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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

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Extensive NMR analysis of the delipidated lipopolysaccharide of *Shigella flexneri* serotype 6 strain MDC 2924-71 confirmed the most recently reported structure of the O-antigen chemical repeating unit as follows {→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→}, while setting into light the non stoichiometric acetylation at O-3_C/4_C. Input from the CASPER program contributed to define the fine distribution of the three possible patterns of *O*-acetylation. Data favored the non *O*-acetylated repeating unit (**ABCD**) corresponding to about 2/3 of the population, whilst 1/4 is acetylated at O-3_C (**$_{3Ac}$CDAB**) and 1/10 at O-4_C (**$_{4Ac}$CDAB**). The corresponding di- to tetrasaccharides, having the Gal*p*A residue (**A**) at their reducing end, were synthesized as their propyl glycosides according to a multistep linear strategy relying on late stage acetylation at O-3_C. Thus, the 3_C-*O*-acetylated and non-acetylated oligosaccharides were synthesized from common protected intermediates comprising a rhamnose **C** residue, in which a 3-*O*-*para*-methoxybenzyl protecting group masked the site of *O*-acetylation. Donors were optimized for high yielding glycosylation. Rhamnosylation was most efficiently achieved by use of imidate donors, also at O-4 of a benzyl galacturonate acceptor. In contrast, a thiophenyl 2-trichloroacetamido-D-galactopyranoside precursor was preferred for chain elongation involving residue **B**. Final Pd/C-mediated deprotection, run under controlled pH, ensured *O*-acetyl stability. All targets represent parts of the O-antigen of *S. flexneri* serotype 6, a prevalent serotype. Non-*O*-acetylated oligosaccharides are shared by the *Escherichia coli* O147 O-antigen.

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Introduction

Shigellosis, an invasive infection of the human colon, is 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 diarrhea.[2] Also known as bacillary dysentery, this highly contagious infection is associated with increased antibiotic-resistance.[3] It is endemic worldwide and remains a major health concern especially in the pediatric population living in the most impoverished areas.[3-4] *S. flexneri* – one out of the four species of *Shigella* – prevails in developing countries, where it accounts for endemic disease.[3, 5] Numerous *S. flexneri* serotypes – varying in geographic and temporal distributions – are isolated from patients. In recent years, *S. flexneri* serotype 6 (SF6) was identified as a serotype of increasing prevalence in several settings worldwide,[3, 6] and evidences strongly support the inclusion of SF6 as one of the key valences to be included 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), that is the polysaccharide part of the bacterial lipopolysaccharide (LPS).[8] Protection against reinfection by the homologous serotype, suggesting serotype-specific natural immunity, was established following *Shigella* infection.[6a, 9] These observations provided strong evidence for *S. flexneri* O-Ags being major targets of the host adaptive immunity. Accordingly, several LPS-based vaccine candidates against shigellosis have been developed and even evaluated during clinical trials.[4, 10] Along this line, we have investigated a promising alternative to the use of material of biological origin. It involves the molecular design of synthetic oligosaccharide haptens to serve as "functional" mimics of the natural O-Ag of interest.[11] The strategy under development relies for an important part on the availability of well-defined synthetic frame-shifted fragments of the O-Ag.[12] In the following, it is addressed for the first time in the case of SF6.

It is of note that knowledge of the exact repeating unit (RU) of the O-Ag of interest is a major pre-requirement to launch such a strategy. Considering the numerous revised structures of *S. flexneri* O-Ags published recently,[13a, 13b , 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 latter.

In this context, the following reports the elucidation by NMR spectroscopy of the RU and acetylation pattern of the O-Ag from SF6 strain MDC 2924-71 on one hand, and the first synthesis of di- to tetrasaccharide fragments thereof on the other hand.

Results and Discussion

Structural investigation on the O-Ag from SF6 strain MDC 2924-71.

The most recent structural investigations on 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 the O-acetylation of the former (Fig. 1). The occurrence and position of O-acetyl (OAc) groups in a PS may influence antigenicity,[17] and more importantly immunogenicity,[18] and thus it is of particular interest to establish their exact location(s). In this regard, acetylation in the SF6 O-Ag was identified at O-3 of rhamnose **C**.[14d] However, the main problem when determining the exact O-acetylation pattern in a native PS is whether acetyl migration or acetyl loss take place, which can occur easily given appropriate spatial arrangements, or in the course of PS extraction, purification, or even during the acid-mediated delipidation procedure of LPS.[19] Consequently, in the latter case in particular, one does not know if 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 not always possible. Nevertheless, often one can at least obtain information about the presence or absence of OAc groups by 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 particular, the population distribution of OAc groups on rhamnose **C** in the SF6 O-Ag.

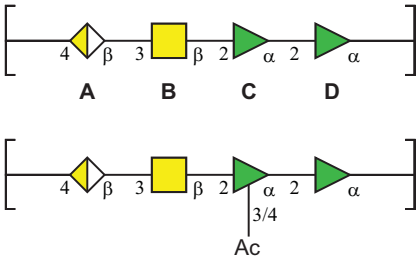


Figure 1. Comparison using CFG-notation of the structures of the O-Ag from *E. coli* O147 (top) and SF6 (bottom). The sugar residues are denoted **A**-**D**.

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 *via* residue **B**, which was purified by gel-permeation chromatography. Different fractions were collected, and the average number of RU per O-Ag was estimated from the ^{1}H NMR spectrum, by integration of the *N*-acetyl signals of residue **B** in the region 2.04 – 2.09 ppm (Fig. 2a) relative to the anomeric signals in the core region for α-Gal*p* (5.86 ppm) and α-Glc*p* (5.63 ppm), as described by Kubler-Kielb *et al.*[14c] From direct inspection of the ^{1}H NMR spectra in the region of 2.14 – 2.21 ppm, different *O*-acetylation patterns were suggested in the lower molecular weight (mw) fraction, consistent with the reported results.[14c] A fraction of intermediate mw showed resonances characteristic from both regions of the PS. As the present study was undertaken to establish the *O*-acetyl location in the O-Ag, a fraction of higher mw, corresponding to about a dozen RUs, was used in the NMR studies. Precautions were taken in order to minimize acetyl migration, such as maintaining the pH not higher than 6 and the sample temperature as low as possible.

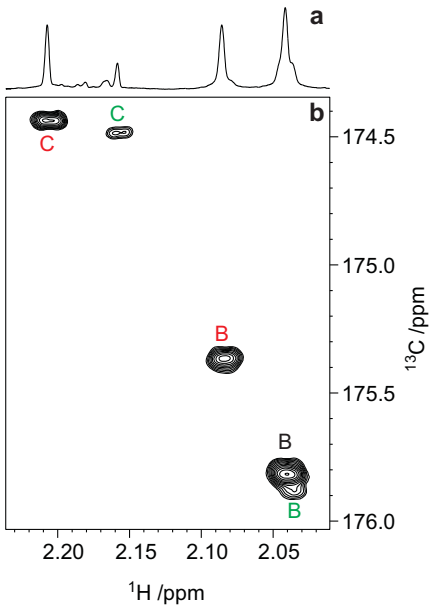


Figure 2. (a) Spectral region of the ^{1}H NMR spectrum of the SF6 PS where the N- and O-acetyl resonances reside. (b) Selected region of the ^{1}H,^{13}C-BS-CT-HMBC NMR spectrum of the PS from SF6 showing correlations from the carbonyl carbons to the methyl protons of the *O*-acetyl and *N*-acetyl groups (residues **C** and **B**, respectively). The capital letters are coloured, where non-*O*-acetylated population is denoted in black, and the *O*-acetylated populations are indicated by red (major) and green (minor).

The ^{1}H NMR spectrum revealed a material of high complexity. Two ^{1}H signals for OAc at 2.158 (minor form) and 2.207 ppm (major form) could be easily identified (Fig. 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 2.037, 2.042 and 2.086 ppm suggest three different populations of residue **B**. The relative 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, whereas 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 174.43 and 174.49 ppm as well as three NAc signals at 175.36, 175.82 and 175.88 ppm (Fig. 2b).

In order 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 re-assigned at pD 5 using 1D and 2D NMR experiments. Due to the pD change, the signals for C-5_A, C-6_A and H-4_A were shifted downfield to δ_C 74.91, 174.42 and δ_H 4.10, while the signals for C-1_D and H-5_D were found at δ_C 100.24 and δ_H 3.72, respectively. The remaining chemical shifts 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 1.23 – 1.27 ppm in the SF6 O-Ag were assigned to H-6 in the non-*O*-acetylated rhamnose **C** and rhamnose **D**. Two conspicuous signals of lower intensity at 1.151 and 1.289 ppm, that are absent in 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 employed 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 the ramnosyl residues **C** and **D** in the RUs, mono-acetylated either at O-3_C or O-4_C, were predicted. The signals from the H-6_C were calculated to resonate at 1.28 ppm in

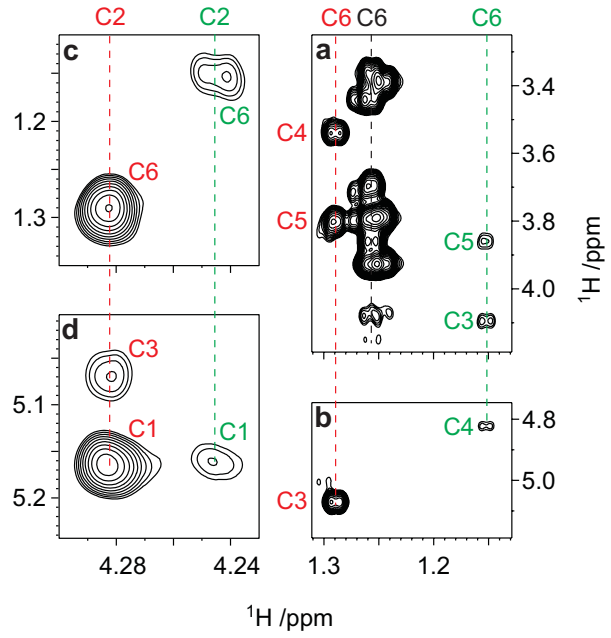


Figure 3. Selected regions of the ^{1}H,^{1}H-TOCSY NMR spectrum (t_{mix}120 ms) of the SF6 PS showing the spin system of residues $_{3Ac}$**C** (red), **C** (black) and $_{4Ac}$**C** (green).

the 3_C-OAc RU and 1.16 ppm in the 4_C-OAc RU, whereas the signals from the H-6_D were calculated to resonate at 1.33 ppm in the 3_C-OAc RU and 1.20 ppm in the 4_C-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 using ^{1}H,^{1}H-TOCSY experiments with increasing mixing times. In both cases, the spins systems could be fully characterized starting from H-6. Subsequent correlations were observed to H-5, H-4 and H-3 (Fig. 3a and 3b) as well to H-2 (Fig. 3c). The resonances from the anomeric protons were then readily tied to their respective H-2 (Fig. 3d). In the spin system originating from the proton at 1.151 ppm (minor *O*-acetylated population, denoted **C** in green color in Fig. 3), the large downfield shift of proton H-4 (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 at 2.158 ppm to the H-6 resonance at 1.151 ppm (Fig. 4a) and to the H-4 resonance at 4.823 ppm (Fig. 4c). Likewise, the large downfield shift of H-3 (5.071 ppm) in the spin system originating from the proton at 1.289 ppm (H-6, major *O*-acetylated population, denoted **C** in red color in Fig. 3), indicates acetylation at O-3_C. Intra-residual NOEs from the OAc at 2.207 ppm to the resonances at 4.282 (H-2) and 5.071 ppm (H-3) supported this substitution pattern (Fig. 4c). Besides residue **B**, present in the non-*O*-acetylated population, two new spin-systems corresponding to minor populations were identified. Both spin-systems were assigned from H-1 to H-4 using ^{1}H,^{1}H-TOCSY experiments with different mixing times, and the respective H-5 protons were traced via intra-residual NOE correlations from the anomeric protons. Based on their relative intensity, the spin systems originating from the H-1 signals at 4.512 ppm and 4.716 ppm were assigned to the major and minor *O*-acetylated populations. The chemical shift displacements, in particular upfield by almost 0.2 ppm in the former case, were attributed to the perturbation by the OAc group in the neighboring residue. ^{1}H chemical shifts of residues **B** and **C** of the *O*-acetylated populations are compiled in Table 1.

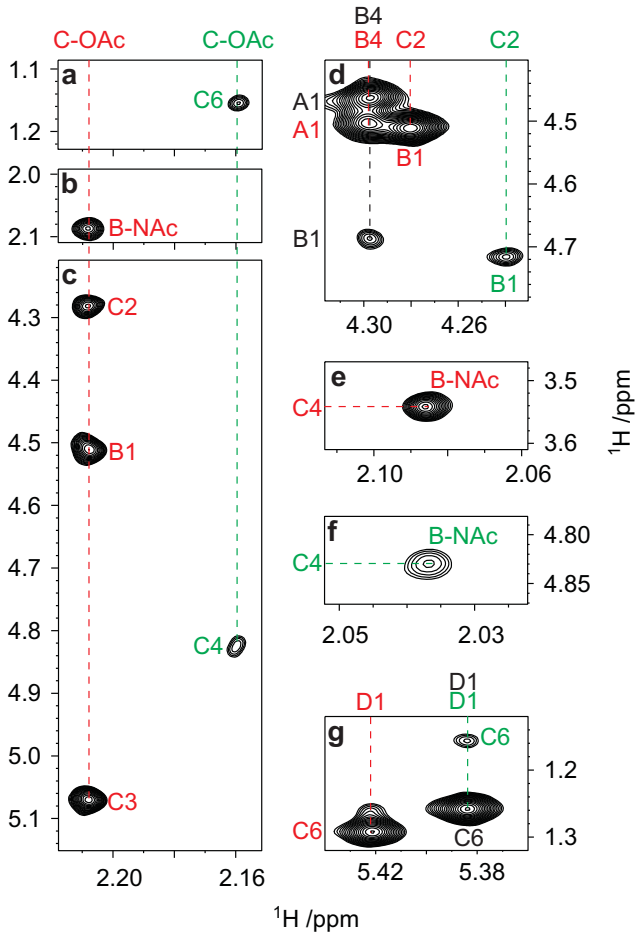


Figure 4. Selected regions of the ^{1}H,^{1}H-NOESY NMR spectrum (t_{mix}150 ms) of the SF6 PS showing intra- and inter-residue NOE correlations in residues $_{3Ac}$**C** (red), **C** (black) and $_{4Ac}$**C** (green).

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
H1	4.512	5.164	4.716	5.159
H2	4.014	4.282	4.055	4.238
H3	3.899	5.071	3.868	4.095
H4	4.300	3.540	4.301	4.823
H5	3.633	3.800	3.685	3.857
H6	n.d.	1.289	n.d.	1.151
NAc	2.086		2.037	
OAc		2.207		2.158

n.d. = not determined

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 H-2_C in the *O*-acetylated **C** to the respective H-1_B (Fig. 4d) and from H-4_C in the *O*-acetylated **C** to the respective NAc in residue **B** (Fig. 4e and 4f), indicating residue **B** substituting the *O*-acetylated rhamnose **C**. NOE correlations were also observed between H-6_C to protons at 5.421 and 5.384 ppm in the major and minor populations, respectively (Fig. 4g). In comparison with the chemical shifts in the PS from *E. coli* O147, those resonances can be attributed to H-1_D, suggesting that the *O*-acetylated residue **C** is substituting rhamnose **D**. The NOE correlations from the NAc at 2.086 and 2.042 ppm, and from the OAc at 2.207 ppm, observed in

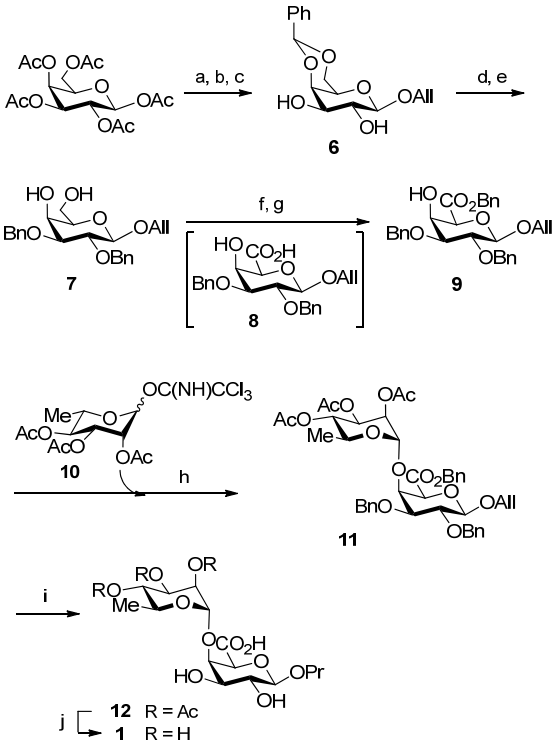
the $^{1}H,^{1}H$-NOESY experiment where also confirmed using DPFGSE CSSF-NOESY experiments.[24] From the experimental data in Table 1, it can be noted that acetylation at O-3_C strongly affects the chemical shifts of the NAc and H-1_B, which can be explained in terms of the close spatial proximity of these groups as shown by the respective NOE correlations in Fig. 4b and 4c (in red color). On the other hand, acetylation at O-4_C does not influence significantly 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 correspond 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 unexpected importance of the population of non-*O*-acetylated RU emphasizes to 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.

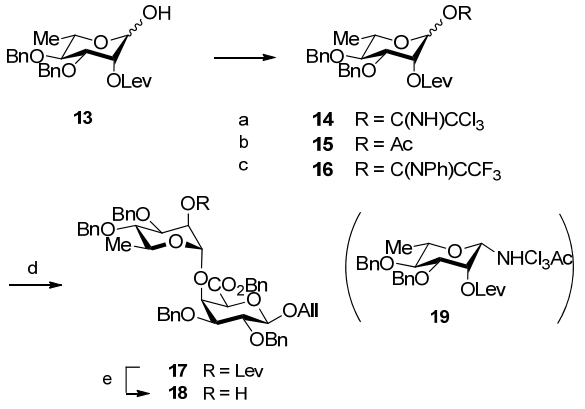
Having confirmed the structure of the RU of the SF6 O-Ag, we turned to the synthesis of the di- to tetrasaccharides having a galacturonide glycoside **A** at the reducing end. These oligosaccharides were synthesized both in their non-*O*-acetylated form **1** (**DA**-Pr), **2** (**CDA**-Pr), **4** (**BCDA**-Pr), 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 block 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 the allyl aglycon would be (i) easily introduced on commercially available D-galactose, (ii) fully orthogonal to most other conventional protecting groups used in glycochemistry, in particular to acetate, (iii) smoothly converted into propyl upon concomitant Pd/C mediated hydrogenolysis of benzyl ether or benzyl esters, and in due course could 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 the corresponding hydroxyl group before functionalization was achieved by use of a *para*-methoxybenzyl ether (PMB). Stepwise chain extension starting from a galactopyranosiduronate acceptor allowed to investigate and to optimize each glycosylation step.

*Synthesis of disaccharide **DA**-Pr **(1)***. Glycosylation at O-4 of a galacturonide glycoside acceptor is thought to be disfavored in comparison to that of the homologous galactopyranoside.[27] In addition, the reactivity of galactopyranosiduronic acid esters possessing a free axial hydroxyl group was shown to depend significantly on the anomeric configuration. Interestingly, experimental data and theoretical calculations converge towards a more nucleophilic OH-4 in the β- than in the α-anomers.[28] Thus, taking advantage of the successful implementation 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[28-29] or rhamnogalacturonans,[29b, 30] we selected benzyl (allyl 2,3-di-*O*-benzyl-β-D-galacto-pyranosid)uronate[31] (**9**) as precursor to residue **A**. The latter was prepared from the 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).[32] Benzylation of acetal **6** and subsequent acid hydrolysis of the



Scheme 1. Synthesis of the **A** acceptor (**9**) and of the target **DA-Pr** disaccharide (**1**). *Reagents and conditions.* a) AllOH, $BF_3{\cdot}Et_2O$, DCM, 0 °C to rt, 24 h; b) NaOMe, MeOH, rt, 18 h; c) benzaldehyde dimethylacetal, CSA, CH_3CN, rt, 2 h, 54% over 3 steps; d) NaH, BnBr, DMF, 0 °C to rt, 2 h; e) 80% aq. AcOH, 80 °C, 2 h, 89% over 2 steps; f) TEMPO, BAIB, DCM/H_2O, rt, 1 h; g) $KHCO_3$, BnBr, DMF, rt, 16 h, 74% over 2 steps; h) TMSOTf, DCM, -40 °C to rt, 1 h, 91%; i) H_2, Pd/C 10%, EtOAc, rt, 16 h; j) NaOH, THF/H_2O, rt, 24 h, 51% over 2 steps.

benzylidene protecting group furnished the 4,6-diol **7** (89%) on a multigram scale.[33] Amongst the numerous well-established oxidation protocols envisioned for the conversion of diol **7** into uronic acid **8**, we favored the use of the 2,2,6,6-tetramethyl-1-piperidinyloxy/[bis(acetoxy)iodo]benzene (TEMPO/BAIB) system,[34] which was thought to be compatible with the allyl aglycon. To our satisfaction, treatment of diol **7** with a catalytic amount of TEMPO and excess BAIB in DCM/water (2:1) furnished the expected carboxylic acid **8**, which was smoothly converted into benzyl ester **9** upon reaction with benzyl bromide in the presence of potassium hydrogenocarbonate. 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[35] **10** using TMSOTf as catalyst (Scheme 1). The α-L-(1→4)-linked disaccharide **11** (NMR data for C-1_D: δ 99.7, $^{1}J_{CH}$ 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 sequential two-step deprotection route was designed so as to prevent any β-elimination at risk upon treatment of uronate intermediates in basic medium. Thus, Pd/C-mediated hydrogenolysis of the benzyl ether protecting groups was run first. The reaction used ethyl acetate, which solubilized uronic acid **12** resulting from concomitant cleavage of the benzyl ester in the fully protected **11**. Saponification of the acetyl protecting groups of the crude acid **12** provided the **DA**-Pr glycoside **1** in 51% isolated yield over two steps, following RP-HPLC purification. Satisfactorily, no side-product related to β-elimination was identified.

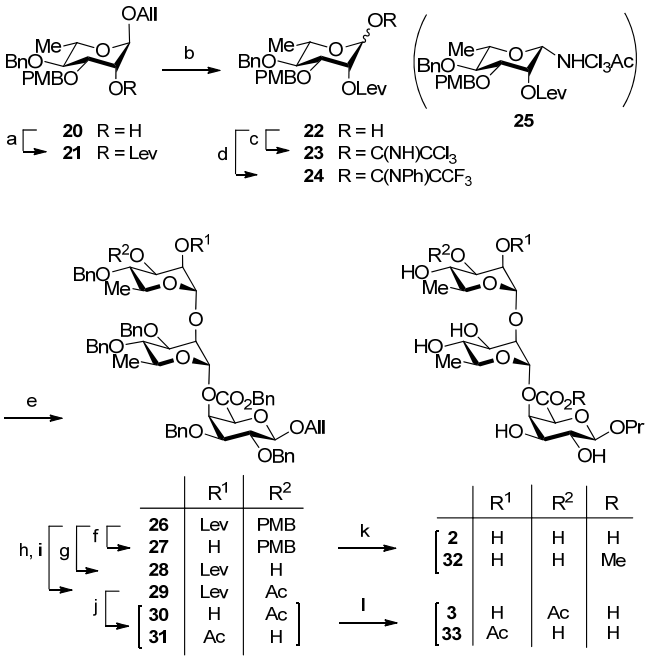


Scheme 2. Synthesis of the **DA** disaccharide acceptor **18**. *Reagents and conditions.* a) see ref 38, 94%; b) Ac_2O, pyridine, 0 °C to rt, 2 h, 96%; c) PTFACl, Cs_2CO_3, acetone, rt, 2 h, 95%; d) **9**, TMSOTf, see Table 2; e) $H_2NNH_2{\cdot}H_2O$, AcOH/Pyridine, 0 °C to rt, 1.5 h, 90%.

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

Entry	Donor (equiv.)	Solvent	T (°C)	**17**
1	**14** (1.3)	DCM	-40 → rt	70%
2	**14** (1.5)	DCM	-40 → rt	84%
3	**14** (1.2)	Toluene	-10 → rt	94%
4	**15** (1.5)	DCM	-10 → rt	89%
5	**16** (1.2)	Toluene	-10 → rt	96%

Synthesis of trisaccharides ***CDA****-Pr (**2**) and* $_{3Ac}$***CDA****-Pr (**3**).* Once ascertained that galacturonate **9** was an efficient acceptor even when considering disarmed donors, the next step consisted in identifying a suitable rhamnosyl donor, precursor to residue **D**, compatible with chain elongation at O-2. As concomitant $CO_2Bn \rightarrow CO_2Me$ transesterification was reported during acetyl removal under methanolysis conditions,[30a] we turned to donors bearing a levulinoyl ester at C-2 in view of its stereodirecting potency[36] and convenient selective removal in the presence of other esters, including benzyl uronates.[36-37] As a result, acceptor **9** was reacted with the known rhamnosyl trichloroacetimidate[38] **14** in DCM containing a catalytic amount of TMSOTf (Scheme 2). To our surprise, the reaction could not be completed even though 1.3 and up to 1.5 equiv. of donor were engaged (Table 2, Entries 1 and 2). This unexpected outcome was in part explained by donor rearrangement into the β-glycosylamide **19** (^{13}C NMR data for C-1: δ 78.4, $^1J_{CH}$ 154.3 Hz), which was isolated as a major side-product. The β-glycosidic linkage in **19** was ascertained from the corresponding NOESY 1D NMR data, indicative of spatial proximity between H-1 (5.32 ppm) and both H-3 (3.74 ppm) and H-5 (3.55 ppm). Although formation of the β-glycosylamide could not be avoided, changing DCM for toluene allowed reaction completion in the presence of only 1.2 equiv. of trichloroacetimidate (TCA) **14**. Diminishing the amount of donor reduced the amount of side-product, thus facilitating the purification to furnish 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 (^{13}C NMR data: $^1J_{CH}$ 172.7 Hz). In the search for improvement, which would avoid glycosylamide formation, we referred to former work by C. Vogel suggesting that β-D-galactopyranosiduronate acceptors could react at O-4 with numerous types of donors.[29b] On one hand, acetylation of the known hemiacetal[38] **13** gave donor **15** as a 4.5:1 mixture of α/β anomers (96%). On the other hand, the same hemiacetal was reacted with (*N*-phenyl)trifluoroacetimidoyl chloride in acetone containing excess cesium carbonate[39] to give the corresponding (*N*-phenyl)trifluoroacetimidate (PTFA) donor **16** as a 4:1 α/β mixture (95%). Both the acetate and PTFA donors - **15** and **16** - were reacted with acceptor **9** in the presence of catalytic TMSOTf (Scheme 3). In the former case, the condensation reached a nice 89% isolated yield of disaccharide **17**, which compared favorably with published data,[29b] when using 1.5 equiv. of donor **15** and 0.15 equiv of TMSOTf in DCM (Table 2, Entry 4). Under conditions optimized for the TCA donor **14** (Table 2, Entry 3), a rewarding 96% condensation yield was reached with the PTFA analogue **16** (Table 2, Entry 5). As already observed,[40] the use of donor **16** (1.2 equiv.), avoided the formation of any unwanted glycosylamide or other side-products hampering purification. Removal of the levulinoyl group by action of hydrazine hydrate in buffered medium gave the target acceptor **18** (90%).



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

Elaboration of the **C**-**D** glycosidic linkage was inspired from the above results. Hence, the 3-*O*-PMB analogues of donors **14** and **16**

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

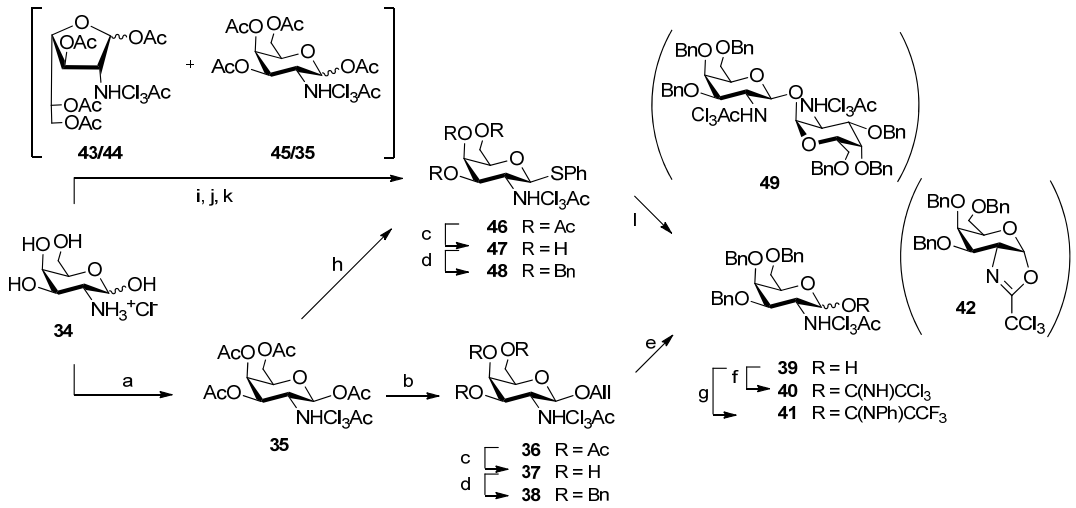
Entry	Donor (equiv.	Solvent	**26**
1	**23** (1.5)	DCE	84%
2	**23** (1.5)	Toluene	91%
3	**23** (1.2)	Toluene	92%
4	**24** (1.2)	Toluene	89%

– the novel 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*-*para*-methoxybenzyl-α-L-rhamnoside[41] (**20**) (Scheme 3). Thus, alcohol **20** was treated with levulinic acid in the presence of DCC and DMAP to give ester **21**, which was converted to hemiacetal **22** following a two-step

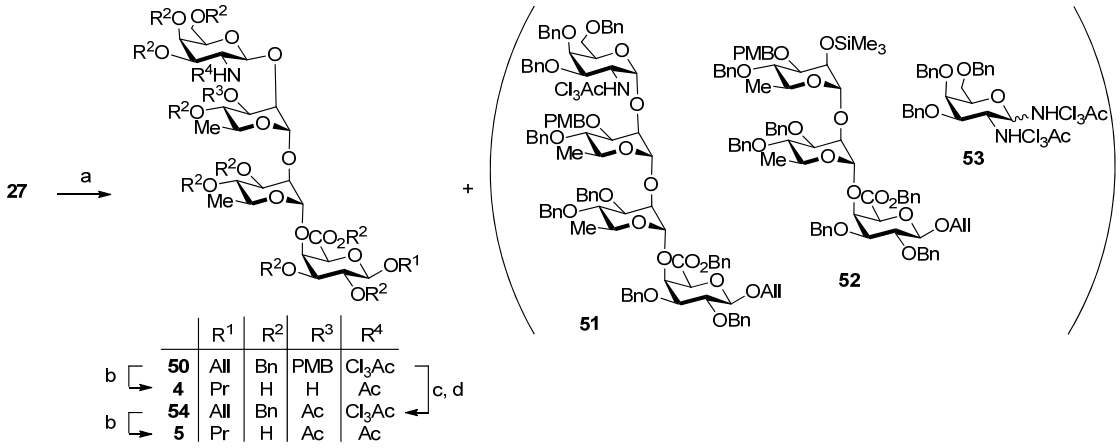
anomeric deallylation procedure involving the isomerisation of the allyl ether into the corresponding prop-1-enyl ether with a cationic iridium complex[42] and its subsequent iodine-mediated hydrolysis (90%).[43] The latter was either turned into TCA **23** (97 %) by reaction with trichloroacetonitrile in the presence of catalytic DBU or to the PTFA donor **24** (98%) under conditions similar to those used to prepare the corresponding 3,4-di-*O*-benzyl derivative **16**. The outcome of the TMSOTf-mediated [**C** + **DA**] assembly was similar to that of the [**D** + **A**] glycosylation. Briefly, trisaccharide **26** (NMR data for C-1_C: δ 98.9, $^1J_{CH}$ 172.5 Hz) was isolated in higher yield – 91% versus 84% – when rhamnosyl **C** (**23**, 1.5 equiv.) and the **DA** acceptor **18** were set to reaction in toluene rather than in a chlorinated solvent (Table 3, Entries 1 and 2). Fortunately, while the lesser proportion of rearranged β-glycosylamide **25** formed as a side-product facilitated purification, reducing the amount of donor **23** to 1.2 equiv. had no influence on the condensation yield (92%, Table 3, Entry 3). To our satisfaction, side-products were minimized and glycosylation at O-2_D of acceptor **18** remained high yielding (89%) when the 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 the condensation product **26** by reaction with hydrazine hydrate in pyridine/AcOH gave alcohol **27** (93%), serving either as an intermediate to the **CDA**-Pr target **2**, or as an acceptor in the synthesis of tetrasaccharides **4** and **5**. The Pd/C-mediated benzyl ether hydrogenolysis, benzyl ester cleavage and concomitant allyl reduction furnished the propyl glycoside **2** in a good 69% yield following RP-HPLC purification. This final deprotection step was run in methanol and it is worthy to note that despite the neutral conditions used, the methyl ester analog **32** was also isolated, albeit in low yield (5%). Added to an independent report of a similar outcome,[44] these observations encouraged the use of the THF/H_2O system as solvent for subsequent hydrogenolysis reactions. Alternatively, acetylation at O-3_C was a pre-requirement to the obtaining of the $_{3Ac}$**CDA**-Pr target **3**. Oxidative removal of the PMB ether was attempted by means of DDQ or CAN. When run in DCM/H_2O in the presence of DDQ (3.0 equiv.), the reaction was slow while degradation increased with time. Alcohol **28** was at best isolated in 45% yield. In contrast, treatment of the fully protected **26** with CAN (3.0 equiv.) in CH_3CN/H_2O led to a faster and cleaner conversion, furnishing 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 to form alcohol **28** only. Under the best conditions, CAN mediated removal of the 3_C-PMB ether and subsequent acetylation of the crude intermediate gave the 3_C-*O*-acetyl trisaccharide **29** in a rewarding 88% yield. The latter was submitted to conventional hydrazinolysis of the levulinoyl ester at O-2_C. Acetyl migration to the vicinal hydroxyl group could not be avoided and a 10:1 mixture of the OH-2_C and OH-3_C regiosiomers – **30** (NMR data for H-3_C: δ 4.96), and **31** (NMR data for H-2_C: δ 5.04), respectively – was isolated (93%). Pd/C-hydrogen mediated final deprotection of the mixture of the two alcohols in THF/H_2O yielded the corresponding mono-*O*-acetylated trisaccharides, $_{3Ac}$**CDA**-Pr (**3**, 78%) and $_{2Ac}$**CDA**-Pr (**33**, 2%) *O*-acetylated at 2_C, while the 4_C-*O*-acetyl isomer was not detected. This outcome suggested that acetyl migration did not occur under the neutral conditions used for hydrogenolysis.

Synthesis of tetrasaccharides ***BCDA****-Pr (****4****) and* ***B*$_{3Ac}$*CDA****-Pr (****5****).* Since chain elongation at residue **B** was not envisioned, an *N*-acetyl-D-galactosamine precursor acting as chain terminator, thus limiting protecting group manipulation, was preferred. In view of our work involving β-linked *N*-acetyl glucosamine residues,[45] a trichloroacetamide moiety was chosen to mask the acetamido function and ensure the required 1,2-*trans* stereoselectivity in glycosylation reactions. It was also hypothesized that Pd/C-mediated hydrodechlorination of the trichloroacetamide function at the final stage of the synthesis would permit full recovery of the acetamido moiety without perturbation of the 3_C-acetate.[38] Since the known 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyl trichloroacetimidate[46] did not meet orthogonality criteria, we turned to perbenzylated analogues in order to (i) facilitate glycosylation by use of an armed donor, (ii) avoid the remote α-stereodirecting effect attributed to esterification at O-4 of galactose and galactosamine donors,[47] 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[48] (**40**), or PTFA (**41**) were evaluated as precursors to residue **B**. Toward this aim, the β-pyranose tetraacetate **35** was prepared in four steps as described[46] in 43% overall yield on a multigram scale from glucosamine hydrochloride **34**. It was adequately turned into the β-allyl glycoside **36** (92%) when treated with allyl alcohol and stoichiometric TMSOTf in DCM (Scheme 4). Transesterification gave triol **37** and subsequent selective *O*-benzylation furnished the protected intermediate **38** (86%). Anomeric allyl cleavage proceeded smoothly to give the key hemiacetal **39** in high yield (93%). The latter is thus readily accessible (74%) in four steps from the pure β-tetracetate **35**. Treatment of hemiacetal **39** under standard conditions furnished, according to needs, either the known TCA donor **40** (94%) or its PTFA equivalent **41** (88%). The concomitant NMR-based identification of oxazoline **42** (5%) 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 **27** (Scheme 5). Glycosylation of acceptor **27** with trichloroacetimidate **40** was found problematic. When the reaction was run at -10 °C in toluene containing catalytic TMSOTf (Table 4, Entry 1), the product of α-glycosylation **51** identified by mass spectrometry analysis and NMR data for C-1_B (δ 96.0, $^1J_{CH}$ 175.2 Hz) was isolated in a meaningful 15% yield, in addition to the required β-linked tetrasaccharide **50** (NMR data for C-1_B: δ 100.7, $^1J_{CH}$ 162.4 Hz). Although the later was the major compound formed according to TLC analysis, it co-eluted with the glycosylamide side-product **53**, as suggested by mass spectrometry data and could not be isolated as a pure material. It is worth mentioning that mass spectrometry analysis also indicated formation of the silylated acceptor **52** under these conditions. Lowering the temperature to -78 °C improved selectivity remarkably since only traces of the unwanted α-linked condensation product remained (Table 4, Entry 2). However, the acceptor reactivity dropped tremendously. Recovery of the unconsumed acceptor was accompanied by the isolation of the rearranged donor. Changing toluene for DCM and TMSOTf for TBSOTf led to the same conclusion (Table 4, Entry 3), whereas the conversion was slow and degradation occurred if $BF_3.OEt_2$ was employed as a promoter in DCM (Table 4, Entry 4). When the best conditions identified for TCA **40** were applied to the PTFA donor **41**, rearrangement of the donor was not observed and the coupling was significantly improved (Table 4, Entry 5). The fully protected **BCDA** tetrasaccharide **50** was obtained in 63% yield as ascertained based on ^{13}C NMR data for C-1_B (δ 100.7, $^1J_{CH}$ 162.4 Hz). Yet, some unreacted acceptor remained despite the use of 1.5 equiv. of donor, while the selectivity was only moderate since the α isomer **51** was also formed (9%).



Scheme 4. Synthesis of the **B** donors (**40**, **41**). *Reagents and conditions.* a) see ref 46, 43% over 4 steps; b) AllOH, TMSOTf, DCM, rt, 14 h, 92%; c) NaOMe, MeOH, rt, 2 h; d) NaH, BnBr, DMF, -10 °C to 0 °C, 1.5 h, 86% for **38**, 77% for **48** over two steps; e) (i) $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+.PF_6^-$, H_2, THF, rt, 2 h; (ii) I_2, THF/H_2O, rt, 1 h, 93%; f) CCl_3CN, DBU, DCE, rt, 1 h, 94%; g) PTFACl, Cs_2CO_3, acetone, rt, 4 h, 88%; h) PhSH, $BF_3{\cdot}Et_2O$, DCM, rt, 2.5 h 89%; i) $(CCl_3CO)_2O$, NaOMe, MeOH, 0 °C, 2 h; j) Ac_2O, Pyridine, 0 °C to rt, 16 h; k) PhSH, $BF_3{\cdot}Et_2O$, DCM, rt, 16 h, 64% over 3 steps; l) NIS/TfOH, DCM/H_2O, 0 °C, 30 min, 77%.



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

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	- / 15%
2[a]	**40** (1.2)	TMSOTf	Toluene	-78	[b]
3[a]	**40** (1.2)	TBSOTf	DCM	-78	[c]
4	**40** (1.2)	$BF_3{\cdot}Et_2O$	DCM	-40	n.d.
5	**41** (1.5)	TMSOTf	DCM	-78	63% / 9%
6	**48** (1.2)	NIS/TMSOTf	DCM	-70 → -55	75% / -
7	**48** (1.5)	NIS/TMSOTf	DCM	-70 → -60	80% / -

[a] TLC analysis; [b] Good β/α ratio, poor conversion; [c] Excellent β/α ratio, poor conversion; n.d. = not determined.

As an attempt to overcome the poor outcome of the imidate-based [**B** + **CDA**] glycosylation, the thiophenyl β-glycoside **48** was investigated as an alternate donor. Treatment of the β-tetraacetate **35** with thiophenol and $BF_3.OEt_2$ gave thioglycoside[49] **46** (89%). Zemplén deacetylation of the later and subsequent benzylation of the resulting triol[50] **47** under controlled conditions gave the expected tribenzyl analog **48** (77%). Besides, NIS/TfOH-mediated hydrolysis of the thioglycosidic linkage in **48** provided another access to hemiacetal **39** (77%). Interestingly, under the conditions used (DCM/H_2O), the α-(1↔1)-β-linked disaccharide **49** (NMR data for H-1 and H-1': δ 5.30, $^1J_{1,2}$ 8.8 Hz, and δ 5.44, $^1J_{1,2}$ 3.8 Hz) issued from the condensation of hemiacetal **39** on thioglycoside **48** – was also isolated, albeit in minimal amount (3%). Even though this hydrolysis step may be improved, this route to hemiacetal **39** was found less efficient than that involving the allyl glycoside intermediate (53% versus 74% over four steps starting from tetraacetate **35**). The preparation of thioglycoside **46** from the crude material, issued 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. Thus, a mixture of α/β-furanose (**43**/**44**) and α/β-pyranose isomers (**45**/**35**), formed in a ratio of 7%/11% and 51%/31%, respectively (Scheme 4), 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 favored over the previous one (5 steps, 38%).

Glycosylation of the trisaccharide alcohol **27** with the thioglycoside donor **48** was attempted next (Scheme 5). In contrast to previous observations involving imidate donors **40** and **41**, when thioglycoside **48** was utilized in combination with the NIS/TMSOTf activator system at -70 → -55 °C in DCM, the pure β-condensation product **50** was isolated in a gratifying 75% yield (Table 4, Entry 6). While maintaining an excellent β-selectivity, use of a larger excess of donor **48** (1.5 versus 1.2 equiv., Table 4, Entry 7) resulted in a slight increase in yield (80% versus 75%). To our satisfaction, the product of α-glycosylation **51** was not detected under these conditions, while the silylated acceptor **52** was occasionally noticed as traces.

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

Conclusions

SF6 was recently identified as an essential serotype to include 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] Interestingly, the latter was recently introduced to differentiate between SF6 strains diverging in terms of O-acetylation ratio.[15] As a direct consequent, the SF6 O-Ag used in this study resembles more to that of *E. coli* O147 than anticipated. Whether this O-acetylation pattern has any influence on pathogenicity and/or antigenicity remains to be established. Towards this aim, 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 glycosylation steps were optimized to reach 80% yield for the formation of the **B**-**C** linkage and 92% and 96% for that of the **D**-**A** and **C**-**D** linkages, respectively. While thioglycoside **48** in DCM was the preferred precursor to residue **B** (80%), the combination of imidate donors and toluene was favored in the case of rhamnosyl donors **C** and **D**. The newly described PTFA donors **16** and **24** were employed advantageously to facilitate purification, without interfering with the yield of the condensation products **17** and **26**. The synthetic strategy was designed to perform *O*-acetylation at 3_C at an advanced stage, which provided an easy access to both the 3_C-OH and 3_C-OAc tri- (**2**, **3**) and tetrasaccharides (**4**, **5**), respectively.

Interestingly, ^{1}H NMR analyses of tetrasaccharides **4** and **5** indicate that a backbone conformational change may be present already within one RU-long O-Ag fragments since the anomeric proton of the aminosugar 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 > 0.2 ppm, which is observed for the C-1_D in the O-Ag of SF6 strain MDC 2924-71 (*vide infra*). This may be contrasted to several other *S. flexneri* O-Ags in which the polymer backbone conformation was essentially independent of substituents.[51]

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 overnight at 37 °C. Thereafter, bacteria were recovered by use of physiological water (2 mL per Petri dish). The resulting bacterial solution (about 400 mL) was centrifuged in glass tubes for 10 min at 7,000 rpm, followed by a washing step of the pellet with physiological water. The bacterial pellet was resuspended in acetone (250 mL) and centrifuged at 7,000 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, 5 g of powdered bacteria were resuspended in glass tubes containing distilled water heated at 68 °C (90 mL). A 68 °C-heated solution of 90% phenol 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 for 10 min on ice, the solution was centrifuged at 7,000 rpm, for 45 min at 4 °C. The aq upper layer was recovered and kept on ice. The phenol treatment was repeated once. The aq layers recovered from the 2 centrifugations were pooled (about 150 mL in total), and dialyzed for 3 days against tap water. The dialyzed solution kept in the dialysis tubing was then concentrated 6 times by use of Aquacide III (Calbiochem). The concentrated solution was recovered from the dialysis tubing, dialyzed overnight in distilled water, and then centrifuged at 5,000 rpm for 15 min. The supernatant was freeze-dried to give some 1.5 to 2 g of the crude LPS extract.

For LPS purification, the resulting freeze-dried crude extract was suspended at a concentration of 3% (w/v) in sterile distilled water and centrifuged at 4 °C, for 7 h at 25,400 rpm (Beckman SW41 rotor, about 80,000g). The pellet was resuspended in distilled water and centrifuged at 29,100 rpm for 3 h. This step was repeated once. The pellet was then resuspended in distilled water and centrifuged at 3,000 rpm for 10 min. The supernatant, which contained the purified LPS, was freeze-dried to give some 70-80 mg of a white powder. 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 acid hydrolysis in 1% AcOH, pH 3 (6 mL) at 100 °C for 90 min.[52] The lipid A was removed by centrifugation at 15000×*g* for 20 min at 4 °C. The clear supernatant was cooled to 0 °C and under stirring 1 M NaOH was added very slowly until pH 5. The solution was dialyzed against distilled water for three days, followed by purification by size exclusion chromatography on a HiLoad™ 16/60 Superdex™ 30 column (GE healthcare) using an ÄKTA™ purifier system (GE healthcare).

NMR experiments

^{1}H and ^{13}C NMR chemical shift assignments of the SF6 PS were performed in D_2O (1 mg in 0.18 mL, 3 mm NMR tube) at pD 6 and 17 °C on 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) and 1,4-dioxane in D_2O (δ_C 67.40).

^{1}H NMR spectra were recorded with 34k data points over a spectral width of 12 ppm, 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 using ^{1}H,^{1}H-TOCSY experiments[53] 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 using multiplicity-edited ^{1}H,^{13}C-HSQC experiments.[54] 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, employing the echo/antiecho method. Adiabatic pulses[55] 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 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 processed in magnitude mode.

A 2D ^{1}H,^{1}H-NOESY experiment with a zero-quantum suppression filter[56] 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 adiabatic smoothed CHIRP pulse was employed during the zero-quantum suppression, accompanied by a gradient pulse of strength 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.

The 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, 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 2.207 ppm and 16 ms for the signals at 2.086 and 2.042 ppm. Zero-filling to 65k data points and an exponential weighting function using a line-broadening factor of 3 Hz were applied prior to Fourier transformation.

Additionally, ^{1}H and ^{13}C NMR chemical shift assignments of the PS from *E. coli* O147[16] were performed in D_2O (5.5 mg in 0.55 mL) at pD 5 and 19 °C on 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 on a Bruker Avance III 600 MHz spectrometer equipped with an 5-mm inverse Z-gradient (55.7 G·cm^{-1}) TXI (^{1}H/^{13}C/^{15}N) probe. The assignments were performed 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 (DCM), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), methanol (MeOH), and pyridine – were delivered on molecular sieves and used as received. Additional solvents cited in the text are abbreviated as Chex (cyclohexane), EtOAc (ethyl acetate), and acetonitrile (MeCN), in addition to Acetone. Reactions requiring anhyd. conditions, were run under an Argon (Ar) atmosphere, using dried glassware. 4Å Molecular sieves (4Å MS) and 4Å acid washed molecular sieves (4Å 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 TLC aluminium foil plates. Compounds were visualized using UV_{254} and/or orcinol (1 mg·mL^{-1}) in 10% aq. H_2SO_4 with charring. Flash column chromatography was carried out using silica gel (Merck, particle size 40-63 µm, unless indicated otherwise). RP-HPLC was carried out using a Kromasil 5 µm C18 100 Å 10 × 250 mm semi-preparative column, eluting with a 0-20% linear gradient of CH_3CN in 0.08% aq. TFA over 20 min at a flow rate of 5.5 mL·min^{-1}. Analytical RP-HPLC analyses 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 0.01 N aq. TFA over 20 min at a flow rate of 0.3 mL·min^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 303 K on 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 ($CDCl_3$), dimethylsulfoxide (DMSO-d_6), 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. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), dd (doublet of doublet), q (quadruplet), qt (quintuplet), sex (sextuplet), dt (doublet of triplet), dq (doublet of quadruplet), ddd (doublet of doublet of doublet), 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 and coupling constants are reported in ppm (δ) relative to residual solvent peak ($CHCl_3$, DMSO, and DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) at 7.28/77.0, 2.50/39.5 and 0.00/0.00 ppm for the ^{1}H and ^{13}C spectra, respectively), and hertz (Hz), respectively. Of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a, and the one at higher field is denoted H-6b. Interchangeable assignments are marked with an asterisk. Sugar residues are lettered according to the lettering 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 on 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[33] (7): NaH (60% in mineral oil, 4.67 g, 116.8 mmol, 4.0 equiv.) was slowly added to a solution of diol **6**[32] (9.0 g, 29.2 mmol) in anhyd. DMF (102 mL) stirred at 0 °C. After stirring for 30 min at this temperature, 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. At this time, a TLC control (Chex/EtOAc 6:4) showed the total transformation of the starting material into a less polar product. The reaction was quenched by adding MeOH at 0 °C, and volatiles were evaporated. The residue was taken up in EtOAc, washed with water and brine, dried over anhyd. Na_2SO_4, filtered and concentrated. The crude material was suspended in 80% aq. AcOH (146 mL) and heated at 80 °C for 2 h, at which time observation by TLC (Chex/EtOAc 1:1) showed the conversion of the starting material to a major more polar product. 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. Analytical data were as published.[33]

Benzyl (allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate[31] (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 DCM/H_2O (2:1, 62 mL) at rt. After 1 h, observation by TLC (DCM/MeOH 9:1) showed the conversion of the starting material into the more polar acid **8**. The reaction was quenched by addition of a 10% aq. $NaHSO_3$ solution, diluted with DCM, and the layers were separated. The aq. phase was acidified with a 10% aq. HCl solution and re-extracted twice with DCM. The combined organic extracts were washed with brine, filtered over a phase separator and concentrated. The 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 and the reaction mixture was stirred under an Ar atmosphere for 16 h. At that time, observation by 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 volatiles were evaporated. The residue was taken up in EtOAc, washed with water and brine, dried over anhyd. Na_2SO_4, 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. Analytical data were as published.[31]

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[35] **10** (1.12 g, 2.58 mmol, 1.3 equiv.) and freshly activated 4Å AW 300 MS (1.5 g) in anhyd. DCM (19.8 mL) was stirred for 1 h at rt under an Ar atmosphere. The reaction mixture was cooled to -40 °C, stirred for 15 min, and TMSOTf (18 µL, 198 µmol, 0.05 equiv.) was added. Stirring went on at that temperature for 1 h, and the bath temperature was allowed to reach rt. A TLC control (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 **11** (1.41 g, 91 %) as a white foam. Disaccharide **11** had R_f = 0.16 (Chex/EtOAc 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44-7.24 (m, 15H, H_{Ar}), 5.98 (m, 1H, $CH{=}_{All}$), 5.52 (dd, $J_{1,2}$ = 1.8 Hz, 1H, $J_{2,3}$ = 3.3 Hz, 1H, H-2_D), 5.41-5.32 (m, 2H, H-3_D, $=CH_{2All}$), 5.32 (d, J = 12.2 Hz, 1H, H_{CO2Bn}), 5.22 (m, J_{cis} = 10.5 Hz, J_{gem} = 1.3 Hz, 1H, $=CH_{2All}$), 5.16 (d, 1H, H_{CO2Bn}), 5.04 (d, 1H, H-1_D), 5.03 (dd, $J_{3,4}$ = 10.0 Hz, $J_{4,5}$ = 9.8 Hz, 1H, H-4_D), 4.98 (d, J = 10.8 Hz, 1H, H_{Bn}), 4.80 (d, J = 10.8 Hz, 1H, H_{Bn}), 4.78 (d, J = 11.7 Hz, 1H, H_{Bn}), 4.67 (d, J = 11.7 Hz, 1H, H_{Bn}), 4.53 (m, 1H, H_{All}), 4.42 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1_A), 4.35 (bd, 1H, H-4_A), 4.18 (m, 1H, H_{All}), 4.07 (bs, 1H, H-5_A), 3.98 (dq, 1H, H-5_D), 3.88 (dd, $J_{2,3}$ = 9.6 Hz, 1H, H-2_A), 3.54 (dd, $J_{3,4}$ = 3.0 Hz, 1H, H-3_A), 2.07, 2.06, 1.99 (3s, 9H, H_{Ac}), 1.21 (d, $J_{5,6}$ = 6.3 Hz, 3H, H-6_D) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3, 169.7 (3C, CO_{Ac}), 167.3 (C-6_A), 138.4, 137.9, 135.0 (3C, C_{IVAr}), 133.9 ($CH{=}_{All}$), 128.7-127.7 (15C, C_{Ar}), 117.6 ($=CH_{2All}$), 102.6 (C-1_A, $^1J_{CH}$ = 154.0 Hz), 99.7 (C-1_D, $^1J_{CH}$ = 170.4 Hz), 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 (3C, CH_{3Ac}), 17.6 (C-6_D) ppm. HRMS (ESI$^+$): *m/z* 777.3004 (calcd for $C_{42}H_{49}O_{14}$ $[M+H]^+$: *m/z* 777.3123), *m/z* 799.2759 (calcd for $C_{42}H_{48}O_{14}Na$ $[M+Na]^+$: *m/z* 799.2942).

Propyl α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic acid (1): To a stirred solution of disaccharide **11** (218 mg, 0.28 mmol) in EtOAc (14 mL), was added 10% Pd/C (150 mg). The suspension was stirred under a hydrogen atmosphere 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 the intermediate uronic acid **12**. The reaction mixture was filtered and evaporated to dryness. The residue was dissolved in THF/H_2O (1:1, 8.4 mL), and treated with 1M aq. NaOH (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 2 steps) as a white solid following extensive freeze-drying. Disaccharide **1** had R_t = 7.6 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.06 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1_D), 4.37 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1_A), 4.33 (d, $J_{4,5}$ = 1.2 Hz, 1H, H-5_A), 4.28 (dd, $J_{3,4}$ = 3.0 Hz, 1H, H-4_A), 4.01 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-2_D), 3.82 (dt_{po}, J = 6.9 Hz, J = 9.9 Hz, 1H, OCH_{2Pr}), 3.78 (dd_{po}, $J_{2,3}$ = 10.0 Hz, 1H, H-3_A), 3.69 (dd, $J_{3,4}$ = 9.8 Hz, 1H, H-3_D), 3.57-3.49 (m, 2H, H-5_D, OCH_{2Pr}), 3.49 (dd, 1H, H-2_A), 3.31 (pt, $J_{4,5}$ = 9.7 Hz, 1H, H-4_D), 1.56 (sex, 2H, CH_{2Pr}), 1.16 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_D), 0.84 (t, 3H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 171.5 (C-6_A), 102.4 (C-1_A, $^1J_{CH}$ = 162.7 Hz), 101.4 (C-1_D, $^1J_{CH}$ = 172.1 Hz), 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$^+$): *m/z* 405.1366 (calcd for $C_{15}H_{26}O_{11}Na$ $[M+Na]^+$: *m/z* 405.1373).

1-*O*-Acetyl-3,4-di-*O*-benzyl-2-*O*-levulinoyl-α/β-L-rhamnopyranose (15): Hemiacetal **13**[38] (3.18 g, 7.19 mmol) and DMAP (88 mg, 719 µmol, 0.1 equiv.) were dissolved in dry pyridine under an Ar atmosphere 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 allowing the bath to reach rt. At that time, a TLC control (Chex/EtOAc 6:4) showed the transformation of the starting material into a mixture of α and β anomeric acetates. 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. The α isomer had R_f = 0.26 (Chex/EtOAc 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.29 (m, 10H, H_{Ar}), 6.02 (d, $J_{1,2}$ = 1.9 Hz, 1H, H-1), 5.36 (dd, $J_{2,3}$ = 3.4 Hz, 1H, H-2), 4.94 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.72 (d, J= 11.2 Hz, 1H, H_{Bn}), 4.66 (d, 1H, H_{Bn}), 4.56 (d, 1H, H_{Bn}), 3.94 (dd, $J_{3,4}$ = 9.4 Hz, 1H, H-3), 3.82 (dq, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-5), 3.48 (pt, 1H, H-4), 2.84-2.69 (m, 4H, CH_{2Lev}), 2.19 (s, 3H, CH_{3Lev}), 2.09 (s, 3H, CH_{3Ac}), 1.35 (d, 3H, H-6) 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 (2C, C_{IVAr}), 128.4-127.8 (10C, C_{Ar}), 91.0 (C-1), 79.5 (C-4), 77.6 (C-3), 75.5, 71.8 (2C, 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. The β isomer had R_f = 0.35 (Chex/EtOAc 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.29 (m, 10H, H_{Ar}), 5.74 (d, $J_{1,2}$ = 0.9 Hz, 1H, H-1), 5.61 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 4.94 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.72 (d, J= 11.2 Hz, 1H, H_{Bn}), 4.66 (d, 1H, H_{Bn}), 4.52 (d, 1H, H_{Bn}), 3.71 (dd, $J_{3,4}$ = 9.0 Hz, 1H, H-3), 3.53 (dq, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, 1H, H-5), 3.45 (pt, 1H, H-4), 2.84-2.69 (m, 4H, CH_{2Lev}), 2.20 (s, 3H, CH_{3Lev}), 2.12 (s, 3H, CH_{3Ac}), 1.39 (d, 3H, H-6) 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 (2C, C_{IVAr}), 128.4-127.8 (10C, 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$^+$): *m/z* 507.1999 (calcd for $C_{27}H_{32}O_8Na$ $[M+Na]^+$: *m/z* 507.1995).

3,4-Di-*O*-benzyl-2-*O*-levulinoyl-α/β-L-rhamnopyranosyl *N*-(phenyl)trifluoroacetimidate (16): Hemiacetal **13** (3.22 g, 7.3 mmol) was dissolved in acetone (24.7 mL). *N*-(phenyl)trifluoroacetimidoyl 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 for 2 h at rt. At this time, a TLC control (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. The α isomer had R_f = 0.50 (Chex/EtOAc 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.26 (m,

12H, H_{Ar}), 5.13 (m, 1H, H_{Ar}), 6.86-6.82 (m, 2H, H_{Ar}), 6.13 (bs, 1H, H-1), 5.48 (dd, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 4.94 (d, J = 10.8 Hz, 1H, H_{Bn}), 4.73 (d, J = 11.1 Hz, 1H, H_{Bn}), 4.66 (d, 1H, H_{Bn}), 4.60 (d, 1H, H_{Bn}), 3.99 (dd, $J_{3,4}$ = 9.3 Hz, 1H, H-3), 3.90 (dq, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-5), 3.51 (pt, 1H, H-4), 2.81-2.69 (m, 4H, CH_{2Lev}), 2.19 (s, 3H, CH_{3Lev}), 1.34 (d, 3H, H-6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, partial): δ = 206.1 (CO_{Lev}), 171.6 (CO_{2Lev}), 143.3, 138.1, 137.6 (3C, C_{IVAr}), 128.8-127.9 (13C, C_{Ar}), 119.4 (2C, C_{Ar}), 94.0 (C-1), 79.2 (C-4), 77.3 (C-3), 75.7, 72.1 (2C, 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 4Å AW 300 MS (450 mg) in anhyd. DCM (5.9 mL) was stirred for 1 h at rt under an Ar atmosphere. 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 allowing the cooling bath to reach rt. A TLC control (Tol/Acetone 9:1) showed the conversion of acceptor **9** to a less polar product. The reaction was quenched by addition of Et_3N, 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 colorless oil.

Route 2: A suspension of acceptor **9** (300 mg, 595 µmol), TCA **14**[38] (419 mg, 713 µmol, 1.2 equiv.) and freshly activated 4Å AW 300 MS (450 mg) in anhyd. toluene (5.9 mL) was stirred for 1 h at rt under an Ar atmosphere. 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 allowing the cooling bath to reach rt. A TLC control (Tol/Acetone 9:1) indicated the absence of acceptor **9** and the presence of a less polar product. The reaction was quenched by adding 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 colorless oil.

Route 3: A suspension of acceptor **9** (375 mg, 743 µmol), PTFA **16** (547 mg, 892 µmol, 1.2 equiv.), and freshly activated 4Å AW 300 MS (560 mg) in anhyd. toluene (7.4 mL) was stirred for 1 h at rt under an Ar atmosphere. 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 allowing the cooling bath to reach rt. A TLC control (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 colorless oil. Disaccharide **17** had: R_f = 0.47 (Tol/Acetone 9:1)**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41-7.23 (m, 25H, H_{Ar}), 5.99 (m, 1H, CH=$_{All}$), 5.55 (dd, $J_{1,2}$ = 2.1 Hz, $J_{2,3}$ = 3.3 Hz, 1H, H-2$_D$), 5.36 (m, J_{trans} = 17.2 Hz, J_{gem} = 1.5 Hz, 1H, =CH_{2All}), 5.28 (d, J = 12.3 Hz, 1H, H_{CO2Bn}) 5.24-5.20 (m, 2H, H-1$_D$, =CH_{2All}), 5.12 (d, 1H, H_{CO2Bn}), 4.94 (d, J = 11.0 Hz, 1H, H_{Bn}), 4.91 (d, J = 11.2 Hz, 1H, H_{Bn}), 4.79 (d, J = 11.0 Hz, 1H, H_{Bn}), 4.77 (d, J = 12.1 Hz, 1H, H_{Bn}), 4.74 (d, J = 12.1 Hz, 1H, H_{Bn}), 4.64 (d, J = 11.1 Hz, 1H, H_{Bn}), 4.61 (d, J = 11.2 Hz, 1H, H_{Bn}), 4.50 (m, 1H, H_{All}), 4.45 (d, J = 11.0 Hz, 1H, H_{Bn}), 4.43-4.39 (m, 2H, H-4$_A$, H-1$_A$), 4.17 (m, 1H, H_{All}), 4.05 (bs, 1 H, H-5$_A$), 3.88 (dd, $J_{3,4}$ = 9.4 Hz, 1H, H-3$_D$), 3.80-3.71 (m, 2H, H-2$_A$, H-5$_D$), 3.56 (dd, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 2.9 Hz, 1H, H-3$_A$), 3.37 (pt, $J_{4,5}$ = 9.4 Hz, 1H, H-4$_D$), 2.72-2.62 (m, 4H, CH_{2Lev}), 2.16 (s, 3H, CH_{3Lev}), 1.30 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_D$) 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 (5C, C_{IVAr}), 133.9 (CH=$_{All}$), 128.6-127.5 (25C, C_{Ar}), 117.6 (=CH_{2All}), 102.6 (C-1$_A$, $^1J_{CH}$ = 161.3 Hz), 98.9 (C-1$_D$, $^1J_{CH}$ = 172.7 Hz), 80.8 (C-3$_A$), 79.7 (C-4$_D$), 78.3 (C-2$_A$), 77.8 (C-3$_D$), 75.3, 75.1 (2C, C_{Bn}), 73.7 (C-4$_A$), 73.6 (C-5$_A$), 73.1, 71.7 (2C, C_{Bn}), 70.6 (CH_{2All}), 69.0 (C-2$_D$), 68.3 (C-5$_D$), 67.4 (C_{CO2Bn}), 38.1 ($COCH_{2Lev}$), 29.9 (CH_{3Lev}), 28.2 (CO_2CH_{2Lev}), 18.1 (C-6$_D$) ppm. HRMS (ESI$^+$): *m/z* 951.3846 (calcd for $C_{55}H_{60}O_{13}Na$ [M+Na]$^+$: *m/z* 951.3932).

***N*-Trichloroacetyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-β-L-rhamnopyranosylamine (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 4Å AW 300 MS (4.5 g) in anhyd. DCM (59.5 mL) was stirred for 1 h at rt under an Ar atmosphere. 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 allowing the cooling bath to reach rt. A TLC control (Tol/Acetone 9:1) indicated the absence of acceptor **9** and the presence of a less polar product. The reaction was quenched by adding 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 by order of elution glycosylamide **19** (530 mg) and disaccharide **17**, contaminated with trichloroacetamide. A second flash chromatography (DCM/EtOAc) afforded pure **17** (3.86 g, 70 %) as a colorless oil. Glycosylamide **19** had 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, 1H, NH), 7.39-7.29 (m, 10H, H_{Ar}), 5.59 (dd, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 5.32 (dd, 1H, H-1), 4.93 (d, J = 11.1 Hz, 1H, H_{Bn}), 4.74 (d, J = 11.4 Hz, 1H, H_{Bn}), 4.65 (d, 1H, H_{Bn}), 4.53 (d, 1H, H_{Bn}), 3.74 (dd, $J_{3,4}$ = 9.1 Hz, 1H, H-3), 3.55 (dq, $J_{4,5}$ = 9.3 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-5), 3.38 (pt, 1H, H-4), 2.85-2.64 (m, 4H, CH_{2Lev}), 2.19 (s, 3H, CH_{3Lev}), 1.37 (d, 3H, H-6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.8 (CO_{Lev}), 172.0 (CO_{2Lev}), 161.5 (NHCO), 138.2, 137.4 (2C, C_{IVAr}), 128.4-127.8 (10C, C_{Ar}), 92.1 (CCl_3), 80.2 (C-3), 79.2 (C-4), 78.4 (C-1, $^1J_{CH}$ = 154.3 Hz), 75.4 (C_{Bn}), 74.0 (C-5), 71.8 (C_{Bn}), 68.9 (C-2), 38.2 ($COCH_{2Lev}$), 29.6 (CH_{3Lev}), 28.2 (CO_2CH_{2Lev}), 18.1 (C-6) ppm. HRMS (ESI$^+$): *m/z* 608.0972 (calcd for $C_{27}H_{30}Cl_3NO_7Na$ [M+Na]$^+$: *m/z* 608.0986).

Benzyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate (18): To a solution of disaccharide **17** (1.25 g, 1.35 mmol) in dry pyridine (8.3 mL) stirred at 0 °C under an Ar atmosphere was slowly added acetic acid (5.6 mL) followed by hydrazine monohydrate (326 µL, 6.73 mmol, 5.0 equiv.). The reaction mixture was stirred at rt for 1.5 h. At this time, a TLC control (Chex/EtOAc 7:3) showed the total transformation of the starting material into a less polar product. Volatiles were evaporated and co-evaporated twice with toluene. The residue was taken up in DCM and washed with water. The aq. layer was re-extracted twice with DCM, and the combined organic phases 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 light yellow oil. Alcohol **18** had: R_f = 0.35 (Chex/EtOAc 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42-7.27 (m, 25H, H_{Ar}), 6.01 (m, 1H, CH=$_{All}$), 5.39 (d$_o$, $J_{1,2}$ = 1.7 Hz, 1H, H-1$_D$), 5.38 (m, J_{trans} = 17.2 Hz, J_{gem} = 1.6 Hz, 1H, =CH_{2All}), 5.32 (d, J = 12.2 Hz, 1H, H_{CO2Bn}), 5.25 (m, J_{cis} = 10.5 Hz, 1H, =CH_{2All}), 5.14 (d, 1H, H_{CO2Bn}), 4.96 (d, J = 11.0 Hz, 1H, H_{Bn}), 4.90 (d, J = 11.3 Hz, 1H, H_{Bn}), 4.80 (d$_{po}$, J = 11.9 Hz, 1H, H_{Bn}), 4.76 (d$_{po}$, J = 10.9 Hz, 1H, H_{Bn}), 4.75 (d$_{po}$, J = 11.9 Hz, 1H, H_{Bn}), 4.67-4.64 (m, 3H, H_{Bn}), 4.54 (m, 1H, H_{All}), 4.49 (dd, 1H, H-4$_A$), 4.43 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1$_A$), 4.23-4.16 (m, 2H, H-2$_D$ H_{All}), 4.08 (d, $J_{4,5}$ = 1.2 Hz, 1H, H-5$_A$), 3.89 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-3$_D$), 3.80-3.71 (m, 2H, H-2$_A$, H-5$_D$), 3.58 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.0 Hz, 1H, H-3$_A$), 3.47 (pt, $J_{4,5}$ = 9.3 Hz, 1H, H-4$_D$), 2.43 (bs, 1H, OH), 1.32 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_D$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3 (C-6$_A$), 138.8, 138.4, 138.2, 137.8, 135.1 (5C, C_{IVAr}), 134.0 (CH=$_{All}$), 128.6-127.5 (25C, C_{Ar}), 117.5 (=CH_{2All}), 102.7 (C-1$_A$, $^1J_{CH}$ = 159.4 Hz), 100.4 (C-1$_D$, $^1J_{CH}$ = 172.7 Hz), 81.2 (C-3$_A$), 79.9 (C-4$_D$), 79.6 (C-3$_D$), 78.4 (C-2$_A$), 75.2, 75.0 (2C, C_{Bn}), 73.7 (C-5$_A$), 73.5 (C-4$_A$), 73.3, 72.2 (2C, 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$^+$): *m/z* 853.3478 (calcd for $C_{50}H_{54}O_{11}Na$ [M+Na]$^+$: *m/z* 853.3564).

Allyl 4-*O*-benzyl-2-*O*-levulinoyl-3-*O*-*para*-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**[41] (15.8 g, 38.1 mmol) in anhyd. DCM (150 mL). The solution was stirred under an Ar atmosphere for 2 h. At that time, a TLC control (Tol/EtOAc 9:1) showed the conversion of the starting alcohol to a less polar product. The reaction mixture was filtered through a pad of Celite. The filtrate was diluted with DCM, and washed successively with a 10 % aq. HCl solution, a satd aq. $NaHCO_3$ solution 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. Rhamnoside **21** had R_f = 0.24 (Tol/EtOAc 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.24 (m, 7H, H_{Ar}), 6.88-6.84 (m, 2H, H_{ArPMB}), 5.89 (m, 1H, $CH=_{All}$), 5.39 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, 1H, H-2), 5.29 (m, J_{trans} = 17.2 Hz, J_{gem} = 1.7 Hz, 1H, $=CH_{2All}$), 5.21 (m, J_{cis} = 10.4 Hz, 1H, $=CH_{2All}$), 4.92 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.78 (d, 1H, H-1), 4.63 (d, J= 10.8 Hz, 1H, H_{Bn}), 4.62 (d, 1H, H_{Bn}), 4.46 (d, 1H, H_{Bn}), 4.16 (m, 1H, H_{All}), 4.02-3.94 (m, 2H, H-3, H_{All}), 3.78 (dq, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.3 Hz, 1H, H-5), 3.81 (s, 3H, CH_{3PMB}), 3.40 (pt, 1H, H-4), 2.82-2.67 (m, 4H, CH_{2Lev}), 2.19 (s, 3H, CH_{3Lev}), 1.33 (d, 3H, H-6) 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 (7C, C_{Ar}), 117.5 ($=CH_{2All}$), 113.7 (2C, 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 ($COCH_{2Lev}$), 29.8 (CH_{3Lev}), 28.2 (CO_2CH_{2Lev}), 18.0 (C-6) ppm. HRMS (ESI^+): *m/z* 535.2281 (calcd for $C_{29}H_{36}Cl_3O_8Na$ $[M+Na]^+$: *m/z* 535.2308).

4-*O*-Benzyl-2-*O*-levulinoyl-3-*O*-*para*-methoxybenzyl-α/β-L-rhamnopyranose (22): 1,5-Cyclooctadiene-bis(methyldiphenylphosphane)iridium hexafluorophosphate (658 mg, 0.78 mmol, 0.03 equiv.) was dissolved in anhyd. THF (130 mL) and hydrogen was bubbled through the solution for 15 min (H-cube, full H_2 mode). The resulting yellow solution was evaporated to dryness. The residue was taken up in anhyd. THF (130 mL) and poured to a solution of the allyl rhamnoside **21** (13.29 g, 25.9 mmol) in anhyd. THF (130 mL). The mixture was stirred under Ar at rt 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 for 1 h at rt. A TLC control (Chex/EtOAc 7:3) showed the conversion of the intermediate compound to a more polar product. The reaction was quenched with 10% aq. sodium bisulfite. The mixture was concentrated to 1/3 volume and the aq. phase was extracted three times with DCM. The organic layers were pooled, 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 ratio 3.5:1). The α isomer had R_f = 0.18 (Chex/EtOAc 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.24 (m, 7H, H_{Ar}), 6.87-6.83 (m, J= 8.7 Hz, 2H, H_{ArPMB}), 5.38 (dd, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 5.13 (d, 1H, H-1), 4.92 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.63 (d, J= 10.9 Hz, 1H, H_{Bn}), 4.62 (d, 1H, H_{Bn}), 4.46 (d, 1H, H_{Bn}), 4.03-3.95 (m, 2H, H-3, H-5), 3.81 (s, 3H, CH_{3PMB}), 3.40 (pt, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.5 Hz, 1H, H-4), 2.93-2.61 (m, 4H, CH_{2Lev}), 2.19 (s, 3H, CH_{3Lev}), 1.32 (d, $J_{5,6}$ = 6.3 Hz, 3H, H-6) 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 (7C, C_{Ar}), 113.8 (2C, C_{ArPMB}), 92.4 (C-1, $^1J_{CH}$ = 171.0 Hz), 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.

The β isomer had R_f = 0.18 (Chex/EtOAc 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.24 (m, 7H, H_{Ar}), 6.87-6.83 (m, J= 8.7 Hz, 2H, H_{ArPMB}), 5.53 (dd, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 4.78 (bs, 1H, H-1), 4.68 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.65-460 ($2d_o$, 2H, H_{Bn}), 4.45 (d, J = 10.9 Hz, 1H, H_{Bn}), 3.81 (s, 3H, CH_{3PMB}), 3.64 (dd, $J_{3,4}$ = 9.1 Hz, 1H, H-3), 3.42-3.38 (dq_o, 1H, H-5), 3.40 (pt, $J_{4,5}$ = 9.4 Hz, 1H, H-4), 2.82-2.67 (m, 4H, CH_{2Lev}), 2.21 (s, 3H, CH_{3Lev}), 1.37 (d, $J_{5,6}$ = 6.1 Hz, 3H, H-6) 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 (7C, C_{Ar}), 113.9 (2C, C_{ArPMB}), 93.1 (C-1, $^1J_{CH}$ = 158.2 Hz), 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^+): *m/z* 495.2004 (calcd for $C_{26}H_{32}O_8Na$ $[M+Na]^+$: *m/z* 495.1995).

4-*O*-Benzyl-2-*O*-levulinoyl-3-*O*-*para*-methoxybenzyl-α/β-L-rhamnopyranosyl trichloroacetimidate (23): Hemiacetal **22** (10.0 g, 21.16 mmol) was dissolved in anhyd. DCE (42 mL) and stirred under an Ar atmosphere. Trichloroacetonitrile (10.6 mL, 105.8 mmol, 5.0 equiv.) and DBU (0.95 mL, 6.3 mmol, 0.3 equiv.) were added at rt. After 20 min, a TLC control showed the conversion of the starting material into a less polar product. The reaction mixture was purified as such by flash chromatography (Chex/EtOAc 7:3 to 1:1 + 1% Et_3N) to give a 7.5:1 α/β mixture of TCA **23** (12.66 g, 97%) as a light yellow oil (α/β ratio 7.5:1). The α isomer had R_f = 0.46 (Chex/EtOAc 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.67 (s, 1H, NH), 7.41-7.26 (m, 7H, H_{Ar}), 6.89-6.84 (m, J= 8.7 Hz, 2H, H_{ArPMB}), 6.19 (d, $J_{1,2}$ = 1.9 Hz, 1H, H-1), 5.47 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 4.94 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.66 (d, J= 10.9 Hz, 1H, H_{Bn}), 4.65 (d, 1H, H_{Bn}), 4.51 (d, 1H, H_{Bn}), 3.98 (dd, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 3.94 (dq, $J_{4,5}$ = 9.4 Hz, 1H, H-5), 3.82 (s, 3H, CH_{3PMB}), 3.50 (pt, 1H, H-4), 2.84-2.72 (m, 4H, CH_{2Lev}), 2.22 (s, 3H, CH_{3Lev}), 1.36 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6) 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 (8C, 7C_{Ar}, $C_{IVArPMB}$),), 113.8 (2C, 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*-*para*-methoxybenzyl-2-*O*-levulinoyl-α/β-L-rhamnopyranosyl *N*-(phenyl)trifluoroacetimidate (24): Hemiacetal **22** (5.0 g, 10.58 mmol) was dissolved in acetone (36 mL). *N*-(phenyl)trifluoroacetimidoyl 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 for 4 h at rt. At that time, TLC (Chex/EtOAc 6:4) showed the total 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 light yellow oil. The α isomer had R_f = 0.48 (Chex/EtOAc 6:4). ^{1}H NMR ($CDCl_3$), δ 7.42-6.81 (m, 14H, H_{Ar}), 6.17 (bs, 1H, H-1), 5.46 (bs, 1H, H-2), 4.94 (d, 1H, J = 10.6 Hz, H_{Bn}), 4.70-4.62 (m, 2H, H_{Bn}), 4.53 (d, 1H, J = 10.6 Hz, H_{Bn}), 3.98 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.90 (dq, 1H, $J_{4,5}$ = 9.1 Hz, $J_{5,6}$ = 6.1 Hz, H-5), 3.83 (s, 3H, CH_{3PMB}), 3.49 (pt, 1H, H-4), 2.82-2.69 (m, 4H, CH_{2Lev}), 2.21 (s, 3H, CH_{3Lev}), 1.32 (d, 3H, H-6). ^{13}C NMR ($CDCl_3$, partial), δ 206.2 (CO_{Lev}), 171.8 (CO_{2Lev}), 159.4 ($C_{IVArPMB}$), 143.3, 138.1 (2C, C_{IVAr}), 130.0-119.4 (13C, 12C_{Ar}, $C_{IVArPMB}$), 113.8 (2C, 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).

***N*-Trichloroacetyl 4-*O*-benzyl-2-*O*-levulinoyl-3-*O*-*para*-methoxybenzyl-β-L-rhamnopyranosylamine (25):** A mixture of acceptor **18** (0.30 g, 361 µmol), TCA **23** (334 mg, 542 µmol, 1.5 equiv.) and freshly activated 4Å powdered MS (750 mg) in anhyd. toluene (10.8 mL) was stirred for 1 h at rt under an Ar atmosphere. 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. A TLC control (Chex/EtOAc 6:4) showed the conversion of acceptor **18** into a more polar product. The reaction was quenched by adding Et_3N. The resulting suspension was filtered and concentrated. The residue was purified by flash chromatography (Tol/EtOAc 95:5 to 85:15) to give by order of elution glycosylamide **25** (59 mg) followed by trisaccharide **26** (391 mg, 84 %) as

a light yellow oil. Glycosylamide **25** had ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (d, $J_{NH,1}$ = 8.8 Hz, 1H, NH), 7.41-7.18 (m, 7H, H_{Ar}), 6.88-6.84 (m, 2H, H_{ArPMB}), 5.57 (dd, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 3.2 Hz, 1H, H-2), 5.31 (dd, 1H, H-1), 4.91 (d, J = 11.1 Hz, 1H, H_{Bn}), 4.67 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.62 (d, 1H, H_{Bn}), 4.45 (d, 1H, H_{Bn}), 3.82 (s, 3H, CH_{3PMB}), 3.72 (dd, $J_{3,4}$ = 9.1 Hz, 1H, H-3), 3.54 (dq, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, 1H, H-5), 3.35 (pt, 1H, H-4), 2.92-2.63 (m, 4H, CH_{2Lev}), 2.21 (s, 3H, CH_{3Lev}), 1.37 (d, 3H, H-6) 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 (2C, C_{Ar}) 129.6 (C_{IVAr}), 128.4-127.6 (5C, C_{Ar}), 113.9 (2C, 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$^+$): *m/z* 638.1140 (calcd for $C_{28}H_{32}Cl_3NO_8Na$ [M+Na]$^+$: *m/z* 638.1091).

Benzyl (4-*O*-benzyl-3-*O*-*para*-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 4Å powdered MS (750 mg) in anhyd. toluene (10.8 mL) was stirred for 1 h at rt under an Ar atmosphere. 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. A TLC control (Chex/EtOAc 6:4) showed the conversion of acceptor **18** to a more polar product. The reaction was quenched by adding Et_3N. The resulting suspension was filtered and concentrated. The residue was purified by flash chromatography (Tol/EtOAc 95:5 to 85:15) to give trisaccharide **26** (426 mg, 91 %) as a light 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 4Å powdered MS (750 mg) in anhyd. toluene (10.8 mL) was stirred for 1 h at rt under an Ar atmosphere. 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. A TLC control (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 light yellow oil. Trisaccharide **26** had R_f = 0.32 (Chex/EtOAc 6:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43-7.17 (m, 32H, H_{Ar}), 6.86 (d, J = 8.5 Hz, 2H, H_{ArPMB}), 5.99 (m, 1H, CH=$_{All}$), 5.52 (dd, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.1 Hz, 1H, H-2$_C$), 5.36 (m, J_{trans} = 17.2 Hz, J_{gem} = 1.6 Hz, 1H, =CH_{2All}), 5.33 (bs$_o$, 1H, H-1$_D$), 5.27 (d, J = 12.2 Hz, 1H, H_{CO2Bn}), 5.22 (m, J_{cis} = 10.5 Hz, 1H, =CH_{2All}), 5.12 (d, 1H, H_{CO2Bn}), 4.94 (d$_{po}$, J = 11.1 Hz, 1H, H_{Bn}), 4.92-4.88 (m$_o$, 3H, H-1$_C$, 2H_{Bn}), 4.80 (d, J = 12.1 Hz, 1H, H_{Bn}), 4.75 (d, J = 12.1 Hz, 1H, H_{Bn}), 4.70-4.57 (m, 6H, H_{Bn}), 4.50 (m$_{po}$, 1H, H_{All}), 4.48 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.39 (m$_o$, 1H, H-4$_A$), 4.38 (d$_o$, $J_{1,2}$ = 7.8 Hz, 1H, H-1$_A$), 4.17 (m, 1H, H_{All}), 4.11 (pt, 1H, H-2$_D$), 4.02 (d, $J_{4,5}$ = 1.0 Hz, 1H, H-5$_A$), 3.94 (dd, $J_{3,4}$ = 9.3 Hz, 1H, H-3$_C$), 3.86 (dd$_{po}$, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 9.4 Hz, 1H, H-3$_D$), 3.82 (dq, 1H, H-5$_C$), 3.78 (s, 3H, CH_{3PMB}), 3.75-3.64 (m, 2H, H-2$_A$, H-5$_D$), 3.51 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.8 Hz, 1H, H-3$_A$), 3.42 (pt, $J_{4,5}$ = 9.5 Hz, 1H, H-4$_D$), 3.37 (pt, $J_{4,5}$ = 9.5 Hz, 1H, H-4$_C$), 2.77-2.66 (m, 4H, CH_{2Lev}), 2.18 (s, 3H, CH_{3Lev}), 1.28 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_D$), 1.20 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_C$) 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 (6C, C_{IVAr}), 134.0 (CH=$_{All}$), 130.3 (C_{IVAr}), 129.8-127.4 (32C, C_{Ar}), 117.4 (=CH_{2All}), 113.7 (2C, C_{ArPMB}), 102.7 (C-1$_A$, $^1J_{CH}$ = 159.4 Hz), 100.0 (C-1$_D$, $^1J_{CH}$ = 174.9 Hz), 99.2 (C-1$_C$, $^1J_{CH}$ = 172.5 Hz), 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 (2C, 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 (3C, 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$^+$): *m/z* 1307.5554 (calcd for $C_{76}H_{84}O_{18}Na$ [M+Na]$^+$: *m/z* 1307.5555).

Benzyl (4-*O*-benzyl-3-*O*-*para*-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 solution of the fully protected **26** (2.60 g, 2.0 mmol) in dry pyridine (16.8 mL) stirred at 0 °C under an Ar atmosphere. The reaction mixture was stirred at rt for 30 min. At that time, TLC (Tol/EtOAc 9:1) showed the total transformation of the starting material into a more polar product. Volatiles were evaporated and co-evaporated twice with toluene. The residue was taken up in DCM and washed with water. The aq. layer was re-extracted twice with DCM, and the combined organic phases 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. Alcohol **27** had R_f = 0.10 (Tol/EtOAc 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45-7.16 (m, 32H, H_{Ar}), 6.89 (d, J = 8.6 Hz, 2H, H_{ArPMB}), 6.00 (m, 1H, CH=$_{All}$), 5.37 (m, J_{trans} = 17.3 Hz, J_{gem} = 1.6 Hz, 1H, =CH_{2All}), 5.35 (bs$_o$, 1H, H-1$_D$), 5.28 (d, J = 12.1 Hz, 1H, H_{CO2Bn}), 5.23 (m, J_{cis} = 10.5 Hz, 1H, =CH_{2All}), 5.13 (d, 1H, H_{CO2Bn}), 5.02 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1$_C$), 4.94 (d$_{po}$, J = 10.9 Hz, 1H, H_{Bn}), 4.90 (d$_{po}$, J = 11.2 Hz, 1H, H_{Bn}), 4.89 (d$_{po}$, J = 10.9 Hz, 1H, H_{Bn}), 4.81 (d, J = 12.1 Hz, 1H, H_{Bn}), 4.75 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.70-4.59 (m, 7H, H_{Bn}), 4.51 (m, 1H, H_{All}), 4.40 (m$_o$, 1H, H-4$_A$), 4.39 (d$_o$, $J_{1,2}$ = 7.7 Hz, 1H, H-1$_A$), 4.17 (m$_{po}$, 1H, H_{All}), 4.15 (m$_o$, 1H, H-2$_D$), 4.12 (m, 1H, H-2$_C$), 4.03 (d, $J_{4,5}$ = 0.9 Hz, 1H, H-5$_A$), 3.91-3.86 (m, 2H, H-3$_C$, H-3$_D$), 3.84 (dq$_{po}$, 1H, H-5$_C$), 3.79 (s, 3H, CH_{3PMB}), 3.76-3.66 (m, 2H, H-2$_A$, H-5$_D$), 3.52 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.8 Hz, 1H, H-3$_A$), 3.45 (pt$_{po}$, $J_{4,5}$ = 9.5 Hz, 1H, H-4$_C$), 3.41 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4$_D$), 1.30 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_D$), 1.21 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_C$) 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 (6C, C_{IVAr}), 134.1 (CH=$_{All}$), 130.2 (C_{IVAr}), 129.5-127.3 (32C, C_{Ar}), 117.4 (=CH_{2All}), 114.0 (2C, C_{ArPMB}), 102.7 (C-1$_A$, $^1J_{CH}$ = 161.1 Hz), 100.8 (C-1$_D$, $^1J_{CH}$ = 169.0 Hz), 100.2 (C-1$_C$, $^1J_{CH}$ = 171.9 Hz), 80.5 (C-3$_A$), 80.2 (2C, C-4$_C$, C-4$_D$), 79.4 (2C, C-3$_C$, C-3$_D$), 78.4 (C-2$_A$), 75.3, 75.2 (3C, 2C$_{Bn}$, C-2$_D$), 74.9 (C_{Bn}), 73.7 (C-5$_A$), 73.6 (C-4$_A$), 72.7, 72.3, 71.7 (3C, 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$^+$): *m/z* 1210.5117 (calcd for $C_{71}H_{78}O_{16}Na$ [M+Na]$^+$: *m/z* 1210.5222), *m/z* 613.2374 (calcd for $C_{71}H_{79}O_{16}K$ [M+H+K]$^{2+}$: *m/z* 613.2502).

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 DCM (1.8 mL). The reaction mixture was stirred at rt for 3 h. At that time, a TLC control (Tol/EtOAc 8:2) showed the transformation of the starting material to a more polar product. The reaction was quenched with satd aq. $NaHCO_3$. The reaction mixture was diluted with water and DCM and the aq. phase was extracted three times with DCM. 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 rt for 1.5 h. At that time, a TLC control (Tol/EtOAc 8:2) showed the transformation of the starting material to a more polar product. The reaction was quenched with satd aq. $NaHCO_3$. The reaction mixture was diluted with water and DCM and the aq. phase was extracted three times with DCM. 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. Alcohol **28** had R_f = 0.21 (Tol/EtOAc 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41-7.19 (m, 30H, H_{Ar}),

6.00 (m, 1H, CH=$_{All}$), 5.40-5.33 (m, 3H, H-2$_C$, =CH$_{2All}$, H-1$_D$), 5.28 (d, J = 12.2 Hz, 1H, H$_{CO2Bn}$), 5.23 (m, J_{cis} = 10.5 Hz, J_{gem} = 1.3 Hz, 1H, =CH$_{2All}$), 5.12 (d, 1H, H$_{CO2Bn}$), 4.94 (d, J = 11.1 Hz, 1H, H$_{Bn}$), 4.92-4.87 (m, 3H, H-1$_C$, 2H$_{Bn}$), 4.81 (d, J = 12.1 Hz, 1H, H$_{Bn}$), 4.75 (d$_{po}$, J = 10.9 Hz, 1H, H$_{Bn}$), 4.70 (d, J = 11.2 Hz, 1H, H$_{Bn}$), 4.66 (d$_{po}$, J = 12.5 Hz, 1H, H$_{Bn}$), 4.63 (d$_{po}$, J = 11.2 Hz, 1H, H$_{Bn}$), 4.61 (bs$_o$, 2H, H$_{Bn}$), 4.51 (m, 1H, H$_{All}$), 4.41 (d$_o$, 1H, H-4$_A$), 4.38 (d$_o$, $J_{1,2}$ = 7.9 Hz, 1H, H-1$_A$), 4.22-4.13 (m, 2H, H$_{All}$, H-3$_C$), 4.11 (pt, 1H, H-2$_D$), 4.01 (s, 1H, H-5$_A$), 3.86 (dd$_{po}$, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3$_D$), 3.82 (dq$_{po}$, $J_{4,5}$ = 9.4 Hz, 1H, H-5$_C$), 3.74-3.64 (m, 2H, H-2$_A$, H-5$_D$), 3.53 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.7 Hz, 1H, H-3$_A$), 3.46 (pt, $J_{4,5}$ = 9.4 Hz, 1H, H-4$_D$), 3.31 (pt, $J_{3,4}$ = 9.4 Hz, 1H, H-4$_C$), 2.89-2.57 (m, 4H, CH$_{2Lev}$), 2.22 (s, 3H, CH$_{3Lev}$), 1.28 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_D$), 1.21 (d, $J_{5,6}$ = 6.3 Hz, 3H, H-6$_C$) 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 (6C, C$_{IVAr}$), 134.0 (CH=$_{All}$), 128.7-127.4 (30C, C$_{Ar}$), 117.6 (=CH$_{2All}$), 102.6 (C-1$_A$, $^1J_{CH}$ = 156.5 Hz), 99.8 (C-1$_D$, $^1J_{CH}$ = 172.4 Hz), 99.1 (C-1$_C$, $^1J_{CH}$ = 170.6 Hz), 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 (2C, C$_{Bn}$), 75.2 (2C, C-2$_D$, C$_{Bn}$), 73.6 (C-5$_A$), 73.0 (C-4$_A$), 72.9 (C-2$_C$), 72.7, 72.2 (2C, 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$^+$): *m/z* 1165.5172 (calcd for $C_{68}H_{77}O_{17}$ [M+H]$^+$: *m/z* 1165.5161); *m/z* 1187.5001 (calcd for $C_{68}H_{76}O_{17}Na$ [M+Na]$^+$: *m/z* 1187.4980).

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 rt for 30 min. At that time, a TLC control (Tol/EtOAc 8:2) showed the complete transformation of the starting material to alcohol **28**. The reaction was quenched with satd aq. $NaHCO_3$. The reaction mixture was diluted with water and DCM, and the aq. phase was extracted three times with DCM. 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 rt. After 2 h, a TLC control (Tol/EtOAc 8:2) indicated that the conversion of intermediate **28** to a less polar product was completed. 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. Acetate **29** had R_f = 0.41 (Tol/EtOAc 8:2). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.44-7.14 (m, 30H, H$_{Ar}$), 5.99 (m, 1H, CH=$_{All}$), 5.48 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, 1H, H-2$_C$), 5.40-5.33 (m, 3H, =CH$_{2All}$, H-1$_D$, H-3$_C$), 5.29 (d, J = 12.2 Hz, 1H, H$_{CO2Bn}$), 5.23 (m, J_{cis} = 10.5 Hz, J_{gem} = 1.5 Hz, 1H, =CH$_{2All}$), 5.12 (d, 1H, H$_{CO2Bn}$), 4.93 (d$_{po}$, J = 10.0 Hz, 1H, H$_{Bn}$), 4.91 (d, J = 10.2 Hz, 1H, H$_{Bn}$), 4.86 (d, 1H, H-1$_C$), 4.80 (d, J = 11.8 Hz, 1H, H$_{Bn}$), 4.74 (d, J = 10.6 Hz, 1H, H$_{Bn}$), 4.71-4.60 (m, 5H, H$_{Bn}$), 4.58 (d, J = 11.9 Hz, 1H, H$_{Bn}$), 4.51 (m, 1H, H$_{All}$), 4.44 (dd, 1H, H-4$_A$), 4.40 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1$_A$), 4.17 (m, 1H, H$_{All}$), 4.12 (pt, 1H, H-2$_D$), 4.04 (d, $J_{4,5}$ = 1.0 Hz, 1H, H-5$_A$), 3.88 (dq$_{po}$, $J_{4,5}$ = 9.5 Hz, 1H, H-5$_C$), 3.83 (dd, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 9.4 Hz, 1H, H-3$_D$), 3.72 (dd$_{po}$, $J_{2,3}$ = 9.8 Hz, 1H, H-2$_A$), 3.67 (dq$_{po}$, $J_{4,5}$ = 9.5 Hz, 1H, H-5$_D$), 3.56 (pt$_{po}$, 1H, H-4$_D$), 3.54 (dd$_{po}$, $J_{3,4}$ = 2.9 Hz, 1H, H-3$_A$), 3.45 (pt, 1H, H-4$_C$), 2.85-2.50 (m, 4H, CH$_{2Lev}$), 2.22 (s, 3H, CH$_{3Lev}$), 2.01 (s, 3H, CH$_{3Ac}$), 1.31 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_D$), 1.16 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_C$) 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 (6C, C$_{IVAr}$), 134.0 (CH=$_{All}$), 129.5-127.2 (30C, C$_{Ar}$), 117.4 (=CH$_{2All}$), 102.7 (C-1$_A$, $^1J_{CH}$ = 159.5 Hz), 99.6 (C-1$_D$, $^1J_{CH}$ = 175.8 Hz), 99.1 (C-1$_C$, $^1J_{CH}$ = 171.9 Hz), 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 (3C, 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 (COCH$_{2Lev}$), 29.9 (CH$_{3Lev}$), 28.0 (CO$_2$CH$_{2Lev}$), 20.9 (CH$_{3Ac}$), 18.0 (C-6$_D$), 17.8 (C-6$_C$) ppm. HRMS (ESI$^+$): *m/z* 1207.5286 (calcd for $C_{70}H_{79}O_{18}$ [M+H]$^+$: *m/z* 1207.5266); 1224.5576 (calcd for $C_{70}H_{82}NO_{18}$ [M+NH$_4$]$^+$: *m/z* 1224.5532); 1229.5131 (calcd for $C_{70}H_{78}O_{18}Na$ [M+Na]$^+$: *m/z* 1229.5085).

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) followed by hydrazine monohydrate (74 µL, 1.53 mmol, 5.0 equiv.) were slowly added to a solution of **29** (0.37 g, 0.31 mmol) in dry pyridine (5.1 mL) stirred at 0 °C under an Ar atmosphere. The reaction mixture was stirred at rt for 1.5 h. At that time, TLC (Tol/EtOAc 8:2) showed the total transformation of the starting material into a more polar product. Following addition of DCM and water, the two phases were separated and the aq. one was re-extracted with DCM. 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 the 2$_C$-acetate **31**, as a white foam. Alcohol **30** had Rf = 0.38 (Tol/EtOAc 8:2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.38-7.12 (m, 30H, H$_{Ar}$), 5.94 (m, 1H, CH=$_{All}$), 5.35 (d$_o$, $J_{OH,2}$ = 5.2 Hz, 1H, OH-2$_C$), 5.34 (m$_{po}$, J_{gem} = 1.5 Hz, 1H, =CH$_{2All}$), 5.22 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1$_D$), 5.20-5.15 (m, 2H, H$_{CO2Bn}$, =CH$_{2All}$), 4.99 (d, J = 12.2 Hz, 1H, H$_{CO2Bn}$), 4.96 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.7 Hz, 1H, H-3$_C$), 4.86 (d, $J_{1,2}$ = 1.2 Hz, 1H, H-1$_C$), 4.82-4.77 (m, 2H, H$_{Bn}$), 4.73 (d, J = 12.3 Hz, 1H, H$_{Bn}$), 4.68-4.55 (m, 6H, H$_{Bn}$), 4.49 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1$_A$), 4.47 (d, J = 11.9 Hz, 1H, H$_{Bn}$), 4.45 (bs, 1H, H-5$_A$), 4.40 (dd, 1H, H-4$_A$), 4.33 (m, 1H, H$_{All}$), 4.13-4.06 (m, 2H, H$_{All}$, H-2$_D$), 3.96 (m, 1H, H-2$_C$), 3.75 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.7 Hz, 1H, H-3$_A$), 3.73-3.66 (m, 2H, H-5$_C$, H-3$_D$), 3.57-3.50 (m, 2H, H-4$_C$, H-5$_D$), 3.45 (dd, 1H, H-2$_A$), 3.39 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, 1H, H-4$_D$), 2.04 (s, 3H, CH$_{3Ac}$), 1.15 (d, $J_{5,6}$ = 6.1 Hz, 3H, H-6$_D$), 1.11 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_C$) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.4 (CO$_{Ac}$), 167.9 (C-6$_A$), 139.0, 138.9, 138.8, 138.7, 135.1 (6C, C$_{IVAr}$), 134.9 (CH=$_{All}$), 129.4-127.8 (30C, 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 (3C, 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 (2C, 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$^+$): *m/z* 1131.4695 (calcd for $C_{65}H_{72}O_{16}Na$ [M+Na]$^+$: *m/z* 1131.4718).

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-galactopyrosid)uronate (32): To a stirred solution of the trisaccharide **27** (100 mg, 84 µmol) in MeOH (5.1 mL), was added 10% Pd/C (100 mg). The suspension was stirred under a hydrogen atmosphere for a day. After this time, MS analysis indicated a molecular weight corresponding to that of the target trisaccharide. The reaction mixture was filtered and the filtrate was rinsed with water. Evaporation of the volatiles, freeze-drying and purification of the crude material by preparative RP-HPLC gave by order of elution trisaccharide **2** (30.8 mg, 69%) and the corresponding methyl ester **32** (2.1 mg, 5%) both as a white solids following repeated freeze-drying. Compound **2** had R_t = 8.5 min. ^{1}H NMR (400 MHz, D$_2$O): δ = 5.24 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1$_D$), 4.88 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1$_C$), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1$_A$), 4.34 (d, $J_{4,5}$ = 1.2 Hz, 1H, H-5$_A$), 4.27 (dd, $J_{3,4}$ = 3.0 Hz, 1H, H-4$_A$), 4.01 (dd, $J_{2,3}$ = 3.2 Hz, 1H, H-2$_D$), 3.98 (dd, $J_{2,3}$ = 3.4 Hz, 1H, H-2$_C$), 3.86-3.75 (m, 3H, H-3$_A$, H-3$_D$, OCH$_{2Pr}$), 3.70 (dd, $J_{3,4}$ = 9.8 Hz, 1H, H-3$_C$), 3.62 (dq, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-5$_C$), 3.57-3.51 (m, 2H, H-5$_D$, OCH$_{2Pr}$), 3.50 (dd, $J_{2,3}$ = 10.0 Hz, 1H, H-2$_A$), 3.36 (2pt$_o$, 2H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4$_D$, H-4$_C$), 1.57 (sex, J = 7.3 Hz, 2H, CH$_{2Pr}$), 1.18 (d, 3H, H-6$_C$), 1.17 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6$_D$), 0.85 (t, 3H, CH$_{3Pr}$) ppm. ^{13}C NMR (100 MHz, D$_2$O): δ = 174.2 (C-6$_A$), 104.9 (C-1$_A$, $^1J_{CH}$ = 163.4 Hz), 104.7 (C-1$_C$, $^1J_{CH}$ = 171.2 Hz), 102.6 (C-1$_D$, $^1J_{CH}$ = 174.0 Hz), 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 (2C, C-4$_D$, C-4$_C$), 72.8 (C-2$_A$),

72.6 (2C, 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 (2C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI^+): *m/z* 551.1940 (calcd for $C_{21}H_{36}O_{15}Na$ $[M+Na]^+$: *m/z* 551.1952).

Methyl ester **32** had: ^1H NMR (400 MHz, D_2O): δ = 5.18 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1_D), 4.85 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1_C), 4.37 (d, $J_{4,5}$ = 0.7 Hz, 1H, H-5_A), 4.34 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1_A), 4.28 (dd, $J_{3,4}$ = 2.8 Hz, 1H, H-4_A), 3.97 (dd, $J_{2,3}$ = 3.0 Hz, 1H, H-2_D), 3.93 (dd, 1H, H-2_C), 3.79 (dt, J = 6.8 Hz, J = 9.8 Hz, 1H, OCH_{2Pr}), 3.76-3.72 (m, 2H, H-3_D, H-3_A), 3.72 (s_o, 3H, CH_{3Me}), 3.66 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.8 Hz, 1H, H-3_C), 3.59 (dq, $J_{3,4}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-5_C), 3.50 (dt, J = 6.8 Hz, 1H, OCH_{2Pr}), 3.45 (dd, $J_{2,3}$ = 9.9 Hz, 1H, H-2_A), 3.34 (m_o, 1H, H-5_D), 3.32 ($2t_o$, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 2H, H-4_C, H-4_D), 1.53 (sex, J = 7.1 Hz, 2H, CH_{2Pr}), 1.15 (d, $J_{5,6}$ = 6.0 Hz, 6H, H-6_C, H-6_D), 0.81 (t, J = 7.4 Hz, 3H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 172.8 (C-6_A), 105.1 (C-1_A, $^1J_{CH}$ = 162.3 Hz), 104.6 (C-1_C, $^1J_{CH}$ = 171.5 Hz), 102.8 (C-1_D, $^1J_{CH}$ = 173.7 Hz), 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 (2C, C-4_C, C-4_D), 72.8 (C-2_A), 72.6 (2C, 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 (2C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI^+): *m/z* 565.2087 (calcd for $C_{22}H_{38}O_{15}Na$ $[M+Na]^+$: *m/z* 565.2108).

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): 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) was added 10% Pd/C (150 mg). The suspension was stirred under a hydrogen atmosphere 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 by order of elution first trisaccharide **3** (67.8 mg, 78%) and then regioisomer **33** (1.8 mg, 2%), both as a white foams following repeated freeze-drying. Compound **3** had R_t = 9.0 min. ^1H NMR (400 MHz, D_2O): δ = 5.29 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1_D), 4.90 (dd_o, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.9 Hz, 1H, H-3_C), 4.90 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1_C), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1_A), 4.35 (d, $J_{4,5}$ = 1.2 Hz, 1H, H-5_A), 4.28 (dd, $J_{3,4}$ = 2.8 Hz, 1H, H-4_A), 4.12 (dd, 1H, H-2_C), 4.03 (dd, $J_{2,3}$ = 3.1 Hz, 1H, H-2_D), 3.83 (dt_{po}, J = 6.8 Hz, J = 9.8 Hz, 1H, OCH_{2Pr}), 3.81-3.76 (m, 2H, H-3_A, H-3_D), 3.74 (dq, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-5_C), 3.59-3.52 (m, 3H, H-4_C, H-5_D, OCH_{2Pr}), 3.50 (dd, $J_{2,3}$ = 10.0 Hz, 1H, H-2_A), 3.41 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4_D), 2.09 (s, 3H, CH_{3Ac}), 1.56 (sex, 2H, CH_{2Pr}), 1.21 (d, 3H, H-6_C), 1.18 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_D), 0.84 (t, 3H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O); δ = 176.2 (CO_{Ac}), 174.1 (C-6_A), 104.9 (C-1_A, $^1J_{CH}$ = 162.9 Hz), 104.3 (C-1_C, $^1J_{CH}$ = 172.3 Hz), 102.5 (C-1_D, $^1J_{CH}$ = 173.4 Hz), 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 (2C, 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 (2C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI^+): *m/z* 593.2044 (calcd for $C_{23}H_{38}O_{16}Na$ $[M+Na]^+$: *m/z* 593.2057).

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

Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (36): A mixture of the β-acetate **35**[46] (2.83 g, 5.74 mmol), allyl alcohol (0.78 mL, 11.5 mmol, 2.0 equiv.) and freshly activated 4Å powdered MS (2.8 g) in anhyd. DCM (57 mL) was stirred for 1 h at rt under an Ar atmosphere. TMSOTf (1.10 mL, 5.74 mmol, 1.0 equiv.) was added at rt, and stirring went on for 14 h at this temperature. A TLC control (Tol/MeCN 7:3) showed the total conversion of donor **35** into a very slightly less polar new 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. Allyl glycoside **36** had R_f = 0.42 (Tol/MeCN 7:3). ^1H NMR (400 MHz, $CDCl_3$): δ = 6.69 (d, $J_{NH,2}$ = 8.6 Hz, 1H, NH), 5.87 (m, 1H, CH=$_{All}$), 5.42 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.0 Hz, 1H, H-4), 5.36 (dd, $J_{2,3}$ = 11.3 Hz, 1H, H-3), 5.31 (m, J_{trans} = 17.3 Hz, J_{gem} = 1.5 Hz, 1H, =CH_{2All}), 5.23 (m, J_{cis} = 10.4 Hz, 1H, =CH_{2All}), 4.78 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1), 4.39 (m, 1H, H_{All}), 4.22 (dd, $J_{5,6a}$ = 6.7 Hz, $J_{6a,6b}$ = 11.3 Hz, 1H, H-6a), 4.13 (dd_{po}, $J_{5,6b}$ = 6.8 Hz, 1H, H-6b), 4.16-4.08 (m, 2H, H_{All}, H-2), 3.96 (pdt, 1H, H-5), 2.19, 2.08, 2.02 (3s, 9H, CH_{3Ac}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.1 (3C, 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 (3C, CH_{3Ac}) ppm. HRMS (ESI^+): m/z 512.0239 (calcd for $C_{17}H_{22}Cl_3NO_9Na$ $[M+Na]^+$: m/z 512.0258).

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 anhyd. 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 rt for 2 h. A TLC control (Chex/EtOAc 6:4) showed the conversion of the starting material into a more polar product. The reaction was quenched with Dowex-H^+ resin, filtered and evaporated, to give quantitatively the intermediate triol **37** as a white solid. The crude material was dissolved in anhyd. 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 an Ar atmosphere while keeping the temperature below 0 °C. After 1.5 h, follow up by 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 layer was dried over anhyd. Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (Tol/EtOAc 95:5 to 9:1) to give the fully protected **38** (2.24 g, 86%) as a white solid. Compound **38** had R_f = 0.44 (Tol/EtOAc 9:1). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.41-6.26 (m, 15H, H_{Ar}), 6.92 (d, $J_{NH,2}$ = 7.0 Hz, NH), 5.88 (m, 1H, CH=$_{All}$), 5.27 (m, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.6 Hz, =CH_{2All}), 5.18 (m, 1H, J_{cis} = 10.4 Hz, 1H, =CH_{2All}), 4.99 (d, $J_{1,2}$ = 8.1 Hz, 1H, H-1), 4.92 (d, J = 11.6 Hz, 1H, H_{Bn}), 4.68 (d, J = 11.4 Hz, 1H, H_{Bn}), 4.62 (d, 1H, H_{Bn}), 4.56 (d, 1H, H_{Bn}), 4.52 (d, J = 11.9 Hz, 1H, H_{Bn}), 4.47 (d, 1H, H_{Bn}), 4.35 (m_o, 1H, H_{All}), 4.32 (dd, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 2.8 Hz, 1H, H-3), 4.09 (m, 1H, H_{All}), 4.04 (d, 1H, H-4), 3.85 (ddd, 1H, H-2), 3.72-3.62 (m, 3H, H-5, H-6a, H-6b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.9 (NHCO), 138.4, 137.9, 137.5 (3C, C_{IVAr}), 133.7 (CH=$_{All}$), 126.6-127.6 (15C, C_{Ar}), 117.8 (=CH_{2All}), 98.2 (C-1, $^1J_{CH}$ = 162.9 Hz), 92.6 (CCl_3), 77.2 (C-3), 74.7, 73.6 (2C, 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^+): *m/z* 656.1321 (calcd for $C_{32}H_{34}Cl_3NO_6Na$ $[M+Na]^+$: *m/z* 656.1349).

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 anhyd. THF (17 mL) and hydrogen was bubbled through the solution for 15 min (H-cube, full H_2 mode). The resulting yellow solution was evaporated to dryness. The residue was taken up in anhyd. THF (17 mL) and poured to a solution of allyl glycoside **38** (2.14 g, 3.36 mmol) in anhyd. THF (17 mL). The mixture was stirred under Ar at rt for 2 h. Follow up by 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 for 1 h at rt. A TLC control (Tol/EtOAc 9:1) showed the conversion of the intermediate compound to a more polar product. The reaction was quenched with 10% aq. sodium bisulfite. The mixture was concentrated to 1/3 volume and the aq. phase was extracted three times with DCM. The organic layers were pooled, 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 DCM (22 mL) and water (2.2 mL). The reaction mixture was stirred at that temperature for 0.5 h. Follow up by TLC (Chex/EtOAc 7:3) showed the conversion of the starting material into a less polar intermediate. The reaction was quenched by adding 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, by order of elution, first the (α1→β1)-linked disaccharide **49** (84 mg, 3%) as a yellow oil, and then the target hemiacetal **39** (998 mg, 77%), as a 9:1 α/β mixture isolated as a white solid. The α hemiacetal **39** had R_f = 0.23 (Tol/EtOAc 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40-6.25 (m, 15H, H_{Ar}), 6.81 (d, $J_{NH,2}$ = 8.9 Hz, 1H, NH), 5.38 (pt, $J_{OH,1} = J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.97 (d, J = 11.6 Hz, 1H, H_{Bn}), 4.72 (d, J = 11.9 Hz, 1H, H_{Bn}), 4.65 (m, 1H, H-2), 4.57 (d, 1H, H_{Bn}), 4.56 (d, 1H, H_{Bn}), 4.53 (d, J = 12.2 Hz, 1H, H_{Bn}), 4.45 (d, 1H, H_{Bn}), 4.15 (pt, 1H, H-5), 3.98 (bd, 1H, H-4), 3.80 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 2.5 Hz, 1H, H-3), 3.67 (dd, $J_{OH,2}$ = 1.6 Hz, 1H, OH-1), 3.62 (dd, $J_{5,6a}$ = 6.8 Hz, $J_{6a,6b}$ = 9.6 Hz, 1H, H-6a), 3.50 (dd, $J_{5,6b}$ = 5.8 Hz, 1H, H-6b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.7 (NHCO), 138.1, 137.5 (3C, C_{IVAr}), 128.6-127.5 (15C, C_{Ar}), 92.7 (CCl_3), 91.6 (C-1), 76.8 (C-3), 74.4, 73.6 (2C, 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$^+$): *m/z* 616.1080 (calcd for $C_{29}H_{30}Cl_3NO_6Na$ [M+Na]$^+$: *m/z* 616.1036).

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

3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranosyl trichloroacetimidate[48] (40): 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 anhyd. DCE (8.3 mL). The mixture was stirred for 1 h at rt under an Ar atmosphere. Follow up by TLC (Chex/EtOAc 8:2) indicated the total conversion of the starting hemiacetal into a less polar product. Following concentration to 1/3 volume (reduced pressure, rt), 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 light yellow oil. Analytical data were as published.[48]

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*-(phenyl)trifluoroacetimidoyl 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 for 4 h at rt. At that time, a TLC control (Chex/EtOAc 8:2) indicated the total transformation of the starting material to a less polar product. The reaction mixture was filtered and concentrated. The residue was purified by flash chromatography (Chex/EtOAc 85:15 to 7:3 + 1% Et_3N) to give, by order of elution, first oxazoline **42** (83 mg, 5%) and second PTFA **41** (2.10 g, 88%), both as light yellow oils.

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

Oxazoline **42** had R_f = 0.46 (Chex/EtOAc 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46-7.27 (m, 15H, H_{Ar}), 6.28 (d, $J_{1,2}$ = 6.6 Hz, 1H, H-1), 4.95 (d, J = 11.6 Hz, 1H, H_{Bn}), 4.92 (d, J = 12.2 Hz, 1H, H_{Bn}), 4.77 (d, 1H, H_{Bn}), 4.64 (d, 1H, H_{Bn}), 4.51 (d, J = 11.8 Hz, 1H, H_{Bn}), 4.64 (d, 1H, H_{Bn}), 4.40 (pt, 1H, H-2), 4.07 (dt, $J_{4,5}$ = 1.7 Hz, $J_{5,6a} = J_{5,6b}$ = 6.5 Hz, 1H, H-5), 3.98 (pt, 1H, H-4), 3.66 (d, 2H, H-6a, H-6b), 3.54 (dd, $J_{2,3}$ = 7.5 Hz, $J_{3,4}$ = 2.6 Hz, 1H, H-3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.2 (NCO), 138.2, 137.9, 137.7 (3C, C_{IVAr}), 128.4-127.7 (15C, 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 (2C, C_{Bn}), 71.6 (C-4), 68.2 (C-6), 67.3 (C-2) ppm. HRMS (ESI$^+$): *m/z* 598.0910 (calcd for $C_{29}H_{28}Cl_3NO_5Na$ [M+Na]$^+$: *m/z* 598.0931).

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside[49] (46): *Route 1:* Thiophenol (3.2 mL, 31.3 mmol, 2 equiv.) and $BF_3.OEt_2$ (12.0 mL, 93.9 mmol, 6 equiv.) were added at rt to a solution of β acetate **35** (7.71 g, 15.6 mmol) in anhyd. DCM (150 mL). The mixture was stirred at this temperature for 2.5 h under an Ar atmosphere. A TLC control (Chex/EtOAc 7:3) showed the total conversion of the starting material into a less polar product. The reaction was quenched with satd. aq. $NaHCO_3$. DCM was added, the layers were separated and 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 anhyd. MeOH (482 mL) stirred at 0 °C under an Ar atmosphere. 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 a TLC control

(iPrOH/H_2O/NH_3 4:1:0.5) showed the complete conversion of the starting material to a less polar product. The reaction was quenched with Dowex-H^+ resin, the resin was filtered and volatiles were evaporated to dryness. The residue was dissolved in anhyd. pyridine (224 mL) and acetic anhydride (100 mL) was slowly added over 30 min at 0 °C. The reaction mixture was stirred at rt for 16 h. At this time, a TLC control (DCM/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, respectively, as ascertained by NMR. Solvents were evaporated and co-evaporated 3 times with toluene. The residue was taken up in DCM (500 mL), washed with water (150 mL), 10% aq. HCl (150 mL), satd. aq. $NaHCO_3$ (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 $BF_3.OEt_2$ (42.8 mL, 336.1 mmol, 3.0 equiv.) were added at rt to a solution of the residue dissolved in anhyd. DCM (390 mL). The mixture was stirred at this temperature for 16 h under an Ar atmosphere. A TLC control (Tol/EtOAc 7:3) showed the conversion of the intermediates into a major more polar product. The reaction was quenched with satd. aq. $NaHCO_3$ (200 mL). DCM (500 mL) was added, the layers were separated and 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. Thioglycoside **46** had R_f = 0.35 (Tol/EtOAc 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.57-7.32 (m, 5H, H_{Ar}), 6.75 (d, $J_{NH,2}$ = 9.1 Hz, 1H, NH), 5.42 (d, 1H, H-4), 5.32 (dd, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 4.88 (d, $J_{1,2}$ = 10.3 Hz, 1H, H-1), 4.20 (dd_o, 1H, H-6a), 4.19 (ddd_o, 1H, H-2), 4.16 (dd_{po}, $J_{5,6b}$ = 6.1 Hz, $J_{6a,6b}$ = 11.4 Hz, 1H, H-6b), 3.99 (dt, $J_{4,5}$ = 0.9 Hz, 1H, H-5), 2.15, 2.06, 2.00 (3s, 9H, $CH3_{Ac}$) ppm. ^{13}C NMR ($CDCl_3$), δ = 170.4, 170.3, 170.0 (3C, CO), 161.7 (NHCO), 133.3 (2C, C_{Ar}), 132.1 (C_{IVAr}), 129.0, 128.4 (3C, 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 (3C, CH_{3Ac}) ppm. HRMS (ESI^+): *m/z* 564.0087 (calcd for $C_{20}H_{22}Cl_3NO_8SNa$ $[M+Na]^+$: *m/z* 564.0029).

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 anhyd. MeOH (100 mL) was treated by MeONa (0.5 M in MeOH, 311 µL, 1.44 mmol, 0.2 equiv.). The solution was stirred for 2 h at rt under an Ar atmosphere. A TLC control (Chex/EtOAc 6:4, DCM/MeOH 8:2) showed the total 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 evaporated to give the crude phenyl 2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside[50] (**47**, 3.83 g) quantitatively. 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 to a solution of the latter in anhyd. DMF (74 mL) cooled to -10 °C. The mixture was stirred under an Ar atmosphere keeping the temperature below 0 °C. After 1.5 h, a follow up by 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 layer was dried over anhyd. Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (Tol/EtOAc 98:2 to 95:5) to give the target tri-*O*-benzyl derivative **48** (4.90 g, 77%), as a white solid. Thioglycoside **48** had R_f = 0.48 (Tol/EtOAc 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56-7.22 (m, 2H, H_{Ar}), 7.40-7.20 (m, 18H, H_{Ar}), 6.84 (d, $J_{NH,2}$ = 7.5 Hz, 1H, NH), 5.30 (d, $J_{1,2}$ = 10.2 Hz, 1H, H-1), 4.90 (d, *J* = 11.4 Hz, 1H, H_{Bn}), 4.68 (d, *J* = 11.2 Hz, 1H, H_{Bn}), 4.58 (d, 1H, H_{Bn}), 4.54 (d, 1H, H_{Bn}), 4.53 (d, *J* = 11.8 Hz, 1H, H_{Bn}), 4.48 (d, 1H, H_{Bn}), 4.28 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 2.7 Hz, 1H, H-3), 4.09 (d, 1H, H-4), 3.94 (pdt, 1H, H-2), 3.78-3.68 (m, 3H, H-5, H-6a, H-6b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.7 (NHCO), 138.4, 137.8, 137.3 (3C, C_{IVAr}), 132.8 (2C, C_{Ar}), 132.2 (C_{IVAr}), 130.0-127.6 (18C, C_{Ar}), 92.5 (CCl_3), 84.3 (C-1), 78.2 (C-3), 77.6 (C-5), 74.5, 73.6 (2C, C_{Bn}), 72.4 (C-4), 72.3 (CBn), 68.4 (C-6), 53.7 (C-2) ppm. HRMS (ESI^+): *m/z* 708.1128 (calcd for $C_{35}H_{34}Cl_3NO_5SNa$ $[M+Na]^+$: *m/z* 708.1121). Analytical data for triol **47** were as published.[50]

Benzyl (3,4,6-tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→2)-(4-*O*-benzyl-3-*O*-*para*-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*-*para*-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 4Å powdered MS (750 mg) in anhyd. DCM (2.9 mL) was stirred for 1 h at rt under an Ar atmosphere. 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 allowing the bath to reach -60 °C. A TLC control (Tol/EtOAc 9:1) indicated the absence of acceptor **27** and the presence of a less polar product. The reaction was quenched by 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 the 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 4Å MS (500 mg) in anhyd. DCM (3.4 mL) was stirred at rt under an Ar atmosphere 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 for 20 min at this temperature and Et_3N was added since a TLC control (Tol/EtOAc 9:1) had shown the presence of a new major compound, whereas neither acceptor nor donor remained. Solids were filtered, and the filtrate was concentrated to dryness. The residue was purified by flash chromatography (Tol/EtOAc 95:5 to 85:15) to give by order of elution the product of α-glycosylation **51** (19 mg, 9%) and the desired tetrasaccharide **50** (186 mg, 63 %), both as light yellow foams. The product of β-glycosylation **50** had R_f = 0.38 (Tol/EtOAc 9:1) ^{1}H NMR (100 MHz, $CDCl_3$): δ = 7.41-7.05 (m, 47H, H_{Ar}), 6.91 (d, $J_{NH,2}$ = 7.5 Hz, 1H, NH), 6.83 (d, *J* = 8.6 Hz, 2H, H_{ArPMB}), 5.97 (m, 1H, $CH{=}_{All}$), 5.35 (m, J_{trans} = 17.3 Hz, J_{gem} = 1.6 Hz, 1H,$=CH_{2All}$), 5.26 (bs, 1H, H-1_D), 5.25 (d_{po}, 1H, H_{CO2Bn}), 5.21 (m, J_{cis} = 10.5 Hz, 1H, $=CH_{2All}$), 5.10 (d, *J* = 12.2 Hz, 1H, H_{CO2Bn}), 4.98-4.92 (m, 4H, H-1_B, H-1_C, $2H_{Bn}$), 4.90 (d_{po}, *J* = 10.9 Hz, 1H, H_{Bn}), 4.84 (d_{po}, *J* = 11.1 Hz, 1H, H_{Bn}), 4.76 (d, *J* = 12.1 Hz, 1H, H_{Bn}), 4.72 (d_{po}, *J* = 10.5 Hz, 1H, H_{Bn}), 4.71-4.67 (m, 2H, H_{Bn}), 4.65-4.55 (m, 3H, H_{Bn}), 4.58-4.45 (m, 6H, $5H_{Bn}$, H_{All}), 4.36 (d_o, $J_{1,2}$ = 7.7 Hz, 1H, H-1_A), 4.34 (bd_o, 1H, H-4_A), 4.27 (d, *J* = 11.6 Hz, 1H, H_{Bn}), 4.22 (d, *J* = 11.6 Hz, 1H, H_{Bn}), 4.18-4.10 (m, 2H, H-2_B, H_{All}), 4.08 (pt, 1H, H-2_C), 4.03-3.96 (m, 4H, H-5_A, H-3_B, H-4_B, H-2_D), 3.87 (dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3_C), 3.81 (dd_{po}, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3_D), 3.79 (dq_{po}, 1H, H-5_C), 3.71 (s, 3H, CH_{3PMB}), 3.70-3.59 (m, 3H, H-2_A, H-$6a_B$, H-5_D), 3.51-3.42 (m, 3H, H-3_A, H-5_B, H-4_C), 3.34 (pt_{po}, $J_{4,5}$ = 9.2 Hz, 1H, H-4_D), 3.31 (m_o, 1H, H-$6b_B$), 1.28 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_D), 1.18 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_C) 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 (9C, C_{IVAr}), 134.1 ($CH{=}_{All}$), 130.7 (C_{IVAr}), 129.8-127.3 (47C, C_{Ar}), 117.4 ($=CH_{2All}$), 113.9 (2C, C_{ArPMB}), 102.7 (C-1_A, $^1J_{CH}$ = 162.0 Hz), 101.3 (C-1_C, $^1J_{CH}$ = 173.5 Hz), 100.7 (C-1_B, $^1J_{CH}$ = 162.4 Hz), 100.4 (C-1_D, $^1J_{CH}$ = 175.4 Hz), 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 (2C, C_{Bn}), 75.2 (C-2_C), 74.9 (2C, 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 (2C, C_{Bn}), 72.4 (C-4_B), 72.3, 71.6 (2C, 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$^+$): *m/z* 1784.6198 (calcd for $C_{100}H_{106}Cl_3NO_{21}Na$ $[M+Na]^+$: *m/z* 1784.6221).

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

The silylated acceptor **52** had ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.20 (m, 32H, H_{Ar}), 6.85 (m, J = 8.6 Hz, 2H, H_{ArPMB}), 5.98 (m, 1H, $CH{=}_{All}$), 5.35 (m, J_{trans} = 17.3 Hz, J_{gem} = 1.6 Hz, 1H, $=CH_{2All}$), 5.32 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1_D), 5.27 (d, 1H, H_{CO2Bn}), 5.21 (m, J_{cis} = 10.5 Hz, 1H, $=CH_{2All}$), 5.11 (d, J = 12.3 Hz, 1H, H_{CO2Bn}), 4.92 (d, J = 10.8 Hz, 1H, H_{Bn}), 4.90 ($2d_o$, J = 11.0 Hz, 2H, H_{Bn}), 4.83 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1_C), 4.81 (d, J = 12.1 Hz, 1H, H_{Bn}), 4.73 (d, J = 10.9 Hz, 1H, H_{Bn}), 4.71-4.57 (m, 7H, H_{Bn}), 4.50 (m, 1H, H_{All}), 4.39 (bd_{po}, 1H, H-4_A), 4.38 (d_{po}, $J_{1,2}$ = 7.7 Hz, 1H, H-1_A), 4.16 (m_{po}, 1H, H_{All}), 4.13 (dd_o, $J_{2,3}$ = 3.0 Hz, 1H, H-2_D), 4.09 (dd, 1H, H-2_C), 4.02 (d, $J_{4,5}$ = 1.0 Hz, 1H, H-5_A), 3.85 (dd, $J_{3,4}$ = 9.4 Hz, 1H, H-3_D), 3.80-3.73 (m, 5H, H-3_C, H-5_C, CH_{3PMB}), 3.73-3.67 (m, 2H, H-2_A, H-5_D), 3.53 (pt_{po}, $J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 9.5 Hz, 1H, H-4_C), 3.52 (dd_o, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 2.9 Hz, 1H, H-3_A), 3.40 (pt, $J_{4,5}$ = 9.6 Hz, 1H, H-4_D), 1.29 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_D), 1.19 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_C), 0.09 (s, 9H, $SiMe_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3 (C-6_A), 159.0 (C_{IVPMB}), 138.9-135.1 (6C, C_{IVAr}), 134.0 ($CH{=}_{All}$), 130.8 (C_{IVAr}), 129.4-127.4 (32C, C_{Ar}), 117.4 ($=CH_{2All}$), 113.6 (2C, 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 (2C, C-4_C, C-4_D), 79.4, 79.2 (2C, C-3_C, C-3_D), 78.5 (C-2_A), 75.3, 75.1 (2C, 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 (3C, C_{Bn}), 70.6 (CH_{2All}), 70.4 (C-2_C), 68.8, 68.6 (2C, 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 (3C, $SiMe_3$) 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 rt for 30 min. At that time, a TLC control (Tol/EtOAc 9:1) showed the complete transformation of the starting material into a more polar product. The reaction was quenched with satd. aq. $NaHCO_3$. The reaction mixture was diluted with water and DCM and the aq. phase was extracted three times with DCM. 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 kept under stirring at rt. After 14 h, a TLC control (Tol/EtOAc 9:1) indicated that the intermediate alcohol had been converted into a less polar product. 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 afford the monoacetylated tetrasaccharide **54** (582 mg, 87 % over two steps) as a white foam. Tetrasaccharide **54** had R_f = 0.38 (Tol/EtOAc 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41-7.04 (m, 45H, H_{Ar}), 7.02 (d, $J_{NH,2}$ = 7.0 Hz, 1H, NH), 5.98 (m, 1H, $CH{=}_{All}$), 5.36 (dd_{po}, $J_{2,3}$ = 3.1 Hz, 1H, H-3_C), 5.35 (m, J_{gem} = 1.6 Hz, 1H, $=CH_{2All}$), 5.32 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1_D), 5.26 (d, J = 12.2 Hz, 1H, H_{CO2Bn}), 5.22 (m, J_{cis} = 10.5 Hz, 1H, $=CH_{2All}$), 5.09 (d, 1H, H_{CO2Bn}), 5.03 (d_{po}, $J_{1,2}$ = 8.4 Hz, 1H, H-1_B), 5.01 (d_{po}, $J_{1,2}$ = 2.0 Hz, 1H, H-1_C), 4.89 (d_{po}, J = 10.8 Hz, 1H, H_{Bn}), 4.88 (d, J = 11.1 Hz, 2H, $2H_{Bn}$), 4.77 (d, J = 11.8 Hz, 1H, H_{Bn}), 4.70 (d, J = 11.2 Hz, 2H, H_{Bn}), 4.66 (d, J = 11.2 Hz, 1H, H_{Bn}), 4.62-4.52 (m_{po}, 7H, H_{Bn}), 4.50 (m_{po}, 1H, H_{All}), 4.40-4.35 (m, 4H, H-1_A, H-4_A, H-3_B, H-2_C), 4.26 (d, J = 11.6 Hz, 1H, H_{Bn}), 4.20 (d, J = 11.6 Hz, 1H, H_{Bn}), 4.16 (m, 1H, H_{All}), 4.05 (d, $J_{3,4}$ = 2.4 Hz, 1H, H-4_B), 4.01 (d, $J_{4,5}$= 0.9 Hz, 1H, H-5_A), 4.00 (m, 1H, H-2_D), 3.92-3.82 (m, 2H, H-2_B, H-5_C), 3.80 (dd, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3_D), 3.70-3.58 (m, 3H, H-2_A, H-$6a_B$, H-5_D), 3.56-3.47 (m, 4H, H-3_A, H-5_B, H-4_C, H-4_D), 3.35 (dd, $J_{5.6b}$ = 4.5 Hz, $J_{6a,6b}$ = 8.3 Hz, 1H, H-$6b_B$), 2.17 (s, 3H, H_{Ac}), 1.29 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_D), 1.14 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_C) 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 (9C, C_{IVAr}), 134.1 ($CH{=}_{All}$), 129.6-127.2 (45C, C_{Ar}), 117. 4 ($=CH_{2All}$), 102.6 (C-1_A, $^1J_{CH}$ = 156.6 Hz), 101.3 (C-1_C, $^1J_{CH}$ = 176.3 Hz), 100.0 (C-1_D, $^1J_{CH}$ = 171.1 Hz), 99.6 (C-1_B, $^1J_{CH}$ = 163.3 Hz), 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 (2C, C_{Bn}), 75.2 (C-2_C), 75.1, 75.0 (2C, 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 (2C, 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$^+$): *m/z* 1684.5983 (calcd for $C_{94}H_{101}Cl_3NO_{21}$ $[M+H]^+$: *m/z* 1684.5931), *m/z* 1706.5928 (calcd for $C_{94}H_{100}Cl_3NO_{21}Na$ $[M+Na]^+$: *m/z* 1706.5751).

Propyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-Dgalactopyranosiduronic acid (4): To a stirred solution of tetrasaccharide **50** (254 mg, 140 µmol) in THF/H_2O (4:1, 10.2 mL), was added 10% Pd/C (200 mg). The suspension was stirred under a hydrogen atmosphere for 2 days. 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. Tetrasaccharide **4** had R_t = 8.5 min. ^{1}H NMR (400 MHZ, D_2O): δ = 5.24 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1_D), 5.08 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1_C), 4.55 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1_B), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1_A), 4.35 (d, $J_{4,5}$ = 1.2 Hz, 1H, H-5_A), 4.27 (dd, $J_{3,4}$ = 2.8 Hz, 1H, H-4_A), 4.05 (dd, $J_{2,3}$ = 3.0 Hz, 1H, H-2_C), 4.02 (dd, $J_{2,3}$ = 3.1 Hz, 1H, H-2_D), 3.87-3.74 (m, 6H, H-4_B, OCH_{2Pr}, H-2_B, H-3_A, H-3_D, H-3_C), 3.74-3.63 (m, 3H, H-$6a_B$, H-$6b_B$, H-3_B), 3.63-3.51 (m, 4H, H-5_B, H-5_C, OCH_{2Pr}, H-5_D), 3.50 (dd, $J_{2,3}$ = 9.9 Hz, 1H, H-2_A), 3.34 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1H, H-4_D), 3.27 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4_C), 1.97 (s, 3H, CH_{3NHAc}), 1.57 (sex, J = 7.4 Hz, 2H, CH_{2Pr}), 1.20-1.15 (m, 6H, H-6_C, H-6_D), 0.84 (t, 3H, CH_{3Pr}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 177.7 (NHCO), 174.2 (C-6_A), 105.7 (C-1_B, $^1J_{CH}$ = 163.2 Hz), 104.9 (C-1_A, $^1J_{CH}$ = 161.7 Hz), 103.6 (C-1_C, $^1J_{CH}$ = 175.8 Hz), 102.5 (C-1_D, $^1J_{CH}$ = 174.7 Hz), 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 (2C, C-4_C, OCH_{2Pr}), 74.5 (C-4_D), 73.4 (C-3_B), 72.8 (C-2_A), 72.3 (2C,

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 (2C, CH_{2Pr}, CH_{3NHAc}), 19.2 (2C, C-6_D, C-6_C), 12.2 (CH_{3Pr}) ppm. HRMS (ESI^+): *m/z* 754.2722 (calcd for $C_{29}H_{49}NO_{20}Na$ $[M+Na]^+$: *m/z* 754.2745).

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): To a stirred solution of tetrasaccharide **54** (187 mg, 110 µmol) in THF/H_2O (4:1, 8.4 mL), was added 10% Pd/C (150 mg). The suspension was stirred under a hydrogen atmosphere for 2 days. 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. Tetrasaccharide **5** had R_t = 9.0 min. ^{1}H NMR (400 MHz, D_2O): δ = 5.23 (d, $J_{1,2}$ = 1.3 Hz, 1H, H-1_D), 5.03 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1_C), 4.92 (dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 10.0 Hz, 1H, H-3_C), 4.33 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1_A), 4.31 (bs_o, 1H, H-5_A), 4.30 (d_{po}, $J_{1,2}$ = 8.3 Hz, 1H, H-1_B), 4.22 (dd, $J_{3,4}$ = 2.8 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-4_A), 4.12 (pt, 1H, H-2_C), 3.99 (dd, $J_{2,3}$ = 3.1 Hz, 1H, H-2_D), 3.83-3.70 (m, 5H, H-4_B, OCH_{2Pr}, H-2_B, H-3_A, H-3_D), 3.70-3.57 (m, 4H, H-5_C, H-$6a_B$, H-$6b_B$, H-3_B), 3.52-3.46 (m, 3H, H-5_B, OCH_{2Pr}, H-5_D), 3.45 (dd_{po}, $J_{2,3}$ = 10.0 Hz, 1H, H-2_A), 3.40 (pt_{po}, J= 9.3 Hz, J= 9.8 Hz, 1H, H-4_C), 3.35 (pt_{po}, J= 9.6 Hz, J= 9.8 Hz, 1H, H-4_D), 2.09 (s, 3H, CH_{3Ac}), 1.97 (s, 3H, CH_{3NHAc}), 1.56 (sex, J = 7.4 Hz, 2H, CH_{2Pr}), 1.16 (d, $J_{5,6}$ = 6.3 Hz, 3H, H-6_D), 1.14 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6_C), 0.80 (t, 3H, 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 (C-1_B, $^1J_{CH}$ = 163.2 Hz), 104.9 (C-1_A, $^1J_{CH}$ = 161.8 Hz), 103.6 (C-1_C, $^1J_{CH}$ = 174.7 Hz), 102.4 (C-1_D, $^1J_{CH}$ = 173.6 Hz), 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 (2C, C-6_D, C-6_C), 12.1 (CH_{3Pr}) ppm. HRMS (ESI^+): *m/z* 796.2866 (calcd for $C_{31}H_{51}NO_{21}Na$ $[M+Na]^+$: *m/z* 796.2852).

Supporting Information (see footnote on the first page of this article): This material contains 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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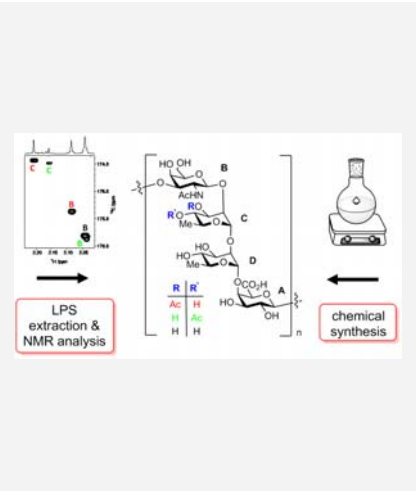
Entry for the Table of Contents

Layout 1:

((Carbohydrates))

The repeating unit of the O-specific polysaccharide (PS) from *Shigella flexneri* serotype 6 (SF6) strain MDC 2924-71 has been studied by NMR spectroscopy, confirming a non-stoichiometric *O*-acetylation at one of the rhamnosyl residues. On this basis, the synthesis of di- to tetrasaccharide derivatives mono-*O*-acetylated or not is reported, leading for the first time to moieties shared by the PS of SF6, SF6a or *Escherichia coli* O147 strains.



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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

Keywords: carbohydrates / glycosylation / lipopolysaccharide / NMR / synthesis design

Supporting Information

Structural studies of the *O*-acetyl containing O-antigen from a *Shigella flexneri* serotype 6 strain and synthesis of oligosaccharide fragments thereof

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NMR spectra for new compounds are provided in the following order.
The ^{1}H, COSY, DEPT, HSQC, ^{13}C spectra are provided for all compounds. Information on J_{CH} are provided when appropriate. In all cases, enlargements are included whenever found useful. The NOESY spectrum for compound **19** is also listed.

30/31:	rhamnopyranosyl)-(1→4)-(allyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate	
Compound **2**:	Propyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic acid	p. 124
Compound **32**:	Methyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-(propyl β-D-galactopyrosid)uronate	p. 134
Compound **3**:	Propyl 3-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic acid	p. 142
Compound **33**:	Propyl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic acid	p. 152
Compound **36**:	Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside	p. 160
Compound **38**:	Allyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside	p. 165
Compound **39**:	3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranose	p. 170
Compound **41**:	3,4,6-Tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α/β-D-galactopyranosyl *N*-phenyltrifluoroacetimidate	p. 175
Compound **42**:	2-Trichloromethyl-(3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-galactopyrano)-[2,1-*d*]-2-oxazoline	p. 180
Compound **48**:	Phenyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside	p. 185
Compound **49**:	(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	p. 190
Compound **50**:	Benzyl (3,4,6-tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→2)-(4-*O*-benzyl-3-*O*-*para*-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	p. 195
Compound **51**:	Benzyl (3,4,6-tri-*O*-benzyl-2-deoxy-2-trichloroacetamido-α-D-galactopyranosyl)-(1→2)-(4-*O*-benzyl-3-*O*-*para*-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	p. 204
Compound **52**:	Benzyl (4-*O*-benzyl-3-*O*-*para*-methoxybenzyl-2-*O*-trimethylsilyl-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	p. 211
Compound **4**:	Propyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosiduronic acid	p. 215
Compound **54**:	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	p. 224
Compound **5**:	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	p. 233