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Novel protecting group strategies in the synthesis of oligosaccharides

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*Novel Protecting Group Strategies in
the Synthesis of Oligosaccharides*

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Novel Protecting Group Strategies in the Synthesis of Oligosaccharides

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"Als ik zou willen dat je het begreep, dan had ik het wel beter uitgelegd."

Johan Cruijff

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Chapter 1

Protecting Group Strategies in Carbohydrate Chemistry

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Introduction

Carbohydrates are the most densely functionalized class of biopolymers in Nature. Every monosaccharide features multiple contiguous stereocenters and bears multiple hydroxyl functionalities. These can in turn be decorated with sulfate groups, acyl esters, lactic acid esters and ethers or phosphate moieties. Amine and carboxylate functions can also be present. Most often the amine groups are acetylated, but different amide functions are also found, as well as *N*-sulfates and alkylated amines. The discrimination of the functional groups on a carbohydrate ring has been and continues to be one of the great challenges in synthetic carbohydrate chemistry.¹⁻³

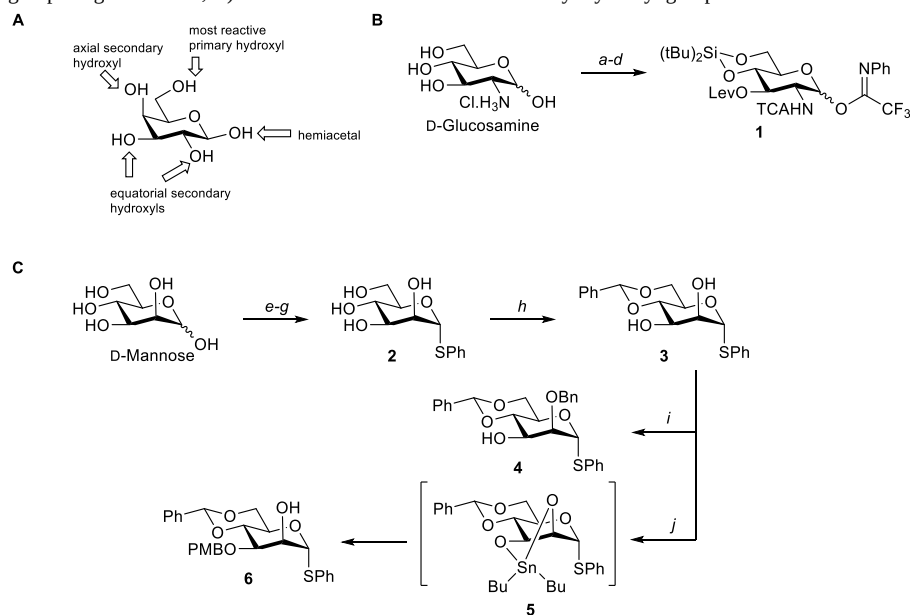
This thesis deals with the development of new protecting group strategies and manipulations in the solution and solid phase assembly of relevant oligosaccharides. This introductory Chapter provides an overview of the difference in reactivity of the various functional groups on a carbohydrate ring and shows how these can be exploited for effective protecting group strategies. The protecting groups on a carbohydrate dictate the reactivity of the (mono)saccharide and this Chapter will describe how protecting group effects can be used to control stereoselective transformations (most importantly glycosylation reactions) and reactivity controlled one-pot synthesis strategies. Applications and strategies in automated synthesis are also highlighted.

Discriminating different functionalities on a carbohydrate ring

The main challenge in the functionalization of a carbohydrate (mono)saccharide is the discrimination of the different hydroxyl functionalities. The - often subtle - differences in reactivity can be capitalized upon to formulate effective protecting group strategies (See Scheme 1A). The primary alcohol functionality is generally the most reactive of the hydroxyl groups because of steric reasons. It can be site selectively addressed using bulky protecting groups such as silyl or trityl ethers. The anomeric hydroxyl group discerns itself from the other secondary hydroxyl groups in that it is part of a hemiacetal functionality. It can therefore be selectively modified using acetal chemistry, and acid catalyzed acetal and mixed thioacetal formations are amongst the most used methods to start a protecting group manipulation sequence. Because it is part of a hemiacetal functionality the anomeric hydroxyl group is also the most acidic alcohol on a carbohydrate ring and it can be chemoselectively modified under basic conditions. Conversely, it is less reactive than the other secondary alcohol groups under acidic conditions. Axial secondary alcohols are generally somewhat less reactive than the equatorial ones on a carbohydrate ring and these reactivity differences can often be exploited in designing an efficient protecting group scheme. Finally, the position of a hydroxyl group on the carbohydrate ring and the nature of its neighboring substituents affect its reactivity. In this regard the use of cyclic protecting groups that engage two hydroxyl groups in a cyclic context has proven to be a very powerful tool.⁴ Benzyldiene acetals and silyldiene ketals can be used to mask C-4 C-6 diols, where isopropylidene groups and orthoesters are commonly employed to protect *cis*-hydroxyl groups in a five membered ring constellation. Butane 2,3-bisacetals and the recently introduced *o*-xylylene groups can be used to protect vicinal diequatorial diols.⁵ To illustrate how the reactivity of various

alcohol groups can be exploited two examples are given in Scheme 1B and 1C. The first example shows a four-step reaction sequence that has been used to site selectively mask all groups of a glucosamine synthon **1**. Thus, the nitrogen functionality in D-glucosamine can be chemoselectively protected with a trichloroacetyl group, by virtue of its higher nucleophilicity with respect to the alcohols present. Next the primary alcohol at C-6 and the hydroxyl group at C-4 can be masked with a di-*tert*-butyl silylidene ketal. The selectivity of this transformation originates from the bulky nature of the protecting group and the fact that a stable *trans*-decalin system can be formed. Next the anomeric hydroxyl group can be selectively addressed using basic conditions to install an imidate group. Finally the remaining alcohol can be masked with a levulinoyl ester.⁶ In the second example the different hydroxyls of D-mannose are discriminated using the following steps (Scheme 1C). First all hydroxyl groups are acetylated, concomitantly locking the mannose monosaccharide in a pyranoside ring. Next the anomeric thioacetal is installed under Lewis acidic conditions. After saponification of the four remaining acetyl groups (**2**), the alcohol groups are diversified through the installation of a benzylidene acetal^{7,8} (**3**) and

Scheme 1. A) Relative reactivity of carbohydrate alcohols; B) Four step reaction sequence to mask all functional groups in glucosamine; C) Site selective modification of mannosyl hydroxyl groups.

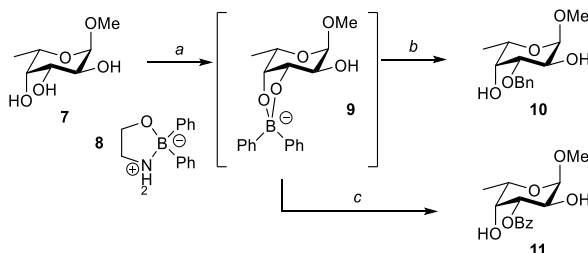


Reagents and conditions: a) Cl_3CCOCl , Et_3N , MeOH; b) $(\text{tBu})_2\text{Si}(\text{OTf})_2$, pyridine, DMF, -40°C (86% over two steps); c) $\text{CF}_3\text{C}(\text{=NPh})\text{Cl}$, Cs_2CO_3 , acetone (98%); d) LevOH, DIC, DMAP, DCM (82%). C) Site selective modification of mannosyl hydroxyl groups; e) Ac_2O , pyridine; f) PhSH , $\text{BF}_3\cdot\text{OEt}_2$, DCM (75% over two steps); g) NaOMe , MeOH (100%); h) $\text{HBF}_4\cdot\text{OEt}_2$, $\text{PhCH}(\text{OMe})_2$, DMF (60%); i) $\text{Bu}_4\text{NH}_2\text{SO}_4$, BnBr , NaOH, DCM (75%); j) i. Bu_2SnO , toluene, reflux; ii. CsF , Bu_4NBr , PMBCl, toluene, reflux (94%).

selective benzylation of the C2-OH using phase transfer conditions (**4**).⁹ The selectivity in the latter transformation can be explained by taking into account the relative mild basic

conditions (as opposed to the use of NaH in DMF) and the slightly higher acidity of the C2-OH because of its closer proximity to the anomeric center. Alternatively, the C3-OH can selectively be protected by exploiting the slightly higher nucleophilicity of this alcohol. Selective acylation is possible as well as regio selective alkylation. To further enhance the reactivity difference between neighboring axial and equatorial hydroxyl groups the use of stannylidene ketals presents a very effective approach.¹⁰ Thus, diol **3** can be transformed into a dibutylstannylidene ketal (**5**) using dibutyltin oxide, after which the tin ketal can react with an appropriate electrophile, such as *para*-methoxybenzyl chloride under the *aegis* of cesium fluoride and tetrabutyl ammonium bromide (**6**).

Scheme 2. Borinic acid catalysis to regioselectively protect alcohol functionalities.

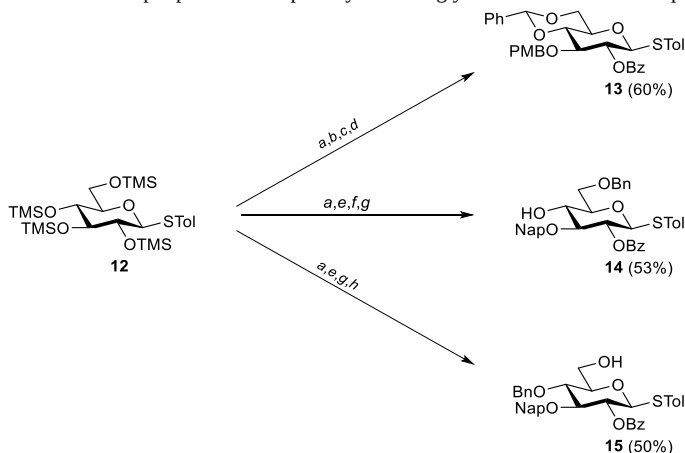


Reagents and conditions: a) **8**; b) BnBr, Ag₂O, MeCN, 40°C, 48h (94%); c) BzCl, iPr₂NEt, MeCN (92%).

Although the use of tin ketals, in stoichiometric and catalytic amounts, represents a very powerful means to discriminate alcohol functionalities, it requires the use of toxic tin species. To circumvent this drawback Taylor and co-workers have introduced borinic acid catalysis to regioselectively protect glycosyl polyols.^{11,12} α -O-methyl-fucopyranoside **7** can be regioselective alkylated or acylated using a catalytic amount of diphenylborinic ethylamine ester **8** and benzylbromide or benzoylchloride (Scheme 2). The reaction proceeds *via* borinate intermediate **9** that reacts in a highly regioselective manner to protect the equatorial alcohol at C3. (**10** and **11**, respectively)

To streamline the introduction of protecting groups, the groups Hung^{13–16} and Beau^{17–19} have devised a strategy to provide fully orthogonal protected building blocks in a one-pot fashion. Key to the strategy is the transformation of all hydroxyl groups into trimethylsilyl (TMS) ethers, which renders the carbohydrate **12** well soluble in an organic solvent, such as dichloromethane, even at low temperature. As shown in Scheme 3, the next steps in Hung's strategy involve the selective TMSOTf mediated formation of a C4-C6-acetal, ensuing installation of a C2-C3 acetal and regioselective opening of the most reactive acetal (which is the acetal at C2-C3). This liberates the C2-O-TMS, which can be benzoylated to provide glucoside **13**. Regioselective, reductive opening of the C4-C6-acetal can then give access to either the C4 (**14**) or C6-alcohol (**15**). Using this strategy the one-pot generation of a large variety of building blocks has been reported.^{13–16}

Scheme 3. One-pot protection of per-silylated thioglycoside to form different protected building blocks **13-15**.



Reagents and conditions: a) TMSOTf, PhCHO, DCM, -86°C; b) 4-MeOPhCHO, Et₃SiH, -86°C; c) TBAF; d) BzCl, Et₃N, 0°C to rt; e) 2-NapCHO, Et₃SiH, -86°C; f) Bz₂O, 0°C; g) 4M HCl/dioxane, NaCHBH₃, 0°C; h) BH₃/THF, 0°C.

Strategies for an (oligo)saccharide synthesis campaign

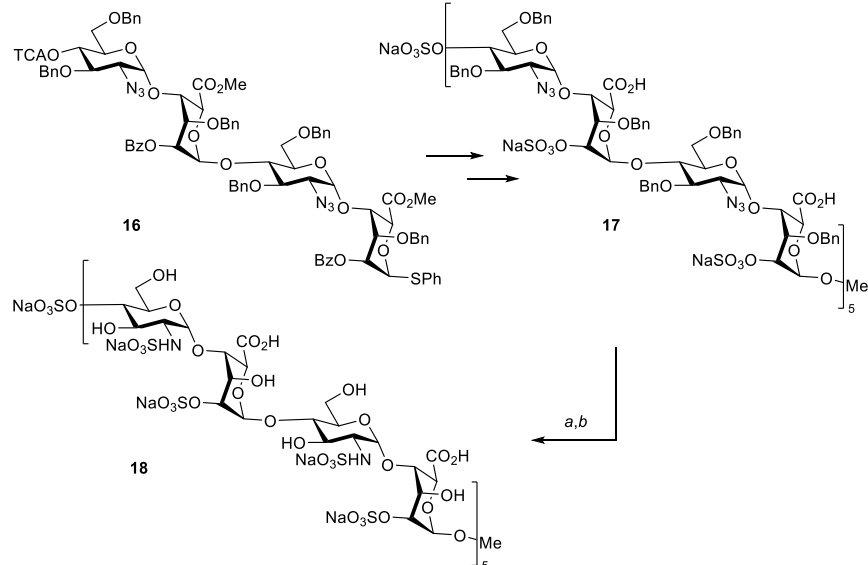
During an (oligo)saccharide synthesis campaign different types of protecting groups can be discerned: those that will be removed during the assembly to allow for the manipulation of the unmasked alcohol: the temporary protecting groups; and those that are only to be removed at the very end of the assembly line: the permanent protecting groups. The latter groups should be stable to all reaction conditions used and be cleavable under mild conditions that do not jeopardize the integrity of the (oligo)saccharide target with all its functionalities. Benzyl ethers are by far the most used permanent protecting used to date, because they are stable to both acidic and basic conditions and can be removed using mild catalytic hydrogenation conditions.

An impressive recent example of a synthesis, featuring benzyl groups for permanent protection, is presented in Scheme 4. Protected heparin eicosasaccharide **17** was built up from tetrasaccharide building block **16**. In the penultimate step 40 benzyl ethers and ten azides were removed simultaneously to give the fully deprotected 20-mer **18** in 89% yield. In the final step the ten liberated amino groups were chemoselectively sulfated.²⁰

It deserves mentioning that the last step(s) in the assembly of an oligosaccharide may be less trivial than they seem. Most oligosaccharide synthesis campaigns are based on a global deprotection event using a palladium catalyzed hydrogenation as the key step to simultaneously remove a multitude of functional groups (benzyl ethers, benzyloxycarbonyl groups, benzylidene acetals, azides).²¹ Because many lipophilic groups are removed from the target compound to expose hydrophilic alcohols or amines, the polarity of the substrates increases tremendously leading to poorly soluble semi-protected intermediates, complicating the full deprotection of the target compounds. The presence of functional

Chapter 1

Scheme 4. Block coupling to heparin-like 20-mers.

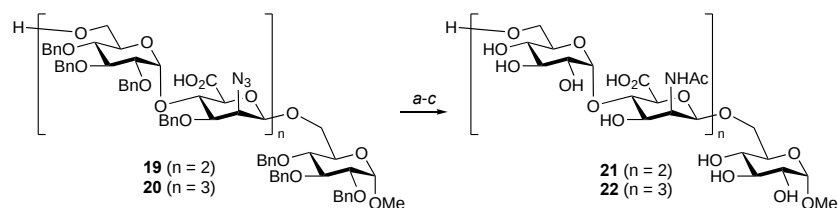


Reagents and conditions: a) $\text{Pd}(\text{OH})_2/\text{C}$, $\text{EtOH}/\text{H}_2\text{O}$ (89%); b) $\text{SO}_3 \cdot \text{Pyridine}$, H_2O .

groups such as amines and thiols that can deactivate the palladium catalyst, renders the final deprotection step(s) even more complicated.

As an alternative to a catalytic hydrogenation, a dissolving metal (Birch) reduction can be employed. For these reductions it also holds that the changing polarity of the substrate during the reaction can be a complication. Although impressive global deprotection events have been described using a Birch reduction, unexpected side reaction may occur. For example, in the final deprotection of *Micrococcus luteus* teicuronic acid stretches, composed of alternating *N*-acetyl mannosaminuronic acid and glucose residues, the unexpected cleavage of glycosidic linkages was encountered leading to fragmentation of the oligosaccharides (see Scheme 5).²² The cleavage occurred chemoselectively at the anomeric center of the mannosaminuronic acid residues, indicating that the cleavage was not the result of a β -elimination caused by the basic conditions of the Birch reduction.

Scheme 5. Birch reduction of teicuronic acid oligosaccharides in which cleavage of the mannosaminuronic acid linkages was encountered.

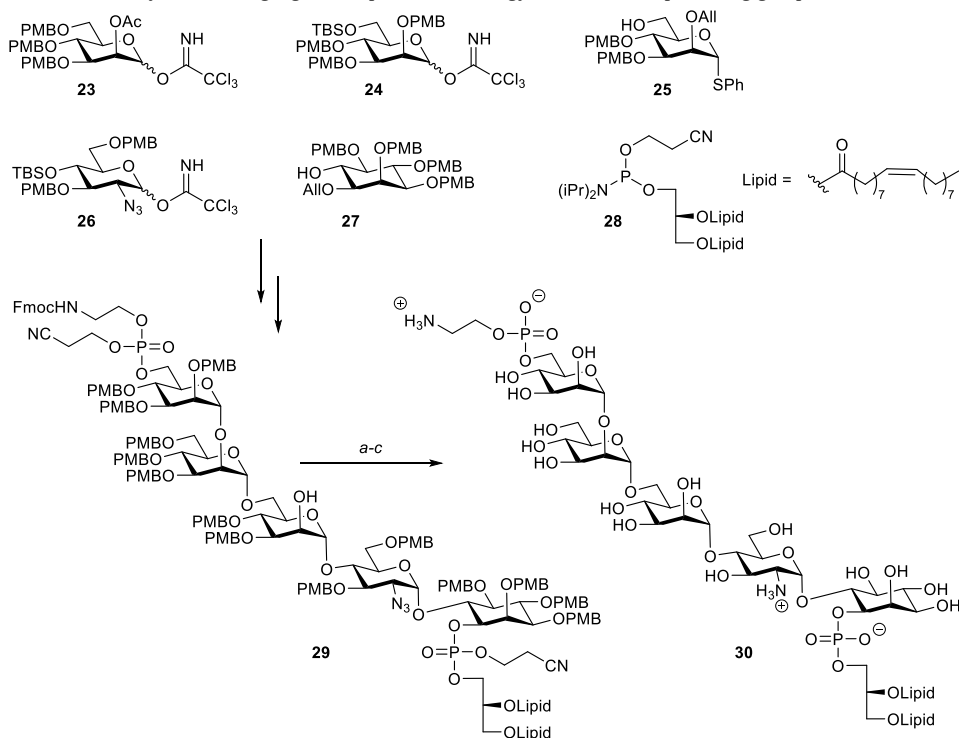


Reagents and conditions: a) Na (s), liquid NH_3 , THF , -60°C ; b) HPLC-purification; c) Ac_2O , NaHCO_3 , $\text{THF}/\text{H}_2\text{O}$ (21:35% over 2 steps; 22: 14% over 2 steps)

Many types of protecting groups have been employed as temporary groups, including silyl ethers, (substituted) acetyl esters, such as the levulinoyl, and chloroacetyl esters, carbamates, carbonates, allyl and substituted benzyl ethers.

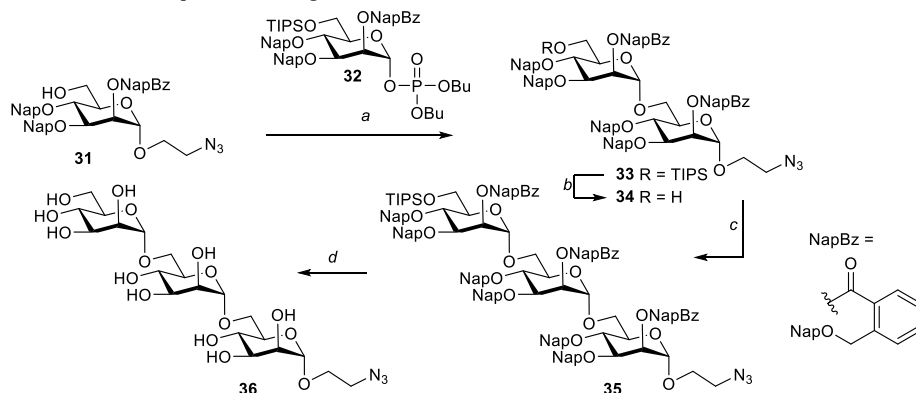
The presence of double bonds precludes the use of catalytic hydrogenation for global deprotection of a target compound and therefore represents a synthetic challenge. Guo and co-workers have reported on the synthesis of a complex glycosyl phosphatidyl inositol (GPI) anchor, bearing unsaturated lipids.²³ They selected PMB ethers to mask the hydroxyl functions throughout the synthesis (scheme 6). With monosaccharides **23**, **24** and **25**, a trisaccharide was assembled, which was coupled to a disaccharide (constructed from **26**, **27** and **28**), to form pentasaccharide **29**. Although PMB groups can be labile under Lewis acidic glycosylation conditions, no side reactions due to PMB cleavage occurred during the glycosylations. Deprotection commences with the reduction of the azide with zinc in acetic acid, followed by base catalyzed removal of the Fmoc and cyanoethyl groups. The last step is the removal of all PMB groups using trifluoroacetic acid. All PMB groups are removed without affecting the glycosidic linkages or the unsaturated lipid bearing phosphatidyl inositol.

Scheme 6. GPI synthesis using a global deprotection strategy based on PMB protecting groups.



Recently Liu and co-workers described the use of TFA in toluene to remove substituted benzyl ethers for the global deprotection of oligosaccharides. They introduced PMB and 2-naphthylmethyl (Nap)-protected hydroxymethylbenzoates as acid labile ester protecting groups for the same purpose.²⁴ Elongation of the reducing end terminus mannoside **31** with dibutylphosphate donor **32** using stoichiometric amounts of TMSOTf provided dimer **33** (Scheme 7). Of note, under these Lewis acidic conditions all protecting groups remained unaffected. Removal of the temporary tri-*iso*-propyl silyl ether (**34**) and ensuing coupling with another copy of **32** provided the target trisaccharide **35**. Global deprotection of this molecule by treatment with TFA in toluene gave the deprotected trisaccharide **36** in quantitative yield. Although it remains to be seen how general this methodology is, it can present a powerful alternative to the use of heterogeneous metal catalyzed hydrogenolysis commonly used.

Scheme7. Global deprotection using TFA in toluene.



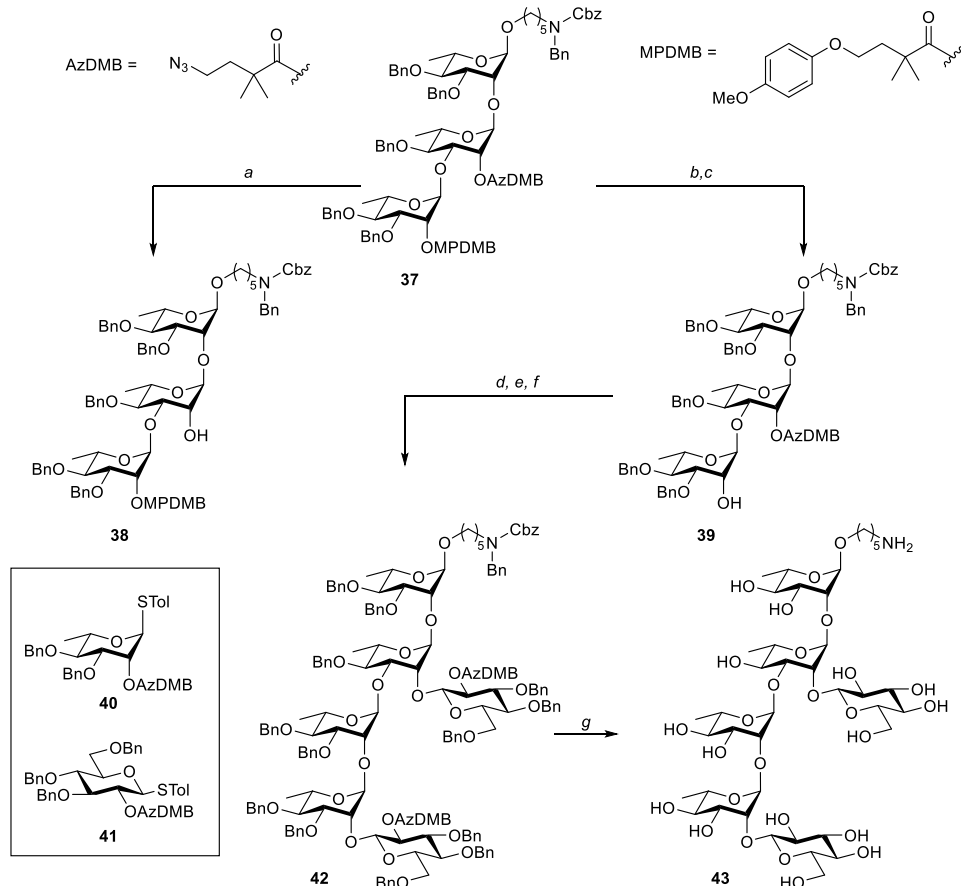
Reagents and conditions: a) TMSOTf, DCM, -20°C (97%); b) HF/Pyridine, pyridine (91%); c) 28, TMSOTf, DCM, -20°C (94%); d) TFA/toluene (10:1, v/v), 0°C to rt (100%).

Permanent acyl protecting groups that are often employed (for example to stereoselectively introduce glycosidic linkages, *vide infra*) are the pivaloyl and benzoyl esters. The former is more stable than the latter, representing an advantage during synthetic manipulations required during the assembly of the target compound. On the other hand its stability necessitates harsh deprotection conditions that may affect other functionalities and linkages in the final product.

In this context, two new pivaloyl-type groups have been introduced, that combine the advantages of the parent pivaloyl ester, *i.e.* stability and suppression of orthoester formation during glycosylation reactions, with ease of cleavage (see Scheme 8).²⁵ These two pivaloyl-based groups bear a reactive functionality appended to the pivaloyl core. The 2,2-dimethyl-4-(4-methoxy-phenoxy)-butanoate ester (MPDMB) and the 2,2-dimethyl-4-azido butanoate (AzDMB) are pivaloyl analogues that can be removed under either mild oxidative (**39**) or reductive (**38**) conditions, respectively. Glycosylation of AzDMB bearing donor **40** with **39** results in a tetrasaccharide, which, after removal of the two AzDMB groups, can be decorated with glucose moieties. An added advantage of the latter protecting group is found

in the fact that it can be removed simultaneously with the commonly used permanent benzyl protecting groups using catalytic hydrogenation conditions. Applying hydrogenation conditions on hexasaccharide **42** results in fully deprotected compound **43**.

Scheme 8. Selective deprotection of AzDMB and MPDMB pivaloyl analogues



Reagents and conditions: a) PMe_3 , THF/ H_2O , then KOH (aq), rt, 24h (78%); b) CAN, 0°C , acetone/ H_2O , 40 min; c) DBU, rt, MeOH, 1h (80% over 2 steps); d) **40**, NIS, TMSOTf, -40°C , DCM (88%); e) PMe_3 , THF/ H_2O , then KOH (aq), rt, 24h (77%); f) **41**, NIS, TMSOTf, 0°C , DCM (69%); g) i. H_2 , $\text{Pd}(\text{OH})_2/\text{C}$, THF/ H_2O /tBuOH; ii. H_2 , $\text{Pd}(\text{OH})_2/\text{C}$, HCl, H_2O , then Et_3N (excess) (75% 2 steps).

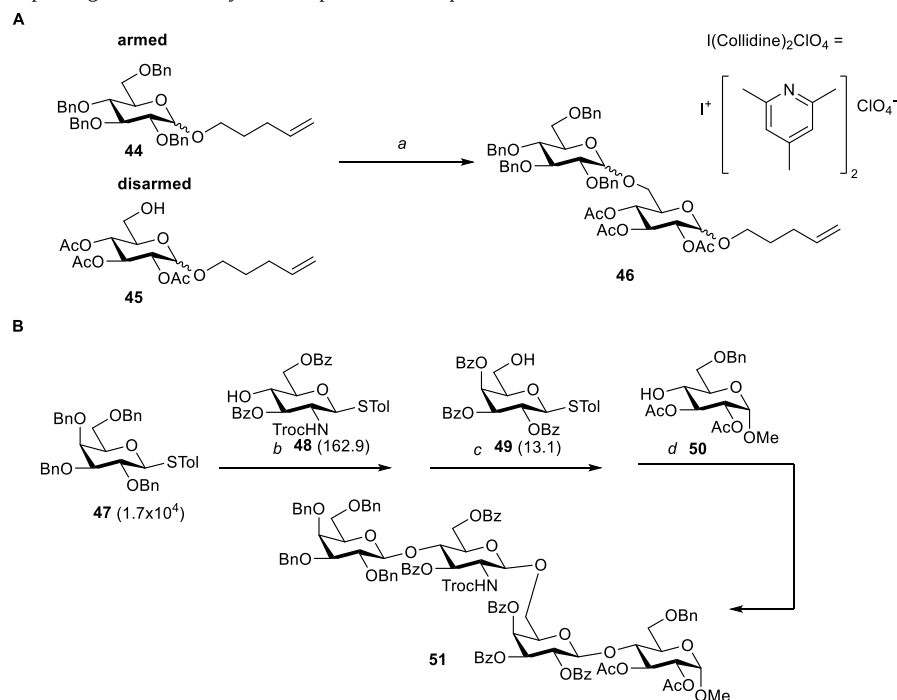
Reactivity and stereochemistry

Protecting groups have a major impact on the reactivity of a carbohydrate synthon. Electron-withdrawing protecting groups, such as acyl groups, deactivate a glycosyl donor, because the electron-withdrawing effect of these groups destabilizes the build up of (partial) positive charge at the anomeric center of the donor upon activation. This effect has been elegantly exploited and conceptualized by Fraser-Reid who introduced the armed-disarmed concept: benzyl ether carrying donors (so-called “armed” donors **44**) can be activated in the presence of acylated ones (termed “disarmed” donors **45**) allowing for the selective condensation of

the armed donor with the disarmed building block (See Scheme 9A).²⁶ Since the introduction of this seminal concept, insight into glycosyl donor reactivity has tremendously increased and it is now clear that, besides the nature of the protecting groups, the configuration and conformation of the donor glycoside, the orientation of the leaving group and the exact position of the protecting groups, all influence the reactivity of a donor building block.²⁷ The groups of Ley and Wong have developed reactivity scales, quantifying the relative reactivity of thioglycosides, setting the stage for effective one-pot assembly procedures involving multiple sequential glycosylation steps.^{28,29}

The one-pot synthesis of tetrasaccharide **51** illustrates the use of relative reactivity values (RRVs) in oligosaccharide synthesis (Scheme 9B). The RRV values as determined by Wong and co-workers have been established with respect to the reactivity of tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio- α -mannopyranoside (RRV = 1). The high RRV of thioglycoside **47** compared to thioglycoside **48** allows for the selective coupling of **47** to acceptor **48** in an NIS/TfOH mediated glycosylation reaction. The obtained disaccharide donor is then treated with thioglycoside **49** and an additional amount of NIS to form a trisaccharide. Tetrasaccharide **51** is obtained after addition of acceptor **50** and a third batch of NIS to the reaction mixture. The synthesis of this tetrasaccharide demonstrates the sophistication of the reactivity scales and their usefulness in the one-pot synthesis of oligosaccharides.

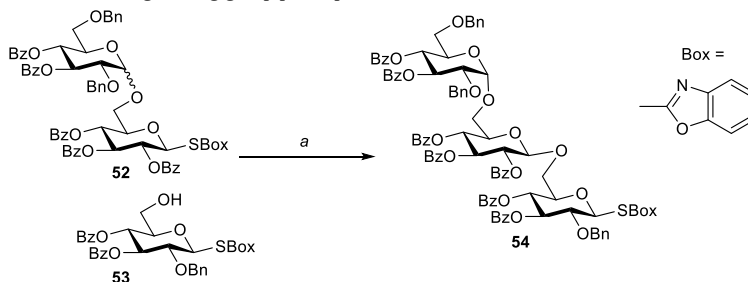
Scheme 9. A) The armed-disarmed concept using *n*-pentenyl glycosides as conceptualized by Fraser-Reid. B) Exploiting donor reactivity in a one-pot reaction sequence.



Reagents and conditions: a) $I(collidine)_2ClO_4$, DCM (62%); a) TfOH, NIS, $-25^\circ C$, DCM; b) NIS, $0^\circ C$, DCM; c) NIS, DCM (40%).

The impact of protecting groups on the stereochemical outcome of a glycosylation reaction is best illustrated by the anchimeric assistance that neighboring groups can provide during a glycosylation reaction. Glycosyl donors equipped with a C2-O or N-acyl group in general provide 1,2-*trans* products with great fidelity (exceptions occur due to stereochemical mismatch situations, or overruling steric requirements).³⁰ This can be explained by the formation of an intermediate dioxolenium ion that is formed by attack of the C2-acyl group on the (developing) oxocarbenium ion. The dioxolenium ion bridge effectively shields one side of the carbohydrate ring, allowing the nucleophile only to approach from the opposite direction. Even though acyl groups are inherently more electronwithdrawing than for example benzyl ethers, their presence can make a glycosyl donor more reactive because it can provide ‘active’ anchimeric assistance (scheme 10). For example, disaccharide **52**, bearing three ‘disarming’ benzoyl groups at C2, C3 and C4 could be selectively activated over building block **53**, carrying an arming benzyl group at C2, next to two disarming benzoates at C3 and C4, with the mild activator Cu(OTf)₂.³¹ Because of the limited reactivity of the activator, expulsion of the S-box aglycons only occurred when anchimeric assistance was provided by the neighboring C2-benzoate.³²

Scheme 10. Neighboring group participation assisted selective activation.



Reagents and conditions: a) Cu(OTf)₂, TfOH, DCM (70%).

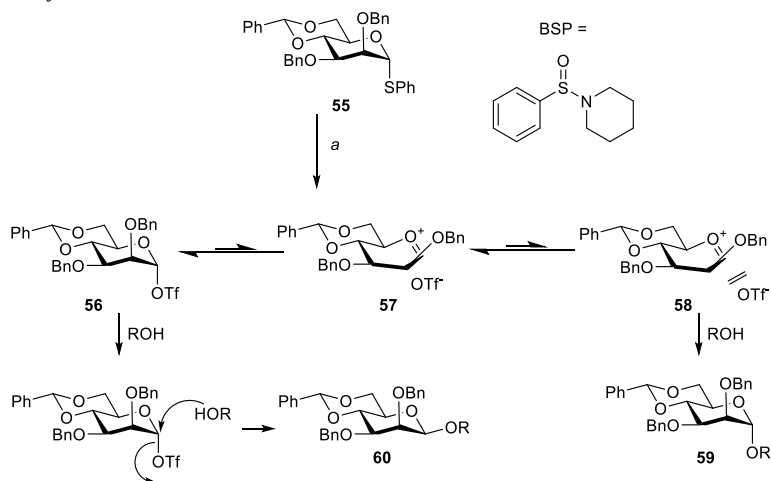
It has been proposed that acyl groups at positions other than C2 can also provide neighboring group participation thereby influencing the stereochemical outcome of a glycosylation reaction.^{33,34} There are various examples describing the beneficial effect of C-6-acyl groups for the stereoselective synthesis of glucosyl, galactosyl and mannosyl donors. Similarly, empirical evidence points to possible participation of ester groups at C4 of galactosyl and fucosyl donors. At the same time, studies with model compounds failed to convincingly demonstrate long-range participation leaving the subject open to further debate and showing that more sophisticated models and deeper insights into the effect of functional groups in glycosylation reactions are needed.

The stereoselective synthesis of 1,2-*cis*- and 2-deoxy glycosidic linkages is considerably more challenging than the construction of 1,2-*trans* bonds, but much progress has been made over the years in the stereoselective syntheses of these difficult linkages.^{35–38} In all these syntheses protecting groups play a key role in determining the overall shape and reactivity of the coupling partners. The overall reactivity of a glycosyl donor is decisive for the

stereochemical outcome of a glycosylation reaction as it determines the stability of reactive intermediates that are formed upon activation. These include both covalent species^{39,40}, such as anomeric triflates, as well as oxocarbenium ion intermediates, be it solvent separated or as part of a contact (or close) ion pair.^{41–43} The equilibrium between these species, their stability and the ease with which these are attacked by an incoming nucleophile determine the overall stereochemical outcome of a glycosylation reaction. Because it is beyond the scope of this introductory Chapter to provide an all-encompassing overview of these stereodirecting protecting group effects, only one –possibly the most prominent, but for sure the best studied one– example will be described here. Mannosyl donors, equipped with a benzylidene acetal spanning C4 and C6 can be used to effectively provide 1,2-*cis* mannosides. Crich and co-workers, who pioneered the method⁴⁴, have rationalized this stereochemical outcome through the intermediacy of the covalent α -triflate as main product forming intermediate (Scheme 11).⁴⁵ The benzylidene acetal serves to limit the conformational freedom of the mannosyl ring, making it more difficult to adopt a flattened structure, which is required to accommodate the positive charge in an oxocarbenium ion intermediate. S_N2 -type substitution on the anomeric triflate leads to the observed β -selectivity. This methodology has been applied in many different syntheses of complex (bacterial) oligosaccharides and glycoconjugates, including the assembly of β -rhamnoside³⁶ and *cis*-linked heptose containing oligomers³⁵. To further investigate the origin of the striking selectivity, Crich and co-workers have conducted a number of seminal studies, including the determination of primary⁴⁶ and secondary⁴⁵ kinetic isotope effects and the development of “cation clock” methodology^{47,48} to discriminate between associative and dissociative product forming pathways. Primary kinetic isotope effects indicated that the β -linked products are formed through an associative pathway, where the α -products in these reactions resulted from an attack of an oxocarbenium ion intermediate.⁴⁹ Secondary isotope effects measured in the glycosylation of between a benzylidene mannose donor and a methyl 2,3,6-tri-*O*-benzyl- α -D-glucopyranoside acceptor revealed that substantial oxocarbenium ion character developed in the transition state leading to the β -linked disaccharide, indicative of an S_N2 -reaction with an exploded transition state. In contrast, C-glycosylation reactions of benzylidene mannose donors proceed through a dissociative pathway presumably via a $B_{2,5}$ -oxocarbenium ion like intermediate.⁵⁰ Overall the benzylidene mannose system has not only developed to become the most direct and effective way to construct 1,2-*cis*-mannosidic linkages, it has also proven to be a rich breeding ground for the development of physical organic chemistry methods to investigate the principles underlying glycosylation stereochemistry.

Many different covalent reactive species have been reported and characterized by spectroscopic techniques such as NMR.⁴⁰ However, in the majority of cases, the stereochemical outcome of glycosylation reactions involving these species, can not be simply traced back to the covalent reactive intermediates. Clearly other reactive intermediates have to be taken into account and more insight is needed how protecting and functional groups control the stability and reactivity of the different reactive intermediates.

Scheme 11. Reaction mechanism manifold to account for the stereoselectivity in glycosylation reactions of benzylidene mannose donors.



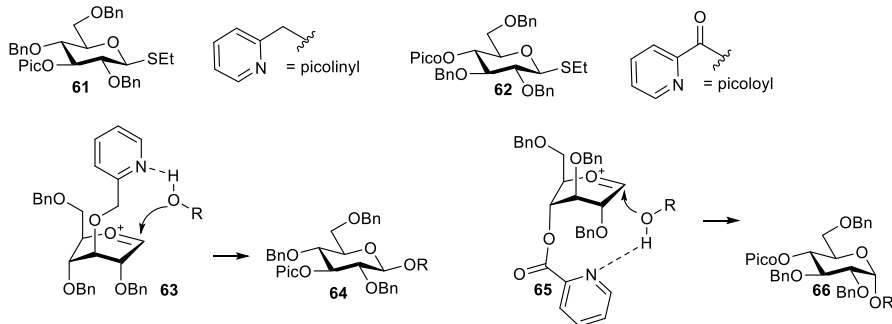
Reagents and conditions: a) BSP, Ti_2O , -80°C , DCM.

Recently several reports have appeared that make use of hydrogen bonding between donor and acceptor to direct glycosylation stereochemistry (Scheme 12). Demchenko and co-workers have used picolinyl ethers (**61**) and picolinoyl esters (**62**) to direct the incoming nucleophile to the activated donor species with excellent facial selectivity.^{51,52} Hoang and Liu have described that glucosyl (**67**) and galactosyl donors bearing an *O*-cyanobenzyl ether at C2 can provide either α - or β -linked products, depending on the reactivity of the acceptor and the solvent system used.⁵³ Reactive acceptors and the use of toluene lead to β -products, where unreactive alcohols and diethyl ether provide the opposite anomers. To account for the latter stereochemistry the authors speculated that a hydrogen bond between the cyano group and the incoming acceptor could guide the nucleophile to the α -face of the donor molecule. How these new hydrogen bonding protecting groups behave in the context of complex oligosaccharide synthesis will have to be shown in the near future.

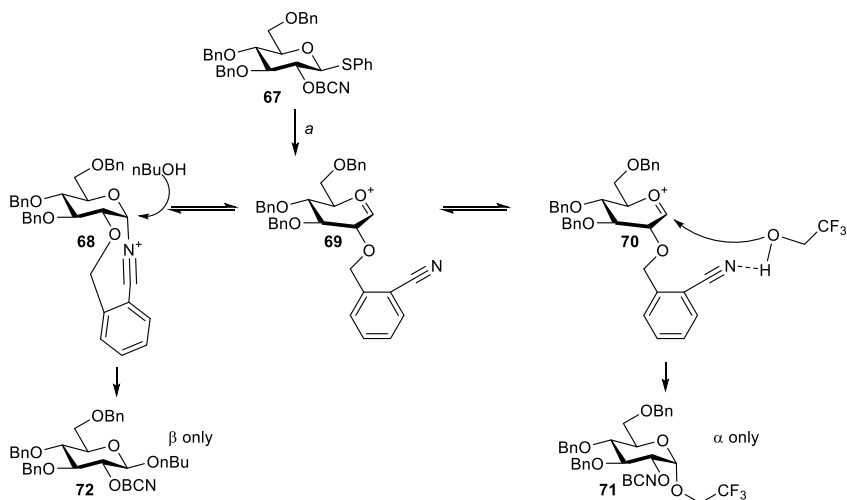
Chapter 1

Scheme 12. A) Hydrogen bonding acceptor delivery by picolinyl ether and picoloyl ester; B) Hydrogen bonding acceptor delivery by cyanobenzyl ethers.

A



B



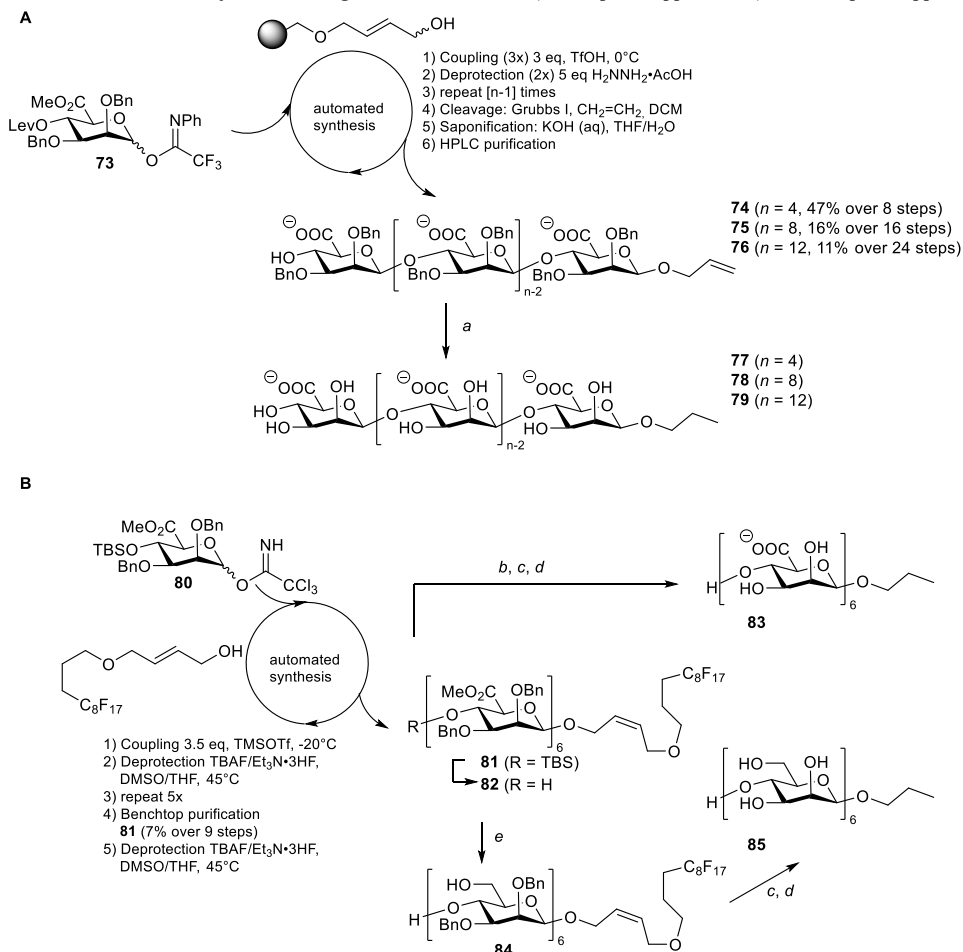
Reagents and conditions: a) Ph_2SO , Tf_2O , -80°C , DCM.

Protecting groups in automated synthesis

To streamline oligosaccharide assembly much effort has been devoted to the development of automated synthesis techniques.^{54–56} The automated solid phase synthesis of peptides and nucleic acids is one of the major contributions of synthetic organic chemistry to the life sciences. However, solid phase automated carbohydrate chemistry is significantly more challenging than the assembly of the other two biopolymers, because one has to deal with all the different functionalities present on the carbohydrate ring and the union of two carbohydrate building blocks involves the creation of a new stereocenter. Different strategies have been developed to automate oligosaccharide assembly based on either solution phase synthesis or solid phase techniques and automated solid phase synthesizers are now commercially available. Both techniques are based on the attachment of the growing oligosaccharide to a support. For the solution phase approach a light fluororous tag is used⁵⁷, where the solid phase methodology commonly employs a polystyrene type resin.⁵⁸ The support makes it possible to separate the target compound from the reagents used by filtration

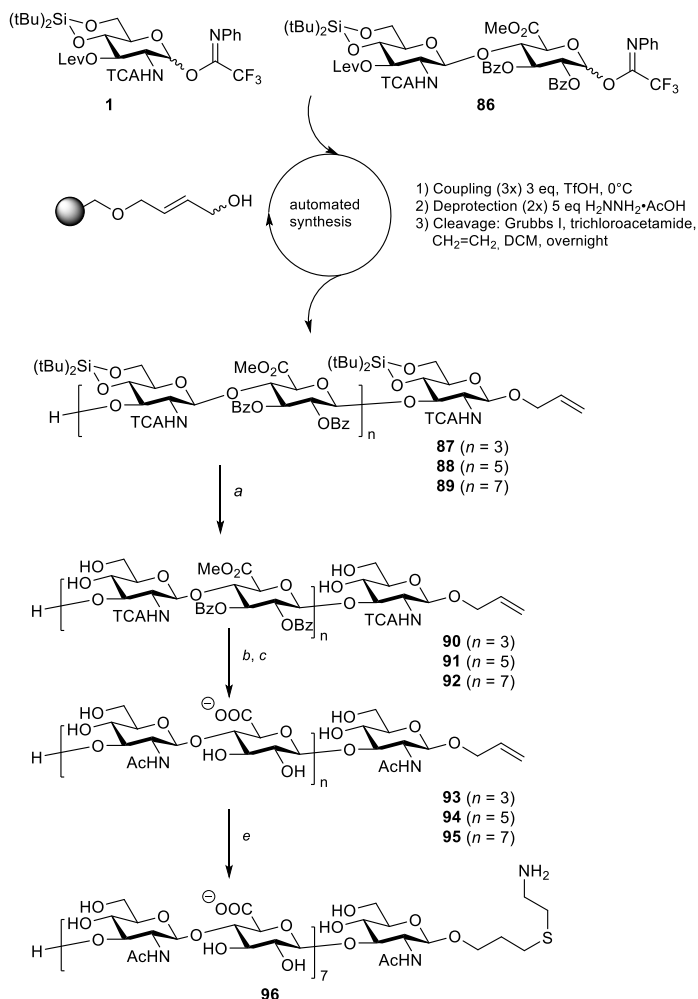
or a relatively simple fluorous solid phase extraction step, thus allowing the use of excess reagents to drive reactions to completion. Other intermediate purification steps are not performed. Overall this makes the process very efficient, but it also puts stringent constraints on the protecting groups used in the assembly. The use of excess reagent makes the reaction conditions employed harsher than the conditions that would be used in an equivalent solution phase step. At the same time cleavage of the temporary protecting groups has to proceed effectively because the build up of deletion sequences leads to complex product mixtures necessitating a difficult, if not impossible, purification at the end of the assembly. Scheme 13 depicts the assembly of two oligomannuronic acid sequences through automated solid phase⁵⁹ and automated fluorous phase synthesis⁶⁰. Both approaches rely on the use of mannuronic acid donor synthons, because these enable the stereoselective formation of the 1,2-*cis* mannosidic linkage with great fidelity.^{61–63} Obviously the generation of epimeric mixtures is highly undesirable because it will generate very complex mixtures at the end of the assembly. Parallels between both approaches are the use of a double bond based linker system (cleavable by cross metathesis) and the use of imidate donors. Using the solid phase approach, mannuronic acid tetramer **74**, octamer **75** and dodecamer **76** were assembled (in 47%, 16% and 11% over 8, 16 and 24 steps, respectively), where the latter approach was used to create hexasaccharide **81** (7% over 9 steps).

Two relevant protecting group related issues deserve mentioning here. Firstly, the methyl ester moieties can be used as precursors for the corresponding alcohol functionalities. It was shown that hexamannuronate **82** could be transformed into protected hexamannoside **84** through DIBAL reduction of the methyl esters in 82% yield. The second issue to note is that during the solid phase assembly of the oligomers deletion sequences were generated because of incomplete glycosylation steps (efficiency ~92% per step, no capping step was included). Saponification of the methyl esters allowed for the easy HPLC separation of the target stretches from their shorter counterparts. In designing automated oligosaccharide

Scheme 13. Automated synthesis of oligomannuronic acids. A) Solid phase approach. B) Fluorous phase approach.

Reagents and conditions: a) Pd/C, Pd black, H₂, THF/H₂O/tBuOH (**77**: 99%, **78**: 99%, **79**: 95%); b) Grubbs II, CH₂=CH₂, DCM; c) KOH (aq), THF/H₂O; d) Pd/C, Pd black, H₂, MeOH/AcOH (**83**: 61% over 3 steps, **85**: 73% over 2 steps); e) DIBAL-H, 0°C, DCM/toluene (83%).

assemblies it can be worthwhile to implement the possibility to purify semi-protected intermediates before the ultimate deprotection event, because compounds featuring both hydrophilic and lipophilic groups allow for effective HPLC procedures, where fully protected compound can be too lipophilic and fully deprotected compound too hydrophilic to efficiently purify. The latter strategy has also been applied in the automated solid phase assembly of a set of hyaluronic acid (HA) oligomers (Scheme 14).⁶⁴

Scheme 14. Automated solid phase assembly of hyaluronic acid oligosaccharides.


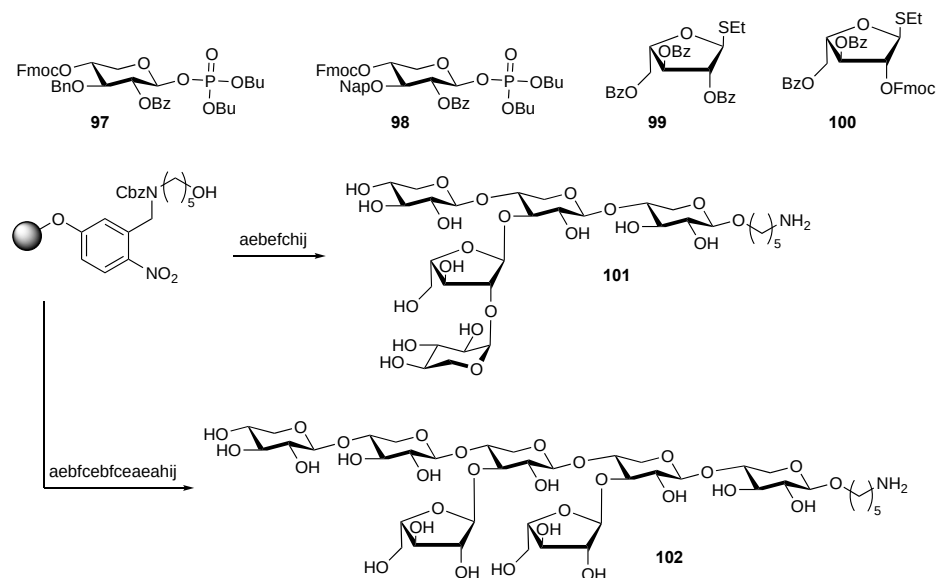
Reagents and conditions: a) Et₃N•3HF, THF (**90** 26% over 10 steps, **91**: 32% over 14 steps, **92**: 18% over 18 steps); b) KOH (aq), THF/H₂O; c) Ac₂O, NaHCO₃, H₂O (**93**: 90% over 2 steps, **94**: 70% over 2 steps, **95**: 69% over 2 steps); e) Cysteamine•HCl, hv, H₂O, (92%).

HA-7-mer, 11-mer and 15-mer were generated on a butanediol functionalized polystyrene resin using monomeric building block **1** (Scheme 1) and disaccharide **86** (Scheme 14). After cleavage of the resin by cross metathesis the fully protected oligomers **87-89** proved to be too lipophilic for purification, but removal of the silylidene ketals liberated two free alcohol groups per dimer repeat providing compounds **90-92** that were readily purified by HPLC. Of note, the silylidene group was employed in these syntheses, because the corresponding benzylidene acetal proved to be too labile to withstand the acidic glycosylation conditions.⁶ Global deprotection of the HA fragments was achieved by the saponification of all methyl and benzoyl esters and the trichloroacetyl amides. Selective *N*-acetylation gave the final

compounds **93-95**. Because the protecting group strategy did not require the use of hydrogenation conditions, the reducing end anomeric allyl functionality could be retained. This in turn allowed the installment of a ligation handle through thiol-ene chemistry to give compound **96**.⁶⁵

Scheme 15 depicts the assembly of two plant arabinoxylans.⁶⁶ These syntheses nicely illustrate the use of the 9-fluorenylmethoxycarbonyl (Fmoc)-Nap couple as a set of orthogonal temporary protecting groups and the use of an UV-cleavable linker system. The former protecting group was used as a base labile protecting group to mask the hydroxyl groups used for the elongation of the xylose backbone. Of note, cleavage of the Fmoc group generates a fulvene, the concentration of which can be measured spectroscopically providing an effective method to monitor the efficiency of the coupling events on-line.

Scheme 15. Automated solid phase assembly of plant cell wall arabinoxylan fragments



Reagents and conditions: a) donor **97**, TMSOTf, DCM, -35°C to -15°C; b) donor **98**, TMSOTf, DCM, -35°C to -15°C; c) donor **99**, NIS/TfOH, DCM/dioxane, -40°C to -20°C; d) donor **100**, NIS/TfOH, DCM/dioxane, -40°C to -20°C; e) 20% Et₃N in DMF, 25°C; f) 0.1M DDQ in DCE/MeOH/H₂O (64:16:1); g) Ac₂O, pyridine, 25°C; h) *hν* (305 nm); i) NaOMe, THF/MeOH; j) H₂, Pd/C, EtOAc/MeOH/H₂O/AcOH (**101**: 42%, **102**: 21%).

The Nap-ether was used at positions on the xylose building blocks where arabinofuranosyl branches were to be introduced. Cleavage of the Nap ethers was affected under oxidative conditions (DDQ) using a DCE/MeOH/H₂O solvent system. Although it is notable that aqueous solvent systems can be employed in combination with the polystyrene resin, the fact that the cleavage of the Nap ethers required seven repetitive reaction cycles illustrates the room for possible improvement. Cleavage of the arabinoxylan fragments from the solid support was affected by exposing the oligosaccharide-bearing resin to 305 nm UV light in a tailor made continuous flow reactor.⁶⁷

Summary and Outlook

Protecting group chemistry can make or break any (oligo)saccharide synthesis effort. Much progress has been made over the years to understand and exploit reactivity differences between the functional groups on a carbohydrate and many efficient protecting group strategies and schemes are now available. Even though these schemes may present multistep synthesis routes, they often involve optimized chemistry, assuring reliable synthetic outcomes. Nonetheless there is a demand for ever-shorter synthetic routes and the development of one-pot operations to introduce multiple protecting groups is therefore of high importance.

The demand for more efficiency can also be met by the development of better and more effective protecting groups. For instance, novel protecting groups and/or cleavage conditions are required to mask amines on carbohydrate rings, especially functionalities that do not provide anchimeric assistance in glycosylation reactions. The only group that is now available for this purpose is the azide and in cases where different orthogonally functionalized amine groups are required the availability of more non-participating amine functionalities would be a valuable asset.⁶⁸⁻⁷¹ In general, protecting groups that are more robust during a solution and/or solid phase synthesis campaign and/or can be removed more easily at the end of these syntheses are needed.

Another important issue is represented by the limited number of possible optimizations for deprotection procedures by the minimal amount of the fully protected target oligosaccharide that is usually available. Insight into why some global deprotection events proceed uneventfully, where others are accompanied by side reactions leading to complex reaction mixtures and difficult purifications would be very valuable indeed. Innovative chromatography procedures to purify the highly polar target compounds, often lacking (UV)-chromophores for detection, would also represent a great addition to the oligosaccharide synthesis toolbox.

Outline of the thesis

This **Chapter** has provided a brief overview of recent developments in the area of protecting group manipulations in carbohydrate chemistry. **Chapter 2** describes a new method to cleave substituted benzyl ethers (PMB and Nap) using catalytic amounts of HCl in DCM/hexafluoro-*iso*-propanol (HFIP).⁷² These conditions were found to effectively cleave both PMB and Nap ethers while leaving other acid labile functionalities (primary TBDPS ethers, glycosidic linkages) intact. In addition, the homogeneous conditions are amendable to a solid phase setting^{73,74} and can therefore provide a more effective use of Nap ethers in solid phase oligosaccharide synthesis. The method described in Chapter 2 was successfully applied in the building block synthesis in Chapter 3. In **Chapter 3**, the new cyanopivaloyl (PivCN) group is introduced, which, in combination with other Piv-type groups should open possibilities in the synthesis of carbohydrate fragments. The PivCN group, bearing a cyano group on one of the methyl groups, possesses all the characteristics of the conventional Piv group, with the additional advantage that it can be removed by hydrogenation. The newly

developed group was used in the synthesis of two bacterial rhamnan structures. The usefulness of the PivCN group was demonstrated by the one step deprotecting leading to the target molecules. The value of the PivCN group is shown in **Chapter 4**, where it is incorporated in a disaccharide donor for automated carbohydrate synthesis. Two donors are synthesized and used on a new automated system. The donor with the PivCN group is applied in the synthesis of a library of rhamnose fragments, corresponding to the Group A *Streptococcus* bacterial backbone. An additional advantage was found, as a catalytic amount of base was sufficient to deprotect all PivCN groups. The automated synthesis method resulted in multimilligram quantities of biologically relevant rhamnose fragments, up to 16 monosaccharides. The first part of **Chapter 5** describes the building block synthesis towards a set of disaccharides leading to a well-defined sulfated mannuronic acid molecule. The deprotection of the fragments was optimized, applying the method developed in Chapter 2, resulting in a selectively sulfated, deprotected mannuronic acid disaccharide. In the second part of Chapter 5, modified donors were synthesized and used in the construction of the target fragments. It was found that the designed molecules show great difference in reactivity depending on their conformation. This leads towards the synthesis of a tetrasaccharide donor to construct a sulfated mannuronic acid tetrasaccharide. **Chapter 6** summarizes the findings of this Thesis and future plans and outlines strategies.

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Chapter 2

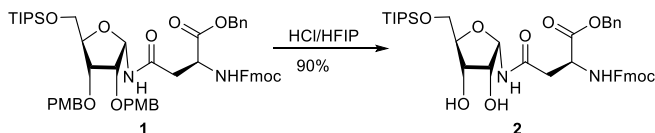
Chemoselective Cleavage of para-Methoxybenzyl and Naphthyl Ethers using a Catalytic Amount of HCl in Hexafluoroisopropanol

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Introduction

Protecting groups play a pivotal role in synthetic organic chemistry.^{1–4} In oligosaccharide synthesis protecting groups are used to (temporarily) mask hydroxyl and amino groups to allow for selective modification of other functionalities on the carbohydrate ring. Besides blocking specific functionalities that otherwise would partake in a glycosylation event, the protective group pattern of carbohydrate building blocks also has a profound effect on the outcome of a glycosylation reaction in terms of yield and stereoselectivity. Various types of protecting groups are available to mask carbohydrate hydroxyls, and amongst the most commonly used groups are the benzyl-type ethers. Besides being robust to a wide variety of reaction conditions, the sterically minimally intrusive benzyl-type ethers stand out because of their non-participating nature. Therefore benzyl-type ethers are often the group of choice to protect the C-2-OH when 1,2-*cis* linkages are to be installed. Substituted benzyl ethers, such as the *p*-methoxybenzyl (PMB) and 2-naphthylmethyl (Nap) ether are attractive, electron rich benzyl ethers, as they can be removed en route to the oligosaccharide using oxidative or acidic cleavage conditions.² For their removal, generally strong oxidizing agents, such as ceric ammonium nitrate or 1,2-dichloro-3,4-dicyano-quinone (DDQ), in combination with biphasic reaction media, are used. These conditions can be disadvantageous when dealing with sensitive compounds or solid phase reactions.^{5–10} Alternatively, the PMB and Nap groups can be split off under acidic conditions, using a large molar excess of rather strong Brønsted or Lewis acids such as TFA^{11,12} or HF.pyridine¹³, the use of which can jeopardize the integrity of acid labile functionalities in the molecule (acetals, silyl ethers etc.). Recently introduced methods to cleave PMB ethers include the use of FeCl₃¹⁴ and AgSbF₆/trimethoxybenzene.¹⁵ Exotic conditions to remove PMB ethers are described,¹⁶ however the applicability remains questionable, since the reagents and conditions require thorough chemical experience. These methods^{14–16} require relatively long reaction times and have not been employed to remove the more stable Nap ethers. The invention of mild, homogeneous and fast reaction conditions to selectively remove PMB or Nap ethers will make these groups even more useful in (carbohydrate) synthesis and open up routine application in both solution and solid phase settings.

Such a reagent can be found in the work of Palladino and Stetsenko, who recently described the use of hydrochloric acid in a fluorinated alcohol, such as hexafluoro-*iso*-propanol (HFIP),¹⁷ to unmask *tert*-butyl protected hydroxyl and carboxylic acid functions in solid phase peptide synthesis.^{18–20} The reactivity of this deprotection system arises from the effective hydrogen bonding of the fluorinated alcohol to the chloride leading to the generation of “naked” protons. In the synthesis of poly adenoside diphosphate ribosylated (poly-ADPR) peptides mild conditions to transform ribosyl glutamine **1** into building block **2** were required, suitable for solid phase synthesis (Scheme 1).

Scheme 1. Deprotection of PMB groups with catalytic HCl.

To this end both PMB ethers at the C2 and C3 positions, installed to allow for the stereoselective construction of the 1,2-*cis* ribosyl linkage, had to be removed. It was found that the use of TFA in DCM rapidly cleaved both ethers but also led to substantial epimerization at the anomeric center. The use of oxidative conditions (DDQ in DCM/H₂O) led to the formation of several side products. In contrast, the use of a catalytic amount of HCl in HFIP prevented these side reactions and resulted in the clean removal of the PMB ethers. Encouraged by this profitable outcome the scope and limitations of the latter cleavage method was explored, the result of which are presented in this chapter. It was found that a catalytic amount of HCl can be sufficient to cleave both PMB and Nap ethers, while chemoselectivity between these two ethers can also be attained. The applicability of the use of Nap-ethers and their HCl/HFIP mediated removal in the synthesis of a sulfated mannuronic acid di- and tetrasaccharide is demonstrated in Chapter 5. The non-participating nature of the Nap-ethers in the building blocks used in this synthesis is crucial for the stereoselective formation of the β -mannuronic acid linkage.^{21–23}

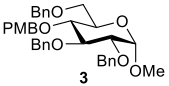
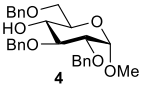
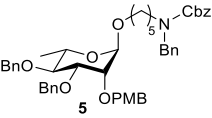
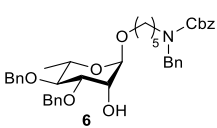
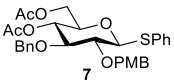
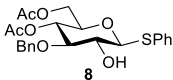
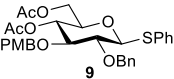
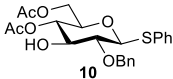
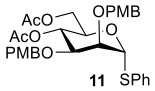
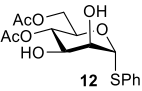
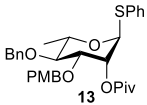
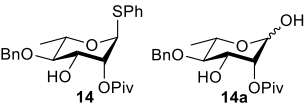
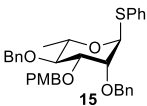
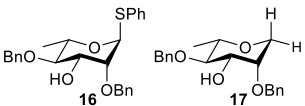
Results and Discussion

A series of substrates varying in protection pattern was tested with the new method. The first substrate that was subjected to a catalytic amount of HCl (0.1 equiv) in DCM/HFIP was O-glycoside **3**, carrying a PMB group at C-4 (Table 1, entry 1). Upon addition of a preformed HCl/HFIP mixture to a solution of **3** in DCM/HFIP, the reaction mixture turned dark purple within seconds, indicative for the formation of *p*-methoxybenzyl cationic species. Within minutes all substrate had been consumed and transformed into a single product (**4**). Besides the formation of the desired alcohol, TLC analysis showed the formation of a lipophilic side product. LC-MS analysis of this side product indicated this to be a PMB derived polymer, indicating that the PMB cations, released during the reaction, are not scavenged by HFIP but instead react with another PMB ether in a Friedel–Crafts manner, resulting in the formation of the polymer, for which a putative mechanism is provided in Scheme 2.^{14,24}

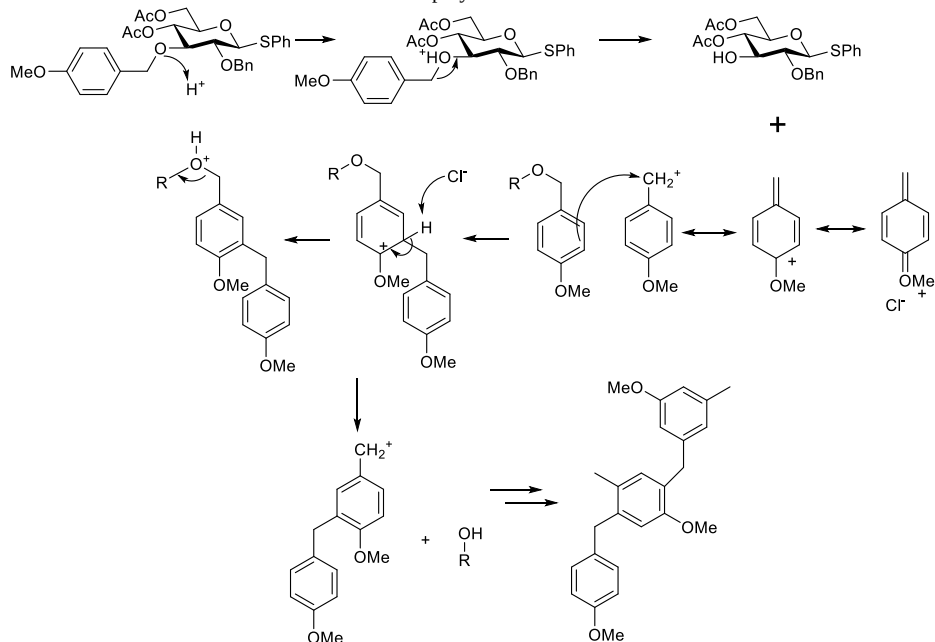
The same conditions (0.1 equiv HCl DCM/HFIP 1:1) also cleanly cleaved the PMB group from the C2-OH in rhamnoside **5** (entry 2), carrying an aminopentanol spacer. The anomeric acetal was completely stable under the conditions used. Next, various thioglycosides were explored. Glucoside **7**, carrying a single PMB group at C2-OH, was subjected to the deprotection mixture to uneventfully afford alcohol **8**. Likewise, the C3-OPMB ether was cleanly removed from glucoside **9** to give compound **10**. Mannoside **11**, carrying two PMB ethers, was deprotected equally efficient leading to diol **12** in 80% yield

(entry 5). When rhamnoside **13** was subjected to the deprotection conditions (0.1 equiv HCl DCM/HFIP 1:1), a complex mixture resulted. Notably, the characteristic purple color was absent, and the reaction required hours to reach completion. Besides the desired product **14**,

Table 1. Deprotection of PMB protected carbohydrates

Entry	Substrate	Conditions	Product	Yield
1		0.1 eq. HCl/HFIP		96%
2		0.1 eq. HCl/HFIP		82%
3		0.1 eq. HCl/HFIP		90%
4		0.1 eq. HCl/HFIP		81%
5		0.1 eq. HCl/HFIP		80%
6		1.0 eq. HCl/HFIP 3.0 eq. TES, 0°C		14: SPh, 85% 14a: OH, n.d.
7		1.0 eq. HCl/HFIP 3.0 eq. TES, 0°C		16: SPh, 75% 17: H, 14%.

anomeric lactol **14a** was formed in this reaction, indicating that alkylation of the anomeric thiofunction by the PMB cation occurred as a side reaction. Expulsion of the activated aglycon then leads to hydrolysis of the thioglycoside.²⁵ To circumvent this side reaction, triethylsilane (TES) was added to the reaction mixture to scavenge the released PMB cations. Because it was reasoned that the addition of a scavenger would necessitate the use of at least an equimolar amount of HCl, 1 equiv of HCl and 3 equiv of scavenger were used. These conditions resulted in clean removal of the PMB group from rhamnoside **13** and the isolation of alcohol **14** in 85% yield (entry 6). When the same conditions were used to cleave the PMB group from rhamnoside **15**, the desired alcohol **16** was obtained in 75%

Scheme 2. Mechanism for the formation of PMB polymer

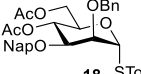
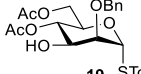
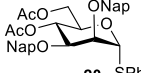
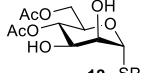
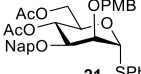
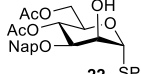
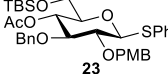
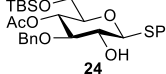
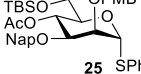
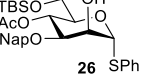
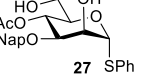
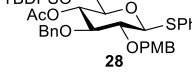
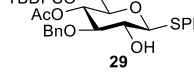
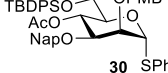
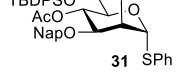
alongside desulfurized compound **17** (entry 7). Here, activation of the thiofunction in **15** or **16** could not be completely suppressed because of the high reactivity of the rhamnose, being a 6-deoxy glycoside featuring solely “arming” benzyl ether protecting groups. Of note, the anomeric linkage in O-rhamnoside **5/6** (entry 2) is completely stable under the acidic conditions.

Since Nap ethers can be removed under acidic conditions, it was investigated whether Nap ethers can also be cleaved using the HCl/HFIP cocktail. Mannoside **18** (Table 2) was subjected to the catalytic cleavage conditions described above (0.1 equiv. HCl DCM/HFIP 1 : 1). These conditions proved not forceful enough to cleave the Nap ether and the reaction progressed very slow and led to a low yield of the desired alcohol. The amount of acid was raised to an equimolar amount. The addition of triethyl silane as a scavenger led to the clean and controllable formation of alcohol **19** (entry 1, Table 2). Similarly, deprotection of bis-Nap ether **20** proceeded uneventfully to give diol **12** (entry 2).

Based on these results it was reasoned that the difference in reactivity of the PMB and Nap ethers towards the HCl/HFIP combination should allow for the selective removal of a PMB ether in the presence of a Nap ether. The addition of a catalytic amount of HCl to mannoside **21** proved this hypothesis and the PMB ether in **21** was selectively cleaved to give alcohol **22** in good yield (entry 3, Table 2). The orthogonality of the PMB ether with respect to commonly used silyl ethers was explored next.^{1,26} Removal of the PMB ether in **23** and **25** was accompanied by partial cleavage of *tert*-butyldimethylsilyl (TBS) groups at the primary hydroxyl function (entries 4 and 5). Although conditions that left the TBS

ethers untouched could not be identified, it was found during the optimization of these reactions that a catalytic amount of HCl could be used in combination with a stoichiometric amount of scavenger (TES). Besides, the more acid stable *tert*-butyldiphenylsilyl (TBDPS) was stable to this catalytic cleavage cocktail and selective deprotection of the PMB ether in **28** in the presence of a TBDPS ether gave glucosyl alcohol **29** in 89% yield (entry 6). Similarly, the PMB ether in mannoside **30** was selectively deblocked, leaving both the primary TBDPS ether and the secondary naphthyl ether unaffected (entry 7). When mannoside **30** was subjected to 5% trifluoroacetic acid in DCM,¹ compound **31** was obtained in 77% yield, where oxidative removal of the C-2-O-PMB using DDQ,¹ resulted in a complex mixture.

Table 2. Nap deprotection and selectivity

Entry	Substrate	conditions	Product	Yield
1	 18 STol	1.0 eq. HCl/HFIP 3.0 eq. TES	 19 STol	86%
2	 20 SPh	2.0 eq. HCl/HFIP 5.0 eq. TES	 12 SPh	67%
3	 21 SPh	0.1 eq. HCl/HFIP 3.0 eq. TES	 22 SPh	80%
4	 23	0.1 eq. HCl/HFIP 1.0 eq. TES	 24	48%
5	 25 SPh	0.1 eq. HCl/HFIP 1.0 eq. TES	 26 SPh	26 : OTBS, 63% 27 : OH, 24%
			 27 SPh	
6	 28	0.1 eq. HCl/HFIP 1.0 eq. TES	 29	89%
7	 30 SPh	0.1 eq. HCl/HFIP 1.0 eq. TES	 31 SPh	88%

Conclusion

In summary, a new, fast, and homogeneous deprotection method for electron-rich benzyl-type ethers is described employing HCl in HFIP. PMB and Nap ethers can be removed with a catalytic amount of acid in a selective manner without affecting other groups. PMB ethers can also be selectively cleaved with respect to Nap ethers by limiting the amount of HCl. The ease of cleavage of these groups under the established conditions is a valuable asset for the utility of the PMB and Nap ethers in synthetic (carbohydrate) chemistry. The

latter is illustrated by the successful application of the HCl/HFIP method in the synthesis of complex fucosazide donors²⁷ as well as its use in constructing *E. faecium* wall teichoic acid fragments.²⁸ The mild, fast, and homogeneous reaction conditions should allow for their use in a solid-phase reaction setting. Also in stereoselective glycosylation reactions that are mediated through external nucleophiles (“moderators”), the use of a protecting group scheme that builds on all-benzyl ether-type protecting groups that can be selectively removed will be very valuable.²⁹

Experimental Section

General experimental procedures. All chemicals were used as received unless stated otherwise. ^1H and ^{13}C NMR spectra were recorded on a 400/100 MHz, 500/125 MHz, 600/150 MHz or a 850/214 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All individual signals were assigned using 2D-NMR spectroscopy, HH-COSY, HSQC and HMBC. IR spectra are reported in cm^{-1} . Flash chromatography was performed on silica gel 60 (0.04 – 0.063 mm). TLC-analysis was followed by detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at $\sim 150^\circ\text{C}$ or by spraying with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot \text{H}_2\text{O}$ (25 g/l) and $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}$ (10 g/l) in 10% sulfuric acid in water followed by charring at 50°C . LC-MS standard eluents used were A: 100% H_2O , B: 100% acetonitrile, C: 1% TFA in H_2O . The column used was a C18 column (4.6 mmD $\times$ 50 mmL, 3μ particle size). All analyses were 13 min, with a flow-rate of 1 ml/min. High-resolution mass spectra were recorded on a LTQ-Orbitrap equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275°C) with resolution $R=60.000$ at $m/z=400$ (mass range = 150–4000) and dioctylphthalate ($m/z=391.28428$) as "lock mass". HCl/HFIP solution were freshly prepared prior to use.

Methyl 2,3,6-tri-*O*-benzyl- α -D-glucopyranoside (4) Compound **3**³⁰ (0.117 g, 0.200 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.1 mL 0.2M HCl/HFIP was added. After 150 seconds the reaction was quenched by addition of sat. aq. NaHCO_3 . The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO_4 and concentrated. Purification by column chromatography (Tol/EtOAc) gave **4** in 96% yield (0.0891 g, 0.19 mmol). TLC R_f 0.35 (Tol/EtOAc, 9/1, v/v); ^1H NMR(CDCl_3 , 500 MHz): δ 7.39 – 7.20 (m, 15H, CH_{arom}), 4.99 (d, 1H, $J = 11.5$ Hz, CHH OBn), 4.78 – 4.70 (m, 2H, CHH OBn, CHH OBn), 4.68 – 4.61 (m, 2H, CHH OBn, H-1), 4.55 (q, 2H, $J = 12.1$, 12.1, 12.1 Hz, CH_2 OBn), 3.78 (t, 1H, $J = 9.2$, 9.2 Hz, H-3), 3.74 – 3.64 (m, 3H, H-5, H-6), 3.59 (t, 1H, $J = 9.2$, 9.2 Hz, H-4), 3.52 (dd, 1H, $J = 9.6$, 3.5 Hz, H-2), 3.37 (s, 3H, OMe), 2.37 (s, 1H, 4-OH); ^{13}C NMR(CDCl_3 , 126 MHz): δ 138.9, 138.2, 138.1 (Cq), 128.6, 128.5, 128.4, 128.2, 128.0, 128.0, 127.9, 127.7, 127.7 (CH_{arom}), 98.3 (C-1), 81.6 (C-3), 79.7 (C-2), 75.5 (CH_2Bn), 73.7 (CH_2Bn), 73.2 (CH_2Bn), 70.9 (C-4), 70.0 (C-5), 69.6 (C-6), 55.3 (CH_3 OMe).

***N*-benzyl-*N*-3,4-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl- α -L-rhamno-pyranoside (5)** *N*-benzyl-*N*-benzyloxycarbonyl-5-aminopentanyl-3,4-di-*O*-benzyl- α -L-rhamno-pyranoside³¹ (0.908 g, 1.39 mmol) was coevaporated twice with anhydrous toluene before being dissolved in DMF (4 mL). The mixture was cooled to 0°C , after which sodium hydride (60% dispersion in mineral oil, 0.08 g, 2.08 mmol) was added. The mixture was stirred for 10 minutes followed by addition of *para*-methoxybenzylchloride (0.28 mL, 2.08 mmol). After 115 minutes, the reaction was quenched with sat. aq. NaHCO_3 , diluted with Et_2O and washed with water. The organic layer was dried over MgSO_4 and concentrated. Purification by column chromatography (Tol/EtOAc) gave **5** in 75% yield (0.802 g, 1.03 mmol). ^1H NMR (CDCl_3 , 400 MHz): δ 7.30 (m, 22H, CH_{arom}), 7.17 (s, 1H, CH_{arom}), 6.83 (d, 2H, $J = 8.6$ Hz, CH_{arom}), 5.17 (d, 2H, $J = 9.4$ Hz, CH_2 Cbz), 4.93 (d, 1H, $J = 10.8$ Hz, CHH OBn), 4.72 – 4.54 (m, 6H, CHH OBn, CH_2 OBn, CH_2 OPMB, H-1), 4.48 (s, 2H, CH_2 Bn), 3.82 – 3.73 (m, 5H, CH_3 OMe, H-2, H-3), 3.66 – 3.50 (m, 3H, H-5, CH_2), 3.33 – 3.10 (m, 1H, H-4, CH_2), 1.66 – 1.37 (m, 5H, CH_2), 1.34 – 1.07 (m, 6H, CH_3 H-6, CH_2); ^{13}C -NMR (CDCl_3 , 101 MHz): δ 159.3, 138.8, 138.0, 130.6 (Cq), 129.6, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 113.9 (CH_{arom}), 98.1 (C-1), 80.7 (C-3), 80.4 (C-4), 75.6 (CH_2Bn), 74.6 (C-2), 72.5, 72.2 (CH_2 PMB/Bn), 68.1 (C-5), 67.3 (CH_2Bn), 55.4 (CH_3 OMe), 50.7, 50.3 (CH_2), 29.3 (CH_2), 23.5 (CH_2), 18.2 (CH_3 C-6); HRMS: $[\text{M}+\text{NH}_4]^+$ calculated for $\text{C}_{48}\text{H}_{59}\text{N}_2\text{O}_8$ 791.42659, found 791.42758.

***N*-benzyl-*N*-benzyloxycarbonyl-5-aminopentanyl-3,4-di-*O*-benzyl- α -L-rhamno-pyranoside (6)** Compound **5** (0.157 g, 0.200 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.1 mL 0.2M HCl/HFIP was added. After 150 seconds the reaction was quenched by addition of sat. aq. NaHCO_3 . The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO_4 and concentrated. Purification by column chromatography (Tol/EtOAc) gave **6** in 82% yield (0.108 g, 0.165 mmol). TLC R_f 0.15 (Tol/EtOAc, 9/1, v/v); IR (neat, cm^{-1}): 694, 731, 910, 984, 1028, 1051, 1069, 1096, 1227, 1304, 1362, 1421,

1452, 1472, 1497, 1695, 1728, 2930; ^1H NMR (CDCl_3 , 500 MHz): δ 7.37 – 7.17 (m, 20H, CH_{arom}), 5.17 (d, 2H, J = 11.1 Hz, CH_2 Cbz), 4.87 (d, 1H, J = 10.9 Hz, CHH OBn), 4.75 (s, 1H, H-1), 4.69 – 4.60 (m, 3H, CH_2 OBn), 4.49 (s, 2H, CH_2 OBn), 3.99 (s, 1H, H-2), 3.81 (d, 1H, J = 7.0 Hz, H-3), 3.68 (m, 1H, H-5), 3.58 (m, 1H, CH_2), 3.44 (t, 1H, J = 9.3, 9.3 Hz, H-4), 3.26 – 3.19 (m, 3H, CH_2), 2.41 (bs, 1H, 2-OH), 1.53 – 1.47 (m, 4H, 2 x CH_2), 1.30 – 1.26 (m, 5H, CH_3 -6, CH_2); ^{13}C -NMR (CDCl_3 , 126 MHz): δ 138.5, 138.1, (C_q), 128.6, 128.6, 128.5, 128.5, 128.1, 128.0, 127.9, 127.9, 127.8, 127.4 (CH_{arom}), 99.0 (C-1), 80.3 (C-3), 80.1 (C-4), 75.5 (CH_2Bn), 72.1 (CH_2), 68.7 (C-2), 67.4 (CH_2), 67.4 (C-5), 67.3 (CH_2 Cbz), 50.6, 50.3 (CH_2Bn), 47.2, 46.2 (CH_2), 29.2 (CH_2), 23.5 (CH_2), 18.0 (CH_3 -6). Analytical data are identical to literature precedence.

Phenyl 4,6-di-O-acetyl-3-O-benzyl-2-O-p-methoxybenzyl-1-thio- β -D-glucopyranoside (7) Phenyl 4,6-O-benzylidene-3-O-benzyl-2-O-p-methoxybenzyl-1-thio- β -D-glucopyranoside³² (1.76 g, 3.00 mmol) was dissolved in DCM/MeOH (15 mL/ 15 mL) and p-toluenesulfonic acid monohydrate (0.06 g, 0.30 mmol) was added. When TLC analysis showed complete consumption of the starting material, the reaction was neutralized with Et_3N . The crude was dissolved in pyridine (12 mL), cooled to 0°C , followed by addition of 1.3 mL Ac_2O . The reaction was stirred overnight after which it was quenched with EtOH and concentrated. The crude mixture was diluted with EtOAc, washed with 1M HCl, sat. aq. NaCl, dried over MgSO_4 and concentrated. Column purification (hexanes/EtOAc) gave compound **7** in 84% yield (1.428 g, 2.52 mmol). ^1H NMR(CDCl_3 , 500 MHz): δ 7.57 (dd, 2H, J = 7.6, 1.9 Hz, CH_{arom}), 7.36 – 7.19 (m, 10H, CH_{arom}), 6.86 (d, 2H, J = 8.6 Hz, CH_{arom}), 5.03 (t, 1H, J = 9.7, 9.7 Hz, H-4), 4.82 (m, 2H, 2x CHH OBn/OPMB), 4.67 – 4.57 (m, 3H, H-1, 2x CHH OBn/OPMB), 4.20 (dd, 1H, J = 12.2, 5.7 Hz, H-6), 4.10 (dd, 1H, J = 12.2, 2.2 Hz, H-6), 3.77 (s, 3H, OMe), 3.64 (t, 1H, J = 9.1, 9.1 Hz, H-3), 3.60 – 3.49 (m, 2H, H-2, H-5), 2.06 (s, 3H, CH_3 Ac), 1.90 (s, 3H, CH_3 Ac); ^{13}C NMR(CDCl_3 , 126 MHz): δ 170.6, 169.6 (C=O Ac), 159.4, 138.0, 133.2 (C_q), 132.1, 130.0 (CH_{arom}), 129.8 (C_q), 128.9, 128.4, 127.8, 127.7, 113.8 (CH_{arom}), 87.5 (C-1), 83.7 (C-3), 80.2 (C-2), 75.8 (C-5), 75.4, 75.2 ($\text{CH}_2\text{Bn/PMB}$), 69.6 (C-4), 62.6 (C-6), 55.2 (CH_3 OMe), 20.7, 20.7 (CH_3 Ac); HRMS: $[\text{M}+\text{NH}_4]^+$ calculated for $\text{C}_{31}\text{H}_{38}\text{NO}_8$ 584.23126, found 584.23151.

Phenyl 4,6-di-O-acetyl-3-O-benzyl-1-thio- β -D-glucopyranoside (8) Compound **7** (0.134 g, 0.236 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.12 mL 0.2M HCl/HFIP was added. After 15 min the reaction was quenched by addition of sat. aq. NaHCO_3 . The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO_4 and concentrated. Purification by column chromatography (Tol/EtOAc) gave **8** in 88% yield (0.093 g, 0.207 mmol). TLC R_f 0.50 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ -6.8 (c 1, DCM); IR (neat, cm^{-1}): 692, 740, 1026, 1220, 1365, 1739, 2885, 2953, 3375; ^1H -NMR (CDCl_3 , 500 MHz): δ 7.58 – 7.52 (m, 3H, CH_{arom}), 7.36 – 7.24 (m, 13H, CH_{arom}), 4.98 (t, 1H, J = 9.8 Hz, H-4), 4.83 (d, 1H, J = 11.8 Hz, CHH Bn), 4.69 (d, 1H, J = 11.8 Hz, CHH Bn), 4.51 (d, 1H, J = 9.3 Hz, H-1), 4.21 – 4.10 (m, 2H, H-6), 3.62 – 3.51 (m, 3H, H-2, H-3, H-5), 2.65 (s, 1H, 2-OH), 2.07 (s, 3H, CH_3 Ac); ^{13}C NMR (CDCl_3 , 126 MHz): δ 170.8, 169.7 (C=O Ac), 138.2 (C_q), 133.3 (CH_{arom}), 131.3 (C_q), 129.1, 128.5, 128.5, 127.9, 127.9 (CH_{arom}), 88.1 (C-1), 82.9 (C-3), 76.2 (C-5), 74.8 (CH_2Bn), 72.5 (C-2), 69.5 (C-4), 62.7 (C-6), 29.8, 20.9 (CH_3 Ac); HRMS: $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{23}\text{H}_{26}\text{O}_7\text{SNa}$ 469.12915, found 469.12830.

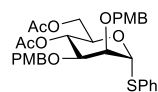
Phenyl 4,6-di-O-acetyl-2-O-benzyl-3-O-p-methoxybenzyl-1-thio- β -D-glucopyranoside (9) Phenyl 4,6-O-benzylidene-3-O-p-methoxybenzyl-1-thio- β -D-glucopyranoside³³ (0.443 g, 0.92 mmol) was coevaporated twice with anhydrous toluene before being dissolved in DMF (5 mL). The mixture was cooled to 0°C , after which sodium hydride (60% dispersion in mineral oil, 0.07 g, 1.84 mmol) was added. The mixture was stirred for 10 minutes followed by addition of benzylbromide (0.21 mL, 1.84 mmol). When TLC analysis showed complete consumption of the starting material, the reaction was quenched with sat. aq. NaHCO_3 . The mixture was diluted with Et_2O , washed with sat. aq. NaCl, dried over MgSO_4 and concentrated. The crude was dissolved in DCM/MeOH (15 mL/ 15 mL), followed by addition of p-toluenesulfonic acid monohydrate until the pH was acidic. The reaction was stirred for 95 minutes after which it was neutralized with Et_3N and concentrated. The diol was dissolved in 5 mL pyridine, cooled to 0°C and 0.35 mL Ac_2O was added. After overnight stirring, the reaction was quenched with MeOH and concentrated. Column purification (Pent/EtOAc) gave compound **9** in 58% yield (0.301 g, 0.53 mmol). ^1H NMR (CDCl_3 , 500 MHz): δ 7.59 – 7.53 (m, 2H, CH_{arom}), 7.43 – 7.20 (m, 8H, CH_{arom}), 7.15 (d, 2H, J = 8.6 Hz, CH_{arom}), 6.84 (d, 2H, J = 8.6 Hz, CH_{arom}), 5.02 (t, 1H, J = 9.7, 9.7 Hz, H-4), 4.87 (d, 1H, J = 10.2 Hz, CHH OBn), 4.72 (m, 2H, CH_2

OBn/OPMB), 4.65 (d, 1H, $J = 9.8$ Hz, H-1), 4.58 (d, 1H, $J = 11.0$ Hz, CHH OBn/OPMB), 4.21 (dd, 1H, $J = 12.2$, 5.7 Hz, H-6), 4.11 (dd, 1H, $J = 12.2$, 2.1 Hz, H-6), 3.77 (s, 3H, CH₃ OMe), 3.65 (t, 1H, $J = 9.1$, 9.1 Hz, H-3), 3.62 – 3.48 (m, 3H, H-2, H-5), 2.07 (s, 3H, CH₃ Ac), 1.95 (s, 3H, CH₃ Ac); ¹³C NMR (CDCl₃, 126 MHz): δ 170.7, 169.6 (C=O Ac), 159.3, 137.8, 133.2 (C_q), 132.3 (CH_{arom}), 130.1 (C_q), 129.5, 129.0, 128.5, 128.3, 128.0, 127.9, 113.9 (CH_{arom}), 87.5 (C-1), 83.4 (C-3), 80.6 (C-2), 76.9 (C-5), 75.6, 75.1 (CH₂ OBn/OPMB), 69.8 (C-4), 62.7 (C-6), 55.3 (CH₃ OMe), 20.9, 20.9 (CH₃ Ac); HRMS: [M+NH₄]⁺ calculated for C₃₁H₃₈NO₈ 584.23126, found 584.23143.

Phenyl 4,6-di-O-acetyl-2-O-benzyl- β -D-glucopyranoside (10) Compound **9** (0.107 g, 0.188 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.1 mL 0.2M HCl/HFIP was added. After 20 min the reaction was quenched by addition of pyridine and the mixture was concentrated.

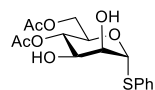
Purification by column chromatography (Tol/EtOAc) gave **10** in 81% yield (0.068 g, 0.152 mmol). TLC: R_f 0.38 (PE/EtOAc, 2/1, v/v); [α]_D²⁰ -45.6 (c 1, DCM); IR (neat, cm⁻¹): 700, 744, 1028, 1043, 1228, 1371, 1739, 2922, 3477; ¹H NMR (CDCl₃, 500 MHz): δ 7.58 – 7.54 (m, 2H, CH_{arom}), 7.37 – 7.26 (m, 8H, CH_{arom}), 4.95 (d, 1H, $J = 10.9$ Hz, H-1), 4.90 (t, 1H, $J = 9.7$, 9.7 Hz, H-4), 4.71 (d, 1H, $J = 10.9$ Hz, CHH Bn), 4.64 (d, 1H, $J = 9.8$ Hz, CHH Bn), 4.22 (dd, 1H, $J = 12.2$, 5.7 Hz, H-6), 4.14 (dd, 1H, $J = 12.2$, 2.3 Hz, H-6), 3.73 (t, 1H, $J = 9.0$, 9.0 Hz, H-3), 3.60 (ddd, 1H, $J = 10.0$, 5.7, 2.3 Hz, H-5), 3.43 – 3.39 (t, 1H, $J = 10$ Hz, 8.5 Hz, H-2), 2.68 (s, 1H, 3-OH), 2.08 (s, 3H), 2.07 (s, 3H) ppm; ¹³C NMR (CDCl₃, 125 MHz): δ 170.8, 170.6 (C=O Ac), 137.9 (C_q), 133.3 (C_q), 132.2, 129.1, 128.7, 128.4, 128.3, 127.9 (CH_{arom}), 87.3 (C-1), 80.7 (C-2), 76.5 (C-3), 75.7 (C-5), 75.5 (CH₂Bn), 70.4 (C-4), 62.8 (C-6), 20.9, 20.9 (CH₃ Ac); HRMS: [M+Na]⁺ calculated for C₂₃H₂₆O₇SnA 469.12915, found 469.12861.

Phenyl 4,6-di-O-acetyl-2,3-di-O-p-methoxybenzyl-1-thio- α -D-mannopyranoside (11) Phenyl 4,6-O-benzylidene-1-thio- α -D-mannopyranoside³⁴ (1.85 g, 5.13 mmol) was coevaporated twice with anhydrous toluene before being dissolved in DMF (13 mL). The mixture was cooled to 0°C,



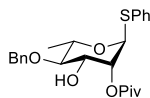
after which sodium hydride (60% dispersion in mineral oil, 0.62 g, 15 mmol) was added. The mixture was stirred for 10 minutes followed by addition of *para*-methoxybenzylchloride (2.16 mL, 15 mmol). When TLC analysis showed complete consumption of the starting material, the reaction was quenched with sat. aq. NaHCO₃. The mixture was diluted with EtOAc, washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. The crude was dissolved in MeOH (50 mL), followed by addition of *p*-toluenesulfonic acid monohydrate (0.09 g, 0.45 mmol). The reaction was stirred for 95 minutes after which it was neutralized with Et₃N and concentrated. The compound was purified by column chromatography (Pent/EtOAc). The diol was dissolved in 20 mL pyridine, cooled to 0°C and 2.17 mL Ac₂O was added. After overnight stirring, the reaction was quenched with EtOH and concentrated. Column purification (Pent/EtOAc) gave compound **11** in 64% yield (1.95 g, 3.26 mmol). ¹H NMR (CDCl₃, 400 MHz): δ 7.45 – 7.38 (m, 2H, CH_{arom}), 7.33 – 7.17 (m, 7H, CH_{arom}), 6.85 (m, 4H, CH_{arom}), 5.52 (d, 1H, $J = 1.6$ Hz, H-1), 5.39 (t, 1H, $J = 9.7$, 9.7 Hz, H-4), 4.66 – 4.54 (m, 2H, CH₂PMB), 4.51 – 4.36 (m, 2H, CH₂PMB), 4.31 (ddd, 1H, $J = 9.6$, 6.1, 2.1 Hz, H-6), 4.23 (dd, 1H, $J = 12.0$, 6.1 Hz, H-5), 4.11 (dd, 1H, $J = 12.0$, 2.2 Hz, H-6), 3.97 – 3.91 (m, 1H, H-2), 3.83 – 3.68 (m, 7H, 2x CH₃ OMe, H-3), 2.04 (s, 3H, CH₃ Ac), 2.01 (s, 3H, CH₃ Ac); ¹³C NMR (CDCl₃, 100 MHz): δ 170.7, 169.7 (C=O Ac), 159.4, 133.8 (C_q), 131.6 (CH_{arom}), 129.9, 129.8 (C_q), 129.6, 129.3, 129.1, 127.7, 113.9, 113.8 (CH_{arom}), 85.9 (C-1), 76.5 (C-3), 75.2 (C-2), 71.9, 71.5 (CH₂PMB), 70.0 (C-5), 68.2 (C-4), 63.0 (C-6), 55.3 (CH₃ OMe), 21.0, 20.8 (CH₃ Ac); HRMS: [M+NH₄]⁺ calculated for C₃₂H₄₀NO₉S 614.24183, found 614.24212.

Phenyl 4,6-di-O-acetyl-1-thio- α -D-mannopyranoside (12) Compound **11** (0.112 g, 0.188 mmol) was dissolved



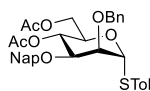
in 1:1 DCM/HFIP (2 mL) and 0.12 mL 0.2M HCl/HFIP was added. After 3 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted DCM and the organic layer was washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (Pent/EtOAc) gave **12** in 79% yield (0.053 g, 0.148 mmol). TLC: R_f 0.21 (PE/EtOAc, 1/1, v/v); [α]_D²⁰ +169.0 (c 1, DCM); IR (neat, cm⁻¹): 744, 1051, 1232, 1735, 2933, 3300; ¹H NMR (CDCl₃, 400 MHz): δ 7.57 – 7.39 (m, 2H, CH_{arom}), 7.39 – 7.19 (m, 3H, CH_{arom}), 5.60 (d, 1H, $J = 1.4$ Hz, H-1), 5.11 (t, 1H, $J = 9.7$ Hz, H-4), 4.46 (ddd, 1H, $J = 10.0$, 5.8, 2.2 Hz, H-5), 4.34 (dd, 1H, $J = 12.1$, 5.9 Hz, H-6), 4.23 (dd, 1H, $J = 3.5$, 1.6 Hz, H-2), 4.08 (dd, 1H, $J = 12.1$, 2.2 Hz, H-6), 3.94 (dd, 1H, $J = 9.4$, 3.4 Hz, H-3), 3.29 (s, 2H, 2-OH, 3-OH), 2.15 (s, 3H, CH₃ Ac), 2.03 (s, 3H, CH₃ Ac); ¹³C NMR (CDCl₃, 100 MHz) δ 171.9, 171.0

(C=O Ac), 133.3 (C_q Sph), 131.7, 129.2, 127.9 (CH_{arom}), 87.6 (C-1), 72.2 (C-2), 70.8 (C-3), 70.2 C-4), 69.1 (C-5), 62.7 (C-6), 21.1, 20.9 (CH₃ Ac); HRMS: [M+Na]⁺ calculated for C₁₆H₂₀O₇SNa 379.08219, found 379.08213.



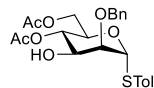
Phenyl 4-O-benzyl-2-O-Pivaloyl-1-thio-α-L-rhamnopyranoside (14) Compound **13'** (0.156 g, 0.276 mmol) was dissolved in a 1:1 DCM/HFIP mixture (2.8 mL) and TES (0.13 mL, 0.84 mmol) was added. The mixture was cooled to 0°C and 1.4 mL of a 0.2M HCl/HFIP solution was added. After TLC and TLC/MS analysis showed complete conversion of the starting material in a lower running spot, the reaction was quenched with sat. aq. NaHCO₃ and diluted with DCM. The aqueous layer was washed with DCM and the combined organic layers were washed with a sat. aq. NaCl solution, dried over MgSO₄ and concentrated. Silica gel column purification afforded compound **14** in 85% yield (0.102 g, 0.23 mmol). TLC: R_f 0.55 (PE/EtOAc, 9/1, v/v); [α]_D²⁰ -123.0 (c 1, DCM); IR (neat, cm⁻¹): 690, 738, 1097, 1151, 1280, 1479, 1730, 2972, 3469; ¹H NMR (CDCl₃, 400 MHz): δ 7.50 – 7.42 (m, 2H, CH_{arom}), 7.40 – 7.21 (m, 8H, CH_{arom}), 5.36 (d, 1H, J = 1.2 Hz, H-1), 5.33 (dd, 1H, J = 3.3, 1.5 Hz, H-2), 4.81 (d, 1H, J = 11.2 Hz, CHH Bn), 4.74 (d, 1H, J = 11.2 Hz, CHH Bn), 4.24 (dq, 1H, J = 9.5, 6.2, 6.2, 6.2 Hz, H-5), 4.09 (d, 1H, J = 10.4 Hz, H-3), 3.38 (t, 1H, J = 9.4, 9.4 Hz, H-4), 2.21 (s, 1H, 3-OH), 1.35 (d, 3H, J = 6.2 Hz, CH₃-6), 1.23 (s, 9H, CH₃-Piv); ¹³C NMR (CDCl₃, 100 MHz): δ 178.1 (C=O Piv), 138.1 (C_q), 133.9 (C_q), 132.1, 129.2, 128.7, 128.3, 128.2, 127.8 (CH_{arom}), 86.0 (C-1), 81.7 (C-4), 75.2 (CH₂ Bn), 74.0 (C-2), 71.1 (C-3), 68.7 (C-5), 39.2 (C_q Piv), 27.2 (CH₃ Piv), 18.1 (CH₃-6); HRMS: [M+Na]⁺ calculated for C₂₄H₃₀O₅SNa 453.17062, found 453.17055.

Phenyl 2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (16) Compound **15'** (0.108 g, 0.194 mmol) was dissolved in a 1:1 DCM/HFIP mixture (2 mL) and TES (0.09 mL, 0.58 mmol) was added. The mixture was cooled to 0°C and 0.97 mL of a 0.2M HCl/HFIP solution was added. After TLC and TLC/MS analysis showed complete conversion of the starting material in a lower running spot, the reaction was quenched with sat. aq. NaHCO₃ and diluted with DCM. The aqueous layer was washed with DCM and the combined organic layers were washed with a sat. aq. NaCl solution, dried over MgSO₄ and concentrated. Silica gel column purification afforded compound **16** in 76% yield (0.064 g, 0.147 mmol). TLC: R_f 0.78 (PE/EtOAc, 2/1, v/v); [α]_D²⁰ -116.0 (c 1, DCM); IR (neat, cm⁻¹): 694, 736, 1026, 1066, 1082, 1583, 2873, 3030, 3061; ¹H NMR (CDCl₃, 400 MHz): δ 7.36 – 7.23 (m, 15H, CH_{arom}), 5.56 (s, 1H, H-1), 4.91 (d, 1H, J = 11.1 Hz, CHH Bn), 4.74 (d, 1H, J = 11.7 Hz, CHH Bn), 4.67 (d, 1H, J = 11.1 Hz, CHH Bn), 4.53 (d, 1H, J = 11.7 Hz, CHH Bn), 4.16 (dq, 1H, J = 9.4, 6.2, 6.2, 6.2 Hz, H-5), 4.00 – 3.95 (m, 2H, H-2, H-3), 3.40 (t, 1H, J = 9.1, 9.1 Hz, H-4), 2.37 (bs, 1H, 3-OH), 1.34 (d, 3H, J = 6.2 Hz, CH₃-6); ¹³C NMR (CDCl₃, 100 MHz): δ 138.5, 137.5, 134.5 (C_q), 131.6, 129.2, 128.7, 128.6, 128.3, 128.2, 128.1, 127.9, 127.5 (CH_{arom}), 85.1 (C-1), 82.5 (C-4), 80.1 (C-2), 75.3 (CH₂Bn), 72.5 (CH₂Bn), 72.2 (C-3), 68.7 (C-5), 18.1 (CH₃-6); HRMS: [M+Na]⁺ calculated for C₂₆H₂₈O₄SNa 459.16005, found 459.15943.

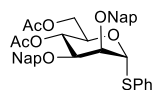


Toly 4,6-di-O-acetyl-2-O-benzyl-3-O-(2-naphthylmethyl)-1-thio-α-D-mannopyranoside (18) Toly 4,6-O-benzylidene-2-O-benzyl-3-O-(2-naphthylmethyl)-1-thio-α-D-mannopyranoside³⁵ (1.37 g, 2.28 mmol) was dissolved in DCM/MeOH (3 mL/ 12 mL) and p-toluenesulfonic acid monohydrate (0.043 g, 0.228 mmol) was added. The reaction was stirred for 5 days after which it was neutralized with Et₃N. The crude was dissolved in pyridine (12 mL), cooled to 0°C, followed by addition of 1.3 mL Ac₂O. The reaction was stirred overnight after which it was quenched with EtOH and concentrated. The crude mixture was diluted with EtOAc, washed with 1M HCl, sat. aq. NaCl, dried over MgSO₄ and concentrated. Column purification (PE/EtOAc) gave compound **18** in 70% yield (0.969 g, 1.61 mmol). ¹H NMR(CDCl₃, 400 MHz): δ 7.87 – 7.79 (m, 3H, CH_{arom}), 7.74 (s, 1H CH_{arom}), 7.53 – 7.48 (m, 1H CH_{arom}), 7.48 – 7.37 (m, 2H CH_{arom}), 7.37 – 7.21 (m, 7H CH_{arom}), 7.12 – 7.05 (m, 2H CH_{arom}), 5.54 – 5.43 (m, 2H, H-1, H-4), 4.84 – 4.51 (m, 4H, CH₂ Bn/Nap), 4.39 – 4.30 (m, 1H, H-5), 4.25 (dd, 1H, J = 12.1, 6.0 Hz, H-6), 4.17 – 4.06 (m, 1H, H-6), 4.05 – 3.98 (m, 1H, H-2), 3.84 (dd, 1H, J = 9.6, 2.9 Hz, H-3), 2.32 (s, 3H, CH₃ Tol), 2.07 – 2.01 (m, 6H, 2x CH₃ Ac); ¹³C NMR(CDCl₃, 101 MHz): δ 170.8, 169.8 (C=O Ac), 138.0, 137.7, 135.3, 133.3, 133.1 (C_q), 132.2, 129.9 (CH_{arom}), 129.9 (C_q), 128.4, 128.3, 128.0, 127.8, 127.8, 126.5, 126.3, 126.1, 125.7, 118.8 (CH_{arom}), 86.1 (C-1), 77.0 (C-3), 75.5 (C-2), 72.2, 71.8 (CH₂ OBn/ONap), 69.9 (C-5), 68.1 (C-4), 63.0 (C-6), 21.2, 21.0, 20.9 (CH₃ Tol, Ac); HRMS: [M+NH₄]⁺ calculated for C₃₅H₄₀NO₇S 618.25200, found 618.25193.

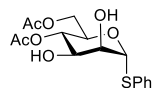
Tolyl 4,6-di-O-acetyl-2-O-benzyl-1-thio- α -D-mannopyranoside (19) Compound **18** (0.117 g, 0.195 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.09 mL TES was added. The solution was treated with 0.97 mL 0.2M HCl/HFIP. After 33 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (hexanes/EtOAc) gave **19** in 86% yield (0.077 g, 0.168 mmol). TLC: *R*_f 0.56 (PE/EtOAc, 2/1, v/v); [α]_D²⁰ +61.6 (c 1, DCM); IR (neat, cm⁻¹): 781, 1051, 1101, 1226, 1739, 2924, 3477; ¹H NMR (CDCl₃, 400 MHz): δ 7.39 – 7.27 (m, 6H, CH_{arom}), 7.12 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.57 (s, 1H, H-1), 5.14 (t, 1H, *J* = 9.9, 9.9 Hz, H-4), 4.74 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.53 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.42 (ddd, 1H, *J* = 9.9, 5.8, 2.0 Hz, H-5), 4.27 (dd, 1H, *J* = 12.1, 5.9 Hz, H-6), 4.12 (dd, 1H, *J* = 12.1, 2.1 Hz, H-6), 4.01 (dd, 1H, *J* = 3.5, 1.1 Hz, H-2), 3.90 (s, 1H, H-3), 2.39 (s, 1H, 3-OH), 2.33 (s, 3H, CH₃ STol), 2.12 (s, 3H, CH₃ Ac), 2.05 (s, 3H, CH₃ Ac); ¹³C-NMR (CDCl₃, 100 MHz): δ 170.8 (C=O Ac), 138.2 (C_q), 137.1 (C_q), 132.5, 130.0 (CH_{arom}), 129.6 (C_q), 128.7, 128.3, 128.1 (CH_{arom}), 85.3 (C-1), 79.3 (C-2), 72.4 (CH₂ Bn), 70.3 (C-3), 69.9 (C-4), 69.2 (C-5), 62.9 (C-6), 21.2 (CH₃ STol), 21.1, 20.9 (CH₃ Ac); HRMS: [M+Na]⁺ calculated for C₂₄H₂₈O₇SNa 483.14480, found 483.14387.



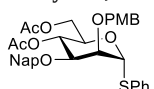
Phenyl 4,6-di-O-acetyl-2,3-di-O-(2-naphthylmethyl)-1-thio- α -D-mannopyranoside (20) 4,6-O-benzylidene-1-thio- α -D-mannopyranoside³⁴ (1.08 g, 3 mmol) was coevaporated twice with anhydrous toluene before being dissolved in DMF (15 mL). The mixture was cooled to 0°C, after which sodium hydride (60% dispersion in mineral oil, 0.48 g, 12 mmol) was added. The mixture was stirred for 10 minutes followed by addition of 2-naphthylmethylbromide (2.65 g, 12 mmol). When TLC analysis showed complete consumption of the starting material, the reaction was quenched with sat. aq. NaHCO₃. The mixture was diluted with EtOAc, the layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. The crude was dissolved in DCM/MeOH (7.5 mL/ 7.5 mL), followed by addition of p-toluenesulfonic acid monohydrate (0.057 g, 0.3 mmol). The reaction was stirred for overnight after which it was neutralized with Et₃N and concentrated. The compound was purified by column chromatography (Pent/EtOAc). The diol was dissolved in 10 mL pyridine, cooled to 0°C and 1.67 mL Ac₂O was added. After stirring for 6 days, the reaction was quenched with EtOH, diluted with EtOAc and washed with 1M HCl. Column purification (Pent/EtOAc) gave compound **20** in 57% yield (1.09 g, 1.71 mmol). ¹H NMR (CDCl₃, 400 MHz): δ 7.84 – 7.61 (m, 8H, CH_{arom}), 7.49 – 7.41 (m, 4H, CH_{arom}), 7.41 – 7.32 (m, 4H, CH_{arom}), 7.26 – 7.17 (m, 3H, CH_{arom}), 5.59 (d, 1H, *J* = 1.6 Hz, H-1), 5.55 (t, 1H, *J* = 9.6, 9.6 Hz, H-4), 4.87 – 4.72 (m, 2H, CH₂ ONap), 4.70 – 4.54 (m, 2H, CH₂ ONap), 4.39 – 4.24 (m, 2H, H-5, H-6), 4.14 (dd, 1H, *J* = 11.8, 1.8 Hz, H-6), 4.09 – 4.01 (m, 1H, H-2), 3.86 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3), 2.02 (m, 6H, 2x CH₃ Ac); ¹³C NMR (CDCl₃, 101 MHz): δ 170.7, 169.7 (C=O Ac), 135.2, 135.1, 133.5, 133.2, 133.1, 133.0, 133.0 (C_q), 131.5, 129.0, 128.2, 128.2, 127.9, 127.9, 127.7, 127.7, 126.8, 126.4, 126.2, 126.1, 126.0, 125.9, 125.6 (CH_{arom}), 85.8 (C-1), 77.0 (C-3), 75.4 (C-2), 72.2, 71.9 (CH₂ Nap), 70.0 (C-5), 68.0 (C-4), 62.8 (C-6), 20.9, 20.8 (CH₃ Ac); HRMS: [M+NH₄]⁺ calculated for C₃₈H₄₀NO₇S 654.25200, found 654.25266.



Phenyl 4,6-di-O-acetyl-1-thio- β -D-mannopyranoside (12) Compound **20** (0.127 g, 0.199 mmol) was dissolved in 1:1 DCM/HFIP (2.0 mL) and 0.16 mL TES was added. The mixture was treated with 3.0 mL 0.2M HCl/HFIP was added. After 20 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (Tol/EtOAc) gave **12** in 68% yield (0.048 g, 0.135 mmol). Spectroscopic data are in full accord with those reported previously.



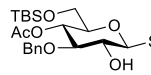
Phenyl 4,6-di-O-acetyl-3-O-(2-naphthylmethyl)-2-O-*p*-methoxybenzyl-1-thio- α -D-mannopyranoside (21) Phenyl 4,6-O-Benzylidene-3-O-(2-naphthylmethyl)-1-thio- α -D-mannopyranoside³⁶ (5.17 g, 10.32 mmol) was coevaporated twice with anhydrous toluene before being dissolved in DMF (25 mL). The mixture was cooled to 0°C, after which sodium hydride (60% dispersion in mineral oil, 1.2 g, 30 mmol) was added. The mixture was stirred for 10 minutes followed by addition of *para*-methoxybenzylchloride (4.1 mL, 30 mmol). When TLC analysis showed complete consumption of the starting material, the reaction was quenched with sat. aq. NaHCO₃. The mixture was diluted with EtOAc,



washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. After column purification (Pent/EtOAc) the compound was dissolved in DCM/MeOH (15 mL/ 15 mL), followed by addition of p-toluenesulfonic acid monohydrate (0.06 g, 0.30 mmol). The reaction was stirred overnight after which it was neutralized with Et₃N and concentrated. The compound was purified by column chromatography (Pent/EtOAc) to yield the diol in 89% yield (2.97 g, 5.57 mmol). The diol (1.425 g, 2.67 mmol) was dissolved in 15 mL pyridine, cooled to 0°C and 1.5 mL Ac₂O was added. After stirring for 3 days, the reaction was quenched with EtOH and concentrated. The crude was taken up in EtOAc, washed with 1M HCl and sat. aq. NaCl, dried over MgSO₄ and concentrated. Column purification (hexanes/EtOAc) gave compound **21** in 75% yield (1.23 g, 1.99 mmol). ¹H NMR (CDCl₃, 400 MHz): δ 7.83 (m, 3H, CH_{arom}), 7.73 (s, 1H CH_{arom}), 7.48 (m, 1H, CH_{arom}), 7.47 – 7.36 (m, 4H, CH_{arom}), 7.25 (m, 6H, CH_{arom}), 6.78 (d, 2H, *J* = 8.2 Hz, CH_{arom}), 5.55 (s, 1H, H-1), 5.47 (t, 1H, *J* = 9.7, 9.7 Hz, H-4), 4.71 – 4.61 (m, 2H, CH₂ ONap/OPMB), 4.58 (m, 2H, CH₂ ONap/OPMB), 4.37 – 4.21 (m, 2H, H-5, H-6), 4.12 (d, 1H, *J* = 11.7 Hz, H-6), 4.01 (s, 1H, H-2), 3.83 (dd, 1H, *J* = 9.6, 2.9 Hz, H-3), 3.73 (s, 3H, CH₃ OMe), 2.06 – 2.00 (m, 6H, 2x CH₃ Ac); ¹³C NMR (CDCl₃, 101 MHz): δ 170.8, 169.8 (C=O Ac), 159.3, 135.3, 133.7, 133.3, 133.0, 131.5 (C_q), 129.7, 129.1, 128.2, 128.0, 127.8, 127.7, 126.4, 126.3, 126.1, 125.6, 113.8 (CH_{arom}), 85.8 (C-1), 77.0 (C-3), 75.0 (C-2), 71.8, 71.8 (CH₂ ONap/OPMB), 70.0 (C-5), 68.1 (C-4), 62.9 (C-6), 55.3 (CH₃ OMe), 21.0, 20.8 (CH₃ Ac); HRMS: [M+NH₄]⁺ calculated for C₃₅H₄₀NO₈S 634.24691, found 634.24718.

Phenyl 4,6-di-O-acetyl-3-O-(2-naphthylmethyl)-1-thio-α-D-mannopyranoside (22) Compound **21** (0.127 g, 0.202 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.1 mL 0.2M HCl/HFIP was added. After 5 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (Tol/EtOAc) gave **22** in 80% yield (0.080 g, 0.162 mmol). TLC: R_f 0.35 (PE/EtOAc, 2/1, v/v); [α]_D²⁰ +132.4 (c 1, DCM); IR (neat, cm⁻¹): 742, 1041, 1099, 1224, 1367, 1739, 2893, 3057, 3460; ¹H NMR (CDCl₃, 500 MHz): δ 7.88 – 7.82 (m, 3H, CH_{arom}), 7.76 (s, 1H, CH_{arom}), 7.55 – 7.47 (m, 2H, CH_{arom}), 7.50 – 7.37 (m, 3H, CH_{arom}), 7.33 – 7.23 (m, 3H, CH_{arom}), 5.63 (d, 1H, *J* = 1.4 Hz, H-1), 5.35 (t, 1H, *J* = 9.7, 9.7 Hz, H-4), 4.86 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.72 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.37 (ddd, 1H, *J* = 9.9, 5.7, 2.2 Hz, H-5), 4.30 (s, 1H, H-2), 4.24 (dd, 1H, *J* = 12.2, 5.8 Hz, H-6), 4.05 (dd, 1H, *J* = 12.2, 2.3 Hz, H-6), 3.86 (dd, 1H, *J* = 9.3, 3.2 Hz, H-3), 2.85 (s, 1H, 2-OH), 2.01 (s, 6H, 2x CH₃ Ac); ¹³C NMR (CDCl₃, 125 MHz): δ 170.9, 169.9 (C=O Ac), 134.7 (C_q), 133.3 (C_q), 133.3 (C_q), 133.2, 131.7, 129.2, 128.6, 128.1, 127.9, 127.8, 127.0, 126.5, 126.4, 125.7 (CH_{arom}), 86.9 (C-1), 77.1 (C-3), 72.2 (CH₂ Nap), 69.6 (C-5), 69.5 (C-2), 67.6 (C-4), 62.7 (C-6), 21.0, 20.9 (CH₃ Ac); HRMS: [M+Na]⁺ calculated for C₂₇H₂₈O₇SNa 519.14480, found 519.14406.

Phenyl 4-O-acetyl-3-O-Benzyl-2-O-p-methoxybenzyl-6-O-tert-butyldimethylsilyl-1-thio-β-D-glucopyranoside (23) Phenyl 3-O-Benzyl-2-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside (from synthesis compound **7**) (2.41 g, 5 mmol) was coevaporated once with anhydrous toluene. The diol was dissolved in DMF (25 mL) and cooled to 0°C. Imidazole (0.35 g, 5.2 mmol) was added followed by TBS-Cl (0.78 g, 5.2 mmol). After 100 minutes the reaction was quenched with MeOH and concentrated. The crude was dissolved in 25 mL pyridine and cooled to 0°C. Ac₂O (1.9 mL) was added and the reaction was stirred for 5 days. The reaction was quenched with EtOH and concentrated. The crude was taken up in EtOAc, washed with 1M HCl and sat. aq. NaCl, dried over MgSO₄ and concentrated. Column purification (hexanes/EtOAc) gave compound **23** in 77% yield (2.45 g, 3.83 mmol). ¹H NMR (CDCl₃, 400 MHz): δ 7.62 – 7.54 (m, 2H, CH_{arom}), 7.38 – 7.18 (m, 10H, CH_{arom}), 6.90 – 6.82 (m, 2H, CH_{arom}), 4.99 (t, 1H, *J* = 9.7, 9.7 Hz, H-4), 4.86 – 4.73 (m, 2H, CHH OBn/OPMB), 4.69 – 4.56 (m, 3H, H-1, CHH OBn/OPMB), 3.78 (s, 3H, CH₃ OMe), 3.73 – 3.59 (m, 3H, H-3, H-6), 3.52 (t, 1H, *J* = 9.6, 9.1 Hz, H-2), 3.44 (ddd, 1H, *J* = 9.9, 4.8, 3.3 Hz, H-5), 1.90 (s, 3H, CH₃ Ac), 0.90 (s, 9H, CH₃ tBu), 0.89 (s, 3H, CH₃ Me), 0.06 (s, 3H, CH₃ Me); ¹³C NMR (CDCl₃, 101 MHz): δ 169.6 (C=O Ac), 159.5, 138.3, 133.9 (C_q), 131.9, 131.8 (CH_{arom}), 130.2 (C_q), 130.0, 129.0, 128.5, 127.9, 127.8, 127.5, 113.9 (CH_{arom}), 87.6 (C-1), 84.3 (C-3), 80.3 (C-2), 79.2 (C-5), 75.5, 75.1 (CH₂ OBn/OPMB), 70.2 (C-4), 62.9 (C-6), 55.3 (CH₃ OMe), 26.0 (CH₃ tBu), 20.9 (CH₃ Ac), 18.4 (C_q tBu), -5.2, -5.4 (CH₃ Me); [M+NH₄]⁺ calculated for C₃₅H₅₀O₇SSiIn 656.30718, found 656.30769.

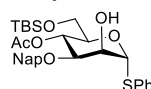
Phenyl 4-O-acetyl-3-O-Benzyl-6-O-tert-butyldimethylsilyl-1-thio-β-D-glucopyranoside (24) Compound **23**

(0.130 g, 0.203 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.033 mL TES was added. The solution was treated with 0.1 mL of a 0.2M HCl/HFIP solution. After 6 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted DCM and

the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (Tol/EtOAc) gave **24** in 48% yield (0.0738 g, 0.142 mmol). TLC: *R_f* 0.33 (PE/EtOAc, 6/1, v/v); [α]_D²⁰ -22.2 (c 1, DCM); IR (neat, cm⁻¹): 734, 1026, 1228, 1741, 2856, 2926, 3288; ¹H NMR (CDCl₃, 400 MHz): δ 7.61 – 7.51 (m, 2H, CH_{arom}), 7.37 – 7.20 (m, 8H, CH_{arom}), 4.93 (t, 1H, *J* = 9.8, 9.8 Hz, H-4), 4.82 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.68 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.50 (d, 1H, *J* = 9.3 Hz, H-1), 3.74 – 3.61 (m, 2H, H-6), 3.60 – 3.43 (m, 3H, H-2, H-3, H-5), 2.46 (s, 1H, 2-OH), 1.96 (s, 3H, CH₃ Ac), 0.90 (s, 9H, CH₃ tBu), 0.07 (s, 3H, CH₃ Me), 0.05 (s, 3H, CH₃ Me); ¹³C NMR (CDCl₃, 100 MHz): δ 169.7 (C=O), 138.4 (C_q), 133.0 (CH_{arom}), 131.6 (C_q), 129.1, 128.6, 128.3, 128.0, 127.9 (CH_{arom}), 88.1 (C-1), 83.3 (C-3), 79.6 (C-5), 74.8 (CH₂ Bn), 72.4 (C-2), 69.9 (C-4), 63.0 (C-6), 26.0 (CH₃ tBu), 21.0 (CH₃ Ac), 18.5 (C_q tBu), -5.1, -5.3 (CH₃ Me); HRMS: [M+Na]⁺ calculated for C₂₇H₃₈O₆SSiNa 541.20506, found 541.20484.

Phenyl 4-O-acetyl-3-O-(2-Naphthylmethyl)-2-O-p-methoxybenzyl-6-O-tert-butyldimethylsilyl-1-thio-α-D-mannopyranoside (25) Phenyl 3-O-(2-Naphthylmethyl)-2-O-p-methoxybenzyl-1-thio-α-D-mannopyranoside (from synthesis compound **21**) (0.37 g, 0.7 mmol) was coevaporated once with anhydrous toluene. The diol was dissolved in DMF (3.5 mL) and cooled to 0°C.

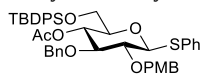
Imidazole (0.05 g, 0.7 mmol) was added followed by TBS-Cl (0.11 g, 0.72 mmol). After 20 minutes the reaction was quenched with MeOH and concentrated. The crude was taken up in Et₂O, washed with H₂O and sat. aq. NaCl, dried over MgSO₄ and concentrated. The compound was dissolved in pyridine (3 mL) and cooled to 0°C, followed by addition of 1 mL Ac₂O. The reaction was stirred overnight after which it was quenched with EtOH. The mixture was concentrated, taken up in EtOAc, washed with 1M HCl, sat. aq. NaHCO₃ and sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography gave compound **25** in 95% yield (0.457 g, 0.66 mmol). ¹H NMR (CDCl₃, 500 MHz): δ 7.75 – 7.70 (m, 3H, CH_{arom}), 7.66 (s, 1H, CH_{arom}), 7.44 – 7.35 (m, 4H, CH_{arom}), 7.21 – 7.08 (m, 6H, CH_{arom}), 6.69 (d, 2H, *J* = 8.5 Hz, CH_{arom}), 5.43 (s, 1H, H-1), 5.28 (t, 1H, *J* = 9.6, 9.6 Hz, H-4), 4.60 (d, 1H, *J* = 12.4 Hz, CHH OPMB/Obn), 4.56 – 4.43 (m, 3H, CHH OPMB/Obn, CH₂ OPMB/Obn), 4.10 (bm, 1H, H-5), 3.91 (s, 1H, H-2), 3.76 – 3.67 (m, 2H, H-3, H-6), 3.63 (m, 4H, CH₃ OMe, H-6), 1.94 (s, 3H, CH₃ Ac), 0.86 – 0.77 (m, 9H, CH₃ tBu), -0.05 (s, 6H, 2x CH₃ Me); ¹³C NMR (CDCl₃, 126 MHz): δ 169.9 (C=O Ac), 159.3, 135.5, 134.3, 133.3, 133.0 (C_q), 131.8 (CH_{arom}), 129.9 (C_q), 129.6, 129.0, 128.2, 128.0, 127.8, 127.5, 126.5, 126.2, 126.0, 125.7, 113.8 (CH_{arom}), 85.9 (C-1), 77.2 (C-3), 75.4 (C-2), 73.3 (C-5), 71.7, 71.7 (CH₂ ONap/OPMB), 68.8 (C-4), 63.3 (C-6), 55.2 (CH₃ OMe), 26.0 (CH₃ tBu), 21.1 (CH₃ Ac), 18.4 (C_q tBu), -5.2, -5.3 (CH₃ Me); [M+NH₄]⁺ calculated for C₃₉H₅₂O₇SSiN 706.32283, found 706.32349.

Phenyl 4-O-acetyl-6-O-tert-butyldimethylsilyl-3-O-(2-Naphthylmethyl)-1-thio-α-D-mannopyranoside (26)

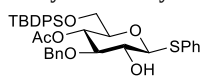
Compound **25** (0.1337 g, 0.194 mmol) was dissolved in 1:1 DCM/HFIP (2 mL) and 0.194 mL TES was added. The solution was treated with 0.095 mL of a 0.2M HCl/HFIP solution. After 3 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted

DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated.

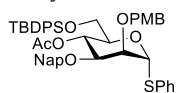
Purification by column chromatography (Tol/EtOAc) gave **26** in 61% yield (0.07 g, 0.123 mmol). TLC: *R_f* 0.48 (PE/EtOAc, 7/1, v/v); [α]_D²⁰ +92.0 (c 1, DCM); IR (neat, cm⁻¹): 740, 777, 835, 1051, 1085, 1228, 1369, 1741, 2854, 2926, 3057, 3640; ¹H NMR (CDCl₃, 500 MHz): 7.83 – 7.77 (m, 3H, CH_{arom}), 7.72 (s, 1H, CH_{arom}), 7.45 – 7.42 (m, 4H, CH_{arom}), 7.38 (dd, 1H, *J* = 8.5, 1.6 Hz, CH_{arom}), 7.24 – 7.19 (m, 3H, CH_{arom}), 5.53 (d, 1H, *J* = 1.7 Hz, H-1), 5.23 (t, 1H, *J* = 9.5, 9.5 Hz, H-4), 4.81 (d, 1H, *J* = 12.2 Hz, CHH Nap), 4.67 (d, 1H, *J* = 12.2 Hz, CHH Nap), 4.27 – 4.22 (m, 1H, H-2), 4.18 (ddd, 1H, *J* = 9.3, 6.2, 2.6 Hz, H-5), 3.79 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3), 3.69 (dd, 1H, *J* = 11.4, 6.2 Hz, H-6), 3.61 (dd, 1H, *J* = 11.4, 2.6 Hz, H-6), 2.74 (s, 1H, 2-OH), 1.96 (s, 3H, CH₃ Ac), 0.82 (s, 9H, CH₃ tBu), -0.03 (s, 3H, CH₃ Me), -0.04 (s, 3H, CH₃ Me); ¹³C NMR (CDCl₃, 125 MHz): δ 170.0 (C=O Ac), 134.9 (C_q), 133.8 (C_q), 133.3 (C_q), 133.2 (C_q), 131.8, 130.5, 129.1, 129.0, 128.6, 128.1, 128.0, 127.9, 127.6, 126.9, 126.5, 126.3, 125.8 (CH_{arom}), 87.2 (C-1), 77.3 (C-3), 72.8 (C-5), 72.0 (CH₂ Nap), 69.6 (C-2), 68.3 (C-4), 63.1 (C-6), 26.0 (CH₃ tBu), 21.1 (CH₃ Ac), 18.5 (C_q tBu), -5.2, -5.3 (CH₃ Me); HRMS: [M+Na]⁺ calculated for C₃₁H₄₀O₆SSiNa 591.22071, found 591.22003.

Phenyl 4-O-acetyl-3-O-Benzyl-2-O-*p*-methoxybenzyl-6-O-*tert*-butyldiphenylsilyl-1-thio- β -D-glucopyranoside (28)

(28) Phenyl 3-O-Benzyl-2-O-*p*-methoxybenzyl-1-thio- β -D-glucopyranoside (from synthesis compound 7) (1.23 g, 2.55 mmol) was coevaporated twice with anhydrous toluene. The diol was dissolved in DMF (13 mL) and cooled to 0°C. Imidazole (0.17 g, 2.55 mmol) was added followed by TBDPS-Cl (0.69 mL, 2.66 mmol). After 15 minutes the icebath was removed and the reaction was stirred overnight. The reaction was quenched with MeOH, concentrated, dissolved in Et₂O and washed twice with H₂O. The organic layer was washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. The crude was dissolved in 15 mL pyridine and cooled to 0°C. Ac₂O (1.2 mL) was added and the reaction was stirred until all starting material was converted in a higher running spot. The reaction was quenched with EtOH and concentrated. The crude was taken up in EtOAc, washed with 1M HCl and sat. aq. NaCl, dried over MgSO₄ and concentrated. Column purification (hexanes/EtOAc) gave compound **27** in 78% yield (1.53 g, 2.00 mmol). ¹H NMR (CDCl₃, 400 MHz): δ 7.75 – 7.64 (m, 5H, CH_{arom}), 7.64 – 7.57 (m, 2H, CH_{arom}), 7.46 – 7.17 (m, 16H, CH_{arom}), 6.92 – 6.83 (m, 2H, CH_{arom}), 5.08 (t, 1H, *J* = 9.6, 9.5 Hz, H-4), 4.80 (m, 2H, CHH OBn/OPMB), 4.70 – 4.57 (m, 3H, H-1, CHH OBn/OPMB), 3.80 (s, 3H, CH₃ OMe), 3.70 (d, 2H, *J* = 3.7 Hz, H-6), 3.65 – 3.50 (m, 2H, H-2, H-3), 3.46 (dt, 1H, *J* = 10.0, 3.7, 3.7 Hz, H-5) 1.75 (s, 3H, CH₃ Ac), 1.06 (s, 9H, CH₃ tBu); ¹³C NMR (CDCl₃, 101 MHz): δ 169.5 (C=O Ac), 159.6, 138.3 (C_q), 135.8, 135.8, 134.9 (CH_{arom}), 133.9, 133.3, 133.2 (C_q), 132.0 (CH_{arom}), 130.3 (C_q), 130.1, 129.8, 129.7, 129.1, 128.6, 128.0, 127.9, 127.8, 127.8, 127.6, 114.0 (CH_{arom}), 87.7 (C-1), 84.4 (C-3), 80.5 (C-5), 79.2 (C-2), 75.5, 75.2 (CH₂ Bn/PMB), 69.8 (C-4), 63.1 (C-6), 55.4 (CH₃ OMe), 26.9 (CH₃ tBu), 20.8 (CH₃ Ac), 19.3 (C_q tBu); [M+NH₄]⁺ calculated for C₄₅H₅₄O₇SiN 780.33848, found 780.33936.

Phenyl 4-O-acetyl-3-O-Benzyl-6-O-*tert*-butyldiphenylsilyl-1-thio- β -D-glucopyranoside (29)

(0.0798 g, 0.104 mmol) was dissolved in 1:1 DCM/HFIP (1 mL) and 0.017 mL TES was added. The solution was treated with 0.05 mL of a 0.2M HCl/HFIP solution. After 18 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (Tol/EtOAc) gave **28** in 90% yield (0.06 g, 0.093 mmol). TLC: R_f 0.37 (PE/EtOAc, 6/1, v/v); [α]_D²⁰ -17.2 (c 1, DCM); IR (neat, cm⁻¹): 740, 1028, 1112, 1228, 1747, 2929, 2954, 3028, 3496; ¹H NMR (CDCl₃, 500 MHz): δ 7.73 – 7.65 (m, 4H, CH_{arom}), 7.62 – 7.56 (m, 2H, CH_{arom}), 7.44 – 7.20 (m, 15H, CH_{arom}), 5.03 (t, 1H, *J* = 9.5, 9.5 Hz, H-4), 4.81 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.67 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.52 (d, 1H, *J* = 9.2 Hz, H-1), 3.73 – 3.68 (m, 2H, H-6), 3.59 – 3.47 (m, 3H, H-2, H-3, H-5), 2.47 (d, 1H, *J* = 1.4 Hz, 2-OH), 1.81 (CH₃ Ac) 1.05 (s, 9H, CH₃ tBu); ¹³C NMR (CDCl₃, 125 MHz): δ 169.5 (C=O Ac), 138.3 (C_q), 135.8, 135.8, 134.9 (CH_{arom}), 133.3 (C_q), 133.2 (CH_{arom}), 133.0 (C_q), 131.7, 129.8, 129.8, 129.2, 128.6 (CH₂), 128.0, 127.9, 127.8, 127.8 (CH_{arom}), 88.2 (C-1), 83.3 (C-3), 79.5 (C-5), 74.7 (CH₂ Bn), 72.5 (C-2), 69.4 (C-4), 63.0 (C-6), 26.8 (CH₃ tBu), 20.9 (CH₃ Ac), 19.3 (C_q tBu); HRMS: [M+Na]⁺ calculated for C₃₇H₄₂O₆SiNa 665.23636, found 665.23572.

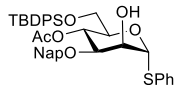
Phenyl 4-O-acetyl-3-O-(2-Naphthylmethyl)-2-O-*p*-methoxybenzyl-6-O-*tert*-butyldiphenylsilyl-1-thio- α -D-mannopyranoside (30)

Compound **21** (0.416 g, 0.6 mmol) was dissolved in MeOH and a catalytic amount of NaOMe was added. After consumption of the starting material in a lower running spot the mixture was neutralized with Amberlite-H⁺ resin, filtered and concentrated. The diol was coevaporated once with anhydrous toluene, dissolved in DMF (5 mL) and cooled to 0°C. Imidazole (0.04 g, 0.6 mmol) was added followed by TBDPS-Cl (0.16 mL, 0.62 mmol). After overnight stirring the reaction was quenched with MeOH and concentrated. The compound was dissolved in pyridine (4 mL) and cooled to 0°C, followed by addition of 2 mL Ac₂O. The reaction was stirred overnight after which it was quenched with EtOH. The mixture was concentrated, taken up in Et₂O, washed with 1M HCl, sat. aq. NaHCO₃ and sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography gave compound **25** in 50% yield (0.25 g, 0.30 mmol). ¹H NMR (CDCl₃, 400 MHz): δ 7.86 – 7.77 (m, 3H, CH_{arom}), 7.74 (s, 1H, CH_{arom}), 7.66 (m, 4H, CH_{arom}), 7.52 – 7.16 (m, 17H, CH_{arom}), 6.80 – 6.73 (m, 2H, CH_{arom}), 5.57 (d, 1H, *J* = 1.8 Hz, H-1), 5.44 (t, 1H, *J* = 9.7, 9.7 Hz, H-4), 4.73 – 4.53 (m, 4H, 2x CH₂ ONap/OPMB), 4.29 – 4.20 (m, 1H, H-5), 4.05 – 3.99 (m, 1H, H-2), 3.85 (dd, 1H, *J* = 11.4, 6.2 Hz, H-6), 3.79 (dd, 1H, *J* = 9.4, 3.0 Hz, H-3), 3.75 – 3.64 (m, 4H, CH₃ OMe, H-6), 1.86 (s, 3H, CH₃ Ac), 1.03 (s, 9H, CH₃ tBu); ¹³C NMR (CDCl₃, 101 MHz): δ 169.7 (C=O), 159.3 (C_q), 135.8, 135.7 (CH_{arom}), 135.5, 134.7, 133.5, 133.4, 133.3,

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133.1 (C_q), 131.3 (CH_{arom}), 129.9 (C_q), 129.7, 129.6, 129.6, 129.1, 128.2, 128.0, 127.8, 127.7, 127.6, 127.4, 126.5, 126.2, 126.0, 125.8, 113.8 (CH_{arom}), 86.0 (C-1), 77.2 (C-3), 75.4 (C-2), 73.3 (C-5), 71.8, 71.6 (CH₂ ONap/OPMB), 68.3 (C-4), 63.5 (C-6), 55.3 (CH₃ OMe), 26.8 (CH₃ tBu), 21.0 (CH₃ Ac), 19.3 (C_q tBu); [M+NH₄]⁺ calculated for C₄₉H₅₆O₇SSiN 830.35413, found 830.35472.

Phenyl 4-O-acetyl-6-O-tert-butylphenylsilyl-3-O-(2-Naphthylmethyl)-1-thio- α -D-mannopyranoside (**31**)



Compound **30** (0.0825 g, 0.101 mmol) was dissolved in 1:1 DCM/HFIP (1 mL) and 0.016 mL TES was added. The solution was treated with 0.05 mL of a 0.2M HCl/HFIP solution. After 11 min the reaction was quenched by addition of sat. aq. NaHCO₃. The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (Tol/EtOAc) gave **30** in 88% yield (0.0614 g, 0.0886 mmol). TLC: R_f 0.23 (PE/EtOAc, 6/1, v/v); [α]_D²⁰ +71.8 (c 1, DCM); IR (neat, cm⁻¹): 740, 821, 1053, 1083, 1228, 1743, 2854, 2927, 3051, 3448; ¹H NMR (CDCl₃, 500 MHz): δ 7.84 (d, 3H, *J* = 7.8 Hz), 7.76 (s, 1H), 7.67 – 7.61 (m, 4H), 7.54 – 7.46 (m, 4H), 7.45 – 7.27 (m, 7H), 7.27 – 7.19 (m, 4H), 5.62 (d, 1H, *J* = 1.6 Hz, H-1), 5.34 (t, 1H, *J* = 9.5, 9.5 Hz, H-4), 4.85 (d, 1H, *J* = 12.2 Hz, CHH Nap), 4.71 (d, 1H, *J* = 12.2 Hz, CHH Nap), 4.31 (s, 1H, H-2), 4.29 – 4.23 (m, 1H, H-5), 3.84 – 3.75 (m, 2H, H-3, H-6), 3.64 (dd, 1H, *J* = 11.5, 2.1 Hz, H-6), 2.74 (s, 1H, 2-OH), 1.85 (s, 3H, CH₃ Ac), 1.01 (s, 9H, CH₃ tBu); ¹³C NMR (CDCl₃, 125 MHz): δ 169.8 (C=O Ac), 135.9, 135.7, 134.8 (C_q), 134.1 (C_q), 133.5 (C_q), 133.3 (C_q), 133.3 (C_q), 133.3 (C_q), 131.4, 129.7, 129.7, 129.2, 128.6, 128.1, 127.9, 127.8, 127.7, 127.5, 127.0, 126.5, 126.3, 125.8 (CH_{arom}), 87.2 (C-1), 77.3 (C-3), 72.8 (C-5), 71.9 (CH₂ Nap), 69.7 (C-2), 67.8 (C-4), 63.3 (C-6), 26.8 (CH₃ tBu), 21.0 (CH₃ Ac), 19.3 (C_q tBu); HRMS: [M+Na]⁺ calculated for HRMS: [M+Na]⁺ calculated for C₄₁H₄₄O₆SSiNa 715.25201, found 715.25149.

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Chapter 3

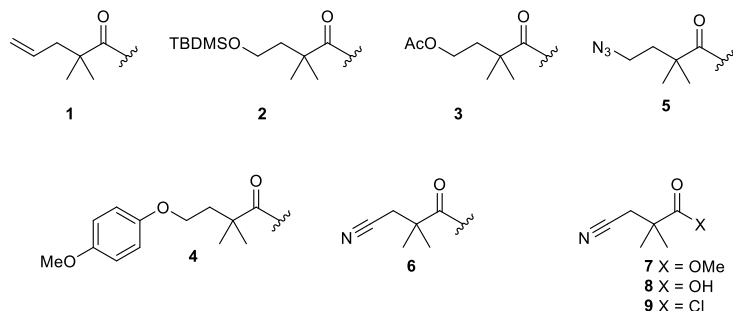
The Cyanopivaloyl Ester: A New Protecting Group in the Assembly of Oligorhamnans

Part of this Chapter has been published: Volbeda, A. G.; Reintjens, N. R. M.; Overkleef, H. S.; van der Marel, G. A.; Codée, J. D. C. *Eur. J. Org. Chem.* **2016**, 2016 (31), 5282–5293.

Introduction

The success of an oligosaccharide or glycoconjugate synthesis campaign hinges on the protecting group strategy followed.^{1,2} Protecting groups are key to discriminate the different hydroxyl and amino groups on the carbohydrate rings and have an important effect on the reactivity of the carbohydrate building block.³⁻⁶ As well, they can be decisive in the stereochemical outcome of a glycosylation. Neighboring group participation by a C-2-O-acyl group is an extremely powerful means to ensure the stereoselective formation of 1,2-trans-glycosidic linkages. Of all ester-type protecting groups, the pivaloyl (Piv) ester is least prone to provide orthoester side products, formed by attack of the nucleophile at the dioxolenium carbon instead of the anomeric center. The neopentyl nature of the pivaloyl dioxolenium ion makes this intermediate significantly less susceptible to nucleophilic attack. Where this steric protection is beneficial during a glycosylation reaction, the bulk of the Piv group can pose a problem during the removal of this protecting group, necessitating harsh nucleophilic conditions for its removal. To enable cleavage of Piv-type esters under less strenuous conditions, various Piv-analogues⁷ have been introduced bearing a masked nucleophile four or five atoms away from the Piv-carbonyl group. Liberating this nucleophile sets the stage for intramolecular attack allowing the smooth deprotection of the Piv ester. Figure 1 depicts some members of the relay-cleavage pivaloyl family.

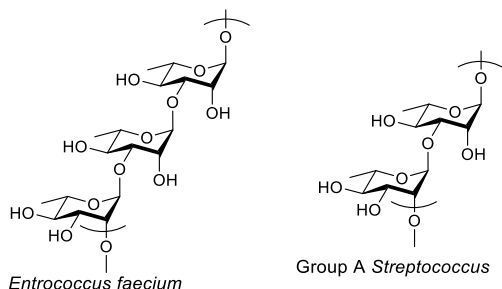
Figure 1. Pivaloyl analogues and the new PivCN.



2,2-Dimethylpentenoate **1**⁸ can be cleaved by hydroboration of the double bond and ensuing base mediated cleavage. Piv-analogues **2**⁹ and **3**^{10,11} bear a distal, masked hydroxyl group that can be liberated by either fluoride or methanolate to release the internal nucleophile. Recently a para-methoxyphenyl based Piv-ester **4**¹² and azido-Piv group **5**¹² were introduced, that can be removed under oxidative (**4**) and reductive (**5**) conditions, respectively. The mutual orthogonality of these Piv-groups was exploited in the assembly a *Streptococcus mutans* oligosaccharide and also the applicability of the azido-Piv group in the solid phase assembly of a β -glucan oligomer was showed.¹³ In this chapter, new relay-cleavage pivaloyl family member is introduced: the cyanopivaloyl (PivCN) group (**6**, Figure 1). This group was developed to streamline the global deprotection of oligosaccharides. It was reasoned that a pivaloyl group that is removable under hydrogenation conditions, commonly employed to remove benzyl esters in the final stage of an oligosaccharide synthesis, could abridge the endgame. As described in this chapter, the

reagents to introduce the PivCN-group, cyanopivalic acid **8**¹⁴ and the corresponding acid chloride (**9**, PivCN-Cl) can be readily synthesized on large scale and the protective group can be easily introduced on carbohydrate building blocks. It is stable under commonly used reaction conditions and can be removed through reduction of the cyano-function. The PivCN group was applied in the assembly of two bacterial rhamnan structures: a tetrasaccharide, representing part of the backbone structure of the exopolysaccharide of Group A *Streptococcus*^{15,16}, and an *Enterococcus faecium*^{17,18} derived hexarhamnoside (figure 2, scheme 2).

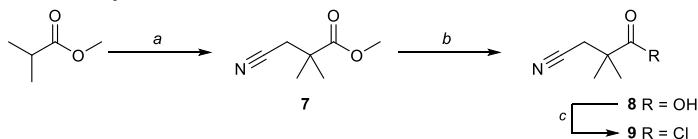
Figure 2. Bacterial rhamnan structures.



Results and Discussion

The synthesis of required reagents, cyanopivalic acid and the corresponding chloride (PivCN-Cl) is depicted in Scheme 1. They can be generated through a three or four step reaction sequence in multigram quantities. Reaction of methyl isobutyrate with bromoacetonitrile under the influence of freshly prepared LDA, resulted in cyanide **7**. Saponification of the methyl ester then provided acid **8**, which can be reacted with oxalylchloride to yield acid chloride **9**. Both reagents can be used without further purification.

Scheme 1. Synthesis of PivCN acid **8** and PivCN chloride **9**.

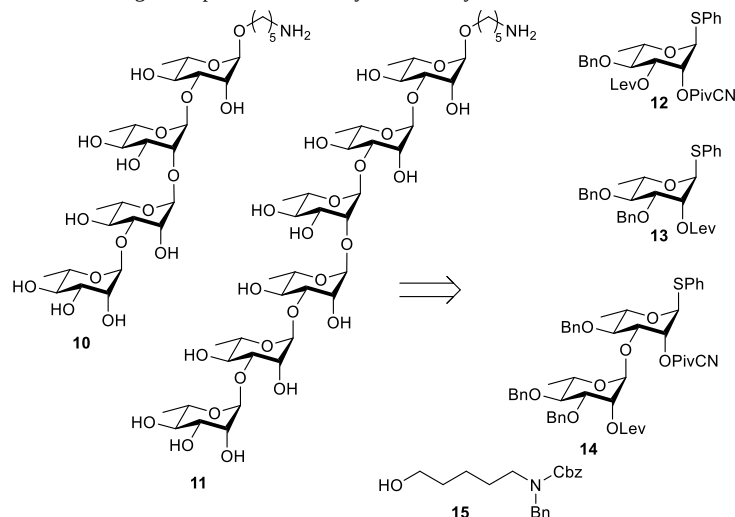


Reagents and conditions: a) i. diisopropylamine, n-BuLi, THF, -78°C, ii. Bromoacetonitrile, THF, -78°C (44% over 2 steps); b) KOH, H₂O/EtOH, 95°C (86%); c) (COCl)₂, DCM, 50°C (100%).

With acid **8** and chloride **9** in hand, the introduction of the PivCN ester and its use as a protecting group in oligosaccharide synthesis was explored. Two bacterial rhamnan structures, the backbone of the exopolysaccharide of Group A *Streptococcus*, and an *Enterococcus faecium* capsular polysaccharide derived hexarhamnoside, were chosen as synthetic targets to validate the performance of the PivCN group in oligosaccharide

assembly.^{19–26} The *Enterococcus faecium* capsular polysaccharide is composed of trisaccharide repeating units featuring α -(1,3)- and α -(1,2)-rhamnosyl linkages, whereas the Group A *Streptococcus* repeating unit is a dirhamnoside with α -(1,3) and α -(1,2)-linkages (figure 2). The target structures for the current study are depicted in Scheme 2.

Scheme 2. Target compounds and retrosynthetic analysis.

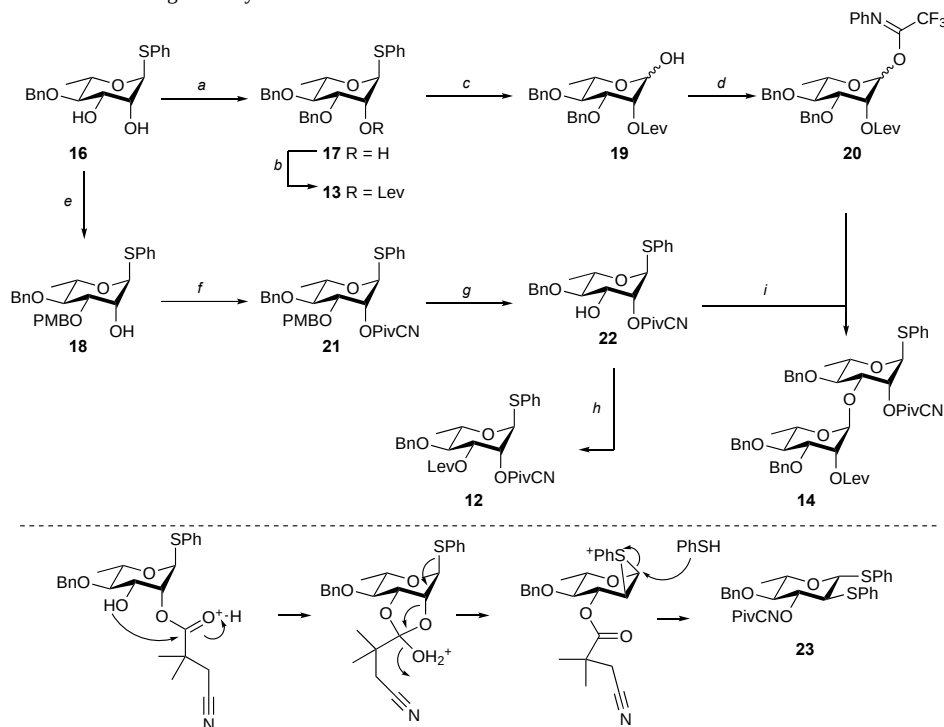


Tetrasaccharide **10** and hexasaccharide **11** each contain two repeating units of the respective capsular polysaccharides. It was envisaged that these two target structures could be assembled from building blocks **12**, **13** and **14**, which are equipped with a levulinoyl group, that serves as a temporary protecting group, to be removed to allow elongation of the rhamnan chains, and/or a PivCN group, which serves as a permanent participating protecting group. Linker **15**²⁷ is used to cap the reducing end of the target rhamnans and can serve as a handle for future conjugation chemistries.

The assembly of the building blocks is depicted in Scheme 3. Known diol **16**²⁸ can be selectively alkylated on the C-3-OH through the intermediacy of the cyclic tin ketal,²⁹ with either benzylbromide or para-methoxybenzylchloride to give rhamnosides **17** and **18** in 86% and 73% yield respectively. Esterification of the C-2-hydroxyl group in **17** with levulinic acid yields donor **13** in excellent yield (96%). Thioglycoside **13** can be converted into the corresponding hemiacetal **19** by *N*-bromosuccinimide driven hydrolysis (78%). Installation of the *N*-phenyltrifluoroacetimidate group³⁰ on the anomeric alcohol then delivers **20** in high yield (99%). para-Methoxybenzyl (PMB) protected rhamnoside **18** is treated with acid chloride **9** and pyridine at elevated temperature to provide fully protected compound **21** in 72% yield. Under standard esterification conditions, applying the PivCN acid **8** in concert with *N,N'*-di-iso-propylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP), compound **21** was obtained in good yield (74%). To remove the PMB ether, deprotection conditions using trifluoroacetic acid and a thiol scavenger were explored.¹²

Besides the desired alcohol **22**, these conditions also provided deoxy-L-glucoside **23**, bearing the PivCN group at its C-3-OH and a C-2-thiophenol group, as a prominent side product. This product is likely the result of the mechanism depicted in Scheme 3. Under the acidic conditions used, the PivCN ester can be protonated and subsequently attacked by the neighboring C-3-alcohol. Migration of the PivCN ester to the C-3 position can be caused by the participating anomeric thiophenol moiety to provide an intermediate episulfonium ion. This is attacked at the anomeric center to provide deoxy-L-glucoside **23**.^{31–33}

Scheme 3. Building block synthesis.



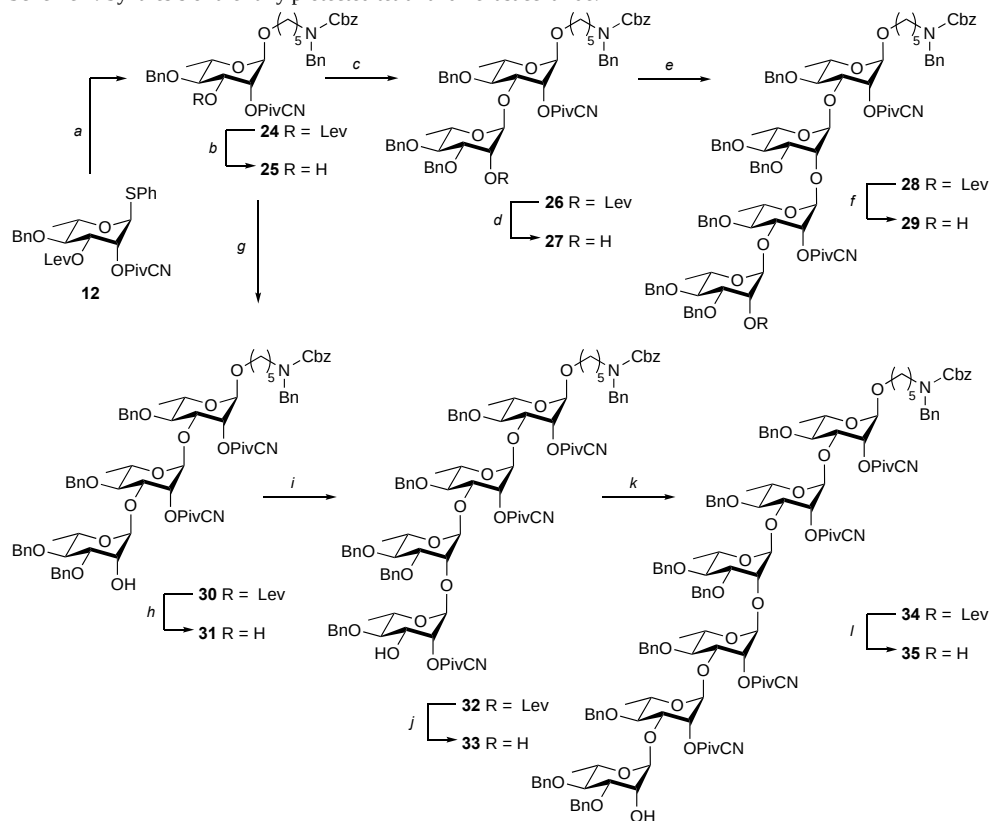
Reagents and conditions: a) i. Bu_2SnO , toluene, 140°C , ii. BnBr , CsF , DMF (86%); b) LevOH , DIC , DMAP , DCM , 0°C (96%); c) NBS , $\text{acetone}/\text{H}_2\text{O}$ (78%); d) ClC(=NPh)CF_3 , Cs_2CO_3 , acetone (99%); e) i. Bu_2SnO , toluene, 140°C , ii. PMBCl , CsF , DMF (73%); f) **8**, DIC , DMAP , DCM (74%) or **9**, 100°C , pyridine (72%); g) TFA , PhSH , DCM , 0°C (73%) or HCl/HFIP , TES , DCM/HFIP , 0°C (76%); h) LevOH , DIC , DMAP , DCM , 0°C (99%); i) TfOH , DCM , 0°C (93%).

As shown in Chapter 2, a catalytic amount of HCl in 2,2,2,3,3-hexafluoro-iso-propanol (HFIP) could be used to cleave electron rich benzyl ethers in a mild and fast manner.³⁴ Application of these conditions proved effective here to remove the C-3-O-PMB from rhamnoside **21**. The reaction time had to be carefully controlled as a prolonged reaction time led to activation of the anomeric thiophenol function. The liberated alcohol in compound **22** was masked with a levulinoyl group to provide building block **12**. Rhamnoside **22** was also used to generate disaccharide building block **14**. To this end, N-phenyltrifluoroacetimidate rhamnoside **20** and thiorhamnoside acceptor **22** were combined

in a chemoselective glycosylation reaction to provide the dirhamnoside **14** in high yield (93%) and with complete stereoselectivity.

Having assembled the required building blocks, the two target oligosaccharides were assembled (Scheme 4). First, the linker-functionalized rhamnoside **24** was generated in a stereoselective, NIS/TMSOTf-mediated glycosylation³⁵ of donor **12** with protected aminohexanol **15**.

Scheme 4. Synthesis of the fully protected tetra- and hexasaccharide.



Reagents and conditions: a) **12**, NIS, TMSOTf, DCM, 0°C (82%); b) $\text{H}_2\text{NNH}_2\cdot\text{AcOH}$, pyridine/AcOH (73%); c) **20**, NIS, TMSOTf, DCM, 0°C (93%); d) $\text{H}_2\text{NNH}_2\cdot\text{AcOH}$, pyridine/AcOH (90%); e) **14**, NIS, TMSOTf, DCM, 0°C (94%); f) $\text{H}_2\text{NNH}_2\cdot\text{AcOH}$, pyridine/AcOH (90%); g) **14**, NIS, TMSOTf, DCM, 0°C (52%); h) $\text{H}_2\text{NNH}_2\cdot\text{AcOH}$, pyridine/AcOH (78%); i) **12**, NIS, TMSOTf, DCM, -40°C (75%); j) $\text{H}_2\text{NNH}_2\cdot\text{AcOH}$, pyridine/AcOH (67%); k) **14**, NIS, TMSOTf, DCM, -40°C (88%); l) $\text{H}_2\text{NNH}_2\cdot\text{AcOH}$, pyridine/AcOH (96%)

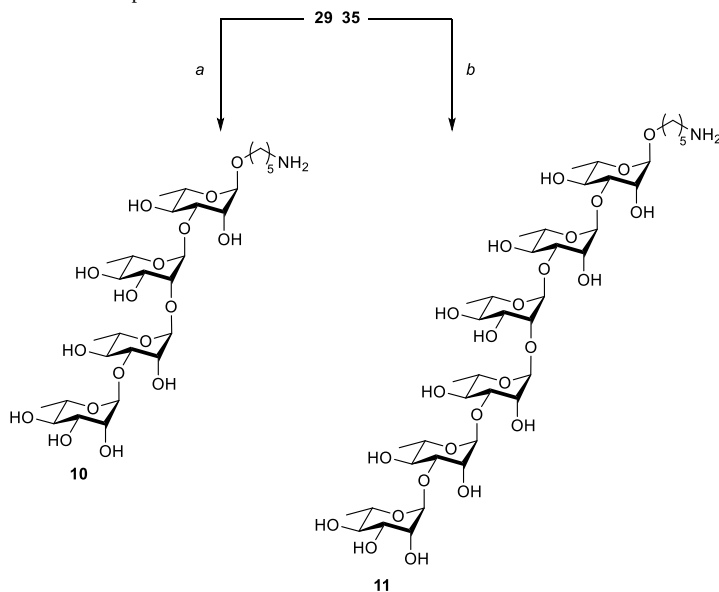
Next the levulinoyl ester was removed to provide the monosaccharide acceptor building block **25**. Surprisingly, the deprotection of the levulinoyl group required optimization. Whereas the Lev group was readily removed from compound **12** in 78% yield, applying the same conditions on rhamnoside **24** resulted in a ~1:1 mixture of **25** and **25a**, in which the PivCN group migrated to the 3-O position. The modified Piv group does seem to have the

tendency to migrate, which could be elaborated to the remote effect of the CN group. When an excess of hydrazine acetate was used, the levulinoyl group was removed in 73% yield. This monosaccharide was elongated with monorhamnoside **13** to give dimer **26** in 93% yield. Liberation of the C-2''-OH by treatment of **26** with hydrazine set the stage for the next glycosylation. In this condensation, the dimers **14** and **27** were united to provide the fully protected target tetrasaccharide **28** in high yield.

Next the assembly of the protected hexarhamnan **34** was undertaken. To this end linker bearing monorhamnose **25** and dirhamnoside **14** were coupled under the agency of NIS/TMSOTf to provide trisaccharide **30** in 52% yield. Delevulinuylation of **30** was followed by a glycosylation with monorhamnose donor **12** to provide the tetrasaccharide **32** in 75% yield. Removal of the Lev-ester then paved the way for the final glycosylation in which tetramer **33** was elongated with dirhamnoside **14** giving the fully protected hexarhamnan in 88% yield.

With the two fragments in hands, global deprotection was commenced. First the levulinoyl groups were removed. Next all benzyl ethers and cyano groups were reduced by hydrogenolysis using $\text{Pd}(\text{OH})_2/\text{C}$.

Scheme 5. Deprotection of the tetra- and hexasaccharide.



Reagents and conditions: a) i. $\text{Pd}(\text{OH})_2/\text{C}$, AcOH, H_2 , $\text{H}_2\text{O}/\text{THF}/t\text{BuOH}$, ii. Et_3N , H_2O (100%); b) i. $\text{Pd}(\text{OH})_2/\text{C}$, AcOH, H_2 , $\text{H}_2\text{O}/\text{THF}/t\text{BuOH}$, ii. Et_3N , H_2O (80%);

The reduction was achieved in two stages, the first of which was executed in $\text{THF}/t\text{BuOH}/\text{H}_2\text{O}/\text{AcOH}$ solvent system. After filtration the partially deprotected hexasaccharide was taken up in water and subjected to a second hydrogen event. During the

Chapter 3

hydrogenation AcOH was added to prevent deactivation of the catalyst. Reduction of the cyano-groups released the primary amines of the Piv-like esters, which under the reaction conditions used are protonated. To affect ring closure, release the 2,2-dimethyl- γ -butyrolactam and complete the removal of the PivCN esters the crude products were treated with triethylamine in water. The release of the 2,2-dimethyl- γ -butyrolactam could be easily followed by NMR analysis using the characteristic triplets of the liberated lactam. After completion of the reaction, the crude tetra- and hexasaccharide were purified by size exclusion chromatography to provide Group A *Streptococcus* tetra- α -D-glucopyranoside **10** and *Enterococcus faecium* hexa- α -D-glucopyranoside **11** in 100% and 80% respectively.

Conclusion

The introduction of the cyanopivaloyl ester as a novel hydroxyl protecting group is described. It features a cyano moiety appended two atoms away from the ester carbonyl to allow for a relay-cleavage of the pivaloyl type ester. This cleavage mechanism alleviated one of the major drawbacks of the pivaloyl ester, that is, its difficult removal.

The applicability of the novel protecting group is shown in the assembly of two bacterial oligosaccharides. It represents a robust protecting group that tolerates many functional group manipulations and withstands both (Lewis) acidic as well as mild basic conditions. It is a new member of the family of pivaloyl-type protecting groups and may be used in combination with other pivaloyl type esters, such as the recently introduced azidopivaloyl group, as a (semi)-orthogonal pair for the streamlined synthesis of carbohydrates and other complex (bio)molecules.

Experimental Section

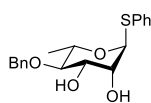
General experimental procedures. All chemicals were used as received unless stated otherwise. ^1H and ^{13}C NMR spectra were recorded on a 400/100 MHz, 500/125 MHz, 600/150 MHz or a 850/214 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All individual signals were assigned using 2D-NMR spectroscopy, HH-COSY, HSQC and HMBC. IR spectra are reported in cm^{-1} . Flash chromatography was performed on silica gel 60 (0.04 – 0.063 mm). TLC-analysis was followed by detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at $\sim 150^\circ\text{C}$ or by spraying with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g/l) and $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot\text{H}_2\text{O}$ (10 g/l) in 10% sulfuric acid in water followed by charring at 50°C . LC-MS standard eluents used were A: 100% H_2O , B: 100% acetonitrile, C: 1% TFA in H_2O . The column used was a C18 column (4.6 mmD $\times$ 50 mmL, 3μ particle size). All analyses were 13 min, with a flow-rate of 1 ml/min. High-resolution mass spectra were recorded on a LTQ-Orbitrap equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275°C) with resolution $R=60.000$ at $m/z=400$ (mass range = 150–4000) and dioctylphthalate ($m/z=391.28428$) as "lock mass". High resolution mass measurements were performed on a Synapt G2-Si MALDI-TOF mass spectrometer equipped with a 355-nm laser. 1 μL samples were spotted on the MALDI-plate, followed by applying 1 μL of the matrix solution (2,5-dihydroxybenzoic acid 100 mg/mL dissolved in H_2O : ACN : dimethylaniline 1 : 1 : 0.02). A laser frequency of 1000 Hz (power set at 60%) was used.

Methyl 3-cyano-2,2-dimethylpropanoate (7) A solution of LDA was prepared by adding *n*-butyllithium in hexanes (1.6 M, 134.8 mL, 215.7 mmol, 1 eq.) drop wise to a solution of diisopropylamine (33.4 mL, 238 mmol, 1.1 eq.) in THF (310 mL) at -78°C under argon. After 45 min no more bubbles appeared and methyl isobutyrate (28.2 mL, 246 mmol, 1.13 eq.) was added. After 1h of stirring at -78°C , bromoacetonitrile (19.5 mL, 280 mmol, 1.26 eq.) was added. The resulting dark mixture was allowed to warm up gradually to room temperature and stirred overnight. The reaction was neutralized by the addition of 1M HCl (90 mL) and 2M HCl (240 mL) at 0°C . The mixture was diluted with sat. aq. NaCl, followed by extraction with Et_2O (3x). The combined organic layers were washed with sat. aq. NaHCO_3 (6x), H_2O (6x) and sat. aq. NaCl (1x), dried over MgSO_4 , and concentrated *in vacuo*. Purification by column chromatography (19:1 PE/EtOAc to 4:1 PE/EtOAc) and coevaporation with CHCl_3 resulted in the title compound as a light yellow oil (13.45 g, 95.3 mmol). Analytical data are identical to literature precedence.³⁶

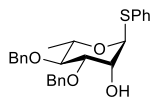
3-cyano-2,2-dimethylpropanoic acid (8) A suspension was formed by the addition of H_2O (205 mL) and EtOH (35 mL) to compound 7 (13.45 g, 95.3 mmol, 1 eq.). Lithium hydroxide (98%) (5.85 g, 244 mmol, 2.5 eq.) was added and the mixture was refluxed at 95°C for 5.5h. The mixture was cooled to $^\circ\text{C}$, quenched with 1M HCl to pH = 1, diluted with H_2O and extracted with EtOAc (2x). The combined organic layers were washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. Coevaporation with DCM and CHCl_3 resulted in an oil that crystallized out as a light yellow solid (10.41 g, 81.89 mmol, 33% in two steps). IR (neat): 750, 974, 1231, 1412, 1585, 1699, 2264, 2544, 2884, 3192, 3368 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 11.12 (bs, 1H, OH), 2.63 (s, 2H, CH_2), 1.42 (s, 6H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ : 181.5 (C=O), 117.4 (CN), 41.0 (Cq), 27.8 (CH_2), 24.7 (CH_3). HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_6\text{H}_{10}\text{NO}_2$ 128.07061, found 128.07055.

3-cyano-2,2-dimethylpropanoyl chloride (9) After coevaporation with anhydrous toluene, acid 8 (5.53 g, 43.5 mmol, 1 eq.) was dissolved in DCM (110 mL). Oxalylchloride (8.4 mL, 97.8 mmol, 2.2 eq.) was added at room temperature and the solution was refluxed at 40°C for 40 min allowing the gases produced during the reaction to be stripped by a stream of argon, after which the water cooling was closed and the DCM was allowed to evaporate at 50°C for 2h. The reaction mixture was cooled and concentrated *in vacuo*. The obtained oil was used directly without further purification. ^1H NMR (400 MHz, CDCl_3) δ : 2.70 (s, 2H, CH_2), 1.49 (s, 6H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ : 177.6 (C=O), 116.3 (CN), 50.6 (Cq), 27.8 (CH_2), 24.5 (CH_3).

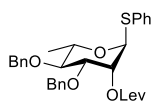
Phenyl 4-O-benzyl-1-thio- α -L-rhamnopyranoside (16) A solution of 80% acetic acid (950 mL) was added to phenyl 4-O-benzyl-2,3-di-O-isopropylidene-1-thio- α -L-rhamnopyranoside²⁸ (73.8 g, 191 mmol) and heated to 70°C and stirred overnight after which TLC analysis showed complete consumption of the starting material. The reaction was cooled down to 0°C, neutralized with Et₃N, diluted with H₂O and extracted with EtOAc (2x). The combined organic layers were washed with sat. aq. NaHCO₃ (5x) and sat. aq. NaCl (1x), dried over MgSO₄ and concentrated *in vacuo*. Crystallization (EtOH) at -20°C resulted in the title compound as a white solid (58.3 g, 168.3 mmol, 88%). TLC: R_f 0.51 (2:1 PE/EtOAc). Analytical data are identical to literature precedence.²⁸



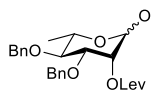
Phenyl 3,4-di-O-benzyl-1-thio- α -L-rhamnopyranoside (17) Diol **16**²⁹ (3.47 g, 10.0 mmol, 1 eq.) was coevaporated with anhydrous toluene two times under argon and dissolved in anhydrous toluene (100 mL). Dibutyltin oxide (3.00 g, 12.1 mmol, 1.2 eq.) was added and the white suspension was heated to 105°C. The reaction was stirred overnight after which the clear solution was cooled down and concentrated *in vacuo*. After three times coevaporation with anhydrous toluene under argon, the oil was dissolved in DMF (100 mL). BnBr (1.6 mL, 13.5 mmol, 1.3 eq.) and CsF (3.05 g, 20.1 mmol, 2 eq.) were added. After 6h, TLC-MS and TLC analysis showed complete reaction and the reaction mixture was diluted with EtOAc, washed with H₂O (2x), sat. aq. NaCl (2x), dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (PE to 2:1 PE/EtOAc) yielded the title compound as a colorless oil (3.76 g, 8.62 mmol, 86%). R_f = 0.77 (2:1 PE/EtOAc). Analytical data are identical to literature precedence.²⁹



Phenyl 3,4-di-O-benzyl-2-O-levulinoyl-1-thio- α -L-rhamnopyranoside (13) After coevaporation with anhydrous toluene (2x) under argon, compound **17** (9.79 g, 22.5 mmol, 1 eq.) was dissolved in freshly distilled DCM and cooled to 0°C. Levulinic acid (6.38 mL, 62.9 mmol, 2.8 eq.), N,N'-diisopropylcarbodiimide (4.92 mL, 31.4 mmol, 1.4 eq.) and 4-dimethylaminopyridine (0.31 g, 2.54 mmol, 0.1 eq.) were added at 0°C. After 1.5h the reaction was allowed to warm up to room temperature and stirred overnight. After TLC-MS showed complete consumption of the starting material, the mixture was filtered over Celite and the filtrate was washed with sat. aq. NaHCO₃ (2x) and sat. aq. NaCl (1x). The organic layer was dried over MgSO₄, concentrated *in vacuo* and coevaporated with anhydrous toluene. Purification by column chromatography (3:1 PE/EtOAc) and coevaporation with CHCl₃ (2x) resulted in the title compound as a yellow oil (11.50 g, 21.51 mmol, 96%). TLC: R_f 0.38 (7:2 PE/EtOAc); IR (neat): 689, 733, 873, 835, 903, 1022, 1051, 1098, 1132, 1198, 1300, 1358, 1439, 1472, 1728, 2968 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 7.44-7.26 (m, 15H, CH_{arom}), 5.59 (m, 1H, H-2), 5.41 (d, 1H, *J* = 1.2 Hz, H-1), 4.93 (d, 1H, *J* = 10.8 Hz, CH₂ Bn), 4.70 (d, 1H, *J* = 11.2 Hz, CH₂ Bn), 4.63 (d, 1H, *J* = 10.8 Hz, CH₂ Bn), 4.54 (d, 1H, *J* = 11.2 Hz, CH₂ Bn), 4.22 (dq, 1H, *J* = 9.4, 6.2 Hz, H-5), 3.90 (dd, 1H, *J* = 9.3, 3.3 Hz, H-3), 3.48 (t, 1H, *J* = 9.4 Hz, H-4), 2.76-2.69 (m, 4H, 2x CH₂ Lev), 2.16 (s, 3H, CH₃ Lev), 1.33 (d, 3H, *J* = 6.2 Hz, CH₃-6); ¹³C NMR (100 MHz, CDCl₃) δ : 206.3 (C=O Lev ketone), 172.1 (C=O Lev), 138.5, 137.9, 134.0 (Cq), 131.9, 129.2, 128.5, 128.5, 128.3, 128.2, 128.0, 127.9, 127.8 (CH_{arom}), 86.1 (C-1), 80.3 (C-4), 78.4 (C-3), 75.6 (CH₂ Bn), 71.8 (CH₂ Bn), 70.9 (C-2), 69.1 (C-5), 38.1 (CH₂ Lev), 30.0 (CH₃ Lev), 28.3 (CH₂ Lev), 18.0 (CH₃-6); HRMS: [M+NH₄]⁺ calcd. for C₃₁H₃₈NO₆ 552.24144, found 552.24165.

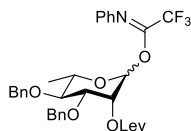


3,4-di-O-benzyl-2-O-levulinoyl- α / β -L-rhamnopyranoside (19) Compound **13** (1.07 g, 2.00 mmol, 1 eq.) was dissolved in acetone/water 3:1 (10 mL) and cooled to 0°C. N-bromosuccinimide (1.08 g, 6.07 mmol, 3 eq.) was added and the mixture was stirred at 0°C. The reaction was allowed to warm up to room temperature after 100 min and stirred overnight. The reaction was quenched by addition of sat. aq. Na₂S₂O₃, diluted with EtOAc and the organic layer was washed with sat. aq. NaHCO₃ (2x), H₂O (1x) and sat. aq. NaCl (1x). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (3:1 to 1:1 PE/EtOAc) yielded hemiacetal **19** (0.692 g, 1.56 mmol, 78%). TLC: R_f 0.53 (1:1 PE/EtOAc); IR (neat): 696, 735, 837, 912, 988, 1028, 1042, 1063, 1155, 1207, 1362, 1717, 1738 cm⁻¹; NMR assignment for the major isomer: ¹H NMR (400 MHz, CDCl₃) δ : 7.33-7.2 (m, 10H, CH_{arom}), 5.25 (m, 1H, H-2), 5.03 (s, 1H, H-1), 4.91-87 (m, 1H, CH₂ Bn), 4.72-4.58 (m, 2H, CH₂ Bn), 4.51-4.45 (m, 1H, CH₂ Bn), 4.00-3.93 (m, 2H, H-3, H-5), 3.40 (t, 1H, *J* = 9.2 Hz, H-4), 2.76-2.60 (m, 4H, 2x CH₂ Lev), 2.11 (s, 3H, CH₃ Lev), 1.27 (d, 3H, *J* = 6.2 Hz, CH₃-6); ¹³C NMR (100 MHz, CDCl₃) δ : 206.9 (C=O Lev



ketone), 172.1 (C=O Lev), 138.3, 137.9 (C_q C_{arom}), 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.7, 127.6, 127.6 (CH_{arom}), 91.9 (C-1), 80.0 (C-4), 77.4 (C-3), 75.2 (CH₂ Bn), 71.4 (CH₂ Bn), 69.6 (C-2), 67.3 (C-5), 37.9 (CH₂ Lev), 29.7 (CH₃ Lev), 28.0 (CH₂ Lev), 18.0 (CH₃-6). HRMS: [M+ NH₄]⁺ calcd. for C₂₅H₃₄NO₇ 460.23298, found 460.23299.

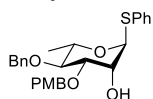
3,4-di-O-benzyl-2-O-levulinoyl-1-(N-[phenyl]-trifluoroacetimidoyl)-α/β-L-rhamnopyranoside (20) To a



solution of compound **19** (0.65 g, 1.47 mmol, 1 eq.) in acetone (7.4 mL), were added ClC(=NPh)CF₃ (0.27 mL, 1.78 mmol, 1.2 eq.) and Cs₂CO₃ (0.72 g, 2.21 mmol, 1.5 eq.) at 0°C. The reaction was allowed to warm up to room temperature after 40 min and stirred for an additional 20 min. TLC analysis showed complete consumption of starting material. The reaction was diluted with EtOAc and the organic layer was washed with

H₂O (2x) and sat. aq. NaCl (1x). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (7:1 PE/EtOAc to EtOAc) yielded the title compound in a 4:1 α/β-ratio (0.894 g, 1.456 mmol, 99%). TLC: R_f 0.54 (4:1 PE/EtOAc); IR (neat): 696, 735, 839, 920, 943, 985, 1028, 1072, 1119, 1153, 1207, 1312, 1364, 1454, 1717, 1740, 2922 cm⁻¹; NMR assignment for the major isomer: ¹H NMR (400 MHz, CDCl₃) δ: 7.35-7.23 (m, 12H, CH_{arom}), 7.09-7.06 (m, 1H, CH_{arom}), 6.86-6.80 (m, 2H, CH_{arom}), 6.17 (bs, 1H, H-1), 5.49 (s, 1H, H-2), 4.92 (d, 1H, J = 10.8 Hz, CH₂ Bn), 4.71 (d, 1H, J = 11.2 Hz, CH₂ Bn), 4.63 (d, 1H, J = 10.8, CH₂ Bn), 4.55 (d, 1H, J = 11.2 Hz, CH₂ Bn), 3.98 (dd, 1H, J = 9.2, 2.8 Hz, H-3), 3.90 (m, 1H, H-5), 3.50 (t, 1H, J = 9.2 Hz, H-4), 2.75-2.63 (m, 4H, 2x CH₂ Lev), 2.08 (s, 3H, CH₃ Lev), 1.36 (d, 1H, J = 6.0 Hz, CH₃-6); ¹³C NMR (100 MHz, CDCl₃) δ: 205.8 (C=O Lev ketone), 171.6 (C=O Lev), 143.2, 138.0, 137.5 (C_q C_{arom}), 128.7, 128.3, 128.3, 128.2, 128.0, 128.0, 127.8, 127.8, 124.4, 199.3 (CH_{arom}), 94.2 (C-1), 79.1 (C-4), 77.2 (C-3), 75.5 (CH₂ Bn), 71.9 (CH₂ Bn), 70.4 (C-5), 67.6 (C-2), 37.7 (CH₂ Lev), 29.6 (CH₃ Lev), 27.9 (CH₂ Lev), 17.9 (CH₃-6). TLC-MS: m/z = 636.35 (M+Na⁺).

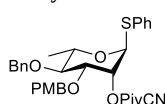
Phenyl 4-O-benzyl-3-O-p-methoxybenzyl-1-thio-α-L-rhamnopyranoside (18) Diol **16** (6.94 g, 20.0 mmol, 1



eq.) was coevaporated with anhydrous toluene two times under argon and dissolved in anhydrous toluene (200 mL). Dibutyltin oxide (5.99 g, 24.1 mmol, 1.2 eq.) was added to the mixture, which was stirred overnight at 105°C. The clear solution was cooled to 0 °C, concentrated *in vacuo* and two times coevaporated with anhydrous toluene under argon. The

oil was dissolved in DMF (200 mL), followed by the addition of *p*-methoxybenzyl chloride (3.1 mL, 22.9 mmol, 1.12 eq.) and CsF (6.08 g, 40.0 mmol, 2 eq.). After heating the mixture to 80 °C for 5h, TLC analysis showed complete reaction, and the reaction mixture was cooled to 0 °C, diluted with H₂O and extracted two times with EtOAc. The combined organic layers were washed with H₂O (2x) and sat. aq. NaCl (2x). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (PE to 2:1 PE/EtOAc) and (5:1 PE/EtOAc to 2:1 PE/EtOAc) resulted in the title compound as a clear oil (6.83 g, 14.6 mmol, 73%). TLC: R_f 0.75 (2:1 PE/EtOAc). Analytical data are identical to literature precedence.²⁹

Phenyl



4-O-benzyl-3-O-p-methoxybenzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-1-thio-α-L-rhamnopyranoside (21)

Method A: Crude chloride **9** (5.53 g, 43.5 mmol, 2 eq.) was cooled to 0°C and dissolved in pyridine (20 mL). A solution of compound **18** (10.14 g, 21.7 mmol, 1 eq.) in pyridine (2x 20 mL) was added to the crude chloride. After an addition of another 50 mL pyridine, the

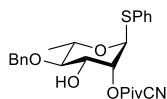
resulting dard mixture was heated to 110°C. After 1h, TLC and TLC-MS analysis showed complete reaction and the mixture was cooled to 0°C. The reaction mixture was diluted with EtOAc and washed with H₂O (1x), 1M HCl (2x) and sat. aq. NaCl (2x). The organic layer was dried over MgSO₄, concentrated *in vacuo* and coevaporated with toluene. Purification by flash column chromatography (8:1 PE/EtOAc → 3:1 PE/EtOAc) and (10:1 PE/EtOAc → EtOAc) and coevaporation with CHCl₃ resulted in the title compound as an oil (9.04 g, 15.7 mmol, 72%), which contained 5% byproduct.

Method B: Acid **8** (3.49 g, 27.5 mmol, 2 eq.) and compound **18** (6.41 g, 13.75 mmol, 1 eq.) were coevaporated twice with anhydrous toluene after which they were dissolved in dry DCM (35 mL). The solution was cooled to 0°C and DIC (2.37 mL, 15.13 mmol, 1.1 eq.) and DMAP (0.17 g, 1.37 mmol, 0.1 eq.) were added. The reaction was allowed to stir overnight, after which TLC analysis showed conversion of the starting material in a higher running spot. Filtration over Celite followed by washing with sat. aq. NaHCO₃, the organic layer was dried over

Chapter 3

MgSO₄ and concentrated. Purification by flash column chromatography (PE/EtOAc 1:0 → 4:1) yielded the fully protected rhamnopyranoside as a yellow oil (5.86 g, 10.2 mmol, 74%). TLC: R_f 0.68 (3:1 PE/EtOAc); IR (neat): 691, 739, 820, 1030, 1084, 1098, 1136, 1246, 1300, 1454, 1472, 1515, 1612, 1734, 2874, 2974 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 7.46-7.43 (m, 2H, CH_{arom}), 7.32-7.22 (m, 10H, CH_{arom}), 6.87-6.84 (m, 2H, CH_{arom}), 5.62 (m, 1H, H-2), 5.37 (d, 1H, *J* = 1.2 Hz, H-1), 4.90 (d, 1H, *J* = 10.8 Hz, CH₂ Bn), 4.64 (d, 1H, *J* = 10.8 Hz, CH₂ Bn), 4.60 (d, 1H, *J* = 10.8 Hz, CH₂ PMB), 4.47 (d, 1H, *J* = 10.4 Hz, CH₂ PMB), 4.24 (dq, 1H, *J* = 9.4, 6.2 Hz, H-5), 3.90 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3), 3.80 (s, 3H, OCH₃ PMB), 3.42 (t, 1H, *J* = 9.2 Hz, H-4), 2.56 (s, 2H, CH₂ PivCN), 1.36 (s, 6H, 2x CH₃ PivCN), 1.32 (d, 3H, *J* = 6.0 Hz, H-6); ¹³C NMR (100 MHz, CDCl₃) δ: 174.1 (C=O PivCN), 159.5, 138.3, 133.7 (C_q C_{arom}), 132.1, 132.1 130.0, 129.8 (CH_{arom}), 129.3 (C_q C_{arom}), 128.5, 128.3, 128.0, 127.9, 127.8 (CH_{arom}), 117.6 (CN), 114.0, 113.9 (CH_{arom}), 86.0 (C-1), 79.9 (C-4), 78.1 (C-3), 75.5 (CH₂ Bn), 71.6 (CH₂ PMB), 71.4 (C-2), 69.1 (C-5), 55.4 (CH₃ PMB), 41.2 (C_q PivCN), 28.1 (CH₂ PivCN), 24.9, 24.8 (CH₃ PivCN), 18.0 (CH₃-6); HRMS: [M+NH₄]⁺ calcd. for C₃₃H₄₁N₂O₆S 593.26798, found 593.26838.

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

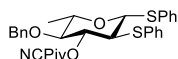


Method A: To a solution of compound **21** (8.47 g, 14.72 mmol, 1 eq.) in DCM (75 mL), thiophenol (1.65 mL, 16.12 mmol, 1.1 eq.) was added and the mixture was cooled down to 0 °C, after which trifluoroacetic acid (7.5 mL) was added. The reaction mixture was stirred for 3h, after which TLC-MS analysis showed complete consumption of the starting material.

The mixture was diluted with Et₂O, washed with sat. aq. NaHCO₃ (4x), H₂O (1x) and sat. aq. NaCl (1x). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (7:1 PE/EtOAc to 3:1 PE/EtOAc) and coevaporation with DCM and CHCl₃ resulted in the title compound as a clear oil (5.38 g, 10.8 mmol, 73%).

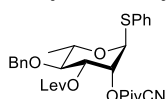
Method B: Compound **21** (3.085 g, 5.35 mmol) is dissolved in 27 mL DCM and 27 mL HFIP. The mixture was cooled to 0 °C and TES (2.56 mL, 16.05 mmol, 3 eq.) was added. 26.8 mL of a freshly prepared HCl/HFIP solution (0.2 M, 1 eq.) was added and the reaction was stirred for 7 minutes after which it was quenched with sat. aq. NaHCO₃. Purification by column chromatography (PE/EtOAc 1:0 → 8:1) yielded **22** as a colorless oil (0.08 g, 0.175 mmol, 76%). TLC: R_f 0.38 (7:2 PE/EtOAc); IR (neat): 691, 739, 772, 847, 968, 1024, 1078, 1136, 1198, 1298, 1471, 1732, 2880, 2974, 3435 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 7.48-7.45 (m, 2H, CH_{arom}), 7.36-7.27 (m, 8H, CH_{arom}), 5.40 (m, 1H, H-2), 5.37 (d, 1H, *J* = 1.2 Hz, H-1), 4.79 (d, 2H, *J* = 4.4 Hz, CH₂ Bn), 4.27 (dq, 1H, *J* = 9.4, 6.2 Hz, H-5), 4.09 (dd, 1H, *J* = 9.2, 2.8 Hz, H-3), 3.44 (t, 1H, *J* = 9.2 Hz, H-4), 2.59 (d, 2H, *J* = 4.8 Hz, CH₂ PivCN), 2.05 (bs, 1H, OH), 1.39 (s, 6H, 2x CH₃ PivCN), 1.38 (s, 3H, CH₃-6). ¹³C NMR (100 MHz, CDCl₃) δ: 174.3 (C=O PivCN), 138.0, 133.7 (C_q C_{arom}), 132.1, 129.2, 128.7, 128.3, 128.2, 127.9 (CH_{arom}), 118.0 (CN), 85.8 (C-1), 81.5 (C-4), 75.3 (CH₂ Bn), 74.9 (C-2), 70.8 (C-3), 68.9 (C-5), 41.3 PivCN), 28.0 (CH₂ PivCN), 25.0, 25.0 (CH₃ PivCN), 18.1 (CH₃-6); HRMS: [M+Na]⁺ calcd. for C₂₅H₂₉NO₅Na 478.16586, found 478.16531.

Phenyl 4-O-benzyl-3-O-(3-cyano-2,2-dimethylpropanoyl)-1-thio-2-thiophenyl-α-L-rhamnopyranoside (23)



TLC: R_f 0.61 (7:1 PE/EtOAc); IR (neat): 689, 733, 783, 1020, 1051, 1096, 1132, 1198, 1300, 1439, 1472, 1582, 1728, 2847, 2968 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 7.67-7.61 (m, 2H, CH_{arom}), 7.47-7.42 (m, 2H, CH_{arom}), 7.35-7.23 (m, 11H, CH_{arom}), 5.28 (dd, 1H, *J* = 10.7, 8.2 Hz, H-3), 4.61 (s, 2H, CH₂ Bn), 4.35 (d, 1H, *J* = 10.6 Hz, H-1), 3.35-3.25 (m, 2H, H-4, H-5), 3.03 (t, 1H, *J* = 10.7 Hz, H-2), 2.54 (d, 2H, *J* = 8.7 Hz, CH₂ PivCN), 1.42 (s, 6H, 2x CH₃ PivCN), 1.29 (d, 3H, *J* = 5.6 Hz, CH₃-6); ¹³C NMR (100 MHz, CDCl₃) δ: 174.2 (C=O PivCN), 137.5 (C_q C_{arom}), 135.0 (CH_{arom}), 132.7 (C_q C_{arom}), 132.5 (CH_{arom}), 130.1 (C_q C_{arom}), 129.2, 129.0, 128.9, 128.6, 128.1, 127.9, 127.5 (CH_{arom}), 117.6 (CN), 86.5 (C-1), 83.0 (C-4), 75.7 (C-3), 75.3 (C-5), 74.8 (CH₂ Bn), 52.1 (C-2), 41.0 (C_q PivCN), 28.0 (CH₂ PivCN), 25.0, 24.8 (CH₃ PivCN), 18.3 (CH₃-6); HRMS: [M+NH₄]⁺ calcd. for C₃₁H₃₇N₂O₄S₂ 565.21893, found 565.21935.

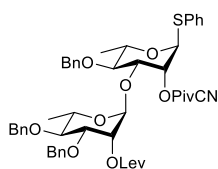
Phenyl 4-O-benzyl-3-O-levulinoyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-1-thio-α-L-rhamnopyranoside (12)



After coevaporation of compound **22** (2.47 g, 5.42 mmol, 1 eq.) with anhydrous toluene (2x) under argon, it was dissolved in distilled DCM (13.5 mL) and cooled to 0°C. Levulinic acid (1.54 mL, 15.2 mmol, 2.8 eq.), *N,N'*-diisopropylcarbodiimide (1.2 mL, 7.7 mmol, 1.4 eq.) and a catalytic amount of 4-dimethylaminopyridine (0.066 g, 0.54 mmol, 0.1 eq.) were added. After 30 min, TLC-MS analysis showed complete reaction and the mixture was filtered over Celite. The filtrate was washed with sat. aq. NaHCO₃ (2x), dried over MgSO₄ and concentrated *in vacuo*. Purification by

column chromatography (2:1 PE/EtOAc) and coevaporation with DCM and CHCl_3 resulted in the title compound as an oil (2.97 g, 5.36 mmol, 99%). TLC: R_f 0.59 (2:1 PE/EtOAc); IR (neat): 692, 741, 912, 1084, 1099, 1132, 1150, 1204, 1300, 1362, 1474, 1717, 1740, 2976 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.46-7.43 (m, 2H, CH_{arom}), 7.37-7.24 (m, 8H, CH_{arom}), 5.54 (m, 1H, H-2), 5.36 (d, 1H, $J = 1.2$ Hz, H-1), 5.31 (dd, 1H, $J = 9.6, 3.2$ Hz, H-3), 4.78 (d, 1H, $J = 11.2$ Hz, CH_2 Bn), 4.34 (dq, 1H, $J = 9.4, 6.0$ Hz, H-5), 3.64 (t, 1H, $J = 9.6$ Hz, H-4), 2.77-2.64 (m, 2H, CH_2 Lev), 2.59 (d, 2H, $J = 1.6$ Hz, CH_2 PivCN), 2.56-2.44 (m, 2H, CH_2 Lev), 2.14 (s, 3H, CH_3 Lev), 1.39 (s, 6H, 2x CH_3 PivCN), 1.36 (s, 3H, CH_3 -6); ^{13}C NMR (100 MHz, CDCl_3) δ : 206.0 (C=O Lev ketone), 173.6, 171.7 (C=O PivCN, Lev), 137.7, 133.1 (C_q C_{arom}), 131.9, 129.0, 128.9, 128.3, 128.0, 127.8 (CH_{arom}), 117.4 (CN), 85.2 (C-1), 78.1 (C-4), 74.8 (CH_2 Bn), 72.2, 72.2 (C-2, C-3), 68.9 (C-5), 41.0 (C_q PivCN), 27.5 (CH_2 Lev), 29.5 (CH_3 Lev), 27.7, 27.6 (CH_2 Lev, CH_2 PivCN), 24.7, 24.6 (CH_3 PivCN), 17.8 (CH_3 -6); HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{30}\text{H}_{35}\text{NO}_7\text{SNa}$ 576.20264, found 576.20209.

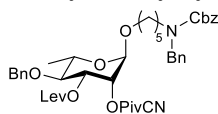
Phenyl



3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-1-thio- α -L-rhamnopyranoside (14) Imidate donor **20** (0.353 g, 0.575 mmol, 1.2 eq.) and acceptor **22** (0.219 g, 0.481 mmol, 1 eq.) were coevaporated two times with anhydrous toluene under an argon atmosphere before being dissolved in distilled DCM (4.8 mL) and the mixture was stirred at room temperature for 15 min over activated molecular sieves ($3\text{\AA}$). The reaction was cooled to 0°C and triflic acid (4.5 μL , 0.051 mmol, 0.1 eq.) was added. After 20 min the reaction was quenched by addition of 0.1 mL Et_3N . The reaction mixture was diluted with Et_2O and washed with

H_2O (2x) and sat. aq. NaCl (2x). The organic layer was dried over MgSO_4 and concentrated *in vacuo*. Purification by size exclusion (1:1 DCM/MeOH) resulted in the title compound as a yellow oil (0.394 g, 0.448 mmol, 93%). TLC: R_f 0.64 (7:2 PE/EtOAc); IR (neat): 700, 731, 845, 922, 989, 1028, 1082, 1117, 1136, 1204, 1364, 1452, 1717, 1740, 2930, 2974 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.45-7.20 (m, 20H, CH_{arom}), 5.40 (m, 1H, H-2'), 5.31 (s, 2H, H-1, H-2), 5.07 (s, 1H, H-1'), 4.93 (d, 1H, $J = 11.2$ Hz, CH_2 Bn), 4.79 (d, 1H, $J = 10.8$ Hz, CH_2 Bn), 4.65-4.59 (m, 3H, CH_2 Bn), 4.49 (d, 1H, $J = 12$ Hz, CH_2 Bn), 4.22 (q, 1H, $J = 6.0$ Hz, H-5), 4.12 (dd, 1H, $J = 9.6, 2.4$ Hz, H-3), 3.80 (dd, 1H, $J = 9.2, 3.2$ Hz, H-3'), 3.60 (q, 1H, $J = 6.0$ Hz, H-5'), 3.53 (t, 1H, $J = 9.6$ Hz, H-4), 3.42 (t, 1H, $J = 9.2$ Hz, H-4'), 2.70-2.63 (m, 4H, 2x CH_2 Lev), 2.45 (q, 2H, $J = 14.0$ Hz, CH_2 PivCN), 2.13 (s, 3H, CH_3 Lev), 1.29 (m, 12H, CH_3 -6, CH_3 -6', 2x CH_3 PivCN); ^{13}C NMR (100 MHz, CDCl_3) δ : 206.0 (C=O Lev ketone), 173.9, 171.7 (C=O PivCN, Lev), 138.5, 137.8, 137.6, 133.2 (C_q C_{arom}), 132.1, 131.9, 129.1, 128.8, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5 (CH_{arom}), 117.2 (CN), 99.7 (C-1'), 85.3 (C-1), 80.2 (C-4), 79.4 (C-4'), 77.05 (C-3), 76.8 (C-3'), 75.5 (CH_2 Bn), 74.9 (CH_2 Bn), 74.6 (C-2), 71.1 (CH_2 Bn), 69.2 (C-5), 68.9 (C-2'), 68.5 (C-5'), 40.9 (C_q PivCN), 37.9 (CH_2 Lev), 29.7 (CH_3 Lev), 28.0 (CH_2 Lev), 27.6 (CH_2 PivCN), 24.7, 24.6 (CH_3 PivCN), 17.8, 17.8 (C-6, C-6'); HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{50}\text{H}_{57}\text{NO}_{11}\text{SNa}$ 902.35445, found 902.35458.

N-benzyl-N-benzylloxycarbonyl-5-aminopentanyl-4-O-benzyl-3-O-levulinoyl-2-O-(3-cyano-2,2-

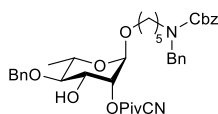


dimethylpropanoyl)- α -L-rhamnopyranoside (24) Donor **12** (0.843 g, 1.52 mmol, 1 eq.) and acceptor **15** (1.552 g, 4.74 mmol, 3 eq.) were coevaporated three times with anhydrous toluene before being dissolved in distilled DCM (17 mL) under an argon atmosphere. Activated molecular sieves ($3\text{\AA}$) were added and the mixture was cooled to 0°C . The mixture was stirred for 15 min, followed by the addition of NIS (0.42 g, 1.9 mmol, 1.2 eq.) and a solution of TMSOTf in distilled DCM (0.221 M, 0.68 mL, 0.15 mmol, 0.1 eq.) were added. After 1h, TLC analysis showed fast conversion of the donor and the mixture was allowed to warm to room temperature for 30 min, after which it was quenched with Et_3N . The mixture was diluted with Et_2O and washed with H_2O (2x) and sat. aq. NaCl (2x). The organic layer was dried over MgSO_4 and concentrated *in vacuo*. Purification by size exclusion (1:1 DCM/MeOH) and coevaporation with CHCl_3 resulted in the title compound as a yellow oil (0.957 g, 1.24 mmol, 82%). TLC: R_f 0.35 (2:1 PE/EtOAc); IR (neat): 696, 733, 912, 976, 1028, 1063, 1126, 1207, 1300, 1360, 1694, 1738 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.36-7.24 (m, 14H, CH_{arom}), 7.18-7.16 (m, 1H, CH_{arom}), 5.30 (dd, 1H, $J = 9.6, 3.2$ Hz, H-3), 5.24 (s, 1H, H-2), 5.17 (d, 2H, $J = 13.2$ Hz, CH_2 Cbz), 4.72 (d, 1H, $J = 11.2$ Hz, CH_2 Bn), 4.65-4.63 (m, 2H, CH_2 Bn, H-1), 4.49-4.48 (m, 2H, CH_2 Bn), 3.80 (m, 1H, H-5), 3.60-3.55 (m, 1H, CH_2), 3.50 (t, 1H, $J = 9.6$ Hz, H-4), 3.34 (m, 1H, CH_2), 3.26 (m, 1H, CH_2), 3.20 (m, 1H, CH_2), 2.76-2.64 (m, 2H, CH_2 Lev), 2.61 (d, 2H, $J = 1.2$ Hz, CH_2 PivCN), 2.53-2.46 (m, 2H, CH_2 Lev) 2.14 (s, 3H, CH_3 Lev), 1.54- 1.48

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(m, 4H, 2x CH₂), 1.40 (s, 6H, 2x CH₃ PivCN), 1.35 (d, 3H, *J* = 12.8 Hz, CH₃-6), 1.26 (m, 2H, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ: 206.3 (C=O Lev ketone), 173.9, 171.8 (C=O PivCN, Lev), 137.9, 137.8 (C_q), 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.2 (CH_{arom}), 117.5 (CN), 97.0 (C-1), 78.3 (C-4), 75.0 (CH₂ Bn), 72.2 (C-3), 71.0 (C-2), 67.7 (CH₂), 67.5 (C-5), 67.1 (CH₂ Cbz), 50.5, 50.2 (CH₂ Bn), 47.1, 46.1 (CH₂), 41.2 (C_q PivCN), 37.8 (CH₂ Lev), 29.8 (CH₃ Lev), 29.0 (CH₂), 27.9 (CH₂ PivCN, Lev), 27.5 (CH₂), 24.9, 24.9 (CH₃ PivCN), 23.3 (CH₂), 18.1 (CH₃-6). HRMS: [M+Na]⁺ calcd. for C₄₄H₅₄N₂O₁₀Na 793.36707, found 793.36653.

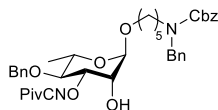
N-benzyl-*N*-benzyloxycarbonyl-5-aminopentanyl-4-*O*-benzyl-2-*O*-(3-cyano-2,2-dimethylpropanoyl)-α-*L*-



rhamnopyranoside (25) Compound **24** (0.212 g, 0.275 mmol, 1 eq.) was dissolved in pyridine (2.2 mL) and AcOH (0.55 mL). Hydrazine acetate (0.130 g, 1.41 mmol, 5 eq.) was added and the mixture was stirred for 30 min, after which TLC analysis showed complete reaction. The reaction mixture was quenched with acetone and diluted with EtOAc. The organic layer were washed with H₂O (3x) and sat. aq. NaCl

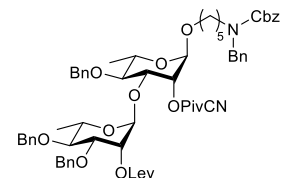
(1x), dried over MgSO₄, concentrated *in vacuo*. Purification by column chromatography (2:1 PE/EtOAc) and coevaporation with CHCl₃ resulted in the title compound as an oil (0.135 g, 0.200 mmol, 73%). TLC: R_f 0.52 (2:1 PE/EtOAc); IR (neat): 696, 734, 976, 1028, 1061, 1128, 1227, 1300, 1368, 1422, 1454, 1472, 1694, 1734, 2934 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 7.35-7.24 (m, 14H, CH_{arom}), 7.17-7.16 (m, 1H, CH_{arom}), 5.16 (d, 2H, *J* = 12.4 Hz, CH₂ Cbz), 5.09 (s, 1H, H-2), 4.82 (d, 1H, *J* = 11.2 Hz, CH₂ Bn), 4.69 (d, 1H, *J* = 11.2 Hz, CH₂ Bn), 4.64 (s, 1H, H-1), 4.48 (d, 2H, *J* = 6.8 Hz, CH₂ Bn), 4.07 (bs, 1H, H-3), 3.70 (m, 1H, H-5), 3.57 (m, 1H, CH₂), 3.32 (t, 1H, *J* = 6.4 Hz, H-4), 3.31 (m, 1H, CH₂), 3.30-3.18 (m, 2H, CH₂), 2.58 (d, 2H, *J* = 6.0 Hz, CH₂ PivCN), 1.54-1.48 (m, 4H, 2x CH₂), 1.39 (s, 6H, 2x CH₃ PivCN), 1.33 (d, 3H, *J* = 6.0 Hz, CH₃-6), 1.26 (m, 2H, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ: 174.5 (C=O PivCN), 138.1, 137.9 (C_q Carom), 128.6, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.3, 127.2 (CH_{arom}), 117.9 (CN), 97.1 (C-1), 81.3 (C-4), 75.2 (CH₂ Bn), 73.5 (C-2), 70.2 (C-3), 67.7 (CH₂), 67.5 (C-5), 67.2 (CH₂ Cbz), 50.5, 50.3 (CH₂ Bn), 47.1, 46.1 (CH₂), 41.2 (C_q PivCN), 29.7, 29.1 (CH₂), 28.0 (CH₂ PivCN), 27.5 (CH₂), 25.0, 24.9 (CH₃ PivCN), 23.4 (CH₂), 18.2 (CH₃-6); HRMS: [M+Na]⁺ calcd. for C₃₉H₄₈N₂O₈Na 695.33358, found 695.32958.

N-benzyl-*N*-benzyloxycarbonyl-5-aminopentanyl-4-*O*-benzyl-3-*O*-(3-cyano-2,2-dimethylpropanoyl)-α-*L*-



rhamnopyranoside (25a) TLC: R_f 0.22 (2:1 PE/EtOAc). ¹H NMR (400 MHz, CDCl₃) δ: 7.36-7.18 (m, 14H, CH_{arom}), 6.99 (m, 1H, CH_{arom}), 5.24 (dd, 1H, *J* = 14.8, 12.4 Hz, H-3), 5.17 (d, 2H, *J* = 12.4 Hz, CH₂ Cbz), 4.70-4.63 (m, 3H, H-1, CH₂ Bn), 4.50 (s, 2H, CH₂ Bn), 4.06 (s, 1H, H-2), 3.80 (bs, 1H, H-5), 3.64-4.59 (m, 2H, H-4, CH₂), 3.40-3.15 (m, 3H, 2x CH₂), 2.53 (d, 2H, *J* = 5.6 Hz, CH₂ PivCN), 1.60-1.43 (m, 4H, 2x CH₂), 1.34-1.28 (m, 11H, 2x CH₃ PivCN, CH₃-6, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ: 174.0 (C=O PivCN), 138.0 (C_q), 128.7, 128.6, 128.0, 127.5, 127.4 (CH_{arom}), 118.4 (CN), 99.6 (C-1), 79.0 (C-4), 75.5 (C-3), 75.0 (CH₂ Bn), 69.5 (C-2), 67.7 (CH₂), 67.7 (C-5), 67.3 (CH₂ Cbz), 50.7, 50.4 (CH₂ Bn), 47.2, 46.3 (CH₂), 41.4 (C_q PivCN), 29.8, 29.2 (CH₂), 28.3 (CH₂ PivCN), 25.1, 24.9 (CH₃ PivCN), 23.5 (CH₂), 18.2 (CH₃-6). HRMS: [M+H]⁺ calcd for C₃₉H₄₉N₂O₈ 673.34834, found 673.34927.

N-benzyl-*N*-benzyloxycarbonyl-5-aminopentanyl-3-*O*-(3,4-di-*O*-benzyl-2-*O*-levulinoyl)-α-*L*-

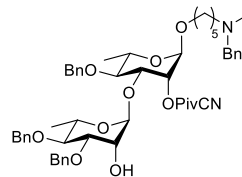


rhamnopyranosyl)-4-*O*-benzyl-2-*O*-(3-cyano-2,2-dimethylpropanoyl)-α-*L*-rhamnopyranoside (26)

Acceptor **25** (0.358 g, 0.533 mmol, 1 eq.) and donor **13** (0.352 g, 0.658 mmol, 1.2 eq.) were coevaporated three times with anhydrous toluene before being dissolved in distilled DCM (7.6 mL) under an argon atmosphere and stirred at room temperature for 20 min over activated molecular sieves (3Å). The reaction was cooled to 0°C, followed by the addition of NIS (0.178 g, 0.791 mmol, 1.44 eq.) and a solution of TMSOTf in distilled DCM (0.221 M, 0.24 mL, 0.053 mmol, 0.1 eq.) were added. After 40 min, TLC and TLC-MS showed complete consumption of the acceptor and the reaction was quenched with 0.1 mL Et₃N. The mixture was diluted with Et₂O and the organic layer was washed with H₂O (2x) and sat. aq. NaCl (2x), dried over MgSO₄ and concentrated *in vacuo*. Purification by size exclusion (1:1 DCM/MeOH) resulted in the title compound as a yellow oil (0.544 g, 0.496 mmol, 93%). TLC: R_f 0.64 (7:2 PE/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ: 7.36-7.20 (m, 24H, CH_{arom}), 7.15 (m, 1H, CH_{arom}), 5.39 (m, 1H, H-2'), 5.15 (d, 2H, *J* = 13.2 Hz, CH₂ Cbz), 5.04 (s, 2H, H-1', H-2),

4.93 (d, 1H, $J = 11.6$ Hz, CH₂ Bn), 4.77 (d, 1H, $J = 10.8$ Hz, CH₂ Bn), 4.64-4.56 (m, 4H, H-1, 2x CH₂ Bn), 4.49-4.46 (m, 3H, CH₂ Bn), 4.11 (d, 1H, $J = 8.8$ Hz, H-3), 3.81 (dd, 1H, $J = 9.2, 3.2$ Hz, H-3'), 3.74 (m, 1H, H-5), 3.68-3.52 (m, 2H, H-5', CH₂), 3.44-3.38 (m, 2H, H-4, H-4'), 3.33-3.18 (m, 3H, CH₂), 2.71-2.63 (m, 4H, 2x CH₂ Lev), 2.48 (q, 2H, $J = 6.8$ Hz, CH₂ PivCN), 2.11 (s, 3H, CH₃ Lev), 1.53-1.43 (m, 4H, CH₂), 1.31-1.27 (m, 14H, CH₃-6, CH₃-6', 2x CH₃ PivCN, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ : 205.9 (C=O Lev ketone), 174.0, 171.6 (C=O PivCN, Lev), 156.6 (C=O Cbz), 138.5, 137.8, 137.7, 137.6, 136.7 (C_q C_{arom}), 128.9, 128.8, 128.4, 128.4, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.1 (CH_{arom}), 117.2 (CN), 99.6 (C-1'), 96.4 (C-1), 80.1 (C-4), 79.4 (C-4'), 77.4 (C-3), 76.7 (C-3'), 75.5, 74.7 (CH₂ Bn), 73.1 (C-2), 71.0 (CH₂ Bn), 68.9 (C-2'), 68.2 (C-5'), 67.5 (C-5), 67.5 (CH₂), 66.9 (CH₂ Cbz), 50.4, 50.1 (CH₂ Bn), 46.9, 46.0 (CH₂), 40.8 (C_q PivCN), 37.8 (CH₂ Lev), 29.6 (CH₃ Lev), 28.9 (CH₂), 28.0, 27.8, 27.5, 27.3 (CH₂ PivCN, CH₂ Lev, CH₂), 24.7, 24.5 (CH₃ PivCN), 23.2 (CH₂), 17.9, 17.7 (CH₃-6, CH₃-6'). HRMS: [M+NH₄]⁺ calcd. for C₆₄H₈₀N₃O₁₄ 1114.56348, found 1114.56494.

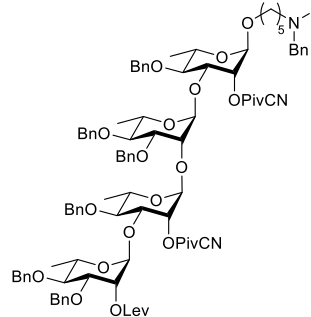
N-benzyl-N-benzyloxycarbonyl-5-aminopentanyl-3-O-(3,4-di-O-benzyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranoside (27)



Compound **26** (0.526 g, 0.479 mmol, 1 eq.) was dissolved in pyridine (3.8 mL), cooled to 0°C and AcOH (0.96 mL) was added, followed by the addition of hydrazine acetate (0.228 g, 2.48 mmol, 5 eq.). The mixture was allowed to warm up to room temperature and stirred for 1h. The reaction mixture was quenched with acetone and diluted with EtOAc. The organic layer were washed with H₂O (3x) and sat. aq. NaCl (1x), dried over MgSO₄, concentrated *in vacuo*.

Purification by column chromatography (2:1 PE/EtOAc) and coevaporation with DCM and CHCl₃ resulted in the title compound as an oil (0.433 g, 0.433 mmol, 90%). TLC: R_f 0.64 (7:2 PE/EtOAc); IR (neat): 696, 733, 837, 914, 1028, 1072, 1126, 1209, 1248, 1300, 1366, 1422, 1452, 1472, 1695, 1734, 2872, 2932, 2972 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 7.36-7.20 (m, 24H, CH_{arom}), 7.15 (m, 1H, CH_{arom}), 5.16 (d, 2H, $J = 12.8$ Hz, CH₂ Cbz), 5.07 (s, 2H, H-1', H-2), 4.88 (d, 1H, $J = 11.6$ Hz, CH₂ Bn), 4.69-4.57 (m, 6H, H-1, 3x CH₂ Bn), 4.48 (d, 2H, $J = 8.4$ Hz, CH₂ Bn), 4.11 (m, 1H, H-3), 3.91 (s, 1H, H-2'), 3.71-3.68 (m, 2H, H-3', H-5), 3.60-3.54 (m, 2H, H-5, CH₂), 3.49-3.38 (m, 2H, H-4, H-4'), 3.37-3.31 (m, 3H, CH₂), 2.64 (bs, 1H, OH), 2.49 (q, 2H, $J = 14.8$ Hz, CH₂ PivCN), 1.53 - 1.22 (m, 16H); ¹³C NMR (100 MHz, CDCl₃) δ : 174.0 (C=O PivCN), 138.6, 137.8, 136.8 (C_q C_{arom}), 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.2 (CH_{arom}), 117.3 (CN), 101.6 (C-1'), 96.6 (C-1), 80.3 (C-4), 79.6 (C-4'), 79.0 (C-3'), 77.3 (C-3), 75.5 (CH₂ Bn), 74.8 (CH₂ Bn), 73.3 (C-2), 71.6 (CH₂ Bn), 68.6 (C-2'), 68.0 (C-5'), 67.7 (CH₂), 67.6 (C-5), 67.1 (CH₂ Cbz), 50.5, 50.2 (CH₂ Bn), 47.0, 46.0 (CH₂), 40.9 (C_q PivCN), 28.9 (CH₂), 27.7 (CH₂ PivCN), 27.4 (CH₂), 24.8, 24.7 (CH₃ PivCN), 23.2 (CH₂), 18.0, 17.7 (CH₃-6, CH₃-6'). HRMS: [M+NH₄]⁺ calcd. for C₅₉H₇₄N₃O₁₂ 1016.52670, found 1016.52807.

N-benzyl-N-benzyloxycarbonyl-5-aminopentanyl-3-O-(2-O-(3-O-(3,4-di-O-benzyl-2-O-levulinoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-3,4-di-O-benzyl- α -L-rhamnopyranoside (28)



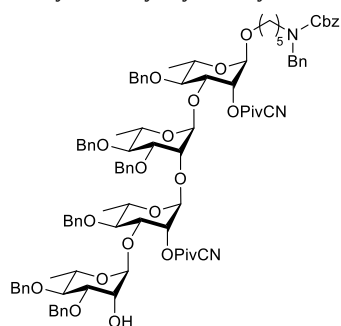
Acceptor **27** (0.191 g, 0.193 mmol, 0.8 eq.) and donor **14** (0.220 g, 0.250 mmol, 1 eq.) were coevaporated three times with anhydrous toluene before being dissolved in distilled DCM (3.6 mL) under an argon atmosphere and stirred at room temperature for 20 min over activated molecular sieves (3Å). The reaction was cooled to 0°C, followed by the addition of NIS (0.0703 g, 0.312 mmol, 1.2 eq.) and a solution of TMSOTf in distilled DCM (0.221 M, 0.12 mL, 0.026 mmol, 0.1 eq.) were added. After 50 min, TLC and TLC-MS showed complete consumption of the acceptor and the reaction

was quenched with 0.1 mL Et₃N. The mixture was diluted with Et₂O and the organic layer was washed with H₂O (1x) and sat. aq. NaCl (2x), dried over MgSO₄ and concentrated *in vacuo*. Purification by size exclusion (1:1 DCM/MeOH) resulted in the title compound as a yellow oil (0.320 g, 0.181 mmol, 94%). TLC: R_f 0.5 (2:1 PE/EtOAc); IR (neat): 696, 733, 893, 914, 980, 1072, 1128, 1206, 1362, 1452, 1697, 1736, 2932 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ : 7.34-7.15 (m, 40H, CH_{arom}), 5.41 (s, 1H, H-2'''), 5.23 (s, 1H, H-2''), 5.16 (d, 2H, $J = 9.6$

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Hz, CH₂ Cbz), 5.07 (s, 1H, H-1'''), 5.0 (s, 1H, H-2), 4.97 (s, 1H, H-1'), 4.96-4.86 (m, 2H, CH₂ Bn), 4.84 (s, 1H, H-1''), 4.75 (d, 1H, *J* = 10.8 Hz, CH₂ Bn), 4.65-4.56 (m, 8H, H-1, 4x CH₂ Bn), 4.54-4.44 (m, 4H, 2x CH₂ Bn), 4.18 (dd, 1H, *J* = 9.6, 3.2 Hz, H-3''), 4.02 (d, 1H, *J* = 9.2 Hz, H-3), 3.83-3.77 (m, 3H, H-2', H-3''', H-5*), 3.72-3.68 (m, 3H, H-3', H-5, H-5), 3.60-3.46 (m, 1H, CH₂), 3.45-3.32 (m, 5H, H-4, H-4', H-4'', H-4''', H-5), 3.31-3.14 (m, 3H, CH₂), 2.71-2.63 (m, 4H, 2x CH₂ Lev), 2.55-2.35 (m, 4H, 2x CH₂ PivCN), 2.14 (s, 3H, CH₃ Lev), 1.58 – 1.16 (m, 28H); ¹³C NMR (100 MHz, CDCl₃): δ 206.1 (C=O Lev ketone), 174.2, 173.8, 171.8 (C=O 2x PivCN, Lev), 138.7, 138.6, 138.2, 138.0, 137.9 (C_q A_{arom}), 128.8, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.3 (CH_{arom}), 117.4 (CN), 101.4 (C-1'), 99.4 (C-1'''), 98.5 (C-1''), 96.5 (C-1), 80.3, 80.0, 79.9, 79.6 (C-4, C-4', C-4'', C-4'''), 78.6 (C-3'), 78.2 (C-3), 77.4, 77.1 (C-2', C-3'''), 75.8 (C-3''), 75.6, 75.5, 75.0, 75.0 (CH₂ Bn), 73.4 (C-2), 73.0 (C-2''), 72.0, 71.2 (CH₂ Bn), 69.0 (C-2'''), 68.7, 68.5, 68.4 (C-5), 67.8 (CH₂), 67.6 (C-5), 67.1 (CH₂ Cbz), 50.6, 50.3 (CH₂ Bn), 47.1, 46.1 (CH₂), 40.9 (C_q PivCN), 38.0 (CH₂ Lev), 29.9 (CH₃ Lev), 29.0, 28.1, 27.9, 27.8, 27.7, 27.5 (2x CH₂ PivCN, CH₂ Lev, 2x CH₂), 24.8, 24.7, 24.7 (CH₃ PivCN), 23.3 (CH₂), 18.1, 18.0, 17.8, 17.8 (CH₃-6, CH₃-6', CH₃-6'', CH₃-6'''); HRMS: [M+NH₄]⁺ calcd. for C₁₀₃H₁₂₅N₄O₂₃ 1786.87623, found 1786.87609.

N-benzyl-N-benzoyloxycarbonyl-5-aminopentanyl-3-O-(2-O-(3-O-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-4-

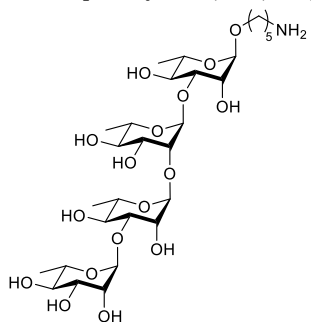


O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-α-L-rhamnopyranosyl)-3,4-di-O-benzyl-α-L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-α-L-rhamnopyranoside (29)

Compound **28** (0.185 g, 0.104 mmol, 1 eq.) was dissolved in pyridine (0.82 mL) and AcOH (0.2 mL). Hydrazine acetate (0.050 g, 0.52 mmol, 5 eq.) was added and the mixture was stirred for 1h. TLC and TLC/MS analysis showed complete consumption of the starting material after which the reaction mixture was quenched with acetone and diluted with EtOAc. The organic layer were washed with H₂O and sat. aq. NaCl (1x), dried over MgSO₄, concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc 4:1 → 2:1) resulted in the title compound as an oil (0.167

g, 0.099 mmol, 96%). TLC: R_f 0.59 (7:2 PE/EtOAc); IR (neat): 696, 733, 837, 912, 982, 1059, 1072, 1126, 1207, 1298, 1364, 1422, 1454, 1472, 1697, 1736, 1926, 2970 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.15 (m, 40H, CH_{arom}), 5.26 (s, 1H, H-2''), 5.16 (d, 2H, *J* = 10.0 Hz, CH₂ Cbz), 5.11 (s, 1H, H-1'''), 4.99 (s, 1H, H-2), 4.97 (s, 1H, H-1'), 4.91-4.87 (m, 3H, H-1'', CH₂ Bn), 4.69-4.54 (m, 10H, H-1, 5x CH₂ Bn), 4.52-4.47 (m, 3H, 2x CH₂ Bn), 4.17 (dd, 1H, *J* = 9.6, 3.2 Hz, H-3''), 4.02 (d, 1H, *J* = 7.2 Hz, H-3), 3.92 (s, 1H, H-2'''), 3.84 (s, 1H, H-2'), 3.82-3.76 (m, 1H, H-5), 3.72-3.66 (m, 4H, H-3', H-3''', H-5, H-5), 3.58-3.49 (m, 1H, CH₂), 3.47-3.31 (m, 5H, H-4, H-4', H-4'', H-4''', H-5), 3.28-3.14 (m, 3H, CH₂), 2.56-2.44 (m, 4H, 2x CH₂ PivCN), 1.57 – 1.16 (m, 28H); ¹³C NMR (100 MHz, CDCl₃): δ: 174.2, 173.7 (C=O 2x PivCN), 138.7, 138.6, 138.3, 137.9, 137.9 (C_q A_{arom}), 128.6, 128.4, 128.4, 128.3, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.3 (CH_{arom}), 117.5, 117.4 (CN), 101.4 (C-1'), 101.2 (C-1'''), 98.5 (C-1''), 96.5 (C-1), 80.4, 80.0, 79.9, 79.7 (C-4, C-4', C-4'', C-4'''), 79.2 (C-3'''), 78.7 (C-3'), 78.3 (C-3), 76.2 (C-3''), 75.7 (C-2'), 75.6, 75.5, 75.1, 75.0 (CH₂ Bn), 73.4 (C-2), 73.1 (C-2''), 72.0, 71.8 (CH₂ Bn), 68.7 (C-2'''), C-5), 68.5, 68.1 (C-5), 67.8 (CH₂), 67.6 (CH₂ Cbz), 50.6, 50.3 (CH₂ Bn), 47.1, 46.2 (CH₂), 41.0, 40.9 (C_q PivCN), 29.8 (CH₂), 29.4, 29.1 (CH₂), 27.8, 27.8 (2x CH₂ PivCN), 24.9, 24.9, 24.8 (CH₃ PivCN), 23.3 (CH₂), 18.1, 18.0, 17.8 (CH₃-6, CH₃-6', CH₃-6'', CH₃-6'''). HRMS: [M+NH₄]⁺ calcd. for C₉₉H₁₁₉N₄O₂₁ 1688.83945, found 1688.84017.

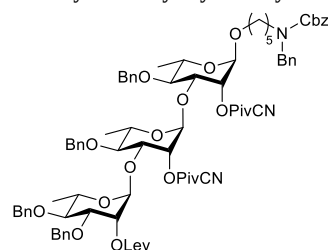
5-aminopentanyl-3-O-(2-O-(3-O-(α -L-rhamnopyranosyl)- α -L-rhamnopyranosyl)- α -L-rhamnopyranosyl)- α -L-rhamnopyranoside (10)



Tetrasaccharide **29** (0.0345 g, 0.0206 mmol) was dissolved in H₂O/THF/tBuOH (3 mL:1.3 mL:1.3 mL) and several drops of acetic acid were added. The solution was purged with argon for 5 minutes after which Pd(OH)₂/C (60 mg, 20% wt loading) was added followed by another purge with argon for 5 minutes. The solution was purged with hydrogen for 5 minutes and kept under a hydrogen atmosphere overnight. The mixture was filtered over a Whatmann filter, which was rinsed 3x with the H₂O/THF/tBuOH mixture. The solution was concentrated and redissolved in 10 mL H₂O. Triethylamine (0.5 mL) was added and the reaction was stirred overnight. Purification using size exclusion chromatography (sephadex LH20 9:1 MeOH/H₂O) followed by two lyophilizations yielded fully deprotected tetrasaccharide **10** in quantitative

yield (16.2 mg) as a white powder. ¹H NMR (D₂O, 500 MHz): δ 5.22 (s, 1H), 5.06 (s, 1H), 4.97 (s, 1H), 4.22 – 4.13 (m, 1H), 4.11 – 4.06 (m, 2H), 4.04 – 3.93 (m, 2H), 3.90 – 3.69 (m, 9H), 3.61 – 3.44 (m, 6H), 3.22 (q, 3H, *J* = 7.3, 7.3, 7.3 Hz), 3.06 – 2.99 (m, 2H), 2.64 (s, 2H), 1.77 – 1.63 (m, 5H), 1.54 – 1.42 (m, 2H), 1.37 – 1.23 (m, 25H); ¹³C NMR (D₂O, 126 MHz): δ 102.4, 102.1, 100.8, 99.7 (4x C-1), 78.1, 77.5, 72.2, 72.0, 71.8, 71.4, 70.2, 70.2, 70.0, 70.0, 69.9, 69.4, 69.3, 69.2, 68.7, 67.5, 46.8, 42.3, 39.4, 28.3, 28.1, 26.6, 25.8, 25.0, 22.5, 16.7, 16.7, 16.7, 16.6, 8.3. HRMS: [M+H]⁺ calcd. for C₂₉H₅₄NO₁₇ 688.33865, found 688.33825.

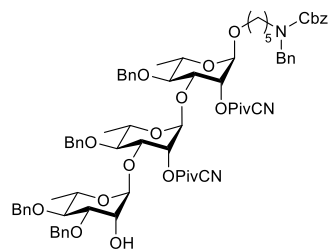
N-benzyl-N-benzyloxycarbonyl-5-aminopentanyl-3-O-(3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranoside (30)



Acceptor **25** (0.204 g, 0.303 mmol, 1.1 eq.) and donor **14** (0.232 g, 0.264 mmol, 1 eq.) were coevaporated three times with anhydrous toluene before being dissolved in distilled DCM (3.8 mL) under an argon atmosphere and stirred at room temperature for 30 min over activated molecular sieves (3Å). The reaction was cooled to 0 °C, followed by the addition of NIS (0.073 g, 0.32 mmol, 1.2 eq.) and a solution of TMSOTf in distilled DCM (0.221

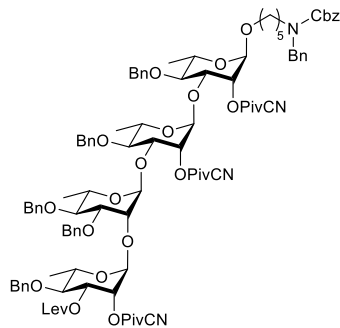
M, 0.12 mL, 0.027 mmol, 0.1 eq.) were added. After 100 min the reaction was quenched with 0.1 mL Et₃N, diluted with Et₂O and the organic layer was washed with H₂O (2x) and sat. aq. NaCl (2x). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Purification by size exclusion (1:1 DCM/MeOH) and coevaporation with CHCl₃ resulted in the title compound as a yellow oil (0.199 g, 0.138 mmol, 52%). TLC: R_f 0.31 (2:1 PE/EtOAc). ¹H NMR (400 MHz, CDCl₃) δ : 7.38-7.24 (m, 29H, CH_{arom}), 7.16 (m, 1H, CH_{arom}), 5.36 (m, 1H, H-2''), 5.17-5.13 (m, 3H, H-2', CH₂ Cbz), 5.06 (s, 1H, H-2), 4.98 (s, 2H, H-1', H-1''), 4.89 (d, 1H, *J* = 11.2 Hz, CH₂ Bn), 4.82 (d, 1H, *J* = 10.8 Hz, CH₂ Bn), 4.76 (d, 1H, *J* = 10.8 Hz, CH₂ Bn), 4.62-4.56 (m, 5H, H-1, 2x CH₂ Bn), 4.46-4.43 (m, 3H, 2x CH₂ Bn), 4.11 (d, 1H, *J* = 7.2 Hz, H-3), 4.02 (dd, 1H, *J* = 9.6, 3.2 Hz, H-3'), 3.73 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3''), 3.66-3.54 (m, 4H, H-5, H-5', H-5'', CH₂), 3.46-3.40 (m, 2H, H-4, H-4'), 3.35 (t, 1H, *J* = 9.2 Hz, H-4''), 3.30-3.18 (m, 3H, CH₂), 2.71-2.62 (m, 4H, 2x CH₂ Lev), 2.59 (q, 2H, *J* = 9.6 Hz, CH₂ PivCN), 2.41 (q, 2H, *J* = 12.4 Hz, CH₂ PivCN), 2.15 (s, 3H, CH₃ Lev), 1.52 – 1.14 (m, 25H); ¹³C NMR (100 MHz, CDCl₃) δ : 206.3 (C=O Lev ketone), 174.4, 173.9, 171.8 (C=O PivCN, PivCN, Lev), 138.7, 138.0, 137.9, 137.9, 137.7 (C_q, C_{arom}), 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4 (CH_{arom}), 117.5, 117.4 (CN), 99.9, 98.9 (C-1', C-1''), 96.7 (C-1), 80.4 (C-4), 79.7 (C-4'), 79.5 (C-4''), 77.4 (C-3''), 77.0 (C-3), 76.7 (C-3'), 75.7, 75.2, 74.9 (CH₂ Bn), 73.2 (C-2), 73.0 (C-2'), 71.2 (CH₂ Bn), 69.0 (C-2''), 68.7, 68.5, 67.8, (C-5, C-5', C-5''), 67.7 (CH₂), 67.2 (CH₂ Cbz), 50.6, 50.2 (CH₂ Bn), 47.1, 46.2 (CH₂), 41.1, 40.9 (C_q PivCN), 38.1 (CH₂ Lev), 29.9 (CH₃ Lev), 29.8, 29.1 (CH₂), 28.1, 27.9, 27.7, 27.5 (2x CH₂ PivCN, CH₂ Lev, CH₂), 25.0, 24.9, 24.8, 24.7 (CH₃ PivCN), 23.4 (CH₂), 18.1, 17.9 (CH₃-6, CH₃-6', CH₃-6''). HRMS: [M+NH₄]⁺ calcd. for C₈₃H₁₀₃N₄O₁₉ 1459.72110, found 1459.72186.

N-benzyl-N-benzyloxycarbonyl-5-aminopentanyl-3-O-(3-O-(3,4-di-O-benzyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranoside (31)



Compound **30** (0.195 g, 0.135 mmol, 1 eq.) was dissolved in pyridine (1.2 mL) and AcOH (0.3 mL). Hydrazine acetate (0.065 g, 0.69 mmol, 5 eq.) was added and the mixture was stirred for 45 min, after which TLC-MS analysis showed complete reaction. The reaction mixture was quenched with acetone and diluted with EtOAc. The organic layer was washed with H₂O (3x) and sat. aq. NaCl (1x), dried over MgSO₄, concentrated *in vacuo*. Purification by column chromatography (2:1 PE/EtOAc) and coevaporation with DCM and CHCl₃ resulted in the title compound as an oil (0.141 g, 0.105 mmol, 78%). *R*_f = 0.59 (2:1 PE/EtOAc). IR (neat): 696, 733, 837, 917, 982, 1028, 1042, 1072, 1128, 1207, 1298, 1362, 1422, 1454, 1695, 1736, 2930, 2970 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 7.39-7.23 (m, 29H, CH_{arom}), 7.15 (m, 1H, CH_{arom}), 5.17-5.15 (m, 3H, H-2', CH₂ Cbz), 5.06 (m, 1H, H-2), 5.03 (s, 1H, H-1''), 4.99 (s, 1H, H-1'), 4.86-4.81 (m, 2H, CH₂ Bn), 4.67 (d, 1H, *J* = 11.2 Hz, CH₂ Bn), 4.61-4.59 (m, 6H, H-1, 3x CH₂ Bn), 4.49-4.47 (m, 2H, CH₂ Bn), 4.13 (dd, 1H, *J* = 9.2, 2.4 Hz, H-3), 4.02 (dd, 1H, *J* = 9.6, 3.2 Hz, H-3'), 3.83 (m, 1H, H-2''), 3.69-3.53 (m, 5H, H-3'', H-5, H-5', H-5'', CH₂), 3.47-3.37 (m, 3H, H-4, H-4', H-4''), 3.27-3.18 (m, 3H, CH₂), 2.58 (q, 2H, *J* = 8.4 Hz, CH₂ PivCN), 2.44 (q, 2H, *J* = 8.8 Hz, CH₂ PivCN), 1.52 - 1.14 (m, 25H). ¹³C NMR (100 MHz, CDCl₃) δ : 174.4, 173.8, (C=O PivCN), 138.6, 138.1, 138.0, 137.9, 137.7 (C_q C_{arom}), 128.5, 128.5, 128.1, 128.0, 127.9, 127.9, 127.7, 127.6, 127.3 (CH_{arom}), 117.4, 117.4 (CN), 101.8 (C-1''), 98.7 (C-1), 80.4, 80.0, 79.5 (C-4, C-4', C-4''), 79.0 (C-3'), 76.9 (C-3'), 75.5, 75.2, 74.9 (CH₂ Bn), 73.2 (C-2, C-2'), 71.8 (CH₂ Bn), 68.7 (C-2''), 68.7, 68.1, 67.8 (C-5, C-5', C-5''), 67.7 (CH₂), 67.2 (CH₂ Cbz), 50.6, 50.3 (CH₂ Bn), 47.1, 46.1 (CH₂), 41.2, 40.9 (C_q PivCN), 29.8, 29.4, 29.1 (CH₂), 27.9, 27.7 (CH₂ PivCN), 25.0, 24.9, 24.8, 24.7 (CH₃ PivCN), 23.4 (CH₂), 18.1, 17.9, 17.8 (CH₃-6, CH₃-6', CH₃-6''). HRMS: [M+NH₄]⁺ calcd. for C₇₈H₉₇N₄O₁₇ 1361.68432, found 1361.68484.

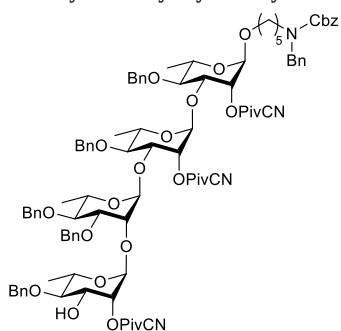
N-benzyl-N-benzyloxycarbonyl-5-aminopentanyl-3-O-(3-O-(2-O-(4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-3-O-levulinoyl- α -L-rhamnopyranosyl)-3,4-di-O-benzyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranoside (32)



Acceptor **31** (1.09 g, 0.81 mmol, 1.0 eq.) and donor **12** (0.68 g, 1.22 mmol, 1.5 eq.) were coevaporated three times with anhydrous toluene before being dissolved in dry DCM. The solution was stirred at room temperature on activated molecular sieves after which NIS (0.309 g, 1.377 mmol, 1.7 eq.) was added. The reaction mixture was cooled to -40 °C and treated with 1.4 mL of a freshly prepared solution of TMSOTf in DCM (0.1 M, 1.4 mL, 0.17 eq.). After 65 minutes, TLC analysis showed complete consumption of the acceptor, and the reaction was quenched with 0.5 mL

Et₃N. The mixture was diluted with EtOAc, washed with sat. aq. Na₂S₂O₃, sat. aq. NaHCO₃ and sat. aq. NaCl, dried over MgSO₄ and concentrated. Column purification (hexanes/acetone 10:1 $\rightarrow$ 4:1) followed by size exclusion purification (DCM/MeOH 1:1) yielded tetrasaccharide **32** as a yellow oil (1.087 g, 0.608 mmol, 75%). TLC: *R*_f 0.62 (PE/EtOAc, 1/1, v/v); IR (neat): 698, 731, 979, 1074, 1128, 1454, 1697, 1737, 2245, 2877, 2935, 2976 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ : 7.37 - 7.13 (m, 35H), 5.44 - 5.36 (m, 1H), 5.33 (dd, 1H, *J* = 9.5, 3.3 Hz), 5.19 - 5.10 (m, 3H), 5.01 - 4.77 (m, 5H), 4.77 - 4.66 (m, 3H), 4.61 (dd, 5H, *J* = 11.0, 5.4 Hz), 4.52 (dd, 3H, *J* = 11.8, 3.7 Hz), 4.48 (s, 1H), 3.91 - 3.76 (m, 2H), 3.70 - 3.64 (m, 2H), 3.64 - 3.54 (m, 2H), 3.47 (m, 4H), 3.42 - 3.11 (m, 5H), 2.81 - 2.34 (m, 13H), 2.20 - 2.09 (m, 3H), 1.58 - 1.07 (m, 34H); ¹³C NMR (CDCl₃, 101 MHz) δ : 206.3, 174.3, 173.8, 173.7, 171.7, 138.8, 138.3, 138.0, 137.9, 137.8, 137.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.6, 127.6, 127.5, 127.4, 127.3, 117.5, 117.3, 117.3, 101.6, 98.7, 98.6, 96.6, 80.3, 79.7, 79.4, 79.0, 78.3, 77.7, 75.6, 75.3, 75.1, 74.9, 73.2, 73.1, 72.2, 72.0, 70.8, 68.8, 68.6, 68.1, 67.7, 67.6, 67.1, 41.1, 40.8, 37.8, 29.8, 29.1, 27.9, 27.9, 27.8, 27.6, 25.0, 24.9, 24.8, 24.8, 24.8, 24.6, 23.3, 18.0, 17.9, 17.8, 17.7; HRMS (MALDI-TOF): [M+Na]⁺ calcd. for C₁₀₂H₁₂₂N₄O₂₄Na 1809.8341, found 1809.8353.

N-benzyl-N-benzyloxycarbonyl-5-aminopentanyl-3-O-(3-O-(2-O-(4-O-benzyl-2-O-(3-cyano-2,2-

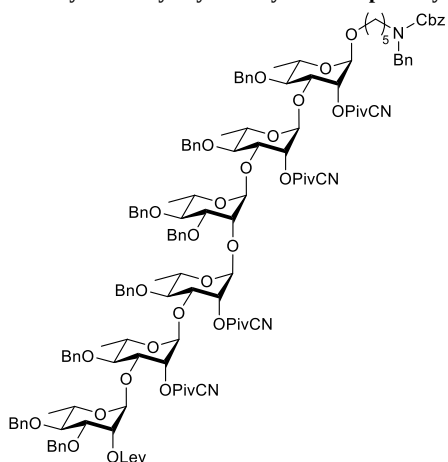


dimethylpropanoyl)- α -L-rhamnopyranosyl)-3,4-di-O-benzyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-

dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranoside (33) A solution of **32** (0.492 g, 0.275 mmol, 1.0 eq.) in pyridine/AcOH (2.9 mL:0.75 mL) was cooled to 0 °C and treated with $\text{H}_2\text{NNH}_2\cdot\text{AcOH}$ (0.124 g, 1.35 mmol, 5 eq.). After 90 minutes the reaction was quenched with acetone, diluted with EtOAc, washed with H_2O (2x) and sat. aq. NaCl, dried over MgSO_4 and concentrated. Purification by column chromatography (hexanes/acetone 9:1 $\rightarrow$ 4:1) yielded the title compound **33** as a colorless oil (0.306 g, 0.18 mmol, 67%). TLC: R_f 0.74 (PE/EtOAc, 1/1, v/v); IR (neat): 696, 732, 979, 1028, 1055, 1128,

1454, 1697, 1735, 2245, 2933, 2974, 3030 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 7.37 – 7.18 (m, 35H), 5.26 (dd, J = 3.1, 1.7 Hz, 1H), 5.19 – 5.13 (m, 3H), 5.13 – 5.02 (m, 3H), 5.00 – 4.93 (m, 3H), 4.90 – 4.79 (m, 4H), 4.78 – 4.62 (m, 5H), 4.62 – 4.59 (m, 2H), 4.59 – 4.42 (m, 8H), 4.11 (dt, J = 9.1, 4.6 Hz, 2H), 3.98 (dd, J = 9.5, 3.1 Hz, 1H), 3.85 – 3.72 (m, 3H), 3.67 (dd, J = 9.0, 2.8 Hz, 2H), 3.65 – 3.48 (m, 4H), 3.48 – 3.12 (m, 11H), 2.66 – 2.38 (m, 9H), 1.64 – 0.94 (m, 34H); ^{13}C NMR (126 MHz, CDCl_3) δ 174.4, 174.2, 174.0, 138.8, 138.7, 138.3, 138.1, 138.1, 138.0, 137.9, 137.7, 137.0, 132.3, 129.3, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.3, 117.9, 117.4, 117.4, 101.7, 98.8, 96.7, 92.6, 91.9, 81.4, 81.3, 81.1, 80.4, 80.1, 79.8, 79.6, 79.5, 79.1, 78.9, 77.9, 76.8, 75.6, 75.3, 75.2, 75.2, 75.1, 74.9, 74.9, 73.8, 73.3, 73.3, 73.2, 73.0, 72.3, 72.1, 71.8, 70.2, 69.8, 68.7, 68.2, 68.2, 67.8, 67.8, 67.6, 67.2, 50.7, 50.3, 47.2, 46.2, 41.2, 41.2, 40.9, 29.2, 28.1, 28.0, 27.9, 27.8, 27.6, 25.1, 25.0, 24.9, 24.9, 24.8, 24.7, 23.4, 18.3, 18.1, 17.9; HRMS (MALDI-TOF): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{97}\text{H}_{116}\text{N}_4\text{O}_{22}\text{Na}$ 1711.7973, found 1711.7979.

N-benzyl-N-benzyloxycarbonyl-5-aminopentanyl-3-O-(3-O-(3,4-di-O-benzyl-2-O-(3-cyano-2,2-



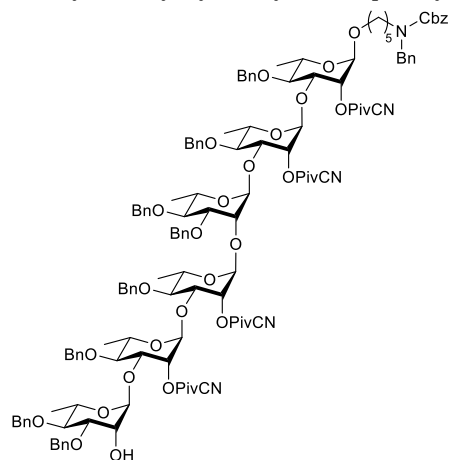
benzyl-2-O-levulinoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranoside (34) Donor **14** (0.15 g, 0.17 mmol, 1.5 eq.) and acceptor **33** (0.19 g, 0.11 mmol, 1.0 eq.) were coevaporated three times with anhydrous toluene and kept under argon, after which they were dissolved in anhydrous DCM. Activated molecular sieves were added and the mixture was stirred for 30 minutes at room temperature. After addition of NIS (0.042 g, 0.19 mmol, 1.7 eq.), the solution was cooled to -40°C, followed by addition of a freshly prepared DCM solution of TMSOTf (0.1 M, 0.18 mL, 0.018 mmol, 0.17 eq.). After TLC and TLC/MS

analysis showed complete consumption of the acceptor, the reaction was quenched by addition of 0.3 mL Et_3N and allowed to warm up to room temperature. The mixture was diluted with EtOAc, washed with sat $\text{Na}_2\text{S}_2\text{O}_3$ (aq), dried over MgSO_4 and concentrated. Column purification (hexanes/EtOAc) followed by size exclusion (1:1 DCM/MeOH) yielded fully protected hexasaccharide **34** as yellow glass like solid (0.237 g, 0.096 mmol, 88%). TLC: R_f 0.74 (PE/EtOAc, 1/1, v/v); IR (neat): 698, 734, 981, 1028, 1043, 1201, 1454, 1697, 1737, 2247, 2875, 2933, 2972 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.36 – 7.13 (m, 50H), 5.43 – 5.30 (m, 1H), 5.29 – 5.08 (m, 5H), 5.08 – 4.92 (m, 5H), 4.92 – 4.75 (m, 6H), 4.75 – 4.41 (m, 16H), 4.20 – 4.06 (m, 2H), 3.99 (ddd, 2H, J = 22.7, 9.5, 3.0 Hz), 3.85 – 3.11 (m, 20H), 2.74 – 2.48 (m, 8H), 2.48 – 2.35 (m, 5H), 2.15 (s, 3H), 1.60 – 1.03 (m, 46H); ^{13}C NMR (CDCl_3 , 101 MHz): δ 206.2, 174.3, 174.0, 173.9, 171.8, 138.7, 138.7, 138.3, 138.0, 137.9, 137.9, 137.7, 137.6, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 127.9, 127.9, 127.8, 127.7, 127.7, 127.7,

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127.5, 127.3, 117.6, 117.4, 117.3, 100.0, 98.8, 98.5, 96.7, 80.6, 80.4, 79.6, 79.5, 77.0, 76.8, 75.6, 75.2, 74.9, 73.3, 73.2, 73.0, 72.8, 72.0, 71.2, 69.0, 68.8, 68.7, 68.5, 68.5, 67.2, 41.2, 41.1, 40.9, 40.9, 38.1, 29.9, 28.2, 27.9, 27.7, 25.0, 24.8, 24.7, 24.7, 23.4, 18.1, 17.9, 17.9; HRMS (MALDI-TOF): $[M+Na]^+$ calculated for $C_{141}H_{167}N_5O_{33}Na$ 2481.1436, found 2481.1436.

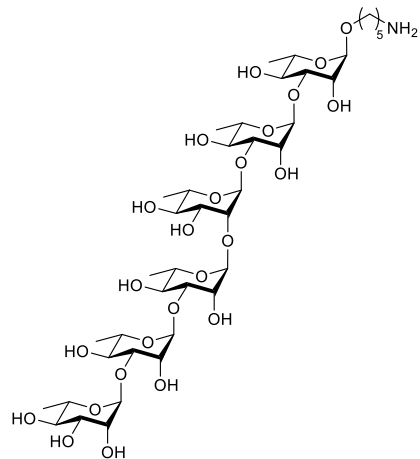
N-benzyl-N-benzylloxycarbonyl-5-aminopentanyl-3-O-(3-O-(3,4-di-O-benzyl-2-O-(3-O-(3-O-(3,4-di-O-benzyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)- α -L-rhamnopyranoside (35)



Compound **34** (0.133 g, 0.054 mmol) was dissolved in 0.8 mL pyridine and 0.2 mL AcOH. The solution was cooled to 0 °C followed by addition of $H_2NNH_2 \cdot AcOH$ (0.025 g, 0.27 mmol, 5 eq.) and stirred for 10 minutes at 0 °C. After stirring at rt for 160 minutes, TLC and TLC/MS analysis showed complete conversion of the starting material and the reaction was quenched by addition of acetone. The mixture was diluted with EtOAc, washed with H_2O and sat. aq. NaCl, dried over $MgSO_4$ and concentrated. Column purification (hexanes/acetone) followed by size exclusion

purification (DCM:MeOH 1/1) yielded the title hexasaccharide as a yellow glass-like solid (0.124 g, 0.052 mmol, 96%). TLC: R_f 0.68 (PE/EtOAc, 1/1, v/v); IR (neat): 698, 734, 983, 1028, 1041, 1454, 1697, 1735, 2247, 2875, 2933, 2974 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.40 – 7.12 (m, 50H), 5.26 – 4.97 (m, 5H), 4.99 – 4.72 (m, 9H), 4.72 – 4.45 (m, 17H), 4.17 (dd, 1H, J = 9.5, 3.1 Hz), 4.13 – 4.05 (m, 1H), 3.99 (ddd, 2H, J = 21.0, 9.5, 3.1 Hz), 3.85 – 3.53 (m, 10H), 3.49 – 3.29 (m, 8H), 2.61 – 2.32 (m, 10H), 1.49 – 1.03 (m, 46H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.4, 174.0, 174.0, 173.9, 138.8, 138.7, 138.3, 138.1, 138.1, 138.0, 137.9, 137.8, 137.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.4, 117.5, 117.4, 117.4, 101.8, 101.7, 98.8, 98.6, 98.5, 96.7, 80.6, 80.4, 80.1, 79.9, 79.6, 79.1, 78.8, 77.8, 76.9, 75.7, 75.5, 75.4, 75.3, 75.2, 75.0, 74.9, 74.7, 73.3, 73.2, 72.8, 72.0, 71.8, 68.9, 68.7, 68.6, 68.2, 67.8, 67.8, 67.3, 50.7, 46.2, 41.2, 40.9, 40.9, 29.2, 28.0, 27.9, 27.8, 27.8, 25.1, 25.0, 24.9, 24.9, 24.9, 24.8, 24.7, 23.4, 18.1, 17.9; HRMS (MALDI-TOF): $[M+Na]^+$ calculated for $C_{136}H_{161}N_5O_{31}Na$ 2383.1068, found 2383.1064.

5-aminopentanyl-3-O-(3-O-(2-O-(3-O-(3-O-(α -L-rhamnopyranosyl)- α -L-rhamnopyranosyl)- α -L-rhamnopyranosyl)- α -L-rhamnopyranosyl)- α -L-rhamnopyranosyl)- α -L-rhamnopyranoside (11)



Compound **35** (0.069 g, 0.029 mmol) was dissolved in a mixture $H_2O/THF/tBuOH$ (3 mL:1.3 mL: 1.3 mL) followed by addition of several drops of acetic acid. The solution was purged for 5 minutes with argon. $Pd(OH)_2/C$ (60 mg, 20% wt loading) was added and the solution was purged for another 5 minutes with argon. The solution was then purged with hydrogen for 5 minutes and kept under a hydrogen atmosphere overnight. The mixture was filtered over a Whatmann filter and concentrated. This procedure was repeated two times after which the residue was dissolved in 10 mL H_2O , followed by addition of 0.5 mL Et_3N . The mixture was stirred for 150 minutes and concentrated. Purification using size exclusion chromatography (sephadex LH20 9:1 MeOH/ H_2O) followed by three lyophilizations yielded fully deprotected hexasaccharide as a white powder (23.8 mg, 24.4 mmol, 84%). 1H NMR (D_2O , 600

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MHz): δ 5.18 (s, 1H, H-1), 5.06 - 4.96 (m, 3H, 3x H-1), 4.92 (d, 1H, J = 4.8 Hz, H-1), 4.73 (s, 1H, H-1), 4.15 - 4.08 (m, 2H, 2 x H-2), 4.04 (s, 2H, 2 x H-2), 3.98 (s, 1H, H-2), 3.91 (m, 1H), 3.89 - 3.83 (m, 3H), 3.83 - 3.78 (m, 4H), 3.78 - 3.62 (m, 8H), 3.59 - 3.35 (m, 8H), 3.00 - 2.94 (m, 2H), 1.65 (m, 4H, CH₂), 1.45 (m, 2H, CH₂), 1.41 - 1.31 (m, 2H, CH₂), 1.25 (m, 18H, 6x CH₃-6); ¹³C NMR (D₂O, 151 MHz): δ 121.8, 103.4, 103.4, 103.2, 103.0, 101.8, 100.5 (6x C-1), 79.3, 79.2, 79.1, 79.0, 78.7 (6x C-2), 73.1, 73.0, 72.9, 72.6, 72.3, 72.2, 71.1, 71.0, 71.0, 70.9, 70.8, 70.7, 70.3, 70.2, 70.2, 70.2, 70.1, 70.0, 69.7, 68.4 (6x C-3, 6x C-4, 6x C-5), 67.5, 47.6, 43.2, 40.4, 40.3 (CH₂), 29.2 (CH₂), 29.0, 27.5 (CH₂), 25.9, 23.4 (CH₂), 17.6, 17.6, 17.5 (6x CH₃), 9.2. HRMS (MALDI-TOF): [M+Na]⁺ calculated for C₄₁H₇₃NO₂₅Na 1002.4364, found 1002.4359.

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Chapter 4

The Cyanopivaloyl Ester in the Automated solid phase synthesis of Oligorhamnans

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Introduction

The advent of automated solid phase synthesis approaches for the assembly of nucleic acids and peptides has transformed the way chemists generate (fragments of) these biopolymers, and the rapid access to these molecules has revolutionized the life sciences. The automated solid phase synthesis of oligosaccharides is significantly more complex than the assembly of the other two biopolymers and as a result its development has been significantly slower. Nonetheless, there has been considerable progress in the field of automated solid phase oligosaccharide synthesis over the last decade.^{1,2} A commercial synthesizer is now available, and there are continuous efforts to build improved machines.^{3,4} Ever more complex molecules are being assembled in an automated manner and recent highlights include the assembly of libraries of plant-derived branched arabino-xylan and xyloglucan structures⁵, hyaluronic acid fragments up to 15 monosaccharides in length⁶, a 50-mer polymannoside⁷, a set of dermatan⁸ and keratan sulfates⁹, a set of α -glucans¹⁰ and a collection of mannuronic acid alginates, built up of up to 12 β -mannuronic acid residues linkages.¹¹ These synthetic successes have shown that linear and branches structures can be assembled in an automated means and that both 1,2-*trans* and 1,2-*cis* linkages can be reliably installed using solid phase chemistry. The method is especially attractive for the generation of libraries of oligosaccharides and oligosaccharides featuring repetitive elements.

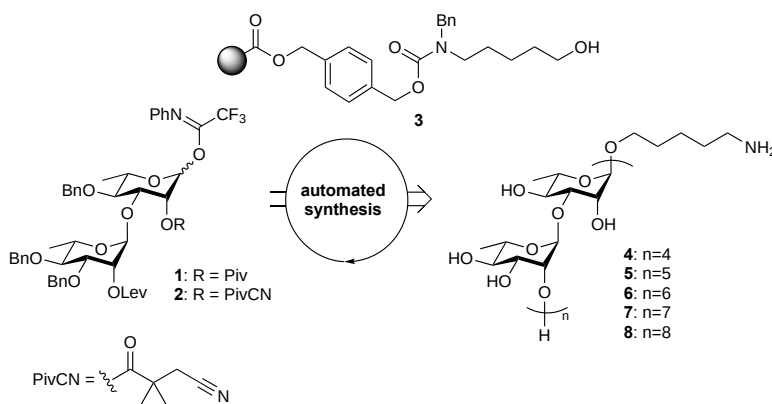
The key to any successful oligosaccharide synthesis campaign is the protection group strategy used. Permanent protecting groups should be able to withstand all conditions used throughout the assembly route, while temporary protecting groups have to be removed selectively without touching any other functionalities in the molecule. The requirements for protecting groups in automated solid phase oligosaccharide synthesis are even more strenuous as they have to withstand glycosylation and deprotection steps repeatedly, under harsher conditions than used in traditional solution phase experiments, because often an excess of reagents is used to drive reactions to completion. The introduction of new protecting groups and protecting group chemistry will be crucial for the further development of automated solid phase oligosaccharide synthesis.

Chapter 3¹² introduced the cyanopivaloyl (PivCN) group as an attractive participating group that allows for the reliable construction of 1,2-*trans*-glycosidic linkages. It features the favourable characteristics of the pivaloyl ester -stability, effective neighboring group participation, minimal orthoester formation and migratory aptitude- while it circumvents the drawbacks of the parent pivaloyl group -its problematic removal at the end of the synthesis- as it can be removed by reduction of the cyano group to the corresponding amine, which can engage in an effective intramolecular ring closure to cleave the ester function. Thus, removal of the cyanopivaloyl group can be effected in tandem with the removal of benzyl ethers, commonly used as permanent protecting groups.

These favourable characteristics should make the cyanopivaloyl group an attractive protecting group to be used in automated synthesis. To probe its effectiveness in an

automated solid phase setting, this Chapter explores its use in the assembly of a set of oligorhamnosides, up to 16 monosaccharides in length (See Scheme 1). These target structures represent fragments of the backbone of the cell wall polysaccharide of Group A streptococcus (GAS), a Gram-positive bacterium, which is the cause of various infections (pharyngitis, necrotizing fasciitis) and which is found responsible for rheumatic fever, causing hundreds of thousands of deaths every year in developing countries.^{13,14} The GAS polyrhamnose backbone is decorated with *N*-acetyl glucosamine appendages at the rhamnosyl C-3 OH.¹⁵ The potential use of this naturally occurring polysaccharide in conjugate vaccines may be thwarted by the potentially autoimmunogenic GlcNAc epitopes and it has been suggested that the non-mammalian “bare” poly rhamnose backbone, devoid of GlcNAc groups, may be an attractive structure for a universal GAS vaccine. Well-defined fragments of the polyrhamnose backbone will be valuable in the generation of semi-synthetic vaccines and therefore represent attractive synthetic targets.¹⁶ The repetitive nature of these molecules makes them very well suited for an automated synthesis approach.

Figure 1. Synthetic approach in this Chapter.



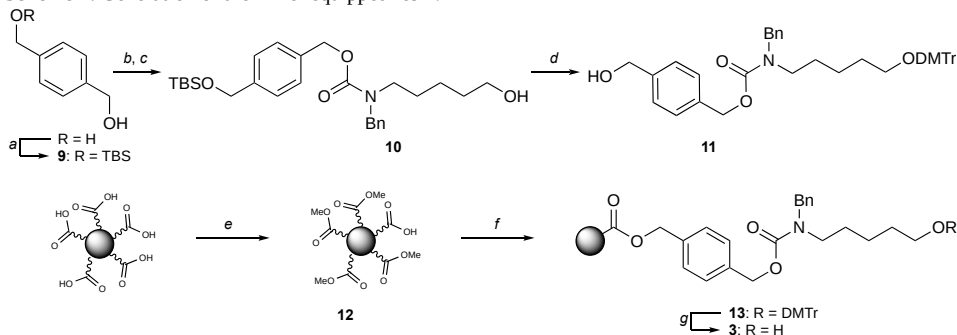
Results and discussion

The synthetic strategy -and test-case for the cyanopivaloyl group- for the assembly of the oligorhamnosides is depicted in Scheme 1. In this study, a commercial Glyconeer 2.1 synthesizer was used for the automated assembly. The oligosaccharides are built on a polystyrene resin equipped with a linker system,¹⁷ that provides the target structures with an aminopentanol spacer after global deprotection. The amine in the linker system is protected with a benzyl and a modified Cbz protecting group. The Cbz-part is connected to the solid support via a base labile ester linkage. Disaccharide building blocks were used in this study bearing an imidate as anomeric leaving group and a levulinoyl group as orthogonal temporary group, as these functionalities have proven very effective in various previous automated solid phase assembly procedures.^{6,11} Dimer donors were to be used

because acyl groups at the axial C-2-hydroxyl of rhamnosides are prone to migrate to the equatorial C-3-hydroxyl group, when this functionality is unmasked during the synthesis. Partial migration of protecting groups will lead to complex and unseparable mixtures after several coupling rounds. Two different dimer building blocks were explored: the first (dimer **1**) carrying a permanent pivaloyl ester at the C-2-hydroxyl, the second (building block **2**) with a cyanopivaloyl at this position.

The linker-functionalized resin **3**, is obtained in 7 steps, from 1,4-benzene-dimethanol, following an improved route of synthesis, originally developed by Czechura *et al.*, as depicted in Scheme 1.¹⁷ After silylation of one hydroxyl group (34% yield), the remaining hydroxyl is transformed into an active carbonate by reaction of compound **9** with *para*-nitrophenylchloroformate and reacted with *N*-benzyl-5-aminopentanol to yield compound **10**. Installation of the dimethoxytrityl group proceeded uneventfully but because purification of the fully protected linked system from excess reagent proved troublesome, the TBS group was directly removed. Compound **11** was obtained pure in quantitative yield over two steps yield on 16 mmol scale.

Scheme 1. Generation of the linker equipped resin.



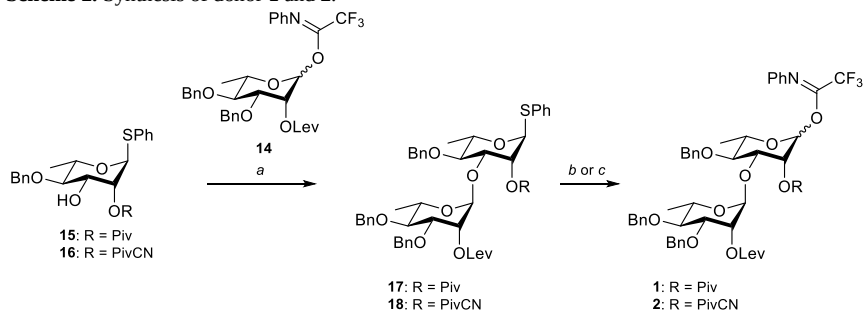
Reagents and conditions: TBDMS-Cl, imidazole, DMF, 0°C (30%); b) *para*-nitrophenylchloroformate, pyridine, 0°C, c) *N*-Benzyl-5-aminopentanol, DIPEA, DMF, 0°C (90%); d) i. DMTr-Cl, pyridine, 0°C, ii. TBAF, THF, 0°C (100%); e) TMSCHN₂, MeOH, THF; f) **11**, DIC, DMAP, DCM, then MeOH; g) TCA, DCM.

Next the linker was conjugated to the carboxylic acid functionalized polystyrene resin. Because the loading of the commercially available resin was too high (2.19 mmol/g), the amount of carboxylic acid groups was first reduced by treatment of the resin with TMS-diazomethane.^{18,19} Afterwards the resulting resin was coupled with the DMTr-protected linker. Removal of the DMTr group was achieved by a TCA/DCM treatment, after which the loading was determined to be 0.44-0.47 mmol/g.

The synthesis of the required dirhamnosyl building blocks is depicted in Scheme 2 and started by coupling imidate donor **14**¹² and acceptor **15**²⁰/**16**¹², using a catalytic amount of TfOH. This led to disaccharides **17** and **18**, which could both be purified by crystallization from hot ethanol. The thioglycosides **17/18** were transformed into the corresponding

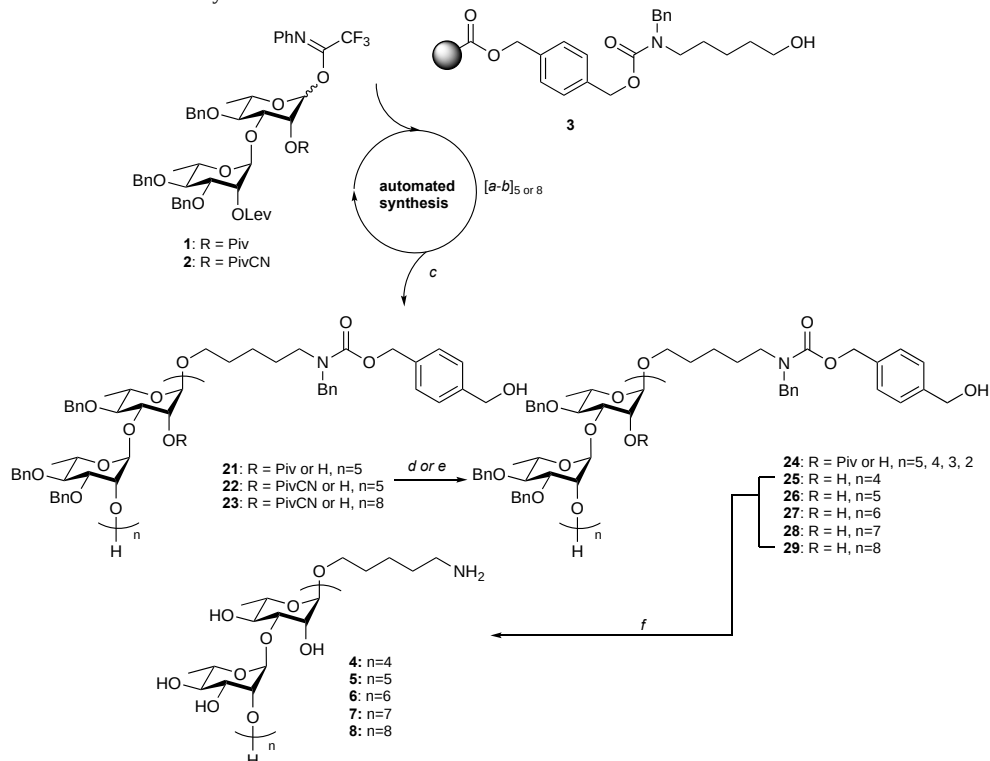
imidate donors by treatment with *N*-bromosuccinimide in acetone/water²¹ and subsequent installation of the *N*-phenyl trifluoroacetimidoyl functionality.

Scheme 2. Synthesis of donor **1** and **2**.



Reagents and conditions: a) **14**, TfOH, DCM, 0°C (**17**: 69%, **18**: 88%); b) NBS, acetone/H₂O (**19**: 65%, **20**: 89%), then ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0°C (**1**: 88%, **2**: 79%); c) NIS, TFA, DCM, 0°C (**20a**: 73%) then ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0°C (**2**: 79%) .

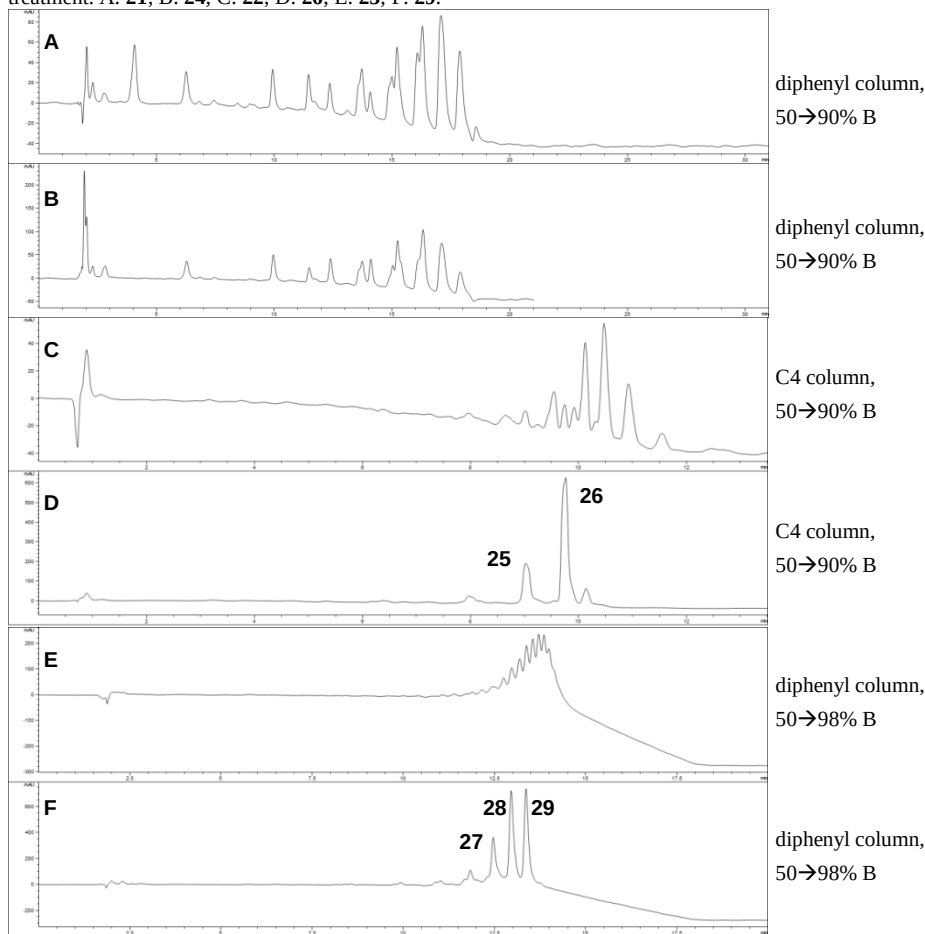
With the required building blocks in hand the assembly of the oligosaccharides was started. As a first research objective, the assembly of a decasaccharide was targeted employing the pivaloyl protected building block **1**. Previously developed glycosylation and deprotection conditions^{6,11} were applied to couple donor **1** to resin **3** (3 x 3 equivalents donor, 0.2 equivalents TfOH with respect to the donor, 30 min at 0°C, Scheme 4), followed by removal of the Lev group (3 x 5 equivalents H₂NNH₂•AcOH, 10 min at 40°C). After five coupling/deprotection cycles, the resin was subjected to cleavage conditions (a catalytic amount of NaOMe in a mixture of THF/MeOH).

Scheme 3. Automated synthesis of decamer **21** and **22** and hexadecamer **23**.

Reagents and conditions: a) 3 eq. **1** or **2**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; b) 8 eq. H₂NNH₂·AcOH, pyr/AcOH, 40°C, 3 cycles; c) NaOMe, MeOH/THF; d) NaOMe, MeOH/THF (**25**: 9%, **26**: 26%, **27**: 6%, **28**: 9%, **29**: 9% starting from resin **3**); e) NaOH (aq), MeOH/Dioxane, 40°C; f) H₂, Pd(OH)₂/C, AcOH, H₂O/THF/tBuOH (**4**: 69%, **5**: 57%, **6**: 27%, **7**: 92%, **8**: 50%).

The crude decasaccharide **21** was analyzed by LC-MS and the obtained LC-spectrum is shown in Figure 2. A complex mixture mixture was obtained, which was the result of incomplete glycosylation reactions and removal of some of the pivaloyl esters. Unfortunately, it proved to be impossible to remove all pivaloyl esters, even under harsh basic conditions,²² and the desired decasaccharide could not be obtained from the complex reaction mixture (Figure 2B). The use of pivaloyl functionalized donor **1** was therefore not further explored and attention was switched to the use of its PivCN counterpart **2**.

Figure 2. LC-chromatograms of the crude products cleaved from the resin before and after prolonged base treatment. A: **21**; B: **24**; C: **22**; D: **26**; E: **23**; F: **29**.



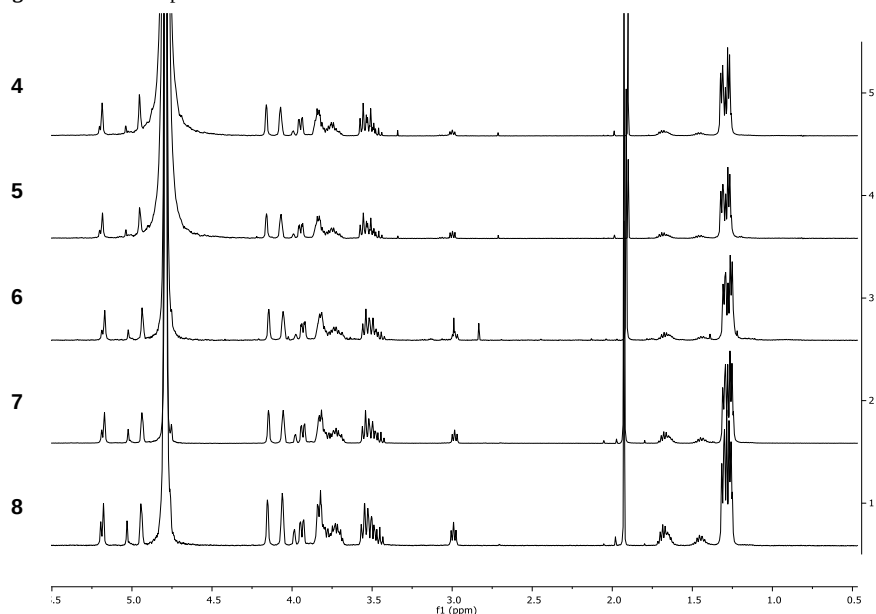
When donor **2** was used for the assembly of decasaccharide **22** again a complex product mixture arose after cleavage of the products from the resin (Figure 2C).²³ It was noted however that a significantly larger portion of the PivCN-groups had been removed from the target structures in comparison to the pivaloyl decasaccharide mixture. This indicates that the cyano group in the PivCN ester render the ester carbonyl more electrophilic, as a result of its electron withdrawing character, even though it is separated from the carbonyl by two carbon atoms. A similar characteristic was employed by Carreira *et al.*, substituting a methyl group for an electronwithdrawing chlorine.²⁴ This also suggested that the PivCN groups could potentially be removed by an additional and/or elongated base treatment. To explore this possibility, the crude mixture was resubjected to basic conditions and progress of the reaction was monitored by LC-MS. The LC-trace of the mixture that was finally obtained is shown in Figure 2D, and it shows the presence of only two products. The major

product in the mixture proved to be the desired decasaccharide **26**, while the minor other peak corresponds to the octasaccharide (**25**). Purification of the target compound was readily achieved from this mixture and the target decasaccharide was obtained in 26% overall yield after 12 steps (89% per step).

Driven by this success, a hexadecasaccharide was synthesized by running 8 coupling/deprotection cycles using donor **2**. After cleavage of the products from the resin, again a complex mixture was obtained (Figure 2E). Subjection of this mixture to an additional base treatment led to complete cleavage of all PivCN groups and Figure 2F depicts the LC chromatogram of the resulting mixture. From this mixture, the target hexadecarhamnoside **29** was obtained in 9% yield (18 steps, 87% per step) alongside the dodeca- and tetradecasaccharide deletion sequences, **27** and **28**, respectively.

To complete the syntheses of the oligorhamnosides, all obtained partially protected oligorhamnosides (**25-29**) were subjected to hydrogenolysis over $\text{Pd}(\text{OH})_2/\text{C}$ in $\text{H}_2\text{O}/\text{THF}/\text{tBuOH}$, to remove all benzyl groups and liberate the alcohols and the amine functionality on the spacer. Gel filtration (HW40, eluted with NH_4OAc) yielded the fully deprotected octa-, deca-, dodeca, tetradeca and hexadecasaccharides (**4-8**) in multimilligram quantities. The ^1H -NMR spectra of rhamnosides **4-8** are depicted in Figure 3. The regular structure of the fragments becomes apparent from the spectra as they are very similar and only differ in relative intensity of the signals.

Figure 3. ^1H NMR spectra of rhamnosides **4-8**.



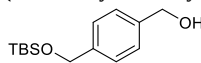
Conclusion

This Chapter has introduced the cyanopivaloyl (PivCN) ester as an effective protecting group for solid phase oligosaccharide synthesis. This novel protecting group was probed in the assembly of a series of oligorhamnosides, alongside its pivaloyl counterpart. It was found that cleavage of the protected oligosaccharides from the resin was accompanied by partial cleavage of the pivaloyl groups. Complete removal of all pivaloyl groups however proved to be difficult, underscoring the problems often encountered with this bulky ester. The cyanopivaloyl ester on the other hand could be effectively cleaved under basic conditions, as a result of the remote electron-withdrawing cyano group, which renders the ester carbonyl group more electrophilic. The favorable cleavage characteristics of the PivCN-group in combination with the favorable properties of the pivaloyl-type esters (minimal orthoester formation during glycosylations, minimal migration, stability) make the PivCN-group an attractive asset in the toolbox of the synthetic chemist. Here, it has proven its merits in the automated solid phase assembly of GAS related oligorhamnosides of considerable length.

Experimental

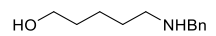
General experimental procedures. All solvents used under anhydrous conditions were stored over 4Å molecular sieves except for methanol which was stored over 3Å molecular sieves. ^1H and ^{13}C NMR spectra were recorded on a 400/100, 500/125, 600/150, or a 850/214 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All individual signals were assigned using 2D-NMR spectroscopy, HH-COSY, HSQC, and HMBC. IR spectra are reported in cm^{-1} , and recorded on a Shimadzu FTIR-8300 or a PerkinElmer universal attenuated total reflectance (UATR; Single Reflection Diamond) Spectrum Two instrument. Solvents used for workup and column chromatography were of technical grade from Sigma Aldrich, Boom, Biosolve or Honeywell and used directly. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40 °C. All chemicals were used as received unless stated otherwise. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F254 with detection by spraying with 20% H_2SO_4 in EtOH, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (25 g/L) and $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}$ (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), or by dipping in anisaldehyde (10 mL in 180 mL EtOH / 10 mL H_2SO_4) followed by charring at approx. 150 °C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). For LC-MS analysis a Agilent Technologies 1260 Infinity LC system (detection simultaneously at 214 and 254 nm) coupled to a Agilent Technologies 6120 Quadrupole LC/MS, using an analytical Vydac C4 column (Alltech, 50 x 4.60 mm, 5 μm) or a Vydac Diphenyl (Alltech, 150 x 4.60 mm, 5 μm) in combination eluents A: H_2O ; B: MeCN and C: 1% aq. TFA. For HPLC, a Gilson HPLC system in combination with eluents A: H_2O (0.1% TFA); B: MeCN as the solvent system using a Vydac C4 HPLC column (Grace, 250 x 10 mm, 5 μm). High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution $R = 60000$ at m/z 400 (mass range $m/z = 150\text{--}2000$) and dioctylphthalate ($m/z = 391.2842$) as a “lock mass”. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Maldi spectra were recorded on an Ultraflex extreme MALDI-TOF (Bruker Daltonics), equipped with Smartbeam-II laser, to measure the samples in reflectron positive ion mode. The MALDI-TOF was calibrated using a peptide calibration standard prior to measurement. 1 μL of 2,5-dihydroxybenzoic acid (2,5-DHB; Bruker Daltonics) matrix (20 mg/mL in ACN/water; 50:50 (v/v)) was applied on a 384-MTP target plate (Bruker Daltonics, Bremen, Germany) and air-dried. Subsequently, 1 μL of compound water solution was spotted on the plate and the spots were left to dry prior MALDI-TOF analysis.

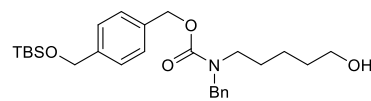
(4-*tert*-Butyl-dimethyl-siloxy)methylphenyl)methanol (9) 1,4-benzene-dimethanol (8.29 g, 60 mmol, 1.0 eq.)

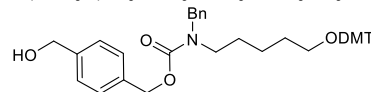


was dissolved in 25 mL DMF and cooled to 0°C followed by the addition of imidazole (10.2 g, 150 mmol, 2.5 eq.). A solution of *tert*-butyldimethylsilyl chloride (9.13 g, 60.6 mmol, 1.01 eq.) in 40 mL DMF was added dropwise and the reaction was allowed to stir overnight. After TLC analysis showed complete consumption of the starting material, the mixture was diluted with Et_2O and washed subsequently with H_2O (2x) and sat. aq. NaCl (1x). The organic phase was dried over MgSO_4 and concentrated *in vacuo*. Purification using flash column chromatography (PE/EtOAc, 9:1 $\rightarrow$ 6:1) yielded the title compound as a colorless oil (4.73 g, 18.7 mmol, 30%). TLC: R_f 0.39 (PE/EtOAc, 6/1, v/v); ^1H NMR (400 MHz, CDCl_3): δ 7.33

(s, 4H), 4.74 (s, 2H), 4.68 (d, 2H, $J=3.1$ Hz), 1.67-1.50 (m, 2H), 0.94 (s, 9H), 0.10 (s, 6H). Analytical data are identical to literature values.²⁵

 **N-Benzyl-5-aminopentanol** Benzaldehyde (10.67 mL, 104.6 mmol, 1.01 eq.) was added to a solution of 5-aminopentanol (11.3 mL, 104.0 mmol, 1.0 eq.) in 150 mL EtOH. The solution was heated to 50°C under reduced pressure until all solvent was removed. The crude mixture was co-evaporated twice with anhydrous toluene, dissolved in MeOH (200 mL) and cooled to 0°C. NaBH₄ (4.82 g, 124.7 mmol, 1.2 eq.) was added in portions and the solution was allowed to stir at 0°C for 70 minutes. After stirring for another 2 hours, the solution was cooled to 0°C followed by addition of 4.5 mL AcOH. A 1.2M K₂CO₃ (aq) solution (135 mL) was added and the mixture was diluted with Et₂O. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Purification using flash column chromatography yielded the linker in 62% yield (12.4 g, 64 mmol); ¹H NMR (400 MHz, CDCl₃): δ 7.41-7.15 (m, 5H), 3.78 (s, 2H), 3.62 (t, 2H, $J=6.4$, 6.4 Hz), 2.64 (t, 2H, $J=7.0$, 7.0 Hz), 1.92 (s, 2H), 1.72-1.47 (m, 4H), 1.47-1.25 (m, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 140.2, 128.5, 128.3, 127.1, 62.7, 54.1, 49.3, 42.0, 32.6, 29.7, 29.1, 23.5. Analytical data are identical to literature values.²⁶

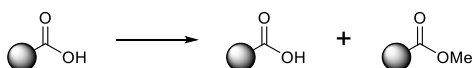
 **5-(benzyl(4-tert-butyltrimethylsilyl)oxymethylbenzyloxycarbonyl)amino)pentanol (10)** Silylether **9** (4.73 g, 18.7 mmol, 1.0 eq.) was dissolved in dry DCM (125 mL) and cooled to 0°C. Pyridine (3.0 mL, 37.5 mmol, 2.0 eq.) was added followed by addition of para-nitrophenylchloroformate (4.53 g, 22.5 mmol, 1.2 eq.) after which the solution was allowed to warm up to RT and stirred overnight. The reaction was concentrated *in vacuo* and coevaporated with toluene. The crude compound was dissolved in DMF (75 mL) and cooled to 0°C. To this mixture was added *N*-benzyl-5-aminopentanol (4.78 g, 23.0 mmol, 1.23 eq.) in DMF (20 mL) followed by addition of DIPEA (4.23 mL, 24.4 mmol, 1.3 eq.) The reaction mixture was stirred overnight, diluted with Et₂O and washed with H₂O. The aqueous layer wash back extracted with Et₂O, and the combined organic layers were washed multiple times with sat. aq. NaHCO₃. The solution was dried over MgSO₄, filtered and concentrated *in vacuo*. Column purification (PE/EtOAc, 8:1 → 3:1) yielded the title compound (8.02 g, 17.0 mmol, 90%). IR (neat): 1083, 1249, 1417, 1454, 1681, 1695, 2856, 2929, 2949, 3062, 3387, 3437 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.21-7.10 (m, 9H, CH_{arom}), 5.06 (s, 2H, CH₂ Cbz), 4.63 (s, 2H, CH₂ Bn), 4.39 (s, 2H, CH₂ Cbz), 3.46 (s, 2H, CH₂), 3.15 (s, 2H, CH₂), 1.41-1.20 (s, 6H, 3x CH₂), 0.85 (s, 9H, 3x CH₃ TBDMS), 0.00 (s, 6H, 2x CH₃ TBDMS); ¹³C NMR (126 MHz, CDCl₃): δ 141.5, 138.3, 135.7 (Cq), 128.7, 128.1, 127.5, 126.4 (CH_{arom}), 67.3 (CH₂), 65.0 (CH₂), 62.9 (CH₂), 50.7 (CH₂), 32.6 (CH₂), 26.1 (3x CH₃ TBDMS), 23.2 (CH₂), -5.1 (2x CH₃ TBDMS); HRMS: [M+H]⁺ calcd. for C₂₇H₄₂NO₄Si 472.28776, found 472.28773

 **5-(benzyl(4-hydroxymethylbenzyloxycarbonyl)amino)pentyl dimethoxytrityl ether (11)** Silylether **10** (7.50 g, 15.9 mmol, 1.0 eq.) was coevaporated twice with pyridine under an argon atmosphere, before being dissolved in pyridine (160 mL) and cooled to 0°C. To the mixture was added DMTr-Cl (5.92 g, 17.5 mmol, 1.1 eq.) and it was allowed to stir overnight. After overnight stirring, TLC analysis (hexans/EtOAc, 4:1) showed conversion of the starting material to a high running spot. The mixture was concentrated, dissolved in EtOAc and washed twice with H₂O, dried over MgSO₄ and concentrated *in vacuo*. The intermediate was coevaporated with toluene, dissolved in THF (160 mL) and cooled to 0°C. TBAF (1.0 M in THF, 25 mL, 1.6 eq.)

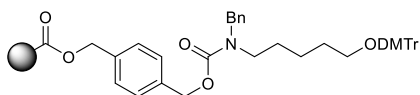
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was added and the green coloured reaction was stirred for 5h after which it was concentrated. The compound was dissolved in EtOAc, washed subsequently with H₂O, sat. aq. NaHCO₃ and sat. aq. NaCl. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (Tol/EtOAc + Et₃N, 9:1 → 4:1) yielded DMTr protected linker (10.2 g, 15.9 mmol, 100%). IR (neat): 1031, 1246, 1300, 1417, 1506, 1606, 1693, 2835, 2864, 2931, 3030, 3059, 3415, 3441 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, T=328K) δ 7.41 (d, *J* = 7.6 Hz, 2H, CH_{arom}), 7.35-7.07 (m, 16H, CH_{arom}), 6.80 (d, *J* = 8.5 Hz, 4H, CH_{arom}), 5.13 (s, 2H, CH₂ Cbz), 4.63 (s, 2H, CH₂ Bn), 4.46 (s, 2H, CH₂ Cbz), 3.76 (s, 6H, 2x CH₃ OMe), 3.21 (s, 2H, CH₂), 3.02 (s, 2H, CH₂), 1.69-1.24 (m, 6H, 3x CH₂); ¹³C NMR (101 MHz, CDCl₃) δ 158.6, 145.6, 140.9, 136.9 (Cq), 130.2, 128.6, 128.4, 128.2, 127.8, 127.4, 127.1, 126.7, 113.2 (CH_{arom}), 67.1 (CH₂), 65.1 (CH₂), 63.4 (CH₂), 55.3 (OMe), 29.9 (CH₂), 23.8 (CH₂); HRMS: [M+Na]⁺ calcd. for C₄₂H₄₅NO₆Na 682.31391, found 682.31390.

Synthesis of aminopentanol-functionalized Polystyrene (12)

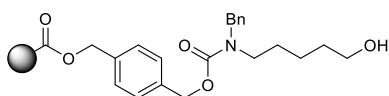


Carboxy Polystyrene (Rapp polymer, 5 g, 2.19 mmol/g, 11 mmol) was added to a fritted syringe and swollen in 32 mL DCM. The resin was purged with argon after which it was washed with DCM (3x), alternating DCM and hexane (3x), and DCM (2x). The resin was dried *in vacuo* at 45°C overnight. The dried resin was suspended in 60 mL THF and MeOH (1.03 mL, 25.4 mmol, 3 eq. with respect to Me₃SiCHN₂) was added. The suspension was shaken for 10 min followed by addition of Me₃SiCHN₂ (4.24 mL of 2.0M solution in hexanes, 8.47 mmol, 0.77 eq. with respect to the resin), whereupon the solution turned yellow. The reaction was allowed to shake overnight, after which it became colorless. The solution was filtered, and resin **12** was washed with DCM (4x), hexanes (4x) and THF (4x), and dried *in vacuo* at 45°C.



Carboxy Polystyrene **12** (Rapp polymer, 5g, ~0.51 mmol/g, 2.54 mmol) was swollen in DCM (60 mL) and the suspension was shaken for 1h. The solution was filtered and DCM (40 mL) was added to the resin. Compound **11** (5.04 g, 7.64

mmol, 3 eq.) was coevaporated twice with toluene under argon, dissolved in DCM (8.5 mL), along with addition of DIC (1.20 mL, 7.64 mmol, 3 eq.) and DMAP (0.03 g, 0.25 mmol, 0.1 eq.). An additional rinse with 5 mL DCM was performed before the resin was allowed to shake overnight. Then, MeOH (0.6 mL) was added and the suspension was shaken again. The mixture was filtered, and resin **13** was washed with alternating DCM and hexanes (4x), followed by DCM (3x). The resin was dried *in vacuo* at 45°C.



Solid support (3) DMT-functionalized resin **13** (5 g) was loaded into a fritted funnel and washed with 3% TCA (w/v in DCM, 60 mL) and shaken for 5 min. The orange solution was filtered and the procedure was repeated 4x. After the TCA washes, the orange

resin was washed 3x with DCM (60 mL), 3x with toluene (60 mL), 3x with DCM/MeOH (60 mL), 1x with MeOH (60 mL) and 4x DCM (60 mL). The resin was dried *in vacuo* to a constant weight of 4.22 g.

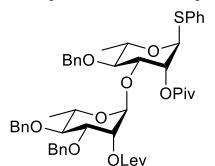
DMTr-assay (performed in duplicate) DMT-functionalized resin **13** (4.1 mg) was added to a 10 mL volumetric flask and treated with 10 mL 3% TCA/DCM (w/v). A 1 mL aliquot was taken and diluted 100x with the 3% TCA/DCM solution. Absorbance read at $\lambda = 503$ nm.

Loading calculation:

$$\begin{aligned} \frac{[(A_{503})(100 \text{ mL})]}{76} &= \text{mmol in final solution} \\ \left(\frac{\text{mmol in final solution}}{\text{volume aliquot}} \right) \times 10 \text{ mL} &= \text{mmol in initial solution} \\ &= \frac{(0.137)(100 \text{ mL})}{76} = 0.180 \mu\text{mol} \\ &= \left(\frac{76 \text{ mL} / \mu\text{mol}}{0.00018 \text{ mmol}} \right) \times 10 \text{ mL} = 0.00180 \text{ mmol} \\ \text{Loading} &= \frac{0.00180 \text{ mmol}}{0.0041 \text{ g}} = 0.44 \text{ mmol/g} \end{aligned}$$

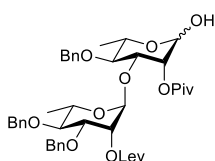
A loading of 0.44-0.47 mmol/g was determined.

Phenyl 4-O-Benzyl-2-O-Pivaloyl-3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)-1-thio- α -L-rhamnopyranoside (17) Compound **15**²⁰ (4.96 g, 11.52 mmol, 1.0 eq.) and imidate donor **14** (7.56 g, 13.32 mmol, 1.2 eq.) were coevaporated twice with anhydrous toluene under an argon atmosphere, after which they were dissolved in dry DCM (56 mL). The mixture was stirred on activated molecular sieves for 20 min at RT, and then cooled to 0°C. TfOH (0.1 mL, 1.12 mmol, 0.1 eq.) was added, and, after 135 min, TLC



analysis showed complete consumption of the acceptor, the reaction was quenched by addition of 0.3 mL Et₃N. The mixture was diluted with EtOAc, washed subsequently with sat. aq. NaHCO₃ and sat. aq. NaCl, dried over MgSO₄, and concentrated *in vacuo*. Crystallization from hot EtOH (5.65 g, 6.61 mmol), followed by a second crystallization of the mother liquid yielded the disaccharide as white crystals (6.65 g, 7.78 mmol, 69%). TLC: R_f 0.69 (PE/EtOAc, 2/1, v/v); IR (neat): 918, 987, 1026, 1039, 1060, 1082, 1138, 1454, 1479, 1732, 2873, 2910, 2933, 2974 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.47-7.41 (m, 2H, CH_{arom}), 7.36 (d, $J = 4.4$ Hz, 4H, CH_{arom}), 7.37-7.16 (m, 13H, CH_{arom}), 5.39 (dd, $J = 3.3, 1.8$ Hz, 1H, H-2'), 5.35 (d, $J = 1.8$ Hz, 1H, H-1), 5.29 (dd, $J = 3.2, 1.8$ Hz, 1H, H-2), 5.06 (d, $J = 1.9$ Hz, 1H, H-1'), 4.91 (d, $J = 11.5$ Hz, 1H, CHH Bn), 4.78 (d, $J = 10.8$ Hz, 1H, CHH Bn), 4.67-4.54 (m, 3H, CHH, CH₂ Bn), 4.46 (d, $J = 11.5$ Hz, 1H, CHH Bn), 4.26-4.16 (m, 1H, H-5), 4.11 (dd, $J = 9.4, 3.1$ Hz, 1H, H-3), 3.81 (dd, $J = 9.3, 3.4$ Hz, 1H, H-3'), 3.73-3.64 (m, 1H, H-5'), 3.49 (t, $J = 9.5$ Hz, 1H, H-4), 3.41 (t, $J = 9.4$ Hz, 1H, H-4'), 2.69-2.65 (m, 4H, 2x CH₂ Lev), 2.13 (s, 3H, CH₃ Lev), 1.33-1.22 (m, 6H, 2x CH₃-6), 1.20 (s, 9H, 3x CH₃ Piv); ¹³C NMR (126 MHz, CDCl₃): δ 206.0 (C=O Lev), 177.3, 171.8 (C=O Lev, Piv), 138.7, 137.8, 137.7, 133.6 (Cq), 132.1, 129.0, 128.5, 128.4, 128.3, 128.2, 127.9, 127.7, 127.6, 127.5, 127.4 (CH_{arom}), 99.7 (C-1'), 85.6 (C-1), 80.4 (C-4), 79.6 (C-4'), 77.7 (C-3), 77.2 (C-3'), 75.5 (CH₂ Bn), 74.8 (CH₂ Bn), 73.6 (C-2), 71.5 (CH₂ Bn), 69.3 (C-5), 69.1 (C-2'), 68.5 (C-5'), 39.0 (Cq Piv), 38.0 (CH₂ Lev), 29.8 (CH₃ Lev), 28.1 (CH₂ Lev), 27.1 (CH₃ Piv), 17.9 (C-6), 17.8 (C-6'). HRMS: [M+H]⁺ calcd. for C₄₉H₆₂NO₁₁S 872.40381, found 872.40453.

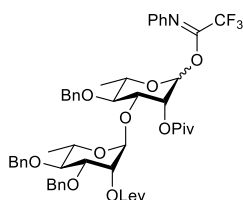
4-O-Benzyl-2-O-Pivaloyl-3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)- α / β -L-rhamnopyranoside (19) Compound **17** (2.76 g, 3.23 mmol, 1.0 eq.) was dissolved in acetone/H₂O (3:1, 16 mL) and cooled to 0°C. NBS (1.73 g, 9.69 mmol, 3.0 eq.) was added and the reaction was stirred overnight. TLC analysis showed conversion of the starting material to a lower running spot and the mixture was quenched with sat. aq. Na₂S₂O₃. The mixture was diluted with EtOAc and the organic layer was washed with



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sat. aq. NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 4:1 → 1:1) yielded the title hemiacetal (1.60 g, 2.10 mmol, 65%). Spectroscopic data are reported for the major (α) isomer. TLC: R_f 0.26 (PE/EtOAc, 2/1, v/v); IR (neat): 1064, 1082, 1134, 1163, 1363, 1708, 1776, 2875, 2933, 2974, 3381 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.39-7.15 (m, 15H, CH_{arom}), 5.36 (dd, *J* = 3.1, 1.8 Hz, 1H, H-2'), 5.05-4.99 (m, 3H, H-1, H-1', H-2), 4.90 (d, *J* = 11.4 Hz, 1H, CHH Bn), 4.74 (d, *J* = 10.8 Hz, 1H, CHH Bn), 4.66-4.51 (m, 3H, CHH Bn, CH₂ Bn), 4.43 (d, *J* = 11.5 Hz, 1H, CHH Bn), 4.16 (dd, *J* = 9.5, 3.0 Hz, 1H, H-3'), 3.97-3.87 (m, 1H, H-5), 3.79 (dd, *J* = 9.3, 3.3 Hz, 1H, H-3), 3.71-3.62 (m, 1H, H-5'), 3.48 (s, 1H, OH), 3.38 (dt, *J* = 9.4, 6.7 Hz, 2H, H-4, H-4'), 2.73-2.60 (m, 4H, 2x CH₂ Lev), 2.15 (s, 3H, CH₃ Lev), 1.29-1.17 (m, 15H, 2x CH₃ C-6, C-6', 3x CH₃ Piv); ¹³C NMR (126 MHz, CDCl₃) δ 206.5 (C=O Lev), 177.7, 171.9 (C=O Lev, Piv), 138.8, 138.8, 137.9, 137.9 (Cq), 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.6, 127.5 (CH_{arom}), 99.7 (C-1'), 91.7 (C-1), 80.4, 79.7 (C-4, C-4'), 77.3 (C-3'), 77.1 (C-3), 75.5 (CH₂ Bn), 74.9 (CH₂ Bn), 72.5 (C-2), 71.6 (CH₂ Bn), 69.4 (C-2'), 68.4, 67.7 (C-5, C-5'), 39.0 (Cq), 38.1 (CH₂ Lev), 29.9 (CH₃ Lev), 28.2 (CH₂ Lev), 27.2 (3x CH₃ Piv), 18.2, 17.9 (2x CH₃ C-6, C-6'); HRMS: [M+Na]⁺ calcd. for C₄₃H₅₄O₁₂Na 785.35075, found 785.35106.

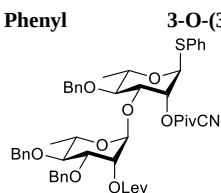
4-O-Benzyl-2-O-Pivaloyl-3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)-1-(N-phenyl-trifluoroacetimidoyl)- α / β -L-rhamnopyranoside (1)



To a solution of hemiacetal **19** (1.66 g, 2.18 mmol, 1.0 eq.) in acetone (11 mL) at 0°C were added *N*-phenyl trifluoroacetimidoyl chloride (0.41 mL, 2.62 mmol, 1.2 eq.) followed by Cs₂CO₃ (1.07 g, 3.27 mmol, 1.5 eq.). The solution was allowed to stir for 3h after which it was filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 6:1 → 2:1) yielded the title compound as a clear yellow oil (1.81 g, 4.15 mmol, 88%). Spectroscopic data are reported for the major

(α) isomer. TLC: R_f 0.67 (PE/EtOAc, 2/1, v/v); IR (neat): 989, 1028, 1116, 1138, 1207, 1454, 1597, 1716, 1737, 2908, 2976 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.49-7.22 (m, 17H, CH_{arom}), 7.08 (t, *J* = 7.5 Hz, 1H, CH_{arom}), 6.81 (d, *J* = 7.7 Hz, 2H, CH_{arom}), 6.08 (s, 1H, H-1), 5.39 (dd, *J* = 3.0, 1.8 Hz, 1H, H-2'), 5.22 (s, 1H, H-2), 5.07 (s, 1H, H-1'), 4.92 (d, *J* = 11.4 Hz, 1H, CHH Bn), 4.77 (d, *J* = 10.7 Hz, 1H, CHH Bn), 4.69-4.54 (m, 3H, CHH Bn, CH₂ Bn), 4.47 (d, *J* = 11.5 Hz, 1H, CHH Bn), 4.22-4.15 (m, 1H, H-3), 3.90-3.75 (m, 2H, H-5, H-3'), 3.75-3.63 (m, 1H, H-5'), 3.49 (t, *J* = 9.5 Hz, 1H, H-4), 3.41 (t, *J* = 9.3 Hz, 1H, H-4'), 2.75-2.60 (m, 4H, 2x CH₂ Lev), 2.15 (s, 3H, CH₃ Lev), 1.39-1.11 (m, 15H, 2x CH₃ C-6, C-6', 3x CH₃ Piv); ¹³C NMR (101 MHz, CDCl₃) δ 206.2 (C=O Lev), 177.3, 171.9 (C=O Lev, Piv), 143.4, 138.8, 137.9, 137.5 (Cq), 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 124.5, 119.4 (CH_{arom}), 99.8 (C-1'), 79.7 (C-4'), 77.3 (C-3), 76.6 (C-3'), 75.8 (CH₂ Bn), 74.9 (CH₂ Bn), 71.7 (CH₂ Bn), 70.5 (C-2), 70.5 (C-5), 69.4 (C-2'), 68.7 (C-5'), 39.1 (Cq), 38.1 (CH₂ Lev), 29.9 (CH₃ Lev), 28.2 (CH₂ Lev), 27.2 (3x CH₃ Piv), 18.2, 17.9 (2x CH₃ C-6, C-6'); HRMS: [M+Na]⁺ calcd. for C₅₁H₅₈F₃NO₁₂Na 956.38033, found 956.38091.

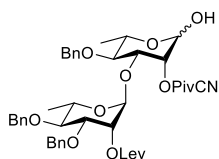
Phenyl



3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)-4-O-benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-1-thio- α -L-rhamnopyranoside (18) Imidate donor **14** (4.00 g, 6.51 mmol, 1.1 eq.) and acceptor **16** (2.70 g, 5.92 mmol, 1.0 eq.) were coevaporated two times with anhydrous toluene under an argon atmosphere before being dissolved in distilled DCM (59 mL) and the mixture was stirred at room temperature for 30 min over activated molecular sieves (3Å). The reaction was cooled to 0 °C and TfOH (0.05 mL, 0.59 mmol, 0.1 eq.) was added. After 50 min the reaction was quenched by addition of 1.0 mL Et₃N.

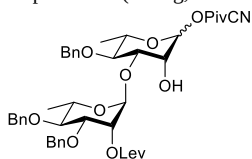
The reaction mixture was diluted with Et₂O and washed with sat. aq. NaHCO₃, H₂O and sat. aq. NaCl. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. A quick column purification (PE/EtOAc, 6:1 → 1:1) followed by crystallization from hot EtOH yielded the target disaccharide as a white powder (4.57 g, 5.19 mmol, 88%).

4-O-Benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-



rhamnopyranosyl)- α/β -L-rhamnopyranoside (20) Compound **18** (0.260 g, 0.295 mmol, 1.0 eq.) was dissolved in acetone/H₂O (1.2 mL/0.4 mL) and cooled to 0°C. NBS (0.16 g, 0.899 mmol, 3.0 eq.) was added and the reaction was stirred for 3h, after which TLC analysis showed conversion of the starting material in a lower running spot. The reaction was quenched by addition of sat. aq. Na₂S₂O₃, and diluted with EtOAc. The organic layer was washed with sat. aq. NaHCO₃, sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (PE/EtOAc, 4:1 → 1:1) yielded the hemiacetal (0.209 g, 0.264 mmol, 89%). Spectroscopic data are reported for the major (α) isomer. ¹H NMR (500 MHz, CDCl₃): δ 7.39-7.20 (m, 15H, CH_{arom}), 5.37 (dd, J = 3.0, 1.9 Hz, 1H, H-2'), 5.06 (dd, J = 3.1, 1.9 Hz, 1H, H-2), 5.02 (m, 2H, H-1, H-1'), 4.91 (d, J = 11.2 Hz, 1H, CHH Bn), 4.76 (d, J = 10.9 Hz, 1H, CHH Bn), 4.68-4.54 (m, 3H, CH₂ Bn, CHH Bn), 4.46 (d, J = 11.9 Hz, 1H, CHH Bn), 4.18 (dd, J = 9.5, 3.2 Hz, 1H, H-2), 4.01-3.89 (m, 1H, H-5 or H-5'), 3.77 (dd, J = 9.2, 3.3 Hz, 1H, H-3'), 3.62-3.51 (m, 1H, H-5 or H-5'), 3.39 (m, 2H, H-4, H-4'), 2.78-2.58 (m, 4H, CH₂ Lev), 2.58-2.40 (m, 2H, CH₂ PivCN), 2.15 (s, 3H, CH₃ Lev), 1.37-1.17 (m, 12H, 2x CH₃ PivCN, 2x CH₃-6); ¹³C NMR (126 MHz, CDCl₃): δ 206.5 (C=O Lev), 174.3, 171.9 (C=O Lev, PivCN), 138.6, 137.9, 137.8 (Cq), 128.6, 128.4, 128.4, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7 (CH_{arom}), 117.4 (CN), 99.8 (C-1'), 91.4 (C-1), 80.3 (C-4 or C-4'), 79.7 (C-4 or C-4'), 77.0 (C-3'), 76.6 (C-3), 75.6 (CH₂ Bn), 75.0 (CH₂ Bn), 73.6 (C-2), 71.3 (CH₂ Bn), 69.1 (C-2'), 68.5 (C-5 or C-5'), 67.8 (C-5 or C-5'), 41.0 (Cq), 38.1 (CH₂ Lev), 29.9 (CH₃ Lev), 29.6 (CH₂ Lev), 28.2 (CH₂ PivCN), 27.8 (CH₂ PivCN), 24.9, 24.8 (2x CH₃ PivCN), 18.1, 17.9 (2x CH₃ C-6, C-6').

Compound **18** (3.65 g, 4.15 mmol, 1 eq.) was dissolved in DCM (40 mL) and cooled to 0°C. NIS (1.03 g, 4.57 mmol, 1.1 eq.) was added followed by the dropwise addition of TFA (0.35 mL, 4.57 mmol, 1.1 eq.), after which the reaction turned purple. After 340 min, the reaction was quenched by addition of 50 mL sat. aq. Na₂S₂O₃. The mixture was diluted with 60 mL DCM and washed with 60 mL sat. aq. NaHCO₃. The aqueous layers were extracted 2x with DCM and the combined organic layers

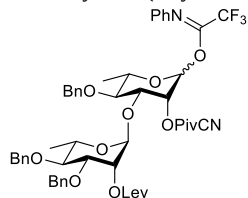


were washed with sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (PE/EtOAc, 4:1 → 1:1) resulted a mixture of **20** and **20a** 73% yield (2.37 g, 3.01 mmol). TLC: R_f 0.35 (PE/EtOAc, 2/1, v/v); IR (neat): 733, 839, 988, 1040, 1063, 1135, 1363, 1454, 1497, 1717, 1737, 2933, 2976; ¹H NMR (500 MHz, Chloroform-d) δ 7.41-7.20 (m, 40H), 6.07 (d, J = 2.2 Hz, 1H), 5.38 (dd, J = 3.3, 1.6 Hz, 3H), 5.13 (t, J = 2.8 Hz, 1H), 5.10-5.05 (m, 3H), 5.05-5.01 (m, 3H), 4.92 (dd, J = 11.2, 2.7 Hz, 3H), 4.81-4.73 (m, 3H), 4.67-4.54 (m, 8H), 4.53-4.43 (m, 3H), 4.18 (dd, J = 9.5, 3.2 Hz, 1H), 4.13 (dd, J = 9.3, 3.3 Hz, 1H), 3.86-3.72 (m, 4H), 3.63-3.49 (m, 4H), 3.45-3.34 (m, 4H), 2.75-2.60 (m, 11H), 2.56-2.40 (m, 5H), 2.16 (d, J = 2.8 Hz, 8H), 1.37-1.22 (m, 33H). ¹³C NMR (126 MHz, CDCl₃) δ 206.4, 206.3, 173.9, 171.9, 171.9, 155.6, 155.2, 138.0, 138.0, 137.9, 137.9, 137.8, 137.3, 128.7, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.7, 127.6, 127.5, 117.8, 117.4, 117.2, 115.4, 113.1, 75.9, 75.8, 75.6, 75.1, 75.1, 74.9, 74.8, 72.1, 71.5, 71.4, 71.4, 71.3, 70.6, 69.7, 69.1, 68.9, 41.1, 41.1, 38.2,

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38.1, 38.1, 29.8, 29.7, 28.2, 28.2, 28.2, 28.0, 27.9, 27.8, 27.8, 18.1, 18.1, 18.0. HRMS: $[M+NH_4]^+$ calcd. for $C_{44}H_{57}N_2O_{12}$ 805.39060, found 805.39077.

4-O-Benzyl-2-O-(3-cyano-2,2-dimethylpropanoyl)-3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-



rhannopyranosyl)-1-(N-phenyl-trifluoroacetimidoyl)-

α/β -L-

rhannopyranoside (2)

To a solution of mixture hemiacetal **20** and **20a** (4.15 g, 5.27 mmol, 1 eq.) in acetone (26 mL) at 0°C were added *N*-phenyl trifluoroacetimidoyl chloride (0.98 mL, 6.32 mmol, 1.2 eq.) followed by Cs_2CO_3 (2.57 g, 7.9 mmol, 1.5 eq.). The solution was allowed to stir over night after which it was diluted with EtOAc and washed subsequently with H_2O and satd. aq.

NaCl. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 6:1 $\rightarrow$ 1:1) yielded the title compound as a clear yellow oil (3.98 g, 4.15 mmol, 79%). TLC: R_f 0.69 (PE/EtOAc, 2/1, v/v); IR (neat): 751, 1044, 1137, 1119, 1137, 1364, 1453, 1597, 1720, 1741, 2935 cm^{-1} ; Spectroscopic data are reported for the major (α) isomer. 1H NMR (500 MHz, $CDCl_3$): δ 7.39-7.18 (m, 17H, CH_{arom}), 7.12-7.04 (m, 1H, CH_{arom}), 6.83-6.77 (m, 2H, CH_{arom}), 6.00 (s, 1H, H-1), 5.38 (dd, J = 3.3, 1.9 Hz, 1H, H-2'), 5.24 (dd, J = 3.3, 2.0 Hz, 1H, H-2), 5.06 (d, J = 2.0 Hz, 1H, H-1), 4.90 (d, J = 11.3 Hz, 1H, CHH Bn), 4.79 (d, J = 10.9 Hz, 1H, CHH Bn), 4.66-4.54 (m, 3H, CHH, CH_2 Bn), 4.48 (dd, J = 11.8, 3.2 Hz, 1H, CHH Bn), 4.18 (dd, J = 9.5, 3.2 Hz, 1H, H-3), 3.92-3.81 (m, 1H, H-5), 3.79 (dd, J = 9.1, 3.4 Hz, 1H, H-3'), 3.67-3.57 (m, 1H, H-5'), 3.53 (t, J = 9.5 Hz, 1H, H-4), 3.41 (t, J = 9.3 Hz, 1H, H-4'), 2.72-2.55 (m, 4H, CH_2 Lev), 2.54-2.40 (m, 2H, CH_2 PivCN), 2.14 (s, 3H, CH_3 Lev), 1.39-1.21 (m, 12H, 2x CH_3 PivCN, 2x CH_3 -6); ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.8 (C=O Lev), 173.9, 171.8 (C=O Lev, PivCN), 143.4, 138.8, 138.2, 137.7 (Cq), 128.9, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 124.7, 124.6, 119.5, 119.4, 117.1 (CH_{arom}), 100.0 (C-1'), 93.7 (C-1), 79.9 (C-4), 79.8 (C-4'), 77.3 (C-3'), 76.9 (C-3), 75.8 (CH_2 Bn), 75.1 (CH_2 Bn), 71.8 (C-2), 71.6 (CH_2 Bn), 70.9 (C-5), 69.4 (C-2'), 68.9 (C-5'), 41.1 (Cq), 38.2 (CH_2 Lev), 29.8 (CH_3 Lev), 28.4 (CH_2 Lev), 28.0 (CH_2 PivCN), 25.0, 24.9 (2x CH_3 PivCN), 18.2 (C-6), 18.0 (C-6'); HRMS: $[M+NH_4]^+$ calcd. $C_{52}H_{61}F_3N_3O_{12}$ 976.42019, found 976.42045.

Methods for automated synthesis

The washing solvents are pre-dried 24 h before use on 4Å molecular sieves and are of HPLC grade. Activator and deblock solutions are freshly prepared using the pre-dried solvents.

Activator: 0.09 M trifluoromethanesulfonic acid in DCE

Deblock: 0.12 M hydrazine acetate in pyridine/AcOH (4/1, v/v)

Method A. Agitation of the resin during washing

After addition of the appropriate solvent, an argon-flow is applied from the bottom of the RV, suspending the resin in solution. The argon-flow is applied for 15 seconds after which the RV is emptied to the waste.

Method B. Agitation of the resin during coupling/deblock

After addition of the solvent, an argon-flow is applied from the bottom of the RV for 10 s, suspending the resin in the solution. After 10 s, the argon flow is interrupted, and the resin is allowed to settle for 20 s.

Method C. Swelling of the resin

Dry resin is applied to the RV and washed with DCM (3x), alternating THF/Hexane (3x), THF (1x) and DCM (3x).

Method D. Coupling cycle

The resin is suspended in DCM. The RV is emptied, followed by addition of the building block solution (1 mL) while being agitated. The delivery line is flushed with an additional 0.5 mL DCM to the RV. The temperature is set to 0°C while employing method B. A 10 min pause is started, after which the activator solution (300 µL) is added, keeping the temperature below 0 °C. The delivery line is flushed with an additional 0.5 mL DCM to the RV. Method B is applied for 1h, after which the RV is emptied and the mixture is collected in the fraction collector. The resin is washed with DCM (3x 2 mL), and the washes are drained to the fraction collector.

Method E. Deblock cycle

The resin is washed with DMF (4x 3 mL), running method A. The deblock solution is added (3 mL) and the temperature is set to 40 °C, followed by a 5 minute incubation applying method B. The temperature is kept at 40 °C, after which the solid support is incubated 10 minutes applying protocol B. Then the RV is emptied to the waste. The resin is washed with DMF (3x 3 mL), running method A.

Method F. Washing of the resin after coupling

The temperature is set to 20 °C. The resin is washed with MeOH (3x 2 mL), alternating THF/Hexane (6x 2 mL), THF (2x 2 mL), DCM (5x 3 mL), all applying method A.

Method G. Washing of the resin after deblock

The temperature is set to 20 °C. The resin is washed with DMF (4x 3 mL), DCM (4x 3 mL), alternating THF/Hexane (6x 3 mL), 0.01M AcOH in THF (6x 3 mL), THF (4x 3 mL) and DCM (8x 5 mL).

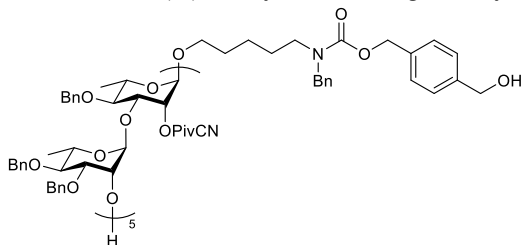
Method H. Suspending the resin for isolation

To the dry resin is added a mixture of DCM/MeOH (3:2; 5 mL), after which the resin is agitated for 15 s. The suspended resin is collected from the RV. The procedure is repeated four times.

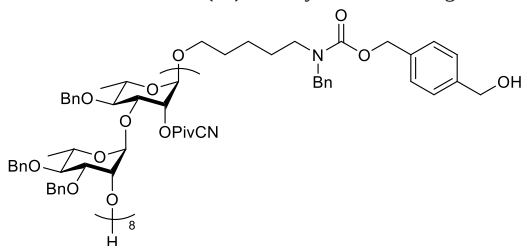
Method	# Cycles	Description	Time	Temperature
C	1	Swelling of the resin		RT
D	1	Coupling: 3 eq. donor, 0.3 eq. TfOH	60 min	0°C
E	3	Washing of the resin after coupling		0°C
F	3	Deblock: 8 eq. H ₂ NNH ₂ •AcOH	15 min	40°C
G	1	Washing of the resin after deblock		RT

Automated synthesis of rhamnose fragments. The reaction vessel is charged with CarboxyPolystyrene **3** (100 mg, 45 µmol) and method C was applied to prepare the resin for synthesis. Then method D and E for coupling and deprotection are repeated 5 times for decasaccharide **22** and 8 times to obtain hexadecasaccharide **23**. Method H was used to isolate the resin from the reaction vessel. The resin was dried overnight. After cleavage from solid support the rhamnose fragments were analyzed by LC/MS.

Decarhamnoside (22) The dry resin was charged in a syringe with screw cap and suspended in THF/MeOH (2 mL, 1:1) followed by addition of NaOMe (0.08 mL, 0.54M NaOMe/MeOH, 1 eq.). The resin was shaken overnight. The solution was filtered and the remaining resin was washed with MeOH (5x 4 mL). The combined filtrate and washes were neutralized with 2-3 drops of AcOH and concentrated *in vacuo*. The cleavage procedure was repeated once to obtain the mixture containing **22** as an amorphous solid (0.161 g).

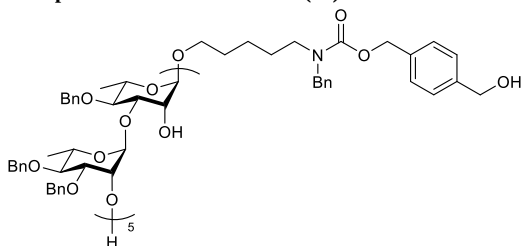


Hexadecarhamnoside (23) The dry resin was charged in a syringe with screw cap and suspended in THF/MeOH (2 mL, 1:1) followed by addition of NaOMe (0.08 mL, 0.54M NaOMe/MeOH, 1 eq.). The resin was shaken overnight. The solution was filtered and the remaining resin was washed with MeOH (5x 4 mL). The combined filtrate and washes were neutralized with 2-3 drops of AcOH and concentrated *in vacuo*. The cleavage procedure was repeated once to obtain the mixture containing **23** as an amorphous solid (0.198 g).



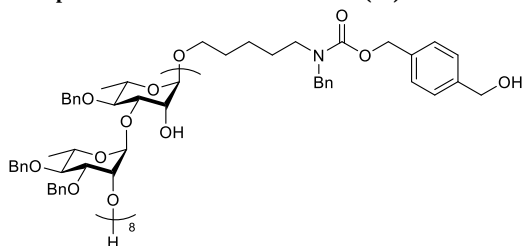
General procedure for complete removal of PivCN groups. The crude rhamnoside mixture was dissolved in THF/MeOH (0.6-2 mL, 1:1), and treated with a 0.54M NaOMe/MeOH (0.7-2 eq.) solution. The reaction was monitored by LC/MS and allowed to stir overnight. Additional 0.54M NaOMe/MeOH was added when LC/MS analysis indicated incomplete removal of the PivCN groups. If the deprotection proceeded slowly, the mixture was neutralized, concentrated *in vacuo* and treated with the conditions mentioned *vide supra*. Purification by size exclusion chromatography (LH20, eluted with DCM/MeOH, 1/1,v/v) or HPLC yielded the target rhamnoside fragments.

Semi-protected decarhamnoside (26) The crude rhamnoside mixture (0.162 g) was dissolved in THF/MeOH (4 mL, 1:1) and treated with 0.16 mL NaOMe (0.54M NaOMe/MeOH). After overnight stirring, LC/MS analysis indicated incomplete removal of the PivCN groups, after which the mixture was neutralized with AcOH and concentrated *in vacuo*. The mixture was redissolved in THF/MeOH (2 mL, 1:1), treated with 0.1 mL NaOMe (0.54M NaOMe/MeOH) and stirred overnight. After overnight stirring, 0.08 mL NaOMe (0.54M NaOMe/MeOH) was added, followed by 0.16 mL NaOMe (0.54M NaOMe/MeOH) after 6.5h, whereafter LC/MS analysis indicated complete removal of the PivCN groups. The mixture was neutralized with AcOH and concentrated *in vacuo* and coevaporated once with toluene. The target



decarhamnoside was isolated using RP-HPLC purification (C4 column, gradient 70 $\rightarrow$ 90 , 20 min per run) as a white solid (37.4 mg, 11.8 μ mol, 26% based on 45 μ mol resin). IR (neat): 736, 1028, 1041, 1070, 1126, 1207, 1361, 1454, 1496, 1681, 2927, 3030, 3377 cm^{-1} ; ^1H NMR (500 MHz, $\text{MeCN-}d_3$, $T=328\text{K}$) δ 7.42-7.18 (m, 84H, CH_{arom}), 5.11-5.06 (m, 4H, CH_2 linker-CBz, 2x H-1), 5.04 (s, 1H, H-1), 4.98 (s, 1H, H-1), 4.87-4.49 (m, 33H), 4.45 (s, 2H, CH_2 linker), 4.05-3.95 (m, 8H), 3.95-3.77 (m, 14H), 3.72 (m, 4H), 3.66-3.51 (m, 2H), 3.52-3.34 (m, 9H), 3.32 (s, 1H), 3.21 (t, $J = 7.3$ Hz, 2H, CH_2 linker), 3.06 (s, 5H), 1.53-1.46 (m, 4H, CH_2 linker), 1.35-1.24 (m, 7H, CH_2 linker, CH_3 -6), 1.24-1.11 (m, 17H, CH_3 -6), 1.10-1.00 (m, 10H, CH_3 -6); ^{13}C NMR (126 MHz, $\text{MeCN-}d_3$, $T=328\text{K}$) δ 140.1 (Cq), 129.6, 129.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 129.0, 129.0, 128.8, 128.8, 128.7, 128.7, 128.3, 128.0 (CH_{arom}), 103.2, 103.0, 103.0, 103.0, 102.4, 101.2, 101.0 (10x C-1), 81.5, 81.4, 81.3, 81.2, 81.0, 80.9, 80.8, 80.8, 80.0 (10x C-3, 10x C-4), 77.4, 77.3, 77.2 (C-2), 76.1, 76.0, 75.9 (CH_2), 72.9, 72.3, 72.2 (CH_2), 72.1 (10 xC-2), 69.6, 69.6, 69.3, 69.0, 68.3, 67.8 (10x C-5), 64.8 (CH_2), 51.5 (CH_2), 30.1 (CH_2), 24.4 (CH_2), 18.8, 18.7, 18.7, 18.6 (10x CH_3 -6). HRMS: $[\text{M} + \text{NH}_4]^+$ calcd. for $\text{C}_{186}\text{H}_{221}\text{N}_2\text{O}_{44}$ 3188.51782, found 3188.51214.

Semi-protected hexadecarhamnoside (29) The crude rhamnoside mixture (0.199 g) was dissolved in

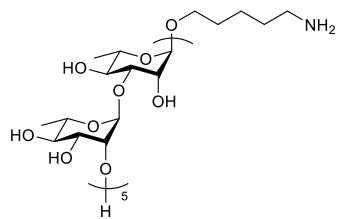


THF/MeOH (2 mL, 1:1) and treated with 0.10 mL NaOMe (0.54M NaOMe/MeOH). After 2h, an additional 0.24 mL NaOMe (0.54M NaOMe/MeOH) was added, followed by another 0.10 mL after 4h. After overnight stirring, LC/MS analysis indicated complete removal of all PivCN groups after which the mixture was neutralized by addition of 2-3 drops AcOH. The mixture was

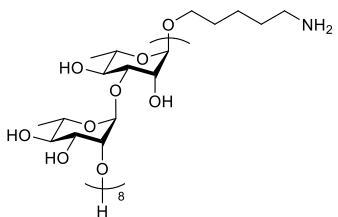
concentrated *in vacuo* and coevapotrated with toluene once. The target hexadecarhamnoside was isolated using RP-HPLC purification (C4 column, gradient 70 $\rightarrow$ 90 , 20 min per run) as a white solid (20.3 mg, 4.2 μ mol, 9.3% based on 45 μ mol resin). IR (neat): 750, 1051, 1129, 1454, 1671, 2917 cm^{-1} ^1H NMR (600 MHz, $\text{MeCN-}d_3$, $T=328\text{K}$): δ 7.39 (d, $J = 7.4$ Hz, 2H), 7.36-7.17 (m, 129H), 5.12-5.04 (m, 8H), 5.03 (d, $J = 1.9$ Hz, 1H), 4.97 (d, $J = 1.7$ Hz, 1H), 4.87-4.47 (m, 60H), 4.44 (s, 2H), 4.08-3.93 (m, 16H), 3.94-3.80 (m, 24H), 3.80-3.63 (m, 8H), 3.63-3.51 (m, 4H), 3.51-3.34 (m, 16H), 3.33-3.24 (m, 2H), 3.20 (t, $J = 7.3$ Hz, 2H), 3.17-2.79 (m, 9H), 1.55-1.43 (m, 4H), 1.36-1.22 (m, 8H), 1.21-1.10 (m, 29H), 1.09-0.95 (m, 21H); ^{13}C NMR (151 MHz, $\text{MeCN-}d_3$, $T=328\text{K}$): δ 140.1, 140.0, 140.0, 129.6, 129.5, 129.4, 129.4, 129.4, 129.3, 129.3, 129.2, 129.1, 129.1, 129.0, 128.9, 128.9, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.2, 127.9, 118.3, 103.1, 103.0, 102.9, 102.9, 102.9, 102.3, 102.3, 100.9, 81.4, 81.3, 81.3, 81.2, 81.2, 81.1, 81.0, 80.8, 80.7, 79.9, 77.3, 77.3, 77.2, 77.0, 76.0, 75.9, 75.9, 72.8, 72.8, 72.2, 72.1, 72.0, 69.5, 69.5, 69.2, 68.9, 64.7, 51.4, 30.0, 24.3, 18.8, 18.7, 18.6, 18.6, 18.6, 18.5, 1.8, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 0.9; MALDI-TOF m/z $[\text{M} + \text{Na}]^+$ calc. for $\text{C}_{285}\text{H}_{331}\text{NO}_{68}\text{Na}$ 4878.2, found 4884.9.

General procedure for the hydrogenation. The oligosaccharide was dissolved in $\text{H}_2\text{O}/\text{THF}/\text{tBuOH}$ (3:1.3:1.3) followed by addition of several drops of AcOH. The solution was purged with N_2 for 5 min, after which $\text{Pd}(\text{OH})_2/\text{C}$ (10-20 mg) was added followed by another purge with N_2 for 5 min. The solution was purged for 5 min with H_2 and kept under a H_2 atmosphere overnight. The mixture was filtered over a Whatmann filter, and rinsed with the $\text{H}_2\text{O}/\text{THF}/\text{tBuOH}$ mixture and H_2O .

Decarhamnoside (5) Compound **26** (19.1 mg, 6 μ mol) was dissolved in H₂O/THF/tBuOH (1.6 mL, 3:1.3:1.3) and 4-5 drops of AcOH were added. The solution was purged with N₂ for 5 min after which Pd(OH)₂/C (20 mg) was added, followed by another purge with N₂ for 5 min. The solution was purged for 5 min with H₂ and kept under a H₂ atmosphere overnight. After overnight stirring, the mixture was filtered through a Whatmann filter and concentrated *in vacuo*. Purification by size exclusion chromatography (LH20, eluted with MeOH/H₂O, 9/1,v/v) and analysis by H-NMR indicated the presence of aromatic signals. The hydrogenation procedure was repeated once. Purification using gel filtration (HW-40, eluted with NH₄OAc) and subsequent lyophilization yielded the target decarhamnoside as a white powder (5.3 mg, 3.4 μ mol, 57%). ¹H NMR (500 MHz, D₂O): δ 5.11-5.06 (m, 4H), 4.93 (d, *J* = 1.8 Hz, 1H), 4.87-4.81 (m, 4H), 4.05 (t, *J* = 2.7 Hz, 4H), 3.99-3.93 (m, 5H), 3.91-3.87 (m, 1H), 3.86-3.80 (m, 4H), 3.78-3.70 (m, 9H), 3.70-3.56 (m, 8H), 3.49-3.31 (m, 11H), 2.89 (t, *J* = 7.6 Hz, 2H), 1.64-1.52 (m, 4H), 1.42-1.29 (m, 2H), 1.24-1.09 (m, 30H); ¹³C NMR (126 MHz, D₂O): δ 102.5, 102.2, 102.2, 101.0, 101.0, 100.9, 99.7, 78.3, 78.2, 78.1, 77.7, 77.6, 77.6, 72.3, 72.1, 71.9, 71.8, 71.5, 70.3, 70.3, 70.1, 70.1, 70.1, 70.0, 69.5, 69.5, 69.4, 69.3, 68.8, 67.6, 39.5, 28.2, 26.7, 22.8, 22.6, 16.9, 16.8, 16.8, 16.7, 16.6; HRMS: [M+H]⁺ calcd. for C₆₅H₁₁₄NO₄₁ 1564.68608, found 1564.68732.

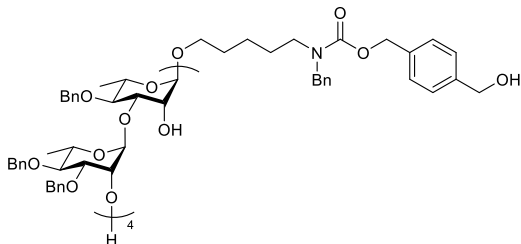


Hexadecarhamnoside (8) Compound **29** (7.2 mg, 1.5 μ mol) H₂O/THF/tBuOH (1.0 mL, 3:1.3:1.3) and 4-5 drops of AcOH were added. The solution was purged with N₂ for 5 min after which Pd(OH)₂/C (8 mg) was added, followed by another purge with N₂ for 5 min. The solution was purged for 5 min with H₂ and kept under a H₂ atmosphere overnight. After overnight stirring, the mixture was filtered through a Whatmann filter and concentrated *in vacuo*. Purification using gel filtration (HW-40, eluted with NH₄OAc) and subsequent lyophilization yielded the target hexadecarhamnoside as a white powder (1.8 mg, 0.75 μ mol, 50%). ¹H NMR (500 MHz, D₂O): δ 5.26-5.12 (m, 7H), 5.04 (d, 2H, *J* = 1.5 Hz), 4.95 (s, 9H), 4.16 (s, 7H), 4.07 (s, 8H), 3.99 (s, 1H), 3.95 (dd, 7H, *J* = 9.8, 2.8 Hz), 3.88-3.68 (m, 25H), 3.59-3.43 (m, 17H), 2.99 (t, 2H, *J* = 7.5 Hz), 1.74-1.59 (m, 4H), 1.52-1.39 (m, 2H), 1.35-1.20 (m, 48H); ¹³C NMR (126 MHz, D₂O): δ 102.4, 102.1, 102.1, 100.9, 100.9, 99.6, 78.2, 78.1, 78.0, 77.6, 77.4, 72.2, 72.0, 71.7, 71.4, 70.2, 70.1, 70.0, 70.0, 69.9, 69.4, 69.4, 69.3, 69.2, 68.7, 67.5, 39.4, 28.1, 26.6, 23.3, 22.5, 16.8, 16.7, 16.7, 16.6, 16.5. HRMS: [M+H]⁺ calcd. for C₁₀₁H₁₇₄NO₆₅ 2442.03693, found 2442.03607.



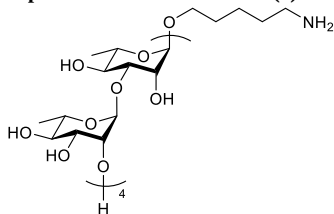
Isolation of deletion fragments

Octarhamnoside (25) Obtained as byproduct from **22**. (10.4 mg, 4.0 μ mol). ¹H NMR (500 MHz, MeCN-*d*₃, T=328K): δ 7.42 – 7.37 (m, 2H), 7.36 – 7.13 (m, 67H), 5.13 – 5.07 (m, 4H), 5.04 (d, *J* = 2.1 Hz, 1H), 4.98 (d, *J* = 1.8 Hz, 1H), 4.88 – 4.73 (m, 10H), 4.72 – 4.52 (m, 20H), 4.45 (s, 2H), 4.06 – 3.96 (m, 7H), 3.95 – 3.78 (m, 13H), 3.78 – 3.67 (m, 3H), 3.63 – 3.51 (m, 2H), 3.51 – 3.36 (m, 8H), 3.37 – 3.28 (m, 1H), 3.22 (t, *J* = 7.3 Hz, 2H), 3.05 (s, 5H), 1.56 – 1.44 (m, 4H), 1.30 (s, 6H), 1.24 –



1.12 (m, 15H), 1.11 – 1.02 (m, 9H); ^{13}C NMR (126 MHz, $\text{MeCN-}d_3$, $T=328\text{K}$): δ 140.1, 129.6, 129.6, 129.5, 129.4, 129.4, 129.3, 129.3, 129.2, 129.2, 129.1, 129.1, 129.0, 129.0, 128.9, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.3, 128.3, 127.9, 111.2, 103.1, 102.9, 102.3, 101.0, 81.4, 81.3, 81.3, 81.3, 81.1, 81.1, 81.0, 80.9, 80.8, 79.9, 77.2, 77.1, 76.1, 75.9, 75.9, 72.8, 72.8, 72.3, 72.1, 72.1, 69.6, 69.5, 69.2, 69.0, 68.2, 67.7, 64.8, 18.8, 18.7, 18.6. HRMS: $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{153}\text{H}_{180}\text{NO}_{36}$ 2608.22459, found 2608.22729.

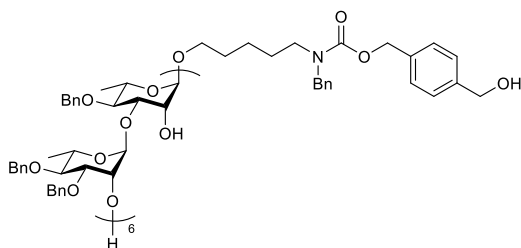
Deprotected octarhamnoside (4) White solid after general hydrogenation procedure (1.35 mg, 1.06 μmol , 69%).



^1H NMR (500 MHz, D_2O) δ 5.24 – 5.13 (m, 3H), 5.03 (s, 1H), 4.94 (s, 3H), 4.15 (s, 3H), 4.06 (s, 4H), 3.98 (s, 1H), 3.97 – 3.90 (m, 3H), 3.87 – 3.66 (m, 14H), 3.60 – 3.39 (m, 9H), 2.99 (t, $J = 7.4$ Hz, 2H), 1.76 – 1.59 (m, 4H), 1.54 – 1.37 (m, 2H), 1.36 – 1.18 (m, 24H); ^{13}C NMR (126 MHz, D_2O) δ 109.8, 102.5, 102.2, 100.9, 99.7, 78.1, 77.6, 72.4, 72.1, 71.9, 71.8, 71.5, 70.3, 70.0, 69.5, 69.4, 69.3, 68.8, 67.6, 59.3, 39.5, 28.2, 26.7, 22.6, 16.8, 16.8, 9.3. HRMS: $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{53}\text{H}_{94}\text{NO}_{33}$

1272.57026, found 1272.57136.

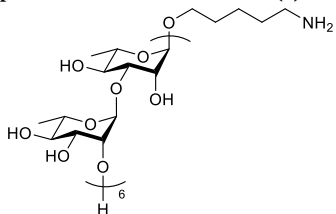
Dodecarhamnoside (27) Obtained as byproduct from **23**. (10.3 mg, 2.8 μmol). ^1H NMR (600 MHz, $\text{MeCN-}d_3$,



$T=328\text{K}$) δ 7.42 – 7.17 (m, 99H), 5.12 – 5.05 (m, 6H), 5.03 (d, $J = 2.1$ Hz, 1H), 4.97 (d, $J = 2.0$ Hz, 1H), 4.87 – 4.48 (m, 44H), 4.45 (s, 2H), 4.05 – 3.94 (m, 12H), 3.93 – 3.76 (m, 19H), 3.76 – 3.66 (m, 6H), 3.64 – 3.51 (m, 3H), 3.50 – 3.35 (m, 13H), 3.33 – 3.26 (m, 1H), 3.26 (s, 1H), 3.21 (t, $J = 7.3$ Hz, 3H), 1.54 – 1.44 (m, 4H), 1.29 (s, 9H), 1.23 – 0.98 (m, 36H); ^{13}C NMR (151 MHz,

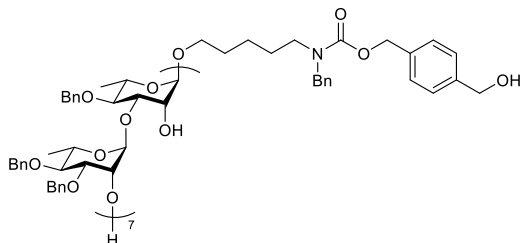
CD_3CN) δ 143.0, 140.3, 140.1, 140.1, 140.0, 140.0, 140.0, 140.0, 139.8, 137.3, 129.6, 129.5, 129.4, 129.4, 129.4, 129.4, 129.3, 129.3, 129.3, 129.3, 129.2, 129.2, 129.1, 129.1, 129.1, 129.0, 129.0, 129.0, 128.9, 128.8, 128.8, 128.7, 128.7, 128.7, 128.7, 128.6, 128.6, 103.1, 103.0, 102.9, 102.9, 102.9, 102.9, 102.9, 102.3, 102.3, 101.0, 81.4, 81.3, 81.2, 81.2, 81.2, 81.1, 81.0, 80.9, 80.8, 76.1, 76.0, 76.0, 75.9, 75.9, 75.9, 72.8, 72.8, 72.7, 72.2, 69.5, 69.5, 68.2, 67.7, 64.7, 30.0, 27.7, 24.3, 18.7, 18.6, 18.6, 18.6, 18.5. MALDI-TOF m/z $[\text{M}+\text{Na}]^+$ calc. for $\text{C}_{219}\text{H}_{255}\text{NO}_{52}\text{Na}$ 3753.7, found 3756.4.

Deprotected dodecarhamnoside (6) White solid after general hydrogenation procedure (0.51 mg, 0.27 μmol ,



27%). ^1H NMR (500 MHz, D_2O) δ 5.24 – 5.15 (m, 5H), 5.05 – 5.00 (m, 1H), 4.94 (s, 6H), 4.14 (d, $J = 2.4$ Hz, 6H), 4.06 (s, 7H), 3.98 (s, 1H), 3.97 – 3.88 (m, 7H), 3.87 – 3.65 (m, 24H), 3.61 – 3.35 (m, 16H), 3.04 – 2.93 (m, 2H), 1.76 – 1.60 (m, 6H), 1.53 – 1.39 (m, 2H), 1.35 – 1.18 (m, 36H). HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{77}\text{H}_{134}\text{NO}_{49}$ 1856.80203, found 1856.80622.

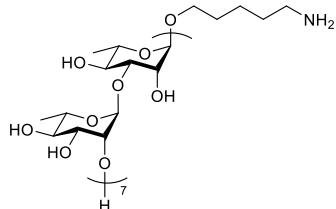
Tetradecarhamnoside (28) Obtained as byproduct from **23**. (20 mg, 4.65 μmol). ^1H NMR (600 MHz, $\text{MeCN-}d_3$,



$T=328\text{K}$): δ 7.48 – 7.14 (m, 114H), 5.10 – 5.05 (m, 6H), 5.03 (d, $J = 2.1$ Hz, 1H), 4.97 (d, $J = 2.0$ Hz, 1H), 4.87 – 4.48 (m, 46H), 4.45 (s, 2H), 4.06 – 3.94 (m, 12H), 3.94 – 3.77 (m, 20H), 3.71 (dtd, $J = 12.2, 9.7, 6.3$ Hz, 6H), 3.64 – 3.50 (m, 3H), 3.50 – 3.35 (m, 13H), 3.30 (s, 1H), 3.21 (t, $J = 7.3$ Hz, 2H), 3.16 – 3.01 (m, 7H), 1.50 (dd, $J = 11.3, 5.3$ Hz, 4H), 1.36 – 1.24 (m, 5H), 1.24 – 0.97 (m,

42H); ^{13}C NMR (151 MHz, CD_3CN) δ 140.3, 140.1, 140.1, 140.0, 140.0, 140.0, 140.0, 139.8, 137.3, 129.5, 129.5, 129.4, 129.4, 129.4, 129.4, 129.3, 129.3, 129.3, 129.2, 129.2, 129.1, 129.1, 129.1, 129.1, 129.0, 129.0, 128.8, 128.8, 128.7, 128.7, 128.7, 128.7, 128.7, 128.6, 128.6, 128.6, 103.1, 103.0, 102.9, 102.9, 102.9, 102.9, 102.3, 102.3, 101.0, 81.3, 81.2, 81.2, 81.1, 80.7, 76.1, 76.0, 75.9, 75.9, 75.9, 75.9, 72.8, 72.8, 72.7, 72.2, 72.1, 69.5, 69.5, 68.2, 67.7, 64.7, 30.0, 24.3, 18.6. MALDI-TOF m/z $[\text{M}+\text{K}]^+$ calc. For $\text{C}_{252}\text{H}_{293}\text{KNO}_{60}$ 4332.0, found 4337.0.

Deprotected Tetradecarhamnoside (7) White solid after general hydrogenation procedure (4.7 mg, 2.2 μmol ,



92%) ^1H NMR (500 MHz, D_2O): δ 5.23 – 5.14 (m, 6H), 5.04 (d, $J = 1.7$ Hz, 1H), 4.98 – 4.90 (m, 7H), 4.17 – 4.13 (m, 6H), 4.07 (s, 7H), 4.02 – 3.98 (m, 1H), 3.98 – 3.90 (m, 7H), 3.89 – 3.68 (m, 26H), 3.61 – 3.38 (m, 16H), 3.04 – 2.95 (m, 2H), 1.77 – 1.57 (m, 5H), 1.54 – 1.37 (m, 3H), 1.36 – 1.18 (m, 42H). ^{13}C NMR (126 MHz, D_2O): δ 102.5, 102.1, 100.9, 99.6, 78.1, 78.0, 77.6, 77.4, 72.2, 72.0, 71.8, 71.7, 71.4, 70.2, 70.1, 69.9, 69.4, 69.3, 69.2, 68.7, 67.5, 39.4, 28.1, 26.6, 22.5, 16.8, 16.7, 16.5.

HRMS: $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{89}\text{H}_{154}\text{N}_{10}\text{O}_{57}$ 2149.92135, found 2149.92197.

Notes and references

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Chapter 4

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Chapter 5

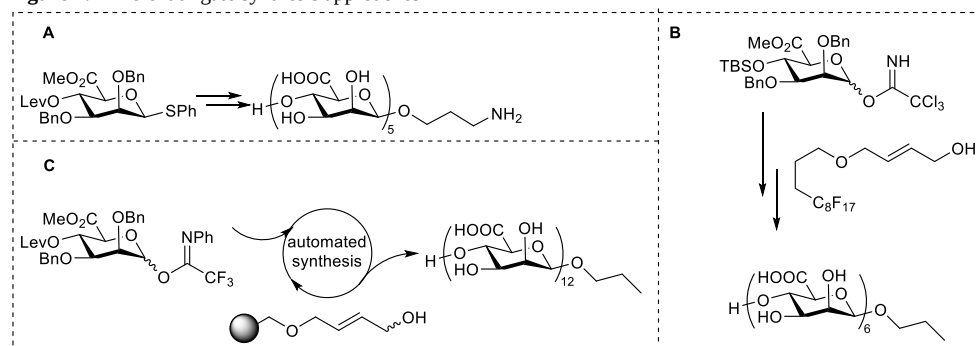
Synthesis of SOMA fragments

Volbeda, A.G.; Van der Vorm, S.; Hogervorst, T.; le Roy, J.; Overkleef, H.S.; Van der Marel, G.A.; Codée, J.D.C. were involved in the research described in this Chapter.

Introduction

Marine animals, plants and algae are an important source of medically and industrially relevant polysaccharides. Brown seaweeds represent a prime example as these provide alginates and fucans, polysaccharides that are being investigated for their biological activity and their attractive physical properties.¹ Alginates are being used in food and cosmetic preparations because of their gelling characteristics, but it has been suggested that they also have immune stimulating activity, through interaction with toll like receptors.^{2,3} Alginate is build up of β -(1-4)-linked D-mannuronic and α -(1-4)-linked L-guluronic acid (ManA and GulA) monosaccharides, and the monomeric composition dictates the properties of the polymer. Modification of these biopolymers, provides semi-synthetic biomaterials with potentially interesting activity. Sulfation of ManA alginates, generates sulfated oligomannuronic alginates, so-called SOMAs, which have been investigated for their glycosaminoglycan (GAG)-like properties, such as anticoagulation activity, but they have also been probed for their anti-cancer, anti-HIV, anti-influenza capacity.^{4,5} The SOMAs used for these studies have been obtained by random sulfation of naturally sourced alginates. For the establishment of structure-activity relationships for this promising class of compounds, synthetic fragments of well-defined length and sulfation pattern would be a very valuable asset. Over the years several syntheses of short alginate fragments have appeared and Figure 1A depicts the different strategies reported to date. Traditional solution phase chemistry has been used to assemble fragments up to five repeating monosaccharides (Figure 1A)^{6,7}, a fluorous supported synthesis (Figure 1B) has led to a ManA hexamer⁸ and an automated solid phase approach has allowed for the generation of fragments with a length of up to 12 monosaccharides (Figure 1C).⁹ In all these syntheses, the unique capacity of mannuronic acid donors to stereoselectively provide the challenging 1,2-*cis* linkages was exploited.^{6,7}

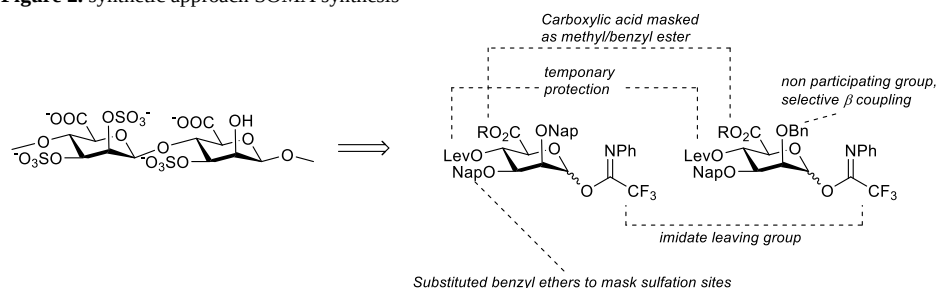
Figure 1. Different alginate synthesis approaches



Building on these precedents, it can be envisaged that the use mannuronic acid donors, bearing a pair of semi-orthogonal protecting groups at the C2 and C3, will allow for the construction of SOMA fragments with pre-defined sulfation patterns. The protecting groups to be used have to meet a selection of stringent criteria, not to compromise the stereoselectivity of the ManA donors. They obviously should not engage in (long-range)

participation since this would lead to the selective formation of 1,2-*trans* ManA linkages. Thus, acyl-type protecting groups are excluded. Bulky silyl ethers are less attractive as these have been shown to cause erosion of stereoselectivity in β -mannosylations reactions.¹⁰ Substituted benzyl ethers, such as the *para*-methoxy benzyl (PMB) and 2-methylnaphthyl (Nap) group, would present an attractive type of masking functionality, as these would maintain all characteristics of the stereoselective 2,3-di-*O*-benzyl ManA donor, yet allow for the regioselective removal to subsequently install the desired sulfate esters. This Chapter describes the development of chemistry required to assemble short SOMAs, building on the use of ManA donors, of which the hydroxyl groups at the C2 and C3 positions are differentiated through the use of Nap and Bn ethers (see Figure 2). In line with the building blocks used by Walvoort *et al.* the donor synthons were equipped with an anomeric *N*-phenyl trifluoroacetimidate latent leaving group and a C-4-*O*-levulinoyl for temporary protection of the alcohol to be elongated during the synthesis.⁹

Figure 2. synthetic approach SOMA synthesis



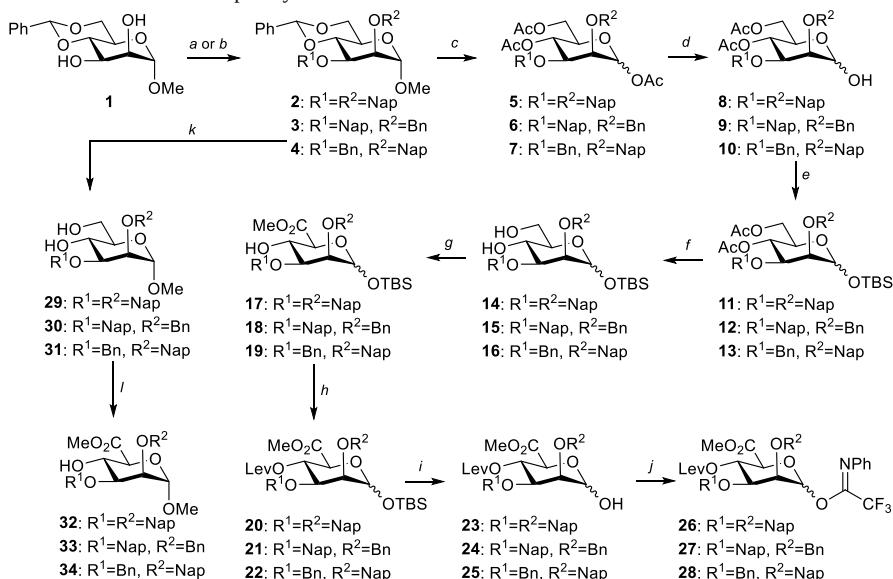
Results and discussion

First, the envisaged protecting group strategy was probed by the assembly of a set of protected ManA dimers and a SOMA disaccharide. Three new donors and acceptors were synthesized from benzylidenemannose **1** as depicted in Scheme 2. This starting compound is readily available and provided ready access to the donors and acceptors to be used in the glycosylations. Starting from compound **1**, the C-2 and C-3 hydroxyls were protected with a 2-methylnaphthyl ether (to give **2**), or the C-3 hydroxyl was regioselectively masked, through the intermediate formation of a stannylene acetal, with either a Nap or a Bn group. The remaining C-2 hydroxyl was then protected with either a Bn (**3**) or a Nap (**4**) ether. The fully protected mannosides were readily transformed into acceptors **32**, **33** and **34**, by acidic removal of the benzylidene acetal, followed by TEMPO/BAIB mediated oxidation of the C-6 hydroxyl and methylation of the newly formed acid.

The set of donor ManA building blocks, comprising donors **26**, **27** and **28**, were also accessed from the benzylidene precursors **2**, **3** and **4**. Thus, acidic cleavage of the anomeric and benzylidene acetals in acetic anhydride, yielded tri-acetates **5**, **6** and **7**. Selective removal of the anomeric acetyl by piperidine and ensuing silylation then afforded lactols **11**, **12** and **13**, which were saponified to give the diols **14**, **15** and **16**. These mannosides were transformed

in their mannuronic acid counterparts **17**, **18** and **19**, through a TEMPO/BAIB mediated oxidation, followed by conversion into the methyl esters. Protection of the C-4 alcohols with a levulinoyl group provided the fully protected ManA **20**, **21** and **22**. Removal of the anomeric silyl group liberated the lactols which were then transformed into donors **26**, **27** and **28**.

Scheme 1. Donor and acceptor synthesis.



Reagents and conditions: a) NapBr, NaH, DMF, 0°C (98%); b) i. $n\text{Bu}_2\text{SnO}$, toluene, reflux; ii. NapBr, CsF, DMF (89%) then NaH, BnBr, DMF (95% for **3**) or BnBr, CsF, DMF (89%) then NaH, NapBr, DMF (94% for **4**); c) $p\text{TsOH}\cdot\text{H}_2\text{O}$, Ac_2O (**5**: 91%, **6**: 84%, **7**: 66%); d) piperidine, THF (**8**: 79%, **9**: 85%, **10**: 88%); e) TBSCl, imidazole, DCM (**11**: 94%, **12**: 77%, **13**: 86%); f) NaOMe, MeOH (**14**: 94%, **15**: 99%, **16**: 98%); g) i. TEMPO, BAIB, DCM/ H_2O ; ii. MeI, K_2CO_3 , DMF, 0°C (**17**: 81%, **18**: 76%, **19**: 79%); h) LevOH, DIC, DMAP, DCM, 0°C (**20**: 87%, **21**: 96%, **22**: 95%); i) TBAF, AcOH, THF, 0°C (**23**: 98%, **24**: 97%, **25**: 99%); j) $\text{ClC}(=\text{NPh})\text{CF}_3$, Cs_2CO_3 , acetone, 0°C (**26**: 99%, **27**: 87%, **28**: 98%); k) $p\text{TsOH}\cdot\text{H}_2\text{O}$, DCM/MeOH (**29**: 88%, **30**: 91%, **31**: 83%); l) i. TEMPO, BAIB, DCM/ H_2O ; ii. MeI, K_2CO_3 , DMF, 0°C (**32**: 55%, **33**: 55%, **34**: 76%).

With the set of donor and acceptor building blocks in hand, a set of glycosylation reactions was performed to assess the selectivity of the reactions and the stability of the Nap-ethers under the conditions used. As summarized in Table 1, all combination of donor and acceptor building blocks provided profitable glycosylation reactions. The combination of donor **27** and acceptor **32** in a TfOH mediated glycosylation reaction at delivered disaccharide **35**, bearing three Nap ethers, in 74% with excellent stereoselectivity. Also, the union of donor **28**, having a Nap ether at C-2 and acceptor **32** proceeded uneventfully, providing dimer **36** in 79% yield. No side reaction originating from the intramolecular attack of the naphthyl on the anomeric center of the activated ManA donor was observed.^{11,12} A similar result was

obtained when donor **26**, bearing two Nap-ethers, was paired with acceptors **33** or **34**. These glycosylations delivered dimannuronic acids **37** and **38** in 85% and 95% yield respectively.

Scheme 2. Glycosylation of donors **26**, **27**, **28** and acceptors **32**, **33** and **34**.

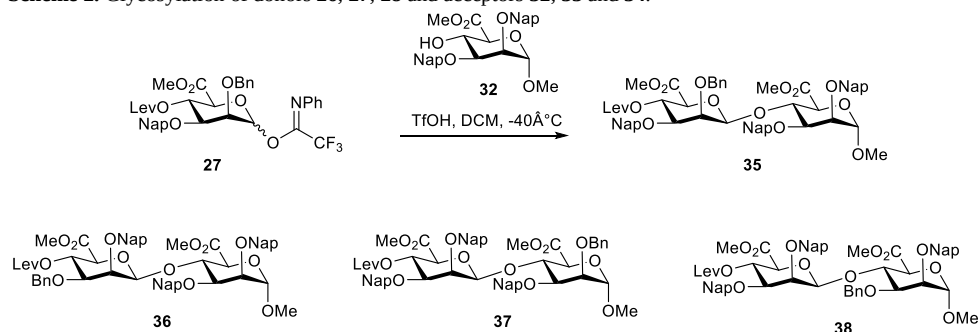


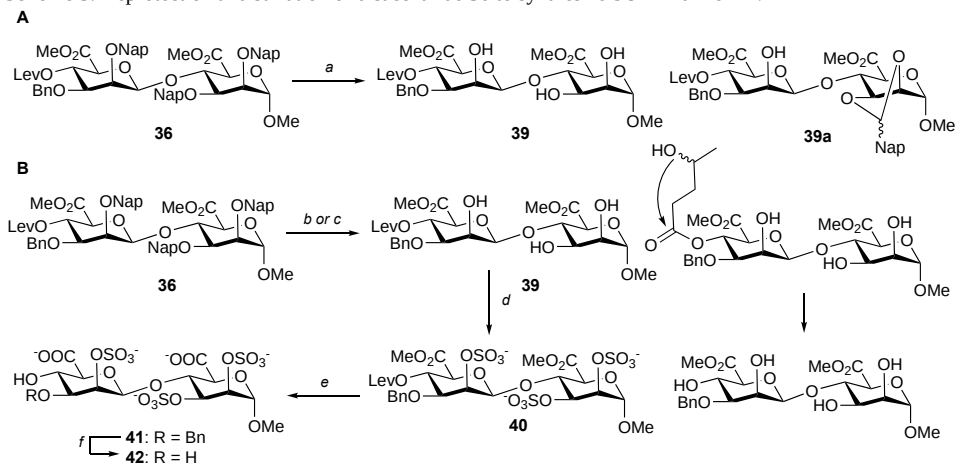
Table 1. Results glycosylation studies

Entry	Donor	Acceptor	TfOH	Product	yield
1	27	32 (1.3 eq.)	0.2 eq.	35	74%
2	28	32 (1.2 eq.)	0.2 eq.	36	79%
3	26	33 (1.3 eq.)	0.2 eq.	37	85%
4	26	34 (1.25 eq.)	0.2 eq.	38	95%

Then the selective removal of the Nap groups was investigated. Initially, oxidative conditions were probed for this purpose.^{13,14} However, when disaccharide **36** was subjected to DDQ, a complex mixture was obtained. Besides the desired triol, undesirable 2,3-naphthylidene formation was observed and the target triol was obtained in a mere 19% (Scheme 3A). Chapter 2 has introduced a new method for the removal of electron rich benzyl ethers using a catalytic amount of HCl in HFIP. When the conditions described in Chapter 2, (3 eq. of HCl (1 eq. per Nap ether) in combination with 5 eq. of triethyl silane (TES) to scavenge the naphthyl cations) were applied to disaccharide **36** (Scheme 3B), rapid removal of the Nap groups was observed via TLC and TLC/MS analysis. However, partial cleavage of the levulinoyl ester was also observed. Likely, the combination of HCl and TES led to reduction of the ketone functionality of the Lev ester, to provide an alcohol group which can attack the nearby carbonyl group to liberate the C-4'-alcohol. To circumvent this side reaction, a more hindered, slightly less reactive hydride donor, tri-*iso*-propylsilane, was examined. The use of this scavenger in combination with 3 eq. HCl in HFIP/DCM resulted in the fast and clean removal of the three Nap-ethers to provide triol **39** in 86% yield. To develop even milder conditions for the removal of multiple Nap ethers from an oligosaccharide, it was attempted to use a catalytic amount of HCl (0.5 eq. per disaccharide substrate, 0.17 eq. for each Nap-ether) acid. Although a slightly longer reaction time was required with respect to the use of stoichiometric amounts of acid, triol **39** was obtained in high yield (86%). The liberated hydroxyls were then decorated with sulfate groups to provide trisulfate **40**. It was observed that concentration of the reaction mixture at elevated temperature, resulted in degradation of the product. Removal of the solvent at room temperature did not jeopardize the integrity of the product and purification of the thus concentrated crude product by LH20 size-exclusion

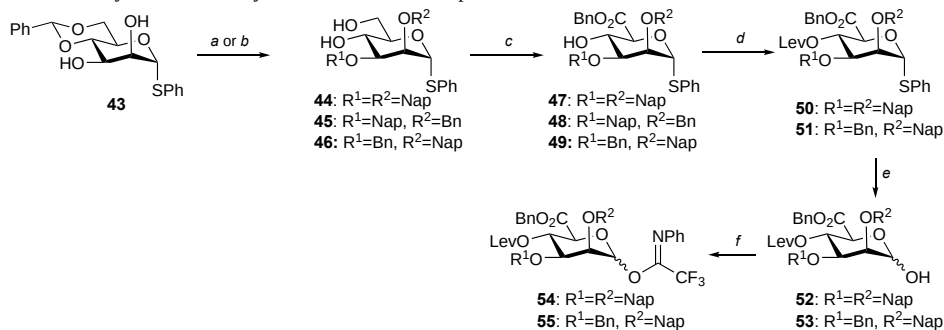
chromatography (using DCM/MeOH as eluent system) provided the trisulfate in quantitative yield. Final saponification of the levulinoyl and methyl esters using an excess of LiOOH (aq), was then followed by removal of the remaining benzyl ether at the C-3' position to deliver SOMA-disaccharide **42**.

Scheme 3. Deprotection and sulfation of disaccharide **36** to synthesize SOMA-dimer **42**.



Reagents and conditions: a) DDQ, DCM/H₂O (19%); b) HCl/HFIP, TES, DCM/HFIP (nd); c) HCl/HFIP, TIS, DCM/HFIP (86%); d) Et₃N·SO₃, DMF, 55°C (quant.); e) LiOH, H₂O₂, THF/H₂O (66%); f) Pd/C, H₂, H₂O (42%).

Aiming for larger SOMA fragments, functionalized with a conjugation handle, a set of ManA thioglycosides was generated as these potentially open the way to a more convergent assembly strategy through the use of chemoselective glycosylation steps. In these synthons, benzyl esters were installed as these can be removed at the end of the synthesis under mild reductive conditions to further streamline the assembly of larger SOMAs. The new building blocks were readily available from 4,6-*O*-benzylidene-1-thio- α -D-mannopyranoside **43**, following chemistry described above. Thus, protection of the C-2 and C-3 acohols with either two Nap-groups or a Nap and Bn-ether, was followed by removal of the benzylidene acetals, chemo- and regioselective oxidation of the primary alcohol and subsequent formation of the benzyl esters (see Scheme 4). This provided ManA thioglycoside acceptors **47**, **48** and **49**. Protection of the C4-OH in **47** and **49** then provided fully protected ManA synthons **49** and **51**. Of note, protection of the C-4-OH generates ManA building blocks that adopt two major conformations in solution: besides the “normal” ⁴C₁ chair conformation they also easily adopt an “inverted” ¹C₄ chair shape. This conformational flexibility leads to significant line broadening in the recorded NMR-spectra of the compounds, necessitating high temperature measurements to obtain NMR spectra with better defined resonances. The fully protected thioglycosides (**50–51**) were hydrolyzed and treated with *N*-phenyltrifluoroacetimidoyl chloride to generate the corresponding imidate donors **54** and **55**.

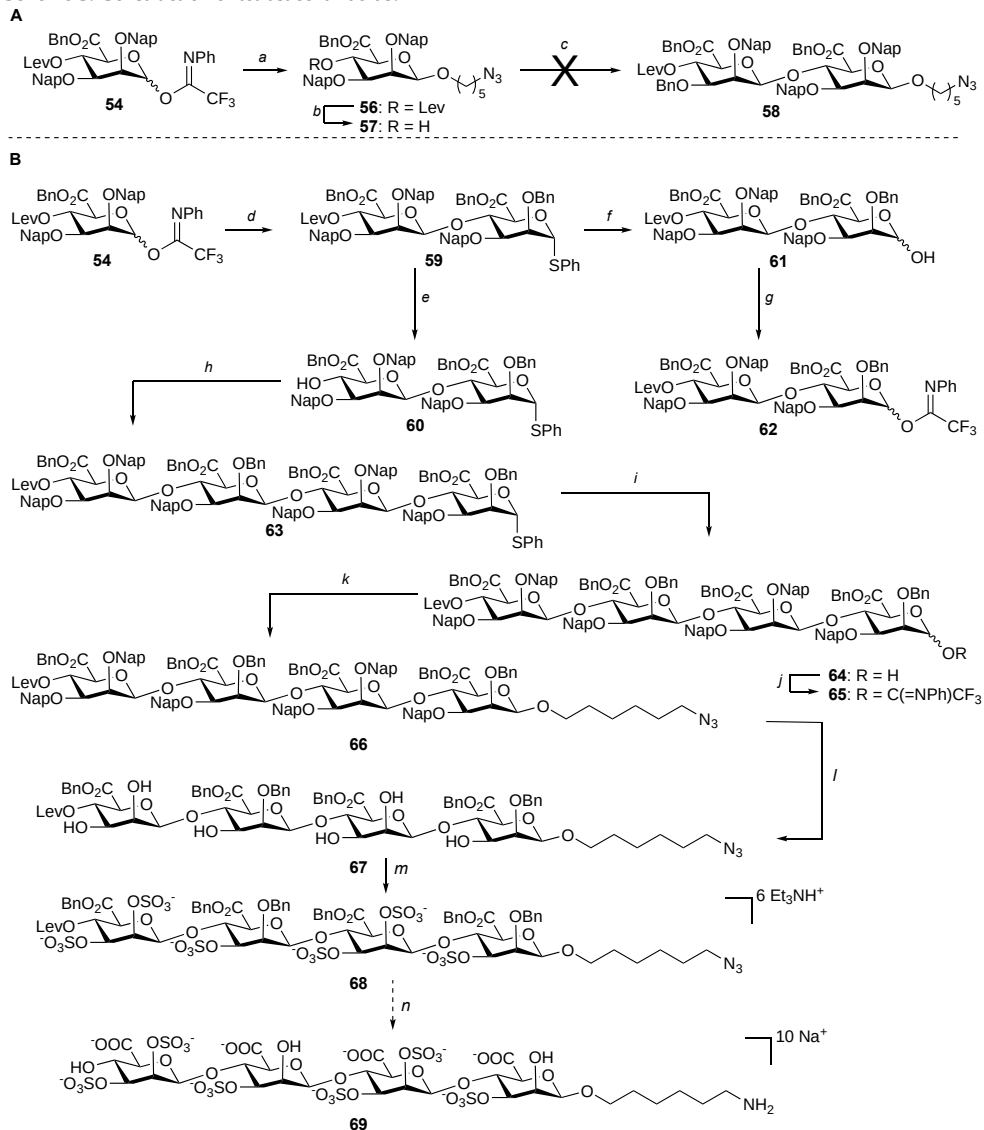
Scheme 4. Synthesis of benzyl ester donors and acceptors.


Reagents and conditions: a) i. NapBr, NaH, DMF, 0°C; ii. pTsOH·H₂O, DCM/MeOH (91% for **44**); b) i. Bu₂SnO, toluene, reflux; ii. CsF, NapBr, toluene, 100°C; then BnBr, NaH, DMF, 0°C (88%) or CsF, BnBr, DMF then NapBr, NaH, DMF (86%); iv. pTsOH·H₂O, DCM/MeOH (75% over 3 steps for **45**, 84% for **46**); c) i. TEMPO, BAIB, DCM/H₂O/tBuOH; ii. BnBr, K₂CO₃, DMF, 0°C (**47**: 68%, **48**: 77%, **49**: 56%); d) LevOH, DIC, DMAP, DCM, 0°C (**50**: 82%, **51**: 97%); e) NIS, TFA, DCM, 0°C (**52**: 80%, **53**: 95%); f) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0°C (**54**: 81%, **55**: 77%).

With the required building blocks in hand, the assembly of SOMA oligomers was started (see Scheme 5). To this end an azide pentanol spacer was first condensed with donor **54** using TMSOTf to provide ManA **56**. Removal of the levulinoyl ester then set the stage to couple the second ManA (Scheme 5A). This reaction however, in sharp contrast to the many successful condensation reactions between various mannuronic acid building blocks (See Figure 1 for a schematic overview) and the dimannuronic acid syntheses described above, did not provide a productive outcome: a complex reaction mixture was obtained, wherefrom the desired disaccharide **58** could not be isolated. Changing the type of ManA donor, the donor/acceptor ratio and the reaction conditions proved to no avail as in each case a complex reaction mixture resulted. The most important difference between the building blocks used here and the previously reported ManA alginate syntheses is the presence of the Nap ethers instead of Bn ethers. Although these protecting groups are very similar they apparently have a major influence on the outcome of the glycosylation reactions. Likely, the electron rich nature (with respect to their Bn-counterparts) of the Nap ethers leads to competitive nucleophilic attack of these functional groups, in an intra- and/or intermolecular fashion, on the activated donor species, to provide the complex reaction mixtures. Apparently, the ManA C-4-OH in this case is not reactive enough to outcompete the Nap ethers and provide a productive glycosylation reaction. This leaves the question why the α -O-methyl mannuronic acid acceptors described above engage in highly efficient glycosylation reactions, while the use of the β -linked mannuronic acids provide complex mixtures. Previously, Zhang *et al.* have described the effect of conformational freedom on the reactivity of guluronic acid-mannuronic acid disaccharide C4'-OH acceptors.¹⁵ In the construction of mixed sequence alginates, they showed that in the condensation of a GulA-ManA-disaccharide donor and a GulA-ManA-dimer acceptor, the nature of the acceptor's "reducing" end moiety played an all-important role. The condensation reaction of the acceptor bearing a β -azidopropanol spacer led to a modest yield of 26%. In contrast, when a disaccharide acceptor was used,

having an α -thiophenol at the reducing end, the yield of the coupling reaction was increased to 91%. It was reasoned that the flexibility of the α -thiophenol disaccharide made this acceptor a better nucleophile by allowing better accommodation of the steric requirements of the crowded glycosylation transition state. Inspired by this result, thioglycoside **48** was probed as an acceptor in a glycosylation reaction with imidate donor **54** (Scheme 5B). This glycosylation reaction proceeded smoothly and the desired disaccharide could now be obtained in 72% yield. Encouraged by this result, dimer **59** was transformed into dimer imidate donor **62** by an anomeric hydrolysis and imidate formation reaction sequence to give dimannuronic acid donor **62**. Removal of the C-4'-levulonoyl ester from dimer **59** provided a disaccharide acceptor **60**. The union of the two latter building blocks under the agency of a catalytic amount of Lewis acid promotor proceeded effectively and tetra-ManA **63** was obtained in 68%. In line with the results of Zhang *et al.*, the effect of the flexible reducing end monosaccharide also here allowed for a productive glycosylation of dimer building blocks. To install a spacer at the reducing end of the tetrasaccharide, the anomeric thiophenyl was again transformed into an *N*-phenyltrifluoroacetimidate to provide donor **65**, which was coupled with azidohexanol to give the fully protected, spacer equipped tetra-mannuronic acid **66**.

With compound **66** in hand, the deprotection conditions described above for the mannuronic acid disaccharide were applied (see Scheme 3). A small molar excess of acid with respect to the tetrasaccharide (1.1 equiv., ± 0.2 equiv. per Nap ether) was used in combination with HFIP and TIS as cation scavenger in DCM to remove the Nap ethers. Using these conditions, all six Nap ethers were cleanly removed to provide the desired hexaol in 94% yield. Sulphation using sulfur trioxide triethylamine complex at 55°C then yielded compound **68**, which could be purified by size exclusion chromatography (DCM/MeOH). The removal of the Lev and benzyl esters was accomplished by LiOH/H₂O₂ mediated saponification, followed by gel filtration. NMR analysis showed removal of the Lev and a reduction of the amount of aromatic resonances. Next, the remaining benzyl ethers and the primary azide were removed by hydrogenolysis. After concentration of the reaction mixture and purification by size exclusion chromatography the desired tetrasaccharide could unfortunately not be obtained in sufficient purity. Because of lack of precursor tetrasaccharide **69**, no further attempts could be made to obtain the final product. Currently, no reason can be provided to explain the failure in obtaining **69** in sufficient yield and purity. It stands in contrast to the successful assembly of dimannuronate **42** and the vast amount of previously accomplished syntheses of various sulfated oligosaccharides,^{14,16,17} that essentially follow a similar protecting group strategy (sulfation, followed by saponification and final hydrogenolysis).

Scheme 5. Construction of tetrasaccharide **68**.


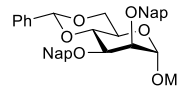
Reagents and conditions: a) 6-azidopentanol, TMSOTf, DCM, -55°C (79%); b) H₂NNH₂·AcOH, pyridine/AcOH, (99%); c) TMSOTf, DCM, -55°C; d) **48**, TMSOTf, DCM, -55°C (72%); e) H₂NNH₂·AcOH, pyridine/AcOH, 0°C (95%); f) NIS, TFA, Et₃N, DCM, 0°C (84%); g) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0°C (70%); h) **62**, TMSOTf, DCM, -55°C (68%); i) NIS, TFA, DCM, 0°C; j) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0°C (83% over 2 steps); k) 6-Azidohexanol, TMSOTf, DCM, -50°C (68%). l) HCl/HFIP, TIS, DCM/HFIP (94%); m) Et₃N·SO₃, DMF, 55°C (74%); n) LiOOH, THF/H₂O, 0°C, then H₂, Pd(OH)₂/C, AcOH, THF/H₂O/tBuOH (n.d.).

Conclusion

This chapter describes a study towards the synthesis of sulfated oligomannuronic acid (SOMA) fragments. First, six new mannuronic acid donors and acceptors were designed and synthesized, bearing a protecting group pattern suited for selective sulfation of the C-2 and/or C-3-hydroxyls. The glycosylating properties of these building blocks were studied in a set of model glycosylations delivering four disaccharides with a varying protecting pattern. One of these disaccharides was transformed into a SOMA fragment, by selective removal of the Nap ethers, sulfation of the liberated hydroxyls and global deprotection. The acidic removal of the Nap ethers using conditions described in Chapter 2, proved superior to the more commonly used oxidative conditions, as these led to significant side reactions. During the assembly of larger SOMA oligosaccharides, it was observed that the union of β -configured Nap-bearing ManA acceptors and ManA donors, also featuring Nap-ethers, posed a significant problem. Notably, the use of more flexible ManA acceptors, bearing an α -thiophenol aglycon, did allow for the construction of larger oligomannuronates, bearing multiple Nap ethers. These results match the finding of Zhang *et al.* that the flexibility of the acceptor nucleophile can have a tremendous impact on the outcome of a glycosylation reaction. They also underscore the need for better insight into the mechanistic details of glycosylation reactions and into the steric requirements of the crowded glycosylation reaction transition states. Finally, a fully protected SOMA tetrasaccharide could be obtained. All six Nap-ethers could be removed from this tetrasaccharide using a catalytic amount of HCl (with respect to the Nap ethers) in HFIP/DCM. The liberated alcohols could be sulfated, but the final deprotection sequence unfortunately did not deliver the desired target SOMA tetrasaccharide in sufficient quantity and purity. The incorporation of a purification/visualization handle, for example mounted on the amino functionalized spacer, may allow for better purification and characterization of synthetic SOMA fragments in the future. Changing the protection of the non-reducing C-4-OH functionality to a benzyl ether (instead of the currently used levulinoyl ester) may streamline the deprotection further.

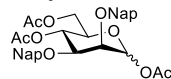
Experimental

General experimental procedures. All solvents used under anhydrous conditions were stored over 4Å molecular sieves except for methanol which was stored over 3Å molecular sieves. ¹H and ¹³C NMR spectra were recorded on a 400/100, 500/125, 600/150, or a 850/214 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All individual signals were assigned using 2D-NMR spectroscopy, HH-COSY, HSQC, and HMBC. IR spectra are reported in cm⁻¹, and recorded on a Shimadzu FTIR-8300 or a PerkinElmer universal attenuated total reflectance (UATR; Single Reflection Diamond) Spectrum Two instrument. Solvents used for workup and column chromatography were of technical grade from Sigma Aldrich, Boom, Biosolve or Honeywell and used directly. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40 °C. All chemicals were used as received unless stated otherwise. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F254 with detection by spraying with 20% H₂SO₄ in EtOH, (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), or by dipping in anisaldehyde (10 mL in 180 mL EtOH / 10 mL H₂SO₄) followed by charring at approx. 150 °C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). For LC-MS analysis a Agilent Technologies 1260 Infinity LC system (detection simultaneously at 214 and 254 nm) coupled to a Agilent Technologies 6120 Quadrupole LC/MS, using an analytical Vydac C4 column (Alltech, 50 x 4.60 mm, 5 μm) or a Vydac Diphenyl (Alltech, 150 x 4.60 mm, 5 μm) in combination eluents A: H₂O; B: MeCN and C: 1% aq. TFA. For HPLC, a Gilson HPLC system in combination with eluents A: H₂O (0.1% TFA); B: MeCN as the solvent system using a Vydac C4 HPLC column (Grace, 250 x 10 mm, 5 μm). High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.2842) as a “lock mass”. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Maldi spectra were recorded on an Ultraflextreme MALDI-TOF (Bruker Daltonics), equipped with Smartbeam-II laser, to measure the samples in reflectron positive ion mode. The MALDI-TOF was calibrated using a peptide calibration standard prior to measurement. 1 μL of 2,5-dihydroxybenzoic acid (2,5-DHB; Bruker Daltonics) matrix (20 mg/mL in ACN/water; 50:50 (v/v)) was applied on a 384-MTP target plate (Bruker Daltonics, Bremen, Germany) and air-dried. Subsequently, 1 μL of compound water solution was spotted on the plate and the spots were left to dry prior MALDI-TOF analysis.

Methyl 4,6-O-benzylidene-2,3-di-O-(2-naphthylmethyl)-α-D-mannopyranoside (2) 2-
 (Bromomethyl)naphthalene (464 mg, 2.1 mmol, 2.1 eq.) was added to a 0°C solution of compound **1** (285 mg, 1.01 mmol) in DMF (5mL) under an argon atmosphere. Sodium hydride (60% dispersion in oil, 100 mg, 2.5 mmol, 2.5 eq.) was added and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by the dropwise addition of H₂O and extracted with EtOAc. The organic layer was dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 8:1 → 4:1) yielded the title compound as a yellow oil (560 mg, 0.99 mmol, 98%). TLC: R_f 0.59 (PE/EtOAc, 4/1, v/v); IR (neat): 698, 748, 813, 1053, 1371 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.78-7.81 (m, 5H, CH_{arom}), 7.72-7.74 (m, 2H, CH_{arom}), 7.63-7.66 (m, 1H, CH_{arom}), 7.52-7.54 (m, 3H, CH_{arom}), 7.35-7.47 (m, 8H, CH_{arom}), 5.68 (s, 1H, CHPh), 4.91-4.98 (m, 3H, CH₂ Nap, CHH Nap), 4.79 (d, 1H, *J* = 12.4 Hz, CHH Nap), 4.73 (d, 1H, *J* = 1.2 Hz, H-1), 4.25-4.35 (m, 2H, H-4, H-6), 4.01 (dd, 1H, *J* = 3.2, 10 Hz, H-3), 3.89-3.94 (m, 2H, H-2, H-6), 3.79 (ddd, 1H, *J* = 4.4, 4.8, 9.6 Hz, H-5), 3.28 (s, 3H, CH₃ OMe); ¹³C NMR (CDCl₃, 100 MHz): δ

137.9, 136.3, 135.6, 133.4, 133.2, 133.0 (Cq CHPh, Nap), 129.0, 128.5, 128.4, 128.3, 128.1, 128.1, 128.0, 127.8, 127.8, 127.1, 126.9, 126.3, 126.3, 126.2, 126.2, 126.1, 126.0, 125.8, 125.8 (CH_{arom}), 101.7 (CHPh), 100.6 (C-1), 79.3 (C-4), 76.5 (C-3), 76.2 (C-2), 73.7, 73.1 (CH₂ Nap), 69.0 (C-6), 64.2 (C-5), 54.9 (OMe); HRMS: [M+Na]⁺ calcd. for C₃₆H₃₄O₆Na 585.22476, found 585.22390.

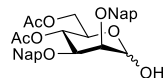
Acetyl 4,6-di-O-acetyl-2,3-di-O-(2-naphthylmethyl)- α -D-mannopyranoside (5) To a solution of compound 2



(12.4 g, 22 mmol) in acetic anhydride (125 mL), pTsOH·H₂O (6.3 g, 33 mmol, 1.5 eq.) was added. The reaction mixture was allowed to stir for 5.5 days at room temperature. The reaction mixture was quenched by pouring it over ice and gradually adding solid NaHCO₃

until all ice had melted and CO₂ evolution had stopped. The aqueous mixture was extracted three times with EtOAc and the combined organic layers were washed once with brine. The organic fraction was dried with MgSO₄, filtered and concentrated *in vacuo*, after which the residue was co-evaporated once with toluene. Column chromatography purification (PE/EtOAc, 9:1 → 2:1) afforded the title compound as an orange oil (11.8 g, 20.1 mmol, 91%, $\alpha >> \beta$). Analytic data for α -anomer of compound 5: TLC: R_f 0.30 (PE/EtOAc, 2/1, v/v); IR (neat): 748, 813, 962, 1051, 1217, 1369, 1732 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): 7.71-7.83 (m, 8H, CH_{arom}), 7.31-7.53 (m, 6H, CH_{arom}), 6.24 (d, 1H, *J* = 1.6 Hz, H-1), 5.56 (t, 1H, *J* = 9.6 Hz, H-4), 4.95 (d, 1H, *J* = 12.6 Hz, CHH Nap), 4.88 (d, 1H, *J* = 12.6 Hz, CHH Nap), 4.66 (d, 1H, *J* = 12.4 Hz, CHH Nap), 4.58 (d, 1H, *J* = 12.4 Hz, CHH Nap), 4.25 (dd, 1H, *J* = 5.2, 11.6 Hz, H-6), 4.14 (dd, 1H, *J* = 2.4, 11.6 Hz, H-6), 3.90-3.94 (m, 1H, H-5), 3.80-3.85 (m, 2H, H-2, H-3), 2.08 (s, 3H, CH₃ Ac), 2.03 (s, 3H, CH₃ Ac), 1.96 (s, 3H, CH₃ Ac); ¹³C NMR (CDCl₃, 400 MHz): δ 171.0, 169.7, 168.7 (C=O Ac), 135.3, 135.1, 133.3, 133.2 (Cq), 128.4, 128.3, 128.0, 127.9, 127.8, 127.2, 127.0, 126.8, 126.5, 126.4, 126.2, 126.1, 125.7, 125.6 (CH_{arom}), 91.9 (C-1), 76.2 (C-3), 72.8 (C-2), 72.7, 72.2 (CH₂ Nap, Nap), 71.6 (C-5), 67.5 (C-4), 62.8 (C-6), 21.0, 21.0, 20.9 (CH₃ Ac); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 91.9 (*J*_{C1,H1} = 174 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₄H₃₈NO₉ 604.25411, found 604.25468.

4,6-Di-O-acetyl-2,3-di-O-(2-naphthylmethyl)- α/β -D-mannopyranose (8) Compound 5 (255 mg, 0.44 mmol) was

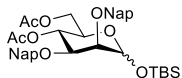


dissolved in 4% piperidine (90 μ L, 0.9 mmol, 2.1 eq.) in THF (2.2 mL). After stirring for 3 days at room temperature H₂O was added and the mixture was extracted with EtOAc. The organic layer was washed once with brine and subsequently dried (MgSO₄), filtered, and

concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 8:1 → 1:1) gave the title compound as a yellow oil (188 mg, 0.35 mmol, 79%, $\alpha : \beta = 5 : 1$). TLC: R_f 0.39 (PE/EtOAc, 1/1, v/v); IR (neat): 746, 812, 1049, 1217, 1367, 1732 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.67-7.82 (m, 9.60H, CH_{arom}), 7.34-7.48 (m, 7.20H, CH_{arom}), 5.50 (t, 1H, *J* = 9.6 Hz, H-4 α), 5.43 (t, 0.20H, *J* = 9.6 Hz, H-4 β), 5.29 (s, 1H, H-1 α), 5.18 (d, 0.20H, *J* = 11.6 Hz, CHH Nap β), 4.75-4.87 (m, 2.40H, CH₂ Nap α , CHH Nap β , CHH Nap β), 4.66-4.70 (m, 1.40H, CHH Nap α , CHH Nap β , H-1 β), 4.58 (d, 1H, *J* = 12.4 Hz, CHH Nap α), 4.21 (dd, 1.20H, *J* = 5.2, 12.0 Hz, H-6 α , H-6 β), 4.11-4.14 (m, 1.40H, H-6 α , H-6 β , 1-OH β), 4.03-4.07 (m, 1H, H-5 α), 3.99 (bs, 1H, 1-OH α), 3.95 (dd, 1H, *J* = 2.8, 9.6 Hz, H-3 α), 3.90 (d, 0.20H, *J* = 1.2 Hz, H-2 β), 3.85 (t, 1H, *J* = 2.4 Hz, H-2 α), 3.60 (dd, 0.20H, *J* = 2.8 Hz, H-3 β), 3.50-3.54 (m, 0.20H, H-5 β), 2.01 (s, 0.60H, CH₃ Ac β), 2.00 (s, 0.60H, CH₃ Ac β), 2.00 (s, 3H, CH₃ Ac α), 1.99 (s, 3H, CH₃ Ac α); ¹³C NMR (CDCl₃, 100 MHz): δ 171.1, 171.1, 170.0, 169.9 (C=O Ac), 135.6, 135.6, 135.1, 135.0, 133.3, 133.2, 133.1, 133.0, 133.0 (Cq), 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.7, 127.3, 126.7, 126.5, 126.4, 126.2, 126.2, 126.1, 126.1, 126.0, 126.0, 125.9, 125.6, 125.5 (CH_{arom}), 93.9 (C-1 β), 92.9 (C-1 α), 79.9 (C-3 β), 76.7 (C-3 α), 75.2 (C-2 β), 74.8 (CH₂ Nap β), 74.5 (C-2 α), 72.9 (CH₂ Nap α), 72.6 (CH₂ Nap β), 72.4 (C-5 β), 72.0 (CH₂ Nap α), 69.1 (C-5 α), 68.3 (C-4 α), 67.8 (C-4 β), 63.3 (C-6), 63.0 (C-6 β), 21.0, 21.0,

20.8 (CH₃ Ac); ¹³C-GATED NMR (CDCl₃, 100 MHz): δ 93.9 (*J*_{C1,H1} = 160 Hz, C-1 β), 92.9 (*J*_{C1,H1} = 169 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₂H₃₆NO₈ 562.24354, found 562.24347.

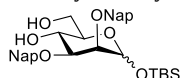
Tert-butyldimethylsilyl 4,6-di-O-acetyl-2,3-di-O-(2-naphthylmethyl)-α/β-D-mannopyranoside (11) TBDMSCl



(3.83 g, 25 mmol, 2 eq.) and imidazole (1.73 g, 25 mmol, 2eq.) were added to a solution of hemiacetal **8** (6.91 g, 12.8 mmol) in dry DCM (65 mL) under an argon atmosphere. After stirring overnight, the reaction was quenched with H₂O and extracted twice with Et₂O.

Combined organic layers were washed with brine, dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 15:1 → 2:1) yielded the title compound as a yellowish oil (8.39 g, 12.7 mmol, 99%, α : β = 1 : 4). TLC: R_f 0.47 (Pentane/EtOAc, 4/1, v/v); IR (neat): 746, 779, 837, 895, 1052, 1055, 1233, 1368, 1742 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.74-7.89 (m, 9H, CH_{arom}), 7.67-7.69 (dd, 1H, *J* = 1.2, 8.4 Hz, CH_{arom}), 7.63 (s, 1H, CH_{arom}), 7.43-7.54 (m, 5.50H, CH_{arom}), 7.30-7.33 (dd, 1H, *J* = 1.2, 8.4 Hz, CH_{arom} β), 5.57 (t, 0.25H, *J* = 10.0 Hz, H-4 α), 5.47 (t, 1H, *J* = 9.6 Hz, H-4 β), 5.23 (d, 1H, *J* = 12.8 Hz, CHH Nap β), 5.16 (d, 0.25H, *J* = 2.0 Hz, H-1 α), 5.10 (d, 1H, *J* = 12.8 Hz, CHH Nap β), 5.03 (d, 0.25H, *J* = 12.8 Hz, CHH Nap α), 4.88 (d, 0.25H, *J* = 12.8 Hz, CHH Nap α), 4.76-4.79 (m, 1.50H, CH₂ Nap α, H-1 β), 4.59 (d, 1H, *J* = 12.4 Hz, CHH Nap β), 4.46 (d, 1H, *J* = 12.4 Hz, CHH Nap β), 4.18-4.34 (m, 2.50H, H-6 α, H-6 α, H-6 β, H-6 β), 3.97-4.03 (m, 1.50H, H-2 β, H-3 α, H-5 α), 3.70 (t, 0.25H, *J* = 2.4 Hz, H-2 α), 3.53-3.61 (m, 2H, H-3 β, H-5 β), 2.12 (s, 1.50H, CH₃ Ac α), 2.11 (s, 3H, CH₃ Ac β), 2.09 (s, 3H, CH₃ Ac β), 1.03 (s, 9H, CH₃ tBu β), 0.79 (s, 2.25H, CH₃ tBu α), 0.26 (s, 3H, CH₃ Me β), 0.22 (s, 3H, CH₃ Me β), 0.05 (s, 0.75H, CH₃ Me α), -0.04 (s, 0.75H, CH₃ Me α); ¹³C NMR (CDCl₃, 100 MHz): δ 170.7, 170.6, 169.8, 169.7 (C=O Ac), 136.1, 135.5, 135.4, 135.3, 133.1, 133.1, 133.0, 132.9, 132.9 (Cq), 128.2, 128.1, 128.0, 127.8, 127.8, 127.6, 127.6, 126.8, 126.7, 126.4, 126.1, 126.1, 126.0, 126.0, 125.9, 125.9, 125.8, 125.8, 125.6, 125.3 (CH_{arom}), 96.6 (C-1 β), 93.2 (C-1 α), 78.9 (C-3 β), 76.1 (C-3 α), 75.6 (C-2 α), 74.6 (C-2 β), 74.1 (CH₂ Nap β), 73.0 (CH₂ Nap α), 72.6 (C-5 β), 72.2 (CH₂ Nap α), 71.2 (CH₂ Nap β), 69.1 (C-5 α), 68.3 (C-4 β), 68.2 (C-4 α), 63.4 (C-6 β), 63.1 (C-6 α), 25.8 (CH₃ tBu β), 25.3 (CH₃ tBu α), 20.9, 20.7 (CH₃ Ac), 18.0 (Cq tBu β), 17.7 (Cq tBu α), -4.0 (CH₃ Me β), -4.9 (CH₃ Me α), -5.4 (CH₃ Me β), -6.0 (CH₃ Me α); ¹³C-GATED NMR (CDCl₃, 100 MHz): δ 96.6 (*J*_{C1,H1} = 153 Hz, C-1 β), 93.2 (*J*_{C1,H1} = 167 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₈H₅₀NO₈Si 676.33002, found 676.33046.

Tert-butyldimethylsilyl 2,3-di-O-(2-naphthylmethyl)-α/β-D-mannopyranoside (14) To a solution of compound



11 (8.3 g, 12.6 mmol) in MeOH (63 mL) a catalytic amount of NaOMe (55 mg, 1.0 mmol, 0.1 eq.) was added. After stirring overnight, the reaction mixture was neutralized with

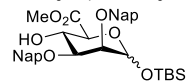
Amberlite H⁺ which was subsequently filtered off. The filtrate was concentrated *in vacuo*

and **14** was obtained as a colourless oil (6.82 g, 11.9 mmol, 94%, α : β = 1 : 3.8). TLC: R_f 0.14 (PE/EtOAc, 2/1, v/v); IR (neat): 745, 779, 814, 835, 1069, 1252, 1362, 3426 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.91 (s, 1H, CH_{arom}), 7.65-7.84 (m, 10.34H, CH_{arom}), 7.45-7.58 (m, 5.30H, CH_{arom}), 7.37 (dd, 1H, *J* = 1.6, 8.4 Hz, CH_{arom}), 5.57 (d, 1H, *J* = 12.4 Hz, CHH Nap β), 5.12 (d, 0.26H, *J* = 1.2 Hz, H-1 α), 5.05 (d, 1H, *J* = 12.4 Hz, CHH Nap β), 4.96 (d, 0.26H, *J* = 12.4 Hz, CHH Nap α), 4.82-4.91 (m, 1.78H, CHH Nap α, CH₂ Nap α, H-1 β), 4.65 (d, 1H, *J* = 12.0 Hz, CHH Nap β), 4.58 (d, 1H, *J* = 12.0 Hz, CHH Nap β), 4.31 (t, 0.26H, *J* = 10.0 Hz, H-4 α), 4.20 (t, 1H, *J* = 9.6 Hz, H-4 β), 3.93-4.06 (m, 3.78H, H-2 β, H-3 α, H-6 α, H-6 α, H-6 β, H-6 β), 3.81-3.85 (m, 0.26H, H-5 α), 3.63-3.65 (m, 0.52H, H-2 α, 4-OH α), 3.53 (bs, 1H, 4-OH β), 3.44 (dd, 1H, 2.8, 9.6 Hz, H-3 β), 3.36-3.41 (m, 1H, H-5 β), 2.88 (bs, 1.26H, 6-OH α, 6-OH β), 1.06 (s, 9H, CH₃ tBu β), 0.78 (s, 2.34H, CH₃ tBu α), 0.28 (s, 3H, CH₃ Me β), 0.24 (s, 3H, CH₃ Me β), 0.05 (s, 0.78H, CH₃ Me α), -0.08 (s, 0.78H, CH₃ Me α); ¹³C NMR (CDCl₃, 100 MHz): δ 136.3, 135.6, 135.4, 135.4, 133.2, 133.0, 133.0, 132.9 (Cq), 128.8, 128.3, 128.2, 128.2, 128.0, 127.9, 127.9, 127.7, 127.0, 126.7, 126.4,

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126.3, 126.1, 126.1, 126.0, 125.9, 125.7, 125.6 (CH_{arom}), 96.8 (C-1 β), 93.3 (C-1 α), 81.5 (C-3 β), 78.8 (C-3 α), 76.0 (C-5 β), 75.9 (C-2 α), 75.2 (C-2 β), 74.5 (CH_2 Nap β), 73.0, 72.6 (CH_2 Nap α), 72.4 (C-5 α), 71.4 (CH_2 Nap β), 67.4 (C-4 β), 67.2 (C-4 α), 62.9 (C-6 β), 62.5 (C-6 α), 25.8 (CH_3 tBu β), 25.4 (CH_3 tBu α), 18.0 (Cq tBu β), 17.7 (Cq tBu α), -3.8 (CH_3 Me β), -4.7 (CH_3 Me α), -5.3 (CH_3 Me β), -6.0 (CH_3 Me α); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 96.8 ($J_{\text{C1,H1}} = 153$ Hz, C-1 β), 93.3 ($J_{\text{C1,H1}} = 165$ Hz, C-1 α); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. for $\text{C}_{34}\text{H}_{46}\text{NO}_6\text{Si}$ 592.30889, found 592.30922.

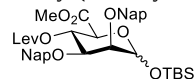
Methyl (*tert*-butyldimethylsilyl 2,3-di-*O*-(2-naphthylmethyl)- α/β -D-mannopyranosyl uronate) (17) Diol 14



(4.58 g, 7.97 mmol) was dissolved in DCM (25 mL) and H_2O (15 mL) was added. To the two phase system TEMPO (250 mg, 1.59 mmol, 0.2 eq.) and BAIB (6.42 g, 19.9 mmol, 2.5 eq.) were added. After stirring vigorously for 6 hours at room temperature, the reaction was

quenched by addition of sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$. EtOAc (50 mL) was added and the layers separated. The organic layer was dried with MgSO_4 , filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene once. The crude mannuronic acid was then dissolved in DMF (40 mL) and put under an argon atmosphere at 0°C . Methyl iodide (1.5 mL, 23.9 mmol, 3 eq.) and K_2CO_3 (3.30 g, 23.9 mmol, 3 eq.) were added and the reaction was stirred overnight. The reaction was quenched with H_2O and extracted twice with EtOAc. The organic layers were collected and dried with MgSO_4 , filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 8:1 $\rightarrow$ 2:1) afforded the title compound as a yellow oil (3.9 g, 6.5 mmol, 81%, $\alpha : \beta = 1 : 5$). TLC R_f 0.27 (PE/EtOAc, 4/1, v/v); IR (neat): 745, 781, 814, 837, 1067, 1250, 1362, 1748, 3472 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.59-7.83 (m, 10.80H, CH_{arom}), 7.39-7.47 (m, 5H, CH_{arom}), 7.30 (dd, 1H, $J = 1.2, 8.4$ Hz, CH_{arom}), 5.19 (d, 1H, $J = 12.8$ Hz, CHH Nap β), 5.12 (d, 0.20H, $J = 2.0$ Hz, H-1 α), 4.98 (d, 1H, $J = 12.8$ Hz, CHH Nap β), 4.77-4.90 (m, 0.80H, CH_2 Nap α , CH_2 Nap α), 4.75 (s, 1H, H-1 β), 4.67 (d, 1H, $J = 12.4$ Hz, CHH Nap β), 4.63 (d, 1H, $J = 12.4$ Hz, CHH Nap β), 4.37 (t, 1.20H, $J = 9.6$ Hz, H-4 α , H-4 β), 4.20 (d, 0.20H, $J = 9.2$ Hz, H-5 α), 3.83-3.87 (m, 1.20H, H-2 β , H-3 α), 3.79 (s, 3.60H, CH_3 CO_2Me α,β), 3.75 (d, 1H, $J = 9.6$ Hz, H-5 β), 3.56 (t, 0.20H, $J = 2.4$ Hz, H-2 α), 3.41 (dd, 1H, $J = 2.8, 9.6$ Hz, H-3 β), 3.21 (bs, 1H, 4-OH β), 3.10 (bs, 0.20H, 4-OH α), 0.95 (s, 9H, CH_3 tBu β), 0.68 (s, 1.80H, CH_3 tBu α), 0.19 (s, 3H, CH_3 Me β), 0.14 (s, 3H, CH_3 Me β), -0.01 (s, 0.60H, CH_3 Me α), -0.14 (s, 0.60H, CH_3 Me α); ^{13}C NMR (CDCl_3 , 100 MHz): δ 171.0 (C=O CO_2Me α), 170.2 (C=O CO_2Me β), 136.4, 135.8, 135.6, 133.3, 133.3, 133.2, 133.1, 133.1 (Cq), 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 128.0, 127.8, 127.1, 127.0, 127.0, 126.9, 126.6, 126.4, 126.2, 126.2, 126.1, 126.1, 126.0, 126.0, 125.9, 125.8, 125.7 (CH_{arom}), 97.3 (C-1 β), 93.8 (C-1 α), 80.4 (C-3 β), 77.8 (C-3 α), 75.9 (C-2 α), 75.3 (C-2 β), 75.0 (C-5 β), 74.6, 73.2, 73.1, 72.1 (CH_2 Nap β , α , α , β), 72.0 (C-5 α), 68.8 (C-4 α), 68.4 (C-4 β), 52.6 (CH_3 CO_2Me), 25.9 (CH_3 tBu β), 25.4 (CH_3 tBu α), 18.1 (Cq tBu β), 17.8 (Cq tBu α), -3.9, -5.4 (CH_3 Me); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 97.3 ($J_{\text{C1,H1}} = 153$ Hz, C-1 β), 93.8 ($J_{\text{C1,H1}} = 167$ Hz, C-1 α); HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{35}\text{H}_{42}\text{O}_7\text{SiNa}$ 625.25920, found 625.25806.

Methyl (*tert*-butyldimethylsilyl 4-*O*-levulinoyl-2,3-di-*O*-(2-naphthylmethyl)- α/β -D-mannopyranosyl uronate) (20)

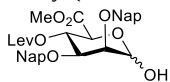


(20) Levulinic acid (2.25 g, 19.4 mmol, 3.0 eq.) and DIC (1.5 mL, 9.7 mmol, 1.5 eq.) were added to a 0°C solution of **17** (3.9 g, 6.47 mmol) in dry DCM (16 mL). A catalytic amount of DMAP (79 mg, 0.65 mmol, 0.1 eq.) was added and the reaction mixture was allowed to

reach room temperature. After 3 hours the reaction mixture was filtered over Celite and the filtrate washed with sat. aq. NaHCO_3 and brine. The organic layer was dried (MgSO_4), filtered, and concentrated *in vacuo*. Column chromatography (PE/EtOAc, 8:1 $\rightarrow$ 2:1) afforded the title compound as an amorphous off-white solid (3.95 g, 5.65 mmol, 87%, $\alpha : \beta = 1 : 5$). TLC: R_f 0.54 (PE/EtOAc, 2/1, v/v); IR (neat): 752, 783, 820, 1030, 1109, 1265, 1368,

1715, 1742, 1753 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.68-7.81 (m, 8.80H, CH_{arom}), 7.59-7.63 (m, 2H, CH_{arom}), 7.41-7.48 (m, 5.20H, CH_{arom}), 7.27-7.29 (m, 1H, CH_{arom}), 5.58 (t, 1.20H, $J = 9.6$ Hz, H-4 α , H-4 β), 5.29 (bs, 0.20H, H-1 α), 5.19 (d, 1H, $J = 12.8$ Hz, CHH Nap β), 5.03 (d, 1H, $J = 12.8$ Hz, CHH Nap β), 4.88 (s, 0.40H, CH_2 Nap α), 4.74-4.77 (m, 1.40H, H-1 β , CH_2 Nap α), 4.57 (d, 1H, $J = 12.4$ Hz, CHH Nap β), 4.49 (d, 1H, $J = 12.4$ Hz, CHH Nap β), 4.30 (d, 0.20H, $J = 7.6$ Hz, H-5 α), 3.92 (dd, 0.20H, $J = 3.2$, 8.0 Hz, H-3 α), 3.88 (d, 1H, $J = 2.8$ Hz, H-2 β), 3.84 (d, 1H, $J = 9.6$ Hz, H-5 β), 3.73 (s, 3H, CH_3 CO_2Me β), 3.67 (s, 0.60H, CH_3 CO_2Me α), 3.51-3.54 (m, 1.20H, H-2 α , H-3 β), 2.68 (t, 2H, $J = 6.8$ Hz, CH_2 Lev β), 2.62-2.65 (m, 0.40H, CH_2 Lev α), 2.51-2.58 (m, 2.40H, CH_2 Lev α,β), 2.13 (s, 3.60H, CH_3 Lev α,β), 0.94 (s, 9H, CH_3 tBu β), 0.76 (s, 1.80H, CH_3 tBu α), 0.17 (s, 3H, CH_3 Me β), 0.12 (s, 3H, CH_3 Me β), 0.05 (s, 0.60H, CH_3 Me α), -0.04 (s, 0.60H, CH_3 Me α); ^{13}C NMR (CDCl_3 , 100MHz): δ 206.4 (C=O Lev), 171.7, 168.0 (C=O Lev, CO_2Me), 136.3, 135.5, 133.3, 133.3, 133.1, 133.1 (Cq), 128.2, 128.1, 128.0, 128.0, 127.8, 127.8, 127.0, 126.8, 126.4, 126.3, 126.1, 126.0, 125.8, 125.7 (CH_{arom}), 97.0 (C-1), 78.6 (C-3), 74.6 (C-2), 74.3 (CH_2 Nap), 73.6 (C-5), 71.8 (CH_2 Nap), 69.1 (C-4), 52.7 (OMe), 37.9 (CH_2 Lev), 30.0 (CH_3 Lev), 28.1 (CH_2 Lev), 25.9 (CH_3 tBu), 18.2 (Cq tBu), -3.9, -5.4 (CH_3 Me); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 97.0 ($J_{\text{C1,H1}} = 154$ Hz, C-1 β); HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{40}\text{H}_{48}\text{O}_9\text{SiNa}$ 723.29598, found 723.29508.

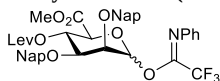
Methyl (4-O-levulinoyl-2,3-di-O-(2-naphthylmethyl)- α/β -D-mannopyranosyl uronate) (23) Acetic acid (70 μL ,



0.7 mmol, 3.5 eq.) was added to a 0°C solution of compound **20** (245 mg, 0.35 mmol) in dry THF (3.5 mL). TBAF (1.0 M solution in THF, 1.0 mL, 1.0 mmol, 3 eq.) was added drop wise over 5 minutes. The reaction mixture was stirred for 4.5 hours at room temperature and

subsequently diluted with EtOAc and washed once with H_2O and brine. The organic layer was dried (MgSO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (Pentane/EtOAc, 8:1 $\rightarrow$ 1:1) furnished the title compound as a yellow oil (216 mg, 0.36 mmol, 98%, $\alpha \gg \beta$). Analytic data is reported for the α -anomer. TLC: R_f 0.17 (PE/EtOAc, 1/1, v/v); IR (neat): 750, 816, 1032, 1123, 1362, 1715, 1742, 3422 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.71-7.81 (m, 5H, CH_{arom}), 7.66-7.69 (m, 3H, CH_{arom}), 7.38-7.49 (m, 6H, CH_{arom}), 5.59-5.62 (m, 2H, H-1, H-4), 4.89 (d, 1H, $J = 12.0$ Hz, CHH Nap), 4.78 (d, 1H, $J = 12.0$ Hz, CHH Nap), 4.73 (s, 2H, CH_2 Nap), 4.48 (d, 1H, $J = 5.2$ Hz, H-5), 4.15 (d, 1H, $J = 4.4$ Hz, 1-OH), 3.98 (dd, 1H, $J = 3.2$, 10.4 Hz, H-3), 3.68-3.70 (m, 1H, H-2), 3.61 (s, 3H, CH_3 CO_2Me), 2.37-2.60 (m, 4H, CH_2 Lev), 2.10 (s, 3H, CH_3 Lev); ^{13}C NMR (CDCl_3 , 100 MHz): δ 206.5 (C=O Lev), 171.8, 169.2 (C=O Lev, CO_2Me), 135.7, 135.3, 133.3, 133.2, 133.0 (Cq), 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 126.8, 126.6, 126.3, 126.2, 126.1, 126.1, 126.0, 126.0, 125.8 (CH_{arom}), 92.7 (C-1), 75.2 (C-2, C-3), 73.1, 72.7 (CH_2 Nap), 71.3 (C-5), 69.7 (C-4), 52.6 (CH_3 CO_2Me), 37.8 (CH_2 Lev), 29.9 (CH_3 Lev), 28.0 (CH_2 Lev); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 92.7 ($J_{\text{C1,H1}} = 171$ Hz, C-1 α); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. for $\text{C}_{34}\text{H}_{38}\text{NO}_9$ 604.25411, found 604.25436.

Methyl (4-O-levulinoyl-2,3-di-O-(2-naphthylmethyl)-1-O-(N-[phenyl]trifluoroacetimidoyl)- α/β -D-mannopyranosyl uronate) (26) Cs_2CO_3 (141 mg, 0.43 mmol, 1.2 eq.) was added to a



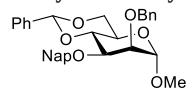
0°C solution of compound **23** (216 mg, 0.36 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (60 μL , 0.4 mmol, 1.1 eq.) in acetone (1.2 mL). After

stirring for 1.5 hours at ambient temperature TLC analysis showed complete conversion of the starting compound to a higher running spot. H_2O was added and the mixture was extracted twice with EtOAc. The organic fraction was washed with brine, dried with MgSO_4 , filtered, and concentrated *in vacuo*. The crude compound was purified using column chromatography (PE/EtOAc, 8:1 $\rightarrow$ 1:1) to yield the title compound as a yellow solid (252 mg, 0.356 mmol, 99%, $\alpha : \beta = 6.7 : 1$). TLC: R_f 0.57 α , 0.47 β (PE/EtOAc, 2/1, v/v); IR (neat): 1125, 1153, 1207, 1717, 1748 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.56-7.81 (m, 9.20H, CH_{arom}), 7.36-7.50 (m, 6.90H, CH_{arom}), 7.22 (t, 2.30H, $J = 8.0$

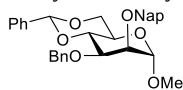
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Hz, NPh), 7.08 (t, 1.15H, $J = 7.6$ Hz, NPh), 6.68 (d, 0.30H, $J = 7.6$ Hz, NPh β), 6.58 (d, 2H, $J = 6.4$ Hz, NPh α), 6.46 (bs, 1H, H-1 α), 6.05 (bs, 0.15H, H-1 β), 5.77 (t, 0.15H, $J = 6.0$ Hz, H-4 β), 5.66 (t, 1H, $J = 7.6$ Hz, H-4 α), 5.00 (d, 0.15H, $J = 12.4$ Hz, CHH Nap β), 4.96 (d, 0.15H, $J = 12.4$ Hz, CHH Nap β), 4.75–4.87 (m, 3.30H, CH₂ Nap α , CH₂ Nap β , CHH Nap α), 4.69 (d, 1H, $J = 12.4$ Hz, CHH Nap α), 4.42 (d, 1H, $J = 7.2$ Hz, H-5 α), 4.16 (bs, 0.15H, H-5 β), 4.09 (bs, 0.15H, H-2 β), 3.95 (dd, 1H, $J = 2.8$, 7.6 Hz, H-3 α), 3.79–3.88 (m, 1.15H, H-2 α , H-3 β), 3.69 (s, 3H, CH₃ CO₂Me α), 3.63 (s, 0.45H, CH₃ CO₂Me β), 2.64–2.67 (m, 2.30H, CH₂ Lev α,β), 2.48–2.60 (m, 2.30H, CH₂ Lev α,β), 2.14 (s, 3H, CH₃ Lev α), 2.13 (s, 0.45H, CH₃ Lev β); ¹³C APT NMR (CDCl₃, 100 MHz, HSQC), only provided for the α -anomer: δ 206.2 (C=O Lev), 171.7, 168.0 (C=O Lev, CO₂Me), 143.2 (Cq NPh), 134.9, 134.9, 133.2, 133.2, 133.2 (Cq), 128.8, 128.7, 128.4, 128.3, 128.1, 128.0, 127.8, 127.2, 127.1, 126.3, 126.3, 126.2, 126.1, 126.0 (CH_{arom} Nap), 124.5, 124.2, 119.4 (CH_{arom} NPh), 94.5 (C-1), 74.8 (C-3), 73.2 (CH₂ Nap), 73.0 (C-2), 72.9 (CH₂ Nap), 72.7 (C-5), 68.9 (C-4), 52.9 (CH₃ CO₂Me), 37.8 (CH₂ Lev), 29.9 (CH₃ Lev), 28.0 (CH₂ Lev); HRMS: [M+Na]⁺ calcd. for C₄₂H₃₈F₃NO₉Na 780.23909, found 780.23981.

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-(2-naphthylmethyl)- α -D-mannopyranoside (**3**)

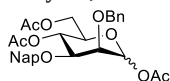


(10.9 g, 43.8 mmol, 1.2 eq.) was added to a solution of compound **1** (10.3 g, 36.6 mmol) in toluene (200 mL) and refluxed overnight under an argon atmosphere. The solution was concentrated to dryness *in vacuo* and DMF (200 mL) was added under argon. Benzyl bromide (9.55 g, 43.2 mmol, 1.18 eq.) and cesium fluoride (6.65 g, 43.8 mmol, 1.2 eq.) were added to the reaction mixture. After stirring overnight, the reaction mixture was quenched with H₂O and extracted with EtOAc. The organic layer was washed with H₂O and brine, dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 8:1 $\rightarrow$ 2:1) yielded the title compound as a yellow oil (13.7 g, 32.5 mmol, 89%). TLC: R_f 0.22 (PE/EtOAc, 4/1, v/v); IR (neat): 748, 972, 1049, 1373, 1452, 3453 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.78–7.82 (m, 3H, CH_{arom}), 7.70–7.73 (m, 1H, CH_{arom}), 7.50–7.52 (m, 2H, CH_{arom}), 7.43–49 (m, 3H, CH_{arom}), 7.36–7.40 (m, 3H, CH_{arom}), 5.63 (s, 1H, CHPh), 4.97 (d, 1H, $J = 12$ Hz, CHH Nap), 4.87 (d, 1H, $J = 12$ Hz, CHH Nap), 4.72 (d, 1H, $J = 1.2$ Hz, H-1), 4.28 (dd, 1H, $J = 4.0$, 12 Hz, H-6), 4.13 (t, 1H, $J = 9.6$ Hz, H-4), 4.05 (dd, 1H, $J = 1.2$, 3.6 Hz, H-2), 3.93 (dd, 1H, $J = 3.6$, 9.6 Hz, H-3), 3.77–3.89 (m, 2H, H-5, H-6), 3.33 (s, 3H, CH₃ OMe), 2.82 (s, 1H, 2-OH); ¹³C NMR (CDCl₃, 400 MHz): δ 137.7, 135.5 (Cq), 133.3, 133.2 (Cq Nap), 129.1, 128.4, 128.1, 127.8, 126.7, 126.3, 126.1, 125.8 (CH_{arom}), 101.8 (CHPh), 101.2 (C-1), 78.9 (C-4), 75.6 (C-3), 73.0 (CH₂ Nap), 69.9 (C-2), 69.0 (C-6), 63.3 (C-5), 55.0 (OMe); HRMS: [M+Na]⁺ calcd. for C₂₅H₂₆O₆Na 445.16216, found 445.16173. The alcohol (10.35 g, 24.5 mmol) was dissolved in DMF (125 mL) and cooled to 0°C. Benzyl bromide (3.2 mL, 27 mmol, 1.1 eq.) and sodium hydride (60% dispersion in oil, 1.08 mg, 27 mmol, 1.1 eq., in three equal parts) were added and the solution left to stir overnight. The reaction mixture was quenched by dropwise addition of MeOH (15 mL) and H₂O (150 mL) and subsequently extracted twice with EtOAc. The organic layer was washed with brine (100 mL) and dried with MgSO₄. After filtration and concentration *in vacuo*, the crude compound was purified by column chromatography (PE/EtOAc, 9:1 to 4:1) to yield the title compound as a yellow oil (12.0 g, 23.4 mmol, 95%). TLC: R_f 0.67 (PE/EtOAc, 4/1, v/v); IR (neat): 750, 1057, 1099, 1126, 1375, 2909 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.76–7.82 (m, 3H, CH_{arom}), 7.68–7.70 (m, 1H, CH_{arom}), 7.51–7.54 (m, 2H, CH_{arom}), 7.23–7.46 (m, 11H, CH_{arom}), 5.67 (s, 1H, CHPh), 4.92 (d, 1H, $J = 12.8$ Hz, CHH Nap), 4.76–4.94 (m, 3H, CH₂ Bn, CHH Nap), 4.70 (d, 1H, $J = 1.2$ Hz, H-1), 4.24–4.31 (m, 2H, H-4, H-6), 3.99 (dd, 1H, $J = 3.2$, 10 Hz, H-3), 3.85–3.92 (m, 2H, H-2, H-6), 3.74–3.80 (m, 1H, H-5), 3.30 (s, 3H, CH₃ OMe); ¹³C NMR (CDCl₃, 100 MHz): δ 138.2, 137.9, 136.2, 133.4, 133.0 (Cq), 129.0, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 126.3, 126.2, 126.1, 125.8, 125.7 (CH_{arom}), 101.7 (CHPh), 100.5 (C-1), 79.2 (C-4), 76.3, 76.2 (C-3, C-2), 73.7, 72.9 (CH₂ Bn, Nap), 69.0 (C-6), 64.1 (C-5), 54.9 (OMe); HRMS: [M+NH₄]⁺ calcd. for C₃₂H₃₆NO₆ 530.25371, found 530.25349.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2-naphthylmethyl)- α -D-mannopyranoside (4)


(5.98 g, 24 mmol, 1.2 eq.) was added to a solution of compound **1** (5.65 g, 20 mmol) in toluene (100 mL) and refluxed overnight under an argon atmosphere. The solution was concentrated to dryness *in vacuo* and DMF (100 mL) was added under argon. Benzyl

bromide (2.6 mL, 22 mmol, 1.1 eq.) and cesium fluoride (3.65 g, 24 mmol, 1.2 eq.) were added to the reaction mixture. After stirring overnight, the reaction mixture was quenched with H₂O and extracted first with Et₂O and then EtOAc, dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 5:1 → 1:2) yielded the title compound as a yellow oil (6.7 g, 18 mmol, 89%). TLC: R_f 0.59 (PE/EtOAc, 1/1, v/v); Spectroscopic data were in accord with those reported previously. IR (neat): 746, 972, 1049, 1373, 1454, 3447 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.48-7.50 (m, 2H, CH_{arom} CHPh), 7.26-7.40 (m, 8H, CH_{arom}), 5.60 (s, 1H, CHPh), 4.84 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.73 (d, 1H, *J* = 1.2 Hz, H-1), 4.70 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.27 (dd, 1H, *J* = 4.0, 9.2 Hz, H-6), 4.09 (t, 1H, *J* = 9.2 Hz, H-4), 4.01 (dd, 1H, *J* = 1.2, 3.6 Hz, H-2), 3.77-3.90 (m, 3H, H-3, H-5, H-6), 3.35 (s, 3H, CH₃ OMe), 2.80 (bs, 1H, 2-OH); ¹³C NMR: (CDCl₃, 100 MHz): δ 138.1, 137.7 (Cq Bn, CHPh), 129.0, 128.6, 128.3, 128.0, 127.9, 126.2 (CH_{arom}), 101.7 (CHPh), 101.2 (C-1), 78.9 (C-4), 75.7 (C-3), 73.1 (CH₂ Bn), 70.0 (C-2), 69.0 (C-6), 63.3 (C-5), 55.0 (OMe); HRMS: [M+Na]⁺ calcd. for C₂₁H₂₄O₆Na 395.14651, found 395.14638. The alcohol (6.48 g, 17.4 mmol) was dissolved in DMF (90 mL) and cooled to 0°C. 2-(Bromomethyl)naphthalene (4.62 g, 20.9 mmol, 1.2 eq.) and sodium hydride (60% dispersion in oil, 867 mg, 20.9 mmol, 1.2 eq.) were added and the solution left to stir for 3.5 hours. The reaction mixture was quenched by dropwise addition of H₂O and subsequently extracted with EtOAc. The organic layer was washed with brine and dried with MgSO₄. After filtration and concentration *in vacuo*, the crude compound was purified by column chromatography (PE/EtOAc, 9:1 to 4:1) to yield the title compound as a yellow oil (8.41 g, 16.4 mmol, 94%). TLC: R_f 0.62 (PE/EtOAc, 4/1, v/v); IR (neat): 696, 746, 1051, 1371, 1452 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.74-7.80 (m, 4H, CH_{arom}), 7.50-7.52 (m, 3H, CH_{arom}), 7.41-7.46 (m, 2H, CH_{arom}), 7.23-7.38 (m, 8H, CH_{arom}), 5.66 (s, 1H, CHPh), 4.96 (d, 1H, *J* = 12.4 Hz, CHH Nap), 4.91 (d, 1H, *J* = 12.4 Hz, CHH Nap), 4.83 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.71 (d, 1H, *J* = 1.2 Hz, H-1), 4.65 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.25-4.31 (m, 2H, H-4, H-6), 3.96 (dd, 1H, *J* = 3.2, 10 Hz, H-3), 3.87-3.93 (m, 2H, H-2, H-6), 3.75-3.81 (m, 1H, H-5), 3.30 (s, 3H, CH₃ OMe); ¹³C NMR (CDCl₃, 100 MHz): δ 138.8, 137.8, 135.6, 133.3, 133.2 (Cq), 129.2, 129.0, 128.4, 128.4, 128.3, 128.1, 127.8, 127.7, 127.6, 127.1, 126.3, 126.2, 126.2, 126.1 (CH_{arom}), 101.6 (CHPh), 100.6 (C-1), 79.3 (C-4), 76.6 (C-3), 76.2 (C-2), 73.8, 73.3 (CH₂ Bn, Nap), 69.0 (C-6), 64.2 (C-5), 54.9 (OMe); ¹³C-GATED NMR (CDCl₃, 100 MHz): δ 100.6 (*J*_{C1,H1} = 168 Hz, C-1); HRMS: [M+Na]⁺ calcd. for C₃₂H₃₂O₆Na 535.20911, found 535.20818.

Acetyl 4,6-di-O-acetyl-2-O-benzyl-3-O-(2-naphthylmethyl)- α -D-mannopyranoside (6)


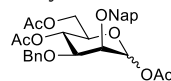
compound **3** (11.5 g, 22.5 mmol) in acetic anhydride (110 mL), pTsOH·H₂O (6.6 g, 35 mmol, 1.5 eq.) was added. The reaction mixture was allowed to stir for four days at room temperature. The reaction mixture was quenched by pouring it over ice and gradually adding

solid NaHCO₃ until all ice had melted and CO₂ evolution had stopped. The aqueous mixture was extracted three times with EtOAc and the combined organic layers were washed once with brine. The organic fraction was dried with MgSO₄, filtered and concentrated *in vacuo*, after which the residue was co-evaporated once with toluene. Column chromatography purification (PE/EtOAc, 9:1 → 2:1) afforded the title compound as an orange oil (10.15 g, 18.9 mmol, 84%, $\alpha \gg \beta$). TLC: R_f 0.45 (PE/EtOAc, 2/1, v/v); IR (neat): 700, 745, 820, 955, 1013, 1043, 1217, 1369, 1738 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.78-7.84 (m, 3H, CH_{arom}), 7.70 (s, 1H, CH_{arom}), 7.46-7.49 (m, 2H, CH_{arom}), 7.27-7.39 (m, 6H, CH_{arom}), 6.18 (d, 1H, *J* = 2.0 Hz, H-1), 5.52 (t, 1H, *J* = 9.6 Hz, H-4), 4.68-4.76 (m, 3H, CH₂, CHH Bn/Nap), 4.58 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap), 4.22 (dd, 1H, *J* = 5.0, 12.4 Hz, H-6), 4.12 (dd, 1H, *J*

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= 2.4, 12.4 Hz, H-6), 3.89-3.93 (m, 1H, H-5), 3.83 (dd, 1H, $J = 2.8, 9.6$ Hz, H-3), 3.78 (t, 1H, $J = 2.4$ Hz, H-2), 2.07 (s, 3H, CH₃ Ac), 2.04 (s, 3H, CH₃ Ac), 1.98 (s, 3H, CH₃ Ac); ¹³C NMR (CDCl₃, 100 MHz): δ 170.9, 169.6, 168.7 (C=O Ac), 137.6, 135.2, 133.2, 133.0 (Cq-_{arom}), 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.8, 126.4, 126.3, 126.1, 125.5 (CH_{arom}), 91.8 (C-1), 75.9 (C-3), 72.8 (C-2), 72.6, 71.7 (CH₂ Bn, Nap), 71.4 (C-5), 67.3 (C-4), 62.6 (C-6), 20.9, 20.9, 20.8 (CH₃ Ac); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 91.8 ($J_{C1,H1} = 175$ Hz, C-1 α); HRMS: $[M+NH_4]^+$ calcd. for C₃₀H₃₆NO₉ 554.23846, found 554.23861.

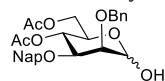
Acetyl 4,6-di-O-acetyl-3-O-benzyl-2-O-(2-naphthylmethyl)- α -D-mannopyranoside (7) To a solution of



compound **4** (7.3 g, 14.2 mmol) in acetic anhydride (70 mL), pTsOH·H₂O (4.0 g, 21.0 mmol, 1.5 eq.) was added. The reaction mixture was allowed to stir for four days at room temperature until TLC analysis showed substantial conversion to the desired product. The

reaction mixture was quenched by pouring it over ice and gradually adding solid NaHCO₃ until all ice had melted and CO₂ evolution had stopped. The aqueous mixture was extracted two times with EtOAc and the combined organic layers were washed once with brine. The organic fraction was dried with MgSO₄, filtered and concentrated *in vacuo*, after which the residue was co-evaporated once with toluene. Column chromatography purification (PE/EtOAc, 6:1 $\rightarrow$ 2:1) afforded the title compound as an orange oil (5.06 g, 9.44 mmol, 66%, $\alpha \gg \beta$). TLC: R_f 0.36 (PE/EtOAc, 2/1, v/v); IR (neat): 733, 955, 1217, 1368, 1740, 2918 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.73-7.82 (m, 4H, CH_{arom}), 7.51 (dd, 1H, $J = 1.6, 8.4$ Hz, CH_{arom}), 7.43-7.47 (m, 2H, CH_{arom}), 7.28-7.33 (m, 3H, CH_{arom}), 7.21-7.27 (m, 2H, CH_{arom}), 6.24 (d, 1H, $J = 1.6$ Hz, H-1), 5.53 (t, 1H, $J = 9.6$ Hz, H-4), 4.92 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap), 4.87 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap), 4.52 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap), 4.44 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap), 4.24 (dd, 1H, $J = 4.8, 12.0$ Hz, H-6), 4.13 (dd, 1H, $J = 2.4, 12.0$ Hz, H-6), 3.91-3.95 (m, 1H, H-5), 3.78-3.81 (m, 2H, H-2, H-3), 2.08 (s, 3H, CH₃ Ac), 2.05 (s, 3H, CH₃ Ac), 2.04 (s, 3H, CH₃ Ac); ¹³C NMR (CDCl₃, 100 MHz): δ 171.0, 169.7, 168.7 (C=O Ac), 137.9, 135.1, 133.2, 133.2 (Cq-_{arom}), 128.6, 128.5, 128.3, 128.0, 127.9, 127.8, 127.5, 127.0, 126.5, 126.2, 126.1 (CH_{arom}), 92.6 (C-1 β) 91.8 (C-1 α), 76.3 (C-3), 72.7 (C-2), 72.7, 72.1 (CH₂ Bn, Nap), 71.5 (C-5), 67.5 (C-4), 62.7 (C-6), 21.1, 21.0, 20.9 (CH₃ Ac); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 91.8 ($J_{C1,H1} = 175$ Hz, C-1 α); HRMS: $[M+Na]^+$ calcd. for C₃₀H₃₂O₉Na 559.19385, found 559.19279.

4,6-Di-O-acetyl-2-O-benzyl-3-O-(2-naphthylmethyl)- α/β -D-mannopyranose (9) Compound **6** (10.14 g, 18.9

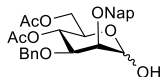


mmol) was dissolved in 4% piperidine (3.75 mL, 38 mmol, 2 eq.) in THF (100 mL). After stirring for 2.5 days at room temperature H₂O was added and the mixture was extracted with EtOAc. The organic layer was washed once with brine and subsequently dried (MgSO₄),

filtered, and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 8:1 $\rightarrow$ 1:1) gave the title compound as an orange oil (8.95 g, 18.1 mmol, 85%, $\alpha : \beta = 3.8 : 1$). TLC: R_f 0.53 (PE/EtOAc, 1/1, v/v); IR (neat): 743, 880, 1042, 1086, 1238, 1371, 1732, 3343 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.78-7.85 (m, 3.78H, CH_{arom}), 7.73-7.75 (m, 1.26H, CH_{arom}), 7.46-7.51 (m, 2.51H, CH_{arom}), 7.25-7.41 (m, 6.56H, CH_{arom}), 5.46 (t, 1H, $J = 9.6$ Hz, H-4 α), 5.38 (t, 0.26H, $J = 9.6$ Hz, H-4 β), 5.27 (d, 1H, $J = 1.6$ Hz, H-1 α), 5.10 (d, 0.26H, $J = 11.6$ Hz, CHH Bn/Nap β), 4.84 (d, 0.26H, $J = 12.4$ Hz, CHH Bn/Nap β), 4.66-4.79 (m, 3.78H, CH₂ Bn/Nap α , CHH Bn β , Nap β , CHH Bn/Nap α , H-1 β), 4.63 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap α), 4.11-4.22 (m, 2.52H, H-6 α , H-6 α , H-6 β , H-6 β), 4.02-4.06 (m, 1H, H-5 α), 3.95 (dd, 1H, $J = 2.8, 9.6$ Hz, H-3 α), 3.88-3.89 (m, 0.26H, H-2 β), 3.84 (t, 1H, $J = 2.4$ Hz, H-2 α), 3.64 (dd, 0.26H, $J = 2.8, 9.6$ Hz, H-3 β), 3.52-3.56 (m, 0.26H, H-5 β), 3.31 (bs, 1H, 1-OH α), 2.07 (s, 3H, CH₃ Ac α), 2.04 (s, 0.42H, CH₃ Ac β), 2.03 (s, 0.42H, CH₃ Ac β), 2.01 (s, 3H, CH₃ Ac α); ¹³C (CDCl₃, 100 MHz): δ 171.1, 170.0, 169.9 (C=O Ac), 138.2, 137.8, 135.7, 133.4, 133.2, 133.1 (Cq Bn Nap), 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 126.6, 126.5, 126.4, 126.3, 126.1, 125.7, 125.6 (CH_{arom}), 93.8 (C-1

β), 93.1 (C-1 α), 79.9 (C-3 β), 76.7 (C-3 α), 75.5 (C-2 β), 74.9 (CH₂ Bn/Nap β), 74.5 (C-2 α), 73.0 (CH₂ Bn/Nap α), 72.7 (CH₂ Bn/Nap β), 72.5 (C-5 β), 72.1 (CH₂ Bn/Nap α), 69.4 (C-5 α), 68.3 (C-4 α), 67.9 (C-4 β), 63.3 (C-6 α), 63.1 (C-6 β), 21.1, 20.9 (CH₃ Ac); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 93.8 (J_{C1,H1} = 158 Hz, C-1 β), 93.1 (J_{C1,H1} = 169 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₂₈H₃₄NO₈ 512.22789, found 512.22750.

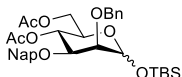
4,6-Di-O-acetyl-3-O-benzyl-2-O-(2-naphthylmethyl)- α/β -D-mannopyranose (10) Compound 7 (5.06 g, 9.44



mmol) was dissolved in 4% piperidine (1.85 mL, 18.9 mmol, 2 eq.) in THF (47 mL). After stirring for 3 days at room temperature H₂O was added and the mixture was extracted with EtOAc. The organic layer was washed once with brine and subsequently dried (MgSO₄),

filtered, and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 9:1 $\rightarrow$ 1:1) gave the title compound as a yellow oil (4.1 g, 8.3 mmol, 88%, α : β = 4.3 : 1). TLC: R_f 0.58 (PE/EtOAc, 1/1, v/v); IR (neat): 1042, 1099, 1238, 1369, 1740, 3428 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.73-7.81 (m, 4.92H, CH_{arom}), 7.42-7.49 (m, 3.69H, CH_{arom}), 7.24-7.34 (m, 6.15H, CH_{arom}), 5.46 (t, 1H, *J* = 10.0 Hz, H-4 α), 5.39 (t, 0.23H, *J* = 9.6 Hz, H-4 β), 5.27 (s, 1H, H-1 α), 5.18 (d, 0.23H, *J* = 11.6 Hz, CHH Bn/Nap β), 4.78-4.90 (m, 2.23H, CHH Bn/Nap β , CH₂ Bn/Nap α), 4.67 (m, 0.46H, CHH Bn/Nap β , H-1 β), 4.54-4.60 (m, 1.23H, CHH Bn/Nap β , CHH Bn/Nap α), 4.55 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap α), 4.09-4.23 (m, 2.46H, H-6 α , H-6 α , H-6 β , H-6 β), 4.03-4.07 (m, 1H, H-5 α), 3.89 (dd, 1.23H, *J* = 2.8, 9.6 Hz, H-2 β , H-3 α), 3.82-3.83 (m, 1H, H-2 α), 3.67 (bs, 1H, 1-OH α), 3.55-3.60 (m, 0.46H, H-3 β , H-5 β), 2.03 (s, 3H, CH₃ Ac β), 2.03 (s, 0.69H, CH₃ Ac α), 2.02 (s, 0.69H, CH₃ Ac α), 1.98 (s, 3H, CH₃ Ac β); ¹³C (CDCl₃, 100 MHz): δ 171.2, 171.1, 170.0, 169.9 (C=O Ac), 138.2, 137.6, 135.7, 135.1, 133.2, 133.2, (Cq), 128.9, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 128.0, 127.8, 127.7, 127.7, 127.5, 127.4, 126.8, 126.3, 126.2, 126.1, 126.1, 126.0 (CH_{arom}), 93.9 (C-1 β), 93.1 (C-1 α), 80.1 (C-3 β), 76.7 (C-3 α), 75.3 (C-2 β), 74.9 (CH₂ Bn/Nap β), 74.4 (C-2 α), 73.0 (CH₂ Bn/Nap α), 72.7 (CH₂ Bn/Nap β), 72.5 (C-5 β), 72.0 (CH₂ Bn/Nap α), 69.2 (C-5 α), 68.3 (C-4 α), 67.9 (C-4 β), 63.3 (C-6), 63.0 (C-6 β), 21.0, 20.9 (CH₃ Ac); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 93.1 (J_{C1,H1} = 169 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₂₈H₃₄NO₈ 512.22789, found 512.22754.

Tert-butyldimethylsilyl 4,6-di-O-acetyl-2-O-benzyl-3-O-(2-naphthylmethyl)- α/β -D-mannopyranoside (12)

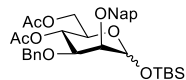


TBDMSCl (5.43 g, 36 mmol, 2.25 eq.) and imidazole (2.45 g, 36 mmol, 2.25 eq.) were added to a solution of hemiacetal 9 (7.9 g, 16 mmol) in DCM (85 mL) under an argon atmosphere. After stirring overnight, the reaction was quenched with H₂O and extracted

twice with EtOAc. Combined organic layers were washed with brine, dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 15:1 $\rightarrow$ 2:1) yielded the title compound as a white solid (7.53 g, 12.37 mmol, 77%, α : β = 1 : 2.8). TLC: R_f 0.51 (Pentane/EtOAc, 4/1, v/v); IR (neat): 839, 1044, 1236, 1368, 1744 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.78-7.84 (m, 4.08H, CH_{arom}), 7.72 (s, 0.36H, CH_{arom} α), 7.66 (s, 1H, CH_{arom} β), 7.43-7.49 (m, 5.04H, CH_{arom}), 7.24-7.34 (m, 5.8H, CH_{arom}), 5.41 (t, 0.36H, *J* = 10.0 Hz, H-4 α), 5.32 (t, 1H, *J* = 10.0 Hz, H-4 β), 5.06 (d, 0.36H, *J* = 2.0 Hz, H-1 α), 5.01 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap β), 4.81-4.88 (m, 1.36H, CHH Bn/Nap β , CHH Bn/Nap α), 4.60-4.75 (m, 3.08H, CHH Bn/Nap α , CH₂ Bn/Nap α , CHH Bn/Nap β , H-1 β), 4.45 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap β), 4.16-4.20 (m, 2.36H, H-6 β , H-6 β , H-6 α), 4.08 (dd, 0.36H, *J* = 2.2, 12.2 Hz, H-6 α), 3.89-3.92 (m, 0.72H, H-3 α , H-5 α), 3.86 (d, 1H, *J* = 2.8 Hz, H-2 β), 3.58 (t, 0.36H, *J* = 2.4 Hz, H-2 α), 3.48-3.53 (m, 2H, H-3 β , H-5 β), 2.06 (s, 1.08H, CH₃ Ac α), 2.04 (s, 3H, CH₃ Ac β), 2.04 (s, 1.08H, CH₃ Ac α), 2.03 (s, 3H, CH₃ Ac β), 0.92 (s, 9H, tBu TBS β), 0.77 (s, 3.24H, tBu TBS α), 0.14 (s, 3H, CH₃ TBS β), 0.11 (s, 3H, CH₃ TBS β), 0.01 (s, 1.08H, CH₃ TBS α), -0.05 (s, 1.08H, CH₃ TBS α); ¹³C (CDCl₃, 100 MHz): δ 170.6, 170.5, 169.7, 169.6 (C=O Ac), 138.6, 138.1, 135.3, 133.1, 133.0, 132.8 (Cq), 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.3, 126.6, 126.1, 126.0, 125.9, 125.8, 125.7, 125.3 (CH_{arom}), 96.5 (C-1 β), 93.2 (C-1 α), 78.8 (C-3

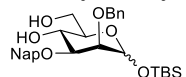
β), 76.0 (C-3 α), 75.7 (C-2 α), 74.7 (C-2 β), 74.0 (CH₂ Bn/Nap β), 72.9 (CH₂ Bn/Nap α), 72.5 (C-5 β), 72.0 (CH₂ Bn/Nap α), 71.0 (CH₂ Bn/Nap β), 69.1 (C-5 α), 68.3 (C-4 β), 68.1 (C-4 α), 63.3 (C-6 β), 63.1 (C-6 α), 25.7 (CH₃ tBu), 25.3 (CH₃ tBu), 20.8 (CH₃ Ac), 20.6 (CH₃ Ac), 17.9 (Cq tBu), 17.6 (Cq tBu), -4.1 (CH₃ Me β), -4.9 (CH₃ Me α), -5.5 (CH₃ Me β), -5.9 (CH₃ Me α); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 96.5 ($J_{C1,H1}$ = 153 Hz, C-1 β), 93.2 ($J_{C1,H1}$ = 168 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₄H₄₈NO₈Si 626.31437, found 626.31427.

Tert-butyldimethylsilyl 4,6-di-O-acetyl-3-O-benzyl-2-O-(2-naphthylmethyl)- α/β -D-mannopyranoside (13)



TBDMSCl (1.5 g, 10 mmol, 2 eq.) and imidazole (0.68 g, 10 mmol, 2eq.) were added to a solution of hemiacetal **10** (2.45 g, 4.95 mmol) in dry DCM (25 mL) under an argon atmosphere. After stirring for 6.5 hours the reaction was quenched with H₂O and extracted twice with EtOAc. Combined organic layers were washed with brine, dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 15:1 $\rightarrow$ 2:1) yielded the title compound as a yellowish oil (2.59 g, 4.25 mmol, 86%, α : β = 1 : 4.5). TLC: R_f 0.49 (PE/EtOAc, 4/1, v/v); IR (neat): 743, 837, 1040, 1233, 1366, 1742 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.73-7.84 (m, 4.88H, CH_{arom}), 7.63 (dd, 1H, J = 1.2, 8.4 Hz, CH_{arom}), 7.52-7.55 (m, 0.22H, CH_{arom}), 7.44-7.49 (m, 2.44H, CH_{arom}), 7.26-7.35 (m, 4.10H, CH_{arom}), 7.18-7.20 (m, 2H, CH_{arom}), 5.47 (t, 0.22H, J = 10.0 Hz, H-4 α), 5.36 (t, 1H, J = 9.8 Hz, H-4 β), 5.17 (d, 1H, J = 12.8 Hz, CHH Bn/Nap β), 5.13 (d, 0.22H, J = 1.6 Hz, H-1 α), 5.05 (d, 1H, J = 12.8 Hz, CHH Bn/Nap β), 4.99 (d, 0.22H, J = 12.4 Hz, CHH Bn/Nap α), 4.83 (d, 0.22H, J = 12.4 Hz, CHH Bn/Nap α), 4.76 (s, 1H, H-1 β), 4.62 (d, 0.22H, J = 12.4 Hz, CHH Bn/Nap α), 4.43 (d, 0.22H, J = 12.4 Hz, CHH Bn/Nap α), 4.46 (d, 1H, J = 12.0 Hz, CHH Nap β), 4.32 (d, 1H, J = 12.0 Hz, CHH Nap β), 4.22-4.26 (m, 2.22H, H-6 α , H-6 β , H-6 β), 4.13 (dd, 0.22H, J = 2.4, 12.0 Hz, H-6 α), 3.94-3.98 (m, 0.22H, H-5 α), 3.90 (dd, 1.22H J = 2.8, 9.2 Hz, H-3 α , H-2 β), 3.64 (t, 0.22H, J = 2.4 Hz, H-2 α), 3.56 (m, 1H, H-5 β), 3.49 (dd, 1H, J = 3.0, 10.0 Hz, H-3 β), 2.10 (s, 0.66H, CH₃ Ac α), 2.09 (s, 3H, CH₃ Ac β), 2.08 (s, 0.66H, CH₃ Ac α), 2.06 (s, 3H, CH₃ Ac β), 1.22 (s, 9H, CH₃ tBu β), 0.82 (s, 1.98H, CH₃ tBu α), 0.20 (s, 3H, CH₃ Me β), 0.17 (s, 3H, CH₃ Me β), 0.04 (s, 0.66H, CH₃ Me α), -0.04 (s, 0.66H, CH₃ Me α); ¹³C (CDCl₃, 100 MHz): δ 170.9, 169.9, 169.9 (C=O Ac), 138.2, 138.0, 136.3, 135.8, 133.3, 133.3, 133.2, (Cq), 128.5, 128.4, 128.3, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.4, 126.9, 126.8, 126.6, 126.2, 126.2, 126.0, 126.0, 125.8 (CH_{arom}), 96.8 (C-1 β), 93.5 (C-1 α), 79.2 (C-3 β), 76.5 (C-3 α), 75.8 (C-2 α), 74.7 (C-2 β), 74.3 (CH₂ Bn/Nap α), 73.2 (CH₂ Bn/Nap β), 72.8 (C-5 β), 72.3 (CH₂ Bn/Nap α), 71.3 (CH₂ Bn/Nap β), 69.2 (C-5 α), 68.5 (C-4 β), 68.3 (C-4 α), 63.6 (C-6 β), 63.3 (C-6 α), 26.0 (CH₃ tBu β), 25.6 (CH₃ tBu α), 21.1, 21.1, 20.9 (CH₃ Ac), 18.2 (Cq tBu β), 17.9 (Cq tBu α), -3.9 (CH₃ Me β), -4.7 (CH₃ Me α), -5.3 (CH₃ Me β), -5.7 (CH₃ Me α); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 96.8 ($J_{C1,H1}$ = 153 Hz, C-1 β), 93.5 ($J_{C1,H1}$ = 166 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₄H₄₈NO₈Si 626.31437, found 626.31523.

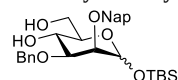
Tert-butyldimethylsilyl 2-O-benzyl-3-O-(2-naphthylmethyl)- α/β -D-mannopyranoside (15)



To a solution of compound **12** (7.5 g, 12.3 mmol) in MeOH (60 ml) a catalytic amount of NaOMe (110 mg, 2.0 mmol, 0.17 eq.) was added. After stirring overnight, the reaction mixture was neutralized with Amberlite H⁺ which was subsequently filtered off. The filtrate was concentrated *in vacuo* and **15** was obtained as a colourless oil (6.45 g, 12.3 mmol, quant., α : β = 1 : 3.5). TLC: R_f 0.19 (PE/EtOAc, 2/1, v/v); IR (neat): 735, 779, 1070, 1252, 3412 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.76-7.84 (m, 4.12H, CH_{arom}), 7.70 (s, 1H, CH_{arom}), 7.45-7.50 (m, 4.84H, CH_{arom}), 7.37-7.39 (m, 1H, CH_{arom}), 7.26-7.33 (m, 4.40H, CH_{arom}), 5.04 (d, 1.28H, J = 12.0 Hz, CHH Bn/Nap β , H-1 α), 4.74-4.83 (m, 2.84H, CHH Bn/Nap β , CH₂ Bn/Nap α , CHH Bn/Nap α , H-1 β), 4.61-4.65 (m, 1.28H, CHH Bn/Nap β , CHH Bn/Nap α), 4.48 (d, 1H, J = 12.0 Hz, CHH Bn/Nap β), 4.14 (t, 0.28H, J = 9.6 Hz, H-4 α), 4.01 (t, 1H, J = 9.6 Hz, H-4 β), 3.81-3.94 (m, 3.84H, H-6

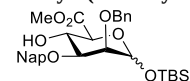
α , H-6 α , H-6 β , H-6 β , H-2 β , H-3 α), 3.70-3.75 (m, 0.28H, H-5 α), 3.58 (t, 0.28H, J = 2.8 Hz, H-2 α), 3.37 (dd, 1H, J = 2.8, 9.6 Hz, H-3 β), 3.29-3.33 (m, 1H, H-5 β), 2.67 (bs, 2.56H, 4-OH α , 4-OH β , 6-OH α , 6-OH β), 0.96 (s, 9H, tBu Me β), 0.78 (s, 2.52H, tBu Me α), 0.17 (s, 3H, CH₃ Me β), 0.15 (s, 3H, CH₃ Me β), 0.02 (s, 0.84H, CH₃ Me α), -0.04 (s, 0.84H, CH₃ Me α); ¹³C (CDCl₃, 100 MHz): δ 138.8, 128.2, 135.5, 135.3, 133.3, 133.2, 133.1 (Cq), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.1, 126.6, 126.3, 126.1, 126.0, 125.7 (CH_{arom}), 96.9 (C-1 β), 93.4 (C-1 α), 81.5 (C-3 β), 78.9 (C-3 α), 76.0 (C-5 β), 75.8 (C-2 α), 75.0 (C-2 β), 74.5 (CH₂ Bn/Nap β), 73.0 (CH₂ Bn/Nap α), 72.3 (CH₂ Bn/Nap α), 72.3 (C-5 α), 71.2 (CH₂ Bn/Nap β), 67.5 (C-4 β), 67.4 (C-4 α), 63.2 (C-6 β), 62.8 (C-6 α), 25.9 (CH₃ tBu β), 25.6 (CH₃ tBu α), 18.1 (Cq tBu β), 17.9 (Cq tBu α), -3.8 (CH₃ Me β), -4.5 (CH₃ Me α), -5.3 (CH₃ Me β), -5.8 (CH₃ Me α); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 96.9 ($J_{C1,H1}$ = 154 Hz, C-1 β), 93.4 ($J_{C1,H1}$ = 166 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₀H₄₄NO₆Si 542.29324, found 542.29320.

Tert-butyldimethylsilyl 3-O-benzyl-2-O-(2-naphthylmethyl)- α/β -D-mannopyranoside (16) To a solution of



compound **13** (907 mg, 1.49 mmol) in MeOH (8 ml) a catalytic amount of NaOMe (8 mg, 0.15 mmol, 0.1 eq.) was added. After stirring overnight, the reaction mixture was neutralized with Amberlite H⁺ which was subsequently filtered off. The filtrate was concentrated *in vacuo* and **16** was obtained as a colourless oil (770 mg, 1.47 mmol, 98%, α : β = 1 : 4.2). TLC: R_f 0.48 (PE/EtOAc, 1/1, v/v); IR (neat): 727, 837, 907, 1070, 1252, 3420 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.74-7.86 (m, 5.20H, CH_{arom}), 7.62 (dd, 1H, J = 1.2, 8.4 Hz, CH_{arom}), 7.47-7.51 (m, 2.73H, CH_{arom}), 7.34-7.37 (m, 1.24H, CH_{arom}), 7.28-7.30 (m, 2.73H, CH_{arom}), 7.22-7.25 (m, 1H, CH_{arom}), 5.20 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 5.12 (d, 0.24H, J = 1.6 Hz, H-1 α), 4.98 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 4.92 (d, 0.24H, J = 12.4 Hz, CHH Bn/Nap α), 4.78-4.82 (m, 1.24H, CHH Bn/Nap α , H-1 β), 4.66 (s, 0.48H, CH₂ Bn/Nap α), 4.49 (d, 1H, J = 12.0 Hz, CHH Bn/Nap β), 4.39 (d, 1H, J = 12.0 Hz, CHH Bn/Nap β), 4.18 (t, 0.24H, J = 9.6 Hz, H-4 α), 4.07 (t, 1H, J = 9.6 Hz, H-4 β), 3.96 (dd, 1H, J = 3.6, 11.6Hz, H-6 β), 3.82-3.94 (m, 2.73, H-6 α , H-6 α , H-6 β , H-2 β , H-3 α), 3.75-3.80 (m, 0.24H, H-5 α), 3.63 (t, 0.24H, J = 2.4 Hz, H-2 α), 3.32-3.38 (m, 2H, H-3 β , H-5 β), 3.18 (bs, 0.24H, 4-OH α), 3.12 (bs, 1H, 4-OH β), 2.62 (bs, 1.24H, 6-OH α/β), 1.02 (s, 9H, CH₃ tBu β), 0.84 (s, 2.16H, CH₃ tBu α), 0.23 (s, 3H, CH₃ Me β), 0.21 (s, 3H, CH₃ Me β), 0.05 (s, 0.72H, CH₃ Me α), -0.04 (s, 0.72H, CH₃ Me α); ¹³C (CDCl₃, 100 MHz): δ 138.1, 137.9, 136.3, 135.6, 133.2, 133.1, 133.0 (Cq), 128.5, 128.4, 128.2, 128.1, 127.9, 127.9, 127.7, 127.6, 126.9, 16.7, 126.4, 126.1, 125.9, 125.7 (CH_{arom}), 96.8 (C-1 β), 93.4 (C-1 α), 81.6 (C-3 β), 78.9 (C-3 α), 76.0 (C-5 β), 75.8 (C-2 α), 75.2 (C-2 β), 74.5 (CH₂ Bn/Nap β), 73.0 (CH₂ Bn/Nap α), 72.4 (C-5 α), 72.3 (CH₂ Bn/Nap α), 71.3 (CH₂ Bn/Nap β), 67.4 (C-4 β), 67.2 (C-4 α), 63.0 (C-6 β), 62.6 (C-6 α), 25.9 (CH₃ tBu β), 25.5 (CH₃ tBu α), 18.0 (Cq tBu β), 17.8 (Cq tBu α), -3.8 (CH₃ Me β), -4.7 (CH₃ Me α), -5.3 (CH₃ Me β), -5.9 (CH₃ Me α); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 96.8 ($J_{C1,H1}$ = 153 Hz, C-1 β), 93.4 ($J_{C1,H1}$ = 166 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₀H₄₄NO₆Si 542.29324, found 542.29370.

Methyl (tert-butyldimethylsilyl 2-O-benzyl-3-O-(2-naphthylmethyl)- α/β -D-mannopyranosyl uronate) (18)

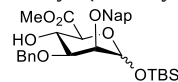


Diol **15** (40 g, 7.6 mmol) was dissolved in DCM (25 mL) and H₂O (13 mL) was added. To the two phase system TEMPO (236 mg, 1.51 mmol, 0.2 eq.) and BAIB (7.08 g, 22 mmol, 2.9 eq.) were added. After stirring vigorously for 6 hours at room temperature, the reaction

was quenched by addition of saturated aqueous Na₂S₂O₃. The mixture was extracted twice with EtOAc. The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene once. The crude mannuronic acid was then dissolved in DMF (40 mL) and put under an argon atmosphere at 0°C. Methyl iodide (1.4 mL, 22.9 mmol, 3 eq.) and K₂CO₃ (3.16 g, 22.9 mmol, 3 eq.) were added and reaction was stirred overnight. The reaction was quenched with H₂O and extracted twice with EtOAc. The organic layers were collected

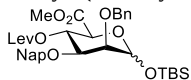
and dried with MgSO_4 , filtered and concentrated *in vacuo*. Purification by column chromatography (Toluene/Acetone, 40:1 $\rightarrow$ 10:1) afforded the title compound as a yellow oil (3.2 g, 5.79 mmol, 76%, $\alpha : \beta = 1 : 5.5$). TLC: R_f 0.62 (PE/EtOAc, 2/1, v/v); IR (neat): 698, 781, 839, 1070, 1200, 1252, 1362, 1748, 3447 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.81-7.77 (m, 3.90H, CH_{arom}), 7.73 (s, 1H, CH_{arom}), 7.44-7.48 (m, 4.54H, CH_{arom}), 7.38-7.40 (m, 1H, CH_{arom}), 7.27-7.32 (m, 3.72H, CH_{arom}), 5.01 (d, 0.18H, $J = 2.8$ Hz, H-1 α), 5.05 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap β), 4.65-4.88 (m, 4.72H, CHH Bn/Nap β , CH_2 Bn α , Nap α , Bn/Nap β , H-1 β), 4.32 (dt, 1.18H, $J = 2.0, 9.6$ Hz, H-4 α , H-4 β), 4.18 (d, 0.18H, $J = 9.2$ Hz, H-5 α), 3.82-3.84 (m, 1.18H, H-2 β , H-3 α), 3.80 (s, 3.54H, CH_3 CO_2Me α, β), 3.73 (d, 1H, $J = 9.6$ Hz, H-5 β), 3.53 (t, 0.18H, $J = 2.8$ Hz, H-2 α), 3.41 (dd, 1H, $J = 2.8, 9.6$ Hz, H-3 β), 3.08 (d, 1H, $J = 2.0$ Hz, 4-OH β), 2.96 (d, 0.18H, $J = 2.4$ Hz, 4-OH α), 0.93 (s, 9H, CH_3 tBu β), 0.73 (s, 1.62H, CH_3 tBu α), 0.18 (s, 3H, CH_3 Me β), 0.13 (s, 3H, CH_3 Me β), 0.02 (s, 0.54H, CH_3 Me α), -0.06 (s, 0.54, CH_3 Me α); ^{13}C (CDCl_3 , 100 MHz): δ 170.7 (C=O CO_2Me), 169.9 (C=O CO_2Me β), 138.7, 138.0, 135.5, 133.1, 133.0, 132.9 (Cq), 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.3, 126.9, 126.8, 126.2, 126.0, 125.9, 125.9, 125.8, 125.6 (CH_{arom}), 97.0 (C-1 β), 93.6 (C-1 α), 80.2 (C-3 β), 77.4 (C-3 α), 75.9 (C-2 α), 75.2 (C-2 β), 74.8 (C-5 β), 74.3 (CH_2 Bn/Nap β), 72.9, 72.8 (CH_2 Bn α , Nap α), 72.0 (C-5 α), 71.7 (CH_2 Bn/Nap β), 68.5 (C-4 α), 68.0 (C-4 β), 52.4 (CH_3 CO_2Me β), 52.2 (CH_3 CO_2Me α), 25.7 (CH_3 tBu β), 25.3 (CH_3 tBu α), 17.9 (Cq tBu β), 17.6 (Cq tBu α), -4.1 (CH_3 Me β), -4.8 (CH_3 Me α), -5.6 (CH_3 Me β), -6.0 (CH_3 Me α); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 97.0 ($J_{\text{C1,H1}} = 153$ Hz, C-1 β), 93.6 ($J_{\text{C1,H1}} = 168$ Hz, C-1 α); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. for $\text{C}_{31}\text{H}_{44}\text{NO}_7\text{Si}$ 570.28816, found 570.28804.

Methyl (tert-butyldimethylsilyl 3-O-benzyl-2-O-(2-naphthylmethyl)- α/β -D-mannopyranosyl uronate) (19)

 Diol **16** (3.7 g, 7.05 mmol) was dissolved in EtOAc (25 mL) and H_2O (10 mL) was added. To the two phase system TEMPO (220 mg, 1.41 mmol, 0.2 eq.) and BAIB (5.68 g, 17.6 mmol, 2.5 eq.) were added. After stirring vigorously for 4.5 hours at room temperature, the reaction was quenched by addition of saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$. EtOAc (50 mL) was added and the layers separated. The organic layer was dried with MgSO_4 , filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene once. The crude mannuronic acid was then dissolved in DMF (22 mL) and put under an argon atmosphere at 0°C . Methyl iodide (1.3 mL, 21.15 mmol, 3.0 eq.) and K_2CO_3 (2.92 g, 21.15 mmol, 3.0 eq.) were added and the reaction was stirred overnight. The reaction was quenched with H_2O and extracted twice with EtOAc. The organic layers were collected and dried with MgSO_4 , filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 10:1 $\rightarrow$ 2:1) afforded the title compound as a yellow solid (3.11 g, 5.63 mmol, 79%, $\alpha : \beta = 1 : 8.3$). TLC: R_f 0.29 (PE/EtOAc, 4/1, v/v); ^1H NMR (CDCl_3 , 400 MHz): δ 7.74-7.82 (m, 4.48H, CH_{arom}), 7.57-7.60 (dd, 1H, $J = 1.6, 8.4$ Hz, CH_{arom}), 7.44-7.48 (m, 2.48H, CH_{arom}), 7.19-7.35 (m, 5.48H, CH_{arom}), 5.17 (d, 1H, $J = 12.8$ Hz, CHH Bn/Nap β), 5.14 (d, 0.12H, $J = 1.2$ Hz, H-1 α), 4.95 (d, 1H, $J = 12.8$ Hz, CHH Bn/Nap β), 4.89 (d, 0.12H, $J = 12.4$ Hz, CHH Bn/Nap α), 4.81 (d, 0.12H, $J = 12.4$ Hz, CHH Bn/Nap α), 4.76 (s, 1H, H-1 β), 4.72 (d, 0.12H, $J = 12.0$ Hz, CHH Bn/Nap α), 4.65 (d, 0.12H, $J = 12.0$ Hz, CHH Bn/Nap α), 4.51 (s, 2H, CH_2 Bn/Nap β), 4.32 (dt, 1.12H, $J = 2.0, 9.6$ Hz, H-4 α , H-4 β), 4.19 (d, 0.12H, $J = 8.8$ Hz, H-5 α), 3.85 (d, 1H, $J = 3.2$ Hz, H-2 β), 3.74-3.83 (m, 4.48H, H-3 α , H-5 β , CH_3 OMe α, β), 3.56 (t, 0.12H, $J = 2.4$ Hz, H-2 α), 3.38 (dd, 1H, $J = 3.2, 9.6$ Hz, H-3 β), 3.04 (d, 1H, $J = 2.0$ Hz, 4-OH β), 2.93 (d, 0.12H, $J = 2.4$ Hz, 4-OH α), 0.95 (s, 9H, CH_3 tBu β), 0.77 (s, 1.08H, CH_3 tBu α), 0.19 (s, 3H, CH_3 Me β), 0.15 (s, 3H, CH_3 Me β), 0.02 (s, 0.36H, CH_3 Me α), -0.08 (s, 0.36H, CH_3 Me α); ^{13}C (CDCl_3 , 100 MHz): δ 170.9 (C=O CO_2Me α), 170.0 (C=O CO_2Me β), 138.3, 128.1, 136.3, 135.6, 133.2, 133.1, 133.0 (Cq), 128.5, 128.4, 128.2, 128.1, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 126.9, 126.7, 126.5, 126.1, 126.1, 125.9, 125.9, 125.8, 125.7 (CH_{arom}), 97.2 (C-1 β), 93.8 (C-1 α), 80.5 (C-3 β), 77.8 (C-3 α), 75.9 (C-2 α), 75.3 (C-2 β), 75.0 (C-5 β), 74.6 (CH_2 Bn/Nap β), 73.1, 72.8 (CH_2 Bn/Nap α), 72.0 (CH_2

Bn/Nap β), 71.9 (C-5 α), 68.6 (C-4 α), 68.2 (C-4 β), 52.5 (CH₃ CO₂Me α,β), 25.9 (CH₃ tBu β), 25.5 (CH₃ tBu α), 18.0 (Cq tBu β), 17.8 (Cq tBu α), -4.0 (CH₃ Me β), -4.7 (CH₃ Me α), -5.5 (CH₃ Me β), -5.9 (CH₃ Me α); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 97.2 ($J_{C1,H1}$ = 154 Hz, C-1 β), 93.8 ($J_{C1,H1}$ = 167 Hz, C-1 α).

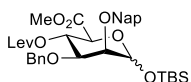
Methyl (tert-butyldimethylsilyl 2-O-benzyl-4-O-levulinoyl-3-O-(2-naphthylmethyl)- α/β -D-mannopyranosyl



uronate) (21) Levulinic acid (2.02 g, 17.4 mmol, 3 eq.) and DIC (1.35 mL, 8.7 mmol, 1.5 eq.) were added to a 0°C solution of **18** (3.2 g, 5.8 mmol) in dry DCM (14 mL). A catalytic amount of DMAP (71 mg, 0.58 mmol, 0.1 eq.) was added and the reaction mixture was

allowed to reach room temperature. After 4 hours, the reaction mixture was filtered over Celite and the filtrate washed with sat. aq. NaHCO₃ and brine. The organic layer was dried (MgSO₄), filtered, and concentrated *in vacuo*. Column chromatography (Toluene/Acetone, 40:1 → 10:1) afforded the title compound as an amorphous off-white solid (3.62 g, 5.56 mmol, 96%, α : β = 1 : 5.5). TLC: R_f 0.47 (PE/EtOAc, 2/1, v/v); IR (neat): 696, 781, 837, 1053, 1152, 1252, 1362, 1717, 1746 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.80-7.82 (m, 3.72H, CH_{arom}), 7.69 (s, 1H, CH_{arom}), 7.45-7.49 (m, 4.72H, CH_{arom}), 7.37 (d, 1H, J = 8.4 Hz, CH_{arom}), 7.25-7.31 (m, 3.72H, CH_{arom}), 5.55 (t, 1.18H, J = 9.6 Hz, H-4 α , H-4 β), 5.28 (s, 0.18H, H-1 α), 5.05 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 4.88 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 4.73-4.77 (m, 1.72H, CH₂ Bn α , Nap α , H-1 β), 4.64 (d, 1H, J = 12.8 Hz, CHH Bn/Nap β), 4.51 (d, 1H, J = 12.8 Hz, CHH Bn/Nap β), 4.30 (d, 0.18H, J = 6.8 Hz, H-5 α), 3.93 (dd, 0.18H, J = 3.6, 8.4 Hz, H-3 α), 3.82-3.86 (m, 2H, H-2 β , H-5 β), 3.72 (s, 3H, CH₃ CO₂Me β), 3.66 (s, 0.54H, CH₃ CO₂Me α), 3.52-3.55 (m, 1.18H, H-2 α , H-3 β), 2.67 (t, 2.36H, J = 6.8 Hz, CH₂ Lev α , β), 2.55 (t, 2.36H, J = 6.8 Hz, CH₂ Lev α , β), 2.15 (s, 0.54H, CH₃ Lev α), 2.12 (s, 3H, CH₃ Lev β), 0.93 (s, 9H, CH₃ tBu β), 0.80 (s, 1.62H, CH₃ tBu α), 0.17 (s, 3H, CH₃ Me β), 0.11 (s, 3H, CH₃ Me β), 0.02 (s, 0.54H, CH₃ Me α), 0.01 (s, 0.54H, CH₃ Me α); ¹³C (CDCl₃, 100 MHz): δ 206.3 (C=O Lev), 171.6, 167.9 (C=O Lev, CO₂Me), 138.7, 135.4, 133.2, 133.0 (Cq), 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.8, 127.4, 126.8, 126.4, 126.2, 126.2, 126.0, 126.0, 125.7 (CH_{arom}), 96.9 (C-1 β), 93.4 (C-1 α), 78.4 (C-3 β), 76.1 (C-2 α), 75.3 (C-3 α), 74.7 (C-2 β), 74.2 (CH₂ Bn/Nap β), 73.5 (C-5 β), 73.1, 72.7 (CH₂ Bn α , Nap α), 71.8 (CH₂ Bn/Nap β), 71.6 (C-5 α), 69.7 (C-4 α), 69.0 (C-4 β), 52.6 (CH₃ CO₂Me), 37.8 (CH₂ Lev), 28.0 (CH₃ Lev), 28.0 (CH₂ Lev), 25.8 (CH₃ tBu), 25.6 (CH₃ tBu α), 18.1 (Cq tBu), -4.0 (CH₃ Me), -5.4 (CH₃ Me); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 96.9 ($J_{C1,H1}$ = 153 Hz, C-1 β), 93.4 ($J_{C1,H1}$ = 167 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₆H₅₀NO₉Si 668.32494, found 668.32529.

Methyl (tert-butyldimethylsilyl 3-O-benzyl-4-O-levulinoyl-2-O-(2-naphthylmethyl)- α/β -D-mannopyranosyl

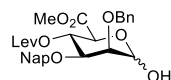


uronate) (22) Levulinic acid (1.08 g, 9.32 mmol, 2.8 eq.) and DIC (0.73 mL, 4.66 mmol, 1.4 eq.) were added to a 0°C solution of **19** (1.84 g, 3.33 mmol) in dry DCM (8.5 mL). A catalytic amount of DMAP (40 mg, 0.3 mmol, 0.1 eq.) was added and the reaction mixture

was allowed to reach room temperature. After 3 hours, the reaction mixture was filtered over Celite and the filtrate washed with sat. aq. NaHCO₃ and brine. The organic layer was dried (MgSO₄), filtered, and concentrated *in vacuo*. Column chromatography (PE/EtOAc, 6:1 → 2:1) afforded the title compound as an amorphous off-white solid (2.07 g, 3.18 mmol 95%, α : β = 1 : 10). TLC: R_f 0.45 (PE/EtOAc, 2/1, v/v); IR (neat): 736, 783, 841, 1055, 115, 1256, 1362, 1722, 1748 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.72-7.81 (m, 4.60H, CH_{arom}), 7.60 (d, 1H, J = 8.4 Hz, CH_{arom}), 7.43-7.48 (m, 2.40H, CH_{arom}), 7.26-7.32 (m, 3.20H, CH_{arom}), 7.17-7.19 (m, 2H, CH_{arom}), 5.56 (t, 1.10H, J = 9.6 Hz, H-4 α , H-4 β), 5.31 (s, 0.10H, H-1 α), 5.17 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 5.01 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 4.88 (s, 0.20H, CH₂ Bn/Nap α), 4.76 (s, 1H, H-1 β), 4.62 (s, 0.20H, CH₂ Bn/Nap α), 4.44 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 4.37 (d, 1H, J = 12.4 Hz, CHH Bn/Nap β), 3.31 (d, 0.10H, J = 7.6 Hz, H-5 α), 3.85-3.92 (m, 2.10H, H-2 β , H-5 β , H-3 α), 3.74 (s, 3H, CH₃ CO₂Me β), 3.67 (s, 0.30H, CH₃ CO₂Me α), 3.55 (t, 0.10H,

$J = 3.2$ Hz, H-2 α), 3.50 (dd, 1H, $J = 2.8, 9.6$ Hz, H-3 β), 2.71 (t, 2H, $J = 6.8$ Hz, CH₂ Lev β), 2.65-2.68 (m, 0.20H, CH₂ Lev α), 2.51-2.57 (m, 2.20H, CH₂ Lev α , β), 2.16 (s, 3H, CH₃ Lev β), 2.04 (s, 0.30H, CH₃ Lev α), 0.95 (s, 9H, CH₃ tBu β), 0.84 (s, 0.90H, CH₃ tBu α), 0.19 (s, 3H, CH₃ Me β), 0.14 (s, 3H, CH₃ Me β), 0.09 (s, 0.30H, CH₃ Me α), 0.01 (s, 0.30H, CH₃ Me α); ¹³C (CDCl₃, 100 MHz): δ 206.3 (C=O Lev), 171.6, 168.0 (C=O Lev, CO₂Me), 138.0, 136.2, 133.3, 133.1 (Cq_{arom}), 128.5, 128.4, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 126.9, 126.8, 126.7, 126.2, 125.9, 125.7 (CH_{arom}), 97.0 (C-1 β), 93.4 (C-1 α), 78.8 (C-3 β), 76.1 (C-2 α), 75.5 (C-3 α), 74.7 (C-2 β), 74.3 (CH₂ Bn/Nap β), 73.6 (C-5 β), 73.2, 72.8, (CH₂ Bn/Nap α), 71.7 (CH₂ Bn/Nap β), 69.9 (C-4 α), 69.1 (C-4 β), 52.7, 52.5 (CH₃ CO₂Me), 37.9, 37.8 (CH₂ Lev), 29.9 (CH₃ Lev), 28.0 (CH₂ Lev), 25.9, 25.7 (CH₃ tBu), 18.1 (Cq tBu), -3.9, -5.3 (CH₃ Me); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 97.0 ($J_{C1,H1} = 154$ Hz, C-1 β); HRMS: [M+NH₄]⁺ calcd. for C₃₆H₅₀NO₅Si 668.32494, found 668.32532.

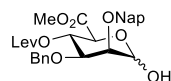
Methyl (2-O-benzyl-4-O-levulinoyl-3-O-(2-naphthylmethyl)- α/β -D-mannopyranosyl uronate) (24) Acetic acid



(0.07 mL, 1.16 mmol, 2 eq.) was added to a 0°C solution of compound **21** (370 mg, 0.57 mmol) in dry THF (5.7 mL). TBAF (1.0 M solution in THF, 0.9 mL, 0.88 mmol, 1.5 eq.) was added drop wise over 5 minutes. The reaction mixture was stirred for 30 minutes at room

temperature and subsequently diluted with EtOAc and washed once with H₂O. The aqueous layer was extracted two more times with EtOAc and the combined organic layers were washed with brine. The organic layer was dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 4:1 → 1:1) furnished the title compound as a colourless oil (300 mg, 0.55 mmol, 97%, $\alpha : \beta = 17 : 1$). Analytic data is reported for the α -anomer. TLC: R_f 0.18 (PE/EtOAc, 1/1, v/v); IR (neat): 698, 748, 820, 1028, 1123, 1364, 1717, 1742, 3437 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.75-7.81 (m, 4H, CH_{arom}), 7.40-7.47 (m, 3H, CH_{arom}), 7.23-7.30 (m, 5H, CH_{arom}), 5.59 (t, 1H, $J = 6.4$ Hz, H-4), 5.54 (bs, 1H, H-1 α), 4.85 (s, 0.06H, H-1 β), 4.60-4.81 (m, 5H, CH₂ Bn, Nap, 1-OH), 4.47 (d, 1H, $J = 6.4$ Hz, H-5), 3.97 (dd, 1H, $J = 3.2, 6.4$ Hz, H-3), 3.65-3.68 (m, 1H, H-2), 3.58 (s, 3H, CH₃ CO₂Me), 2.65 (t, 2H, $J = 6.4$ Hz, CH₂ Lev), 2.49-2.55 (m, 2H, CH₂ Lev), 2.11 (s, 3H, CH₃ Lev); ¹³C (CDCl₃, 100 MHz): δ 206.5 (C=O Lev), 171.8, 169.2 (C=O Lev, CO₂Me), 138.2, 135.3, 133.2, 133.0 (Cq), 129.1, 128.6, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 126.7, 126.4, 126.1, 125.9, 125.7 (CH_{arom}), 93.8 (C-1 β), 92.6 (C-1 α), 75.4, 75.3 (C-2, C-3), 72.9, 72.6 (CH₂ Bn, Nap), 71.7 (C-5), 69.6 (C-4), 52.5 (CH₃ CO₂Me), 37.8 (CH₂ Lev), 29.8 (CH₃ Lev), 28.0 (CH₂ Lev); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 92.6 ($J_{C1,H1} = 170$ Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₀H₃₆NO₉ 559.19385, found 559.19282.

Methyl (3-O-benzyl-4-O-levulinoyl-2-O-(2-naphthylmethyl)- α/β -D-mannopyranosyl uronate) (25) Acetic acid



(0.36 mL, 6.36 mmol, 2 eq.) was added to a 0°C solution of compound **22** (2.07 g, 3.18 mmol) in dry THF (30 mL). TBAF (1.0 M solution in THF, 4.8 mL, 4.8 mmol, 1.5 eq.) was added drop wise over 5 minutes. The reaction mixture was stirred for 2.5 hours at room

temperature and subsequently diluted with EtOAc and washed once with H₂O and brine. The organic layer was dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (Pentane/DCM/EtOAc, 4:1:1 → 1:1:2) furnished the title compound as a colourless oil (1.7 g, 3.17 mmol, 99%, $\alpha : \beta = 8.3 : 1$). TLC: R_f 0.18 (PE/EtOAc, 1/1, v/v); IR (neat): 698, 739, 820, 1028, 11.23, 1362, 1717, 1744, 3402 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.72-7.83 (m, 4.48H, CH_{arom}), 7.42-7.49 (m, 3.36H, CH_{arom}), 7.27-7.37 (m, 5.60H, CH_{arom}), 5.63 (t, 0.12H, $J = 7.2$ Hz, H-4 β), 5.55-5.58 (m, 2H, H-1 α , H-4 α), 5.05 (d, 0.12H, $J = 12.0$ Hz, CHH Bn/Nap β), 4.88-4.94 (m, 1.12H, CHH Bn/Nap α , H-1 β), 4.81 (d, 1.12H, $J = 12.4$ Hz, CHH Bn/Nap α , CHH Bn/Nap β), 4.74 (d, 0.12H, $J = 12.0$ Hz, CHH Bn/Nap β), 4.68 (d, 0.12H, $J = 12.0$ Hz, CHH Bn/Nap β), 4.62 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap α), 4.59 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap α), 4.47 (d, 1H, $J = 5.2$ Hz, H-5 α), 4.08 (d, 0.12H, $J = 6.8$ Hz,

H-5 β), 3.94 (dd, 1H, $J = 3.0, 6.4$ Hz, H-3 α), 3.85 (t, 0.12H, $J = 2.6$ Hz, H-2 β), 3.78 (dd, 0.12H, $J = 2.4, 8.0$ Hz, H-3 β), 3.68 (s, 0.36H, CH₃ CO₂Me β), 3.65-3.67 (m, 1H, H-2 α), 3.61 (s, 3H, CH₃ CO₂Me α), 3.47 (d, 1H, $J = 4.0$ Hz, 1-OH α), 2.68-2.72 (m, 0.24H, CH₂ Lev β), 2.63 (t, 2H, $J = 6.4$ Hz, CH₂ Lev α), 2.39-2.56 (m, 2.24H, CH₂ Lev α,β), 2.17 (s, 0.36H, CH₃ Lev β), 2.14 (s, 3H, CH₃ Lev α); ¹³C (CDCl₃, 100 MHz): δ 206.5 (C=O Lev), 171.7, 169.3 (C=O Lev, CO₂Me), 137.8, 135.7, 135.0 133.2, 133.0 (Cq-_{arom}), 128.6, 128.4, 128.1, 127.9, 127.9, 127.7, 127.3, 126.7, 126.2, 126.1, 126.0, 125.9 (CH_{arom}), 93.9 (C-1 β), 92.6 (C-1 α), 77.9 (C-3 β), 75.3, 75.2 (C-2 α , C-3 α), 73.9 (C-2 β , C-5 β), 73.8, 73.1 (CH₂ Bn β , Nap β), 72.9, 72.6 (CH₂ Bn α , Nap α), 71.7 (C-5 α), 69.6, 69.5 (C-4 α,β), 52.8 (CH₃ CO₂Me β), 52.5 (CH₃ CO₂Me α), 37.7 (CH₂ Lev), 29.8 (CH₃ Lev), 28.0 (CH₂ Lev); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 92.6 ($J_{C1,H1} = 170$ Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₀H₃₆NO₉ 554.23846, found 554.23850.

Methyl (2-O-benzyl-4-O-levulinoyl-3-O-(2-naphthylmethyl)-1-O-(N-[phenyl]trifluoroacetimidoyl)- α/β -D-mannopyranosyl uronate) (27) Cs₂CO₃ (215 mg, 0.66 mmol, 1.2 eq.) was added to a 0°C solution of compound **24** (300 mg, 0.55 mmol) and 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (92 μ L, 0.61 mmol, 1.1 eq.) in acetone (1.8 mL). After

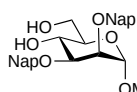
stirring overnight at ambient temperature TLC analysis showed complete conversion of the starting compound to a higher running spot. H₂O was added and the mixture was extracted two times with EtOAc. The organic fraction was washed with brine, dried with MgSO₄, filtered, and concentrated *in vacuo*. The crude compound was purified using column chromatography (PE/EtOAc, 8:1 $\rightarrow$ 2:1) to yield the title compound as a yellow oil (340 mg, 0.48 mmol, 87%, $\alpha : \beta = 6.7 : 1$). TLC: R_f 0.63 (PE/EtOAc, 1/1, v/v); IR (neat): 696, 752, 1124, 1153, 1207, 1717, 1749 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.76-7.84 (m, 4.45H, CH_{arom}), 7.39-7.50 (m, 3.60H, CH_{arom}), 7.24-7.33 (m, 8.05H, CH_{arom}), 7.08-7.11 (m, 1.15H, NPh α,β), 6.79 (d, 0.30H, $J = 7.6$ Hz, NPh β), 6.68 (d, 2H, $J = 7.6$ Hz, NPh α), 6.44 (bs, 1H, H-1 α), 6.05 (bs, 0.15H, H-1 β), 5.77 (t, 0.15H, $J = 6.2$ Hz, H-4 β), 5.64 (t, 1H, $J = 7.2$ Hz, H-4 α), 4.62-4.83 (m, 4.60H, CH₂ Bn α,β , CH₂ Nap α,β), 4.41 (d, 1H, $J = 7.2$ Hz, H-5 α), 4.05 (bs, 0.15H, H-2 β), 3.97 (dd, 1H, $J = 3.0, 7.8$ Hz, H-3 α), 3.80-3.85 (m, 0.15H, H-3 β), 3.78 (bs, 1H, H-2 α), 3.67 (s, 3H, CO₂Me α), 3.62 (s, 0.45H, CO₂Me β), 2.70 (t, 2.30H, $J = 6.4$ Hz, CH₂ Lev α,β), 2.53-2.60 (m, 2.30H, CH₂ Lev α,β), 2.15 (s, 3.45H, CH₃ Lev α,β); ¹³C NMR (CDCl₃, 100 MHz): δ 206.2 (C=O Lev), 171.7, 168.0 (C=O Lev, CO₂Me), 143.2 (Cq NPh), 137.5, 135.0, 133.2, 133.1 (Cq), 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.0, 126.3, 126.2, 126.1, 125.9, 125.5 (CH_{arom}), 124.5, 124.2, 119.4 (CH_{arom} NPh), 94.4 (C-1 α), 74.8 (C-3 α), 73.2 (C-2 α), 73.1, 73.0 (CH₂ Bn, Nap), 72.9 (C-5 α), 71.8 (C-2 β), 69.5 (C-4 β), 68.9 (C-4 α), 52.8 (CH₃ CO₂Me α), 52.6 (CH₃ CO₂Me β), 37.8 (CH₂ Lev), 29.8 (CH₃ Lev), 28.0 (CH₂ Lev); HRMS: [M+Na]⁺ calcd. for C₃₈H₃₆F₃NO₉Na 730.22344, found 730.22384.

Methyl (3-O-benzyl-4-O-levulinoyl-2-O-(2-naphthylmethyl)-1-O-(N-[phenyl]trifluoroacetimidoyl)- α/β -D-mannopyranosyl uronate) (28) 2,2,2-Trifluoro-N-phenylacetimidoyl chloride (0.82 mL, 5.4 mmol, 1.1 eq.) was added drop wise to a 0°C solution of **25** (2.6 g, 4.8 mmol) and Cs₂CO₃ (1.9 g, 5.86 mmol 1.2 eq.) in acetone (16 mL). After stirring overnight at

ambient temperature TLC analysis showed complete conversion of the starting compound to a higher running spot. H₂O was added and the mixture was extracted two times with EtOAc. The organic fraction was washed with brine, dried with MgSO₄, filtered, and concentrated *in vacuo*. The crude compound was purified using column chromatography (PE/EtOAc, 8:1 $\rightarrow$ 1:1) to yield the title compound as a yellow oil (3.39 g, 4.79 mmol, 98%, $\alpha : \beta = 8.3 : 1$). TLC: R_f 0.69 α , 0.63 β (PE/EtOAc, 1/1, v/v); IR (neat): 1123, 1153, 1207, 1717, 1748 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.71-7.81 (m, 2.24H, CH_{arom}), 7.44-7.50 (m, 3.36H, CH_{arom}), 7.21-7.30 (m, 10.20H, CH_{arom}), 7.11 (t, 1H, $J = 7.6$ Hz, CH_{arom} NPh), 6.67-6.71 (m, 2.24H, CH_{arom} NPh), 6.47 (bs, 1H, H-1 α), 6.04 (bs, 0.12H, H-1

β), 5.74 (t, 0.12H, $J = 6.4$ Hz, H-4 β), 5.61 (t, 1H, $J = 7.6$ Hz, H-4 α), 4.97 (s, 0.24H, CH₂ Bn/Nap β), 4.86 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap α), 4.80 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap α), 4.68 (d, 0.12H, $J = 12.4$ Hz, CHH Bn/Nap β), 4.60-4.63 (m, 1.12H, CHH Bn/Nap α , CHH Bn/Nap β), 4.55 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap α), 4.40 (d, 1H, $J = 7.2$ Hz, H-5 α), 4.14 (bs, 0.12H, H-5 β), 4.07 (bs, 0.12H, H-2 β), 3.89 (dd, 1H, $J = 3.2, 7.6$ Hz, H-3 α), 3.80-3.82 (m, 1.12H, H-2 α , H-3 β), 3.69 (s, 3H, CH₃ CO₂Me α), 3.64 (s, 0.36H, CH₃ CO₂Me β), 2.69 (t, 2.24H, $J = 6.4$ Hz, CH₂ Lev α,β), 2.46-2.62 (m, 2.24H, CH₂ Lev α,β), 2.17 (s, 3H, CH₃ Lev α), 2.16 (s, 0.36H, CH₃ Lev β); ¹³C (CDCl₃, 100 MHz): δ 206.2 (C=O Lev), 171.6, 168.0 (C=O Lev, CO₂Me), 143.2, 142.5, 142.2, 141.8, 137.9, 137.5, 135.2, 134.9, 133.2 (Cq), 128.8, 128.7, 128.5, 128.4, 128.1, 128.0, 128.0, 127.8, 127.7, 127.4, 127.2, 126.2, 126.1, 126.1, 124.5, 124.2, 119.5 (CH_{arom}), 94.5 (C-1 α), 74.8 (C-3 α), 73.3, 73.1, 73.0, 72.9, 72.9, 72.7, 72.6, 71.6 (CH₂ Bn α,β , Nap α,β , C-2 α , C-3 β , C-5 α , C-5 β), 69.5 (C-2 β) (C-4 α), 68.9 (C-4 β), 52.8 (CH₃ CO₂Me α), 52.6 (CH₃ CO₂Me β), 37.7 (CH₂ Lev), 29.8 (CH₃ Lev), 27.9 (CH₂ Lev); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 94.5 ($J_{C1,H1} = 186$ Hz, C-1 α); HRMS: [M+Na]⁺ calcd. for C₃₈H₃₆F₃NO₉Na 730.22344, found 730.22372.

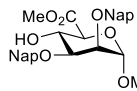
Methyl 2,3-di-O-(2-naphthylmethyl)- α -D-mannopyranoside (29) To a solution of compound 2 (5.4 g, 9.6 mmol)



in MeOH/DCM (1/1, 50 mL) pTsOH·H₂O (1.2 g, 6.25 mmol, 0.65 eq.) was added and allowed to stir overnight. After quenching with sat. aq. NaHCO₃, the mixture was extracted with EtOAc and the layers separated. The organic layer was washed with H₂O and brine, dried with MgSO₄,

filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 7:1 $\rightarrow$ 1:3) afforded the title compound as a yellowish oil (4.0 g, 8.44 mmol, 88%). TLC: R_f 0.20 (Pentane/EtOAc, 1/2, v/v); IR (neat): 748, 812, 1047, 1261, 2922, 3412 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.70-7.80 (m, 8H, CH_{arom}), 7.37-7.51 (m, 6H, CH_{arom}), 4.83 (d, 1H, $J = 12.4$ Hz, CHH Nap), 4.76-4.79 (m, 2H, CHH Nap, H-1), 4.69 (d, 1H, $J = 12.0$ Hz, CHH Nap), 4.63 (d, 1H, $J = 12.0$ Hz, CHH Nap), 4.13 (t, 1H, $J = 9.6$ Hz, H-4), 3.83-3.91 (m, 3H, H-2, H-6, H-6), 3.76 (dd, 1H, $J = 3.2, 9.6$ Hz, H-3), 3.59-3.63 (m, 1H, H-5), 3.30 (s, 3H, CH₃ OMe), 2.85 (bs, 1H, 4-OH), 2.50 (bs, 1H, 6-OH); ¹³C NMR (CDCl₃, 100 MHz): δ 135.6, 135.6, 133.3, 133.3, 133.1, 133.1 (Cq), 128.4, 128.4, 128.0, 128.0, 127.8, 127.8, 126.8, 126.5, 126.3, 126.2, 126.1, 126.1, 126.0, 125.7 (CH_{arom}), 99.4 (C-1), 79.9 (C-3), 73.9 (C-2), 73.0 (CH₂ Nap), 72.3 (C-5), 72.0 (CH₂ Nap), 67.4 (C-4), 62.9 (C-6), 55.0 (OMe); HRMS: [M+NH₄]⁺ calcd. for C₂₉H₃₄NO₆ 492.23806, found 492.23821.

Methyl (methyl 2,3-di-O-(2-naphthylmethyl)- α -D-mannopyranosyl uronate) (32) Diol 29 (2.77 g, 5.84 mmol)



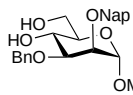
was dissolved in DCM (20 mL) and H₂O (10 mL). To the two phase system TEMPO (228 mg, 1.46 mmol, 0.25 eq.) and BAIB (230 mg, 0.713 mmol, 2.5 eq.) were added. After stirring vigorously for 5 hours at room temperature, the reaction was quenched by addition of sat. aq.

Na₂S₂O₃. The mixture was extracted twice with Et₂O and the layers separated. The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene once. The crude mannuronic acid was then dissolved in DMF (30 mL) and put under an argon atmosphere at 0°C. Methyl iodide (1.1 mL, 17.5 mmol, 3 eq.) and K₂CO₃ (2.4 g, 17.5 mmol, 3 eq.) were added and the reaction was stirred overnight. The reaction was quenched with H₂O and extracted two times with EtOAc. The organic layers were collected and dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (Pentane/EtOAc, 10:1 $\rightarrow$ 3:2) afforded the title compound as a yellow oil (1.6 g, 3.2 mmol, 55%). TLC: R_f 0.24 (Pentane/EtOAc, 2/1, v/v); IR (neat): 750, 818, 1059, 1172, 1748, 3480 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.68-7.81 (m, 8H, CH_{arom}), 7.40-7.49 (m, 6H, CH_{arom}), 4.87-4.90 (m, 2H, H-1 CHH Nap), 4.80-4.84 (m, 2H, CHH Nap, CHH Nap), 4.75 (d, 1H, $J = 12.0$ Hz, CHH Nap), 4.40 (t, 1H, $J = 9.4$ Hz, H-4), 4.14 (d, 1H, $J = 9.4$ Hz, H-5), 3.80-3.84 (m, 2H, H-2, H-3), 3.79 (s, 3H, CH₃ CO₂Me), 3.69 (bs, 1H, 4-OH), 3.37 (s, 3H, CH₃ OMe); ¹³C NMR (CDCl₃, 100 MHz): δ 170.6

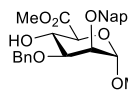
(C=O CO₂Me), 135.8, 135.6, 133.2, 133.1, 132.9, 132.9 (Cq), 128.1, 128.0, 127.8, 127.8, 127.6, 126.5, 126.2, 126.0, 125.9, 125.8, 125.8, 125.6 (CH_{arom}), 99.8 (C-1), 78.6 (C-3), 74.2 (C-2), 72.9, 72.6 (CH₂ Nap), 72.1 (C-5), 68.4 (C-4), 55.3 (CH₃ OMe), 52.5 (CH₃ CO₂Me); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 99.8 (*J*_{C1,H1} = 169 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₃₀H₃₄NO₇ 520.23298, found 520.23331.

Methyl 2-O-benzyl-3-O-(2-naphthylmethyl)-α-D-mannopyranoside (30) To a solution of compound **3** (2.84 g, 5.54 mmol) in MeOH/DCM (3/2, 25 mL) pTsOH·H₂O (185 mg, 0.97 mmol, 0.18 eq.) was added and allowed to stir overnight. After quenching with sat. aq. NaHCO₃, the mixture was extracted with EtOAc and the layers separated. The organic layer was washed with H₂O and brine, dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 4:1 → 1:3) afforded the title compound as a colourless oil (2.16 g, 5.08 mmol, 91%). TLC: R_f 0.22 (Pentane/EtOAc, 1/2, v/v); IR (neat): 698, 737, 814, 1049, 1454, 3404 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.73-7.79 (m, 4H, CH_{arom}), 7.39-7.45 (m, 3H, CH_{arom}), 7.21-7.32 (m, 5H, CH_{arom}), 4.73 (s, 1H, H-1), 4.61-4.71 (m, 4H, CH₂ Bn, Nap), 4.08 (t, 1H, *J* = 10.0 Hz, H-4), 3.72-3.84 (m, 4H, H-2, H-3 H-6, H-6), 3.55-3.59 (m, 1H, H-5), 3.27 (s, 3H, CH₃ OMe), 3.20 (bs, 1H, 4-OH), 2.80 (bs, 1H, 6-OH); ¹³C NMR (CDCl₃, 100 MHz): δ 138.1, 135.7, 133.3, 133.1 (Cq_{arom}), 128.4, 128.3, 128.3, 127.9, 127.8, 127.8, 127.7, 127.6, 126.4, 126.2, 126.1, 126.0, 125.7 (CH_{arom}), 99.3 (C-1), 79.7 (C-3), 74.2 (C-2), 72.8 (CH₂ Bn/Nap), 72.4 (C-5), 72.0 (CH₂ Bn/Nap), 67.2 (C-4), 62.7 (C-6), 54.9 (OMe); ¹³C-GATED NMR (CDCl₃, 100MHz): δ 99.3 (*J*_{C1,H1} = 169 Hz, C-1 α); HRMS: [M+NH₄]⁺ calcd. for C₂₅H₃₂NO₆ 442.22241, found 442.22214.

Methyl (Methyl 2-O-benzyl-3-O-(2-naphthylmethyl)-α-D-mannopyranosyl uronate) (33) Diol **30** (1.27 g, 3.0 mmol) was dissolved in EtOAc (10 mL) and H₂O (5 mL). To the two phase system TEMPO (102 mg, 0.65 mmol, 0.2 eq.) and BAIB (2.62 g, 8.13 mmol, 2.5 eq.) were added. After stirring vigorously for 6 hours at room temperature, the reaction was quenched by addition of sat. aq. Na₂S₂O₃. The mixture was extracted twice with EtOAc. The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene once. The crude mannuronic acid was then dissolved in DMF (18 mL) and put under an argon atmosphere at 0°C. Methyl iodide (0.6 mL, 9.75 mmol, 3 eq.) and K₂CO₃ (1.35 g, 9.75 mmol, 3 eq.) were added and the reaction was stirred overnight. The reaction was quenched with H₂O and extracted two times with EtOAc. The organic layers were collected, washed with brine and dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (Toluene/Acetone, 20:1 → 7:1) afforded the title compound as a yellow oil (748 mg, 1.65 mmol, 55%). TLC: R_f 0.25 (Toluene/Acetone, 8/1, v/v); IR (neat): 698, 737, 816, 1051, 1125, 1202, 1439, 1746, 3478 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.76-7.82 (m, 4H, CH_{arom}), 7.43-7.47 (m, 3H, CH_{arom}), 7.22-7.35 (m, 5H, CH_{arom}), 4.82 (d, 1H, *J* = 1.6 Hz, H-1), 4.80 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap), 4.75 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap), 4.72 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap), 4.67 (d, 1H, *J* = 12.4 Hz, CHH Bn/Nap), 4.35 (dt, 1H, *J* = 2.4, 9.2 Hz, H-4), 4.10 (d, 1H, *J* = 9.2 Hz, H-5), 3.75-3.81 (m, 5H, CH₃ CO₂Me, H-2, H-3), 3.36 (s, 3H, CH₃ OMe), 3.11 (d, 1H, *J* = 2.4 Hz, 4-OH); ¹³C NMR (CDCl₃, 100 MHz): δ 170.8 (C=O CO₂Me), 138.1, 135.8, 133.3, 133.0 (Cq), 128.4, 128.2, 128.0, 127.9, 127.8, 126.4, 126.2, 125.9, 125.7 (CH_{arom}), 99.9 (C-1), 78.5 (C-3), 74.2 (C-2), 73.0, 72.6 (CH₂ Bn, Nap), 71.8 (C-5), 68.5 (C-4), 55.5 (OMe), 52.7 (CH₃ CO₂Me); HRMS: [M+NH₄]⁺ calcd. for C₂₆H₃₂NO₇ 470.21733, found 470.21690.

Methyl 3-O-benzyl-2-O-(2-naphthylmethyl)- α -D-mannopyranoside (31)

7.4 mmol) in MeOH/DCM (1/1, 40 mL) pTsOH $\cdot$ H₂O (222 mg, 1.17 mmol, 0.15 eq.) was added and allowed to stir over the weekend. After quenching with sat. aq. NaHCO₃, the mixture was extracted with EtOAc and the layers separated. The organic layer was washed with H₂O and brine, dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 4:1 $\rightarrow$ 1:4) afforded the title compound as a yellow oil (2.63 g, 6.2 mmol, 83%). TLC: R_f 0.25 (PE/EtOAc, 1/2, v/v); IR (neat): 734, 820, 1053, 1454, 2913, 3393 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.72- 7.79 (m, 4H, CH_{arom}), 7.42- 7.48 (m, 3H, CH_{arom}), 7.25-7.32 (m, 5H, CH_{arom}), 4.75-4.83 (m, 3H, H-1, CH₂ Bn/Nap), 4.56 (d, 1H, *J* = 11.8 Hz, CHH Bn/Nap), 4.48 (d, 1H, *J* = 11.8 Hz, CHH Bn/Nap), 4.08 (t, 1H, *J* = 9.6 Hz, H-4), 3.80-3.89 (m, 3H, H-2, H-6, H-6), 3.70 (dd, 1H, *J* = 2.8, 9.6 Hz, H-3), 3.57- 3.61 (m, 1H, H-5), 3.29 (s, 3H, CH₃ OMe), 2.94 (bs, 1H, 4-OH), 2.63 (bs, 1H, 6-OH); ¹³C NMR (CDCl₃, 100 MHz): δ 138.2, 135.6, 133.3, 133.1 (Cq), 128.6, 128.3, 128.0, 127.9, 127.8, 126.8, 126.2, 126.0 (CH_{arom}), 99.4 (C-1), 79.9 (C-3), 74.0 (C-2), 73.0 (CH₂ Bn/Nap), 72.4 (C-5), 72.0 (CH₂ Bn/Nap), 67.3 (C-4), 62.8 (C-6), 54.9 (OMe); HRMS: [M+NH₄]⁺ calcd. for C₂₅H₃₂NO₆ 442.22241, found 442.22236.

Methyl (Methyl 3-O-benzyl-2-O-(2-naphthylmethyl)- α -D-mannopyranosyl uronate) (34)

0.285 mmol) was dissolved in DCM (1.0 mL) and H₂O (0.5 mL). To the two phase system TEMPO (9 mg, 0.057 mmol, 0.2 eq.) and BAIB (230 mg, 0.713 mmol, 2.5 eq.) were added.

After stirring vigorously for 6 hours at room temperature, the reaction was quenched by addition of sat. aq. Na₂S₂O₃. The mixture was transferred to a separatory funnel, EtOAc was added and the layers were separated. The organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene once. The crude mannuronic acid was then dissolved in DMF (1.5 mL) and put under an argon atmosphere at 0°C. Methyl iodide (53 μ L, 0.855 mmol, 3.0 eq.) and K₂CO₃ (118 mg, 0.855 mmol, 3.0 eq.) were added and the reaction was stirred overnight. The reaction was quenched with H₂O and extracted three times with EtOAc. The organic layers were collected and dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 9:1 $\rightarrow$ 2:1) afforded the title compound as a yellow oil (98 mg, 0.217 mmol, 76%). TLC: R_f 0.24 (PE/EtOAc, 2/1, v/v); IR (neat): 698, 737, 1051, 1125, 1201, 1439, 1746, 3476 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.75-7.82 (m, 4H, CH_{arom} Bn/Nap), 7.44-7.49 (m, 3H, CH_{arom} Bn/Nap), 7.26-7.32 (m, 5H, CH_{arom} Bn/Nap), 4.81-4.88 (m, 3H, H-1, CH₂ Bn/Nap), 4.66 (d, 1H, *J* = 11.8 Hz, CHH Bn/Nap), 4.61 (d, 1H, *J* = 11.8 Hz, CHH Nap), 4.35 (t, 1H, *J* = 9.2 Hz, H-4), 4.10 (d, 1H, *J* = 9.2 Hz, H-5), 3.73-3.82 (m, 5H, H-2, H-3, CH₃ CO₂Me), 3.37 (s, 3H, CH₃ OMe), 2.92 (d, 1H, *J* = 2.4 Hz, 4-OH); ¹³C NMR (CDCl₃, 100 MHz): δ 170.8 (C=O CO₂Me), 138.4, 135.6, 133.3, 133.1 (Cq), 128.5, 128.3, 128.0, 127.8, 127.7, 126.8, 126.2, 126.1, 126.0 (CH_{arom}), 100.0 (C-1), 78.7 (C-3), 74.1 (C-2), 73.1 (CH₂ Bn), 72.7 (CH₂ Nap), 71.8 (C-5), 68.6 (C-4), 55.5 (OMe), 52.7 (CH₃ CO₂Me); HRMS: [M+NH₄]⁺ calcd. for C₂₆H₃₂NO₇ 470.21733, found 470.21745.

General procedure for TfOH-mediated glycosylations. A mixture of the donor (1 eq.) and acceptor (1.2 eq.) was co-evaporated with dry toluene twice. While the mixture was under an argon atmosphere, freshly distilled DCM (0.1 M, based on combined amounts of donor and acceptor) was added, followed by the addition of activated molecular sieves (3 Å). The resulting mixture was stirred at room temperature for 30 minutes and then cooled to -40°C. TfOH (0.2 eq.) was added and the reaction was monitored by TLC analysis. After TLC analysis showed the complete consumption of donor material, the reaction was quenched by addition of Et₃N (1 % v/v). The mixture was transferred to a separatory funnel with EtOAc and washed with brine twice. After drying with MgSO₄ and

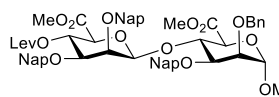
concentrating *in vacuo*, the crude disaccharide was passed through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) which gave the purified product.

Methyl (methyl 4-O-[methyl 2-O-benzyl-4-O-levulinoyl-3-O-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-2,3-di-O-(2-naphthylmethyl)-α-D-mannopyranosyl uronate) (35)

Donor **27** (135 mg, 0.19 mmol) and acceptor **32** (123 mg, 0.245 mmol, 1.2 eq.) were condensed using the general protocol for TfOH-mediated glycosylations to yield disaccharide **35** (145 mg, 0.142 mmol, 74%, $\alpha \ll \beta$) as an off-white foam. TLC: R_f 0.24 (PE/EtOAc, 3/2, v/v); IR (neat): 747, 816, 1051, 1125, 1360, 1717, 1746 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.63–7.82 (m, 12H, CH_{arom}), 7.35–7.48 (m, 11H, CH_{arom}), 7.20–7.27 (m, 3H, CH_{arom}), 5.52 (t, 1H, $J = 9.6$ Hz, H-4'), 5.06 (bs, 1H, H-1), 4.91 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap), 4.63–4.87 (m, 7H, CHH Bn/Nap, CH_2 Nap, CH_2 Bn/Nap, CHH Bn/Nap, H-1'), 4.51–4.54 (m, 2H, CHH Bn/Nap, H-4), 4.27 (d, 1H, $J = 5.2$ Hz, H-5), 4.12 (bs, 1H, H-3), 3.88 (d, 1H, $J = 2.4$ Hz, H-2'), 3.80 (d, 1H, $J = 9.6$ Hz, H-5'), 3.74 (dd, 1H, $J = 2.8, 5.2$, H-2), 3.48–3.53 (m, 10H, CH_3 CO_2Me , $\text{CO}_2\text{Me}'$, OMe, H-3), 2.64 (t, 2H, $J = 6.4$ Hz, CH_2 Lev), 2.53 (t, 2H, $J = 6.4$ Hz, CH_2 Lev), 2.11 (s, 3H, CH_3 Lev); ^{13}C (CDCl_3 , 100 MHz): δ 206.3 (C=O Lev), 171.7, 169.9, 167.8 (C=O CO_2Me , Lev), 138.4, 136.1, 135.8, 135.3, 133.3, 133.2, 133.1, 133.0, 132.9 (Cq), 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.5, 127.4, 127.4, 126.6, 126.5, 126.3, 126.2, 126.1, 126.0, 125.9, 125.9, 125.8, 125.8, 125.5 (CH_{arom}), 101.1 (C-1'), 99.7 (C-1), 77.9 (C-3'), 77.0 (C-3), 76.5 (C-4), 75.1 (C-2), 74.2 (CH_2 Bn/Nap), 74.1 (C-2'), 73.5 (C-5'), 73.1, 73.0 (CH_2 Bn/Nap), 71.8 (C-5, CH_2 Bn/Nap), 69.1 (C-4'), 56.2 (OMe), 52.5, 52.3 (CH_3 CO_2Me), 37.8 (CH_2 Lev), 29.9 (CH_3 Lev), 28.0 (CH_2 Lev); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 101.1 ($J_{\text{C1,H1}} = 161$ Hz, C-1' β), 99.7 ($J_{\text{C1,H1}} = 167$ Hz, C-1 α); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. for $\text{C}_{60}\text{H}_{64}\text{NO}_{15}$ 1038.42705, found 1038.42941.

Methyl (methyl 4-O-[methyl 3-O-benzyl-4-O-levulinoyl-2-O-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-2,3-di-O-(2-naphthylmethyl)-α-D-mannopyranosyl uronate) (36)

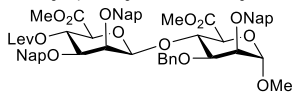
Donor **28** (139 mg, 0.196 mmol) and acceptor **32** (119 mg, 0.237 mmol, 1.2 eq.) were condensed using the general protocol for TfOH-mediated glycosylations to yield disaccharide **36** (159 mg, 0.156 mmol, 79%, $\alpha \ll \beta$) as an off-white foam. TLC: R_f 0.23 (PE/EtOAc, 3/2, v/v); IR (neat): 750, 820, 1055, 1126, 1364, 1719, 1748 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.60–7.80 (m, 12H, CH_{arom}), 7.36–7.50 (m, 9H, CH_{arom}), 7.23–7.36 (m, 3H, CH_{arom}), 7.17–7.19 (m, 2H, CH_{arom}), 5.53 (t, 1H, $J = 9.6$ Hz, H-4'), 5.08 (bs, 1H, H-1), 4.68–4.94 (m, 7H, CH_2 Nap, CH_2 Nap, CH_2 Bn/Nap, H-1'), 4.53 (t, 1H, $J = 5.6$ Hz, H-4), 4.45 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap), 4.39 (d, 1H, $J = 12.0$ Hz, CHH Bn/Nap), 4.28 (d, 1H, $J = 5.6$ Hz, H-5), 4.14 (bs, 1H, H-3), 3.89 (d, 1H, $J = 2.8$ Hz, H-2'), 3.83 (d, 1H, $J = 9.6$ Hz, H-5'), 3.76 (dd, 1H, $J = 2.8, 5.2$ Hz, H-2), 3.52–3.54 (m, 9H, CH_3 CO_2Me , $\text{CO}_2\text{Me}'$, OMe), 3.46 (dd, 1H, $J = 2.8, 9.6$ Hz, H-3'), 2.68 (t, 2H, $J = 6.4$ Hz, CH_2 Lev), 2.51–2.55 (m, 2H, CH_2 Lev), 2.13 (s, 3H, CH_3 Lev); ^{13}C (CDCl_3 , 100 MHz): δ 206.3 (C=O Lev), 171.7, 169.8, 167.8 (C=O CO_2Me , Lev), 137.8, 136.1, 135.8, 135.7, 133.3, 133.2, 133.2, 133.0, 133.0, 132.9 (Cq), 128.4, 128.4, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.0, 126.6, 126.2, 126.1, 126.0, 126.0, 125.9, 125.9, 125.8, 125.7, 125.6 (CH_{arom}), 101.2 (C-1'), 99.8 (C-1), 78.1 (C-3'), 76.9 (C-3), 76.7 (C-4), 75.2 (C-2), 74.2 (CH_2 Bn/Nap), 73.9 (C-2'), 73.5 (C-5'), 73.0, 72.9 (CH_2 Bn/Nap), 72.0 (CH_2 Bn/Nap), 71.9 (C-5), 71.8 (CH_2 Bn/Nap), 69.2 (C-4'), 56.2 (OMe), 52.5, 52.3 (CH_3 CO_2Me), 37.8 (CH_2 Lev), 29.9 (CH_3 Lev), 27.9 (CH_2 Lev); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 101.2 ($J_{\text{C1,H1}} = 156$ Hz, C-1' β), 99.8 ($J_{\text{C1,H1}} = 169$ Hz, C-1 α); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. for $\text{C}_{60}\text{H}_{64}\text{NO}_{15}$ 1038.42705, found 1038.42936.

Methyl (methyl 4-O-[methyl 4-O-levulinoyl-2,3-di-O-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-2-O-**benzyl-3-O-(2-naphthylmethyl)-α-D-mannopyranosyl uronate) (37) Donor**

26 (140 mg, 0.185 mmol) and acceptor **33** (112 mg, 0.247 mmol, 1.2 eq.) were

condensed using the general protocol for TfOH-mediated glycosylations to

yield disaccharide **37** (160 mg, 0.157 mmol, 85%, $\alpha << \beta$) as an yellowish foam. TLC: R_f 0.11 (PE/EtOAc, 2/1, v/v); IR (neat): 748, 818, 1053, 1125, 1362, 1717, 1748 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.69-7.81 (m, 11H, CH_{arom}), 7.61 (s, 1H, CH_{arom}), 7.55 (dd, 1H, $J = 1.6, 8.4$ Hz, CH_{arom}), 7.39-7.46 (m, 7H, CH_{arom}), 7.31 (dd, 1H, $J = 1.2, 8.4$ Hz, CH_{arom}), 7.19-7.22 (m, 5H, CH_{arom}), 5.56 (t, 1H, $J = 9.6$ Hz, H-4'), 5.04 (bs, 1H, H-1), 5.00 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap), 4.89 (d, 2H, $J = 12.4$ Hz, CHH Bn/Nap , CHH Bn/Nap), 4.76 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap), 4.53-4.67 (m, 5H, $\text{CH}_2 \text{ Bn/Nap}$, $\text{CH}_2 \text{ Bn/Nap}$, H-1'), 4.49 (t, 1H, $J = 5.6$ Hz, H-4), 4.26 (d, 1H, $J = 5.6$ Hz, H-5), 4.11 (d, 1H, $J = 2.8$ Hz, H-3), 3.91 (d, 1H, $J = 2.8$ Hz, H-2'), 3.82 (d, 1H, $J = 9.6$ Hz, H-5'), 3.71 (dd, 1H, $J = 2.8, 5.2$ Hz, H-2), 3.54 (s, 3H, $\text{CH}_3 \text{ CO}_2\text{Me}$), 3.49-3.53 (m, 4H, $\text{CH}_3 \text{ OMe}$, H-3'), 3.44 (s, 3H, $\text{CH}_3 \text{ CO}_2\text{Me}$), 2.65 (t, 2H, $J = 6.4$ Hz, $\text{CH}_2 \text{ Lev}$), 2.54 (t, 2H, $J = 6.4$ Hz, $\text{CH}_2 \text{ Lev}$), 2.11 (s, 3H, $\text{CH}_3 \text{ Lev}$); ^{13}C (CDCl_3 , 100 MHz): δ 206.3 (C=O Lev), 171.7, 169.8, 167.8 (C=O CO_2Me , Lev), 138.3, 136.1, 135.9, 135.6, 135.3, 133.3, 133.2, 133.2, 133.0, 133.0, 132.9 (Cq), 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 127.5, 127.0, 126.6, 126.5, 126.3, 126.2, 126.1, 126.0, 125.9, 125.8, 125.8, 125.7 (CH_{arom}), 101.2 (C-1'), 99.7 (C-1), 78.1 (C-3'), 76.9 (C-3), 76.7 (C-4), 75.2 (C-2), 74.2 ($\text{CH}_2 \text{ Bn/Nap}$), 73.9 (C-2'), 73.5 (C-5'), 73.0, 72.9 ($\text{CH}_2 \text{ Bn/Nap}$), 72.0 ($\text{CH}_2 \text{ Bn/Nap}$), 71.9 (C-5), 69.2 (C-4'), 56.2 (OMe), 52.5, 52.2 ($\text{CH}_3 \text{ CO}_2\text{Me}$), 37.8 ($\text{CH}_2 \text{ Lev}$), 29.9 ($\text{CH}_3 \text{ Lev}$), 28.0 ($\text{CH}_2 \text{ Lev}$); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 101.2 ($J_{\text{C1,H1}} = 156$ Hz, C-1' β), 99.7 ($J_{\text{C1,H1}} = 168$ Hz, C-1 α); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. for $\text{C}_{60}\text{H}_{64}\text{NO}_{15}$ 1038.42705, found 1038.42888

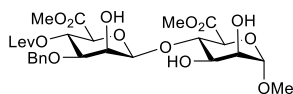
Methyl (methyl 4-O-[methyl 4-O-levulinoyl-2,3-di-O-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-3-O-**benzyl-2-O-(2-naphthylmethyl)-α-D-mannopyranosyl uronate) (38) Donor**

26 (130 mg, 0.172 mmol) and acceptor **34** (96 mg, 0.217 mmol, 1.2 eq.) were

condensed using the general protocol for TfOH-mediated glycosylations to

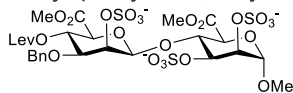
yield disaccharide **38** (167 mg, 0.164 mmol, 95%, $\alpha << \beta$) as an off-white foam. TLC: R_f 0.12 (PE/EtOAc, 2/1, v/v); IR (neat): 750, 820, 1057, 1126, 1364, 1719, 1748 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.67-7.80 (m, 12H, CH_{arom}), 7.60 (s, 1H, CH_{arom}), 7.51 (dd, 1H, $J = 1.2, 8.4$ Hz, CH_{arom}), 7.32-7.46 (m, 7H, CH_{arom}), 7.23-7.30 (m, 5H, CH_{arom}), 5.56 (t, 1H, $J = 9.6$ Hz, H-4'), 5.06 (bs, 1H, H-1), 4.90 (d, 1H, $J = 12.8$ Hz, CHH Bn/Nap), 4.83 (d, 1H, $J = 12.8$ Hz, CHH Bn/Nap), 4.69-4.80 (m, 3H, $\text{CH}_2 \text{ Bn/Nap}$, CHH Bn/Nap), 4.57-4.63 (m, 3H, CHH Bn/Nap , CHH Bn/Nap , H-1'), 4.52 (d, 1H, $J = 12.4$ Hz, CHH Bn/Nap), 4.46 (t, 1H, $J = 5.6$ Hz, H-4), 4.26 (d, 1H, $J = 5.6$ Hz, H-5), 4.09 (d, 1H, $J = 2.8$ Hz, H-3), 3.90 (d, 1H, $J = 2.4$ Hz, H-2'), 3.82 (d, 1H, $J = 9.6$ Hz, H-5'), 3.74 (dd, 1H, $J = 2.8, 5.2$ Hz, H-2), 3.61 (s, 3H, $\text{CH}_3 \text{ CO}_2\text{Me}$), 3.49-3.53 (m, 4H, $\text{CH}_3 \text{ OMe}$, H-3'), 3.45 (s, 3H, $\text{CH}_3 \text{ CO}_2\text{Me}$), 2.66 (t, 2H, $J = 6.4$ Hz, $\text{CH}_2 \text{ Lev}$), 2.55 (t, 2H, $J = 6.4$ Hz, $\text{CH}_2 \text{ Lev}$), 2.12 (s, 3H, $\text{CH}_3 \text{ Lev}$); ^{13}C (CDCl_3 , 100 MHz): δ 206.3 (C=O Lev), 171.7, 169.8, 167.8 (C=O CO_2Me , Lev), 138.6, 135.8, 135.8, 135.2, 133.2, 133.2, 133.2, 133.0, 133.0, 133.0 (Cq), 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.1, 126.6, 126.5, 126.5, 126.3, 126.2, 126.1, 126.0, 125.9, 125.9, 125.8, 125.8, 125.7 (CH_{arom}), 101.2 (C-1'), 99.8 (C-1), 78.1 (C-3'), 76.9 (C-3), 76.7 (C-4), 75.0 (C-2), 74.1 ($\text{CH}_2 \text{ Bn/Nap}$), 73.8 (C-2'), 73.6 (C-5'), 73.1, 72.8 ($\text{CH}_2 \text{ Bn/Nap}$), 71.9 ($\text{CH}_2 \text{ Bn/Nap}$, C-5), 69.2 (C-4'), 56.2 (OMe), 52.6, 52.2 ($\text{CH}_3 \text{ CO}_2\text{Me}$), 37.8 ($\text{CH}_2 \text{ Lev}$), 29.9 ($\text{CH}_3 \text{ Lev}$), 28.0 ($\text{CH}_2 \text{ Lev}$); ^{13}C -GATED NMR (CDCl_3 , 100MHz): δ 101.2 ($J_{\text{C1,H1}} = 152$ Hz, C-1' β), 99.8 ($J_{\text{C1,H1}} = 169$ Hz, C-1 α); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. for $\text{C}_{60}\text{H}_{64}\text{NO}_{15}$ 1038.42705, found 1038.42877.

Methyl (methyl 4-O-[methyl 3-O-benzyl-4-O-levulinoyl-β-D-mannopyranosyl uronate]-α-D-mannopyranosyl uronate) (39)



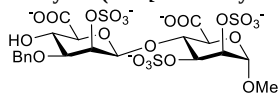
Mannuronic acid disaccharide **36** (0.0825 g, 0.0807) was dissolved in 1:1 DCM/HFIP (2 mL). Triisopropylsilane (0.082 mL, 0.4 mmol) was added and the mixture was treated with 1.2 mL 0.2M HCl/HFIP. After stirring for 10 minutes, the reaction was quenched with sat. aq. NaHCO₃. The mixture was diluted DCM and the organic layer is washed with sat. aq. NaCl, dried over MgSO₄ and concentrated. Purification by column chromatography (2:1 pentanes/EtOAc → 19:1 EtOAc/MeOH) yielded the triol **39** 86% yield (0.0421 g, 0.070 mmol). TLC: R_f 0.43 (EtOAc/MeOH, 19/1, v/v); ¹H-NMR (CDCl₃, 400 MHz): δ 7.40 – 7.29 (m, 5H, CH_{arom}), 5.47 (t, 1H, J = 8.7, 8.7 Hz, H-4'), 4.83 – 4.74 (m, 3H, H-1, H-1', OH), 4.71 (d, 1H, J = 12.2 Hz, CHH Bn), 4.65 (d, 1H, J = 12.2 Hz, CHH Bn), 4.21 – 4.06 (m, 3H, H-5, H-2, H-2'), 4.06 – 3.94 (m, 3H, H-3, H-4, H-5'), 3.77 (s, 3H, CH₃ CO₂Me), 3.72 – 3.63 (m, 4H, CH₃ CO₂Me, H-3'), 3.43 (s, 3H, CH₃ OMe), 3.28 (bs, 1H, OH), 2.99 (bs, 1H, OH), 2.73 (t, 2H, J = 6.5, 6.5 Hz, CH₂ Lev), 2.56 (dt, 2H, J = 13.3, 6.5, 6.5 Hz, CH₂ Lev), 2.19 (s, 3H, CH₃ Lev); ¹³C-NMR (CDCl₃, 100 MHz): δ 206.3 (C=O Lev), 171.7, 169.8, 168.1 (C=O Lev, CO₂Me), 137.5 (C_q), 128.4, 127.9, 127.7 (CH_{arom}), 100.9 (C-1), 100.4 (C-1'), 79.9 (C-4), 77.3 (C-3'), 72.0 (CH₂ Bn), 71.9 (C-5'), 69.5 (C-2), 69.3 (C-3), 69.1 (C-5), 67.9 (C-4'), 67.4 (C-2'), 55.4 (CH₃ OMe), 52.9, 52.5 (CH₃ CO₂Me), 37.6 (CH₂ Lev), 29.8 (CH₃ Lev), 27.8 (CH₂ Lev); HRMS: [M+NH₄]⁺ calculated for C₂₇H₄₀NO₁₅ 618.23925, found 618.23972.

Methyl (methyl 4-O-[methyl 3-O-benzyl-4-O-levulinoyl-2-O-sulfo-β-D-mannopyranosyl uronate]-2,3-di-O-sulfo-α-D-mannopyranosyl uronate) (40)



Triol **39** (0.061 g, 0.101 mmol) was co-evaporated twice with DMF and dissolved in DMF. Sulfur trioxide triethylamine complex (0.276 g, 1.52 mmol) was added and the temperature is raised to 55°C. The septum is replaced with a stopper and the flask is sealed, allowed to stir overnight at 55°C. After TLC analysis showed conversion of the starting material in a lower running spot, the mixture was cooled to 0°C and NaCO₃ (0.14 g, 1.67 mmol) in 10 mL H₂O was added and stirred for 30 minutes at 0°C. The mixture was concentrated at 25°C and purified using size exclusion chromatography (eluted with DCM/MeOH, 1/1, v/v) to yield sulfated disaccharide **40** in 100% yield as the triethylammonium salt (0.124 g, 0.108 mmol). TLC: R_f 0.43 (DCM/MeOH, 3/1, v/v); ¹H-NMR (MeOD, 850 MHz): δ 7.38 (d, 2H, J = 7.6 Hz, CH_{arom}), 7.30 (t, 2H, J = 7.6, 7.6 Hz, CH_{arom}), 7.23 (t, 1H, J = 7.4, 7.4 Hz, CH_{arom}), 5.16 – 5.09 (m, 2H, H-1', H-4'), 5.01 – 4.97 (m, 2H, H-1, H-2'), 4.94 – 4.86 (m, 2H, H-2, H-3), 4.84 (d, 1H, J = 12.0 Hz, CHH Bn), 4.45 (d, 1H, J = 12.0 Hz, CHH Bn), 4.41 (s, 2H, H-4, H-5), 4.05 (d, 1H, J = 9.9 Hz, H-5'), 3.78 (s, 3H, CH₃ CO₂Me), 3.74 (dd, 1H, J = 9.8, 2.9 Hz, H-3'), 3.66 (s, 3H, CH₃ CO₂Me), 3.41 (s, 3H, CH₃ OMe), 3.20 (q, 18H, J = 7.3, 7.3, 7.2 Hz, 3xCH₂ Et₃N), 2.65 (td, 2H, J = 6.5, 6.4, 2.1 Hz, CH₂ Lev), 2.47 (q, 2H, J = 6.8, 6.8, 6.6 Hz, CH₂ Lev), 2.10 (s, 3H, CH₃ Lev), 1.28 (t, 27H, J = 7.4, 7.4 Hz, 3xCH₃ Et₃N); ¹³C-NMR (MeOD, 214 MHz): δ 207.9 (C=O Lev), 172.7, 170.2, 169.5 (C=O Lev, CO₂Me), 138.9 (C_q), 128.6, 128.5, 128.5, 128.5, 127.9 (CH_{arom}), 100.1 (C-1), 99.6 (C-1'), 77.4 (C-3'), 76.8 (C-4 or C-5), 74.9 (C-2), 74.2 (C-2'), 73.4 (C-3 and C-5'), 71.9 (C-4 or C-5), 71.4 (CH₂ Bn), 69.1 (C-4'), 55.2 (CH₃ OMe), 52.4, 52.3 (CH₃ CO₂Me), 47.3 (CH₂ Et₃N), 37.7 (CH₂ Lev), 28.9 (CH₃ Lev), 28.2 (CH₂ Lev), 8.6 (CH₃ Et₃N); HRMS: [M+H]⁺ calculated for C₄₅H₈₁N₃O₂₄S₃ 1144.44591, found 1144.44449.

Methyl (4-O-[3-O-benzyl-2-O-sulfo-β-D-mannopyranosyl uronate]-2,3-di-O-sulfo-α-D-mannopyranosyl uronate) (41)

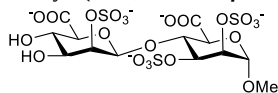


Sulfated disaccharide **40** (0.0567 g, 0.05 mmol) was dissolved in 1:1 THF/H₂O (2 mL) and cooled to 0°C. A 0.5M LiOH/H₂O₂ (0.74 mL, 5 eq. per ester) solution was added and the reaction was allowed to warm up to room temperature. After overnight stirring, the reaction was neutralized with 1M HCl (aq). The mixture was concentrated

Chapter 5

in vacuo and purified using HW-40 size-exclusion chromatography (eluted with NH_4OAc) to give the oligosaccharide after lyophilization. The compound was taken up in a small amount of H_2O and passed through a column of Dowex 50 WX-4 (Na^+ form) to yield the saponified disaccharide after lyophilization (23.8 mg, 28.9 μmol , 66%). $^1\text{H-NMR}$ (D_2O , 600 MHz, $T=313\text{K}$): δ 7.54 – 7.49 (m, 2H, CH_{arom}), 7.45 – 7.41 (m, 2H, CH_{arom}), 7.40 – 7.36 (m, 1H, CH_{arom}), 5.12 (d, 1H, $J=3.8$ Hz, H-1'), 5.05 (d, 1H, $J=2.8$ Hz, H-2'), 4.91 (d, 1H, $J=11.2$ Hz, CHH Bn), 4.86 – 4.78 (m, 3H, H-1, H-2, H-3), 4.54 (d, 1H, $J=11.2$ Hz, CHH Bn), 4.33 (s, 1H, H-4), 4.21 (d, 1H, $J=7.0$ Hz, H-5), 3.77 (t, 1H, $J=9.7, 9.7$ Hz, H-4'), 3.70 (d, 1H, $J=9.9$ Hz, H-5'), 3.65 (dd, 1H, $J=9.7, 2.9$ Hz, H-3'), 3.49 (s, 3H, CH_3 OMe); $^{13}\text{C-NMR}$ (D_2O , 150 MHz, $T=313\text{K}$): δ 176.5, 175.8 (2x COO^-), 138.3 (C_q), 130.7, 129.8, 129.6, 129.4, 129.0 (CH_{arom}), 99.4 (C-1), 98.5 (C-1'), 80.0 (C-3'), 77.7 (C-5'), 76.8 (C-4), 75.6 (C-2'), 75.2 (C-2), 74.9 (C-3), 74.7 (C-5), 72.1 (CH_2 Bn), 68.5 (C-4'), 56.6 (CH_3 OMe); HRMS: $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{20}\text{H}_{33}\text{O}_{22}\text{S}_3\text{Na}_2$ 749.06816, found 749.06891.

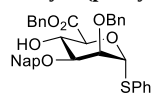
Methyl (4-O-[2-O-sulfo- β -D-mannopyranosyl uronate]-2,3-di-O-sulfo- α -D-mannopyranosyl uronate) (42)



Saponified disaccharide **41** (3.98 mg, 4.84 μmol) was dissolved in H_2O (1.5 mL) and purged with argon for 5 minutes. Pd/C (10% palladium on carbon, 8.3 mg) was added and the resulting black suspension was purged with argon for 5

minutes. A hydrogen balloon was applied and the suspension was purged for 5 minutes after which it was allowed to stir overnight at room temperature. The mixture was filtered through a Whatmann-filter and concentrated *in vacuo*. This procedure was repeated followed by HW-40 size-exclusion chromatography (eluted with NH_4OAc). The product fractions were pooled, concentrated, dissolved in a small amount of H_2O and passed through a column of Dowex 50 WX-4 (Na^+ form) to yield the fully deprotected disaccharide as a white solid after lyophilization (1.49 mg, 2.03 μmol , 42%). $^1\text{H-NMR}$ (D_2O , 600 MHz, $T=313\text{K}$): δ 5.10 (d, 1H, $J=3.3$ Hz, H-1'), 4.86 – 4.77 (m, 3H, H-1, H-2, H-3), 4.73 (d, 2H, $J=3.3$ Hz, H-2'), 4.29 (s, 1H, H-4), 4.17 (d, 1H, $J=7.3$ Hz, H-5'), 3.76 – 3.66 (m, 3H, H-3', H-4', H-5'), 3.47 (s, 3H CH_3 OMe); $^{13}\text{C-NMR}$ (D_2O , 150 MHz): δ 176.5, 175.8 (2x COO^-), 99.2 (C-1), 98.6 (C-1'), 79.2 (C-2'), 77.6 (C-3'), 76.6 (C-4), 75.3 (C-2), 74.8 (C-3), 74.6 (C-5), 73.1 (C-5'), 69.8 (C-4'), 56.5 (CH_3 OMe); HRMS: $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{13}\text{H}_{17}\text{O}_{22}\text{S}_3\text{Na}_3$ 712.89589, found 712.89593.

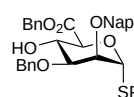
Benzyl (phenyl 2-O-benzyl-3-O-(2-naphthylmethyl)-1-thio- α -D-mannopyranosyl uronate) (48) 4,6-O-



benzylidene-1-thio- α -D-mannopyranoside (7.2 g, 20 mmol) was dissolved in toluene and Bu_2SnO (5.48 g, 22 mmol, 1.1 eq.) was added. The mixture was refluxed overnight, after which CsF (4.56 g, 30 mmol, 1.5 eq.) was added followed by addition of 2-(Bromomethyl)naphthalene (6.63 g, 30 mmol, 1.5 eq.) and the mixture was heated to 100°C . After overnight heating, the mixture was concentrated, coevaporated twice with toluene and dissolved in DMF (100 mL) and cooled to 0°C . The mixture was treated with sodium hydride (60% dispersion in mineral oil, 1.6 g, 40 mmol, 2 eq.), and, after 10 minutes, with Benzyl bromide (4.8 mL, 40 mmol, 2 eq.). After stirring overnight, the reaction was quenched by addition of sat. aq. NaHCO_3 . The mixture was diluted with Et_2O , washed with sat. aq. NaCl , dried over MgSO_4 and concentrated *in vacuo*. The crude was dissolved in MeOH/DCM (50 mL : 50 mL) and pTsOH (0.38 g, 2 mmol, 0.1 eq.) was added. After conversion to a lower running spot, the reaction was neutralized with Et_3N , concentrated and purified by column purification (PE/EtOAc , 4:1 $\rightarrow$ 1:1) yielding diol **45** (7.57 g, 15.1 mmol, 75%). Diol **45** (4.77 g, 9.498 mmol, 1.0 eq.) was dissolved in $t\text{BuOH}/\text{DCM}/\text{H}_2\text{O}$ (4:4:1, 95 mL) and cooled to 0°C . TEMPO (0.30 g, 1.92 mmol, 0.2 eq.) and BAIB (7.64 g, 23.72 mmol, 2.5 eq.) were added and the reaction was stirred vigorously for 230 min. at which it was allowed to warm up to RT. TLC analysis showed conversion of the starting material to a low running spot, and the reaction was quenched with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$. The mixture was diluted with EtOAc and the

layers were separated. The aqueous layer was acidified and extracted with EtOAc. The combined organic layers were washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. The resulting oil was coevaporated twice with anhydrous toluene and dissolved in DMF (95 mL). Benzyl bromide (2.26 mL, 18.98 mmol, 2 eq.) was added, followed by K_2CO_3 (2.63 g, 18.98 mmol, 2 eq.). The reaction was stirred over night, after which it was quenched with H_2O . The mixture was diluted with Et_2O and washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. Column purification (PE/EtOAc, 4:1 $\rightarrow$ 1:1) yielded the title compound (4.44 g, 7.32 mmol, 77%). IR (neat): 966, 1026, 1114, 1440, 1583, 1732, 2870, 1933, 3061, 3394, 3471 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3 , T=328K): δ 7.80 – 7.73 (m, 3H), 7.45 – 7.39 (m, 5H), 7.31 – 7.09 (m, 11H), 5.62 (d, 1H, $J = 2.0$ Hz, H-1), 5.21 – 5.08 (m, 2H, CH_2 Bn), 4.80 (d, 1H, $J = 12.0$ Hz, CHH), 4.74 (d, $J = 12.0$ Hz, 1H, CHH), 4.72 – 4.62 (m, 2H, H-5, CHH), 4.56 (d, 1H, $J = 12.1$ Hz, CHH), 4.48 (t, 1H, $J = 8.6$ Hz, H-4), 3.96 (s, 1H, H-2), 3.80 (dd, 1H, $J = 8.7$, 2.6 Hz, H-3), 3.02 (s, 1H, OH); ^{13}C NMR (126 MHz, CDCl_3 , T=328K): δ 169.6 (C=O), 137.9, 135.7, 135.4, 133.8, 133.5, 133.2 (Cq), 131.8, 129.1, 128.6, 128.5, 128.4, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.8, 127.6, 126.7, 126.2, 126.0, 125.9 (CH_{arom}), 86.1 (C-1), 78.6 (C-3), 76.3 (C-2), 73.2 (C-5), 72.9 (CH_2 Bn/Nap), 72.6 (CH_2 Bn/Nap), 68.9 (C-4), 67.2 (CH_2 Bn/Nap); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd. $\text{C}_{37}\text{H}_{38}\text{NO}_6\text{S}$ 624.24144, found 624.24128.

Benzyl (phenyl 3-O-benzyl-2-O-(2-naphthylmethyl)-1-thio- α -D-mannopyranosyl uronate) (49) 4,6-O-

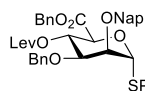


benzylidene-1-thio- α -D-mannopyranoside (3.6 g, 10 mmol) was dissolved in toluene and Bu_2SnO (2.54 g, 10.2 mmol, 1.02 eq.) was added. The mixture was refluxed for 1.5 h, after the mixture was concentrated and coevaporated with toluene, and redissolved in DMF (50 mL).

Benzyl bromide (1.31 mL, 11.0 mmol, 1.1 eq.) and CsF (1.55 g, 10.2 mmol, 1.0 eq.) were added and the mixture was stirred overnight at RT. After overnight stirring, H_2O was added, the mixture was diluted with EtOAc and washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. Column purification (PE/EtOAc, 8:1 $\rightarrow$ 1:1) yielded alcohol intermediate (3.96 g, 8.8 mmol, 88%). The compound was coevaporated twice with toluene and dissolved in DMF (50 mL) and cooled to 0°C . The mixture was treated with sodium hydride (60% dispersion in mineral oil, 0.42 g, 10.6 mmol, 1.2 eq.), and, after 10 minutes, with Naphthyl bromide (2.14 g, 9.7 mmol, 1.1 eq.). After stirring overnight, the reaction was quenched by addition of H_2O . The mixture was diluted with Et_2O , washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. Purification by column chromatography yielded protected intermediate (4.43 g, 7.5 mmol, 86%). The mannoside was dissolved in MeOH/DCM (25 mL : 25 mL) and pTsOH (0.084 g, 0.44 mmol, 0.05 eq.) was added. After conversion to a lower running spot, the reaction was neutralized with Et_3N , concentrated and purified by column purification (PE/EtOAc, 9:1 $\rightarrow$ 1:1) yielding diol **46** (3.17 g, 6.5 mmol, 84%). Diol **46** (3.16 g, 6.3 mmol, 1.0 eq.) was dissolved in EtOAc/ H_2O /tBuOH (4:4:1, 40 mL) and cooled to 0°C . AcOH (0.04 mL, 0.7 mmol, 0.1 eq.) was added followed by addition of TEMPO (0.21 g, 1.3 mmol, 0.2 eq.) and BAIB (5.39 g, 16.2 mmol, 2.5 eq.) and the reaction was stirred vigorously for 230 min. at which it was allowed to warm up to RT. TLC analysis showed conversion of the starting material to a low running spot, and the reaction was quenched with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$. The mixture was diluted with EtOAc and the layers were separated. The aqueous layer was acidified and extracted with EtOAc. The combined organic layers were washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. The resulting oil was coevaporated twice with anhydrous toluene and dissolved in DMF (50 mL) and cooled to 0°C . Benzyl bromide (1.5 mL, 12.4 mmol, 2 eq.) was added, followed by K_2CO_3 (1.72 g, 12.4 mmol, 2 eq.). The reaction was stirred over night, after which it was quenched with H_2O . The mixture was diluted with Et_2O and washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. Column purification (PE/EtOAc, 10:1 $\rightarrow$ 5:1) yielded the title compound (2.12 g, 3.5 mmol, 56%). TLC: R_f 0.30 (PE/EtOAc, 4/1, v/v); ^1H NMR (400 MHz, $\text{CDCl}_3/\text{CDCl}_3$): δ 7.88 – 7.65 (m, 5H, CH_{arom}), 7.58 – 7.37 (m, 6H, CH_{arom}), 7.37 – 7.27 (m, 7H, CH_{arom}), 7.25 – 7.11 (m, 4H, CH_{arom}), 5.64 (d, 1H, $J = 2.4$ Hz, H-1), 5.30

– 5.08 (m, 2H, CH₂ Bn/Nap), 4.93 – 4.53 (m, 5H, 2xCH₂ Bn/Nap, H-5), 4.47 (t, 1H, *J* = 8.7 Hz, H-4), 3.99 (t, 1H, *J* = 2.7 Hz, H-2), 3.73 (dd, 1H, *J* = 8.9, 3.0 Hz, H-3); ¹³C NMR (101 MHz, CDCl₃): δ 169.8 (C=O), 138.0 (Cq), 135.3, 135.2, 133.6, 133.3, 133.1 (Cq), 131.8 (CH_{arom}), 129.1, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 126.9, 126.2, 126.0 (CH_{arom}), 86.1 (C-1), 78.3 (C-3), 75.7 (C-2), 72.8 (C-5), 72.7 (CH₂ Bn/Nap), 72.5 (CH₂ Bn/Nap), 68.7 (C-4), 67.3 (CH₂ Bn). HRMS: [M+Na]⁺ calcd. for C₃₇H₃₄O₆SNa 629.19683, found 629.19619.

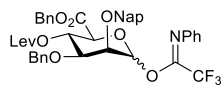
Benzyl (phenyl 3-O-benzyl-4-O-levulinoyl-2-O-(2-naphthylmethyl)-1-thio- α -D-mannopyranosyl uronate) (51)



Compound **49** (2.13 g, 3.5 mmol, 1.0 eq.) was coevaporated with toluene twice, dissolved in DCM (15 mL) and cooled to 0°C. LevOH (0.84 mL, 8.2 mmol, 2.3 eq), DIC (0.6 mL, 3.9 mmol, 1.2 eq) and DMAP (0.04 g, 0.33 mmol, 0.1 eq) were added and the reaction was stirred

at RT overnight. After overnight stirring, the mixture was filtrated over Celite® and concentrated *in vacuo*. The residue was dissolved in EtOAc and washed with sat. aq. NaHCO₃ twice, dried over MgSO₄ and concentrated *in vacuo*. Column chromatography (PE/EtOAc 7/1 → 7/3) yielded compound **51** (2.40 g, 3.4 mmol, 97%) as a yellow oil. TLC: R_f 0.26 (PE/EtOAc, 7/3 v/v); ¹H NMR (500 MHz, CDCl₃, T=328K): δ 7.84 – 7.64 (m, 4H, CH_{arom}), 7.58 – 7.36 (m, 7H, CH_{arom}), 7.35 – 7.15 (m, 11, CH_{arom}), 5.79 (d, 1H, *J* = 7.0 Hz, H-1), 5.60 (t, 1H, *J* = 5.4 Hz, H-4), 5.09 – 4.93 (m, 2H, CH₂ Bn/Nap), 4.83 – 4.64 (m, 2H, CH₂ Bn/Nap), 4.57 (d, 1H, *J* = 4.7 Hz, H-5), 4.54 – 4.51 (m, 2H, CH₂ Bn/Nap), 3.87 (dd, 1H, *J* = 6.0, 2.6 Hz, H-3), 3.80 (dd, 1H, *J* = 6.9, 2.3 Hz, H-2), 2.52 (m, 2H, CH₂ Lev), 2.44 – 2.28 (m, 2H, CH₂ Lev), 2.09 (s, 3H, CH₃ Lev); ¹³C NMR (126 MHz, CDCl₃, T=328K): δ 205.8 (C=O Lev), 171.4 (C=O lev), 168.1 (C=O Bn), 137.8 (Cq), 135.4, 135.3, 133.9, 133.4, 133.3 (Cq), 131.6 (CH_{arom}), 131.4, 129.0, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.2, 127.0, 126.9, 126.2, 126.0 (CH_{arom}), 88.8 (C-1), 74.6 (C-2), 74.4 (C-3), 73.1 (C-5), 72.8 (CH₂ Bn/Nap), 72.7 (CH₂ Bn/Nap), 70.2 (C-4), 67.4 (CH₂ Bn), 37.9 (CH₂ Lev), 29.7 (CH₃ Lev), 28.1 (CH₂ Lev). HRMS: [M+Na]⁺ calcd. for C₄₂H₃₆O₆SNa 727.23361, found 727.23328.

Benzyl (3-O-benzyl-4-O-levulinoyl-3-O-(2-naphthylmethyl)-O-(N-phenyl-trifluoroacetimidoyl)- α / β -D-mannopyranosyl uronate) (55)

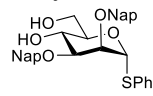


Compound **51** (1.40 g, 2.0 mmol, 1.0 eq.) was coevaporated with toluene twice, dissolved in dry DCM (20 mL) and cooled to 0°C. NIS (0.50 g, 2.2 mmol, 1.1 eq) and TFA (0.17 mL, 2.2 mmol, 1.1 eq) were added and

the reaction mixture was stirred at 0°C for 15 minutes after which it was allowed to warm up to RT. After 1h, full conversion was observed by TLCMS (masses: 635, product and 731 product + TFA), the reaction was quenched with sat. aq. Na₂S₂O₃ (20 mL) followed by addition of sat. aq. NaHCO₃ (20 mL) and the reaction was stirred for 1 h during which it turned from deep purple to yellow. The mixture was diluted with Et₂O, washed with sat. aq. NaHCO₃, dried over MgSO₄, concentrated *in vacuo* and purified by column chromatography (PE/EtOAc 3/1 to 1/1) which yielded the hemiacetal (1.16 g, 1.9 mmol, 95%) as a yellow oil. Compound **53** (1.16 g, 1.9 mmol, 1.0 eq.) was dissolved in acetone (20 mL) and cooled to 0°C, followed by addition of Cs₂CO₃ (0.78 g, 2.4 mmol, 1.2 eq) and ClC(=NPh)CF₃ (0.48 mL, 3.0 mmol, 1.5 eq) and the reaction was stirred at 0°C for 1.5 h. The mixture was diluted with EtOAc and washed with sat. aq. NaCl (2x), dried over MgSO₄, concentrated *in vacuo* and then purified using column chromatography (PE/EtOAc, 4/1 → 1/1) which yielded compound **55** (1.18 g, 1.5 mmol, 77%) as a yellow oil. TLC: R_f 0.41 (PE/EtOAc, 2/1 v/v); ¹H NMR (500 MHz, CDCl₃, T=328K): δ 7.86 – 7.61 (m, 4H, CH_{arom}), 7.49 – 7.38 (m, 3H, CH_{arom}), 7.31 – 7.16 (m, 12H, CH_{arom}), 7.06 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.68 (d, 2H, *J* = 7.5 Hz, CH_{arom}), 6.47 (s, 1H, H-1), 5.65 (t, 1H, *J* = 7.1 Hz, H-4), 5.16 – 5.00 (m, 2H, CH₂ Bn/Nap), 4.89 – 4.72 (m, 2H, CH₂ Bn/Nap), 4.64 – 4.49 (m, 2H, CH₂ Bn/Nap), 4.43 (d, 1H, *J* = 6.8 Hz, H-5), 3.91 (dd, 1H, *J* = 7.4, 2.8 Hz, H-3), 3.84 (t, 2H, *J* = 3.7, 3.1 Hz, H-2), 2.71 – 2.27 (m, 4H, 2xCH₂ Lev), 2.08 (s, 3H, CH₃ Lev); ¹³C NMR (126 MHz, CDCl₃, T=328K): δ 205.7 (C=O Lev), 171.3 (C=O Lev), 167.5 (C=O Bn), 143.4 (Cq), 142.6, 142.3, 137.7, 135.4,

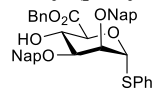
135.2, 135.1, 133.3, 133.2 (Cq), 129.1 (CH_{arom}), 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.5, 127.0, 126.4, 126.2, 126.1, 126.0, 124.4, 124.2, 119.5 (CH_{arom}), 94.8 (C-1), 75.0 (C-3), 73.4 (C-2), 73.3 (C-5), 73.0 (2xCH₂ Bn/Nap), 69.2 (C-4), 67.6 (CH₂ Bn), 37.8 (CH₂ Lev), 29.6 (CH₃ Lev), 28.1 (CH₂ Lev). HRMS: [M+Na]⁺ calcd. for C₄₄H₄₀F₃NO₉Na 806.25474, found 806.25496.

Phenyl 2,3-O-di-(2-naphthylmethyl)-1-thio- α -D-mannopyranoside (44) 4,6-O-benzylidene-1-thio- α -D-

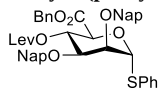


mannopyranoside **43** (8.65 g, 24 mmol) was coevaporated twice with anhydrous toluene before being dissolved in DMF (15 mL). The mixture was cooled to 0°C, after which sodium hydride (60% dispersion in mineral oil, 2.4 g, 60 mmol) was added. The mixture was stirred for 10 minutes followed by addition of 2-naphthylmethylbromide (13.27 g, 60 mmol). When, after overnight stirring, TLC analysis showed complete consumption of the starting material, the reaction was quenched with sat. aq. NaHCO₃. The mixture was diluted with Et₂O and the organic layer was washed with H₂O, dried over MgSO₄ and concentrated *in vacuo*. After a quick column purification/filtration, the crude was dissolved in MeOH/DCM (60 mL : 60 mL) and the mixture was treated with pTsoH (0.5 g, 2.4 mmol, 0.1 eq.). After overnight stirring, the reaction was neutralized with Et₃N and concentrated. Column purification (PE/EtOAc, 4:1 → 1:1) yielded the diol (12.03 g, 21.8 mmol, 91%). TLC: R_f 0.43 (PE/EtOAc, 1/1, v/v); IR (neat): 817, 1039, 1103, 1344, 1508, 2873, 3053, 3354 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.88 – 7.62 (m, 8H, CH_{arom}), 7.53 – 7.31 (m, 8H, CH_{arom}), 7.28 – 7.17 (m, 3H, CH_{arom}), 5.54 (s, 1H, H-1), 4.81 (d, 1H, *J* = 12.3 Hz, CHH), 4.74 – 4.62 (m, 3H, CH₂, CHH), 4.23 – 4.15 (m, 1H, H-4), 4.16 – 4.08 (m, 1H, H-5), 4.07 (dd, 1H, *J* = 3.0, 1.6 Hz, H-2), 3.94 – 3.81 (m, 2H, 2x H-6), 3.77 (dd, 1H, *J* = 9.4, 3.0 Hz, H-3), 2.67 (s, 2H, 2x OH); ¹³C NMR (126 MHz, CDCl₃): δ 135.2, 135.2, 133.9, 133.4, 133.3, 133.2 (Cq), 132.1, 132.1, 129.3, 128.6, 128.5, 128.1, 128.1, 127.9, 127.8, 127.0, 126.9, 126.4, 126.3, 126.2, 126.2, 126.1, 125.8 (CH_{arom}), 86.3 (C-1), 79.9 (C-3), 75.7 (C-2), 73.5 (C-5), 72.5, 72.1 (CH₂), 67.5 (C-4), 62.9 (C-6); HRMS: [M+NH₄]⁺ calcd. for C₃₄H₃₆NO₅S 570.23087, found 570.23102.

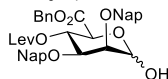
Benzyl (phenyl 2,3-O-di-(2-naphthylmethyl)-1-thio- α -D-mannopyranosyl uronate) (48) Diol **45** (9.23 g, 16.7



mmol, 1.0 eq.) was dissolved in tBuOH/DCM/H₂O (4:4:1, 152 mL) and cooled to 0°C. TEMPO (3.34 g, 0.52 mmol, 0.2 eq.) and BAIB (13.45 g, 41.75 mmol, 2.5 eq.) were added and the reaction was stirred vigorously for 255 min. at which it was allowed to warm up to RT. TLC analysis showed conversion of the starting material to a low running spot, and the reaction was quenched with sat. aq. Na₂S₂O₃. The mixture was diluted with EtOAc and the layers were separated. The aqueous layer was acidified and extracted with EtOAc. The combined organic layers were washed with sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. The resulting oil was coevaporated twice with anhydrous toluene and dissolved in DMF (150 mL). Benzyl bromide (3.97 mL, 33.4 mmol, 2 eq.) was added, followed by K₂CO₃ (4.62 g, 33.4 mmol, 2 eq.). The reaction was stirred over night, after which it was quenched with H₂O. The mixture was diluted with Et₂O and washed with sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (PE/EtOAc, 4:1 → 1:1) yielded the title compound (7.41 g, 11.28 mmol, 68%). TLC: R_f 0.59 (PE/EtOAc, 3/1, v/v); IR (neat) 817, 1026, 1099, 1271, 1693, 1747, 2866, 3057, 3423 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.91 – 7.58 (m, 10H, CH_{arom}), 7.55 – 7.35 (m, 10H, CH_{arom}), 7.26 – 7.06 (m, 4H, CH_{arom}), 5.64 (d, 1H, *J* = 2.1 Hz, H-1), 5.32 – 5.08 (m, 2H, CH₂ Bn), 4.89 – 4.77 (m, 2H, CH₂ Nap), 4.75 – 4.62 (m, 3H, CH₂ Nap, H-5), 4.51 (t, 1H, *J* = 8.9 Hz, H-4), 4.00 (t, 1H, *J* = 2.6 Hz, H-2), 3.80 (dd, 1H, *J* = 8.9, 3.0 Hz, H-3); ¹³C NMR (100 MHz, CDCl₃): δ 169.8 (C=O), 135.5 (Cq), 135.2, 135.2, 133.6, 133.3, 133.2, 133.1 (Cq), 131.8 (CH_{arom}), 129.1, 128.7, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 126.9, 126.7, 126.2, 126.0, 125.9 (CH_{arom}), 86.1 (C-1), 78.4 (C-3), 75.7 (C-2), 72.9 (CH₂ Nap), 72.8 (C-5), 72.5 (CH₂ Nap), 68.7 (C-4), 67.3 (CH₂ Bn). HRMS: [M+Na]⁺ calcd. for C₄₁H₃₆O₆SN 674.21248, found 674.25715.

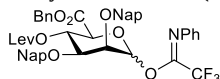
Benzyl (phenyl 4-O-levulinoyl-2,3-O-di-(2-naphthylmethyl)-1-thio- α -D-mannopyranosyl uronate) (50)

Compound **47** (7.41 g, 11.3 mmol) was coevaporated twice with anhydrous toluene before being dissolved in DCM (30 mL). The mixture was cooled to 0°C, after which LevOH (3.22 mL, 31.6 mmol, 2.8 eq.), DIC (2.47 mL, 15.8 mmol, 1.4 eq.) and DMAP (0.14 g, 1.13 mmol, 0.1 eq.) were added and the reaction was stirred overnight. The mixture was filtered over Celite, washed with sat. aq. NaHCO₃ and sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (PE/EtOAc, 5:1 $\rightarrow$ 2:1) yielded the title compound (6.96 g, 9.22 mmol, 82%). TLC: R_f 0.36 (PE/EtOAc, 3/1, v/v); IR (neat): 896, 1082, 1151, 1361, 1716, 1743, 2870, 2916, 3057 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.80 – 7.51 (m, 10H, CH_{arom}), 7.47 – 7.11 (m, 14H, CH_{arom}), 5.87 (d, 1H, *J* = 6.6 Hz, H-1), 5.65 (t, 1H, *J* = 5.2 Hz, H-4), 5.03 (d, 1H, *J* = 12.2 Hz, CHH), 4.97 (d, 1H, *J* = 12.2 Hz, CHH), 4.75 (d, 1H, *J* = 12.1 Hz, CHH), 4.69 – 4.57 (m, 4H, CHH, CH₂, H-5), 3.89 (dd, 1H, *J* = 5.8, 2.8 Hz, H-3), 3.80 (d, 1H, *J* = 5.7 Hz, H-2), 2.53 – 2.21 (m, 4H, 2x CH₂ Lev), 2.04 (s, 3H, CH₃ Lev); ¹³C NMR (126 MHz, CDCl₃, T=328K): δ 205.8 (C=O Lev), 171.4 (C=O lev), 168.1 (C=O), 135.4, 135.3, 133.9, 133.5, 133.4, 133.3 (Cq), 131.7, 131.4, 129.0, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.3, 127.0, 126.9, 126.2, 126.1, 126.0 (CH_{arom}), 83.5 (C-1), 74.7 (C-2), 74.4 (C-3), 73.2 (C-5), 72.8 (CH₂ Nap), 70.2 (C-4), 67.4 (CH₂ Bn), 37.9 (CH₂ Lev), 29.7 (CH₃ Lev), 28.1 (CH₂ Lev). HRMS: [M+H]⁺ calcd. for C₄₆H₄₃O₈S 755.26732, found 755.26848.

Benzyl (4-O-levulinoyl-2,3-O-di-(2-naphthylmethyl)- α / β -D-mannopyranosyl uronate) (52)

Compound **50** (6.96 g, 9.22 mmol) was dissolved in DCM (90 mL) and cooled to 0°C. NIS (2.3 g, 10.1 mmol, 1.1 eq.) was added, followed by addition of TFA (0.78 mL, 10.1 mmol, 1.1 eq.). After 225 min., piperidine (3.0 mL, 30.4 mmol, 3.3 eq.) was added and the mixture was stirred for 30 min.,

allowing to warm up to RT. The mixture was diluted with DCM, and the organic layer was washed with 1M HCl, H₂O and sat. aq. NaCl. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Column purification (PE/EtOAc, 4:1 $\rightarrow$ 1:1) yielded the hemiacetal as a white foam (4.89 g, 7.38 mmol, 80%). TLC: R_f 0.33 (PE/EtOAc, 3/1, v/v); IR (neat): 1031, 1153, 1361, 1716, 1747, 2872, 2924, 3055, 3458 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.81 – 7.59 (m, 8H, CH_{arom}), 7.49 – 7.31 (m, 6H, CH_{arom}), 7.30 – 7.15 (m, 5H, CH_{arom}), 5.65 – 5.57 (m, 2H, H-1, H-4), 5.07 (d, 1H, *J* = 12.2 Hz, CHH), 4.95 (d, 1H, *J* = 12.2 Hz, CHH), 4.87 – 4.68 (m, 2H, CH₂), 4.67 – 4.55 (m, 3H, CH₂, OH), 4.52 (d, 1H, *J* = 5.6 Hz, H-5), 3.96 (dd, 1H, *J* = 6.6, 2.9 Hz, H-3), 3.69 (dd, 1H, *J* = 4.9, 2.9 Hz, H-2), 2.49 – 2.22 (m, 4H, 2x CH₂ Lev), 2.01 (s, 3H, CH₃ Lev); ¹³C NMR (101 MHz, CDCl₃): δ 206.5 (C=O Lev), 171.6, 168.8, (2x C=O), 135.6, 135.3, 135.1, 135.0, 134.8, 133.2, 133.1, 133.1, 133.0, 132.9 (Cq), 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.2, 126.7, 126.6, 126.5, 126.3, 126.1, 126.1, 126.0, 126.0, 125.9, 125.8, 125.7 (CH_{arom}), 92.5 (C-1), 75.2 (C-2, C-3), 72.9, 72.4 (CH₂), 71.7 (C-5), 69.5 (C-4), 67.4 (CH₂), 37.6 (CH₂ Lev), 29.7 (CH₃ Lev), 27.9 (CH₂ Lev); HRMS: [M+NH₄]⁺ calcd. for C₄₀H₄₂NO₉ 680.28541 found 680.28550.

Benzyl (4-O-levulinoyl-2,3-O-di-(2-naphthylmethyl)-O-(N-phenyl-trifluoroacetimidoyl)- α / β -D-mannopyranosyl uronate) (54)

Hemiacetal **52** (4.82 g, 7.27 mmol) was dissolved in acetone and cooled to 0°C. The mixture was treated with ClC(=NPh)CF₃ (1.35 mL, 8.73 mmol, 1.2 eq.) followed by addition of Cs₂CO₃ (3.55 g, 10.91 mmol, 1.5 eq.). The

reaction was stirred for 6 h, when TLC analysis showed complete reaction, and the mixture was diluted with EtOAc, washed with H₂O, dried over MgSO₄ and concentrated *in vacuo*. Column purification (PE/EtOAc, 4:1 $\rightarrow$ 1:1) yielded the imide donor (4.93 g, 5.91 mmol, 81%). TLC: R_f 0.43 (PE/EtOAc, 3/1, v/v); IR (neat): 819, 1043, 1151, 1205, 1597, 1714, 1747, 2872, 2922, 3055 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, T=328K): δ 7.84 – 7.59 (m, 9H), 7.49

– 7.34 (m, 6H), 7.29 – 7.14 (m, 6H), 7.05 (t, 1H, $J = 7.2$ Hz), 6.60 (d, 2H, $J = 7.3$ Hz), 6.48 (s, 1H), 5.69 (t, 1H, $J = 7.1$ Hz), 5.13 – 5.04 (m, 2H), 4.84 – 4.69 (m, 3H), 4.65 (d, 1H, $J = 12.2$ Hz), 4.44 (d, 1H, $J = 6.7$ Hz), 3.96 (dd, 1H, $J = 7.2, 2.8$ Hz), 3.85 – 3.81 (m, 1H), 2.61 – 2.32 (m, 4H, 2x CH₂ Lev), 2.05 (s, 3H, CH₃ Lev); ¹³C NMR (126 MHz, CDCl₃): δ 205.8 (C=O Lev), 171.4, 167.5 (2x C=O), 143.4, 142.7, 142.4, 135.3, 135.2, 135.1, 133.4, 133.4, 133.3, 133.3 (Cq), 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.1, 128.1, 127.8, 127.1, 127.0, 126.3, 126.2, 126.2, 126.1, 126.1, 126.0, 125.6, 124.5, 124.2, 119.5 (CH_{arom}), 94.9 (C-1), 75.1 (C-3), 73.5 (C-2), 73.4 (C-5), 73.2 (CH₂), 73.1 (CH₂), 69.2 (C-4), 67.6 (CH₂), 37.8 (CH₂ Lev), 29.7 (CH₃ Lev), 28.1 (CH₂ Lev); HRMS: [M+Na]⁺ calcd. C₄₈H₄₂F₃NO₉Na 856.27039, found 856.27040.

Benzyl (5-azido-pentyl-4-O-Levulinoyl-2,3-O-di-(2-naphthylmethyl)-β-D-mannopyranosyl uronate) (56)

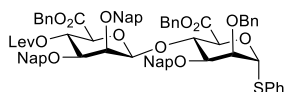
Compound **54** (0.164 g, 0.20 mmol) and 5-azidopentanol (0.039 g, 0.30 mmol, 1.5 eq) were coevaporated with toluene under argon three times, dissolved in dry DCM (2 mL), activated molsieves 3 Å were added and the reaction was stirred under an argon atmosphere at RT for 30 min. After 30 min the reaction was cooled to -40 °C, followed by addition of TMSOTf (0.09 mL of a 0.1M TMSOTf/DCM, 0.2 eq.) and the reaction was stirred at -40 °C for 1.5 hours. The reaction was quenched by addition of Et₃N (0.2 mL) at -40 °C and was allowed to warm to RT. The mixture was diluted with EtOAc, washed with sat. aq. NaHCO₃, sat. aq. NaCl, dried over MgSO₄, and concentrated *in vacuo*. Column purification (PE/EtOAc 3/1 to 1/1) yielded product **56** (0.124 g, 0.16 mmol 79%) as a white solid. TLC: R_f 0.61 (toluene/EtOAc, 4/1, v/v); ¹H NMR (400 MHz, CDCl₃): δ 7.90 – 7.53 (m, 8H, CH_{arom}), 7.53 – 7.19 (m, 11H, CH_{arom}), 5.64 (t, 1H, $J = 9.4$ Hz, H-4), 5.27 – 4.94 (m, 4H, 2x CH₂ Nap/ Bn), 4.71 – 4.46 (m, 2H, CH₂ Nap/ Bn), 4.41 (s, 1H, H-1), 3.98 (m, 1H, CHH Linker), 3.92 (d, 1H, $J = 2.4$ Hz, H-2), 3.90 (d, 1H, $J = 9.5$ Hz, H-5), 3.51 (dd, 1H, $J = 9.5, 2.8$ Hz, H-3), 3.46 – 3.33 (m, 1H, CHH Linker), 3.21 (m, 2H, CH₂ Linker), 2.65 – 2.19 (m, 4H, 2x CH₂ Lev), 2.05 (s, 3H, CH₃ Lev), 1.85 – 1.52 (m, 4H, 2xCH₂ Linker), 1.52 – 1.35 (m, 1H, CHH Linker), 1.25 (t, $J = 7.1$ Hz, 1H, CHH Linker); ¹³C NMR (101 MHz, CDCl₃): δ 206.3 (C=O Lev), 171.5 (C=O Lev), 167.5 (C=O Bn), 136.0 (Cq), 135.3, 133.1 (Cq), 128.7 (CH_{arom}), 128.6, 128.5, 128.2, 128.0, 127.9, 127.8, 127.2, 126.8, 126.4, 126.2, 126.0, 125.9, 125.8 (CH_{arom}), 101.1 (C-1), 78.3 (C-3), 73.8 (CH₂ Bn/Nap), 73.6 (C-5), 73.0 (C-2), 71.9 (CH₂ Bn/Nap), 70.1 (CH₂ Linker), 69.2 (C-4), 67.5 (CH₂ Bn/Nap), 51.4 (CH₂ Linker), 37.8 (CH₂ Linker), 29.9 (CH₃ Lev), 29.2 (CH₂ Lev), 28.7 (CH₂ Lev), 28.0 (CH₂ Linker), 23.4 (CH₂ Linker). HRMS: [M+Na]⁺ calcd. for C₄₅H₄₇N₃O₉Na 796.32045, found 796.31981.

Benzyl (5-azido-pentyl-2,3-O-di-(2-naphthylmethyl)-β-D-mannopyranosyl uronate) (57)

Compound **56** (0.155 g, 0.20 mmol) was co-evaporated with toluene twice under an argon atmosphere, dissolved pyridine/AcOH (2 mL, 4:1) and H₂NNH₂·AcOH (0.092 g, 1.0 mmol, 5 eq.) was added. The reaction was stirred for 1 hour at RT, after which it was quenched by addition of acetone. The mixture was diluted with EtOAc, washed with 1M HCl (aq), dried over MgSO₄ and concentrated *in vacuo* followed by column chromatography (PE/EtOAc 3/1 to 2/1) which resulted in product **57** (0.128 g, 0.19 mmol, 99%) as a white solid. TLC: R_f 0.81 (PE/EtOAc, 1/1, v/v); ¹H NMR (400 MHz, CDCl₃): δ 7.89 – 7.55 (m, 9H, CH_{arom}), 7.51 – 7.20 (m, 10H, CH_{arom}), 5.35 – 5.18 (m, 2H, CH₂ Bn/Nap), 5.19 – 4.91 (m, 2H, CH₂ Bn/Nap), 4.65 (s, 2H, CH₂ Bn/Nap), 4.47 – 4.36 (m, 2H, H-1, H-4), 4.05 – 3.93 (m, 1H, CHH Linker), 3.94 – 3.90 (m, 1H, H-3), 3.79 (d, 1H, $J = 9.6$ Hz, H-5), 3.51 – 3.33 (m, 2H, H-2, CHH Linker), 3.21 (t, 2H, $J = 6.6$ Hz, CH₂ Linker), 1.62 (m, 4H, 2xCH₂ Linker), 1.45 (dd, 1H, $J = 13.8, 6.0$ Hz, CHH Linker), 1.26 (s, 1H, CHH Linker); ¹³C NMR (101 MHz, CDCl₃): δ 169.1 (C=O), 136.1 (Cq), 135.4, 133.3 (Cq), 128.7 (CH_{arom}), 128.5, 128.4, 128.3, 127.9, 127.8, 127.2, 126.7, 126.5, 126.2, 126.0, 125.9, 125.7 (CH_{arom}), 102.3 (C-1), 80.3 (C-3), 75.3 (C-5), 74.3

(CH₂ Bn/Nap), 73.6 (C-2), 72.1 (CH₂ Bn/Nap), 70.0 (CH₂ Linker), 68.5 (C-4), 67.3 (CH₂ Bn/Nap), 51.4 (CH₂ Linker), 29.3 (CH₂ Linker), 28.7 (CH₂ Linker), 23.4 (CH₂ Linker). HRMS: [M+Na]⁺ calcd. for C₄₀H₄₁N₃O₇Na 698.28367, found 698.28314.

Benzyl (phenyl-4-O-[benzyl 4-O-levulinoyl-2,3-O-di-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-

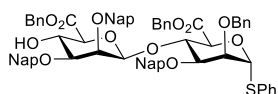


2-O-benzyl-3-O-(2-naphthylmethyl)-1-thio-α-D-mannopyranosyl uronate)

(59) Donor **54** (2.527 g, 3.03 mmol, 1.0 eq.) and acceptor **48** (2.02 g, 3.33 mmol, 1.1 eq.) were coevaporated 3 times under an argon atmosphere. The compounds were dissolved in dry DCM (30 mL) and stirred on activated molecular sieves

for 30 min. The mixture was cooled to -55°C and TMSOTf (6.1 mL of 0.1M TMSOTf/DCM, 0.2 eq.) was added. After 110 min., TLC: indicated complete reaction and the reaction was quenched with Et₃N (1 mL) and allowed to warm up to RT. The mixture was diluted with DCM, washed with sat. aq. NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. Column purification (hexanes/EtOAc, 6:1 → 2:1) yielded the disaccharide (2.696 g, 2.154 mmol, 72%). TLC: R_f 0.42 (PE/EtOAc, 5/2, v/v); IR (neat) 740, 1026, 1058, 1151, 1363, 1716, 1747, 2868, 3057 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.84 – 7.00 (m, 36H, CH_{arom}), 5.80 (d, 1H, *J* = 7.8 Hz, H-1), 5.64 (t, 1H, *J* = 9.3 Hz, H-4'), 5.08 – 4.81 (m, 6H, 3x CH₂), 4.75 – 4.49 (m, 7H, H-1', H-4, H-5, 2x CH₂), 4.38 (s, 2H, CH₂), 4.23 (s, 1H, H-3), 3.91 (s, 1H, H-2'), 3.89 – 3.79 (m, 2H, H-2, H-5'), 3.48 (d, 1H, *J* = 9.2 Hz, H-3'), 2.54 – 2.27 (m, 4H, 2x CH₂ Lev), 2.00 (s, 3H, CH₃ Lev); ¹³C NMR (126 MHz, CDCl₃): δ 205.7 (C=O Lev), 171.3, 169.0, 167.1 (3x C=O), 138.3, 137.9, 136.0, 135.8, 135.4, 135.3, 135.3, 134.1, 133.4, 133.3, 133.1, 133.1 (Cq), 131.5, 131.3, 131.2, 128.9, 128.9, 128.7, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.1, 126.9, 126.9, 126.8, 126.7, 126.6, 126.5, 126.4, 126.3, 126.2, 126.1, 126.0, 126.0, 125.9, 125.8, 125.7, 125.6 (CH_{arom}), 101.1 (C-1'), 83.0 (C-1), 78.4 (C-3'), 76.8 (C-4), 75.2 (C-3'), 75.1 (C-2), 74.2 (CH₂), 74.2 (C-2'), 73.8 (C-5), 73.7 (C-5'), 73.3 (CH₂), 72.1, 72.0 (CH₂), 69.3 (C-4'), 67.4, 67.1 (CH₂), 37.8 (CH₂ Lev), 29.6 (CH₃ Lev), 28.1 (CH₂ Lev); HRMS: [M+NH₄]⁺ calcd. C₇₇H₇₄O₁₄SN 1268.48245, found 1268.48297.

Benzyl (phenyl-4-O-[Benzyl 2,3-O-di-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-2-O-benzyl-3-O-(2-naphthylmethyl)-1-thio-α-D-mannopyranosyl uronate) (60)

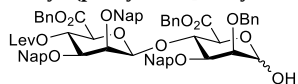


(60) Disaccharide **59** (1.263 g, 1.0 mmol, 1.0 eq.) was dissolved in pyridine/AcOH (10 mL, 4:1) and cooled to 0°C, followed by addition of H₂NNH₂·AcOH (0.46 g, 5 mmol, 5.0 eq.). The reaction was quenched with acetone after 85 min, when TLC analysis

showed complete conversion of the starting material into a higher running spot. The mixture was diluted with EtOAc, washed with aq. 1M HCl, H₂O and sat. aq. NaCl. Column purification (hexanes/EtOAc, 6:1 → 2:1) yielded the title compound (1.098 g, 0.952 mmol, 95%). TLC: R_f 0.65 (PE/EtOAc, 2/1, v/v); IR (neat): 902, 1062, 1122, 1454, 1730, 1743, 2862, 2924, 3053 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.88 – 7.55 (m, 14H, CH_{arom}), 7.54 – 7.28 (m, 14H, CH_{arom}), 7.26 – 7.02 (m, 23H, CH_{arom}), 5.78 (d, 1H, *J* = 8.1 Hz, H-1), 5.14 (d, 1H, *J* = 12.3 Hz, CHH), 5.08 (d, 1H, *J* = 12.3 Hz, CHH), 4.94 (d, *J* = 12.3 Hz, 1H, CHH), 4.89 (d, 1H, *J* = 12.2 Hz, CHH), 4.82 (m, 2H, 2x CHH), 4.70 (m, 3H, CH₂, CHH), 4.62 – 4.52 (m, 5H, H-1, H-4, H-5, CH₂), 4.43 – 4.35 (m, 3H, H-4', CH₂), 4.22 – 4.18 (m, 1H, H-3), 3.90 (d, 1H, *J* = 2.8 Hz, H-2'), 3.83 – 3.73 (m, 2H, H-2, H-5'), 3.35 (dd, 1H *J* = 9.4, 2.9 Hz, H-3'), 3.11 (s, 1H, 4-OH); ¹³C NMR (126 MHz, CDCl₃, T=328K): δ 169.0, 169.0 (2x C=O), 138.0, 136.2, 135.8, 135.6, 135.3, 135.3, 134.1, 133.4, 133.4, 133.2, 133.2, 133.2 (Cq), 131.3, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.0, 126.9, 126.7, 126.5, 126.4, 126.2, 126.1, 126.1, 126.0, 126.0, 125.9, 125.8, 125.7, 101.9 (C-1'), 82.9 (C-1), 80.6 (C-3'), 76.9 (C-5), 75.6 (C-3), 75.5 (C-5'), 75.2 (C-2), 74.7 (C-

2'), 74.7 (CH₂), 74.1 (C-4), 73.4 (CH₂), 72.4 (CH₂), 72.2 (CH₂), 68.5 (C-4'), 67.4, 67.2; HRMS: [M+NH₄]⁺ calcd. C₇₂H₆₈NO₁₂S 1170.44567, found 1170.44656.

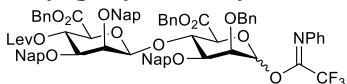
Benzyl (phenyl-4-O-[benzyl 2,3-O-di-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-2-O-benzyl-3-O-(2-naphthylmethyl)-α/β-D-mannopyranosyl uronate) (61)



To a solution of compound **59** (1.798 g, 1.436 mmol, 1.0 eq.) in DCM (15 mL) at 0°C, was added NIS (0.36 g, 1.580 mmol, 1.1 eq.) and TFA (0.12 mL, 1.580 mmol, 1.1

eq.) after which the reaction mixture turned dark purple. After 3h, the reaction was quenched with Et₃N (0.4 mL), 15 mL sat. aq. Na₂S₂O₃ was added and the reaction was stirred for 30 min. after which the color changed from purple to colorless. The mixture was diluted with DCM and washed with sat. aq. NaHCO₃. The aqueous layer was extracted 2x with DCM, and the combined organic layers were washed with sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (hexanes/EtOAc) yielded the hemiacetal (1.41 g, 1.22 mmol, 84%). TLC: R_f 0.33 (PE/EtOAc, 3/1, v/v); IR (neat): 731, 1055, 1122, 1361, 1716, 1747, 2875, 3030, 3057 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.86 – 7.57 (m, 12H), 7.49 (dd, 1H, *J* = 8.5, 1.8 Hz), 7.47 – 7.02 (m, 23H), 5.68 – 5.43 (m, 2H), 5.11 – 4.75 (m, 6H), 4.75 – 4.42 (m, 8H), 4.20 (dd, 1H, *J* = 5.4, 2.8 Hz), 3.91 (d, 1H, *J* = 2.9 Hz), 3.82 (d, 1H, *J* = 9.2 Hz), 3.44 (dd, 1H, *J* = 9.4, 3.0 Hz), 3.23 (d, 1H, *J* = 5.3 Hz), 2.63 – 2.22 (m, 4H), 2.00 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 205.9, 171.4, 169.3, 167.2, 138.5, 136.2, 136.1, 135.9, 135.5, 135.5, 135.4, 133.5, 133.4, 133.4, 133.2, 133.2, 133.1, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.7, 127.5, 126.9, 126.8, 126.8, 126.7, 126.6, 126.6, 126.5, 126.4, 126.4, 126.3, 126.2, 126.1, 126.1, 126.0, 126.0, 125.9, 125.9, 125.8, 125.8, 125.7, 125.6, 101.3, 101.1, 94.1, 93.0, 92.9, 78.6, 78.4, 78.1, 76.8, 76.8, 76.4, 75.9, 75.5, 74.7, 74.5, 74.5, 74.4, 74.3, 73.7, 73.7, 73.4, 73.3, 73.1, 72.8, 72.5, 72.5, 72.4, 72.1, 72.0, 70.1, 69.3, 67.4, 67.3, 67.1, 60.4, 37.9, 37.9, 29.7, 28.2, 21.0, 14.3; HRMS: [M+NH₄]⁺ calcd. C₇₁H₇₀NO₁₅ 1176.47400, found 1176.47406.

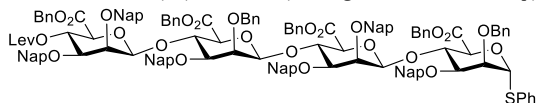
Benzyl (phenyl-4-O-[benzyl 2,3-O-di-(2-naphthylmethyl)-β-D-mannopyranosyl uronate]-2-O-benzyl-3-O-(2-naphthylmethyl)-O-(N-phenyl-trifluoroacetimidoyl)-α/β-D-mannopyranosyl uronate) (62)



Hemiacetal **61** (0.871 g, 0.751 mmol, 1.0 eq.) was dissolved in acetone and cooled to 0°C. The mixture was

treated with ClC(=NPh)CF₃ (0.14 mL, 0.901 mmol, 1.2 eq.) followed by addition of Cs₂CO₃ (0.367 g, 1.126 mmol, 1.5 eq.). The reaction was stirred for 110 min., after which it was diluted with EtOAc, washed with H₂O, dried over MgSO₄ and concentrated *in vacuo*. Column purification (PE/EtOAc, 6:1 → 2:1) yielded the imidate donor (0.696 g, 0.522 mmol, 70%). TLC: R_f 0.53 (PE/EtOAc, 2/1, v/v); IR: 732, 817, 1051, 1151, 1205, 1323, 1597, 1716, 1749, 2873, 3034, 3059 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.83 – 7.59 (m, 14H), 7.51 (dd, 1H, *J* = 8.5, 1.6 Hz), 7.49 – 6.97 (m, 30H), 6.68 (d, 2H, *J* = 7.8 Hz), 6.47 (s, 1H), 5.63 (t, 1H, *J* = 9.3 Hz), 5.09 – 4.83 (m, 7H), 4.75 – 4.37 (m, 10H), 4.22 (dd, 1H, *J* = 5.8, 2.8 Hz), 3.90 (d, 1H, *J* = 2.8 Hz), 3.88 – 3.79 (m, 2H), 3.45 (dd, 1H, *J* = 9.4, 2.8 Hz), 2.60 – 2.22 (m, 4H), 2.03 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 205.8, 171.4, 168.6, 167.9, 167.5, 167.2, 167.2, 143.7, 143.4, 143.0, 142.7, 138.4, 138.1, 137.9, 136.1, 135.9, 135.5, 135.5, 135.4, 135.3, 135.2, 135.2, 135.1, 133.4, 133.4, 133.2, 133.2, 128.8, 128.7, 128.6, 128.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.1, 127.0, 126.9, 126.7, 126.6, 126.4, 126.4, 126.3, 126.2, 126.2, 126.1, 126.1, 126.0, 125.9, 125.8, 125.8, 125.7, 124.4, 124.3, 119.7, 119.5, 115.1, 101.6, 101.5, 78.6, 76.7, 76.1, 75.9, 75.1, 74.8, 74.5, 74.4, 74.4, 74.4, 74.4, 73.8, 73.6, 73.5, 73.5, 73.4, 73.2, 73.1, 72.8, 72.6, 72.2, 69.3, 69.2, 67.6, 67.5, 67.4, 67.3, 67.1, 60.4, 53.4, 37.9, 37.8, 29.7, 29.6, 28.2, 28.1, 21.0, 14.3; HRMS: [M+NH₄]⁺ calcd. C₇₉H₇₄F₃N₂O₁₅ 1347.50358, found 1347.50430.

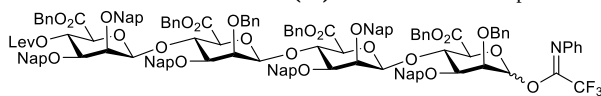
Tetrasaccharide (63) Donor **62** (0.527 g, 0.396 mmol, 1.0 eq.) and acceptor **60** (0.502 g, 0.440 mmol, 1.1 eq.) were



coevaporated 3 times with anhydrous toluene under an N₂ atmosphere. The mixture was dissolved in DCM (4 mL) and stirred on activated molecular sieves for 30 min. at RT. The mixture

was cooled to -65°C and treated with TMSOTf (0.8 mL of 0.1M TMSOTf/DCM, 0.2 eq.). When TLC analysis indicated complete consumption of the donor, the reaction was quenched with Et₃N (0.3 mL) and allowed to warm up to RT. The mixture was diluted with DCM, washed with sat. aq. NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. Purification by size exclusion chromatography (LH20, eluted with DCM/MeOH, 1/1, v/v), yielded the title tetrasaccharide as a white foam (0.617 g, 0.269 mmol, 68%). TLC: R_f 0.52 (PE/EtOAc, 5/4, v/v); IR (neat): 742, 1026, 1103, 1153, 1361, 1720, 1747, 2875, 2926, 3034, 3055 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.88 – 6.94 (m, 77H), 5.77 (d, 1H, J = 7.3 Hz), 5.54 (t, 1H, J = 9.6 Hz), 5.05 – 4.23 (m, 38H), 4.15 (s, 1H), 3.97 – 3.55 (m, 8H), 3.55 – 3.18 (m, 3H), 2.61 – 2.14 (m, 4H), 1.99 (s, 3H); ¹³C NMR (126 MHz, CDCl₃): δ 205.8 (C=O Lev), 171.4, 169.1, 168.3, 168.0, 167.3 (5x C=O), 139.2, 138.1, 136.8, 136.6, 136.6, 136.2, 135.9, 135.7, 135.5, 135.4, 135.3, 135.3, 134.2, 133.5, 133.5, 133.4, 133.2, 133.2, 133.1, 133.1, 133.0 (Cq), 131.3, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.3, 126.9, 126.8, 126.6, 126.5, 126.4, 126.3, 126.3, 126.2, 126.1, 126.1, 126.0, 126.0, 125.9, 125.9, 125.9, 125.9, 125.7, 125.7, 125.7, 125.6 (CH_{arom}), 102.3, 102.2, 101.1 (3x C-1), 83.1 (C-1), 79.3, 79.1, 78.8, 78.0, 77.8, 76.5, 76.4, 75.5, 75.4, 74.7, 74.5, 74.3, 73.7, 73.3, 73.1, 72.8, 72.3, 71.9, 69.3 (C-4'''), 67.2, 67.1, 67.1, 66.9, 37.9 (CH₂ Lev), 29.6 (CH₃ Lev), 28.1 (CH₂ Lev); HRMS: [M+H]⁺ calcd. C₁₄₃H₁₂₉O₂₆S 2294.85210, found 2294.85237.

Tetrasaccharide imidate donor (65) To a solution of compound **63** (0.345 g, 0.156 mmol, 1.0 eq.) in DCM (1.5

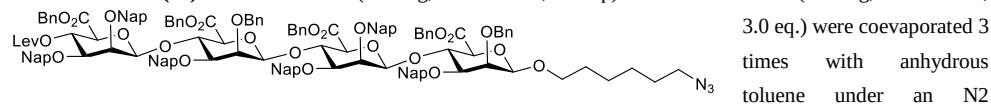


mL) at 0°C, was added NIS (0.04 g, 0.180 mmol, 1.2 eq.) and TFA (0.014 mL, 0.180 mmol, 1.2 eq.) after which the reaction

mixture turned dark purple. After 35 min, the reaction was quenched with Et₃N (0.04 mL), 3 mL sat. aq. Na₂S₂O₃ was added and the reaction was stirred for 30 min. after which the color changed from purple to colorless. The mixture was diluted with DCM and washed with sat. aq. NaHCO₃. The aqueous layer was extracted 2x with DCM, and the combined organic layers were washed with sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (hexanes/EtOAc) yielded the hemiacetal as a difficult to concentrate foam. R_f 0.30 (PE/EtOAc, 2/1, v/v); HRMS: [M+NH₄]⁺ calcd. C₁₃₇H₁₂₈NO₂₇ 2219.87020, found 2219.87251. The hemiacetal (**63**) was dissolved in acetone (1 mL) and cooled to 0°C. Then, ClC(=NPh)CF₃ (0.03 mL, 0.18 mmol, 1.2 eq.) was added, followed by Cs₂CO₃ (0.074 g, 0.23 mmol, 1.5 eq.), after which the reaction was allowed to stir overnight. TLC analysis indicated complete conversion of the hemiacetal, after which the mixture was diluted with EtOAc, washed with H₂O and sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (Hexanes/EtOAc, 4:1 → 1:1) yielded the imidate donor (0.298 g, 0.125 mmol, 83% over 2 steps). R_f 0.57 (PE/EtOAc, 2/1, v/v); IR (neat): 752, 1157, 1212, 1365, 1724, 1745, 2849, 3059 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, T=328K): δ 7.89 – 6.94 (m, 96H, CH_{arom}), 6.67 (d, 2H J = 7.5 Hz), 6.46 (s, 1H, H-1), 5.54 (t, 1H, J = 9.7 Hz, H-4'''), 5.04 – 4.24 (m, 39H), 4.24 – 4.13 (m, 1H), 3.86 – 3.74 (m, 5H), 3.74 – 3.56 (m, 4H), 3.56 – 3.39 (m, 2H), 3.36 – 3.24 (m, 2H), 2.51 – 2.18 (m, 4H, 2x CH₂ Lev), 2.00 (s, 3H, CH₃ Lev); ¹³C NMR (126 MHz, CDCl₃, T=328K): δ 205.9 (C=O Lev), 171.4, 168.6, 168.3, 168.0, 167.4 (5x C=O), 143.8, 143.0, 142.7, 139.3, 138.0, 136.8, 136.7, 136.6, 136.3, 135.9, 135.7,

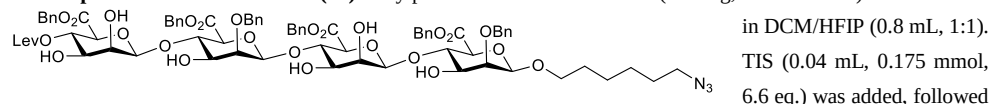
135.5, 135.5, 135.3, 135.2, 133.5, 133.5, 133.5, 133.5, 133.2, 133.2, 133.1, 133.1 (Cq), 129.4, 129.2, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 126.8, 126.8, 126.7, 126.5, 126.4, 126.4, 126.3, 126.2, 126.1, 126.1, 126.1, 126.0, 126.0, 126.0, 125.9, 125.9, 125.8, 125.7, 125.7, 124.3, 119.7 (CH_{arom}), 102.3, 102.3, 101.5 (3x C-1), 95.0 (C-1), 79.3, 79.3, 78.8, 78.0, 77.8, 76.5, 76.4, 75.6, 75.5, 75.4, 75.1, 74.7, 74.4, 74.3, 73.7, 73.6, 73.2, 72.8, 72.1, 71.9 (4x C-2, 4x C-3, 3x C-4, 4x C-5, 9x CH₂), 69.3 (C-4'''), 67.2, 67.1, 66.9 (CH₂), 37.9 (CH₂ Lev), 29.7 (CH₃ Lev), 28.2 (CH₂ Lev); HRMS: [M+Na]⁺ calcd. C₁₄₅H₁₂₈F₃NO₂₇Na 2395.85518, found 2395.85657.

Tetrasaccharide (66) Imidate donor **65** (0.298 g, 0.125 mmol, 1.0 eq.) and 6-azidoheptanol (0.054 g, 0.375 mmol,



3.0 eq.) were coevaporated 3 times with anhydrous toluene under an N₂ atmosphere. The mixture was dissolved in DCM (1 mL) and stirred on activated molecular sieves for 30 min. The mixture was cooled to -50°C, followed by addition of TMSOTf (0.25 mL of 0.1M TMSOTf/DCM, 0.2 eq.). The reaction was stirred for 80 min., quenched with Et₃N and allowed to warm up to RT. The mixture was diluted with DCM, washed with sat. aq. NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. Purification by size exclusion chromatography (LH-20, eluted with DCM/MeOH, 1/1, v/v) followed by column purification (Hexanes/EtOAc) yielded the tetrasaccharide (0.197 g, 0.085 mmol, 68%). TLC: R_f 0.6 (PE/EtOAc, 2/1, v/v); IR (neat): 749, 819, 1059, 1124, 1274, 1363, 1455, 1746, 2095, 2933, 3056 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.83 – 6.95 (m, 72H), 5.47 (t, 1H, J = 9.8 Hz), 5.08 – 4.18 (m, 38H), 3.92 – 3.44 (m, 16H), 3.43 – 3.36 (m, 1H), 3.36 – 3.12 (m, 7H), 2.48 – 2.27 (m, 4H), 2.02 (s, 3H), 1.59 (s, 8H), 1.42 – 1.27 (m, 7H); ¹³C NMR (126 MHz, CDCl₃): δ 206.2, 171.5, 168.2, 168.2, 168.1, 167.9, 167.3, 139.1, 138.7, 136.8, 136.6, 136.5, 136.5, 136.2, 135.5, 135.3, 133.4, 133.3, 133.1, 133.0, 132.9, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.5, 127.3, 126.7, 126.6, 126.4, 126.3, 126.3, 126.2, 126.1, 126.0, 125.9, 125.8, 125.8, 125.7, 125.6, 102.5, 102.2, 102.0, 79.0, 78.4, 77.8, 77.7, 76.0, 75.1, 75.0, 74.8, 74.6, 73.8, 73.4, 73.1, 72.9, 72.8, 72.3, 71.6, 70.1, 69.0, 67.2, 67.0, 66.9, 66.7, 51.4, 37.8, 29.8, 29.6, 29.5, 28.9, 28.8, 28.0, 26.6, 25.7; HRMS: [M+Na]⁺ calcd. C₁₄₃H₁₃₅N₃O₂₇Na 2349.92086, found 2349.92411.

Semi deprotected tetrasaccharide (67) Fully protected tetrasaccharide **66** (0.062 g, 0.0266 mmol) was dissolved

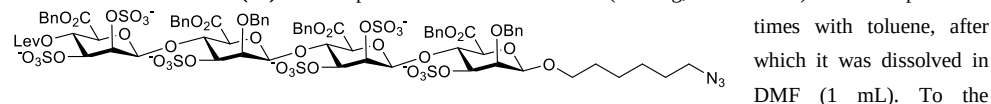


in DCM/HFIP (0.8 mL, 1:1). TIS (0.04 mL, 0.175 mmol, 6.6 eq.) was added, followed by addition of HCl/HFIP (0.33 mL of 0.2M HCl/HFIP, 2.5 eq.). After 100 min., an additional portion HCl/HFIP (0.13 mL of 0.2M HCl/HFIP, 1.0 eq.) was added and the reaction was quenched with sat. aq. NaHCO₃ (4 mL) after 30 min. The mixture was diluted with DCM, and the layers were separated. The aqueous layer was extracted with DCM and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. Column purification (Hexanes/DCM 2:1 → 1:1 → DCM/MeOH 15:1) yielded the semi deprotected tetrasaccharide (0.0372 g, 0.025 mmol, 94%). TLC: R_f 0.61 (DCM/MeOH, 9/1, v/v); IR (neat): 698, 751, 1041, 1104, 1364, 1455, 1744, 2096, 2933, 3475 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.37 – 7.27 (m, 30H, CH_{arom}), 5.26 – 4.99 (m, 8H, 4x CH₂), 4.87 (d, J = 12.3 Hz, 1H, CHH), 4.80 (s, 1H, H-1), 4.75 – 4.59 (m, 3H, CHH, CH₂), 4.49 – 4.36 (m, 2H), 4.22 – 4.13 (m, 2H), 4.05 – 3.57 (m, 9H), 3.50 – 3.27 (m, 6H), 3.23 (t, J = 6.9 Hz, 2H, linker CH₂), 2.71 – 2.22 (m, 4H, 2x CH₂ Lev), 2.13 (s, 3H, CH₃ Lev), 1.60 – 1.49 (m, 4H), 1.43 – 1.11 (m, 6H); ¹³C NMR (126 MHz, CDCl₃): δ 206.8 (C=O Lev), 172.3, 169.1, 167.9, 167.4, 167.3 (C=O), 138.8, 138.7, 135.5, 135.0, 135.0, 134.9, 134.9, 134.8 (Cq), 129.4, 129.2,

Chapter 5

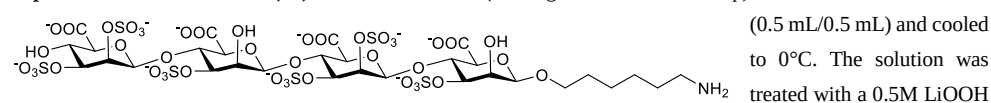
129.1, 128.9, 128.9, 128.8, 128.7, 128.4, 128.2, 128.1, 128.0, 127.7, 127.7 (CH_{arom}), 102.1, 101.2, 100.5, 99.4 (4x C-1), 80.3, 79.3, 79.1, 76.6, 76.1 (4x C-3, C-5), 75.1 (CH₂), 74.0 (CH₂), 73.5, 73.4, 72.4, 72.2, 71.9, 71.5, 70.6, 70.1, 69.7, 69.4, 69.4 (4x C-4, 4x C-2, 3x C-5), 68.3 (CH₂), 68.1 (CH₂), 68.0 (CH₂), 67.8 (CH₂), 67.3 (CH₂), 51.5 (CH₂), 38.1 (CH₂ Lev), 29.9 (CH₃ Lev), 29.3 (CH₂), 28.9 (CH₂), 28.0 (CH₂), 26.6 (CH₂), 25.8 (CH₂); HRMS: [M+NH₄]⁺ calcd. C₇₇H₉₁N₄O₂₇ 1503.58652, found 1503.58865.

Sulfated tetrasaccharide (68) Semi deprotected tetrasaccharide **67** (0.024 g, 0.016 mmol) was coevaporated 3



solution, Et₃N·SO₃ (0.09 g, 0.49 mmol, 30 eq.) was added and the solution was heated to 55°C. The septum was replaced with a stopper and sealed, after which the reaction was stirred at 55°C overnight. The reaction was cooled to 0°C, and quenched with NaHCO₃ (0.045 g, 0.54 mmol, 33 eq.) in 1 mL H₂O, and the mixture was stirred for 1h after which the solvents were removed *in vacuo* at 22°C. The crude compound was taken up in a small volume of DCM/MeOH and applied on a Sephadex LH-20 column and eluted with DCM/MeOH (1:1), resulting in the sulfated tetrasaccharide (0.0304 g, 0.012 mol, 74%); IR (neat): 699, 750, 807, 1040, 1217, 1365, 1455, 1747, 2096, 2942, 3474 cm⁻¹; ¹H NMR (500 MHz, MeOD) δ 7.63 – 7.12 (m, 30H, CH_{arom}), 5.39 – 4.92 (m, 17H), 4.82 – 3.91 (m, 15H), 3.63 – 3.38 (m, 6H), 3.21 (t, J = 6.9 Hz, 2H), 3.14 (q, J = 7.3 Hz, 36H, 18x CH₂ Et₃N), 2.75 – 2.27 (m, 4H, 2x CH₂ Lev), 2.10 (s, 3H, CH₃ Lev), 1.64 – 1.44 (m, 4H, CH₂ linker), 1.31 (d, J = 8.8 Hz, 6H, CH₂ linker), 1.24 (t, J = 7.3 Hz, 54H, 18x CH₃ Et₃N); ¹³C NMR (126 MHz, MeOD) δ 209.2 (C=O Lev), 173.4 (C=O Lev), 169.9, 169.9, 169.8, 169.7 (4x CO₂Bn), 140.5, 137.4, 136.6 (Cq), 129.8, 129.6, 129.6, 129.5, 129.4, 129.1, 128.3 (CH_{arom}), 100.8, 100.7, 100.5, 100.5 (4x C-1), 78.5, 77.7, 77.5, 77.4, 76.7, 76.6, 75.8, 73.7, 68.7, 68.7, 68.4, 68.3, 68.2, 68.2, 52.4, 49.5, 49.3, 49.2, 49.0, 48.8, 48.7, 48.5, 47.8, 38.6, 30.7, 29.9, 29.6, 29.2, 27.6, 26.7, 9.3. ESI-MS: m/z [M+2H]⁴⁺ calc. for C₇₇H₈₃N₃O₄₅S₆ 490.8, found 490.8.

Deprotected tetrasaccharide (69) Tetrasaccharide **68** (0.031 g, 0.012 mmol, 1.0 eq.) was dissolved in H₂O/THF



solution (0.6 mL, 5.0 eq. per ester) and the reaction was allowed to stir overnight during which at warmed up to RT. After overnight stirring, the reaction was neutralized with 1M HCl and concentrated at 25°C. The crude was purified by gel filtration (HW40 eluted with NH₄HCO₃) and passed through a Dowex Na⁺ column. NMR analysis indicated removal of the Lev and several benzyl esters. The tetrasaccharide was dissolved in H₂O/THF/tBuOH (3 mL, 3:1.3:1.3), 2-3 drops of AcOH were added and the solution was purged with N₂ for 5 minutes. Pd(OH)₂/C (20 mg) was added, after which the solution was purged with N₂ for 5 minutes, H₂ for 5 minutes after which the reaction was kept under a H₂ atmosphere overnight. The mixture was passed through a Whatmann filter, rinsed several times with H₂O/THF/tBuOH and H₂O, after which it was concentrated at 25°C. The compound was purified by gel filtration (HW40 eluted with NH₄HCO₃), lyophilized 4 times and passed through a Dowex Na⁺ column. (2.22 mg, 1.46 μmol, 12% over 2 steps). ¹H NMR (D₂O, 850 MHz): δ 5.08 – 5.02 (m, 2H), 4.98 (s, 1H), 4.90 (s, 1H), 4.58 – 4.48 (m, 5H), 4.40 – 4.33 (m, 4H), 4.18 – 4.01 (m, 6H), 3.99 – 3.92 (m, 3H), 3.89 – 3.81 (m, 3H), 3.67 (s, 2H), 3.15 – 3.08 (m, 2H), 3.03 – 2.92 (m, 2H), 1.76 – 1.70 (m, 2H), 1.70 – 1.57 (m, 4H), 1.40 (s, 3H); ¹³C NMR (D₂O, 214 MHz): δ 173.7, 173.7, 100.6, 100.5, 78.4, 78.3, 77.7, 77.4, 77.4, 77.0, 76.7, 76.5, 76.5, 76.3, 75.9, 75.8, 75.5, 74.7, 69.6, 67.3, 62.7, 45.5, 40.3, 29.1, 27.4, 25.9, 23.1, 22.4.

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Chapter 6

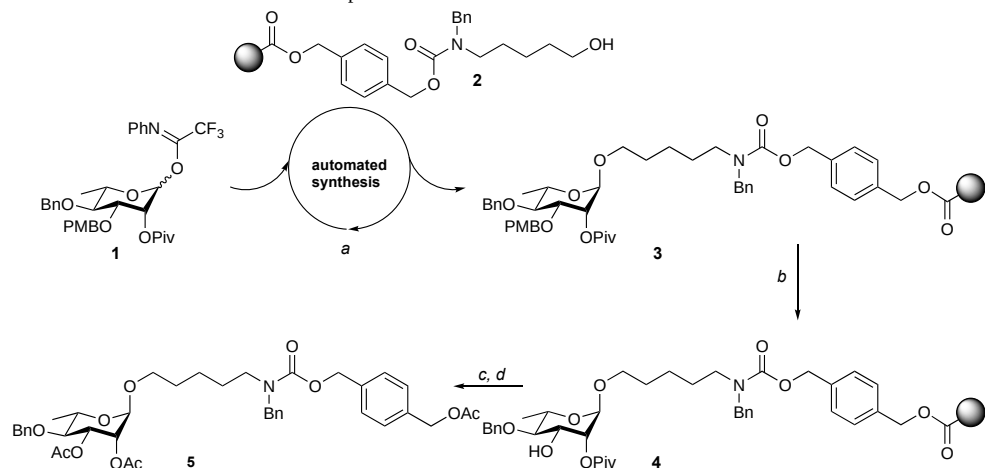
Summary & future prospects

Chapter 6

The chemical synthesis of complex oligosaccharides can provide well-defined carbohydrate fragments which are essential for the in depth understanding of glycochemistry/glycobiology. The success of a synthesis campaign relies on the protecting group pattern. The stereochemical outcome of the glycosylation, the introduction of certain functionalities, e.g. amines or sulphates, and the ease of deprotection all depend on the correct set of protecting groups. In Chapter 1, an overview on how protecting groups influence carbohydrate synthesis is presented. A wide spectrum of subjects are highlighted, from mechanistic explanations of glycosylations to deprotection of complex oligosaccharides.

In Chapter 2, a new method for the chemoselective removal of *para*-methoxybenzyl- and naphthylmethylethers is described. It is shown that a catalytic amount of hydrochloric acid in hexafluoroisopropanol can be used to rapidly cleave *para*-methoxybenzyl (PMB) and naphthylmethyl (Nap) ethers. The scope and limitations of the method have been tested by the selective removal of PMB groups on several carbohydrate building blocks in the presence of other acid labile functionalities, such as Nap ethers and commonly used silyl ethers. For the removal of Nap ethers, a scavenger (triethylsilane, TES) was required but also the removal of this functional group could be achieved using a catalytic amount of acid. The developed method proved to be essential for the synthesis of sulfated mannuronic acids described in Chapter 5.

The possibility to use the PMB group as a temporary protecting group in automated solid phase oligosaccharide assembly should be considered, given the fact that PMB groups can be cleaved rapidly using only a catalytic amount of HCl. In addition the method has already found application in solid phase peptide and DNA synthesis.^{1,2} Of note, the use of Nap-ethers as temporary protecting groups in automated solid phase oligosaccharide has been reported, but the cleavage of these groups required 7 cycles of an oxidative treatment with DDQ in a DCE/MeOH/H₂O (64:16:1) mixture to allow for complete removal.³ In an initial approach, shown in Scheme 1, a single PMB group is removed from a resin bound rhamnose fragment. After cleavage of the monosaccharide from the solid support, with concomitant removal of the Piv group - a phenomenon also reported on in Chapter 4 - and ensuing acetylation, the linker equipped rhamnoside **5** was obtained in 53% yield.

Scheme 1. HCl/HFIP test case on solid phase.

Reagents and conditions: a) 3 eq. 1, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; b) HCl/HFIP, DCM, 3 cycles; c) NaOMe/MeOH, DCM, 2 cycles; d) Ac₂O, pyridine, 0°C (53% over 4 steps from resin 2).

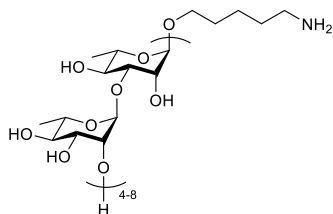
These results indicate that the PMB can be applied on solid phase as temporary protecting group. A disadvantage encountered in the test case described above is the loss of the Piv group. As the Piv-group was completely cleaved, it is impossible to establish whether any migration had occurred during the cleavage of the PMB group. An extra elongation step to provide a disaccharide will provide more insight in the applicability of the PMB group. The use of a nucleophilic scavenger (such as TES) has to be investigated as well.

In Chapter 3, a new pivaloyl-based protecting group is designed and synthesized. The cyanopivaloyl (PivCN) group, with one of the methyl groups substituted for a cyanomethyl moiety, features the characteristics of the conventional Piv group and acts as an effective participating group. However, the PivCN group can be removed under mild hydrogenation conditions: transformation of the cyano group into the corresponding primary amine, leads to intramolecular attack on the ester carbonyl to produce a gamma lactam and liberate the alcohol. The normal Piv group requires harsh deprotection conditions, which can harm other parts of the molecule and present a challenge at the end of the synthesis. The PivCN group proved its strength in the synthesis of a hexarhamnan that represents a fragment of the capsular polysaccharide of *Enterococcus faecium* and a tetrarhamnan that is part of the polyrhamnose backbone of the Lancefield Group A carbohydrate (GAC).

In Chapter 4 two new dirhamnoside imidate donors were synthesized and used in automated solid phase oligosaccharide synthesis. The donors were equipped with a Piv or a PivCN group at the C-2-OH and applied to construct bacterial polyrhamnose fragments. A new automated carbohydrate synthesizer was used and its methods were optimized. Global deprotection of the Piv groups proved to be impossible, after which fragments were synthesized bearing the PivCN group. After base mediated cleavage of the fragments from

the solid support, partial removal of the PivCN group was observed. Prolonged and repetitive cleavage cycles, with a catalytic amount of base, resulted in complete removal of all PivCN groups from the oligosaccharides. Multimilligram quantities of rhamnose fragments, up to the hexadecasaccharide level, were obtained after global deprotection.

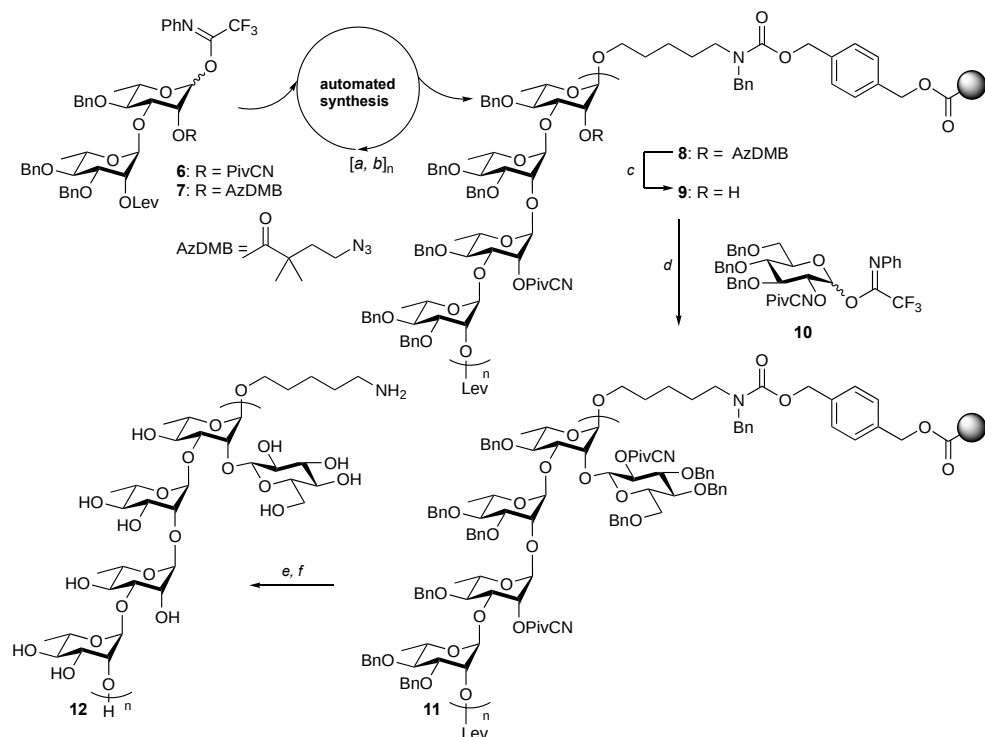
Figure 1. Structures synthesized in Chapter 4.



The success of the automated synthesis of the oligorhamnans combined with the PivCN and the previously developed 2,2-dimethyl-4-azido-butanoate (AzDMB) and 2,2-dimethyl-4-(4-methoxy-phenoxy)butanoate (MPDMB),⁴ invites a combination of these protecting groups in the automated solid phase assembly of various oligosaccharides. The applicability of the PivCN in a solid phase setting is shown in Chapter 4, and the AzDMB has already been successfully applied in the automated construction of short β -1,3 glucans.⁵ The AzDMB can be removed using phosphine based reagents under mild conditions as previously shown.⁴ As described in Chapter 4, the Group A *Streptococcus* (GAS) polyrhamnose backbone is decorated on the rhamnosyl C-3 OH with *N*-acetyl glucosamine. In the case of *Streptococcus mutans*, a polyrhamnose backbone is decorated at certain C-2 OH positions with a glucose moiety, while *S. flexneri* carries an *N*-acetyl glucosamine on the rhamnose C-2 OH on a polyrhamnose backbone.⁶ Synthetic fragments of these polysaccharides may be useful in the generation of semi-synthetic vaccines directed at these bacteria.

A possible route towards oligorhamnose fragments, decorated on specific hydroxyls with relevant carbohydrate fragments, is shown in Scheme 2. The potential donors **6** and **7**, are equipped with AzDMB and Lev as temporary, orthogonal, protecting groups, and can be used to access a variety of bacterial rhamnose targets. The first priority would be to test the orthogonality of the AzDMB to the PivCN, during on-resin deprotection conditions. To this end, the disaccharide donors **6** and **7** can be combined and the automated synthesis depicted in Scheme 2 of a rhamnose fragment (for example $n=1$ as test substrate) will provide insight in the mutual applicability of both protecting groups. The deprotection of the AzDMB by reduction of the azide can be achieved with PMe_3 and KOH. It has to be established whether these mildly basic conditions affect the PivCN group. Once liberated, the C-2 hydroxyl may be glycosylated by a glucose donor. Cleavage from the solid support followed by hydrogenolysis then results in branched saccharide **12**.

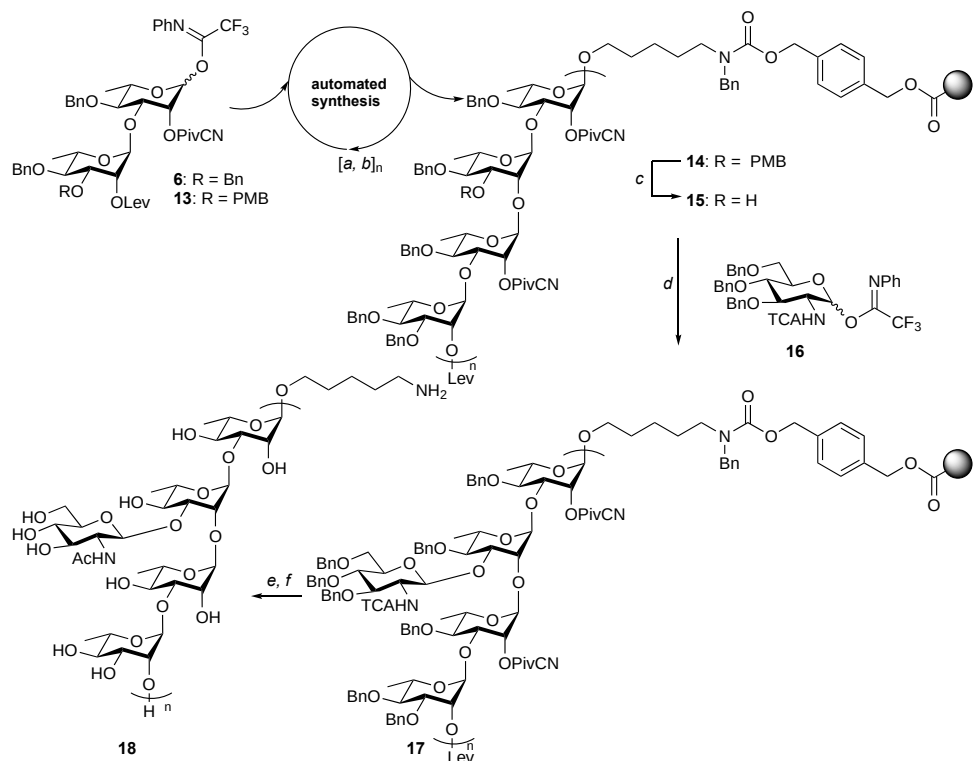
Scheme 2.



Reagents and conditions: a) 3 eq. **6** or **7**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; b) 8 eq. $\text{H}_2\text{NNH}_2 \cdot \text{AcOH}$, pyr/AcOH, 40°C, 3 cycles; c) PMe_3 , KOH, THF/ H_2O ; d) 3 eq. **10**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; e) NaOMe, MeOH/THF; f) H_2 , Pd(OH) $_2$ /C, AcOH, H_2O /THF/tBuOH.

To broaden the pallet of orthogonal protecting groups the use of an PMB-ether may be attractive as an addition. The automated synthesis of a branched rhamnoside with donors **6** and **13**, featuring a PMB or Bn on the C-3"-OH, may be achieved using similar synthesis methods as described before (Scheme 3). The oligosaccharide can be treated with HCl/HFIP, to remove the C-3-O-PMB group, liberating the C-3 hydroxyl, creating a new branching position. Glycosylation with a glucosamine donor, followed by cleavage from the solid support and subsequent hydrogenolysis, yields a branched saccharide **18**, which represents part of Group A *Streptococcus* capsular polysaccharide.

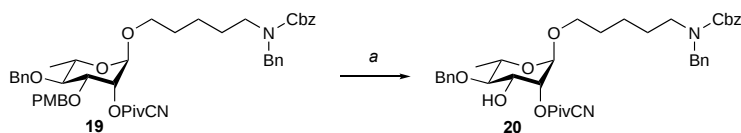
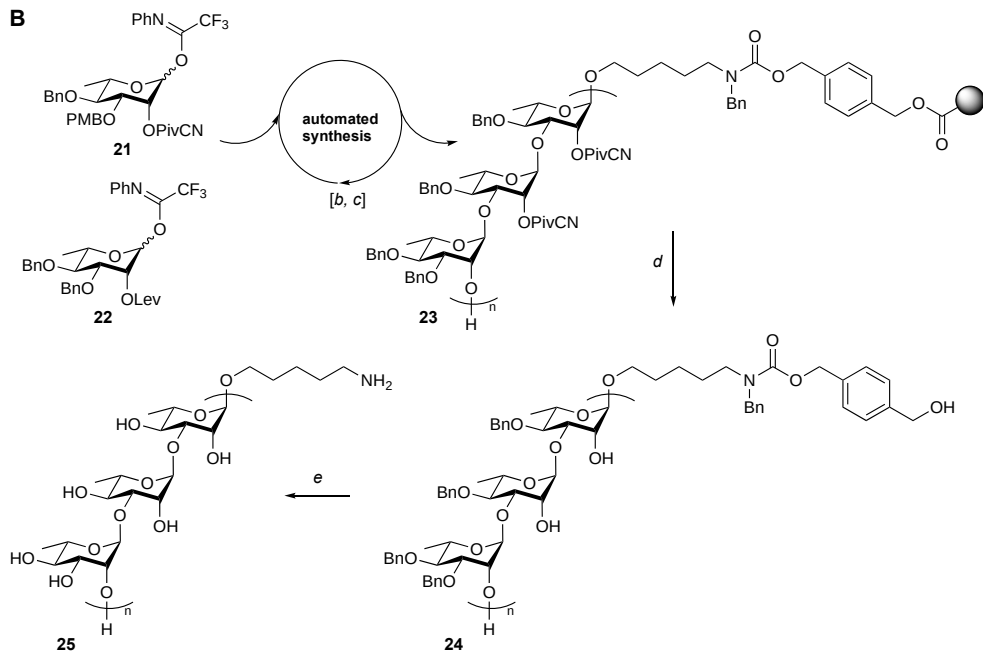
Scheme 3.



Reagents and conditions: a) 3 eq. **16** or **13**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; b) 8 eq. H₂NNH₂·AcOH, pyr/AcOH, 40°C, 3 cycles; c) HCl/HFIP, TES, HFIP/DCM; d) 3 eq. **16**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; e) NaOMe, MeOH/THF; f) H₂, Pd(OH)₂/C, AcOH, H₂O/THF/tBuOH.

These new donors combined with the on-resin deprotection methods could result in a streamlined access to libraries of well-defined, bacterial polyrhamnose fragments. These fragments should be within reach with the above described chemistry. The automated assembly of the *Enterococcus faecium* oligorhamnosides, of which the solution phase synthesis is described in Chapter 3, may be undertaken as well, as shown in Scheme 4B. In Chapter 3 it was found that the PivCN group can migrate during the removal of the Lev-ester. Substitution of the Lev for a PMB could overcome this problem, giving access to the [α-1,3-α-1,3-α-1,2]-rhamnosides. Preliminary, solution phase results have proven the possibility to remove the 3-O-PMB with catalytic acid, in the presence of a 2-O-PivCN (Scheme 4A).

Scheme 4.

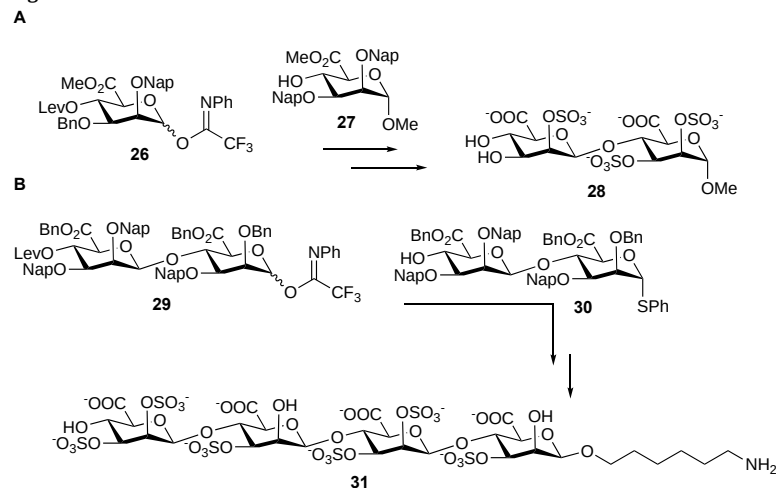
A**B**

Reagents and conditions: a) HCl/HFIP, HFIP/DCM (76%); b) 3 eq. **21** or **22**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; then: c) 8 eq. $\text{H}_2\text{NNH}_2 \cdot \text{AcOH}$, pyr/AcOH, 40°C, 3 cycles; or HCl/HFIP, TES, HFIP/DCM, 3 cycles; d) NaOMe, MeOH/THF; e) H_2 , $\text{Pd}(\text{OH})_2/\text{C}$, AcOH, $\text{H}_2\text{O}/\text{THF}/\text{tBuOH}$.

In Chapter 5 a study towards the synthesis of sulfated mannuronic acid fragments is described. A series of donors and acceptors was constructed and tested in glycosylation studies (Figure 3A). Thereafter, the Nap groups were removed under oxidative conditions. These conditions resulted in complex mixtures and a new deprotection method was explored. The method described in Chapter 2 was applied which, after optimization, resulted in a fast and efficient removal of the Nap groups. The sulfation- and deprotection conditions were optimized, resulting in a sulfated ManA disaccharide **28**. Next, the donors were redesigned to allow for the generation of longer oligosaccharides and these were tested in glycosylation studies. The reactivity of the β -configured spacer containing mannuronic acid acceptor alcohols proved to be too low to allow for production of the oligosaccharides from the reducing end. An alternative approach was developed in which a tetrasaccharide was built up from the non-reducing end, and transformed into an imide

donor, which could then be coupled efficiently to the primary alcohol spacer (Figure 3B). Sulfation and deprotection resulted in the sulfated tetrasaccharide **31**. Unfortunately, the saccharide was not obtained in pure enough form.

Figure 2.

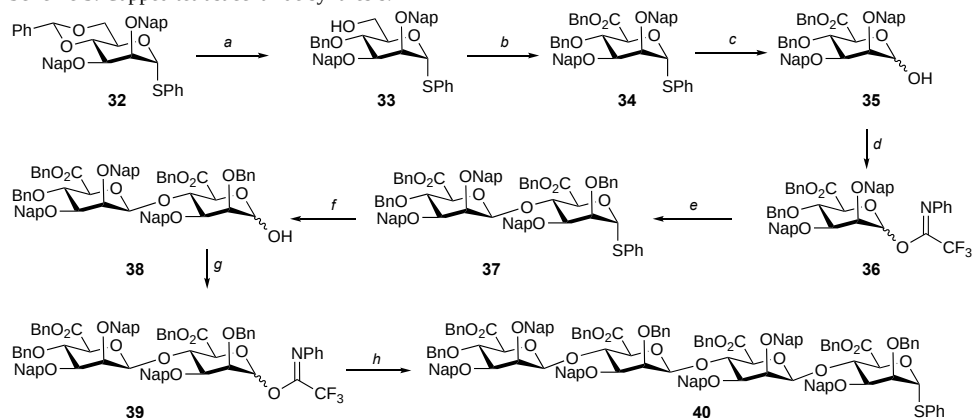


In principle, with the developed donors and deprotection methods, bigger fragments with a specified sulfation pattern are within reach. However, the deprotection procedure requires optimization, specifically the saponification/hydrogenation sequence. The removal of the Nap groups proceeds smoothly, followed by the introduction of the sulfate groups. Nonetheless, the amphiphilic character of the resulting compound made it poorly soluble and consequently difficult to purify. A possible alternative is to reverse the last two deprotection steps. The first hydrogenolysis step reduces the azide to an amine and liberates the benzyl protected carboxylic acids. Hereafter, mild basic conditions are required to remove the Lev group.

To streamline the deprotection sequence a ‘capping’ building block, bearing a benzyl ether instead of an Lev group at the C-4-OH could be used. Scheme 6 depicts an initial study towards this alternative. Hereto, donor **36** was synthesized as follows. Mannoside **32** was treated with catalytic acid to remove the benzylidene, after which the C-6 hydroxyl was protected with a TBDPS ether. Benzylation of the C-4 hydroxyl followed by deprotection of the silyl ether yielded compound **33**. Oxidation and subsequent benzylation of the C-6 hydroxyl proceeded in good yield to give **34**, which was treated with NIS/TFA to produce the hemiacetal **35**, which was transformed into imidate donor **36**. This donor was used to construct disaccharide **37**, applying similar conditions as described in Chapter 5. Unfortunately this reaction also produced a minor amount of the undesired anomer (1:10 α/β), which could not be separated from the desired compound. The mixture of disaccharides was transformed into the corresponding imidate donor **39** that was applied for the production of tetrasaccharide **40** in good yield. Unfortunately, also at this stage removal of the undesired (α,β,β,β)-anomer could not be achieved. A more elaborate

reactivity study with donor **3** should provide more insight into the glycosylating properties of this donor and its potential use for this synthesis.

Scheme 5. Capped tetrasaccharide synthesis.



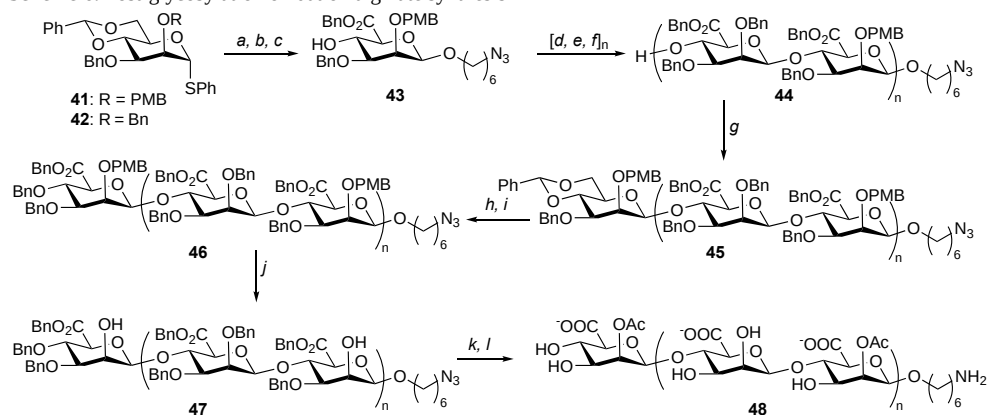
Reagents and conditions: a) i. pTsoH, DCM/MeOH; ii. Imidazole, TBDPS-Cl, DMF; iii. NaH, BnBr, DMF, 0°C; iv. TBAF (1.0 M in THF), THF (74% over 4 steps); b) i. TEMPO, BAIB, tBuOH/DCM/H₂O; ii. BnBr, K₂CO₃, DMF (99%); c) NIS, TFA, DCM/H₂O (74%); d) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0°C (86%); e) TMSOTf, DCM, -40°C (90%); f) NIS, TFA, Et₃N, DCM, 0°C (73%); g) ClC(=NPh)CF₃, Cs₂CO₃, acetone, 0°C (88%); h) TMSOTf, DCM, -45°C (98%).

Other biologically relevant alginate fragments are those with an acetyl group on the C-2 O- or C-3 OH.^{11,12} For instance, acetylated alginate plays an important role in the biofilm consistency of *P. aeruginosa*.¹³

A possible route towards these acetylated alginate is depicted in Scheme 6. The well established β -selective mannosylation, described in depth by Crich^{14,15}, employing 4,6-O-benzylidene thioglycosides as donors can give access to the selectively protected β -mannosides. Activation of donor **41** with triflic anhydride and Ph₂SO, in the presence of TTBP, followed by addition of a acceptor (e.g. azidohexanol spacer), should provide the benzylidene mannoside in β -selective fashion. Removal of the benzylidene, followed by a TEMPO/BAIB oxidation-benzylation procedure, yields acceptor **43**. Glycosylation with **42**, followed by benzylidene removal and oxidation/esterification, results in disaccharide **44**, which can then be elongated by repetition of these steps. It will be of interest to find out whether the inflexible β -configured spacer containing mannuronic acid acceptor can be combined with a benzylidene-protected mannose donor to provide a productive coupling reaction. If the ManA fragment has reached the desired length, the benzylidene is opened to the C-4 position, creating a 4-O-Bn capped alginate fragment. The C-6 hydroxyl is then oxidized and protected following the conditions described above. Removal of the PMB, leading to compound **47**, is followed by acetylation of the liberated hydroxyls. Hydrogenolysis removes the remaining benzyl groups, resulting in a selective acetylated deprotected alginate fragment.

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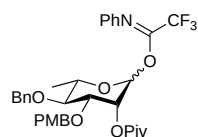
Scheme 6. Post-glycosylation oxidation alginate synthesis



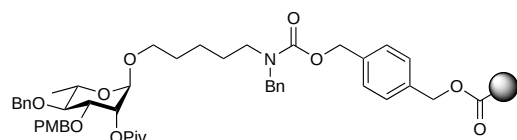
Reagents and conditions: a) 6-azidohexanol, TiF_2O , TTBP, Ph_2SO , -78°C ; b) pTsOH, DCM/MeOH; c) i. TEMPO, BAIB, tBuOH/DCM/ H_2O ; ii. BnBr, K_2CO_3 , DMF; d) **41** or **42**, TiF_2O , TTBP, BSP, -78°C ; e) pTsOH, DCM/MeOH; f) i. TEMPO, BAIB, tBuOH/DCM/ H_2O ; ii. BnBr, K_2CO_3 , DMF; g) **54**, TiF_2O , TTBP, BSP, -78°C ; h) $\text{BH}_3\cdot\text{THF}$, Bu_2BOTf , DCM, 0°C ; i) i. TEMPO, BAIB, tBuOH/DCM/ H_2O ; ii. BnBr, K_2CO_3 , DMF; j) HCl/HFIP, DCM/HFIP; k) Ac_2O , pyridine; l) $\text{Pd}(\text{OH})_2/\text{C}$, $\text{H}_2\text{O}/\text{THF}/\text{tBuOH}$.

Experimental

4-O-benzyl-3-O-(4-methoxybenzyl)-2-O-Pivaloyl- α/β -L-rhamnopyranoside S-Phenyl 4-O-Benzyl-3-O-(4-methoxybenzyl)-2-O-pivaloyl- α -L-thiorhamnopyranoside¹⁶ (1.38 g, 2.51 mmol, 1.0 eq.) was dissolved in acetone/H₂O (50 mL, 10:1) and cooled to 0°C. NBS (1.12 g, 6.28 mmol, 2.5 eq.) was added and the reaction was quenched with sat. aq. Na₂S₂O₃ after 5 min. The mixture was diluted with EtOAc, the layers were separated and the organic layer was washed with sat. aq. NaHCO₃ and sat. aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Column purification (hexanes/EtOAc) yielded the hemiacetal **xx** (0.93 g, 2.02 mmol, 80%). ¹H NMR (CDCl₃, 500 MHz): δ 7.46 – 7.12 (m, 7H), 6.82 (d, 2H, *J* = 8.4 Hz), 5.36 (s, 1H), 5.05 (s, 1H), 4.88 (d, 1H, *J* = 10.9 Hz), 4.67 – 4.49 (m, 2H), 4.41 (d, 1H, *J* = 10.8 Hz), 4.07 – 3.92 (m, 2H), 3.77 (s, 3H), 3.75 – 3.63 (m, 1H), 3.54 – 3.17 (m, 1H), 1.40 – 1.07 (m, 12H); ¹³C NMR (CDCl₃, 126 MHz): δ 178.5, 178.0, 159.3, 159.1, 138.4, 130.4, 129.9, 129.9, 129.7, 128.4, 128.4, 128.3, 127.9, 127.8, 113.7, 113.7, 93.1, 92.5, 80.1, 79.8, 79.1, 77.5, 75.3, 71.7, 71.1, 71.1, 69.5, 68.9, 67.7, 60.6, 55.3, 39.0, 27.4, 27.3, 18.2, 18.1, 14.3.

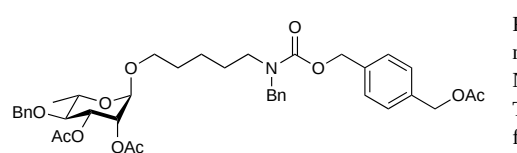


4-O-benzyl-3-O-(4-methoxybenzyl)-2-O-Pivaloyl-1-(N-phenyl-trifluoroacetimidoyl)- α/β -L-rhamnopyranoside (1) The hemiacetal (0.44 g, 0.96 mmol, 1.0 eq.) was dissolved in acetone, cooled to 0°C, followed by addition of ClC(=NPh)CF₃ (0.18 mL, 1.16 mmol, 1.2 eq.) and Cs₂CO₃ (0.47 g, 1.44 mmol, 1.5 eq.). The reaction was stirred for 55 min. after which it was filtered over Celite and concentrated *in vacuo*. Column purification (hexanes/EtOAc, 8:1 $\rightarrow$ 2:1) yielded the imide donor (0.59 g, 0.93 mmol, 97%). ¹H NMR (CDCl₃, 500 MHz): δ 7.26 (m, 10H), 7.08 (t, 1H, *J* = 7.4, 6.1, 6.1 Hz), 6.83 (s, 4H), 6.05 (s, 1H), 5.55 – 5.33 (m, 1H), 4.87 (dd, 1H, *J* = 11.0, 5.6 Hz), 4.72 – 4.52 (m, 2H), 4.47 (d, 1H, *J* = 10.7 Hz), 3.94 (dd, 1H, *J* = 9.3, 3.2 Hz), 3.91 – 3.82 (m, 1H), 3.77 (s, 3H), 3.43 (t, 1H, *J* = 9.4, 9.4 Hz), 1.31 (d, 3H, *J* = 6.2 Hz), 1.22 (s, 9H); ¹³C NMR (CDCl₃, 126 MHz): δ 177.3, 159.7, 143.6, 138.5, 130.2, 129.9, 129.8, 129.5, 128.9, 128.5, 128.3, 128.3, 127.9, 126.5, 124.6, 124.5, 120.7, 119.6, 119.6, 114.1, 114.0, 94.9, 94.7, 79.7, 79.4, 79.3, 77.6, 75.5, 75.4, 72.9, 71.8, 71.3, 70.7, 67.5, 66.2, 60.4, 55.4, 39.2, 27.3, 18.3, 18.2.



Automated synthesis using donor **1** (3x 3 eq.) and activator TfOH (0.09 M TfOH in DCE, 3x 0.2 eq.) at 0°C, as described in Chapter 4.

On-resin PMB deprotection. Dry resin bound rhamnoside **3** is swollen in 1 mL DCM. The suspension is treated with 0.09 mL HCl/HFIP (0.2 eq, 0.009 mmol, 0.1 M HCl/HFIP) after which the reaction turned deep purple immediately, and the fritted syringe is shaken for 10 min. The resin is washed with DCM (6x) and the acidic treatment is repeated 2x.



Resin bound rhamnoside **4** was swollen in DCM (2 mL), purged with argon, and treated with 0.1 mL NaOMe/MeOH (0.22 M, 0.5 eq) and shaken for 4 h. The reaction was neutralized with Amberlite H⁺, filtered and concentrated. The cleavage procedure was repeated and the combined fractions were combined

and dissolved in a chilled Ac₂O/pyridine mixture (2 mL, 1:1). The reaction was stirred overnight, quenched with MeOH and concentrated *in vacuo*. The mixture was purified by column chromatography (hexanes/EtOAc) yielding the triacetate (0.0173 g, 0.030 mmol, 53%). ¹H NMR (CDCl₃, 500 MHz): δ 7.38 – 7.20 (m, 12H), 7.17 (d,

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2H, $J = 6.7$ Hz), 5.30 (dd, 1H, $J = 9.7, 3.4$ Hz), 5.22 (s, 1H), 5.16 (d, 1H, $J = 19.2$ Hz), 5.09 (d, 1H, $J = 7.3$ Hz), 4.75 – 4.60 (m, 2H), 4.49 (d, 1H, $J = 5.3$ Hz), 3.80 (d, 1H, $J = 6.6$ Hz), 3.68 – 3.53 (m, 1H), 3.49 (t, 1H, $J = 9.6, 9.6$ Hz), 3.43 – 3.22 (m, 2H), 3.19 (t, 1H, $J = 7.1, 7.1$ Hz), 2.14 (s, 3H), 2.10 (s, 3H), 1.97 (s, 3H), 1.81 – 1.36 (m, 5H), 1.36 – 1.08 (m, 6H); ^{13}C NMR (CDCl_3 , 126 MHz): δ 170.3, 170.0, 138.1, 138.0, 137.1, 135.7, 128.7, 128.6, 128.2, 128.0, 127.8, 127.5, 127.4, 127.3, 97.5, 79.0, 75.3, 75.2, 71.8, 70.6, 67.9, 67.8, 67.7, 66.9, 66.2, 50.7, 50.4, 47.3, 46.3, 29.2, 28.1, 27.6, 23.5, 21.2, 21.1, 21.1, 18.1.

Mannoside **32** (12.35 g, 19.28 mmol, 1.0 eq.) was dissolved in DCM/MeOH (50 mL/50 mL) and treated with pTsOH (0.25g, 1.93 mmol, 0.1 eq.) and the reaction was stirred overnight. After TLC analysis showed complete consumption of the starting material, the reaction was neutralized with Et_3N and concentrated *in vacuo*. Column purification (PE/EtOAc, 4:1 $\rightarrow$ 1:1) yielded the diol (7.81 g, 14.13 mmol, 73%). The diol was coevaporated twice with toluene and dissolved in DMF (60 mL). Imidazole (1.92 g, 28.3 mmol, 2.0 eq.) was added followed by TBDPS-Cl (4.8 mL, 18.4 mmol, 1.3 eq.). The reaction was quenched with MeOH after stirring for 2h, the mixture was diluted with Et_2O and washed with sat. aq. NaCl, dried over MgSO_4 and concentrated *in vacuo*. The crude was coevaporated with toluene, dissolved in DMF (50 mL) and cooled to 0°C , followed by addition of NaH (60% dispersion in oil, 1.41 g, 35.3 mmol, 2.5 eq.). After 10 min, benzylbromide (3.7 mL, 32 mmol, 2.2 eq.) was added and the reaction was stirred until TLC analysis showed conversion to a higher running spot. The reaction was quenched with H_2O , diluted with Et_2O and the organic layer was washed with sat. aq. NaCl. The organic layer was dried over MgSO_4 and concentrated *in vacuo* after which it was dissolved in THF (70 mL). TBAF (1.0 M in THF, 28 mL, 2.0 eq.) was added and the reaction was stirred overnight. After overnight stirring, the reaction was diluted with Et_2O , washed with sat. aq. NaHCO_3 , dried over MgSO_4 and concentrated *in vacuo*. Column purification yielded alcohol **33** (6.7 g, 10.5 mmol, 74%). ^1H NMR (CDCl_3 , 500 MHz): δ 7.91 – 7.59 (m, 8H), 7.53 – 7.17 (m, 16H), 5.50 (d, 1H, $J = 1.9$ Hz), 5.02 (d, 1H, $J = 10.9$ Hz), 4.93 – 4.61 (m, 6H), 4.17 – 4.02 (m, 2H), 3.97 (dd, 1H, $J = 9.1, 3.1$ Hz), 3.88 – 3.80 (m, 2H); ^{13}C NMR (CDCl_3 , 126 MHz): δ 132.0, 129.2, 128.6, 128.5, 128.4, 128.2, 128.1, 128.1, 127.8, 127.0, 126.7, 126.3, 126.1, 86.3, 76.4, 75.5, 72.6, 62.4; HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{41}\text{H}_{42}\text{O}_5\text{SN}$ 660.27782, found 660.27829.

Alcohol **33** (6.7 g, 10.39 mmol, 1.0 eq.) was dissolved in tBuOH/DCM/ H_2O (42 mL/42 mL, 10.5 mL) and cooled to 0°C . TEMPO (0.32 g, 2.08 mmol, 0.2 eq.) and BAIB (8.37 g, 25.98 mmol, 2.5 eq.) were added and after 90 min, the reaction was allowed to warm up to RT. The reaction was quenched with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ after stirring for 90 min at RT, and diluted with EtOAc. The aqueous layer were extracted with EtOAc and the combined organic layers were washed with sat. aq. NaCl, dried over MgSO_4 in concentrated *in vacuo*. The crude was coevaporated twice with toluene, dissolved in DMF (95 mL), followed by the addition of benzylbromide (2.47 mL, 20.8 mmol, 2 eq.) and K_2CO_3 (2.87 g, 20.8 mmol, 2 eq.). The reaction was stirred overnight, quenched with H_2O , diluted with Et_2O , and the organic layer was washed with sat. aq. NaCl. The mixture was dried over MgSO_4 and concentrated *in vacuo* followed by column purification (PE/EtOAc, 9:1 $\rightarrow$ 6:1) to yield mannuronic acid **34** (7.19 g, 10.3 mmol, 99%). ^1H NMR (CDCl_3 , 400 MHz): δ 7.84 – 7.57 (m, 8H), 7.55 – 7.02 (m, 19H), 5.74 (d, 1H, $J = 5.8$ Hz), 5.20 (s, 1H), 5.08 (d, 2H, $J = 4.3$ Hz), 4.81 (d, 1H, $J = 12.2$ Hz), 4.73 – 4.46 (m, 6H), 4.31 (t, 1H, $J = 6.5, 6.5$ Hz), 3.96 (dd, 1H, $J = 5.7, 2.9$ Hz), 3.84 (dd, 1H, $J = 6.9, 2.9$ Hz); ^{13}C NMR (101 MHz, CDCl_3) δ 169.2, 137.8, 135.3, 133.8, 133.2, 133.1, 131.4, 128.9, 128.6, 128.4, 128.3, 128.3, 128.2, 128.0, 127.8, 127.8, 127.6, 127.3, 127.0, 126.8, 126.7, 126.2, 126.1, 126.1, 126.0, 77.5, 77.2, 76.8, 76.0, 73.2, 72.6, 67.1; HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{48}\text{H}_{46}\text{O}_6\text{SN}$ 764.30404, found 764.30414.

Compound **34** (7.0 g, 9.37 mmol, 1.0 eq.) was dissolved in DCM/ H_2O (100 mL/ 10 mL) and cooled to 0°C . NIS (2.32 g, 10.31 mmol, 1.1 eq.) was added, followed by addition of TFA (0.79 mL, 10.31 mmol, 1.1 eq.). The reaction was stirred overnight and quenched with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ and sat. aq. NaHCO_3 . Column purification (PE/EtOAc) yielded the hemiacetal **35** (4.51 g, 6.89 mmol, 74%) as a α/β mixture. ^1H NMR (CDCl_3 , 400 MHz): δ 7.85 – 7.61 (m, 10H), 7.52 – 7.38 (m, 6H), 7.37 – 7.33 (m, 1H), 7.30 – 7.00 (m, 11H), 5.50 (d, 1H, $J = 4.6$ Hz), 5.17 – 5.01 (m, 3H), 4.94 – 4.71 (m, 3H), 4.71 – 4.42 (m, 6H), 4.34 – 4.20 (m, 1H), 4.12 (dd, 0.24H, $J = 11.1, 7.0$ Hz), 3.94 (dd, 1H, $J = 7.1, 3.0$ Hz), 3.89 (t, 0.15H, $J = 2.5, 2.5$ Hz), 3.78 (dd, 1H, $J = 4.6, 2.8$ Hz), 3.71 (dd, 0.16H, $J = 8.1, 2.6$ Hz), 3.41 (s, 1H); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{42}\text{H}_{42}\text{O}_7\text{N}$ 672.29558, found 672.29565.

Hemiacetal **35** (4.27 g, 6.52 mmol, 1.0 eq.) was dissolved in acetone and cooled to 0°C. The mixture was treated with ClC(=NPh)CF_3 (1.2 mL, 7.84 mmol, 1.2 eq.) followed by addition of Cs_2CO_3 (3.2 g, 9.78 mmol, 1.5 eq.). The reaction was stirred overnight, after which it was diluted with EtOAc, washed with H_2O , dried over MgSO_4 and concentrated *in vacuo*. Column purification (PE/EtOAc, 10:1 $\rightarrow$ 6:1) yielded the imidate donor **36** as a α/β mixture (4.65 g, 5.63 mmol, 86%). ^1H NMR (CDCl_3 , 400 MHz): δ = 7.84 – 7.02 (m, 29H), 6.50 (d, 2H, J = 7.7 Hz), 6.39 (s, 1H), 5.23 – 5.12 (m, 2H), 4.94 – 4.71 (m, 4H), 4.59 (dd, 2H, J = 19.1, 11.4 Hz), 4.44 (d, 1H, J = 8.1 Hz), 4.34 (t, 1H, J = 8.1, 8.1 Hz), 3.90 (dd, 1H, J = 8.2, 3.1 Hz), 3.81 (t, 1H, J = 3.3, 3.3 Hz); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{50}\text{H}_{42}\text{F}_3\text{NO}_7\text{Na}$ 848.28056, found 848.28076.

Donor **36** (1.69 g, 2.06 mmol, 1.0 eq.) and benzyl (phenyl 2-O-benzyl-3-O-(2-naphthylmethyl)-1-thio- α -D-mannopyranosyl uronate) (1.49 g, 2.46 mmol, 1.2 eq.) were coevaporated 3x with toluene, dissolved in dry DCM (2 mL), activated molsieves 3Å were added and the reaction was stirred under an argon atmosphere at RT for 30 min. After 30 min the reaction was cooled to -60°C, followed by addition of TMSOTf (4.12 mL of a 0.1M TMSOTf/DCM, 0.2 eq.) and the reaction was stirred at -40°C overnight. The reaction was quenched with Et_3N (0.8 mL), diluted with EtOAc and washed with sat. aq. NaHCO_3 , dried over MgSO_4 in concentrated *in vacuo*. Column purification (PE/Et₂O) yielded the disaccharide as a 1:10 α/β mixture (2.31 g, 1.86 mmol, 90%). ^1H NMR (CDCl_3 , 500 MHz): δ 7.89 – 7.03 (m, 58H), 5.78 (d, 1H, J = 8.2 Hz), 5.71 (d, 0.11H, J = 6.5 Hz), 5.61 (d, 0.18H, J = 2.8 Hz), 5.44 (d, 0.13H, J = 4.4 Hz), 5.20 – 4.73 (m, 9H), 4.73 – 4.26 (m, 14H), 4.18 (dd, 1H, J = 5.1, 2.9 Hz), 4.03 – 3.73 (m, 4H), 3.67 (t, 0.12H, J = 3.7, 3.7 Hz), 3.51 (dd, 1H, J = 9.3, 3.2 Hz); ^{13}C NMR (CDCl_3 , 126 MHz): δ = 169.1, 168.2, 138.6, 138.1, 136.3, 135.9, 135.7, 135.5, 135.4, 134.2, 133.5, 133.5, 133.3, 132.0, 131.8, 131.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.2, 127.0, 126.9, 126.8, 126.8, 126.6, 126.6, 126.5, 126.5, 126.3, 126.2, 126.1, 126.0, 125.9, 125.9, 125.8, 125.8, 101.8, 99.4, 86.4, 81.3, 78.7, 76.8, 76.4, 76.2, 75.8, 75.5, 75.3, 75.1, 74.7, 74.5, 74.0, 73.4, 73.4, 73.1, 73.0, 72.7, 72.4, 72.3, 72.2, 69.0, 67.3, 67.3, 67.2, 67.1; HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{79}\text{H}_{74}\text{O}_{12}\text{SN}$ 1260.49262, found 1260.49329.

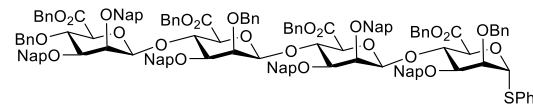
Compound **37** (0.23 g, 0.19 mmol, 1.0 eq.) was dissolved in DCM (1.9 mL) and cooled to 0°C. NIS (0.05 g, 0.22 mmol, 1.2 eq.) was added, followed by addition of TFA (0.02 mL, 0.22 mmol, 1.2 eq.). The reaction was stirred for 3h and quenched with Et_3N (0.05 mL, 0.372 mmol, 2 eq.). A solution of sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ was added, the mixture was diluted with DCM and the organic layer was washed with sat. aq. NaCl. Column purification (PE/EtOAc, 5:1 $\rightarrow$ 1:1) yielded the hemiacetal **38** (0.16 g, 0.139 mmol, 73%). ^1H NMR (CDCl_3 , 500 MHz): δ 7.89 – 7.52 (m, 20H), 7.47 – 7.04 (m, 50H), 5.62 (t, 1H, J = 5.5, 5.5 Hz), 5.17 – 4.74 (m, 10H), 4.79 (d, 2H, J = 12.2 Hz), 4.71 – 4.35 (m, 16H), 4.31 (t, 2H, J = 9.3, 9.3 Hz), 4.25 – 4.03 (m, 1H), 3.92 (d, 1H, J = 2.8 Hz), 3.87 (d, 2H, J = 9.4 Hz), 3.73 – 3.55 (m, 2H), 3.47 (dd, 1H, J = 9.3, 2.9 Hz), 3.43 – 3.26 (m, 2H); ^{13}C NMR (CDCl_3 , 126 MHz): δ 169.3, 168.1, 138.3, 136.0, 135.8, 135.4, 135.3, 135.2, 133.3, 133.3, 133.1, 133.0, 129.1, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.0, 126.5, 126.4, 126.2, 126.1, 126.1, 126.0, 125.9, 125.9, 125.7, 101.4, 92.7, 80.9, 77.4, 77.2, 76.9, 76.5, 76.3, 76.2, 75.9, 75.5, 75.1, 74.3, 74.0, 73.4, 73.1, 72.4, 71.9, 67.3, 67.2.

Hemiacetal **38** (0.16 g, 0.139 mmol, 1.0 eq.) was dissolved in acetone and cooled to 0°C. The mixture was treated with ClC(=NPh)CF_3 (0.03 mL, 0.17 mmol, 1.2 eq.) followed by addition of Cs_2CO_3 (0.07 g, 0.21 mmol, 1.5 eq.). The reaction was stirred overnight, after which it was diluted with EtOAc, washed with H_2O , dried over MgSO_4 and concentrated *in vacuo*. Column purification (PE/EtOAc, 5:1 $\rightarrow$ 3:1) yielded the imidate donor **39** (0.162 g, 0.122 mmol, 88%). ^1H NMR (CDCl_3 , 500 MHz): δ 7.88 – 7.47 (m, 16H), 7.47 – 7.29 (m, 10H), 7.29 – 6.96 (m, 32H), 6.81 – 6.47 (m, 2H), 6.48 (s, 1H), 6.39 (s, 1H), 5.52 (d, 0.1H, J = 4.1 Hz), 5.20 – 4.76 (m, 9H), 4.76 – 4.37 (m, 13H), 4.38 – 4.28 (m, 1H), 4.20 (s, 1H), 4.04

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– 3.82 (m, 3H), 3.77 (s, 0.15H), 3.71 (s, 0.11H), 3.48 (dd, 1H, $J = 9.2, 2.7$ Hz); ^{13}C NMR (CDCl₃, 126 MHz): δ 169.3, 168.6, 168.2, 167.9, 143.8, 143.0, 142.7, 138.6, 138.2, 137.9, 137.8, 136.3, 136.0, 135.9, 135.9, 135.7, 135.6, 135.5, 135.3, 135.2, 133.5, 133.5, 133.4, 133.2, 133.2, 131.0, 130.5, 130.4, 130.3, 130.2, 129.3, 129.2, 129.1, 129.0, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.8, 127.7, 127.3, 127.2, 127.1, 126.9, 126.8, 126.7, 126.6, 126.6, 126.4, 126.4, 126.3, 126.2, 126.1, 126.1, 126.0, 126.0, 126.0, 125.9, 125.9, 125.8, 125.8, 125.7, 125.1, 124.4, 124.3, 124.1, 119.7, 117.5, 115.2, 102.1, 95.1, 81.4, 81.2, 77.3, 76.7, 76.4, 76.3, 76.2, 76.2, 76.0, 75.8, 75.7, 75.2, 75.0, 75.0, 74.8, 74.6, 74.5, 74.5, 74.4, 74.2, 73.7, 73.6, 73.4, 73.3, 73.0, 72.9, 72.9, 72.8, 72.7, 72.7, 72.6, 72.3, 72.2, 67.4, 67.3, 67.3, 67.2, 67.1.

Donor **39** (0.153 g, 0.12 mmol, 1.2 eq.) and acceptor **30** (0.12 g, 0.1 mmol, 1.0 eq.) were coevaporated 3x with toluene, dissolved in dry DCM (1 mL), activated molsieves 3 Å were added and the reaction was stirred under an argon atmosphere at RT for 30 min. After 30 min the reaction was cooled to -60 °C, followed by addition of TMSOTf (0.2 mL of



a 0.1M TMSOTf/DCM, 0.2 eq.) and the reaction was stirred at -45 °C over 2h. The reaction was quenched with Et₃N (0.2 mL), diluted with DCM and washed with sat. aq. NaHCO₃, dried over MgSO₄ in concentrated *in vacuo*. Size exclusion yielded the tetrasaccharide as a mixture (0.225 g, 0.098 mmol, 98%). ^1H NMR (CDCl₃, 500 MHz): δ 7.92 – 6.94 (m, 106H), 5.75 (d, 1H, $J = 7.7$ Hz), 5.49 (s, 0.13H), 5.25 (d, 0.13H, $J = 5.4$ Hz), 5.15 – 4.19 (m, 44H), 4.14 (s, 1H), 3.89 – 3.69 (m, 6H), 3.67 (d, 3H, $J = 9.4$ Hz), 3.47 (d, 1H, $J = 8.6$ Hz), 3.30 (td, 2H, $J = 9.1, 8.8, 2.4$ Hz); ^{13}C NMR (CDCl₃, 126 MHz): δ 169.1, 168.4, 168.4, 168.1, 139.3, 138.8, 138.2, 136.9, 136.9, 136.7, 136.3, 136.0, 135.9, 135.6, 135.4, 135.4, 134.3, 133.6, 133.5, 133.5, 133.2, 133.2, 133.1, 131.4, 128.8, 128.7, 128.7, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.6, 127.5, 127.4, 127.0, 126.9, 126.7, 126.5, 126.4, 126.3, 126.2, 126.1, 126.1, 126.0, 126.0, 125.9, 125.8, 125.8, 125.7, 102.7, 102.3, 101.1, 81.6, 79.6, 79.1, 77.8, 76.7, 76.6, 76.2, 75.8, 75.6, 75.6, 75.4, 75.0, 74.8, 74.8, 74.3, 73.7, 73.4, 73.2, 73.1, 72.3, 71.9, 67.2, 67.1, 67.0, 67.0; HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for C₁₄₅H₁₃₂O₂₄SN 2302.88545, found 2302.88772.

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Samenvatting

De organisch chemische synthese maakt goed gedefinieerde complexe oligosacchariden beschikbaar, die gebruikt kunnen worden voor het begrijpen van de rol van koolhydraten in de biologie. Een juiste keuze van beschermgroepen is doorslaggevend voor het succes van de synthese van elk oligosaccharide. De uitkomst van een glycosylering, met name de stereochemie, het introduceren van functionele groepen als amines en sulfaten en het ontschermen tijdens en aan het eind van de synthese is bijvoorbeeld afhankelijk van de correcte keus van beschermgroepen. In Hoofdstuk 1 wordt een overzicht gegeven van de invloed die beschermgroepen kunnen hebben op verschillende onderdelen van oligosaccharide synthese, variërend van mechanistische verklaringen voor de uitkomst van glycosyleringen tot de volledige ontscherming van complexe oligosacchariden.

In Hoofdstuk 2 wordt een nieuwe, chemoselectieve methode om *para*-methoxybenzyl (PMB) en naphthylmethyl (Nap) ethers te verwijderen beschreven. Een katalytische hoeveelheid zoutzuur in hexafluoro-*iso*-propanol blijkt zeer effectief voor het snel en efficiënt verwijderen van PMB en Nap ethers. De toepasbaarheid en beperkingen van deze methode werd geëvalueerd met een serie van beschermde koolhydraat derivaten. Er werden condities gevonden om de PMB beschermgroep selectief te verwijderen in aanwezigheid van andere zuurlabiele beschermgroepen, zoals Nap ethers en veel gebruikte silyl groepen. Tevens bleek dat de verwijdering van Nap groep effectiever verliep in combinatie met triethylsilane (TES) dat reageert met de gevormde PMB kationen. Ook werd gevonden dat Nap ethers met katalytisch zuur kunnen worden verwijderd, maar dat het gebruik van een stoichiometrische hoeveelheid zuur de reactie sneller doet verlopen. Deze nieuwe methode bleek essentieel voor het synthetiseren van gesulfateerd mannuronzuur, beschreven in hoofdstuk 5.

Het efficiënt verwijderen van beschermgroepen is in Hoofdstuk 3 wederom een belangrijk onderwerp. Aandacht wordt besteed aan een nieuwe op de pivaloyl gebaseerde beschermgroep, de cyanopivaloyl (PivCN). Het reagens om de PivCN in te voeren is op eenvoudige wijze te verkrijgen en de PivCN groep bezit alle gunstige karakteristieken van de oorspronkelijke Piv groep maar is daarentegen gemakkelijker te verwijderen. PivCN is sterisch gehinderd en een uitstekende participerende groep, maar kan verwijderd worden onder milde hydrogenerings condities. Immers, de cyano groep van de PivCN wordt tijdens de hydrogenering omgezet in een amine, die vervolgens een intramoleculaire nucleofiele aanval op de carbonylgroep van de ester bewerkstelligd, resulterend in een ontschermd alcohol

en een γ -lactam. Daarentegen, heeft de normale Piv groep sterk basische ontschermingscondities nodig, wat nevenreacties kan veroorzaken of kan leiden tot onvolledige ontscherming aan het eind van een syntheseroute. De voordelige eigenschappen worden geïllustreerd door de succesvolle toepassing van de PivCN groep in de synthese van een rhamnose hexasaccharide dat deel uitmaakt van het capsulaire polysaccharide van *Enterococcus faecium* en een tetrasaccharide dat voorkomt in de polyrhamnose hoofdketen van de Lancefield Group A koolhydraat.

In Hoofdstuk 4 behandelt de geautomatiseerde vaste drager synthese van oligorhamnose fragmenten, die een onderdeel vormen van een polysaccharide uit de celwand van Group A streptococcus. Deze Gram-positieve bacterie is de oorzaak van verschillende infecties en is verantwoordelijk voor een reumatische koorts, die veel slachtoffers eist in ontwikkelingslanden. Om deze fragmenten te verkrijgen werd besloten om de oligorhamnose keten te verlengen met rhamnose dimeren. Daartoe werden twee nieuwe dirhamnose donoren gesynthetiseerd met of een Piv of een PivCN groep op de C-2-OH positie van de rhamnose aan het reducerende uiteinde. De geautomatiseerde synthese werd uitgevoerd met behulp van een nieuwe commerciële geautomatiseerde vaste drager machine, de zogenoemde Glyconeer 2.0. Allereerst werd een rhamnose 10-meer gemaakt, voorzien van Piv beschermgroepen. Ondanks meerdere pogingen bleek het onmogelijk om alle Piv groepen te verwijderen. Vervolgens werden dezelfde rhamnose oligomeren op identieke wijze gesynthetiseerd met de donor voorzien van de PivCN groep. De basische afsplitsing van de vaste drager van deze oligorhamnose fragmenten bleek vergezeld te gaan van gedeeltelijke verwijdering van de PivCN groepen. Door de base behandeling te verlengen en te herhalen konden alle PivCN groepen worden afgesplitst. Met deze methode werden multimilligram hoeveelheden van biologische relevante rhamnose fragmenten, tot een lengte van 16 repeterende eenheden, verkregen.

In Hoofdstuk 5 wordt een studie beschreven, die gericht is op de synthese van gesulfateerde mannuronzuur fragmenten. Goed gedefinieerde, gesulfateerde mannuronzuur oligomeren kunnen gebruikt worden om structuur- activiteits relaties vast te stellen van de mogelijke anti-virale en anti-kanker eigenschappen van deze fragmenten. Om een degelijk syntheseplan naar deze moleculen op te kunnen stellen werd een aantal nieuwe donoren en acceptoren ontworpen en gesynthetiseerd en vervolgens getest in een reeks glycosylerings reacties. Naast de stereoselectiviteit van de glycosyleringsreactie is the orthogonaliteit van de beschermgroepen essentieel. Een selectief te verwijderen beschermgroep is nodig om de nodige sulfaten esters te introduceren aan het eind van de synthese. De daartoe gekozen Nap groepen werden eerst verwijderd onder oxidatieve condities.

Samenvatting

Deze condities zorgde echter voor incomplete verwijdering van de Nap groepen en resulteerde in complexe mengsels van producten. Optimalisatie van de methode met katalytisch zoutzuur in hexafluoro-iso-propanol, zoals beschreven in Hoofdstuk 2, resulteerde uiteindelijk in een snelle en schone ontscherming van de Nap groepen. Met een gesynthetiseerd mannuronzuur dimeer, werden de condities voor sulfatering en de ontschermingsprocedure voor de overige beschermgroepen geoptimaliseerd, waarna een goed gedefinieerd, gesulfateerd mannuronzuur disaccharide werd verkregen. De mannuronzuur bouwstenen werden vervolgens aangepast met als doel langere fragmenten te synthetiseren. Evaluatie van deze nieuwe donoren en acceptoren in glycosylerings reacties liet zien dat acceptoren, vastgezet in een β -configuratie, een dermate lage reactiviteit bezaten dat het onmogelijk was om vanaf het “niet-reducerende” einde een lange mannuronzuur keten op te bouwen. Met behulp van een alternatieve benadering, waarbij thioglycoside acceptoren werden verlengd aan het “reducerende” einde werd een tetrasaccharide verkregen, dat kon worden omgezet in een imidaat donor. Dit tetrasaccharide-imidaat werd aan de linker gekoppeld tot het gewenste volledig beschermde tetrasaccharide. De ontschermingsmethode uit Hoofdstuk 2 verwijderde zeer effectief de Nap ethers waarna het tetrasaccharide werd onderworpen aan de sulfaterings condities. Uit NMR en LCMS analyse bleek dat deze reactie succesvol was maar het verkregen molecuul bleek moeilijk te hanteren door het amphifiele karakter van de verbinding. Dit maakte de zuivering zeer uitdagend en het volledig ontschermde gesulfateerde tetrasaccharide kon helaas niet worden verkregen.

List of publications

- **Automated Solid-Phase Synthesis of Hyaluronan Oligosaccharides**
Walvoort, M. T. C.; Volbeda, A. G.; Reintjens, N. R. M.; van den Elst, H.; Plante, O. J.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *Org. Lett.* **2012**, 60, 1–66.
- **A Second-Generation Tandem Ring-Closing Metathesis Cleavable Linker for Solid-Phase Oligosaccharide Synthesis**
de Jong, A. R.; Volbeda, A. G.; Hagen, B.; van den Elst, H.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *Eur. J. Org. Chem.* **2013**, 2013, 6644–6655.
- **Synthesis of β -(1→3)-mannobiose**
Picard, S.; Thomas, M.; Volbeda, A. G.; Guinchard, X.; Crich, D. Carbohydrate Chemistry: Proven Synthetic Methods Volume 2, Eds. van der Marel, G. A.; Codée, J. D. C., **2014**, 161–174.
- **Chemoselective Cleavage of *p*-Methoxybenzyl and 2-Naphthylmethyl Ethers Using a Catalytic Amount of HCl in Hexafluoro-2-propanol**
Volbeda, A. G.; Kistemaker, H. A. V.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V.; Codée, J. D. C. *J. Org. Chem.* **2015**, 80, 8796–8806.
- **The Cyanopivaloyl Ester: A Protecting Group in the Assembly of Oligorhamnans**
Volbeda, A. G.; Reintjens, N. R. M.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *European J. Org. Chem.* **2016**, 2016, 5282–5293.
- **Controlling Multivalent Binding through Surface Chemistry: Model Study on Streptavidin**
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- **Cyanopivaloyl Ester in the Automated Solid-Phase Synthesis of Oligorhamnans**
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Curriculum Vitae

The autor was born in Leiden on July 20th 1988. In 2006, he graduated from the high school Pieter Groen (VWO) and subsequently started the bachelor Molecular Science and Technology at Leiden University and the Technological University Delft. He obtained his bachelor degree in 2010 (Major chemistry) after finishing his bachelor research internship “Adamantane based protecting groups” in the Bio-organic synthesis group, under the supervision of prof. dr. G.A. van der Marel and prof. dr. H.S. Overkleeft. Thereafter, he started his master Chemistry – Research, Design and Synthesis. He performed his master research internship on the automated synthesis of Hyaluronic Acid oligosaccharides, in the Bio-organic synthesis group, under the guidance of dr. M.T.C. Walvoort, prof. dr. G.A. van der Marel and dr. J.D.C. Codée. A library of well-defined hyaluronic acid fragments was constructed, representing the first automated solid phase synthesis of a glycosaminoglycan (GAG) family member.

In March 2012, he commenced his PhD studies in the Bio-organic synthesis group under the supervision of prof. dr. G.A. van der Marel and dr. J.D.C. Codée. Parts of this research were orally presented at the CHAINS symposium in Veldhoven (2014) and the 18th European Carbohydrate Symposium in Moscow (2015). Posters were presented at the Glycan Forum Berlin (2012), NWO Design and Synthesis meeting in Lunteren (2013). The Holland Research School for Molecular Chemistry (HRSMC) Summer School ‘New Vistas in Organic Synthesis’ was attended in July 2013. The author is currently scientist analytical chemistry and bioanalysis at ProQR.

De auteur van dit proefschrift werd op 20 juli 1988 geboren te Leiden. In 2006 werd het eindexamen VWO, profiel Natuur & Techniek, met goed gevolg afgelegd. Datzelfde jaar begon hij aan de Bachelor Molecular Science and Technology aan de Universiteit Leiden en Technische Universiteit Delft. In 2010 behaalde hij zijn Bachelor graad na het afronden van de onderzoeksstage “Adamantane based protecting groups” in de Bio-organische synthese groep, onder de supervisie van prof. dr. G.A. van der Marel en prof. dr. H.S. Overkleeft. In datzelfde jaar begon hij aan de Master Chemistry, met de afstudeerrichting Research – Design & Synthesis. De onderzoeksstage, die zich richtte op het ontwikkelen van een automatische vaste drager synthese van hyaluronan fragmenten, werd afgerond binnen de Bio-organische synthese groep, onder de directe begeleiding van dr. M.T.C. Walvoort, prof. dr. G.A. van der Marel en dr. J.D.C. Codée. Deze afstudeerstage resulteerde in een bibliotheek van goed gedefinieerde hyaluronzuur moleculen en de succesvolle synthese is bovendien de eerste geautomatiseerde vaste drager synthese van een glycosaminoglycan (GAG) gebleken.

In maart 2012 begon hij aan zijn promotie-onderzoek in de Bio-organische synthese groep onder de supervisie van dr. J.D.C. Codée en prof. dr. G.A. van der Marel. Delen van zijn onderzoek werden gepresenteerd tijdens het CHAINS-symposium te Veldhoven (2014) en tijdens het 18^e European Carbohydrate Symposium in Moskou (2015). Posters werden gepresenteerd op het Glycan Forum Berlin (2012), en de NWO Design and Synthesis

meeting in Lunteren (2013). De auteur is momenteel werkzaam bij ProQR als scientist analytische chemie en bioanalytiek.