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Convergent synthesis of the methyl glycosides of a tetra- and a pentasaccharide fragment of the *Shigella flexneri* serotype 2a *O*-specific polysaccharide†

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Abstract—The branched pentasaccharide, α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→2)-α-L-Rha*p* (B(E)CDA) and the corresponding linear tetrasaccharide (ECDA), which are part of the *Shigella flexneri* serotype 2a *O*-antigen, were synthesized as their methyl glycosides according to a convergent strategy. The syntheses rely on the use of suitable B(E)C and EC trichloroacetimidate donors, respectively, and involve an appropriate DA acceptor which bears an isopropylidene acetal to block OH-4 and OH-6 of residue **D**. The preparation of the related linear CDA-OMe trisaccharide, which was used as a model, is also described. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Shigella flexneri serotype 2a, a Gram negative bacterium which is pathogenic in humans only, is the major causative agent of the endemic form of shigellosis or bacillary dysentery.[2] To date there is no licensed vaccine against this pathogen, which is resistant to the first line of available antibiotics in several countries.[2] As for other non-capsulated Gram negative bacteria, it is believed that the *O*-specific polysaccharide moiety (O-SP) of its surface lipopolysaccharide (LPS) serves as a protective antigen and that serum antibodies directed to it may provide protection against homologous infections.[3] That carbohydrates could be used as vaccines to elicit type-specific protection has been demonstrated previously.[4] At present, vaccination with purified capsular polysaccharides (CPs) protects adults and older children from infections such as those caused by *Streptococcus pneumoniae*, *Neisseria meningitidis* or *Salmonella typhi*.[5] Bacterial O-SPs are much shorter than CPs and often behave as haptens. They are not immunogenic in humans as such. However, the safety and immunogenicity in humans of detoxified LPS conjugated to protein carriers were demonstrated for several bacterial strains,[6] including *Shigella flexneri* 2a.[7] There is evidence that conjugates incorporating carbohydrate structures shorter than the native bacterial polysaccharide may be immunogenic as well.[8] Indeed, haptens mimicking the average conformation of 'protective' epitopes may be the minimal structures required for the corresponding conjugates to induce homologous protective antibodies.

As part of an ongoing project aimed at characterizing the carbohydrate epitopes recognized by a set of monoclonal antibodies protective against *S. flexneri* 2a infection,[9] the molecular interaction between *S. flexneri* 2a O-SP and such antibodies is under investigation in the laboratory. Our approach is based on the use of synthetic oligosaccharides representative of the native O-SP. The latter is a regular heteropolysaccharide whose biological repeating unit is the branched pentasaccharide **I**, composed of α-L-rhamnoses, 2-acetamido-2-deoxy-β-D-glucose, and a branched α-D-glucose.[10,11]

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

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→
(1↑4)
E α-D-Glc*p* **I**

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The preparation of the required frame-shifted di-, tri-, tetra- and pentasaccharides, all bearing the characteristic EC ramification, was undertaken.[12,13] Herein, we describe the convergent syntheses of the linear tetrasaccharide ECDA and the branched pentasaccharide B(E)CDA. The target compounds were synthesized as their methyl glycosides **1** and **2**, respectively, with the natural α-anomeric configuration at their reducing end terminus to allow both conformational analysis and binding studies in solution. The known linear trisaccharide **3**,[14] common to all *S. flexneri O*-antigens was synthesized as a model compound.

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

ECDA-OMe 1

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

B(E)CDA-OMe 2

α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→2)-α-L-Rha*p*-OMe

CDA-OMe 3

2. Results and discussion

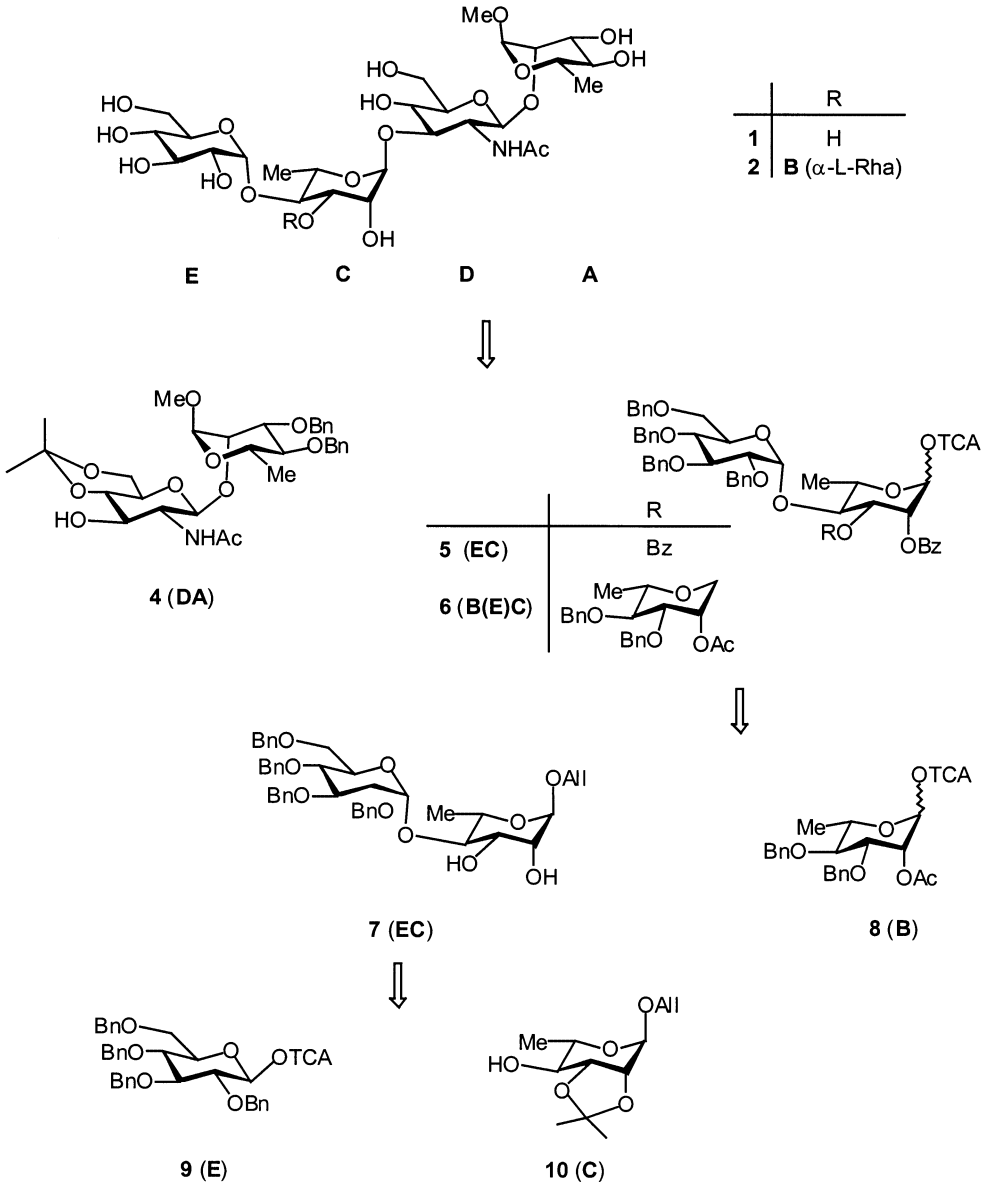
The syntheses of the targets **1** and **2** follow a common disconnection approach (Scheme 1) involving the known DA acceptor[15] **4** and two distinct donors having a participating group at position 2 of residue **C**, namely the trichloroacetimidate[12] **5** and the trisaccharide **6**, respectively. The efficacy of such a disconnection approach involving donors possessing at their reducing end a rhamnopyranose residue non-glycosylated at position 2, was demonstrated earlier in the synthesis of linear penta- and heptasaccharide fragments of the *S. flexneri* Y O-SP, which features the tetrasaccharide ABCD as its repeating unit.[16] Additionally, in order to prevent any extensive loss of material during the final deprotection steps, we reasoned that the use of a DA acceptor involving a **D** residue bearing an unmasked *N*-acetamido function was advantageous.[15] Such a strategy may also prevent unsatisfactory glycosylation yields as was reported earlier in closely related situations.[17] In the present case, the overall synthesis is based on the use of the trichloroacetimidate (TCA) chemistry.[18] Indeed, the key EC disaccharide[12] **7**, which was used as a common intermediate for the preparation of both trichloroacetimidates **5** and **6**, derived from the condensation of the perbenzylated glucopyranosyl donor[19,20] **9** with a suitable rhamnopyranosyl acceptor[21] **10** bearing orthogonal protecting groups. Moreover, in the case of the branched pentasaccharide **2**, the rhamnopyranosyl donor[22] **8**, eventually allowing further extension at position 2, was chosen as a readily available precursor to residue **B**.

2.1. Synthesis of the linear trisaccharide CDA-OMe, 3 (Scheme 2)

Considering the structure of the targets, we reasoned that the synthesis of the known trisaccharide **3** could help as a model in the design of the blockwise strategy to **1** and **2**. For this reason, the preparation of **12** was studied rather closely. Indeed, when performed in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf), the condensation of the disaccharide acceptor **4** and donor[23] **11** resulted in a mixture from which the target **12** (28%) and a trisaccharide contaminant (13%) were isolated in a 2:1 ratio. Based on mass spectrometry and NMR data, the latter was assumed to be the βC-anomer **13**. The use of boron trifluoride etherate complex ($BF_3{\cdot}OEt_2$), known to be a milder glycosylation promoter, was more satisfactory: The yield of the targeted αC-anomer **12** was 80% and that of the contaminant βC-anomer **13** was 8%. Deprotection of **12** involved aqueous trifluoroacetic acid (TFA) mediated hydrolysis of the isopropylidene group to give the diol **14** (95%), subsequent Zemplén deacetylation into the intermediate **15** (98%), and final hydrogenolysis to give the targeted CDA-OMe trisaccharide **3** (82%).

2.2. Synthesis of the linear tetrasaccharide ECDA-OMe, 1 (Scheme 3)

The fully protected disaccharide **18** is a key intermediate in the preparation of both **1** and **2**. In preceding reports, it was synthesized according to two methodologies involving either the trichloroacetate donor[24] **16** or the fluoride donor[12] **17** (Fig. 1). Both approaches yielded the required α-anomer **18** in yields close to 55%. Further investigation of this compound showed that under appropriate conditions based on the use of a catalytic amount of TMSOTf, condensation of the trichloroacetimidate donor **9** with the rhamnopyranoside acceptor **10** gave the target **18** in over 70% isolated yield. Nevertheless, the β-anomer **19** was also formed, indeed the estimated α/β ratio was approximately 4/1. This latter approach, involving the inverted procedure[25,26] in order to prevent extensive degradation



Scheme 1. Retrosynthetic approach to the targets **1** and **2**.

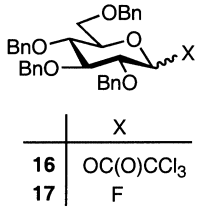
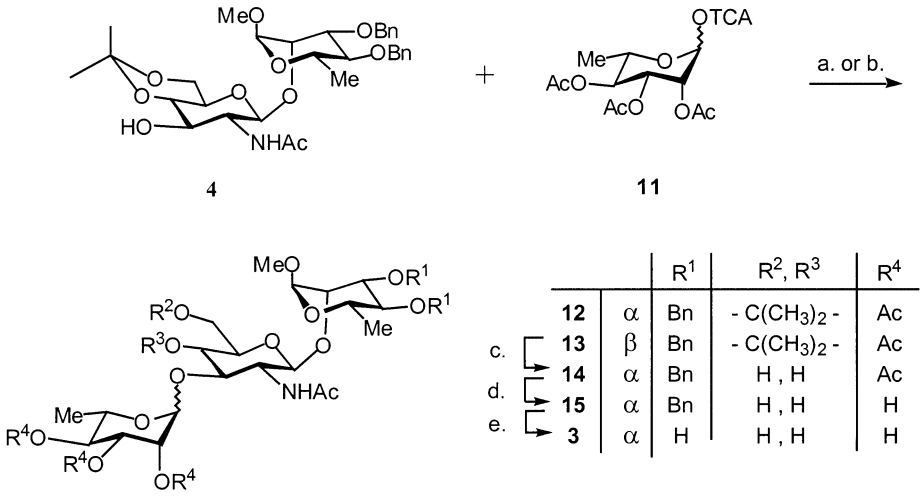
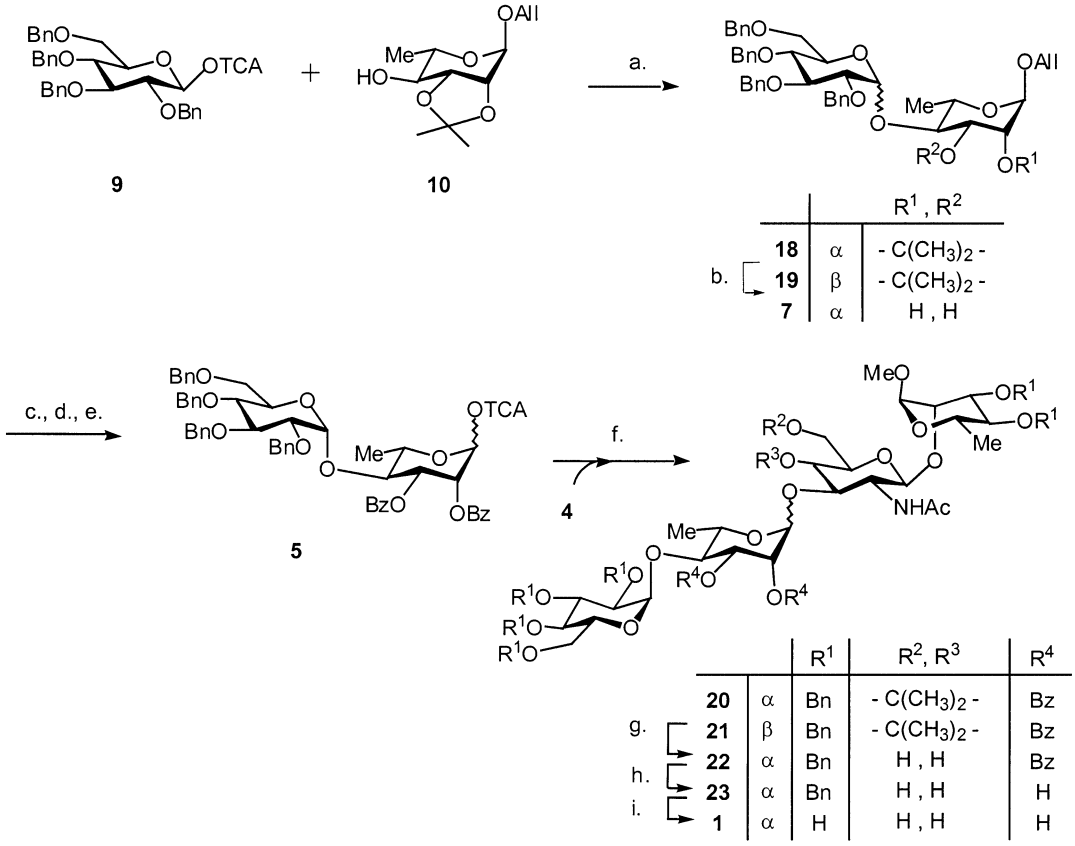


Figure 1.

of the reactive donor **9**, was thus definitely adopted. As described previously,[12] acidic hydrolysis of the isopropylidene acetal **18** gave the diol **7**, which was next benzoylated, deallylated and subsequently activated into the known trichloroacetimidate disaccharide **5**. When performed in the presence of a catalytic amount of TMSOTf, the condensation of precursors **4** and **5** was rather slow and did not go to completion. The fully protected tetrasaccharide **20** was, at best, isolated in 10 and 27% yield when CH_2Cl_2 and Et_2O were used as the solvent, respectively. In the latter case, a side-product to which structure **21** was tentatively assigned based on mass spectrometry analysis and experience gained in the CDA-OMe series, was also isolated (7%). As already observed in the preparation of the related trisaccharide **3**, the use of $BF_3{\cdot}OEt_2$ as the Lewis acid in the glycosylation process proved to be more satisfactory. Indeed, when run in Et_2O, the glycosidation of **4** and **5** proceeded with exclusive α-stereochemistry to give **20** in 88% yield. The stereochemistry of the linkages in **20** was ascertained based on the $^1J_{C,H}$ heteronuclear coupling constants,[27,28] which were 164, 171, 167, and 172 Hz for carbons 1_D, 1_A, 1_E, and 1_C, respectively. Complete deprotection of **20** was performed as described for the preparation of **3**, to afford sequentially the partially deblocked diol **22** (92%), the debenzoylated **23** (98%), and finally the targeted ECDA-OMe tetrasaccharide **1** (85%).



Scheme 2. *Reagents and conditions*: (a) $BF_3{\cdot}OEt_2$, Et_2O, −78→0°C (80%); (b) TMSOTf, Et_2O, −78→0°C (28%); (c) 50% aq. TFA, CH_2Cl_2, 0°C (95%); (d) MeONa cat., $MeOH/CH_2Cl_2$, rt (98%); (e) H_2, Pd/C, MeOH/AcOH, rt (82%).



Scheme 3. *Reagents and conditions*: (a) TMSOTf, Et_2O, −78→−55°C (73%); (b)–(e) see Ref. 12; (f) $BF_3{\cdot}OEt_2$, Et_2O (88%); (g) 50% aq. TFA, CH_2Cl_2, 0°C (92%); (h) MeONa cat., $MeOH/CH_2Cl_2$ (98%); (i) H_2, Pd/C, MeOH/AcOH (85%).

2.3. Synthesis of the branched pentasaccharide B(E)CDA-OMe, 2 (Scheme 4)

The encouraging results obtained in the condensation of the disaccharide donor **5** and acceptor **4** prompted the investigation of the use of the trisaccharide donor **6** in the synthesis of **2**. Thus, the crude diol **7**, resulting from the selective hydrolysis of the isopropylidene protecting group in disaccharide **18** (Scheme 3), was regioselectively benzoylated at position 2_C according to a two-step procedure involving (i) reaction with trimethyl orthobenzoate in the presence of a catalytic amount of camphorsulfonic acid (CSA), and (ii) regioselective opening of the resulting orthoester using 50% aq. TFA, to give **24** in 87% yield from the key intermediate **18**. Condensation of the appropriately

functionalized acceptor **24** with the trichloroacetimidate donor **8**, which was readily available by standard protecting group/activation strategies,[22] was performed in Et_2O, in the presence of a catalytic amount of TMSOTf, to afford the α-linked trisaccharide **25** (97%). The fully protected **25** was de-*O*-allylated into the hemiacetal **26** (75%) using $PdCl_2$ in acetic acid.[29] The selected trichloroacetimidate anomeric leaving group was introduced by treatment of **26** with trichloroacetonitrile in the presence of DBU, which resulted in the formation of **6** (83%). Several signals in the corresponding ^{13}C NMR spectrum were highly distorted, or even absent as in the case of C-3_C and C-4_C, indicating that the donor **6** was most probably sterically hindered. Nevertheless, $BF_3{\cdot}OEt_2$-mediated glycosylation of donor **6** with acceptor **4** resulted in the isolation of the fully protected pentasaccharide **27** in high yield (81%). Deacylation of the latter to give **28** (90%) was performed under Zemplén conditions. However, debenzoylation at position 2_C of **27** was slow and required heating in order to be completed satisfactorily. Similar behaviour was observed previously in related series,[1] and most probably derived from steric hindrance in **27**. Indeed, other authors have pointed out that the classical Zemplén procedure failed to remove hindered or isolated *O*-acetyl groups.[30] More conveniently, acidic hydrolysis of **27** allowing the selective removal of the isopropylidene group and subsequent transesterification gave **29** (82%) which was finally converted to the target B(E)CDA-OMe pentasaccharide **2** after conventional hydrogenolysis (76%).

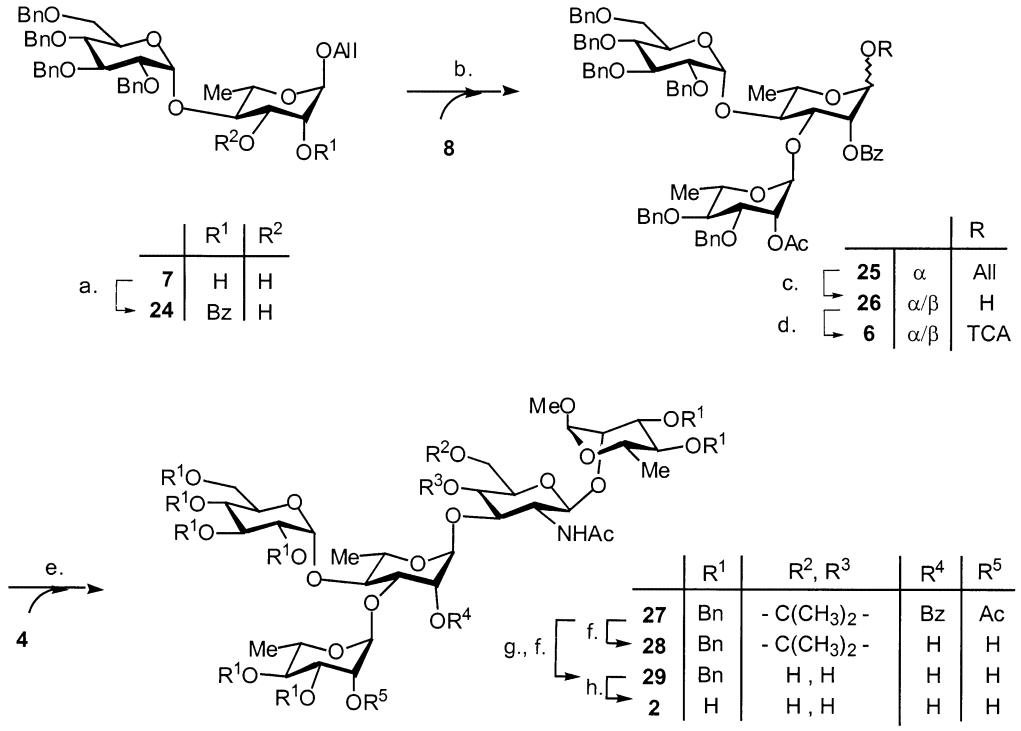
3. Conclusion

The strategy described here demonstrates the advantage of using $BF_3{\cdot}OEt_2$ as the Lewis acid instead of TMSOTf when using 'bulky' trichloroacetamidate donors. Moreover, it shows that, as demonstrated earlier in the *S. flexneri* Y series, the CD linkage is a suitable position to target in the construction of oligosaccharides of higher order in the *S. flexneri* 2a series.[31,32]

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 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, cyclohexane–acetone; B, cyclohexane–EtOAc; C, toluene–acetone; D, CH_2Cl_2–MeOH; E, *iso*-propanol–ammonia–water; F, water–acetonitrile. Detection was effected when applicable, with UV light, and/or by charring with orcinol (35 mM) in aqueous H_2SO_4 (4N). Preparative chromatography was performed by elution from columns of silica gel 60 (particle size 0.040–0.063 mm). Reverse-phase column chromatography was performed by elution from columns of Lichoprep® RP-18 (25–40 μm). The NMR spectra were recorded at 25°C



Scheme 4. *Reagents and conditions*: (a) i. $PhC(OMe)_3$, CSA, CH_2Cl_2, ii. 50% aq. TFA, CH_2Cl_2; (b) TMSOTf cat., Et_2O, −78→−30°C (97%); (c) $PdCl_2$, NaOAc, AcOH, rt (75%); (d) CCl_3CN, DBU cat., CH_2Cl_2 (83%); (e) $BF_3{\cdot}OEt_2$, Et_2O, −78→−30°C (81%); (f) MeONa cat., MeOH/CH_2Cl_2 (90%); (g) 50% aq. TFA (82% (g+f)); (h) H_2, Pd/C, MeOH/AcOH (76%).

for solutions in $CDCl_3$, unless stated otherwise, on a Bruker AC 300P spectrometer (300 MHz for ^1H, 75 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 of the glucopyranosyl moieties, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. Abbreviations are as follows: p, b, s, d, t, q stand for pseudo, broad, singlet, doublet, triplet, and quadruplet, respectively. 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. Fast atom bombardment mass spectra (FABMS) were recorded in the positive-ion mode using dithioerythritol/dithio-L-threitol (4/1, MB) as the matrix, in the presence of NaI, and Xenon as the gas. Anhydrous CH_2Cl_2, sold on molecular sieves, was used as such. Et_2O and THF were distilled over sodium/benzophenone. CH_3CN, suitable for DNA synthesis and kept on Trap-Pack molecular sieves bags, was used as such. Solutions in organic solvents were dried by passing through phase separator filters.

4.2. Methyl (2,3,4-tri-*O*-acetyl-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-rhamnopyranoside, 12

(a) A 0.1 M solution of TMSOTf in anhydrous Et_2O (250 μL, 271 μmol) was added at −78°C to a stirred solution of the rhamnosyl donor[23] **11** (158 mg, 364 μmol) and the disaccharide acceptor[15] **4** (105 mg, 175 μmol) in Et_2O (10 mL). The mixture was stirred at this temperature for 7 h while the bath temperature reached 4°C, and was stirred at this temperature overnight. Although TLC (solvent C, 4:1) showed some **4** still remained, Et_3N (20 μL) was added and after 15 min, the suspension was concentrated. Chromatography of the residue (solvent B, 7:3→1:1) gave first the α-anomer **13** (42 mg, 28%) as a white foam and then the β-anomer **12** (20 mg, 13%).

(b) Activated powdered 4 Å molecular sieves (1 g) were added to a solution of the disaccharide acceptor **4** (402 mg, 0.67 mmol) and the rhamnopyranosyl donor **11** (523 mg, 1.2 mmol) in anhydrous Et_2O (35 mL) and the suspension was stirred for 30 min at −78°C. $BF_3{\cdot}OEt_2$ (700 μL, 5.5 mmol) was added and the mixture was stirred for 5 h while the bath temperature was slowly coming back to 0°C. TLC (solvent B, 3:2) showed that no **4** remained. Et_3N (2.0 mL) 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, 7:3→1:1) gave the β-anomer **13** (50 mg, 8%) as the first isolated product and the desired α-anomer **12** (466 mg, 80%) as a white foam; $[\alpha]_D$ −15 (*c* 1.0); ^1H NMR: δ 7.39–7.30 (m, 10H, Ph), 5.68 (d, 1H, $J_{NH,2}=7.5$ Hz, NH), 5.28 (dd, 1H, $J_{2,3}=3.4$, $J_{3,4}=10.1$ Hz, H-3_C), 5.06 (dd, 1H, H-2_C), 5.03 (pt, 1H, H-4_C), 4.87 (d, 1H, $J=10.8$ Hz, OCH_2), 4.80 (d, 1H, $J_{1,2}=8.4$ Hz, H-1_D), 4.73 (d, 1H, $J_{1,2}=1.4$ Hz, H-1_C), 4.68 (d, 1H, $J=11.2$ Hz, OCH_2), 4.65 (d, 1H, $J_{1,2}=1.4$ Hz, H-1_A), 4.62 (d, 1H, OCH_2), 4.60 (d, 1H, OCH_2), 4.16 (dq, 1H, $J_{4,5}=9.9$ Hz, H-5_C), 3.97 (pt, 1H, $J_{2,3}=J_{3,4}=9.5$ Hz, H-3_D), 3.89–3.83 (dd, 1H, $J_{5,6a}=5.4$, $J_{6a,6b}=10.8$ Hz, H-$6a_D$), 3.86–3.73 (m, 3H, H-2_A, 3_A, $6b_D$), 3.64 (dq, 1H, H-5_A), 3.60 (pt, 1H, $J_{4,5}=9.3$ Hz, H-4_D), 3.50 (m, 1H, H-2_D), 3.68 (pt, 1H, $J_{3,4}=J_{4,5}=9.3$ Hz, H-4_A), 3.30 (s, 3H, OCH_3), 3.25 (m, 1H, H-5_D), 2.13, 2.04, 1.99, 1.78 (4s, 12H, $C(O)CH_3$), 1.50, 1.40 (2s, 6H, $C(CH_3)_2$), 1.32 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A), and 1.16 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C); ^{13}C NMR: δ 171.2–170.0 (4C, C(O)), 138.4–138.1 (Ph), 102.3 (C-1_D, $J_{C,H}=163$ Hz), 100.1 (C-1_A, $J_{C,H}=171$ Hz), 99.4 ($C(CH_3)_2$), 97.7 (C-1_C, $J_{C,H}=173$ Hz), 80.8 (C-4_A), 79.6 (C-3_A), 77.4 (2C, C-2_A, 3_D), 75.5, 73.0 (2C, OCH_2), 72.7 (C-4_D), 71.4 (C-4_C), 70.6 (C-2_C), 68.9 (C-3_C), 67.6 (C-5_A), 67.4 (C-5_D), 66.3 (C-5_C), 62.3 (C-6_D), 57.8 (C-2_D), 54.6 (OCH_3), 29.2 ($C(CH_3)_2$), 23.4, 21.0, 20.9, 20.8, (4C, $C(O)CH_3$), 19.3 ($C(CH_3)_2$), 17.9 (C-6_A), and 17.3 (C-6_C). ESMS for $C_{44}H_{59}NO_{17}$ (M, 873.38) *m*/*z* 874.5 $[M+H]^+$. Anal. calcd for $C_{44}H_{59}NO_{17}$: C, 60.47; H, 6.80; N, 1.60. Found: C, 60.39; H, 6.92; N, 1.64%.

4.2.1. Available analytical data for 13. ^1H NMR: δ 7.40–7.20 (m, 10H, Ph), 5.55 (d, 1H, $J_{NH,2}=7.5$ Hz, NH), 5.50 (bd, 1H, $J_{2,3}=2.6$ Hz, H-2_C), 5.01 (pt, 1H, $J_{3,4}=J_{4,5}=9.1$ Hz, H-4_C), 4.96 (d overlapped, 1H, H-1_D), 4.94 (dd overlapped, 1H, H-3_C), 4.87 (d, 1H, $J=10.8$ Hz, OCH_2), 4.75 (bs, 1H, H-1_C), 4.66 (d, 1H, $J_{1,2}=1.5$ Hz, H-1_A), 4.63 (m, 3H, OCH_2), 4.20 (pt, 1H, $J_{2,3}=J_{3,4}=9.5$ Hz, H-3_D), 3.90–3.77 (m, 4H, H-2_A, H-$6a_D$, 3_A, $6b_D$), 3.63 (dq, 1H, $J_{4,5}=9.4$ Hz, H-5_A), 3.55 (pt, 1H, $J_{4,5}=9.4$ Hz, H-4_D), 3.47 (dq, 1H, H-5_C), 3.44 (pt, 1H, $J_{3,4}=9.5$ Hz, H-4_A), 3.35 (m, 1H, H-2_D), 3.30 (s, 3H, OCH_3), 3.25 (m, 1H, H-5_D), 2.14, 2.06, 1.99, 1.74 (4s, 12H, $C(O)CH_3$), 1.49, 1.45 (2s, 6H, $C(CH_3)_2$), 1.31 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A), and 1.24 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C); ^{13}C NMR: δ 170.5, 170.2, 170.0 (4C, C(O)), 138.5–127.0 (Ph), 102.3 (C-1_D, $J_{C,H}=156$ Hz), 100.2 (C-1_A, $J_{C,H}=175$ Hz), 99.8 ($C(CH_3)_2$), 99.0 (C-1_C, $J_{C,H}=164$ Hz), 80.4 (C-4_A), 79.5 (C-3_A), 77.6 (C-3_D), 76.8 (C-2_A), 75.3 (OCH_2), 74.0 (C-4_D), 72.4 (OCH_2), 71.1 (C-3_C), 70.6 (C-4_C), 70.4 (C-5_C), 69.2 (C-2_C), 67.8 (C-5_A), 66.6 (C-5_D), 62.1 (C-6_D), 56.7 (C-2_D), 54.6 (OCH_3), 28.9 ($C(CH_3)_2$), 23.5, 20.8, 20.7 (4C, $C(O)CH_3$), 18.9 ($C(CH_3)_2$), 17.9 (C-6_A), and 17.7 (C-6_C). ESMS for $C_{44}H_{59}NO_{17}$ (M, 873.38) *m*/*z* 834 $[M-C(CH_3)_2+3H]^+$.

4.3. Methyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside, 14

50% aq. TFA (10 mL) was added at 0°C to a solution of the fully protected trisaccharide **12** (450 mg, 515

μmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at this temperature for 1.25 h. TLC (solvent D, 19:1) showed that no starting **12** remained. The organic phase was separated, washed successively with a satd aq. solution of $NaHCO_3$, water, and a satd aq. solution of NaCl, then concentrated. Chromatography of the residue (solvent D, 39:1) gave diol **14** (410 mg, 95%) as a white foam; $[\alpha]_D$ +4 (*c* 1.0); ^{1}H NMR: δ 7.37–7.30 (m, 10H, Ph), 5.87 (d, 1H, $J_{NH,2}=7.2$ Hz, NH), 5.26 (dd, 1H, $J_{2,3}=3.3$, $J_{3,4}=10.1$ Hz, H-3_C), 5.15 (dd, 1H, H-2_C), 5.09 (pt, 1H, $J_{4,5}=10.0$ Hz, H-4_C), 4.90 (d, 1H, $J_{1,2}=8.4$ Hz, H-1_D), 4.88 (d, 1H, $J=10.8$ Hz, OCH_2), 4.79 (d, 1H, $J_{1,2}=1.4$ Hz, H-1_C), 4.72 (d, 1H, $J_{1,2}=1.4$ Hz, H-1_A), 4.70 (d, 1H, $J=11.4$ Hz, OCH_2), 4.63 (d, 1H, OCH_2), 4.61 (d, 1H, OCH_2), 4.14 (dq, 1H, H-5_C), 3.95 (pt, 1H, $J_{2,3}=8.3$ Hz, H-3_D), 3.91 (m, 1H, H-2_A), 3.86 (m, 2H, H-6a_D, 6b_D), 3.81 (m, 1H, H-3_A), 3.66 (dq, 1H, $J_{4,5}=9.4$ Hz, H-5_A), 3.55 (pt, 1H, $J_{3,4}=J_{4,5}=8.9$ Hz, H-4_D), 3.36 (m, 3H, H-4_A, 2_D, 5_D), 3.31 (s, 3H, OCH_3), 2.15, 2.05, 1.99, 1.81 (4s, 12H, C(O)CH_3), 1.33 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A), and 1.16 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C); ^{13}C NMR: δ 171.1–170.1, 170.0 (4C, C(O)), 138.4–127.7 (Ph), 101.5 (C-1_D), 100.0 (C-1_A), 99.1 (C-1_C), 83.5 (C-3_D), 80.7 (C-4_A), 79.5 (C-3_A), 77.1 (C-2_A), 75.4 (OCH_2), 75.3 (C-5_D), 72.7 (OCH_2), 70.7 (C-4_C), 70.1 (C-4_D), 69.9 (C-2_C), 68.8 (C-3_C), 67.7 (C-5_A), 67.5 (C-5_C), 62.2 (C-6_D), 56.6 (C-2_D), 54.6 (OCH_3), 23.4, 21.0, 20.8, (4C, C(O)CH_3), 19.0 (C-6_A), and 17.5 (C-6_C). FABMS for $C_{41}H_{55}NO_{17}$ (M, 833.35) *m*/*z* 856.4 [M+Na]$^+$. Anal. calcd for $C_{41}H_{55}NO_{17}$: C, 59.05; H, 6.65; N, 1.68. Found: C, 58.91; H, 6.79; N, 1.52%.

4.4. Methyl α-L-rhamnopyranosyl-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside, 15

Methanolic MeONa (1 M) was added to a solution of diol **14** (299 mg, 359 μmol) in a 1:1 mixture of CH_2Cl_2 and MeOH (5 mL) until the pH reached 10. The mixture was stirred overnight at rt and neutralized with Amberlite IR-120 (H^+). The crude material was purified by column chromatography (solvent D, 10:1) to give the pentanol **15** (250 mg, 98%) as a white foam; $[\alpha]_D$ −25 (*c* 1.0); ^{1}H NMR: (DMSO-d_6); δ 7.70 (d, 1H, $J_{NH,2}=8.4$ Hz, NH), 7.43–7.27 (m, 10H, Ph), 4.87–4.50 (m, 12H, H-1_C, 1_D, 1_A, OH, OCH_2), 4.01 (bs, 1H, H-2_A), 3.86 (dq, 1H, $J_{4,5}=9.4$, $J_{5,6}=6.2$ Hz, H-5_C), 3.70 (dd, 1H, $J_{6a,6b}=11.3$, $J_{5,6a}=9.3$, H-6a_D), 3.61 (dd, 1H, $J_{2,3}=2.8$, $J_{3,4}=9.2$ Hz, H-3_A), 3.59–3.50 (m, 4H, H-3_D, 2_D, 2_C, 6b_D), 3.48–3.30 (m, 6H, H-5_A, 3_C, 4_A, OCH_3), 3.21–3.14 (m, 3H, H-5_D, 4_C, 4_D), 2.50 (s, 3H, C(O)CH_3), and 1.10 (m, 6H, H-6_A, 6_C); ^{13}C NMR: (DMSO-d_6); δ 169.0 (C(O)), 138.7–127.3 (Ph), 102.2 (C-1_D), 101.0 (C-1_C), 99.6 (C-1_A), 80.1 (C-3_D), 79.1 (C-4_A), 78.6 (C-3_A), 76.6 (C-5_D^*), 74.8 (C-2_A), 74.1 (OCH_2), 71.9 (C-4_C^*), 70.6 (C-2_C), 70.5 (C-3_C), 70.0 (OCH_2), 69.1 (C-4_D^*), 68.3 (C-5_A), 67.0 (C-5_C), 61.0 (C-6_D), 55.2 (C-2_D), 54.0 (OCH_3), 23.0 (C(O)CH_3), 17.9 (2C, C-6_A, 6_C). FABMS for $C_{35}H_{49}NO_{14}$ (M, 707.32) *m*/*z* 730.4 [M+Na]$^+$. Anal. calcd for $C_{35}H_{49}NO_{14}$: C, 59.39; H, 6.98; N, 1.98. Found: C, 59.22; H, 7.15; N, 1.85%.

4.5. Methyl α-L-rhamnopyranosyl-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-α-L-rhamnopyranoside, 3

10% Pd–C catalyst (100 mg) was added to a degassed solution of the pentaol **15** (146 mg, 206 μmol) in a mixture of methanol (9 mL) and acetic acid (1 mL). The suspension was saturated with hydrogen at atmospheric pressure and stirred overnight at rt. The mixture was filtered on a pad of Celite and the crude material was purified by reverse-phase chromatography (solvent F, 100:0→49:1) to give the trisaccharide **3** as a lyophilized powder (89 mg, 82%). Analytical data were as described.[14] $[\alpha]_D$ −51 (*c* 0.65, MeOH). ^{1}H NMR: (D_2O); δ 4.79 (d, 1H, $J_{1,2}=1.8$ Hz, H-1_C), 4.77 (d, 1H, $J_{1,2}=1.6$ Hz, H-1_A), 4.67 (d, 1H, $J_{1,2}=7.9$ Hz, H-1_D), 3.93 (dd, 1H, $J_{2,3}=3.2$ Hz, H-2_A), 3.91 (dq, 1H, $J_{4,5}=9.0$ Hz, H-5_C), 3.84 (dd, 1H, $J_{5,6a}=2.2$, $J_{6a,6b}=12.2$ Hz, H-6a_D), 3.75 (dd, 1H, $J_{2,3}=10.1$ Hz, H-2_D), 3.74–3.65 (m, 4H, H-2_C, 3_A, 6b_D, 3_C), 3.60 (dq, partially overlapped, 1H, $J_{4,5}=9.7$ Hz, H-5_A), 3.53 (pt, partially overlapped, 1H, H-3_D), 3.44 (pt, 1H, $J_{3,4}=J_{4,5}=8.6$ Hz, H-4_D), 3.39 (m, 1H, H-5_D), 3.35 (pt, 1H, $J_{3,4}=9.7$ Hz, H-4_C), 3.31 (s, 3H, OCH_3), 3.24 (pt, 1H, $J_{3,4}=9.7$ Hz, H-4_A), 2.00 (s, 3H, C(O)CH_3), 1.25 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A), and 1.16 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C); ^{13}C NMR: (D_2O); δ 174.9 (C(O)), 102.2 (C-1_D, $J_{C-H}=165$ Hz), 101.6 (C-1_C, $J=170$ Hz), 100.0 (C-1_A, $J_{C-H}=172$ Hz), 81.5 (C-3_D), 78.9 (C-2_A), 76.2 (C-5_D), 72.6 (C-4_A), 72.2 (C-4_C), 71.1 (C-2_C), 70.5 (C-3_C), 70.3 (C-3_A), 69.3 (C-5_C), 68.9 (C-5_A), 68.7 (C-4_D), 61.0 (C-6_D), 56.0 (C-2_D), 55.2 (OCH_3), 22.6 (C(O)CH_3), 17.0 (C-6_A), 16.8 (C-6_C). High-resolution ESMS for $C_{21}H_{37}NO_{14}$ (M+Na, 550.21117) *m*/*z* 550.21040 [M+Na]$^+$.

4.6. Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside, 18

TMSOTf (40 μL, 0.02 equiv.) was added to a solution of the rhamnose acceptor[21] **10** (2.74 g, 11.2 mmol) in anhydrous Et_2O (20 mL) and the mixture was stirred at −78°C for 15 min. A solution of the glucopyranosyl donor[19,20] **9** (9.49 g, 13.9 mmol) in a mixture of CH_2Cl_2 (10 mL) and Et_2O (80 mL) was added dropwise for 3.5 h while the bath was slowly coming back to −55°C. The mixture was stirred for 3 h more, at which time TLC (solvent A, 1:1) showed the total disappearance of **10**. Et_3N (150 μL) was added, and the mixture was stirred for 0.5 h, then volatiles were evaporated. Chromatography of the residue (solvent A, 19:1) gave first a mixture of the condensation products **18** and **19** in a ~3:2 ratio (3.28 g, 38%), then the pure α-disaccharide **18** (4.38 g, 50.9%). In an analogous experiment run on 4.78 g (18.6 mmol) of acceptor **10**, repeated chromatography (solvents A and C) of the crude reaction mixture yielded 11.03 g (73%) of the pure α-anomer **18**. Analytical data for **18**, isolated as a colourless oil, were identical to that reported previously.[12]

4.7. 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-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside, 20

(a) A 0.11 M ethereal solution of TMSOTf (250 μL, 27 μmol) was added, at −78°C, to a mixture of the disaccharide acceptor[15] **4** (105 mg, 175 μmol) and the disaccharide donor[12] **5** (307 mg, 295 μmol) in anhydrous Et_2O (11 mL). The mixture was stirred at 4°C for 7 h, at which time Et_3N (20 μL, 142 μmol) was added, and the mixture was concentrated. Column chromatography of the residue (solvent B, 4:1) gave the expected tetrasaccharide **20** (71 mg, 27%, corrected yield 41%), then the supposedly corresponding β-glycosidation product **21** (19 mg, 7%, corrected yield 11%), and finally the unreacted acceptor **4** (36 mg, conversion rate 65%). ESMS data for **21** *m*/*z* 1479 $[M+H]^+$corresponded to $C_{86}H_{95}NO_{21}$ (M, 1478.71).

(b) $BF_3{\cdot}OEt_2$ (1.3 mL, 10.3 mmol) was added portionwise, at −78°C, to a mixture of the disaccharides **4** (825 mg, 1.37 mmol) and **5** (2.21 g, 2.13 mmol) in anhydrous Et_2O (50 mL) containing activated powered 4 Å molecular sieves (2 g). The mixture was stirred for 3 h while the cooling bath was slowly coming back to −10°C. TLC (solvent B, 2.3:1) showed the complete disappearance of **4**. Et_3N (4 mL, 28.7 mmol) was added and after 30 min, the mixture was filtered through a pad of Celite, and the filtrate was concentrated. Chromatography of the residue (solvent B, 4:1) gave **20** (1.78 g, 88%) as a white foam; $[\alpha]_D$ +85 (*c* 1.0); ^{1}H NMR: δ 8.05–7.01 (m, 40H, Ph), 5.80 (d, 1H, $J_{NH,2}=7.4$ Hz, NH), 5.61 (dd, 1H, $J_{2,3}=3.5$, $J_{3,4}=9.5$ Hz, H-3_C), 5.39 (dd, 1H, $J_{1,2}=1.7$ Hz, H-2_C), 5.00 (d, 1H, $J_{1,2}=3.3$ Hz, H-1_E), 4.94 (d, 1H, $J_{1,2}=8.4$ Hz, H-1_D), 4.92 (bs, 1H, H-1_C), 4.69 (bs, 1H, H-1_A), 4.91–4.62 (m, 9H, OCH_2), 4.34 (d, 1H, $J=10.9$ Hz, OCH_2), 4.32–4.20 (m, 3H, H-5_C, 3_D, OCH_2), 3.95–3.79 (m, 7H, H-6a$_D$, 3_E, 4_C, 2_A, OCH_2, 3_A, 6b$_D$), 3.68–3.61 (m, 4H, H-5_E, 5_A, 4_E, 4_D), 3.53–3.39 (m, 3H, H-2_E, 2_D, 4_A), 3.39–3.25 (m, 5H, H-5_D, 6a$_E$, OCH_3), 3.05 (bd, 1H, $J_{6a,6b}=10.9$ Hz, H-6b$_E$), 1.78 (s, 3H, C(O)CH_3), 1.49 (s, 3H, $C(CH_3)_2$), 1.43 (d, 3H, $J_{5,6}=6.0$ Hz, H-6_C), 1.35 (s, 3H, $C(CH_3)_2$), and 1.34 (d, 3H, $J_{5,6}=6.3$ Hz, H-6_A); ^{13}C NMR: δ 171.3, 165.5, 165.4 (3C, C(O)), 138.7–127.4 (Ph), 102.0 (C-1_D, $J_{C-H}=164$ Hz), 100.1 (C-1_A, $J_{C-H}=171$ Hz), 99.5 ($C(CH_3)_2$), 99.1 (C-1_E, $J_{C-H}=167$ Hz), 97.5 (C-1_C, $J_{C-H}=172$ Hz), 81.6 (C-3_E), 80.7 (C-4_A), 80.4 (C-2_E), 79.6 (C-3^*_A), 79.5 (C-4^*_C), 77.3 (2C, C-4_E, 2_A), 77.0 (C-3_D), 75.5, 75.4, 74.6, 74.0, 73.3 (5C, OCH_2), 72.8 (C-4_D), 72.7 (OCH_2), 71.4 (C-2_C), 71.2 (C-5_E), 71.1 (C-3_C), 67.7 (C-5_A), 67.6 (C-6_E), 67.5 (C-5_C), 67.3 (C-5_D), 62.3 (C-6_D), 58.4 (C-2_D), 54.5 (OCH_3), 29.1 ($C(CH_3)_2$), 23.4 (C(O)CH_3), 19.3 ($C(CH_3)_2$), 18.2 (C-6_C), and 17.8 (C-6_A). ESMS for $C_{86}H_{95}NO_{21}$ (M, 1478.71) *m*/*z* 1479 $[M+H]^+$. Anal. calcd for $C_{86}H_{95}NO_{21}$: C, 69.85; H, 6.48; N, 0.95. Found: C, 69.75; H, 6.56; N, 0.97%.

4.8. 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-rhamnopyranoside, 22

A solution of the fully protected tetrasaccharide **20** (1.95 g, 1.32 mmol) in CH_2Cl_2 (25 mL) was treated, at 0°C, with 50% aq. TFA (25 mL) for 2 h. Solid Na_2CO_3 (10 g) was added portionwise to the reaction mixture to which CH_2Cl_2 (25 mL) had been added. The organic phase was separated and washed with a 2 M aq. solution of Na_2CO_3, then with a satd aq. solution of NaCl. Evaporation of the volatiles and chromatography (solvent B, 1:1) of the residue gave diol **22** (1.75 g, 92%) as a white foam; $[\alpha]_D$ +79 (*c* 1.0); ^{1}H NMR: δ 8.03–7.01 (m, 40H, Ph), 5.87 (d, 1H, $J_{NH,2}=7.1$ Hz, NH), 5.60 (dd, 1H, $J_{2,3}=3.4$, $J_{3,4}=9.1$ Hz, H-3_C), 5.48 (dd, 1H, $J_{1,2}=2.2$ Hz, H-2_C), 5.06 (d, 1H, $J_{1,2}=8.3$ Hz, H-1_D), 4.95 (m, 2H, H-1_C, 1_E), 4.74 (bs, 1H, H-1_A), 4.90–4.60 (m, 9H, OCH_2), 4.35 (d, 1H, $J=11.0$ Hz, OCH_2), 4.37–4.18 (m, 3H, H-5_C, 3_D, OCH_2), 3.96–3.83 (m, 7H, H-2_A, 3_E, 6a$_D$, 6b$_D$, OCH_2, 4_C, 3_A), 3.72–3.56 (m, 4H, H-5_E, 5_A, 4_E, 4_D), 3.50 (dd, 1H, $J_{1,2}=3.3$, $J_{2,3}=9.7$ Hz, H-2_E), 3.45 (pt, 1H, $J_{3,4}=J_{4,5}=9.7$ Hz, H-4_A), 3.43–3.35 (m, 2H, H-2_D, 5_D), 3.32 (m, 4H, H-6a$_E$, OCH_3), 3.06 (bd, 1H, $J_{6a,6b}=10.6$ Hz, H-6b$_E$), 1.81 (s, 3H, C(O)CH_3), 1.51 (d, 3H, $J_{5,6}=6.0$ Hz, H-6_C), and 1.35 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A); ^{13}C NMR: δ 171.1, 165.5, 165.3 (3C, C(O)), 138.8–127.4 (Ph), 101.2 (C-1_D), 100.2 (C-1_A), 99.5 (2C, C-1_C, 1_E), 84.2 (C-3_D), 81.7 (C-3_E), 80.6 (C-4_A), 80.2 (C-2_E), 79.5 (C-3_A), 79.3 (C-4_C), 77.3 (C-4_E), 77.0 (C-2_A), 75.6, 75.5 (2C, OCH_2), 75.2 (C-5_D), 74.7, 74.1, 73.3, 72.4 (4C, OCH_2), 71.3 (C-5_E), 70.8 (C-4_D), 70.7 (C-3_C), 70.6 (C-2_C), 69.0 (C-5_C), 67.7 (C-5_A), 67.6 (C-6_E), 62.7 (C-6_D), 57.1 (C-2_D), 54.7 (OCH_3), 23.4 (C(O)CH_3), 18.5 (C-6_C), and 17.1 (C-6_A). ESMS for $C_{83}H_{91}NO_{21}$ (M, 1437.61) *m*/*z* 1438.5 $[M+H]^+$. Anal. calcd for $C_{83}H_{91}NO_{21}$: C, 69.30; H, 6.38; N, 0.97. Found: C, 69.15; H, 6.42; N, 1.01%.

4.9. 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-rhamnopyranoside, 23

Methanolic MeONa (1 M) was added dropwise to a solution of diol **22** (700 mg, 487 μmol) in a mixture of CH_2Cl_2 (2 mL) and MeOH (2 mL) until the pH reached 10, and the mixture was stirred overnight at rt. TLC showed that **22** had turned into a more polar product. After neutralization with Amberlite IR-120 (H^+), filtration and evaporation of the solvent, the crude product was purified by column chromatography (solvent D, 32:1) to give the tetraol **23** (589 mg, 98%) as a white foam; $[\alpha]_D$ +38 (*c* 1.0); ^{1}H NMR: δ 7.40–7.14 (m, 30H, Ph), 5.61 (d, 1H, $J_{NH,2}=7.5$ Hz, NH), 4.97–4.39 (m, 12H, OCH_2), 4.88 (d, partially overlapped, 1H, $J_{1,2}=4.2$ Hz, H-1_E), 4.75 (bs, 1H, H-1_C), 4.72 (bs, 1H, H-1_A), 4.60 (d, partially overlapped, 1H, $J_{1,2}=8.0$ Hz, H-1_D), 4.07–3.78 (m, 9H, H-5_E, 3_E, 5_C, 2_A, 6a$_D$, 3_A, 2_C, 3_C, 6b$_D$), 3.72–3.44 (m,

8H, H-5_A, 2_D, $6a_E$, $6b_E$, 2_E, 4_E, 4_D, 3_D), 3.42–3.33 (m, 6H, H-4_A, 4_C, 5_D, OCH_3), 2.91 (bs, 1H, OH), 2.27 (bs, 1H, OH), 1.73 (s, 3H, C(O)CH_3), 1.41 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C), and 1.33 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A); ^{13}C NMR: δ 170.2 (C(O)), 138.5–127.6 (Ph), 102.5 (C-1_D), 101.3 (C-1_C), 100.0 (C-1_A), 99.1 (C-1_E), 86.1 (C-3_D), 84.3 (C-4^*_A), 81.6 (C-3_E), 81.0 (C-4^*_C), 79.9 (C-3_A), 79.7 (C-2^*_E), 77.7 (C-4^*_E), 77.4 (C-2_A), 75.7 (OCH_2), 75.5 (C-5_D), 75.4, 74.9, 73.6, 73.5, 73.2 (OCH_2), 71.2 (C-5_E), 70.8 (C-2_C), 70.3 (C-4_D), 69.4 (C-3_C), 68.6 (C-6_E), 67.9 (C-5_C), 67.7 (C-5_A), 62.6 (C-6_D), 55.3 (C-2_D), 54.6 (OCH_3), 23.4 (C(O)CH_3), 17.8 (C-6_A), and 17.7 (C-6_C). FABMS for $C_{69}H_{83}NO_{19}$ (M, 1229.56) m/z 1252.6 $[M+Na]^+$. Anal. calcd for $C_{69}H_{83}NO_{19}$: C, 67.36; H, 6.80; N, 1.14. Found: C, 67.25; H, 6.95; N, 0.95%.

4.10. Methyl α-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside, 1

The benzylated tetrasaccharide **23** (484 mg, 394 μmol) was dissolved in a mixture of methanol (10 mL) and AcOH (1 mL), treated with 10% Pd–C catalyst (200 mg), and the suspension was stirred overnight at rt under an atmospheric pressure of hydrogen. TLC monitoring (solvent D, 3:2) 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 coevaporated repeatedly with cyclohexane. Reverse-phase chromatography of the residue (solvent F, 100:0→49:1), followed by freeze-drying, gave the targeted tetrasaccharide **1** as an amorphous powder (230 mg, 85%); $[\alpha]_D$ +3 (*c* 1.0, water); ^{1}H NMR: (D_2O); δ 5.04 (d, 1H, $J_{1,2}=3.8$ Hz, H-1_E), 4.87 (bs, 1H, H-1_C), 4.84 (bs, 1H, H-1_A), 4.76 (d, overlapped, 1H, H-1_D), 4.10 (dq, 1H, $J_{4,5}=9.5$ Hz, H-5_C), 4.01 (m, 1H, H-2_A), 4.00 (m, 1H, H-5_E), 3.92 (dd, 1H, $J_{6a,6b}=12.0$, $J_{5,6a}=1.8$ Hz, H-$6a_D$), 3.87–3.73 (m, 7H, H-3_C, 3_A, $6a_E$, $6b_E$, 2_D, 2_C, $6b_D$), 3.73–3.61 (m, 3H, H-3_E, 3_D, 5_A), 3.59–3.43 (m, 5H, H-2_E, 4_D, 4_C, 5_D, 4_E), 3.39 (s, 3H, OCH_3), 3.32 (pt, 1H, $J_{3,4}=J_{4,5}=9.6$ Hz, H-4_A), 2.07 (s, 3H, C(O)CH_3), 1.32 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C), and 1.28 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A); ^{13}C NMR: (D_2O); δ 175.3 (C(O)), 102.7 (C-1_D, $J_{C-H}=163$ Hz), 102.0 (C-1_C, $J_{C-H}=170$ Hz), 100.5 (2C, C-1_A, 1_E, $J_{C-H}=170$ Hz), 82.3 (C-3_D), 81.8 (C-4_C), 79.3 (C-2_A), 76.7 (C-4_E), 73.6 (C-3_E), 73.1 (C-4_A), 72.6 (C-5_E), 72.4 (C-2_E), 71.8 (C-2_C), 70.7 (C-3_A), 70.1 (C-5_D), 69.7 (C-3_C), 69.3 (C-5_A), 69.2 (C-4_D), 68.9 (C-5_C), 61.4 (C-6_D), 60.9 (C-6_E), 56.4 (C-2_D), 55.6 (OCH_3), 23.0 (C(O)CH_3), 17.5 (C-6_A), and 17.3 (C-6_C). High-resolution ESMS for $C_{27}H_{47}NO_{19}$ (M−H, 688.26640) m/z 688.26880 $[M-H]^+$.

4.11. Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-α-L-rhamnopyranoside, 24

A solution of the fully protected disaccharide **18** (6.27 g, 8.18 mmol) in 80% aq. AcOH (88 mL) was heated for 4.5 h at 70°C. TLC monitoring (solvent B, 2.5:1) showed the presence of one more polar product. The mixture was concentrated and coevaporated repeatedly with cyclohexane and toluene. The resulting crude diol **7** (6.11 g) was solubilized in anhydrous CH_2Cl_2 (20 mL) and treated with trimethyl orthobenzoate (10 mL, 58.2 mmol) and CSA (60 mg, 2.58 mmol) for 2 h at rt, then cooled to 0°C. 50% aq. TFA (11 mL) was added and the biphasic mixture was stirred for 50 min at this temperature. At this time TLC (solvent B, 2.5:1) showed that no intermediate orthoester remained. The mixture was treated with Et_3N, volatiles were evaporated, and the residue was purified by column chromatography (solvent B, 9:1 containing 1‰ Et_3N) to give **24** as a white foam (5.91 g, 87%); $[\alpha]_D$ +32 (*c* 1.0); ^{1}H NMR: δ 8.16–7.19 (m, 25H, Ph), 5.99 (m, 1H, CH=CH_2), 5.49 (m, 1H, H-2_C), 5.49–5.28 (m, 2H, CH=CH_2), 5.05 (d, 1H, $J_{1,2}=3.6$ Hz, H-1_E), 5.04 (d, 1H, $J=10.6$ Hz, OCH_2), 5.00 (d, 1H, $J_{1,2}=1.2$ Hz, H-1_C), 4.93–4.75 (m, 3H, OCH_2), 4.64–4.49 (m, 4H, OCH_2), 4.30–4.16 (m, 4H, OCH_2, H-3_C, $6a_E$, $6b_E$), 4.11–4.04 (m, 2H, H-3_E, OCH_2), 3.96 (dq, 1H, $J_{4,5}=9.3$ Hz, H-5_C), 3.74–3.62 (m, 3H, H-2_E, 4_E, 5_E), 3.58 (pt, 1H, $J_{3,4}=9.3$ Hz, H-4_C), and 1.54 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C); ^{13}C NMR: δ 166.0 (C(O)), 138.7–127.7 (Ph, All), 117.7 (All), 98.7 (C-1_E), 96.6 (C-1_C), 85.4 (C-4_C), 81.7 (C-3_E), 80.1 (C-4_E), 77.8 (C-5_E), 75.7, 75.2, 73.8, 73.5 (4C, OCH_2), 72.7 (C-2_C), 71.3 (C-3_C), 68.5 (2C, C-2_E, 6_E), 68.3 (OCH_2), 66.7 (C-5_C), and 18.1 (C-6_C). ESMS for $C_{50}H_{54}O_{11}$ (M, 830.37) m/z 831.4 $[M+H]^+$. Anal. calcd for $C_{50}H_{54}O_{11}\cdot H_2O$: C, 70.74; H, 6.65. Found: C, 70.80; H, 6.56%.

4.12. Allyl (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-rhamnopyranoside, 25

TMSOTf (180 μL, 99 μmol) was added to a solution of donor[22] **8** (3.82 g, 7.2 mmol) and acceptor **24** (4.32 g, 5.2 mmol) in anhydrous Et_2O (260 mL) at −78°C. The mixture was stirred for 3 h, at which time the bath temperature was −30°C. As no starting acceptor remained, Et_3N (800 μL) was added and the mixture was stirred for 15 min. Evaporation of the volatiles followed by column chromatography of the residue (solvent B, 1‰ Et_3N) gave pure **25** as a white foam (6.08 g, 97%); $[\alpha]_D$ +24 (*c* 1.0); ^{1}H NMR: δ 8.07–7.12 (m, 35H, Ph), 5.93 (m, 1H, CH=CH_2), 5.70 (dd, 1H, $J_{1,2}=2.2$ Hz, H-2_C), 5.41 (dd, 1H, $J_{2,3}=3.1$ Hz, H-2_B), 5.33 (dd, 1H, $J=17.2$, $J=1.5$ Hz, CH=CH_2), 5.24 (dd, 1H, $J=10.3$, $J=1.3$ Hz, CH=CH_2), 5.04 (d, 1H, $J_{1,2}=3.2$ Hz, H-1_E), 5.01 (d, 1H, $J_{1,2}=1.6$ Hz, H-1_C), 4.94 (d, 1H, $J=11.0$ Hz, OCH_2), 4.92 (d, 1H, $J_{1,2}=1.8$ Hz, H-1_B), 4.91–4.78 (m, 5H, OCH_2), 4.69–4.33 (m, 6H, OCH_2), 4.20–4.14 (m, 2H, H-3_B, OCH_2), 4.02 (m, 3H, H-5_E, 3_E, OCH_2), 3.89 (bd, 1H, $J_{6a,6b}=9.4$ Hz, H-$6a_E$), 3.83–3.70 (m, 5H, H-5_B, $6b_E$, 4_B, 4_E, 3_C), 3.64 (dq, 1H, $J_{4,5}=9.4$ Hz, H-5_C), 3.50 (dd, 1H, $J_{2,3}=9.5$ Hz, H-2_E), 3.31 (pt, 1H, $J_{3,4}=9.4$ Hz, H-4_C), 2.14 (s, 3H, C(O)CH_3), 1.38 (d, 3H, $J_{5,6}=5.9$ Hz, H-6_B), and 0.99 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C); ^{13}C NMR: δ 170.1, 165.8 (2C, C(O)), 138.9–127.4 (Ph, All), 117.8 (All), 99.4 (bs, C-1_C, $J_{C,H}=169$ Hz), 98.3 (C-1_E), 96.1 (C-1_B, $J_{C,H}=169$ Hz), 81.9 (C-3_E), 81.3 (C-2_E), 79.9 (C-4_C), 79.8 (bs, C-3_B), 78.9 (C-4_B), 77.9 (C-4_E), 77.5 (C-3_C), 75.7, 75.2, 75.0, 74.1, 73.0 (5C, OCH_2), 72.2 (C-2_B), 71.7 (C-5_E), 70.6 (OCH_2), 68.8 (C-5_C), 68.7 (C-2_C), 68.6 (C-6_E), 68.5 (OCH_2), 67.5 (C-5_B), 21.3 (C(O)CH_3), 18.9 (C-6_B), and 17.7 (C-6_C). ESMS for $C_{72}H_{78}O_{16}$ (M, 1198.4) m/z 1199.5 $[M+H]^+$. Anal. calcd for $C_{72}H_{78}O_{16}$: C, 72.10; H, 6.55. Found: C, 72.19; H, 6.49%.

4.13. (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/β-L-rhamnopyranose, 26

Water (35 drops) was added to a suspension of palladium dichloride (2.07 g, 11.66 mmol), sodium acetate (3.41 g, 25 mmol) and the fully protected pentasaccharide **25** (5.93 g, 4.95 mmol) in acetic acid (31.2 mL). The mixture was stirred overnight at rt. TLC (solvent B, 2.3:1) showed that the starting material had turned into a major more polar product. Volatiles were evaporated and the residue was taken up in AcOEt and washed successively with satd aq. $NaHCO_3$, and satd aq. NaCl. Column chromatography of the residue (solvent B, 4:1) gave pure hemiacetal **26** (4.31 g, 75%) as a white foam; ^{1}H NMR: δ 8.06–7.12 (m, 35H, Ph), 5.68 (dd, 1H, $J_{2,3}=2.5$ Hz, H-2_B), 5.39 (dd, 1H, H-2_C), 5.21 (dd, 1H, $J_{1,2}=2.4$ Hz, H-1_C), 5.03 (d, 1H, H-1_E), 5.01 (d, 1H, $J_{1,2}=1.8$ Hz, H-1_B), 4.98–4.40 (m, 11H, OCH_2), 4.23 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=8.5$ Hz, H-3_C), 4.07–3.98 (m, 4H, H-3_E, 5_C, 5_E, OCH_2), 3.89 (bd, 1H, $J_{6a,6b}=10.3$ Hz, H-$6a_E$), 3.83–3.69 (m, 4H, H-$6b_E$, 4_E, 3_B, 4_C), 3.66 (dq, 1H, $J_{4,5}=9.5$ Hz, H-5_B), 3.50 (dd, 1H, $J_{1,2}=3.3$, $J_{2,3}=9.7$ Hz, H-2_E), 3.32 (pt, 1H, $J_{3,4}=9.4$ Hz, H-4_B), 3.04 (d, 1H, $J_{1,OH}=4.2$ Hz, OH-1_C), 2.13 (s, 3H, $C(O)CH_3$), 1.36 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C), and 0.99 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_B); ^{13}C NMR: δ 170.2, 165.8 (2C, C(O)), 133.3–127.4 (Ph), 99.0 (C-1_B), 98.4 (C-1_E), 91.3 (C-1_C), 81.9 (C-3_E), 81.3 (C-2_E), 79.9 (C-4_B), 79.2 (C-4_C), 79.1 (C-3_C), 77.8 (C-4_E), 77.3 (C-3_B), 75.7, 75.3, 75.0, 74.0, 73.0 (5C, OCH_2), 72.4 (C-2_C), 71.6 (C-5_E), 70.6 (OCH_2), 68.7 (2C, C-5_B, 2_B), 68.5 (C-6_E), 67.4 (C-5_C), 21.3 ($C(O)CH_3$), 18.9 (C-6_C), and 17.7 (C-6_B). ESMS for $C_{69}H_{74}NO_{16}$ (M, 1158.50) m/z 1159.6 $[M+H]^+$. Anal. calcd for $C_{69}H_{74}NO_{16}$: C, 71.49; H, 6.62; N, 6.43. Found: C, 71.38; H, 6.47%.

4.14. Trichloroacetimidate (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/β-L-rhamnopyranosyl, 6

Trichloroacetonitrile (1.2 mL, 11.96 mmol) and DBU (24 μL, 160 μmol) were added to a solution of hemiacetal **26** (368 mg, 318 μmol) in CH_2Cl_2 (2 mL) and the mixture was stirred at rt for 4 h. The reaction mixture was concentrated and the residue was purified by flash-column chromatography (solvent B, 4:1 containing 1‰ Et_3N) to give **6** as a white foam (343 mg, 83%); ^{1}H NMR: δ 8.73 (s, 1H, NH), 8.07–7.13 (m, 35H, Ph), 6.35 (d, 1H, $J_{1,2}=2.8$ Hz, H-1_C), 5.68 (bs, 1H, H-2_B), 5.58 (dd, 1H, $J_{2,3}=3.0$ Hz, H-2_C), 5.06 (d, 1H, $J_{1,2}=3.3$ Hz, H-1_E), 5.04 (bs, 1H, H-1_B), 4.94 (d, 1H, $J=11$ Hz, OCH_2), 4.82 (m, 3H, OCH_2), 4.66–4.34 (m, 8H, OCH_2), 4.25 (bs, 1H, H-3_C), 4.06–3.97 (m, 3H, H-3_E, 5_C, 5_E), 3.83–3.72 (m, 5H, H-$6a_E$, $6b_E$, 4_C, 4_E, 3_B), 3.67 (dq, 1H, $J_{4,5}=9.5$ Hz, H-5_B), 3.53 (dd, 1H, $J_{2,3}=9.7$ Hz, H-2_E), 3.32 (pt, 1H, $J_{3,4}=9.4$ Hz, H-4_B), 2.13 (s, 3H, $C(O)CH_3$), 1.42 (d, 3H, $J_{5,6}=6.0$ Hz, H-6_C), and 0.98 (bs, 3H, H-6_B); ^{13}C NMR: δ 170.0, 165.5 (2C, C(O)), 160.3 (C=NH), 138.8–127.5 (Ph), 98.8 (bs, 2C, C-1_B, 1_E), 94.4 (bs, C-1_C), 91.0 (CCl_3), 81.8 (C-3_E), 81.0 (bs, C-2_E), 79.7 (C-4_B), 77.7 (C-4_E^*), 77.5 (C-3_B^*), 75.6, 75.2, 74.9, 74.1, 73.1 (5C, OCH_2), 71.7 (C-5_E), 71.0 (bs, OCH_2), 70.8 (C-2_C), 70.2 (bs, C-5_C), 68.8 (bs, C-5_B), 68.6 (C-2_B), 68.4 (C-6_E), 21.2 ($C(O)CH_3$), 18.8 (bs, C-6_C), and 17.7 (C-6_B). Due to signal broadness, C-3_C and C-4_C could not be extracted from any of the 1D or 2D spectra. Anal. calcd for $C_{71}H_{74}Cl_3NO_{16}$: C, 65.41; H, 5.72; N, 1.07. Found: C, 65.26; H, 6.86; N, 1.02%.

4.15. 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-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside, 27

$BF_3{\cdot}OEt_2$ (1.25 mL, 9.86 mmol) was added to a mixture of the disaccharide acceptor **4** (794 mg, 1.32 mmol) and the trisaccharide donor **6** (2.61 g, 2.0 mmol) in anhydrous Et_2O (70 mL) containing activated powered 4 Å molecular sieves (2 g) and the mixture was processed as described for the preparation of **20**. Chromatography of the residue (solvent B, 4:1→2.3:1) gave the fully protected pentasaccharide **27** (1.86 g, 81%) as a white foam; $[\alpha]_D$ +26 (c 1.0); ^{1}H NMR: δ 8.04–7.10 (m, 45H, Ph), 5.72 (pt, 1H, H-2_C), 5.66 (d, 1H, $J_{NH,2}=7.4$ Hz, NH), 5.25 (dd, 1H, $J_{1,2}=1.9$ Hz, H-2_B), 5.07 (d, 1H, $J_{1,2}=3.2$ Hz, H-1_E), 5.02 (d, 1H, $J_{1,2}=1.6$ Hz, H-1_C), 4.97–4.33 (m, 16H, OCH_2), 4.77 (bs, 2H, H-1_D, 1_B), 4.68 (bs, 1H, H-1_A), 4.11 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=9.1$ Hz, H-3_B), 4.07–3.93 (m, 3H, H-5_B, 5_E, 3_E), 3.89–3.69 (m, 10H, H-$6a_E$, 2_A, 3_A, $6a_D$, $6b_E$, $6b_D$, 4_B, 3_C, 4_E, 3_D), 3.67–3.60 (m, 3H, H-5_A, 2_D, 5_C), 3.57 (pt, 1H, $J_{3,4}=J_{4,5}=9.2$ Hz, H-4_D), 3.48 (dd, 1H, $J_{2,3}=9.6$ Hz, H-2_E), 3.42 (pt, 1H, $J_{3,4}=J_{4,5}=9.3$ Hz, H-4_A), 3.32 (s, 3H, OCH_3), 3.30–3.20 (m, 2H, H-4_C, 5_D), 2.14, 1.91 (2s, 6H, $C(O)CH_3$), 1.47, 1.38 (2s, 6H, $C(CH_3)_2$), 1.35 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_A), 1.30 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_B), and 0.95 (d, 3H, $J_{5,6}=6.2$ Hz, H-6_C); ^{13}C NMR: δ 171.2, 170.1, 165.6 (3C, C(O)), 138.8–127.3 (Ph), 102.7 (C-1_D, $J_{C,H}=161$ Hz), 100.1 (C-1_A, $J_{C,H}=171$ Hz), 99.5 ($C(CH_3)_2$), 99.2 (C-1_C, $J_{C,H}=168$ Hz), 98.0 (C-1_E, $J_{C,H}=169$ Hz), 97.9 (C-1_B, $J_{C,H}=169$ Hz), 81.8 (C-3_E), 81.4 (C-2_E), 80.9 (C-4_A), 79.9 (C-4_C), 79.7 (C-3_A), 79.6 (bs, C-3_B), 78.8 (bs, C-4_B^*), 78.5 (bs, C-3_C^*), 77.8 (C-4_E^*), 77.5 (C-2_A), 77.3 (C-3_D^*), 75.5, 75.4, 75.0, 74.9, 73.9, 73.0, 72.9 (7C, OCH_2), 72.5 (C-4_D), 72.3 (C-2_B), 71.6 (C-5_E), 70.5 (OCH_2), 68.6 (3C, C-2_C, 5_C, 6_E), 67.7 (2C, C-5_A, 5_D), 67.3 (C-5_B), 62.2 (C-6_D), 57.3 (C-2_D), 54.5 (OCH_3), 29.2 ($C(CH_3)_2$), 23.5, 21.3 (2C, $C(O)CH_3$), 19.3 ($C(CH_3)_2$), 18.5 (C-6_B), 17.9 (C-6_A), and 17.7 (C-6_C). ESMS for $C_{101}H_{115}NO_{25}$ (M, 1741.78) m/z 1742.9 $[M+H]^+$. Anal. calcd for $C_{101}H_{115}NO_{25}$: C, 69.60; H, 6.65; N, 0.89. Found: C, 69.40; H, 6.68; N, 0.83%.

4.16. Methyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-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-rhamnopyranoside, 28

Methanolic MeONa (1 M) was added to a solution of **27** (150 mg, 86 μmol) in MeOH (3 mL) until the pH reached 10, and the mixture was stirred at 60°C overnight. TLC (solvent C, 5:1) showed the presence of a more polar product. The mixture was neutralized by addition of Amberlite IR 120 (H^+), filtered, and volatiles were evaporated. The crude residue was purified by column chromatography (solvent C, 9:1) to give the diol **28** (124 mg, 90%) as a white foam; $[\alpha]_D$ +21 (*c* 1.0); ^{1}H NMR: δ 7.39–7.25 (m, 40H, Ph), 5.44 (d, 1H, $J_{NH,2}$ = 8.0 Hz, NH), 5.07 (d, 1H, $J_{1,2}$ = 2.2 Hz, H-1$_C$), 5.04 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1$_E$), 4.94 (d, 1H, *J* = 10.8 Hz, OCH_2), 4.86–4.44 (m, 15H, OCH_2), 4.68 (m, 2H, H-1$_A$, 1$_B$), 4.48 (d, partially overlapped, 1H, H-1$_D$), 4.20 (bs, 1H, H-2$_C$), 4.06 (dq, 1H, $J_{4,5}$ = 9.4, $J_{5,6}$ = 6.2 Hz, H-5$_B$), 3.89–3.69 (m, 13H, H-5$_E$, 3$_E$, 2$_A$, 3$_A$, 3$_B$, 6a$_D$, 6b$_D$, 2$_B$, 5$_C$, 2$_D$, 3$_C$, 6a$_E$, 6b$_E$), 3.66–3.58 (m, 4H, H-5$_A$, 4$_E$, 4$_D$, 3$_D$), 3.53–3.46 (m, 3H, H-2$_E$, 4$_C$, 4$_B$), 3.35 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-4$_A$), 3.32 (s, 3H, OCH_3), 3.23 (m, 1H, H-5$_D$), 3.13 (bs, 1H, OH), 2.35 (bs, 1H, OH), 1.66 (s, 3H, $C(O)CH_3$), 1.46 (s, 3H, $C(CH_3)_2$), 1.39–1.36 (m, 6H, $C(CH_3)_2$, H-6$_C$), and 1.32 (m, 6H, H-6$_A$, 6$_B$); ^{13}C NMR: δ 170.1 (C(O)), 138.6–127.6 (Ph), 103.4 (C-1$_D$), 101.4 (C-1$_C$), 100.0, 99.8 (C-1$_A$, 1$_B$), 99.5 ($C(CH_3)_2$), 98.1 (C-1$_E$), 81.4 (C-3$_E$), 81.1, 81.0 (C-2$_E$, 4$^*_A$), 80.4, 79.9 (C-3$_B$, 3$_A$), 79.8, 79.5 (C-4$^*_B$, 4$_C$), 79.1 (C-3$_C$), 77.8, 77.7, 77.6 (C-4$_E$, 2$_A$, 3$_D$), 75.5, 75.4, 75.0, 74.8, 73.5, 73.4, 73.1 (7C, OCH_2), 72.2 (C-4$_D$), 72.0 (OCH_2), 71.2 (C-5$_E$), 70.7 (C-2$_B$), 69.2 (C-5$_C$), 68.4 (C-2$_C$), 68.3 (C-6$_E$), 67.8 (C-5$_D$), 67.6 (C-5$_A$), 66.9 (C-5$_B$), 62.2 (C-6$_D$), 56.5 (C-2$_D$), 54.5 (OCH_3), 29.1 ($C(CH_3)_2$), 23.2 ($C(O)CH_3$), 19.2 ($C(CH_3)_2$), 18.3 (C-6$_C$), 17.8 and 17.7 (2C, C-6$_B$, 6$_A$). FABMS for $C_{92}H_{109}NO_{23}$ (M, 1595.74) *m*/*z* 1618.8 $[M+Na]^+$. Anal. calcd for $C_{92}H_{109}NO_{23}$: C, 69.20; H, 6.88; N, 0.88. Found: C, 69.09; H, 7.04; N, 0.96%.

4.17. Methyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(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-rhamnopyranoside, 29

50% aq. TFA (1 mL) was added to a solution of the fully protected **27** (162 mg, 93 μmol) in CH_2Cl_2 (3 mL) and the mixture was stirred at 0°C for 50 min. TLC (solvent C, 6:1) showed that the complete disappearance of the starting material. Toluene was added and volatiles were evaporated. The crude mixture was solubilized in MeOH (3 mL), 1N methanolic MeONa was added dropwise until the pH was 10, and the mixture was stirred overnight at 60°C. TLC (solvent C, 9:1) showed that the material had been transformed into a more polar product. Neutralization with Amberlite IR 120 (H^+), filtration and evaporation of the volatiles resulted in a crude product which was purified by column chromatography (solvent D, 49:1) to give **29** (119 mg, 82%) as a white foam; $[\alpha]_D$ +22 (*c* 1.0); ^{1}H NMR: δ 7.37–7.26 (m, 40H, Ph), 5.70 (d, 1H, $J_{NH,2}$ = 7.2 Hz, NH), 5.01 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1$_D$), 4.96–4.58 (m, 18H, H-1$_E$, 1$_A$, 1$_B$, 1$_C$, 14 OCH_2), 4.46 (m, 2H, OCH_2), 4.16 (bs, 1H, H-2$_C$), 3.98–3.85 (m, 9H, H-3$_E$, 5$_A$, 5$_E$, 3$_A$, 2$_A$, 5$_C$, 3$_B$, 2$_B$, 6a$_D$), 3.77–3.58 (m, 7H, H-6b$_D$, 3$_C$, 6a$_E$, 6b$_E$, 5$_B$, 2$_D$, 4$_E$), 3.53–3.42 (m, 6H, H-2$_E$, 4$_A$, 4$_C$, 3$_D$, 5$_D$, 4$_B$), 3.36 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4$_D$), 3.33 (s, 3H, OCH_3), 2.98 (bs, 1H, OH-2$_C$), 2.69 (bs, 1H, OH-2$_B$), 2.69, 1.84 (2bs, 2H, OH-6$_D$, OH-4$_D$), 1.72 (s, 3H, $C(O)CH_3$), and 1.38–1.30 (m, 9H, H-6$_A$, 6$_B$, 6$_C$); ^{13}C NMR: δ 170.9 (C(O)), 138.7–128.0 (Ph), 102.3 (C-1$_D$), 101.2 (C-1$^*_B$), 100.8 (C-1$_C$), 100.0 (C-1$^*_A$), 98.4 (C-1$_E$), 86.4 (C-3$_D$), 81.5 (C-3$_E$), 81.0 (C-4$^*_B$), 80.6 (C-2$^*_E$), 80.0 (C-3$^*_B$), 79.8 (C-4$^*_C$), 79.2 (2C, C-3$^*_A$, 3$_C$), 79.1 (C-4$^*_A$), 77.6 (C-4$_E$), 77.4 (C-2$_A$), 75.7 (OCH_2), 75.5 (C-4$_D$), 75.4, 75.0, 74.9, 73.6, 73.2, 72.9, (7C, OCH_2), 71.3 (C-5$_E$), 70.5 (C-5$_D$), 70.0 (C-2$_B$), 69.1 (C-5$^*_A$), 68.6 (C-5$_C$), 68.4 (C-2$_C$), 68.3 (C-6$_E$), 67.6 (C-5$^*_B$), 62.9 (C-6$_D$), 55.4 (C-2$_D$), 54.6 (OCH_3), 23.4 ($C(O)CH_3$), and 18.4, 17.9, 17.8 (3C, C-6$_B$, 6$_A$, 6$_C$). FABMS for $C_{89}H_{105}NO_{23}$ (M, 1555.71) *m*/*z* 1578.8 $[M+Na]^+$. Anal. calcd for $C_{89}H_{105}NO_{23}$: C, 68.66; H, 6.80; N, 0.90. Found: C, 68.64; H, 6.89; N, 0.81%.

4.18. Methyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-α-L-rhamnopyranoside, 2

A solution of tetraol **29** (310 mg, 199 μmol) in a mixture of MeOH (9 mL) and AcOH (1 mL) was treated with 10% Pd–C catalyst (200 mg) as described for the preparation of **1**. Reverse phase chromatography (solvent F, gradient), followed by lyophilization, gave the targeted pentasaccharide **2** as a lyophilized powder (126 mg, 76%); $[\alpha]_D$ −12 (*c* 1.0, water); ^{1}H NMR: (D_2O); δ 5.10 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1$_E$), 4.90 (bs, 1H, H-1$_B$), 4.76 (bs, 1H, H-1$_A$), 4.75 (bs, 1H, H-1$_C$), 4.65 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1$_D$), 4.08 (dq, 1H, $J_{4,5}$ = 9.0 Hz, H-5$_C$), 4.03 (m, 1H, H-2$_B$), 3.99 (m, 1H, H-2$_A$), 3.96–3.65 (m, 12H, H-3$_C$, 2$_C$, 6a$_D$, 6a$_E$, 6b$_E$, 5$_E$, 2$_D$, 5$_B$, 3$_A$, 3$_B$, 4$_C$, 6b$_D$), 3.60–3.33 (m, 8H, H-3$_E$, 5$_A$, 3$_D$, 4$_D$, 2$_E$, 5$_D$, 4$_E$, 4$_B$), 3.31 (s, 3H, OCH_3), 3.13 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4$_A$), 1.99 (s, 3H, $C(O)CH_3$), 1.27 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_A$), 1.21 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6$_C$), and 1.20 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6$_B$); ^{13}C NMR: (D_2O); δ 175.1 (C(O)), 103.7 (C-1$_D$, $J_{C,H}$ = 161 Hz), 102.9 (C-1$_A$, $J_{C,H}$ = 171 Hz), 101.8 (C-1$_C$, $J_{C,H}$ = 168 Hz), 100.5 (C-1$_E$, $J_{C,H}$ = 169 Hz), 99.0 (C-1$_B$, $J_{C,H}$ = 169 Hz), 82.2 (C-3$_D$), 79.4 (C-3$_C$), 79.2 (C-2$_A$), 77.0 (bs, C-4$_C$), 76.6 (C-4$_E$), 73.3 (C-3$_E$), 73.0 (C-4$_A$), 72.7 (2C, C-4$_B$, 5$^*_E$), 72.2 (C-2$_E$), 71.5 (C-2$_C$), 70.9 (2C, C-3$^*_A$, 2$_B$), 70.0 (C-3$^*_B$), 70.1 (C-5$^*_D$), 70.0 (C-5$_B$), 69.6 (C-5$_C$), 69.3 (C-5$_A$), 69.1 (C-4$^*_D$), 61.4 (C-6$_D$), 61.2 (C-6$_E$), 56.3 (C-2$_D$), 55.5 (OCH_3), 23.1 ($C(O)CH_3$), 18.4 (C-6$_C$), and 17.4, 17.3 (2C, C-6$_A$, 6$_B$). High-resolution ESMS for $C_{33}H_{57}NO_{23}$ (M−H, 834.32431) *m*/*z* 834.32135 $[M+Na]^+$.

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