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Development of Synthetic Procedures Towards Immunostimulating Carbohydrates

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“Le véritable voyage de découverte ne consiste pas à chercher de nouveaux paysages, mais à avoir de nouveaux yeux”

Marcel Proust

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List of Abbreviations

Ac	acetyl	ESI	electrospray ionization
AGP	aminoalkyl glucosamine 4-phosphate	Et	ethyl
AcOH	acetic acid	Et ₃ N	triethylamine
AIBN	2,2'-azobis(2-methyl- propionitrile)	Et ₂ O	diethyl ether
All	allyl	EtOAc	ethyl acetate
APT	attached proton test	Fmoc	(9 <i>H</i> -fluoren-9-yl) methoxycarbonyl
aq.	aqueous	GAG	glycosaminoglycan
arom.	aromatic	Glc	D-glucose
AzDMB	4-azido-2,2- dimethylbutanoyl	GlcA	D-glucuronic acid
BAIB	bis(acetoxy)iodobenzene	GlcN	D-glucosamine
Bn	benzyl	h	hour(s)
bs	broad singlet	HA	hyaluronic acid
Bu	butyl	HRMS	high-resolution mass Spectroscopy
Bz	benzoyl	HSQC	heteronuclear single quantum coherence
calcd	calculated	Hz	hertz
cat.	catalytic	IgM	Immunoglobulin M
COSY	correlation spectroscopy	IR	infrared
C _q	quaternary carbon atom	<i>J</i>	coupling constant
δ	chemical shift	LC-MS	liquid chromatography- mass spectroscopy
d	doublet	Lev	levulinyl
DAMP	damage-associated molecular pattern	Lev ₂ O	levulinic anhydride
DBU	1,8-diazabicycloundec-7-ene	LPS	lipopolysaccharides
DC	dendritic cell	m	multiplet
DCM	dichloromethane	<i>m</i> -CPBA	3-chloroperbenzoic acid
dd	doublet of doublets	MDP	<i>N</i> -acetylmuramyl-L-alanine- D-isoglutamine
DIC	<i>N,N</i> -diisopropyl- carbodiimide	Me	methyl
DMAP	4-(dimethylamino)pyridine	MeCN	acetonitrile
DMF	<i>N,N</i> -dimethylformamide	mg	milligram(s)
DMSO	dimethylsulfoxide	MHC	major histocompatibility complex
eq	molar equivalents		

min	minute	sat.	saturated
NAP	naphthylmethyl	SEC	size-exclusion chromatography
NaH	sodium hydride	t	triplet
NaOMe	sodium methoxide	TBAF	tetrabutylammonium fluoride
NIS	<i>N</i> -iodosuccinimide	TBAI	tetrabutylammonium iodide
NLR	NOD-like receptor	TBDMS	<i>tert</i> -butyldimethylsilyl
NOD	Nucleotide-binding Oligomerization Domain	<i>t</i> Bu	<i>tert</i> -butyl
NMR	Nuclear Magnetic Resonance	TCA	trichloroacetyl
PAMP	pathogen associated molecular pattern	TEA	triethylamine
<i>p</i> TsOH	<i>para</i> -toluenesulfonic acid	TEMPO	2,2,6,6-tetramethylpiperidinyl-oxy
PE	petroleum ether (40-60)	<i>tert</i>	tertiary
Ph	phenyl	TES	triethylsilane
Piv	pivaloyl	Tf	trifyl
PMB	<i>para</i> -methoxybenzyl	TFA	trifluoroacetic acid
ppm	parts per million	THF	tetrahydrofuran
PRR	pattern recognition receptor	TLC	thin layer chromatography
q	quartet	TLR	Toll-like receptor
RCM	ring-closing metathesis	TMS	trimethylsilyl
R _f	retention factor	TOCSY	total correlation spectroscopy
RRV	relative reactivity value	Troc	2,2,2-trichloroethyloxycarbonyl
rT	room temperature	UV	ultraviolet
RV	reaction vessel	ZP	zwitterionic polysaccharide
s	singlet		
SAR	structure-activity relationship		

Chapter 1

General introduction: synthetic carbohydrates and the immune system

Introduction

Carbohydrates play a role in a vast array of biological processes such as cell proliferation and cell differentiation. Besides, different classes of carbohydrates influence the functioning of the mammalian immune system. This is a highly evolved defense system that protects us from invading pathogenic microorganisms.¹ It can be divided into two classes: the innate and the adaptive (or acquired) immune system. The innate immune system is the first line of defense that provides an immediate response after detection of invading microorganisms.² Pathogens are recognized through pathogen-associated molecular patterns (PAMPs) by various pattern recognition receptors (PRRs).³ These receptors are abundantly expressed on cells of the immune system such as dendritic cells (DCs) and macrophages. Members of the PRRs are the Toll-like receptors (TLRs), the nucleotide-binding oligomerization domain receptors (NLRs), lectin receptors and retinoic acid-inducible gene (RIG)-I-like receptors (RLRs). Since carbohydrates are among the most abundant molecules found on bacterial, parasitic and viral cell surfaces, it is not surprising that specific carbohydrates are ligands for PRRs.⁴ Well known examples are lipopolysaccharide (LPS) which is recognized by TLR4 and muramyl dipeptide, a

NOD-2 ligand. Microbial pathogens generally present a number of PAMPs, which activate multiple PRRs. For example, TLR2 forms heterodimers with TLR6 upon the recognition of Pam₂CSK₄ and TLR1 and TLR2 dimerize upon sensing Pam₃CSK₄. Furthermore, damage-associated molecular patterns (DAMPs), which are released upon cell damage, can also excite the innate immune system.⁵ Cross talk between several PRRs has also been established, which results in the collaboration between different receptors, cells and signal mediators.⁶

The adaptive immune system, on the other hand, is a more specialized defense mechanism and is developed during life.⁷ It exhibits immunological memory and as a result, a faster and more effective immune response is induced after a second encounter with a pathogen, thus preventing reinfection. Although carbohydrates influence the adaptive immune system, antibody responses to carbohydrates are generally poor. Polysaccharide antigens directly activate B lymphocytes, which subsequently differentiate into plasma cells to produce Immunoglobulin M (IgM) antibodies. Proteins, on the other hand, interact with antigen-presenting cells (APCs) such as dendritic cells and macrophages, resulting in internalization and presentation to T-lymphocytes via major histocompatibility complex (MHC) class II molecules. This induces B cells to differentiate into memory B cells. This class switching process (IgM to IgG) and the creation of immunological memory is excluded for polysaccharides.⁸ To increase the immunogenicity of carbohydrates, several strategies have been employed, including conjugation to immunogenic carrier proteins and covalent attachment of carbohydrates to peptide epitopes. For the development of new vaccine modalities and immunomodulating therapeutics, knowledge on the interaction of polysaccharides with the immune system at the molecular level is essential. Conventional isolation of carbohydrates from their natural sources is hampered by the (micro-)heterogeneity of the oligo- or polysaccharide targets. In addition possible contaminants such as LPS or lipopeptides can complicate proper biological evaluation. As a consequence, the availability of well-defined carbohydrates largely depends on robust synthetic methods. Recent advances in synthetic carbohydrate chemistry such as one-pot strategies and automated oligosaccharide synthesis⁹ have contributed to faster access to complex oligosaccharides.

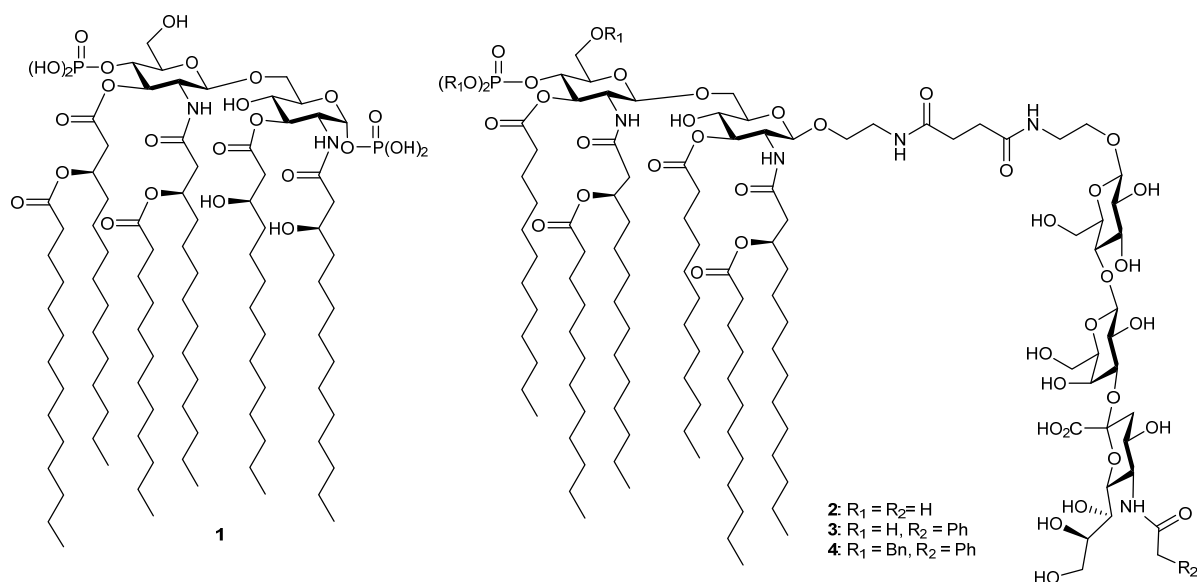
This chapter provides a selection of synthetic carbohydrates that have been used to explore their interaction with the immune system. First, a selection of synthetic immunostimulating carbohydrates is presented. Zwitterionic polysaccharides (ZPs) and adjuvant QS-21 exhibit interesting immunostimulating properties and these molecules are highlighted in the second part of this chapter. Finally, a series of synthetic carbohydrates used in the development of vaccine modalities is presented.

Synthetic carbohydrates and the innate immune system

Toll-like receptors were the first PRRs identified¹⁰ and a total of 13 TLRs are currently discovered in mammals, of which 11 are found in humans. TLRs recognize structurally diverse PAMPs, including bacterial lipoproteins, lipoteichoic acids, virus-derived RNA, CpG DNA fragments, and lipopolysaccharide (LPS).¹¹ LPS is found in the outer membrane of Gram-negative bacteria and is anchored in the membrane with Lipid A (Figure 1). Lipid A is not only responsible for toxic effects of gram-negative infections but it is also identified as the actual ligand of TLR4.¹² Lipid A is a β -1,4-linked glucosamine disaccharide with, depending on the

bacterium, phosphates at the reducing end and the C-4' position and four to eight fatty acids attached to the C-3, C-3', N-2, and N-2' positions. Recognition of lipid A by TLR4 is initiated by LPS-binding protein (LBP) which transfers lipid A to another accessory protein CD14 that in turn transfers lipid A to MD2, a secreted glycoprotein that interacts with the extracellular domain of TLR4. As a result, the TLR4-MD2 complex dimerizes and an immune response is initiated. To explore the immunostimulating properties of lipid A, many derivatives and analogues have been synthesized and evaluated.¹³ The outcome of these studies led to structure-activity relationships (SARs) and revealed that both the number and the length of the fatty acids and the phosphorylation pattern have a huge impact on its endotoxicity and immunostimulating activity.¹⁴ Overall, it was found that lipid A structures with six fatty acids and two phosphates, such as *Escherichia coli* lipid A (Figure 1, **1**) are optimally recognized by TLR4.

Figure 1. Lipid A structure of *E.coli* **1** prepared by Imoto and coworkers¹⁵ and three synthetic glycoconjugate vaccines with MPLA (**2-4**).

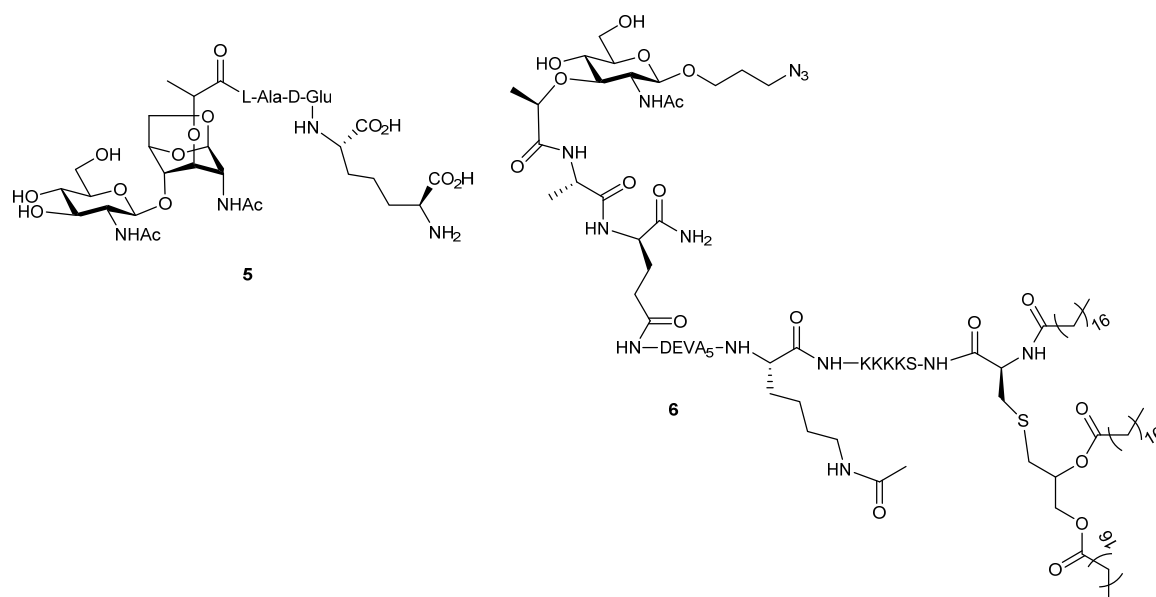


It was also assessed that changes in the fatty acid structures are accompanied by reduction of the activity. For example, replacement of the natural 3-hydroxy/acyloxytetradecanoic acid moieties by tetradecanoic acids reduces the activity. A similar effect was observed by esterification of the hydroxyl of the 3-hydroxytetradecanoyl group with fatty acids.¹⁶ On the contrary, removal of the anomeric phosphate group in lipid A was found to be beneficial: the endotoxicity was lowered without reducing the immunostimulating activity.¹⁷ Monophosphoryl lipid A (MPLA) has proven to be clinically safe and has been explored as part of potential vaccines against bacterial infections and cancer. Wang and coworkers conjugated MPLA to GM3, a tumor-associated carbohydrate antigen expressed by human melanoma and other cancers (Figure 1, **2-4**).¹⁸ Biological evaluation showed that conjugate **3** is a promising immunostimulant because the induced antibodies were shown to bind selectively to target tumor cells. Removal of the phosphate at the C-4' position of lipid A, is accompanied by loss of the immunostimulating activity, showing that this phosphate is essential for activity.¹⁹ The structure-activity relationships were strengthened by the elucidation of several

crystal structures of lipid A and its analogues with MD2.²⁰ Lipid A derivatives with six fatty acids and two phosphates show agonistic activity, whereas lipid A derivatives with 4 acyl chains, such as Eritoran, exhibit antagonist activity. The opposite mode of action was confirmed with the crystal structures of lipid A and Eritoran with the MD2-TLR4 complex. In the Lipid A-MD2 complex, 5 of the 6 fatty acid chains bind to MD2 whereas the final fatty acid is exposed to the surface and contacts TLR4. The phosphate groups contribute to TLR4-MD2 dimerization by interacting with lysine and arginine on both TLR4 and MD2. In the Eritoran-MD2 complex no direct contacts between Eritoran and MD2 are present and as a result, dimerization cannot be induced.

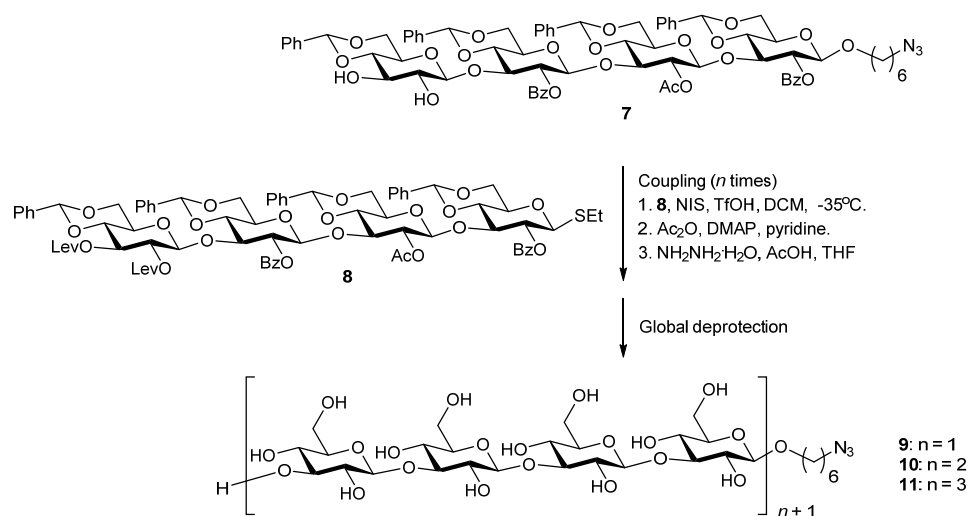
The NLR family in humans contains twenty-two proteins and they recognize several PAMPs of invading pathogens, such as bacterial cell wall peptidoglycan and flagellin. NLRs have gained much interest since it was recognized that polymorphisms in certain NLR genes were related to inflammatory disorders such as Crohn's disease and Blau syndrome.²¹ Two well-characterized members, NOD1 and NOD2, recognize peptidoglycan and subsequently form homo-oligomers and finally regulate the expression of signalling molecules, such as pro-inflammatory cytokines and type 1 interferons.²² NOD1 recognizes diaminopimelic acid (DAP) found in peptidoglycan whereas muramyl dipeptide (MDP) binds to NOD2. A minimal structural element for MDP binding of these ligands was identified.²³ The group of Fukase also synthesized diaminopimelic acid (DAP)-type peptidoglycan fragments. For example, DAP-containing PGN fragment **5** (Figure 2) was shown to be highly NOD1 stimulatory.²⁴

The collaboration of PRRs and the synergistic effect of mixtures of PAMPs was an incentive to explore the combination of two ligands within one structure. Willems *et al.*²⁵ synthesized tri-component antigen bisconjugates (*e.g.* **6**, Figure 2) in which the antigenic peptide DEVA₅K was attached to both the well-known TLR2 ligand Pam₃CysSK₄ and the NOD2 ligand MDP. Interestingly, this bisconjugate showed enhanced immunostimulating properties in comparison with the corresponding monovalent conjugates.

Figure 2. DAP-type peptidoglycan fragment **5** and a Pam₃Cys-MDP bisconjugate **6**.

Lectins are a large family of receptors that recognize (pathogen derived) carbohydrate structures. Examples are galectins, C-type lectin receptors (CLRs) and siglecs. Their carbohydrate-recognition domains (CRDs) most often have calcium ions in their binding pocket to promote the binding between the protein and the corresponding carbohydrate by forming a bridge between the hydroxyl groups of the carbohydrate and glutamates of the lectin.²⁶ The mannose receptor and dectin-1 are well-known CLRs. Dectin-1 is a receptor for β -1,3-glucans and is a key player in antifungal immunity. Upon binding of β -1,3-glucans to the extracellular part of Dectin-1, the intracellular domain is phosphorylated and dimerization occurs.²⁷ Dectin-1 has been shown to synergize with TLR2.²⁸ To study Dectin-1 signalling, the group of Takahashi synthesized an octasaccharide, a dodecasaccharide and a hexadecasaccharide (both linear and branched) using block couplings of tetrasaccharides.²⁹ The synthesis of the linear fragments is presented in Scheme 1. The 2,3-diol acceptor **7** was coupled with tetrameric donor **8** followed by acetylation of the free hydroxyl and removal of the levulinoyl protecting groups. This strategy provided the next acceptor in a similar glycosylation event. The target fragments were obtained by global deprotection. The biological evaluation of these compounds showed that at least an undecamer is required to activate Dectin-1 signalling.

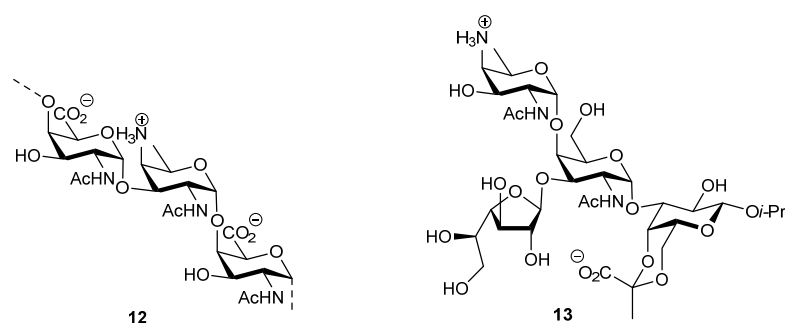
Scheme 1. Synthesis of a octameric, dodecameric and hexadecameric β -1,3-glucan fragment to study Dectin-1 signalling.



Zwitterionic polysaccharides

As discussed above, carbohydrate antigens are usually not capable of eliciting humoral immunity because they are T cell-independent and therefore cannot promote immunoglobulin class switching. However, the unique class of bacterial zwitterionic polysaccharides (ZPs) can evoke T-cell dependent immune responses without conjugation to a carrier protein and the use of adjuvants. In addition, ZPs have also been recognized as TLR2 ligands. To explore their mode of action at the molecular levels, well-defined ZPs are crucial. Both the group of Bundle³⁰ and Christina *et al.*³¹ explored the synthesis of fragments of Sp1 polysaccharides (Figure 3, **12**), having a trisaccharide repeating unit, originating from *Streptococcus pneumoniae*. The group of Seeberger published the total synthesis of the zwitterionic polysaccharide A1 repeating unit of the commensal bacteria *Bacteroides fragilis* (Figure 3, **13**).³²

Figure 3. The zwitterionic Sp1 polysaccharide of *S. Pneum* (**12**) and the A1 repeating unit found on *B. Fragilis* (**13**).

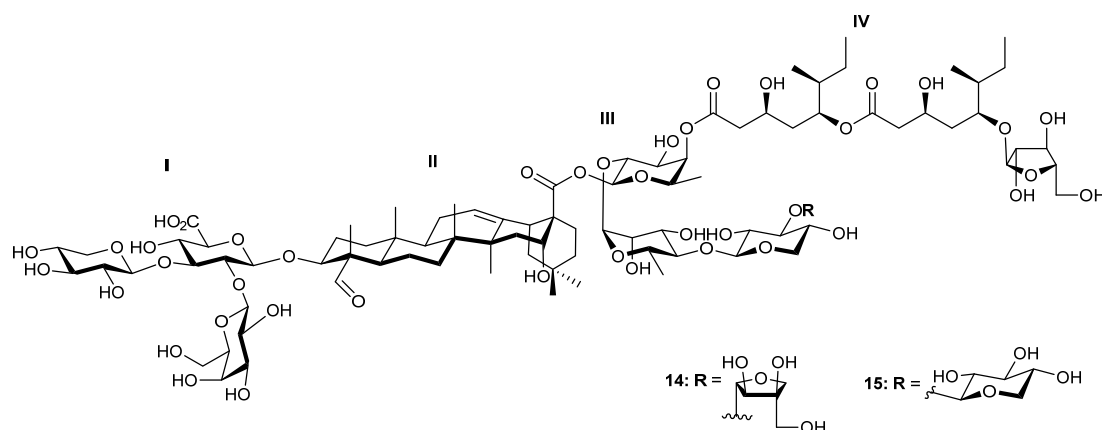


The immunostimulating profile of ZPs has attracted attention as a guideline to develop new vaccine modalities. Gallorini and coworkers³³ explored the introduction of zwitterionic motifs into isolated bacterial polysaccharides by chemical modifications and it was found that human and mouse APCs were activated upon introduction of a zwitterionic motif.

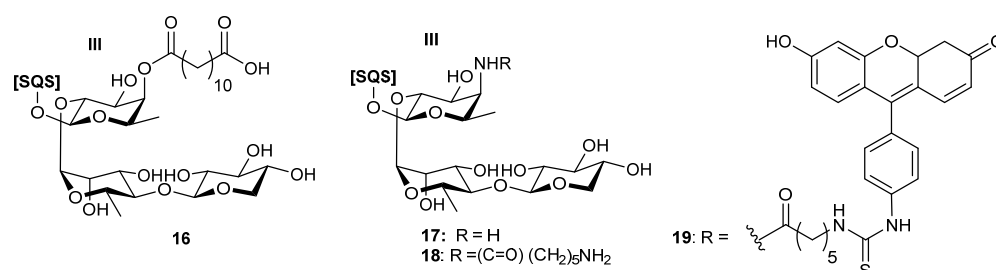
Adjuvant QS-21

The use of adjuvants is a popular method to augment the immune response against pathogens. An adjuvant itself is not necessarily immunogenic, but significantly enhances the immune response to a coadministered antigen. The most commonly used adjuvant in human vaccinations is Alum. Alum consists of aluminium salts and is believed to bind lipid moieties on dendritic cells and the resulting altered membrane lipid structures trigger DC responses.³⁴ Extracts from the bark of the South American tree *quillaja saponaria* (QS) were also shown to possess adjuvant activity. The active compound of this extract, QS-21 (Figure 4), consists of a branched trisaccharide (I), a quillaic acid triterpene (II), a linear tetrasaccharide (III) and an acyl chain (IV). Two isomeric forms are found that differ in substitution of the terminal carbohydrate (III). The major isomer **14** has an abundance of 65% and is known as QS-21-apiose and the minor isomer **15** is known as QS-21-xylose.³⁵ The group of Gin developed a synthetic route towards QS-21.³⁶

Figure 4. Structure of the saponin adjuvant QS, which consists of a branched trisaccharide (I), a quillaic acid triterpene (II), a linear tetrasaccharide (III) and an acyl chain (IV).



Assessment of the triterpene aldehyde in II showed that the aldehyde function is essential for adjuvant activity.³⁷ It is proposed that this aldehyde forms a Schiff base with a free amino group on an immune cell to stabilize the interactions. The glucuronic acid moiety in the branched trisaccharide part (I) does not seem to be crucial for its activity, since modifications are tolerated, which makes this a potential conjugation site. As the total synthesis of QS-21 is lengthy, the group of Gin made a series of shortened QS-21 derivatives to assess the adjuvant activity of simplified QS-21 compounds.³⁸ For example, they replaced the acyl chain IV in QS-21 for a carboxyacyl group (as in **16**, Figure 5). The increased polarity of the acyl chain caused by the carboxylate improved the water solubility. The adjuvant activity of this compound was comparable to QS-21. With this dodecanoyl variant, Gin and coworkers further explored the influence of the linear tetrasaccharide domain III in a series of compounds. The optimal balance between toxicity and efficacy was achieved with a linear trisaccharide fragment (Figure 5, **16**). Fragment **16** exhibits comparable activity as QS-21, but is more readily accessible (26 vs 76 synthetic steps for QS-21 apiose). Since the acyl chain domain provides a good conjugation site, the introduction of two-amine containing saponins with the trisaccharide moiety was explored (Figure 5, **17** and **18**).

Figure 5. Saponin derivatives: variation in the acyl domain (SQS= synthetic QS, domain I and II).

Both of these saponins (**17** and **18**) showed loss of the adjuvant activity. This indicated that the positively charged ammonium group weakens the saponin adjuvant activity and that the carboxylate anion (at physiological pH) in **16** contributes to the adjuvant activity. The adjuvant activity of **18** could be restored when the free amine was functionalized with a fluorophore (**19**). In addition to its adjuvant activity, compound **19** also exhibited less toxicity. Subcellular localization showed that saponin **19** entered the cell and analysis of the cellular lysate revealed that the saponin remained intact. Encouraged by this favorable activity profile, two other variants functionalized with a BODIPY or Cascade Blue label were prepared (not shown). The BODIPY and the Cascade Blue labeled derivatives, however, exhibited low adjuvant activity and subcellular localization revealed that, these derivatives were not internalized by the dendritic cells. These results indicate that internalization is a prerequisite for adjuvant activity. Although these synthetic analogues shed some light on the structural requirements of QS-21 for adjuvant activity, the exact mechanism of the immunogenicity of QS-21 is still unknown. QS-21 is believed to assist the uptake of antigens into APCs through lectin-mediated interaction with the cell membrane. This can provoke cytokine profiles that augment the T- and/or B-cell responses.

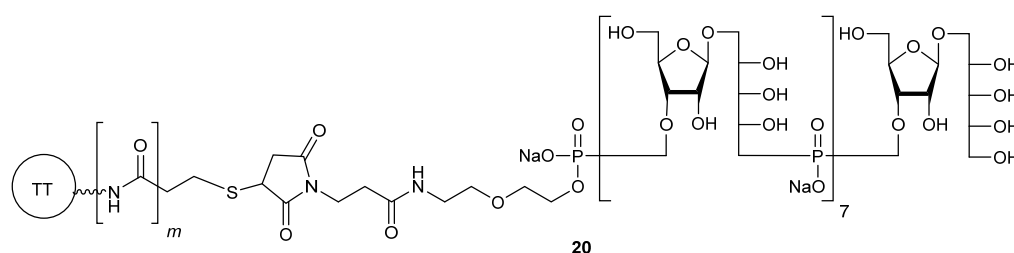
Synthetic carbohydrate-based vaccines

Vaccination with the ability to create long-term protective immunity is regarded as the most cost-effective strategy for controlling infectious diseases. The cell surfaces of bacteria and viruses are coated with characteristic carbohydrate structures, which are crucial for the pathogen to interact with the host cells. In addition, the expression of these cell surface carbohydrates is not under genetic control and therefore the surface oligosaccharides do not change upon genetic variation.³⁹ Several issues have to be addressed in carbohydrate vaccine design and these have been extensively reviewed.⁴⁰ The first request is the availability of carbohydrate antigens. Although numerous examples are reported to be successful, vaccines consisting of isolated polysaccharides do not always lead to effective vaccines. A viable alternative approach is presented by synthetic carbohydrate-based vaccines. These vaccines provide several advantages over those based on carbohydrates from natural sources. Synthetically prepared well-defined carbohydrates are homogeneous antigens and thus batch-to-batch heterogeneity during the production is minimal. Furthermore, as chemistry is generally able to provide larger quantities and the production costs of synthetic carbohydrate antigens might be lower than isolation from the natural sources, which is a tedious and often low yielding process. Another advantage of the use of synthetic carbohydrates is the possibility

to incorporate conjugation sites for the attachment of carrier proteins and higher control over the carbohydrate/protein ratio can be attained.

An example of a synthetic anti-bacterial vaccine is a polyribosylribitol conjugate developed by Verez-Bencomo *et al.* (Figure 6, **20**)⁴¹ as a vaccine against the *Haemophilus influenzae* type b bacterium which can cause meningitis, epiglottitis and pneumonia. The conjugate consists of a synthetic fragment of the capsular polysaccharide of the *H. influenzae* type b conjugated to carrier protein tetanus toxoid (TT) and it matched the efficacy of the conjugate vaccine derived from the native bacterial polysaccharide. Nowadays, the synthetic vaccine is used in various countries, including Cuba and Vietnam.

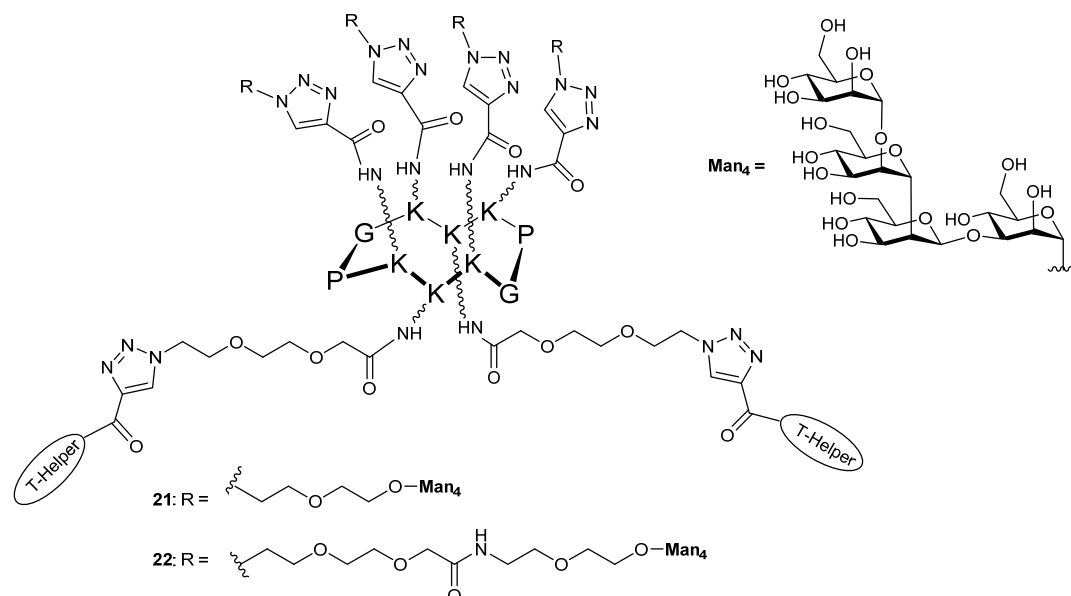
Figure 6. Polyribosylribitol phosphate-TT conjugate for protection against *H. influenzae* type b.



Viruses express glycans on their envelope and protein glycosylation is crucial for their survival and virulence. Therefore, viral glycans have become interesting molecular targets for the development of antiviral vaccines.⁴² An important threat to human health is the human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS). The virus destroys memory T cells and as a result, the immune system of HIV-infected people is severely weakened leading to infections by opportunistic pathogens.⁴³ The envelope of the HIV-1 virus consists of the glycoproteins (gps) gp120 and gp41 of which gp120 is exposed on the envelope surface. This gp is densely glycosylated with mannose and *N*-glycans and mediates the attachment of the virus to the host cells, indicating that it could function as a candidate antigen. A few monoclonal antibodies (MAbs) have been shown to be able to neutralize infection by HIV-1. For example, human MAb 2G12 recognizes the multiple high-mannose structures of gp120 and inhibits the interactions of HIV-1 with its host cells.⁴⁴ Vaccine modalities based on this phenomenon aim to evoke an immune response of 2G12-like antibodies with structural mimics of the 2G12 epitope. Several research groups including the groups of Danishefsky⁴⁵ and Wang⁴⁶ set out to explore this type of vaccine modality. Wang *et al.* focused on the assembly of oligomannose clusters on molecular scaffolds mimicking the 2G12 epitope. Initially they explored cholic acid⁴⁷ and galactose⁴⁸ as scaffolds, but these constructs did not result in the immunostimulating properties of gp120. It was hypothesized that the oligomannose chains required more glycan spacing or flexibility. Therefore, they changed their strategy and used a conformationally stabilized cyclic decapeptide as scaffold. The upper face of the peptide was decorated with oligomannose clusters and the other face was functionalized with T-helper peptide epitopes.⁴⁹ Two clusters studied are depicted in Figure 7. Cluster **22** with the extended spacer showed higher affinity to 2G12 than cluster **21**, indicating that high affinity binding requires appropriate spatial

orientation of the mannose chains. These clusters are now being evaluated for their ability to elicit carbohydrate specific neutralizing antibodies against HIV-1.

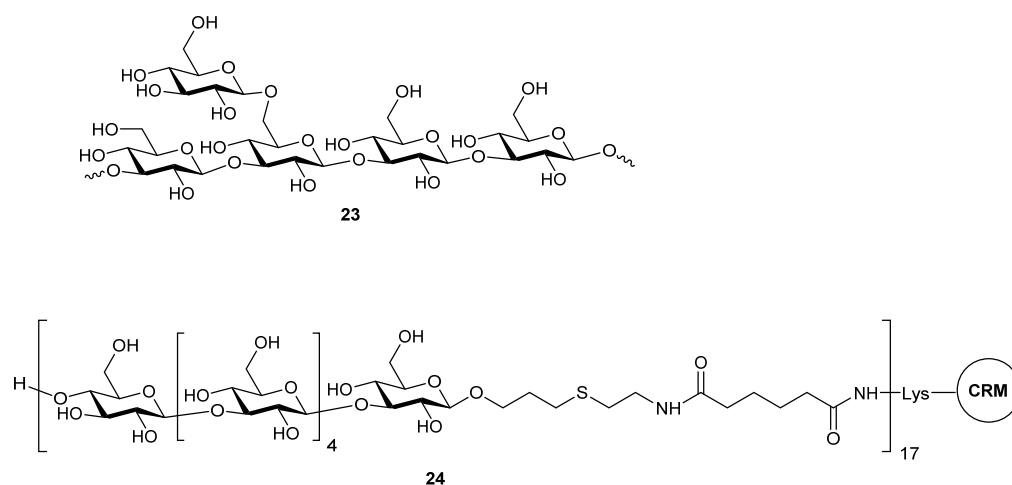
Figure 7. Template-assisted oligomannose clusters as mimics of 2G12 (T-Helper = QYIKANSKFIGITEL).



Fungi are eukaryotic organisms and among them are yeasts, molds and mushrooms. *Candida* is a well-known yeast and a harmless commensal. However, in organisms with a compromised immune system, it can cause several diseases such as thrush and esophagitis.⁵⁰ The cell wall of fungi is abundantly covered with polysaccharides and glycoproteins. Glucans with both β -1,3 and β -1,6-linkages are found on most if not all pathogenic fungi, making these polysaccharides an interesting target for vaccine development. As described in the first section of this Chapter, β -glucans have also been identified as Dectin-1 ligands.

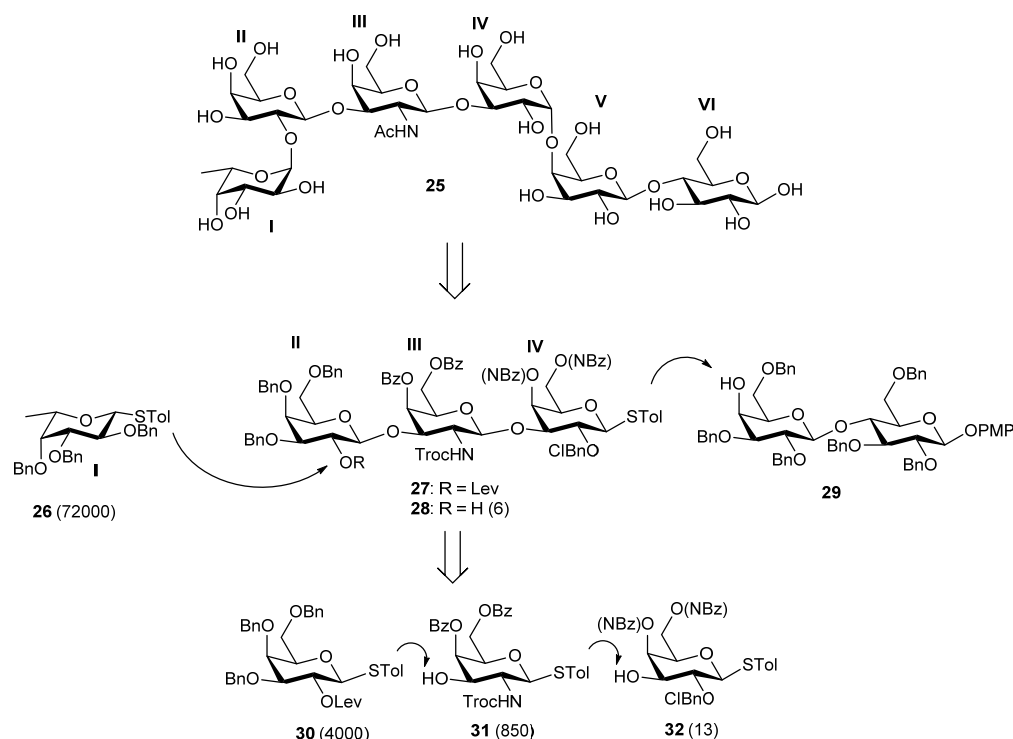
Laminarin (Lam) (Figure 8, **23**) has been proven to be immunostimulating and provide protection against *Candida albicans*, a prevalent and virulent *Candida* species, in mice. This polysaccharide is built up from a β -1,3-glucosyl-backbone with β -1,6-branches interspaced by about 10 residues. Adamo and coworkers⁵¹ studied the immunogenicity of several synthetic short linear and branched glucan fragments. A linear hexameric fragment turned out to be the most active and was conjugated to CRM₁₉₇, a well-known carrier protein (Figure 8, **24**).⁵² This conjugate proved to be more immunogenic than the Laminarin-CRM₁₉₇ conjugate, which indicates that the linear hexasaccharide fragment might be of sufficient length to mimic the *Candida albicans* epitope.

Figure 8. Laminarin (**23**) and glucan hexasaccharide-CRM₁₉₇ conjugate **24** as vaccine candidate against *Candida albicans*.



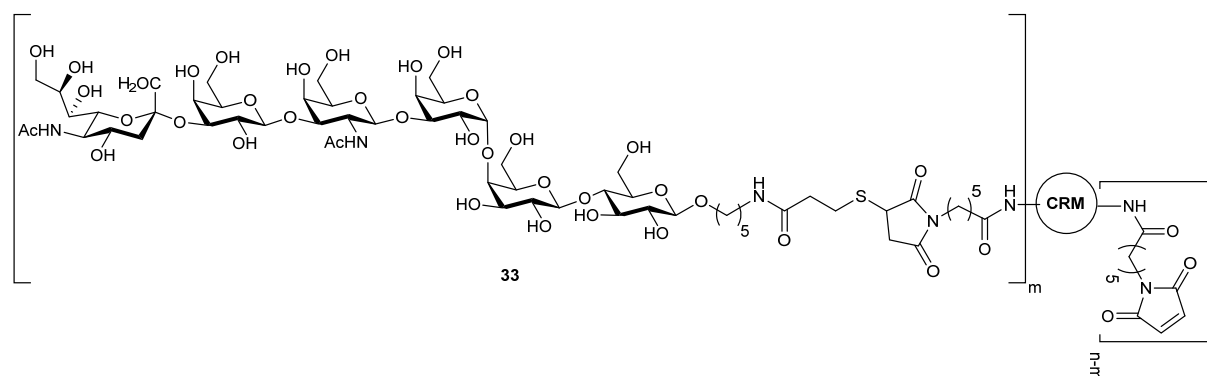
A lot of effort has been devoted to the development of anti-cancer vaccine modalities. Cancer is often accompanied by changes in the expression of cell surface carbohydrates. For instance, glycosphingolipid Globo H (GH) is a famous antigenic carbohydrate which is found on several cancer cell lines, such as ovarian, pancreatic, lung, prostate, and breast cancers.⁵³ Because of the overexpression of the hexasaccharide, Globo H is an important target for cancer immunotherapy. The group of Wong published an efficient synthesis of Globo H hexasaccharide **25** (Scheme 2)⁵⁴ using two one-pot reaction sequences in which they exploited the relative reactivity of the building blocks. The relative reactivities of the thio-building blocks used were tuned by the use of electron-donating groups (benzyl ether and Troc) and electron-withdrawing groups (Bz, NBz, ClBz) thus allowing chemoselective glycosylations. First, they constructed trisaccharide **27**, which contains the II, III and IV carbohydrate rings. Galactoside **30**, being almost five times more reactive than the galactosamine **31**, served as the first donor in this one-pot synthesis. Then the obtained disaccharide with the glucosamine at the reducing end served as the second donor in the coupling with galactoside **32**, yielding trisaccharide **27** in 67% yield. The temporary levulinoyl protective group was removed to give trisaccharide acceptor **28**. Fucose donor **26** reacts chemoselectively with this acceptor to provide a tetrasaccharide donor, which is subsequently condensed with disaccharide acceptor **29**. Reduction of the amides and acetylation of the free amines followed by global deprotection afforded the Globo H hexasaccharide **25**.

Scheme 2. Synthesis of Globo H hexasaccharide using relative reactivity-based one-pot strategies (values indicate relative reactivity values).



The group of Wong further explored their Globo H research by preparing conjugates with several carrier proteins.⁵⁵ Evaluation of their immunogenicity against breast cancer resulted in the development of glycoconjugate **33** (Figure 9) as a vaccine candidate.

Figure 9. Glycoconjugates with several proteins.



Conclusions

The abundance of carbohydrate structures on pathogens led to recognition of the important role carbohydrates play as PRR ligands. Well-defined carbohydrates are valuable tools in unraveling the structure-relationships of these biomolecules at the molecular level. Advances in synthetic chemistry such as automated oligosaccharide synthesis and one-pot strategies, have accelerated the access to carbohydrate antigens and analogues. The availability of synthetic oligosaccharides has allowed numerous promising carbohydrate-based vaccine

candidates to be developed. Undoubtedly, future research in glycoimmunology will lead to a better understanding of the interactions between carbohydrates and our immune system and eventually lead to improved vaccination programs against specific infections and diseases.

Outline of this Thesis

As outlined above, well-defined carbohydrates are essential tools to study the interaction of these biomolecules with our immune system. The research described in the present thesis is devoted to fundamental studies making immunologically active carbohydrate fragments more readily accessible. **Chapter 2** presents the study of the relative reactivity of glucuronic acid donors. Glucuronic acids are widespread in nature and therefore interesting synthetic targets. More insight into the reactivity of these carbohydrates can contribute to successful syntheses of glucuronic acid containing oligosaccharides. **Chapter 3** deals with a study on the application of glucuronic acid donors in the synthesis of *Streptococcus pneumoniae* type 3 polysaccharide fragments. **Chapter 4** describes the preparation of a new tandem ring-closing metathesis cleavable linker for solid phase oligosaccharide synthesis. The linker is an addition to the already established olefin metathesis cleavable linkers and its applicability is shown in the synthesis of two frame-shifted hyaluronic acid fragments. **Chapter 5** deals with the synthesis of β -1,3-glucan fragments, which are found on the cell wall of fungi and certain bacteria. Solution phase synthesis of small fragments is studied and initiatives to translate this chemistry to a solid phase format are presented. The new RCM cleavable linker discussed in Chapter 4 is applied here. Lipid A has been identified as the active principle of LPS of Gram-negative bacteria. Many natural lipid A compounds have been synthesized and evaluated, nonetheless the conjugation of lipid A analogues is still a research field to be explored. **Chapter 6** describes a synthetic route towards a lipid A analogue for conjugation. The final Chapter of this Thesis summarizes the research described in this Thesis and future considerations are presented.

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Chapter 2

Reactivity of glucuronic acid donors

Introduction

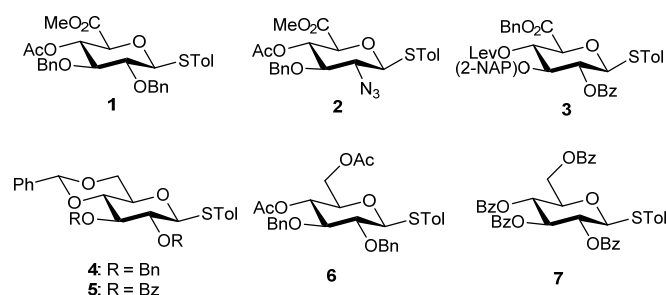
D-Glucuronic acid (GlcA) is a prime constituent of many biologically relevant oligosaccharides and glycoconjugates. For example, the human body uses glucuronylation as a key step in detoxification processes, in which xenobiotic or endobiotic substances are rendered (more) water-soluble by the appendage of a glucuronic acid to allow secretion.¹ Glucuronic acids are also frequently encountered on saponins, a very diverse class of glycosylated steroid and triterpene based compounds, forming the active ingredient of many traditional folk medicine.² The glycosaminoglycans (GAGs), a family of linear, anionic polysaccharides, which fulfills a plethora of biological functions, are an important example of D-glucuronic acid containing polysaccharides.^{3,4} Furthermore, various bacterial capsular polysaccharides feature glucuronic acids as prominent constituents.⁵ Because of their biological relevance, the synthesis of D-glucuronic acid containing oligosaccharides has received considerable attention.⁶ For the preparation of glucuronic acid containing oligosaccharides two strategies can be used. In a post-glycosylation-oxidation approach, the glycosidic linkages are constructed using non-

oxidized glucosyl building blocks and the introduction of the carboxylic acid moiety takes place at the oligosaccharide stage.⁷ In a pre-glycosylation-oxidation approach, glucuronic acid building blocks are used for the construction of the oligosaccharide. Due to presence of the electron-withdrawing carboxylic acid moiety, glucuronic acids are often considered to be unreactive glycosyl donors. Therefore, the post-glycosylation-oxidation approach is usually favoured over the alternative strategy. The reactivity of a large number of thioglycoside donors⁸ has been quantified by the groups of Ley,⁹ Wong,¹⁰ and Bols.¹¹ Recently, the relative reactivity of thio-mannuronic acid esters was studied by Walvoort *et al.*¹² In this study it became apparent that mannuronic acid donors are reactive donors. The relative reactivity of a galacturonic acid has been reported by Christina *et al.*,¹³ showing that galacturonic acid possesses modest reactivity compared to a set of galactosides. The relative reactivity of thioglucuronic acid esters, however, has never been explored. The establishment of the relative reactivity of carbohydrate building blocks not only provides important insight into the glycosylation capacity of a given donor, but it can also pave the way for chemoselective glycosylation reaction sequences. Here, the relative reactivity of glucuronic acid ester donors in comparison with the reactivity of a set of relevant thioglucosides is presented. The relative reactivity of glucuronic acid compared to mannuronic acid and galacturonic acid, the respective C-2 and C-4 epimers, is also discussed.

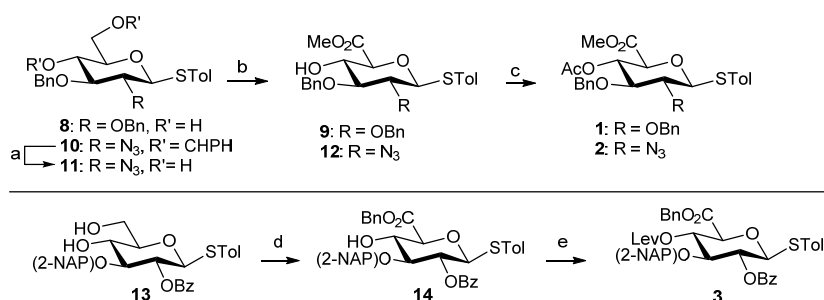
Results & Discussion

Competition experiments with glucuronic acid donors

The relative reactivity of three glucuronic acid donors **1**, **2** and **3** can be determined by competition experiments (Figure 1). Since Wong and co-workers have quantified the reactivity of a large series of *p*-methylphenylthio (*S*-tolyl)-glycosides using the NIS-TfOH promoter system, *S*-Tol glucuronic acid donors and the same activator were chosen. The reactivity of the selected glucuronic acid donors was set against a series of reference thioglucosides (Figure 1, **4-7**). Di-*O*-benzyl glucuronic acid ester **1** was studied as a model glucuronic acid ester having two 'arming' benzyl protecting groups.¹⁴ Azido-glucuronate **2** was selected to investigate the effect on the reactivity of an electron withdrawing group at the C-2 position. Glucuronic acid ester **3** was studied because it represents a potentially useful building block in the preparation of fragments of the *Streptococcus pneumoniae* type 3 capsular oligosaccharide (See Chapter 3). Thioglucosides **4-7** were used as reference compounds, representing commonly used glucosyl donors, featuring arming (benzyl ethers), semi-disarming (benzylidene acetal)¹⁵ and disarming (benzoyl and acetyl esters) protecting groups.

Figure 1. Donors used in this study.

The synthesis of the glucuronic acid donors **1**, **2** and **3** is presented in Scheme 1. Glucuronic acid **1** was constructed from di-benzyl glucoside **8**¹⁶ by regioselective oxidation of the primary alcohol using the 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and [bis(acetoxy)iodo]benzene (BAIB),¹⁷ followed by methylation of the carboxylic acid with methyl iodide and potassium carbonate. The C-4-hydroxyl was protected with an acetyl group using acetic anhydride in pyridine. Azido-glucuronate **2** was prepared from benzylidene protected glucosazide **10**,¹⁸ which was treated with *p*TsOH in MeOH to cleave the acetal protective group. The donor was accessed through the oxidation-methylation-acetylation sequence as described above. The synthesis of glucuronic acid donor **3** started with glucoside **13**.¹⁹ The primary alcohol was also oxidized with the TEMPO/BAIB system described above and the carboxylic acid was protected with a benzyl group under the agency of benzyl bromide and potassium carbonate. Unfortunately, this oxidation-acylation sequence turned out to be low yielding. It is hypothesized that the anomeric *S*-tolyl group might be too reactive for the chemoselective oxidation, because the oxidation-esterification sequence with its *S*-phenyl counterpart led to glucuronic acid benzyl ester in good yield. The free hydroxyl at the C-4 position was protected with a levulinyl group to give donor **3**.

Scheme 1. Synthesis of the glucuronic acid donors.

Reagents and conditions: (a) *p*TsOH, MeOH; (b) (i) TEMPO, BAIB, DCM/H₂O, (ii) MeI, K₂CO₃, DMF, **9**: 69%/2 steps, **12**: 61%/3 steps; (c) Ac₂O, Pyr., **1**: quant., **2**: 82%; (d) (i) TEMPO, BAIB, DCM/H₂O, (ii) BnBr, K₂CO₃, DMF, 17%/2 steps; (e) LevOH, DIC, DMAP, DCM, 78%.

In the competition experiments (depicted in Scheme 2), two thioglucuronic acid esters and/or thioglycosides competed for a limited amount of activator (NIS, 1 eq.) in the presence of a catalytic amount of TfOH (0.1 eq.) and an excess of the model glycosyl acceptor methyl 2,3,4-tri-*O*-benzyl- α -D-glucopyranoside **15** (3 eq.). The ratio of formed disaccharides²⁰ was established by NMR spectroscopy of the disaccharide mixtures, obtained after size exclusion

chromatography of the reaction mixtures. Table 1 compiles the results of the competition experiments. The high yields obtained (>84%) confirm the reliability of the executed experiments.

Scheme 2. Competition experiment of donor A and donor B.

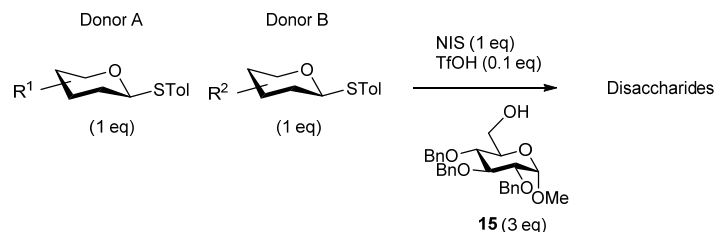


Table 1. Results of the competition experiments

Entry	Donor A	Donor B	Product ^a ratio donor A:B	Yield (%)
1	1	4	1:1	90%
2	1	6	1:8	87%
3	4	6	1:8	84%
4	1	5	2.3:1	90%
5	1	2	1:0	95%
6	1	3	9:1	89%
7	2	3	0:1	84%
8	2	7	1:0	94%

^a For structures of the disaccharides, see Experimental section.

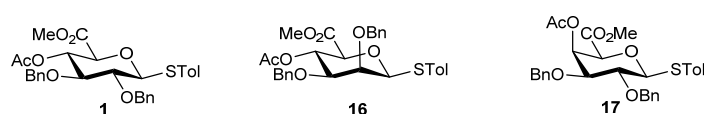
In the first competition experiment, glucuronic acid ester **1** competed with benzylidene-protected glucoside **4** leading to 1:1 mixture of the corresponding disaccharides (Entry 1). This shows that the combined deactivating effect of the C-5 carboxylic acid ester and the C-4-O-acetate in **1** is similar to the disarming effect of the benzylidene group in **4**. The equal reactivity of glucuronic acid **1** and benzylidene glucoside **4** was confirmed in two separate competition experiments, in which both donors competed with donor **6** (entry 2 and 3). Diacetyl donor **6** turned out to be eight times more reactive than both donor **1** and donor **4**. In line with expectations, the C-5 carboxylic acid ester reduces the reactivity of a glucosyl donor more than a CH₂OAc moiety at C-5 (Entry 2).²¹ The conformationally and electronically disarming 4,6-benzylidene group in **4** reduces the glucosyl donor reactivity more than the two acetyls at the C-4 and C-6 position (entry 3). This is in line with the relative reactivity of the analogous mannosyl donors reported by Walvoort *et al.*¹² The introduction of stronger electron withdrawing benzoyl groups at C-2 and C-3 of the 4,6-O-benzylidene glucoside leads to a donor (**5**) that is less reactive than glucuronic acid methyl ester **1** (Entry 4). The introduction of an azide at C-2 of the glucuronic acid donor, as in azideglucuronate **2**, significantly reduces the reactivity of glucuronic acid. In Entry 5 it is shown that donor **1** completely outcompeted donor **2**. The reactivity of glucuronate **3**,²² a building block to be used in the assembly of the *S. pneumoniae* oligomers, was assessed in competition experiments with both glucuronides **1** and **2**. Donor **3** was less reactive than dibenzyl donor **1** and more reactive than **2** (Entries 6 and 7). To establish the relative reactivity of the uronic acid donors with respect to a glucosyl donor of known reactivity, the least reactive glucuronide, *i.e.* **2**, was competed with tetra-O-benzoyl glucose donor **7**. Glucosyl donor **7** has a relative reactivity

value (RRV) of 1.3, on a scale in which peracetyl- α -*D*-tolyl-mannose has a RRV of 1 and perbenzyl- β -*D*-tolyl-glucose a RRV of 2656, as established by Wong and co-workers.¹⁰ In this competition reaction (Entry 8), the 2-azidoglucuronic acid donor completely outcompeted the tetrabenzoyl glucosyl donor leading to the formation of the glucuronic acid derived disaccharide as the sole product in 94% yield. Taken together, these results show that, although the C-5 carboxylic acid ester has a disarming effect on glucosyl donor reactivity (Table 1, entry 2), glucuronic acids should not by definition be classified as highly unreactive donors. Rather, they are placed in the continuum of reactivity amongst the 4,6-*O*-benzylidene glucosides.

Uronic acid reactivity

To determine the relative reactivity of glucuronic acid with respect to its C-2 and C-4 epimers, glucuronic acid **1** was competed with mannuronic acid (ManA, Figure 2, **16**) and galacturonic acid (GalA, Figure 2, **17**). The study presented above showed that the reactivity of glucuronic acid donors is moderate with respect to “non-oxidized” glucosyl donors. Walvoort *et al.*¹² showed that mannuronic acid donors are rather reactive donors²³ and Christina and *et al.*¹³ reported that galacturonic acid donor **17** possesses relatively low reactivity in comparison to a set of galactosides.

Figure 2. Glucuronic acid and its C-2 and C-4 epimeric counterparts (ManA **16** and GalA **17**, respectively).



In order to obtain the relative reactivity of donor **1**, **16** and **17**, two competition experiments were performed (conditions as described in Scheme 2) between donors **1** and **16** and **1** and **17**, respectively (Table 2). Analysis of the obtained disaccharide mixtures showed that mannuronic acid **16** completely outcompeted glucuronic acid **1** and glucuronic acid **1** in turn proved to be seven times more reactive than galacturonic acid **17**, thereby establishing a relative reactivity order ManA > GlcA > GalA. Notably this order contrasts the relative reactivity order of the “non-oxidized” pyranosides which has been established to be Gal > Man > Glc.²³

Table 2. Results of the competition experiments.

Entry	Donor A	Donor B	Product ratio donor A:B	Yield (%)
1	1	16	0:1	90%
2	1	17	7:1	89%

Conclusions

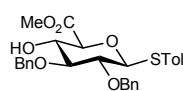
The relative reactivities of three glucuronic acid donors have been investigated in comparison with a set of glucosyl donors. It turned out that the reactivity of glucuronic acid is higher than often assumed. This is illustrated by the finding that 2,3-di-*O*-benzyl glucuronic acid methyl ester **1** is of equal reactivity as the 2,3-di-*O*-benzyl-4,6-*O*-benzylidene glucosyl donor **4**. Furthermore, the azidoglucuronate **2**, the least reactive among the glucuronic acid donors

studied, outcompeted the perbenzoylated glucose **7** completely. Competition experiments with the common uronic acids, having an equal protective group pattern, showed that mannuronic acid is more reactive than glucuronic acid, which in turn is more reactive than galacturonic acid. This reactivity order differs from the relative reactivity order established for the non-oxidized donor glycosides.

Experimental section

General experimental procedures. Chemical shifts (δ) are given in ppm relative to TMS as internal standard. All ^{13}C APT spectra are proton decoupled. Reactions were performed at rT unless stated otherwise and were followed by TLC analysis with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulphuric acid in EtOH or with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ (25 g/L), $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot 2\text{H}_2\text{O}$ (10 g/L) and 10% H_2SO_4 in H_2O , followed by charring at 150 °C. Flash column chromatography was performed on silica gel (0.04-0.063 nm) and size exclusion chromatography (SEC) was performed on SephadexTM LH-20. Experiments which required an inert atmosphere were carried out under dry argon. Dichloromethane (p.a.) was distilled over P_2O_5 prior to use. Molecular sieves (3Å) were flame-dried before use.

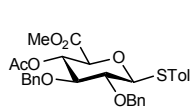
General procedure for competition experiments. Donor A (0.1 mmol, 1 eq.), donor B (0.1 mmol, 1 eq.) and the acceptor (Methyl 2,3,4-tri-*O*-benzyl- α -D-glucopyranoside, 3 eq.) were co-evaporated with toluene (2x). Freshly distilled DCM (4 mL, donor concentration: 0.05M), a teflon stirrer bar and activated (flame-dried) molecular sieves 3Å were added and the mixture was stirred under argon for 30 minutes at rT. NIS (1 eq) was added and the mixture was cooled to -40 °C. TfOH (0.1 eq, 0.1 mL of a 0.1M stock solution in distilled DCM) was added and the mixture was allowed to warm to 0 °C in ~3 h. Triethylamine (0.1 mL) was added and the mixture was diluted with EtOAc, washed with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ (1x) and sat. aq. NaCl (2x), dried over MgSO_4 and concentrated *in vacuo*. Elution over Sephadex LH-20 (DCM/MeOH, 1/1, v/v, 500 mL) enabled isolation of the disaccharide products, which were analysed with NMR spectroscopy. The yield of the disaccharide fraction and the ratio of the disaccharides were determined.



Methyl (*p*-tolyl 2,3-di-*O*-benzyl-1-thio- β -D-glucopyranosyluronate) (**9**):

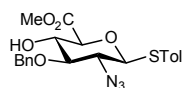
Glucoside **8** (1.0 g, 2.14 mmol) was dissolved in a mixture of DCM/ H_2O (2/1, 10.7 mL, 0.2M). The reaction mixture was cooled to 0 °C, followed by addition of BAIB (1.72 g, 5.35 mmol, 2.5 eq.) and TEMPO (67 mg, 0.43 mmol, 0.2 eq.). The reaction mixture was allowed to warm to rT and after 2 h., TLC analysis (PE/EtOAc: 1/1, v/v) showed total conversion of the starting material. The reaction mixture was quenched by addition of $\text{Na}_2\text{S}_2\text{O}_3$ (aq., sat.), followed by separation of the layers. The aq. layer was extracted with EtOAc (2x) and the combined organic layers were dried over MgSO_4 , filtered, concentrated, and co-evaporated with toluene. The yellow oil obtained was dissolved in DMF (10.7 mL, 0.2M) followed by addition of MeI (0.4 mL, 6.42 mmol, 3 eq.) and K_2CO_3 (0.89 g, 6.42 mmol, 3 eq.). After 30 min., TLC analysis (PE/EtOAc: 1/1, v/v) showed total conversion of the glucuronic acid. The reaction mixture was quenched with MeOH, diluted with DCM, washed with brine, dried over MgSO_4 , filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 6/4) gave the target compound in 69% yield over 2 steps (0.73 g, 1.48 mmol). $[\alpha]_{\text{D}}^{20}$: -38.8 ($c = 1$, DCM). IR (neat, cm^{-1}): 2870, 1746, 1493, 1454, 1439, 1398, 1356, 1279, 1265, 1238, 1209, 1175, 1130, 1059, 1018, 986, 908, 839, 808, 733, 696, 633, 615, 604. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.47 (d, 2H, $J = 8.0$ Hz, H_{arom}), 7.41-7.28 (m, 10H, H_{arom}), 7.11 (d, 2H, $J =$

8.0 Hz, H_{arom}), 4.88 (d, 1H, $J = 10.8$ Hz, $CHHPh$), 4.85 (m, 2H, CH_2 Bn), 4.74 (d, 1H, $J = 10.4$ Hz, $CHHPh$), 4.63 (d, 1H, $J = 9.6$ Hz, H-1), 3.88 (t, 1H, $J = 8.8$ Hz, H-4), 3.82-3.80 (m, 4H, H-5, OCH_3), 3.57 (t, 1H, $J = 8.8$ Hz, H-3), 3.45 (t, 1H, $J = 9.2$ Hz, H-2), 2.93 (bs, 1H, OH), 2.33 (s, 3H, CH_3 STol). ^{13}C APT NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC): 169.4 (C=O CO_2Me), 138.0 (C_q C_{arom}), 137.7 (C_q C_{arom}), 132.7 (CH_{arom}), 129.6 (CH_{arom}), 129.1 (C_q C_{arom}), 128.4-127.7 (CH_{arom}), 88.5 (C-1), 85.1 (C-3), 79.5 (C-2), 77.4 (C-5), 75.5 (CH_2 Bn), 75.3 (CH_2 Bn), 71.7 (C-4), 52.6 (OCH_3), 21.0 (CH_3 STol). HRMS: $[M+Na]^+$ calcd for $C_{28}H_{30}O_6SNa$: 517.16553, found 517.16477.



Methyl (p-tolyl) 4-O-acetyl-2,3-di-O-benzyl-1-thio-β-D-glucopyranosyluronate (1): Glucuronic acid methyl ester **9** (264 mg, 0.53 mmol)

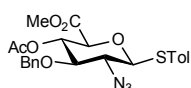
was dissolved in pyridine (1.8 mL, 0.3M). The reaction mixture was cooled to 0 °C, followed by addition of acetic anhydride (0.15 mL, 1.60 mmol, 3 eq.). After 15 min., TLC analysis (PE/EtOAc: 1/1, v/v) showed total conversion into a higher running spot. The reaction mixture was quenched with MeOH, diluted with EtOAc, washed with 1M HCl, $NaHCO_3$ (aq., sat.), dried over $MgSO_4$, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 8/2) gave the title compound as a white solid in quantitative yield (286 mg, 0.53 mmol). $[\alpha]_D^{20}$: + 21.2 ($c = 1$, DCM). IR (neat, cm^{-1}): 2874, 1753, 1732, 1495, 1454, 1435, 1369, 1356, 1310, 1234, 1206, 1179, 1121, 1090, 1078, 1045, 1028, 1018, 986, 962, 947, 899, 820, 806, 743, 698, 671, 646, 629, 617. ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): 7.49 (d, 2H, $J = 7.6$ Hz, H_{arom}), 7.40-7.22 (m, 10H, H_{arom}), 7.12 (d, 2H, $J = 8.0$ Hz, H_{arom}), 5.11 (t, 1H, $J = 9.6$ Hz, H-4), 4.87 (d, 1H, $J = 10.0$ Hz, $CHHPh$), 4.79 (d, 1H, $J = 11.6$ Hz, $CHHPh$), 4.69 (d, 1H, $J = 10.8$ Hz, $CHHPh$), 4.66 (d, 1H, $J = 11.6$ Hz, $CHHPh$), 4.57 (d, 1H, $J = 9.6$ Hz, H-1), 3.88 (d, 1H, $J = 10.0$ Hz, H-5), 3.73 (s, 3H, CO_2CH_3), 3.67 (t, 1H, $J = 9.2$ Hz, H-3), 3.52 (t, 1H, $J = 9.6$ Hz, H-2), 2.33 (s, 3H, CH_3 STol), 1.91 (s, 3H, CH_3 Ac). ^{13}C APT NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC): 169.5 (C=O), 167.6 (C=O), 138.3 (C_q C_{arom}), 137.9 (C_q C_{arom}), 137.8 (C_q C_{arom}), 133.2-132.8 (CH_{arom}), 129.8 (CH_{arom}), 129.6 (C_q C_{arom}), 128.6-127.7 (CH_{arom}), 87.9 (C-1), 83.3 (C-3), 79.7 (C-2), 76.3 (C-5), 75.4 (CH_2 Bn), 75.2 (CH_2 Bn), 69.3 (C-4), 52.7 (OCH_3), 21.1 (CH_3 STol), 20.6 (CH_3 Ac). HRMS: $[M+Na]^+$ calcd for $C_{30}H_{32}O_7SNa$: 559.17610, found 559.17561.



Methyl (p-tolyl) 2-azido-3-O-benzyl-2-O-deoxy-1-thio-β-D-glucopyranosyluronate (12): Benzylidene protected glucosamine **10** (2.83 g, 5.78 mmol) was suspended in MeOH (19.3 mL, 0.3M) and a catalytic amount of $pTsOH$

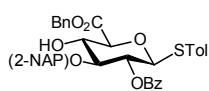
was added. After 4 h., TLC analysis showed total conversion into a lower running spot (PE/EtOAc: 3/1, v/v, R_f 0.2). The reaction mixture was neutralized with TEA and concentrated. The residue was dissolved in EtOAc and washed with H_2O (2x), dried over $MgSO_4$, filtered and concentrated. Crude ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): 7.41 (d, 2H, $J = 8.0$ Hz, H_{arom}), 7.35-7.25 (m, 5H, H_{arom}), 7.09 (d, 2H, $J = 8.0$ Hz, H_{arom}), 4.86 (d, 1H, $J = 10.8$ Hz, $CHHPh$), 4.76 (d, 1H, $J = 11.2$ Hz, $CHHPh$), 4.35 (d, 1H, $J = 10.0$ Hz, H-1), 3.81 (d, 1H, $J = 11.6$ Hz, H-6), 3.71 (d, 1H, $J = 11.6$ Hz, H-6), 3.51 (t, 1H, $J = 8.8$ Hz, H-3), 3.44 (d, 1H, $J = 3.6$ Hz, C-3-OH), 3.29 (t, 1H, $J = 9.2$ Hz, H-4), 3.26-3.21 (m, 2H, H-2, H-5), 2.95 (bs, 1H, C-6-OH), 2.30 (s, 3H, CH_3 STol). Crude ^{13}C APT NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC): 138.6 (C_q C_{arom}), 137.6 (C_q C_{arom}), 133.6 (CH_{arom}), 129.7 (CH_{arom}), 128.4 (CH_{arom}), 128.0 (CH_{arom}), 127.1 (C_q C_{arom}), 86.2 (C-1), 84.4 (C-4), 79.3 (C-2), 75.3 (CH_2 Bn), 69.8 (C-3), 64.5 (C-5), 61.8 (C-6), 21.0 (CH_3 STol). The crude residue was dissolved in a mixture of DCM/ H_2O (2/1, 28.9 mL, 0.2M). The reaction mixture was cooled to 0 °C and BAIB (4.66 g, 14.45mmol, 2.5 eq.) and TEMPO (181 mg, 1.16 mmol, 0.2 eq) were added.⁸ After 2 h., TLC analysis (PE/EtOAc: 1/1, v/v) showed total consumption of the starting material. The reaction mixture was quenched by addition of $Na_2S_2O_3$ (aq., sat.) and $NaHCO_3$ (aq., sat.). The reaction mixture was extracted with EtOAc (2x) and the combined organic layers were dried over $MgSO_4$, filtered, concentrated, and co-concentrated with toluene. The obtained yellow oil was dissolved in DMF (29.0 mL, 0.2M), followed by addition of methyl iodide (1.08 mL, 17.34 mmol, 3 eq.) and K_2CO_3 (2.4 g,

17.34 mmol, 3 eq.). After 20 min., TLC analysis (PE/EtOAc: 1/1, v/v) showed total conversion of the glucuronate. The reaction mixture was quenched by addition of MeOH and extracted with Et₂O (2x). The combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 1/1) gave the title compound in 61% yield over 3 steps (1.51 g, 3.51 mmol). $[\alpha]_{\text{D}}^{20}$: +82.8 (*c* = 1, DCM). IR (neat, cm⁻¹): 2874, 2108, 1744, 1493, 1454, 1439, 1379, 1354, 1267, 1236, 1207, 1175, 1065, 1018, 957, 908, 837, 810, 737, 698, 677, 617. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.46 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.37-7.25 (m, 5H, H_{arom}), 7.11 (d, 2H, *J* = 8.0 Hz, H_{arom}), 4.88 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.82 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.35 (d, 1H, *J* = 10.0 Hz, H-1), 3.78-3.75 (m, 5H, H-4, H-5, OCH₃), 3.38 (t, 1H, *J* = 8.4 Hz, H-3), 3.26 (t, 1H, *J* = 9.6 Hz, H-2), 2.31 (s, 3H, CH₃ STol). ¹³C NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 168.9 (C=O), 138.6 (C_q C_{arom}), 137.3 (C_q C_{arom}), 133.9 (CH_{arom}), 129.4 (CH_{arom}), 128.1 (CH_{arom}), 127.8 (CH_{arom}), 127.6 (CH_{arom}), 126.2 (C_q C_{arom}), 86.2 (C-1), 83.2 (C-3), 77.3 (C-4 or C-5), 75.1 (CH₂ Bn), 71.3 (C-4 or C-5), 63.6 (C-2), 60.0 (C-6), 52.4 (OCH₃), 20.7 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₂₁H₂₃N₃O₅SNa: 452.12506, found 452.12362.



Methyl (*p*-tolyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-*O*-deoxy-1-thio-β-D-glucopyranosyluronate) (2): Azide glucuronate **12** (1.07 g, 2.50 mmol) was dissolved in pyridine (8.33 mL, 0.3M). The reaction mixture was cooled to 0 °C and

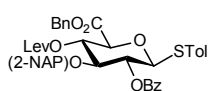
acetic anhydride (1.0 mL, 10.58 mmol, 4.2 eq.) was added. After 15 min., TLC analysis (PE/EtOAc: 1/1, v/v) showed total conversion of the starting material. The reaction mixture was quenched by subsequent addition of MeOH and H₂O, diluted with EtOAc, washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated yielding the target compound as a colourless oil in 82% (0.97 g, 2.1 mmol). $[\alpha]_{\text{D}}^{20}$: -10.7 (*c* = 1.5, DCM). IR (neat, cm⁻¹): 2108, 1746, 1356, 1277, 1233, 1213, 1186, 1163, 1126 1111, 1074, 1053, 1007, 986, 955, 903, 841, 812, 752, 698, 677, 650. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.51 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.34-7.24 (m, 6H, H_{arom}), 7.19-7.15 (m, 2H, H_{arom}), 5.00 (t, 1H, *J* = 9.6 Hz, H-4), 4.80 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.66 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.36 (d, 1H, *J* = 10.4 Hz, H-1), 3.87 (d, 1H, *J* = 10.0 Hz, H-5), 3.74 (s, 3H, OCH₃), 3.53 (t, 1H, *J* = 9.2 Hz, H-3), 3.45 (t, 1H, *J* = 9.6 Hz, H-2), 2.36 (s, 3H, CH₃ STol), 1.94 (s, 3H, CH₃ Ac). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 134.7 (CH_{arom}), 130.0 (CH_{arom}), 128.5 (CH_{arom}), 128.1 (CH_{arom}), 128.0 (CH_{arom}), 86.0 (C-1), 81.8 (C-3), 76.3 (C-5), 75.5 (CH₂ Bn), 70.8 (C-4), 63.9 (C-2), 52.8 (OCH₃), 21.2 (CH₃ STol), 20.6 (CH₃ Ac). [M+Na]⁺ calcd for C₂₃H₂₅N₃O₆SNa: 494.13563, found 494.13521.



Benzyl (*p*-tolyl 2-*O*-benzoyl-3-*O*-naphthylmethyl-1-thio-β-D-glucopyranosyluronate) (14): Glucoside **13** (1.47 g, 2.37 mmol) was dissolved in DCM/H₂O (2/1, 11.8 mL, 0.2M) and chilled to 0 °C. To the reaction mixture,

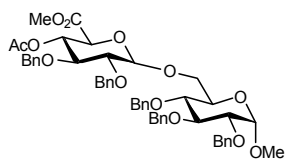
BAIB (1.91 g, 5.93 mmol, 2.5 eq.) and TEMPO (74 mg, 0.47 mmol, 0.2 eq.) were added and the reaction mixture was allowed to warm to rT. After 1 h., TLC analysis (PE/EtOAc: 3/1 + 1% AcOH, v/v) showed total conversion into a lower running spot. The reaction mixture was quenched with Na₂S₂O₃ (aq., sat.) and stirred for 30 min. Then, the reaction mixture was diluted with DCM, washed with brine, dried over MgSO₄, filtered, concentrated, and co-evaporated with toluene. An orange oil was obtained, which was dissolved in DMF (11.84 mL, 0.2M), followed by addition of BnBr (0.56 mL, 4.74 mmol, 2 eq.) and K₂CO₃ (0.66 g, 4.74 mmol, 2 eq.). TLC analysis showed total conversion into a higher running spot (PE/EtOAc: 1/1, v/v, R_f 0.48). The reaction mixture was quenched with MeOH, diluted with DCM, washed with H₂O, brine, dried over MgSO₄, filtered, and concentrated. A yellow solid was obtained, which was purified by column chromatography (PE/EtOAc: 1/0 → 8/2) yielding the title compound as a white solid in 17% over 2 steps (0.31 g, 0.43 mmol). $[\alpha]_{\text{D}}^{20}$: +18.6 (*c* = 1, DCM). IR (neat, cm⁻¹): 1728, 1452, 1398, 1352, 1315, 1263, 1204, 1175, 1144, 1090, 1069, 1026, 972, 955, 812, 750, 710, 698. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.94 (d, 2H, *J* = 7.6 Hz,

H_{arom}), 7.66-7.49 (m, 16H, H_{arom}), 7.47-7.06 (m, 6H, H_{arom}), 7.10 (d, 2H, $J = 8.0$ Hz, H_{arom}), 5.24 (t, 1H, $J = 9.6$ Hz, H-2), 5.20 (m, 2H, CH₂ CO₂Bn), 4.88 (d, 1H, $J = 10.8$ Hz, CHHAr), 4.79-4.68 (m, 2H, CH₂Ar, H-1), 4.53 (d, 1H, $J = 10.0$ Hz, CHHAr), 4.11 (d, 1H, $J = 9.6$ Hz, H-5), 3.97 (t, 1H, $J = 9.6$ Hz, H-4), 3.87 (t, 1H, $J = 8.8$ Hz, H-3), 2.30 (s, 3H, CH₃ STol). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 167.7 (C=O), 164.9 (C=O), 138.4 (C_q C_{arom}), 137.5 (C_q C_{arom}), 135.0-133.1 (CH_{arom}), 133.0 (C_q C_{arom}), 129.7-125.7 (CH_{arom}), 86.9 (C-1), 83.1 (C-3), 79.2 (C-4), 78.3 (C-5), 75.3 (CH₂Ar), 75.0 (CH₂Ar), 71.6 (C-2), 67.3 (CH₂ CO₂Bn), 21.1 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₄₅H₄₀O₇SNa: 747.23870, found 747.23875.



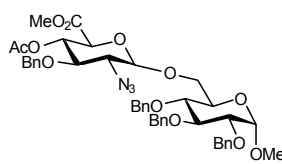
Benzyl (p-tolyl 2-O-benzoyl-4-O-benzyl-3-O-levulinyl-1-thio-β-D-glucopyranosyluronate) (3):

Glucuronic acid ester **14** (0.44 g, 0.75 mmol) was dissolved in DCM (7.5 mL, 0.1M) followed by subsequent addition of LevOH (98 μL, 0.97 mmol, 1.3 eq.), DIC (114 μL, 0.97 mmol, 1.3 eq.) and DMAP (cat.). After 30 min., TLC analysis showed total conversion into a lower running spot (PE/EtOAc: 12/8, v/v, R_f 0.48). The reaction mixture was filtered, diluted with EtOAc, washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 7/3) gave the title compound as a white solid in 78% yield (398 mg, 0.58 mmol). [α]_D²⁰: +1.56 (c = 5.6, DCM). IR (neat, cm⁻¹): 1742, 1719, 1452, 1402, 1360, 1315, 1263, 1206, 1177, 1155, 1090, 1069, 1026, 997, 970, 910, 847, 808, 750, 712, 698. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.05 (d, 2H, $J = 7.6$ Hz, H_{arom}), 7.57 (t, 1H, $J = 7.6$ Hz, H_{arom}), 7.46-7.42 (m, 2H, H_{arom}), 7.36-7.21 (m, 10H, H_{arom}), 7.16-7.14 (m, 2H, H_{arom}), 7.02 (d, 2H, $J = 8.0$ Hz, H_{arom}), 5.43 (t, 1H, $J = 9.2$ Hz, H-3), 5.20 (s, 2H, CH₂ Bn ester), 5.14 (t, 1H, $J = 9.6$ Hz, H-2), 4.79 (d, 1H, $J = 10.0$ Hz, H-1), 4.54 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.49 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.08 (d, 1H, $J = 10.0$ Hz, H-5), 3.98 (t, 1H, $J = 9.6$ Hz, H-3), 2.50-2.40 (m, 2H, CH₂ Lev), 2.39-2.25 (CH₂ Lev), 1.97 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.6 (C=O Lev ketone), 171.6 (C=O), 167.2 (C=O), 165.0 (C=O), 138.6 (C_q C_{arom}), 137.3 (C_q C_{arom}), 134.8 (C_q C_{arom}), 133.7 (CH_{arom}), 133.3 (CH_{arom}), 129.8 (CH_{arom}), 129.6 (CH_{arom}), 129.1 (C_q C_{arom}), 128.5-127.7 (CH_{arom}), 127.4 (C_q C_{arom}), 86.5 (C-1), 78.1 (C-5), 77.0 (C-4), 75.3 (C-3), 74.6 (CH₂ Bn ether), 70.2 (C-2), 67.4 (CH₂ Bn ester), 37.5 (CH₂ Lev), 29.4 (CH₃ Lev), 29.7 (CH₂ Lev), 21.1 (CH₃ STol). HRMS: [M+K]⁺ calcd for C₃₉H₃₈O₉SK: 721.18681, found 721.18661.



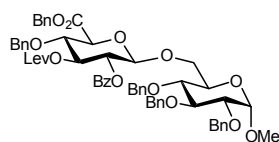
Methyl 2,3,4-tri-O-benzyl-6-O-(methyl 4-O-acetyl-2,3-di-O-benzyl-α/β-D-glucopyranosyluronate)-α-D-glucopyranoside (1a):

The title compound was isolated as a white solid and an anomeric mixture (α/β: 1/3). IR (neat, cm⁻¹): 2920, 2909, 2872, 1748, 1454, 1362, 1292, 1265, 1231, 1156, 1138, 1090, 1047, 1028, 908, 733, 696. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.90 (d, 2H, $J = 7.2$ Hz, H_{arom}), 7.43 (t, 1H, $J = 7.2$ Hz, H_{arom}), 7.35-7.22 (m, 23H, H_{arom}), 7.15-7.13 (m, 2H, H_{arom}), 7.03-7.01 (m, 2H, H_{arom}), 5.40 (dd, 1H, $J = 3.6$ Hz, $J = 9.2$ Hz, H-3'), 5.27 (t, 1H, $J = 9.6$ Hz, H-2'), 5.19 (s, 2H, CH₂ Bn ester), 4.88 (d, 1H, $J = 12.4$ Hz, CHHPh), 4.72 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.63 (d, 1H, $J = 8.0$ Hz, H-1'), 4.58 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.55-4.47 (m, 2H, CH₂ Bn), 4.46 (d, 1H, $J = 3.2$ Hz, H-1), 4.25 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.08 (d, 1H, $J = 9.2$ Hz, H-6), 4.05-4.02 (m, 2H, H-4', H-5'), 3.86 (t, 1H, $J = 9.2$ Hz, H-3), 3.69-3.63 (m, 2H, H-5, H-6), 3.40 (dd, 1H, $J = 3.6$ Hz, $J = 9.6$ Hz, H-2), 3.32 (t, 1H, $J = 9.6$ Hz, H-4), 3.17 (s, 3H, OCH₃), 2.52-2.41 (m, 2H, CH₂ Lev), 2.38-2.27 (m, 2H, CH₂ Lev), 1.98 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.7 (C=O Lev ketone), 171.8 (C=O), 167.5 (C=O), 165.0 (C=O), 138.7 (C_q C_{arom}), 138.1 (C_q C_{arom}), 138.0 (C_q C_{arom}), 137.4 (C_q C_{arom}), 134.9 (C_q C_{arom}), 133.2 (CH_{arom}), 129.8 (CH_{arom}), 129.2 (C_q C_{arom}), 128.6-127.5 (CH_{arom}), 101.3 (C-1'), 97.8 (C-1), 81.8 (C-3), 79.7 (C-2), 77.2 (C-4, C-4', C-5'), 75.5 (CH₂ Bn), 74.7 (CH₂ Bn), 74.7 (CH₂ Bn), 74.1 (C-3'), 73.3 (CH₂ Bn), 71.7 (C-2'), 69.4 (C-5), 68.4 (C-6), 67.5 (CH₂ Bn ester), 54.9 (OCH₃), 37.7 (CH₂ Lev), 29.5 (CH₃ Lev), 27.9 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₄₄H₄₉O₁₂Na: 770.32968, found 770.33962.



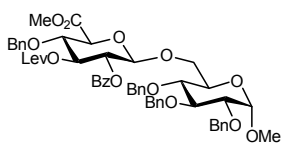
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 3-*O*-acetyl-2-azido-2-deoxy-3-*O*-benzyl- α/β -D-glucopyranosyluronate)- α -D-glucopyranoside (2a):

The title compound was isolated as a clear oil and as an anomeric mixture (α/β : 1/7.7). IR (neat, cm^{-1}): 2112, 1748, 1265, 1229, 1161, 1136, 1086, 1047, 1029, 908, 733, 696. ^1H NMR β anomer (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.37-7.26 (m, 20H, H_{arom}), 5.09 (t, 1H, $J = 9.6$ Hz, H-4'), 4.99 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.93 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.85-4.77 (m, 3H, CHHPh, CH_2Ph), 4.69-4.61 (m, 3H, CHHPh, CH_2Ph), 4.60 (d, 1H, $J = 3.6$ Hz, H-1), 4.18 (d, 1H, $J = 8.0$ Hz, H-1'), 4.10 (dd, 1H, $J = 1.6$ Hz, $J = 10.8$ Hz, H-6), 4.00 (t, 1H, $J = 9.2$ Hz, H-4), 3.82-3.80 (m, 1H, H-5), 4.76 (d, 1H, $J = 10.0$ Hz, H-5'), 3.67 (s, 3H, CO_2Me), 3.66-3.64 (m, 1H, H-6), 3.57-3.48 (m, 3H, H-2, H-2', H-3), 3.43 (t, 1H, $J = 9.6$ Hz, H-3'), 3.37 (s, 3H, CH_3 Ac), 1.96 (s, 3H, OCH_3). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 169.4 (C=O), 167.3 (C=O), 138.7-137.4 (C_q C_{arom}), 128.4-127.6 (CH_{arom}), 102.0 (C-1'), 98.1 (C-1), 82.0 (C-4), 79.8 (C-3'), 77.7 (C-2', C-2, C-3), 75.7 (CH_2 Bn), 75.1 (CH_2 Bn), 74.8 (CH_2 Bn), 73.4 (CH_2 Bn), 72.8 (C-5), 70.9 (C-4'), 69.7 (C-5), 68.8 (C-6), 65.4 (C-2, C-2', C-3), 55.2 (OCH_3), 52.7 (CO_2Me), 20.6 (CH_3 Ac). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{48}\text{H}_{51}\text{O}_{10}\text{Na}$: 810.33744, found 810.32286.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(benzyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-levulinyl- β -D-glucopyranosyluronate)- α -D-glucopyranoside (3a):

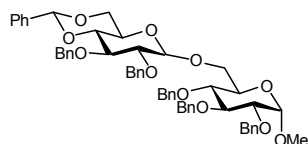
The title compound was isolated as a white solid. $[\alpha]_{\text{D}}^{25}$: +0.8 ($c = 0.8$, DCM). IR (neat, cm^{-1}): 2918, 1744, 1454, 1360, 1265, 1213, 1177, 1155, 1092, 1070, 1028, 999, 735, 712, 698. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.91-7.89 (m, 2H, H_{arom}), 7.43 (t, 1H, $J = 7.2$ Hz, H_{arom}), 7.35-7.22 (m, 23H, H_{arom}), 7.15-7.03 (m, 2H, H_{arom}), 7.02-7.01 (m, 2H, H_{arom}), 5.40 (t, 1H, $J = 9.2$ Hz, H-3'), 5.27 (t, 1H, $J = 9.6$ Hz, H-2'), 5.19 (s, 2H, CH_2 Bn ester), 4.88 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.72 (d, 1H, $J = 12.4$ Hz, CHHPh), 4.68 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.63 (d, 1H, $J = 8.0$ Hz, H-1'), 4.58 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.55-4.47 (m, 3H, CH_2Ph , CHHPh), 4.46 (d, 1H, $J = 3.2$ Hz, H-1), 4.25 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.08 (d, 1H, $J = 9.2$ Hz, H-6'), 4.05-3.95 (m, 2H, H-4', H-5'), 3.86 (t, 1H, $J = 9.2$ Hz, H-3), 3.69-3.63 (m, 2H, H-5, H-6), 3.40 (dd, 1H, $J = 3.6$ Hz, $J = 9.6$ Hz, H-2), 3.53 (t, 1H, $J = 9.6$ Hz, H-4), 3.17 (s, 3H, OCH_3), 2.51-2.41 (m, 2H, CH_2 Lev), 2.38-2.24 (m, 2H, CH_2 Lev), 1.98 (s, 3H, CH_3 Lev). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 205.7 (C=O Lev ketone), 171.8 (C=O), 167.5 (C=O), 165.0 (C=O), 138.7 (C_q C_{arom}), 138.1 (C_q C_{arom}), 138.0 (C_q C_{arom}), 137.4 (C_q C_{arom}), 134.9 (C_q C_{arom}), 133.2 (CH_{arom}), 129.8 (CH_{arom}), 129.2 (C_q C_{arom}), 128.6-127.5 (CH_{arom}), 101.3 (C-1'), 97.8 (C-1), 81.8 (C-3), 79.7 (C-2), 77.2 (C-4, C-4', C-5'), 75.5 (CH_2 Bn), 74.7 (CH_2 Bn), 74.7 (CH_2 Bn), 74.1 (C-3'), 73.3 (CH_2 Bn), 71.7 (C-2'), 69.4 (C-5), 68.4 (C-6), 67.5 (CH_2 Bn ester), 54.9 (OCH_3), 37.7 (CH_2 Lev), 29.5 (CH_3 Lev), 27.9 (CH_2 Lev). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{55}\text{H}_{54}\text{O}_{13}\text{Na}$: 945.34566, found 945.34717.



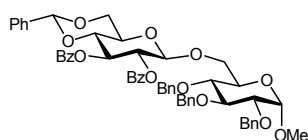
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-benzyl-2-*O*-benzoyl-3-*O*-levulinyl- β -D-glucopyranosyluronate)- α -D-glucopyranoside (3b):

The title compound was isolated as a transparent oil. $[\alpha]_{\text{D}}^{25}$: +3.6 ($c = 1.2$, DCM). IR (neat, cm^{-1}): 2913, 1748, 1452, 1404, 1358, 1315, 1265, 1225, 1202, 1175, 1155, 1092, 1070, 1028, 1001, 910, 933, 912, 698. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.90 (d, 2H, H_{arom}), 7.46 (t, 1H, $J = 7.6$ Hz, H_{arom}), 7.34-7.23 (m, 20H, H_{arom}), 7.05-7.02 (m, 2H, H_{arom}), 5.42 (t, 1H, $J = 8.8$ Hz, H-3'), 5.26 (t, 1H, $J = 9.6$ Hz, H-2'), 4.88 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.72 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.68 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.65-4.62 (m, 3H, CHHPh, CHHPh, H-1'), 4.58 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.50 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.59 (d, 1H, $J = 3.6$ Hz, H-1), 4.27 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.09-4.06 (m, 1H, H-6), 4.03-3.98 (m, 2H, H-4', H-5'), 3.86 (t, 1H, $J = 9.2$ Hz, H-3), 3.73 (s, 3H, CO_2Me), 3.69-3.63 (m, 1H, H-6), 3.40 (dd, 1H, $J = 3.2$ Hz, $J = 9.6$ Hz, H-2), 3.32 (t, 1H, $J = 9.6$ Hz, H-4), 3.17 (s, 3H, OCH_3),

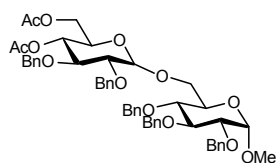
2.54-2.46 (m, 2H, CH₂ Lev), 2.44-2.38 (m, 2H, CH₂ Lev), 2.00 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.6 (C=O Lev ketone), 171.8 (C=O), 168.1 (C=O), 165.0 (C=O), 138.7 (C_q C_{arom}), 138.1 (C_q C_{arom}), 138.1 (C_q C_{arom}), 133.2 (CH_{arom}), 129.8 (CH_{arom}), 129.3 (C_q C_{arom}), 128.4-127.5 (CH_{arom}), 101.3 (C-1'), 97.8 (C-1), 81.8 (C-3), 79.7 (C-2'), 77.4 (C-4), 77.2 (C-5'), 75.5 (CH₂ Bn), 74.7 (CH₂ Bn), 74.7 (CH₂ Bn), 74.5 (C-4'), 74.2 (C-3'), 73.3 (CH₂ Bn), 71.7 (C-2'), 69.5 (C-5'), 68.5 (C-6), 54.9 (OCH₃), 52.6 (CO₂Me), 37.7 (CH₂ Lev), 29.5 (CH₃ Lev), 27.9 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₅₄H₅₈O₁₅Na: 969.36679, found 969.36717.



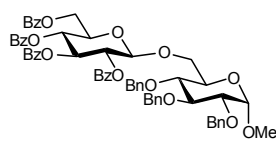
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene- α/β -D-glucopyranosyl)- α -D-glucopyranoside (4a): Disaccharide **4a** was produced as an anomeric mixture (α/β : 1.4/1). Spectroscopic data were in accord with those reported previously.²⁴



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-benzoyl- β -D-glucopyranosyl)- α -D-glucopyranoside (5a): The title compound was isolated as a white solid. [α]_D: +12.0 (*c* = 1, DCM). IR (neat): 2930, 1728, 1452, 1362, 1315, 1265, 1209, 1179, 1159, 1090, 1069, 1053, 1026, 999, 914, 853, 733, 696. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.93 (d, 2H, *J* = 6.8 Hz, H_{arom}), 7.90 (d, 2H, *J* = 7.2 Hz, H_{arom}), 7.46-7.37 (m, 5H, H_{arom}), 7.35-7.17 (m, 19H, H_{arom}), 7.01-6.99 (m, 2H, H_{arom}), 5.77 (t, 1H, *J* = 9.6 Hz, H-3'), 5.54 (t, 1H, *J* = 9.2 Hz, H-2'), 5.52 (s, 1H, CHPh), 4.89 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.78 (d, 1H, *J* = 7.6 Hz, H-1'), 4.75 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.68 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.65 (d, 1H, *J* = 9.6 Hz, CHHPh), 4.50 (d, 1H, *J* = 3.2 Hz, H-1), 4.45 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.41 (dd, 1H, *J* = 5.2 Hz, *J* = 10.4 Hz, H-6'), 4.23 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.12 (d, 1H, *J* = 9.6 Hz, H-6), 3.96-3.82 (m, 3H, H-6', H-3, H-4'), 3.75-3.62 (m, 3H, H-6, H-4, H-5'), 3.44 (dd, 1H, *J* = 3.2 Hz, *J* = 9.6 Hz, H-2), 3.39-3.36 (m, 1H, H-5), 3.23 (s, 3H, OCH₃). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 165.6 (C=O Bz), 165.0 (C=O Bz), 138.7 (C_q C_{arom}), 138.1 (C_q C_{arom}), 138.1 (C_q C_{arom}), 136.9 (C_q C_{arom}), 136.7 (C_q C_{arom}), 133.4-133.0 (CH_{arom}), 129.9-129.0 (CH_{arom}), 128.7 (C_q C_{arom}), 128.4 (C_q C_{arom}), 128.4 (C_q C_{arom}), 128.3-127.4 (CH_{arom}), 126.1-125.1 (CH_{arom}), 101.6 (C-1), 101.4 (CHPh), 98.0 (C-1'), 81.8 (C-3' or C-4'), 79.4 (C-2), 78.6 (C-3' or C-4'), 77.3 (C-5), 75.5 (CH₂ Bn), 74.7 (CH₂ Bn), 73.3 (CH₂ Bn), 72.3 (C-2'), 72.0 (C-3'), 69.4 (C-4), 68.5 (C-6'), 68.3 (C-6), 66.6 (C-5'), 55.0 (OCH₃). HRMS: [M+Na]⁺ calcd for C₅₅H₅₄O₁₃Na: 945.34566, found 945.34537.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl- α/β -D-glucopyranosyl)- α -D-glucopyranoside (6a): The title compound was isolated as a clear oil and as an anomeric mixture (α/β : 1/1.2). IR (neat): 2930, 2907, 2872, 1744, 1497, 1454, 1362, 1329, 1265, 1237, 1194, 1157, 1136, 1090, 1044, 1028, 910, 845, 822, 733, 696, 635, 604. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): Diagnostic peaks: 4.35 (d, 1H, *J* = 7.2 Hz, H-1 β), 3.36 (2.68H OCH₃ α), 3.33 (3.07H OCH₃ β). HRMS: [M+Na]⁺ calcd for C₅₂H₅₈O₁₃Na: 913.37696, found 913.37733.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl- β -D-glucopyranosyl)- α -D-glucopyranoside (7a): Disaccharide **7a** was produced as β -fused product only. Spectroscopic data were in accord with those reported previously.²⁵

References and notes

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22. The effect of the nature of the ester (methyl versus benzyl) on the reactivity of the glucuronyl donor was found to be very small: the methyl ester was 1.3 times more reactive than the benzyl ester (1.3:1, 94% yield).
23. The high reactivity of mannuronic acid **16** has been related to the formation of the relatively stable $^3\text{H}_4$ oxacarbenium ion. The relative stability of this oxacarbenium ion originates from the favorable orientations of the substituents in the mannuronic acid core, that all contribute to the stabilization of the positive charge.
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Chapter 3

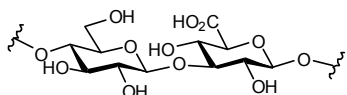
Synthesis of *Streptococcus pneumoniae* type 3 capsular polysaccharide fragments

Introduction

Streptococcus pneumoniae is an anaerobic Gram-positive bacterium which exclusively inhabits human beings.¹ Harmlessly it resides within the upper respiratory tract, specifically in the nasal passages. Normally it represents no significant health threat, but it is the leading cause of invasive bacterial disease in people with weakened immune system such as children and the elderly. The bacterium can cause diseases such as pneumonia, acute otitis media, meningitis, and brain abscesses.² A tridecavalent capsular polysaccharide vaccine, licenced under the name of Prevnar/Prevenar-13[®],³ which is routinely administered to infants and young children, presents one of the most powerful tools to prevent infections by this pathogen, and polyvalent vaccines providing protection against more *S. pneumoniae* serotypes are currently being introduced on the market. To study the immune response to this type of vaccines and the virulence factors of the parent pathogen at the molecular level, well-defined oligo- or polysaccharide structures are valuable tools.⁴

The type 3 capsular polymer of *S. pneumoniae*, built up from 1,3-linked [β -D-glucuronic acid-(1 $\rightarrow$ 4)- β -D-glucose] disaccharide repeats (Figure 1) is one of the prominent *S. pneumoniae* capsular polysaccharides.⁵

Figure 1. Capsular polysaccharide of *Streptococcus pneumoniae* type 3 repeating unit [$\rightarrow$ 4)- β -D-Glc-(1 $\rightarrow$ 3)- β -D-GlcA-(1 $\rightarrow$].

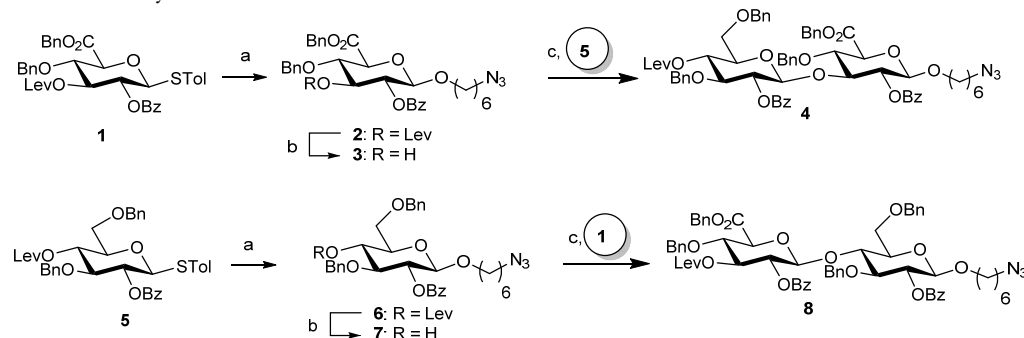


In the construction of fragments of this polysaccharide two types of linkages have to be installed: a β -glucuronic acid bond to the C-4-OH of a glucose acceptor and a β -glucosyl bond to a glucuronic acid C-3-OH. Although previous syntheses of fragments of this polysaccharide have employed a post-glycosylation oxidation approach⁶ to circumvent the assumed low reactivity of glucuronic acid building blocks, the results described in Chapter 2 indicate that the reactivity of glucuronic acid donors is sufficient to be of use in the synthesis of oligosaccharides. Encouraged by these results, glucuronic acid donors were probed for their use in the construction of *Streptococcus pneumoniae* type 3 di- and trisaccharides. The construction of these glycosidic bonds was investigated using both monomeric and dimeric glucuronic acid donor building blocks.

Results & Discussion

First, the two relevant spacer-containing monomeric acceptors **3** and **7** were prepared as depicted in Scheme 1. Glucuronic acid donor **1** (described in Chapter 2) and *S*-tolyl glucose **5** were condensed with 6-azidoheptanol using NIS and TfOH yielding **2** in 85% yield and **6** in 80% yield. Delevulinoylation gave the acceptor molecules **3** and **7** in 91% and 99% yield, respectively. The glycosylation of glucoside **5** (1 eq.) with glucuronic acid ester acceptor **3** (1.3 eq.) yielded disaccharide **4** in 62% yield. Glucuronic acid ester donor **1** (1.3 eq.) was glycosylated with glucosyl acceptor **7** (1 eq.) to give the alternative disaccharide **8** in slightly higher yield (74%). These results show that the glucose donor-glucuronic acid acceptor combination does not outperform the glucuronic acid donor-glucose acceptor pair.

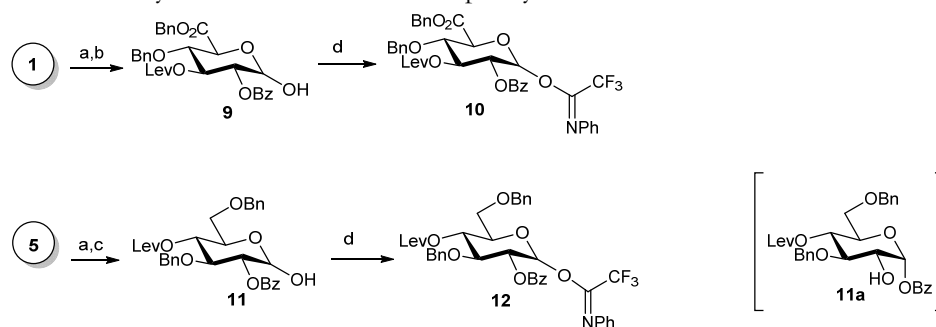
Scheme 1. Synthesis of both frame-shifted disaccharides.



Reagents and conditions: (a) 6-azidoheptanol, NIS, TfOH, DCM, 0 °C, **2**: 85%, **6**: 80%; (b) Hydrazine hydrate, pyridine/AcOH, **3**: 91%, **7**: 99%; (c) NIS, TfOH, DCM, 0 °C, **4**: 62%, **8**: 74%.

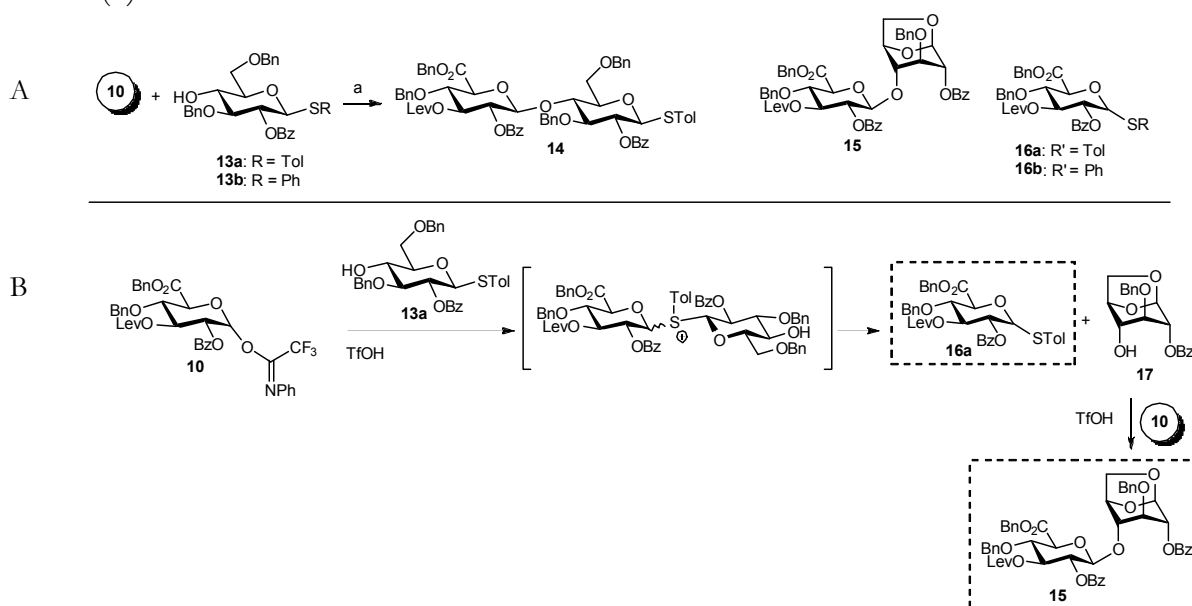
Next, the applicability of dimer donors in the synthesis of two complementary trisaccharides was explored. The required dimeric GlcA-Glc and Glc-GlcA donors were synthesized via an orthogonal glycosylation reaction between a *S*-tolyl acceptor and a *N*-phenyltrifluoro-imidate donor.⁷ The latter were in turn obtained from the thioglycoside precursor **1** or **5** (Scheme 2). The preparation of imidate **10** started by substitution of the anomeric thio-functionality in **1** for a trifluoroacetyl function using NIS and TFA in dry DCM.⁸ Subsequent hydrolysis of the anomeric trifluoroacetate with aqueous NaHCO₃ led to hemiacetal **9** in 88%. These conditions, however, were not efficient for the hydrolysis of donor **5**, as a result of intramolecular benzoyl migration (**11a**).⁹ To prevent the formation of the anomeric benzoate, the anomeric trifluoroacetate was cleaved with piperidine, producing hemiacetal **11** in 84%. The hemiacetals **9** and **11** were converted into the corresponding *N*-phenyl trifluoroacetimidate donors¹⁰ **10** and **12** in 83% and 68% yield, respectively.

Scheme 2. Synthesis of the trifluoro-*N*-phenylimidate donors.



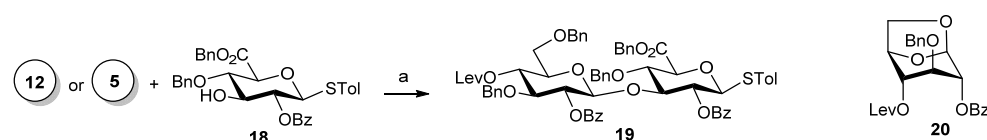
Reagents and conditions: (a) NIS, TFA, DCM, 0 °C; (b) NaHCO₃ (aq., sat.), **9**: 88%; (c) piperidine, **11**: 84%; (d) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0 °C, **10**: 83%; **12**: 68%.

The orthogonal glycosylations to provide disaccharides are depicted in Scheme 3 (A). The condensation of glucuronic acid ester *N*-phenyl trifluoroacetimidate **10** (1.3 eq.) with acceptor **13a** (1 eq.)¹¹ using a catalytic amount of TfOH in DCM only led to a modest yield of disaccharide **14** (12%). Increasing the concentration of the reaction (0.05M to 0.5M) did not give an improved yield, but led to formation of two major side products instead. Disaccharide **15**, featuring an anhydroglucose reducing end, and *S*-tolyl glucuronic acid ester **16a** were isolated in 29% and 53% yield, respectively. The formation of glucuronic acid ester **16a** can be explained by aglycon transfer¹² from glucoside **13a** to the activated glucuronic acid donor (Scheme 3, B). The activated glucoside, formed in this event, collapses via the formation of the 1,6-anhydro-ring into a glucosyl acceptor **17**, with an accessible axial hydroxyl group.¹³ This 1,6-anhydro-glucoside reacts with an activated glucuronic acid ester, leading to the formation of disaccharide **15**. To explore whether these side reactions were due to the nucleophilicity of the anomeric *S*-tolyl moiety in **13a**, we prepared acceptor **13b**, bearing the somewhat less nucleophilic thiophenyl aglycon. However, use of this thioglucosyl acceptor also resulted in aglycon transfer, yielding glucuronic acid ester **16b**, and disaccharide **15** (41% isolated yield). On the basis of these results it was reasoned that activation of glucuronic acid donor **10** was not the problem, but that acceptors **13a** and **13b** were too reactive.

Scheme 3. Synthesis of dimer thio-donor **14** (A) and mechanistic explanation for the side products formed (B).

Reagents and conditions: (a): TfOH, DCM, 0 °C, **14**: 12%, **15**: 29%; **16a**: 53%; **16b**: 45%.

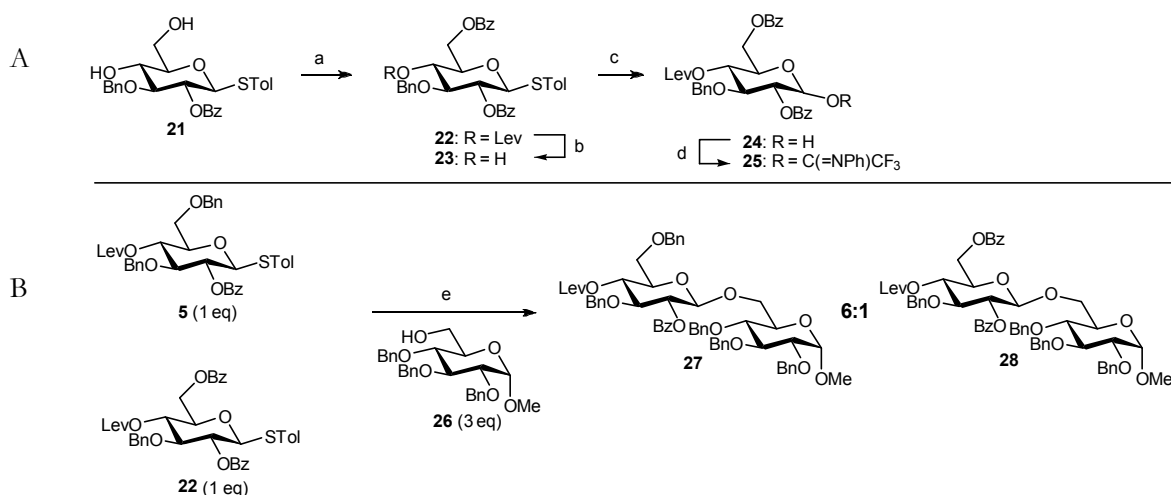
The high reactivity of the glucoside building block was confirmed in the attempted synthesis of dimer **19** through the condensation of glycosyl *N*-phenyl trifluoroacetimidate **12** (1.3 eq.) and glucuronic acid ester acceptor **18** (1 eq.), as depicted in Scheme 4. No disaccharide could be obtained from this reaction. Instead only anhydroglucoside **20** was isolated in 34% yield. A chemoselective glycosylation with donor **5** and acceptor **18** (Scheme 4) also proved to be unproductive due to formation of the same anhydroglucoside.

Scheme 4. Attempted synthesis of dimer thio-donor **19**.

Reagents and conditions: (a) TfOH, DCM, 0 °C, **19**: 0%, **20**: 34%.

In order to prevent these side reactions, the donor reactivity of the *S*-tolyl glucosyl building block was down-tuned by replacing the C-6-*O*-benzyl for a more electron withdrawing C-6-*O*-benzoyl group as in glucoside donor **22** (Scheme 5, A). Glucoside **22** was prepared from diol **21**¹⁴ by treatment with dibutyltin oxide in refluxing toluene followed by addition of benzoyl chloride to result in selective acylation of the C-6-OH. The remaining free C-4 hydroxyl was subsequently protected with a levulinoyl group, yielding glucoside **22** in 75% over 2 steps. The synthesis of the corresponding imidate donor **25** from thioglucoside **22** was performed according to the same protocol described above for donors **12**. The relative reactivity of glucoside donor **22** compared to donor **6** was assessed in a competition experiment (Scheme 5, B) as described in Chapter 2. Analysis of the obtained disaccharides showed that the C-6-*O*-Bn glucoside **5** is six times more reactive than its C-6-*O*-Bz counterpart.¹⁵

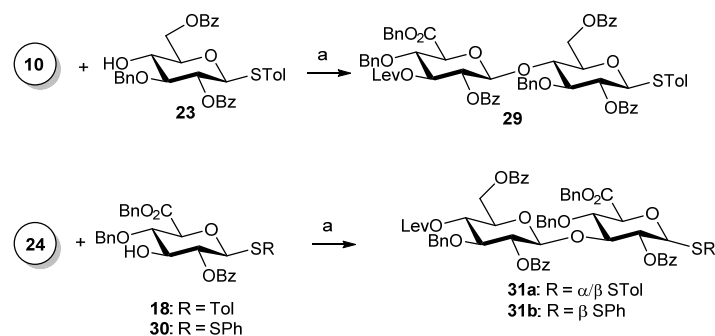
Scheme 5. Synthesis of glucosyl donor **22** and **25** and the competition experiment with glucosyl donor **6** and its C-6-O-Bn counterpart **22**.



Reagents and conditions: (a) (i) Bu₂SnO, toluene, reflux, (ii) BzCl, (iii) LevOH, DIC, DMAP, 75%/2 steps.; (b) hydrazine acetate, pyridine/AcOH, 95%; (c) NIS, TFA, DCM, 0 °C, then piperidine, 85%; (d) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0 °C, 77%; (e) NIS, TfOH, DCM, -40 °C → 0 °C, 94%.

Having down-tuned glucoside **22** in hand, the use of donor **10** and acceptor **23** was probed (Scheme 6). The condensation of glucuronic acid imidate **10** with acceptor glucoside **28** yielded disaccharide **29** without any side products. Similarly, anhydroglucoside formation was prevented in the reaction between glucosyl imidate **25** (1 eq.)¹⁶ and glucuronic acid acceptor **18** (1.3 eq.), which gave disaccharide **31a** in 51% yield as a mixture of α/β -thioglucuronides.¹⁷ To circumvent the anomerization, glucuronic acid acceptor **18** was exchanged for acceptor **30**, having a somewhat less electron rich thiophenyl function.¹⁸ Condensation of glucosyl donor **24** with glucuronic acid acceptor **30** led to the formation of **31b** without anomerization of the anomeric thio-functionality.

Scheme 6. Synthesis of the disaccharide thio-donors.

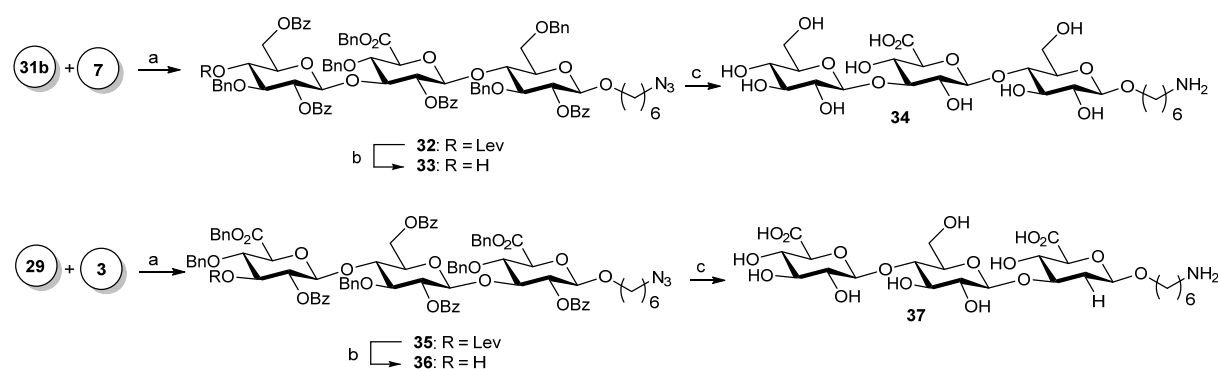


Reagents and conditions: (a) TfOH, DCM, 0 °C, **29**: 68%; **31a**: 51%, **31b**: 64%.

Next, the disaccharide donors **29** and **31b** were used as donors for the construction of both framed-shifted trisaccharides **32** and **35** (Scheme 7). Donor **31b**, having the glucuronic acid donor moiety (1 eq.) was coupled to spacer containing glucoside **7** (1.3 eq.), leading to the

formation of trisaccharide **32** in 75% yield. The condensation of dimer **29** (1 eq.), featuring the glucosyl donor moiety, and acceptor **3** (1.3 eq.) led to the alternative trimer **35** in 54% yield. In line with the results obtained with the monomeric donors, the glucuronic acid donor-glucosyl acceptor combination performed better than the corresponding glucosyl donor-glucuronic acid acceptor couple, indicating that the former disaccharide donor is the building block of choice for the assembly of larger oligomers of *S. pneumoniae* type 3.

Scheme 7. Preparation of both trisaccharides and deprotection.



Reagents and conditions (a) NIS, TfOH, DCM, 0 °C, **32**: 75%, **35**: 54%; (b) Hydrazine acetate, pyridine/AcOH, **33**: 95%, **36**: 93%; (c) *i*) KOH, dioxane/ H₂O, *ii*) H₂, Pd/C, *t*BuOH/H₂O, **34**: 44%/2 steps; **37**: 28%/2 steps.

Finally, the obtained trisaccharides were deprotected as follows. First, the levulinyl group was removed from trisaccharide **32** and **35** by treatment with hydrazine acetate in a pyridine/AcOH mixture, yielding trisaccharides **33** and **36** in 95% and 93% yield, respectively. Then, the benzoyl esters and benzyl esters were saponified using KOH in dioxane/H₂O and the azides en benzyl ethers were reduced with H₂ gas and Pd/C in a mixture of *t*BuOH and H₂O. The deprotected trisaccharides **34** and **37** were obtained in 44% and 28% yield over 2 steps, respectively.

Conclusions

The glucuronic acid donors used in this study have shown to be very efficient in the construction of dimeric and trimeric fragments of the capsular polysaccharide of *Streptococcus pneumoniae* type 3. These results pave the way for the construction of larger oligomers using glucuronic acid donor building blocks.

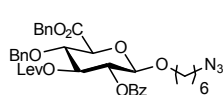
Experimental section

General experimental procedures. Chemical shifts (δ) are given in ppm relative to TMS as internal standard. All ¹³C APT spectra are proton decoupled. Reactions were performed at rT unless stated otherwise and were followed by TLC analysis with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulphuric acid in EtOH or with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L), (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) and 10% H₂SO₄ in H₂O, followed by

charring at 150 °C. Flash column chromatography was performed on silica gel (0.04-0.063 nm) and size exclusion chromatography (SEC) was performed on Sephadex™ LH-20. Experiments which required an inert atmosphere were carried out under dry argon. Dichloromethane (p.a.) was distilled over P₂O₅ prior to use. Molecular sieves (3Å) were flame-dried before use.

General procedure for glycosylations using NIS/TfOH. The donor and acceptor glucosides and/or glucuronic acid esters were co-evaporated with toluene (2x), dissolved in freshly distilled DCM, followed by addition of activated (flame-dried) molecular sieves 3Å. The reaction mixture was stirred for 30 min., followed by addition of NIS (1.3 eq. relative to the donor). The reaction mixture was cooled to 0 °C, followed by addition of TfOH (0.1 eq.) and the reaction mixture was allowed to warm to rT. When TLC analysis showed completion, the reaction mixture was neutralized with TEA and quenched with Na₂S₂O₃ (aq., sat). The organic layer was isolated, dried over MgSO₄, filtered, and concentrated. Size Exclusion chromatography (DCM/MeOH, 1/1, v/v) and/or column chromatography resulted in the isolation of the products formed.

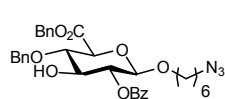
Procedure for the competition experiment. Donor **5** (0.1 mmol, 1 eq.), donor **22** (0.1 mmol, 1 eq.) and the acceptor (methyl 2,3,4-tri-*O*-benzyl- α -D-glucopyranoside, 3 eq.) were co-evaporated with toluene (2x). Freshly distilled DCM (4 mL, donor concentration 0.05 M), a teflon stirrer bar and activated (flame-dried) molecular sieves 3Å were added and the mixture was stirred under argon for 30 minutes at rT. NIS (1 eq) was added and the mixture was cooled to -40 °C. TfOH (0.1 eq, 0.1 mL of a 0.1M stock solution in distilled DCM) was added and the mixture was allowed to warm to 0 °C in ~3 h. Triethylamine (0.1 mL) was added and the mixture was diluted with EtOAc, washed with sat. aq. Na₂S₂O₃ (1x) and sat. aq. NaCl (2x), dried over MgSO₄ and concentrated *in vacuo*. Elution over Sephadex LH-20 (DCM/MeOH, 1/1, v/v, 500 mL) enabled isolation of the disaccharide products, which were analysed with NMR spectroscopy. The yield of the disaccharide fraction and the ratio of the disaccharides were determined.



Benzyl (6-azido-hexyl 4-*O*-benzyl-2-*O*-benzoyl-3-*O*-levulinyl- β -D-glucopyranosyluronate) (2): Glucuronic acid ester **1** (410 mg, 0.6 mmol) and 6-azido-hexanol (102 mg, 0.71 mmol, 1.18 eq.) were co-evaporated with toluene

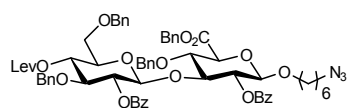
(2x) and dissolved in freshly distilled DCM (24 mL, 0.03M). Activated MS 3Å were added and the reaction mixture was stirred for 30 min., followed by addition of NIS (202 mg, 0.9 mmol, 1.5 eq.). The reaction mixture was cooled to 0 °C, followed by addition of TfOH (0.6 mL of a 0.1M stock solution, 0.06 mmol, 0.1 eq.). The reaction mixture was allowed to warm to rT and after 3 h., TLC analysis (PE/EtOAc: 12/8, v/v) showed total conversion into a higher running spot. The reaction mixture was neutralized with TEA, quenched with Na₂S₂O₃ (aq., sat.), diluted with EtOAc, followed by separation of the layers. The organic layer was washed with NaHCO₃ (aq., sat.), brine, dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 17/3) gave the title compound in 85% yield (358 mg, 0.51 mmol). $[\alpha]_{\text{D}}^{20}$: +24.2 (c = 0.7, DCM). IR (neat, cm⁻¹): 2940, 2862, 2097, 1746, 1724, 1454, 1360, 1315, 1265, 1215, 1179, 1153, 1096, 1070, 1028, 1001, 735, 712, 700. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.99 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.57 (t, 1H, *J* = 7.6 Hz, H_{arom}), 7.46-7.42 (m, 2H, H_{arom}), 7.36-7.24 (m, 7H, H_{arom}), 7.18-7.14 (m, 3H, H_{arom}), 5.43 (t, 1H, *J* = 9.6 Hz, H-3), 5.25-5.18 (m, 3H, H-2, CH₂ CO₂Bz), 4.62 (d, 1H, *J* = 7.6 Hz, H-1), 5.54 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.81 (d, 1H, CHHPh), 4.01-4.013 (m, 2H, H-4, H-5), 3.90-3.85 (m, CHH N₃(CH₂)₆OH), 3.49-3.41 (CHH N₃(CH₂)₆OH), 3.04 (t, 2H, *J* = 7.2 Hz, N₃(CH₂)₆OH), 2.52-2.47 (m, 2H, CH₂ Lev), 2.40-2.35 (m, 2H, CH₂ Lev), 2.00 (s, 3H, CH₃ Lev), 1.50-1.45 (m, 2H, CH₂ N₃(CH₂)₆OH), 1.34-1.28 (m, 4H, 2x CH₂ N₃(CH₂)₆OH), 1.28-1.15 (m, 2H, CH₂ N₃(CH₂)₆OH). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.9 (C=O Lev ketone), 171.9 (C=O Lev), 168.0 (C-6), 165.3 (C=O Bz), 137.6 (C_q

C_{arom}), 135.0 ($C_{\text{q}} C_{\text{arom}}$), 133.4 (CH_{arom}), 129.9 (CH_{arom}), 129.5 ($C_{\text{q}} C_{\text{arom}}$), 129.2-128.0 (CH_{arom}), 125.4 (CH_{arom}), 101.4 (C-1), 77.6 (C-4 or C-5), 77.4 (C-4 or C-5), 74.2 (C-3), 72.0 (C-2), 70.2 ($CH_2 N_3(CH_2)_6OH$), 67.7 ($CH_2 CO_2Bn$), 51.3 ($CH_2 N_3(CH_2)_6OH$), 37.8 ($CH_2 Lev$), 29.2 ($CH_2 N_3(CH_2)_6OH$), 28.7 ($CH_2 N_3(CH_2)_6OH$), 28.0 ($CH_2 Lev$), 26.4 ($CH_2 N_3(CH_2)_6OH$), 25.5 ($CH_2 N_3(CH_2)_6OH$). HRMS: $[M+Na]^+$ calcd for $C_{38}H_{43}N_3O_{10}Na$: 724.28407, found 724.28369.

**Benzyl****(6-azido-hexyl****2-O-benzoyl-4-O-benzyl-β-D-**

glucopyranosyluronate) (3): Glucuronic acid ester **2** (211 mg, 0.3 mmol) was

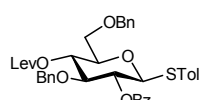
dissolved in a mixture of pyridine/AcOH (4/1, 6 mL) and hydrazine monohydrate (90 μL, 1.50 mmol, 5 eq.) was added. The reaction mixture was stirred for 2 h. The reaction mixture was diluted with EtOAc, washed with 1M HCl, $NaHCO_3$ (aq., sat.), dried over $MgSO_4$, filtered, and concentrated. Column chromatography (PE/EtOAc: 9/1) gave the title compound as a transparent oil in 91% yield (165 mg, 0.27 mmol). $[\alpha]_D^{20}$: -1.5 ($c = 1.6$, DCM). IR (neat, cm^{-1}): 2932, 2884, 2860, 2095, 1732, 1601, 1497, 1454, 1396, 1362, 1315, 1267, 1215, 1177, 1098, 1069, 1028, 1009, 1001, 978, 908, 897, 750, 712, 698, 619. ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): 8.04 (d, 2H, $J = 7.6$ Hz, H_{arom}), 7.58 (t, 1H, $J = 7.2$ Hz, H_{arom}), 7.45-7.22 (m, 10H, H_{arom}), 7.20-7.18 (m, 2H, H_{arom}), 5.25 (d, 1H, $J = 12.4$ Hz, $CHHPh$), 5.20 (d, 1H, $J = 12.0$ Hz, $CHHPh$), 5.10 (t, 1H, $J = 8.2$ Hz, H-2), 4.70 (d, 1H, $J = 11.2$ Hz, $CHHPh$), 4.60 (d, 1H, $J = 7.6$ Hz, H-1), 5.54 (d, 1H, $J = 11.2$ Hz, $CHHPh$), 4.02 (d, 1H, $J = 8.8$ Hz, H-5), 3.95-3.86 (m, 3H, H-3, H-4, $CHH N_3(CH_2)_6OH$), 3.48-3.23 (m, 1H, $CHH N_3(CH_2)_6OH$), 3.06 (t, 2H, $J = 7.0$ Hz, $CH_2 N_3(CH_2)_6OH$), 2.72 (bs, 1H, C-3-OH), 1.54-1.44 (m, 2H, $CH_2 N_3(CH_2)_6OH$), 1.35-1.13 (m, 6H, 3x $CH_2 N_3(CH_2)_6OH$). ^{13}C APT NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC): 168.4 (C=O), 166.3 (C=O), 137.9 ($C_{\text{q}} C_{\text{arom}}$), 135.1 ($C_{\text{q}} C_{\text{arom}}$), 133.6 (CH_{arom}), 130.0 (CH_{arom}), 129.6 ($C_{\text{q}} C_{\text{arom}}$), 128.7-128.1 (CH_{arom}), 101.2 (C-1), 79.8 (C-3 or C-4), 75.3 (C-3 or C-4), 75.0 ($CH_2 Bn$), 74.7 (C-2 or C-5), 74.6 (C-2 or C-5), 70.1 ($CH_2 N_3(CH_2)_6OH$), 67.6 ($CH_2 CO_2Bn$), 51.3 ($CH_2 N_3(CH_2)_6OH$), 29.3 ($CH_2 N_3(CH_2)_6OH$), 28.7 ($CH_2 N_3(CH_2)_6OH$), 26.4 ($CH_2 N_3(CH_2)_6OH$), 25.5 ($CH_2 N_3(CH_2)_6OH$). HRMS: $[M+Na]^+$ calcd for $C_{33}H_{37}N_3O_8Na$: 626.24729, found: 626.24673



Benzyl (6-azido-hexyl 2-O-benzoyl-4-O-benzyl-3-O-(2-O-benzoyl-3,6-di-O-benzyl-4-O-levulinyl-β-D-glucopyranoside)-β-D-glucopyranosyluronate) (4):

Glucuronic acid ester acceptor **3** (175 mg, 0.26 mmol, 1.3 eq.) and glucoside donor **5** (119 mg, 0.20 mmol, 1 eq.) were condensed according to the general procedure for glycosylations and yielded the title compound as a white solid in 62% yield (142 mg, 0.12 mmol). R_f 0.29 (toluene/EtOAc: 3/1, v/v). $[\alpha]_D^{20}$: +5.4 ($c = 1$, DCM). IR (neat, cm^{-1}): 2938, 2095, 1721, 1452, 1362, 1315, 1263, 1211, 1177, 1146, 1094, 1069, 1026, 1001, 737, 710, 700. ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): 8.01 (d, 2H, $J = 7.2$ Hz, H_{arom}), 7.94 (d, 2H, $J = 7.6$ Hz, H_{arom}), 7.84 (d, 2H, $J = 7.6$ Hz, H_{arom}), 7.59-7.52 (m, 2H, H_{arom}), 7.50-7.30 (m, 7H, H_{arom}), 7.28-7.26 (m, 6H, H_{arom}), 7.17-7.10 (m, 7H, H_{arom}), 7.10-7.01 (m, 2H, H_{arom}), 5.33 (t, 1H, $J = 8.8$ Hz, H-2'), 5.21 (t, 1H, $J = 9.6$ Hz, H-2), 5.14-5.10 (m, 2H, $CH_2 CO_2Bn$), 4.98 (d, 1H, $J = 10.8$ Hz, $CHHPh$), 4.93 (d, 1H, $J = 8.0$ Hz, H-1'), 4.51-4.47 (m, 3H, $CH_2 Bn$, H-6), 4.46 (d, 1H, $J = 6.0$ Hz, H-1), 4.44 (d, 1H, $J = 12.0$ Hz, $CHHPh$), 4.24-4.17 (m, 2H, H-6', H-5'), 4.03 (t, 1H, $J = 9.6$ Hz, H-4), 3.98 (d, 1H, $J = 8.8$ Hz, H-5), 3.77-3.69 (m, 3H, H-3, H-3', $CHH CH_2 N_3(CH_2)_6OH$), 3.23-3.18 (m, 1H, $CHH CH_2 N_3(CH_2)_6OH$), 3.04 (t, 2H, 7.2 Hz, $CH_2 N_3(CH_2)_6OH$), 2.65-2.60 (m, 2H, $CH_2 Lev$), 2.50-2.42 (m, 1H, $CHH Lev$), 2.38-2.32 (m, 1H, $CHH Lev$), 2.09 (s, 3H, $CH_3 Lev$), 1.30-1.22 (m, 4H, 2x $CH_2 N_3(CH_2)_6OH$), 1.11-1.00 (m, 4H, 2x $CH_2 N_3(CH_2)_6OH$). ^{13}C APT NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC): 206.1 (C=O Lev ketone), 171.4 (C=O), 168.2 (C=O), 166.1 (C=O Bz), 165.0 (C=O), 164.4 (C=O), 138.0 ($C_{\text{q}} C_{\text{arom}}$), 137.4 ($C_{\text{q}} C_{\text{arom}}$), 135.1 ($C_{\text{q}} C_{\text{arom}}$), 133.5 (CH_{arom}), 133.0 (CH_{arom}), 129.9-129.8 (CH_{arom}), 129.7-129.5 ($C_{\text{q}} C_{\text{arom}}$), 128.6-127.11 (CH_{arom}), 100.5 (C-1), 100.2 (C-1'), 80.0 (C-3'), 76.6 (C-5'), 76.8 (C-4), 74.8 ($CH_2 Bn$), 74.5 (C-5), 74.1 ($CH_2 Bn$), 73.6 (C-4'), 73.3 (C-2'),

72.2 (C-3'), 70.5 (C-2), 69.3 (CH₂ N₃(CH₂)₆OH), 67.3 (CH₂ CO₂Bn), 63.0 (C-6'), 51.2 (CH₂ N₃(CH₂)₆OH), 37.8 (CH₂ Lev), 29.7 (CH₃ Lev), 28.9 (CH₂ N₃(CH₂)₆OH), 28.5 (CH₂ Lev), 27.8 (CH₂ N₃(CH₂)₆OH), 26.2 (CH₂ N₃(CH₂)₆OH), 25.2 (CH₂ N₃(CH₂)₆OH). HRMS: [M+Na]⁺ calcd for C₆₅H₆₇N₃O₁₇Na: 1184.43627, found 1184.43563.



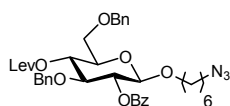
***p*-Tolyl**

glucopyranoside

2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-levulinyl-1-thio-β-D-

glucopyranoside (5):

p-Tolyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (1.14 g, 1.99 mmol) was dissolved in DCM (10.0 mL, 0.2M) followed by subsequent addition of LevOH (301 μL, 2.99 mmol, 1.5 eq.), DIC (319 μL, 2.99 mmol, 1.5 eq.) and a catalytic amount of DMAP. After 10 min., TLC analysis (PE/EtOAc: 3/1, v/v) showed total conversion of the starting material into a lower running spot. The reaction mixture was filtered, diluted with EtOAc, washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 7/3) gave the title compound in 75% yield (1.0 g, 1.50 mmol). [α]_D: +23.1 (c = 0.3, DCM). IR (neat, cm⁻¹): 2864, 1740, 1717, 1495, 1452, 1418, 1400, 1362, 1317, 1287, 1260, 1213, 1179, 1153, 1112, 1090, 1063, 1040, 1028, 1007, 982, 964, 932, 908, 883, 843, 820, 804, 748, 708, 694, 685, 619. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.05 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.57-7.55 (m, 1H, H_{arom}), 7.46-7.42 (m, 2H, H_{arom}), 7.38-7.22 (m, 7H, H_{arom}), 7.19-7.11 (m, 5H, H_{arom}), 7.98 (d, 2H, *J* = 7.6 Hz, H_{arom}), 5.29 (t, 1H, *J* = 9.6 Hz, H-2), 5.11 (t, 1H, *J* = 9.6 Hz, H-4), 4.78 (d, 1H, *J* = 10.0 Hz, H-1), 4.57 (s, 2H, CH₂ Bn), 4.53 (s, 2H, CH₂ Bn), 3.89 (t, 1H, *J* = 9.2 Hz, H-3), 3.72-3.60 (m, 3H, H-5, H-6), 2.71-2.51 (m, 2H, CH₂ Lev), 2.47-2.31 (m, 2H, CH₂ Lev), 2.26 (s, 3H, CH₃ STol), 2.10 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.1 (C=O Lev ketone), 171.3 (C=O Lev), 164.8 (C=O Bz), 138.0 (C_q C_{arom}), 137.9 (C_q C_{arom}), 137.4 (C_q C_{arom}), 133.1 (CH_{arom}), 132.8 (CH_{arom}), 129.7 (CH_{arom}), 129.5 (CH_{arom}), 129.4 (C_q C_{arom}), 128.7 (C_q C_{arom}), 127.3-127.4 (CH_{arom}), 86.3 (C-1), 81.3 (C-3), 77.7 (C-5), 74.0 (CH₂ Bn), 73.3 (CH₂ Bn), 72.0 (C-2), 70.7 (C-4), 69.5 (C-6), 37.6 (CH₂ Lev), 29.5 (CH₃ Lev), 27.7 (CH₂ Lev), 20.9 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₃₉H₄₀O₈SNa: 691.23361, found 691.23359.



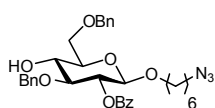
6-Azido-hexyl

glucopyranoside (6):

2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-levulinyl-β-D-

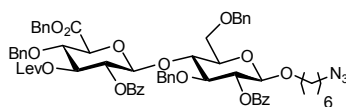
Glucoside donor **5** (654 mg, 1 mmol) and 6-azido-hexanol (214 mg, 1.5 mmol, 1.5 eq.) were co-evaporated with toluene (2x) and dissolved in freshly distilled DCM (4.0 mL, 0.25M). Activated MS 3Å were added and the reaction mixture was stirred for 30 min., followed by addition of NIS (338 mg, 1.5 mmol, 1.5 eq.). The reaction mixture was cooled to -0 °C, followed by addition of TfOH (1 mL of 0.1M stock solution, 0.1 mmol, 0.1 eq.). The reaction mixture was allowed to warm to rT. TLC analysis (PE/EtOAc: 3/2, v/v) showed total consumption of the thio-glucoside. The reaction mixture was quenched with TEA and Na₂S₂O₃ (aq., sat.), diluted with DCM, followed by separation of the layers. The organic layer was washed with NaHCO₃ (aq., sat.), brine, dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 9/1 → 7/3) gave a mixture of the title compound and 6-azido-hexanol. This mixture was dissolved in a mixture of pyridine/Ac₂O (1/1, 10 mL) and stirred overnight (acetylation of 6-azido-hexanol). The reaction mixture was quenched with MeOH, diluted with EtOAc, washed with 2M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 9/1 → 3/1) gave the title compound as a transparent oil in 80% yield (550 mg, 0.80 mmol). [α]_D²⁰: +16.0 (c = 1.1, DCM). IR (neat, cm⁻¹): 2936, 2862, 2095, 1721, 1452, 1362, 1314, 1267, 1206, 1177, 1148, 1094, 1069, 1026, 988, 970, 910, 748, 712, 698. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.03-8.01 (m, 2H, H_{arom}), 7.59 (t, 1H, *J* = 7.6 Hz, H_{arom}), 7.77-7.73 (m, 2H, H_{arom}), 7.35-7.26 (m, 5H, H_{arom}), 7.14-7.10 (m, 5H, H_{arom}), 5.30 (t, 1H, *J* = 9.2 Hz, H-2), 5.14 (t, 1H, *J* = 9.6 Hz, H-4), 4.60 (s, 2H, CH₂ Bn), 4.55 (s, 2H, CH₂ Bn), 5.54 (d, 1H, *J* = 8.0 Hz, H-1), 3.91-3.85 (m, 2H, H-3, CHH N₃(CH₂)₆OH), 3.70-3.58 (m, 3H, H-5, H-6), 3.47-3.41 (m, 1H, CHH N₃(CH₂)₆OH), 3.02 (t, 1H, *J* = 6.8 Hz, CH₂ N₃(CH₂)₆OH), 2.63-2.58 (m, 2H, CH₂ Lev),

2.43-2.36 (m, 2H, CH₂ Lev), 2.10 (s, 3H, CH₃ Lev), 1.50-1.44 (m, 2H, CH₂ N₃(CH₂)₆OH), 1.31-1.15 (m, 6H, 3x CH₂ N₃(CH₂)₆OH). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.3 (C=O Lev ketone), 171.6 (C=O Lev), 165.0 (C=O Bz), 138.1 (C_q C_{arom}), 137.8 (C_q C_{arom}), 133.3 (CH_{arom}), 129.9 (C_q C_{arom}), 129.8 (CH_{arom}), 128.5-127.6 (CH_{arom}), 101.2 (C-1), 79.8 (C-3), 73.8 (CH₂ Bn), 76.7 (C-5), 76.6 (CH₂ Bn), 73.4 (C-2), 71.2 (C-4), 69.7 (CH₂ N₃(CH₂)₆OH), 69.7 (C-6), 51.2 (CH₂ N₃(CH₂)₆OH), 37.8 (CH₂ Lev), 29.8 (CH₃ Lev), 29.3 (CH₂ N₃(CH₂)₆OH), 29.3 (CH₂ N₃(CH₂)₆OH), 27.9 (CH₂ Lev), 26.3 (CH₂ N₃(CH₂)₆OH), 25.4 (CH₂ N₃(CH₂)₆OH). HRMS: [M+Na]⁺ calcd for C₃₈H₄₅N₃O₉Na: 710.30480, found 710.30474.



6-Azido-hexyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (7):

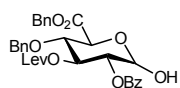
Glucoside **7** (523 mg, 0.76 mmol) was dissolved in pyridine/AcOH (4/1, 15 mL, 0.05M) followed by addition of hydrazine monohydrate (0.22 mL, 3.8 mmol, 5 eq.). After 1 h., TLC analysis (PE/EtOAc: 4/1, v/v) showed total conversion of the starting material. The reaction mixture was diluted with EtOAc, washed with 2M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 7/3) gave the target compound in 99% yield (442 mg, 0.75 mmol). [α]_D²⁰: +2.6 (c = 0.7, DCM). IR (neat, cm⁻¹): 2938, 2866, 2095, 1726, 1452, 1362, 1314, 1265, 1209, 1179, 1110, 1069, 1026, 984, 733, 698, 648. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): ¹H NMR: 8.08-8.03 (m, 2H, H_{arom}), 7.60-7.57 (m, 1H, H_{arom}), 7.47-7.44 (m, 2H, H_{arom}), 7.37-7.18 (m, 10H, H_{arom}), 5.24 (t, 1H, *J* = 8.8 Hz, H-2), 4.73 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.69 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.63 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.58 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.50 (d, 1H, *J* = 8.0 Hz, H-1), 3.94-3.85 (m, 1H, CHH N₃(CH₂)₆OH), 3.84-3.73 (m, 3H, H-4, H-6), 3.68 (t, 1H, *J* = 9.2 Hz, H-3), 3.57-3.52 (m, 1H, H-5), 3.44-3.38 (m, CHH N₃(CH₂)₆OH), 3.01 (t, 1=2H, *J* = 6.8 Hz, CH₂ N₃(CH₂)₆OH), 1.52-1.41 (m, 2H, CH₂ N₃(CH₂)₆OH), 1.13-1.15 (m, 6H, 3x CH₂ N₃(CH₂)₆OH). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 165.2 (C=O Bz), 138.0 (C_q C_{arom}), 137.8 (C_q C_{arom}), 133.2 (CH_{arom}), 130.0 (C_q C_{arom}), 130.0-129.8 (CH_{arom}), 128.6-127.9 (CH_{arom}), 101.3 (C-1), 82.1 (C-3), 74.5 (CH₂ Bn), 74.2 (C-5), 73.8 (CH₂ Bn), 73.5 (C-2), 72.3 (C-4), 70.4 (C-6), 69.7 (CH₂ N₃(CH₂)₆OH), 51.2 (CH₂ N₃(CH₂)₆OH), 29.3 (CH₂ N₃(CH₂)₆OH), 28.6 (CH₂ N₃(CH₂)₆OH), 26.3 (CH₂ N₃(CH₂)₆OH), 25.5 (CH₂ N₃(CH₂)₆OH). HRMS: [M+Na]⁺ calcd for C₃₃H₃₉N₃O₇Na: 612.26802, found 612.26814.



6-Azido-hexyl 3,6-di-*O*-benzyl-4-*O*-(benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-*O*-levulinyl-β-D-glucopyranosyluronate))-2-*O*-benzoyl-β-D-glucopyranoside (8): Glucuronic acid ester donor **1** (151 mg, 0.22 mmol, 1.3 eq.) and glucoside acceptor **7** (100 mg, 0.17 mmol, 1 eq.) were glycosylated according to the general procedure for glycosylations and yielded disaccharide **8**

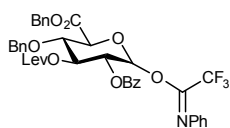
in 74% yield (144 mg, 0.13 mmol) as a transparent oil, which crystallized on standing. *R_f* 0.46 (PE/EtOAc: 12/8, v/v). [α]_D: +26.0 (c = 1, DCM). IR (neat, cm⁻¹): 2940, 2095, 1721, 1452, 1364, 1315, 1263, 1211, 1177, 1148, 1094, 1069, 1026, 1001, 735, 710, 700. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.94-7.91 (m, 4H, H_{arom}), 7.58-7.54 (m, 2H, H_{arom}), 7.45-7.24 (m, 17H, H_{arom}), 7.15-7.14 (m, 4H, H_{arom}), 7.14-7.04 (m, 3H, H_{arom}), 5.31 (t, 1H, *J* = 9.2 Hz, H-3'), 5.23 (t, 1H, *J* = 10.0 Hz, H-2'), 5.17 (t, 1H, *J* = 8.8 Hz, H-2), 5.06 (d, 1H, *J* = 12.0 Hz, CHH CO₂Bn), 5.01 (d, 1H, *J* = 12.0 Hz, CHH CO₂Bn), 4.86 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.84 (d, 1H, *J* = 7.2 Hz, H-1'), 4.64 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.59 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.49 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.49 (d, 1H, *J* = 12.4 Hz, CHHPh), 4.37 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.34 (d, 1H, *J* = 8.4 Hz, H-1), 4.11 (t, 1H, *J* = 9.2 Hz, H-4), 4.00 (d, 1H, *J* = 9.6 Hz, H-4'), 3.89 (d, 1H, *J* = 9.6 Hz, H-5'), 3.80-3.70 (m, 2H, H-3, CHH CH₂ N₃(CH₂)₆OH), 3.60 (dd, 1H, *J* = 3.6 Hz, *J* = 11.2 Hz, H-6), 3.49 (d, 1H, *J* = 10.4 Hz, H-6), 3.35-3.27 (m, 1H, CHH CH₂ N₃(CH₂)₆OH), 3.26-3.23 (m, 1H, H-5), 3.03-3.96 (m, 2H, CH₂ CH₂ N₃(CH₂)₆OH), 2.47-2.44 (m, 2H, CH₂ Lev), 2.34-2.31 (m, 2H, CH₂ Lev), 1.97 (s, 3H, CH₃ Lev), 1.50-1.28 (m, 2H, CH₂ N₃(CH₂)₆OH), 1.28-1.26 (m, 2H, CH₂ N₃(CH₂)₆OH), 1.24-1.11 (m, 4H, 2x CH₂ CH₂ N₃(CH₂)₆OH).

$\text{N}_3(\text{CH}_2)_6\text{OH}$). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 205.5 (C=O Lev ketone), 171.5 (C=O, 167.5 (C=O), 165.0 (C=O), 164.9 (C=O), 138.3 (C_q C_{arom}), 137.8 (C_q C_{arom}), 137.5 (C_q C_{arom}), 136.6 (C_q C_{arom}), 134.8 (C_q C_{arom}), 133.4 (CH_{arom}), 132.9 (CH_{arom}), 130.0 (C_q C_{arom}), 129.8-129.1 (CH_{arom}), 128.6 (C_q C_{arom}), 128.5-127.1 (CH_{arom}), 101.1 (C-1), 100.4 (C-1'), 80.2 (C-3), 77.6 (C-4'), 77.3 (C-4), 74.6 (C-5), 74.5 (CH_2 Bn), 74.5 (C-5'), 74.4 (CH_2 Bn), 74.0 (C-3'), 73.5 (CH_2 Bn), 73.1 (C-2), 72.2 (C-2'), 69.3 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 67.5 (C-6), 67.4 (CH_2 CO_2Bn), 51.1 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 37.7 (CH_2 Lev), 29.4 (CH_3 Lev), 29.1 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 28.5 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 27.8 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 26.2 (CH_2 Lev), 25.3 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{65}\text{H}_{69}\text{N}_3\text{O}_{16}\text{Na}$: 1170.45700, found 1170.45688.



Benzyl (2-O-benzoyl-4-O-benzyl-3-O-levulinyl- α/β -D-glucopyranosyluronate)

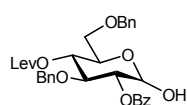
(9): Glucuronic acid ester **1** (3074 mg, 0.45 mmol) was dissolved in freshly distilled DCM (4.5 mL, 0.1M). The reaction mixture was cooled to 0 °C, followed by addition of NIS (111 mg, 0.50 mmol, 1.1 eq.) and TFA (38 μL , 0.50 mmol, 1.1 eq.). The reaction mixture was allowed to warm to rT and after 30 min., TLC analysis (toluene/EtOAc: 4/1, v/v) showed total conversion into a lower running spot. The reaction mixture was quenched by addition of $\text{Na}_2\text{S}_2\text{O}_3$ (aq., sat.) and NaHCO_3 (aq., sat.) and stirred for 30 min. Then the reaction mixture was diluted with DCM, followed by separation of the layers. The organic layer was dried over MgSO_4 , filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 6/4) gave hemiacetal **16** as a transparent oil and as anomeric mixture (α/β : 4/1) in 88% yield (229 mg, 0.40 mmol). IR (neat, cm^{-1}): 3443, 1744, 1721, 1601, 1497, 1452, 1404, 1360, 1339, 1315, 1265, 1213, 1179, 1155, 1109, 1098, 1070, 1061, 1028, 986, 962, 908, 853, 750, 714, 698, 635, 621. α isomer: ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 8.01 (d, 2H, J = 7.6 Hz, H_{arom}), 7.44 (t, 1H, J = 5.2 Hz, H_{arom}), 7.41-7.22 (m, 10H, H_{arom}), 7.17-7.13 (m, 2H, H_{arom}), 5.78 (t, 1H, J = 10.0 Hz, H-3), 5.59 (d, 1H, J = 3.2 Hz, OH), 5.18 (s, 2H, CH_2 Bn ester), 5.02 (dd, 1H, J = 3.6 Hz, J = 10.0 Hz, H-2), 4.63 (d, 1H, J = 10.0 Hz, H-5), 4.53 (d, 1H, J = 11.2 Hz, CHHPh), 4.50 (d, 1H, J = 11.2 Hz, CHHPh), 3.96 (t, 1H, J = 9.6 Hz, H-4), 3.62 (bs, OH), 2.54-2.48 (m, 2H, CH_2 Lev), 2.38-2.32 (m, 2H, CH_2 Lev), 1.99 (s, 3H, CH_3 Lev). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 206.0 (C=O Lev ketone), 171.8 (C=O Bn ester), 169.0 (C=O Lev), 165.8 (C=O Bz), 137.5 (C_q C_{arom}), 134.8 (C_q C_{arom}), 133.5 (CH_{arom}), 130.0 (CH_{arom}), 129.0 (CH_{arom}), 128.7 (C_q C_{arom}), 128.6-127.8 (CH_{arom}), 90.6 (C-1), 77.6 (C-4), 74.5 (CH_2 Bn ether), 71.8 (C-2), 71.2 (C-3), 70.2 (C-5), 67.5 (C-6), 37.7 (CH_2 Lev), 29.6 (CH_3 Lev), 27.9 (CH_2 Lev), 27.9 (CH_3 STol). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{32}\text{H}_{32}\text{O}_{10}\text{Na}$: 599.18877, found 599.18776



Benzyl (2-O-benzoyl-4-O-benzyl-3-O-levulinyl-1-O-(N-[phenyl]-trifluoroacetimidoyl)- α/β -D-glucopyranosyluronate (10):

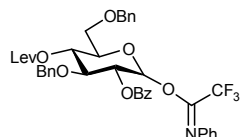
(10): Hemiacetal **9** (190 mg, 0.33 mmol) was dissolved in acetone (1.65 mL, 0.2M) and the reaction mixture was cooled to 0 °C. To this mixture, *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.1 mL, 0.66 mmol, 2 eq.) and Cs_2CO_3 (108 mg, 0.33 mmol, 1 eq.) were added. The reaction mixture was allowed to warm to rT and after 2 h., TLC analysis showed total conversion into a higher running spot (PE/EtOAc: 12/8, v/v, R_f 0.55). The reaction mixture was filtered over celite and concentrated. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 6/4) gave the title compound as a transparent oil in 83% yield (α/β : 3.4/1). IR (neat, cm^{-1}): 1746, 1719, 1452, 1404, 1358, 1317, 1263, 1209, 1153, 1107, 1096, 1070, 1040, 1028, 1003, 984, 908, 754, 739, 712, 696. α isomer: ^1H NMR (50 °C, 400 MHz, CDCl_3 , HH-COSY, HSQC): 8.00 (d, 2H, J = 7.6 Hz, H_{arom}), 7.59-7.50 (m, 2H, H_{arom}), 7.41-7.16 (m, 12H, H_{arom}), 7.11-7.07 (m, 3H, H_{arom}), 6.99 (t, 1H, J = 7.2 Hz, H_{arom}), 6.68 (bs, 1H, H-1), 5.81 (t, 1H, J = 10.0 Hz, H-3), 5.31 (dd, 1H, J = 2.4 Hz, J = 10.0 Hz, H-2), 5.23 (s, 2H, CH_2 Bn ester), 4.56-4.50 (m, 3H, H-5, CH_2 Bn ether), 4.08 (t, 1H, J = 9.6 Hz, H-4), 2.55-2.50 (m, 2H, CH_2 Lev), 2.42-2.38 (m, 2H, CH_2 Lev), 2.02 (s, 3H, CH_3 Lev). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 171.6 (C=O), 167.8 (C=O), 165.3 (C=O), 142.9 (C_q C_{arom}), 137.4 (C_q C_{arom}), 134.9 (C_q C_{arom}), 133.6

(CH_{arom}), 130.1 (CH_{arom}), 128.9 (C_q C_{arom}), 128.7-127.9 (CH_{arom}), 124.4 (CH_{arom}), 119.1 (CH_{arom}), 92.4 (C-1), 77.2 (C-4), 74.9 (CH₂ Bn ether), 72.8 (C-5), 71.3 (C-3), 70.4 (C-2), 67.8 (CH₂ Bn ester), 37.8 (CH₂ Lev), 29.4 (CH₃ Lev), 28.0 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₄₀H₃₆F₃NO₁₀Na: 770.21835, found 770.21895



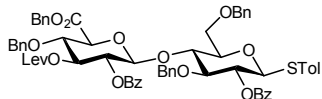
2-O-benzoyl-3,6-di-O-benzyl-4-O-levulinyl-α/β-D-glucopyranose (11):

Glucoside **6** (263 mg, 0.39 mmol) was dissolved in freshly distilled DCM (39.3 mL, 0.1M). The reaction mixture was cooled to 0 °C, followed by addition of NIS (97 mg, 0.43 mmol, 1.1 eq.) and TFA (33 μL, 0.43 mmol, 1.1 eq.). The reaction mixture was allowed to warm to rT. After 3 h., piperidine (128 μL, 1.29 mmol, 3 eq.) was added and the reaction mixture turned orange. The reaction was quenched by addition of Na₂S₂O₃ (aq., sat.), diluted with DCM, followed by separation of the layers. The organic layer was dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 6/4) gave hemiacetal **11** as a transparent oil in 84% yield (186 mg, 0.33 mmol). IR (neat, cm⁻¹): 1717, 1452, 1404, 1362, 1271, 1207, 1177, 1153, 1098, 1067, 1026, 1001, 937, 914, 746, 712, 698. α anomer: ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.05-8.03 (m, 2H, H_{arom}), 7.59-7.18 (m, 13H, H_{arom}), 5.52 (t, 1H, *J* = 3.2 Hz, H-1), 5.15-5.04 (m, 2H, H-2, H-4), 4.71 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.63 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.50 (s, 2H, CH₂ Bn), 4.26-4.16 (m, 2H, H-3, H-5), 3.53-3.49 (m, 2H, H-6), 2.67-2.48 (m, 2H, CH₂ Lev), 2.44-2.23 (m, 2H, CH₂ Lev), 2.11 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.4 (C=O Lev ketone), 171.5 (C=O Lev), 165.6 (C=O Bz), 138.0 (C_q C_{arom}), 137.5 (C_q C_{arom}), 133.2 (CH_{arom}), 129.8 (CH_{arom}), 129.7 (CH_{arom}), 129.5 (C_q C_{arom}), 128.5-127.5 (CH_{arom}), 92.2 (C-1), 76.8 (C-3 or C-5), 74.7 (CH₂ Bn), 73.8 (C-2 or C-4), 73.4 (CH₂ Bn), 70.9 (C-2 or C-4), 69.0 (C-6), 68.5 (C-3 or C-5), 37.7 (CH₂ Lev), 29.7 (CH₃ Lev), 27.7 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₃₂H₃₄O₉Na: 585.20950, found 585.20905.



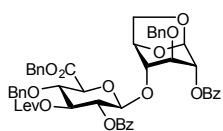
2-O-benzoyl-3,6-di-O-benzyl-4-O-levulinyl-1-O-(N-phenyl)-trifluoroacetimidoyl-α/β-D-glucopyranoside (12):

Hemiacetal **11** (465 mg, 0.83 mmol) was dissolved in acetone (5.5 mL, 0.2 M) and the reaction mixture was cooled to 0 °C. To this mixture, *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.25 mL, 1.66 mmol, 2 eq.) and Cs₂CO₃ (270 mg, 0.83 mmol, 1 eq.) were added. The reaction mixture was allowed to warm to rT and after 2 h., TLC analysis showed total conversion into a higher running spot (PE/EtOAc: 12/8, v/v, R_f 0.5). The reaction mixture was filtered over celite and concentrated. Column chromatography (PE/EtOAc: 1/0 → 7/3) gave the title compound as a transparent oil in 68% yield (α/β: 9/1). IR (neat, cm⁻¹): 1717, 1364, 1315, 1265, 1207, 1152, 1119, 1098, 1070, 1026, 924, 885, 737, 712, 696. α anomer: ¹H NMR (50 °C, 400 MHz, CDCl₃, HH-COSY, HSQC): 8.02-7.99 (m, 2H, H_{arom}), 7.61-7.58 (m, 1H, H_{arom}), 7.47-7.43 (m, 3H, H_{arom}), 7.35-7.07 (m, 12H, H_{arom}), 7.00-6.96 (m, 2H, H_{arom}), 6.62 (bs, 1H, H-1), 6.43 (d, 2H, *J* = 7.6 Hz, H_{arom}), 5.36 (dd, 1H, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2), 5.29 (t, 1H, *J* = 9.6 Hz, H-4), 4.73 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.68 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.56 (d, 1H, *J* = 12.4 Hz, CHHPh), 4.53 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.22 (t, 1H, *J* = 9.6 Hz, H-3), 4.14-4.11 (m, 1H, H-5), 3.66-3.57 (m, 2H, H-6), 2.67-2.53 (m, 2H, CH₂ Lev), 2.50-2.35 (m, 2H, CH₂ Lev), 2.12 (s, 3H, CH₃ Lev). ¹³C NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.8 (C=O Lev ketone), 171.3 (C=O Lev ketone), 165.1 (C=O Bz), 143.1 (C_q C_{arom}), 138.0 (C_q C_{arom}), 133.5 (CH_{arom}), 129.8 (CH_{arom}), 129.3 (C_q C_{arom}), 128.7-127.6 (CH_{arom}), 124.2 (CH_{arom}), 119.2 (CH_{arom}), 92.9 (C-1), 77.1 (C-3), 74.8 (CH₂ Bn), 73.7 (CH₂ Bn), 72.2 (C-2), 72.1 (C-5), 70.3 (C-4), 68.8 (C-6), 37.8 (CH₂ Lev), 29.6 (CH₃ Lev), 28.0 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₄₀H₃₈F₃NO₉Na: 756.23909, found 756.23982.



***p*-Tolyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-(benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-*O*-levulinyl-β-D-glucopyranosyluronate))-2-*O*-benzoyl-1-thio-β-D-glucopyranoside (14):** Donor **10** (256 mg, 0.34 mmol, 1.3 eq.) and acceptor **13a** (151 mg, 0.26 mmol) were co-evaporated with

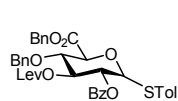
toluene (2x) and dissolved in freshly distilled DCM (6.8 mL, 0.05M). Activated MS 3Å were added and the reaction mixture was stirred for 30 min. The reaction mixture was cooled to 0 °C, followed by addition of TfOH (0.2 mL of 0.1M stock solution, 0.02 mmol, 0.1 eq.). The reaction mixture was allowed to warm to rT. After 4 h., TLC analysis (PE/EtOAc: 12/8, v/v) showed disappearance of the acceptor. The reaction mixture was neutralized with TEA, diluted with DCM, washed with H₂O, dried over MgSO₄, filtered, and concentrated. A yellow oil was obtained, which was purified by SEC (DCM/MeOH: 1/1), yielding disaccharide **14** in 12% yield (35 mg, 0.031 mmol). $[\alpha]_{\text{D}}^{20}$: +2.0 (*c* = 0.6, DCM). IR (neat, cm⁻¹): 2914, 2872, 1744, 1728, 1495, 1452, 1400, 1360, 1315, 1263, 1215, 1177, 1144, 1092, 1069, 1051, 1026, 1001, 910, 845, 810, 750, 710, 698. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.95-7.92 (m, 4H, H_{arom}), 7.60-7.55 (m, 2H, H_{arom}), 7.45-7.25 (m, 19H, H_{arom}), 7.19-7.03 (m, 7H, H_{arom}), 6.95 (d, 2H, *J* = 7.6 Hz, H_{arom}), 5.33 (t, 1H, *J* = 9.2 Hz, H-3'), 5.23 (t, 1H, *J* = 9.6 Hz, H-2'), 5.16 (t, 1H, *J* = 9.6 Hz, H-2), 5.03 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.96 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.87 (d, 1H, *J* = 8.4 Hz, H-1'), 4.87 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.58-4.53 (m, 3H, CH₂ Bn, H-1), 4.47 (s, 2H, CH₂ Bn), 4.35 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.11 (t, 1H, *J* = 9.6 Hz, H-4), 4.04 (t, 1H, *J* = 9.2 Hz, H-4'), 3.90 (d, 1H, *J* = 9.6 Hz, H-5'), 3.73 (t, 1H, *J* = 8.8 Hz, H-3), 3.61 (dd, 1H, *J* = 3.6 Hz, *J* = 11.6 Hz, H-6), 3.53 (d, 1H, *J* = 11.2 Hz, H-6), 3.28-3.25 (m, 1H, H-5), 2.46-2.44 (m, 2H, CH₂ Lev), 2.35-2.29 (m, 2H, CH₂ Lev), 2.24 (s, 3H, CH₃ STol), 1.97 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.6 (C=O Lev ketone), 171.6 (C=O), 167.5 (C=O Bz), 165.0 (C=O Bz), 138.2 (C_q Carom), 138.1 (C_q Carom), 137.9 (C_q Carom), 137.5 (C_q Carom), 134.8 (C_q Carom), 133.4-133.0 (CH_{arom}), 130.0 (C_q Carom), 129.8 (CH_{arom}), 129.5 (CH_{arom}), 129.0 (C_q Carom), 128.6-127.2 (CH_{arom}), 100.5 (C-1'), 86.2 (C-1), 81.6 (C-3), 78.8 (C-5), 77.5 (C-4'), 77.1 (C-4), 74.8 (CH₂ Bn), 74.5 (CH₂ Bn), 74.5 (C-5'), 74.1 (C-3'), 73.5 (CH₂ Bn), 72.2 (C-2'), 71.9 (C-2), 67.6 (C-6), 67.5 (CH₂ CO₂Bn), 37.7 (CH₂ Lev), 29.5 (CH₃ Lev), 27.8 (CH₂ Lev), 21.1 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₆₆H₆₂O₁₆SN_a: 1165.36508, found 1165.36484



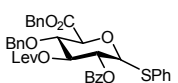
1,6-anhydro-2-*O*-benzyl-4-*O*-(benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-*O*-levulinyl-β-D-glucopyranosyluronate))-2-*O*-benzoyl-β-D-glucopyranoside (15): Donor **10** (1.15 g mg, 1.54 mmol, 1.8 eq.) and acceptor **13a** (491 mg, 0.86 mmol) were co-evaporated with toluene (2x) and dissolved in freshly distilled

DCM (1.72 mL, 0.5M). Activated MS 3Å were added and the reaction mixture was stirred for 30 min. The reaction mixture was cooled to 0 °C, followed by addition of TfOH (0.14 μL, 154 μmol, 0.1 eq.). After 5 min., TLC analysis (PE/EtOAc: 12/8, v/v) showed total conversion of the acceptor.. The reaction mixture was neutralized with TEA, diluted, washed with H₂O, dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 7/3), followed by SEC (DCM/MeOH: 1/1) gave the title compound as a clear oil in 10% yield (81 mg, 0.089 mmol). $[\alpha]_{\text{D}}^{20}$: -6.0 (*c* = 1, DCM). IR (neat, cm⁻¹): 1746, 1719, 1362, 1316, 1265, 1215, 1179, 1150, 1094, 1070, 1026, 1009, 1001, 750, 712, 698. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.05 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.95 (d, 2H, *J* = 7.2 Hz, H_{arom}), 7.61-7.06 (m, 21H, H_{arom}), 5.45 (s, 1H, H-1), 5.37 (t, 1H, *J* = 8.4 Hz, H-3'), 5.26 (t, 1H, *J* = 9.6 Hz, H-2'), 5.06 (d, 1H, *J* = 12.0 Hz, CHHPh), 5.01 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.91 (s, 1H, H-2), 4.74 (d, 1H, *J* = 13.6 Hz, CHHPh), 4.71 (d, 1H, *J* = 7.6 Hz, H-1'), 4.54-4.44 (m, 4H, CH₂ Bn, CHHPh, H-5), 3.99 (d, 1H, *J* = 7.6 Hz, H-6), 4.02-3.95 (m, 2H, H-4', H-5'), 3.78 (s, 1H, H-3 or H-4), 3.74 (s, 1H, H-3 or H-4), 3.68 (t, 1H, *J* = 6.4 Hz, H-6), 2.52-2.40 (m, 2H, CH₂ Lev), 2.38-2.34 (m, 2H, CH₂ Lev), 2.18 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.7 (C=O Lev ketone), 171.7 (C=O), 167.3 (C=O), 165.7 (C=O), 165.1 (C=O), 137.7 (C_q Carom), 137.4 (C_q Carom), 134.7 (C_q Carom), 133.3-133.2 (CH_{arom}), 131.9-129.3 (CH_{arom}), 128.8-128.5 (C_q Carom),

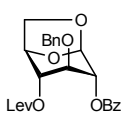
128.5-127.5 (CH_{arom}), 100.2 (C-1'), 99.4 (C-1), 77.0 (C-4' or C-5'), 76.9 (C-3 or C-4), 76.4 (C-3 or C-4), 74.7 (CH₂ Bn), 74.6 (C-4' or C-5'), 74.1 (C-3), 73.6 (C-5), 72.4 (CH₂ Bn), 71.9 (C-2'), 68.9 (C-2), 67.5 (CH₂ Bn ester), 64.8 (C-6), 37.6 (CH₂ Lev), 29.6 (CH₃ Lev), 27.8 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₅₂H₅₀O₁₅Na: 937.30419, found 937.30413.



Benzyl (tolyl 2-O-benzoyl-4-O-benzyl-3-O-levulinyl-1-thio-α/β-D-glucopyranosyluronate) (16a): The title compound was isolated during the glycosylation of glucuronic acid ester **10** with glucoside **13a** as a transparent oil (341 mg, 0.46 mmol, α/β: 1/3, 53% yield with respect to acceptor **13a**). IR (neat, cm⁻¹): 1744, 1721, 1493, 1452, 1402, 1358, 1315, 1263, 1206, 1177, 1153, 1090, 1069, 1026, 997, 972, 908, 847, 810, 735, 710, 696, 640. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.05-8.00 (m, H_{arom}), 7.58-7.56 (m, H_{arom}), 7.47-7.14 (m, H_{arom}), 7.04-6.95 (m, H_{arom}), 5.89 (d, *J* = 5.6 Hz, H-1 α), 5.66 (t, *J* = 9.2 Hz, H-3 β), 5.42 (t, *J* = 9.2 Hz, H-3 β), 5.26 (dd, *J* = 4.8 Hz, *J* = 10.0 Hz, H-2 α), 5.21 (s, CH₂ Bn ester), 5.14 (t, *J* = 9.6 Hz, H-2 β), 5.00 (d, *J* = 9.2 Hz, H-5 α), 4.79 (d, *J* = 10.0 Hz, H-1 β), 4.95 (s, CH₂ Bn ether α), 5.54 (d, *J* = 11.2 Hz, CHHPh β), 4.49 (d, *J* = 11.2 Hz, CHHPh β), 4.08 (d, *J* = 9.6 Hz, H-5 β), 4.04 (t, *J* = 9.2 Hz, H-4 α), 3.98 (t, *J* = 9.6 Hz, H-4 β), 2.59-2.26 (m, 2x CH₂ Lev, CH₃ Lev), 2.02 (s, CH₃ Lev α), 1.98 (s, CH₃ Lev β). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.7 (C=O Lev ketone β), 205.6 (C=O Lev ketone α), 171.6 (C=O), 171.5 (C=O), 168.5 (C=O), 165.4 (C=O), 165.1 (C=O), 165.0 (C=O), 138.7-137.4 (C_q Carom), 134.9 (C_q Carom), 133.5-132.3 (CH_{arom}), 131.9 (CH_{arom}), 130.0-129.1 (CH_{arom}), 128.9-128.8 (C_q Carom), 128.6-127.4 (CH_{arom}), 127.2 (C_q Carom), 86.6 (C-1 β), 86.3 (C-1 α), 74.1 (C-5 β), 77.2 (C-4 α), 77.0 (C-4 β), 75.1 (C-3 β), 74.6 (CH₂ Bn β), 74.6 (CH₂ Bn α), 71.3 (C-3 α), 70.8 (C-2 α), 70.7 (C-5 α), 70.3 (C-2 β), 67.4 (CH₂ CO₂Bn β), 67.4 (CH₂ CO₂Bn α), 37.7 (CH₂ Lev), 29.5 (CH₃ Lev), 27.8 (CH₂ Lev), 21.0 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₃₉H₃₈O₉Na: 705.21287, found 705.21230.

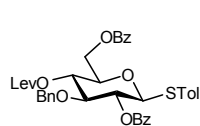


Benzyl (phenyl 2-O-benzoyl-4-O-benzyl-3-O-levulinyl-1-thio-α/β-D-glucopyranosyluronate) (16b): The title compound was isolated during the glycosylation of glucuronic acid ester **10** with glucoside **13b** in (86 mg, 0.13 mmol, 45% yield with respect to acceptor **13b**) as a clear oil and as an anomeric mixture (α/β: 1/2.2). IR (neat, cm⁻¹): 1744, 1721, 1404, 1358, 1315, 1265, 1207, 1179, 1155, 1092, 1070, 1026, 999, 972, 908, 733, 712, 698. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.04-8.00 (m, 3H, H_{arom}), 7.59-7.57 (m, 1.6H, H_{arom}), 7.47-7.15 (m, 27.6H, H_{arom}), 5.97 (d, 1H, 0.4H, *J* = 5.6 Hz, H-1 α), 5.66 (t, 1H, *J* = 9.2 Hz, H-3 α), 5.43 (t, 1H, *J* = 9.2 Hz, H-3 β), 5.29-5.15 (m, 4.3H, H-2 α, CH₂ CO₂Bn, H-2 β), 4.97 (d, 1H, *J* = 9.6 Hz, H-5 α), 4.86 (d, 1H, *J* = 10.0 Hz, H-1 β), 4.59 (s, 1H, CH₂ Bn α), 4.54 (d, 1H, *J* = CHHPh β), 5.50 (d, 1H, *J* = CHHPh β), 4.10 (d, 1H, *J* = 9.6 Hz, H-5 β), 4.07-4.05 (m, 1.53H, H-4 α, H-4 β), 2.57-2.55 (m, 1H, CHH Lev), 2.46-2.32 (m, 5H, CH₂ Lev α and β), 2.03 (s, 1.36H, CH₃ Lev α), 1.97 (s, 2.85H, CH₃ Lev β). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.6 (C=O Lev ketone), 171.7 (C=O), 167.3 (C=O), 165.3 (C=O), 137.4 (C_q Carom), 133.6-132.6 (CH_{arom}), 131.6 (C_q Carom), 130.1-130.0 (CH_{arom}), 129.0 (C_q Carom), 128.9-127.8 (CH_{arom}), 86.6 (C-1 α), 86.0 (C-1 β), 78.2 (C-5 β), 77.3 (C-4 α), 77.2 (C-4 β), 75.4 (C-3 β), 74.7 (CH₂ Bn β), 74.7 (CH₂ Bn α), 71.7 (C-3 α), 71.2 (C-2 α), 71.0 (C-5 α), 70.4 (C-2 β), 67.5 (CH₂ CO₂Bn β), 67.4 (CH₂ CO₂Bn α), 37.8 (CH₂ Lev α), 37.7 (CH₂ Lev β), 29.5 (CH₃ Lev), 27.9 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₃₈H₃₆O₉Na: 691.19722, found 691.19684.



1,6-Anhydro-2-O-benzoyl-3-O-benzyl-4-O-levulinyl-β-D-glucopyranoside (20): The title compound was isolated as a transparent oil (185 mg, 0.41 mmol, 34% yield with respect to the donor **12**). [α]_D²⁰: +30.9 (*c* = 3.6, DCM). IR (neat, cm⁻¹): 1717, 1362, 1337, 1315, 1265, 1207, 1179, 1148, 1109, 1098, 1070, 1047, 1026, 1009, 1001, 932, 903, 885, 733, 712, 700, 617. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.11-8.00 (m, 2H, H_{arom}), 7.59-7.57 (m, 1H, H_{arom}), 7.49-7.44 (m, 2H, H_{arom}), 7.38-7.11 (m, 5H, H_{arom}), 5.60 (s, 1H, H-1), 5.00 (s, 1H, H-2 or H-4), 4.84 (s, 1H, H-5), 4.83 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.69 (d, 1H, *J* = 11.2 Hz, CHHPh),

4.65 (d, 1H, $J = 5.2$ Hz, H-2 or H-4), 4.25 (d, 1H, $J = 7.2$ Hz, H-6), 3.80 (t, 1H, $J = 6.8$ Hz, H-6), 3.65 (d, 1H, H-3), 2.79-2.54 (m, 4H, 2x CH₂ Lev), 2.11 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.1 (C=O Lev ketone), 171.9 (C=O), 165.3 (C=O), 137.4 (C_q C_{arom}), 133.4 (CH_{arom}), 129.7 (CH_{arom}), 129.4 (C_q C_{arom}), 128.4-127.5 (CH_{arom}), 99.5 (C-1), 75.6 (C-3), 73.6 (C-2 or C-4), 72.3 (CH₂ Bn), 71.0 (C-5), 69.3 (C-2 or C-4), 65.1 (C-6), 37.7 (CH₂ Lev), 29.6 (CH₃ Lev), 28.0 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₂₅H₂₈O₇Na: 463.17272, found 463.17281.

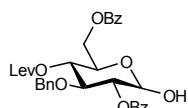


***p*-Tolyl**

glucopyranoside

2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-β-D-glucopyranoside (22):

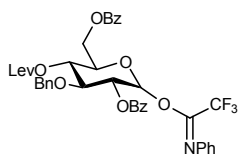
p-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-glucopyranoside **21** (2.40 g, 4.86 mmol) was suspended in toluene (16.2 mL, 0.3M) followed by addition of Bu₂SnO (1.21 g, 4.86 mmol, 1 eq.). The reaction mixture was heated to reflux and stirred for 3 h. A clear reaction mixture was obtained, which was concentrated and co-evaporated with toluene (2x), yielding a transparent oil. The oil obtained was dissolved in toluene (16.2 mL, 0.3M) followed by addition of BzCl (622 μL, 5.35 mmol, 1.1 eq.). After 3 h., TLC analysis (PE/EtOAc: 1/1, v/v) showed total conversion into higher running spot. The reaction mixture was diluted with EtOAc, washed with H₂O, dried over MgSO₄, filtered, and concentrated. The white residue was dissolved in DCM (24.3 mL, 0.2M) followed by addition of DIC (0.74 mL, 6.32 mmol, 1.3 eq.), LevOH (0.22 mL, 2.17 mmol, 1.3 eq.) and DMAP (cat.). After 1h., TLC analysis showed total conversion into a lower running spot (PE/EtOAc: 12/8, v/v, R_f 0.33). The reaction mixture was filtered, diluted with DCM, washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 6/4) gave the title compound as a white solid in 75% yield (2.52 g, 3.65 mmol). [α]_D: +14.2 (c = 1, DCM). IR (neat, cm⁻¹): 1742, 1717, 1493, 1450, 1402, 1377, 1364, 1315, 1261, 1233, 1204, 1175, 1157, 1123, 1111, 1084, 1067, 1038, 1028, 976, 964, 914, 804, 750, 710, 700, 687. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.08-8.04 (m, 4H, H_{arom}), 7.61-7.57 (m, 2H, H_{arom}), 7.61-7.57 (m, 2H, H_{arom}), 7.48-7.44 (m, 4H, H_{arom}), 7.43-7.31 (m, 2H, H_{arom}), 7.24-7.10 (m, 5H, H_{arom}), 6.83 (d, 2H, $J = 8.0$ Hz, H_{arom}), 5.28 (t, 1H, $J = 9.2$ Hz, H-2), 5.23 (t, 1H, $J = 9.6$ Hz, H-4), 4.77 (d, 1H, $J = 10.0$ Hz, H-1), 4.64 (dd, 1H, $J = 2.4$ Hz, $J = 12.4$ Hz, H-6), 4.61 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.58 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.35 (dd, 1H, $J = 6.0$ Hz, $J = 12.4$ Hz, H-6), 3.93 (t, 1H, $J = 9.2$ Hz, H-3), 3.88-3.84 (m, 1H, H-5), 2.73-2.59 (m, 2H, CH₂ Lev), 2.57-2.51 (m, 1H, CHH Lev), 2.50-2.37 (m, 1H, CHH Lev), 2.20 (s, 3H, CH₃ STol), 2.10 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.0 (C=O Lev ketone), 171.3 (C=O Lev), 165.9 (C=O Bz), 164.8 (C=O Bz), 138.1 (C_q C_{arom}), 137.3 (C_q C_{arom}), 133.7-133.0 (CH_{arom}), 129.7 (CH_{arom}), 129.7 (CH_{arom}), 129.5 (C_q C_{arom}), 129.5 (CH_{arom}), 129.4 (CH_{arom}), 128.3 (CH_{arom}), 128.2 (CH_{arom}), 128.1 (C_q C_{arom}), 128.0 (CH_{arom}), 127.8 (CH_{arom}), 127.5 (CH_{arom}), 86.1 (C-1), 81.2 (C-3), 76.1 (C-5), 74.3 (CH₂ Bn), 71.9 (C-2), 70.1 (C-4), 62.8 (C-6), 37.6 (CH₂ Lev), 29.5 (CH₃ Lev), 27.7 (CH₂ Lev), 20.9 (CH₃ STol). HRMS: [M+H]⁺ calcd for C₃₂H₃₁O₉H: calcd: 560.20408, found 560.19972.



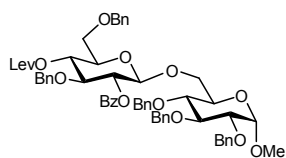
2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinyl-α/β-D-glucopyranose (24):

Glucoside **23** (1.23 g, 1.80 mmol) was dissolved in DCM (18 mL, 0.1 M) and the reaction mixture was cooled to 0 °C. NIS (445 mg, 1.98 mmol, 1.1 eq.) and TFA (152 μL, 1.98 mmol) were added and the reaction mixture was allowed to warm to rT. After 3 h., the reaction mixture was cooled to 0 °C, followed by addition of piperidine (535 μL, 5.40 mmol, 3 eq.). The reaction mixture was allowed to warm to rT and TLC analysis (PE/EtOAc: 12/8, v/v) showed total conversion into a lower running spot. The reaction mixture was quenched with Na₂S₂O₃ (aq., sat.), diluted with DCM, followed by separation of the layers. The organic layer was washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. The yellow oil obtained was purified by column chromatography (PE/EtOAc: 1/0 → 6/4) yielding the title compound as a transparent oil in 85% yield (882 mg, 1.53 mmol). IR (neat, cm⁻¹): 3412, 1719, 1601, 1452, 1362, 1315, 1267, 1207, 1177, 1152, 1110, 1098, 1069, 1049, 1026, 989, 972, 937, 764, 750, 712,

689. α anomer: ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 8.08-8.01 (m, 5H, H_{arom}), 7.60-7.53 (m, 2H, H_{arom}), 7.48-7.26 (m, 4H, H_{arom}), 7.21-7.18 (m, 4H, H_{arom}), 5.59 (t, 1H, $J = 3.6$ Hz, H-1), 5.31 (t, 1H, $J = 9.2$ Hz, H-4), 5.13 (dd, 1H, $J = 3.2$ Hz, $J = 10.0$ Hz, H-2), 4.76 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.69 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.57 (d, 1H, $J = 10.0$ Hz, H-6), 4.38-4.31 (m, 2H, H-5, H-6), 4.28 (t, 1H, $J = 9.6$ Hz, H-3), 3.31 (d, 1H, $J = 3.2$ Hz, OH), 2.70-2.64 (m, 2H, CH_2 Lev), 2.57-2.35 (m, 2H, CH_2 Lev), 2.11 (s, 3H, CH_3 Lev). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 206.3 (C=O ketone), 171.5 (C=O Lev), 166.4 (C=O Bz), 165.7 (C=O Bz), 138.0 (C_q C_{arom}), 133.6-133.1 (CH_{arom}), 129.9 (CH_{arom}), 129.8 (C_q C_{arom}), 129.4-127.6 (CH_{arom}), 90.4 (C-1), 76.9 (C-3), 75.0 (CH_2 Bn), 73.9 (C-2), 70.4 (C-4), 67.9 (C-5), 62.6 (C-6), 37.8 (CH_2 Lev), 29.7 (CH_3 Lev), 27.9 (CH_2 Lev). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{32}\text{H}_{32}\text{O}_{10}\text{Na}$: 599.18877, found 599.18785.

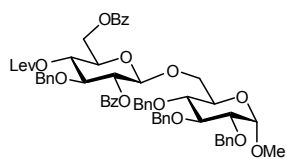


2,6-di-O-benzoyl-3-O-benzyl-4-O-levulinyl-1-O-(N-phenyl)-trifluoroacetimidoyl- α/β -D-glucopyranoside (25): Hemiacetal **24** (149 mg, 0.26 mmol) was dissolved in acetone (2.6 mL, 0.1M) and the reaction mixture was cooled to 0 °C. To this mixture, N-phenyl-2,2,2-trifluoroacetimidoyl chloride (80 μL , 0.52 mmol, 2 eq.) and Cs_2CO_3 (85 mg, 0.26 mmol, 1 eq.) were added. The reaction mixture was allowed to warm to rT and after 2 h., TLC analysis showed total conversion into a higher running spot (PE/EtOAc: 12/8, v/v, R_f 0.76). The reaction mixture was filtered over celite and concentrated. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 6/4) gave the title compound as a transparent oil in 77% yield (α/β : 8/1). IR (neat, cm^{-1}): 1717, 1452, 1364, 1315, 1265, 1207, 1152, 1109, 1098, 1069, 1026, 926, 908, 895, 773, 735, 710, 696, 633, 617. α anomer: ^1H NMR (50 °C, 400 MHz, CDCl_3 , HH-COSY, HSQC): ^1H NMR: 8.07-7.93 (m, 4H, H_{arom}), 7.60-7.23 (m, 7H, H_{arom}), 7.19-6.96 (m, 8H, H_{arom}), 6.59 (bs, 1H, H-1), 6.45-6.43 (m, 1H, H_{arom}), 5.39-5.31 (m, 2H, H-2, H-4), 4.722 (s, 2H, CH_2 Bn), 5.54 (d, 1H, $J = 12.0$ Hz, H-6), 4.39 (dd, 1H, $J = 5.2$ Hz, $J = 12.4$ Hz, H-6), 4.28-4.24 (m, 2H, H-3, H-5), 2.69-2.44 (m, 4H, 2x CH_2 Lev), 2.08 (s, 3H, CH_3 Lev). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 205.4 (C=O Lev ketone), 171.3 (C=O Lev), 166.1 (C=O Bz), 165.2 (C=O Bz), 143.0 (C_q C_{arom}), 137.9 (C_q C_{arom}), 133.5 (CH_{arom}), 133.3 (CH_{arom}), 133.0 (CH_{arom}), 130.1 (C_q C_{arom}), 129.9 (CH_{arom}), 129.8 (C_q C_{arom}), 129.4-127.7 (CH_{arom}), 124.4 (CH_{arom}), 119.2 (CH_{arom}), 93.0 (C-1), 77.2 (C-3 or C-5), 75.0 (CH_2 Bn), 72.3 (C-2), 71.1 (C-3 or C-5), 70.1 (C-4), 62.6 (C-6), 37.9 (CH_2 Lev), 29.4 (CH_3 Lev), 28.1 (CH_2 Lev). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{40}\text{H}_{36}\text{F}_3\text{NO}_{10}\text{Na}$: 770.21835, found 770.21854

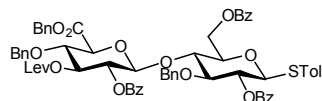


Methyl 2,3,4-tri-O-benzyl-6-O-(2-O-benzoyl-3,6-di-O-benzyl-4-O-levulinyl- β -D-glucopyranosyl)- β -D-glucopyranoside (27): The title compound was isolated as a transparent oil, which crystallized on standing. $[\alpha]_D^{25}$: +23.6 ($c = 0.9$, DCM). IR (neat, cm^{-1}): 2874, 1721, 1452, 1362, 1315, 1265, 1207, 1152, 1092, 1061, 1028, 912, 935, 696. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.92 (d, 1H, $J = 6.4$ Hz, H_{arom}), 7.46-7.43 (m, 1H, H_{arom}), 7.32-7.21 (m, 15H, H_{arom}), 7.14-7.13 (m, 5H, H_{arom}), 6.99-6.97 (m, 2H, H_{arom}), 5.39 (t, 1H, $J = 9.6$ Hz, H-2'), 5.12 (t, 1H, $J = 9.2$ Hz, H-4'), 4.87 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.73 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.65 (d, 1H, CHHPh), 4.60-4.53 (m, 6H, 2x CH_2 Bn, CHHPh, H-1'), 4.49 (d, 1H, $J = 3.6$ Hz, H-1), 4.41 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.23 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.13 (d, 1H, $J = 12.4$ Hz, H-6), 3.91-3.84 (m, 2H, H-3, H-3'), 3.69-3.62 (m, 4H, H-6, H-5, H-6', H-5'), 3.44 (dd, 1H, $J = 3.6$ Hz, $J = 9.6$ Hz, H-2), 3.38 (t, 1H, $J = 9.2$ Hz, H-4), 3.20 (s, 3H, OCH_3), 2.61-2.57 (m, 2H, CH_2 Lev), 2.41-2.36 (m, CH_2 Lev), 2.11 (s, 3H, CH_3 Lev). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 206.1 (C=O Lev ketone), 171.5 (C=O), 164.7 (C=O Bz), 138.8-137.6 (C_q C_{arom}), 133.0 (CH_{arom}), 129.7 (CH_{arom}), 129.5 (C_q C_{arom}), 128.4-127.3 (CH_{arom}), 101.0 (C-1'), 97.9 (C-1), 81.8 (C-3 or C-3'), 79.8 (C-3 or C-3'), 79.6 (C-2), 77.2 (C-4), 75.4 (CH_2 Bn), 74.5 (CH_2 Bn), 73.7 (C-5'), 73.6 (CH_2 Bn), 73.5 (CH_2 Bn), 73.3

(CH₂ Bn), 72.2 (C-2'), 71.1 (C-4'), 69.7 (C-6'), 69.3 (C-5), 67.9 (C-6), 54.9 (OCH₃), 37.6 (CH₂ Lev), 29.7 (CH₃ Lev), 27.8 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₆₀H₆₄O₁₄Na: 1031.41883, found 1031.41796.



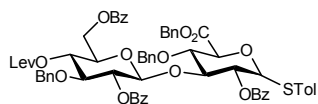
Methyl 2,3,4-tri-O-benzyl-6-O-(2,6-di-O-benzoyl-3-O-benzyl-4-levulinyl- β -D-glucopyranosyl)- β -D-glucopyranoside (28): The title compound was isolated as a transparent oil, which crystallized on standing. $[\alpha]_D^{20}$: +17.4 (c = 1, DCM). IR (neat, cm⁻¹): 3057, 2928, 1719, 1452, 1420, 1362, 1315, 1263, 120, 1177, 1152, 1092, 1069, 1026, 1013, 912, 897, 731, 698. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.05 (d, 2H, J = 8.4 Hz, H_{arom}), 7.93 (d, 2H, J = 8.4 Hz, H_{arom}), 7.54 (t, 1H, J = 7.6 Hz, H_{arom}), 7.46-7.40 (m, 3H, H_{arom}), 7.32-7.22 (m, 16H, H_{arom}), 7.21-7.14 (m, 4H, H_{arom}), 7.00-6.98 (m, 2H, H_{arom}), 5.42 (t, 1H, J = 9.6 Hz, H-2'), 5.28 (t, 1H, J = 9.6 Hz, H-4'), 4.86 (d, 1H, J = 11.2 Hz, CHHPh), 4.71 (d, 1H, J = 12.0 Hz, CHHPh), 4.65 (d, 1H, J = 11.2 Hz, CHHPh), 4.60-4.57 (m, 3H, H-1', CH₂Ph), 4.56-4.53 (m, 2H, CHHPh, H-6'), 4.47 (d, 1H, J = 3.6 Hz, H-1), 4.41 (d, 1H, J = 11.2 Hz, H-6'), 4.36 (dd, 1H, J = 5.6 Hz, J = 11.2 Hz, H-6'), 4.22 (d, 1H, J = 11.2 Hz, CHHPh), 4.08 (d, 1H, J = 8.8 Hz, H-6), 3.90 (t, 1H, J = 9.6 Hz, H-3'), 3.84 (t, 1H, J = 9.6 Hz, H-3), 3.81-3.77 (m, 1H, H-5'), 3.68-3.63 (m, 2H, H-5, H-6), 3.41 (dd, 1H, J = 3.6 Hz, J = 9.6 Hz, H-2), 3.33 (t, 1H, J = 9.6 Hz, H-4), 3.18 (s, 3H, OCH₃), 2.67-2.59 (m, 2H, CH₂ Lev), 2.51-2.45 (m, 2H, CH₂ Lev), 2.11 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.2 (C=O Lev ketone), 171.5 (C=O Lev), 166.2 (C=O Bz), 164.8 (C=O Bz), 138.9 (C_q Carom), 138.2 (C_q Carom), 138.2 (C_q Carom), 137.6 (C_q Carom), 133.1 (CH_{arom}), 129.9 (CH_{arom}), 129.8 (C_q Carom), 129.6 (C_q Carom), 128.4-127.4 (CH_{arom}), 101.3 (C-1'), 97.9 (C-1), 81.9 (C-3), 79.8 (C-3'), 79.7 (C-2), 77.1 (C-4), 75.5 (CH₂ Bn), 74.6 (CH₂ Bn), 73.9 (CH₂ Bn), 73.4 (CH₂ Bn), 73.2 (C-2'), 72.3 (C-5'), 70.5 (C-4'), 69.3 (C-5), 68.1 (C-6), 63.1 (C-6'), 55.0 (OCH₃), 37.8 (CH₂ Lev), 29.7 (CH₃ Lev), 27.9 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₆₀H₆₂O₁₅Na: 1045.39809, found 1045.39751.



p-Tolyl 2,6-di-O-benzoyl-3-O-benzyl-4-O-(benzyl (2-O-benzoyl-4-O-benzyl-3-O-levulinyl- β -D-glucopyranosyluronate))-2,6-di-O-benzoyl-1-thio- β -D-glucopyranoside (29): Glucuronic acid ester

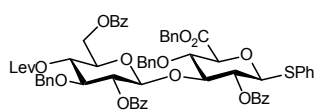
donor **10** (260 mg, 0.35 mmol, 1 eq.) and glucoside acceptor **23** (275 mg, 0.46 mmol, 1.3 eq.) were co-evaporated with toluene (2x) and dissolved in freshly distilled DCM (3.5 mL, 0.1M). Activated MS 3Å were added and the reaction mixture was stirred for 30 min. The reaction mixture was cooled to 0 °C, followed by addition of TfOH (175 μ L of 0.2M stock solution, 0.035 mmol, 0.1 eq.). The reaction mixture was allowed to warm to rT. After 10 min., TLC analysis (toluene/EtOAc: 16/4, v/v) showed total consumption of the donor. The reaction mixture was neutralized with TEA, diluted with DCM, washed with H₂O, dried over MgSO₄, filtered, and concentrated, yielding a yellow oil. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 6/4) followed by SEC (MeOH/DCM: 1/1) gave the title compound as a white solid in 68% (274 mg, 0.24 mmol). $[\alpha]_D^{20}$: +20.6 (c = 1.6, DCM). IR (neat, cm⁻¹): 1724, 1452, 1400, 1360, 1315, 1263, 1215, 1177, 1144, 1092, 1069, 1051, 1026, 980, 737, 710, 700. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.99-7.95 (m, 4H, H_{arom}), 7.88 (d, 2H, J = 7.6 Hz, H_{arom}), 7.59-7.51 (m, 2H, H_{arom}), 7.45-7.33 (m, 7H, H_{arom}), 7.28-7.14 (m, 10H, H_{arom}), 7.12-7.05 (m, 7H, H_{arom}), 6.78 (d, 2H, J = 8.0 Hz, H_{arom}), 5.37 (t, 1H, J = 9.2 Hz, H-3'), 5.30 (t, 1H, J = 9.6 Hz, H-2'), 5.13 (t, 1H, J = 9.6 Hz, H-2), 5.05 (d, 1H, J = 12.0 Hz, CHHPh), 4.97 (d, 1H, J = 12.0 Hz, CHHPh), 4.90 (d, 1H, J = 7.6 Hz, H-1'), 4.89 (d, 1H, J = 11.6 Hz, CHHPh), 4.64-4.53 (m, 2H, CHHPh, H-6), 4.48 (d, 1H, J = 11.2 Hz, CHHPh), 4.44 (d, 1H, J = 11.2 Hz, CHHPh), 4.29 (dd, 1H, J = 4.4 Hz, J = 12.0 Hz, H-6), 4.05-4.00 (m, 2H, H-4, H-4'), 3.96 (d, 1H, J = 9.6 Hz, H-5'), 3.82 (t, 1H, J = 8.8 Hz, H-3), 3.56-3.53 (m, 1H, H-5), 2.56-2.32 (m, 4H, 2x CH₂ Lev), 2.16 (s, 3H, CH₃ STol), 1.95 (s, 3H, CH₃ STol). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.6 (C=O Lev ketone), 171.5 (C=O), 167.3 (C=O), 165.6 (C=O), 165.0 (C=O), 164.9 (C=O), 138.1 (C_q Carom), 137.8 (C_q Carom), 137.2 (C_q Carom), 134.7 (C_q Carom), 134.0 (CH_{arom}), 133.9

(CH_{arom}), 133.4 (CH_{arom}), 133.0 (CH_{arom}), 129.8-127.8 (CH_{arom}), 127.5 (C_q Carom), 127.3 (CH_{arom}), 100.9 (C-1'), 85.6 (C-1), 81.7 (C-3), 77.8 (C-4 or C-4'), 77.3 (C-4 or C-4'), 76.7 (C-5), 75.0 (CH₂ Bn), 74.6 (C-5'), 74.6 (CH₂ Bn), 73.8 (C-3'), 72.2 (C-2'), 71.7 (C-2), 67.5 (CH₂ CO₂Bn), 62.3 (C-6), 37.6 (CH₂ Lev), 29.4 (CH₃ Lev), 27.8 (CH₂ Lev), 21.0 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₆₆H₆₂O₁₆SNa: 1165.36508, found 1165.36540.



Benzyl (*p*-tolyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(3,6-di-*O*-benzyl-2-*O*-benzoyl-4-*O*-levulinyl-1-thio-β-D-glucopyranosyl)-α/β-D-glucopyranosyluronate)) (31a):

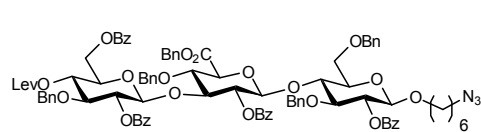
Glucoside donor **22** (88 mg, 0.12 mmol) and glucuronic acid acceptor **18** (90 mg, 0.15 mmol, 1.3 eq.) were co-evaporated with toluene (2x), dissolved in freshly distilled DCM (1.2 mL, 0.1M) followed by addition of activated MS 3Å. The reaction mixture was stirred for 30 min. and then cooled to 0 °C. TfOH (120 μL of 0.1M stock solution, 0.012 mmol, 0.1 eq.) and the reaction mixture was allowed to warm to rT. After 15 min., TLC analysis (PE/EtOAc: 12/8, v/v) showed total consumption of the donor. The reaction mixture was neutralized with TEA, diluted with DCM, washed with H₂O, dried over MgSO₄, filtered, and concentrated. Size Exclusion chromatography (DCM/MeOH: 1/1) gave the disaccharide **31a** as a transparent oil in 51% yield (69 mg, 0.06 mmol, α/β: 1/1). IR (neat, cm⁻¹): 2920, 1719, 1601, 1493, 1452, 1402, 1632, 1315, 1263, 1209, 1177, 1148, 1092, 1067, 1026, 1001, 910, 845, 810, 737, 710, 698, 662, 637. The identification was based on NMR analysis (400 MHz, CDCl₃, HSQC): **31α**: 5.76 (d, *J* = 4.8 Hz, H-1), **31β**: 4.59 (d, *J* = 9.6 Hz, H-1). ¹³C NMR (100 MHz, CDCl₃, HH-COSY, HSQC): **31α**: 84.7 (C-1), **31β**: 87.5 (C-1). HRMS: [M+Na]⁺ calcd for C₆₆H₆₂O₁₆SNa: 1165.36508, found 1165.36444.



Benzyl (phenyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinyl-β-D-glucopyranoside)-1-thio-β-D-glucopyranosyluronate)) (31b):

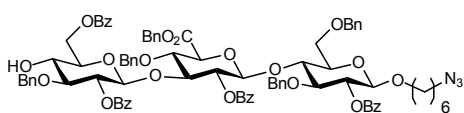
Glucoside donor **22** (593 mg, 0.79 mmol, 1.3 eq.) and glucuronic acid ester acceptor **30** (354 mg, 0.61 mmol) were co-evaporated with toluene (2x), dissolved in freshly distilled DCM (6.1 mL, 0.1M) followed by addition of activated MS 3Å. The reaction mixture was stirred for 30 min. and then cooled to 0 °C. TfOH (305 μL of 0.2M stock solution, 0.061 mmol, 0.1 eq.) was added and the reaction mixture was allowed to warm to rT. After 15 min., TLC analysis (PE/EtOAc: 12/8, v/v) showed total consumption of the acceptor. The reaction mixture was neutralized with TEA, diluted with DCM, washed with H₂O, brine, dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 1/1) followed by SEC gave disaccharide **31b** as a colourless oil in 64% yield (444 mg, 0.39 mmol). [α]_D²⁰: +19.2 (*c* = 1, DCM). IR (neat, cm⁻¹): 1719, 1452, 1362, 1315, 1263, 1204, 1177, 1148, 1090, 1067, 1026, 991, 972, 910, 845, 933, 698, 638, 617. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 8.01 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.96 (d, 2H, *J* = 7.2 Hz, H_{arom}), 7.92 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.67-7.65 (m, 1H, H_{arom}), 7.59-7.57 (m, 1H, H_{arom}), 7.54-7.50 (m, 2H, H_{arom}), 7.45-7.35 (m, 3H, H_{arom}), 7.33-7.23 (m, 10H, H_{arom}), 7.19-7.08 (m, 11H, H_{arom}), 7.00-6.98 (m, 2H, H_{arom}), 5.31 (t, 1H, *J* = 8.8 Hz, H-2'), 5.22 (t, 1H, *J* = 8.8 Hz, H-2), 5.19 (t, 1H, *J* = 9.2 Hz, H-4'), 5.13 (d, 1H, *J* = 12.8 Hz, CHH CO₂Bn), 5.09 (d, 1H, *J* = 12.8 Hz, CHH CO₂Bn), 4.98 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.86 (d, 1H, *J* = 8.0 Hz, H-1'), 4.67 (d, 1H, *J* = 10.0 Hz, H-1), 4.51 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.51-4.47 (m, 1H, H-6'), 4.44 (s, 2H, CH₂ Bn), 4.24 (t, 1H, *J* = 8.8 Hz, H-3), 4.21 (dd, 1H, *J* = 6.0 Hz, *J* = 12.4 Hz, H-6'), 3.99 (t, 1H, *J* = 9.2 Hz, H-4), 3.90 (d, 1H, *J* = 9.6 Hz, H-5), 3.74-3.69 (m, 1H, H-5'), 3.62 (t, 1H, *J* = 9.2 Hz, H-3'), 2.62-2.58 (m, 2H, CH₂ Lev), 2.50-2.38 (m, 1H, CHH Lev), 2.38-2.29 (m, 1H, CHH Lev), 2.07 (s, 3H, CH₃ Lev). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.0 (C=O Lev ketone), 171.3 (C=O), 167.2 (C=O), 166.0 (C=O), 165.0 (C=O), 164.3 (C=O), 137.9 (C_q Carom), 137.3 (C_q Carom), 135.0 (C_q Carom), 133.5 (CH_{arom}), 133.0 (CH_{arom}), 132.9 (C_q Carom), 132.1 (CH_{arom}), 129.8 (CH_{arom}), 129.7 (CH_{arom}), 129.7 (CH_{arom}), 129.4-129.3 (C_q Carom), 128.8-127.4 (CH_{arom}), 100.4 (C-1'),

87.2 (C-1), 80.3 (C-3), 79.8 (C-3'), 78.1 (C-5), 76.7 (C-4), 74.9 (CH₂ Bn), 74.0 (CH₂ Bn), 73.3 (C-2'), 72.3 (C-2), 72.2 (C-3'), 70.5 (C-4'), 67.2 (CH₂ CO₂Bn), 63.0 (C-6'), 37.7 (CH₂ Lev), 29.6 (CH₃ Lev), 27.7 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₆₅H₆₀O₁₆SNa: 1151.34943, found 1151.34865.



6-Azido-hexyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-[benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-{2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinyl-β-D-glucopyranosyl}-β-D-glucopyranosyluronate)]-β-D-glucopyranoside (32):

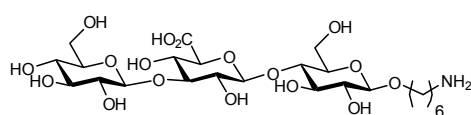
Disaccharide **31b** (102 mg, 0.09 mmol) and acceptor glucoside **7** (69 mg, 0.117 mmol, 1.3 eq.) were glycosylated according to the general procedure for glycosylations using NIS/TfOH. Trisaccharide **32** was obtained as a transparent oil in 75% yield (108 mg, 0.067 mmol). *R*_f 0.49 (toluene/EtOAc: 4/1, v/v). [α]_D²⁰: +11.0 (c = 1, DCM, cm⁻¹). IR (neat): 2872, 2095, 1722, 1452, 1418, 1402, 1362, 1315, 1263, 1209, 1177, 1140, 1092, 1069, 1026, 1001, 910, 847, 733, 700, 638, 617. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC, HMBC): 7.99 (d, 2H, *J* = 7.2 Hz, H_{arom}), 7.88 (d, 2H, *J* = 7.2 Hz, H_{arom}), 7.75 (d, 3H, *J* = 7.2 Hz, H_{arom}), 7.74-7.57 (m, 1H, H_{arom}), 7.56-6.96 (m, 37H, H_{arom}), 5.30 (t, 1H, *J* = 9.2 Hz, H-2''), 5.21-5.16 (m, 2H, H-4'', H-2), 5.11 (t, 1H, *J* = 9.2 Hz, H-2'), 4.97 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.96 (d, 1H, *J* = 12.0 Hz, CHH CO₂Bn), 4.91 (d, 1H, *J* = 12.0 Hz, CHH CO₂Bn), 4.79 (d, 1H, *J* = 10.4 Hz, CHHPh), 4.77 (d, 1H, *J* = 7.6 Hz, H-1''), 4.65 (d, 1H, *J* = 8.0 Hz, H-1), 4.57 (d, 1H, CHHPh), 4.51 (dd, 1H, *J* = 2.8 Hz, *J* = 12.0 Hz, H-6''), 4.47 (d, 2H, *J* = 10.8 Hz, CH₂ Bn), 4.43 (s, 2H, CH₂ Bn), 4.25 (d, 1H, *J* = 8.0 Hz, H-1'), 4.24-4.17 (m, 2H, CHHPh, H-6''), 4.06 (t, 1H, *J* = 8.8 Hz, H-3), 3.98 (t, 2H, *J* = 8.4 Hz, H-4, H-4'), 3.86 (d, 1H, *J* = 9.2 Hz, H-5'), 3.78 (dt, 1H, *J* = 3.2, *J* = 9.6 Hz, H-5''), 3.73-3.65 (m, 1H, CHH N₃(CH₂)₆OH), 3.65-3.59 (m, 2H, H-3'', H-3'), 3.43 (dd, 1H, *J* = 3.6 Hz, *J* = 11.2 Hz, H-6), 4.44 (d, 1H, *J* = 11.2 Hz, H-6), 3.33-3.21 (m, 1H, CHH N₃(CH₂)₆OH), 3.04-3.02 (m, 1H, H-5), 3.00-2.95 (m, 2H, CH₂ N₃(CH₂)₆OH), 2.65-2.60 (m, 2H, CH₂ Lev), 2.45-2.44 (m, 1H, CHH Lev), 2.38-2.32 (m, 1H, CHH Lev), 2.08 (s, 3H, CH₃ Lev), 1.50-1.20 (m, 4H, 2x CH₂ N₃(CH₂)₆OH), 1.19-1.05 (m, 4H, 2x CH₂ N₃(CH₂)₆OH). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC, HMBC): 206.2 (C=O Lev ketone), 171.4 (C=O), 167.5 (C=O), 166.3 (C=O), 165.0 (C=O), 165.0 (C=O), 163.9 (C=O), 138.4 (C_q Carom), 138.1 (C_q Carom), 137.4 (C_q Carom), 135.0 (C_q Carom), 133.5-132.9 (CH_{arom}), 130.1 (C_q Carom), 130.0-129.2 (CH_{arom}), 128.7 (C_q Carom), 128.6 (C_q Carom), 128.5 (C_q Carom), 128.4-127.1 (CH_{arom}), 101.1 (C-1'), 100.9 (C-1''), 100.4 (C-1), 80.3 (C-3'), 80.0 (C-3''), 79.9 (C-3), 77.5 (C-4 or C-4'), 77.1 (C-4 or C-4'), 75.0 (CH₂ Bn), 74.8 (C-5'), 74.7 (C-5), 74.6 (CH₂ Bn), 74.0 (CH₂ Bn), 73.8 (C-2), 73.5 (CH₂ Bn), 73.4 (C-2''), 73.1 (C-2'), 72.3 (C-5''), 70.6 (C-4''), 69.3 (CH₂ N₃(CH₂)₆OH), 67.4 (CH₂ CO₂Bn), 67.3 (C-6), 63.2 (C-6''), 51.2 (CH₂ N₃(CH₂)₆OH), 37.8 (CH₂ Lev), 29.7 (CH₃ Lev), 29.2 (CH₂ Lev), 28.6 (CH₂ N₃(CH₂)₆OH), 27.8 (CH₂ N₃(CH₂)₆OH), 26.2 (CH₂ N₃(CH₂)₆OH), 26.3 (CH₂ N₃(CH₂)₆OH), 25.4 (CH₂ N₃(CH₂)₆OH). HRMS: [M+Na]⁺ calcd for C₉₂H₉₃N₃O₂₃Na: 1630.60921, found 1630.60872



6-Azido-hexyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-[benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-{2,6-di-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl}-β-D-glucopyranosyluronate)]-β-D-glucopyranoside (33):

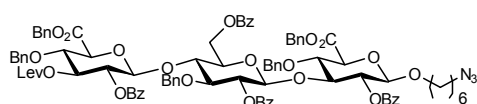
Trisaccharide **32** (82 mg, 0.051 mmol) was dissolved in a mixture of pyridine/AcOH (4/1, 0.51 mL, 0.1M) and the reaction mixture was cooled to 0 °C. Hydrazine acetate (24 mg, 0.26 mmol, 5 eq.) was added and the reaction mixture was allowed to warm to rT. After 15 min., TLC analysis showed total conversion into a higher running spot (toluene/EtOAc: 4/1, v/v, *R*_f 0.5). The reaction mixture was cooled to 0 °C, quenched with acetone, diluted with EtOAc, washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 6/4) gave the title compound as a colourless oil in 95% yield (73mg, 0.048 mmol). [α]_D²⁰: +16.7 (c = 0.86, DCM). IR (neat, cm⁻¹): 3065, 3034, 2928, 2869, 2253, 2099, 1728, 1603, 1497, 1452, 1418, 1362, 1315, 1265,

1213 1179, 1157, 1086, 1069, 1026, 905, 849. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC, HMBC): 8.00 (d, 2H, $J = 7.2$ Hz, H_{arom}), 4.94 (d, 2H, $J = 7.2$ Hz, H_{arom}), 7.92-7.78 (m, 3H, H_{arom}), 7.58-7.51 (m, 1H, H_{arom}), 7.51-7.40 (m, 5H, H_{arom}), 7.40-6.97 (m, 33H, H_{arom}), 5.23-5.16 (m, 2H, H-2, H-2''), 5.13 (t, 1H, $J = 9.2$ Hz, H-2''), 5.03 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.94 (d, 1H, CHH CO_2Bn), 4.88 (d, 1H, CHH CO_2Bn), 4.78 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.76 (d, 1H, $J = 8.0$ Hz, H-1''), 4.69 (d, 1H, $J = 8.0$ Hz, H-1), 4.67-4.54 (m, 4H, H-6'', CH_2Ph), 4.54-4.45 (m, 4H, 2x CH_2Ph), 4.25 (d, 1H, $J = 10.0$ Hz, H-5''), 4.25 (d, 1H, $J = 8.0$ Hz, H-1'), 4.12 (t, 1H, $J = 8.8$ Hz, H-3), 4.02-3.95 (m, 2H, H-4', H-4), 3.90 (d, 1H, $J = 9.6$ Hz, H-5'), 3.91-3.55 (m, 3H, CHH $\text{N}_3(\text{CH}_2)_6\text{OH}$, H-4'', H-3''), 3.46 (dd, 1H, $J = 3.2$ Hz, $J = 8.0$ Hz, H-6^a), 3.41 (t, 1H, $J = 9.2$ Hz, H-3''), 3.30 (d, 1H, $J = 10.0$ Hz, H-6^b), 3.28-3.24 (m, 1H, CHH $\text{N}_3(\text{CH}_2)_6\text{OH}$), 3.08-3.05 (m, 1H, H-5), 3.0 (dt, 2H, $J = 2.0$ Hz, $J = 6.8$ Hz, CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 2.81 (d, 1H, $J = 3.2$ Hz, OH), 1.58-1.26 (m, 4H, 2x CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 1.16-1.09 (m, 4H, 2x CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC, HMBC): 167.5 (C=O CO_2Bn), 167.2 (C=O Bz), 165.2 (C=O Bz), 164.9 (C=O Bz), 163.8 (C=O Bz), 138.4-137.5 (C_q C_{arom}), 134.9 (C_q C_{arom}), 133.6-132.9 (CH_{arom}), 130.1 (C_q C_{arom}), 130.0-129.7 (CH_{arom}), 129.7-129.6 (C_q C_{arom}), 129.3-127.0 (CH_{arom}), 101.3 (C-1'), 100.9 (C-1''), 100.4 (C-1), 82.0 (C-3''), 80.3 (C-3'), 79.7 (C-3), 77.5 (C-4), 77.3 (C-4'), 74.8 (CH_2 Bn), 74.8 (C-5'), 74.8 (CH_2 Bn), 74.7 (C-5), 74.6 (CH_2 Bn), 74.1 (C-3'), 73.9 (C-2 and C-5''), 73.5 (CH_2 Bn), 73.4 (C-2''), 73.1 (C-2'), 70.3 (C-4''), 69.3 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 67.4 (C-6), 67.3 (CH_2 CO_2Bn), 63.5 (C-6''), 51.1 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 29.1 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 28.5 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 26.2 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 25.3 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{87}\text{H}_{87}\text{N}_3\text{O}_{21}\text{Na}$: 1532.57243, found 1532.57327.



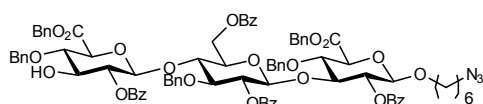
6-Aminohexyl 4-O-(3-O-(β -D-glucopyranosyl)- β -D-glucopyranosyluronic acid)- β -D-glucopyranoside (34):

Trisaccharide **33** (66 mg, 43.7 μmol) was dissolved in dioxane (874 μL , 0.05M) followed by addition of KOH (0.5M KOH in H_2O , 1.05 mL, 0.52 mmol, 12 eq.). After stirring overnight, more KOH was added portionwise (52 μL , 0.26 mmol, 6 eq., then 26 μL , 0.13 mmol, 3 eq.). After 6 days, LC-MS analysis confirmed that the esters were saponified. The reaction mixture was neutralized with H^+ Amberlite, filtered, and concentrated. Size exclusion chromatography (DCM/MeOH: 1/1) gave a white solid, which was dissolved in H_2O /BuOH mixture (1/2, v/v, 486 μL , 0.09 M). A drop of 1M HCl (aq.) was added followed by addition of Pd/C. The reaction mixture was purged with H_2 gas and stirred overnight. The reaction mixture was filtered over a Whatman filter and concentrated. The obtained residue was subjected to gel filtration (HW-40, 0.15M NH_4HCO_3 in H_2O) and subsequent lyophilisation. The deprotected trimer was obtained as a white solid in 44% yield over 2 steps (12 mg, 19.4 μmol). ^1H NMR (600 MHz, CDCl_3 , HH-COSY, HSQC, TOCSY, HMBC): 4.75 (d, 1H, $J = 7.8$ Hz, H-1''), 4.49 (d, 1H, $J = 7.8$ Hz, H-1'), 3.93 (d, 1H, $J = 11.4$ Hz, H-6''), 3.91-3.77 (m, 2H, H-6^a, CHH $\text{N}_3(\text{CH}_2)_6\text{OH}$), 3.77-3.72 (m, 3H, H-5, H-3', H-6^b), 3.67 (dd, 1H, $J = 6.0$ Hz, $J = 12.4$ Hz, H-6^b), 3.67-3.63 (m, 1H, CHH $\text{N}_3(\text{CH}_2)_6\text{OH}$), 3.61-3.54 (m, 4H, H-3, H-4, H-4', H-5'), 5.53 (t, 1H, $J = 9.0$ Hz, H-2), 3.47 (t, 1H, $J = 9.6$ Hz, H-3''), 3.45-3.40 (m, 1H, H-5''), 3.35 (t, 1H, $J = 9.6$ Hz, H-4''), 3.30 (t, 1H, $J = 9.0$ Hz, H-2''), 3.24 (t, 1H, $J = 9.0$ Hz, H-2), 2.94 (t, 2H, $J = 7.8$ Hz, CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 1.64-1.59 (m, 4H, 2x CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 1.37-1.36 (m, 4H, 2x CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$). ^{13}C NMR (150 MHz, CDCl_3 , HH-COSY, HSQC, TOCSY, HMBC): 176.3 (C=O), 103.5 (C-1''), 103.0 (C-1'), 102.9 (C-1), 83.5 (C-5), 79.8 (C-4 or C-4' or C-5'), 77.0 (C-5''), 76.7 (C-3'), 76.5 (C-3''), 75.7 (C-4 or C-4' or C-5'), 75.3 (C-3), 74.4 (C-2''), 74.1 (C-2'), 73.8 (C-2), 71.4 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 71.2 (C-4 or C-4' or C-5'), 70.5 (C-4''), 61.7 (C-6''), 61.0 (C-6), 40.3 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 29.4 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 27.6 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 26.2 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$), 25.5 (CH_2 $\text{N}_3(\text{CH}_2)_6\text{OH}$). HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{44}\text{NO}_{17}$: 618.26038, found 618.26016.



Benzyl (6-azido-hexyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-{2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-[benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-*O*-levulinyl-β-D-glucopyranosyluronate)]-β-D-glucopyranosyl}-β-D-glucopyranosyluronate) (35):

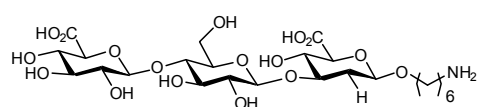
Disaccharide **29** (90 mg, 0.08 mmol) and glucuronic acid ester acceptor **3** (61 mg, 0.10 mmol, 1.3 eq.) were condensed according to the general procedure for glycosylations using NIS/TfOH. The title compound was isolated as a white solid in 54% yield. R_f 0.36 (PE/EtOAc: 13/7, v/v). $[\alpha]_D^{20}$: +6.1 (c = 1, DCM). IR (neat, cm^{-1}): 2095, 1728, 1452, 1362, 1315, 1261, 1215, 1177, 1142, 1094, 1069, 1026, 978, 910, 750, 710, 698. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC, HMBC): 8.00-7.88 (m, 2H, H_{arom}), 7.86-7.76 (m, 4H, H_{arom}), 7.61-7.56 (m, 2H, H_{arom}), 7.48-7.35 (m, 11H, H_{arom}), 7.28-7.09 (m, 15H, H_{arom}), 7.08-7.01 (m, 8H, H_{arom}), 7.08-6.95 (m, 3H, H_{arom}), 5.33-5.20 (m, 3H, H-3'', H-2', H-2''), 5.10-5.03 (m, 3H, H-2, $\text{CH}_2 \text{CO}_2\text{Bn}$), 5.02 (d, 1H, J = 12.4 Hz, $\text{CHH CO}_2\text{Bn}$), 4.94 (d, 1H, J = 12.4 Hz, $\text{CHH CO}_2\text{Bn}$), 4.85 (d, 1H, J = 11.2 Hz, CHHPh), 4.82 (d, 1H, J = 8.0 Hz, H-1'), 4.76 (d, 1H, J = 10.8 Hz, CHHPh), 4.76 (d, 1H, J = 8.0 Hz, H-1''), 4.53 (d, 1H, J = 11.6 Hz, H-6), 4.48-4.34 (m, 4H, 2x $\text{CH}_2 \text{Bn}$), 4.35 (d, 1H, J = 7.6 Hz, H-1), 4.19 (dd, 1H, J = 4.4 Hz, J = 12.0 Hz, H-6), 4.12 (t, 1H, J = 8.0 Hz, H-3), 4.05 (t, 1H, J = 8.8 Hz, H-4'), 3.97 (t, 1H, J = 9.2 Hz, H-4''), 3.93 (t, 1H, J = 8.4 Hz, H-4), 3.88 (d, 1H, J = 8.4 Hz, H-5 or H-5''), 3.84 (d, 1H, J = 9.6 Hz, H-5 or H-5''), 3.71-3.64 (m, 2H, H-3, $\text{CHH N}_3(\text{CH}_2)_6\text{OH}$), 3.49-3.47 (m, 1H, H-5'), 3.20-3.14 (m, 1H, $\text{CHH N}_3(\text{CH}_2)_6\text{OH}$), 3.02 (t, 2H, J = 6.8 Hz, $\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$), 2.47-2.38 (m, 2H, $\text{CH}_2 \text{Lev}$), 2.36-2.23 (m, 2H, $\text{CH}_2 \text{Lev}$), 1.97 (s, 3H, $\text{CH}_3 \text{Lev}$), 1.26-1.17 (m, 4H, 2x $\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$), 1.14-0.96 (m, 4H, 2x $\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC, HMBC): 205.6 (C=O Lev ketone), 171.5 (C=O), 168.2 (C=O), 167.3 (C=O), 165.6 (C=O), 165.1 (C=O), 164.4 (C=O), 137.9 ($\text{C}_q \text{Carom}$), 137.8 ($\text{C}_q \text{Carom}$), 137.2 ($\text{C}_q \text{Carom}$), 135.0 ($\text{C}_q \text{Carom}$), 134.6 ($\text{C}_q \text{Carom}$), 133.4 (CH_{arom}), 133.1 (CH_{arom}), 132.9 (CH_{arom}), 129.9 ($\text{C}_q \text{Carom}$), 129.8 ($\text{C}_q \text{Carom}$), 129.7-127.2 (CH_{arom}), 100.8 (C-1''), 100.5 (C-1), 99.7 (C-1'), 80.7 (C-3'), 79.1 (C-3), 77.8 (C-4'), 77.3 (C-4''), 76.9 (C-4), 74.7 ($\text{CH}_2 \text{Bn}$), 74.6 ($\text{CH}_2 \text{Bn}$), 74.6 ($\text{CH}_2 \text{Bn}$), 74.5 (C-5 or C-5''), 74.4 (C-5 or C-5''), 73.9 (C-3''), 73.5 (C-2), 73.3 (C-2'), 72.6 (C-5'), 72.1 (C-2''), 69.3 ($\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$), 67.5 ($\text{CH}_2 \text{CO}_2\text{Bn}$), 67.2 ($\text{CH}_2 \text{CO}_2\text{Bn}$), 62.4 (C-6'), 51.2 ($\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$), 37.6 ($\text{CH}_2 \text{Lev}$), 29.5 ($\text{CH}_3 \text{Lev}$), 28.9 ($\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$), 28.5 ($\text{CH}_2 \text{Lev}$), 27.8 ($\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$), 26.1 ($\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$), 25.2 ($\text{CH}_2 \text{N}_3(\text{CH}_2)_6\text{OH}$). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{92}\text{H}_{91}\text{N}_3\text{O}_{24}\text{Na}$: 1644.58847, found 1644.58818



Benzyl (6-azido-hexyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-{2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-[benzyl (2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyluronate)]-β-D-glucopyranosyl}-β-D-glucopyranosyluronate) (36):

Trisaccharide **35** (24 mg, 0.015 mmol) was dissolved in a mixture of pyridine/AcOH (4/1, 300 μL , 0.05M) and the resulting mixture was cooled to 0 °C. Hydrazine acetate (7 mg, 0.075 mmol, 5 eq.) was added and the reaction mixture was allowed to warm to rT. After 30 min., TLC analysis showed total conversion into a slightly higher running spot (PE/EtOAc: 12/8, v/v, R_f 0.82). The reaction mixture was cooled to 0 °C and quenched with acetone. The reaction mixture was diluted with EtOAc, washed with 1M HCl, NaHCO_3 (aq., sat.), dried over MgSO_4 , filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 7/3) gave the title compound as an transparent oil which crystallized on standing in 93% yield (21 mg, 0.014 mmol). $[\alpha]_D^{20}$: +16.2 (c = 0.4, DCM). IR (neat, cm^{-1}): 3696, 303, 2986, 2307, 1732, 1603, 1558, 1452, 1421, 1398, 1373, 1263, 1217, 1179, 1157, 1096, 1070, 1028, 1001, 986, 949, 895. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC, HMBC): 8.09 (d, 2H, J = 7.8 Hz, H_{arom}), 7.91-7.86 (m, 4H, H_{arom}), 7.75 (d, 2H, J = 8.4 Hz, H_{arom}), 7.55-7.11 (m, 29 H, H_{arom}), 7.05-6.95 (m, 8H, H_{arom}), 5.25 (t, 1H, J = 8.8 Hz, H-2'), 5.15 (t, 1H, J = 8.8 Hz, H-2''), 5.04 (t, 1H, J = 8.8 Hz, H-2), 5.02 (s, 2H, $\text{CH}_2 \text{CO}_2\text{Bn}$), 5.01 (d, 1H, J = 12.0 Hz, $\text{CHHPh CO}_2\text{Bn}$), 4.96 (d, 1H, J = 12.0 Hz, $\text{CHHPh CO}_2\text{Bn}$), 4.89 (d, 1H, J = 11.2 Hz, CHHPh), 4.81 (d, 1H, J = 10.4 Hz,

CHHPh), 4.81 (d, 1H, $J = 8.4$ Hz, H-1'), 4.69 (d, 1H, $J = 7.6$ Hz, H-1''), 4.54 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.49-4.44 (m, 3H, H-6^a, 2x CHHPh), 4.43 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.40 (d, 1H, $J = 8.4$ Hz, H-1), 4.33 (dd, 1H, $J = 4.8$ Hz, $J = 11.6$ Hz, H-6^b), 4.10 (t, 1H, $J = 9.6$ Hz, H-3), 4.08 (t, 1H, $J = 9.6$ Hz, H-4'), 3.95 (t, 1H, $J = 10.0$ Hz, H-4), 3.92 (d, 1H, $J = 10.8$ Hz, H-5), 3.80-3.68 (m, 4H, H-3'', H-4'', H-5'', CHH N₃(CH₂)₆OH), 3.63 (t, 1H, $J = 8.8$ Hz, H-3'), 3.49-3.46 (m, 1H, H-5'), 3.21-3.15 (m, 1H, CHH N₃(CH₂)₆OH), 3.02 (t, 2H, $J = 6.8$ Hz, CH₂ N₃(CH₂)₆OH), 1.28-1.24 (m, 4H, 2x CH₂ N₃(CH₂)₆OH), 1.23-1.08 (m, 4H, 2x CH₂ spacer). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC, HMBC): 168.1 (C=O CO₂Bn), 167.6 (C=O CO₂Bn), 165.8 (C=O Bz or CO₂Bn), 165.7 (C=O or CO₂Bn), 165.1 (C=O Bz), 164.4 (C=O Bz), 138.0-137.5 (C_q C_{arom}), 135.0-134.8 (C_q C_{arom}), 133.5-132.8 (CH_{arom}), 130.0-129.4 (CH_{arom}), 128.9-128.7 (C_q C_{arom}), 128.6-127.2 (CH_{arom}), 100.8 (C-1''), 100.5 (C-1), 100.0 (C-1'), 80.6 (C-3'), 79.6 (C-3''), 79.4 (C-3), 77.8 (C-4'), 76.9 (C-4), 74.9 (CH₂ Bn), 74.9 (C-4''), 74.8 (CH₂ Bn), 74.6 (CH₂ Bn), 74.5 (C-5 and C-5''), 74.1 (C-2''), 73.5 (C-2), 73.2 (C-2'), 72.8 (C-5), 69.3 (CH₂ N₃(CH₂)₆OH), 67.4 (CH₂ CO₂Bn), 67.2 (CH₂ CO₂Bn), 62.6 (C-6'), 51.2 (CH₂ N₃(CH₂)₆OH), 28.9 (CH₂ N₃(CH₂)₆OH), 28.5 (CH₂ N₃(CH₂)₆OH), 26.2 (CH₂ N₃(CH₂)₆OH), 25.2 (CH₂ N₃(CH₂)₆OH). HRMS: [M+Na]⁺ calcd for C₈₇H₈₅N₃O₂₂Na: 1546.55169, found 1546.55288.



6-Aminoethyl 3-O-(4-O-(β-D-glucopyranosyluronic acid)-β-D-glucopyranosyl)-β-D-glucopyranosyluronic acid (37): Trisaccharide **36** (61 mg, 40 μmol) was dissolved in dioxane (800 μL, 0.05M) followed by addition of KOH

(0.5M KOH in H₂O, 0.96 mL, 0.48 mmol, 12 eq.). After stirring overnight, more KOH was added portionwise (48 μL, 0.26 mmol, 6 eq., then 24 μL, 0.13 mmol, 3 eq., then 24 μL, 0.13 mmol, 3 eq.). After 6 days, LC-MS analysis confirmed that the esters were saponified. The reaction mixture was neutralized with H⁺ Amberlite, filtered, and concentrated. Size exclusion chromatography (DCM/MeOH: 1/1) gave a white solid, which was dissolved in H₂O/BuOH mixture (1/2, v/v, 500 μL, 0.08M). A drop of 1M HCl (aq.) was added followed by addition of Pd/C. The reaction mixture was purged with H₂ gas and stirred overnight. The reaction mixture was filtered over a Whatman filter and concentrated. The obtained residue was subjected to gel filtration (HW-40, 0.15M NH₄HCO₃ in H₂O) and subsequent lyophilisation. The deprotected trimer was obtained as a fine white powder in 28% yield over 2 steps (7 mg, 11 μmol). ¹H NMR (600 MHz, D₂O, 283 K, HH-COSY, HSQC, HMBC): 4.77 (d, 1H, $J = 7.8$ Hz, H-1'), 4.46 (d, 1H, $J = 8.4$ Hz, H-1''), 4.44 (d, 1H, $J = 7.8$ Hz, H-1), 3.94 (d, 1H, $J = 11.4$ Hz, H-6^a), 3.90-3.80 (m, 1H, CHH N₃(CH₂)₆OH), 3.77-3.68 (m, 4H, H-3, H-4', H-5, H-6^b), 3.65-3.62 (m, 2H, H-3'', CHH N₃(CH₂)₆OH), 3.60-3.55 (m, 2H, H-5', H-5''), 3.48-3.44 (m, 3H, H-2, H-3', H-4), 3.35-3.31 (m, 2H, H-2', H-2''), 2.94 (t, 2H, $J = 7.2$ Hz, CH₂ N₃(CH₂)₆OH), 1.6-1.58 (m, 4H, 2x CH₂ N₃(CH₂)₆OH), 1.37-1.36 (m, 4H, 2x CH₂ N₃(CH₂)₆OH). ¹³C APT NMR (150 MHz, D₂O, HH-COSY, HSQC, HMBC): 176.7 (C=O), 176.6 (C=O), 103.4 (C-1'), 103.3 (C-1''), 102.9 (C-1), 84.3 (C-3 or C-4'), 79.9 (C-5' or C-5''), 77.2 (C-5), 76.8 (C-3 or C-4'), 76.2 (C-2 or C-3'), 75.8 (C-5' or C-5''), 75.1 (C-3''), 74.1 (C-2''), 73.9 (C-4), 76.9 (C-2'), 72.7 (C-2 or C-3'), 71.7 (CH₂ N₃(CH₂)₆OH), 71.2 (C-4''), 61.0 (C-6), 40.4 (CH₂ N₃(CH₂)₆OH), 29.4 (CH₂ N₃(CH₂)₆OH), 27.6 (CH₂ N₃(CH₂)₆OH), 26.1 (CH₂ N₃(CH₂)₆OH), 25.4 (CH₂ N₃(CH₂)₆OH). HRMS: [M+Na]⁺ calcd for C₂₄H₄₁NO₁₈Na: 654.22158, found 654.22136

References and notes

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11. The *S*-tolyl acceptors **6** and **18** were obtained *en route* to donors **5** and **1**, respectively.
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15. A competition experiment with donor **5** and tolyl 4,6-*O*-benzylidene-2,3-di-*O*-benzyl-1-thio- β -D-glucopyranoside (Donor **4** in Chapter 2) revealed that donor **5** was 19 times more reactive, where donor 6-Bz **22** was threefold less reactive than this thioglycoside.
16. Synthesis of the corresponding imidate donor **24** from thioglucoside **22** was according to the protocol used for donors **10** and **12**.
17. The anomerization of thio-glycosides in the presence of Lewis acids has been reported before. See for example: a) Connolly, D.T., Roseman, S., Lee, Y.C., *Carbohydr. Res.*, **1980**, *87*, 227-239; b) Satoh, H., Manabe, S., Ito, Y., Lüthi, H.P., Laino, T., Hutter, J., *J. Am. Chem. Soc.*, **2011**, *133*, 5610-5619.
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Chapter 4

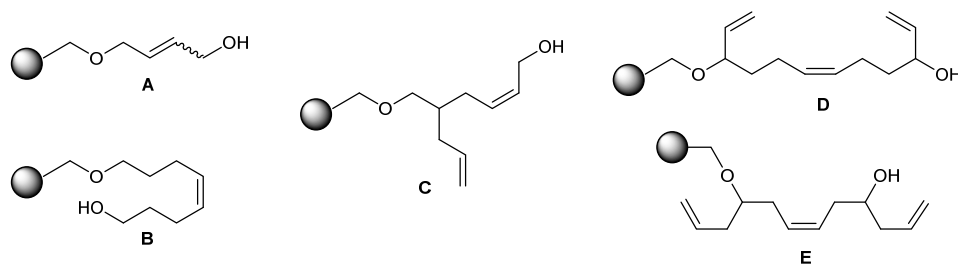
A second-generation tandem RCM cleavable linker for solid phase oligosaccharide synthesis

Introduction

Carbohydrates are involved in many fundamental biological processes, but in many cases relatively little is known about their exact modes of action at molecular level.¹ Well-defined oligosaccharides have been recognized as crucial tools to study the mechanism of these processes, but the synthesis of oligosaccharides is still complex and time consuming. Carbohydrates possess several similar hydroxyl functionalities that need to be addressed independently for assembly of the oligomers. Moreover, the glycosidic bond can have two configurations and oligosaccharide chains can be branched, contributing to even more synthetic complexity.² Whereas oligopeptides³ and oligonucleotides⁴ can now be obtained using (automated) solid phase techniques, the development of such a method for carbohydrates is significantly more challenging due to their structural complexity.⁵ Nevertheless, considerable progress has been made in the development of (automated) solid phase synthesis techniques to access complex oligosaccharides.⁶

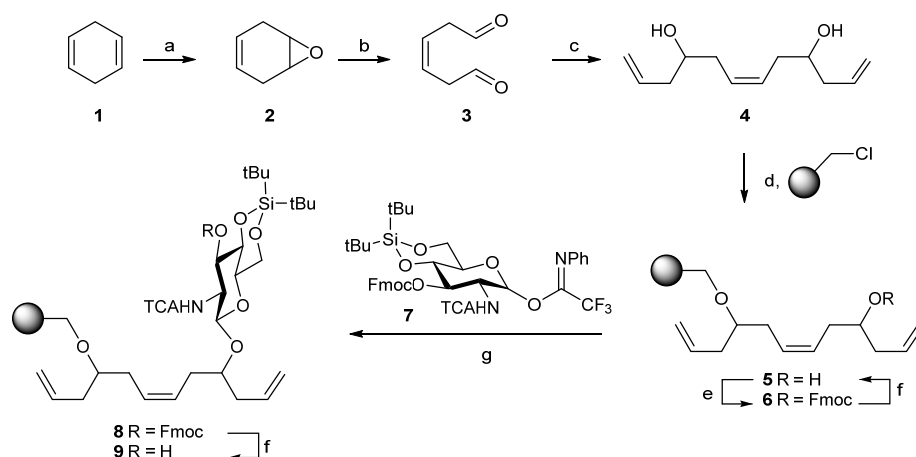
Notable recent achievements include the synthesis of a branched sialic acid-containing complex-type *N*-glycan octasaccharide,⁷ an all-*cis*-linked pentaglucan,⁸ the automated synthesis of β -mannuronic acid alginate fragments,⁹ featuring up to 12 *cis*-mannosidic linkages, the automated synthesis of glycosaminoglycan fragments¹⁰ and the assembly of a polymannoside composed of 30 monomers in length.¹¹ To allow for the synthesis of more complex oligosaccharides on the solid support, new synthetic tools and methods, including glycosylation reactions, new protecting groups, and linker systems have to be developed.

The linker moiety, by which the first building block is connected to the solid support, is key to the success of a solid phase synthesis campaign.¹² A prerequisite is that the linker is stable to all reaction conditions employed during the assembly of the oligomers and the anchoring functionality has to be effectively cleaved at the end of the solid phase synthesis. Over the years various linker systems have been developed for the (automated) solid phase synthesis of oligosaccharides,¹³ including a hydrogenolysis-labile linker,¹⁴ acid¹⁵- and base-labile linkers,¹⁶ silyl ether based systems,¹⁷ photo-cleavable anchors¹⁰ and thioglycoside based linker systems.¹⁸ Olefin based linkers, which can be selectively cleaved using a metathesis reaction, are attractive since they are stable to both the (Lewis) acidic glycosylation conditions and the basic conditions required to remove temporary protecting groups. The metathesis linker systems developed to date are shown in Figure 1 and include: i) 1,4-butanediol linker **A**,¹⁹ which can be cleaved in a cross metathesis event using ethylene gas to provide the target oligosaccharides with an allyl moiety at their reducing end; ii) octenediol linker **B**,¹⁹ which delivers pentenyl oligosaccharides in an analogous process; iii) ring-closing metathesis (RCM) linker **C**,²⁰ which can be cleaved without the use of an additional olefin; and iv) tandem RCM linker **D**,²¹ that allows for the release of the target compounds through a double RCM sequence. Recently the first linker system (**A**) was successfully applied to the automated solid phase synthesis of a set of β -mannuronic acid alginates⁹ and a collection of hyaluronic acid (HA)²² oligomers. Drawbacks of this system are the relatively slow release of the target structures from the resin (typically, an overnight reaction is required) and the necessity to repeat the cleavage at least once to ensure optimal recovery of the products from the resin. In addition it was found that the reactive primary allylic alcohol system in **A** was not compatible with relatively unreactive donor glycoside building blocks, such as the glucuronic acid donor synthons employed in the assembly of the HA fragments. Here a substantial amount of acyl migration occurs during the glycosylation reaction leading to a complex mixture on the support. To circumvent these drawbacks a new tandem ring closing metathesis cleavable linker (**E**) has been designed. This linker system allows rapid liberation of the solid support bound oligomers without the need for any additives. Linker **E** represents an improvement to the reported tandem RCM linker **D** because the latter provides a set of diastereomeric glycosides upon cleavage, where linker **E** generates a symmetrical cyclopentene moiety at the reducing end of the target structures. This Chapter shows the application of linker system **E** in two solid phase synthesis sequences. Both syntheses are directed at the assembly of HA oligomers, the structurally simplest member of the glycosaminoglycan superfamily and a major constituent of the extracellular matrix of mammalian cells.²³

Figure 1. Metathesis cleavable linker systems for solid phase oligosaccharide synthesis.

Results and Discussion

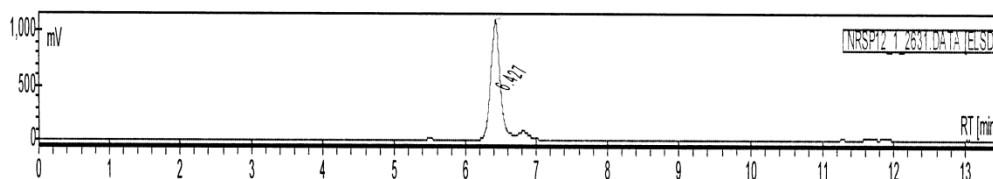
The preparation of tandem RCM linker system **E** started with the synthesis of triene **4** from cyclohexa-1,4-diene (**1**) as depicted in Scheme 1. Diene **1** is selectively oxidized to provide the monoepoxide **2**. This crude epoxide was used in the next step in which the epoxide ring in **2** was opened under oxidative conditions to provide the crude dialdehyde **3**.²⁴ Direct subjection of this labile compound to a double Grignard reaction with allylmagnesium bromide gave diol **4** in 8% yield over the three steps. This low yield can be explained by the lability of the dialdehyde **3**, which can engage in various (self-) condensation reactions. Because diol **4** could be readily purified from the crude reaction mixture, obtained in good quantities for further studies and the used chemicals are readily available at low cost, no attempts were made to further optimize the procedure. Merrifield resin (loading 0.75 mmol/g) was functionalized with diol **4**, by shaking the resin in the presence of KO^tBu, 18-crown-6 and tetrabutylammonium iodide (TBAI) for three days. Capping of the unreacted chlorides on the resin was achieved by subsequent treatment of the resin with a large excess of potassium methoxide (KOMe). The loading of resin **5** was determined by Fmoc quantification after treatment of the resin with 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl). The loading was established to be 0.6 mmol/g. In a parallel approach resin **5** was glycosylated with glucosamine imidate **7** (3x 3 equivalents), bearing a Fmoc group at its C-3-hydroxyl group. Fmoc quantification of the resulting resin (**8**) corroborated the loading found by the above described “direct” Fmocylation protocol.

Scheme 1: Synthesis of the triene linker system and determination of the loading on resin.

Reagents and conditions: (a) *m*-CPBA, K_2HPO_4 , DCM/ H_2O , 0 °C; (b) Periodic acid, H_2O /dioxane, 0 °C; (c) AllMgBr (1M in Et_2O), THF, 0 °C, 8%/3 steps; (d) KOtBu, 18-crown-6, TBAI, THF, 3 d., (ii) KOMe, 1d., 80%; (e) Fmoc-Cl, pyridine, DCM; (f) 20% piperidine/DMF, loading of **5** and **9**: 0.6 mmol/g; (g) TFOH, DCM, 0 °C (3x).

Having resin **5** in hand, its application in solid phase oligosaccharide synthesis was explored. Two frame shifted HA oligomers (Scheme 2) were chosen as model targets. First, a sequence of reactions that was proven to be successful with butene-1,4-diol linker **A** was studied. Thus, resin **5** was treated first with monomeric glucosamine *N*-phenyltrifluoroacetimidate donor **10**,²⁵ bearing a trichloroacetamide group to mask the C-2 amine functionality, a C-4,6-di-*tert*-butylsilylidene ketal to mask the C-4 and C-6 hydroxyls and a levulinoyl ester as a temporary protecting group to mask the C-3 hydroxyl, which is to be glycosylated in the next glycosylation event. The glycosylation was promoted by a catalytic amount of trifluoromethanesulfonic acid (TfOH) and the reaction was repeated two times to ensure a complete glycosylation. After cleavage of the levulinoyl group using hydrazine acetate in an acetic acid/pyridine mixture, the resin-bound monosaccharide **13** was elongated with disaccharide building block **11**, featuring a glucuronic acid donor moiety. After removal of the levulinoyl group from the trisaccharide, an aliquot of resin (**15**) was taken and subjected to a catalytic amount of Grubbs second-generation catalyst in the presence of trichloroacetimide. The latter additive was added to prevent potential dehalogenation from the TCA groups.²² After a cleavage reaction of three hours, the resulting mixture was analyzed by LC-MS to reveal the presence of HA trisaccharide product **22** without any deletion sequence products. Here, the value of the new linker system becomes apparent, in that a small aliquot of resin can be readily and rapidly cleaved to inform on the progress of the synthesis. After the ring closing metathesis reaction of 3 hours the resin was subjected to another RCM event, but this did not lead to the isolation of more product. Encouraged by the successful assembly of the resin bound trisaccharide (**15**), the synthesis was continued by elongation with disaccharide synthon **11** and subsequent delevulinoylation. A single three-hour cleavage event led to the release of target pentasaccharide **23** from the resin. After filtration of the resin and purification of the product over a short plug of silica the protected HA-pentasaccharide **23** was obtained in 63% yield over the seven solid phase steps, equaling 94% per step. This pentasaccharide was

Figure 2. LC-MS trace of crude disaccharide **24** after cleavage of an aliquot of resin **18** (C4 column, linear gradient 50 → 90% in 13.5 min.).



Conclusions

A new tandem RCM cleavable linker system for solid phase oligosaccharide synthesis has been prepared and evaluated in the synthesis of two frame-shifted hyaluronic acids fragments. The linker can be cleaved without the use of alkene additives and the reaction time required for the release of the compounds from the resin is significantly reduced with respect to the cross metathesis linker reported by Walvoort *et al.*²² As a result aliquots of resin can be interrogated efficiently, which can provide valuable additional information on the progress of the solid phase synthesis.

Experimental section

General experimental procedures. Chemical shifts (δ) are given in ppm relative to TMS as internal standard. All ^{13}C -APT spectra are proton decoupled. Reactions were performed at rT unless stated otherwise and were followed by TLC analysis with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulphuric acid in EtOH or with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g L⁻¹), followed by charring at 150 °C. Flash column chromatography was performed on silica gel (0.04-0.063 nm) and size exclusion chromatography (SEC) was performed on Sephadex™ LH-20. Experiments that required an inert atmosphere were carried out under dry argon. Dichloromethane (p.a.) was distilled over P₂O₅ prior to use. Molecular sieves (3Å) were flame-dried before use. LC-MS analysis was performed using a C4 column with the following solvent systems: A = 100% water, B = 100% acetonitrile, C = 1% TFA.

General experimental procedures for solid phase synthesis. The synthesizer instrument used was supplied by Ancora Pharmaceuticals. The synthesizer's solvent bottles are filled with commercially acquired solvents, which are pre-dried 24 h. before use on 4Å molecular sieves. The solutions containing building block, activator and deblocking reagents are freshly prepared directly before use with pre-dried solvents. During the coupling, deblocking, and reduction steps the reagent solutions were added manually.

Reagent solutions:

Building block solution: Donor **10** or **11** in DCM (0.09M)

Activator solution: Trifluoromethanesulfonic acid in DCM (0.09M)

Deblocking solution: Hydrazine acetate in pyridine/AcOH (4/1, v/v, 0.12M)

Protocol A: Agitation of the resin during washing. After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the reaction vessel (RV) for 15 sec. to agitate the resin suspension. Then the RV is emptied.

Protocol B: Agitation of the resin during reaction. After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the RV for 10 sec. to agitate the resin suspension. Then the purging is halted and the suspension is allowed to settle for 20 sec.

Protocol C: Swelling of new resin. The RV is charged with dry resin. The resin is washed with DCM (3x), alternating THF and hexane (3x), THF (1x) and DCM (3x). Every wash step involves protocol A.

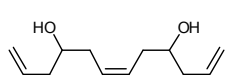
Protocol D: Washing of the resin before or after the reaction. If applicable, the chiller is set to ambient temperature. The pre-swollen resin is washed with alternating THF and hexane (3x), followed by THF (1x) and DCM (3x). Every wash step involves protocol A.

Protocol E: Coupling cycle. The resin is washed with DCM (3x) before the building block solution (1.5 mL, 0.09M) is added. Then, the temperature is set to -5 °C to ensure an actual temperature of 0 °C in the RV. Simultaneously a pause of 10 min. is started. When the temperature of the chiller has reached its target point, the activator solution (300 µL) is added. Protocol B is applied for 30 min. Then the RV is emptied and the solution is collected in a mixture of DCM/H₂O/TEA (50/5/1, v/v/v). The resin is washed with DCM (3x) and the solutes are similarly collected.

Protocol F: Deblocking. The resin is washed with DMF (3x) before the deblock solution (2.5 mL, 0.12M) is added. The temperature is raised to 40 °C and the resin is agitated using protocol B for 10 min. Then the RV is emptied and the resin is washed with DMF (3x).

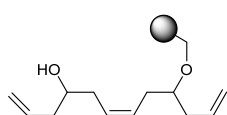
Protocol G: Washing of the resin after deblocking. The temperature of the chiller is set to ambient. The resin is successively washed with DMF (3x), DCM (3x), alternating THF and hexane (6x), 0.01M AcOH in THF (6x) and THF (3x). Every wash step involves protocol A.

Protocol H: Suspending of the resin for isolation: The resin is washed with alternating DCM and MeOH (2x), followed by a mixture of DCM/MeOH (7/1, v/v, 2x), both employing protocol A. Then a mixture of DCM/MeOH (7/1, v/v) is added, the resin is agitated for 15 sec. after which time the gas-flow was halted and the program was paused. The suspended resin is isolated and this last procedure is repeated two times.

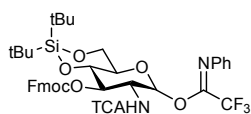


(Z)-dodeca-1,6,11-triene-4,9-diol (4): 1,4-Cyclohexadiene (4 mL, 42 mmol) was dissolved in a mixture of DCM/H₂O (151/1, v/v, 300 mL, 0.14M). The reaction mixture was cooled to 0 °C followed by addition of K₂HPO₄ (7.46 g, 42.8 mmol, 1.02 eq.) and *m*-CPBA (7.40 g, 42.8 mmol, 1.02 eq.). The reaction mixture was allowed to warm to rT and stirred overnight. A white suspension was obtained. The reaction mixture was washed with NaHCO₃ (aq., sat., 2x), dried over MgSO₄, filtered, and concentrated. A colorless oil was obtained, which was dissolved in dioxane (105 mL, 0.4M). The reaction mixture was cooled to 0 °C, followed by addition of a periodic acid solution (0.8M in H₂O, 53 mL, 42 mmol, 1 eq.). The reaction mixture was allowed to warm to rT. TLC analysis showed total conversion into a lower running spot (PE/EtOAc: 1/1, v/v, R_f 0.3). The reaction mixture was diluted with DCM, followed by separation of the layers. The organic layer was washed with NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. A yellow oil was obtained, which was directly dissolved in THF (140 mL, 0.3M). The reaction mixture was cooled to 0 °C, followed by addition of allylmagnesium bromide (1M in Et₂O, 96.6 mL, 96.6 mmol, 2.3 eq.). Precipitation of salts was observed. TLC analysis showed total conversion into a slightly higher running spot (PE/EtOAc: 12/8, v/v, R_f 0.3). The reaction mixture was slowly quenched with NH₄Cl (aq., sat.) until the reaction mixture turned transparent. The reaction mixture was diluted with Et₂O, followed by separation of the layers. The organic layer was washed

with NH_4Cl (aq., sat.), dried over MgSO_4 , filtered, and concentrated. A brown oil was obtained. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 7/3) gave the diol **4** as a colorless oil in 8% yield over 3 steps (659 mg, 3.36 mmol). IR (neat, cm^{-1}): 3417, 3346, 2927, 1722, 1705, 1436, 1406, 1315, 1265, 1178, 1043, 997, 918, 918, 736, 705. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 5.87-5.78 (m, 2H, H-2, H-11), 5.69-5.58 (m, 2H, H-6, H-7), 5.18-5.12 (m, 4H, H-1, H-12), 3.77-3.66 (m, 2H, H-4, H-9), 2.46 (bs, 2H, OH), 2.41-2.20 (m, 8H, H-3, H-5, H-8, H-10). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 134.8 (C-2, C-11), 128.8 (C-6 or C-7), 128.3 (C-6 or C-7), 118.1 (C-1 or C-12), 118.12 (C-1 or C-12), 70.3 (C-4 or C-9), 70.2 (C-4 or C-9), 41.9 (C-3 or C-5 or C-8 or C-10), 41.4 (C-3 or C-5 or C-8 or C-10), 34.7 (C-3 or C-5 or C-8 or C-10), 34.3 (C-3 or C-5 or C-8 or C-10). HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{12}\text{H}_{21}\text{O}_2$: 197.15361, found 197.15361.



10-polystyrenyloxy-(Z)-dodeca-1,6,11-triene-4-ol (5): Merrifield peptide resin 1% cross linked (5.04 g, 0.75 mmol/g, 3.78 mmol) was washed and swollen in THF (3x 60 mL, 20 min.) in a frit. The frit was purged with argon. (Z)-dodeca-1,6,11-triene-4,9-diol (1.10 g, 5.6 mmol) was dissolved in THF (40 mL, 0.14M) and cooled to 0 °C, followed by addition of KOtBu (0.63 g, 5.9 mmol, 1.05 eq. relative to diol). The reaction mixture turned brown and was stirred for 1 h. Then, the alkoxide solution was transferred to the frit with resin. 18-crown-6 (98 mg, 0.37 mmol, 0.1 eq.) and TBAI (137 mg, 0.37 mmol, 0.1 eq.) were added and the frit was shaken for 3 days. KOMe (1.73 g, 24.64 mmol, 6.6 eq.) was added and the frit was shaken for another 24 h. The resin was subsequently washed with: MeOH (250 mL, 5x), THF (250 mL, 3x), THF/MeOH (10/1, v/v, 250 mL, 3x), MeOH (250 mL, 3x), THF (250 mL, 3x), THF/*t*-BuOH (10/1, v/v, 250 mL, 3x), THF (250 mL, 3x), DCM (250 mL, 8x). The resin was dried in a vacuum oven at 45 °C overnight. Dry resin was obtained (5.57 g).



4,6-*O*-Di-*tert*-butylsilylidene-3-*O*-(*N*-[phenyl]trifluoroacetimidoyl)-2-*N*-trichloroacetamido- α -D-glucopyranoside (7): 4,6-*O*-Di-*tert*-butylsilylidene-1-*O*-(*N*-[phenyl]trifluoroacetimidoyl)-2-*N*-trichloroacetamido- α -D-glucopyranoside (642 mg, 0.75 mmol)²² was dissolved in DCM (5.34 mL, 0.14M) followed by addition of Fmoc-Cl (349 mg, 1.35 mmol, 1.8 eq.) and pyridine (0.6 mL, 7.5 mmol, 10 eq.). After 30 min., TLC analysis (Hexane/EtOAc: 9/1, v/v, R_f 0.9) showed total conversion into a higher running spot. The reaction mixture was diluted with EtOAc, washed with H_2O , brine, dried over MgSO_4 , filtered, and concentrated. A yellowish oil was obtained. Column chromatography (Hexane/EtOAc: 1/0 $\rightarrow$ 19/1) gave the title compound in 98% yield (630 mg, 0.73 mmol) as a foam. $[\alpha]_D$: -69 (c = 1.5, DCM). IR (neat, cm^{-1}): 2933, 1722, 1514, 1473, 1450, 1388, 1344, 1315, 1267, 1209, 1161, 1114, 1087, 1060, 1002, 966, 943, 906, 887, 825, 758, 729, 692, 651, 621. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.79-7.73 (m, 2H, H_{arom}), 7.57-7.53 (m, 2H, H_{arom}), 7.39-7.33 (m, 2H, H_{arom}), 7.30-7.26 (m, 3H, H_{arom}), 7.20-7.18 (d, 1H, J = 7.6 Hz, H_{arom}), 7.12-7.09 (m, 1H, H_{arom}), 6.79 (d, 2H, J = 7.2 Hz, H_{arom}), 6.46 (bs, 1H, H-1), 5.15 (t, 1H, J = 10.0 Hz, H-3), 4.49-4.39 (m, 2H, H-2, CHH Fmoc), 4.33-4.27 (m, 2H, CHH Fmoc, CH Fmoc), 4.22-4.20 (m, 1H, H-6a), 4.14 (t, 1H, J = 8.8 Hz, H-4), 4.03-3.93 (m, 2H, H-5, H-6b), 1.09 (s, 9H, *tert*-butyl silylidene), 1.00 (s, 9H, *tert*-butyl silylidene). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 162.1 (C=O), 155.9 (C=O), 143.3-141.1 (C_q C_{arom}), 129.2-128.5 (CH_{arom}), 128.0-127.1 (CH_{arom}), 125.1-124.8 (CH_{arom}), 120.4 (CH_{arom}), 120.1 (CH_{arom}), 119.2 (CH_{arom}), 93.6 (C-1), 75.9 (C-3), 74.0 (C-4), 70.8 (CH_2 Fmoc), 69.0 (C-5), 66.0 (C-6), 53.8 (C-2), 46.4 (CH Fmoc), 27.3 (CH_3 *tert*-butyl silylidene), 26.7 (CH_3 *tert*-butyl silylidene), 22.6 (C_q *tert*-butyl silylidene), 19.9 (C_q *tert*-butyl silylidene). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{39}\text{H}_{42}\text{Cl}_3\text{F}_3\text{N}_2\text{O}_8\text{SiNa}$: 879.16203, found 879.16281.

Fmoc loading assays:

Fmoc loading assay A (Scheme 1, conditions *e* and *f*): Functionalized resin **5** (100 mg) was suspended in DCM (3 mL) in a frit, followed by addition of pyridine (60 μ L) and Fmoc-Cl (100 mg, 0.387 mmol). The frit was shaken for 3 days. Then, the resin was washed with alternating MeOH (5 mL) and DCM (5 mL) ten times, followed by washing with DCM (5 mL, 3x). Then the resin was dried in a vacuum oven overnight. The resin was dissolved in 20% piperidine in DMF (3 mL) and shaken for 2 h. 100 μ L of this sample was taken and diluted to 10 mL with 20% piperidine in DMF. The absorbance was read at 301 nm and a loading of 0.6 mmol/g was obtained.

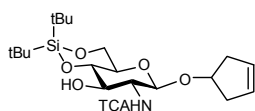
Loading calculation: (Extinction coefficient = 7800);

Loading (mmol/g) = $[(\text{Abs}_{301}/7800) \times 0.01 \text{ L} \times [3 \text{ mL}/0.1 \text{ mL}] \times 1000]/0.1 \text{ g}$.

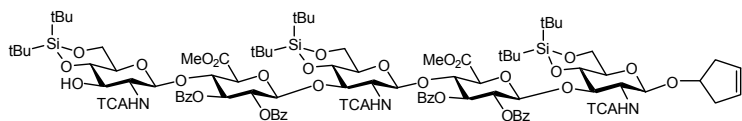
Fmoc loading assay B (Scheme 1, conditions *g* and *f*): The RV was charged with functionalized Merrifield polystyrene **5** (100 mg) and prepared for the coupling with Fmoc-functionalized donor **7** using protocol C. Then, the coupling cycle was run to couple monosaccharide donor **7**. After the synthesis was complete, protocol H was used to isolate the resin. The obtained resin was suspended in 20% piperidine in DMF (3 mL) for 2 h. 100 μ L of this sample was taken and diluted to 10 mL with 20% piperidine in DMF. The absorbance was read at 301 nm and a loading of 0.6 mmol/g was determined.

Loading calculation: (Extinction coefficient = 7800);

Loading (mmol/g) = $[(\text{Abs}_{301}/7800) \times 0.01 \text{ L} \times [3 \text{ mL}/0.1 \text{ mL}] \times 1000]/0.1 \text{ g}$.

**Cyclopentenyl 4,6-di-*tert*-butylsilylidene-2-*N*-trichloroacetamido- β -D-glucopyranoside (**7a**):**

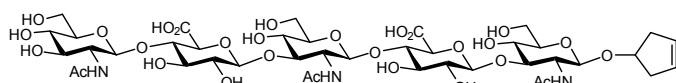
The resin used in the Fmoc assay (resin **9**) was washed with DCM (5x), suspended in DCM (6 mL, 0.01M) followed by addition of trichloroacetamide (52 mg, 0.32 mmol, 46 eq. relative to Grubbs catalyst). The solution was purged with argon, followed by addition of Grubbs second-generation catalyst (6 mg, 7 μ mol, 0.12 eq.). After 3 h., the reaction mixture was filtered and concentrated. Column chromatography (hexane/EtOAc: 8/2) gave the title compound as a white solid in 91% yield (28 mg, 52.7 μ mol). $[\alpha]_D$: -13 (c = 0.5, DCM). IR (neat, cm^{-1}): 2931, 2858, 1691, 1531, 1471, 1161, 1072, 1006, 767, 750, 650. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.08 (d, 1H, J = 6.8 Hz, NH), 5.68-5.62 (m, 1H, H-3 cyclopentenyl), 5.61-5.60 (m, 1H, H-3 cyclopentenyl), 5.09 (d, 1H, J = 8.4 Hz, H-1), 4.54-4.51 (m, 1H, H-1 cyclopentenyl), 4.24 (t, 1H, J = 10.4 Hz, H-3), 4.19 (dd, 1H, J = 10.0 Hz, J = 4.8 Hz, H-6a), 3.94 (t, 1H, J = 10.4 Hz, H-6b), 3.68 (t, 1H, J = 8.8 Hz, H-4), 3.52-3.48 (m, 1H, H-5), 3.32-3.25 (m, 1H, H-2), 2.86 (bs, 1H, OH), 2.58-2.45 (m, 3H, 3xH-2 cyclopentenyl), 2.35-2.31 (m, 1H, H-2 cyclopentenyl), 1.52 (s, 9H, *tert*-butyl silylidene), 1.44 (s, 9H, *tert*-butyl silylidene). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 162.0 (C=O TCA), 128.4 (C-3 cyclopentenyl), 127.7 (C-3 cyclopentenyl), 98.0 (C-1), 79.0 (C-1 cyclopentenyl), 77.9 (C-4), 71.7 (C-3), 70.2 (C-5), 66.1 (C-6), 59.0 (C-2), 40.1 (C-2 cyclopentenyl), 39.3 (C-2 cyclopentenyl), 27.4 (CH_3 *tert*-butyl silylidene), 26.9 (CH_3 *tert*-butyl silylidene), 22.7 (C_q *tert*-butyl silylidene), 19.9 (C_q *tert*-butyl silylidene). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{21}\text{H}_{34}\text{Cl}_3\text{NO}_6\text{SiNa}$: 552.11132, found 552.11109.



Pentamer (23**):** The RV was charged with functionalized Merrifield polystyrene **5** (75 mg, 45 μ mol) and prepared for the

synthesis using protocol C. Then, the coupling/deprotection cycle was run to couple first monosaccharide donor **10**, and then repeated two times with disaccharide donor **11**. After the synthesis was complete, protocol H was used to isolate the resin. The resin was washed with dry DCM (5x),

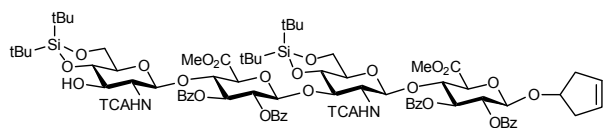
suspended in DCM (4.5 ml, 0.01M) followed by addition of trichloroacetamide (40 mg, 0.28 mmol, 46 eq. relative to Grubbs cat.). The suspension was purged with argon, followed by addition of Grubbs second-generation catalyst (4.6 mg, 5.4 μ mol, 0.12 eq.). After 3 h., the reaction mixture was filtered and concentrated. The brown residue was filtered over a plug of silica (Hexane/EtOAc: 8/2, v/v) and concentrated. A white solid was obtained (69 mg). LC-MS analysis showed the presence of pentamer. The residue was purified by SEC (DCM/MeOH, 1/1), which afforded pentameric hyaluronic acid in 63% yield over 7 steps (63 mg, 28.4 μ mol). $[\alpha]_D$: -39 (c = 0.1, ACN). IR (neat, cm^{-1}): 2860, 2555, 2156, 1732, 1716, 1521, 267, 1161, 1161, 1028, 825, 711, 626. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.92-7.87 (m, 8H, H_{arom}), 7.55-7.49 (m, 4H, H_{arom}), 7.48-7.33 (m, 8H, H_{arom}), 7.10 (d, 1H, J = 7.6 Hz, NH), 6.83 (d, 2H, J = 7.6 Hz, 2x NH), 5.65-5.64 (m, 1H, H-3 cyclopentenyl), 5.61-5.51 (m, 3H, H-3 cyclopentenyl, 2x H-3 GlcA), 5.37 (d, 1H, J = 4.0 Hz, H-1 GlcA), 5.03 (t, 1H, J = 9.2 Hz, H-2 GlcA), 5.19 (d, 1H, J = 5.6 Hz, H-1 GlcA), 5.11 (d, 1H, J = 8.8 Hz, H-1 GlcN), 5.10 (d, 1H, J = 8.8 Hz, H-1 GlcN), 4.95 (d, 1H, J = 8.8 Hz, H-1 GlcN), 4.48-4.43 (m, 1H, H-A cyclopentenyl), 4.43 (t, 1H, J = 8.8 Hz, H-4 GlcN), 4.37 (t, 1H, J = 8.8 Hz, H-4 GlcA), 4.30 (t, 1H, J = 9.6 Hz, H-4 GlcA), 4.18-4.16 (m, 2H, H-5 GlcA , H-6a GlcN), 4.12-4.05 (m, 2H, H-5 GlcN , H-5 GlcA), 3.93-3.89 (m, 2H, H-3 GlcN , H-6b GlcN), 3.82 (s, 3H, CO_2CH_3), 3.82 (s, 3H, CO_2CH_3), 3.69 (t, 1H, J = 8.8 Hz, H-3 GlcN), 3.61-3.57 (m, 3H, H-2 GlcN , H-4 GlcA , H-6a GlcN), 3.53 (dd, 1H, J = 10.4 Hz, J = 4.8 Hz, H-6a GlcN), 3.47-3.37 (m, 3H, H-2 GlcN , H-3 GlcN , H-5 GlcN), 2.73 (bs, 1H, OH), 2.65-2.60 (m, 2H, 2x H-6b GlcN), 2.60-2.44 (m, 3H, 3x H-2 cyclopentenyl), 2.31-2.29 (m, 1H, 1x H-2 cyclopentenyl), 1.04 (s, 9H, *tert*-butyl silylidene), 1.02-0.91 (m, 36H, 4x *tert*-butyl silylidene), 0.77 (s, 9H, *tert*-butyl silylidene). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 170.9 (C=O, CO_2Me), 169.5 (C=O, CO_2Me), 165.8 (C=O Bz), 165.4 (C=O Bz), 165.3 (C=O Bz), 165.1 (C=O Bz), 162.4 (C=O TCA), 161.8 (C=O TCA), 161.4 (C=O TCA), 133.5-133.0 (CH_{arom}), 130.0 (C_q C_{arom}), 129.9-129.6 (CH_{arom}), 128.9 (C_q C_{arom}), 128.5-127.7 (CH_{arom} and H-3 cyclopentenyl), 100.3 (C-1 GlcN), 99.8 (H-1 GlcA), 99.6 (C-1 GlcA), 99.4 (C-1 GlcN), 98.0 (C-1 GlcN), 92.7 (2x C_q TCA), 92.4 (C_q TCA), 79.1 (C-1 cyclopentenyl), 78.3 (C-5 GlcN), 77.2 (C-4 GlcN), 77.2 (C-4 GlcN), 76.3 (C-4 GlcA), 75.9 (C-4 GlcN), 75.8 (C-4 GlcA), 75.7 (C-3 GlcN), 74.9 (C-3 GlcN), 74.8 (C-2 GlcN), 74.5 (C-5 GlcN), 74.2 (C-5 GlcN), 73.0 (C-2 GlcA), 71.9 (C-3 GlcA), 71.4 (C-3 GlcA), 70.4 (C-5 GlcN), 70.2 (C-3 GlcN), 69.9 (C-5 GlcN), 66.2 (C-6 GlcN), 65.1 (2x C-6 GlcN), 58.7 (C-2 GlcN), 57.9 (C-2 GlcN), 57.3 (C-2 GlcN), 53.1 (CO_2CH_3), 52.8 (CO_2CH_3), 40.0 (C-2 cyclopentenyl), 39.3 (C-2 cyclopentenyl), 27.3 (CH_3 silylidene), 27.3 (CH_3 silylidene), 27.2 (CH_3 silylidene), 26.9 (CH_3 silylidene), 26.7 (CH_3 silylidene), 26.6 (CH_3 silylidene), 22.6 (C_q silylidene), 22.5 (C_q silylidene), 22.5 (C_q silylidene), 19.7 (C_q silylidene), 19.6 (C_q silylidene). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{95}\text{H}_{122}\text{Cl}_9\text{N}_3\text{O}_{32}\text{Si}_3\text{Na}$: 1397.27312, found 1397.27375. LC-MS: R_f 11.45 min (C4 column, linear gradient 70 $\rightarrow$ 90% B in 13.5 min.).



Deprotected pentasaccharide (26):

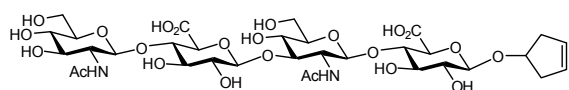
Pentamer **23** (58 mg, 26.2 μ mol) was dissolved in THF (1 mL, 0.03M). The reaction mixture was stirred followed by addition 3HF/TEA (39 μ L, 0.24 mmol, 9 eq.). After 30 min., TLC analysis showed total conversion into a lower running spot (DCM/MeOH: 9/1, v/v, R_f 0.3). the reaction mixture was quenched by addition of NaHCO_3 (aq., sat.), diluted with EtOAc, washed with brine, dried over MgSO_4 , filtered, and concentrated. A white solid was obtained, which was purified by SEC (DCM/MeOH: 1/1). The desilylated compound was obtained as a white solid in 83% yield (39 mg, 22 μ mol). $[\alpha]_D$: -21 (c = 0.1, ACN). IR (neat, cm^{-1}): 3055, 1421, 1263, 731, 702. ^1H NMR (400 MHz, CD_3CN , HH-COSY, HSQC): 7.90-7.83 (m, 7H, H_{arom}), 7.57-7.51 (m, 5H, H_{arom}), 7.50-7.33 (m, 8H, H_{arom}), 5.60-5.52 (m, 4H, 2x H-3 GlcA , H-3 cyclopentenyl), 5.29-5.22 (m, 2H, 2x H-2 GlcA), 5.04 (d, 1H, J = 7.2 Hz, H-1 GlcA), 5.01 (d, 1H, J = 7.23 Hz, H-1 GlcA), 4.67 (d, 1H, J = 8.4 Hz, H-1 GlcN), 4.64 (d, 1H, J = 8.4 Hz, H-1 GlcN), 4.57 (d, 1H, J = 8.4 Hz, H-1 GlcN), 4.47-4.39 (m, 3H, H-1 cyclopentenyl, 2x H-4 GlcA), 4.31 (d, 2H, J = 8.4 Hz, 2x H-5 GlcA), 3.91-3.84 (m, 2H, 2x H-3 GlcN), 3.77-3.75 (m, H-6a GlcN), 3.75 (s, 3H, CO_2CH_3), 3.73 (s, 3H, CO_2CH_3), 3.64-3.61 (m, 1H, H-6b GlcN), 3.60-3.77 (m, 7H, H-6 GlcN , 3x H-

2_{GlcN}, 2x H-4_{GlcN}), 3.33-3.18 (m, 1H, H-5_{GlcN}), 3.20-3.17 (m, 1H, H-5_{GlcN}), 3.12-3.08 (m, 1H, H-5_{GlcN}), 3.07-2.89 (m, 4H, H-6_{GlcN}, H-3_{GlcN}, H-4_{GlcN}), 2.52-2.20 (m, 4H, H-2 cyclopentenyl). ¹³C APT NMR (100 MHz, CD₃CN, HH-COSY, HSQC): 169.5 (C=O), 169.5 (C=O), 166.2 (C=O), 166.1 (C=O), 163.1 (C=O), 162.7 (C=O), 162.7 (C=O), 134.5-134.4 (CH_{arom}), 130.9-130.5 (CH_{arom}), 130.5-130.4 (C_q C_{arom}), 130.1-128.9 (CH_{arom}, C-3 cyclopentenyl), 100.9 (C-1_{GlcN}), 100.8 (C-1_{GlcA}), 100.7 (C-1_{GlcA}), 100.4 (C-1_{GlcN}), 100.3 (C-1_{GlcN}), 93.6 (C_q TCA), 93.5 (C_q TCA), 93.4 (C_q TCA), 83.3 (C-3_{GlcN}), 82.5 (C-3_{GlcN}), 79.5 (C-1 cyclopentenyl), 77.3 (C-5_{GlcN}), 77.2 (C-5_{GlcN}), 77.0 (C-5_{GlcN}), 75.7 (C-4_{GlcA}), 75.0 (C-4_{GlcA}), 74.7 (C-5_{GlcA}), 74.6 (C-5_{GlcA}), 74.5 (C-4_{GlcN}), 73.3 (C-3_{GlcA}), 73.2 (C-3_{GlcA}), 72.6 (C-2_{GlcA}), 72.6 (C-2_{GlcA}), 72.2 (C-4_{GlcN}), 70.5 (C-3_{GlcN}), 70.3 (C-4_{GlcN}), 62.7 (C-6_{GlcN}), 62.7 (2x C-6_{GlcN}), 58.8 (C-2_{GlcN}), 57.6 (C-2_{GlcN}), 57.5 (C-2_{GlcN}), 54.0 (CO₂CH₃), 53.9 (CO₂CH₃), 40.8 (C-2 cyclopentenyl), 40.1 (C-2 cyclopentenyl). HRMS: [M+Na]⁺ calcd for C₇₁H₇₄Cl₉N₃O₃₂Na 1822.12854, found 1822.12959. Desilylated pentasaccharide (30 mg, 16.7 μmol) was dissolved in THF (1 mL, 0.017M). The reaction mixture was cooled to 0 °C, followed by slow addition of 0.5M KOH (0.6 mL, 0.30 mmol, 18 eq.). The reaction mixture was stirred overnight, followed by addition of a second portion of 0.5M KOH (0.6 mL, 0.30 mmol, 18 eq.). After 4 days, the reaction mixture was neutralized with AcOH and concentrated. A white powder was obtained, which was purified by gel filtration (HW-40, 0.15M NH₄OAc in H₂O). The pooled fractions were lyophilized (3x) yielding the fully deprotected pentamer in 46% yield (7.0 mg, 7.6 μmol). ¹H NMR (600 MHz, D₂O, 322 K, HH-COSY, HSQC): 5.78-5.77 (m, 2H, H-3 cyclopentenyl), 4.83-4.80 (m, 3H, 3x H-1_{GlcN}), 4.71 (d, 1H, *J* = 7.8 Hz, H-1_{GlcA}), 4.71 (m, 2H, H-1_{GlcA}, H-1 cyclopentenyl), 4.01 (t, 1H, *J* = 6.8 Hz, H-3_{GlcN}), 3.96-3.90 (m, 6H, H-3_{GlcN}, 2x H-4_{GlcA}, 3x H-6a_{GlcN}), 3.79-3.66 (m, 13H, 3x H-6b_{GlcN}, 2x H-5_{GlcA}, 2x H-3_{GlcA}, 3x H-4_{GlcN}, H-3_{GlcN}), 3.54-3.48 (m, 5H, 2x H-2_{GlcA}, 3x H-5_{GlcN}), 3.31 (t, 1H, *J* = 10.2 Hz, H-2_{GlcN}), 3.18 (t, 1H, *J* = 10.2 Hz, H-2_{GlcN}), 3.10 (t, 1H, *J* = 10.8 Hz, H-2_{GlcN}), 2.68-2.65 (m, 2H, 2x H-2 cyclopentenyl), 2.53-2.48 (m, 1H, H-2 cyclopentenyl), 2.42-2.38 (m, 1H, H-2 cyclopentenyl). ¹³C APT NMR (125 MHz, D₂O, 322 K, HH-COSY, HSQC): 175.4 (C=O), 129.6 (C-3 cyclopentenyl), 129.1 (C-3 cyclopentenyl), 102.1 (C-1_{GlcA}), 102.1 (C-1_{GlcA}), 99.8 (C-1_{GlcN}), 99.5 (C-1_{GlcN}), 98.3 (C-1_{GlcN}), 81.8 (C-3_{GlcN}), 81.0 (C-1 cyclopentenyl), 81.7, 81.1, 80.9, 77.2, 76.8, 76.8, 75.4, 75.3, 75.1, 75.0, 73.4, 73.2, 72.9, 70.5, 69.9, 68.7 (2x C-3_{GlcN}, 3x C-4_{GlcN}, 3x C-5_{GlcN}, 2x C-2_{GlcA}, 2x C-3_{GlcA}, 2x C-4_{GlcA}, 2x C-5_{GlcA}), 61.4 (C-6_{GlcN}), 61.2 (C-6_{GlcN}), 61.2 (C-6_{GlcN}), 56.6 (C-2_{GlcN}), 55.8 (C-2_{GlcN}), 55.6 (C-2_{GlcN}), 40.3 (C-2 cyclopentenyl), 39.6 (C-2 cyclopentenyl). HRMS: [M+H]⁺ calcd for C₃₅H₅₈N₃O₂₅ 920.33539, found 920.33563. The obtained saponified pentasaccharide (7 mg, 7.6 μmol) was dissolved in a mixture of THF/H₂O (1/10, 1.57 mL, 0.005M) followed by addition of Ac₂O (11 μL, 114 μmol, 15 eq.). Solid NaHCO₃ was added until a pH of 8-9 was obtained. After 4 h., the reaction mixture was acidified with AcOH until pH~3 and concentrated. A white solid was obtained, which was subjected to gel filtration (HW 40, 0.15M NH₄OAc in H₂O). Lyophilization of the pooled fractions afforded pentamer **26** in 86% yield. ¹H NMR (600 MHz, D₂O, HH-COSY, HSQC): 5.84-5.82 (m, 2H, H-3 cyclopentenyl), 4.71-4.70 (m, 1H, H-1 cyclopentenyl), 4.69-4.65 (m, 3H, 3x H-1_{GlcN}), 4.63 (d, 2H, *J* = 7.2 Hz, 2x H-1_{GlcA}), 4.05-4.02 (m, 5H, 2x H-5_{GlcA}, 3x H-6a_{GlcN}), 4.97 (t, 1H, *J* = 10.2 Hz, H-2_{GlcN}), 4.95-4.80 (m, 11H, 3x H-6b_{GlcN}, 3x H-4_{GlcN}, 2x H-2_{GlcN}, 2x H-4_{GlcA}), 3.74 (t, 2H, *J* = 8.4 Hz, 2x H-3_{GlcA}), 3.69-3.56 (m, 6H, 3x H-5_{GlcN}, 3x H-3_{GlcN}), 3.48 (t, 2H, *J* = 9.6 Hz, 2x H-2_{GlcA}), 2.74-2.69 (m, 2H, 2x H-2 cyclopentenyl), 2.54-2.51 (m, 1H, H-2 cyclopentenyl), 2.43-2.40 (m, 1H, H-2 cyclopentenyl), 2.15 (s, 3H, CH₃ Ac), 2.13 (s, 3H, CH₃ Ac), 2.11 (s, 3H, CH₃ Ac). ¹³C APT NMR (125 MHz, D₂O, HH-COSY, HSQC): 175.7 (C=O), 175.5 (C=O), 173.2 (C=O), 173.1 (C=O), 129.5 (C-3 cyclopentenyl), 128.8 (C-3 cyclopentenyl), 103.9 (C-1_{GlcA}), 103.8 (C-1_{GlcA}), 102.1 (C-1_{GlcN}), 101.8 (C-1_{GlcN}), 100.7 (C-1_{GlcN}), 83.4, 83.2, 80.9, 80.9, 76.7, 76.4, 76.2, 74.5, 73.1, 73.1, 70.6 (3x C-3_{GlcN}, 3x C-4_{GlcN}, 3x C-5_{GlcN}, 2x C-2_{GlcA}), 81.0 (C-1 cyclopentenyl), 75.4 (C-5_{GlcA}), 75.4 (C-5_{GlcA}), 74.5 (C-3_{GlcA}), 74.5 (C-3_{GlcA}), 69.4 (C-2_{GlcA}), 69.1 (C-2_{GlcA}), 61.6 (C-6_{GlcN}), 61.4 (C-6_{GlcN}), 56.3 (C-2_{GlcN}), 55.6 (C-2_{GlcN}), 55.2 (C-2_{GlcN}), 40.3 (C-2 cyclopentenyl), 39.9 (C-2 cyclopentenyl), 23.3 (CH₃ Ac), 23.2 (CH₃ Ac), 23.0 (CH₃ Ac). HRMS: [M+H]⁺ calcd for C₄₁H₆₄Cl₉N₃O₂₈: 1046.36708, found 1046.36907.



Tetramer (25): The RV was charged with functionalized Merrifield polystyrene **5** (75 mg, 45 μmol) and prepared for the synthesis using protocol C. Then, the coupling/deblocking

cycle was run to couple disaccharide donor **11** twice. After the synthesis was complete, protocol H was used to isolate the resin. The resin was washed with dry DCM (5x), suspended in DCM (4.5 ml, 0.01M) followed by addition of trichloroacetamide (40 mg, 0.28 mmol, 46 eq. relative to Grubbs cat.). The suspension was purged with argon, followed by addition of Grubbs' second/generation catalyst (4.6 mg, 5.4 μmol , 0.12 eq.). After 3 h., the reaction mixture was filtered and concentrated. The brown residue was filtered over a plug of silica (hexane/EtOAc: 8/2, v/v) and concentrated. A white solid was obtained. LC-MS analysis showed the presence of the target tetramer. The residue was purified by SEC (DCM/MeOH, 1/1), which afforded the title compound in 73% yield over 5 steps (58 mg, 32.7 μmol). $[\alpha]_{\text{D}}^{25}$: -27 ($c = 0.3$, DCM). IR (neat, cm^{-1}): 1728, 1265, 1161, 1111, 1093, 1028, 829, 709. ^1H NMR (400 MHz, CD_3Cl_3 , HH-COSY, HSQC): 7.94-4.91 (m, 8H, H_{arom}), 7.56-7.49 (m, 4H, H_{arom}), 7.43-7.27 (m, 8H, H_{arom}), 7.12 (d, 1H, $J = 7.6$ Hz, NH), 6.82 (d, 1H, $J = 8.4$ Hz, NH), 5.61-5.57 (m, 1H, H-3 cyclopentenyl), 5.57 (t, 2H, $J = 9.6$ Hz, H-3, H-3''), 5.37 (d, 1H, $J = 3.8$ Hz, H-1), 5.28 (t, 1H, $J = 9.6$ Hz, H-2), 5.11 (dd, 1H, $J = 8.4$ Hz, $J = 3.6$ Hz, H-2''), 5.02 (d, 1H, $J = 8.4$ Hz, H-1''), 4.96 (d, 1H, $J = 8.4$ Hz, H-1'), 4.82 (d, 1H, $J = 6.8$ Hz, H-1), 4.58-4.54 (m, 1H, H-1 cyclopentenyl), 4.36 (t, 1H, $J = 10.0$ Hz, H-4''), 4.27 (t, 1H, $J = 9.2$ Hz, H-4), 4.18 (d, 1H, $J = 8.8$ Hz, H-5''), 4.11-4.06 (m, 2H, H-5, H-3'), 3.86 (s, 3H, CO_2CH_3), 3.84 (s, 3H, CO_2CH_3), 3.69 (t, 1H, $J = 8.0$ Hz, H-3''), 3.60-3.58 (m, 2H, H-4', H-2''), 3.56-3.45 (m, 3H, H-2', H-6'a, H-6''a), 3.40 (t, 1H, $J = 9.2$ Hz, H-4''), 3.24-3.20 (m, 2H, H-5', H-5''), 2.64-2.48 (m, 5H, H-6'b, H-6''b, 3x H-2 cyclopentenyl), 2.35-2.29 (m, 1H, H-2 cyclopentenyl), 0.91-0.88 (m, 27H, 3x *t*-butyl silylidene), 0.77 (s, 9H, *t*-butyl silylidene). ^{13}C APT NMR (100 MHz, CD_3Cl_3 , HH-COSY, HSQC): 175.0 ($\text{C}=\text{O}$ CO_2CH_3), 169.3 ($\text{C}=\text{O}$ CO_2CH_3), 165.8 ($\text{C}=\text{O}$ Bz), 165.3 ($\text{C}=\text{O}$ Bz), 165.2 ($\text{C}=\text{O}$ Bz), 165.1 ($\text{C}=\text{O}$ Bz), 162.4 ($\text{C}=\text{O}$ TCA), 161.4 ($\text{C}=\text{O}$ TCA), 133.5-133.0 (CH_{arom}), 130.0-129.9 (C_q C_{arom}), 129.8-129.7 (CH_{arom}), 129.1 (C_q C_{arom}), 128.8 (C_q C_{arom}), 128.4-127.4 (CH_{arom} , C-3 cyclopentenyl), 100.4 (C-1''), 100.3 (C-1), 99.6 (C-1''), 99.4 (C-1'), 92.7 (C_q TCA), 92.6 (C_q TCA), 79.5 (C-1 cyclopentenyl), 78.4 (C-3''), 77.3 (C-4''), 76.4 (C-4''), 76.0 (C-4'), 75.9 (C-4), 74.9 (C-3''), 74.8 (C-2''), 74.5 (C-5''), 73.9 (C-5), 72.2 (C-3 or C-3''), 72.0 (C-2), 71.3 (C-3 or C-3''), 70.1 (C-5' or C-5''), 69.9 (C-5' or C-5''), 65.1 (C-6' or C-6''), 65.0 (C-6' or C-6''), 57.8 (C-2''), 57.1 (C-2), 53.2 (CO_2CH_3), 52.9 (CO_2CH_3), 39.8 (C-2 cyclopentenyl), 39.2 (C-2 cyclopentenyl), 27.3-26.6 (CH_3 silylidene), 22.5 (C_q *tert*-butyl silylidene), 22.5 (C_q *tert*-butyl silylidene), 19.7 (C_q *tert*-butyl silylidene), 19.5 (C_q *tert*-butyl silylidene). HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{95}\text{H}_{123}\text{Cl}_9\text{N}_3\text{O}_{32}\text{Si}_3$: 2217.46223, found 2217.46201.



Deprotected tetrasaccharide (27):

Tetrasaccharide **25** (51 mg, 28.7 μmol) was dissolved in THF (1 mL, 0.03 M), followed by addition of 3HF/TEA (28 μL , 172 μmol , 6 eq.). The reaction mixture was stirred and after 30 min., TLC analysis showed total conversion into a lower running spot (DCM/MeOH, 9/1, v/v, R_f 0.3). The reaction mixture was quenched by addition of NaHCO_3 (aq., sat.), diluted with EtOAc, washed with brine, dried over MgSO_4 , filtered, and concentrated. Size exclusion chromatography (DCM/MeOH, 1/1) gave the desilylated compound as a white solid in 72% yield (31 mg, 20.8 μmol). $[\alpha]_{\text{D}}^{25}$: -15 ($c = 0.15$, ACN). IR (neat, cm^{-1}): 1728, 1529, 1315, 1265, 1070, 1028, 819, 734. ^1H NMR (400 MHz, CD_3CN , HH-COSY, HSQC): 7.95-7.85 (m, 8H, H_{arom}), 7.59-7.54 (m, 4H, H_{arom}), 7.46-7.34 (m, 8H, H_{arom}), 5.91-5.56 (m, 2H, H-3 cyclopentenyl, H-3 $_{\text{GlcA}}$), 5.50 (t, 1H, $J = 8.8$ Hz, H-3 $_{\text{GlcA}}$), 5.45-5.41 (m, 1H, H-3 cyclopentenyl), 5.25 (t, 1H, $J = 8.0$ Hz, H-2 $_{\text{GlcA}}$), 5.18 (t, 1H, $J = 9.2$ Hz, H-2 $_{\text{GlcA}}$), 5.02 (d, 1H, $J = 6.8$ Hz, H-1 $_{\text{GlcA}}$), 4.97 (d, 1H, $J = 7.6$ Hz, H-1 $_{\text{GlcA}}$), 4.67-4.63 (m, 2H, 2x H-1 $_{\text{GlcN}}$), 4.50-4.45 (m, 1H, H-4 $_{\text{GlcA}}$, H-1 cyclopentenyl), 4.41 (t, 1H, $J = 8.0$, H-4 $_{\text{GlcA}}$), 4.32 (d, 1H, $J = 8.8$ Hz, H-5 $_{\text{GlcA}}$), 4.17 (d, 1H, $J = 9.2$ Hz, H-5 $_{\text{GlcA}}$), 3.86 (t, 1H, $J = 8.8$ Hz, H-3 $_{\text{GlcN}}$), 3.77-3.75 (m, 6H, 2x CO_2CH_3), 3.52-3.32

(m, 5H, H-3_{GlcN}, H-6_{GlcN}, 2x H-2_{GlcN}), 3.17-3.15 (m, 1H, H-5_{GlcN}), 3.10-3.07 (m, 1H, H-5_{GlcN}), 3.02-2.88 (m, 4H, 2x H-4_{GlcN}, H-6_{GlcN}), 2.38-2.19 (m, 4H, H-2 cyclopentenyl). ¹³C APT NMR (100 MHz, CD₃CN, HH-COSY, HSQC): 169.5 (C=O CO₂Me), 169.5 (C=O CO₂Me), 166.2 (C=O Bz), 166.1 (C=O Bz), 166.1 (C=O Bz), 165.9 (C=O Bz), 163.0 (C=O TCA), 162.5 (C=O TCA), 134.5-134.1 (CH_{arom}), 130.8-130.4 (C_q C_{arom}), 130.1-128.5 (CH_{arom}, C-3 cyclopentenyl), 100.8 (C-1_{GlcN}), 100.7 (C-1_{GlcA}), 100.3 (C-1_{GlcA}), 99.7 (C-1_{GlcN}), 93.4 (C_q TCA), 93.4 (C_q TCA), 82.6 (C-3_{GlcN}), 79.9 (C-1 cyclopentenyl), 77.3 (C-5_{GlcA}), 77.3 (C-5_{GlcA}), 75.6 (C-4_{GlcA}), 74.8 (C-5_{GlcA}), 74.5 (C-2_{GlcN}, C-5_{GlcA}), 73.6 (C-3_{GlcA}), 76.2 (C-3_{GlcA}), 72.7 (C-2_{GlcA}), 72.5 (C-2_{GlcA}), 72.1 (C-4_{GlcN}), 70.5 (C-4_{GlcN}), 62.7 (C-6_{GlcN}), 62.6 (C-6_{GlcN}), 58.8 (C-2_{GlcN}), 57.6 (C-2_{GlcN}), 53.9 (CO₂CH₃), 53.8 (CO₂CH₃), 40.6 (C-2 cyclopentenyl), 40.2 (C-2 cyclopentenyl). HRMS: [M+H]⁺ calcd for C₆₃H₆₅Cl₆N₂O₂₇ 1493.18709, found 1493.19327. The obtained desilylated tetrasaccharide (25 mg, 16.7 μmol) was dissolved in THF (1 mL, 0.017M) and the reaction mixture was cooled to 0 °C. Then, 0.5M KOH (0.54 mL, 0.27 mmol, 16 eq.) was added. The reaction mixture was allowed to warm to rT and was stirred overnight. Another portion of 0.5M KOH (0.54 mL, 0.27 mmol, 16 eq.) was added. After 4 d., the reaction mixture was neutralized with AcOH and concentrated. A yellowish powder was obtained, which was purified by gel filtration (HW-40, 0.15M NH₄OAc in H₂O). The pooled fractions were lyophilized (3x) yielding the deprotected pentamer in 52% yield (6.6 mg, 8.7 μmol). ¹H NMR (600 MHz, D₂O, 288 K, HH-COSY, HSQC): 5.74-5.7 (m, 2H, H-3 cyclopentenyl), 4.77 (d, 1H, *J* = 8.4 Hz, H-1_{GlcN}), 4.77 (d, 1H, *J* = 8.4 Hz, H-1_{GlcN}), 4.68 (d, 1H, *J* = 7.8 Hz, H-1_{GlcA}), 4.63-4.61 (m, 1H, H-1 cyclopentenyl), 4.54 (d, 1H, *J* = 7.8 Hz, H-1_{GlcA}), 3.97 (t, 1H, *J* = 10.8 Hz, H-3_{GlcN}), 3.93 (d, 1H, *J* = 10.2 Hz, H-5_{GlcA}), 3.88-3.85 (m, 3H, H-5_{GlcA}, 2x H-6a_{GlcN}), 3.77-3.64 (m, 7H, H-3_{GlcA}, 2x H-4_{GlcA}, H-3_{GlcN}, H-4_{GlcN}, 2x H-6b_{GlcN}), 3.60 (t, 1H, *J* = 9.0 Hz, H-3_{GlcA}), 3.51-3.44 (m, 4H, H-4_{GlcN}, 2x H-5_{GlcN}, H-2_{GlcA}), 3.28 (t, 1H, *J* = 10.8 Hz, H-2_{GlcN}), 3.25 (t, 1H, *J* = 9.6 Hz, H-2_{GlcA}), 3.06 (t, 1H, *J* = 10.2 Hz, H-2_{GlcN}), 2.64-2.59 (m, 2H, 2x H-2 cyclopentenyl), 2.46-2.61 (m, 2H, 2x H-2 cyclopentenyl). ¹³C APT NMR (150 MHz, D₂O, 288K, HH-COSY, HSQC): 176.2 (C=O), 175.7 (C=O), 129.5 (C-3 cyclopentenyl), 129.1 (C-3 cyclopentenyl), 102.4 (C-1_{GlcA}), 101.6 (C-1_{GlcA}), 99.6 (C-1_{GlcN}), 99.2 (C-1_{GlcN}), 82.0 (C-3_{GlcN}), 80.8 (C-1 cyclopentenyl), 75.7 (C-5_{GlcA}), 75.3 (C-5_{GlcA}), 75.1 (C-3_{GlcA}), 73.5 (C-2_{GlcA}), 80.9, 80.6, 77.1, 76.7, 74.9, 73.4, 73.8, 70.3, 68.5 (C-2_{GlcA}, C-3_{GlcA}, 2x C-4_{GlcA}, C-3_{GlcN}, 2x C-4_{GlcN}, 2x C-5_{GlcN}), 61.1 (C-6_{GlcN}), 61.0 (C-6_{GlcN}), 56.5 (C-2_{GlcN}), 55.6 (C-2_{GlcN}), 40.4 (C-2 cyclopentenyl), 39.9 (C-2 cyclopentenyl). HRMS: [M+H]⁺ calcd for C₂₉H₄₇N₂O₂₁ 759.26658, found 759.26789. The obtained saponified tetrasaccharide (4 mg, 5.27 μmol) was dissolved THF/H₂O (1/10, v/v, 1.1 mL, 0.005M) followed by addition of Ac₂O (5 μL, 52.7 μmol, 10 eq.). Solid NaHCO₃ was added until pH~8-9. After 4 h., the reaction mixture was acidified with AcOH until pH~3 and concentrated. A white powder was obtained, which was purified by gel filtration (HW-40, 0.15M NH₄OAc in H₂O). The pooled fractions were lyophilized (3x) yielding the target tetramer in 91% yield (4.0 mg, 4.8 μmol). ¹H NMR(600 MHz, D₂O, HH-COSY, HSQC): 5.88-5.78 (m, 2H, H-3 cyclopentenyl), 4.65-4.64 (m, 1H, H-1 cyclopentenyl), 4.72 (d, 1H, *J* = 7.8 Hz, H-1_{GlcA}), 4.69 (d, 1H, *J* = 9.0 Hz, H-1_{GlcN}), 4.66 (d, 1H, *J* = 8.4 Hz, H-1_{GlcN}), 4.64 (d, 1H, *J* = 7.8 Hz, H-1_{GlcA}), 4.05-4.02 (m, 3H, 2x H-6a_{GlcN}, H-5_{GlcA}), 3.99 (d, 1H, *J* = 10.2 Hz, H-5_{GlcA}), 3.96 (t, 1H, *J* = 8.4 Hz, H-2_{GlcN}), 3.92-3.80 (m, 6H, 2x H-6b_{GlcN}, 2x H-4_{GlcA}, H-2_{GlcN}, H-3_{GlcN}), 3.76-3.72 (m, 2H, 2x H-3_{GlcA}), 3.68-3.64 (m, 2H, H-3_{GlcN}, H-4_{GlcN}), 3.62-3.58 (m, 3H, H-4_{GlcN}, 2x H-5_{GlcN}), 3.48 (t, 1H, *J* = 9.0 Hz, H-2_{GlcA}), 3.40 (t, 1H, *J* = 9.0 Hz, H-2_{GlcA}), 2.78-2.73 (m, 2H, 2x H-2 cyclopentenyl), 2.58-2.55 (m, 2H, 2x H-2 cyclopentenyl), 2.16 (s, 3H, CH₃ Ac), 2.13 (s, 3H, CH₃ Ac). ¹³C APT NMR (150 MHz, D₂O, HH-COSY, HSQC): 175.7 (C=O), 175.7 (C=O), 173.6 (C=O), 173.1 (C=O), 129.5 (C-3 cyclopentenyl), 129.1 (C-3 cyclopentenyl), 103.9 (C-1_{GlcA}), 102.3 (C-1_{GlcN}), 102.0 (C-1_{GlcN}), 101.9 (C-1_{GlcN}), 83.1 (C-3_{GlcA}), 81.1 (C-1 cyclopentenyl), 81.1 (C-4_{GlcA}), 80.9 (C-4_{GlcA}), 76.8 (C-5_{GlcN}), 76.2 (C-5_{GlcN}), 75.7 (C-5_{GlcA}), 75.6 (C-5_{GlcA}), 74.7 (C-3_{GlcA}), 74.5 (C-3_{GlcA}), 74.5 (C-3_{GlcN} or C-4_{GlcN}), 73.4 (C-2_{GlcA}), 73.2 (C-2_{GlcA}), 70.6 (C-4_{GlcN}), 69.2 (C-3_{GlcN} or C-4_{GlcN}), 61.4 (C-6_{GlcN}), 56.3 (C-2_{GlcN}), 55.2 (C-2_{GlcN}), 40.3 (C-2 cyclopentenyl), 39.9 (C-2 cyclopentenyl), 23.3 (CH₃ Ac), 23.3 (CH₃ Ac). HRMS: [M+H]⁺ calcd for C₃₃H₅₁N₂O₂₃: 843.28771, found 843.28875.

References & Notes

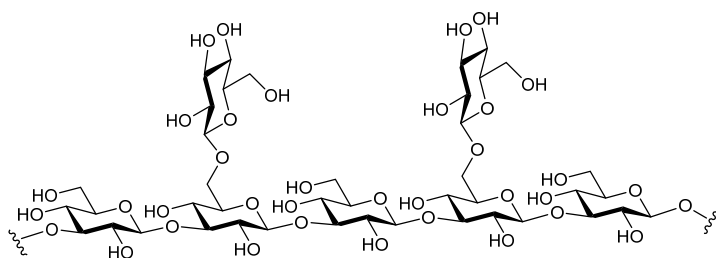
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Chapter 5

Synthesis of β -1,3-glucan fragments

Introduction

β -(1,3)-glucans are naturally occurring polysaccharides and were originally identified as essential constituents of the cell wall in fungi and as major storage source in brown seaweed. These glucans are also found on several pathogenic bacteria.¹ The healing and immunostimulating role of these polysaccharides has long been known and β -1,3-glucans have nowadays been identified as Dectin-1 ligands.² From a structural point of view these glucans have a linear β -1,3-linked backbone can be substituted in a random fashion with β -1,6-glucosyl branches. A well-known example is Lentinan (Figure 1),³ which is isolated from Shiitake and Maitake mushrooms and probed as anti-cancer drug. Research towards the elucidation of the immunostimulating properties of these polysaccharides requires sufficient amounts of pure, well-defined oligomers. The availability of a row of β -(1,3)-glucans of variable length can be very valuable to elucidate structure-activity relationships of these polysaccharides.

Figure 1. Lentinan, a glucan with β -(1,3)-linkages, known as antitumor agent.

Several syntheses of β -(1,3)-glucans, both branched and linear, have been reported.^{4,5} To allow access to a library of β -(1,3)-glucans it is desirable to prepare these (functionalized) fragments of oligosaccharides in a more convenient, and possibly automated fashion.⁶ In this Chapter, the solution phase synthesis of a β -1,3-glucan trisaccharide is explored and initial experiments to bring the synthesis to a solid phase synthesis platform are described.

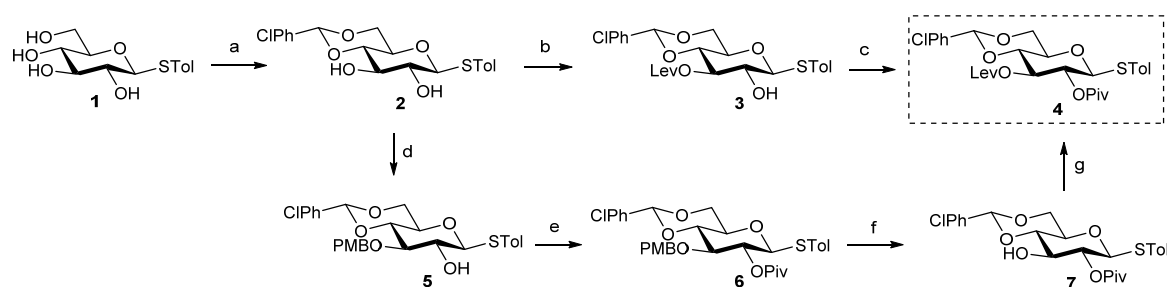
Results & Discussion

Solution phase synthesis of a β -1,3-glucan trisaccharide.

For the synthesis of β -(1,3)-glucan fragments, thioglucoside **4** (Scheme 1) was selected as precursor for the synthesis of the corresponding trifluoro-*N*-phenylimidate donor (Scheme 2, **9**).⁷ The thio-functionality in **4** acts as a temporary masking group for the anomeric hydroxyl group, which in turn can be converted to the trifluoro-*N*-phenylimidate functionality in **9**. For the introduction of β -glycosidic linkages, the participating and sterically demanding C-2-*O*-pivaloyl (Piv) group was chosen instead of the commonly used benzoyl group. Due to steric bias of the *tert*-butyl group, possible orthoester formation during the glycosylation is minimized. The use of the benzoyl protective group for the introduction of *trans* glycosidic linkages can lead to *cis*-fused product and orthoester formation.^{5b} The 4,6-*O*-*p*-chlorobenzylidene diol protective group was described to be more stable than the unsubstituted benzylidene acetal as the electron-withdrawing *p*-chloro substituent limits susceptibility towards acidic conditions.⁸ The extra stability is desirable in a solid phase synthesis, in which a relatively large amount of strong acid is repetitively used during the glycosylation step. The levulinoyl group serves as a temporary protecting group for the C-3 hydroxyl, which is to be elongated. The synthesis of target thioglucoside started with the introduction of the 4,6-*O*-chlorobenzylidene acetal. Condensation of glycoside **1** with *p*-chlorobenzaldehyde and a catalytic amount of *p*-TsOH in DMF proved to be ineffective under atmospheric pressure at elevated temperatures. Performing the reaction under reduced pressure (30 mbar) at 50 °C gave glucoside **2** in 90% yield. Glucoside **2** was converted to a tin acetal by treatment with Bu₂SnO in refluxing toluene. After cooling down to room temperature, levulinic anhydride was added to the reaction mixture. This resulted in the regioselective introduction of the levulinoyl ester at the C-3 position. According to the conditions described by Ishihara *et al.*,⁹ the pivaloyl group was installed with pivalic anhydride and a catalytic amount of Sc(OTf)₃ to give the fully protected glucoside **4** in 81% yield. Unfortunately, this route gave difficulties in scaling up. In particular, the regioselective introduction of the levulinoyl group at the C-3 position could not be achieved on a larger

scale. Therefore, a more robust route in which the *para*-methoxybenzyl (PMB) group was used for interim protection of the C-3 hydroxyl was employed. First, the free hydroxyl groups in diol **2** were silylated by treatment with TMSCl in pyridine. Then, the C-3 position was regioselectively *p*-methoxybenzylated using *p*-anisaldehyde and triethylsilane (TES) in the presence of a catalytic amount of TMSOTf at $-86\text{ }^{\circ}\text{C}$.¹⁰ Finally, addition of TBAF resulted in desilylation of the intermediate and glucoside **5** was isolated in 73% yield. The introduction of the PMB group was successfully accomplished on 51 mmol scale, which shows the robustness of this reaction on big scale. The pivaloyl group was introduced at the C-2 position utilizing pivaloyl chloride and DMAP in refluxing pyridine to give fully protected **6** in 82% yield. The high temperature was required for the installation of the pivaloyl ester, because no conversion was attained at room temperature. The *para*-methoxybenzyl ether in **6** was cleaved with DDQ in a mixture of DCM and H_2O , resulting in glucoside **7**. The hydroxyl at the C-3 position was protected with a levulinoyl group using LevOH and DIC in the presence of DMAP, yielding central building block **4**.

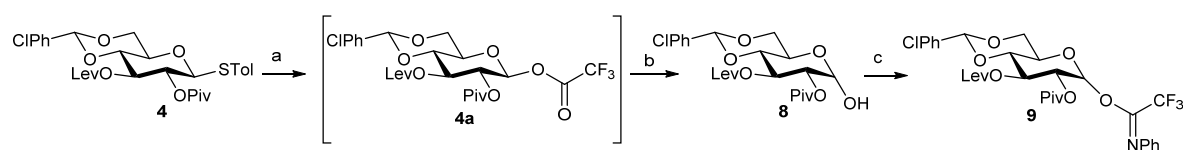
Scheme 1. Synthesis of glucoside building block **4**.



Reagents and conditions: (a) *p*-chlorobenzaldehyde, *p*-TsOH, DMF, $50\text{ }^{\circ}\text{C}$, 30 mbar, 90%; (b) Bu_2SnO , toluene, reflux, then 2.5M Lev₂O in THF, 86%/2 steps; (c) Piv_2O , $\text{Sc}(\text{OTf})_3$, MeCN, 81%; (d) (i) TMSCl, pyridine, $0\text{ }^{\circ}\text{C}$, (ii) *p*-anisaldehyde, TES, TMSOTf, DCM, $-86\text{ }^{\circ}\text{C}$, then TBAF, 73%/2 steps; (e) PivCl , DMAP, pyridine, reflux, 82%; (f) DDQ, DCM/ H_2O , 78%; (g) LevOH, DIC, DMAP, DCM, 90%.

Having thioglucoside **4** in hand, the corresponding trifluoro-*N*-phenyl imidate donor **9** was prepared (Scheme 2). Donor **9** can be directly activated with triflic acid (TfOH) making this a convenient donor in an automated synthesis. To prepare donor **9** from thioglucoside **4** the anomeric thiotoluyll group is to be hydrolyzed. The presence of the acid-labile chlorobenzylidene protective group in donor **4** required an anhydrous method. Therefore, glucoside **4** was treated with a combination of NIS and TFA¹¹ to provide the intermediate anomeric trifluoroacetate **4a**.

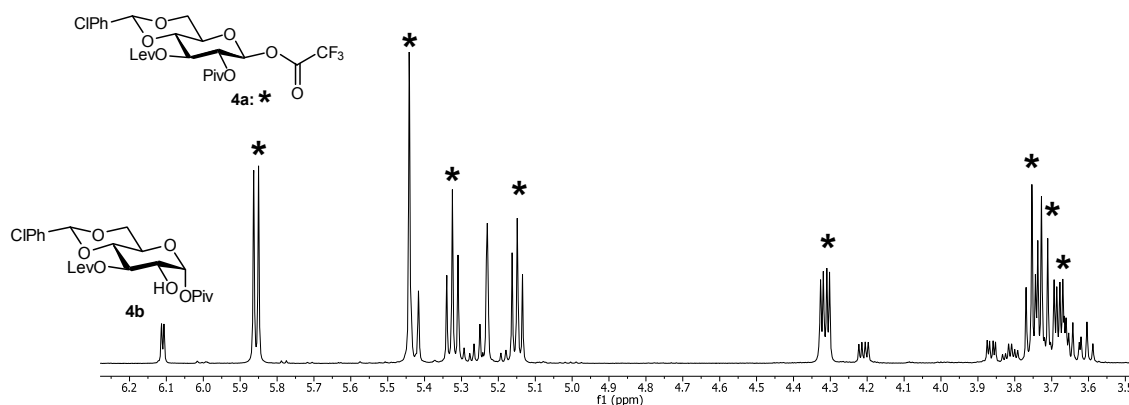
Scheme 2. Synthesis of glucoside donor **9**.



Reagents and conditions: (a) NIS, TFA, DCM, $0\text{ }^{\circ}\text{C}$; (b) piperidine, 85%; (c) $\text{ClC}(\text{=NPh})\text{CF}_3$, DBU, acetone, $0\text{ }^{\circ}\text{C}$, 94%.

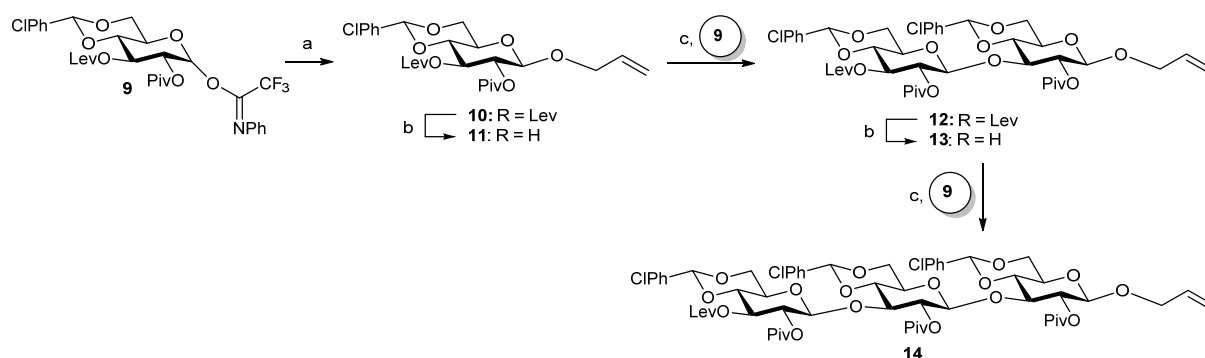
NMR spectroscopy revealed the anomeric trifluoroacetate to be the β anomer only ($J_{1,2} = 7.8$ Hz). In the ^1H NMR spectrum of **4a** a smaller set of signals was also observed, corresponding to pivaloyl-migration product **4b** (Figure 2, $J_{1,2} = 3.6$ Hz, $\sim 15\%$).¹² Cleavage of the anomeric trifluoroacetate group with piperidine led to hemiacetal **8**. The corresponding trifluoro-*N*-phenyl imidate donor **9** was obtained by treatment of **8** with *N*-phenyl trifluoroacetimidoyl chloride¹³ and DBU in acetone.

Figure 2. Part of the ^1H NMR spectrum of glucoside **4** after treatment with NIS/TFA in $\text{DCM}-d_2$ at 0°C .



With glucosyl donor **9** in hand, its glycosylating properties were studied by the solution phase synthesis of a trimeric β -1,3-glucan fragment (Scheme 3). Allyl alcohol was selected as the first acceptor to obtain the same allyl glucoside product as produced by executing an automated synthesis using the 1,4-butanediol linker. Glucosyl donor **9** was reacted with allyl alcohol and TfOH (cat.) yielding allyl glucoside **10** in 70% yield. The temporary levulinoyl group was removed with hydrazine hydrate in a mixture of pyridine and acetic acid giving glucosyl acceptor **11** in 96%. Next, donor **9** and acceptor **11** were coupled with a catalytic amount of TfOH in DCM, which led to the isolation of disaccharide **12** in 99% yield. Subsequent deleverinylation of the disaccharide afforded disaccharide acceptor **13**, which was glycosylated with donor **9** to give trisaccharide **14** in 80% yield.

Scheme 3. Synthesis of trisaccharide **14**.



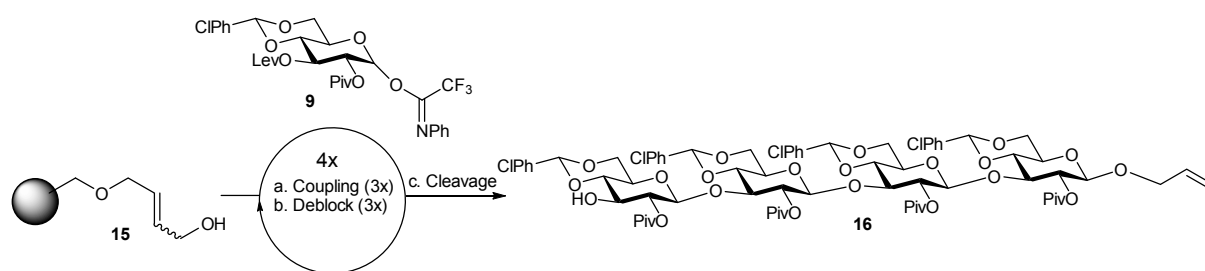
Reagents and conditions: (a) Allyl alcohol, TfOH, DCM, 0°C , 70%; (b) hydrazine hydrate, pyridine/AcOH, **11**: 96%, **13**: 94%; (c) TfOH, DCM, 0°C , **12**: 99%, **14**: 80%.

NMR analysis of the trisaccharide revealed several remarkable features. First, the anomeric proton of the middle glucoside showed a very small coupling constant ($J_{1,2'} = 2.1$ Hz), which is unexpected for a β -glucosyl linkage in a ${}^4C_1$ conformation. This small coupling was not observed in the NMR analysis of the disaccharide precursor. Also, the H-2' signal of the middle residue was not split into the expected triplet. Instead, a broad singlet was observed. H-H COSY NMR showed only a weak correlation between H-2' and H-3'. Furthermore, TOCSY NMR analysis showed two distinct spin systems in the middle glucoside: a spin system between H-1' and H-2' and a spin system of H-3' through H-6', indicating that the middle glucoside does not adopt the expected ${}^4C_1$ chair conformation. Another noteworthy observation is the shift of the benzylidene proton of the middle glucoside. This proton is about 1 ppm upfield compared to the other two benzylidene signals, which suggests a high degree of electron shielding. These results are in line with structural effects reported for 4,6-O-benzylidinated β -1,3-glucans. Vetricka and coworkers¹⁴ performed a series of NOESY NMR experiments, which suggest that the affected glucosides uptake a pseudorotational itinerary between the ${}^4C_1$ and ${}^1,4B$ or $B_{2,5}$ conformation rather than the ${}^4C_1$ chair which is the unaffected glucoside residue. The crystal structure of a 4,6-O-benzylidene protected hexasaccharide reported by Ensley *et al.*⁴ confirmed that glucoside residues in between (third and fifth) adopt a twist boat conformation whereas all other units were found in a ${}^4C_1$ conformation.

Taken together, the above described results show that the trifluoro-*N*-phenyl imidate donor **9** is a suitable donor for the construction of the β -1,3-glycosidic linkages. The 4,6-O-chlorobenzylidene functionality remains intact during the glycosylations and the C-2-pivaloyl ester ensures stereoselective couplings. Encouraged by the successful solution phase synthesis of trisaccharide **14**, the solution phase chemistry was translated to an automated solid phase format.

Automated synthesis of β -1,3-glucan fragments.

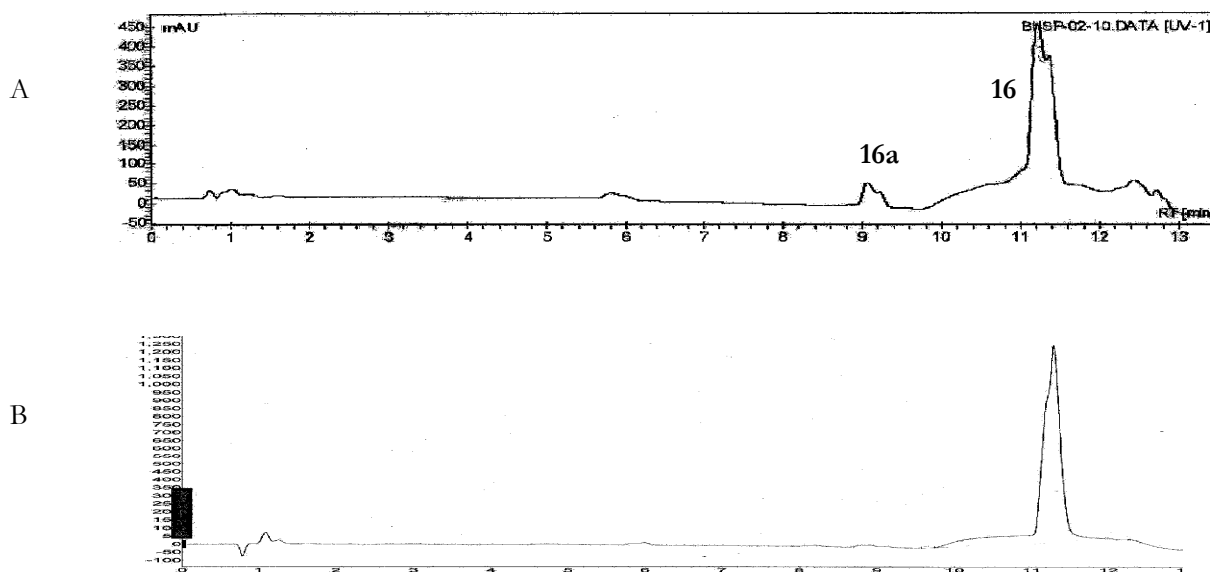
The automated synthesis of a tetrameric β -glucan fragment¹⁵ was explored using the above described donor **9** and 1,4-butene-diol linker **15** (Scheme 4).¹⁶ This linker is stable towards the reaction conditions applied and the product can be cleaved via a cross metathesis reaction with ethene and Grubbs first-generation catalyst at the end of the synthesis.¹⁷ Donor **9** was used in excess (3 eq. per coupling cycle) and since the glycosylation proceeded fast in solution phase, a short coupling time (3x 15 minutes) was employed. The amount of triflic acid (0 °C and 0.1 eq. per donor, respectively) and the temperature were not changed with respect to the solution phase synthesis. The temporary levulinoyl group was cleaved at 40 °C to ensure rapid and complete unmasking of the C-3-hydroxyl.

Scheme 4. Automated synthesis of tetrasaccharide **16**.

Reagents and conditions: (a) Donor **9** (3 eq.), TfOH (0.3 eq.), 0 °C, 15 min.; (b) hydrazine acetate (10 eq.), pyridine/AcOH, 40 °C, 10 min.; (c) Grubbs first-generation catalyst, ethene, DCM, overnight, 44%.

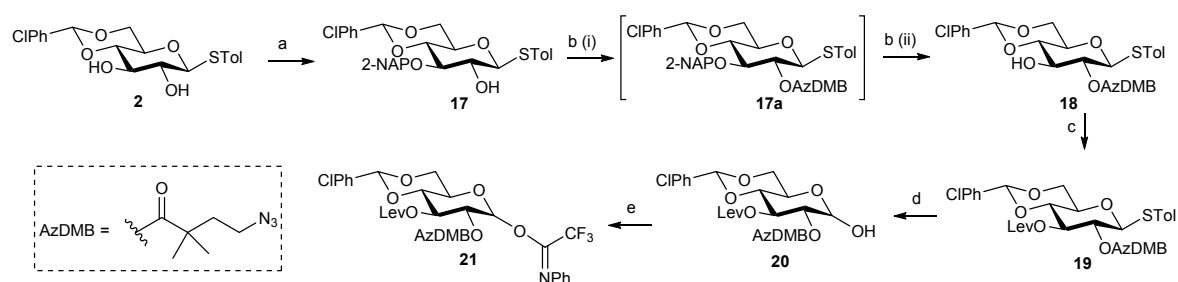
After cleavage from the resin and filtration over a plug of silica, LC-MS analysis showed the predominant formation of tetrasaccharide **16**, along with presence of a minor amount trisaccharide **16a** (tetramer: trimer, ~26:1) and trace amounts of disaccharide (Figure 3). Preparative HPLC was employed to isolate tetrasaccharide **16** in 44% yield (~91% yield/step). In an attempt to further improve the efficiency of the synthesis of tetrasaccharide **16**, the couplings were performed at 20 °C. LC-MS analysis of the so-obtained product however revealed a higher proportion of trisaccharide relative to the tetrasaccharide. Therefore, the glycosylation temperature was set to 0 °C for further syntheses. Although the automated preparation of the tetrameric β -1,3-glucan fragment was successful, deprotection of the pivaloyl esters turned out to be troublesome. Treatment with bases such as ammonia and KOH did not result in deprotection of the C-2 position. Therefore, a pivaloyl-like group that can be cleaved in a milder and faster fashion was investigated.

Figure 3. LC-MS chromatograms of the crude tetrasaccharide after cleavage from the resin (A, C4 column, linear gradient 70 $\rightarrow$ 90% B) and after HPLC purification (B).



Synthesis of β -1,3-glucan fragments with the AzDMB-protective group.

To circumvent the troublesome deprotection of the pivaloyl group the recently reported 4-azido-2,2-dimethyl-butanoyl (AzDMB) protective group (Scheme 5) was implemented.¹⁸ The appended azido functionality allows the cleavage of the ester by a Staudinger reduction reaction or hydrogenation. The synthesis of the glucosyl donor **21** starts with diol **2** which was regioselectively 2-methylnaphthylated at the C-3 position. This was achieved by first preparation of the tin ketal followed by treatment with 2-naphthylmethyl bromide and CsF in DMF to yield alcohol **17** in 69% yield. Subsequent treatment of **17** with freshly prepared AzDMBCl in pyridine gave fully protected glucoside **17a**. Because this product could not be separated from 4-azido-2,2-dimethyl-butanolic acid, the crude product was subjected to a mixture of DDQ in DCM/H₂O to give glucoside **18** in 78% yield over 2 steps. The free hydroxyl was protected with a levulinoyl group to give fully protected glucoside **19** in 90% yield. Thio-donor **19** was transformed into imidate **21** using the same sequence of reactions, as described for the synthesis of pivaloyl-donor **9** from hemiacetal **4**.

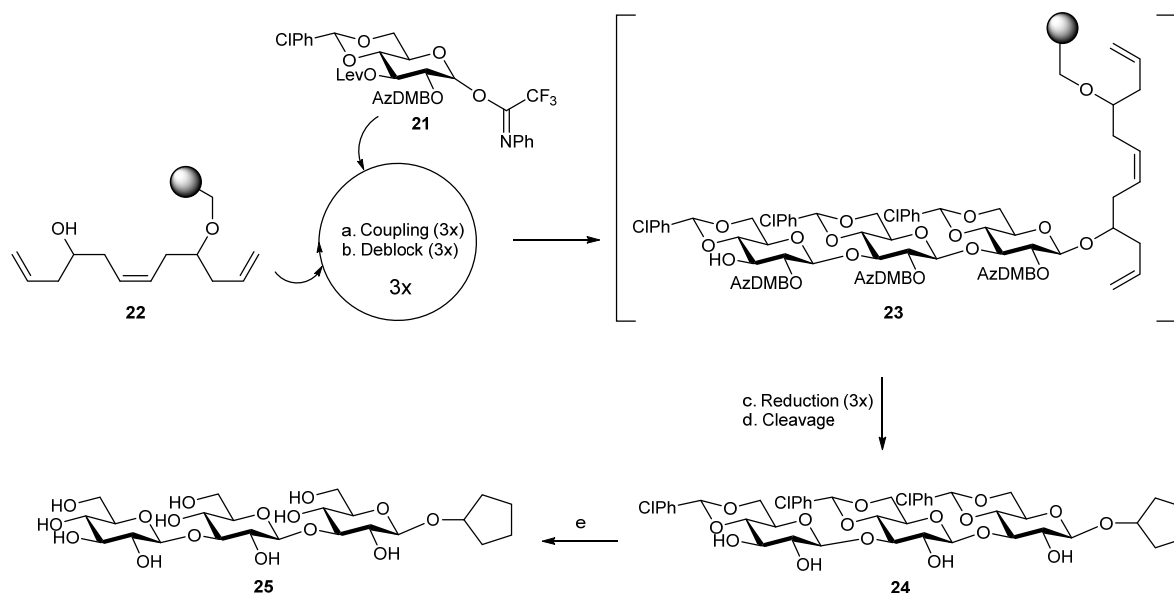
Scheme 5. Synthesis of AzDMB-donor **21**.

Reagents and conditions: (a) (i) Bu₂SnO, toluene, reflux, (ii) 2-NAP-Br, CsF, DMF, overnight, 69%/2 steps; (b) (i) AzDMBCl, pyridine, 110 °C, (ii) DDQ, DCM/H₂O, 78%/2 steps; (c) LevOH, DIC, DMAP, DCM, 90%; (d) NIS, TFA, DCM, 0 °C, then piperidine, 86%; (e) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 96%.

The AzDMB protected donor **21** was explored in the solid phase synthesis of trisaccharide **25** (Scheme 6). The tandem RCM cleavable linker **22**, described in Chapter 4, was applied. Since the coupling (3x 3 eq. of donor, 3x 0.1 eq. of TfOH) and deblocking conditions (3x 6.7 eq. of hydrazine hydrate) described above were shown to be effective, the same conditions were applied. Before cleaving the product from the resin the AzDMB groups were removed on-resin by Staudinger reduction of the azides¹⁹ with tributylphosphine in the presence of aqueous KOH. Some optimization was required because the hydrophobic polystyrene resin significantly shrinks in aqueous solutions, making the azides on the resin inaccessible for the reagents. Using a mixture of 0.5M aqueous KOH in dioxane (1/19, v/v) and 9 eq. of tributylphosphine at elevated temperature (50 °C) led to the desired triple deprotection and set the stage to release the trisaccharide from the resin by tandem RCM. The resin was treated with Grubbs second-generation catalyst for 2 hours, after which the trisaccharide was isolated. LC-MS analysis of the crude product showed that the whole synthetic procedure had proceeded uneventfully and no benzylidene cleavage products or diastereomers resulting from non-selective glycosylation reactions could be observed. After purification of the trisaccharide over a small plug of silica the target trisaccharide **24** was isolated in 36% yield (~88% per

step). Cleavage of the *p*-chlorobenzylidene functions was readily accomplished by hydrogenolysis in a THF/H₂O/*tert*-BuOH solvent mixture to provide β -glucan trisaccharide **25** in 91% yield.

Scheme 6. Solid phase synthesis of a trisaccharide β -1,3-glucan fragment.



Reagents and conditions: (a) Donor **21** (3 eq.), TfOH (0.3 eq.), DCM, 0 °C, 15 min.; (b) hydrazine hydrate (6.7 eq.), pyridine/AcOH, 40 °C, 10 min.; (c) Bu₃P (9 eq.), dioxane/0.5M KOH, 50 °C, 90 min.; (d) Grubbs second-generation catalyst (0.12 eq.), DCM, 2 h., 36%/8 steps; (e) H₂, Pd/C, THF/H₂O/*tert*-BuOH, 2 h., 91%.

Conclusions

The successful solution phase synthesis of a β -(1,3)-glucan trisaccharide formed the basis for the automated solid synthesis of a partly protected tetrameric β -(1 $\rightarrow$ 3)-glucan fragment. However, cleavage of the pivaloyl esters in this tetrasaccharide turned out to be troublesome and did not give the fully unprotected targets. Replacement of the pivaloyl by the mildly cleavable AzDMB protective group led to the first successful solid phase synthesis of a β -(1,3)-glucan trisaccharide fragment. Having established the glycosylation properties and deprotection conditions, the (automated) solid phase synthesis of larger β -(1,3)-glucan fragments can now be explored.

Experimental section

General experimental procedures. Chemical shifts (δ) are given in ppm relative to TMS as internal standard. All ¹³C APT NMR spectra are proton decoupled. Reactions were performed at rT unless stated otherwise and were followed by TLC analysis with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulphuric acid in EtOH or with a solution of (NH₄)₆Mo₇O₂₄·H₂O (25 g L⁻¹), followed by charring at 150 °C. Flash column chromatography was performed on silica gel (0.04-0.063 nm) and size exclusion chromatography (SEC) was performed on Sephadex™ LH-20. Experiments that required an inert atmosphere were carried out under dry argon. Dichloromethane

(p.a.) was distilled over P_2O_5 prior to use. Molecular sieves (3Å) were flame-dried before use. LC-MS analysis was performed using a C4 column with the following solvent systems: A = 100% water, B = 100% acetonitrile, C = 1% TFA.

General experimental procedures for (automated) solid phase synthesis. The synthesizer used was supplied by Ancora Pharmaceuticals. The synthesizer's solvent bottles are filled with commercially acquired solvents, which are pre-dried 24 h. before use on 4Å molecular sieves. The solutions containing building block, activator and deblocking reagents are freshly prepared directly before use with pre-dried solvents. During the synthesis of trisaccharide **24**, the coupling, deblocking, and reduction reagents were added manually.

Reagent solutions:

Building block: Donor **9** or **21** in DCM (0.09M)

Activator: Trifluoromethanesulfonic acid in DCM (0.09M)

Deblocking: Synthesis of tetramer **16**: Hydrazine acetate in pyridine/AcOH (4/1, v/v, 0.12M)

Synthesis of trisaccharide **24**: Hydrazine hydrate in pyridine/AcOH (4/1, v/v, 0.12M)

Reduction Bu_3P in THF dioxane/0.5M KOH (19/1, v/v, 0.25M)

Protocol A: Agitation of the resin during washing. After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the reaction vessel (RV) for 15 seconds to agitate the resin suspension. Then the RV is emptied.

Protocol B: Agitation of the resin during reaction. After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the RV for 10 seconds to agitate the resin suspension. Then the purging is halted and the suspension is allowed to settle for 20 sec.

Protocol C: Swelling of new resin. The RV is charged with dry resin. The resin is washed with DCM (3x), alternating THF and hexane (3x), THF (1x) and DCM (3x). Every wash step involves protocol A.

Protocol D: Washing of the resin before or after the reaction. If applicable, the chiller temperature is set to ambient. The pre-swollen resin is washed with alternating THF and hexane (3x), followed by THF (1x) and DCM (3x). Every wash step involves protocol A.

Protocol E: Coupling cycle. The resin is washed with DCM (3x) before the building block solution (1.5 mL, 0.09M) is added. Then, the temperature is set to -5 °C to ensure an actual temperature of 0 °C in the RV. Simultaneously a pause of 10 min is started. When the temperature of the chiller has reached its target point, the activator solution (300 μ L for the synthesis of tetrasaccharide **15**, 150 μ L for the synthesis of trisaccharide **24**) is added. Protocol B is applied for 15 min. Then the RV is emptied and the solution is collected in a mixture of DCM/H₂O/triethylamine (50/5/1, v/v/v). The resin is washed with DCM (3x) and the solutes are similarly collected.

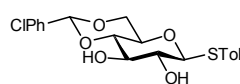
Protocol F: Deblocking. The resin is washed with DMF (3x) before the deblock solution (2.5 mL, 0.12M) is added. The temperature was raised to 40 °C and the resin was agitated using protocol B for 10 min. Then the RV is emptied and the resin is washed with DMF (3x).

Protocol G: Washing of the resin after deblocking. The temperature of the chiller is set to ambient. The resin is successively washed with DMF (3x), DCM (3x), alternating THF and hexane (6x), 0.01M AcOH in THF (6x) and THF (3x). Every wash step involves protocol A.

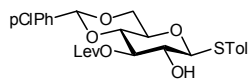
Protocol H: Reduction. The resin is washed with THF (3x) before the reduction solution is added (2.13 mL) and the resin is agitated using protocol B for 90 min. while the temperature is raised to 50 °C. Then the RV is emptied and the resin is washed with DMF (6x) and THF (6x).

Protocol I: Washing of the resin after reduction. The temperature of the chiller is set to ambient. The resin is successively washed with DMF (6x), DCM (3x), alternating THF and hexane (6x), 0.01M AcOH in THF (6x) and THF (3x). Every wash step involves protocol A.

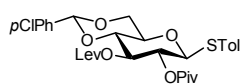
Protocol J: Suspending of the resin for isolation. The resin is washed with alternating DCM and MeOH (2x), followed by a mixture of DCM/MeOH (7/1, v/v, 2x), both employing protocol A. Then a mixture of DCM/MeOH (7/1, v/v) is added, the resin is agitated for 15 sec. after which time the gas-flow was halted and the program was paused. The suspended resin is isolated and this last procedure is repeated two times.



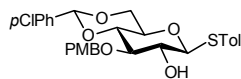
***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-1-thio- β -D-glucopyranoside (2):** To a solution of thioglucoside **1** (20 g, 69.8 mmol) in DMF (0.6 M, 120 mL) was added *p*-chlorobenzaldehyde (210 mmol, 29.4 g, 3 eq.) and a catalytic amount of *p*-toluenesulfonic acid. The mixture was stirred on a rotary evaporator (50 °C, 30 mbar) for 3.5 hours. The reaction was quenched with TEA until pH ~7. The mixture was concentrated in vacuo, co-evaporated with toluene and crystallized from EtOAc/PE to give the title compound in 90% yield (62.9 mmol, 25.7 g). Melting point: 188 °C. $[\alpha]_D$: -44.4 (*c* = 1, MeCN). IR (neat, cm^{-1}): 3362, 2914, 2870, 2852, 1668, 1653, 1601, 1493, 1449, 1402, 1371, 1350, 1296, 1281, 1269, 1240, 1215, 1167, 1140, 1107, 1086, 1072, 1040, 4013, 988, 976, 947, 930, 908, 893, 872, 833, 808. ^1H NMR (400 MHz, DMSO- d_6 , HH-COSY, HSQC): 7.45 (s, 4H, H_{arom}), 7.35 (d, 2H, J = 8.4 Hz, H_{arom}), 7.16 (d, 2H, J = 8.0 Hz, H_{arom}), 5.59-5.58 (m, 2H, CH benzylidene, C-2-OH), 4.46 (d, 1H, J = 5.2 Hz, C-3-OH), 4.74 (d, 1H, J = 9.6 Hz, H-1), 4.18 (dd, 1H, J = 10.0 Hz, J = 4.8 Hz, H-6a), 3.66 (t, 1H, J = 10.0 Hz, H-6b), 3.49 (m, 2H, H-3, H-5), 3.14 (m, 2H, H-2, H-4), 2.29 (s, 3H, CH_3 STol). ^{13}C APT NMR (100 MHz, DMSO- d_6), HH-COSY, HSQC): 136.7 (C_q C_{arom}), 133.4 (C_q C_{arom}), 131.4-128.2 (CH_{arom}), 99.7 (CH benzylidene), 87.6 (C-1), 80.3 (C-3), 74.2 (C-2 or C-4), 72.9 (C-2 or C-4), 69.6 (C-5), 67.8 (C-6), 20.9 (CH_3 STol). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{21}\text{ClO}_5\text{SNa}$: 431.06904, found 431.06893.



***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-levulinoyl-1-thio- β -D-glucopyranoside (3):** To a solution of glucoside **2** (5.0 g, 12.2 mmol) in toluene (0.3 M, 40 mL) was added Bu_2SnO (3.2 g, 12.8 mmol, 1.1 eq.). The mixture was refluxed for 18 hours, after which it was cooled to room temperature. Next levulinic anhydride (26.8 mmol, 10.8 mL of a 2.5M solution in THF) was added. After TLC analysis (DCM/acetone, 19/1, v/v) confirmed formation of a higher running compound, the mixture was quenched with MeOH, washed with H_2O and brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. Crystallization from PE/EtOAc gave the product in 86% yield, (5.3 g, 10.5 mmol). Melting point: 141 °C. $[\alpha]_D$: -44.4 (*c* = 1, DCM). IR (neat, cm^{-1}): 3397, 2982, 2878, 1742, 1709, 1601, 1493, 1456, 1418, 1402, 1360, 1304, 1267, 1186, 1155, 1105, 1070, 1030, 1015, 991, 972, 937, 908, 883, 810. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.44 (d, 2H, J = 8.0 Hz, CH arom), 7.34 (m, 4H, H_{arom}), 7.15 (d, 2H, J = 8.0 Hz, H_{arom}), 5.44 (s, 1H, CH benzylidene), 5.23 (t, 1H, J = 9.2 Hz, H-3), 4.63 (d, 1H, J = 9.6 Hz, H-1), 4.36 (dd, 1H, J = 10.4 Hz, J = 4.8 Hz, H-6a), 3.75 (t, 1H, J = 10.0 Hz, H-6b), 3.54 (m, 3H, H-2, H-4, H-5), 3.05 (d, 1H, J = 2.8 Hz, C-2-OH), 2.78 (m, 2H, CH_2 Lev), 2.60 (m, 2H, CH_2 Lev), 2.36 (s, 3H, CH_3 Lev), 2.15 (s, 3H, CH_3 STol). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 207.1 (C=O Lev ketone), 172.7 (C=O Lev), 139.0 (C_q C_{arom}), 135.5 (C_q C_{arom}), 135.0 (C_q C_{arom}), 134.0 (CH_{arom}), 130.0 (CH_{arom}), 128.5 (CH_{arom}), 127.6 (CH_{arom}), 127.4 (C_q C_{arom}), 100.7 (CH benzylidene), 89.2 (C-1), 78.2 (C-2), 75.2 (C-3), 71.5 (C-4 or C-5), 70.7 (C-4 or C-5), 68.6 (C-6), 38.3 (CH_2 Lev), 29.9 (CH_3 Lev), 28.2 (CH_2 Lev), 21.3 (CH_3 STol). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{25}\text{H}_{29}\text{ClO}_6\text{SNa}$: 529.10582, found 529.10553.

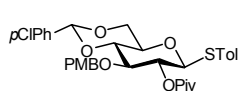


***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-levulinoyl-2-*O*-pivaloyl-1-thio- β -D-glucopyranoside (4):** *Method A:* To a solution of glucoside **3** (0.9 g, 1.8 mmol) in MeCN (9 mL, 0.2 M) was added Piv₂O (1.7 mL, 8.9 mmol, 5 eq.) and Sc(OTf)₃ (1.5 mL of a 60mM solution in MeCN, 0.09 mmol, 0.08 eq.). After 60 minutes, TLC analysis (DCM/acetone, 19/1, v/v) confirmed complete consumption of the starting material, and the mixture was quenched with sat. aq. NaHCO₃ and diluted in Et₂O. The organic layer was washed three times with sat. aq. NaHCO₃ and once with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Crystallization from MeOH gave the title compound in 81% yield (0.85 g, 1.44 mmol). *Method B:* To an ice-cooled solution of glucoside **7** (2.85 g, 5.8 mmol) in DCM (20 mL, 0.3M) was added LevOH (1.6 mL, 15.1 mmol, 2.6 eq.), DIC (1.2 mL, 7.5 mmol, 1.3 eq.) and DMAP (0.07 g, 0.6 mmol, 0.1 eq.). The mixture was allowed to warm to room temperature. After TLC analysis (DCM/acetone, 19/1, v/v) indicated complete conversion, the reaction was quenched with MeOH. The organic phase was washed with sat. aq. NaHCO₃ (3x), water and brine. The organic phase was dried over MgSO₄, filtered and concentrated *in vacuo*. After column chromatography (PE/EtOAc: 9/1 $\rightarrow$ 7/3), the product was obtained in 90% yield (5.2 mmol, 3.09 g). Melting point: 146 °C. $[\alpha]_D$: -4.6 (c = 1, CHCl₃). IR (neat, cm⁻¹): 2974, 2916, 2862, 1749, 1740, 1719, 1441, 1479, 1422, 1375, 1364, 1354, 1281, 1273, 1209, 1180, 1157, 1148, 1125, 1092, 1067, 1016, 978, 949, 941, 879, 841, 826, 806, 797, 770. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.37-7.31 (m, 6H, H_{arom}), 7.14 (d, 2H, *J* = 8.0 Hz, H_{arom}), 5.46 (s, 1H, CH benzylidene), 5.35 (t, *J* = 9.4 Hz, H-3), 4.99 (t, 1H, *J* = 9.6 Hz, H-2); 4.73 (d, 1H, *J* = 10 Hz, H-1); 4.37 (dd, 1H, *J* = 10.4 Hz, *J* = 4.8 Hz, H-6a), 3.78 (t, 1H, *J* = 9.8 Hz, H-6b), 3.65 (t, 1H, *J* = 9.6 Hz, H-4), 3.54 (dt, 1H, *J* = 9.6 Hz, *J* = 4.8 Hz, H-5), 2.69-2.66 (m, 2H, CH₂ Lev), 2.57-2.52 (m, 2H, CH₂ Lev), 2.35 (s, 3H, CH₃ STol), 2.14 (s, 3H, CH₃ Lev), 1.26 (s, 9H, 3x CH₃ Piv). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.9 (C=O Lev ketone), 176.8 (C=O Piv), 171.7 (C=O Lev), 138.9-135.1 (C_q Carom), 133.6-128.5 (CH_{arom}), 128.3 (C_q Carom), 127.8 (CH_{arom}), 100.7 (CH benzylidene), 87.5 (C-1), 78.4 (C-4), 73.0 (C-3), 70.6 (C-5), 70.0 (C-2), 68.6 (C-6), 38.9 (C_q Piv), 37.9 (CH₂ Lev), 29.9 (CH₃ Lev), 28.0 (CH₂ Lev), 27.2 (CH₃ Piv), 21.3 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₃₀H₃₅ClO₈SNa: 613.16389, found 613.16375.



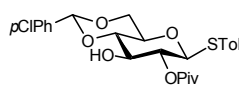
***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-(*p*-methoxybenzyl)-1-thio- β -D-glucopyranoside (5)** To a stirred solution of glucoside **2** (25.0 g, 51.5 mmol) in pyridine (100 mL, 0.5M) was added TMSCl (19.5 mL, 153.5 mmol, 3.0 eq.) at 0 °C. The mixture was gradually warmed to room temperature, and after TLC analysis (DCM/acetone, 19/1, v/v) indicated complete conversion of the starting material, the mixture was diluted in EtOAc and washed with H₂O (2x). The combined aqueous phases were extracted with EtOAc (2x). The combined organics were dried over MgSO₄, filtered and concentrated *in vacuo*. The crude product was coevaporated with toluene. The silyl ether was dissolved in DCM (350 mL, 0.15M), *p*-anisaldehyde (7.0 mL, 11.3 mmol, 1.1 eq.) was added and the mixture was cooled to -86 °C. Added was Et₃SiH (1.8 mL, 56.5 mmol, 1.1 eq.) and TMSOTf (0.8 mL, 4.5 mmol, 0.09 eq.) and the mixture was stirred at -86 °C overnight, after which TLC analysis (PE/EtOAc, 3/1, v/v) indicated complete conversion of the starting material. The mixture was quenched with MeOH and NaOMe (up to pH ~ 9) and the mixture was warmed to room temperature. The mixture was diluted with DCM, washed with sat. aq. NaHCO₃. The aqueous phase was extracted with DCM (2x). The combined organics were dried over MgSO₄, filtered and concentrated *in vacuo*. The crude compound was treated with TBAF (1M solution in THF, 55 mL, 55 mmol, 1.07 eq.) and stirred at room temperature until TLC analysis (PE/EtOAc, 3/1, v/v) indicated complete conversion. The reaction mixture was diluted with DCM and washed with sat. aq. NaHCO₃. The aqueous phase was extracted with DCM (2x). The combined organics were dried over MgSO₄, filtered and concentrated *in vacuo*. Crystallization from hot EtOH gave the product as a white solid in 73% yield (23.8 g, 37.6 mmol). Melting point: 163 °C. $[\alpha]_D$: -46.6 (c = 1, DCM). IR (neat, cm⁻¹): 3487, 2953, 2920, 2882, 2862, 1614, 1603, 1514, 1491, 1466, 1400, 1379, 1348, 1304, 1250, 1227, 1171,

1130, 1148, 1084, 1070, 1013, 982, 972, 943, 883, 856, 810. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.43-7.34 (m, 6H, H_{arom}), 7.26 (d, 2H, $J = 8.4$ Hz, H_{arom}), 7.12 (d, 2H, $J = 8.0$ Hz, H_{arom}), 6.84 (d, 2H, $J = 8.4$ Hz, H_{arom}), 5.51 (s, 1H, CH benzyldiene), 4.83 (d, 1H, $J = 11.2$ Hz, CHH PMB), 4.71 (d, 1H, $J = 11.2$ Hz, CHH PMB), 4.54 (d, 1H, $J = 9.6$ Hz, H-1), 4.36 (dd, 1H, $J = 10.8$ Hz, $J = 4.8$ Hz, H-6a), 3.81-3.73 (m, 4H, H-6b, CH_3 PMB), 3.66-3.56 (m, 2H, H-3, H-4), 3.49-3.43 (m, 2H, H-2, H-5), 2.52 (d, 1H, $J = 2.0$ Hz, C-2-OH), 2.34 (s, 3H, CH_3 STol). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 159.5 (C_q C_{arom}), 138.9, 135.9, 135.0 (C_q C_{arom}), 134.0 (CH_{arom}), 130.3 (C_q C_{arom}), 130.0 (CH_{arom}), 129.9 (CH_{arom}), 128.6 (CH_{arom}), 127.6 (CH_{arom}), 127.3 (C_q C_{arom}), 114.0 (CH_{arom}), 100.6 (CH benzyldiene), 88.7 (C-1), 81.3 (C-3 or C-4), 81.2 (C-3 or C-4), 74.6 (CH_2 PMB), 72.2 (C-2 or C-5), 70.8 (C-2 or C-5), 68.7 (C-6), 55.4 (CH_3 PMB), 21.3 (CH_3 STol). HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{28}\text{H}_{33}\text{ClO}_6\text{SN}$: 546.17116, found 546.19636.



***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-(*p*-methoxybenzyl)-2-*O*-pivaloyl-1-thio- β -D-glucopyranoside (6)** To a solution of glucoside **5** (15.9 g, 30.0 mmol) in pyridine (75 mL, 0.4M) was added PivCl (7.20 mL, 60.0 mmol, 2

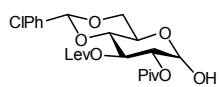
eq.) and a catalytic amount of DMAP (0.37 g, 3.0 mmol, 0.1 eq.). The mixture was heated to reflux and stirred until TLC (PE/EtOAc, 4/1, v/v) showed complete consumption of the starting material. The mixture was cooled to room temperature, diluted with EtOAc and washed with aqueous 1M HCl solution (3x), water (1x) and brine. The organic phase was dried over MgSO_4 , filtered and concentrated *in vacuo*. After crystallization from hot EtOH, the title compound was obtained in 82% yield (15.1 g, 24.6 mmol). Melting point: 143 $^\circ\text{C}$. $[\alpha]_{\text{D}}^{25}$: -29.4 ($c = 1$, DCM). IR (neat, cm^{-1}): 2974, 2959, 2932, 2905, 2872, 2832, 1724, 1614, 1512, 1493, 1477, 1468, 1425, 1398, 1371, 1329, 1302, 1279, 1244, 1213, 1173, 1150, 1082, 1067, 1051, 1042, 1003, 982, 962, 943, 928, 889, 880, 824, 812, 802. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.37-7.33 (m, 6H, H_{arom}), 7.17-7.11 (m, 4H, H_{arom}), 6.81 (d, 2H, $J = 8.8$ Hz, H_{arom}), 5.51 (s, 1H, CH benzyldiene), 5.03 (dd, 1H, $J = 10.0$ Hz, $J = 8.4$ Hz, H-2), 4.72 (d, 1H, $J = 11.2$ Hz, CHH PMB), 4.67 (d, 1H, $J = 10.0$ Hz, H-1), 4.58 (d, 1H, $J = 11.2$ Hz, CHH PMB), 4.37 (dd, 1H, $J = 10.8$ Hz, $J = 4.8$ Hz, H-6a), 3.81-3.78 (m, 5H, H-3, H-6b, OCH_3 PMB), 3.70 (t, 1H, $J = 9.2$ Hz, H-4), 3.47 (dt, 1H, $J = 9.6$ Hz, $J = 4.8$ Hz, H-5), 2.34 (s, 3H, CH_3 STol), 1.24 (s, 9H, CH_3 Piv). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 176.6 (C=O Piv), 159.3 (C_q C_{arom}), 138.6-135.0 (C_q C_{arom}), 133.4 (CH_{arom}), 130.2 (C_q C_{arom}), 129.9-129.3 (CH_{arom}), 128.7 (C_q C_{arom}), 128.6-113.8 (CH_{arom}), 100.6 (CH benzyldiene), 87.7 (C-1), 81.2 (C-4), 80.1 (C-3), 74.4 (CH_2 PMB), 71.2 (C-2), 70.5 (C-5), 68.7 (C-6), 55.4 (CH_3 PMB), 38.9 (C_q Piv), 27.3 (CH_3 Piv), 21.3 (CH_3 STol). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{33}\text{H}_{37}\text{ClO}_7\text{SNa}$: 635.18407, found 635.18401.



***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-pivaloyl-1-thio- β -D-glucopyranoside (7):** To a solution of thioglucoside **6** (9.19 g, 15 mmol) in DCM/ H_2O (9/1, v/v, 150 mL, 0.1M) was added DDQ (4.09 g, 18 mmol, 1.2

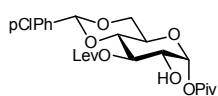
eq.), and the resulting dark mixture was stirred vigorously at room temperature. After TLC analysis (toluene/EtOAc, 9/1, v/v) indicated complete conversion of the starting material, the reaction was quenched with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ solution, diluted with EtOAc and washed with sat. aq. NaHCO_3 solution, water and brine. The organic phase was dried over MgSO_4 , filtered and concentrated *in vacuo*. The title compound was obtained after crystallization from hot EtOH in 78% yield. (5.75 g, 11.7 mmol). Melting point: 144 $^\circ\text{C}$. $[\alpha]_{\text{D}}^{25}$: -46 ($c = 1.5$, DCM). IR (neat, cm^{-1}): 2957, 2878, 2855, 1726, 1647, 1601, 1491, 1479, 1458, 1423, 1396, 1371, 1325, 1279, 1254, 1213, 1165, 1150, 1045, 1013, 995, 984, 964, 941, 928, 916, 878, 864, 835, 822, 810. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.37-7.32 (m, 6H, CH arom), 7.14 (d, 2H, $J = 8.0$ Hz, CH arom), 5.50 (s, 1H, CH benzyldiene), 4.88 (dd, 1H, $J = 10.0$ Hz, $J = 8.8$ Hz, H-2), 4.70 (d, 1H, $J = 10.0$ Hz, H-1), 4.37 (dd, 1H, $J = 10.4$ Hz, $J = 4.8$ Hz, H-6a), 3.91 (dt, 1H, $J = 8.8$ Hz, $J = 3.2$ Hz, H-3), 3.78 (t, 1H, $J = 10.2$ Hz, H-6b), 3.56 (t, 1H, $J = 9.2$ Hz, H-4), 3.47 (dt, 1H, $J = 10.0$ Hz, $J = 4.8$ Hz, H-5), 2.57 (d, 1H, $J = 3.2$ Hz, OH), 2.35 (s, 3H,

CH₃ STol), 1.26 (s, 9H, 3x CH₃ Piv). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 177.8 (C=O Piv), 138.8, 135.5, 135.2 (C_q C_{arom}), 133.5 (CH_{arom}), 129.9 (CH_{arom}), 128.6 (CH_{arom}), 128.5 (C_q C_{arom}), 127.9 (CH_{arom}), 101.1 (CH benzylidene), 87.2 (C-1), 80.7 (C-4), 73.9 (C-3), 72.4 (C-2), 70.3 (C-5), 68.6 (C-6), 39.0 (C_q Piv), 27.3 (CH₃ Piv), 21.3 (CH₃ STol). HRMS: [M+Na]⁺ calcd for C₂₅H₂₉ClO₆Na: 515.12656, found 515.12628.



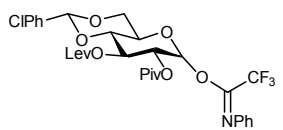
4,6-O-(*p*-chlorobenzylidene)-3-O-levulinoyl-2-O-pivaloyl-D-glucopyranose

(8): To a solution of glucoside **4** (296 mg, 0.5 mmol) in DCM (5.0 mL, 0.1M) was added NIS (124 mg, 0.55 mmol, 1.1 eq.) and TFA (0.42 mL, 0.55 mmol, 1.1 eq.) at 0 °C. After TLC analysis (toluene/EtOAc, 4/1, v/v) showed total conversion of the starting material, piperidine (0.15 mL, 1.5 mmol, 3.0 eq.) was slowly added. After TLC analysis (PE/EtOAc, 7/3, v/v) indicated total consumption of the trifluoroacetyl glucoside, the reaction was quenched with Na₂S₂O₃ (aq., sat.), diluted in DCM and washed with 1M HCl solution, water and brine. The organic phase was dried with MgSO₄, filtered and concentrated *in vacuo*. Column chromatography (PE/EtOAc 1:0 → 7:3) gave the title compound as a mixture of α - and β -isomers (1.7/1), in 85% yield. IR (neat, cm⁻¹): 3300, 2979, 2870, 1746, 1722, 1703, 1601, 1479, 1368, 1323, 1306, 1281, 1219, 1184, 1152, 1090, 1053, 1013, 986, 968, 943, 837, 820, 77. α isomer: ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.39 (d, 2H, *J* = 8.4 Hz, H_{arom}), 7.32 (d, 2H, *J* = 8.4 Hz, CH_{arom}), 5.65 (t, 1H, *J* = 10.0 Hz, H-3), 5.48 (s, 1H, CH benzylidene), 5.44 (t, 1H, *J* = 3.2 Hz, H-1), 5.84 (dd, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-2), 4.27 (dd, *J* = 10.2 Hz, *J* = 4.8 Hz, H-6a), 4.14 (dt, 1H, *J* = 9.8 Hz, *J* = 4.8 Hz, H-5), 3.73 (t, 1H, *J* = 10.0 Hz, H-6b), 3.65 (t, *J* = 9.8 Hz, H-4), 3.24 (d, *J* = 3.2 Hz, C-1-OH), 2.73-2.69 (m, 2H, CH₂ Lev), 2.61-2.54 (m, 2H, CH₂ Lev), 2.15 (s, 3H, CH₃ Lev), 1.20 (s, 9H, 3x CH₃ Piv). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.3 (C=O Lev ketone), 178.0 (C=O Piv), 171.7 (C=O Lev), 135.4-135.0 (C_q C_{arom}), 128.5 (CH_{arom}), 127.8 (CH_{arom}), 100.8 (CH benzylidene), 91.1 (C-1), 79.3 (C-4), 71.7 (C-2), 69.0 (C-3), 68.9 (C-6), 62.5 (C-5), 38.9 (C_q Piv), 37.9 (CH₂ Lev), 30.0 (CH₃ Lev), 28.0 (CH₂ Lev), 27.0 (CH₃ Piv). HRMS: [M+Na]⁺ calcd for C₂₃H₂₉ClO₉Na: 507.13978, found 507.13923.



Pivaloyl 4,6-O-(*p*-chlorobenzylidene)-3-O-levulinoyl- α -D-glucopyranoside

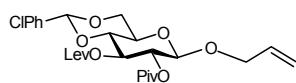
(4b) The title compound was isolated as a side-product in thioglycoside hydrolysis, in varying amounts. [α]_D: +50 (c = 1, DCM). IR (neat, cm⁻¹): 3495, 2972, 2932, 2914, 2870, 1751, 1713, 1601, 1493, 1477, 1458, 1414, 1398, 1366, 1319, 1287, 1234, 1204, 1179, 1152, 1136, 1101, 1080, 1024, 1016, 988, 966, 943, 908, 893, 810. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.42 (d, 2H, *J* = 8.4 Hz, CH_{arom}), 7.34 (d, 2H, *J* = 8.4 Hz, CH_{arom}), 6.22 (d, 1H, *J* = 4.0 Hz, H-1), 5.50 (s, 1H, CH benzylidene), 5.35 (t, 1H, *J* = 9.6 Hz, H-3), 4.32 (dd, 1H, *J* = 10.4 Hz, *J* = 4.8 Hz, H-6a), 3.93 (dd, 1H, *J* = 9.6 Hz, *J* = 4.0 Hz, H-2), 3.88 (dt, 1H, *J* = 9.8 Hz, *J* = 4.8 Hz, H-5), 3.73 (t, 1H, *J* = 10.0 Hz, H-6b), 3.68 (t, 1H, *J* = 9.6 Hz, H-4), 2.81 (m, 2H, CH₂ Lev), 2.65 (m, 2H, CH₂ Lev), 2.17 (s, 3H, CH₃ Lev), 1.28 (s, 9H, 3x CH₃ Piv). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 207.2 (C=O Lev ketone), 176.9 (C=O Piv), 173.5 (C=O Lev), 135.4 (C_q C_{arom}), 135.1 (C_q C_{arom}), 128.6 (CH_{arom}), 127.8 (C_q C_{arom}), 100.8 (CH benzylidene), 91.7 (C-1), 78.2 (C-4), 72.8 (C-3), 71.1 (C-2), 68.8 (C-6), 65.0 (C-5), 39.4 (C_q Piv), 38.3 (CH₂ Lev), 30.0 (CH₃ Lev), 28.2 (CH₂ Lev), 27.2 (CH₃ Piv). HRMS: [M + Na]⁺ calcd for C₂₃H₂₉ClO₉Na 507.13978, found 507.13953.



4,6-O-(*p*-chlorobenzylidene)-3-O-levulinoyl-1-O-(*N*-phenyl trifluoroacetimidoyl)-2-O-pivaloyl- α/β -D-glucopyranoside

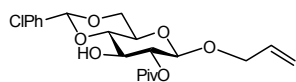
(9): To an ice-cooled solution of hemiacetal **8** (1.6 g, 3.3 mmol) in acetone (17 mL, 0.2M) was added *N*-phenyl trifluoroacetimidoyl chloride (0.61 mL, 4.0 mmol, 1.2 eq.) and DBU (0.64 mL, 3.3 mmol, 1 eq.). After 2.5 hours at 0 °C, TLC (toluene/EtOAc, 4/1, v/v) indicated complete conversion of the starting material. The mixture was diluted with H₂O and extracted with EtOAc (3x). The combined organics were washed with H₂O, brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Column chromatography (PE/EtOAc/TEA 100/0/1 →

85/15/1) afforded the glucosyl imidate in 94% yield (2.0 g, 3.1 mmol, α/β : 1/2). IR (neat, cm^{-1}): 2972, 2936, 2914, 2864, 2847, 2826, 1746, 1715, 1599, 1491, 1481, 1398, 1368, 1325, 1310, 1279, 1250, 1204, 1177, 1153, 1125, 1101, 1084, 1061, 1016, 974, 941, 904, 895, 881, 835, 810. β -isomer: ^1H NMR (400 MHz, CDCl_3 , 323 K, HH-COSY, HSQC): 7.41-7.25 (m, 6H, H_{arom}), 7.14-7.08 (m, 1H, H_{arom}), 6.82-6.76 (m, 2H, H_{arom}), 5.86 (bs, 1H, H-1), 5.46 (s, 1H, CH benzyldiene), 5.33 (t, 1H, J = 8.8 Hz, H-3), 5.23 (t, 1H, J = 8.4 Hz, H-2), 4.34-4.31 (m, 1H, H-6a), 3.79-3.71 (m, 2H, H-4, H-6b), 3.50 (bs, 1H, H-5), 2.69-2.66 (m, 2H, CH_2 Lev), 2.61-2.56 (m, 2H, CH_2 Lev), 2.12 (s, 3H, CH_3 Lev), 1.20 (s, 9H, 3x CH_3 Piv). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 205.5 (C=O Lev ketone), 177.5 (C=O Piv), 171.6 (C=O Lev), 143.1 (C=N), 135.5-135.2 (C_q C_{arom}), 129.0-119.4 (CH_{arom}), 101.1 (CH benzyldiene), 92.6 (C-1), 78.8 (C-4), 70.1 (C-2), 69.2 (C-3), 68.6 (C-6), 65.2 (C-5), 39.0 (C_q C_{arom}), 37.9 (CH_2 Lev), 28.0 (CH_3 Lev), 27.0 (CH_2 Lev), 27.0 (CH_3 Piv). HRMS: $[\text{M}+\text{Na}]^+$ calcd $\text{C}_{23}\text{H}_{29}\text{ClO}_9\text{Na}$: 507.13978, found 507.13923.



Allyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-levulinoyl-2-*O*-pivaloyl- β -D-glucopyranoside (10) To a solution of glucosyl imidate **9** (0.13 g, 0.2 mmol) in DCM (4.0 mL, 50mM) were added flame-dried 3Å molecular

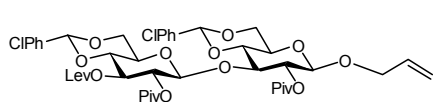
sieves and the mixture was cooled to 0 °C. Allyl alcohol (dried over K_2CO_3 , 0.04 mL, 0.6 mmol, 3 eq.) was added and the reaction was stirred for 30 minutes. TfOH (1.8 μL , 0.02 mmol, 0.1 eq.) was added and the reaction was gradually warmed to room temperature. After 120 minutes, the reaction was neutralized with TEA, washed with brine, dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc: 19/1 $\rightarrow$ 4/1) afforded the title compound in 70% yield (73 mg, 0.14 mmol). $[\alpha]_D$: -63.2 (c = 1.1, DCM). IR (neat, cm^{-1}): 2972, 2955, 2924, 2909, 2893, 2874, 1740, 1715, 1603, 1495, 1479, 1468, 1425, 1396, 1362, 1319, 1304, 1277, 1209, 1177, 1150, 1125, 1086, 1061, 1030, 986, 976, 951, 937, 922, 908, 878, 829. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.39 (d, 2H, J = 8.4 Hz, H_{arom}), 7.33 (d, 2H, J = 8.4 Hz, H_{arom}), 5.83 (ddd, 1H, J = 22.8 Hz, J = 11.2 Hz, J = 5.6 Hz, CH allyl), 5.48 (s, 1H, CH benzyldiene), 5.34 (t, 1H, J = 9.6 Hz, H-3), 5.27 (dd, 1H, J = 17.4 Hz, J = 1.4 Hz, C=CHH allyl), 5.20 (dd, 1H, J = 10.4, J = 1.4 Hz, C=CHH allyl), 5.05 (dd, 1H, J = 9.2 Hz, J = 8.0 Hz, H-2), 4.62 (d, 1H, J = 7.6 Hz, H-1), 4.38-4.32 (m, 2H, H-6a, CHH allyl), 4.07 (dd, 1H, J = 12.8 Hz, J = 6.4 Hz, CHH allyl), 3.80 (t, 1H, J = 10.4 Hz, H-6b), 3.70 (t, 1H, J = 9.6 Hz, H-4), 3.51 (dt, 1H, J = 9.6 Hz, J = 4.8 Hz, H-5), 2.71-2.68 (m, 2H, CH_2 Lev), 2.59-2.54 (m, 2H, CH_2 Lev), 2.15 (s, 3H, CH_3 Lev), 1.18 (s, 9H, 3x CH_3 Piv). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 206.0 (C=O Lev ketone), 177.0 (C=O Piv), 171.2 (C=O Lev), 135.5 (C_q C_{arom}), 135.0 (C_q C_{arom}), 133.2 (CH allyl), 128.5-127.5 (CH_{arom}), 118.1 (C=CH₂ allyl), 100.7 (C-1, CH benzyldiene), 78.6 (C-4), 71.7 (C-2 or C-3), 71.6 (C-2 or C-3), 70.7 (CH_2 allyl), 68.7 (C-6), 66.4 (C-5), 38.9 (C_q Piv), 37.9 (CH_2 Lev), 29.9 (CH_3 Lev), 28.0 (CH_2 Lev), 27.1 (CH_3 Piv). HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{26}\text{H}_{33}\text{ClO}_9\text{Na}$: 547.17053, found 547.17029.



Allyl 4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-pivaloyl- β -D-glucopyranoside (11) To a stirred solution of allyl glucoside **10** (210 mg, 0.38 mmol) in argon-flushed pyridine/AcOH (4/1, 4 mL, 0.1M),

hydrazine hydrate (0.11 mL, 2.0 mmol, 5 eq.) was added and the mixture was stirred for 30 minutes at room temperature. The reaction was quenched with acetone, diluted with 1M HCl solution and extracted three times with DCM. The combined organic layers were washed with brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. Column chromatography (PE/EtOAc: 9/1 $\rightarrow$ 4/1) gave the product in 96% yield. $[\alpha]_D$: -68.6 (c = 1, DCM). IR (neat, cm^{-1}): 3470, 3368, 2974, 2963, 2932, 2874, 1746, 1722, 1493, 1477, 1458, 1398, 1371, 1352, 1281, 1267, 1229, 1182, 1171, 1152, 1130, 1088, 1074, 1047, 1013, 980, 926, 885, 812. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.42 (d, 2H, J = 8.4 Hz, H_{arom}), 7.32 (d, 2H, J = 8.4 Hz, H_{arom}), 5.84 (ddd, 1H, J = 22.8 Hz, J = 11.2 Hz, J = 5.6 Hz, CH allyl), 5.49 (s, 1H, CH benzyldiene), 5.27 (dd, 1H, J = 17.2 Hz, J = 1.6 Hz, C=CHH allyl), 5.19 (d, 1H,

$J = 10.4$ Hz, $J = 1.4$ Hz, C=CHH allyl), 4.91 (t, 1H, $J = 8.8$ Hz, H-2), 4.55 (d, 1H, $J = 7.6$ Hz, H-1), 4.33 (m, 2H, H-6a, CHH allyl), 4.06 (dd, $J = 13.0$ Hz, $J = 6.2$ Hz, CHH allyl), 3.85 (t, 1H, $J = 9.2$ Hz, H-3), 3.76 (t, 1H, $J = 10.2$ Hz, H-6b), 3.57 (t, 1H, $J = 9.4$ Hz, H-4), 3.40 (dt, 1H, $J = 9.6$ Hz, $J = 4.8$ Hz, H-5), 1.22 (s, 9H, 3x CH₃ Piv). ¹³C NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 178.2 (C=O Piv), 135.6-135.2 (C_q C_{arom}), 133.4 (CH allyl), 128.6-127.9 (CH_{arom}), 118.0 (CH=CH₂ allyl), 101.1 (CH benzylidene), 100.5 (C-1), 81.1 (C-4), 74.4 (C-2), 72.7 (C-3), 70.5 (CH₂ allyl), 68.7 (C-6), 66.2 (C-5), 39.1 (C_q Piv), 27.2 (CH₃ Piv). HRMS: [M+Na]⁺ calcd for C₂₁H₂₇ClO₇Na: 449.13375, found 449.13362.



Allyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-[4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-levulinoyl-2-*O*-pivaloyl- β -D-glucopyranosyl]-2-*O*-pivaloyl- β -D-glucopyranoside (12)

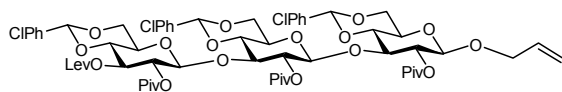
To a stirred solution of donor **9** (250 mg, 0.38 mmol, 1.2 eq.) and acceptor **11** (136 mg, 0.32 mmol) in DCM (10 mL, 0.03M) were added 3Å MS. After 30 minutes at 0 °C, TfOH (2.6 μ L, 0.03 mmol) was added and the mixture was gradually warmed to room temperature. After 4 hours TLC analysis (DCM/acetone: 49/1, v/v) indicated complete conversion of the starting material and the reaction was quenched with TEA (0.3 mL) and washed with brine. The organic phase was dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by size-exclusion chromatography (DCM/MeOH: 1/1) gave the product as a white solid in 99% yield (0.29 g, 0.32 mmol). $[\alpha]_D^{25}$: -51.6 (c = 0.9, DCM). IR (neat, cm⁻¹): 2974, 2932, 2911, 2874, 1742, 1721, 1603, 1495, 1481, 1462, 1398, 1369, 1302, 1275, 1230, 1207, 1180, 1125, 1088, 1059, 1034, 1017, 1003, 941, 881, 818. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.45 (d, 2H, $J = 8.4$ Hz, H_{arom}), 7.35-7.31 (m, 6H, H_{arom}), 5.82 (ddd, 1H, $J = 22.0$ Hz, $J = 10.8$ Hz, $J = 6.0$ Hz, CH allyl), 5.53 (s, 1H, CH benzylidene), 5.28-5.12 (m, 3H, C=CH₂ allyl, H-3'), 5.10 (s, 1H, CH benzylidene'), 5.04 (t, 1H, $J = 7.8$ Hz, H-2), 4.96 (t, 1H, $J = 6.4$ Hz, H-2'), 4.87 (d, 1H, $J = 6.0$ Hz, H-1'), 4.52 (d, 1H, $J = 7.6$ Hz, H-1), 4.37-4.29 (m, 2H, H-6a, CHH allyl), 4.25 (dd, 1H, $J = 10.4$ Hz, $J = 4.8$ Hz, H-6a'), 4.14 (t, 1H, $J = 8.8$ Hz, H-3), 4.00 (dd, 1H, $J = 12.8$ Hz, $J = 6.0$ Hz, CHH allyl), 3.88 (t, 1H, $J = 9.4$ Hz, H-4'), 3.78 (t, 1H, $J = 10.4$ Hz, H-6b), 3.74 (t, 1H, $J = 9.6$ Hz, H-4), 3.64 (t, 1H, $J = 10.2$ Hz, H-6b'), 3.52-3.46 (m, 2H, H-5, H-5'), 2.73-2.55 (m, 4H, 2x CH₂ Lev), 2.16 (s, 3H, CH₃ Lev), 1.24-1.17 (m, 18H, 6x CH₃ Piv). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.1 (C=O Lev ketone), 177.0 (C=O Piv), 176.3 (C=O Piv, C=O Lev), 135.8 (C_q C_{arom}), 135.4 (C_q C_{arom}), 135.3 (C_q C_{arom}), 134.9 (C_q C_{arom}), 133.2 (CH allyl), 128.7 (CH_{arom}), 128.5 (CH_{arom}), 128.0 (CH_{arom}), 127.7 (CH_{arom}), 118.0 (C=CH₂ allyl), 101.1 (CH benzylidene), 100.5 (C-1), 100.3 (CH benzylidene'), 98.9 (C-1'), 78.8 (C-4), 77.9 (C-4'), 75.1 (C-3), 73.8 (C-2), 72.4 (C-2' or C-3'), 72.2 (C-2' or C-3'), 70.3 (CH₂ allyl), 68.9 (C-6 or C-6'), 68.7 (C-6 or C-6'), 66.5 (C-5 or C-5'), 66.0 (C-5 or C-5'), 38.9 (C_q Piv), 37.9 (CH₂ Lev), 30.0 (CH₃ Lev), 28.0 (CH₂ Lev), 27.2 (CH₃ Piv). HRMS: [M+Na]⁺ calcd for C₄₄H₅₄Cl₂O₁₅Na: 915.27320, found 915.27375.



1-*O*-allyl-4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-[4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-pivaloyl- β -D-glucopyranosyl]-2-*O*-pivaloyl- β -D-glucopyranoside (13)

To a solution of disaccharide **12** (0.19 g, 0.21 mmol) in argon-flushed pyridine/AcOH (4/1, v/v, 2 mL, 0.1M) was added argon-flushed hydrazine hydrate (0.057 mL, 1.0 mmol, 5 eq.). After TLC analysis (DCM/acetone: 19/1, v/v) indicated complete conversion of the starting product, the reaction mixture was quenched with acetone, diluted with EtOAc, washed with 1M HCl (3x) and brine. The organics were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 9/1 $\rightarrow$ 7/3, v/v) afforded the title compound in 94% yield (0.15 g, 0.20 mmol). $[\alpha]_D^{25}$: -30.8 (c = 0.5, DCM). IR (neat, cm⁻¹): 3447, 2974, 2934, 2907, 2874, 1738, 1603, 1495, 1479, 1462, 1398, 1369, 1302, 1277, 1229, 1209, 1171, 1132, 1090, 1059, 1034, 1016, 1003, 941, 930, 883, 816. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.44 (d, 2H, $J = 8.4$ Hz, H_{arom}), 7.33-7.27 (m, 6H, H_{arom}), 5.82 (ddd, 1H, $J = 22.0$ Hz, $J = 10.6$ Hz, $J = 5.6$ Hz, CH allyl), 5.51 (s, 1H, CH benzylidene), 5.26 (dd,

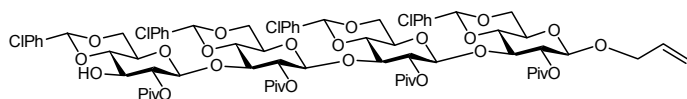
1H, $J = 17.2$ Hz, $J = 1.6$ Hz, CH=CHH allyl), 2.19 (dd, 1H, $J = 10.4$ Hz, $J = 1.2$ Hz, CH=CHH allyl), 5.10 (s, 1H, CH benzylidene), 5.06 (t, 1H, $J = 8.0$ Hz, H-2), 4.91 (d, 1H, $J = 5.6$ Hz, H-1'), 4.83 (t, 1H, $J = 5.6$ Hz, H-2''), 4.53 (d, 1H, $J = 7.2$ Hz, H-1), 4.37-4.29 (m, 2H, H-6a, CHH allyl), 4.23 (dd, 1H, $J = 10.2$ Hz, $J = 4.8$ Hz, H-6a'), 4.15 (t, 1H, $J = 8.8$ Hz, H-3), 4.01 (dd, 1H, $J = 12.8$ Hz, $J = 6.4$ Hz, CHH allyl), 3.85-3.71 (m, 4H, H-3', H-4, H-4', H-6b), 3.63 (t, 1H, $J = 10.4$ Hz, H-6b'), 3.53-3.46 (m, 2H, H-5, H-5'), 2.78 (bs, 1H, 3-OH'), 1.23-1.20 (m, 18H, 6x CH₃ Piv). ¹³C NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 178.3 (C=O Piv), 176.4 (C=O Piv), 135.8 (C_q C_{arom}), 135.5 (C_q C_{arom}), 135.3 (C_q C_{arom}), 135.1 (C_q C_{arom}), 133.2 (CH allyl), 128.6 (CH_{arom}), 128.5 (CH_{arom}), 127.9 (CH_{arom}), 127.7 (CH_{arom}), 117.9 (C=CH₂ allyl), 101.1 (CH benzylidene), 100.5 (CH benzylidene), 100.4 (C-1), 98.7 (C-1'), 80.4 (C-4 or C-4'), 78.9 (C-4 or C-4'), 75.2, 75.1 (C-2', C-3), 73.9 (C-2), 73.0 (C-3'), 70.2 (CH₂ allyl), 68.8 (C-6 of C-6'), 68.7 (C-6 or C-6'), 66.4 (C-5 or C-5'), 65.8 (C-5 or C-5'), 38.9 (C_q Piv), 38.9 (C_q Piv), 27.1 (CH₃ Piv), 27.1 (CH₃ Piv). HRMS: [M+Na]⁺ calcd for C₃₉H₄₈Cl₂O₁₃Na: 817.23642, found 817.23677.



Allyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-[4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-{4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-levulinoyl-2-*O*-

pivaloyl-β-D-glucopyranosyl}-2-*O*-pivaloyl-β-D-glucopyranosyl]-2-*O*-pivaloyl-β-D-

glucopyranoside (14) To a stirred solution of donor **9** (131 mg, 0.20 mmol) and acceptor **13** (110 mg, 0.14 mmol) in DCM (4 mL, 50mM) were added 3Å MS at 0 °C. After 30 minutes, TfOH (2 μL, 0.02 mmol) was added and the mixture was warmed to room temperature. After two hours, TLC analysis (DCM/acetone: 19/1, v/v) indicated consumption of the starting material, and the reaction was stopped by addition of TEA (0.2 mL). The organic phase was washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by size-exclusion chromatography (DCM/MeOH, 1/1) gave the title trisaccharide in 80% yield (141 mg, 0.11 mmol). [α]_D: -104.4 (c = 1, DCM). IR (neat, cm⁻¹): 2972, 2932, 2911, 2872, 1738, 1603, 1495, 1479, 1462, 1398, 1369, 1319, 1302, 1275, 1207, 1180, 1123, 1088, 1051, 1032, 1015, 99, 941, 912, 880, 814. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.54 (d, 2H, $J = 8.4$ Hz, H_{arom}), 7.36-7.26 (m, 6H, H_{arom}), 7.23 (d, 2H, $J = 8.0$ Hz, H_{arom}), 7.18 (d, 2H, $J = 8.4$ Hz, H_{arom}), 5.82 (ddd, $J = 22.4$ Hz, $J = 10.8$ Hz, $J = 5.6$ Hz, CH allyl), 5.62 (s, 1H, CH benzylidene), 5.40 (s, 1H, CH benzylidene), 5.34 (t, 1H, $J = 9.0$ Hz, H-3''), 5.28-5.17 (m, 2H, C=CH₂ allyl), 5.05-4.98 (m, 3H, H-2, H-1'', H-2''), 4.92 (d, 1H, $J = 2.4$ Hz, H-1'), 4.79 (bs, 1H, H-2'), 4.50 (d, 1H, $J = 7.8$ Hz, H-1), 4.41 (s, 1H, CH benzylidene), 4.39-4.26 (m, 4H, H-6a, H-4', H-6a'', CHH allyl), 4.20 (t, 1H, $J = 9.2$ Hz, H-3), 4.10 (dd, 1H, $J = 9.8$ Hz, $J = 4.8$ Hz, H-6a'), 4.01 (dd, $J = 12.8$ Hz, $J = 6.4$ Hz, CHH allyl), 3.79 (t, 1H, $J = 10.4$ Hz, H-6b), 3.76-3.71 (m, 3H, H-3', H-4'', H-6b''), 3.67 (t, 1H, $J = 9.4$ Hz, H-4), 3.57-3.51 (m, 4H, H-5, H-5', H-5'', H-6b'), 2.71-2.68 (m, 2H, CH₂ Lev), 2.59-2.55 (m, 2H, CH₂), 2.14 (s, 3H, CH₃), 1.41-1.17 (m, 27H, 9x CH₃ Piv). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.0 (C=O Lev ketone), 177.1 (C=O Piv), 177.0 (C=O Piv), 176.2 (C=O Piv), 171.7 (C=O Lev), 135.8-134.7 (C_q C_{arom}), 133.2 (CH allyl), 128.7-127.5 (CH_{arom}), 118.1 (C=CH₂ allyl), 101.8 (CH benzylidene), 100.7 (C-1, CH benzylidene), 99.5 (CH benzylidene), 99.0 (C-1''), 96.7 (C-1'), 79.2 (C-4), 78.6 (C-4''), 77.2 (C-3'), 76.6 (C-4'), 74.3 (C-2''), 73.4 (C-2'), 72.6 (C-3), 72.2 (C-3''), 71.7 (C-2), 70.5 (CH₂ allyl), 68.9-68.7 (C-6, C-6', C-6''), 66.8-65.0 (C-5, C-5', C-5''), 39.0-38.8 (C_q Piv), 37.9 (CH₂ Lev), 29.9 (CH₃ Lev), 28.0 (CH₂ Lev), 27.4-27.1 (CH₃ Piv). HRMS: [M+Na]⁺ calcd for C₆₂H₇₅Cl₃O₂₁Na: 1283.37746, found 1283.37586.

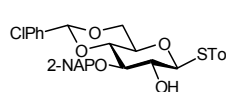


Allyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-[4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-{4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-

***O*-(4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-**

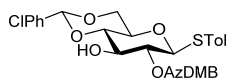
glucopyranosyl}-2-*O*-pivaloyl-β-D-glucopyranosyl]-2-*O*-pivaloyl-β-D-glucopyranoside (16): The RV was charged with functionalized Merrifield polystyrene **15** (100 mg, 45 μmol) and prepared for the

synthesis using protocol C. Then, the coupling/deprotection cycle was run to couple donor **9** four times. After the synthesis was complete, protocol J was used to isolate the resin. After cleavage with Grubbs first-generation catalyst and filtration over a plug of silica, preparative RP-HPLC yielded the pure tetrasaccharide in 44% yield (18 mg, 22.6 μ mol). $[\alpha]_D$: -20 (c = 0.26, DCM). IR (neat, cm^{-1}): 2972, 2934, 2909, 2872, 1740, 1603, 1495, 1479, 1462, 1398, 1371, 1300, 1275, 1261, 1207, 1173, 1123, 1126, 1088, 1051, 1034, 1016, 1001, 941, 912, 881, 816. ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.57 (d, 2H, J = 8.4 Hz, CH arom), 7.37-7.24 (m, 12H, H_{arom}), 7.19 (d, 2H, J = 8.4 Hz, H_{arom}), 5.83 (ddd, 1H, J = 22.0 Hz, J = 10.8 Hz, J = 6.0 Hz, CH allyl), 5.74 (s, 1H, CH benzyldiene), 5.29-5.17 (m, 3H, CH benzyldiene, $\text{C}=\text{CH}_2$ allyl), 5.03 (t, 1H, J = 8.4 Hz, H-2), 4.98 (d, 1H, J = 4.8 Hz, H-1'), 4.94-4.91 (m, 2H, H-1'', H-2'), 4.90 (d, 1H, J = 3.2 Hz, H-1'''), 4.86 (s, 1H, CH benzyldiene), 4.83-4.80 (m, 2H, H-2'', H-2'''), 4.62 (s, 1H, CH benzyldiene), 4.51 (d, 1H, J = 7.6 Hz, H-1), 4.38 (dd, 1H, J = 10.4 Hz, J = 4.8 Hz, H-6a), 4.35-4.20 (m, 4H, CHH allyl, H-3, 2x H-6), 4.16-4.14 (m, 2H, 2x H-6), 4.06-3.99 (m, 2H, CHH allyl, H-4'), 3.92 (dd, 1H, J = 4.8 Hz, J = 8.6 Hz, H-3'), 3.86-3.79 (m, 4H, H-3'', H-3''', H-4, 2x H-6), 3.68-3.44 (m, 7H, H-4'', H-4''', 4x H-5, H-6), 1.28-1.18 (m, 36H, 12x CH_3 Piv). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 178.4 ($\text{C}=\text{O}$ Piv), 176.9 ($\text{C}=\text{O}$ Piv), 176.7 ($\text{C}=\text{O}$ Piv), 176.4 ($\text{C}=\text{O}$ Piv), 136.0 (C_q Carom), 135.8 (C_q Carom), 135.7 (C_q Carom), 135.6 (C_q Carom), 135.2 (C_q Carom), 135.1 (C_q Carom), 135.0 (C_q Carom), 133.3 (CH allyl), 128.7 (CH_{arom}), 128.6 (CH_{arom}), 128.5 (CH_{arom}), 128.4 (CH_{arom}), 127.9 (CH_{arom}), 127.8 (CH_{arom}), 127.8 (CH_{arom}), 118.1 ($\text{C}=\text{CH}_2$ allyl), 101.5 (CH benzyldiene), 100.9 (CH benzyldiene), 100.7 (CH benzyldiene), 100.5 (CH benzyldiene), 100.2 (C-1), 99.6 (C-1'' or C-1'''), 97.3 (C-1'), 97.0 (C-1'' or C-1'''), 80.7 (C-4'' or C-4'''), 79.0 (C-3'' or C-3''' or C-4), 77.9 (C-4'), 77.5 (C-3), 76.8 (C-4'' or C-4'''), 75.3 (C-3'' or C-3''' or C-4), 74.8 (C-2', C-2'' or C-2'''), 74.4 (C-2), 74.2, 74.0 (2x C-2' or C-2'' or C-2'''), 73.0 (C-3'' or C-3''' or C-4), 72.6 (C-3), 70.5 (CH_2 allyl), 68.9, 68.9, 68.8, 68.7 (4x C-6), 66.9, 65.8, 65.6, 65.5 (4x C-5), 39.0-38.9 (4x C_q Piv), 27.4, 27.4, 27.4, 27.1 (4x CH_3 Piv). HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{75}\text{H}_{94}\text{Cl}_4\text{O}_{25}\text{N}$: 1548.48635, found 1548.48791.



***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-(naphthalen-2-methyl)-1-thio-**

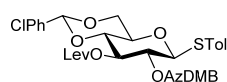
β -D-glucopyranoside (17): Glucoside **2** (7.0 g, 25.7 mmol) was suspended in toluene (64 mL, 0.4M) followed by addition of Bu_2SnO (6.40 g, 25.7 mmol, 1 eq.). The reaction mixture was heated to reflux and stirred for 2 h. The reaction mixture was cooled to rT , concentrated, and co-concentrated with toluene. The obtained white solid was dissolved in DMF (86 mL, 0.3M) followed by addition of 2-(bromomethyl)naphthalene (6.85 g, 30.9 mmol, 1.2 eq.) and CsF (5.08 g, 33.4 mmol, 1.3 eq.). The reaction mixture was stirred overnight. A white suspension was obtained. The reaction mixture was diluted with EtOAc , washed with H_2O , brine, dried over MgSO_4 , filtered, and concentrated. A white solid was obtained, which was recrystallized from EtOH . The title compound was obtained as a white powder in 69% yield over 2 steps (9.7 g, 17.63 mmol). Melting point: 172 $^{\circ}\text{C}$. $[\alpha]_D$: -33 (c = 1, DCM). IR (neat, cm^{-1}): 3300, 2912, 1367, 1265, 1107, 1085, 1008, 810, 734, 705, 611. ^1H NMR (300 MHz, CDCl_3 , HH-COSY, HSQC): 7.92-7.63 (m, 4H, H_{arom}), 7.53-7.34 (m, 9H, H_{arom}), 7.12 (d, 2H, J = 8.1 Hz, H_{arom}), 5.52 (s, 1H, CH chlorobenzylidene), 5.03 (d, 1H, J = 12.0 Hz, CHH naphthylmethyl), 4.96 (d, 1H, J = 12.0 Hz, CHH naphthylmethyl), 4.55 (d, 1H, J = 9.6 Hz, H-1), 4.36 (dd, 1H, J = 10.5 Hz, J = 4.8 Hz, H-6a), 3.79-3.42 (m, 5H, H-6b, H-2, H-3, H-4, H-5), 2.57 (d, 1H, J = 2.8 Hz, C-2-OH), 2.34 (s, 3H, CH_3 STol). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 135.7 (C_q Carom), 133.8 (CH_{arom}), 129.8 (CH_{arom}), 128.4 (C_q Carom), 128.2-125.9 (CH_{arom}), 100.6 (CH benzyldiene), 88.8 (C-1), 81.4 (C-3 or C-4), 80.9 (C-3 or C-4), 74.8 (CH_2 naphthylmethyl), 72.4 (C-2), 70.6 (C-5), 68.6 (C-6), 21.2 (CH_3 STol). HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{31}\text{H}_{30}\text{ClO}_5\text{S}$ 549.14970, found 549.14958.



***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-(4-azido-2,2-dimethylbutanoyl)-**

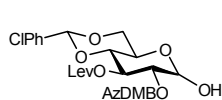
1-thio- β -D-glucopyranoside (18): 4-Azido-2,2-dimethylbutanoic acid (1.90 g, 12.14 mmol, 2 eq. relative to glucoside) was dissolved in DCM (30.4 mL, 0.4M)

followed by addition of oxalyl chloride (2.60 mL, 30.4 mmol, 2.5 eq. relative to 4-Azido-2,2-dimethylbutanoic acid). The reaction mixture was heated to reflux and stirred for 1 h. The reaction mixture was concentrated and co-evaporated with toluene (2x) and chloroform (1x). The obtained acid chloride was dissolved in pyridine (30.4 mL, 0.2M) and cooled to 0 °C, followed by slow addition of glucoside **17** (3.25 g, 6.07 mmol). The reaction mixture was heated to 110 °C and the reaction mixture was stirred overnight. TLC analysis (hexane/ EtOAc: 3/1, v/v) showed total conversion into a higher running spot (hexane/EtOAc: 3/1, v/v, R_f 0.81) and the reaction mixture was cooled to rT, concentrated in vacuo and co-concentrated with toluene (2x). The obtained yellowish oil (crude **17a**) was dissolved in a DCM/H₂O mixture (9/1, 91.1 mL, 0.1M) and the resulting mixture was cooled to 0 °C. DDQ (3.10 g, 13.67 mmol, 1.5 eq.) was added and the reaction mixture was allowed to warm to rT. After 1 h., TLC analysis showed total conversion into a lower running spot (hexane/EtOAc: 3/1, R_f 0.75). The reaction mixture was quenched by addition of Na₂S₂O₃ (aq., sat.), diluted with DCM, followed by separation of the layers. The organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated. A yellow oil was obtained. Column chromatography (pentane/EtOAc: 1/0 → 7/3) gave the title compound as a colorless oil, which crystallized on standing. Glucoside **18** was obtained in 78% yield over 2 steps (3.20 g, 4.75 mmol). $[\alpha]_D$: -34 (c = 1, DCM). IR (neat, cm⁻¹): 2926, 2096, 1737, 1263, 1089, 813, 731, 702, 665. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.40-7.24 (m, 6H, H_{arom}), 7.13-7.11 (m, 2H, H_{arom}), 5.49 (s, 1H, CHPh chlorobenzylidene), 4.92 (t, 1H, J = 10.0 Hz, H-2), 4.767 (d, 1H, J = 10.0 Hz, H-1), 4.32 (dd, 1H, J = 10.8 Hz, J = 5.2 Hz, H-6a), 3.89 (t, 1H, J = 9.6 Hz, H-3), 3.73 (t, 1H, J = 10.4 Hz, H-6b), 3.51 (t, 1H, J = 9.6 Hz, H-4), 3.45-3.36 (m, 3H, H-5, CH₂ AzDMB), 3.14 (bs, 1H, OH), 2.33 (s, 3H, CH₃ STol), 2.00-1.80 (m, 2H, CH₂ AzDMB), 1.27 (s, 3H, CH₃ AzDMB), 1.23 (s, 3H, CH₃ AzDMB). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 175.6 (C=O), 138.4 (C_q C_{arom}), 135.3-134.8 (C_q C_{arom}), 133.0 (CH_{arom}), 129.7-128.3 (CH_{arom}), 128.2 (C_q C_{arom}), 128.2-127.7 (CH_{arom}), 100.8 (CHPh chlorobenzylidene), 86.8 (C-1), 80.2 (C-4), 73.3 (C-3), 72.2 (C-2), 70.1 (C-5), 68.2 (C-6), 47.8 (CH₂ AzDMB), 40.9 (C_q AzDMB), 38.7 (CH₂ AzDMB), 25.6 (CH₃ AzDMB), 24.6 (CH₃ AzDMB), 21.0 (CH₃ STol). HRMS: [M+H]⁺ calcd for C₂₆H₃₁ClN₃O₆S: 548.16166, found 548.16149.



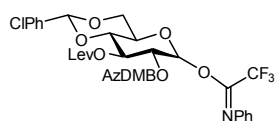
***p*-Tolyl 4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-(4-azido-2,2-dimethylbutanoyl)-3-*O*-levulinyl-1-thio-β-D-glucopyranoside (**19**):**

Glucoside **18** (2.34 g, 6.25 mmol) was dissolved in DCM (31 mL, 0.2M) followed by addition of LevOH (823 μL, 8.13 mmol, 1.3 eq.), DIC (950 μL, 8.13 mmol, 1.3 eq.) and DMAP (cat.). The reaction mixture was stirred for 15 min, after which TLC analysis showed total conversion into a slightly lower running spot (PE/EtOAc: 3/1, v/v, R_f 0.63). The reaction mixture was filtered, diluted with DCM, washed with H₂O, brine, dried over MgSO₄, filtered, and concentrated. A white solid was obtained. Column chromatography (hexane/EtOAc: 1/0 → 7/3) gave the title compound as a white solid in 90% yield (3.48 g, 5.63 mmol). $[\alpha]_D$: -112 (c = 2). IR (neat, cm⁻¹): 2098, 1745, 1718, 1421, 1367, 1265, 1089, 1014, 731, 702, 663. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.36-7.26 (m, 6H, H_{arom}), 7.15-7.13 (m, 2H, H_{arom}), 5.45 (s, 1H, CH benzylidene), 5.35 (t, 1H, J = 9.2 Hz, H-3), 5.02 (t, 1H, J = 9.6 Hz, H-2), 4.74 (d, 1H, J = 10.0 Hz, H-1), 4.36 (dd, 1H, J = 10.8 Hz, J = 5.2 Hz, H-6a), 3.77 (t, 1H, J = 10.4 Hz, H-6b), 3.64 (t, 1H, J = 9.6 Hz, H-4), 3.56-3.50 (m, 1H, H-5), 3.39-3.33 (m, 2H, CH₂ AzDMB), 2.93-2.66 (m, 2H, CH₂ Lev), 2.59-2.50 (m, 2H, CH₂ Lev), 2.35 (s, 3H, CH₃ STol), 2.13 (s, 3H, CH₃ Lev), 1.94-1.86 (m, 6H, 2x CH₃ AzDMB). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 205.7 (C=O Lev ketone), 175.2 (C=O AzDMB), 171.6 (C=O Lev), 137.8 (C_q C_{arom}), 135.2 (C_q C_{arom}), 134.8 (C_q C_{arom}), 133.3 (CH_{arom}), 128.3 (CH_{arom}), 127.8 (C_q C_{arom}), 127.6 (CH_{arom}), 100.5 (CH benzylidene), 87.0 (C-1), 78.2 (C-4), 72.8 (C-3), 70.3 (C-5), 70.2 (C-2), 68.3 (C-6), 47.6 (CH₂ AzDMB), 41.1 (C_q AzDMB), 38.3 (CH₂ AzDMB), 37.5 (CH₂ Lev), 29.7 (CH₃ Lev), 27.7 (CH₂ Lev), 25.3 (CH₃ AzDMB), 25.0 (CH₃ AzDMB), 21.1 (CH₃ STol). HRMS: [M+K]⁺ calcd for C₃₁H₃₆ClN₃O₈SK: 684.15405, found 684.15432.



4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-(4-azido-2,2-dimethylbutanoyl)-3-*O*-levulinyl- α/β -D-glucopyranose (20**):** Glucoside **19** (372 mg, 0.60 mmol) was dissolved in freshly distilled DCM (3 mL, 0.2M) and the reaction mixture was

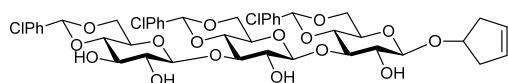
stirred under argon and cooled to 0 °C. NIS (148 mg, 0.66 mmol, 1.1 eq.) and TFA (51 μ L, 0.66 mmol, 1.1. eq.) were added. TLC analysis (PE/EtOAc: 12/8, v/v) did not show any R_f difference but an UV positive spot (indicating *p*-thiocresol being formed) was observed. After 1 h., the reaction mixture was cooled to 0 °C, followed by piperidine (178 μ L, 1.80 mmol, 3 eq.). After 30 min., TLC analysis showed total conversion of the anomeric trifluoroacetate into a lower running spot (PE/EtOAc: 12/8, v/v, R_f 0.41). The reaction mixture was quenched by subsequent addition of triethylamine (reaction mixture turned from dark purple to yellow) and Na₂S₂O₃ (aq., sat.) (yellow reaction mixture turned colorless). The reaction mixture was diluted with DCM, washed with H₂O, dried over MgSO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 4/6) gave hemiacetal **20** as an anomeric mixture (α/β : 1/0.6) and a colourless oil in 86% yield (278 mg, 0.51 mmol). IR (neat, cm⁻¹): 2096, 1720, 1419, 1365, 1265, 1089, 1014, 815, 732, 702. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.40-7.36 (m, 3.2H, H_{arom}), 7.33-7.28 (m, 3.2H, H_{arom}), 5.64 (t, 1H, J = 9.6 Hz, H-3 α), 5.48 (s, 0.6H, CH benzylidene β), 5.45 (s, 1H, CH benzylidene α), 5.36 (t, 0.6H, J = 9.6 Hz, H-2 β), 4.91 (t, 0.6H, J = 0.6H, H-3 β), 4.82-4.79 (m, 1.6H, H-1 β , H-2 α), 4.34 (dd, 0.6H, J = 10.4 Hz, J = 4.8 Hz, H-6a β), 4.27 (dd, 1H, J = 10.4 Hz, J = 4.8 Hz, H-6a α), 4.16-4.10 (m, 1H, H-5 α), 3.79-3.67 (m, 1.6H, H-6a α , H-6b β), 3.67-3.62 (m, 1.6H, H-4 α , H-4 β), 3.56-3.52 (m, 0.6H, H-5 β), 3.34-3.26 (m, 3.2H, CH₂ AzDMB α , CH₂ AzDMB β), 2.76-2.69 (m, 3.2H, CH₂ Lev α , CH₂ Lev β), 2.60-2.55 (m, 3.2H, CH₂ Lev α , CH₂ Lev β), 2.15 (s, 4.8H, CH₃ Lev α , CH₃ Lev β), 2.04-1.88 (m, 1.6H, CHH AzDMB α , CHH AzDMB β), 1.86-1.76 (m, 1.6H, CHH AzDMB α , CHH AzDMB β), 1.21 (9.6 H, 2x CH₃ AzDMB α , 2x CH₃ AzDMB β). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 206.3 (C=O Lev ketone α), 206.1 (C=O Lev ketone β), 176.7 (C=O AzDMB β), 176.4 (C=O AzDMB α), 171.6 (C=O Lev α , C=O Lev β), 135.4-134.7 (CH_{arom}), 128.3-127.5 (CH_{arom}), 100.5 (CH benzylidene α), 100.5 (CH benzylidene β), 96.0 (C-1 β), 90.5 (C-1 α), 78.9 (C-4 α), 78.4 (C-4 β), 73.8 (C-2 β), 72.1 (C-2 α), 71.7 (C-3 β), 68.7 (C-3 α), 68.6 (C-6 α), 68.2 (C-6 β), 66.4 (C-5 β), 62.2 (C-5 α), 47.8 (CH₂ AzDMB α), 47.6 (CH₂ AzDMB β), 41.1 (C_q AzDMB β), 40.8 (C_q AzDMB α), 38.4 (CH₂ AzDMB α , CH₂ AzDMB β), 37.6 (CH₂ Lev α), 37.5 (CH₂ Lev β), 29.7 (CH₃ Lev α), 29.7 (CH₃ Lev β), 27.7 (CH₂ Lev), 27.6 (CH₂ Lev), 25.3 (CH₃ AzDMB α), 24.4 (CH₃ AzDMB β). HRMS: [M+H]⁺ calcd for C₂₄H₃₁ClN₃O₉: 540.17433, found 540.17418.



4,6-*O*-(*p*-chlorobenzylidene)-2-*O*-(4-azido-2,2-dimethylbutanoyl)-3-*O*-levulinyl-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)- α/β -D-glucopyranoside (21**):** Hemiacetal **20** (1.71 g, 3.17 mmol) was dissolved in

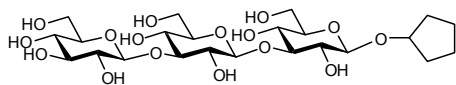
acetone (21.1 mL, 0.15M), and the reaction mixture was cooled to 0 °C. *N*-phenyl-trifluoroacetimidoyl chloride (0.66 mL, 4.78 mmol, 1.5 eq.) and Cs₂CO₃ (1.03 g, 3.17 mmol, 1 eq.) were added. The reaction mixture was allowed to warm to rT. After 1 h., TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 3/1, v/v, R_f 0.41). The reaction mixture was filtered over celite and concentrated. Column chromatography (hexane/EtOAc + 1% TEA: 1/0 $\rightarrow$ 7/3) gave the title compound as an anomeric mixture (α/β : 1/2) and as a transparent oil in 96% yield (1.99 g, 3.03 mmol). IR (neat, cm⁻¹): 2096, 1745, 1716, 1367, 1321, 1267, 1207, 1153, 1116, 1087, 1014, 906, 815, 777, 734, 694, 665. ¹H NMR (400 MHz, 50 °C, CDCl₃, HH-COSY, HSQC): 7.41-7.24 (m, 9H, H_{arom}), 7.14-7.10 (m, 1.5H, H_{arom}), 6.83-6.77 (m, 3H, H_{arom}), 6.51 (s, 0.5H, H-1 α), 5.86 (s, 1H, H-1 β), 5.67 (t, 0.5H, J = 10.0 Hz, H-3 α), 5.49 (s, 0.5H, CH benzylidene α), 5.46 (s, 1H, CH *p*-chlorobenzylidene β), 5.33 (t, 1H, J = 9.2 Hz, H-3 β), 5.24 (t, 1H, J = 8.0 Hz, H-2 β), 5.10 (dd, 0.5H, J = 10.0 Hz, J = 4.0 Hz, H-2 α), 4.37-4.31 (m, 1.5 H, H-6a α , H-6a β), 4.04-3.95 (m, 0.5H, H-5 α), 3.80-3.72 (m, 3H, H-4 α , H-4 β , H-6b α , H-6b β), 3.50 (m, 1H, H-5 β), 3.31-.26 (m, 3H, CH₂ AzDMB α , CH₂ AzDMB β), 2.71-2.66 (m, 3H, CH₂ Lev α , CH₂ Lev β), 2.61-2.56 (m, 2H, CH₂ Lev α , CH₂ Lev β),

2.18 (s, 4.5H, CH₃ Lev α , CH₃ Lev β). ¹³C APT NMR (100 MHz, 50 °C, CDCl₃, HH-COSY, HSQC): 205.3 (C=O Lev ketone α , C=O Lev ketone β), 175.0 (C=O AzDMB α , C=O AzDMB β), 171.5 (C=O Lev α), 171.4 (C=O Lev ketone β), 142.8 (C_q C_{arom}), 135.3-135.0 (C_q C_{arom}), 128.8 (CH_{arom}), 128.3 (CH_{arom}), 127.7 (CH_{arom}), 127.6 (CH_{arom}), 124.8 (CH_{arom}), 124.6 (CH_{arom}), 119.3 (CH_{arom}), 119.1 (CH_{arom}), 100.9 (CH *p*-chlorobenzylidene α), 100.8 (CH *p*-chlorobenzylidene β), 94.7 (C-1 β), 92.3 (C-1 α), 78.5 (H-4 α), 78.0 (C-4 β), 71.6 (C-3 β), 71.1 (C-2 β), 70.2 (C-2 α), 68.9 (C-3 α), 68.3 (C-6 α), 68.2 (C-6 β), 66.9 (C-5 β), 64.8 (C-5 α), 47.7 (CH₂ AzDMB α), 47.6 (CH₂ AzDMB β), 41.3 (C_q AzDMB α , C_q AzDMB β), 38.4 (CH₂ AzDMB α), 38.2 (CH₂ AzDMB β), 37.6 (CH₂ Lev α , CH₂ Lev β), 29.6 (CH₃ Lev α), 29.5 (CH₃ Lev β), 29.8 (CH₂ Lev α , CH₂ Lev β), 25.0 (CH₃ AzDMB β), 24.8 (CH₃ AzDMB α). HRMS: [M+Na]⁺ calcd for C₃₂H₃₄ClF₃N₄O₉Na: 733.18586, found 733.18592



Cyclopentenyl 4,6-*O*-(*p*-chlorobenzylidene)-3-*O*-[(4,6-*O*-(*p*-chlorobenzylidene)-(3-*O*-(4,6-*O*-(*p*-chlorobenzylidene)- β -D-glucopyranosyl)]-(β -D-glucopyranosyl)- β -D-glucopyranoside (24):

The resin was washed with dry DCM (8x), suspended in DCM (4.5 mL, 0.01M) and purged with argon. Grubbs second-generation catalyst (~3 mg) was added and the resulting brown suspension was allowed to stand at rT for 2 h. The solution was filtered off and the resin was washed with DCM and MeOH. The filtrates were concentrated and the obtained residue was filtered over a plug of silica (DCM/MeOH, 99/1, v/v) and concentrated. The title compound was isolated as a white solid in 36% yield over 8 steps (15 mg, 16 μ mol). [α]_D: -6.0 (c = 0.1, ACN). IR (neat, cm⁻¹): 2924, 2854, 1421, 1379, 1265, 1172, 1074, 1014, 915, 732, 702, 621. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.43 (d, 6H, *J* = 8.0 Hz, H_{arom}), 7.34 (d, 6H, *J* = 8.4 Hz, H_{arom}), 5.71-5.69 (m, 1H, H-3 cyclopentenyl), 5.53 (s, 2H, 2x CHPh chlorobenzylidene), 5.50 (s, 1H, CHPh chlorobenzylidene), 4.65-4.57 (m, 3H, H-1 cyclopentenyl, 2x H-1), 4.50 (d, 1H, *J* = 8.0 Hz, H-1), 3.35 (dd, 1H, *J* = 10.8 Hz, *J* = 6.0 Hz, H-6a), 4.29-4.24 (m, 2H, 2x H-6a), 3.81-3.75 (m, 7H, 3x H-6b, 3x H-3, H-2), 3.69-3.54 (m, 5H, 2x H-2, 3x H-4), 3.49-3.38 (m, 3H, 3x H-5), 2.70-2.58 (m, 2H, 2x H-2 cyclopentenyl), 2.55-2.47 (m, 2H, 2x H-2 cyclopentenyl). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 135.5-135.0 (C_q C_{arom}), 130.9-127.4 (CH_{arom}, C-3 cyclopentenyl), 105.6 (C-1), 105.5 (C-1), 101.8 (C-1), 101.1 (CHPh chlorobenzylidene), 100.3 (CHPh chlorobenzylidene), 100.2 (CHPh chlorobenzylidene), 84.5, 83.5, 80.1, 78.7, 78.6, 75.2, 74.5, 73.9, 73.2 (3x C-2, 3x C-3, 3x C-4), 79.0 (C-1 cyclopentenyl), 68.5 (2x C-6), 68.3 (C-6), 66.9 (C-5), 66.8 (C-5), 66.6 (C-5), 40.2 (C-2 cyclopentenyl), 39.4 (C-2 cyclopentenyl). HRMS: [M+Na]⁺ calcd for C₄₅H₅₁Cl₃O₁₅Na: 959.18219, found 959.18249.



Cyclopentanylyl 3-*O*-[(3-*O*-(β -D-glucopyranosyl)]-(β -D-glucopyranosyl)- β -D-glucopyranoside (25):

Trisaccharide **24** (9 mg, 9.59 μ mol) was dissolved in a mixture of THF/H₂O/*tert*-BuOH (2/1/1, v/v/v, 1 mL, 0.01M). The reaction mixture was purged with argon followed by addition of Pd/C (cat.). After stirring under argon for 15 min., the reaction mixture was purged with H₂ (g) and stirred for 2 h. TLC analysis showed total conversion into a single lower running spot (DCM/MeOH, 3/1, v/v, R_f 0.2). The reaction mixture was filtered over a Whatmann filter, washed with H₂O, neutralized with ammonia,²⁰ and concentrated. The obtained residue was subjected to gel filtration (HW-40, 0.15M NH₄OAc in H₂O) and subsequent lyophilization. The deprotected trimer was obtained as a fine white powder in 91% yield (5.0 mg, 8.7 μ mol). ¹H NMR (600 MHz, 284 K, D₂O, HH-COSY, HSQC): 4.74 (d, 1H, *J* = 8.4 Hz, H-1), 4.72 (d, 1H, *J* = 7.8 Hz, H-1), 4.50 (d, 1H, *J* = 8.4 Hz, H-1), 4.42-4.40 (m, 1H, H-1 cyclopentanylyl), 3.90-3.87 (m, 2H, 2x H-6a), 3.75-3.66 (m, 5H, 3x H-6b, H-3, H-4), 3.52-3.38 (m, 9H, 2x H-2, 2x H-4, 2x H-3, 3x H-5), 3.31 (t, 1H, *J* = 7.8 Hz, H-2), 1.86-1.52 (m, 8H, 4x H-2 cyclopentanylyl, 2x H-3 cyclopentanylyl). ¹³C NMR (150 MHz, 284 K, D₂O, HH-COSY, HSQC): 103.8 (C-1), 103.5 (C-1), 101.6 (C-1), 85.0 (C-3 or C-4), 84.9 (C-3 or C-4), 83.4 (C-1 cyclopentanylyl), 77.0, 76.6, 76.5, 76.5, 74.4, 70.5, 69.1, 69.1 (C-2, 2x C-3, 3x C-4, 3x C-5),

74.3 (C-2), 74.0 (C-2), 61.7 (C-6), 61.7 (C-6), 61.6 (C-6), 33.4 , 32.6 (C-2, C-3 cyclopentanyl). HRMS: $[M+Na]^+$ calcd for $C_{23}H_{40}O_{16}Na$: 595.22086, found 595.22052

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20. If the reaction mixture is not neutralized before concentration, fragmentation can take place.

Chapter 6

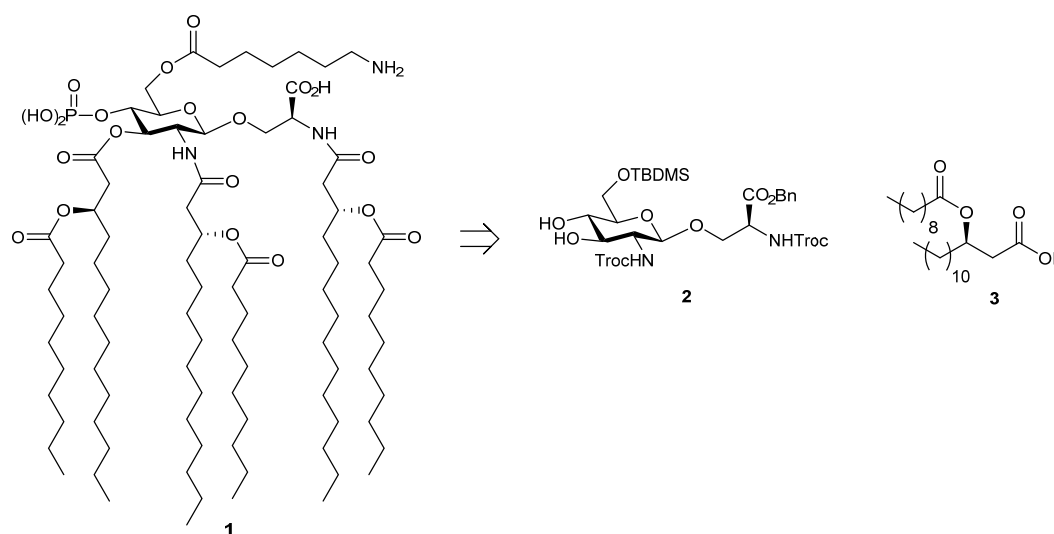
Towards a lipid A analogue suitable for conjugation

Introduction

Lipopolysaccharides (LPS) are found anchored in the outer membrane of Gram-negative bacteria and are responsible for a variety of pathophysiological activities in mammals. Stimulation of the innate immune system by LPS during a bacterial infection leads to physiological events and can eventually result in septic shock for which no treatment is available to date. Lipid A, the membrane anchor of LPS, is the active principle of LPS and structure-activity relationship studies opened the door for analogues with an improved activity profile (as discussed in Chapter 1).¹ The group of Johnson introduced a new class of potent monosaccharide immunomodulators, aminoalkyl glucosamine 4-phosphates (AGPs), in which the reducing end glucosamine of lipid A is replaced for a N-acylated aminoalkyl residue.² The flexible AGP motif can accommodate close packing of the fatty acid moieties, which promotes the interaction of the lipid chains into the hydrophobic pocket of the TLR4 accessory molecule MD-2. Seryl based AGPs with a N-acyloxyacyl residue at the anomeric center have also been reported³ and the carboxylate in these analogues serves as a stable substitute for the labile anomeric phosphate of lipid A, which interacts with positively charged

lysines at the edge of the hydrophobic pocket of the TLR4-MD2 complex.⁴ Lipid A derivatives with maintained immunostimulating properties and negligible toxicity can be a valuable asset for the development of new vaccine modalities. Relevant examples are the lipid A conjugates of Wang and coworkers discussed in Chapter 1. The favorable properties of the AGP immunomodulators of Johnson was an incentive to design AGP derivative **1** (Scheme 1) that can be used for the preparation of conjugates. The primary amine functionality in **1** allows the conjugation of molecules such as carbohydrate or peptide epitopes and other TLR ligands. In this Chapter, first the preparation of chiral fatty acid **3** is described. Then the synthesis is continued with the incorporation of these chiral fatty acids in glucosamine **2** followed by deprotection to obtain an AGP building block for conjugation.

Scheme 1. Retrosynthetic overview of the synthesis of lipid A analogue **1**.

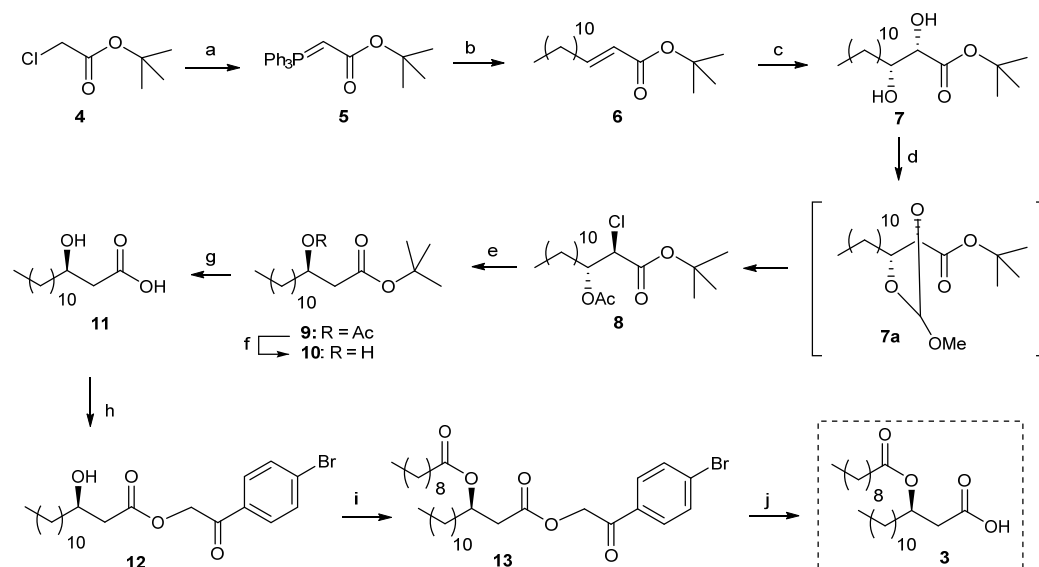


Results & Discussion

The synthesis of β -hydroxytetradecanoic acid **11**, the key building block towards lipid A derived fatty acids, has been reported by several groups.⁵ Exploring of some of these synthetic routes led to the choice for the synthesis as developed by Oikawa and Kusumoto.⁶ The 10-step route to chiral fatty acid **3** (Scheme 2) starts with the conversion of chlorine **4** into Horner-Wittig reagent **5** by a reaction with triphenylphosphine and subsequent treatment with base. Wittig reaction of acetate **5** with dodecyl aldehyde (or lauric aldehyde) yielded α,β -unsaturated ester **6**. The double bond in ester **6** was oxidized in an asymmetric dihydroxylation reaction, affording diol **7** as the single product. The desired chirality was confirmed by optical rotation and NMR analysis. For the removal of the α -hydroxyl group, the following steps were conducted. First, diol **7** was treated with TMSCl and trimethyl orthoacetate to give the orthoester intermediate **7a**, the formation of which can be detected by TLC analysis. This intermediate is subsequently attacked by a chlorine anion at the α -carbon next to the *tert*-butyl ester resulting in opening of the orthoester ring to give fatty acid ester **8**. The chlorine substituent is then reductively removed via a radical reaction with tributyltin hydride and azobisisobutyronitrile in toluene. The acetyl group is cleaved with an equimolar amount of sodium methoxide affording hydroxytetradecanoic acid ester **10**. To prevent acid mediated ester cleavage in a later stage of the synthesis, the *tert*-butyl ester was replaced for a 4-

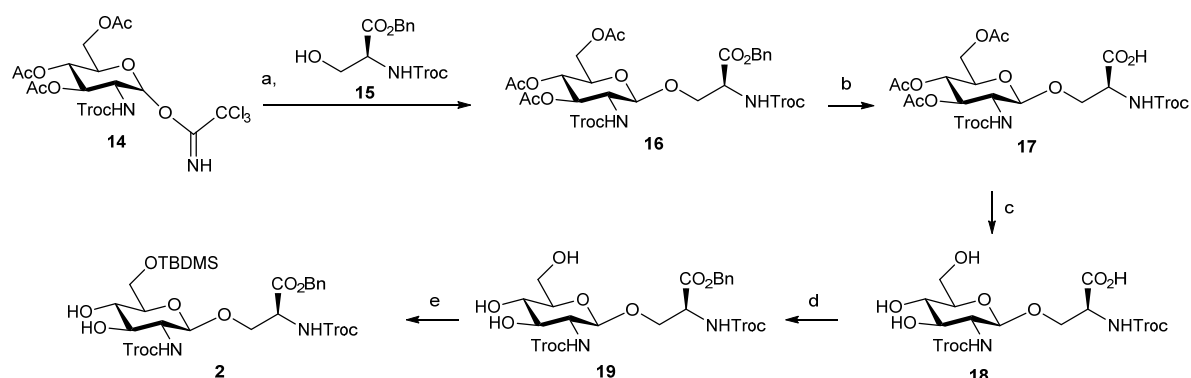
bromoacetophenone ester, that can be selectively removed under mild conditions. The *tert*-butyl ester was removed by treatment with TFA in DCM to give fatty acid **11**. Reprotection by treatment with 2,4'-dibromoacetophenone and triethylamine gave tetradecanoic ester **12**. Final steps in the synthetic route to target **3** entailed acylation with decanoyl chloride and pyridine followed by deprotection by zinc dust in acetic acid. The above described synthetic route provides access to chiral fatty acid **3** in good overall yield.

Scheme 2. Synthesis of chiral fatty acid **3**.



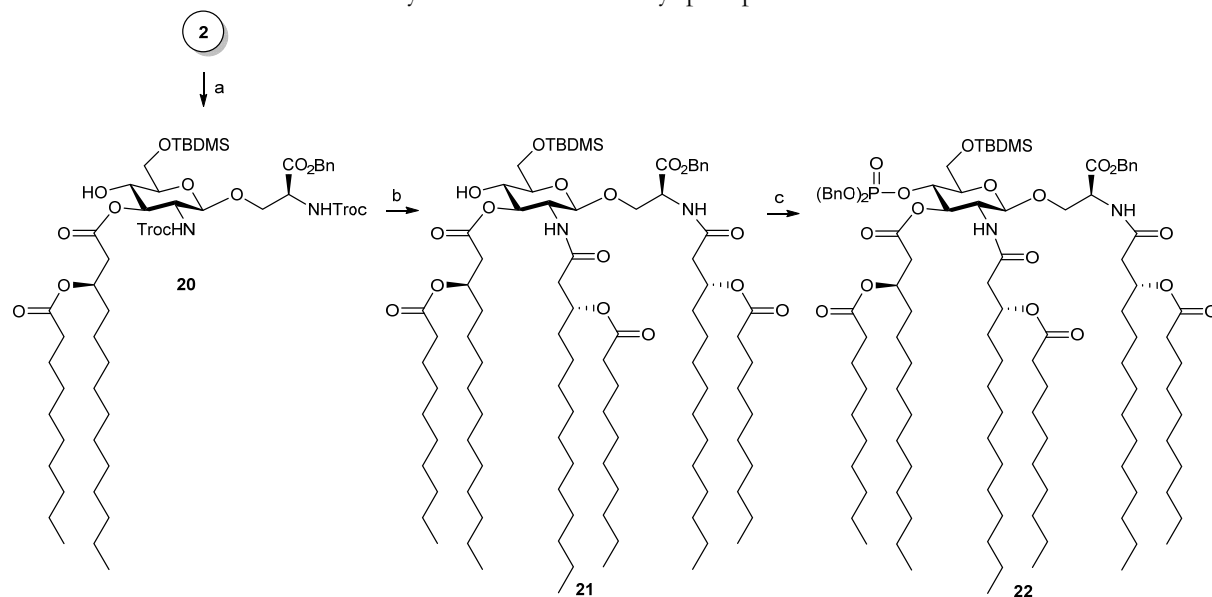
Reagents and conditions: (a) (i) triphenyl phosphine, toluene, 82°C, (ii) NaOH, H₂O, 86%; (b) dodecyl aldehyde, DCM, 93%; (c) AD-mix-β, methanesulfonamide, H₂O/*tert*-BuOH, 68%; (d) trimethyl orthoacetate, TMSCl, DCM, 63%; (e) Bu₃SnH, AIBN, toluene, reflux, 98%; (f) NaOMe, MeOH, 70%; (g) TFA, DCM, 85%; (h) 2,4'-dibromoacetophenone, TEA, EtOAc; (i) decanoyl chloride, pyridine; (j) Zn dust, AcOH, 60 °C, 80%/3 steps.

The next stage in the synthesis of lipid A analogue **1** entails the synthesis of glucosamine **2** (Scheme 3), amenable for the installation of the fatty acids. Glucosamine imidate **14**⁷ was glycosylated with Troc-protected serine **15**⁸ with the aid of triflic acid in DCM to give glucosamine **16** in 87%. This glycosylation gave reproducible yields (~87%) both on small scale (1 mmol) and large scale (up to 30 mmol). Then the acetyl groups were to be removed. Prior to deacetylation, the benzyl ester in the anomeric serine was removed in order to prevent any side reactions during the deacetylation of **16** with base. The benzyl ester was deprotected by hydrogenolysis with Pd/C in the presence of H₂ (g) to afford glucosamine **17**. Then the three acetyl groups were removed with ammonia in methanol to obtain triol **18**. Noteworthy, it was observed that removal of the acetyl groups with sodium methoxide can lead to cleavage of the Troc protective groups leading to inferior yields. Then the benzyl ester was restored by treatment of **18** with benzyl bromide using phase transfer conditions (DCM/NaHCO₃ (aq., sat.)) to give glucosamine **19** in 66% yield over 3 steps. Conveniently, these three reactions can be performed without intermediate column purification. The primary alcohol in **19** was protected with a *tert*-butyldimethylsilyl (TBDMS) group by treatment with TBDMSCl in pyridine.

Scheme 3. Synthesis of building block **18**.

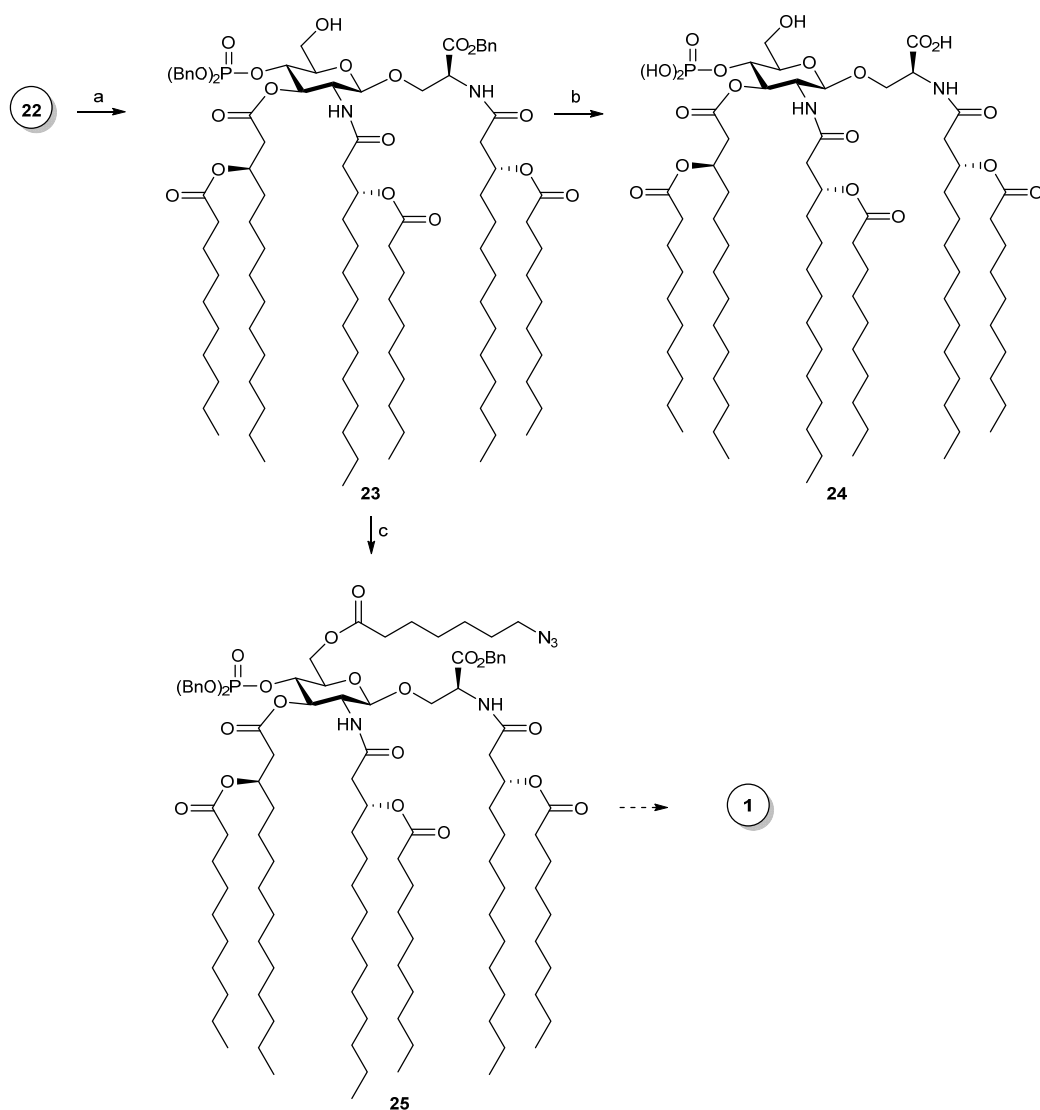
Reagents and conditions: (a) TfOH , DCM , $0\text{ }^\circ\text{C}$, 87%; (b) H_2 (g), Pd/C , EtOAc ; (c) NH_4OH , MeOH ; (d) BnBr , Bu_4NHSO_4 , $\text{DCM}/\text{NaHCO}_3$ (aq. sat.), 66%/3 steps; (e) TBDSMCl , pyridine, 83%.

Since the C-4 position of glucosamine is known to be of low reactivity⁹ silylated glucosamine **2** provides a suitable building block for the selective introduction of the fatty acid at the C-3 alcohol. Glucosamine **2** was treated with fatty acid **3** and EDC, DMAP in DCM at $0\text{ }^\circ\text{C}$ (Scheme 4) to provide glucosamine **20** in 69% yield. The reaction temperature turned out to be very important to attain regioselectivity, since the esterification at room temperature led to regio-isomeric mixtures with fatty acid esters at C-3 and C-4. For the installation of fatty acid **3** on the amine groups, the Troc protecting groups were cleaved with zinc dust in aqueous THF. After workup, the obtained diamine was treated with fatty acid **3** in the presence of EDC in DCM to afford glucosamine **21**. Although building block **21** can be obtained in good yield, the yields of this acylation turned out to be variable. To complete the synthesis of the fully protected lipid A analogue, the C-4 alcohol in **21** was phosphorylated by treatment with dibenzyl diisopropyl aminephosphoramidite and tetrazole in DCM . After 15 minutes, total conversion to the phosphite was observed as monitored by TLC analysis. Subsequent oxidation with *tert*-butyl hydroperoxide gave phosphotriester **22**.

Scheme 4. Introduction of the fatty acids and the dibenzyl-phosphate.

Reagents and conditions: (a) Fatty acid **3**, EDC·HCl, DCM, DMAP, 0 °C, 69%; (b) (i) Zn dust, AcOH, THF/H₂O, (ii) Fatty acid **3**, EDC·HCl, DCM, 60%/2 steps; (c) (i) (BnO)₂P(N*i*-Pr₂), tetrazole, DCM, 0 °C, (ii) *tert*-butyl hydroperoxide, 54%/2 steps.

In the final stage of the synthesis the azide-containing spacer was introduced and the removal of all protecting groups was undertaken (Scheme 5). To this end, the C-6 hydroxyl of glucosamine **22** was liberated via desilylation with TFA in DCM. Hydrogenation of the thus obtained alcohol **23** resulted in removal of the benzyl groups from the serine carboxylate and the C-4-O-phosphate. The obtained compound **24** is necessary as reference compound during the evaluation of the projected conjugates. To convert glucosamine **23** into the protected precursor of target **1**, the azide-containing spacer was introduced by reacting glucosamine **23** with 7-azidohexanoic acid¹⁰ in the presence of EDC and DMAP in DCM. The reaction turned out to be relatively slow. After 5 hours, total consumption of **23** was observed by TLC analysis and compound **25** was isolated in 46% yield. Unfortunately, an attempt to remove the protecting groups in **25** in a single hydrogenation event was unsuccessful and therefore, the synthesis of target compound **1** was not achieved. Exploration of other conditions are needed to attain suitable deprotection conditions.

Scheme 5. Deprotection and introduction of an azide-spacer.

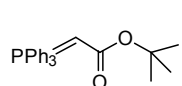
Reagents and conditions: (a) TFA, DCM, 0 °C, 85%; (b) (i) H₂, Pd/C, THF, (ii) Chelex sodium form, 84%, (c) 7-azidoheptanoic acid, EDC·HCl, DCM, 46%.

Conclusions

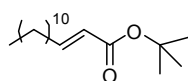
A lipid A analogue, AGP **1**, was designed with the aim to develop an immunostimulating lipid A analogue for conjugation. AGP **1** is provided with an amine-containing spacer allowing the conjugation with specific carbohydrate or peptide epitopes. En route to target **1** the synthesis of the chiral fatty acid **3** and AGP **24**, a reference compound for future immunological studies were achieved. In addition, 7-azidoheptanoyl functionalized AGP **25**, the precursor of target **1**, was synthesized. Unfortunately, the deprotection of **25** was unsuccessful to provide target **1**.

Experimental section

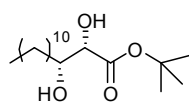
General experimental procedures. Chemical shifts (δ) are given in ppm relative to TMS as internal standard. All ^{13}C APT NMR spectra are proton decoupled. Reactions were performed at rT unless stated otherwise and were followed by TLC analysis with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulphuric acid in EtOH or with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g L^{-1}), followed by charring at 150 °C. Flash column chromatography was performed on silica gel (0.04–0.063 nm) and size exclusion chromatography (SEC) was performed on SephadexTM LH-20. Experiments which required an inert atmosphere were carried out under dry argon. Dichloromethane (p.a.) was distilled over P_2O_5 prior to use. Molecular sieves (3Å) were flame-dried before use.



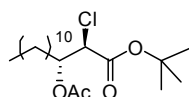
***tert*-Butyl (triphenylphosphoranylidene)acetate (5):** triphenyl phosphine (47.2 g, 0.18 mol, 1.13 eq.) was dissolved in toluene (200 mL, 0.9 M relative to triphenyl phosphine) followed by addition of *tert*-butyl chloroacetate (22.9 mL, 0.16 mol). The reaction mixture was heated to 82 °C¹¹ and stirred overnight. A white salt precipitated, which was filtered off, washed with toluene and dried in a vacuum oven at 50 °C overnight. The salt was dissolved in H_2O (1.2 L, 0.15M) and the mixture was cooled to 5 °C, followed by addition of 12M NaOH (14.9 mL, 179 mmol, 1.12 eq.). After 1 h., a sticky precipitate was formed. Rotating the flask on the rotavap at 50 °C resulted in the formation of white crystals which were filtered off and dried in the vacuum oven. The title compound was isolated as a white powder in 86% yield (51.8 g, 138 mmol). Analytic data were in agreement with reported data.



***tert*-Butyl (E)-2-tetradecanoate (6):** Dodecanal (10 mL, 45.08 mmol) and *tert*-butyl (triphenylphosphoranylidene)acetate (22.6 g, 59.16 mmol, 1.33 eq.) were dissolved in DCM (200 mL, 0.23M). TLC analysis showed total conversion into a slightly higher running spot (toluene/EtOAc: 3/1, v/v, R_f 0.72). The reaction mixture was concentrated and the residue was suspended in a mixture of Et_2O /hexane, filtered (removal of triphenylphosphine oxide) and concentrated. The obtained oil was further purified by column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 19/1 $\rightarrow$ 9/1) to give the title compound as a transparent oil in 93% yield (11.86 g, 41.98 mmol). Analytic data were in agreement with reported data.¹²

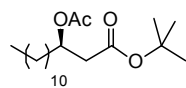


***tert*-Butyl (2*S*, 3*R*)-2,3-dihydroxytetradecanoate (7):** A mixture of *tert*-BuOH/ H_2O (186 mL, 0.2M) was prepared and to this the following chemicals were subsequently added: potassium ferricyanide (III) (36.79 g, 111.8 mmol, 3 eq.), K_2CO_3 (15.44 g, 111.8 mmol, 3 eq.), [(DHQD)₂PHAL] (290 mg, 0.37 mmol, 0.01 eq.), aq. OsO_4 (0.14 M, 0.54 mL, 75 μmol , 0.002 eq.), and methanesulfonamide (3.54 g, 37.3 mmol, 1 eq.). The reaction mixture was thoroughly stirred for 30 minutes, followed by addition of *tert*-butyl (E)-2-tetradecanoate (10.52 g, 37.3 mmol) in DCM¹³ (4.1 mL, 9M). After 7 hours, TLC analysis showed total conversion into a lower running product (PE/EtOAc: 4/1, v/v, R_f 0.25).¹⁴ The reaction was quenched by addition of $\text{Na}_2\text{S}_2\text{O}_3$ (56 g, 443.3 mmol, 12 eq.). The reaction mixture turned yellow and was extracted with EtOAc (2x). The combined organic layers were washed with 2M KOH, dried over MgSO_4 , filtered, and concentrated. A yellow residue was obtained. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 8/2) gave the diol **4** in 68% yield (8.07 g, 25.5 mmol). Analytic data were in agreement with reported data.¹²

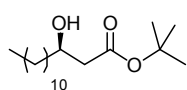


***tert*-Butyl (2*S*, 3*R*)-3-acetoxy-2-chlorotetradecanoate (8):** *tert*-Butyl (2*S*, 3*R*)-2,3-dihydroxytetradecanoate **7** (20.4 g, 64.4 mmol) was dissolved in DCM (430 mL, 0.15M) followed by addition of trimethyl orthoacetate (12.4 mL, 96.6 mmol, 1.5

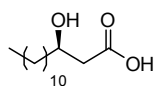
eq.) and TMSCl (12.4 mL, 96.6 mmol, 1.5 eq.). TLC analysis showed first conversion into a higher running spot (PE/EtOAc: 4/1, R_f 0.45, **4a**), which was further converted into a higher running spot (R_f 0.6) after 6 hours. The reaction mixture was concentrated and the obtained yellow oil was purified by column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 19/1) to give the title compound in 63% yield (17.5 g, 46.5 mmol). Analytic data were in agreement with reported data.¹²



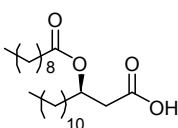
tert-Butyl (*R*)-3-acetoxytetradecanoate (9): Tetradecanoate **8** (9.5 g, 25.21 mmol) was dissolved in toluene (36 mL, 0.7M) followed by addition of tributyltin hydride (8.82 mL, 32.8 mmol, 1.3 eq.) and AIBN (207 mg, 1.26 mmol, 0.05 eq.). The reaction mixture was heated to reflux under argon. TLC analysis showed total conversion into a lower running spot (toluene/EtOAc: 19/1, v/v, R_f 0.56). The reaction mixture was cooled to room temperature and concentrated. The obtained transparent oil was purified by column chromatography (toluene/EtOAc: 1/0 $\rightarrow$ 8/2) yielding the title compound in 98% yield (8.49 g, 24.78 mmol). Analytic data were in agreement with reported data.¹²



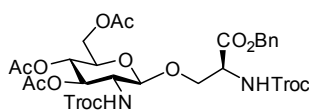
tert-Butyl (*R*)-3-hydroxytetradecanoate (10): Compound **9** (4.1 g, 11.97 mmol) was dissolved in MeOH (40 mL, 0.3M) and NaOMe (0.65 g, 11.97 mmol, 1 eq.) was added. After 5 hours, TLC analysis showed total conversion into a lower running spot (PE/EtOAc: 9/1, v/v, R_f 0.5). The reaction mixture was neutralized with Amberlite H⁺, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 $\rightarrow$ 19/1) gave alcohol **10** in 70% yield (2.87 g, 8.38 mmol). Analytic data were in agreement with reported data.¹²



(*R*)-3-hydroxytetradecanoic acid (11): *tert*-butyl (*R*)-3-hydroxytetradecanoate **10** (8.62 g, 28.7 mmol) was dissolved in DCM (57 mL, 0.5M) followed by slow addition of trifluoroacetic acid (11 mL, 143.5 mmol, 5 eq.). The reaction mixture was stirred overnight. TLC analysis showed total conversion into a lower running spot (toluene/EtOAc: 4/1, v/v, R_f 0.2). The reaction mixture was slowly quenched by addition of H₂O, extracted with EtOAc (2x). The combined organic layers were dried over MgSO₄, filtered, concentrated, and co-evaporated with toluene (2x). A yellow oil was obtained. Column chromatography (toluene/EtOAc: 1/0 $\rightarrow$ 6/4) gave the title compound as a white solid in 85% yield (5.93 g, 24.3 mmol). Analytic data were in agreement with reported data.¹²

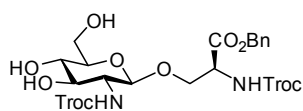


(*R*)-3-(tetradecanoyloxy)tetradecanoic acid (3): Tetradecanoic acid **11** (1.38 g, 5.66 mmol) was dissolved in EtOAc (28.3 mL, 0.2M) followed by addition of 2,4'-dibromoacetophenone (1.29 g, 5.66 mmol, 1 eq.) and TEA (0.79 mL, 5.66 mmol, 1 eq.). After 1 h., TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 1/1, v/v, R_f 0.85). The reaction mixture was diluted with DCM, washed with H₂O, dried over MgSO₄, filtered, and concentrated. The obtained residue was dissolved in pyridine (18.9 mL, 0.3 M) and the reaction mixture was cooled to 0 °C. Decanoyl chloride (1.32 mL, 6.79 mmol, 1.2 eq.) was added and the reaction mixture was allowed to warm to rT. After 30 min., TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 3/1, v/v, R_f 0.8). The reaction mixture was diluted with EtOAc, washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. The obtained yellow oil was dissolved in AcOH (14.2 mL, 0.4M) followed by addition of zinc (2.22 g, 34 mmol, 6 eq.). The reaction mixture was heated to 60 °C. After 4 h., TLC analysis showed total conversion into a lower running spot (hexane/EtOAc: 3/1, v/v, R_f 0.4). The reaction mixture was cooled to rT, filtered over celite, washed with DCM, and co-concentrated with toluene. A yellow oil was obtained, which was purified by column chromatography (hexane/EtOAc: 1/0 $\rightarrow$ 8/2). The title compound was obtained as a transparent oil in 80% yield over 3 steps (1.80 g, 4.53 mmol). Analytic data were in agreement with reported data.¹²



Benzyl *N*-(2,2,2-trichloro-ethoxycarbonylamino)-*O*-[3,4,6-*O*-acetyl-2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)-β-D-glucopyranosyl]-L-serinate (16):

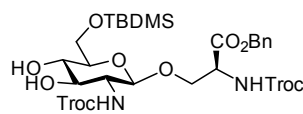
N-Troc-L-serine benzyl ester **15** (8.0 g, 21.6 mmol) and donor **14** (17.1 g, 27.39 mmol, 1.27 eq.) were co-evaporated with toluene (2x) and dissolved in DCM (216 mL, 0.1M). Activated MS 3Å were added and the reaction mixture was stirred for 30 min. Then, the reaction mixture was cooled to 0 °C, followed by addition of TfOH (0.24 mL, 2.74 mmol, 0.1 eq. relative to donor). The reaction mixture turned purple and was allowed to warm to rT. After 1 h., TLC analysis showed total conversion into a slightly lower running spot (PE/EtOAc: 1/1, v/v, R_f 0.46). The reaction mixture was neutralized with TEA, filtered, diluted with DCM, washed with H₂O, brine, dried over MgSO₄, filtered, and concentrated. A yellow oil was obtained. Column chromatography (PE/EtOAc: 1/0 → 6/4) gave the title compound as a white foam in 87% yield (15.6 g, 18.8 mmol). $[\alpha]_D^{25}$: + 8.4 (c = 0.62, DCM). IR (neat, cm⁻¹): 3313, 2954, 1737, 1523, 1456, 1433, 1367, 1336, 1222, 1168, 1099, 1080, 950, 815, 767, 732, 698, 634. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.40-7.28 (m, 5H, H_{arom}), 6.07 (d, 1H, J = 7.6 Hz, 1H, NH), 5.38 (d, 1H, J = 8.0 Hz, NH), 5.25-5.17 (m, 3H, H-4, CH₂ Bn), 5.03 (t, 1H, J = 9.6 Hz, H-3), 4.84-4.79 (m, 2H, 2x CHH Troc), 4.74-4.62 (m, 3H, 2x CHH Troc, H-1), 4.58-4.56 (m, 1H, H_{α} serine), 4.32-4.22 (m, 2H, H-6a, CHH serine), 4.11-4.09 (m, 1H, H-6b), 3.93-3.91 (m, 1H, CHH serine), 3.65-3.55 (m, 2H, H-2, H-5), 2.06 (s, 3H, CH₃ Ac), 2.03-2.00 (m, 6H, 2x CH₃ Ac). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 170.6 (C=O Ac at C-6), 169.4 (C=O Ac at C-3), 169.0 (C=O Ac at C-4), 154.3 (C=O Troc), 154.1 (C=O Troc), 135.0 (C_q C_{arom}), 128.6-128.3 (CH_{arom}), 100.6 (C-1), 95.3 (C_q Troc), 95.2 (C_q Troc), 74.6 (CH₂ Troc), 74.5 (CH₂ Troc), 71.9 (C-4), 71.5 (C-5), 68.9 (C_{β} serine), 68.3 (C-3), 67.7 (CH₂ CO₂Bn), 61.8 (C-6), 56.0 (C-2), 54.3 (C_{α} serine), 20.6 (CH₃ Ac), 20.5 (CH₃ Ac). ¹³C GATED NMR (100 MHz): 100.6 ($J_{C-1',H-1'} = 156$ Hz, C-1'). HRMS: $[M+H]^+$ calcd for C₂₈H₃₃Cl₆N₂O₁₄: 833.00428, found 833.00280.



Benzyl *N*-(2,2,2-trichloro-ethoxycarbonylamino)-*O*-[2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)-β-D-glucopyranosyl]-L-serinate (19):

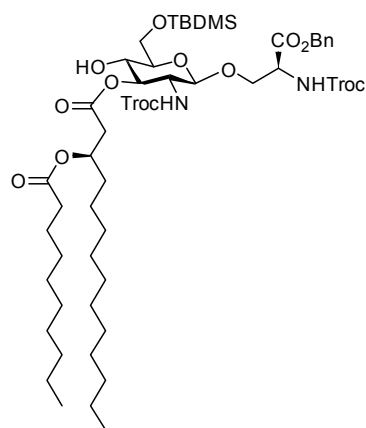
Serinate **16** (8.8 g, 10.6 mmol) was dissolved in EtOAc (35 mL, 0.3M) and the reaction mixture was purged with argon. Pd/C (cat.) was added and the reaction mixture was purged with H₂ (g). After 6 h., TLC analysis (PE/EtOAc, 1/1, v/v) showed total consumption of the starting material and the reaction mixture was filtered over a Whatmann filter and concentrated. The obtained residue was dissolved in MeOH (118 mL, 0.09M) followed by addition of NH₄OH (14.5M, 6.7 mL, 97.2 mmol, 9.2 eq.). After 2 d., TLC analysis showed total conversion of the starting material. The reaction mixture was concentrated. The obtained yellow oil was dissolved in a DCM/NaHCO₃ (aq. sat.) mixture (2/1, 53 mL, 0.2M). Bu₄NHSO₄ (356 mg, 1.06 mmol, 0.1 eq.) and benzyl bromide (3.8 mL, 31.8 mmol, 3 eq.) were added. The reaction mixture was stirred overnight. TLC analysis showed total conversion into a higher running spot (EtOAc/MeOH, 9/1, R_f 0.22). The layers were separated and the aq. layer was extracted with DCM. The combined organic layers were dried over Mg₂SO₄, filtered, and concentrated. Column chromatography (PE/EtOAc: 1/0 → 0/1, then: EtOAc/MeOH: 1/0 → 9/1) gave the title compound as a white solid in 66% yield over 3 steps (4.92 g, 6.96 mmol). $[\alpha]_D^{25}$: +24 (c = 0.3, DCM). IR (neat, cm⁻¹): 1728, 1722, 1263, 1165, 1074, 1049, 1035, 896, 732, 702, 673, 659. ¹H NMR (400 MHz, MeOD, HH-COSY, HSQC): 7.36-7.31 (m, 5H, H_{arom}), 5.23 (d, 1H, J = 12.4 Hz, CHH Bn), 5.17 (d, 1H, J = 12.4 Hz, CHH Bn), 4.85 (d, 1H, J = 11.6 Hz, CHH Troc), 4.78-4.77 (m, 2H, 2x CHH Troc), 4.72 (d, 1H, J = 11.6 Hz, CHH Troc), 4.50 (t, 1H, J = 4.0 Hz, C_{α} serine), 4.46 (d, 1H, J = 8.4 Hz, H-1), 4.25 (dd, 1H, J = 10.0 Hz, J = 5.2 Hz, H_{β} serine), 3.92-3.87 (m, 2H, H_{β} serine, H-6a), 3.68 (dd, 1H, J = 12.0 Hz, J = 5.6 Hz, H-6b), 3.46 (t, 1H, J = 8.4 Hz, H-3), 3.38 (t, 1H, J = 8.4 Hz, H-2), 3.30-3.25 (m, 2H, H-4, H-5). ¹³C APT NMR (100 MHz, MeOD, HH-COSY, HSQC): 171.2 (C=O), 156.6 (C=O Troc), 156.5 (C=O Troc), 137.0 (C_q C_{arom}), 129.5 (CH_{arom}), 129.2 (CH_{arom}), 129.1 (CH_{arom}), 102.8 (C-1), 96.8 (2x C_q Troc),

78.0 (C-5), 75.6 (2x CH₂ Troc), 75.5 (C-3), 71.9 (C-4), 69.5 (C_β serine), 68.2 (CH₂ Bn), 62.7 (C-6), 58.9 (C-2), 56.2 (C_α serine). HRMS: [M+H]⁺ calcd for C₂₂H₂₇Cl₆N₂O₁₁: 706.97110, found 706.97127.



***N*-(2,2,2-trichloroethoxy-carbonyl)-*O*-[3-*O*-[(*R*)-3-decanoyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-*L*-serine benzyl ester (**2**):**

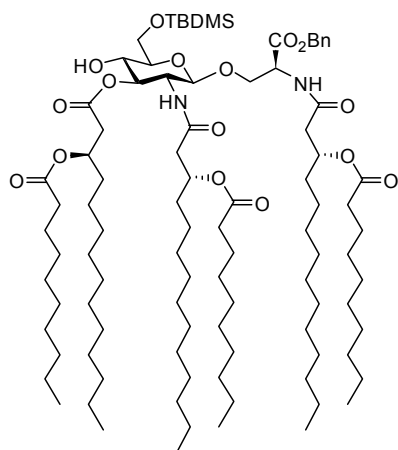
Serinate **19** (4.90 g, 6.93 mmol) was dissolved in pyridine (46 mL, 0.15M) followed by addition of TBDMSCl (1.57 g, 10.4 mmol, 1.5 eq.). After 2 h., TLC analysis showed total conversion into a higher running spot (PE/EtOAc: 1/3, v/v, R_f 0.64). The reaction mixture was diluted with EtOAc, washed with 1M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, concentrated, and co-concentrated with toluene (2x). Column chromatography (PE/EtOAc: 1/0 → 4/6) gave silylated **2** as a transparent oil in 83% yield (4.95 g, 5.73 mmol). [α]_D: +13.3 (c = 1.1 DCM). IR (neat, cm⁻¹): 3053, 1421, 1263, 894, 796, 731, 702, 648. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.37-7.35 (m, 5H, H_{arom}), 6.14 (d, 1H, *J* = 7.2 Hz, *NH*), 5.59 (d, 1H, *J* = 7.2 Hz, *NH*), 5.21 (s, 2H, CH₂ Bn), 4.82-4.70 (m, 4H, 2x CH₂ Troc), 4.57-4.54 (m, 2H, H-1, H_α serine), 4.24 (dd, 1H, *J* = 10.4 Hz, *J* = 4.4 Hz, H_β serine), 3.91-3.87 (m, 2H, H_β serine, H-6a), 3.84-3.80 (m, 1H, H-6b), 3.72-3.68 (m, 1H, H-3 or H-4), 3.55 (t, 1H, *J* = 8.8 Hz, H-3 or H-4), 3.34-3.31 (m, 2H, H-2, H-5), 0.89 (s, 9H, 3x CH₃ *tert*-butyl), 0.08 (s, 6H, 2x CH₃). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 169.2 (C=O CO₂Bn), 155.0 (C=O Troc), 154.5 (C=O Troc), 135.0 (C_q C_{arom}), 128.5 (CH_{arom}), 128.4 (CH_{arom}), 128.2 (CH_{arom}), 100.4 (C-1), 95.2 (2x C_q Troc), 74.7 (CH₂ Troc), 74.6 (CH₂ Troc), 74.1 (C-5, C-3 or C-4), 73.4 (C-3 or C-4), 68.5 (C_β serine), 67.6 (CH₂ CO₂Bn), 64.3 (C-6), 57.4 (C-2), 54.3 (C_α serine), 25.8 (3x CH₃ *tert*-butyl), -5.5 (2x CH₃ TBDMS). HRMS: [M+Na]⁺ calcd for C₂₈H₄₀Cl₆N₂O₁₁SiNa: 843.03952, found 843.03935.



***N*-(2,2,2-trichloroethoxy-carbonyl)-*O*-[6-*O*-*tert*-butyltrimethylsilyl-3-*O*-[(*R*)-3-decanoyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-*L*-serine benzyl ester (**20**):**

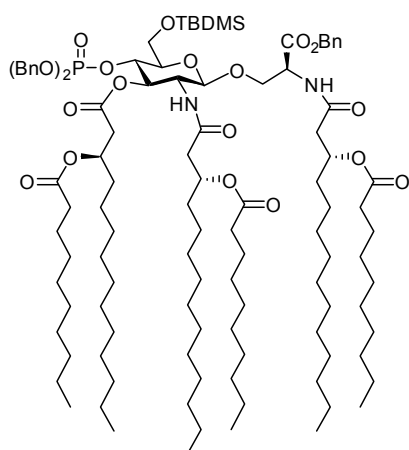
Compound **2** (345 mg, 0.43 mmol) was dissolved in DCM (4.3 mL, 0.1M), followed by addition of (*R*)-3-decanoyloxytetradecanoic acid **3** (197 mg, 0.49 mmol, 1.15 eq.). The reaction mixture was cooled to 0 °C. *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (99 mg, 0.52 mmol, 1.2 eq.) and DMAP (3 mg, 21.5 μmol, 0.05 eq.) were added. After 1 hour, TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 12/8, v/v, R_f 0.7). The reaction mixture was diluted, washed with 1 M HCl, NaHCO₃ (aq., sat.), dried over MgSO₄, filtered, and concentrated. A transparent oil was obtained. Purification by column chromatography (hexane/EtOAc: 1/0 → 8/2) gave the title compound as a transparent oil in 69% yield (359 mg, 0.30 mmol). [α]_D: -4.3 (c = 0.14, DCM). IR (neat, cm⁻¹): 2926, 2854, 1737, 1263, 1163, 1101, 1062, 835, 732, 702. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.36-7.33 (m, 5H, H_{arom}), 6.08 (d, 1H, *J* = 7.6 Hz, *NH* Serine), 5.48 (d, 1H, *J* = 7.2 Hz, *NH* GlcNTroc), 5.16 (s, 2H, CH₂ CO₂Bn), 5.6 (m, 1H, CH fatty acid), 4.96 (t, 1H, *J* = 10.0 Hz, H-3), 4.87-4.80 (m, 2H, 2x CHH Troc), 4.69 (d, 1H, *J* = 12.0 Hz, CHH Troc), 4.63 (d, 1H, *J* = 12.0 Hz, CHH Troc), 4.55-4.51 (m, 1H, H_α serine), 4.49 (d, 1H, *J* = 8.4 Hz, H-1), 4.32-4.31 (m, 1H, H_β serine), 3.90 (dd, 1H, *J* = 10.8 Hz, *J* = 4.8 Hz, H-6a), 3.85-3.81 (m, 2H, H_β serine, H-6b), 3.67 (t, 1H, *J* = 9.2 Hz, H-4), 3.58-3.54 (m, 2H, H-2, C-3-OH), 3.72-3.33 (m, 1H, H-5), 2.58-2.55 (m, 2H, CH₂ fatty acid), 2.31-2.27 (m, 2H, CH₂ fatty acid), 1.59-1.58 (m, 4H, 2x CH₂ fatty acid), 1.26-1.20 (m, 30H, 15x CH₂ fatty acid), 0.89-0.86 (m, 15H, 2x CH₃ fatty acid, 3x CH₃ *tert*-butyl TBDMS), 0.07-0.03 (m, 6H, 2x CH₃ TBDMS). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 174.2 (C=O), 171.5 (C=O), 169.1 (C=O), 154.2 (C=O amide),

135.2 (C_q C_{arom}), 128.6-128.1 (CH_{arom}), 101.3 (C-1), 95.5 (C_q Troc), 95.3 (C_q Troc), 75.2 (C-3), 74.7 (C-5), 74.6 (CH_2 Troc), 74.5 (CH_2 Troc), 70.9 (CH fatty acid), 70.7 (C-4), 68.6 (C_β serine), 67.5 (CH_2 CO_2Bn), 63.9 (C-6), 55.6 (C-2), 54.4 (C_α serine), 40.0 (CH_2 fatty acid), 34.5 (CH_2 fatty acid), 31.9 (CH_2 fatty acid), 29.6-29.1 (CH_2 fatty acid), 25.8 (CH_3 *tert*-butyl), 25.1 (CH_2 fatty acid), 25.0 (CH_2 fatty acid), 22.7 (CH_2 fatty acid), 22.7 (CH_2 fatty acid), 18.2 (CH_2 fatty acid), 14.1 (CH_3 fatty acid), -5.44 (CH_3 TBDMS), -5.48 (CH_3 TBDMS). HRMS: $[M+H]^+$ calcd for $C_{52}H_{85}Cl_6N_2O_{14}Si$: 1201.38662, found 1201.38783.



***N*-[*(R)*-3-decanoyloxytetradecanoyl]-*O*-[6-*O*-*tert*-butyldimethylsilyl]-2-deoxy-2-[*(R)*-3-decanoyloxytetradecanoylamino]-3-*O*-[*(R)*-decanoyloxytetradecanoyl]-β-D-glycopyranosyl]-L-serine benzyl ester (**21**):**

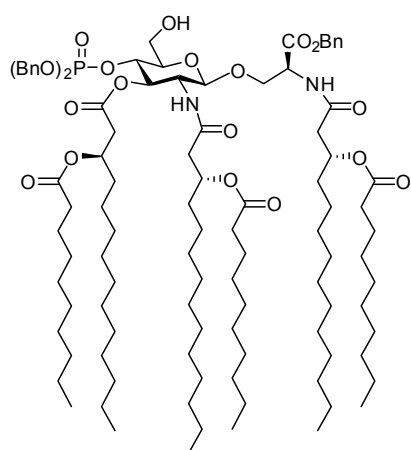
Compound **20** (336 mg, 0.28 mmol) was dissolved in 20% aq. THF (4.7 mL, 0.06M) followed by addition of zinc dust (183 mg, 2.8 mmol, 10 eq.) and acetic acid (35 μ mol, 0.62 mmol, 2.2 eq.). After 3 hours, TLC analysis showed total consumption of the starting material. The reaction mixture was filtered over celite, diluted with $CHCl_3$, washed with 0.1 M HCl, $NaHCO_3$ (aq., sat.), H_2O , dried over $MgSO_4$, filtered, and concentrated. A transparent oil was obtained, which was dissolved in DCM (5.6 mL, 0.05M). To this reaction mixture, fatty acid **3** (246 mg, 0.62 mmol, 2.2 eq.) and EDC·HCl (119 mg, 0.62 mmol, 2.2 eq.) were added. After 1h., TLC analysis (hexane/EtOAc: 12/8, v/v, R_f 0.68) showed total consumption of the starting material. The reaction mixture was diluted with $CHCl_3$, washed with 1M HCl, $NaHCO_3$ (aq., sat.), dried over $MgSO_4$, filtered and concentrated. The obtained transparent oil was purified by column chromatography (hexane/EtOAc: 1/0 $\rightarrow$ 3/1) to give the title compound as a transparent oil in 60% yield (278 mg, 0.17 mmol). $[\alpha]_D$: 7.14 ($c = 0.09$, $CHCl_3$). IR (neat, cm^{-1}): 2954, 2924, 2852, 1735, 1649, 1377, 1251, 1161, 1134, 1064, 837. ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): 7.34-7.23 (m, 5H, H_{arom}), 7.08 (d, 1H, $J = 7.6$ Hz, NH serine), 6.32 (d, 1H, $J = 8.4$ Hz, NH GlcNTroc), 5.20-5.11 (m, 5H, CH_2 CO_2Bn , 3x CH fatty acid), 4.98 (t, 1H, $J = 9.6$ Hz, H-3), 4.72-4.71 (m, 1H, H_α serine), 4.59 (d, 1H, $J = 8.0$ Hz, H-1), 4.22 (dd, 1H, $J = 10.8$ Hz, $J = 3.2$ Hz, H_β serine), 3.92-3.67 (m, 4H, H_β serine, H-6, H-2), 3.65 (t, 1H, $J = 9.2$ Hz, H-4), 3.36-3.33 (m, 1H, H-5), 2.69-2.50 (m, 4H, 2x CH_2 fatty acid), 2.49-2.26 (m, 3x CH_2 fatty acid), 1.70-1.60 (m, 8H, 4x CH_2 fatty acid), 1.60-1.26 (m, 90H, 45x CH_2 fatty acid), 0.90-0.86 (m, 27H, 6x CH_3 fatty acid, 3x CH_3 *tert*-butyl TBDMS), 0.07-0.02 (m, 6H, 2x CH_3 TBDMS). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC): 175.0 (C=O), 174.0 (C=O), 174.0 (C=O), 171.4 (C=O), 170.3 (C=O), 169.9 (C=O), 169.4 (C=O), 135.4 (C_q C_{arom}), 135.2 (C_q C_{arom}), 128.7-128.0 (CH_{arom}), 101.2 (C-1), 75.6 (C-3), 74.6 (C-5), 71.7 (CH fatty acid), 71.5 (CH fatty acid), 70.7 (CH fatty acid or C-4), 70.6 (CH fatty acid or C-4), 68.5 (C_β serine), 67.2 (CH_2 CO_2Bn), 64.0 (C-6), 54.1 (C-2), 52.7 (C_α serine), 42.0 (CH_2 fatty acid), 41.2 (CH_2 fatty acid), 40.0 (CH_2 fatty acid), 39.9 (CH_2 fatty acid), 38.7 (CH_2 fatty acid), 36.8-34.0 (CH_2 fatty acid), 31.9-31.8 (CH_2 fatty acid), 29.7-29.1 (CH_2 fatty acid), 25.6 (CH_3 *tert*-butyl TBDMS), 25.4-24.0 (CH_2 fatty acid), 22.7 (CH_2 fatty acid), 18.2 (CH_2 fatty acid), 14.2 (CH_3 fatty acid), -5.5 (CH_3 TBDMS), -5.5 (CH_3 TBDMS). HRMS: $[M+Na]^+$ calcd for $C_{94}H_{170}N_2O_{16}SiNa$: 635.22562, found 1635.22562.



***N*-[(*R*)-3-decanoyloxytetradecanoyl]-*O*-[6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-decanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-decanoyloxytetradecanoyl]-β-*D*-glucopyranosyl]-*L*-serine benzyl ester (**22**):**

Compound **21** (80 mg, 49.6 μmol) was dissolved in DCM (0.71 mL, 0.07M) followed by addition of dibenzyl diisopropylaminephosphoramidite (29 μL, 86.8 μmol, 1.75 eq.) and tetrazole (9 mg, 0.12 mmol, 2.5 eq.). After 15 min., TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 12/8, v/v, *R_f* 0.8). The reaction mixture was cooled to 0 °C, followed by dropwise addition of hydrogen peroxide (5.5M in decane, 25 μL, 139 μmol, 2.8 eq.). After 2 hours, TLC analysis showed total conversion into the phosphate

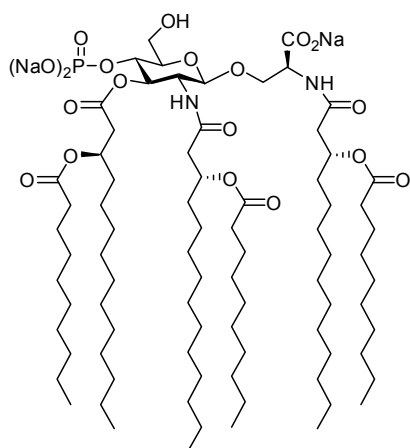
(hexane/EtOAc: 12/8, v/v, *R_f* 0.54). The reaction mixture was neutralized with NaHCO₃ (aq., sat.), diluted with DCM, washed with H₂O, dried over Na₂SO₄, filtered, and concentrated. Subsequent column chromatography (hexane/EtOAc: 1/0 → 7/3), size exclusion chromatography (THF), column chromatography (hexane/EtOAc: first 19/1, then 7/3) gave the title compound as a transparent oil in 54% yield (50 mg, 26.7 μmol). [α]_D: +48 (c = 0.1, DCM). IR (neat, cm⁻¹): 2953, 2922, 2852, 1734, 1654, 1543, 1379, 1247, 1213, 1163, 1105, 1078, 1014, 734. ¹H NMR (600 MHz, CDCl₃, HH-COSY, HSQC): 7.36-7.26 (m, 15H, H_{arom}), 7.11 (d, 1H, *J* = 7.8 Hz, NH serine), 6.39 (d, 1H, *J* = 7.2 Hz, NH GlcN), 5.32 (t, 1H, *J* = 9.0 Hz, H-3), 5.21-5.14 (m, 5H, CH₂ CO₂Bn, 3x CH fatty acid), 4.99-4.97 (m, 2H, 2x CHH Bn), 4.93-4.90 (m, 2H, 2x CHH Bn), 4.74-4.72 (m, 1H, H_α serine), 4.60 (d, 1H, *J* = 7.9 Hz, H-1), 4.36 (t, 1H, *J* = 9.0 Hz, H-4), 4.25 (dd, 1H, *J* = 11.4 Hz, *J* = 3.0 Hz, H_β serine), 3.90 (d, 1H, *J* = 10.8 Hz, H-6a), 3.81 (dd, 1H, *J* = 10.8 Hz, *J* = 2.4 Hz, H_β serine), 3.72 (dd, 1H, *J* = 12.0 Hz, *J* = 5.41 Hz, H-6b), 3.46-3.41 (m, 2H, H-2, H-5), 2.69-2.21 (m, 10H, 5x CH₂ fatty acid), 1.68-1.54 (m, 12H, 6x CH₂ fatty acid), 1.49-1.28 (m, 86H, 43x CH₂ fatty acid), 0.88-0.84 (m, 27H, 6x CH₃ fatty acid, 3x CH₃ *tert*-butyl TBDMS), -0.01- -0.03 (m, 6H, 2x CH₃ TBDMS). ¹³C APT NMR (150 MHz, CDCl₃, HH-COSY, HSQC): 173.7 (C=O), 173.6 (C=O), 173.5 (C=O), 170.9 (C=O), 170.2 (C=O), 169.4 (C=O), 169.4 (C=O), 135.6-135.2 (C_q C_{arom}), 128.4-127.8 (CH_{arom}), 100.3 (C-1), 75.3, 75.3 (2x C-5), 73.9, 73.8 (2x C-4), 72.9 (C-3), 71.3 (CH fatty acid), 71.1 (CH fatty acid), 71.0 (CH fatty acid), 69.6-69.4 (CH₂ Bn phosphate), 68.9 (C_β serine), 67.1 (CH₂ CO₂Bn), 61.9 (C-6), 55.8 (C-2), 52.8 (C_α serine), 41.6 (CH₂ fatty acid), 41.0 (CH₂ fatty acid), 39.6 (CH₂ fatty acid), 34.7-34.5 (CH₂ fatty acid), 31.9-31.8 (CH₂ fatty acid), 29.8-29.2 (CH₂ fatty acid), 25.9 (CH₃ *tert*-butyl TBDMS), 25.4-25.0 (CH₂ fatty acid), 22.7-22.7 (CH₂ fatty acid), 18.3 (CH₂ fatty acid), 14.6 (CH₃ fatty acid), -5.2 (CH₃ TBDMS), -5.3 (CH₃ TBDMS). ³¹P NMR (400 MHz, CDCl₃): -1.66. HRMS: [M+Na]⁺ calcd for C₁₀₈H₁₈₃N₂O₁₉PSiNa: 1895.28477, found 1895.28822.



***N*-[(*R*)-3-decanoyloxytetradecanoyl]-*O*-[2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-decanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-decanoyloxytetradecanoyl]-β-*D*-glucopyranosyl]-*L*-serine benzyl ester (**23**):**

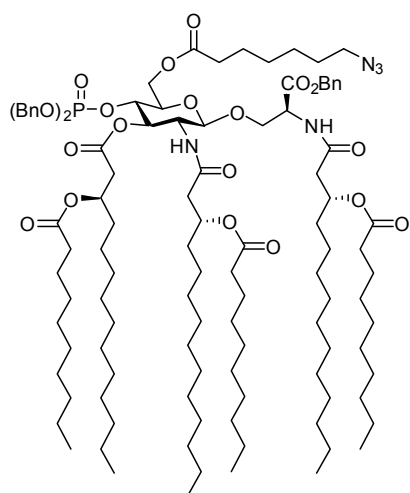
Serinate **22** (19 mg, 10 μmol) was dissolved in DCM (0.67 mL, 0.015 M). The reaction mixture was cooled to 0 °C, followed by addition of TFA (8 μL, 0.1 mmol, 10 eq.). The reaction mixture was allowed to warm to room temperature. TLC analysis showed total conversion into a lower running spot (hexane/EtOAc: 1/1, v/v, *R_f* 0.6). The reaction mixture was cooled to 0 °C and neutralized by slow addition of NaHCO₃, followed by separation of the layers. The organic layer

was dried over Na₂SO₄, filtered, and concentrated. A transparent oil was obtained, which was purified by column chromatography (hexane/EtOAc: 1/0 → 1/1), yielding the title compound as a transparent oil in 85% yield (15 mg, 8.5 μmol). [α]_D: +21.6 (c = 0.1, DCM/MeOH, 1/1). IR (neat, cm⁻¹): 2953, 2924, 2852, 1734, 1662, 1463, 1456, 1377, 1263, 1249, 1203, 1159, 1105, 1014, 655, 636. ¹H NMR (600 MHz, CDCl₃, HH-COSY, HSQC): 7.38-7.29 (m, 15H, H_{arom}), 7.01 (d, 1H, *J* = 7.8 Hz, *NH* serine), 6.31 (d, 1H, *J* = 7.2 Hz, *NH* GlcN), 5.30 (t, 1H, *J* = 9.2 Hz, H-3), 5.22-5.05 (m, 5H, 3x CH fatty acid, CH₂ CO₂Bn), 5.03-4.89 (m, 5H, 2x CH₂ dibenzyl phosphate, H-1), 4.72-4.71 (m, 1H, H_α serine), 4.42 (dd, 1H, *J* = 9.0 Hz, *J* = 9.0 Hz, H-4), 4.23 (dd, 1H, *J* = 11.4 Hz, *J* = 3.0 Hz, H_β serine), 3.81 (dd, 1H, *J* = 11.4 Hz, *J* = 3.0 Hz, H_β serine), 3.77-3.75 (m, 2H, H-6), 3.53-3.50 (m, 1H, H-2), 3.34-3.33 (m, 1H, H-3), 2.54-2.21 (m, 10H, 5x CH₂ fatty acid), 1.66-1.59 (m, 10H, 5x CH₂ fatty acid), 1.43-1.13 (m, 88H, 44x CH₂ fatty acid), 0.89-0.87 (m, 18H, 6x CH₃ fatty acid). ¹³C APT NMR (150 MHz, CDCl₃, HH-COSY, HSQC): 173.8 (C=O), 173.8 (C=O), 173.4 (C=O), 169.9 (C=O), 169.9 (C=O), 169.5 (C=O), 169.5 (C=O), 135.4-135.2 (C_q C_{arom}), 128.2-124.0 (CH_{arom}), 119.1 (CH_{arom}), 100.5 (C-1), 74.8, 74.8 (2x C-5), 73.1, 73.0 (2x C-4), 72.3, 72.3 (2x C-3), 71.3 (CH fatty acid), 71.0 (CH fatty acid), 70.1 (CH₂ dibenzyl phosphate), 70.1 (CH₂ dibenzyl phosphate), 70.0 (CH fatty acid), 68.9 (C_β serine), 67.3 (CH₂ CO₂Bn), 60.4 (C-6), 55.4 (C-2), 52.8 (C_α serine), 41.7 (CH₂ fatty acid), 39.3 (CH₂ fatty acid), 35.1 (CH₂ fatty acid), 34.6-34.4 (CH₂ fatty acid), 31.9-31.4 (CH₂ fatty acid), 30.3-29.1 (CH₂ fatty acid), 25.4 (CH₂ fatty acid), 25.3 (CH₂ fatty acid), 23.0 (CH₂ fatty acid), 14.2 (CH₃ fatty acid). ³¹P NMR (400 MHz, CDCl₃): 0.21. HRMS: [M+H]⁺ calcd for C₁₀₂H₁₇₀N₂O₁₉P: 1758.21299, found 1758.21262.



***N*-[(*R*)-3-decanoyloxytetradecanoyl]-*O*-[2-deoxy-4-*O*-phosphono-2-[(*R*)-3-decanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-decanoyloxytetradecanoyl]-β-D-glucopyranosyl]-L-serine sodium salt (**24**):**

Serinate **23** (23 mg, 13.1 μmol) was dissolved in distilled THF (1 mL, 13.1mM) and purged with argon. Pd/C was added and the reaction mixture was stirred for 15 min. Then, the reaction mixture was purged with H₂ (g) for 10 min. and stirred overnight. The reaction mixture was filtered over a Whatmann filter and concentrated. The obtained residue was subsequently filtered over Chelex sodium form resulting in the isolation of compound **24** in 84% yield. ¹H NMR (600 MHz, CDCl₃/MeOD/D₂O, HH-COSY, HSQC): 5.24-5.07 (m, 4H, 3x CH fatty acid, H-3), 4.48-4.07 (m, 4H, H-1, H_β serine, H_α serine), 4.19-4.05 (m, 1H, H-4), 3.88-3.74 (m, 1H, H-6a), 3.73-3.67 (m, 2H, H-2, H-6b), 3.43-3.39 (m, 1H, H-5), 2.68-2.18 (m, 10H, 5x CH₂ fatty acid), 1.59-1.45 (m, 10H, 5x CH₂ fatty acid), 1.33-1.13 (m, 88H, 44x CH₂ fatty acid), 0.88-0.84 (m, 18H, 6x CH₃ fatty acid). ¹³C APT NMR (150 MHz, CDCl₃/MeOD/D₂O, HH-COSY, HSQC): 175.0 (C=O), 175.0 (C=O), 174.7 (C=O), 174.6 (C=O), 172.3 (C=O), 171.9 (C=O), 171.4 (C=O), 101.6 (C-1), 76.1 (C-5), 74.1 (C-3), 71.8 (CH fatty acid), 71.6 (CH fatty acid), 71.3 (C-4), 71.2 (CH fatty acid), 69.6 (C-6), 54.6 (C-2), 48.9 (CH₂ fatty acid), 41.6 (CH₂ fatty acid), 41.3 (CH₂ fatty acid), 35.3-34.6 (CH₂ fatty acid), 32.6-32.5 (CH₂ fatty acid), 30.4-29.2 (CH₂ fatty acid), 26.5-25.6 (CH₂ fatty acid), 23.3 (CH₂ fatty acid), 23.3 (CH₂ fatty acid), 14.1 (CH₃ fatty acid). ³¹P NMR (300 MHz, CDCl₃): 0.21. ESI-MS: 1488.9.



***N*[(*R*)-3-decanoyloxytetradecanoyl]-*O*-[6-*O*-(6-azidoheptanoyl)-2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-decanoyloxytetradecanoylamino]-3-*O*[(*R*)-3-decanoyloxytetradecanoyl]- β -D-glucopyranosyl]-L-serine benzyl ester (**25**):** Serinate **23** (12 mg, 6.8 μ mol) was dissolved in DCM (1.0 mL, 6.8mM) followed by addition of 7-azidoheptanoic acid (3 mg, 17 μ mol, 2.5 eq.). The reaction mixture was cooled to 0 $^{\circ}$ C, followed by addition of EDC $\cdot$ HCl (3.3 mg, 17 μ mol, 2.5 eq.). After 2h., no conversion of the starting material was observed. The reaction mixture was allowed to warm to room temperature. Only partial conversion of the starting material was observed (TLC analysis: hexane/EtOAc, 1/1, v/v, R_f 0.7). The reaction mixture was cooled to 0 $^{\circ}$ C, followed by addition of another portion of 7-

azidoheptanoic acid (3 mg, 17 μ mol, 2.5 eq.) and EDC $\cdot$ HCl (3.3 mg, 17 μ mol, 2.5 eq.). The reaction mixture was allowed to warm to room temperature. After 5 hours, the starting material was fully consumed. The reaction mixture was diluted with CHCl₃, washed with H₂O, dried over Na₂SO₄, filtered, and concentrated. Column chromatography (Hexane/EtOAc: 1/0 $\rightarrow$ 6/4) gave the title compound with some 7-azidoheptanoic acid. The compound was further purified by SEC (THF) and column chromatography (hexane/EtOAc: 19/1 $\rightarrow$ 6/4), which gave serinate **25** in 46% yield (6 mg, 3.1 μ mol). $[\alpha]_D^{25}$: +21.6 (c = 0.1, DCM/MeOH, 1/1). IR (neat, cm⁻¹): 2924, 2852, 2088, 1737, 1656, 1566, 1548, 1527, 1462, 1454, 1261, 1247, 1165, 1109, 1016, 696. ¹H NMR (600 MHz, CDCl₃, HH-COSY, HSQC): 7.37-7.26 (m, 15H, H_{arom}), 7.08 (d, 1H, J = 7.8 Hz, NH serine), 6.41 (d, 1H, J = 7.6 Hz, NH GlcN), 5.33 (t, 1H, J = 9.0 Hz, H-3), 5.19-5.13 (m, 5H, 3x CH fatty acid, CH₂ CO₂Bn), 4.98-4.96 (m, 3H, H-1, CH₂ dibenzyl phosphate), 4.92-4.89 (m, 2H, CH₂ dibenzyl phosphate), 4.74-4.73 (m, 1H, H_x serine), 4.42-4.36 (m, 2H, H-4, H-6a), 4.26 (dd, 1H, J = 11.4 Hz, J = 3.0 Hz, H $_{\beta}$ serine), 4.14-4.10 (m, 1H, H-6b), 3.85-3.82 (m, 1H, H $_{\beta}$ serine), 3.58-3.57 (m, 1H, H-5), 3.47-3.43 (m, 1H, H-2), 3.23 (t, 7.2 Hz, 2H, CH₂ 7-azidoheptanoyl), 2.28-2.24 (m, 2H, CH₂ fatty acid), 2.24-2.22 (m, 8H, 4x CH₂ fatty acid), 1.67-1.48 (m, 10H, 5x CH₂ fatty acid), 1.43-1.11 (m, 88H, 44x CH₂ fatty acid), 1.05-0.87 (m, 18H, 6x CH₃ fatty acid). ¹³C APT NMR (150 MHz, CDCl₃, HH-COSY, HSQC): 173.8 (C=O), 173.7 (C=O), 173.6 (C=O), 170.9 (C=O), 170.2 (C=O), 170.0 (C=O), 169.4 (C=O), 135.4-135.2 (C_q C_{arom}), 128.7-128.0 (CH_{arom}), 100.4 (C-1), 73.8 and 73.7 (C-4), 72.4 (C-3 or C-5), 72.4 (C-3 or C-5), 71.3 (CH fatty acid), 71.0 (CH fatty acid), 70.2 (CH fatty acid), 69.7-69.6 (CH₂ dibenzyl phosphate), 69.1 (C $_{\beta}$ serine), 67.2 (CH₂ CO₂Bn), 62.0 (C-6), 55.6 (C-2), 52.7 (C $_{\alpha}$ serine), 51.3 (CH₂ azidoheptanoyl), 48.3 (CH₂ fatty acid), 51.6 (CH₂ fatty acid), 41.6-41.1 (CH₂ fatty acid), 39.7 (CH₂ fatty acid), 35.1-33.7 (CH₂ fatty acid, CH₂ 7-azidoheptanoyl), 32.0-26.4 (CH₂ fatty acid, CH₂ 7-azidoheptanoyl), 25.8-22.8 (CH₂ fatty acid, CH₂ 7-azidoheptanoyl), 14.2-14.1 (CH₃ fatty acid). ³¹P NMR (300 MHz, CDCl₃): 2.60. HRMS: $[M+H]^+$ calcd for C₁₀₉H₁₈₁N₅O₂₀P: 1912.30321, found 1912.30430.

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13. *tert*-Butyl (*E*)-2-tetradecanoate did not dissolve in *tert*-BuOH/H₂O and therefore it was added as a solution in DCM.
14. Both the starting material and the title compound stain with molybdenum TLC spray.

Chapter 7

Summary & Future prospects

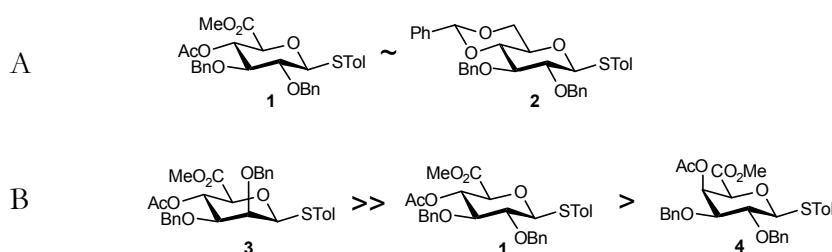
Introduction

Carbohydrates take part in various essential biological processes, including immune responses against microbial infections. Pathogens are characterized by specific carbohydrate structures at their cell walls and the mammalian immune system takes advantage of the presence of these unique molecules to combat these microbes. In **Chapter 1**, selected examples of carbohydrate structures interacting with the innate and adaptive immune systems are presented. In order to be able to accurately influence the immune system, knowledge on these interactions and the accompanying processes at a molecular level is required. A viable approach to achieve this comprises the design, synthesis, and evaluation of tailor-made oligosaccharides and glycoconjugates. The current state of the art in the field of carbohydrate chemistry is of such a level that within certain limits the majority of relevant oligosaccharide fragments are accessible. These advances and those in the field of immunology have resulted in the development of several promising glycoconjugate vaccine candidates. The present chapter

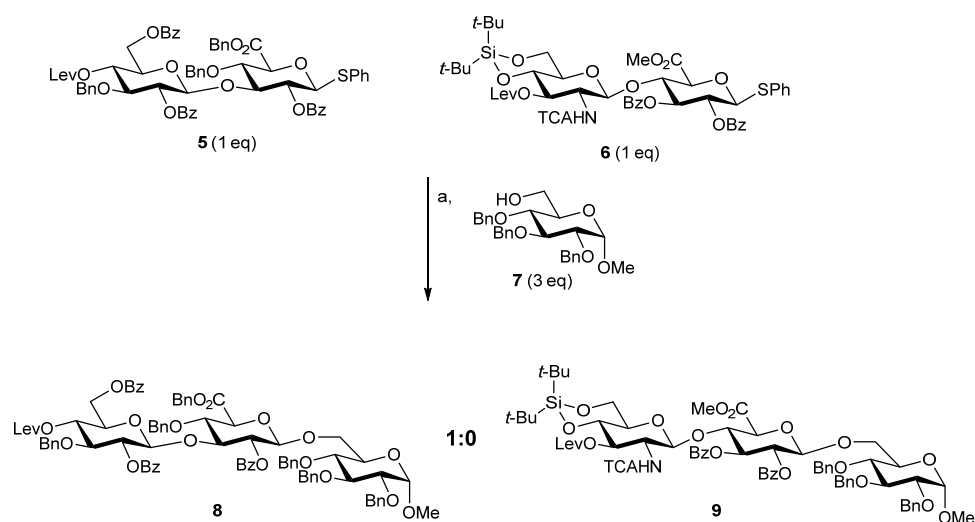
summarizes the work described in this thesis and some prospects for future research are laid out.

Chapter 2 evaluates the relative reactivity of three glucuronic acid donors. Glucuronic acids are generally believed to be inactive due to the presence of the electron withdrawing carboxylate substituent. This assumption often disfavors glucuronic acid donors over glucoside donors in the construction of glucuronic acid containing oligosaccharides. The relative reactivity was studied by a series of competition experiments in which two donors compete for a limiting amount of activator. Glucuronic acid **1** proved to be of equal reactivity as benzylidene protected glucoside **2** (Figure 1, A), which shows that glucuronic acids are more reactive than often presumed. In competition with the epimeric mannuronic acid (Figure 1B, **3**) and galacturonic acid (Figure 1B, **4**) donors, glucuronic acid **1** was completely outcompeted by mannuronic acid **3**, but was found more reactive than galacturonic acid **4**.

Figure 1. Relative reactivity of glucuronic acid.

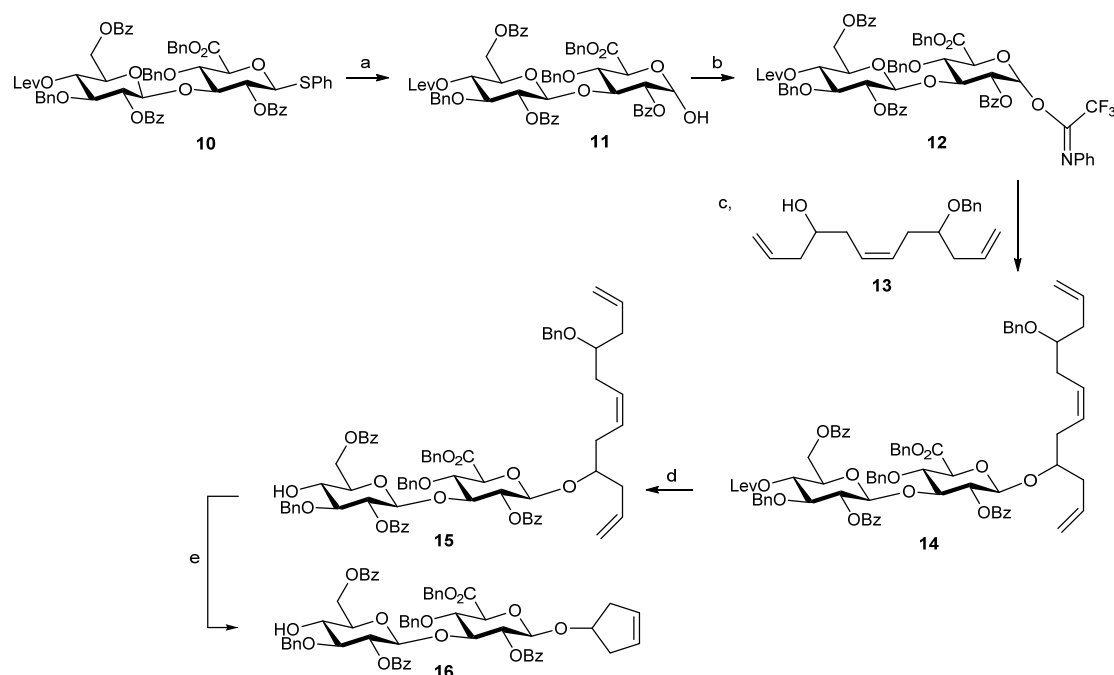


As described in **Chapter 3** the reactivity and applicability of glucuronic acid donors was further explored in the synthesis of frame-shifted *Streptococcus pneumoniae* type 3 capsular polysaccharide disaccharides and trisaccharides. It was found that glucuronic acid mono- and disaccharide donors are more productive in the glycosylations than the corresponding glucosides. This shows that glucuronic acid derivatives should be regarded as useful donors in the construction of oligosaccharides. Since the capsular polysaccharide of *Streptococcus pneumoniae* type 3 has a disaccharide as repeating unit, the assembly of larger oligosaccharide fragments via block couplings of dimers is the most obvious strategy. Hyaluronic acid (HA), a member of the glycosaminoglycan family, also consists of a repetitive core disaccharide. The successful automated synthesis of fragments of HA¹ makes an analogous synthetic approach using the *Streptococcus pneumoniae* type 3 disaccharide attractive. To gain more insight into the reactivity of donor **5** (Scheme 1), in light of its future application in a solid phase synthesis campaign, a competition experiment between HA donor **6** and *Streptococcus pneumoniae* disaccharide **5** was executed (Scheme 1). The *Streptococcus pneumoniae* dimer **5** completely outcompeted HA donor **6**.

Scheme 1. Competition experiment between *Strept. Pneum.* **5** and HA **6**.

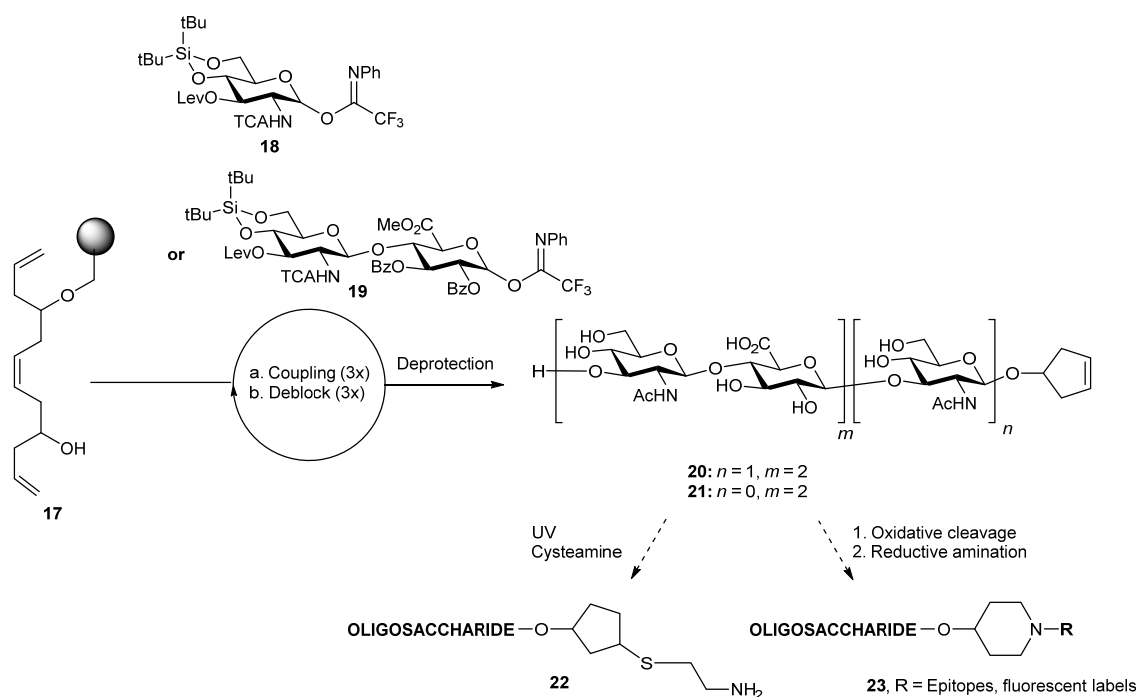
Reagents and conditions: (a) NIS, TfOH, -40 °C → 0 °C, 92%.

To further explore a possible future solid phase synthesis of fragments of the *Streptococcus pneumoniae* type 3 capsular polysaccharide a solution phase pilot study was executed (Scheme 2). First, disaccharide **10** was converted into imidate donor **12** by hydrolysis of the anomeric thioacetal followed by treatment with *N*-phenyl-trifluoroacetimidoyl chloride and cesium carbonate. Donor **12** was then condensed with triene **13**, the tandem RCM cleavable linker discussed in Chapter 4. The glycosylation to **14** proceeded fast (5 minutes) and in good yield (92%). Gratifying, no benzoyl migration was observed during the condensation of **12** with linker **13**. The temporary levulinoyl ester in **14** was cleaved by treatment with hydrazine acetate in a mixture of pyridine and acetic acid, affording disaccharide **15**. This alcohol was subjected to a ring closing metathesis reaction with Grubbs second-generation catalyst in DCM. After 30 minutes, TLC analysis showed total conversion into **16**, which was isolated in 92% yield. With the translation of the glycosylation and delevulinoylation to a solid phase format, the construction of large fragments via a solid phase approach comes within reach.

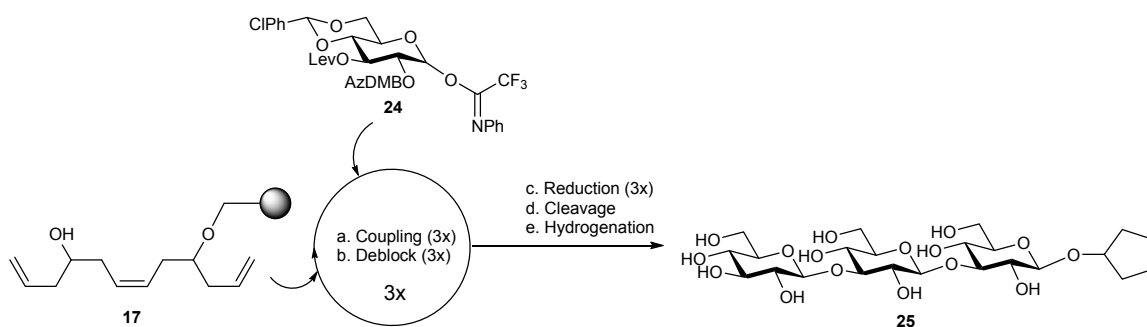
Scheme 2. Synthesis of disaccharide donor and glycosylation.

Reagents and conditions: (a) NIS, TFA, DCM, 0 °C, 72%; (b) ClC(=NPh)CF_3 , Cs_2CO_3 , acetone, 84%; (c) TfOH , DCM, 0 °C, 83%; (d) Hydrazine acetate, pyridine/AcOH, 84%, (e) Grubbs second-generation, DCM, 92%.

A second-generation tandem ring closing metathesis cleavable linker is presented in **Chapter 4** (Scheme 3, **17**). This acid- and base stable linker allows cleavage with Grubbs second-generation catalyst without the use of alkene additives to liberate the assembled oligosaccharides from the solid support. The linker was successfully applied in the synthesis of two frame-shifted hyaluronic acid fragments **20** and **21**. The reaction time required for the release of the immobilized compounds from the resin was significantly reduced in comparison with commonly employed cross metathesis linker systems. This allows aliquots of resin to be interrogated efficiently to monitor the progress of the solid phase synthesis. Furthermore, the symmetrical cyclopentene moiety provides a handle for further modification such as the installation of a fluorescent label. For example, irradiation with UV of alkene **20** in the presence of cysteamine can give functionalized oligosaccharides (**22**). The double bond in **20** can also be oxidatively cleaved to provide two aldehydes. Subsequent reductive amination then leads to another type of functionalized hyaluronic acid oligosaccharides (**23**).

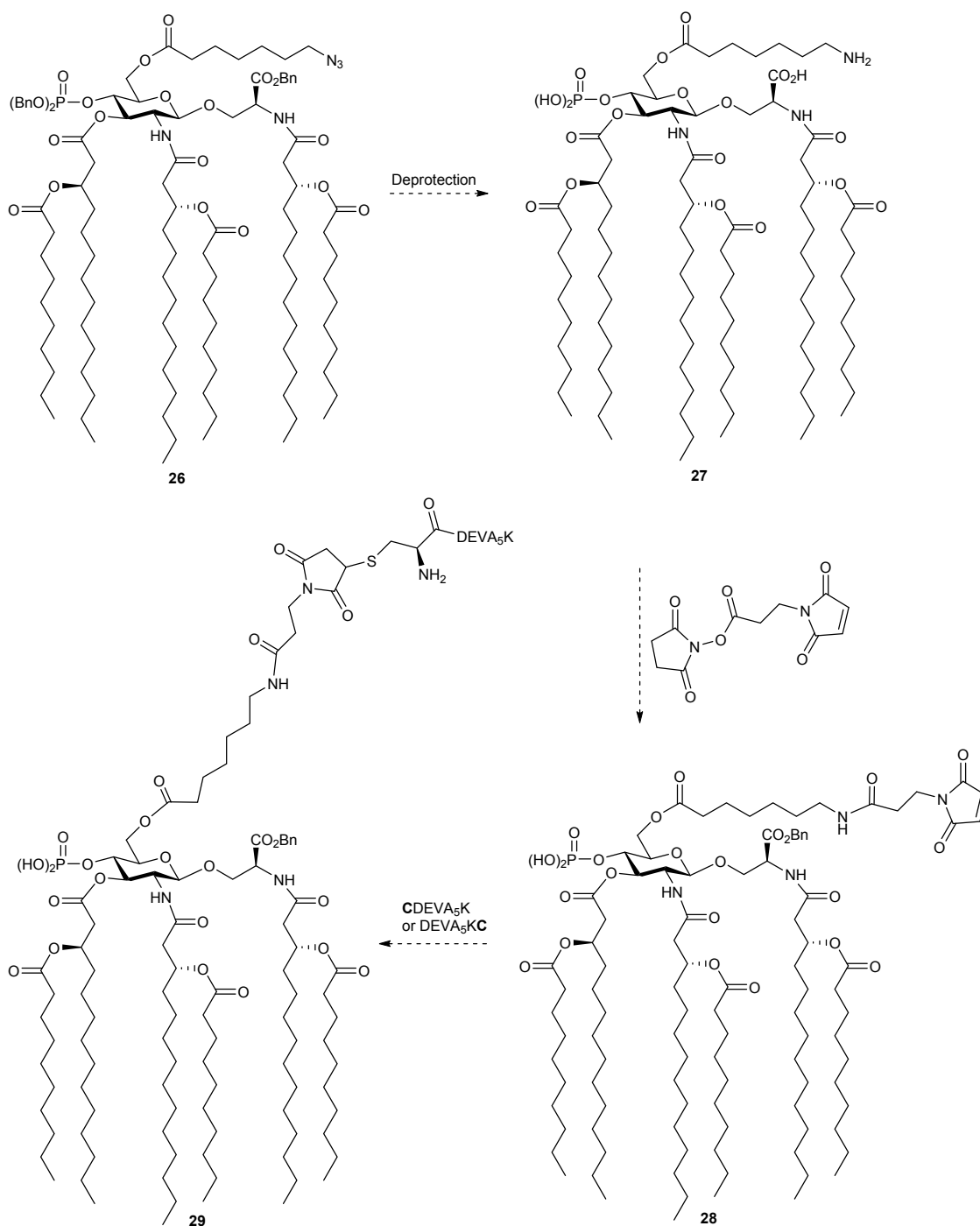
Scheme 3. Application of the ring closing metathesis cleavable linker.

Chapter 5 describes the synthesis of a tetrameric β -1,3-glucan. Translation of the chemistry developed in solution to a solid phase format led to the successful synthesis of a protected tetrasaccharide. Unfortunately, this tetramer could not be deprotected due to harshness of the conditions required for the removal of pivaloyl esters present at the C-2 positions. Therefore the pivaloyl group in the donor was replaced with the 4-azido-2,2-dimethylbutanoyl (AzDMB) group, as in donor **24** (Scheme 4). Using a similar solid phase methodology and linker **17** (described in Chapter 4), a trisaccharide fragment was assembled and the AzDMB groups were successfully cleaved on-resin. With these conditions in hand, the road is paved for the construction of larger linear fragments. The use of an orthogonal protective group at the C-6 position (such as a silyl-based protective group) can open the door towards the preparation of 1,6-branched fragments.

Scheme 4. Solid phase synthesis of a β -1,3-glucan trisaccharide.

In **Chapter 6** a synthetic route towards a lipid A analogue suitable for conjugation with other relevant molecules such as epitopes and labels is presented. A monosaccharide lipid A analogue, designed by the group of Johnson² was selected as lipid A analogue suitable for

functionalization with a conjugation handle. Key steps in the synthesis are the condensation of the serine building block with the glucosamine donor, the synthesis and introduction of the chiral fatty acid and the functionalization of the C-6 position with a 7-azidoheptanoyl group. Although the unfunctionalized analogue described by Johnson was reached, the preparation of the functionalized target **27** was not achieved by a single hydrogenation event (Scheme 5). Suitable deprotection conditions should lead to lipid A analogue **27** with a free amine as conjugation handle. It would be interesting to conjugate this lipid A analogue to an ovalbumin derived peptide comprising the MHC I epitope SIINFKL, incorporated in a longer peptide motif (DEVSGLEQLESIINFELAAAAAK, DEVA₅K). It would be desirable to prepare conjugates in which the epitope is connected to the spacer of the lipid A analogue both at its *N*- and *C*-terminus. A plausible route of synthesis entails the introduction of a cysteine at either side of the epitope to allow a maleimide conjugation (**29**). Biological evaluation will provide information on the immunostimulating activities of these conjugates.

Scheme 5. Synthetic plan for conjugation.

Conclusions

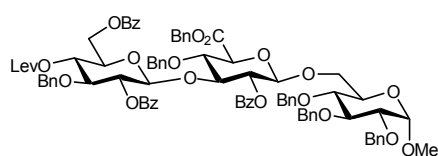
Altogether, the work described in this thesis presents a step forward in the development of synthetic procedures towards several immunostimulating carbohydrates. Whereas solution phase syntheses will remain necessary to explore and optimize reaction conditions, (automated) solid phase oligosaccharide syntheses will accelerate the access to libraries of (longer) oligosaccharides. The availability of well-defined (functionalized) immunostimulating

carbohydrates will lead to a better knowledge of the interactions of these saccharides with the mammalian immune system culminating in the development of more efficient vaccines.

Experimental section

General experimental procedures. Chemical shifts (δ) are given in ppm relative to TMS as internal standard. All ^{13}C APT NMR spectra are proton decoupled. Reactions were performed at rT unless stated otherwise and were followed by TLC analysis with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulphuric acid in EtOH or with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g L $^{-1}$), followed by charring at 150 °C. Flash column chromatography was performed on silica gel (0.04–0.063 nm) and size exclusion chromatography (SEC) was performed on SephadexTM LH-20. Experiments which required an inert atmosphere were carried out under dry argon. Dichloromethane (p.a.) was distilled over P_2O_5 prior to use. Molecular sieves (3Å) were flame-dried before use.

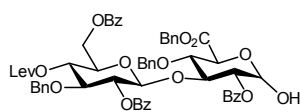
Competition experiment. Donor **5** (0.1 mmol, 1 eq.), donor **6** (0.1 mmol, 1 eq.) and the acceptor **7** (Methyl 2,3,4-tri-*O*-benzyl- α -D-glucopyranoside, 3 eq.) were co-evaporated with toluene (2x). Freshly distilled DCM (4 mL, donor concentration: 0.05M), a teflon stirrer bar and activated (flame-dried) molecular sieves 3Å were added and the mixture was stirred under argon for 30 minutes at rT. NIS (1 eq) was added and the mixture was cooled to -40 °C. TfOH (0.1 eq, 0.1 mL of a 0.1M stock solution in distilled DCM) was added and the mixture was allowed to warm to 0 °C in ~3 h. Triethylamine (0.1 mL) was added and the mixture was diluted with EtOAc, washed with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ (1x) and brine (2x), dried over MgSO_4 and concentrated *in vacuo*. Elution over a Sephadex LH-20 (DCM/MeOH, 1/1, v/v, 500 mL) enabled isolation of the trisaccharide products and were analyzed with NMR spectroscopy. The yield of the trisaccharide fraction and the ratio of the trisaccharides were determined.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*- (Benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinyl- β -D-glucopyranoside))- α/β -D-glucopyranosyluronate)))- α -D-glucopyranoside (8**):**

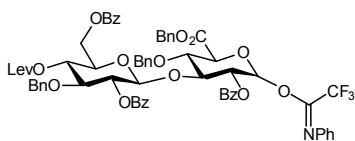
Trisaccharide **8** was obtained as a white solid. IR (neat, cm $^{-1}$): 3032, 1732, 1600, 1494, 1452, 1361, 1315, 1263, 1205, 1176, 1145, 1091, 1068, 1026, 896, 844, 810, 731, 698, 640, 630, 615, 603. $[\alpha]_D$: +17 (DCM, c = 0.5). ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 8.02–8.00 (m, 2H, H_{arom}), 7.89–7.87 (m, 2H, H_{arom}), 7.78–7.76 (m, 2H, H_{arom}), 7.57–7.48 (m, 3H, H_{arom}), 7.38–6.99 (m, 36H, H_{arom}), 5.30 (t, 1H, J = 8.0 Hz, H-2''), 5.21–5.16 (m, 2H, H-2', H-4''), 5.09–5.08 (m, 2H, CH_2 CO_2Bn), 4.97 (d, 1H, J = 10.8 Hz, CHH Bn), 4.83–4.85 (m, 2H, CHH Bn, H-1''), 4.70 (d, 1H, J = 12.0 Hz, CHH Bn), 4.64 (d, 1H, J = 10.8 Hz, CHH Bn), 4.58 (d, 1H, J = 12.0 Hz, CHH Bn), 4.50–4.36 (m, 8H, H-1', H-1, H-6a, 4x CHH Bn), 4.21–4.16 (m, 3H, H-3', H-6b, CHH Bn), 4.00–3.96 (m, 2H, H-6''a, H-4'), 3.97 (d, 1H, J = 8.4 Hz, H-5'), 3.82 (d, 1H, J = 9.2 Hz, H-3), 3.79–3.71 (m, 1H, H-5''), 3.66 (t, 1H, J = 8.8 Hz, H-3''), 3.61–3.57 (m, 1H, H-5), 3.47 (dd, 1H, J = 10.8 Hz, J = 4.8 Hz, H-6''b), 3.37 (dd, 1H, J = 9.6 Hz, J = 3.6 Hz, H-2), 3.20 (t, 1H, J = 9.6 Hz, H-4), 3.19 (s, 3H, OCH_3), 2.70–2.50 (m, 2H, CH_2 Lev), 2.49–2.41 (m, 1H, CHH Lev), 2.38–2.27 (m, 1H, CHH Lev), 2.07 (s, 3H, CH_3 Lev). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 206.0 (C=O Lev ketone), 171.3 (C=O), 167.6 (C=O), 166.1 (C=O), 165.0 (C=O), 164.0 (C=O), 138.8–138.0 (C_q C_{arom}), 137.3 (C_q C_{arom}), 135.0–133.0 (CH_{arom}), 129.4 (C_q C_{arom}), 128.5–127.4 (CH_{arom}), 101.0 (C-1''), 100.5 (C-1'), 97.6 (C-1), 81.7 (C-3), 79.9 (C-3''), 79.7 (C-2), 79.5 (C-3'), 77.4 (C-4), 77.0 (C-4'), 75.4 (CH_2 Bn), 74.9 (CH_2 Bn),

74.8 (C-5'), 74.5 (CH₂ Bn), 74.0 (CH₂ Bn), 73.6 (C-2' or C-4'), 73.3 (C-2''), 73.2 (CH₂ Bn), 72.2 (C-5''), 70.5 (C-2' or C-4''), 69.4 (C-5), 67.9 (C-6''), 67.2 (CH₂ CO₂Bn), 63.0 (C-6), 54.8 (CH₃), 37.7 (CH₂ Lev), 29.6 (CH₃ Lev), 27.7 (CH₂ Lev). HRMS: [M+H]⁺ calcd for C₈₇H₈₇O₂₂: 1483.56835, found 1483.56822.



Benzyl (2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinyl-β-D-glucopyranoside)-α/β-D-glucopyranosyluronate)

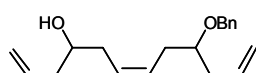
(11): Disaccharide **10** (1.0 g, 0.89 mmol) was dissolved in freshly distilled DCM (8.9 mL, 1M) and the resulting mixture was cooled to 0 °C. NIS (220 mg, 0.98 mmol, 1.1 eq.) and TFA (75 μL, 0.98 mmol, 1.1 eq.) were added and the reaction mixture was allowed to warm to rT. The reaction mixture turned dark red. After 1 hour, TLC analysis showed the appearance of a UV positive spot (indicating the thiophenol being released), but no R_f difference for the disaccharide was observed (hexane/EtOAc: 12/8, v/v). The reaction mixture was cooled to 0 °C followed by addition of piperidine (264 μL, 2.67 mmol, 3 eq.). After 20 minutes, TLC analysis showed total conversion into a lower running spot (hexane/EtOAc: 12/8, v/v, R_f 0.40). The reaction mixture was diluted, quenched with Na₂S₂O₃ (aq., sat.), followed by separation of the layers. The aq. layer was extracted with DCM and the combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated. A yellow oil was obtained, which was purified by column chromatography (hexane/EtOAc: 1/0 → 1/1). Hemiacetal **11** was obtained as an anomeric mixture and as a transparent oil in 72% yield (666 mg, 0.64 mmol, α/β: 10/1). IR (neat, cm⁻¹): 3446, 1718, 1600, 1450, 1400, 1363, 1315, 1269, 1176, 1147, 1107, 1062, 1026, 985, 711, 698. ¹H NMR α anomer (400 MHz, CDCl₃, HH-COSY, HSQC): 8.10-7.99 (m, 4H, H_{arom}), 7.69-7.67 (t, 1H, *J* = 7.6 Hz, H_{arom}), 7.56-7.49 (m, 5H, H_{arom}), 7.48-7.34 (m, 2H, H_{arom}), 7.29-7.18 (m, 8H, H_{arom}), 7.15-6.99 (m, 8H, H_{arom}), 5.42 (t, 1H, *J* = 3.2 Hz, H-1), 5.35 (t, 1H, *J* = 8.8 Hz, H-2'), 5.30 (t, 1H, *J* = 8.4 Hz, H-4'), 5.08-4.96 (m, 4H, CH₂ CO₂Bn, H-1', CHH Bn), 4.81 (dd, 1H, *J* = 9.6 Hz, *J* = 3.2 Hz, H-2), 4.58-4.45 (m, 6H, CHH Bn, CH₂ Bn, H-3, H-5, H-6'a), 4.23 (dd, 1H, *J* = 8.8 Hz, *J* = 5.2 Hz, H-6'b), 3.91-3.85 (m, 2H, H-4, H-5'), 3.73 (t, 1H, *J* = 9.2 Hz, H-3'), 2.65-2.59 (m, 2H, CH₂ Lev), 2.51-2.34 (m, 2H, CH₂ Lev), 2.06 (s, 3H, CH₃ Lev). ¹³C APT NMR α anomer (100 MHz, CDCl₃, HH-COSY, HSQC): 206.4 (C=O Lev ketone), 171.3 (C=O), 168.9 (C=O), 166.2 (C=O), 164.9 (C=O), 137.9 (C_q C_{arom}), 137.2 (C_q C_{arom}), 134.9 (C_q C_{arom}), 133.5 (CH_{arom}), 132.9 (CH_{arom}), 129.7-129.5 (CH_{arom}), 129.2-129.0 (C_q C_{arom}), 128.6-127.3 (CH_{arom}), 101.3 (C-1'), 90.0 (C-1), 79.7 (C-3'), 77.2 (C-3 or C-5), 75.4 (CH₂ Bn), 74.0 (C-2), 73.9 (C-2'), 72.3 (C-4), 72.1 (C-5'), 70.4 (C-4', C-3 or C-5), 67.2 (CH₂ CO₂Bn), 62.9 (C-6'), 37.7 (CH₂ Lev), 29.6 (CH₃ Lev), 27.7 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₅₉H₅₆O₁₇Na: 1059.34097, found 1059.34146.



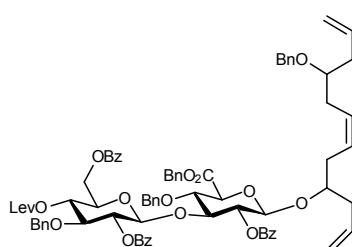
Benzyl (phenyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(2,6-di-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinyl-β-D-glucopyranoside)-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-glucopyranosyluronate)

(12): Hemiacetal **11** (660 mg, 0.64 mmol) was dissolved in acetone (5 mL, 0.15 M). The reaction mixture was cooled to 0 °C, followed by addition of *N*-phenyl-trifluoroacetimidoyl chloride (134 μL, 0.96 mmol, 1.5 eq.) and Cs₂CO₃ (209 mg, 0.64 mmol, 1 eq.). The reaction mixture was allowed to warm to rT. After stirring for 2 hours, TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 1/1, v/v, R_f 0.44). The reaction mixture was filtered over celite and concentrated. Column chromatography (hexane/EtOAc: 1/0 → 1/1) gave disaccharide **12** as an anomeric mixture (α/β: 4/1) and a transparent oil in 84% yield (649 mg, 0.54 mmol). IR (neat, cm⁻¹): 3066, 2956, 1722, 1600, 1452, 1404, 1361, 1315, 1265, 1205, 1176, 1149, 1091, 1026, 991, 972, 744, 709, 700, 611. ¹H NMR α isomer (400 MHz, CDCl₃, 50 °C, HH-COSY, HSQC): 8.03 (d, 2H, *J* = 7.6 Hz, H_{arom}), 7.98 (d, 2H, *J* = 8.0 Hz, H_{arom}), 7.69 (t, 1H, *J* = 7.6 Hz, H_{arom}), 7.61-7.59 (m, 2H, H_{arom}), 7.54-7.34 (m, 6H, H_{arom}), 7.26-6.91 (m, 20H, H_{arom}), 6.56 (bs, 1H, H-1), 6.28 (d, 2H, *J* = Hz, H_{arom}), 5.36 (t, 1H, *J* = 8.8 Hz, H-2'), 5.22 (t, 1H, *J* = 9.6 Hz, H-4'), 5.14-5.00 (m, 4H, H-2, H-1', CH₂ CO₂Bn), 4.56-4.45 (m, 6H, H-3, H-6'a, 2x CH₂ Bn), 4.39 (d, 1H, *J* = 9.6 Hz, H-5),

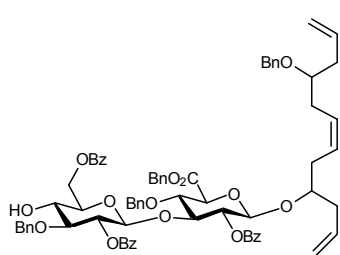
4.26 (dd, 1H, $J = 11.6$ Hz, $J = 5.6$ Hz, H-6'b), 3.99 (t, 1H, $J = 9.6$ Hz, H-4), 3.96-3.88 (m, 1H, H-5'), 3.77 (t, 1H, $J = 9.2$ Hz, H-3'), 2.64-2.62 (m, 2H, CH₂ Lev), 2.60-2.42 (m, 2H, CH₂ Lev), 2.07 (s, 3H, CH₃ Lev). ¹³C APT NMR α isomer (100 MHz, CDCl₃, HH-COSY, HSQC): 205.6 (C=O Lev ketone), 171.3 (C=O), 167.5 (C=O), 166.1 (C=O), 164.8 (C=O), 164.4 (C=O), 142.7 (C_q C_{arom}), 137.5-137.4 (C_q C_{arom}), 134.9 (C_q C_{arom}), 133.6 (CH_{arom}), 132.9 (CH_{arom}), 129.8-129.5 (CH_{arom}), 129.2-128.8 (C_q C_{arom}), 128.7-127.5 (CH_{arom}), 124.2 (CH_{arom}), 119.2-118.9 (CH_{arom}), 101.4 (C-1), 92.3 (C-1'), 79.9 (C-3'), 77.4 (C-3), 76.6 (C-4), 72.2 (CH₂ Bn), 74.0 (CH₂ Bn), 73.4 (H-2'), 72.9 (H-5), 72.5 (H-2, H-5'), 70.8 (C-4'), 67.4 (CH₂ CO₂Bn), 63.1 (C-6'), 37.7 (CH₂ Lev), 29.4 (CH₃ Lev), 27.9 (CH₂ Lev). HRMS: [M+Na]⁺ calcd for C₆₇H₆₀O₁₇F₃NO₁₇Na: 1230.37055, found 1230.37133.



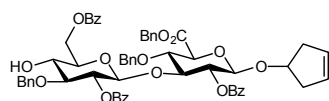
(Z)-9-(benzyloxy)dodeca-1,6,11-trien-4-ol (13): (Z)-dodeca-1,6,11-trien-4,9-diol³ (1.0 g, 5.10 mmol) was dissolved in DMF (25.5 mL, 0.2M) followed by addition of benzyl bromide (0.6 mL, 5.1 mmol, 1 eq.). The reaction mixture was cooled to 0 °C and NaH (60% dispersion in oil, 224 mg, 5.6 mmol, 1.1 eq.) was added. The reaction mixture was allowed to warm to room temperature and the reaction mixture was stirred overnight. TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 12/8, v/v, R_f 0.8). The reaction mixture was cooled to 0 °C, quenched with MeOH, diluted with EtOAc, washed with H₂O (2x), dried over MgSO₄, filtered, and concentrated. Column chromatography (hexane/EtOAc: 1/0 → 9/1) gave the title compound as a transparent oil in 66% yield (964 mg, 3.37 mmol). IR (neat, cm⁻¹): 3408, 2924, 2873, 1714, 1641, 1450, 1435, 1350, 1315, 1273, 1176, 1111, 1095, 1068, 1026, 995, 916, 844, 713, 698, 642, 630. ¹H NMR (400 MHz, CDCl₃, HH-COSY, HSQC): 7.37-7.24 (m, 5H, H_{arom}), 5.89-5.76 (m, 2H, H-2, H-11), 5.64-5.51 (m, 2H, H-6, H-7), 5.27-5.06 (m, 4H, H-1, H-12), 4.56-4.46 (m, 2H, CH₂ Bn), 3.68-3.62 (m, 1H, H-9), 3.52-3.46 (m, 1H, H-4), 2.41-2.12 (m, 8H, H-3, H-5, H-8, H-10). ¹³C APT NMR (100 MHz, CDCl₃, HH-COSY, HSQC): 134.9-134.7 (C-2, C-11), 128.8-127.2 (C-6, C-7, CH_{arom}), 117.8-117.1 (C-1, C-12), 78.2 and 78.0 (C-4), 71.0 and 70.9 (CH₂ Bn), 70.4 and 70.4 (C-9), 41.4, 41.3, 38.1, 38.1, 34.8, 34.7, 31.8, 31.7 (C-3, C-5, C-8, C-10). HRMS: [M+Na]⁺ calcd for C₁₉H₂₆O₂Na: 309.18250, found 309.18259.



(Z)-9-(benzyloxy)dodeca-1,6,11-trienyl [Benzyl (2-O-benzoyl-4-O-benzyl-3-O-(2,6-di-O-benzoyl-3-O-benzyl-4-O-levulinyl- β -D-glucopyranoside)- β -D-glucopyranosyluronate)] (14): (Z)-9-(benzyloxy)-dodeca-1,6,11-trien-4-ol (20 mg, 71 μ mol) **13** and disaccharide donor **12** (107 mg, 89 μ mol, 1.25 eq.) were dissolved in freshly distilled DCM (0.89 mL, 0.08M) followed by addition of activated MS 3Å. The reaction mixture was stirred for 30 min. before cooling to 0 °C and subsequent addition of TfOH (45 μ L of a 0.2 M stock solution, 8.9 μ mol, 0.1 eq relative to donor). TLC-MS analysis showed total conversion of the starting material after 30 minutes. The reaction mixture was neutralized with TEA, diluted with DCM, washed with H₂O, dried over MgSO₄, filtered, and concentrated. Purification by column chromatography (hexane/EtOAc: 1/0 → 1/1) gave the title compound as a transparent oil in 83% yield (83 mg, 64 μ mol). IR (neat, cm⁻¹): 3062, 2960, 2927, 1720, 1600, 1496, 1452, 1402, 1361, 1315, 1263, 1209, 1176, 1145, 1093, 1068, 1026, 92, 802, 734, 709, 700. HRMS: [M+Na]⁺ calcd for C₇₈H₈₀O₁₈Na: 1327.52369, found 1327.52424.



(Z)-9-(benzyloxy)dodeca-1,6,11-trienyl [Benzyl (2-O-benzoyl-4-O-benzyl-3-O-(2,6-di-O-benzoyl-3-O-benzyl- β -D-glucopyranoside- β -D-glucopyranosyluronate)] (15): Disaccharide **14** (52 mg, 40 μ mol) was dissolved in a mixture of pyridine/AcOH (4/1, 0.5 mL, 0.08 M), followed by addition of hydrazine acetate (18 mg, 0.20 mmol, 5 eq.). After 30 min., TLC analysis showed total conversion into a higher running spot (hexane/EtOAc: 1/1, v/v, R_f 0.5). The reaction mixture was quenched with acetone, diluted with toluene, and concentrated. Column chromatography (hexane/EtOAc: 1/0 $\rightarrow$ 6/4) gave the title compound as a transparent oil in 84% yield (41 mg, 34 μ mol). IR (neat, cm^{-1}): 3495, 3066, 2922, 2864, 1726, 1600, 1452, 1359, 1348, 1265, 1176, 1091, 1068, 1026, 914, 844, 736, 709, 698. HRMS: $[M+Na]^+$ calcd for $C_{73}H_{74}O_{16}Na$: 1229.48690, found 1229.48681.



Cyclopentenyl [Benzyl (2-O-benzoyl-4-O-benzyl-3-O-(2,6-di-O-benzoyl-3-O-benzyl- β -D-glucopyranoside- β -D-glucopyranosyluronate)] (16): Disaccharide **15** (33 mg, 27 μ mol) was dissolved in DCM (2.7 mL, 0.01M) and was purged with argon. Grubbs second-generation catalyst (2.8 mg, 3.2 μ mol, 0.12 eq.) was added. After 1 h., TLC analysis (toluene/EtOAc: 3/1, v/v, R_f 0.67) showed total conversion into a lower running spot. The reaction mixture was filtered over celite and a Whatmann filter and concentrated. Column chromatography (hexane/EtOAc: 1/0 $\rightarrow$ 7/3) gave the title compound as a transparent oil in 92% yield (25 mg, 25 μ mol). IR (neat, cm^{-1}): 2924, 2852, 1722, 1600, 1496, 1450, 1361, 1315, 1263, 1176, 1066, 1026, 989, 910, 846, 802, 734, 707, 698, 632. $[\alpha]_D^{+25}$ (DCM, c = 0.1). ^1H NMR (400 MHz, CDCl_3 , HH-COSY, HSQC): 7.98-7.95 (m, 4H, H_{arom}), 7.89-7.87 (m, 2H, H_{arom}), 7.64 (t, 1H, J = 7.6 Hz, H_{arom}), 7.58-7.47 (m, 4H, H_{arom}), 7.42-7.23 (m, 9H, H_{arom}), 7.18-7.08 (m, 10H, H_{arom}), 5.46-5.45 (m, 1H, H-3 cyclopentenyl), 5.27-5.13 (m, 2H, H-3 cyclopentenyl, H-2'), 5.13-5.02 (m, 4H, $\text{CH}_2 \text{CO}_2\text{Bn}$, H-2, CHH Bn), 4.91 (d, 1H, J = 8.0 Hz, H-1'), 4.65 (dd, 1H, J = 12.4 Hz, J = 4.0 Hz, H-6'a), 4.64-4.47 (m, 5H, H-1, H-6'b, CHH Bn, CH_2 Bn), 4.40-4.38 (m, 1H, H-1 cyclopentenyl), 4.26 (t, 1H, J = 7.6 Hz, H-3), 4.08-4.00 (m, 2H, H-4, H-5), 3.69 (t, 1H, J = 9.2 Hz, H-4'), 3.56-3.49 (m, 2H, H-3', H5'), 2.92 (bs, 1H, OH), 2.44-2.39 (m, 1H, 1x H-2 cyclopentenyl), 2.30-2.27 (m, 2H, 2x H-2 cyclopentenyl), 1.98-1.94 (m, 1H, 1x H-2 cyclopentenyl). ^{13}C APT NMR (100 MHz, CDCl_3 , HH-COSY, HSQC): 168.3 (C=O), 167.1 (C=O), 165.3 (C=O), 164.4 (C=O), 138.2 (C_q C_{arom}), 137.6 (C_q C_{arom}), 133.50 (C_q C_{arom}), 133.3-133.1 (CH_{arom}), 129.9-129.4 (C_q C_{arom}), 128.6-127.1 (CH_{arom} , C-3 cyclopentenyl), 100.3 (C-1'), 99.5 (C-1), 82.0 (C-3'), 79.5 (C-3), 78.6 (C-1 cyclopentenyl), 76.8 (C-4 or C-5), 74.8 (CH_2 Bn), 74.6 (C-4 or C-5), 74.1 (C-5', C-2), 73.4 (C-2'), 70.2 (C-4'), 67.3 ($\text{CH}_2 \text{CO}_2\text{Bn}$), 63.4 (C-6'), 39.8 (C-2 cyclopentenyl), 38.8 (C-2 cyclopentenyl). HRMS: $[M+Na]^+$ calcd for $C_{59}H_{56}O_{15}Na$: 1027.35114, found 1027.35119.

References and notes

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- a) Bazin, H.G., Murray, T.J., Bowen, W.S., Mozaffarian, A., Fling, S.P., Bess, L.S., Livesay, M.T., Arnold, J.S., Johnson, C.L., Ryter, K.T., Cluff, C.W., Evans, J.T., Johnson, D.A., *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 5350-5354; b) US 7,288,640 B2, October 30, 2007, Johnson, D.A., Johnson, C.L., Bazin-Lee, H.G., Gregory, C., Sowell, M., *Processes for the Production of Aminoalkyl Glucosaminide Phosphate and Disaccharide Immunoeffectors, and Intermediates Thereof*.
- The synthesis of (Z)-dodeca-1,6,11-trien-4,9-diol is described in Chapter 4.

Samenvatting

“Ontwikkeling van synthetische procedures naar immuunstimulerende koolhydraten”

Koolhydraten (of suikers) vervullen verschillende belangrijke biologische functies waaronder celgroei, celdifferentiatie en communicatie tussen cellen. Hiernaast beïnvloeden verschillende koolhydraten het functioneren van ons immuunsysteem. Om deze interacties beter te begrijpen op moleculair niveau, zijn synthetische, goed gedefinieerde koolhydraten een vereiste. Het verkrijgen van deze suikers in hoge zuiverheid uit hun natuurlijke bron is namelijk lastig en vaak zelfs onmogelijk. Dit proefschrift beschrijft de ontwikkeling van synthetische procedures voor het verkrijgen van gedefinieerde immuunstimulerende suikers.

In de algemene inleiding (**Hoofdstuk 1**) wordt een selectie van synthetische immuunstimulerende suikers gepresenteerd. Een aantal op koolhydraat-gebaseerde conjugaten, die als toekomstig nieuw vaccin kunnen dienen worden besproken.

Hoofdstuk 2 omvat een studie naar de relatieve reactiviteit van glucuronzuur donoren. Glucuronzuren komen in veel natuurlijke oligosacchariden voor en verschillende syntheses zijn beschreven. Hierin heeft de introductie van de carboxyl groep na de constructie van de glycosidische banden de voorkeur boven het gebruik van glucuronzuur donoren. Deze keuze is gebaseerd op de aanname dat glucuronzuur donoren niet erg reactief zijn. De relatieve reactiviteit van glucuronzuur donoren werd verkregen met behulp van competitie experimenten met verschillende glucuronzuur en glucose donoren. Uit de competitie experimenten bleek dat de relatieve reactiviteit van glucuronzuren groter is dan vaak wordt aangenomen. Uit competitie experimenten tussen glucuronzuur, mannuronzuur en galacturonzuur bleek dat mannuronzuur de reactiefste uronzuur epimeer is, gevolgd door respectievelijk glucuronzuur en galacturonzuur.

De relatieve reactiviteit van glucuronzuur staat ook centraal in **Hoofdstuk 3** waar het gebruik beschreven wordt van glucuronzuur donoren in de synthese van fragmenten van capsulaire polysaccharide van de *Streptococcus pneumoniae* type 3 bacterie. Dit polysaccharide bestaat uit een repeterende disaccharide eenheid van glucose en glucuronzuur. Dit maakt een synthese met behulp van disaccharide donoren een aantrekkelijke strategie en om dit te bewerkstelligen werd de reactiviteit van de twee mogelijke repeterende dimeren vergeleken. De donoren met glucuronzuur aan de reducerende zijde bleken productiever in de glycosyleringen te zijn dan de corresponderende glucose donoren. Dit toont aan dat glucuronzuren nuttige bouwstenen kunnen zijn voor de constructie van glucuronzuur bevattende oligosacchariden.

De vaste-drager synthese van oligosacchariden is een relatief nieuwe synthetische benadering, die in vergelijking met de geautomatiseerde synthese van oligopeptiden en oligonucleotiden, nog in haar kinderschoenen staat. In **Hoofdstuk 4** worden het ontwerp en het gebruik van een tweede generatie trieen linker voor vaste drager oligosacchariden synthese beschreven. Het (beschermde) oligosaccharide, dat met behulp van deze linker is verankerd aan de vaste drager, kan met een ringsluiting metathese afgesplitst worden. De toepasbaarheid van deze linker is aangetoond met de synthese van twee alternerende hyaluronan fragmenten. Het gemak waarmee deze linker kan worden gemaakt en de efficiëntie waarmee het gesynthetiseerde oligosaccharide kan worden afgesplitst, maken deze linker een waardevolle aanwinst voor de vaste drager synthese van oligosacchariden.

Hoofdstuk 5 behandelt de vaste-drager synthese van β -1,3-glucaan fragmenten. Hiertoe is een glucose bouwsteen gesynthetiseerd met de relatief weinig gebruikte 4,6-*O*-*p*-chlorobenzylideen acetaal en een tijdelijke levulinoyl beschermgroep. De stereoselectiviteit tijdens de glycosyleringen werd bewerkstelligd met een pivaloyl ester op de C-2 positie. De pivaloyl esters konden echter niet ontschermd worden. Vervanging voor de recentelijk ontwikkeld en mild afsplitsbare 4-azido-2,2-dimethylbutanoyl (AzDMB) groep resulteerde in de vaste drager synthese van een β -1,3-glucaan trisaccharide.

Lipide A is geïdentificeerd als Toll-like receptor 4 ligand en veel natuurlijke lipide A verbindingen en derivaten zijn gesynthetiseerd en geëvalueerd op hun biologische activiteit. Conjugatie van lipide A verbindingen om zo een sterk immuunstimulerend vaccin te verkrijgen is echter nog niet vaak toegepast. **Hoofdstuk 6** beschrijft een synthese route naar de chirale vetstaarten in lipide A, een ontschermd lipide A analoog en een lipide A bouwsteen die gebruikt kan worden voor conjugatie.

List of Publications

Chirality of TLR-2 ligand Pam₃CysSK₄ in fully synthetic peptide conjugates critically influences the induction of specific CD8⁺ T-cells

Selina Khana, Jimmy J. Weterings, Cedrik M. Britten, Ana R. de Jong, Dirk Graafland, Cornelis J. M. Melief, Sjoerd H. van der Burg, Gijsbert A. van der Marel, Herman S. Overkleeft, Dmitri V. Filippov, Ferry Ossendorp.
Molecular Immunology, **2009**, *46*, 1084–1091.

The Stereodirecting Effect of the Glycosyl C5-Carboxylate Ester: Stereoselective Synthesis of β -Mannuronic Acid Alginates

Jeroen D. C. Codée, Leendert J. van den Bos, Ana R. de Jong, Jasper Dinkelaar, Gerrit Lodder, Herman S. Overkleeft, and Gijsbert A. van der Marel.
Journal of Organic Chemistry, **2009**, *74*, 38–47.

Stereodirecting Effect of the Pyranosyl C-5 Substituent in Glycosylation Reactions

Jasper Dinkelaar, Ana R. de Jong, Robert van Meer, Mark Somers, Gerrit Lodder, Herman S. Overkleeft, Jeroen D. C. Codée, and Gijsbert A. van der Marel.
Journal of Organic Chemistry, **2009**, *74*, 4982–4991.

Stereoselectivity of glycosylations of conformationally restricted mannuronate esters

Jeroen D. C. Codée, Ana R. de Jong, Jasper Dinkelaar, Herman S. Overkleeft, Gijsbert A. van der Marel.
Tetrahedron, **2009**, *65*, 3780–3788.

Mannuronic Acids: Reactivity and Selectivity

Jeroen D. C. Codée, Marthe T. C. Walvoort, Ana R. de Jong, Gerrit Lodder, Herman S. Overkleeft, and Gijsbert A. van der Marel.

Journal of Carbohydrate Chemistry, **2011**, 30, 438-457.

Exploring and Exploiting the Reactivity of Glucuronic Acid Donors

Ana R. de Jong, Bas Hagen, Vincent van der Ark, Herman S. Overkleeft, Jeroen D. C. Codée, and Gijsbert A. van der Marel.

Journal of Organic Chemistry, **2012**, 77, 108-125.

A Second-Generation Tandem Ring-Closing Metathesis Cleavable Linker for Solid Phase Oligosaccharide Synthesis

Ana R. de Jong, Anne G. Volbeda, Bas Hagen, Hans van den Elst, Herman S. Overkleeft, Gijsbert A. van der Marel, Jeroen D. C. Codée, *European Journal of Organic Chemistry*, **2013**, 29, 6644-6655.

Curriculum Vitae

Ana Rae de Jong werd geboren op 23 september 1986 te Inchun-Kun (Zuid-Korea). Na het behalen van het gymnasium diploma aan 't Visser het Hooft lyceum te Leiden in 2004 werd in dat jaar begonnen met de studie Scheikunde aan de Universiteit Leiden. Het propedeutisch examen werd in augustus 2005 afgelegd, gevolgd door de bachelor en masteropleiding Chemistry (Design & Synthesis). In 2006 werd een bachelorstage gedaan in de vakgroep Bio-organische chemie, onder leiding van dr. J.J. Weterings en dr. D.V. Filippov en behelsde de synthese van Pam₃Cys conjugaten en een 7-hydro-8-oxo-adenine analoog. Tijdens de daaropvolgende master stage werd er onder begeleiding van dr. J.D.C. Codée en prof. dr. G.A. van der Marel onderzoek gedaan aan conformationeel vastgelegde mannuronzuur donoren en de stereoselectiviteit van een serie glycosiden en de corresponderende glycuronzuur esters. Een industriële bijvakstage werd uitgevoerd bij MSD (toen Schering-Plough) op de afdeling Medicinal Chemistry Oss (MCO) in de groep van dr. J.C.H.M Wijkmans, onder begeleiding van dr. C.E.P. Maljaars en ing. I.A.M. Huisman en omvatte de synthese van designer TLR-4 liganden.

Van februari 2009 tot juli 2013 werd als assistent in opleiding gewerkt aan het in dit proefschrift beschreven onderzoek binnen de vakgroep Bio-organische synthese onder supervisie van Prof. dr. G.A. van der Marel en dr. J.D.C. Codée.

Gedeelten van het onderzoek werden gepresenteerd in de vorm van posters op de jaarlijkse NWO Design and Synthesis bijeenkomsten, op het 16th European Carbohydrate symposium 2011 in Sorrento en op het 6th Glycan forum te Berlijn. Tevens werd er in juli 2009 deelgenomen aan de HRSMC summer school "New horizons in synthetic methodology" in Maastricht. In april 2013 werd een mondelinge presentatie gegeven tijdens het Organische Chemie Symposium georganiseerd door de KNCV Organische Chemie divisie en NWO-CW studiegroepen te Wageningen.

Sinds 16 september 2013 is de auteur van dit proefschrift werkzaam als junior recruiter bij BIRD Recruitment.

