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## **Novel protecting group strategies in the synthesis of oligosaccharides**

Volbeda, A.G.

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**Author:** Volbeda, Anne Geert

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## Chapter 6

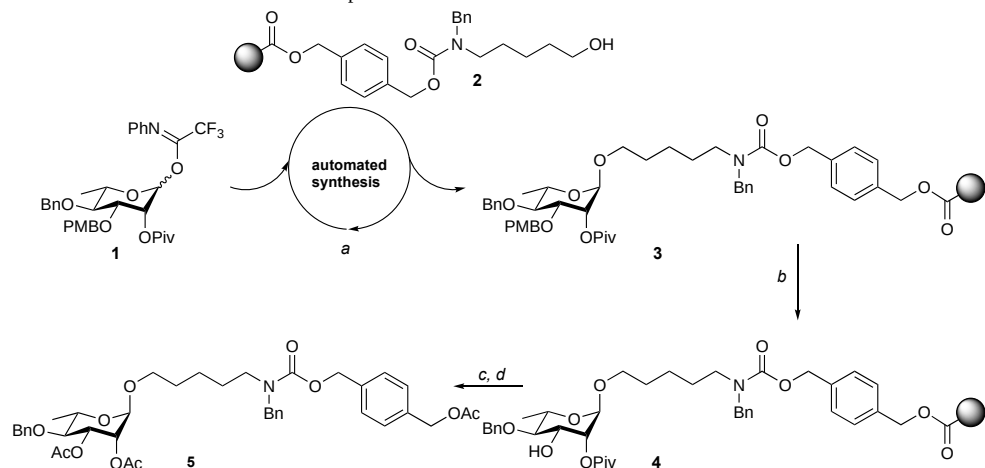
*Summary & future prospects*

## 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.<sup>1,2</sup> 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/H<sub>2</sub>O (64:16:1) mixture to allow for complete removal.<sup>3</sup> 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) Ac<sub>2</sub>O, pyridine, 0°C (53% over 4 steps from resin **2**).

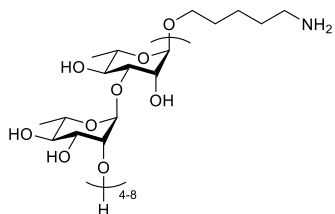
These results indicate that the PMB can be applied on solid phase as temporary protecting group. A disadvantage encountered in the test case described above is the loss of the Piv group. As the Piv-group was completely cleaved, it is impossible to establish whether any migration had occurred during the 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.

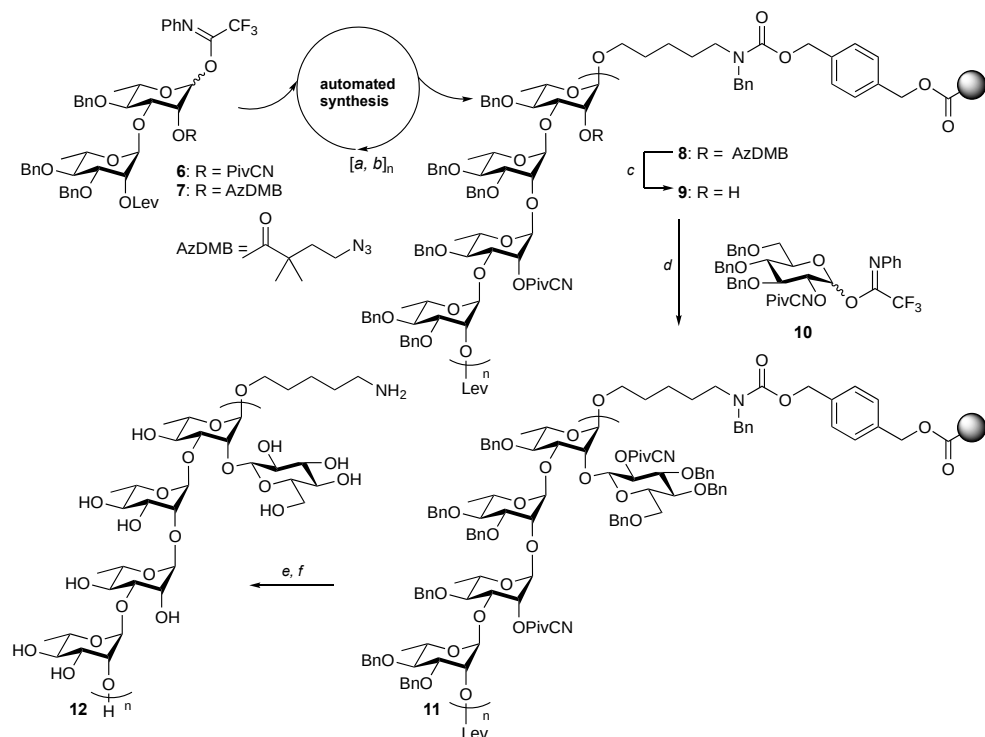
**Figure 1.** Structures synthesized in Chapter 4.



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),<sup>4</sup> 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  $\beta$ -1,3 glucans.<sup>5</sup> The AzDMB can be removed using phosphine based reagents under mild conditions as previously shown.<sup>4</sup> As described in Chapter 4, the Group A *Streptococcus* (GAS) polyrrhamnose backbone is decorated on the rhamnosyl C-3 OH with *N*-acetyl glucosamine. In the case of *Streptococcus mutans*, a polyrrhamnose 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 polyrrhamnose backbone.<sup>6</sup> 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  $\text{PMe}_3$  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**.

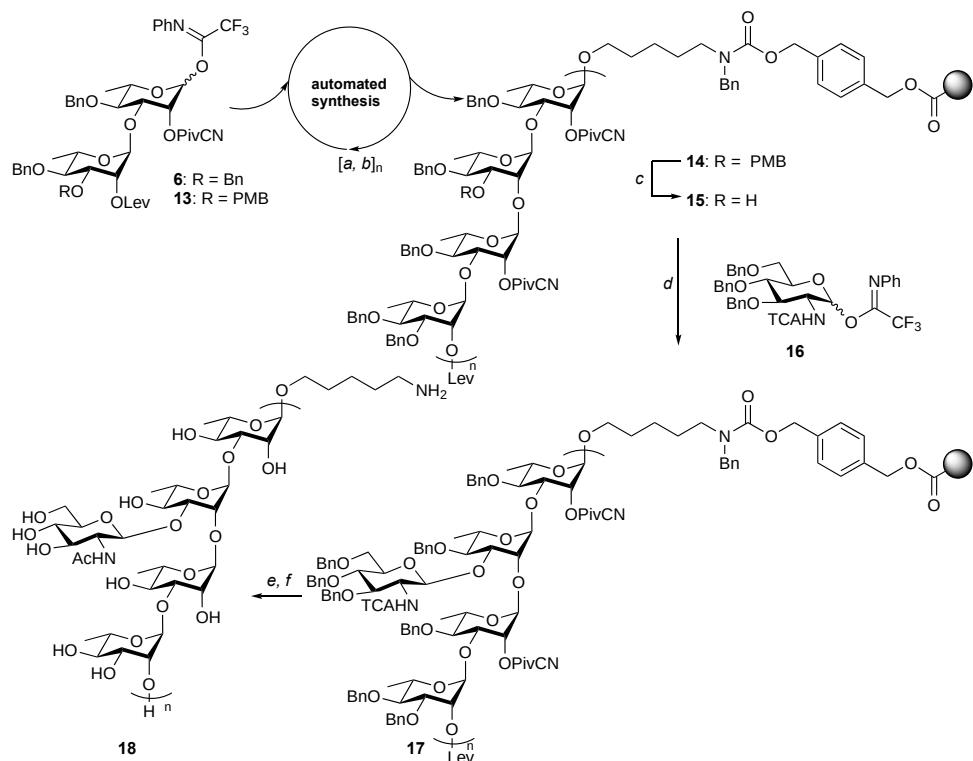
Scheme 2.



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)  $\text{PMe}_3$ , KOH, THF/ $\text{H}_2\text{O}$ ; d) 3 eq. **10**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; e) NaOMe, MeOH/THF; f)  $\text{H}_2$ , Pd(OH) $_2$ /C, AcOH,  $\text{H}_2\text{O}$ /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.

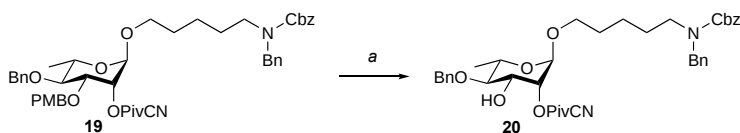
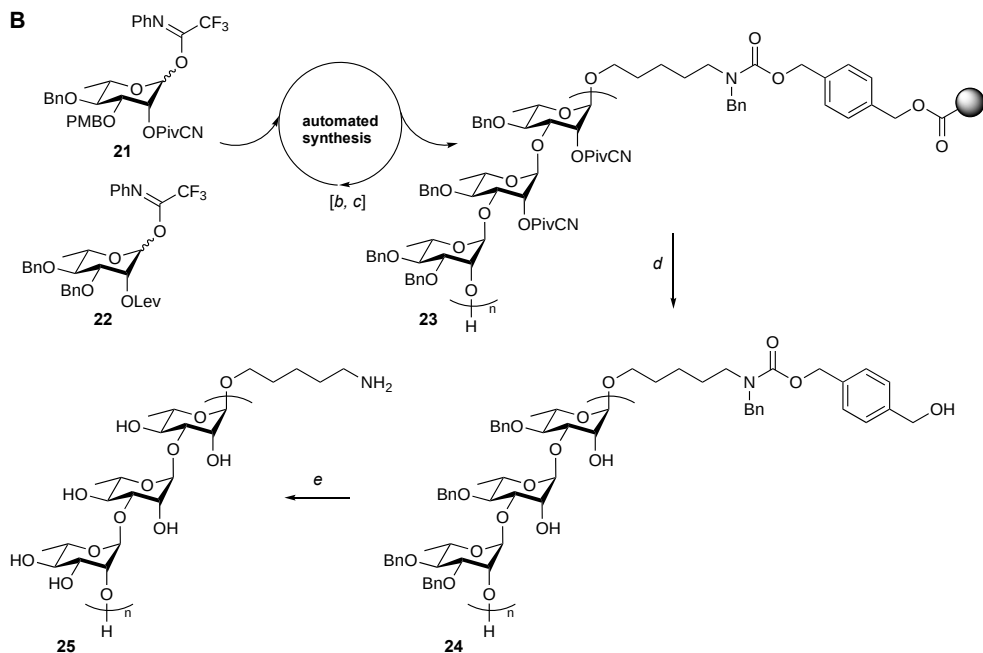


**Reagents and conditions:** a) 3 eq. **16** or **13**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; b) 8 eq. H<sub>2</sub>NNH<sub>2</sub>·AcOH, pyr/AcOH, 40°C, 3 cycles; c) HCl/HFIP, TES, HFIP/DCM; d) 3 eq. **16**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; e) NaOMe, MeOH/THF; f) H<sub>2</sub>, Pd(OH)<sub>2</sub>/C, AcOH,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

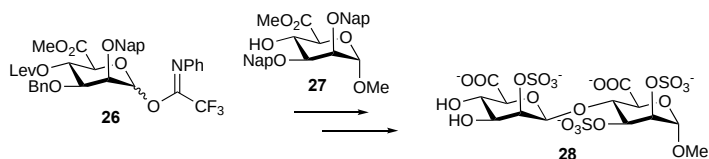
**Reagents and conditions:** a) HCl/HFIP, HFIP/DCM (76%); b) 3 eq. **21** or **22**, 0.3 eq. TfOH, DCM, 0°C, 3 cycles; then: c) 8 eq.  $\text{H}_2\text{NNH}_2 \cdot \text{AcOH}$ , pyr/AcOH, 40°C, 3 cycles; or HCl/HFIP, TES, HFIP/DCM, 3 cycles; d) NaOMe, MeOH/THF; e)  $\text{H}_2$ ,  $\text{Pd}(\text{OH})_2/\text{C}$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

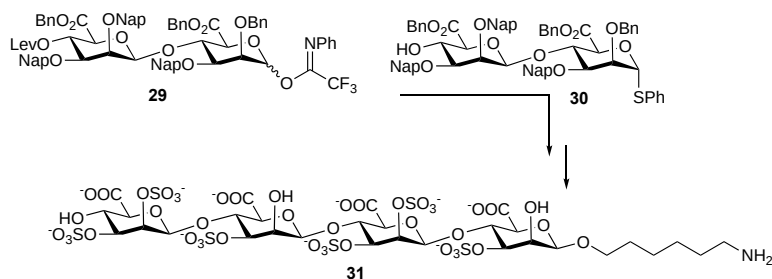
donor, which could then be coupled efficiently to the primary alcohol spacer (Figure 3B). Sulfation and deprotection resulted in the sulfated tetrasaccharide **31**. Unfortunately, the saccharide was not obtained in pure enough form.

Figure 2.

A



B

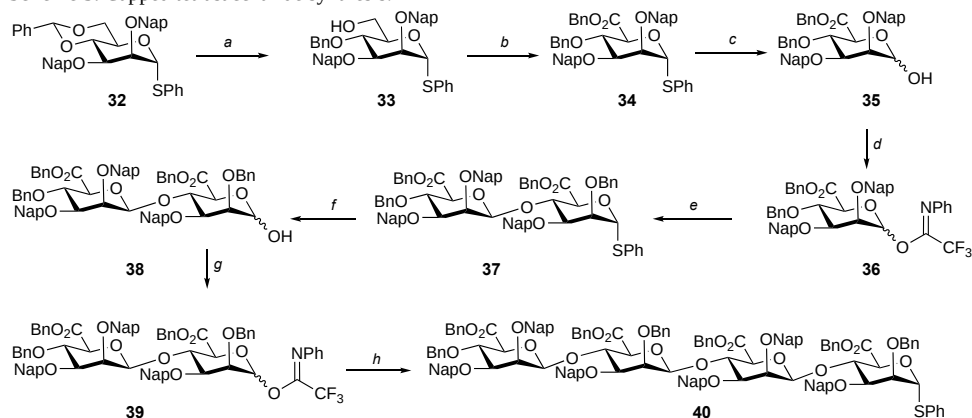


In principle, with the developed donors and deprotection methods, bigger fragments with a specified sulfation pattern are within reach. However, the deprotection procedure requires optimization, specifically the saponification/hydrogenation sequence. The removal of the Nap groups proceeds smoothly, followed 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  $\alpha/\beta$ ), 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 ( $\alpha,\beta,\beta,\beta$ )-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.



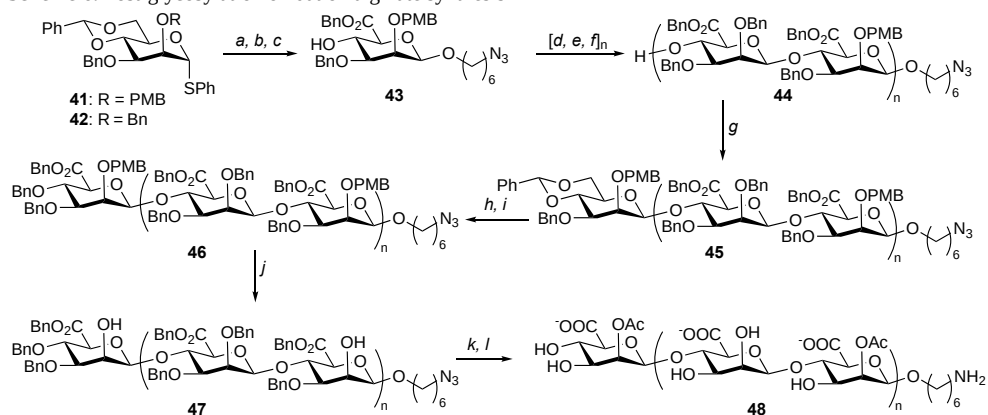
**Reagents and conditions:** a) i. pTsoH, DCM/MeOH; ii. Imidazole, TBDPS-Cl, DMF; iii. NaH, BnBr, DMF, 0°C; iv. TBAF (1.0 M in THF), THF (74% over 4 steps); b) i. TEMPO, BAIB, tBuOH/DCM/H<sub>2</sub>O; ii. BnBr, K<sub>2</sub>CO<sub>3</sub>, DMF (99%); c) NIS, TFA, DCM/H<sub>2</sub>O (74%); d) ClC(=NPh)CF<sub>3</sub>, Cs<sub>2</sub>CO<sub>3</sub>, acetone, 0°C (86%); e) TMSOTf, DCM, -40°C (90%); f) NIS, TFA, Et<sub>3</sub>N, DCM, 0°C (73%); g) ClC(=NPh)CF<sub>3</sub>, Cs<sub>2</sub>CO<sub>3</sub>, 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*.<sup>11,12</sup> For instance, acetylated alginate plays an important role in the biofilm consistency of *P. aeruginosa*.<sup>13</sup>

A possible route towards these acetylated alginate is depicted in Scheme 6. The well established  $\beta$ -selective mannosylation, described in depth by Crich<sup>14,15</sup>, employing 4,6-*O*-benzylidene thioglycosides as donors can give access to the selectively protected  $\beta$ -mannosides. Activation of donor **41** with triflic anhydride and Ph<sub>2</sub>SO, in the presence of TTBP, followed by addition of a acceptor (e.g. azidohexanol spacer), should provide the benzylidene mannoside in  $\beta$ -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  $\beta$ -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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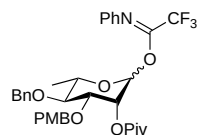
**Scheme 6.** Post-glycosylation oxidation alginate synthesis



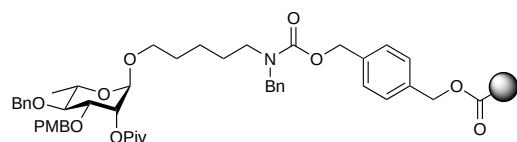
**Reagents and conditions:** a) 6-azido-6-deoxy-6-phenyl- $\alpha$ -D-glucopyranoside,  $\text{TiF}_2\text{O}$ , TTBP,  $\text{Ph}_2\text{SO}$ ,  $-78^\circ\text{C}$ ; b) pTsOH, DCM/MeOH; c) i. TEMPO, BAIB, tBuOH/DCM/ $\text{H}_2\text{O}$ ; ii. BnBr,  $\text{K}_2\text{CO}_3$ , DMF; d) **41** or **42**,  $\text{TiF}_2\text{O}$ , TTBP, BSP,  $-78^\circ\text{C}$ ; e) pTsOH, DCM/MeOH; f) i. TEMPO, BAIB, tBuOH/DCM/ $\text{H}_2\text{O}$ ; ii. BnBr,  $\text{K}_2\text{CO}_3$ , DMF; g) **54**,  $\text{TiF}_2\text{O}$ , TTBP, BSP,  $-78^\circ\text{C}$ ; h)  $\text{BH}_3\cdot\text{THF}$ ,  $\text{Bu}_2\text{BOTf}$ , DCM,  $0^\circ\text{C}$ ; i) i. TEMPO, BAIB, tBuOH/DCM/ $\text{H}_2\text{O}$ ; ii. BnBr,  $\text{K}_2\text{CO}_3$ , DMF; j) HCl/HFIP, DCM/HFIP; k)  $\text{Ac}_2\text{O}$ , 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- $\alpha/\beta$ -L-rhamnopyranoside** S-Phenyl 4-O-Benzyl-3-O-(4-methoxybenzyl)-2-O-pivaloyl- $\alpha$ -L-thiorhamnopyranoside<sup>16</sup> (1.38 g, 2.51 mmol, 1.0 eq.) was dissolved in acetone/H<sub>2</sub>O (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. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> after 5 min. The mixture was diluted with EtOAc, the layers were separated and the organic layer was washed with sat. aq. NaHCO<sub>3</sub> and sat. aq. NaCl, dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Column purification (hexanes/EtOAc) yielded the hemiacetal **xx** (0.93 g, 2.02 mmol, 80%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  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); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  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.

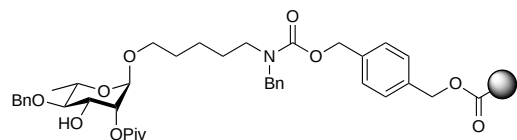


**4-O-benzyl-3-O-(4-methoxybenzyl)-2-O-Pivaloyl-1-(N-phenyl-trifluoroacetimidoyl)- $\alpha/\beta$ -L-rhamnopyranoside (1)** The hemiacetal (0.44 g, 0.96 mmol, 1.0 eq.) was dissolved in acetone, cooled to 0°C, followed by addition of ClC(=NPh)CF<sub>3</sub> (0.18 mL, 1.16 mmol, 1.2 eq.) and Cs<sub>2</sub>CO<sub>3</sub> (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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  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); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  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<sup>+</sup>, filtered and concentrated. The cleavage procedure was repeated and the combined fractions were combined and dissolved in a chilled Ac<sub>2</sub>O/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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  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);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  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  $\text{Et}_3\text{N}$  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  $\text{Et}_2\text{O}$  and washed with sat. aq. NaCl, dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The crude was coevaporated with toluene, dissolved in DMF (50 mL) and cooled to  $0^\circ\text{C}$ , 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  $\text{H}_2\text{O}$ , diluted with  $\text{Et}_2\text{O}$  and the organic layer was washed with sat. aq. NaCl. The organic layer was dried over  $\text{MgSO}_4$  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  $\text{Et}_2\text{O}$ , washed with sat. aq.  $\text{NaHCO}_3$ , dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. Column purification yielded alcohol **33** (6.7 g, 10.5 mmol, 74%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  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);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  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  $\text{tBuOH}/\text{DCM}/\text{H}_2\text{O}$  (42 mL/42 mL, 10.5 mL) and cooled to  $0^\circ\text{C}$ . 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  $\text{MgSO}_4$  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  $\text{K}_2\text{CO}_3$  (2.87 g, 20.8 mmol, 2 eq.). The reaction was stirred overnight, quenched with  $\text{H}_2\text{O}$ , diluted with  $\text{Et}_2\text{O}$ , and the organic layer was washed with sat. aq. NaCl. The mixture was dried over  $\text{MgSO}_4$  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%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  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);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  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/ $\text{H}_2\text{O}$  (100 mL/ 10 mL) and cooled to  $0^\circ\text{C}$ . 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.  $\text{NaHCO}_3$ . Column purification (PE/EtOAc) yielded the hemiacetal **35** (4.51 g, 6.89 mmol, 74%) as a  $\alpha/\beta$  mixture.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  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  $\text{ClC(=NPh)CF}_3$  (1.2 mL, 7.84 mmol, 1.2 eq.) followed by addition of  $\text{Cs}_2\text{CO}_3$  (3.2 g, 9.78 mmol, 1.5 eq.). The reaction was stirred overnight, after which it was diluted with EtOAc, washed with  $\text{H}_2\text{O}$ , dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. Column purification (PE/EtOAc, 10:1  $\rightarrow$  6:1) yielded the imidate donor **36** as a  $\alpha/\beta$  mixture (4.65 g, 5.63 mmol, 86%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  = 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- $\alpha$ -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  $\text{Et}_3\text{N}$  (0.8 mL), diluted with EtOAc and washed with sat. aq.  $\text{NaHCO}_3$ , dried over  $\text{MgSO}_4$  in concentrated *in vacuo*. Column purification (PE/Et<sub>2</sub>O) yielded the disaccharide as a 1:10  $\alpha/\beta$  mixture (2.31 g, 1.86 mmol, 90%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  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);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  = 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.

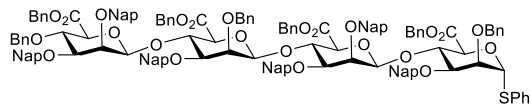
Compound **37** (0.23 g, 0.19 mmol, 1.0 eq.) was dissolved in DCM (1.9 mL) and cooled to 0°C. NIS (0.05 g, 0.22 mmol, 1.2 eq.) was added, followed by addition of TFA (0.02 mL, 0.22 mmol, 1.2 eq.). The reaction was stirred for 3h and quenched with  $\text{Et}_3\text{N}$  (0.05 mL, 0.372 mmol, 2 eq.). 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%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  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);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  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  $\text{ClC(=NPh)CF}_3$  (0.03 mL, 0.17 mmol, 1.2 eq.) followed by addition of  $\text{Cs}_2\text{CO}_3$  (0.07 g, 0.21 mmol, 1.5 eq.). The reaction was stirred overnight, after which it was diluted with EtOAc, washed with  $\text{H}_2\text{O}$ , dried over  $\text{MgSO}_4$  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%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  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);  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  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 Et<sub>3</sub>N (0.2 mL), diluted with DCM and washed with sat. aq. NaHCO<sub>3</sub>, dried over MgSO<sub>4</sub> in concentrated *in vacuo*. Size exclusion yielded the tetrasaccharide as a mixture (0.225 g, 0.098 mmol, 98%).  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  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);  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  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 C<sub>145</sub>H<sub>132</sub>O<sub>24</sub>SN 2302.88545, found 2302.88772.



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