosulphate was added and diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DCM (2 mL), followed by addition of 0.2M phenyldiazomethane ($PhCHN_2$) in Et_2O (1 mL) at RT. After the mixture was allowed to stir overnight at rt and TLC showed complete consumption of the starting material, the reaction was diluted with EtOAc and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by preparative TLC plates (Macherey-Nagel, pre-coated TLC plates SIL G-100 UV254) (DCM/Acetone/MeOH 16:4:0.4) yielded **47** (7.4 mg, 3.0 µmol, 64%). ^{1}H NMR (850 MHz, Chloroform-*d*) δ 7.41 – 7.14 (m, 60H), 5.94 (s, 1H), 5.74 (d, *J* = 9.3 Hz, 1H), 5.32 – 5.28 (m, 2H), 5.27 – 5.22 (m, 3H), 5.20 – 5.14 (m, 2H), 5.13 – 5.10 (m, 1H), 5.02 (d, *J* = 11.8 Hz, 1H), 5.00 – 4.95 (m, 2H), 4.95 – 4.90 (m, 3H), 4.88 – 4.81 (m, 2H), 4.76 (d, *J* = 11.5 Hz, 1H), 4.72 (d, *J* = 12.2 Hz, 1H), 4.67

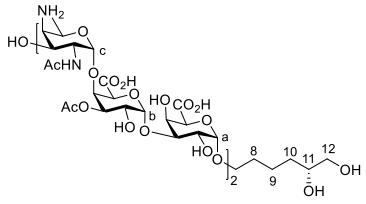
(d, *J* = 11.7 Hz, 2H), 4.63 (s, 1H), 4.60 – 4.45 (m, 14H), 4.40 – 4.36 (m, 2H), 4.30 – 4.20 (m, 4H), 4.13 – 4.07 (m, 2H), 4.06 – 4.01 (m, 1H), 3.98 – 3.90 (m, 4H), 3.89 – 3.82 (m, 2H), 3.82 – 3.75 (m, 2H), 3.68 – 3.46 (m, 9H), 3.40 – 3.30 (m, 3H), 2.09 (s, 3H), 2.03 – 1.96 (m, 9H), 1.59 – 1.28 (m, 6H), 1.06 – 0.99 (m, 6H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 173.09, 170.88, 170.19, 169.90, 168.56, 168.51, 167.84, 167.73, 157.59, 156.83, 138.97, 138.48, 138.31, 138.03, 137.00, 136.65, 136.49, 136.23, 135.51, 135.38, 134.13, 134.06, 129.22, 128.99, 128.97, 128.95, 128.87, 128.82, 128.78, 128.76, 128.73, 128.64, 128.59, 128.57, 128.56, 128.54, 128.52, 128.48, 128.45, 128.39, 128.37, 128.06, 127.94, 127.86, 127.83, 127.74, 127.73, 127.64, 127.45, 98.62, 98.09, 97.09, 95.48, 94.53, 77.99, 76.23, 76.14, 74.82, 74.32, 74.10, 74.02, 73.86, 73.46, 73.12, 72.84, 72.76, 72.14, 72.12, 72.06, 70.55, 70.34, 70.28, 70.16, 69.94, 69.45, 68.84, 67.74, 67.63, 67.49, 67.26, 67.19, 67.11, 66.61, 65.91, 55.32, 50.99, 31.89, 29.85, 29.49, 23.52, 23.33, 22.85, 21.98, 21.45, 17.18, 17.12. HR-MS: Calculated for $C_{136}H_{150}N_4O_{39}$ [M+Na$^+$]: 2485.97694, found: 2485.97705. $[\alpha]^{20}_D$ = + 117° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (DCM/Acetone/MeOH = 16/4/0.4, v/v/v).

Side-product from the oxidation 47e



^{1}H NMR (850 MHz, Chloroform-*d*) δ 7.42 – 7.16 (m, 30H), 5.90 (d, *J* = 8.4 Hz, 1H, N*H*Ac), 5.42 (d, *J* = 3.5 Hz, 1H, H-1b), 5.24 (dd, *J* = 10.8, 2.7 Hz, 1H, H-3b), 5.17 (d, *J* = 11.8 Hz, 1H, C*H*$_2$), 5.14 – 4.99 (m, 6H, C*H*$_2$), 4.92 (d, *J* = 11.8 Hz, 1H, C*H*$_2$), 4.86 (d, *J* = 9.8 Hz, 1H, N*H*Cbz), 4.81 (d, *J* = 5.9 Hz, 1H, H-3a), 4.71 – 4.66 (m, 2H, C*H*$_2$), 4.60 (s, 1H, H-5b), 4.54 – 4.48 (m, 1H, H-2a), 4.43 (d, *J* = 11.1 Hz, 1H, C*H*$_2$), 4.35 (d, *J* = 12.3 Hz, 1H, C*H*$_2$), 4.21 – 4.17 (m, 1H, H-4b), 4.08 (d, *J* = 3.8 Hz, 1H, , H-1c), 3.92 – 3.88 (m, 2H, H-5c, H-4c), 3.82 – 3.77 (m, 1H, H-2b), 3.70 – 3.52 (m, 2H, H-3c, H-3c), 3.19 (d, *J* = 6.5 Hz, 1H, 3c-OH), 2.11 – 2.07 (m, 3H, NHAc), 2.03 (s, 3H, OAc), 1.02 (d, *J* = 6.3 Hz, 3H, H-6c). ^{13}C NMR (214 MHz, $CDCl_3$) δ 172.79 (NHAc), 170.08 (OAc), 169.21, 168.24, 168.02, 157.67 (Cbz), 137.58, 136.74, 136.27, 135.20, 134.86, 134.27, 129.01, 128.98, 128.93, 128.85, 128.83, 128.82, 128.76, 128.73, 128.65, 128.58, 128.57, 128.53, 128.52, 128.47, 128.45, 128.43, 128.36, 128.23, 128.15, 127.94, 127.75, 98.36 (C-1c), 95.70 (C-1b), 78.58 (C-2a), 76.15 (C-4b), 75.09 (C-3a), 73.67, 71.73, 71.06 (C-2b), 70.26 (C-5b), 69.79 (C-3b, C-3c), 67.66, 67.61, 67.49, 67.40, 65.92 (C-5c), 55.44 (C-4c), 50.95 (C-2c), 23.38 (NHAc), 21.46 (OAc), 17.08 (C-6c). HR-MS: Calculated for $C_{63}H_{66}N_2O_{18}$ [M+H$^+$]: 1139.43834, found: 1139.43641. $[\alpha]^{20}_D$ = + 63° (c = 0.1, $CHCl_3$). TLC: Rf = 0.5 (DCM/Acetone/MeOH = 16/4/0.4, v/v/v).

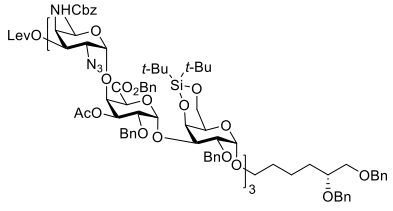
The deprotection of Hexamer 2



The protected hexamer **47** (9.8 mg, 4.3 µmol, 1.0 eq) was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (2 mL). After $Pd(OH)_2$/C (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo* to yield compound **2** (5.1 mg, 3.94 µmol, 91%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.30 – 5.21 (m, 4H, H-3b, H-1b, H-1b$_1$, H-3b$_1$), 5.04 (d, *J* = 4.2 Hz, 1H, H-1a$_1$), 5.00 (d, *J* = 4.0 Hz, 1H, H-1c), 4.92 (dd, *J* = 12.0, 3.9 Hz, 2H, H-1a, H-1c$_1$), 4.66 (s, 1H, H-5b$_1$), 4.62 – 4.49 (m, 5H, H-5c, H-5b, H-4b, H-5c$_1$, H-4b$_1$),

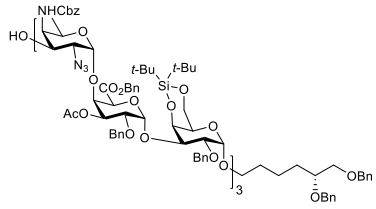
4.47 – 4.41 (m, 2H, H-4a, H-4a$_1$), 4.25 – 4.19 (m, 2H, H-3c, H-5a), 4.16 – 4.06 (m, 5H, H-2b, H-2b$_1$, H-3c$_1$, H-2c, H-5a$_1$), 4.04 – 3.97 (m, 3H, H-3a, H-3a$_1$, H-2c$_1$), 3.94 (dd, *J* = 10.3, 4.1 Hz, 1H, H-2a$_1$), 3.89 (dd, *J* = 10.3, 3.9 Hz, 1H, H-2a), 3.74 (s, 1H, H-4c), 3.69 – 3.63 (m, 2H, H-7, H-11), 3.55 – 3.49 (m, 2H, H-12, H-7), 3.49 – 3.43 (m, 1H, H-4c$_1$), 3.44 – 3.39 (m, 1H, H-12), 2.19 – 2.14 (m, 6H, OAc), 2.05 (s, 3H, 2c$_1$-NHAc), 1.99 (s, 3H, 2c-NHAc), 1.68 – 1.34 (m, 6H, H-8, H-9, H-10), 1.32 – 1.23 (m, 6H, H-6c, H-6c$_1$). ^{13}C NMR (214 MHz, D_2O) δ 176.51 (CO_2H), 176.01 (NHAc), 175.87 (CO_2H), 175.57 (NHAc), 175.16 (CO_2H), 175.01 (CO_2H), 174.51 (OAc), 174.40 (OAc), 99.42 (C-1a$_1$, C-1c$_1$), 99.26 (C-1a), 98.62 (C-1c), 97.11 (C-1b$_1$), 96.93 (C-1b), 78.25 (C-4b$_1$), 77.51 (C-4b), 76.83 (C-3a), 76.54 (C-3a$_1$), 74.05 (C-3c), 73.12 (C-5a$_1$), 72.90 (C-3b), 72.81 (C-3b$_1$), 72.59 (C-11), 72.13 (C-5a), 71.92 (C-5b$_1$), 71.74 (C-5b), 69.22 (C-7), 68.69 (C-4a, C-4a$_1$), 67.43 (C-2a), 67.19 (C-2b$_1$), 67.16 (C-2b), 66.62 (C-2a$_1$), 66.31 (C-12), 66.01 (C-3c$_1$), 65.29 (C-5c$_1$), 64.18 (C-5c), 55.82 (C-4c$_1$), 53.59 (C-4c), 50.20 (C-2c$_1$), 48.48 (C-2c), 32.85, 29.46, 23.32, 23.22, 22.40, 21.75, 21.74, 16.99, 16.95 (C-6c, C-6c$_1$). HR-MS: Calculated for $C_{50}H_{78}N_4O_{35}$ [M+2H$^+$]/2: 648.22961, found: 648.22942.

Nomamer of three repeating units 48



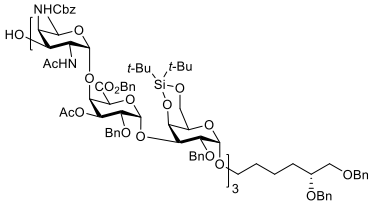
The donor **7** (248 mg, 0.18 mmol, 2.0 eq) and the acceptor **45** (224 mg, 0.09 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (3 mL) and 4Å molecular sieves were added and then the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and TBSOTf (5 µL, 19.5 µmol, 0.2 eq) was added. After stirred 2 hours and TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography (Tol/EA 8:1 - 8:3) to yield desired α anomer compound **48** (283 mg, 76.5 µmol, 85%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 – 7.12 (m, 72H), 5.66 – 5.54 (m, 3H), 5.53 – 5.42 (m, 3H), 5.26 – 4.90 (m, 14H), 4.90 – 4.62 (m, 19H), 4.62 – 4.37 (m, 12H), 4.32 – 3.86 (m, 23H), 3.71 (s, 2H), 3.63 – 3.46 (m, 5H), 3.45 – 3.35 (m, 1H), 3.20 – 3.02 (m, 3H), 2.86 – 2.36 (m, 4H), 2.16 (s, 3H), 2.08 – 1.95 (m, 9H), 1.62 – 1.26 (m, 6H), 1.12 – 0.71 (m, 63H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.35, 171.99, 170.27, 170.17, 170.01, 167.36, 167.33, 167.08, 156.92, 156.57, 138.90, 138.40, 138.26, 137.99, 137.97, 137.94, 137.91, 136.32, 136.14, 135.00, 134.93, 134.82, 128.84, 128.69, 128.66, 128.64, 128.56, 128.52, 128.46, 128.42, 128.37, 128.32, 128.30, 128.24, 128.11, 128.04, 127.83, 127.79, 127.76, 127.69, 127.64, 127.62, 127.55, 127.38, 98.20, 97.95, 97.57, 93.48, 91.99, 91.67, 91.62, 78.08, 77.36, 77.04, 76.63, 73.50, 73.36, 73.04, 72.99, 72.94, 72.91, 72.87, 72.81, 72.60, 72.30, 72.12, 71.99, 71.36, 71.31, 71.15, 70.50, 70.32, 70.27, 70.15, 69.89, 69.47, 69.34, 68.18, 68.08, 67.42, 67.22, 67.14, 66.84, 65.90, 64.75, 59.90, 59.81, 57.65, 52.51, 50.43, 37.97, 31.76, 29.83, 29.37, 27.94, 27.90, 27.82, 27.23, 27.14, 27.12, 23.30, 23.28, 21.91, 21.35, 21.31, 20.62, 20.53, 16.68, 16.60, 16.56. HR-MS: Calculated for $C_{196}H_{242}N_{12}O_{53}Si_3$ [M+H$^+$+NH$_4^+$]/2: 1857.31645, found: 1857.32031. $[\alpha]^{20}_D$ = + 161.8 ° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (Tol/EA = 8:3, v/v).

Nonamer of three repeating units 49



The compound **48** (128 mg, 34.6 µmol, 1.0 eq) was dissolved in pyridine (2 mL) and acetic acid (0.5 mL). After cooled to 0 °C, hydrazine hydrate (N_2H_4 • H_2O 50-60 %) (8.4 µL, 0.17 mmol, 5 eq) was added slowly. After stirred 20 min at RT, checked by TLC complete consumption of the starting material, quenched by acetone. 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 (DCM/Acetone 25:1 – 15:1) to yield compound **49** (111.4 mg, 30.9 µmol, 89%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.11 (m, 70H), 5.65 – 5.54 (m, 3H), 5.49 (d, *J* = 10.6, 2.2 Hz, 3H), 5.25 – 4.92 (m, 15H), 4.89 – 4.36 (m, 31H), 4.30 – 3.87 (m, 24H), 3.71 (s, 2H), 3.67 – 3.45 (m, 6H), 3.44 – 3.34 (m, 1H), 3.23 – 3.08 (m, 2H), 2.99 (dd, *J* = 10.6, 3.8 Hz, 1H), 2.10 – 1.94 (m, 9H), 1.64 – 1.28 (m, 6H), 1.12 – 0.97 (m, 36H), 0.92 – 0.73 (m, 27H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.29, 170.17, 170.02, 167.36, 167.07, 158.16, 156.98, 152.90, 138.89, 138.39, 138.25, 138.03, 137.96, 137.92, 137.88, 136.16, 135.90, 135.01, 134.91, 134.81, 128.79, 128.69, 128.63, 128.60, 128.54, 128.52, 128.46, 128.44, 128.42, 128.37, 128.32, 128.29, 128.25, 128.21, 128.09, 127.83, 127.79, 127.76, 127.71, 127.64, 127.62, 127.56, 127.45, 127.37, 98.57, 98.30, 97.99, 97.56, 93.41, 91.99, 91.65, 91.58, 78.08, 77.36, 77.06, 76.77, 73.49, 73.36, 73.04, 72.99, 72.90, 72.88, 72.79, 72.74, 72.62, 72.40, 72.28, 72.11, 71.98, 71.37, 71.13, 71.04, 70.50, 70.28, 69.95, 69.91, 69.47, 69.41, 69.32, 68.70, 68.08, 67.61, 67.42, 67.30, 67.21, 67.13, 66.84, 65.91, 65.09, 60.78, 59.93, 59.81, 55.77, 50.44, 31.75, 29.37, 27.95, 27.91, 27.82, 27.23, 27.12, 23.30, 23.28, 21.90, 21.35, 21.31, 20.62, 20.53, 16.83, 16.60. HR-MS: Calculated for $C_{191}H_{236}N_{12}O_{51}Si_3$ $[M+H^+ + H^+]/2$: 1799.78479, found: 1799.78658. $[\alpha]^{20}_D$ = + 139.5 ° (c = 1, $CHCl_3$). TLC: Rf = 0.5 (DCM/MeOH = 60/1, v/v).

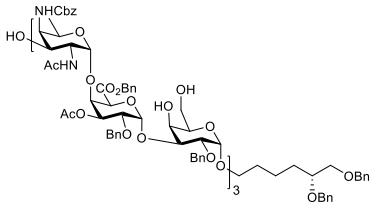
Nonamer of three repeating units 50



The compound **49** (105 mg, 29.2 µmol, 1.0 eq) was dissolved in THF (2 mL) and water (24 µL). Pyridine (106 µL, 1.31 mmol, 45 eq) and Ph_3P (92 mg, 0.35 mmol, 12.0 eq) were added and the reaction was allowed to stir for 7 h at 70 °C. After TLC showed complete consumption of the starting material, the reaction mixture was concentrated *in vacuo* and co-evaporated by toluene. The residue was dissolved in THF (3 ml) and water (1 mL), then sodium bicarbonate (30 mg, 0.36 mmol, 12.0 eq) and acetic anhydride (17 µL, 0.18 mmol, 6.0 eq) were added and stirred for overnight. After TLC showed complete consumption of the starting material, the reaction mixture was diluted with EtOAc and then washed with saturated aqueous sodium bicarbonate 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 (DCM/MeOH 60:1 – 30:1) to yield compound **50** (106 mg, 29.0 µmol, 99%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.42 – 7.09 (m, 70H), 6.21 – 6.06 (m, 1H), 5.72 (d, *J* = 9.4 Hz, 1H), 5.67 – 5.37 (m, 9H), 5.23 – 5.13 (m, 4H), 5.13 – 4.89 (m, 9H), 4.89 – 4.72 (m, 13H), 4.72 – 4.60 (m, 6H), 4.57 – 4.42 (m, 6H), 4.36 – 4.23 (m, 5H), 4.23 – 3.86 (m, 23H), 3.78 – 3.63 (m, 4H),

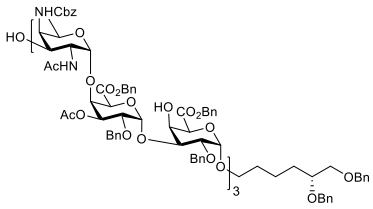
3.62 – 3.46 (m, 7H), 3.45 – 3.35 (m, 1H), 2.90 (s, 2H), 2.12 (s, 3H), 2.09 – 1.93 (m, 15H), 1.64 – 1.28 (m, 6H), 1.12 – 0.73 (m, 63H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.89, 170.66, 170.43, 170.17, 169.95, 169.84, 168.44, 168.39, 157.56, 157.10, 138.94, 138.39, 138.19, 138.11, 138.05, 137.84, 137.78, 137.77, 136.79, 136.65, 136.33, 134.53, 134.29, 133.88, 129.14, 129.04, 129.01, 128.95, 128.93, 128.87, 128.83, 128.78, 128.62, 128.57, 128.49, 128.45, 128.43, 128.39, 128.35, 128.32, 128.25, 128.22, 128.01, 127.95, 127.85, 127.80, 127.79, 127.76, 127.69, 127.66, 127.57, 127.54, 127.49, 127.43, 99.54, 99.13, 98.47, 97.43, 95.86, 92.81, 91.56, 91.34, 78.07, 77.36, 77.01, 76.68, 73.49, 73.43, 73.41, 73.25, 73.15, 72.85, 72.82, 72.79, 72.40, 72.35, 72.13, 72.10, 72.02, 71.56, 71.28, 71.14, 71.01, 70.39, 70.24, 70.07, 69.86, 69.78, 69.65, 69.15, 68.25, 68.14, 67.78, 67.72, 67.69, 67.26, 67.19, 66.86, 66.68, 66.59, 66.46, 65.95, 55.42, 51.55, 51.26, 51.03, 48.54, 31.82, 29.42, 27.90, 27.86, 27.78, 27.26, 27.13, 27.11, 23.43, 23.36, 23.32, 23.31, 21.96, 21.46, 21.38, 20.65, 20.62, 20.61, 17.04, 17.01, 16.95. HR-MS: Calculated for $C_{197}H_{248}N_6O_{54}Si_3$ $[M+H^+ + H^+]/2$: 1823.81489, found: 1823.81474. $[\alpha]^{20}_D$ = + 130.8° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (DCM/MeOH = 50/1, v/v).

Nonamer of three repeating units 6



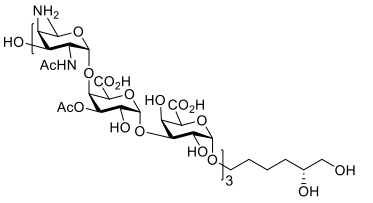
The compound **50** (102 mg, 28.0 µmol, 1.0 eq) was dissolved in THF (2 mL) and pyridine (2 mL), then cooled to 0 °C and hydrogen fluoride (HF)/pyridine (70%) (0.15 mL) was added dropwise. The solution was stirred for overnight. After TLC showed complete consumption of the starting material, the reaction 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 (DCM/Acetone/MeOH 10:3:0.2 – 10:3:0.3) to yield compound **6** (86.2 mg, 26.7 µmol, 96%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.42 – 7.07 (m, 70H), 6.14 – 5.93 (m, 2H), 5.38 – 4.97 (m, 15H), 4.97 – 4.89 (m, 3H), 4.88 – 4.28 (m, 27H), 4.28 – 3.48 (m, 42H), 3.46 – 3.16 (m, 7H), 2.78 (d, *J* = 7.0 Hz, 1H), 2.51 (s, 1H), 2.14 – 1.94 (m, 18H, OAc, NHAc), 1.73 – 1.31 (m, 6H), 1.11 – 0.90 (m, 9H, H-6c). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.03, 171.13, 170.87, 170.01, 169.92, 169.86, 167.77, 167.72, 167.59, 157.53, 156.94, 138.80, 138.43, 138.36, 138.33, 138.14, 136.97, 136.90, 136.73, 136.43, 136.30, 134.21, 134.00, 133.95, 129.09, 128.96, 128.91, 128.89, 128.85, 128.80, 128.72, 128.68, 128.61, 128.59, 128.51, 128.46, 128.44, 128.42, 128.40, 128.36, 128.17, 128.09, 127.87, 127.74, 127.72, 127.70, 127.67, 127.23, 127.02, 98.69, 98.40, 98.18, 96.71, 95.23, 94.42, 94.23, 78.50, 78.00, 76.52, 76.15, 75.72, 75.23, 74.55, 74.26, 74.16, 74.05, 73.93, 73.40, 72.85, 72.81, 72.74, 72.61, 72.53, 72.47, 72.37, 72.00, 71.96, 71.82, 71.64, 70.77, 70.64, 70.40, 70.29, 70.12, 69.43, 68.83, 67.70, 67.61, 67.55, 67.33, 67.24, 66.97, 66.88, 66.56, 66.41, 65.98, 63.25, 63.01, 62.91, 55.28, 50.85, 48.41, 31.54, 29.73, 29.26, 23.60, 23.52, 23.28, 21.95, 21.39, 21.35, 17.01, 16.97, 16.91. HR-MS: Calculated for $C_{173}H_{200}N_6O_{54}$ $[M+2H^+]/2$: 1613.66170, found: 1613.66372. $[\alpha]^{20}_D$ = + 147.6 ° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (DCM/Acetone/MeOH 10:3:0.3, v/v/v).

Nonamer of three repeating units 51



The compound **6** (16.1 mg, 4.99 µmol, 1.0 eq) was dissolved in MeCN/*tert*-BuOH/H_2O (700 µL, 4/1/2, v/v/v). The mixture was cooled to 0 °C and treated with TEMPO (1.9 mg, 12.2 µmol, 2.4 eq), BAIB (20 mg, 0.06 mmol, 12 eq) and $NaHCO_3$ (6.3 mg, 75 µmol, 15 eq). After stirring for 24 hours at 4 °C and TLC showed complete consumption of the starting material, saturated aqueous sodium thiosulphate was added and diluted with EtOAc, washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DCM (2 mL), followed by addition of 0.2M phenyldiazomethane ($PhCHN_2$) in Et_2O (1 mL) at RT. After the mixture was allowed to stir overnight at rt and TLC showed complete consumption of the starting material, the reaction was diluted with EtOAc and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by preparative TLC plates (Macherey-Nagel, pre-coated TLC plates SIL G-100 UV254) (DCM/Acetone/MeOH 10:2:0.5) yielded **51** (11.6 mg, 3.28 µmol, 66%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.49 – 6.99 (m, 85H), 6.03 – 5.79 (m, 3H), 5.40 – 5.20 (m, 10H), 5.20 – 5.09 (m, 4H), 5.07 – 4.80 (m, 10H), 4.77 (d, *J* = 11.8 Hz, 1H), 4.74 – 4.41 (m, 19H), 4.41 – 4.33 (m, 3H), 4.31 – 4.15 (m, 7H), 4.15 – 3.72 (m, 20H), 3.71 – 3.44 (m, 14H), 3.43 – 3.27 (m, 3H), 2.09 (s, 3H), 2.05 – 1.92 (m, 15H), 1.65 – 1.36 (m, 6H), 1.07 – 0.90 (m, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.10, 171.16, 170.96, 170.18, 169.98, 169.89, 168.56, 168.49, 167.83, 167.71, 167.49, 157.60, 156.94, 138.96, 138.46, 138.21, 138.01, 136.95, 136.71, 136.62, 136.52, 136.25, 135.49, 135.43, 135.38, 134.17, 134.06, 134.02, 129.22, 129.06, 129.00, 128.96, 128.87, 128.86, 128.81, 128.77, 128.74, 128.72, 128.64, 128.61, 128.58, 128.55, 128.53, 128.51, 128.45, 128.36, 128.34, 128.09, 128.06, 127.93, 127.91, 127.86, 127.79, 127.77, 127.73, 127.67, 127.63, 127.46, 127.44, 98.65, 98.59, 98.10, 97.08, 95.48, 95.25, 94.52, 77.98, 76.31, 76.24, 76.11, 74.85, 74.28, 74.11, 73.96, 73.83, 73.71, 73.46, 73.12, 73.01, 72.83, 72.72, 72.62, 72.12, 72.06, 70.67, 70.46, 70.26, 70.17, 69.98, 69.81, 69.46, 68.84, 67.75, 67.65, 67.46, 67.27, 67.18, 67.13, 66.59, 65.92, 64.75, 55.33, 51.96, 51.28, 50.94, 48.50, 32.06, 31.87, 29.50, 29.48, 23.48, 23.33, 22.84, 21.97, 21.44, 19.25, 17.15, 17.10. HR-MS: Calculated for $C_{194}H_{212}N_6O_{57}$ $[M+NH_4^+ + NH_4^+]/2$: 1778.21429, found: 1778.21433. $[\alpha]^{20}_D$ = + 127.1° (c = 1, $CHCl_3$). TLC: Rf = 0.35 (DCM/Acetone/MeOH 10:2:0.5, v/v/v).

The deprotection of Nonamer 3



The protected nonamer **51** (13 mg, 3.68 µmol, 1.0 eq) was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (2 mL). After $Pd(OH)_2$/C (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo* to yield compound **3** (6.2 mg, 3.3 µmol, 90%). ^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.38 – 5.26 (m, 6H), 5.13 – 5.08 (m, 2H), 5.08 – 5.02 (m, 2H), 5.02 – 4.96 (m, 2H), 4.75 – 4.69 (m, 2H), 4.68 – 4.55 (m, 7H), 4.54 – 4.47 (m, 3H), 4.34 – 4.11 (m, 11H), 4.10 – 3.92 (m, 7H), 3.85 – 3.78 (m, 2H), 3.76 – 3.68 (m, 2H), 3.62 – 3.53 (m, 3H), 3.50 – 3.44 (m, 1H), 2.27 – 2.21 (m, 9H), 2.14 – 2.09 (m, 4H), 2.08 – 2.02 (m, 5H), 1.76 – 1.45

(m, 6H), 1.39 – 1.32 (m, 9H). ^{13}C NMR (151 MHz, D_2O) δ 176.02, 175.55, 175.13, 175.03, 174.51, 174.43, 100.17, 99.79, 99.38, 99.26, 98.71, 98.62, 97.37, 97.04, 96.92, 78.32, 77.67, 77.60, 76.82, 76.45, 74.18, 73.89, 73.17, 72.91, 72.84, 72.67, 72.59, 72.14, 71.89, 71.78, 71.65, 69.24, 68.83, 68.68, 67.45, 67.18, 67.15, 66.67, 66.58, 66.32, 65.63, 64.80, 63.94, 55.99, 53.86, 53.71, 50.25, 48.53, 35.38, 32.85, 29.46, 23.31, 23.22, 22.38, 21.74, 21.73, 16.92. HR-MS: Calculated for $C_{72}H_{110}N_6O_{51}$ $[M+2H^+]/2$: 938.31720, found: 938.31596.

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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.

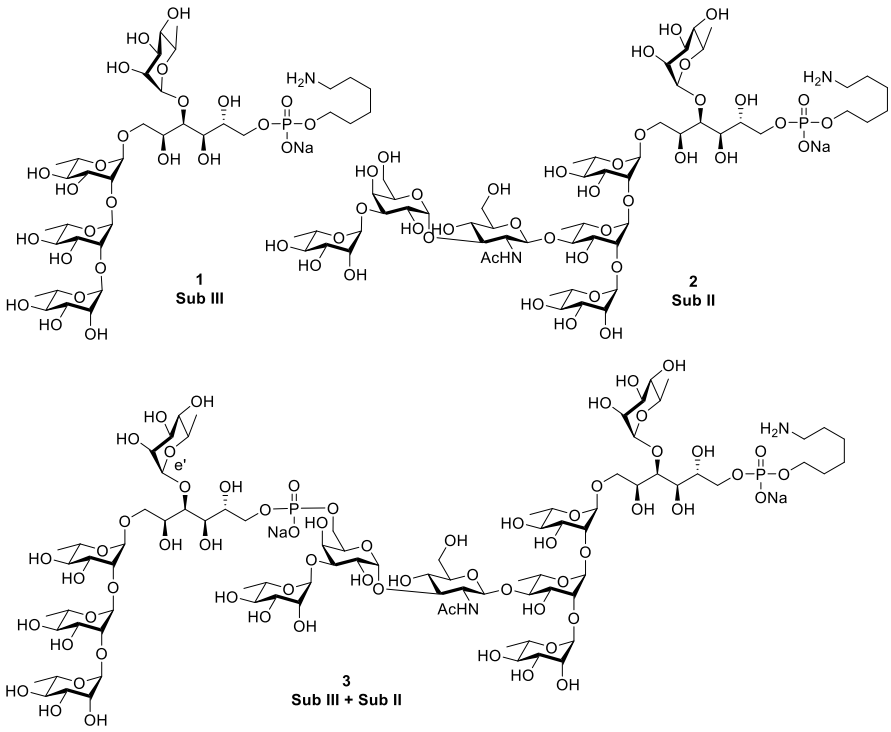
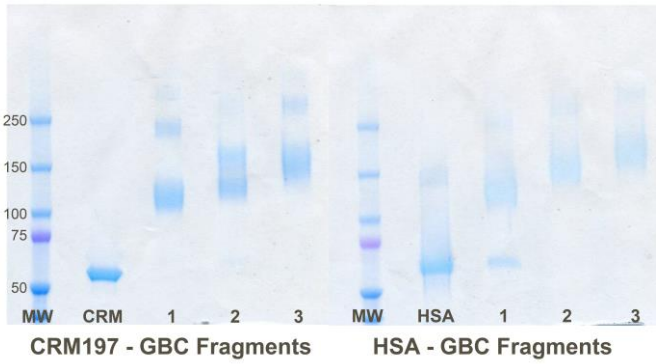


Figure 1. Three conjugation-ready GBC fragments.



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.

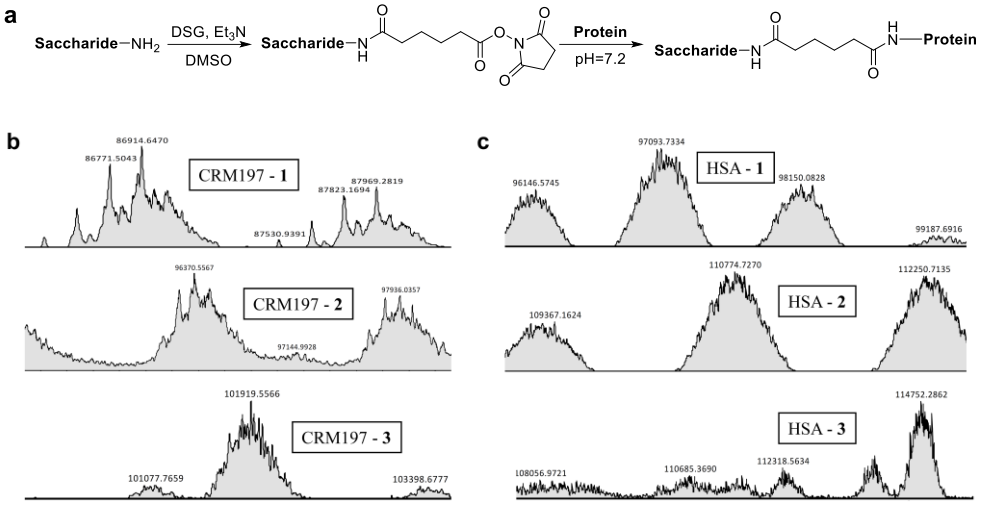


Figure 3. Conjugation of oligosaccharides with CRM197 and HSA. **a**, Oligosaccharides **1** - **3** were conjugated with CRM197 and HSA proteins using the DSG derivatization method; **b**, CE-MS analysis of CRM197 conjugates measure the average molecular weight to estimate the carbohydrate/protein molar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

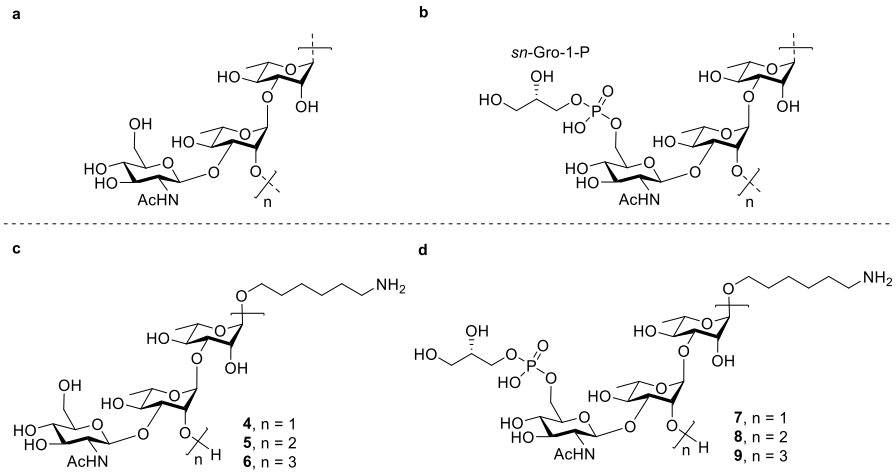


Figure 4. **a**, The structure of GAC. **b**, the structure of glycerol phosphate modified GAC. **c**, the designed fragments of GAC **4** - **6**. **d**, the designed fragments of GroP GAC **7** - **9**.

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.

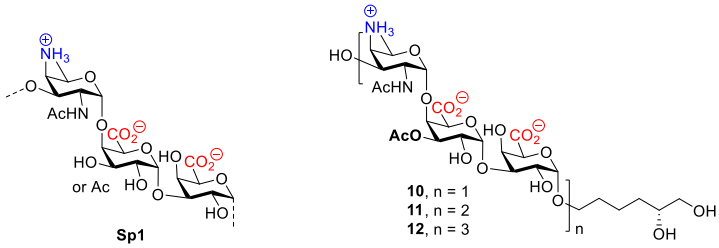


Figure 5. The structure of Sp1 and designed fragments of *O*-Ac-Sp1 **10** – **12**.

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**.

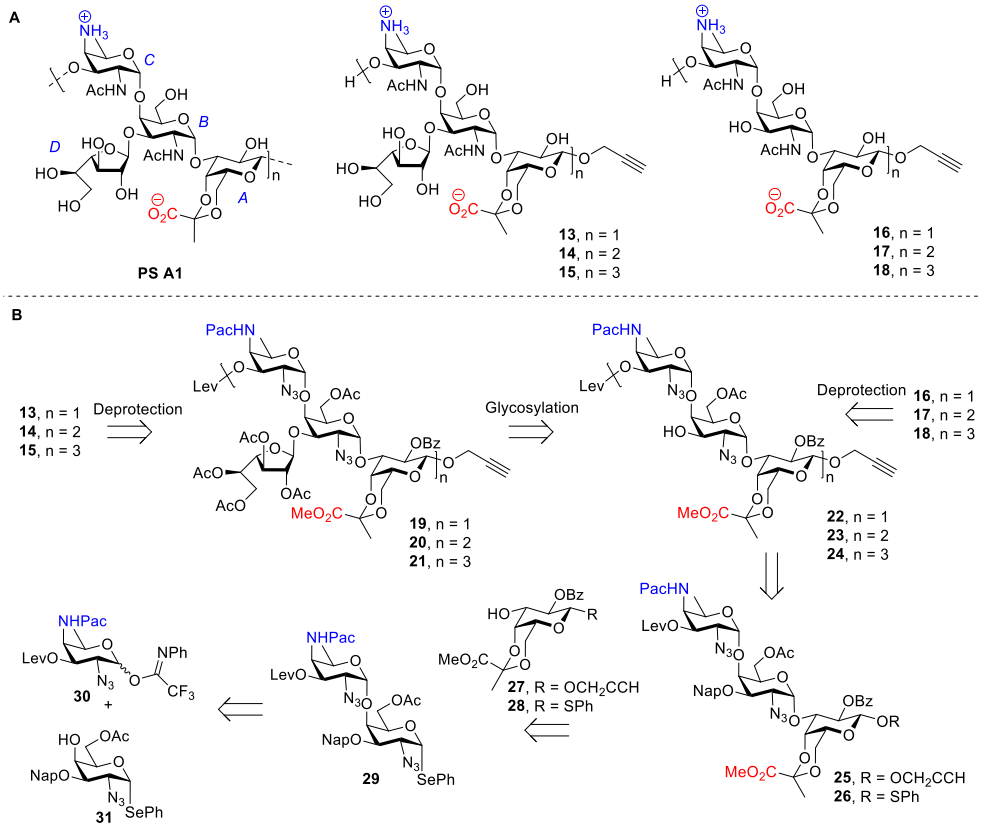
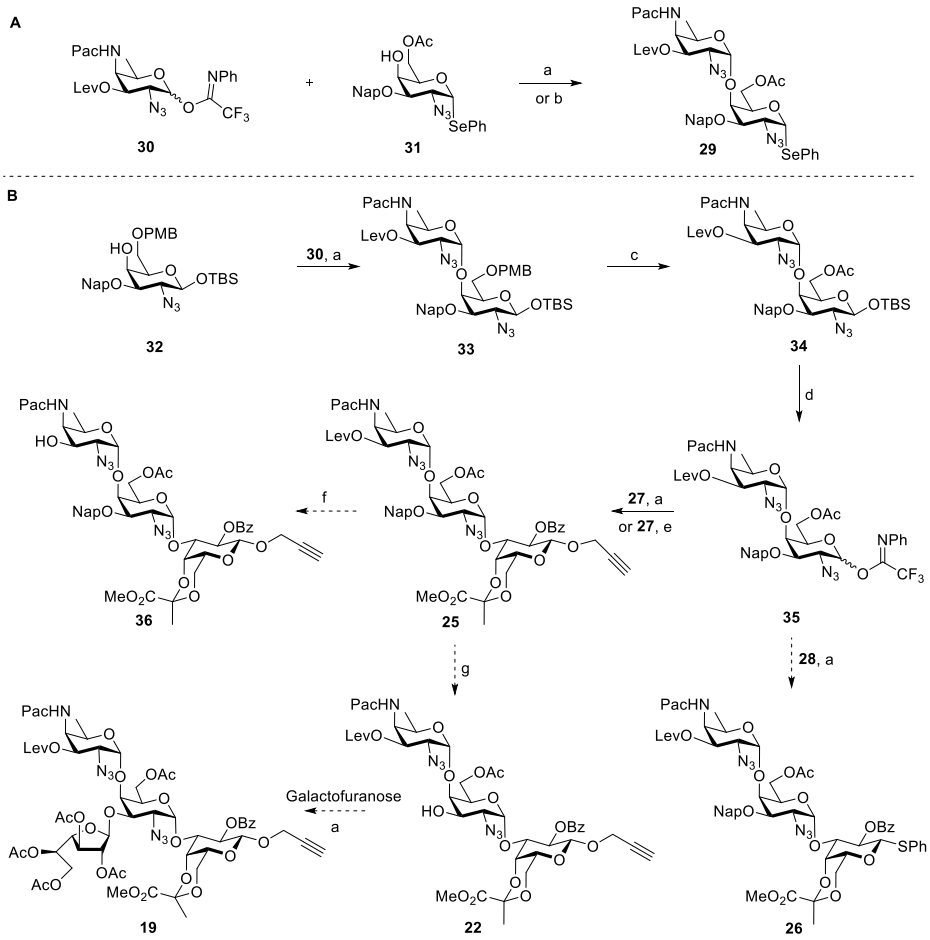


Figure 6. A) Structure of PS A1 repeating unit and the designed fragments. B) the first retrosynthetic analysis of the fragments **13** – **18**.

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.

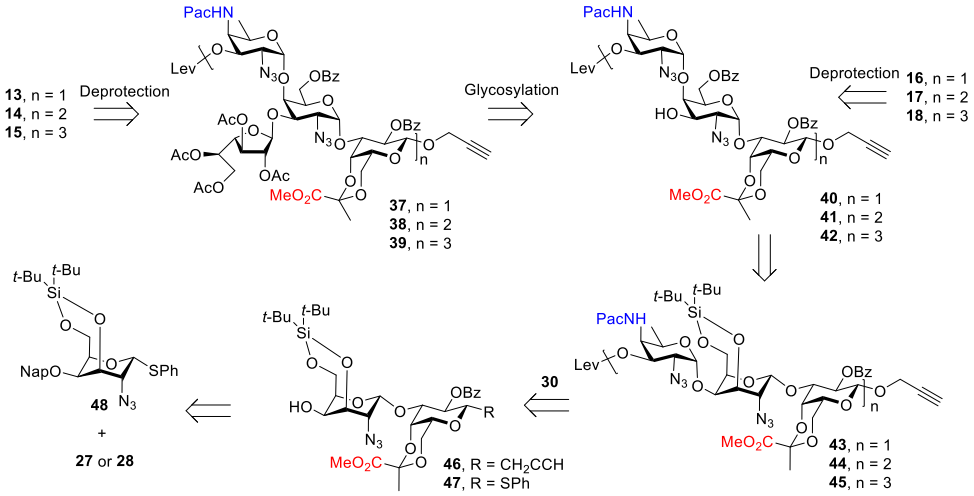


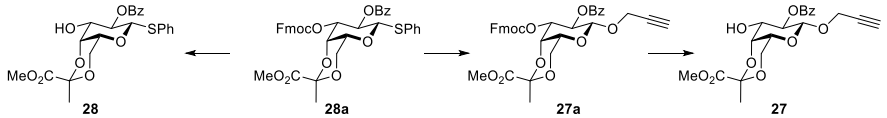
Figure 7. The second generation retrosynthetic analysis of the fragments **13** – **18**.

Experimental section

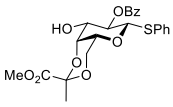
General experimental procedures

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

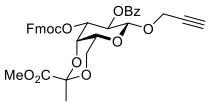


Phenyl 2-*O*-benzoyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-thio-β-D-galactopyranoside (28)



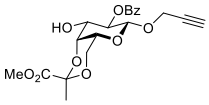
Phenyl 2-*O*-benzoyl-3-*O*-fluorenylmethyloxycarbonyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-thio-β-D-galactopyranoside **28a**[3] (4.93 g, 7,23 mmol, 1 eq) was dissolved in DCM and triethyl amine (60 mL, 434 mmol, 60 eq) is added and the solution stirred for four hours and thirty minutes. 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)

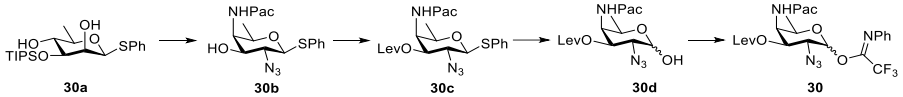


Compound **28a** (1.08 g, 1.59 mmol, 1 eq), diphenyl sulfoxide (0.41 g, 2.02 mmol, 1.27 eq) and 2,4,6-Tri-*tert*-butylpyrimidine (TTBP) (0.99 g, 3.99 mmol, 2.5 eq) were added to a flask and co-evaporated with toluene (3×) under an argon atmosphere. Dry DCM (3×) (36 mL) and molecular sieves (3Å) were 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).

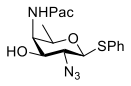
Propynyl 2-*O*-benzoyl-4,6-di-*O*-[1-(*R*)-(methoxycarbonyl)-ethyldiene]-β-D-galactopyranoside (27)



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.

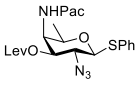


Phenyl 2-azido-6-deoxy-4-*N*-phenoxyacetimide-1-thio-β-D-galactopyranoside (30b)



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).

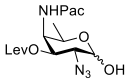
Phenyl 2-azido-6-deoxy-3-*O*-levulinoyl-4-*N*-phenoxyacetimide-1-thio-β-D-galactopyranoside (30c)



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* =

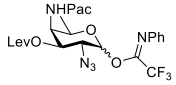
9.5 Hz, 1H, NH), 4.78 (dd, *J* = 10.2, 3.9 Hz, 1H, H-3), 4.64 – 4.48 (m, 2H), 4.47 – 4.37 (m, 2H, H-4, H-1), 3.86 – 3.76 (m, 1H, H-5), 3.04 (t, *J* = 10.2 Hz, 1H, H-2), 2.90 – 2.45 (m, 4H, Lev), 2.19 (s, 3H, Lev), 1.15 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.60 (Lev), 172.07 (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)

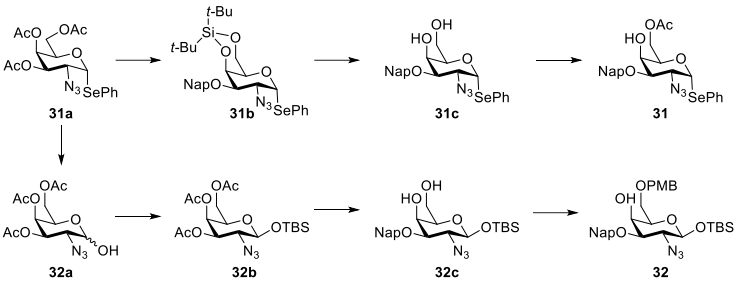


Compound **30c** (0.949 g, 1.85 mmol, 1 eq) was dissolved in DCM (10 mL) and reduced to 0 °C. NIS (0.626 g, 2.78 mmol, 1.5 eq) and TFA (0.17 mL, 2.22 mmol, 1.2 eq) were added and the solution stirred for 1 hour. NIS (0.2 g, 0.925 mmol, 0.5 eq) and TFA (0.01 mL, 1.3 mmol, 0.7 eq) were added and the solution 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).

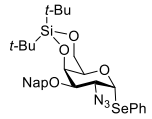
***N*-phenyl-trifluoroacetimidoyl 2-*N*-azido-3-*O*-levulinoyl-5-methyl-4-*N*-phenoxyacetimide-β-D-galactopyranoside (30)**



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).

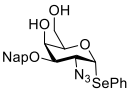


Phenyl 2-*N*-azido-4,6-*O*-di-*tert*-butylsilylidene-3-*O*-(2-methylnaphthyl)-1-seleno-β-D-galactopyranoside (31b)



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.

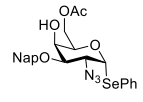
Phenyl 2-*N*-azido-3-*O*-(2-methylnaphthyl)-1-seleno-β-D-galactopyranoside (31c)



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,

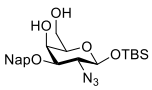
1H, H-4), 4.45 – 4.36 (m, 2H, 4-OH, H-2), 4.29 – 4.19 (m, 1H, H-5), 3.93 – 3.77 (m, 3H, 6-OH, H-6, H-3), 3.71 (dd, *J* = 10.7, 5.9 Hz, 1H, H-6). ^{13}C NMR (101 MHz, Acetone) δ 136.66, 135.40, 134.20, 133.90, 129.85, 129.82, 128.73, 128.66, 128.49, 128.39, 127.18, 126.95, 126.74, 126.73, 86.69 (C-1),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

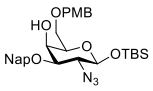
***Tert*-butyldiphenylsilyl 2-*N*-azido-3-*O*-(2-methylnaphthyl)-β-D-galactopyranoside (32c)**



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

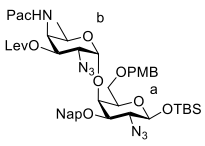
flash chromatography (PE/DCM/EA 10:10:1 - 2:1:1) to yield compound **32c** (1.21 g, 3.1 mmol, 20% over 4 steps,). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 – 7.73 (m, 4H), 7.60 – 7.40 (m, 3H), 4.86 (s, 2H, Nap), 4.45 (d, *J* = 7.7 Hz, 1H, H-1), 4.03 – 3.86 (m, 2H, H-4, H-6), 3.83 – 3.73 (m, 1H, H-6), 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).

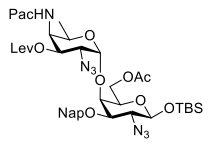
***Tert*-butyldiphenylsilyl 4-*O*-(2-*N*-azido-3-*O*-levulinoyl-5-methyl-4-*N*-phenoxyacetimide-α-D-galactopyranoside) 2-*N*-azido-6-*O*-*p*-methoxybenzyl-3-*O*-(2-methylnaphthyl)-β-D-galactopyranoside (33)**



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

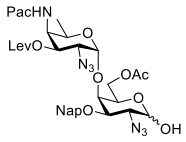
concentrated *in vacuo*. The compound was purified first by size exclusion (DCM/MeOH 1:1) and then flash chromatography (PE/EA 8:1 - 2:1 - PE/DEA 2:1:1) to yield compound **33** (0.460 g, 0.469 mmol, 68%).[1]H NMR (400 MHz, Acetone-d6) δ 7.98 – 7.94 (m, 1H, Nap), 7.93 – 7.84 (m, 3H), 7.63 (dd, *J* = 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)**



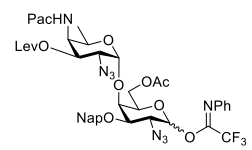
Compound **33** (115 mg, 0.12 mmol, 1.0 eq) and triethylsilane were dissolved in DCM (1.2 mL) and hexafluoro-*iso*-propanol (HFIP) (1.2 mL). Then 0.1M HCl in HFIP (0.12 mL, 0.012 mmol, 0.1 eq) was added to the mixture. The reaction was stirred at rt for 30 mins. After analysis by TLC showed complete 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)



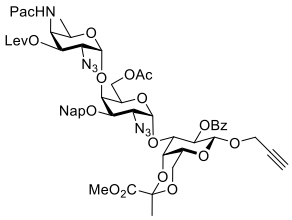
Compound **34** (100 mg, 0.111 mmol, 1.0 eq) was dissolved in THF (1.0 mL) and pyridine (2.0 mL), then cooled to 0 °C. Hydrogen fluoride (HF)/pyridine (70%) (0.2 mL) was added dropwise. The solution was stirred for overnight. After TLC showed complete consumption of the starting material, the reaction 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.

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Chinese Summary

中文小结

Chemical Synthesis of Fragments of Streptococcal Cell Wall Polysaccharides
链球菌细胞壁多糖片段的化学合成研究

本论文描述了链球菌细胞壁中各种各样的多糖片段的合成设计，其中包含 B 组链球菌中枝状的 B 组特异性抗原（GBC），磷酸甘油（GroP）修饰的 A 组链球菌多糖（GAC）以及 *O*-乙酰基修饰的肺炎链球菌 1 型荚膜多糖（*O*-Ac SP1）。所有合成分子的还原端上都被修饰上了一个连接点，以方便与蛋白质或者活性小分子结合修饰，去探讨糖作为疫苗的免疫响应机理以及探究新的疫苗开发。任一多糖的不同长度的寡糖片段被组装出来用以下一步的构效关系研究。

第一章简要介绍糖在生物体内的多样性以及不同结构的糖所表现出来的各式各样的生物作用，回顾天然产物分离的糖疫苗与化学合成的糖疫苗的发展历史及最新进展。总结了不同类型的糖疫苗与免疫系统作用的机理，其中包括纯的寡糖，糖缀合物以及双性离子多糖。列举了一些针对链球菌的合成糖疫苗，其中包括 A 组链球菌，B 组链球菌以及肺炎链球菌。

第二章描述了利用高度汇聚性的化学路径合成具有代表性的 B 组特异性抗原寡糖片段，其中包含一个【3＋5】的糖苷化反应和一个【5＋8】的亚磷酰胺偶联反应。含有支链的三个寡糖被成功合成了几十个毫克量级，其中包括一个五糖，一个八糖以及一个十三糖。所有合成的化合物上都安装有一个氨基连接点为实现进一步的结合修饰（图 1）。来自莱顿大学的 Jacopo Enotarpi 已经完成它们分别与两个不同蛋白质的生物偶联反应，白喉毒素的无毒突变体 CRM197 和人血清白蛋白 HAS，为下一步验证这些合成寡糖的免疫活性做好了准备（图 2）。接下来这些糖缀合物将会被用来与免疫血清和（单克隆）抗体进行结合实验研究以及在小鼠体内进行免疫研究。

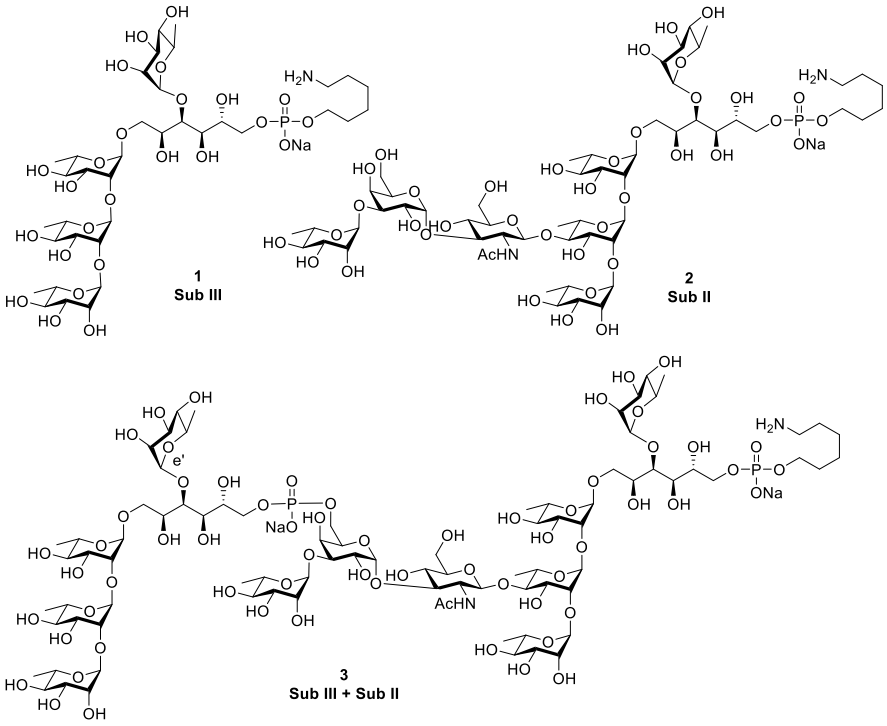
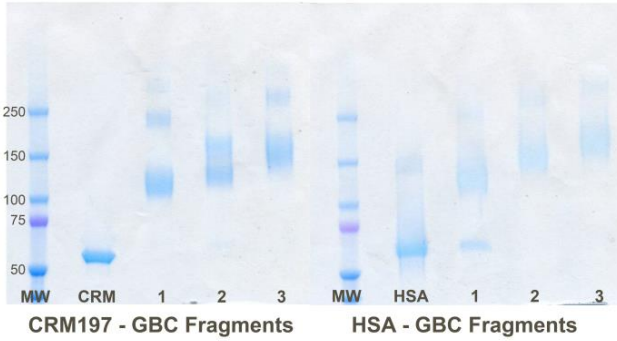


图 1. 三个准结合的 GBC 寡糖目标分子



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*

图 2. 合成寡糖与载体蛋白 CRM197 和 HSA 的缀合物

第三章描述了首次合成最近被发现的磷酸甘油（GroP）修饰的 A 组链球菌多糖的寡糖片段，简称 GroP GAC。相对应的不含 GroP 的 GAC 寡糖片段同时也被合成了，这六个合成的寡糖化合物末端都接有一个氨基连接点为以后的偶联做准备（图 3）。在合成的过程中，一个关键的三糖重复单元切块被用去构建六个目标化合物，其中包括两个三糖，两个六糖和两个九糖。接下来，所有的化合物都会与载体蛋白结合形成糖缀合物进行疫苗开发研究。

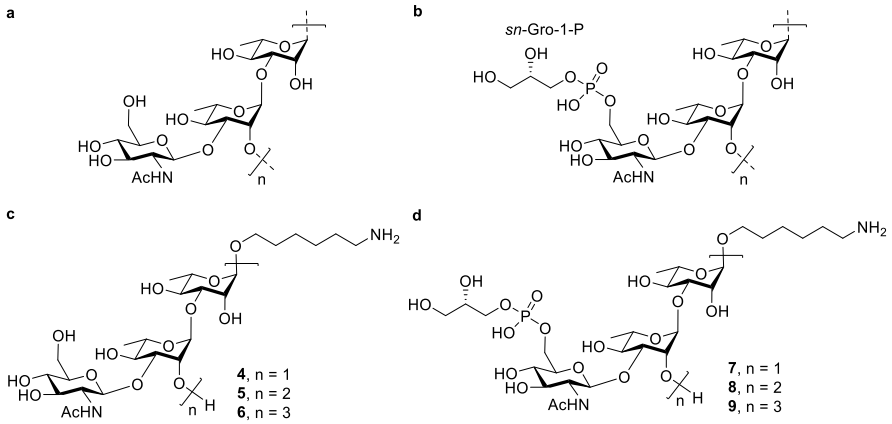


图 4. a, GAC 的重复单元结构。**b**, 磷酸甘油修饰的 GAC 重复单元结构。**c**, 设计的 GAC 寡糖目标分子 **4 - 6**。**d**, 设计的 GroP GAC 寡糖目标分子 **7 - 9**。

第四章描述了 *O*-乙酰基修饰的肺炎链球菌 1 型荚膜多糖（*O*-Ac Sp1）中寡糖片段的首次合成，其中三糖、六糖和九糖各一个（图 4）。Sp1 是双性离子多糖中一员，是一类罕见的免疫调节剂，它可以被抗原递呈细胞（APC）处理加工后与主要组织相容性复合体 II（MHC II）结合，实现 T 细胞介导的免疫反应。考虑到进一步接合修饰需求以及目标分子中已经存在的氨基，一个新颖的邻二醇连接点被设计安装在寡糖的还原端。接下来会利用分子动力学模拟和核磁共振光谱学去探究乙酰基在寡糖三维结构中所扮演的角色；利用酶联免疫吸附试验（ELISA）和饱和转移差谱（STD）NMR 实验去揭露乙酰基在寡糖与抗-Sp1 抗体结合研究中所发挥的作用。

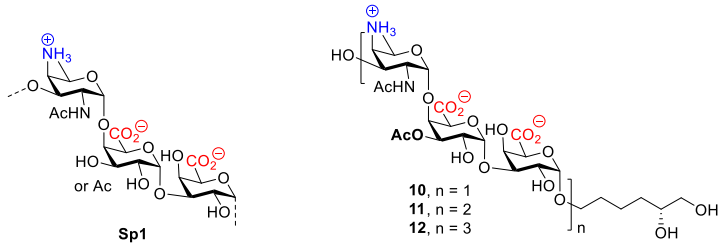


图 5. Sp1 的重复单元结构和设计的 *O*-Ac Sp1 寡糖目标分子 **10 - 12**。

第五章对本论文进行总结以及对未来要进行的实验进行展望。介绍了已经进行了的一些生物活性测试实验结果以及全新设计的一个双性离子寡糖化合物 PS A1。

Curriculum Vitae

Zhen Wang was born on 5th Dec. 1989 in Shangqiu, Henan province, China. After finishing high school education in Shangqiu, he was enrolled in Zhengzhou University in 2007, majoring in chemistry. He obtained his Bachelor of Science in 2011 and joined in Renmin University of China in the same year, majoring in organic chemistry. In 2014, he received his Master of Science degree after finishing the thesis "Diazoacetate's Carbene Transfer Reaction via NHC-Ag^+ Catalysis" under the supervision of Prof. Dr. Zili Chen. Then, he moved to National Institute of Biological Sciences as a research assistant in Prof. Dr. Xiaoguang Lei lab to do some total synthesis work focusing on *Incarviatone* and *Jungermannenones* until Aug. 2015.

He started his Ph.D. study on "Chemical Synthesis of Fragments of Streptococcal Cell Wall Polysaccharides", presenting in this thesis, in the bio-organic synthesis group of Leiden University from September 2015. His doctoral studies were under the supervision of Dr. Jeroen Codée and Prof. Dr. Gijs van der Marel sponsored by the Chinese Scholarship Council (CSC) – Leiden University joint scholarship. Parts of his research were presented as posters at the annual Dutch chemistry conference "CHAINS" (2016, 2017, 2018). A poster was presented at the 19th European Carbohydrate Symposium 2017 in Barcelona, Spain. A poster was presented at the 29th International Carbohydrate Symposium 2018 in Lisbon, Portugal. And Parts of his work were presented as an oral presentation at the 20th European Carbohydrate Symposium 2019 in Leiden, the Netherlands, and the Chains 2019 in Veldhoven, the Netherlands.

From Sept. 2020, he will continue his postdoctoral career, focusing on the synthesis of complex carbohydrates under the supervision of Prof. Dr. Jeroen Codée and Prof. Dr. Gijs van der Marel in Bio-organic Synthesis group of Leiden University.

List of Publications

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Zhen