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

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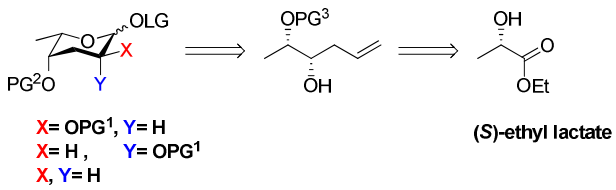
Summary

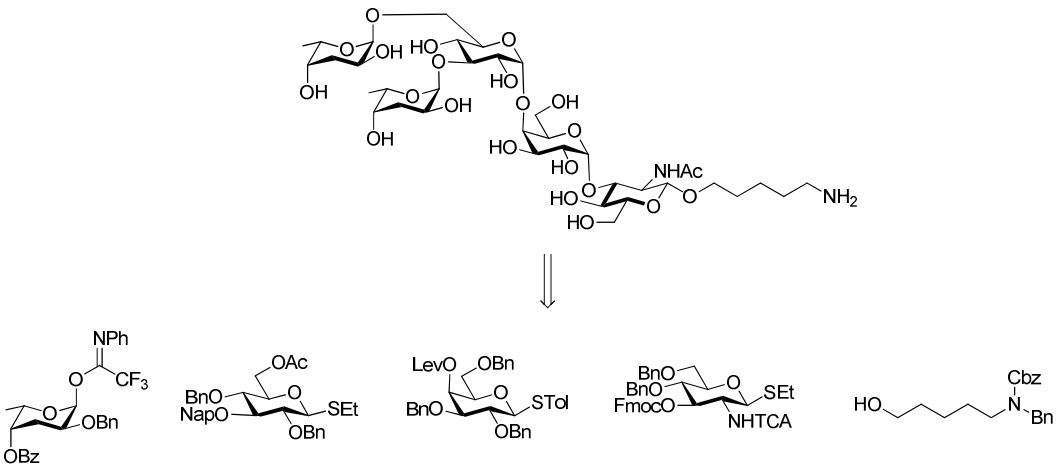
Besides nucleic acids and proteins, carbohydrates play an essential role in different biological events. Their interaction with lectins and antibodies mediate processes involved in immune response. Carbohydrates like lipopolysaccharides, major components of the outer membrane of Gram negative bacteria, 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.

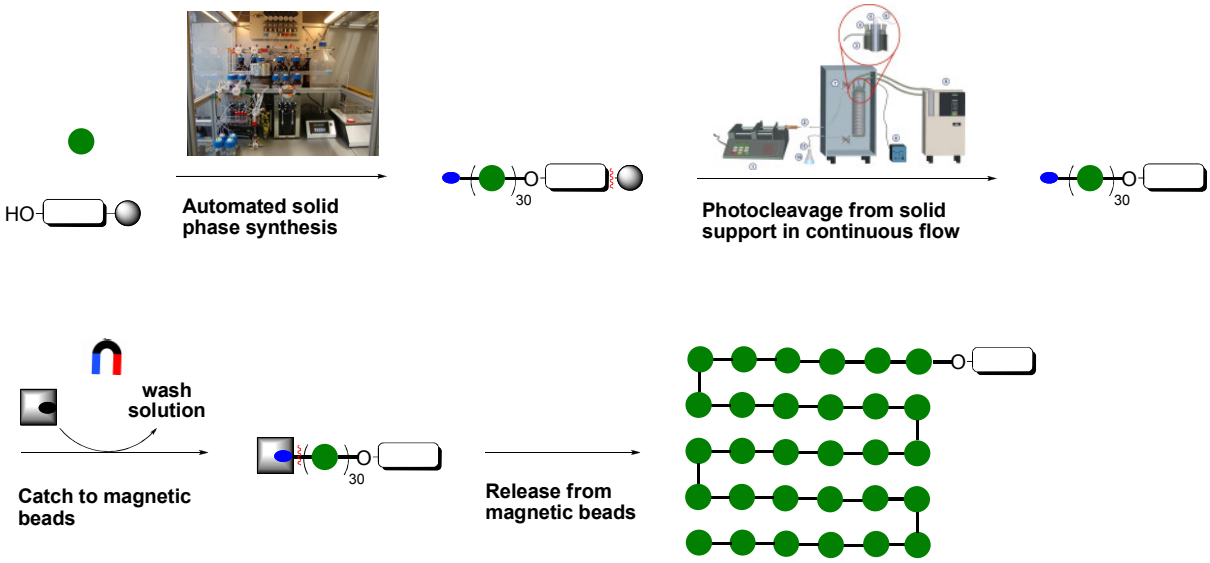


Taking advantage of the synthetic route established in the second chapter, Chapter 3 describes the assembly of pentasaccharide repeating unit of the *O*-antigen of *Escherichia coli*

O111, serotype that causes enteropathogenic, enterotoxigenic and enterohemorrhagic disease in humans. Since the excessive use of antibiotic in live stock 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 α-(1,6)-oligomannoside, the longest carbohydrate ever made by chemical synthesis.



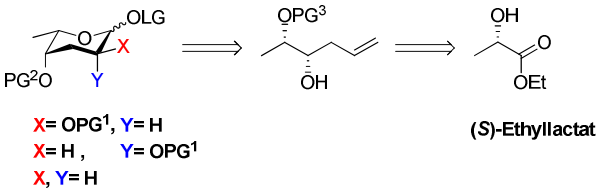
This study was performed in an effort to test whether oligosaccharides that could be required for amplifying the weak carbohydrate-carbohydrate recognition receptor interaction, could be

quickly accessed by automated solid phase synthesis. To aid in the purification of the α-(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.

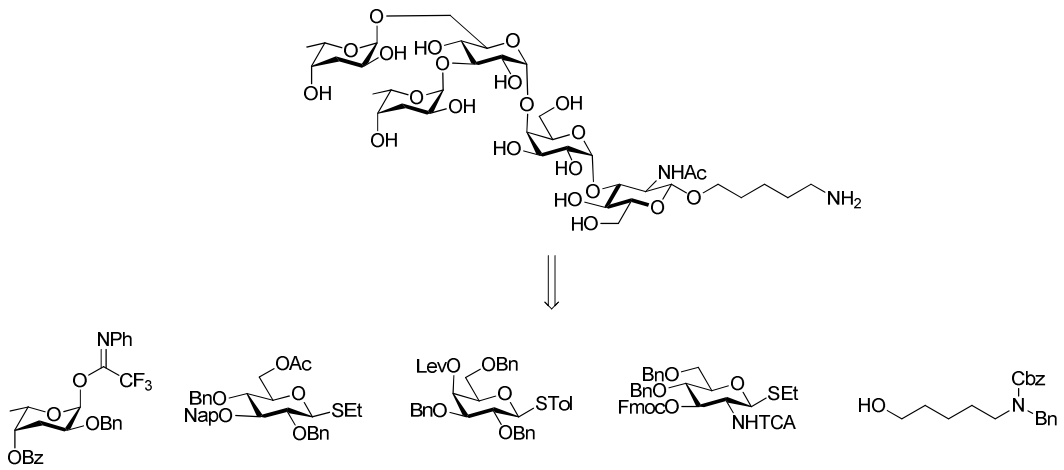
Zusammenfassung

Neben Nukleinsäuren und Proteinen spielen Kohlenhydrate eine wichtige Rolle in den verschiedensten biologischen Vorgängen. Durch die Wechselwirkungen mit Lektinen und Antikörpern sind sie zum Beispiel an Immunprozessen beteiligt und wirken als Antigene auf der Oberfläche von Viren und Bakterien.
Das Lipopolysaccharid, der Hauptbestandteil der äußeren Membran Gram-negativer Bakterien ist aus drei Teilen aufgebaut: das Lipid A, das Polysaccharid der Kernregion und das *O*-Antigen. Während Lipid A und Kernregion nicht besonders artenspezifisch sind, zeigt das *O*-Anitgen eine große strukturelle Vielfalt und enthält sehr oft seltene Zucker, die nicht beim Menschen vorkommen und deshalb durch das menschliche Immunsystem erkannt werden können. Der erste Teil dieser Dissertation konzentriert sich auf die *de novo* Synthese von Zuckern durch die stereokontrollierte Herstellung aus kleineren Substraten durch C-C-Verknüpfungsreaktionen. Das Interesse an den seltenen Zuckern erklärt sich durch die Verwendung dieser als Bausteine für die Herstellung der *O*-Antigene Gram-negativer Bakterien. Der zweite Teil dieser Dissertation widmet sich Machbarkeitsstudien für die automatisierte Festphasensynthese von Oligosacchariden. Diese Methode vereinfacht den Herstellungsprozess synthetischer Kohlenhydrate.

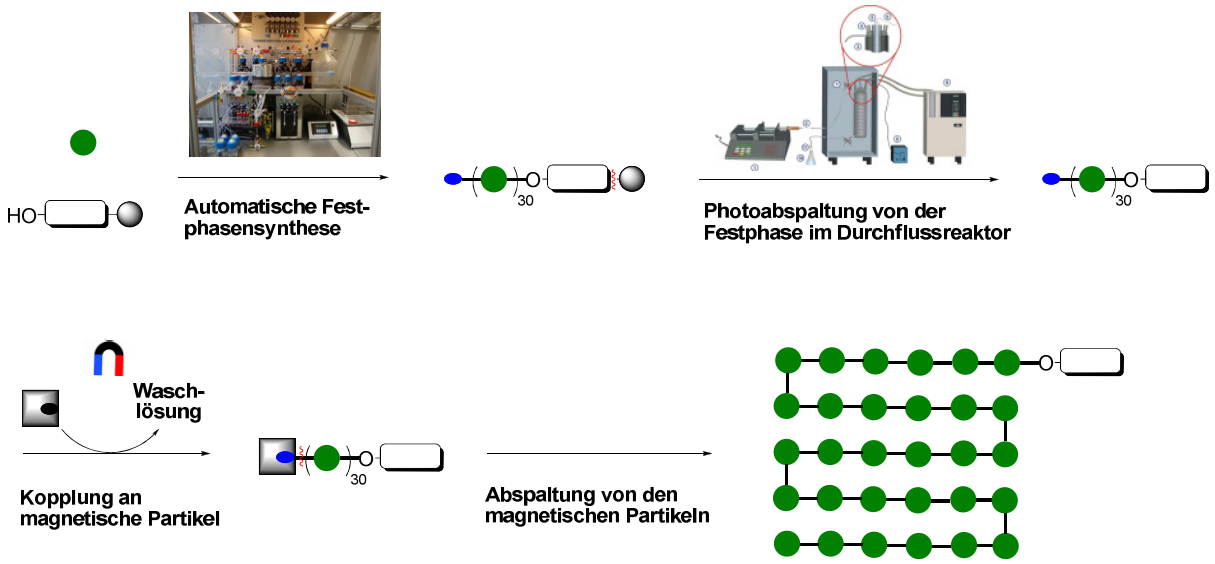
Nach dem einleitenden Kapitel 1 über die Oligosaccharidsynthese wird in Kapitel 2 die *de novo*-Synthese zur Herstellung voll funktionalisierter L-Colitose-Bausteine (3,6-Didesoxy-L-Xylo-Hexapyranose) behandelt. Die Synthese geht von kostengünstigem (*S*)-Ethyllaktat aus und liefert ein Produkt, das in den *O*-Antigen Gram-negativer Bakterien, wie *Escherichia coli* O111, *Salmonella enterica*, *Salmonella adelaide*, *Salmonella greenside* oder *Vibrio cholerae* vorkommt.
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*-chelatkomplexierte Allylierung die zur gemeinsamen Zwischenstufe eines homoallylischen Alkohols führte. Durch Oxidation dieser Zwischenstufe konnten die drei Monosaccharidbausteine hergestellt werden.



Nach der Synthese dieser seltenen Zuckermonomere beschreibt Kapitel 3 die Herstellung einer Pentasaccharid-Widerholungseinheit des *O*-Antigens von *Escherichia coli* O111, ein Serotyp der für enteropathogene, enterotoxigene und enterohämorrhagische Krankheiten von Menschen verantwortlich gemacht wird. Die exzessive Anwendung von Antibiotika in der Viehzucht führt zu vielerlei multiresistenter bakterieller Infektionen und lässt deswegen Impftherapien gegen *E. coli* O111 attraktiv erscheinen. Der Schlüsselschritt der Synthese ist eine [3+1+1]-Glycosylierung das die säaurelabilen Colitosereste auf der letzten Stufe einführt. Zur weiteren immunologischen Untersuchung wurde das Pentasaccharid am reduzierenden Ende mit einem Aminolinker versehen um die Konjugation an Trägerproteine zu erlauben.



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.

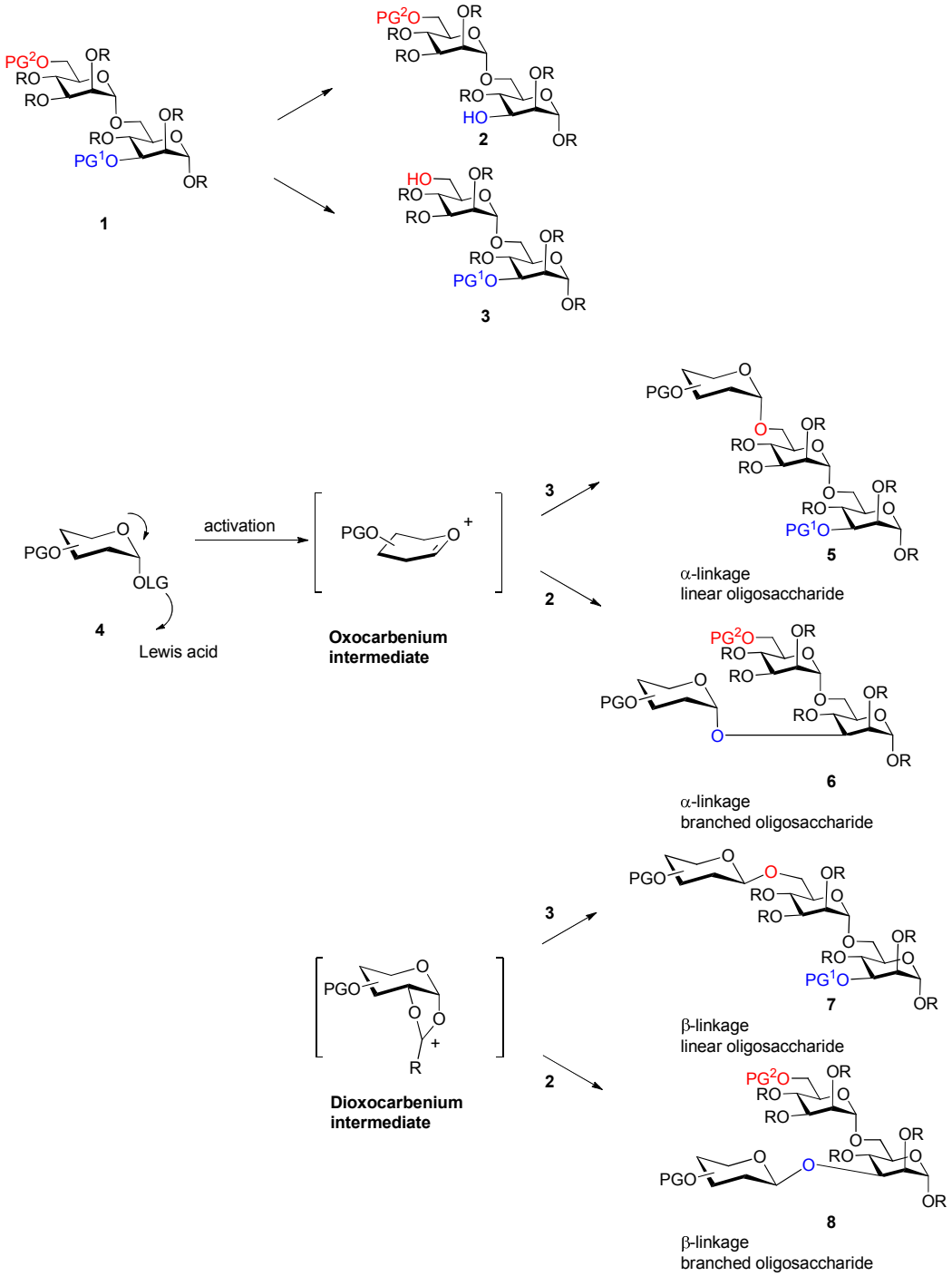


Ziel dieser Studie war es, die Zugänglichkeit von großen Kohlenhydraten durch automatiserte Festphasensynthese zu untersuchen. Diese großen Strukturen können eventuell die schwachen Wechselwirkungen von Rezeptoren mit Kohlenhydraten verstärken. Um die Reinigung dieses Oligomannosids gewährleisten zu können, wurde eine *catch-release*-Strategie entwickelt, die es erlaubte, das Volllängenkohlenhydrat mit Hilfe einer selektiven Funktionalisierung an magnetische Partikel zu binden. Mit Hilfe eines Magenten konnte die Zielsubstanz mit wenig Aufwand von Nebenprodukten aus der Festphasensynthese getrennt werden.

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.[1] 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 de 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**.



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

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.

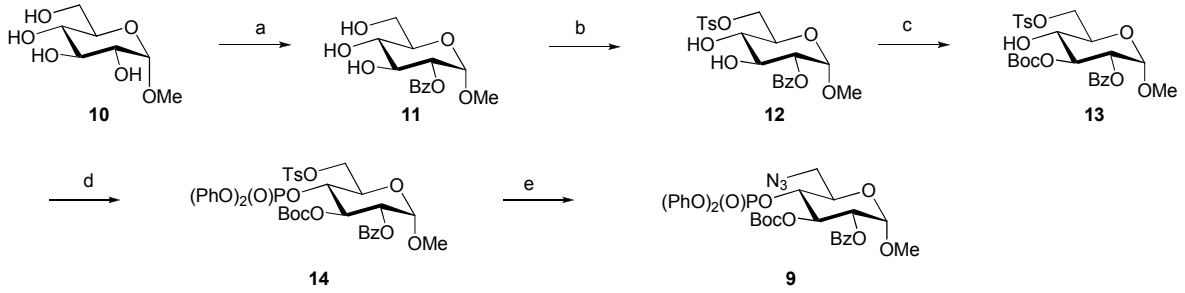
1.1.1. Regioselective Protection of Monosaccharides

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

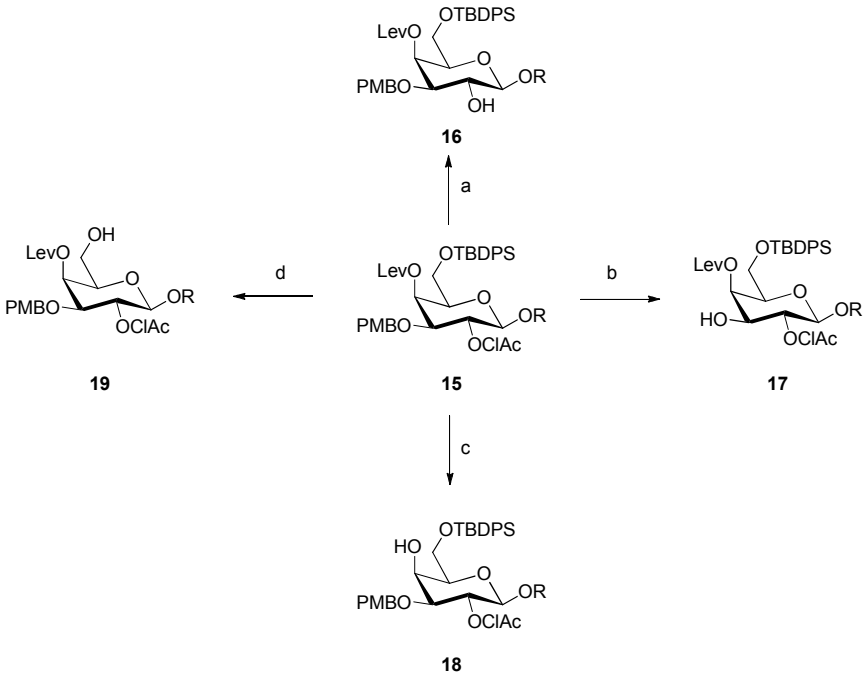
The traditional synthesis of monosaccharide building blocks relies on tedious protection/deprotection steps. To facilitate the construction of libraries of building blocks and to shorten the synthetic process, new strategies for the regioselective protection of hydroxyl groups have been developed.

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).[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 Me_2SnCl_2. 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.



Scheme 2. Me_2SnCl_2 catalyzed regioselective protection of monosaccharides: a) BzCl, DIPEA, Me_2SnCl_2, THF, 82%; b) TsCl, DIPEA, Me_2SnCl_2, THF, 88%; c) Boc_2O, DIPEA, Me_2SnCl_2, DMAP, THF, 93%; d) $ClP(O)(OPh)_2$, pyridine, DMAP, CH_2Cl_2, 95% e) NaN_3, 15-crown-5, DMF, 50 °C, 95%.

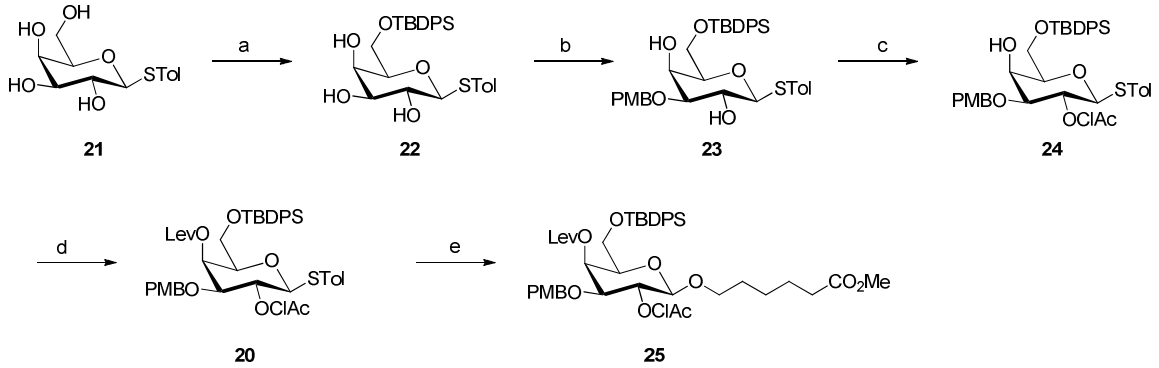
Another orthogonal protection-deprotection strategy for synthesis of oligosaccharides libraries was introduced by Wong and co-workers.[3] They described the synthesis of galactoside **15** functionalized with four selectively removable protecting groups, thereby enabling the branching of these positions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



Scheme 3. Selective removal of the protecting groups on galactoside **15**: a) $NaHCO_3$, $MeOH/H_2O$ (5/1), 60 °C, 99%; b) TFA, CH_2Cl_2, -20 °C, 97%; c) N_2H_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 *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.



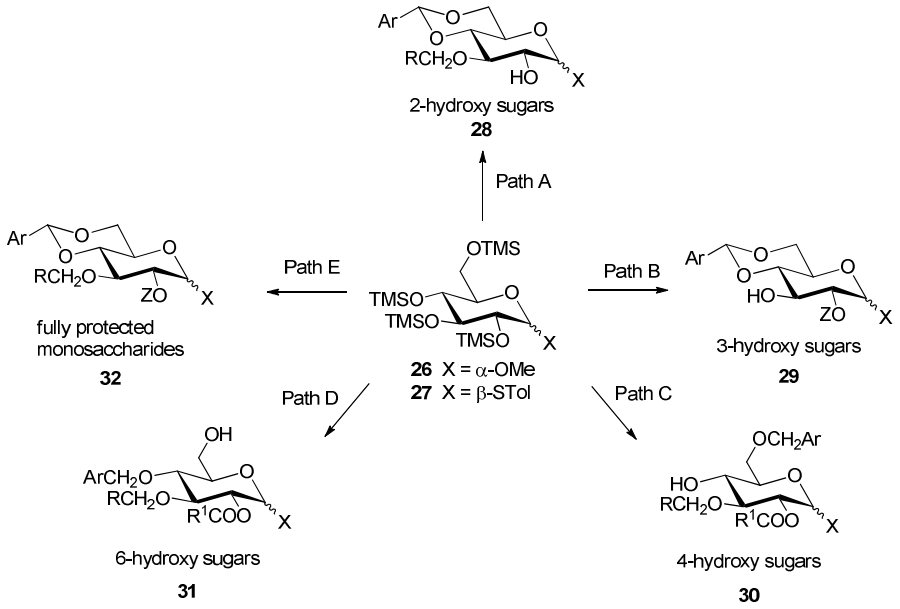
Scheme 4. Synthesis of orthogonally protected galactoside **20**: a) *t*-$BuPh_2SiCl$, imidazole, DMF, 100%; b) i. Bu_2SnO, toluene/benzene, reflux; ii. PMB-Cl, TBAI, DMF, 60 °C, 49%; c) $ClCH_2C(O)Cl$, Et_3N, CH_2Cl_2, -20 °C to RT, 52%; d) LevOH, DCC, DMAP, CH_2Cl_2, 83%; e) i. $HO(CH_2)_5CO_2Me$, NIS, TMSOTf, 4 Å MS, CH_3CN, -20 °C to RT; ii. $HgBr_2$, toluene/CH_3NO_2, 60 °C, 85%.

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 **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

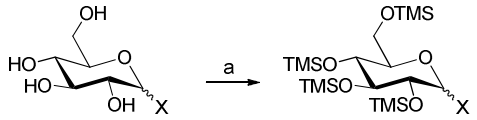
sugars **28** (Scheme 5, Path A); b) selective protection of C4- and C6-hydroxyls as an arylidene acetal, followed by regioselective reductive *p*-methoxybenzylation or 2-naphtylmethylation at C3-hydroxyl, C2-hydroxyl-etherification or C2-hydroxyl-acylation and cleavage of the ethers at C3 by treatment 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 arylidenation, 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 arylidenation, 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, Et_3SiH; c) TBAF; **Path B.** a) cat. TMSOTf, ArCHO; b) 4-MeOPhCHO, Et_3SiH; c) TBAF; d) base, electrophile; e) DDQ; or a) cat. TMSOTf, ArCHO; b) 2-$C_{10}H_7CHO$, Et_3SiH; c) acid anhydride; d) DDQ; **Path C.** a) cat. TMSOTf, ArCHO; b) RCHO, Et_3SiH; c) $(R^1CO)_2O$; d) $HCl_{(g)}$, $NaCNBH_3$; **Path D.** a) cat. TMSOTf, ArCHO; b) RCHO, Et_3SiH; c) $(R^1CO)_2O$; d) BH_3·THF; **Path E.** a) cat. TMSOTf, ArCHO; b) RCHO, Et_3SiH; c) TBAF; d) base, electrophile; or a) cat. TMSOTf, ArCHO; b) RCHO, Et_3SiH; c) acid anhydride.

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



33 X = α-OMe
34 X = β-STol

26 X = α-OMe
27 X = β-STol

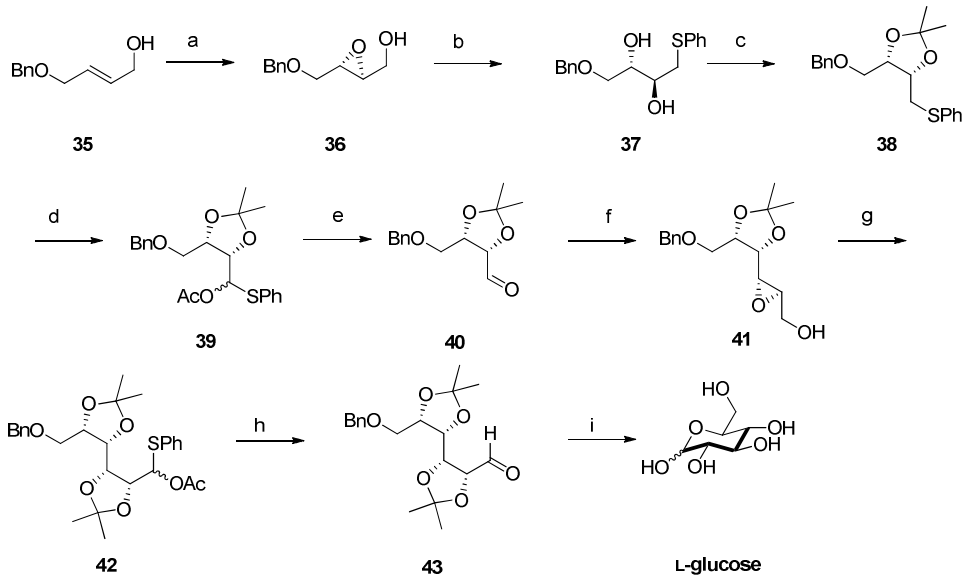
Scheme 6. TMSOTf-catalyzed silylation of glucosides with HMDS: a) cat. TMSOTf, HMDS, CH_2Cl_2, quant..

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

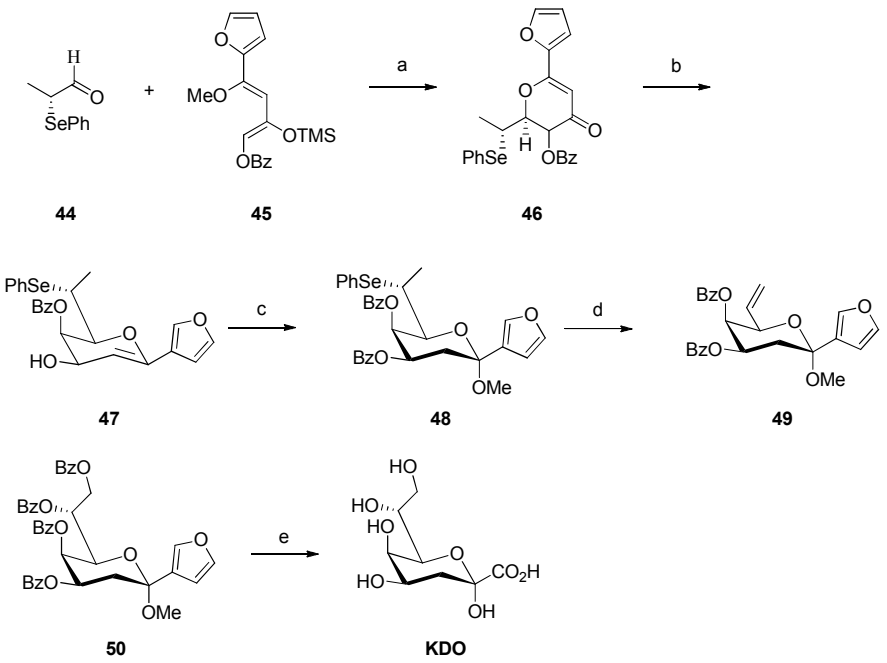
section highlights ingenious methods that have been developed to access differentially protected monosaccharides.

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 α-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.



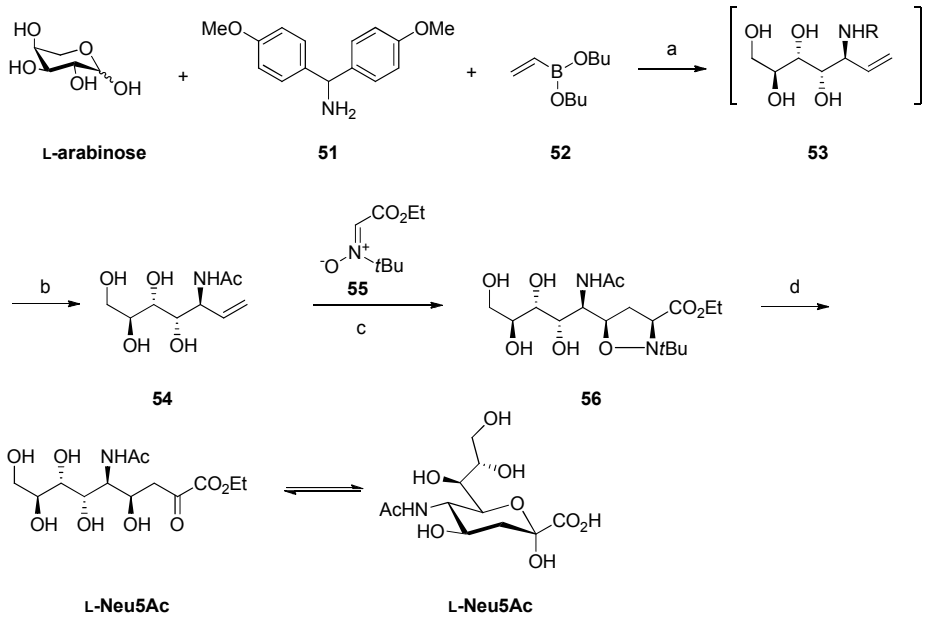
Scheme 7. *Sharpless* asymmetric synthesis of L-glucose: a) (+)-DIPT, $Ti(O^iPr)_4$, TBHP, CH_2Cl_2, -20 °C, 92%; b) 0.5 N aq. NaOH, *t*-BuOH, PhSH, 71%; c) i. $Me_2C(OMe)_2$, $POCl_3$, CH_2Cl_2, quant.; ii. *m*-CPBA, CH_2Cl_2, -78 °C; iii. NaOAc, Ac_2O, 93%; e) DIBAL, CH_2Cl_2, -78 °C, 91%; f) i. Ph_3P=CHCHO, benzene, 88%; ii. $NaBH_4$, MeOH, -40 °C, 91%; iii. (-)-DIPT, $Ti(O^iPr)_4$, TBHP, CH_2Cl_2, -20 °C, 84%; g) i. 0.5 N aq. NaOH, *t*-BuOH, PhSH, 63%; ii. CSA, $Me_2C(OMe)_2$, CH_2Cl_2; 100%; iii. *m*-CPBA, CH_2Cl_2, -78 °C; iv. NaOAc, Ac_2O, 87%; h) K_2CO_3, MeOH, overnight, 65%; i) i. 90% TFA; ii. H_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*[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 benzoylation intermediate **48**. Oxidative elimination furnished alkene **49** in 93% yield that was further submitted to diastereoselective *Upjohn* dihydroxylation and subsequent benzoylation to afford KDO precursor **50**. Oxidation of the furane cycle to the carboxylic acid and final deprotection completed the synthesis of the KDO monosaccharide.



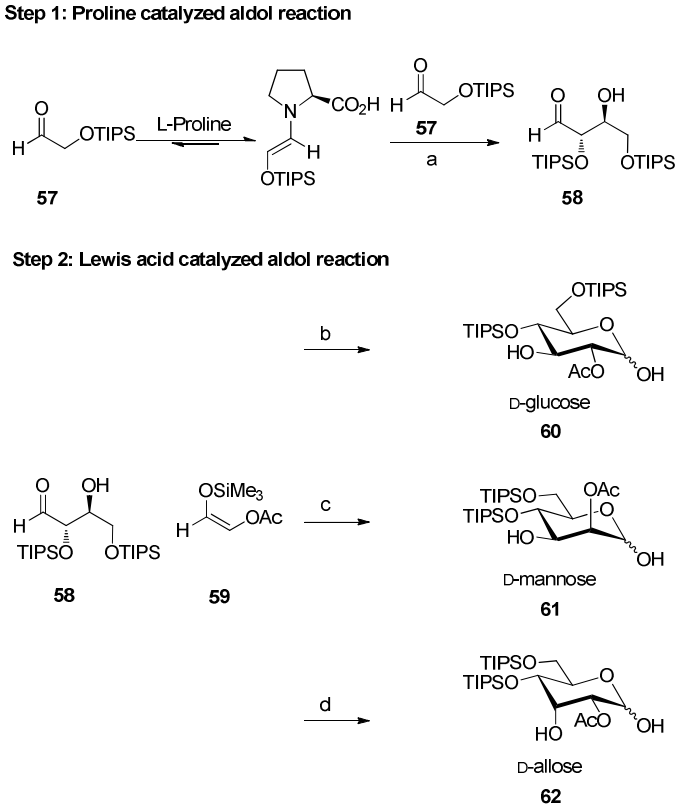
Scheme 8. Hetero-*Diels-Alder* synthesis of KDO: a) i. $BF_3 \cdot Et_2O$; ii. AcOH, 76%, 5:1 (*cis*/*trans*); b) $NaBH_4$; c) i. MeOH; ii. BzCl, Et_3N; d) H_2O_2, pyridine, 0 °C, 93%; e) i. NMO, cat. OsO_4; ii. BzCl, pyridine, DMAP; f) i. $RuO_2 \cdot H_2O$, $NaIO_4$, MeCN, CCl_4, H_2O; ii. CH_2N_2, 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*-acetylneuraminic 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 nitrone **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.



Scheme 9. Three-step synthesis of L-Neu5Ac: a) EtOH/H_2O (4/1), 50 °C; b) i. TFA, 50 °C; ii. Ac_2O, MeOH, 55%, 99% dr; c) dioxane, 30 °C, 90%, 10:1 dr; d) NaOMe, MeOH, then H_2O, 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).

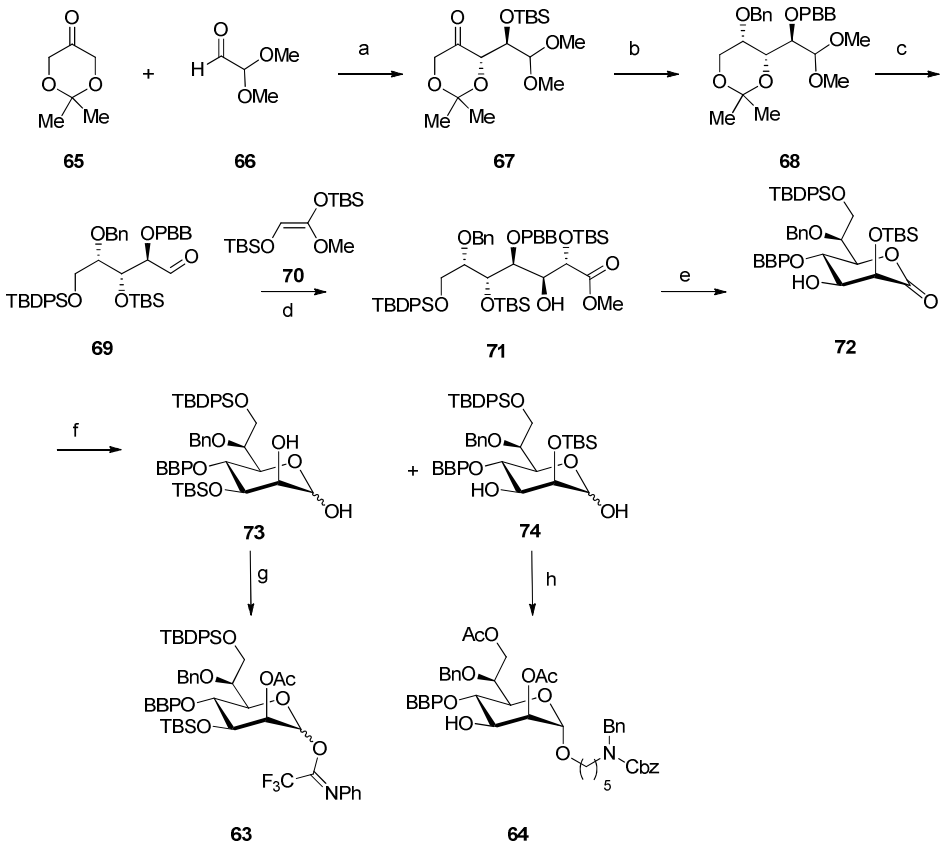


Scheme 10. Two-step synthesis of carbohydrates by selective aldol reactions: a) L-proline (10%), DMF, RT, 92%, dr 4:1 (*anti*/*syn*); b) $MgBr_2 \cdot Et_2O$, Et_2O, -20 °C to 4 °C, 79%, dr 10:1; c) $MgBr_2 \cdot Et_2O$, CH_2Cl_2, -20 °C to 4 °C, 87%, dr 19:1; d) $TiCl_4$, CH_2Cl_2, -78 °C to -40 °C, 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_2O led to the formation of D-glucose **60**. The analogous reaction performed in CH_2Cl_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

elegant pathway for accessing L-*glycero*-D-manno-heptose building blocks **63** and **64** (Scheme 11) required for the total synthesis of the *Yernisia pestis* cell wall polysaccharide.

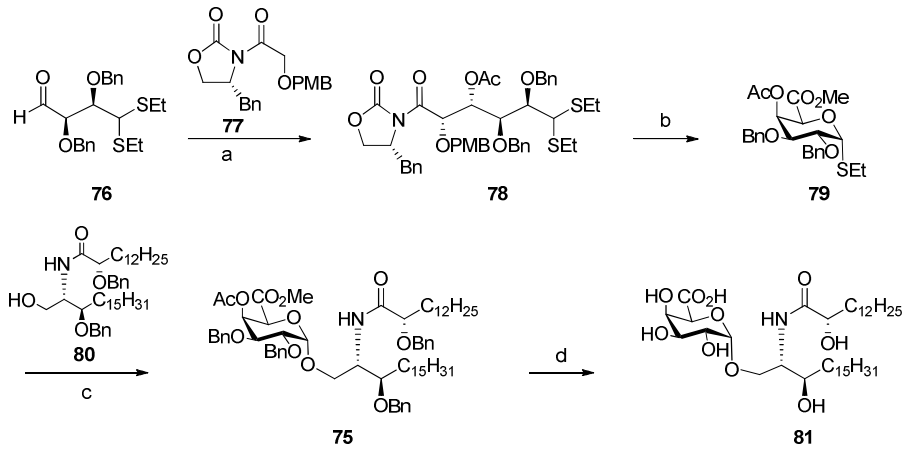


Scheme 11. *De novo* synthesis of L-*glycero*-D-manno-heptoses **63** and **64**: a) i. 30% L-proline, DMF, 2 °C, 69%, 98:2 (*anti*/*syn)*; ii. TBSOTf, lutidine, CH_2Cl_2, -78 °C; 98%; b) i. L-selectride, 79%; ii. PBBBr, NaH; iii. TBAF, 82% (two steps); iv. BnBr, NaH, 95%; c) i. CSA, MeOH; ii. TBDPSCl, imidazole, DMAP, 93% (two steps); iii. *p*-TsOH H_2O, acetone, 97%; d) $MgBr_2{\cdot}Et_2O$, CH_2Cl_2, 61%; e) i. TFA, CH_2Cl_2, 56%; f) $LiAlH(O^tBu)_3$, **73**: 47%, **74**: 38%; g) i. Ac_2O, pyridine, quant.; ii. N_2H_4, AcOH, 89%; iii. $CF_3C(=NPh)Cl$, Cs_2CO_3, 96%; h) i. LevOH, DIPC, DMAP, 89%; ii. HO-$(CH_2)_5$-NBnCbz, $BF_3{\cdot}OEt_2$; iii. HF/pyridine; iv. Ac_2O, pyridine, 76% (three steps); iv. N_2H_4, 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*-toluenesulfonic 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 anomeric 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 *Evan*s aldol reaction as a key step for the synthesis of galacturonic acid building block **75** that is specific to the glycosphingolipid from *Sphingomonas yonoikuyae* (Scheme 12).

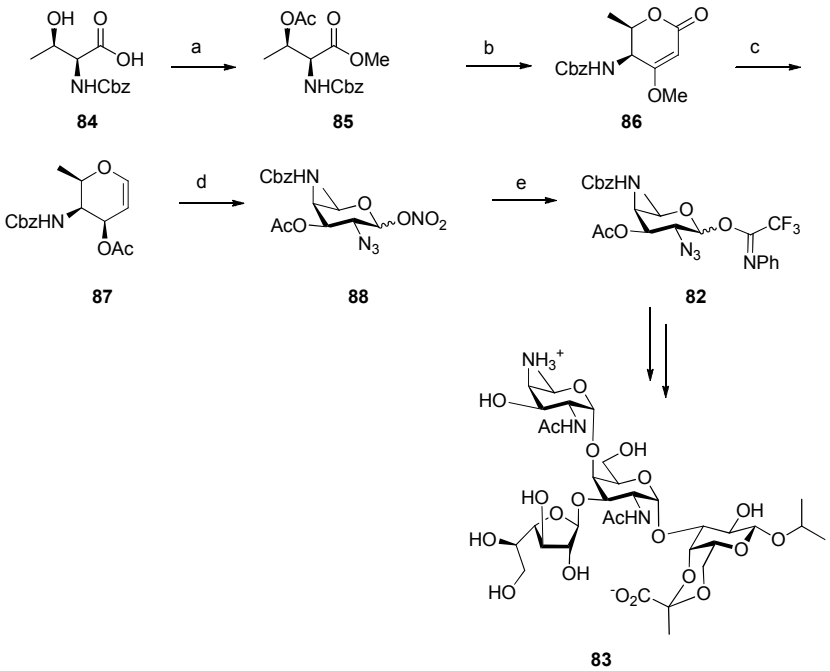


Scheme 12. *De novo* synthesis of glycosphingolipid from *Sphingomonas yanoikuyae*: a) i. LDA, THF, 90%, dr 4.9:1; ii. Ac_2O, pyridine, quant.; b) TFA, CH_2Cl_2, anisole, 92%, α/β = 4:1; ii. $Sm(OTf)_3$, MeOH; c) NIS, TfOH, dioxane/toluene 3:1, 85%, α/β = 4.2:1; d) i. $Pd(OH)_2/C$, H_2, EtOH, $CHCl_3$, 74%; ii. LiOH, H_2O_2, 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

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**.

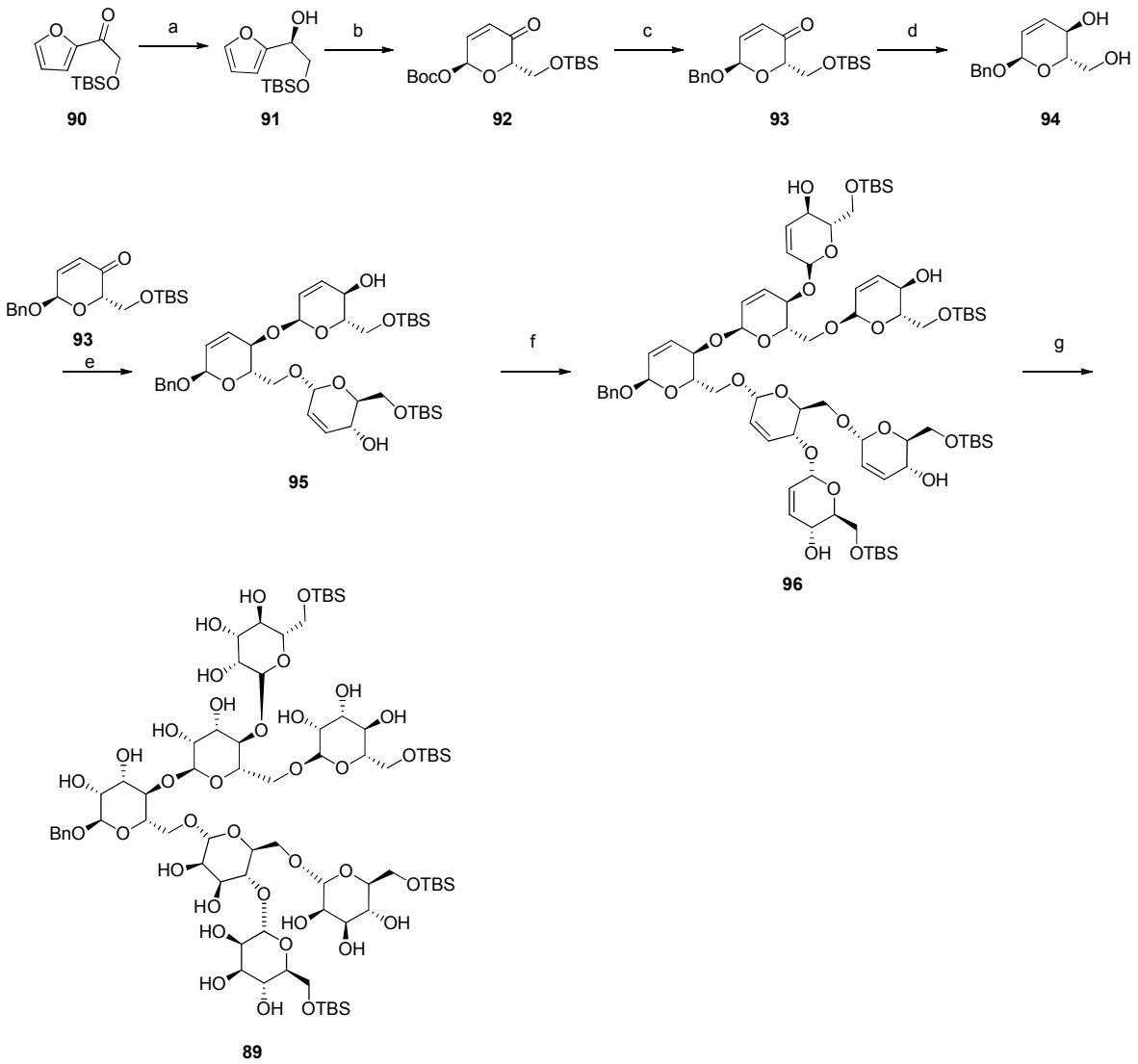
In another *de novo* approach stemming from the Seeberger group, the of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose building block **82**[17] required for the synthesis of *Bacteroides fragilis* A1 polysaccharide fragment **83**[18] was accessed via a *Dieckmann* cyclization (Scheme 13). The synthesis 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 stereoeselective *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. Ac_2O, Et_3N, DMAP, 95% (two steps); b) i. LHMDS, THF, -78 °C to

23 °C; ii. $NaHCO_3$, Me_2SO_4, acetone, 73% (two steps); c) i. DIBAL, THF, -78 °C, H^+; ii. $NaBH_4$, $CeCl_3$, MeOH, -78 °C, 77% (two steps); iii. Ac_2O, Et_3N, DMAP, CH_2Cl_2, 90%; d) CAN, NaN_3, CH_3CN, -20 °C, 67%; e) i. *p*-TolSH, DIPEA, CH_3CN, 87%; ii. $CF_3C(=NPh)Cl$, Cs_2CO_3, CH_2Cl_2, 79% (2:1 α/β).

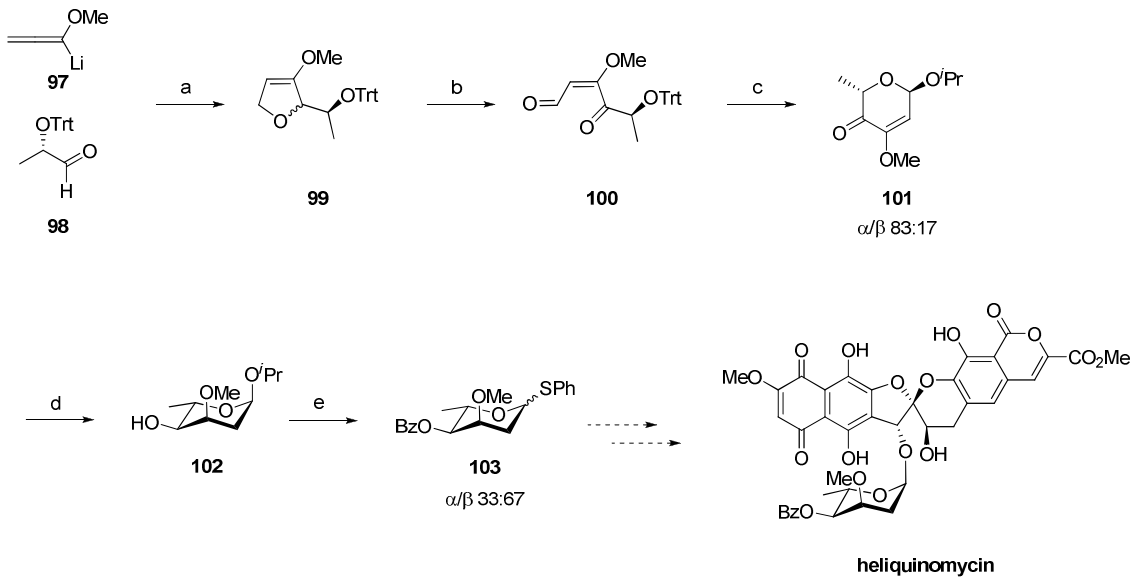
The *Achmatowicz* rearrangement[19] is another valuable tool for *de novo* chemistry that allows for the conversion of furfuryl alcohols to the corresponding pyranones with high degree of stereoselectivity. By varying the stereochemistry of the alcohol group on furanol, both the D- and L- sugars series can be accessed. Based on this transformation, *O´Doherty* developed *de novo* routes to a variety of building blocks including L-sugars,[20] 6-amino-deoxy-sugars[21] and 2-deoxy and 2,3-dideoxyhexoses[22]. An elegant application of this methodology is the 12-step synthesis of highly branched all-L-α-manno-heptapyranoside **89**[23] (Scheme 14). L-α-Manno-heptapyranoside **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 **93**, necessary for the assembly of the heptamannoside **89** was prepared via a *de novo* route starting from acyl furan **90**. Enantioselective *Noyori* reduction provided chiral alcohol **91**, that was further submitted to *Achmatowicz* oxidation and subsequent *tert*-butyl carbonate formation to afford pyranone **92**. Palladium catalyzed glycosylation of the pyranone with benzylic alcohol furnished β-benzyloxy pyranone **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 **94** in 45% yield over six steps. Palladium catalyzed coupling of diol nucleophile **94** with pyranone **93** and subsequent ketones reduction afforded trisaccharide **95** in 65% yield over two steps. After removal of the silyl ether on **95** with tetrabutylammonium fluoride, the glycosylation/reduction sequence provided the heptasaccharide/tetraallylic alcohol **96** in 41% yield over three steps. Dihydroxylation under *Upjohn* conditions gave the α-L-manno-heptasaccharide **89** in 82% yield, thus completing the *de novo* assembly of this molecule.



Scheme 14. *De novo* asymmetric synthesis of all-α-L-heptamannoside **89**: a) Noyori (*S*,*S*); b) i. NBS, H_2O; ii. Boc_2O, 5% DMAP, -78 °C; c) BnOH, $Pd(0)(PPh_3)_4$; d) i. $NaBH_4$, -78 °C; ii. TBAF, 45% (six steps); e) i. $Pd(0)(PPh_3)_4$; ii. $NABH_4$, 65% (two steps); f) i. TBAF, 91%; ii. **93**, $Pd(0)(PPh_3)_4$; iii. $NABH_4$, 45% (two steps); g) OsO_4, 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.[24] Reißig and coworkers[25] 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**. Benzoylation 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. Et_2O, -78 °C; ii. cat. AuCl, pyridine, 86% (two steps); b) DDQ, CH_2Cl_2, H_2O, 83%; c) I_2, $CH(O^iPr)_3$, $^iPrOH/CH_2Cl_2$, 90%; d) i. H_2, Rh/Al_2O_3; ii. L-selectride, 60% (two steps); e) i. Bz_2O, DMAP, pyridine; ii. PhSH, $BF_3 \cdot OEt_2$, 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.

1.2. Oligosaccharide Synthesis Strategies

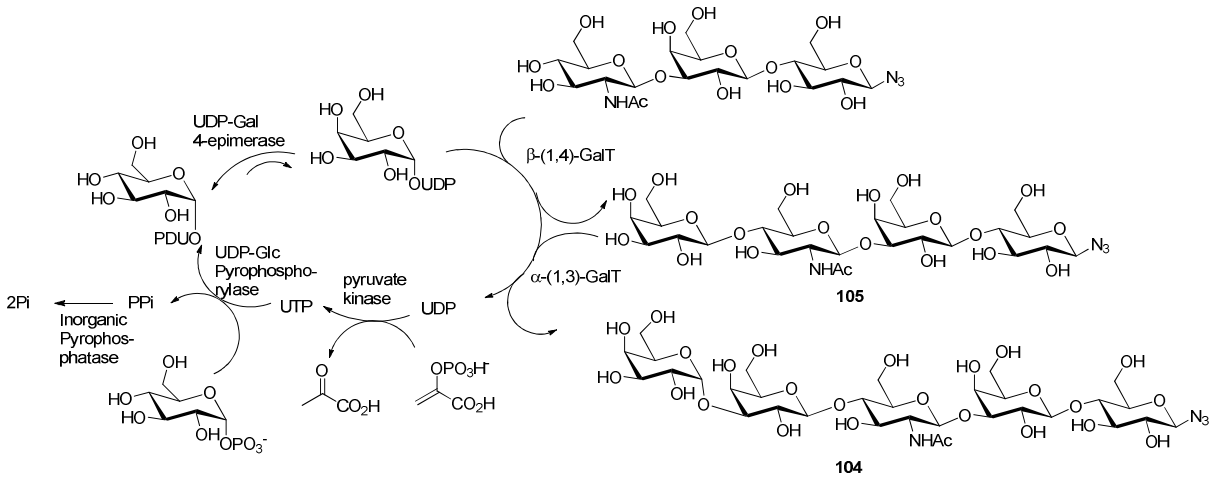
1.2.1 Enzymatic Synthesis of Oligosaccharides

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.[26]

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"[27] 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.[28]

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

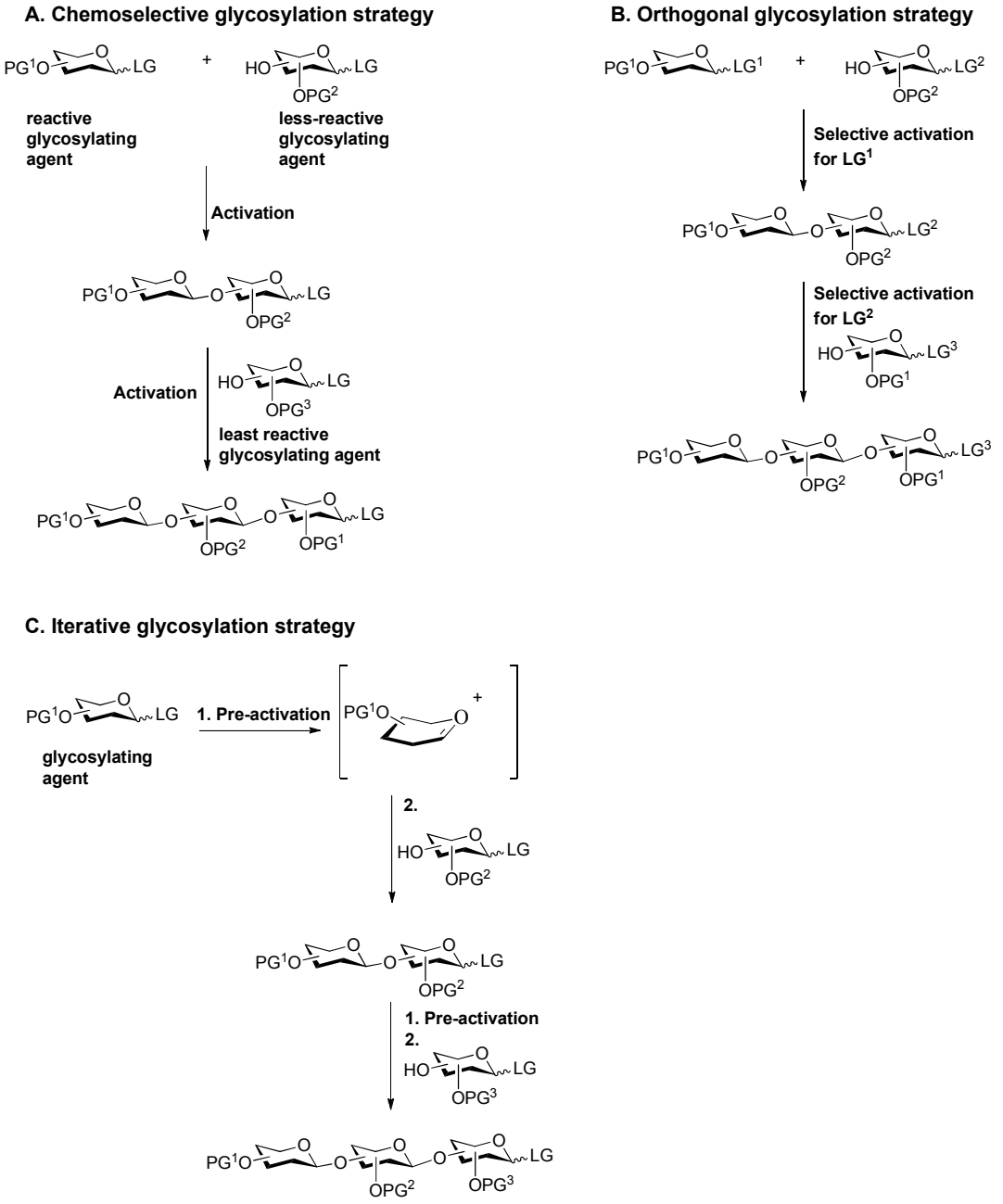
pentasaccharide **104** (Scheme 16).[29] Pentasaccharide **104** is one of the α-Gal epitopes binding specifically to human *anti*-Gal antibodies during xenotransplantation. The one-pot enzymatic route provided tetrasaccharide **105** and pentasaccharide **104**, in 53% and 35% yields, respectively, thus providing a good alternative for chemical syntheses.



Scheme 16. Synthesis of pentasaccharide **104** with *in situ* sugar nucleotide regeneration.

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 anomeric 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.



Scheme 17. One-pot glycosylation strategies.

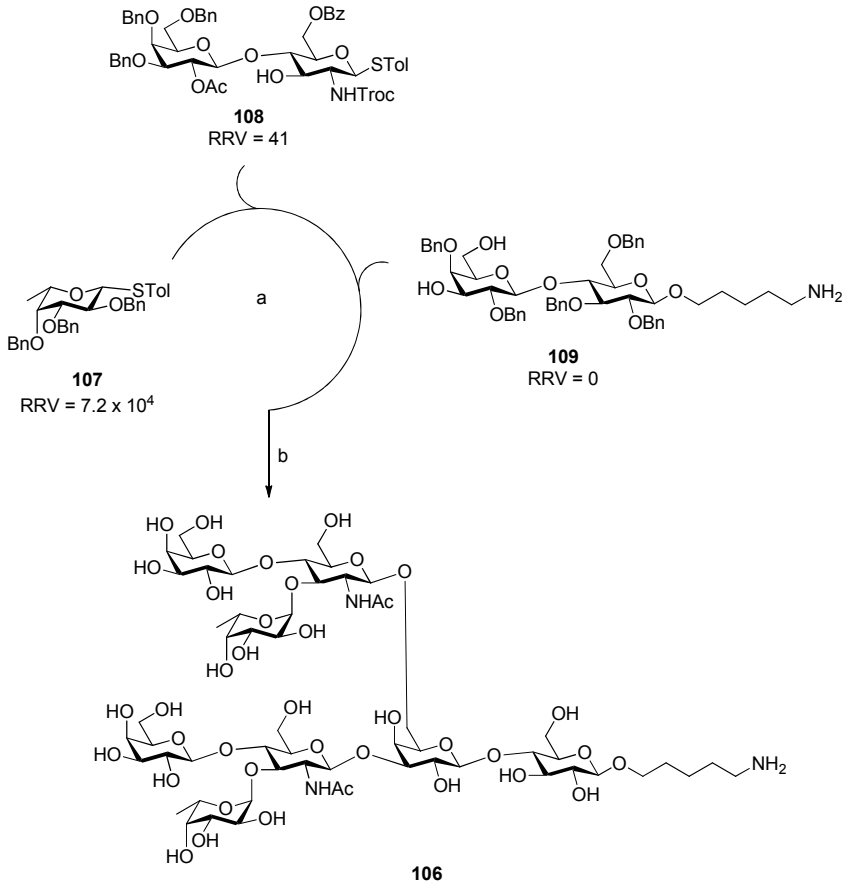
Chemoselective One-Pot Oligosaccharide Synthesis

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:[30] 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).[31] 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.[32] 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).[33] The difference of reactivity between fucosyl donor **107** (RRV = 7.2 x 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.



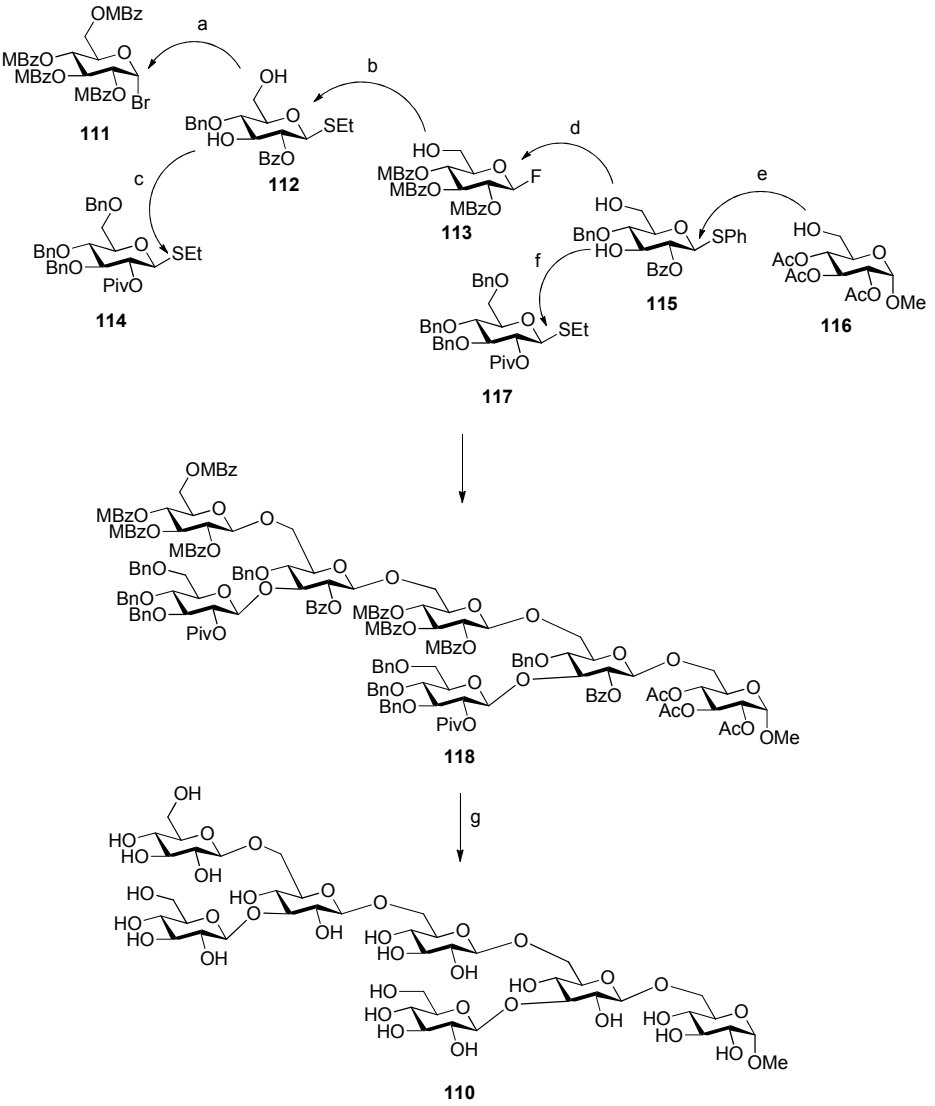
Scheme 18. Chemoselective one-pot synthesis of tumor-associated antigen **106**: a) i. BSP, Tf_2O, CH_2Cl_2, -70 °C to -10 °C; ii. NIS, TfOH, 0 °C to RT; b) i. Zn, AcOH, RT; ii. Ac_2O, DMAP, pyridine, 0 °C to RT; iii. NaOMe, MeOH, RT; iv. Pd/C, H_2, MeOH, HCO_2H, 11% overall yield.

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**[34] by using seven independent building blocks (Scheme 19).

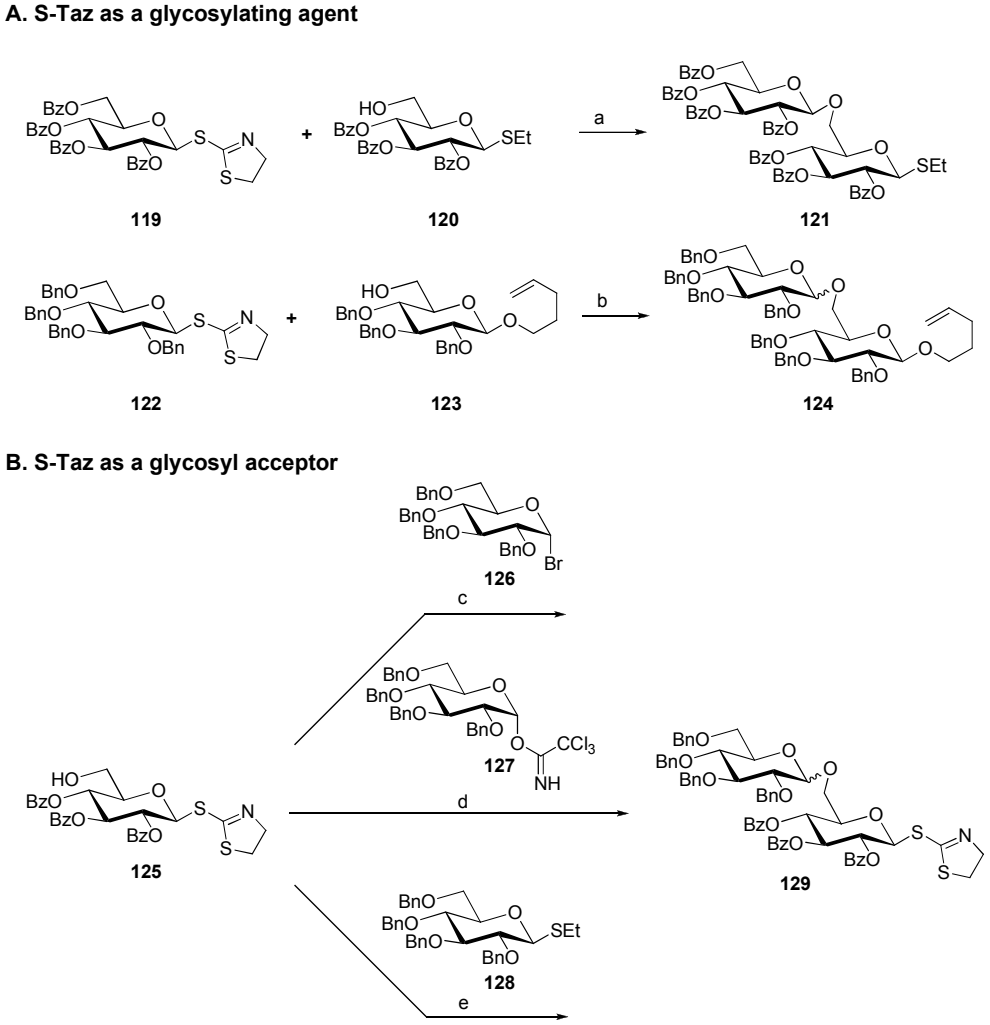


Scheme 19. Orthogonal one-pot synthesis of heptasaccharide **110**: a) AgOTf, CH_2Cl_2; b) MeOTf, CH_2Cl_2; c) MeOTf, CH_2Cl_2; d) $HfCp_2Cl_2$/AgOTf, CH_2Cl_2; e) DMTST, CH_2Cl_2; f) DMTST, CH_2Cl_2, 24% (six steps); g) i. H_2, $Pd(OH)_2$, MeOH, H_2O; ii. NaOMe, MeOH, H_2O, 52% (two steps).

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.[35] 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).[36] Furthermore, *S*-thiazolyl glycosides withstand reaction conditions associated with the activation of the thioglycosylating agents (*N*-iodosuccinime/cat. trifluoromethanesulfonic acid), bromoglycosides (mercury (II) oxide/ mercury (II) bromide) and trichloroacetaimidates (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.[36]

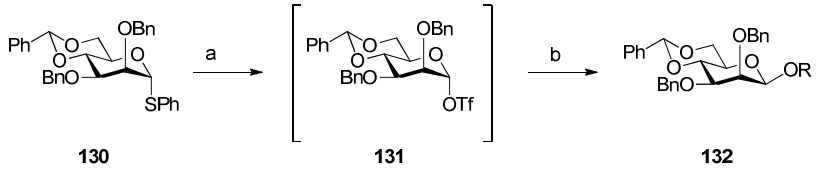


Scheme 20. Orthogonality of *S*-Taz leaving group: a) AgOTf, 81%; b) AgOTf, 91%, 2:1 α/β; c) HgO/$HgBr_2$, 45%, 1.2:1 α/β; d) $BF_3{\cdot}OEt_2$, 50%, 1.1:1 α/β; e) NIS, cat. TfOH, 98%, 1.1:1 α/β.

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.

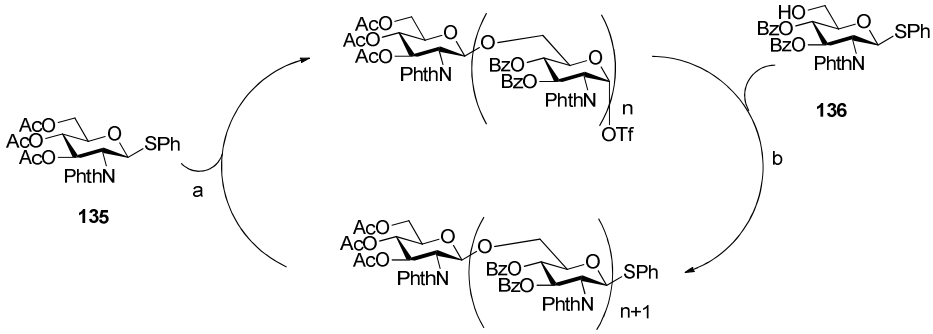
The first step towards the development of this strategy were performed by *Crich*,[37] who reported the pre-activation of thioglycosides using benzenylsulfenyl triflate and 2,6-di-*tert*-butyl-4-methylpyridine as an activating system. Thus, thiomannoside **130** is converted to the more reactive glycosyl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



130 **131** **132**

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.[39] Repetition of the same procedure twice provided tetrasaccharide **134** in 46% overall yield.



135 **136**

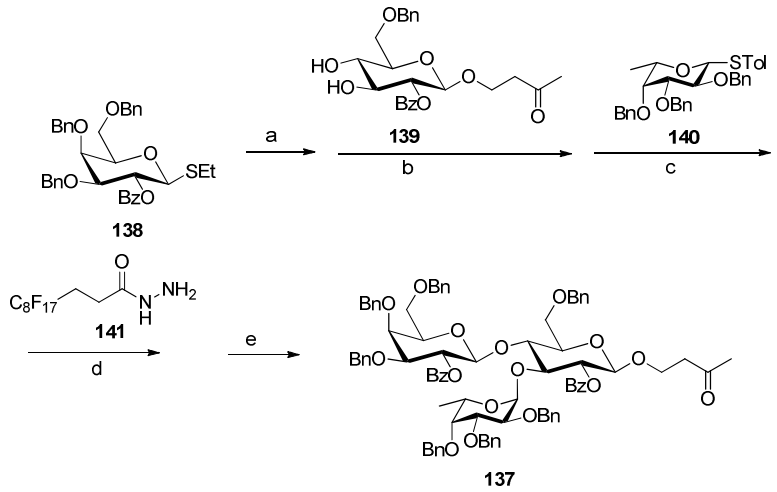
n=0, 1, 2

133 n = 0
134 n = 2

Scheme 22. Iterative synthesis of oligoglucosamines: a) activation: BSP, Tf_2O, -60 °C; b) coupling: -60 °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).[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.

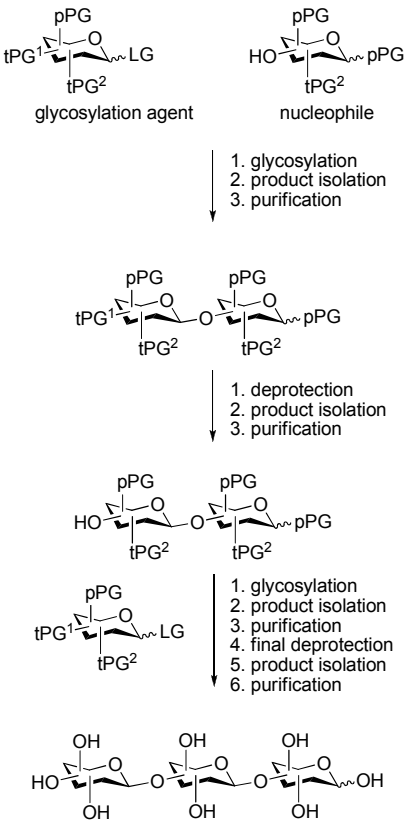


Scheme 23. FSPE-assisted purification of Lewis X trisaccharide: a) AgOTf, *p*-TolSCl, MS 4Å, -78 °C; b) -78 °C to RT; c) AgOTf, *p*-TolSCl, -78 °C to RT; d) CH_2Cl_2/MeOH then FSPE, 62% (five steps).

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

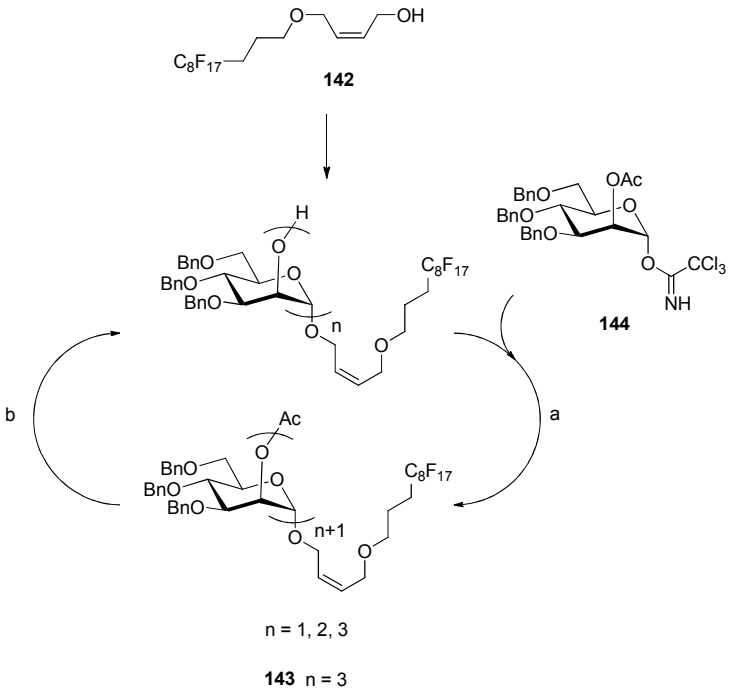
To access libraries of oligosaccharides in a rapid manner, the development of general automated approaches is crucial. The repetitive nature of the oligosaccharide synthesis consisting of a glycosylation step, followed by a deprotection step (Scheme 24), makes it suitable to be performed in an automated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



Scheme 24. Traditional assembly of oligosaccharides.

Fluoruos Assisted Oligosaccharides Synthesis

Fluorous assisted solution-phase oligosaccharides synthesis is a relatively new method[41] that relies upon the solubility properties conferred to a growing oligosaccharide by a fluorous 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 fluorous solid-phase extraction (FSPE) column. In this regard the *Pohl* group designed a fluorous linker **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 fluorous assisted oligosaccharide synthesis, the assembly of a α-(1,2)-tetramannoside **143** was achieved in 79% overall yield. The synthetic cycle consisted of a glycosylation reaction performed with mannosyl imidate **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.



Scheme 25. Fluorous assisted oligomannoside synthesis: a) i. coupling: TMSOTf, CH_2Cl_2, 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.

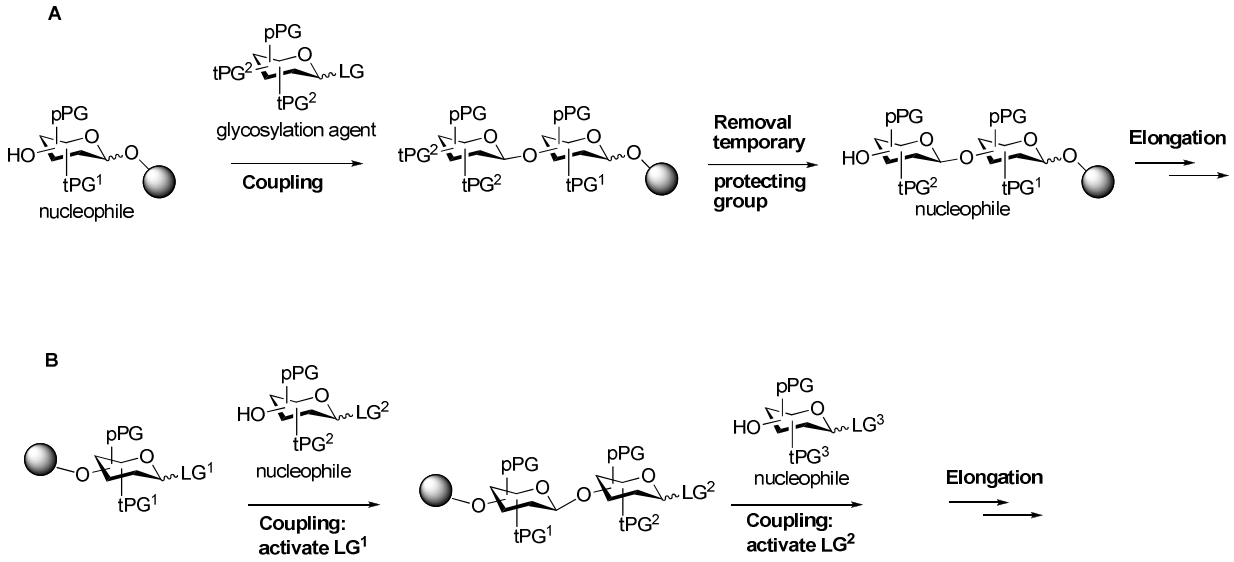
Automated Solid-Phase Synthesis

Inspired by *Merrifield´*s solid-phase peptide synthesis, in 1971 *Schuerch* and *Frechet* introduced the concept of solid-phase oligosaccharide synthesis.[42] 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.[43] In the acceptor-bound strategy

(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.

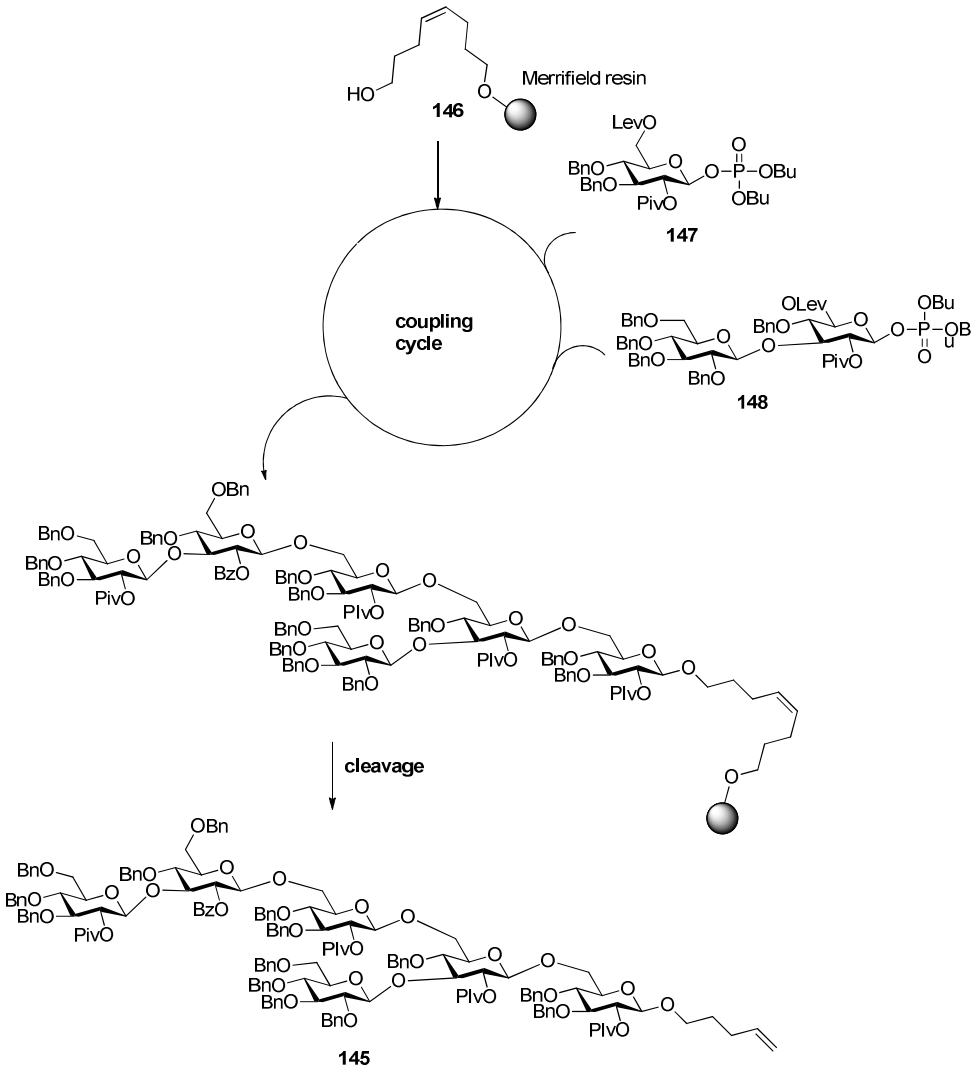
In the donor bound strategy (Scheme 26B), the glycosyl donor linked to the solid support via a suitable hydroxyl group is reacted with the solution-phase building block having a nucleophilic group and an orthogonal leaving group for chain elongation. This approach does not require removal of the temporary 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[44] 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.[45]



Scheme 27. Automated solid-phase synthesis of hexasaccharide **145**: Coupling cycle: i. **147** or **148**, TMSOTf, CH_2Cl_2, -15 °C, 15 min; ii. N_2H_4, pyridine, AcOH, 15 °C, 15 min; Cleavage: Grubbs catalyst, ethylene, CH_2Cl_2, 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]

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.

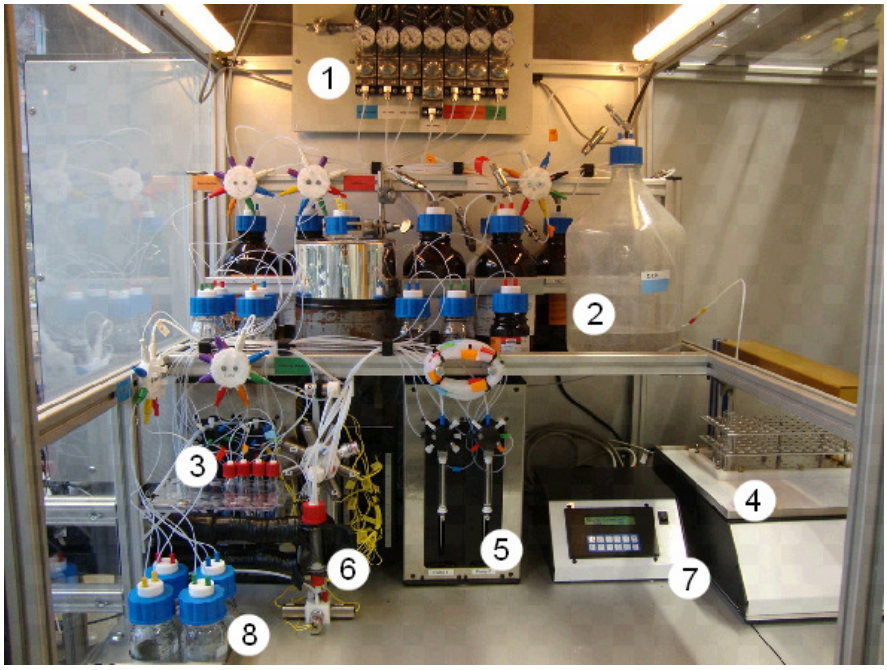


Figure 1. Fully automated synthesizer: (1) reaction vessel; (2) solvent reservoirs; (3) building block vessels; (4) fraction collector; (5) syringe pumps; (6) reaction vessel; (7) controller; (8) reagents.

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

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.

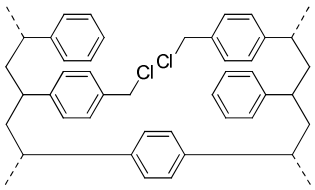
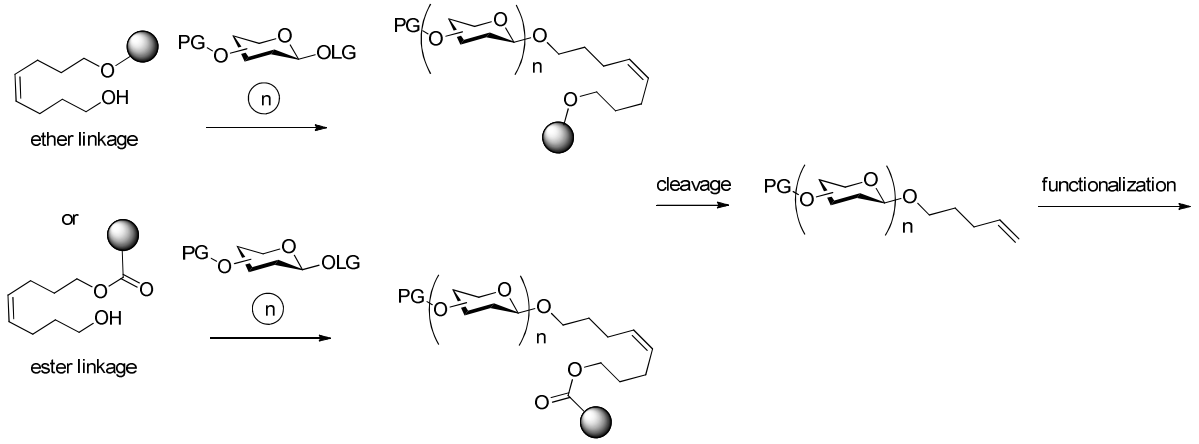


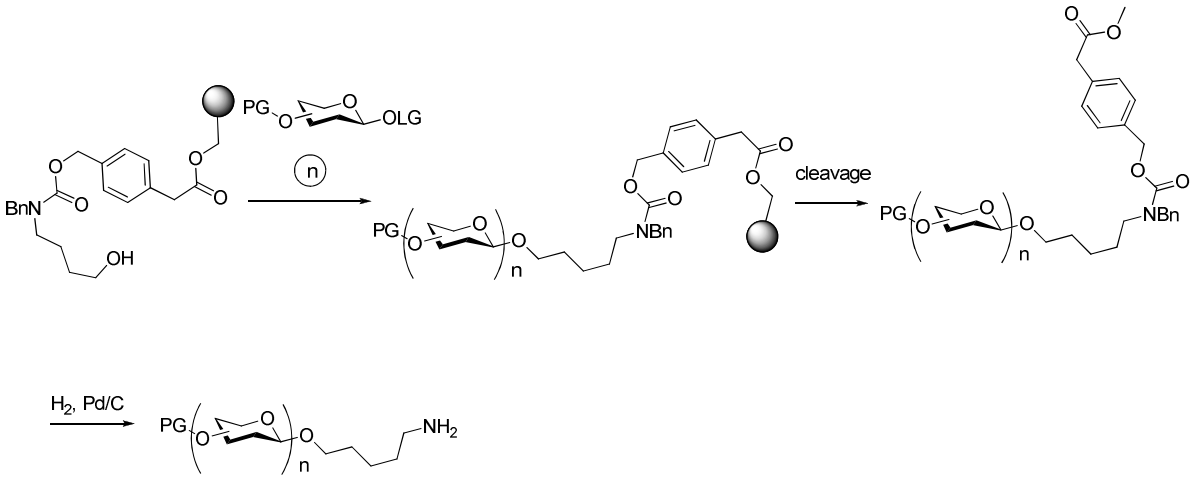
Figure 2. Structure of *Merrifield* resin

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).



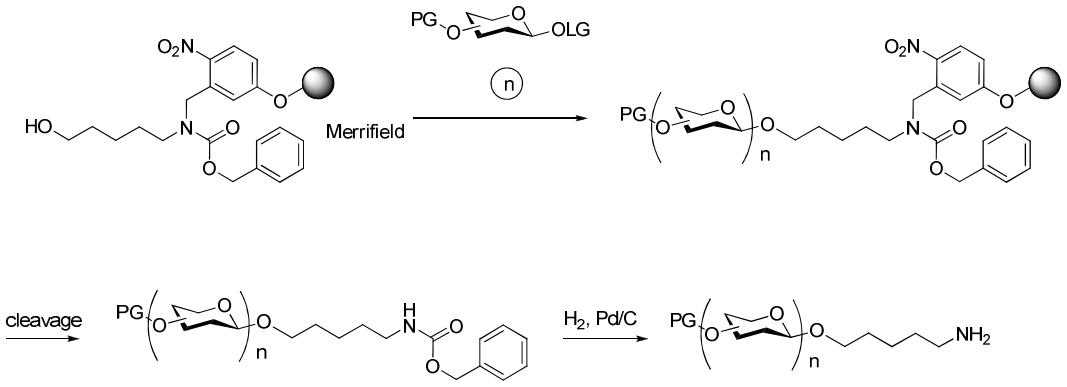
Scheme 28. Octenediol linker.

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).[52] 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.



Scheme 29. Basic labile linker: cleavage: NaOMe, MeOH.

Finally, a photocleavable linker was recently developed (Scheme 30).[53] 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.



Scheme 30. Photocleavable linker: cleavage: hυ, CH_2Cl_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 benzyloxycarbonyl protecting group by hydrogenolysis releases the terminal amino group for further functionalization.

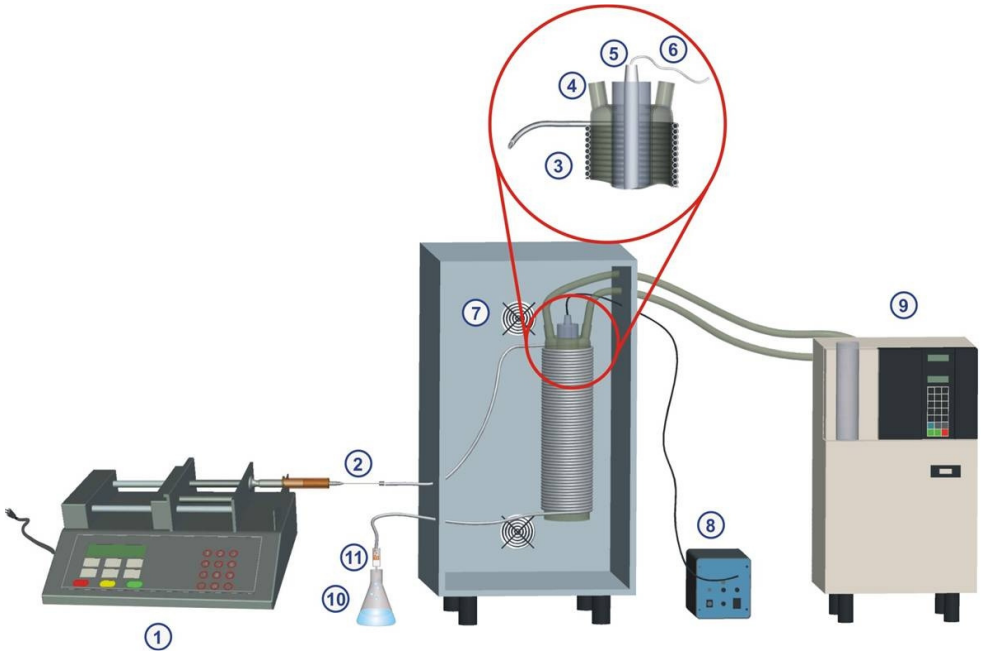
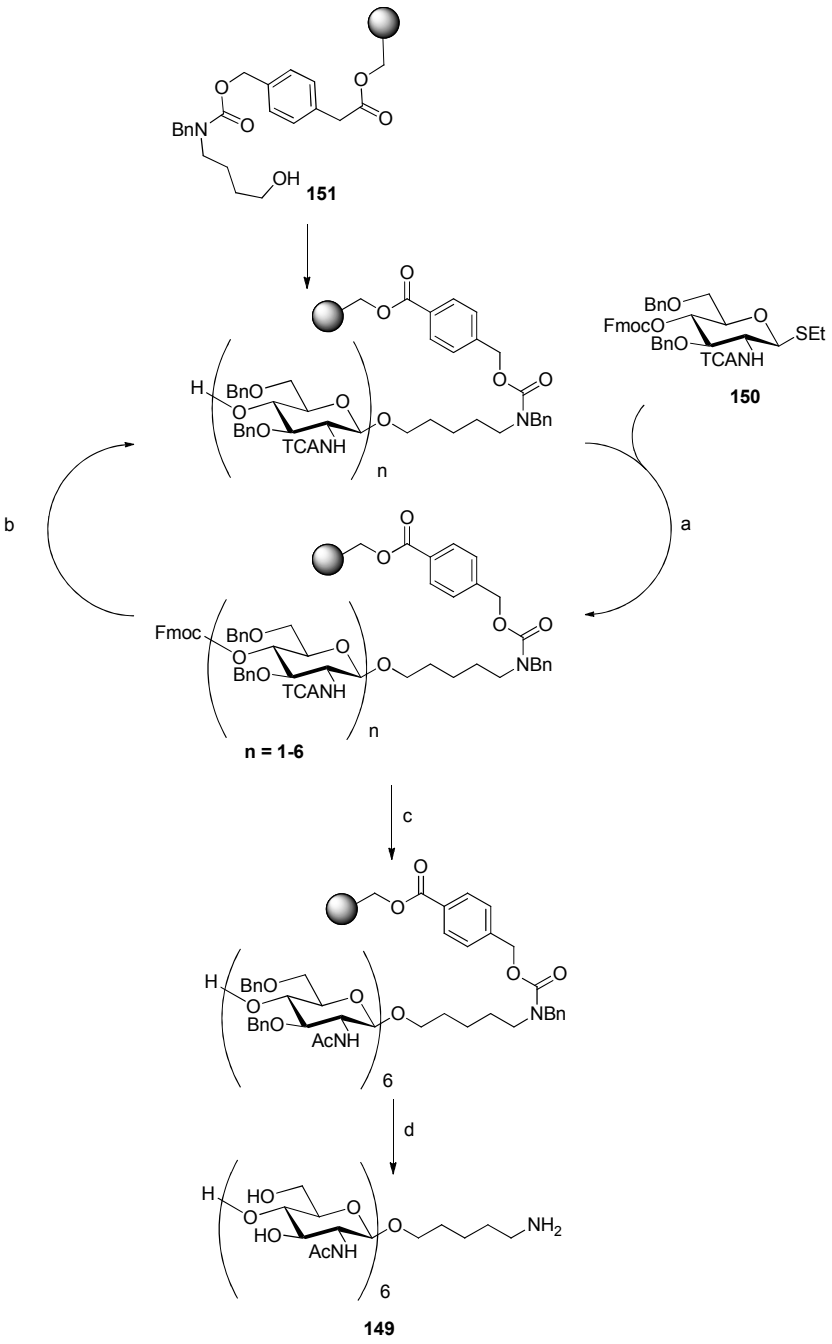


Figure 3. Cleavage of the linker from the solid support by exposure to UV light in continuous flow: (1) syringe pump; (2) inlet; (3) FEP (fluorinated-ethylene-propylene) tubing; (4) cooling system; (5) UV filter (pyrex); (6) UV lamp; (7) fan; (8) power supply; (9) cryostat; (10) outlet; (11) frit.

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[47] reported the synthesis of β-(1,4)-hexaglucosamine **149** (Scheme 31), a compound found on the surface of pathogenic bacteria, starting from glucosamine thioglycoside **150** and *Merrifield* resin functionalized with the base labile linker **151**. To ensure the β-selectivity, the thioglucoside **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.[55] Other hydroxyl protecting groups such as nitrophtalimidobutyric acid were developed to help for colorimetric monitoring of the coupling yields in solid-phase synthesis of carbohydrates.[56] The coupling cycle involved a glycosylation step performed with thioglycoside **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 **149** in 24% overall yield.

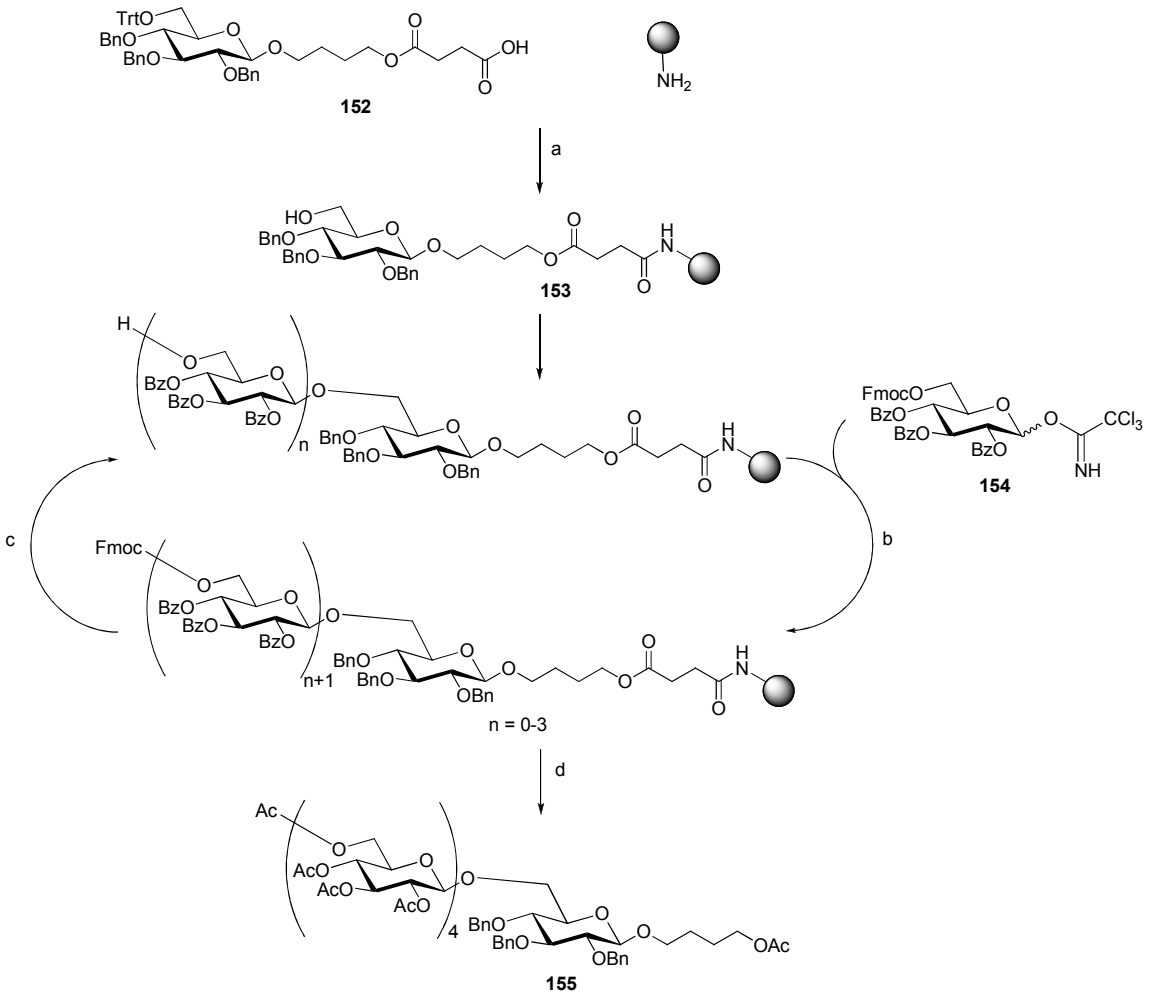


Scheme 31. Automated solid-phase synthesis of β-(1,4)-oligoglucosamine **149**: a) NIS/TfOH, dioxane, CH_2Cl_2, -40 °C to -20 °C; b) piperidine, DMF; c) Bu_3SnH, AIBN; d) i. NaOMe, MeOH; ii. H_2, Pd/C, 24% overall yield.

Sialylated oligosaccharides,[57] β-(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-NH_2 was connected to the HPLC-system.

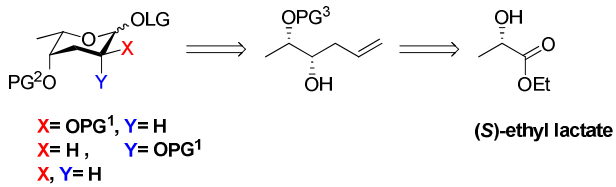


Scheme 32. HPLC-assisted automated solid phase synthesis: a) i. EDC, DMAP, CH_2Cl_2; ii. TFA, wet CH_2Cl_2; b) TMSOTF, CH_2Cl_2; c) piperidine, DMF; d) NaOMe, MeOH, CH_2Cl_2; e) Ac_2O, pyridine, 62% overall yield.

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

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.

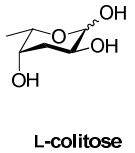
2. *De Novo* Synthesis of L-Colitose, 2-*epi*-L-Colitose and L-Rhodinose Building Blocks



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-*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.

2.1 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[61], *Salmonella enterica*[62], *Salmonella adelaide*[63], *Salmonella greenside* and *Vibrio cholerae*[64] 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*.[65] 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.

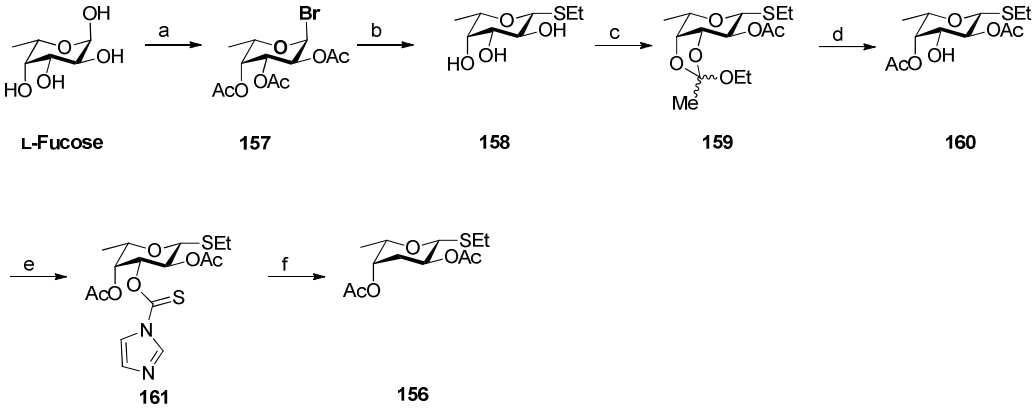


L-colitose

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.[66]
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).[67] 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

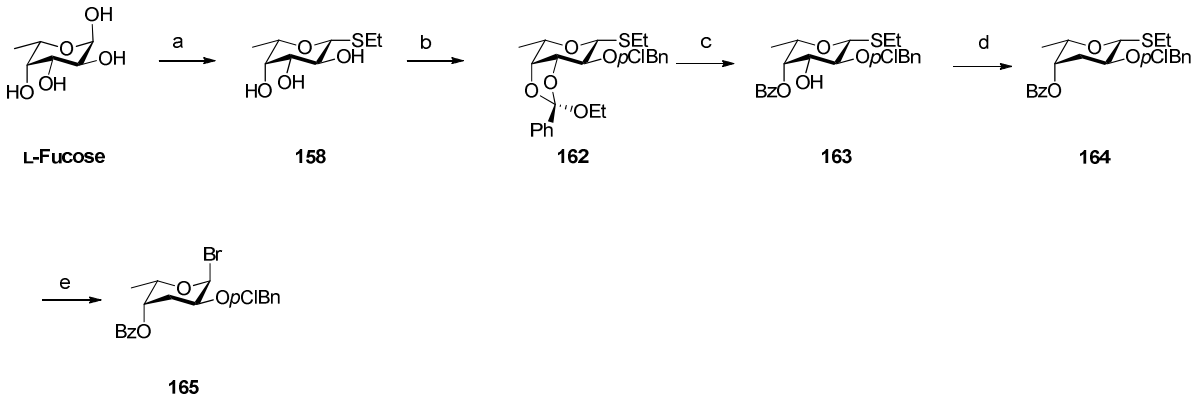
68% overall yield over seven steps. Deoxygenation of the alcohol at C3 via a *Barton-McCombie* procedure provided, in two steps and 88% yield, L-colitose building block **156**. Thus, *Kováč* completed in nine steps and 60% overall yield the synthesis of disarmed L-colitose glycosylating agent **156** starting from α-L-fucose. Although, the below described synthesis is high yielding, the cost of the starting material[68] 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.



Scheme 33. *Kováč´*s route towards L-colitose building block: a) i. Ac_2O, pyridine, DMAP, 0 °C; ii. HBr-AcOH, CH_2Cl_2, 0 °C to RT; b) i. EtSH, NaH, DME, 0 °C to RT; ii. anhydr. K_2CO_3, MeOH; c) i. $CH_3C(OEt)_3$, CSA, CH_2Cl_2, RT; ii. Ac_2O, pyridine, DMAP, 0 °C to RT; d) 90% aq. AcOH, RT, 3 h, 68% (seven steps); e) TCDI, DMAP, DMF, RT, 96%; f) Bu_3SnH, AIBN, toluene, reflux, 92%.

*Oscarson´*s group has also developed routes towards L-colitose building blocks.[69] 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*-chlorobenzylation 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**.

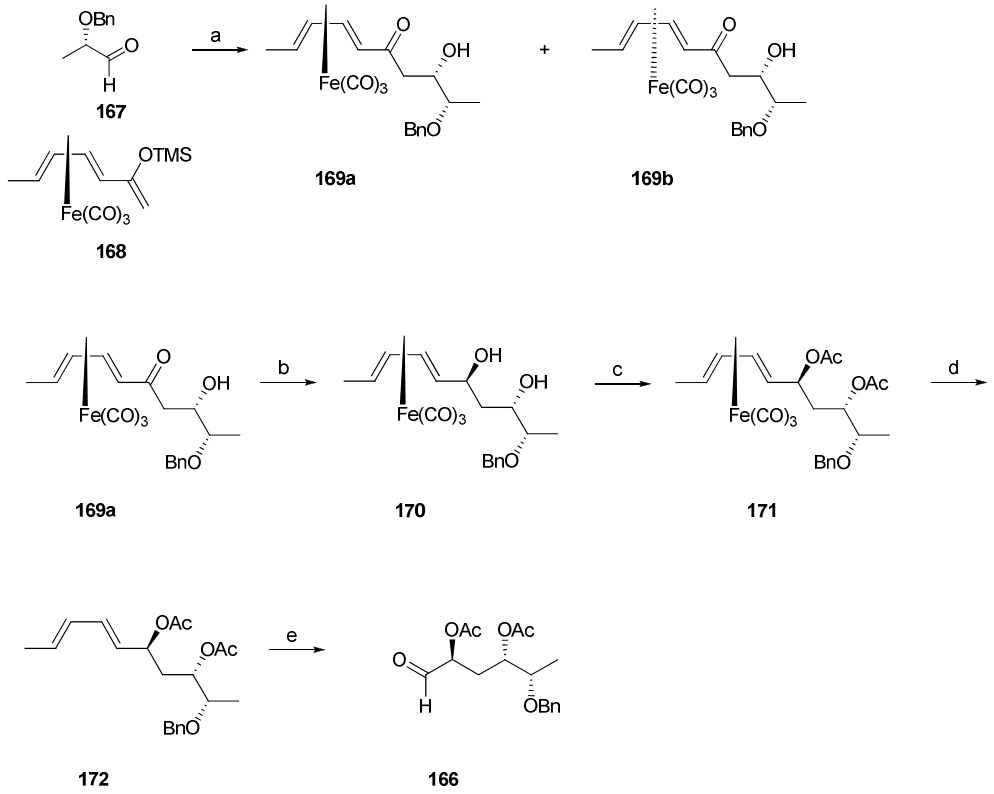
*Oscarson´*s route provided in nine steps a differentially protected colitose building block that was successfully used in the assembly of oligosaccharides. However, the synthetic methodology was low yielding (**164**, 26% over eight steps) and used expensive starting material.



Scheme 34. *Oscarson´*s route towards L-colitose building block: a) i. Ac_2O, pyridine, DMAP, 0 °C; ii. EtSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, RT; iii. K_2CO_3, MeOH, 64% (three steps); b) i. $PhC(OEt)_3$, *p*-TsOH, CH_3CN; ii. *p*-ClBnCl, NaH, DMF; c) TFA (90% aq.), CH_3CN, 61% (three steps); d) i. $C(S)Im_2$, imidazole, $(CH_2Cl)_2$; ii. Bu_3SnH, AIBN, toluene, reflux, 67% (two steps); e) Br_2, CH_2Cl_2.

Similar synthetic routes starting from L-fucose were also reported by *Bundle*[70] and *Thiem*[71]. 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*)-benzyloxylacetaldehyde **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*)-benzyloxylacetaldehyde **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.



Scheme 35. Total synthesis of L-colitose building block **166**: a) $TiCl_4$, CH_2Cl_2, -78 °C, **169a** (38%), **169b** (38%); b) $BH_3{\cdot}Me_2S$, THF, 20 °C, 87%; c) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 20 °C, 82%; d) CAN, MeCN, H_2O, 0 °C, 91%; e) i. O_3, MeOH, -78 °C; ii. Me_2S, 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[15a,16,17,73] I decided to develop a *de novo* synthesis of a L-colitose glycosylating agent starting from inexpensive commercially available (*S*)-ethyl lactate.[74] 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).

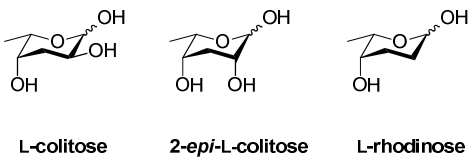
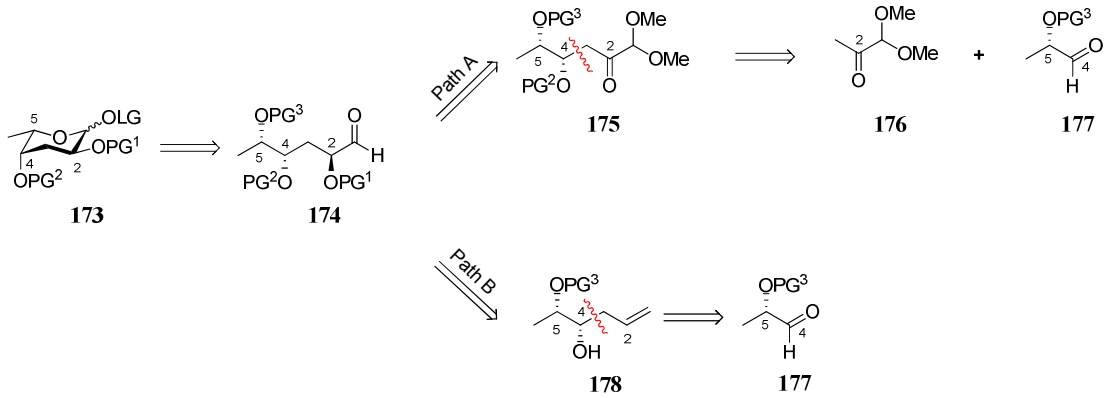


Figure 5. Rare monosaccharide targets.

2.2 Results and Discussions

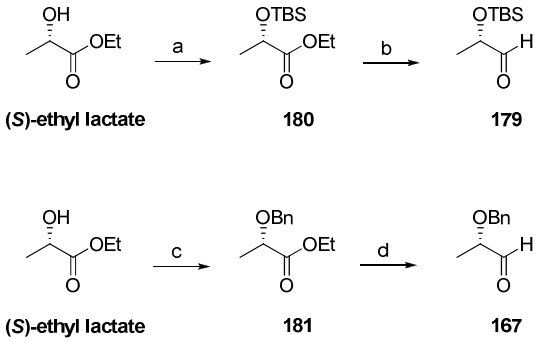
The retrosynthetic design targeted functionalized L-colitose building block **173** (Scheme 36) to be generated from acyclic precursor aldehyde **174**. Two approaches were considered to access differentially protected aldehyde **174**. Path A involved disconnection of β-hydroxy ketone intermediate **175** 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.[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-colitose building block **173**.

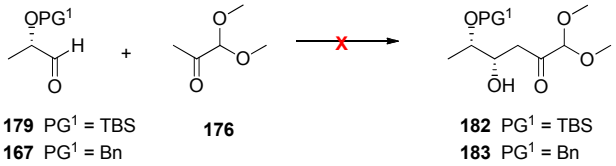
Aldol-based construction of the carbon backbone (Path A)

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**.



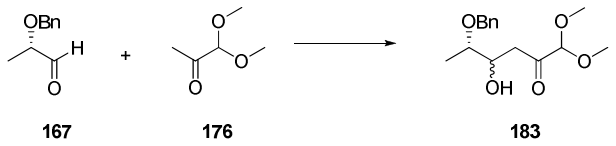
Scheme 37. Synthesis of the aldol donors **179** and **167**: a) TBSCl, DMAP, imidazole, CH_2Cl_2, RT, 3 h, 98%; b) DIBAL, CH_2Cl_2, -78 °C, 5 h, 70%; c) BnBr, NaH, CH_2Cl_2, 0 °C, 4 h, 57%; d) DIBAL, CH_2Cl_2, -78 °C, 4 h, 87%.

The proline-catalyzed aldol reaction was previously used for the synthesis of ulosonic acid precursors with moderate yields, but good diastereoselectivities.[76] Thus, this aldol-based method was explored to approach 1,3-hydroxy ketones **182** and **183**. For this purpose, aldehydes **179** and **167** were treated with commercially available ketone **176** 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 **167** with ketone **176** at room temperature resulted in a 1:1 inseparable diasteroisomeric mixture (12%). The poor yield and distarereoselectivity 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.

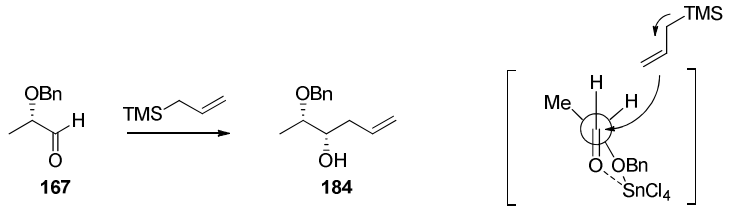
An alternative aldol reaction, using a lithium enolate generated *in situ* with lithium bis(trimethylsilyl)amide, was attempted. This method didn't prove more efficient producing an inseparable mixture of adduct **183** in 35% yield with 1:1 dr (Scheme 39).



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

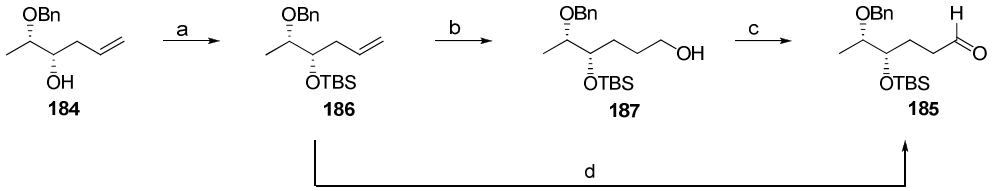
Allylation-based construction of the carbon backbone (Path B)

Due to difficulties encountered with aldol-based C-C bond forming reactions, an allylation[77] strategy was considered. Initial attempts used known (*S*)-benzyloxyaldehyde **167**[78] as substrate. Treatment of the aldehyde **167** with allyltrimetylsilane in presence of tin tetrachloride as a coordinating agent provided 4,5-*syn* diol **184** 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[75] transition state depicted in Scheme 40.



Scheme 40. Stereoselective synthesis of homoallylic alcohol **184**: $SnCl_4$, CH_2Cl_2, -78 °C, 2 h, 7:1 (*syn*/*anti*), 74%.

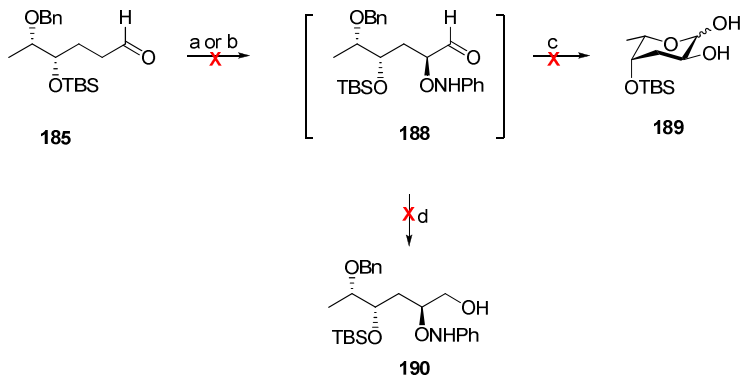
Having established a route to 4,5-*syn* diol **184**, the stereoselective introduction of the hydroxyl at position 2 was the next goal. At this level, several routes were investigated. Initially, the stereoselective organocatalytic α-aminoxylation of aldehydes[79] was explored. To test this method, aldehyde substrate **185** was prepared. Firstly, homoallylic alcohol **184** was treated with *tert*-butyldimethylsilyl trifluoromethansulfonate 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 procedure[80] 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(I).[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. Synthesis of aldehyde **185**: a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 3 h, 100%; b) catechol borane, $Rh(PPh_3)_3Cl$, THF, 0 °C, 90 min then H_2O_2, RT, 62%; c) SO_3·pyridine, DMSO, 0 °C, 1 h, 92%; d) 9-BBN, CH_2Cl_2, 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*)-

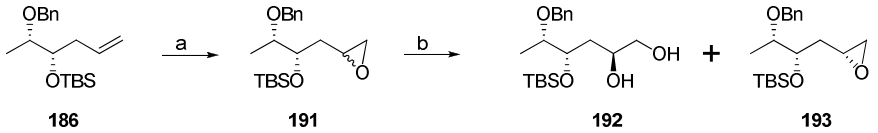
proline in dimethylsulfoxide.[83] After complete consumption of the starting material and work-up, the reaction mixture was directly submitted to standard hydrogenation conditions leading to a mixture of products from which the target hemiacetal **189** could not be isolated (Scheme 42). To simplify 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,[79a,79c,84] 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. 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), CH_3CN, -20 °C, 24 h; c) H_2, Pd/C, AcOEt, 24 h; d) $NaBH_4$.

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*-butyldimethylsilyl 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 diasteroisomeric 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)[85] 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.

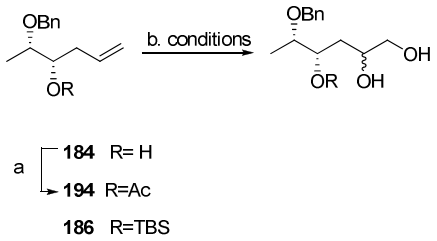


186 **191** **192** **193**

Scheme 43. Hydroxyl introduction at C2 via hydrolytic kinetic resolution: a) *m*-CPBA, CH_2Cl_2, 24 h, RT; b) (*S*, *S*)-(salen)-Co(II), AcOH, H_2O, THF, 24 h, RT, **192** (20%), **193** (38%).

Finally, *Sharpless* asymmetric dihydroxylation (SAD)[86] method was explored (AD, Table 1). At first, homoallylic alcohol **184** was treated with the appropriate reagent AD-mix α in a mixture *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[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.[88]

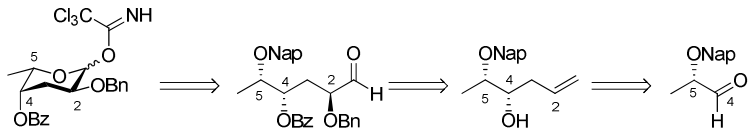
Table 1. Attempted dihydroxylation reactions: a) Ac_2O, pyridine, 12 h, RT, 99%; b) See conditions.



Entry	Substrate	Conditions	dr[a,b]
1	**184**	AD-mix β, *tert*-BuOH/H_2O (1:1), 0 °C, 60 h	1.5:1
2	**184**	AD-mix α, *tert*-BuOH/H_2O (1:1), 0 °C, 60 h	1.5:1
3	**194**	AD-mix β, *tert*-BuOH/H_2O (1:1), 0 °C, 60 h	2.5:1
4	**194**	AD-mix α, *tert*-BuOH/H_2O (1:1), 0 °C, 60 h	2:1
5	**186**	AD-mix β, *tert*-BuOH/H_2O (1:1), 0 °C, 60 h	1:1
6	**186**	AD-mix α, *tert*-BuOH/H_2O (1:1), 0 °C, 60 h	1:1

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

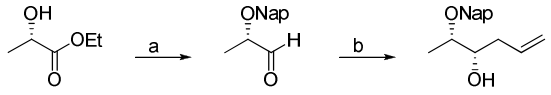
Although the levels of diastereoselectivity were low, the possibility to separate the 1,3-*anti* triol from its 1,3-*syn* diastereoisomer by simple chromatography on silica gel convinced me to retain this route in order to access L-colitose target building block. Since colitose is naturally found only α-linked to other sugars, strategically a building block with a non-participating protecting group PG^1 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^3) is orthogonal to both C2-PG^1 and C4-PG^2. 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.



195 **196** **197** **198**

Scheme 44. Retrosynthetic analysis of L-colitose building block **195**.

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]

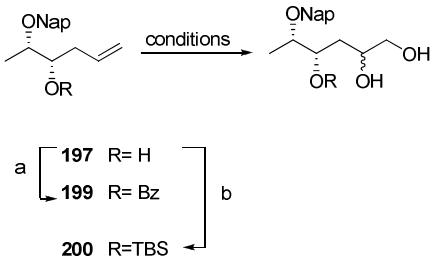


(*S*)-ethyl lactate **198** **197**

Scheme 45. Synthesis of homoallylic alcohol **197**: a) i. NapBr, NaH, DMF, 2 h, 0 °C to RT; ii. DIBAL, CH_2Cl_2, -78 °C, 90 min, 77% (two steps); b) $SnCl_4$, CH_2Cl_2, -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.

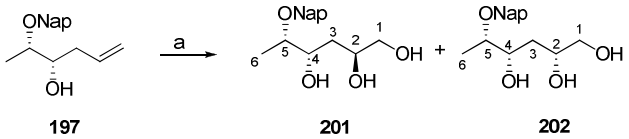
Table 2. Survey of the dihydroxylation attempts: a) BzCl, pyridine, 0 °C to RT, 2 h, 100%; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 2.5 h, 95%.



Entry	Substrate	Conditions	dr[a,b]
1	**197**	AD-mix β, *tert*-BuOH/H_2O (1:1), 0 °C, 12 h	1:1
2	**197**	AD-mix α, *tert*-BuOH/H_2O (1:1), 0 °C, 12 h	1.5:1
3	**199**	AD-mix β, *tert*-BuOH/H_2O (1:1), 0 °C, 48 h	2:1
4	**199**	AD-mix α, *tert*-BuOH/H_2O (1:1), 0 °C, 12 h	2:1
5	**200**	AD-mix β, *tert*-BuOH/H_2O (1:1), 0 °C, 12 h	1.3:1
6	**200**	AD-mix α, *tert*-BuOH/H_2O (1:1), 0 °C, 12 h	1.7:1

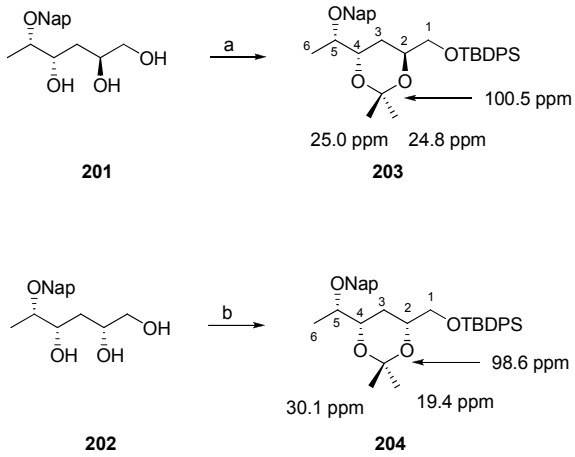
[a.] Ratios determined by ^{1}H NMR. [b.] Major and minor diastereomers of the product mixtures were not determined

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_2O (2/1, v/v), RT, 24 h, **201** (49%), **202** (44%).

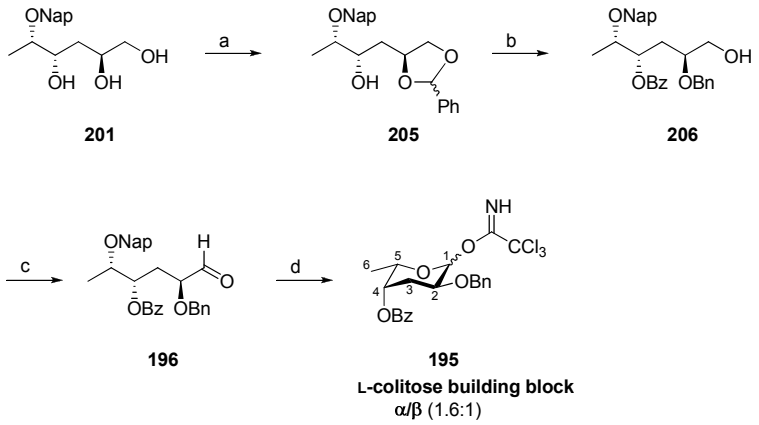
The absolute configuration of the newly introduced hydroxyl at C2 was established using *Rychnovsky´*s acetonide method[92] (Scheme 47). Selective protection of the primary alcohol on substrate **201** as a *tert*-butyldiphenylsilyl ether, followed by treatment of the intermediate diol with 2,2-dimethoxypropane in the presence of pyridinium *para*-toluenesulfonate afforded acetonide **203**. According to the ^{13}C chemical shifts (see Experimental Section) of the acetonide methyl groups and quaternary carbon, it was determined that **201** had a 2,4-*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 *S*. The same procedure was applied to triol **202** to afford acetonide **204**. The ^{13}C NMR data (see Experimental Section) was consistent with the assignment of a 2,4-*syn* configuration for **202** and thus, an *R*-configured C2 stereocenter.



Scheme 47. Synthesis of the C2-C4 acetonides **203** and **204**: a) i. TBDPSCl, imidazole, DMAP, CH_2Cl_2, RT, 18 h; ii. 2,2-DMP, PPTS, CH_2Cl_2, RT, 4 h, 83%; b) i. TBDPSCl, imidazole, DMAP, CH_2Cl_2, RT, 18 h; ii. 2,2-DMP, PPTS, $CuSO_4$, acetone, RT, 15 min, 95%.

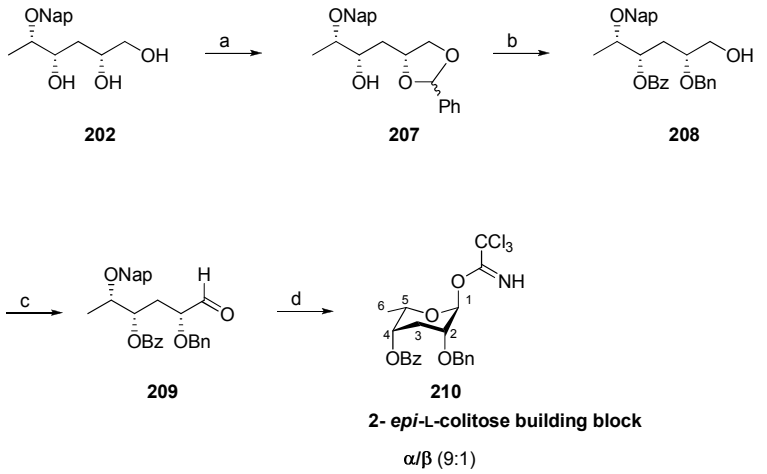
Having set and defined all the necessary stereocenters in triol **201**, the synthesis of L-colitose **195** was completed (Scheme 48). Thus, triol **201** was converted selectively to the 5-membered benzylidene acetal **205**. The remaining hydroxyl group in **205** was benzoylated and the benzylidene 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*[93] or *Parikh-Doering* conditions, *Dess-Martin* periodinane[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 L-colitose

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**.



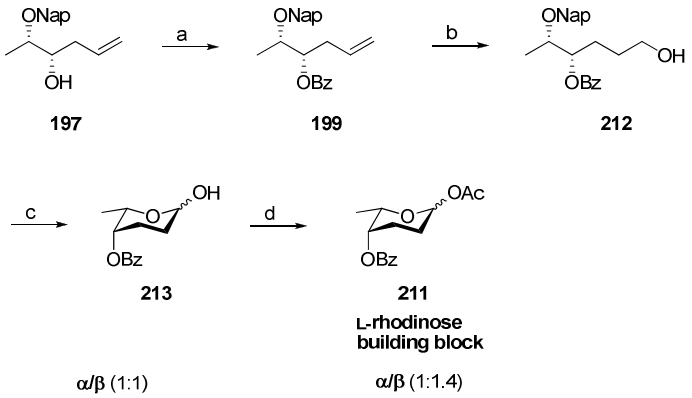
Scheme 48. Synthesis of L-colitose building block **195**: a) $PhCH(OMe)_2$, CSA, $CuSO_4$, CH_3CN/THF (1/1, v/v), RT, 10 min; b) i. BzCl, pyridine, RT, 2 h; ii. BH_3·THF, TMSOTf, CH_2Cl_2, 0 °C to RT, 2 h, 71% (three steps); c) Dess-Martin periodinane, pyridine, CH_2Cl_2, RT, 1 h, 92%; d) i. DDQ, MeOH, CH_2Cl_2, 0 °C to RT, 2 h; ii. CCl_3CN, DBU, CH_2Cl_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.[95] 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.



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.



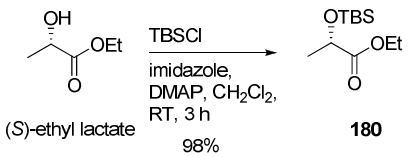
Scheme 50. Synthesis of L-rhodinose building block **211**: a) BzCl, pyridine, RT, 2 h, quant.; b) catechol borane, $Rh(PPh_3)_3Cl$, THF, 0 °C, 90 min then NaOH, H_2O_2, 95%; c) i. $Py{\cdot}SO_3$, DMSO, DIPEA, CH_2Cl_2, 0 °C; 1 h; ii. DDQ, MeOH, CH_2Cl_2, 0 °C to RT, 2 h, 85% (two steps); d) Ac_2O, 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 usefull for the synthesis of natural products and for the basis of oligosaccharide conjugate vaccines against bacteria that contain the respective glycans on their surface.

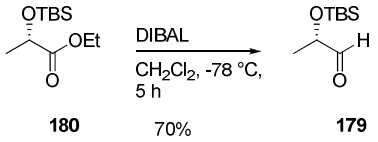
2.4 Experimental Section

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 qaudruplet 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.



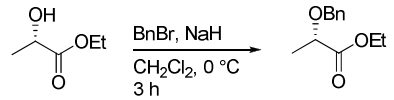
(*S*)-Ethyl-2-(*tert*-butyldimethylsilyloxy)propanoate (180): To a solution of (*S*)-ethyl-lactate (1.0 g, 8.5 mmol) in CH_2Cl_2 (10 mL), DMAP (0.1 g, 0.85 mmol, 0.1 equiv), imidazole (0.70 g, 10.1 mmol, 1.2 equiv) and TBSCl (1.4 g, 9.3 mmol, 1.2 equiv) were added. After stirring at RT for 3 h, the reaction was diluted with H_2O and CH_2Cl_2. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude

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 consisted with the reported literature.[78]



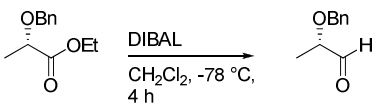
180 70% **179**

(*S*)-2-(*tert*-Butyldimethylsilyloxy)propanal (179): A solution of silyl ether **180** (1.6 g, 6.8 mmol) in CH_2Cl_2 (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 CH_2Cl_2. The combined organic extracts were washed with H_2O, brine, dried over $MgSO_4$ 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 consisted with the reported literature.[78]



(*S*)-ethyl lactate 57% **181**

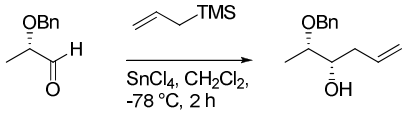
(*S*)-Ethyl-2-(benzyloxy) propanoate (181): At 0 °C, a solution of (*S*)-ethyl-lactate (9.7 mL, 85 mmol) and benzyl bromide (11.0 mL, 93 mmol, 1.1 equiv) in CH_2Cl_2 (120 mL) was treated with NaH (60% in mineral oil, 3.7 g, 93 mmol, 1.1 equiv). The reaction was stirred at 0 °C for 3 h and then quenched with ethanol and extracted with CH_2Cl_2. After separation, the organic layer was washed with H_2O, brine, dried over $MgSO_4$ 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 consisted with the reported literature.[78]



181 87% **167**

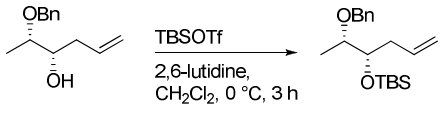
(*S*)-2-(Benzyloxy) propanal (167): A solution of benzyl ether **181** (840 mg, 4.0 mmol) in CH_2Cl_2 (13 mL) was cooled to -78 °C. 1 M DIBAL in cyclohexane (8.1 mL, 8.1 mmol, 2

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 CH_2Cl_2. The combined organic extracts were washed with H_2O, brine, dried over $MgSO_4$ 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.[78]



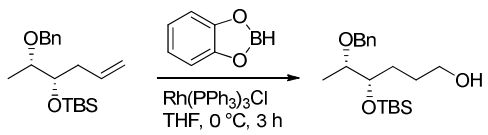
167 74% **184**

(*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 CH_2Cl_2 (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 H_2O and warmed slowly to RT. The mixture was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over $MgSO_4$ 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.[98]



184 100% **186**

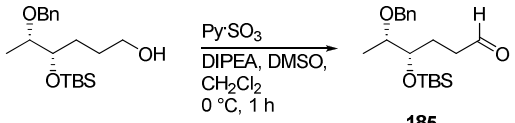
((2*S*, 3*S*)-2-(Benzyloxy)hex-5-en-3-yloxy)(*tert*-butyl)dimethylsilane (186): At 0 °C, a solution of (2*S*, 3*S*)-2-(benzyloxy)hex-5-en-3-ol (**184**) (1.0 g, 4.85 mmol) in CH_2Cl_2 (50 mL) was treated with 2,6-lutidine (1.1 mL, 9.7 mmol, 2 equiv) and TBSOTf (1.7 mL, 7.3 mmol, 1.5 equiv). After being stirred at 0 °C for 3 h, the reaction was quenched with a saturated aq. solution of $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic extracts were washed with H_2O, brine, dried on $MgSO_4$ 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.[98]



186 **187**

62%

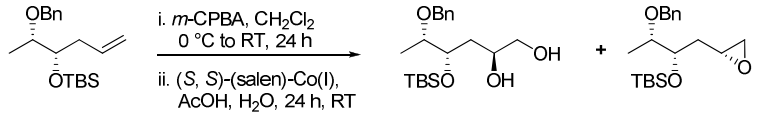
(*4S*, *5S*)-5-(Benzyloxy)-4-(*tert*-butyldimethylsilyloxy)hexan-1-ol (187): A solution of ((*2S*, *3S*)-2-(benzyloxy)hex-5-en-3-yloxy)(*tert*-butyl)dimethylsilane (**186**) (0.5 g, 1.56 mmol) and $Rh(PPh_3)_3Cl$ (50 mg, 55 µmol, 0.035 equiv) in THF (12 mL) was cooled to 0 °C. The catecholborane (4.7 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% H_2O_2 (6.5 mL) were added and the reaction was stirred vigorously overnight. The reaction was diluted with Et_2O, washed with a saturated aq. solution of $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under reduced pressure. Flash column chromatography (Cyclohexane/AcOEt = 80:20, 1% Et_3N) afforded alcohol **187** (325 mg, 960 µmol, 62%) as a colorless oil. The spectral data are consisted with the reported literature.[98]



187 **185**

92%

(*4S*, *5S*)-5-(Benzyloxy)-4-(*tert*-butyldimethylsilyloxy)hexan-1-al (185): At 0 °C, a solution of (*4S*, *5S*)-5-(benzyloxy)-4-(*tert*-butyldimethylsilyloxy)hexan-1-ol (**187**) (320 mg, 946 µmol) and *N*,*N*-diisopropylethylamine (0.82 mL, 4.8 mmol, 5 equiv) in CH_2Cl_2 (4.8 mL) and DMSO (0.7 mL) was treated with $Py{\cdot}SO_3$ 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 $Na_2S_2O_3$ (30 mL) and warmed to RT. The organic layer was washed with H_2O, brine, dried over $MgSO_4$ 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.[98]



186 **192** **193**

20% 39%

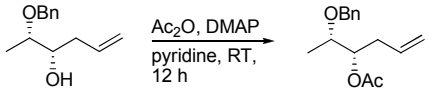
(2*S*, 4*S*, 5*S*)-5-(Benzyloxy)-4-(*tert*-butyldimethylsilyloxy)hexane-1,2-diol (192) and (2*R*, 4*S*, 5*S*)-5-(benzyloxy)-1,2-(epoxy)-4-(*tert*-butyldimethylsilyloxy)hexane (193): At 0 °C, *m*-CPBA (70%, 845 mg, 3.43 mmol, 1.2 equiv) was added to a solution of alkene **186** (916 mg, 2.86 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at RT for 24 h and quenched with a saturated aq. solution of $Na_2S_2O_3$. After 10 min of vigorous stirring, the mixture was extracted with CH_2Cl_2. The organic layer was washed with a saturated aq. solution of $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (Cyclohexane/AcOEt = 80:20) on silica gel afforded 673 mg of epoxide **191** (70%) with a dr *syn*/*anti* = 2:1. ESI m/z calcd. for $(M+Na)^+$ $C_{19}H_{32}O_3SiNa$: 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 H_2O (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.

(2*S*, 4*S*, 5*S*)-5-(Benzyloxy)-4-(*tert*-butyldimethylsilyloxy)hexane-1,2-diol (192): R_f = 0.3 (Cyclohexane/AcOEt = 50:50); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{19}H_{34}O_4SiNa$: 377.2, found 377.2.

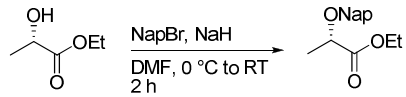
(2*R*, 4*S*, 5*S*)-5-(Benzyloxy)-1,2-(epoxy)-4-(*tert*-butyldimethylsilyloxy)hexane (193): R_f = 0.5 (Cyclohexane/AcOEt = 50:50); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 – 7.27 (m, 5H), 4.58 (d, *J* = 12.0 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.01 (ddd, *J* = 9.6, 4.4, 2.4 Hz, 1H), 3.55 –

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 $(M+Na)^+$ $C_{19}H_{32}O_3SiNa$: 359.2, found 359.2.



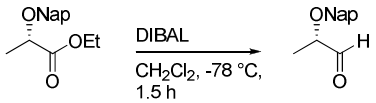
184 99% **194**

(*2S*, 3*S*)-2-(Benzyloxy)hex-5-en-3-yl acetate (194): To a solution of (2*S*, 3*S*)-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_2O (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_2Cl_2 and washed with H_2O 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 consisted with the reported literature.[98]



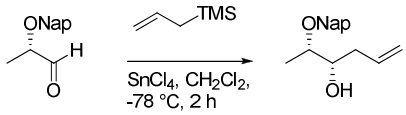
(*S*)-ethyl lactate 94% **SI1**

(*S*)-Ethyl 2-(naphthalen-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_2O. The combined organic extracts were washed with H_2O, 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: $[\alpha]^D_{20}$ = –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 $(M+Na)^+$ $C_{16}H_{18}O_3Na$: 281.1154, found 281.1158.



SI1 82% **198**

(*S*)-2-(Naphthalen-2-ylmethoxy)propanal (198): To a solution of 2-naphthylmethyl ether **SI1** (8.1 g, 31 mmol) in CH_2Cl_2 (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 CH_2Cl_2. The combined organic extracts were washed with H_2O, dried over $MgSO_4$ 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: $[\alpha]_D^{20}$ = –34.5 (c = 0.58, $CHCl_3$); R_f = 0.75 (Toluene/Acetone = 80:20); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{14}H_{14}O_2Na$: 237.0891, found 237.0885.

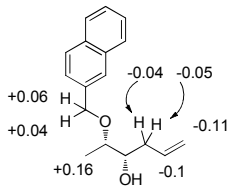


198 91% **197**

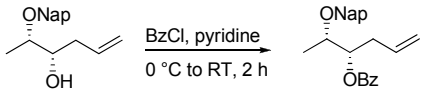
(2*S*, 3*S*)-2-(Naphthalen-2-ylmethoxy)hex-5-en-3-ol (197): $SnCl_4$ (2.6 mL, 23 mmol) was added at –78 °C to a solution of aldehyde **198** (4.4 g, 20 mmol) in CH_2Cl_2 (100 mL). After 15 min, allyltrimethylsilane (3.6 mL, 23 mmol) was added and the mixture was stirred at –78 °C for 2 h, quenched with H_2O and warmed to RT. The mixture was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over $MgSO_4$ 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: $[\alpha]_D^{20}$ = +39.6 (c = 0.42, $CHCl_3$); R_f = 0.67 (Toluene/Acetone = 80:20); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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

(thin film) 3249, 2976, 2924, 2853, 1670, 1641, 1605, 1509, 1374, 1325 cm^{-1}; HRMS(ESI) m/z calcd for $(M+Na)^+$ $C_{17}H_{20}O_2Na$: 279.1361, found 279.1358.

The absolute configuration of the C4-hydroxyl group for compound **197** was assigned by ^1H NMR analysis of the Mosher ester.

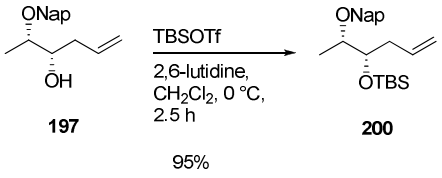


Mosher Ester Analysis (δS-δR)

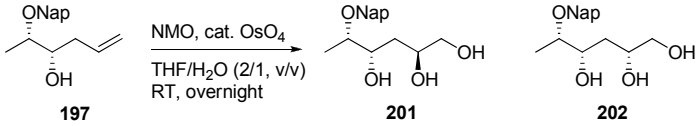


197 100% **199**

(4*S*, 5*S*)-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 CH_2Cl_2 and washed with HCl, water and brine. The organic layer was dried over $MgSO_4$ 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: $[\alpha]_D^{20} = -6.5$ (c = 1.0, $CHCl_3$); R_f = 0.68 (Cyclohexane/AcOEt = 70:30); ^1H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{24}H_{24}O_3Na$: 383.1623, found 383.1598.



(4*S*, 5*S*)-4-*O*-(*tert*-Butyldimethylsilyl)-5-(naphthalen-2-ylmethoxy)hexene (200): A solution of alcohol **197** (150 mg, 0.58 mmol) in CH_2Cl_2 (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 $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ 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: $[\alpha]_D^{20}$ = +1.4 (c = 1.0, $CHCl_3$); R_f = 0.48 (Hexanes/AcOEt = 90:10); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{23}H_{34}O_2SiNa$: 393.2226, found 393.2231.

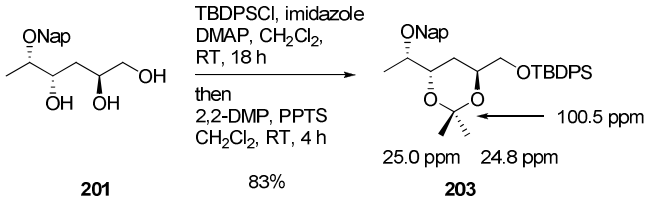


(2*S*, 4*S*, 5*S*)-5-(Naphthalen-2-ylmethoxy)hexane-1,2,4-triol (201) and **(2*R*, 4*S*, 5*S*)-5-(naphthalen-2-ylmethoxy)hexane-1,2,4-triol (202)**: At 0 °C, *N*-methylmorpholin-*N*-oxide (2.5 g, 21 mmol) was added to a solution of **197** (2.7 g, 10 mmol) in THF/H_2O (53 mL, 2:1). After 15 min, OsO_4 (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 $Na_2S_2O_3$, HCl and water, dried over $MgSO_4$ 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.

(2*S*, 4*S*, 5*S*)-5-(Naphthalen-2-ylmethoxy)hexane-1,2,4-triol (201): $[\alpha]_D^{20}$ = +35.2 (c = 0.45, $CHCl_3$); R_f = 0.50 (Toluene/Acetone = 30:70); ^{1}H NMR (400 MHz, $CDCl_3$): 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,

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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{17}H_{22}O_4Na$: 313.1416, found 313.1435.

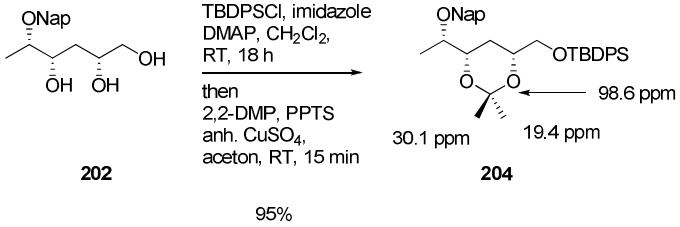
(2*R*, 4*S*, 5*S*)-5-(Naphthalen-2-ylmethoxy)hexane-1,2,4-triol (202): $[\alpha]_D^{20}$ = +25.0 (c = 0.65, $CHCl_3$); R_f = 0.59 (Toluene/Acetone = 30:70); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 – 7.77 (m, 4H), 7.51 – 7.43 (m, 3H), 4.84 (d, *J* = 11.6 Hz, 1H), 4.60 (d, *J* = 11.6 Hz, 1H), 3.99 – 3.94 (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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{17}H_{22}O_4Na$: 313.1416, found 313.1426.



201 83% **203**

Stereochemical proof of **201**: Synthesis of **(2*S*, 4*S*, 5*S*)-1-(*tert*-butyldiphenylsilyloxy)-2,4-isopropanedioxy-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 CH_2Cl_2 (3.4 mL), was added TBDPSCl (100 µL, 0.38 mmol). The mixture was stirred for 18 h at RT, then diluted with CH_2Cl_2, washed with a saturated aq. solution of NH_4Cl, water and brine and dried over $MgSO_4$. 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 CH_2Cl_2 (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 $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated. Flash column chromatography on silica gel (Hexanes/AcOEt = 80:20) gave acetonide **203** (163 mg, 0.29 mmol, 83%) as colorless oil: $[\alpha]^D_{20}$ = –11.7 (c = 2.0, $CHCl_3$); R_f = 0.23 (Hexanes/AcOEt = 90:10); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.83 – 7.80 (m,

4H), 7.71 – 7.69 (m, 3H), 7.50 – 7.36 (m, 10H), 4.83 (d, *J* = 12.0 Hz, 1H), 4.79 (d, *J* = 12.0 Hz, 1H), 3.99 – 3.93 (m, 1H), 3.89 – 3.86 (m, 1H), 3.74 (dd, *J* = 10.8, 6.0 Hz, 1H), 3.64 (dd, *J* = 10.8, 4.2 Hz, 1H), 3.60 – 3.54 (m, 1H), 1.74 (ddd, *J* = 15.6, 10.2, 6.6 Hz, 1H), 1.53 (ddd, *J* = 15.6, 9.6, 6.0 Hz, 1H), 1.41 (2s, 6H), 1.18 (d, *J* = 6.6 Hz, 3H), 1.08 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 136.7, 135.85 (2C), 135.81 (2C), 133.9, 133.8, 133.4, 133.0, 129.73, 129.71, 128.1, 127.9, 127.8, 127.73 (2C), 127.71 (2C), 126.3, 126.1, 126.0, 125.8, 100.5, 76.4, 71.8, 70.4, 67.9, 66.9, 30.5, 26.9 (3C), 25.0, 24.8, 19.4, 15.6; IR (thin film) 3069, 3051, 2989, 2958, 2930, 2858, 1112 cm^{-1}; HRMS(ESI) m/z calcd for $(M+Na)^+$ $C_{36}H_{44}O_4SiNa$: 591.2907, found 591.2894.

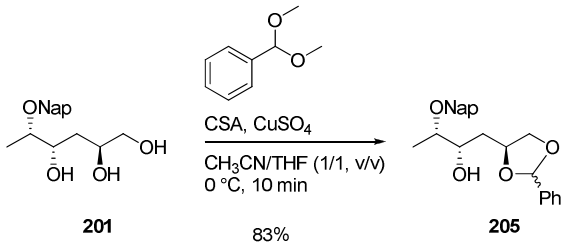


202 **204**

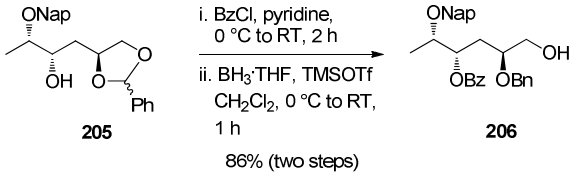
95%

Stereochemical proof of **202**: Synthesis of **(2*R*, 4*S*, 5*S*)-1-(*tert*-butyldiphenylsilyloxy)-2,4-isopropanedioxy-5-(naphthalen-2-ylmethoxy)-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_2Cl_2 (2.5 mL), was added TBDPSCl (70 µL, 0.26 mmol). The mixture was stirred for 18 h at RT, then diluted with CH_2Cl_2, washed with a saturated aq. solution of NH_4Cl, water and brine and dried over $MgSO_4$. 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 125 mg of crude silyl ether. The crude silyl ether dissolved in acetone (3.5 mL) was treated with $CuSO_4$ (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 $NaHCO_3$ and filtered through celite. The mixture was extracted with CH_2Cl_2 and the combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated. Flash column chromatography on silica gel (Hexanes/AcOEt = 80:20) gave acetonide **204** (130 mg, 0.23 mmol, 95%) as colorless oil: $[\alpha]^D_{20}$ = –0.7 (c = 1.0, $CHCl_3$); R_f = 0.23 (Hexanes/AcOEt = 90:10); ^{1}H NMR (400 MHz, $CDCl_3$): δ 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}C NMR (100 MHz, $CDCl_3$): δ 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)

3069, 3051, 2989, 2958, 2930, 2858, 1112 cm^{-1}; HRMS(ESI) m/z calcd for $(M+Na)^+$ $C_{36}H_{44}O_4SiNa$: 591.2907, found 591.2953.

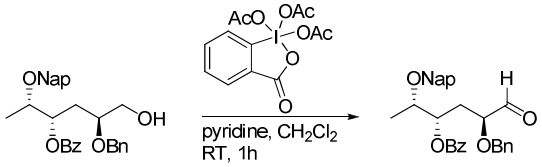


(2*S*, 3*S*)-3-(Naphthalen-2-ylmethoxy)-1-((*S*)-2-phenyl-1,3-dioxolan-4-yl)butan-2-ol (**205**): Triol **201** (1.30 g, 4.5 mmol), $CuSO_4$ (2.10 g, 14 mmol) and CSA (52 mg, 0.22 mmol) were suspended in CH_3CN/THF (46 mL, 1/1, v/v). At 0 °C, benzaldehyde dimethyl acetal (6.7 mL, 45 mmol) was added and the mixture was stirred for 10 min, neutralized with $NaHCO_3$ and filtered through celite. The mixture was extracted with CH_2Cl_2 and the combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated. Flash column chromatography on silica gel (Hexanes/AcOEt/Et_3N = 80:19:1) gave 1,2-hemiacetal **205** (1.42 g, 3.7 mmol, 83%) as a 9:1 mixture of diastereoisomers: $[\alpha]_D^{20}$ = +1.44 (c = 1.0, $CHCl_3$); R_f = 0.25 (Hexanes/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{24}H_{26}O_4Na$: 401.1729, found 401.1734.



(2*S*, 4*S*, 5*S*)-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 CH_2Cl_2 and washed with HCl and water. The organic layer was dried over $MgSO_4$ and concentrated to give the ester. The crude ester in CH_2Cl_2 (35 mL) was treated at 0 °C with a 1 M BH_3 in THF (10.2 mL, 10.2 mmol). After 15 min, TMSOTf (62 µL,

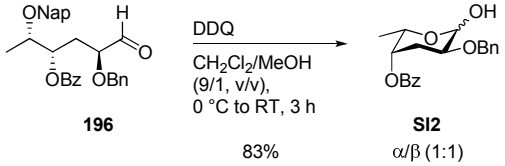
0.34 mmol) was added and the mixture was warmed slowly at RT. 1 h later, the reaction mixture was cooled to 0 °C, quenched with MeOH (8.5 mL) and Et_3N (0.35 mL) and concentrated. The crude oil was diluted in CH_2Cl_2, washed with water and brine, dried over $MgSO_4$ and concentrated. Flash column chromatography (Hexanes/AcOEt = 70:30) on silica gel afforded alcohol **206** (1.4 g, 2.9 mmol, 86% over 2 steps) as white foam: $[\alpha]_D^{20}$ = +29.4 (c = 1.2, $CHCl_3$); R_f = 0.31 (Hexanes/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+H)^+$ $C_{31}H_{33}O_5$: 485.2328, found 485.2326.



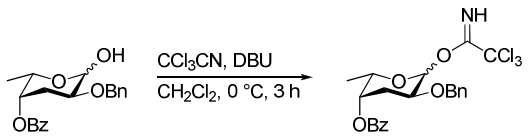
206 92% **196**

(2*S*, 3*S*, 5*S*)-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 CH_2Cl_2 (9 mL) were stirred at RT for 1 h. After addition of a saturated aq. solution of $Na_2S_2O_3$ (10 mL) and a saturated aq. solution of $NaHCO_3$ (10 mL), the mixture was stirred for additional 15 min. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with H_2O, dried over $MgSO_4$ 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: $[\alpha]_D^{20}$ = –19.4 (c = 0.64, $CHCl_3$); R_f = 0.57 (Cyclohexane/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.4, 166.1, 137.0, 135.8, 133.38, 133.30, 133.1, 130.0, 129.90 (2C),

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, 1601, 1585, 1509, 1495, 1452, 1377 cm^{-1}; HRMS(ESI) m/z calcd for $(M+Na)^+$ $C_{31}H_{30}O_5Na$: 505.1991, found 505.1970.

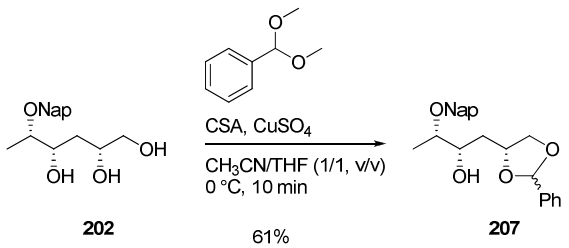


4-*O*-Benzoyl-2-*O*-benzyl-3,6-dideoxy-L-galactohexopyranose (SI2): At 0 °C, DDQ (917 mg, 4.04 mmol) was added to a solution of aldehyde **196** (650 mg, 1.34 mmol) in CH_2Cl_2/MeOH (135 mL, 9:1). The mixture was slowly warmed to RT and stirred for 3 h, then diluted with Et_2O and quenched with a saturated aq. solution of $NaHCO_3$ and a saturated aq. solution of $Na_2S_2O_3$. After separation of the layers, the organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated. Flash column chromatography (Hexanes/AcOEt = 70:30) on silica gel gave a 1:1 α/β mixture of the target hemiacetal (**SI2**) (383 mg, 1.12 mmol) in 83% yield as a colorless oil: $[\alpha]_D^{20}$ = –29.6 (c = 2.0, $CHCl_3$); R_f = 0.28 (Hexanes/AcOEt = 60:40); ^{1}H NMR (400 MHz, $CDCl_3$): 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, C*H*$_2$Ph-β), 4.70 – 4.58 (m, 2H, C*H*$_2$Ph-β, C*H*$_2$Ph-α), 4.55 (d, *J* = 11.6 Hz, 1H, C*H*$_2$Ph-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-β_{eq}), 2.24 (dddd, *J* = 13.6, 3.6, 4.8, 0.8 Hz, 1H, H3-α_{eq}), 2.15 (ddd, *J* = 13.6, 11.6, 3.2 Hz, 1H, H3-α_{ax}), 1.81 (ddd, *J* = 14.4, 11.6, 3.2 Hz, 1H, H3-β_{ax}), 1.26 (d, *J* = 6.4 Hz, 3H, H6-β), 1.18 (d, *J* = 6.4 Hz, 3H, H6-α); ^{13}C NMR (100 MHz, $CDCl_3$): 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 ($^1J_{C1\text{-}H1}$ = 163.9 Hz, C1-β,), 91.0 ($^1J_{C1\text{-}H1}$ = 175.0 Hz, C1-α), 74.5 (C2-β), 72.8 (C2-α), 72.7 (CH_2-β), 71.6 (C4-α), 71.3 (C4-β), 71.0 (*C*H_2-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 $(M+Na)^+$ $C_{20}H_{22}O_5Na$: 365.1365, found 365.1363.



SI2 α/β 1:1 — 95% — **195** α/β (1.6:1)

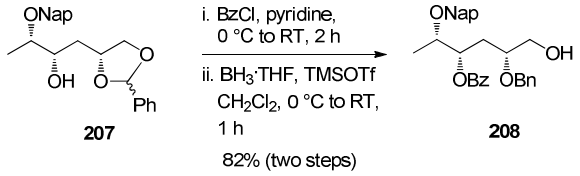
4-*O*-Benzoyl-2-*O*-benzyl-3,6-dideoxy-L-galactohexopyranosyl trichloroacetimidate (195): At 0 °C, DBU (11 µL, 0.07 mmol) and Cl_3CCN (190 µL, 1.89 mmol) were added to a solution of lactol **SI2** (130 mg, 0.38 mmol) in CH_2Cl_2 (1.5 mL). The reaction mixture was stirred for 3 h at 0 °C and concentrated. Flash column chromatography (Hexanes/AcOEt/Et_3N = 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: $[\alpha]_D^{20}$ = –24.4 (c = 0.40, $CHCl_3$); $R_f1\alpha$ = 0.68 (Hexanes/AcOEt/Et_3N = 70:29:1); $R_f1\beta$ = 0.50 (Hexanes/AcOEt/Et_3N = 70:29:1); ^{1}H NMR (400 MHz, $CDCl_3$): 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, C*H*$_2$-β), 4.67 – 4.62 (m, 2H, C*H*$_2$-α, C*H*$_2$-β), 4.57 (d, *J* = 12.0 Hz, 1H, C*H*$_2$-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, $H3_{eq}$-β), 2.38 – 2.27 (m, 2H, $H3_{eq}$-α, $H3_a$-α), 1.96 (ddd, *J* = 14.4, 11.2, 3.6 Hz, 1H, $H3_a$-α), 1.29 (d, *J* = 6.4 Hz, 3H, H6-β), 1.20 (d, *J* = 6.8 Hz, 3H, H6-α); ^{13}C NMR (150 MHz, $CDCl_3$): 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 ($^1J_{C1\text{-}H1}$ = 168.1 Hz, C1-β), 94.1 ($^1J_{C1\text{-}H1}$ = 178.4 Hz, C1-α), 91.6 (*C*Cl_3), 91.1 (*C*Cl_3), 73.5 (C2-β), 73.0 (*C*H_2-β), 72.2 (C2-α), 71.3 (*C*H_2-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 $(M+Na)^+$ $C_{22}H_{22}Cl_3NO_5Na$: 508.0461, found 508.0434.



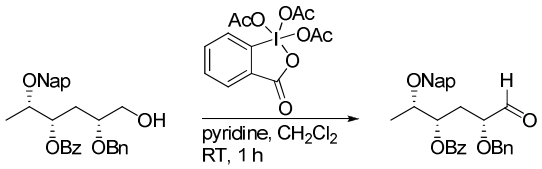
202 — 61% — **207**

(2*S*, 3*S*)-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 diatereoisomeric mixture:

$[\alpha]_D^{20}$ = +17.0 (c = 1.0, $CHCl_3$); R_f = 0.37 (Hexanes/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{24}H_{26}O_4Na$: 401.1729, found 401.1750.

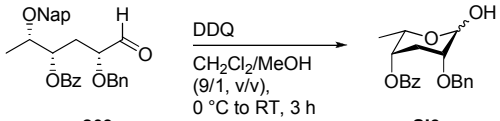


(2*S*, 4*S*, 5*R*)-5-(Benzyloxy)-6-hydroxy-2-(naphthalen-2-ylmethoxy)hexan-3-yl benzoate (208): Using the same procedure as for compound **206**, alcohol **208** was synthesized starting from benzylidene acetal **207** (0.90 g, 2.4 mmol) in 82% yield as a white foam: $[\alpha]_D^{20}$ = +3.6 (c = 0.8, $CHCl_3$); R_f = 0.25 (Cyclohexane/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{31}H_{32}O_5Na$: 507.2147, found 507.2164.



208 85% **209**

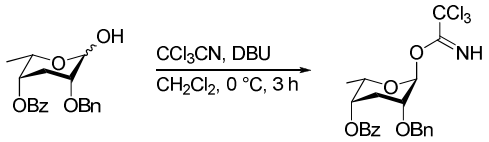
(2*S*, 3*S*, 5*R*)-5-(Benzyloxy)-2-(naphthalen-2-ylmethoxy)-6-oxohexan-3-yl benzoate (209): Using the same procedure as for compound **196**, aldehyde **209** was synthesized starting from alcohol **208** (720 mg, 1.48 mmol) in 85% yield as white foam: $[\alpha]_D^{20}$ = +4.0 (c = 0.62, $CHCl_3$); R_f = 0.53 (Cyclohexane/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.58 (d, *J* = 1.2 Hz, 1H), 8.06 – 7.95 (m, 2H), 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na+MeOH)^+$ $C_{32}H_{34}O_6Na$: 537.2253, found 537.2243.



209 94% **SI3** α/β (1:1)

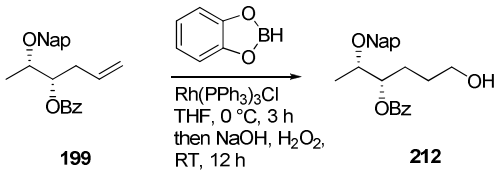
4-*O*-Benzoyl-2-*O*-benzyl-3,6-dideoxy-L-talohexopyranose (SI3): At 0 °C, DDQ (520 mg, 2.3 mmol) was added to a solution of aldehyde **209** (370 mg, 0.76 mmol) in CH_2Cl_2/MeOH (76 mL, 9/1). The mixture was slowly warmed to RT and stirred for 3 h, then diluted with Et_2O and quenched with a saturated aq. solution of $NaHCO_3$ and a saturated aq. solution of $Na_2S_2O_3$. After separation of the layers, the organic layer was washed with H_2O, dried over $MgSO_4$ 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: $[\alpha]_D^{20}$ = –49.9 (c = 1.0, $CHCl_3$); R_f = 0.28 (Hexanes/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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, C*H*$_2$-β), 4.58 (d, *J* = 11.6 Hz, 1H, C*H*$_2$-α), 4.54 (d, *J* = 11.6 Hz, 1H, C*H*$_2$-α), 4.47 (qd, *J* = 6.4, 1.6 Hz, 1H, H5-α), 4.42

(d, J =11.6 Hz, 1H, CH_2-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-β_{eq}), 2.41 (adtd, J = 15.4, 3.2, 1.2 Hz, 1H, H3-α_{eq}), 2.24 (adt, J = 15.4, 3.6 Hz, 1H, H3-α_{ax}), 1.91 (adt, J = 16.0, 3.6 Hz, 1H, H3-β_{ax}), 1.36 (d, J = 6.4 Hz, 3H, H6-β), 1.30 (d, J = 6.4 Hz, 3H, H6-α); ^{13}C NMR (100 MHz, $CDCl_3$): 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 (4C), 128.39 (2C), 128.32 (2C), 128.1 (2C), 127.9, 127.8 (2C), 127.5, 94.7 ($^1J_{C1\text{-}H1}$ = 164.8 Hz, C1-β), 93.1 ($^1J_{C1\text{-}H1}$ = 173.7 Hz, C1-α), 72.9 (C5-β), 71.9 (C2-β), 71.7 (C2-α), 71.5 (*C*H_2-β), 71.1 (*C*H_2-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 $(M+Na)^+$ $C_{20}H_{22}O_5Na$: 365.1365, found 365.1387.



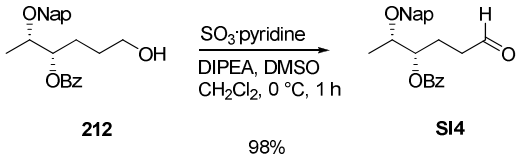
SI3 α/β (1:1) — 89% — **210** α/β (9:1)

4-*O*-Benzoyl-2-*O*-benzyl-3,6-dideoxy-L-talohexopyranosyl trichloroacetimidate (210): At 0 °C, DBU (9 μL, 0.06 mmol) and Cl_3CCN (146 μL, 1.46 mmol) were added to a solution of lactol **SI3** (100 mg, 0.29 mmol) in CH_2Cl_2 (1.1 mL). The reaction mixture was stirred for 3 h at 0 °C and concentrated. Flash column chromatography (Hexanes/AcOEt/Et_3N = 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: $[\alpha]_D^{20}$ = –31.4 (c = 0.75, $CHCl_3$); R_f = 0.56 (Hexanes/AcOEt/Et_3N = 70:29:1); ^{1}H NMR (400 MHz, $CDCl_3$): 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, CH_2Ph), 4.48 (d, J = 11.6 Hz, 1H, CH_2Ph), 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, H3$_{eq}$), 2.17 (ddd, J = 15.6, 4.0, 3.6 Hz, 1H, H3$_a$), 1.21 (d, J = 6.8 Hz, 3H, H6); ^{13}C NMR (150 MHz, $CDCl_3$): 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 ($^1J_{C1\text{-}H1}$ = 180.9 Hz, C1), 90.9 (*C*Cl_3), 71.5 (C4), 70.0 (C5), 68.1 (C2), 67.9 (*C*H_2), 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 $(M+Na)^+$ $C_{22}H_{22}Cl_3NO_5Na$: 508.0461, found 508.0434.



95%

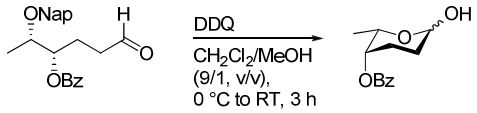
(2*S*, 3*S*)-6-Hydroxy-2-(naphthalen-2-ylmethoxy)hexan-3-yl benzoate (212): A solution of alkene **199** (620 mg, 1.72 mmol) and $Rh(PPh_3)_3Cl$ (56 mg, 0.06 mmol) in THF (14 mL) was cooled to 0 °C. 1 M catecholborane in THF (5.1 mL, 5.1 mmol) was added and the reaction was stirred at 0 °C for 3 h. After dilution with THF/MeOH (7 mL, 1/1, v/v) and addition of 3 M NaOH (4 mL) and an aq. solution 35% H_2O_2 (4 mL), the reaction was stirred overnight and quenched with a saturated aq. solution of Na_2SO_3 (10 mL). After dilution with Et_2O, the organic layer was washed with a saturated aq. solution of $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated. Flash column chromatography (Cyclohexane/AcOEt/Et_3N = 80:19:1) on silica gel afforded alcohol **212** (620 mg, 1.63 mmol, 95%) as a colorless oil: $[\alpha]_D^{20} = -5.2$ (c = 1.0, $CHCl_3$); $R_f = 0.28$ (Cyclohexane/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 calcd for $(M+Na)^+$ $C_{24}H_{26}O_4Na$: 401.1729, found 401.1737.



98%

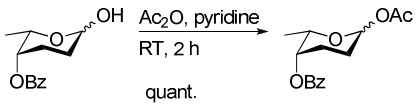
(2*S*, 3*S*)-2-(Naphthalen-2-ylmethoxy)-6-oxohexan-3-yl benzoate (SI4): To a solution of alcohol **212** (450 mg, 1.19 mmol), DIPEA (1 mL, 5.9 mmol) and DMSO (0.8 mL, 11.9 mmol) in CH_2Cl_2 (6 mL) at 0 °C was added SO_3·pyridine complex (568 mg, 3.60 mmol). After 1 h, the reaction mixture was quenched with a saturated aq. solution of $Na_2S_2O_3$ (12 mL) and warmed to RT. The aq. layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated. Flash column chromatography (Hexanes/AcOEt = 80:20) on silica gel afforded aldehyde **SI4** (439 mg, 1.16 mmol, 98%) as colorless oil: $[\alpha]_D^{20} = -2.1$ (c = 0.80, $CHCl_3$); $R_f = 0.44$ (Hexanes/AcOEt = 70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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), 5.34 – 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{24}H_{24}O_4Na$: 399.1572, found 399.1565.



SI4 — 87% — **213** α/β (1:1)

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 **SI4** (439 mg, 1.10 mmol) in CH_2Cl_2/MeOH (22 mL, 9/1, v/v). The mixture was slowly warmed to RT and stirred for 3 h, diluted with Et_2O and quenched with a saturated aq. solution of $NaHCO_3$ and a saturated aq. solution of $Na_2S_2O_3$. After separation of the layers, the organic layer was washed with H_2O, dried over $MgSO_4$ 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: $[\alpha]_D^{20}$ = –14.7 (c = 1.0, $CHCl_3$); R_f = 0.45 (Hexanes/AcOEt = 50:50); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{13}H_{16}O_4Na$: 259.0946, found 259.0983.

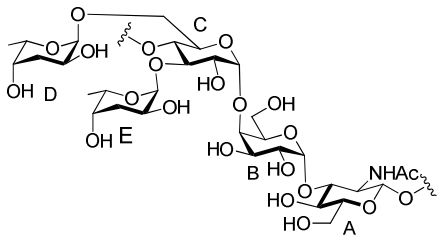


213 α/β (1:1) **211** α/β (41:59)

4-*O*-Benzoyl-L-rhodinosyl acetate (211): Ac_2O (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: $[\alpha]_D^{20}$ = –8.4 (c = 0.95, $CHCl_3$); R_f = 0.28 (Hexanes/AcOEt =70:30); ^{1}H NMR (400 MHz, $CDCl_3$): 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, C*H*$_3$CO-β), 2.13 (s, 3H, C*H*$_3$CO-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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 ($^1J_{C1\text{-}H1}$ = 161.7 Hz, C1-β), 92.1 ($^1J_{C1\text{-}H1}$ = 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, *C*H$_3$CO), 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 $(M+Na)^+$ $C_{15}H_{18}O_5Na$: 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.

3.2 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 Shinga-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 Shinga-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]

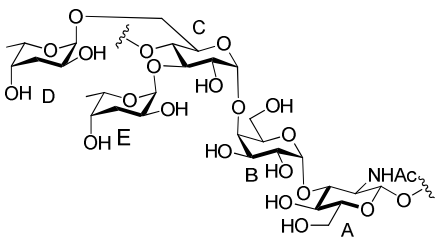


Figure 6. Pentasaccharide repeating unit of the *O*-specific polysaccharide of *E. coli* O111.

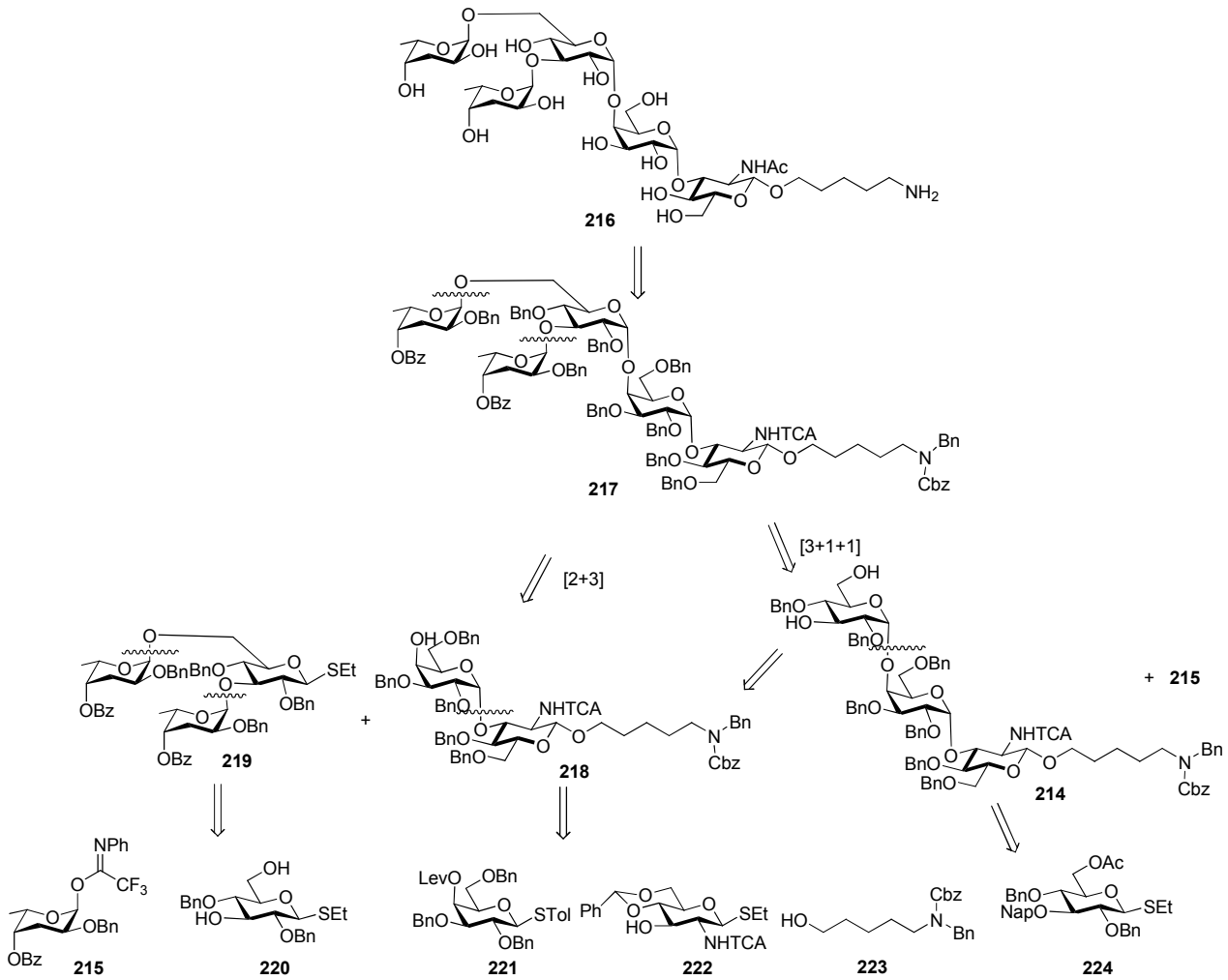
Pure pentasaccharide **216** is required to evaluate the immunological properties of the *O*-specific polysaccharide of *E. coli* O111 and to explore the potential of an oligosaccharide-conjugate vaccine (Scheme 51). This chapter describes the first total synthesis of the

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.

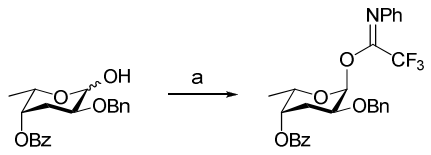
Pentasaccharide **216** reveals several synthetic challenges including one being the α-linkages of colitoses. Retrosynthetic analysis of pentasaccharide **216** reveals a convergent [2+3] approach as attractive (Scheme 51). The reducing end aminopentanol linker will allow for straightforward conjugation. 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*-benzyl-benzyloxycarbonyl-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**.



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

3.3.2 Building Blocks Synthesis

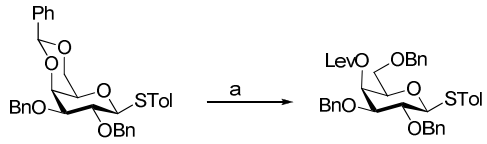
Most of the building blocks needed for the synthesis of pentasaccharide repeating unit **216** are accessed via modification of published methods. The synthesis of colitose building block **215** benefits from the *de novo* synthesis established in Chapter 2 that is more efficient than other procedures.[67,69,70b,71,72] 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 isoamide to amide rearrangement at the anomeric center[108].



225 **215**

Scheme 52. Synthesis of colitose glycosylating agent **215**: a) $CF_3C(=NPh)Cl$, Cs_2CO_3, CH_2Cl_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.



226 **221**

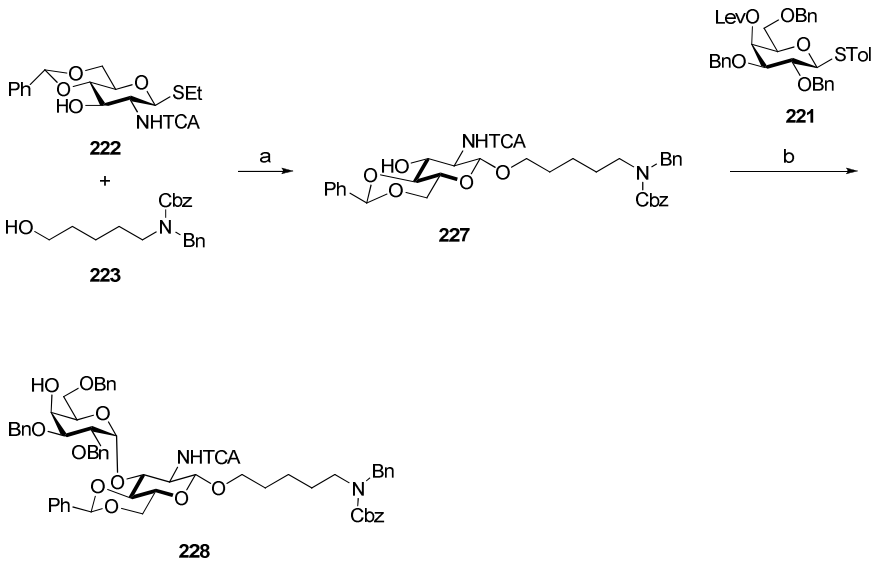
Scheme 53. Synthesis of galactose building block **221**: a) i. Et_3SiH, TfOH, Tf_2O, CH_2Cl_2, 0 °C to RT, 3 h; ii. LevOH, DCC, DMAP, CH_2Cl_2, 0 °C to RT, 15 h, 69% (two steps).

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

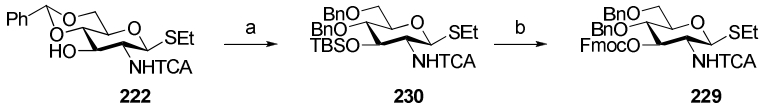
linkage. Furthermore, at -20 °C, only the primary alcohol acts as a nucleophile in the coupling reaction. To aid purification, excess linker was tritylated to afford after flash column chromatography on silica gel, β-glucosamine **227** in 70% yield. Galactopyranose **221** was appended to β-glucosamine **227** using a dimethylformamide-modulated glycosylation procedure[113] 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[114] 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, CH_2Cl_2, MS 4Å, -20 °C, 30 min; ii. TrtCl, pyridine, overnight, 70% (two steps); b) i. DMF, NIS, TMSOTf, MS 4Å, -10 °C, CH_2Cl_2, 12 h; ii. NaOMe, MeOH, 14% (two steps).

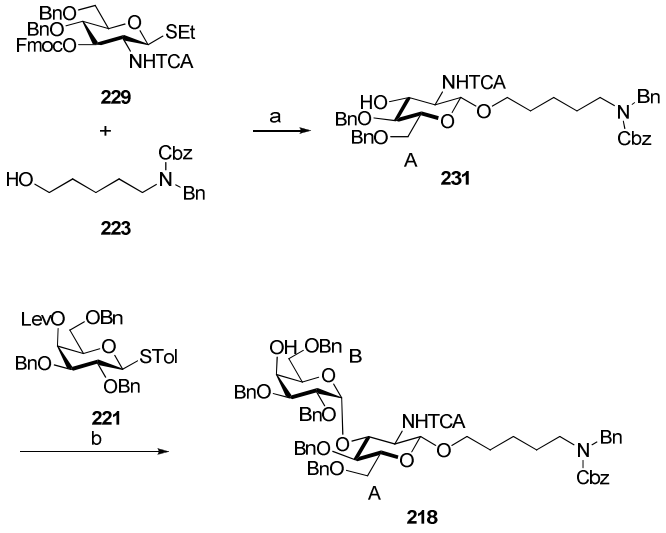
To circumvent the problems encountered in the synthesis of disaccharide **228**, glucosamine building block **222** was converted to glycosylating agent **229**, presenting a 9-fluorenylmethyloxycarbonyl protecting group at C3 for chain elongation and stable benzyl ethers at C4 and C6 (Scheme 55). It 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-fluorenylmethyloxycarbonyl provided the new glucosamine glycosylating agent **229** in 92% yield over two steps.



Scheme 55. Synthesis of glucosamine building block **229**: a) i. TBSCl, imidazole, CH_2Cl_2; ii. BH_3·THF, TMSOTf, CH_2Cl_2; iii. BnBr, NaH, DMF, 77% (three steps); b) i. BF_3·OEt_2, CH_3CN; ii. FmocCl, pyridine, CH_2Cl_2, 92% (two steps).

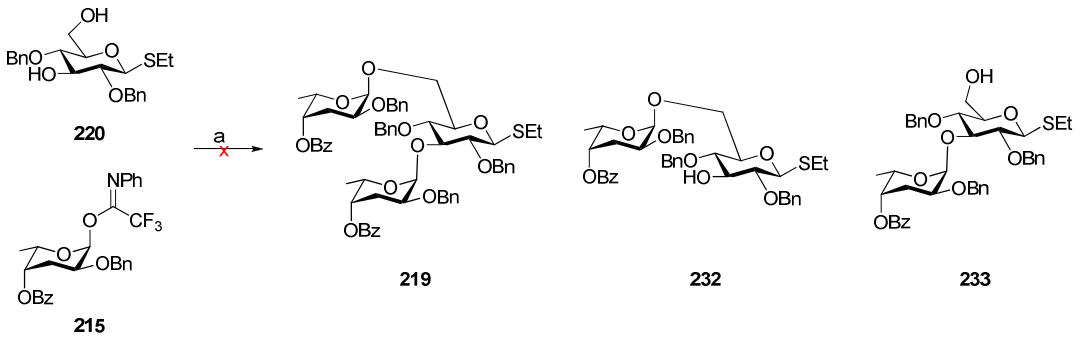
Thioglycoside **229** was successfully coupled to *N*-benzyl-benzyloxycarbonyl-5-aminopentanol **223** at -20 °C upon activation by *N*-iodosuccinimide and triflic acid in CH_2Cl_2 (Scheme 56). 9-Fluorenylmethyloxycarbonyl-cleavage by treatment with triethylamine afforded β-glucosamine nucleophile **231** in 71% yield over two steps. The $^1J_{C1A\text{-}H1A}$ 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 ($^1J_{C1B\text{-}H1B}$ of 171.3 Hz).



Scheme 56. Synthesis of the reducing end disaccharide **218**: a) i. NIS, TfOH, CH_2Cl_2, MS 3Å AW, -20 °C, 1 h; ii Et_3N, CH_2Cl_2, RT, 3 h, 71% (two steps); b) i. NIS, TfOH, Et_2O/CH_2Cl_2 (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.



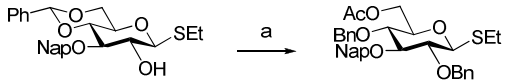
Scheme 57. Attempts at the synthesis of trisaccharide **219**: a) **215** (2.5 equiv.), TMSOTf, CH_2Cl_2, -50 °C.

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-thioglucoside 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 thioglucoside **234**[116] (Scheme 58). Building block **224**

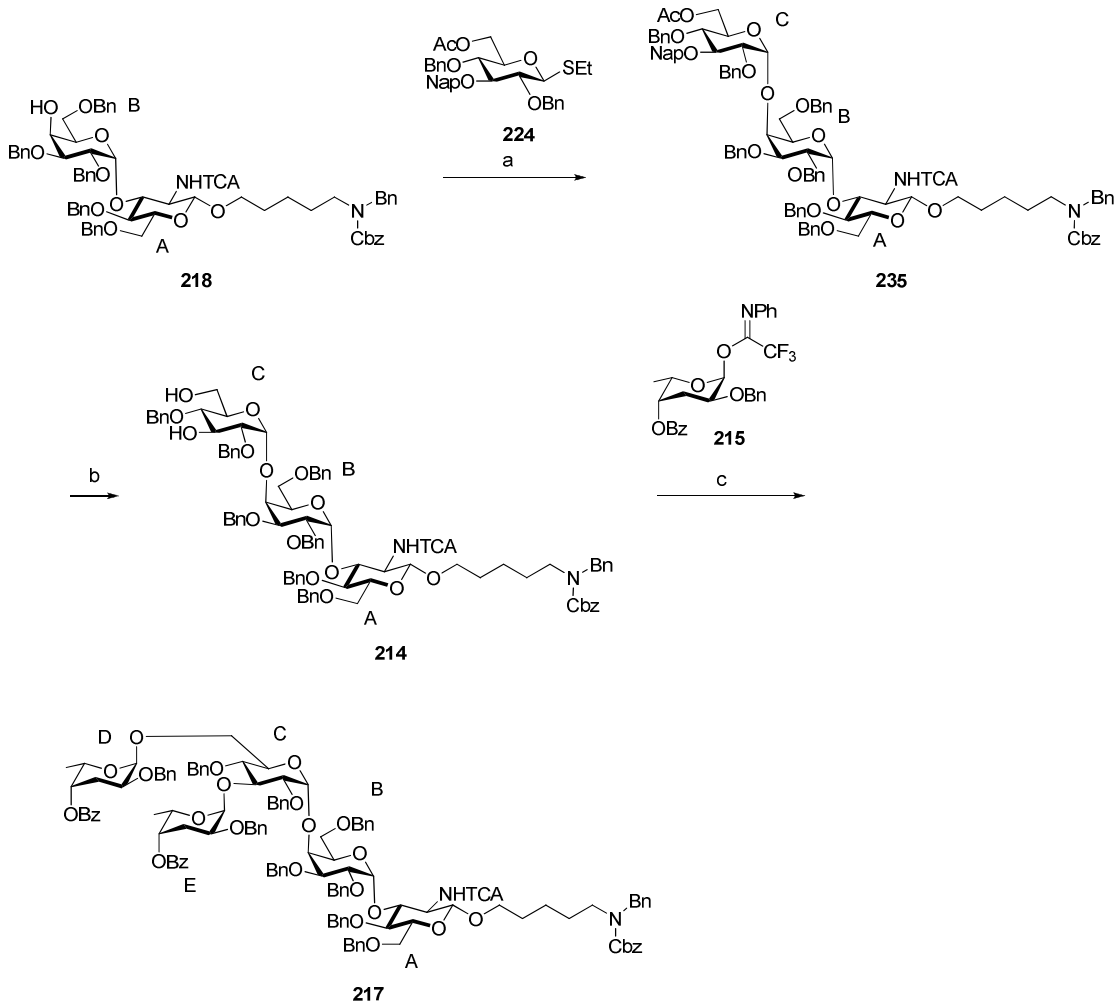
presents non-participating ether groups at C2, C3 and C4 as well as a participating acetate ester at C6 to favor the formation of the α-glycosidic linkage. The orthogonal 2-naphthylmethyl-ether at C3 will allow for branching at this position. To access fully functionalized glucose **224**, the free hydroxyl 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.



234 **224**

Scheme 58. Synthesis of thioglucoside **224**: a) i. BnBr, NaH, DMF ; ii. BH_3·THF, TMSOTf , CH_2Cl_2; iii. Ac_2O, pyridine, 74% (three steps).

Coupling of thioglucoside **224** with disaccharide **218** was performed at -50 °C by activation with *N*-iodosuccinimide in the presence of triflic acid, in diethyl ether/dichoromethane (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 ($^1J_{C1C\text{-}H1C}$ 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, Et_2O/CH_2Cl_2 (3/1, v/v), MS 4Å, -50 °C, 1 h, 97%; b) i. NaOMe, MeOH, RT, overnight; ii. DDQ, CH_2Cl_2/MeOH (9/1, v/v), 0 °C, 4 h, 73% (two steps); c) TMSOTf, CH_2Cl_2, MS 4Å, -50 °C, 15 min, 63%.

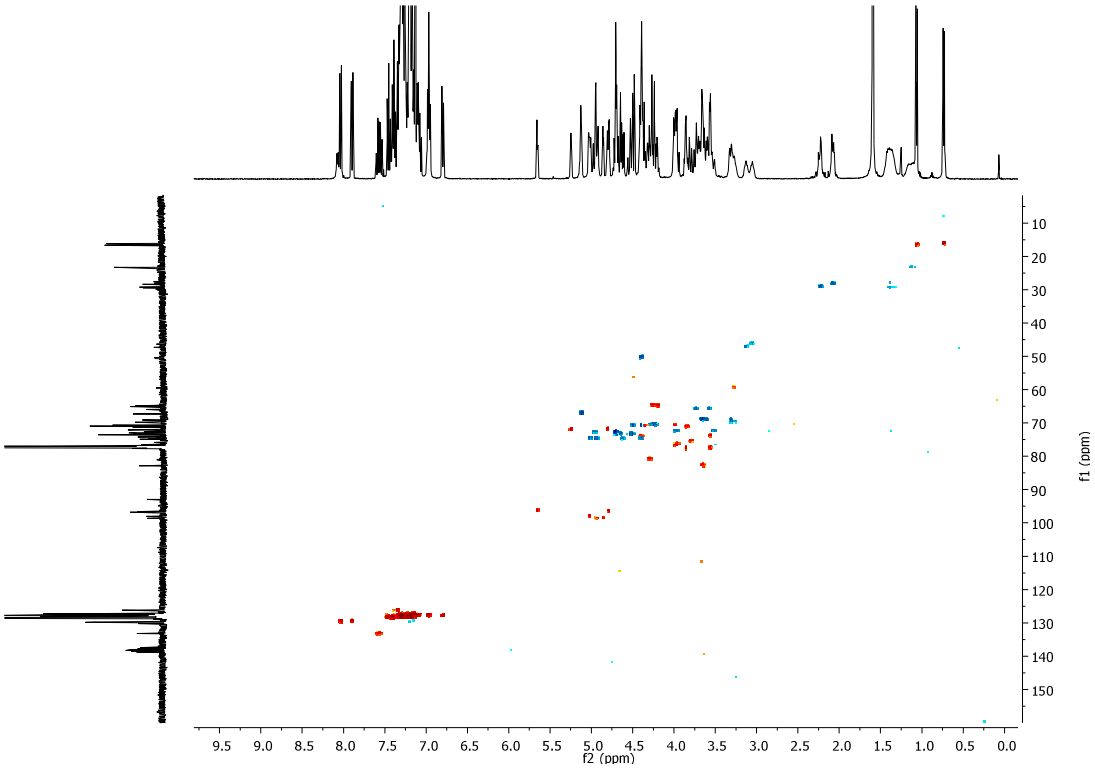
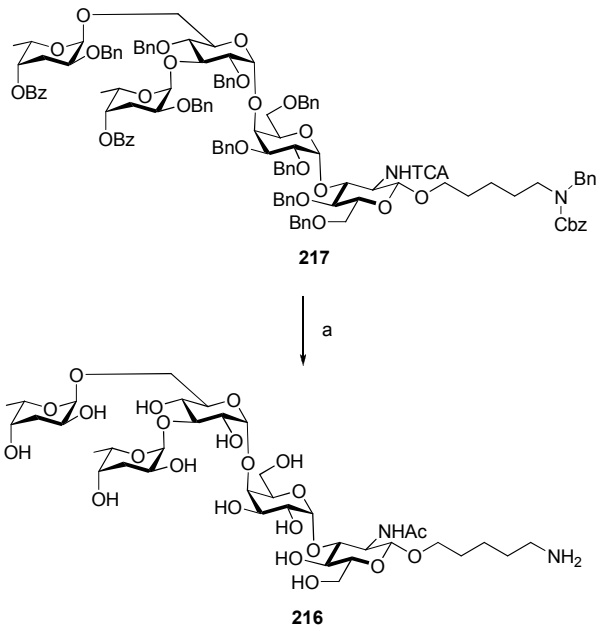


Figure 7. HSQC (600 MHz, $CDCl_3$) of protected pentasaccharide **217**.

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 (H_2, $Pd(OH)_2$, 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.



Scheme 60. Deprotection of pentasaccharide **217**: a) i. Bu_3SnH, AIBN, toluene, 90 °C, 2 h; ii. NaOMe, MeOH, 40 °C, overnight; c) $Pd(OH)_2$, THF/H_2O (1/1, v/v), 24 h, 64% (three steps).

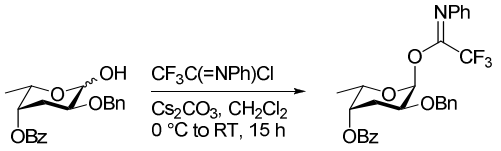
3.4 Conclusions

The first total synthesis of the pathogenic *E. coli* 0111 *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 (CH_2Cl_2), 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

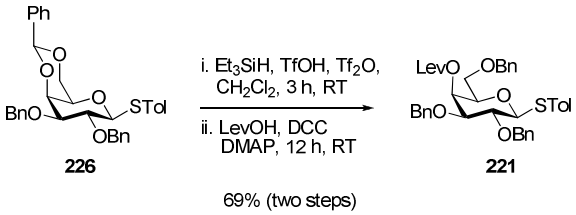
UV or by dipping the plate in a cerium sulfate ammonium molybdate (CAM) solution or in a 1:1 mixture of H_2SO_4 (2 N) and resorcine monomethylether (0.2%) in ethanol. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). Preparative 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_2O with chemical shifts referenced to internal standards ($CDCl_3$: ^{1}H 7.26 ppm, ^{13}C 77.0 ppm; D_2O 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 qaudruplet 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.



225 82% **215**

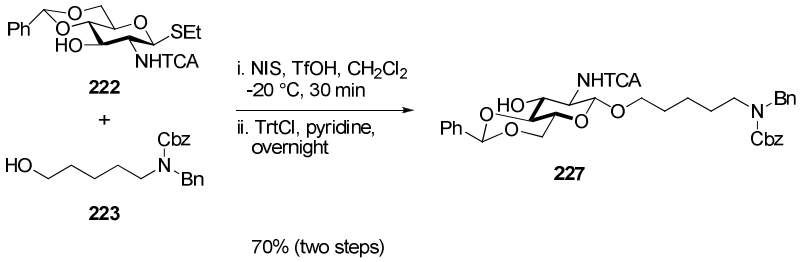
4-*O*-Benzoyl-2-*O*-benzyl-3,6-dideoxy-L-galactohexopyranosyl-*N*-phenyl-trifluoroacetimidate (215): A solution of colitose lactol **225**[106] (130 mg, 0.38 mmol) in CH_2Cl_2 (7.5 mL) was treated at 0 °C with Cs_2CO_3 (250 mg, 0.76 mmol, 2 equiv) and $CF_3C(=NPh)Cl$ (75 µL, 0.57 mmol, 1.5 equiv). The 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_3N = 90:9:1) to yield α-*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, $CDCl_3$): δ 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, $CDCl_3$): δ 165.9, 143.7, 137.8, 133.4 (2C), 129.9 (2C),

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 $(M+Na)^+$ $C_{28}H_{26}F_3NNaO_5$: 536.1655, found 536.1631.



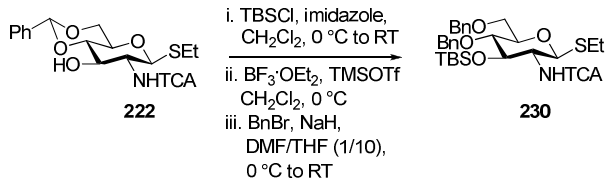
***p*-Tolyl 2,3,6-tri-*O*-benzyl-4-levulinoyl-1-thio-β-D-galactopyranoside (221)**: Trifluoroacetic anhydride (0.5 mL, 3.6 mmol, 1 equiv), followed by trifluoroacetic acid (1.7 mL, 21.6 mmol, 6 equiv) was added dropwise at 0 °C to a solution of benzylidene acetal **226**[111] (2.0 g, 3.6 mmol) and triethylsilane (3.5 mL, 21.6 mmol, 6 equiv) in CH_2Cl_2 (36 mL). The mixture was stirred for 3 h at RT, and then quenched at 0 °C with a saturated aq. solution of $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to obtain 2.0 g of the crude alcohol.

The crude product was then taken up in CH_2Cl_2 (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: R_f (Hexanes/EtOAc = 70:30) = 0.34; $[\alpha]_D^{20}$ = +7.5 (c = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (150 MHz, $CDCl_3$): 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 ($^1J_{C1\text{-}H1}$ = 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 $(M+Na)^+$ $C_{39}H_{42}O_7Na$: 677.2549, found 677.2571.

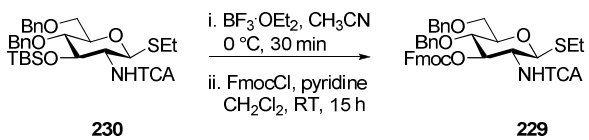


***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 4,6-di-*O*-benzylidene-2-trichloroacetamido-β-D-glucopyranoside (227)**: A mixture of glucosamine donor **222** (0.60 g, 1.31 mmol), linker **223** (0.65 g, 1.97 mmol, 1.5 equiv), freshly activated molecular sieves 3Å AW (1.5 g) and NIS (0.59 g, 2.63 mmol, 2 equiv) in CH_2Cl_2 (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 CH_2Cl_2 and quenched with a saturated aq. solution of $NaHCO_3$. The organic layer was washed with a saturated aq. solution of $Na_2S_2O_3$, water, dried over $MgSO_4$ 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: R_f (Hexanes/AcOEt = 70:30) = 0.25; ^{1}H NMR (400 MHz, $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, 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 (*C*H_2 rotamers), 47.25 and 46.23 (*C*H_2 rotamers), 29.4, 27.9, 23.4; HRMS(ESI) m/z calcd for $(M+Na)^+$ $C_{35}H_{39}Cl_3N_2O_8Na$: 743.1670, found 743.1661.



Ethyl 4,6-di-*O*-benzyl-2-deoxy-3-*tert*-butyldimethylsilyl-2-trichloroacetamido-1-thio-β-D-glucopyranoside (230): TBSCl (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 **222**[110] (6.0 g, 13.1 mmol) in CH_2Cl_2 (66 mL). After completion, the reaction mixture was quenched with MeOH at 0 ºC, diluted with CH_2Cl_2 and washed with a saturated aq. solution of NH_4Cl. Following separation of layers, the organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude hemiacetal was dissolved in CH_2Cl_2 (66 mL) and cooled to 0 °C. After addition of 1 M solution of BH_3·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 $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over $MgSO_4$ 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 CH_2Cl_2 and washed with a saturated aq. solution of NH_4Cl. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (Hexanes/EtOAc/CH_2Cl_2 = 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/CH_2Cl_2 = 7:1:2) = 0.41; $[\alpha]_D^{20}$ = –2.7 (c = 3.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 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 (2d, *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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{30}H_{42}Cl_3NNaO_5SSi$: 684.1516, found 684.1507.

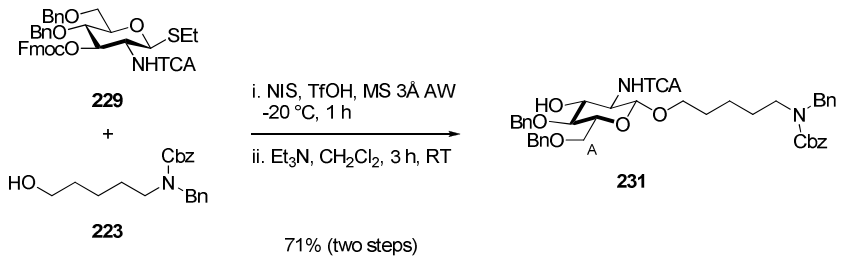


230 **229**

92% (two steps)

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

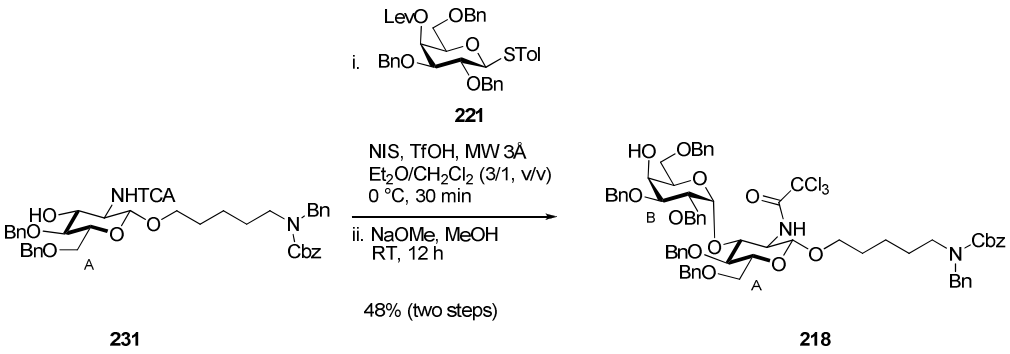
anhydrous CH_3CN (59 mL) was added $BF_3{\cdot}OEt_2$ (0.75 mL, 5.94 mmol, 1.2 equiv). The reaction mixture was stirred at 0 °C for 30 min and quenched with a saturated aq. solution of $NaHCO_3$. The precipitated solid was filtered and dried *in vacuo*. The crude alcohol was taken in CH_2Cl_2 (16 mL) 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 CH_2Cl_2 and the organic layer was washed with a saturated aq. solution of $NaHCO_3$ and brine, dried over Mg_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (Hexanes/EtOAc/CH_2Cl_2 = 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: R_f (Hexanes/EtOAc/CH_2Cl_2 = 8:1:2) = 0.29; $[\alpha]_D^{20}$ = –27.5 (c = 2.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{39}H_{38}Cl_3NNaO_7S$: 792.1332, found 792.1347.



***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 4,6-di-*O*-benzyl-2-trichloroacetamido-β-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 CH_2Cl_2 (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 CH_2Cl_2 and washed with a saturated aq. solution of $NaHCO_3$. The

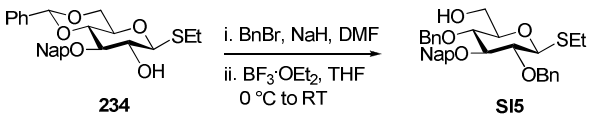
organic layer was washed with a saturated aq. solution of $Na_2S_2O_3$ and water, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was run through a silica column with (Hexanes/AcOEt = 80:20 to 50:50) to yield 0.61 g of crude product.

The crude material was then dissolved in CH_2Cl_2 (12 mL) and treated with Et_3N (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; $[\alpha]_D^{20}$ = –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 ($^1J_{C1A\text{-}H1A}$ = 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 calcd for $(M+Na)^+$ $C_{42}H_{47}Cl_3N_2O_8Na$: 835.2296, found 835.2271.



***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-trichloroacetamido-β-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_2O/CH_2Cl_2 (16 mL, 3/1, v/v) was stirred at RT for 30 min. The mixture was cooled to 0 °C and the TfOH (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_2O and washed with a saturated aq. solution of $Na_2S_2O_3$. After separation of the layers, the organic layer was washed with a saturated aq.

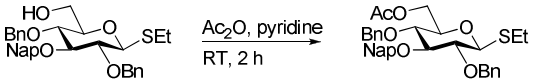
solution of $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was run through a silica column with (Hexanes/AcOEt = 90:10 to 70:30) to yield 0.35 g of crude disaccharide. The crude material was then dissolved in MeOH (12 mL) and treated with 0.5 M solution of NaOMe 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 75:25) to give the target disaccharide **218** (280 mg, 0.224 mmol, 48% over two steps) as an oil: R_f (Hexanes/EtOAc = 70:30) = 0.35; $[a]_D^{20}$ = +27.8 (c = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 8.02 (d, *J* = 6.4 Hz, 1H, N*H*COCl$_3$), 7.31 – 7.22 (m, 30H), 7.16 – 7.09 (m, 5H), 5.14 (s, 2H, CO_2CH_2), 5.08 – 4.93 (m, 3H, C*H*, H_1A, H_1B), 4.77 – 4.67 (m, 3H, CH_2), 4.63 – 4.35 (m, 8H, CH_2), 4.35 – 4.28 (m, 1H, H_3A), 4.17 – 4.14 (m, 1H, H_5B), 3.92 (bs, 1H, H_4B), 3.85 – 3.78 (m, 2H, H_2B, H_3B), 3.77 – 3.59 (m, 6H, OCH_2, H_{6a}A, H_{6b}A, H_{6a}B, H_4A, H_5A), 3.55 (d, *J* = 9.2 Hz, 1H, H_{6b}B), 3.39 (bs, 2H, H_2A, OCH_2), 3.17 – 3.10 (m, 2H, NCH_2), 2.49 (s, 1H, OH), 1.51 – 1.36 (m, 4H, CH_2), 1.30 – 1.12 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, rotamers): δ 161.4 (*C*O), 138.3 (2C), 138.1, 137.9, 137.8, 137.7, 137.4, 128.4 (3C), 128.4 (3C), 128.4 (3C), 128.4 (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 ($^1J_{C1A\text{-}H1A}$ = 166.0 Hz, C_1A-β), 97.7 ($^1J_{C1B\text{-}H1B}$ = 171.3 Hz, C_1B-α), 92.7 (NH*C*OCl$_3$), 80.5 (C_3A), 77.1 (C_4A), 76.7 (C_2B), 75.9 (C_3B), 74.4 (*C*H_2), 74.0 (C_5A), 73.5 (2C, *C*H_2), 73.4 (2C, *C*H_2), 72.5 (*C*H_2), 70.0 (C_6B), 69.6 (O*C*H_2), 69.3 (C_5B), 68.9 (C_6A), 68.2 (C_4B), 67.0 (CO_2CH_2Ph), 59.0 (C_2A), 50.4 and 50.1 (N*C*H_2Ph, rotamers), 47.0 and 46.1 (*C*H_2 rotamers), 27.9 (*C*H_2), 27.4 (*C*H_2), 23.1 (*C*H_2); IR (thin film) 3484, 3340, 2923, 2856, 1701, 1523, 1468, 1454, 1091, 1028, 698 cm^{-1}; HRMS(ESI) m/z calcd for $(M+Na)^+$ $C_{69}H_{75}Cl_3N_2O_{13}Na$ 1267.4232, found 1267.4163.



75% (two steps)

Ethyl 2,4-di-*O*-benzyl-3-*O*-2-(naphtylmethyl)-1-thio-β-D-glucopyranoside (SI5): A solution of thioglucoside **234**[116] (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 NH_4Cl and the formed precipitate was filtered and dried. The crude acetal was taken in CH_2Cl_2 (23 mL) and reacted at 0 °C with a 1 M solution of BH_3·THF (15.4 mL, 15.4

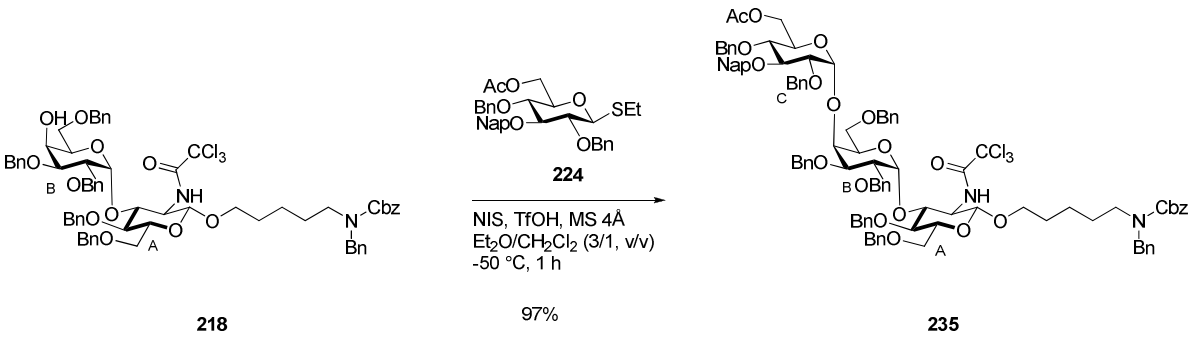
mmol, 4.0 equiv), followed by TMSOTf (0.35 mL, 1.9 mmol, 0.5 equiv). After the reaction was completed, the reaction mixture was quenched with a saturated aq. solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude was purified by column chromatography on silica gel (Hexanes/EtOAc/CH_2Cl_2 = 7:2:1 to 5:2:1) to give alcohol **SI5** (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, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 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); ^{13}C NMR (100 MHz, $CDCl_3$): δ 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 calcd for $(M+Na)^+$ $C_{33}H_{36}NaO_5S$: 567.2181, found 567.2201.



SI5 99% **224**

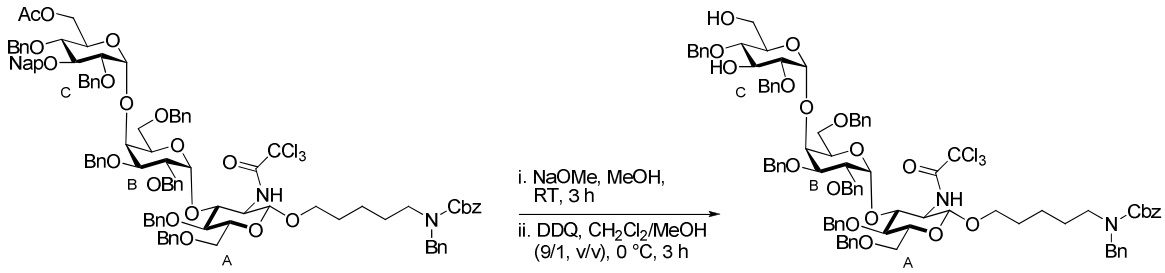
Ethyl 6-*O*-acetyl-2,4-di-*O*-benzyl-3-*O*-2-(naphtylmethyl)-1-thio-β-D-glucopyranoside (224): Acetic anhydride (50 µL, 551 µmol, 3 equiv) was added to a solution of **SI5** (100 mg, 184 µmol) in pyridine (6 mL). The mixture was stirred at RT for 2 h and concentrated *in vacuo*. The crude was purified 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 , $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 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); ^{13}C NMR (100 MHz, $CDCl_3$): δ 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,

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 $(M+Na)^+$ $C_{35}H_{38}NaO_6S$: 609.2287, found 609.2338.



***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 6-*O*-acetyl-2,4-di-*O*-benzyl-3-*O*-2-(naphthylmethyl)-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 (235)**: Acceptor **218** (40 mg, 32 µmol) and thioglucoside **224** (28 mg, 48 µmol, 1.5 equiv) were azeotroped with toluene and dried *in vacuo*. After addition of NIS (14 mg, 64 µmol, 2.0 equiv) and freshly activated molecular sieves 4Å (100 mg), the reaction mixture was solubilized in Et_2O/CH_2Cl_2 (320 µL, 3/1, v/v), cooled to -50 °C and stirred for 15 min. A solution of TfOH in CH_2Cl_2 (30 µL, 3 µmol, 0.1 equiv) was added dropwise and the mixture was stirred for 1 h at -50 °C: After filtration of the molecular sieves on a pad of celite, the reaction mixture was diluted with Et_2O and washed with a saturated aq. solution of $Na_2S_2O_3$. After extraction with Et_2O, the organic layer was washed with a saturated aq. solution of $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by NP-HPLC on YMC Pack silica, eluents AcOEt/Hexanes (gradient: 5% AcOEt (5 min) → 30% AcOEt (30 min)) to afford the target trisaccharide **235** (55 mg, 31 µmol) in 97% yield as a colorless oil: R_f (Hexanes/EtOAc = 70:30) = 0.36; $[\alpha]_D^{20}$ = +18.3 (c = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.90 (d, *J* = 7.6 Hz, 1H, N*H*$COCl_3$), 7.86 – 7.76 (m, 2H), 7.73 – 7.70 (m, 2H), 7.51 – 7.41 (m, 3H), 7.36 – 7.04 (m, 45H), 5.14 (d, *J* = 6.0 Hz, 2H, CO_2CH_2Ph), 5.09 – 5.07 (m, 2H, CH_2Ph, H_1B), 5.00 – 4.95 (m, 2H, CH_2Ph), 4.88 (d, *J* = 10.8 Hz, 1H, CH_2Ph), 4.85 (d, *J* = 3.2 Hz, 1H, H_1C), 4.84 – 4.77 (m, 2H, H_1A, CH_2Ph), 4.71 – 4.69 (m, 2H, CH_2Ph), 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, NCH_2Ph), 4.30 – 4.23 (m, 2H, H_5B, H_3A), 4.20 – 4.15 (m, 2H, CH_2Ph, $H_{6a}C$), 4.12 – 4.09 (m, 1H, H_5C), 4.05 (dd, *J* = 9.2, 9.6 Hz, 1H, H_3C), 3.98 – 3.87 (m, 4H, CH_2Ph, H_4B, $H_{6b}C$, H_2B), 3.83 (dd, *J* = 10.4, 2.8 Hz, 1H, H_3B), 3.77 – 3.56 (m, 7H, OCH_2, $H_{6a}A$, $H_{6b}A$, H_4A, $H_{6a}B$, H_5A, H_4C), 3.53 (dd, *J* = 10.0, 3.2 Hz, 1H, H_2C), 3.51 – 3.40 (m, 2H, $H_{6b}B$, H_2A), 3.35 –

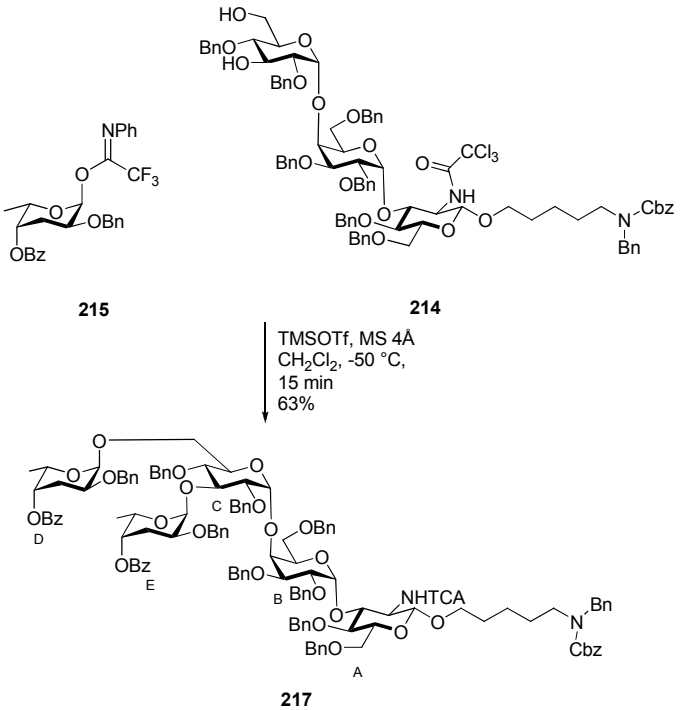
3.32 (m, 1H, OCH_2), 3.16 – 3.08 (m, 2H, NCH_2), 1.97 (s, 3H, COCH_3), 1.50 – 1.33 (m, 4H, CH_2), 1.25 – 1.09 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, rotamers): δ 170.7, 161.5, 138.4, 138.3, 138.2, 138.0, 138.0, 137.7, 136.1, 133.4, 133.0, 128.6, 128.61, 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.2, 126.6, 126.1, 126.0, 126.0, 99.3 ($^1J_{C1C\text{-}H1C}$ = 172.4 Hz, C_1C-α), 99.1 ($^1J_{C1A\text{-}H1A}$ = 164.4 Hz, C_1A-β), 97.8 ($^1J_{C1B\text{-}H1B}$ = 169.1 Hz, C_1B-α), 92.9 (NH*C*OCl_3), 81.9 (C_3C), 80.77 (C_2C), 79.9 and 79.7 (C_3A), 77.6 (C_2B), 77.5 (C_4A), 77.3 (C_4C), 76.4 (C_3B), 75.9 (C_4B), 75.7 (*C*H_2Ph), 75.1 (*C*H_2Ph), 74.4 (*C*H_2Ph), 74.2 (C_5A), 73.9 (*C*H_2Ph), 73.5 (*C*H_2Ph), 73.5 (*C*H_2Ph), 73.1 (*C*H_2Ph), 72.7 (*C*H_2Ph), 70.6 (C_5C), 69.7 (O*C*H_2), 69.5 (C_6B), 69.4 (C_5B), 69.0 (C_6A), 67.2 (CO_2*C*H_2Ph), 62.8 (C_6C), 58.6 (C_2A), 50.6 and 50.2 (N*C*H_2Ph), 47.2 and 46.2 (N*C*H_2), 29.3 (*C*H_2), 28.0 and 27.5 (*C*H_2), 23.2 (*C*H_2), 21.0 (CO*C*H_3); IR (thin film) 3340, 3062, 3030, 2930, 2867, 1739, 1702, 1454, 1234, 1091, 1060 cm^{-1}; HRMS(ESI) m/z calcd for $(M+Na)^+$ $C_{102}H_{107}Cl_3N_2O_{19}$: 1791.6431, found 1791.6380.



235 73% (two steps) **214**

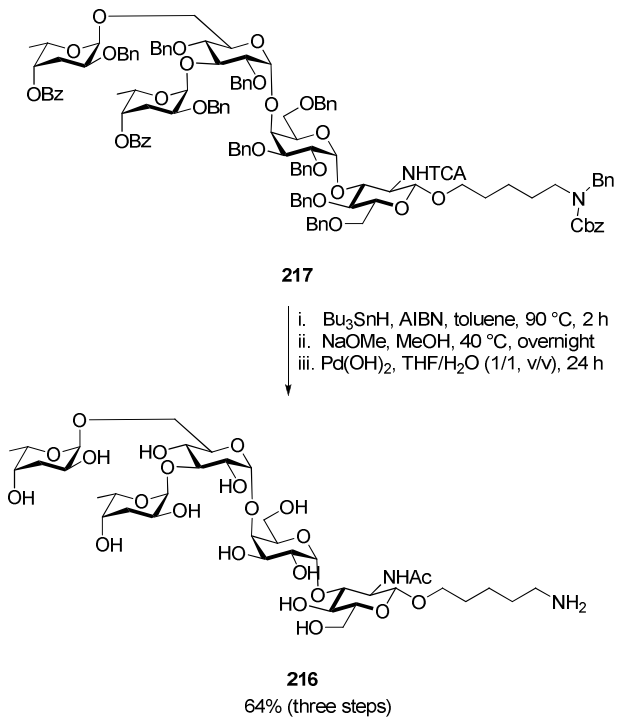
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,4-di-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-trichloroacetamido-β-D-glucopyranoside (214)**: A solution of trisaccharide **235** (50 mg, 28 μmol) in MeOH (0.3 mL) was treated with a 0.5 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 CH_2Cl_2/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 $Na_2S_2O_3$ and a saturated aq. solution of $NaHCO_3$. The mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ 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: R_f (Hexanes/EtOAc =

50:50) = 0.46; $[\alpha]_D^{20}$ = +70.9 (c = 0.25, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, rotamers): δ 7.86 (d, *J* = 6.6 Hz, 1H), 7.42 – 7.04 (m, 45H), 5.15 (d, *J* = 6.6 Hz, 2H, CO_2CH_2Ph), 5.02 (s, 1H, H_1B), 4.96 (d, *J* = 11.0 Hz, 1H, CH_2), 4.84 (d, *J* = 6.0 Hz, 1H, CH_2), 4.81 (s, 2H, H_1C, H_1A), 4.72 – 4.65 (m, 4H, CH_2Ph), 4.65 – 4.58 (m, 2H, CH_2Ph), 4.56 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.50 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.45 – 4.41 (m, 3H, CH_2Ph, NCH_2Ph), 4.31 – 4.27 (m, 1H, CH_2Ph), 4.23 (dd, *J* = 7.8, 7.8 Hz, 1H, H_3A), 4.14 – 4.08 (m, 2H, H_5B, H_3C), 4.04 – 4.02 (m, 2H, H_5C, CH_2Ph), 3.86 –3.81 (m, 3H, H_4B, H_3B, H_2B), 3.77 – 3.44 (m, 10H, OCH_2, $H_{6a}A$, $H_{6b}A$, $H_{6a}B$, H_4A, H_5A, $H_{6a}C$, $H_{6b}C$, $H_{6b}B$, H_2A), 3.41 (dd, *J* = 9.0, 9.6 Hz, 1H, H_4C), 3.37 – 3.25 (m, 2H, H_2C, OCH_2), 3.17 – 3.09 (m, 2H, NCH_2), 2.37 (s, 1H, OH), 1.45 – 1.40 (m, 4H, CH_2), 1.22 – 1.15 (m, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$, rotamers): δ 161.6, 138.4, 138.3, 138.2, 138.1, 137.9, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.3, 99.20 ($^1J_{C1A\text{-}H1A}$ = 165.5 Hz, C_1A-β), 99.1 ($^1J_{C1C\text{-}H1C}$ = 173.0 Hz, C_1C-α), 97.9 ($^1J_{C1B\text{-}H1B}$ = 173.5 Hz, C_1B-α), 92.9 ($NHCOCCl_3$), 80.5 (C_2C), 79.7 (C_3A), 78.5 (C_2B), 78.0 (C_4C), 77.5 (C_4A), 77.1 (C_3B), 76.2 (C_4B), 74.8 (CH_2Ph), 74.4 (CH_2Ph), 74.3 (C_5A), 73.8 (CH_2Ph), 73.57 (CH_2Ph), 73.56 (CH_2Ph), 73.52 (C_3C), 73.2 (CH_2Ph), 73.0 (CH_2Ph), 71.4 (C_5C), 70.6 (C_5B), 69.8 (C_6B), 69.7 (OCH_2), 69.1 (C_6A), 67.2 (CO_2CH_2Ph), 61.9 (C_6C), 58.7 and 58.5 (C_2A), 50.6 and 50.3 (NCH_2Ph), 47.2 and 46.3 (NCH_2), 29.4 (CH_2), 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 calcd for $(M+Na)^+$ $C_{89}H_{97}Cl_3N_2NaO_{18}$: 1609.5700, found 1609.5709.



***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 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 CH_2Cl_2 (340 µL) under Ar atmosphere. The mixture was cooled to -50 °C and stirred for 15 min. After dropwise addition of TMSOTf in CH_2Cl_2 (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 CH_2Cl_2 and washed with a saturated aq. solution of $NaHCO_3$ and brine, and dried over $MgSO_4$. 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: R_f (Hexanes/AcOEt = 70:30) = 0.20; $[\alpha]_D^{20}$ = +10.3 (c = 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 8.07 (d, *J* = 8.0 Hz, 1H, N*H*COCl$_3$), 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 (d, *J* = 7.2 Hz, 2H), 5.65 (d, *J* = 3.2 Hz, 1H, H_1D), 5.26 – 5.22 (m, 1H, H_4E), 5.13 (s, 2H, CO_2CH_2Ph), 5.05 – 5.00 (m, 2H, H_1B, C*H*$_2$Ph), 4.97 – 4.91 (m, 3H, 2 C*H*$_2$Ph, H_1A), 4.86 (d, *J* = 3.2 Hz, 1H, H_1C), 4.82 – 4.77 (m, 2H, H_4D, H_1E), 4.74 – 4.66 (m, 4H, C*H*$_2$Ph), 4.65 – 4.60 (m, 2H, C*H*$_2$Ph), 4.56 – 4.47 (m, 4H, CH_2Ph), 4.44 – 4.17 (m, 12H, C*H*$_2$Ph, NC*H*$_2$Ph, H_3C, H_5B, H_3A, H_5D, H_5E), 4.03 – 3.92 (m, 4H, H_{6a}B, H_5C, H_2B, H_4B), 3.87 – 3.84 (m, 2H, H_2E, H_4C), 3.80 (dd, *J* = 10.2, 3.2 Hz, 1H, H_3B), 3.77 – 3.50 (m, 10H, H_{6a}C, H_2D, H_{6a}A, H_{6b}A, OC*H*$_2$, H_2C, H_4A, H_{6b}C, H_5A, H_{6b}B), 3.37 – 3.22 (m, 2H, OC*H*$_2$, H_2A), 3.19 – 2.95 (m, 2H, NC*H*$_2$), 2.27 – 2.20 (m, 1H, 2H, H_3E), 2.14 – 2.04 (m, 2H, H_3D), 1.41 – 1.35 (m, 4H, C*H*$_2$), 1.18 – 1.12 (m, C*H*$_2$), 1.06 (d, *J* = 6.6 Hz, 3H, H_6D), 0.73 (d, *J* = 6.4 Hz, 3H, H_6E); ^{13}C NMR (150 MHz, $CDCl_3$, rotamers): δ 166.1, 166.0, 161.7, 138.8, 138.6, 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, 128.5, 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 ($^1J_{C1A\text{-}H1A}$ = 166.9 Hz, C_1A-β), 98.6 ($^1J_{C1C\text{-}H1C}$ = 170.8 Hz, C_1C-α), 97.9 ($^1J_{C1B\text{-}H1B}$ = 171.5 Hz, C_1B-α), 96.7 ($^1J_{C1E\text{-}H1E}$ = 171.3 Hz, C_1E-α), 96.5 ($^1J_{C1D\text{-}H1D}$ = 173.6 Hz, C_1D-α), 93.1 (NH*C*OCCl$_3$), 82.8 (C_2C), 80.9 (C_3A), 77.6 (C_4C), 77.3 (C_4A), 76.8 (C_2B), 76.4 (C_4B), 75.8 (C_3B), 74.8, 74.7, 74.1, 74.1, 73.5 (C_5A), 73.4 (C_3C), 73.0, 72.9, 72.6 (C_6B), 72.1 (C_4E), 72.0 (C_4D), 71.3 (C_2E), 71.0, 70.9

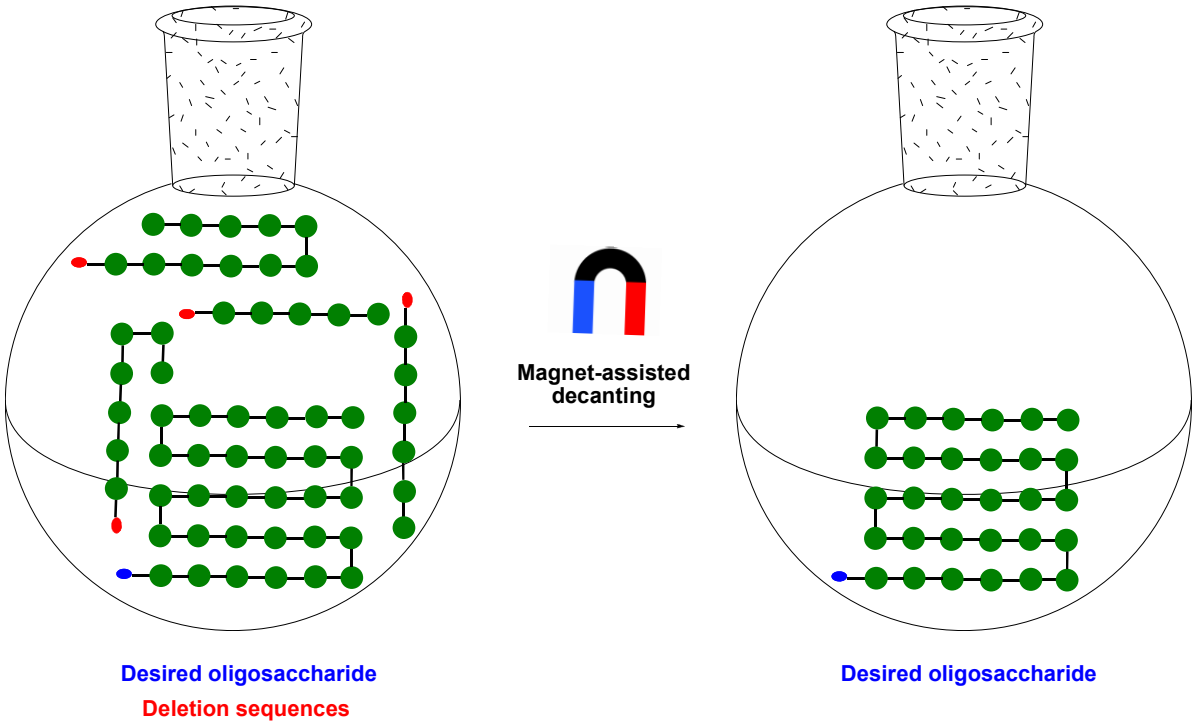
(C_5B), 70.6 (C_5C), 69.8 (C_2D), 69.2, 69.1 (C_6A), 67.2 (NHCO$_2$*C*H$_2$Ph), 65.9 (C_6C), 65.0 (C_5D), 64.8 (C_5E), 59.4 (C_2A), 50.6 and 50.3 (N*C*H$_2$Ph), 47.2 and 46.3 (N*C*H$_2$), 29.4 (*C*H$_2$), 29.1 (C_3E), 28.3 (C_3D), 28.0 and 27.6 (*C*H$_2$), 23.2 (*C*H$_2$), 16.5 (C_6E), 16.1 (C_6D); IR (thin film) 3347, 2925, 2855, 1716, 1454, 1270, 1093, 1061, 1027, 984 cm^{-1}; HRMS(ESI) m/z calcd for (M+Na)$^+$ $C_{129}H_{137}Cl_3N_2NaO_{26}$: 2257.8423, found 2257.8471.



5-Amino-pentanyl 3,6-dideoxy-α-L-galactopyranosyl-(1→3)-[3,6-dideoxy-α-L-galactopyranosyl-(1→6)]-α-L-galactopyranosyl)-α-D-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 Bu_3SnH (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)$^+$ $C_{129}H_{140}N_2NaO_{26}$: 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)$^+$ $C_{115}H_{132}N_2NaO_{24}$: 1947.90, found 1947.92. The oil was solubilized in a mixture of THF/H_2O (6 mL, 1/1, v/v). $Pd(OH)_2$ 20% (20 mg) was added and the suspension was stirred under H_2 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 (^{i}PrOH/1 M aq. NH_4OAc = 1:2) = 0.50; $[\alpha]_D^{20}$ = +29.3 (c = 0.06, $CHCl_3$); ^{1}H NMR (600 MHz, D_2O): δ 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_6CO): δ 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 calcd for $(M+H)^+$ $C_{37}H_{67}N_2O_{22}$: 891.4185, found 891.4208.

4. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside Assisted by Catch-and-Release Purification



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.

4.1 Introduction

Carbohydrates are structurally complex and diverse biopolymers[117] that serve a host of biological functions.[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[118] and oligonucleotides[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.[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.[120]

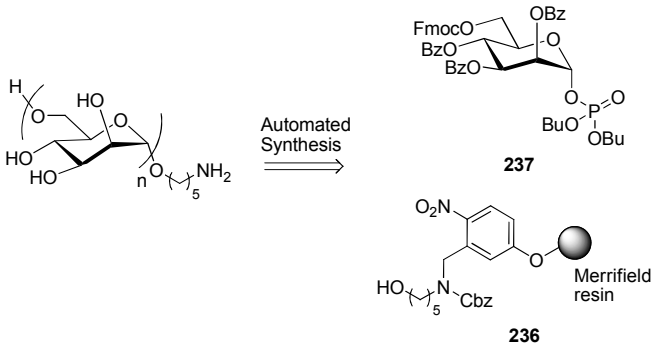
Herein, we report the automated synthesis of a 30mer α-(1,6)-oligomannoside as a proof-of-principle that automated oligosaccharide synthesis can provide access to long carbohydrate chains. Since the interaction carbohydrate-carbohydrate recognition receptor is weak, access to large defined oligosaccharides 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

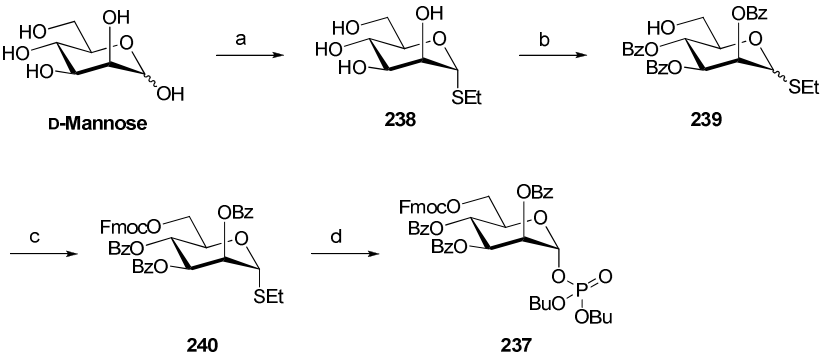
α-(1,6)-Oligomannosides could be assembled on the functionalized Merrifield solid support **236** in a regio- and diastereoselective manner using mannosyl phosphate building block **237** that has a C2 participating benzoyl protecting group to ensure the formation of the trans-

glycosidic linkages and an orthogonal Fmoc protected hydroxyl at C6 for chain elongation (Scheme 61). The anomeric dibutyl phosphate leaving group allows for fast and efficient glycosylation by Lewis acid activation.[121] Based on our experience, Merrifield´s resin[48] 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).[53,54,122]



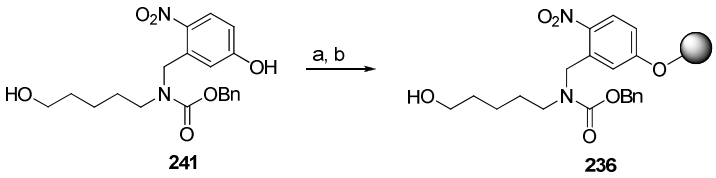
Scheme 61. Retrosynthetic analysis of α-(1,6)-oligomannosides.

Mannosyl phosphate **237** 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[114] gave thiomannoside **238**. 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 **239** in 58% yield over five steps. Intermediate **239** was then treated with 9-fluorenylmethyloxycarbonyl chloride in the presence of pyridine to give building block **240**. Due to the high reactivity of phosphate glycosylating agents, thiomannoside **240** was converted to the corresponding dibutyl phosphate **237** in 78% yield by treatment with *N*-iodosuccinimide and dibutyl phosphate in the presence of a catalytic amount of triflic acide.



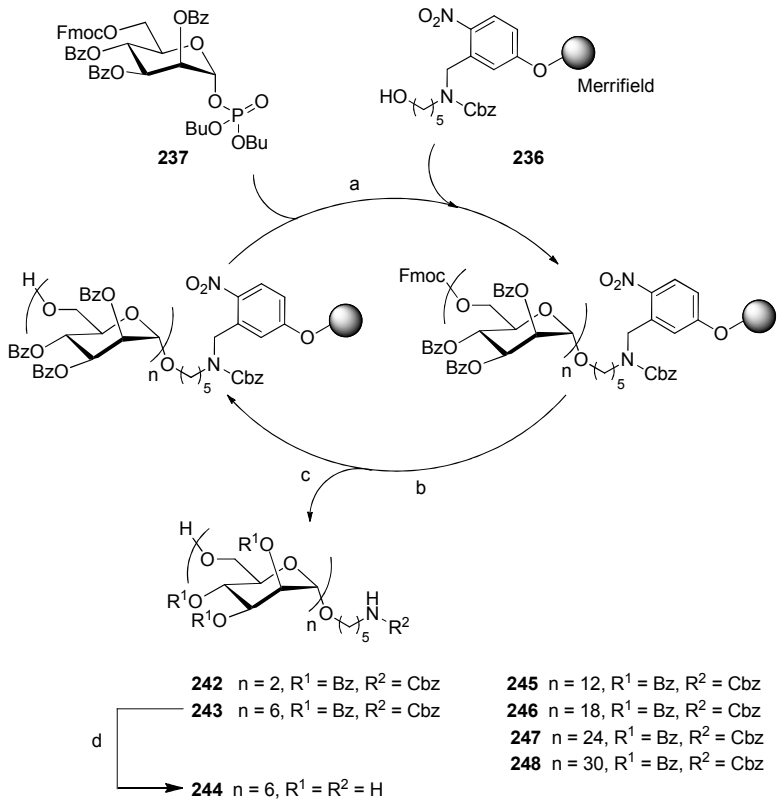
Scheme 62. Synthesis of mannosyl phosphate building block **237**: a) i. Ac_2O, $HClO_4$, 0 °C; ii. EtSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C to RT; iii. NaOMe, MeOH, RT; b) i. TrtCl, pyridine, 80 °C then BzCl, 0 °C to RT; ii. *p*-TSA, CH_2Cl_2, MeOH, **239α** 51% (five steps), **239β** 7% (five steps), 35 g scale; c) FmocCl, pyridine, 0 °C to RT, 87%; d) $(BuO)_2P(=O)OH$, NIS, TfOH, CH_2Cl_2, 0 °C, 88%.

Merrifield resin **236** functionalized with the photolabile linker **241** was prepared according to a known procedure (Scheme 63).[53]



Scheme 63. Synthesis of functionalized Merrifield resin **236**: Reactions and Conditions: a) Merrifield resin, Cs_2CO_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).[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.[55,123]



Scheme 64. Automated synthesis of α-(1,6)-polysaccharides: Reactions and conditions: a) Glycosylation: **237**, TMSOTf, CH_2Cl_2, -15 °C (45 min) - 0 °C (15 min); b) Fmoc deprotection: piperidine, DMF, 25 °C (5 min); c) Cleavage from solid support: hν, CH_2Cl_2; d) i. NaOMe cat., MeOH; ii. Pd/C, H_2, H_2O.

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).

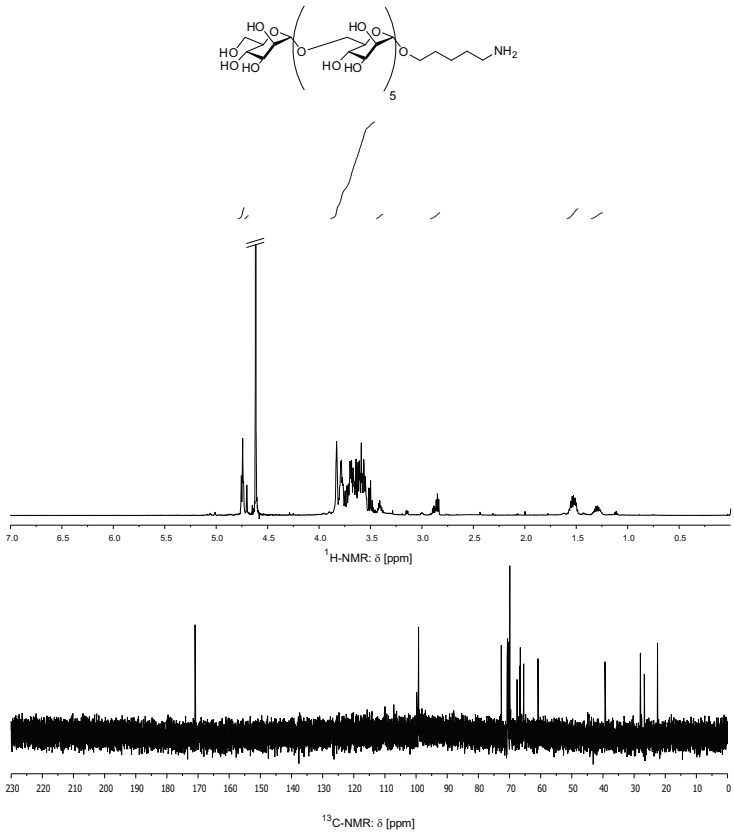


Figure 8. Characterization of hexasaccharide **244**: A) ^{1}H NMR (D_2O, 400 MHz) of α-(1,6)-hexamannoside **244**; B) ^{13}C NMR (D_2O, 100 MHz) of α-(1,6)-hexamannoside **244**.

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).

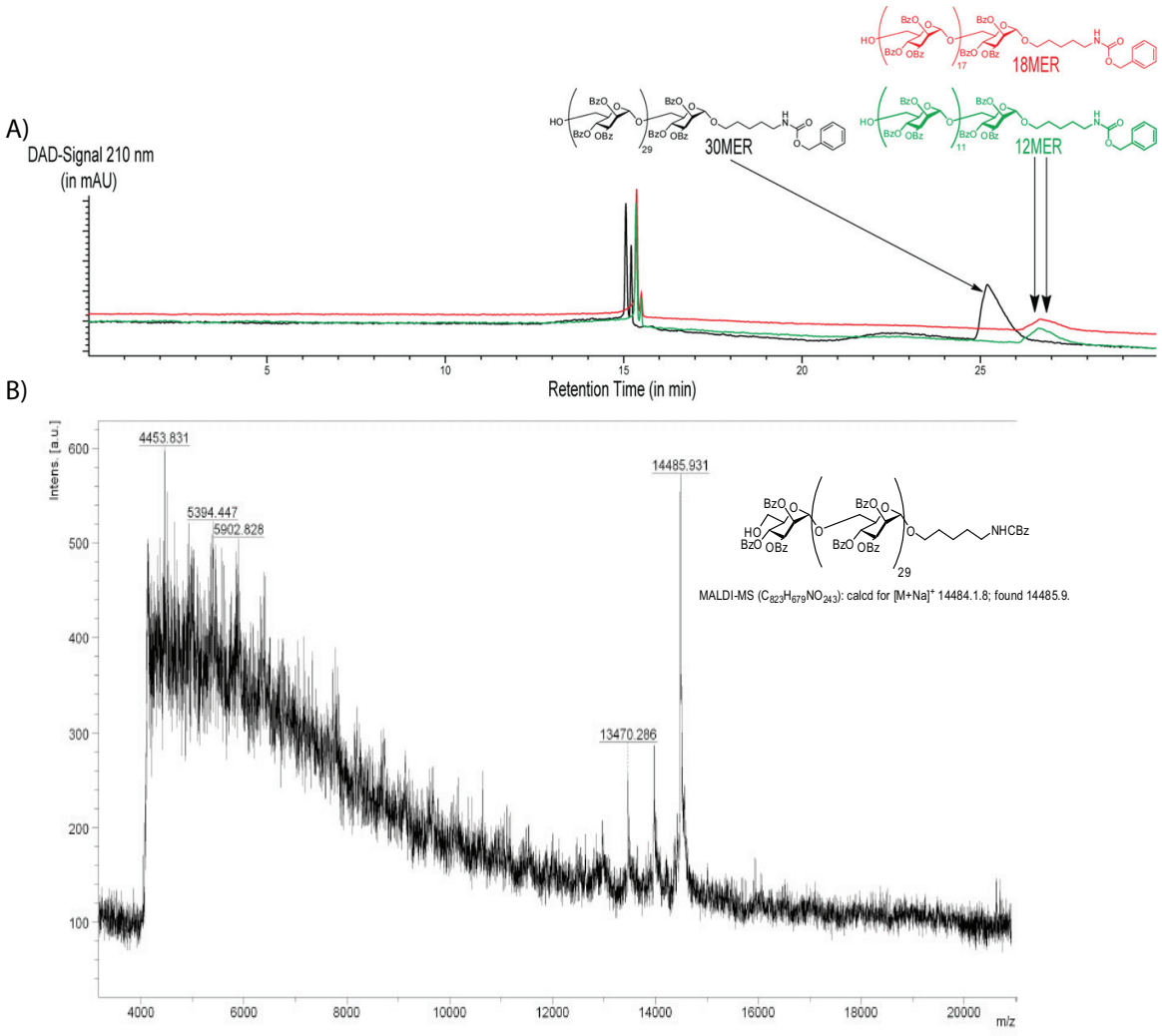
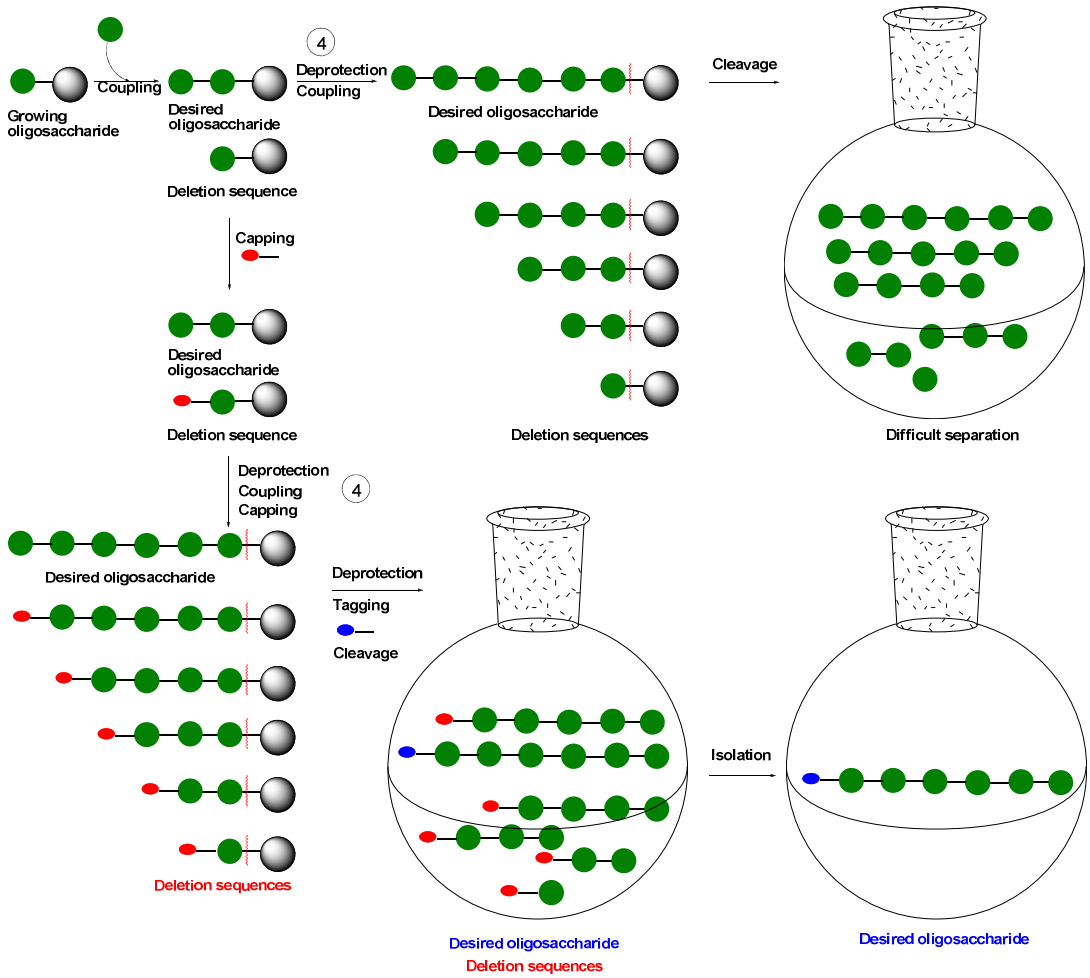


Figure 9. Challenges in purification of large α-(1,6)-oligomannosides: A) NP-HPLC comparative chromatogram of the crude reaction mixture containing the α-(1,6)-30 mer **248**, α-(1,6)-24 mer **247** and α-(1,6)-12 mer **245** (Nucleosil 100-5OH, eluents CH_2Cl_2/MeOH, gradient: 0% MeOH (5 min) → 30% 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[124] and oligohistidine.[125] This strategy cannot be adopted for the purification of protected oligomannosides since aqueous solvents are required as eluents. Fluorous tags have helped to

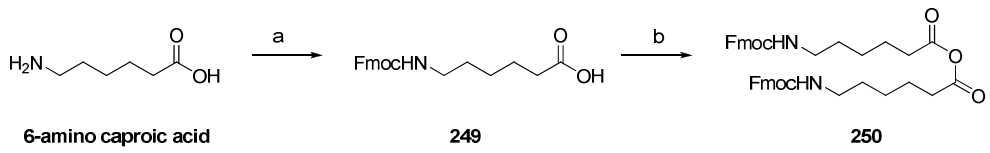
separate oligonucleotides up to 100mers[126] using fluorous solid phase extraction and liquid-liquid extraction.[127] Solubility issues associated with fluorous labels rendered them useless in the context of the isolation of longer α-(1,6)-oligomannosides. Therefore, we considered capture-release techniques (Scheme 65)[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 attachement and disattachement from a solid suport.

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.

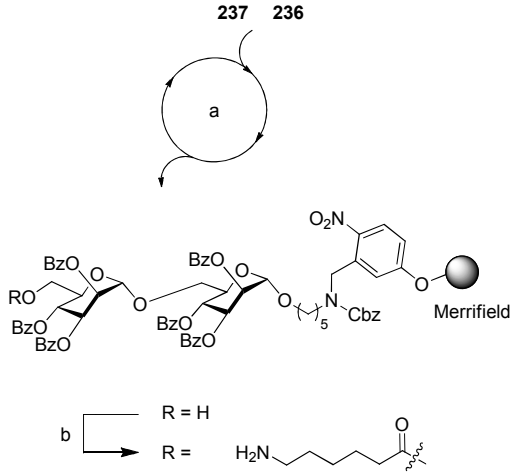
Preliminary tests required the synthesis of the tagging agents **249** and **250**. Protection of the primary amine on the 6-amino caproic acid as an Fmoc-carbamate afforded **249** in 67% yield that was further converted to the corresponding anhydride **250** by treatment with *N*,*N*´-dicyclohexylcarbodiimide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



Scheme 66. Synthesis of the tagging reagents **249** and **250**: a) FmocOSuc, $NaHCO_3$, water/acetone (1/1, v/v), 67%; b) DCC, CH_2Cl_2, quant..

To test the introduction of the cap-tag strategy in the automated solid phase synthesis of α-(1,6)-oligomannosides, the synthesis of an α-(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 attachement of the tag on the molecule was attempted. Treatment of the target disaccharide with anhydride **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

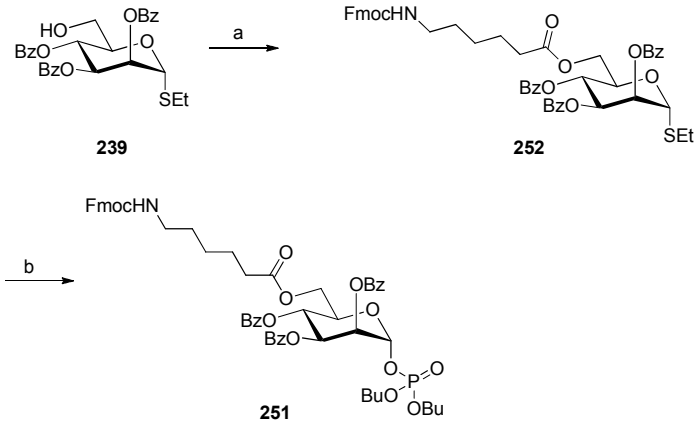
the desired disaccharide was reacted with 6-amino hexanoic acid **249** in presence of *N*,*N´*-diisopropylcarbodiimide and 4-(*N*,*N*-dimethyl)aminopyridin (Scheme 67, Table, entry 2). However, this second trial didn´t proove either efficient leading to only 22% coupling efficiency (Table, entry 2). Applying such as inefficient labelling method to the automated solid phase synthesis will result in a lost of considerable quantities of the target oligosaccharide.



entry	conditions	efficiency
1	5 equiv. **250**, pyridine, DCM, 25 °C (4 h)	13%
2	5 equiv. **249**, DIC, DMAP, DMF, DCM, 25 °C (3 h)	22%

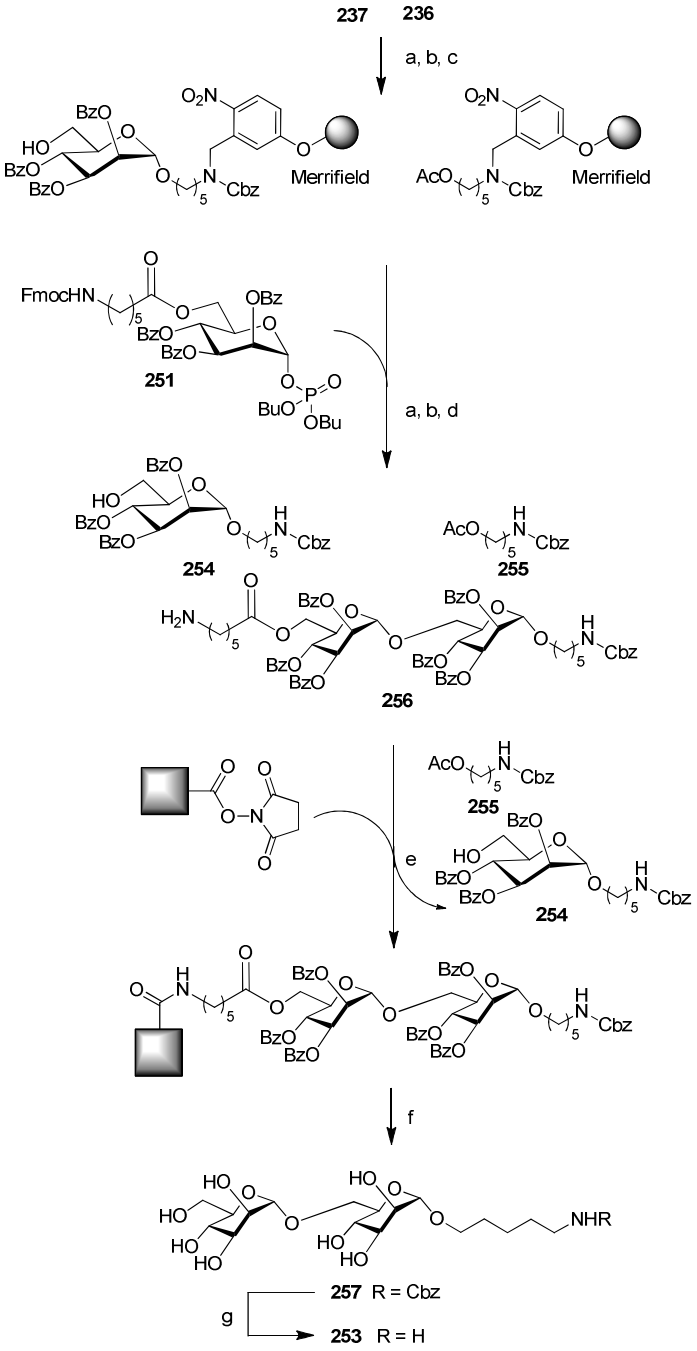
Scheme 67. Initial attempts to introduce the tag to an automatically synthesized disaccharide: Reactions and conditions: a) i. Glycosylation: **237**, TMSOTf, CH_2Cl_2, -15 °C (45 min) - 0 °C (15 min); ii. Capping: Ac_2O, pyridine 25 °C (60 min); iii. Fmoc deprotection: piperidine, DMF, 25 °C (5 min); 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.



Scheme 68. Synthesis of glycosyl based tagging agent **251**: a) **249**, DIC, pyridine, CH_2Cl_2, 0 °C to RT, 73%; b) $(BuO)_2P(=O)OH$, NIS, TfOH, CH_2Cl_2, 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 CH_2Cl_2 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.



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, CH_2Cl_2, -15 °C (45 min) - 0 °C (15 min); b) Capping: Ac_2O, pyridine 25 °C (60 min); c) Fmoc deprotection: piperidine, DMF, 25 °C (5 min); d) Cleavage from solid support: hν, CH_2Cl_2; e) Immobilization on magnetic beads: i. Et_3N, CH_2Cl_2, 25 °C, 12 h; ii. Washing with CH_2Cl_2 to remove deletion sequences of the type **254** and **255**; f) Release from magnetic beads: NaOMe, MeOH, 12 h; g) Pd/C, H_2, H_2O, 12 h.

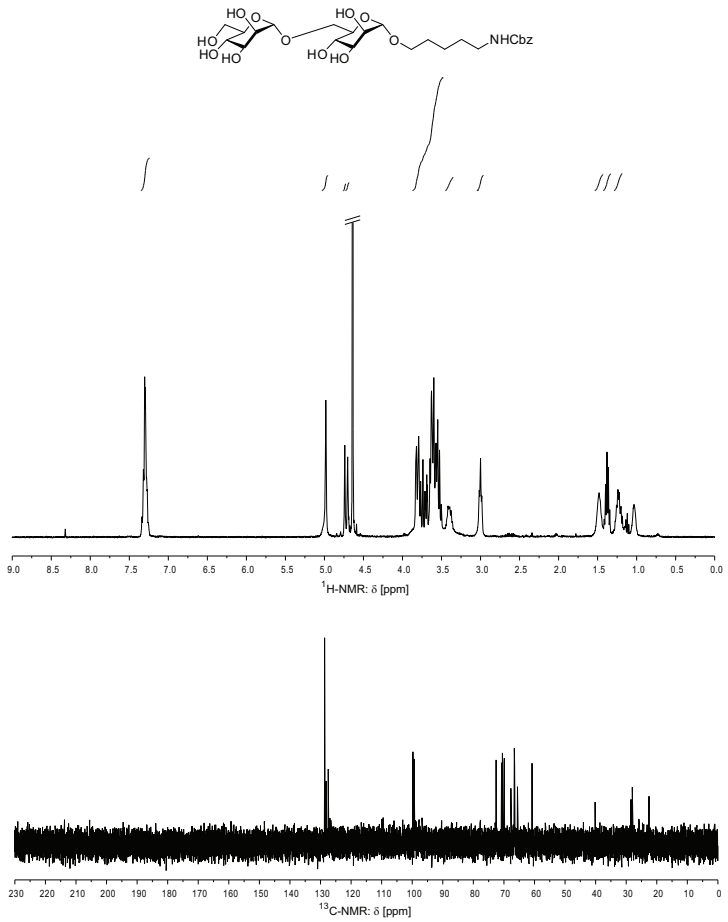
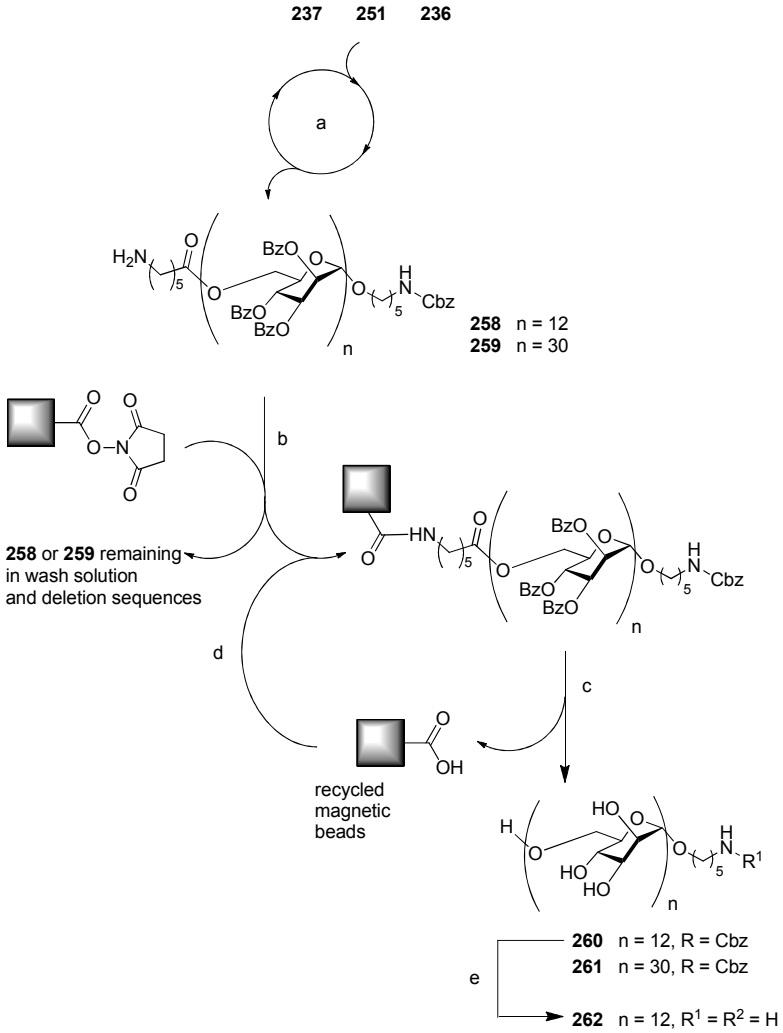


Figure 10. Characterization of disaccharide **257**: A) ^{1}H NMR (D_2O, 400 MHz) of α-(1,6)-dimannoside **257**; B) ^{13}C NMR (D_2O, 100 MHz) of α-(1,6)-dimannoside **257**.

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-oxytripyrrolidinophosphonium hexafluorophosphate and *N*,*N*-diisopropylethylamine (Scheme 70).



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. Et_3N, CH_2Cl_2, 25 °C, 12 h; ii. Remove deletion sequences by CH_2Cl_2 wash; c) Release from magnetic beads: NaOMe, MeOH, 12 h; d) Magnetic beads recycling: **258**, **259**, PyBOP, DIPEA, CH_2Cl_2, 25 °C, 12 h; e) Pd/C, H_2, H_2O.

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.

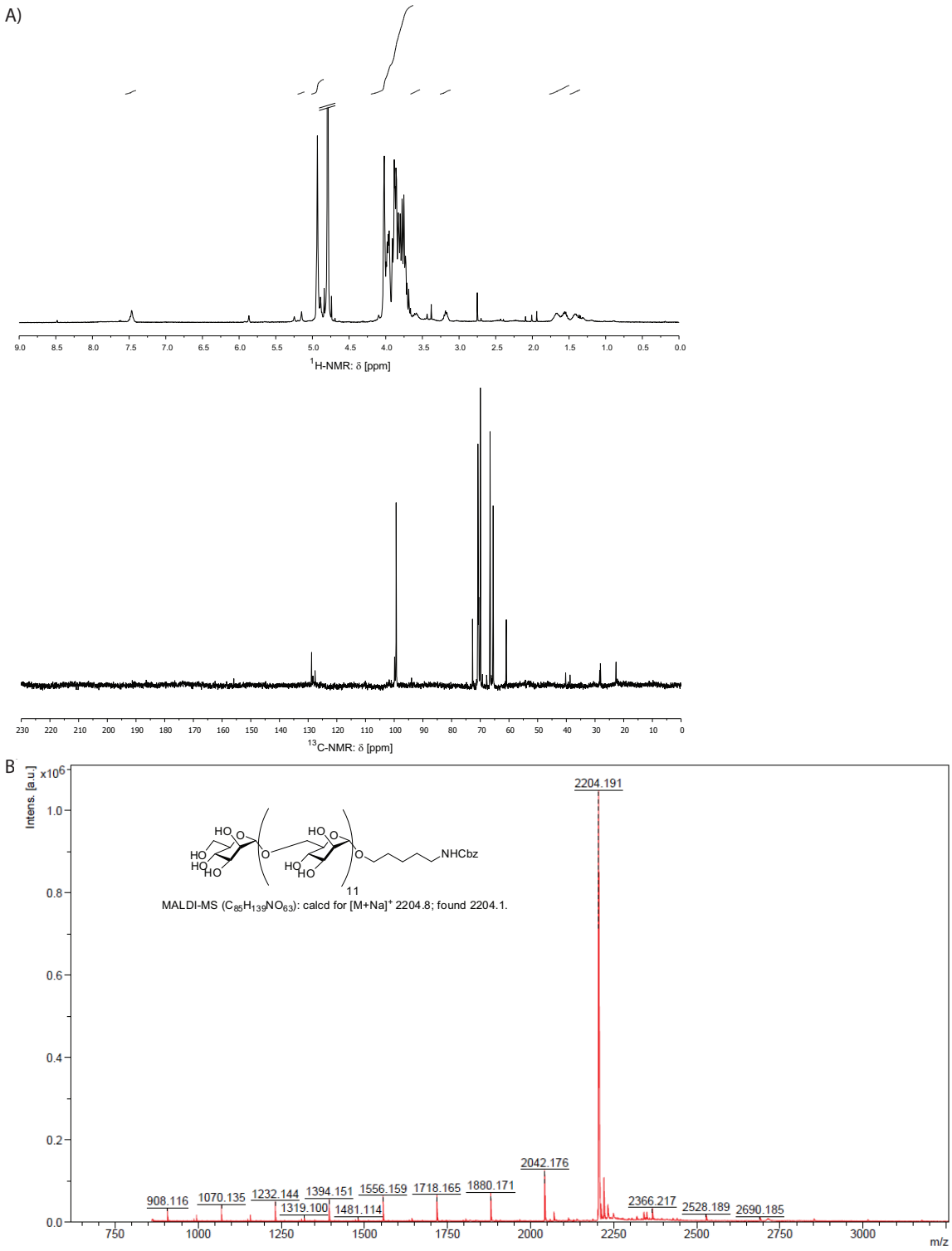


Figure 11. Characterization of dodecamannoside **260**: A) ^{1}H NMR (D_2O, 600 MHz) and ^{13}C NMR (D_2O, 150 MHz) of α-(1,6)-dodecamanoside **260**; B) MALDI-MS analysis of the α-(1,6)-dodecamanoside **260**.

The automated synthesis of an α-(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).

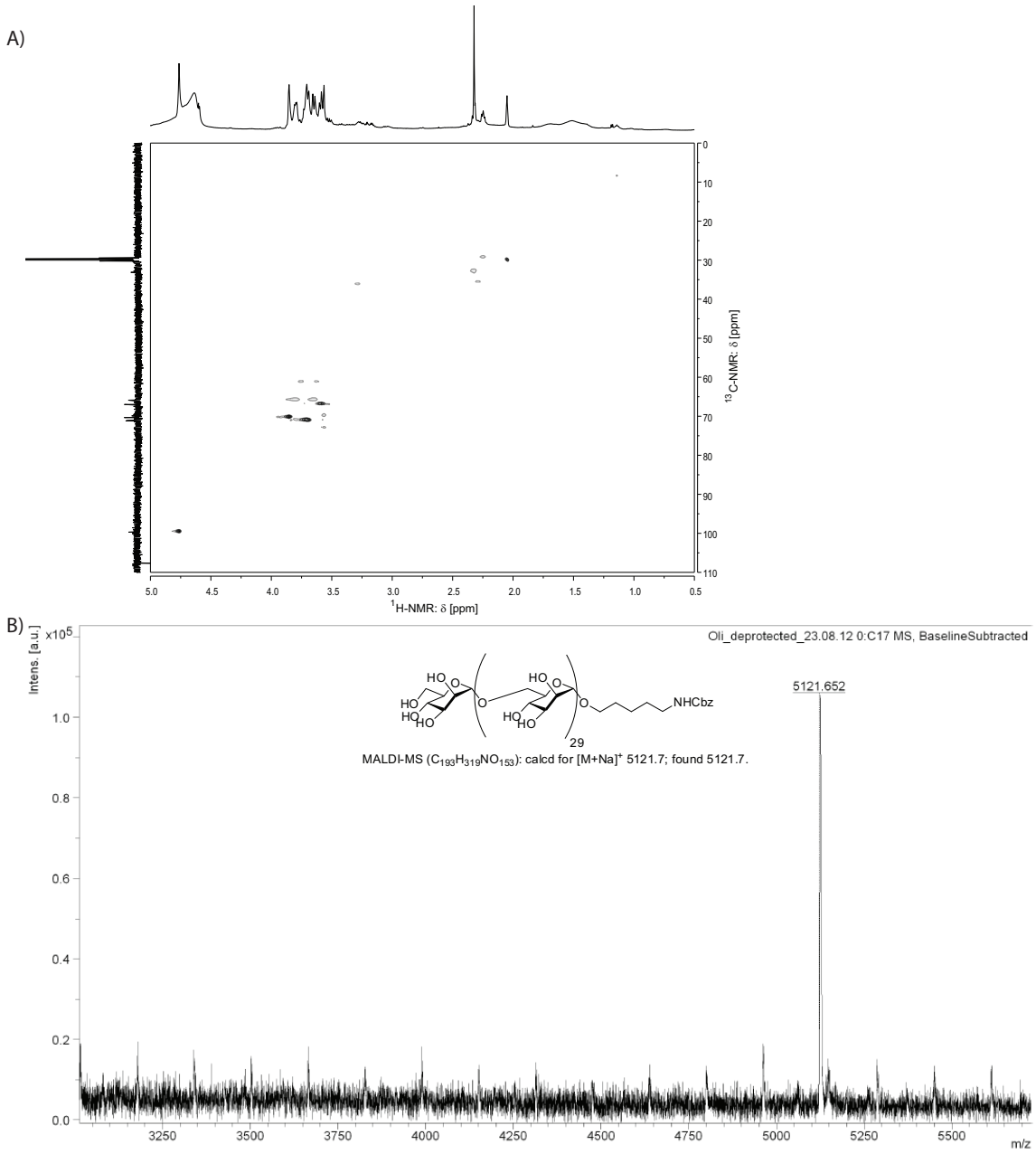


Figure 12. Characterization of polymannoside **261**. A) HSQC NMR (D_2O, 600MHz) of α-(1,6)-30mer **261**; B) MALDI-MS analysis of the α-(1,6)-30mer **261**.

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.

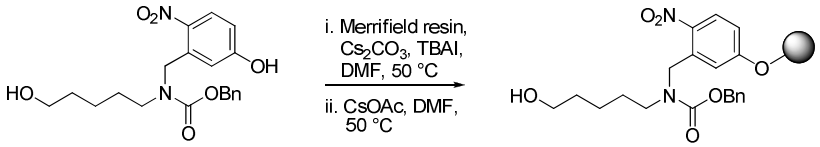
4.4 Experimental Section

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_2Cl_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_2SO_4 (2 N) and resorcine 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). All automated glycosylations were performed on an automated oligosaccharide synthesizer prototype[47] 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. ^1H, ^{13}C spectra were recorded on a Varian Mercury 400 (400 MHz) or 600 (600 MHz) spectrometer in $CDCl_3$ or CD_3OD with chemical shifts referenced to internal standards ($CDCl_3$: ^1H 7.26 ppm, ^{13}C 77.0 ppm; D_2O with acetone as internal standard: ^1H 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 ^1H-NMR data. NMR chemical shifts (δ) are reported in ppm and coupling

constants (*J*) are reported in Hz. High resolution mass spectral (HRMS) analyses were performed by the MS-service 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[130]



241 **236**

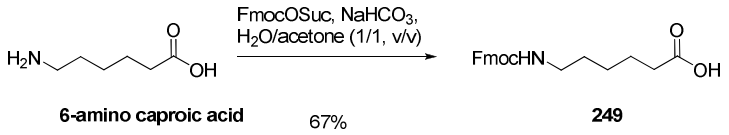
Merrifield resin (6.40 g, loading: 0.74 mmol/g, 4.74 mmol) was swollen overnight in CH_2Cl_2 in a flask while being gently shaken on a rotavap. A solution of phenol **241** (2.2 g, 5.67 mmol) in CH_2Cl_2 was added to the resin. DMF (60 mL) was added to the solution followed by Cs_2CO_3 (2.31 g, 7.095 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_2Cl_2, MeOH, CH_2Cl_2 (six times each) and then swollen in CH_2Cl_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_2Cl_2, MeOH, CH_2Cl_2 (six times each), the solvent was drained, and the resin was dried under vacuum.

Loading Determination[55b]

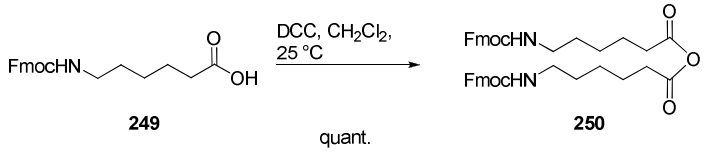
Dry resin **236** (51 mg, theoretical loading: 0.74 mmol/g, 0.037 mmol) was placed in a syringe (5 mL) equipped with a frit. CH_2Cl_2 (2-3 mL) was added to swell the resin for 1 h. The CH_2Cl_2 was drained and a solution of FmocCl (100 mg, 0.39 mmol) and pyridine (0.1 mL, 1.24 mmol) in CH_2Cl_2 (1 mL) was added to the resin. The reaction mixture was shaken for 6 h, the solution was drained and the resin was washed with CH_2Cl_2, MeOH and CH_2Cl_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: $Absorption_{304\ nm}$×16.4/mass of resin used in mg (for 304 nm) and $Absorption_{294\ nm}$×14.2/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

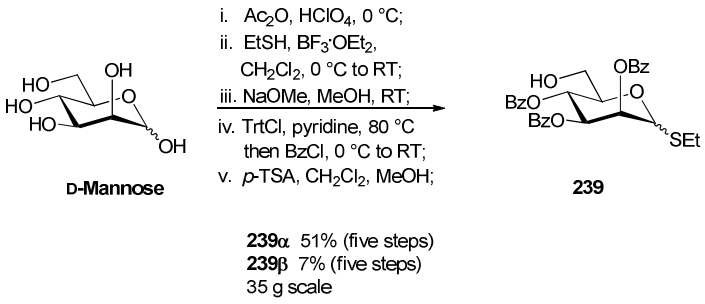


***N*-Fluorenylmethoxycarbonyl-6-aminohexanoic acid (249)**: $NaHCO_3$ (3.3 g, 39.6 mmol) was added to a suspension of caproic acid (4.0 g, 30.5 mmol) and FmocOSuc (10.3 g, 30.5 mmol) in a mixture H_2O/acetone (150 mL, 1/1, v/v). The mixture was stirred at RT for 12 h, cooled to 0 °C and quenched with 1 M aq. solution of HCl. After extraction with AcOEt, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The white residue was suspended in water and treated with triethylamine until complete solubilization. The mixture was extracted with Et_2O and a mixture of Et_2O/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 $MgSO_4$ and concentrated *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.[131]



***N*-Fluorenylmethoxycarbonyl-6-aminohexanoyl anhydride (250)**: A solution of Fmoc-protected 6-amino caproic acid **249** (0.72 g, 2.04 mmol) in CH_2Cl_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 *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 $NaHCO_3$ and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was used without further purification for the next step.

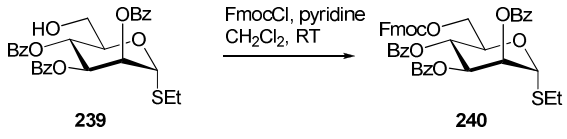
The per-acetylated mannose was co-evaporated three times with toluene and dissolved in CH_2Cl_2 (360 ml). After addition of ethanethiol (12.3 mL, 167 mmol), the solution was cooled to 0 °C and boron trifluoride diethyl etherate (42.2 mL, 333 mmol) was dropwise added. The solution was allowed to warm to RT and after complete conversion of the starting material (tlc: *cyclo*-hexane/AcOEt, 1.5:1) was diluted with CH_2Cl_2, extracted with a saturated aq. solution of $NaHCO_3$ and dried over $MgSO_4$. 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: CH_2Cl_2/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: CH_2Cl_2/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 CH_2Cl_2. The solution was extracted with 1 M aq. solution of HCl and a saturated aq. solution of $NaHCO_3$, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was used without further purification for the next step.

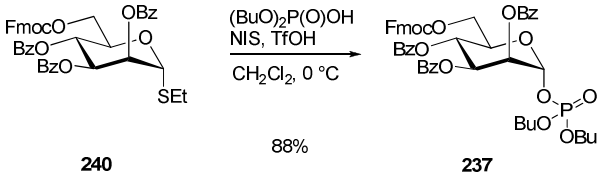
Trityl protected mannose was dissolved in a mixture MeOH/CH_2Cl_2 (700 mL, 2/1, v/v). *p*-TSA (4.60 g, 24.18 mmol) was added and the reaction was stirred at RT. After complete conversion (tlc: *cyclo*-hexane/AcOEt, 2:1), Et_3N (4.6 ml, 33.3 mmol) was added and the volatiles were removed *in vacuo*. The remainder was dissolved in CH_2Cl_2, washed with brine, dried over $MgSO_4$ 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**: R_f (*cyclo*-hexane/AcOEt = 2:1) = 0.32; $[\alpha]_D^{22}$ = –83,6 (c = 0.56, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $(M+Na)^+$ $C_{29}H_{28}O_8NaS$: 559.1403, found 559.1393.



87%

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 CH_2Cl_2 (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 CH_2Cl_2 and extracted with 1 M aq. solution of HCl 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 = 6:1→4:1→2:1) affording the Fmoc-protected mannose **240** (37.4 g, 49.3 mmol, 87%): R_f (*cyclo*-

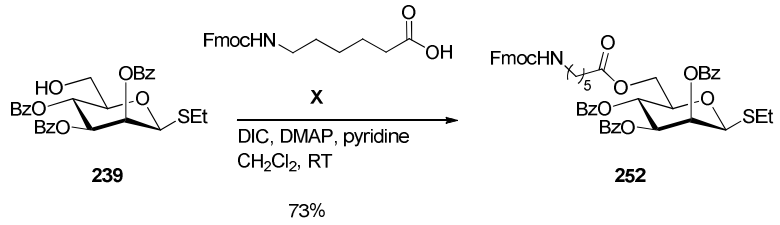
hexane/AcOEt = 2:1) = 0.70; $[\alpha]_D^{22}$ = –46,9 (c = 0.46, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 – 8.09 (m, 2H), 7.99 – 7.97 (m, 2H), 7.83 – 7.81 (m, 2H), 7.76 (d, *J* = 7.2 Hz, 2H), 7.53 – 7.36 (m, 9H), 7.30 – 7.24 (m, 6H), 5.97 (dd, *J* = 10.0, 10.0 Hz, 1H), 5.83 (dd, *J* = 3.6, 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 calcd for $(M+Na)^+$ $C_{44}H_{38}O_{10}NaS$: 781.2083, found 781.2031.



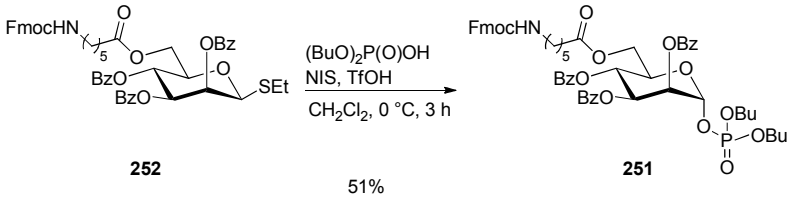
240 **237**

2,3,4-Tri-*O*-benzoyl-6-*O*-fluorenylmethoxycarbonyl-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_2Cl_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 $Na_2S_2O_3$ 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_f (Hexanes/AcOEt = 70:30) = 0.27; $[\alpha]_D^{22}$ = –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 $(M+Na)^+$ $C_{50}H_{51}O_{14}NaP$: 929.2914, found 929.2911.



Ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*N*-fluorenylmethoxycarbonyl-6-aminohexanoyl)-1-thio-β-D-mannosylpyranoside (252): To a solution of thioglycoside **239β** (3.3 g, 6.15 mmol) in CH_2Cl_2 (512 mL), hexanoic acid **249** (6.45 g, 18.26 mmol), pyridine (26.5 mL, 328 mmol) and 4-(*N*,*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: *cyclo*-hexane/AcOEt, 1:1), it was diluted with CH_2Cl_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 (*cyclo*-hexane/AcOEt = 50:50); $[\alpha]_D^{20}$ = –86.3 (c = 0.48, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 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, $CDCl_3$): δ 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 $(M+Na)^+$ $C_{50}H_{49}NO_{11}NaS$: 894.2924, found 894.2912.



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 CH_2Cl_2 (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 CH_2Cl_2 and washed with 10% aq. solution of $Na_2S_2O_3$ 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 flash column chromatography on silica gel (Hexanes/AcOEt = 100:0→50:50) affording the phosphate **251** (0.6 g, 0.6 mmol, 51%). R_f (Hexanes/AcOEt = 50:50) = 0.32; $[\alpha]_D^{20}$ = –60.7 (c = 0.38, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 165.5, 165.4, 165.1, 156.5, 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 $(M+Na)^+$ $C_{56}H_{62}NO_{15}NaP$: 1042.3755, found 1042.3737.

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_2Cl_2. To start the synthetic sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH_2Cl_2 and CH_2Cl_2 (six times each with 2 mL for 25 s). The building blocks were co-evaporated three times with toluene, dissolved in CH_2Cl_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_2Cl_2 and the temperature of the reaction vessel was adjusted to -15 °C. For glycosylations, the CH_2Cl_2 was drained and a solution of phosphate building block (3 equiv in 1.0 mL CH_2Cl_2) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by the addition of TMSOTf in CH_2Cl_2 (3 equiv in 1.0 mL CH_2Cl_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_2Cl_2 and CH_2Cl_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_2Cl_2 and extracted with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed *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_2Cl_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 CH_2Cl_2 and CH_2Cl_2 (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:

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

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

4.4.6. Cleavage from Solid Support[53]

At the end of the run, the resin is washed with CH_2Cl_2 (six times with 2 mL for 25 s), swollen in 2 mL CH_2Cl_2 and transferred into a disposable syringe (2 mL). To prepare the photoreactor (Figure 3)[54] the fluorinated-ethylene-propylene (FEP) tubing is washed with 15 mL MeOH and 15 mL CH_2Cl_2 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 CH_2Cl_2 (flow rate: 300 µL×min^{-1}). In order to shrink and wash out the remaining resin, the tubing is washed with 15 mL CH_2Cl_2/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 CH_2Cl_2/MeOH (1/1, v/v), MeOH and CH_2Cl_2. The tubing is re-equilibrated with 15 mL CH_2Cl_2 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[129] 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_2Cl_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_2Cl_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.

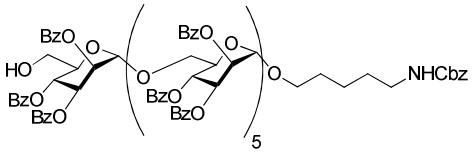
For deprotection and cleavage, the beads were suspended in MeOH/water (6 mL, 1/1, v/v) and the pH was adjusted to 12 by adding sodium methoxide. The reaction was shaken overnight at RT. The solution was magnetically separated from the beads. The magnetic particles were washed with MeOH and water until 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_2Cl_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_2Cl_2 (5 mL). The magnetic beads were washed with CH_2Cl_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_2Cl_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

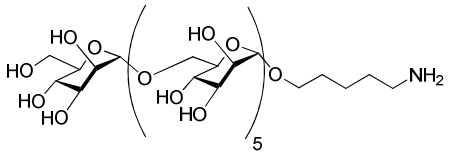


***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (6-*O*-(6-aminohexanoyl)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-**

benzoyl-α-D-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_2Cl_2. To start the synthetic sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH_2Cl_2 and CH_2Cl_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.

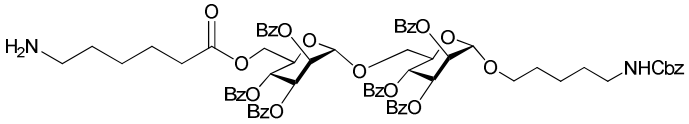
glycosylation	building block and activator (all solutions in 1 mL CH_2Cl_2)	relative Fmoc value
1	**237** (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)	100%
2	**237** (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)	100%
3	**237** (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)	100%
4	**237** (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)	100%
5	**237** (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)	100%
6	**237** (3 × 64 mg; 0.0705 mmol) TMSOTf (3 × 13 µL; 0.0705 mmol)	100%

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-mannopyranosyl-(1→6)-α-D-mannopyranoside (hexamer) (244): Hexasaccharide **243** was dissolved in a mixture of CH_2Cl_2/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). $[\alpha]_D^{20}$ = +58.6 (c = 0.15, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 4.76 – 4.70 (m, 6H), 3.87 – 3.37 (m, 38H), 2.90 – 2.82 (m, 2H), 1.59 – 1.47 (m, 4H), 1.36 – 1.24 (m, 2H); ^{13}C NMR (150 MHz, D_2O): δ 99.8, 99.3, 99.2, 72.6, 70.8, 70.8, 70.7, 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, 65.5, 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 $(M+Na)^+$ $C_{41}H_{73}NO_{31}Na$: 1076.4245, found 1076.4264.

Use of Catch-Release Strategy

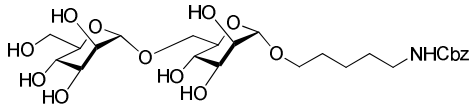


***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (6-*O*-(6-aminohexanoyl)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (dimer) (256)**: Functionalized resin **236** (50 mg, loading 0.47 mmol/g, 0.0235 mmol) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH_2Cl_2. To start the synthetic sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH_2Cl_2 and CH_2Cl_2 (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.

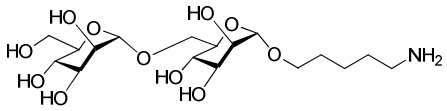
For the last glycosylation, building block **251** and module 1 were used. Subsequent removal of Fmoc was performed using module 3 without prior capping.

glycosylation	building block and activator (all solutions in 1 mL CH_2Cl_2)	relative Fmoc value
1	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
2	**251** (3 × 72 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	

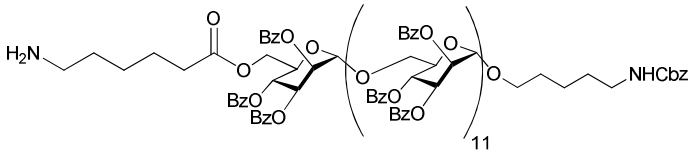
After completing the synthesis of the disaccharide, the target compound was cleaved from the solid support under standard UV light irradiation conditions (see section: Cleavage from Solid Support). The continuous flow reaction afforded 29 mg that was used for the next step without any purification. MS(ESI) m/z calcd for $(M+H)^+$ $C_{73}H_{76}N_2O_{20}$: 1299.3, found 1299.3.



***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl α-D-mannopyranosyl-(1→6)-α-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, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 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}C NMR (100 MHz, D_2O): δ 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, 66.5, 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 $(M+Na)^+$ $C_{25}H_{39}NO_{13}Na$: 584.2319, found 584.2304.



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%). $[\alpha]_D^{20}$ = +20.6 (c = 0.09, H_2O); ^{1}H NMR (600 MHz, D_2O): 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); ^{13}C 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 calcd for $(M+Na)^+$ $C_{17}H_{33}NO_{11}Na$: 450.1951, found 450.1913.



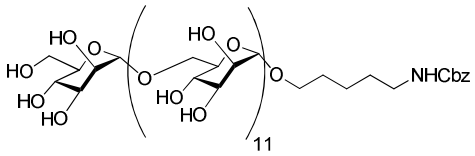
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (6-*O*-(6-aminohexanoyl)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→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-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 CH_2Cl_2. To start the synthesis sequence, the resin was washed with DMF, THF, 0.2 M acetic acid in CH_2Cl_2 and CH_2Cl_2 (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.

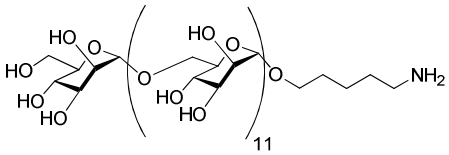
glycosylation	building block and activator (all solutions in 1 mL CH_2Cl_2)	relative Fmoc value
1	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
2	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
3	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
4	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
5	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
6	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
7	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
8	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
9	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
10	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
11	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
12	**251** (3 × 72 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	

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 $(M+Na)^+$ $C_{343}H_{294}N_2O_{100}Na$: 6062.8, found 6060.6.

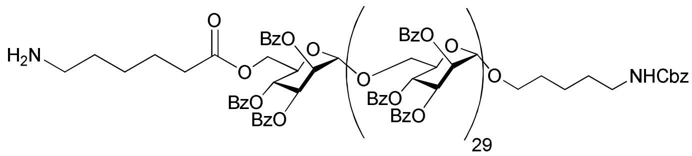


***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (dodecamer) (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_2O); ^1H NMR (600 MHz, D_2O): δ 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_2O): δ 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 $(M+Na)^+$ $C_{85}H_{139}NO_{63}Na$: 2204.8, found 2204.1.



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-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-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, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 4.78 – 4.76 (m, 12H), 3.89 – 3.49 (m, 74H), 2.12 – 2.07 (m, 2H), 1.56 – 1.37 (m, 6H); ^{13}C NMR (taken from HSQC spectrum): δ 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.



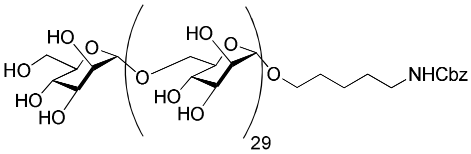
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (6-*O*-(6-aminohexanoyl)-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (30mer) (259)**: Functionalized resin **236** (50 mg, loading 0.47 mmol/g, 0.0235 mmol) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH_2Cl_2. To start the synthetic sequence, it was washed with DMF, THF, 0.2 M acetic acid in CH_2Cl_2 and CH_2Cl_2 (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 module 3 without prior capping.

glycosylation	building block and activator (all solutions in 1 mL CH_2Cl_2)	relative Fmoc value
1	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
2	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
3	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
4	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
5	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
6	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
7	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
8	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
9	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
10	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
11	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
12	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
13	**237** (3 × 64 mg; 0.0705 mmol)	100%
	TMSOTf (3 × 13 µL; 0.0705 mmol)	
14	**237** (3 × 64 mg; 0.0705 mmol)	100%

	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 $(M\text{-}Cbz)^+$ $C_{829}H_{690}N_2O_{244}$ 14440.1:, found 14400.2.



***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl α-D-mannopyranosyl-(1→6)-α-D-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 NMR (600 MHz, D_2O): 4.95 – 4.91 (m, 30H), 3.88 – 3.57 (m, 181H); ^{13}C NMR (taken from HSQC spectrum): δ 100.1, 73.5, 71.6, 70.8, 70.4, 67.4, 66.4, 61.8; MALDI-MS m/z calcd for $(M+Na)^+$ $C_{193}H_{319}NO_{153}Na$: 5121.7, found 5121.6.

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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

DMTST	dimethylsulfonium triflate
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
DMSO	dimethylsulfoxide
EDC	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide
equiv	equivalent
ESI	electrospray ionization
Et	ethyl
Fmoc	9-fluorenylmethyloxycarbonyl
FSPE	fluorous solid-phase extraction
GTs	glycosyltransferases
h	hour(s)
HPLC	high pressure liquid chromatography
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HRMS	high resolution mass spectroscopy
Hz	herz
IR	infrared spectroscopy
Im	imidazole
J	coupling constant
KDO	3-deoxy-D-manno-2-octulosonic acid
LCMS	liquid chromatography mass spectroscopy
LDA	lithium diisopropylamide
Lev	levulinoyl
LevOH	levulinic acid
LHMDS	lithium bis(trimethylsilyl) amide
L-Neu5Ac	L-*N*-acetylneuraminic acid
M	multiplet
M	molar
MALDI	matrix assisted laser desorption/ionization
Me	methyl
min	minute(s)
MS	molecular sieves
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide

NMR	nuclear magentic resonance
NP	normal phase
NPB	nitrophtalimidobutyric
PBB	*para*-bromobenzyl
PG	protecting group
Ph	phenyl
PMB	*para*-methoxybenzyl
ppm	parts per milion
Pr	propyl
PPTS	pyridinium *para*-toluenesulfonate
PS	polystyrene
quant.	quantitative
RT	room temperature
RRV	known relative reactivity value
s	singulet
SAE	*Sharpless* asymmetric epoxidation
*S*Taz	*S*-thiazolyl
t	triplet
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TBHP	butyl hydroperoxide
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetyl
TCDI	*N*,*N´*-thiocarbonyldiimidazole
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Tol	toluene
Ts	Tosyl
p-TsOH	*para*-toluenesulfonic acide
UV	ultraviolet

Curriculum vitae

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the curriculum vitae is not included in the online version