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Novel protecting group strategies in the synthesis of oligosaccharides

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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.

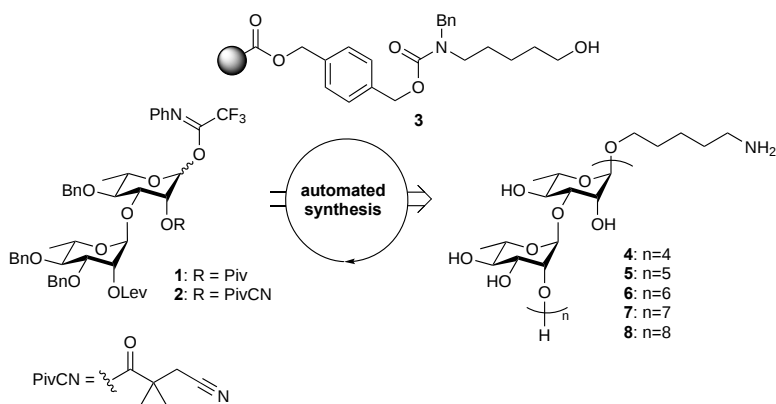
The key to any successful oligosaccharide synthesis campaign is the protection group strategy used. Permanent protecting groups should be able to withstand all conditions used throughout the assembly route, while temporary protecting groups have to be removed selectively without touching any other functionalities 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.



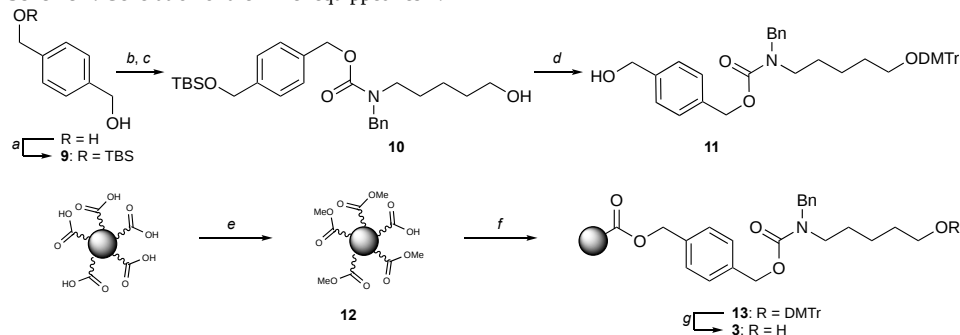
Results and discussion

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.



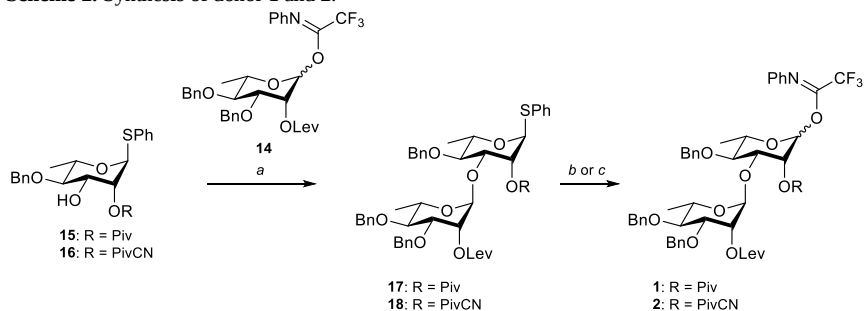
Reagents and conditions: TBDMS-Cl, imidazole, DMF, 0°C (30%); b) *para*-nitrophenylchloroformate, pyridine, 0°C, c) *N*-Benzyl-5-aminopentanol, DIPEA, DMF, 0°C (90%); d) i. DMTr-Cl, pyridine, 0°C, ii. TBAF, THF, 0°C (100%); e) TMSCHN₂, MeOH, THF; f) **11**, DIC, DMAP, DCM, then MeOH; g) TCA, DCM.

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

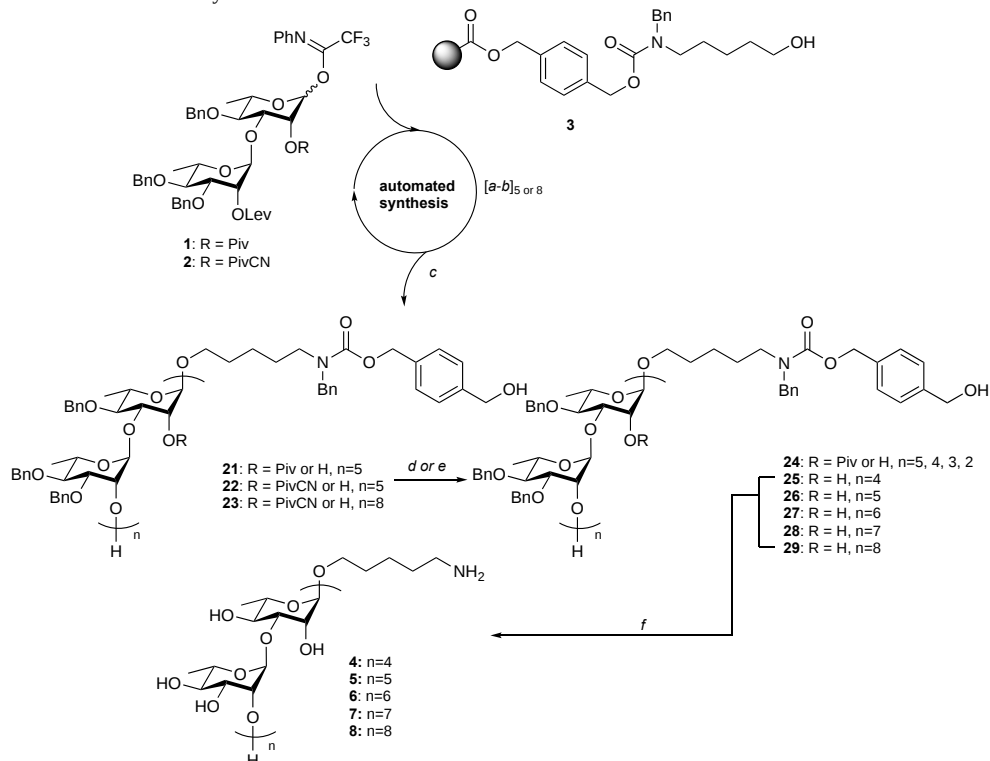
imidate donors by treatment with *N*-bromosuccinimide in acetone/water²¹ and subsequent installation of the *N*-phenyl trifluoroacetimidoyl functionality.

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%) .

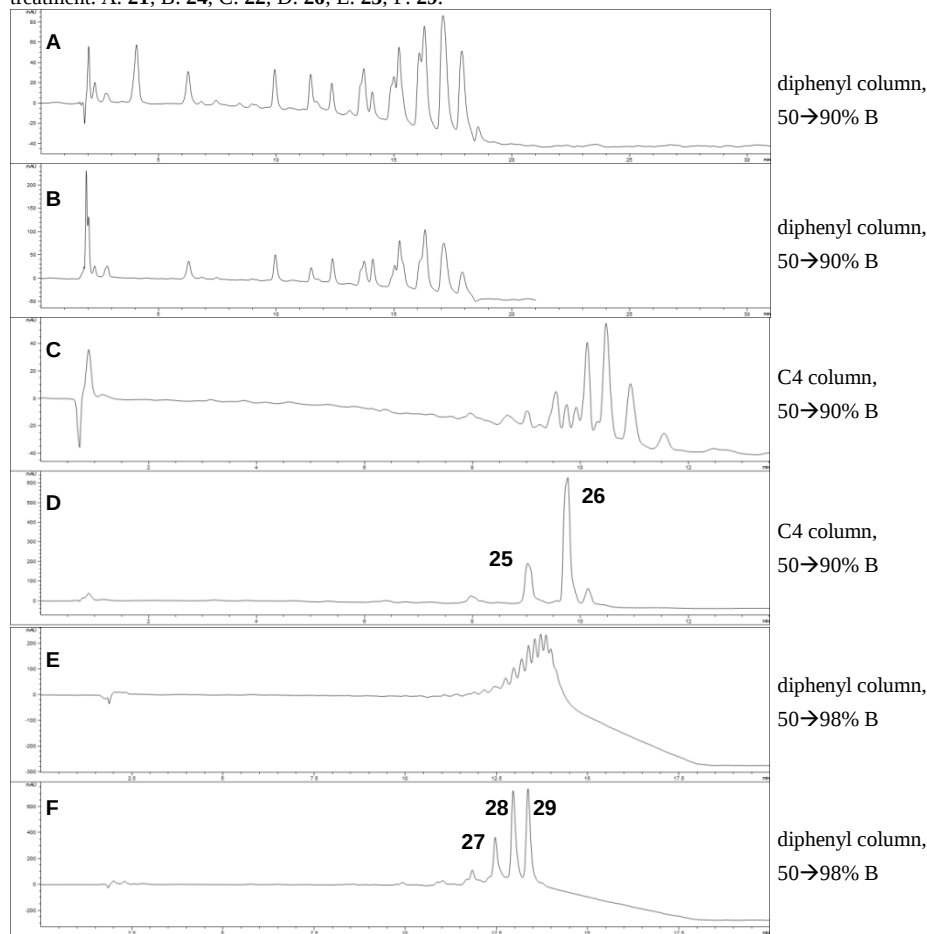
With the required building blocks in hand the assembly of the oligosaccharides was started. As a first research objective, the assembly of a decasaccharide was targeted employing the pivaloyl protected building block **1**. Previously developed glycosylation and deprotection conditions^{6,11} were applied 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**.



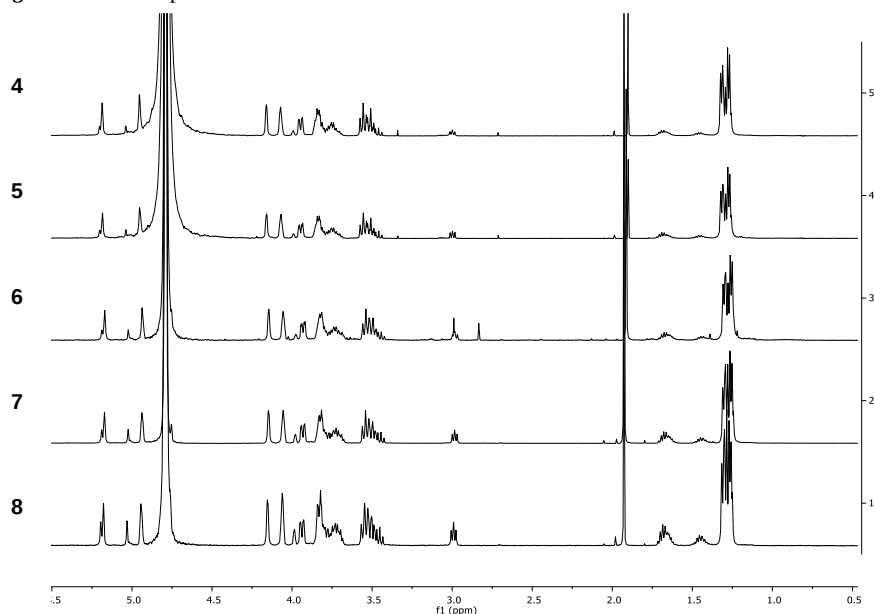
When donor **2** was used for the assembly of decasaccharide **22** again a complex product mixture arose after cleavage of the products from the resin (Figure 2C).²³ It was noted however that a significantly larger portion of the PivCN-groups had been removed from the target structures in comparison 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**.



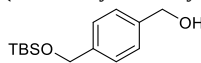
Conclusion

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

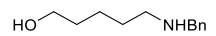
General experimental procedures. All solvents used under anhydrous conditions were stored over 4Å molecular sieves except for methanol which was stored over 3Å molecular sieves. ^1H and ^{13}C NMR spectra were recorded on a 400/100, 500/125, 600/150, or a 850/214 MHz spectrometer. Chemical shifts (δ) 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.

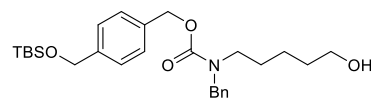
(4-*tert*-Butyl-dimethyl-siloxy)methylphenyl)methanol (9) 1,4-benzene-dimethanol (8.29 g, 60 mmol, 1.0 eq.)

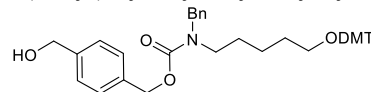


was dissolved in 25 mL DMF and cooled to 0°C followed by the addition of imidazole (10.2 g, 150 mmol, 2.5 eq.). A solution of *tert*-butyldimethylsilyl chloride (9.13 g, 60.6 mmol, 1.01 eq.) in 40 mL DMF was added dropwise and the reaction was allowed to stir overnight. After TLC analysis showed complete consumption of the starting material, the mixture was dilute 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

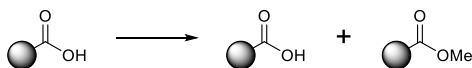
 **5-(benzyl(4-tert-butyltrimethylsilyl)oxymethylbenzyloxycarbonyl)amino)pentanol (10)** Silylether **9** (4.73 g, 18.7 mmol, 1.0 eq.) was dissolved in dry DCM (125 mL) and cooled to 0°C. Pyridine (3.0 mL, 37.5 mmol, 2.0 eq.) was added followed by addition of para-nitrophenylchloroformate (4.53 g, 22.5 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.)

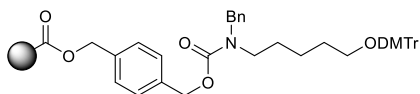
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was added and the green coloured reaction was stirred for 5h after which it was concentrated. The compound was dissolved in EtOAc, washed subsequently with H₂O, sat. aq. NaHCO₃ and sat. aq. NaCl. The organic layer was dried over MgSO₄ and concentrated *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)

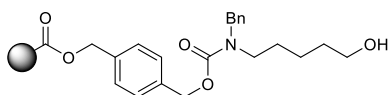


Carboxy Polystyrene (Rapp polymer, 5 g, 2.19 mmol/g, 11 mmol) was added to a fritted syringe and swollen in 32 mL DCM. The resin was purged with argon after which it was washed with DCM (3x), alternating DCM and hexane (3x), and DCM (2x). The resin was dried *in vacuo* at 45°C overnight. The dried resin 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.

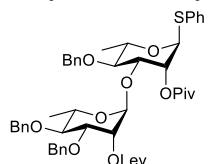
DMTr-assay (performed in duplicate) DMT-functionalized resin **13** (4.1 mg) was added to a 10 mL volumetric flask and treated with 10 mL 3% TCA/DCM (w/v). A 1 mL aliquot was taken and diluted 100x with the 3% TCA/DCM solution. Absorbance read at $\lambda = 503$ nm.

Loading calculation:

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

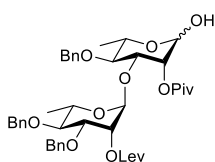
A loading of 0.44-0.47 mmol/g was determined.

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.

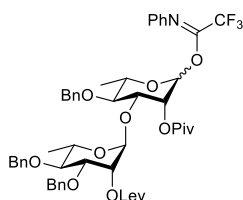
4-O-Benzyl-2-O-Pivaloyl-3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)- α/β -L-



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

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.

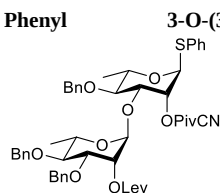
4-O-Benzyl-2-O-Pivaloyl-3-O-(3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl)-1-(N-phenyl-trifluoroacetimidoyl)- α / β -L-rhamnopyranoside (**1**)



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.

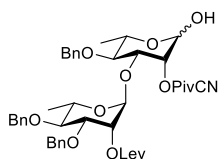
Phenyl



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.

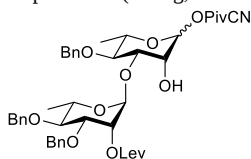
The reaction mixture was diluted with Et₂O and washed with sat. aq. NaHCO₃, H₂O and sat. aq. NaCl. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. A quick column purification (PE/EtOAc, 6:1 → 1:1) followed by crystallization from hot EtOH yielded the target disaccharide as a white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



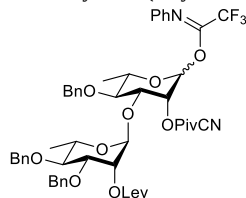
mmol, 1.1 eq.) was added followed by the dropwise addition of TFA (0.35 mL, 4.57 mmol, 1.1 eq.), after which the reaction turned purple. After 340 min, the reaction was quenched by addition of 50 mL sat. aq. Na₂S₂O₃. The mixture was diluted with 60 mL DCM and washed with 60 mL sat. aq. NaHCO₃. The aqueous 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-



rhamnopyranosyl)-1-(N-phenyl-trifluoroacetimidoyl)-

α/β -L-

rhamnopyranoside (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).

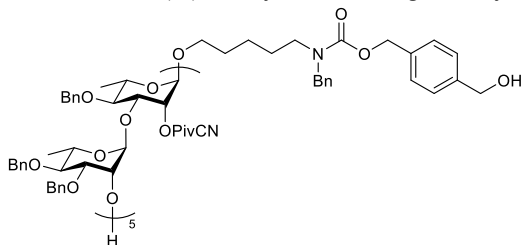
Method H. Suspending the resin for isolation

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.

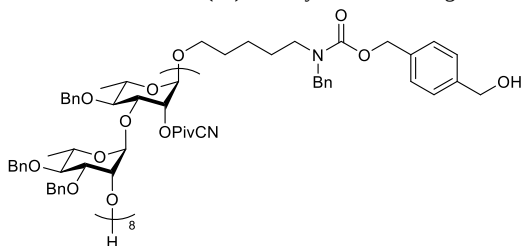
Method	# Cycles	Description	Time	Temperature
C	1	Swelling of the resin		RT
D	1	Coupling: 3 eq. donor, 0.3 eq. TfOH	60 min	0°C
E	3	Washing of the resin after coupling		0°C
F	3	Deblock: 8 eq. H ₂ NNH ₂ •AcOH	15 min	40°C
G	1	Washing of the resin after deblock		RT

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).

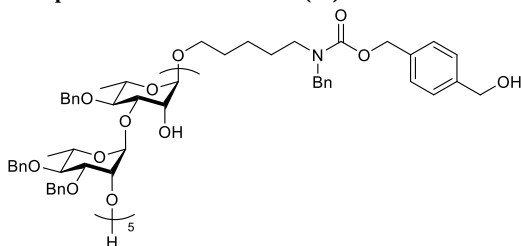


Hexadecarhamnoside (23) The dry resin was charged in a syringe with screw cap and suspended in THF/MeOH (2 mL, 1:1) followed by addition of NaOMe (0.08 mL, 0.54M NaOMe/MeOH, 1 eq.). The resin was shaken overnight. The solution was filtered and the remaining resin was washed with MeOH (5x 4 mL). The combined 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).



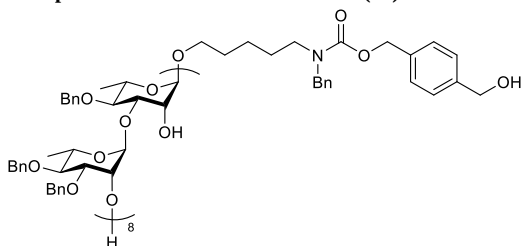
General procedure for complete removal of PivCN groups. The crude rhamnoside mixture was dissolved in THF/MeOH (0.6-2 mL, 1:1), and treated with a 0.54M NaOMe/MeOH (0.7-2 eq.) solution. The reaction was monitored by LC/MS and allowed to stir overnight. Additional 0.54M NaOMe/MeOH was added when LC/MS 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 → 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, 8H, 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

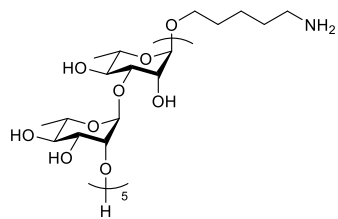


THF/MeOH (2 mL, 1:1) and treated with 0.10 mL NaOMe (0.54M NaOMe/MeOH). After 2h, an additional 0.24 mL NaOMe (0.54M NaOMe/MeOH) was added, followed by another 0.10 mL after 4h. After overnight stirring, LC/MS analysis indicated complete removal of all PivCN groups after which the mixture was neutralized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

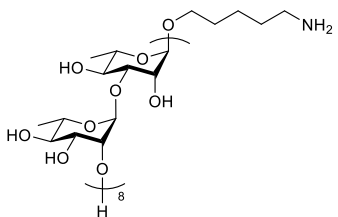
concentrated *in vacuo* and coevapotrated with toluene once. The target hexadecarhamnoside was isolated using RP-HPLC purification (C4 column, gradient 70 → 90 , 20 min per run) as a white solid (20.3 mg, 4.2 μ mol, 9.3% based on 45 μ mol resin). IR (neat): 750, 1051, 1129, 1454, 1671, 2917 cm^{-1} ^1H 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, 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, 72.2, 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.

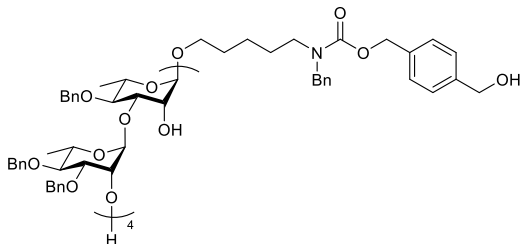


Hexadecarhamnoside (8) Compound **29** (7.2 mg, 1.5 μ mol) H₂O/THF/tBuOH (1.0 mL, 3:1.3:1.3) and 4-5 drops of AcOH were added. The solution was purged with N₂ for 5 min after which Pd(OH)₂/C (8 mg) was added, followed by another purge with N₂ for 5 min. The solution was purged for 5 min with H₂ and kept 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.



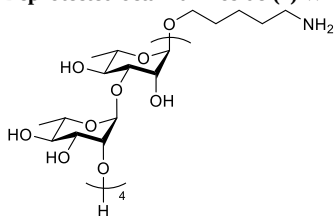
Isolation of deletion fragments

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.

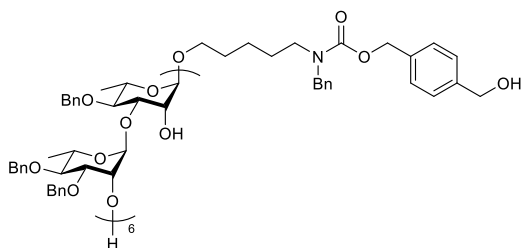
Deprotected octarhamnoside (4) White solid after general hydrogenation procedure (1.35 mg, 1.06 μmol , 69%).



^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.

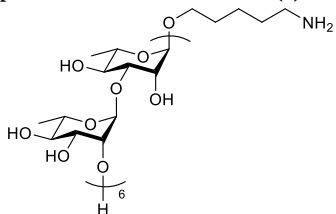
Dodecarhamnoside (27) Obtained as byproduct from **23**. (10.3 mg, 2.8 μmol). ^1H NMR (600 MHz, $\text{MeCN-}d_3$,



$T=328\text{K}$) 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,

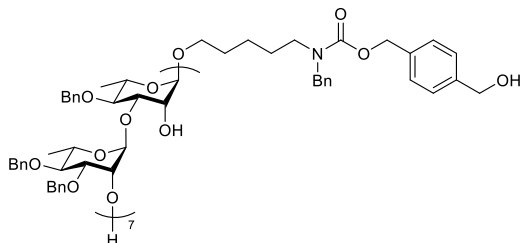
CD_3CN) δ 143.0, 140.3, 140.1, 140.1, 140.0, 140.0, 140.0, 140.0, 139.8, 137.3, 129.6, 129.5, 129.4, 129.4, 129.4, 129.4, 129.3, 129.3, 129.3, 129.3, 129.2, 129.2, 129.1, 129.1, 129.1, 129.0, 129.0, 129.0, 128.9, 128.8, 128.8, 128.7, 128.7, 128.7, 128.7, 128.6, 128.6, 103.1, 103.0, 102.9, 102.9, 102.9, 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.

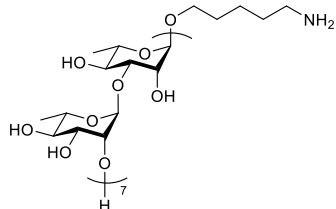
Tetradecarhamnoside (28) Obtained as byproduct from **23**. (20 mg, 4.65 μmol). ^1H NMR (600 MHz, $\text{MeCN-}d_3$,



$T=328\text{K}$): 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 $\text{C}_{89}\text{H}_{154}\text{NO}_{57}$ 2149.92135, found 2149.92197.

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Chapter 4

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