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Linear synthesis of the branched pentasaccharide repeats of O-antigens from *Shigella flexneri* 1a and 1b demonstrating major steric hindrance associated to type-specific glucosylation

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Jason M. Hargreaves,‡§[a,b] Yann Le Guen,‡[a,b,c] Catherine Guerreiro,[a,b] Karine Descroix#[a,b] and Laurence A. Mulard*[a,b]

Shigella flexneri serotypes 1b and 1a are Gram-negative enteroinvasive bacteria causing shigellosis in humans. The O-antigen from *S. flexneri* 1b is a {→2)-[3Ac/4Ac]-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[2Ac]-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-β-D-Glc*p*NAc-(1→}$_n$ branched polysaccharide ($\{_{Ac}AB_{Ac}C(E)D\}_n$). It is identical to that from *S. flexneri* 1a, except for the 2_C-acetate. A concise synthesis of disaccharide ED, trisaccharides $_{Ac}C(E)D$ and C(E)D, tetrasaccharides $B_{Ac}C(E)D$ and BC(E)D, pentasaccharides $AB_{Ac}C(E)D$ and ABC(E)D, is described starting from a 2-*N*-acetyl-D-glucosaminide acceptor and using the imidate glycosylation chemistry. The E residue was efficiently introduced via a potent stereoselective [E + D] coupling. In contrast, harsh conditions and appropriate tuning of the donor were required for a high yielding [C + ED] glycosylation. Irrespective of the level of steric bulk at residue C, glycosylation at O-3_D of the ED acceptor generated a major change of conformation of the D residue within the obtained C(E)D trisaccharide, as attested by NMR data. Proper manipulation of the constrained C(E)D trisaccharide was necessary to proceed with the stepwise chain elongation at O-3_C of an acceptor having the 2_C-*O*-acetyl already in place. The protected intermediates went through a one- to three-step deprotection sequence to give the propyl glycoside targets, as portions of the O-antigens from both *S. flexneri* 1a and 1b. Protecting group removal was clearly associated with conformational relief, yielding oligosaccharides, for which NMR data were consistent with a $^4C_1$ conformation for the 3,4-di-*O*-glycosylated residue D, as in the native bacterial polymers.

Introduction

Shigella flexneri are gram-negative enteroinvasive bacteria. As the major agents of endemic shigellosis – an acute rectocolitis otherwise known as bacillary dysentery – in human, they represent an important cause of diarrhoeal disease worldwide, especially among children in developing countries.[1] Renewed awareness of the burden of shigellosis in this population boosted by the growing spread of resistant strains has accelerated the search for novel vaccine strategies.[2] The ongoing development of synthetic carbohydrate-based immunogens targeting the most prevalent *S. flexneri* serotypes is part of this effort.[3] The strategy in place entails complete identification of the protective carbohydrate epitopes, that is to say the saccharide structures recognized by antibodies protective against infection, prior to any *in vivo* study.[4] At least 17 *S. flexneri* serotypes and subtypes have been identified.[5] They differ by the chemical nature of the lipopolysaccharide (LPS) embedded in their outer membrane, and in particular by the structure of their O-antigen (O-Ag), that is the polysaccharide component of their LPS. The latter contributes to virulence. It is also a major target of the acquired immunity stimulated by clinical infection.[2]

Although exceptions do exist, most *S. flexneri* O-Ags have a common linear backbone defined by a tetrasaccharide unit comprising three L-rhamnose residues (**A**, **B**, **C**) and a *N*-acetyl-D-glucosamine residue (**D**). Differences between *S. flexneri* serotypes are mainly associated with the phage-encoded site selective modification of the **ABCD** unit with α-D-glucopyranosyl residues (**E**) and/or O-acetyl groups (Ac).[6, 7] Moreover, several recently revised structures from *S. flexneri* O-Ags have outlined the frequent occurrence of non-stoichiometric O-acetylation in this family of bacterial polysaccharide antigens.[5] Yet, the function of this substitution pattern is not clear. As part of an ongoing study on *S. flexneri* O-Ag chemical diversity in relation to antigenicity and protective epitope identification, we have previously synthesized panels of *S. flexneri*-related oligosaccharides α-D-glucosylated at either OH-3_A,[8, 9] OH-4_C,[10, 11] or OH-3_B.[12] Owing to their glucosylation pattern, the corresponding

synthetic oligosaccharides represent portions from *S. flexneri* O-Ags featuring group factor 7,8, and type factors II and V, respectively. Moreover, oligosaccharides related to the O-Ags from *S. flexneri* serotypes 2a (SF2a),[13] 3a (SF3a),[14] and 6 (SF6),[15, 16] were designed so as to also encompass the O-acetylation patterns found in the natural polysaccharides, when appropriate. In particular, O-acetylation at position 2_C, as found in SF3a, characterizes group factor 6.[5]

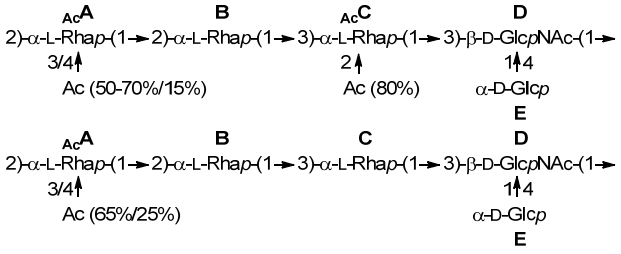


Fig. 1 Structure of the biological repeating units of the O-Ags from SF1b (top) and SF1a (bottom), $_{Ac}AB_{Ac}C(E)D$ (**I**) and $_{Ac}ABC(E)D$ (**II**), respectively.[17]

Herein, we tackle for the first time the synthesis of fragments of the O-Ags from *S. flexneri* 1a (SF1a) and 1b (SF1b), two relevant serotypes in the field.[18-21] The two surface polysaccharides of interest have the α-D-Glc*p*-(1→4)-β-D-Glc*p*NAc branching pattern in common (Fig. 1), a feature related to type factor I in the *S. flexneri* classification.[5, 22] On the one hand, the branched pentasaccharide **I** ($_{Ac}AB_{Ac}C(E)D$), whereby residue C is O-acetylated in a non-stoichiometric manner at position 2, defines the biological repeating unit of the O-Ag from SF1b.[17] On the other hand, the branched pentasaccharide analogue **II** ($_{Ac}ABC(E)D$), which has a free 2_C-OH, reflects the biological repeating unit of the O-Ag from SF1a.[17] It is of note that residue A is O-acetylated at position 3 in a similar non-stoichiometric extent in both pentasaccharide repeats **I** and **II**.

In this context, we report the first synthesis of *S. flexneri* di- to pentasaccharides having the α-D-Glc*p*-(1→4)-β-D-Glc*p*NAc (ED) moiety at their reducing end. All synthetic targets, including the ED disaccharide, represent parts of the SF1b O-Ag. They are synthesized as their propyl glycosides. Moreover, oligosaccharides synthesized with a free 2_C-OH also exemplify segments of the SF1a O-Ag. In particular, the pentasaccharides $AB_{Ac}C(E)D$-Pr and ABC(E)D-Pr correspond to the biological repeating units of the O-Ags from SF1b and SF1a, albeit non-O-acetylated at rhamnose A, respectively. The corresponding 3_A-*O*-acetyl oligosaccharides were not considered herein owing to the known propensity for acetyl migration at all positions within a terminal rhamnose residue.

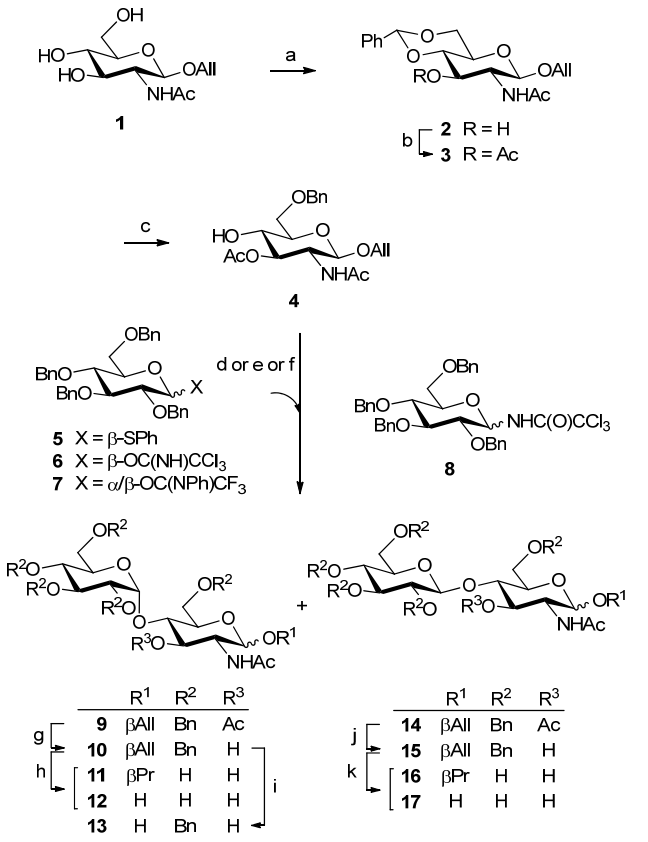
Results and discussion

The 2_C-acetate, 1,2-*cis* stereochemistry at the E-D linkage, and 3,4-di-*O*-glycosylation of the *N*-acetyl-D-glucosamine residue (D) occurring in pentasaccharide **I** were identified as key features in the SF1b targets and as possible synthetic challenges. Both SF1b and SF3a O-Ags express group factor 6, which is associated to the 2_C-Ac. Therefore, with regards to the first point, the synthetic design was inspired from the successful strategy previously established for the synthesis of SF3a-specific oligosaccharides.[8, 9, 14] With a view to the early stage installation of the 2_C-*O*-acetyl group, the common precursor to rhamnoses A and B was protected at OH-2 with the orthogonal levulinyl (Lev) ester, to ensure anchimeric assistance during glycosylation. When considering the E-D linkage, a rapid literature survey evidenced that several syntheses of α-D-Glc*p*-(1→4)-β-D-Glc*p*NAc disaccharides had been reported. Original attempts by enzymatic routes[23] were followed by the development of diverse chemical pathways, which substantiated some limitations. For instance, a modest yield of the α-D-glucosylation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside was initially observed. It was tentatively explained by a strong steric compression at the acceptor site and successfully circumvented by use of a 2-acetamido-3-*O*-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose acceptor.[24] Yet, an excellent 84% yield of the α-D-glucosylation product of the aforementioned benzyl glucosaminide acceptor was subsequently reported using a 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose precursor and different reaction conditions.[25] In the same years, an alternative route to α-D-Glc*p*-(1→4)-β-D-Glc*p*NAc glycosides was proposed in the context of SF1a. Azidonitration of maltal hexaacetate, which has the α-(1→4)-linkage already in place, into the corresponding 2-azido-1-α/β-nitrate (47%) was the key step.[26] More recently, the synthesis of two pentasaccharides related to the O-Ag from SF1a, the aminopentyl glycosides of segments C(E)DAB and BC(E)DA, was described.[27] The construction of the E-D linkage involved a sophisticated intramolecular glycosylation strategy. Thus, using appropriately prearranged glycosides, the exemplified α-(1→4)-linked ED disaccharides were obtained stereospecifically, in a good 70% yield. On the basis of this overview and convincing literature data,[25] we chose to reinvestigate a more straightforward route to SF1a and SF1b-related oligosaccharides. It features commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucose as precursor to residue E, as already successfully exploited in the synthesis of SF2a,[28] SF3a[8] and SF5a[29] oligosaccharides. We examined first the glycosylation of an orthogonally protected 2-acetamido-2-deoxy-β-D-glucopyranoside acceptor (D) with easily accessible 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl donors, next the coupling of the corresponding ED acceptor with suitable rhamnosyl C donors, and finally possible stepwise chain elongation strategies with rhamnoses B and A toward the propyl glycosides of pentasaccharides $AB_{Ac}C(E)D$ and ABC(E)D.

Synthesis of the ED-Pr disaccharide (11)

Despite possible interference of the 2-acetamido moiety during glycosylation, allyl glycoside[30] **4** (Scheme 1), benzylated at O-6 and orthogonally protected with an acetyl group at O-3, was the precursor to the D reducing end residue common to all targets. It was prepared from glucosamine hydrochloride via the key triol[31-34] **1**. Thus, regioselective protection of the latter by treatment with benzaldehyde dimethyl acetal in the presence of catalytic camphor-10-sulfonic acid (CSA) in MeCN gave partially protected[35] **2** (93%). Acetylation of the remaining hydroxyl group gave the fully protected[36] **3** (89%) and subsequent reductive regioselective opening of the benzylidene acetal provided the 6-*O*-benzyl derivative[30] **4** (86%). The stepwise transformation of allyl glycoside **1** into acceptor **4** was performed on a 5.0 g scale with an acceptable overall 71% yield. We favoured this strategy for its robustness and crystalline intermediates – **2** and **3** – when working on a 10 g scale. In this case, the yield of the conversion of triol **1** into acceptor **4** reached 77%.

Glycosylation of acceptor **4** was first attempted with the available perbenzylated thiophenyl glucopyranoside donor[37] **5**



Scheme 1 Synthesis of disaccharides **11** and **16**. *Reagents and conditions:* a) $PhCH(OMe)_2$, CSA, MeCN, rt, 93%; b) Ac_2O, Pyr, rt, 89%; c) Et_3SiH, TfOH, DCM, 86%; d) **5**, NIS, DCM/Et_2O, see Table 1; e) **6**, TMSOTf, DCM/Et_2O, 0°C, 85% of **9**/**14** 9:1; f) **7**, TMSOTf, DCM/Et_2O, rt, 76% for **9** and 11% for **14**; g) NaOMe, MeOH, rt, 97%; h) *route 1.* H_2, Pd/C, 90% aq EtOH, rt, 50% for **11** and 12% for **12** or *route 2.* H_2, Pd/C, HCl, 96% aq EtOH, rt, 2 days, 70% for **11** or *route 3*. H_2, $Pd(OH)_2$/C, 96% aq EtOH, rt, 85% for **11**; i) Pd/C, 90% aq EtOH, HCl, rt; j) MeONa, MeOH, rt, 8% from **9**/**14** 9:1; k) H_2, Pd/C, 90% aq EtOH, HCl, rt, 67% for **16** and 19% for **17**. rt: room temperature.

Table 1 Attempts at α-D-glucosylation at OH-4 of acceptor **4**.

Entry	Donor (equiv.)	Promoter	Solvent DCM/Et_2O	Temperature	**9** and **14**	α/β ratio[§]
1[#]	**5** (1.25)	TfOH / NIS	2:5	0°C	< 51%	7:1
2[#]	**5** (1.25)	TMSOTf / NIS	2:5	0°C	< 44% (< 81%)	7:1
3[#]	**5** (5.0)	TMSOTf / NIS	2:5	0°C	< 64%	7:1
4[*]	**6** (1.3)	TMSOTf	2:3 → 1:2	-78°C → 4°C	85%	9:1
5[*]	**7** (1.2)	TMSOTf	1:5 → 1:4	rt	87%	9:1

[#] Overestimated yields due to contamination with succinimide ; * Use of the inverse procedure. [§] Calculated based on NMR data.

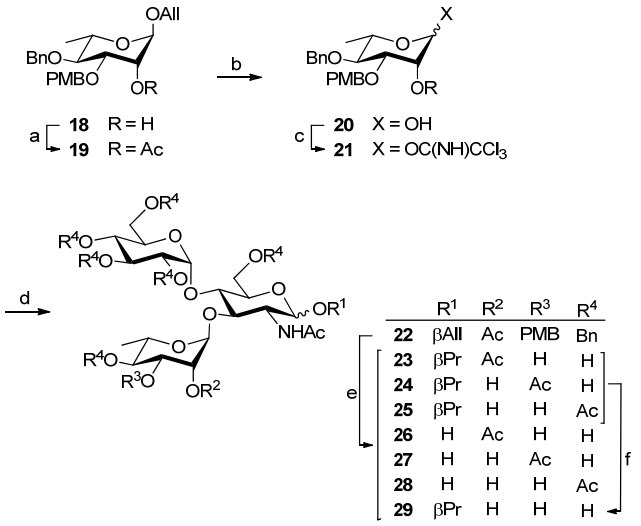
in the presence of stoichiometric *N*-iodosuccinimide (NIS) in combination with trifluoromethanesulfonic acid (TfOH) or trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the promoter. Glycosidic couplings were achieved in modest yields when using a slight excess of donor **5** at 0°C in 2:5 DCM/Et_2O (Table 1, entries 1 and 2). Under these conditions, large quantities of acceptor **4** were recovered. An increase in the amount of donor **5** to 5 equiv. resulted in an improved yield of the condensation (Table 1, entry 3). However, glycosylation at OH-4 of acceptor **4** *via* this methodology proceeded with a maximum observed α/β selectivity of 7:1. Changing donor **5** for the more readily available glucopyranosyl trichloroacetimidate[38] (TCA) **6** (Scheme 1) was the next step. The glycosylation was run in a mixture of DCM and Et_2O, the ratio of which (3:2 to 2:1) was optimized to comply with solubility requirements on the one hand and glycosylation stereoselectivity on the other hand. Owing to the high donor propensity of TCA **6** as opposed to the poor reactivity of acceptor **4**, the inverse procedure[39] was applied. Pleasingly, proper tuning of the reaction conditions (Table 1, entry 4) permitted isolation of the coupling products as a 9:1 α/β mixture of diastereoisomers in a good 85% yield. Subsequent treatment of the glycosylation product with methanolic sodium methoxide gave the expected alcohol **10** in 70% yield over two steps and the β-linked diastereoisomer **15** (7% over two steps). This product distribution was in agreement with the good α/β stereoselectivity of the coupling step. The kinetics of deacetylation of the α-linked and β-linked disaccharides differed, with a faster reaction in the case of the former. Attempts to exploit this difference were unsuccessful and completion of the transesterification step for the two isomers was necessary for a proper isolation of acceptor **10**. Moreover, partial conversion of donor **6** into the corresponding *N*-glycoside[40] **8** could not be avoided during glycosylation. For that reason, conditions optimized for TCA **6** were applied to the more recently disclosed *N*-phenyltrifluoroacetimidoyl (NPTFA) analogue[41] (**7**). Advantageously, the reaction was run at room temperature in this case (Table 1, entry 5). These conditions were compatible with the use of an enhanced Et_2O/DCM ratio, in favour of the required α-D-glucosylation. Moreover, while the separation of the α/β condensation products was initially found problematic, optimized coupling conditions using donor **7** allowed to achieve an excellent 76% yield of the pure α-linked disaccharide **9**, as ascertained by NMR analysis (NMR spectroscopic data for C-1_E: δ = 97.3 ppm, $^1J_{CH}$ = 169.5 Hz). In this case, the β-linked isomer **14** (NMR spectroscopic data for C-1_E: δ = 102.8 ppm, $^1J_{CH}$ = 162.7 Hz) was isolated in a meaningful 11% yield. These data suggested that a good 9:1 α/β ratio was again reached at the coupling step. Deacetylation of the major glycosylation product **9** readily gave acceptor **10** (97%). Gratifyingly, despite appearing somewhat less stereoselective than published procedures,[25] appropriate control of the well-established imidate chemistry[42, 43] allowed to reach a reproducible 70-73% isolated yield of the α-linked disaccharide **10** over two steps. Whether by means of crystalline TCA **6** or of the more stable NPTFA **7**, it provides a promising route with regards to multigram diasetereoselective formation of an ED intermediate ready for chain elongation.

Conventional hydrogenolysis of disaccharide **10**, and concomitant reduction of the allyl aglycon, provided the ED target **11** as its propyl glycoside (50%) following RP-MPLC purification. This somewhat low yield was in part explained by the isolation of a meaningful amount of the corresponding hemiacetal[25] **12** (12%), suggesting that partial degradation had occurred. A similar, albeit more controlled, procedure was adopted to convert the β-linked disaccharide **15** to the propyl glycoside isomer **16**. While the yield of the expected **16** reached 67%, loss of the aglycon could not be avoided, and hemiacetal **17** was also isolated (19%) following RP-MPLC purification. Under the above conditions, the conversion of

disaccharide **10** into the ED-Pr disaccharide **11** was attained in a good 70% yield.

Synthesis of the $_{Ac}$C(E)D-Pr (23) and C(E)D-Pr (29) trisaccharides.

In an attempt to minimize the number of deprotection steps to reach the target trisaccharides **23** and **29**, the orthogonally protected rhamnosyl donor **21**, having an acetyl group at position 2 and a *para*-methoxybenzyl (PMB) ether at position 3, was selected as a precursor to residue C (Scheme 2). It was easily prepared in 8 steps from L-rhamnose monohydrate via allyl 4-*O*-benzyl-3-*O*-*para*-methoxybenzyl-α-L-rhamnoside[16] (**18**). Acetylation of the remaining hydroxyl group gave the fully protected **19** (91%), which was converted to hemiacetal **20** following a two-step anomeric deallylation procedure (97%). Finally, the latter was transformed into the TCA donor **21** (94%) by reaction with trichloroacetonitrile in the presence of catalytic DBU (1,8-diazabicyclo[5.4.0]undec-7-ene).



Scheme 2 Synthesis of the C(E)D-Pr trisaccharides O-acetylated at residue C or not. *Reagents and conditions:* a) Ac_2O, Pyr, rt, 91%; 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, 97%; c) CCl_3CN, DBU, DCE, rt, 1 h, 94%; d) **10**, Toluene, see Table 2; e) H_2, Pd/C, 96% aq. EtOH, HCl, rt, 2 days, 47% for **23/24/25** as a 4:5:1 mix, 23% for **26/27/28** as a complex mix; f) MeONa, MeOH, rt, 69%.

Table 2 Attempts at α-L-rhamnosylation at OH-3_D of acceptor **10**.

Entry	**21** (equiv.)	Temperature	TMSOTf (equiv.)	Reaction time (h)	Yield of **22** (brsm)*
1	2.1	-10°C → rt	0.3	6	-
2	1.3	-10°C → 40°C	0.3	3	40% (68%)
3	1.5	40°C	0.1	19	43% (87%)
4	1.5	40°C	0.3	1.5	81% (90%)

* brsm: yield based on recovered starting material **10**.

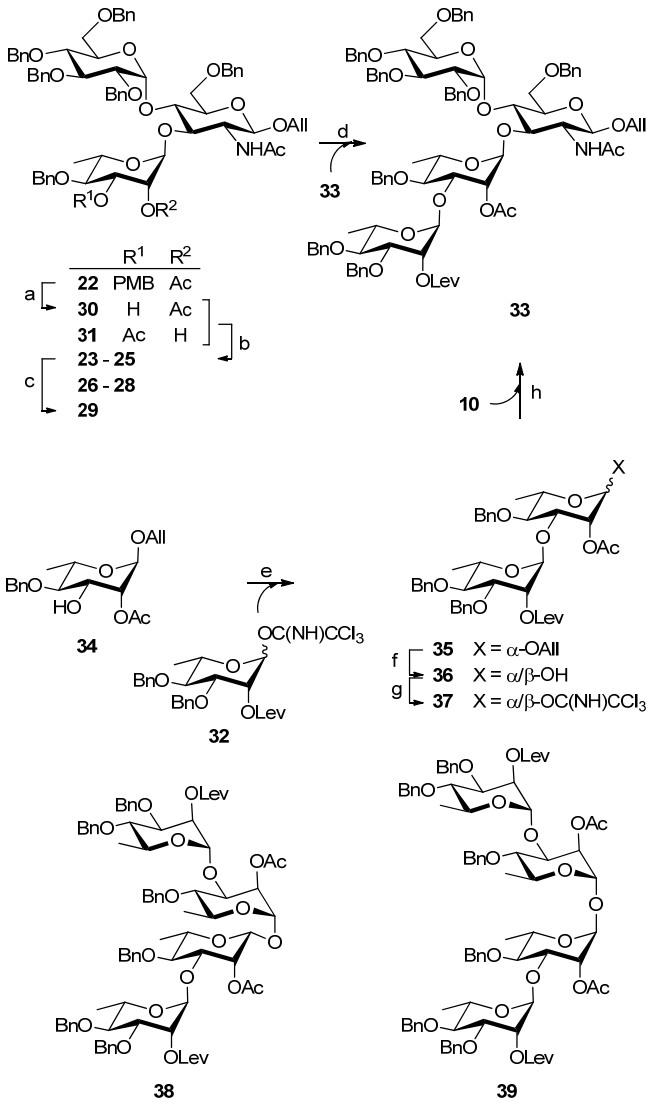
Attempts at the [ED + C] condensation used an established methodology for coupling to rhamnose donors, somewhat adapted to the inherent steric hindrance of the reactive centre. Thus, TMSOTf-mediated glycosylation of a toluene solution containing acceptor **10** and a two-fold excess of donor **21** was performed at -10°C to rt (Table 2, entry 1). It disappointingly resulted in decomposition of the donor. Gratifyingly, a gradual increase of the reaction temperature from -10°C to 40°C allowed the isolation of the desired trisaccharide together with some recovered acceptor (Table 2, entry 2). Setting the reaction temperature to 40°C, and diminishing the amount of promoter (Table 2, entry 3) had no visible effect. Again, a large amount of acceptor **10** was recovered. In contrast, performing the condensation at 40°C in the presence of 0.3 equiv. of TMSOTf generated a rewarding 81% glycosylation yield (Table 2, entry 4), while increasing the amount of donor led to a diminished yield of trisaccharide **22** (not shown). Next, Pd/C-mediated hydrogenolysis of the fully protected trisaccharide intermediate **22** in a slightly acidic alcoholic medium furnished the C(E)D-Pr target O-acetylated at rhamnose C (47%). Owing to the propensity of acetyl moieties to migrate to vicinal hydroxyl groups, the hydrogenolysis product was isolated following RP-MPLC purification as a 4:5:1 mixture of regioisomers **23**, **24** and **25**, O-acetylated at position 2_C, 3_C, and 4_C, respectively (Scheme 2). As in the case of the hydrogenolysis of disaccharides **10** and **15**, degradation occurred during this transformation. The corresponding hemiacetals **26-28** were isolated (23%) as a complex mixture of α and β O-acetylated regioisomers. As for disaccharides **10** and **15**, it was hypothesized that the cleavage of the allyl aglycon occurring during the hydrogenolysis of trisaccharide **22** into **23** was caused by an unfavourable competition between alkene reduction and propen-1-yl hydrolysis. Gratifyingly however, there was no trace of acetate loss. In order to investigate further the outcome of the deprotection step yielding the desired $_{Ac}$C(E)D-Pr trisaccharide, an available fraction of mixed monoacetates **30** and **31** (3:2 based on ^{1}H NMR data, see below, Scheme 3) was submitted to Pd/C hydrogenolysis using the above conditions. In this case, the target trisaccharide was obtained in a good 74% yield as a 4:5:1 mixture of regioisomers **23**, **24** and **25**, following RP-MPLC purification. In support to our previous observation, there was no trace of any acetate loss in this case. However, acetyl migration was still observed as expected. Interestingly, the final distributions of the O-acetylated regioisomers **23-25** were identical whether starting from the fully protected **22** or from a 3:2 mix of alcohols **30** and **31**, suggesting that it is independent of the trisaccharide precursor. Subsequent treatment of a sample of mixed monoacetates **23-25** under Zemplén conditions provided propyl glycoside **29** in a non optimized 69% yield following RP-HPLC purification.

The isolation of hemiacetals upon conventional hydrogenolysis of allyl glycoside precursors has no precedent in our hands despite frequent use of the Pd/C-induced anomeric allyl to propyl conversion, whether in the case of allyl rhamnosides,[14, 16] (allyl galactopyranosid)uronates,[15] or in the case of allyl glucosaminides.[9] Puzzled by this phenomenon, we questioned the origin of the aglycon loss. It could not be explained by the quality of the catalyst neither by any peculiar experimental feature. However, anomeric deallylation in the presence of Pd/C catalyst in acidic alcohol medium has precedent. For example, the high yielding selective Pd/C-mediated conversion in acidic methanol of an allyl rhamnoside to the corresponding hemiacetal was reported.[44] Referring to established procedures for Pd/C-mediated anomeric allyl cleavage, it is assumed that following Pd/C-induced allyl conversion into a propen-1-yl, which often requires forcing conditions,[45, 46] acid alcoholysis of the latter reveals the hemiacetal.[44, 46, 47] Nevertheless, partial loss of the allyl moiety during isomerisation under neutral conditions, as observed during the final deprotection of disaccharide **10**, has been occasionally reported.[45] On that basis, our experimental observations were tentatively explained by a competition

between the kinetics of the Pd/C-mediated hydrogenation of the allyl moiety and that of the cleavage of a propenyl ether intermediate under the hydrogenolysis conditions. In the present case, this competition strongly impaired the formation of the propyl glycosides **11** and **16**. To test this hypothesis, disaccharide **10** was reacted under conditions used for hydrogenolysis (Pd/C in 90% aq. EtOH, 1M aq HCl 0.1 equiv., overnight, rt) in a saturated hydrogen atmosphere or in an Ar atmosphere. The former condition led to the clean formation of the ED-Pr (74%). In contrast, in the absence of hydrogen disaccharide **10** evolved into a complex mixture of products. HRMS and NMR data demonstrated that the allyl moiety was strongly affected. Furthermore, in support to our observations, HRMS data (HRMS (ESI^+): *m/z* 856.3798, calcd for $C_{49}H_{55}NO_{11}Na$ $[M+Na]^+$ *m/z* 856.3723) clearly indicated the presence of detectable amounts of the corresponding hemiacetal **13**. Undoubtedly, the hemiacetal/glycosides ratio increased with time (not described). As a general trend, under the conditions in use, dilution and reaction duration were identified as factors possibly interfering with allyl to propyl conversion. Running the reaction in a buffered medium[48] may prevent hydrolysis while retaining the efficiency of the reduction process. Alternatively, the use of $Pd(OH)_2$, which is known to be more selective than Pd on charcoal, was considered to be a relevant option. Satisfactorily, under conventional hydrogenolysis conditions whereby Pd/C was replaced by $Pd(OH)_2$/C as the catalyst, the model compound **10** was converted to disaccharide **11** in a very good 85% yield, while no traces of hemiacetal **12** were detected.

Synthesis of the $B_{Ac}C(E)D$-Pr (47) and BC(E)D-Pr (48) tetrasaccharides.

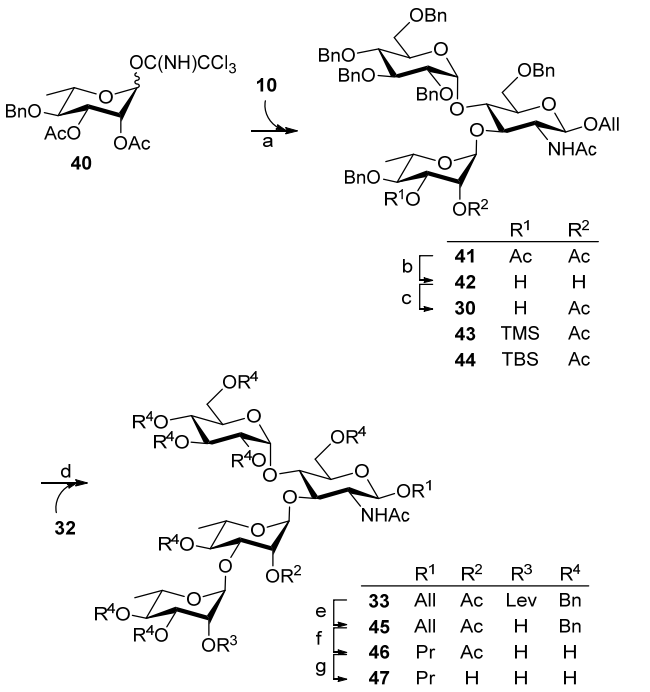
Alternatively, oxidative removal of the PMB ether of the orthogonally protected trisaccharide **22** using ceric ammonium nitrate (CAN) in $MeCN/H_2O$ enabled a fast and clean quantitative unmasking of OH-3_C, giving alcohol **30** as the sole regioisomer, as ascertained by NMR analysis of the crude material (Scheme 3). Owing to the propensity of acetyl groups occurring in *cis*-diol systems to migrate to the vicinal hydroxyl moiety during column chromatography, acceptor **30** was used without further purification in the reaction with the known 3,4-di-*O*-benzyl donor[9] **32**. Conventional TMSOTf-mediated glycosylation furnished tetrasaccharide **33** in an acceptable 58% yield from the fully protected **22**. Attempts to improve the yield of this two step transformation were met with complete failure (not described), and suggested poor reproducibility. Instead, we placed more confidence in the [BC + ED] coupling reaction. Thus, the rhamnosyl TCA **32** was reacted with allyl rhamnoside[49] **34** to furnish rhamnobioside[9] **35**, which in turn was converted to the corresponding TCA donor **37**, *via* hemiacetal **36**, as described.[14] In agreement with the best conditions identified for the [C + ED] coupling, the condensation of the latter with disaccharide **10** was set up in toluene at 55°C in the presence of 0.3 equiv. of TMSOTf. Despite these harsh conditions, the reaction did not go to completion, showing only a 50% conversion. Moreover, tetrasaccharide **33** could not be isolated as pure material. This route was therefore abandoned. Evidence for the poor reactivity of OH-3_D in acceptor **10** was provided by the formation of the α-(1↔1)-β-linked tetrasaccharide **38** (HRMS (ESI^+): *m/z* 1445.5952, calcd for $C_{80}H_{94}O_{23}Na$ $[M+Na]^+$ *m/z* 1445.6136; NMR spectroscopic data for C-1_C and C-$1_{C'}$: δ = 91.6 ppm, $^1J_{CH}$ = 161.9 Hz and 97.5 ppm, $^1J_{CH}$ = 175.2 Hz, respectively) arising as a major side reaction from the glycosylation of



Scheme 3 Synthesis of tetrasaccharide **33** via a [B + C(E)D] coupling or a [BC + ED] coupling. *Reagents and conditions:* a) CAN, $MeCN/H_2O$, rt; b) H_2, Pd/C, HCl, 90% aq EtOH, rt, 1 day, 74%; c) MeONa, MeOH, 69%; d) TMSOTf, Toluene, 55°C, 58% over two steps; e,f,g) see ref. 14; h) TMSOTf, Toluene, 55°C, 50% conversion at the most.

hemiacetal **36** with TCA **37**. The corresponding α-(1↔1)-α-linked tetrasaccharide **39** (HRMS (ESI^+): *m/z* 1445.5952, calcd for $C_{80}H_{94}O_{23}Na$ $[M+Na]^+$ *m/z* 1445.6084; NMR spectroscopic data for both C-1_C and C-$1_{C'}$: δ = 92.2 ppm, $^1J_{CH}$ = 173.5 Hz) was also isolated, albeit in a minimal amount. These recurrent discouraging outcomes led us to consider an alternative route.

Thus, chain elongation at OH-3_D of disaccharide **10** was next attempted with the 3,4-di-*O*-acetyl-4-*O*-benzyl-α/β-L-rhamnosyl trichloroacetimidate[50] (**40**). Donor **40** does not feature the required orthogonal set of protecting groups. However, it is more stable and more readily available (3 steps, 82%) than its 3-*O*-PMB counterpart (4 steps, 72%) starting from allyl 4-*O*-benzyl-α-L-rhamnoside,[51] a common precursor in our laboratory. Moreover, an easy differentiation between the 3_C-OH and 2_C-OH at the C(E)D trisaccharide level proceeded as anticipated (Scheme 4). To our satisfaction, the reaction conditions developed for the condensation of acceptor **10** and orthogonally protected donor **21** were successfully applied to



Scheme 4 Synthesis of tetrasaccharides $B_{Ac}C(E)D$-Pr (**46**) and BC(E)D-Pr (**47**) via a [B + C + (E)D] coupling. *Reagents and conditions:* a) TMSOTf, Toluene, 50°C, 81%; b) MeONa, MeOH, rt, 99%; c) i. $MeC(OMe)_3$, PTSA, MeCN, rt; ii. 80% aq AcOH, rt, 99%; d) See Table; e) $H_2NNH_2{\cdot}H_2O$, Pyr, AcOH, rt, 96%; f) H_2, Pd/C, HCl, 90% aq. EtOH, rt, 90%; g) MeONa, MeOH, rt, 60%.

Table 3 Study on the condensation of acceptor **30** and donor **32**.

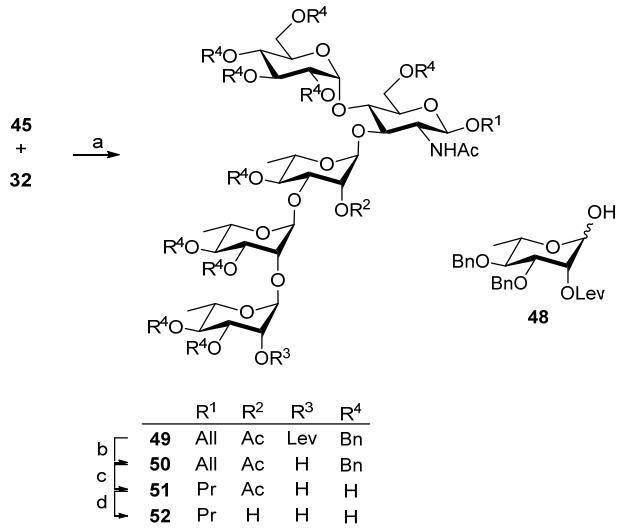
Entry	**32** equiv.	Promoter (0.2 equiv.)	Solvent / Temperature	**33** (corrected yield)	Side-products (yield)
1	1.2	TMSOTf	Et_2O / -15°C	34%	**43** (23%) **30**/**31** (29%)
2	1.2	TMSOTf	Toluene / rt	81%	- [#]
3	1.3	TMSOTf	Toluene / rt	74%	**43** (6%) **30**/**31** (11%)
4	1.3	TBSOTf	Toluene / rt	90%	- [#]
5	1.3	TBSOTf	Toluene / rt	83% (89%)	**44** (6%)

[#] The reaction was run on a small scale (150 mg of acceptor **30**).

the glycosylation of the same acceptor and donor **40**. Whether the condensation was performed on milligram or gram amounts of acceptor, trisaccharide **41** was isolated in an acceptable 81% yield. Conventional deacetylation gave diol **42** (99%), which was in turn regioselectively acetylated at the axial hydroxyl group to give acceptor **30**. This two-step conversion of the fully protected **41** into the 2_C-*O*-acetylated **30** (99% over two steps, no purification) can be compared advantageously with the former strategy (Scheme 2), especially since trisaccharide **30** is this time free of any interfering contaminants. Having identified a feasible route to a C(E)D acceptor having the 2_C-acetate already in place, its condensation with donor **32** was the next step (Table 3). Running the reaction at low temperature in diethyl ether in the presence of TMSOTf gave the coupling product in 34% yield, while silylation of the acceptor to give the unreactive trisaccharide **43** (23%) was identified as a major side reaction (Table 3, entry 1). To our satisfaction, changing diethyl ether for toluene and performing the reaction at room temperature resulted in an improved yield (81%) of tetrasaccharide **33** (Table 3, entry 2). However, the robustness of the conditions was questioned since large quantities of the silylated acceptor **43** (6%), and of the remaining **30** in combination with regioisomer **31** (11%), were isolated when working an a larger scale (Table 3, entry 3). Gratifyingly, when *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) was used as the promoter, the yield of tetrasaccharide **33** reached 90% (Table 3, entry 4). However, silylation of the acceptor resulting in trisaccharide **44** (6%) could not be avoided when working on gram amounts and the yield of the condensation dropped to 83% (Table 3, entry 5). As in the case of the trimethyl silyl derivative **43**, the silylated **44** was isolated as a single regioisomer.While the yield of the glycosylation was thought acceptable, the fact that silylation at the acceptor site occurs despite the use of room temperature and of the bulky TBSOTf as promoter suggests interference with glycosylation at the acceptor site. Removal of the 2_B-levulinyl ester of the glycosylation product **33** by reaction with hydrazine hydrate in pyridine/AcOH was high yielding (96%). The resulting alcohol **45** could serve either as an intermediate for the synthesis of $B_{Ac}C(E)D$-Pr and BC(E)D-Pr targets, or as an acceptor in the synthesis of pentasaccharide **51** (Scheme 5). In the first place, conventional Pd/C-promoted benzyl ether hydrogenolysis and concomitant allyl reduction gave the propyl glycoside **46** in an excellent 90% yield after RP-MPLC purification. As expected, the isolated 2_C-acetate was stable in conventional hydrogenolysis conditions. Moreover, although the same hydrogenolysis conditions were used for the final deprotection of trisaccharides **30**/**31** and tetrasaccharide **45**, no traces of the product of anomeric deallylation was detected in the later case. Subsequent treatment of the acetylated **46** with methanolic sodium methoxide gave the BC(E)D-Pr target **47** in a unoptimized 60% yield.

Synthesis of the $AB_{Ac}C(E)D$-Pr (51) and ABC(E)D-Pr (52) pentasaccharides.

Alternatively, alcohol **45** was reacted with the rhamnosyl TCA **32** under conditions adapted for the [B + C(E)D] coupling, except that trifluoromethanesulfonic acid (TfOH) replaced TMSOTf in order to avoid any parasitic silylation at the reactive hydroxyl group. The condensation evolved as desired. Nevertheless, contamination with hemiacetal **48** was observed repeatedly and pentasaccharide **49** could not be isolated as pure material (not shown). Thus, the polluted glycosylation product was treated under conventional delevulination conditions to give pentasaccharide **50**, whose 2_A hydroxyl group was unmasked, in an acceptable 63% yield over two steps (Scheme 5). Next, the one-step Pd/C-mediated benzyl ether hydrogenolysis and concomitant allyl reduction gave the $AB_{Ac}C(E)D$-Pr pentasaccharide (**51**) in an excellent 92% yield after RP-MPLC purification. To our satisfaction, no trace of the product of anomeric deallylation was detected at the pentasaccharide stage. This outcome substantiates the result of the hydrogenolysis of tetrasaccharide **45**. However, the differences in the behaviours of disaccharide **10** and trisaccharide **15** in comparison to the performances of trisaccharides **30**/**31**, tetrasaccharide **45** and pentasaccharide **50**, when submitted to similar Pd/C mediated deprotection conditions, remain unexplained. In particular, available data indicate that the change in the conformation of the pyranoside ring of a 3,4-di-*O*-substituted residue D does not account for the disparity in the results of the hydrogenolysis reactions. Last, treatment of the 2_C-acetylated **51** with methanolic sodium



	R1	R2	R3	R4
49	All	Ac	Lev	Bn
50	All	Ac	H	Bn
51	Pr	Ac	H	H
52	Pr	H	H	H

Scheme 5 Synthesis of the target pentasaccharides **51** and **52**. *Reagents and conditions:* a) TfOH, Toluene, rt; b) $H_2NNH_2{\cdot}H_2O$, Pyr, AcOH, rt, 63% over two steps; f) H_2, Pd/C, HCl, 90% aq. EtOH, rt, 92%; g) MeONa, MeOH, rt, 85%.

methoxide resulted in its smooth conversion to the corresponding ABC(E)D-Pr target **52**, which was isolated in a non optimized 85% yield following RP-MPLC purification.

The SF1a/SF1b-specific 3,4-di-*O*-glucosylation pattern of *N*-acetyl-glucosamine: C(E)D.

β- and α-glycosides of 3,4-di-*O*-glycosyl-2-acetamido-2-deoxy-D-glucopyranose have been synthesized in various instances, owing to the implication of such trisaccharides as components of carbohydrates of biological importance. They have been identified as part of plant *N*-glycans,[52] glycosphingolipids from parasites,[53, 54] lipopolysacharides others than those from *S. flexneri*,[55, 56] human milk oligosaccharides,[57] and more generally as key constituents of the human glycome, including Lewis determinants.[58] The latter have generated the most attractiveness in terms of synthesis. As a general trend, no synthetic difficulties associated to the 3,4-di-*O*-glycosylation of *N*-acetyl glucosamine have emerged, apart from observations related to the clearly established poor reactivity of the 4-OH group in a *N*-acetyl-β-D-glucosaminide acceptor. This phenomenon was studied exhaustively and even tentatively explained by use of computational analysis.[59] It did not occur as a problem herein, likely owing to the excellent reactivity of the involved glucosyl donors. In contrast, α-L-rhamnosylation at OH-3_D of the ED acceptor **10** was found problematic in our hands. Reaction temperatures of 50°C at least were necessary for the glycosylation to proceed. As a reward for our perseverance in the quest for optimization, a good 81% yield was achieved in the presence of minor excess of donor (1.2 equiv. for TCA **40**). The need for such uncommon reaction temperatures is supported by previous reports of low fucosylation yields at the 3-OH group of (1→4)-*O*-glycosyl-*N*-acetyl-β-D-glucosaminide acceptors,[60, 61] which were tentatively explained by steric hindrance at the reactive site. Moreover, analysis of the ^1H and ^{13}C NMR spectra collected for trisaccharides **22**, **30**, **41** and **42** revealed some unconventional features for several signals assigned to the *N*-acetyl-D-glucosamine residue D (Table 4). In contrast to data characterizing the βED acceptor **15** (Table 4, entry 3), which were consistent with the expected $^4C_1$ conformation for residue D, the coupling constants $J_{1,2}$ and $^1J_{CH}$ measured for *N*-acetyl-glucosamine D within the protected trisaccharides (Table 4, Entries 4 - 7) strongly differed from those predictable for a 2-*N*-acetyl-β-D-glucopyranoside.[62] The measured values suggested that the successful α-L-rhamnosylation at the 3_D-OH of disaccharide **10** had caused a major conformational change at the D ring. It can be hypothesized that the glycosylation reaction required heating to overcome the energy barrier associated to this change in conformation. Removal of the benzyl protecting groups resulted in conformational release at the vicinal glycosidic linkages, as attested by the $J_{1,2}$ and $^1J_{CH}$ values measured for trisaccharides **23** and **29** (Table 4, Entries 8 and 9).

Table 4 Partial NMR data measured for residue D as part of the ED disaccharides to ABC(E)D pentaccharides (400 MHz, $CDCl_3$ or D_2O, 298K).

Entry	Compound	$J_{1,2}$ (Hz)	$^1J_{CH}$ (Hz)
1	**10**	8.8*	163.8
2	**16**	8.6	161.0
3	**15**	8.3	162.8
4	**41**	3.7*	170.7
5	**42**	3.7*	169.5
6	**33**	3.2*	171.4
7	**50**	3.1*	170.7
8	**23**	7.4	163.9
9	**29**	7.8	162.9

* The H-1 signal partially overlapped with signals from benzylic protons. The $J_{1,2}$ coupling constant was measured using Jres NMR experiments.

Non-chair conformations of 3,4-di-*O*-glycosyl *N*-acetyl glucosamine have precedence. They are most often associated to poor experimental outcomes.[63] In complement to extensive support from NMR spectroscopy analysis,[64] and subsequent computational investigations,[59] convincing evidence for these unlikely conformations have more recently emerged from structural data.[65]

Conclusion

This study is part of a program aimed at a better understanding of the molecular specificity of recognition of *Shigella flexneri* LPS by protective mAbs by use of synthetic fragments of the O-Ags of interest. For the first time we have dealt with the synthesis of oligosaccharides specific for SF1a and SF1b. Except for the ED disaccharides, all novel oligosaccharides described herein have the 3,4-di-*O*-glycosyl-*N*-acetyl-β-D-glucosaminide moiety (C(E)D) in common. As previously observed in the course of the synthesis of Lewis determinants and analogues thereof, our findings on protected intermediates encompassing the C(E)D moiety clearly demonstrate that the *N*-acetyl-D-glucosamine ring (D) adopts a non conventional conformation. Conformational changes have occurred as a result of all successful ED to C(E)D conversions, independently of the protecting groups at the rhamnose donor (C). It is hypothesized that steric hindrance generated at OH-3_D by the vicinal tetra-*O*-benzyl-D-glucopyranosyl residue governs the glycosylation outcomes. Nevertheless, conditions involving only a slight excess (1.2 equiv.) of TCA donor **40** were found for a high yielding [C + ED] coupling. Satisfactorily, the change of conformation of residue D did not interfere with subsequent chain elongation at OH-3_C, with residues B and A, respectively. Unfavourable kinetics led to partial aglycon

cleavage upon final protecting group removal at the di- and trisaccharide level. To our satisfaction, no such side-reaction was observed in the case of the tetra- and pentasaccharides. Besides, the final deprotection step led to conformational release at the D ring of all oligosaccharides bearing the C(E)D branching pattern. The novel di- to pentasaccharides described herein – compounds **11**, **23**-**25**, **29**, **46**, **47**, **51**, and **52** – will serve as probes to clarify the structural requirements for SF1b and/or SF1a O-Ag molecular recognition by protective mAbs.

Experimental Section

General

Anhydrous (anhyd.) solvents – including toluene (Tol), dichloromethane (DCM), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), methanol (MeOH), acetonitrile (MeCN), and pyridine – were delivered on molecular sieves and used as received. Additional solvents cited in the text are abbreviated as cHex (cyclohexane), and EtOAc (ethyl acetate), in addition to acetone. Reactions requiring anhyd. conditions were run under an argon (Ar) atmosphere, using dried glassware. 4Å Molecular sieves (4 Å 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 (particle size 40-63 µm). RP-HPLC purification was carried out using a Kromasil 5 µm C18 100 Å 10 × 250 mm semi-preparative column. RP-MPLC purification was carried out using a NucleoPrep 20 µm C18 100 Å 26 × 313 cm semi-preparative column). Unless stated otherwise, analytical RP-HPLC of the final compounds (λ = 215 nm) was carried out using an Aeris Peptide 3.6 µm C_{18} 100 Å 2.1 × 100 mm analytical column, eluting with a 0-20% linear gradient of MeCN in 0.08% aq. TFA over 20 min at a flow rate of 0.3 mL•min^{-1}. 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 ($CDCl_3$), deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated water (D_2O). Chemical shifts are reported in ppm (δ) relative to residual solvent peak $CHCl_3$ in he case of $CDCl_3$, HOD and DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) in the case of D_2O, at 7.28/77.0, and 4.70/0.00 ppm for the ^{1}H and ^{13}C spectra, respectively. Coupling constants are reported in hertz (Hz). Elucidations of chemical structures were based on ^{1}H, COSY, DEPT-135, HSQC, decoupled HSQC, ^{13}C, decoupled ^{13}C, HMBC, and Jres. Signals are reported as 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 $_{po}$). 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 SF1b O-Ag and identified by a subscript in the listing of signal assignments. HRMS spectra were recorded on a WATERS QTOF Micromass instrument in the positive-ion electrospray ionisation (ESI$^+$) mode. Solutions were prepared using 1:1 MeCN/H_2O containing 0.1% formic acid. In the case of sensitive compounds, solutions were prepared using 1:1 MeOH/H_2O to which was added 10 mM ammonium acetate.

Allyl 2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (4): To a solution of acetal[36] **3** (6.54 g, 16.7 mmol) in anhyd. DCM (220 mL) containing activated 4Å MS (12.0 g), stirred at -78°C, were added dropwise first triethylsilane (8.3 mL, 52.0 mmol, 3.1 equiv.) over 5 min, then TMSOTf (3.1 mL, 35.0 mmol, 2.1 equiv.) over 12 min. The mixture was stirred for 2 h at -78°C. A TLC control (DCM/EtOAc 3:7) showed the conversion of the starting material (Rf 0.46) into a more polar product (Rf 0.20). The reaction was quenched by addition of solid $NaHCO_3$ (15.0 g) and MeOH (50 mL). The suspension was filtered over a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (300 mL), washed with 5% aq. citric acid (150 mL), 5% aq. $NaHCO_3$ (150 mL) and brine (150 mL), then dried over anhyd. Na_2SO_4 and concentrated to dryness. The crude was purified by flash chromatography (DCM/EtOAc 3:7 to 15:85) to give alcohol **4** (5.65 g, 86%) as a white foam. Compound **4** had ^{1}H NMR ($CDCl_3$) δ 7.39-7.29 (m, 5H, H_{Ar}), 5.87 (m, 1H, C*H*=CH_2), 5.73 (d, 1H, $J_{2,NH}$ = 9.2 Hz, NH), 5.27 (m, 1H, J_{trans} = 17.1 Hz, J_{gem} = 1.5 Hz, CH=CH_2), 5.18 (m, 1H, J_{cis} = 10.5 Hz, CH=CH_2), 5.08 (dd, 1H, $J_{3,4}$ = 9.1 Hz, $J_{2,3}$ = 10.6 Hz, H-3), 4.63 (d, 1H, J = 12.0 Hz, H_{Bn}), 4.59 (d_{po}, 1H, H_{Bn}), 4.56 (d_{po}, 1H, H-1), 4.34 (m, 1H, H_{All}), 4.08 (m, 1H, H_{All}), 3.97 (pdt, 1H, $J_{1,2}$ = 8.7 Hz, H-2), 3.83-3.78 (m, 2H, H-6a, H-6b), 3.74 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.56 (pdt, 1H, $J_{4,5}$ = 8.9 Hz, $J_{5,6a}$ = 10.6 Hz, H-5), 3.14 (bs, 1H, OH), 2.11 (s, 3H, CH_{3Ac}), 1.96 (s, 3H, CH_{3NHAc}) ; ^{13}C NMR ($CDCl_3$) δ 172.0 (CO_{Ac}), 170.2 (NHCO), 137.6 (C_{IVAr}), 133.8 (*C*H=CH_2), 128.5-127.7 (5C, C_{Ar}), 117.4 (CH=*C*H_2), 100.1 (C-1, $^1J_{C,H}$ = 160.5 Hz), 75.5 (C-3), 74.1 (C-5), 73.8 (C_{Bn}), 70.8 (C-4), 70.4 (C-6), 69.6 (C_{All}), 54.1 (C-2), 23.3 (CH_{3Ac}), 21.0 (CH_{3NHAc}) ; HRMS (ESI$^+$): *m*/*z* 416.1688 (calcd for $C_{20}H_{27}NO_7Na$ [M+Na]$^+$ *m*/*z* 416.1788).

Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (9) and Allyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (14): *Route 1.* MS 4 Å (162 mg) were added to a solution of acceptor **4** (100 mg, 253 µmol) and thioglycoside **5** (800 mg, 1.27 mmol, 5.0 equiv.) in Et_2O/DCM (2:5, 5.0 mL) at rt, under an atmosphere of Ar. The reaction mixture was cooled to 0°C and stirred at this temperature for 25 min, then NIS (341 mg, 152 mmol, 6.0 equiv.) and TMSOTf (23 µL, 126 µmol, 0.5 equiv.) were added. After stirring for 2.5 h at this temperature, TLC analyses revealed the conversion of acceptor **4** (DCM/ Me_2CO 1:1, R_f 0.53) to one major new product (DCM/Me_2CO 1:1, R_f 0.77 and cHex/EtOAc 1:1, R_f 0.18). The reaction was quenched with Et_3N (500 µL) and the reaction mixture was warmed to rt. The reaction mixture was filtered over a pad of Celite and concentrated *in vacuo* to a yellow oil, which was purified by flash column chromatography (cHex/EtOAc 4:1 → 1:1) to yield the coupling product as an inseparable 7:1 α/β mixture (164 mg, 64%).

Route 2. MS 4 Å (2.5 g) were added to a solution of acceptor **4** (1.0 g, 2.54 mmol) in Et_2O/DCM (3:2, 12.5 mL) at rt, under an atmosphere of Ar. After 15 min of stirring, the reaction mixture was cooled to -78°C, and TMSOTf (140 µL, 0.76 mmol, 0.3 equiv.) was added. After an additional 30 min of stirring, a solution of the TCA donor[38] **6** in a Et_2O/DCM (5:2, 7 mL) was added at a constant rate to the reaction mixture

at -78°C, over a period of 35 min. After stirring for 1 h at this temperature, the reaction mixture was allowed to reach 4°C overnight. After this time, TLC analyses revealed the conversion of acceptor **4** (DCM/Me_2CO 1:1, R_f 0.53) and donor **6** (cHex/EtOAc 1:1, R_f 0.61) to one major new product (DCM/Me_2CO 1:1, R_f 0.77 and cHex/EtOAc 1:1, R_f 0.18). The reaction was quenched with Et_3N (500 µL) and the reaction mixture was warmed to rt. The reaction mixture was filtered and concentrated *in vacuo* to a yellow oil, which was purified by flash column chromatography (cHex/EtOAc 4:1 → DCM/Me_2CO 7:3) to yield the coupling product as an inseparable 9:1 α/β mixture (1.97 g, 85%).

Route 3. Alcohol **4** (4.8 g, 12.2 mmol) was dissolved in DCM/Et_2O (1:5 180 mL) containing 4Å MS (10.0 g). The suspension was stirred for 25 min at rt under an atmosphere of Ar, then TMSOTf (470 µL, 2.6 mmol, 0.2 equiv.) was added. After 5 min, donor[41] **7** (11.1 g, 15.6 mmol, 1.2 equiv.) in DCM/Et_2O (1:3, 70 mL) was added dropwise over 50 min. The reaction was stirred for 2 h at rt. A TLC control (Tol/EtOAc 5:5) showed the disappearance of the acceptor **4** (Rf 0.05) and the presence of a less polar product (Rf 0.3). The reaction was quenched by addition of Et_3N (2 mL) and the suspension was filtered over a pad of Celite. Volatiles were removed under reduced pressure and the residue was purified by flash chromatography (Tol/EtOAc 7:3 to 65:35) to give in order of elution first the α-linked disaccharide **9** (8.50 g, 76%) and then the β-linked isomer **14** (1.2 g, 11%) both as white foams. The α anomer **9** had ^{1}H NMR ($CDCl_3$) δ 7.37-7.27 (m, 23H, H_{Ar}), 7.16-7.14 (m, 2H, H_{Ar}), 6.40 (d, 1H, $J_{2,NH}$ = 8.6 Hz, NH), 5.90 (m, 1H, C*H*=CH_2), 5.30 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.6 Hz, CH=C*H*$_2$), 5.20 (m, 1H, J_{cis} = 10.4 Hz, CH=C*H*$_2$), 5.08 (t_{po}, 1H, H-3_D), 5.07 (d_{po}, 1H, H-1_E), 4.90 (d, 1H, *J* = 10.9 Hz, H_{Bn}), 4.84 (d_{po}, 1H, H_{Bn}), 4.82 (d_{po}, 1H, *J* = 10.8 Hz, H_{Bn}), 4.74 (d, 1H, *J* = 11.8 Hz, H_{Bn}), 4.69 (d, 1H, H_{Bn}), 4.61 (d, 1H, $J_{1,2}$ = 5.0 Hz, H-1_D), 4.56 (d_{po}, 1H, H_{Bn}), 4.55 (d_{po}, 1H, *J* = 12.0 Hz, H_{Bn}), 4.49 (d, 2H, H_{Bn}), 4.38 (d, 1H, *J* = 12.2 Hz, H_{Bn}), 4.33 (m, 1H, H_{All}), 4.19 (ddd, 1H, $J_{2,3}$ = 5.7 Hz, H-2_D), 4.11-4.04 (m, 2H, H_{All}, H-4_D), 3.94 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3_E), 3.87-3.79 (m, 4H, H-$6a_D$, H-$6b_D$, H-5_D, H-5_E), 3.68 (pt_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4_E), 3.64 (dd_{po}, 1H, $J_{5,6a}$ = 3.3 Hz, $J_{6a,6b}$ = 11.0 Hz, H-$6a_E$), 3.58 (dd, 1H, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 9.8 Hz, H-2_E), 3.48 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, H-$6b_E$), 1.98 (s, 3H, CH_3), 1.83 (s, 3H, CH_{3NHAc}) ; ^{13}C NMR ($CDCl_3$) δ 170.3 (CO_{Ac}), 169.7 (NHCO), 138.6-137.4 (5C, C_{IVAr}), 133.9 (*C*H=CH_2), 128.7-127.6 (25C, C_{Ar}), 116.8 (CH=*C*H$_2$), 99.2 (C-1_D, $^1J_{C,H}$ = 164.7 Hz), 97.3 (C-1_E, $^1J_{C,H}$ = 169.5 Hz), 81.8 (C-3_E), 79.5 (C-2_E), 77.6 (C-4_E), 75.7 (C_{Bn}), 75.0 (2C, C_{Bn}, C-5_D), 73.9, 73.5, 73.2 (3C, C_{Bn}), 71.7 (C-4_D), 71.4 (C-5_E), 70.6 (C-3_D), 69.5 (C-6_D), 69.1 (C_{All}), 68.2 (C-6_E), 51.1 (C-2_D), 22.9 (CH_{3NHAc}), 20.9 (CH_{3Ac}) ; HRMS (ESI^+): *m/z* 938.4050 (calcd for $C_{54}H_{61}NO_{12}Na$ $[M+Na]^+$ *m/z* 938.4091).

The β anomer **14** had ^{1}H NMR ($CDCl_3$) δ 7.35-7.27 (m, 23H, H_{Ar}), 7.18.16 (m, 2H, H_{Ar}), 5.88 (m, 1H, C*H*=CH_2), 5.42 (d, 1H, $J_{2,NH}$ = 9.1 Hz, NH), 5.28 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.6 Hz, CH=C*H*$_2$), 5.19 (m, 1H, J_{cis} = 10.4 Hz, CH=C*H*$_2$), 5.11 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 9.0 Hz, H-3_D), 4.89 (d, 1H, *J* = 11.0 Hz, H_{Bn}), 4.83-4.77 (m, 3H, H_{Bn}), 4.70 (d, 1H, *J* = 11.2 Hz, H_{Bn}), 4.59 (d, 1H, *J* = 11.2 Hz, H_{Bn}), 4.69 (d, 1H, H_{Bn}), 4.57-4.51 (m, 3H, H_{Bn}, H-1_D), 4.47 (d_{po}, 1H, *J* = 11.8 Hz, H_{Bn}), 4.45 (d_{po}, 1H, *J* = 12.2 Hz, H_{Bn}), 4.37 (d_{po}, 1H, $J_{1,2}$ = 7.7 Hz, H-1_E), 4.35 (m, 1H, H_{All}), 4.08 (m, 1H, H_{All}), 4.03 (ddd_{po}, 1H, $J_{1,2}$ = 8.6 Hz, H-2_D), 4.11-4.04 (pt, 1H, $J_{4,5}$ = 9.2 Hz, H-4_D), 3.81 (dd, 1H, $J_{5,6a}$ = 4.0 Hz, $J_{6a,6b}$ = 11.0 Hz, H-$6a_D$), 3.78-3.67 (m, 3H, H-$6a_E$, H-$6b_E$, H-$6b_D$), 3.67 (pt_o, 1H, $J_{4,5}$ = 9.6 Hz, H-4_E), 3.53 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3_E), 3.48 (ddd, 1H, $J_{5,6b}$ = 1.9 Hz, H-5_D), 3.32 (dd_{po}, 1H, H-2_E), 3.30 (m_{po}, 1H, H-5_E), 2.01 (s, 3H, CH_3), 1.97 (s, 3H, CH_{3NHAc}) ; ^{13}C NMR ($CDCl_3$) δ 171.7 (CO_{Ac}), 170.0 (NHCO), 138.7-137.9 (5C, C_{IVAr}), 133.9 (*C*H=CH_2), 128.4-127.5 (25C, C_{Ar}), 117.3 (CH=*C*H$_2$), 102.8 (C-1_E, $^1J_{C,H}$ = 162.7 Hz), 100.2 (C-1_D, $^1J_{C,H}$ = 161.0 Hz), 84.8 (C-3_E), 82.5 (C-2_E), 77.6 (C-4_E), 75.5 (C_{Bn}), 75.3 (C-5_D), 75.0 (C_{Bn}), 74.9 (C-4_D), 74.8 (C_{Bn}), 74.4 (C-5_E), 73.3, 73.2 (2C, C_{Bn}), 72.7 (C-3_D), 69.5 (C_{All}), 68.8 (C-6_E), 67.8 (C-6_D), 56.0 (C-2_D), 23.4 (CH_{3NHAc}), 20.7 (CH_{3Ac}) ; HRMS (ESI^+): *m/z* 938.4114 (calcd for $C_{54}H_{61}NO_{12}Na$ $[M+Na]^+$ *m/z* 938.4091).

Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (10): Methanolic sodium methoxide (25% w/w, 350 µL, 1.53 mmol, 0.3 equiv.) was added to a solution of allyl glycoside **9** (6.23 g, 6.80 mmol) in anhyd. MeOH (100 mL), the solution was stirred at rt for 6 h, at which time a TLC control (Tol/EtOAc 5:5) showed the total conversion of the starting material (Rf 0.41) into a more polar product (Rf 0.29). The reaction was quenched with Dowex H^+ ion-exchange resin. Following filtration of the resin and concentration to dryness, the crude was purified by flash chromatography (Tol/EtOAc 55:45 to 3:7) to give alcohol **10** (5.77 g, 97%) as a white foam. Acceptor **10** had ^{1}H NMR ($CDCl_3$) δ 7.39-7.24 (m, 23H, H_{Ar}), 7.20-7.16 (m, 2H, H_{Ar}), 5.93 (m, 1H, C*H*=CH_2), 5.84 (d, 1H, $J_{2,NH}$ = 7.0 Hz, NH), 5.30 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.4 Hz, CH=C*H*$_2$), 5.21 (m, 1H, J_{cis} = 10.4 Hz, CH=C*H*$_2$), 5.03 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1_E), 4.95-4.82 (m, 5H, $J_{1,2}$ = 8.8 Hz, 4H_{Bn}, H-1_D), 4.74 (d, 1H, *J* = 12.0 Hz, H_{Bn}), 4.58 (d_{po}, 1H, *J* = 12.0 Hz, H_{Bn}), 4.56 (d_o, 1H, H_{Bn}), 4.50 (d_{po}, 1H, *J* = 10.9 Hz, H_{Bn}), 4.48 (d_{po}, 1H, *J* = 12.2 Hz, H_{Bn}), 4.43 (d_{po}, 1H, *J* = 12.2 Hz, H_{Bn}), 4.37 (m_{po}, 1H, H_{All}), 4.20-4.10 (m, 2H, H-3_D, H_{All}), 3.98 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3_E), 3.86-3.78 (m, 2H, H-5_E, H-$6a_D$), 3.71 (pdd, 1H, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 10.5 Hz, H-$6b_D$), 3.64-3.60 (m, 4H, H-4_E, H-4_D, H-5_D, H-$6a_E$), 3.56 (dd_{po}, 1H, H-2_E), 3.51-3.45 (m, 2H, H-2_D, H-$6b_E$), 2.01 (s, 3H, CH_{3NHAc}) ; ^{13}C NMR ($CDCl_3$) δ 170.8 (NHCO), 138.5-137.1 (5C, C_{IVAr}), 133.9 (*C*H=CH_2), 128.6-127.5 (25C, C_{Ar}), 117.6 (CH=*C*H$_2$), 100.1 (C-1_E, $^1J_{C,H}$ = 170.9 Hz), 99.0 (C-1_D, $^1J_{C,H}$ = 163.8 Hz), 82.2 (C-3_E), 81.3 (C-3_D), 79.3 (C-2_E), 77.7 (C-4_E), 75.7, 75.0 (2C, C_{Bn}), 74.5 (C-5_D), 74.0, 73.5, 73.2 (3C, C_{Bn}), 72.6 (C-4_D), 71.4 (C-5_E), 69.9 (C_{All}), 69.4 (C-6_D), 68.4 (C-6_E), 57.1 (C-2_D), 23.6 (CH_{3NHAc}) ; HRMS (ESI^+): *m/z* 896.3914 (calcd for $C_{52}H_{59}NO_{11}Na$ $[M+Na]^+$ *m/z* 896.3686).

Allyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (15): Methanolic sodium methoxide (25% w/w, 203 µL, 0.90 mmol, 0.3 equiv.) was added to a solution of a 9:1 mixture of allyl glycosides **9** and **14** (2.89 g, 3.00 mmol) in anhyd. MeOH (22.5 mL), at 0°C, under an atmosphere of Ar. The solution was stirred at rt overnight, at which time TLC analysis (cHex/EtOAc 3:7) showed the total conversion of the starting material (Rf 0.48) into one major more polar product (Rf 0.28) and one minor more polar product (Rf 0.22). The reaction was quenched with Dowex H^+ ion-exchange resin. Following filtration of the resin and concentration to dryness, the crude was purified by extensive flash chromatography (cHex/EtOAc 3:7) to give first the above described α-linked disaccharide **10** (2.18 g, 82%) as a white foam, then the β-linked analogue **15** (203 mg, 8%) as a solid. The latter was subsequently crystallized from MeOH. The crystalline material had mp = 198°C ; ^{1}H NMR ($CDCl_3$) δ 7.36-7.24 (m, 23H, H_{Ar}), 7.17-7.15

(m, 2H, H_{Ar}), 5.93 (m, 1H, C*H*=CH_2), 5.65 (d, 1H, $J_{2,NH}$ = 7.7 Hz, NH), 5.29 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.4 Hz, CH=C*H*$_2$), 5.21 (m, 1H, J_{cis} = 10.7 Hz, CH=C*H*$_2$), 5.01 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.92 (d, 1H, *J* = 10.9 Hz, H_{Bn}), 4.83 (d_{po}, 1H, H_{Bn}), 4.82 (d_o, 1H, H_{Bn}), 4.78 (d_{po}, 1H, H_{Bn}), 4.58 (d, 1H, *J* = 12.0 Hz, H_{Bn}), 4.51 (d_o, 1H, *J* = 10.4 Hz, H_{Bn}), 4.50 (d_{po}, 1H, H_{Bn}), 4.42 (d_{po}, 1H, H_{Bn}), 4.40 (d_{po}, 1H, *J* = 10.7 Hz, H_{Bn}), 4.37 (d_o, 1H, $J_{1,2}$ = 8.0 Hz, H-1_E), 4.36-4.33 (m, 2H, H_{Bn}, H_{All}), 4.17 (pdd_{po}, 1H, $J_{2,3}$ = 7.9 Hz, $J_{3,4}$ = 9.4 Hz, H-3_D), 4.12 (m_{po}, 1H, H_{All}), 3.72-3.55 (m, 8H, H-$6a_D$, H-$6b_D$, H-$6a_E$, H-$6b_E$, H-3_E, H-4_E, H-4_D, H-5_D), 3.50 (m, 1H, H-5_E), 3.42 (m, 1H, $J_{2,3}$ = 8.5 Hz, H-2_E), 3.30 (ddd, 1H, H-2_D), 2.02 (s, 3H, CH_{3NHAc}); ^{13}C NMR ($CDCl_3$) δ 170.6 (NHCO), 138.3-137.8 (5C, C_{IVAr}), 134.1 (*C*H=CH_2), 128.6-127.5 (25C, C_{Ar}), 117.6 (CH=*C*H$_2$), 103.3 (C-1_E, $^1J_{C,H}$ = 162.2 Hz), 98.9 (C-1_D, $^1J_{C,H}$ = 162.8 Hz), 84.6 (C-3_E), 81.9 (C-2_E), 81.5 (C-4_D), 77.7 (C-4_E), 75.8, 75.2, 75.0 (3C, C_{Bn}), 74.5 (C-5_D), 74.4 (C-5_E), 73.5, 73.2 (2C, C_{Bn}), 71.2 (C-3_D), 70.0 (C_{All}), 68.7 (2C, C-6_D, C-6_E), 58.0 (C-2_D), 23.8 (CH_{3NHAc}) ; HRMS (ESI^+): *m/z* 896.3940 (calcd for $C_{52}H_{59}NO_{11}Na$ $[M+Na]^+$ *m/z* 896.3986).

Propyl α-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (11) and α-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-α/β-D-glucopyranose (12): *Route 1.* Pd/C (670 mg) was added to a stirred solution of disaccharide **10** (584 mg, 668 µmol) in 90% aq. EtOH (67 mL). The suspension was stirred under an H_2 atmosphere for 2 days at rt, and filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-50% linear gradient of 80% aq. MeCN over 60 min at a flow rate of 20 mL•min^{-1}) gave first hemiacetal **12** (31 mg, 12%) as a 1.0:0.7 α/β mixture eluting at the solvent front, then disaccharide **11** (141 mg, 50%). Both compounds were isolated as white solids following repeated freeze-drying.

Route 2. Pd/C (50 mg) was added to a stirred solution of disaccharide **10** (50 mg, 57 µmol) in 96% aq. EtOH (5 mL) containing 1M aq. HCl (5 µL, 0.1 equiv.). The suspension was stirred under an H_2 atmosphere for 15 h, and filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-HPLC (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}) gave disaccharide **11** (17 mg, 70%) as a white solid following repeated freeze-drying.

Route 3. $Pd(OH)_2$/C (50 mg) was added to a stirred solution of disaccharide **10** (50 mg, 57 µmol) in 96% aq. EtOH (5 mL). The suspension was stirred under an H_2 atmosphere for 16 h, and filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-HPLC (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}) gave disaccharide **11** (21 mg, 85%) as a white solid following repeated freeze-drying.

The propyl glycoside **11** had ^{1}H NMR (D_2O) δ 5.40 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 4.51 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1_D), 3.92 (d, 1H, $J_{6a,6b}$ = 12.0 Hz, H-$6a_D$), 3.85-3.66 (m, 7H, H-$6a_E$, OCH_{2Pr}, H-3_D, H-$6b_D$, H-$6b_E$, H-2_D, H-5_E), 3.63 (pt_{po}, 2H, H-3_E, H-4_D), 3.58-3.50 (m, 3H, H-2_E, H-5_D, OCH_{2Pr}), 3.40 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4_E), 2.01 (s, 3H, CH_{3NHAc}), 1.53 (psex, 2H, *J* = 6.9 Hz, CH_{2Pr}), 0.85 (t, 3H, *J* = 7.4 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) δ 177.2 (NHCO), 103.6 (C-1_D, $^1J_{C,H}$ = 162.5 Hz), 102.2 (C-1_E, $^1J_{C,H}$ = 173.5 Hz), 79.5 (C-4_D), 77.2 (C-5_D*), 77.0 (C-3_D), 75.5 (C-3_E), 75.4 (C-5_E), 74.9 (OCH_{2Pr}), 74.3 (C-2_E*), 72.0 (C-4_E), 63.4 (C-6_D), 63.2 (C-6_E), 58.2 (C-2_D), 24.8, 24.7 (2C, CH_{3NHAc}, CH_{2Pr}), 12.2 (CH_{3Pr}) ; HRMS (ESI^+): *m/z* 448.1792 (calcd for $C_{17}H_{31}NO_{11}Na$ $[M+Na]^+$ *m/z* 448.1795) ; RP-HPLC (215 nm): R_t = 9.6 min.

For hemiacetal **12**, the α anomer had ^{1}H NMR (partial, D_2O) δ 5.39 (d_{po}, 1H, H-1_E), 5.17 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_D), 3.99 (dd, 1H, *J* = 9.1 Hz, *J* = 10.3 Hz, H-3_D), 3.93 (m_o, 1H, H-5_D), 3.89 (m_o, 1H, H-2_D), 4.84-3.70 (m, 4H, H-$6a_D$, H-$6a_E$, H-$6b_D$, H-$6b_E$), 3.72-3.62 (m_o, H-5_E, H-4_D, H-3_E), 3.57-3.53 (m_o, 1H, H-5_E), 3.39 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4_E), 2.01 (s, 3H, CH_{3NHAc}) ; ^{13}C NMR (D_2O) δ 177.2 (NHCO), 102.4 (C-1_E, $^1J_{C,H}$ = 171.6 Hz), 93.3 (C-1_D, $^1J_{C,H}$ = 171.6 Hz), 80.1 (C-4_D), 75.5 (C-3_E), 75.4 (C-5_E), 74.4 (C-2_E), 73.8 (C-3_D), 72.8 (C-5_D), 72.0 (C-4_E), 63.3 (C-6_E), 63.2 (C-6_D), 56.6 (C-2_D), 24.5 (CH_{3NHAc}) ; HRMS (ESI^+): *m/z* 406.1273 (calcd for $C_{14}H_{25}NO_{11}Na$ $[M+Na]^+$ *m/z* 406.1325) ; RP-HPLC (215 nm): R_t = 0.9 min

For hemiacetal **12**, the β anomer had ^{1}H NMR (D_2O) δ 5.40 (d_{po}, 1H, H-1_E), 4.71 (d_o, 1H, H-1_D), 4.84-3.70 (m, 4H, H-$6a_D$, H-$6a_E$, H-$6b_D$, H-$6b_E$), 3.72-3.62 (m_o, H-5_E, H-4_D, H-3_E), 3.57-3.53 (m_o, 2H, H-2_E, H-5_E), 3.39 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4_E), 2.01 (s, 3H, CH_{3NHAc}) ; ^{13}C NMR (D_2O) δ 177.4 (NHCO), 102.2 (C-1_E, $^1J_{C,H}$ = 172.7 Hz), 97.4 (C-1_D, $^1J_{C,H}$ = 161.8 Hz), 79.4 (C-4_D), 77.3 (C-5_D*), 77.0 (C-3_D), 75.5 (C-3_E), 75.4 (C-5_E), 74.3 (C-2_E), 72.0 (C-4_E), 63.4 (C-6_D), 63.2 (C-6_E), 59.3 (C-2_D), 24.8 (CH_{3NHAc}) ; HRMS (ESI^+): *m/z* 406.1273 (calcd for $C_{14}H_{25}NO_{11}Na$ $[M+Na]^+$ *m/z* 406.1325) ; RP-HPLC (215 nm): R_t = 0.9 min.

Propyl β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (16) and β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-α/β-D-glucopyranose (17): Pd/C (300 mg) was added to a stirred solution of disaccharide **14** (298 mg, 341 µmol) in 90% aq. EtOH (33 mL) containing 1M aq. HCl (38 µL, 0.1 equiv.). The suspension was stirred under an H_2 atmosphere for 2 days at rt. The suspension was filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-40% linear gradient of 80% aq. MeCN over 60 min at a flow rate of 20 mL•min^{-1}) gave first hemiacetal **17** (25 mg, 19%) and then disaccharide **16** (89 mg, 67%), both as white fluffy powders following repeated freeze-drying. The propyl glycoside **16** had ^{1}H NMR (D_2O) δ 4.51 (d, 2H, $J_{1,2}$ = 8.5 Hz, H-1_E, H-1_D), 3.96 (dd, 1H, $J_{5,6a}$ = 2.2 Hz, $J_{6a,6b}$ = 12.3 Hz, H-$6a_D$), 3.88 (dd, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{6a,6b}$ = 12.4 Hz, H-$6a_E$), 3.86-3.79 (m, 2H, H-$6b_D$, OCH_{2Pr}), 3.73-3.65 (m, 4H, H-$6b_E$, H-2_D, H-3_D, H-4_D), 3.59-3.44 (m, 4H, OCH_{2Pr}, H-5_E, H-3_E, H-5_E), 3.39 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_E), 3.28 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.2 Hz, H-2_E), 2.01 (s, 3H, CH_{3NHAc}), 1.53 (pβsex, 2H, *J* = 7.3 Hz, CH_{2Pr}), 0.85 (t, 3H, *J* = 7.4 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) δ 177.2 (NHCO), 105.2 (C-1_E, $^1J_{C,H}$ = 163.1 Hz), 103.6 (C-1_D, $^1J_{C,H}$ = 161.6 Hz), 81.5 (C-4_D), 78.7 (C-5_E), 78.2 (C-3_E), 77.4 (C-5_D), 75.8 (C-2_E), 75.1 (C-3_D), 75.0 (OCH_{2Pr}), 72.1 (C-4_E), 63.2 (C-6_E), 62.7 (C-6_D), 57.9 (C-2_D), 24.8 (CH_{3NHAc}), 24.7 (CH_{2Pr}), 12.2 (CH_{3Pr}) ; HRMS (ESI^+): *m/z* 448.1807 (calcd for $C_{17}H_{31}NO_{11}Na$ $[M+Na]^+$ *m/z* 448.1795) ; RP-HPLC (215 nm): R_t = 8.7 min.

Hemiacetal **17** had ^{1}H NMR (D_2O) δ 5.18 (d, 0.6H, $J_{1,2}$ = 2.4 Hz, H-$1_{D\alpha}$), 4.70 (d, 0.4H, H-$1_{D\beta}$), 4.51 (2d, 1H, $J_{1,2}$ = 7.9 Hz, H-1_E), 3.98-3.92 (m, 1H, H-$3_{D\alpha}$, H-$6a_{D\beta}$), 3.90-3.85 (m, 3.4H, H-$6a_E$, H-$6a_{D\beta}$, H-$6b_D$, H-$2_{D\alpha}$, H-$3_{D\beta}$), 3.81 (dd, 0.4H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 5.0 Hz, H-$6b_{D\beta}$), 3.73-3.65 (m, 3H, H-$6b_E$, H-$2_{D\beta}$, H-4_D, H-$5_{D\alpha}$), 3.59-3.44 (m, 4H, H-5_E, H-3_E, H-5_E), 3.57 (m, 0.4H, H-$5_{D\alpha}$), 3.52-3.45 (m, 2H, H-3_E, H-5_E), 3.42-3.36 (m, 1H, H-4_E), 3.32-3.26 (m, 1H, H-2_E), 2.02 (s, 3H, CH_{3NHAc}) ; ^{13}C NMR (D_2O) δ 177.4, 177.1 (NHCO), 105.2 (C-1_E, $^1J_{C,H}$ = 162.2 Hz), 97.5 (C-$1_{D\beta}$, $^1J_{C,H}$ = 161.7 Hz), 93.2 (C-

$1_{D\alpha}$, $^1J_{C,H}$ = 173.0 Hz), 81.8, 81.4 (C-$5_{D\alpha}$), 78.6 (C-5_E), 78.2 (C-3_E), 77.4 (C-$5_{D\beta}$), 75.8 (C-2_E), 75.0 (C-4_D), 72.9 (C-$3_{D\alpha}$), 72.1 (C-4_E), 71.9 (C-$3_{D\beta}$), 63.2 (C-6_E), 62.7 (C-$6_{D\beta}$), 62.6 (C-$6_{D\alpha}$), 59.0 (C-$2_{D\beta}$), 56.5 (C-$2_{D\alpha}$), 24.9, 24.6 (CH_{3NHAc}) ; HRMS (ESI^+): *m/z* 406.1281 (calcd for $C_{14}H_{25}NO_{11}Na$ $[M+Na]^+$ *m/z* 406.1325) ; RP-HPLC (215 nm): R_t = 1.1 min.

Allyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-*para*-methoxybenzyl-α-L-rhamnopyranoside (19): Alcohol[16] **18** (10.3 g, 25.1 mmol) was stirred overnight in Ac_2O/pyridine (1:1, 20 mL). TLC analysis (cHex/EtOAc 7/3) showed the total conversion of the starting material into a less polar product (Rf 0.5). MeOH (40 mL) was added dropwise to the suspension stirred at 0°C. After being stirred at rt for an additional 3 h, the reaction mixture was concentrated under vacuum. Volatiles were coevaporated repeatedly with cyclohexane and toluene. The residue was dissolved in EtOAc and the organic phase was washed twice with 5% aq HCl, water, and satd aq. $NaHCO_3$ and then dried over Na_2SO_4. Volatiles were evaporated. Column chromatography (cHex/EtOAc 9:1 to 6:4) of the crude material gave the fully protected rhamnoside **19** as a yellow oil (10.26 g, 91%). Allyl glycoside **19** had ^{1}H NMR ($CDCl_3$), 7.39-7.27 (m, 7H, H_{Ar}), 6.88-6.85 (m, 2H, H_{ArPMB}), 5.90 (m, 1H, $CH{=}_{All}$), 5.40 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 5.29 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.6 Hz, $=CH_{2All}$), 5.24 (m, 1H, J_{cis} = 10.4 Hz, $=CH_{2All}$), 4.93 (d, 1H, *J* = 10.9 Hz, H_{Bn}), 4.79 (d, 1H, H-1), 4.66 (d, 1H, *J* = 10.9 Hz, H_{PMB}), 4.62 (d, 1H, H_{Bn}), 4.48 (d, 1H, H_{PMB}), 4.17 (m, 1H, H_{All}), 4.01-3.96 (m, 2H, H_{All}, H-3), 3.81 (s_{po}, 3H, CH_{3PMB}), 3.80 (dq_{po}, 1H, H-5), 3.44 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 2.17 (s, 3H, CH_{3Ac}), 1.35 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6) ; ^{13}C NMR ($CDCl_3$), 170.4 (CO), 159.3 (C_{IVPMB}), 138.6 (C_{IVBn}), 133.6 ($CH{=}_{All}$), 130.2 (C_{IVPMB}), 129.7, 128.3, 128.2, 127.6 (7C, C_{Ar}), 117.5 ($=CH_{2All}$), 113.9 (2C, C_{ArPMB}), 96.8 (C-1), 80.1 (C-4), 77.8 (C-3), 75.4 (C_{Bn}), 71.4 (C_{PMB}), 69.1 (C-2), 68.0 (CH_{2All}), 67.8 (C-5), 55.2 (CH_{3PMB}), 21.1 (CH_{3Ac}), 18.2 (C-6) ; HRMS (ESI+): *m/z* 479.2036 (calcd for $C_{26}H_{32}O_7Na$ $[M+Na]^+$: *m/z* 479.2046).

2-*O*-Acetyl-4-*O*-benzyl-3-*O*-*para*-methoxybenzyl-α/β-L-rhamnopyranose (20): The [Ir] complex (560 mg, 66 µmol, 0.03 equiv.) was dissolved in anhyd. THF (90 mL) and the solution was degassed repeatedly. Hydrogen was bubbled through the solution for 15 min, resulting in a yellow coloration. The solution was again degassed repeatedly, before being poured into a solution of allyl rhamnoside **19** (10.14 g, 22.0 mmol) in anhyd. THF (90 mL). The mixture was stirred under Ar at rt for 2.5 h. Follow up by TLC (Tol/EtOAc 9:1) showed the conversion of the starting glycoside into a slightly less polar intermediate. A solution of iodine (12.4 g, 49 mmol, 2.0 equiv.) in THF/H_2O (4:1, 80 mL) was added, and the mixture was stirred for 2 h at rt. A TLC control (cHex/EtOAc 6:4) showed the conversion of the intermediate compound (Rf 0.68) to a more polar product (Rf 0.37). The reaction was quenched with 10% aq. sodium bisulfate (100 mL). The reaction mixture was concentrated to 1/3rd of its volume and DCM (500 mL) was added. The organic layer was washed twice with water and twice with brine, dried by passing through a phase separator filter and concentrated to dryness. Column chromatography (cHex/EtOAc 7:3 to 1:1) of the crude material gave hemiacetal **20** (α/β 4:1) as a yellow oil (8.14 g, 97%). The □□anomer had ^{1}H NMR ($CDCl_3$), *δ* 7.36-7.23 (m, 7H, H_{Ar}), 6.86 (m, 2H, H_{ArPMB}), 5.41 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.17 (d, 1H, H-1), 4.93 (d, 1H, *J* = 11.0 Hz, H_{Bn}), 4.66 (d, 1H, *J* = 10.9 Hz, H_{PMB}), 4.63 (d, 1H, H_{Bn}), 4.49 (d, 1H, H_{PMB}), 4.04-3.92 (m, 2H, H-3, H-5), 3.81 (s, 3H, CH_{3PMB}), 3.44 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 2.75 (bs, 1H, OH), 2.17 (s, 3H, CH_{3Ac}), 1.34 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6). ^{13}C NMR ($CDCl_3$), *δ* 165.8 (CO), 159.2 (C_{IVPMB}), 138.5 (C_{IVBn}), 130.3 (C_{IVPMB}), 129.9, 129.6, 128.4, 128.3, 128.1, 127.6, (12C, 11C_{Ar}), 113.7 (2C, C_{ArPMB}), 92.5 (C-1), 80.0 (C-4), 77.2 (C-3), 75.3 (C_{Bn}), 71.3 (C_{PMB}), 69.5 (C-2), 67.8 (C-5), 55.2 (CH_{3PMB}), 21.2 (CH_{3Ac}), 18.0 (C-6). HRMS (ESI+): *m/z* 439.1730 (calcd for $C_{23}H_{28}O_7Na$ $[M+Na]^+$: *m/z* 439.1733).

The β anomer had ^{1}H NMR (partial, $CDCl_3$), *δ* 7.36-7.23 (m, 7H, H_{Ar}), 6.86 (m, 2H, H_{ArPMB}), 5.52 (dd, 1H, $J_{1,2}$ = 1.2 Hz, H-2), 4.92 (d,1H, *J* = 10.9 Hz, H_{Bn}), 4.84 (d, 1H, H-1), 4.71 (d, 1H, *J* = 10.8 Hz, H_{PMB}), 4.46 (d, 1H, H_{PMB}), 3.81 (s, 3H, CH_{3PMB}), 3.64 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 3.40 (pt, 1H, $J_{4,5}$ = 9.1 Hz, H-4), 2.52 (bs, 1H, OH), 2.24 (s, 3H, CH_{3Ac}), 1.38 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6). ^{13}C NMR (partial, $CDCl_3$), *δ* 171.0 (CO), 159.4 (C_{IVPMB}), 138.3 (C_{IVBn}), 129.6, 128.3, 127.6 (7C, 7C_{Ar}), 114.0 (2C, C_{ArPMB}), 92.9 (C-1), 79.7 (C-3), 79.1 (C-4), 77.2 (C-5), 75.2 (C_{Bn}), 71.3 (C_{PMB}), 69.8 (C-2), 55.2 (CH_{3PMB}), 21.3 (CH_{3Ac}), 18.2 (C-6). HRMS (ESI^+): *m/z* 439.1730 (calcd for $C_{23}H_{28}O_7Na$ $[M+Na]^+$: *m/z* 439.1733).

2-*O*-Acetyl-4-*O*-benzyl-3-*O*-*para*-methoxybenzyl-α/β-L-rhamnopyranosyl trichloroacetimidate (21): Hemiacetal **20** (8.14 g, 19.5 mmol) was dissolved in anhyd. DCE (30 mL). Trichloroacetonitrile (10.7 mL, 107 mmol, 5.5 equiv.) and DBU (1.0 mL, 6.7 mmol, 0.34 equiv.) were added at -5°C. The mixture was stirred under an Ar atmosphere for 3 h. Follow up by TLC (cHex/EtOAc 7:3 + 1% Et_3N) showed that the conversion of the starting material (Rf 0.12) into a less polar product (Rf 0.32) was completed. The mixture was concentrated to half of its volume and directly purified by flash chromatography (cHex/EtOAc 8:2 + 1% Et_3N) to give TCA **21** (10.25 g, 94 %) as a brownish oil (α/β 4:1). The α anomer had ^{1}H NMR ($CDCl_3$), *δ* 8.67 (s, 1H, NH), 7.39-7.26 (m, 7H, H_{Ar}), 6.89 (m, 2H, H_{ArPMB}), 6.19 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.48 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 4.94 (d, 1H, *J* = 10.8 Hz, H_{Bn}), 4.68 (d, 1H, *J* = 10.9 Hz, HPMB), 4.64 (d, 1H, H_{Bn}), 4.53 (d, 1H, H_{PMB}), 3.99 (dd_{po}, 1H, H-3), 3.95 (dq, 1H, H-5), 3.81 (s, 3H, CH_{3PMB}), 3.52 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 2.21 (s, 3H, CH_{3Ac}), 1.36 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6) ; ^{13}C NMR ($CDCl_3$), *δ* 170.0 (CO), 160.1 (C=NH), 159.4 (C_{IVPMB}), 138.2 (C_{IVBn}), 129.7 (C_{IVPMB}), 129.9, 128.4, 128.1, 127.9 (7C, 7C_{Ar}), 113.9 (2C, C_{ArPMB}), 95.3 (C-1), 90.9 (CCl_3), 79.3 (C-4), 76.8 (C-3), 75.6 (CBn), 71.6 (C_{PMB}), 70.8 (C-5), 67.7 (C-2), 55.2 (CH_{3PMB}), 21.0 (CH_{3Ac}), 18.0 (C-6). HRMS (ESI^+): *m/z* 582.0862 (calcd for $C_{25}H_{28}NO_7Na$ $[M+Na]^+$: *m/z* 582.0829).

Allyl (2-*O*-acetyl-4-*O*-benzyl-3-*O*-*para*-methoxybenzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (22): Activated MS 4 Å (418 mg) were added to a solution of disaccharide **10** (167 mg, 0.19 mmol) and orthogonally protected donor **21** (161 mg, 0.29 mmol, 1.5 equiv.) in toluene (2.9 mL). The suspension was stirred at rt, under an atmosphere of Ar, for 30 min, then heated to 40°C. Trimethylsilyl trifluoromethanesulfonate (10 µL, 0.06 mmol, 0.3 equiv.) was added and the reaction mixture was stirred for 1.5 h at this temperature. TLC analysis (cHex/EtOAc 1:1) indicated consumption of acceptor **10** (Rf 0.19) and donor **19** (Rf 0.77) and the formation of one new major product (Rf 0.57). The reaction was quenched with Et_3N (500 µL) and warmed to rt. The reaction mixture was filtered over a pad of Celite and the filtrate was concentrated *in vacuo* to a yellow oil.

Purification of the crude by flash column chromatography (cHex/EtOAc 4:1 to 1:1) gave trisaccharide **22** (197 mg, 81%) as a white foam, followed by the unreacted acceptor **10** (14 mg, 9%). The fully protected trisaccharide **22** had ^{1}H NMR ($CDCl_3$) δ 7.37-7.26 (m, 33H, NH, H_{Ar}), 6.80 (m, 2H, H_{ArPMB}), 5.99 (m, 1H, C*H*=CH_2), 5.49 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.1 Hz, H-2_C), 5.35 (m, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.7 Hz, CH=CH_2), 5.23 (m, 1H, J_{cis} = 10.5 Hz, CH=CH_2), 5.07 (d, 1H, H-1_C), 4.93 (d_{po}, 1H, H_{Bn}), 4.89 (bs, 2H, H_{Bn}), 4.82 (d_o, 1H, H-1_E), 4.81 (d_o, 1H, H_{Bn}), 4.80 (d_o, 1H, H_{Bn}), 4.78 (d_o, 1H, $J_{1,2}$ = 1.6 Hz, H-1_D), 4.67 (d_{po}, 1H, J = 12.0 Hz, H_{Bn}), 4.64 (d_{po}, 1H, H_{Bn}), 4.62 (d_{po}, 1H, J = 11.6 Hz, H_{Bn}), 4.55 (d_o, 1H, H_{Bn}), 4.45 (d, 1H, J = 10.7 Hz, H_{Bn}), 4.35 (d, 1H, J = 10.5 Hz, H_{Bn}), 4.34 (bs, 2H, H_{Bn}), 4.33-4.29 (m_o, 2H, H-2_D, H_{All}), 4.28 (d_{po}, 1H, J = 12.1 Hz, H_{Bn}), 4.09-3.90 (m, 6H, H-5_D, H_{All}, H-3_D, H-3_E, H-4_D, H-$6a_D$), 3.82 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3_C), 3.78-3.70 (m, 6H, CH_{3PMB}, H-$6b_D$, H-4_E, H-5_E), 3.67-3.60 (m, 3H, H-5_C, H-$6a_E$, H-2_E), 3.45 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_C), 3.35 (bd, 1H, $J_{6a,6b}$ = 10.0 Hz, H-$6b_E$), 2.22 (s, 3H, CH_{3Ac}), 1.76 (s, 3H, CH_{3NHAc}), 1.33 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C) ; ^{13}C NMR ($CDCl_3$) δ 169.9 (2C, CO_{Ac}, NHCO), 159.4 (C_{IVPMB}), 138.5-137.2 (6C, C_{IVAr}), 134.1 (*C*H=CH_2), 130.0 (C_{IVPMB}), 129.7-127.5 (32C, C_{Ar}), 116.8 (CH=*C*H_2), 113.8 (2C, CH_{PMB}), 98.0 (C-1_D, $^1J_{C,H}$ = 169.3 Hz), 97.0 (C-1_E, $^1J_{C,H}$ = 170.6 Hz), 95.9 (C-1_C, $^1J_{C,H}$ = 172.4 Hz), 82.1 (C-3_E), 79.7 (C-4_C), 78.9 (C-2_E), 77.7 (C-3_C), 77.5 (C-4_E), 75.9, 75.3, 75.0 (3C, C_{Bn}), 74.8 (C-5_D), 74.4, 73.4, 73.1 (3C, C_{Bn}), 71.4 (C-5_E), 71.1 (C_{Bn}), 70.9 (C-6_D), 70.8 (C-3_D), 69.4 (C_{All}), 68.9 (C-4_D), 68.8 (C-5_C), 68.2 (C-2_C), 67.6 (C-6_E), 55.2 (CH_{3PMB}), 46.5 (C-2_D), 22.7 (CH_{3NHAc}), 21.2 (CH_{3Ac}), 18.2 (C-6_C) ; HRMS (ESI$^+$): *m/z* 1294.5577 (calcd for $C_{75}H_{85}NO_{17}Na$ [M+Na]$^+$ *m/z* 1294.5715).

Propyl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (23), Propyl 3-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (24), and Propyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (25):

Route 1. To a stirred solution of trisaccharide **20** (281 mg, 221 µmol) in 96% aq. EtOH (35 mL) containing 1M aq. HCl (38 µL), were added Pd/C (284 mg). The suspension was stirred under an H_2 atmosphere for 2 days at rt. The reaction mixture was filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-40% linear gradient of 80% aq. MeCN over 60 min at a flow rate of 20 mL•min^{-1}) gave by order of elution a complex mixture of monoacetylated hemiacetals **26**, **27** and **28** (29 mg, 23%) and then a 4:5:1 mix of the acetylated propyl glycosides **23**, **24**, and **25** (63 mg, 47%), all as white powders following repeated freeze-drying.

Route 2. To a stirred solution of trisaccharide **31**/**32** (150 mg, 130 µmol) in 90% aq. EtOH (7.0 mL), were added Pd/C (213 mg) and 1M aq. HCl (20 µL). The suspension was stirred under H_2 atmosphere for a day at rt. After this time, MS analysis revealed a molecular weight corresponding to that of the target trisaccharide and the absence of any benzylated intermediates. The reaction mixture was filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-50% linear gradient of MeCN over 60 min at a flow rate of 20 mL•min^{-1}) gave the deprotected material (59 mg, 74%) as a white powder following repeated freeze-drying. NMR analysis indicated that the isolated product was a 4:5:1 mix of 3 regioisomers, corresponding to trisaccharides **23**, **24** and **25** respectively.

The 2$_C$-*O*-acetyl product **23** had ^{1}H NMR (D_2O) δ 5.42 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1_E), 5.11 (bs, 1H, H-1_C), 5.06 (bs, 1H, H-2_C), 4.55 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1_D), 4.07 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.2 Hz, H-3_D), 3.96-3.77 (m, 7H, H-4_D, H-$6a_D$, OCH_{2Pr}, H-3_C, H-5_C, H-$6b_D$, H-$6a_E$), 3.75 (dd_{po}, 1H, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 12.8 Hz, H-$6b_E$), 3.70 (m, 1H, H-5_D), 3.66-3.48 (m, 6H, H-5_E, H-3_E, H-2_E, OCH_{2Pr}), 3.46 (pt_{po}, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4_C), 3.44 (pt_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_E), 2.14 (s, 3H, CH_{3Ac}), 1.99 (s, 3H, CH_{3NHAc}), 1.54 (psex, 2H, J = 6.4 Hz, CH_{2Pr}), 1.27 (d_{po}, 3H, $J_{5,6}$ = 5.6 Hz, H-6_C), 0.85 (t, 3H, J = 7.4 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) δ 177.0 (NHCO), 175.8 (CO_{Ac}), 103.5 (C-1_D, $^1J_{C,H}$ = 163.9 Hz), 100.3 (C-1_C, $^1J_{C,H}$ = 171.6 Hz), 100.0 (C-1_E, $^1J_{C,H}$ = 173.8 Hz), 81.4 (C-3_D), 78.4 (C-5_D), 75.4 (2C, C-5_E, C-3_E), 75.2 (C-2_C), 74.8 (2C, OCH_{2Pr}, C-4_C), 74.3 (C-4_D), 73.8 (C-2_E), 72.6 (C-5_C), 71.9 (C-4_E), 71.1 (C-3_C), 63.9 (C-6_D), 63.0 (C-6_E), 56.9 (C-2_D), 25.0 (CH_{3NHAc}), 24.8 (CH_{2Pr}), 23.1 (CH_{3Ac}), 19.4 (C-6_C), 12.3 (CH_{3Pr}) ; HRMS (ESI$^+$): *m/z* 614.2626 (calcd for $C_{25}H_{44}NO_{16}$ [M+H]$^+$ *m/z* 614.2660), HRMS (ESI$^+$): *m/z* 636.2496 (calcd for $C_{25}H_{43}NO_{16}Na$ [M+Na]$^+$ *m/z* 636.2479), HRMS (ESI$^+$): *m/z* 1249.5182 (calcd for $C_{50}H_{86}N_2O_{32}Na$ [2M+Na]$^+$ *m/z* 1249.5061) ; RP-HPLC (215 nm): R_t = 11.7 min.

The 3$_C$-*O*-acetyl product **24** had ^{1}H NMR (D_2O) δ 5.41 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1_E), 5.06 (bs, 1H, H-1_C), 4.97 (dd, 1H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.9 Hz, H-3_C), 4.54 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1_D), 4.02 (bdd, 1H, $J_{1,2}$ = 2.0 Hz, H-2_C), 4.00 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.7 Hz, H-3_D), 3.91-3.77 (m, 6H, H-4_D, H-$6a_D$, H-5_C, OCH_{2Pr}, H-$6b_D$), 3.75 (dd_{po}, 1H, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 12.8 Hz, H-$6a_E$), 3.66-3.61 (m, 5H, H-5_E, OCH_{2Pr}, H-5_D, H-4_C, H-3_E), 3.59 (dd_{po}, 1H, H-2_E), 3.43 (pt_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_E), 2.14 (s, 3H, CH_{3Ac}), 2.02 (s, 3H, CH_{3NHAc}), 1.54 (psex, 2H, J = 6.4 Hz, CH_{2Pr}), 1.28 (d_{po}, 3H, $J_{5,6}$ = 5.8 Hz, H-6_C), 0.85 (t, 3H, J = 7.4 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) δ 176.6 (NHCO), 176.1 (CO_{Ac}), 103.5 (C-1_D, $^1J_{C,H}$ = 163.9 Hz), 101.6 (C-1_C, $^1J_{C,H}$ = 171.6 Hz), 99.9 (C-1_E, $^1J_{C,H}$ = 173.8 Hz), 83.6 (C-3_D), 77.9 (C-5_D), 75.9 (C-3_C),75.5 (C-4_D), 75.4 (2C, C-5_E, C-3_E), 74.9 (OCH_{2Pr}), 73.9 (C-2_E), 72.6 (C-5_C), 72.2 (C-4_C), 71.9 (C-4_E), 71.2 (C-2_C), 63.7(C-6_D), 63.0 (C-6_E), 56.5 (C-2_D), 24.8 (2C, CH_{3NHAc}, CH_{2Pr}), 22.9 (CH_{3Ac}), 19.5 (C-6_C), 12.3 (CH_{3Pr}) ; HRMS (ESI$^+$): *m/z* 614.2626 (calcd for $C_{25}H_{44}NO_{16}$ [M+H]$^+$ *m/z* 614.2660), HRMS (ESI$^+$): *m/z* 636.2496 (calcd for $C_{25}H_{43}NO_{16}Na$ [M+Na]$^+$ *m/z* 636.2479), HRMS (ESI$^+$): *m/z* 1249.5182 (calcd for $C_{50}H_{86}N_2O_{32}Na$ [2M+Na]$^+$ *m/z* 1249.5061) ; RP-HPLC (215 nm): R_t = 11.7 min.

The 4$_C$-*O*-acetyl product **25** had ^{1}H NMR (D_2O) (partial NMR) δ 5.38 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1_E), 5.09 (bs, 1H, H-1_C), 4.85 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4_C), 2.14 (s, 3H, CH_{3Ac}), 2.02 (s, 3H, CH_{3NHAc}), 1.54 (psex, 2H, J = 6.4 Hz, CH_{2Pr}), 1.15(d_{po}, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C), 0.85 (t, 3H, J = 7.4 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) (partial NMR) δ 176.7 (NHCO), 176.4 (CO_{Ac}), 103.5 (C-1_D, $^1J_{C,H}$ = 163.9 Hz), 102.6 (C-1_C, $^1J_{C,H}$ = 170.3 Hz), 82.0 (C-3_D), 78.4 (C-5_D), 75.1 (OCH_{2Pr}), 73.9 (C-2_E), 72.9 (C-5_C), 70.8 (C-2_C), 70.5 (C-3_C), 63.8 (C-6_D), 63.0 (C-6_E), 56.8 (C-2_D), 24.8 (CH_{2Pr}), 23.1 (CH_{3Ac}), 19.3 (C-6_C), 12.3 (CH_{3Pr}) ; HRMS (ESI$^+$): *m/z* 614.2626 (calcd for $C_{25}H_{44}NO_{16}$ [M+H]$^+$ *m/z* 614.2660) ; HRMS (ESI$^+$): *m/z* 636.2496 (calcd for $C_{25}H_{43}NO_{16}Na$ [M+Na]$^+$ *m/z* 636.2479) ; HRMS (ESI$^+$): *m/z* 1249.5182 (calcd for $C_{50}H_{86}N_2O_{32}Na$ [2M+Na]$^+$ *m/z* 1249.5061) ; RP-HPLC (215 nm): R_t = 11.7 min.

Regioisomers **26-28** had ^{1}H NMR (D_2O, partial) δ 5.45-5.40 (m, 1H, H-$1_{E26\alpha}$, H-$1_{E27\alpha}$, H-$1_{E28\alpha}$, H-$1_{E26\beta}$, H-$1_{E27\beta}$, H-$1_{E28\beta}$),

5.20 (d, 0.7H, $J_{1,2}$ = 3.3 Hz, H-$1_{D26\alpha}$, H-$1_{D27\alpha}$, H-$1_{D28\alpha}$), 5.17 (d, 0.4H, $J_{1,2}$ = 1.6 Hz, H-$1_{C26\alpha}$), 5.13-5.10 (m, 0.9H, H-$2_{C26\alpha}$, H-$1_{C27\alpha}$, H-$1_{C28\alpha}$, H-$1_{C26\beta}$, H-$1_{C28\beta}$), 5.08-5.06 (m, 0.3H, H-$2_{C26\beta}$, H-$1_{C27\beta}$), 4.98 (dd, 0.1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.8 Hz, H-$3_{C27\beta}$), 4.96 (dd, 0.25H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 8.3 Hz, H-$3_{C27\alpha}$), 4.83 (dd, 0.2H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-$4_{C28\alpha}$), 4.76-4.72 (m, 0.34H, H-$1_{D26\beta}$, H-$1_{D27\beta}$, H-$1_{D28\beta}$), 2.14, 2.05, 2.04, 2.02, 2.00 (5s, 6H, CH_{3Ac}**26**, CH_{3Ac}**27**, CH_{3Ac}**28**, CH_{3NHAc}**26**, CH_{3NHAc}**27**, CH_{3NHAc}**28**), 1.29-1.24, 1.16-1.13 (m, 3H, H-6_{C26}, H-6_{C27}, H-6_{C28}) ; ^{13}C NMR (D_2O) δ 176.9, 176.8, 176.1, 175.9 ($NHCO_{26}$, $NHCO_{27}$, $NHCO_{28}$, CO_{Ac26}, CO_{Ac27}, CO_{Ac28}), 102.8 (C-$1_{C26\beta*}$), 102.5 (C-$1_{C28\alpha*}$, $^1J_{C,H}$ = 169.2 Hz), 101.9 (C-$1_{C26\beta*}$, $^1J_{C,H}$ = 171.0 Hz), 101.5 (C-$1_{C27\alpha*}$, $^1J_{C,H}$ = 169.8 Hz), 100.5 (C-$1_{E26\alpha*}$, C-$1_{E26\beta*}$, $^1J_{C,H}$ = 172.9 Hz), 100.3 (C-$1_{C27\beta*}$, $^1J_{C,H}$ = 171.7 Hz), 100.1 (C-$1_{E28\alpha*}$, C-$1_{E28\beta*}$, $^1J_{C,H}$ = 173.5 Hz), 99.8 (C-$1_{E27\alpha*}$, C-$1_{E27\beta*}$, $^1J_{C,H}$ = 172.6 Hz), 99.2 (C-$1_{C26\alpha}$, $^1J_{C,H}$ = 170.7 Hz), 97.4 (C-$1_{D26\beta}$, C-$1_{D25\beta}$, C-$1_{D28\beta}$, $^1J_{C,H}$ = 167.5 Hz), 92.3 (C-$1_{D26\alpha}$, C-$1_{D27\alpha}$, C-$1_{D28\alpha}$, $^1J_{C,H}$ = 172.6 Hz), 63.8, 63.2, 63.0 (C-$6_{D\alpha}$, C-$6_{D\beta}$, C-$6_{E\alpha}$, C-$6_{E\beta}$), 58.2 (C-$2_{D\beta}$), 54.8 (C-$2_{D\alpha}$), 24.9, 24.8, 24.7 ($CH_{3NHAc\beta}$, $CH_{3NHAc\alpha}$), 19.5, 19.4 (C-$6_{C\alpha}$, C-$6_{C\beta}$) ; HRMS (ESI$^+$): *m/z* 594.1964 (calcd for $C_{22}H_{37}NO_{16}Na$ $[M+Na]^+$ *m/z* 594.2010) ; RP-HPLC (215 nm): R_t = 1.97, 4.26 min.

Propyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (29): To a stirred solution of triccharide **23**, **24** and **25** (28 mg, 46 µmol) in MeOH (5 mL), was added methanolic sodium methoxide (25% w/w, 12 µL). The solution was stirred at rt for 3 h. After this time, MS analysis of the reaction mixture revealed a molecular weight of corresponding to that of the target tetrasaccharide. The reaction solution was neutralized by addition of Dowex H^+ resin, and the suspension was filtered over a 0.2 µm filter. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-HPLC (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}) gave the fully deprotected trisaccharide **29** (18 mg, 69%) as a white powder following repeated freeze-drying. Propyl glycoside **29** had ^{1}H NMR (D_2O) δ 5.38 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 5.05 (bs, 1H, H-1_C), 4.57 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1_D), 4.00 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.3 Hz, H-3_D), 3.92 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 7.1 Hz, H-4_D), 3.90-3.87 (m, 3H, H-2_C, H-2_D, H-$6a_D$), 3.85-3.73 (m, 5H, H-$6a_E$, OCH_{2Pr}, H-$6b_D$, H-$6b_E$, H-5_C), 3.69-3.64 (m, 4H, H-3_C, H-5_D, H-5_E, H-3_E), 3.58 (dd$_{po}$, 1H, $J_{2,3}$ = 9.9 Hz, H-2_E), 3.53 (dt, 1H, *J* = 6.6 Hz, *J* = 9.6 Hz, OCH_{2Pr}), 3.44 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_E), 3.43 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4_C), 2.03 (s, 3H, CH_{3NHAc}), 1.54 (psex, 2H, *J* = 6.4 Hz, CH_{2Pr}), 1.26 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C), 0.86 (t, 3H, *J* = 7.4 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) δ 176.7 (NHCO), 103.5 (C-1_D, $^1J_{C,H}$ = 162.9 Hz), 102.9 (C-1_C, $^1J_{C,H}$ = 169.1 Hz), 99.9 (C-1_E, $^1J_{C,H}$ = 172.2 Hz), 82.1 (C-3_D), 78.5 (C-3_C), 75.4 (C-3_E*), 75.3 (C-5_E*), 74.9 (C-4_D), 74.8 (OCH_{2Pr}), 74.6 (C-4_C), 73.9 (C-2_E), 73.1 (C-2_C), 72.7 (C-5_D), 72.6 (C-5_C), 71.9 (C-4_E), 63.9 (C-6_D), 63.0 (C-6_E), 56.8 (C-2_D), 24.8 (CH_{3NHAc}), 24.7 (CH_{2Pr}), 19.4 (C-6_C), 12.3 (CH_{3Pr}) ; HRMS (ESI$^+$): *m/z* 572.2556 (calcd for $C_{23}H_{42}NO_{15}$ $[M+H]^+$ *m/z* 572.2554), HRMS (ESI$^+$): *m/z* 594.2404 (calcd for $C_{23}H_{41}NO_{15}Na$ $[M+Na]^+$ *m/z* 594.2374), HRMS (ESI$^+$): *m/z* 1165.4824 (calcd for $C_{46}H_{82}N_2O_{30}Na$ $[2M+Na]^+$ *m/z* 1165.4850) ; RP-HPLC (215 nm): R_t = 8.6 min.

Allyl (2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (30): *Route 1.* CAN (110 mg, 200 µmol, 4 equiv.) was added to a solution of the fully protected trisaccharide **22** (64 mg, 50 µmol) in MeCN/H_2O (10:1, 2.2 mL) at 0°C, under an atmosphere of Ar. After 3 h of stirring at this temperature, TLC analysis (cHex/EtOAc 4:6) indicated the conversion of **22** (Rf 0.66) to a more polar product (Rf 0.30). At this time, the reaction mixture was diluted with DCM (30 mL) and washed with satd aq. $NaHCO_3$ (2 x 10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* to a pale yellow residue (**30**, 58 mg), which was used without further purification.

Route 2. Methanolic sodium methoxide (25% w/w, 400 µL, 1.75 mmol, 0.4 equiv.) was added to allyl trisaccharide **41** (5.6 g, 4.69 mmol) in anhyd. MeOH (150 mL). The solution was stirred at rt for 2h30, at which time a TLC control (Tol/EtOAc 5:5) showed the total conversion of the starting material (Rf 0.7) into a more polar product (Rf 0.28). The reaction was quenched with H^+ Dowex resin. The suspension was filtered over a pad of Celite and the filtrate was concentrated to dryness under reduced pressure to give crude diol **42** (5.0 g, 97%) as a white foam. The material (5.0 g, 4.50 mmol) was dissolved in anhyd. MeCN (100 mL) under an atmosphere of Ar, then trimethyl orthoacetate (240 µL, 1.89 mmol, 2.0 equiv.) and PTSA (21 mg, 0.11 mmol, 0.1 equiv.) were added. The mixture was stirred at rt for 25 min, at which time a TLC control (Tol/EtOAc 8:2) showed the total conversion of the starting material (Rf 0.28) into a less polar product (Rf 0.78). 80% aq. acetic acid (4.5 mL) was then added and the solution was stirred for 5 min at rt. A TLC control (Tol/EtOAc 8:2) showed the presence of a more polar product (Rf 0.52). The mixture was diluted with water (50 mL) and extracted with EtOAc (300 mL). The organic phase was washed with brine (150 mL), dried over anhyd. Na_2SO_4 and concentrated to dryness to give alcohol **30** (5.32 g, 99%) as a white foam. Acceptor **30** had ^{1}H NMR ($CDCl_3$) δ 7.39-7.13 (m, 31H, NH, H_{Ar}), 5.92 (m, 1H, C*H*=CH_2), 5.29 (m, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.7 Hz, CH=C*H*$_2$), 5.21-5.18 (m, 2H, H-2_C, CH=C*H*$_2$), 5.05 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1_C), 4.89 (bs, 2H, H_{Bn}), 4.85 (d$_o$, 1H, H_{Bn}), 4.85 (d$_o$, 1H, H-1_E), 4.81 (d$_{po}$, 1H, H_{Bn}), 4.80 (d$_{po}$, 1H, H_{Bn}), 4.75 (d$_o$, 1H, $J_{1,2}$ = 1.5 Hz, H-1_D), 4.72 (d, 1H, *J* = 11.2 Hz, H_{Bn}), 4.68 (d$_{po}$, 1H, *J* = 11.9 Hz, H_{Bn}), 4.57 (d$_{po}$, 1H, H_{Bn}), 4.48 (bs, 2H, H_{Bn}), 4.47 (d$_o$, 1H, *J* = 10.8 Hz, H_{Bn}), 4.32 (d$_{po}$, 1H, *J* = 12.1 Hz, H_{Bn}), 4.30-4.25 (m$_o$, 2H, H-2_D, H_{All}), 4.10-3.97 (m, 5H, H-5_D, H_{All}, H-3_C, H-4_D, H-3_E), 3.93 (bd, 1H, $J_{6a,6b}$ = 9.0 Hz, H-$6a_D$), 3.89 (bs, 1H, H-3_D), 3.77-3.67 (m, 5H, H-4_E, H-5_E, H-$6b_D$, H-5_C, H-$6a_E$), 3.62 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.7 Hz, H-2_E), 3.42 (bd$_{po}$, 1H, $J_{6a,6b}$ = 10.9 Hz, H-$6b_E$), 3.38 (pt$_{po}$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4_C), 2.21 (s, 3H, CH_{3Ac}), 1.73 (s, 3H, CH_{3NHAc}), 1.34 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6_C) ; ^{13}C NMR ($CDCl_3$) δ 170.6 (CO_{Ac}), 169.7 (NHCO), 138.4-137.2 (5C, C_{IVAr}), 134.0 (*C*H=CH_2), 129.9 (C_{IVAr}), 128.8-127.5 (30C, C_{Ar}), 116.8 (CH=*C*H$_2$), 97.9 (C-1_D, $^1J_{C,H}$ = 170.9 Hz), 96.9 (C-1_E, $^1J_{C,H}$ = 170.3 Hz), 95.6 (C-1_C, $^1J_{C,H}$ = 171.9 Hz), 82.1 (C-3_E), 81.3 (C-4_C), 79.0 (C-2_E), 77.5 (C-4_E), 75.8, 75.2, 75.0 (3C, C_{Bn}), 74.8 (C-5_D), 74.3, 73.5, 73.2 (3C, C_{Bn}), 72.2 (C-2_C), 71.4 (C-5_E), 71.0 (C-4_D), 70.8 (C-6_D), 70.6 (C-3_C), 69.2 (C_{All}), 69.1 (C-3_D), 68.4 (C-5_C), 67.7 (C-6_E), 46.3 (C-2_D), 22.7 (CH_{3NHAc}), 21.0 (CH_{3Ac}), 18.2 (C-6_C) ; HRMS (ESI$^+$): *m/z* 1174.5121 (calcd for $C_{67}H_{77}NO_{16}Na$ $[M+Na]^+$ *m/z* 1174.5140).

(2-*O*-Levulinoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1↔1)-(2-*O*-levulinoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-acetyl-4-*O*-benzyl-β-L-rhamnopyranoside (38) and (2-*O*-Levulinoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-

benzyl-α-L-rhamnopyranosyl)-(1↔1)-(2-*O*-levulinoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside (39): Activated MS 4Å (300 mg) were added to a solution of the disaccharide acceptor **10** (236 mg, 270 µmol) and disaccharide donor[14] **37** (316 mg, 365 µmol, 1.4 equiv.) in anhyd. Tol (6.0 mL), under an atmosphere of Ar. The reaction mixture was stirred at rt for 10 min, and TMSOTf (15 µL, 83 µmol, 0.3 equiv.) was added. The reaction mixture was heated to 55°C and stirred at this temperature for 3 h. After this time, TLC analysis (Tol/EtOAc 6:4) indicated the presence of a major new product (Rf 0.56), and some remaining acceptor (Rf 0.21). Stirring at this temperature for an additional 3 h did not result in any improvement and the reaction was quenched with Et_3N. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to a yellow residue, which was purified by flash column chromatography (Tol/EtOAc 9:1 to 0:10), to give by order of elution the α-(1↔1)-α-linked dimer **39** (10 mg), the α-(1↔1)-β-linked dimer **38** (155 mg) both as colorless oils, and the somewhat contaminated tetrasaccharide **33** (160 mg), and finally the unreacted acceptor **10** (126 mg, 50%), both as white foams. Dimer **39** had Rf = 0.83 (Tol/EtOAc 6:4) ; ^{1}H NMR ($CDCl_3$) δ 7.23-7.04 (m, 12H, H_{Ar}), 5.44 (dd, 2H, H-2_B, H-$2_{B'}$), 5.06 (dd, 2H, H-2_C, H-$2_{C'}$), 5.03 (d, 2H, $J_{1,2}$ = 1.6 Hz, H-1_B, H-$1_{B'}$), 5.01 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_C, H-$1_{C'}$), 4.90 (d, 2H, J = 11.0 Hz, H_{Bn}), 4.82 (d, 2H, J = 11.0 Hz, H_{Bn}), 4.64 (d, 2H, J = 10.5 Hz, H_{Bn}), 4.61 (d, 2H, J = 11.0 Hz, H_{Bn}), 4.60 (d, 2H, J = 11.0 Hz, H_{Bn}), 4.62 (d_o, 1H, J = 9.6 Hz, H_{Bn}), 4.43 (d, 2H, J = 11.3 Hz, H_{Bn}), 4.07 (dd, 2H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.4 Hz, H-3_C, H-$3_{C'}$), 3.86 (dd, 2H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.2 Hz, H-3_B, H-$3_{B'}$), 3.82-3.73 (m, 4H, H-5_C, H-$5_{C'}$, H-5_B, H-$5_{B'}$), 3.46 (pt, 2H, $J_{4,5}$ = 9.5 Hz, H-4_C, H-$4_{C'}$), 3.39 (pt, 2H, $J_{4,5}$ = 9.4 Hz, H-4_B, H-$4_{B'}$), 2.72-2.65 (m, 8H, H_{Lev}), 2.16 (s, 6H, 2 CH_{3Ac}), 2.12 (s, 6H, 2 CH_{3Lev}), 1.30 (d_o, 6H, $J_{5,6}$ = 6.2 Hz, H-6_C, H-$6_{C'}$), 1.27 (d, 6H, $J_{5,6}$ = 6.3 Hz, H-6_B, H-$6_{B'}$) ; ^{13}C NMR ($CDCl_3$) δ 206.1 (2C, CO_{Lev}), 171.7 (2C, CO_{2Lev}), 170.1 (2C, CO_{Ac}), 138.5, 138.0, 137.9 (6C, C_{IVAr}), 128.5-127.6 (30C, C_{Ar}), 99.6 (2C, C-1_B, C-$1_{B'}$, $^1J_{C,H}$ = 170.8 Hz), 92.2 (2C, C-1_C, C-$1_{C'}$, $^1J_{C,H}$ = 173.5 Hz), 79.9 (2C, C-4_C, C-$4_{C'}$), 79.8 (2C, C-4_B, C-$4_{B'}$), 77.6 (2C, C-3_B, C-$3_{B'}$), 77.2 (2C, C-3_C, C-$3_{C'}$), 75.5, 75.2 (4C, C_{Bn}), 71.9 (2C, C-2_C, C-$2_{C'}$), 71.5 (2C, C_{Bn}), 69.2 (2C, C-2_B, C-$2_{B'}$), 68.7 (2C, C-5_B, C-$5_{B'}$), 68.6 (2C, C-5_C, C-$5_{C'}$), 38.1 (2C, CH_{2Lev}), 29.8 (2C, CH_{3Lev}), 28.2 (2C, CH_{2Lev}), 21.0 (2C, CH_{3Ac}), 18.0 (2C, C-6_B, C-$6_{B'}$), 17.8 (2C, C-6_C, C-$6_{C'}$) ; HRMS (ESI^+): *m/z* 1445.6136 (calcd for $C_{92}H_{105}NO_{22}Na$ $[M+Na]^+$ *m/z* 1445.6084).

Dimer **38** had Rf = 0.60 (Tol/EtOAc 6:4) ; ^{1}H NMR ($CDCl_3$) δ 7.38-7.21 (m, 12H, H_{Ar}), 5.66 (bs, 1H, H-1_C), 5.54 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-$2_{B'}$), 5.45 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2_B), 5.32 (dd, 1H, H-$2_{C'}$), 5.21 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-$1_{C'}$), 5.15 (d, 1H, H-$1_{B'}$), 5.09 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 4.90-4.82 (m, 4H, H_{Bn}), 4.72 (d, 1H, J = 10.9 Hz, H_{Bn}), 4.67-4.60 (m, 5H, H_{Bn}), 4.57 (d, 1H, J = 11.2 Hz, H_{Bn}), 4.40 (d, 1H, J = 11.3 Hz, H_{Bn}), 4.28 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, H-$3_{C'}$), 4.19 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-$5_{C'}$), 4.02 (m, 1H, J = 1.6 Hz, H-2_C), 3.95 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.3 Hz, H-$3_{B'}$), 3.88 (dd_{po}, 1H, H-3_B), 3.87-3.82 (m, 2H, H-3_C, H-5_C), 3.76 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-$5_{B'}$), 3.61 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_C), 3.49 (pt_{po}, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-$4_{C'}$), 3.47 (m_o, 1H, H-5_C), 3.43 (pt_{po}, 1H, $J_{4,5}$ = 9.2 Hz, H-4_B), 3.38 (pt_{po}, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-$4_{B'}$), 2.74-2.55 (m, 8H, H_{Lev}), 2.17 (s, 3H, CH_{3Lev}), 2.12 (s, 6H, 2 CH_{3Lev}), 2.11 (s, 3H, CH_{3Ac}), 1.95 (s, 3H, CH_{3Ac}), 1.36-1.25 (m, 12H, H-6_B, H-$6_{B'}$, H-6_C, H-$6_{C'}$) ; ^{13}C NMR ($CDCl_3$) δ 206.0 (2C, CO_{Lev}), 171.7, 171.5 (2C, CO_{2Lev}), 169.9, 168.9 (2C, CO_{Ac}), 138.8, 138.7, 138.4, 138.3, 138.0, 137.7 (6C, C_{IVAr}), 129.0-127.4 (30C, C_{Ar}), 97.5 (C-$1_{C'}$, $^1J_{C,H}$ = 175.2 Hz), 91.6 (C-1_C, $^1J_{C,H}$ = 161.9 Hz), 80.4 (C-4_C), 80.1 (C-$4_{C'}$), 79.8 (C-$4_{B'}$), 79.7 (C-4_B), 78.5 (C-3_B), 78.0 (C-$3_{B'}$), 77.7 (C-3_C), 77.5 (C-$3_{C'}$), 75.4, 75.3, 75.2 (3C, C_{Bn}), 74.8 (2C, C-2_C, C_{Bn}), 72.9 (C-5_C), 72.2 (C-$2_{C'}$), 71.6, 71.4 (2C, C_{Bn}), 69.3 (C-2_B), 69.1 (C-$2_{B'}$), 68.6 (C-5_B, C-$5_{B'}$), 67.9 (C-$5_{C'}$), 38.1, 38.0 (2C, CH_{2Lev}), 29.8, 29.7 (2C, CH_{3Lev}), 28.1, 28.0 (2C, CH_{2Lev}), 21.0, 20.9 (2C, CH_{3Ac}), 18.1, 18.0, 17.7 (4C, C-6_B, C-$6_{B'}$, C-6_C, C-$6_{C'}$) ; HRMS (ESI^+): *m/z* 1445.5952 (calcd for $C_{80}H_{94}O_{23}Na$ $[M+Na]^+$ *m/z* 1445.6084).

Allyl (2,3-di-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (41): Alcohol **10** (5.47 g, 6.26 mmol) and TCA **40** (3.63 g, 7.52 mmol, 1.2 equiv.) were dissolved in anhyd. toluene (150 mL), and activated 4Å MS (5.2 g) was added. The suspension was stirred for 15 min at rt under an atmosphere of Ar, and TMSOTf (200 µL, 1.10 mmol, 0.2 equiv.) was added. After stirring for 5 h at 50°C, a TLC control (Tol/EtOAc 6:4) showed the disappearance of the acceptor **10** (Rf 0.21) and the presence of a major less polar product (Rf 0.50). The reaction was quenched by addition of Et_3N (1 mL), and the suspension was filtered over a pad of Celite. Volatiles were removed under reduced pressure and the crude material was purified by flash chromatography (Tol/EtOAc 8:2 to 2:8) to give trisaccharide **41** (5.6 g, 81%) as a white foam. The fully protected trisaccharide **41** had ^{1}H NMR ($CDCl_3$) δ 7.39-7.11 (m, 31H, NH, H_{Ar}), 5.92 (m, 1H, C*H*=CH_2), 5.37 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.3 Hz H-2_C), 5.31-5.25 (m, 2H, H-3_C, CH=CH_2), 5.21 (m, 1H, J_{cis} = 10.5 Hz, J_{gem} = 1.6 Hz, CH=CH_2), 5.07 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1_C), 4.89 (bs, 2H, H_{Bn}), 4.82 (d_{po}, 1H, H-1_E), 4.80 (d_{po}, 1H, J = 11.1 Hz, H_{Bn}), 4.78 (d_{po}, 1H, H_{Bn}), 4.76 (d_o, 1H, $J_{1,2}$ = 3.7 Hz, H-1_D), 4.72 (d, 1H, J = 11.3 Hz, H_{Bn}), 4.66 (d_{po}, 1H, J = 12.1 Hz, H_{Bn}), 4.64 (d_{po}, 1H, H_{Bn}), 4.62 (d_{po}, 1H, J = 11.6 Hz, H_{Bn}), 4.56 (d_{po}, 1H, J = 12.3 Hz, H_{Bn}), 4.53 (d_{po}, 1H, H_{Bn}), 4.46 (d_{po}, 1H, H_{Bn}), 4.31-4.26 (m_o, 2H, H-2_D, H_{All}), 4.27 (d_o, 1H, H_{Bn}), 4.13-3.98 (m, 5H, H-5_D, H-6a_D, H-4_D, H_{All}, H-3_E), 3.94 (bs, 1H, H-3_D), 3.83-3.74 (m, 4H, H-6b_D, H-5_C, H-4_E, H-5_E), 3.67 (bd, 1H, $J_{6a,6b}$ = 10.5 Hz, H-6a_E), 3.61 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.7 Hz, H-2_E), 3.54 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4_C), 3.37 (bd, 1H, H-6b_E), 2.19 (s, 3H, CH_{3Ac}), 1.99 (s, 3H, CH_{3Ac}), 1.76 (s, 3H, CH_{3NHAc}), 1.36 (d, 3H, $J_{5,6}$ = 6.7 Hz, H-6_C) ; ^{13}C NMR ($CDCl_3$) δ 169.8 (NHCO), 169.6, 169.5 (2C, CO_{Ac}), 138.7-137.3 (6C, C_{IVAr}), 133.8 (*C*H=CH_2), 129.0-127.3 (30C, C_{Ar}), 117.0 (CH=*C*H_2), 97.5 (C-1_D, $^1J_{C,H}$ = 170.7 Hz), 96.7 (C-1_E, $^1J_{C,H}$ = 169.5 Hz), 94.9 (C-1_C, $^1J_{C,H}$ = 173.2 Hz), 82.1 (C-3_E), 79.0 (C-2_E), 78.6 (C-4_C), 77.5 (C-4_E), 75.8, 75.0 (2C, C_{Bn}), 74.9 (2C, C_{Bn}, C-5_D), 74.3, 73.4, 73.1 (3C, C_{Bn}), 71.7 (C-3_C), 71.4 (C-5_E), 70.8 (2C, C-6_D, C-4_D), 69.8 (C-2_C), 69.2 (C_{All}), 68.8 (C-5_C), 68.2 (C-3_D), 67.6 (C-6_E), 45.8 (C-2_D), 22.7 (CH_{3NHAc}), 20.9, 20.8 (2C, CH_{3Ac}), 18.2 (C-6_C) ; HRMS (ESI^+): *m/z* 1216.5172 (calcd for $C_{75}H_{85}NO_{17}Na$ $[M+Na]^+$ *m/z* 1216.5246).

Allyl (4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (42): Methanolic sodium methoxide (25% w/w, 400 µL, 1.75 mmol, 0.4 equiv.) was added to allyl trisaccharide **41** (5.6 g, 4.69 mmol) in anhyd. MeOH (150 mL). The solution was stirred at rt for 2h30, at which time a TLC control (Tol/EtOAc 5:5) showed the total conversion of the starting material (Rf 0.7) into a more polar product (Rf 0.28). The reaction was quenched with H^+ Dowex resin. The suspension was filtered over a pad of Celite and the

filtrate was concentrated to dryness under reduced pressure. Flash chromatography of the crude material (Tol/EtOAc 6:4 to 3:7) gave trisaccharide **42** (200 mg, 99%) as a white foam. The diol had ^{1}H NMR ($CDCl_3$) δ 7.38-7.13 (m, 31H, NH, H_{Ar}), 5.88 (m, 1H, C*H*=CH_2), 5.28 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.7 Hz, CH=CH_2), 5.21 (m, 1H, J_{cis} = 10.4 Hz, CH=CH_2), 5.07 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1_C), 4.89 (bs, 2H, H_{Bn}), 4.85 (d_{po}, 1H, H-1_E), 4.82 (d_{po}, 1H, J = 10.7 Hz, H_{Bn}), 4.78 (d_o, 2H, H_{Bn}), 4.75 (d_o, 1H, $J_{1,2}$ = 3.7 Hz, H-1_D), 4.72 (d_{po}, 1H, H_{Bn}), 4.68 (d_{po}, 1H, J = 11.9 Hz, H_{Bn}), 4.57 (d_{po}, 1H, J = 12.1 Hz, H_{Bn}), 4.48 (d_{po}, 1H, J = 10.6 Hz, H_{Bn}), 4.47 (bs, 2H, H_{Bn}), 4.33 (d_{po}, 1H, H_{Bn}), 4.30-4.24 (m, 2H, H-2_D, H_{All}), 4.09-3.97 (m, 5H, H-5_D, H_{All}, H-4_D, H-3_E, H-2_C), 3.93-3.89 (m, 2H, H-3_D, H-$6a_D$), 3.84 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-3_C), 3.78-3.69 (m, 4H, H-5_E, H-4_E, H-$6b_D$, H-5_C), 3.67 (d_{po}, 1H, $J_{5,6a}$ = 1.9 Hz, $J_{6a,6b}$ = 10.5 Hz, H-$6a_E$), 3.62 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.7 Hz H-2_E), 3.37 (bd_{po}, 1H, H-$6b_E$), 3.41 (pt_{po}, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.9 Hz, H-4_C), 2.82 (bs, 1H, OH), 2.47 (bs, 1H, OH), 1.74 (s, 3H, CH_{3NHAc}), 1.34 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6_C) ; ^{13}C NMR ($CDCl_3$) δ 169.9 (NHCO), 138.4-137.1 (6C, C_{IVAr}), 133.9 (*C*H=CH_2), 128.9-127.5 (30C, C_{Ar}), 116.9 (CH=*C*H_2), 98.1 (C-1_D, $^1J_{C,H}$ = 169.5 Hz), 97.6 (C-1_C, $^1J_{C,H}$ = 169.8 Hz), 97.1 (C-1_C, $^1J_{C,H}$ = 169.2 Hz), 82.1 (C-3_E), 81.5 (C-4_C), 79.1 (C-2_E), 77.6 (C-4_E), 75.8, 75.0 (2C, C_{Bn}), 74.9 (C-5_D), 74.8, 74.4, 73.5, 73.2 (4C, C_{Bn}), 71.4 (2C, C-3_C, C-5_E), 71.2 (C-4_D), 70.8 (2C, C-6_D, C-2_C), 69.4 (C-3_D), 69.2 (C_{All}), 68.5 (C-5_C), 67.8 (C-6_E), 46.9 (C-2_D), 22.6 (CH_{3NHAc}), 18.2 (C-6_C) ; HRMS (ESI^+): *m/z* 1132.4995 (calcd for $C_{65}H_{75}NO_{15}Na$ $[M+Na]^+$ *m/z* 1132.5034).

Allyl (2-*O*-levulinoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (33), Allyl (2-*O*-acetyl-4-*O*-benzyl-3-*O*-trimethylsilyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (43) and Allyl (2-*O*-acetyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (44): *Route 1.* Activated MS 4Å (159 mg) were added to a solution of the crude alcohol **30** (obtained from the PMB-protected precursor **22**, see above) (46 mg, 40 µmol) and donor[9] **32** (28 mg, 48 µmol, 1.2 equiv.) in anhyd. toluene (610 µL), under an atmosphere of Ar. The reaction mixture was stirred at rt for 30 min before being cooled to -10°C. TMSOTf (0.7 µL, 4 µmol, 0.1 equiv.) was added, and the reaction mixture was allowed to warm to rt while stirring for 18 h. After this time, TLC analysis (cHex/EtOAc 1:1) indicated the complete conversion of acceptor **30** (Rf 0.17) and donor **32** (Rf 0.55) to a new product (Rf 0.42). The reaction was quenched with Et_3N (100 µL). The reaction mixture was filtered and concentrated *in vacuo* to a yellow residue (60 mg), which was purified by flash column chromatography (Tol/EtOAc 8:2 to 4:6), to give tetrasaccharide **33** (36 mg, 58% over two steps) as a pale yellow residue.

Route 2. Alcohol **30** (prepared from diacetate **41**, see above) (540 mg, 0.47 mmol) and TCA **32** (330 mg, 0.56 mmol, 1.2 equiv.) were dissolved in anhyd. Et_2O (20 mL), and activated 4Å MS (1 g) was added. The suspension was stirred for 25 min at rt, then cooled to -15°C. TMSOTf (17 µL, 94 µmol, 0.2 equiv.) was added. After stirring for 6 h at -15°C, a TLC control (Tol/EtOAc 6:4) showed the presence of the acceptor (Rf 0.23) and that of a major new product (Rf 0.56). In the absence of any observed evolution, the suspension was filtered over a pad of Celite following neutralization by addition of Et_3N. Volatiles were removed under reduced pressure and the crude material was purified by flash chromatography (Tol/EtOAc 8:2 to 0:1) to give by order of elution first the silylated acceptor **43** (145 mg, 23%) as a colorless oil, then tetrasaccharide **33** (254 mg, 34%) as a white foam, and finally some remaining trisaccharide **30** (155 mg, 29%) as a white foam. The trimethylsilyl derivative **43** had Rf = 0.58 (Tol/EtOAc 6:4) ; ^{1}H NMR ($CDCl_3$) δ 7.37-7.12 (m, 31H, NH, H_{Ar}), 5.95 (m, 1H, C*H*=CH_2), 5.31 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.7 Hz, CH=CH_2), 5.21-5.18 (m, 2H, H-2_C, CH=CH_2), 5.00 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1_C), 4.91 (d_{po}, 1H, H_{Bn}), 4.89 (bs, 2H, H_{Bn}), 4.86 (d_o, 1H, H-1_E), 4.81 (d_{po}, 1H, J = 10.8 Hz, H_{Bn}), 4.78 (d_{po}, 1H, H_{Bn}), 4.77 (bs, 1H, H-1_D), 4.67 (d, 1H, J = 11.8 Hz, H_{Bn}), 4.62 (d, 1H, J = 11.2 Hz, H_{Bn}), 4.56 (d, 1H, J = 12.1 Hz, H_{Bn}), 4.53-4.46 (m, 3H, H_{Bn}), 4.31-4.26 (m_o, 2H, H-2_D, H_{All}), 4.30 (d_o, 1H, H_{Bn}), 4.10 (dd_{po}, 1H, $J_{4,5}$ = 8.7 Hz, H-5_D), 4.06 (dd_o, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.2 Hz, H-3_C), 4.05-3.98 (m, 4H, H_{All}, H-3_E, H-4_D, H-$6a_D$), 3.91 (bs, 1H, H-3_D), 3.81 (dd_{po}, 1H, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 9.0 Hz, H-$6b_D$), 3.77-3.67 (m, 4H, H-4_E, H-5_E, H-5_C, H-$6a_E$), 3.62 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.7 Hz, H-2_E), 3.42-3.38 (m, 2H, H-$6b_E$, H-4_C), 2.21 (s, 3H, CH_{3Ac}), 1.72 (s, 3H, CH_{3NHAc}), 1.30 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C), 0.16 (bs, 9H, $SiCH_3$) ; ^{13}C NMR ($CDCl_3$) δ 169.8 (CO_{Ac}), 169.7 (NHCO), 138.4-137.1 (6C, C_{IVAr}), 134.0 (*C*H=CH_2), 129.0-127.4 (30C, C_{Ar}), 116.8 (CH=*C*H_2), 97.7 (C-1_D, $^1J_{C,H}$ = 170.9 Hz), 97.0 (C-1_E, $^1J_{C,H}$ = 169.3 Hz), 95.7 (C-1_C, $^1J_{C,H}$ = 170.8 Hz), 82.1 (C-3_E), 81.1 (C-4_C), 79.0 (C-2_E), 77.5 (C-4_E), 75.8, 75.5, 75.0 (3C, C_{Bn}), 74.7 (C-5_D), 74.3, 73.5, 73.0 (3C, C_{Bn}), 72.2 (C-2_C), 71.5 (C-5_E), 71.2 (C-3_C), 71.0 (2C, C-4_D, C-6_D), 69.4 (C_{All}), 68.7 (C-5_C), 68.5 (C-3_D), 67.7 (C-6_E), 45.9 (C-2_D), 22.6 (CH_{3NHAc}), 21.0 (CH_{3Ac}), 18.1 (C-6_C), 0.1 (3C, CH_{3Si}) ; HRMS (ESI^+): *m/z* 1224.5702 (calcd for $C_{70}H_{86}NO_{16}Si$ $[M+H]^+$ *m/z* 1224.5716), *m/z* 1224.5590 (calcd for $C_{70}H_{85}NO_{16}SiNa$ $[M+Na]^+$ *m/z* 1246.5535).

Route 3. Alcohol **30** (4.0 g, 3.47 mmol) prepared from diacetate **41** and TCA **32** (2.7 g, 4.60 mmol, 1.3 equiv.) were dissolved in anhyd. Tol (150 mL), and activated 4Å MS (12 g) was added. The suspension was stirred for 25 min at rt, then TBSOTf (170 µL, 0.74 mmol, 0.2 equiv.) was added. After stirring for 3 h at rt, a TLC control (Tol/EtOAc 6:4) showed the disappearance of the acceptor (Rf 0.23) and the presence of a major less polar product (Rf 0.56). The mixture was neutralized by addition of Et_3N, and the suspension was filtered over a pad of Celite. Volatiles were removed under reduced pressure and the crude material was purified by flash chromatography (Tol/EtOAc 8:2 to 6:4) to give first the silylated acceptor **44** (300 mg, 6%) as a colorless oil, then tetrasaccharide **33** (4.54 g, 83%) as a white foam. The *tert*-butyldimethylsilyl trisaccharide **44** had Rf = 0.66 (Tol/EtOAc 6:4) ; ^{1}H NMR ($CDCl_3$) δ 7.37-7.11 (m, 31H, NH, H_{Ar}), 5.95 (m, 1H, C*H*=CH_2), 5.31 (m, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.7 Hz, CH=CH_2), 5.21-5.18 (m, 2H, H-2_C, CH=CH_2), 4.99 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1_C), 4.91 (d_{po}, 1H, H_{Bn}), 4.89 (bs, 2H, H_{Bn}), 4.86 (d_o, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 4.81 (d_{po}, 1H, J = 11.0 Hz, H_{Bn}), 4.77 (d_{po}, 1H, H_{Bn}), 4.77 (bs, 1H, H-1_D), 4.67 (d, 1H, J = 11.8 Hz, H_{Bn}), 4.62 (d, 1H, J = 11.2 Hz, H_{Bn}), 4.57 (d, 1H, J = 12.1 Hz, H_{Bn}), 4.51 (d_{po}, 1H, J = 11.4 Hz, H_{Bn}), 4.47 (d_{po}, 1H, H_{Bn}), 4.45 (d_{po}, 1H, H_{Bn}), 4.30-4.26 (m_o, 2H, H-2_D, H_{All}), 4.29 (d_o, 1H, H_{Bn}), 4.10 (ddd_{po}, 1H, $J_{5,6a}$ = 5.5 Hz, $J_{4,5}$ = 8.8 Hz, H-5_D), 4.05-3.98 (m, 5H, H_{All}, H-3_C, H-3_E, H-4_D, H-$6a_D$), 3.91 (bs, 1H, H-3_D), 3.81 (dd_{po}, 1H, $J_{6a,6b}$ = 8.9 Hz, H-$6b_D$), 3.77-3.61 (m, 5H, H-4_E, H-5_E, H-5_C, H-$6a_E$, H-2_E), 3.41 (pt_{po}, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4_C), 3.38 (bd_{po}, 1H, $J_{6a,6b}$ = 10.3

Hz, H-6b$_E$), 2.20 (s, 3H, CH_{3Ac}), 1.72 (s, 3H, CH_{3NHAc}), 1.29 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_C$), 0.91 (bs, 9H, CH_{3tBuSi}), 0.14 (bs, 3H, $SiCH_3$), 0.05 (bs, 3H, $SiCH_3$) ; ^{13}C NMR ($CDCl_3$) δ 169.8 (2C, CO_{Ac}, NHCO), 138.4-137.1 (6C, C_{IVAr}), 134.0 (*C*H=CH$_2$), 129.0-125.3 (30C, C_{Ar}), 116.9 (CH=*C*H$_2$), 97.6 (C-1$_D$, $^1J_{C,H}$ = 170.6 Hz), 97.0 (C-1$_E$, $^1J_{C,H}$ = 169.7 Hz), 95.6 (C-1$_C$, $^1J_{C,H}$ = 171.1 Hz), 82.1 (C-3$_E$), 81.1 (C-4$_C$), 79.0 (C-2$_E$), 77.5 (C-4$_E$), 75.9, 75.5, 75.0 (3C, C_{Bn}), 74.6 (C-5$_D$), 74.3, 73.4, 73.0 (3C, C_{Bn}), 72.1 (C-2$_C$), 71.4 (C-5$_E$), 71.1 (C-4$_D$), 71.0 (2C, C-3$_C$, C-6$_D$), 69.4 (C_{All}), 68.8 (C-5$_C$), 68.3 (C-3$_D$), 67.7 (C-6$_E$), 45.8 (C-2$_D$), 25.8 (3C, CH_{3tBuSi}), 22.6 (CH_{3NHAc}), 21.0 (CH_{3Ac}), 18.1 (C_{IVSi}), 17.8 (C-6$_C$), -4.5 (CH_{3Si}), -4.8 (CH_{3Si}) ; HRMS (ESI$^+$): *m/z* 1266.5991 (calcd for $C_{73}H_{92}NO_{16}Si$ [M+H]$^+$ *m/z* 1266.6185), *m/z* 1288.5845 (calcd for $C_{73}H_{91}NO_{16}SiNa$ [M+Na]$^+$ *m/z* 1288.6005).

The coupling product **33** had: ^{1}H NMR ($CDCl_3$) δ 7.36-7.11 (m, 41H, 40H$_{Ar}$, NH), 5.95 (m, 1H, C*H*=CH$_2$), 5.42 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.2 Hz, H-2$_B$), 5.31 (m, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.7 Hz, CH=C*H*$_2$), 5.21-5.16 (m, 2H, H-2$_C$, CH=C*H*$_2$), 5.04 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1$_C$), 4.94 (d, 1H, H-1$_B$), 4.90 (d$_{po}$, 1H, J = 11.3 Hz, H$_{Bn}$), 4.88 (bs$_{po}$, 2H, H$_{Bn}$), 4.82 (d$_o$, 1H, H-1$_E$), 4.81 (d$_o$, 1H, J = 11.4 Hz, H$_{Bn}$), 4.80 (d$_o$, 1H, J = 11.3 Hz, H$_{Bn}$), 4.77 (d$_o$, 1H, H$_{Bn}$), 4.76 (d$_o$, 1H, $J_{1,2}$ = 3.2 Hz, H-1$_D$), 4.66 (d$_o$, 1H, J = 10.2 Hz, H$_{Bn}$), 4.64 (d$_o$, 1H, H$_{Bn}$), 4.62 (d$_o$, 1H, J = 9.6 Hz, H$_{Bn}$), 4.59 (d$_o$, 1H, H$_{Bn}$), 4.55 (d$_o$, 1H, H$_{Bn}$), 4.54 (d$_o$, 1H, J = 11.6 Hz, H$_{Bn}$), 4.49 (d$_o$, 1H, J = 11.4 Hz, H$_{Bn}$), 4.47 (d$_o$, 1H, J = 11.2 Hz, H$_{Bn}$), 4.44 (d$_o$, 1H, J = 11.6 Hz, H$_{Bn}$), 4.30-4.24 (m, 2H, H-2$_D$, H$_{All}$), 4.26 (d$_o$, 1H, J = 12.0 Hz, H$_{Bn}$), 4.09-3.97 (m, 6H, H-6a$_D$, H-5$_D$, H$_{All}$, H-3$_C$, H-4$_D$, H-3$_E$), 3.89-3.83 (m, 3H, H-3$_D$, H-3$_B$, H-5$_B$), 3.77-3.65 (m, 5H, H-6b$_D$, H-5$_E$, H-4$_E$, H-5$_C$, H-6a$_E$), 3.60 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.7 Hz, H-2$_E$), 3.49 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4$_C$), 3.40 (t$_{po}$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4$_B$), 3.36 (bd$_{po}$, 1H, $J_{6a,6b}$ = 10.7 Hz, H-6b$_E$), 2.63-2.61 (m, 4H, H$_{Lev}$), 2.15 (s, 3H, CH_{3Ac}), 2.12 (s, 3H, CH_{3Lev}), 1.74 (s, 3H, CH_{3NHAc}), 1.29 (d$_o$, 3H, $J_{5,6}$ = 6.0 Hz, H-6$_C$), 1.28 (d$_o$, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_B$) ; ^{13}C NMR ($CDCl_3$) δ 206.0 (CO_{Lev}), 171.7 (CO_{2Lev}), 169.7 (NHCO), 169.6 (CO_{Ac}), 138.6-137.2 (8C, C_{IVAr}), 134.0 (*C*H=CH$_2$), 129.1-127.3 (40C, C_{Ar}), 116.9 (CH=*C*H$_2$), 99.5 (C-1$_B$, $^1J_{C,H}$ = 171.7 Hz), 97.6 (C-1$_D$, $^1J_{C,H}$ = 171.4 Hz), 96.9 (C-1$_E$, $^1J_{C,H}$ = 170.7 Hz), 95.1 (C-1$_C$, $^1J_{C,H}$ = 172.3 Hz), 82.1 (C-3$_E$), 80.4 (C-4$_C$), 79.7 (C-4$_B$), 79.0 (C-2$_E$), 77.5 (2C, C-3$_B$, C-4$_E$), 76.4 (C-3$_C$), 75.8, 75.5, 75.0 (3C, C_{Bn}), 74.9 (C-5$_D$), 74.7, 74.3, 73.5, 73.1 (4C, C_{Bn}), 71.6 (2C, C-2$_C$, C_{Bn}), 71.4 (C-5$_E$), 70.9 (C-6$_D$), 70.7 (C-4$_D$), 69.4 (C-2$_B$), 69.3 (C_{All}), 68.8 (C-5$_C$), 68.6 (C-3$_D$), 68.5 (C-5$_B$), 67.7 (C-6$_E$), 46.0 (C-2$_D$), 38.0 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.1 (CH_{2Lev}), 22.7 (CH_{3NHAc}), 21.0 (CH_{3Ac}), 18.1 (2C, C-6$_B$, C-6$_C$) ; HRMS (ESI$^+$): *m/z* 1598.6995 (calcd for $C_{92}H_{105}NO_{22}Na$ [M+Na]$^+$ *m/z* 1598.7026).

Allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (45): To a solution of tetrasaccharide **33** (960 mg, 0.61 mmol) in anhyd. pyridine (30 mL) stirred at 0°C under an Ar atmosphere were added dropwise AcOH (10 mL) and hydrazine monohydrate (215 µL, 4.42 mmol, 7.3 equiv.). The reaction mixture was stirred at rt for 90 min. A TLC control (Tol/EtOAc 6:4) showed the conversion of the starting material (Rf 0.46) into a more polar product (Rf 0.38). Following addition of DCM (80 mL) and water (35 mL), the two layers were separated and the aq. one was extracted twice with DCM. The combined organic extracts were washed with 5% aq. citric acid (35 mL), and brine (100 mL), then dried over anhyd. Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (Tol/EtOAc 8:2 to 0:1) to give alcohol **45** (0.86 g, 96%) as a white foam. Tetrasaccharide **45** had ^{1}H NMR ($CDCl_3$) δ 7.37-7.12 (m, 41H, 40H$_{Ar}$, NH), 5.96 (m, 1H, C*H*=CH$_2$), 5.33 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.7 Hz, CH=C*H*$_2$), 5.23 (dd$_{po}$, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.2 Hz, H-2$_C$), 5.20 (m$_o$, 1H, CH=C*H*$_2$), 5.06 (d, 1H, H-1$_C$), 5.00 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1$_B$), 5.02-4.87 (m, 3H, H$_{Bn}$), 4.84 (d$_o$, 1H, H-1$_E$), 4.81 (d$_o$, 2H, J = 10.9 Hz, H$_{Bn}$), 4.78 (bs, 1H, H-1$_D$), 4.77 (d$_o$, 1H, J = 11.9 Hz, H$_{Bn}$), 4.73 (d, 1H, J = 11.0 Hz, H$_{Bn}$), 4.70-4.57 (m, 5H, H$_{Bn}$), 4.56 (d$_o$, 1H, J = 12.1 Hz, H$_{Bn}$), 4.54 (d$_o$, 1H, J = 11.6 Hz, H$_{Bn}$), 4.47 (d$_o$, 2H, H$_{Bn}$), 4.32-4.25 (m, 3H, H-2$_D$, H$_{Bn}$, H$_{All}$), 4.11-3.98 (m, 6H, H-6a$_D$, H-5$_D$, H$_{All}$, H-3$_C$, H-4$_D$, H-3$_E$), 3.94 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2$_B$), 3.90 (bs, 1H, H-3$_D$), 3.82 (dq$_o$, 1H, $J_{4,5}$ = 9.5 Hz, H-5$_B$), 3.80-3.66 (m, 6H, H-3$_B$, H-6b$_D$, H-5$_E$, H-4$_E$, H-5$_C$, H-6a$_E$), 3.61 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.7 Hz, H-2$_E$), 3.47 (t$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4$_C$), 3.46 (t$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4$_B$), 3.36 (bd, 1H, $J_{6a,6b}$ = 10.4 Hz, H-6b$_E$), 2.34 (bs, 1H, OH), 2.17 (s, 3H, CH_{3Ac}), 1.74 (s, 3H, CH_{3NHAc}), 1.31-1.29 (bd, 6H, $J_{5,6}$ = 6.2 Hz, H-6$_B$, H-6$_C$) ; ^{13}C NMR ($CDCl_3$) δ 169.8 (NHCO), 169.7 (CO_{Ac}), 138.5-137.2 (8C, C_{IVAr}), 134.0 (*C*H=CH$_2$), 128.9-127.4 (40C, C_{Ar}), 116.9 (CH=*C*H$_2$), 101.3 (C-1$_B$, $^1J_{C,H}$ = 173.0 Hz), 97.6 (C-1$_D$, $^1J_{C,H}$ = 168.7 Hz), 96.9 (C-1$_E$, $^1J_{C,H}$ = 170.9 Hz), 95.0 (C-1$_C$, $^1J_{C,H}$ = 170.0 Hz), 82.1 (C-3$_E$), 80.5 (C-4$_C$), 79.7 (C-4$_B$), 79.5 (C-3$_B$), 78.9 (C-2$_E$),77.5 (C-4$_E$), 76.8 (C-3$_C$), 75.9, 75.5 (2C, C_{Bn}), 75.0 (2C, C_{Bn}), 74.7 (C-5$_D$), 74.3, 73.5, 73.1, 72.1 (4C, C_{Bn}), 71.8 (C-2$_C$), 71.4 (C-5$_E$), 70.9 (C-6$_D$), 70.6 (C-4$_D$), 69.3 (C_{All}), 69.1 (C-2$_B$), 68.8 (C-5$_C$), 68.2 (2C, C-3$_D$, C-5$_B$), 67.6 (C-6$_E$), 45.8 (C-2$_D$), 22.7 (CH_{3NHAc}), 21.0 (CH_{3Ac}), 18.1, 18.0 (2C, C-6$_B$, C-6$_C$) ; HRMS (ESI$^+$): *m/z* 1500.6599 (calcd for $C_{87}H_{99}NO_{20}Na$ [M+Na]$^+$ *m/z* 1500.6658).

Propyl α-L-rhamnopyranosyl-(1→3)-(2-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (46): To a stirred solution of tetrasaccharide **45** (299 mg, 202 µmol) in 90% aq. EtOH (14.9 mL), were added Pd/C (299 mg) and 1M aq. HCl (38 µL). The suspension was stirred under an H_2 atmosphere for a day at rt. After this time, MS analysis of revealed a molecular weight corresponding to that of the target tetrasaccharide and the absence of any molecular weight corresponding to the benzylated intermediates. The reaction mixture was filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-50% linear gradient of 80% aq. MeCN over 60 min at a flow rate of 20 mL•min^{-1}) gave tetrasaccharide **46** (140 mg, 90%) as a white powder following repeated freeze-drying. ^{1}H NMR (D_2O) δ 5.40 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1$_E$), 5.15 (d, 1H, $J_{2,3}$ = 2.5 Hz, H-2$_C$), 5.13 (bs, 1H, H-1$_C$), 4.98 (bs, 1H, H-1$_B$), 4.53 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1$_D$), 4.06 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.6 Hz, H-3$_D$), 3.99-3.77 (m, 9H, H-2$_B$, H-4$_D$, H-3$_C$, H-6a$_D$, H-2$_D$, H-5$_C$, OCH$_{2Pr}$, H-6b$_D$, H-6a$_E$), 3.75 (dd$_{po}$, 1H, $J_{5,6b}$ = 4.6 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6b$_E$), 3.69-3.60 (m, 5H, H-5$_D$, H-3$_B$, H-3$_E$, H-5$_E$, H-5$_B$), 3.59 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4$_C$), 3.56 (dd$_{po}$, 1H, $J_{2,3}$ = 9.8 Hz, H-2$_E$), 3.50 (dt, 1H, J = 6.6 Hz, J = 12.8 Hz, OCH$_{2Pr}$), 3.43 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4$_E$), 3.40 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4$_B$), 2.16 (s, 3H, CH_{3Ac}), 2.01 (s, 3H, CH_{3NHAc}), 1.52 (psex, 2H, J = 7.0 Hz, CH_{2Pr}), 1.27 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_C$), 1.23 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_B$), 0.85 (t, 3H, J = 7.4 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) δ 176.9 (NHCO), 175.6 (CO_{Ac}), 104.6 (C-1$_B$, $^1J_{C,H}$ = 169.4 Hz), 103.6 (C-1$_D$, $^1J_{C,H}$ = 163.4 Hz), 99.8 (C-1$_E$, $^1J_{C,H}$ = 173.4 Hz), 99.3 (C-1$_C$, $^1J_{C,H}$ = 170.7 Hz), 82.7 (C-3$_D$), 78.7 (C-4$_D$), 78.1 (C-5$_D$), 75.5 (C-3$_E$), 75.4 (C-5$_E$), 74.8

(OCH_{2Pr}), 74.7 (C-2_C), 74.6 (C-4_B), 74.5 (C-3_C), 74.1 (C-4_C), 73.9 (C-2_E), 72.7 (2C, C-2_B, C-3_B), 72.5 (C-5_C), 72.0 (C-5_B), 71.9 (C-4_E), 63.9 (C-6_D), 63.1 (C-6_E), 56.5 (C-2_D), 25.1 (CH_{3NHAc}), 24.8 (CH_{2Pr}), 22.9 (CH_{3Ac}), 19.5 (C-6_C), 19.4 (C-6_B), 12.3 (CH_{3Pr}) ; HRMS (ESI^+): *m/z* 760.3248 (calcd for $C_{31}H_{53}NO_{20}$ $[M+H]^+$ *m/z* 760.3239), HRMS (ESI^+): *m/z* 782.3068 (calcd for $C_{31}H_{53}NO_{20}Na$ $[M+Na]^+$ *m/z* 782.3058), HRMS (ESI^+): *m/z* 1541.6234 (calcd for $C_{62}H_{106}N_2O_{40}Na$ $[2M+Na]^+$ *m/z* 1541.6219) ; RP-HPLC (215 nm): R_t = 12.8 min.

Propyl α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (47): To a stirred solution of slightly contaminated tetrasaccharide **46** (82 mg, 108 µmol), issued from RP-HPLC purification, in MeOH (8 mL), was added methanolic sodium methoxide to reach pH 10 (25% w/w, 25 µL). The solution was stirred at rt for 15 h. After this time, MS analysis revealed the presence of the target tetrasaccharide and the absence of any starting **46**. The reaction was neutralized by addition of Dowex H^+ resin, and the suspension was filtered over a 0.2 µm filter. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-50% linear gradient of 80% aq. MeCN over 60 min at a flow rate of 20 $mL•min^{-1}$) gave tetrasaccharide **47** (47 mg, 60%) as a white powder following repeated freeze-drying. The fully deprotected tetrasaccharide **47** had ^1H NMR (D_2O) δ 5.35 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1_E), 5.04 (d_{po}, 1H, $J_{1,2}$ = 1.7 Hz, H-1_C), 4.98 (d_{po}, 1H, $J_{1,2}$ = 1.5 Hz, H-1_B), 4.55 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1_D), 4.05 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 6.7 Hz, H-3_D), 4.02 (dd_{po}, 1H, $J_{2,3}$ = 3.4 Hz, H-2_B), 3.96 (bd_{po}, 1H, $J_{2,3}$ = 3.6 Hz, H-2_C), 3.94 (pt_{po}, $J_{3,4}$ = $J_{4,5}$ = 7.1 Hz, H-4_D), 3.91 (pt_{po}, $J_{2,3}$ = 7.0 Hz, H-2_D), 3.86 (dd_o, 1H, $J_{5,6a}$ = 7.9 Hz, $J_{6a,6b}$ = 11.8 Hz, H-$6a_D$), 3.83-3.71 (m, 9H, H-$6a_E$, H-5_C, H-5_B, OCH_{2Pr}, H-3_C, H-3_B, H-$6b_D$, H-$6b_E$, H-5_D), 3.66 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3_E), 3.62 (ddd_{po}, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{5,6b}$ = 4.6 Hz, $J_{4,5}$ = 10.0 Hz, H-5_E), 3.57 (dd_{po}, 1H, $J_{2,3}$ = 9.8 Hz, H-2_E), 3.53 (pt_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4_C), 3.50 (dt, 1H, *J* = 6.6 Hz, *J* = 9.6 Hz, OCH_{2Pr}), 3.43 (pt_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4_E), 3.42 (pt_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, H-4_B), 2.00 (s, 3H, CH_{3NHAc}), 1.53 (psex, 2H, *J* = 7.0 Hz, CH_{2Pr}), 1.28 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C), 1.25 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B), 0.85 (t, 3H, *J* = 7.5 Hz, CH_{3Pr}) ; ^{13}C NMR (D_2O) δ 176.5 (NHCO), 104.7 (C-1_B, $^1J_{C,H}$ = 172.4 Hz), 103.6 (C-1_D, $^1J_{C,H}$ = 163.4 Hz), 101.6 (C-1_C, $^1J_{C,H}$ = 170.0 Hz), 99.6 (C-1_E, $^1J_{C,H}$ = 173.0 Hz), 80.8 (C-3_D), 79.9 (C-3_C), 78.7 (C-5_D), 75.5 (C-3_E), 75.4 (C-5_E), 74.8 (OCH_{2Pr}), 74.7 (C-4_B), 74.1 (C-4_D), 73.8 (2C, C-2_E, C-4_C), 73.0 (C-2_C), 72.8 (C-2_B), 72.7 (C-3_B), 72.5 (C-5_C), 71.9 (C-5_B), 71.7 (C-4_E), 64.1 (C-6_D), 63.1 (C-6_E), 56.3 (C-2_D), 24.9 (CH_{3NHAc}), 24.8 (CH_{2Pr}), 22.9 (CH_{3Ac}), 19.4 (2C, C-6_C, C-6_B), 12.3 (CH_{3Pr}) ; HRMS (ESI^+): *m/z* 718.3145 (calcd for $C_{29}H_{52}NO_{19}$ $[M+H]^+$ *m/z* 718.3134) ; HRMS (ESI^+): *m/z* 740.2953 (calcd for $C_{29}H_{51}NO_{19}Na$ $[M+Na]^+$ *m/z* 740.2953) ; RP-HPLC (215 nm): R_t = 9.9 min.

Allyl (2-*O*-levulinyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (49): Alcohol **45** (350 mg, 0.24 mmol) and trichloroacetimidate **32** (210 mg, 0.36 mmol, 1.5 equiv.) were dissolved in anhyd. DCE (7 mL), and activated 4Å MS (0.31 g) was added. The suspension was stirred for 25 min at rt, then TfOH (7 µL, 79 µmol, 0.3 equiv.) was added. After stirring for 5 h at rt, a TLC control (Tol/EtOAc 6:4) showed the disappearance of the acceptor (Rf 0.29) and the presence of a major less polar product (Rf 0.60). The mixture was neutralized by addition of Et_3N, and the suspension was filtered over a pad of Celite. Volatiles were removed under reduced pressure and the crude material was purified by flash chromatography (Tol/EtOAc 8:2 to 0:1) to give a 3:7 mixture of the hydrolyzed donor **48** and of the desired pentasaccharide **49** as a white foam. The coupling product **49** had ^1H NMR ($CDCl_3$) δ 7.36-7.11 (m, 51H, 50 H_{Ar}, NH), 5.93 (m, 1H, C*H*=CH_2), 5.50 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.1 Hz, H-2_A), 5.31 (m, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.7 Hz, CH=CH_2), 5.19-5.16 (m, 2H, H-2_C, CH=CH_2), 5.03 (bs, 1H, H-1_C), 4.99 (bs, 1H, H-1_B), 4.92 (d_o, 1H, H-1_A), 4.89-4.86 (m, 4H, H_{Bn}), 4.83 (d_o, 1H, H-1_E), 4.80 (d_{po}, 1H, *J* = 11.2 Hz, H_{Bn}), 4.76-4.74 (m, 3H, H-1_D, H_{Bn}), 4.70-4.62 (m, 4H, H_{Bn}), 4.60-4.49 (m, 5H, H_{Bn}), 4.47-4.42 (m, 3H, H_{Bn}), 4.30-4.23 (m, 2H, H-2_D, H_{All}), 4.26 (d_o, 1H, *J* = 12.1 Hz, H_{Bn}), 4.09-3.97 (m, 6H, H-$6a_D$, H-5_D, H_{All}, H-3_C, H-4_D, H-3_E), 3.95-3.86 (m, 3H, H-2_B, H-3_A, H-3_D), 3.80 (dd_{po}, 1H, $J_{2,3}$ = 2.7 Hz, H-3_B), 3.78- 3.71 (m, 5H, H-5_B, H-5_A, H-$6b_D$, H-5_E, H-4_E), 3.73-3.66 (m, 2H, H-5_C, H-$6a_E$), 3.60 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.7 Hz, H-2_E), 3.47 (t_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_C), 3.46 (t_o, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4_B), 3.39-3.32 (m, 2H, H-4_A, H-$6b_E$), 2.77-2.65 (m, 4H, H_{Lev}), 2.17 (s, 3H, CH_{3Lev}), 2.14 (s, 3H, CH_{3Ac}), 1.74 (bs, 3H, CH_{3NHAc}), 1.19 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B), 1.13 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6_C), 1.09 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A) ; ^{13}C NMR ($CDCl_3$) δ 206.1 (CO_{Lev}), 171.7 (CO_{2Lev}), 169.7 (NHCO), 169.6 (CO_{Ac}), 138.6-137.2 (10C, C_{IVAr}), 134.0 (*C*H=CH_2), 129.0-125.3 (50C, C_{Ar}), 116.9 (CH=*C*H_2), 100.9 (C-1_B, $^1J_{C,H}$ = 171.2 Hz), 99.1 (C-1_A, $^1J_{C,H}$ = 171.7 Hz), 97.5 (C-1_D, $^1J_{C,H}$ = 170.6 Hz), 96.9 (C-1_E, $^1J_{C,H}$ = 171.2 Hz), 95.2 (C-1_C, $^1J_{C,H}$ = 172.9 Hz), 82.1 (C-3_E), 80.2 (C-4_C), 80.0 (C-4_A), 79.7 (C-4_B), 79.2 (C-3_B), 79.0 (C-2_E), 77.6 (C-3_A), 77.5 (C-4_E), 77.2 (C-3_C), 75.8 (C_{Bn}), 75.7 (C-2_B), 75.3 (2C, C_{Bn}), 74.9, 74.7 (2C, C_{Bn}), 74.6 (C-5_D), 74.3, 73.4, 73.1, 72.2 (4C, C_{Bn}), 71.8 (C-2_C), 71.6 (2C, C_{Bn}, C-5_E), 70.8 (2C, C-6_D, C-4_D), 69.3 (C_{All}), 69.2 (C-2_A), 68.9 (C-5_B), 68.7 (C-5_C), 68.5 (C-3_D), 68.3 (C-5_A), 67.6 (C-6_E), 46.0 (C-2_D), 38.1 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 22.6 (CH_{3NHAc}), 21.0 (CH_{3Ac}), 18.1 (2C, C-6_B, C-6_C), 17.9 (C-6_A) ; HRMS (ESI^+): *m/z* 1924.8542 (calcd for $C_{112}H_{127}NO_{26}Na$ $[M+Na]^+$ *m/z* 1924.8544) ; HRMS (ESI^+): *m/z* 1902.8735 (calcd for $C_{112}H_{128}NO_{26}$ $[M+H]^+$ *m/z* 1903.8724) ; HRMS (ESI^+): *m/z* 973.9128 (calcd for $C_{112}H_{127}NO_{26}Na_2$ $[M+2Na]^+$ *m/z* 973.9221).

Allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (50): To a solution of pentasaccharide **49** (400 mg, 0.21 mmol) in anhyd. pyridine (11 mL) stirred at rt under an atmosphere of Ar, were added dropwise AcOH (7 mL) and hydrazine monohydrate (70 µL, 1.44 mmol, 7.0 equiv.). The reaction mixture was stirred at rt for 3 h. Following addition of DCM (35 mL) and water (35 mL), the two layers were separated and the aq. one was extracted twice with DCM (2 x 35 mL). The combined organic extracts were washed with 5% aq. citric acid (75 mL), brine (75 mL), then dried over anhyd. Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (Tol/EtOAc 8:2 to 0:1) to give product of delevulination **50** (0.27 g, 63% over 2 steps) as a white foam. Alcohol **50** had ^1H NMR ($CDCl_3$) δ 7.37-7.12 (m, 51H, 50 H_{Ar}, NH), 5.95 (m, 1H, C*H*=CH_2), 5.32 (m, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.7 Hz,

CH=CH_2), 5.22-5.17 (m, 2H, H-2$_C$, CH=CH_2), 5.05 (d$_{po}$, 1H, $J_{1,2}$ = 1.5 Hz, H-1$_A$), 5.04 (d$_{po}$, 1H, $J_{1,2}$ = 1.6 Hz, H-1$_C$), 5.02 (d$_{po}$, 1H, $J_{1,2}$ = 1.5 Hz, H-1$_B$), 4.88 (bs$_{po}$, 2H, H$_{Bn}$), 4.87 (d$_{po}$, 1H, J = 10.7 Hz, H$_{Bn}$), 4.85 (d$_{po}$, 1H, J = 11.1 Hz, H$_{Bn}$), 4.84 (d$_o$, 1H, $J_{1,2}$ = 3.7 Hz, H-1$_E$), 4.81 (d$_o$, 1H, H$_{Bn}$), 4.79 (d$_o$, 1H, J = 11.6 Hz, H$_{Bn}$), 4.78 (d$_o$, 1H, $J_{1,2}$ = 3.1 Hz, H-1$_D$), 4.77 (d$_o$, 1H, J = 11.0 Hz, H$_{Bn}$), 4.75 (d, 1H, J = 11.0 Hz, H$_{Bn}$), 4.68-4.62 (m, 6H, H$_{Bn}$), 4.58 (d$_o$, 1H, J = 12.0 Hz, H$_{Bn}$), 4.54 (d$_o$, 1H, H$_{Bn}$), 4.52 (d$_o$, 1H, H$_{Bn}$), 4.47 (d$_o$, 1H, J = 10.9 Hz, H$_{Bn}$), 4.45 (d$_o$, 1H, J = 11.5 Hz, H$_{Bn}$), 4.31-4.24 (m, 2H, H-2$_D$, H$_{All}$), 4.27 (d$_{po}$, 1H, J = 12.1 Hz, H$_{Bn}$), 4.11 (dd$_{po}$, 1H, $J_{1,2}$ = 1.8 Hz, H-2$_A$), 4.07-4.00 (m, 6H, H-5$_D$, H$_{All}$, H-6a$_D$, H-3$_C$, H-4$_D$, H-3$_E$), 3.94 (pt$_{po}$, 1H, $J_{1,2}$ = $J_{2,3}$ = 2.2 Hz, H-2$_B$), 3.89 (bs, 1H, H-3$_D$), 3.82 (dd$_o$, 2H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.7 Hz, H-3$_A$, H-3$_B$), 3.79-3.71 (m, 5H, H-5$_A$, H-5$_B$, H-6b$_D$, H-5$_E$, H-4$_E$), 3.70-3.65 (m, 2H, H-5$_C$, H-6a$_E$), 3.61 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.7 Hz, H-2$_E$), 3.45 (t$_{po}$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4$_C$), 3.43 (t$_o$, 2H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4$_A$, H-4$_B$), 3.35 (bd, 1H, $J_{6a,6b}$ = 10.4 Hz, H-6b$_E$), 2.16 (s, 3H, CH$_{3Ac}$), 1.72 (s, 3H, CH$_{3NHAc}$), 1.30 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_B$), 1.20 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_C$), 1.09 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_A$) ; ^{13}C NMR (CDCl$_3$) δ 169.7 (NHCO), 169.6 (CO$_{Ac}$), 138.7-137.2 (10C, C$_{IVAr}$), 134.0 (*C*H=CH$_2$), 129.0-127.4 (50C, C$_{Ar}$), 116.9 (CH=*C*H$_2$), 101.1 (C-1$_B$, $^1J_{C,H}$ = 171.8 Hz), 100.6 (C-1$_A$, $^1J_{C,H}$ = 171.1 Hz), 97.5 (C-1$_D$, $^1J_{C,H}$ = 170.7 Hz), 96.9 (C-1$_E$, $^1J_{C,H}$ = 172.5 Hz), 95.2 (C-1$_C$, $^1J_{C,H}$ = 172.5 Hz), 82.1 (C-3$_E$), 80.2 (C-4$_C$), 80.1 (2C, C-4$_A$, C-4$_B$), 79.5 (C-3$_A$), 79.2 (C-3$_B$), 79.0 (C-2$_E$), 77.5 (C-4$_E$), 76.9 (C-3$_C$), 75.8 (C$_{Bn}$), 75.6 (C-2$_B$), 75.4, 75.3, 74.9, 74.7 (4C, C$_{Bn}$), 74.6 (C-5$_D$), 74.3, 73.4, 73.1, 72.4, 72.2 (5C, C$_{Bn}$), 71.9 (C-2$_C$), 71.4 (C-5$_E$), 70.9 (C-6$_D$), 70.8 (C-4$_D$), 69.3 (C$_{All}$), 68.9 (C-5$_B$), 68.8 (2C, C-5$_C$, C-2$_A$), 68.7 (C-3$_D$), 68.0 (C-5$_A$), 67.6 (C-6$_E$), 46.0 (C-2$_D$), 22.6 (CH$_{3NHAc}$), 21.0 (CH$_{3Ac}$), 18.1 (2C, C-6$_B$, C-6$_C$), 17.8 (C-6$_A$) ; HRMS (ESI$^+$): *m/z* 1826.7903 (calcd for $C_{107}H_{119}NO_{24}Na$ [M+Na]$^+$ *m/z* 1826.8176), HRMS (ESI$^+$): *m/z* 924.9005 (calcd for $C_{107}H_{119}NO_{24}Na_2$ [M+2Na]$^{2+}$ *m/z* 924.9037), HRMS (ESI$^+$): *m/z* 921.8867 (calcd for $C_{107}H_{120}NO_{24}K$ [M+H+K]$^{2+}$ *m/z* 921.8997).

Propyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-(2-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (51): To a stirred solution of pentasaccharide **50** (322 mg, 178 µmol) in 90% aq. EtOH (13.5 mL), were added Pd/C (300 mg) and 1M aq. HCl (33.5 µL). The suspension was stirred under H_2 atmosphere for a day at rt. After this time, MS analysis of the reaction mixture revealed a molecular weight of corresponding to that of the target pentasaccharide. The reaction mixture was filtered over a pad of Celite. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-50% linear gradient of 80% aq. MeCN over 60 min at a flow rate of 20 mL•min^{-1}) gave pentasaccharide **51** (149 mg, 92%) as a white powder following repeated freeze-drying. Monoacetate **51** had ^{1}H NMR (D$_2$O) δ 5.38 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1$_E$), 5.17 (d, 1H, $J_{2,3}$ = 2.5 Hz, H-2$_C$), 5.14 (bs, 1H, H-1$_B$), 5.12 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1$_C$), 4.93 (bs, 1H, H-1$_A$), 4.54 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1$_D$), 4.08 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.2 Hz, H-3$_D$), 4.04 (dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.1Hz, H-2$_A$), 3.97-3.94 (m, 2H, H-2$_B$, H-4$_D$), 3.93 (dd$_o$, 1H, $J_{2,3}$ = 3.0Hz, $J_{3,4}$ = 9.2 Hz, H-3$_C$), 3.92-3.76 (m, 9H, H-2$_D$, H-6a$_D$, H-5$_C$, OCH$_{2Pr}$, H-6b$_D$, H-6a$_E$, H-6b$_E$, H-3$_B$, H-3$_A$), 3.74-3.61 (m, 5H, H-5$_D$, H-5$_A$, , H-3$_E$, H-5$_B$, H-5$_E$), 3.60 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4$_C$), 3.57 (dd$_{po}$, 1H, $J_{2,3}$ = 9.9 Hz, H-2$_E$), 3.50 (dt, 1H, J = 6.2 Hz, J = 10.1 Hz, OCH$_{2Pr}$), 3.43 (pt$_o$, 2H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4$_B$, H-4$_E$), 3.41 (pt$_{po}$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4$_A$), 2.15 (s, 3H, CH$_{3Ac}$), 1.99 (s, 3H, CH$_{3NHAc}$), 1.53 (psex, 2H, J = 6.9 Hz, CH$_{2Pr}$), 1.30 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_C$), 1.27 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6$_B$), 1.24 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6$_A$), 0.85 (t, 3H, J = 7.4 Hz, CH$_{3Pr}$) ; ^{13}C NMR (D$_2$O) δ 176.7 (NHCO), 175.4 (CO$_{Ac}$), 104.9 (C-1$_B$, $^1J_{C,H}$ = 171.6 Hz), 103.6 (C-1$_D$, $^1J_{C,H}$ = 162.7 Hz), 103.2 (C-1$_B$, $^1J_{C,H}$ = 172.3 Hz), 99.6 (C-1$_E$, $^1J_{C,H}$ = 172.3 Hz), 99.1 (C-1$_C$, $^1J_{C,H}$ = 171.6 Hz), 81.9 (C-3$_D$), 80.5 (C-2$_B$), 78.4 (C-5$_D$), 77.7 (C-3$_C$), 75.5 (C-3$_E$), 75.4 (C-5$_E$), 74.8 (OCH$_{2Pr}$), 74.7 (3C, C-4$_B$, C-4$_A$, C-4$_C$), 74.6 (C-2$_C$), 73.9 (2C, C-4$_D$, C-2$_E$), 72.7 (2C, C-2$_A$, C-3$_A$), 72.5 (2C, C-3$_B$, C-5$_C$), 72.1 (C-5$_B$), 71.9 (C-4$_E$), 71.8 (C-5$_A$), 64.0 (C-6$_D$), 63.1 (C-6$_E$), 56.2 (C-2$_D$), 25.0 (CH$_{3NHAc}$), 24.8 (CH$_{2Pr}$), 22.9 (CH$_{3Ac}$), 19.5 (C-6$_C$), 19.3 (2C, C-6$_B$,C-6$_A$), 12.4 (CH$_{3Pr}$) ; HRMS (ESI$^+$): *m/z* 906.3813 (calcd for $C_{37}H_{63}NO_{24}$ [M+H]$^+$ *m/z* 906.3818), HRMS (ESI$^+$): *m/z* 928.3572 (calcd for $C_{37}H_{63}NO_{24}$ Na [M+Na]$^+$ *m/z* 928.3638), HRMS (ESI$^+$): *m/z* 1833.7378 (calcd for $C_{37}H_{63}NO_{24}$ $C_{37}H_{63}NO_{24}$ Na [2M+Na]$^+$ *m/z* 1833.7412) ; RP-HPLC (215 nm): R$_t$ = 13.2 min.

Propyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (52): To a stirred solution of pentasaccharide **51** (91 mg, 100 µmol) in MeOH (8 mL), was added methanolic sodium methoxide (25% w/w, 25 µL). The solution was stirred at rt for 15 h. After this time, MS analysis of the reaction mixture revealed a molecular weight of corresponding to that of the target tetrasaccharide. The reaction solution was neutralized by addition of Dowex H$^+$ resin, and the suspension was filtered over a 0.2 µm filter. Evaporation of the volatiles, freeze-drying and purification of the residue by RP-MPLC (0-50% linear gradient of 80% aq. MeCN over 60 min at a flow rate of 20 mL•min^{-1}) gave pentasaccharide **52** (73 mg, 85%) as a white powder following repeated freeze-drying. The fully deprotected **52** had ^{1}H NMR (D$_2$O) δ 5.29 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1$_E$), 5.11 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1$_B$), 4.98 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1$_C$), 4.87 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1$_A$), 4.49 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1$_D$), 3.99 (t$_{po}$, 1H, $J_{2,3}$ = $J_{3,4}$ = 6.3 Hz, H-3$_D$), 3.98 (dd$_o$, 1H, $J_{2,3}$ = 3.2 Hz, H-2$_A$), 3.96 (dd$_{po}$, 1H, $J_{2,3}$ = 3.2 Hz, H-2$_B$), 3.89 (pt$_{po}$, $J_{3,4}$ = $J_{4,5}$ = 6.5 Hz, H-4$_D$), 3.88 (dd$_o$, 1H, $J_{2,3}$ = 2.4 Hz, H-2$_C$), 3.86-3.82 (m, 2H, H-2$_D$, H-3$_B$), 3.80 (dd$_{po}$, 1H, $J_{5,6a}$ = 7.9 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a$_D$), 3.78-3.65 (m, 8H, OCH$_{2Pr}$, H-6a$_E$, H-6b$_D$, H-3$_A$, H-6b$_E$, H-5$_C$, H-3$_C$, H-5$_D$, H-5$_B$), 3.64 (dq$_{po}$, 1H, H-5$_A$), 3.60 (pt$_{po}$, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3$_E$), 3.54 (ddd$_{po}$, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 4.6 Hz, $J_{4,5}$ = 10.0 Hz, H-5$_E$), 3.50 (dd$_{po}$, 1H, $J_{2,3}$ = 9.8 Hz, H-2$_E$), 3.48 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4$_C$), 3.44 (dt, 1H, J = 6.6 Hz, J = 9.6 Hz, OCH$_{2Pr}$), 3.39 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4$_B$), 3.36 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4$_E$), 3.35 (pt$_o$, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4$_A$), 1.93 (s, 3H, CH$_{3NHAc}$), 1.46 (psex, 2H, J = 7.3 Hz, CH$_{2Pr}$), 1.23 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_B$), 1.19 (d$_{po}$, 3H, $J_{5,6}$ = 6.1 Hz, H-6$_C$), 1.17 (d$_{po}$, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_A$), 0.85 (t, 3H, J = 7.4 Hz, CH$_{3Pr}$) ; ^{13}C NMR (D$_2$O) δ 176.4 (NHCO), 104.9 (C-1$_A$, $^1J_{C,H}$ = 171.4 Hz), 103.6 (C-1$_D$, $^1J_{C,H}$ = 162.2 Hz), 103.2 (C-1$_B$, $^1J_{C,H}$ = 173.1 Hz), 101.5 (C-1$_C$, $^1J_{C,H}$ = 170.2 Hz), 99.4 (C-1$_E$, $^1J_{C,H}$ = 171.9 Hz), 80.6 (2C, C-2$_B$, C-3$_D$), 79.1 (C-3$_C$), 78.7 (C-5$_D$), 75.4 (C-3$_E$), 75.3 (C-5$_E$), 74.7 (2C, C-4$_B$, OCH$_{2Pr}$), 74.6 (C-4$_A$), 74.4 (C-4$_C$), 73.8 (C-2$_E$), 73.3 (C-4$_D$), 73.0 (C-2$_C$), 72.6 (2C, C-2$_A$,C-3$_A$), 72.5 (C-5$_C$), 72.4 (C-3$_B$), 71.8 (2C, C-4$_E$, C-5$_B$), 71.7 (C-5$_A$), 64.0 (C-6$_D$), 62.9 (C-6$_E$), 56.2 (C-2$_D$), 24.7 (2C, CH$_{3NHAc}$, CH$_{2Pr}$), 19.4 (C-6$_B$), 19.3 (2C, C-6$_C$, C-6$_A$), 12.2 (CH$_{3Pr}$) ; HRMS (ESI$^+$): *m/z* 451.6599 (calcd for $C_{35}H_{61}NO_{23}Na$ [M+H+Na]$^{2+}$ *m/z* 451.6675), HRMS (ESI$^+$): *m/z* 864.3688 (calcd for $C_{35}H_{61}NO_{23}$ [M+H]$^+$ *m/z* 864.3713), HRMS (ESI$^+$): *m/z* 886.3514 (calcd for

$C_{35}H_{60}NO_{23}Na$ $[M+Na]^+$ *m/z* 886.3532), HRMS (ESI^+): *m/z* 1750.7321 (calcd for $C_{70}H_{120}N_2O_{46}Na$ $[2M+Na]^+$ *m/z* 1750.6932) ; RP-HPLC (215 nm): R_t = 10.2 min.

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Notes and references

[a] *Institut Pasteur, Unité de Chimie des Biomolécules, 28 rue du Dr Roux, 75724 Paris Cedex 15, France. Fax: +33 1 45 68 84 04; Tel: +33 1 40 61 38 20; E-mail: laurence.mulard@pasteur.fr*

[b] *CNRS UMR3523, Institut Pasteur, 75015 Paris, France*

[c] *Université Paris Descartes Sorbonne Paris Cité, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France.*

[§] *These authors contributed equally.*

[‡] *Present address: University of Calgary 2500 University Drive N.W. Calgary, Alberta, Canada, T2N 1N4.*

[#] *Present address: Arkema Saint-Auban, 04600 Saint-Auban, France.*

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