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Structural Studies of the *O*-Acetyl-Containing O-Antigen from a *Shigella flexneri* Serotype 6 Strain and Synthesis of Oligosaccharide Fragments Thereof

Pierre Chassagne,[a,b,c][‡] Carolina Fontana,[d] Catherine Guerreiro,[a,b] Charles Gauthier,[a,b][‡‡] Armelle Phalipon,[e,f] Göran Widmalm,[d] and Laurence A. Mulard*[a,b]

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Extensive analysis by NMR spectroscopy of the delipidated lipopolysaccharide of *Shigella flexneri* serotype 6 strain MDC 2924-71 confirmed the most recently reported structure of the O-antigen repeating unit as {→4)-β-D-Gal*p*A-(1→3)-β-D-Gal*p*NAc-(1→2)-α-L-Rha$p_{3Ac/4Ac}$-(1→2)-α-L-Rha*p*-(1→}, and revealed the non-stoichiometric acetylation at O-3_C/4_C. Input from the CASPER program helped to ascertain the fine distribution of the three possible patterns of *O*-acetylation. The non-*O*-acetylated repeating unit (ABCD) corresponded to about 2/3 of the population, while 1/4 was acetylated at O-3_C ($_{3Ac}$CDAB), and 1/10 at O-4_C ($_{4Ac}$CDAB). Di- to tetrasaccharides with a Gal*p*A residue (A) at their reducing end were synthesized as their propyl glycosides following a multistep linear strategy relying on late-stage acetylation at O-3_C. Thus, the 3_C-*O*-acetylated and non-*O*-acetylated targets were synthesized from common protected intermediates. Rhamnosylation was most efficiently achieved by using imidate donors, including at O-4 of a benzyl galacturonate acceptor. In contrast, a thiophenyl 2-deoxy-2-trichloroacetamido-D-galactopyranoside precursor was preferred for chain elongation involving residue B. Final Pd/C-mediated deprotection ensured *O*-acetyl stability. All of the target molecules represent parts of the O-antigen of *S. flexneri* 6, a prevalent serotype. Non-*O*-acetylated oligosaccharides are also fragments of the *Escherichia coli* O147 O-antigen.

Introduction

Shigellosis, an invasive infection of the human colon, has been identified as one of the major diarrhoeal diseases worldwide.[1] In its most classical expression, it is characterized by a triad of fever, intestinal cramps, and bloody diarrhoea.[2] Also known as bacillary dysentery, this highly contagious infection is associated with increased antibiotic resistance.[3] It is endemic worldwide, and it remains a major health concern, especially in the child population of the most impoverished areas.[3,4] *S. flexneri* – one of the four species of *Shigella* – is prevalent in developing countries, where it accounts for the endemic disease.[3,5] Numerous *S. flexneri* serotypes – varying in their geographic and temporal distributions – have been isolated from patients. In recent years, *S. flexneri* serotype 6 (SF6) has been identified as an increasingly prevalent serotype in several settings worldwide,[3,6] and evidence strongly supports the inclusion of SF6 as one of the key valences in a broad-coverage *Shigella* vaccine.[4,7]

S. flexneri serotypes are defined on the basis of the carbohydrate repeating unit of the surface O-antigen (O-Ag), the polysaccharide part of the bacterial lipopolysaccharide (LPS).[8] Protection against reinfection by the homologous serotype, suggesting serotype-specific natural immunity, has been established following *Shigella* infection.[6a,9] These observations provided strong evidence that *S. flexneri* O-Ags are major targets of the host's adaptive immune system. Accordingly, several LPS-based vaccine candidates against shigellosis have been developed and even evaluated in clinical trials.[4,10] Along these lines, we have investigated a promising alternative to the use of material of biological origin. It involves the design of synthetic oligosaccharide haptens to serve as functional mimics of the natural O-Ag of interest.[11] The strategy relies on the availabil-

[a] Institut Pasteur, Unité de Chimie des Biomolécules, 28 rue du Dr Roux, 75015 Paris, France
Fax: +33-1-45688404
E-mail: laurence.mulard@pasteur.fr
[b] CNRS UMR3523, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France
[c] Université Paris Descartes Sorbonne Paris Cité, Institut Pasteur, rue du Dr Roux, 75015 Paris, France
[d] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, 10691 Stockholm, Sweden
[e] Institut Pasteur, Pathogénie Microbienne Moléculaire, 28 rue du Dr Roux, 75015 Paris, France
[f] INSERM U786, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France
[‡] Present address: Glycom A/S, DTU, Bld 201, 2800 Kgs Lyngby, Denmark
[‡‡] Present address: Université de Poitiers, Institut de Chimie IC2MP UMR-CNRS 7285, 86022 Poitiers, France
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ity of well-defined synthetic frame-shifted fragments of the O-Ag.[12] In this paper, this is addressed for the first time for SF6.

Knowledge of the exact repeating unit (RU) of the O-Ag of interest is a major prerequisite to launching such a strategy. Considering the numerous revised structures of *S. flexneri* O-Ags published recently,[13a–13c] in addition to the various structures reported for the SF6 O-Ag,[14] our first concern was to ascertain the exact molecular composition of the O-Ag of SF6.

This paper describes the elucidation by NMR spectroscopy of the RU and acetylation pattern of the O-Ag from SF6 strain MDC 2924-71, and also the first synthesis of di- to tetrasaccharide fragments of the O-Ag.

Results and Discussion

Structural Investigation of the O-Ag from SF6 Strain MDC 2924-71

The most recent structural investigations of the full-length SF6 O-Ag[14d,15] reported a structure similar to that of the O-Ag of *Escherichia coli* O147.[16] The basic RU is a linear tetrasaccharide made of one D-galacturonic acid (A), one *N*-acetyl-D-galactosamine (B) and two L-rhamnose residues (C, D). The only difference between these two polysaccharides (PS) is that the O-Ag of SF6 is *O*-acetylated (Figure 1). The occurrence and position of *O*-acetyl (OAc) groups in a PS may influence its antigenicity,[17] and more importantly, its immunogenicity,[18] and thus it is particularly important to establish the exact location(s) of such OAc groups. Acetylation in the SF6 O-Ag was identified at O-3 of rhamnose C.[14d] However, the main problem when determining the *O*-acetylation pattern in a native PS is whether acetyl migration or deacetylation has occurred; these processes can easily take place given appropriate spatial arrangements, either in the course of PS extraction or purification, or even during the acid-mediated delipidation procedure of the LPS.[19] Consequently, when deacetylation occurs during the delipidation procedure, one does not know whether OAc groups were present in the native LPS. It has been possible to determine the locations of OAc groups by NMR spectroscopy directly on the intact LPS,[20] but this is highly dependent on the preparation, and is not always feasible. Nevertheless, one can often obtain information at least about the presence or absence of OAc groups from a one-dimensional ^{1}H NMR spectrum of the LPS in D_2O as solvent. In the first part of this study, we confirm the acetylation at O-3_C, as was previously reported,[14d,15] and describe in detail the population distribution of OAc groups on rhamnose C in the SF6 O-Ag.

The LPS was isolated from SF6 strain MDC 2924-71 according to a known protocol.[21] It was delipidated under mild acidic conditions to yield a PS corresponding to the O-Ag covalently linked to the core through residue B. The PS was purified by gel-permeation chromatography. Different fractions were collected, and the average number of RU per O-Ag was estimated from their ^{1}H NMR spectra

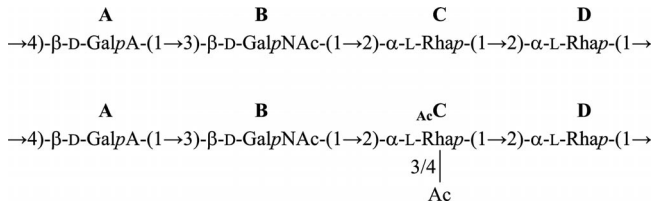


Figure 1. Comparison of the structures of the repeating units of the O-Ag from *E. coli* O147 (top) and SF6 (bottom). The sugar residues are denoted A–D.

by integration of the *N*-acetyl signals of residue B in the region δ = 2.04–2.09 ppm (Figure 2a) relative to the anomeric signals in the core region for α-Gal*p* (δ = 5.86 ppm) and α-Glc*p* (δ = 5.63 ppm), as described by Kubler-Kielb et al.[14c] A direct inspection of the ^{1}H NMR spectra in the region of δ = 2.14–2.21 ppm suggested that different *O*-acetylation patterns were present in the lower molecular weight (mw) fraction, which is consistent with reported results.[14c] A fraction of intermediate mw showed resonances characteristic of both regions of the PS. As the present study was undertaken to establish the location of *O*-acetyl groups in the O-Ag, a fraction of higher mw, corresponding to about a dozen RUs, was used in the NMR studies. Precautions were taken to minimize acetyl migration, such as keeping the pH at 6 or below, and keeping the sample temperature as low as possible, between 0 and 17 °C.

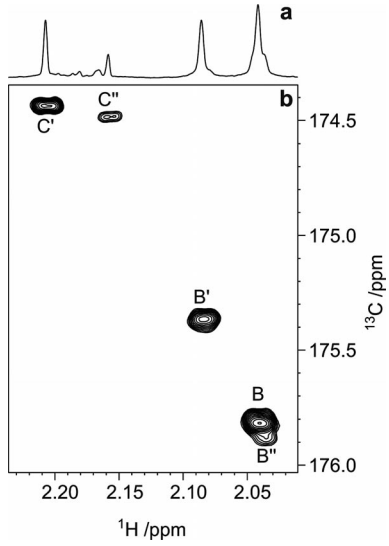


Figure 2. (a) Spectral region of the ^{1}H NMR spectrum of the SF6 PS containing the *N*- and *O*-acetyl resonances. (b) Selected region of the ^{1}H,^{13}C-BS-CT-HMBC NMR spectrum of the PS from SF6 showing correlations from the carbonyl carbon atoms to the methyl protons of the *O*-acetyl and *N*-acetyl groups (residues C and B, respectively). The residues of the non-*O*-acetylated population are denoted by the respective non-primed capital letters, whereas the *O*-acetylated populations are denoted by primed (major) and double-primed capital letters (minor).

The ^{1}H NMR spectrum revealed a material of high complexity. Two ^{1}H signals for OAc at δ = 2.158 (minor form) and 2.207 ppm (major form) could be easily identified (Figure 2a). As a consequence, several sets of signals were found

for all the residues due to partial *O*-acetylation. For instance, the *N*-acetyl (NAc) signals at δ = 2.037, 2.042, and 2.086 ppm suggest three different populations of residue B. The ratio of the different populations was estimated by integration of the OAc signals with respect to the NAc signals. The major and minor *O*-acetylated populations corresponded to about 1/4 and 1/10 of the total, respectively, and the population without OAc corresponded to the remaining 2/3. A band-selective constant-time ^{1}H,^{13}C-HMBC experiment[22] confirmed the presence of two OAc signals at δ = 174.43 and 174.49 ppm, as well as three NAc signals at δ = 175.36, 175.82, and 175.88 ppm (Figure 2b).

To facilitate the identification of the resonances corresponding to the population of the non-*O*-acetylated O-Ag, the ^{1}H and ^{13}C chemical shifts of the *E. coli* O147 O-Ag[16] were reassigned at pD = 5 by using 1D and 2D NMR experiments. Due to the pD change, the signals for C-5$_A$, C-6$_A$, and 4$_A$-H were shifted downfield to δ_C = 74.91 and 174.42 ppm, and δ_H = 4.10 ppm, while the signals for C-1$_D$ and 5$_D$-H were found at δ_C = 100.24 ppm and δ_H = 3.72 ppm, respectively. The remaining chemical shift differences were less than 0.04 ppm and 0.25 ppm for ^{1}H and ^{13}C resonances, respectively.

By comparison with the *E. coli* O147 O-Ag, the resonances at δ = 1.23–1.27 ppm in the SF6 O-Ag were assigned to 6-H in the non-*O*-acetylated rhamnose C and rhamnose D. Two conspicuous signals of lower intensity at δ = 1.151 and 1.289 ppm, which are absent from the ^{1}H NMR spectrum of the *E. coli* O147 PS, corresponded to the minor and major *O*-acetylated forms, respectively. At this point we used the CASPER program,[23] which is able to predict ^{1}H and ^{13}C NMR chemical shifts of oligo- and polysaccharides. The ^{1}H chemical shifts of rhamnosyl residues C and D in the RUs, mono-acetylated either at O-3$_C$, O-4$_C$, O-3$_D$, or O-4$_D$, were predicted. The signals from the 6$_C$-H were calculated to resonate at δ = 1.28 ppm in the 3$_C$-OAc RU and at δ = 1.16 ppm in the 4$_C$-OAc RU, whereas the signals from the 6$_D$-H were calculated to resonate at δ = 1.33 ppm in the 3$_D$-OAc RU and at δ = 1.20 ppm in the 4$_D$-OAc RU. These predictions suggest that the major and minor *O*-acetylated populations corresponded to RUs of the O-Ags acetylated at O-3$_C$ or O-4$_C$, respectively.

The ^{1}H chemical shifts of the two variants of the *O*-acetylated residue C were assigned by using ^{1}H,^{1}H-TOCSY experiments with increasing mixing times. In both cases, the spin systems could be fully characterized by starting from 6-H. Subsequent correlations were observed to 5-H, 4-H, and 3-H (Figure 3a and b) as well to 2-H (Figure 3c). The resonances from the anomeric protons were then readily associated with their respective 2-H signals (Figure 3d). In the spin system originating from the proton signal at δ = 1.151 ppm (minor *O*-acetylated population, denoted C″ in Figure 3), the large downfield shift of proton 4-H signal (δ = 4.823 ppm) suggests acetylation at O-4$_C$. This was confirmed by intra-residual NOE correlations in the ^{1}H,^{1}H-NOESY spectrum from the OAc signal at δ = 2.158 ppm to the 6-H resonance at δ = 1.151 ppm (Figure 4a) and to the 4-H resonance at δ = 4.823 ppm (Figure 4c). Likewise, the large downfield shift of the 3-H signal (δ = 5.071 ppm) in the spin system originating from the proton signal at δ = 1.289 ppm (6-H, major *O*-acetylated population, denoted C′ in Figure 3), indicates acetylation at O-3$_C$. Intra-residual NOEs from the OAc signal at δ = 2.207 ppm to the resonances at δ = 4.282 (2-H) and 5.071 ppm (3-H) supported this substitution pattern (Figure 4c). In addition to the signals due to residue B in the non-*O*-acetylated population, two new spin systems corresponding to minor populations were identified. Both spin systems were assigned from 1-H to 4-H by using ^{1}H,^{1}H-TOCSY experiments with different mixing times, and the respective 5-H protons were identified by intra-residual NOE correlations from the anomeric protons. Based on their relative intensities, the spin systems originating from the 1-H signals at δ = 4.512 and 4.716 ppm were assigned to the major and minor *O*-acetylated populations, respectively. The chemical shift displacements, in particular upfield by 0.2 ppm in the former case, were attributed to the perturbation by the OAc group in the neighboring residue. The ^{1}H chemical shifts of residues B and C of the *O*-acetylated populations are compiled in Table 1.

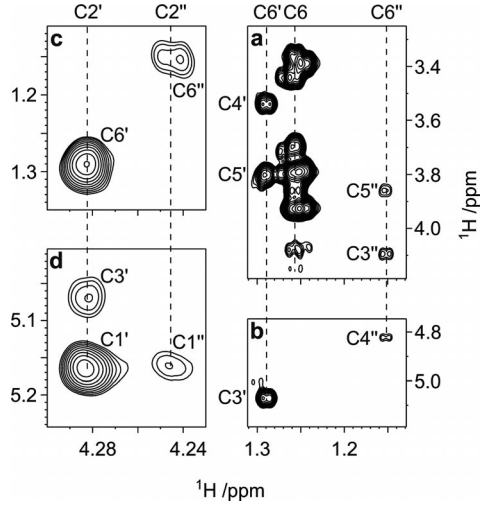


Figure 3. Selected regions of the ^{1}H,^{1}H-TOCSY NMR spectrum (t_{mix} = 120 ms) of the SF6 PS showing the spin system of residues $_{3Ac}$C (denoted C′), C (denoted C), and $_{4Ac}$C (denoted C″).

Inter-residue correlations observed in the ^{1}H,^{1}H-NOESY spectrum were consistent with acetylation at O-3$_C$ and O-4$_C$. In both cases, long-range NOE correlations were observed from 2$_C$-H in the *O*-acetylated residue C to the respective 1$_B$-H (Figure 4d) and from 4$_C$-H in the *O*-acetylated residue C to the respective NAc in residue B (Figure 4e and f), indicating that residue B is linked to the *O*-acetylated rhamnose C. NOE correlations were also observed between 6$_C$-H and the proton signals at δ = 5.421 and 5.384 ppm in the major and minor populations, respectively (Figure 4g). By comparison with the chemical shifts in the PS from *E. coli* O147, those resonances can be attributed to 1$_D$-H, suggesting that the *O*-acetylated residue C is linked to rhamnose D. The NOE correlations observed in the ^{1}H,^{1}H-NOESY spectrum from the NAc signal at δ =

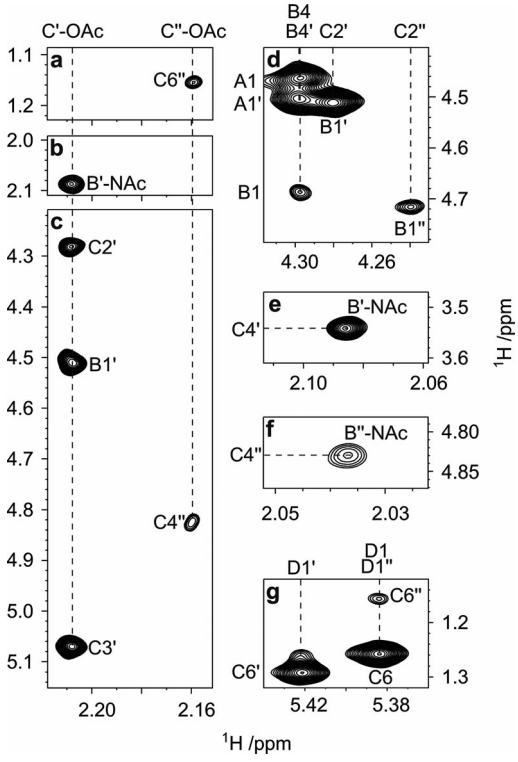


Figure 4. Selected regions of the ^{1}H,^{1}H-NOESY NMR spectrum (t_{mix} = 150 ms) of the SF6 PS showing intra- and inter-residue NOE correlations in residues $_{3Ac}$C (denoted C′), C (denoted C), and $_{4Ac}$C (denoted C′′).

Table 1. ^{1}H NMR chemical shifts (ppm) of selected resonances of the *O*-acetylated populations from the SF6 O-Ag.

Atom	Major		Minor	
	B	C	B	C
1-H	4.512	5.164	4.716	5.159
2-H	4.014	4.282	4.055	4.238
3-H	3.899	5.071	3.868	4.095
4-H	4.300	3.540	4.301	4.823
5-H	3.633	3.800	3.685	3.857
6-H	n.d.[a]	1.289	n.d.	1.151
NAc	2.086		2.037	
OAc		2.207		2.158

[a] n.d. = not determined.

2.086 and 2.042 ppm, and from the OAc signal at δ = 2.207 ppm, were also confirmed by using DPFGSE CSSF-NOESY experiments.[24] From the data in Table 1, it can be seen that acetylation at O-3$_C$ strongly affects the chemical shifts of the NAc group and 1$_B$-H. This can be explained in terms of the close spatial proximity of these groups, as shown by the NOE correlations in Figure 4b and c (denoted by primed capital letters). On the other hand, acetylation at O-4$_C$ does not significantly influence the chemical shifts of residue B.

All these results are consistent with the structure →2)-α-L-Rha*p*3Ac/4Ac-(1→2)-α-L-Rha*p*-(1→4)-β-D-Gal*p*A-(1→3)-β-D-Gal*p*NAc-(1→, in which about 2/3 corresponds to the non-*O*-acetylated form (CDAB), 1/4 is acetylated at O-3$_C$ ($_{3Ac}$CDAB), and 1/10 at O-4$_C$ ($_{4Ac}$CDAB). This substitution pattern is in agreement with that reported.[14d] Interestingly, the dominance of the population of non-*O*-acetylated RU is, to the best of our knowledge, a new finding, and suggests that the *S. flexneri* strain MDC 2924-71, used in this study, belongs to the newly introduced type 6.[15] The observed NOEs are in good agreement with a 3D model generated by CarbBuilder.[25]

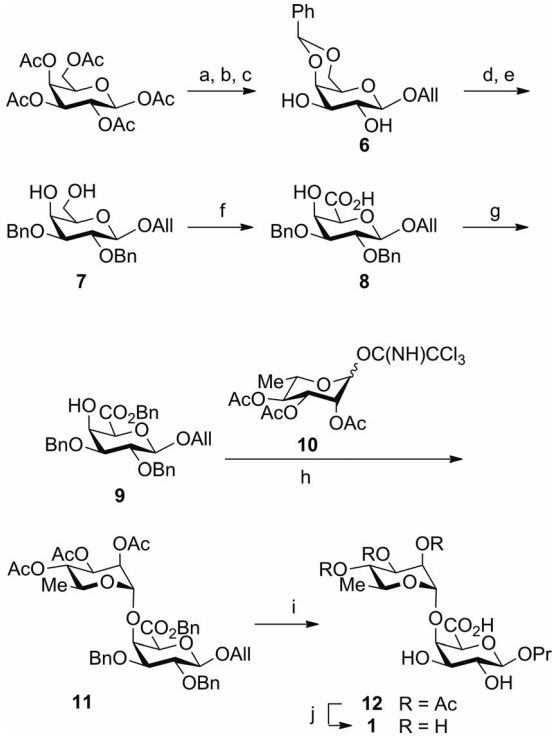
Chemical Synthesis of Di- to Tetrasaccharides Representing SF6 O-Ag Fragments Bearing Residue A at Their Reducing End

Having confirmed the structure of the RU of the SF6 O-Ag, we turned to the synthesis of di- to tetrasaccharides having a galactopyranosiduronic acid residue A at the reducing end. These oligosaccharides were synthesized both in their non-*O*-acetylated form **1** (DA-Pr), **2** (CDA-Pr), **4** (BCDA-Pr), and as their mono-*O*-acetylated counterparts **3** ($_{3Ac}$CDA-Pr) and **5** (B$_{3Ac}$CDA-Pr). All were isolated as β-propyl glycosides in order to lock their reducing end in a form mimicking the natural linkages found in the O-Ag. The choice of propyl glycosides derived from our assumptions that an allyl aglycon would (i) be easily introduced into commercially available D-galactose, (ii) be fully orthogonal to most other conventional protecting groups used in glycochemistry, in particular to acetate, (iii) be smoothly converted into a propyl group upon concomitant Pd/C-mediated hydrogenolysis of benzyl ether or benzyl esters, and (iv) in due course, have the potential to serve as an anchor for chemoselective modification,[26] opening the way to a variety of SF6-related glycoconjugates. To reduce the number of synthetic intermediates, the 3$_C$-*O*-acetyl moiety was introduced at a late stage of the synthesis. Masking of the corresponding hydroxy group before functionalization was achieved by using a *p*-methoxybenzyl (PMB) ether. Stepwise chain extension starting from a galactopyranosiduronate acceptor allowed the investigation and optimization of each glycosylation step by using appropriate monosaccharide precursors selected from trichloroacetimidate (TCA),[27] thioglycoside,[28] or *N*-phenyltrifluoroacetimidate[29] (PTFA) donors.

Synthesis of Disaccharide DA-Pr (1)

Glycosylation at O-4 of a galacturonide acceptor is thought to be disfavored compared to the corresponding galactopyranoside.[30] In addition, the reactivity of galactopyranosiduronic acid esters possessing a free axial hydroxy group has been shown to depend significantly on the anomeric configuration. Interestingly, experimental data and theoretical calculations agree that OH-4 is more nucleophilic in the β than in the α anomers.[31] Thus, taking advantage of the successful use of 2,3-di-*O*-benzyl-D-galactopyranosiduronic acid esters as acceptors in α-(1→4)- or β-(1→4)-glycosylation reactions involved in the synthesis of homogalacturonans[31,32] or rhamnogalacturonans,[32b,33] we selected benzyl (allyl 2,3-di-*O*-benzyl-β-D-galactopyr-

anosid)uronate[34] (**9**) as the precursor to residue A. This precursor was prepared from commercially available β-D-galactose pentaacetate via allyl glycoside **6**, obtained as a crystalline material in a non-optimized 54% yield over three steps (Scheme 1).[35] Benzylation of acetal **6** and subsequent acidic hydrolysis of the benzylidene protecting group gave 4,6-diol **7** (89%) on a multigram scale.[36] Of the numerous well-established oxidation protocols envisioned for the conversion of diol **7** into known uronic acid **8**,[37] we favored the 2,2,6,6-tetramethyl-1-piperidinyloxy/[bis(acetoxy)iodo]-benzene (TEMPO/BAIB) system,[38] which we thought would be compatible with the allyl aglycon. To our satisfaction, treatment of diol **7** with a catalytic amount of TEMPO and excess BAIB in CH_2Cl_2/water (2:1) gave the expected carboxylic acid (i.e., **8**), which was smoothly converted into benzyl ester **9** upon reaction with benzyl bromide in the presence of potassium hydrogen carbonate. When running the oxidation/protection sequence on a multigram scale, acceptor **9** was isolated in a good 74% yield over the two steps. Next, allyl glycoside **9** was condensed with the readily accessible 2,3,4-tri-*O*-acetyl-L-rhamnosyl trichloroacetimidate[39] (**10**) by using TMSOTf (TMS = trimethylsilyl) as catalyst (Scheme 1). The α-L-(1→4)-linked disaccharide (i.e., **11**; NMR spectroscopic data for C-1_D: δ = 99.7 ppm, $^1J_{CH}$ = 170.4 Hz) was isolated in a pleasing 91% yield, which confirmed the good nucleophilic properties of OH-4 of β-D-galactopyranosiduronic acid esters. A two-step deprotection sequence was designed so as to prevent any risk of β-elimination upon treatment of uronate intermediates with base. Thus, Pd/C-mediated hydrogenolysis of the benzyl ether protecting groups was run first. Ethyl acetate was used in this reaction to solubilize uronic acid **12** resulting from concomitant cleavage of the benzyl ester in fully protected **11**. Saponification of the acetyl protecting groups of crude acid **12** provided DA-Pr glycoside **1** in 51% isolated yield over two steps, following RP-HPLC purification. Satisfactorily, no by-products related to β-elimination were identified.

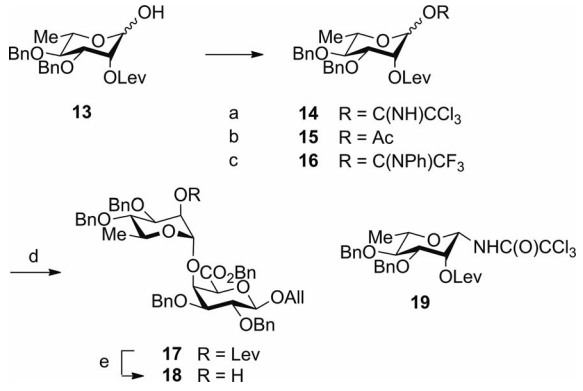


Scheme 1. Synthesis of A acceptor **9** and of target DA-Pr disaccharide **1**. Reagents and conditions: (a) AllOH, BF_3·OEt_2, CH_2Cl_2, 0 °C to room temperature, 24 h; (b) NaOMe, MeOH, room temperature, 18 h; (c) benzaldehyde dimethyl acetal, CSA (camphorsulfonic acid), MeCN, room temperature, 2 h, 54% over 3 steps; (d) NaH, BnBr, DMF, 0 °C to room temperature, 2 h; (e) AcOH (80% aq.), 80 °C, 2 h, 89% over 2 steps; (f) TEMPO, BAIB, CH_2Cl_2/H_2O, room temperature, 1 h; (g) $KHCO_3$, BnBr, DMF, room temperature, 16 h, 74% over 2 steps; (h) TMSOTf, CH_2Cl_2, –40 °C to room temperature, 1 h, 91%; (i) H_2, Pd/C 10%, EtOAc, room temperature, 16 h; (j) NaOH, THF/H_2O, room temperature, 24 h, 51% over 2 steps.

Synthesis of Trisaccharides CDA-Pr (2) and $_{3Ac}$CDA-Pr (3)

Having ascertained that galacturonate **9** was an efficient acceptor even when using disarmed donors, the next step consisted of identifying a suitable rhamnosyl donor, a precursor to residue D, that would be compatible with chain elongation at O-2. As concomitant $CO_2Bn \rightarrow CO_2Me$ transesterification has been observed during acetyl removal under methanolysis conditions,[33a] we turned to donors bearing a levulinoyl (Lev) ester at C-2 in view of its stereodirecting potency[40] and convenient selective removal in the presence of other esters, including benzyl uronates.[40,41] Thus, acceptor **9** was treated with known rhamnosyl trichloroacetimidate[42] **14** in CH_2Cl_2 containing a catalytic amount of TMSOTf (Scheme 2). To our surprise, the reaction did not go to completion, even when 1.3 or up to 1.5 equiv. of donor were used (Table 2, Entries 1 and 2). This unexpected outcome was, in part, explained by rearrangement of the donor into β-glycosylamide **19** (^{13}C NMR spectroscopic data for C-1: δ = 78.4 ppm, $^1J_{CH}$ = 154.3 Hz), which was isolated as a major by-product. The β configuration of the glycosidic linkage in **19** was ascertained from NOESY 1D NMR spectroscopic data, which indicated spatial proximity between 1-H (δ = 5.32 ppm) and both 3-H (δ = 3.74 ppm) and 5-H (δ = 3.55 ppm). Although formation of the β-glycosylamide could not be avoided, changing CH_2Cl_2 for toluene resulted in completion of the reaction in the presence of only 1.2 equiv. of TCA **14**. Reducing the amount of donor resulted in the formation of less of the by-product, thus facilitating the purification to give disaccharide **17** in 94% yield (Table 2, Entry 3). As with donor **10**, the α stereochemistry of the newly formed glycosidic linkage was obvious from the $^1J_{CH}$ coupling constant at C-1_D ($^1J_{CH}$ = 172.7 Hz). In our search for an improved method that would avoid glycosylamide formation, we referred to previous work by C. Vogel, which suggested that β-D-galactopyranosiduronate acceptors could react at O-4 with numerous types of donors.[32b] In one approach, acetylation of known hemiacetal[42] **13** gave donor **15** as a 4.5:1 mixture of α/β anomers (96%). In a second approach,

the same hemiacetal was treated with *N*-phenyltrifluoroacetimidoyl chloride in acetone containing excess cesium carbonate[43] to give the corresponding PTFA donor (i.e., **16**) as a 4:1 α/β mixture (95%). Both the acetate and PTFA donors – **15** and **16** – were treated with acceptor **9** in the presence of catalytic TMSOTf (Scheme 2). With the acetate donor, the glycosylation resulted in an 89% isolated yield of disaccharide **17**, which compared favorably with published data,[32b] by using 1.5 equiv. of donor **15** and 0.15 equiv. of TMSOTf in CH_2Cl_2 (Table 2, Entry 4). Under conditions optimized for TCA donor **14** (Table 2, Entry 3), a rewarding 96% glycosylation yield was obtained with PTFA donor **16** (Table 2, Entry 5). As already observed,[44] the use of donor **16** (1.2 equiv.) avoided the formation of any unwanted glycosylamide or other by-products that hampered the purification. Removal of the levulinoyl group by using hydrazine hydrate in buffered medium gave target acceptor **18** (90%).



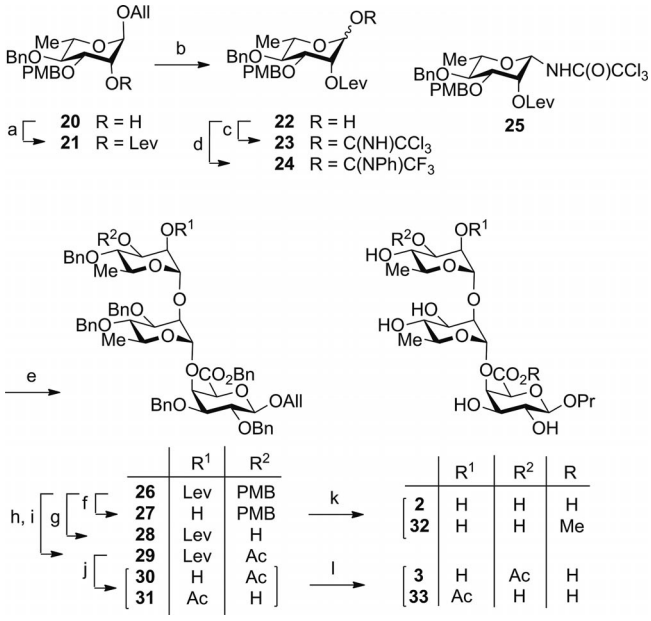
Scheme 2. Synthesis of DA disaccharide acceptor **18**. Reagents and conditions: (a) see ref.[42], 94%; (b) Ac_2O, pyridine, 0 °C to room temperature, 2 h, 96%; (c) PTFACl, Cs_2CO_3, acetone, room temperature, 2 h, 95%; (d) **9**, TMSOTf, see Table 2; (e) $H_2NNH_2{\cdot}H_2O$, AcOH/pyridine, 0 °C to room temperature, 1.5 h, 90%.

Table 2. Conditions for the synthesis of disaccharide **17** from acceptor **9**.

Entry	Donor (equiv.)	Solvent	*T* [°C]	Yield of **17** [%]
1	**14** (1.3)	CH_2Cl_2	–40 → room temperature	70
2	**14** (1.5)	CH_2Cl_2	–40 → room temperature	84
3	**14** (1.2)	toluene	–10 → room temperature	94
4	**15** (1.5)	CH_2Cl_2	–10 → room temperature	89
5	**16** (1.2)	toluene	–10 → room temperature	96

The formation of the C–D glycosidic linkage was inspired by the above results. Hence, the 3-*O*-PMB analogs of donors **14** and **16** – rhamnosyl TCA **23** and PTFA **24**, respectively – were examined as precursors to residue C. They were prepared in three steps from allyl 4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnoside[45] (**20**) (Scheme 3). Thus, alcohol **20** was treated with levulinic acid in the presence of DCC (*N*,*N*′-dicyclohexylcarbodiimide) and DMAP [4-(dimethylamino)pyridine] to give ester **21**, which was converted into hemiacetal **22** following a two-step anomeric deallylation procedure involving isomerisation of the allyl ether into the corresponding prop-1-enyl ether with a cationic iridium complex[46] and its subsequent iodine-mediated hydrolysis (90%).[47] The hemiacetal was transformed either into TCA **23** (97%) by reaction with trichloroacetonitrile in the presence of catalytic DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), or into PTFA donor **24** (98%) under conditions similar to those used to prepare the corresponding 3,4-di-*O*-benzyl derivative (i.e., **16**). The outcome of the TMSOTf-mediated [C + DA] assembly was similar to that of the [D + A] glycosylation. Briefly, trisaccharide **26** (NMR spectroscopic data for C-1_C: δ = 98.9 ppm, $^1J_{CH}$ = 172.5 Hz) was isolated in higher yield – 91 vs. 84% – when rhamnosyl C donor **23** (1.5 equiv.) and DA acceptor **18** were set to react in toluene rather than in a chlorinated solvent (Table 3, Entries 1 and 2). Fortunately, while a lower proportion of rearranged β-glycosylamide **25** formed as a by-product, which facilitated the purification, reducing the amount of donor **23** to 1.2 equiv. had no influence on the glycosylation yield (92%, Table 3, Entry 3). To our satisfaction, the formation of by-products was minimal, and glycosylation at O-2_D of acceptor **18** remained high-yielding (89%) when TCA donor **23** was substituted by its PTFA equivalent **24** (Scheme 3, Table 3, Entry 4). The high-yielding removal of the 2_C-levulinoyl ester of glycosylation product **26** by reaction with hydrazine hydrate in pyridine/AcOH gave alcohol **27** (93%), which could serve either as an intermediate for the synthesis of CDA-Pr target **2**, or as an acceptor in the synthesis of tetrasaccharides **4** and **5**; Pd/C-mediated benzyl ether hydrogenolysis, benzyl ester cleavage, and concomitant allyl reduction gave propyl glycoside **2** in a good 69% yield after RP-HPLC purification. This final deprotection step was run in methanol, and it is noteworthy that despite the neutral conditions used, methyl ester **32** was also isolated, albeit in low yield (5%). Taken together with an independent report with a similar outcome,[48] this encouraged us to use a THF/H_2O solvent for subsequent hydrogenolysis reactions. Alternatively, acetylation at O-3_C was a prerequisite for obtaining $_{3Ac}$CDA-Pr target **3**. Oxidative removal of the PMB ether was attempted by using DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) or CAN (ceric ammonium nitrate). When run in CH_2Cl_2/H_2O in the presence of DDQ (3.0 equiv.), the reaction was slow, and degradation increased with time. The best isolated yield of alcohol **28** was 45%. In contrast, treatment of fully protected **26** with CAN (3.0 equiv.) in MeCN/H_2O resulted in a faster and cleaner conversion, giving alcohol **28** in a good 71% yield. When the amount of CAN was increased to 4.0 equiv., the oxidative unmasking of OH-3_C was accelerated, and only alcohol **28** was formed. Under the best conditions, CAN-mediated removal of the 3_C-PMB ether, and subsequent acetylation of the crude intermediate gave 3_C-*O*-acetyl trisaccharide **29** in a rewarding 88% yield. This trisaccharide was subjected to conventional hydrazinolysis of the levulinoyl ester at O-2_C. Acetyl migration to the vicinal hydroxy group could not be avoided, and a 10:1 mixture

of the OH-2_C and OH-3_C regiosiomers – **30** (NMR spectroscopic data for 3_C-H: δ = 4.96 ppm), and **31** (NMR spectroscopic data for 2_C-H: δ = 5.04 ppm), respectively – was isolated (93%); Pd/C/hydrogen-mediated final deprotection of the mixture of the two alcohols in THF/H_2O gave the corresponding mono-*O*-acetylated trisaccharides, $_{3Ac}$CDA-Pr (**3**, 78%) and $_{2Ac}$CDA-Pr (**33**, 2%; *O*-acetylated at 2_C). The 4_C-*O*-acetyl isomer was not detected. This outcome suggests that acetyl migration did not occur under the neutral conditions used for hydrogenolysis.



Scheme 3. Synthesis of C donors **23** and **24**, and of CDA trisaccharides **2** and **3**. Reagents and conditions: (a) LevOH, DCC, DMAP, CH_2Cl_2, room temperature, 2 h, quantitative; (b) (i) $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$ (COD = 1,5-cyclooctadiene), H_2, THF, room temperature, 2 h; (ii) I_2, THF/H_2O, room temperature, 1 h, 90%; (c) CCl_3CN, DBU, DCE (1,2-dichloroethane), room temperature, 20 min, 97%; (d) PTFACl, Cs_2CO_3, acetone, room temperature, 4 h, 98%; (e) **18**, TMSOTf, –10 °C, see Table 3; (f) $H_2NNH_2{\cdot}H_2O$, AcOH/pyridine, 0 °C to room temperature, 30 min, 93%; (g) DDQ, CH_2Cl_2/H_2O, room temperature, 3 h, 45%; or CAN, MeCN/H_2O, room temperature, 1.5 h, 71%; (h) CAN, MeCN/H_2O, room temperature, 30 min; (i) Ac_2O, DMAP, pyridine, room temperature, 2 h, 88% over 2 steps; (j) $H_2NNH_2{\cdot}H_2O$, AcOH/pyridine, 0 °C to room temperature, 1.5 h, 93%; (k) Pd/C (10%), H_2, MeOH, room temperature, 24 h, 69% for **2**, 5% for **32**; (l) Pd/C (10%), H_2, THF/H_2O, room temperature, 20 h, 78% for **3**, 2% for **33**.

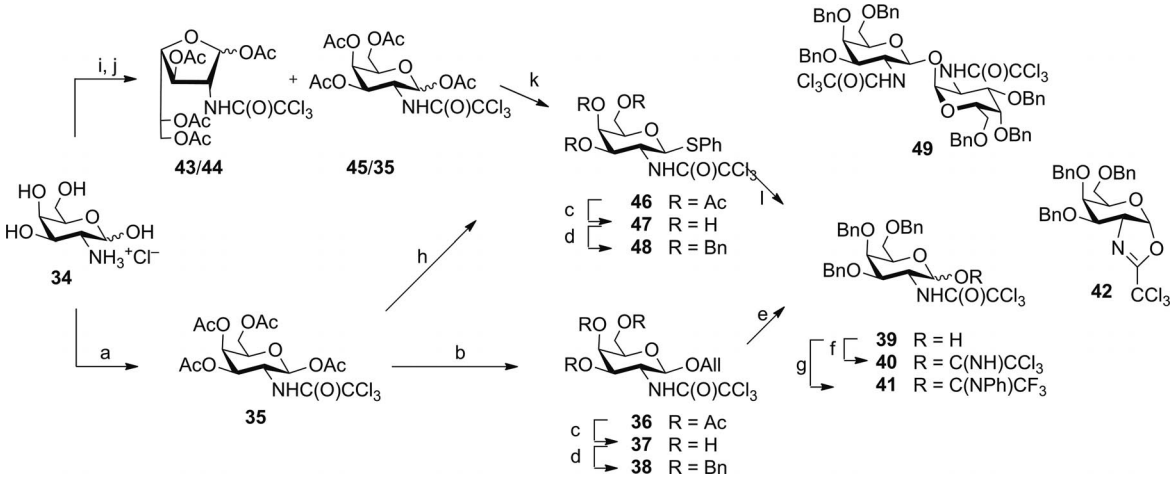
Table 3. Conditions for the synthesis of trisaccharide **26**.

Entry	Donor (equiv.)	Solvent	Yield of **26** [%]
1	**23** (1.5)	DCE	84
2	**23** (1.5)	toluene	91
3	**23** (1.2)	toluene	92
4	**24** (1.2)	toluene	89

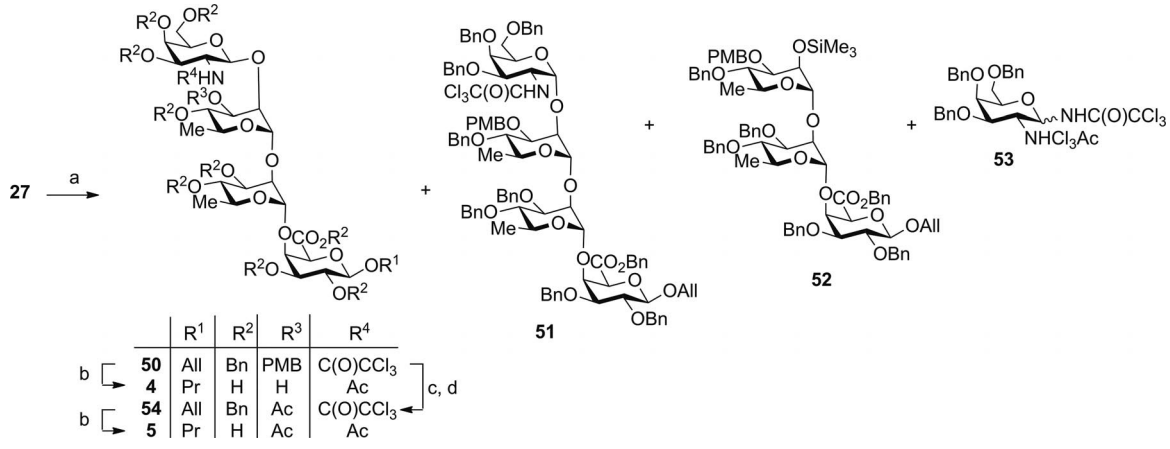
Synthesis of Tetrasaccharides BCDA-Pr (4) and B_{3Ac}CDA-Pr (5)

Since chain elongation at residue B was not planned, an *N*-acetyl-D-galactosamine precursor that would act as a chain terminator, thus limiting the number of protecting group manipulations, was preferred. In view of our work involving β-linked *N*-acetylglucosamine residues,[49] a trichloroacetamide moiety was chosen to act as a masked acetamido functionality and ensure the required 1,2-*trans* stereoselectivity in the glycosylation reactions. It was also hypothesized that Pd/C-mediated hydrodechlorination of the trichloroacetamide functionality at the final stage of the synthesis would permit full recovery of the acetamido moiety without perturbation of the 3_C-acetate.[42] Since the known 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyl trichloroacetimidate[50] did not meet orthogonality criteria, we turned to perbenzylated analogs in order to (i) facilitate glycosylation by using an armed donor, (ii) avoid the remote α-stereodirecting effect attributed to esterification at O-4 of galactose and galactosamine donors,[51] and (iii) minimize the number of final deprotection steps. As for the construction of the C–D and D–A linkages, donors activated in the form of TCA[52] (**40**) or PTFA (**41**) were evaluated as precursors to residue B. Towards this aim, β-pyranose tetraacetate **35** was prepared in four steps as described[50] in 43% overall yield on a multigram scale from galactosamine hydrochloride **34**. It was adequately transformed into β-allyl glycoside **36** (92%) when treated with allyl alcohol and stoichiometric TMSOTf in CH_2Cl_2 (Scheme 4). Transesterification gave triol **37**, and subsequent selective *O*-benzylation gave protected intermediate **38** (86%). Anomeric allyl cleavage proceeded smoothly to give key hemiacetal **39** in high yield (93%). This hemiacetal was thus readily accessible (74%) in four steps from pure β-tetraacetate **35**. Treatment of hemiacetal **39** under standard conditions gave either known TCA donor **40** (94%) or its PTFA equivalent **41** (88%). The identification of oxazoline **42** (5%) by NMR spectroscopy could explain the lower isolated yield in the latter case.

With the two galactosaminyl donors in hand, we set out to explore the best conditions to form tetrasaccharide **50** from the above-mentioned trisaccharide alcohol (i.e., **27**; Scheme 5). Glycosylation of acceptor **27** with trichloroacetimidate **40** was found to be problematic. When the reaction was run at –10 °C in toluene containing catalytic TMSOTf (Table 4, Entry 1), the product of α-glycosylation (i.e., **51**), identified by mass spectrometry analysis and NMR spectroscopic data for C-1_B (δ = 96.0 ppm, $^1J_{CH}$ = 175.2 Hz), was isolated in a significant 15% yield, in addition to the required β-linked tetrasaccharide (i.e., **50**; NMR spectroscopic data for C-1_B: δ = 100.7 ppm, $^1J_{CH}$ = 162.4 Hz). Although the β-glycoside was the major product according to TLC analysis, it co-eluted with the glycosylamide by-product **53**, as suggested by mass spectrometry data, and could not be isolated as a pure material. Mass spectrometry analysis also indicated the formation of the silylated acceptor (i.e., **52**) under these conditions. Lowering the temperature to –78 °C improved the selectivity remarkably, since only traces of the unwanted α-linked glycosylation product remained according to TLC observation. However, the reactivity of the acceptor dropped tremendously. Changing toluene for CH_2Cl_2 and TMSOTf for



Scheme 4. Synthesis of B donors **40** and **41**. Reagents and conditions: (a) see ref.[50], 43% over 4 steps; (b) AllOH, TMSOTf, CH_2Cl_2, room temperature, 14 h, 92%; (c) NaOMe, MeOH, room temperature, 2 h; (d) NaH, BnBr, DMF, –10 °C to 0 °C, 1.5 h, 86% for **38**, 77% for **48** over 2 steps; (e) (i) $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$, H_2, THF, room temperature, 2 h; (ii) I_2, THF/H_2O, room temperature, 1 h, 93%; (f) CCl_3CN, DBU, DCE, room temperature, 1 h, 94%; (g) PTFACl, Cs_2CO_3, acetone, room temperature, 4 h, 88%; (h) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, room temperature, 2.5 h, 89%; (i) $(CCl_3CO)_2O$, NaOMe, MeOH, 0 °C, 2 h; (j) Ac_2O, pyridine, 0 °C to room temperature, 16 h; (k) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, room temperature, 16 h, 64% over 3 steps; (l) NIS/TfOH (NIS = *N*-iodosuccinimide), CH_2Cl_2/H_2O, 0 °C, 30 min, 77%.



	R^1	R^2	R^3	R^4
50	All	Bn	PMB	$C(O)CCl_3$
4	Pr	H	H	Ac
54	All	Bn	Ac	$C(O)CCl_3$
5	Pr	H	Ac	Ac

Scheme 5. Synthesis of tetrasaccharides **4** and **5**. Reagents and conditions: (a) see Table 4; (b) Pd/C (10%), H_2, THF/H_2O, room temperature, 48 h, 59% for **4**, 53% for **5**; (c) CAN, MeCN/H_2O, room temperature, 30 min; (d) Ac_2O, DMAP, pyridine, room temperature, 2 h, 87% over 2 steps.

TBDMSOTf (TBDMS = *tert*-butyldimethylsilyl) gave similar results, whereas when $BF_3{\cdot}OEt_2$ was used as a promoter in CH_2Cl_2, the conversion was slow, and degradation occurred. When the best conditions identified for TCA **40** were used with PTFA donor **41**, rearrangement of the donor was not observed, and the coupling reaction was significantly improved (Table 4, Entry 2). The fully protected BCDA tetrasaccharide **50** was obtained in 63% yield. However, some unreacted acceptor remained, despite the use of 1.5 equiv. of donor, and the selectivity was only moderate, since α isomer **51** was also formed (9%).

In an attempt to overcome the poor outcome of the imidate-based [B + CDA] glycosylation, thiophenyl β-glycoside **48** – prepared from a peracylated precursor (Scheme 4) – was investigated as an alternative donor. Thus, treatment of β-tetraacetate **35** with thiophenol and $BF_3{\cdot}OEt_2$ gave thioglycoside[53] **46** (89%). Zemplén deacetylation of this compound and subsequent benzylation of the resulting triol[54] (i.e., **47**) under controlled conditions gave the expected tribenzyl analog (i.e., **48**; 77%). In addition, NIS/TfOH-mediated hydrolysis of the thioglycosidic linkage in **48** provided another access to hemiacetal **39** (77%). Interestingly, under

Table 4. Conditions for the synthesis of tetrasaccharide **50** from acceptor **27**.

Entry	Donor (equiv.)	Promotor	Solvent	*T* [°C]	**50/51** [%]
1	**40** (1.2)	TMSOTf	toluene	–10	n.d.[a]/15
2	**41** (1.5)	TMSOTf	CH_2Cl_2	–78	63/9
3	**48** (1.2)	NIS/TMSOTf	CH_2Cl_2	–70 → –55	75/–
4	**48** (1.5)	NIS/TMSOTf	CH_2Cl_2	–70 → –60	80/–

[a] n.d. = not determined.

the conditions used (CH_2Cl_2/H_2O), α-(1↔1)-β-linked disaccharide **49** (NMR spectroscopic data for 1-H and 1′-H: δ = 5.30 ppm, $^1J_{1,2}$ = 8.8 Hz, and δ = 5.44 ppm, $^1J_{1,2}$ = 3.8 Hz) – arising from the glycosylation of hemiacetal **39** with thioglycoside **48** – was also isolated, albeit in a minimal amount (3%). Even though this hydrolysis step may be improved, this route to hemiacetal **39** was found to be less efficient than that involving the allyl glycoside intermediate (53% compared with 74% over four steps starting from tetraacetate **35**). The preparation of thioglycoside **46** from the crude material resulting from the two-step *N*-trichloroacetylation/per-*O*-acetylation of D-galactosamine hydrochloride **34** was attempted so as to reduce the number of synthetic steps (Scheme 4). Thus, a mixture of α/β-furanose (**43**/**44**) and α/β-pyranose isomers (**45**/**35**), in a ratio of 7%:11% and 51%:31%, respectively, was treated with thiophenol and $BF_3{\cdot}OEt_2$. Thioglycoside **46** was isolated in a satisfactory 64% yield over three steps. Undoubtedly, this route is better than the previous one (five steps, 38%).

Glycosylation of trisaccharide alcohol **27** with thioglycoside donor **48** was attempted next (Scheme 5). In contrast to previous observations involving imidate donors **40** and **41**, when thioglycoside **48** was used in combination with the NIS/TMSOTf activator system at –70 → –55 °C in CH_2Cl_2, the pure β-glycosylation product **50** was isolated in a gratifying 75% yield (Table 4, Entry 3). The use of a larger excess of donor **48** (1.5 vs. 1.2 equiv., Table 4, Entry 4) resulted in a slight increase in yield (80 vs. 75%) while maintaining an excellent β selectivity. To our satisfaction, the product of α-glycosylation (**51**) was not detected under these conditions, while the formation of traces of the silylated acceptor (i.e., **52**) was occasionally noticed.

As planned, the one-step Pd/C-catalyzed hydrogenolysis of the benzyl protecting groups, allyl reduction, and concomitant hydrodechlorination of the 2_B-trichloroacetamide moiety in glycosylation product **50** was best performed in THF/H_2O to give the target BCDA propyl glycoside **4** in 59% yield after RP-HPLC purification. Alternatively, oxidative cleavage of the PMB ether in tetrasaccharide **50** and subsequent acetylation of the released hydroxy group provided the 3_C-*O*-acetyl analog **54** (87% over two steps). A solution of the latter tetrasaccharide in THF/H_2O was similarly treated with Pd/C under hydrogen to give the B_{3Ac}CDA-Pr tetrasaccharide **5** in 53% yield after RP-HPLC purification. In this case, the regioisomer resulting from acetyl migration onto the vicinal hydroxy group was not isolated.

Conclusions

SF6 was recently identified as an essential serotype for inclusion in a broad-coverage *Shigella* vaccine preparation. In the search for a synthetic carbohydrate-based SF6 immunogen, we first analyzed the composition of the O-Ag from strain MDC 2924-71, which was identified as a representative strain amongst those available at the Institut Pasteur. The structure of the RU of strain MDC 2924-71 O-Ag, including its *O*-acetylation pattern, is consistent with published data available at the start of this study. In contrast, the extent of the non-*O*-acetylated population was unexpected. It suggests that the *S. flexneri* strain MDC 2924-71 belongs to type 6 and not to type 6a.[15] This distinction was recently introduced to differentiate between SF6 strains diverging in terms of their *O*-acetylation ratios.[15] As a direct consequence, the SF6 O-Ag used in this study resembles that of *E. coli* O147 more than anticipated. Whether this *O*-acetylation pattern has any influence on pathogenicity and/or antigenicity remains to be established. To help establish this, di- to tetrasaccharides bearing a residue A at the reducing end were synthesized starting from benzyl (allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (**9**) by stepwise chain extension with monosaccharide donors. All the glycosylation steps were optimized to reach 80% yield for the formation of the B–C linkage, and 92 and 96% yields for the formation of the D–A and C–D linkages, respectively. While thioglycoside **48**, used in CH_2Cl_2, was the preferred precursor to residue B (80%), the use of imidate donors in toluene gave better results with rhamnosyl donors representing residues C and D. Newly described PTFA donors **16** and **24** were used advantageously to facilitate purification, without adversely affecting the yield of glycosylation products **17** and **26**. The synthetic strategy was designed such that *O*-acetylation at 3_C would be performed at an advanced stage, so providing easy access to both the 3_C-OH and 3_C-OAc tri- (**2** and **3**) and tetrasaccharides (**4** and **5**), respectively.

Interestingly, ^{1}H NMR analysis of tetrasaccharides **4** and **5** indicates that a backbone conformational change may already be present within one RU-long O-Ag fragments, since the signal of the anomeric proton of the amino sugar in the structural element β-D-Gal*p*NAc-(1→2)-α-L-Rha*p* (BC) is shifted upfield by 0.25 ppm upon *O*-acetylation at O-3 of the vicinal rhamnosyl residue, consistent with the upfield chemical shift of > 0.2 ppm observed for the signal of 1_D-H in the O-Ag of SF6 strain MDC 2924-71 (vide infra). This contrasts with the behavior of several other *S. flexneri* O-Ags in which the conformation of the polymer backbone was essentially independent of the substituents.[55]

Experimental Section

Bacteria and Cultivation: For the selection of invasive bacteria, the SF6 strain MCDC 2924-71 (Institut Pasteur, Centre National de Référence des Entérobactéries) was grown on Congo Red agar plate. A Congo-Red-positive colony was selected and cultured in Trypticase Soy Broth (TCS) medium for 30 min at 37 °C with shaking. Then, TCS agar Petri dishes (about 200 with a 100 mm diameter) were filled each with 2 mL of the bacterial subculture and left at 37 °C overnight. Thereafter, bacteria were recovered by using physiological water (2 mL per Petri dish). The resulting bacterial solution (about 400 mL) was centrifuged in glass tubes at 7000 rpm for 10 min, and then the pellet was washed with physiological water. The bacterial pellet was resuspended in acetone (250 mL) and centrifuged at 7000 rpm for 10 min. This step was repeated once. The acetone-treated bacteria were left under the hood overnight for drying and mashed to obtain about 5 g of powder.

Preparation of LPS Material: To obtain the LPS crude extract, powdered bacteria (5 g) were resuspended in glass tubes containing distilled water (90 mL) heated to 68 °C. A 68 °C-heated solution of phenol (90% in distilled water; 90 mL) was added, and the suspension was incubated for 30 min at 68 °C (water bath) with shaking time to time. After being kept on ice for 10 min, the solution was centrifuged at 7000 rpm at 4 °C for 45 min. The aqueous upper layer was recovered and kept on ice. The phenol treatment was repeated once. The aqueous phases recovered from the two centrifugations were combined (about 150 mL in total), and dialyzed against tap water for 3 d. The dialyzed solution kept in the dialysis tubing was then concentrated six times by using Aquacide III (Calbiochem). The concentrated solution was recovered from the dialysis tubing, dialyzed in distilled water overnight, and then centrifuged at 5000 rpm for 15 min. The supernatant was freeze-dried to give the crude LPS extract (1.5–2 g). To purify the LPS, the freeze-dried crude extract was suspended at a concentration of 3% (w/v) in sterile distilled water and centrifuged at 25400 rpm (Beckman SW41 rotor, about 80000 *g*) at 4 °C for 7 h. The pellet was resuspended in distilled water and centrifuged at 29100 rpm for 3 h. This step was repeated once. The pellet was then resuspended in distilled water and centrifuged at 3000 rpm for 10 min. The supernatant, which contained the purified LPS, was freeze-dried to give a white powder (70–80 mg). The purified LPS was tested in SDS-PAGE followed by silver staining.

Preparation of Lipid-Free Polysaccharide: Lipid-free polysaccharide (PS) was obtained by subjecting the LPS (30 mg) to weak acidic hydrolysis in AcOH (1%; pH = 3; 6 mL) at 100 °C for 90 min.[56] The lipid A was removed by centrifugation at 15000 *g* at 4 °C for 20 min. The clear supernatant was cooled to 0 °C and stirred, while NaOH (1 M) was added very slowly until pH = 5. The solution was dialyzed against distilled water for 3 d, then it was purified by size exclusion chromatography on a HiLoad™ 16/60 Superdex™ 30 column (GE healthcare) using an ÄKTA™ purifier system (GE healthcare).

NMR Experiments: Spectra for the ^{1}H and ^{13}C NMR chemical shift assignment of the SF6 PS were recorded in D_2O (1 mg in 0.18 mL, 3 mm NMR tube) at pD = 6 and 17 °C by using a Bruker Avance III 700 MHz spectrometer equipped with a 5 mm Z-Gradient (53.0 G cm^{-1}) TCI ($^{1}H/^{13}C/^{15}N$) CryoProbe. Chemical shifts are reported in ppm relative to external sodium 3-trimethylsilyl-(2,2,3,3-$^{2}H_4$)propanoate (TSP, δ_H = 0.00 ppm) or 1,4-dioxane in D_2O (δ_C = 67.40 ppm). ^{1}H NMR spectra were recorded with 34k data points over a spectral width of 12 ppm, with 128 scans and a repetition time of 12.0 s. Zero-filling to 128k data points and an exponential weighting function using a line-broadening factor of 0.3 Hz were applied prior to Fourier transformation. ^{1}H chemical shift assignments were performed by using $^{1}H,^{1}H$-TOCSY experiments[57] recorded over 6.0 ppm with 2048 × 256 data points and 8 scans per t_1 increment, using the States-TPPI method. An MLEV-17 spin-lock of 10 kHz and four different mixing times (20, 40, 80, and 120 ms) were used. Zero-filling was performed to 4096 × 1024 points. Prior to Fourier transformation, 90°-shifted squared sine-bell functions were applied in both dimensions. ^{13}C chemical shifts were assigned by using multiplicity-edited $^{1}H,^{13}C$-HSQC experiments.[58] The experiments were recorded with 1024 × 512 data points and 16 scans per t_1 increment over a spectral region of 6.0 ppm for ^{1}H and 110 ppm for ^{13}C by using the echo/antiecho method. Adiabatic pulses[59] were used for ^{13}C inversion (smoothed CHIRP, 20%, 80 kHz, 500 μs, Q = 5.0) and refocusing (composite smoothed CHIRP, 80 kHz, 2.0 ms). Prior to Fourier transformation, forward linear prediction to 1024 points in the F_1 dimension and zero-filling to 4096 × 4096 points were performed; 90°-shifted squared sine-bell functions were applied in both dimensions. The carbonyl resonances were assigned by using a band-selective constant-time $^{1}H,^{13}C$-HMBC experiment[22] over a spectral region of 6.0 ppm in the direct dimension and 9.0 ppm in the indirect dimension, with 2048 × 256 data points and 224 scans per t_1 increment. A 60 ms delay for the evolution of long-range couplings, and a selective ^{13}C excitation pulse (Q3 Gaussian cascade) of 2.5 ms centered at the carbonyl resonances were used. Zero-filling was performed to 4096 × 2048 points. Prior to Fourier transformation, 90°-shifted squared sine-bell functions were applied in both dimensions, and the spectrum was processed in magnitude mode. A 2D $^{1}H,^{1}H$-NOESY experiment with a zero-quantum suppression filter[60] was recorded over a spectral width of 6.0 ppm, with 2048 × 512 data points and 16 scans per t_1 increment. A mixing time of 150 ms was used. A 40 kHz broad and 20 ms long adiabatically smoothed CHIRP pulse was used during the zero-quantum suppression, accompanied by a gradient pulse with a strength of 4% of the maximum. Prior to Fourier transformation, zero-filling was performed to 4096 × 2048 points, and a 90°-shifted squared sine-bell function was applied in both dimensions. DPFGSE CSSF-NOESY experiments[24] with two zero-quantum suppression filters were performed to selectively excite the protons of the *N*-acetyl and *O*-acetyl methyl groups. The spectra were acquired with 43008 data points, 256 scans per increment by using a mixing time of 150 ms and a total recycle time of 5 s. A 39 Hz broad and 22.5 ms long Gaussian 180° pulse with truncation at 1% of the total height was used for selective excitation. WURST inversion pulses were used for the adiabatic sweeps in the zero-quantum filters, accompanied by a gradient pulse of 3.2% of the maximum. The increment Δ was 2 ms in all the cases, and t_{max} was 36 ms for the signal at δ = 2.207 ppm and 16 ms for the signals at δ = 2.086 and 2.042 ppm. Zero-filling to 65k data points and an exponential weighting function by using a line-broadening factor of 3 Hz were applied prior to Fourier transformation. Additionally, spectra for the ^{1}H and ^{13}C NMR chemical shift assignment of the PS from *E. coli* O147[16] were recorded in D_2O (5.5 mg in 0.55 mL) at pD = 5 and 19 °C with a Bruker Avance 500 MHz spectrometer equipped with a 5 mm Z-Gradient (53.0 G cm^{-1}) TCI ($^{1}H/^{13}C/^{15}N$) CryoProbe and with a Bruker Avance III 600 MHz spectrometer equipped with a 5-mm inverse Z-gradient (55.7 G cm^{-1}) TXI ($^{1}H/^{13}C/^{15}N$) probe. The assignments were performed by using 1D and 2D NMR experiments such as ^{1}H, ^{13}C, $^{1}H,^{13}C$-HSQC, $^{1}H,^{1}H$-TOCSY (t_{mix} = 40 and 120 ms) and $^{1}H,^{13}C$-BS-CT-HMBC. Chemical shifts were referenced relative to TSP and 1,4-dioxane as described above.

Chemical Synthesis: Anhydrous solvents – including toluene (Tol), dichloromethane, 1,2-dichloroethane (DCE), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), methanol (MeOH), and pyridine – were supplied over molecular sieves, and were used as received. Other solvents referred to in the text are abbreviated as Chex (cyclohexane), EtOAc (ethyl acetate), and MeCN (acetonitrile), in addition to acetone. Reactions requiring anhydrous conditions were run under argon (Ar) by using dried glassware; 4 Å molecular sieves (4 Å MS) and 4 Å acid-washed molecular sieves (4 Å AW 300 MS) were activated before use by heating under high vacuum. Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254} 0.25 mm pre-coated aluminium foil plates. Compounds were visualized by using UV_{254} and/or orcinol (1 mg mL^{-1}) in H_2SO_4 (10% aq.) with charring. Flash column chromatography was carried out by using silica gel (Merck, particle size 40–63 μm, unless otherwise indicated). Reverse-phase (RP) HPLC was carried out by using a Kromasil 5 μm C18 100 Å 10 × 250 mm semi-preparative column, eluting with a 0–20% linear gradient of MeCN in TFA (0.08% aq.) over 20 min at a flow rate

of 5.5 mL min^{-1}. Analytical RP-HPLC analysis of the final compounds (λ = 215 nm) used a Symmetry 3.5 µm C_{18} 100 Å 2.1 × 100 mm analytical column eluting with a 0–35% linear gradient of MeCN in TFA (0.01 N aq.) over 20 min at a flow rate of 0.3 mL min^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 303 K by using a Bruker Avance spectrometer equipped with a BBO probe at 400 MHz (^{1}H) and 100 MHz (^{13}C). Spectra were recorded in deuterated chloroform ($CDCl_3$), dimethyl sulfoxide ([D_6]DMSO), and water (D_2O). Elucidations of chemical structures were based on ^{1}H, COSY, TOCSY, DEPT-135, decoupled DEPT-90, HSQC, decoupled HSQC, ^{13}C, decoupled ^{13}C, and HMBC spectra. Signals are reported as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), q (quadruplet), sext (sextuplet), dt (doublet of triplets), dq (doublet of quadruplets), ddd (doublet of doublet of doublets), and m (multiplet). The signals can also be described as broad (prefix b), pseudo (prefix p), overlapped (suffix $_o$), or partially overlapped (suffix $_{po}$). Chemical shifts are reported in ppm (δ) relative to the residual solvent peak [$CHCl_3$, DMSO, and DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) at δ = 7.28/77.0, 2.50/39.5, and 0.00/0.00 ppm for the ^{1}H and ^{13}C spectra, respectively], and coupling constants are given in Hertz (Hz). Of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted 6a-H, and the one at higher field is denoted 6b-H. Interchangeable assignments are marked with an asterisk. Sugar residues are labelled according to the labelling of the RU of the SF6 O-Ag and identified by a subscript in the listing of signal assignments. LC-MS and HRMS spectra were recorded in the positive-ion electrospray ionisation (ESI$^+$) mode with a Waters Q-TOFmicro mass spectrometer coupled to an Alliance HPLC. Solutions were prepared in 1:1 MeCN/H_2O containing 0.1% formic acid.

Allyl 2,3-Di-*O*-Benzyl-β-D-galactopyranoside (7):[36] NaH (60% in mineral oil; 4.67 g, 116.8 mmol, 4.0 equiv.) was slowly added to a solution of diol **6**[35] (9.0 g, 29.2 mmol) in anhydrous DMF (102 mL) stirred at 0 °C. After stirring at this temperature for 30 min, benzyl bromide (13.9 mL, 116.8 mmol, 4.0 equiv.) was added, keeping the temperature close to 0 °C. The reaction mixture was stirred for 2 h, while the bath temperature reached ambient temperature. After this time, TLC (Chex/EtOAc, 6:4) showed the complete transformation of the starting material into a less polar product. The reaction was quenched by adding MeOH at 0 °C, and the volatiles were evaporated. The residue was dissolved in EtOAc, and the resulting solution was washed with water and brine, dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was suspended in AcOH (80% aq.; 146 mL) and heated at 80 °C for 2 h, after which time, TLC (Chex/EtOAc, 1:1) showed the conversion of the starting material to a major more polar product. The volatiles were evaporated and co-evaporated twice with toluene. The residue was purified by flash chromatography (Chex/EtOAc, 1:1 to 3:7) to give diol **7** (10.42 g, 89%) as a white solid. The analytical data were as published.[36]

Benzyl (Allyl 2,3-Di-*O*-benzyl-β-D-galactopyranosid)uronate[34] (9): TEMPO (390 mg, 2.50 mmol, 0.2 equiv.) and BAIB (10.05 g, 31.2 mmol, 2.5 equiv.) were added to a vigorously stirred solution of diol **7** (5.00 g, 12.49 mmol) in CH_2Cl_2/H_2O (2:1, 62 mL) at room temperature. After 1 h, TLC (CH_2Cl_2/MeOH, 9:1) showed the conversion of the starting material into the more polar acid **8**.[37] The reaction was quenched by the addition of $NaHSO_3$ (10% aq.), the mixture was diluted with CH_2Cl_2, and the phases were separated. The aqueous phase was acidified with HCl (10% aq.) and re-extracted twice with CH_2Cl_2. The combined organic extracts were washed with brine, filtered through a phase separator, and concentrated. Crude acid **8** was dissolved in dry DMF (240 mL), then benzyl bromide (2.97 mL, 25.0 mmol, 2.0 equiv.) and $KHCO_3$ (4.75 g, 47.5 mmol, 4.0 equiv.) were added. The reaction mixture was stirred under Ar for 16 h. After that time, TLC (Chex/EtOAc, 7:3) showed the conversion of the intermediate acid into a major less polar product. The reaction was quenched by addition of MeOH, and the volatiles were evaporated. The residue was dissolved in EtOAc, the resulting solution was washed with water and brine, dried with anhydrous Na_2SO_4, and filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 9:1 to 6:4) to give uronate **9** (4.69 g, 74%) as a white solid. The analytical data were as published.[34]

Benzyl (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyrosid)uronate (11): A suspension of acceptor **9** (1.00 g, 1.98 mmol), donor[39] **10** (1.12 g, 2.58 mmol, 1.3 equiv.), and freshly activated 4 Å AW 300 MS (1.5 g) in anhydrous CH_2Cl_2 (19.8 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –40 °C and stirred for 15 min, and then TMSOTf (18 µL, 198 µmol, 0.05 equiv.) was added. Stirring was continued at that temperature for 1 h, and the bath temperature was allowed to reach room temperature. TLC (Chex/EtOAc, 7:3) showed the conversion of acceptor **9** into a less polar product. The reaction was quenched with Et_3N, filtered, and concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 8:2 to 6:4) to give disaccharide **11** (1.41 g, 91%) as a white foam. R_f = 0.16 (Chex/EtOAc, 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.24 (m, 15 H, H_{Ar}), 5.98 (m, 1 H, CH=$_{All}$), 5.52 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, 1 H, 2_D-H), 5.41–5.32 (m, 2 H, 3_D-H, =CH_{2All}), 5.32 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 5.22 (m, J_{cis} = 10.5, J_{gem} = 1.3 Hz, 1 H, =CH_{2All}), 5.16 (d, 1 H, H_{CO2Bn}), 5.04 (d, 1 H, 1_D-H), 5.03 (dd, $J_{3,4}$ = 10.0, $J_{4,5}$ = 9.8 Hz, 1 H, 4_D-H), 4.98 (d, J = 10.8 Hz, 1 H, H_{Bn}), 4.80 (d, J = 10.8 Hz, 1 H, H_{Bn}), 4.78 (d, J = 11.7 Hz, 1 H, H_{Bn}), 4.67 (d, J = 11.7 Hz, 1 H, H_{Bn}), 4.53 (m, 1 H, H_{All}), 4.42 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1_A-H), 4.35 (bd, 1 H, 4_A-H), 4.18 (m, 1 H, H_{All}), 4.07 (bs, 1 H, 5_A-H), 3.98 (dq, 1 H, 5_D-H), 3.88 (dd, $J_{2,3}$ = 9.6 Hz, 1 H, 2_A-H), 3.54 (dd, $J_{3,4}$ = 3.0 Hz, 1 H, 3_A-H), 2.07, 2.06, 1.99 (3 s, 9 H, H_{Ac}), 1.21 (d, $J_{5,6}$ = 6.3 Hz, 3 H, 6_D-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3, 169.7 (3 C, CO_{Ac}), 167.3 (C-6_A), 138.4, 137.9, 135.0 (3 C, C_{IVAr}), 133.9 (CH=$_{All}$), 128.7–127.7 (15 C, C_{Ar}), 117.6 (=CH_{2All}), 102.6 ($^1J_{CH}$ = 154.0 Hz, C-1_A), 99.7 ($^1J_{CH}$ = 170.4 Hz, C-1_D), 80.4 (C-3_A), 78.8 (C-2_A), 77.2 (C-4_A), 75.6 (C_{Bn}), 73.5 (C_{Bn}), 73.4 (C-5_A), 73.0 (C-4_D), 70.6 (CH_{2All}), 69.8 (C-2_D), 69.0 (C-3_D), 67.3 (C_{CO2Bn}), 67.1 (C-5_D), 21.0, 20.9, 20.8 (3 C, CH_{3Ac}), 17.6 (C-6_D) ppm. HRMS (ESI$^+$): calcd. for $C_{42}H_{49}O_{14}$ [M + H]$^+$ 777.3123; found 777.3004; calcd. for $C_{42}H_{48}O_{14}Na$ [M + Na]$^+$ 799.2942; found 799.2759.

Propyl α-L-Rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic Acid (1): Pd/C (10%; 150 mg) was added to a stirred solution of disaccharide **11** (218 mg, 0.28 mmol) in EtOAc (14 mL). The suspension was stirred under hydrogen for 16 h. After this time, MS analysis of the crude reaction mixture indicated the presence of a single compound, with a molecular weight corresponding to that of intermediate uronic acid **12**. The reaction mixture was filtered, and the solvents were evaporated to dryness. The residue was dissolved in THF/H_2O (1:1, 8.4 mL), and treated with NaOH (1 M aq.; 4.21 mL, 4.21 mmol, 15 equiv.) for 24 h. The reaction was quenched with Dowex-H$^+$ resin, and the suspension was filtered. Evaporation of the volatiles, freeze drying, and purification of the crude material by preparative RP-HPLC gave disaccharide **1** (55.1 mg, 51% over two steps) as a white solid following extensive freeze-drying. t_R = 7.6 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.06 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 1_D-H), 4.37 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1_A-H), 4.33 (d, $J_{4,5}$ = 1.2 Hz, 1 H, 5_A-H), 4.28 (dd, $J_{3,4}$ = 3.0 Hz, 1 H, 4_A-H), 4.01 (dd, $J_{2,3}$ = 3.3 Hz, 1 H, 2_D-H), 3.82 (dt_{po}, J = 6.9, J =

9.9 Hz, 1 H, OCH_{2Pr}), 3.78 (dd_{po}, $J_{2,3}$ = 10.0 Hz, 1 H, 3_A-H), 3.69 (dd, $J_{3,4}$ = 9.8 Hz, 1 H, 3_D-H), 3.57–3.49 (m, 2 H, 5_D-H, OCH_{2Pr}), 3.49 (dd, 1 H, 2_A-H), 3.31 (pt, $J_{4,5}$ = 9.7 Hz, 1 H, 4_D-H), 1.56 (sext, 2 H, CH_{2Pr}), 1.16 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H), 0.84 (t, 3 H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 171.5 (C-6_A), 102.4 ($^1J_{CH}$ = 162.7 Hz, C-1_A), 101.4 ($^1J_{CH}$ = 172.1 Hz, C-1_D), 76.6 (C-4_A), 73.0 (C-3_A), 72.8 (C-5_A), 72.3 (OCH_{2Pr}), 71.8 (C-4_D), 70.2 (C-2_D), 70.1 (C-2_A), 69.9 (C-3_D) 69.1 (C-5_D), 22.1 (CH_{2Pr}), 16.5 (C-6_D), 9.6 (CH_{3Pr}) ppm. HRMS (ESI^+): calcd. for $C_{15}H_{26}O_{11}Na$ $[M + Na]^+$ 405.1373; found 405.1366.

1-*O*-Acetyl-3,4-di-*O*-benzyl-2-*O*-levulinoyl-α/β-L-rhamnopyranose (15): Hemiacetal **13**[42] (3.18 g, 7.19 mmol) and DMAP (88 mg, 719 μmol, 0.1 equiv.) were dissolved in dry pyridine under Ar, and the solution was cooled to 0 °C. Acetic anhydride (13.6 mL, 143.7 mmol) was added dropwise, and the reaction mixture was stirred for 2 h, while the bath reached room temperature. After that time, TLC (Chex/EtOAc, 6:4) showed the transformation of the starting material into a mixture of α- and β-anomeric acetates. The volatiles were evaporated and co-evaporated twice with toluene. The residue was purified by flash chromatography (Chex/EtOAc, 7:3 to 6:4) to give a 4.5:1 α/β mixture of acetate **15** (3.33 g, 96%) as a yellow oil. Data for the α isomer: R_f = 0.26 (Chex/EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.29 (m, 10 H, H_{Ar}), 6.02 (d, $J_{1,2}$ = 1.9 Hz, 1 H, 1-H), 5.36 (dd, $J_{2,3}$ = 3.4 Hz, 1 H, 2-H), 4.94 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.72 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.66 (d, 1 H, H_{Bn}), 4.56 (d, 1 H, H_{Bn}), 3.94 (dd, $J_{3,4}$ = 9.4 Hz, 1 H, 3-H), 3.82 (dq, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.2 Hz, 1 H, 5-H), 3.48 (pt, 1 H, 4-H), 2.84–2.69 (m, 4 H, CH_{2Lev}), 2.19 (s, 3 H, CH_{3Lev}), 2.09 (s, 3 H, CH_{3Ac}), 1.35 (d, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 205.8 (CO_{Lev}), 171.7 (CO_{2Lev}), 168.4 (CO_{2Ac}), 138.2, 137.8 (2 C, C_{IVAr}), 128.4–127.8 (10 C, C_{Ar}), 91.0 (C-1), 79.5 (C-4), 77.6 (C-3), 75.5, 71.8 (2 C, C_{Bn}), 70.0 (C-5), 68.1 (C-2), 38.0 ($COCH_{2Lev}$), 29.7 (CH_{3Lev}), 28.0 (CO_2CH_{2Lev}), 20.8 (CH_{3Ac}), 18.0 (C-6) ppm. Data for the β isomer: R_f = 0.35 (Chex/EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.29 (m, 10 H, H_{Ar}), 5.74 (d, $J_{1,2}$ = 0.9 Hz, 1 H, 1-H), 5.61 (dd, $J_{2,3}$ = 3.3 Hz, 1 H, 2-H), 4.94 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.72 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.66 (d, 1 H, H_{Bn}), 4.52 (d, 1 H, H_{Bn}), 3.71 (dd, $J_{3,4}$ = 9.0 Hz, 1 H, 3-H), 3.53 (dq, $J_{4,5}$ = 9.4, $J_{5,6}$ = 6.1 Hz, 1 H, 5-H), 3.45 (pt, 1 H, 4-H), 2.84–2.69 (m, 4 H, CH_{2Lev}), 2.20 (s, 3 H, CH_{3Lev}), 2.12 (s, 3 H, CH_{3Ac}), 1.39 (d, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.03 (CO_{Lev}), 172.2 (CO_{2Lev}), 168.8 (CO_{2Ac}), 138.2, 137.5 (2 C, C_{IVAr}), 128.4–127.8 (10 C, C_{Ar}), 91.2 (C-1), 79.6 (C-3), 79.3 (C-4), 75.4 (C_{Bn}), 72.8 (C-5), 71.5 (C_{Bn}), 67.8 (C-2), 38.0 ($COCH_{2Lev}$), 29.7 (CH_{3Lev}), 28.0 (CO_2CH_{2Lev}), 20.7 (CH_{3Ac}), 17.9 (C-6) ppm. HRMS (ESI^+): calcd. for $C_{27}H_{32}O_8Na$ $[M + Na]^+$ 507.1995; found 507.1999.

3,4-Di-*O*-benzyl-2-*O*-levulinoyl-α/β-L-rhamnopyranosyl *N*-Phenyltrifluoroacetimidate (16): Hemiacetal **13** (3.22 g, 7.3 mmol) was dissolved in acetone (24.7 mL). *N*-Phenyltrifluoroacetimidoyl chloride (3.02 g, 14.6 mmol, 2.0 equiv.) followed by Cs_2CO_3 (2.61 g, 8.0 mmol, 1.1 equiv.) were added to the solution. The mixture was stirred at room temperature for 2 h. After this time, TLC (Chex/EtOAc, 6:4) showed the transformation of the starting material into a less polar product. The reaction mixture was filtered and concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 75:25 to 65:35 + 1% Et_3N) to give a 4:1 α/β mixture of PTFA **16** (4.27 g, 95%) as a yellow oil. Data for the α isomer: R_f = 0.50 (Chex/EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.26 (m, 12 H, H_{Ar}), 5.13 (m, 1 H, H_{Ar}), 6.86–6.82 (m, 2 H, H_{Ar}), 6.13 (bs, 1 H, 1-H), 5.48 (dd, $J_{1,2}$ = 2.0, $J_{2,3}$ = 3.3 Hz, 1 H, 2-H), 4.94 (d, J = 10.8 Hz, 1 H, H_{Bn}), 4.73 (d, J = 11.1 Hz, 1 H, H_{Bn}), 4.66 (d, 1 H, H_{Bn}), 4.60 (d, 1 H, H_{Bn}), 3.99 (dd, $J_{3,4}$ = 9.3 Hz, 1 H, 3-H), 3.90 (dq, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.2 Hz, 1 H, 5-H), 3.51 (pt, 1 H, 4-H), 2.81–2.69 (m, 4 H, CH_{2Lev}), 2.19 (s, 3 H, CH_{3Lev}), 1.34 (d, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, partial): δ = 206.1 (CO_{Lev}), 171.6 (CO_{2Lev}), 143.3, 138.1, 137.6 (3 C, C_{IVAr}), 128.8–127.9 (13 C, C_{Ar}), 119.4 (2 C, C_{Ar}), 94.0 (C-1), 79.2 (C-4), 77.3 (C-3), 75.7, 72.1 (2 C, C_{Bn}), 70.4 (C-5), 67.7 (C-2), 38.0 ($COCH_{2Lev}$), 29.9 (CH_{3Lev}), 28.0 (CO_2CH_{2Lev}), 18.0 (C-6) ppm.

Benzyl (3,4-Di-*O*-benzyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (17)

Route 1: A suspension of acceptor **9** (300 mg, 0.60 mmol), acetate **15** (432 mg, 0.89 mmol, 1.5 equiv.), and freshly activated 4 Å AW 300 MS (450 mg) in anhydrous CH_2Cl_2 (5.9 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –10 °C, and TMSOTf (14 μL, 83 μmol, 0.14 equiv.) was added. The reaction mixture was stirred for 1 h, while the cooling bath was allowed to reach room temperature. TLC (Tol/acetone, 9:1) showed the conversion of acceptor **9** into a less polar product. The reaction was quenched by the addition of Et_3N, the solids were filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Tol/acetone, 95:5) to give disaccharide **17** (472 mg, 89%) as a colourless oil.

Route 2: A suspension of acceptor **9** (300 mg, 595 μmol), TCA **14**[42] (419 mg, 713 μmol, 1.2 equiv.), and freshly activated 4 Å AW 300 MS (450 mg) in anhydrous toluene (5.9 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –10 °C, and TMSOTf (5 μL, 30 μmol, 0.05 equiv.) was added. The reaction mixture was stirred for 30 min, while the cooling bath was allowed to reach room temperature. TLC (Tol/acetone, 9:1) indicated the absence of acceptor **9** and the presence of a less polar product. The reaction was quenched by the addition of Et_3N. The resulting mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Tol/acetone, 97:3 to 96:4) to give disaccharide **17** (519 mg, 94%) as a colourless oil.

Route 3: A suspension of acceptor **9** (375 mg, 743 μmol), PTFA **16** (547 mg, 892 μmol, 1.2 equiv.), and freshly activated 4 Å AW 300 MS (560 mg) in anhydrous toluene (7.4 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –10 °C, and TMSOTf (5 μL, 30 μmol, 0.05 equiv.) was added. The reaction mixture was stirred for 30 min, while the cooling bath was allowed to reach room temperature. TLC (Tol/acetone, 9:1) showed the absence of acceptor **9** and the presence of a less polar product. The reaction was quenched by addition of Et_3N. The resulting mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Tol/acetone, 95:5) to give disaccharide **17** (663 mg, 96%) as a colourless oil. R_f = 0.47 (Tol/acetone, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41–7.23 (m, 25 H, H_{Ar}), 5.99 (m, 1 H, $CH{=}_{All}$), 5.55 (dd, $J_{1,2}$ = 2.1, $J_{2,3}$ = 3.3 Hz, 1 H, 2_D-H), 5.36 (m, J_{trans} = 17.2, J_{gem} = 1.5 Hz, 1 H, $=CH_{2All}$), 5.28 (d, J = 12.3 Hz, 1 H, H_{CO2Bn}) 5.24–5.20 (m, 2 H, 1_D-H, $=CH_{2All}$), 5.12 (d, 1 H, H_{CO2Bn}), 4.94 (d, J = 11.0 Hz, 1 H, H_{Bn}), 4.91 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.79 (d, J = 11.0 Hz, 1 H, H_{Bn}), 4.77 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.74 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.64 (d, J = 11.1 Hz, 1 H, H_{Bn}), 4.61 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.50 (m, 1 H, H_{All}), 4.45 (d, J = 11.0 Hz, 1 H, H_{Bn}), 4.43–4.39 (m, 2 H, 4_A-H, 1_A-H), 4.17 (m, 1 H, H_{All}), 4.05 (bs, 1 H, 5_A-H), 3.88 (dd, $J_{3,4}$ = 9.4 Hz, 1 H, 3_D-H), 3.80–3.71 (m, 2 H, 2_A-H, 5_D-H), 3.56 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 2.9 Hz, 1 H, 3_A-H), 3.37 (pt, $J_{4,5}$ = 9.4 Hz, 1 H, 4_D-H), 2.72–2.62 (m, 4 H, CH_{2Lev}), 2.16 (s, 3 H, CH_{3Lev}), 1.30 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.4 (CO_{Lev}), 171.6 (CO_{2Lev}), 167.4 (C-6_A), 138.8, 138.3, 138.2, 137.9, 135.0 (5 C, C_{IVAr}), 133.9 ($CH{=}_{All}$),

128.6–127.5 (25 C, C_{Ar}), 117.6 (=CH_{2All}), 102.6 ($^1J_{CH}$ = 161.3 Hz, C-1_A), 98.9 ($^1J_{CH}$ = 172.7 Hz, C-1_D), 80.8 (C-3_A), 79.7 (C-4_D), 78.3 (C-2_A), 77.8 (C-3_D), 75.3, 75.1 (2 C, C_{Bn}), 73.7 (C-4_A), 73.6 (C-5_A), 73.1, 71.7 (2 C, C_{Bn}), 70.6 (CH_{2All}), 69.0 (C-2_D), 68.3 (C-5_D), 67.4 (C_{CO2Bn}), 38.1 (CO*C*H_{2Lev}), 29.9 (CH_{3Lev}), 28.2 (CO_2*C*H_{2Lev}), 18.1 (C-6_D) ppm. HRMS (ESI$^+$): calcd. for $C_{55}H_{60}O_{13}Na$ [M + Na]$^+$ 951.3932; found 951.3846.

(3,4-Di-*O*-benzyl-2-*O*-levulinoyl-β-L-rhamnopyranosyl)(trichloroacetyl)amine (19): A suspension of acceptor **9** (3.00 g, 5.95 mmol), TCA **14** (4.54 g, 7.73 mmol, 1.3 equiv.), and freshly activated 4 Å AW 300 MS (4.5 g) in anhydrous CH_2Cl_2 (59.5 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –40 °C, and TMSOTf (54 μL, 0.30 mmol, 0.05 equiv.) was added. The reaction mixture was stirred for 30 min, while the cooling bath was allowed to reach room temperature. TLC (Tol/acetone, 9:1) indicated the absence of acceptor **9** and the presence of a less polar product. The reaction was quenched by the addition of Et_3N. The resulting mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 95:5 to 9:1) to give, in order of elution, glycosylamide **19** (530 mg) and disaccharide **17**, contaminated with trichloroacetamide. A second purification by flash chromatography (CH_2Cl_2/EtOAc) gave pure **17** (3.86 g, 70%) as a colourless oil. Data for **19**: R_f = 0.23 (Tol/EtOAc, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.54 (d, $J_{NH,1}$ = 8.8 Hz, 1 H, NH), 7.39–7.29 (m, 10 H, H_{Ar}), 5.59 (dd, $J_{1,2}$ = 1.3, $J_{2,3}$ = 3.3 Hz, 1 H, 2-H), 5.32 (dd, 1 H, 1-H), 4.93 (d, J = 11.1 Hz, 1 H, H_{Bn}), 4.74 (d, J = 11.4 Hz, 1 H, H_{Bn}), 4.65 (d, 1 H, H_{Bn}), 4.53 (d, 1 H, H_{Bn}), 3.74 (dd, $J_{3,4}$ = 9.1 Hz, 1 H, 3-H), 3.55 (dq, $J_{4,5}$ = 9.3, $J_{5,6}$ = 6.2 Hz, 1 H, 5-H), 3.38 (pt, 1 H, 4-H), 2.85–2.64 (m, 4 H, CH_{2Lev}), 2.19 (s, 3 H, CH_{3Lev}), 1.37 (d, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.8 (CO_{Lev}), 172.0 (CO_{2Lev}), 161.5 (NHCO), 138.2, 137.4 (2 C, C_{IVAr}), 128.4–127.8 (10 C, C_{Ar}), 92.1 (CCl_3), 80.2 (C-3), 79.2 (C-4), 78.4 ($^1J_{CH}$ = 154.3 Hz, C-1), 75.4 (C_{Bn}), 74.0 (C-5), 71.8 (C_{Bn}), 68.9 (C-2), 38.2 (CO*C*H_{2Lev}), 29.6 (CH_{3Lev}), 28.2 (CO_2*C*H_{2Lev}), 18.1 (C-6) ppm. HRMS (ESI$^+$): calcd. for $C_{27}H_{30}Cl_3NO_7Na$ [M + Na]$^+$ 608.0986; found 608.0972.

Benzyl (3,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (18): Acetic acid (5.6 mL) and then hydrazine monohydrate (326 μL, 6.73 mmol, 5.0 equiv.) were slowly added to a stirred solution of disaccharide **17** (1.25 g, 1.35 mmol) in dry pyridine (8.3 mL) at 0 °C under Ar. The reaction mixture was stirred at room temperature for 1.5 h. After this time, TLC (Chex/EtOAc, 7:3) showed the complete transformation of the starting material into a less polar product. The volatiles were evaporated and co-evaporated twice with toluene. The residue was dissolved in CH_2Cl_2 and washed with water. The aqueous phase was re-extracted twice with CH_2Cl_2, and the combined organic extracts were washed with brine, passed through a phase-separator filter, and concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 8:2 to 7:3) to give alcohol **18** (1.05 g, 90%) as a pale yellow oil. R_f = 0.35 (Chex/EtOAc, 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42–7.27 (m, 25 H, H_{Ar}), 6.01 (m, 1 H, CH=$_{All}$), 5.39 (d_o, $J_{1,2}$ = 1.7 Hz, 1 H, 1_D-H), 5.38 (m, J_{trans} = 17.2, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.32 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 5.25 (m, J_{cis} = 10.5 Hz, 1 H, =CH_{2All}), 5.14 (d, 1 H, H_{CO2Bn}), 4.96 (d, J = 11.0 Hz, 1 H, H_{Bn}), 4.90 (d, J = 11.3 Hz, 1 H, H_{Bn}), 4.80 (d_{po}, J = 11.9 Hz, 1 H, H_{Bn}), 4.76 (d_{po}, J = 10.9 Hz, 1 H, H_{Bn}), 4.75 (d_{po}, J = 11.9 Hz, 1 H, H_{Bn}), 4.67–4.64 (m, 3 H, H_{Bn}), 4.54 (m, 1 H, H_{All}), 4.49 (dd, 1 H, 4_A-H), 4.43 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1_A-H), 4.23–4.16 (m, 2 H, 2_D-H, H_{All}), 4.08 (d, $J_{4,5}$ = 1.2 Hz, 1 H, 5_A-H), 3.89 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.0 Hz, 1 H, 3_D-H), 3.80–3.71 (m, 2 H, 2_A-H, 5_D-H), 3.58 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 3.0 Hz, 1 H, 3_A-H), 3.47 (pt, $J_{4,5}$ = 9.3 Hz, 1 H, 4_D-H), 2.43 (bs, 1 H, OH), 1.32 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3 (C-6_A), 138.8, 138.4, 138.2, 137.8, 135.1 (5 C, C_{IVAr}), 134.0 (CH=$_{All}$), 128.6–127.5 (25 C, C_{Ar}), 117.5 (=CH_{2All}), 102.7 ($^1J_{CH}$ = 159.4 Hz, C-1_A), 100.4 ($^1J_{CH}$ = 172.7 Hz, C-1_D), 81.2 (C-3_A), 79.9 (C-4_D), 79.6 (C-3_D), 78.4 (C-2_A), 75.2, 75.0 (2 C, C_{Bn}), 73.7 (C-5_A), 73.5 (C-4_A), 73.3, 72.2 (2 C, C_{Bn}), 70.6 (CH_{2All}), 68.7 (C-2_D), 68.1 (C-5_D), 67.3 (C_{CO2Bn}), 18.1 (C-6_D) ppm. HRMS (ESI$^+$): calcd. for $C_{50}H_{54}O_{11}Na$ [M + Na]$^+$ 853.3564; found 853.3478.

Allyl 4-*O*-Benzyl-2-*O*-levulinoyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranoside (21): Levulinic acid (7.81 mL, 76.2 mmol, 2.0 equiv.), DCC (15.7 g, 76.2 mmol, 2.0 equiv.), and DMAP (931 mg, 7.6 mmol, 0.2 equiv.) were added to a solution of alcohol **20**[45] (15.8 g, 38.1 mmol) in anhydrous CH_2Cl_2 (150 mL). The solution was stirred under Ar for 2 h. After that time, TLC (Tol/EtOAc, 9:1) showed the conversion of the starting alcohol into a less polar product. The reaction mixture was filtered through a pad of Celite. The filtrate was diluted with CH_2Cl_2, and washed successively with HCl (10% aq.), $NaHCO_3$ (satd. aq.), and brine, then passed through a phase-separator filter, and concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 8:2 to 7:3) to give **21** (19.5 g, quantitative) as a yellow oil. R_f = 0.24 (Tol/EtOAc, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.24 (m, 7 H, H_{Ar}), 6.88–6.84 (m, 2 H, H_{ArPMB}), 5.89 (m, 1 H, CH=$_{All}$), 5.39 (dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.4 Hz, 1 H, 2-H), 5.29 (m, J_{trans} = 17.2, J_{gem} = 1.7 Hz, 1 H, =CH_{2All}), 5.21 (m, J_{cis} = 10.4 Hz, 1 H, =CH_{2All}), 4.92 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.78 (d, 1 H, 1-H), 4.63 (d, J = 10.8 Hz, 1 H, H_{Bn}), 4.62 (d, 1 H, H_{Bn}), 4.46 (d, 1 H, H_{Bn}), 4.16 (m, 1 H, H_{All}), 4.02–3.94 (m, 2 H, 3-H, H_{All}), 3.78 (dq, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.3 Hz, 1 H, 5-H), 3.81 (s, 3 H, CH_{3PMB}), 3.40 (pt, 1 H, 4-H), 2.82–2.67 (m, 4 H, CH_{2Lev}), 2.19 (s, 3 H, CH_{3Lev}), 1.33 (d, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.2 (CO_{Lev}), 172.1 (CO_{2Lev}), 159.2 ($C_{IVArPMB}$), 138.5 (C_{IVAr}), 133.6 (CH=$_{All}$), 130.3 ($C_{IVArPMB}$), 130.0–127.7 (7 C, C_{Ar}), 117.5 (=CH_{2All}), 113.7 (2 C, C_{ArPMB}), 96.8 (C-1), 80.0 (C-4), 79.7 (C-3), 75.4 (C_{Bn}), 71.3 (C_{PMB}), 69.2 (C-2), 68.0 (C_{All}), 67.7 (C-5), 55.1 (CH_{3PMB}), 38.0 (CO*C*H_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CO_2*C*H_{2Lev}), 18.0 (C-6) ppm. HRMS (ESI$^+$): calcd. for $C_{29}H_{36}Cl_3O_8Na$ [M + Na]$^+$ 535.2308; found 535.2281.

4-*O*-Benzyl-2-*O*-levulinoyl-3-*O*-*p*-methoxybenzyl-α/β-L-rhamnopyranose (22): (1,5-Cyclooctadiene)bis(methyldiphenylphosphane)iridium hexafluorophosphate (658 mg, 0.78 mmol, 0.03 equiv.) was dissolved in anhydrous THF (130 mL), and hydrogen was bubbled through the solution for 15 min (H-cube, full H_2 mode). The resulting yellow solution was concentrated to dryness. The residue was dissolved in anhydrous THF (130 mL), and the resulting solution was poured into a solution of allyl rhamnoside **21** (13.29 g, 25.9 mmol) in anhydrous THF (130 mL). The mixture was stirred under Ar at room temperature for 2 h. A solution of iodine (13.16 g, 51.85 mmol, 2.0 equiv.) in THF/H_2O (4:1, 156 mL) was added, and the mixture was stirred at room temperature for 1 h. TLC (Chex/EtOAc, 7:3) showed the conversion of the intermediate compound into a more polar product. The reaction was quenched with sodium bisulfite (10% aq.). The mixture was concentrated to a third of its volume, and the aqueous phase was extracted three times with CH_2Cl_2. The organic extracts were combined, washed with brine, dried by passing through a phase-separator filter, and concentrated to dryness. The residue was purified by flash chromatography (Chex/EtOAc, 1:1) to give hemiacetal **22** (11.46 g, 90%) as a yellow oil (α/β, 3.5:1). Data for the α isomer: R_f = 0.18 (Chex/EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.24 (m, 7 H, H_{Ar}), 6.87–6.83 (m, J = 8.7 Hz, 2 H, H_{ArPMB}), 5.38 (dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 3.3 Hz, 1 H, 2-H), 5.13 (d, 1 H, 1-H), 4.92 (d, J

= 10.9 Hz, 1 H, H_{Bn}), 4.63 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.62 (d, 1 H, H_{Bn}), 4.46 (d, 1 H, H_{Bn}), 4.03–3.95 (m, 2 H, 3-H, 5-H), 3.81 (s, 3 H, CH_{3PMB}), 3.40 (pt, $J_{3,4}$ = 9.3, $J_{4,5}$ = 9.5 Hz, 1 H, 4-H), 2.93–2.61 (m, 4 H, CH_{2Lev}), 2.19 (s, 3 H, CH_{3Lev}), 1.32 (d, $J_{5,6}$ = 6.3 Hz, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.3 (CO_{Lev}), 172.1 (CO_{2Lev}), 159.3 ($C_{IVArPMB}$), 138.6 (C_{IVAr}), 130.3 ($C_{IVArPMB}$), 129.8–127.6 (7 C, C_{Ar}), 113.8 (2 C, C_{ArPMB}), 92.4 ($^1J_{CH}$ = 171.0 Hz, C-1), 80.0 (C-4), 77.2 (C-3), 75.3 (C_{Bn}), 71.3 (C_{PMB}), 69.6 (C-2), 67.7 (C-5), 55.2 (CH_{3PMB}), 38.1 ($COCH_{2Lev}$), 29.8 (CH_{3Lev}), 28.2 (CO_2CH_{2Lev}), 18.1 (C-6) ppm. Data for the β isomer: R_f = 0.18 (Chex/EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.24 (m, 7 H, H_{Ar}), 6.87–6.83 (m, J = 8.7 Hz, 2 H, H_{ArPMB}), 5.53 (dd, $J_{1,2}$ = 1.0, $J_{2,3}$ = 3.3 Hz, 1 H, 2-H), 4.78 (bs, 1 H, 1-H), 4.68 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.65–460 (2d_o, 2 H, H_{Bn}), 4.45 (d, J = 10.9 Hz, 1 H, H_{Bn}), 3.81 (s, 3 H, CH_{3PMB}), 3.64 (dd, $J_{3,4}$ = 9.1 Hz, 1 H, 3-H), 3.42–3.38 (dq_o, 1 H, 5-H), 3.40 (pt, $J_{4,5}$ = 9.4 Hz, 1 H, 4-H), 2.82–2.67 (m, 4 H, CH_{2Lev}), 2.21 (s, 3 H, CH_{3Lev}), 1.37 (d, $J_{5,6}$ = 6.1 Hz, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.5 (CO_{Lev}), 172.6 (CO_{2Lev}), 159.4 ($C_{IVArPMB}$), 138.4 (C_{IVAr}), 130.3 ($C_{IVArPMB}$), 129.8–127.6 (7 C, C_{Ar}), 113.9 (2 C, C_{ArPMB}), 93.1 ($^1J_{CH}$ = 158.2 Hz, C-1), 79.7 (C-3), 79.5 (C-4), 75.3 (C_{Bn}), 71.7 (C-5), 71.2 (C_{PMB}), 70.2 (C-2), 55.1 (CH_{3PMB}), 38.6 ($COCH_{2Lev}$), 29.8 (CH_{3Lev}), 28.2 (CO_2CH_{2Lev}), 18.0 (C-6) ppm. HRMS (ESI$^+$): calcd. for $C_{26}H_{32}O_8Na$ [M + Na]$^+$ 495.1995; found 495.2004.

4-*O*-Benzyl-2-*O*-levulinoyl-3-*O*-*p*-methoxybenzyl-α/β-L-rhamnopyranosyl Trichloroacetimidate (23): Hemiacetal **22** (10.0 g, 21.16 mmol) was dissolved in anhydrous DCE (42 mL) and stirred under Ar. Trichloroacetonitrile (10.6 mL, 105.8 mmol, 5.0 equiv.) and DBU (0.95 mL, 6.3 mmol, 0.3 equiv.) were added at room temperature. After 20 min, TLC showed the conversion of the starting material into a less polar product. The reaction mixture was purified directly by flash chromatography (Chex/EtOAc, 7:3 to 1:1 + 1% Et_3N) to give TCA **23** (12.66 g, 97%) as a pale yellow oil (α/β, 7.5:1). Data for the α isomer: R_f = 0.46 (Chex/EtOAc, 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.67 (s, 1 H, NH), 7.41–7.26 (m, 7 H, H_{Ar}), 6.89–6.84 (m, J = 8.7 Hz, 2 H, H_{ArPMB}), 6.19 (d, $J_{1,2}$ = 1.9 Hz, 1 H, 1-H), 5.47 (dd, $J_{2,3}$ = 3.3 Hz, 1 H, 2-H), 4.94 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.66 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.65 (d, 1 H, H_{Bn}), 4.51 (d, 1 H, H_{Bn}), 3.98 (dd, $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 3.94 (dq, $J_{4,5}$ = 9.4 Hz, 1 H, 5-H), 3.82 (s, 3 H, CH_{3PMB}), 3.50 (pt, 1 H, 4-H), 2.84–2.72 (m, 4 H, CH_{2Lev}), 2.22 (s, 3 H, CH_{3Lev}), 1.36 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.3 (CO_{Lev}), 171.9 (CO_{2Lev}), 160.1 (C=NH), 159.4 ($C_{IVArPMB}$), 138.1 (C_{IVAr}), 130.0–127.9 (8 C, 7 C_{Ar}, $C_{IVArPMB}$), 113.8 (2 C, C_{ArPMB}), 95.1 (C-1), 90.8 (CCl_3), 79.2 (C-4), 76.6 (C-3), 75.6 (C_{Bn}), 71.5 (C_{PMB}), 70.7 (C-5), 67.8 (C-2), 55.3 (CH_{3PMB}), 38.0 ($COCH_{2Lev}$), 29.9 (CH_{3Lev}), 28.1 (CO_2CH_{2Lev}), 18.0 (C-6) ppm.

4-*O*-Benzyl-3-*O*-*p*-methoxybenzyl-2-*O*-levulinoyl-α/β-L-rhamnopyranosyl *N*-Phenyltrifluoroacetimidate (24): Hemiacetal **22** (5.0 g, 10.58 mmol) was dissolved in acetone (36 mL). *N*-Phenyltrifluoroacetimidoyl chloride (4.39 g, 21.2 mmol, 2.0 equiv.) and Cs_2CO_3 (3.79 g, 11.6 mmol, 1.1 equiv.) were added to the solution. The mixture was stirred at room temperature for 4 h. After that time, TLC (Chex/EtOAc, 6:4) showed the complete transformation of the starting material into a less polar product. The reaction mixture was filtered and concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 9:1 to 7:3 + 1% Et_3N) to give a 5:1 α/β mixture of PTFA **24** (6.70 g, 98%) as a pale yellow oil. Data for the α isomer: R_f = 0.48 (Chex/EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42–6.81 (m, 14 H, H_{Ar}), 6.17 (bs, 1 H, 1-H), 5.46 (bs, 1 H, 2-H), 4.94 (d, J = 10.6 Hz, 1 H, H_{Bn}), 4.70–4.62 (m, 2 H, H_{Bn}), 4.53 (d, J = 10.6 Hz, 1 H, H_{Bn}), 3.98 (dd, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 3.90 (dq, $J_{4,5}$ = 9.1, $J_{5,6}$ = 6.1 Hz, 1 H, 5-H), 3.83 (s, 3 H, CH_{3PMB}), 3.49 (pt, 1 H, 4-H), 2.82–2.69 (m, 4 H, CH_{2Lev}), 2.21 (s, 3 H, CH_{3Lev}), 1.32 (d, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, partial): δ = 206.2 (CO_{Lev}), 171.8 (CO_{2Lev}), 159.4 ($C_{IVArPMB}$), 143.3, 138.1 (2 C, C_{IVAr}), 130.0–119.4 (13 C, 12C_{Ar}, $C_{IVArPMB}$), 113.8 (2 C, C_{ArPMB}), 93.9 (C-1), 79.2 (C-4), 76.9 (C-3), 75.6 (C_{Bn}), 71.7 (C_{PMB}), 70.4 (C-5), 67.8 (C-2), 55.3 (CH_{3PMB}), 38.0 ($COCH_{2Lev}$), 29.9 (CH_{3Lev}), 28.0 (CO_2CH_{2Lev}), 18.0 (C-6) ppm.

(4-*O*-Benzyl-2-*O*-levulinoyl-3-*O*-*p*-methoxybenzyl-β-L-rhamnopyranosyl)(trichloroacetyl)amine (25): A mixture of acceptor **18** (0.30 g, 361 μmol), TCA **23** (334 mg, 542 μmol, 1.5 equiv.), and freshly activated 4 Å powdered MS (750 mg) in anhydrous toluene (10.8 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –10 °C, and TMSOTf (3.3 μL, 18 μmol, 0.05 equiv.) was added. The reaction mixture was stirred at that temperature for 20 min. TLC (Chex/EtOAc, 6:4) showed the conversion of acceptor **18** into a more polar product. The reaction was quenched by the addition of Et_3N. The resulting suspension was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 95:5 to 85:15) to give, in order of elution, glycosylamide **25** (59 mg) followed by trisaccharide **26** (391 mg, 84%) as a pale yellow oil. Data for **25**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (d, $J_{NH,1}$ = 8.8 Hz, 1 H, NH), 7.41–7.18 (m, 7 H, H_{Ar}), 6.88–6.84 (m, 2 H, H_{ArPMB}), 5.57 (dd, $J_{1,2}$ = 1.1, $J_{2,3}$ = 3.2 Hz, 1 H, 2-H), 5.31 (dd, 1 H, 1-H), 4.91 (d, J = 11.1 Hz, 1 H, H_{Bn}), 4.67 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.62 (d, 1 H, H_{Bn}), 4.45 (d, 1 H, H_{Bn}), 3.82 (s, 3 H, CH_{3PMB}), 3.72 (dd, $J_{3,4}$ = 9.1 Hz, 1 H, 3-H), 3.54 (dq, $J_{4,5}$ = 9.4, $J_{5,6}$ = 6.1 Hz, 1 H, 5-H), 3.35 (pt, 1 H, 4-H), 2.92–2.63 (m, 4 H, CH_{2Lev}), 2.21 (s, 3 H, CH_{3Lev}), 1.37 (d, 3 H, 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.9 (CO_{Lev}), 172.0 (CO_{2Lev}), 161.5 (NHCO), 159.5 (C_{IVPMB}), 138.2 (C_{IVAr}), 129.8 (2 C, C_{Ar}) 129.6 (C_{IVAr}), 128.4–127.6 (5 C, C_{Ar}), 113.9 (2 C, C_{ArPMB}), 92.0 (CCl_3), 79.9 (C-3), 79.2 (C-4), 78.4 (C-1), 75.3 (C_{Bn}), 74.0 (C-5), 71.4 (C_{Bn}), 69.0 (C-2), 55.3 (CH_{3PMB}), 38.2 ($COCH_{2Lev}$), 29.6 (CH_{3Lev}), 28.2 (CO_2CH_{2Lev}), 18.1 (C-6) ppm. HRMS (ESI$^+$): calcd. for $C_{28}H_{32}Cl_3NO_8Na$ [M + Na]$^+$ 638.1091; found 638.1140.

Benzyl (4-*O*-Benzyl-3-*O*-*p*-methoxybenzyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (26)

Route 1: A mixture of acceptor **18** (0.30 g, 361 μmol), TCA **23** (267 mg, 433 μmol, 1.2 equiv.), and freshly activated 4 Å powdered MS (750 mg) in anhydrous toluene (10.8 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –10 °C, and TMSOTf (3.3 μL, 18 μmol, 0.05 equiv.) was added. The reaction mixture was stirred at that temperature for 20 min. TLC (Chex/EtOAc, 6:4) showed the conversion of acceptor **18** into a more polar product. The reaction was quenched by the addition of Et_3N. The resulting suspension was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 95:5 to 85:15) to give trisaccharide **26** (426 mg, 92%) as a pale yellow oil.

Route 2: A mixture of acceptor **18** (0.30 g, 361 μmol), PTFA **24** (279 mg, 433 μmol, 1.2 equiv.), and freshly activated 4 Å powdered MS (750 mg) in anhydrous toluene (10.8 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –10 °C, and TMSOTf (3.3 μL, 18 μmol, 0.05 equiv.) was added. The reaction mixture was stirred at that temperature for 20 min. TLC (Chex/EtOAc, 6:4) showed the conversion of acceptor **18** into a more polar product. The reaction was quenched with Et_3N. The

resulting mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 7:3 to 6:4) to give trisaccharide **26** (415 mg, 89%) as a pale yellow oil. R_f = 0.32 (Chex/EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43–7.17 (m, 32 H, H_{Ar}), 6.86 (d, J = 8.5 Hz, 2 H, H_{ArPMB}), 5.99 (m, 1 H, CH=$_{All}$), 5.52 (dd, $J_{1,2}$ = 1.9, $J_{2,3}$ = 3.1 Hz, 1 H, 2$_C$-H), 5.36 (m, J_{trans} = 17.2, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.33 (bs$_o$, 1 H, 1$_D$-H), 5.27 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 5.22 (m, J_{cis} = 10.5 Hz, 1 H, =CH_{2All}), 5.12 (d, 1 H, H_{CO2Bn}), 4.94 (d$_{po}$, J = 11.1 Hz, 1 H, H_{Bn}), 4.92–4.88 (m$_o$, 3 H, 1$_C$-H, 2 H_{Bn}), 4.80 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.75 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.70–4.57 (m, 6 H, H_{Bn}), 4.50 (m$_{po}$, 1 H, H_{All}), 4.48 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.39 (m$_o$, 1 H, 4$_A$-H), 4.38 (d$_o$, $J_{1,2}$ = 7.8 Hz, 1 H, 1$_A$-H), 4.17 (m, 1 H, H_{All}), 4.11 (pt, 1 H, 2$_D$-H), 4.02 (d, $J_{4,5}$ = 1.0 Hz, 1 H, 5$_A$-H), 3.94 (dd, $J_{3,4}$ = 9.3 Hz, 1 H, 3$_C$-H), 3.86 (dd$_{po}$, $J_{2,3}$ = 2.9, $J_{3,4}$ = 9.4 Hz, 1 H, 3$_D$-H), 3.82 (dq, 1 H, 5$_C$-H), 3.78 (s, 3 H, CH_{3PMB}), 3.75–3.64 (m, 2 H, 2$_A$-H, 5$_D$-H), 3.51 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 2.8 Hz, 1 H, 3$_A$-H), 3.42 (pt, $J_{4,5}$ = 9.5 Hz, 1 H, 4$_D$-H), 3.37 (pt, $J_{4,5}$ = 9.5 Hz, 1 H, 4$_C$-H), 2.77–2.66 (m, 4 H, CH_{2Lev}), 2.18 (s, 3 H, CH_{3Lev}), 1.28 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_D$-H), 1.20 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_C$-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.1 (CO_{Lev}), 171.7 (CO_{2Lev}), 167.3 (C-6$_A$), 159.2 (C_{IVPMB}) 138.9, 138.6, 138.4, 137.8, 135.1 (6 C, C_{IVAr}), 134.0 (CH=$_{All}$), 130.3 (C_{IVAr}), 129.8–127.4 (32 C, C_{Ar}), 117.4 (=CH_{2All}), 113.7 (2 C, C_{ArPMB}), 102.7 ($^1J_{CH}$ = 159.4 Hz, C-1$_A$), 100.0 ($^1J_{CH}$ = 174.9 Hz, C-1$_D$), 99.2 ($^1J_{CH}$ = 172.5 Hz, C-1$_C$), 80.5 (C-3$_A$), 80.1 (C-4$_C$), 79.9 (C-4$_D$), 79.3 (C-3$_D$), 78.4 (C-2$_A$), 77.4 (C-3$_C$), 75.4, 75.3 (2 C, C_{Bn}), 75.1 (C-2$_D$), 75.0 (C_{Bn}), 73.6 (C-5$_A$), 73.4 (C-4$_A$), 72.7, 72.1, 71.2 (3 C, C_{Bn}), 70.6 (CH_{2All}), 69.1 (C-2$_C$), 68.6 (C-5$_D$), 68.2 (C-5$_C$), 67.3 (C_{CO2Bn}), 55.1 (CH_{3PMB}), 38.2 ($COCH_{2Lev}$), 29.8 (CH_{3Lev}), 28.3 (CO_2CH_{2Lev}), 18.1 (C-6$_D$), 17.9 (C-6$_C$) ppm. HRMS (ESI$^+$): calcd. for $C_{76}H_{84}O_{18}Na$ [M + Na]$^+$ 1307.5555; found 1307.5554.

Benzyl (4-*O*-Benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (27): Acetic acid (11.3 mL) and hydrazine monohydrate (492 μL, 10.1 mmol, 5.0 equiv.) were slowly added to a stirred solution of fully protected **26** (2.60 g, 2.0 mmol) in dry pyridine (16.8 mL) at 0 °C under Ar. The reaction mixture was stirred at room temperature for 30 min. After that time, TLC (Tol/EtOAc, 9:1) showed the complete transformation of the starting material into a more polar product. The volatiles were evaporated and co-evaporated twice with toluene. The residue was dissolved in CH_2Cl_2 and washed with water. The aqueous phase was re-extracted twice with CH_2Cl_2, and the combined organic extracts were washed with brine, passed through a phase-separator filter, and concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 9:1 to 7:3) to give alcohol **27** (2.23 g, 93%) as a white foam. R_f = 0.10 (Tol/EtOAc, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45–7.16 (m, 32 H, H_{Ar}), 6.89 (d, J = 8.6 Hz, 2 H, H_{ArPMB}), 6.00 (m, 1 H, CH=$_{All}$), 5.37 (m, J_{trans} = 17.3, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.35 (bs$_o$, 1 H, 1$_D$-H), 5.28 (d, J = 12.1 Hz, 1 H, H_{CO2Bn}), 5.23 (m, J_{cis} = 10.5 Hz, 1 H, =CH_{2All}), 5.13 (d, 1 H, H_{CO2Bn}), 5.02 (d, $J_{1,2}$ = 1.4 Hz, 1 H, 1$_C$-H), 4.94 (d$_{po}$, J = 10.9 Hz, 1 H, H_{Bn}), 4.90 (d$_{po}$, J = 11.2 Hz, 1 H, H_{Bn}), 4.89 (d$_{po}$, J = 10.9 Hz, 1 H, H_{Bn}), 4.81 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.75 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.70–4.59 (m, 7 H, H_{Bn}), 4.51 (m, 1 H, H_{All}), 4.40 (m$_o$, 1 H, 4$_A$-H), 4.39 (d$_o$, $J_{1,2}$ = 7.7 Hz, 1 H, 1$_A$-H), 4.17 (m$_{po}$, 1 H, H_{All}), 4.15 (m$_o$, 1 H, 2$_D$-H), 4.12 (m, 1 H, 2$_C$-H), 4.03 (d, $J_{4,5}$ = 0.9 Hz, 1 H, 5$_A$-H), 3.91–3.86 (m, 2 H, 3$_C$-H, 3$_D$-H), 3.84 (dq$_{po}$, 1 H, 5$_C$-H), 3.79 (s, 3 H, CH_{3PMB}), 3.76–3.66 (m, 2 H, 2$_A$-H, 5$_D$-H), 3.52 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 2.8 Hz, 1 H, 3$_A$-H), 3.45 (pt$_{po}$, $J_{4,5}$ = 9.5 Hz, 1 H, 4$_C$-H), 3.41 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1 H, 4$_D$-H), 1.30 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_D$-H), 1.21 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_C$-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3 (C-6$_A$), 159.2 (C_{IVPMB}) 139.0, 138.6, 138.5, 137.8, 135.2 (6 C, C_{IVAr}), 134.1 (CH=$_{All}$), 130.2 (C_{IVAr}), 129.5–127.3 (32 C, C_{Ar}), 117.4 (=CH_{2All}), 114.0 (2 C, C_{ArPMB}), 102.7 ($^1J_{CH}$ = 161.1 Hz, C-1$_A$), 100.8 ($^1J_{CH}$ = 169.0 Hz, C-1$_D$), 100.2 ($^1J_{CH}$ = 171.9 Hz, C-1$_C$), 80.5 (C-3$_A$), 80.2 (2 C, C-4$_C$, C-4$_D$), 79.4 (2 C, C-3$_C$, C-3$_D$), 78.4 (C-2$_A$), 75.3, 75.2 (3 C, 2 C_{Bn}, C-2$_D$), 74.9 (C_{Bn}), 73.7 (C-5$_A$), 73.6 (C-4$_A$), 72.7, 72.3, 71.7 (3 C, C_{Bn}), 70.5 (CH_{2All}), 68.8 (C-2$_C$), 68.6 (C-5$_D$), 67.9 (C-5$_C$), 67.3 (C_{CO2Bn}), 55.2 (CH_{3PMB}), 18.2 (C-6$_D$), 17.8 (C-6$_C$) ppm. HRMS (ESI$^+$): calcd. for $C_{71}H_{78}O_{16}Na$ [M + Na]$^+$ 1210.5222; found 1210.5117; calcd. for $C_{71}H_{79}O_{16}K$ [M + H + K]$^{2+}$ 613.2502; found 613.2374.

Benzyl (4-*O*-Benzyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (28)

Route 1: Water (0.2 mL) and DDQ (27 mg, 120 μmol, 3.0 equiv.) were added to a solution of trisaccharide **26** (49 mg, 38 μmol) in CH_2Cl_2 (1.8 mL). The reaction mixture was stirred at room temperature for 3 h. After that time, TLC (Tol/EtOAc, 8:2) showed the transformation of the starting material into a more polar product. The reaction was quenched with $NaHCO_3$ (satd. aq.). The reaction mixture was diluted with water and CH_2Cl_2, and the aqueous phase was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, passed through a phase-separator filter, and concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 8:2) to give alcohol **28** (20 mg, 45%) as a yellow oil.

Route 2: Water (0.2 mL) and CAN (68 mg, 120 μmol, 3.0 equiv.) were added to a solution of trisaccharide **26** (53 mg, 41 μmol) in MeCN (1.8 mL). The reaction mixture was stirred at room temperature for 1.5 h. After that time, TLC (Tol/EtOAc, 8:2) showed the transformation of the starting material into a more polar product. The reaction was quenched with $NaHCO_3$ (satd. aq.). The reaction mixture was diluted with water and CH_2Cl_2, and the aqueous phase was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, passed through a phase-separator filter, and concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 9:1 to 8:2) to give alcohol **28** (34 mg, 71%) as a yellow oil. R_f = 0.21 (Tol/EtOAc, 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41–7.19 (m, 30 H, H_{Ar}), 6.00 (m, 1 H, CH=$_{All}$), 5.40–5.33 (m, 3 H, 2$_C$-H, =CH_{2All}, 1$_D$-H), 5.28 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 5.23 (m, J_{cis} = 10.5, J_{gem} = 1.3 Hz, 1 H, =CH_{2All}), 5.12 (d, 1 H, H_{CO2Bn}), 4.94 (d, J = 11.1 Hz, 1 H, H_{Bn}), 4.92–4.87 (m, 3 H, 1$_C$-H, 2 H_{Bn}), 4.81 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.75 (d$_{po}$, J = 10.9 Hz, 1 H, H_{Bn}), 4.70 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.66 (d$_{po}$, J = 12.5 Hz, 1 H, H_{Bn}), 4.63 (d$_{po}$, J = 11.2 Hz, 1 H, H_{Bn}), 4.61 (bs$_o$, 2 H, H_{Bn}), 4.51 (m, 1 H, H_{All}), 4.41 (d$_o$, 1 H, 4$_A$-H), 4.38 (d$_o$, $J_{1,2}$ = 7.9 Hz, 1 H, 1$_A$-H), 4.22–4.13 (m, 2 H, H_{All}, 3$_C$-H), 4.11 (pt, 1 H, 2$_D$-H), 4.01 (s, 1 H, 5$_A$-H), 3.86 (dd$_{po}$, $J_{2,3}$ = 2.9, $J_{3,4}$ = 9.5 Hz, 1 H, 3$_D$-H), 3.82 (dq$_{po}$, $J_{4,5}$ = 9.4 Hz, 1 H, 5$_C$-H), 3.74–3.64 (m, 2 H, 2$_A$-H, 5$_D$-H), 3.53 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 2.7 Hz, 1 H, 3$_A$-H), 3.46 (pt, $J_{4,5}$ = 9.4 Hz, 1 H, 4$_D$-H), 3.31 (pt, $J_{3,4}$ = 9.4 Hz, 1 H, 4$_C$-H), 2.89–2.57 (m, 4 H, CH_{2Lev}), 2.22 (s, 3 H, CH_{3Lev}), 1.28 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_D$-H), 1.21 (d, $J_{5,6}$ = 6.3 Hz, 3 H, 6$_C$-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 207.2 (CO_{Lev}), 172.3 (CO_{2Lev}), 172.2 (C-6$_A$), 138.9, 138.6, 138.5, 138.4, 137.8, 135.0 (6 C, C_{IVAr}), 134.0 (CH=$_{All}$), 128.7–127.4 (30 C, C_{Ar}), 117.6 (=CH_{2All}), 102.6 ($^1J_{CH}$ = 156.5 Hz, C-1$_A$), 99.8 ($^1J_{CH}$ = 172.4 Hz, C-1$_D$), 99.1 ($^1J_{CH}$ = 170.6 Hz, C-1$_C$), 81.8 (C-4$_C$), 80.6 (C-3$_A$), 80.0 (C-4$_D$), 79.3 (C-3$_D$), 78.4 (C-2$_A$), 75.4, 75.3 (2 C, C_{Bn}), 75.2 (2 C, C-2$_D$, C_{Bn}), 73.6 (C-5$_A$), 73.0 (C-4$_A$), 72.9 (C-2$_C$), 72.7, 72.2 (2 C, C_{Bn}), 70.7 (C-3$_C$), 70.6 (CH_{2All}), 68.6 (C-5$_D$), 68.0 (C-5$_C$), 67.4 (C_{CO2Bn}), 38.4 (CH_{2Lev}), 29.9 (CH_{3Lev}), 28.3 (CH_{2Lev}),

18.2 (C-6_D), 17.9 (C-6_C) ppm. HRMS (ESI$^+$): calcd. for $C_{68}H_{77}O_{17}$ [M + H]$^+$ 1165.5161; found 1165.5172; calcd. for $C_{68}H_{76}O_{17}Na$ [M + Na]$^+$ 1187.4980; found 1187.5001.

Benzyl (3-*O*-Acetyl-4-*O*-benzyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (29): Water (2.2 mL) and CAN (1.57 g, 2.86 mmol, 4.0 equiv.) were added to a solution of trisaccharide **26** (920 mg, 716 μmol) in MeCN (21.5 mL). The reaction mixture was stirred at room temperature for 30 min. After that time, TLC (Tol/EtOAc, 8:2) showed the complete transformation of the starting material into alcohol **28**. The reaction was quenched with $NaHCO_3$ (satd. aq.). The reaction mixture was diluted with water and CH_2Cl_2, and the aqueous phase was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, passed through a phase-separator filter, and concentrated to give the intermediate alcohol. The crude oil was dissolved in pyridine (18 mL). Acetic anhydride (9.45 mL) and DMAP (12.2 mg, 72 μmol, 0.1 equiv.) were added at room temperature. After 2 h, TLC (Tol/EtOAc, 8:2) indicated that conversion of intermediate **28** into a less polar product was complete. The volatiles were evaporated and co-evaporated three times with toluene. The residue was purified by flash chromatography (Tol/EtOAc, 9:1 to 85:15) to give acetate **29** (762 mg, 88%) as a white foam. R_f = 0.41 (Tol/EtOAc, 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.14 (m, 30 H, H_{Ar}), 5.99 (m, 1 H, CH=$_{All}$), 5.48 (dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.4 Hz, 1 H, 2$_C$-H), 5.40–5.33 (m, 3 H, =CH_{2All}, 1$_D$-H, 3$_C$-H), 5.29 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 5.23 (m, J_{cis} = 10.5, J_{gem} = 1.5 Hz, 1 H, =CH_{2All}), 5.12 (d, 1 H, H_{CO2Bn}), 4.93 (d_{po}, J = 10.0 Hz, 1 H, H_{Bn}), 4.91 (d, J = 10.2 Hz, 1 H, H_{Bn}), 4.86 (d, 1 H, 1$_C$-H), 4.80 (d, J = 11.8 Hz, 1 H, H_{Bn}), 4.74 (d, J = 10.6 Hz, 1 H, H_{Bn}), 4.71–4.60 (m, 5 H, H_{Bn}), 4.58 (d, J = 11.9 Hz, 1 H, H_{Bn}), 4.51 (m, 1 H, H_{All}), 4.44 (dd, 1 H, 4$_A$-H), 4.40 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1$_A$-H), 4.17 (m, 1 H, H_{All}), 4.12 (pt, 1 H, 2$_D$-H), 4.04 (d, $J_{4,5}$ = 1.0 Hz, 1 H, 5$_A$-H), 3.88 (dq_{po}, $J_{4,5}$ = 9.5 Hz, 1 H, 5$_C$-H), 3.83 (dd, $J_{2,3}$ = 2.9, $J_{3,4}$ = 9.4 Hz, 1 H, 3$_D$-H), 3.72 (dd_{po}, $J_{2,3}$ = 9.8 Hz, 1 H, 2$_A$-H), 3.67 (dq_{po}, $J_{4,5}$ = 9.5 Hz, 1 H, 5$_D$-H), 3.56 (pt_{po}, 1 H, 4$_D$-H), 3.54 (dd_{po}, $J_{3,4}$ = 2.9 Hz, 1 H, 3$_A$-H), 3.45 (pt, 1 H, 4$_C$-H), 2.85–2.50 (m, 4 H, CH_{2Lev}), 2.22 (s, 3 H, CH_{3Lev}), 2.01 (s, 3 H, CH_{3Ac}), 1.31 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_D$-H), 1.16 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_C$-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 205.9 (CO_{Lev}), 171.4 (CO_{2Lev}), 169.6 (CO_{Ac}), 167.3 (C-6$_A$), 139.0, 138.7, 138.5, 138.2, 137.7, 135.1 (6 C, C_{IVAr}), 134.0 (CH=$_{All}$), 129.5–127.2 (30 C, C_{Ar}), 117.4 (=CH_{2All}), 102.7 ($^1J_{CH}$ = 159.5 Hz, C-1$_A$), 99.6 ($^1J_{CH}$ = 175.8 Hz, C-1$_D$), 99.1 ($^1J_{CH}$ = 171.9 Hz, C-1$_C$), 81.0 (C-3$_A$), 80.1 (C-4$_D$), 79.5 (C-3$_D$), 78.9 (C-4$_C$), 78.5 (C-2$_A$), 75.4 (C-2$_D$), 75.3, 75.2, 75.0 (3 C, C_{Bn}), 73.7 (C-5$_A$), 72.9 (C_{Bn}), 72.5 (C-4$_A$), 72.3 (C_{Bn}), 71.8 (C-3$_C$), 70.6 (CH_{2All}), 70.4 (C-2$_C$), 68.9 (C-5$_D$), 68.0 (C-5$_C$), 67.3 (C_{CO2Bn}), 37.8 (CO*C*H_{2Lev}), 29.9 (CH_{3Lev}), 28.0 (CO_2*C*H_{2Lev}), 20.9 (CH_{3Ac}), 18.0 (C-6$_D$), 17.8 (C-6$_C$) ppm. HRMS (ESI$^+$): calcd. for $C_{70}H_{79}O_{18}$ [M + H]$^+$ 1207.5266; found 1207.5286; calcd. for $C_{70}H_{82}NO_{18}$ [M + NH_4]$^+$ 1224.5532; found 1224.5576; calcd. for $C_{70}H_{78}O_{18}Na$ [M + Na]$^+$ 1229.5085; found 1229.5131.

Benzyl (3-*O*-Acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (30): Acetic acid (3.4 mL) and then hydrazine monohydrate (74 μL, 1.53 mmol, 5.0 equiv.) were slowly added to a stirred solution of **29** (0.37 g, 0.31 mmol) in dry pyridine (5.1 mL) at 0 °C under Ar. The reaction mixture was stirred at room temperature for 1.5 h. After that time, TLC (Tol/EtOAc, 8:2) showed the complete transformation of the starting material into a more polar product. After addition of CH_2Cl_2 and water, the two phases were separated and the aqueous phase was re-extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried by passing through a phase-separator filter, and concentrated to dryness. The residue was purified by flash chromatography (Tol/EtOAc, 9:1 to 8:2) to give alcohol **30** (318 mg, 93%), slightly contaminated by 2$_C$-acetate **31**, as a white foam. R_f = 0.38 (Tol/EtOAc, 8:2). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.38–7.12 (m, 30 H, H_{Ar}), 5.94 (m, 1 H, CH=$_{All}$), 5.35 (d_o, $J_{OH,2}$ = 5.2 Hz, 1 H, 2$_C$-OH), 5.34 (m_{po}, J_{gem} = 1.5 Hz, 1 H, =CH_{2All}), 5.22 (d, $J_{1,2}$ = 1.4 Hz, 1 H, 1$_D$-H), 5.20–5.15 (m, 2 H, H_{CO2Bn}, =CH_{2All}), 4.99 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 4.96 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.7 Hz, 1 H, 3$_C$-H), 4.86 (d, $J_{1,2}$ = 1.2 Hz, 1 H, 1$_C$-H), 4.82–4.77 (m, 2 H, H_{Bn}), 4.73 (d, J = 12.3 Hz, 1 H, H_{Bn}), 4.68–4.55 (m, 6 H, H_{Bn}), 4.49 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1$_A$-H), 4.47 (d, J = 11.9 Hz, 1 H, H_{Bn}), 4.45 (bs, 1 H, 5$_A$-H), 4.40 (dd, 1 H, 4$_A$-H), 4.33 (m, 1 H, H_{All}), 4.13–4.06 (m, 2 H, H_{All}, 2$_D$-H), 3.96 (m, 1 H, 2$_C$-H), 3.75 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 2.7 Hz, 1 H, 3$_A$-H), 3.73–3.66 (m, 2 H, 5$_C$-H, 3$_D$-H), 3.57–3.50 (m, 2 H, 4$_C$-H, 5$_D$-H), 3.45 (dd, 1 H, 2$_A$-H), 3.39 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, 1 H, 4$_D$-H), 2.04 (s, 3 H, CH_{3Ac}), 1.15 (d, $J_{5,6}$ = 6.1 Hz, 3 H, 6$_D$-H), 1.11 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_C$-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 170.4 (CO_{Ac}), 167.9 (C-6$_A$), 139.0, 138.9, 138.8, 138.7, 135.1 (6 C, C_{IVAr}), 134.9 (CH=$_{All}$), 129.4–127.8 (30 C, C_{Ar}), 117.1 (=CH_{2All}), 102.1 (C-1$_A$), 101.6 (C-1$_C$), 100.1 (C-1$_D$), 80.6 (C-3$_A$), 80.0 (C-4$_D$), 79.3 (C-3$_D$), 78.6 (C-4$_C$), 78.5 (C-2$_A$), 74.8, 74.7, 74.6 (3 C, C_{Bn}), 74.4 (C-3$_C$), 74.1 (C-4$_A$), 73.8 (C-2$_D$), 73.1 (C-5$_A$), 72.1, 71.4 (2 C, C_{Bn}), 70.0 (CH_{2All}), 68.4 (C-2$_C$), 68.3 (C-5$_D$), 68.2 (C-5$_C$), 66.9 (C_{CO2Bn}), 21.6 (CH_{3Ac}), 18.5 (C-6$_D$), 18.1 (C-6$_C$) ppm. HRMS (ESI$^+$): calcd. for $C_{65}H_{72}O_{16}Na$ [M + Na]$^+$ 1131.4718; found 1131.4695.

Propyl α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic acid (2) and Methyl α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-(propyl β-D-galactopyranosid)uronate (32): Pd/C (10%; 100 mg) was added to a stirred solution of trisaccharide **27** (100 mg, 84 μmol) in MeOH (5.1 mL). The suspension was stirred under hydrogen for 1 d. After this time, MS analysis indicated a molecular weight corresponding to that of the target trisaccharide. The reaction mixture was filtered. Evaporation of the volatiles, freeze-drying, and purification of the crude material by preparative RP-HPLC gave, in order of elution, trisaccharide **2** (30.8 mg, 69%) and methyl ester **32** (2.1 mg, 5%), both as white solids after repeated freeze-drying.

Data for Compound 2: t_R = 8.5 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.24 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1$_D$-H), 4.88 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1$_C$-H), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1$_A$-H), 4.34 (d, $J_{4,5}$ = 1.2 Hz, 1 H, 5$_A$-H), 4.27 (dd, $J_{3,4}$ = 3.0 Hz, 1 H, 4$_A$-H), 4.01 (dd, $J_{2,3}$ = 3.2 Hz, 1 H, 2$_D$-H), 3.98 (dd, $J_{2,3}$ = 3.4 Hz, 1 H, 2$_C$-H), 3.86–3.75 (m, 3 H, 3$_A$-H, 3$_D$-H, OCH_{2Pr}), 3.70 (dd, $J_{3,4}$ = 9.8 Hz, 1 H, 3$_C$-H), 3.62 (dq, $J_{4,5}$ = 9.5, $J_{5,6}$ = 6.2 Hz, 1 H, 5$_C$-H), 3.57–3.51 (m, 2 H, 5$_D$-H, OCH_{2Pr}), 3.50 (dd, $J_{2,3}$ = 10.0 Hz, 1 H, 2$_A$-H), 3.36 ($2pt_o$, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 2 H, 4$_D$-H, 4$_C$-H), 1.57 (sext, J = 7.3 Hz, 2 H, CH_{2Pr}), 1.18 (d, 3 H, 6$_C$-H), 1.17 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6$_D$-H), 0.85 (t, 3 H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 174.2 (C-6$_A$), 104.9 ($^1J_{CH}$ = 163.4 Hz, C-1$_A$), 104.7 ($^1J_{CH}$ = 171.2 Hz, C-1$_C$), 102.6 ($^1J_{CH}$ = 174.0 Hz, C-1$_D$), 80.9 (C-2$_D$), 79.2 (C-4$_A$), 75.5 (C-3$_A$), 75.4 (C-5$_A$), 74.9 (OCH_{2Pr}), 74.7, 74.6 (2 C, C-4$_D$, C-4$_C$), 72.8 (C-2$_A$), 72.6 (2 C, C-2$_C$, C-3$_C$), 72.4 (C-3$_D$) 71.8 (C-5$_D$), 71.6 (C-5$_C$), 24.7 (CH_{2Pr}), 19.3, 19.2 (2 C, C-6$_D$, C-6$_C$), 12.2 (CH_{3Pr}) ppm. HRMS (ESI$^+$): calcd. for $C_{21}H_{36}O_{15}Na$ [M + Na]$^+$ 551.1952; found 551.1940.

Data for Methyl Ester 32: ^{1}H NMR (400 MHz, D_2O): δ = 5.18 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 1$_D$-H), 4.85 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1$_C$-H), 4.37 (d, $J_{4,5}$ = 0.7 Hz, 1 H, 5$_A$-H), 4.34 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1$_A$-H), 4.28 (dd, $J_{3,4}$ = 2.8 Hz, 1 H, 4$_A$-H), 3.97 (dd, $J_{2,3}$ = 3.0 Hz, 1 H, 2$_D$-H), 3.93 (dd, 1 H, 2$_C$-H), 3.79 (dt, J = 6.8, J = 9.8 Hz, 1 H,

OCH_{2Pr}), 3.76–3.72 (m, 2 H, 3_D-H, 3_A-H), 3.72 (s_o, 3 H, CH_{3Me}), 3.66 (dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.8 Hz, 1 H, 3_C-H), 3.59 (dq, $J_{3,4}$ = 9.5, $J_{5,6}$ = 6.2 Hz, 1 H, 5_C-H), 3.50 (dt, J = 6.8 Hz, 1 H, OCH_{2Pr}), 3.45 (dd, $J_{2,3}$ = 9.9 Hz, 1 H, 2_A-H), 3.34 (m_o, 1 H, 5_D-H), 3.32 ($2t_o$, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 2 H, 4_C-H, 4_D-H), 1.53 (sext, J = 7.1 Hz, 2 H, CH_{2Pr}), 1.15 (d, $J_{5,6}$ = 6.0 Hz, 6 H, 6_C-H, 6_D-H), 0.81 (t, J = 7.4 Hz, 3 H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 172.8 (C-6_A), 105.1 ($^1J_{CH}$ = 162.3 Hz, C-1_A), 104.6 ($^1J_{CH}$ = 171.5 Hz, C-1_C), 102.8 ($^1J_{CH}$ = 173.7 Hz, C-1_D), 80.8 (C-2_D), 79.7 (C-4_A), 75.7 (C-5_A), 75.4 (C-3_A), 75.0 (OCH_{2Pr}), 74.6, 72.5 (2 C, C-4_C, C-4_D), 72.8 (C-2_A), 72.6 (2 C, C-2_C, C-3_C), 72.2 (C-3_D), 71.8 (C-5_D), 71.6 (C-5_C), 55.7 (CH_{3Me}), 24.7 (CH_{2Pr}), 19.5, 19.3 (2 C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI$^+$): calcd. for $C_{22}H_{38}O_{15}Na$ [M + Na]$^+$ 565.2108; found 565.2087.

Propyl 3-*O*-Acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic Acid (3) and Propyl 2-*O*-Acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic Acid (33): Pd/C (10%; 150 mg) was added to a stirred solution of trisaccharides **30** and **31** (10:1; 169 mg, 0.15 mmol) in THF/H_2O (4:1, 7.6 mL). The suspension was stirred under hydrogen for 20 h. After this time, MS analysis indicated a molecular weight corresponding to that of the target trisaccharide. The reaction mixture was filtered. Evaporation of the volatiles, freeze-drying, and purification by preparative RP-HPLC gave, in order of elution, first trisaccharide **3** (67.8 mg, 78%) and then regioisomer **33** (1.8 mg, 2%), both as white foams after repeated freeze-drying.

Data for Compound 3: t_R = 9.0 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.29 (d, $J_{1,2}$ = 1.4 Hz, 1 H, 1_D-H), 4.90 (dd_o, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.9 Hz, 1 H, 3_C-H), 4.90 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1_C-H), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1_A-H), 4.35 (d, $J_{4,5}$ = 1.2 Hz, 1 H, 5_A-H), 4.28 (dd, $J_{3,4}$ = 2.8 Hz, 1 H, 4_A-H), 4.12 (dd, 1 H, 2_C-H), 4.03 (dd, $J_{2,3}$ = 3.1 Hz, 1 H, 2_D-H), 3.83 (dt_{po}, J = 6.8, J = 9.8 Hz, 1 H, OCH_{2Pr}), 3.81–3.76 (m, 2 H, 3_A-H, 3_D-H), 3.74 (dq, $J_{4,5}$ = 9.6, $J_{5,6}$ = 6.2 Hz, 1 H, 5_C-H), 3.59–3.52 (m, 3 H, 4_C-H, 5_D-H, OCH_{2Pr}), 3.50 (dd, $J_{2,3}$ = 10.0 Hz, 1 H, 2_A-H), 3.41 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1 H, 4_D-H), 2.09 (s, 3 H, CH_{3Ac}), 1.56 (sext, 2 H, CH_{2Pr}), 1.21 (d, 3 H, 6_C-H), 1.18 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H), 0.84 (t, 3 H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 176.2 (CO_{Ac}), 174.1 (C-6_A), 104.9 ($^1J_{CH}$ = 162.9 Hz, C-1_A), 104.3 ($^1J_{CH}$ = 172.3 Hz, C-1_C), 102.5 ($^1J_{CH}$ = 173.4 Hz, C-1_D), 81.3 (C-2_D), 79.3 (C-4_A), 76.2 (C-3_C), 75.5 (C-3_A), 75.4 (C-5_A), 74.9 (OCH_{2Pr}), 74.5 (C-4_D), 72.8 (C-2_A), 72.4, 72.3 (2 C, C-3_D, C-4_C), 71.9 (C-5_D), 71.6 (C-5_C), 70.7 (C-2_C), 24.7 (CH_{2Pr}), 23.1 (CH_{3Ac}), 19.3, 19.2 (2 C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI$^+$): calcd. for $C_{23}H_{38}O_{16}Na$ [M + Na]$^+$ 593.2057; found 593.2044.

Data for Compound 33: t_R = 9.4 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.25 (d, 1 H, 1_D-H), 5.11 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 2_C-H), 4.91 (d, 1 H, 1_C-H), 4.34 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1_A-H), 4.25 (s, 1 H, 5_A-H), 4.24 (dd, 1 H, 4_A-H), 3.99 (dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 2.9 Hz, 1 H, 2_D-H), 3.87 (dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.8 Hz, 1 H, 3_C-H), 3.80 (dt_{po}, J = 6.8, J = 9.7 Hz, 1 H, OCH_{2Pr}), 3.77 (dd_o, 1 H, 3_D-H), 3.74 (dd_{po}, $J_{3,4}$ = 3.0 Hz, 1 H, 3_A-H), 3.68 (dq, $J_{3,4}$ = 9.6 Hz, 1 H, 5_C-H), 3.44 (dq_{po}, 1 H, 5_D-H), 3.50 (dt_o, 1 H, OCH_{2Pr}), 3.46 (dd, $J_{2,3}$ = 9.9 Hz, 1 H, 2_A-H), 3.38 (t, 1 H, 4_C-H), 3.34 (t, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1 H, 4_D-H), 2.05 (s, 3 H, CH_{3Ac}), 1.53 (sext, J = 7.2 Hz, 2 H, CH_{2Pr}), 1.19 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_C-H) 1.16 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H), 0.81 (t, J = 7.4 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 175.8 (CO_{Ac}), 174.6 (C-6_A), 104.9 ($^1J_{CH}$ = 161.4 Hz, C-1_A), 102.3 ($^1J_{CH}$ = 175.9 Hz, C-1_C), 101.8.1 ($^1J_{CH}$ = 174.6 Hz, C-1_D), 81.5 (C-2_D), 79.2 (C-4_A), 75.7 (C-5_A), 75.6 (C-3_A), 75.0 (C-2_C), 74.8 (2 C, C-4_C*, OCH_{2Pr}), 74.6 (C-4_D*), 72.8 (C-2_A), 72.2 (C-3_D), 71.8 (C-5_D), 71.7 (C-5_C), 71.1 (C-3_C), 24.7 (CH_{2Pr}), 22.9 (CH_{3Ac}) 19.2 (2 C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI$^+$): calcd. for $C_{23}H_{38}O_{16}Na$ [M + Na]$^+$ 593.2057; found 593.2051.

Allyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (36): A mixture of β-acetate **35**[50] (2.83 g, 5.74 mmol), allyl alcohol (0.78 mL, 11.5 mmol, 2.0 equiv.), and freshly activated 4 Å powdered MS (2.8 g) in anhydrous CH_2Cl_2 (57 mL) was stirred at room temperature under Ar for 1 h. TMSOTf (1.10 mL, 5.74 mmol, 1.0 equiv.) was added at room temperature, and stirring was continued for 14 h at this temperature. TLC (Tol/MeCN, 7:3) showed the complete conversion of donor **35** into a very slightly less polar product. The reaction was quenched with Et_3N. The resulting mixture was filtered and concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 6:4) to give allyl galactopyranoside **36** (2.60 g, 92%) as a white foam. R_f = 0.42 (Tol/MeCN, 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.69 (d, $J_{NH,2}$ = 8.6 Hz, 1 H, NH), 5.87 (m, 1 H, CH=$_{All}$), 5.42 (dd, $J_{3,4}$ = 3.4, $J_{4,5}$ = 1.0 Hz, 1 H, 4-H), 5.36 (dd, $J_{2,3}$ = 11.3 Hz, 1 H, 3-H), 5.31 (m, J_{trans} = 17.3, J_{gem} = 1.5 Hz, 1 H, =CH_{2All}), 5.23 (m, J_{cis} = 10.4 Hz, 1 H, =CH_{2All}), 4.78 (d, $J_{1,2}$ = 8.3 Hz, 1 H, 1-H), 4.39 (m, 1 H, H_{All}), 4.22 (dd, $J_{5,6a}$ = 6.7, $J_{6a,6b}$ = 11.3 Hz, 1 H, 6a-H), 4.13 (dd_{po}, $J_{5,6b}$ = 6.8 Hz, 1 H, 6b-H), 4.16–4.08 (m, 2 H, H_{All}, 2-H), 3.96 (pdt, 1 H, 5-H), 2.19, 2.08, 2.02 (3 s, 9 H, CH_{3Ac}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.1 (3 C, CO_{Ac}), 162.0 (NHCO), 133.1 (CH=$_{All}$), 118.3 (=CH_{2All}), 99.5 (C-1), 92.3 (CCl_3), 71.3 (C-5), 70.4 (CH_{2All}), 69.3 (C-3), 66.7 (C-4), 61.3 (C-6), 53.2 (C-2), 20.7, 20.5 (3 C, CH_{3Ac}) ppm. HRMS (ESI$^+$): calcd. for $C_{17}H_{22}Cl_3NO_9Na$ [M + Na]$^+$ 512.0258; found 512.0239.

Allyl 3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (38): Allyl glycoside **36** (2.02 g, 4.11 mmol) was dissolved in anhydrous MeOH (20.6 mL), and NaOMe (0.5 M in MeOH; 2.47 mL, 1.24 mmol, 0.3 equiv.) was added. The solution was stirred at room temperature for 2 h. TLC (Chex/EtOAc, 6:4) showed the conversion of the starting material into a more polar product. The reaction was quenched with Dowex-H^+ resin, the mixture was filtered, and the solvents were evaporated to give intermediate triol **37** quantitatively as a white solid. The crude material was dissolved in anhydrous DMF (41 mL), and the solution was cooled to –10 °C. Benzyl bromide (4.4 mL, 37.0 mmol, 9.0 equiv.) and NaH (60% in oil, 0.99 g, 24.7 mmol, 6.0 equiv.) were successively added. The reaction mixture was stirred under Ar with the temperature kept below 0 °C. After 1.5 h, TLC (Tol/EtOAc, 9:1) showed the conversion of the triol into a major less polar compound. The reaction was quenched by adding the minimum amount of MeOH. The solution was diluted with EtOAc and washed with water and brine. The organic phase was dried with anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 95:5 to 9:1) to give fully protected **38** (2.24 g, 86%) as a white solid. R_f = 0.44 (Tol/EtOAc, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41–6.26 (m, 15 H, H_{Ar}), 6.92 (d, $J_{NH,2}$ = 7.0 Hz, NH), 5.88 (m, 1 H, CH=$_{All}$), 5.27 (m, J_{trans} = 17.3, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.18 (m, J_{cis} = 10.4 Hz, 1 H, 1 H,=CH_{2All}), 4.99 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1-H), 4.92 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.68 (d, J = 11.4 Hz, 1 H, H_{Bn}), 4.62 (d, 1 H, H_{Bn}), 4.56 (d, 1 H, H_{Bn}), 4.52 (d, J = 11.9 Hz, 1 H, H_{Bn}), 4.47 (d, 1 H, H_{Bn}), 4.35 (m_o, 1 H, H_{All}), 4.32 (dd, $J_{2,3}$ = 10.9, $J_{3,4}$ = 2.8 Hz, 1 H, 3-H), 4.09 (m, 1 H, H_{All}), 4.04 (d, 1 H, 4-H), 3.85 (ddd, 1 H, 2-H), 3.72–3.62 (m, 3 H, 5-H, 6a-H, 6b-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.9 (NHCO), 138.4, 137.9, 137.5 (3 C, C_{IVAr}), 133.7 (CH=$_{All}$), 126.6–127.6 (15 C, C_{Ar}), 117.8 (=CH_{2All}), 98.2 ($^1J_{CH}$ = 162.9 Hz, C-1), 92.6 (CCl_3), 77.2 (C-3), 74.7, 73.6 (2 C, C_{Bn}), 73.5 (C-5), 72.4 (C_{Bn}), 72.3 (C-4), 70.3

(CH_{2All}), 68.6 (C-6), 56.3 (C-2) ppm. HRMS (ESI^+): calcd. for $C_{32}H_{34}Cl_3NO_6Na$ $[M + Na]^+$ 656.1349; found 656.1321.

3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranose (39) and (3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α-D-galactopyranosyl)-(1↔1)-3,4,6-tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (49)

Route 1: (1,5-Cyclooctadiene)bis(methyldiphenylphosphane)iridium hexafluorophosphate (82 mg, 100 μmol, 0.03 equiv.) was dissolved in anhydrous THF (17 mL), and hydrogen was bubbled through the solution for 15 min (H-cube, full H_2 mode). The resulting yellow solution was concentrated to dryness. The residue was dissolved in anhydrous THF (17 mL), and the resulting solution was poured into a solution of allyl glycoside **38** (2.14 g, 3.36 mmol) in anhydrous THF (17 mL). The mixture was stirred under Ar at room temperature for 2 h. TLC (Tol/EtOAc, 9:1) indicated the conversion of the starting glycoside into a less polar intermediate. A solution of iodine (1.71 g, 6.73 mmol, 2.0 equiv.) in THF/H_2O (4:1, 20 mL) was added, and the mixture was stirred at room temperature for 1 h. TLC (Tol/EtOAc, 9:1) showed the conversion of the intermediate compound into a more polar product. The reaction was quenched with sodium bisulfite (10% aq.). The mixture was concentrated to a third of its volume, and the aqueous phase was extracted three times with CH_2Cl_2. The organic phases were combined, washed with brine, dried by passing through a phase-separator filter, and concentrated to dryness. The residue was purified by flash chromatography (Chex/EtOAc, 75:25 to 7:3) to give hemiacetal **39** (1.86 g, 93%) as a yellow solid.

Route 2: NIS (737 mg, 3.28 mmol, 1.5 equiv.) and TfOH (48 μL, 0.55 mmol, 0.25 equiv.) were added at 0 °C to a solution of thiophenyl glycoside **48** (1.50 g, 2.18 mmol) in CH_2Cl_2 (22 mL) and water (2.2 mL). The reaction mixture was stirred at that temperature for 0.5 h. TLC (Chex/EtOAc, 7:3) showed the conversion of the starting material into a less polar intermediate. The reaction was quenched by the addition of Et_3N. The organic phase was washed with brine, dried by passing through a phase-separator filter, and concentrated to dryness. The residue was purified by flash chromatography (Chex/EtOAc, 75:25 to 7:3) to give, in order of elution, first α-(1↔1)-β-linked disaccharide **49** (84 mg, 3%) as a yellow oil, and then target hemiacetal **39** (998 mg, 77%) as a 9:1 α/β mixture, isolated as a white solid.

Data for α-Hemiacetal 39: R_f = 0.23 (Tol/EtOAc, 7:3). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.40–6.25 (m, 15 H, H_{Ar}), 6.81 (d, $J_{NH,2}$ = 8.9 Hz, 1 H, NH), 5.38 (pt, $J_{OH,1} = J_{1,2}$ = 3.5 Hz, 1 H, 1-H), 4.97 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.72 (d, J = 11.9 Hz, 1 H, H_{Bn}), 4.65 (m, 1 H, 2-H), 4.57 (d, 1 H, H_{Bn}), 4.56 (d, 1 H, H_{Bn}), 4.53 (d, J = 12.2 Hz, 1 H, H_{Bn}), 4.45 (d, 1 H, H_{Bn}), 4.15 (pt, 1 H, 5-H), 3.98 (bd, 1 H, 4-H), 3.80 (dd, $J_{2,3}$ = 10.8, $J_{3,4}$ = 2.5 Hz, 1 H, 3-H), 3.67 (dd, $J_{OH,2}$ = 1.6 Hz, 1 H, 1-OH), 3.62 (dd, $J_{5,6a}$ = 6.8, $J_{6a,6b}$ = 9.6 Hz, 1 H, 6a-H), 3.50 (dd, $J_{5,6b}$ = 5.8 Hz, 1 H, 6b-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.7 (NHCO), 138.1, 137.5 (3 C, C_{IVAr}), 128.6–127.5 (15 C, C_{Ar}), 92.7 (CCl_3), 91.6 (C-1), 76.8 (C-3), 74.4, 73.6 (2 C, C_{Bn}), 72.5 (C-4), 71.8 (C_{Bn}), 69.9 (C-5), 69.5 (C-6), 51.4 (C-2) ppm. HRMS (ESI^+): calcd. for $C_{29}H_{30}Cl_3NO_6Na$ $[M + Na]^+$ 616.1036; found 616.1080.

Data for Disaccharide 49: R_f = 0.44 (Tol/EtOAc, 7:3). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.50 (d, $J_{NH,2}$ = 6.7 Hz, 1 H, NH′), 7.29–7.11 (m, 30 H, H_{Ar}), 6.69 (d, $J_{NH,2}$ = 9.5 Hz, 1 H, NH), 5.44 (d, $J_{1,2}$ = 3.8 Hz, 1 H, 1′-H), 5.30 (d, $J_{1,2}$ = 8.8 Hz, 1 H, 1-H), 4.84 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.79 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.63 (ddd, $J_{2,3}$ = 10.6 Hz, 1 H, 2′-H), 4.58 (d, J = 11.8 Hz, 1 H, H_{Bn}), 4.50 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.48 (d, J = 11.6 Hz, 2 H, H_{Bn}), 4.43 (d, J = 11.7 Hz, 1 H, H_{Bn}), 4.38 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.37 (d, J = 11.4 Hz, 1 H, H_{Bn}), 4.44 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.33–4.25 (m, 3 H, 2 H_{Bn}, 3-H), 4.13 (m, 1 H, 5-H), 3.96 (ddd, $J_{2,3}$ = 10.8 Hz, 1 H, 2-H), 3.91 (d, $J_{3,4}$ = 2.3 Hz, 1 H, 4-H), 3.77 (bd, 1 H, 4′-H), 3.66 (dd, $J_{3,4}$ = 2.4 Hz, 1 H, 3′-H), 3.63 (m, 1 H, 5′-H), 3.53–3.46 (m, 2 H, 6a-H, 6a′-H), 3.38 (dd, $J_{5,6b}$ = 5.3, $J_{6a,6b}$ = 9.0 Hz, 1 H, 6b′-H), 3.29 (dd, $J_{5,6b}$ = 4.2, $J_{6a,6b}$ = 9.6 Hz, 1 H, 6b-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.5 (2 C, NHCO, NHCO′), 138.4–137.4 (6 C, C_{IVAr}), 128.8–127.5 (30 C, C_{Ar}), 95.3 (C-1), 93.2 (C-1′), 92.8, 92.6 (2 C, CCl_3), 77.8 (C-3′), 76.6 (C-3) 74.6, 74.2, 73.9 (3 C, C_{Bn}), 73.8 (C-5′), 73.5 (C_{Bn}), 72.3 (2 C, C-4′, C_{Bn}), 72.2 (C_{Bn}), 72.1 (C-4), 71.0 (C-5), 70.1 (C-6), 68.1 (C-6′), 55.7 (C-2), 50.6 (C-2′) ppm. HRMS (ESI^+): calcd. for $C_{58}H_{58}Cl_6N_2O_{11}Na$ $[M + Na]^+$ 1191.2069; found 1191.1906.

3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranosyl Trichloroacetimidate (40):[52] CCl_3CN (834 μL, 8.3 mmol, 5.0 equiv.) and DBU (75 μL, 500 μmol, 0.3 equiv.) were successively added to a solution of hemiacetal **39** (990 mg, 1.66 mmol) in anhydrous DCE (8.3 mL). The mixture was stirred at room temperature under Ar for 1 h. TLC (Chex/EtOAc, 8:2) indicated the complete conversion of the starting hemiacetal into a less polar product. Following concentration to a third of its volume (reduced pressure, room temperature), the reaction mixture was directly purified by flash chromatography (Chex/EtOAc, 8:2 + 1% Et_3N) to give a 9:1 α/β mixture of trichloroacetimidate **40** (1.16 g, 94%) as a pale yellow oil. The analytical data were as published.[52]

3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranosyl *N*-Phenyltrifluoroacetimidate (41) and 2-Trichloromethyl-(3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-galactopyrano)-[2,1-*d*]-2-oxazoline (42): Hemiacetal **39** (1.85 g, 3.11 mmol) was dissolved in acetone (31 mL). *N*-Phenyltrifluoroacetimidoyl chloride (1.29 g, 6.22 mmol, 2.0 equiv.) and Cs_2CO_3 (1.12 g, 3.42 mmol, 1.1 equiv.) were added to the solution. The mixture was stirred at room temperature for 4 h. After that time, TLC (Chex/EtOAc, 8:2) indicated the complete transformation of the starting material into a less polar product. The reaction mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (Chex/EtOAc, 85:15 to 7:3 + 1% Et_3N) to give, in order of elution, first oxazoline **42** (83 mg, 5%) and then PTFA **41** (2.10 g, 88%), both as pale yellow oils.

Data for Donor 41: R_f = 0.44 (Chex/EtOAc, 8:2). ^1H NMR (100 MHz, $CDCl_3$): δ = 7.61–7.09 (m, 18 H, H_{Ar}), 6.77 (d, J = 7.6 Hz, 2 H, H_{Ar}), 6.48 (d_o, $J_{NH,2}$ = 7.7 Hz, 1 H, NH), 6.47 (bs_o, 1 H, 1-H), 4.97 (d, J = 11.4 Hz, 1 H, H_{Bn}), 4.76 (d_{po}, J = 11.9 Hz, 1 H, H_{Bn}), 4.73 (m, 1 H, 2-H), 4.64 (d, 1 H, H_{Bn}), 4.57–4.48 (m, 3 H, H_{Bn}), 4.20 (bs, 1 H, 4-H), 4.07 (pt, 1 H, 5-H), 3.88 (dd, $J_{2,3}$ = 11.0, $J_{3,4}$ = 1.4 Hz, 1 H, 3-H), 3.72 (pt, $J_{5,6a}$ = 7.8 Hz, 1 H, 6a-H), 3.62 (dd, $J_{5,6b}$ = 5.7, $J_{6a,6b}$ = 9.1 Hz, 1 H, 6b-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.8 (NHCO), 143.1, 138.0, 137.7, 137.0 (4 C, C_{IVAr}), 129.4–119.3 (20 C, C_{Ar}), 94.3 ($^1J_{CH}$ = 162.9 Hz, C-1), 92.3 (CCl_3), 75.5 (C-3), 74.8, 73.7 (2 C, C_{Bn}), 72.5 (C-5), 71.6 (C-4), 71.3 (C_{Bn}), 68.1 (C-6), 50.6 (C-2) ppm.

Data for Oxazoline 42: R_f = 0.46 (Chex/EtOAc, 8:2). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.46–7.27 (m, 15 H, H_{Ar}), 6.28 (d, $J_{1,2}$ = 6.6 Hz, 1 H, 1-H), 4.95 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.92 (d, J = 12.2 Hz, 1 H, H_{Bn}), 4.77 (d, 1 H, H_{Bn}), 4.64 (d, 1 H, H_{Bn}), 4.51 (d, J = 11.8 Hz, 1 H, H_{Bn}), 4.64 (d, 1 H, H_{Bn}), 4.40 (pt, 1 H, 2-H), 4.07 (dt, $J_{4,5}$ = 1.7, $J_{5,6a} = J_{5,6b}$ = 6.5 Hz, 1 H, 5-H), 3.98 (pt, 1 H, 4-H), 3.66 (d, 2 H, 6a-H, 6b-H), 3.54 (dd, $J_{2,3}$ = 7.5, $J_{3,4}$ = 2.6 Hz, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.2 (NCO), 138.2, 137.9, 137.7 (3 C, C_{IVAr}), 128.4–127.7 (15 C, C_{Ar}), 106.8 (C-1), 92.3 (CCl_3), 79.3 (C-3), 74.4 (C_{Bn}), 73.6 (C-5), 73.5, 72.0 (2 C,

C_{Bn}), 71.6 (C-4), 68.2 (C-6), 67.3 (C-2) ppm. HRMS (ESI^+): calcd. for $C_{29}H_{28}Cl_3NO_5Na$ $[M + Na]^+$ 598.0931; found 598.0910.

Phenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside (46)[53]

Route 1: Thiophenol (3.2 mL, 31.3 mmol, 2 equiv.) and $BF_3{\cdot}OEt_2$ (12.0 mL, 93.9 mmol, 6 equiv.) were added at room temperature to a solution of β-acetate **35** (7.71 g, 15.6 mmol) in anhydrous CH_2Cl_2 (150 mL). The mixture was stirred at this temperature for 2.5 h under Ar. TLC (Chex/EtOAc, 7:3) showed the complete conversion of the starting material into a less polar product. The reaction was quenched with $NaHCO_3$ (satd. aq.). CH_2Cl_2 was added, and the phases were separated. The organic phase was washed with H_2O and brine, then dried by passing through a phase-separator filter, and concentrated to dryness. The residue was purified by flash chromatography (Chex/EtOAc, 9:1 to 1:1) to give thioglycoside **46** (7.43 g, 87%) as a white solid.

Route 2: NaOMe (25% in MeOH, 76.9 mL, 336.1 mmol, 3.0 equiv.) was slowly added to a solution of D-galactosamine hydrochloride (**34**; 24.16 g, 112.0 mmol) in anhydrous MeOH (482 mL) stirred at 0 °C under Ar. After 15 min, trichloroacetic anhydride (30.7 mL, 168.1 mmol, 1.5 equiv.) was added dropwise, keeping the reaction temperature close to 0 °C. The reaction mixture was stirred for 2 h, until TLC (*i*PrOH/H_2O/NH_3, 4:1:0.5) showed the complete conversion of the starting material into a less polar product. The reaction was quenched with Dowex-H^+ resin, the resin was removed by filtration, and the volatiles were evaporated to dryness. The residue was dissolved in anhydrous pyridine (224 mL), and acetic anhydride (100 mL) was slowly added at 0 °C over 30 min. The reaction mixture was stirred at room temperature for 16 h. After this time, TLC (CH_2Cl_2/EtOAc, 9:1) indicated that the intermediate had been converted into less polar compounds corresponding to the α and β anomers of the furanose (**43**, 7% and **44**, 11%) and pyranose forms (**45**, 51% and **35**, 31%) of the peracylated galactosamine, as ascertained by NMR spectroscopy. The solvents were evaporated and co-evaporated three times with toluene. The residue was dissolved in CH_2Cl_2 (500 mL), and the resulting solution was washed with water (150 mL), HCl (10% aq.; 150 mL), $NaHCO_3$ (satd. aq.; 100 mL) and brine (3× 50 mL). The organic phase was dried by passing through a phase-separator filter, and concentrated to dryness. Thiophenol (23.0 mL, 224 mmol, 2.0 equiv.) and $BF_3{\cdot}OEt_2$ (42.8 mL, 336.1 mmol, 3.0 equiv.) were added at room temperature to a solution of the residue in anhydrous CH_2Cl_2 (390 mL). The mixture was stirred at this temperature under Ar for 16 h. TLC (Tol/EtOAc, 7:3) showed the conversion of the intermediates into a major more polar product. The reaction was quenched with $NaHCO_3$ (satd. aq.; 200 mL). CH_2Cl_2 (500 mL) was added, and the phases were separated. The organic phase was washed with H_2O (150 mL) and brine (150 mL), then dried by passing through a phase-separator filter, and concentrated to dryness. The residue was purified by flash chromatography (Chex/EtOAc, 9:1 to 1:1) to give thioglycoside **46** (39.0 g, 64%) as a white solid. R_f = 0.35 (Tol/EtOAc, 7:3). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.57–7.32 (m, 5 H, H_{Ar}), 6.75 (d, $J_{NH,2}$ = 9.1 Hz, 1 H, NH), 5.42 (d, 1 H, 4-H), 5.32 (dd, $J_{2,3}$ = 10.6, $J_{3,4}$ = 3.2 Hz, 1 H, 3-H), 4.88 (d, $J_{1,2}$ = 10.3 Hz, 1 H, 1-H), 4.20 (dd_o, 1 H, 6a-H), 4.19 (ddd_o, 1 H, 2-H), 4.16 (dd_{po}, $J_{5,6b}$ = 6.1, $J_{6a,6b}$ = 11.4 Hz, 1 H, 6b-H), 3.99 (dt, $J_{4,5}$ = 0.9 Hz, 1 H, 5-H), 2.15, 2.06, 2.00 (3 s, 9 H, $CH3_{Ac}$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.0 (3 C, CO), 161.7 (NHCO), 133.3 (2 C, C_{Ar}), 132.1 (C_{IVAr}), 129.0, 128.4 (3 C, C_{Ar}), 92.3 (CCl_3), 86.7 (C-1), 74.7 (C-5), 70.6 (C-3), 66.9 (C-4), 61.7 (C-6), 51.4 (C-2), 20.7, 20.6, 20.5 (3 C, $CH_{3\,Ac}$) ppm. HRMS (ESI^+): calcd. for $C_{20}H_{22}Cl_3NO_8SNa$ $[M + Na]^+$ 564.0029; found 564.0087.

Phenyl 3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside (48): A solution of thioglycoside **46** (5.00 g, 9.21 mmol) in anhydrous MeOH (100 mL) was treated with MeONa (0.5 M in MeOH; 311 μL, 1.44 mmol, 0.2 equiv.). The solution was stirred at room temperature under Ar for 2 h. TLC (Chex/EtOAc, 6:4, CH_2Cl_2/MeOH, 8:2) showed the complete conversion of thioglycoside **46** into a more polar product. The reaction was quenched with Dowex-H^+ resin. The resin was filtered, and the filtrate was concentrated to give crude phenyl 2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside[54] (**47**, 3.83 g) quantitatively. This residue was dissolved in anhydrous DMF (74 mL), the solution was cooled to –10 °C, and benzyl bromide (6.6 mL, 55.3 mmol, 6.0 equiv.) and NaH (60% in oil; 2.21 g, 55.3 mmol, 6.0 equiv.) were successively added. The mixture was stirred under Ar keeping the temperature below 0 °C. After 1.5 h, TLC (Tol/EtOAc, 95:5) showed the conversion of triol **47** into a major less polar compound. The reaction was quenched with the minimum amount of MeOH. The reaction mixture was diluted with EtOAc, and washed with water and brine. The organic phase was dried with anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 98:2 to 95:5) to give target tri-*O*-benzyl derivative **48** (4.90 g, 77%) as a white solid. R_f = 0.48 (Tol/EtOAc, 95:5). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.56–7.22 (m, 2 H, H_{Ar}), 7.40–7.20 (m, 18 H, H_{Ar}), 6.84 (d, $J_{NH,2}$ = 7.5 Hz, 1 H, NH), 5.30 (d, $J_{1,2}$ = 10.2 Hz, 1 H, 1-H), 4.90 (d, J = 11.4 Hz, 1 H, H_{Bn}), 4.68 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.58 (d, 1 H, H_{Bn}), 4.54 (d, 1 H, H_{Bn}), 4.53 (d, J = 11.8 Hz, 1 H, H_{Bn}), 4.48 (d, 1 H, H_{Bn}), 4.28 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 2.7 Hz, 1 H, 3-H), 4.09 (d, 1 H, 4-H), 3.94 (pdt, 1 H, 2-H), 3.78–3.68 (m, 3 H, 5-H, 6a-H, 6b-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.7 (NHCO), 138.4, 137.8, 137.3 (3 C, C_{IVAr}), 132.8 (2 C, C_{Ar}), 132.2 (C_{IVAr}), 130.0–127.6 (18 C, C_{Ar}), 92.5 (CCl_3), 84.3 (C-1), 78.2 (C-3), 77.6 (C-5), 74.5, 73.6 (2 C, C_{Bn}), 72.4 (C-4), 72.3 (CBn), 68.4 (C-6), 53.7 (C-2) ppm. HRMS (ESI^+): calcd. for $C_{35}H_{34}Cl_3NO_5SNa$ $[M + Na]^+$ 708.1121; found 708.1128.

The analytical data for triol **47** were as published.[54]

Benzyl (3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→2)-(4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-Di-*O*-benzyl-β-D-galactopyranosid)uronate (50) and Benzyl (3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α-D-galactopyranosyl)-(1→2)-(4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (51)

Route 1: A mixture of acceptor **27** (300 mg, 253 μmol), thioglycoside **48** (260 mg, 379 μmol, 1.5 equiv.), and freshly activated 4 Å powdered MS (750 mg) in anhydrous CH_2Cl_2 (2.9 mL) was stirred at room temperature under Ar for 1 h. The reaction mixture was cooled to –78 °C, then NIS (114 mg, 505 μmol, 2.0 equiv.) and TMSOTf (4.6 μL, 25 μmol, 0.1 equiv.) were added. The reaction mixture was stirred for 30 min, while the bath was allowed to reach –60 °C. TLC (Tol/EtOAc, 9:1) indicated the absence of acceptor **27** and the presence of a less polar product. The reaction was quenched by the addition of Et_3N. The resulting suspension was filtered and concentrated. The residue was purified by flash chromatography (Tol/EtOAc, 98:2 to 92:8) to give tetrasaccharide **50** (356 mg, 80%) as a white foam. Traces of silylated acceptor **52** were also recovered.

Route 2: A mixture of acceptor **27** (203 mg, 168 μmol), PTFA **41** (194 mg, 253 μmol, 1.5 equiv.), and powdered 4 Å MS (500 mg) in anhydrous CH_2Cl_2 (3.4 mL) was stirred at room temperature under Ar for 1 h. The suspension was cooled to –78 °C, and TMSOTf

(1.5 μL, 8 μmol, 0.05 equiv.) was added. The reaction mixture was stirred at this temperature for 20 min, and Et_3N was added since TLC (Tol/EtOAc, 9:1) had shown the presence of a new major compound, whereas neither acceptor nor donor remained. The solids were removed by filtration, and the filtrate was concentrated to dryness. The residue was purified by flash chromatography (Tol/EtOAc, 95:5 to 85:15) to give, in order of elution, the product of α-glycosylation **51** (19 mg, 9%) and desired tetrasaccharide **50** (186 mg, 63%), both as pale yellow foams.

Data for β-Glycoside 50: R_f = 0.38 (Tol/EtOAc, 9:1) ^{1}H NMR (100 MHz, $CDCl_3$): δ = 7.41–7.05 (m, 47 H, H_{Ar}), 6.91 (d, $J_{NH,2}$ = 7.5 Hz, 1 H, NH), 6.83 (d, J = 8.6 Hz, 2 H, H_{ArPMB}), 5.97 (m, 1 H, CH=$_{All}$), 5.35 (m, J_{trans} = 17.3, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.26 (bs, 1 H, 1_D-H), 5.25 (d_{po}, 1 H, H_{CO2Bn}), 5.21 (m, J_{cis} = 10.5 Hz, 1 H, =CH_{2All}), 5.10 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 4.98–4.92 (m, 4 H, 1_B-H, 1_C-H, 2 H_{Bn}), 4.90 (d_{po}, J = 10.9 Hz, 1 H, H_{Bn}), 4.84 (d_{po}, J = 11.1 Hz, 1 H, H_{Bn}), 4.76 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.72 (d_{po}, J = 10.5 Hz, 1 H, H_{Bn}), 4.71–4.67 (m, 2 H, H_{Bn}), 4.65–4.55 (m, 3 H, H_{Bn}), 4.58–4.45 (m, 6 H, 5 H_{Bn}, H_{All}), 4.36 (d_o, $J_{1,2}$ = 7.7 Hz, 1 H, 1_A-H), 4.34 (bd_o, 1 H, 4_A-H), 4.27 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.22 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.18–4.10 (m, 2 H, 2_B-H, H_{All}), 4.08 (pt, 1 H, 2_C-H), 4.03–3.96 (m, 4 H, 5_A-H, 3_B-H, 4_B-H, 2_D-H), 3.87 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.6 Hz, 1 H, 3_C-H), 3.81 (dd_{po}, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.5 Hz, 1 H, 3_D-H), 3.79 (dq_{po}, 1 H, 5_C-H), 3.71 (s, 3 H, CH_{3PMB}), 3.70–3.59 (m, 3 H, 2_A-H, $6a_B$-H, 5_D-H), 3.51–3.42 (m, 3 H, 3_A-H, 5_B-H, 4_C-H), 3.34 (pt_{po}, $J_{4,5}$ = 9.2 Hz, 1 H, 4_D-H), 3.31 (m_o, 1 H, $6b_B$-H), 1.28 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H), 1.18 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_C-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3 (C-6_A), 161.6 (NHCO), 159.4 (C_{IVPMB}), 138.9, 138.8, 138.7, 138.6, 138.5, 137.8, 137.7, 135.1 (9 C, C_{IVAr}), 134.1 (CH=$_{All}$), 130.7 (C_{IVAr}), 129.8–127.3 (47 C, C_{Ar}), 117.4 (=CH_{2All}), 113.9 (2 C, C_{ArPMB}), 102.7 ($^1J_{CH}$ = 162.0 Hz, C-1_A), 101.3 ($^1J_{CH}$ = 173.5 Hz, C-1_C), 100.7 ($^1J_{CH}$ = 162.4 Hz, C-1_B), 100.4 ($^1J_{CH}$ = 175.4 Hz, C-1_D), 92.9 (CCl_3), 80.9 (C-4_C), 80.3 (C-3_A), 80.2 (C-4_D), 79.2 (C-3_C), 78.9 (C-3_D), 78.8 (C-2_D), 78.4 (C-2_A), 75.8 (C-5_A), 75.6, 75.3 (2 C, C_{Bn}), 75.2 (C-2_C), 74.9 (2 C, C_{Bn}), 74.1 (C-4_A), 73.7 (C-3_B), 73.4 (C_{Bn}), 73.3 (C-5_B), 72.7, 72.5 (2 C, C_{Bn}), 72.4 (C-4_B), 72.3, 71.6 (2 C, C_{Bn}), 70.5 (CH_{2All}), 68.5 (C-5_D), 68.3 (C-5_C), 68.0 (C-6_B), 67.3 (C_{CO2Bn}), 55.4 (C-2_B), 55.2 (CH_{3PMB}), 18.2 (C-6_D), 17.8 (C-6_C) ppm. HRMS (ESI^+): calcd. for $C_{100}H_{106}Cl_3NO_{21}Na$ $[M + Na]^+$ 1784.6221; found 1784.6198.

Data for α Anomer 51: R_f = 0.40 (Tol/EtOAc, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42–7.17 (m, 47 H, H_{Ar}), 6.83–6.76 (m, 3 H, NH, 2 H_{ArPMB}), 5.98 (m, 1 H, CH=$_{All}$), 5.35 (m, J_{trans} = 17.3, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.27 (bd, $J_{1,2}$ = 1.2 Hz, 1 H, 1_D-H), 5.26 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 5.22 (m, J_{cis} = 10.5 Hz, 1 H, =CH_{2All}), 5.10 (d, 1 H, H_{CO2Bn}), 5.02 (d, J = 11.4 Hz, 1 H, H_{Bn}), 4.94 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.90 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.85 (d_{po}, J = 10.8 Hz, 1 H, H_{Bn}), 4.84 (bs_o, 1 H, 1_C-H), 4.79 (d_{po}, J = 12.7 Hz, 1 H, H_{Bn}), 4.75 (d_{po}, J = 12.7 Hz, 1 H, H_{Bn}), 4.74 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.69 (d_{po}, J = 11.6 Hz, 1 H, H_{Bn}), 4.68–4.45 (m, 11 H, 9 H_{Bn}, H_{All}, 2_B-H), 4.43 (d, $J_{1,2}$ = 3.5 Hz, 1 H, 1_B-H), 4.40–4.34 (m, 4 H, H_{Bn}, 1_A-H, 4_A-H, 5_B-H), 4.15 (m, 1 H, H_{All}), 4.11 (bd, 1 H, 4_B-H), 4.07 (pt, 1 H, 2_C-H), 4.05 (pt, 1 H, 2_D-H), 4.00 (d, $J_{4,5}$ = 1.0 Hz, 1 H, 5_A-H), 3.85 (dd, $J_{2,3}$ = 2.8, $J_{3,4}$ = 9.5 Hz, 1 H, 3_C-H), 3.81 (dd, $J_{2,3}$ = 2.8, $J_{3,4}$ = 9.4 Hz, 1 H, 3_D-H), 3.75 (dd, $J_{2,3}$ = 10.6, $J_{3,4}$ = 2.0 Hz, 1 H, 3_B-H), 3.72–3.66 (6 H, 5_C-H, 2_A-H, $6a_B$-H, CH_{3PMB}), 3.64 (dq_{po}, $J_{4,5}$ = 9.6 Hz, 1 H, 5_D-H), 3.52–3.46 (m, 2 H, 3_A-H, $6b_B$-H), 3.29 (pt, 1 H, 4_D-H), 3.17 (pt, $J_{4,5}$ = 9.4 Hz, 1 H, 4_C-H), 1.24 (d, $J_{5,6}$ = 6.1 Hz, 3 H, 6_D-H), 1.07 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_C-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.4 (C-6_A), 161.5 (NHCO), 159.1 ($C_{IVArPMB}$), 138.8, 138.7, 138.5, 138.4, 137.9, 137.9, 137.8, 137.7, 135.0 (9 C, C_{IVAr}), 134.0 (CH=$_{All}$), 130.4 (C_{IVAr}), 129.3–127.4 (47 C, C_{Ar}), 117.5 (=CH_{2All}), 113.7 (2 C, C_{ArPMB}), 102.7 ($^1J_{CH}$ = 160.2 Hz, C-1_A), 99.7 ($^1J_{CH}$ = 172.0 Hz, C-1_D), 98.3 ($^1J_{CH}$ = 172.4 Hz, C-1_C), 96.0 ($^1J_{CH}$ = 175.2 Hz, C-1_B), 92.9 (CCl_3), 80.5 (C-4_C), 80.2 (2 C, C-3_A, C-4_D), 79.4 (C-3_D), 78.5 (C-2_A), 77.2 (2 C, C-3_C, C-3_B), 75.4, 75.3 (2 C, C_{Bn}), 75.1 (C-2_D), 74.9, 74.5 (2 C, C_{Bn}), 73.6 (C-5_A), 73.6, 73.1 (2 C, C_{Bn}), 72.8 (C-5_B), 72.4 (2 C, C-2_C, C_{Bn}), 72.1 (C-4_B), 71.2 (C_{Bn}), 70.9 (CH_{2All}), 70.6 (C_{Bn}), 70.1 (C-4_A), 68.6 (2 C, C-6_B, C-5_D), 68.3 (C-5_C), 67.3 (C_{CO2Bn}), 55.1 (CH_{3PMB}), 50.9 (C-2_B), 18.2, 18.1 (2 C, C-6_D, C-6_C) ppm. HRMS (ESI^+): calcd. for $C_{100}H_{106}Cl_3NO_{21}Na$ $[M + Na]^+$ 1784.6221; found 1784.6265.

Data for Silylated Acceptor 52: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.20 (m, 32 H, H_{Ar}), 6.85 (m, J = 8.6 Hz, 2 H, H_{ArPMB}), 5.98 (m, 1 H, CH=$_{All}$), 5.35 (m, J_{trans} = 17.3, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.32 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1_D-H), 5.27 (d, 1 H, H_{CO2Bn}), 5.21 (m, J_{cis} = 10.5 Hz, 1 H, =CH_{2All}), 5.11 (d, J = 12.3 Hz, 1 H, H_{CO2Bn}), 4.92 (d, J = 10.8 Hz, 1 H, H_{Bn}), 4.90 ($2d_o$, J = 11.0 Hz, 2 H, H_{Bn}), 4.83 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1_C-H), 4.81 (d, J = 12.1 Hz, 1 H, H_{Bn}), 4.73 (d, J = 10.9 Hz, 1 H, H_{Bn}), 4.71–4.57 (m, 7 H, H_{Bn}), 4.50 (m, 1 H, H_{All}), 4.39 (bd_{po}, 1 H, 4_A-H), 4.38 (d_{po}, $J_{1,2}$ = 7.7 Hz, 1 H, 1_A-H), 4.16 (m_{po}, 1 H, H_{All}), 4.13 (dd_o, $J_{2,3}$ = 3.0 Hz, 1 H, 2_D-H), 4.09 (dd, 1 H, 2_C-H), 4.02 (d, $J_{4,5}$ = 1.0 Hz, 1 H, 5_A-H), 3.85 (dd, $J_{3,4}$ = 9.4 Hz, 1 H, 3_D-H), 3.80–3.73 (m, 5 H, 3_C-H, 5_C-H, CH_{3PMB}), 3.73–3.67 (m, 2 H, 2_A-H, 5_D-H), 3.53 (pt_{po}, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.5 Hz, 1 H, 4_C-H), 3.52 (dd_o, $J_{2,3}$ = 9.9, $J_{3,4}$ = 2.9 Hz, 1 H, 3_A-H), 3.40 (pt, $J_{4,5}$ = 9.6 Hz, 1 H, 4_D-H), 1.29 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H), 1.19 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_C-H), 0.09 (s, 9 H, $SiMe_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3 (C-6_A), 159.0 (C_{IVPMB}), 138.9–135.1 (6 C, C_{IVAr}), 134.0 (CH=$_{All}$), 130.8 (C_{IVAr}), 129.4–127.4 (32 C, C_{Ar}), 117.4 (=CH_{2All}), 113.6 (2 C, C_{ArPMB}), 102.7 (C-1_A), 102.5 (C-1_C), 100.3 (C-1_D), 80.5 (C-3_A), 80.3, 80.2 (2 C, C-4_C, C-4_D), 79.4, 79.2 (2 C, C-3_C, C-3_D), 78.5 (C-2_A), 75.3, 75.1 (2 C, C_{Bn}), 75.0 (C-2_D), 74.9 (C_{Bn}), 73.7 (C-5_A), 73.4 (C-4_A), 72.6, 72.3, 72.1 (3 C, C_{Bn}), 70.6 (CH_{2All}), 70.4 (C-2_C), 68.8, 68.6 (2 C, C-5_C, C-5_D), 67.3 (C_{CO2Bn}), 55.2 (CH_{3PMB}), 18.2 (C-6_D), 17.9 (C-6_C), 0.4 (3 C, $SiMe_3$) ppm.

Benzyl (3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→2)-(3-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (54): Water (1.6 mL) and CAN (870 mg, 1.59 mmol, 4.0 equiv.) were added to a solution of tetrasaccharide **50** (700 mg, 397 μmol) in MeCN (15.9 mL). The reaction mixture was stirred at room temperature for 30 min. After that time, TLC (Tol/EtOAc, 9:1) showed the complete transformation of the starting material into a more polar product. The reaction was quenched with $NaHCO_3$ (satd. aq.). The reaction mixture was diluted with water and CH_2Cl_2, and the aqueous phase was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, passed through a phase-separator filter, and concentrated. The resulting crude oil was dissolved in pyridine (20 mL), and excess acetic anhydride (3.75 mL) and DMAP (48 mg, 397 μmol, 1.0 equiv.) were added to the solution whilst stirring at room temperature. After 14 h, TLC (Tol/EtOAc, 9:1) indicated that the intermediate alcohol had been converted into a less polar product. The volatiles were evaporated and co-evaporated three times with toluene. The residue was purified by flash chromatography (Tol/EtOAc, 95:5 to 9:1) to give monoacetylated tetrasaccharide **54** (582 mg, 87% over two steps) as a white foam. R_f = 0.38 (Tol/EtOAc, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41–7.04 (m, 45 H, H_{Ar}), 7.02 (d, $J_{NH,2}$ = 7.0 Hz, 1 H, NH), 5.98 (m, 1 H, CH=$_{All}$), 5.36 (dd_{po}, $J_{2,3}$ = 3.1 Hz, 1 H, 3_C-H), 5.35 (m, J_{gem} = 1.6 Hz, 1 H, =CH_{2All}), 5.32 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1_D-H), 5.26 (d, J = 12.2 Hz, 1 H, H_{CO2Bn}), 5.22 (m, J_{cis} = 10.5 Hz, 1 H,

=CH_{2All}), 5.09 (d, 1 H, H_{CO2Bn}), 5.03 (d_{po}, $J_{1,2}$ = 8.4 Hz, 1 H, 1_B-H), 5.01 (d_{po}, $J_{1,2}$ = 2.0 Hz, 1 H, 1_C-H), 4.89 (d_{po}, J = 10.8 Hz, 1 H, H_{Bn}), 4.88 (d, J = 11.1 Hz, 2 H, 2 H_{Bn}), 4.77 (d, J = 11.8 Hz, 1 H, H_{Bn}), 4.70 (d, J = 11.2 Hz, 2 H, H_{Bn}), 4.66 (d, J = 11.2 Hz, 1 H, H_{Bn}), 4.62–4.52 (m_{po}, 7 H, H_{Bn}), 4.50 (m_{po}, 1 H, H_{All}), 4.40–4.35 (m, 4 H, 1_A-H, 4_A-H, 3_B-H, 2_C-H), 4.26 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.20 (d, J = 11.6 Hz, 1 H, H_{Bn}), 4.16 (m, 1 H, H_{All}), 4.05 (d, $J_{3,4}$ = 2.4 Hz, 1 H, 4_B-H), 4.01 (d, $J_{4,5}$ = 0.9 Hz, 1 H, 5_A-H), 4.00 (m, 1 H, 2_D-H), 3.92–3.82 (m, 2 H, 2_B-H, 5_C-H), 3.80 (dd, $J_{2,3}$ = 2.9, $J_{3,4}$ = 9.5 Hz, 1 H, 3_D-H), 3.70–3.58 (m, 3 H, 2_A-H, $6a_B$-H, 5_D-H), 3.56–3.47 (m, 4 H, 3_A-H, 5_B-H, 4_C-H, 4_D-H), 3.35 (dd, $J_{5.6b}$ = 4.5, $J_{6a,6b}$ = 8.3 Hz, 1 H, $6b_B$-H), 2.17 (s, 3 H, H_{Ac}), 1.29 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_D-H), 1.14 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_C-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7 (CO_{Ac}) 167.2 (C-6_A), 161.5 (NHCO), 139.0, 138.9, 138.6, 138.5, 138.4, 137.8, 137.7, 137.6, 135.1 (9 C, C_{IVAr}), 134.1 (CH=$_{All}$), 129.6–127.2 (45 C, C_{Ar}), 117. 4 (=CH_{2All}), 102.6 ($^1J_{CH}$ = 156.6 Hz, C-1_A), 101.3 ($^1J_{CH}$ = 176.3 Hz, C-1_C), 100.0 ($^1J_{CH}$ = 171.1 Hz, C-1_D), 99.6 ($^1J_{CH}$ = 163.3 Hz, C-1_B), 92.9 (CCl_3), 80.7 (C-3_A), 80.2 (C-4_D), 79.6 (C-4_C), 79.0 (C-3_D), 78.5 (C-2_A), 76.7 (C-3_B), 76.1 (C-2_D), 75.4, 75.3 (2 C, C_{Bn}), 75.2 (C-2_C), 75.1, 75.0 (2 C, C_{Bn}), 73.6 (C-5_A), 73.4 (C-3_C), 73.3 (C_{Bn}), 73.1 (C-4_A), 73.0 (C-5_B), 72.7 (C_{Bn}), 72.6 (C-4_B), 72.5, 71.8 (2 C, C_{Bn}), 70.5 (CH_{2All}), 68.8 (C-5_D), 68.2 (C-5_C), 67.6 (C-6_B), 67.3 (C_{CO2Bn}), 56.4 (C-2_B), 21.3 (CH_{3Ac}), 18.0 (C-6_D), 17.7 (C-6_C) ppm. HRMS (ESI^+): calcd. for $C_{94}H_{101}Cl_3NO_{21}$ $[M + H]^+$ 1684.5931; found 1684.5983; calcd. for $C_{94}H_{100}Cl_3NO_{21}Na$ $[M + Na]^+$ 1706.5751; found 1706.5928.

Propyl 2-Acetamido-2-deoxy-β-D-galactopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic Acid (4): Pd/C (10%; 200 mg) was added to a stirred solution of tetrasaccharide **50** (254 mg, 140 μmol) in THF/H_2O (4:1, 10.2 mL). The suspension was stirred under hydrogen for 2 d. After this time, MS analysis indicated a single molecular weight corresponding to that of the target tetrasaccharide. The reaction mixture was filtered. Evaporation of the volatiles, freeze-drying, and purification of the crude material by preparative RP-HPLC gave tetrasaccharide **4** (61.8 mg, 59%) as a white foam following repeated freeze-drying. t_R = 8.5 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.24 (d, $J_{1,2}$ = 1.4 Hz, 1 H, 1_D-H), 5.08 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1_C-H), 4.55 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1_B-H), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1_A-H), 4.35 (d, $J_{4,5}$ = 1.2 Hz, 1 H, 5_A-H), 4.27 (dd, $J_{3,4}$ = 2.8 Hz, 1 H, 4_A-H), 4.05 (dd, $J_{2,3}$ = 3.0 Hz, 1 H, 2_C-H), 4.02 (dd, $J_{2,3}$ = 3.1 Hz, 1 H, 2_D-H), 3.87–3.74 (m, 6 H, 4_B-H, OCH_{2Pr}, 2_B-H, 3_A-H, 3_D-H, 3_C-H), 3.74–3.63 (m, 3 H, $6a_B$-H, $6b_B$-H, 3_B-H), 3.63–3.51 (m, 4 H, 5_B-H, 5_C-H, OCH_{2Pr}, 5_D-H), 3.50 (dd, $J_{2,3}$ = 9.9 Hz, 1 H, 2_A-H), 3.34 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1 H, 4_D-H), 3.27 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1 H, 4_C-H), 1.97 (s, 3 H, CH_{3NHAc}), 1.57 (sext, J = 7.4 Hz, 2 H, CH_{2Pr}), 1.20–1.15 (m, 6 H, 6_C-H, 6_D-H), 0.84 (t, 3 H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 177.7 (NHCO), 174.2 (C-6_A), 105.7 ($^1J_{CH}$ = 163.2 Hz, C-1_B), 104.9 ($^1J_{CH}$ = 161.7 Hz, C-1_A), 103.6 ($^1J_{CH}$ = 175.8 Hz, C-1_C), 102.5 ($^1J_{CH}$ = 174.7 Hz, C-1_D), 81.2 (C-2_C), 81.1 (C-2_D), 79.3 (C-4_A), 77.6 (C-5_B), 75.5 (C-3_A), 75.4 (C-5_A), 74.9 (2 C, C-4_C, OCH_{2Pr}), 74.5 (C-4_D), 73.4 (C-3_B), 72.8 (C-2_A), 72.3 (2 C, C-3_D, C-3_C), 71.8 (C-5_D), 71.7 (C-5_C), 70.3 (C-4_B), 63.5 (C-6_B), 55.4 (C-2_B), 25.0, 24.7 (2 C, CH_{2Pr}, CH_{3NHAc}), 19.2 (2 C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI^+): calcd. for $C_{29}H_{49}NO_{20}Na$ $[M + Na]^+$ 754.2745; found 754.2722.

Propyl 2-Acetamido-2-deoxy-β-D-galactopyranosyl-(1→2)-(3-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic Acid (5): Pd/C (10%; 150 mg) was added to a stirred solution of tetrasaccharide **54** (187 mg, 110 μmol) in THF/H_2O (4:1, 8.4 mL). The suspension was stirred under hydrogen for 2 d. After this time, MS analysis indicated a molecular weight corresponding to that of the target tetrasaccharide. The reaction mixture was filtered. Evaporation of the volatiles, freeze-drying, and purification by preparative RP-HPLC gave tetrasaccharide **5** (45.3 mg, 53%) as a white solid following repeated freeze-drying. t_R = 9.0 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.23 (d, $J_{1,2}$ = 1.3 Hz, 1 H, 1_D-H), 5.03 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 1_C-H), 4.92 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 10.0 Hz, 1 H, 3_C-H), 4.33 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1_A-H), 4.31 (bs_o, 1 H, 5_A-H), 4.30 (d_{po}, $J_{1,2}$ = 8.3 Hz, 1 H, 1_B-H), 4.22 (dd, $J_{3,4}$ = 2.8, $J_{4,5}$ = 1.1 Hz, 1 H, 4_A-H), 4.12 (pt, 1 H, 2_C-H), 3.99 (dd, $J_{2,3}$ = 3.1 Hz, 1 H, 2_D-H), 3.83–3.70 (m, 5 H, 4_B-H, OCH_{2Pr}, 2_B-H, 3_A-H, 3_D-H), 3.70–3.57 (m, 4 H, 5_C-H, $6a_B$-H, $6b_B$-H, 3_B-H), 3.52–3.46 (m, 3 H, 5_B-H, OCH_{2Pr}, 5_D-H), 3.45 (dd_{po}, $J_{2,3}$ = 10.0 Hz, 1 H, 2_A-H), 3.40 (pt_{po}, J = 9.3, J = 9.8 Hz, 1 H, 4_C-H), 3.35 (pt_{po}, J = 9.6, J = 9.8 Hz, 1 H, 4_D-H), 2.09 (s, 3 H, CH_{3Ac}), 1.97 (s, 3 H, CH_{3NHAc}), 1.56 (sext, J = 7.4 Hz, 2 H, CH_{2Pr}), 1.16 (d, $J_{5,6}$ = 6.3 Hz, 3 H, 6_D-H), 1.14 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6_C-H), 0.80 (t, 3 H, CH_{3Pr}) ppm. ^{13}C NMR (400 MHz, D_2O): δ = 177.1 (NHCO), 176.1 (CO_{Ac}), 174.1 (C-6_A), 105.8 ($^1J_{CH}$ = 163.2 Hz, C-1_B), 104.9 ($^1J_{CH}$ = 161.8 Hz, C-1_A), 103.6 ($^1J_{CH}$ = 174.7 Hz, C-1_C), 102.4 ($^1J_{CH}$ = 173.6 Hz, C-1_D), 81.4 (C-2_D), 79.3 (C-4_A), 79.0 (C-2_C), 77.4 (C-5_B), 75.4 (C-3_A), 75.3 (C-5_A), 75.2 (C-3_C), 74.8 (OCH_{2Pr}), 74.4 (C-4_D), 72.8 (C-3_B), 72.7 (C-2_A), 72.6 (C-4_C), 72.2 (C-3_D), 71.8 (C-5_D), 71.7 (C-5_C), 70.2 (C-4_B), 63.4 (C-6_B), 55.0 (C-2_B), 24.9 (CH_{3NHAc}), 24.7 (CH_{2Pr}), 23.1 (CH_{3Ac}), 19.2, 19.1 (2 C, C-6_D, C-6_C), 12.1 (CH_{3Pr}) ppm. HRMS (ESI^+): calcd. for $C_{31}H_{51}NO_{21}Na$ $[M + Na]^+$ 796.2852; found 796.2866.

Supporting Information (see footnote on the first page of this article): Copies of the NMR spectra (^{1}H, ^{13}C, COSY and HSQC) of all new compounds, including key intermediates and final products.

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[1] M. M. Levine, *Vaccine* **2006**, *24*, 3865–3873.
[2] S. K. Niyogi, *J. Microbiol.* **2005**, *43*, 133–143.
[3] L. von Seidlein, D. R. Kim, M. Ali, H. Lee, X. Wang, V. D. Thiem, G. Canh do, W. Chaicumpa, M. D. Agtini, A. Hossain, Z. A. Bhutta, C. Mason, O. Sethabutr, K. Talukder, G. B. Nair, J. L. Deen, K. Kotloff, J. Clemens, *PLoS Med.* **2006**, *3*, e353.
[4] a) M. M. Levine, K. L. Kotloff, E. M. Barry, M. F. Pasetti, M. B. Sztein, *Nat. Rev. Microbiol.* **2007**, *5*, 540–553; b) M. N. Kweon, *Curr. Opin. Infect. Dis.* **2008**, *21*, 313–318.
[5] K. L. Kotloff, J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak, M. M. Levine, *Bull. W. H. O.* **1999**, *77*, 651–666.
[6] a) C. Ferreccio, V. Prado, A. Ojeda, M. Cayyazo, P. Abrego, L. Guers, M. M. Levine, *Am. J. Epidemiol.* **1991**, *134*, 614–627; b) J. H. Passwell, S. Ashkenzi, Y. Banet-Levi, R. Ramon-Saraf, N. Farzam, L. Lerner-Geva, H. Even-Nir, B. Yerushalmi, C. Y. Chu, J. Shiloach, J. B. Robbins, R. Schneerson, I. S. S. Grp, *Vaccine* **2010**, *28*, 2231–2235.

[7] F. R. Noriega, F. M. Liao, D. R. Maneval, S. Ren, S. B. Formal, M. M. Levine, *Infect. Immun.* **1999**, *67*, 782–788.
[8] D. A. Simmons, E. Romanowska, *J. Med. Microbiol.* **1987**, *23*, 289–302.
[9] H. L. DuPont, R. B. Hornick, M. J. Snyder, J. P. Libonati, S. B. Formal, E. J. Gangarosa, *J. Infect. Dis.* **1972**, *125*, 12–16.
[10] R. W. Kaminski, E. V. Oaks, *Expert Rev. Vaccines* **2009**, *8*, 1693–1704.
[11] A. Phalipon, M. Tanguy, C. Grandjean, C. Guerreiro, F. Belot, D. Cohen, P. J. Sansonetti, L. A. Mulard, *J. Immunol.* **2009**, *182*, 2241–2247.
[12] L. Mulard, A. Phalipon, in *Carbohydrate-Based Vaccines* (Ed.: R. Roy), American Chemical Society, Washington DC, **2008**, vol. 989, pp. 105–136.
[13] a) J. Kubler-Kielb, E. Vinogradov, C. Chu, R. Schneerson, *Carbohydr. Res.* **2007**, *342*, 643–647; b) A. V. Perepelov, V. L. L′vov, B. Liu, S. N. Senchenkova, M. E. Shekht, A. S. Shashkov, L. Feng, P. G. Aparin, L. Wang, Y. A. Knirel, *Carbohydr. Res.* **2009**, *344*, 687–692; c) A. V. Perepelov, S. D. Shevelev, B. Liu, S. N. Senchenkova, A. S. Shashkov, L. Feng, Y. A. Knirel, L. Wang, *Carbohydr. Res.* **2010**, *345*, 1594–1599.
[14] a) D. A. Simmons, *Eur. J. Biochem.* **1969**, *11*, 554–575; b) E. Katzenellenbogen, M. Mulczyk, E. Romanowska, *Eur. J. Biochem.* **1976**, *61*, 191–197; c) J. Kubler-Kielb, E. Vinogradov, C. Mocca, V. Pozsgay, B. Coxon, J. B. Robbins, R. Schneerson, *Carbohydr. Res.* **2010**, *345*, 1600–1608; d) B. A. Dmitriev, Y. A. Knirel, O. K. Sheremet, A. A. Shashkov, N. K. Kochetkov, I. L. Hofman, *Eur. J. Biochem.* **1979**, *98*, 309–316.
[15] A. V. Perepelov, M. E. Shekht, B. Liu, S. D. Shevelev, V. A. Ledov, S. N. Senchenkova, V. L. L′vov, A. S. Shashkov, L. Feng, P. G. Aparin, L. Wang, Y. A. Knirel, *FEMS Immunol. Med. Microbiol.* **2012**, *66*, 201–210.
[16] K. Hygge Blakeman, A. Weintraub, G. Widmalm, *Eur. J. Biochem.* **1998**, *251*, 534–537.
[17] C. Gauthier, P. Chassagne, F. X. Theillet, C. Guerreiro, F. Thouron, F. Nato, M. Delepierre, P. J. Sansonetti, A. Phalipon, L. A. Mulard, manuscript in preparation.
[18] a) E. Konadu, J. Shiloach, D. A. Bryla, J. B. Robbins, S. C. Szu, *Infect. Immun.* **1996**, *64*, 2709–2715; b) D. S. Berry, F. Lynn, C. H. Lee, C. E. Frasch, M. C. Bash, *Infect. Immun.* **2002**, *70*, 3707–3713.
[19] M. U. Roslund, O. Aitio, J. Warna, H. Maaheimo, D. Y. Murzin, R. Leino, *J. Am. Chem. Soc.* **2008**, *130*, 8769–8772.
[20] T. Ali, A. Weintraub, G. Widmalm, *Carbohydr. Res.* **2006**, *341*, 1878–1883.
[21] O. Westphal, J. Jann, *Methods Carbohydr. Chem.* **1965**, *5*, 83–91.
[22] T. D. Claridge, I. Perez-Victoria, *Org. Biomol. Chem.* **2003**, *1*, 3632–3634.
[23] a) M. Lundborg, C. Fontana, G. Widmalm, *Biomacromolecules* **2011**, *12*, 3851–3855; b) M. Lundborg, G. Widmalm, *Anal. Chem.* **2011**, *12*, 3851–3855.
[24] a) P. T. Robinson, T. N. Pham, D. Uhrin, *J. Magn. Reson.* **2004**, *170*, 97–103; b) S. J. Duncan, R. Lewis, M. A. Bernstein, P. Sandor, *Magn. Reson. Chem.* **2007**, *45*, 283–288.
[25] M. Kuttel, Y. Mao, G. Widmalm, M. Lundborg, *Proc. 7th IEEE Int. Conf. eScience* **2011**, 395–402.
[26] A. Dondoni, A. Marra, *Chem. Soc. Rev.* **2012**, *41*, 573–586.
[27] R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.
[28] P. J. Garegg, *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179–205.
[29] B. A. Yu, J. S. Sun, *Chem. Commun.* **2010**, *46*, 4668–4679.
[30] Y. Nakahara, T. Ogawa, *Tetrahedron Lett.* **1987**, *28*, 2731–2734.
[31] D. Magaud, R. Dolmazon, D. Anker, A. Doutheau, Y. L. Dory, P. Deslongchamps, *Org. Lett.* **2000**, *2*, 2275–2277.
[32] a) D. Magaud, C. Grandjean, A. Doutheau, D. Anker, V. Shevchik, N. Cotte-Pattat, J. Robert-Baudouy, *Carbohydr. Res.* **1998**, *314*, 189–199; b) B. Nolting, H. Boye, C. Vogel, *J. Carbohydr. Chem.* **2001**, *20*, 585–610.
[33] a) D. Reiffarth, K. B. Reimer, *Carbohydr. Res.* **2008**, *343*, 179–188; b) N. Nemati, G. Karapetyan, B. Nolting, H. U. Endress, C. Vogel, *Carbohydr. Res.* **2008**, *343*, 1730–1742.
[34] S. Kramer, B. Nolting, A. J. Ott, C. Vogel, *J. Carbohydr. Chem.* **2000**, *19*, 891–921.
[35] M. Rommel, A. Ernst, U. Koert, *Eur. J. Org. Chem.* **2007**, 4408–4430.
[36] V. A. Olah, A. Liptak, J. Harangi, P. Nanasi, *Acta Chim. Hung.* **1985**, *118*, 25–29.
[37] M. Barbier, T. Breton, K. Servat, E. Grand, B. Kokoh, J. Kovensky, *J. Carbohydr. Chem.* **2006**, *25*, 253–266.
[38] L. J. van den Bos, J. D. C. Codee, J. C. van der Toorn, T. J. Boltje, J. H. van Boom, H. S. Overkleeft, G. A. van der Marel, *Org. Lett.* **2004**, *6*, 2165–2168.
[39] A. M. P. van Steijn, J. P. Kamerling, J. F. G. Vliegenthart, *Carbohydr. Res.* **1991**, *211*, 261–277.
[40] J. Boutet, C. Guerreiro, L. A. Mulard, *J. Org. Chem.* **2009**, *74*, 2651–2670.
[41] M. Petitou, P. Duchaussoy, G. Jaurand, F. Gourvenec, I. Lederman, J. M. Strassel, T. Barzu, B. Crepon, J. P. Herault, J. C. Lormeau, A. Bernat, J. M. Herbert, *J. Med. Chem.* **1997**, *40*, 1600–1607.
[42] J. Boutet, L. A. Mulard, *Eur. J. Org. Chem.* **2008**, 5526–5542.
[43] S. Hanashima, B. Castagner, D. Esposito, T. Nokami, P. H. Seeberger, *Org. Lett.* **2007**, *9*, 1777–1779.
[44] S. Serna, B. Kardak, N. C. Reichardt, M. Martin-Lomas, *Tetrahedron: Asymmetry* **2009**, *20*, 851–856.
[45] M. K. Gurjar, A. S. Mainkar, *Tetrahedron* **1992**, *48*, 6729–6738.
[46] J. J. Oltvoort, C. A. A. Van Boeckel, J. H. De Koning, J. H. Van Boom, *Synthesis* **1981**, 305–308.
[47] M. A. Nashed, L. Anderson, *J. Chem. Soc., Chem. Commun.* **1982**, 1274–1276.
[48] E. M. Scanlan, M. M. Mackeen, M. R. Wormald, B. G. Davis, *J. Am. Chem. Soc.* **2010**, *132*, 7238–7329.
[49] a) C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, *19*, 1131–1150; b) J. Boutet, T. H. Kim, C. Guerreiro, L. A. Mulard, *Tetrahedron Lett.* **2008**, *49*, 5339–5342.
[50] J. Bartek, R. Muller, P. Kosma, *Carbohydr. Res.* **1998**, *308*, 259–273.
[51] A. V. Demchenko, E. Rousson, G. J. Boons, *Tetrahedron Lett.* **1999**, *40*, 6523–6526.
[52] K. Pekari, D. Tailler, R. Weingart, R. R. Schmidt, *J. Org. Chem.* **2001**, *66*, 7432–7442.
[53] F. Belot, J. C. Jacquinet, *Carbohydr. Res.* **2000**, *325*, 93–106.
[54] F. Belot, J. C. Jacquinet, *Carbohydr. Res.* **1996**, *290*, 79–86.
[55] F. X. Theillet, C. Simenel, C. Guerreiro, A. Phalipon, L. A. Mulard, M. Delepierre, *Glycobiology* **2011**, *21*, 109–121.
[56] R. A. Foster, N. I. A. Carlin, M. Majcher, H. Tabor, L. K. Ng, G. Widmalm, *Carbohydr. Res.* **2011**, *346*, 872–876.
[57] A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *65*, 355–360.
[58] J. Schleucher, M. Schwendinger, M. Sattler, P. Schmidt, O. Schedletzky, S. J. Glaser, O. W. Sørensen, C. Griesinger, *J. Biomol. NMR* **1994**, *4*, 301–306.
[59] a) Ē. Kupče, in *Methods Enzymol.* (Eds.: T. L. James, V. Dotsch, U. Schmitz), Academic Press, San Diego, CA, USA, **2002**, vol. 338, pp. 82–111; b) A. Tannus, M. Garwood, *NMR Biomed.* **1997**, *10*, 423–434.
[60] M. J. Thrippleton, J. Keeler, *Angew. Chem.* **2003**, *115*, 4068–4071; *Angew. Chem. Int. Ed.* **2003**, *42*, 3938–3941.

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