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Total synthesis of a tetra- and two pentasaccharide fragments of the O-specific polysaccharide of *Shigella flexneri* serotype 2a☆

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Abstract—The synthesis of the methyl glycoside of the branched pentasaccharide biological repeating unit of the O-antigen of *Shigella flexneri* serotype 2a is described together with that of the methyl glycoside of the corresponding tetrasaccharide and frame-shifted linear pentasaccharide. All the strategies disclosed herein involve a key disaccharide corresponding to the branching point and otherwise appropriate monosaccharide building blocks activated as their trichloroacetimidate. Our data suggest partial lack of conformational flexibility at the branched residue.

1. Introduction

Shigellosis, also known as bacillary dysentery, is a major enteric disease which accounts for some 165 million annual episodes, among which 1.1 million deaths, occurring mostly in developing countries.[12] Young children and immunocompromised individuals are the main victims. Some 15 years ago, vaccination was defined as a priority by the WHO in its program on enteric diseases. However, there is still no license vaccine against this bacterial infection although intensive research is ongoing in the field.[11] *Shigellae* are Gram negative bacteria. As for other bacterial pathogens, their lipopolysaccharide (LPS) is an important virulence factor. It is also a major target of the host's protective immunity against infection.

Shigella flexneri 2a is the prevalent serotype in developing countries, where it is responsible for the endemic form of the disease.[12] Based on the early hypothesis that a critical level of serum IgG antibodies specific for the O-specific polysaccharide (O-SP) moiety of the LPS was sufficient to confer protection against homologous infections,[26,27] several *S. flexneri* 2a O-SP-protein conjugates were prepared. They were found safe and immunogenic in both adults and children.[2,22]

Allowing a better control of the various structural parameters possibly involved in the immunogenicity of glycoconjugate vaccines, oligosaccharide-protein conjugates were proposed as alternatives to polysaccharide–protein conjugate vaccines against bacteria.[24] Indeed, such constructs were found immunogenic on several occasions, including examples whereby the oligosaccharide portion was made of one repeating unit only.[5,18] We reasoned that glycoconjugates incorporating chemically synthesized oligosaccharides, appropriately selected for their ability to mimic the native O-SP in terms of both antigenicity and solution conformation, may offer an alternative to the *S. flexneri* 2a O-SP-protein conjugates currently under study. Our approach relies on a rational basis. Indeed, in order to select the best oligosaccharide mimic, we have undertaken the characterization of the antigenic determinants of *S. flexneri* 2a O-SP recognized by serotype-specific protective monoclonal antibodies. A panel of methyl glycosides representative of fragments of *S. flexneri* 2a O-SP was thus synthesized to be used as probes in the study of antibody recognition.

A **B** **E** **C** **D**

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*(1→

I

The O-SP of *S. flexneri* 2a is a heteropolysaccharide defined by the pentasaccharide repeating unit **I**.[15,29] It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-antigens and comprises a *N*-acetyl

☆ See Ref. 1.

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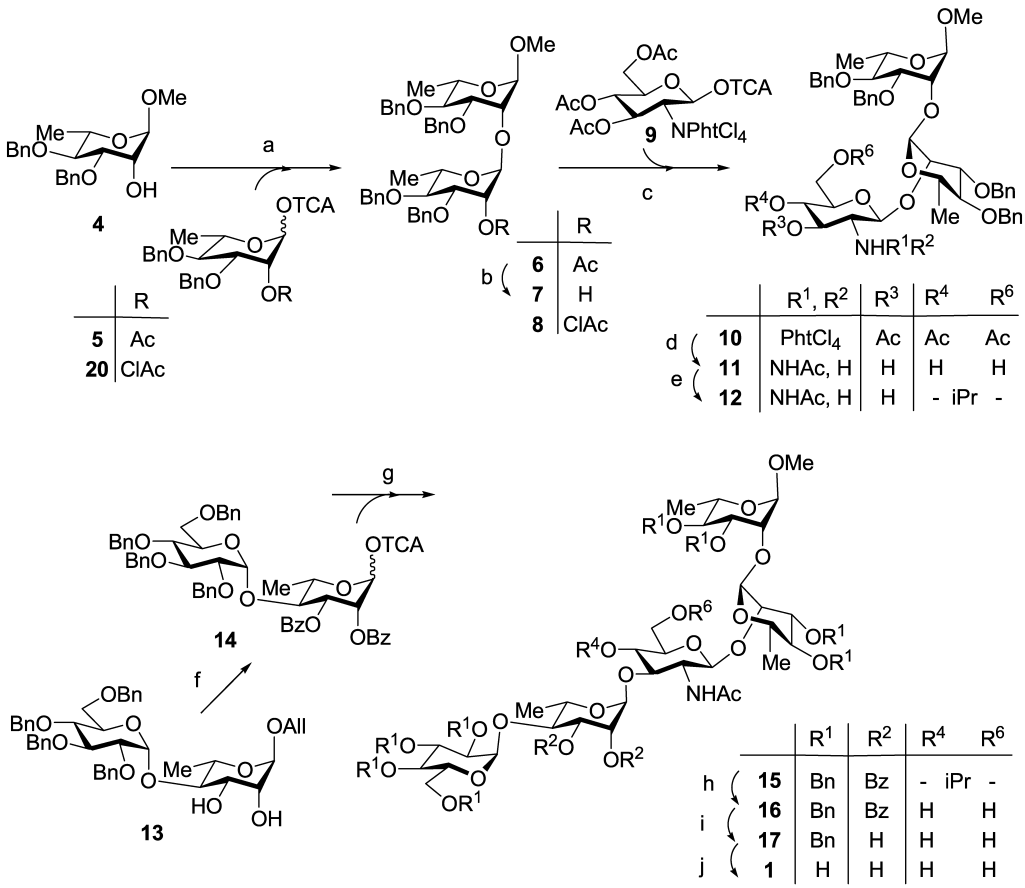
glucosamine and three rhamnose residues, together with an α-D-glucopyranose residue branched at position 4 of one of the rhamnoses. We have already reported on the synthesis of the methyl glycosides of various fragments of the O-SP, including the known **EC** disaccharide,[6,16,19] the **ECD**[19] and **B(E)C**[19] trisaccharides, the **ECDA**[28] and **AB(E)C**[9] tetrasaccharides, the **B(E)CDA**[28] and **DAB(E)C**[9] pentasaccharides, the **B′(E′)C′DAB(E)C** octasaccharide[3] and more recently the **D′A′B′(E′)C′DAB(E)C** decasaccharide.[4] However, in order to complete the full set of frame-shifted fragments of the repeating unit, the methyl glycosides of the **ECDAB**, **AB(E)CD** pentasaccharides and that of the **B(E)CD** tetrasaccharide, **1**, **2** and **3**, respectively, were missing. Their synthesis is reported in the following.

2. Results and discussion

Analysis of the targets shows that all the glycosylation reactions to set up involve 1,2-*trans* glycosidic linkages except for that at the **E**–**C** junction which is 1,2-*cis*. Consequently, the syntheses described herein rely on key **EC** disaccharide building blocks as well as on appropriate **A**, **B** and **D** monosaccharide synthons.

2.1. Synthesis of the linear ECDAB-OMe pentasaccharide 1

Earlier findings in the series have demonstrated that the **C**–**D** linkage was an appropriate disconnection site.[3,4,28] Consequently, the synthesis of **1** was designed (Scheme 1) based on the glycosylation of the known **EC** trichloroacetimidate donor **14**,[19] obtained in three steps (69%) from the key diol **13**,[28] and the **DAB** trisaccharide acceptor **12**. The latter was obtained by the stepwise condensation of known monosaccharide precursors, readily available by selective protection, deprotection and activation sequences. Thus, TMSOTf-catalysed condensation of the rhamnopyranoside acceptor **4**[25] with the trichloroacetimidate donor **5**[7] in diethyl ether to give the fully protected rhamnobioside **6**,[23] and subsequent de-*O*-acetylation gave the **AB** disaccharide acceptor **7**[25] in 91% overall yield, which compares favourably with the previously described preparation using the corresponding 1-*O*-acetyl donor.[25] Analogously to previous work in a related series,[4] the known glucosaminyl trichloroacetimidate donor **9**,[8] was chosen as the precursor to residue **D**. Conventional glycosylation of **7** with **9** was best performed in acetonitrile using tin trifluoromethanesulfonate ($Sn(OTf)_2$) as the catalyst[17] to give the fully protected trisaccharide **10** in 72% yield (extracted from the ^1H NMR spectrum). When TMSOTf was used instead of $Sn(OTf)_2$, **10** was formed in lower yield (52%) outlining the sensitivity of the tetrachlorophtaloyl group to these stronger conditions, as previously noted.[14] A three step process including heating **10** with ethylenediamine in dry ethanol,[10] ensuing *N*-acetylation with acetic anhydride, and de-*O*-acetylation under Zemplén conditions, furnished the triol **11** (51% from **7**). It



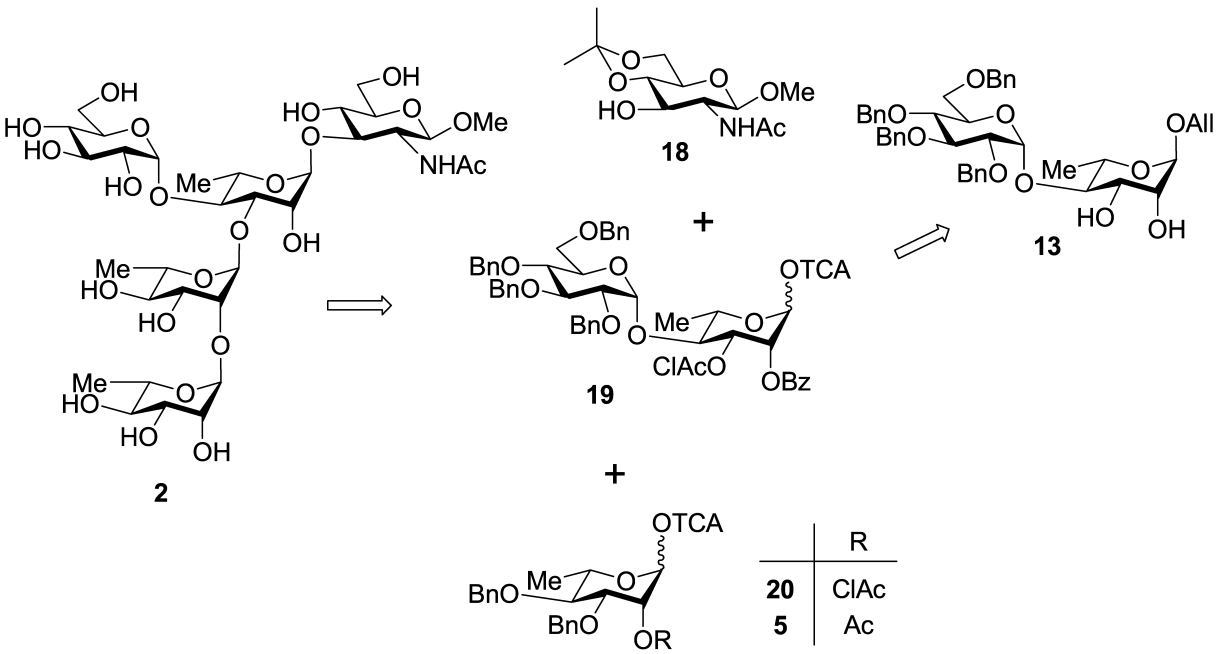
Scheme 1. (a) **6** from **5**, **8** from **20**, TMSOTf, Et_2O, −35 °C→rt; (b) cat. MeONa, MeOH–CH_2Cl_2, rt; (c) from **7**, $Sn(OTf)_2$, CH_3CN, rt; (d) (i) $H_2NCH_2CH_2NH_2$, EtOH, 60 °C, (ii) Ac_2O, EtOH; (iii) MeONa, MeOH–CH_2Cl_2, rt; (e) $Me_2C(OMe)_2$, PTSA, acetone, rt; (f) see Ref. 19; (g) 4 Å molecular sieves, TfOH, CH_2Cl_2, −15 °C→rt; (h) 90% aq. TFA, 0 °C; (i) cat. MeONa, MeOH–CH_2Cl_2, rt; (j) H_2, 10% Pd/C, EtOH–AcOH, rt.

was next protected at positions 4_D and 6_D by regioselective introduction of an isopropylidene acetal upon reaction with 2,2-dimethoxypropane under acid-catalysis to give **12** (96%). The latter acetal-protecting group was selected based on data previously obtained when synthesizing shorter fragments in the series which had outlined the interest of using 4,6-*O*-isopropylidene–glucosaminyl intermediates instead of the more common benzylidene analogues.[19] Once the two key building blocks were made available, their condensation was performed in dichloromethane in the presence of a catalytic amount of triflic acid to give the fully protected pentasaccharide **15** (84%). Conventional stepwise deprotection involving (i) acidic hydrolysis of the isopropylidene acetal using 90% aq. TFA to give diol **16** (95%), (ii) conversion of the latter into the corresponding tetraol **17** under Zemplén conditions (86%), and (iii) final hydrogenolysis of the benzyl protecting groups, gave the linear pentasaccharide target **1** in 81% yield.

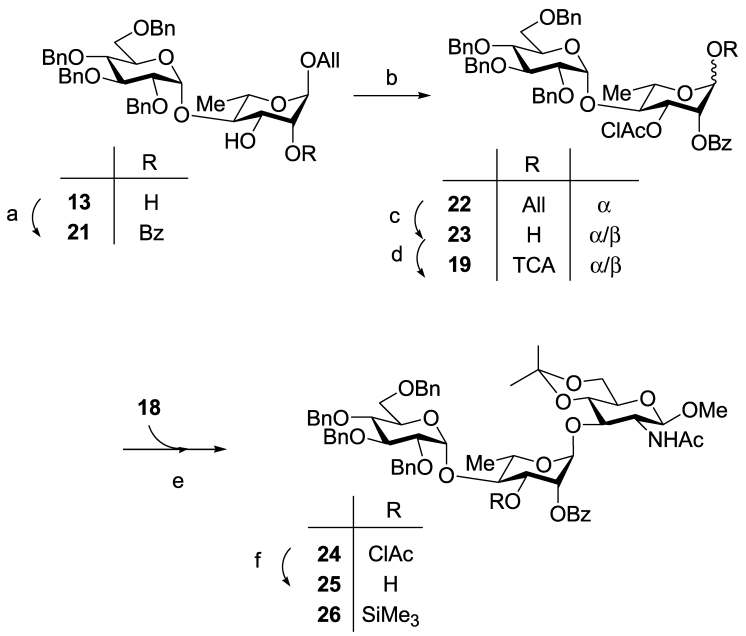
2.2. Synthesis of the AB(E)CD pentasaccharide 2 and of the B(E)CD tetrasaccharide 3

For reasons mentioned above, the glucosaminyl acceptor **18**,[19] protected at its 4 and 6 hydroxyl groups by an isopropylidene acetal was the precursor of choice for residue **D** (Scheme 2). In the past, introduction of residue **B** at position 3_C was performed on a 2_C-*O*-benzoylated **EC** acceptor resulting from the regioselective acidic hydrolysis of the corresponding 2,3-orthoester intermediate.[9,28] It rapidly occurred to us that opening of the intermediate phenyl orthoester was not compatible with the presence of the 4_D,6_D-*O*-isopropylidene acetal. For that reason, the trichloroacetimidate donor **19**, suitably benzoylated at position 2_C and orthogonally protected by a chloroacetyl group at position 3_C was used as the **EC** building block instead of the previously used **14**. Protection at the 2-OH of the rhamnosyl precursor to residue **B** was also crucial in the synthesis of **2**. Indeed, most of our previous work in the series relied on the use of the known 2-*O*-acetyl rhamnopyranosyl donor **5**. In the reported syntheses,[9] selective de-*O*-acetylation at position 2_B in the presence of a 2_C-*O*-benzoate was best performed by treatment with methanolic $HBF_4{\cdot}OEt_2$ for 5 days. Clearly, such de-*O*-acetylation conditions are not compatible with the presence of an isopropylidene acetal on the molecule. To overcome this limitation, the corresponding 2-*O*-chloroacetyl rhamnopyranosyl trichloroacetimidate **20** was selected as an alternate donor. In theory, the latter could also serve as an appropriate precursor to residue **A**.

Regioselective conversion of diol **13** into its 2-*O*-benzoylated counterpart **21** was performed as described (Scheme 3).[28] Treatment of the latter with chloroacetic anhydride and pyridine gave the orthogonally protected **22** (95%), which was smoothly de-*O*-allylated to yield the corresponding hemiacetal **23** (91%) by a two-step process, involving (i) iridium (I)-promoted isomerisation[21] of the allyl glycoside and (ii) subsequent hydrolysis in the presence of iodine.[20] The selected trichloroacetimidate leaving group was successfully introduced by treatment of **23** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[4.3.0]undec-7-ene (DBU), which resulted in the formation of **19** (84%) together with the recovery of some starting hemiacetal (14%) since partial hydrolysis during column chromatography could not be avoided. TMSOTf-mediated glycosylation of donor **19** and acceptor **18** furnished the fully protected **ECD** trisaccharide (**24**, 80%), which was readily converted to the required acceptor **25** upon selective deblocking of the chloroacetyl protecting group with thiourea (97%). Following the two-step protocol described above for the preparation of **19**, the known allyl rhamnopyranoside **27**,[33] bearing a 2-*O*-chloroacetyl protecting group, was converted to the hemiacetal **28** (85%) (Scheme 4). Next, treatment of the latter with trichloroacetonitrile and a slight amount of DBU gave at best donor **20**

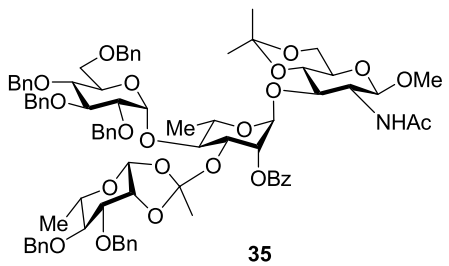


Scheme 2. Retrosynthetic analysis of pentasaccharide **2**.



Scheme 3. (a) see Ref. 28; (b) $(ClAc)_2O$, Pyridine–CH_2Cl_2, 0 °C; (c) (i) $(COD)Ir^+(P(MePh_2)_2)PF_6^-$, THF; (ii) I_2, THF/H_2O, rt; (d) CCl_3CN, DBU, CH_2Cl_2, 0 °C; (e) 4 Å molecular sieves, TMSOTf, CH_2Cl_2, −60 °C→rt; (f) thiourea, MeOH–pyridine, 65 °C.

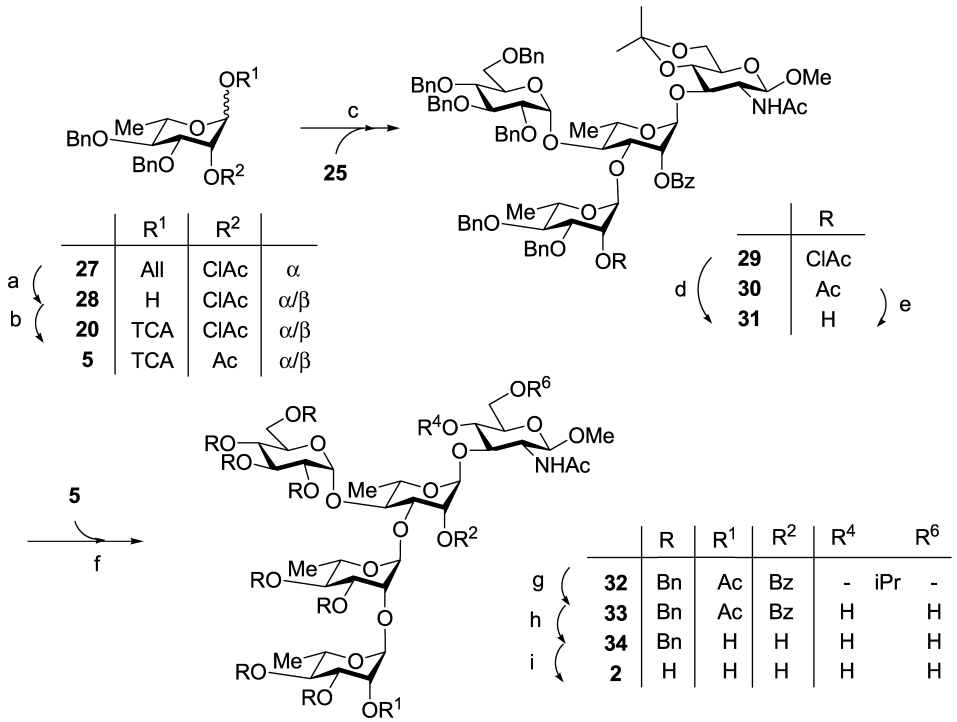
in a yield of 73%. Although the isolated yield of **20** was not better (72%), running the activation step in the presence of K_2CO_3 instead of DBU resulted in a more reproducible isolated yield of the activated donor. Glycosylation of the **ECD** acceptor **25** and the **B** donor **20** was attempted under various conditions of solvent and catalyst. Whatever the conditions, hardly separable mixtures of compounds were obtained, among which the yield of the target tetrasaccharide reached 45–50%. Running the condensation in Et_2O in the presence of TMSOTf as the promoter were the best conditions tested, although the expected tetrasaccharide **29** was often slightly contaminated with glycosylation intermediates such as the silylated **26** or the orthoester **35**, as suggested from mass spectroscopy analysis and NMR data. In fact, the nature of the latter was fully ascertained at the next step in the synthesis. Indeed, full recovery of the starting material was observed upon treatment of **35** with thiourea (Fig. 1). On the contrary, treatment of a mixture of the condensation products **29** and supposedly **26** under the same conditions led to the expected tetrasaccharide acceptor **31** and the trisaccharide acceptor **25** (not described). The β**B**-tetrasaccharide isomer could not be detected at this stage, indicating that the corresponding chloroacetylated β**B**-anomer was probably not part of the initial mixture. Formation of the starting **25** during the dechloroacetylation step was not unexpected, since loss of a trimethylsilyl group under similar treatment was observed for a model compound (not described). Although the fluoride analog corresponding to donor **20** has been used successfully in a prior report,[33] the poor yield of **29** may be, in part, associated to the sensitivity of the chloroacetyl group to the glycosylation conditions. Thus, in order to investigate the poor outcome of the condensation reaction, the donor properties of the chloroacetylated **20** were compared to that of the more common acetylated **5**. When methyl rhamnopyranoside **4** was condensed with **20** as described for the preparation of **6**, the rhamnobioside **8** was isolated in 67% yield. This result tends to suggest that indeed the acetylated **5** is a more powerful donor than **20**.



35

Figure 1.

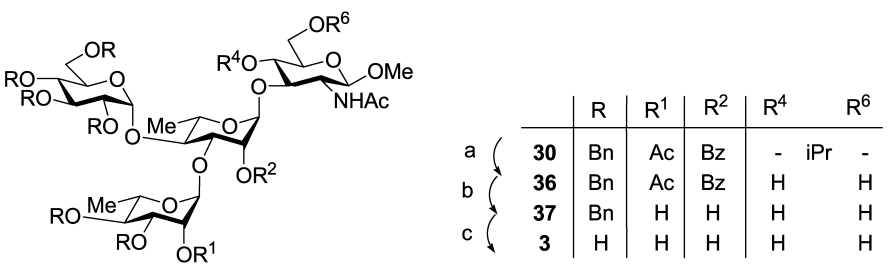
Starting from **20** and **25**, the isolated yield of the tetrasaccharide acceptor **31** was 34%, which encouraged us to reconsider the use of **5** as a precursor to residues **B** and **A** in the synthesis of **2**. Condensation of **5** and **25** in CH_2Cl_2 using TMSOTf as the promoter furnished the corresponding tetrasaccharide **30** (72%). However, even though the yield of **30** was better than that of **29**, slight contamination by the silylated side-product **26** was again apparent, outlining the somewhat poor reactivity of the **ECD** acceptor. Subsequent treatment of **30** with a 4 mM ethanolic solution of guanidine[13] resulted, as expected, in selective 2_B-*O*-deacetylation to give **31** in a satisfactory 83% yield, which outlined the interest of the method. However, previous experience in other closely related series has shown that the selectivity of the method was highly dependent on the nature of the substrate. Nevertheless, the 2-*O*-acetylated donor **5** was clearly preferred to the chloroacetate analogue **20**. Condensation of the tetrasaccharide acceptor **31** and donor **5** in the presence of TMSOTf gave the fully protected pentasaccharide **32** in a yield of 52%. TFA-mediated hydrolysis of the isopropylidene acetal followed by transesterification of the ester groups and subsequent conventional hydrogenolysis of the benzyl ethers finally gave the target pentasaccharide **2** (88%).



Scheme 4. (a) (i) $(COD)Ir^{+}(P(MePh_2)_2)PF_6^{-}$, THF; (ii) I_2, THF/H_2O, rt; (b) CCl_3CN, K_2CO_3, CH_2Cl_2, 0 °C; (c) **29** from **20**, **30** from **5**, TMSOTf, Et_2O, −60 °C→0 °C; (d) thiourea, MeOH–pyridine, 65 °C; (e) guanidine, EtOH–CH_2Cl_2, rt; (f) 4 Å molecular sieves, TMSOTf, Et_2O, −60 °C→rt; (g) 50% aq. TFA, CH_2Cl_2, 0 °C; (h) 0.5% MeONa, MeOH, 55 °C; (i) 10% Pd/C, EtOH–AcOEt, 1 N aq. HCl, rt.

Alternatively, the fully protected tetrasaccharide **30** was converted to the diol **36** by acidic removal of the isopropylidene acetal (85%), and subsequently to the corresponding tetraol **37** upon transesterification (83%). Final hydrogenolysis of the benzyl groups furnished the target tetrasaccharide **3** (71%) (Scheme 5).

Noteworthy, in the case of intermediates **33** and **36**, successful sodium methoxide-mediated transesterification of the acyl groups required heating of the reaction mixture.[31] Isolation of the esters to be cleaved may best explain the phenomenon. Indeed, the above-mentioned procedure may be seen as an alternative to the use of K_2CO_3 in dioxane/methanol[30] or that of *t*BuOK in methanol,[32] which were found appropriate in related cases. Steric hindrance may account for the poor outcome of the condensation of the **ECD** acceptor **25** with the **B** donors **20** and **5**. Interestingly, ^{13}C NMR data support this hypothesis. Although no altered signals could be seen in the ^{13}C NMR spectrum of the **ECD** acceptor **25** or in the ^{13}C NMR spectra of the fully protected precursor **24**, significant disturbance of several signals in the ^{13}C NMR spectra of the tetra- and pentasaccharides were observed repeatedly. At the protected and partially protected stage, major altered signals are those tentatively assigned to C-3_C and C-4_C. Besides, signals assigned to C-2_D, C-3_D as well as to C-1_B are significantly broader than expected. Loss of conformational flexibility at the **C** ring is not totally unexpected especially since the carbons involved are those corresponding to the branching points. Of particular interest, however, was the observation that residue **D**, the *N*-acetyl-glucosaminyl residue, was also partially constrained. Full conformational freedom of residue **D** is recovered when the **B(E)CD** and **AB(E)CD** oligosaccharides are in their free form. However, this observation does not stand true for residue **C** since characteristic broad signals for C-3_C and C-4_C as well for C-1_B and C-1_E are still present in the ^{13}C NMR spectra of compounds **2** and **3**, respectively. Overall, these observations suggest a somewhat compact organisation at the branching point of the **B(E)CD** structure. It is worth mentioning that none of these disturbed signals are seen in the ^{13}C NMR spectra of the oligosaccharides corresponding to the linear **ECDAB** fragment.



Scheme 5. (a) 50% aq. TFA, CH_2Cl_2, 0 °C; (b) 0.1% MeONa, MeOH, 55 °C; (c) 10% Pd/C, EtOH–AcOEt, 1 N aq. HCl, rt.

3. Conclusion

The synthesis of the methyl glycoside (**2**) of the repeating unit **I** of the *S. flexneri* 2a O-SP, together with that of the corresponding frame-shifted pentasaccharide **1** and tetrasaccharide **3** were described. All the methyl glycosides of the di- to pentasaccharides obtained by circular permutation of the monosaccharide residues partaking in the linear backbone of **I**, and comprising the **EC** portion, are now available in the laboratory. Their binding to a set of protective monoclonal IgG antibodies will be reported elsewhere.

4. Experimental

4.1. General methods

Melting points were determined in capillary tubes with an electrothermal apparatus and are uncorrected. Optical rotations were measured for $CHCl_3$ solutions at 25 °C, except where indicated otherwise, with a Perkin–Elmer automatic polarimeter, Model 241 MC. TLC on precoated slides of Silica Gel 60 F_{254} (Merck) was performed with solvent mixtures of appropriately adjusted polarity consisting of *A*, dichloromethane–methanol; *B*, cyclohexane–ethyl acetate, *C*, cyclohexane–acetone, *D*, water–acetonitrile, *E*, *iso*-propanol–ammonia–water; *F*, 0.01 M aq. TFA–acetonitrile. Detection was effected when applicable, with UV light, and/or by charring with orcinol (35 mM) in 4 N aq. H_2SO_4. Preparative chromatography was performed by elution from columns of Silica Gel 60 (particle size 40–63 μm). RP-HPLC (215 nm) used a Kromasil 5 μm C18 100 Å 4.6×250 mm analytical column (1 mL min^{-1}). The NMR spectra were recorded at 20 °C for solution in $CDCl_3$, unless stated otherwise, on a Brucker Avance 400 spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C). External references: for solutions in $CDCl_3$, TMS (0.00 ppm for both ^1H and ^{13}C); for solutions in D_2O, dioxane (67.4 ppm for ^{13}C) and trimethylsilyl-3-propionic acid sodium salt (0.00 ppm for ^1H). Proton signal assignments were made by first-order analysis of the spectra, as well as analysis of two-dimensional $^1H-^1H$ correlation maps (COSY) and selective TOCSY experiments. 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. The ^{13}C NMR assignments were supported by two-dimensional $^{13}C-^1H$ correlation maps (HETCOR). Interchangeable assignments are marked with an asterisk in the listing of signal assignments. Sugar residues in oligosaccharides are serially lettered according to the lettering of the repeating unit of the O-SP and identified by a subscript in the listing of signal assignments. Low-resolution mass spectra were obtained by either chemical ionisation (CIMS) using NH_3 as the ionising gas, by electrospray mass spectrometry (ESMS), or by fast atom bombardment mass spectrometry (FABMS). HRMS were obtained by Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDIMS).

4.1.1. Methyl (3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (8). Activated powered 4 Å molecular sieves (200 mg) was added to a solution of alcohol[25] **4** (60 mg, 167 μmol) and trichloroacetimidate donor **20** (113 mg, 0.2 mmol) in dry Et_2O (2 mL) and the solution was stirred at rt for 30 min then cooled to −40 °C. TMSOTf (9 μL, 50 μmol) was added and the mixture was stirred for 1 h at −30 °C, then for 2 h while the bath temperature was coming back to rt. TLC (solvent B, 4:1) showed the presence of a major product less polar than **4**. The mixture was neutralized by addition of Et_3N, and filtered on a pad of Celite. Concentration of the filtrate and column chromatography of the residue (solvent B, 4:1) gave 86 mg of **8** as a colourless oil (67%). $[\alpha]_D$ −13.6 (*c* 1.0); ^1H NMR δ 7.42–7.32 (m, 20H, Ph), 5.64 (dd, 1H, $J_{1,2}$=1.9 Hz, $J_{2,3}$=3.2 Hz, H-2_A), 5.07 (d, 1H, H-1_A), 4.98–4.93 (m, 2H, OCH_2), 4.83–4.61 (m, 6H, OCH_2), 4.64 (brs, 1H, H-1_B), 4.18 (d, 1H, *J*=15.2 Hz, CH_2Cl), 4.13 (d, 1H, OCH_2Cl), 3.90 (dd, 1H, $J_{3,4}$=9.3 Hz, H-3_B), 3.89 (m, 1H, partially overlapped, $J_{5,6}$=6.3 Hz, H-5_A), 3.73 (dq, 1H, $J_{4,5}$=9.5 Hz, $J_{5,6}$=6.2 Hz, H-5_B), 3.48 (pt, 1H, $J_{3,4}$=9.4 Hz, H-4_B), 3.45 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-4_A), 3.36 (s, 3H, OCH_3), 1.37 (d, 3H, H-6_A), 1.35 (d, 3H, H-6_B); ^{13}C NMR δ 165.5 (C=O), 137.4–126.4 (Ph), 100.2 (C-1_A), 99.2 (C-1_B), 80.4, 80.3, 80.2 (3C, C-4_A, 4_B, 3_B), 77.9 (C-3_A), 75.8, 75.7 (2C, OCH_2), 74.8 (C-2_B), 72.6, 72.5 (2C, OCH_2), 71.2 (C-2_A), 68.7 (C-5_A), 68.2 (C-5_B), 55.0 (OCH_3), 41.4 (CH_2Cl), 18.4 (2C, C-6_A, 6_B). FABMS for $C_{43}H_{49}ClNO_{10}$ (M, 760.3) *m/z* 783.3 $[M+Na]^+$. Anal. calcd for $C_{43}H_{49}ClNO_{10}$: C, 67.84; H, 6.49%. Found: C, 68.03; H, 7.02.

4.1.2. Methyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (7). Activated powered 4 Å molecular sieves was added to a solution of alcohol **4** (322 mg, 0.90 mmol) and trichloroacetimidate donor[7] **5** (573 mg, 1.08 mmol) in dry Et_2O (9 mL) and the solution was stirred at rt for 30 min then cooled to −35 °C. TMSOTf (48 μL, 266 μmol) was added and the mixture was stirred for 4 h, while the bath temperature was coming back to rt. TLC (solvent B, 23:2) showed that only little starting material remained and the mixture was neutralized by addition of Et_3N, and filtered on a pad of Celite. Concentration of the filtrate and column chromatography of the residue (solvent B, 9:1) gave 647 mg of slightly contaminated **6**. The later (626 mg) was dissolved in a mixture of CH_2Cl_2 (2 mL) and MeOH (5 mL), and 1 M methanolic sodium methoxide (300 μL) was added. The mixture was stirred overnight, neutralized with Amberlite IR 120 (H^+), filtered and concentrated. Chromatography of the residue (solvent G, 89:11) gave syrupy **7** (554 mg, 91% from **4**). Analytical data were as described.[25]

4.1.3. Methyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophtalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (10). A solution of disaccharide **7** (179 mg, 0.26 mmol) and trichloroacetimidate donor[8] **9** (436 mg, 0.60 mmol) in dry CH_3CN (9 mL) was stirred at rt for 30 min in the presence of activated 4 Å molecular sieves (1.2 g). Tin(II) trifluoromethanesulfonate $[Sn(OTf)_2]$ (75 mg, 180 μmol) was added and the mixture was stirred at rt for 4 h, then neutralized with Et_3N. Filtration on a pad of Celite, concentration of the filtrate and column chromatography of the residue (solvent B, 87:13) gave **10** (324 mg) as a slightly contaminated white foam (72% as

estimated from the ^{1}H NMR spectrum). An analytical sample had $[\alpha]_D$ +23.3 (*c* 1.0); ^{1}H NMR δ 7.43–7.17 (m, 20H, Ph), 5.92 (d, 1H, *J*=9.2, 10.5 Hz, H-3$_D$), 5.24 (d, 1H, $J_{1,2}$=8.4 Hz, H-1$_D$), 5.14 (dd, 1H, *J*=9.7, 9.4 Hz, H-4$_D$), 5.00 (brs, 1H, H-1$_A$), 4.79 (d, 1H, *J*=10.8 Hz, OCH$_2$), 4.65 (s, 2H, OCH$_2$), 4.55 (d, 1H, *J*=11.2 Hz, OCH$_2$), 4.53 (brs, 1H, H-1$_B$), 4.46–4.36 (m, 3H, H-2$_D$, OCH$_2$), 4.28 (d, 1H, *J*=12.4 Hz, OCH$_2$), 4.26 (d, 1H, *J*=10.6 Hz, OCH$_2$), 4.06 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{5,6a}$=6.8 Hz, H-6a$_D$), 3.91 (brs, 1H, H-2$_B$), 3.85–3.69 (m, 5H, H-2$_A$, 3$_B$, 3$_A$, 6b$_D$, 5$^*_A$), 3.59 (dq, 1H, $J_{4,5}$=9.4 Hz, $J_{5,6}$=6.2 Hz, H-5$^*_B$), 3.40 (m, 1H, H-5$_D$), 3.27 (s, 3H, OCH$_3$), 3.18 (m, 2H, H-4$_A$, 4$_B$), 2.03, 2.01, 1.94 (3s, 9H, C(O)CH$_3$), 1.27, 1.25 (2d, 6H, H-6$_A$, 6$_B$); ^{13}C NMR δ 170.5, 170.4, 170.3, 163.8, 162.6 (5C, C=O), 140.3–128.0 (Ph), 101.1 (C-1$_A$), 100.0 (C-1$_D$), 99.8 (C-1$_B$), 80.7 (2C, C-4$_A$, 4$_B$), 79.7 (C-2$_A$), 78.9 (C-3$_B$), 78.1 (C-3$_A$), 76.2 (C-2$_B$), 75.3, 75.2, 72.7, 71.4 (4C, OCH$_2$), 71.3 (C-5$_D$), 70.1 (C-3$_D$), 68.5 (C-5$^*_A$), 68.4 (C-4$_D$), 67.4 (C-5$^*_B$), 61.3 (C-6$_D$), 55.4 (C-2$_D$), 54.6 (OCH$_3$), 20.7, 20.6 (3C, C(O)CH$_3$), 18.0, 17.7 (2C, C-6$_A$, 6$_B$). FABMS for $C_{61}H_{63}Cl_4NO_{18}$ (M, 1237.3) *m/z* 1259.9 [M+Na]$^+$. Anal. calcd for $C_{61}H_{63}Cl_4NO_{18}\cdot H_2O$: C, 58.24; H, 5.21; N, 1.11%. Found: C, 58.21; H, 4.91; N, 1.01%.

4.1.4. Methyl (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (11). A solution of disaccharide **7** (179 mg, 0.26 mmol) and trichloroacetimidate donor **9** (436 mg, 0.60 mmol) in dry CH_3CN (9 mL) was stirred at rt for 30 min in the presence of activated 4 Å molecular sieves (1.2 g). Tin(II) trifluoromethanesulfonate [Sn(OTf)$_2$] (75 mg, 180 μmol) was added and the mixture was stirred at rt for 4 h, then neutralized with Et_3N. Filtration on a pad of Celite, concentration of the filtrate and column chromatography of the residue (solvent B, 87:13) gave **10** (324 mg) as a slightly contaminated product. The latter was solubilized in dry ethanol (13 mL) and diethylamine (200 μL, 3.0 mmol) was added and the mixture was stirred overnight at 60 °C. The mixture was cooled to rt and acetic anhydride (1.0 mL, 10.6 mmol) was added and the mixture was stirred at this temperature for 2 h. The suspension was filtered and volatiles were evaporated and co-evaporated repeatedly with toluene and cyclohexane. The crude residue was taken up in a minimum of CH_2Cl_2 and MeOH (10 mL). 1 N methanolic sodium methoxide was added until the pH was 10 and the solution was stirred overnight at rt, neutralized with IR 120 (H$^+$), filtered and concentrated. Chromatography of the residue (solvent A, 24:1) gave foamy **11** (135 mg, 51% from **7**). $[\alpha]_D$ −15.0 (*c* 1.0); ^{1}H NMR δ 7.44–7.28 (m, 20H, Ph), 8.88 (brs, 1H, NH$_D$), 5.28 (brs, 1H, H-1$_A$), 4.93–4.61 (m, 8H, OCH$_2$), 4.59 (s, 1H, $J_{1,2}$=1.3 Hz, H-1$_B$), 4.41 (d, 1H, $J_{1,2}$=8.3 Hz, H-1$_D$), 4.06 (m, 2H, H-2$_A$, 2$_B$), 4.00 (dd, 1H, $J_{2,3}$=3.3 Hz, $J_{3,4}$=9.4 Hz, H-3$_A$), 3.86 (dd, 1H, $J_{2,3}$=2.9 Hz, $J_{3,4}$=9.4 Hz, H-3$_B$), 3.79 (dq, 1H, $J_{4,5}$=9.4 Hz, $J_{5,6}$=6.2 Hz, H-5$^*_A$), 3.67 (m, 2H, H-5$^*_B$, 6a$_D$), 3.51 (m, 1H, H-2$_D$), 3.49–3.38 (m, 6H, H-6b$_D$, 4$_D$, 3$_D$, 4$_B$, 4$_A$), 3.31 (s, 3H, OCH$_3$), 3.29 (m, 1H, H-5$_D$), 1.55 (s, 3H, C(O)CH$_3$), 1.35 (d, 6H, H-6$_A$, 6$_B$); ^{13}C NMR δ 173.6 (C=O), 138.5–127.6 (Ph), 103.2 (C-1$_D$), 100.2 (C-1$_A$), 99.9 (C-1$_B$), 81.3, 80.7 (2C, C-4$_A$, 4$_B$), 79.9 (2C, C-3$_A$, 3$_B$), 79.0 (C-2$_A$), 77.2 (C-3$_D$), 75.8 (C-5$_D$), 75.7, 75.2, 74.6 (3C, OCH$_2$), 73.4 (C-2$_B$), 72.3 (OCH$_2$), 71.8 (C-4$_D$), 68.2, 67.7 (2C, C-5$_A$, 5$_B$), 62.5 (C-6$_D$), 58.9 (C-2$_D$), 54.6 (OCH$_3$), 22.3 (C(O)CH$_3$), 17.9, 17.7 (2C, C-6$_A$, 6$_B$). FABMS for $C_{49}H_{61}NO_{14}$ (M, 887.44) *m/z* 910.1 [M+Na]$^+$. Anal. calcd for $C_{49}H_{61}NO_{14}H_2O$: C, 64.96; H, 7.01; N, 1.55%. Found: C, 65.19; H, 6.83; N, 1.51%.

4.1.5. Methyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (12). 2,2-Dimethoxypropane (4.9 mL, 39.8 mmol) and *para*-toluenesulfonic acid (18 mg, 95 μmol) were added to a solution of the triol **11** (964 mg, 1.09 mmol) in acetone (3 mL) and the mixture was stirred at rt for 1 h. Et_3N was added, and volatiles were evaporated. Column chromatography of the residue (solvent A, 99:1) gave the acceptor **12** as a white solid (969 mg, 96%) which could be crystallized from AcOEt/iPr_2O; mp 164–165 °C $[\alpha]_D$ −25.9 (*c* 1.0); ^{1}H NMR δ 7.45–7.31 (m, 20H, Ph), 6.98 (d, 1H, $J_{NH,2}$=2.4 Hz, NH), 6.37 (brs, 1H, OH), 5.07 (d, 1H, $J_{1,2}$=1.9 Hz, H-1$_A$), 4.90 (d, 1H, *J*=10.8 Hz, OCH$_2$), 4.85 (d, 1H, *J*=10.1 Hz, OCH$_2$), 4.84 (d, 1H, *J*=10.8 Hz, OCH$_2$), 4.76 (d, 1H, OCH$_2$), 4.69 (d, 1H, OCH$_2$), 4.68 (s, 2H, OCH$_2$), 4.65 (d, 1H, OCH$_2$), 4.61 (d, 1H, $J_{1,2}$=1.6 Hz, H-1$_B$), 4.48 (d, 1H, $J_{1,2}$=8.3 Hz, H-1$_D$), 4.09 (dd, 1H, H-2$_A$), 4.01 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=9.4 Hz, H-3$_A$), 3.91 (dd, 1H, H-2$_B$), 3.89–3.84 (m, 2H, $J_{5,6}$=6.3 Hz, $J_{4,5}$=9.4 Hz, $J_{2',3'}$=3.3 Hz, $J_{3',4'}$=9.4 Hz, H-5$_A$, 3$_B$), 3.68 (dq, partially overlapped, $J_{5,6}$=6.2 Hz, $J_{4,5}$=9.5 Hz, H-5$_B$), 3.66–3.58 (m, 5H, H-6a$_D$, 6b$_D$, 2$_D$, 3$_D$, 4$_D$), 3.44 (pt, 1H, H-4$_A$), 3.41 (pt, 1H, H-4$_B$), 3.32 (s, 3H, OCH$_3$), 3.16 (m, 1H, H-5$_D$), 1.60 (s, 3H, C(O)CH$_3$), 1.54, 1.48 (2s, 6H, C(CH$_3$)$_2$), 1.35 (d, 6H, H-6$_A$, 6$_B$); ^{13}C NMR δ 173.9 (C=O), 138.8–128.0 (Ph), 103.7 (C-1$_D$), 101.3 (C-1$_A$), 100.3 (C(CH$_3$)$_2$), 100.2 (C-1$_B$), 81.9 (C-4$_A$), 80.8 (C-4$_B$), 80.5 (C-3$_A$), 79.7 (C-3$_B$), 79.4 (C-2$_A$), 76.2 (OCH$_2$), 76.0 (C-2$_B$), 75.6, 75.1 (2C, OCH$_2$), 74.7 (C-4$_D$), 74.4 (C-3$_D$), 72.6 (OCH$_2$), 68.6 (C-5$_A$), 68.0, 67.9 (2C, C-5$_B$, 5$_D$), 62.2 (C-6$_D$), 60.6 (C-2$_D$), 55.1 (OCH$_3$), 29.5 (C(CH$_3$)$_2$), 22.7 (C(O)CH$_3$), 19.4 (C(CH$_3$)$_2$), 18.5, 18.2 (2C, C-6$_A$, 6$_B$). FAB-MS for $C_{52}H_{65}NO_{14}$ (M, 927.44) *m/z* 950.1 [M+Na]$^+$. Anal. calcd for $C_{52}H_{65}NO_{14}$: C, 67.30; H, 7.06; N, 1.51%. Found: C, 67.12; H, 6.98; N, 1.44%.

4.1.6. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (15). Activated powdered 4 Å molecular sieves were added to a solution of the trisaccharide acceptor **12** (202 mg, 0.22 mmol) and the disaccharide donor **14**[19] (263 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (5 mL) and the suspension was stirred for 30 min at −15 °C. TfOH (7 μL, 34 μmol) was added and the mixture was stirred for 2 h while the bath temperature was slowly coming back to 10 °C. TLC (solvent D, 49:1) showed that no **12** remained. Et_3N was added and after 30 min, the suspension was filtered through a pad of Celite. Concentration of the filtrate and chromatography of the residue (solvent *B*, 9:1→17:5) gave the fully protected pentasaccharide **15** (330 mg, 84%) as a white foam; $[\alpha]_D$ +63.3 (*c* 1.0); ^{1}H NMR δ 8.07–6.96 (m, 50H, Ph), 5.82 (d, 1H, $J_{NH,2}$=7.4 Hz, NH), 5.63 (dd, 1H, $J_{2,3}$=3.5 Hz, $J_{3,4}$=9.5 Hz, H-3$_C$), 5.43 (dd, 1H, $J_{1,2}$=1.6 Hz,

H-2_C), 5.09 (brs, 1H, H-1_A), 5.02 (d, 1H, $J_{1,2}$=3.4 Hz, H-1_E), 4.99 (d, 1H, $J_{1,2}$=8.3 Hz, H-1_D), 4.95 (d, 1H, $J_{1,2}$=1.1 Hz, H-1_C), 4.94–4.63 (m, 13H, OCH_2), 4.63 (s, 1H, H-1_B), 4.37 (d, 1H, J=11.0 Hz, OCH_2), 4.29 (dq, 1H, $J_{4,5}$=9.5 Hz, $J_{5,6}$=6.2 Hz, H-5_C), 4.25 (d, 1H, J=9.5 Hz, OCH_2), 4.23 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.5 Hz, H-3_D), 4.01 (m, 1H, H-2_A), 3.97–3.86 (m, 5H, H-3_A, 2_B, 3_E, 4_C, OCH_2), 3.82 (m, 1H, H-3_B, 5_A), 3.71–3.57 (m, 6H, H-5_D, 4_E, 5_B, 4_D, $6a_D$, $6b_D$), 3.54–3.41 (m, 3H, H-2_E, 4_A, 2_D) 3.38–3.31 (m, 2H, H-4_B, $6a_E$), 3.31 (s, 3H, OCH_3), 3.17 (m, 1H, H-5_D), 3.08 (d, 1H, $J_{6a,6b}$=10.1 Hz, H-$6b_E$), 1.84 (s, 3H, C(O)CH_3), 1.46 (s, 3H, C(CH_3)$_2$), 1.45 (d, 3H, $J_{5,6}$=5.9 Hz, H-6_C), 1.35 (m, 6H, $J_{5,6}$=5.9 Hz, H-6_A, C(CH_3)$_2$), 1.31 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_B); ^{13}C NMR δ 171.7, 165.9, 165.8 (3C, C=O), 138.9–127.9 (Ph), 102.3 (C-1_D, J=167 Hz), 101.5 (C-1_A, J=170 Hz), 100.3 (C-1_B, J=170 Hz), 99.8 (C(CH_3)$_2$), 99.6 (C-1_E, J=172 Hz), 98.2 (C-1_C, J=172 Hz), 82.0 (C-3_E), 81.2, 80.9, 80.7 (3C, C-4_A, 4_B, 2_E), 80.0, 79.7, 79.3 (3C, C-3_B, 3_A, 4_C), 78.1, 77.8, 77.4 (3C, C-2_A, 4_E, 3_D), 75.9, 75.8, 75.6 (3C, OCH_2), 75.5 (C-2_B), 75.0, 74.4, 73.7 (3C, OCH_2), 73.2 (2C, C-4_D, OCH_2), 72.2 (OCH_2), 71.7, 71.6 (3C, C-2_C, 3_C, 5_E), 68.8 (C-5_B), 68.0 (C-6_E), 68.0 (2C, C-5_A, 5_B), 67.6 (C-5_D), 62.5 (C-6_D), 58.9 (C-2_D), 55.0 (OCH_3), 29.5 (C(CH_3)$_2$), 23.8 (C(O)CH_3), 19.8 (C(CH_3)$_2$), 18.6 (C-6_C), 18.5 (C-6_A), 18.3 (C-6_B). FAB-MS for $C_{106}H_{117}NO_{25}$ (M, 1803.79) *m/z* 1826.4 $[M+H]^+$. Anal. calcd for $C_{106}H_{117}NO_{25}\cdot H_2O$: C, 69.83; H, 6.58; N, 0.77%. Found: C, 69.86; H, 6.33; N, 0.71%.

4.1.7. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (16). Aq. TFA (750 μL) was added at 0 °C to a solution of the fully protected **15** (588 mg, 326 μmol) in CH_2Cl_2 (6.7 mL) and the mixture was stirred at this temperature for 1 h TLC (solvent *B*, 1.5:1) showed that no **15** remained. Volatiles were evaporated by repeated addition of toluene. Chromatography of the residue (solvent *B*, 4:1→1:1) gave **16** (544 mg, 95%) as a white foam; $[\alpha]_D$ +58.8 (*c* 1.0); ^{1}H NMR δ 8.06–7.06 (m, 50H, Ph), 5.82 (d, 1H, $J_{NH,2}$=7.1 Hz, NH), 5.65 (dd, 1H, $J_{2,3}$=3.8 Hz, $J_{3,4}$=9.0 Hz, H-3_C), 5.53 (m, 1H, H-2_C), 5.34 (brs, 1H, H-1_A), 5.04 (d, 1H, $J_{1,2}$=8.3 Hz, H-1_D), 5.00 (m, 2H, H-1_C, 1_E), 4.97–4.63 (m, 13H, OCH_2), 4.48 (brs, 1H, H-1_B), 4.40 (d, 1H, J=8.4 Hz, OCH_2), 4.29 (d, 1H, J=8.0 Hz, OCH_2), 4.28–4.21 (m, 2H, H-3_D, 5_C), 4.10 (m, 1H, H-2_B), 4.04 (m, 1H, H-2_A), 3.99 (d, 1H, OCH_2), 3.95–3.89 (m, 3H, H-3_A, 3_E, 4_C), 3.87 (dd, 1H, $J_{2,3}$=2.7 Hz, $J_{3,4}$=9.7 Hz, H-3_B), 3.81–3.64 (m, 5H, H-5_E, 5_A, $6a_D$, 4_E, 5_B), 3.54 (dd, 1H, $J_{1,2}$=3.2 Hz, $J_{2,3}$=9.7 Hz, H-2_E), 3.51 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.5 Hz, H-4_A), 3.45–3.37 (m, 4H, H-4_B, 4_D, $6a_E$, 2_D), 3.33 (m, 5H, H-5_D, $6b_D$, OCH_3), 3.12 (d, 1H, $J_{6a,6b}$=10.6 Hz, H-$6b_E$), 2.28 (brs, 1H, OH),1.97 (brs, 1H, OH), 1.84 (s, 3H, C(O)CH_3), 1.54 (d, 3H, $J_{5,6}$=6.1 Hz, H-6_C), 1.37 (m, 6H, H-6_B, 6_A); ^{13}C NMR δ 171.5, 165.8, 165.6 (3C, C=O), 138.8–127.9 (Ph), 101.6 (C-1_D), 100.8 (C-1_A), 100.5 (C-1_B), 100.1 (C-1_E^*), 99.9 (C-1_C^*), 84.9 (C-3_D), 82.1 (C-3_E), 80.9, 80.7, 80.6, 80.5 (4C, C-4_B, 3_B, 4_A, 2_E), 79.7 (C-4_C), 79.3 (C-3_A), 77.8 (2C, C-2_A, 4_E), 76.0, 75.9 (2C, OCH_2), 75.8 (C-5_D), 75.6, 75.1, 74.6, 73.7, 73.1 (5C, OCH_2), 72.8 (C-2_B), 72.6 (OCH_2), 71.8 (C-5_E), 71.6 (C-4_D), 71.3 (C-3_C), 71.1 (C-2_C), 69.4 (C-5_C), 68.8 (C-5_A), 68.3 (C-5_B), 68.1 (C-6_E), 63.0 (C-6_D), 57.6 (C-2_D), 55.0 (OCH_3), 23.8 (C(O)CH_3), 18.8 (C-6_C), 18.6, 18.5 (2C, C-6_A, 6_B). FAB-MS for $C_{103}H_{113}NO_{25}$ (M, 1763.76) *m/z* 1786.2 $[M+H]^+$. Anal. calcd for $C_{103}H_{113}NO_{25}\cdot 2H_2O$: C, 68.69; H, 6.55; N, 0.78%. Found: C, 68.74; H, 6.45; N, 0.65%.

4.1.8. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-α-L-rhamnopyranosyl-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (17). 1 M Methanolic sodium methoxide was added to a solution of **16** (277 mg, 157 μmol) in a 1:1 mixture of CH_2Cl_2 and MeOH (6 mL) until the pH was 10. The mixture was stirred overnight at rt and neutralized with Amberlite IR-120 (H^+). The crude material was chromatographed (solvent *A*, 49:1) to give **17** (211 mg, 86%) as a white foam; $[\alpha]_D$ +23.8 (*c* 1.0); ^{1}H NMR δ 7.33–7.16 (m, 40H, Ph), 5.34 (d, 1H, $J_{NH,2}$=7.6 Hz, NH), 5.18 (brs, 1H, H-1_A), 4.79 (d, partially overlapped, 1H, H-1_E), 4.67 (brs, 1H, H-1_C), 4.50 (d, partially overlapped, 1H, H-1_D), 4.49 (brs, 1H, H-1_B), 4.88–4.33 (m, 16H, OCH_2), 3.98–3.81 (m, 6H, H-2_A, 2_B, 5_E, 3_A, 3_E, 5_B^*), 3.77–3.70 (m, 3H, H-3_B, 2_C, 5_C^*), 3.65 (dq, 1H, $J_{4,5}$=9.4 Hz, $J_{5,6}$=6.2 Hz, H-5_A^*), 3.62–3.51 (m, 4H, H-2_D, $6a_D$, $6a_E$, $6b_E$), 3.48–3.27 (m, 7H, H-2_E, 4_E, 3_D, 4_A, 4_B, 3_C, 4_C), 3.23–3.12 (m, 6H, H-4_D, $6b_D$, 5_D, OCH_3), 2.76 (brs, 1H, OH), 1.72 (brs, 3H, OH), 1.65 (s, 3H, NHAc), 1.32, 1.25 (2d, 9H, H-6_C, 6_B, 6_A); ^{13}C NMR δ 170.6 (C=O), 138.5–128.0 (Ph), 103.0 (C-1_D), 101.8 (C-1_C), 100.7 (C-1_A), 100.4 (C-1_B), 99.6 (C-1_E), 87.3 (C-3_D), 85.0 (C-4_C^*), 82.0 (C-3_E), 81.2, 80.7, 80.5, 80.2, 79.7, 78.1, 77.9 (7C, C-2_B, 3_A, 3_B, 4_A, 4_B, 2_E, 4_E), 76.2 (C-5_D), 76.1, 75.9, 75.6, 75.4, 74.0, 73.9, 73.6 (7C, OCH_2), 73.0 (C-2_A), 72.8 (OCH_2), 71.7, 71.2, 71.1, 69.8 (4C, C-4_D, 5_E, 2_C, 3_C), 68.8, 68.2 (3C, C-5_A, 5_B, 5_C), 63.1 (C-6_D), 55.6 (C-2_D), 55.0 (OCH_3), 23.7 (C(O)CH_3), 18.6, 18.3, 18.1 (3C, C-6_A, 6_B, 6_C). FAB-MS for $C_{89}H_{105}NO_{23}$ (M, 1555.71) *m/z* 1578.2 $[M+H]^+$. Anal. calcd for $C_{89}H_{105}NO_{23}$: C, 68.66; H, 6.80; N, 0.90%. Found: C, 68.41; H, 6.78; N, 0.61%.

4.1.9. Methyl α-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (1). The benzylated tetrasaccharide **17** (352 mg, 226 μmol) was dissolved in a mixture of ethanol (14 mL) and AcOH (1 mL), treated with 10% Pd–C catalyst (200 mg), and the suspension was stirred for 5 days at rt. TLC (solvent A, 1:1) showed that the starting material had been transformed into a more polar product. The suspension was filtered on a pad of Celite. The filtrate was concentrated and co-evaporated repeatedly with cyclohexane. Reverse phase chromatography of the residue (solvent D, 100:0→49:1), followed by freeze-drying, gave the target tetrasaccharide **1** as an amorphous powder (153 mg, 81%). RP-HPLC gave a single product eluting at rt: 15.21 min (solvent F, 1:0→80:20 over 20 min); $[\alpha]_D$ −3.2 (*c* 1.0, methanol); ^{1}H NMR (D_2O) δ 5.08 (d, 1H, $J_{1,2}$=1.2 Hz, H-1_A), 4.97 (d, 1H, $J_{1,2}$=3.9 Hz, H-1_E), 4.79 (d, 1H, $J_{1,2}$=1.3 Hz, H-1_C), 4.69 (m, 2H, H-1_B, 1_D), 4.07 (dd, 1H, $J_{2,3}$=3.3 Hz, H-2_A), 4.02 (dq, 1H, $J_{4,5}$=9.3 Hz, $J_{5,6}$=6.2 Hz, H-5_C), 3.93 (m, 1H, H-5_E), 3.86 (m, 2H, H-2_B, 3_A), 3.82–3.73 (m, 7H, H-3_C, 2_D, $6a_E$, $6b_E$, 3_B, 2_C, $6a_D$), 3.70–3.59 (m, 4H, H-5_A, 3_E, $6b_D$, 5_B), 3.56 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz,

H-3_D), 3.49 (dd, 1H, $J_{2,3}$=9.6 Hz, H-2_E), 3.46–3.38 (m, 5H, H-4_C, 4_B, 4_D, 5_D, 4_E), 3.32 (s, 3H, OCH_3), 3.24 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.6 Hz, H-4_A), 2.00 (s, 3H, C(O)CH_3), 1.25 (d, 3H, partially overlapped, H-6_C), 1.23 (d, 3H, partially overlapped, H-6_B), 1.18 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A); ^{13}C NMR (D_2O) δ 175.0 (C=O), 102.3 (C-1_D, J=162 Hz), 101.5 (C-1_C, J=170 Hz), 101.3 (C-1_A, J=173 Hz), 100.0 (C-1_E, J=170 Hz), 99.9 (C-1_B, J=172 Hz), 81.9 (C-3_D), 81.4 (C-4_C), 79.2 (C-2_A), 79.0 (C-2_B), 76.2, 73.1, 72.6, 72.2, 72.0, 71.4, 70.4, 70.0, 69.8, 69.7, 69.6, 69.3, 68.9, 68.7 (14C, 3_A, 4_A, 5_A, 3_B, 4_B, 5_B, 2_C, 3_C, 4_D, 5_D, 2_E, 3_E, 4_E, 5_E), 68.4 (C-5_C), 60.5 (2C, C-6_D, 6_E), 56.0 (C-2_D), 55.3 (OCH_3), 22.6 (C(O)CH_3), 17.0 (3C, C-6_A, 6_B, 6_C). HRMS (MALDI) calcd for $C_{27}H_{47}NO_{19}$ +Na: 858.3214. Found: 858.3206.

4.1.10. 3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α/β-L-rhamnopyranose (28). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (Ir(I), 25 mg) was dissolved in dry THF (5 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of rhamnopyranoside[33] **27** (3.28 g, 7.12 mmol) in THF (30 mL) was degassed and added. The mixture was stirred overnight at rt, and a solution of iodine (3.6 g, 14.2 mmol) in a mixture of THF (70 mL) and water (20 mL) was added. The mixture was stirred at rt for 1 h, then concentrated. The residue was taken up in CH_2Cl_2 and washed twice with 5% aq. $NaHSO_4$. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 9:1) to give **28** (2.53 g, 85%). ^{1}H NMR δ 7.40–7.28 (m, 10H, Ph), 5.57 (brd, 0.2H, H-2β), 5.45 (dd, 0.8H, $J_{1,2}$=2.0 Hz, H-2α), 5.13 (brd, 0.8H, H-1α), 4.92 (d, 1H, J=10.9 Hz, OCH_2α, OCH_2β), 4.79 (d, 0.2H, J=11.2 Hz, OCH_2β), 4.74 (d, 1H, J=11.2 Hz, OCH_2α, H-1β), 4.65 (d, 0.8H, OCH_2α), 4.64 (d, 0.2H, OCH_2β), 4.58 (d, 0.8H, OCH_2α), 4.54 (d, 0.2H, OCH_2β), 4.30 (d, 0.2H, J=15.1 Hz, CH_2Clβ), 4.26 (d, 0.2H, CH_2Clβ), 4.20 (s, 1.6H, CH_2Clα), 4.08 (dd, 0.8H, $J_{2,3}$=3.3 Hz, $J_{3,4}$=9.6 Hz, H-3α), 4.04 (dq, 0.8H, $J_{4,5}$=9.5 Hz, H-5α), 3.66 (dd, 0.2H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=8.7 Hz, H-3β), 3.44 (pt, 2H, H-4α, 5β, OH-1α, 1β), 3.38 (pt, 0.2H, $J_{4,5}$=9.5 Hz, H-4β), 1.37 (d, 0.6H, $J_{5,6}$=5.7 Hz, H-6β), 1.34 (d, 2.4H, $J_{5,6}$=6.2 Hz, H-6α); ^{13}C NMR δ 167.8 (C=Oβ), 167.4 (C=Oα), 138.6–128.2 (Ph), 93.0 (C-1β), 92.4 (C-1α), 80.3 (C-4α), 80.2 (C-3β), 79.6 (C-4β), 77.8(C-3α), 75.9 (OCH_2β), 75.8 (OCH_2α), 72.5 (OCH_2α), 72.3 (0.4C, C-5β, OCH_2β), 71.9 (C-2-β), 71.7 (C-2α), 68.2(C-5α), 41.3 (CH_2Clα, CH_2Clβ), 18.3 (C-6α, 6β); FAB-MS for $C_{22}H_{25}ClO_6$ (M, 420.5) *m/z* 443.1 $[M+Na]^+$. Anal. calcd for $C_{22}H_{25}ClO_6$: C, 62.78; H, 5.94%. Found: C, 62.92; H, 6.11%.

4.1.11. 3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α/β-L-rhamnopyranosyl trichloroacetimidate (20). (a) The hemiacetal **28** (700 mg, 1.66 mmol) was dissolved in CH_2Cl_2 (6 mL) and the solution was cooled to 0 °C. Trichloroacetonitrile (1.7 mL) and DBU (26 μL) were added. The mixture was stirred at rt for 2 h. Toluene was added, and co-evaporated twice from the residue. The crude material was purified by flash chromatography (solvent *B* 4:1+0.1% Et_3N) to give **20** as a white foam (687 mg, 73%, α/β: 4:1).

(b) The hemiacetal **28** (858 mg, 2.04 mmol) was dissolved in CH_2Cl_2 (11 mL) and freshly activated K_2CO_3 (1.1 g, 8.0 mmol) was added. The suspension was cooled to 0 °C, and trichloroacetonitrile (1.0 mL) was added. The mixture was stirred vigorously at rt for 5 h. The suspension was filtered on a pad of Celite, and concentrated. The crude material was purified by flash chromatography (solvent *B*, 9:1+0.1% Et_3N) to give **20** as a white foam (840 mg, 72%, α/β: 9:1 from the ^{1}H NMR spectrum). ^{1}H NMR (α-anomer) δ 8.71 (s, 1H, NH), 7.40–7.30 (m, 10H, Ph), 6.24 (d, 1H, $J_{1,2}$=1.8 Hz, H-1), 5.57 (dd, 1H, H-2), 4.94 (d, 1H, J=10.8 Hz, OCH_2), 4.76 (d, 1H, J=11.2 Hz, OCH_2), 4.67 (d, 1H, OCH_2), 4.62 (d, 1H, OCH_2), 4.22 (s, 2H, CH_2Cl), 4.04 (dd, 1H, $J_{2,3}$=3.2 Hz, H-3), 3.99 (dq, 1H, $J_{4,5}$=9.6 Hz, H-5), 3.53 (pt, 1H, H-4), 1.37 (d, 3H, $J_{5,6}$=6.2 Hz, H-6); ^{13}C NMR (α-anomer) δ 166.9 (C=O), 160.4 (C=NH), 138.4–128.3 (Ph), 95.2 (C-1), 91.1 (CCl_3), 79.5 (C-4), 77.6 (C-3), 76.1, 72.9 (2C, OCH_2), 71.2 (C-5), 69.8 (C-2), 41.1 (CH_2Cl), 18.3 (C-6).

4.1.12. Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranoside (22). To a solution of the known **21**[28] (7.10 g, 8.55 mmol) in a mixture of CH_2Cl_2 (40 mL) and pyridine (5 mL) at 0 °C was added chloroacetic anhydride (3.65 g, 21.3 mmol), and the mixture was stirred at this temperature for 2 h. TLC (solvent C, 9:1) showed the complete disappearance of the starting material. MeOH (10 mL) was added, and after 30 min, volatiles were evaporated. Column chromatography (solvent *B*, 1:0→4:1) of the crude yellow oil afforded **22** as a colourless foam (7.34 g, 95%). $[\alpha]_D$ +47.5 (*c* 1.0); ^{1}H NMR δ 8.12–7.13 (m, 25H, Ph), 5.95 (m, 1H, CH=), 5.50–5.42 (m, 2H, $J_{2,3}$=3.6 Hz, H-2_C, 3_C), 5.37 (m, 1H, =CH_2), 5.28 (m, 1H, =CH_2), 4.96 (d, 1H, J=11.0 Hz, OCH_2), 4.93 (d, 1H, $J_{1,2}$=1.5 Hz, H-1_C), 4.90 (d, 1H, $J_{1,2}$=3.3 Hz, H-1_E), 4.87–4.81 (m, 3H, OCH_2), 4.67 (d, 1H, J=12.1 Hz, OCH_2), 4.64 (d, 1H, J=12.8 Hz, OCH_2), 4.47 (d, 1H, J=10.8 Hz, OCH_2), 4.43 (d, 1H, J=12.0 Hz, OCH_2), 4.25 (m, 2H, OCH_2), 4.09 (d, 1H, J=15.5 Hz, CH_2Cl), 3.99–3.93 (m, 3H, CH_2Cl, H-5_C, 3_C), 3.84 (m, 1H, H-5_E), 3.78–3.74 (m, 2H, H-$6a_E$, 4_E), 3.70 (pt, 1H, $J_{4,5}$=$J_{3,4}$=9.3 Hz, H-4_C), 3.58–3.54 (m, 2H, H-$6b_E$, 2_E), 1.50 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C); ^{13}C NMR δ 167.0, 166.0 (2C, C=O), 139.1–128.0 (Ph, All), 118.5 (All), 99.5 (C-1_E), 96.8 (C-1_C), 81.9 (C-3_E), 81.0 (C-2_E), 79.7 (C-4_C), 77.7 (C-4_E), 76.0, 75.4, 74.1, 73.8 (4C, OCH_2), 73.5 (C-3_C), 71.8 (C-5_E), 70.9 (C-2_C), 68.8 (OCH_2), 68.1 (C-6_E), 67.7 (C-5_C), 41.5 (CH_2Cl), 18.6 (C-6_C); FAB-MS for $C_{52}H_{55}O_{12}$ (M, 906.5) *m/z* 929.3 $[M+Na]^+$. Anal. calcd for $C_{52}H_{55}ClO_{12}$: C, 68.83; H, 6.11%. Found: C, 68.74; H, 6.19%.

4.1.13. (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-chloroacetyl-α/β-L-rhamnopyranose (23). A solution of **22** (7.21 g, 7.95 mmol) in THF (80 mL) containing activated iridium complex (60 mg) was treated as described for the preparation of **28**. The mixture was stirred at rt for 3 h, at which point a solution of iodine (4.0 g, 15.7 mmol) in a mixture of THF (90 mL) and water (24 mL) was added. The mixture was stirred at rt for 30 min, then concentrated. The residue was taken up in CH_2Cl_2 and washed twice with 5% aq. $NaHSO_4$, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent *B*, 4:1) to give

23 (6.7 g, 97%) as a slightly yellow foam. ^{1}H NMR δ 8.10–7.09 (m, 25H, Ph), 5.47 (dd, 1H, $J_{2,3}$=3.5 Hz, $J_{3,4}$=9.3 Hz, H-3$_C$), 5.41 (brs, 1H, H-2$_C$), 5.03 (brs, 1H, H-1$_C$), 4.94 (d, 1H, J=10.9 Hz, OCH$_2$), 4.87 (d, 1H, $J_{1,2}$=3.4 Hz, H-1$_E$), 4.85 (d, 1H, OCH$_2$), 4.80 (m, 2H, OCH$_2$), 4.64 (m, 2H, OCH$_2$), 4.45 (d, 1H, J=10.7 Hz, OCH$_2$), 4.41 (d, 1H, J=12.1 Hz, OCH$_2$), 4.16 (dq, 1H, $J_{4,5}$=9.3 Hz, H-5$_C$), 4.09 (d, 1H, J=15.6 Hz, CH$_2$Cl), 3.96 (d, 1H, CH$_2$Cl), 3.93 (pt, 1H, H-3$_E$), 3.83 (m, 1H, H-5$_E$), 3.77–3.68 (m, 2H, H-4$_E$, 6a$_E$), 3.65 (pt, 1H, H-4$_C$), 3.54 (m, 2H, H-6b$_E$, 2$_E$), 1.48 (d, 3H, $J_{5,6}$=6.2 Hz, H-6$_C$); ^{13}C NMR δ 167.0, 166.0 (2C, C=O), 139.1–127.9 (Ph), 99.5 (C-1$_E$), 92.3 (C-1$_C$), 81.9 (C-3$_E$), 81.0 (C-2$_E$), 79.9 (C-4$_C$), 77.6 (C-4$_E$), 76.0, 75.6, 74.2, 74.1 (4C, OCH$_2$), 72.1 (C-3$_C$), 71.7 (C-4$_E$), 71.1 (C-2$_C$), 68.0 (C-6$_E$), 67.5 (C-5$_C$), 41.5 (CH$_2$Cl), 18.9 (C-6$_C$); FAB-MS for $C_{49}H_{51}ClO_{12}$ (M, 866.3) *m/z* 889.3 [M+Na]$^+$. Anal. calcd for $C_{49}H_{51}ClO_{12}$: C, 67.85; H, 5.93%. Found: C, 67.72; H, 6.00%.

4.1.14. (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl trichloroacetimidate (19). Trichloroacetonitrile (1.1 mL, 10.9 mmol) and DBU (17 μL) were added to a solution of the hemiacetal **23** (950 mg, 1.09 mmol) in dry CH_2Cl_2 (8 mL), and the mixture was stirred at 0 °C for 1.5 h. Toluene was added, and volatiles were evaporated. The residue was purified by flash chromatography (solvent *B*, 3:2 containing 0.1% Et_3N) to give **19** (930 mg, 84%) as a colourless foam. Further elution gave some remaining starting material **23** (136 mg, 14%). $[\alpha]_D$ +39.3 (*c* 1.0); ^{1}H NMR δ 8.76 (s, 1H, NH), 8.12–7.17 (m, 25H, Ph), 6.34 (d, 1H, $J_{1,2}$=1.5 Hz, H-1$_C$), 5.67 (dd, 1H, H-2$_C$), 5.54 (dd, 1H, $J_{2,3}$=3.4 Hz, $J_{3,4}$=8.8 Hz, H-3$_C$), 4.98 (d, 1H, OCH$_2$), 4.88 (d, 1H, $J_{1,2}$=3.4 Hz, H-1$_E$), 4.84 (d, 1H, J=11.1 Hz, OCH$_2$), 4.82 (d, 1H, J=11.2 Hz, OCH$_2$), 4.65 (d, 1H, OCH$_2$), 4.62 (d, 1H, OCH$_2$), 4.44 (d, 1H, J=11.4 Hz, OCH$_2$), 4.41 (d, 1H, J=11.8 Hz, OCH$_2$), 4.14 (dq, 1H, $J_{4,5}$=9.5 Hz, H-5$_C$), 4.11 (d, 1H, J=15.5 Hz, CH$_2$Cl), 3.98 (d, 1H, CH$_2$Cl), 3.94 (pt, 1H, H-3$_E$), 3.83–3.71 (m, 4H, H-5$_E$, 6a$_E$, 4$_E$, 4$_C$), 3.56–3.51 (m, 2H, H-6b$_E$, 2$_E$), 1.51 (d, 3H, $J_{5,6}$=6.2 Hz, H-6$_C$); ^{13}C NMR δ 167.1, 165.7, 160.6 (3C, C=), 139.0–127.9 (Ph), 99.9 (C-1$_E$), 95.2 (C-1$_C$), 82.1 (C-3$_E$), 80.9 (C-2$_E$), 79.0 (C-4$_C$), 77.6 (C-4$_E$), 76.0, 75.6, 74.2, 73.8 (4C, OCH$_2$), 73.0 (C-3$_C$), 71.9 (C-5$_E$), 70.7 (C-5$_C$), 69.2 (C-2$_C$), 68.0 (C-6$_E$), 67.7 (C-5$_C$), 41.4 (CH$_2$Cl), 18.6 (C-6$_C$). Anal. calcd for $C_{51}H_{51}Cl_4NO_{12}$: C, 60.54; H, 5.08; N, 1.38%. Found: C, 60.49; H, 5.01; N, 1.34%.

4.1.15. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (24). The acceptor[19] **18** (500 mg, 1.82 mmol) was dissolved in CH_2Cl_2 (5.5 mL) and 4 Å molecular sieves (300 mg) were added. The mixture was cooled to −60 °C and stirred for 15 min. TMSOTf (35 μL, mmol) and a solution of the disaccharide donor **19** (2.39 g, 2.36 mmol) in CH_2Cl_2 (7.5 mL) were added. The mixture was stirred for 45 min while the cooling bath was coming back to rt, and for more 3 h at rt. The mixture was then heated at 65 °C for 1 h 30 min. Et_3N was added and the mixture was stirred at rt for 20 min, then diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent *B*, 85:15→1:1) to give **24** (1.64 g, 80%) as a white powder. $[\alpha]_D$ +55.1 (*c* 1.0); ^{1}H NMR δ 8.06–6.93 (m, 25H, Ph), 6.18 (d, 1H, $J_{NH,2}$=7.3 Hz, NH$_D$), 5.40 (dd, 1H, $J_{2,3}$=3.5 Hz, H-3$_C$), 5.38 (brs, 1H, H-2$_C$), 4.98 (d, 1H, $J_{1,2}$=8.3 Hz, H-1$_D$), 4.94 (brs, 1H, H-1$_C$), 4.94 (d, 1H, OCH$_2$), 4.93 (d, 1H, $J_{1,2}$=3.4 Hz, H-1$_E$), 4.83 (d, 2H, J=10.7 Hz, OCH$_2$), 4.81 (d, 1H, J=10.6 Hz, OCH$_2$), 4.67 (d, 1H, J=11.7 Hz, OCH$_2$), 4.62 (d, 1H, J=11.4 Hz, OCH$_2$), 4.47 (m, 3H, H-3$_D$, OCH$_2$), 4.22 (dq, 1H, $J_{4,5}$=9.4 Hz, $J_{5,6}$=6.2 Hz, H-5$_C$), 4.10 (d, 1H, J=15.5 Hz, CH$_2$Cl), 3.96 (m, 2H, H-6a$_D$, CH$_2$Cl), 3.91 (pt, 1H, H-3$_E$), 3.82 (m, 2H, H-5$_E$, 6b$_D$), 3.72 (m, 3H, H-6a$_E$, 4$_E$, 4$_C$), 3.62 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-4$_D$), 3.55 (m, 2H, H-6b$_E$, 2$_E$), 3.51 (s, 3H, OCH$_3$), 3.41 (m, 1H, H-5$_D$), 3.15 (m, 1H, H-2$_D$), 2.04 (s, 3H, C(O)CH$_3$), 1.51 (s, 3H, C(CH$_3$)$_2$), 1.42 (m, 6H, H-6$_C$, C(CH$_3$)$_2$), 1.51 (d, 3H, $J_{5,6}$=6.2 Hz, H-6$_C$); ^{13}C NMR δ 171.8, 167.3, 166.1 (3C, C=O), 139.0–128.0 (Ph), 101.1 (C-1$_D$, J_{CH}<164 Hz), 99.9 (C(CH$_3$)$_2$), 99.4 (C-1$_E$, J_{CH}>165 Hz), 98.2 (C-1$_C$, J_{CH}=172 Hz), 81.8 (C-3$_E$), 80.9 (C-2$_E$), 79.0 (C-4$^*_C$), 77.7 (C-4$^*_E$), 76.7 (C-3$_D$), 75.9, 75.3, 74.2, 73.9 (4C, OCH$_2$), 73.7 (C-4$_D$), 73.4 (C-3$_C$), 71.9 (C-5$_E$), 71.2 (C-2$_C$), 68.2 (C-6$_E$), 67.8 (C-5$_C$), 67.4 (C-5$_D$), 62.7 (C-6$_D$), 59.6 (C-2$_D$), 57.6 (OCH$_3$), 41.5 (CH$_2$Cl), 29.5 (C(CH$_3$)$_2$), 27.3 (C(O)CH$_3$), 19.7 (C(CH$_3$)$_2$), 18.6 (C-6$_C$); FAB-MS for $C_{61}H_{70}ClNO_{17}$ (M, 1123.4) *m/z* 1146.5 [M+Na]$^+$. Anal. calcd for $C_{61}H_{70}ClNO_{17}$: C, 65.15; H, 6.27; N, 1.25%. Found: C, 65.13; H, 6.23; N, 1.22%.

4.1.16. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (25). To a solution of the fully protected **24** (1.40 g, 1.25 mmol) in a mixture of methanol (18 mL) and pyridine (18 mL) was added thiourea (951 mg, 12.5 mmol). The mixture was stirred at 65 °C for 5 h, at which time TLC (solvent C, 4:1) showed that no starting material remained. Evaporation of the volatiles and co-evaporation of petroleum ether from the residue resulted in a crude solid, which was taken up in a minimum of methanol. A large excess of CH_2Cl_2 was added and the mixture was left to stand at 0 °C for 1 h. The precipitate was filtrated on a pad of Celite and the filtrate was concentrated. Column chromatography of the residue (solvent C, 4:1) gave the trisaccharide acceptor **25** (1.28 g, 97%) as a white powder. $[\alpha]_D$ +33.5 (*c* 1.0); ^{1}H NMR δ 8.10–6.96 (m, 25H, Ph), 6.09 (d, 1H, $J_{NH,2}$=7.9 Hz, NH$_D$), 5.26 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.4 Hz, H-2$_C$), 4.97 (m, 3H, H-1$_C$, 1$_E$, OCH$_2$), 4.86 (m, 3H, H-1$_D$, OCH$_2$), 4.81 (d, 1H, OCH$_2$), 4.72 (d, 1H, OCH$_2$), 4.58 (d, 1H, J=12.2 Hz, OCH$_2$), 4.51 (d, 1H, J=10.9 Hz, OCH$_2$), 4.48 (d, 1H, J=12.2 Hz, OCH$_2$), 4.23 (pt, 1H, $J_{2,3}$=$J_{3,4}$=9.4 Hz, H-3$_D$), 4.18–4.10 (m, 2H, H-5$_C$, 5$_E$), 4.06–3.95 (m, 3H, H-3$_C$, 3$_E$, 6a$_D$), 3.80 (pt, 1H, $J_{5,6b}$=$J_{6a,6b}$=10.4 Hz, H-6b$_D$), 3.66 (m, 2H, H-6a$_E$, 6b$_E$), 3.62 (dd, 1H, $J_{2,3}$=9.8 Hz, $J_{1,2}$=4.1 Hz, H-2$_E$), 3.59 (pt, 1H, $J_{3,4}$=$J_{4,5}$=8.9 Hz, H-4$_E$), 3.55 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.2 Hz, H-4$_D$), 3.51 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.3 Hz, H-4$_C$), 3.49 (s, 3H, OCH$_3$), 2.22 (s, 3H, C(O)CH$_3$), 1.90 (brs, 1H, OH), 1.49 (s, 3H, CMe$_2$), 1.43 (s, 3H, CMe$_2$), 1.40 (s, 3H, $J_{5,6}$=6.2 Hz, H-6$_C$); ^{13}C NMR δ 171.8, 166.6 (2C, C=O), 138.9–128.1 (Ph), 101.6 (C-1$_D$), 99.8 (C(CH$_3$)$_2$), 98.6 (C-1$^*_E$), 98.3 (C-1$^*_C$), 85.4 (C-4$_C$), 82.0 (C-3$_E$), 80.4 (C-2$_E$), 78.2 (C-4$_E$), 77.1 (C-3$_D$), 75.9, 75.5, 74.2, 73.9 (4C, OCH$_2$), 73.6 (C-4$^*_D$), 73.5 (C-2$^*_C$), 71.7 (C-5$_E$), 69.0 (C-6$_E$), 68.3 (C-3$_C$), 67.5

(C-5_D), 66.9 (C-5_C), 62.7 (C-6_D), 58.9 (C-2_D), 57.5 (OCH_3), 29.5 ($C(CH_3)_2$), 24.0 ($C(O)CH_3$), 19.7 ($C(CH_3)_2$), 18.2 (C-6_C); FAB-MS for $C_{59}H_{69}NO_{16}$ (M, 1047.5) *m/z* 1070.4 $[M+Na]^+$. Anal. calcd for $C_{70}H_{76}O_{16}$: C, 67.61; H, 6.64; N, 1.34%. Found: C, 67.46; H, 6.78; N, 1.24%.

4.1.17. Methyl (3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (29). (a) The trisaccharide acceptor **25** (615 mg, 0.58 mmol) was dissolved in Et_2O (10 mL) and the solution was cooled to −60 °C. TMSOTf (32 μL) and donor **20** (497 mg, 0.88 mmol) in Et_2O (12 mL) were added, and the mixture was stirred for 1 h while the bath was slowly coming back to −20 °C. The mixture was stirred for 4 h at this temperature, then at 0 °C overnight. More **20** (50 mg, 88 μmol) was added, and the mixture was stirred at rt for 3 h more at 0 °C. Et_3N was added, and the mixture was concentrated. Column chromatography of the residue (solvent B, 9:1→1:1) gave the orthoester **35** (44 mg, 5%) then the fully protected **29** (445 mg, 52%) contaminated with the trimethylsilyl side product **26** (**29/26**: 9:1) together with a mixture of **29** and **35** (65 mg, 8%), and the starting **25** (27 mg, 4%). An analytical sample of compound **29** had $[\alpha]_D$ +17.9 (*c* 1.0); ^1H NMR δ 8.07–7.12 (m, 35H, Ph), 5.96 (d, 1H, $J_{NH,2}$=7.9 Hz, NH), 5.82 (m, 1H, H-2_B), 5.33 (dd, 1H, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.2 Hz, H-2_C), 5.07 (d, 1H, $J_{1,2}$=3.2 Hz, H-1_E), 5.05 (d, 1H, $J_{1,2}$=1.7 Hz, H-1_B), 4.98 (d, 1H, OCH_2), 4.97 (brs, 1H, H-1_C), 4.91–4.78 (m, 5H, H-1_D, OCH_2), 4.64 (d, 1H, *J*=11.6 Hz, OCH_2), 4.60–4.45 (m, 5H, OCH_2), 4.36 (d, 1H, *J*=11.9 Hz, OCH_2), 4.26 (pt, 1H, $J_{2,3}$=$J_{3,4}$=9.5 Hz, H-3_D), 4.17 (dd, 1H, $J_{2,3}$=3.4 Hz, H-3_C), 4.16 (d, 1H, *J*=15.1 Hz, CH_2Cl), 4.11 (d, 1H, CH_2Cl), 4.10 (dq, 1H, $J_{4,5}$=9.1 Hz, $J_{5,6}$=6.3 Hz, H-5_C), 4.06 (m, 1H, H-5_E), 4.00 (pt, 1H, $J_{3,4}$=$J_{2,3}$=9.4 Hz, H-3_E), 3.97 (dd, 1H, $J_{5,6a}$=5.3 Hz, $J_{6a,6b}$=10.8 Hz, H-$6a_D$), 3.89 (m, 1H, H-$6a_E$), 3.88–3.68 (m, 4H, H-$6b_E$, $6b_D$, 4_C, 3_B), 3.67 (m, 1H, H-5_B), 3.58 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-4_D), 3.52 (dd, 1H, $J_{1,2}$=3.3 Hz, $J_{2,3}$=9.8 Hz, H-2_E), 3.49 (s, 3H, OCH_3), 3.39 (m, 1H, H-5_D), 3.30 (m, 2H, H-2_D, 4_B), 2.12 (s, 3H, $C(O)CH_3$), 1.52 (s, 3H, $C(CH_3)_2$), 1.42 (s, 3H, $C(CH_3)_2$), 1.33, 0.96 (2d, 6H, $J_{5,6}$=6.2 Hz, H-6_B, 6_C); ^{13}C NMR δ 171.9, 167.0, 166.3 (3C, C=O), 138.8–128.0 (Ph), 101.4 (C-1_D, J_{CH}=164 Hz), 99.9 ($C(CH_3)_2$), 99.3 (C-1_C, J_{CH}=168 Hz), 98.3 (C-1_E, J_{CH}=168 Hz), 97.9 (C-1_B, J_{CH}=171 Hz), 82.1 (C-3_E), 81.8 (C-2_E), 80.4 (brs, C-3_B), 80.0 (C-4_C), 78.8 (brs, C-4^*_E), 78.3 (C-4^*_B), 77.7 (C-3^*_C), 76.9 (C-3_D), 75.9, 75.5, 75.3, 74.3 (4C, OCH_2), 73.4 (C-4_D), 73.2 (OCH_2), 72.7 (C-2_B), 72.1 (C-5_E), 69.1 (C-5_C), 67.7 (C-5^*_D), 67.6 (C-5^*_B), 62.7 (C-6_D), 59.1 (C-2_D), 57.5 (OCH_3), 41.4 (CH_2Cl), 29.5 ($C(CH_3)_2$), 24.0 ($C(O)CH_3$), 19.7 ($C(CH_3)_2$), 18.8, 18.2 (2C, C-6_B, 6_C); FAB-MS for $C_{81}H_{92}NClO_{21}$ (M, 1449.5) *m/z* 1472.7 $[M+Na]^+$. Anal. calcd for $C_{81}H_{92}NClO_{21}$: C, 67.05; H, 6.39; N, 0.97%. Found: C, 66.21; H, 6.46; N, 1.01%.

Compound **35** had $[\alpha]_D$ +26.7 (*c* 0.8); ^1H NMR δ 8.07–7.15 (m, 35H, Ph), 5.47 (d, 1H, $J_{NH,2}$=7.4 Hz, NH_D), 5.45 (brs, 1H, H-2_C), 5.42 (d, 1H, $J_{1,2}$=2.3 Hz, H-1_B), 5.24 (d, 1H, $J_{1,2}$=3.4 Hz, H-1_E), 4.94 (d, 1H, $J_{1,2}$=8.2 Hz, H-1_D), 4.91–4.82 (m, 7H, H-1_C, OCH_2), 4.80 (d, 1H, *J*=11.0 Hz, OCH_2), 4.75 (d, 1H, *J*=11.6 Hz, OCH_2), 4.68 (dd, 1H, $J_{1,2}$=2.4 Hz, $J_{2,3}$=4.0 Hz, H-2_B), 4.65–4.47 (m, 4H, OCH_2), 4.44–4.32 (m, 4H, H-5_E, 3_D, 3_C, OCH_2), 4.15 (m, 1H, H-5_C), 4.05 (pt, 1H, $J_{2,3}$=$J_{3,4}$=9.5 Hz, H-3_E), 4.03 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-4_C), 3.94 (dd, 1H, $J_{5,6a}$=5.3 Hz, $J_{6a,6b}$=10.7 Hz, H-$6a_D$), 3.83–3.75 (m, 4H, H-$6a_E$, $6b_D$, CH_2Cl), 3.74–3.70 (m, 3H, H-4_E, 6_E, 3_B), 3.65 (dd, 1H, $J_{1,2}$=3.4 Hz, $J_{2,3}$=9.4 Hz, H-2_E), 3.48 (pt, 2H, H-4_B, 4_D), 3.46 (s, 3H, OCH_3), 3.38 (m, 1H, H-5_D), 3.22 (dq, 1H, $J_{4,5}$=9.5 Hz, $J_{5,6}$=6.2 Hz, H-5_B), 2.88 (m, 1H, H-2_D), 1.90 (s, 3H, $C(O)CH_3$), 1.42 (s, 3H, $C(CH_3)_2$), 1.36 (s, 6H, $C(CH_3)_2$, H-6_C), 1.30 (d, 3H, $J_{5,6}$=6.3 Hz, H-6_B); ^{13}C NMR δ 171.8, 166.4 (2C, C=O), 139.1–122.5 (Ph), 101.0 (C-1_D, J_{CH}=165 Hz), 99.7 ($C(CH_3)_2$), 98.3 (C-1_C, J_{CH}=172 Hz), 97.8 (brs, C-1_E, J_{CH}=170 Hz), 97.5 (C-1_B, J_{CH}=176 Hz), 82.2 (C-3_E), 80.7 (C-2_E), 79.3 (brs, C-4_B), 78.8 (C-3_B), 78.1 (brs, C-4_E), 77.3 (C-2_B), 76.2 (brs, C-3_C), 75.8, 75.6, 74.9, 74.6, 73.9 (6C, C-4_C, OCH_2), 73.5 (2C, C-4_D, 2_C), 71.4 (OCH_2), 71.0 (C-3_D), 70.7 (2C, C-5_E, 5_B), 69.0 (C-5_C), 68.8 (C-6_E), 67.2 (C-5_D), 62.5 (C-6_D), 60.0 (C-2_D), 57.6 (OCH_3), 46.9 (CH_2Cl), 29.5 ($C(CH_3)_2$), 23.9 ($C(O)CH_3$), 19.7 ($C(CH_3)_2$), 19.0 (C-6_B), 18.4 (C-6_C); FAB-MS for $C_{81}H_{92}NClO_{21}$ (M, 1449.5) *m/z* 1472.7 $[M+Na]^+$. Anal. calcd for $C_{81}H_{92}NClO_{21}\cdot H_2O$: C, 66.23; H, 6.34; N, 0.96%. Found: C, 66.11; H, 6.62; N, 0.85%.

4.1.18. Methyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (30). The trisaccharide acceptor **25** (500 mg, 0.47 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was cooled to −40 °C. TMSOTf (21 μL) and donor **5** (328 mg, 0.62 mmol) were added and the mixture was left under stirring while the bath was slowly coming back to rt. After 5 h, more **5** (50 mg, 94 μmol) was added and the mixture was stirred at rt for 1 h more. Et_3N was added and the mixture was concentrated. Column chromatography of the residue (solvent B, 4:1→1:1) gave the fully protected **30** (484 mg, 72%) slightly contaminated with the corresponding trimethylsilyl side product **26**. The **30/26** ratio was estimated to be 85:15 from the ^1H NMR spectrum. Eluting next was some residual starting **25** (45 mg, 9%). Thus, based on the consumed acceptor, the estimated yield of contaminated **30** was 79%. An analytical sample of **30** had $[\alpha]_D$ +15.9 (*c* 0.8); ^1H NMR δ 8.09–7.14 (m, 35H, Ph), 6.04 (brs, 1H, NH_D), 5.76 (m, 1H, H-2_B), 5.37 (dd, 1H, $J_{1,2}$=1.9 Hz, $J_{2,3}$=2.8 Hz, H-2_C), 5.11 (d, 1H, $J_{1,2}$=3.1 Hz, H-1_E), 5.06 (d, 1H, H-1_B), 4.96 (brs, 1H, H-1_C), 5.02–4.82 (m, 7H, H-1_D, OCH_2), 4.69–4.37 (m, 6H, OCH_2), 4.28 (pt, 1H, $J_{2,3}$=$J_{3,4}$=9.5 Hz, H-3_D), 4.15 (dd, 1H, $J_{2,3}$=3.3 Hz, $J_{3,4}$=9.4 Hz, H-3_C), 4.13–3.93 (m, 5H, H-5_E, $6a_E$, 3_E, 5_C, $6a_D$), 3.87–3.76 (m, 5H, H-4_E, $6b_E$, 3_B, 4_C, $6b_D$), 3.68 (dq, 1H, $J_{4,5}$=9.5 Hz, H-5_B), 3.57 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-4_D), 3.54 (dd, 1H, $J_{2,3}$=3.2 Hz, H-2_E), 3.48 (s, 3H, OCH_3), 3.40 (m, 1H, H-5_D), 3.34 (pt, 1H, $J_{3,4}$=9.7 Hz, H-4_B), 3.27 (m, 1H, H-2_D), 2.18, 2.13 (2s, 6H, $C(O)CH_3$), 1.51, 1.42 (2s, 6H, $C(CH_3)_2$), 1.33 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C), 0.98 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_B); ^{13}C NMR δ 171.9, 170.5, 166.3 (3C, C=O), 139.3–127.7 (Ph), 101.3 (C-1_D), 99.9 ($C(CH_3)_2$), 99.6 (C-1_B), 98.4 (C-1_E), 98.0 (C-1_C), 82.1 (C-3_E), 81.8 (C-2_E), 80.3 (2C, C-3_C, 4_B), 78.7 (brs, C-4_C), 78.2 (C-3^*_B), 77.7 (C-4^*_E), 76.9 (brs, C-3_D), 75.9, 75.4, 75.3, 74.3 (4C, OCH_2), 73.4 (C-4_D), 73.3 (OCH_2), 72.7 (C-2_C),

72.1 (C-5_E), 70.9 (OCH_2), 69.0 (3C, C-2_B, 5_B, 6_E), 67.8 (C-5_C), 67.6 (C-5_D), 62.7 (C-6_D), 59.2 (C-2_D), 57.5 (OCH_3), 29.5 ($C(CH_3)_2$), 24.0, 21.6 (2C, $C(O)CH_3$), 19.7 ($C(CH_3)_2$), 18.9 (C-6_C), 18.2 (C-6_B). FAB-MS for $C_{81}H_{93}NO_{21}$ (M, 1415) *m/z* 1438.6 $[M+Na]^+$.

4.1.19. Methyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (31). (a) Thiourea (22 mg, 0.29 mmol) was added to the chloroacetylated **29** (83 mg, 57 μmol) in MeOH/pyridine (1:1, 2.8 mL), and the mixture was heated overnight at 65 °C. Volatiles were evaporated, and the solid residue thus obtained was taken up in the minimum of MeOH. CH_2Cl_2 was added, and the suspension was left standing at 0 °C for 1 h. The precipitate was filtered on a pad of Celite, and the filtrate was concentrated. Column chromatography of the residue (solvent B, 9:1→1:1) gave the tetrasaccharide acceptor **31** (74 mg, 94%).

(b) The monoacetylated **30** (52 mg, 37 μmol) was dissolved in a mixture of EtOH (10 mL) and CH_2Cl_2 (100 μL). A freshly prepared 0.4 M ethanolic solution of guanidine (92 μL, 37 μmol) was added and the mixture was stirred at rt overnight. Volatiles were evaporated, and the residue taken up in CH_2Cl_2 was washed with water. The organic phase was dried and concentrated. Column chromatography of the crude product gave **31** (42 mg, 83%) as a glassy solid. Compound **31** had $[\alpha]_D$ +27.3 (*c* 1.0); ^{1}H NMR δ 8.24–6.88 (m, 35H, Ph), 5.90 (brs, 1H, NH_D), 5.29 (brs, 1H, H-2_C), 5.14 (d, 1H, $J_{1,2}$=3.0 Hz, H-1_E), 5.06 (d, 1H, $J_{1,2}$=1.6 Hz, H-1_B), 5.00–4.95 (m, 3H, H-1_D, 1_C, OCH_2), 4.88–4.46 (m, 9H, OCH_2), 4.31 (pt, 1H, $J_{2,3}$=$J_{3,4}$=9.4 Hz, H-3_D), 4.24 (brs, 1H, H-2_B), 4.14–3.08 (m, 3H, H-3_C, 5_C, 5_E), 4.02 (pt, 1H, $J_{2,3}$=$J_{3,4}$=9.3 Hz, H-3_E), 3.97 (dd, 1H, $J_{5,6a}$=5.2 Hz, $J_{6a,6b}$=10.7 Hz, H-$6a_D$), 3.80 (m, 2H, H-4_C, $6b_D$), 3.71 (m, 2H, H-$6a_E$, $6b_E$), 3.66 (pt, 1H, $J_{4,5}$=9.5 Hz, H-4_E), 3.61–3.55 (m, 4H, H-3_B, 2_E, 5_B, 4_D), 3.50 (s, 3H, OCH_3), 3.42–3.36 (m, 2H, H-5_D, 4_B), 3.20 (m, 1H, H-2_D), 2.85 (brs, 1H, OH), 2.10 (s, 3H, $C(O)CH_3$), 1.51, 1.41 (2s, 6H, $C(CH_3)_2$), 1.33 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C), 1.15 (s, 3H, $J_{5,6}$=6.2 Hz, H-6_B); ^{13}C NMR δ 171.7, 166.3 (2C, C=O), 139.0–127.8 (Ph), 103.1 (C-1_B), 101.2 (C-1_D), 99.8 ($C(CH_3)_2$), 98.2, 98.1 (2C, C-1_E, 1_C), 82.0 (C-3_E), 81.5 (C-3_B^*), 80.6 (C-4_B), 79.4 (C-2_E^*), 79.1 (2C, C-4_C, 3_C), 78.2 (C-4_B), 76.8 (C-3_D), 76.0, 75.5, 74.5, 74.2 (4C, OCH_2), 73.9 (C-2_C), 73.7 (OCH_2), 73.5 (C-4_D), 72.1 (OCH_2), 71.6 (C-5_E), 69.0 (C-6_E), 68.7 (2C, C-2_B, 5_B), 67.9 (C-5_C), 67.5 (C-5_D), 62.7 (C-6_D), 59.4 (C-2_D), 57.5 (OCH_3), 29.5 ($C(CH_3)_2$), 24.0 ($C(O)CH_3$), 19.7 ($C(CH_3)_2$), 19.0 (C-6_C), 18.3 (C-6_B); FAB-MS for $C_{79}H_{91}NO_{20}$ (M, 1373) *m/z* 1396.5 $[M+Na]^+$. Anal. calcd for $C_{79}H_{91}NO_{20}0.5H_2O$: C, 68.56; H, 6.65; N, 1.01%. Found: C, 68.53; H, 6.71; N, 1.01%.

4.1.20. Methyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (32). Activated 4 Å molecular sieves and TMSOTf (16 μL) were added to a solution of the tetrasaccharide acceptor **31** (406 mg, 0.29 mmol) in Et_2O (10 mL), and the mixture was stirred at −60 °C for 30 min. The donor **5** (234 mg, 0.44 mmol) in CH_2Cl_2 (7 mL) was added, and the mixture was stirred for 1 h while the bath temperature was reaching rt. After a further 1 h at this temperature, more **5** (50 mg, 94 μmol) was added, and the mixture was stirred for 1 h before Et_3N was added. Filtration through a pad of Celite and evaporation of the volatiles gave a residue which was column chromatographed twice (solvent B, 4:1; then solvent A, 17:3) to give **32** (262 mg, 52%) as a white powder. $[\alpha]_D$ +25.9 (*c* 1.0); ^{1}H NMR δ 8.07–7.13 (m, 45H, Ph), 6.03 (brs, 1H, NH_D), 5.59 (brs, 1H, H-2_A), 5.35 (brs, 1H, H-2_C), 5.16 (brs, 1H, H-1_E), 5.13 (brs, 1H, H-1_A), 5.06 (brs, 1H, H-1_B), 5.02–4.97 (m, 4H, H-1_D, 1_C, OCH_2), 4.91–4.50 (m, 12H, OCH_2), 4.44–4.32 (m, 4H, H-2_B, 3_D, OCH_2), 4.20–3.96 (m, 7H, H-5_E, 5_A, 3_C, 3_E, $6a_D$, 5_C, 3_A), 3.87–3.68 (m, 6H, H-4_E, $6a_E$, $6b_E$, $6b_D$, 4_C, 3_B), 3.64–3.47 (m, 7H, H-5_B, 4_D, 2_E, 4_A, OCH_3), 3.42 (m, 1H, H-5_D), 3.34 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.3 Hz, H-4_B), 3.17 (m, 1H, H-2_D), 2.13 (s, 3H, $C(O)CH_3$), 1.49 (s, 3H, $C(CH_3)_2$), 1.43 (s, 6H, $C(CH_3)_2$, H-6_C), 1.33 (d, 3H, $J_{5,6}$=6.1 Hz, H-6_A), 1.01 (d, 3H, $J_{5,6}$=5.8 Hz, H-6_B); ^{13}C NMR δ 171.9, 170.3, 166.3 (3C, C=O), 139.2–127.6 (Ph), 101.5 (brs, C-1_B, J_{CH}=171 Hz), 101.2 (C-1_D, J_{CH}=163 Hz), 99.8 ($C(CH_3)_2$), 99.7 (C-1_A, J_{CH}=171 Hz), 97.9 (2C, C-1_E, 1_C, J_{CH}=172, 169 Hz), 82.4 (C-3_E), 82.1 (C-2_E), 80.5 (C-4_A), 80.2 (brs, C-3_C), 80.1 (C-4_B), 79.4, 78.1, 78.0 (4C, C-3_B, 4_E, 3_A, 4_C), 76.6 (brs, C-3_D), 75.9, 75.8, 75.4 (3C, OCH_2), 74.8 (2C, C-2_B, OCH_2), 73.5 (C-4_D), 73.4 (OCH_2), 73.2 (C-2_C), 72.1 (OCH_2), 71.8 (C-5_A), 71.2 (OCH_2), 69.4 (C-2_A), 69.2 (C-5_B), 68.9 (C-6_E), 68.7 (C-5_C), 67.8 (C-5_E), 67.5 (C-5_D), 62.7 (C-6_D), 59.6 (brs, C-2_D), 57.6 (OCH_3), 29.5 ($C(CH_3)_2$), 24.0, 21.4 (2C, $C(O)CH_3$), 19.7 ($C(CH_3)_2$), 19.1 (C-6_A), 18.8 (C-6_C), 18.2 (C-6_B); FAB-MS for $C_{101}H_{115}NO_{25}$ (M, 1741.7) *m/z* 1765.9 $[M+Na]^+$. Anal. calcd for $C_{101}H_{115}NO_{25}$: C, 69.60; H, 6.65; N, 0.80%. Found: C, 69.56; H, 6.75; N, 0.73%.

4.1.21. Methyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (2). 50% aq. TFA (1 mL) was added at 0 °C to a solution of the fully protected pentasaccharide **32** (155 mg, 89 μmol) dissolved in CH_2Cl_2 (4 mL) After 1 h at this temperature, volatiles were evaporated. The residue, containing diol **33**, was taken up in 0.5% methanolic sodium methoxide (8 mL) and the mixture was heated overnight at 55 °C. Neutralisation with Dowex X8 (H^+), evaporation of the volatiles and column chromatography of the residue gave **34** (121 mg, 98%). Compound **34** (111 mg, 81 μmol) was dissolved in a mixture of ethanol (13 mL) and ethyl acetate (2.6 mL) containing 1 N aq. HCl (130 μL). Palladium on charcoal (130 mg) was added, and the suspension was stirred under a hydrogen atmosphere for 2 h. Filtration of the catalyst and reverse phase chromatography gave the target pentasaccharide (60 mg, 88%) as a slightly yellow foam. RP-HPLC purification followed by freeze-drying gave pure **2** (36 mg). Compound **2** had rt: 9.63 min (solvent F, 100:0→80:20 over 20 min); $[\alpha]_D$ −18.6 (*c* 1.0, methanol); ^{1}H NMR δ 5.13 (d, 1H, $J_{1,2}$=3.7 Hz, H-1_E), 4.98 (brs, 1H, H-1_B), 4.90 (d, 1H, $J_{1,2}$=1.4 Hz, H-1_A), 4.72 (d, 1H, $J_{1,2}$=1.4 Hz, H-1_C), 4.39 (d, 1H, $J_{1,2}$=8.6 Hz, H-1_D), 4.09 (dq, 1H, $J_{4,5}$=9.2 Hz, H-5_C), 4.00 (m, 2H, H-2_B, 2_A), 3.94–3.79 (m, 7H, H-5_E, 2_C,

3_C, $6a_E$, $6a_D$, 2_D, 3_A), 3.76–3.65 (m, 7H, H-4_C, 3_E, $6b_E$, $6b_D$, 5_A, 5_B, 3_B), 3.52 (pt, 1H, $J_{3,4}$=8.8 Hz, H-3_D), 3.49–3.33 (m, 9H, H-4_D, 2_E, 4_A, 4_B, 5_D, 4_E, OCH_3), 1.98 (s, 3H, $C(O)CH_3$), 1.27 (d, 3H, $J_{5,6}$=6.3 Hz, H-6_C), 1.24, 1.23 (d, 6H, H-6_A, 6_B); ^{13}C NMR δ 172.3 (C=O), 100.7 (C-1_A, J_{CH}=171 Hz), 99.6 (2C, C-1_D, 1_B, J_{CH}=163, 170 Hz), 99.2 (C-1_C, J_{CH}=170 Hz), 95.7 (brs, C-1_E, J_{CH}=170 Hz), 82.0 (C-3_D), 79.1 (C-2_B), 79.4 (brs, C-3_C), 76.4 (C-5_D^*), 75.4 (brs, C-4_C), 73.0 (C-3_E), 72.4 (2C, C-4_A, 4_B), 72.2 (C-5_E), 71.7 (C-2_E), 71.1 (C-2_C), 70.4, 70.1, 70.0 (4C, C-2_A, 3_A, 3_B, 4_E), 69.7, 69.6, 69.3 (3C, C-5_A, 5_B, 5_C), 68.8 (C-4_D), 61.2, 61.0 (2C, C-6_D, 6_E), 57.4 (OCH_3), 55.4 (C-2_D), 22.6 ($C(O)CH_3$), 18.2 (C-6_C), 17.2, 17.0 (2C, C-6_A, 6_B); HRMS (MALDI) calcd for $C_{33}H_{57}NO_{23}$ +Na: 858.3219. Found: 858.3089.

4.1.22. Methyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (36). 50% aq. TFA (400 μL) was added to a solution of the fully protected tetrasaccharide **30** (57 mg, 40 μmol) in CH_2Cl_2 (1 mL) at 0 °C, and the mixture was stirred overnight at this temperature. Volatiles were evaporated and the residue was purified by column chromatography (solvent B, 1:1) to give diol **36** (47 mg, 85%). $[\alpha]_D$ +19.5 (*c* 0.9); ^1H NMR δ 8.10–7.16 (m, 35H, Ph), 5.80 (d, 1H, *J*=8.8 Hz, NH_D), 5.66 (m, 1H, H-2_B), 5.39 (pt, 1H, $J_{1,2}$=2.8 Hz, H-2_C), 5.01 (m, 2H, H-1_B, 1_E), 4.96 (m, 2H, H-1_C, OCH_2), 4.90–4.81 (m, 5H, H-1_D, OCH_2), 4.66–4.41 (m, 7H, OCH_2), 4.18 (dd, 1H, $J_{2,3}$=2.9 Hz, $J_{3,4}$=7.4 Hz, H-3_C), 4.10 (pt, 1H, H-3_D), 4.08–3.95 (m, 5H, H-5_E, 3_E, 5_C), 3.89–3.64 (m, 8H, H-$6a_D$, $6b_D$, $6a_E$, $6b_E$, 3_B, 4_C, 4_E, 5_B), 3.54–3.49 (m, 5H, H-2_E, 4_D, OCH_3), 3.45 (m, 1H, H-5_D), 3.33 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-4_B), 3.27 (m, 1H, H-2_D), 2.26 (brs, 1H, OH), 2.17 (s, 6H, $C(O)CH_3$), 1.99 (brs, 1H, OH), 1.39 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C), 0.95 (d, 3H, $J_{5,6}$=6.1 Hz, H-6_B); ^{13}C NMR δ 171.5, 170.4, 166.1 (3C, C=O), 139.1–127.8 (Ph), 100.9 (C-1_D), 99.7 (2C, C-1_B^*, 1_C), 99.2 (brs, C-1_E), 85.0 (C-3_D), 82.1 (C-3_E), 81.3 (brs, C-3_E), 80.1 (C-4_B), 78.0, 77.8 (4C, C-3_C, 4_C, 3_B, 4_E), 76.0 (OCH_2), 75.6 (C-5_D), 75.3, 75.2, 74.4, 73.4 (4C, OCH_2), 72.3 (C-2_C), 72.1 (C-5_C^*), 71.3 (C-4_D), 71.2 (OCH_2), 69.2 (C-5_B), 69.0 (C-5_E, 2_B), 68.4 (C-6_E), 63.2 (C-6_D), 57.4 (2C, C-2_D, OCH_3), 23.9, 21.0 (2C, $C(O)CH_3$), 19.1 (C-6_C), 18.0 (C-6_B). FAB-MS for $C_{78}H_{89}NO_{21}$ (M, 1375.6) *m/z* 1398.6 $[M+Na]^+$. Anal. calcd for $C_{78}H_{89}NO_{21}$: C, 68.06; H, 6.52; N, 1.02%. Found: C, 68.10; H, 6.62; N, 0.98%.

4.1.23. Methyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (3). 1% Methanolic sodium methoxide (255 μL) was added to a suspension of diol **36** (68 mg, 49 μmol) in MeOH (2 mL) and the mixture was heated overnight at 55 °C TLC (solvent A, 19:1) showed that the starting material had been converted to a more polar product. Neutralisation with Dowex X8 (H^+), evaporation of the volatiles, and column chromatography (solvent A, 24:1) gave tetraol **37** (52 mg, 85%). The latter (48 mg, 39 μmol) was dissolved in a mixture of ethanol (5 mL) and ethyl acetate (2 mL) containing 1 N aq. HCl (50 μL). Palladium on charcoal (50 mg) was added and the suspension was stirred under a hydrogen atmosphere overnight. TLC (solvent E, 4:1:2) showed the presence of a single product. Filtration of the catalyst and reverse phase chromatography, followed by RP-HPLC purification and freeze-drying gave pure **3** (19 mg, 71%). Rt: 9.35 min (solvent F, 100:0→80:20 over 20 min); $[\alpha]_D$ +12.5 (*c* 0.8, methanol); ^1H NMR δ 5.09 (d, 1H, $J_{1,2}$=3.7 Hz, H-1_E), 4.89 (brs, 1H, H-1_B), 4.71 (d, 1H, $J_{1,2}$=1.1 Hz, H-1_C), 4.39 (d, 1H, $J_{1,2}$=8.6 Hz, H-1_D), 4.08 (dq, 1H, $J_{4,5}$=9.3 Hz, H-5_C), 3.96 (dd, 1H, $J_{1,2}$=1.4 Hz, $J_{2,3}$=3.2 Hz, H-2_B), 3.88–3.80 (m, 4H, H-2_C, 3_C, $6a_E$, $6b_E$, 5_D), 3.77–3.62 (m, 6H, H-$6a_D$, $6b_D$, 3_B, 5_B, 2_D, 4_C), 3.59 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-3_E), 3.50 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H-3_E), 3.50 (pt, 1H, $J_{3,4}$=$J_{4,5}$=8.7 Hz, H-3_D), 3.47–3.34 (m, 8H, H-2_E, 4_E, 4_B, 4_D, 5_E, OCH_3), 1.98 (s, 3H, $C(O)CH_3$), 1.27 (d, 3H, $J_{5,6}$=6.3 Hz, H-6_C), 1.21 (d, 3H, $J_{5,6}$=6.3 Hz, H-6_B); ^{13}C NMR δ 174.5 (C=O), 103.2 (brs, C-1_B, J_{CH}=172 Hz), 101.8 (C-1_D, J_{CH}=160 Hz), 101.5 (C-1_C, J_{CH}=170 Hz), 98.0 (C-1_E, J_{CH}=170 Hz), 82.2 (C-3_D), 79.1 (brs, C-3_C), 76.6 (brs, C-4_C), 76.4 (C-4_B^*), 72.9 (C-3_E), 72.3, 72.2 (2C, C-4_D, 5_D), 71.87 (C-2_E), 71.1 (brs, C-2_C), 70.6 (2C, C-2_B, 3_B), 69.7, 69.6 (2C, C-5_E, 5_B), 69.2, 68.9 (2C, C-6_D, 6_E), 57.4 (OCH_3), 55.4 (C-2_D), 22.6 ($C(O)CH_3$), 18.0 (C-6_C), 17.0 (C-6_B). HRMS (MALDI) calcd for $C_{27}H_{47}NO_{19}$ +Na: 712.2635. Found: 712.2635.

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