Doctoral Thesis

Automated solid-phase oligosaccharide synthesis of conjugation-ready glycans

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Automated Solid-Phase Oligosaccharide Synthesis of Conjugation-Ready Glycans

A dissertation submitted to
ETH Zurich

for the degree of
Doctor of Sciences

presented by

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2011
to my brothers, to my sisters...
Acknowledgments

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Summary

Among all classes of biopolymers, carbohydrates remain the major synthetic challenge. In this thesis, the development of a fully automated oligosaccharides synthesis platform as a tool for the rapid and efficient assembly of complex glycans is described. The ultimate goal is to enable the evaluation of structure-activity relationships of carbohydrates in biological systems. This required investigation into three distinct but complementary areas:

1. Monosaccharide building blocks design and synthesis
2. Development of automated methodologies for oligosaccharides assembly
3. Development of molecular tools for biological evaluation

In Chapter 2, the synthesis of a small library of differentially protected monosaccharide building blocks is reported. In addition, the use of some of the obtained building blocks in the solution phase synthesis of the tumor-associated antigen Sialyl Lewis\(^x\) is described.

Chapter 3 deals with the solid phase synthesis of a set of biologically relevant oligosaccharides using a new automated platform. Targets were chosen to challenge the chemical strategy while exploring the installation of different glycosidic linkages. In addition, application of the obtained oligosaccharide to the construction of microarrays and protein conjugates is described.

Chapter 4 reports on the construction of carbohydrate capped quantum dots as probe for the study of carbohydrate-carbohydrate interactions at cellular level.
Riassunto

Tra le varie classi di biopolimeri, i carboidrati rappresentano la sfida più grande per la sintesi organica. Questa tesi descrive lo sviluppo di una piattaforma automatizzata per un rapido ed efficiente assemblaggio di oligosaccaridi complessi. Il fine ultimo è la semplificazione dello studio delle relazioni tra struttura e attività che caratterizzano i carboidrati nei sistemi biologici. Il raggiungimento di tali obiettivi necessita ricerca e sviluppo in tre aree differenti e complementari:

1. Progettazione e sintesi di monosaccaridi donatori per l’assemblaggio di polisaccaridi.
2. Sviluppo di metodologie automatizzate per l’assemblaggio di oligosaccharidi.
3. Creazione di sonde molecolari per la valutazione biologica di carboidrati.

Il Capitolo 2 illustra la sintesi di una piccola libreria di monosaccaridi donatori. Inoltre, si riporta l’uso di alcuni di questi monosaccaridi per la sintesi dell’antigene tumorale Sialyl LewisX.

Il Capitolo 3 descrive la sintesi automatizzata in fase solida, effettuata con una piattaforma automatizzata di nuova generazione, di una serie di oligosaccharidi di rilevanza biologica. Questi composti sono stati scelti per verificare i limiti della strategia chimica utilizzata e verificare contemporaneamente la possibilità di installare legami glicosidici di differente natura. In aggiunta, i composti ottenuti sono stati utilizzati per la creazione di microarrays e proteine glicoconjugate.

Il Capitolo 4 descrive la preparazione di quantum dots funzionalizzati con oligosaccaridi per lo studio di interazioni di tipo “carboidrato-carboidrato” a livello cellulare.
1

General Introduction

1.1 Biologically Inspired Chemistry

The concept of a “central dogma” in molecular biology was introduced during the second half of the twentieth century.\(^{(1)}\) The idea that a stream of biological information shuffling from DNA to RNA and on to protein was capable of explaining the basic principles of biological life, continuously inspired discoveries and rapid development in the field of molecular biology. This, in turn had a strong influence on the development of chemical disciplines. As the need to access pure and well defined nucleic acids and proteins became crucial for the study of their biological properties, synthetic chemists, in parallel, designed and developed the tools necessary for the preparation of such compounds. A notable example in this direction is the development of automated solid phase platforms for the synthesis of biomacromolecules. The first achievement in this field is represented by the work of Merrifield on peptide automated solid phase synthesis. Today this work, which earned Merrifield the Nobel Price in 1984, is still considered a milestone.\(^{(2)}\) In 1980 Caruthers demonstrated the possibility of exploiting this approach for the synthesis of oligonucleotides.\(^{(3)}\)

This synergy between biological discovery and chemical innovation became more and more evident with time. In particular, automated solid phase synthesis
platforms became widely available, making it possible for non experts to perform oligonucleotide and peptide syntheses on a routine basis. This has led to several fundamental breakthroughs in genomics and proteomics like the development of the polymerase chain reaction (PCR) for the amplification of the number of copies of a DNA fragment (4), or the spreading of native chemical ligation techniques for protein synthesis. (5) In recent decades, molecular approaches involving DNA, RNA and proteins are inadequate to fully describe cellular maintenance and communication. These processes require at least two additional major classes of biomacromolecules, namely lipids and carbohydrates (Figure 1). (6) Several discoveries have shown how a higher order of complexity can be introduced by posttranslational modifications once a gene has been translated into the corresponding protein. (7) Carbohydrates, in particular, are important in this respect as they account for a considerable percentage of all posttranslational modifications and play critical roles in cellular communication through interaction with carbohydrate binding proteins (lectins).

Figure 1. Flow of biological information between biomolecules.
Despite their importance, understanding the biological role of sugars has been limited due to the difficulty of accessing pure and defined oligosaccharide motifs. The factors that are responsible for this are: (i): the rather heterogeneous nature of carbohydrates which makes their isolation from natural sources difficult; (ii): for carbohydrates there are no techniques analogous to that of polymerase chain reaction (PCR) of nucleic acids or the recombinant DNA technologies used for the expression of proteins; (iii): structural information on naturally occurring oligosaccharides has been limited due to the lack of reliable sequencing platforms; 4) the sheer structural complexity of carbohydrates renders their synthesis far more challenging than that of peptides and oligonucleotides. Nevertheless, the need for a better understanding of the structure-function relationship of carbohydrates in biological systems has required that the field of carbohydrate chemistry, and oligosaccharide synthesis in particular, develop rapidly.

1.2 Classical Carbohydrate Synthesis

Glycosylations are central to the understanding of carbohydrate synthesis. These transformations, which belong to the category of substitution reactions, generally involve the reaction between a selectively functionalized “donor” sugar, with an anomeric leaving group, and a suitably functionalized “glycosyl acceptor”. The former serves as an electrophile that, upon activation by a promoter, generates a glycosidic linkage transferring the “glycosyl moiety” to the glycosyl acceptor (Figure 2). The latter, in turn, acts as the nucleophile and contains at least one free hydroxyl group.

Glycosylation methods are classified on the basis of the nature of the leaving group at the anomeric carbon of the glycosyl donor and the activation conditions.
The protecting group patterns on both the donor and acceptor play a crucial role in the outcome of the reaction. The participating or nonparticipating nature of the “directing” protective groups, usually located on position C-2 of the glycosyl donor (Figure 2), influences the stereochemistry of the glycosidic linkage. Accordingly, a participating group at C-2 will favor the formation of 1,2 \textit{trans} linkages. A non-participating group is exploited at C-2 when a 1,2 \textit{cis} linkage in the product is desired. Generally, ester or amide functionalities are chosen as participating groups due to their ability to perform anchimeric assistance. Otherwise, ether, amino or azido (non participating) protecting group are used. Permanent protecting groups are used to block hydroxyl groups that do not play an active role during glycosylations, whereas, a temporary protecting group will be uncovered at an appropriate point to access nucleophilic hydroxyl groups for subsequent glycosylations. For the preparation of branched structures, building blocks functionalized with orthogonal protecting groups that can be cleaved selectively in the presence of the remaining functionalities are required. Fundamental aspects on the chemistry of glycosidic bond formation have been previously described \cite{9 and references therein}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{glycosylation_diagram.png}
\caption{Schematic description of glycosylation reactions.}
\end{figure}
The principles described so far are central for understanding carbohydrate synthesis. Nevertheless, it is worth considering that the notion of glycosylation alone is rather incomplete to describe the whole process of synthesizing biologically active oligosaccharides. The preparation of properly functionalized building blocks and deprotection strategies are also key components in developing high yielding and reproducible synthetic routes to oligosaccharides. In the past, the development of new and more efficient glycosylation methods, combined with the discovery of new leaving and protecting groups represented the main focus of several different research groups working in the area of carbohydrate synthesis. More recently, significant attention has been devoted to the creation of new strategies to reduce the complexity of building block syntheses, assembly and deprotection protocols crucial for the synthesis of complex sugars. In the section below, crucial advancements are highlighted.

1.3 Building Blocks for Carbohydrate Synthesis

The first obvious difference between carbohydrates and other biopolymers is the number of building blocks that nature utilizes to generate polymeric structures. While DNA is synthesized from a pool of four different nucleotides and proteins are assembled from a library of twenty amino acids, carbohydrate diversity is achieved starting from an undefined collection of monosaccharides building blocks. As previously mentioned, the connection of two monomeric residues can occur in different ways depending on the regio- and stereo-specificity of the glycosidic linkage of interest. In this way, additional complexity is added to oligosaccharides. It was recently estimated that a minimal set of twenty sugar building blocks would generate hexameric structures in number up to three orders of magnitude higher than in the case of peptides (Figure 3).
Introduction

<table>
<thead>
<tr>
<th>Oligomer Size</th>
<th>Nucleotides</th>
<th>Peptides</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>400</td>
<td>1 360</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
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<tr>
<td>6</td>
<td>4096</td>
<td>64 000 000</td>
<td>192 780 943 360</td>
</tr>
</tbody>
</table>

Figure 3. Diversity space of oligonucleotides, peptides and mammalian oligosaccharides (adapted from reference 10).

However this number is misleading since nature utilizes only a limited selection of all possible theoretical structures. A clear understanding of the biological relevance of any given linkage is therefore crucial to identify the minimal set of building blocks required to assemble diverse carbohydrate structures. A systematic structural analysis of mammalian oligosaccharides catalogued in glycan databases highlight the fact that, amongst all possible structures that can be generated by the ten mammalian oligosaccharides, only a limited selection is actually observed in nature (Figure 4). For example, a study of the database “glycoscience.de”, which is regarded as one of the most comprehensive glycan databases, showed that 75% of the catalogued 3299 mammalian oligosaccharides could be constructed from a minimal set of 36 building blocks.(10)
Once relevant sugar building blocks are identified, one can proceed by providing them with the proper protecting group pattern. Generally, protecting groups are installed on a monosaccharide through iterative alcohol protection-deprotection strategies aimed at differentiating between several chemically similar hydroxyl groups. Starting from the naturally occurring sugars, 8 to 14 chemical steps are required to prepare monosaccharides for glycan assembly (Figure 5a). Alternative methods for the preparation of building blocks have been generated in order to reduce the complexity of traditional strategies. It is worth mentioning in this regard the *de novo* approach (Figure 5b), in which orthogonally protected monosaccharides are generated from non-carbohydrate precursors generally by stereoselective synthesis.\((11, 12)\)
Introduction

Figure 5. Possible approaches for building block preparation.

One-pot methods (Figure 5c) for the streamlined synthesis of differentially protected building blocks have also been introduced. (13) Using this approach, the regioselective protection of polyhydroxyls is achieved in a single reaction setup. Different orthogonal functionalities can be introduced without the need of lengthy and time-consuming purifications in intermediate steps. Recently, chemoenzymatic approaches have been reported, which make use of promiscuous enzymes with broad tolerance for substrate size and shape. In one such example, differentially protected 3-deoxy sugars were generated by aldolase catalyzed addition of a pyruvate enolate to a wide range of structurally complex aldehydes (Figure 5d). (14)
1.4 Assembly Strategies

Performing glycosylations in solution phase remains, at present, one of the most robust methods for accessing complex oligosaccharides. This kind of approach is complicated by a relatively long series of purification-deprotection steps needed for sugar elongation. In this regard, one-pot glycosylation methods were developed.\(^\text{15}\) In a one pot strategy, several glycosyl donors are allowed to react sequentially in the same flask to generate a single oligosaccharide as the major product, while avoiding intermediate isolation and deprotection steps. One-pot methodologies can be divided into three different subclasses: (i): “chemoselective strategy”; (ii): “orthogonal glycosylation”; and (iii): “pre-activation strategy”. The chemoselective strategy exploits the differences in the intrinsic reactivity of donors and acceptors generated. This difference is determined by the nature of different protecting groups.\(^\text{16}\) In general, electron-donating groups have the tendency to activate the glycosyl donor (armed) whereas electron withdrawing groups deactivate it (disarmed). The resulting difference in the reactivity enables chemoselective glycosylations. Thus, in the chemoselective one-pot glycosylation, the most reactive donor is activated to undergo glycosylation with a second, less reactive donor (Figure 6a). This results in the formation of a new glycoside that can subsequently undergo a second glycosylation reaction. The orthogonal glycosylation strategy exploits the orthogonality of different anomeric groups (e.g. thioglycoside and fluoride).\(^\text{17}\) Functionalities on position C-1 are utilized either as anomeric protecting groups or as leaving groups. The basic requirement is the respective stability of a first glycosyl donor to the conditions used to activate the anomeric functionality on a second donor (Figure 6b). In the case of pre-activation strategy \(^\text{18}\) the glycosyl donor is activated in the absence of the acceptor. In this way, a reactive species is generated which can react rapidly with a glycosyl acceptor with an identical leaving group at the reducing
end. The resulting saccharide can then be activated again for a successive glycosylation (Figure 6c).

![Figure 6. One pot glycosylation strategies.](image)

One-pot methods have found significant applications in the synthesis of complex carbohydrates. For example, the armed-disarmed approach was used by Wong and coworkers in their programmable one pot strategy.\(^{(19)}\) Here, a software guides the selection of building blocks from a library containing over 50 thioglycosides. Glycosyl donors are identified based on their relative reactivity and their ability to be sequentially activated. As another example, Takahashi’s automated orthogonal leaving group method was used to synthesize a combinatorial library of 72 trisaccharides.\(^{(20)}\) Although these methodologies are based on solid principles, they suffer from the limitation of being difficult to implement for the synthesis of higher oligosaccharides. This is due to the requirement of large libraries of differentially protected building blocks, as in the armed-disarmed approach, or to the limited number of orthogonal leaving groups, as illustrated with the orthogonal glycosylation approach. An additional limiting factor is the accumulation of undesired byproducts in the reaction mixture.

As a consequence, only short oligosaccharides (tri-, tetra-saccharides) can be prepared using short sequences of three to four glycosylations,\(^{(15)}\) while only
few reports on the one pot preparation of higher oligosaccharides have been reported in the literature.\(^\text{(21)}\)

Alternative solution-phase strategies for carbohydrates assembly incorporate molecular tagging methods to simplify purification.\(^\text{(22)}\) Tagging was shown to simplify the purification of short oligosaccharides. Using this approach, the capping trisaccharide from *Leishmania*’s lipoarabinomannan was synthesized by Pohl and coworkers.\(^\text{(23)}\) However, this method proved less effective with longer structures. Attempts to automate this approach have been complicated by the limited availability of machinery engineered to execute the complicated liquid or solid phase extractions required for purification of tag-linked intermediates and products.

Solid phase oligosaccharide synthesis became appealing since the early 1970s.\(^\text{(24)}\) The main advantage of solid phase methodology is that after glycosylation of a proper linker bound to an insoluble solid support, purification is performed by filtration and washing. This allows the use of excess reagents to drive reactions to completion. Deprotection of the temporary protecting group exposes a new building block acceptor. Reiteration generates polymer-linked oligosaccharide that finally can be cleaved from the solid support, purified and deprotected.

Two possible approaches have been reported for solid phase oligosaccharide synthesis.\(^\text{(25)}\) In the acceptor-bound strategy, a growing oligosaccharide chain is bound to the resin via its reducing end and exposure of a free hydroxyl on the terminal residue provides a nucleophile for the next step. In the donor-bound strategy the ‘non-reducing end’ of the oligosaccharide is anchored to the solid support. A stable anomeric leaving group precursor is added to the activated resin-bound donor generating a glycosyl transferring moiety. The attracting feature of a solid phase approach is the possibility of automation that results in a substantial shortening of the synthetic cycles.
1.5 Automated Oligosaccharide Synthesis

The first example of automated oligosaccharide synthesis was reported in 2001. (26) In this study, an Applied Biosystems peptide synthesizer ABI 433 was adapted for the synthesis of oligosaccharides with a few modifications. Merrifield resin, which exhibits good swelling properties in the organic solvents commonly employed in glycosylation reactions and is also stable under acidic conditions, was functionalized with an octenediol linker 1.13 (Figure 7). The synthesis began with the glycosylation of the support-bound octenediol linker 1.13 with a monosaccharide building block. In order to force the reaction to completion, excess glycosyl donor was used at temperatures dependent on the nature of the building blocks. Subsequent removal of the temporary protecting group exposed the hydroxyl group for the subsequent coupling. Subsequent building blocks were installed via this iterative, stepwise glycosylation/deprotection sequence. Once the elongation process was complete, the protected oligosaccharide was detached from the solid support via olefin cross metathesis (27) yielding an oligosaccharide functionalized with a pentenyl glycoside at the reducing end. The latter could then be employed as a leaving group for further glycosylations (28) or converted into a functional linker by oxidation of the double bond. (29) Finally, standard deprotection steps were established to afford fully deprotected oligosaccharides ready for biological evaluation. Glycosyl phosphate or imidate building blocks were successfully employed in these preliminary studies. The use of thioglycosides was precluded due to the presence of a double bond in linker 1.13 which is incompatible with the electrophilic activation protocols necessary for this class of glycosylating agents.
In this setup, the overall assembly strategy became much simpler, as no intermediate purification steps were required besides the filtration and washes that were automated and performed by the machine.

Several examples presented in a series of investigations corroborated the efficiency of this platform. The synthesis of a poly α-(1→2) mannosides is one such example (Figure 8). Polymannosides were assembled using mannosyl trichloroacetimidate building block 1.16. The strategy used a ten-fold excess of building block 1.16 to glycosylate the octenediol-functionalized resin 1.13 in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate for 30 minutes. After glycosylation the resin was washed several times with dichloromethane and methanol-dichloromethane mixtures.
Deprotection of hydroxyl at C2 was achieved by treatment with excess sodium methoxide in a methanol/dichloromethane mixture. This cycle of glycosylation-deprotection was repeated to obtain penta-, hepta- and deca-saccharides 1.19, 1.20 and 1.21 in good overall yields after cross-metathesis with ethylene. The coupling-deprotection cycle is described in detail in Table 1. The duration of each cycle was 2.6 hours. In order to incorporate each residue of the pentasaccharide, this cycle has to be repeated five times resulting in an overall duration of 14 h.

The technology developed in this preliminary investigation enabled in short time the synthesis of a diverse collection of protected oligosaccharides by simple variations of building blocks, leaving groups and temporary protecting groups. Some representative examples are shown in Figure 9. For example, the tumor associated marker Le'–Le nonasaccharide has been currently explored as conjugate cancer vaccine candidate and served to showcase the efficient installation on the solid phase of challenging α-fucosidic linkages (Figure 9a).
The preparation of a protected phytoalexin elicitor (Figure 9b) confirmed that longer oligosaccharides can be obtained by an automated approach. Furthermore, accomplishing the synthesis of the tumor antigen Globo H (Figure 9c) corroborated the versatility of the platform since other difficult linkages (α-galactose) were successfully installed on the solid support.

<table>
<thead>
<tr>
<th>Step</th>
<th>Function</th>
<th>Reagent</th>
<th>Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Couple</td>
<td>10 eq. Building Block and 0.5 eq. TMSOTf</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Wash</td>
<td>CH₂Cl₂</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Couple</td>
<td>10 eq. Building Block and 0.5 eq. TMSOTf</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Wash</td>
<td>CH₂Cl₂</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Wash</td>
<td>1:9 Methanol: CH₂Cl₂</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Deprotection</td>
<td>2 x 10 eq. NaOMe (1:9 Methanol : CH₂Cl₂)</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>Wash</td>
<td>1:9 Methanol : CH₂Cl₂</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Wash</td>
<td>0.2 M Acetic acid in THF</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Wash</td>
<td>THF</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Wash</td>
<td>CH₂Cl₂</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1. Coupling cycle for automated synthesis of polymannosides 1.19, 1.20, 1.21 using trichloroacetimidate building blocks 1.16 with acetate as temporary protecting group.
Figure 9. Successful examples of automated solid phase synthesis of complex oligosaccharides by the Seeberger group.
One of the most interesting aspects shown in the previous examples is that the assembly of higher structures could be completed within one day due to the speed of glycosylation-deprotection cycles. The advantages of an automated solid phase approach over a classical solution synthesis become clear with a qualitative comparison. The synthesis of a generic trisaccharide 1.22 (Figure 10a) is considered as an example. The approach chosen makes use of three sequential glycosylations with three different sugar building blocks (1.23, 1.26, 1.28). Common hexose building blocks can be prepared with an average of seven synthetic steps. Five steps (glycosylation-deprotection) will be used to assemble the protected oligosaccharide 1.29 and a standard deprotection strategy will provide the desired compound 1.22 in three steps.

If it is assumed that any given synthetic step performed in traditional solution phase requires a single day of work, the targeted trisaccharide 1.22 will be obtained in 29 days. The time contribution for building block preparation, assembly and deprotection of the trisaccharide has been plotted in Figure 10b (solution phase). Approximately 17% of the overall time is dedicated to the assembly; assembly and deprotection phases together account for ca. 27% of the overall time. Performing the assembly in an automated fashion takes one single day, hence 13% of time can be saved (Figure 10b, automation 2001).

Despite the advantages, automated oligosaccharide synthesis platforms have found only limited application. The reason is that, at the end of the synthesis, complex synthetic manipulations are required to transform the obtained n-pentenyl glycoside into a compound ready for conjugation to surfaces or carrier proteins.

An ideal automated platform should be able to implement also the deprotection sequences leading to additional time savings, providing direct access to glycans that are ready for biological evaluation (Figure 10b, automation ideal). This, combined with newer and faster strategies for building blocks preparation, could improve tremendously the performance of the platform.
1.5 Aims and Outline of the Thesis

The work described in this thesis encompasses the development of a new automated oligosaccharide synthesis platform for the rapid assembly of biologically relevant oligosaccharides (Figure 11). Specifically, we focused our
efforts on meeting the following challenges: (i): generating a collection of stable, differentially protected building blocks that can be prepared in short time; (ii): synthesizing a new linker 3.1 that supports glycosylation reactions and deprotection sequences; (iii): developing a standardized glycosylation protocol for each unique building block; (iv): integrating these components to devise a strategy that initially generates partially-protected oligosaccharides, for ease of purification, then releases glycans that are ready for conjugation after a single simple chemical manipulation.

Chapter 2 deals with the preparation of building blocks for the efficient installation of statistically relevant glycosidic linkages during oligosaccharide synthesis. Chapter 3 describes the development of a new linker for solid phase synthesis and automated protocols for the generation of complex carbohydrates. Moreover,
the successful use of thioglycosides in the synthesis of complicated structures will be described. Application of oligosaccharide libraries prepared via solid phase synthesis to the generation of microarrays and conjugated vaccine candidates will be discussed. Chapter 4 presents a preliminary investigation on the possibility to exploit carbohydrate-carbohydrate interactions for the targeting of melanoma cells. Finally, Chapter 5 summarizes the findings presented in this thesis providing conclusions and future prospects.

References

Introduction


Acknowledgments

Part of the work presented in this chapter was performed in collaboration with Dr. Cheng-Chung Wang (synthesis of glucosamine building blocks), Dr. Shinia Hanashima and Dr. Bastien Castagner (synthesis of Sialyl Lewis\(^x\)).
2.1 Introduction

The construction of a series of differentially protected carbohydrate building blocks is central to the synthesis of glycans. The wide structural diversity imparted to sugars by chain branching and the formation of new stereogenic centers with each glycosidic linkage, requires that a large number of building blocks be available for the synthesis of diverse oligosaccharide libraries. As mentioned in Chapter 1, a bioinformatic analysis of the currently known mammalian glycospace helped to identify the most prevalent monosaccharide building blocks. These sugars were then synthesized based on prevalence, resulting in the generation of a small library of building blocks amenable to oligosaccharide assembly. This chapter describes the synthesis of sialic acid, galactose, fucose, glucosamine and glucose building blocks (Figure 1) and their preliminary evaluation in a modular solution phase synthesis of Sialyl Lewis\(^x\) (SLx). For each monomer, the selection of protecting group patterns and anomeric leaving groups were guided by reactivity, ease of synthesis and stability during prolonged storage.
2.2 Sialic Acids

The term “sialic acids” relates to a vast class of $\alpha$-keto acids derived from $N$-acetylneuraminic acid (Neu5Ac). This class of compounds, which were isolated from submaxillary mucin for the first time in 1930 by Klenk and Blix, all share a nine carbon backbone. Later, several additional derivatives of neuraminic acids were extracted from brain glycolipids (neuro-amine-acid).

The biosynthesis of these compounds is based on the enzymatic condensation of a phosphoenolpyruvate unit with a six carbon scaffold (typically acetylmannosamine [ManNAc] in the case of Neu5Ac, or mannose [Man] in the case of...
3-deoxy-\textit{d}-\textit{glycero-d}-galacto-nonulosonic acid [KDN]).(4) Structural diversity in sialic acids can be generated by modification at the C5 position. Moreover, biosynthetic modification of one or more hydroxyl groups at positions C4 to C9 with acetates, lactates, sulfates, phosphate esters or methyl esters further expands the functionality of this family of sugars, of which there are currently 50 known derivatives.(1)

![Figure 2](image-url) Natural connectivity of sialic acids.

In humans, Neu5Ac is often linked to the C3- or C6-position of galactose or \textit{N}-acetylgalactosamine (Figure 2, 2.12, 2.13). Polymeric structures of sialic acids have also been reported (Figure 2, 2.14, 2.15).

At physiological pH, the carboxylic group of sialic acid (pKa = 2.6) is deprotonated, conferring to the residue a negative charge. As a consequence, until recent times, it was considered that the most interesting feature of sialic acids was their ability to provide hydrophilicity and charge to the cell surface.(5) More recently, it has been extensively reported that these sugars, located at the non-reducing terminus of glycolipids and glycoproteins, often serve as possible
point of attachment for viral and bacterial receptors.\(^6\) Sialic acids have also been shown to mediate several other important biological processes. For example, in mammals, “siglecs”, a branch of the immunoglobulin superfamily (IgSF) exclusively devoted to interacting with sialic acids (sialic-acid binding-immunoglobulin-like-lectins)\(^7\) mediate signaling functions in cell communication, with different siglecs showing considerable differences in specificity for sialylated ligands.

Interestingly, sialic acids are not distributed homogeneously in nature, and are only found in eukaryotes, in particular in the deuterostome lineage of animals, and in some gram negative bacteria (Figure 3).\(^1\) In general, nearly all bacteria that express Neu5Ac are causative agents of serious illnesses in humans and domestic animals. For example, \emph{Neisseria meningitides}, which causes meningitis, and \emph{Campylobacter jejuni}, which causes gastroenteritis, both contain sialic acid residues.\(^1\) This can be explained based on the consideration that Neu5Ac is the outermost component of membrane oligosaccharides and serves as regulator of innate immunity mechanisms. Therefore, microbes, which during host invasion are in close contact with sialic acid, have probably evolved a way to incorporate this residue on their surface, in a form of molecular mimicry that is crucial to avoid attack from the host’s immune system.
Figure 3. Sialic acid occurrence in evolutionary perspective. (1) Expression of Neu5Ac (determined by physical methods) is indicated with a close circle, while putative expression with an open circle.

Although sialic acids play a central role in glycobiology, sialylation (glycosylation with sialic acid donors) by chemical means remains a challenge and enzymatic glycosylations are often used as an alternative to construct sialylated oligosaccharides. (8) Direct exploitation of Neu5Ac building blocks, especially in the glycosylation of the C3-position of galactose, one of the most important linkages found in sialylated glycans, often results in low yields and anomeric mixtures. This is due to the hindered nature of the tertiary anomeric position of sialic acid and the lack of a participating group on the neighboring C3 (Figure 4). In addition, a highly favored competing elimination reaction, which generates $\alpha-\beta$ unsaturated ester 2.19, further lowers the potential efficiency of the method. (9)
Several strategies aimed at improving the efficiency of sialylation have been reported.\(\text{(9, 10)}\) The introduction of auxiliary participating groups at C1 or C3 has been proposed as a way to address the lack of selectivity, while different leaving groups have been screened in an effort to maximize glycosylation yields.\(\text{(9)}\) With respect to the latter, the “fixed dipole” concept was introduced to explain how the dipole generated by the different protecting groups on position C5 can have a remote “through space” influence on the stability of the oxocarbenium ion generated during glycosylation.\(\text{(11)}\) In this regard, different N-5 protecting moieties, including carbamate,\(\text{(12)}\) N-Troc,\(\text{(13, 14)}\) TFA,\(\text{(15)}\) azide,\(\text{(16)}\) and imide\(\text{(11)}\) have been evaluated in glycosylation reactions and have shown improved yields and selectivities. The only drawback associated with these methods is the introduction of a deprotection step at the end of the synthesis to generate the natural occurring acetamido moiety.

Given the ubiquity of terminal \(\alpha\)-(2,3)-linked sialyl galactose capped oligosaccharides,\(\text{(1)}\) we sought to discover a highly efficient sialylation route to access a disaccharide building block for the synthesis of glycans containing these functional residues. We rationalized that the ideal building block would provide products in high yield and with selectivity in glycosylation reactions with a complex oligosaccharide partner, and would be amenable to assembly on solid
phase. Moreover, it should be obtainable through a rapid, streamlined and scalable synthesis.

2.3 N-Troc-Protected Sialic Acid $\alpha$-(2-3) Galactose Building Block

We began the synthesis of disaccharide building block 2.1 by evaluating different nucleophiles in sialylation reactions (Table 1).(17) We considered at first the use of glycals, because they are sterically less hindered than the corresponding saturated monosaccharide. In this regard, it was reasoned that galactal 2.24 (Table 1) may be well suited for coupling with sialic acid because the hydroxyl group on the C3-position is sterically less hindered due to the absence of a C2-hydroxyl. In addition, the C3-hydroxyl group is more nucleophilic than a typical galactose C3-hydroxyl group, due to its allylic character. Finally, it has been shown that glycals are excellent precursors for the preparation of glycosylating agents.(18, 19).
Literature reports on the use of galactals as acceptors in sialylation reactions have been limited.\((20,21)\) This is mainly due to the instability of the olefin under the activation conditions required for the more commonly accessed sialyl thioglycoside building blocks. For our synthesis, we employed phosphite\((22)\) or \(N\)-phenyl trifluoroacetimidate\((11)\), which were shown to be competent leaving groups for sialic acid building blocks.\((23,24,25)\) In addition, \(N\)-Troc protection was chosen for its favorable “fixed dipole” effect\((13)\).

Sialic acid building blocks \(2.20\) and \(2.21\) were activated with TMSOTf (0.15 eq.) in propionitrile\((26)\) at \(-78 \, ^\circ\text{C}\) (Table 1). Diol acceptors similar to \(2.22\) and \(2.23\) have been previously exploited for the generation of sialosides.\((21)\) In this cases, the most nucleophilic hydroxyl, usually 3-OH, was found to react almost exclusively. With this in mind, compounds \(2.22\) and \(2.23\) were used as model

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>Nucleophile</th>
<th>Product</th>
<th>(\alpha/\beta ) ratio</th>
<th>Yield of (\alpha) product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(2.20) (1.5 eq.)</td>
<td>(2.22)</td>
<td>(2.25)</td>
<td>3/1</td>
<td>44%</td>
</tr>
<tr>
<td>2</td>
<td>(2.21) (1.0 eq.)</td>
<td>(2.23)</td>
<td>(2.26)</td>
<td>8/1</td>
<td>56%</td>
</tr>
<tr>
<td>3</td>
<td>(2.20) (1.0 eq.)</td>
<td>(2.24)</td>
<td>(2.27)</td>
<td>9.5/1</td>
<td>76%</td>
</tr>
<tr>
<td>4</td>
<td>(2.20) (1.2 eq.)</td>
<td>(2.24)</td>
<td>(___)</td>
<td>11/1</td>
<td>80%</td>
</tr>
<tr>
<td>5</td>
<td>(2.20) (1.5 eq.)</td>
<td>(2.24)</td>
<td>(___)</td>
<td>11/1</td>
<td>86%</td>
</tr>
</tbody>
</table>

* Determined by \(^1\)H NMR after size exclusion chromatography.

\(2.20\) \(R = \text{P(OBn)}_2\)
\(2.21\) \(R = \text{C(NPh)}\text{CF}_3\)

**Table 1.** Glycosylation of galactose nucleophiles \(2.22-2.24\) with sialic acid building blocks \(2.20\) and \(2.21\).
substrates for comparison. As expected, the desired disaccharides 2.25 and 2.26 were obtained in yields and regioselectivities comparable to published data (entries 1 and 2, Table 1). In contrast, galactal 2.24 showed a remarkable improvement in both yield and stereoselectivity (entry 3-5, Table 1). The use of 1.5 equivalents of galactal 2.24 with glycosylating agent 2.20 provided disaccharide 2.27 with good selectivity (α/β = 9.5/1, entry 3, Table 1). The α-anomer was isolated in 76% yield. The use of 1.5 equivalents of glycosylating agent 2.20 provided 2.27 in 86% yield with the best selectivity (α/β = 11:1, entry 5, Table 1). The use of N-phenyl trifluoroacetimidate 2.21 gave only low yields while elimination product was accumulated in the reaction mixture.

The galactal portion of disaccharide 2.27 was subsequently equipped with a suitable anomic leaving group for further functionalization (Scheme 1). Treatment of disaccharide 2.27 with PhI(OAc)₂ and catalytic amount of BF₃·Et₂O (18) followed by in situ acetylation using acetic anhydride provided diacetate 2.28. In this particular case, the acetylation step was required since treatment with PhI(OAc)₂ and BF₃·Et₂O produced a mixture of 2-OH and 2-OAc products. The anomic acetate was cleaved with hydrazine acetate to afford the corresponding hemiacetal, which was further derivatized as N-phenyl trifluoroacetimidate (27, 28) to give building block 2.1 in good yield.

Scheme 1. Transformation of 2.27 into sialyl galactose building block 2.1. Reagents and conditions: i: PhI(OAc)₂, BF₃·Et₂O, CH₂Cl₂, -40 ºC; then Ac₂O, pyridine; ii: N₂H₄·AcOH, DMF; iii: CF₃C(NPh)Cl, Cs₂CO₃, CH₂Cl₂, DCM. 76% over three steps.
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2.4 N-Acetyl-Sialic Acid α-(2-3) Galactose Disaccharide Building Block

The possible exploitation of simple N-acetyl sialyl glycosylating agents for the glycosylation of galactals in place of N-Troc protected donors was further investigated. It was envisaged that using N-acetyl sialic acids glycosylating agents, which are easier to access when compared to N-Troc protected donors, would provide more rapid access to valuable disaccharide building blocks (Scheme 2). In this regard, modification of the N-acetamido moiety on 2.10, requires a N-deacetylation step preceded by protection of the anomeric hydroxyl (Scheme 2a). These complicated steps can be avoided starting from intermediate 2.33 (Scheme 2b). As an additional advantage, avoiding N-modification eliminates the need for a low yielding deprotection step at the end of the synthesis.

Scheme 2. Retrosynthetic analysis for the preparation of sialic acid building blocks 2.32.
Most of the \(N\)-Acetyl sialic acid donors reported in literature can be accessed from the common intermediate \(2.33\), \((29)\) which is prepared in two steps starting from the commercially available neuraminic acid (scheme 3).

**Scheme 3.** Synthesis of different sialic acid glycosyl donors \(2.35-2.37\).

Reaction of \(2.33\) with different electrophiles afforded respectively the \(N\)-phenyl trifluoroacetimidoyl glycoside \(2.35\) and the phosphites \(2.36\) and \(2.37\) as previously described in the literature.\((23, 24, 25)\) The reactivity of the readily obtained glycosyl donors was evaluated in the glycosylation of galactal \(2.24\) (Table 3, entries 1-4). In the case of compound \(2.36\), no traces of product were detected upon activation with TMSOTf at -78 °C (Table 3, entry 1). However, donors \(2.35\) and \(2.37\) afforded the desired disaccharide \(2.40\), but with poor yields (Table 3, entry 2, 3). Increasing the reaction temperature caused a considerable increase in reactivity (entry 4) resulting in \(2.40\) being isolated in 72% yield. The desired anomere could be easily isolated by column chromatography and efficiently derivatized as in the case of compound \(2.27\) through oxidation of the double bond (Scheme 4). Thus, treatment with PhI(OAc)\(_2\) and a catalytic amount of BF\(_3\)-Et\(_2\)O, followed by complete acetylation produced diacetate \(2.43\). Hydrazine acetate mediated removal of the anomeric acetate provided the hemiacetal, followed by introduction of the anomeric \(N\)-phenyl trifluoroacetimidate to furnish building block \(2.2\) in good yield. Although the sialylation of galactal \(2.24\) with
donor 2.20 proved to be much more efficient, the reduced number of steps for the preparation of 2.37 makes it possible to obtain the sialyl containing disaccharide building block in a higher overall yield (ca. 26% in the case of 2.1, ca. 40% for 2.2).

Donor 2.37 also performed efficiently in combination with nucleophiles 2.38 and 2.39, illustrating the importance of this method for the production of α-(2-6) derivatives (Table 2, entry 5-6). In particular, compound 2.41 could be further derivatized as previously described to afford the hemiacetal 2.45 (Scheme 4), (30) a useful intermediate for the preparation of imidates building blocks.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>Nucleophile</th>
<th>Conditions</th>
<th>Product</th>
<th>Yield of product α/β ratio (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.36</td>
<td>OPEt₂</td>
<td>(1.5 eq.) a</td>
<td>2.40</td>
<td>30% 9/1</td>
</tr>
<tr>
<td>2</td>
<td>2.35</td>
<td>O,N,NPh</td>
<td>(1.5 eq.) a</td>
<td>*</td>
<td>27% 9/1</td>
</tr>
<tr>
<td>3</td>
<td>2.37</td>
<td>OP(OBn)₂</td>
<td>(1.7 eq.) b</td>
<td>*</td>
<td>72% 2.5/1</td>
</tr>
<tr>
<td>4</td>
<td>2.37</td>
<td>OAcHN</td>
<td>(2.0 eq.) b</td>
<td>2.41</td>
<td>75% 2.5/1</td>
</tr>
<tr>
<td>5</td>
<td>2.37</td>
<td>OAcHN</td>
<td>(1.3 eq.) b</td>
<td>2.42</td>
<td>70% 2.5/1</td>
</tr>
</tbody>
</table>

Table 3. Reagents and Conditions: a:TMSOTf (0.2 eq.), EtCN, AW-4Å MS, -78° C; b: TMSOTf
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(0.2 eq.), CH₃CN, AW-4Å MS, -42 °C: Determined by ¹H NMR after size exclusion chromatography.

Scheme 4. Reagents and conditions: i: PhI(OAc)₂, BF₃·Et₂O, CH₂Cl₂, -40 ºC; then Ac₂O, pyridine; ii: N₂H₄·AcOH, DMF; iii: CF₃C(NPh)Cl, Cs₂CO₃, CH₂Cl₂, DCM.

2.5 (1-3)-β-Galactose and Capping Galactose Building Blocks

According to statistical analysis, 3-linked β-galactose is one of the most frequently occurring residues in mammalian oligosaccharides. (31) Therefore, a building block of general applicability for the introduction of this sugar in synthetic oligosaccharides was designed (Scheme 5). A thioethyl functionality was chosen for the anomic position for two reasons. On one hand, thioethyl glycosides are strong glycosylating agents characterized by a high reactivity upon activation with an electrophile in acidic conditions. On the other side, the inherent stability of the thioglycosidic linkage guaranties prolonged stability of the building block upon storage. The fluorenylmethyloxycarbonyl (Fmoc) group, very stable under acidic conditions and removable with mild bases, was employed for position O-3. Due to its fluorescence, Fmoc has been intensively used in solid phase synthesis to monitor the efficiency of coupling steps. Therefore, we decided to adopt this functional group as standard temporary protecting group, anticipating the use of our building blocks for solid phase synthesis. Due to our interest in β-
galactosides, position O-2 was provided with a pivaloyl ester, very stable under acidic conditions and capable of performing anchimeric assistance.

Our synthesis commenced with the conversion of the peracetylated β-galactose 2.46 (Scheme 5) to the corresponding thioethyl glycoside. Treatment of 2.46 with thioethanol in the presence of catalytic amounts of BF$_3$·Et$_2$O, followed by deacetylation under Zemplen conditions afforded thioglycoside polyol 2.47. The C2-C3 trans diol was then masked as a diacetal, following the work of Ley and co-workers who reported the use of 1,2-diketones under acidic conditions for the selective protection of trans-diequatorial-1,2-diols on monosaccharides. (32) Thus, reaction of 2.47 with butane-2,3-dione in presence of camphorsulphonic acid and trimethyl orthoformate afforded compound 2.48 in 68% yield. Benzylation of the remaining alcohols under standard conditions followed by acid cleavage of the diacetal moiety afforded 2.50 in 56% yield over two steps. (33) It is worth mentioning that a diol with characteristics similar to those of compound 2.50 could be obtained in a single step, derivatizing intermediate 2.47 as a 4,6-benzylidene acetal. Nevertheless, in order to avoid conformational constraint on the final building block, this approach was not pursued.

Compound 2.50 was further reacted with dibutylstannoxide resulting in the in situ formation of a stannylidene that upon treatment with FmocCl yielded derivative 2.51 in excellent yield. Finally, reaction with PivCl in presence of pyridine gave the desired building block 2.3. Terminal galactose building block 4 was also generated by treatment of compound 2.51 with benzylbromide and sodium hydride in 80% yield.
2.6 Capping Fucose Building Block

Fucose is an important constituent of different mammalian and bacterial carbohydrate antigens. (3) In bacterial polysaccharides it is generally connected via O-3 or O-4 to different oligosaccharide residues. (34) In human oligosaccharides and glycoproteins it is usually found at the non-reducing end of oligosaccharides. Fucose containing carbohydrates are involved in several important biological processes including tissue development, cell adhesion and tumor metastasis. In particular, high levels of expression of fucosylated oligosaccharides are generally observed on malignant cancer cells. (34) In the context of mammalian glycans, our goal was to generate a capping building block
for the introduction of naturally occurring 1,2-cis linkages. This requires the installation of a non participating group at the C-2 position.

The synthesis commenced from the known intermediate 2.54 (35) (Scheme 6), which was prepared from compound 2.52 by reaction with thiophenol in presence of BF₃·Et₂O, followed by methoxide mediated deacetylation, and finally installation of a 3,4 acetonide upon reaction with 2,2-dimethoxypropane in the presence of catalytic p-toluensulfonic acid. Benzylolation in the presence of sodium hydride and benzyl bromide, followed by acid cleavage of the acetonide afforded 2.56. Position O-3 and O-4 on compound 2.56 were successfully acetylated by treatment with acetic anhydride in pyridine to afford the thiophenyl glycoside 2.57. Although the latter compound represents a potential glycosylating agent, we decided to derivatize the building block also with a different anomeric leaving group for comparative studies. Therefore, in order to generate glycosyl imidate 2.5, 2.57 was reacted with NBS in presence of water to yield lactol 2.58. Reaction of 2.58 with N-phenyl trifluoroacetimidoyl chloride in the presence of cesium carbonate afforded the desired building block 2.5.

**Scheme 6.** Reagents and conditions: i: a) BF₃·Et₂O, PhSH, CH₂Cl₂, overnight, 0 °C. b) NaOMe, MeOH, rt; ii: 2,2-dimethoxypropane, p-TSA, 48 h, rt; iii: NaH, BnBr, DMF, 2 h, rt; iv: HCl(aq)/MeOH, 2.5 h, 50 °C; v: Ac₂O, pyridine, rt; vi: NBS, acetone/H₂O, rt; vii: N-phenyltrifluoroacetimidoyl chloride, Cs₂CO₃, DCM, rt.
2.7 Glucosamine Building Blocks

(This work was completed in collaboration with Dr. C.-C. Wang.)

\(N\text{-Acetyl glucosamine}\) is an essential constituent of mammalian glycans. Our library of glycan building blocks was therefore expanded to include 3,4-\(\beta\), 6-\(\beta\)- and 4-\(\beta\)-\(N\)-acetyl glucosamine building blocks. These compounds were prepared on a multi-gram scale, expanding on a recently-introduced one-pot method\(^{36}\) for the streamlined synthesis of differentially protected thiogluco\(\)sides.\(^{37}\) Our synthetic strategies are outlined in Scheme 7.

\begin{align*}
\text{Scheme 7: Reagents and conditions.} & \quad \text{i: EtSH, BF}_3\cdot\text{Et}_2\text{O, CH}_2\text{Cl}_2, 85\%; \quad \text{ii: a) NaOMe, MeOH, quant. b) TMSCl, Et}_3\text{N, CH}_2\text{Cl}_2, 90\%; iii: a) cat. TMSOTf, PhCHO, 4A MS, CH}_2\text{Cl}_2, 0 ^\circ \text{C. b) TBAF. c) LevOH, DIC, DMAP, 86\%; iv: Et}_3\text{SiH, TFA, DCM, 94\%; v: FmocCl, pyr. 62\% for 2.6, 81\% for 2.7, 80\% for 2.8; vi: 1) cat. TMSOTf, PhCHO, 4A MS, CH}_2\text{Cl}_2, 0 ^\circ \text{C; 2) PhCHO, Et}_3\text{SiH, -78 }^\circ \text{C; 3) BH}_3/\text{THF, TMSOTf, 0 }^\circ \text{C}, 3.5 \text{ h. 95\%. vii: a) cat. TMSOTf, PhCHO, 4A MS, CH}_2\text{Cl}_2, 0 ^\circ \text{C; b) PhCHO, Et}_3\text{SiH, -78 }^\circ \text{C; c) Et}_3\text{SiH, TFA, CH}_2\text{Cl}_2, -78 ^\circ \text{C, 87\%;}}
\end{align*}
Glucosamine 2.59 \((38)\) was converted to thioglycoside 2.60 by reaction with thioethanol in the presence of BF\(_3\)·Et\(_2\)O. Methoxide mediated deacetylation, followed by treatment with trimethylsilyl chloride and triethylamine gave rise to the common precursor 2.61, which underwent three different one-pot reactions to produce 2.6, 2.7 and 2.8. The common sequence between these one-pot reactions included the generation of a 4,6-\textit{O}-benzylidene, followed by protection of position \textit{O}-3 and stereoselective benzylidene opening. Key transformation was the stereoselective opening of the benzylidene, which can be tuned to generate hydroxyl nucleophiles at positions \textit{O}-4 or \textit{O}-6, alternatively. Benzyl ethers were chosen as permanent protecting groups, while a Fmoc functionality was used as temporary protecting group. In addition, in the case of compound 2.6, a levulinoyl (Lev) ester was chosen as orthogonal group to the Fmoc.\((39)\) The Lev group, in fact, can be cleaved under almost neutral conditions with hydrazine buffered with acetic acid, but is stable under the mildly basic conditions typically employed for Fmoc cleavage.

A one pot reaction of 2.61 with benzaldehyde and trimethylsilyl triflate at 0 °C, followed by treatment with TBAF and then with levulinic acid in the presence of diisopropylcarbodiimide and dimethylaminopyridine provided compound 2.62 in 86% yield. Benzylidene opening in the presence of triethylsilane and trifluoroacetic acid afforded compound 2.63 in 94% yield, which was converted to compound 2.6 by treatment with FmocCl in pyridine.

A similar strategy was employed for the generation of non-branching building blocks. When 2.61 was reacted with benzaldehyde and trimethylsilyl triflate at 0 °C, followed by excess benzaldehyde in presence of triethylsilane, and then finally with tetrahydrofuran-borane complex in presence of trimethylsilyl triflate at 0 °C, compound 2.65 was obtained. On the other hand, when treatment with tetrahydrofuran-borane complex and trimethylsilyl triflate was replaced by \textit{in situ} treatment of 2.61 with an excess of triethylsilane and trifluoroacetic acid, compound 2.66 was obtained in excellent yield. Reaction of 2.65 or 2.66 with FmocCl in pyridine afforded building blocks 2.7 and 2.8, respectively.
2.8 (1-4)-β-Glucose Building Block

(This work was completed in collaboration with Dr. C.-C. Wang.)

Glucose can be found in mammalian oligosaccharides with a relative abundance of 1.6%. One pot methods for the preparation of glucose building blocks have recently been reported (36) but the applicability has been limited to thiotoluyl glycosides. We were able to extend this methodology to include the more reactive thioethyl anomeric leaving group.(37) This synthesis is given in Scheme 8.

Scheme 8. Reagents and conditions. i: 1) cat. TMSOTf, PhCHO, 4Å MS, CH₂Cl₂, 0 °C; 2) PhCHO, Et₃SiH, -78 °C;3) Bz₂O (2.0 eq.), BzOH (0.2 eq.); 4) Et₃SiH, TFA, 51%; ii) FmocCl, pyr. CH₂Cl₂, 76%

One pot treatment of derivative 2.67 with benzaldehyde and trimethylsilyl triflate at 0 °C, followed by sequential addition of excess benzaldehyde in presence of triethylsilane, benzylic anhydride, benzylic alcohol and finally excess of triethylsilane and trifluoroacetic acid gave compound 2.68 in 51% yield. An Fmoc group was introduced under standard conditions to afford the desired compound 2.9.
2.9 Solution Phase Synthesis of Sialyl Lewis\textsuperscript{x}

(In collaboration with Dr. S. Hanashima and Dr. B. Castager)

The building blocks introduced in the previous section constitute the starting point for the synthesis of a diverse array of mammalian oligosaccharides. Having established robust routes that enable the preparation of glycosylating agents on a multi gram scale, we decided to further evaluate the reactivity of such compounds during a complex multistep synthesis. In this regard, Sialyl Lewis\textsuperscript{x} (SL\textsubscript{x}) hexasaccharide 2.69 was selected as a target (Scheme 7).\textsuperscript{(40, 41)} As shown retrosynthetically, compound 2.70 can be obtained by glycosylating trisaccharide 2.71 with the capping building blocks 2.1 and 2.5. In turn, compound 2.71 can be assembled using building blocks 2.72, 2.73 and 2.74, as was recently shown (Scheme 7).\textsuperscript{(42)} Interestingly, the protecting group patterns of monomers 2.72, 2.73 and 2.74 closely resemble those that have been chosen for glycosylating agents 2.6, 2.3 and 2.9, respectively. As a consequence, trisaccharide 2.71 constituted a good model for the evaluation of the protecting group patterns of building blocks 2.3, 2.6 and 2.9. In particular, the nucleophilic properties of the branching glucosamine 2.6 could be inferred upon glycosylation with compounds 2.1 and 2.5. More generally, the efficient removal of the different protecting groups was evaluated during global deprotection.
The synthesis started with glycosylation of compound 2.71 using building block 2.1. The desired pentasaccharide 2.75 was obtained as a single anomer in excellent yield, demonstrating that even in combination with a complex nucleophile like 2.71, a sterically demanding disaccharide building block can perform well during glycosylation. Deprotection of the levulinoyl group using hydrazine monohydrate in AcOH/pyridine in the presence of allyl alcohol set the stage for the final fucosylation. Correspondingly, building block 2.5, when treated with catalytic amounts of Yb(OTf)₃ as a mild activating agent, resulted in the efficient glycosylation of the sterically hindered hydroxyl on pentasaccharide 2.76 to give 2.77 in 88% yield.

Global deprotection of hexasaccharide 2.77 required several steps. Since the Alloc group is not compatible with the conditions required for the removal of Troc and TCA functionalities, it was exchanged for a Cbz group. At this point, treatment with Zn-Cu in acetic acid resulted in the simultaneous removal of the Troc group and reduction of the trichloroacetate (TCA). Thus, acetylation under standard conditions afforded 2.78. Finally, treatment of 2.78 with sodium methoxide, followed by addition of water to hydrolyze the methyl ester and hydrogenolysis with 10% Pd(OH)₂/C gave the desired deprotected SLx 2.69 with

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**Scheme 7. Retrosynthetic analysis for the synthesis of Sialyl Lewis X 2.69**

[Diagram of the synthesis process]
an amino handle for conjugation or for printing on microarrays.\(^{(44)}\) The overall yield was of 17% over nine steps.

The synthesis of \(\text{2.69}\) represents the first successful application for some of the building blocks described in this chapter, showing in particular, the efficiency of glycosylating agents \(\text{2.1} \) and \(\text{2.5}\). Furthermore, the terminal branching glucosamine on trisaccharide \(\text{2.71}\) performed well as a nucleophile corroborating at the same time the choice of levulinoyl and fluorenylmethyloxycarbonyl moieties as an efficient combination of orthogonal protecting groups for building blocks \(\text{2.72} \) and \(\text{2.6}\).

Scheme 7. Total synthesis of Sialyl Lewis X. Reagents and conditions: \(i:\) \(\text{2.1} \) (1.5 eq.), TMSOTf, \(\text{CH}_2\text{Cl}_2\), -15 °C, 93%; \(ii:\) \(\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}\), AcOH, pyridine, allyl alcohol, 95%; \(\text{iii:} \) \(\text{2.5}\), \(\text{Yb(OTf)}_3\), MS \(4\text{Å}\), \(\text{CH}_2\text{Cl}_2\), dioxane, Et\(\text{2} \)O, 88%; \(\text{iv:}\) a) Pd(PPh\(\text{3}\))\(_4\), \(p\)-toluenesulfonic acid, acid, \(\text{CH}_2\text{Cl}_2\). b) Cbz-OSu, Et\(\text{3} \)N; \(\nu:\) a) Zn-Cu couple, AcOH, 50 °C; b) Ac\(\text{2} \)O, pyridine 42% (from \(\text{2.77}\)). \(\text{vii:}\) a) 0.1 M NaOMe/MeOH; \(\text{H}_2\text{O}\), b) \(\text{H}_2\text{Pd(OH)}\(_2\)/C, MeOH, \(\text{H}_2\text{O}\), AcOH, 53%.
2.10 Conclusions

In summary, the synthesis of a series of mammalian monosaccharide building blocks has been achieved (Figure 1). Emphasis was given to the description of novel sialic acid α-(2-3) galactose building blocks obtained by combining galactal nucleophile 2.24 and phosphite sialylating agents 2.20 and 2.27. The galactal moiety was readily oxidized and functionalized to afford N-phenyl trifluoroacetimidoyl glycosides. In addition, galactose, fucose, glucosamine and glucose building blocks were prepared. Some of these building blocks were evaluated during the synthesis of SLx hexasaccharide, 2.69. In particular, compound 2.1 and 2.5 performed well in the glycosylation of trisaccharide 2.71 and pentasaccharide 2.76 respectively. In general, the protecting group patterns explored ensured robust reactivity during glycosylations and enabled straightforward deprotection at the end of the synthesis. Having shown that this set of building blocks is useful for the solution phase synthesis of a complex oligosaccharide it is possible to anticipate the successful application of these compounds to the solid phase synthesis of complex oligosaccharides. The following chapters highlight the efficient use of these building blocks for the chemical synthesis of different complex oligosaccharides, with emphasis on automated solid supported syntheses.
2.11 Experimental Procedures

**General Material and Methods.** $^1$H, $^{13}$C spectra were recorded on a Varian Mercury 300 (300 MHz), Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), Bruker ECX (400 MHz), Bruker DRX500 (500 MHz), or, Bruker DRX700 (700 MHz) spectrometer in CDCl$_3$ with chemical shifts referenced to the solvent signal (CDCl$_3$: 7.26 ppm $^1$H, 77.0 ppm $^{13}$C) unless otherwise stated. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for $^1$H NMR data. NMR chemical shifts ($\delta$) are reported in ppm and coupling constants ($J$) are reported in Hz. High resolution mass spectral (HRMS) analyses were performed by the MS-service at the Laboratory for Organic Chemistry (LOC) at ETH Zürich. High-resolution MALDI and ESI mass spectra were run on an IonSpec Ultra instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin-Elmer 241 and Unipol L1000 polarimeter. A recycling Preparative HPLC system (GPC: LC-9101 Japan Analytical Industry Co. Ltd.) was used for size exclusion chromatography. Analytical and preparative thin layer chromatography was performed using glass plates pre-coated with silica gel 60 F$_{254}$ (Merck) or silica gel GF (Analtech.).

**Synthetic Procedures**

*Methyl 4,7,8,9-tetra-0-acetyl-3,5-dideoxy-5-trichloroethoxycarbonylamino-D-glycero-\(\alpha\)-D-galacto-2-nonulopyranosylonate N-phenyl trifluoroacetimidate 2.21.*

![Diagram of the synthetic procedure]
To a solution of hemiketal 2.76(42) (291 mg, 0.466 mmol) in acetone (5 mL) was added CF₃C(NPh)Cl (290 mg, 1.40 mmol) and Cs₂CO₃ (309 mg, 0.947 mmol). After stirring for 20 min under Ar atmosphere, the mixture was filtered through Celite, and the filtrate was concentrated. Purification with flash column silica gel chromatography (toluene:ethylacetate = 10:1 to 6:1) gave N-phenyl trifluoroacetimidate 2.21 (235 mg, 0.295 mmol 63%; α:β = 1:3). [a]D²³ = +10 (c 1.9, chloroform); ¹H NMR (300 MHz, CDCl₃): δ 7.30-7.17 (m, 2H), 7.10 (m, 1H), 6.18-6.72 (2H), 5.71 (t, 1H, J = 3.7 Hz, a), 5.48 (dd, 1H, J = 2.2, 5.6 Hz, a), 5.41 (dd, 1H, J = 1.6, 6.9 Hz, b), 5.35-5.06, 4.92 (d, 1H, J = 12.1 Hz, b), 4.91 (s, 1H, J = 12.1 Hz, a), 4.84-4.21, 4.14-4.03 (m, 1H), 3.93-3.73, 3.82 (s, 1H, a), 3.80 (s, 3H, b), 2.93 (dd, 1H, J = 4.7, 13.4 Hz, a), 2.78 (dd, 1H, J = 5.0, 13.4 Hz, b), 2.35-2.24, 2.17-1.97; ¹³C NMR (75 MHz, CDCl₃): δ 170.5, 170.2, 170.1, 169.9, 169.7, 167.1, 154.0, 142.4, 128.7, 128.7, 128.6, 124.6, 119.3, 119.0 (br), 99.8, 97.7, 95.4, 77.9, 77.3, 74.9, 74.8, 74.5, 73.5, 73.1, 71.3, 70.8, 69.9, 67.9, 67.6, 62.2, 61.7, 58.6, 53.2, 53.0, 51.5, 51.2, 36.7, 35.3, 20.9, 20.8, 20.6; HR-MALDI MS: m/z [M+Na]+ calcd for C₂₉H₃₂Cl₃F₃N₂O₁₄Na: 817.0769, found 817.0773.

Thexyldimethylsilyl (methyl 4,7,8,9-tetra-0-acetyl-3,5-dideoxy-5-trichloroethoxy carbonyl amino-0-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2-O-benzoyl-6-O-benzyl-β-D-galactopyranoside 2.25.
To a solution of 2.22 (37 mg, 0.061 mmol) and phosphite donor 2.20 (78 mg, 0.092 mmol) (22) in EtCN (1.5 mL) was added molecular sieves (MS) 4Å (200 mg). The mixture was stirred for 15 min at room temperature under Ar atmosphere. Then, the reaction mixture was cooled to -78˚C and TMSOTf (2.5 mL, 0.013 mmol) was added. After stirring for 2 h at -78˚C, the mixture was neutralized with a few drops of Et₃N, and molecular sieves were removed by filtration through Celite. The filtrate was then concentrated, and purified with GPC (chloroform) to yield an α/β mixture of 2.25 (42 mg, 0.037 mmol, 61%; α:β = 3:1).

Flash column silica gel chromatography (toluene: acetone = 5:1) gave each anomers of compound 2.25. (2.25α: 31 mg, 0.027 mmol, 2.25β: 9 mg, 0.008 mmol 58%). 2.25: [α]D23 = +13 (c 1.4, chloroform); ¹H NMR (300 MHz, CDCl₃): δ 8.11 (m, 2H), 7.57-7.25 (m, 8H), 5.52 (m, 1H), 5.25 (m, 2H), 4.90-4.78 (m, 3H), 4.72 (d, 1H, J = 10.0 Hz), 4.62 (d, 1H, J = 11.5 Hz), 4.57 (d, 1H, J = 11.8 Hz), 4.42 (d, 1H, J = 12.1 Hz), 4.37 (dd, 1H, J = 3.4, 10.0 Hz), 4.28 (dd, 1H, J = 2.2, 12.5 Hz), 4.01-3.93 (m, 2H), 3.85-3.70 (m, 4H), 3.73 (s, 3H), 3.64 (m, 1H), 3.47 (dd, 1H, J = 10.3, 20.9 Hz), 2.62 (dd, 1H, J = 4.5, 12.5 Hz), 2.55 (d, 1H, J = 2.2 Hz,OH), 2.14 (s, 3H), 2.05 (s, 3H), 1.95 (s, 3H), 1.83 (t, 1H, J = 12.5 Hz), 1.45 (m, 1H), 0.66 (m, 12H), 0.15 (s, 3H), 0.06 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 170.5, 170.1, 169.9, 168.3, 165.0, 153.9, 138.1, 132.8, 130.3, 130.0, 128.3-127.4, 96.9, 96.1, 95.2, 77.2, 74.5, 74.0, 73.6, 72.8, 72.7, 71.9, 69.2, 68.4, 67.6, 67.5, 67.0, 62.5, 53.1, 51.2, 37.8, 33.8, 24.7, 21.4-18.4, -1.6, -3.2; Elemental analysis: calcd (%) for C₄₉H₆₆Cl₃NO₂₀Si: C 52.38; H 5.92; N 1.25 Found: C 52.39; H 5.96; N 1.31.
Chapter 2

Thexyldimethylsilyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy- 5-
trichloroethoxycarbonylamino-D-glycero-α-D-galacto-2-
onulopyranosylonate)-(2→3)-4,6-O-benzylidne-β-D-galactopyranoside
2.26.

![Chemical Structure](image)

To a solution of 2.23 (41) (31 mg, 0.076 mmol) and trifluoroacetimidate donor 2.21 (40 mg, 0.050 mmol) in EtCN (1 mL) was added MS 4Å (160 mg). The mixture was stirred for 15 min at room temperature under Ar atmosphere. Then, the reaction mixture was cooled to -78°C and TMSOTf (1.5 mL, 0.0078 mmol) was added. After stirring for 2 h at -78°C, the mixture was neutralized with a few drops of Et₃N, and molecular sieves were removed by filtration through Celite. The filtrate was then concentrated, and purified with GPC (chloroform) to yield an a/b mixture of 2.26 (34 mg, 0.034 mmol, 68 %; α:β = 8:1). Preparative TLC (toluene: ethylacetate = 3:1) gave each anomers of disaccharide 2.26. (2.26: 28 mg, 0.028 mmol 56%; 2.26β: 3.5 mg, 0.0034 mmol 7%). 2.26; [α]D²² = +19 (c, 1.27 chloroform); ¹H NMR (300 MHz, CDCl₃): δ 7.49 (m, 2H), 7.34 (m, 3H), 5.40 (m, 2H), 5.35 (s, 2H), 5.05- 4.91 (m, 2H), 4.90 (d, 1H, J = 12.1Hz), 4.67 (d, 1H, J = 7.5 Hz), 4.68 (d, 1H, J = 12.1 Hz), 4.27 (dd, 1H, J = 12.1, 14.0 Hz), 4.23-4.18 (m, 3H), 3.97 (d, 1H, J = 3.4 Hz), 3.76 (m, 1H), 3.58 (s, 3H), 3.42 (s, 1H), 2.76 (dd, 1H, J = 8.4, 12.8 Hz), 2.56 (s, 1H), 2.19 (s, 3H), 2.17 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.95 (t, 1H, J = 12.5 Hz), 1.68 (m, 1H), 0.91-0.85 (m, 1H), 0.21 (s, 3H), 0.20 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 170.6, 170.3, 170.0, 169.9, 168.2, 153.9, 138.0, 128.8, 128.0, 126.4, 100.8, 97.9, 97.0, 95.3, 74.9, 74.4, 73.8, 71.9, 70.4, 69.2, 67.8, 67.7, 67.0, 66.1, 62.0, 60.3, 52.7, 51.6, 38.5, 34.0, 24.9, 21.2,
20.7, 20.1, 20.0, 18.5, 18.4, 14.1, -1.9, -2.7; HR-MALDI-MS: m/z [M+Na]+ calcd for C₄₂H₆₀NO₁₉Cl₃SiNa: 1138.2492, found 1138.2469.

**Methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroethoxycarbonylamino-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)-4,6-O-benzyl-D-galactal 2.27.**

![Structure of 2.27](image)

To a solution of 4,6-O-benzyl-galactal 2.24 (44) (41 mg, 0.13 mmol) and phosphite donor 2.20 (128 mg, 0.150 mmol) in EtCN (2 mL) was added MS 4Å (350 mg). The mixture was stirred for 15 min at room temperature under Ar atmosphere, and then cooled to -78˚C. After the addition of TMSOTf (4.3 mL, 0.022 mmol), the mixture was stirred for 2 h at -78˚C. Then, the mixture was neutralized with a few drops of Et₃N, molecular sieves were removed by filtration through Celite. The filtrate was concentrated, and purified with GPC (chloroform) to yield α/β mixture of 2.27 (109 mg, 0.117 mmol, 93%; α:β = 10:1). Then, flash column silica gel chromatography (toluene:CH₂Cl₂:acetone with 0.25 % Et₃N = 5:5:0.5 to 2:8:0.5) gave each anomers of 2.27. (2.27: 94 mg, 0.101 mmol, 2.27β: 10 mg, 0.011 mmol 89%). 2.27; [α]D²³ = -21 (c 1.6, chloroform); ¹H NMR (300 MHz, CDCl₃): δ 7.35-7.22 (m, 10H), 6.37 (dd, 1H, J = 1.6, 6.2 Hz), 5.37 (m, 2H), 5.05 (m, 1H), 4.94 (m, 1H), 4.91 (d, 1H, J = 12.1 Hz), 4.86-4.79 (m, 1H), 4.58-4.44 (m, 4H), 4.36 (d, 1H, J = 11.8 Hz), 4.25 (dd, 1H, J = 12.1, 14.0 Hz), 4.16-4.08 (m, 5H), 3.76-3.57 (m, 4H), 3.73 (s, 3H), 3.52 (dd, 1H, J = 5.9, 10.0 Hz), 2.69 (dd, 1H, J = 5.0, 13.1 Hz), 2.14 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.96 (t, 1H, J = 12.5 Hz); ¹³C NMR (75 MHz, CDCl₃): δ 170.5, 170.3, 170.0, 169.6, 168.4, 153.9, 144.1, 138.3, 137.8, 128.3-127.5, 102.1, 99.0, 95.4, 77.2, 75.6, 74.5, 74.0, 73.4, 71.9, 70.9, 68.5, 68.4, 68.3, 67.4, 62.2, 52.9, 51.7, 38.3,
22.0-20.9; HR-MALDI MS: m/z [M+Na]⁺ calcd for C₄₁H₄₈Cl₃N₂O₁₇Na: 954.1886, found 954.1864.

**Acetyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroethoxycarbonylamino-α-D-galacto-2-nonulopyranosylate)-(2→3)-2-O-acetyl-4,6-di-O-benzyl-D-galactopyranoside 2.28.**

To a solution of 2.27 (131 mg, 0.140 mmol) in CH₂Cl₂ (2 mL) was added PhI(OAc)₂ (57 mg, 0.18 mmol) and BF₃·Et₂O (4.0 mL, 0.033 mmol) at -40°C. After stirring for 2 h at -40°C to -15°C under Ar atmosphere, pyridine (2 mL) and Ac₂O (1 mL) were added. The mixture was stirred at room temperature for 13 h, and then concentrated. This resulting residue was dissolved in EtOAc and washed with 10% citric acid, H₂O, NaHCO₃ solution, and brine. The organic phase was dried over Na₂SO₄, filtered, and concentrated. Purification with flash column silica gel chromatography (hexane:acetone = 6:1 to 3:1) gave 2.28 (101 mg, 0.0962 mmol, 67%). ¹H NMR (300 MHz, CDCl₃): δ 7.34-7.12 (m, 10H), 6.27 (d, 1H, J = 3.7 Hz, a anomeric), 5.58 (d, 1H, J = 8.1 Hz, b anomeric), 5.59 (m, 1H, a), 5.01 (m, 1H, b), 5.52-5.35 (m), 4.95-4.82, 4.54-4.37, 4.24-3.51, 3.74 (s, 3H, a), 3.70 (s, 3H, b), 2.67 (m, 1H), 2.20-1.98, 1.87 (t, 1H, J = 12.5 Hz); ¹³C NMR (75 MHz, CDCl₃): δ 170.6-169.2, 167.7, 154.0, 138.3, 137.8, 128.3-127.4, 97.5, 97.4, 95.3, 92.3, 90.3, 77.2, 75.5-74.3, 73.4-73.2, 72.1-71.4, 69.0-67.3, 62.5, 61.8, 53.0, 51.6, 51.4, 37.9, 21.5-20.8; HR-MALDI MS: m/z [M+Na]⁺ calcd for C₄₃H₅₂Cl₃NO₂₀Na: 1030.2046, found 1030.2088.

To a solution of 2.28 (100 mg, 0.0954 mmol) in DMF (1.5 mL) was added hydrazine acetate (19 mg, 0.20 mmol). After stirring for 15 h at room temperature under N₂ atmosphere, the mixture was diluted with EtOAc and 10% citric acid solution. The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated. Purification with flash column silica gel chromatography (hexane:ethylacetate = 2:1 to 1:1) gave the corresponding hemiacetal (89 mg, 0.088 mmol, 92%). The latter (89 mg, 0.088 mmol) was dissolved in CH₂Cl₂ (1 mL) and Cs₂CO₃ (57 mg, 0.17 mmol) and CF₃C(NPh)Cl (56 mg, 0.27 mmol) were added. After stirring for 2 h at room temperature under Ar atmosphere, the mixture was filtered through Celite, and concentrated. Purification with flash column silica gel chromatography (hexane:ethylacetate = 6:1 to 2:1) gave trifluoroacetimidate 2.1 (95 mg, 0.081 mmol, 92%). [α]D₂₃ = +16 (c 0.77, chloroform); ¹H NMR (300 MHz, CDCl₃): δ 7.33-7.23 (m, 12H), 7.05 (m, 1H), 6.83 (m, 1H), 5.81 (br, 1H), 5.60 (m, 1H), 5.50 (t, 1H, J = 9.6 Hz), 5.42 (dd, 1H, J = 2.5, 8.8 Hz), 4.95 (m, 1H), 4.91-4.80 (m, 3H), 4.52-4.37 (m, 6H), 4.06 (ddd, 1H, J = 5.0, 11.0 Hz), 3.89 (ddd, 1H, J = 2.2, 10.7 Hz), 3.77-3.55 (m, 4H), 3.71 (s, 3H), 3.52 (brs, 1H), 2.72 (dd, 1H, J = 4.7, 12.9 Hz), 2.23 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.86 (t, 1H, J = 12.6 Hz); ¹³C NMR (75 MHz, CDCl₃): δ 170.5, 170.3, 170.0, 169.6, 169.3, 167.8, 154.1, 143.4, 138.2, 137.9, 128.5-127.5, 124.1, 119.4, 97.4, 95.2, 95.1, 74.6, 74.4, 74.2, 73.6, 73.1, 72.0,
68.9, 68.5, 67.9, 67.5, 62.4, 52.9, 51.2, 37.6, 21.2-20.5; HR-MALDI-MS: m/z [M+Na]+ calcd for C_{51}H_{56}F_{3}Cl_{3}N_{2}O_{20}Na: 1201.2342, found 1201.2361.

\[(N\text{-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-}\alpha\text{-neuraminosyl)-(2→3)-O-1,5-anhydro-4,6-O-benzyl-2-deoxy-D-\text{lyxo-hex-1-enopyranose 2.40.}}\]

To a solution of 4,6-O-benzyl-galactal 2.24 (45) (3 g, 9.2 mmol) and methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-(dibenzylphosphityl)-3,5-dideoxy-\(\beta\)-glycero-D-galacto-2-nonulopyranosonate 2.37 (29) (4.2 g, 5.6 mmol) in anhydrous acetonitrile (40 mL) were added 4Å-AW MS (ca. 100 rods). The mixture was stirred for 30 min at room temperature under an argon atmosphere. The solution was then cooled to \(-42\) °C and TMSOTf (198 \(\mu\)L, 1.1 mmol) was added. After 2 h the mixture was neutralized with a few drops of Et\(_3\)N. Molecular sieves were removed by filtration through celite. The filtrate was concentrated and purified by silica gel chromatography (cyclohexane/EtOAc = 4:1 to 0:1) to afford 2.40 in 64% yield (4.7 g). \([\alpha]_{D}^{20} -2.85 (c 1.8, \text{CHCl}_3)\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.38-7.23 (m, 10H), 6.38 (dd, \(J = 1.4, 6.2\) Hz, 1H), 5.40 (ddd, \(J = 2.6, 5.7, 8.4,\) Hz, 1H), 5.31 (dd, \(J = 1.6, 8.4,\) Hz, 1H), 5.18 (d, \(J = 9.0\) Hz, 1H), 4.94 (ddd, \(J = 4.7, 9.9, 12.2,\) Hz, 1H), 4.83 (m, 2H), 4.62 (s, 1H), 4.57 (d, \(J = 12.0\) Hz, 1H), 4.48 (d, \(J = 11.8\) Hz, 1H), 4.38 (d, \(J = 11.8\) Hz, 1H), 4.31 (dd, \(J = 2.6, 12.5\) Hz, 1H), 4.14 (m, 1H), 4.07 (m, 3H), 3.73 (s, 3H), 3.68 (t, \(J = 8.4\) Hz, 1H), 3.55 (dd, \(J = 5.6, 9.9\) Hz, 1H), 2.64 (dd, \(J = 4.7, 12.9\) Hz, 1H), 2.14 (s, 6H), 2.09-1.99 (m, 7H), 1.90 (s, 3H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 171.2, 170.8, 170.4, 170.3, 170.0, 168.9, 144.4, 138.7, 138.2, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 102.3, 99.4, 75.8, 74.1, 73.6, 72.8, 71.2, 69.2, 68.9, 68.8, 67.6, 62.6, 53.0, 49.8, 38.3, 23.4, 21.3, 21.1, 21.00,
20.97; HR-MALDI MS: $m/z$ [M+Na$^+$] calcd for C$_{40}$H$_{48}$NO$_{16}$Na: 822.2949, found 822.2961. IR (thin film) $\nu$ = 2927, 1744, 1664, 1540, 1454, 1369, 1223, 1125, 1038, 752 cm$^{-1}$.

**Acetyl (N-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-α-neuraminosyl)-(2→3)-α-2-O-acetyl-4,6-di-O-benzyl-D-galactopyranoside 2.43.**

![Structure 2.43](image)

A solution of acetoxyiodobenzene in dichloromethane (18.5 mL, 1.93 g, 5.99 mmol) was added at $-40 \, ^\circ\text{C}$ to a cold solution of **2.40** (2.4 g, 3.00 mmol) in CH$_2$Cl$_2$ (18.5 mL) under an argon atmosphere followed by BF$_3$·Et$_2$O (280 μL, 2.2 mmol). After stirring for 2 h, pyridine (2 mL) and Ac$_2$O (2 mL) were added and the mixture was stirred at room temperature for 12 h and concentrated. The crude residue was dissolved in EtOAc and washed with 10% citric acid, water, NaHCO$_3$ solution and brine. The combined organic phase was dried over MgSO$_4$, filtered and concentrated. Purification with flash column silica gel chromatography (cyclohexane/EtOAc = 3:2 to 1:4) gave **2.43** (2.31 g, 2.52 mmol, 84%). $[\alpha]_{D}^{20}$ $-2.81$ (c 1.5, CHCl$_3$); $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.32-7.16 (m, 10H), 5.72 (dd, $J$ = 1.9, 8.2, Hz, 1H), 5.53 (t, $J$ = 7.9 Hz, 1H), 5.34 (t, $J$ = 9.2 Hz, 1H), 5.32-5.28 (m, 2H), 4.82-4.76 (m, 2H), 4.46-4.41 (m, 3H), 4.41-4.34 (q, 2H), 4.11-3.99 (m, 1H), 3.94 (dd, $J$ = 6.8, 12.3 Hz, 1H), 3.78 (m, $J$ = 4.7, 7.8, 13.2 Hz, 2H), 3.65 (s, 3H), 3.63-3.58 (m, 1H), 3.53 (dd, $J$ = 5.4, 9.1 Hz, 1H), 3.48 (s, 1H), 2.62 (dd, $J$ = 4.5, 12.8 Hz, 1H), 2.17 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.03 (m, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.82 (s, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 170.9, 170.7, 170.4, 170.2, 169.8, 169.8, 169.3, 168.2, 138.5, 138.1, 128.4, 128.2, 127.8, 127.7, 127.5, 97.6, 92.4, 74.8, 74.7, 74.4, 73.4, 73.2, 72.5, 69.2, 69.0, 68.6, 68.6, 67.6, 67.5, 62.7, 52.9, 49.2, 37.8, 23.2, 21.4, 21.1, 21.0, 21.0, 20.9, 20.8, 20.8, 20.8, 20.7; HR-MALDI MS: $m/z$ [M+Na$^+$] calcd for C$_{44}$H$_{55}$NO$_{20}$Na:
940.3215, found 940.3227. IR (thin film) ν = 2934, 1744, 1370, 1217 cm⁻¹.

\((N\text{-}acetyl\text{-}4,7,8,9\text{-}tetra\text{-}O\text{-}acetyl\text{-}1\text{-}methyl\text{-}\alpha\text{-}neuraminosyl})\text{-(2}\rightarrow\text{3})\text{-}\alpha\text{-}2\text{-}O\text{-}acetyl\text{-}4,6\text{-}di\text{-}O\text{-}benzyl\text{-}D\text{-}galactopyranosyl\text{-}N\text{-}phenyl trifluoroacetimidate 2.2\)

To a solution of \textbf{2.43} (2.6 g, 2.8 mmol) in DMF (44 mL) was added hydrazine acetate (580 mg, 6.30 mmol). After stirring for 15 h at room temperature under argon atmosphere, the mixture was diluted with EtOAc and washed twice with a 10% citric acid solution (100 mL). The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated. Purification by flash column silica gel chromatography (cyclohexane/EtOAc = 5:1 to 0:1) gave the corresponding hemiacetal (2.25 g, 92%). The latter (1.95 g, 2.23 mmol) was dissolved in dichloromethane (30 mL) and Cs₂CO₃ (2.18 g, 6.67 mmol) and CF₃C(NPh)Cl (1.45 g, 7.05 mmol) were added. After stirring overnight at room temperature under argon atmosphere, the mixture was filtered through celite and concentrated. Silica column chromatography (cyclohexane/EtOAc = 7:3 to 9:1) afforded the desired product \textbf{2.2} (2.06 g, 86%). ¹H NMR (600 MHz, CDCl₃) δ 7.34-7.23 (m, 12H), 7.08 (t, \(J = 7.5\) Hz, 1H), 6.84 (d, \(J = 7.2\) Hz, 2H), 5.59 (ddd, \(J = 2.6, 6.9, 8.2\) Hz, 1H), 5.54-5.48 (m, 1H), 5.37 (dd, \(J = 2.6, 8.1\) Hz, 1H), 5.32 (d, \(J = 10.2\) Hz, 1H), 4.90-4.83 (m, 2H), 4.52-4.40 (m, 6H), 4.11-4.05 (m, 1H), 4.02 (dd, \(J = 6.7, 12.4\) Hz, 2H), 3.84 (dd, \(J = 6.0, 8.5\) Hz, 1H), 3.71 (s, 3H), 3.67 (d, \(J = 6.2\) Hz, 1H), 3.53 (s, 1H), 2.68 (dd, \(J = 4.6, 12.7\) Hz, 1H), 2.25 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.07 (m, 1H), 2.03 (s, 3H), 1.95 (s, 3H), 1.87 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.9, 170.6, 170.4, 170.2, 169.8, 169.4, 168.2, 168.2, 138.4, 138.1, 128.7, 128.4, 128.3, 127.8, 127.7, 127.7, 127.6, 125.5, 124.2, 119.5, 97.7, 74.8, 74.5, 74.5, 73.8, 73.3, 72.6, 69.1, 69.1, 68.6, 67.7, 62.7, 60.5, 53.0, 49.2, 37.7, 23.2,
21.4, 20.8, 20.8, 20.7, 14.2; HR-MALDI MS: m/z [M+Na⁺] calcd for C₅₀H₆₇F₃N₂O₁₉Na: 1069.3405, found 1069.3420. IR (thin film) ν = 3328, 1745, 1666, 1551, 1453, 1371, 1040, 699 cm⁻¹.

**(N-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-α-neuraminosyl)-(2→6)-O-1,5-anhydro-3,4-O-benzyl-2-deoxy-D-lyxo-hex-1-enopyranose 2.41.**

To a solution of 4,6-O-benzyl-galactal 2.38 (30) (1.35 g, 4.16 mmol) and methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-(dibenzylphosphityl)-3,5-dideoxy-β-glycero-d-galacto-2-nonulopyranosonate 2.37 (29) (1.7 g, 2.31 mmol) in anhydrous acetonitrile (15 mL) were added 4Å-AW MS (ca. 30 rods). The mixture was stirred for 30 min at room temperature under an argon atmosphere. The solution was then cooled to –42 °C and TMSOTf (80 μL, 0.45 mmol) was added. After 2 h the mixture was neutralized with a few drops of Et₃N. Molecular sieves were removed by filtration through celite. The filtrate was concentrated and purified by silica gel chromatography (cyclohexane/EtOAc = 4:1 to 0:1) to afford 2.41 (1.38 g, 75%) Spectroscopic data were consistent with previously reported data. (30)

**Acetyl (N-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-α-neuraminosyl)-(2→6)-α-2-O-acetyl-3,4-di-O-benzyl-α-galactopyranoside 2.44.**
A solution of acetoxyiodobenzene in dichloromethane (5 mL, 451 mg, 1.40 mmol) was added at \(-40^\circ C\) to a cold solution of 2.41 (700 mg, 0.875 mmol) in CH\(_2\)Cl\(_2\) (5 mL) under an argon atmosphere followed by BF\(_3\)·Et\(_2\)O (111 μL, 0.870 mmol). After stirring for 2 h, pyridine (1 mL) and Ac\(_2\)O (1 mL) were added and the mixture was stirred at room temperature for 12 h and concentrated. The crude residue was dissolved in EtOAc and washed with 10% citric acid, water, NaHCO\(_3\) solution and brine. The combined organic phase was dried over MgSO\(_4\), filtered and concentrated. Purification with flash column silica gel chromatography (chlorophorm/MeOH = 99:1) gave 2.44 (640 mg, 80%). Spectroscopic data were consistent with previously reported data.(30)

\((N\text{-acetyl-4,7,8,9-tetra-}O\text{-acetyl-1-methyl-}\alpha\text{-neuraminosyl)-(2}\rightarrow6\alpha\text{-2-O-}}\text{acetyl-3,4-di-}O\text{-benzyl-D-galactopyranoside 2.45.}\)

To a solution of 2.44 (240 mg, 261 μmol) in DMF (4 mL) was added hydrazine acetate (52 mg, 575 μmol). After stirring for 15 h at room temperature under argon atmosphere, the mixture was diluted with EtOAc and washed twice with a 10% citric acid solution (100mL). The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with brine, dried over MgSO\(_4\), filtered and concentrated. Purification by flash column silica gel chromatography (cyclohexane/EtOAc = 5:1 to 0:1) gave the corresponding hemiacetal 2.45 (205 mg, 90%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.55 – 7.16 (m, 10H), 5.86 – 5.65 (m, 1H), 5.44 – 5.41 (m, 1H), 5.38 – 5.26 (m, 2H), 4.98 – 4.90 (m, 1H), 4.89 – 4.82 (m, 1H), 4.75 – 4.56 (m, 3H), 4.48 – 4.37 (m, 2H), 4.20 – 4.00 (m, 5H), 3.91 (m, 1H), 3.75 – 3.49 (m, 5H), 2.61 (dd, \(J = 12.5, 4.8\) Hz, 1H), 2.16 – 2.00 (m, 15H),
1.87 (s, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 171.98, 171.01, 170.70, 170.57, 170.25, 168.19, 138.71, 138.50, 128.43, 128.20, 127.97, 127.65, 127.45, 127.34, 98.75, 96.08, 90.92, 76.47, 74.71, 74.09, 73.44, 72.73, 71.48, 70.71, 69.19, 68.64, 68.26, 63.64, 62.41, 52.91, 49.12, 38.06, 23.14, 21.15, 21.12, 21.09, 20.95, 20.91, 20.84.

**Ethyl 4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-thio-β-D-galactopyranoside 2.51.**

![Structure of 2.51](image)

Ethyl 4,6-di-O-benzyl-thio-β-D-galactopyranoside **2.50** (33) (2.92 g, 7.24 mmol) was coevaporated three times with toluene (30 mL), then dissolved in toluene (91 mL) in a round bottom flask equipped with a dean stark apparatus. Dibutyltin oxide (2.07 g, 8.33 mmol) was added and the solution was heated to 150 °C. Toluene (20 mL) was collected over a period of 1 h when fresh toluene was added and the reaction was cooled to room temperature. FmocCl (2.06 g, 7.97 mmol) was added and the reaction was stirred for another 2 h. Dichloromethane was added (100 mL) and the organic layer was extracted twice with HCl (0.5 M, 100 mL), dried over MgSO$_4$, filtered and concentrated under reduced pressure. Silica column chromatography (cyclohexane/EtOAc = 19:1 to 3:1) afforded **2.51** in 87% yield (3.94 g). $[\alpha]_D^{20}$ 3.24 (c 1, CHCl$_3$); $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.73 (t, $J = 7.4$ Hz, 2H), 7.61 (dd, $J = 7.4$, 13.1 Hz, 2H), 7.33-7.12 (m, 14H), 4.68 (dd, $J = 3.1$, 9.7 Hz, 1H), 4.57 (d, $J = 11.3$ Hz, 1H), 4.46-4.39 (m, 2H), 4.36 (dt, $J = 4.7$, 7.6 Hz, 3H), 4.29 (d, $J = 9.7$ Hz, 1H), 4.19 (t, $J = 7.2$ Hz, 1H), 3.94 (ddd, $J = 2.8$, 7.5, 9.7 Hz, 2H), 3.66 (dd, $J = 3.7$, 10.0 Hz, 1H), 3.56-3.50 (m, 2H), 3.25 (qq, $J = 7.4$, 12.7 Hz, 2H), 3.33 (dd, $J = 2.5$ Hz, 1H), 1.22 (dd, $J = 5.2$, 9.7 Hz, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 154.7, 143.5, 142.9, 141.3, 141.2, 138.0, 137.7, 128.4, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.1, 127.1, 125.2, 125.0,
Chapter 2

Ethyl 4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-2-O-pivaloyl-thio-β-D-galactopyranoside 2.3.

Compound 2.51 (950 mg, 1.51 mmol) was dissolved in dichloromethane (15mL) under an argon atmosphere and the solution was cooled to −15 °C. Pivaloyl chloride (373μL, 3.04 mmol) was added and the reaction was stirred for 40 min. The reaction was diluted with hexane/ethyl acetate (1/1 v/v, 100mL) and the formed precipitate was filtered through a silica pad. The organic layer was washed with HCl (0.3 M, 3 x 50 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. Silica column chromatography (cyclohexane/EtOAc = 19:1 to 4:1) afforded 2.3 in 90% yield (964 mg). [α]D²⁰ 1.81 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.80-7.68 (m, 2H), 7.58 (d, J = 7.6 Hz, 2H), 7.41-7.10 (m, 16H), 5.46 (t, J = 10.0 Hz, 1H), 4.92 (dd, J = 3.0, 10.0, Hz, 1H), 4.76 (d, J = 11.5 Hz, 1H), 4.55-4.40 (m, 4H), 4.37-4.27 (m, 2H), 4.21 (t, J = 7.3 Hz, 1H), 4.07 (d, J = 2.6 Hz, 1H), 3.75 (t, J = 6.5 Hz, 1H), 3.63 (d, J = 6.6 Hz, 2H), 2.81-2.61 (m, 2H), 1.28-1.21 (m, 3H), 1.16 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 176.9, 154.6, 143.3, 143.2, 141.40, 141.39, 138.1, 137.9, 128.6, 128.4, 128.2, 128.08, 128.06, 127.98, 127.96, 127.8, 127.33, 127.32, 125.30, 125.26, 120.21, 120.18, 83.8, 79.1, 77.3, 75.1, 74.1, 73.7, 70.3, 68.2, 67.5, 46.8, 38.8, 27.1, 23.8, 15.0; ESI-MS: m/z [M+NH₄⁺] calcd for C₄₂H₄₅NO₁₀S⁺: 728.32, found 728.3. IR (thin film) ν = 3511, 3035, 1746, 1259, 1153, 1073, 1027, 740, 698 cm⁻¹.
6-Deoxy-3,4-di-O-acetyl-2-O-benzyl-L-galactopyranose N-phenyl trifluoroacetimidate 2.5.

![Image of chemical structure]

To a solution of thiophenyl fucoside 2.57 (46) (1.50 g, 2.58 mmol) in acetone (20 mL) and H₂O (5 mL) was added NBS (1.61 g, 9.05 mmol). After stirring for 1 h at room temperature, the mixture was diluted with 5% Na₂S₂O₃ solution. The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated. Purification with flash column silica gel chromatography (hexane:ethylacetate = 3:1 to 3:2) gave hemiacetal 2.58 (841 mg, 2.49 mmol, 96%). Produced hemiacetal 2.58 (832 mg, 2.46 mmol) was dissolved in CH₂Cl₂ (6 mL) and added CF₃C(NPh)Cl (1.17 g, 5.64 mmol) and Cs₂CO₃ (1.25 g, 3.84 mmol). After stirring for 100 min, the mixture was filtered through Celite, and concentrated. Purification with flash column silica gel chromatography (hexane:ethylacetate = 6:1 to 4:1) gave 2.5 (1.24 g, 2.44 mmol, 99%: a:b = 1:1). [a]_D^25 = -103 (c 0.97, chloroform); ¹H NMR (300 MHz, CDCl₃): δ 7.39-7.24, (7H), 7.14-7.06 (1H), 6.90-6.72 (2H), 6.55 (br, 1H), 5.69 (br, 1H), 5.36-5.32 (2H), 5.21 (br, 1H), 5.00 (brd, 1H, J = 8.1 Hz), 4.84-4.65 (3H), 4.30 (m, 1H), 4.11 (m, 1H), 3.88 (m, 2H), 2.17 (s, 3H), 2.14 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.28-1.16; ¹³C NMR (75 MHz, CDCl₃): δ 170.3, 170.1, 169.8, 169.7, 143.3, 143.1, 137.5, 128.6-127.3, 124.2, 119.3, 119.1, 97.0, 95.4, 75.3, 75.1, 73.2, 72.6, 75.1, 73.2, 72.6, 72.5, 70.8, 70.3, 70.1, 69.8, 67.4, 20.9, 20.8, 16.0; Elemental analysis: calcd (%) for C₃₃H₂₆NO₇F₃: C58.94; H5.14; N2.75 Found: C58.65; H5.34; N2.73.
**Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside 2.60.**

To a solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-N-trichloroacetamido-D-glucopyranose 2.59 (38) (16.0 g, 32.45 mmol) in CH$_2$Cl$_2$ (35 mL) was added ethanethiol (2.7 mL, 35.70 mmol) and boron trifluoride etherate (3.1 mL, 24.34 mmol) at 0 °C. The reaction was allowed to warm to room temperature and was stirred overnight. The mixture was diluted with EtOAc (100 mL) and washed with water (150 mL), aqueous saturated NaHCO$_3$ solution (2 x 75 mL) and brine (50 mL). The organic layer was dried over MgSO$_4$, filtered and concentrated. Crystallization from EtOAc/hexanes gave 2.60 (13.6 g, 85%). [α]$_D^{24}$ = −65.8 (c 2.0, CHCl$_3$); mp. 133-134 °C; $^1$H NMR (400 MHz, CDCl$_3$) δ 6.98 (d, $J$ = 9.3 Hz, 1H), 5.35 (dd, $J$ = 9.5, 10.3 Hz, 1H), 5.12 (t, $J$ = 9.7 Hz, 1H), 4.69 (d, $J$ = 10.3 Hz, 1H), 4.25 (dd, $J$ = 5.2, 12.4 Hz, 1H), 4.22-4.03 (m, 2H), 3.77 (ddd, $J$ = 2.4, 5.2, 10.0 Hz, 1H), 2.86-2.61 (m, 2H), 2.08 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.27 (t, $J$ = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 171.0, 170.6, 169.2, 92.2, 83.8, 76.1, 73.1, 68.5, 62.3, 54.7, 24.3, 20.7, 20.6, 20.5, 14.9; HR-MALDI-MS: m/z [M+Na$^+$] calcd for C$_{16}$H$_{22}$Cl$_3$NO$_8$Na 516.0024, found 516.0018. IR (thin film) ν = 3336, 2968, 1749, 1529, 1368, 1229, 1039, 821 cm$^{-1}$.

**Ethyl 2-deoxy-2-N-trichloroacetamido-3,4,6-tri-O-trimethylsilyl-thio-β-D-glucopyranoside 2.61.**
To a solution of 2.60 (13.5 g, 27.4 mmol) in MeOH (130 mL) was added sodium methoxide (148 mg, 2.74 mmol) at room temperature. The solution was stirred overnight, and was neutralized using acidic Amberlite resin IR-120. The resin was filtered off and washed with MeOH (50 mL). The filtrate was evaporated in vacuo to afford the triol intermediate (10.1 g, quant.). The triol (10.1 g, 27.4 mmol) was suspended in CH$_2$Cl$_2$ (50 mL) and Et$_3$N (76.2 mL, 548 mmol). The mixture was cooled to 0 °C and chlorotrimethylsilane (14.0 mL, 109.6 mmol) was added dropwise at the same temperature. The reaction was allowed to warm to room temperature and was stirred overnight. The solvent was evaporated in vacuo. The solid residue was suspended with hexanes/EtOAc (9/1, 200 mL) and filtered. The filtrate was evaporated and purified by flash column chromatography on silica gel (hexanes/EtOAc = 19:1 with 0.1% triethylamine) to provide 2.61 (14.5 g, 90%). $\left[\alpha\right]_D^{25}$ −18.3 (c 1.0, CHCl$_3$); mp. 142-143 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.94 (d, $J = 7.8$ Hz, 1H), 4.54 (d, $J = 8.2$ Hz, 1H), 3.76 (dd, $J = 4.9$, 10.9 Hz, 1H), 3.71-3.63 (m, 2H), 3.63-3.53 (m, 2H), 3.30 (dd, $J = 4.9$, 10.9 Hz, 1H), 2.68-2.38 (m, 2H), 1.09 (t, $J = 7.4$ Hz, 3H), 0.00 (s, 9H), −0.00 (s, 9H), −0.05 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.9, 92.2, 81.7, 80.8, 74.3, 70.3, 61.93, 56.5, 24.4, 14.6, 0.1, −0.0, −0.8; HR-MALDI-MS: m/z [M+2+Na+] calcd for C$_{19}$H$_{40}$Cl$_3$NO$_5$SSi$_3$Na 608.0865, found 608.0858. IR (thin film) $\nu$ = 3309, 2966, 1694, 1532, 1248, 1157, 837 cm$^{-1}$.

**Ethyl 4,6-O-benzylidene-3-O-levulinoyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside 2.62.**

A mixture of 2.61 (500 mg, 0.854 mmol), benzaldehyde (91 mL, 0.897 mmol), and freshly dried 4Å molecular sieves (500 mg) in CH$_2$Cl$_2$ (7.5 mL) was stirred at
0 °C for 1 h. TMSOTf (23 μL, 0.128 mmol) was added to the solution and the mixture was kept stirring at 0 °C for another 2 h. A TBAF solution (1 M in THF, 0.95 mL, 0.95 mmol) was added to the mixture, the reaction was warmed up to room temperature and was kept stirring for another 6 h. DMAP (104.3 mg, 0.854 mmol), LevOH (281 μL, 2.734 mmol) and DIC (319 μL, 2.050 mmol) were consecutively added and the mixture was stirred at room temperature overnight before the mixture was filtered through a pad of celite. The filtrate was washed with water (20 mL) and the aqueous layer was extracted with EtOAc (40 mL). The organic layers were combined, dried over MgSO₄, filtered and evaporated. Flash column chromatography on silica gel (hexanes/EtOAc = 5:2) gave product 2.62 (408 mg, 86%). [α]D₂⁵ –0.1 (c 0.7, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.48-7.46 (m, 2H), 7.39-7.35 (m, 3H), 7.16 (d, J = 9.5 Hz, 1H), 5.53 (s, 1H), 5.47 (t, J = 10.0 Hz, 1H), 4.66 (d, J = 10.5 Hz, 1H), 4.20 (dd, J = 5.0, 10.5 Hz, 1H), 4.11 (q, J = 9.5 Hz, 1H), 3.73 (td, J = 4.9, 9.5 Hz, 1H), 3.65 (td, J = 5.0, 9.5 Hz, 1H), 2.74-2.55 (m, 6H), 2.13 (s, 3H), 1.24 (t, J = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 205.5, 173.2, 162.0, 137.0, 129.0, 128.24, 128.21, 128.18, 126.0, 101.12, 101.10, 92.4, 84.6, 78.7, 72.7, 70.6, 68.3, 55.1, 37.9, 29.6, 28.1, 24.5, 14.9; HR-ESI-MS: m/z [M+2+Na+] calcd for C₂₂H₂₆Cl₃NO₇SNa 578.0360, found 578.0391. IR (thin film) ν = 3342, 2924, 1717, 1529, 1082, 820 cm⁻¹.

**Ethyl 6-O-benzyl-3-O-levulinoyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside 2.63.**

To a solution of 2.62 (8.36 g, 15.07 mmol) and Et₃SiH (14.4 mL, 90 mmol) in CH₂Cl₂ (80 mL) was added trifluoroacetic anhydride (0.63 mL, 4.52 mmol) and trifluoroacetic acid (5.8 mL, 75 mmol) at 0 °C. The mixture was stirred at the
same temperature for 6 h. The reaction was quenched by pouring it into saturated aqueous NaHCO₃ (300 mL). The mixture was extracted three times with EtOAc (200 mL). The organic layers were combined, washed with brine (100 mL), dried over MgSO₄, filtered and evaporated. Purification with flash column chromatography on silica gel (hexanes/EtOAc = 3:2 to 1:1) gave 2.63 (7.89 g, 94%). [α]D²⁰ –33.4 (c 4.9, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.33–7.27 (m, 5H), 6.90 (d, J = 9.4 Hz, 1H), 5.18 (dd, J = 9.0, 10.4 Hz, 1H), 4.57 (ABq, J = 12.1 Hz, 2H), 4.57 (d, J = 10.3 Hz, 1H), 4.03 (q, J = 9.4 Hz, 1H), 3.81–3.76 (m, 3H), 3.63–3.59 (m, 1H), 2.79-2.47 (m, 6H), 2.15 (s, 3H), 1.24 (t, J = 7.5 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 207.6, 173.5, 161.9, 137.8, 128.4, 127.8, 127.7, 92.4, 83.6, 78.4, 76.6, 73.6, 70.1, 69.8, 54.3, 38.3, 29.7, 28.2, 24.0, 14.0; HR-ESI-MS: m/z [M+Na⁺] calcd for C₂₂H₂₈Cl₃NO₇SNa 578.0550, found 578.0534. IR (thin film) ν = 3341, 2927, 1705, 1704, 1527, 1069, 820 cm⁻¹.

**Ethyl 6-O-benzyl-4-O-fluorenylmethoxycarbonyl-3-O-levulinoyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside 2.6.**

![Compound 2.6](image)

Compound 2.63 (7.83 g, 14.06 mmol) was coevaporated three times with toluene (100 mL) and was dissolved in pyridine (40 mL). To the mixture was added FmocCl (4.36 g, 16.87 mmol) at room temperature, and stirred for 24 h. Methanol (50 mL) was added, and the mixture was poured into water (100 mL). The aqueous layer was extracted three times with EtOAc (100 mL). The organic layers were combined, dried over MgSO₄, filtered and evaporated. Flash column chromatography on silica gel (hexanes/EtOAc = 9:1 to 2:1 to 1:1) provided the desired product 2.6 (6.78 g, 62%) along with recovered 2.63 (2.65 g, 34%). [α]D²⁰ –6.9 (c 3.0, CHCl₃); mp. 139-140 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.74 (d, J = 7.5
Hz, 2H), 7.54 (t, J = 7.3 Hz, 2H), 7.38 (t, J = 7.6 Hz, 2H), 7.30-7.19 (m, 7H), 6.81 (d, J = 8.3 Hz, 1H), 5.40 (t, J = 9.6 Hz, 1H), 5.01 (t, J = 9.6 Hz, 1H), 4.71 (d, J = 10.4 Hz, 1H), 4.51 (ABq, J = 12.3 Hz, 2H), 4.44-4.40 (m, 1H), 4.27 (td, J = 1.2, 8.8 Hz, 1H), 4.20 (t, J = 7.2 Hz, 1H), 4.02 (q, J = 10.2 Hz, 1H), 3.81-3.76 (m, 1H), 3.65-3.63 (m, 2H), 2.80-2.45 (m, 6H), 2.04 (s, 3H), 1.28 (t, J = 6.2 Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 205.7, 172.9, 161.9, 154.0, 143.5, 143.2, 141.3, 141.2, 137.7, 128.3, 127.9, 127.62, 127.56, 127.2, 125.2, 125.1, 120.0, 92.3, 83.6, 77.1, 73.6, 73.4, 73.1, 70.5, 69.0, 54.8, 46.9, 37.7, 29.5, 28.1, 24.1, 14.9; HR-ESI-MS: \(m/z\) [M+1+Na\(^+\)] calcd for C\(_{37}\)H\(_{38}\)Cl\(_3\)NO\(_9\)SNa 802.1201, found 802.1157. IR (thin film) \(\nu\) = 3344, 2927, 1755, 1718, 1528, 1257, 1159, 819 cm\(^{-1}\).

**Ethyl 3,4-di-O-benzyl-2-deoxy-2-N-trichloroacetamido-thio-\(\beta\)-D-glucopyranoside 2.65.**

A mixture of compound 2.61 (15 g, 25.6 mmol), benzaldehyde (6.25 mL, 61.5 mmol), and freshly dried 4Å molecular sieves (10 g) in CH\(_2\)Cl\(_2\) (300 mL) was stirred using an over-head stirrer at 0 °C for 1 h. TMSOTf (695 \(\mu\)L, 3.84 mmol) was added to the solution, and the mixture was kept stirring at the same temperature for another 1 h. The reaction was cooled to −78 °C, and triethylsilane (4.5 mL, 28.2 mmol) and TMSOTf (347 \(\mu\)L, 1.92 mmol) were sequentially added to the reaction solution. The mixture was stirred for an additional 16 h at −78 °C. Additional triethylsilane (2.25 mL, 14.1 mmol) and TMSOTf (347 \(\mu\)L, 1.92 mmol) were added to the reaction solution and the mixture was stirred at −78 °C for another 8 h. The reaction was warmed up to 0 °C. A solution of borane in THF (1 M, 120.0 mL, 120.0 mmol) and TMSOTf (2.3 mL, 12.8 mmol) were sequentially added to the reaction at the same temperature, and
the mixture was stirred for another 6 h. The reaction was slowly quenched by MeOH (50 mL) at 0 °C. The mixture was filtered through a pad of celite, and the filtrate was coevaporated with MeOH (200 mL) *in vacuo*. The residue was dissolved in EtOAc (200 mL), washed with water (100 mL) and the aqueous layer was extracted three times with EtOAc (100 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, filtered and concentrated *in vacuo*. Purification with flash column chromatography on silica gel (cyclohexane/EtOAc = 2:1 to 3:2 to 1:1) gave **2.65** (13.40 g, 95%). [α]D24 −3.3 (c 1.0, CHCl3); mp. 147-148 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.23-7.11 (m, 10H), 6.70 (d, J = 8.2 Hz, 1H), 4.78 (d, J = 10.2 Hz, 1H), 4.68 (d, J = 10.9 Hz, 2H), 4.58 (d, J = 10.8 Hz, 1H), 4.53 (d, J = 11.1 Hz, 1H), 3.90 (dd, J = 8.6, 9.7 Hz, 1H), 3.74 (dd, J = 2.4, 12.1 Hz, 1H), 3.63-3.45 (m, 3H), 3.34 (ddd, J = 2.7, 4.5, 9.5 Hz, 1H), 2.67-2.46 (m, 2H), 1.77 (s, 1H), 1.12 (t, J = 7.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 161.7, 137.7, 137.6, 128.5, 127.92, 127.86, 127.82, 92.4, 82.9, 81.8, 79.7, 78.2, 75.3, 74.9, 61.9, 57.3, 24.6, 15.1; HR-MALDI-MS: m/z [M+Na⁺] calcd for C₂₉H₂₈Cl₃NO₅SNa 570.0646, found 570.0655. IR (thin film) ν = 3324, 1687, 1527, 1084, 823 cm⁻¹.

**Ethyl 3,4-di-O-benzyl-6-O-fluorenylmethoxycarbonyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside 2.7.**

Compound **2.65** (13.40 g, 24.41 mmol) was coevaporated three times with toluene (100 mL), and was dissolved in pyridine (44 mL) and CH₂Cl₂ (67 mL). FmocCl (7.58 g, 29.3 mmol) was added at room temperature and the reaction mixture was stirred for 24 h. Methanol (50 mL) was added, and the mixture was poured into water (100 mL). The aqueous layer was extracted three times with
EtOAc (100 mL). The organic layers were combined, dried (MgSO₄), filtered, and evaporated. Crystallization from EtOAc/EtOH gave 2.7 (14.2 g, 75%). Purification of the mother liquor by flash column chromatography on silica gel (hexanes/EtOAc = 10:1 to 3:1 to 2:1 to 1:1) provided additional 2.7 (2.27 g, 12%) as well as recovered starting material 2.65 (1.13 g, 8%). 

\[ \alpha \] D \text{24} = -15.0 (c 2.0, CHCl₃); mp. 172-173 °C; \textsuperscript{1}H NMR (400 MHz, CDCl₃) \( \delta \) 7.75 (dd, \( J = 0.7, 7.6 \) Hz, 2H), 7.63-7.55 (m, 2H), 7.39 (td, \( J = 0.6, 7.5 \) Hz, 2H), 7.35-7.25 (m, 11H), 6.85 (d, \( J = 8.2 \) Hz, 1H), 4.91 (d, \( J = 10.1 \) Hz, 1H), 4.87-4.78 (m, 2H), 4.74 (d, \( J = 10.8 \) Hz, 1H), 4.60 (d, \( J = 11.0 \) Hz, 1H), 4.45 (dd, \( J = 2.3, 11.6 \) Hz, 1H), 4.43-4.33 (m, 2H), 4.30 (dd, \( J = 5.3, 11.6 \) Hz, 1H), 4.23 (t, \( J = 7.4 \) Hz, 1H), 4.06 (dd, \( J = 8.2, 9.6 \) Hz, 1H), 3.80-3.65 (m, 2H), 3.61 (dd, \( J = 8.2, 9.4 \) Hz, 1H), 2.70 (m, 2H), 1.24 (t, \( J = 7.4 \) Hz, 3H); \textsuperscript{13}C NMR (100 MHz, CDCl₃) \( \delta \) 161.6, 154.9, 143.4, 143.3, 141.30, 141.29, 137.5, 137.4, 128.6, 128.1, 128.0, 127.90, 127.86, 127.2, 125.1, 120.1, 92.4, 82.6, 81.7, 78.2, 75.4, 75.0, 70.0, 66.6, 57.4, 46.7, 24.7, 15.1; HR-MALDI-MS: \( m/z \) [M+2+Na\textsuperscript{+}] calcd. for C\textsubscript{39}H\textsubscript{38}Cl\textsubscript{3}NO\textsubscript{7}SNa 794.1303, found 794.1291. IR (thin film) \( \nu \) = 3309, 3031, 1749, 1689, 1534, 1434, 1343, 1413, 14129, 1375, 1374, 1286, 1281, 1280, 12790, 12786, 1272, 1252, 1251, 1201, 924, 826, 817, 782, 754, 750, 700, 666, 574, 467, 247, 151.

**Ethyl 3,6-di-O-benzyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside 2.66.**

A mixture of 2.61 (15 g, 25.6 mmol), benzaldehyde (6.25 mL, 61.5 mmol), and freshly dried 4Å molecular sieves (10 g) in CH\textsubscript{2}Cl\textsubscript{2} (300 mL) was stirred using an over-head stirrer at 0 °C for 1 h. TMSOTf (695 μL, 3.84 mmol) was added to the solution, and the mixture was kept stirring at the same temperature for another hour. The reaction was cooled to −78 °C, and triethylsilane (4.5 mL, 28.2 mmol) and TMSOTf (347 μL, 1.92 mmol) were sequentially added to the reaction.
solution. The mixture was stirred for an additional 16 h at –78 °C. Additional triethylsilane (2.25 mL, 14.1 mmol) and TMSOTf (347 μL, 1.92 mmol) were added to the reaction solution, and the mixture was stirred at –78 °C for 8 h. Triethylsilane (49.1 mL, 307.2 mmol) and trifluoroacetic acid (9.8 mL, 128.0 mmol) were then sequentially added to the reaction at the same temperature, and the mixture was stirred for 4 h. The reaction flask was slowly warmed to room temperature and the mixture was filtered through a pad of celite. The filtrate was carefully washed twice with saturated aqueous NaHCO₃ (150 mL). The aqueous layer was extracted three times with EtOAc (150 mL), and the combined organic layers were washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated in vacuo. Purification by flash column chromatography on silica gel (cyclohexane/EtOAc = 4:1) provided 2.66 (12.3 g, 87%). [α]D²³ –16.0 (c 1.0, CHCl₃); mp. 55-56 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.22-7.08 (m, 10H), 6.70 (d, J = 8.3 Hz, 1H), 4.69 (d, J = 10.2 Hz, 1H), 4.60 (ABq, J = 11.2 Hz, 2H), 4.39 (ABq, J = 12.0, 2H), 3.69 (dd, J = 8.5, 9.9 Hz, 1H), 3.63-3.46 (m, 4H), 3.37 (dt, J = 4.9, 9.7 Hz, 1H), 2.70 (d, J = 2.6 Hz, 1H), 2.62-2.42 (m, 2H), 1.07 (t, J = 7.4, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 161.6, 137.9, 137.54, 128.5, 128.5, 128.0, 127.9, 127.9, 127.7, 92.4, 82.8, 81.4, 77.9, 74.7, 73.7, 73.3, 70.6, 56.8, 24.4, 15.1; HR-MALDI-MS: m/z [M+Na⁺] calcd for C₂₄H₂₈Cl₃NO₅SNa 570.0646, found 570.0653. IR (thin film) ν = 3323, 2870, 1690, 1528, 1060, 821 cm⁻¹.

Ethyl 3,6-di-O-benzyl-4-O-fluorenylmethoxycarbonyl-2-deoxy-2-N-trichloroacetamido-thio-β-D-glucopyranoside 2.8.

![Structural formula of compound 2.8](image)

Compound 2.66 (4.92 g, 8.96 mmol) was coevaporated three times with toluene (30 mL) and dissolved in pyridine (49 mL). To the mixture was added FmocCl.
(4.64 g, 17.93 mmol) at room temperature and stirred for 5 h. Methanol (20 mL) was added and the mixture was poured into water (50 mL). The aqueous layer was extracted three times with EtOAc (50 mL). The organic layers were combined, dried (MgSO\(_4\)), filtered, and evaporated. Purification by flash column chromatography on silica gel (cyclohexane/EtOAc = 9:1 to 3:1 to 2:1 to 1:1) provided 2.8 (5.50 g, 80%) and recovered starting material (0.42 g, 9%). \([\alpha]D^{27}_{27} −5.2 (c 1.0, \text{CHCl}_3); \text{mp.} 126-127 °C; ^1\text{H NMR (400 MHz, CDCl}_3) \delta 7.67 (dt, J = 1.0, 7.6 Hz, 2H), 7.48 (dd, J = 0.7, 7.2 Hz, 1H), 7.43 (d, J = 7.5 Hz, 1H), 7.30 (t, J = 7.5 Hz, 2H), 7.26-7.18 (m, 5H), 7.18-7.09 (m, 7H), 6.82 (d, J = 7.9 Hz, 1H), 4.95 (d, J = 10.3 Hz, 1H), 4.86 (dd, J = 9.1, 9.8 Hz, 1H), 4.56 (s, 2H), 4.45 (s, 2H), 4.29-4.19 (m, 2H), 4.15 (t, J = 9.8 Hz, 1H), 4.03 (t, J = 7.2 Hz, 1H), 3.74-3.63 (m, 1H), 3.64-3.50 (m, 3H), 2.77-2.53 (m, 2H), 1.21 (t, J = 7.4 Hz, 3H); ^13\text{C NMR (100 MHz, CDCl}_3) \delta 161.6, 154.3, 143.3, 143.1, 141.3, 141.3, 137.9, 137.3, 128.41, 128.3, 127.9, 127.9, 127.8, 127.6, 127.2, 127.2, 125.1, 125.0, 120.07, 120.06, 92.3, 82.5, 78.8, 76.2, 74.7, 73.6, 70.1, 69.6, 57.7, 46.7, 24.7, 15.2; HR-MALDI-MS: \textit{m/z} [M+2+Na\(^+\)] calcd for C\(_{39}\)H\(_{38}\)Cl\(_3\)NO\(_7\)SNa 794.1303, found 794.1297. IR (thin film) \(\nu = 3316, 2929, 1754, 1690, 1531, 1257, 738 \text{ cm}^{-1}\).

**Ethyl 2,3,4,6-tetra-\(\text{O}\)-trimethylsilyl-thio-\(\beta\)-D-glucopyranoside 2.67.**

![2.67]

To a solution of ethyl 2,3,4,6-tetra-\(\text{O}\)-acetyl-thio-\(\beta\)-D-glucopyranoside (9.5 g, 24.2 mmol) in MeOH (90 mL) was added sodium methoxide (131 mg, 2.42 mmol) at room temperature. The solution was stirred overnight, and was neutralized using acidic Amberlite resin IR-120. The resin was filtered off and washed with MeOH (50 mL). The filtrate was evaporated \textit{in vacuo} to afford the tetraol intermediate (5.41 g, quant.). The tetraol (5.41 g, 24.2 mmol) was suspended in CH\(_2\)Cl\(_2\) (30
mL) and Et₃N (70.0 mL, 506 mmol), and the mixture was cooled to 0 °C. Chlorotrimethylsilane (16.2 mL, 126.5 mmol) was added dropwise at the same temperature, and the reaction was allowed to warm up to room temperature and was stirred overnight. The solvent was evaporated in vacuo. The solid residue was suspended with 10% EtOAc/hexane (200 mL) and filtered. The filtrate was evaporated, and purified by flash column chromatography on silica gel (EtOAc/hexanes 5:95 with 0.1% triethylamine) to provide the desired product 2.67 (12.1 g, 93%) as colorless syrup. [α]Dⁿ⁻²₂⁻₂₂.₃ (c 1.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 4.35 (d, J = 9.0 Hz, 1H), 3.77 (dd, J = 2.2, 11.3 Hz, 1H), 3.62 (dd, J = 5.8, 11.6 Hz, 1H), 3.43-3.30 (m, 3H), 3.23-3.19 (m, 1H), 1.27 (t, J = 7.5 Hz, 3H), 0.19 (s, 9H), 0.15 (s, 9H), 0.14 (s, 9H), 0.09 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 86.2, 81.2, 79.8, 75.4, 71.7, 62.6, 26.9, 25.1, 15.0, 1.6, 1.4, 0.9, –0.2; HR-ESI-MS: m/z [M+Na⁺] calcd for C₂₀H₄₈O₅SSi₄Na 535.2197, found 535.2195. IR (thin film) ν = 2957, 2900, 1249, 1161, 1090, 839 cm⁻¹.

Ethyl 2-O-benzoyl-3,6-di-O-benzyl-thio-β-D-glucopyranoside 2.68.

To a solution of 2.67 (500 mg, 0.975 mmol), PhCHO (217 μL, 2.144 mmol), and freshly dried 4Å molecular sieves (250 mg) in CH₂Cl₂ (7.5 mL) was added TMSOTf (27 μL, 0.146 mmol) at –78 °C under argon atmosphere. After stirring at -78 °C for 2 h, Et₃SiH (171 μL, 1.072 mmol) and TMSOTf (14 μL, 0.073 mmol) were sequentially added to the solution, and the mixture was kept stirring for another 16 h. Benzoic anhydride (478 mg, 2.144 mmol), benzoic acid (24 mg, 0.195 mmol) and TMSOTf (90 μL, 0.487 mmol) were consecutively added to the solution. The reaction flask was warmed up to 0 °C and the mixture was stirred at the same temperature for 3 days. The mixture was then cooled to –78 °C, and
Et$_3$SiH (1.86 mL, 11.69 mmol) followed by TFA (0.375 mL, 4.873 mmol) were added under argon at −78 °C and the mixture was kept stirring for 3 h. The reaction was warmed up to 0 °C and slowly quenched by saturated aqueous NaHCO$_3$ (10 mL). The mixture was filtered through a pad of celite, and the filtrate was extracted three times with EtOAc (20 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO$_4$, filtered, evaporated, and purified by flash column chromatography on silica gel (hexanes/EtOAc = 3:1) to give **2.68** (255 mg, 51%). \[\alpha\]$_D$\textsubscript{20} 13.1 (c 0.7, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.04-8.01 (m, 2H), 7.58-7.54 (m, 1H), 7.44 (t, $J$ = 7.9 Hz, 2H), 7.35-7.28 (m, 5H), 7.17 (s, 5H), 5.26 (dd, $J$ = 9.2, 10.0 Hz, 1H), 4.70 (ABq, $J$ = 11.5 Hz, 2H), 4.59 (ABq, $J$ = 12.0 Hz, 2H), 4.54 (d, $J$ = 10.0 Hz, 1H), 3.82-3.77 (m, 3H), 3.68 (t, $J$ = 8.9 Hz, 1H), 3.56 (dt, $J$ = 9.5, 4.6 Hz, 1H), 2.80 (s, 1H), 2.76 (m, 2H), 1.21 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 165.2, 137.9, 137.7, 133.2, 129.8, 128.45, 128.40, 128.36, 128.0, 127.82, 127.76, 127.74, 83.6, 83.5, 78.2, 74.6, 73.7, 72.3, 72.0, 70.5, 23.9, 14.9; HR-ESI-MS: m/z [M+Na$^+$] calcd for C$_{29}$H$_{32}$O$_6$SNa 531.1817, found 531.1807. IR (thin film) $\nu$ = 3463, 3031, 2870, 1723, 1451, 1266, 1066 cm$^{-1}$.

**Ethyl 2-O-benzoyl-3,6-di-O-benzyl-4-O-fluorenlymethoxycarbonyl-thio-β-D-glucopyranoside 2.9.**

Compound **2.68** (795 mg, 1.563 mmol) was coevaporated three times with toluene (50 mL) and was dissolved in CH$_2$Cl$_2$ (6 mL) and pyridine (4 mL). To the mixture was added FmocCl (485 mg, 1.876 mmol) at room temperature under argon atmosphere and the mixture was stirred for 24 h. Methanol (5 mL) was added before and the mixture was poured into water (20 mL). The aqueous layer
was extracted three times with EtOAc (30 mL). The organic layers were combined, washed with brine, dried (\(\text{MgSO}_4\)), filtered, and evaporated. Flash column chromatography on silica gel (hexanes/EtOAc = 10:1 to 6:1 to 2:1) provided \(2.9\) (872 mg, 76%) and recovered \(2.68\) (93 mg, 12%). \([\alpha]^20\) 32.3 (c 0.75, CHCl_3); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.00 (dd, \(J = 1.2, 8.3\) Hz, 2H), 7.73 (dd, \(J = 4.0, 7.6\) Hz, 2H), 7.59-7.50 (m, 3H), 7.43 (t, \(J = 8.0\) Hz, 2H), 7.37 (td, \(J = 2.7, 7.5\) Hz, 2H), 7.32-7.18 (m, 7H), 7.03 (s, 5H), 5.32 (t, \(J = 9.9\) Hz, 1H), 4.97 (t, \(J = 9.8\) Hz, 1H), 4.60-4.53 (m, 5H), 4.31 (d, \(J = 6.9\) Hz, 2H), 4.10 (t, \(J = 7.1\) Hz, 1H), 3.89 (t, \(J = 9.2\) Hz, 1H), 3.76-3.71 (m, 1H), 3.66-3.65 (m, 2H), 2.77-2.63 (m, 2H), 1.22 (t, \(J = 7.4\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 165.0, 154.2, 143.3, 143.1, 141.29, 141.26, 137.9, 137.4, 133.2, 129.9, 129.7, 128.40, 128.36, 128.32, 128.13, 128.10, 128.0, 127.8, 127.7, 127.6, 127.5, 127.2, 125.1, 125.0, 120.1, 83.7, 81.1, 77.4, 75.6, 74.4, 73.6, 71.9, 70.0, 69.7, 46.7, 24.1, 14.9; HR-ESI-MS: \(m/z\) [M+Na\(^+\)] calcd for \(C_{44}H_{42}O_8SNa\) 753.2498, found 753.2479. IR (thin film) \(\nu\) = 3031, 2871, 1753, 1728, 1451, 1252, 1070, 739 cm\(^{-1}\).

**Allyloxy carbonylaminohexyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroethoxycarbonylamino-D-glycero-\(\alpha\)-D-galacto-2-nonulopyranosylonate)-(2\(\rightarrow\)3)-2-O-acetyl-4,6-di-O-benzyl-\(\beta\)-D-galactopyranosyl-(1\(\rightarrow\)4)-2-deoxy-6-O-benzyl-3-O-levulinoyl-2-trichloroacetimidyl-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)3)-2-O-benzoyl-4,6-di-O-benzyl-\(\beta\)-D-galactopyranosyl-(1\(\rightarrow\)4)-2,3-di-O-benzoyl-6-O-benzyl-\(\beta\)-D-glucopyranoside 2.75.**

![Image](2.75)

To a solution of sialyl galactose donor \(2.1\) (135 mg, 0.115 mmol) and trisaccharide \(2.71\) (122 mg, 0.0763 mmol) in CH\(_2\)Cl\(_2\) (2.0 mL) was added TMSOTf
(3.0 mL, 0.016 mmol) at -15˚C under Ar atmosphere. After stirring for 1.5 h at -15˚C, the mixture was neutralized with a few drops of Et,N, and then concentrated. Purification with flash column silica gel chromatography (hexane:ethylacetate = 2:1 to 1:1) gave pentasaccharide 2.75 (184 mg, 0.0710 mmol, 93%). $[\alpha]_{D}^{23} = -7.3$ (c 0.43, chloroform); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.92-7.80 (m, 7H), 7.60-7.10 (m, 32H), 6.36 (d, 1H, $J = 9.3$ Hz), 5.89 (m, 1H), 5.60 (m, 1H), 5.53 (t, 1H, $J = 9.7$ Hz), 5.42-5.15 (m, 7H), 5.10 (dd, 1H, $J = 7.8$, 10.3 Hz), 4.94-4.75 (m, 5H), 4.69 (d, 1H, $J = 7.8$ Hz), 4.57-3.28 (m, overlapped), 3.65 (s, 3H), 2.96 (m, 2H), 2.84 (m, 2H), 2.66 (dd, 1H, $J = 4.7$, 12.8 Hz), 2.52-2.31 (m, 4H), 2.12 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.91 (s, 3H), 1.79 (t, 1H, $J = 10.9$ Hz), 1.41 (br, 2H), 1.11 (br, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 205.7, 172.1, 170.3, 169.3, 167.7, 165.0, 164.1, 161.8, 156.0, 154.0, 138.8, 138.3, 138.0, 133.4, 132.8, 132.2, 130.3-127.0, 117.4, 100.8, 100.6, 100.5, 97.3, 95.2, 91.8, 78.6, 77.2, 75.8, 75.1, 75.0, 74.8, 74.5, 74.1, 73.3, 73.3, 73.1, 73.0, 72.7, 72.6, 72.0, 71.8, 70.5, 69.7, 68.7, 67.4, 67.1, 65.3, 62.3, 56.0, 52.9, 51.3, 40.8, 37.8, 29.7, 29.2, 27.8, 26.3, 25.5, 21.4-20.8; HR-MALDI-MS: m/z [M+Na]$^+$ calcd for C$_{127}$H$_{141}$Cl$_6$N$_3$O$_{42}$Na: 2616.7017, found 2616.7101.

Allyloxycarbonylaminoheptyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroethoxycarbonylamino-O-glycero-O-D-galacto-2-nonulopyranosylonate)-(2$\rightarrow$3)-2-O-acetyl-4,6-di-O-benzyl-\(\beta\)-D-galactopyranosyl-(1$\rightarrow$4)-2-deoxy-6-O-benzyl-2-trichloroacetamidyl-\(\beta\)-D-glucopyranosyl-(1$\rightarrow$3)-2-O-benzoyl-4,6-di-O-benzyl-\(\beta\)-D-galactopyranosyl-(1$\rightarrow$4)-2,3-di-O-benzoyl-6-O-benzyl-\(\beta\)-D-glucopyranoside 2.76.
To a solution of pentasaccharide 2.75 (140 mg, 0.0538 mmol) in pyridine (3 mL) and AcOH (0.75 mL) with allyl alcohol (0.18 mL, 2.7 mmol) was added hydrazine monohydrate (13 ml, 0.27 mmol) at 0 °C. After stirring for 2 h at room temperature under N₂ atmosphere, the mixture was diluted with EtOAc. The organic layer was washed with 10% citric acid solution, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification with flash column silica gel chromatography (toluene:acetone = 6:1) produced 2.76 (127.5 mg, 0.05108 mmol, 95%). [a]D²⁰ = -1.6 (c 1.54, chloroform); ¹H NMR (300 MHz, CDCl₃): δ 7.92-7.81 (m, 7H), 7.60-7.10 (m, 38H), 6.30 (d, 1H, J = 8.4 Hz), 5.90 (m, 1H), 5.54 (m, 2H), 5.54-5.17 (m, 6H), 4.95-4.78 (m, 6H), 4.65 (dd, 1H, J = 8.1, 10.0 Hz), 4.51-4.22 (m, 20H), 4.04-3.29 (30H), 3.71 (s, 3H), 2.95 (m, 2H), 2.81 (m, 2H), 2.69 (dd, 1H, J = 4.7, 12.8 Hz), 2.16 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H), 2.96 (s, 3H), 1.80 (t, 1H, J = 12.5 Hz), 1.61 (dd, 1H, J = 6.9, 14.1 Hz), 1.42 (m, 2H), 1.11 (br, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 170.3, 169.9, 169.4, 167.7, 167.7, 165.1, 164.3, 161.6, 154.1, 139.0, 138.0, 137.3, 133.3, 132.9, 132.3, 130.4-127.4, 117.5, 101.5, 100.9, 100.2, 97.4, 95.2, 92.3, 82.2, 78.7, 77.3, 75.8, 75.0-74.4, 73.5-73.0, 72.6, 72.0-71.8, 70.0, 69.8, 69.2, 68.7, 68.4, 67.5, 67.3, 66.3, 65.4, 62.4, 57.8, 53.1, 51.3, 40.9, 37.8, 29.8, 29.3, 26.3, 25.6, 22.5, 21.4-20.9; HR-MALDI-MS: m/z [M+Na]⁺ calcd for C₁₂₅H₁₃₅Cl₆N₃O₄₀Na: 2514.6645, found 2514.6596.

Allyloxycarbonylaminohexyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trichloroethoxycarbonylamino-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2-O-acetyl-4,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)[(1→3)-6-deoxy-3,4-di-O-acetyl-2-O-benzyl-α-L-galactopyranosyl]-2-deoxy-6-O-benzyl-2-trichloroacetamidyl-β-D-glucopyranosyl-(1→3)-2-O-benzoyl-4,6-di-O-benzyl-β-D-
To a solution of pentasaccharide acceptor 2.76 (48 mg, 0.019 mmol) and fucose donor 2.5 (30 mg, 0.058 mmol) in CH₂Cl₂ (1 mL), Et₂O (0.25 mL), and dioxane (0.25 mL) was added MS 4Å (108 mg). The mixture was stirred for 10 min at room temperature under Ar atmosphere. Then, Yb(OTf)₃ (19 mg, 0.030 mmol) was added at 0 °C. After stirring for 5 h at room temperature under Ar atmosphere, the mixture was neutralized with a few drops of Et₃N, filtered through Celite, and then filtrate was concentrated. Purification with preparative TLC (hexane:acetone = 2:1) gave hexasaccharide 2.77 (48 mg, 0.017 mmol, 88%). [α]D²³ = -28 (c 1.4, chloroform); ¹H NMR (300 MHz, CDCl₃): δ 7.94-7.79 (m, 6H), 7.60-6.91 (m, 54H), 6.54 (d, 1H, J = 8.2 Hz), 5.84 (m, 1H), 5.63-5.51 (3H), 5.46-5.03, 4.96-4.78, 4.69-3.28, 3.64 (s, 3H), 2.95 (m, 1H), 2.84 (m, 1H), 2.75 (t, 1H, J = 8.5 Hz), 2.66 (dd, 1H, J = 4.4, 12.4 Hz), 2.20 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.95 (s, 3H), 1.86 (s, 3H), 1.41 (br, 2H), 1.10 (br, 6H), 0.77 (d, 3H, J = 6.3 Hz); ¹³C NMR (75 MHz, CDCl₃): δ 170.3, 170.2, 169.8, 169.6, 169.3, 167.6, 165.0, 164.4, 160.9, 154.0, 140.0-137.9, 133.0, 132.8, 132.2, 130.3, 129.8-127.0, 117.4, 100.8, 99.7, 97.5, 96.8, 95.2, 91.8, 78.6, 77.2, 75.7-70.2, 70.1-67.3, 64.5, 62.1, 58.9, 52.9, 51.4, 40.5, 39.2, 29.7, 29.2, 26.3, 25.6, 22.5, 2.13-20.8, 16.4, 15.2, 10.5; HR-MALDI-MS: m/z [M+Na]⁺ calcd for C₁₃₉H₁₅₅Cl₁₆N₆O₄₆Na: 2834.7951, found 2834.7905.

Aminohexyl 3,5-dideoxy-5-acetamidyl-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)-6-deoxy-α-
L-galactopyranosyl]-2-deoxy-2-acetamidyl-β-D-glucopyranosyl-(1→3)-β-
D-galactopyranosyl-(1→4)-β-D-glucopyranoside 2.69.

To a solution of 2.77 (48 mg, 0.017 mmol) in degassed THF/MeOH (1.5 mL, 2/1) was added Pd(PPh₃)₄ (2 mg, 0.002 mmol) and p-toluenesulfinic acid sodium salt (9 mg, 0.05 mmol). The resulting mixture was stirred for 5 h at room temperature under Ar atmosphere, and then, Cbz-OSu (11 mg, 0.042 mmol) and Et₃N (15 mL, 0.086 mmol) were added to the reaction mixture. After stirring for an additional 5 h, the mixture was concentrated and dried under reduced pressure to give crude 2.70, which was dissolved in AcOH (3 mL) and heated at 50 °C. Zn-Cu couple (404 mg) was added in five portions at 1 h intervals, and then stirred for an additional 19 h under N₂ atmosphere. After the reaction, the mixture was cooled to room temperature and filtered through Celite. Then, the filtrate was concentrated and dried under reduced pressure. The residue was then suspended in pyridine (3 mL) and Ac₂O (1.5 mL) was added. After stirring for 18 h, the solvent was evaporated and the residue was dissolved in EtOAc. The organic layer was washed with 10% citric acid solution, H₂O, NaHCO₃ solution, and brine, dried over Na₂SO₄, filtered, and concentrated. Purification with preparative TLC (hexane:ethylacetate = 1:5) provided 2.78 (19 mg, 0.0072 mmol, 42%). Then, compound 2.78 (8.1 mg, 0.0030 mmol) was dissolved in 0.1 M NaOMe in MeOH solution (3 mL), and stirred for 20 h at room temperature. Then, H₂O (0.3 mL) was added and the mixture was stirred for an additional 18 h. After the completion of the reaction, the mixture was neutralized with Amberlite IR-120 resin. The resin was then removed by filtration, and the filtrate was concentrated and dried under reduced pressure. Finally, the resulting residue was dissolved in MeOH (1.5 mL)/H₂O (0.5 mL)/ AcOH (50 mL) and 20% Pd(OH)₂/C (15 mg) was
added. After stirring for 22 h under H₂ atmosphere, the catalyst was removed by filtration through Celite, and the filtrate was concentrated. Purification with size exclusion chromatography (Sephadex C-15, H₂O) followed by solid-phase extraction (Sep-pak C-18, MeOH/H₂O) gave 2.69 (2.0 mg, 0.0016 mmol, 53%).

¹H NMR (300 MHz, CDCl₃): δ 5.08 (s, 1H, J = 2.4 Hz), 4.51 (d, 1H, J = 8.4 Hz), 4.46 (d, 1H, J = 7.8 Hz), 4.41 (d, 1H, J = 8.4 Hz), 4.15 (brs, 1H), 4.06 (dd, 1H, J = 3.1, 12.6 Hz), 4.01-3.33, 3.27 (m, 1H), 2.97 (t, 2H, J = 7.5 Hz), 2.73 (dd, 1H, J = 3.0, 11.5 Hz), 2.01 (s, 3H), 2.00 (s, 3H), 1.77 (t, 1H, J = 11.2 Hz), 1.63 (br, 4H), 1.39 (br, 4H), 1.14 (d, 3H, J = 6.6 Hz); HR-ESI-MS: m/z [M+Na]+ calcd for C₄₉H₈₅Cl₆N₃O₃₃Na: 1266.4963, found 1266.4987.
2.12 References

37) Unpublished data from Dr. C.-C. Wang. See “Synthetic Procedures".
Acknowledgments

Portions of the work presented in this chapter were performed in collaboration with Dr. Lenz Kröck (synthesis instrument, linker synthesis and oligoglucosamine), Dr. Cheng-Chung Wang (oligoglucosamines synthesis), Dr. Pascal Bindschälder (linker synthesis) and Dr. Bastien Castagner (linker synthesis and development of synthetic protocols).
3.1 Introduction

The development of a new fully automated platform for oligosaccharide synthesis is crucial for the development of glycobiology. Ideally, such a platform should be able to perform as a processing system that receives building blocks as an input, and generates biologically active oligosaccharides as an output. Central to the performance of a such platform is: 1) a fully automated instrument (synthesizer) capable of performing coupling-deprotection cycles; 2) a linker system conjugated to a proper solid support, which is stable to all reaction conditions exploited during the synthesis of targeted compounds; 3) versatile protocols for coupling-deprotection cycles that can be applied to different classes of building blocks; 4) deprotection-cleavage protocols to deprotect and detach the oligosaccharide on and from the support, minimizing manual operation on the final compound. Proof of the efficiency of such a platform would ultimately lie in the synthesis of a diverse library of biologically active oligosaccharides containing a collection of statistically relevant linkages starting from a library of readily accessible and differentially protected monosaccharide building blocks.

This chapter describes the first synthesis of a library of structurally unrelated oligosaccharides exploiting an improved automated solid phase synthesis platform and a new solid phase linker 3.1 (Figure 2). The development of automated synthetic protocols and their optimization will be discussed in the context of the synthesis of several important carbohydrate antigens, including oligoglucosamines and sialylated antigens. The ultimate goal of the platform is to investigate structure-activity relationships of carbohydrates in biological systems. The development of simple molecular tools for biological evaluation showcases the applicability of the new platform.
3.2 Development of a New Platform for Automated Oligosaccharide Synthesis

In order to match a solution phase reaction set-up, an automated instrument should be able to perform, in a reliable way, a series of simple operations (Figure 1) including the ability: 1) to deliver solvents and reagents into a reaction vessel capable of retaining a solid support under inert atmosphere; 2) to control the temperature of the reaction vessel and maintain efficient solution mixing; 3) to drain and collect washing solutions and reaction mixtures. In this context, points 1) and 2) summarize the actions that are required to put a reaction into operation, while point 3) refers to typical work up or analytic steps.

Figure 1. Schematic view of key features of the reaction vessel in an automated synthesizer.

The modified ABI synthesizer utilized in the initial work on automated oligosaccharide synthesis (Chapter 1) presented some limitations. In particular, manual control of the temperature as well as manual collection of the reaction mixture was needed. In addition, precise delivery of small quantities of reagent
was not achievable due to hardware limitations. Furthermore, solution mixing was achieved through agitation of the whole reaction vessel. Since the latter was made up of double jacketed glass connected to a cryostat via plastic tubing, shaking could result in rupture or leakages.

Recently, a new synthesizer has been developed in the Seeberger laboratory. (1,2) Some of the limiting features characteristic of the first instrument have been overcome using the new design. The notable changes are: (i) a more accurate solvent delivery has been achieved through integration of syringe pumps; (ii) automatic regulation of reaction vessel temperature has been implemented by use of a computer controllable chiller unit; (iii) a more efficient strategy for reaction mixing has been developed that is based on bubbling inert gas through the reaction mixture and (iv) an automated fraction collector has been integrated for the “in-line” analysis of reaction mixtures.

3.3 Linker System

The experience gained during the first synthetic work on automated carbohydrate synthesis(3) was inspiring for the development of a new linker system. In Chapter 1, the octenediol linker 1.13 was introduced. While this linker was efficient for the synthesis of oligosaccharides using glycosyl phosphate and glycosyl imidate building blocks, a significant limitation was the exclusion of thioglycosides due to the presence of an electrophilic double bond, which is unstable under the reaction condition required to activate these building blocks. Furthermore, a non-trivial cross metathesis step was required to detach the target molecule from the solid support, and the resulting n-penteny l linker was not suitable for direct conjugation to surfaces or carrier proteins. Ideally, a linker should be cleavable under mild conditions and should incorporate a chemical handle ready for direct conjugation with minimal chemical manipulation. Based on these considerations,
linker 3.1 (Figure 2) was designed (1,2) and attached to Merrifield solid support via an ester linkage. This compound is amenable to removal from the solid support by simple treatment with sodium methoxide. The reducing end of the glycoside is attached on a five carbon spacer containing a latent amine released at the end of the synthesis in one single step through hydrogenolysis.

As emerged in preliminary solution phase studies, the linker was compatible with the most commonly utilized glycosylating agents, including thioglycosides (4) and glycosyl acetimidates (5). Additionally, reaction conditions for cleavage of standard temporary protecting groups (6) including 9-fluorenemethoxycarbonate (Fmoc), levulinate ester (Lev), and silyl ethers, as well as N-trichloroacetate (TCA) did not compromise linker integrity.

![Figure 2. The new linker for oligosaccharide solid phase synthesis.](image)

### 3.3 Reaction Modules

The synthesizer is instructed with a series of computer programs or modules that contain all the information relevant to reagent addition, solvent washing, and the draining and collection of reaction mixtures for in-line analysis. Several modules have been created for different types of building blocks (i.e. thioglycoside module,
imidate module) and different operations (i.e. glycosylation module, deprotection module, cleavage module). Most of the parameters, including the scale of the reaction or amounts of activator required, have been standardized for each module. For example, all automated syntheses were performed on a 25 μmol scale with respect to linker 3.1 with resin loading varying between 0.120 and 0.20 mmol/g. Each module also contains predefined inputs relative to temperature and reaction time. In addition, modules were generated such that the last two parameters could be easily modified to enable the optimization of reaction conditions for each particular building block.

3.4 Initial Studies

The automated synthesis of glucosamine 3.4 (Scheme 1) was explored as a case study to streamline the new platform. One of our goals was to use the automated synthesizer to perform some of the deprotection steps in order to minimize subsequent manual operations. We aimed at the creation of modules for reduction of TCA groups, ester removal and linker cleavage from the solid support. In this way, the automated platform delivers sugars that are protected only by benzyl ethers and benzyl carbamates, both cleavable by a single hydrogenolysis.

The synthesis commenced with the glycosylation of the support-bound linker 3.1 with monosaccharide building block 3.2. As a general approach, glycosylation reactions were driven to completion by treating the resin twice with five equivalents of glycosylating agent. For glycosyl imidate 3.2,(5) trimethylsilyl triflate was used as the activator at 0 °C. This was followed by washing of the solid support with dichloromethane and tetrahydrofuran, alternatively. Subsequent
removal of the 9-fluorenemethoxycarbonate (Fmoc) temporary protecting group by treatment with piperidine in dimethylformamide exposed the hydroxyl nucleophile for the next coupling. The solution containing the dibenzofulvene-piperidine adduct produced during the cleavage reaction was delivered to the fraction collector. The distinct UV absorption maximum of this byproduct was used to monitor the coupling efficiency, analogous to the protocol used to monitor peptide assembly.(7)

A module was also developed for the on resin reduction of trichloroacetyl (TCA) groups. Thus, treatment of the solid support bound sugar with AIBN as radical initiator and tributyltin hydride as hydride source at 90 °C readily converted the TCA amino protecting group into the naturally occurring acetamido group. Cleavage of the product from the solid support was also automated and achieved by treatment with sodium methoxide in a methanol-dichloromethane mixture to provide compound 3.3 with a yield of 35% over four steps.

Since the purification of partially protected oligosaccharides by HPLC is not yet a common practice in comparison to synthetic peptides and oligonucleotides, compound 3.3 served as a standard for analytical and preparative reverse phase analysis. The partially protected monosaccharide was purified by reverse-phase HPLC followed by hydrogenolysis to yield the final compound 3.4 in 93% yield. Based on design, upon hydrogenolysis the carbamate group on the linker was cleaved to reveal an amino pentanol group that can be employed for conjugation.
Scheme 1. Automated synthesis of glucosamine 3.4. Reagents and conditions: i (Imidate-Fmoc module): a) TMSOTf, DCM, -10 °C, 1 h; b) Piperidine, DMF. ii (Reduction module): AIBN (cat.), Bu3SnH (10 eq.), Xylene, 90 °C. iii (Cleavage module): NaOMe, MeOH, DCM, 1.5 h, 35%. iv: Pd/C, H2, MeOH, H2O, THF, AcOH, 93%.

The principles described for the synthesis of 3.4 are of general use, and were applied to a series of biologically-relevant oligosaccharide molecules to illustrate the power and versatility of this new approach. Target selection was guided by biological significance, and by taking into account the structural considerations required to generate challenging glycosidic linkages of chemical and biological importance.

3.5 Synthesis of Oligo-glucosamines

Oligo β-(1-6) glucosamines are found on the surface of pathogenic bacteria of many clinically important strains of Staphylococci. (8) The potential application of these compounds in vaccine development has been recently described. (9) The
bacterial $\beta$-(1→6)-glucosamine hexamer 3.6 (Scheme 2) was assembled using 6-Fmoc protected glucosamine thioglycoside building block 2.7. Each cycle involved two additions of glycosylating agent (double coupling) using five equivalents of building block 2.7 in combination with $N$-iodo succinimide as activator, in the presence of catalytic amounts of triflic acid at -20 °C. (4) The temporary Fmoc protecting group was cleaved using piperidine. Following assembly of the oligosaccharide chain, the C2 amino-trichloroacetyl protective groups were reduced to the corresponding $N$-acetyl groups by six-fold exposure to tin hydride in the presence of the radical initiator azobisisobutyronitrile (AIBN). Release of the partially-protected oligosaccharide product was achieved by cleavage of the linker with sodium methoxide. Preparative HPLC purification yielded 40 mg of the partially-protected hexasaccharide 3.5 with 58% overall yield over 19 steps, based on resin loading. Hydrogenation as a single solution phase chemical transformation resulted in quantitative global deprotection to give fully deprotected hexasaccharide 3.6 in 58% yield.

Scheme 2. Automated synthesis of $\beta$-(1→6)-glucosamine hexamer 3.6. Reagents and conditions:  

i (Thio-Fmoc module): a) NIS, TfOH, dioxane, DCM, -40 °C to -20 °C, 40 min; b) Piperidine, DMF. ii (Reduction module): AIBN (cat.), Bu$_3$SnH (10 eq.), Xylene, 90 °C. iii (Cleavage module): NaOMe, MeOH, DCM, 1.5 h, 58%. iv: Pd/C, H$_2$, MeOH, H$_2$O, THF, AcOH, 58% (solution phase).
Fmoc quantification for the synthesis of 3.6 was consistent with an average step-wise yield of 96% for each of the six glycosylations. Based on this result, the possibility of synthesizing higher order β-(1→6)-oligo-glucosamines was envisioned. In this regard, dodecaglucosamine 3.8 (Scheme 3) served as the next synthetic challenge. Employing a similar automated protocol composed of twelve thioglycoside couplings, followed by dechlorination of the TCA groups and methanolysis, dodecasaccharide 3.7 was produced in 43% overall yield.

Unexpectedly, hydrogenation of 3.7 using palladium on carbon and hydrogen atmosphere under standard conditions was sluggish and resulted in dodecasaccharides containing three to six cyclohexyl ether residues. It was therefore speculated that some of the benzyl ethers were inaccessible due to the possible formation of secondary structures in the particular solvent system used (methanol-tetrahydrofuran-water), thereby resulting in over reduction of the benzyl ring rather than cleavage of the ethereal bond. When the more reactive Pearlman’s catalyst (10) was used in slight excess in a mixture of tert-butanol, tetrahydrofuran and aqueous hydrochloric acid, and the polarity of the solution was increased by addition of water as the reaction proceeded, dodecasaccharide 3.8 was obtained in 53% yield after reverse phase HPLC purification (Figure 3).
Scheme 3. Automated synthesis of dodecaglucosamine 3.8. Reagents and condition: i (Thio-Fmoc module): a) NIS, TfOH, dioxane, DCM, -40 °C to -20 °C, 40 min; b) Piperidine, DMF. ii (Reduction module): AIBN (cat.), Bu$_3$SnH (10 eq.), Xylene, 90 °C. iii (Cleavage module): NaOMe, MeOH, DCM, 1.5 h, 43%. iv: Pd(OH)$_2$/C, H$_2$, t-BuOH, THF, aq. HCl, 53% (solution phase).

Figure 3. LC-MS of the 1,6-dodecaglucosamine 3.8. Conditions: 0 to 25% A in B, A = CH$_3$CN + 1% Formic Acid, B = water + 1% Formic Acid, over 25 min.

In collaboration with Dr. C.-C. Wang and Dr Lenz Kröck, the synthesis of a β-(1→4)-glucosamine hexamer 3.10 (Scheme 4) was also undertaken by adopting essentially the same strategy exploited for the synthesis of 3.6. Thus, six glycosylation-deprotection modules with building block 2.8 followed by the same number of reduction cycles and cleavage from the support gave 3.9 in 31% overall yield. Hydrogenation with palladium on carbon as catalyst yielded the deprotected hexasaccharide 3.10 (79%). The overall yield for the two-step process was 25%.
Scheme 4. Automated synthesis of dodecaglucosamine 3.10. Reagents and conditions:  

i (Thio-Fmoc module): a) NIS, TIOH, dioxane, DCM, -40 °C to -20 °C, 40 min; b) Piperidine, DMF.  

ii (Reduction module): AIBN (cat.), Bu$_3$SnH (10 eq.), Xylene, 90 °C.  

iii (Cleavage module): NaOMe, MeOH, DCM, 1.5 h, 31%.  

iv: Pd/C, H$_2$, MeOH, H$_2$O, THF, AcOH, 79% (solution phase).

It is noteworthy that the behavior of imidate 3.2 (Scheme 1) has been also initially evaluated during the synthesis of β-(1→4)-oligoglucosamines by Dr. C.-C. Wang and Dr. Lenz Kröck. (1) Using the modules described in section 3.4, β-(1→4)-glucosamine hexamer 3.10 could be assembled starting from building block 3.2. However, the desired product could only be isolated in 7% yield, while conspicuous amounts of deletion sequences (penta- and tetra-saccharides) were found in the final mixture. These findings suggested thioglycosides as better glycosylating agents for the synthesis glucosamine homopolymers, and strongly influenced our choice of building block 2.7 for the assembly of the β-(1→6)-oligoglucosamines introduced in this section.
3.6 Automated Synthesis of iGb3

Glycosphingolipid iGb3 is involved in stimulating the response of invariant natural killer T cells (NKT) during infection and malignancy (11, 12). This glycoconjugate is composed of a trisaccharide (glycan 3.11, Scheme 5) connected to a ceramide unit (aglycon). Besides its biological relevance, trisaccharide 3.11 served as an attractive target to illustrate the possibility of using the improved automated platform to perform the stereoselective installation of cis-glycosidic linkages, which are notoriously difficult linkages to achieve in a stereoselective fashion.

The automated solid phase synthesis of Globo H, (13) which contains an α-galactoside residue, was recently reported. Inspired by this work, galactose 2.4 carrying a non-participating benzyl ether at position C-2 was chosen as a building block for the installation of the difficult α-galactoside linkage in 3.12 (Scheme 5). In turn, building blocks 2.9 and 2.4, functionalized with participating groups at position C-2, were utilized to construct the beta linkage of the lactose fragment 3.13.

Installation of building blocks 2.9 and 2.3 proceeded smoothly under standard reaction conditions (Scheme 6). This was followed by glycosylation with building
block 2.4 in a mixture of ether and dioxane. Generally, ethereal solvents favor the formation of alpha isomers by reverse anomic effect. Finally, linker cleavage gave the partially protected trisaccharide 3.14 in 80% overall yield following reverse phase HPLC purification. Installation of the alpha 1→3 galactoside was almost completely stereoselective as judged by the HPLC chromatogram (Figure 4a). The anomeric configuration was confirmed by HSQC-NMR analysis of the final product 3.11 (Figure 4b), which was readily obtained by hydrogenation in 82% yield.

Scheme 6. Automated synthesis of 3.11. Reagents and conditions: 

- **i (Thio-Fmoc module):** NIS, TfOH, dioxane, DCM, -40 °C to -20 °C, 40 min; b) Piperidine, DMF.
- **i (ether module):** NIS, TfOH, dioxane, Et2O, -40 °C to -20 °C, 40 min. b) Piperidine, DMF.
- **ii (Cleavage module):** NaOMe, MeOH, DCM, 1.5 h, 80%
- **iii:** KOH, MeOH, THF, H2O, 60 °C, quant (solution phase).
- **iv:** Pd/C, H2, MeOH, H2O, cat. AcOH, 82% (solution phase).
Figure 4.  a) HPLC chromatogram of compound 3.14.  b) Proton coupled HSQC-NMR of compound 3.11.  Expansion of the anomeric region.
3.7 Automated Synthesis of Sialosides

Many naturally-occurring N- and O-glycans contain a terminal sialic acid residue. The synthesis of these compounds is particularly challenging because glycosylations with direct exploitation of Neu5Ac building blocks in solution phase synthesis often result in low yields and anomeric mixtures. As such, synthetic chemists often enlist the help of enzymes to overcome this problem.\(14, 15\) An automated oligosaccharide platform could be used as an alternative to overcome these problems by efficiently installing the sialic acid capping building blocks.

Reactions on solid phase can be driven to completion by using excess reagents. While mass action ensures high reaction yields, it can constitute a disadvantage when the reagent or building block to be used in excess is relatively expensive or difficult to prepare, as in the case of sialic acid. In this regard, compound \(2.2, (16)\) introduced in Chapter 2, was particularly appealing for use as a building block in automated solid phase synthesis since it can be obtained rapidly and in an efficient manner (six steps with 43% overall yield). Sialyl lactosamine \(3.16\) (Scheme 7) and sialyl lactose (Gm3) \(3.18\) (Scheme 8) are two important receptors for viral infections \(17, 18\) and were chosen as initial targets to monitor the reactivity of building block \(2.2\) on the solid phase.

The automated synthesis of compound \(3.16\) (Scheme 7) began with the glycosylation of resin \(3.1\) using building block \(2.8\) (2 x 5 eq.) in presence of \(N\)-iodosuccinamide and triflic acid. Following Fmoc cleavage, treatment with building block \(2.2\) (2 x 5 eq.) for one hour at -10 °C followed by reduction of the trichloroacetamide moiety and by methoxide mediated cleavage provided compound \(3.15\) in low yield together with a linker bound glucosamine. The latter was likely generated due to incomplete glycosylation upon treatment with building block \(2.2\). After optimization, the best results were obtained when imidate \(2.2\) was reacted for two hours at 0 °C. The corresponding trisaccharide \(3.15\) was
isolated in 33% overall yield with respect to resin loading. Standard hydrogenolysis afforded desired 3.16 in 78%.

Scheme 7. Automated synthesis of 3.16. Reagents and conditions: i (Thio-Fmoc module): a) NIS, TfOH, dioxane, DCM, -40 °C to -20 °C, 40 min; b) Piperidine, DMF. ii (Imidate-Fmoc module): a) TMSOTf, DCM, 0 °C, 2 h. b) Piperidine, DMF. iii (Reduction module): AIBN (cat.), Bu3SnH (10 eq.), Xylene, 90 °C. iv (Cleavage module): NaOMe, MeOH, DCM, 1.5 h, 33% v: Pd/C, H2, MeOH/H2O, cat. AcOH, 78% (solution phase).

Disaccharide building block 2.2 performed in an analogous way when glucose 2.9 served as a nucleophile during the synthesis of Gm3 3.18 (Scheme 8). In particular, a sequence of thioglycoside and imidate couplings followed by cleavage and saponification afforded protected 3.17 in 40% overall yield before hydrogenolysis gave 3.18 in 91% yield.
The branching nature of oligosaccharides is a characteristic feature when compared to oligopeptides and oligonucleotides.\(^{(18)}\) Regiocontrol for the installation of glycosidic linkages is exercised by the placement of orthogonal protective groups on the building blocks to allow for unmasking of a particular hydroxyl group on the sugar ring for glycoside formation at the respective position. One of the major concerns associated with the assembly of branched oligosaccharides is the steric hindrance of the branching sites, which can sensibly affect glycosylation yields. Working in a solid phase environment could, in principle, additionally reduce the accessibility of a sterically hindered nucleophile. We decided, therefore, to test the possibility of synthesizing branched structures on the solid support.

Sialyl Lewis\(^{X}\) hexasaccharide 2.69, which has been implicated in inflammation and cancer metastasis,\(^{(20)}\) was previously used to illustrate the synthesis of branched compounds in solution and could serve as a basis for comparison for the solid phase synthesis of Sialyl Lewis\(^{X}\) tetrasaccharide 3.20 (Scheme 9). The shorter sequence was chosen in order to exclusively focus on aspects related to the construction of the branching core of Sialyl Lewis\(^{X}\).
Glucosamine 2.6 containing C3-Lev and C4-Fmoc protecting groups, was first installed on resin 3.1. This was followed by unveiling of the C4 position by the addition of piperidine to cleave the Fmoc protecting group. Glycosylation using building block 2.2 followed by removal of the levulinate ester by treatment with hydrazine hydrate and acetic acid, exposed the second nucleophilic site on the central glucosamine. Glycosylation with 2.5 resulted in the desired, branched, partially protected tetrascarcase 3.19 with an overall yield of 51% after cleavage from the resin, ester saponification and HPLC purification.


It is worth mentioning that when thiophenyl fucoside 2.57 was used instead of 2.5, complex product mixtures were obtained as confirmed by LCMS analysis. In particular, deletion sequences were diagnostic of inefficient fucosylation. During fucosylation with building block 2.5, the use of dichloromethane as a standard solvent for Imidate-Fmoc modules, resulted in the formation of mixture of anomers of compounds 3.19 as confirmed by NMR analysis (Figure 5b). Only
permanently the reaction in ether, a strong α-directing solvent, ensured stereoselective introduction of the fucose residue (Figure 5a). Finally, a solution phase hydrogenation produced tetrasaccharide 3.20.

Figure 5. Expansion of the anomeric region in the $^{13}$C NMR of tetrasaccharide 3.19
Fucosylation was performed in: a) ether; b) dichloromethane.

Even taking into account the differences, it is possible to identify, for both the synthesis of hexasaccharide 2.69 and tetrasaccharide 3.20, a common strategy built on a core set of transformations. Key steps in both cases were the glycosylation of the branching glucosamine by a sialic acid-galactose disaccharide building block, followed by fucosylation and global deprotection. The synthesis of tetrasaccharide 3.20 could be completed in nine steps with an overall yield of 15%. Interestingly, Sialyl Lewis$^x$ hexasaccharide 2.69 was
obtained in the same number of steps and a similar overall yield (17%). Based on these results, it is possible to conclude that the solid phase approach introduced in this chapter is at least comparable to solution phase methodologies on the level of synthetic efficiency. All things being equal, the solid phase approach remained superior in terms of time efficiency, since, starting from the same number of building blocks, the synthesis of 3.20 could be completed within days, while the solution phase assembly of 2.69 required weeks.

The last two general considerations can be made since even synthesis of high molecular weight compounds like dodecasaccharide 3.8 could be completed with high yields (23% over 26 steps) in relatively short time. These features confirm the potential of an automated solid phase approach for the synthesis of oligosaccharides.

3.8 Oligosaccharide Based Molecular Probes

Synthetic oligosaccharides have found application in different screening platforms as well as in the formation of protein conjugates for antibody production or vaccine development. A key advancement with the novel automated platform described herein is that by design, all glycans released at the end of a synthesis can be immediately conjugated via the amino group on the C5-linker that is incorporated at the reducing terminus. In this way, access to glycan microarrays and glycoconjugates is simplified.

To corroborate our statement, a glycan microarray containing a selection of synthetic structures produced by automation was printed using a piezoelectric robotic printer. Figure 5 shows the results that were obtained when a slide containing equimolar concentrations of compounds 3.4, 3.6 and 3.10, was incubated with an Alexa Fluor (594) labeled wheat germ agglutinin (WGA), a lectin specific for glucosamine residues in oligosaccharide chains. Microarray
platforms are generally utilized to characterize binding events by extrapolating information on specificity and concentration dependence.\(^{(22)}\) In our experiment the interaction proved to be specific, as no binding was detected with galactose (Figure 6, line a) or linker controls (line b). Furthermore, a clear concentration profile was deducible. Additionally, monoglucosamine (line c) bound less intensively than hexaglucosamines 1,6 and 1,4. The last observation seems to suggest that WGA is not specific for terminal glucosamines residues, but can probably bind simultaneously, in a multivalent fashion, to one or more sugar residues organized in oligomers.

The results obtained clearly demonstrate the applicability of the new automated platform to the preparation of carbohydrate microarrays.

**Figure 6.** Glycan microarray incubated with Alexa Fluor (594) labeled WGA. Every row in the array contains a double replica (in a gradient of three different concentrations) of the compounds indicated to the right of the slide.

A second family of tools for glycobiology can be obtained by linking the synthetic glycans to proteins, forming in this way “neoglycoconjugates”.\(^{(23)}\) Such conjugates are important for the production of anti-glycan antibodies and are the
basic constituents for the development of conjugate vaccines.\(^{(23)}\) To illustrate this approach, oligosaccharide \(3.10\) was conjugated to bovine serum albumin (BSA) using the squarate ester method (Scheme 10).\(^{(24)}\) Compound \(3.10\) was treated with two equivalents of diethyl squarate in aqueous buffer to afford compound \(3.21\) after reverse phase HPLC purification. Reaction of \(3.21\) with bovinum serum albumin (BSA) in basic buffer provided glyconjugate \(3.22\). Gel electrophoresis and MALDI analysis revealed a shift of twenty KDa in the molecular weight of the protein consistent with an average percentage of loading of ten antigens per protein molecule (Figure 7).

**Scheme 10.** Synthesis of a BSA conjugate \(3.22\). \(i:\) EtOH, Phosphate Buffer (50 mM), pH 7.2, rt. \(ii:\) 0.1 M NaHCO\(_3\) Buffer, pH 9.0, rt.
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3.9 Conclusions

In summary, a general strategy for the automated solid-phase synthesis of oligosaccharides based on a novel linker and several of the building blocks mentioned in Chapter 2 was realized. The new fully automated platform accommodates most of the commonly used methods for glycoside formation, and allows for the removal of temporary protective groups in situ. Since all stereochemical and regiochemical information is encoded in the building blocks and tuned with solvent conditions, the repetitive elongation process can be executed by the instrument once reaction parameters have been introduced by the operator. Purification of the synthetic oligosaccharides produced in

Figure 7. a) MALDI analysis of compound 3.22. b) Electrophoresis of glycoconjugate 3.22: Left, molecular weight markers; center, BSA control; right, 3.22.
automation required establishing general protocols for HPLC analysis, and for the separation of complex oligosaccharide mixtures. Following automated assembly, hydrogenolysis was the only solution phase chemical transformation used as a mean of global deprotection. This greatly accelerated and simplified our approach for accessing in an efficient manner a diverse family of glycans. By design, our synthetic strategy incorporates an amino spacer at the reducing terminus which can be used for the construction of molecular tools for glycobiology. In this regard, conjugation of the synthetic glycans to the surface of glycan microarrays, as well as to carrier proteins, was also demonstrated using a set of representative oligosaccharide structures.

3.10 Experimental Procedures.

**General Materials and Methods.** All chemicals used were reagent grade and used as supplied except where noted. All reactions were performed in oven-dried glassware under an inert atmosphere unless noted otherwise. Reagent grade N,N-dimethylformamide (DMF) was dried over activated molecular sieves prior to use. Pyridine, triethylamine (NEt₃) and acetonitrile (MeCN) were distilled over CaH₂ prior to use. Dichloromethane (CH₂Cl₂), toluene and tetrahydrofuran (THF) were purified by a Cycle-Tainer Solvent Delivery System unless noted otherwise. All solvents used on the automated synthesizer are extra dry grade without molecular sieves, purchased from Acros in sure seal bottles except DCM and THF, which are dried using a dry still. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution. Flash column chromatography was carried out using
forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). Purification by size exclusion recycling HPLC was carried out using JAI LC 9101 equipped with JAI GEL-1H and 2-H column in a series (CHCl₃). Purification by reverse phase HPLC was performed using Agilent 1200 series equipped with a Macherey-Nagel Nucleodur Pyramid C-18 column (length 250 mm, 40 mm i.d., flow 10 mL/min) unless noted otherwise.¹H, ¹³C spectra were recorded on a Varian Mercury 300 (300 MHz), Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), Bruker ECX (400 MHz), Bruker DRX500 (500 MHz), or, Bruker DRX700 (700 MHz) spectrometer in CDCl₃ with chemical shifts referenced to solvent signals (CDCl₃: 7.26 ppm ¹H, 77.0 ppm ¹³C) unless otherwise stated. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for ¹H NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. High resolution mass spectral (HRMS) analyses were performed by the MS-service at the Laboratory for Organic Chemistry (LOC) at ETH Zürich and the MS-service at Department of Organic Chemistry at Free University Berlin. High-resolution MALDI and ESI mass spectra were run on an IonSpec Ultra instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

**Automated Oligosaccharide Synthesis Instrument**

The instrument consists of a syringe pump driven part (utilized for the delivery of washing solvents to the reaction vessel) and a solenoid valve driven part (utilized for the delivery of reagents and building blocks to the reaction vessel). A double-jacketed glass reaction vessel, a cryostat, reagent, solvent and waste vessels, a fraction collector and an argon manifold complete the parts list. Details on the set up of the instrument have been given elsewhere.¹(1,2)

Solvents and stock solutions utilized during the syntheses are:
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**TMSOTf**: TMSOTf (364 μl) in DCM (40 mL)

**Piperidine**: 20% in DMF (v/v)

**Hydrazine**: hydrazine monohydrate (680 μl, 0.56 M) in pyridine (15 mL) and HOAc (10 mL).

**NaOMe**: 0.25 M solution of NaOMe in MeOH.

**Bu₃SnH**: Bu₃SnH (135 μl) and AIBN (4 mg) in xylene (1.9 mL degassed)

**NIS**: N-Iodosuccinimide (1.48 g, 6.66 mmol) and TfOH (60 μl, 0.66 mmol) in DCM (20 mL) and dioxane (20 mL).

**Programming and Operation**

The programming language of the terminal program uses commands that are grouped into modules.

**Modules**

The combination of commands to modules allows for operations such as washes or glycosylations or deprotections.

**Module “Wash”**

Washing with a solvent involves the following steps: delivery of the solvent to the reaction vessel, mixing and delivery of the soiled washing solvent to waste. Delivery of solvents is pressure driven and takes place by opening a solenoid valve at the reaction vessel top connected to the exhaust. The exhaust has to be opened to prevent overpressure in the reaction vessel. Mixing takes place by simultaneous opening of an argon line at the bottom of the reaction vessel and
the exhaust line on the top of the vessel. Thereby a flow of argon passes via the porous glass filter through the reaction or washing solution. Mixing by gas flow avoids mechanical shaking. Waste removal is achieved by opening an argon line on the top of the vessel to pressurize; upon pressurizing the vessel, a valve at the bottom of the vessel is opened. The argon pressure expels the solution from the vessel via the glass filter that retains the resin in the vessel. The expelled solutions can be directed to waste or to a fraction collector by selecting the appropriate valve either leading to the waste container or to the fraction collector.

**Reaction Modules**

Glycosylation, deprotection and linker cleavage are performed automatically. The corresponding modules require the combination of syringe pump reagent delivery, solenoid mixing and delivery to waste as well as cryostat temperature control. Delivery of a reagent is preceded by setting the appropriate cryostat temperature and priming of the loops utilized in the reagent delivery with dichloroethane. Once the cryostat has reached the appropriate temperature the reagent is taken up into a Teflon loop. If the reagent is a building block it is directly delivered via the respective reaction vessel line to the reaction vessel. For stock solutions the loop is first primed with the respective dispensable solution, a second aliquot of stock solution is then taken up into the loop and delivered to the reaction vessel. Upon delivery the reaction vessel line is emptied and the respective loop is primed. Once the reaction time is exceeded the reaction mixture is delivered to waste or to fraction collector via the respective outlet. Finally, the reaction vessel lines are cleaned by priming with solvent from the loop.

For general glycosylations: "Glycosylation (type of leaving group, number of building block equivalents used, number of activator equivalents used, reaction time, temperature, send to (W)aste or (F)raction collector)" e.g.
"Glycosylation(Imidate, 5 eq bb X, 0.5 eq TMSOTf, 60 min., -10 °C, F)". For thioglycoside glycosylations two reaction temperatures and times are indicated as the reagent addition generally takes place at -40 °C, after 5 minutes the temperature is raised to -20 °C and maintained there for further 40 minutes: "Glycosylation(Thio, 5 eq X, 5 eq NIS, 0.5 eq TfOH, 10 min., -40 °C, 40 min., -20 °C, W)" For deprotections: "Deprotection(temporary protecting group cleaved, reaction time, reaction temperature, send to (W)aste or (F)raction collector)" e.g. Deprotection(Fmoc, 3x, 5 min., 25 °C, F). For linker cleavage "Cleavage(linker, 1x, 25 °C, F)".

**Temperature Module**

Resetting the temperature after a glycosylation generally is preceded by washing steps at low temperature, which are represented by separate modules. A temperature module is therefore required to reset the temperature. This module sets a temperature (i.e. a set temperature is sent to the cryostat) and then the program monitors the actual cryostat temperature (i.e. the temperature of the cryostat cooling fluid) until a preset threshold temperature is exceeded (PTEMPHIGH command), or under-run (PTEMPLOW command). The resulting intermission can take place either in the presence ("Temperature(25 °C, 24 °C, DCM)") or absence ("Temperature(25 °C, 20 °C, none)") of solvents in the reaction vessel. The first temperature indicates the temperature the chiller is set to, while the second temperature indicates the temperature the controller waits for before continuing with the program execution.

**Combination of Modules**

The combination of the wash and reaction modules results in a coupling cycle. A detailed description of the sequence of modules that are used can be found in the following.


**Module Sequences**

**Glycosyl Imidate Double coupling - Fmoc deprotection**

Temperature (25 °C, 20 °C, none)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hexane, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Glycosylation (Imidate, 5 eq BB X, 0.5 eq TMSOTf, 60 min., -10 °C, F)

add DCM (2 mL), set temperature(-10 °C).

Prime Loop 3 and empty reaction vessel Line 3, wait till set temperature is reached, DCM to waste, delivery of bb in DCM (0.75 mL), emty rv line 3, prime loop 3, push back bb to storage vessel.

Prime Loop 6 and Empty RV Line 6, delivery of TMSOTf (0.25 mL) stock solution empty rv line 6, prime loop 6, push back TMSOTf to storage vessel.

Glycosylation reaction (60 min.), while continuously purging with high Ar flow; collect glycosylation mix to fraction collector, clean RV lines 3 and 6 and prime loops 3 and 6.

Wash(DCM, 6x, -10 °C, F)

Glycosylation(Imidate, 5 eq BB X, 0.5 eq TMSOTf, 60 min., -10 °C, F)

Wash (DCM, 6x, -10 °C, F)
Temperature (25 °C, 24 °C, DCM)

Wash (THF, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DMF, 3x, 25 °C, W)

Deprotection (Fmoc, 3x, 5 min., 25 °C, F)

Add DMF, Prime Loop 4 and Empty RV Line 4, repeat 3x: delivery of piperidine stock solution, deprotection reaction 5 min, while continuously purging with high Ar flow; collect deprotection mix to fraction collector, empty and clean RV line 4, prime loop 4, push back.

Wash (DMF, 3x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (HOAc, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hexane, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

**Thioglycoside Double Coupling - Fmoc Deprotection**

Temperature (25 °C, 20 °C, none)

Wash (THF, 6x, 25 °C, W)
Wash (DCM/hexane, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Glycosylation (Thio, 5 eq X, 5 eq NIS, 0.5 eq TfOH, 10 min., -40 °C, 40 min., -20 °C, W)

Add DCM (2 mL), set temperature(-40 °C). Prime Loop 3 and Empty RV Line 3,

Wait till set temperature is reached, DCM to waste, delivery of bb in DCM (1 mL),
empty rv line 3, prime loop 3, push back bb to storage vessel. Prime Loop 6 and
Empty RV Line 6, delivery of NIS stock solution (0.75 mL) (incl prior, afterwards).
Empty rv line 6, prime loop 6, push back NIS to storage vessel. Glycosylation
reaction 5 min at -40 °C followed by 40 min at -20 °C , while continously purging
with high Ar flow. Clean RV lines 3 and 6 and prime loops 3 an 6.

Wash (THF, 6x, -20 °C, W)

Wash (DCM, 6x, -20 °C, W)

Glycosylation (Thio, 5 eq X, 5 eq NIS, 0.5 eq TfOH, 10 min., -40 °C, 40 min., -20 °C, W)

Wash (THF, 6x, -20 °C, W)

Wash (DCM, 6x, -20 °C, W)

Temperature (25 °C, 24 °C, DCM)

Wash (THF, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (DMF, 3x, 25 °C, F)
Deprotection (Fmoc, 3x, 5 min., 25 °C, F)

Wash (DMF, 3x, 25 °C, F)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (HOAc, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

Wash (THF, 6x, 25 °C, W)

Wash (DCM/hex, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

**TCA-Reduction 1 cycle (treatment with 10 eq Bu₃SnH and cat. AIBN)**

Temperature (25 °C, 20 °C, dry)

Wash (THF, 6x, 25 °C, W)

Deprotection (TCA, 1x, 120 min., 90 °C, W)

Prime Loop 5 and Empty RV Line 5, deliver Bu₃SnH, AIBN stock solution (2 mL), empty rv line 5, prime loop 5, push back Bu₃SnH, AIBN, degass at 25 °C for 9 min. by high Ar flow. Set temperature 90 °C, deprotection reaction for 2 h while purging with Ar every 2 min for 5 s.

Wash (DCM, 6x, 25 °C, W)

Wash (DCM/hex, 6x, 25 °C, W)
Wash (DCM, 6x, 25 °C, W)

Wash (DCM/MeOH, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, W)

**Linker Cleavage by Treatment With 10 eq NaOMe**

Wash (DCM, 6x, 25 °C, W)

Wash (DCM, 6x, 25 °C, F)

Idle till depaused

Cleavage (linker, 1x, 25 °C, F), Prime Loop 4 and Empty RV Line 4. Deliver DCM (3 mL) to reaction vessel, deliver NaOMe stock solution (1 mL), empty rv line 4, prime loop 4, push back stock NaOMe solution to storage vessel, cleavage reaction 1.5 h, collect mix to fraction collector. Wash resin with 2 x DCM : MeOH (2:1), 5 x DCM, collect all washes to fraction collector clean NaOMe delivery line.

**Synthetic Procedures**

**Synthesis of Linker 3.1.** Synthesis of the linker (1, 2) started from acid SI-1 that was converted to hydroxymethylated acid SI-2 by chloromethylation and hydrolysis (Scheme 11). Methyl ester SI-5 was synthesized by treatment of SI-2 with diazomethane, followed by carbamate formation with 5-(benzylamino)penten-1-ol SI-4. The ester of resulting linker construct SI-5 was hydrolyzed with aqueous potassium hydroxide. Acid SI-6 was installed on solid support by reaction of the corresponding cesium-salt with Merrifield resin. The resin was capped with excess acetic acid cesium-salt under the same conditions.
Subsequently the loading of resin 3.1 was determined by reaction with Fmoc-chloride, washing and Fmoc cleavage. Loading was determined by UV-quantification of the cleavage mixture.

**Scheme 11. Synthesis of linker 3.1**

**Methyl 3-(4-(hydroxymethyl)phenyl)propanoate SI-3.** *Caution:* The generation and handling of diazomethane requires special precautions. A solution of 3-(4-(hydroxymethyl)phenyl)propionic acid SI-2 (11.7 g, 65 mmol) (25) in THF (150 mL) was treated at room temperature with a solution of diazomethane in diethyl ether until a strong yellow color persisted. Then, acetic acid was added dropwise until the solution turned colorless. The solution was diluted with ethyl acetate, and washed with saturated aqueous NaHCO₃ solution and brine. The aqueous layers were sequentially re-extracted once with ethyl acetate. The combined organic phases were dried over MgSO₄, filtered and concentrated. Flash column chromatography on silica gel (cyclohexane/EtOAc = 7:3) afforded SI-3 (11.1 g, 88%). ¹H NMR (CDCl₃, 300 MHz) δ 7.29-7.26 (m, 2H), 7.20-7.17 (m, 2H), 4.64 (d,
\( J = 5.9 \text{ Hz}, 2H \), 3.66 (s, 3H), 2.94 (t, \( J = 7.8 \text{ Hz}, 2H \)), 2.62 (t, \( J = 7.8 \text{ Hz}, 2H \)), 1.93 (t, \( J = 5.9 \text{ Hz}, 1H \)); \(^{13}\text{C} \) NMR (CDCl\(_3\), 75 MHz) \( \delta \) 173.2, 139.8, 138.8, 128.4, 127.2, 65.1, 51.7, 35.8, 30.7; EI-HRMS: \( m/z \) calcd for C\(_{11}\)H\(_{14}\)O\(_3\) [M\(^{+}\)] 194.0938, found 194.0937. IR (thin film) \( \nu \) = 3607, 3446, 3008, 2953, 2872, 1732, 1514, 1438, 1366, 1292, 1036, 1003 cm\(^{-1}\).

**Methyl 3-(4-((benzyl(5-hydroxypentyl)-carbamoyloxy)methyl)phenyl)propanoate SI-5.** A solution of SI-3 (8.0 g, 41 mmol) in acetonitrile (210 mL) was treated at room temperature with triethylamine (17.4 mL, 124 mmol) and cooled to 0 °C. At 0 °C, \( N, N' \)-disuccinimidyl carbonate (DSC) (15.9 g, 62 mmol) was added. The solution was warmed to 18° C over 1 h, diluted with ethyl acetate (800 mL), and washed with saturated aqueous NaHCO\(_3\) solution. The aqueous layer was re-extracted once with ethyl acetate. The combined organic phases were dried over MgSO\(_4\), filtered and concentrated in vacuo to afford the mixed carbonate that was used without further purification. A solution of the crude carbonate in CH\(_2\)Cl\(_2\) (82 mL) was added at 0 °C to a solution of SI-4 (16.0 g, 83 mmol) (S3) and triethylamine (14.4 mL, 103 mmol) in CH\(_2\)Cl\(_2\) (210 mL). The solution was stirred for 50 min at room temperature, diluted with CH\(_2\)Cl\(_2\) and washed with saturated aqueous NaHCO\(_3\) solution. The aqueous layer was re-extracted twice with CH\(_2\)Cl\(_2\). The combined organic phases were washed with brine, dried over MgSO\(_4\), filtered and concentrated. Flash column chromatography on silica gel (cyclohexane/EtOAc = 2:1 to 1:1) afforded SI-5 (15.1 g, 88%) as a colorless oil. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 7.38-7.09 (m, 9H), 5.13 (s, 2H), 4.49 (s, 2H), 3.67 (s, 3H), 3.67-3.46 (m, 2H), 3.34-3.13 (m, 2H), 2.95 (s, 2H), 2.63 (s, 2H), 1.64-1.40 (m, 4H), 1.40-1.19 (m, 2H); \(^{13}\text{C} \) NMR (CDCl\(_3\), 75 MHz) \( \delta \) 173.2, 156.7, 156.2, 140.2, 137.8, 134.7, 134.6, 128.4, 128.3, 128.1, 127.7, 127.2, 66.9, 62.5, 51.6, 50.4, 50.1, 46.9, 46.0, 35.5, 32.2, 30.5, 27.8, 27.3, 22.8; MALDI-HRMS: \( m/z \) calcd for C\(_{24}\)H\(_{31}\)NO\(_5\)Na [M+Na\(^{+}\)] 436.2094, found 436.2097. IR (thin film) \( \nu \) = 3610, 3467, 3008, 2938, 1732, 1690, 1468, 1438, 1364, 1303, 1072 cm\(^{-1}\).
3-(4-((Benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propionic acid SI-6. SI-5 (3 g) was dissolved in THF (30 mL), aqueous KOH (1 M, 30 mL) was added and the solution was stirred for 13 h. The pH was adjusted to 7 by addition of acidic Amberlite IR-120. The Amberlite was filtered off and rinsed with THF/H₂O (1:1). The filtrate was concentrated, the residue was coevaporated with toluene purified by flash column chromatography (CH₂Cl₂/MeOH = 99:1 to 9:1 with 1% HOAc) and dried under high vacuum to afford the SI-6 (3 g, quant.) as a colorless oil. ¹H NMR (CDCl₃, 300 MHz) δ 7.38-7.10 (m, 9H), 5.21 (s, 2H), 4.56 (s, 2H), 3.68-3.52 (m, 2H), 3.3-3.15 (m, 2H), 3.03 (s, 2H), 2.74 (s, 2H), 1.70-1.15 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 177.5, 177.1, 156.8, 156.4, 140.2, 137.8, 134.8, 128.6, 128.55, 128.4, 128.2, 127.8, 127.4, 127.2, 67.1, 62.6, 50.6, 50.2, 46.9, 46.2, 30.4, 27.4, 22.8; MALDI-HRMS: m/z calcd for C₉₅H₆₅NO₅Na [M+Na⁺] 422.1938, found: 422.1940. IR (thin film) ν = 3685, 3602, 3436, 3008, 2936, 1690, 1602, 1515, 1474, 1453, 1426, 1364, 1248, 1127, 1074, 1042, 908, 816 cm⁻¹.

3-(4-((Benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propionic ester resin 3.1.

**Functionalization.** Merrifield resin (3g, 1.14 mmol, copolymerized, Polymer Labs, 0.38 mmol/g free sites) was swollen in CH₂Cl₂ (30 mL). Acid SI-6 (690 mg, 1.71 mmol) was dissolved in EtOH (6 mL) and H₂O (1.5 mL) before aqueous Cs₂CO₃ (0.85 mL, 1 M) was added to adjust to pH 7. The solution was concentrated and coevaporated three times with dioxane. The resin was drained and washed twice with DMF (10 mL). The Cs-salt of the acid was dissolved in DMF (10 mL) and added to the resin followed by a catalytic amount of sodium iodide. The resulting mixture was heated to 50 °C for 13 h. Upon cooling to room temperature, the resin was washed consecutively three times each with DMF, H₂O/DMF (1:1), DMF, DCM, MeOH, DCM. This procedure was repeated twice.
Capping of Unreacted Sites. The functionalized resin was preswollen in CH$_2$Cl$_2$ (30 mL). Acetic acid (652 µL, 11.4 mmol) was dissolved in EtOH (6 mL) and H$_2$O (1.5 mL) before aqueous Cs$_2$CO$_3$ (5.7 mL, 1 M) was added to adjust to pH 7. The solution was concentrated and coevaporated three times with dioxane. The resin was drained and washed twice with DMF (10 mL). The Cs-salt of the acid was dissolved in DMF (17 mL) and added to the resin followed by a catalytic amount of sodium iodide. The resulting mixture was heated to 50°C for 24 h. Upon cooling to room temperature the resin was washed consecutively three times each with DMF, H$_2$O/DMF (1:1), DMF, DCM, MeOH, DCM. The resin was dried under high vacuum.

Loading Determination. An aliquot of functionalized and capped resin (29.8 mg) was swollen in CH$_2$Cl$_2$ (1 mL) for 1 h. Pyridine (100 µL) and fluorenylmethoxycarbonyl chloride (100 mg) were added and the mixture was shaken overnight. The resin was drained, washed with CH$_2$Cl$_2$ and MeOH (six alternating washes), swollen in CH$_2$Cl$_2$, drained and dried. A solution of piperidine in DMF (20%, 2 mL) was added and the resin was shaken for 4 h. The solution was drained and a 100 µL aliquot was taken. This aliquot was diluted to 10 mL and the UV absorption at 301 nm was determined: A$_{301}$ = 0.206. The loading was calculated according to the following formula, using a reported value for the extinction coefficient of the dibenzofulvene-piperidine adduct (27):

$$\text{Loading} \left[ \text{mmol} \text{ g}^{-1} \right] = \frac{\text{Absorption} \times \text{Dilution} \times \text{Volume} \times 1000}{\text{Extinction Coefficient} \times \text{Weight resin}} = \frac{0.206 \times 100 \times 0.002 \times 1000}{7800 \times 0.0298} = 0.177 \text{ mmol g}^{-1}$$

Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl 2-acetamido-2-deoxy-3,6-dibenzyl-ß-D-glucopyranoside 3.3
The following module sequences were performed on 25 μmol scale with respect to resin 3.1

1) Glycosyl Imidate Double coupling - Fmoc deprotection using building block 3.2

2) TCA-reduction one cycle (treatment with 10 eq Bu₃SnH and cat. AIBN)

3) Linker cleavage (treatment with 10 eq NaOMe)

The eluted solution was neutralized with acidic Amberlite IR-120 and concentrated under vacuum. The crude residue (16.9 mg, 85%) was purified by preparative HPLC over 40 min using a gradient of 60 to 95% of acetonitrile (0.1% TFA) in water (0.05% TFA) to yield 3.3 (7 mg, 35% yield based on resin). [α]D²⁵ - 9.0 (c 0.75, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.60-7.08 (m, 19H), 6.12-5.05 (m, 1H), 5.18-5.06 (m, 2H), 4.88-4.41 (m, 7H), 4.06-3.10 (m, 13H), 3.01-2.86 (m, 2H), 2.72-2.57 (m, 2H), 2.90-1.73 (m, 3H), 1.68-1.10 (m, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 140.3, 138.6, 137.9,137.8, 128.5, 128.6, 128.4, 128.13, 128.1, 127.9, 127.8, 127.3, 127.2, 100.0, 80.8, 76.6, 74.3, 73.7, 73.6, 70.8, 69.5, 67.0, 57.3, 51.7, 50.3, 35.6, 30.6, 28.8, 27.3, 23.5, 23.4; MALDI-HRMS: m/z calcd for [M+Na⁺], 819.3827 found 819.3811. IR (thin film) ν = 3458, 3007, 2940, 2868, 1731, 1683, 1517, 1473, 1453, 1437, 1366, 1248, 1073, 986, 906 cm⁻¹.
**Figure 7.** LC-MS of the crude monogucosamine 3.3. Conditions: 40 to 100% A in B, A = CH$_3$CN + 20% iso-propanol + 0.1% TFA, B = water + 20% iso-propanol + 0.1% TFA from 2 to 32 min.

**Aminopentyl-2-acetamido-2-deoxy-β-D-glucopyranoside 3.4**

![Image of 3.4](image)

To a solution of 3.3 (17.0 mg, 23.9 µmol) in THF/MeOH/water/AcOH (2 mL/1 mL/1 mL/0.05 mL) was added 10% Pd/C (20 mg). The mixture was stirred under an atmosphere of hydrogen for 66 h and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated and the residue was purified by solid phase extraction (Sep-pak C-18, water) to give 3.4 (6.8 mg, 93%) The purity of 3.4 was assessed by analytical HPLC using a 0 to 10% gradient (acetonitrile in water, Pyramid nucleodur C-18 column, SI Figure 6). $^1$H NMR (400 MHz, D$_2$O) δ 4.45 (d, J = 8.4 Hz, 1H), 3.94-3.80 (m, 2H), 3.74-3.44 (m, 4H), 3.44-3.33 (m, 2H), 3.00-2.88 (m, 2H), 1.98 (s, 3H), 1.70-1.48 (m, 4H), 1.34 (m, 2H); $^{13}$C NMR (100 MHz, D$_2$O) δ 174.5, 101.2, 75.9, 73.9, 70.1, 70.0, 60.8, 55.7, 39.4, 28.1, 26.5, 23.3, 22.20, 22.16; ESI-HRMS: m/z calcd for C$_{13}$H$_{26}$N$_{2}$O$_{6}$Na [M+Na$^+$], 329.1683 found 329.1679.

**Figure 8.** LC-MS of 3.4. Conditions: 0 to 10% A in B, A = CH$_3$CN, B = water, over 20 min.
Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl [2-acetamido-2-deoxy-3,4-dibenzyl-β-D-glucopyranoside(1→6)]ε-2-acetamido-2-deoxy-3,4-di-O-benzyl-β-D-glucopyranoside 3.5

![Chemical Structure](image)

The following modules were performed on 25 µmol scale based on resin 1.

1) Six cycles Thioglycoside Double Coupling - Fmoc deprotection using building block 2.7

2) Six cycles TCA-reduction (treatment with 10 eq Bu3SnH and cat. AIBN)

3) Linker Cleavage (treatment with 10 eq NaOMe)

The eluate was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. The crude residue was purified by preparative HPLC using a gradient of 60 to 70% over 40 min of MeCN/i-PrOH (4:1) in water/i-PrOH (4:1) to yield 3.5 (39.4 mg, 58% overall yield from resin). [α]D26 -33.4 (c 1.0, CHCl3); 1H NMR (600 MHz, CDCl3) δ 8.93 (s, 2H), 8.73 (s, 1H), 8.45 (s, 1H), 8.34-8.14 (m, 1H), 7.35-6.79 (m, 69H), 6.78-6.62 (m, 2H), 5.65-2.93 (m, 73H), 2.93-2.82 (m, 2H), 2.63-2.49 (m, 2H), 2.40-1.86 (m, 18H), 1.66-0.98 (m, 10H); 13C NMR (150 MHz, CDCl3) δ 173.2, 172.9, 171.5, 171.4, 171.2, 170.5, 159.9, 159.6, 156.8, 156.2, 140.2, 139.0, 138.8, 138.4, 138.1, 138.0, 137.5, 135.0, 134.6, 129.5, 129.2, 128.6, 128.3, 128.2, 128.1, 128.0, 127.7, 127.5, 127.3, 127.1, 126.9, 126.6, 103.3, 102.6, 102.0, 100.2, 85.2, 83.4, 83.2, 82.3, 81.8, 79.9, 76.5, 76.1, 75.6, 74.8, 74.4, 72.6, 72.1, 66.7, 56.8, 56.1, 51.6, 50.3, 47.6, 46.6, 35.6, 33.9, 32.0, 30.6, 29.70, 29.67, 29.63, 29.5, 29.4, 29.2, 29.0, 28.7, 28.2, 27.7, 23.6,
23.5, 23.3, 23.2, 22.7, 22.3, 14.1; HR-MALDI-MS: m/z [M+1+Na+] calcd for C_{156}H_{181}N_{7}O_{35}Na: 2736.2524, found: 2736.259. IR (thin film) \nu = 3281, 1654, 1556, 1071, 699 \text{ cm}^{-1}.

**Figure 9.** LC-MS of crude (1\(\rightarrow\)6)-\(\beta\)-\(N\)-acetyl-hexaglucosamine 3.5 synthesized from thioglycoside 2.7. Conditions: 60 to 80% A in B, A = CH_3CN + 20% iso-propanol + 0.1% TFA, B = water + 20% iso-propanol + 0.1% TFA from 2 to 32 min.

**Aminopentyl (acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)6)- (acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)6)-(acetamido-2-deoxy-2-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)6)-acetamido-2-deoxy-2-\(\beta\)-D-glucopyranoside 3.6**

![Image of chemical structure]

To a solution of 3.5 (15 mg, 5.5 \(\mu\)mol) in THF/MeOH/water/AcOH (2 mL/ 1 mL/1 mL/0.05 mL) was added 10\% Pd/C (20 mg). The mixture was stirred under a hydrogen atmosphere for 36 h and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated, and the residue was purified by size exclusion chromatography (Superdex, 5\% EtOH in water) and solid phase extraction (Sep-Pak 1g cartridge, C-18, water) to give 3.6 (4.2 mg, 58%).
purity of the compound was assessed by analytical HPLC using a 0 to 10% gradient (acetonitrile in water, Pyramid nucleodur C-18 column) as shown in SI Figure 11. $^1$H NMR (600 MHz, D$_2$O) $\delta$ 4.56-4.39 (m, 6H), 4.21-4.08 (m, 5H), 3.93-3.85 (m, 1H), 3.84-3.78 (m, 1H), 3.77-3.62 (m, 11H), 3.61-3.45 (m, 13H), 3.44-3.29 (m, 7H), 2.97-2.91 (m, 2H), 2.03-1.99 (m, 12H), 1.99 (s, 3H), 1.97 (s, 3H), 1.62 (m, 2H), 1.58-1.50 (m, 2H), 1.36 (m, 2H); $^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 174.48, 174.45, 101.61, 101.57, 101.1, 75.9, 74.6, 74.51, 74.48, 74.47, 74.41, 73.9, 73.7, 69.99, 69.97, 69.91, 69.86, 68.6, 68.5, 68.3, 60.7, 55.6, 55.5, 55.4, 39.4, 28.0, 26.4, 22.30, 22.28, 22.25, 22.14, 22.13; HR-MALDI-MS: $m/z$ [M+H$^+$] calcd for C$_{53}$H$_{91}$N$_7$O$_{31}$ 1322.5832, found 1322.584.

Figure 10. LC-MS of unprotected 1,6-hexaglucosamine 15. Conditions: 0 to 10% A in B, A = CH$_3$CN, B = water, over 20 min.

Automated Synthesis of Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl [2-acetamido-2-deoxy-3,4-dibenzyl-$\beta$-D-glucopyranoside(1→6)]$_{11}$-2-acetamido-2-deoxy-3,4-dibenzyl-$\beta$-D-glucopyranoside 3.7
The following modules were performed on 25 μmol scale based on resin 3.1.

1) Twelve cycles Thioglycoside double coupling - Fmoc deprotection using building block 2.7

2) Sixteen cycles TCA-reduction (treatment with 20 eq Bu₃SnH and cat. AIBN)

3) Cleavage of linker (treatment with 10 eq NaOMe)

The eluate solution was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. The crude residue was purified by preparative HPLC using a 0 to 10% gradient (MeOH in DCM, YMC-pack silica column) over 35 min to afford 3.7 (54.4 mg, 43% overall yield from resin). ¹H NMR (700 MHz, CDCl₃) δ 7.48-6.67 (m, 129H), 5.18-4.65 (m, 49H), 4.55 (s, 11H), 4.30 (s, 19H), 4.06 (s, 19H), 3.84 (s, 21H), 3.66 (s, 12H), 3.24 (s, 33H), 2.94 (t, J = 7.7 Hz, 4H), 2.62 (t, 4H), 2.51-1.97 (m, 36H), 1.58 (s, 3H), 1.38-1.09 (m, 22H), 0.95-0.86 (m, 6H); ¹³C NMR (176 MHz, CDCl₃) δ 171.2 (m), 140.2, 139.5 (m), 138.8, 137.6 (m), 129.7, 129.0, 128.5 (m), 128.3 (m), 128.8 (m), 127.4 (m), 127.2 (m), 126.9 (m), 101.9 (m), 85.1 (m), 81.9 (m), 71.1 (m), 66.9, 56.9 (m), 51.5, 35.6, 31.9, 30.6, 29.7, 29.3, 23.2, 22.7, 14.0; MALDI-MS: m/z [M+Na⁺] calcd for C₂₈₈H₃₃¹N₁₃NaO₆₅ 5034.29, found 5034.80.

Figure 11. HPLC of the crude protected 1,6-N-acetyl-glucosamine dodecasaccharide 3.7.
Conditions: 0 to 10% A in B, A = MeOH, B = DCM, over 35 min.

**Aminopentyl (acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)- (acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)-(acetamido-2-deoxy-2-β-D-**
glucopyranosyl)-(1→6)-(acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)-
(acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)-(acetamido-2-deoxy-2-β-D-
glucopyranosyl)-(1→6)-(acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)-
(acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)-(acetamido-2-deoxy-2-β-D-
glucopyranosyl)-(1→6)-(acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)-
(acetamido-2-deoxy-2-β-D-glucopyranosyl)-(1→6)-(acetamido-2-deoxy-2-β-D-
glucopyranosyl)-(1→6)-acetamido-2-deoxy-2-β-D-glucopyranoside 3.8

To a solution of 3.7 (9 mg, 1.7 µmol) in t-BuOH/THF/1 M HCl (0.75 mL/ 1.25 mL/
0.02 mL) was added 20% Pd(OH)$_2$/C (10 mg). The mixture was stirred under an
atmosphere of hydrogen for 36 h. Pd(OH)$_2$/C (15 mg) was added followed by
several drops of water and the mixture was stirred for additional 3 days. The
catalyst was removed by filtration through a pad of celite. The filtrate was
concentrated, and the residue was purified by reverse phase HPLC using a 0 to
25% gradient (acetonitrile in water with 1% formic acid, Pyramid nucleodur C-18
column) to give 3.8 (2.3 mg, 53%) as shown in SI Figure 13. $^1$H NMR (600 MHz,
D$_2$O) δ 4.60-4.48 (m, 12H), 4.21-4.08 (m, 12H), 3.96 (m, 1H), 3.87 (m, 1H), 3.81-
3.70 (m, 26H), 3.64-3.52 (m, 34H), 3.49-3.34 (m, 21H), 3.07-2.99 (m, 2H), 2.14-
2.04 (m, 36H), 1.70 (m, 2H), 1.62 (m, 2H), 1.43 (m, 2H); $^{13}$C NMR (extracted from
HSQC; 176 MHz, D$_2$O) δ 101.6, 101.1, 75.9, 74.5, 73.73, 73.70, 70.0, 69.92,
69.90, 69.8, 68.4, 60.68, 60.67, 55.5, 55.4, 39.3, 28.0, 26.3, 22.2, 22.0; HR-
MALDI-MS: $m/z$ [M+Na$^+$] calcd for C$_{101}$H$_{169}$N$_{13}$O$_{61}$ 2563.042, found 2563.306.
Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl [2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside(1→4)]$_5$-2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside 3.9

The following module sequences were performed on 25 μmol scale based on resin 3.1:

1) Six cycles Thioglycoside Double Coupling - Fmoc deprotection with building block 2.8

2) Eight cycles TCA-reduction (treatment with 10 eq Bu$_3$SnH and cat. AIBN)

3) Linker cleavage (treatment with 10 eq NaOMe)

The eluate was neutralized with acidic Amberlite IR-120 resin and concentrated under vacuum. The crude residue was purified by preparative HPLC using a gradient of 60 to 70% over 40 min of MeCN + 20% i-PrOH in water + 20% i-PrOH to yield 3.9 (21.1 mg, 31% overall yield from resin). [$\alpha$]$_D^{25}$ $-$16.9 (c 1.0, CHCl$_3$); $^1$H NMR (600 MHz, CDCl$_3$) δ 7.42-7.10 (m, 69H), 6.70-6.60 (m, 1H), 6.59-6.47 (m, 1H), 5.89-5.75 (m, 1H), 5.60-5.47 (m, 1H), 5.44-5.34 (m, 1H), 5.31-5.17 (m, 1H),
5.12 (m, 2H), 4.83-4.31 (m, 25H), 4.31-4.09 (m, 7H), 4.09-3.81 (m, 10H), 3.80-3.67 (m, 4H), 3.65 (s, 3H), 3.63-3.07 (m, 23H), 2.93 (t, \(J = 7.7\) Hz, 2H), 2.61 (t, \(J = 7.8\) Hz, 2H), 1.95 (s, 3H), 1.84 (s, 3H), 1.78 (s, 3H), 1.77 (s, 3H), 1.76 (s, 3H), 1.72 (s, 3H), 1.59-1.40 (m, 4H), 1.35-1.12 (m, 6H); \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\) 173.4, 173.3, 171.2, 171.8, 170.8, 156.7, 156.2, 140.2, 139.3, 138.8, 138.7, 138.6, 138.5, 138.4, 138.1, 138.0, 137.9, 137.8, 137.3, 134.9, 134.7, 128.8, 128.6, 128.6, 128.5, 128.49, 128.4, 128.32, 128.29, 128.27, 128.20, 128.17, 128.14, 128.07, 127.99, 127.97, 127.92, 127.85, 127.81, 127.77, 127.70, 127.66, 127.5, 127.4, 127.3, 127.2, 114.0, 100.27, 100.21, 99.4, 80.8, 80.1, 79.7, 79.5, 79.3, 75.5, 75.4, 75.2, 75.1, 75.0, 74.8, 74.4, 74.1, 73.8, 73.7, 73.5, 73.43, 73.37, 73.2, 72.9, 72.7, 70.9, 69.9, 69.8, 69.0, 68.9, 66.9, 54.8, 54.3, 54.1, 54.0, 53.8, 51.6, 50.4, 50.2, 47.2, 46.1, 45.9, 35.6, 33.8, 31.9, 30.6, 29.7, 29.6, 29.5, 29.3, 29.1, 28.9, 27.9, 27.3, 23.4, 23.2, 23.1, 23.0, 22.8, 22.6, 14.1, 8.5; HR-MALDI-MS: \(m/z\) [M+Na\(^+\)] calcd for C\(_{156}\)H\(_{181}\)N\(_7\)O\(_{35}\)Na 2735.2491, found 2735.254. IR (thin film) \(\nu\) = 3351, 3081, 1664, 1550, 1063, 736 cm\(^{-1}\).

**Figure 11.** LC-MS of crude protected \((1\rightarrow4)-\beta-N\text{-acetyl-hexaglucosamine}\) 3.9 synthesized from thioglycoside 2.8. Conditions: 60 to 80% A in B, A = CH\(_3\)CN + 20% iso-propanol + 0.1% TFA, B = water + 20% iso-propanol + 0.1% TFA from 2 to 32 min.

**Aminopentyl** (2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-(2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-(2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-(2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-(2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-(2-acetamido-2-deoxy-\(\beta\)-D-glucopyranoside) 3.10
To a solution of 3.9 (9.8 mg, 3.6 µmol) in THF/MeOH/water/AcOH (2 mL/1 mL/1 mL/0.05 mL) was added 10% Pd/C (15 mg). The mixture was stirred under an atmosphere of hydrogen for 70 h and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated, and the residue was purified by solid phase extraction (Sep-pak 1g cartridge, C-18, water) to give 3.10 (3.8 mg, 79%). The purity was assessed by analytical HPLC using a 0 to 10% gradient (acetonitril in water, Pyramid nucleodur C-18 column) as shown in SI Figure 9. \(^1\)H NMR (600 MHz, D\(_2\)O) \(\delta\) 4.48 (m, 5H), 4.40 (d, \(J = 8.0\) Hz, 1H), 3.90-3.72 (m, 9H), 3.72-3.32 (m, 30H), 2.95-2.84 (m, 2H), 1.99-1.95 (m, 15H), 1.94 (s, 3H), 1.65-1.54 (m, 2H), 1.53-1.47 (m, 2H), 1.33-1.27 (m, 2H); \(^{13}\)C NMR (150 MHz, D\(_2\)O) \(\delta\) 174.6, 174.4, 101.4, 101.2, 101.0, 79.2, 79.1, 78.9, 75.9, 74.5, 73.4, 72.4, 72.1, 72.0, 70.1, 69.7, 39.3, 28.0, 26.3, 22.1, 22.1; HR-MALDI-MS: \(m/z\) [M+H\(^+\)] calcd for C\(_{53}\)H\(_{91}\)N\(_7\)O\(_{31}\) 1322.5832, found 1322.581.

**Figure 12.** LC-MS of unprotected 1,4-hexa-glucosamine 3.10. Conditions: 0 to 10% A in B, A = CH\(_3\)CN, B = water, over 20 min.
Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (2,3,4,6-tetra-O-benzyl-a-D-galactopyranosyl)-(1→3)-4,6-di-O-benzyl-[b]-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-[b]-D-glucopyranoside 3.14

The following modules were performed on 25 μmol scale based on resin 3.1:

1) Thioglycoside Double coupling–Fmoc deprotection using building block 2.9
2) Thioglycoside Double coupling–Fmoc deprotection using building block 2.3
3) Thioglycoside Double coupling–Fmoc deprotection using perbenzylated thioethyl galactose 2.4
4) Linker Cleavage (treatment with 10eq NaOMe)

The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was dissolved in methanol (1 mL), THF (1 mL) and aqueous KOH (3N, 200 μL, ca. 24 eq) was added. The reaction was stirred at 60 °C overnight, quenched with Amberlite IR-120 resin, filtered through a pad of celite and concentrated. The crude residue was purified by preparative HPLC using a 40 to 100% gradient of A in B (A= acetonitrile + 0.1% TFA, B= water + 0.1% TFA, Pyramid nucleodur C-18 column) to afford 3.14 (32 mg, 80% overall yield from resin). H NMR (600 MHz, CDCl₃) δ 7.35-7.11 (m, 49H), 5.12 (s, 2H), 5.01-4.90 (m, 4H), 4.85 (d, J = 11.5 Hz, 1H), 4.76 (t, J = 13.3 Hz, 1H), 4.67 (dd, J = 11.8, 20.6 Hz, 2H), 4.61-4.52 (m, 3H), 4.50-4.35 (m, 7H), 4.31-4.26 (m, 1H), 4.23-4.14 (m, 3H), 4.11 (dd, J = 3.7, 10.0 Hz, 1H), 3.92 (dd, J = 6.6, 16.1 Hz, 1H), 3.89-3.83 (m, 3H), 3.81-3.70 (m,
4H), 3.66 (d, J = 5.8 Hz, 1H), 3.58-3.49 (m, 2H), 3.47-3.37 (m, 5H), 3.36-3.25 (m, 4H), 3.16 (s, 3H), 2.97-2.88 (m, 2H), 2.68-2.55 (m, 2H), 1.65-1.53 (m, 2H), 1.50-1.39 (m, 2H), 1.15 (m, 2H); \(^{13}\text{C} \text{ NMR (151 MHz, CDCl}_3 \) \( \delta \) 130.1, 128.6, 128.50, 128.48, 128.3, 128.2, 127.98, 127.97, 127.9, 127.7, 127.4, 103.4, 102.9, 99.8, 85.5, 83.4, 78.7, 75.2, 75.0, 74.9, 74.8, 74.6, 74.1, 74.0, 73.6, 73.5, 73.3, 73.1, 71.7, 70.4, 69.74, 69.73, 69.2, 68.7, 68.4, 67.2, 51.8, 50.6, 50.4, 50.3, 47.2, 46.9, 46.2, 35.8, 35.1, 33.6, 32.1, 30.8, 30.5, 29.9, 29.3, 27.9, 27.3, 24.9, 23.4, 23.1, 22.9, 20.0, 14.3; HR-ESI MS: \( m/z \) [M+Na\(^+\)] calcd for C\(_{97}\)H\(_{107}\)NNaO\(_{20}\): 1628.7284; Found: 1628.7333.

*Figure 13.* LC-MS of crude 3.14. Conditions 40 to 100% A in B; A = CH\(_3\)CN + 0.1% TFA, B = water + 0.1% TFA over 31 min.

**Aminopentyl (**\(\alpha\)-d-galactopyranosyl)-(1→3)-**β**-d-galactopyranosyl-(1→4)**-**β**-d-glucopyranoside 3.11.**

![Structure of Aminopentyl](image)

3.11
To a solution of 3.14 (4 mg, 2.49 µmol) in methanol (1.5 mL), THF (1 mL), water (200 µL), toluene (200 µL) and acetic acid (100 µL) was added Pd/C (10 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by solid phase extraction (Sep-pak C-18, water + 0.1% TFA) to give 3.14 (1.2 mg, 80%).

\[ ^1\text{H NMR (600 MHz, D}_2\text{O)} \delta 5.16 (d, J = 3.7 Hz, 1H), 4.54 (d, J = 7.8 Hz, 1H), 4.51 (d, J = 8.1 Hz, 1H), 4.23-4.19 (m, 2H), 4.04 (s, 1H), 4.01 (d, J = 12.2 Hz, 1H), 3.99-3.93 (m, 2H), 3.88 (dd, J = 3.8, 10.4 Hz, 1H), 3.86-3.78 (m, 4H), 3.78-3.73 (m, 3H), 3.72-3.65 (m, 4H), 3.64-3.59 (m, 1H), 3.33 (t, J = 7.8 Hz, 1H), 3.03 (t, J = 7.5 Hz, 2H), 1.75-1.65 (m, 4H), 1.52-1.44 (m, 2H); ^13\text{C NMR (176 MHz, D}_2\text{O)} \delta 102.9, 102.1, 95.5, 78.8, 77.3, 75.1, 74.8, 74.6, 72.7, 70.9, 70.1, 69.6, 69.3, 69.2, 68.2, 64.9, 61.03, 60.98, 60.2, 39.4, 28.2, 26.4, 22.1; HR-MALDI-MS: m/z [M+H+] calcd for C_{23}H_{44}NO_{16} 590.266, found 590.454.\]

**Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (5-Acetamido-3,5-dideoxy-\textalpha-D-galacto-non-2-ulopyranosonic-acid)-(2\rightarrow3)-\beta-D-4,6-di-O-benzyl-galactopyranosyl-(1\rightarrow4)-2-acetamido-2-deoxy-3,6-di-O-benzyl-\beta-D-glucopyranoside 3.15**

![3.15](image)

The following modules sequences were performed on 25 µmol scale with respect to resin 3.1:
1) Glycosyl Imidate Double coupling - Fmoc deprotection with building block 2.8

2) Glycosyl Imidate Double coupling (0 °C) – Fmoc deprotection with building block 2.2

3) TCA-reduction (treatment with 20 eq Bu₃SnH and cat. AIBN)

4) Linker Cleavage (treatment with 10 eq NaOMe)

The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was purified by preparative HPLC using a 30 to 90% gradient over 90 min (CH₃CN + 0.05% TFA in water + 0.1% TFA; 10 mL/min, C8 30mm) affording \(\text{3.15} \) in 33% (overall yield from resin): \(^1\)H NMR (500 MHz, MeOD) \(\delta \) 7.49-7.10 (m, 29H), 5.15 (d, \(J = 9.6 \text{ Hz}, 2H\)), 5.01 (d, \(J = 11.4 \text{ Hz}, 2H\)), 4.63 (dd, \(J = 11.7, 43.9 \text{ Hz}, 2H\)), 4.55 (d, \(J = 2.3 \text{ Hz}, 1H\)), 4.53 (s, 2H), 4.48-4.43 (m, 2H), 4.26 (ABq, \(J = 11.8, 87.9, \text{ Hz}, 2H\)), 4.11 (dd, \(J = 3.1, 9.7 \text{ Hz}, 1H\)), 4.04 (dd, \(J = 4.4, 11.5 \text{ Hz}, 1H\)), 3.99 (t, \(J = 9.2 \text{ Hz}, 1H\)), 3.97-3.95 (m, 1H), 3.93-3.87 (m, 2H), 3.87-3.77 (m, 4H), 3.76-3.69 (m, 3H), 3.67 (s, 3H), 3.66 (s, 1H), 3.64 (m, 2H), 3.55 (d, \(J = 9.3 \text{ Hz}, 1H\)), 3.46 (dd, \(J = 6.6, 9.0 \text{ Hz}, 1H\)), 3.41-3.37 (m, 1H), 3.34-3.31 (m, 1H), 3.31-3.22 (m, 2H), 2.96 (t, \(J = 7.6 \text{ Hz}, 2H\)), 2.87 (dd, \(J = 4.6, 12.9 \text{ Hz}, 1H\)), 2.67 (t, \(J = 7.6 \text{ Hz}, 2H\)), 2.07 (s, 3H), 1.99 (t, \(J = 12.8 \text{ Hz}, 1H\)), 1.90 (s, 3H), 1.55 (m, 4H), 1.32 (m, 2H); \(^{13}\)C NMR (126 MHz, CDCl₃) \(\delta \) 175.3, 175.0, 173.1, 172.4, 159.2, 158.5, 158.0, 156.9, 154.5, 152.0, 142.0, 140.50, 140.46, 139.8, 139.6, 139.3, 136.0, 129.6, 129.5, 129.3, 129.2, 129.1, 129.00, 128.95, 128.7, 128.5, 128.5, 128.2, 111.4, 106.8, 104.0, 102.7, 100.2, 82.0, 78.8, 77.5, 77.4, 76.4, 76.3, 75.5, 75.1, 74.6, 74.3, 74.3, 72.8, 71.7, 70.3, 69.8, 69.7, 69.7, 68.8, 68.30, 68.25, 68.23, 68.18, 68.17, 68.16, 68.1, 64.5, 56.3, 53.8, 52.1, 49.6, 49.5, 49.3, 49.1, 41.3, 36.5, 31.6, 30.2, 28.8, 28.4, 24.2, 23.0, 22.7; HR-MALDI MS: \(m/z\) \([\text{M+Na}^+]\) calcd for C\(_{77}\)H\(_{95}\)N\(_3\)NaO\(_{23}\): 1452.6254, found: 1452.6249.
Aminopentyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside 3.16

To a solution of 3.15 (13 mg, 9.09 μmol) in methanol/water (2 mL, 8/2) acetic acid (50 μL) was added followed by Pd/C (13 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by solid phase extraction (Sep-Pak C-18, water + 0.1% TFA) to give 3.16 (5.37 mg, 7.09 μmol, 78% yield). The purity of the compound was assessed by analytical HPLC using a 95 to 5% gradient (CH₃CN in water, TSKgel amide-80 TOSOH) as shown in SI Figure 18. ¹H NMR (500 MHz, D₂O) δ 4.57 (d, J = 7.9 Hz, 1H), 4.54 (d, J = 7.9 Hz, 1H), 4.15 (dd, J = 3.1, 9.9 Hz, 1H), 4.03-3.99 (m, 1H), 3.98 (d, J = 3.2 Hz, 1H), 3.95-3.84 (m, 5H), 3.78-3.72 (m, 6H), 3.71-3.68 (m, 2H), 3.65 (dd, J = 6.3, 12.8 Hz, 1H), 3.62-3.56
(m, 4H), 3.01 (t, J = 4.6, 12.6 Hz, 1H), 2.05 (d, J = 1.5 Hz, 6H), 1.85 (t, J = 12.1 Hz, 1H), 1.69 (m, 2H), 1.61 (m, 2H), 1.42 (m, 2H); \(^{13}\)C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) 175.0, 174.4, 173.2, 102.6, 101.1, 99.5, 78.4, 75.5, 75.1, 74.8, 73.0, 72.4, 71.5, 70.1, 69.4, 68.1, 68.1, 67.5, 62.7, 61.0, 60.0, 55.1, 51.7, 48.8, 39.3, 28.1, 26.4, 22.1, 22.1, 22.0; MALDI-MS: \(m/z\) [M+Na\textsuperscript{+}] calcd for C\(_{30}\)H\(_{53}\)N\(_3\)NaO\(_{19}\) 782.317, found 782.329.

Figure 15. LC-MS of 3.16. Conditions: 95 to 5% A in B; A = CH\textsubscript{3}CN, B = water, over 30 min.

Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (5-Acetamido-3,5-dideoxy-D-glycero-\(\alpha\)-D-galacto-non-2-ulopyranosylic-acid)-(2\(\rightarrow\)3)-4,6-di-O-benzyl-\(\beta\)-D-galactopyranosyl-(1\(\rightarrow\)4)-3,6-di-O-benzyl-\(\beta\)-D-glucopyranoside 3.17

The following modules sequences were performed on 25 \(\mu\)mol scale with respect to resin 3.1:

1) Thioglycoside Double Coupling - Fmoc deprotection with building block 2.9
2) Glycosyl Imidate Double coupling (0 °C) - Fmoc deprotection with building block 2.2

3) Linker Cleavage (treatment with 10eq NaOMe)

The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was dissolved in methanol (1.6 mL), water (0.4 mL), THF (0.5 mL) and aqueous KOH (1N, 300 μL, ca. 14 eq) was added. The reaction was stirred at 60 °C overnight, quenched with Amberlite IR-120 resin, filtered through a pad of celite and concentrated. Preparative HPLC using 60% MeCN in water (with 0.1% TFA, C-8, 10 mL/min) as eluent afforded 13.7 mg of 3.17 (40% overall yield from resin).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.42-7.07 (m, 29H), 5.12 (d, $J = 17.3$ Hz, 2H), 4.99 (dd, $J = 11.0, 13.1$ Hz, 2H), 4.69 (d, $J = 10.6$ Hz, 1H), 4.63 (d, $J = 11.5$ Hz, 1H), 4.57-4.48 (m, 4H), 4.43 (d, $J = 7.7$ Hz, 1H), 4.35 (d, $J = 11.8$ Hz, 1H), 4.29-4.22 (m, 1H), 4.16 (d, $J = 11.8$ Hz, 1H), 4.05 (dd, $J = 3.1, 9.7$ Hz, 1H), 4.00 (dd, $J = 3.4, 10.9$ Hz, 1H), 3.95-3.91 (m, 2H), 3.88 (t, $J = 10.1$ Hz, 2H), 3.83-3.75 (m, 4H), 3.73-3.65 (m, 3H), 3.62 (dd, $J = 1.3, 9.0$ Hz, 1H), 3.56-3.42 (m, 4H), 3.36-3.30 (m, 2H), 3.25-3.20 (m, 1H), 2.91 (t, $J = 7.1$ Hz, 2H), 2.82 (dd, $J = 4.6, 13.0$ Hz, 1H), 2.60 (t, $J = 7.6$ Hz, 2H), 2.03 (s, 3H), 1.99 (t, $J = 12.6$ Hz, 1H), 1.64-1.47 (m, 4H), 1.41-1.27 (m, 2H); $^{13}$C NMR (151 MHz, CD$_3$OD) $\delta$ 177.4, 176.1, 173.0, 141.32, 141.30, 140.7, 140.5, 130.5, 130.3, 130.23, 130.17, 130.04, 129.98, 129.8, 129.8, 129.5, 129.4, 129.3, 129.0, 105.5, 104.9, 100. 9, 85.4, 79.6, 78.2, 78.0, 77.3, 77.0, 76.6, 76.4, 75.8, 75.3, 75.2, 75.1, 73.7, 72.5, 71.5, 70.6, 70.4, 69.6, 69.1, 65.3, 54.6, 42.0, 37.5, 32.5, 31.2, 25.2, 23.5; HR-ESI MS: m/z: [M+Na$^+$] calcd for C$_{74}$H$_{90}$N$_2$NaO$_{23}$ 1397.5832, found 1397.5814.
Aminopentyl \((5\text{-Acetamido-3,5-dideoxy-D-glycero-}\alpha\text{-D-galacto-non-2-}
ulopyranosylonic-acid)-(2\rightarrow3)\beta\text{-D-galactopyranosyl-(1}\rightarrow4)\beta\text{-D-
glucopyranoside 3.18}

Acetic acid (50 μL) was added to a solution of 3.17 (13 mg, 9.09 μmol) in methanol/water (2 mL, 8/2) followed by Pd/C (13 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by preparative HPLC using a 0 to 20% gradient (CH₃CN in water + 0.1% TFA, Pyramid nucleodur C-18 column) over 35 min to afford the 3.18 (6 mg) in 91% yield. The purity of 3.18 was assessed by analytical HPLC using a 95 to 5% gradient (CH₃CN in water, TSKgel amide-80 TOSOH) as shown in SI Figure 16. \(^1\)H NMR (600 MHz, D₂O) δ 4.53 (dd, \(J = 7.9, 25.1\) Hz, 2H), 4.14 (dd, \(J = 2.9, 9.8\) Hz, 1H), 4.03-3.93 (m, 3H), 3.92-3.81 (m, 4H), 3.80-3.64 (m, 9H), 3.63-3.55 (m, 3H), 3.32 (t, \(J = 12.0\) Hz, 1H), 3.03 (t, \(J = 7.4\) Hz, 2H), 2.78 (dd, \(J = 4.5, 12.4\) Hz, 1H), 2.05 (s, 3H), 1.83 (t, \(J = 12.2\) Hz, 2H). 

Figure 16. LC-MS of crude 3.17. Conditions: 60% A in B; A = CH₃CN + 0.1% TFA, B = water + 0.1%TFA over 30 min.
Hz, 1H), 1.75-1.65 (m, 4H), 1.51-1.44 (m, 2H); $^{13}$C NMR (151 MHz, D$_2$O) δ 174.8, 173.4, 102.6, 102.0, 99.6, 78.3, 75.5, 75.1, 74.7, 74.4, 72.9, 72.8, 71.6, 70.0, 69.3, 68.2, 68.1, 67.4, 62.6, 61.0, 60.0, 51.6, 39.5, 39.3, 28.1, 26.3, 22.00, 21.98;
MALDI-MS: m/z [M+Na$^+$] calcd for C$_{28}$H$_{50}$N$_2$NaO$_{19}$ 741.290, found 741.383.

Figure 17. LC-MS of 3.18. Conditions: 95 to 5% A in B; A = CH$_3$CN, B = water, over 30 min.

Automated Synthesis of Protected Methyl 3-(4-((benzyl(5-hydroxypentyl)carbamoyloxy)methyl)phenyl)propanoyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic-acid)-(2→3)-4,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-[2-O-benzyl-α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside 3.19

The following modules sequences were performed on 25 μmol scale with respect to resin 3.1:

1) Thioglycoside Double coupling–Fmoc deprotection with building block 2.6
2) Glycosyl Imidate Double coupling (0 °C)-Lev deprotection with building block 2.2
3) Glycosyl Imidate Double coupling (-10 °C) with building block 2.5
4) TCA Reduction by Treatment with 10 eq of Bu_3SnH/AIBN
5) Linker Cleavage by Treatment with 10 eq NaOMe

The eluate from the synthesizer was neutralized with acidic Amberlite IR-120 resin and concentrated under reduced pressure. The crude residue was dissolved in methanol (1.6 mL), water (0.4 mL), THF (0.5 mL) and aqueous KOH (1N, 300 μL, ca. 14 eq) was added. The reaction was stirred at 60 °C overnight, quenched with Amberlite IR-120 resin, filtered through a pad of celite and concentrated. The crude residue was purified by preparative HPLC using a 35 to 60% gradient (CH_3CN in water + 20% i-PrOH, + 0.1% TFA) over 35 min to afford the desired compound 3.19 (20 mg) in 51% yield from the resin. ^1H NMR (600 MHz, CD_3OD) δ 7.48-7.16 (m, 29H), 5.32 (s, 1H), 5.12 (d, J = 18.3 Hz, 2H), 4.75 (d, J = 11.7 Hz, 1H), 4.68-4.63 (m, 2H), 4.54-4.46 (m, 5H), 4.42-4.32 (m, 2H), 4.09 (d, J = 10.4 Hz, 1H), 4.04-3.97 (m, 3H), 3.95-3.90 (m, 2H), 3.88-3.84 (m, 2H), 3.83-3.80 (m, 1H), 3.77 (m, 4H), 3.71 (dd, J = 5.9, 10.7 Hz, 2H), 3.62 (dd, J = 4.1, 9.7 Hz, 2H), 3.55 (m, 4H), 3.48 (d, J = 2.8 Hz, 1H), 3.41 (s, 1H), 3.27-3.19 (m, 3H), 2.92 (t, J = 7.6 Hz, 2H), 2.83 (dd, J = 4.5, 12.9 Hz, 1H), 2.60 (t, J = 7.6 Hz, 2H), 2.03 (s, 3H), 1.94 (t, J = 15.2 Hz, 1H), 1.90 (d, J = 22.6 Hz, 3H), 1.50-1.38 (m, 4H), 1.35-1.19 (m, 2H), 0.94 (d, J = 6.6 Hz, 3H); ^13C NMR (151 MHz, CD_3OD) δ 177.4, 176.1, 173.84, 173.83, 173.82, 141.0, 140.8, 140.7, 140.6, 130.7, 130.5, 130.29, 130.27, 130.2, 130.1, 129.9, 129.7, 129.5, 129.39, 129.37, 104.4, 103.8, 97.7, 79.7, 78.2, 77.4, 77.2, 77.1, 76.4, 76.3, 75.9, 75.1, 75.0, 74.9, 74.6, 73.6, 73.3, 72.2, 71.1, 70.62, 70.59, 70.5, 70.4, 69.6, 69.1, 67.9, 65.3, 62.4, 58.6, 54.6, 50.4, 42.3, 37.5, 32.5, 31.1, 25.1, 24.3, 23.5, 21.7; HR-ESI MS: m/z [M-H] calcd for C_82H_102N_3O_27 1560.6706, found 1560.6757.
Figure 18. LC-MS of crude 3.19. Conditions: 20 to 60% A in B; A = CH₃CN + 0.1% TFA + 20% i-PrOH, B = water + 0.1% TFA + 20% i-PrOH over 20 min.

Aminopentyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside 3.20

To a solution of 3.19 (13 mg, 14.3 μmol) in methanol/water (2 mL, 8/2) acetic acid (30 μL) was added followed by Pd/C (13 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by HPLC using a 0 to 20% gradient (CH₃CN in water + 0.1% TFA, Pyramid nucleodur C-18 column) over 35 min to afford the desired compound 3.20 (3.3 mg) in 30% yield. ¹H NMR (600 MHz, D₂O) δ 5.11 (d, J = 3.7 Hz, 1H), 4.83 (d, J = 6.5 Hz, 1H), 4.53 (d, J = 7.8 Hz, 2H), 4.10 (d, J = 9.9 Hz, 1H), 4.03 (d, J = 12.3 Hz, 1H), 3.97-3.83 (m, 10H), 3.79 (s, 1H), 3.73-3.63 (m, 6H), 3.63-3.57 (m, 4H), 3.54 (m, 1H), 3.00 (t, J = 7.5 Hz, 2H),
2.78 (dd, $J = 4.9$, 12.6 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.81 (t, $J = 12.0$ Hz, 1H), 1.72-1.65 (m, 2H), 1.64-1.57 (m, 2H), 1.45-1.37 (m, 2H), 1.18 (d, $J = 6.5$ Hz, 3H); $^{13}$C NMR (151 MHz, D$_2$O) $\delta$ 175.0, 174.1, 173.8, 101.6, 101.0, 99.6, 98.5, 75.6, 75.2, 74.9, 74.8, 73.3, 72.9, 71.8, 71.8, 70.1, 69.2, 69.1, 68.2, 68.1, 68.1, 67.7, 67.3, 66.6, 62.6, 61.4, 59.6, 55.8, 51.6, 39.7, 39.3, 28.0, 26.3, 22.1, 22.0, 15.2; HR-ESI MS: $m/z$ [M+Na]$^+$ calcd for C$_{38}$H$_{63}$N$_3$NaO$_{23}$: 928.3750; Found: 928.3762.

Figure 19. LC-MS of 3.20. Conditions: 0 to 10% A in B; A = CH$_3$CN, B = water, over 20 min.

Preparation of 1,4-Hexaglucosamine-BSA-Conjugate 3.22

Compound 3.10 (0.9 mg, 0.66 µmol) was dissolved in 100 µL 50 mM sodium phosphate buffer pH7.2. Diethyl squarate (0.335 mg, 2.0 µmol) dissolved in 100 µL ethanol was added. The mixture was agitated overnight at room temperature. The mixture was purified by reverse phase HPLC (several injections) on a Merck KGaA LiChroCART 250 mm x 4 mm column with LiChrospher 100 RP-18 (5 µm) solid phase using a gradient of 0 to 50% methanol/water.

The isolated fraction of squarate-sugar conjugate (0.2 mg, 0.14 µmol) and BSA (0.2 mg, 3.0 µmol) were dissolved in sodium bicarbonate buffer (pH 9.0, 0.1 M,
80 µL). The mixture was shaken overnight at room temperature. SDS-PAGE on a 12.5% acrylamide gel shows a diffuse band at approximately 80 kDa (SI Figure 22 b). The mixture was agitated for an additional day. The conjugate 3.22 was purified using a Microcon centrifugal filter device by Millipore with a molecular weight cutoff of 30kDa. The protein was centrifuged twice against 320 µL of ultrapure water (14000 g at 25°C for 12min) and lyophilized.

**Glycan Microarray Printing**

Oligosaccharide solutions (10 mM solutions were prepared in sodium phosphate buffer, pH 9) were printed on N-hydroxysuccinimide (NHS)-activated CodeLink glass slides, using a piezoelectric spotting device (S11, Scienion) to place 1 nL of solution at each spot. Each compound was spotted in six replicas of three different concentrations (1 mM, 0.25 mM, 0.1 mM). After printing, the slides were sealed in a humidity chamber and incubated overnight to covalently couple.

**Glycan Microarray Screening**

The slides were washed three times with water in a 50 mL centrifugation tube, quenched with 45 mL of quenching solution for 1 h at 50 °C. Three washes with water were used to clean the surface of the slide, thus the residual water was removed by centrifugation [2500 rpm for 5 min (r.t.)]. The slides were incubated with BSA (1% in PBS, 100 µL) in a closed, moisturized chamber for 1 h, in order to block unspecific interactions. The slides were washed (2 x 10 min in 20 mL PBS buffer) and dried by centrifugation (200 rpm for 5 min, r.t.). The slides were incubated with biotinylated wheat germ agglutinin (Vector Laboratories) solutions (84.5 µL water, 0.5 µL 2% Tween20, 10µL PBS, 5 µg lectin, 100 µL) in a sealed humidity chamber for 1 h, washed with PBS and dried by centrifugation. A last incubation step was performed with an alexa fluor 594 labeled streptavidin solution (Invitrogen) (77.5 µL water, 0.5 µL 2% Tween20,
10 µL PBS, 10 µL 5% BSA, 2 µg streptavidin marker, 100 µL) under exclusion of light. The slides were washed (PBS), rapidly rewashed with water and dried by centrifugation. A fluorescent microarray scanner (Tecan LS400 scanner analysis by Array-Pro Analyzer) was used for detection (Figure 20 b). Alternatively, after blocking with BSA, slides were incubated with alexa fluor (594) labeled wheat germ agglutinin (Sigma) solutions (84.5 µL water, 0.5 µL 2% Tween20, 10µL PBS, 5 µg lectin, 100 µL) in a sealed humidity chamber for 1 h, washed (PBS), dried (centrifugation) and scanned (Figure 20 a).
Figure 20. Incubation with lectin WGA (specificity for N-acetyl-glucosamine and N-acetyl-neuraminic).
3.11 References

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Exploiting Carbohydrate-Carbohydrate Interactions for *In Vitro* targeting of Melanoma Cells

Acknowledgments

Part of the work presented in this chapter was performed in collaboration with Dr. Raghavendra Kikkeri (synthesis of Quantum Dots), Mr. Heung Sik Hahm (scale up synthesis of carbohydrate antigens), and Mrs Magdalena Eriksson (biological assays).
4.1 Introduction

Cell surface carbohydrates modulate important biological phenomena through specific bindings events. (1) The binding partners are usually proteins that recognize specific oligosaccharide sequences. (2) In some cases however, the binding partner in these specific recognition processes can be another oligosaccharide. This results in a carbohydrate-carbohydrate interaction (CCI's). The specific interaction between ganglioside Gm3 (NeuAca2→3Galb1→4Glcb1→Cer) and glycolipid Gg3 (GalNAcb1→4Galb1→4Glcb1→Cer) is one such example. (3) In a preliminary study by Hakomori 1989, it was demonstrated that a Gm3 liposome can interact with Gg3 coated on a plastic surface. The interaction was shown to be highly dependent on the density of Gg3 on the surface, and was mediated by the presence of divalent cations. Furthermore, either anti-Gg3 or anti-Gm3 antibodies could inhibit the interaction. Based on these findings, it was also possible to show how the specific interaction between Gm3 and Gg3 was capable of modulating the adhesion between mouse lymphoma L5178 cells (overexpressing Gg3 on the cell membrane) and mouse B16 melanoma cells (overexpressing Gm3 on the cell membrane). Following these initial studies, a similar, yet homotopic interaction between Lewis X trisaccharide antigens was reported. (4) These examples led to the conclusion that CCI's share some general features, including specificity, concentration dependency and the presence of divalent cations. (5, 6) CCI's have also been shown to be enhanced by glycan clustering. For example, oligosaccharides that are not arrayed on a carrier do not show CCI's as a result of the randomization of their orientation in solution. (5)

Several reports have suggested the involvement of CCI's in a wide range of phenomena including the formation of a compact myelin sheath, (7) the aggregation of sponge cells, (8) and sperm-egg cell adhesion. (9) As a consequence, a range of techniques have been exploited in an attempt to
characterize these interactions, including surface plasmon resonance (SPR), nuclear magnetic resonance (NMR) spectroscopy and atomic force microscopy. Interestingly, atomic force microscopy studies on Lewis X determinants using gold self-assembled monolayers revealed that the adhesion force between two Lewis X antigen molecules ranges around 20 pN. While such an isolated interaction can be classified as relatively weak and would not be able to generate any significant biological effect, the collective adhesion force of 16 pairs of Lewis X (320 pN) would be sufficient to hold T and B lymphocyte cells together.

Despite the success of several studies, the role of CCI’s as possible mediators of cell adhesion and recognition is still a matter of debate among glycoscientists. Therefore, we became interested in gaining additional insights on these phenomena, with the intention of exploring possible applications. Alteration of cell surface carbohydrates in malignancy has been extensively reported. Typically, tumor associated modifications are characterized by specific carbohydrate epitopes overexpression. The increased level of clustering of these epitopes, also results in multivalency.

Methods for detection of malignancy rely on the targeting of specific biomarkers. We surmised, therefore, the possibility of using specific CCI’s for the in vitro targeting of carbohydrate biomarkers. Antigens could, in principle, be targeted via CCI’s using glycoprobes composed of a ligand presented in a multivalent fashion and linked to a photoactive moiety. This chapter describes how carbohydrates capped quantum dots were designed as probes to capitalize on the specific interaction between gangliosides Gm3 and Gg3 for targeting mouse B16 melanoma cell line. This particular cell line was chosen because it is known to express high levels of Gm3.
4.2 Preparation of Glycoprobes

Quantum dots are inorganic nanocrystals characterized by a broad excitation profile and narrow emission spectra. (18) These materials have found broad application in the field of molecular imaging, showing several advantages with respect to small organic fluorophores. For instance, they are brighter than organic dyes, thanks to higher extinction coefficients and quantum yields. Additionally, they are ideal for long term observations, due to their exceptional photostability. (18) The development of coating strategies that make QD’s stable to water environment has been crucial for the development of biocompatible QD conjugates. Seeberger and coworkers recently demonstrated the use of carbohydrate-capped quantum dots (QDs) as probes for selectively targeting ASGPR on hepatocytes in vitro and in vivo. (19) In this contest, QD present the additional advantage of having a large surface area, which permits the assembly of a multivalent ligand display. Following these successful reports, we decided to exploit quantum dots with the double function of fluorescent probe and physical carrier to generate a multivalent display of carbohydrates, as necessary for CCI.

We commenced our investigation with the synthesis of compound 4.1 (Scheme 1), which we hoped to employ as a probe for studying carbohydrate-carbohydrate interactions between Gm3 and Gg3, and compound 3.18, which serves as proper specificity control for interaction studies. The trisaccharide portion of Gg3 was constructed by elongating at the non-reducing terminus using building block 4.2 provided with a Wong linker (20) at the anomeric position. Glycosylation with galactosyl phosphate 4.3 (21) followed by Fmoc removal in situ afforded 4.4 in 65% yield, providing in this way the nucleophile required for the last elongation step. Reaction of N-phenyltrifluoroacetamidoyl protected galactosamine 4.5 with disaccharide 4.4 in presence of catalytic trimethylsilyl trifluoromethanesulfonate yielded compound 4.6 in 62% yield. At this stage, radical reduction of the trichloroacetamide on the terminal sugar, followed by saponification of the ester
protecting groups and hydrogenolysis, afforded the saccharide portion of ganglioside Gg3 4.1 (55% over three steps) containing an amino linker at the reducing end for further functionalization.

A streamlined solution phase synthesis for the preparation of compound 3.18 was then designed. A key step in the route was the glycosylation of building block 2.2 with glucose building block 4.2 (Scheme 2), which proceeded efficiently in presence of trimethylsilyl triflate as promoter at -10 °C to yield 80% of trisaccharide 4.7. At this point, deacetylation under Zemplen conditions, followed by saponification and hydrogeolysis resulted in trisaccharide 3.18, which was ready for conjugation with quantum dots (QDs).
Scheme 2. Synthesis of 3.18. Reagents and conditions. i: TMSOTf, DCM, 0 °C, 80%; ii: NaOMe, MeOH; then KOH, MeOH, 60 °C; then Pd/C, H2, AcOH, MeOH, THF, H2O, rt, 76% over three steps.

CdSe/ZnS nanoparticles with an absorption maximum at 450nm were prepared by pyrolysis of organometallic precursors as described previously.\(^{(19)}\) The hydrophilicity of these particles was improved by functionalization with a polyethylene glycol (PEG) linker.\(^{(22)}\) The PEG linker also served as a spacer between the glycan and the photoactive probe. As described previously,\(^{(22)}\) PEG 2000 4.8 (Scheme 3) was mesylated and then converted to the di-azido derivative 4.10 by reaction with sodium azide. Staudinger reduction produced diamino compound 4.11, which was selectively monosubstituted with lipoic acid in presence of N,N‘-diisopropylcarbodiimide to afford 4.12 in 50% yield. Reaction with succinic anhydride and triethylamine gave compound 4.13, which was successively reduced with sodium borohydride to yield dithiol derivative 4.14. Compound 4.14 was used to functionalize the surface of QDs by ligand exchange, performed by treating the particle in a water-ethanol suspension with excess ligand at 60 °C to provide the water-soluble probe 4.15.
Scheme 3: Reagents and Conditions. 

- **i**: MsCl, TEA, THF, 0 °C to rt; 
- **ii**: NaN₃, H₂O, reflux; 
- **iii**: PPh₃, H₂O; 
- **iv**: DL-thioctic acid, DIC, DCM; 
- **v**: Succinic anhydride, TEA, DCM, 30%; 
- **vi**: NaBH₄, EtOH/H₂O, 40%; 
- **vii**: EtOH, CHCl₃, 60°C.

The terminal carboxylic acid group on 4.15 was then activated for coupling with trisaccharides 4.1 or 3.18 (Scheme 4) by treatment with water soluble coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinamide to afford carbohydrate capped quantum dots 4.16 or 4.17, respectively.

Scheme 4: Reagents and conditions: 

- **i**: EDC, NHS, aminopentyl-Gg3 4.1 or aminopentyl-Gm3 3.18.
The particles obtained in this study were shown to be monodispersed, as judged by TEM images (see experimental procedures). In addition, the glycan loading was estimated at 400 sugars per particle, using a quantitative NMR method with formic acid employed as the internal standard (see experimental procedures).

### 4.3 In vitro Studies with B16-F10 Melanoma Cells

(In collaboration with M. Eriksson)

Having completed the synthesis of glycoprobes 4.16 and 4.17, we proceeded with *in vitro* analysis on murine B16-F10 melanoma cell line. This cell line is known to express high levels of Gm3 on its surface. The increased level of expression of this epitope should also result in multivalency. Under the hypothesis that CCI’s can take place between multivalent displays of Gg3 and Gm3, we speculated that the collective adhesion force between the dense layer of Gg3 on 4.16 and Gm3 on the cell surface could be sufficient to anchor 4.16 on the cell membrane. Furthermore, if such interaction is specific, Gm3 coating on 4.17 should not produce any interaction with B16 cells.

Murine B16-F10 melanoma cells were incubated with compounds 4.16 or 4.17 in DMEM medium for 30 min. Flow cytometry analysis showed a shift of the fluorescence emission (660 nm) of cells incubated with 4.16 (Gg3-QDs) in comparison with cells incubating with medium only. Interestingly, cells incubated with the 4.17 (Gm3-QDs) did not show such a shift. This demonstrates the capability of 4.16 to bind specifically to the cell surface of these melanoma cells.
Figure 1. Specific interaction between 4.16 (Gg3-QDs) and the B16-F10 melanoma cells. Melanoma cells were incubated for 30 min with 1µM of 4.16 (Gg3-QDs, red), 4.17 (Gm3-QDs, blu) or cell medium only (gray).

Since 4.16 (Gg3-QDs) interact with the melanoma cells, but 4.17 (Gm3-QDs) do not, the interacting cell surface molecule expressed on the cells is suggested to be Gm3 as indicated by previous findings.\(^3\) As determined by preliminary analysis, the interaction appears to be modulated as a function of the QDs’ concentration (Figure 2) in the micromolar concentration range. Additional data points are currently being acquired to clearly establish the concentration profile of the interaction.
To further corroborate our data we decided to image the specific interaction of compound 4.16 by confocal microscopy using the optical properties of QDs. Based on the results obtained by flow cytometry, cells were incubated at micromolar concentrations of QD’s for 1h and observed. The images supported the specificity of the interaction with Gg3 capped QD’s. Indeed, control compound 4.17 did not show any significant interaction with the cell membrane. Interestingly, the picture seems to suggest that Gg3 particles lay on the cellular membrane, in line with the hypothesis that CCIs should be regarded as surface interactions. (5)
Figure 3. B16 melanoma cells incubated for 30 min with either 1 µM of 4.16 (Gg3-QDs, left row) or 1µM of 4.17 (Gm3-QD, right row) analyzed under fluorescence microscope. A = C, E, G merged; B = D, F, H merged. C and D: DAPI staining E and F: Annexin V staining. G and H: detection of QDs. Scale bar: 10µm

Additional experiments are currently under way to rule out the possibility that receptors on the cell membrane, other than Gm3, are mediating the interaction of 4.16 with B16 melanoma cells. In this regard, it is worth mentioning that an inhibition experiment based on the use of a neuraminidase from *Vibrio cholerae* (VCN) for the cleavage of sialic acid from Gm3 on the cell surface did not give positive results. This could be explained by considering that treatment with VCN did not result in complete removal of sialic acid from the surface of B16 cells, in line with previous findings. (23)

Alternatively, an inhibition experiment based on the blocking of Gm3 residues on the cell membrane by antibodies could clearly show that the process is mediated by the specific interaction between Gm3 (on the cell) and Gg3 (on the particle). In
this regard, the characterization of the DH2 IgG antibody (24) (provided by Prof. Hakomori), specific for Gm3 is ongoing.

It is noteworthy that galectins, such as galectin-1 and -3, which are known to bind to terminal galactose residues, are expressed on the cell surface of B16 melanoma cells. (25) Therefore, it is possible that the interaction between glycoprobe 4.16 and the cells is mediated by these lectins, by interaction with the galactosamine residue at the non reducing terminus of Gg3 on 4.16. In order to exclude this possibility, an additional control experiment based on a galectin-1 knockdown model (26) should be performed.

Finally, it is worth considering the possibility of characterizing the CCI's of carbohydrates capped QD's by physical methods, including surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). Such studies can provide information on binding energies improving our understanding on the biological significance of CCI's.

4.4 Conclusions

In conclusion, this chapter described a preliminary investigation on the possibility of targeting carbohydrate biomarkers via CCI's. Two glycans (Gg3 and Gm3) were synthesized and conjugated to QDs to produce bifunctional constructs 4.16 and 4.17. These compounds combine a multivalent glycan display and an optically active probe. Our preliminary results suggest, in line with previous findings, that CCI's can be regarded as a form of “binding”, since they show specificity for given ligands as well as concentration dependency. Nevertheless, additional work needs to be performed in order to unambiguously elucidate the mechanism of the interaction between carbohydrates capped QD's and B16
melanoma cells. This work represents a first attempt to understand the possible application of CCI’s to cellular imaging. Studies aiming at the identification of specific cancer carbohydrate markers that could be targeted by use of specific CCIs are currently ongoing.
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4.5 Experimental Procedure.

**General Materials and Methods.** All chemicals used were reagent grade and used as supplied except where noted. All reactions were performed in oven-dried glassware under an inert atmosphere unless noted otherwise. Reagent grade $N,N$-dimethylformamide (DMF) was dried over activated molecular sieves prior to use. Pyridine, triethylamine (NEt₃) and acetonitrile (MeCN) were distilled over CaH₂ prior to use. Dichloromethane (CH₂Cl₂), toluene and tetrahydrofuran (THF) were purified by a Cycle-Tainer Solvent Delivery System unless noted otherwise. All solvents used on the automated synthesizer are extra dry grade without molecular sieves, purchased from Acros in sure seal bottles except DCM and THF, which are dried using a dry still. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). Purification by size exclusion recycling HPLC was carried out using JAI LC 9101 equipped with JAIGEL-1H and 2-H column in a series (CHCl₃). Purification by reverse phase HPLC was performed using Agilent 1200 series equipped with a Macherey-Nagel Nucleodur Pyramid C-18 column (length 250 mm, 40 mm i.d., flow 10 mL/min) unless noted otherwise.¹H, ¹³C spectra were recorded on a Varian Mercury 300 (300 MHz), Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), Bruker ECX (400 MHz), Bruker DRX500 (500 MHz), or, Bruker DRX700 (700 MHz) spectrometer in CDCl₃ with chemical shifts referenced to internal standards CDCl₃ (7.26 ppm ¹H, 77.0 ppm ¹³C) unless otherwise stated. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for ¹H NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. High resolution mass spectral (HRMS)
analyses were performed by the MS-service at the Laboratory for Organic Chemistry (LOC) at ETH Zürich and the MS-service at Department of Organic Chemistry at Free University Berlin. High-resolution MALDI and ESI mass spectra were run on an IonSpec Ultra instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

\[ \text{\(N\)-(Benzyl)-Benzyloxycarbonyl-5-aminopropentyl 2-Acetyl-3,6-di-O-benzyl-\alpha-D-glucopyranoside 4.2} \]

To a cold solution (0 °C) of glucose building block 2.6 (190 mg, 0.241 mmol) and \(N\)-(benzyl)-benzyloxycarbonyl-5-aminopentane-1-ol (12) (134 mg, 0.327 mmol) in DCM (1.5 mL) under an atmosphere of argon and in the presence of 4 Å AW-MS (5 rods), NIS (65 mg, 0.290 mmol) and TfOH (4.29 \(\mu\)L, 0.048 mmol) were added. The reaction was stirred for 2 hours at 0 °C, then quenched with triethylamine (100 \(\mu\)L). After warming up to room temperature, the reaction was stirred for one hour at room temperature, then filtered over celite and concentrated in vacuum. Purification with flash column silica gel chromatography (hexane:ethylacetate = 9:1 to 2:1) gave compound 4.2 (137 mg, 0.192 mmol, 80% yield). \(4.2: [\alpha]_D^{20} = 12.10 (c 1, \text{CHCl}_3)\). \(^1\text{H NMR} (600 MHz, \text{CDCl}_3) \delta 7.40 – 7.02 (m, 20H), 5.09 (d, J = 17.1 Hz, 2H), 4.87 (t, J = 8.7 Hz, 1H), 4.75 – 4.56 (m, 2H), 4.50 (dd, J = 27.6, 12.0 Hz, 2H), 4.40 (d, J = 11.5 Hz, 2H), 4.25 (dd, J = 19.6, 7.5 Hz, 1H), 3.75 – 3.58 (m, 4H), 3.46 – 3.38 (m, 2H), 3.34 – 3.24 (m, 1H), 3.12 (d, J = 41.2 Hz, 2H), 1.89 (d, J = 18.3 Hz, 3H), 1.51 – 1.35 (m, 4H), 1.24 – 1.09 (m, 2H). \(^{13}\text{C NMR} (151 MHz, \text{CDCl}_3) \delta 169.48, 138.46, 138.04, 137.85, 128.65, 128.62, 128.59, 127.95, 127.95, \]

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127.94, 127.86, 101.13, 82.50, 74.42, 74.11, 73.88, 72.86, 72.48, 70.65, 67.28, 29.29, 23.26, 21.02. ESI HR-MS: m/z [M+Na]⁺ calcd for C₄₂H₄₉NNaO₉: 734.3305; Found: 734.3274. IR (thin film) ν = 3457, 2910, 2323, 1746, 1691, 1420, 1367, 1240, 1059.

N-(Benzyl)- Benzyloxycarbonyl-5-aminopropentyl (3,4,6-tri-O-Benzyl-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-O-pivaloyl-α-D-galactopyranosyl-(1→4)-2-O-acetyl-3,6-di-O-benzyl-α-D-glucopyranoside 4.7

<Diagram>

Compound 4.2 (111 mg, 0.156 mmol) and 4.3 (170 mg, 0.198 mmol) were combined in a flask, co-evaporated three times with toluene and dried. The mixture was dissolved in CH₂Cl₂ (3 mL) and cooled to -40 °C. TMSOTf (35 µL, 0.197 mmol) was added and the reaction was stirred 2 h at the same temperature, then quenched with 100 µL of Et₃N and allowed to warm up to rt. After 1 h the solution was filtered through a pad of celite and the solvent was removed under reduced pressure. Purification by flash silica column chromatography gave disaccharide 4.4 (120 mg, 0.105 mmol) that was taken to the next step. 4.4: ¹H NMR (500 MHz, CDCl₃) δ 7.38 – 7.23 (m, 30H), 5.24 – 5.13 (m, 3H), 4.93 (dd, J = 19.2, 9.9 Hz, 2H), 4.74 (d, J = 12.2 Hz, 1H), 4.62 (dd, J = 28.1, 11.7 Hz, 2H), 4.54 – 4.38 (m, 6H), 4.31 – 4.24 (m, 1H), 4.07 – 4.00 (m, 2H), 3.83 – 3.64 (m, 4H), 3.55 (td, J = 10.2, 7.5 Hz, 2H), 3.40 (t, J = 6.0 Hz, 1H), 3.34 (dt, J = 12.1, 6.1 Hz, 2H), 3.27 – 3.14 (m, 2H), 2.40 (s, 1H), 1.92 (d, J = 10.7 Hz, 3H), 1.57 – 1.46 (m, 4H), 1.31 – 1.24 (m, 2H), 1.19 (s, 9H). Compound 4.4 (60
mg, 0.052 mmol) and **4.5** (70 mg, 0.092 mmol) were combined in a flask, co-evaporated three times with toluene and dried. 4Å AW-MS (7 rods) were added to the flask. Then, the compounds were dissolved in DCM (3 mL) and the solution was cooled to -40 °C and stirred for 3 h after the addition of TMSOTf (2 µL, 0.011 mmol). The reaction was quenched by addition of a few drops of Et₃N, filtered through a pad of celite and concentrated in vacuo. Purification with flash column silica gel chromatography (hexane:ethylacetate = 9:1 to 1:1) afforded trisaccharide **4.7** (55 mg, 0.032 mmol, 62%). ¹H NMR (700 MHz, CDCl₃) δ 7.40 – 7.18 (m, 45H), 5.19 (d, J = 19.1 Hz, 2H), 5.12 (dd, J = 10.1, 8.0 Hz, 1H), 4.99 – 4.92 (m, 3H), 4.90 (t, J = 8.7 Hz, 1H), 4.70 (d, J = 12.1 Hz, 1H), 4.66 (d, J = 11.3 Hz, 1H), 4.61 – 4.55 (m, 4H), 4.61 – 4.48 (m, 4H), 4.54 – 4.49 (m, 1H), 4.45 (d, J = 11.8 Hz, 3H), 4.39 – 4.37 (m, 1H), 4.34 (d, J = 12.1 Hz, 1H), 4.32 – 4.26 (m, 1H), 4.16 (d, J = 12.0 Hz, 1H), 4.12 (d, J = 10.7 Hz, 2H), 4.00 (d, J = 2.4 Hz, 1H), 3.94 (d, J = 3.0 Hz, 2H), 3.79 (d, J = 27.1 Hz, 1H), 3.70 – 3.66 (m, 2H), 3.66 – 3.63 (m, 1H), 3.58 (dt, J = 17.6, 7.1 Hz, 1H), 3.51 (dd, J = 7.7, 6.0 Hz, 2H), 3.45 – 3.41 (m, 1H), 3.37 (t, J = 4.9 Hz, 1H), 3.36 – 3.32 (m, 3H), 3.28 – 3.24 (m, 1H), 3.21 – 3.17 (m, 1H), 2.00 (d, J = 18.6 Hz, 3H), 1.59 – 1.46 (m, 4H), 1.37 – 1.24 (m, 2H), 1.19 (s, 9H). ¹³C NMR (176 MHz, CDCl₃) δ 176.57, 162.12, 138.86, 138.83, 137.96, 137.86, 137.80, 128.70, 128.66, 128.62, 128.58, 128.53, 128.45, 128.43, 128.40, 128.37, 128.34, 128.26, 128.20, 128.09, 128.07, 128.01, 127.97, 127.90, 127.87, 127.78, 127.73, 127.50, 127.45, 127.37, 101.07, 100.21, 99.62, 92.74, 80.62, 79.28, 78.15, 75.44, 75.41, 75.11, 74.99, 74.16, 73.62, 73.61, 73.40, 73.27, 72.81, 72.77, 72.72, 72.52, 72.20, 72.09, 69.15, 68.48, 68.29, 67.27, 55.86, 38.91, 29.27, 27.50, 23.24, 21.00. ESI HR-MS: m/z [M+Na]⁺ calcd for C₉₀H₅₁Cl₃N₂NaO₂₀: 1735.6380; Found: 1735.6472.
Aminopentyl \((2\text{-acetamido}-2\text{-deoxy-}\beta\text{-d-galactopyranoside})-(1\rightarrow4)-\beta\text{-d-galactopyranosyl}-(1\rightarrow4)-\beta\text{-d-glucopyranoside} \; 4.1\)

Compound \textbf{4.7} (133 mg, 0.076 mmol) was dissolved in xylene (2.5 mL). Bu\textsubscript{3}SnH (100 mL, 0.37 mmol) and AIBN (3 mg, 0.018 mmol) were added and the solution was heated to 90 °C for 2 h. The mixture was then allowed to cool to room temperature and the solvent was removed in vacuum. Silica column chromatography (hexane:ethylacetate = 9:1 to 1.5:1) afforded the trisaccharide (130 mg, 0.0807 mmol) that was taken to the next step. The compound was dissolved in THF/MeOH/H\textsubscript{2}O (4 mL, 2/1.8/0.2) and the reaction was heated to 60 °C. Thus, KOH (500 \(\mu\)L of a 1M solution in water) was added and the mixture was stirred overnight. The reaction was cooled to room temperature, quenched with amberlyte IR-120, filtered through celite and concentrated. Finally, a portion of the crude residue (56 mg, ~0.038 mmol) was dissolved in MeOH/H\textsubscript{2}O/THF (11 mL 8/2/1) and Pd/C (50 mg) was added. The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by u.e was purified by solid phase extraction (Sep-pak C-18, water) to give \textbf{4.1} (17 mg, 0.027 mmol, 55% over three steps). \(^1\)H NMR (600 MHz, D\textsubscript{2}O) \(\delta\) 4.64 (d, \(J = 8.3\) Hz, 1H), 4.50 (d, \(J = 8.2\) Hz, 1H), 4.45 (d, \(J = 7.8\) Hz, 1H), 4.11 (s, 1H), 3.99 (d, \(J = 12.2\) Hz, 1H), 3.96 – 3.89 (m, 3H), 3.86 – 3.55 (m, 14H), 3.44 – 3.40 (m, 1H), 3.31 (t, \(J = 8.6\) Hz, 1H), 2.96 (t, \(J = 7.5\) Hz, 2H), 2.07 (s, 3H), 1.72 – 1.62 (m, 4H), 1.51 – 1.43 (m, 2H). \(^{13}\)C NMR (151 MHz, D\textsubscript{2}O) \(\delta\) 102.91, 102.61, 101.91, 78.46, 76.08, 74.77, 74.72, 74.31, 70.08, 60.99,
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60.62, 59.98, 39.45, 28.14, 27.18, 22.07. ESI-MS: \( m/z \) [M+H]\(^+\) calcd for \( \text{C}_{25}\text{H}_{47}\text{N}_{2}\text{O}_{16} \): 631.292; Found: 631.2, 653.2 [M+Na]\(^+\).

\[
N-\text{(Benzyl)- Benzylloxycarbonyl-5-aminopropetyl} \quad (5\text{-Acetamido-3,5-dideoxy-D-glycero-D-galacto-non-2-ulopyranosylonic-acid)-(2→3)4,6-di-O-benzyl-\alpha-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-\alpha-D-glucopyranoside} \quad 4.8
\]

To a solution of sialyl galactose donor \( 2.2 \) (220 mg, 0.210 mmol) and glucose \( 4.2 \) (93 mg, 0.131 mmol) in \( \text{CH}_2\text{Cl}_2 \) (2.0 mL) was added TMSOTf (4.7 \( \mu \)L, 0.026 mmol) at 0 °C under Ar atmosphere, in presence of 4Å AW-MS (5 rods). After stirring for 1.5 h at 0 °C, the mixture was neutralized with a few drops of Et\(_3\)N and then concentrated. Purification with flash column silica gel chromatography (hexane:ethylacetate = 2:1 to 1:1) gave trisaccharide \( 4.8 \) (120 mg, 0.16 mmol, 80%). \(^1\)H NMR (600 MHz, CDCl\(_3\)) \( \delta \) 7.40 – 7.10 (m, 28H), 5.59 (ddd, \( J = 8.8, 6.2, 2.7 \) Hz, 1H), 5.36 (ddd, \( J = 8.6, 2.4, 1 \) Hz, 1H), 5.26 (dd, \( J = 10.1, 7.8 \) Hz, 1H), 5.21 – 5.12 (m, 3H), 4.95 (d, \( J = 11.6 \) Hz, 1H), 4.92 (t, 1H), 4.87 – 4.78 (m, 3H), 4.62 (q, \( J = 12.1 \) Hz, 2H), 4.56 (d, \( J = 11.6 \) Hz, 1H), 4.47 (d, \( J = 10.8 \) Hz, 2H), 4.40 – 4.33 (m, 2H), 4.30 – 4.25 (m, 3H), 4.16 (d, \( J = 11.7 \) Hz, 1H), 4.06 (dd, \( J = 20.8, 10.4 \) Hz, 1H), 3.99 (dd, \( J = 12.4, 6.2 \) Hz, 1H), 3.91 – 3.84 (m, 2H), 3.82 (dd, \( J = 10.8, 2.4 \) Hz, 1H), 3.70 (dd, \( J = 11.0, 5.7 \) Hz, 1H), 3.67 (s, 3H), 3.60 – 3.54 (m, 2H), 3.47 (t, \( J = 8.7 \) Hz, 2H), 3.43 (d, \( J = 2.4 \) Hz, 1H), 3.37 (m, 1H), 3.25 (dd, \( J = 9.2, 5.2 \) Hz, 2H), 3.16 (s, 1H), 2.64 (dd, \( J = 12.7, 4.5 \) Hz, 1H), 2.17 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.94 – 1.88 (m, 4H), 1.86 (s, 3H). \(^{13}\)C
NMR (151 MHz, CDCl₃) δ 171.00, 170.56, 170.46, 170.03, 169.79, 169.77, 168.19, 139.13, 138.94, 138.69, 138.34, 128.62, 128.34, 128.32, 128.21, 128.11, 127.92, 127.64, 127.57, 127.47, 127.41, 100.90, 100.85, 97.73, 81.37, 75.58, 75.13, 74.79, 74.74, 74.70, 73.34, 73.15, 72.93, 72.57, 72.39, 71.20, 69.20, 68.90, 68.16, 67.77, 67.49, 67.25, 62.44, 52.95, 49.30, 37.75, 29.25, 23.27, 23.23, 21.38, 21.17, 20.95, 20.91, 20.84, 20.81. ESI-MS: $m/z$ [M+NH₄]⁺ calcd for C₈₄H₁₀₄N₃O₂₇: 1586.69; Found: 1586.1, 792.5 [M+H+NH₄]²⁺.

Aminopentyl (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic-acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside

![Chemical structure of Aminopentyl](image)

Compound 4.7 (150 mg, 0.076 mmol) was dissolved in methanol (1mL) and a few drops of dichloromethane. A solution of Na in methanol (250 µL, 0.015 mmol) was added and the reaction was stirred overnight at room temperature. The reaction was quenched with amberlite IR-120, filtered over celite and
concentrated. The crude residue was dissolved in methanol/water (7 mL, 8/2) and 1M aqueous KOH (300 μL) was added. The reaction was stirred at 60 °C overnight, quenched with amberlite IR-120, filtered over celite and concentrated under reduced pressure to give 84 mg (0.064 mmol) of compound. Finally, a portion of the crude residue (70 mg, 0.053 mmol) was dissolved in methanol/water (7.5 mL, 8/2) and acetic acid (120 μL) was added to the solution followed by Pd/C (60 mg). The mixture was sonicated under argon flow for 20 min, then under hydrogen flow for an additional 20 min and finally stirred under an atmosphere of hydrogen for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated and lyophilized. The crude residue was purified by preparative HPLC using a 0 to 20% gradient (CH₃CN in water + 0.1% TFA, Pyramid nucleodur C-18 column) over 35 min to afford the 3.18 (34 mg) in 76% yield over three steps. Characterization data were consistent with data reported in Chapter 2 for the automated synthesis of 3.18.

**TA-PEG$_{2000}$-COOH 4.13**

A solution of TA-PEG$_{2000}$-NH$_2$ (2.35 g, ~1.07 mmol) (11) succinic anhydride (213 mg, 2.128 mmol) and triethylamine (375 μL, 2.69 mmol) in CH$_2$Cl$_2$ (10 mL) was stirred at room temperature overnight under an atmosphere of Ar. The solvent was removed in vacuum and the crude was purified by flash column silica gel chromatography (chloroform:methanol = 9:1) to provide compound 4.13 (700 mg, 0.3 mmol, ~30%). $^1$H NMR (400 MHz, CDCl$_3$) δ 6.77 (s, 1H), 6.14 (s, 1H), 6.14 (s, 1H), 3.87 – 3.55 (m), 3.56 – 3.52 (m, 5H), 3.44 (dd, J = 9.9, 5.0 Hz, 4H), 3.14 (m, 2H), 2.72 –
2.61 (m, 2H), 2.57 – 2.49 (m, 2H), 2.45 (dt, J = 12.5, 6.5 Hz, 1H), 2.18 (t, J = 7.4 Hz, 2H), 1.90 (m, 1H), 1.76 – 1.60 (m, 4H), 1.51 – 1.39 (m, 2H).

**DHLA-PEG\textsubscript{2000}-COOH**

Compound **4.13** (420 mg, 0.20 mmol) was dissolved in ethanol (4 mL). The solution was cooled to 0 °C then NaBH\(_4\) (34 mg, 0.89 mmol) in \(\text{H}_2\text{O}\) (1.5 mL) was added drop wise with stirring. The solution was warmed up to room temperature and stirred for additional 6 h. 1M HCl was added until pH became acidic (pH~2). The solution was extracted with chloroform (4 times), then the organic layers were combined, dried over MgSO\(_4\), filtered and concentrated under reduced pressure. The residue was purified on silica gel (chloroform:methanol = 8:1) to give compound **4.14** (170 mg, 0.080 mmol, ~40%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.76 (s, 1H), 6.17 (s, 2H), 3.63 – 3.56 (m), 3.53 – 3.48 (m, 4H), 3.42 – 3.36 (m, 4H), 2.94 – 2.82 (m, 1H), 2.64 – 2.56 (m, 4H), 2.49 (m, 2H), 2.15 (t, J = 7.3 Hz, 2H), 1.86 (m, 1H), 1.74 – 1.49 (m, 7H), 1.35 – 1.24 (m, 2H).

**QD\textsubscript{645}-DHLA-PEG\textsubscript{2000}-COOH 4.15**
CdSe/ZnS quantum dots with an emission maximum at 645 nm were synthesized using published procedure. (11) Cadmium oxide (0.1 g, 0.78 mmol) and lauric acid (0.6 g, 3.0 mmol) were mixed at 160° to form an homogeneous and clear solution. Trioctylphosphine oxide (TOPO) (4 g, 10 mmol) and hexadecylamine (4 g, 16.5 mmol) were added and the mixture was heated to 260 °C, under nitrogen flow, for 4 min. A selenium stock solution, prepared from selenium (0.06 g 0.78 mmol) in trioctylphosphine (TOP) (4 mL, 8.9 mmol), was rapidly injected into the reaction mixture at 260 °C. Temperature was kept at 260 °C and after 5 min the reaction mixture was cooled to 180 °C while stirring. The forming CdSe particles were coated in situ with ZnS layers by dropwise addition of a mixture of TOP (4 mL), diethylzinc (1M solution in hexane, 4.0 mL), and hexamethyldisilathiane (0.35 mL, 1.6 mmol) over 2h. The solution was then cooled to room temperature and the particles were purified by precipitation in CHCl₃/MeOH. Thus, 50-100 mg of particles were mixed with ~120 mg of compound 4.14 in ethanol (500 µL) under an atmosphere of argon and the suspension was heated to 65 °C for several hours. Finally, the particle were precipitated using a mixture of ethanol/chloroform/hexane (1:0.5:5) and the supernatant was discarded. The procedure was repeated a few times to remove excess of unreacted ligand and obtain pure 4.15.

QD-DHLA-PEG₂₀₀₀-Gg₃ 4.16

\( N \)-Hydroxysuccinimide (3 mg, 29 µmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (6 mg, 26 µmol) were added to a solution of compound 4.15 (0.5 µM) in 1mL of millipore water and agitated at room temperature for 40 min before Gg3 4.1 (2.4 mg, 3.8 µmol) was added. The solution was agitated at room temperature overnight then ethanol amine (5 µL) was added to quench the reaction. The solution was purified by dialysis with a
cutoff range of 1KDa. The final sample concentration was estimated by using a previously published procedure.

QD-DHLA-PEG$_{2000}$-Gm3 4.17

$N$-Hydroxysuccinimide (3 mg, 29 $\mu$mol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (6 mg, 26 $\mu$mol) were added to a solution of compound 4.15 (0.5 $\mu$M) in 1mL of millipore water and agitated at room temperature for 40 min before Gm3 3.18 (3.5 mg, 4.8 $\mu$mol) was added. The solution was agitated at room temperature overnight then ethanol amine (5 $\mu$L) was added to quench the reaction. The solution was purified by dialysis with cutoff range of 1KDa. The final sample concentration was estimated by using a previously published procedure.

Photophysical Properties

The emission spectra of QDs were recorded and are depicted in Table 1. Upon excitation at 400 nm a maximum emissions were observed at 645-648 nm. Absorbance measurement of QD solution were performed by a Cary 100 UV-visible scan spectrophotometer (Varian, Australia).
Transmission Electron Microscopy (TEM)

A drop of the QD-DHLA-PEG<sub>2000</sub>-Gg3 4.16 solution was deposited on a 400 mesh copper grid coated with nitrocellulose followed by carbon evaporation. The grids were observed in a Philips CM 120 microscope at an accelerating voltage of 200 kV. Figure 1 shows typical TEM images of monodispersed QD-DHLA-PEG<sub>2000</sub>-Gg3.
Estimation of Antigen Loading per QD Particle

The loading of Gg3 (or Gm3) CdSe-ZnS was determined by quantitative NMR(27) using formic acid as internal standard. A sugar functionalized-QD solution (500 µL, 1.4 µM) in D₂O containing formic acid (2.6 mM) was prepared. A T₁ measure experiment was performed to estimate the relaxation time (T₁max). Thus, a ¹H was performed (number of scans 512, relaxation delay 10s) and the antigen concentration was estimated by comparing the integral value for the Gg3 acetamido signal at 2.15 ppm with the integral value for the aldehydic formic acid proton signal at 8ppm. The number of sugar molecule per QD particle was calculated from the ratio of the concentration of sugar and the concentration of CdSe-ZnS QD particles. Compounds 4.16 and 4.17 showed an average number of 400 sugars molecules per quantum dot.
In vitro Interaction Analysis

Murine B16-F10 melanoma cells (ATCC, CRL-6475, ~10^5 cells) were grown on a 24 well plate overnight in fully supplemented DMEM (PAN biotech). Compounds 4.16 or 4.17 in DMEM were added in different concentrations and incubated for 30 min. Cells were thus detached by shear force and washed in phosphate buffered saline (PBS) (PAN biotech) and analyzed by flow cytometry (BD Canton II). Emission was measured at 660 nm (APC channel).

For confocal fluorescence microscopy, 10^5 murine B16-F10 melanoma cells were grown in a 1µl-Slide VI ibiTreat (ibidi) overnight and the two compounds were added for 30 min. Nucleus was stained with DAPI and the cells were fixed in 4% parafomaldehyde and then the phospholipid phosphatidylserine (PS) on the fixed cells membrane was stained with Annexin V-FITC (bioscience) to. Confocal fluorescence photos were taken under 40X magnification (LSM 700 Carl Zeiss).
4.6 References


Automated solid phase synthesis platforms have the great potential to advance biological science since they can contribute, in a rapid and efficient manner, pure molecules for biological studies. In Chapter 1, the synthesis of carbohydrates was introduced, followed by the description of the first automated solid phase synthesis platform for oligosaccharide assembly.\(^{(1)}\)

A simple analysis was performed to understand the advantages of an automated solid phase approach over classical solution phase strategies. In this context, the limits of the current automated oligosaccharide synthesis platform were also evaluated. With these limitations in mind, we identified several aspects of the current technology that require improvement in order to approach the generality of oligopeptide and oligonucleotide automated assembly.

Investigation into the following areas was undertaken: i) identification of a library of stable building blocks amenable to automated assembly; ii) development of new strategies for the automated assembly with a focus on a new solid phase linker that could simplify the cleavage and deprotection of resin bound oligosaccharides; iii) application of deprotected oligosaccharides to the preparation of useful molecular glycoprobes.
5.1 Building Block Design and Synthesis

In the automated assembly of oligosaccharides, the stereochemistry and regiochemistry of the glycosidic linkage to be generated is influenced by the nature and positioning of the protecting groups on the monosaccharide (or disaccharide) building blocks. In Chapter 2 we proposed a collection of general building blocks functionalized with certain protecting groups, which would account for the most common “glycosidic linkages”, as suggested by statistical analysis.\(^{(2)}\)

Particular relevance was given to the development of building blocks for sialic acids. N-Acetylneuraminic acid (Neu5Ac) is the most abundant member of the sialic acid family in mammals.\(^{(3)}\) In humans, Neu5Ac is mostly linked to either the C3- or C6-position of galactose. Glycosylations with Neu5Ac building blocks are generally troublesome, especially in the context of solid-phase synthesis. However, we demonstrated that galactal 2.24 (Figure 1), due to its steric and electronic properties, can be used for highly efficient couplings with different sialic acid glycosyl phosphites (2.20 is provided as an example in Figure 1). The disaccharides obtained were further manipulated at the anomeric position to afford disaccharide building blocks that can be subsequently used in solution phase\(^{(4)}\) as well as solid phase synthesis for the generation of important antigens.
Conclusions

Figure 1. Reagents and conditions: i: TMSOTf, -78 °C, DCM; then Phl(OAc)₂, BF₃·Et₂O, CH₂Cl₂, -40 °C; then Ac₂O, pyridine, 90%. ii: N₂H₄·AcOH, DMF, 92%. iii: CF₃C(NPh)Cl, Cs₂CO₃, CH₂Cl₂, 92%.

A number of additional monosaccharide building blocks were prepared by sequential protection-deprotection strategies as described in Chapter 2 (Figure 2). For example, galactose and fucose building blocks were generated using conventional routes in 7 to 12 steps on a multigram scale (2.3, 2.4, 2.5, Figure 2). For the preparation of glucose-based building blocks, including glucosamines (2.6 to 2.9, Figure 2) we expanded on a recently introduced one-pot method for the streamlined synthesis of differentially protected thioglycoside building blocks. (5)

Figure 2. Building blocks for oligosaccharide synthesis.
5.2 Automated Solid Phase Synthesis and Glycoprobe Construction

Chapter 3 describes the development of an improved platform for automated synthesis of oligosaccharides. An ideal fully automated synthesis platform utilizes building blocks as an input for the generation of active biomolecules, possibly provided with a proper spacer. With these points in mind, linker 3.1 was designed (Scheme 1). Linker 3.1 is tolerant to different glycosylation promoters and deprotection reagents. In addition, it is removable from the solid support under mild conditions. Glycosylation cycles were performed using a new synthesizer developed in the laboratory. The reactivity of each building block was evaluated in order to create reliable automated glycosylation-deprotection protocols of general applicability. The creation of modules for the partial deprotection of the oligosaccharide already on the solid support was emphasized in order to minimize any manual solution phase post-processing. As an example, trichloroacetyl (TCA) reduction and ester hydrolysis were automated and performed on the solid support as showcased in the synthesis of Sialyl Lewis\textsuperscript{x} (Scheme 1).

A series of three glycosylation-deprotection modules with building blocks 2.6, 2.2 and 2.5 afforded the branched tetrasaccharide 3.19 after radical mediated TCA reduction and methanolysis in only 1.5 days. The latter step simultaneously cleaves the product from the solid support. Significant effort was directed towards the establishment of general HPLC protocols for the purification of partially deprotected oligosaccharides. Thus, pure 3.19 could be easily isolated (peak at 10.3 min in the chromatogram) and the final compound 3.20 was obtained by hydrogenolysis. Based on design, during hydrogenolysis, the carbamate group on the linker is cleaved revealing an amino group on a five-carbon atom spacer that can be employed in conjugation.
Conclusions

Scheme 1. Automated synthesis of SLX 3.18. Reagents and conditions: i (Thio-Fmoc module): a) NIS, TIOH, dioxane, DCM, -40 °C to -20 °C, 40 min; b) Piperidine, DMF. ii (Imidate-Lev module): a) TMSOTf, DCM, 0 °C, 2 h. b) NH₂NH₂·H₂O, AcOH, pyridine, DCM. iii (Imidate-ether module): TMSOTf, Et₂O, -10 °C, 1 h. iv (Reduction module): AIBN (cat.), Bu₃SnH (10 eq.), Xylene, 90 °C. v (Cleavage module): NaOMe, MeOH, DCM, 1.5 h. vi: KOH, MeOH, H₂O, THF, 60 °C, 51% (solution phase). vii: Pd/C, H₂, MeOH/H₂O, cat. AcOH, 30% (solution phase).

Figure 3. LC-MS of crude 3.19. Conditions: 20 to 60% A in B; A = CH₃CN + 0.1% TFA + 20% i-ProOH, B = water + 0.1% TFA + 20% i-ProOH over 20 min.

The principles described for the synthesis of 3.20 are of general applicability. Using similar strategies, we synthesized a small library of diverse oligosaccharides that comprises 1,4 and 1,6-hexaglucosamines (3.10, 3.6, bacterial antigens) as well as 1,6-dodecaglucosamines (3.8), Gm3, sialolactosamine (3.18, 3.15, Sialosides) and the iGb3 trisaccharide 3.11 belonging to the globo series (Figure 4). All these biologically relevant antigens required the generation of difficult linkages or were chosen to show the versatility of the new synthetic platform for the preparation of very large compounds. In this
Conclusions

regard, compound 3.8 is to date one of the biggest oligosaccharides obtained by automated solid phase synthesis.

![Figure 4. Oligosaccharides library prepared by automated solid phase synthesis.](image)

Having demonstrated that the automated platform is capable of providing antigens ready for applications in rapid and efficient manner, we directed our attention to the construction of molecular glyco-tools, in order to fully prove the usefulness of the platform.(6) The first application was the preparation of a sugar-conjugate vaccine candidate (Scheme 2). Using squarate chemistry, the immunogenic carrier protein BSA was functionalized with the bacterial antigen 1,4-Hexaglucosamine 3.10 to afford the conjugate 3.22, that will be successively used for immunization studies.
Glycan microarrays constitute a powerful tool for glycobiologists. A selection of the obtained oligosaccharide library was printed on NHS activated glass slides and binding with WGA (wheat germ agglutinin) lectin, specific for GluNAc was evaluated (Figure 5).

A preliminary investigation on the possible use of carbohydrate-carbohydrate interactions (CCI’s) for cellular imaging was presented in Chapter 4. CCI’s can take place between two multivalent displays of sugars. It was shown for example that Gm3 gangliosides can specifically interact with Gg3 gangliosides.\(^7\) In order to explore the applicability of this phenomenon, the sugar portions of Gg3 and
Gm3 gangliosides were synthesized and conjugated to polyethylene glycol (PEG)-coated quantum dots (QDs) prepared by modifying established procedures.\(^8\) The specific interaction of sugar capped QDs with murine B16 melanoma cells, which over express Gm3, was evaluated (Figure 6). The results obtained by flow cytometry and confocal microscopy seemed to be in accordance with the hypothesis that Gg3 capped QDs bind selectively to Gm3 on the cell surface, anticipating for the first time the possibility of exploiting CCIs for cellular imaging purposes.

![Figure 6. CCI's binding assay.](image)

### 5.3 Outlook

The automated platform introduced in this thesis proved to be competent for the fast synthesis of a diverse library of oligosaccharides that are ready for biological applications. In order to expand the scope of the methodology, the synthesis of additional oligosaccharides related to the ones depicted in Figure 4 should be undertaken. A particular emphasis could be given to the family of sialosides,
which mediate very important physiological processes.\(^{(9)}\) In this context, using the approach described in Chapter 3, an automated solid phase synthesis of a full Sialyl Lewis\(^x\) hexasaccharide 5.3 (Figure 7) represents the first challenge. Slight modifications of the synthetic strategy *en route* to compound 5.3, can afford related classes of biologically active compounds like 5.1 and 5.2, which are given as examples.

Expanding the library of available building blocks will further widen the applicability of the automated solid phase platform. In Chapter 2 the synthesis of a capping sialic acid \(\alpha-(2-6)\) galactose building block 5.4 was described. A number of different important oligosaccharides related to viral infection \(^{(10)}\) could be generated employing compound 5.4 during automated solid phase synthesis of different glycans.

**Figure 7.** Possible sialic acid containing targets for automated synthesis.

Great opportunities can arise connecting the technology disclosed in the present work with the well-established platforms for solid phase peptide synthesis. The synthesis of glycoproteins is the objective of an intense area of research nowadays, since mammalian oligosaccharides are mostly found in the form of
protein glycoconjugates, and access to pure forms of these compounds is particularly difficult. (11) Currently, glycopeptides are obtained introducing presynthesized “glycosylated amino acids” during peptide elongation. Since most of the standard methodologies for the synthesis of peptides are compatible with sugar assembly protocols, one can speculate that a single synthesis instrument could be used to perform peptide elongation, site selective glycosylation and finally sugar elongation, resulting in a more streamlined and versatile platform for glycoprotein synthesis.

As the ultimate goal of automated oligosaccharide synthesis is to enable the evaluation of structure-activity relationships of carbohydrates in biological systems, one of the future goals should be the generation of new molecular glyco-tools and vaccine candidates starting from the identification of interesting oligosaccharide system targets.

Our preliminary investigations on the use of CCI’s for cellular imaging with glycan capped QDs revealed a series of interesting questions on the nature of these interactions. Additional experiments are request to corroborate our results and in particular, to exclude the possibility that some protein receptor like galectin-1, specific for galactose residues contained in Gg3, could mediate the specific interaction with the surface of B16 surface, instead of ganglioside Gm3. Inhibition experiments based on the blocking of Gm3 residues on the cell membrane with blocking antibodies will be performed to rule this out. An additional galectin-1 knockout model for B-16 melanoma cells can be envisaged in order to definitely exclude the interaction with a protein receptor. This work, could ultimately result in a deeper understanding of the very unique phenomenon of CCI’s that could successively be exploited for biomedical applications like cellular imaging.
References

Appendix

Acknowledgments

The work presented in this chapter was performed by Dr. Takafumi Ohara (synthesis), Dr. Alexander Adibekian (synthesis and design of the synthetic route), Mr. Davide Esposito (design of the synthetic route, optimization of the organocatalytic reaction, compounds purifications).
Towards the Synthesis of a *Yersinia pestis* Cell Wall Polysaccharide: Enantioselective Synthesis of an L-*glycero*-D-\*manno*-heptose Building Block (6)

Plague is considered to be a re-emerging disease. Furthermore, plague is also recognized as a potential vector of bio-terrorism. It has been used, or considered for use, as a biologic weapon on several occasions.\(^1,2\) We sought therefore to access a candidate vaccine against plague using a synthetic approach. In this direction, in collaboration with Dr. T. Ohara and Dr. A. Adibekian, a program was started for the synthesis of the core pentasaccharide related to the cell wall lipopolysaccharide (LPS) extracted from different bacterial strains of *Yersinia pestis* (Figure 1), the causative agent of plague. Indeed, similar oligosaccharides showed promising immunogenic properties and, moreover, it has been shown that carbohydrates are able to induce specific antibody response, if properly conjugated to a suitable immunogenic carrier.\(^3,4\)

**Figure 1.** Structure of the *Yersinia pestis* core pentasaccharide.
The capping trisaccharide part is entirely composed of α-linked L-glycero-D-manno-heptoses and represents synthetically the most challenging part of the core. Our first effort towards the synthesis of the full core pentasaccharide was the establishment of a robust synthesis of differentially protected L-glycero-D-manno-heptoses. A de novo strategy\(^\text{(5)}\) that used asymmetric reactions to construct the monosaccharide carbon skeleton and set the sugar absolute stereochemistry was chosen. In this regard, two enantioselective aldol reactions provided a seven carbon backbone in which all the hydroxyl groups are already functionalized (Scheme 1). A few additional steps were used to functionalize compound A.4 yielding a building block\(^\text{(6)}\) that is currently used in the total synthesis of a bacterial core pentasaccharide.

**Scheme 1.** Key steps in the synthesis of L-glycero-D-manno-heptose.
References


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