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Featured Article

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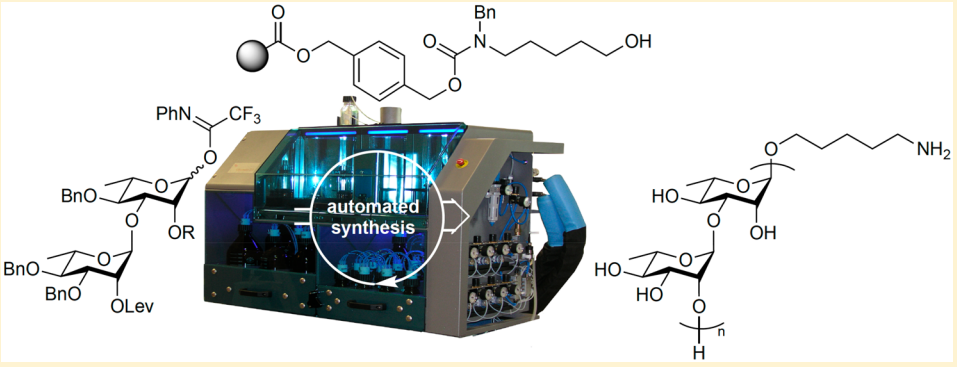
Cyanopivaloyl Ester in the Automated Solid-Phase Synthesis of Oligorhamnans

Anne Geert Volbeda, Jeanine van Mechelen, Nico Meeuwenoord, Herman S. Overkleeft, Gijsbert A. van der Marel, and Jeroen D. C. Codée*

Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Supporting Information



ABSTRACT: The development of effective protecting group chemistry is an important driving force behind the progress in the synthesis of complex oligosaccharides. Automated solid-phase synthesis is an attractive technique for the rapid assembly of oligosaccharides, built up of repetitive elements. The fact that (harsh) reagents are used in excess in multiple reaction cycles makes this technique extra demanding on the protecting groups used. Here, the synthesis of a set of oligorhamnan fragments is reported using the cyanopivaloyl (PivCN) ester to ensure effective neighboring group participation during the glycosylation events. The PivCN group combines the favorable characteristics of the parent pivaloyl (Piv) ester, stability, minimal migratory aptitude, minimal orthoester formation, while it can be cleaved under mild conditions. We show that the remote CN group in the PivCN renders the PivCN carbonyl more electropositive and thus susceptible to nucleophilic cleavage. This feature is built upon in the automated solid-phase assembly of the oligorhamnan fragments. Where the use of a Piv-protected building block failed because of incomplete cleavage, PivCN-protected synthons performed well and allowed the generation of oligorhamnans, up to 16 monosaccharides in length.

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 significant progress in the field of automated solid-phase oligosaccharide synthesis over the past 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 structures,[5] hyaluronic acid fragments up to 15 monosaccharides in length,[6] a 50-mer polymannoside,[7] a set of dermatan[8] and keratan sulfates,[9] a set of α-glucans,[10] and a collection of mannuronic acid alginates, built 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 conditions harsher than those used in traditional solution-phase experiments, because often an excess of reagents is used

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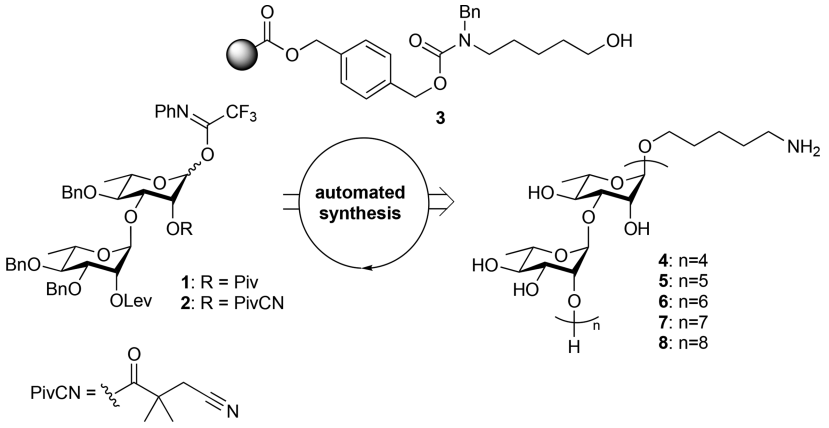
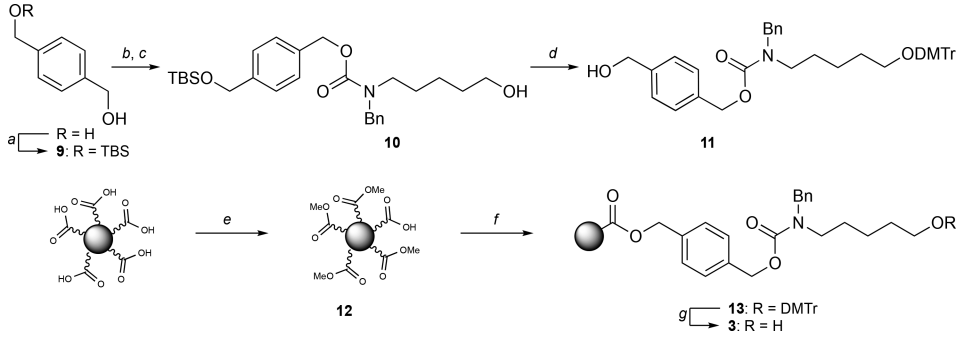


Figure 1. Synthetic approach described in this work.

Scheme 1. Generation of the Linker-Equipped Resin[a]



[a]Reagents and conditions: (a) 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) $TMSCHN_2$, MeOH, THF; (f) **11**, DIC, DMAP, DCM, then MeOH; (g) TCA, DCM.

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.

We introduced the cyanopivaloyl (PivCN) group as an attractive participating group that allows for the reliable construction of 1,2-*trans*-glycosidic linkages.[12] It features the favorable characteristics of the pivaloyl ester—stability, effective neighboring group participation, minimal othoester 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 favorable 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, we explored its use in the assembly of a set of oligorhamnosides, up to 16 monosaccharides in length (see Figure 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 (pharyngitidis, 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 hydroxyl.[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 nonmammalian "bare" polyrhamnose backbone, devoid of GlcNAc groups, may be an attractive structure for a GAS vaccine. Well-defined fragments of the polyrhamnose backbone will be valuable in the generation of semisynthetic vaccines and therefore represent attractive synthetic targets.[16−22] The repetitive nature of these molecules makes them very well suited for an automated synthesis approach.

■ RESULTS AND DISCUSSION

The synthetic strategy and test case for the cyanopivaloyl group for the assembly of the oligorhamnosides are depicted in Scheme 1. In this study, a commerical Glyconeer 2.1 synthesizer was used for the automated assembly. The oligosaccharides are built on a polystyrene resin equipped with a linker system[23] 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 proce-

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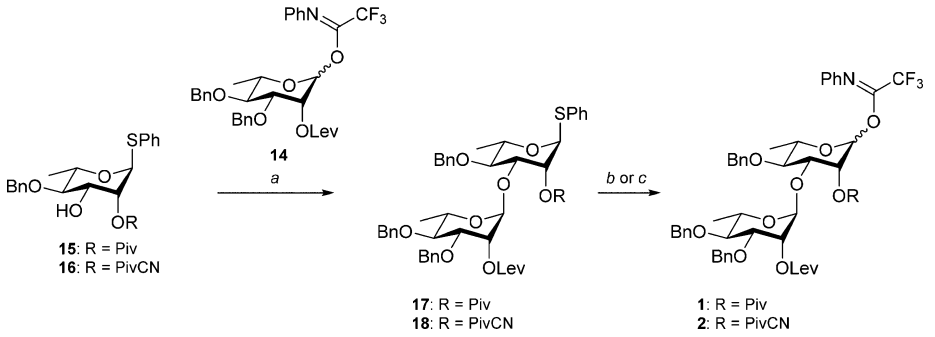
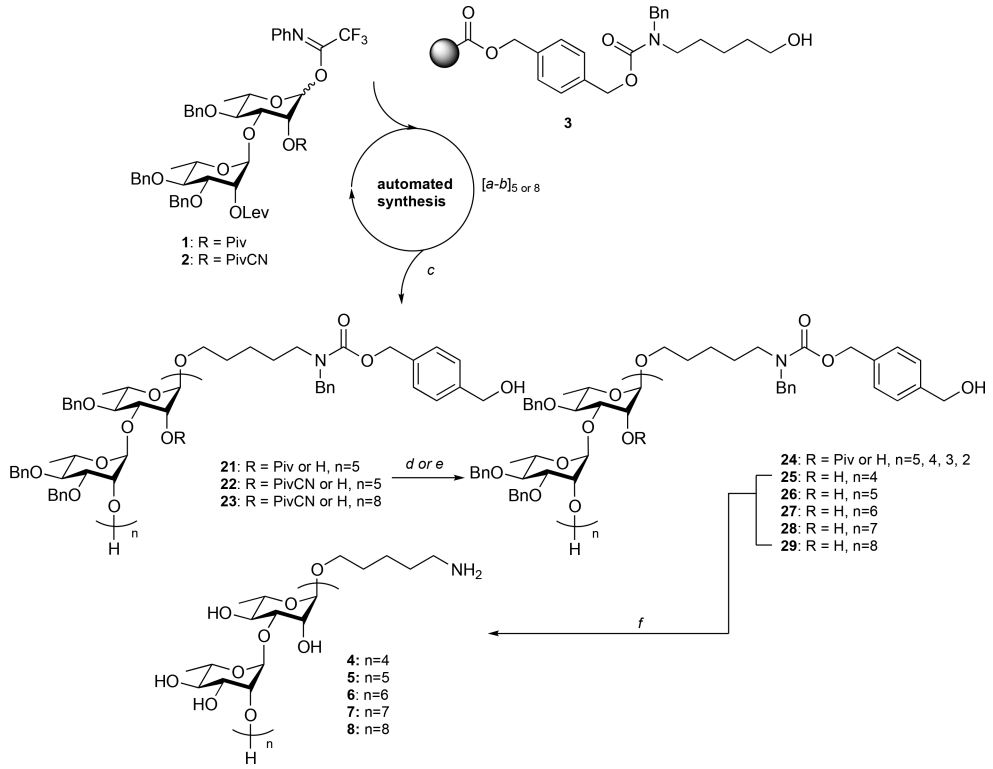
Scheme 2. Synthesis of Donors 1 and 2[a]



[a]Reagents and conditions: (a) **14**, TfOH, DCM, 0 °C (**17**, 69%, **18**, 88%); (b) NBS, acetone/H_2O (**19**, 65%, **20**, 89%), then $ClC(=NPh)CF_3$, Cs_2CO_3, acetone, 0 °C (**1**, 88%, **2**, 79%); (c) NIS, TFA, DCM, 0 °C (**20a**, 73%), then $ClC(=NPh)CF_3$, Cs_2CO_3, acetone, 0 °C (**2**, 79%).

Scheme 3. Automated Synthesis of Decamers 21 and 22 and Hexadecamer 23[a]



[a]Reagents and conditions: (a) 3 equiv of **1** or **2**, 0.3 equiv of TfOH, DCM, 0 °C, 3 cycles; (b) 8 equiv of H_2NNH_2·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) H_2, $Pd(OH_2)$/C, AcOH, H_2O/THF/*t*BuOH (**4**, 69%, **5**, 57%, **6**, 27%, **7**, 92%, **8**, 50%).

dures.[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 inseparable 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 and the second (building block **2**) with a cyanopivaloyl at this position.

The linker-functionalized resin **3** is obtained in seven steps from 1,4-benzenedimethanol, following an improved route of synthesis, originally developed by Czechura et al., as depicted in Scheme 1.[23] After silylation of one hydroxyl group (30% 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 on 16 mmol scale.

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.[24,25] Afterward, the resulting resin was coupled with the DMT-protected linker. Removal of the DMTr group was achieved by a TCA/DCM

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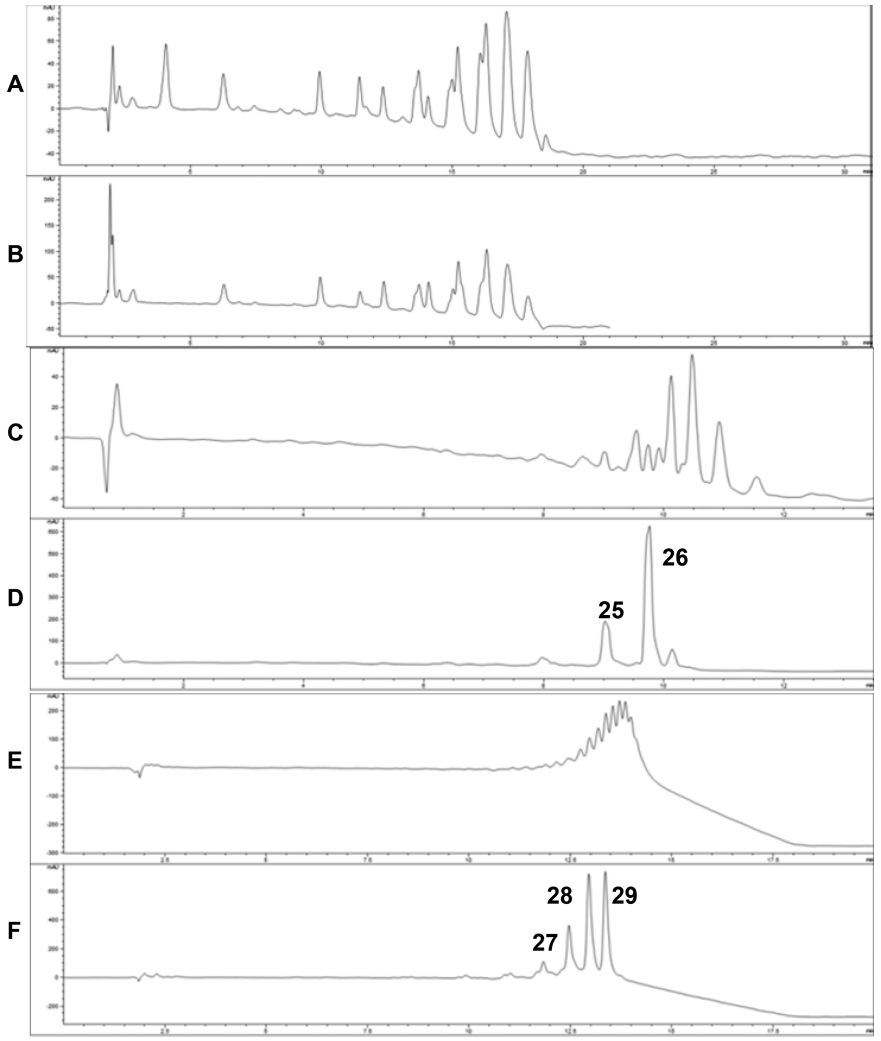

Figure 2. LC chromatogram of the crude products cleaved from the resin before and after prolonged base treatment: (A) **21**; (B) **24**; (C) **22**; (D) **26**; (E) **23**; (F) **29**. Reagents and conditions: (A) diphenyl column, 50 → 90% B; (B) diphenyl column, 50 → 90% B; (C) C4 column, 50 → 90% B; (D) C4 column, 50 → 90% B; (E) diphenyl column, 50 → 98% B; (F) diphenyl column, 50 → 98% B.

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**[26]/**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/water[21] and subsequent installation of the *N*-phenyl trifluoroacetimidoyl functionality.[27]

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 × 3 equiv of donor, 0.2 equiv of TfOH with respect to the donor, 30 min at 0 °C, Scheme 3), followed by removal of the Lev group (3 × 5 equiv of H_2NNH_2·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).

The crude decasaccharide **21** was analyzed by LC-MS, and the obtained LC-spectrum is shown in Figure 2. A complex 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 (NaOH in MeOH/dioxane at elevated temperature (conditions e, Scheme 3),[28] and the desired decasaccharide could not be obtained from the complex reaction mixture (Figure 2B). The use of pivaloyl-funtionalized donor **1** was therefore not further explored, and attention was switched to the use of its PivCN counterpart **2**.

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).[29] 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 renders 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.[30] This also suggested that the PivCN groups could potentially be removed by an additional and/or elongated base

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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 chromatogram 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**, whereas the other minor 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 eight coupling/deprotection cycles using donor **2**. After cleavage of the products from the resin, 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 $Pd(OH)_2/C$ in H_2O/THF/*t*BuOH 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.

CONCLUSION

We have 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 SECTION

General Experimental Procedures. All solvents used under anhydrous conditions were stored over 4 Å molecular sieves except for methanol, which was stored over 3 Å 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 parts per million relative to tetramethylsilane as internal standard. Coupling constants are given in hertz. 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, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L), and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulfuric acid, 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 approximately 150 °C. Column chromatography was performed on Fluka silica gel (0.04−0.063 mm). For LC-MS analysis, an 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 × 4.60 mm, 5 μm) or a Vydac Diphenyl (Alltech, 150 × 4.60 mm, 5 μm) in combination eluents A (H_2O), (B) MeCN, and (C) 1% aqueous TFA. For HPLC, a Gilson HPLC system in combination with eluents A (H_2O, 0.1% TFA) and B (MeCN as the solvent system using a Vydac C4 HPLC column; Grace, 250 × 10 mm, 5 μm). High-resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150−2000) and dioctyl phthalate (m/z = 391.2842) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). MALDI spectra were recorded on an Ultraflextreme MALDI-TOF (Bruker Daltonics), equipped with Smartbeam-II laser, to measure the samples in reflectron positive ion mode. The MALDI-TOF was calibrated using a peptide calibration standard prior to measurement. One microliter of 2,5-dihydroxybenzoic acid (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 to MALDI-TOF analysis.

(4-*tert*-Butyldimethylsiloxylmethylphenyl)methanol (9). 1,4-Benzenedimethanol (8.29 g, 60 mmol, 1.0 equiv) was dissolved in 25 mL of DMF and cooled to 0 °C followed by the addition of imidazole (10.2 g, 150 mmol, 2.5 equiv). A solution of *tert*-butyldimethylsilyl chloride (9.13 g, 60.6 mmol, 1.01 equiv) in 40 mL of DMF was added dropwise, and the reaction was allowed to stir overnight. After TLC analysis showed complete consumption of the starting material, the mixture was diluted with Et_2O and washed subsequently with H_2O (2×) and saturated aqueous NaCl (1×). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification using flash column chromatography (PE/EtOAc, 9:1 → 6:1) yielded the title compound as a colorless oil (4.73 g, 18.7 mmol, 30%): 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.[31]

***N*-Benzyl-5-aminopentanol.** Benzaldehyde (10.67 mL, 104.6 mmol, 1.01 equiv) was added to a solution of 5-aminopentalnol (11.3 mL, 104.0 mmol, 1.0 equiv) in 150 mL of EtOH. The solution was heated to 50 °C under reduced pressure until all solvent was removed. The crude mixture was coevaporated twice with anhydrous toluene, dissolved in MeOH (200 mL), and cooled to 0 °C. $NaBH_4$ (4.82 g, 124.7 mmol, 1.2 equiv) was added in portions, and the solution was allowed to stir at 0 °C for 70 min. After being stirred for another 2 h, the solution was cooled to 0 °C followed by addition of 4.5 mL of AcOH. A 1.2 M K_2CO_3 (aq) solution (135 mL) was added, and the mixture was diluted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification using flash column chromatography yielded the linker in 62% yield (12.4 g, 64 mmol): ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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.[32]

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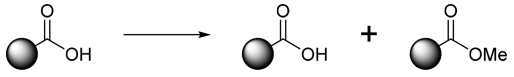
5-(Benzyl(4-*tert*-butyldimethylsilyl)oxymethylbenzyloxycarbonyl)amino)pentanol (10). Silylether **9** (4.73 g, 18.7 mmol, 1.0 equiv) was dissolved in dry DCM (125 mL) and cooled to 0 °C. Pyridine (3.0 mL, 37.5 mmol, 2.0 equiv) was added followed by addition of *para*-nitrophenylchloroformate (4.53 g, 22.5 mmol, 1.2 equiv), after which the solution was allowed to warm 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 equiv) in DMF (20 mL) followed by addition of DIPEA (4.23 mL, 24.4 mmol, 1.3 equiv) The reaction mixture was stirred overnight, diluted with Et_2O, and washed with H_2O. The aqueous layer was back extracted with Et_2O, and the combined organic layers were washed multiple times with saturated aqueous $NaHCO_3$. The solution was dried over $MgSO_4$, 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, $CDCl_3$) δ 7.21−7.10 (m, 9H, CH_{arom}), 5.06 (s, 2H, CH_2 Cbz), 4.63 (s, 2H, CH_2 Bn), 4.39 (s, 2H, CH_2 Cbz), 3.46 (s, 2H, CH_2), 3.15 (s, 2H, CH_2), 1.41−1.20 (s, 6H, 3 × CH_2), 0.85 (s, 9H, 3 × CH_3 TBDMS), 0.00 (s, 6H, 2 × CH_3 TBDMS); ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.5, 138.3, 135.7 (Cq), 128.7, 128.1, 127.5, 126.4 (CH_{arom}), 67.3 (CH_2), 65.0 (CH_2), 62.9 (CH_2), 50.7 (CH_2), 32.6 (CH_2), 26.1 (3 × CH_3 TDBDMS), 23.2 (CH_2), −5.1 (2 × CH_3 TBDMS); HRMS $[M + H]^+$ calcd for $C_{27}H_{42}NO_4Si$ 472.2878, found 472.2877.

5-(Benzyl(4-hydroxymethylbenzyloxycarbonyl)amino)-pentyldimethoxytrityl ether (11). Silylether **10** (7.50 g, 15.9 mmol, 1.0 equiv) 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 equiv), and it was allowed to stir overnight. After being stirred overnight, TLC analysis (hexanes/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 H_2O, dried over $MgSO_4$, 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 equiv) was added, and the green colored reaction was stirred for 5 h, after which it was concentrated. The compound was dissolved in EtOAc, washed subsequently with H_2O, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (Tol/EtOAc + Et_3N, 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, $CDCl_3$, T = 328 K) 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, CH_2 Cbz), 4.63 (s, 2H, CH_2 Bn), 4.46 (s, 2H, CH_2 Cbz), 3.76 (s, 6H, 2 × CH_3 OMe), 3.21 (s, 2H, CH_2), 3.02 (s, 2H, CH_2), 1.69−1.24 (m, 6H, 3 × CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) 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 (CH_2), 65.1 (CH_2), 63.4 (CH_2), 55.3 (OMe), 29.9 (CH_2), 23.8 (CH_2); HRMS $[M + Na]^+$ calcd for $C_{42}H_{45}NO_6Na$ 682.3139, found 682.3139.

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 of DCM. The resin was purged with argon, after which it was washed with DCM (3×), alternating DCM and hexane (3×), and DCM (2×). The resin was dried in vacuo at 45 °C overnight. The dried resin was suspended in 60 mL of THF, and MeOH (1.03 mL, 25.4 mmol, 3 equiv with respect to Me_3SiCHN_2) was added. The suspension was shaken for 10 min followed by addition of Me_3SiCHN_2 (4.24 mL of 2.0 M solution in hexanes, 8.47 mmol, 0.77 equiv 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 (4×), hexanes (4×), and THF (4×) and dried in vacuo at 45 °C.

Carboxy polystyrene **12** (Rapp polymer, 5 g, ∼0.51 mmol/g, 2.54 mmol) was swollen in DCM (60 mL), and the suspension was shaken for 1 h. The solution was filtered, and DCM (40 mL) was added to the resin. Compound **11** (5.04 g, 7.64 mmol, 3 equiv) was coevaporated twice with toluene under argon, dissolved in DCM (8.5 mL), with addition of DIC (1.20 mL, 7.64 mmol, 3 equiv) and DMAP (0.03 g, 0.25 mmol, 0.1 equiv). An additional rinse with 5 mL of 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 (4×), followed by DCM (3×). 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 4×. After the TCA washes, the orange resin was washed 3× with DCM (60 mL), 3× with toluene (60 mL), 3× with DCM/MeOH (60 mL), 1× with MeOH (60 mL), and 4× 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 of 3% TCA/DCM (w/v). A 1 mL aliquot was taken and diluted 100× with the 3% TCA/DCM solution. Absorbance read at λ = 503 nm.

Loading calculation:

$$\frac{[(A_{503})(100\ \text{mL})]}{76} = \text{mmol in final solution}$$

$$\left(\frac{\text{mmol in final solution}}{\text{volume aliquot}}\right) \times 10\ \text{mL} = \text{mmol in initial solution}$$

$$= \frac{(0.137)(100\ \text{mL})}{76\ \text{mL/μmol}} = 0.180\ \text{μmol}$$

$$= \left(\frac{0.00018\ \text{mmol}}{1\ \text{mL}}\right) \times 10\ \text{mL} = 0.00180\text{mmol}$$

$$\text{loading} = \frac{0.00180\ \text{mmol}}{0.0041\ \text{g}} = 0.44\ \text{mmol/g}$$

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**[26] (4.96 g, 11.52 mmol, 1.0 equiv) and imidate donor **14** (7.56 g, 13.32 mmol, 1.2 equiv) 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 equiv) was added, and after 135 min, TLC analysis showed complete consumption of the acceptor, and the reaction was quenched by addition of 0.3 mL of Et_3N. The mixture was diluted with EtOAc, washed subsequently with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$, 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%): mp 145 °C; 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, $CDCl_3$) 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, CH*H* Bn), 4.78 (d, J = 10.8 Hz, 1H, CH*H* Bn), 4.67−4.54 (m, 3H, C*H*H, CH_2 Bn), 4.46 (d, J = 11.5 Hz, 1H, C*H*H Bn), 4.26−4.16 (m, 1H, H-5), 4.11 (dd, J = 9.4, 3.1 Hz, 1H, H-3), 3.81 (dd,

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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, 2 × CH_2 Lev), 2.13 (s, 3H, CH_3 Lev), 1.33−1.22 (m, 6H, 2 × CH_3-6), 1.20 (s, 9H, 3 × CH_3 Piv); ^{13}C NMR (126 MHz, $CDCl_3$) 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 (CH_2 Bn), 74.8 (CH_2 Bn), 73.6 (C-2), 71.5 (CH_2 Bn), 69.3 (C-5), 69.1 (C-2′), 68.5 (C-5′), 39.0 (Cq Piv), 38.0 (CH_2 Lev), 29.8 (CH_3 Lev), 28.1 (CH_2 Lev), 27.1 (CH_3 Piv), 17.9 (C-6), 17.8 (C-6′); HRMS [M + H]$^+$ calcd for $C_{49}H_{62}NO_{11}S$ 872.4038, found 872.4045.

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 equiv) was dissolved in acetone/H_2O (3:1, 16 mL) and cooled to 0 °C. NBS (1.73 g, 9.69 mmol, 3.0 equiv) 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 saturated aqueous $Na_2S_2O_3$. The mixture was diluted with EtOAc, and the organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, 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: 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}; ^{1}H NMR (500 MHz, $CDCl_3$) 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, CH*H* Bn), 4.74 (d, J = 10.8 Hz, 1H, CH*H* Bn), 4.66−4.51 (m, 3H, C*H*H Bn, CH_2 Bn), 4.43 (d, J = 11.5 Hz, 1H, C*H*H 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, 2 × CH_2 Lev), 2.15 (s, 3H, CH_3 Lev), 1.29−1.17 (m, 15H, 2 × CH_3 C-6, C-6′, 3 × CH_3 Piv); ^{13}C NMR (126 MHz, $CDCl_3$) 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 (CH_2 Bn), 74.9 (CH_2 Bn), 72.5 (C-2), 71.6 (CH_2 Bn), 69.4 (C-2′), 68.4, 67.7 (C-5, C-5′), 39.0 (Cq), 38.1 (CH_2 Lev), 29.9 (CH_3 Lev), 28.2 (CH_2 Lev), 27.2 (3 × CH_3 Piv), 18.2, 17.9 (2 × CH_3 C-6, C-6′); HRMS [M + Na]$^+$ calcd for $C_{43}H_{54}O_{12}Na$ 785.3508, found 785.3511.

4-*O*-Benzyl-2-*O*-pivaloyl-3-*O*-(3,4-di-*O*-benzyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl)-1-(*N*-phenyltrifluoroacetimidoyl)-α/β-L-rhamnopyranoside (1). To a solution of hemiacetal **19** (1.66 g, 2.18 mmol, 1.0 equiv) in acetone (11 mL) at 0 °C were added *N*-phenyltrifluoroacetimidoyl chloride (0.41 mL, 2.62 mmol, 1.2 equiv) followed by Cs_2CO_3 (1.07 g, 3.27 mmol, 1.5 equiv). The solution was allowed to stir for 3 h, 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: 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}; ^{1}H NMR (400 MHz, $CDCl_3$) 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, CH*H* Bn), 4.77 (d, J = 10.7 Hz, 1H, CH*H* Bn), 4.69−4.54 (m, 3H, C*H*H Bn, CH_2 Bn), 4.47 (d, J = 11.5 Hz, 1H, C*H*H 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, 2 × CH_2 Lev), 2.15 (s, 3H, CH_3 Lev), 1.39−1.11 (m, 15H, 2 × CH_3 C-6, C-6′, 3 × CH_3 Piv); ^{13}C NMR (101 MHz, $CDCl_3$) 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 (CH_2 Bn), 74.9 (CH_2 Bn), 71.7 (CH_2 Bn), 70.5 (C-2), 70.5 (C-5), 69.4 (C-2′), 68.7 (C-5′), 39.1 (Cq), 38.1 (CH_2 Lev), 29.9 (CH_3 Lev), 28.2 (CH_2 Lev), 27.2 (3 × CH_3 Piv), 18.2, 17.9 (2 × CH_3 C-6, C-6′); HRMS [M + Na]$^+$ calcd for $C_{51}H_{58}F_3NO_{12}Na$ 956.3803, found 956.3809.

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 equiv) and acceptor **16** (2.70 g, 5.92 mmol, 1.0 equiv) 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 (3 Å). The reaction was cooled to 0 °C, and TfOH (0.05 mL, 0.59 mmol, 0.1 equiv) was added. After 50 min, the reaction was quenched by addition of 1.0 mL of Et_3N. The reaction mixture was diluted with Et_2O and washed with saturated aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$ 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%):[12] mp 106 °C.

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 equiv) was dissolved in acetone/H_2O (1.2 mL/0.4 mL) and cooled to 0 °C. NBS (0.16 g, 0.899 mmol, 3.0 equiv) was added, and the reaction was stirred for 3 h, after which TLC analysis showed conversion of the starting material in a lower running spot. The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ and diluted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried over $MgSO_4$, 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: ^{1}H NMR (500 MHz, $CDCl_3$) 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, CH*H* Bn), 4.76 (d, J = 10.9 Hz, 1H, CH*H* Bn), 4.68−4.54 (m, 3H, CH_2 Bn, C*H*H Bn), 4.46 (d, J = 11.9 Hz, 1H, C*H*H 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, CH_2 Lev), 2.58−2.40 (m, 2H, CH_2 PivCN), 2.15 (s, 3H, CH_3 Lev), 1.37−1.17 (m, 12H, 2 × CH_3 PivCN, 2 × CH_3-6); ^{13}C NMR (126 MHz, $CDCl_3$) 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 (CH_2 Bn), 75.0 (CH_2 Bn), 73.6 (C-2), 71.3 (CH_2 Bn), 69.1 (C-2′), 68.5 (C-5 or C-5′), 67.8 (C-5 or C-5′), 41.0 (Cq), 38.1 (CH_2 Lev), 29.9 (CH_3 Lev), 29.6 (CH_2 Lev), 28.2 (CH_2 PivCN), 27.8 (CH_2 PivCN), 24.9, 24.8 (2 × CH_3 PivCN), 18.1, 17.9 (2 × CH_3 C-6, C-6′).

Anomeric PivCN (20a). Compound **18** (3.65 g, 4.15 mmol, 1 equiv) was dissolved in DCM (40 mL) and cooled to 0 °C. NIS (1.03 g, 4.57 mmol, 1.1 equiv) was added followed by the dropwise addition of TFA (0.35 mL, 4.57 mmol, 1.1 equiv), after which the reaction turned purple. After 340 min, the reaction was quenched by addition of 50 mL of saturated aqueous $Na_2S_2O_3$. The mixture was diluted with 60 mL of DCM and washed with 60 mL of saturated aqueous $NaHCO_3$. The aqueous layers were extracted 2× with DCM, and the combined organic layers were washed with saturated aqueous NaCl, dried over $MgSO_4$, 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): 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; ^{1}H 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); ^{13}C NMR (126 MHz, $CDCl_3$) 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,

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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, 128.4, 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, 127.8, 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, 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 $C_{44}H_{57}N_2O_{12}$ 805.3906, found 805.3908.

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*-phenyltrifluoroacetimidoyl)-*α*/*β*-L-rhamnopyranoside (2). To a solution of mixture hemiacetal **20** and **20a** (4.15 g, 5.27 mmol, 1 equiv) in acetone (26 mL) at 0 °C were added *N*-phenyltrifluoroacetimidoyl chloride (0.98 mL, 6.32 mmol, 1.2 equiv) followed by Cs_2CO_3 (2.57 g, 7.9 mmol, 1.5 equiv). The solution was allowed to stir overnight, after which it was diluted with EtOAc and washed subsequently with H_2O and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (PE/EtOAc, 6:1 → 1:1) yielded the title compound as a clear yellow oil (3.98 g, 4.15 mmol, 79%): 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, CH*H* Bn), 4.79 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.66−4.54 (m, 3H, C*H*H, CH_2 Bn), 4.48 (dd, *J* = 11.8, 3.2 Hz, 1H, C*H*H 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, 2 × CH_3 PivCN, 2 × 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 (2 × CH_3 PivCN), 18.2 (C-6), 18.0 (C-6′); HRMS $[M + NH_4]^+$ calcd $C_{52}H_{61}F_3N_3O_{12}$ 976.4202, found 976.4205.

Methods for Automated Synthesis. The washing solvents were predried 24 h before use on 4 Å molecular sieves and were of HPLC grade. Activator and deblock solutions were freshly prepared using the predried 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 was applied from the bottom of the RV, suspending the resin in solution. The argon flow was applied for 15 s, after which the RV was emptied to the waste.

Method B. *Agitation of the resin during coupling/deblock.* After addition of the solvent, an argon-flow was applied from the bottom of the RV for 10 s, suspending the resin in the solution. After 10 s, the argon flow was interrupted, and the resin was allowed to settle for 20 s.

Method C. *Swelling of the resin.* Dry resin was applied to the RV and washed with DCM (3×), alternating THF/hexane (3×), THF (1×), and DCM (3×).

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

Method E. *Deblock cycle.* The resin was washed with DMF (4 × 3 mL), running method A. The deblock solution was added (3 mL), and the temperature was set to 40 °C, followed by a 5 min incubation applying method B. The temperature was kept at 40 °C, after which the solid support was incubated 10 min applying protocol B. Then the RV was emptied to the waste. The resin was washed with DMF (3 × 3 mL), running method A.

Method F. *Washing of the resin after coupling.* The temperature was set to 20 °C. The resin was washed with MeOH (3 × 2 mL), alternating THF/hexane (6 × 2 mL), THF (2 × 2 mL), and DCM (5 × 3 mL), all applying method A.

Method G. *Washing of the resin after deblock.* The temperature was set to 20 °C. The resin was washed with DMF (4 × 3 mL), DCM (4 × 3 mL), alternating THF/hexane (6 × 3 mL), 0.01 M AcOH in THF (6 × 3 mL), THF (4 × 3 mL), and DCM (8 × 5 mL).

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

Table 1

method	no. of cycles	description	time	temperature
C	1	swelling of the resin		rt
D	1	coupling: 3 equiv of donor, 0.3 equiv of TfOH	60 min	0 °C
E	3	washing of the resin after coupling		20 °C
F	3	deblock: 8 equiv of H_2NNH_2·AcOH	15 min	40 °C
G	1	washing of the resin after deblock		20 °C

Automated Synthesis of Rhamnose Fragments. The reaction vessel was charged with carboxy polystyrene **3** (100 mg, 45 *μ*mol), and method C was applied to prepare the resin for synthesis. Then methods D and E for coupling and deprotection were 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 the solid support, the rhamnose fragments were analyzed by LC/MS.

Decarhamnoside (22). The dry resin was charged in a syringe with a screw cap and suspended in THF/MeOH (2 mL, 1:1) followed by addition of NaOMe (0.08 mL, 0.54 M NaOMe/MeOH, 1 equiv). The resin was shaken overnight. The solution was filtered, and the remaining resin was washed with MeOH (5 × 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 a screw cap and suspended in THF/MeOH (2 mL, 1:1) followed by addition of NaOMe (0.08 mL, 0.54 M NaOMe/MeOH, 1 equiv). The resin was shaken overnight. The solution was filtered, and the remaining resin was washed with MeOH (5 × 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 0.54 M NaOMe/MeOH (0.7−2 equiv) solution. The reaction was monitored by LC/MS and allowed to stir overnight. An additional 0.54 M 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

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supra. Purification by size exclusion chromatography (LH20, eluted with DCM/MeOH, 1/1, v/v) or HPLC yielded the target rhamnoside fragments.

Semiprotected Decarhamnoside (26). The crude rhamnoside mixture (0.162 g) was dissolved in THF/MeOH (4 mL, 1:1) and treated with 0.16 mL of NaOMe (0.54 M 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 of NaOMe (0.54 M NaOMe/MeOH), and stirred overnight. After overnight stirring, 0.08 mL of NaOMe (0.54 M NaOMe/MeOH) was added, followed by 0.16 mL of NaOMe (0.54 M NaOMe/MeOH) after 6.5 h, 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 μmol, 26% based on 45 μmol resin): IR (neat) 736, 1028, 1041, 1070, 1126, 1207, 1361, 1454, 1496, 1681, 2927, 3030, 3377 cm^{-1}; ^1H NMR (500 MHz, MeCN-d_3, T = 328 K) δ 7.42−7.18 (m, 84H, CH_{arom}), 5.11−5.06 (m, 4H, CH_2 linker-CBz, 2 × 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, MeCN-d_3, T = 328 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 (10 × C-3, 10 × 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 × C-2), 69.6, 69.6, 69.3, 69.0, 68.3, 67.8 (10 × 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 (10 × CH_3-6); HRMS $[M + NH_4]^+$ calcd for $C_{186}H_{221}N_2O_{44}$ 3188.5173, found 3188.5121.

Semiprotected Hexadecarhamnoside (29). The crude rhamnoside mixture (0.199 g) was dissolved in THF/MeOH (2 mL, 1:1) and treated with 0.10 mL of NaOMe (0.54 M NaOMe/MeOH). After 2 h, an additional 0.24 mL of NaOMe (0.54 M NaOMe/MeOH) was added, followed by another 0.10 mL after 4 h. 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 μmol, 9.3% based on 45 μmol resin): IR (neat) 750, 1051, 1129, 1454, 1671, 2917 cm^{-1}; ^1H NMR (600 MHz, MeCN-d_3, T = 328 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, MeCN-d_3, T = 328 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, 72.8, 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 $[M + Na]^+$ calcd for $C_{285}H_{331}NO_{68}Na$ 4878.2, found 4884.9.

General Procedure for the Hydrogenation. The oligosaccharide was dissolved in H_2O/THF/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 $Pd(OH)_2$/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 H_2O/THF/tBuOH mixture and H_2O.

Decarhamnoside (5). Compound **26** (19.1 mg, 6 μmol) was dissolved in H_2O/THF/tBuOH (1.6 mL, 3:1.3:1.3), and 4−5 drops of AcOH were added. The solution was purged with N_2 for 5 min, after which $Pd(OH)_2$/C (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. After overnight stirring, the mixture was filtered through a Whatmann filter and concentrated in vacuo. Purification by size exclusion chromatography (LH20, eluted with MeOH/H_2O, 9/1,v/v) and analysis by ^1H NMR indicated the presence of aromatic signals. The hydrogenation procedure was repeated once. Purification using gel filtration (HW-40, eluted with NH_4OAc) and subsequent lyophilization yielded the target decarhamnoside as a white powder (5.3 mg, 3.4 μmol, 57%): ^1H NMR (500 MHz, D_2O) δ 5.11−5.06 (m, 4H), 4.93 (d, J = 1.8 Hz, 1H), 4.87−4.81 (m, 4H), 4.05 (m, 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); ^{13}C NMR (126 MHz, D_2O) 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 $C_{65}H_{114}NO_{41}$ 1564.6861, found 1564.6873.

Hexadecarhamnoside (8). Compound **29** (7.2 mg, 1.5 μmol) was dissolved in H_2O/THF/tBuOH (1.0 mL, 3:1.3:1.3), and 4−5 drops of AcOH were added. The solution was purged with N_2 for 5 min, after which $Pd(OH)_2$/C (8 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. After overnight stirring, the mixture was filtered through a Whatmann filter and concentrated in vacuo. Purification using gel filtration (HW-40, eluted with NH_4OAc) and subsequent lyophilization yielded the target hexadecarhamnoside as a white powder (1.8 mg, 0.75 μmol, 50%): ^1H NMR (500 MHz, D_2O) 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 (m, 7H), 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); ^{13}C NMR (126 MHz, D_2O) 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 $C_{101}H_{174}NO_{65}$ 2442.0369, found 2442.0361.

Isolation of Deletion Fragments. *Octarhamnoside (25).* Obtained as byproduct from **22** (10.4 mg, 4.0 μmol): ^1H NMR (500 MHz, MeCN-d_3, T = 328 K) 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, MeCN-d_3, T = 328 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 $[M + H]^+$ calcd For $C_{153}H_{180}NO_{36}$ 2608.2246, found 2608.2273.

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 $[M + H]^+$ calcd For $C_{53}H_{94}NO_{33}$ 1272.5703, found 1272.5714.

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*Dodecarhamnoside (**27**).* Obtained as byproduct from **23** (10.3 mg, 2.8 μmol): ^{1}H NMR (600 MHz, MeCN-d_3, T = 328 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, 139.8, 137.3, 129.6, 129.5, 129.4, 129.4, 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 $[M + Na]^+$ calcd for $C_{219}H_{255}NO_{52}Na$ 3753.7, found 3756.4.

*Deprotected Dodecarhamnoside (**6**).* White solid after general hydrogenation procedure (0.51 mg, 0.27 μmol, 27%): ^{1}H 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 $[M + H]^+$ calcd for $C_{77}H_{134}NO_{49}$ 1856.8019, found 1856.8064.

*Tetradecarhamnoside (**28**).* Obtained as byproduct from **23** (20 mg, 4.65 μmol): ^{1}H NMR (600 MHz, MeCN-d_3, T = 328 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 (m, 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 (m, 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, 129.4, 129.3, 129.3, 129.3, 129.2, 129.2, 129.1, 129.1, 129.1, 129.1, 129.0, 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 $[M + K]^+$ calcd for $C_{252}H_{293}KNO_{60}$ 4332.0, found 4337.0.

*Deprotected Tetradecarhamnoside (**7**).* White solid after general hydrogenation procedure (4.7 mg, 2.2 μmol, 92%): ^{1}H 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 $[M + H]^+$ calcd For $C_{89}H_{154}NO_{57}$ 2149.9211, found 2149.9220.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b02511.

^{1}H and ^{13}C NMR spectra of all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jcodee@chem.leidenuniv.nl.

ORCID

Herman S. Overkleeft: 0000-0001-6976-7005

Jeroen D. C. Codée: 0000-0003-3531-2138

Notes

The authors declare no competing financial interest.

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