Assembly of Oligosaccharides: from the *De novo* Synthesis of Building Blocks to the Automated Solid Phase Synthesis

Inaugural-Dissertation
to obtain the academic degree
Doctor rerum naturalium (Dr. rer. nat.)
submitted to the Department of Biology, Chemistry and Pharmacy
of Freie Universität Berlin

by

Oliviana Sabina Calin
from Craiova, Romania

2012
Acknowledgments

The following work was completed from September 2009 until December 2012 at the Department for Biomolecular Systems of the Max Planck Institute of Colloids and Interfaces and at the Department of Biology, Chemistry and Pharmacy of Freie Universität Berlin under the supervision of Professor Dr. Peter H. Seeberger.

1st Reviewer: Professor Dr. Peter H. Seeberger

2nd Reviewer: Professor Dr. Hans-Ulrich Reißig

Date of Defence: 23.04.2013
Acknowledgments

I would like to express my deepest appreciation and gratitude to everyone that has inspired, supported and encouraged me in these last three years. First, I would like to thank my supervisor, Professor Dr. Peter H. Seeberger for his trust and support throughout my thesis. Through his guidance, Professor Seeberger promoted a unique research environment that was very fruitful for the achievement of integrity, scientific development and generation of new ideas.

I am grateful to Professor Dr. Hans-Ulrich Reißig for generously agreeing to co-chair my PhD examination.

I would like to thank to Marie Curie ITN FP7 project CARMUSYS (PITN-GA-2008-213592) for financial support.

Dr. Claney Lebev-Perreira, Dr. Kerry Gilmore, Dr. Mattan Hurevich and Dr. Steffen Eller are kindly acknowledged for proof reading parts of this thesis.

I would like to express my gratefulness to all the members of the three groups I integrated during my Ph.D.: the de novo group, the automation group and the vaccine group. In particular, I would like to thank Dr. Rajan Pragani for his introduction to asymmetric synthesis and Dr. Steffen Eller for helping me to familiarize with solution and automated solid phase synthesis of oligosaccharides. Our fruitful discussions about chemical and non chemical problems led not only to succesfull collaborations, but also to strong friendships.

Thanks to Dr. Mark Schlegel for the help with HPLC and MALDI. Ms. Kathrin Stavenhagen, Mr. Kathirvel Alagesan, Mr. Kay-Marcus Oetter are acknowledged for the help with MALDI measurements.

An important help and back-up over the years were my friends here at MPI. In particular, I would like to mention: Ms. Maha Maglinao, Dr. Sharavathi Parameswarappa, Dr. Srisuda Tirakarn, Dr. Daniel Kopetzki, Dr. Kerry Gilmore, Dr. Steffen Eller, Dr. Mattan Hurevich, Dr. Claney Lebev-Perreira, Mr. Bopanna Monnanda, Mr. Kathirvel Alagesan, Dr. Francois Lévesque, Dr. Mark Schlegel, Dr. Rajan Pragani and Dr. Yoshikazu Suzuki. Thank you for
the great time we had working together, the adventures we had out of the lab and the support you offered to me over the years. These moments made this experience at MPI unforgettable.

Finally, but most importantly, I would like to thank my parents, Viorica and Ion Calin for all their unconditional support and encouragement throughout these years. Thank you.
Publications

Parts of this thesis have been published and communicated:

**Publications:**


**Oral Presentations:**


**Poster Presentations:**

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Summary

Besides nucleic acids and proteins, carbohydrates play an essential role in different biological events. Their interaction with lectins and antibodies mediate processes involved in immune response. Carbohydrates like lipopolysaccharides, major components of the outer membrane of Gram negative bacteria, consist of lipid A, core polysaccharide and O-antigen. While the structures of lipid A and the core-polysaccharide are not particularly bacteria specific, the structure of the O-antigen does vary dramatically and often contains rare sugars. These rare sugars are not found in humans and hence presumed as threat by the immune system. The first part of this thesis focuses on the stereocontrolled synthesis of such rare sugars from smaller precursors via carbon-carbon bond forming reactions, referred to as de novo synthesis. These rare sugars are highly desireable as they are required for the assembly of the O-antigen repeating unit of Gram negative bacteria. The second part of this thesis is dedicated to study the upper limits of the automated solid phase synthesis of oligosaccharides, a technique that significantly facilitates the synthetic process of carbohydrates.

After an exhaustive introduction on oligosaccharide synthesis in Chapter 1, Chapter 2 describes a practical de novo approach towards fully functionnalized L-colitose (3,6-dideoxy-L-xylo-hexopyranose) glycosylating agent starting from inexpensive, commercially available (S)-ethyl lactate. This rare sugar is specific to the O-antigen of a large spectrum of Gram negative bacteria such as *Escherichia coli* O111, *Salmonella enterica*, *Salmonella adelaide*, *Salmonella greenside* and *Vibrio cholerae*. The divergent route allowed for the preparation of two other congeners of L-colitose: a 2-epi-L-colitose building block and a L-rhodinose building block. The synthetic route centered on a diastereoselective Cram-chelated allylation that provided a common homoallylic alcohol intermediate. Oxidation of this common intermediate completed the synthesis of these three monosaccharide building blocks.

Taking advantage of the synthetic route established in the second chapter, Chapter 3 describes the assembly of pentasaccharide repeating unit of the O-antigen of *Escherichia coli*
O111, serotype that causes enteropathogenic, enterotoxigenic and enterohemorrhagic disease in humans. Since the excessive use of antibiotics in livestock industry resulted in problems related to multidrug-resistance, a vaccination therapy against *E. coli* O111 would be desirable. The key step to the synthetic approach was a [3+1+1] glycosylation that introduced the acid-labile colitose residues at a late stage of the synthesis. In anticipation of immunological studies, the pentasaccharide was functionalized at the reducing end with an amino-spacer to provide a handle for subsequent conjugation to a carrier protein.

The fourth chapter of this dissertation presents the automated solid phase assembly of a 30mer \( \alpha-(1,6) \)-oligomannoside, the longest carbohydrate ever made by chemical synthesis.

This study was performed in an effort to test whether oligosaccharides that could be required for amplifying the weak carbohydrate-carbohydrate recognition receptor interaction, could be
quickly accessed by automated solid phase synthesis. To aid in the purification of the \( \alpha-(1,6) \)-oligomannosides, a catch-and-release strategy was developed allowing the tagging and selective attachment of the full-length product on magnetic beads. Following magnet-assisted decantation, release of the oligosaccharide from the solid support provided the target compound with a minimum of effort.
Zusammenfassung


Eine divergente Syntheseroute ermöglichte neben der Herstellung von L-Colitose auch die Zugänglichkeit eines 2-epi-L-Colitose und eines L-Rhodinosebausteins. Der zentrale Syntheseschritt war eine Cram-chelatkomplizierte Allylierung die zur gemeinsamen Zwischenstufe eines homoallylischen Alkohols führte. Durch Oxidation dieser Zwischenstufe konnten die drei Monosaccharidbausteine hergestellt werden.

Im vierten Kapitel dieser Dissertation wird die automatisierte Festphasensynthese eines α-1,6-verknüpften Oligomannosids mit 30 Monomeren behandelt. Bei dieser Verbindung handelt es sich um die längste Polysaccharidkette, die bis jetzt durch chemische Synthese erhalten werden konnte.
1. Oligosaccharide Synthesis

Carbohydrates, one of the three major classes of biopolymers, have been shown to be crucial for numerous biological processes including viral and bacterial infection, toxin interaction, angiogenesis and tumor cell metastasis, cell growth and proliferation, inflammation and immune response. In comparison with proteins and nucleic acids, the high degree of diversity and complexity presented by oligo- and polysaccharides, glycolipids and glycoproteins as well as glyco-containing antibiotics and natural products allows for the encoding of required information for a specific molecular recognition. To understand the biological functions involving carbohydrates, pure and well defined oligosaccharides and glycoconjugates are required. However, complex carbohydrates cannot be easily obtained from natural sources in acceptable purity and quantity due to microheterogeneity issues. Therefore, chemical and enzymatic syntheses provide a good alternative for accessing well defined oligosaccharides in sufficient quantities for biological studies.

Unlike oligopeptides/proteins and nucleic acids/oligonucleotides that can now be easily accessed by chemical and biological techniques, oligosaccharides are more difficult to synthesize because of variables like branched rather than linear and the type of connectivity α or β (Scheme 1). For example, starting from disaccharide 1 protected with the orthogonal protective groups PG\(^1\) and PG\(^2\), the two nucleophiles 2 and 3 (glycosyl acceptors) can be accessed. Both nucleophiles can react with the intermediate oxocarbenium, generated by activation of glycosylating agent (glycosyl donor) 4 in presence of a Lewis acid, leading mainly to the thermodynamically favored α-linear and α-branched trisaccharides 5 and 6. The presence of a participating group, such as an ester at C2, favors the formation of the dioxocarbenium intermediate directing the attack of the nucleophile towards the formation of the β-glycosidic linkages and providing trisaccharides 7 and 8.
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Scheme 1. Challenges in oligosaccharide synthesis.

1.1. Synthesis of Building Blocks

The design and synthesis of building blocks presenting a strategically positioned hydroxyl group as a nucleophile for glycosylation, an appropriate leaving group at the anomeric position and suitable protecting groups on the remaining hydroxyls constitute crucial steps in the assembly of oligosaccharides. Fully functionalized monosaccharide building blocks can be
1. Oligosaccharide Synthesis

prepared via either a protection/deprotection pathway starting from cheap naturally occurring carbohydrates such as D-mannose, D-glucose, D-glucosamine, D-galactose and D-arabinose or a de novo chemistry from inexpensive commercially available linear molecules by stereoselective C-C bond formation. This section highlights recent regioselective protection strategies and relevant de novo synthetic pathways for accessing important building blocks.

1.1.1. Regioselective Protection of Monosaccharides

The protecting group pattern on a monosaccharide building block has a great influence on: a) the reactivity of the donor/acceptor, b) the stereoselectivity of glycosidic bond formation and c) the ease of final deprotection. The difficulty in differentiating the hydroxyl groups on a monosaccharide increases as the difference between their intrinsic reactivity decreases. While the reactivity of the primary and the secondary hydroxyl is sufficient different, the differentiation between secondary hydroxyl groups can be a challenge. Therefore, a lot of effort has been directed towards establishing efficient regioselective protection strategies.

Stepwise Protection Strategies

The traditional synthesis of monosaccharide building blocks relies on tedious protection/deprotection steps. To facilitate the construction of libraries of building blocks and to shorten the synthetic process, new strategies for the regioselective protection of hydroxyl groups have been developed.

For example, the dimethyltin dichloride catalyzed protection of sugars allowed the synthesis of orthogonally protected glucoside 9 to proceed in high yield and with high level of regioselectivity (Scheme 2). After installing regioselectively the benzoyl ester at C2 on methyl glucoside 10, monotosylation was achieved exclusively at C6 in the presence of a catalytic amount of Me₂SnCl₂. Metal-sugar interaction allowed further differentiation between C3 and C4 on diol 12 leading to tert-butoxycarbonylation of the C3-hydroxyl and phosphorylation of the C4-hydroxyl. Fully protected glucoside 14 can be further converted to 6-azido sugar 9 by simple treatment with sodium azide. This strategy shows how the relative reactivities of hydroxyl groups can be modulated by coordination with metal ions, thereby leading to orthogonally protected monosaccharides.
1. Oligosaccharide Synthesis

**Scheme 2.** Me$_2$SnCl$_2$ catalyzed regioselective protection of monosaccharides: a) BzCl, DIPEA, Me$_2$SnCl$_2$, THF, 82%; b) TsCl, DIPEA, Me$_2$SnCl$_2$, THF, 88%; c) Boc$_2$O, DIPEA, Me$_2$SnCl$_2$, DMAP, THF, 93%; d) ClP(O)(OPh)$_2$, pyridine, DMAP, CH$_2$Cl$_2$, 95% e) NaN$_3$, 15-crown-5, DMF, 50 °C, 95%.

Another orthogonal protection-deprotection strategy for synthesis of oligosaccharides libraries was introduced by Wong and co-workers. They described the synthesis of galactoside 15 functionalized with four selectively removable protecting groups, thereby enabling the branching of these positions (Scheme 3).

**Scheme 3.** Selective removal of the protecting groups on galactoside 15: a) NaHCO$_3$, MeOH/H$_2$O (5/1), 60 °C, 99%; b) TFA, CH$_2$Cl$_2$, -20 °C, 97%; c) N$_2$H$_4$/AcOH, THF/MeOH (10/1), 90%; d) HF-pyridine, AcOH/THF (1/4), 96%.

Orthogonally protected building block 20 was prepared in four steps starting from thiogalactoside 21 (Scheme 4). After masking the primary alcohol as tert-butyldiphenylsilyl
ether, the \textit{para}-methoxybenzyl ether was selectively installed at C3 in presence of dibutyltinoxide. The intermediate diol 23 was then acetylated at C2 by treatment with chloroacetyl chloride in presence of triethylamine and further treated with levulinic acid, \(N,N\)-dicyclohexylcarbodiimide and 4-(\(N,N\)-dimethylamino)pyridine to afford the fully protected thiogalactoside 20 in 21\% overall yield. Thiogalactoside 20 was then coupled with methyl-6-hydroxyhexanoate to give compound 25 that can be selectively deprotected at each position.

\textbf{Scheme 4.} Synthesis of orthogonally protected galactoside 20: a) \(t\)-BuPh\(_2\)SiCl, imidazole, DMF, 100\%; b) i. Bu\(_2\)SnO, toluene/benzene, reflux; ii. PMB-Cl, TBAI, DMF, 60 °C, 49\%; c) ClCH\(_2\)C(O)Cl, Et\(_3\)N, CH\(_2\)Cl\(_2\), -20 °C to RT, 52\%; d) LevOH, DCC, DMAP, CH\(_2\)Cl\(_2\), 83\%; e) i. HO(CH\(_2\))\(_3\)CO\(_2\)Me, NIS, TMSOTf, 4 Å MS, CH\(_3\)CN, -20 °C to RT; ii. HgBr\(_2\), toluene/CH\(_3\)NO\(_2\), 60 °C, 85\%.

\textbf{One-Pot Strategies}

Although a lot of improvement has been done in the preparation of functionalized monosaccharides by stepwise protection, this methodology still suffers from: a) an independent and multi-step protection-deprotection sequence to access each compound (4 – 6 steps), b) a tedious work-up after each reaction, c) a time-consuming purification to separate the different regioisomers. Furthermore, when regioselectivity problems are encountered, a low overall yield of the target compound is expected (see \textbf{Section Stepwise Protection Strategies}). In an effort to overcome these problems, a combinatorial, regioselective, orthogonal, and sequential one-pot procedure was developed to access either the fully protected monosaccharide or the respective individual alcohols (Scheme 5).\(^{4}\) The trimethylsilyl trifluoromethansulfonate-catalyzed functionalization of glucopyranosides 26 and 27 involves: a) selective protection of C4- and C6-hydroxyls as an arylidene acetal, followed by regioselective reductive arylmethylation at C3-hydroxyl to furnish the 2-hydroxy
1. Oligosaccharide Synthesis

sugars 28 (Scheme 5, Path A); b) selective protection of C4- and C6-hydroxyls as an arylidene acetal, followed by regioselective reductive p-methoxybenzylolation or 2-naphtylmethylation at C3-hydroxyl, C2-hydroxyl-etherification or C2-hydroxyl-acylation and cleavage of the ethers at C3 by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone yielding the 3-hydroxy sugars 29 (Scheme 5, Path B); c) C4- and C6-hydroxyls arylideneation, followed by C3-hydroxyl reductive arylmethylation, C2-acylation and reductive opening of the arylidene acetal at C4 (Path C) or C6 (Scheme 5, Path D) to afford 4- and 6-hydroxy sugars 30 and 31, respectively; d) C4- and C6-hydroxyls arylideneation, followed by C3 reductive arylmethylation and C2 acylation or etherification to afford fully functionalized building blocks 32 (Scheme 5, Path E).

By tuning the reaction conditions in order to obtain only one regioisomer, differentially protected monosaccharides can be obtained with good yield and on large scale with only one work-up and one column purification.

Scheme 5. Regioselective one-pot protection of glucopyranosides 26 and 27: Path A. a) cat. TMSOTf, ArCHO; b) RCHO, Et3SiH; c) TBAF; Path B. a) cat. TMSOTf, ArCHO; b) 4-MeOPhCHO, Et3SiH; c) TBAF; d) base, electrophile; e) DDQ; or a) cat. TMSOTf, ArCHO; b) 2-C10H7CHO, Et3SiH; c) acid anhydride; d) DDQ; Path C. a) cat. TMSOTf, ArCHO; b) RCHO, Et3SiH; c) (R1CO)2O; d) HCl(g), NaCNBH3; Path D. a) cat. TMSOTf, ArCHO; b) RCHO, Et3SiH; c) (R1CO)2O; d) BH3·THF; Path E. a) cat. TMSOTf, ArCHO; b) RCHO, Et3SiH; c) TBAF; d) base, electrophile; or a) cat. TMSOTf, ArCHO; b) RCHO, Et3SiH; c) acid anhydride.
1. Oligosaccharide Synthesis

The protocol was recently improved by implementing a trimethylsilyl trifluoromethansulfonate-catalyzed silylation\(^5\) that allows the one-pot preparation of the persilylated glucopyranosides 26 and 27 starting material by treatment of deprotected methoxyglucoside 33 or thioglucoside 34 with 1,1,3,3,3-hexamethyldisilazane (Scheme 6).

![Scheme 6](image)

**Scheme 6.** TMSOTf-catalyzed silylation of glucosides with HMDS: a) cat. TMSOTf, HMDS, CH\(_2\)Cl\(_2\), quant..

1.1.2. De novo Synthesis of Carbohydrates

The advent in the field of chemo- and stereoselective methods in organic chemistry enabled the development of de novo synthesis of carbohydrates as an excellent alternative to the traditional synthesis of sugar building blocks. The de novo synthesis (total synthesis) of monosaccharides refers to the stereoselective construction of carbohydrates by carbon-carbon bond formation starting from inexpensive commercially available substrates, mainly derived from chiral pool. The synthesis of sugars from non-carbohydrate substrates has attracted the attention of chemists since 1861, when Butlerov\(^6\) reported the “formose reaction” that generated racemic aldoses and ketoses by oligomerisation of formaldehyde in presence of Ca(OH)\(_2\). However, only the last 30 years have witnessed a remarkable development of strategies leading to differentially protected or “naked” monosaccharides. The contribution of de novo chemistry is highly appreciated for the synthesis of “rare sugars”, which are specific to the bacterial lipopolysaccharides\(^7\) or as precursors of glycosyl-based drugs with anti-cancer or anti-inflammatory activity.\(^8\) The access of rare sugars such as deoxy, aminodeoxy or alkoxy-sugars starting from naturally occurring carbohydrates such as D-mannose, D-glucose, D-glucosamine, D-galactose or D-arabinose requires time-consuming and expensive routes involving selective removal of hydroxyl groups, incorporation of new functional groups or a switch from D- to L- series. To overcome these drawbacks, a plethora of different de novo routes have been reported. However, only few of these routes delivered protected carbohydrate building blocks suitable for direct use in oligosaccharides syntheses. This
section highlights ingenious methods that have been developed to access differentially protected monosaccharides. The discovery of the Sharpless asymmetric epoxidation (SAE)\(^9\) in 1983 constituted a milestone not only for asymmetric synthesis, but also for the de novo synthesis of monosaccharides. For example, an iterative two-carbon homologation/ Sharpless asymmetric epoxidation allowed the divergent access of eight \(L\)-hexoses\(^{10}\). Scheme 7 summarizes the SAE-based total synthesis of \(L\)-glucose. The sequence starts with the enantioselective epoxidation of \(E\)-butene allylic alcohol 35 using tert-butyl hydroperoxide in the presence of (+)-diisopropyltartrate and titanium isopropoxide. Newly formed chiral epoxide 36 was submitted to base mediated rearrangement to give the terminal epoxide, which was in situ regioselectively opened with thiophenoxide to give diol 37. Through this sequence, two of the four stereogenic centers of the target hexose were introduced. Oxidation of the thiol to the corresponding sulfoxide, followed by Pummerer rearrangement and subsequent diisobutylaluminum hydride reduction afforded aldehyde 40. The rest of the carbon backbone was introduced via a Wittig olefination with triphenylphosponium \(\alpha\)-formylmethylide. Reduction of the newly formed aldehyde to the corresponding allylic alcohol, followed by SAE in presence of (-)-diisopropyltartrate furnished diastereoselectively epoxide 41, which was converted to open chain \(L\)-glucose 43 as previously described. The main advantage of this methodology is that the stereoselectivity could be easily introduced at each center by simple variations of the two carbons homologation/ SAE cycles, therefore allowing the access of the eight possible \(L\)-hexoses.
Scheme 7. Sharpless asymmetric synthesis of L-glucose: a) (+)-DIPT, Ti(O\textsuperscript{4}Pr\textsubscript{4}), TBHP, CH\textsubscript{2}Cl\textsubscript{2}, -20 °C, 92%; b) 0.5 N aq. NaOH, i-BuOH, PhSH, 71%; c) i. Me\textsubscript{2}C(OMe)\textsubscript{2}, POCl\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}, quant.; ii. m-CPBA, CH\textsubscript{2}Cl\textsubscript{2}, -78 °C; iii. NaOAc, Ac\textsubscript{2}O, 93%; c) DIBAL, CH\textsubscript{2}Cl\textsubscript{2}, -78 °C, 91%; f) i. Ph\textsubscript{3}P=CHCHO, benzene, 88%; ii. NaBH\textsubscript{4}, MeOH, -40 °C, 91%; iii. (-)-DIPT, Ti(O\textsuperscript{4}Pr\textsubscript{4}), TBHP, CH\textsubscript{2}Cl\textsubscript{2}, -20 °C, 84%; g) i. 0.5 N aq. NaOH, i-BuOH, PhSH, 63%; ii. CSA, Me\textsubscript{2}C(OMe)\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}; 100%; iii. m-CPBA, CH\textsubscript{2}Cl\textsubscript{2}, -78 °C; iv. NaOAc, Ac\textsubscript{2}O, 87%; h) K\textsubscript{2}CO\textsubscript{3}, MeOH, overnight, 65%; i) i. 90% TFA; ii. H\textsubscript{2}, Pd/C, MeOH, 38% (two steps).

Other valuable transformations that are intensively used for the total synthesis of monosaccharides are pericyclic reactions. The enantioselective hetero-Diels-Alder total synthesis of 3-deoxy-d-manno-2-octulosonic acid (KDO) reported by Danishefsky\textsuperscript{11} (Scheme 8) is widely recognized as one of the most elegant de novo routes to carbohydrates. The key step of the synthesis is the [4+2] cycloaddition of Z-furyl diene 45 to the (R)-seleno aldehyde 44 in presence of boron trifluoride diethyl etherate affording a 5:1 mixture of cis/trans dihydropyrones 46 in 76% yield. Reduction of the ketone 46 with sodium borohydride furnished allylic alcohol 47, which served as a substrate for the selective addition of methanol, providing after benzylation intermediate 48. Oxidative elimination furnished alkene 49 in 93% yield that was further submitted to diastereoselective Upjohn dihydroxylation and subsequent benzylation to afford KDO precursor 50. Oxidation of the furane cycle to the carboxylic acid and final deprotection completed the synthesis of the KDO monosaccharide.
Scheme 8. Hetero-Diels-Alder synthesis of KDO: a) i. BF$_3$Et$_2$O; ii. AcOH, 76%, 5:1 (cis/trans); b) NaBH$_4$; c) i. MeOH; ii. BzCl, Et$_3$N; d) H$_2$O$_2$, pyridine, 0 °C, 93%; e) i. NMO, cat. OsO$_4$; ii. BzCl, pyridine, DMAP; f) i. RuO$_2$H$_2$O, NaIO$_4$, MeCN, CCl$_4$, H$_2$O; ii. CH$_2$N$_2$, 95% (two steps); iii. NaOMe, MeOH, THF; iv. DOWEX 50 W H$^+$. The potential of the de novo synthesis was also demonstrated by Wong$^{12}$ who developed a three-step expedient synthesis of sialic acids that relies upon a three-component Petasis coupling and a diastereoselective 1,3-dipolar cycloaddition reaction. For example, L-N-acetyleneuraminic acid (L-Neu5Ac) was easily accessed starting from inexpensive commercially available L-arabinose (Scheme 9). A Petasis coupling reaction allowed the simultaneous introduction of the amino and vinyl groups on the molecule. Cleavage of the bis(4-methoxy-phenyl)methyl group on the amine and subsequent selective acetylation afforded terminal alkene 54 in 55% yield and 99% dr. The introduction of the last stereocenter and the rest of the carbon backbone was accomplished via a diastereoselective 1,3-dipolar cycloaddition between alkene 54 and N-tert-butyl nitron 55. The pericyclic reaction proceeded with high levels of diastereoselectivity affording intermediate 56 in 90% yield. Base-catalyzed β-elimination on intermediate 56 provided L-Neu5Ac in 60% yield. The methodology is versatile and can be applied on other aldehyde substrates for accessing different sialic acids and their derivatives.
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**Scheme 9.** Three-step synthesis of L-Neu5Ac: a) EtOH/H₂O (4/1), 50 °C; b) i. TFA, 50 °C; ii. Ac₂O, MeOH, 55%, 99% dr; c) dioxane, 30 °C, 90%, 10:1 dr; d) NaOMe, MeOH, then H₂O, 60%.

In the ascent of synthetic chemistry, the aldol reaction is a powerful tool for the stereoselective construction of the carbon-carbon bonds. Based on this reaction, Macmillan and coworkers\(^{13}\) developed a two step-synthetic route the production of differentiated hexoses in a *de novo* manner (Scheme 10).
Scheme 10. Two-step synthesis of carbohydrates by selective aldol reactions: a) L-proline (10%), DMF, RT, 92%, dr 4:1 (anti/syn); b) MgBr$_2$/Et$_2$O, Et$_2$O, -20 °C to 4 °C, 79%, dr 10:1; c) MgBr$_2$/Et$_2$O, CH$_2$Cl$_2$, -20 °C to 4 °C, 87%, dr 19:1; d) TiCl$_4$, CH$_2$Cl$_2$, -78 °C to -40 °C, 97%, dr 19:1.

The first step in the synthesis is L-proline catalyzed stereoselective dimerization of protected α-oxyaldehyde 57, which provides differentially protected anti-1,2 triol 58 in good yield and a dr anti/syn of 4:1 (Scheme 10). The condensation product is relatively inert to further proline-catalyzed enolization or enamine addition, hence preventing unwanted oligo and polymerization. The second step of the route is a tandem Mukaiyama aldol/cyclisation reaction between Z-enolsilane 59 and the β-hydroxy-aldehyde 58, catalyzed by a Lewis acid. It is during this step that the divergence was introduced by varying the solvent or the Lewis acid thereby accessing different hexoses building blocks. Thus, the use of magnesium bromide diethyl etherate in Et$_2$O led to the formation of D-glucose 60. The analogous reaction performed in CH$_2$Cl$_2$ afforded with good yields and stereoselectivity D-manno-configured compound 61. By changing the nature of the Lewis acid from magnesium bromide diethyl etherate to titanium tetrachloride, the formation of D-allose 62 was favored.

Combining both asymmetric Mukaiyama aldol reaction and Enders proline-catalyzed condensation of 2,2-dimethyl-1,3-dioxan-5-one with aldehydes$^{14}$, Seeberger$^{15}$ reported an
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elegant pathway for accessing L-glycero-D-manno-heptose building blocks 63 and 64 (Scheme 11) required for the total synthesis of the Yersinia pestis cell wall polysaccharide.

![Diagram](image)

**Scheme 11.** *De novo* synthesis of L-glycero-D-manno-heptoses 63 and 64: a) i. 30% L-proline, DMF, 2 °C, 69%, 98:2 (*anti*/syn); ii. TBSOTf, lutidine, CH₂Cl₂, -78 °C; 98%; b) i. L-selectride, 79%; ii. PBBBr, NaH; iii. TBAF, 82% (two steps); iv. BnBr, NaH, 95%; c) i. CSA, MeOH; ii. TBDPSCl, imidazole, DMAP, 93% (two steps); iii. p-TsOH H₂O, acetone, 97%; d) MgBr₂Et₂O, CH₂Cl₂, 61%; e) i. TFA, CH₂Cl₂, 56%; f) LiAlH(OᵗBu)₃, 73: 47%, 74: 38%; g) i. Ac₂O, pyridine, quant.; ii. N₂H₄, AcOH, 89%; iii. CF₃C(=NPh)Cl, Cs₂CO₃, 96%; h) i. LevOH, DIPC, DMAP, 89%; ii. HO-(CH₂)₅-NBnCbz, BF₃·OEt₂; iii. HF/pyridine; iv. Ac₂O, pyridine, 76% (three steps); iv. N₂H₄, AcOH, 85%.

The synthesis of L-glycero-D-manno-heptose building blocks 63 and 64 commenced with the *anti*-selective L-proline catalyzed condensation of ketone 65 with aldehyde 66, followed by protection of the new formed alcohol as a the tert-butyldimethylsilyl ether (Scheme 11). After L-selectride reduction of the ketone on 67 and concomitant 1,3-migration of the tert-butyldimethylsilyl protecting group, the C2-hydroxyl was protected as a *para*-bromobenzyl ether and a benzyl ether was installed at C4 after removal of the silyl ether. Selective removal of the isopropylidene acetal, followed by regioselective protection of the primary alcohol as
the tert-butyldiphenylsilyl ether and the secondary alcohol as tert-butyldimethylsilyl ether, resulted in the intermediate dimethylacetal, which was hydrolyzed using a catalytic amount of para-toluene sulfonic acid affording aldehyde 69. At this point, the rest of the carbon backbone was introduced via a Mukaiyama aldol reaction. Thus, treatment of the aldehyde 69 with silylenolether 70 in presence of a chelating activator as magnesium bromide diethyl etherate furnished stereoselectively aldol 71 in 61% yield. Cleavage of the tert-butyldimethylsilyl-group in acid media and concomitant cyclization afforded lactone 72. Reduction of the lactone with tri-tert-(butoxy)aluminium hydride led to the partial migration of the tert-butyldimethylsilyl protecting group during the reduction step leading to the formation of regioisomers 73 (47%) and 74 (38%). After separation, diol 73 was converted in a few steps into the imidate donor 63 and its regioisomer 74 was functionalized with a linker on the anomic position and regioselectively deprotected at C3 to give the glycosyl acceptor 64. Thus, using divergent de novo synthesis the necessary building blocks for the assembly of the Y. pestis core pentasaccharide were accessed.

In a similar manner, the Seeberger group\(^{16}\) made use of the Evans aldol reaction as a key step for the synthesis of galacturonic acid building block 75 that is specific to the glycosphingolipid from Sphingomonas yanoikuyae (Scheme 12).

**Scheme 12.** De novo synthesis of glycosphingolipid from Sphingomonas yanoikuyae: a) i. LDA, THF, 90%, dr 4.9:1; ii. Ac₂O, pyridine, quant.; b) TFA, CH₂Cl₂, anisole, 92%, α/β = 4:1; ii. Sm(OTf)₃, MeOH; c) NIS, TfOH, dioxane/toluene 3:1, 85%, α/β = 4.2:1; d) i. Pd(OH)₂/C, H₂, EtOH, CHCl₃, 74%; ii. LiOH, H₂O₂, THF, 75%.

Fully differentiated galacturonic acid 75 was synthesized starting from aldehyde 76 and Evans oxazolidinone 77 (Scheme 12). A lithium diisopropylamide-promoted aldol reaction, followed by acetylation of the newly formed alcohol provided the target adduct in a 90% yield
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with a diastereoselectivity of 4.9:1 syn/anti. Cleavage of para-methoxybenzyl ether and simultaneous cyclization in presence of anisole and trifluoroacetic acid, followed by removal of the chiral auxiliary with samarium (III) triflate in methanol completed the de novo synthesis of ethyl-thiogalactoglycoside 79. N-iodosuccinimide/triflic acid activated coupling of the thioglycoside 79 with the lipid 80 afforded the fully protected glycosphingolipid 75, which was converted in two steps into the desired product 81.

In another de novo approach stemming from the Seeberger group, the of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose building block 8217 required for the synthesis of Bacteroides fragilis A1 polysaccharide fragment 8318 was accessed via a Dieckmann cyclization (Scheme 13). The synthesis started from commercially available N-Cbz-L-threonine 84. After acid mediated methyl ester formation, the free alcohol was protected as acetate to give threonine derivative 85. Lithium bis(trimethylsilyl) amide induced Dieckmann cyclization, followed by methylation provided enone 86 in 73% yield. Diisobutylaluminium hydride reduction in acid media of methoxy enone 86, followed by stereoselective Luche reduction and subsequent protection of the free alcohol as an acetate afforded glycal 87. At this point the equatorial azide was introduced on the molecule via an azido nitration reaction with ceric ammonium nitrate and sodium azide in 67% yield. Nitrate 88 was further converted in two steps into the imidate 82 which was used as glycosylating agent for the total synthesis of tetrasaccharide fragment of Bacteroides fragilis A1.

**Scheme 13.** De novo synthesis of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT): a) i. AcCl, MeOH; ii. Ac₂O, Et₃N, DMAP, 95% (two steps); b) i. LHMDS, THF, -78 °C to
The Achmatowicz rearrangement\textsuperscript{19} is another valuable tool for de novo chemistry that allows for the conversion of furfuryl alcohols to the corresponding pyranones with high degree of stereoselectivity. By varying the stereochemistry of the alcohol group on furanol, both the D- and L-sugars series can be accessed. Based on this transformation, O’Doherty developed de novo routes to a variety of building blocks including L-sugars,\textsuperscript{20} 6-amino-deoxy-sugars\textsuperscript{21} and 2-deoxy and 2,3-dideoxyhexoses\textsuperscript{22}. An elegant application of this methodology is the 12-step synthesis of highly branched all-L-\(\alpha\)-manno-heptapyranoside \textsuperscript{89} (Scheme 14). L-\(\alpha\)-Manno-heptapyranoside \textsuperscript{89} is of great help for structure-activity relationship studies in comparison with the natural Man-9, which is involved in a variety of biological functions. The pyranone building block \textsuperscript{93}, necessary for the assembly of the heptamannoside \textsuperscript{89} was prepared via a de novo route starting from acyl furan \textsuperscript{90}. Enantioselective Noyori reduction provided chiral alcohol \textsuperscript{91}, that was further submitted to Achmatowicz oxidation and subsequent tert-butylicarbonate formation to afford pyranone \textsuperscript{92}. Palladium catalyzed glycosylation of the pyranone with benzylic alcohol furnished \(\beta\)-benzylxy pyranone \textsuperscript{93} that was further submitted to a diastereoselective reduction with sodium borohydride, followed by tert-butyldimethylsilyl ether cleavage with tetrabutylammonium fluoride to give the 1,2-anti diol \textsuperscript{94} in 45\% yield over six steps. Palladium catalyzed coupling of diol nucleophile \textsuperscript{94} with pyranone \textsuperscript{93} and subsequent ketones reduction afforded trisaccharide \textsuperscript{95} in 65\% yield over two steps. After removal of the silyl ether on \textsuperscript{95} with tetrabutylammonium fluoride, the glycosylation/reduction sequence provided the heptasaccharide/tetraallylic alcohol \textsuperscript{96} in 41\% yield over three steps. Dihydroxylation under Upjohn conditions gave the \(\alpha\)-L-manno-heptasaccharide \textsuperscript{89} in 82\% yield, thus completing the de novo assembly of this molecule.
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Scheme 14. De novo asymmetric synthesis of all-α-L-heptamannoside 89: a) Noyori (S,S); b) i. NBS, H₂O; ii. Boc₂O, 5% DMAP, -78 °C; c) BnOH, Pd(0)(PPh₃)₄; d) i. NaBH₄, -78 °C; ii. TBAF, 45% (six steps); e) i. Pd(0)(PPh₃)₄; ii. NABH₄, 65% (two steps); f) i. TBAF, 91%; ii. 93, Pd(0)(PPh₃)₄; iii. NABH₄, 45% (two steps); g) OsO₄, NMO, 86%.

A transformation similar to Achmatowicz rearrangement is the oxidative cleavage of 2,5-dihydrofurans to the corresponding α,β-unsaturated γ-keto aldehydes, which proved valuable intermediates for the synthesis of monosaccharides. Reißig and coworkers applied this transformation to the synthesis of L-cymarose (Scheme 15), rare sugar present in the structure of the DNA-helicase inhibitor heliquinomycin. Thus, addition of lithiated alkoxyallene 97 to (S)-lactaldehyde 98, followed by gold(I) catalyzed 5-endo-trig-cyclization provided 3-methoxy-2,5-dihydrofuran 99 in 86% yield over two steps. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone mediated oxidation of substrate 99 afforded α,β-unsaturated aldehyde 100 in 83% yield. Cleavage of trityl ether on intermediate 100 with iodine in isopropanol and in situ
cyclization provided pyranoside 101 that was further subjected to hydrogenation with rhodium on aluminium oxide followed by stereoselective reduction of the ketone with L-selectride to give L-cymarose acetal 102. Benzylation of the free hydroxyl at C4, followed by thioglycosylation provided L-cymarose glycosylating agent 103 that could be directly used for accessing target heliquinomycin.

Scheme 15. De novo synthesis of L-cymarose: a) i. Et₂O, -78 °C; ii. cat. AuCl, pyridine, 86% (two steps); b) DDQ, CH₂Cl₂, H₂O, 83%; c) I₂, CH(O’Pr)₃, iPrOH/CH₂Cl₂, 90%; d) i. H₂, Rh/Al₂O₃; ii. L-selectride, 60% (two steps); e) i. Bz₂O, DMAP, pyridine; ii. PhSH, BF₃OEt₂, 67% (two steps).

The few de novo examples described in this section highlight the degree of advancement reached in carbohydrate chemistry thus enabling the synthesis of polyfunctionalized systems with a high degree of stereoselectivity and versatility. To add to this repertoire, the development of general, cost efficient and scalable de novo approaches towards rare sugars building blocks suitable for the assembly of complex biomolecules, such as vaccines or antibiotics, will be of great help in supporting and facilitating the progress in the fields of chemical biology and drug discovery.
1. Oligosaccharide Synthesis

1.2. Oligosaccharide Synthesis Strategies

1.2.1 Enzymatic Synthesis of Oligosaccharides

The enzymatic assembly of oligosaccharides is a powerful method for accessing naturally occurring carbohydrates. In comparison with traditional syntheses of oligosaccharides that require tedious protection/deprotection steps to achieve both regio- and stereoselectivity, enzyme catalyzed glycosylation occurs with perfect control of the anomeric configuration and high regioselectivity without requiring any protective group. The two classes of enzymes used in carbohydrate assembly are glycosyltransferases and glycosylhydrolases (glycosidases). Glycosyltransferases (GTs) transfer a given carbohydrate from the corresponding sugar nucleotide donor substrate to a specific hydroxyl group of the acceptor sugar. Since GTs are highly regio- and stereospecific and furthermore provide the target compound with high yield, they are generally the catalysts of choice for oligosaccharides synthesis. Nevertheless, restricted substrate specificity and enzyme availability and the high cost of both sugar nucleotides and enzymes limit their application. Moreover, the enzyme can be inhibited by the in situ formation of nucleoside phosphate. To circumvent the expense of the sugar nucleotides and to reduce the concentration of nucleotide phosphate, multi-enzymatic systems able to regenerate the sugar nucleotides have been reported.

Compared to GT-catalyzed reactions, glycosidase-catalyzed transglycosylation use inexpensive readily available donor substrates (such as para-nitrophenyl glycosides and halides) and a large variety of acceptors. Furthermore, the enzymes are stable, inexpensive and readily available. However, the glycosidase-catalyzed reaction presents two main drawbacks: the low transglycosylation yield and the hydrolysis of the target compound. To overcome these drawbacks, a new class of glycosidase mutants was introduced in 1998. These mutant glycosidases, termed “glycosynthases” are able to catalyze the formation of the new glycosidic linkages without hydrolyzing the newly formed linkages, thus driving the reaction forward. Furthermore, to improve or alter catalytic activity and substrate specificity and to modify optimum reaction conditions, glycosidases and glycosyltransferases were further engineered by means of random mutagenesis and directed evolution.

The progress in the engineering field has allowed also the development of ingenious chemoenzymatic routes towards complex oligosaccharides. For example, a one-pot enzymatic route, including two enzymatic glycosylation steps catalyzed by recombinant α-(1,3)-GalT and β-(1,4)-GalT with in situ regeneration of the sugar nucleotide allowed the preparation of
pentasaccharide 104 (Scheme 16).\textsuperscript{29} Pentasaccharide 104 is one of the $\alpha$-Gal epitopes binding specifically to human \textit{anti}-Gal antibodies during xenotransplantation. The one-pot enzymatic route provided tetrasaccharide 105 and pentasaccharide 104, in 53\% and 35\% yields, respectively, thus providing a good alternative for chemical syntheses.

\textbf{Scheme 16. Synthesis of pentasaccharide 104 with \textit{in situ} sugar nucleotide regeneration.}

\textbf{1.2.2 One-Pot Oligosaccharide Synthesis}

The development of one-pot glycosylation strategies facilitates the assembly of oligosaccharides by eliminating the tedious isolation/purification steps of intermediates. Three general one-pot strategies could be differentiated: a) the chemoselective glycosylations in which the most reactive glycosylating agent is activated and treated with a less-reactive glycosylating agent to provide a new glycoside that could be immediately condensed with the least reactive glycosylating agent (Scheme 17A); b) the orthogonal glycosylation strategies that employ two glycosides with different anomic leaving groups in which one glycosylating agent is activated selectively in the presence of the other (Scheme 17B); c) the iterative (pre-activation) glycosylation strategies that exploit the pre-activation of a glycosylating agent to provide the reactive glycosylating species, which is subsequently treated with a second glycosylating agent, bearing an identical aglycon at the reducing end to provide the new glycosylating agent that could be again pre-activated for further elongation of the oligosaccharide (Scheme 17C). In this section, the advantages of each of the one-pot strategies will be highlighted and examples presented to illustrate their contributions to oligosaccharides assembly.
1. Oligosaccharide Synthesis

Scheme 17. One-pot glycosylation strategies.
Chemoselective One-Pot Oligosaccharide Synthesis

The reactivity of different glycosylating agents within a class (for example: thioglycosides) can be modulated through protecting groups manipulation. By varying the electrodonating and electrowithdrawing nature of the protecting groups, the character of the leaving group can be controlled and the reactivities of building blocks can be discriminated allowing for the assembly of the corresponding carbohydrates by chemoselective one-pot synthesis. By assembling oligosaccharides via chemoselective one-pot synthesis, the manipulation of the protective groups between the coupling cycles is eliminated.

Fraser-Reid established the basics for chemoselective one-pot strategies by introducing the concept of armed-disarmed glycosylating agents: benzylated \( n \)-pentenyl glycosides are electron-rich building blocks, which are more reactive (armed) than their acetylated analogues, which are electron-poor (disarmed).

A few years later, Wong quantified the relative reactivity of a library of tolyl thioglycosides by performing competitive glycosylation assays between the glycosylating agents of interest and a reference donor of known relative reactivity value (RRV). The determined RRVs of the glycosyl donors were collected in a database (Optimer) and with the help of an algorithm, which ranks the suitability of specific donor molecules for the synthesis of a target molecule, provided a synthetic protocol that can be performed in a single pot without the need to isolate intermediates or cleave temporary protective groups. The study revealed reactivity trends among sugars (RRVs pattern: fucose > galactose > glucose > mannose). Furthermore, general trends in reactivity, in line with the ones reported earlier by Fraser-Reid, have emerged to facilitate the design of an effective synthetic strategy.

The reactivity-based one-pot synthesis allowed for expedient access of different oligosaccharides such as the tumor-associated antigen N3 minor octasaccharide 106 (Scheme 18). The difference of reactivity between fucosyl donor 107 (RRV = 7.2 \times 10^4), the lactosaminyl disaccharide 108 (RRV = 41) and the reducing end disaccharide 109 (RRV = 0) made the one-pot assembly of oligosaccharide 106 possible. Thus, fucosyl thioglycoside 107 was coupled with the less reactive lactosaminyl disaccharide 108 by activation with 1-benzenesulfinyl piperidine/trifluoromethanesulfonic anhydride. Subsequently, lactosyl building block 109 and \( N \)-iodosuccinimide/trifluoromethanesulfonic acid were added respectively to the reaction mixture resulting in the introduction of the previously formed trisaccharide at the positions C3 and C6 of the lactosyl building block 109. Removal of the protective groups provided the target 106 in 11% overall yield.
Scheme 18. Chemoselective one-pot synthesis of tumor-associated antigen 106: a) i. BSP, Tf$_2$O, CH$_2$Cl$_2$, -70 °C to -10 °C; ii. NIS, TfOH, 0 °C to RT; b) i. Zn, AcOH, RT; ii. Ac$_2$O, DMAP, pyridine, 0 °C to RT; iii. NaOMe, MeOH, RT; iv. Pd/C, H$_2$, MeOH, HCO$_2$H, 11% overall yield.

Although the Optimer database currently covers the RRVs of a large number of building blocks (circa 600), this method is limited by the reactivity differences between the coupling partners.

Orthogonal One-Pot Oligosaccharide Synthesis

The orthogonal one-pot strategy allows for the assembly of the carbohydrates in one flask starting from building blocks having different leaving groups at the anomeric position that could be selectively activated. In comparison with the chemoselective strategy, where the reactivity of the building blocks needs to be modulated through the protecting group in order to meet the requirements of the synthetic pathways, the orthogonal one-pot assembly of carbohydrates is totally independent of the protection group pattern, relying only on the glycosylation conditions that activate exclusively one building block.
An elegant application of the orthogonal one-pot synthesis of carbohydrates was the assembly of phytoalexin elicitor active heptasaccharide 110 by using seven independent building blocks (Scheme 19).

**Scheme 19.** Orthogonal one-pot synthesis of heptasaccharide 110: a) AgOTf, CH₂Cl₂; b) MeOTf, CH₂Cl₂; c) MeOTf, CH₂Cl₂; d) Hf(Cp₂)Cl₂/AgOTf, CH₂Cl₂; e) DMTST, CH₂Cl₂; f) DMTST, CH₂Cl₂, 24% (six steps); g) i. H₂, Pd(OH)₂, MeOH, H₂O; ii. NaOMe, MeOH, H₂O, 52% (two steps).

The sequence involved: a) the regioselective glycosylation of the primary alcohol of 112 with α-bromide 111 by activation with silver triflate, b) glycosidation of the newly formed thiodisaccharide with acceptor 113 without self-condensation in presence of an excess of methyl triflate, c) elongation of the trisaccharide at C3 by coupling with ethylthioglucoside 114, d) activation of the fluoride-tetrasaccharide with bis(cyclopentadienyl)hafnium.
dichloride/silver triflate and coupling with the primary alcohol of thioglucoside 115, e) addition of the terminal glucoside moiety by activation of the pentasaccharide with dimethylsulfonium triflate, and finally f) branching of the hexasaccharide at C3 in the presence of a large excess of dimethylsulfonium triflate. The six-step one-pot synthesis provided heptasaccharide 118 in 24% overall yield and proved the potential of this methodology.

The orthogonal one-pot technique was expanded by Demchenko by introducing a new class of leaving groups: the S-thiazolyl that can be activated by methyl triflate, silver triflate, copper (II) triflate or N-iodosuccinimide in combination with a stoechiometric amount of trifluoromethanesulfonic acid. The activation conditions allowed for the S-thiazolyl glycosides to be more reactive than ethyl- and phenyl-thioglycosides (silver triflate) or O-pentenyl glycosides (silver triflate) (Scheme 20A). Furthermore, S-thiazolyl glycosides withstand reaction conditions associated with the activation of the thioglycosylating agents (N-iodosuccinimine/cat. trifluoromethanesulfonic acid), bromoglycosides (mercury (II) oxide/mercury (II) bromide) and trichloroacetimidates (boron trifluoride diethyl etherate) (Scheme 20B). Via this new class of leaving groups, new orthogonal pathways were developed resulting in the one-pot synthesis of different oligosaccharides.
A. S-Taz as a glycosylating agent

\[
\begin{align*}
\text{119} & \quad + \quad \text{120} & \quad \rightarrow \quad \text{121} \\
\text{122} & \quad + \quad \text{123} & \quad \rightarrow \quad \text{124}
\end{align*}
\]

B. S-Taz as a glycosyl acceptor

\[
\begin{align*}
\text{125} & \quad \rightarrow \quad \text{126} \\
\text{127} & \quad \rightarrow \quad \text{128} \\
\text{126} & \quad + \quad \text{127} & \quad \rightarrow \quad \text{129}
\end{align*}
\]

**Scheme 20.** Orthogonality of S-Taz leaving group: a) AgOTf, 81%; b) AgOTf, 91%, 2:1 $\alpha/\beta$; c) HgO/HgBr$_2$, 45%, 1.2:1 $\alpha/\beta$; d) BF$_3$OEt$_2$, 50%, 1.1:1 $\alpha/\beta$; e) NIS, cat. TfOH, 98%, 1.1:1 $\alpha/\beta$.

**Iterative One-Pot Oligosaccharides Synthesis**

Compared to the orthogonal and chemoselective one-pot strategies, the iterative or preactivation strategy is one of the most straightforward approaches towards the synthesis of oligosaccharides, since it is independent of the glycosylation conditions (as in the orthogonal strategy) and the nature of the protecting group (as in a chemoselective strategy). As mentioned, this strategy relies upon the pre-activation of the glycosylating agent in the absence of the acceptor. Upon addition of the second building block to the preactivated glycosylating agent, a disaccharide building block is formed with an identical aglycon at the reducing end. This process can be repeated in the same reaction flask allowing for expedient oligosaccharide assembly.
1. Oligosaccharide Synthesis

The first step towards the development of this strategy were performed by Crich,\textsuperscript{37} who reported the pre-activation of thioglycosides using benzenylsulfenyl triflate and 2,6-di-tert-butyl-4-methylpyridine as an activating system. Thus, thiomannoside 130 is converted to the more reactive glycosyl triflate 131, that can easily react with a nucleophile ROH to afford the corresponding β-mannoside 132\textsuperscript{38} (Scheme 21).

\[ \text{Scheme 21. Preactivation of thiomannosides: a) PhSOTf, DTBMP; b) ROH.} \]

Yamago later described the combinatorial synthesis of linear oligoglucosamines 133 and 134 using 2-deoxy-2-aminothioglycosides both as a glycosylating agent and nucleophile (Scheme 22). Activation of the thioglycoside 135 with 1-benzenesulfinyl piperidine and triflic anhydride led to the formation of the intermediate “glycosyl triflate” that was further treated at low temperature with nucleophile 136 to give disaccharide 133 in 90% yield.\textsuperscript{39} Repetition of the same procedure twice provided tetrasaccharide 134 in 46% overall yield.

\[ \text{Scheme 22. Iterative synthesis of oligoglucosamines: a) activation: BSP, Tf}_2\text{O, -60 }^\circ\text{C; b) coupling: -60 }^\circ\text{C.} \]

To help in purification of the oligosaccharides obtained from these one-pot iterative methods, Huang labeled the full-length oligosaccharide with a fluorous tag (Scheme 23).\textsuperscript{40} The tagged compound could be easily separated from the byproducts by fluorous solid-phase extraction (FSPE). Removal of the tag provided the desired oligosaccharide. Thus, by combining the
advantages of one-pot synthesis and fluorous separation, Lewis X trisaccharide 137 was synthesized in only 4 h and in 62% overall yield.

![Scheme 23](image)

**Scheme 23.** FSPE-assisted purification of Lewis X trisaccharide: a) AgOTf, p-TolSCI, MS 4Å, -78 °C; b) -78 °C to RT; c) AgOTf, p-TolSCI, -78 °C to RT; d) CH₂Cl₂/MeOH then FSPE, 62% (five steps).

Despite various advantages of this method, particular attention needs to be paid to the control of the reaction conditions. Furthermore, this method is limited by the dilution of the reaction mixture, which decreases with the sequential addition of building blocks and activating agents and with the accumulation of the reaction byproducts.

### 1.2.3 Automated Oligosaccharide Synthesis

To access libraries of oligosaccharides in a rapid manner, the development of general automated approaches is crucial. The repetitive nature of the oligosaccharide synthesis consisting of a glycosylation step, followed by a deprotection step (Scheme 24), makes it suitable to be performed in an automated way. This section presents the most relevant automated solution- and solid-phase syntheses that have been reported in the literature.
1. Oligosaccharide Synthesis

**Scheme 24.** Traditional assembly of oligosaccharides.

**Fluoruous Assisted Oligosaccharides Synthesis**

Fluorous assisted solution-phase oligosaccharides synthesis is a relatively new method\(^\text{11}\) that relies upon the solubility properties conferred to a growing oligosaccharide by a fluoruous tag. The solvophobic interactions allow for the isolation of the labeled carbohydrate from the deletion sequences and reagents by filtration of the crude product on a fluoruous solid-phase extraction (FSPE) column. In this regard the *Pohl* group designed a fluoruous linker\(^\text{142}\) to be attached at the reducing end of the growing oligosaccharide (Scheme 25). The linker is stable during the glycosylation cycle and maintains the solubility of the labeled carbohydrates both in organic solvents that are used during glycosylation and in aqueous-organic mixtures used for purification by FSPE. By using fluoruous assisted oligosaccharide synthesis, the assembly of a \(\alpha\)-(1,2)-tetramannoside\(^\text{143}\) was achieved in 79% overall yield. The synthetic cycle consisted of a glycosylation reaction performed with mannosyl imidate\(^\text{144}\) by activation with trimethylsilyltriflate, followed by acetate cleavage at C2 for chain elongation. After each synthetic step, the intermediate product is purified by filtration on FSPE column. This method has been extended for the synthesis of branched oligomannosides.
1. Oligosaccharide Synthesis

Scheme 25. Fluorous assisted oligomannoside synthesis: a) i. coupling: TMSOTf, CH₂Cl₂, 5 °C; ii. filtration: FSPE; b) i. deprotection: NaOMe; ii. filtration: FSPE, 79% overall yield.

Although the fluorous assisted assembly of carbohydrates is suitable for being performed in an automated way, the FSPE purification is difficult to be executed using an instrument. Furthermore, it is expected that this methodology cannot be applied for accessing large size carbohydrates as the influence of the fluorous tags is being reduced.

Automated Solid-Phase Synthesis

Inspired by Merrifield’s solid-phase peptide synthesis, in 1971 Schuerch and Frechet introduced the concept of solid-phase oligosaccharide synthesis. The main advantage of the solid-phase method compared to the solution phase approach is that the reactions can be driven to completion by using an excess of reagent that can be easily removed at the end of each step by filtration. Furthermore, the tedious and time-consuming work-up and purification steps following each reaction are completely eliminated. Only one purification of the compound is performed, after the sugar is cleaved from the solid support. Drawbacks of this method are that either complete stereocontrol has to be ensured, or the impurities resulting from incomplete stereocontrol need to be separated from the target compound. Since building blocks are used in excess, they need to be available also on large scale.

The solid-phase synthesis of carbohydrates can be accomplished by attaching the first sugar either through the reducing end (acceptor-bound strategy - Scheme 26A) or the non-reducing end (donor-bound strategy- Scheme 26B) to the resin. In the acceptor-bound strategy
1. Oligosaccharide Synthesis

(Scheme 26A), the first carbohydrate is anchored to the solid support via its reducing end and presents a nucleophilic group that is reacted with an excess of glycosylating agent in a coupling reaction. Removal of the temporary protecting group provides a new nucleophile that can further undergo the next coupling step thereby completing the synthetic cycle. In this strategy, the oligosaccharide is built from the reducing end to the non-reducing end.

In the donor bound strategy (Scheme 26B), the glycosyl donor linked to the solid support via a suitable hydroxyl group is reacted with the solution-phase building block having a nucleophilic group and an orthogonal leaving group for chain elongation. This approach does not require removal of the temporary protective groups. However, a major drawback of this strategy is the fact that most side reactions during glycosylation involve the glycosylating agent, which will result in the termination of chain elongation.

Scheme 26. A. Acceptor-bound strategy; B. Donor-bound strategy.

Bi-directional strategies, in which the carbohydrate bound to the solid support acts both as a nucleophile and as an electrophile, have been also described and are of particular use when branched structures are targeted.

Thirty years after Schuerch’s pioneering work, Seeberger and co-workers reported the first automated oligosaccharide synthesizer and showed that oligosaccharide synthesis is suitable to be performed in an automated fashion.
1. Oligosaccharide Synthesis

Scheme 27. Automated solid-phase synthesis of hexasaccharide 145: Coupling cycle: i. 147 or 148, TMSOTf, CH₂Cl₂, -15 °C, 15 min; ii. N₂H₄, pyridine, AcOH, 15 °C, 15 min; Cleavage: Grubbs catalyst, ethylene, CH₂Cl₂, 89% overall yield. The fully protected hexasaccharide β-glucan 145 corresponding to a fragment of a dodecasaccharide phytoalexin elicitor, was assembled on a Merrifield resin functionalized with an octenediol linker by using a modified peptide synthesizer (Scheme 27). The synthesis of the hexasaccharide 145 was accomplished using two glycosyl phosphate building blocks: monosaccharide 147 and disaccharide 148 that were used alternately for coupling by activation with trimethylsilyl trifluoromethanesulfonate. Levulinoyl ester was used a temporary protecting group on both building blocks, providing after removal with hydrazine, the nucleophile for the next glycosylation step. Once the hexasaccharide was assembled, it was cleaved from the solid support by cross metathesis to give fully protected hexasaccharide 145 in 89% overall yield determined by HPLC. The terminal olefin can be further functionalized to provide a nucleophilic conjugation handle.46
1. Oligosaccharide Synthesis

This first example of assembling oligosaccharides by automated solid-phase synthesis proved the applicability of this technique, but revealed also challenges regarding the instrument and the synthetic methodology. These challenges needed to be overcome to enable straightforward and facile access to defined carbohydrates.

To meet the requirements of fully automated oligosaccharide synthesis, a new instrument was designed (Figure 1).47 The new instrument was equipped with a solenoid unit, a cryostat to control the temperature and a fraction collector. The solenoid system enables for the fast delivery of solvents, good mixing as well as fast and reliable removal of waste. The cryostat allows for an accurate control of the temperature of the reaction vessel in a range from -50 °C to 90 °C. Solutions from the reaction vessel can be collected at any time of the synthesis with the help of a fraction collector. The accuracy of reagents delivery is achieved using a system of syringe pumps.

Figure 1. Fully automated synthesizer: (1) reaction vessel; (2) solvent reservoirs; (3) building block vessels; (4) fraction collector; (5) syringe pumps; (6) reaction vessel; (7) controller; (8) reagents.

To access defined oligosaccharides simply, reliably and efficiently, a standardized synthetic strategy that integrates key elements such as solid support, linker, building blocks, assembly and post-assembly manipulations was established.

In order to minimize the side-reactions related to the glycosylation step (such as donor hydrolysis) that could lead to the formation of byproducts and decrease of the yield, the
1. Oligosaccharide Synthesis

automated solid-phase synthesis of oligosaccharides was constructed on an acceptor-base strategy, implying that the reducing-end sugar is bound to the solid support. Among the commercially available solid supports, Merrifield’s resin\(^48\) (Figure 2) was found to be the most suitable for the use in automated solid-phase synthesis of oligosaccharides.\(^49\) Merrifield resin is a polystyrene cross-linked with divinylbenzene and typically functionalized with chloride groups. This solid support provided good chemical and mechanical stability and performed best in terms of permeability for the reagents, reproducible loadings and swelling behavior in organic solvents such as dichloromethane, tetrahydrofuran and toluene. The main disadvantage of this support in comparison with the glass plates used in DNA synthesis resides in the high number of steps required to remove the reagents from the porous polystyrene matrix.

Figure 2. Structure of Merrifield resin

The resin needs to be functionalized with a linker on which the oligosaccharide will be constructed. The linker has to be stable during the synthetic cycle and be efficiently cleaved at the end of the synthesis. To allow for the attachment to a carrier protein or a glass surface, the cleaved linker should present a terminal functional group such as an amine or a thiol. Three linker systems compatible with Merrifield resin were designed to be used for the automated solid phase synthesis of oligosaccharides. The previously mentioned octenediol linker can be attached to the solid support either by ether\(^50\) or an ester linkage.\(^51\) The linker is stable during the coupling conditions and can be cleaved from the solid support by olefin cross metathesis providing a terminal olefin that can be further functionalized (Scheme 28).
The presence of the double bond on the octenediol linker prohibits the use of some glycosylating agents such as thioglycosides. To eliminate this drawback, a new linker based on a 4-hydroxymethylbenzyl N-(5-hydroxypentyl)-N-benzyl carbamate spacer was designed and attached via an ester linkage to the polystyrene solid support (Scheme 29). The linker is stable under Lewis acid, mild basic and nucleophilic conditions and is cleaved under basic conditions providing an amine protected as a carbamate. Standard hydrogenolysis conditions on palladium catalysts release the terminal amine that can be used as an attachment point for the synthesis of glycoarrays and glycoconjugates.

Finally, a photocleavable linker was recently developed (Scheme 30). Compared to the previously reported examples, the o-nitro-benzyl linker proved stable both in basic and acid media and compatible with all classes of glycosylating agents.
1. Oligosaccharide Synthesis

Scheme 30. Photocleavable linker: cleavage: $h\nu$, CH$_2$Cl$_2$.

The linker is connected to the solid support via an ether linkage and can be cleaved efficiently in continuous flow$^{54}$ by exposure to UV light (Figure 3). Due to reducing light scattering by the resin beads and the decrease of the light intensity with the distance from the light source, this synthetic set-up enhances the cleavage efficiency to batch reactions. Following cleavage, removal of the benzylxycarbonyl protecting group by hydrogenolysis releases the terminal amino group for further functionalization.

Figure 3. Cleavage of the linker from the solid support by exposure to UV light in continuous flow: (1) syringe pump; (2) inlet; (3) FEP (fluorinated-ethylene-propylene) tubing; (4) cooling system; (5) UV filter (pyrex); (6) UV lamp; (7) fan; (8) power supply; (9) cryostat; (10) outlet; (11) frit.
With these tools in hand and with a set of building blocks that are available on large scale, the assembly of biologically interesting oligosaccharides was achieved by automated solid-phase synthesis. For example, the Seeberger group\textsuperscript{47} reported the synthesis of $\beta$-(1,4)-hexaglucosamine\textsuperscript{149} (Scheme 31), a compound found on the surface of pathogenic bacteria, starting from glucosamine thioglycoside\textsuperscript{150} and Merrifield resin functionalized with the base labile linker\textsuperscript{151}. To ensure the $\beta$-selectivity, the thioglucoside\textsuperscript{150} was equipped with a trichloroacetimidate participating group at C2. The orthogonal 9-fluorenylmethyloxycarbonyl protecting group at C4 allowed for elongation at this position and real-time monitoring of the coupling yields by spectroscopic measurements of the piperidine-dibenzofulvene adduct released during the 9-fluorenylmethyloxycarbonyl cleavage.\textsuperscript{55} Other hydroxyl protecting groups such as nitroptalimidobutyric acid were developed to help for colorimetric monitoring of the coupling yields in solid-phase synthesis of carbohydrates.\textsuperscript{56} The coupling cycle involved a glycosylation step performed with thioglycoside\textsuperscript{150} by activation with $N$-iodosuccinimide/trifluoromethanesulfonic acid and removal of the 9-fluorenylmethyloxycarbonyl protecting group for chain elongation. Once the target compound was assembled, the $N$-trichloroacetyl protecting group was converted to the corresponding $N$-acetyl by treatment with tributyltin hydride and azobisisobutyronitrile. The hexamer was cleaved from the solid support by exposure to sodium methoxide and subsequently submitted to hydrogenolysis to give the target glucosamine product\textsuperscript{149} in 24% overall yield.
1. Oligosaccharide Synthesis

Scheme 31. Automated solid-phase synthesis of \( \beta -(1,4) \)-oligoglucosamine 149: a) NIS/TfOH, dioxane, CH\(_2\)Cl\(_2\), -40 °C to -20 °C; b) piperidine, DMF; c) Bu\(_3\)SnH, AIBN; d) i. NaOMe, MeOH; ii. H\(_2\), Pd/C, 24% overall yield.

Sialylated oligosaccharides, \(^{57}\) \( \beta -(1,4) \)-mannuronic acids, \(^{58}\) hyaluronic acids\(^{59}\) and chondroitin sulfates\(^{53}\) constitute other remarkable molecules with interesting biological properties that were successfully assembled using automated solid-phase synthesis and highlight the potential of this emerging method.

Recently, Demchenko reported the HPLC-assisted automated solid-phase oligosaccharide synthesis.\(^{60}\) The new setup is based on an unmodified HPLC instrument presenting a pump, a
UV detector and a computer with standard HPLC-operating software installed. A chromatography column packed with preswelled TentaGel MB-NH₂ was connected to the HPLC-system.

Scheme 32. HPLC-assisted automated solid phase synthesis: a) i. EDC, DMAP, CH₂Cl₂; ii. TFA, wet CH₂Cl₂; b) TMSOTF, CH₂Cl₂; c) piperidine, DMF; d) NaOMe, MeOH, CH₂Cl₂; e) Ac₂O, pyridine, 62% overall yield.

The oligosaccharides were assembled from the reducing to the non-reducing end. Thus, the resin was functionalized with glycosyl acceptor precursor 152 in presence of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide and 4-(N,N-dimethylamino)pyridine (Scheme 32). Acid treatment revealed the glycosyl acceptor 153. Simultaneous delivery of glycosylating agent 154 and trimethylsilyl trifluoromethanesulfonate activator, followed by removal of the 9-fluorenylmethyloxycarbonyl temporary protecting group allowed for chain elongation. The progress of the reactions was monitored by UV absorbance of the mixture eluting from the column. Once, the desired β-(1,6)-glucose pentamer was obtained, it was
1. Oligosaccharide Synthesis

cleaved from the solid support under Zemplén conditions, and then per-acetylated to give target compound 155 in 62% yield. The yield was comparable with the one obtained by manual oligosaccharides assembly, however the experimental time was considerably reduced. A feature that differentiates this technique from the previously presented ones is that the reaction conditions can be easily optimized by real-time monitoring with the help of UV absorbance.
2. *De Novo* Synthesis of L-Colitose, 2-\textit{epi}-L-Colitose and L-Rhodinose Building Blocks

3,6-Dideoxyhexoses are a class of deoxysugars characteristic to the O-specific side chain of the lipopolysaccharides from Gram negative bacteria, where they serve as antigenic determinants and are crucial for bacterial survival. A divergent, practical and efficient *de novo* synthesis of fully functionalized L-colitose (3,6-dideoxy-L-xylo-hexopyranose), 2-\textit{epi}-colitose (3,6-dideoxy-L-lyxo-hexopyranose) and L-rhodinose (2,3,6-trideoxy-L-threo-hexopyranose) building blocks has been achieved using inexpensive, commercially available (S)-ethyl lactate as the starting material. The synthetic route centered around a diastereoselective Cram-chelated allylation that provided a common homoallylic alcohol intermediate. Oxidation of this common intermediate finally resulted in the synthesis of the three monosaccharide building blocks.
2. De Novo Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

2.1 Introduction

Deoxysugars are commonly found in nature as a component of plants, fungi and bacteria. One particular class of deoxysugars, the 3,6-dideoxyhexoses, are widely spread in the O-specific side chain of lipopolysaccharides of Gram negative bacteria. These sugars serve as antigenic determinants and are vital for bacterial survival. L-Colitose (3,6-dideoxy-L-xylo-hexopyranose; Figure 4) is a 3,6-dideoxyhexose of particular interest since this monosaccharide is specific to the O-antigen of a large range of Gram negative bacteria such as Escherichia coli O111, Salmonella enterica, Salmonella adelaide, Salmonella greenside and Vibrio cholerae that are enteric pathogens responsible for many epidemics. Furthermore, L-colitose is present in the O-specific polysaccharides of aerobic heterotrophic marine prokaryotes of the genera Pseudoalteromonas. These prokaryotes produce a range of biologically active products including antibiotics, antitoxins, antitumor and antiviral agents. To evaluate the immunological properties of L-colitose-containing bacterial oligosaccharides and the potential of the corresponding glycoconjugates as vaccines against bacteria, the development of an efficient synthesis for functionalized L-colitose building blocks is critical.

![L-colitose](image)

Figure 4. L-Colitose, a rare sugar.

Literature describing the synthesis of L-colitose building block is scarce. However, several routes towards L-colitose derivatives and L-colitose glycosylating agents have been reported. These routes start mainly from expensive L-fucose and involve formation of a cyclic 3,4-acetal or 3,4-orthoester, followed by the protection of the hydroxyl at C2 and selective cleavage of the 1,3-dioxolane. Removal of the hydroxyl at C3 is generally achieved via a Barton-McCombie deoxygenation.

For example, Kováč has described the synthesis of disarmed β-colitose thioglycoside 156 in nine steps and 60% overall yield starting from α-L-fucose (Scheme 33). α-L-Fucose was converted in four steps to the corresponding β-thiofucoside 158. After masking the hydroxyls at C3 and C4 as an orthoester, an acetate protecting group was installed at C2 to afford fucose intermediate 159. Regioselective opening of the orthoester provided secondary alcohol 160 in
2. De Novo Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

68% overall yield over seven steps. Deoxygenation of the alcohol at C3 via a Barton-McCombie procedure provided, in two steps and 88% yield, L-colitose building block 156. Thus, Kovič completed in nine steps and 60% overall yield the synthesis of disarmed L-colitose glycosylating agent 156 starting from α-L-fucose. Although, the below described synthesis is high yielding, the cost of the starting material and the presence of a participating protective group at C2, which is not compatible with the occurrence of L-colitose as α-sugar in nature are main drawbacks of this route.

\[ \text{Scheme 33. Kovič's route towards L-colitose building block: a) i. Ac}_2\text{O, pyridine, DMAP, 0 °C; ii. HBr-AcOH, CH}_2\text{Cl}_2, 0 °C \text{ to RT; b) i. EtSH, NaH, DME, 0 °C to RT; ii. anhyd. K}_2\text{CO}_3, \text{MeOH; c) i. CH}_3\text{C(OEt)}_3, \text{CSA, CH}_2\text{Cl}_2, \text{RT; ii. Ac}_2\text{O, pyridine, DMAP, 0 °C to RT; d) 90% aq. AcOH, RT, 3 h, 68% (seven steps); e) TCDI, DMAP, DMF, RT, 96%; f) Bu}_3\text{SnH, AIBN, toluene, reflux, 92%}} \]

Oscarson’s group has also developed routes towards L-colitose building blocks. They reported that the outcome of the glycosylation reaction with colitose is highly influenced by the protective group pattern. Thus, a non-participating protecting group needs to be placed at C2 to favor the formation of the α-glycosidic linkage and an electron withdrawing group has to be installed at C4 to stabilize the electron-rich dideoxysugars. As a result, particular care was taken in functionalizing the L-colitose building blocks (Scheme 34). The synthesis started from ethyl-β-L-thiofucoside 158 that was prepared in three steps from L-fucose. Formation of the 3,4-orthobenzoate, followed by O-para-chlorobenzylolation and subsequent opening of the orthoester gave alcohol 163 in 61% overall yield. The alcohol was converted to the corresponding thiocarbonylimidazole derivative that was reduced with tin hydride to give the thiocolitose 164 in 67% yield over two steps. Since, this building block doesn’t perform well as a glycosylating agent, it was transformed to the bromide glycosylating agent 165.
2. De Novo Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

Oscarson’s route provided in nine steps a differentially protected colitose building block that was successfully used in the assembly of oligosaccharides. However, the synthetic methodology was low yielding (164, 26% over eight steps) and used expensive starting material.

![Scheme 34](image)

**Scheme 34.** Oscarson’s route towards L-colitose building block: a) i. Ac₂O, pyridine, DMAP, 0 °C; ii. EtSH, BF₃·OEt₂, CH₂Cl₂, RT; iii. K₂CO₃, MeOH, 64% (three steps); b) i. PhC(OEt)₃, p-TsOH, CH₃CN; ii. p-ClBnCl, NaH, DMF; c) TFA (90% aq.), CH₃CN, 61% (three steps); d) i. C(S)Im₂, imidazole, (CH₂Cl)₂; ii. Bu₃SnH, AIBN, toluene, reflux, 67% (two steps); e) Br₂, CH₂Cl₂.

Similar synthetic routes starting from L-fucose were also reported by Bundle⁷⁰ and Thiem⁷¹. The routes provided in lower yields colitose derivatives that required further steps to be converted to the corresponding colitose donors.

A total synthesis of open chain colitose 166 was developed starting from (S)-benzyloxyacetaldehyde 167 and the silyl enol ether of (2-acetyl butadiene)-tricarbonyl iron 168 (Scheme 35).⁷² Addition of silyl enol 168 generated *in situ* from the corresponding ketone to the preformed aldehyde-titanium tetrachloride complex provided a mixture of 1,2-syn diastereoisomers 169a and 169b. Reduction of the ketone group on 169a with borane dimethyl sulfide complex introduced the last stereogenic center on the molecule. Per-acetylation of 170, followed by decomplexation in the presence of ceric ammonium nitrate gave intermediate diene 172, which was further submitted to reductive ozonolysis to give colitose derivative 166 in six steps and 19% overall yield starting from (S)-benzyloxyacetaldehyde 167 that can be easily obtained in two steps from inexpensive (S)-ethyl lactate. To convert the opened chain sugar 166 to an efficient glycosyl donor, further protection-deprotection steps were required making this route lengthy.
2. De Novo Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

Scheme 35. Total synthesis of L-colitose building block 166: a) TiCl₄, CH₂Cl₂, -78 °C, 169a (38%), 169b (38%); b) BH₃·Me₂S, THF, 20 °C, 87%; c) Ac₂O, Et₃N, DMAP, CH₂Cl₂, 20 °C, 82%; d) CAN, MeCN, H₂O, 0 °C, 91%; e) i. O₃, MeOH, -78 °C; ii. Me₂S, 80%.

The different methodologies established so far for accessing L-colitose building blocks start mainly from expensive L-fucose and/or require many steps. As part of our ongoing program aimed at establishing efficient synthetic pathways for accessing fully protected natural and non-natural monosaccharides, I decided to develop a de novo synthesis of a L-colitose glycosylating agent starting from inexpensive commercially available (S)-ethyl lactate. Furthermore, I would like to extend this synthetic methodology to the preparation of 2-epi-colitose (3,6-dideoxy-L-lyxo-hexopyranose) and L-rhodinose (2,3,6-trideoxy-L-threo-hexopyranose) that are closely related to L-colitose (Figure 5).

Figure 5. Rare monosaccharide targets.
2.2 Results and Discussions

The retrosynthetic design targeted functionalized L-cotise building block 173 (Scheme 36) to be generated from acyclic precursor aldehyde 174. Two approaches were considered to access differentially protected aldehyde 174. Path A involved disconnection of β-hydroxy ketone intermediate 175 to commercially available methylglyoxal dimethyl acetal 176 and aldehyde 177 via an aldol-type reaction. Approach B was based on the allylation of (S)-α-hydroxy aldehyde 177 under Cram-chelation control.\textsuperscript{75} Aldehyde 177 with an inherent chirality at C5 was synthesized in two steps starting from (S)-ethyl lactate.

Scheme 36. Retrosynthetic analysis of L-cotise building block 173.

Aldol-based construction of the carbon backbone (Path A)
To investigate the aldol-based approach shown in Scheme 36, aldehydes 179 and 167 were prepared in two steps starting from (S)-ethyl lactate (Scheme 37). After protection of the hydroxyl group as silyl ether 180 and benzyl ether 181 respectively, reduction of the ester function with diisobutylaluminium hydride at -78 °C provided aldehydes 179 and 167.

Scheme 37. Synthesis of the aldol donors 179 and 167: a) TBSCI, DMAP, imidazole, CH\textsubscript{2}Cl\textsubscript{2}, RT, 3 h, 98%; b) DIBAL, CH\textsubscript{2}Cl\textsubscript{2}, -78 °C, 5 h, 70%; c) BnBr, NaH, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C, 4 h, 57%; d) DIBAL, CH\textsubscript{2}Cl\textsubscript{2}, -78 °C, 4 h, 87%.
The proline-catalyzed aldol reaction was previously used for the synthesis of ulosonic acid precursors with moderate yields, but good diastereoselectivities. Thus, this aldol-based method was explored to approach 1,3-hydroxy ketones and . For this purpose, aldehydes and were treated with commercially available ketone in the presence of catalytic amounts of (R)-proline at 4 °C (Scheme 38). Unfortunately, after one week, no aldol adducts were observed. Performing the condensation of benzyloxyacetaldehyde with ketone at room temperature resulted in a 1:1 inseparable diasteroisomeric mixture (12%). The poor yield and diastereoselectivity could be the result of the low reactivity of the aldehyde partner or of the high instability of the target adduct, which could undergo a retro-aldol reaction.

Scheme 38. Proline catalyzed aldol condensation: (R)-proline (30 mol %), DMSO, 4 °C, 7 days.

An alternative aldol reaction, using a lithium enolate generated in situ with lithium bis(trimethylsilyl)amide, was attempted. This method didn’t prove more efficient producing an inseparable mixture of adduct in 35% yield with 1:1 dr (Scheme 39).

Scheme 39. Aldol reaction via lithium enolate: LHMDS, THF, -78 °C, 2 h, 35%, dr 1:1.

**Allylation-based construction of the carbon backbone (Path B)**

Due to difficulties encountered with aldol-based C-C bond forming reactions, an allylation strategy was considered. Initial attempts used known (S)-benzyloxyaldehyde as substrate. Treatment of the aldehyde with allyltrimethylsilane in presence of tin tetrachloride as a coordinating agent provided 4,5-syn diol in 74% yield on gram scale (Scheme 40). The preference of the allylation reaction towards the formation of the 4,5-syn diol is favored by the Cram-chelated transition state depicted in Scheme 40.
2. *De Novo* Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

![Scheme 40](image)

**Scheme 40.** Stereoselective synthesis of homoallylic alcohol 184: SnCl4, CH2Cl2, -78 °C, 2 h, 7:1 (syn/anti), 74%.

Having established a route to 4,5-*syn* diol 184, the stereoselective introduction of the hydroxyl at position 2 was the next goal. At this level, several routes were investigated. Initially, the stereoselective organocatalytic α-aminoxylation of aldehydes79 was explored. To test this method, aldehyde substrate 185 was prepared. Firstly, homoallylic alcohol 184 was treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate in presence of 2,6-lutidine to install an orthogonal *tert*-butyldimethylsilyl protecting group at C4 (Scheme 41). Alkene 186 was regioselectively transformed via a hydroboration reaction into primary alcohol 187 that was further oxidized to the corresponding aldehyde 185. To optimize the two steps-sequence, various reactions were performed. In my hands, the use of borane tetrahydrofuran complex and the 9-borabicyclo[3.3.1]nonane as hydroborating agents provided the target alcohol 187 in 40% and 38% yield, respectively. A one pot hydroboration-pyridinium chlorochromate oxidation procedure80 on the other hand gave aldehyde 185 in only 20% yield. Better results were obtained using catechol borane as hydroborating agent in presence of a catalytic amount of chlorotris(triphenylphosphine)rhodium(II).81 Alcohol 187 was isolated in 62% yield and further oxidized to the corresponding aldehyde 185 by applying *Parikh-Doering* oxidation conditions.82

![Scheme 41](image)

**Scheme 41.** Synthesis of aldehyde 185: a) TBSOTf, 2,6-lutidine, CH2Cl2, 0 °C, 3 h, 100%; b) catechol borane, Rh(PPh3)3Cl, THF, 0 °C, 90 min then H2O2, RT, 62%; c) SO3pyridine, DMSO, 0 °C, 1 h, 92%; d) 9-BBN, CH2Cl2, reflux, 4 h then PCC, -50 °C to RT, 4 h, 20%.

With aldehyde 185 in hand, the (R)-proline-catalyzed α-oxidation reaction was investigated. Aldehyde 185 was reacted with nitrosobenzene in presence of a catalytic amount of (R)-
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proline in dimethylsulfoxide. After complete consumption of the starting material and work-up, the reaction mixture was directly submitted to standard hydrogenation conditions leading to a mixture of products from which the target hemiacetal 189 could not be isolated (Scheme 42). To simplify the system, in the next α-aminoxylation trials, the conversion of the intermediate 188 to the more stable diol 190 by reduction with sodium borohydride was attempted without success. Various conditions described in the literature differing in solvent, temperature or number of equivalents of aldehyde substrate were applied, but without any success. The target product could be identified only by MS, when the reaction was performed at -20 °C with two equiv of aldehyde 185 per equiv of nitrosobenzene using acetonitrile as a solvent. Unfortunately, product 190 could not be isolated by flash column chromatography on silica gel. Due to this issue, this approach was abandoned.

![Scheme 42](image)

**Scheme 42.** α-Aminoxylation attempts: a) 185 (1 equiv), nitrosobenzene (1 equiv), (R)-proline (0.4 equiv), DMSO, RT, 1 h; b) 185 (2 equiv), nitrosobenzene (1 equiv), (R)-proline (0.4 equiv), CH₃CN, -20 °C, 24 h; c) H₂, Pd/C, AcOEt, 24 h; d) NaBH₄.

An alternative method for stereoselectively introducing the hydroxyl group at C2 relies upon an epoxidation/hydrolytic kinetic resolution sequence. Thus, available alkene 186 with the homoallylic alcohol protected as bulky tert-butylimethylsilyl ether was submitted to an epoxidation reaction using meta-chloroperoxybenzoic acid as oxidizing reagent to give epoxide 191 (Scheme 43). When the reaction was performed at 0 °C epoxide 191 was isolated as a 3:1 diastereoisomeric mixture in 47% yield. The yield was improved by increasing the temperature to 25 °C. This attempt resulted in a 2:1 diastereoisomeric mixture (70% yield) that was directly submitted to Jacobsen hydrolytic kinetic resolution (HKR) using (S, S)-(salen)-Co(III)-OAc as catalyst to provide the 2,4-anti diol 192 in 20% yield and 2,4-syn epoxide 193 in 39% yield. At this level, we realized that although a bulky silyl ether was placed on the homoallylic position to avoid coordination during the epoxidation step, the
undesired 2,4-syn epoxide is the major diastereoisomer. Thus, target 2,4-anti diol 192 was obtained only in 20% yield. The poor yield at such late stage of the synthesis convinced us to abandon this route as well.

**Scheme 43.** Hydroxyl introduction at C2 via hydrolytic kinetic resolution: a) \textit{m}-CPBA, CH₂Cl₂, 24 h, RT; b) (S, S)-(salen)-Co(II), AcOH, H₂O, THF, 24 h, RT, 192 (20%), 193 (38%).

Finally, Sharpless asymmetric dihydroxylation (SAD)\textsuperscript{86} method was explored (AD, Table 1). At first, homoallylic alcohol 184 was treated with the appropriate reagent AD-mix α in a mixture \textit{tert}-butanol/water to afford the corresponding 2,4-anti triol (Table 1, entry 1). Surprisingly, the reaction was not stereoselective, and the target triol was obtained as a 1.5:1 diastereoisomeric mixture that was separated by silica gel chromatography purification. The use of AD-mix β that should favor the formation of 2,4-syn triol, exhibited similar levels of diastereoselectivity (Table 1, entry 2). At least modest diastereoselectivity in line with the selectivity achieved in the Sharpless dihydroxylation reaction of simple terminal olefins\textsuperscript{87} was expected. With the hope to increase the diastereoselectivity of the dihydroxylation reaction, substrates 186 and 194 presenting a silyl ether and an acetyl ester at C4 were subjected to SAD. Unfortunately, these trials resulted in inseparable diol mixtures with very poor diastereoselectivities ranging from 1:1 to 2.5:1 (Table 1, entries 3-6). These results showed that for this particular class of terminal alkenes, the diastereoisotopic faces could not be differentiated by the chiral catalyst, allowing thereby the non-selective mechanism to be dominant. Similar poor diastereoselectivities for the Sharpless AD of homoallylic alcohol, ether and ester substrates were previously reported.\textsuperscript{88}
2. De Novo Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

Table 1. Attempted dihydroxylation reactions: a) Ac₂O, pyridine, 12 h, RT, 99%; b) See conditions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Conditions</th>
<th>dr&lt;sup&gt;a,b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>184</td>
<td>AD-mix β, tert-BuOH/H₂O (1:1), 0 °C, 60 h</td>
<td>1.5:1</td>
</tr>
<tr>
<td>2</td>
<td>184</td>
<td>AD-mix α, tert-BuOH/H₂O (1:1), 0 °C, 60 h</td>
<td>1.5:1</td>
</tr>
<tr>
<td>3</td>
<td>194</td>
<td>AD-mix β, tert-BuOH/H₂O (1:1), 0 °C, 60 h</td>
<td>2.5:1</td>
</tr>
<tr>
<td>4</td>
<td>194</td>
<td>AD-mix α, tert-BuOH/H₂O (1:1), 0 °C, 60 h</td>
<td>2:1</td>
</tr>
<tr>
<td>5</td>
<td>186</td>
<td>AD-mix β, tert-BuOH/H₂O (1:1), 0 °C, 60 h</td>
<td>1:1</td>
</tr>
<tr>
<td>6</td>
<td>186</td>
<td>AD-mix α, tert-BuOH/H₂O (1:1), 0 °C, 60 h</td>
<td>1:1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratios determined by <sup>1</sup>H NMR. <sup>b</sup> Major and minor diastereomers of the product mixtures were not determined

Although the levels of diastereoselectivity were low, the possibility to separate the 1,3-<i>anti</i> triol from its 1,3-<i>syn</i> diastereoisomer by simple chromatography on silica gel convinced me to retain this route in order to access L-colitose target building block. Since colitose is naturally found only α-linked to other sugars, strategically a building block with a non-participating protecting group PG<sup>1</sup> at C2 was required (Scheme 36). To enable the cyclization of the aldehyde precursor 174 (Scheme 36), I needed to ensure that the protecting group at C5 (PG<sup>3</sup>) is orthogonal to both C2-PG<sup>1</sup> and C4-PG<sup>2</sup>. Furthermore, an electron-withdrawing protecting group was to be placed in the C4 position to stabilize the electron rich deoxysugar building block. To fulfill these requirements, it was envisioned to access fully functionalized colitose 195 from linear aldehyde 196 presenting a non-participating benzyl ether at C2, an electron withdrawing benzoate ester at C4 and an orthogonal 2-naphthylmethyl at C5 (Scheme 44). The aldehyde intermediate 196 could be generated via a dihydroxylation/oxidation sequence from homoallylic alcohol 197 that could be prepared as previously, by allylation of (S)-α-hydroxy aldehyde 198 under Cram-chelation control.
2-Naphthylmethyl ether protection of the alcohol in (S)-ethyl lactate, followed by reduction of the ester moiety with diisobutylaluminium hydride afforded aldehyde 198 in 77% yield over two steps (Scheme 45). Introduction of the remaining L-colitose carbon backbone and the C4 stereocenter was accomplished as previously via Cram-chelated controlled allylation of 198 with allyl trimethyl silane in the presence of tin tetrachloride. The allylation reaction proceeded with better levels of diastereoselectivity than before (dr 14:1 compared to 7:1 dr obtained for the benzylic analogue 184) providing the desired homoallylic alcohol 197 in 91% yield. The absolute stereochemistry of the newly formed chiral center was assigned by Mosher ester analysis (see Experimental Section).89

Scheme 45. Synthesis of homoallylic alcohol 197: a) i. NapBr, NaH, DMF, 2 h, 0 °C to RT; ii. DIBAL, CH₂Cl₂, -78 °C, 90 min, 77% (two steps); b) SnCl₄, CH₂Cl₂, -78 °C, 2 h, 91%.

With olefin 197 and its benzoylated and silylated analogues 199 and 200 in hand, I investigated whether the outcome of the SAD was different from the previous tested substrates (AD; Table 2). To our disappointment, the new protecting group pattern didn’t have any significant influence on the diastereoselectivity of the dihydroxylation reaction.
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Table 2. Survey of the dihydroxylation attempts: a) BzCl, pyridine, 0 °C to RT, 2 h, 100%; b) TBSOTf, 2,6-lutidine, CH$_2$Cl$_2$, 2.5 h, 95%.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Conditions</th>
<th>dr$^{a,b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>197</td>
<td>AD-mix β, tert-BuOH/H$_2$O (1:1), 0 °C, 12 h</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>197</td>
<td>AD-mix α, tert-BuOH/H$_2$O (1:1), 0 °C, 12 h</td>
<td>1.5:1</td>
</tr>
<tr>
<td>3</td>
<td>199</td>
<td>AD-mix β, tert-BuOH/H$_2$O (1:1), 0 °C, 48 h</td>
<td>2:1</td>
</tr>
<tr>
<td>4</td>
<td>199</td>
<td>AD-mix α, tert-BuOH/H$_2$O (1:1), 0 °C, 12 h</td>
<td>2:1</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>AD-mix β, tert-BuOH/H$_2$O (1:1), 0 °C, 12 h</td>
<td>1.3:1</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>AD-mix α, tert-BuOH/H$_2$O (1:1), 0 °C, 12 h</td>
<td>1.7:1</td>
</tr>
</tbody>
</table>

$^a$ Ratios determined by $^1$H NMR. $^b$ Major and minor diastereomers of the product mixtures were not determined

The lack of selectivity when enlisting the Sharpless catalyst prompted me to use the Upjohn dihydroxylation$^{90}$ due its lower cost (Scheme 46).$^{91}$ Consequently, homoallylic alcohol 197 was treated at room temperature with osmium tetroxide/N-methylmorpholine-N-oxide in tetrahydrofuran/water to furnish an 1:1 mixture of 2,4-anti triol 201 and 2,4-syn triol 202. Triols 201 and 202 were separated by silica gel column chromatography to afford triol 201 in 49% yield and diastereoisomer 202 in 44% yield.

Scheme 46. Upjohn dihydroxylation of homoallylic alcohol 197: NMO, cat. OsO$_4$ THF/H$_2$O (2/1, v/v), RT, 24 h, 201 (49%), 202 (44%).
The absolute configuration of the newly introduced hydroxyl at C2 was established using Rychnovsky’s acetonide method\textsuperscript{92} (Scheme 47). Selective protection of the primary alcohol on substrate 201 as a tert-butylidiphenylsilyl ether, followed by treatment of the intermediate diol with 2,2-dimethoxypropane in the presence of pyridinium para-toluenesulfonate afforded acetonide 203. According to the \textsuperscript{13}C chemical shifts (see Experimental Section) of the acetonide methyl groups and quaternary carbon, it was determined that 201 had a 2,4-\textit{anti} configuration. Having established the absolute configuration of C4 through Mosher ester analysis of alcohol 197, the absolute configuration of C2 in triol 201 was thus determined to be \textit{S}. The same procedure was applied to triol 202 to afford acetonide 204. The \textsuperscript{13}C NMR data (see Experimental Section) was consistent with the assignment of a 2,4-\textit{syn} configuration for 202 and thus, an \textit{R}-configured C2 stereocenter.

![Scheme 47. Synthesis of the C2-C4 acetonides 203 and 204:](image-url)

Having set and defined all the necessary stereocenters in triol 201, the synthesis of \textit{L}-colitose 195 was completed (Scheme 48). Thus, triol 201 was converted selectively to the 5-membered benzylidene acetal 205. The remaining hydroxyl group in 205 was benzyolated and the benzylidene regioselectively opened using borane tetrahydrofuran complex/trimethylsilyl trifluoromethanesulfonate to afford primary alcohol 206 in 71\% yield over three steps. Although alcohol 206 could not be converted to corresponding aldehyde 196 using Swern\textsuperscript{93} or Parikh-Doering conditions, Dess-Martin periodinane\textsuperscript{94} provided aldehyde 196 in 92\% yield. Removal of the 2-naphthylmethyl protecting group in 196 with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone led to the corresponding hemiacetal, which was treated with trichloroacetonitrile in presence of 1,8-diazabicyclo[5.4.0]undec-7-ene to give \textit{L}-colitose.
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Trichloroacetimidate 195 as a 1.6:1 α/β mixture in 79% yield over two steps. Coupling constant analysis of the α-anomer ($^3J_{H1,H2} = 3.2$ Hz) and β-anomer ($^3J_{H1,H2} = 7.6$ Hz) of imidate 195 are consistent with an axially-oriented H2, confirming the $^{13}$C acetonide analysis of intermediate 201.

Scheme 48. Synthesis of L-colitose building block 195: a) PhCH(OCH$_3$)$_2$, CSA, CuSO$_4$, CH$_3$CN/THF (1/1, v/v), RT, 10 min; b) i. BzCl, pyridine, RT, 2 h; ii. BH$_3$THF, TMSOTf, CH$_2$Cl$_2$, 0 °C to RT, 2 h, 71% (three steps); c) Dess-Martin periodinane, pyridine, CH$_2$Cl$_2$, RT, 1 h, 92%; d) i. DDQ, MeOH, CH$_2$Cl$_2$, 0 °C to RT, 2 h; ii. CCl$_3$CN, DBU, CH$_2$Cl$_2$, 0 °C, 3 h, 79% (two steps).

As a means to access derivatives of carbohydrates that are not found in nature, the de novo approach was applied towards the synthesis of 2-epi-colitose (3,6-dideoxy-L-lyxo-hexopyranose) building block 210 (Scheme 49). Such non-natural analogs serve as important probes when determining the epitope of oligosaccharide antigens. Thus, 2,4-syn triol 202 was converted in three steps to primary alcohol 208 in 50% overall yield. A major undesired byproduct of this reaction sequence was the formation of the C2-C4 6-membered benzylidene acetal. The 6-membered ring forms more easily here (in contrast to the conversion of the 2,4-anti triol 201 to 205) due to the formation of an 6-membered acetal where all substituents occupy equatorial positions. Oxidation of primary alcohol 208 gave aldehyde 209 in 85% yield. Following 2-naphthylmethyl cleavage in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, treatment of the intermediate acetal with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene afforded 2-epi-colitose trichloroacetimidate 210 in 84% overall yield as a 9:1 α/β mixture. Coupling constant analysis of the major α-anomer ($^3J_{H1,H2} < 1.0$ Hz) of 210 is consistent with an equatorial-equatorial proton coupling.
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**Scheme 49.** Synthesis of 2-epi-colitose building block 210: a) PhCH(OMe)_2, CSA, CuSO_4_, CH_3CN/THF (1/1, v/v), RT, 10 min; b) i. BzCl, pyridine, RT, 2 h; ii. BH_3·THF, TMSOTf, CH_2Cl_2, 0 °C to RT, 2 h, 50% (three steps); c) Dess-Martin periodinane, pyridine, CH_2Cl_2, RT, 1 h, 85%; d) i. DDQ, MeOH, CH_2Cl_2, 0 °C to RT, 2 h; ii. CCl_3CN, DBU, CH_2Cl_2, 0 °C, 3 h, 84% (two steps).

L-Rhodinose building block 211 was prepared due to the presence of this motif in different classes of polyketides with antitumor and antibacterial activity\(^96\) (Scheme 50). After protection of secondary alcohol 197 as a benzoyl ester, rhodium-catalyzed hydroboration in the presence of catecholborane furnished alcohol 212 in 95% yield over two steps. Parikh-Doering oxidation of alcohol 212 to the corresponding aldehyde, followed by cleavage of the 2-naphthylmethyl protecting group in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and in situ cyclization afforded intermediary hemiacetal 213 as an 1:1 α/β anomeric mixture in 85% yield over two steps. Since L-rhodinosyl acetates have been shown to be efficient glycosylating agents,\(^97\) hemiacetal 213 was quantitatively converted into the corresponding glycosylating agent by treatment with acetic anhydride and pyridine. Thus, L-rhodinosyl acetate 211 was prepared in eight steps and 57% overall yield starting from inexpensive (S)-ethyl lactate.
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Scheme 50. Synthesis of L-rhodinose building block 211: a) BzCl, pyridine, RT, 2 h, quant.; b) catechol borane, Rh(PPh₃)₃Cl, THF, 0 °C, 90 min then NaOH, H₂O₂, 95%; c) i. PySO₃, DMSO, DIPEA, CH₂Cl₂, 0 °C; 1 h; ii. DDQ, MeOH, CH₂Cl₂, 0 °C to RT, 2 h, 85% (two steps); d) Ac₂O, pyridine, RT, 2 h, quant..

2.3 Conclusion

In summary, Chapter 2 describes a practical synthesis of a fully functionalized L-colitose glycosylating agent in ten steps with 18% overall yield starting from inexpensive commercially available (S)-ethyl lactate. Particular emphasis was given to install the appropriate protective group pattern on the glycosylating agent 195 in order to fulfill both stereoselectivity and reactivity requirements for the following glycosylation steps. The divergent route also allowed for preparation of two other congeners of L-colitose: a 2-epi-colitose building block (11% overall yield) and a L-rhodinose building block (57% overall yield) from advanced intermediate 197. The key step of this synthesis is the allylation reaction under Cram-chelated control that introduces the hydroxyl group at C4 in a stereoselective manner. This approach was extended to the preparation of other natural and non-natural 3,6-dideoxy L- and D-hexoses. This methodology will be particularly useful for the synthesis of natural products and for the basis of oligosaccharide conjugate vaccines against bacteria that contain the respective glycans on their surface.
2.4 Experimental Section

**General Experimental Details:** Commercial grade reagents and solvents were used without further purification except as indicated below. All reactions were conducted under an Ar atmosphere. The term “concentrated” refers to the removal of solvents and other volatile material using a rotary evaporator while maintaining a water bath temperature under 40 °C. The compounds purified by flash chromatography are further concentrated by the removal of residual solvent under high vacuum (<0.2 mbar). Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or potassium permanganate solution (potassium permanganate in basic aqueous solution). Column chromatography was performed using Kieselgel 60 (230-400 mesh) silica gel with a typical 50-100:1 weight ratio of silica gel to crude product. $^1$H, $^{13}$C spectra were recorded on a Varian Mercury 400 (400 MHz) or 600 (600 MHz) spectrometer in CDCl$_3$ with chemical shifts referenced to internal standards (CDCl$_3$: $^1$H 7.26 ppm, $^{13}$C 77.0 ppm) unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet; as, apparent singulet; ad, apparent doublet; at, apparent triplet, aq, apparent quadruplet for $^1$H-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 in the Department of Organic Chemistry at Free University Berlin using a Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a UniPol L 1000 polarimeter (Schmidt & Haensch, Berlin, Germany), with concentrations expressed in g/100 mL.

![Chemical Reaction](image)

**(S)-Ethyl-2-(tert-butyldimethylsilyloxy)propanoate (180):** To a solution of (S)-ethyl-lactate (1.0 g, 8.5 mmol) in CH$_2$Cl$_2$ (10 mL), DMAP (0.1 g, 0.85 mmol, 0.1 equiv), imidazole (0.70 g, 10.1 mmol, 1.2 equiv) and TBSCl (1.4 g, 9.3 mmol, 1.2 equiv) were added. After stirring at RT for 3 h, the reaction was diluted with H$_2$O and CH$_2$Cl$_2$. The organic layer was separated, washed with brine, dried over MgSO$_4$ and concentrated under reduced pressure. The crude
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Material was purified by flash column chromatography on silica gel (Cyclohexane/ACOEt = 15:1) to afford ester 180 (1.9 g, 8.3 mmol, 98%) as an oil. The spectral data are consistent with the reported literature.⁷⁸

(S)-2-(tert-Butyldimethylsilyloxy)propanal (179): A solution of silyl ether 180 (1.6 g, 6.8 mmol) in CH₂Cl₂ (23 mL) was cooled to -78 °C. 1 M DIBAL in cyclohexane (13.5 mL, 13.5 mmol, 2 equiv) was added and the reaction was stirred at -78 °C for 5 h. The reaction was quenched by adding methanol and was warmed up to RT. A saturated aq. solution of potassium sodium tartrate was added and the mixture was stirred for 30 min. The reaction was extracted with CH₂Cl₂. The combined organic extracts were washed with H₂O, brine, dried over MgSO₄ and concentrated under reduced pressure. Flash column chromatography on silica gel (Cyclohexane/ACOEt = 20:1) gave aldehyde 179 (0.9 g, 4.7 mmol, 70%) as an oil. The spectral data are consistent with the reported literature.⁷⁸

(S)-Ethyl-2-(benzoyloxy) propanoate (181): At 0 °C, a solution of (S)-ethyl-lactate (9.7 mL, 85 mmol) and benzyl bromide (11.0 mL, 93 mmol, 1.1 equiv) in CH₂Cl₂ (120 mL) was treated with NaH (60% in mineral oil, 3.7 g, 93 mmol, 1.1 equiv). The reaction was stirred at 0 °C for 3 h and then quenched with ethanol and extracted with CH₂Cl₂. After separation, the organic layer was washed with H₂O, brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (Cyclohexane/ACOEt = 1:0 to 15:1) to yield benzyl ether 181 (10.0 g, 49 mmol, 57%) as a yellow oil. The spectral data are consistent with the reported literature.⁷⁸

(S)-2-(Benzoyloxy) propanal (167): A solution of benzyl ether 181 (840 mg, 4.0 mmol) in CH₂Cl₂ (13 mL) was cooled to -78 °C. 1 M DIBAL in cyclohexane (8.1 mL, 8.1 mmol, 2
equiv) was added and the reaction was stirred at -78 °C for 4 h. The reaction was quenched by adding methanol and was warmed up to RT. A saturated aq. solution of potassium sodium tartrate was added and the mixture was stirred for 30 min. The reaction was extracted with CH₂Cl₂. The combined organic extracts were washed with H₂O, brine, dried over MgSO₄ and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (Cyclohexane/AcOEt = 20:1) afforded aldehyde 167 (580 g, 3.5 mmol, 87%) as an oil. The spectral data are consisted with the reported literature.  

(2S, 3S)-2-(Benzyloxy)hex-5-en-3-ol (184): To a solution of (S)-2-(benzyloxy)propanal 167 (1.3 g, 7.8 mmol) in CH₂Cl₂ (37 mL) was added at -78 °C, tin tetrachloride (1.0 mL, 8.6 mmol, 1.1 equiv). After 15 min, allyltrimethylsilane (1.4 mL, 8.6 mmol, 1.1 equiv) was added and the mixture was stirred at -78 °C for 2 h. The reaction was quenched by adding H₂O and warmed slowly to RT. The mixture was extracted with CH₂Cl₂ and the combined organic extracts were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Cyclohexane/AcOEt = 100:0 to 90:10) to give homoallylic alcohol 184 (1.18 g, 5.7 mmol, 74%). The spectral data are consisted with the reported literature.  

((2S, 3S)-2-(Benzyloxy)hex-5-en-3-yloxy)(tert-butyl)dimethylsilane (186): At 0 °C, a solution of (2S, 3S)-2-(benzyloxy)hex-5-en-3-ol (184) (1.0 g, 4.85 mmol) in CH₂Cl₂ (50 mL) was treated with 2,6-lutidine (1.1 mL, 9.7 mmol, 2 equiv) and TBSOTf (1.7 mL, 7.3 mmol, 1.5 equiv). After being stirred at 0 °C for 3 h, the reaction was quenched with a saturated aq. solution of NaHCO₃ and extracted with CH₂Cl₂. The combined organic extracts were washed with H₂O, brine, dried on MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (Cyclohexane/AcOEt = 100:0 to 90:10) to afford quantitatively silyl ether 186 (1.55 g, 4.85 mmol) as an oil. The spectral data are consisted with the reported literature.
(4S, 5S)-5-(Benzylxy)-4-(tert-butyldimethylsilyloxy)hexan-1-ol (187): A solution of ((2S, 3S)-2-(benzylxy)hex-5-en-3-yloxy)(tert-butyl)dimethylsilane (186) (0.5 g, 1.56 mmol) and Rh(PPh₃)₃Cl (50 mg, 55 µmol, 0.035 equiv) in THF (12 mL) was cooled to 0 °C. The catecholborane (4.7 mL, 4.7 mmol, 3 equiv) was added and the reaction was stirred at 0 °C for 3 h. After diluting the reaction with a mixture THF/MeOH (1/1, v/v) (6.5 mL), a buffer solution pH=8.8 (6.5 mL) followed by an aq. solution 35% H₂O₂ (6.5 mL) were added and the reaction was stirred vigorously overnight. The reaction was diluted with Et₂O, washed with a saturated aq. solution of NaHCO₃ and brine, dried over MgSO₄ and concentrated under reduced pressure. Flash column chromatography (Cyclohexane/AcOEt = 80:20, 1% Et₃N) afforded alcohol 187 (325 mg, 960 µmol, 62%) as a colorless oil. The spectral data are consisted with the reported literature.

(4S, 5S)-5-(Benzylxy)-4-(tert-butyldimethylsilyloxy)hexan-1-ol (185): At 0 °C, a solution of (4S, 5S)-5-(benzylxy)-4-(tert-butyldimethylsilyloxy)hexan-1-ol (187) (320 mg, 946 µmol) and N,N-diisopropylethylamine (0.82 mL, 4.8 mmol, 5 equiv) in CH₂Cl₂ (4.8 mL) and DMSO (0.7 mL) was treated with PySO₃ complex (460 mg, 2.9 mmol, 3 equiv). The reaction was stirred at 0 °C for 1 h, quenched with a saturated aq. solution of Na₂S₂O₃ (30 mL) and warmed to RT. The organic layer was washed with H₂O, brine, dried over MgSO₄ and concentrated under reduced pressure. Flash column chromatography (Cyclohexane/AcOEt = 100:0 to 80:20) afforded aldehyde 185 (295 mg, 873 µmol, 92%). The spectral data are consisted with the reported literature.
(2S, 4S, 5S)-5-(Benzylxylo)-4-(tert-butylidemethylsilylxylo)hexane-1,2-diol (192) and (2R, 4S, 5S)-5-(benzylxylo)-1,2-(epxy)-4-(tert-butylidemethylsilylxylo)hexane (193): At 0 °C, m-CPBA (70%, 845 mg, 3.43 mmol, 1.2 equiv) was added to a solution of alkene 186 (916 mg, 2.86 mmol) in CH₂Cl₂ (10 mL). The reaction mixture was stirred at RT for 24 h and quenched with a saturated aq. solution of Na₂S₂O₃. After 10 min of vigorous stirring, the mixture was extracted with CH₂Cl₂. The organic layer was washed with a saturated aq. solution of NaHCO₃, brine, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography (Cyclohexane/AcOEt = 80:20) on silica gel afforded 673 mg of epoxide 191 (70%) with a dr synlanti = 2:1. ESI m/z calcd. for (M+Na)⁺ C₁₉H₃₂O₃SiNa: 359.2, found 359.2. The diastereoisomeric mixture was directly submitted to Jacobsen’s kinetic resolution.

(S, S)-(−)-N,N’-bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexane diaminocobalt (II) (8 mg, 13 μmol, 0.01 equiv) and AcOH (3.2 mL, 56 μmol, 0.04 equiv) were added to a solution of epoxide 191 (dr 2:1, 470 mg, 1.4 mmol) in THF (1 mL). The reaction flask was cooled to 0 °C and H₂O (14 μL, 770 μmol, 0.55 equiv) was added. The reaction mixture was stirred for 24 h at RT and then concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (Hexanes/AcOEt = 80:20 to 50:50) to afford 1,2-anti diol 192 (140 mg, 395 μmol, 20% over two steps) and the 1,2-syn epoxide 193 (260 mg, 773 μmol, 39% over two steps) as colorless oils.

(2S, 4S, 5S)-5-(Benzylxylo)-4-(tert-butylidemethylsilylxylo)hexane-1,2-diol (192): Rᵣ = 0.3 (Cyclohexane/AcOEt = 50:50); ¹H NMR (400 MHz, CDCl₃): δ 7.40 – 7.27 (m, 5H), 4.63 (d, J = 12.0 Hz, 1H), 4.45 (d, J = 12.0 Hz, 1H), 4.03 – 3.98 (m, 1H), 3.91 – 3.85 (m, 1H), 3.65 – 3.51 (m, 2H), 3.50 – 3.44 (m, 1H), 3.30 (d, J = 2.0 Hz, 1H), 2.12 (s, 1H), 1.75 (ddd, J = 14.4, 4.8, 3.2 Hz, 1H), 1.63 – 1.59 (m, 1H), 1.17 (d, J = 6.4 Hz, 3H), 0.87 (s, 9H), 0.06 (s, 3H), -0.01 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 138.43, 128.59 (2C), 127.89, 127.80 (2C), 76.91, 73.18, 71.06, 70.90, 67.02, 34.03, 25.89 (3C), 18.03, 13.09, -4.21, -4.72; ESI m/z calcd. for (M+Na)⁺ C₁₉H₃₄O₃SiNa: 377.2, found 377.2.

(2R, 4S, 5S)-5-(Benzylxylo)-1,2-(epxy)-4-(tert-butylidemethylsilylxylo)hexane (193): Rᵣ = 0.5 (Cyclohexane/AcOEt = 50:50); ¹H NMR (400 MHz, CDCl₃): δ 7.42 – 7.27 (m, 5H), 4.58 (d, J = 12.0 Hz, 1H), 4.51 (d, J = 12.0 Hz, 1H), 4.01 (ddd, J = 9.6, 4.4, 2.4 Hz, 1H), 3.55 –
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3.49 (m, 1H), 3.08 – 2.99 (m, 1H), 2.80 (dd, J = 5.2, 4.0 Hz, 1H), 2.51 (dd, J = 5.2, 2.8 Hz, 1H), 1.72 (m, 1H), 1.61 (m, 1H), 1.12 (d, J = 6.4 Hz, 3H), 0.87 (s, 9H), 0.05 (s, 3H), -0.00 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 138.86, 128.47 (2C), 127.71 (2C), 127.66, 76.95, 71.08, 70.71, 50.39, 48.27, 34.31, 25.91 (3C), 18.10, 13.47, -4.39, -4.76; ESI m/z calcd. for (M+Na)$^+$ C$_{19}$H$_{32}$O$_3$SiNa: 359.2, found 359.2.

(2S, 3S)-2-(Benzyloxy)hex-5-en-3-yl acetate (194): To a solution of (2S, 3S)-2-(benzyloxy)hex-5-en-3-ol (184) (670 mg, 3.25 mmol) in pyridine (11 mL), DMAP (4 mg, 32 µmol, 0.01 equiv), followed by Ac$_2$O (0.7 mL, 7.2 mmol, 2.0 equiv) were added. The reaction was stirred overnight at RT and concentrated under reduced pressure. The crude oil is diluted with CH$_2$Cl$_2$ and washed with H$_2$O and brine, dried over MgSO$_4$, concentrated under reduced pressure and co-evaporated with toluene to yield ester 194 (800 mg, 3.21 mmol, 99%) as oil. The spectral data are consistent with the reported literature.

(S)-Ethyl 2-(naphthal-2-ylmethoxy)propanoate (SI1): At 0 °C, a solution of (S)-ethyl-lactate (5.0 mL, 44 mmol) and 2-naphthylmethyl bromide (10.6 g, 48 mmol, 1.1 equiv) in DMF (146 mL) was treated with NaH (60% in mineral oil, 1.9 g, 48 mmol). The reaction mixture was stirred at RT for 2 h and quenched by adding ethanol. The reaction was extracted with Et$_2$O. The combined organic extracts were washed with H$_2$O, brine, dried over MgSO$_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (Cyclohexane/AcOEt = 100:0 to 90:10) to yield the 2-naphthylmethyl ether SI1 (10.6 g, 41 mmol, 94%) as a yellow oil: [α]$_{D}^{20}$ = -62.6° (c = 0.96, CHCl$_3$); R$_f$ = 0.50 (Cyclohexane/AcOEt = 80:20); $^1$H NMR (400 MHz, CDCl$_3$): δ 7.90 – 7.77 (m, 4H), 7.54 – 7.39 (m, 3H), 4.86 (d, J = 12.0 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.28 – 4.15 (m, 2H), 4.10 (q, J = 6.8 Hz, 1H), 1.46 (d, J = 6.8 Hz, 3H), 1.30 (t, J = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 173.4, 135.2, 133.3, 133.2, 128.3, 128.0, 127.8, 126.9, 126.2, 126.08, 126.07, 74.1, 72.2, 61.0, 18.8, 14.4; IR (thin film) 3055, 2982, 2936, 1742, 1445, 1369, 1270 cm$^{-1}$; HRMS(ESI) m/z calcd. for (M+Na)$^+$ C$_{16}$H$_{18}$O$_3$Na: 281.1154, found 281.1158.
(S)-2-(Naphthalen-2-ylmethoxy)propanal (198): To a solution of 2-naphthylmethyl ether SI1 (8.1 g, 31 mmol) in CH₂Cl₂ (314 mL), 1 M DIBAL in cyclohexane (34.5 mL, 34 mmol) was added at -78 °C. After 1.5 h, the reaction was quenched with methanol (80 mL) and was warmed up to RT. A solution of potassium sodium tartrate was added and the mixture was stirred overnight and extracted with CH₂Cl₂. The combined organic extracts were washed with H₂O, dried over MgSO₄ and concentrated. Flash column chromatography (Cyclohexane/AcOEt = 100:0 to 90:10) on silica gel gave aldehyde 198 (5.5 g, 25.6 mmol, 82%) as a colorless oil: [α]D⁺²₀ = -34.5 (c = 0.58, CHCl₃); Rf = 0.75 (Toluene/Acetone = 80:20); ¹H NMR (400 MHz, CDCl₃): δ 9.70 (d, J = 1.6 Hz, 1H), 7.93 – 7.75 (m, 4H), 7.58 – 7.43 (m, 3H), 4.83 (d, J = 12.0 Hz, 1H), 4.76 (d, J = 12.0 Hz, 1H), 3.95 (qd, J = 6.8, 1.6 Hz, 1H), 1.36 (d, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 203.4, 134.9, 133.3, 133.2, 128.5, 128.0, 127.8, 127.0, 126.4, 126.2, 125.8, 79.5, 72.2, 15.4; IR (thin film) 3448, 3055, 2980, 2932, 2854, 2801, 2706, 1733, 1602, 1509, 1445, 1373, 1330 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₁₄H₁₄O₂Na: 237.0891, found 237.0885.

(2S, 3S)-2-(Naphthalen-2-ylmethoxy)hex-5-en-3-ol (197): SnCl₄ (2.6 mL, 23 mmol) was added at -78 °C to a solution of aldehyde 198 (4.4 g, 20 mmol) in CH₂Cl₂ (100 mL). After 15 min, allyltrimethylsilane (3.6 mL, 23 mmol) was added and the mixture was stirred at -78 °C for 2 h, quenched with H₂O and warmed to RT. The mixture was extracted with CH₂Cl₂ and the combined organic extracts were washed with brine, dried over MgSO₄ and concentrated. Flash column chromatography on silica gel (Hexanes/AcOEt = 90:10) afforded alcohol 197 (4.8 g, 18.7 mmol, 91%) as a colorless oil: [α]D⁺²₀ = +39.6 (c = 0.42, CHCl₃); Rf = 0.67 (Toluene/Acetone = 80:20); ¹H NMR (400 MHz, CDCl₃): δ 7.84 – 7.78 (m, 4H), 7.51 – 7.45 (m, 3H), 5.93 – 5.83 (m, 1H), 5.13 – 5.07 (m, 2H), 4.83 (d, J = 11.6 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 3.58 – 3.54 (m, 1H), 3.53 – 3.47 (m, 1H), 2.54 (bs, 1H), 2.40 – 2.34 (m, 1H), 2.26 – 2.19 (m, 1H), 1.25 (d, J = 6.0 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 135.9, 134.9, 133.4, 133.1, 128.4, 128.0, 127.8, 126.6, 126.3, 126.0, 125.9, 117.4, 77.7, 74.4, 71.3, 37.7, 15.6; IR
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(4S, 5S)-4-O-(Benzoyl)-5-(naphthalen-2-ylmethoxy)hexene (199): A solution of alcohol 197 (827 mg, 3.23 mmol) in pyridine (16 mL) was treated at 0 °C with BzCl (0.75 mL, 6.45 mmol). After 2 h of stirring at RT the mixture was concentrated. The crude was diluted with CH₂Cl₂ and washed with HCl, water and brine. The organic layer was dried over MgSO₄ and concentrated. Flash column chromatography (Cyclohexane/AcOEt = 100:0 to 70:30) gave benzoate 199 (1.16 g, 3.2 mmol, 100%) as a colorless oil: [α]D₂⁰ = −6.5 (c = 1.0, CHCl₃); Rf = 0.68 (Cyclohexane/AcOEt = 70:30); ¹H NMR (400 MHz, CDCl₃): δ 8.06 – 8.04 (m, 2H), 7.82 – 7.76 (m, 4H), 7.58 – 7.54 (m, 1H), 7.46 – 7.41 (m, 5H), 5.86 – 5.76 (m, 1H), 5.32 – 5.27 (m, 1H), 5.11 (dd, J = 17.2 Hz, 1.2 Hz, 1H), 5.03 (m, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 3.82 (dq, J = 1.2, 6.4 Hz, 1H), 2.63 – 2.57 (m, 1H), 2.53 – 2.45 (m, 1H), 1.27 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 166.3, 136.1, 133.9, 133.3, 133.09, 133.04, 130.5, 129.8 (2C), 128.4 (2C), 128.2, 128.0, 127.8, 126.5, 126.1, 125.99, 125.94, 117.9, 75.5, 74.8, 71.4, 34.5, 15.6; IR (thin film) 3059, 2979, 2934, 2867, 1716, 1643, 1602, 1584, 1509, 1491, 1451, 1373, 1349, 1314, 1272 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₂₃H₂₄O₃Na: 383.1623, found 383.1598.
(4S, 5S)-4-O-(tert-Butyldimethylsilyl)-5-(naphthalen-2-ylmethoxy)hexene (200): A solution of alcohol 197 (150 mg, 0.58 mmol) in CH2Cl2 (6 mL) was treated at 0 °C with 2,6-lutidine (0.14 mL, 1.1 mmol) and TBSOTf (0.2 mL, 0.9 mmol). After 2.5 h, the reaction mixture was quenched with a saturated aq. solution of NaHCO3 (20 mL) and extracted with CH2Cl2. The combined organic extracts were dried over MgSO4 and concentrated. Flash column chromatography on silica gel (Hexanes/AcOEt = 100:0 to 90:10) afforded compound 200 (200 mg, 0.55 mmol, 95%) as colorless oil: [α]D20 = +1.4 (c = 1.0, CHCl3); Rf = 0.48 (Hexanes/AcOEt = 90:10); 1H NMR (400 MHz, CDCl3): δ 7.91 – 7.71 (m, 4H), 7.48 – 7.45 (m, 3H), 5.90 – 5.70 (m, 1H), 5.09 – 5.00 (m, 2H), 4.76 (d, J = 12.0 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 3.80 – 3.76 (m, 1H), 3.60 – 3.50 (m, 1H), 2.46 – 2.39 (m, 1H), 2.19 – 2.12 (m, 1H), 1.17 (d, J = 6.4 Hz, 3H), 0.62 (s, 9H), 0.01 (s, 3H), -0.02 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 136.6, 136.2, 133.4, 133.0, 128.1, 127.9, 127.8, 126.2, 126.1, 125.9, 125.8, 116.7, 77.5, 74.0, 71.3, 36.5, 26.0 (3C), 18.2, 14.2, -4.33, -4.36; IR (thin film) 3058, 2953, 2926, 2855, 1729, 1642, 1603, 1509, 1462, 1377 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C23H34O3SiNa: 393.2226, found 393.2231.

(2S, 4S, 5S)-5-(Naphthalen-2-ylmethoxy)hexane-1,2,4-triol (201) and (2R, 4S, 5S)-5-(naphthalen-2-ylmethoxy)hexane-1,2,4-triol (202): At 0 °C, N-methylmorpholin-N-oxide (2.5 g, 21 mmol) was added to a solution of 197 (2.7 g, 10 mmol) in THF/H2O (53 mL, 2:1). After 15 min, OsO4 (2.5% wt solution in tert-butanol, 130 μL, 0.010 mmol) was added and the mixture was stirred at RT overnight. After dilution with AcOEt, the organic layer was washed with Na2S2O3, HCl and water, dried over MgSO4 and concentrated. Flash column chromatography (Cyclohexane/AcOEt = 80:20) on silica gel afforded 2,4-anti triol 201 (1.5 g, 5.1 mmol, 49%) and the 2,4-syn triol 202 (1.3 g, 4.6 mmol, 44%) as white foams.

(2S, 4S, 5S)-5-(Naphthalen-2-ylmethoxy)hexane-1,2,4-triol (201): [α]D20 = +35.2 (c = 0.45, CHCl3); Rf = 0.50 (Toluene/Acetone = 30:70); 1H NMR (400 MHz, CDCl3): δ 7.84 – 7.76 (m, 4H), 7.49 – 7.38 (m, 3H), 4.84 (d, J = 11.6 Hz, 1H), 4.60 (d, J = 11.6 Hz, 1H), 4.01 – 3.96 (m,
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1H), 3.77 – 3.74 (m, 1H), 3.63 (dd, J = 11.2, 3.6 Hz, 1H), 3.54 – 3.46 (m, 2H), 2.64 (bs, 3H), 1.70 (ddd, J = 14.4, 8.8, 3.2 Hz, 1H), 1.57 (ddd, J = 14.4, 9.2, 3.6 Hz, 1H), 1.23 (d, J = 6.0 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 135.6, 133.3, 133.1, 128.5, 128.0, 127.8, 126.7, 126.3, 126.1, 125.9, 78.5, 72.5, 71.3, 69.6, 67.0, 35.4, 15.6; IR (thin film) 3372, 3054, 2923, 2871, 1712, 1633, 1602, 1509, 1453, 1401, 1375 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C17H22O4Na: 313.1416, found 313.1435.

(2R, 4S, 5S)-5-(Naphthalen-2-ylmethoxy)hexane-1,2,4-triol (202): [α]D²⁰ +25.0 (c = 0.65, CHCl₃); Rf = 0.59 (Toluene/Acetone = 30:70); ¹H NMR (400 MHz, CDCl3): δ 7.86 – 7.77 (m, 4H), 7.51 – 7.43 (m, 3H), 4.84 (d, J = 11.6 Hz, 1H), 4.60 (d, J = 11.6 Hz, 1H), 3.99 – 3.94 (m, 1H), 3.79 – 3.74 (m, 1H), 3.63 (dd, J = 11.2, 3.6 Hz, 1H), 3.52 – 3.42 (m, 2H), 3.24 (s, 1H), 2.36 (s, 2H), 1.66 – 1.59 (m, 2H), 1.22 (d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 135.5, 133.3, 133.1, 128.5, 128.0, 127.8, 126.7, 126.4, 126.2, 125.9, 78.5, 75.2, 71.8, 71.3, 66.7, 35.3, 15.4; IR (thin film) 3367, 3054, 2923, 2870, 1712, 1633, 1602, 1509, 1449, 1400, 1374 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C17H22O4Na: 313.1416, found 313.1426.

Stereochemical proof of 201: Synthesis of (2S, 4S, 5S)-1-(tert-butyldiphenylsilyloxy)-2,4-isopropanediol-5-(naphthalen-2-ylmethoxy)-hexan (203): To a mixture of triol 201 (100 mg, 0.34 mmol), imidazole (28 mg, 0.41 mmol) and DMAP (9 mg, 0.07 mmol) in CH₂Cl₂ (3.4 mL), was added TBDPSCI (100 µL, 0.38 mmol). The mixture was stirred for 18 h at RT, then diluted with CH₂Cl₂, washed with a saturated aq. solution of NH₄Cl, water and brine and dried over MgSO₄. Following removal of the solvents in vacuo, the crude product was run through a plug of silica gel (Hexanes/AcOEt = 100:0 to 70:30) to furnish 180 mg of crude silyl ether. The crude silyl ether dissolved in anhydrous CH₂Cl₂ (3.5 mL) was treated with PPTS (17 mg, 0.07 mmol) and 2,2-DMP (220 µL, 1.7 mmol). The mixture was stirred at RT for 4 h and neutralized with an aq. saturated solution of NaHCO₃. The mixture was extracted with CH₂Cl₂ and the combined organic layers were washed with water and brine, dried over MgSO₄ and concentrated. Flash column chromatography on silica gel (Hexanes/AcOEt = 80:20) gave acetonide 203 (163 mg, 0.29 mmol, 83%) as colorless oil: [α]D²⁰ = -11.7 (c = 2.0, CHCl₃); Rf = 0.23 (Hexanes/AcOEt = 90:10); ¹H NMR (600 MHz, CDCl3): δ 7.83 – 7.80 (m,
Stereochemical proof of 202: Synthesis of \((2R, 4S, 5S)-1-(\text{tert-butylidiphenylsilyloxy})-2,4\text{-isopropanedi}xy-5-(\text{naphthalen-2-ylmethoxy})\text{-hexan} (204):\) To a mixture of triol 202 (70 mg, 0.24 mmol), imidazole (20 mg, 0.28 mmol) and DMAP (6 mg, 0.05 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (2.5 mL), was added TBDPSCI (70 µL, 0.26 mmol). The mixture was stirred for 18 h at RT, then diluted with CH\textsubscript{2}Cl\textsubscript{2}, washed with a saturated aq. solution of NH\textsubscript{4}Cl, water and brine and dried over MgSO\textsubscript{4}. Following removal of the solvents in vacuo, the crude product was run through a plug of silica gel (Hexanes/AC\textsubscript{2}OEt = 100:0 to 70:30) to furnish 125 mg of crude silyl ether. The crude silyl ether dissolved in acetone (3.5 mL) was treated with CuSO\textsubscript{4} (115 mg, 0.72 mmol), PPTS (12 mg, 0.05 mmol) and 2,2-DMP (150 µL, 1.2 mmol). The mixture was stirred at RT for 10 min, neutralized with an aq. saturated solution of NaHCO\textsubscript{3} and filtered through celite. The mixture was extracted with CH\textsubscript{2}Cl\textsubscript{2} and the combined organic layers were washed with water and brine, dried over MgSO\textsubscript{4} and concentrated. Flash column chromatography on silica gel (Hexanes/AC\textsubscript{2}OEt = 80:20) gave acetonide 204 (130 mg, 0.23 mmol, 95%) as colorless oil: [\(\alpha\)]\textsubscript{D}\textsuperscript{20} = -0.7 (c = 1.0, CHCl\textsubscript{3}); \(R_{f} = 0.23\) (Hexanes/AC\textsubscript{2}OEt = 90:10); \(^1\text{H NMR (400 MHz, CDCl}_3\}): \(\delta\) 7.85 – 7.80 (m, 4H), 7.72 – 7.65 (m, 3H), 7.54 – 7.32 (m, 10H), 4.80 (2d, \(J = 12.4, 12.4\) Hz, 2H), 4.01 – 3.95 (m, 2H), 3.74 (dd, \(J = 10.4, 5.2\) Hz, 1H), 3.62 – 3.52 (m, 2H), 1.59 – 1.51 (m, 1H), 1.42 (2s, 6H), 1.35 – 1.26 (m, 1H), 1.17 (d, \(J = 6.4\) Hz, 3H), 1.07 (s, 9H); \(^{13}\text{C NMR (100 MHz, CDCl}_3\}): \(\delta\) 136.7, 135.8 (3C), 133.8, 133.4, 133.0, 129.75, 129.73, 128.1, 127.9, 127.8, 127.74 (3C), 127.73 (3C), 126.3, 126.1, 126.0, 125.8, 98.6, 77.0, 72.3, 71.9, 69.8, 67.7, 30.1, 28.9, 27.0 (3C), 19.9, 19.4, 15.3; IR (thin film)
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3069, 3051, 2989, 2958, 2930, 2858, 1112 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₃₆H₄₄O₅SiNa: 591.2907, found 591.2953.

(2S, 3S)-3-(Naphthalen-2-ylmethoxy)-1-((S)-2-phenyl-1,3-dioxolan-4-yl)butan-2-ol (205): Triol 201 (1.30 g, 4.5 mmol), CuSO₄ (2.10 g, 14 mmol) and CSA (52 mg, 0.22 mmol) were suspended in CH₃CN/THF (46 mL, 1/1, v/v). At 0 °C, benzaldehyde dimethyl acetal (6.7 mL, 45 mmol) was added and the mixture was stirred for 10 min, neutralized with NaHCO₃ and filtered through celite. The mixture was extracted with CH₂Cl₂ and the combined organic layers were washed with water and brine, dried over MgSO₄ and concentrated. Flash column chromatography on silica gel (Hexanes/AcOEt/Et₃N = 80:19:1) gave 1,2-hemiacetal 205 (1.42 g, 3.7 mmol, 83%) as a 9:1 mixture of diastereoisomers: [α]D²⁰ = +1.44 (c = 1.0, CHCl₃); Rf = 0.25 (Hexanes/AcOEt = 70:30); ¹H NMR (400 MHz, CDCl₃): δ 7.91 – 7.68 (m, 4H), 7.53 – 7.42 (m, 5H), 7.41 – 7.33 (m, 3H), 5.82 (s, 1H), 4.84 (d, J = 12.0 Hz, 1H), 4.61 (d, J = 12.0 Hz, 1H), 4.51 – 4.48 (m, 1H), 4.18 (dd, J = 8.0, 6.8 Hz, 1H), 3.81 – 3.71 (m, 2H), 3.49 – 3.43 (m, 1H), 2.65 (dd, J = 4.0, 0.8 Hz, 1H), 1.96 – 1.86 (m, 1H), 1.76 (ddd, J = 15.6, 10.4, 5.2 Hz, 1H), 1.24 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 137.8, 135.6, 133.2, 132.9, 129.2, 128.3 (2C), 128.2, 127.8, 127.7, 126.5 (2C), 126.4, 126.1, 125.9, 125.7, 103.7, 78.3, 75.0, 72.14, 71.10, 70.6, 37.0, 15.5; IR (thin film) 3474, 3053, 2952, 2924, 2874, 1087, 1067 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₂₄H₂₆O₄Na: 401.1729, found 401.1734.

(2S, 4S, 5S)-5-(Benzyloxy)-6-hydroxy-2-(naphthalen-2-ylmethoxy)hexan-3-yl benzoate (206): At 0 °C, BzCl (0.8 mL, 6.8 mmol) was added to the solution of hemiacetal 205 (1.3 g, 3.43 mmol) in pyridine (16 mL). The mixture was stirred at RT for 2 h and concentrated. The crude was dissolved in CH₂Cl₂ and washed with HCl and water. The organic layer was dried over MgSO₄ and concentrated to give the ester. The crude ester in CH₂Cl₂ (35 mL) was treated at 0 °C with 1 m BH₃ in THF (10.2 mL, 10.2 mmol). After 15 min, TMSOTf (62 µL,
0.34 mmol) was added and the mixture was warmed slowly at RT. 1 h later, the reaction mixture was cooled to 0 °C, quenched with MeOH (8.5 mL) and Et₃N (0.35 mL) and concentrated. The crude oil was diluted in CH₂Cl₂, washed with water and brine, dried over MgSO₄ and concentrated. Flash column chromatography (Hexanes/AcOEt = 70:30) on silica gel afforded alcohol 206 (1.4 g, 2.9 mmol, 86% over 2 steps) as white foam: [α]ᴰ²⁰ = +29.4 (c = 1.2, CHCl₃); Rₜ = 0.31 (Hexanes/AcOEt = 70:30); ¹H NMR (400 MHz, CDCl₃): δ 8.07 (d, J = 7.6 Hz, 2H), 7.81 – 7.75 (m, 4H), 7.58 (t, J = 7.6 Hz, 1H), 7.47 – 7.41 (m, 5H), 7.37 – 7.21 (m, 5H), 5.60 (ddd, J = 10.4, 4.4, 2.4 Hz, 1H), 4.80 (d, J = 12.0 Hz, 1H), 4.71 (d, J = 12.0 Hz, 1H), 4.56 (d, J = 10.8 Hz, 1H), 4.51 (d, J = 10.8 Hz, 1H), 3.85 – 3.76 (m, 2H), 3.63 – 3.57 (m, 1H), 3.57 – 3.48 (m, 1H), 2.13 (ddd, J = 14.8, 9.2, 2.4 Hz, 1H), 1.94 (dd, J = 14.8, 10.4, 3.6 Hz, 1H), 1.79 (bs, 1H), 1.26 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 166.2, 139.1, 138.2, 133.3, 133.1, 133.0, 130.3, 129.8 (2C), 128.6 (2C), 128.5 (2C), 128.21 (2C), 128.20, 128.0, 127.9, 127.7, 126.5, 126.1, 126.0, 125.9, 76.6, 75.2, 72.8, 72.4, 71.3, 64.4, 32.0, 15.4; IR (thin film) 3470, 3060, 2976, 2927, 2871, 1714, 1621, 1601, 1584, 1509, 1487, 1458, 1450, 1376, 1343 cm⁻¹; HRMS(ESI) m/z calcd for (M+H)⁺ C₃₁H₃₅O₅: 485.2328, found 485.2326.

(2S, 3S, 5S)-5-(Benzyloxy)-2-(naphthalen-2-ylmethoxy)-6-oxohexan-3-yl benzoate (196):
Alcohol 206 (1.00 g, 2.0 mmol), Dess–Martin periodinane (2.63 g, 6.2 mmol) and pyridine (1.7 mL, 20.6 mmol) in CH₂Cl₂ (9 mL) were stirred at RT for 1 h. After addition of a saturated aq. solution of Na₂S₂O₅ (10 mL) and a saturated aq. solution of NaHCO₃ (10 mL), the mixture was stirred for additional 15 min. The mixture was extracted with CH₂Cl₂ and the organic layer was washed with H₂O, dried over MgSO₄ and concentrated. Flash column chromatography (Cyclohexane/AcOEt = 100:0 to 80:20) on silica gel afforded aldehyde 196 (886 mg, 1.84 mmol, 92%) as white foam: [α]ᴰ²⁰ = −19.4 (c = 0.64, CHCl₃); Rₜ = 0.57 (Cyclohexane/AcOEt = 70:30); ¹H NMR (400 MHz, CDCl₃): δ 9.66 (d, J = 2.0 Hz, 1H), 8.06 – 8.02 (m, 2H), 7.83 – 7.73 (m, 4H), 7.61 – 7.55 (m, 1H), 7.48 – 7.41 (m, 5H), 7.34 – 7.19 (m, 5H), 5.61 (ddd, J = 10.4, 4.4, 2.4 Hz, 1H), 4.79 (d, J = 12.4 Hz, 1H), 4.70 (d, J = 12.4 Hz, 1H), 4.59 (d, J = 11.2 Hz, 1H), 4.53 (d, J = 11.2 Hz, 1H), 3.89 – 3.80 (m, 2H), 2.17 (ddd, J = 14.4, 10.4, 3.2 Hz, 1H), 2.04 (ddd, J = 14.4, 10.4, 2.4 Hz, 1H), 1.17 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 203.4, 166.1, 137.0, 135.8, 133.38, 133.30, 133.1, 130.0, 129.90 (2C),
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128.6 (2C), 128.5 (4C), 128.28, 128.27, 128.0, 127.8, 126.6, 126.1, 126.0, 125.9, 80.6, 74.8, 73.5, 71.5, 71.4, 30.5, 15.3; IR (thin film) 3061, 3032, 2976, 2926, 2864, 2714, 1718, 1718, 1601, 1585, 1509, 1495, 1452, 1377 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C₃₁H₃₀O₅Na: 505.1991, found 505.1970.

![Chemical structure](image)

**4-O-Benzoyl-2-O-benzyl-3,6-dideoxy-1-galactohexopyranose (S12):** At 0 °C, DDQ (917 mg, 4.04 mmol) was added to a solution of aldehyde 196 (650 mg, 1.34 mmol) in CH₂Cl₂/MeOH (135 mL, 9:1). The mixture was slowly warmed to RT and stirred for 3 h, then diluted with Et₂O and quenched with a saturated aq. solution of NaHCO₃ and a saturated aq. solution of Na₂S₂O₃. After separation of the layers, the organic layer was washed with H₂O, dried over MgSO₄ and concentrated. Flash column chromatography (Hexanes/ACOEt = 70:30) on silica gel gave a 1:1 α/β mixture of the target hemiacetal (S12) (383 mg, 1.12 mmol) in 83% yield as a colorless oil: [α]D²⁰ = −29.6 (c = 2.0, CHCl₃); Rf = 0.28 (Hexanes/ACOEt = 60:40); ¹H NMR (400 MHz, CDCl₃): δ 8.08 – 8.03 (m, 4H), 7.61 – 7.57 (m, 2H), 7.48 – 7.44 (m, 4H), 7.33 – 7.24 (m, 10H), 5.35 (d, J = 2.8 Hz, 1H, H1-α), 5.24 – 5.21 (m, 1H, H4-α), 5.19 – 5.17 (m, 1H, H4-β), 4.78 (2d, J = 7.2 Hz, J = 11.6 Hz, 2H, H1-β, CH₂Ph-β), 4.70 – 4.58 (m, 2H, CH₂Ph-β, CH₂Ph-α), 4.55 (d, J = 11.6 Hz, 1H, CH₂Ph-α), 4.36 (qd, J = 6.4, 1.2 Hz, H5-α), 3.93 – 3.58 (m, 2H, H5-β, H2-α), 3.57 (ddd, J = 5.2, 11.6, 7.6 Hz, 1H, H2-β), 3.08 (bs, 1H, OH-β), 2.85 (bs, 1H, OH-α), 2.45 (ddd, J = 14.4, 5.2, 3.2 Hz, 1H, H3-βeq), 2.24 (ddddd, J = 13.6, 3.6, 4.8, 0.8 Hz, 1H, H3-αeq), 2.15 (ddd, J = 13.6, 11.6, 3.2 Hz, 1H, H3-αα), 1.81 (ddd, J = 14.4, 11.6, 3.2 Hz, 1H, H3-βα), 1.26 (d, J = 6.4 Hz, 3H, H6-β), 1.18 (d, J = 6.4 Hz, 3H, H6-α); ¹³C NMR (100 MHz, CDCl₃): δ 166.1, 166.0, 138.2, 137.6, 133.3, 133.2, 130.1, 129.99, 129.93 (2C), 129.8 (2C), 128.58 (2C), 128.56 (3C), 128.50 (2C), 128.06 (2C), 128.04 (2C), 127.96 (2C), 127.8, 98.9 (¹JC1-H1 = 163.9 Hz, C1-β), 91.0 (¹JC1-H1 = 175.0 Hz, C1-α), 74.5 (C2-β), 72.8 (C2-α), 72.7 (CH₂-β), 71.6 (C4-α), 71.3 (C4-β), 71.0 (CH₂-α), 70.9 (C5-β), 65.2 (C5-α), 34.0 (C3-β), 28.4 (C3-α), 16.8 (C6-β), 16.5 (C6-α); IR (thin film) 3417, 3064, 3032, 2982, 2937, 2874, 1715, 1267 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C₂₀H₂₂O₅Na: 365.1365, found 365.1363.
4-O-Benzoyl-2-O-benzyl-3,6-dideoxy-L-galactohexopyranosyl trichloroacetimidate (195): At 0 °C, DBU (11 μL, 0.07 mmol) and Cl₃CCN (190 μL, 1.89 mmol) were added to a solution of lactol S12 (130 mg, 0.38 mmol) in CH₂Cl₂ (1.5 mL). The reaction mixture was stirred for 3 h at 0 °C and concentrated. Flash column chromatography (Hexanes/AcOEt/ Et₃N = 80:19:1) on silica gel yielded imidate 195 (176 mg, 0.36 mmol) in 95% yield in a ratio α/β 1.6:1 as a colorless oil: [α]D²⁰ = −24.4 (c = 0.40, CHCl₃); Rf 1α = 0.68 (Hexanes/AcOEt/ Et₃N = 70:29:1); Rf 1β = 0.50 (Hexanes/AcOEt/ Et₃N = 70:29:1); ¹H NMR (400 MHz, CDCl₃): δ 8.66 (s, 1H, NH-β), 8.61 (s, 1H, NH-α), 8.08 – 8.02 (m, 4H), 7.62 – 7.57 (m, 2H), 7.51 – 7.45 (m, 4H), 7.31 – 7.20 (m, 10H), 6.56 (d, J = 3.2 Hz, 1H, H1-α), 5.87 (d, J = 7.6 Hz, 1H, H1-β), 5.33 – 5.30 (m, 1H, H4-α), 5.25 – 5.23 (m, 1H, H4-β), 4.80 (d, J = 11.6 Hz, 1H, CH₂-β), 4.67 – 4.62 (m, 2H, CH₂-α, CH₂-β), 4.57 (d, J = 12.0 Hz, 1H, CH₂-α), 4.32 (qd, J = 6.8, 2.0 Hz, 1H, H5-α), 4.09 – 4.03 (m, 2H, H2-α, H5-β), 3.87 (ddd, J = 11.2, 7.6, 4.8 Hz, 1H, H2-β), 2.47 (ddd, J = 14.4, 4.8, 3.6 Hz, 1H, H3eq-β), 2.38 – 2.27 (m, 2H, H3eq-α, H3a-α), 1.96 (ddd, J = 14.4, 11.2, 3.6 Hz, 1H, H3a-α), 1.29 (d, J = 6.4 Hz, 3H, H6-β), 1.20 (d, J = 6.8 Hz, 3H, H6-α); ¹³C NMR (150 MHz, CDCl₃): δ 166.1, 165.9, 161.59, 161.57, 138.0, 137.8, 133.42, 133.40, 130.0, 129.9 (2C), 129.8 (4C), 128.66 (3C), 128.61, 128.53 (3C), 128.50, 127.92, 127.90 (2C), 127.8 (2C), 100.1 (1J_C1-H1 = 168.1 Hz, C1-β), 94.1 (1J_C1-H1 = 178.4 Hz, C1-α), 91.6 (CCl₃), 91.1 (CCl₃), 73.5 (C2-β), 73.0 (CH₂-β), 72.2 (C2-α), 71.3 (CH₂-α), 71.2 (C4-β), 70.7 (C4-α), 70.1 (C5-β), 68.0 (C5-α), 34.0 (C3-β), 29.8 (C3-α), 16.69 (C6-β), 16.62 (C6-α); IR (thin film) 3406, 3344, 2926, 2857, 1718, 1671, 1601, 1497, 1452, 1359 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₂₂H₂₂Cl₃NO₂Na: 508.0461, found 508.0434.

(2S, 3S)-3-(Naphthalen-2-ylmethoxy)-1-((R)-2-phenyl-1,3-dioxolan-4-yl)butan-2-ol (207): Using the same procedure as for compound 205, benzylidene acetal 207 was synthesized starting from triol 202 (1.2 g, 4.1 mmol) in 61% yield as a 1.6:1 diastereoisomeric mixture:
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[α]_D^{20} = +17.0 (c = 1.0, CHCl₃); R_f = 0.37 (Hexanes/AcOEt = 70:30); _1^H NMR (400 MHz, CDCl₃): δ 7.85 – 7.78 (m, 8H), 7.50 – 7.44 (m, 10H), 7.38 – 7.36 (m, 6H), 5.96 (s, 1H), 5.80 (s, 1H), 4.84 (d, J = 11.6 Hz, 2H), 4.63 (d, J = 11.6 Hz, 2H), 4.47 – 4.40 (m, 2H), 4.28 (dd, J = 8.0, 6.0 Hz, 1H), 4.13 (dd, J = 7.6, 6.8 Hz, 1H), 3.78 – 3.67 (m, 4H), 3.65 – 3.59 (m, 2H), 2.99 (d, J = 2.4 Hz, 1H), 2.89 (d, J = 2.8 Hz, 1H), 2.00 – 1.80 (m, 4H), 1.27 (2d, J = 6.4 Hz, J = 6.8 Hz, 6H); _1^3C NMR (100 MHz, CDCl₃): δ 138.2, 137.6, 135.89, 135.88, 133.3 (2C), 133.1 (2C), 129.4, 129.2, 128.5 (2C), 128.4 (2C), 128.3 (2C), 127.9 (2C), 127.8 (2C), 126.7, 126.67, 126.66, 126.4 (3C), 126.3 (2C), 126.1 (2C), 125.96, 125.95, 104.2, 103.3, 77.6, 77.4, 75.4, 74.7, 72.9, 71.2 (2C), 70.9 (2C), 70.2, 35.9, 35.5, 15.25, 15.22; IR (thin film) 3496, 3056, 2924, 2871, 1087, 1068 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)^+ C_{24}H_{26}O_{4}Na: 401.1729, found 401.1750.

(2S, 4S, 5R)-5-(Benzyloxy)-6-hydroxy-2-(naphthalen-2-ylmethoxy)hexan-3-yl benzoate (208): Using the same procedure as for compound 206, alcohol 208 was synthesized starting from benzylidene acetal 207 (0.90 g, 2.4 mmol) in 82% yield as a white foam: [α]_D^{20} = +3.6 (c = 0.8, CHCl₃); R_f = 0.25 (Cyclohexane/AcOEt = 70:30); _1^H NMR (400 MHz, CDCl₃): δ 8.03 (m, 2H), 7.85 – 7.72 (m, 4H), 7.60 – 7.54 (m, 1H), 7.50 – 7.40 (m, 5H), 7.26 – 7.21 (m, 5H), 5.42 – 5.35 (m, 1H), 4.81 (d, J = 12.0 Hz, 1H), 4.66 (d, J = 12.0 Hz, 1H), 4.52 (d, J = 11.6 Hz, 1H), 4.47 (d, J = 11.6 Hz, 1H), 3.85 – 3.70 (m, 2H), 3.64 – 3.50 (m, 2H), 2.17 – 2.08 (m, 2H), 1.87 (bs, 1H), 1.24 (d, J = 6.4 Hz, 3H); _1^3C NMR (100 MHz, CDCl₃): δ 166.2, 138.1, 135.9, 133.3, 133.19, 133.10, 130.2, 129.8 (2C), 128.5 (4C), 128.2, 128.0, 127.9 (2C), 127.85, 127.81, 126.6, 126.2, 126.03, 126.02, 76.7, 74.7, 73.0, 71.3, 71.2, 63.7, 30.7, 15.3; IR (thin film) 3469, 3060, 2926, 2871, 1714, 1602, 1584, 1509, 1495, 1451, 1375, 1345 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)^+ C_{31}H_{32}O_{8}Na: 507.2147, found 507.2164.
(2S, 3S, 5R)-5-(Benzyloxy)-2-(naphthalen-2-ylmethoxy)-6-oxohexan-3-yl benzoate (209): Using the same procedure as for compound 196, aldehyde 209 was synthesized starting from alcohol 208 (720 mg, 1.48 mmol) in 85% yield as white foam: [α]_D^20 = +4.0 (c = 0.62, CHCl₃); R₉ = 0.53 (Cyclohexane/AcOEt = 70:30); ¹H NMR (400 MHz, CDCl₃): δ 9.58 (d, J = 1.2 Hz, 1H), 7.87 – 7.70 (m, 4H), 7.54 (t, J = 7.6 Hz, 1H), 7.47 – 7.38 (m, 5H), 7.23 – 7.19 (m, 5H), 5.54 – 5.50 (m, 1H), 4.78 (d, J = 12.0 Hz, 1H), 4.65 (d, J = 12.0 Hz, 1H), 4.56 (d, J = 11.6 Hz, 1H), 4.44 (d, J = 11.6 Hz, 1H), 3.86 (td, J = 5.6, 1.2 Hz, 1H), 3.78 – 3.72 (m, 1H), 2.29 (ddd, J = 14.8, 5.6, 4.4 Hz, 1H), 2.17 (ddd, J = 14.8, 8.8, 5.6 Hz, 1H), 1.21 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 203.3, 165.8, 137.1, 135.8, 133.3, 133.2, 133.1, 130.12, 129.8 (2C), 128.6 (2C), 128.5 (2C), 128.3, 128.14, 128.12 (2C), 128.0, 127.8, 126.6, 126.2, 126.04, 126.02, 80.5, 74.1, 72.4, 71.8, 71.3, 30.3, 15.0; IR (thin film) 3061, 2978, 2930, 2866, 2714, 1718, 1601, 1584, 1509, 1495, 1452, 1377, 1338 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na+MeOH)+ C₃₂H₃₄O₆Na: 537.2253, found 537.2243.

4-O-Benzoyl-2-O-benzyl-3,6-dideoxy-1-talohexopyranose (SI3): At 0 °C, DDQ (520 mg, 2.3 mmol) was added to a solution of aldehyde 209 (370 mg, 0.76 mmol) in CH₂Cl₂/MeOH (76 mL, 9/1). The mixture was slowly warmed to RT and stirred for 3 h, then diluted with Et₂O and quenched with a saturated aq. solution of NaHCO₃ and a saturated aq. solution of Na₂S₂O₃. After separation of the layers, the organic layer was washed with H₂O, dried over MgSO₄ and concentrated. Flash column chromatography (Hexanes/AcOEt = 70:30) on silica gel gave a 1:1 α/β mixture of hemiacetal SI3 (247 mg, 0.72 mmol) in 94% yield as colorless oil: [α]_D^20 = –49.9 (c = 1.0, CHCl₃); R₉ = 0.28 (Hexanes/AcOEt = 70:30); ¹H NMR (400 MHz, CDCl₃): δ 8.16 – 8.12 (m, 4H), 7.61 – 7.54 (m, 2H), 7.45 – 7.35 (m, 4H), 7.27 – 7.18 (m, 10H), 5.43 (s, 1H, H1-α), 5.13 – 5.10 (m, 1H, H4-α), 5.05 – 5.03 (m, 1H, H4-β), 4.91 – 4.80 (dd, J = 12.4 Hz, J < 1.0 Hz, 1H, H1-β), 4.64 (d, J = 11.6 Hz, 1H, CH₂-β), 4.58 (d, J = 11.6 Hz, 1H, CH₂-α), 4.54 (d, J = 11.6 Hz, 1H, CH₂-α), 4.47 (qd, J = 6.4, 1.6 Hz, 1H, H5-α), 4.42
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(d, J = 11.6 Hz, 1H, CH₂-β), 4.26 (d, J = 12.4 Hz, 1H, OH-β), 3.95 (qd, J = 6.4, 1.6 Hz, 1H, H5-β), 3.65 – 3.63 (m, 1H, H2-β), 3.56 – 3.54 (m, 1H, H2-α), 3.15 (bs, 1H, OH-α), 2.74 (adt, J = 16.0, 2.4 Hz, 1H, H3-βeq), 2.41 (addt, J = 15.4, 3.2, 1.2 Hz, 1H, H3-αeq), 2.24 (adt, J = 15.4, 3.6 Hz, 1H, H3-αax), 1.91 (adt, J = 16.0, 3.6 Hz, 1H, H3-βax), 1.36 (d, J = 6.4 Hz, 3H, H6-β), 1.30 (d, J = 6.4 Hz, 3H, H6-α); ¹³C NMR (100 MHz, CDCl₃): δ 166.63, 166.61, 138.0, 137.2, 133.1, 132.9, 130.3, 130.1, 130.08 (2C), 130.04 (2C), 128.4 (2C), 128.39 (2C), 128.32 (2C), 128.1 (2C), 127.9, 127.8 (2C), 127.5, 94.7 (J_C1-H1 = 164.8 Hz, C1-β), 93.1 (J_C1-H1 = 173.7 Hz, C1-α), 72.9 (C5-β), 71.9 (C2-β), 71.7 (C2-α), 71.5 (CH₂-β), 71.1 (CH₂-α), 68.7 (C4-α), 67.7 (C4-β), 65.2 (C5-α), 29.6 (C3-β), 26.4 (C3-α), 17.2 (C6-β), 16.8 (C6-α); IR (thin film) 3427, 3064, 3032, 2982, 2936, 2874, 1709, 1271, 1068 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₂₀H₂₂O₅Na: 365.1365, found 365.1387.

4-O-Benzoyl-2-O-benzyl-3,6-dideoxy-l-talohexopyranosyl trichloroacetimidate (210): At 0 °C, DBU (9 µL, 0.06 mmol) and Cl₃CCN (146 µL, 1.46 mmol) were added to a solution of lactol SI₃ (100 mg, 0.29 mmol) in CH₂Cl₂ (1.1 mL). The reaction mixture was stirred for 3 h at 0 °C and concentrated. Flash column chromatography (Hexanes/AcOEt/Et₃N = 80:19:1) on silica gel yielded imidate 210 (127 mg, 0.26 mmol) in 89% yield in a ratio α/β 9:1 as colorless oil: [α]D²⁰ = −31.4 (c = 0.75, CHCl₃); Rf = 0.56 (Hexanes/AcOEt/Et₃N = 70:29:1); ¹H NMR (400 MHz, CDCl₃): δ 8.55 (s, 1H, NH), 8.03 – 7.96 (m, 2H), 7.49 – 7.41 (m, 1H), 7.30 – 7.21 (m, 2H), 7.19 – 7.09 (m, 5H), 6.39 (s, 1H, H1), 5.06 – 5.04 (m, 1H, H4), 4.56 (d, J = 11.6 Hz, 1H, CH₂Ph), 4.48 (d, J = 11.6 Hz, 1H, CH₂Ph), 4.30 (qd, J = 6.8, 1.6 Hz, 1H, H5), 3.63 – 3.61 (m, 1H, H2), 2.40 (dddd, J = 15.6, 3.2, 2.8, 1.6 Hz, 1H, H3eq), 2.17 (ddd, J = 15.6, 4.0, 3.6 Hz, 1H, H3ax), 1.21 (d, J = 6.8 Hz, 3H, H6); ¹³C NMR (150 MHz, CDCl₃): δ 166.6, 160.6, 137.8, 133.0, 130.2, 130.0 (2C), 128.4 (2C), 128.3 (2C), 127.7 (2C), 127.6, 96.3 (J_C1-H1 = 180.9 Hz, C1), 90.9 (CCl₃), 71.5 (C4), 70.0 (C5), 68.1 (C2), 67.9 (CH₂), 27.5 (C3), 16.9 (C6); IR (thin film) 3336, 2925, 2854, 1715, 1670, 1602, 1492, 1452, 1366 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₂₂H₂₂Cl₃NO₅Na: 508.0461, found 508.0434.
(2S, 3S)-6-Hydroxy-2-(naphthalen-2-ylmethoxy)hexan-3-yl benzoate (212): A solution of alkene 199 (620 mg, 1.72 mmol) and Rh(PPh3)3Cl (56 mg, 0.06 mmol) in THF (14 mL) was cooled to 0 ºC. 1 M catecholborane in THF (5.1 mL, 5.1 mmol) was added and the reaction was stirred at 0 ºC for 3 h. After dilution with THF/MeOH (7 mL, 1/1, v/v) and addition of 3 M NaOH (4 mL) and an aq. solution 35% H2O2 (4 mL), the reaction was stirred overnight and quenched with a saturated aq. solution of Na2SO3 (10 mL). After dilution with Et2O, the organic layer was washed with a saturated aq. solution of NaHCO3 and brine, dried over MgSO4 and concentrated. Flash column chromatography (Cyclohexane/AcOEt/Et3N = 80:19:1) on silica gel afforded alcohol 212 (620 mg, 1.63 mmol, 95%) as a colorless oil: [α]D20 = −5.2 (c = 1.0, CHCl3); Rf = 0.28 (Cyclohexane/AcOEt = 70:30); 1H NMR (400 MHz, CDCl3): δ 8.07 – 8.04 (m, 2H), 7.81 – 7.75 (m, 4H), 7.58 – 7.54 (m, 1H), 7.47 – 7.42 (m, 5H), 5.31 – 7.27 (m, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 3.83 – 3.77 (m, 1H), 3.66 (t, J = 6.4 Hz, 2H), 3.37 – 3.83 (m, 1H), 1.94 – 1.77 (m, 2H), 1.69 – 1.54 (m, 2H), 1.27 (d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 166.5, 136.0, 133.3, 133.1, 133.0, 130.4, 129.8 (2C), 128.5 (2C), 128.2, 128.0, 127.8, 126.5, 126.1, 126.0, 125.9, 76.0, 75.1, 71.4, 62.7, 28.8, 26.1, 15.5; IR (thin film) 3422, 3058, 2952, 2933, 2870, 1715, 1601, 1584, 1450, 1376, 1341 cm⁻¹; HRMS(ESI) m/z calc'd for (M+Na)+ C24H26O4Na: 401.1729, found 401.1737.

(2S, 3S)-2-(Naphthalen-2-ylmethoxy)-6-oxohexan-3-yl benzoate (SI4): To a solution of alcohol 212 (450 mg, 1.19 mmol), DIPEA (1 mL, 5.9 mmol) and DMSO (0.8 mL, 11.9 mmol) in CH2Cl2 (6 mL) at 0 ºC was added SO3-pyridine complex (568 mg, 3.60 mmol). After 1 h, the reaction mixture was quenched with a saturated aq. solution of Na2S2O3 (12 mL) and warmed to RT. The aq. layer was extracted with CH2Cl2. The combined organic layers were dried over MgSO4 and concentrated. Flash column chromatography (Hexanes/AcOEt = 80:20) on silica gel afforded aldehyde SI4 (439 mg, 1.16 mmol, 98%) as colorless oil: [α]D20 = −2.1 (c = 0.80, CHCl3); Rf = 0.44 (Hexanes/AcOEt = 70:30); 1H NMR (400 MHz, CDCl3): δ 9.75 (s, 1H), 8.04 (d, J = 7.2 Hz, 2H), 7.85 – 7.72 (m, 4H), 7.58 (t, J = 7.2 Hz, 1H), 7.47 – 7.42 (m, 5H), 5.31 – 7.27 (m, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 3.83 – 3.77 (m, 1H), 3.66 (t, J = 6.4 Hz, 2H), 3.37 – 3.83 (m, 1H), 1.94 – 1.77 (m, 2H), 1.69 – 1.54 (m, 2H), 1.27 (d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 166.5, 136.0, 133.3, 133.1, 133.0, 130.4, 129.8 (2C), 128.5 (2C), 128.2, 128.0, 127.8, 126.5, 126.1, 126.0, 125.9, 76.0, 75.1, 71.4, 62.7, 28.8, 26.1, 15.5; IR (thin film) 3422, 3058, 2952, 2933, 2870, 1715, 1601, 1584, 1450, 1376, 1341 cm⁻¹; HRMS(ESI) m/z calc'd for (M+Na)+ C24H26O4Na: 401.1729, found 401.1737.
2. De Novo Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

5H), 5.34 – 5.23 (m, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 3.83 – 3.77 (m, 1H), 2.53 (t, J = 7.6 Hz, 2H), 2.20 – 2.03 (m, 2H), 1.28 (d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 201.3, 166.3, 135.9, 133.36, 133.30, 133.1, 130.09, 129.8 (2C), 128.5 (2C), 128.2, 128.0, 127.8, 126.6, 126.2, 126.0, 125.9, 75.2, 75.0, 71.4, 40.3, 22.3, 15.3; IR (thin film) 3057, 2935, 2976, 2863, 2735, 1716, 1271 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C24H24O4Na: 399.1572, found 399.1565.

![Chemical structure](image)

4-O-Benzoyl-2,3,6-trideoxy-L-galactohexopyranose (213): At 0 °C, DDQ (760 mg, 3.30 mmol) was added to a solution of aldehyde S14 (439 mg, 1.10 mmol) in CH2Cl2/MeOH (22 mL, 9:1, v/v). The mixture was slowly warmed to RT and stirred for 3 h, diluted with Et2O and quenched with a saturated aq. solution of NaHCO3 and a saturated aq. solution of Na2S2O3. After separation of the layers, the organic layer was washed with H2O, dried over MgSO4 and concentrated. Flash column chromatography (Hexanes/AcOEt = 70:30) on silica gel gave an inseparable mixture 1:1 α/β of hemiacetal 213 (230 mg, 0.97 mmol) in 87% yield as a colorless oil: [α]D²⁰ = −14.7 (c = 1.0, CHCl3); Rf = 0.45 (Hexanes/AcOEt = 50:50); 1H NMR (400 MHz, CDCl3): δ 8.14 – 8.10 (m, 4H), 7.60 – 7.55 (m, 2H), 7.47 – 7.43 (m, 4H), 5.42 (d, J = 2.4 Hz, 1H, H1-α), 5.09 – 5.07 (m, 1H, H4-α), 5.01 – 4.98 (m, 1H, H4-β), 4.88 (dd, J = 9.2, 2.4 Hz, 1H, H1-β), 4.38 (qd, J = 6.4, 1.2 Hz, 1H, H5-α), 3.87 (qd, J = 6.4, 1.2 Hz, 1H, H5-β), 3.15 (bs, 1H, OH-β), 2.66 (bs, 1H, OH-α), 2.31 – 2.14 (m, 2H, H3-α, H3-β), 2.08 – 1.61 (m, 6H, H2-α, H3-α, H3-β, H2-β, H2-β, H2-α), 1.27 (d, J = 6.4 Hz, 3H, H6-β), 1.18 (d, J = 6.4 Hz, 3H, H6-α); 13C NMR (100 MHz, CDCl3): 166.31, 166.30, 133.2, 133.1, 130.4, 130.2, 129.9 (2C), 129.8 (2C), 128.55 (2C), 128.54 (2C), 96.3 (C1-β), 91.8 (C1-α), 73.1 (C5-β), 70.1 (C4-α), 68.7 (C4-β), 65.4 (C5-α), 27.8 (C2-β), 27.4 (C3-β), 24.4 (C2-α), 22.4 (C3-α), 17.48 (C6-β), 17.47 (C6-α); IR (thin film) 3416, 3064, 2980, 2938, 2863, 1715, 1601, 1584, 1450, 1384, 1358 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C13H16O4Na: 259.0946, found 259.0983.
2. De Novo Synthesis of L-Colitose, 2-epi-L-Colitose and L-Rhodinose Building Blocks

4-O-Benzoyl-L-rhodinosyl acetate (211): Ac₂O (3.4 mL, 36 mmol) was added at 0 °C to a solution of hemiacetal 213 (172 mg, 0.72 mmol) in pyridine (6 mL). The mixture was stirred at RT for 2 h and the solvents were removed. The crude product was co-evaporated with toluene to give quantitatively rhodinosyl acetate 211 (200 mg, 0.72 mmol) as an oil in a ratio α/β 41:59: [α]D<sup>20</sup> = −8.4 (c = 0.95, CHCl₃); R<sub>f</sub> = 0.28 (Hexanes/ACOEt =70:30); <sup>1</sup>H NMR (400 MHz, CDCl₃): δ 8.14 – 8.09 (m, 4H), 7.60 – 7.56 (m, 2H), 7.48 – 7.44 (m, 4H), 6.25 (s, 1H, H1-α), 5.80 (dd, J = 9.6, 2.4 Hz, 1H, H1-β), 5.11 (as, 1H, H4-α), 5.03 (ad, J = 1.6 Hz, 1H, H4-β), 4.22 (qd, J = 6.4, 1.2 Hz, 1H, H5-α), 3.97 (qd, J = 6.4, 1.6 Hz, 1H, H5-β), 2.27 – 2.19 (m, 1H, H3-β), 2.15 (s, 3H, CH₂CO-β), 2.13 (s, 3H, CH₃CO-α), 2.08 – 1.99 (m, 2H, H2-α, H3-α), 1.96 – 1.85 (m, 3H, H3-α, H2-β, H3-β), 1.79 – 1.72 (m, 1H, H2-β), 1.68 – 1.65 (m, 1H, H2-α), 1.28 (d, J = 6.4 Hz, 3H, H6-β), 1.20 (d, J = 6.4 Hz, 3H, H6-α); <sup>13</sup>C NMR (100 MHz, CDCl₃): δ 169.8, 169.5, 166.2, 166.2, 133.3, 133.2, 130.2, 130.1, 129.9 (2C), 129.8 (2C), 128.6 (2C), 128.5 (2C), 94.4 (J<sub>C1-H1</sub> = 161.7 Hz, C1-β), 92.1 (J<sub>C1-H1</sub> = 177.5 Hz, C1-α), 73.9 (C5-β), 69.4 (C4-α), 68.5 (C4-β), 67.8 (C5-α), 27.1 (C3-β), 25.0 (C2-β), 23.2 (C2-α), 22.8 (C3-α), 21.4 (2C, CH₃CO), 17.4 (C6-α), 17.3 (C6-β); IR (thin film) 3064, 2983, 2963, 2939, 2868, 1745, 1714, 1601, 1584, 1491, 1450, 1366, 1329 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)<sup>+</sup> C₁₅H₁₈O₅Na: 301.1052, found 301.1064.
3. Total Synthesis of the *Escherichia coli* O111 *O*-Specific Polysaccharide Repeating Unit

3.1 Abstract

The first total synthesis of the *O*-antigen pentasaccharide repeating unit from Gram negative bacteria *Escherichia coli* O111 was achieved starting from four monosaccharide building blocks. Key to the synthetic approach is a [3+1+1] glycosylation combining trisaccharide 214 and colitose 215. The colitose building block 215 was obtained via the *de novo* route established in Chapter 2. The pentasaccharide was equipped at the reducing end with an amino-spacer to provide a handle for the subsequent conjugation to a carrier protein in anticipation of immunological studies.
3. Total Synthesis of the *Escherichia coli* O111 *O*-Specific Polysaccharide

3.2 Introduction

A new pathogenic strain of the bacteria *Escherichia coli*, serotype O111, has recently emerged as a significant cause for enteropathogenic, enterotoxigenic and enterohemorrhagic disease in humans. For example, enteropathogenic *E. coli* O111 (EPEC) causes diarrhea in children, particularly in the developing world. Enterotoxigenic *E. coli* (ETEC) O111 is responsible for watery diarrhea in infants and is associated with heat-labile (cholera-like) toxin, while enterohemorrhagic *E. coli* (EHEC) O111 generally carrying at least one Shiga-toxin gene, is one of the most common non-O157 causes of bloody diarrhea and hemolytic-uremic syndrome in developed countries.\(^99\) The largest outbreak of Shiga-toxin producing *E. coli* O111 in the U.S. history occurred in 2008 and affected 341 persons of all age groups. One patient out of the 70 hospitalized died.\(^100\)

The problem with antibiotic resistance of EHEC O111 is evident due to the excessive use in the live stock industry.\(^101\) Prevention by vaccination against *E. coli* O111 would circumvent the multidrug-resistance problem. Serum antibodies to the *O*-specific polysaccharide of O111 lipopolysaccharide protect mice and dogs against infection with this pathogen.\(^102\) The structure of the *O*-specific polysaccharide of O111 *E. coli* contains a pentasaccharide repeating unit with two rare colitose (3,6-dideoxy-L-xylo-hexopyranose) residues as a distinguishing feature (Figure 6).\(^103\) The *O*-specific polysaccharide from *E. coli* O111 is also found on other enteric pathogens such as *Salmonella adelaide*\(^63\) and *Salmonella enterica* O35.\(^104\)

![Figure 6. Pentasaccharide repeating unit of the *O*-specific polysaccharide of *E. coli* O111.](image)

Pure pentasaccharide 216 is required to evaluate the immunological properties of the *O*-specific polysaccharide of *E. coli* O111 and to explore the potential of an oligosaccharide-conjugate vaccine (Scheme 51). This chapter describes the first total synthesis of the
3. Total Synthesis of the *Escherichia coli* O111 O-Specific Polysaccharide

pentasaccharide repeating unit bearing an aminopentanol handle at the reducing end for attachment to any surface and carrier proteins.
3.3 Results and Discussion

3.3.1 Initial Attempt to Synthesize Pentasaccharide 216.

Pentasaccharide 216 reveals several synthetic challenges including one being the α-linkages of colitoses. Retrosynthetic analysis of pentasaccharide 216 reveals a convergent [2+3] approach as attractive (Scheme 51). The reducing end aminopentanol linker will allow for straightforward conjugation. Target structure 216 will be obtained from fully protected pentasaccharide 217 via a three steps deprotection sequence, including conversion of the trichloroacetamide to the corresponding acetamide, cleavage of the ester groups followed by hydrogenolysis. Pentasaccharide 217 in turn will be assembled from disaccharide building block 218 and trisaccharide 219. Disaccharide 218 can be derived from N-benzylbenzyloxycarbonyl-5-aminopentanol 223, glucosamine thioglycoside 222 and galactose thioglycoside 221. The synthesis of a colitose-containing trisaccharide analogue of 216 was already reported by Bundle and coworkers\(^{70a}\) employing halide-assisted glycosylation\(^{105}\). The lability of dideoxyglycosyl halides and the need for a reaction time of ten days suggested the use of N-phenyltrifluoroacetimidate colitose 215 as a precursor of trisaccharide 219. Thus, it was envisaged to access trisaccharide 219 from glucose 220 and colitose imidate 215.
3. Total Synthesis of the *Escherichia coli* O111 O-Specific Polysaccharide

![Chemical structure](image)

**Scheme 51.** Retrosynthetic analysis of pentasaccharide 216.

### 3.3.2 Building Blocks Synthesis

Most of the building blocks needed for the synthesis of pentasaccharide repeating unit 216 are accessed via modification of published methods. The synthesis of colitose building block 215 benefits from the *de novo* synthesis established in Chapter 2 that is more efficient than other procedures.\(^{67,69,70b,71,72}\) This time the colitose hemiacetal 225\(^{106}\) was converted to the corresponding *N*-trifluorophenylacetimidate 215 (Scheme 52) that is an attractive alternative to the previously synthesized colitose trichloroacetimidate, due to its more moderate reactivity\(^{107}\) and the reduced tendency to undergo O→N isomerase to amide rearrangement at the anomeric center\(^{108}\).
Scheme 52. Synthesis of colitose glycosylating agent 215: a) CF$_3$C(=NPh)Cl, Cs$_2$CO$_3$, CH$_2$Cl$_2$, 0 °C to RT, 15 h, 82%.

N-Benzyl-benzyloxycarbonyl-5-aminopentanol 223 and glucosamine building block 222 (Scheme 51) were prepared in collaboration with Heung-Sik Hahm, following literature procedures.$^{109,110}$ Galactose building block 221 equipped with an orthogonal levulinic ester at C4 in anticipation of chain elongation was synthesized from known benzylidene galactoside 226$^{111}$ presenting non-participating benzyl ethers at C2 and C3 (Scheme 53). Regioselective reductive opening of the benzylidene group on 226 with triethylsilane in the presence of triflic acid and triflic anhydride, followed by the protection of the C4 hydroxyl group with levulinic acid, N,N′-dicyclohexylcarbodiimide and 4-(N,N-dimethylamino)pyridine afforded galactose building block 221 in 69% yield over two steps.

Scheme 53. Synthesis of galactose building block 221: a) i. Et$_3$SiH, TfOH, Tf$_2$O, CH$_2$Cl$_2$, 0 °C to RT, 3 h; ii. LevOH, DCC, DMAP, CH$_2$Cl$_2$, 0 °C to RT, 15 h, 69% (two steps).

Finally, known glucose building block 220$^{112}$ (Scheme 51) presenting free hydroxyl groups at C3 and C6 served as branching building block.

3.3.3 Synthesis of Disaccharide Nucleophile 218

With all required building blocks in hand, the assembly of the pentasaccharide repeating unit via a [2+3] strategy was explored, commencing from the reducing end (Scheme 54). Thioglycoside 222 was treated, at -20 °C, with an excess of linker 223 in the presence of N-iodosuccinimide and triflic acid to afford the target glucosamine 227. The trichloroacetamide as a participating group at the C2 position ensured the selective formation of the β-glycosidic
linkage. Furthermore, at -20 °C, only the primary alcohol acts as a nucleophile in the coupling reaction. To aid purification, excess linker wastritylated to afford after flash column chromatography on silica gel, β-glucosamine 227 in 70% yield. Galactopyranose 221 was appended to β-glucosamine 227 using a dimethylformamide-modulated glycosylation procedure to direct the stereochemical course of the reaction. In this case, the dimethylformamide-modulated glycosylation, followed by removal of the levulinoyl ester under Zemplén conditions provided the target disaccharide 228 in only 14% yield. The low yield was explained by the long glycosylation time that favors the cleavage of the benzylidene acetal on the target molecule 228 and the formation of byproducts.

Scheme 54. Initial synthetic attempts towards disaccharide 228: a) i. NIS, TfOH, CH₂Cl₂, MS 4Å, -20 °C, 30 min; ii. TrtCl, pyridine, overnight, 70% (two steps); b) i. DMF, NIS, TMSOTf, MS 4Å, -10 °C, CH₂Cl₂, 12 h; ii. NaOMe, MeOH, 14% (two steps).

To circumvent the problems encountered in the synthesis of disaccharide 228, glucosamine building block 222 was converted to glycosylating agent 229, presenting a 9-fluorenylmethoxy carbonyl protecting group at C3 for chain elongation and stable benzyl ethers at C4 and C6 (Scheme 55). It was expected that the use of building block 229 would facilitate the isolation of compound 231 that will perform better as acceptor in the coupling with galactopyranose 221. Following tert-butyldimethylsilyl protection of the hydroxyl group at C3 on 222, regioselective reductive opening of the benzylidene acetal and subsequent benzylation of the hydroxyl group at C6 provided glucosamine 230 in 77% yield over three steps. Removal of the silyl ether at C3 in the presence of boron trifluoride diethyl etherate
and protection of the free hydroxyl group with 9-fluorenlymethoxy carbonyl provided the new glucosamine glycosylating agent 229 in 92% yield over two steps.

**Scheme 55.** Synthesis of glucosamine building block 229: a) i. TBSCI, imidazole, CH₂Cl₂; ii. BH₃·THF, TMSOTf, CH₂Cl₂; iii. BnBr, NaH, DMF, 77% (three steps); b) i. BF₃·OEt₂, CH₃CN; ii. FmocCl, pyridine, CH₂Cl₂, 92% (two steps).

Thioglycoside 229 was successfully coupled to N-benzyl-benzylxoyarbonyl-5-aminopentanol 223 at -20 °C upon activation by N-iodosuccinimide and triflic acid in CH₂Cl₂ (Scheme 56). 9-Fluorenlymethoxy carbonyl cleavage by treatment with triethylamine afforded β-glucosamine nucleophile 231 in 71% yield over two steps. The $^1J_{\text{C1H1A}}$ coupling constant of 164.8 Hz was characteristic for the formation of a β-glycosidic linkage. Coupling of nucleophile 231 with thiogalactoside 221 by activation with N-iodosuccinimide and triflic acid in a mixture diethyl ether/dichloromethane (3/1, v/v), followed by levulinic ester-removal afforded target disaccharide 218 in 48% yield over two steps. The configuration of the newly formed glycosidic bond was assigned based on the coupling constant between C1B and the H1B of the galactopyranose residue ($^1J_{\text{C1B-H1B}}$ of 171.3 Hz).

**Scheme 56.** Synthesis of the reducing end disaccharide 218: a) i. NIS, TfOH, CH₂Cl₂, MS 3Å AW, -20 °C, 1 h; ii Et₃N, CH₂Cl₂, RT, 3 h, 71% (two steps); b) i. NIS, TfOH, Et₂O/CH₂Cl₂ (3/1, v/v), MS 3Å AW, 0 °C, 30 min; ii. NaOMe, MeOH, 48% (two steps).
3.3.4 Attempts to Synthesize Trisaccharide 219

With ethyl-thioglycoside 220 and L-colitose N-trifluorophenylacetimidate 215 in hand, the synthesis of trisaccharide 219 was attempted (Scheme 57). Due to the high reactivity of deoxysugars,107 the reaction was performed at -50 °C using 1.25 equivalents of glycosylating agent 215 per nucleophile. After one hour, mainly target trisaccharide was observed along with deletion sequences. Prolonged reaction time (2 h) produced α-(1,6)-disaccharide 232 at the expense of the desired trisaccharide 219. The degradation of electron-rich colitose residues in acid media has been reported also by Oscarson.69b The instability of 3,6-dideoxysugars in acidic media rendered the assembly of the target pentasaccharide via a [2+3] approach not feasible since the colitose residues would be exposed to acidic conditions twice.

![Scheme 57. Attemps at the synthesis of trisaccharide 219: a) 215 (2.5 equiv.), TMSOTf, CH₂Cl₂, -50 °C.](image)

3.3.5 Total Synthesis of the *Escherichia coli* O111 O-Antigen

To circumvent the degradation pathways, an alternative retrosynthetic analysis utilizing a linear approach was designed. The colitose residues will be introduced at a late-stage of the synthesis via a bis-glycosylation reaction with trisaccharide 214 (Scheme 51). Trisaccharide 214 could be further disconnected to disaccharide 218 and ethyl-thioglucooside building block 224, equipped with an acetate ester at C6 as a remote protecting group favoring the formation of the α-linkage.115

To install residue C on the disaccharide 218 (Figure 6), glucose building block 224 was synthesized starting from known thioglucooside 234116 (Scheme 58). Building block 224
presents non-participating ether groups at C2, C3 and C4 as well as a participating acetate ester at C6 to favor the formation of the α-glycosidic linkage. The orthogonal 2-naphthylmethyl-ether at C3 will allow for branching at this position. To access fully functionalized glucose 224, the free hydroxyl group of 234 was benzylated by treatment with benzyl bromide and sodium hydride. Regioselective opening of the benzylidene acetal with borane tetrahydrofuran complex and trimethylsilyl trifluoromethanesulfonate afforded as an intermediate the primary alcohol, which was further acetylated to yield glycosylating agent 224 in 74% over three steps.

**Scheme 58.** Synthesis of thioglycoside 224: a) i. BnBr, NaH, DMF; ii. BH₃·THF, TMSOTf, CH₂Cl₂; iii. Ac₂O, pyridine, 74% (three steps).

Coupling of thioglycoside 224 with disaccharide 218 was performed at -50 °C by activation with N-iodosuccinimide in the presence of triflic acid, in diethyl ether/dichloromethane (3/1, v/v) to promote α-selectivity (Scheme 59). This procedure afforded α-trisaccharide 235 in 97% yield. Analysis of the coupling constant of the appended glucose moiety (J₁C₁H₁C₂ of 172.4 Hz) allowed the assignment of the α-stereochemistry at the newly formed center. Cleavage of the acetate protecting group at C6 under Zemplén conditions, followed by removal of the 2-naphthylmethyl ether at C3 by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone afforded diol 214 in 73% yield over two steps. Finally, conversion of diol 214 to the corresponding fully protected pentasaccharide 217 (Figure 7) was achieved via a bis-glycosylation using four equivalents of colitose N-phenyltrifluoroacetimidate 215 in the presence of trimethylsilyl trimethanesulfonate at -50 °C. Quenching of the reaction after 15 min resulted in the formation of the target pentasaccharide 217 in 63% yield. The formation of the two α-linkages was confirmed via analysis of the anomeric C-H coupling constants (See Experimental Section).
Scheme 59. Synthesis of the colitose containing pentasaccharide 217: a) NIS, TfOH, Et₂O/CH₂Cl₂ (3/1, v/v), MS 4Å, -50 °C, 1 h, 97%; b) i. NaOMe, MeOH, RT, overnight; ii. DDQ, CH₂Cl₂/MeOH (9/1, v/v), 0 °C, 4 h, 73% (two steps); c) TMSOTf, CH₂Cl₂, MS 4Å, -50 °C, 15 min, 63%.
Figure 7. HSQC (600 MHz, CDCl₃) of protected pentasaccharide 217.

Final deprotection of the target pentasaccharide 217 was achieved in three steps (Scheme 60). To reduce the number of transformations, conversion of trichloroacetamide to the corresponding acetamide was attempted by hydrogenolysis (H₂, Pd(OH)₂, THF) resulting in incomplete conversions. Finally, reduction of trichloroacetamide to the N-acetyl group was achieved by treatment of the pentasaccharide 217 with tributylstannane and 2,2′-azoisobutyronitrile at 90 °C. Removal of the benzoate esters on the colitose residues proceeded slowly under Zemplén conditions, but heating the intermediary pentasaccharide with sodium methoxide at 40 °C overnight drove the reaction to completion. Particular care was taken during the final hydrogenolysis in order to avoid acidic conditions that could result in the degradation of the colitose residue. Cleavage of the benzyl ethers and benzyloxycarbonyl protecting group was achieved by hydrogenolysis on palladium hydroxide to provide target pentasaccharide 216 in 64% yield over three steps.
3. Conclusions

The first total synthesis of the pathogenic *E. coli* O111 O-antigen repeating unit pentasaccharide starting from inexpensive (S)-ethyl lactate has been achieved in 21 linear steps and 1.5% overall yield. The key step of this approach was a bis-glycosylation to install the acid-labile L-colitoses (Scheme 59). The pentasaccharide repeating unit presents a terminal amine linker at the reducing end for immobilization on any surface or for conjugation to carrier proteins for subsequent immunological evaluation.

3.5 Experimental Section

**General Experimental Details:** All chemicals used were reagent grade and used as supplied, unless noted otherwise. Molecular sieves were activated prior to use by heating and drying under high vacuum. All reactions were performed in oven-dried glassware under argon atmosphere, unless noted otherwise. *N,N*-Dimethylformamide (DMF), dichloromethane (CH₂Cl₂), toluene and tetrahydrofuran (THF) were used from a Cycle-Tainer Solvent Delivery System, unless noted otherwise. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). The TLC plates were visualized by
3. Total Synthesis of the *Escherichia coli* O111 O-Specific Polysaccharide

UV or by dipping the plate in a cerium sulfate ammonium molybdate (CAM) solution or in a 1:1 mixture of H$_2$SO$_4$ (2 N) and resorcin monomethylether (0.2%) in ethanol. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). Preparative HPLC purifications were performed on an Agilent 1200 Series. $^1$H, $^{13}$C spectra were recorded on a Varian Mercury 400 (400 MHz) or 600 (600 MHz) spectrometer in CDCl$_3$ or D$_2$O with chemical shifts referenced to internal standards (CDCl$_3$: $^1$H 7.26 ppm, $^{13}$C 77.0 ppm; D$_2$O with acetone as internal standard: $^1$H 2.05 ppm, $^{13}$C 29.84 or 206.260 ppm) unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet; as, apparent singulet; ad, apparent doublet; at, apparent triplet, aq, apparent quadruplet 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 in the Department of Organic Chemistry at Free University Berlin using a Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a UniPol L 1000 polarimeter (Schmidt & Haensch, Berlin, Germany), with concentrations expressed in g/100 mL.

4-O-Benzoyl-2-O-benzyl-3,6-dideoxy-L-galactohexopyranosyl-N-phenyl-trifluoroacetimidate (215): A solution of colitose lactol 225$^{106}$ (130 mg, 0.38 mmol) in CH$_2$Cl$_2$ (7.5 mL) was treated at 0 °C with Cs$_2$CO$_3$ (250 mg, 0.76 mmol, 2 equiv) and CF$_3$C(=NPh)Cl (75 µL, 0.57 mmol, 1.5 equiv). The mixture was heated to RT and vigorously stirred overnight, then diluted with cyclohexane and decanted to afford a turbid solution, which was concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (Hexanes/EtOAc/Et$_3$N = 90:9:1) to yield $\alpha$-N-phenyl trifluoroacetimidate 215 (160 mg, 0.31 mmol) in 82% yield as an oil: R$_f$ (Hexanes/EtOAc = 90:10) = 0.22; [\alpha]$_D^{20}$ = −50.6 (c = 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.08 − 7.97 (m, 2H), 7.61 − 7.51 (m, 1H), 7.43 (t, $J$ = 7.6 Hz, 2H), 7.31 − 7.16 (m, 7H), 7.13 − 7.01 (m, 1H), 6.82 (d, $J$ = 7.6 Hz, 2H), 5.82 − 5.54 (m, 1H), 5.13 (s, 1H), 4.69 (d, $J$ = 11.6 Hz, 1H), 4.59 (d, $J$ = 11.6 Hz, 1H), 3.79 (bs, 2H), 2.42 (ad, $J$ = 14.0 Hz, 1H), 1.81 (as, 1H), 1.20 (d, $J$ = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.9, 143.7, 137.8, 133.4 (2C), 129.9 (2C),...
3. Total Synthesis of the *Escherichia coli* O111 *O*-Specific Polysaccharide

129.4, 128.7, 128.6 (2C), 128.5 (2C), 127.98, 127.93 (2C), 126.4, 124.3, 120.6, 119.5 (2C), 119.3 (q, *J* = 285 Hz ), 99.0, 73.6, 73.0, 72.2, 70.6, 34.0, 16.5; IR (thin film) 3071, 2938, 1718, 1598, 1314, 1270, 1208, 1161, 1091, 1027 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₂₈H₆₆F₃NNaO₅: 536.1655, found 536.1631.

\[
\text{p-Tolyl \quad 2,3,6-tri-}O\text{-benzyl-4-levulinoyl-1-thio-β-d-galactopyranoside (221):} \]

Trifluoroacetic anhydride (0.5 mL, 3.6 mmol, 1 equiv), followed by trifluoroacetic acid (1.7 mL, 21.6 mmol, 6 equiv) was added dropwise at 0 °C to a solution of benzylidene acetal 226¹¹ (2.0 g, 3.6 mmol) and triethylsilane (3.5 mL, 21.6 mmol, 6 equiv) in CH₂Cl₂ (36 mL). The mixture was stirred for 3 h at RT, and then quenched at 0 °C with a saturated aq. solution of NaHCO₃. The mixture was extracted with CH₂Cl₂ and the organic layer was washed with brine, dried over MgSO₄ and concentrated *in vacuo* to obtain 2.0 g of the crude alcohol.

The crude product was then taken up in CH₂Cl₂ (170 mL) and treated with DCC (1.9 g, 8.9 mmol, 2.5 equiv), LevOH (2.0 g, 17.9 mmol, 5 equiv) and DMAP (88 mg, 0.7 mmol, 0.2 equiv). The mixture was stirred overnight at RT and then filtered. The organic layer was concentrated *in vacuo* and the crude product was purified by flash column chromatography on silica gel (Hexanes/AcOEt = 100:0 to 70:30) to give galactose 221 (1.6 g, 2.5 mmol, 69%) as a white foam: Rf (Hexanes/EtOAc = 70:30) = 0.34; [α]D²⁰ = +7.5 (c = 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 7.47 (d, *J* = 8.0 Hz, 2H), 7.43 – 7.27 (m, 15H), 7.06 (d, *J* = 8.0 Hz, 2H), 5.63 (d, *J* = 2.0 Hz, 1H), 4.78 – 4.71 (m, 3H), 4.62 (d, *J* = 9.2 Hz, 1H), 4.51 (s, 2H), 4.47 (d, *J* = 10.8 Hz, 1H), 3.72 (at, *J* = 6.4 Hz, 1H), 3.68 – 3.52 (m, 4H), 2.79 – 2.56 (m, 4H), 2.31 (s, 3H), 2.17 (s, 3H); ¹³C NMR (150 MHz, CDCl₃): δ 206.3, 172.1, 138.3, 137.9, 137.8 (2C), 132.7 (2C), 129.9, 129.7 (2C), 128.5 (2C), 128.49 (2C), 128.47 (2C), 128.38 (2C), 128.32 (2C), 128.22 (2C), 127.91 (3C), 88.2 (¹JCl₁-H₁ = 154.8 Hz, C1-β), 81.3, 77.0, 76.1, 75.8, 73.8, 71.9, 68.4, 67.2, 38.1, 30.0, 28.1, 21.2; IR (thin film) 3651, 2981, 2912, 2883, 1739, 1719, 1494, 1454, 1363, 1156, 1102, 738 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₃₉H₄₂O₇Na: 677.2549, found 677.2571.
N-(Benzyl)benzyloxy carbonyl-5-amino-pentanly 4,6-di-O-benzylidene-2-trichloroacet-ami do-β-D-glucopyranoside (227): A mixture of glucosamine donor 222 (0.60 g, 1.31 mmol), linker 223 (0.65 g, 1.97 mmol, 1.5 equiv), freshly activated molecular sieves 3Å AW (1.5 g) and NIS (0.59 g, 2.63 mmol, 2 equiv) in CH₂Cl₂ (26 mL) was stirred for 15 min, then cooled to -20 °C. After addition of TfOH (12 μL, 130 μmol, 0.1 equiv), the mixture was stirred at -20 °C for 30 min. After filtration of molecular sieves on a pad of celite, the reaction mixture was diluted with CH₂Cl₂ and quenched with a saturated aq. solution of NaHCO₃. The organic layer was washed with a saturated aq. solution of Na₂S₂O₃, water, dried over MgSO₄ and concentrated in vacuo.

The crude material was taken in pyridine (26 mL) and treated with trityl chloride (0.190 g, 0.657 mmol, 0.5 equiv). The solution was stirred at RT overnight and then all volatiles were removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel (Hexanes/AcOEt = 100:10 to 70:30) to give 0.66 g (0.92 mmol, 70 % over two steps) of target compound 227 as an oil: Rf (Hexanes/AcOEt = 70:30) = 0.25; ¹H NMR (400 MHz, CDCl₃, rotamers): δ 7.53 – 7.43 (m, 2H), 7.42 – 7.27 (m, 11H), 7.22 – 7.14 (m, 2H), 6.89 (bs, 1H), 5.55 (s, 1H), 5.17 (d, J = 10.4 Hz, 2H), 4.92 – 4.87 (m, 1H), 4.48 (s, 2H), 4.36 – 4.33 (m, 2H), 3.85 – 3.76 (m, 2H), 3.56 – 3.47 (m, 4H), 3.24 – 3.17 (m, 2H), 1.71 – 1.44 (m, 4H), 1.40 – 1.14 (m, 2H); ¹³C NMR (100 MHz, CDCl₃, rotamers): δ 162.3, 137.98, 137.94, 137.0, 129.4, 128.6, 128.6, 128.5, 128.4, 128.0, 127.9, 127.4, 127.3, 126.4, 101.9, 100.0, 92.6, 81.7, 70.3, 69.6, 68.7, 67.3, 66.2, 59.7, 50.6 and 50.4 (CH₂ rotamers), 47.25 and 46.23 (CH₂ rotamers), 29.4, 27.9, 23.4; HRMS(ESI) m/z calcd for (M+Na)+ C₃₅H₃₉Cl₃N₂O₈Na: 743.1670, found 743.1661.
Ethyl 4,6-di-O-benzyl-2-deoxy-3-tert-butyldimethylsilyl-2-trichloroacetamido-1-thio-β-D-glucopyranoside (230): TBSCI (3.96 g, 26.3 mmol, 2 equiv) and imidazole (2.14 g, 31.5 mmol, 2.4 equiv) were added at 0 °C to a solution of 2-deoxy-2-trichloroacetamido-1-thio-β-D-glucopyranoside 222110 (6.0 g, 13.1 mmol) in CH₂Cl₂ (66 mL). After completion, the reaction mixture was quenched with MeOH at 0 °C, diluted with CH₂Cl₂ and washed with a saturated aq. solution of NH₄Cl. Following separation of layers, the organic layer was washed with brine, dried over MgSO₄ and concentrated in vacuo. The crude hemiacetal was dissolved in CH₂Cl₂ (66 mL) and cooled to 0 °C. After addition of 1 M solution of BH₃·THF (52.5 mL, 52.5 mmol, 4 equiv) and TMSOTf (1.2 mL, 6.5 mmol, 0.5 equiv), the reaction was stirred at 0 °C till completion, then quenched with a saturated aq. solution of NaHCO₃. The mixture was extracted with CH₂Cl₂ and the organic layer was washed with brine, dried over MgSO₄ and concentrated in vacuo. Then the crude product was taken in a mixture DMF/THF (66 mL, 1/10, v/v) and treated at 0 °C with BnBr (4.7 mL, 39.4 mmol, 3 equiv) and NaH (60 % in mineral oil, 1.6 g, 39.4 mmol, 3.0 equiv). After the reaction was completed, it was quenched with MeOH, diluted with CH₂Cl₂ and washed with a saturated aq. solution of NH₄Cl. The organic layer was washed with brine, dried over MgSO₄ and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (Hexanes/EtOAc/CH₂Cl₂ = 9:0.5:1 to 9:1:1) to give thioglucoside 230 (6.7 g, 10.0 mmol, 77% over three steps) as a white foam: R_f (Hexanes/EtOAc/CH₂Cl₂ = 7:1:2) = 0.41; [α]D^20 = -2.7 (c = 3.5, CHCl₃); 1H NMR (400 MHz, CDCl₃): δ 7.37 – 7.21 (m, 10H), 7.04 (d, J = 9.2 Hz, 1H), 4.75 (d, J = 8.8 Hz, 1H), 4.72 (d, J = 11.6 Hz, 1H), 4.65 (d, J = 11.6 Hz, 1H), 4.56 (d, J = 12.0 Hz, J = 12.0 Hz, 2H), 3.97 (at, J = 7.2 Hz, 1H), 3.91 – 3.81 (m, 1H), 3.82 (dd, J = 10.4, 3.6 Hz, 1H), 3.76 (dd, J = 10.6, 4.4 Hz, 1H), 3.73 – 3.69 (m, 1H), 3.59 (at, J = 7.2 Hz, 1H), 2.83 – 2.65 (m, 2H), 1.28 (t, J = 7.6 Hz, 3H), 0.90 (s, 9H), 0.10 (s, 3H), 0.03 (s, 3H); 13C NMR (100 MHz, CDCl₃): δ 161.5, 138.2, 137.9, 128.4 (4C), 127.9 (2C), 127.7, 127.7, 127.5 (2C), 92.6, 82.7, 78.6, 78.3, 74.0, 74.0, 73.5, 69.5, 57.4, 25.9 (3C), 24.8, 18.0, 15.0, -3.9, -4.1; IR (thin film) 3340, 2928, 2856, 1684, 1522, 1252, 1085, 836 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)^+ C₃₀H₄₂Cl₃NaO₅S₅: 684.1516, found 684.1507.

![Ethyl 4,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-3-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (229):](image)

Ethyl 4,6-di-O-benzyl-2-deoxy-2-trichloroacetamido-3-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (229): To a solution of silylated glucosamine 230 (3.3 g, 5.0 mmol) in
anhydrous CH₃CN (59 mL) was added BF₃·OEt₂ (0.75 mL, 5.94 mmol, 1.2 equiv). The reaction mixture was stirred at 0 °C for 30 min and quenched with a saturated aq. solution of NaHCO₃. The precipitated solid was filtered and dried in vacuo. The crude alcohol was taken in CH₂Cl₂ (16 mL) and treated at 0 °C with FmocCl (2.6 g, 9.9 mmol, 2.0 equiv) and pyridine (1.2 mL, 4.6 mmol, 3.0 equiv). The reaction mixture was stirred at RT for 15 h and then quenched with a 1 M aq. solution of HCl. The mixture was extracted with CH₂Cl₂ and the organic layer was washed with a saturated aq. solution of NaHCO₃ and brine, dried over MgSO₄ and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (Hexanes/EtOAc/CH₂Cl₂ = 70:20:1 to 70:30:10) to give glucosamine 229 (3.50 g, 4.6 mmol, 92% over two steps) as a white foam: Rf (Hexanes/EtOAc/CH₂Cl₂ = 8:1:2) = 0.29; [α]D²⁰ = -27.5 (c = 2.6, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 7.75 (dd, J = 7.6, 3.2 Hz, 2H), 7.55 (ddd, J = 10.2, 7.6, 0.6 Hz, 2H), 7.45 – 7.17 (m, 12H), 7.14 – 7.07 (m, 2H), 6.99 (d, J = 9.6 Hz, 1H), 5.14 (dd, J = 10.2, 9.2 Hz, 1H), 4.70 – 4.50 (m, 4H), 4.48 (d, J = 10.2 Hz, 1H), 4.30 (m, 2H), 4.22 – 4.09 (m, 2H), 3.85 (at, J = 9.6 Hz, 1H), 3.67 (dd, J = 11.2, 3.8 Hz, 1H), 3.60 (dd, J = 11.2, 1.6 Hz, 1H), 3.52 (ddd, J = 9.6, 3.6, 1.8 Hz, 1H), 2.67 (qd, J = 7.2, 2.4 Hz, 2H), 1.21 (t, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): 162.0, 155.6, 143.1, 142.9, 141.37, 141.32, 138.1, 137.5, 128.56 (3C), 128.54 (2C), 128.16, 128.14, 128.10 (2C), 127.9 (2C), 127.8, 127.4, 127.3, 125.3, 125.2, 120.23, 120.21, 92.2, 83.7, 80.2, 79.1, 75.7, 75.1, 73.5, 70.8, 68.3, 54.9, 46.4, 24.0, 14.8; IR (thin film) 3361, 3032, 2921, 2868, 1720, 1526, 1451, 1390, 1265, 1085, 1057, 967, 819, 738 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₉₉H₃₈Cl₃NaN₃O₇S: 792.1332, found 792.1347.

\[ \text{N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl \ 4,6-di-O-benzyl-2-trichloroacetamido-\(\beta\)-D-glucopyranoside (231)} \]: A mixture of glucosamine building block 229 (0.6 g, 0.78 mmol), linker 223 (0.38 g, 1.17 mmol, 1.5 equiv), freshly activated molecular sieves 3Å AW (1.8 g) and NIS (0.35 g, 1.56 mmol, 2 equiv) in CH₂Cl₂ (16 mL) was stirred for 15 min, then cooled to -20 °C. After addition of TFOH (7 µL, 78 µmol, 0.1 equiv), the mixture was stirred at -20 °C for 1 h. After filtration of the molecular sieves on a pad of celite, the reaction mixture was diluted with CH₂Cl₂ and washed with a saturated aq. solution of NaHCO₃. The
organic layer was washed with a saturated aq. solution of Na$_2$S$_2$O$_3$ and water, dried over MgSO$_4$ and concentrated in vacuo. The crude product was run through a silica column with (Hexanes/AcOEt = 80:20 to 50:50) to yield 0.61 g of crude product. The crude material was then dissolved in CH$_2$Cl$_2$ (12 mL) and treated with Et$_3$N (1.6 mL, 11.7 mmol). The solution was stirred for 3 h at RT and then concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (Hexanes/EtOAc = 90:10 to 50:50) to give 0.45 g (0.553 mmol, 71% over two steps) of target compound 231 as an oil: R$_f$ (Hexanes/EtOAc = 70:30) = 0.25; [α]$_D^{20}$ = −2.4 (c = 0.35, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): δ 7.36 – 7.23 (m, 20H), 7.17 (s, 1H), 5.16 (s, 2H), 4.74 (d, J = 11.2 Hz, 1H), 4.69 – 4.61 (m, 3H), 4.55 (d, J = 12.0 Hz, 1H), 4.48 (bs, 2H), 4.13 – 4.04 (m, 1H), 3.85 – 3.71 (m, 3H), 3.63 – 3.47 (m, 3H), 3.43 – 3.37 (m, 1H), 3.23 – 3.17 (m, 2H), 2.99 (s, 1H), 1.56 – 1.49 (m, 4H), 1.43 – 1.14 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 162.3, 138.06, 138.03, 137.8, 128.6 (3C), 128.5 (3C), 128.5, 128.4 (3C), 128.0 (3C), 127.9, 127.8 (4C), 127.7, 127.3, 127.2, 99.66 (J$_{C1A-H1A}$ = 164.8 Hz, C1-β), 92.6, 92.0, 78.3, 74.9, 74.5, 73.5 (2C), 72.9, 69.5, 69.1, 67.2, 58.4, 50.6, 50.3, 47.1, 46.2, 29.1, 27.9, 27.3, 23.2; IR (thin film) 3436, 3327, 2924, 2862, 1697, 1101, 1062 cm$^{-1}$; HRMS(ESI) m/z caled for (M+Na)$^+$ C$_{42}$H$_{47}$Cl$_3$N$_2$O$_8$Na: 835.2296, found 835.2271.

$N$-(Benzyl)benzylxycarbonyl-5-amino-pentanyl 2,3,6-tri-$O$-benzyl-$\alpha$-D-galactopyranosyl-(1→3)-4,6-di-$O$-benzyl-2-trichloroacetamido-$\beta$-D-glucopyranoside (218): A mixture of β-thiogalactoside 221 (0.40 g, 0.60 mmol, 1.3 equiv), glucosamine acceptor 231 (0.38 g, 0.47 mmol), NIS (0.16 g, 0.7 mmol, 1.5 equiv) and freshly activated molecular sieves 3Å AW (1 g) in Et$_2$O/CH$_2$Cl$_2$ (16 mL, 3/1, v/v) was stirred at RT for 30 min. The mixture was cooled to 0 °C and the TiOH (4 µL, 47 µmol, 0.1 equiv) was added. The mixture was stirred for 30 min at 0 °C. After filtration of the molecular sieves on a pad of celite, the reaction mixture was diluted with Et$_2$O and washed with a saturated aq. solution of Na$_2$S$_2$O$_3$. After separation of the layers, the organic layer was washed with a saturated aq.
solution of NaHCO₃ and brine, dried over MgSO₄ and concentrated in vacuo. The crude product was run through a silica column with (Hexanes/AcOEt = 90:10 to 70:30) to yield 0.35 g of crude disaccharide. The crude material was then dissolved in MeOH (12 mL) and treated with 0.5 M solution of NaOMe in MeOH (90 µL, 45 µmol, 0.2 equiv). The mixture was stirred at RT overnight and then acidified till pH 6 with Amberlite IR 120-H⁺ resin. The solvents were concentrated in vacuo and the crude material was purified by flash column chromatography on silica gel (Hexanes/AcOEt = 90:10 to 70:30) = 0.35; [α]D⁰ = +27.8 (c = 0.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃, rotamers): δ 8.02 (d, J = 6.4 Hz, 1H, NHCOCl₃), 7.31 – 7.22 (m, 30H), 7.16 – 7.09 (m, 5H), 5.14 (s, 2H, CO₂CH₂), 5.08 – 4.93 (m, 3H, CH, H₁A, H₁B), 4.77 – 4.67 (m, 3H, CH₂), 4.63 – 4.35 (m, 8H, CH₂), 4.35 – 4.28 (m, 1H, H₃A), 4.17 – 4.14 (m, 1H, H₂B), 3.92 (bs, 1H, H₂B), 3.85 – 3.78 (m, 2H, H₂B, H₃B), 3.77 – 3.59 (m, 6H, OCH₂, H₆aA, H₆bA, H₆bB, H₄A, H₅A), 3.55 (d, J = 9.2 Hz, 1H, H₆bB), 3.39 (bs, 2H, H₂A, OCH₂), 3.17 – 3.10 (m, 2H, NCH₂), 2.49 (s, 1H, OH), 1.51 – 1.36 (m, 4H, CH₂), 1.30 – 1.12 (m, 2H, CH₂); ¹³C NMR (100 MHz, CDCl₃, rotamers): δ 161.4 (CO), 138.3 (2C), 138.1, 137.9, 137.8, 137.7, 137.4, 128.4 (3C), 128.4 (3C), 128.4 (3C), 128.3 (3C), 128.3 (3C), 128.2 (3C), 128.0 (3C), 127.9, 127.8 (3C), 127.7 (4C), 127.7 (4C), 127.5, 127.4, 98.6 (¹JC₁A-H₁A = 166.0 Hz, C₁A-β), 97.7 (¹JC₁B-H₁B = 171.3 Hz, C₁B-α), 92.7 (NHCOCl₃), 80.5 (C₃A), 77.1 (C₄A), 76.7 (C₃B), 75.9 (C₃B), 74.4 (CH₂), 74.0 (C₅A), 73.5 (2C, CH₂), 73.4 (2C, CH₂), 72.5 (CH₂), 70.0 (C₆B), 69.6 (OCH₂), 69.3 (C₅B), 68.9 (C₆A), 68.2 (C₄B), 67.0 (CO₂CH₂Ph), 59.0 (C₂A), 50.4 and 50.1 (NCH₂Ph, rotamers), 47.0 and 46.1 (CH₂ rotamers), 27.9 (CH₂), 27.4 (CH₂), 23.1 (CH₂); IR (thin film) 3484, 3340, 2923, 2856, 1701, 1523, 1468, 1454, 1091, 1028, 698 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₆₉H₆₁Cl₂N₂O₁₃Na 1267.4232, found 1267.4163.

**Ethyl 2,4-di-Ø-benzyl-3-O-2-(naphtylmethyl)-1-thio-β-D-glucopyranoside (SI5):** A solution of thioglucoide 234¹¹⁶ (1.74 g, 3.8 mmol) in DMF (20 mL) was treated at 0 °C with BnBr (0.7 mL, 5.7 mmol, 1.5 equiv) and NaH (60% in mineral oil, 0.23 g, 5.7 mmol, 1.5 equiv). After completion, the reaction was quenched at 0 °C with MeOH and a saturated solution of NH₄Cl and the formed precipitate was filtered and dried. The crude acetal was taken in CH₂Cl₂ (23 mL) and reacted at 0 °C with a 1 M solution of BH₃·THF (15.4 mL, 15.4
mmol, 4.0 equiv), followed by TMSOTf (0.35 mL, 1.9 mmol, 0.5 equiv). After the reaction was completed, the reaction mixture was quenched with a saturated aq. solution of NaHCO₃ and extracted with CH₂Cl₂. The organic layer was washed with brine, dried over MgSO₄, and concentrated in vacuo. The crude was purified by column chromatography on silica gel (Hexanes/EtOAc/CH₂Cl₂ = 7:2:1 to 5:2:1) to give alcohol S15 (1.6 g, 2.85 mmol, 75% over two steps) as a white foam: \( R_f \) (Hexanes/EtOAc = 70:30) = 0.33; \( [\alpha]_D^{20} = +13.5 \) (c = 0.15, CHCl₃); \(^1^H\) NMR (400 MHz, CDCl₃): \( \delta \) 7.90 – 7.64 (m, 4H), 7.55 – 7.40 (m, 3H), 7.37 – 7.26 (m, 10H), 5.08 (d, J = 11.2 Hz, 1H), 5.02 (d, J = 11.2 Hz, 1H), 4.94 (d, J = 10.4 Hz, 1H), 4.89 (d, J = 11.2 Hz, 1H), 4.77 (d, J = 10.4 Hz, 1H), 4.68 (d, J = 11.2 Hz, 1H), 4.53 (d, J = 9.6 Hz, 1H), 3.89 (dd, J = 12.0, 2.0 Hz, 1H), 3.78 – 3.69 (m, 2H), 3.62 (t, J = 9.6 Hz, 1H), 3.52 – 3.32 (m, 2H), 2.93 – 2.54 (m, 2H), 1.33 (t, J = 7.6 Hz, 3H); \(^1^C\) NMR (100 MHz, CDCl₃): \( \delta \) 138.0, 138.0, 136.0, 133.4, 133.1, 128.6 (2C), 128.5 (2C), 128.4 (2C), 128.3, 128.1 (2C), 128.0 (2C), 128.0, 127.8, 126.5, 126.2, 126.0, 125.9, 86.6, 85.4, 81.9, 79.4, 77.8, 75.9, 75.7, 75.3, 62.3, 25.3, 15.3; IR (thin film) 3748, 3343, 3030, 2959, 2905, 2871, 1454, 1399, 1348, 1087, 1067, 1038, 1022 cm⁻¹; HRMS(ESI) m/z calc'd for (M+Na)⁺ C₃₃H₃₆NaO₇S: 567.2181, found 567.2201.

Ethyl 6-O-acetyl-2,4-di-O-benzyl-3-O-2-(naphtylmethyl)-1-thio-β-D-glucopyranoside (224): Acetic anhydride (50 μL, 551 μmol, 3 equiv) was added to a solution of S15 (100 mg, 184 μmol) in pyridine (6 mL). The mixture was stirred at RT for 2 h and concentrated in vacuo. The crude was purified by flash column chromatography on silica gel (Hexanes/AcOEt = 100:0 to 70:30) to give acetylated glucose 224 (107 mg, 182 μmol, 99%) as a white foam: \( R_f \) (Hexanes/AcOEt = 80:20) = 0.46; \( [\alpha]_D^{20} = +17.3 \) (c = 0.90 , CHCl₃); \(^1^H\) NMR (400 MHz, CDCl₃): \( \delta \) 7.87 – 7.72 (m, 4H), 7.52 – 7.46 (m, 2H), 7.44 (dd, J = 8.4, 1.6 Hz, 1H), 7.40 – 7.36 (m, 2H), 7.34 – 7.27 (m, 5H), 7.26 – 7.23 (m, 3H), 5.11 (d, J = 11.2 Hz, 1H), 5.01 (d, J = 11.2 Hz, 1H), 4.97 (d, J = 10.4 Hz, 1H), 4.90 (d, J = 10.8 Hz, 1H), 4.78 (d, J = 10.4 Hz, 1H), 4.61 (d, J = 10.8 Hz, 1H), 4.51 (d, J = 9.6 Hz, 1H), 4.37 (dd, J = 12.0, 2.0 Hz, 1H), 4.22 (dd, J = 11.6, 4.8 Hz, 1H), 3.78 (dd, J = 8.8, 8.4 Hz, 1H), 3.61 – 3.52 (m, 2H), 3.49 (dd, J = 10.0, 9.2 Hz, 1H), 2.86 – 2.58 (m, 2H), 2.05 (s, 3H), 1.35 (t, J = 7.6 Hz, 3H); \(^1^C\) NMR (100 MHz, CDCl₃): \( \delta \) 170.8, 137.9, 137.7, 135.9, 133.4, 133.1, 128.6 (2C), 128.5 (2C), 128.4 (2C), 128.3, 128.17 (2C), 128.12, 128.0, 128.0, 127.8, 126.5, 126.2, 126.0, 125.9, 86.7,
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85.4, 81.8, 77.8, 77.0, 75.9, 75.6, 75.2, 63.5, 25.4, 21.0, 15.2; IR (thin film) 3060, 3030, 2956, 2924, 2869, 1741, 1454, 1235, 1080, 1069, 1030 cm\(^{-1}\); HRMS(ESI) m/z calcd for (M+Na\(^+\))\_ C\(_{35}\)H\(_{38}\)NaO\(_{6}\)S: 609.2287, found 609.2338.

\[ N-(\text{Benzyl})\text{benzyloxycarbonyl-5-amino-pentanyl} \quad 6-O\text{-acetyl-2,4-di-O-benzyl-3-O-2-}
\quad (\text{naphthylmethyl})-\alpha-d\text{-glucopyranosyl}(1\rightarrow4)-2,3,6\text{-tri-O-benzyl-}\alpha-d\text{-galactopyranosyl-}
\quad (1\rightarrow3)-4,6\text{-di-O-benzyl-2-trichloroacetamido-}\beta-d\text{-glucopyranoside} \quad (235): \text{Acceptor 218}
\quad (40 \text{ mg}, 32 \text{ µmol}) \text{ and thioglucoside 224 (28 mg, 48 µmol, 1.5 equiv) were azeotroped}
\quad \text{with toluene and dried in vacuo. After addition of NIS (14 mg, 64 µmol, 2.0 equiv) and freshly}
\quad \text{activated molecular sieves 4Å (100 mg), the reaction mixture was solubilized in Et}_2\text{O/CH}_2\text{Cl}_2
\quad (320 \mu\text{L, 3/1, v/v}), \text{cooled to -50 °C and stirred for 15 min. A solution of TfOH in CH}_2\text{Cl}_2 (30
\quad \mu\text{L, 3 µmol, 0.1 equiv) was added dropwise and the mixture was stirred for 1 h at -50 °C:}
\quad \text{After filtration of the molecular sieves on a pad of celite, the reaction mixture was diluted}
\quad \text{with Et}_2\text{O and washed with a saturatedaq. solution of Na}_2\text{SO}_4. \text{After extraction with Et}_2\text{O,}
\quad \text{the organic layer was washed with a saturatedaq. solution of NaHCO}_3, \text{brine, dried over MgSO}_4
\quad \text{and concentrated in vacuo. The crude product was purified by NP-HPLC on YMC}
\quad \text{Pack silica, eluents AcOEt/Hexanes (gradient: 5% AcOEt (5 min) → 30% AcOEt (30 min))}
\quad \text{to afford the target trisaccharide 235 (55 mg, 31 µmol) in 97% yield as a colorless oil: Rf}
\quad \text{(Hexanes/EtOAc = 70:30) = 0.36; [α]_D^{20} = +18.3 (c = 1.0, CHCl}_3; \text{^1H NMR (400 MHz,}
\quad \text{CDCl}_3, \text{rotamers): δ 7.90 (d, J = 7.6 Hz, 1H, NJHCOCl}_3, 7.86 - 7.76 (m, 2H), 7.73 - 7.70 (m,}
\quad 2H), 7.51 - 7.41 (m, 3H), 7.36 - 7.04 (m, 45H), 5.14 (d, J = 6.0 Hz, 2H, CO}_2\text{CH}_2\text{Ph), 5.09 -}
\quad 5.07 (m, 2H, CH}_2\text{Ph, H}_2\text{B}, 5.00 - 4.95 (m, 2H, CH}_2\text{Ph), 4.88 (d, J = 10.8 Hz, 1H, CH}_2\text{Ph),}
\quad 4.85 (d, J = 3.2 Hz, 1H, H}_2\text{C), 4.84 - 4.77 (m, 2H, H}_1\text{A, CH}_2\text{Ph), 4.71 - 4.69 (m, 2H, CH}_2\text{Ph),}
\quad 4.65 (d, J = 12.0 Hz, 1H), 4.63 - 4.46 (m, 4H), 4.46 (d, J = 10.4 Hz, 1H), 4.42 (bs, 2H,}
\quad \text{NCH}_2\text{Ph), 4.30 - 4.23 (m, 2H, H}_2\text{B, H}_3\text{A), 4.20 - 4.15 (m, 2H, CH}_2\text{Ph, H}_6\text{A,C), 4.12 - 4.09 (m,}
\quad 1H, H}_3\text{C), 4.05 (dd, J = 9.2, 9.6 Hz, 1H, H}_5\text{C), 3.98 - 3.87 (m, 4H, CH}_2\text{Ph, H}_2\text{B, H}_6\text{B, H}_7\text{B),}
\quad 3.83 (dd, J = 10.4, 2.8 Hz, 1H, H}_3\text{B), 3.77 - 3.56 (m, 7H, OCH}_2\text{, H}_6\text{A, H}_6\text{B, H}_4\text{A, H}_6\text{A, H}_6\text{B,}
\quad H}_5\text{A, H}_4\text{C), 3.53 (dd, J = 10.0, 3.2 Hz, 1H, H}_2\text{C), 3.51 - 3.40 (m, 2H, H}_6\text{B, H}_2\text{A), 3.35 -}
N-(Benzyl)benzylxoycarbonyl-5-amino-pentany 2,4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-O-benzyl-2-trichloroacetamido-β-D-glucopyranoside (214): A solution of trisaccharide 235 (50 mg, 28 µmol) in MeOH (0.3 mL) was treated with a 0.5 M solution of NaOMe in MeOH (6 µL, 3 µmol, 0.1 equiv). The solution was stirred at RT for 3 h, diluted with MeOH and quenched with Amberlite IR-120 H⁺ resin until pH 6 was reached. The resin was filtered off and washed several times with MeOH. The filtrate was concentrated in vacuo to give an oil, which was further dissolved in a mixture of CH₂Cl₂/MeOH (3.1 mL, 9/1, v/v). The mixture was cooled at 0 °C and treated with DDQ (21 mg, 84 µmol, 3 equiv). After 3 h of stirring at 0 °C, the reaction was quenched by addition of a saturated aq. solution of Na₂S₂O₃ and a saturated aq. solution of NaHCO₃. The mixture was extracted with CH₂Cl₂. The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (Hexanes/AcOEt = 70:30) on silica gel to give 33 mg (21 µmol, 73% over two steps) of target diol 214 as a yellow oil: Rf (Hexanes/EtOAc =
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\[ \text{[α]_D}^{20} = +70.9 \ (c = 0.25, \text{CHCl}_3) \]

\[ 1^H \text{NMR (600 MHz, CDCl}_3, \text{rotamers): } \delta 7.86 \ (d, J = 6.6 \ Hz, 1H), 7.42 - 7.04 \ (m, 45H), 5.15 \ (d, J = 6.6 \ Hz, 2H, CO}_2\text{CH}_2\text{Ph), 5.02 \ (s, 1H, H}_1\text{B), 4.96 \ (d, J = 11.0 \ Hz, 1H, }\text{CH}_2\), 4.84 \ (d, J = 6.0 \ Hz, 1H, }\text{CH}_2\), 4.81 \ (s, 2H, H}_1\text{C, H}_1\text{A), 4.72 - 4.65 \ (m, 4H, }\text{CH}_2\text{Ph), 4.65 - 4.58 \ (m, 2H, }\text{CH}_2\text{Ph), 4.56 \ (d, J = 12.2 \ Hz, 1H, }\text{CH}_2\text{Ph), 4.50 \ (d, J = 12.0 \ Hz, 1H, }\text{CH}_2\text{Ph), 4.45 - 4.41 \ (m, 3H, }\text{CH}_2\text{Ph, NCH}_2\text{Ph), 4.31 - 4.27 \ (m, 1H, }\text{CH}_2\text{Ph), 4.23 \ (dd, J = 7.8, 7.8 Hz, 1H, H}_3\text{A), 4.14 - 4.08 \ (m, 2H, H}_5\text{B, H}_3\text{C), 4.04 - 4.02 \ (m, 2H, H}_5\text{C, CH}_2\text{Ph), 3.86 - 3.81 \ (m, 3H, H}_4\text{B, H}_3\text{B, H}_2\text{B), 3.77 - 3.44 \ (m, 10H, }\text{OC}_2\text{H}_2\text{, H}_6\text{aA, H}_6\text{bA, H}_6\text{aC, H}_6\text{bC, H}_6\text{bB, H}_2\text{A), 3.41 \ (dd, J = 9.0, 9.6 Hz, 1H, H}_4\text{C), 3.37 - 3.25 \ (m, 2H, H}_2\text{C, CH}_2\text{Ph), 3.17 - 3.09 \ (m, 2H, }\text{NCH}_2\text{Ph, NCH}_2\text{Ph), 2.37 \ (s, 1H, }\text{OH}, 1.45 - 1.40 \ (m, 4H, }\text{CH}_2\), 1.22 - 1.15 \ (m, 2H, }\text{CH}_2\); 13C NMR (150 MHz, CDCl}_3, \text{rotamers): } \delta 161.6, 138.4, 138.3, 138.2, 138.1, 137.9, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.3, 99.20 (J_{C1A-H1A} = 165.5 \ Hz, C}_1\text{A-β), 99.1 (J_{C1C-H1C} = 173.0 \ Hz, C}_1\text{C-α), 97.9 (J_{C1B-H1B} = 173.5 \ Hz, C}_1\text{B-α), 92.9 (NHCOCCl}_3\text{), 80.5 (C}_2\text{C), 79.7 (C}_3\text{A), 78.5 (C}_2\text{B), 78.0 (C}_4\text{C), 77.5 (C}_4\text{A), 77.1 (C}_3\text{B), 76.2 (C}_4\text{B), 74.8 (CH}_2\text{Ph), 74.4 (CH}_2\text{Ph), 74.3 (C}_5\text{A), 73.8 (CH}_2\text{Ph), 73.57 (CH}_2\text{Ph), 73.56 (CH}_2\text{Ph), 73.52 (C}_3\text{C), 73.2 (CH}_2\text{Ph), 73.0 (CH}_2\text{Ph), 71.4 (C}_5\text{C), 70.6 (C}_6\text{B), 69.8 (C}_6\text{B), 69.7 (OCH}_2\text{), 69.1 (C}_6\text{A), 67.2 (CO}_2\text{CH}_2\text{Ph), 61.9 (C}_6\text{C), 58.7 and 58.5 (C}_2\text{A), 50.6 and 50.3 (NCH}_2\text{Ph), 47.2 and 46.3 (NCH}_2\text{), 29.4 (CH}_2\text{), 28.0 and 27.6 (CH}_2\), 23.3 (CH}_2\); IR (thin film) 3339, 2835, 1678, 1637, 1091, 1027 cm\(^{-1}\); HRMS(ESI) m/z calcld for (M+Na\(^+\))\(^+\) C\(_{89}\)H\(_{97}\)Cl\(_3\)N\(_2\)NaO\(_{18}\): 1609.5700, found 1609.5709.
N-(Benzyl)benzyloxycarbonyl-5-amino-pentany 3,6-dideoxy-4-O-benzoyl-2-O-benzyl-α-L-galactopyranosyl-(1→3)-[3,6-dideoxy-4-O-benzoyl-2-O-benzyl-α-L-galactopyranosyl-(1→6)]-2,4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-O-benzyl-2-trichloroacetamido-β-D-glucopyranoside (217): Colitose imidate 215 (35 mg, 68 µmol, 4 equiv) and nucleophile trisaccharide 214 (27 mg, 17 µmol) were azeotroped three times with toluene and dried in vacuo. After addition of freshly activated molecular sieves 4Å (80 mg), the mixture was suspended in anhydrous CH₂Cl₂ (340 µL) under Ar atmosphere. The mixture was cooled to -50 °C and stirred for 15 min. After dropwise addition of TMSOTf in CH₂Cl₂ (30 µL, 1.7 µmol, 0.1 equiv), the reaction was stirred for 15 min at -50 °C. After filtration of the molecular sieves on a pad of celite, the reaction mixture was diluted with CH₂Cl₂ and washed with a saturated aq. solution of NaHCO₃ and brine, and dried over MgSO₄. After removal of solvents in vacuo, the crude was purified by NP-LCMS on YMC Pack silica, eluents AcOEt/Hexanes (gradient: 5% AcOEt (5 min) → 30% AcOEt (40 min)) to afford target pentasaccharide 217 (24 mg, 11 µmol, 63%) as an oil: Rf (Hexanes/AcOEt = 70:30) = 0.20; [α]D²⁰ = +10.3 (c = 0.30, CHCl₃); ¹H NMR (400 MHz, CDCl₃, rotamers): δ 8.07 (d, J = 8.0 Hz, 1H, NHCOCl₂), 8.04 – 8.02 (m, 2H), 7.90 – 7.88 (m, 2H), 7.60 – 7.51 (m, 3H), 7.49 – 7.26 (m, 22H), 7.24 – 7.04 (m, 21H), 6.99 – 6.94 (m, 4H), 6.80 (dd, J = 7.2 Hz, 2H), 5.65 (d, J = 3.2 Hz, 1H, H₁D), 5.26 – 5.22 (m, 1H, H₄E), 5.13 (s, 2H, CO₂CH₂Ph), 5.05 – 5.00 (m, 2H, H₂B, CH₂Ph), 4.97 – 4.91 (m, 3H, 2CH₂Ph, H₁A), 4.86 (d, J = 3.2 Hz, 1H, H₁C), 4.82 – 4.77 (m, 2H, H₂D, H₁E), 4.74 – 4.66 (m, 4H, CH₂Ph), 4.65 – 4.60 (m, 2H, CH₂Ph), 4.56 – 4.47 (m, 4H, CH₂Ph), 4.44 – 4.17 (m, 12H, CH₂Ph, NCH₂Ph, H₃C, H₂B, H₃A, H₂D, H₃E), 4.03 – 3.92 (m, 4H, H₆aB, H₅C, H₂B, H₂B), 3.87 – 3.84 (m, 2H, H₂E, H₄C), 3.80 (dd, J = 10.2, 3.2 Hz, 1H, H₃B), 3.77 – 3.50 (m, 10H, H₆aC, H₂D, H₆aA, H₆bA, OCH₂, H₂C, H₄A, H₆bC, H₅aA, H₆bB), 3.37 – 3.22 (m, 2H, OCH₂, H₂A), 3.19 – 2.95 (m, 2H, NCH₂), 2.27 – 2.20 (m, 1H, H₃E), 2.14 – 2.04 (m, 2H, H₂D), 1.41 – 1.35 (m, 4H, CH₂), 1.18 – 1.12 (m, CH₂), 1.06 (d, J = 6.6 Hz, 3H, H₆D), 0.73 (d, J = 6.4 Hz, 3H, H₆E); ¹³C NMR (150 MHz, CDCl₃, rotamers): δ 166.1, 166.0, 161.7, 138.8, 138.6, 138.3, 138.0, 137.8, 137.4, 133.2, 133.1, 130.3, 130.2, 129.8, 129.7, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.3, 126.2, 98.8 (¹JC1A,H1A = 166.9 Hz, C₁A-β), 98.6 (¹JC1C,H1C = 170.8 Hz, C₁C-α), 97.9 (¹JC1B,H1B = 171.5 Hz, C₁B-α), 96.7 (¹JC1E,H1E = 171.3 Hz, C₁E-α), 96.5 (¹JC1D,H1D = 173.6 Hz, C₁D-α), 93.1 (NHCOCl₃), 82.8 (C₂C), 80.9 (C₃A), 77.6 (C₄C), 77.3 (C₄A), 76.8 (C₂B), 76.4 (C₄B), 75.8 (C₂B), 74.8, 74.7, 74.1, 74.1, 73.5 (C₅A), 73.4 (C₃C), 73.0, 72.9, 72.6 (C₄B), 72.1 (C₄E), 72.0 (C₄D), 71.3 (C₂E), 71.0, 70.9
3. Total Synthesis of the *Escherichia coli* O111 O-Specific Polysaccharide

(C₃B), 70.6 (C₅C), 69.8 (C₄D), 69.2, 69.1 (C₆A), 67.2 (NHCO₂CH₂Ph), 65.9 (C₆C), 65.0 (C₅D), 64.8 (C₅E), 59.4 (C₄A), 50.6 and 50.3 (NCH₂Ph), 47.2 and 46.3 (NCH₂), 29.4 (CH₂), 29.1 (C₃E), 28.3 (C₃D), 28.0 and 27.6 (CH₂), 23.2 (CH₂), 16.5 (C₆E), 16.1 (C₅D); IR (thin film) 3347, 2925, 2855, 1716, 1270, 1093, 1061, 1027, 984 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C₁₂₉H₁₃₁Cl₃N₂O₂₆: 2257.8423, found 2257.8471.

![Pentasaccharide Structure](image)

**5-Amino-pentanyl 3,6-dideoxy-α-L-galactopyranosyl-(1→3)-(3,6-dideoxy-α-L-galactopyranosyl-(1→6)-α-L-galactopyranosyl-(1→3)-2-N-acetyl-β-glucopyranosyl-(1→4)-α-D-galactopyranosyl-(1→3)-2-N-acetyl-β-glucopyranoside (216):**

A degassed solution of AIBN (15 mg, 89 µmol, 10 equiv) in toluene (2 mL) was added to a solution of pentasaccharide 217 (20 mg, 8.9 µmol) and Bu₃SnH (24 µL, 89 µmol, 10 equiv) in toluene (2 mL). The mixture was stirred at 90 °C for 2 h and then passed through a pad of silica (Hexanes/AcOEt = 90:10 to 50:50) to give 17 mg of the crude intermediate N-acetyl pentasaccharide: MALDI-MS m/z calcd for (M+Na)+ C₁₂₉H₁₄₀N₂O₂₆: 2155.95, found 2155.89. The crude N-acetyl pentasaccharide was dissolved in MeOH (4 mL) and treated with a 0.5 M solution of NaOMe in MeOH (18 µL, 9 µmol, 1 equiv). The mixture was stirred at 40 °C overnight and then acidified till pH 6 with Amberlite IR 120-H⁺ resin. After filtration, the mixture was concentrated in vacuo to give an oil corresponding to the intermediate diol: MALDI-MS m/z calcd for (M+Na)+ C₁₁₅H₁₃₂N₂NaO₂₄: 1947.90, found 1947.92. The oil was solubilized in a mixture of THF/H₂O (6 mL, 1/1, v/v). Pd(OH)₂ 20% (20 mg) was added and the suspension was stirred under H₂ atmosphere for 24 h. After removal of the solvents, the
crude deprotected crude was solubilized in water and filtered on a cotton pad. Removal of the water by lyophilization, followed by gel filtration chromatography (Sephadex LH-20, eluent: water) afforded target pentasaccharide 216 (5 mg, 5.6 µmol, 64% over three steps) as a white foam: $R_f$ (PrOH/1 M aq. NH$_4$OAc = 1:2) = 0.50; $[\alpha]_D^{20} = +29.3$ (c = 0.06, CHCl$_3$); $^1$H NMR (600 MHz, D$_2$O): $\delta$ 5.45 (d, $J$ = 2.4 Hz, 1H), 5.15 (d, $J$ = 3.0 Hz, 1H), 4.95 (d, $J$ = 3.6 Hz, 1H), 4.82 (d, $J$ = 3.6 Hz, 1H), 4.54 (d, $J$ = 8.4 Hz, 1H), 4.36 – 4.28 (m, 2H), 4.09 (s, 1H), 4.05 – 3.98 (m, 3H), 3.92 – 3.89 (m, 6H), 3.84 – 3.58 (m, 13H), 3.47 – 3.44 (m, 1H), 3.01 – 2.95 (t, $J$ = 5.2 Hz, 2H), 2.04 (s, 3H), 2.01 – 1.94 (m, 4H), 1.69 – 1.64 (m, 2H), 1.62 – 1.57 (m, 2H), 1.44 – 1.36 (m, 2H), 1.15 (d, $J$ = 6.6 Hz, 3H), 1.12 (d, $J$ = 6.6 Hz, 3H); $^{13}$C NMR (150 MHz, CD$_6$CO): $\delta$ 174.5, 101.3, 100.5, 99.7, 99.1, 98.9, 80.3, 79.7, 78.4, 75.8, 72.6, 71.6, 71.5, 70.9, 70.4, 69.9, 69.1, 68.9, 68.1, 66.93, 66.90, 66.7, 63.76, 63.73, 60.8, 59.9, 54.5, 39.6, 33.27, 33.21, 28.4, 26.7, 22.6, 22.5, 15.6 (2C); IR (thin film) 3369, 1649, 1563, 1377, 1347, 1139, 1076 cm$^{-1}$; HRMS(ESI) m/z calcld for (M+H)$^+$ C$_{37}$H$_{67}$N$_2$O$_{22}$: 891.4185, found 891.4208.

Herein is described the automated solid-phase synthesis of a 30mer oligosaccharide. This synthesis pushes the limits of carbohydrate assembly, particularly when considering rapid automated protocols of any kind. To facilitate the purification, a catch-and-release strategy is employed whereby the full-length product was tagged and immobilized on magnetic beads. Simple magnet-assisted decanting furnished the desired product.
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

4.1 Introduction

Carbohydrates are structurally complex and diverse biopolymers\textsuperscript{117} that serve a host of biological functions.\textsuperscript{1c} Chemical synthesis provides access to well-defined oligosaccharides, since the purification of carbohydrates from natural sources is often difficult or even impossible. While peptides\textsuperscript{118} and oligonucleotides\textsuperscript{119} are now routinely assembled by automated solid phase synthesis, methods for automated oligosaccharide synthesis have been evolving more slowly. The need to exercise both regio- and stereocontrol during the glycosidic linkage formation poses significant synthetic challenges. These challenges aside, automated solid phase oligosaccharide synthesis has dramatically accelerated the assembly of increasingly complex carbohydrates.\textsuperscript{43a,45,47,53,58,59}

While polysaccharides are common in nature, syntheses of oligosaccharide sequences past 20 units have been very rare. Automated oligonucleotide synthesis that now can routinely reach 200mers paving the way to assemble entire genes serves as an inspiration.\textsuperscript{120}

Herein, we report the automated synthesis of a 30mer α-(1,6)-oligomannoside as a proof-of-principle that automated oligosaccharide synthesis can provide access to long carbohydrate chains. Since the interaction carbohydrate-carbohydrate recognition receptor is weak, access to large defined oligosaccharides would be of particular use for designing biologically active sugar-based structures. Although automated solid phase synthesis of oligosaccharides accelerates drastically the assembly process by eliminating the intermediate purifications, the final purification of the target compound from the putative deletion sequences could pose considerable problems. To facilitate the final purification, a catch-and-release strategy is employed whereby the full-length oligosaccharide was tagged and immobilized on magnetic beads. Following separation from deletion sequences by magnet-assisted decanting, the desired product is released from the beads.

4.2 Results and Discussion

4.2.1 Synthesis of Large α-(1,6)-Oligomannosides by Automated Solid Phase Synthesis

α-(1,6)-Oligomannosides could be assembled on the functionalized Merrifield solid support \textsuperscript{236} in a regio- and diastereoselective manner using mannosyl phosphate building block \textsuperscript{237} that has a C2 participating benzoyl protecting group to ensure the formation of the trans-
glycosidic linkages and an orthogonal Fmoc protected hydroxyl at C6 for chain elongation (Scheme 61). The anomic dibutyl phosphate leaving group allows for fast and efficient glycosylation by Lewis acid activation. Based on our experience, Merrifield’s resin is the solid support of choice for the assembly of carbohydrates, since it has a good chemical and mechanical stability and performs best in terms of permeability for reagents, reproducibility of loadings and swelling behaviour in organic solvents. The Merrifield resin was equipped with a photolabile ortho-nitrobenzyl alcohol derivatized linker, which is stable in both basic and acid conditions and can be efficiently cleaved in continuous flow, by exposure to pyrex filtered UV light from a mercury lamp (450 W).

![Scheme 61. Retrosynthetic analysis of α-(1,6)-oligomannosides.](image)

Mannosyl phosphate was synthesized in seven steps starting from D-mannose (Scheme 62). Per-acetylation of mannose, followed by glycosylation with ethanethiol in presence of boron trifluoride diethyl etherate and cleavage of the acetate esters using Zemplén conditions gave thiomannoside. A one-pot procedure allowed for the regioselective protection of the primary alcohol as the trityl ether, while the remaining free hydroxyl were protected via benzoylation. The trityl group was then cleaved under acidic conditions furnishing primary alcohol in 58% yield over five steps. Intermediate was then treated with 9-fluorenylmethyloxycarbonyl chloride in the presence of pyridine to give building block. Due to the high reactivity of phosphate glycosylating agents, thiomannoside was converted to the corresponding dibutyl phosphate in 78% yield by treatment with N-iodosuccinimide and dibutyl phosphate in the presence of a catalytic amount of triflic acide.
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

![Scheme 62. Synthesis of mannosyl phosphate building block 237: a) i. Ac$_2$O, HClO$_4$, 0 °C; ii. EtSH, BF$_3$OEt$_2$, CH$_2$Cl$_2$, 0 °C to RT; iii. NaOMe, MeOH, RT; b) i. TrtCl, pyridine, 80 °C then BzCl, 0 °C to RT; ii. p-TSA, CH$_2$Cl$_2$, MeOH, 239α 51% (five steps), 239β 7% (five steps), 35 g scale; c) FmocCl, pyridine, 0 °C to RT, 87%; d) (BuO)$_2$P(=O)OH, NIS, TfOH, CH$_2$Cl$_2$, 0 °C, 88%.]

Merrifield resin 236 functionalized with the photolabile linker 241 was prepared according to a known procedure (Scheme 63).\textsuperscript{53}

![Scheme 63. Synthesis of functionalized Merrifield resin 236: Reactions and Conditions: a) Merrifield resin, Cs$_2$CO$_3$, TBAI, DMF, 50 °C; b) CsOAc, DMF, 50 °C.]

With mannosyl phosphate 237 and the functionalized Merrifield resin 236 in hand, α-(1,6)-oligomannosides were assembled using an automated oligosaccharide synthesizer (Scheme 64).\textsuperscript{47} Each glycosylation, using three equivalents of mannosyl building block 237 was repeated three times at -15 °C, while trimethylsilyl triflate served as a promoter. Removal of the temporary 9-fluorenylmethyloxycarbonyl protecting group with piperidine freed the C6 hydroxyl group for chain elongation. The coupling efficiency was assessed by UV-Vis measurement of the piperidine-dibenzofulvene adduct released during Fmoc cleavage.\textsuperscript{55,123}
Scheme 64. Automated synthesis of α-(1,6)-polysaccharides: Reactions and conditions: a) Glycosylation: 237, TMSOTf, CH$_2$Cl$_2$, -15 °C (45 min) - 0 °C (15 min); b) Fmoc deprotection: piperidine, DMF, 25 °C (5 min); c) Cleavage from solid support: hv, CH$_2$Cl$_2$; d) i. NaOMe cat., MeOH; ii. Pd/C, H$_2$, H$_2$O.

Initially the automated procedure was applied for accessing short structures, such disaccharide 242 and hexasaccharide 243 that could be easily separated from the deletion sequences by flash chromatography on silica gel. Global deprotection using base to remove the benzoate esters and subsequent hydrogenolysis on Pd/C afforded fully deprotected hexasaccharide 244 in 25% yield over 15 steps (Figure 8).
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

Encouraged by these results, the automated procedure was expanded towards longer α-(1,6)-oligomannosides ranging in length from a 12mer to a 30mer. The purification of polymannosides longer than 12mers (245, 246, 247 and 248) proved more challenging than expected, due to their changing solubility. Since the crude products dissolved only in chlorinated solvents, reverse-phase, normal phase and size exclusion HPLC became very challenging (Figure 9).

Figure 8. Characterization of hexasaccharide 244: A) $^1$H NMR (D$_2$O, 400 MHz) of α-(1,6)-hexamannoside 244; B) $^{13}$C NMR (D$_2$O, 100 MHz) of α-(1,6)-hexamannoside 244.
Figure 9. Challenges in purification of large α-(1,6)-oligomannosides: A) NP-HPLC comparative chromatogram of the crude reaction mixture containing the α-(1,6)-30 mer 248, α-(1,6)-24 mer 247 and α-(1,6)-12 mer 245 (Nucleosil 100-5OH, eluents CH₂Cl₂/MeOH, gradient: 0% MeOH (5 min) → 30% MeOH (in 25 min)); B) MALDI/MS analysis of the 30-mer 248 after isolation of the main compound by NP-HPLC.

4.2.2 Development of a Catch-Release Strategy to Assist Separation and Purification of Oligosaccharides Assembled by Automated Solid Phase Synthesis.

To circumvent the problems encountered for polymannosides purification, a cap-and-tag strategy needed to be developed. Peptides, oligonucleotides and oligosaccharides have been purified using affinity chromatography that relies on labels such as biotin and oligohistidine. This strategy cannot be adopted for the purification of protected oligomannosides since aqueous solvents are required as eluents. Fluorous tags have helped to
separate oligonucleotides up to 100mers\textsuperscript{126} using fluorous solid phase extraction and liquid-liquid extraction.\textsuperscript{127} Solubility issues associated with fluorous labels rendered them useless in the context of the isolation of longer $\alpha$-(1,6)-oligomannosides. Therefore, we considered capture-release techniques (Scheme 65)\textsuperscript{128} that rely on the covalent attachment of the labeled target molecule to a solid support to separate the desired oligosaccharide from any deletion sequences. A capping step was included in the synthesis cycle to block any unreacted hydroxyl groups prior to the subsequent glycosylation reaction. After completing the oligosaccharide sequence, the tag was attached on the hydroxyl group at C6, thus allowing for facile separation of the desired product from the deletion sequences and byproducts.

**Scheme 65.** Schematic overview of a cap-tag strategy: Isolation differs with tag: a) biotin and oligohistidine tags require isolation by affinity column chromatography; b) fluorous tags require isolation by solid-phase extraction or liquid-liquid extraction; c) catch-release techniques separate the target product from byproducts by attachment and disattachment from a solid support.
For a successful catch-release approach to be applied to automated solid phase oligosaccharide synthesis, a fast and efficient capping reaction is required. The caps introduced after each glycosylation have to be stable during subsequent synthetic steps and the tag has to contain a unique handle for facile separation of the product. Based on these considerations, an acetylation step was implemented after each glycosylation to render silent the unreacted hydroxyl groups and 6-amino caproic acid was to be introduced as a tag on the full-length oligosaccharide to “fish” the desired oligosaccharide from the reaction mixture. 6-Amino caproic acid has on its structure two functionalities: the carboxylic acid that could be reacted with the free hydroxyl group of the full length oligosaccharide and the amino function that will allow the isolation of the desired oligosaccharide by attachment on the magnetic beads decorated with carboxylic acid moieties. Once, the oligosaccharide is photocleaved from the Merrifield solid support, the primary amine will allow for selective immobilization of the target compound on magnetic beads having NHS activated carboxylic acids\(^{129}\) on the surface, providing therefore a facile method for the separation of the desired structure. The target structure attached on the magnetic beads will be directly submitted to Zemplén conditions to give the deprotected oligomer.

Preliminary tests required the synthesis of the tagging agents \textbf{249} and \textbf{250}. Protection of the primary amine on the 6-amino caproic acid as an Fmoc-carbamate afforded \textbf{249} in 67% yield that was further converted to the corresponding anhydride \textbf{250} by treatment with \textit{N,N’-}\text{dicyclohexylcarbodiimide} (Scheme 66).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{a} \quad \text{Fmoc} & \quad \text{HN} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{b} \quad \text{Fmoc} & \quad \text{HN} \\
\text{6-amino caproic acid} & \quad \text{249} & \quad \text{Fmoc} & \quad \text{OSuc} \quad \text{NaHCO}_3 \quad \text{water/acetone (1/1, v/v), 67%; b) DCC, CH}_2\text{Cl}_2, \text{quant.}
\end{align*}
\]

Scheme 66. Synthesis of the tagging reagents \textbf{249} and \textbf{250}: a) FmocOSuc, NaHCO\(_3\), water/acetone (1/1, v/v), 67%; b) DCC, CH\(_2\)Cl\(_2\), quant..

To test the introduction of the cap-tag strategy in the automated solid phase synthesis of \textit{\alpha-}\text-(1,6)-oligomannosides, the synthesis of an \textit{\alpha-}\text-(1,6)-dimannoside was approached (Scheme 67). After each glycosylation step, the free hydroxyl groups were blocked by treatment with acetic anhydride in pyridine for one hour at 25 °C. Once the disaccharide was assembled, the attachment of the tag on the molecule was attempted. Treatment of the target disaccharide with anhydride \textbf{250} in presence of pyridine for four hours resulted in only 13% tagging efficiency (Scheme 67, Table, entry 1). A slightly coupling improvement was observed when
the desired disaccharide was reacted with 6-amino hexanoic acid 249 in presence of \(N,N'\)-diisopropylcarbodiimide and 4-(\(N,N\)-dimethyl)aminopyridin (Scheme 67, Table, entry 2). However, this second trial didn’t prove either efficient leading to only 22% coupling efficiency (Table, entry 2). Applying such as inefficient labelling method to the automated solid phase synthesis will result in a lost of considerable quantities of the target oligosaccharide.

Scheme 67. Initial attempts to introduce the tag to an automatically synthesized disaccharide: Reactions and conditions: a) i. Glycosylation: 237, TMSOTf, CH\(2\)Cl\(2\), -15 °C (45 min) - 0 °C (15 min); ii. Capping: Ac\(2\)O, pyridine 25 °C (60 min); iii. Fmoc deprotection: piperidine, DMF, 25 °C (5 min); b) Different conditions (see Table).

To circumvent previously mentioned problems and based on our experience with glycosylation reactions performed by automated solid phase synthesis, a glycosyl based labeling building block was synthesized. Therefore, building block 251 was prepared as a label for the synthesis starting from carboxylic acid 249 and primary alcohol 239 (Scheme 68). Following esterification in presence of \(N,N'\)-diisopropylcarbodiimide and pyridine, thiomannoside 252 was converted to the phosphate glycosylating agent 251 in 51% yield.
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Scheme 68. Synthesis of glycosyl based tagging agent 251: a) 249, DIC, pyridine, CH₂Cl₂, 0 °C to RT, 73%; b) (BuO₂)₂P(=O)OH, NIS, TfOH, CH₂Cl₂, 0 °C, 3 h, 51%.

The glycosyl based tagging agent 251 was evaluated in the context of the automated synthesis of the α-(1,6)-dimannoside 253 (Scheme 69). Following completion of the automated synthesis, building block 251, equipped with an amino caproic ester at the C6 position was used. The disaccharide product 256 was cleaved from the resin in continuous flow by exposure to UV light before the crude product mixture was reacted with magnetic beads functionalized with NHS-activated carboxylic acid. The unique amine group on the disaccharide resulted in covalent attachment to the beads before CH₂Cl₂ and methanol washes. Treatment of the magnetic beads with sodium methoxide in methanol released deprotected disaccharide 257 that was purified by gel filtration chromatography to afford dimer 257 in 22% yield over nine steps (Figure 10). Cleavage of the benzyl carbamate by hydrogenation provided target disaccharide 253 in 74% yield.
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Scheme 69. Catch-release purification applied to the automated solid phase synthesis of α-(1,6)-dimannoside 253. Reactions and conditions: a) Glycosylation: 237 or 251, TMSOTf, CH₂Cl₂, -15 °C (45 min) - 0 °C (15 min); b) Capping: Ac₂O, pyridine 25 °C (60 min); c) Fmoc deprotection: piperidine, DMF, 25 °C (5 min); d) Cleavage from solid support: hv, CH₂Cl₂; e) Immobilization on magnetic beads: i. Et₃N, CH₂Cl₂, 25 °C, 12 h; ii. Washing with CH₂Cl₂ to remove deletion sequences of the type 254 and 255; f) Release from magnetic beads: NaOMe, MeOH, 12 h; g) Pd/C, H₂, H₂O, 12 h.
To improve the coupling efficiency of the target saccharide to the activated magnetic beads, and thus the overall yield, a second coupling step was introduced. Any oligosaccharides remaining after the first coupling were reacted with the magnetic beads from the first “release” in the presence of benzotriazol-1-yl-oxytrypyrrolidinophosphonium hexafluorophosphate and N,N-diisopropylethylamine (Scheme 70).

**Figure 10.** Characterization of disaccharide 257: A) $^1$H NMR (D$_2$O, 400 MHz) of $\alpha$-(1,6)-dimannoside 257; B) $^{13}$C NMR (D$_2$O, 100 MHz) of $\alpha$-(1,6)-dimannoside 257.
Scheme 70. Catch-release purification applied to α-(1,6)-oligomannosides 258 and 259 prepared by automated solid phase assembly. Reactions and conditions: a) Automated synthesis; b) Immobilization on magnetic beads: i. Et₃N, CH₂Cl₂, 25 °C, 12 h; ii. Remove deletion sequences by CH₂Cl₂ wash; c) Release from magnetic beads: NaOMe, MeOH, 12 h; d) Magnetic beads recycling: 258, 259, PyBOP, DIPEA, CH₂Cl₂, 25 °C, 12 h; e) Pd/C, H₂, H₂O.

Implementing the cap-and-tag combined with catch-release techniques in the automated assembly of a medium large α-(1,6)-dodecamannoside provided, after gel filtration chromatography, deprotected 12mer 260 in 13% overall yield without the need of time consuming HPLC purification (Figure 11). Removal of the Cbz protecting group by hydrogenolysis on Pd/C in water provided α-(1,6)-dodecamannoside 262 in 62% yield.
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Figure 11. Characterization of dodecamannoside 260: A) $^1$H NMR (D$_2$O, 600 MHz) and $^{13}$C NMR (D$_2$O, 150 MHz) of $\alpha$-(1,6)-dodecamanoside 260; B) MALDI-MS analysis of the $\alpha$-(1,6)-dodecamanoside 260.

The automated synthesis of an $\alpha$-(1,6)-oligomannoside 30mer presented the next challenge as this polysaccharide is by far the largest carbohydrate ever made by automation and amongst the largest ever synthesized by any method. Executing the method described above, the crude
protected 30mer 261 was obtained in less than one week. Analysis of the crude product by MALDI mass spectroscopy confirmed the presence of the target compound. Catch-release purification separated the deprotected 30mer 261 from any deletion sequences, providing after gel filtration chromatography the pure 30mer 261 in 1% overall yield (96% per step). The identity of the product was confirmed with the help of NMR and MALDI mass spectroscopy (Figure 12).

**Figure 12.** Characterization of polymannoside 261. A) HSQC NMR (D₂O, 600MHz) of α-(1,6)-30mer 261; B) MALDI-MS analysis of the α-(1,6)-30mer 261.
4.3 Conclusion

In conclusion, this chapter describes the automated solid-phase assembly of the largest synthetic glycan ever made. To streamline the purification of the product, a catch-release method combining the cap-tag and magnetic-beads catch-release technique was developed. Rapid access to synthetic polysaccharides of lengths previously not accessible has now become possible. Applications of these defined polysaccharide products as biological probes and even for the construction of novel materials are currently being pursued.

4.4 Experimental Section

4.4.1 General Experimental Details

All chemicals used were reagent grade and used as supplied, unless noted otherwise. Molecular sieves were activated prior to use by heating and drying under high vacuum. All reactions were performed in oven-dried glassware under an argon atmosphere, unless noted otherwise. N,N-Dimethylformamide (DMF), dichloromethane (CH$_2$Cl$_2$), toluene and tetrahydrofuran (THF) were purified by a Cycle-Tainer Solvent Delivery System, unless noted otherwise. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). TLC plates were visualized by UV irradiation or by dipping the plate in a cerium sulfate ammonium molybdate (CAM) solution or in a 1:1 mixture of H$_2$SO$_4$ (2 N) and resorcinol monomethyl ether (0.2%) in ethanol. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). All automated glycosylations were performed on an automated oligosaccharide synthesizer prototype with either anhydrous solvents of the Cycle-Tainer Solvent Delivery System or solvents (Acros Organics: AcroSeal Dry Solvents). LCMS chromatograms were recorded on an Agilent 1100 Series spectrometer. Preparative HPLC purifications were performed on an Agilent 1200 Series. Loading determination of functionalized resins was obtained using a Shimadzu UV-MINI-1240 UV spectrometer. $^1$H, $^{13}$C spectra were recorded on a Varian Mercury 400 (400 MHz) or 600 (600 MHz) spectrometer in CDCl$_3$ or CD$_3$OD with chemical shifts referenced to internal standards (CDCl$_3$: $^1$H 7.26 ppm, $^{13}$C 77.0 ppm; D$_2$O with acetone as internal standard: $^1$H 2.05 ppm, $^{13}$C 29.84 or 206.260 ppm) unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet for $^1$H-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 in the Department of Organic Chemistry at Free University Berlin using a Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a UniPol L 1000 polarimeter (Schmidt & Haensch, Berlin, Germany), with concentrations expressed in g/100 mL.

4.4.2 Solid Support Functionalization

Merrifield resin (6.40 g, loading: 0.74 mmol/g, 4.74 mmol) was swollen overnight in CH\(_2\)Cl\(_2\) in a flask while being gently shaken on a rotavap. A solution of phenol 241 (2.2 g, 5.67 mmol) in CH\(_2\)Cl\(_2\) was added to the resin. DMF (60 mL) was added to the solution followed by Cs\(_2\)CO\(_3\) (2.31 g, 7.095 mmol) and TBAI (2.62 g, 7.095 mmol). The solution was stirred overnight on the rotavap at 60 °C and 200 mbar. The resin was washed successively with DMF/water (1/1), DMF, MeOH, CH\(_2\)Cl\(_2\), MeOH, CH\(_2\)Cl\(_2\) (six times each) and then swollen in CH\(_2\)Cl\(_2\) for 1 h. The swollen resin was placed in a flask with DMF (60 mL) and CsOAc (1.81 g, 9.47 mmol). The suspension was stirred overnight on a rotavap at 60 °C and 200 mbar. The resin was then washed successively with DMF/water (1/1), DMF, MeOH, CH\(_2\)Cl\(_2\), MeOH, CH\(_2\)Cl\(_2\) (six times each), the solvent was drained, and the resin was dried under vacuum.

**Loading Determination**

Dry resin 236 (51 mg, theoretical loading: 0.74 mmol/g, 0.037 mmol) was placed in a syringe (5 mL) equipped with a frit. CH\(_2\)Cl\(_2\) (2.3 mL) was added to swell the resin for 1 h. The CH\(_2\)Cl\(_2\) was drained and a solution of FmocCl (100 mg, 0.39 mmol) and pyridine (0.1 mL, 1.24 mmol) in CH\(_2\)Cl\(_2\) (1 mL) was added to the resin. The reaction mixture was shaken for 6 h, the solution was drained and the resin was washed with CH\(_2\)Cl\(_2\), MeOH and CH\(_2\)Cl\(_2\) (six times each). A 2% (v/v) solution of DBU in DMF (2 mL) was added and the resin was shaken for 1 h. The solution was drained in a vial. An aliquot of this solution (160 µL) was diluted with acetonitrile to a total volume of 10 mL and the UV absorption of this solution was measured at 294 and 304 nm. The loading of the resin was calculated as an average of the values resulting
from the formulas: Absorption_{304 \text{ nm}} \times 16.4 \text{/mass of resin used in mg (for 304 nm)} and Absorption_{294 \text{ nm}} \times 14.2 \text{/mass of resin used in mg (for 294 nm)}. Loading of 236: 0.47 mmol/g.

4.4.3 Synthesis of Aminohexanoic Acid Derivatives 249 and 250

\[ \text{6-aminocapric acid} \xrightarrow{\text{FmocOSuc, NaHCO}_3, \text{H}_2\text{O/acetone (7/1, v/v)}} \text{Fmoc-NH-249} \]

\text{N-Fluorenylmethoxycarbonyl-6-aminohexanoic acid (249)}: NaHCO\textsubscript{3} (3.3 g, 39.6 mmol) was added to a suspension of caproic acid (4.0 g, 30.5 mmol) and FmocOSuc (10.3 g, 30.5 mmol) in a mixture H\textsubscript{2}O/acetone (150 mL, 1/1, v/v). The mixture was stirred at RT for 12 h, cooled to 0 °C and quenched with 1 M aq. solution of HCl. After extraction with AcOEt, the organic layer was dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The white residue was suspended in water and treated with triethylamine until complete solubilization. The mixture was extracted with Et\textsubscript{2}O and a mixture of Et\textsubscript{2}O/hexanes (1/1, v/v). The pH of the aqueous layer was adjusted to 1 by adding 1 M aq. solution of HCl and the solution was extracted with AcOEt. The combined organic layers were washed with brine, dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo} to give Fmoc-protected aminohexanoic acid 249 (7.2 g, 20.3 mmol, 67%) that was used without further purification in the next step. The analytical data were in agreement with those reported in the literature.\textsuperscript{131}

\[ \text{Fmoc-NH-249} \xrightarrow{\text{DCC, CH}_2\text{Cl}_2, 25 \degree \text{C}} \text{Fmoc-NH-250} \]

\text{N-Fluorenylmethoxycarbonyl-6-aminohexanoyl anhydride (250)}: A solution of Fmoc-protected 6-aminocapric acid 249 (0.72 g, 2.04 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (21 mL) was treated at 0 °C with DIC (0.21 g, 1.02 mmol, 0.5 equiv). The mixture was stirred at 0 °C for 1 h, then concentrated \textit{in vacuo} to give the target anhydride 250 as a white solid that was used directly as tagging agent.
4.4.4 Synthesis of Mannose Building Blocks 237 and 251

Ethyl 2,3,4-tri-O-benzoyl-1-thio-α/β-D-mannosylpyranoside (239): Acetic anhydride (100 mL, 1.06 mol) was cooled to 0 °C and perchloric acid (0.38 mL, 4.44 mmol) was added. Mannose (20.0 g, 111 mmol) was added portionwise such that the temperature of the reaction solution did not exceed 30 °C. The reaction was stirred at 0 °C and after complete conversion of the starting material (tlc: cyclo-hexane/AcOEt, 1.5:1) the solution was poured into ice. After phase separation, the organic layer was extracted with a saturated aq. solution of NaHCO₃ and dried over MgSO₄. The solvent was removed in vacuo and the crude product was used without further purification for the next step.

The per-acetylated mannose was co-evaporated three times with toluene and dissolved in CH₂Cl₂ (360 ml). After addition of ethanethiol (12.3 mL, 167 mmol), the solution was cooled to 0 °C and boron trifluoride diethyl etherate (42.2 mL, 333 mmol) was dropwise added. The solution was allowed to warm to RT and after complete conversion of the starting material (tlc: cyclo-hexane/AcOEt, 1.5:1) was diluted with CH₂Cl₂, extracted with a saturated aq. solution of NaHCO₃ and dried over MgSO₄. The solvent was removed in vacuo and the crude product was used without further purification for the next step. The thioglycoside was suspended in MeOH (220 mL) and sodium methoxide (1.8 g, 33.3 mmol) was added. The solution was stirred at RT and after complete conversion (tlc: CH₂Cl₂/MeOH, 95:5), it was neutralized with Amberlite-120 H⁺ ion exchange resin. The resin was filtered off and the solvent was removed in vacuo. The crude product was used without further purification for the next step.

The crude thioglycoside was co-evaporated three times with toluene and dissolved in pyridine (220 mL, 2.72 mol). The solution was cooled to 0 °C and trityl chloride (46.4 g, 167 mmol) was added. The reaction was stirred at 80 °C overnight. After complete conversion of the starting material (tlc: CH₂Cl₂/MeOH, 95:5) the solution was cooled to 0 °C and benzoyl chloride (58.0 mL, 500 mmol) was added. The reaction was allowed to warm to RT. After complete conversion (tlc: cyclo-hexane/AcOEt, 2:1), all volatiles were removed in vacuo and
the remainder was dissolved in CH₂Cl₂. The solution was extracted with 1 M aq. solution of HCl and a saturated aq. solution of NaHCO₃, dried over MgSO₄ and the solvent was removed in vacuo. The crude product was used without further purification for the next step. Trityl protected mannose was dissolved in a mixture MeOH/CH₂Cl₂ (700 mL, 2/1, v/v). p-TSA (4.60 g, 24.18 mmol) was added and the reaction was stirred at RT. After complete conversion (tlc: cyclo-hexane/ACOEt, 2:1), Et₃N (4.6 ml, 33.3 mmol) was added and the volatiles were removed in vacuo. The remainder was dissolved in CH₂Cl₂, washed with brine, dried over MgSO₄ and the solvent was removed in vacuo. The crude product was purified by flash column chromatography on silica gel (Hexanes/ACOEt = 6:1 → 4:1 → 2:1) affording the primary alcohol (239α: 30.5 g, 51% over five steps; 239β: 4.3 g, 7% over five steps). Compound 239α: Rₜ (cyclo-hexane/ACOEt = 2:1) = 0.32; [α]D₂²² = −83.6 (c = 0.56, chloroform); ¹H NMR (400 MHz, CDCl₃): δ 8.11 (dd, J = 8.0, 1.2 Hz, 2H), 8.03 – 7.96 (m, 2H), 7.86 – 7.79 (m, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.56 – 7.47 (m, 3H), 7.45– 7.38 (m, 3H), 7.31 – 7.24 (m, 2H), 5.96 – 5.83 (m, 2H), 5.78 (dd, J = 2.8, 1.6 Hz, 1H), 5.58 (d, J = 1.2 Hz, 1H), 4.47– 4.41 (m, 1H), 3.87 – 3.76 (m, 2H), 2.83 – 2.66 (m, 2H), 2.61 (t, J = 6.0 Hz, 1H), 1.37 (t, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 166.7, 165.6, 165.5, 133.8, 133.7, 133.3, 130.1 (2C), 130.0 (2C), 129.8 (2C), 129.4, 129.1, 128.82, 128.8 (2C), 128.6 (2C), 128.4 (2C), 82.5, 72.5, 71.6, 70.2, 67.6, 61.5, 25.8, 14.9; IR (thin film) 3528, 2964, 2928, 1725, 1601, 1451, 1281, 1261, 1093, 1070, 1027, 708 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)+ C₂₀H₂₈O₆NaS: 559.1403, found 559.1393.

**Ethyl 2,3,4-tri-O-benzoyl-6-O-fluorenylmethoxycarbonyl-1-thio-α-D-mannosylpyranoside (239):** Mannose 239α (30.4 g, 56.7 mmol) was dissolved in CH₂Cl₂ (567 mL) and 9-fluorenylmethyl chloroformate (22.0 g, 85 mmol) and pyridine (9.2 mL, 113 mmol) were added. The reaction was stirred at RT overnight and after complete conversion of the starting material (tlc: cyclo-hexane/ACOEt, 2:1) the solution was diluted with CH₂Cl₂ and extracted with 1 M aq. solution of HCl and a saturated aq. solution of NaHCO₃. The organic phase was dried over MgSO₄ and the solvent was removed in vacuo. The crude product was purified by silica gel flash column chromatography (Hexanes/ACOEt = 6:1 → 4:1 → 2:1) affording the Fmoc-protected mannose 240 (37.4 g, 49.3 mmol, 87%): Rₜ (cyclo-
hexane/AcOEt = 2:1) = 0.70; [α]_D$^{22}$ = −46.9 (c = 0.46, chloroform); $^1$H NMR (400 MHz, CDCl$_3$): δ 8.17 – 8.09 (m, 2H), 7.99 – 7.97 (m, 2H), 7.83 – 7.81 (m, 2H), 7.76 (d, $J = 7.2$ Hz, 2H), 7.53 – 7.36 (m, 9H), 7.30 – 7.24 (m, 6H), 5.97 (dd, $J = 10.0$, 10.0 Hz, 1H), 5.83 (dd, $J = 3.6$, 10.0 Hz, 1H), 5.79 (dd, $J = 1.6$, 3.6 Hz, 1H), 5.59 (d, $J = 1.6$ Hz, 1H), 4.80 – 4.76 (m, 1H), 4.51 (dd, $J = 12.0$, 6.0 Hz, 1H), 4.44 – 4.38 (m, 2H), 4.32 (dd, $J = 10.4$, 7.6 Hz, 1H), 4.23 (t, $J = 7.6$ Hz, 1H), 2.76 (m, 2H), 1.37 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 165.7, 165.6, 165.5, 155.1, 143.5, 143.4, 141.4, 141.4, 133.7, 133.6, 133.4, 130.08 (2C), 130.01 (2C), 129.8 (2C), 129.4, 129.09, 129.04, 128.8 (2C), 128.6 (2C), 128.4 (2C), 128.04, 128.01, 127.33, 127.31, 125.4, 125.3, 120.18, 120.16, 82.4, 72.2, 70.5, 70.3, 69.2, 67.4, 66.5, 46.8, 25.8, 15.0; IR (thin film) 3064, 2964, 2930, 1729, 1281, 1260, 1158, 1094, 1069, 709 cm$^{-1}$; HRMS(ESI) m/z calc'd for (M+Na)$^+$ C$_{44}$H$_{38}$O$_{10}$NaS: 781.2083, found 781.2031.

2,3,4-Tri-O-benzoyl-6-O-fluorenlymethoxycarbonyl-α-D-mannopyranosylphosphate (237): Thioglycoside 240 (2.0 g, 2.6 mmol) was co-evaporated twice with toluene. The remainder, freshly activated molecular sieves 4Å (4 g) and NIS (0.71 g, 3.2 mmol) were suspended in CH$_2$Cl$_2$ (26 mL) under an Ar atmosphere and the solution was cooled to 0 °C. Dibutyl hydrogen phosphate (1.6 mL, 7.9 mmol) and triflic acid (23 µL, 0.2 mmol) were added and the reaction was stirred at 0 °C for 3 h. After complete conversion of the starting material (tlc: Hexanes/AcOEt, 70:30) the solution was diluted with chloroform and extracted with 10% aq. solution of Na$_2$S$_2$O$_3$ and a saturated aq. solution of NaHCO$_3$. The organic phase was dried over MgSO$_4$ and the solvent was removed in vacuo. The crude product was purified by silica gel flash column chromatography (Hexanes/AcOEt, 1:0 to 4:1) affording phosphate 237 (2.1 g, 2.3 mmol, 88%). $R_t$ (Hexanes/AcOEt = 70:30) = 0.27; [α]_D$^{22}$ = −64.3 (c = 0.5, chloroform); $^1$H NMR (400 MHz, CDCl$_3$): δ 8.13 – 8.11 (m, 2H), 7.99 – 7.96 (m, 2H), 7.83 (dd, $J = 8.0$, 0.8 Hz, 2H), 7.76 (d, $J = 7.6$ Hz, 2H), 7.61 – 7.55 (m, 3H), 7.53 – 7.49 (m, 1H), 7.47 – 7.36 (m, 7H), 7.32 – 7.23 (m, 4H), 6.03 (t, $J = 10.0$ Hz, 1H), 5.97 – 5.91 (m, 2H), 5.79 – 5.78 (m, 1H), 4.62 – 4.57 (m, 1H), 4.50 – 4.39 (m, 3H), 4.33 (dd, $J = 10.0$, 7.6 Hz, 1H), 4.26 – 4.17 (m, 5H), 1.81 – 1.68 (m, 4H), 1.46 (m, 4H), 1.10 – 0.84 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 165.5, 165.4, 165.2, 155.0, 143.5, 143.3, 141.4, 141.3, 133.8, 133.7, 133.4, 130.1 (3C), 130.0 (2C), 129.9 (2C), 129.0, 129.0, 128.9, 128.8 (3C), 128.6 (2C), 128.4 (2C), 128.0,
128.0, 127.3, 127.3, 125.4, 125.3, 120.1, 120.1, 95.0 (d, \( J = 6.1 \) Hz, 1C), 70.5, 70.4, 69.8 (d, \( J = 10.1 \) Hz, 1C), 69.2, 68.5 (d, \( J = 5.1 \) Hz, 1C), 68.4 (d, \( J = 5.1 \) Hz, 1C), 66.4, 66.1, 46.8, 32.4 (d, \( J = 4.0 \) Hz, 1C), 32.3 (d, \( J = 3.0 \) Hz, 1C), 18.8, 13.7; IR (thin film) 2961, 2933, 2874, 1730, 1256, 1162, 1093, 1069, 1025, 990, 963, 709 cm\(^{-1}\); HRMS(ESI) m/z calcd for (M+Na\(^+\))\(\text{C}_{50}\text{H}_{51}\text{O}_{14}\text{NaP}: 929.2914, \) found 929.2911.

**Ethyl 2,3,4-tri-O-benzoyl-6-O-(N-fluorenymethoxycarbonyl-6-aminohexanoyl)-1-thio-\(\beta\)-d-mannosylpyranoside** (252): To a solution of thioglycoside 239\(\beta\) (3.3 g, 6.15 mmol) in \(\text{CH}_2\text{Cl}_2\) (512 mL), hexanoic acid 249 (6.45 g, 18.26 mmol), pyridine (26.5 mL, 328 mmol) and 4-(\(N,\text{N}\)-dimethyl)aminopyridine (226 mg, 1.85 mmol) were added. The solution was cooled to 0 °C and diisopropylcarbodiimide (2.85 mL, 18.26 mmol) was added. The solution was allowed to warm to RT and after complete conversion of the starting material (tlc: cyclohexane/AcOEt, 1:1), it was diluted with \(\text{CH}_2\text{Cl}_2\) and washed with 1 m aq. solution of HCl and a saturated aq. solution of NaHCO\(_3\). The organic layer was dried over MgSO\(_4\) and the solvent was removed in vacuo. The crude product was purified by silica gel flash column chromatography (Hexanes/AcOEt = 4:1→3:1→2:1→1.5:1) affording aminohexanoic ester 252 (3.8 g, 4.49 mmol, 73%). \(R_f = 0.59\) (cyclohexane/AcOEt = 50:50); [\(\alpha\)]\(^{20}\)_D = −86.3 (c = 0.48, dichloromethane); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.09 (dd, \( J = 8.3, 1.3 \) Hz, 2H), 7.92 (dd, \( J = 8.4, 1.3 \) Hz, 2H), 7.82 – 7.73 (m, 4H), 7.62 – 7.55 (m, 2H), 7.52 – 7.18 (m, 13H), 5.99 (dd, \( J = 3.4, 0.8 \) Hz, 1H), 5.85 (t, \( J = 10.0 \) Hz, 1H), 5.63 (dd, \( J = 10.1, 3.5 \) Hz, 1H), 5.06 (d, \( J = 0.9 \) Hz, 1H), 4.83 (t, \( J = 5.7 \) Hz, 1H), 4.46 – 4.28 (m, 4H), 4.20 (t, \( J = 6.8 \) Hz, 1H), 4.04 (ddd, \( J = 9.7, 5.3, 3.1 \) Hz, 1H), 3.23 – 3.11 (m, 2H), 2.78 (q, \( J = 7.5 \) Hz, 2H), 2.32 (t, \( J = 7.0 \) Hz, 2H), 1.69 – 1.57 (m, 2H), 1.54 – 1.42 (m, 2H), 1.42 – 1.21 (m, 5H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 173.2, 165.7, 165.6, 165.5, 156.5, 144.1 (2C), 141.4, 133.7, 133.6, 133.4, 130.2 (2C), 129.9 (2C), 129.8 (2C), 129.3, 128.9, 128.9, 128.6 (2C), 128.6 (2C), 128.4 (2C), 127.7 (2C), 127.1 (3C), 125.1, 120.08 (3C), 83.1, 76.6, 72.8, 71.5, 67.1, 66.6, 63.2, 47.4, 40.9, 33.9, 29.7, 26.3, 26.1, 24.4, 15.1; IR (thin film) 3395, 2935, 1723, 1601, 1585, 1521, 1451, 1315, 1247, 1177, 1091, 1068, 1026, 802, 708 cm\(^{-1}\); HRMS(ESI) m/z calcd for (M+Na\(^+\))\(\text{C}_{50}\text{H}_{49}\text{NO}_{13}\text{NaS}: 894.2924, \) found 894.2912.
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

2,3,4-Tri-O-benzoyl-6-O-(N-fluorenylmethoxycarbonyl-6-aminohexanoyl)-α-D-mannopyranosylphosphate (251): Thioglycoside 252 (1.0 g, 1.2 mmol) was co-evaporated twice with toluene. The remainder and NIS (310 mg, 1.38 mmol) were dissolved in CH₂Cl₂ (12 mL) under Ar atmosphere and the solution was cooled to 0 °C. Dibutyl hydrogen phosphate (0.7 mL, 3.5 mmol) and triflic acid (10 µL, 0.1 mmol) were added and the reaction was stirred at 0 °C for 3 h. After complete conversion of the starting material (tlc: Hexanes/AcOEt, 70:30), the solution was diluted with CH₂Cl₂ and washed with 10% aq. solution of Na₂S₂O₃ and a saturated aq. solution of NaHCO₃. The organic layer was dried over MgSO₄ and the solvent was removed in vacuo. The crude product was purified by flash column chromatography on silica gel (Hexanes/AcOEt = 100:0→50:50) affording the phosphate 251 (0.6 g, 0.6 mmol, 51%). R₆(Hexanes/AcOEt = 50:50) = 0.32; [α]D₂₀ = –60.7 (c = 0.38, dichloromethane); H NMR (400 MHz, CDCl₃): δ 8.11 – 8.05 (m, 2H), 7.96 – 7.94 (m, 2H), 7.83 – 7.81 (m, 2H), 7.75 (d, J = 7.6 Hz, 2H), 7.64 – 7.55 (m, 3H), 7.54 – 7.34 (m, 8H), 7.32 – 7.23 (m, 4H), 6.01 (t, J = 10.2 Hz, 1H), 5.92 – 5.88 (m, 2H), 5.75 (dd, J = 2.2, 3.3 Hz, 1H), 4.93 (t, J = 5.6 Hz, 1H), 4.57 – 4.48 (m, 1H), 4.41 – 4.35 (m, 3H), 4.30 (dd, J = 12.3, 2.8 Hz, 1H), 4.25 – 4.14 (m, 5H), 3.27 – 3.08 (m, 2H), 2.44 – 2.25 (m, 2H), 1.80 – 1.70 (m, 4H), 1.70 – 1.61 (m, 2H), 1.53 – 1.41 (m, 6H), 1.38 – 1.29 (m, 2H), 0.98 (t, J = 7.4 Hz, 6H); C NMR (100 MHz, CDCl₃): δ 173.1, 165.5, 165.4, 165.1, 156.5, 156.1, 144.1, 141.4, 133.9, 133.7, 133.4, 130.0 (3C), 129.9 (2C), 129.8 (2C), 129.1, 129.0, 128.9, 128.7 (2C), 128.6 (2C), 128.4 (2C), 127.7 (2C), 127.1 (3C), 125.1, 120.0 (3C), 95.09 (d, J = 5.4 Hz), 70.4, 69.8 (d, J = 10.7 Hz), 69.3, 68.5 (d, J = 5.9 Hz), 68.4 (d, J = 5.9 Hz), 66.6, 66.4, 62.3, 47.4, 40.9, 33.9, 32.4 (d, J = 4.5 Hz), 32.4 (d, J = 4.7 Hz), 29.7, 26.3, 24.5, 18.8 (2C), 13.75, 13.74; IR (thin film) 3391, 3067, 2934, 2872, 1725, 1451, 1315, 1247, 1177, 1157, 1092, 1069, 1025, 1001, 962, 741, 708, 686 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₅₆H₆₂NO₁₅NaP: 1042.3755, found 1042.3737.
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

4.4.5 Automated Synthesis

Preparation of the Resin and the Synthesizer for Automated Synthesis: The functionalized resin was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH\textsubscript{2}Cl\textsubscript{2}. To start the synthetic sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH\textsubscript{2}Cl\textsubscript{2} and CH\textsubscript{2}Cl\textsubscript{2} (six times each with 2 mL for 25 s). The building blocks were co-evaporated three times with toluene, dissolved in CH\textsubscript{2}Cl\textsubscript{2} under an Ar atmosphere and transferred into the vials that were placed on the corresponding port in the synthesizer. Reagents were dissolved in the corresponding solvents under an Ar atmosphere in bottles that were placed on the corresponding port in the synthesizer.

Module 1: Phosphate Glycosylation: The resin was swollen in 2 mL CH\textsubscript{2}Cl\textsubscript{2} and the temperature of the reaction vessel was adjusted to -15 °C. For glycosylations, the CH\textsubscript{2}Cl\textsubscript{2} was drained and a solution of phosphate building block (3 equiv in 1.0 mL CH\textsubscript{2}Cl\textsubscript{2}) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by the addition of TMSOTf in CH\textsubscript{2}Cl\textsubscript{2} (3 equiv in 1.0 mL CH\textsubscript{2}Cl\textsubscript{2}). The glycosylation was performed for 45 min at -15 °C and for 15 min at 0 °C. After the reaction was completed, the solution was drained to the fraction collector and the resin was washed with 0.2 M acetic acid in CH\textsubscript{2}Cl\textsubscript{2} and CH\textsubscript{2}Cl\textsubscript{2} (six times each with 2 mL for 25 s). This procedure was repeated two more times. After the third glycosylation, no acetic acid wash was performed. To recover the building blocks, the glycosylation solutions were pooled, diluted with CH\textsubscript{2}Cl\textsubscript{2} and extracted with saturated aqueous NaHCO\textsubscript{3}. The organic phase was dried over MgSO\textsubscript{4} and the solvent was removed \textit{in vacuo}.

Module 2: Capping - Acetylation of Free Hydroxyl Groups: After the glycosylation cycle, the resin is washed with pyridine (six times each with 2 mL for 25 s), swollen in 2 mL pyridine and the temperature of the reaction vessel is adjusted to 25 °C. The reaction is started by addition of 1 mL of acetic anhydride to the reaction vessel. After 60 min the reaction solution is drained and the resin is washed with pyridine (six times with 2 mL for 25 s).

Module 3: Fmoc Deprotection: The resin is washed with CH\textsubscript{2}Cl\textsubscript{2} and DMF (six times each with 2 mL for 25 s), swollen in 2 mL DMF and the temperature of the reaction vessel is adjusted to 25 °C. For Fmoc deprotection, the DMF is drained and 2 mL of a solution of 20% piperidine in DMF is delivered to the reaction vessel. After 5 min the reaction solution is
collected in the fraction collector of the oligosaccharide synthesizer and 2 mL of a solution of 20% piperidine in DMF is delivered to the resin. This procedure is repeated for a third time. For the next glycosylation, the resin is washed with DMF (six times with 3 mL for 25 s), THF, 0.2 M acetic acid in CH₂Cl₂ and CH₂Cl₂ (six times each with 2 mL for 25 s). For Fmoc quantification the reaction solutions are combined and a 50 µL aliquot is taken. This aliquot is diluted to 5 mL and the UV absorption at λ = 301 nm is determined. The coupling efficiency is calculated according to the following formula:

\[
\text{coupling efficiency [%]} = \frac{\text{amount of deprotected Fmoc [mmol]}}{\text{amount of reactive sites on resin [mmol]}} \times 100
\]

\[
= \frac{A (301 \text{ nm}) \times \text{volume of aliquot [µL]} \times \text{volume of Fmoc solution [mL]}}{\text{extinction coefficient} \times \text{amount of resin [g]} \times \text{amount of reactive sites on resin [mmol]}} \times 100
\]

4.4.6. Cleavage from Solid Support

At the end of the run, the resin is washed with CH₂Cl₂ (six times with 2 mL for 25 s), swollen in 2 mL CH₂Cl₂ and transferred into a disposable syringe (2 mL). To prepare the photoreactor (Figure 3) the fluorinated-ethylene-propylene (FEP) tubing is washed with 15 mL MeOH and 15 mL CH₂Cl₂ subsequently using a flow rate of 4 mL×min⁻¹. For the cleavage, the resin is slowly injected from the disposable syringe (2 mL) into the reactor and pushed through the tubing with 15 mL CH₂Cl₂ (flow rate: 300 µL×min⁻¹). In order to shrink and wash out the remaining resin, the tubing is washed with 15 mL CH₂Cl₂.MeOH (1/1; flow rate: 300 µL×min⁻¹ for 8 mL and 4 mL×min⁻¹ for 7 mL) and finally with 15 mL MeOH (flow rate: 4 mL×min⁻¹). The suspension is led into a filter where the resin is filtered off and washed with CH₂Cl₂/MeOH (1/1, v/v), MeOH and CH₂Cl₂. The tubing is re-equilibrated with 15 mL CH₂Cl₂ using a flow rate of 4 mL×min⁻¹. The entire procedure is performed twice and the resulting solution is evaporated in vacuo.

4.4.7 Coupling to Magnetic Beads and Deprotection for Purification

The coupling reaction and the Zemplén deprotection were performed twice. The magnetic beads were reused after cleavage and deprotection of the target compound by Zemplén reaction for an additional immobilization reaction (Scheme 70).
Prior to the first coupling step, the magnetic beads (loading ca. 0.35 – 0.55 mmol/g) were suspended in CH$_2$Cl$_2$ (5 mL), vigorously shaken and magnetically separated from the solvent. After draining the supernatant, the oligomannoside solution was added to the beads. The coupling reaction was started by adding six equivalents of triethylamine and the suspension was shaken at RT. After 12 h, the beads were washed with CH$_2$Cl$_2$ and MeOH (five times, 5 mL each). The washing solutions were combined and the solvent was removed in vacuo. The remainder was used for an additional coupling reaction.

For deprotection and cleavage, the beads were suspended in MeOH/water (6 mL, 1/1, v/v) and the pH was adjusted to 12 by adding sodium methoxide. The reaction was shaken overnight at RT. The solution was magnetically separated from the beads. The magnetic particles were washed with MeOH and water until the pH of the washing solution was neutral. The combined solutions were neutralized with Amberlite-120 H$^+$ ion exchange resin and the solvent was removed in vacuo. The remainder was dissolved in water and extracted with CH$_2$Cl$_2$. After phase separation, the aqueous phase was concentrated by lyophilization to afford the oligomannosides.

For the second immobilization, the protected oligomannosides were dissolved in anhydrous CH$_2$Cl$_2$ (5 mL). The magnetic beads were washed with CH$_2$Cl$_2$ (five times, 5 mL), the solvent was drained and the oligosaccharide solution, PyBOP (5 equiv) and DIPEA (5 equiv) were added. After shaking the suspension for 12 h at RT, the beads were magnetically separated and washed with CH$_2$Cl$_2$ and MeOH (five times each with 5 mL).

The second cleavage and deprotection reaction employing the Zemplén reaction was performed as described above.

### 4.4.8 Synthesis of α-(1,6)-Mannosyl Oligosaccharides

#### Synthesis Without Catch-Release Strategy

\[
N\text{-}(\text{Benzyl)benzyloxy carbonyl-5-amino-pentanyl})(6-O\text{-(6-aminohexanoyl)-(2,3,4-tri-O-benzoyl-\(\alpha\)-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-\(\alpha\)-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-\(\alpha\)-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-\(\alpha\)-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-\(\alpha\)-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-\(\alpha\)-d-mannopyranosyl)-(1→6)-2,3,4-tri-O-}
\]
**4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside**

**benzoyl-α-d-mannopyranoside (hexamer) (243):** Functionalized resin **236** (50 mg, loading 0.47 mmol/g, 0.0235 mmol) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH$_2$Cl$_2$. To start the synthetic sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH$_2$Cl$_2$ and CH$_2$Cl$_2$ (six times each with 2 mL for 25 s). For the assembly of the oligosaccharide chain, module 1 for phosphate glycosylation with building block **237** and module 3 for Fmoc deprotection were alternatingly performed.

<table>
<thead>
<tr>
<th>glycosylation</th>
<th>building block and activator (all solutions in 1 mL CH$_2$Cl$_2$)</th>
<th>relative Fmoc value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>237</strong> (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td><strong>237</strong> (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
<tr>
<td>3</td>
<td><strong>237</strong> (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
<tr>
<td>4</td>
<td><strong>237</strong> (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
<tr>
<td>5</td>
<td><strong>237</strong> (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
<tr>
<td>6</td>
<td><strong>237</strong> (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
</tbody>
</table>

After completing the synthesis of the hexasaccharide, the target compound was cleaved from solid support under standard UV light irradiation conditions (see section: Cleavage from Solid Support). The continuous flow reaction afforded 61 mg of crude compound that was passed over a plug of silica (Hexanes/AcOEt = 1:0→1:1) to give 30 mg of crude protected hexasaccharide **243** MALDI-MS m/z calcd for (M+Na)$^+$ C$_{175}$H$_{151}$NO$_{51}$Na: 3104.9, found 3105.7.
5-Amino-pentanyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (hexamer) (244): Hexasaccharide 243 was dissolved in a mixture of CH₂Cl₂/MeOH (4 mL, 1/1, v/v) and treated with NaOMe (0.1 mg, 1.95 μmol). The mixture was stirred at RT overnight and quenched with Amberlite-120 H⁺ ion exchange resin. The resin was filtered off and the filtrate was concentrated in vacuo to give a white remainder that was dissolved in water (2 mL). Palladium (12 mg, Pd on carbon) was added and the suspension was stirred under a hydrogen atmosphere overnight. After complete conversion, the catalyst was removed by centrifugation and washed with water. The solutions were combined and the solvents were removed by lyophilization. Gelfiltration chromatography (Sephadex LH-20, eluent: water) afforded 244 (6.4 mg, 5.95 μmol, 25% over 15 steps). [α]D²⁰ = +58.6 (c = 0.15, H₂O); ¹H NMR (600 MHz, D₂O): δ 4.76 – 4.70 (m, 6H), 3.87 – 3.37 (m, 12H), 2.90 – 2.82 (m, 2H), 1.59 – 1.47 (m, 4H), 1.36 – 1.24 (m, 2H); ¹³C NMR (150 MHz, D₂O): δ 99.8, 99.3, 99.2, 72.6, 70.8, 70.7, 70.7, 70.6, 70.6, 70.4, 69.9, 69.9, 69.8, 67.5, 66.6, 66.5, 66.4, 65.5, 65.4, 65.4, 60.8, 39.3, 27.9, 26.7, 22.4; IR (thin film) 3385, 2932, 1592, 1383, 1351, 1131, 1068, 975 cm⁻¹; HRMS(ESI) m/z calcd for (M+Na)⁺ C₄₁H₇₁NO₃₁Na: 1076.4245, found 1076.4264.

Use of Catch-Release Strategy

N-(Benzyl)benzyloxy carbonyl-5-amino-pentanyl (6-O-(6-aminohexanoyl)-(2,3,4-tri-O-benzyol-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-O-benzyol-α-D-mannopyranoside (dimer) (256): Functionalized resin 236 (50 mg, loading 0.47 mmol/g, 0.0235 mmol) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH₂Cl₂. To start the synthetic sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH₂Cl₂ and CH₂Cl₂ (six times each with 2 mL for 25 s). For the assembly of the oligosaccharide chain, module 1 for phosphate glycosylation was performed with building block 237. For the capping of unreacted hydroxyl groups module 2 was carried out before running module 3 for Fmoc deprotection.
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

For the last glycosylation, building block 251 and module 1 were used. Subsequent removal of Fmoc was performed using module 3 without prior capping.

<table>
<thead>
<tr>
<th>glycosylation</th>
<th>building block and activator</th>
<th>relative Fmoc value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 μL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>251 (3 × 72 mg; 0.0705 mmol) TMSOTf (3 × 13 μL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
</tbody>
</table>

After completing the synthesis of the disaccharide, the target compound was cleaved from the solid support under standard UV light irradiation conditions (see section: Cleavage from Solid Support). The continuous flow reaction afforded 29 mg that was used for the next step without any purification. MS(ESI) m/z calcd for (M+H)+ C73H76N2O20: 1299.3, found 1299.3.

\[N\text{-}(\text{Benzyl})\text{benzyloxycarbonyl-5-amino-pentanyl}\quad \alpha\text{-}\text{D-mannopyranosyl-(1→6)-}\alpha\text{-}\text{D-mannopyranoside (dimer) (257)}\]

Separation from deletion sequences with magnetic beads (140 mg, 0.049 mmol) was performed as described in section: Coupling to Beads and Deprotection for Purification. For compound 256, the catch-release cycle was performed only one time. Gel filtration chromatography (Sephadex LH-20, eluent: 5% MeOH in water) afforded 257 (2.9 mg, 5.16 μmol, 22% over 9 steps). \([\alpha]_D^{20} = +37.1 (c = 0.30, \text{H}_2\text{O})\); \(^1\text{H}\) NMR (400 MHz, D2O): \(\delta 7.49 – 7.39 (m, 5H), 5.11 (s, 2H), 4.88 (J < 1.0 Hz, 1H), 4.84 (J < 1.0 Hz, 1H), 4.04 – 3.64 (m, 13H), 3.60 – 3.49 (m, 1H), 3.15 (t, J = 6.5 Hz, 2H), 1.70 – 1.48 (m, 4H), 1.44 – 1.33 (m, 2H); \(^{13}\text{C}\) NMR (100 MHz, D2O): \(\delta 179.4, 128.6, 128.2, 127.4, 99.7, 99.3, 72.5, 72.5, 70.7, 70.5, 69.9, 69.9, 67.7, 66.6, 66.6, 65.6, 65.6, 60.7, 40.2, 28.4, 28.0, 22.5; IR (thin film) 3346, 2933, 1700, 1130, 1063, 973 cm\(^{-1}\); HRMS(ESI) m/z calcd for (M+Na)+ C25H39NO13Na: 584.2319, found 584.2304.
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

5-Amino-pentanyl α-d-mannopyranosyl-(1→6)-α-d-mannopyranoside (dimer) (253):

Disaccharide 257 (2.9 mg, 5.16 μmol) was dissolved in water (0.5 mL). Palladium (3 mg, Pd on carbon) was added and the suspension was stirred under a hydrogen atmosphere overnight. After complete conversion, the catalyst was removed by centrifugation and washed with water. The solutions were combined and the solvents were removed by lyophilization. Gel filtration chromatography (Sephadex LH-20, eluent: 5% MeOH in water) afforded 253 (1.6 mg, 3.8 μmol, 74%). [α]D20 = +20.6 (c = 0.09, H2O); 1H NMR (600 MHz, D2O): δ 4.96 (J < 1.0 Hz, 1H), 4.91 (J < 1.0 Hz, 1H), 3.89 – 3.84 (m, 3H), 3.82 – 3.79 (m, 1H), 3.74 (dd, J = 9.2, 3.2 Hz, 1H), 3.71 – 3.53 (m, 8H), 3.64 – 3.60 (m, 1H), 3.04 (t, J = 7.5 Hz, 2H), 1.79 – 1.67 (m, 4H), 1.55 – 1.45 (m, 2H); 13C NMR (taken from HSQC spectrum): δ 99.9, 99.5, 72.7, 70.9, 70.6, 70.6, 70.0, 70.0, 67.7, 66.8, 66.6, 65.7, 60.9, 39.4, 28.0, 26.7, 22.5; IR (thin film) 3371, 2929, 1643, 1566, 1407, 1332, 1025 cm⁻¹; HRMS (ESI) m/z calc'd for (M+Na)⁺ C17H35NO11Na: 450.1951, found 450.1913.

N-(Benzyl)benzyloxy carbonyl-5-amino-pentanyl (6-O-(6-aminohexanoyl)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoylα-d-mannopyranoside (dodecamer) (258): The functionalized resin 236 (50 mg, loading 0.47 mmol/g, 0.0235 mmol) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH2Cl2. To start the synthesis sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH2Cl2 and CH2Cl2 (six times each with 2 mL for 25 s). For the assembly of the sugar chain, module 1 for phosphate glycosylation was performed with building block 237. To cap the unreacted hydroxyl groups, module 2 was carried out before running module 3 for Fmoc deprotection.
For the last glycosylation, building block 251 and module 1 were used. Subsequent removal of Fmoc was performed using with module 3 without prior capping.

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<td>4</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<tr>
<td>5</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
<tr>
<td>6</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
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<td>7</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
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<td>8</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<td>9</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<td>10</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<td>11</td>
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<td>12</td>
<td>251 (3 × 72 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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After completing the synthesis of the 12mer, the target compound was cleaved from solid support under standard UV light irradiation conditions (see section: Cleavage from Solid Support). The continuous flow reaction afforded 145 mg that was used for the next step.
without any purification. MALDI-MS m/z calcd for (M+Na)$^+$ C$_{343}$H$_{294}$N$_2$O$_{100}$Na: 6062.8, found 6060.6.

\[ \text{N-}(\text{Benzyl)benzylxycarbonyl}-5\text{-amino-pentanyl} \quad \alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranoside (dodecamer) (260):} \]

Separation from deletion sequences with magnetic beads (140 mg, 0.049 mmol) was performed as described in section: Coupling to Magnetic Beads and Deprotection for Purification. Gel filtration chromatography (Sephadex LH-20, eluent: 5% MeOH in water) afforded 260 (6.7 mg, 3.07 µmol, 13% over 38 steps, average: ca. 95% per step). $[\alpha]_D^{20} = +14.1$ (c = 0.08, H$_2$O); $^1$H NMR (600 MHz, D$_2$O): $\delta$ 7.53 – 7.42 (m, 5H), 5.15 (s, 2H), 4.96 – 4.87 (m, 12H), 4.11 – 3.65 (m, 73H), 3.64 – 3.54 (m, 1H), 3.18 (t, $J = 7.4$ Hz, 2H), 1.75 – 1.50 (m, 4H), 1.48 – 1.37 (m, 2H); $^{13}$C NMR (150 MHz, D$_2$O): $\delta$ 128.7, 128.3, 127.5, 99.8, 99.4, 99.4, 99.3, 99.2, 72.7, 70.9, 70.8, 70.6, 70.5, 70.0, 69.9, 66.7, 66.5, 65.6, 65.5, 60.9, 40.2, 28.3, 28.1, 22.6; IR (thin film) 3346, 2933, 1700, 1130, 1063, 973 cm$^{-1}$; MALDI-MS m/z calcd for (M+Na)$^+$ C$_{383}$H$_{319}$NO$_{63}$Na: 2204.8, found 2204.1.

\[ \text{5-amino-pentanyl} \quad \alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranosyl-(1→6)-}\alpha\text{-D-mannopyranoside (dodecamer) (262):} \]

Dodecasaccharide 260 (1.2 mg, 0.55 µmol) was dissolved in water (1 mL). Palladium (2 mg, Pd on carbon) was added and the suspension was stirred under a hydrogen atmosphere overnight. After complete conversion the catalyst was removed by centrifugation and washed with water. The solutions were combined and the solvents were removed by lyophilization. Gel filtration chromatography (Sephadex LH-20,
eluent: 5% MeOH in water) afforded 262 (0.7 mg, 0.34 μmol, 62%). \([\alpha]_D^{20} = +18.7\) (c = 0.07, H₂O); \(^1H NMR (600 MHz, D₂O): \delta \) 4.78 – 4.76 (m, 12H), 3.89 – 3.49 (m, 74H), 2.12 – 2.07 (m, 2H), 1.56 – 1.37 (m, 6H); \(^13C NMR (taken from HSQC spectrum): \delta \) 99.4, 72.9, 71.0, 70.1, 66.8, 65.7, 61.1; IR (thin film) 3296, 2917, 1625, 1354, 1110, 1032, 979, 833, 704 cm\(^{-1}\); MALDI-MS m/z calcd for (M+Na\(^+\)) \(C_{77}H_{133}NO_{61}Na\): 2070.7, found 2070.7.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (6-O-(6-aminohexanoyl)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-benzoyl-α-D-mannopyranoside (30mer) (259):

Functionalized resin 236 (50 mg, loading 0.47 mmol/g, 0.0235 mmol) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH₂Cl₂. To start the synthetic sequence, it was washed with DMF, THF, 0.2 M acetic acid in CH₂Cl₂ and CH₂Cl₂ (six times each with 2 mL for 25 s). For the assembly of the sugar chain, module 1 for phosphate glycosylation was performed with building block 237. To cap the unreacted hydroxyl groups, module 2 was
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

carried out before running module 3 for Fmoc deprotection. For the last glycosylation, building block 251 and module 1 were used. Subsequent removal of Fmoc was performed using module 3 without prior capping.

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<th>building block and activator (all solutions in 1 mL CH₂Cl₂)</th>
<th>relative Fmoc value</th>
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<td>2</td>
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<td>3</td>
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<td>4</td>
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<td>6</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
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<td>7</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
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<tr>
<td>8</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<tr>
<td>9</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<td>11</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
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<td>12</td>
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<td>13</td>
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<td>14</td>
<td>237 (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)</td>
<td>100%</td>
</tr>
</tbody>
</table>
4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside

TMSOTf (3 × 13 µL; 0.0705 mmol)

15  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

16  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

17  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

18  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

19  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

20  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

21  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

22  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

23  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

24  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

25  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

26  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

27  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

28  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

29  237 (3 × 64 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)

30  251 (3 × 72 mg; 0.0705 mmol) 100%

TMSOTf (3 × 13 µL; 0.0705 mmol)
After completing the synthesis of the 30mer, the target compound was cleaved from solid support under standard UV light irradiation conditions (see section: Cleavage from Solid Support). The continuous flow reaction afforded 90 mg that was used for the next step without any purification. MALDI-MS m/z calcd for (M-Cbz)+ C_{829}H_{690}N_{2}O_{244} 14440.1; found 14400.2.

\[
\begin{align*}
N(\text{Benzyl})\text{benzyloxycarbonyl-5-amino-pentanyl} & \quad \alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranosyl-(1→6)-}\alpha-D-\text{mannopyranoside (30mer) (261): Separation of deletion sequences with magnetic beads (140 mg, 0.049 mmol) was performed as described in section: Coupling to Magnetic Beads and Deprotection for Purification. Gel filtration chromatography (Sephadex LH-20, eluent: 5% MeOH in water) afforded 261 (1.2 mg, 0.235 μmol, 1%).} \\
^1H\text{ NMR (600 MHz, D}_2O): 4.95 – 4.91 (m, 30H), 3.88 – 3.57 (m, 181H); ^13C\text{ NMR (taken from HSQC spectrum): }\delta\ 100.1, 73.5, 71.6, 70.8, 70.4, 67.4, 66.4, 61.8; \text{MALDI-MS m/z calcd for (M+Na)+ C}_{193}H_{319}NO_{153}Na: 5121.7, found 5121.6.}
\end{align*}
\]
5. References

5. References

5. References

5. References

68. 25 g of L-fucose cost 596 € at Sigma-Aldrich.
74. At Sigma Aldrich, L-fucose costs 227 times more than (S)-ethyl lactate per gram.
5. References


91. Upjohn dihydroxylation performed on substrate 199 (C4-OBz) afforded inseparable mixture of diastereoisomers with poor levels of diastereoselectivity ranging from 1.3:1 to 1.6:1 when the temperature was decreasing from RT to 0 °C.


95. The undesired 6 membered ring benzylidene acetal was isolated in 12% yield.

5. References


5. References

123. Quantification of the piperidine-dibenzofulvene adduct was achieved by measurement of the absorbance at 301 nm in DMF/piperidine (4/1, v/v).
129. The magnetic beads were provided by PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany. These superparamagnetic beads, presenting 50-60% magnetite, consist of a matrix of carboxylated polyvinyl alcohol, which is subsequently activated with N-hydroxysuccinimidyl (NHS) ester functionalities.
5. References

6. Abbreviations

Å Angstrom, 10^{-10} m
Ac acetyl
AIBN azobisisobutyronitrile
aq. aqueous
Ar aromatic
9-BBN 9-borabicyclo[3.3.1]nonane
Bn benzyl
br broad
Boc tert-butyloxycarbonyl
BSP 1-benzenesulfinyl piperidine
Bu butyl
Bz benzoyl
c concentration
CAN ceric (IV) ammonium nitrate
cat. catalytic
Cbz benzyloxycarbonyl
m-CPBA 3-chloroperbenzoic acid
CSA camphorsulfonic acid
δ chemical shift
d doublet
DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
DCC N,N’-dicyclohexylcarbodiimide
DDQ 2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL diisobutylaluminium hydride
DIPC N,N’-diisopropylcarbodiimide
DIPEA diisopropylethylamine
DIPT diisopropyltartrate
DMAP 4-(N,N-dimethylamino)pyridine
DME dimethylether
DMF N,N-dimethylformamide
2,2-DMP 2,2-dimethoxypropane
6. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMTST</td>
<td>dimethylsulfonium triflate</td>
</tr>
<tr>
<td>DTBMP</td>
<td>2,6-di-tert-butyl-4-methylpyridine</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>EDC</td>
<td>N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide</td>
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<td>equiv</td>
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</table>
Curriculum vitae

For reasons of data protection,
the curriculum vitae is not included in the online version