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Synthesis and NMR study of a linear pentasaccharide fragment of the *Shigella flexneri* 5a *O*-specific polysaccharide☆

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Abstract—A convergent chemical synthesis of the methyl glycoside of the linear epitope α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→2)-α-L-Rha*p* (EBCDA) corresponding to the ramification of the *O*-antigen of *Shigella flexneri* serotype 5a is described. The strategy relies on the preparation of a key EB trichloroacetimidate donor and that of an appropriate CDA trisaccharide acceptor. Trichloroacetimidate chemistry was used for the construction of all glycosidic linkages except that of DA, where a bromide donor was preferred. In depth analysis of the pentasaccharide EBCDA ^{1}H and ^{13}C NMR spectra shows that its conformation approaches that of the corresponding fragment in the native polysaccharide. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

The use of polysaccharide–protein conjugates for the prevention of infection by encapsulated bacteria is now well-established.[2] In the case of non-encapsulated bacteria, such as *Shigella*, a Gram-negative enterobacterium that can cause diarrhea and dysentery in humans, the bacterial *O*-specific polysaccharide (*O*-SP), a component of their LPS, is both an essential virulence factor and a critical antigen for host immunity. The *O*-SP of *S. flexneri* behaves as a hapten, which acts as a T-dependent antigen when conjugated to an immunogenic protein carrier. In the case of *S. flexneri* 2a and *S. sonnei*, such *O*-SP/protein conjugates elicited high levels of anti-LPS IgG in healthy adults.[3,4]

A program to develop chemically defined vaccines as a possible alternative approach to the use of *O*-SP/protein conjugates as human anti-bacterial vaccines is in progress on the model bacterium *S. flexneri* serotype 5a. The approach focuses on the use of mimics of the antigenic polysaccharide.[5] Optimization of the mimics is supported, in part, by a comparative study of their solution conformations and that of the native carbohydrate epitopes. Such a study implies that ligands representative of the major structural features of the *O*-SP of *S. flexneri* serotype 5a be available in rather large amounts.

The repeating unit of the *O*-SP of *S. flexneri* serotype 5a is the branched pentasaccharide[6,7] **I**, composed of α-linked L-rhamnoses, β-linked 2-acetamido-2-deoxy-D-glucose, and α-D-glucose branches. In spite of the large amount of work on *S. flexneri* 5a reported previously in the literature,[8,9] the preparation of the required frame-shifted oligosaccharides was undertaken.[10–12] In this paper, we describe a convergent synthesis of the linear pentasaccharide EBCDA prepared as its methyl glycoside (**1**) that has the natural anomeric

A E B C D

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

I

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

EBCDA-OMe 1

☆ See Ref. 1.

Keywords: bacterial oligosaccharides; *Shigella flexneri*; chemical glycosylation.
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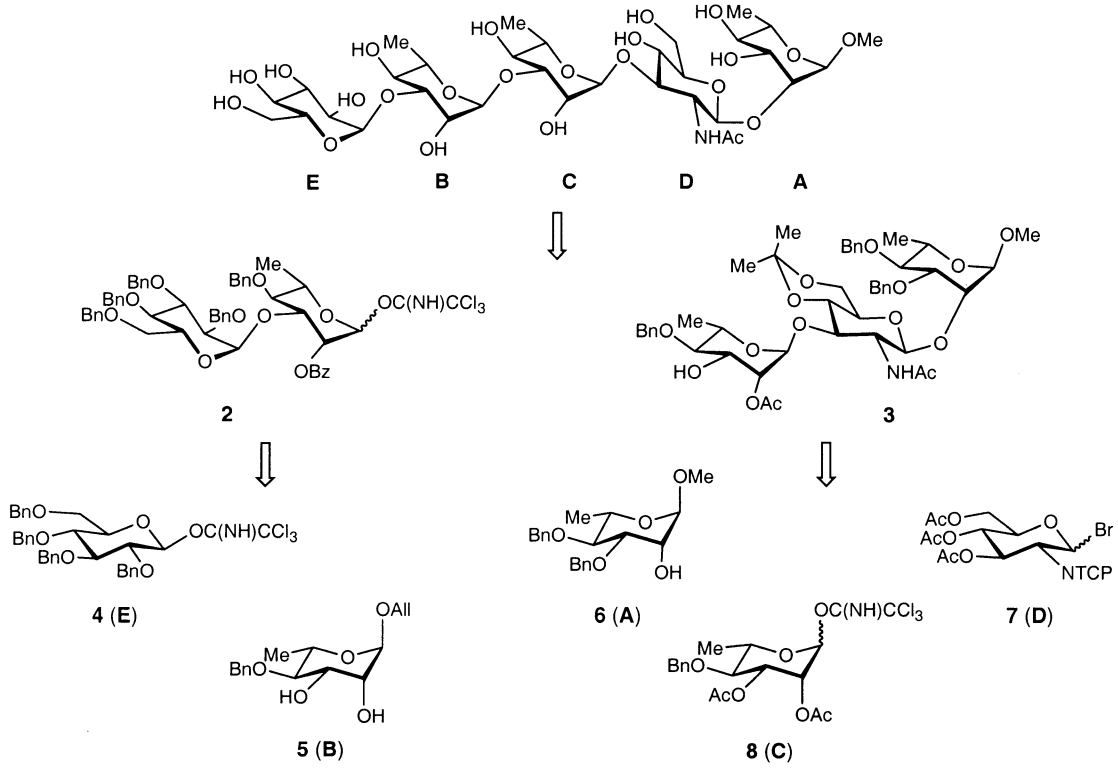


Scheme 1. Retrosynthetic approach to pentasaccharide **1**.

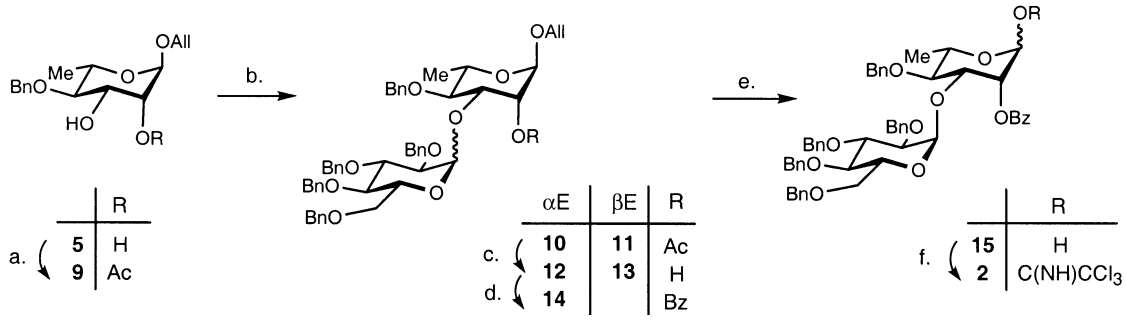
configuration at its reducing end. The NMR study of **1** using a combination of one- and two-dimensional (1D and 2D) techniques is also presented with emphasis on its characteristics in relation to the native *O*-SP.

2. Results and discussion

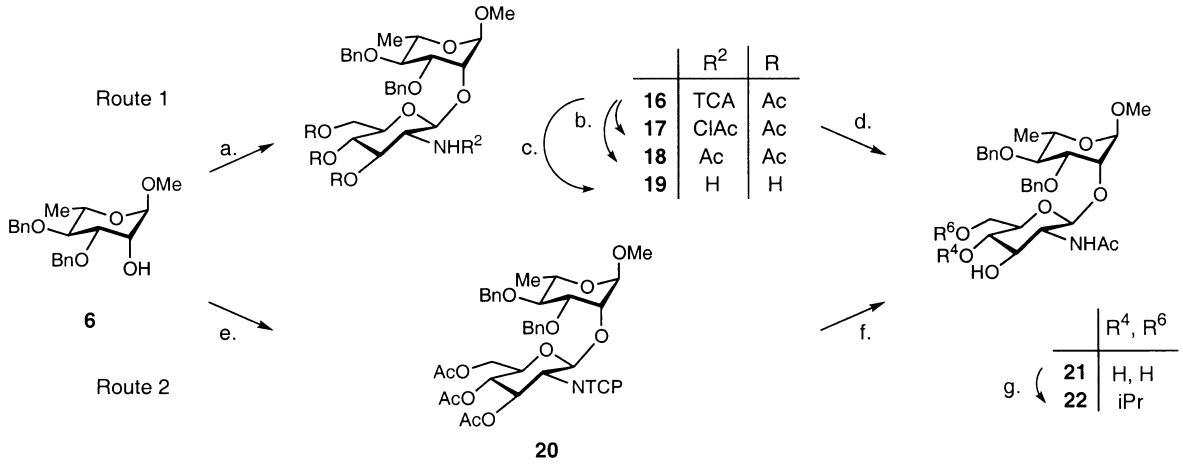
The linear pentasaccharide **1** was prepared following a block synthetic strategy (Scheme 1), using the disaccharide trichloroacetimidate **2** as the donor and the trisaccharide acceptor **3** derived from α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→2)-α-L-Rha*p*-OMe. In designing the synthesis, attention was paid to the fact that the construction of the EB moiety, which involves a 1,2-*cis* glycosidic linkage, was the most demanding. Based on previous experience in the methyl glycoside series,[10] the known trichloroacetimidate donor[13,14] **4** and the allyl rhamnopyranoside[15,16] **5**, which can be blocked selectively at position 2, were selected as suitable precursors to the intermediate **2**. Trisaccharide **3** was readily accessible from the known methyl glycoside[17,18] **6**, glucosaminyl bromide[19] **7** and trichloroacetimidate donor[12] **8**, which were chosen as appropriate precursors for residues A, D, and C, respectively. Compound **8** features easily removable protecting groups at positions 2 and 3, allowing subsequent chain extension at position 3.

2.1. Preparation of the disaccharide donor EB

Conventional regioselective opening of the intermediate orthoester issued from diol **5** gave a 97:3 mixture of the target 2-*O*-acetyl derivative[20] **9** and the corresponding 3-*O*-acetyl regioisomer, as seen by ^{1}H NMR spectroscopy. In order to prevent any potential migration of the acetyl group to the vicinal hydroxyl group, the crude mixture was used as such. Condensation of acceptor **9** with the



Scheme 2. *Reagents and conditions*: (a) (i) MeC(OMe)$_3$ 3 equiv., PTSA cat., CH_3CN, rt, 1.5 h; (ii) AcOH 80% aq., 0°C, 30 min, 99%; (b) **4** 1.3 equiv., TMSOTf 0.05 equiv., Et_2O, −78°C→rt, overnight; (c) MeONa 1.5 equiv., MeOH, rt, 3 days, 57% for two steps; (d) BzCl 1.5 equiv., Pyr, 70°C, overnight, 95%; (e) (i) $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$ 0.09 equiv., THF, rt, overnight; (ii) HgO 1.90 equiv., $HgCl_2$ 1.59 equiv., H_2O/Me_2CO, rt, 2.5 h, 86%; (f) CCl_3CN 10 equiv., DBU cat., CH_2Cl_2, 0°C, 1 h, 95%.

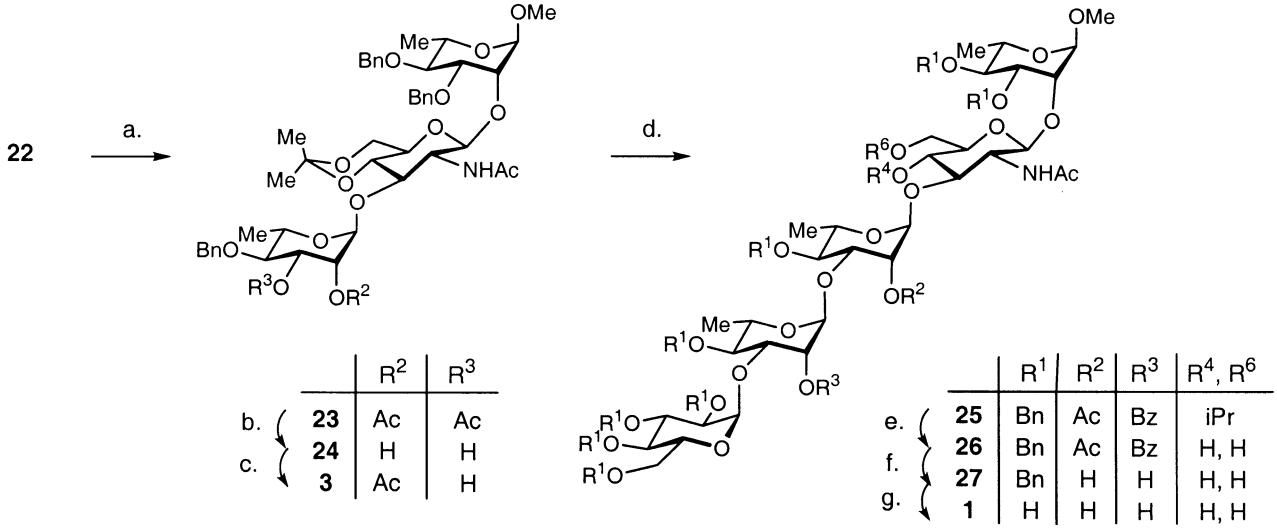


Scheme 3. *Reagents and conditions*: (a) see Ref. 10; (b) Bu_3SnH, AIBN, Toluene/DMA, 100°C; (c) MeONa, MeOH/CH_2Cl_2; (d) Ac_2O, MeOH (59% from **16**); (e) **7** 1.38 equiv., AgOTf, DBMP, CH_2Cl_2, −50°C→rt, 89%; (f) (i) $(H_2NCH_2)_2$, EtOH, 70°C; (ii) Ac_2O, EtOH (83%); (g) $Me_2C(OMe)_2$, PTSA, acetone (87% from **20**).

trichloroacetimidate donor **4** was performed in diethyl ether in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to afford the condensation products **10**[21] and **11** as an inseparable mixture. Treatment of the anomeric mixture with an excess of methanolic sodium methoxide afforded the monohydroxylated disaccharide **12** (57%) and contaminated β-anomer **13**, which could be separated at this stage (Scheme 2). The stereochemistry of the EC linkage in **12** and **13** was ascertained based on the $^1J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants. Data for compound **12** were 168 and 170 Hz, data for compound **13** were 159 and 169 Hz for residues E and B, respectively. As observed in the methyl glycoside series,[10] position 2_B is most probably sterically hindered due to the presence of the 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose residue at *O*-3_B, which renders the deacylation step an unusually slow process. For this reason, benzoylation of **12** by treatment with benzoyl chloride in pyridine was performed at 70°C to give the fully protected **14** (95%). In that case, benzoylation was preferred to acetylation in order to avoid potential acetylation of the acceptor, known to occur during TMSOTf catalyzed glycosylations.[17] Next, disaccharide **14** was converted to the hemiacetal **15** (86%), following a two-step selective deallylation procedure involving: (i) isomerisation of the allyl ether into the corresponding prop-1-enyl ether using a cationic iridium complex[22] and (ii) subsequent hydrolysis with mercury(II) chloride/mercury(II) oxide.[15,23] Finally, the EB disaccharide donor **2** was obtained in 95% yield as an anomeric mixture by treatment of the hemiacetal **15** with trichloroacetonitrile and a catalytic amount of DBU.

2.2. Preparation of the disaccharide acceptor DA

Two types of amine participating groups were considered in the construction of the DA disaccharide (Scheme 3), the selectively removable *N*-trichloroacetyl group (TCA, route 1) and the *N*-tetrachlorophtaloyl group (TCP, route 2). In route 1, the easily accessible disaccharide[10] **16** was the key intermediate to the triol **21**. Conversion of the *N*-trichloroacetyl group to the *N*-acetyl group was first attempted through tributyltin hydride-mediated reductive cleavage of



Scheme 4. *Reagents and conditions*: (a) **8** 1.55 equiv., TMSOTf 0.09 equiv., Et_2O; (b) MeONa, MeOH (81% from **22**); (c) (i) $MeC(OMe)_3$, PTSA, CH_3CN; (ii) AcOH 80% aq.; (d) **2** 1.4 equiv., TMSOTf cat., Et_2O (67% from **24**); (e) TFA 50% aq. (94%); (f) MeONa, MeOH (97%); (g) H_2, Pd/C, AcOH/EtOH (67%).

Table 1. ^{1}H NMR data for EBCDA-OMe (**1**)

Residue	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
α-L-Rha*p* (A)[a]	4.82 (0.32)	3.98 (−0.16)	3.77 (−0.12)	3.29 (0.10)	3.62 (−0.08)	1.25 (0.00)	
α-L-Rha*p* (B)	5.05 (−0.17)	4.24 (−0.01)	3.89 (−0.12)	3.56 (−0.05)	3.80 (0.01)	1.30 (−0.04)	
α-L-Rha*p* (C)	4.84 (−0.04)	3.87 (0.00)	3.79 (−0.01)	3.51 (−0.05)	4.00 (0.01)	1.22 (−0.05)	
β-D-GlcNAc*p* (D)[b]	4.71 (−0.07)	3.82 (−0.01)	3.60 (−0.06)	3.51 (0.02)	3.43 (−0.05)	3.90 (−0.04)	3.73 (−0.03)
α-D-Glc*p* (E)	5.08 (−0.01)	3.56 (−0.04)	3.77 (−0.03)	3.44 (0.02)	3.96 (−0.04)	3.79 (−0.07)	3.76 (−0.02)

Chemicals shifts measured in ppm with an accuracy of ±0.01 ppm are referenced to external DSS (δ_H 0.00). The numbers in parentheses represent the difference in ppm between the chemical shifts of the protons in **1** and the chemical shifts of the respective protons in the O-SP of *S. flexneri* serotype 5a.[37]

[a] The chemical shift of the aglyconic methyl is 3.37 ppm.

[b] The chemical shift of the *N*-acetyl group is 2.05 ppm.

the chlorine atoms.[24] Often yielding the *N*-chloroacetyl disaccharide **17** together with the target **18**, this approach failed to give satisfactory results when applied to **16**. That the *N*-trichloroacetyl moiety was sensitive to treatment with sodium methoxide[10] or sodium hydroxide[25] was reported previously. Indeed, treatment of **16** with sodium methoxide gave the aminotriol **19**, which was next selectively *N*-acetylated into **21** (59% for two steps). In route 2 to DA, the glucosaminyl bromide **7** was preferred to the corresponding trichloroacetimidate,[26] which was found more difficult to obtain. Condensation of the rhamnose acceptor **6** and donor **7** was performed under base-deficient conditions,[27] using silver trifluoromethanesulfonate (AgOTf) as the promoter, to give the fully protected disaccharide **20** (89%). Treatment with ethylenediamine and subsequent selective *N*-acetylation allowed the conversion of the *N*-tetrachlorophthalimido disaccharide to the corresponding *N*-acetamido triol **21** (83%). Overall, the yield of **21** compares favorably with that described previously[28] for an analogous compound when using the corresponding *N*-phthalimido glucosamine donor. Subsequent isopropylidenation of **21** afforded the acceptor **22** (87% from **20**).

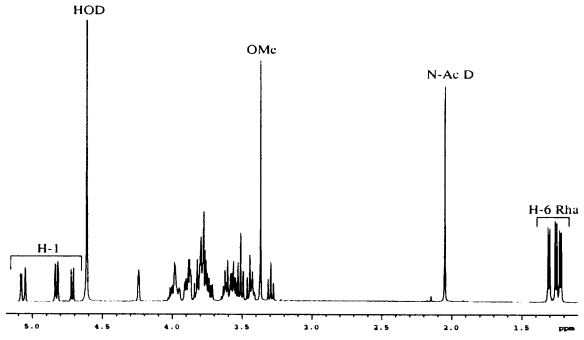


Figure 1. ^{1}H NMR spectrum of the pentasaccharide EBCDA-OMe (**1**) in D_2O at 38°C at 500 MHz.

2.3. Assembly of the pentasaccharide EBCDA-OMe (1)

Condensation of **22** with the trichloroacetimidate donor **8** in the presence of a catalytic amount of TMSOTf gave **23** (Scheme 4). Transesterification gave the diol **24** (81% from **22**) which was next regioselectively acetylated at position 2_C, by acidic hydrolysis of the intermediate orthoacetate, to give the CDA trisaccharide acceptor **3**. TMSOTf promoted glycosylation of this alcohol with the trichloroacetimidate donor **2** provided the fully protected pentasaccharide **25** (67% from **24**). The α-anomery of residues A, B, C and E in compound **25** was indicated by the $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants, whose values were of 170, 172, 170, and 169 Hz, respectively. The $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constant for residue D was 161 Hz, confirming the β-anomery. Acetal cleavage of **25** gave diol **26** (94%), Zemplén deacylation of which afforded the tetraol **27** (97%), which was finally converted to the target **1** (67%) by conventional debenzylation, using palladium on charcoal as the catalyst.

2.4. NMR study of the pentasaccharide EBCDA-OMe (1) in solution

Complete ^{1}H NMR assignments of pentasaccharide EBCDA-OMe (**1**) (Table 1) were obtained from 1D and 2D ^{1}H NMR spectroscopy including DQF-COSY,[29] and TOCSY.[30] The ^{1}H chemical shifts were assigned to individual rings based on straightforward analysis of characteristic regions of the 1D proton spectrum (Fig. 1), namely the methyl groups and the anomeric proton region.[31,32] ^{13}C resonances were further assigned (Table 2) from ^{1}H–^{13}C heteronuclear NMR experiments such as gHSQC[33] and gHSQC-TOCSY.[33,34] Quaternary carbons as well as

Table 2. ^{13}C NMR data for EBCDA-OMe (**1**)

Residue	C-1	C-2	C-3	C-4	C-5	C-6	$^{1}J_{C\text{-}1,H\text{-}1}$[a]
α-L-Rha*p* (A)[b]	102.5 (−0.1)	81.0 (−0.2)	72.6 (−0.3)	74.9 (−0.4)	71.2 (−0.9)	19.1 (−0.1)	172.5
α-L-Rha*p* (B)	104.6 (1.3)	69.5 (−7.8)	78.2 (1.3)	73.0 (−0.4)	71.8 (−0.1)	19.3 (−0.3)	171.7
α-L-Rha*p* (C)	103.9 (0.1)	73.2 (−0.2)	80.7 (−0.6)	74.0 (−0.4)	71.6 (−0.1)	19.1 (−0.3)	169.6
β-D-GlcNAc*p* (D)[c]	104.7 (0.1)	58.1 (−0.3)	84.1 (−0.1)	71.1 (−0.4)	78.4 (−0.3)	63.3 (−0.5)	163.4
α-D-Glc*p* (E)	98.3 (0.6)	74.0 (−0.1)	75.6 (−0.3)	72.1 (−0.5)	74.4 (−0.2)	63.0 (−0.5)	169.6

Chemicals shifts measured in ppm with an accuracy of ±0.2 ppm are referenced to external DSS (δ_C 0.00). The numbers in parentheses represent the difference in ppm between the chemical shifts of the protons in **1** and the chemical shifts of the respective protons in the O-SP of *S. flexneri* 5a.[38]

[a] Anomeric $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants is measured in Hz with a digital resolution of 0.5 Hz.

[b] The chemical shift of the aglyconic methyl is 57.4 ppm.

[c] The chemical shifts of the carboxyl and *N*-acetyl groups are 177.3 and 24.9 ppm, respectively.

Table 3. ^3J coupling constants measured for EBCDA-OMe (**1**) (n.d.: not determined)

Residue	$^3J_{1,2}$	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$	$^3J_{5,6a}$	$^3J_{5,6b}$	$^3J_{6a,6b}$
α-L-Rha*p* (A)	1.3[a]	3.6[a]	9.7[a]	9.7[a]	6.3[a]	–	–
α-L-Rha*p* (B)	1.4[a]	3.2[a]	9.7[a]	n.d.	6.3[a]	–	–
α-L-Rha*p* (C)	1.3[a]	3.2[b]	9.7[b]	9.7[b]	6.3[a]	–	–
β-D-GlcNAc*p* (D)	8.5[a]	9.5[b]	9.7[b]	9.7[b]	n.d.	6.4[b]	12.3[b]
α-D-Glc*p* (E)	3.7[a]	9.7[b]	9.6[b]	9.2[b]	n.d.	n.d.	12.4[b]

[a] Directly measured on the 1D ^{1}H NMR spectrum (Hz±0.1 Hz).
[b] Measured on the DQF-COSY spectrum (Hz±0.5 Hz).

glycosidic linkages H–C–O–C were then obtained from gHMBC.[33] The glycosidic linkages were confirmed from ^{1}H–^{1}H dipolar interactions observed in the off-resonance ROESY experiment.[35] The vicinal coupling constants (Table 3) of the ring protons in the monosaccharide units within the pentasaccharide were found to be consistent with a $^1C_4$ conformation for the L-rhamnopyranosyl rings and a $^4C_1$ conformation for the D-glucopyranosyl and the *N*-acetyl-D-glucosaminyl units. The α and β configurations were corroborated by heteronuclear one-bond $^1J_{C\text{-}1,H\text{-}1}$ coupling constants (Table 2) obtained from the gHMBC experiment.[36] Data in Tables 1 and 2 show that the ^{1}H and ^{13}C chemical shifts of units A, B, C, D, and E in the synthetic **1** are very close to those of the corresponding residues in the native *O*-SP,[37,38] although major differences were observed for residues A and B. In the case of the former, the observed divergences are likely due to the presence of the anomeric *O*-methyl group and to the external position of this residue in the pentasaccharide. The substitution pattern may account for the differences in the chemical shifts associated with residue B. Indeed, B is monosubstituted in **1** whereas it is disubstituted at positions 2 and 3 in the *O*-SP. However, the large chemical shift difference observed for $C\text{-}2_B$ can most likely be attributed to a direct influence of the upstream residue D present in the *O*-SP but absent in the linear **1**. This phenomenon has been

Table 4. Inter-residue ^{1}H–^{1}H distances for EBCDA-OMe (**1**)

Atom pairs	Distance[a] (Å) from ROE intensity	Distance[a] (Å) from minimized structure with constraints
D-Ac/A-6[b]	6.13	6.50
E-1/B-2	2.46	2.44
E-1/B-3	2.51	2.66
E-5/B-3	3.06	3.00
E-5/B-4	3.74	3.82
B-1/C-2	3.44	3.18
B-1/C-3	2.24	2.15
B-1/D-Ac	5.05	5.00
B-2/C-3	3.74	3.84
B-6/C-2	4.41	3.88
B-6/D-Ac	5.48	4.93
C-1/D-3	2.27	2.32
C-1/D-Ac	4.53	4.35
C-2/D-Ac	4.66	3.46
D-1/A-1	3.26	3.55
D-1/A-2	2.27	2.30
D-2/A-1	4.73	4.05
D-5/A-2	3.18	3.53
D-Ac/A-1	5.80	6.01
D-Ac/A-4	4.57	4.68

[a] The errors on distance values are ~10%.
[b] Labeling: D-Ac, *N*-acetyl of residue D; A-6, H-6 of residue A.

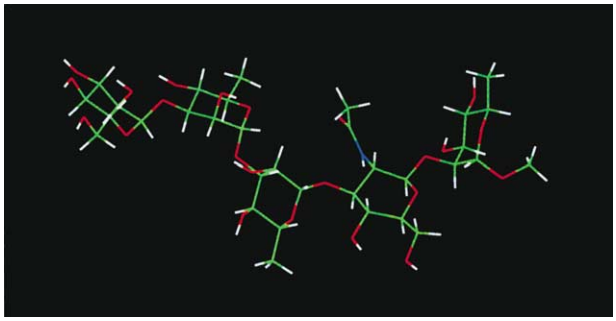


Figure 2. The structure of EBCDA-OMe (**1**) obtained by energy minimization with ROE-derived distances constraints. The methyl aglycone is oriented towards the lower right.

observed earlier for other oligo- and polysaccharides containing a 1→2 linkage.[39,40] Interestingly, the chemical shifts corresponding to residue E which is at the non-reducing end of the linear **1** match with the corresponding resonances in the *O*-SP, although E is a branched residue in the latter. Residue A is involved in a 2,3-*cis* vicinal glycosylation pattern, but it does not appear to interfere with the conformational behavior of residue E. Comparison with data corresponding to the branched synthetic trisaccharide A(E)B-OMe[41] (Clément, unpublished data), shows that there is no direct influence of the downstream units C or D on the ^{1}H and ^{13}C signals of residue E. Consequently, it is most probable that residue E is well-exposed to the solvent in the *O*-SP.

The solution conformation of **1** was then investigated by examination of NOEs determined from the off-resonance ROESY experiment. Thus, based on the ROE-derived distances (Table 4), energy minimization using DISCOVER resulted in an overview of the average structure of **1** (Fig. 2). Due to the high flexibility of such oligosaccharides, this structure is provided as a representation that agrees with NMR data, and which could be used as a good starting point for further studies. Indeed, the only difference between calculated and measured distances involves either long distances or distances implying methyl groups.

Taken as a whole, all the NMR results described herein suggest a similar distribution of conformations in solution for **1** and for the *O*-SP, which makes the former a good candidate for studying the recognition pattern of monoclonal antibodies raised against the *O*-SP of *S. flexneri* serotype 5a.

3. Experimental

3.1. NMR experiments for compound 1

The solution concentration was 28 mM (14 mg in 0.6 mL of D_2O). All NMR spectra were recorded at 38°C on a Varian Unity 500 spectrometer operating at a proton frequency of 500 MHz and a ^{13}C frequency of 125 MHz and equipped with a *z*-gradient triple resonance (^{1}H, ^{15}N, ^{13}C) probe. Chemical shifts in ppm are referenced to external sodium 4,4-dimethyl-4-silapentane-1 sulfonate (DSS) (δ 0). DQF-COSY, TOCSY and off-resonance ROESY spectra were

recorded using a spectral width of 2.2 kHz in both dimensions, a 90° pulse of 4.3 μs, 2K ($F1$)×2K ($F2$) points data sets, zero-filled to 4K in $F1$ dimension and using 16, 8 and 32 scans per increment, respectively. The TOCSY experiment was acquired using a mixing time of 80 ms. The off-resonance ROESY experiment was recorded with a 11.6 kHz effective spin lock field generated by a series of 30° pulses during 400 ms. To avoid Hartmann–Hahn artifacts, the offset of the spin lock carrier was shifted by approximately 8 kHz from the center of the spectrum in order to create an angle of 54.7° between the effective spin lock axis and the static magnetic field. These ^{1}H NMR experiments were processed using shifted sine-bells windows in both dimensions. Gradient-enhanced HSQC, gHSQC-TOCSY and gHMBC experiments were performed with spectral width of 2.2 and 24 kHz in the ^{1}H and ^{13}C dimensions, respectively, with ^{1}H and ^{13}C 90° pulse width of 4.3 and 15.9 μs, respectively, 16 scans per increment and a recycle delay of 1.5 s. Zero-filling was set to 4K in $F1$ dimension and 2K in $F2$ dimension. For gHSQC experiment, a delay corresponding to a $^{1}H–^{13}C$ one-bond coupling constant value of 150 Hz was used. The gHSQC-TOCSY experiment was recorded with a mixing time of 150 ms. The gHMBC experiment was performed with a delay of 60 ms for evolution of long-range couplings. For these heteronuclear experiments, shifted Gaussian windows were applied in both dimensions. All 2D data, except gHMBC were collected in the phase sensitive mode using the States–Haberkorn method.[42] The $^{3}J_{H,H}$ coupling constants were obtained from 1D spectrum with a digital resolution of 0.1 Hz/point, or from the DQF-COSY experiment with a digital resolution of 0.5 Hz/point. The $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants were measured from the gHMBC spectrum with a digital resolution of 0.5 Hz/point.

Inter-proton distances from cross-peak volumes. The cross-peak volumes from off-resonance ROESY were measured with the VNMR software. The intra-residue distance of 2.52 Å between H-1 and H-2 protons of the α-rhamnose unit B was used as the reference for distance calibration. The proton–proton distances were calculated using the usual $1/r^{6}$ NOE/distance relationship.[43]

3.2. Energy minimization

Energy minimization was performed on Silicon Graphics Octane workstation running under the IRIX 6.5 operating system using MSI INSIGHT II/DISCOVER software package. The computations were performed using AMBER forcefield with Homans extensions for the anomeric atoms,[44] with a distance-dependent dielectric constant of 4.0 and 5000 steps of energy minimization. Twenty inter-residual distance constraints were used.

3.3. 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) toluene–acetone, (D) toluene–ethyl acetate, (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 aq. H_2SO_4 (4N). Preparative chromatography was performed by elution from columns of Silica Gel 60 (particle size 0.040–0.063 mm). For all compounds excepts the target **1**, the NMR spectra were recorded at 25°C for solutions in $CDCl_3$, on a Bruker AC 300P spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C). External references: for solutions in $CDCl_3$, TMS (0.00 ppm for both ^{1}H 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 ^{1}H). Proton-signal assignments were made by first-order analysis of the spectra, as well as analysis of 2D $^{1}H–^{1}H$ 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 2D $^{13}C–^{1}H$ 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 chemical ionization mass spectra (CIMS) were obtained using NH_3 as the ionizing gas. 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. Before use, AgOTf was dried at 133 Pa/50°C for 2 h. 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.

3.3.1. Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-4-*O*-benzyl-α-L-rhamnopyranoside 12 and allyl (2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-4-*O*-benzyl-α-L-rhamnopyranoside 13. Allyl 4-*O*-benzyl-α-L-rhamnopyranoside[16] (**5**, 1.47 g, 5.0 mmol) was dissolved in MeCN (2.5 mL) containing methyl orthoacetate (1.85 mL, 14.5 mmol). *p*-Toluenesulphonic acid (13 mg) was added, the mixture was stirred for 1.5 h at rt TLC (solvent D, 9:1) showed that no starting material remained. The reaction mixture was cooled to 0°C, and 80% aq. AcOH (2.5 mL) was added. After 30 min at rt, TLC (solvent D, 9:1) showed that the intermediate orthoester had turned into a more polar product. CH_2Cl_2 was added, and the organic phase was washed with ice-water and satd aq. NaCl, dried and evaporated to dryness to give a yellow syrup. ^{1}H NMR showed that the product **9** was identical to that described[20] and that it was only slightly contaminated by the regiosiomer acetylated at position 3 (2-OAc/3-OAc, 97:3).

TMSOTf (45 μL, 0.23 mmol) was added to a solution of the crude **9** and donor[13,14] **4** (4.5 g, 6.57 mmol) in Et_2O (50 mL), at −78°C. The reaction mixture was stirred overnight while slowly coming back to rt. TLC (solvent B, 3:1) showed the complete disappearance of **5**. Et_3N (1 mL) was added and

volatiles were evaporated. Chromatography of the residue (solvent E, 24:1) gave the condensation products as a contaminated syrupy mixture of α- (**10**)[21] and β- (**11**) isomers (5.82 g). The syrup was dissolved in CH_2Cl_2 and MeOH (8:11) to which 1 M MeONa (5 mL) was added, and the mixture was kept for 5 days at rt under N_2. The mixture was neutralized by addition of resin IR-120 (H^+), and filtered. Evaporation of the filtrate gave a syrup which was purified by chromatography (solvent D, 47:3) to give **12** (2.31 g, 57%) as a colorless oil, and **13** (1.19 g) contaminated by a 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose derivative as the second eluting product. Compound **12** had $[\alpha]_D$=+32° (*c* 1.0); NMR: ^{1}H, δ 7.57–7.13 (m, 25H, Ph), 5.92 (m, 1H, C*H*=CH$_2$), 5.30 (m, 1H, CH=C*H*$_2$), 5.22 (m, 1H, CH=C*H*$_2$), 4.94 (m, 4H, H-1$_E$, 1$_B$, OCH$_2$), 4.87–4.48 (m, 7H, OCH$_2$), 4.31 (d, 1H, *J*=12.0 Hz, OCH$_2$), 4.19 (m, 1H, OCH$_2$), 4.11–3.94 (m, 5H, H-3$_E$, 3$_B$, 5$_E$, 2$_B$, OCH$_2$), 3.80 (m, 1H, $J_{4,5}$=9.6 Hz, H-5$_B$), 3.75 (dd, 1H, $J_{3,4}$=9.2 Hz, $J_{4,5}$=9.8 Hz, H-4$_E$), 3.62 (dd, 1H, $J_{1,2}$=3.6 Hz, $J_{2,3}$=9.6 Hz, H-2$_E$), 3.52 (t, 1H, $J_{3,4}$=9.4 Hz, H-4$_B$), 3.48 (dd, 1H, $J_{5,6a}$=2.3 Hz, H-6a$_E$), 3.44 (bs, 1H, OH-2), 3.36 (dd, 1H, $J_{6a,6b}$=10.6 Hz, H-6b$_E$), and 1.40 (d, 3H, $J_{5,6}$=6.2 Hz, H-6$_B$); ^{13}C, δ 134.0–127.7 (Ph, *C*H=CH$_2$), 117.5 (*C*H=CH$_2$), 98.2 (C-1$_B$, $J_{C,H}$=170 Hz), 93.9 (C-1$_E$, $J_{C,H}$=168 Hz), 82.4 (C-3$_E$), 79.3 (C-4$_B$), 78.9 (C-2$_E$), 77.7 (C-4$_E$), 76.6 (C-3$_B$), 75.6, 74.9, 74.3, 73.4 (5C, OCH$_2$), 70.7 (C-5$_E$), 67.8 (2C, C-6$_E$, OCH$_2$), 67.4 (C-2$_B$), 67.2 (C-5$_B$), and 18.0 (C-6$_B$). FABMS for $C_{50}H_{56}O_{10}$ (M, 816.39) *m/z* 839.5 ([M+Na]$^+$). Anal. Calcd for $C_{50}H_{56}O_{10}$: C, 73.51; H, 6.91%. Found: C, 73.42; H, 7.04%.

Analytical data for compound **13** were $[\alpha]_D$=−12° (*c* 1.0); NMR: ^{1}H, δ 7.35–7.13 (m, 25H, Ph), 5.90 (m, 1H, C*H*=CH$_2$), 5.33–5.18 (m, 2H, CH=C*H*$_2$), 4.99–4.47 (m, 12H, H-1$_E$, 1$_B$, OCH$_2$), 4.18 (m, 1H, OCH$_2$), 4.13 (m, 2H, H-3$_B$, 2$_B$), 3.98 (m, 1H, OCH$_2$), 3.80 (dq, 1H, $J_{4,5}$=9.4 Hz, H-5$_B$), 3.74–3.55 (m, 6H, H-6a$_E$, 3$_E$, 6b$_E$, 4$_E$, 4$_B$, 2$_E$), 3.53 (m, 1H, H-5$_E$), 3.25 (bs, 1H, OH), and 1.34 (d, 3H, $J_{5,6}$=6.2 Hz, H-6$_B$); ^{13}C, δ 138.4–127.5 (Ph, CH=CH$_2$), 117.5 (CH=CH$_2$), 102.6 (C-1$_E$, $J_{C,H}$=159 Hz), 98.6 (C-1$_B$, $J_{C,H}$=169 Hz), 84.8 (C-3$_E$), 82.0 (C-2$_E$), 80.9 (C-3$_B$), 80.0 (C-4$_B^*$), 77.7 (C-4$_E^*$), 75.6, 75.0, 74.7 (4C, OCH$_2$), 74.4 (C-5$_E$), 73.6 (OCH$_2$), 69.9 (C-2$_B$), 68.8 (C-6$_E$), 67.4 (OCH$_2$), 52.6 (C-5$_B$), and 18.0 (C-6$_B$). FABMS for $C_{50}H_{56}O_{10}$ (M, 816.39) *m/z* 839.4 ([M+Na]$^+$). Anal. Calcd for $C_{50}H_{56}O_{10}$: C, 73.51; H, 6.91%. Found: C, 73.41; H, 6.89%.

3.3.2. Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside 14. A solution of disaccharide **12** (1.85 g, 2.27 mmol) in anhydrous pyridine (5 mL) containing benzoyl chloride (390 μL, 3.44 mmol) was stirred overnight at 70°C. TLC (solvent D, 23:2) indicated that the reaction was essentially quantitative. Methanol (5 mL) was added to the cooled mixture, and the solution was stirred at rt for a further time of 1 h. Volatiles were evaporated and the residue was taken up in CH_2Cl_2. The organic phase was washed successively with 5% aq. HCl, water, and satd aq NaCl. Concentration of the organic phase followed by chromatography of the residue (solvent B, 9:1) gave pure **14** (1.86 g, 89%) as a colorless oil, together with a slightly contaminated fraction (180 mg, 8.6%) which could be used as such in the next step (the estimated yield of pure **14** is 95%). Compound **14** had $[\alpha]_D$=+43° (*c* 1.0); NMR: ^{1}H, δ 8.08–7.05 (m, 30H, Ph), 5.89 (m, 1H, C*H*=CH$_2$), 5.63 (dd, 1H, $J_{1,2}$=2.3 Hz, $J_{2,3}$=2.5 Hz, H-2$_B$), 5.29 (m, 1H, CH=C*H*$_2$), 5.25 (d, 1H, $J_{1,2}$=3.6 Hz, H-1$_E$), 5.19 (m, 1H, CH=C*H*$_2$), 4.97 (d, 1H, *J*=10.2 Hz, OCH$_2$), 4.93 (d, 1H, $J_{1,2}$=1.5 Hz, H-1$_B$), 4.86–4.35 (m, 9H, OCH$_2$), 4.36 (dd, 1H, H-3$_B$), 4.16 (m, 1H, OCH$_2$), 4.06–4.00 (m, 3H, H-3$_E$, 5$_E$, OCH$_2$), 3.87 (dq, 1H, $J_{4,5}$=9.4 Hz, H-5$_B$), 3.70 (m, 2H, H-4$_B$, 4$_E$), 3.64–3.52 (m, 3H, H-6a$_E$, 2$_E$, 6b$_E$), and 1.43 (d, 3H, $J_{5,6}$=6.2 Hz, H-6$_B$); ^{13}C, δ 166.1 (C=O), 138.8–127.5 (Ph, *C*H=CH$_2$), 117.5 (CH=*C*H$_2$), 96.6 (C-1$_B$), 92.7 (C-1$_E$), 82.0 (C-3$_E$), 79.9 (C-4$_E^*$), 79.0 (C-2$_E$), 77.5 (C-4$_B^*$), 76.2, 75.4, 74.9, 73.3, (4C, OCH$_2$), 72.6 (C-3$_B$), 72.2 (OCH$_2$), 70.2 (C-5$_E$), 68.6 (C-2$_B$), 68.1 (2C, C-6$_E$, OCH$_2$), 68.0 (C-5$_B$), and 18.0 (C-6$_B$); CIMS for $C_{57}H_{60}O_{11}$ (M, 920.4) *m/z* 943.4 ([M+Na]$^+$). Anal. Calcd for $C_{57}H_{60}O_{11}$: C, 74.33; H, 6.57%. Found: C, 74.18; H, 6.71%.

3.3.3. (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α/β-L-rhamnopyranose 15. A solution of 1,5-cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (150 mg, 0.18 mmol, Ir(COD){PCH$_3$(C$_6$H$_5$)$_2$}$^-$PF$_6^-$) in anhydrous THF (20 mL) was degassed, and the catalyst was activated by passing a stream of hydrogen until the solution had turned yellow (ca. 3 min). The solution was degassed again, and a degassed solution of compound **14** (21.0 g, 22.8 mmol) in anhydrous THF (100 mL) was added. The reaction was stirred overnight under Ar. TLC (solvent D, 23:2) showed that only little starting material remained, and the mixture was concentrated to dryness. Mercuric oxide (9.0 g, 41.9 mmol) and mercuric chloride (10 g, 37.2 mmol) were added to a solution of the residue in a mixture of acetone and water (1.5 L, 9:1). The suspension, protected from light, was stirred at rt for 1.5 h, and acetone was evaporated. The resulting suspension was taken up in CH_2Cl_2, washed twice with 50% KI, water and satd aq NaCl, dried and concentrated. Purification of the crude material was effected by silica gel chromatography (solvent B, 4:1) to furnish the hemiacetal **15** (17.2 g, 86%) as a mixture of α and β anomers. NMR (α-anomer): ^{1}H, δ 8.08–7.07 (m, 30H, Ph), 5.65 (dd, 1H, $J_{1,2}$=$J_{2,3}$=2.6 Hz, H-2$_B$), 5.29 (bs, 1H, H-1$_B$), 5.28 (d, 1H, $J_{1,2}$=3.6 Hz, H-1$_E$), 4.97 (d, 1H, *J*=10.2 Hz, OCH$_2$), 4.86–4.35 (m, 10H, H-3$_B$, OCH$_2$), 4.06–4.00 (m, 3H, H-3$_E$, 5$_E$, 5$_B$), 3.75–3.65 (m, 2H, H-4$_B$, 4$_E$), 3.64–3.52 (m, 3H, H-6a$_B$, 2$_B$, 6b$_B$), 3.18 (bs, 1H, OH-1$_B$), and 1.41 (d, 3H, $J_{5,6}$=6.2 Hz, H-6$_B$); ^{13}C, δ 166.1 (C=O), 138.7–127.3 (Ph), 92.5 (C-1$_B^*$), 92.2 (C-1$_E^*$), 82.0 (C-3$_E$), 79.8 (C-4$_B$), 79.0 (C-2$_E$), 77.6 (C-4$_E$), 76.1, 75.4, 74.9, 73.2, 72.2 (5C, OCH$_2$), 72.0 (C-3$_B$), 70.2 (C-5$_E$), 68.8 (C-2$_B$), 68.3 (C-6$_E$), 68.0 (C-5$_B$), and 18.2 (C-6$_B$); CIMS for $C_{54}H_{56}O_{11}$ (M, 880.4) *m/z* 903.4 ([M+Na]$^+$). Anal. Calcd for $C_{54}H_{56}O_{11}$·0.5H$_2$O: C, 72.88; H, 6.46%. Found: C, 72.82; H, 6.60%.

3.3.4. (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α/β-L-rhamnopyranosyl trichloroacetimidate 2. Trichloroacetonitrile (1.2 mL, 12.0 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 18 μL) were added to a solution of the hemiacetal **15** (1.06 g, 1.20 mmol) in anhydrous CH_2Cl_2 (600 μL). The solution was kept for 1 h at rt, at which time TLC (solvent B containing Et$_3$N 2‰, 3:1) showed that the reaction was

over. Volatiles were evaporated and the residue was flash-chromatographed (solvent B containing Et_3N 5‰) to give **2** (1.17 g, 95%) as a sticky foam. Compound **2** was isolated as a mixture of α and β anomers. NMR (α anomer): ^{1}H, δ 8.71 (s, 1H, NH), 8.09–7.06 (m, 30H, Ph), 6.35 (d, 1H, $J_{1,2}$=1.7 Hz, H-1_B), 5.82 (dd, 1H, $J_{2,3}$=2.5 Hz, H-2_B), 5.20 (d, 1H, H-1_E), 5.01–4.36 (m, 10H, OCH_2), 4.93 (dd, 1H, $J_{3,4}$=9.6 Hz, H-3_B), 4.06–4.00 (m, 3H, H-5_B, 3_E, 5_E), 3.79 (pt, 1H, $J_{4,5}$=9.6 Hz, H-4_B), 3.74 (m, 1H, $J_{3,4}$=9.4 Hz, H-4_E), 3.66 (dd, 1H, $J_{5,6}$=2.6 Hz, $J_{6a,6b}$=10.9 Hz, H-$6a_E$), 3.60 (dd, 1H, $J_{1,2}$=3.3 Hz, $J_{2,3}$=9.6 Hz, H-2_E), 3.48 (d, 1H, H-$6b_E$), and 1.45 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_B); ^{13}C, δ 165.7 (C=O), 160.2 (C=NH), 138.6–127.4 (Ph), 95.0 (C-1_B), 92.9 (C-1_E), 90.8 (CCl_3), 82.0 (C-3_E), 79.1 (C-4_B), 78.8 (C-2_E), 77.4 (C-4_E), 76.4, 75.5, 75.0, 73.4, (4C, OCH_2), 72.3 (C-3_B), 72.2 (OCH_2), 70.9 (C-5_B), 70.4 (C-5_E), 68.1 (C-6_E), 66.9 (C-2_B), and 18.2 (C-6_B). Anal. Calcd for $C_{56}H_{56}Cl_3NO_{11}$: C, 65.59; H, 5.50; N, 1.37%. Found: C, 65.49; H, 5.68; N, 1.28%.

3.3.5. Methyl (3,4,6-tri-*O*-acetyl-2-chloroacetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 17 and methyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 18. Compound **16**[10] (4.86 g, 6.15 mmol) in a mixture of toluene (175 mL) and *N*,*N*-dimethylacetamide (60 mL) was stirred for 20 min under a flow of dry Ar. Tributyltin hydride (7.3 mL, 27.1 mmol) and α,α′-azobisisobutyronitrile (AIBN, 196 mg)) were added and the reaction mixture was heated at 100°C for 45 min under a flow of Ar. TLC (solvent C, 4:1; solvent B, 1:1) showed that no starting material remained and that one more major polar product had been formed. The reaction mixture was concentrated into an oily residue which was triturated in petroleum ether to give a white solid. Chromatography of the latter (solvent C, 22:3) gave the slightly contaminated chloroacetamide **17** (2.06 g, 41% yield from the ^{1}H NMR spectrum) as the first eluting product and the target **18** as the second eluting product (1.95 g, 30% from the ^{1}H NMR spectrum).

An analytical sample of **17**, isolated as a white solid, had $[\alpha]_D$=0° (*c* 1.0); NMR: ^{1}H, δ 7.39–7.29 (m, 10H, Ph), 6.55 (d, 1H, $J_{NH,2}$=8.2 Hz, NH), 5.06 (dd, 1H, $J_{2,3}$=10.4 Hz, $J_{3,4}$=9.4 Hz, H-3_D), 5.05 (t, 1H, $J_{4,5}$=9.7 Hz, H-4_D), 4.87 (d, 1H, *J*=10.8 Hz, OCH_2), 4.72 (d, 1H, OCH_2), 4.71 (d, 1H, H-1_D), 4.69 (bs, 1H, H-1_A), 4.61 (d, 1H, OCH_2), 4.60 (d, 1H, OCH_2), 4.23 (dd, 1H, $J_{5,6a}$=4.7 Hz, $J_{6a,6b}$=12.2 Hz, H-$6a_D$), 4.12 (dd, 1H, $J_{5,6b}$=2.6 Hz, H-$6b_D$), 3.95–3.82 (m, 3H, H-2_D, 2_A, 3_A), 3.83 (d, 1H, CH_2Cl), 3.76 (d, 1H, *J*=13.0 Hz, CH_2Cl), 3.65 (dq, 1H, H-5_A), 3.59 (m, 1H, H-5_D), 3.37 (t, 1H, $J_{3,4}$=$J_{4,5}$=9.3 Hz, H-4_A), 3.32 (s, 3H, OCH_3), 2.08, 2.03, 2.02 (3s, 9H, C(O)CH_3), and 1.31 (d, 3H, $J_{5,6}$=6.3 Hz, H-6_A); ^{13}C, δ 170.6, 170.5, 169.4, 169.2 (4C, C=O), 138.4–127.7 (Ph), 101.8 (C-1_D), 99.9 (C-1_A), 80.7 (C-4_A), 80.0 (C-3_A), 77.3 (C-2_A), 75.4, 73.3 (2C, OCH_2), 72.1 (C-3_D), 71.8 (C-5_D), 68.5 (C-4_D), 67.7 (C-5_A), 62.0 (C-6_D), 55.1 (C-2_D), 54.6 (OCH_3), 42.4 (CH_2Cl), 20.8, 20.7, 20.6 (3C, C(O)CH_3), and 17.9 (C-6_A).

An analytical sample of **18** had $[\alpha]_D$=−3° (*c* 1.0); NMR: ^{1}H, δ 7.42–7.35 (m, 10H, Ph), 5.48 (d, 1H, $J_{NH,2}$=8.6 Hz, NH), 5.06 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.6 Hz, H-4_D), 4.97 (pt, 1H, $J_{2,3}$=9.6 Hz, H-3_D), 4.82 (d, 1H, *J*=10.6 Hz, OCH_2), 4.75 (d, 1H, *J*=10.7 Hz, OCH_2), 4.71 (bs, 1H, H-1_A), 4.61 (d, 2H, OCH_2), 4.53 (d, 1H, $J_{1,2}$=8.5 Hz, H-1_D), 4.22 (dd, 1H, $J_{5,6a}$=4.4 Hz, $J_{6a,6b}$=12.2 Hz, H-$6a_D$), 4.13 (bd, 1H, H-$6b_D$), 4.01 (bq, 1H, H-2_D), 3.90 (bs, 1H, H-2_A), 3.86 (bd, 1H, $J_{3,4}$=9.6 Hz, H-3_A), 3.63 (m, 2H, H-5_D, 5_A), 3.37 (pt, 1H, $J_{4,5}$=9.7 Hz, H-4_A), 3.33 (s, 3H, OCH_3), 2.08, 2.03, 2.00 (3s, 9H, C(O)CH_3), 1.62 (s, 3H, NC(O)CH_3), and 1.32 (d, 3H, $J_{5,6}$=5.9 Hz, H-6_A); ^{13}C, δ 170.8, 170.7, 169.9, 169.3 (4C, C=O), 138.3–127.7 (Ph), 103.1 (C-1_D), 99.8 (C-1_A), 80.9 (C-4_A), 80.1 (C-3_A), 78.1 (C-2_A), 75.5, 73.6 (2C, OCH_2), 73.3 (C-3_D), 72.0 (C-5_D), 68.3 (C-4_D), 67.6 (C-5_A), 62.0 (C-6_D), 54.6 (OCH_3), 54.1 (C-2_D), 23.1, 20.8, 20.7, 20.6 (4C, C(O)CH_3), and 17.9 (C-6_A); ESMS for $C_{35}H_{45}NO_{13}$ (M, 687.29) *m*/*z* 688.3 ($[M+H]^+$). Anal. Calcd for $C_{35}H_{45}NO_{13}\cdot H_2O$: C, 60.34; H, 6.51; N, 2.01%. Found: C, 60.49; H, 6.71; N, 1.94%.

3.3.6. Methyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 20. AgOTf (16.0 g, 62.2 mmol) was added in one portion to a solution of donor **7** (28.1 g, 44.2 mmol), alcohol **6** (11.47 g, 32.0 mmol) and 2,6-di-*tert*-butyl-4-methyl pyridine (11.04 g, 53.8 mmol) in CH_2Cl_2 (600 mL) stirred at −50°C. Stirring was continued overnight, at which time the bath temperature had reached 20°C. TLC (solvent D, 17:3) showed the complete disappearance of **6**. The mixture was filtered through a bed of Celite. The filtrate was washed with a 1:1 mixture of 5% aq. $NaHCO_3$ and 5% aq. $Na_2S_2O_3$, then water and satd aq. NaCl. The organic phase was dried and concentrated. The crude product was chromatographed (solvent D, 9:1) to give pure disaccharide **20** (12.17 g, 89%) as a crystalline material. Compound **20** had, mp 154–155°C (from AcOEt); $[\alpha]_D$=+6° (*c* 1.0); NMR: ^{1}H, δ 7.61–7.10 (m, 10H, Ph), 5.97 (dd, 1H, $J_{2,3}$=9.1 Hz, $J_{3,4}$=10.5 Hz, H-3_D), 5.28 (d, 1H, $J_{1,2}$=8.4 Hz, H-1_D), 5.17 (dd, 1H, $J_{4,5}$=9.4 Hz, H-4_D), 4.68 (bs, 1H, H-1_A), 4.44 (dd, 1H, H-2_D), 4.40–4.21 (m, 5H, OCH_2, H-$6a_D$), 4.15 (dd, 1H, $J_{5,6}$=2.2 Hz, $J_{6a,6b}$=12.1 Hz, H-$6b_D$), 3.83 (m, 1H, H-5_D), 3.64 (bs, 1H, H-2_A), 3.61 (dd, 1H, $J_{2,3}$=2.9 Hz, H-3_A), 3.54 (dq, 1H, $J_{4,5}$=9.4 Hz, H-5_A), 3.26 (s, 3H, OCH_3), 3.11 (m, 1H, $J_{3,4}$=9.4 Hz, H-4_A), 2.11, 2.04, 1.93 (3s, 9H, C(O)CH_3), and 1.25 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A); ^{13}C, δ 170.7, 170.5, 169.5 (C=O), 138.0–127.2 (Ph), 99.9 (C-1_D), 99.6 (C-1_A), 80.6 (C-4_A), 78.8 (2C, C-3_A, 2_A), 75.1, 72.4 (2C, OCH_2), 71.5 (C-5_D), 70.0 (C-3_D), 68.8 (C-4_D), 67.7 (C-5_A), 61.9 (C-6_D), 55.4 (C-2_D), 54.6 (OCH_3), 20.8, 20.7 (3C, C(O)CH_3), and 17.8 (C-6_A). FABMS for $C_{41}H_{41}Cl_4NO_{14}$ (M, 913.57) *m*/*z* 936.2 ($[M+Na]^+$). Anal. Calcd for $C_{41}H_{41}Cl_4NO_{14}$: C, 53.90; H, 4.52; N, 1.53%. Found: C, 53.75; H, 4.56; N, 1.46%.

3.3.7. Methyl (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 21. (a) Ethylenediamine (8.77 mL, 131 mmol) was added to a suspension of the fully protected disaccharide **20** (12.0 g, 13.1 mmol) in absolute ethanol (300 mL), and the reaction mixture was heated at 70°C for 9 h. TLC (solvent A, 9:1) showed that no starting material remained and that two more polar products had been formed. The reaction mixture was cooled to 0°C, acetic anhydride (50 mL, 503 mmol) was slowly added, and stirring went on for 2 h at rt. TLC (solvent A, 9:1) showed that the more polar compound had been

fully transformed into the less polar one. Volatiles were eliminated by repeated coevaporations with cyclohexane. The residue was refluxed in EtOAc and filtered on a pad of Celite. More salts crystallized out of an EtOAc/MeOH solution. Chromatography of the mother liquor (solvent A, 9:1) gave the triol **21** (6.11 g, 83%); $[\alpha]_D=-13°$ (*c* 1.0); NMR: ^{1}H, δ 7.42–7.27 (m, 10H, Ph), 7.05 (d, 1H, $J_{2,NH}$= 2.7 Hz, NH), 4.84–4.65 (m, 4H, OCH_2), 4.73 (d, 1H, $J_{1,2}$=1.0 Hz, H-1_A), 4.42 (d, 1H, $J_{1,2}$=8.1 Hz, H-1_D), 3.96–3.86 (m, 3H, H-2_A, 3_A, $6a_D$), 3.82 (m, 1H, H-$6b_D$), 3.70 (dq, 1H, $J_{4,5}$=9.4 Hz, H-5_A), 3.60–3.51 (m, 2H, H-2_D, 5_D), 3.43–3.34 (m, 6H, H-4_A, 3_D, 4_D, OCH_3), 2.50 (t, 1H, $J_{OH,6}$=6.2 Hz, OH-6_D), 1.58 (s, 3H, C(O)CH_3), and 1.34 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A); ^{13}C, δ 173.5 (C=O), 138.0–127.4 (Ph), 103.2 (C-1_D), 99.7 (C-1_A), 81.2 (C-4_A), 80.4 (C-3_A^*), 78.8 (C-2_A), 77.1 (C-3_D^*), 75.6 (OCH_2), 75.4 (C-4_D^*), 74.4 (OCH_2), 71.4 (C-5_D), 67.4 (C-5_A), 62.3 (C-6_D), 58.5 (C-2_D), 54.6 (OCH_3), 22.3 (C(O)CH_3), and 17.7 (C-6_A). FABMS for $C_{29}H_{39}NO_{10}$ (M, 561.26) *m/z* 584.3 ([M+ Na]$^+$). Anal. Calcd for $C_{29}H_{39}NO_{10}\cdot1.5H_2O$: C, 59.17; H, 7.19; N, 2.38%. Found: C, 58.95; H, 7.08; N, 2.34%.

(b) 1N sodium methoxide (15 mL, 15 mmol) was added to a solution of the fully protected disaccharide **16** (273 mg, 345 μmol) in CH_2Cl_2 (1 mL), and the reaction mixture was stirred at rt for 17 h. TLC (solvent A, 9:1) showed that no starting material remained and that two more polar products had been formed. No further evolution was observed if stirring was pursued. Resin IR 120 (H^+) was added until the pH was 7–8. The resin was filtered and washed with methanol. Concentration of the filtrate, and chromatography of the residue (solvent A, 9:1) gave methyl (2-amino-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (**19**, 162 mg), which was dissolved in methanol (7 mL). Acetic anhydride (700 μL, 7.4 mmol) was slowly added, and stirring went on for 1.25 h at rt. TLC (solvent A, 9:1) showed that the aminotriol intermediate had been fully transformed into **21**. Volatiles were eliminated by repeated coevaporations with cyclohexane and toluene. Chromatography of the residue (solvent A, 19:1) gave the triol **21** (107 mg, 59%).

Compound **19** had NMR (CD_3OD): ^{1}H, δ 7.40–7.27 (m, 10H, Ph), 4.87 (d, 1H, OCH_2), 4.85 (d, 1H, $J_{1,2}$=1.7 Hz, H-1_A), 4.74 (d, 1H, *J*=11.6 Hz, OCH_2), 4.66 (d, 1H, *J*= 11.0 Hz, OCH_2), 4.63 (d, 1H, OCH_2), 4.49 (d, 1H, $J_{1,2}$= 8.0 Hz, H-1_D), 4.12 (dd, 1H, H-2_A), 3.86 (bd, 1H, $J_{6a,6b}$= 11.4 Hz, H-$6a_D$), 3.82 (dd, 1H, $J_{2,3}$=3.2 Hz, H-3_A), 3.67 (m, 1H, H-$6b_D$), 3.61 (dq, 1H, H-5_A), 3.51 (pt, 1H, $J_{3,4}$= $J_{4,5}$= 9.4 Hz, H-4_A), 3.35–3.26 (m, 6H, H-3_D, 4_D, 5_D, OCH_3), 2.63 (m, 1H, H-2_D), and 1.27 (d, 3H, $J_{5,6}$=6.1 Hz, H-6_A); ^{13}C, δ 139.8–128.6 (Ph), 105.6 (C-1_D), 101.5 (C-1_A), 81.3 (C-4_A), 80.5 (C-3_A), 78.1 (C-4_D), 77.7 (C-2_A), 77.2 (C-3_D), 76.1, 73.1 (2C, OCH_2), 71.5 (C-5_D), 68.8 (C-5_A), 62.5 (C-6_D), 58.7 (C-2_D), 55.2 (OCH_3), and 18.2 (C-6_A); ESMS for $C_{27}H_{37}NO_9$ (M, 519.60) *m/z* 520.3 ([M+H]$^-$).

3.3.8. Methyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 22. A suspension of the fully protected disaccharide **20** (12.0 g, 13.1 mmol) in absolute ethanol (300 mL) was treated with ethylenediamine (8.77 mL, 131 mmol) as described for the preparation of **21**. The residue was refluxed in EtOAc and filtered on a pad of Celite. 2,2-Dimethoxypropane (30 mL) and *p*-toluenesulfonic acid (200 mg) was added to a suspension of the resulting crude **21** in acetone (20 mL), and the mixture was stirred at rt overnight. TLC (solvent A, 23:2) showed that no starting material remained. Et_3N was added, and volatiles were evaporated. Chromatography of the residue (solvent A, 99:1) gave monohydroxylated **22** as a white foam (6.88 g, 87%); $[\alpha]_D=-27°$ (*c* 1.0); NMR: ^{1}H, δ 7.42–7.27 (m, 10H, Ph), 6.98 (d, 1H, $J_{2,NH}$=2.0 Hz, NH), 4.84–4.64 (m, 4H, OCH_2), 4.68 (bs, 1H, H-1_A), 4.42 (d, 1H, $J_{1,2}$=8.3 Hz, H-1_D), 3.96–3.91 (m, 3H, H-2_A, 3_A, $6a_D$), 3.80 (t, 1H, $J_{6a,6b}$=10.4 Hz, H-$6b_D$), 3.69 (dq, 1H, $J_{4,5}$=9.4 Hz, H-5_A), 3.64–3.50 (m, 3H, H-2_D, 3_D, 4_D), 3.38 (pt, 1H, $J_{3,4}$= 9.1 Hz, H-4_A), 3.35 (s, 3H, OCH_3), 3.23 (m, 1H, H-5_D), 1.57 (s, 3H, C(O)CH_3), 1.54, 1.47 (2s, 6H, C(CH_3)$_2$), and 1.34 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A); ^{13}C, δ 173.6 (C=O), 138.0–127.5 (Ph), 103.5 (C-1_D), 99.9 (C(CH_3)$_2$), 99.7 (C-1_A), 81.4 (C-4_A), 80.6 (C-3_A^*), 78.8 (C-2_A^*), 75.7, 74.7 (2C, OCH_2), 74.4 (C-3_D^*), 74.0 (C-4_D^*), 67.6 (C-5_D), 67.5 (C-5_A), 61.9 (C-6_D), 60.1 (C-2_D), 54.6 (OCH_3), 29.1 (C(CH_3)$_2$), 22.4 (C(O)CH_3), 19.0 (C(CH_3)$_2$), and 17.8 (C-6_A); ESMS for $C_{32}H_{43}NO_{10}$ (M, 601.29) *m/z* 602.4 ([M+H]$^+$). Anal. Calcd for $C_{32}H_{43}NO_{10}$: C, 63.88; H, 7.20; N, 2.33%. Found: C, 63.72; H, 7.35; N, 2.28%.

3.3.9. Methyl (4-*O*-benzyl-2,3-di-*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 23. TMSOTf (190 μL, 100 μmol) was added to a solution of disaccharide **22** (689 mg, 1.14 mmol) and donor **8** (880 mg, 1.62 mmol) in Et_2O (10 mL) at −78°C. The mixture was stirred overnight while the bath temperature slowly came back to rt. TLC (solvent C, 4:1) showed that no acceptor remained, Et_3N was added and volatiles were evaporated. Chromatography of the residue (solvent D, 22:3) provided the fully protected trisaccharide **23** (971 mg) as a slightly contaminated white foam. Available data for **23** are NMR: ^{1}H, δ 7.37–7.27 (m, 15H, Ph), 5.86 (d, 1H, $J_{NH,2}$=7.5 Hz, NH), 5.27 (dd, 1H, $J_{2,3}$= 3.3 Hz, $J_{3,4}$=9.8 Hz, H-3_C), 5.10 (dd, 1H, $J_{1,2}$=1.6 Hz, H-2_C), 4.87 (d, 1H, *J*=10.8 Hz, OCH_2), 4.85 (d, 1H, $J_{1,2}$= 8.4 Hz, H-1_D), 4.73–4.59 (m, 7H, H-1_A, 1_C, OCH_2), 4.11 (dq, 1H, $J_{4,5}$=9.7 Hz, H-5_C), 4.03 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.5 Hz, H-3_D), 3.92–3.73 (m, 4H, H-2_A, 3_A, $6a_D$, $6b_D$), 3.63 (dq, 1H, $J_{4,5}$=9.4 Hz, H-5_A), 3.59 (pt, 1H, $J_{4,5}$=9.4 Hz, H-4_D), 3.52–3.42 (m, 2H, H-4_C, 2_D), 3.39 (pt, 1H, H-4_A), 3.29 (m, 4H, H-5_D, OCH_3), 2.12, 1.97, 1.78 (3s, 9H, C(O)CH_3), 1.48, 1.40 (2s, 6H, C(CH_3)$_2$), 1.33 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A), and 1.28 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C); ^{13}C, δ 171.3, 170.1, 170.0 (3C, C(O)), 138.4–127.5 (Ph), 102.0 (C-1_D), 100.1 (C-1_A^*), 99.5 (C(CH_3)$_2$), 97.9 (C-1_C^*), 80.8 (C-4_A), 79.5 (C-3_A), 78.8 (C-4_C), 77.3 (C-3_D), 77.2 (C-2_A), 75.4, 74.7, 72.8 (3C, OCH_2), 72.7 (C-4_D), 71.3 (C-3_C), 71.0 (C-2_C), 67.6 (2C, C-5_A, 5_C), 67.3 (C-5_D), 62.2 (C-6_D), 58.0 (C-2_D), 54.5 (OCH_3), 29.2 (CCH_3), 23.4, 21.5, 20.9 (3C, C(O)CH_3), 19.3 (CCH_3), and 17.9 (2C, C-6_A, 6_C); CIMS for $C_{49}H_{63}NO_{16}$ (M, 921.41) *m/z* 922.0 ([M+H]$^-$).

3.3.10. Methyl (4-*O*-benzyl-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 24. A mixture of disaccharide **22** (1.45 g,

mmol) and donor[12] **8** (1.80 g, 2.41 mmol) in anhydrous Et_2O was treated with TMSOTf as described above for the preparation of **23**. The crude mixture was roughly purified by chromatography yielding contaminated **23** (2.19 g). 1N methanolic sodium methoxide was added dropwise to a solution of the latter (2.04 g) in methanol (20 mL) until pH 10 was reached. The mixture was stirred at rt for 7 h when TLC (solvent C, 4:1) showed that no starting material remained. Neutralization with resin IR 120 (H^+) followed by column chromatography (solvent A, 49:1) of the crude material gave diol **24** (1.53 g, 81%) as a white foam; $[\alpha]_D=-28°$ (*c* 1.0); NMR: ^1H, δ 7.36–7.21 (m, 15H, Ph), 6.29 (bd, 1H, $J_{NH,2}$=6.6 Hz, NH), 4.86 (d, 2H, *J*=11.8 Hz, OCH_2), 4.75 (d, 1H, $J_{1,2}$=1.0 Hz, H-1_A), 4.71 (bs, 1H, H-1_C), 4.70–4.56 (m, 5H, H-1_D, OCH_2), 4.07 (dq, 1H, $J_{4,5}$=9.6 Hz, H-5_C), 3.97–3.71 (m, 7H, H-2_C, 3_D, 3_C, 2_A, 3_A, $6a_D$, $6b_D$), 3.70 (m, 1H, H-2_D), 3.66 (m, 1H, H-5_A), 3.58 (dd, 1H, H-4_D), 3.38 (m, 2H, H-4_A, 4_C), 3.32 (s, 3H, OCH_3), 3.27 (m, 2H, H-5_D, OH), 2.92 (d, 1H, *J*=4.7 Hz, OH), 2.06 (s, 3H, C(O)CH_3), 1.39, 1.38 (2s, 6H, $C(CH_3)_2$), 1.31 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A), and 1.27 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C); ^{13}C, δ 170.9 (C=O), 139.1–127.3 (Ph), 103.1 (C-1_D), 100.5 (C-1_C), 99.7 (C-1_A), 99.4 ($C(CH_3)_2$), 80.9 (C-4_A), 80.6 (C-4_C), 79.4 (C-3_A), 77.9 (C-2_A), 75.9 (C-3_D), 75.4, 75.6, 73.1 (3C, OCH_2), 72.3 (C-4_D), 72.0 (C-3_C), 71.1 (C-2_C), 67.9 (C-5_A), 67.5 (C-5_D), 67.4 (C-5_C), 61.8 (C-6_D), 57.5 (C-2_D), 54.7 (OCH_3), 29.6 (CCH_3), 23.1 (NHAc), 18.9 (CCH_3), and 17.8, 17.6 (2C, C-6_C, 6_A); CIMS for $C_{45}H_{59}NO_{14}$ (M, 837.39) *m/z*838.4 ($[M+H]^-$). Anal. Calcd for $C_{45}H_{59}NO_{14}$: C, 64.50; H, 7.10; N, 1.67%. Found: C, 64.50; H, 7.17; N, 1.61%.

3.3.11. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-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 25. *p*-Toluenesulphonic acid (10 mg) was added to a suspension of diol **24** (950 mg, 1.07 mmol) in MeCN (8 mL) containing methyl orthoacetate (550 μL, 4.3 mmol). The mixture was stirred for 45 min at rt. TLC (solvent A, 19:1) showed that no starting material remained. The reaction mixture was cooled to 0°C, and 80% aq. AcOH (1.5 mL) was added. After 30 min at rt, TLC (solvent A, 19:1) showed that the intermediate orthoester had turned into a more polar product. CH_2Cl_2 was added, and the organic phase was washed with ice-water and satd aq. NaCl, dried and evaporated to dryness to give **3** quantitatively, as a white foam; NMR: ^1H, δ 7.40–7.27 (m, 15H, Ph), 5.74 (bd, 1H, $J_{NH,2}$=7.7 Hz, NH), 4.90 (dd, 1H, $J_{1,2}$= 1.5 Hz, $J_{2,3}$=3.3 Hz, H-2_C), 4.86 (d, 2H, *J*=11.3 Hz, OCH_2), 4.74–4.57 (m, 5H, H-1_D, OCH_2), 4.65 (bs, 2H, H-1_A, 1_C), 4.08 (dd, 1H, $J_{3,4}$=9.5 Hz, H-3_C), 3.99 (dq, 1H, $J_{4,5}$=9.6 Hz, H-5_C), 3.92–3.84 (m, 4H, H-2_A, 3_A, $6a_D$, $6b_D$), 3.70 (m, 1H, H-2_D), 3.65 (m, 1H, H-5_A), 3.61 (pt, 1H, $J_{2,3}$=$J_{3,4}$=8.7 Hz, H-3_D), 3.55 (dd, 1H, $J_{4,5}$=8.9 Hz, H-4_D), 3.36 (pt, 1H, H-4_A), 3.35 (pt, 1H, $J_{3,4}$=$J_{4,5}$=9.5 Hz, H-4_C), 3.30 (s, 3H, OCH_3), 3.22 (m, 1H, H-5_D), 2.13, 1.83 (2s, 6H, C(O)CH_3), 1.47, 1.41 (2s, 6H, $C(CH_3)_2$), 1.32 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A), and 1.27 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C); ^{13}C, δ 171.6, 170.6 (2C, C=O), 138.7–127.6 (Ph), 102.8 (C-1_D), 99.9 (C-$1_A{}^*$), 99.5 ($C(CH_3)_2$), 98.3 (C-$1_C{}^*$), 81.3 (C-4_C), 80.9 (C-4_A), 79.7 (C-3_A), 79.2 (C-3_D), 77.6 (C-2_A), 75.4, 74.8 (2C, OCH_2), 73.6 (C-2_C), 73.2 (OCH_2), 72.4 (C-4_D), 69.5 3.73 (C-3_C), 67.7 (C-5_D), 67.6 (C-5_A), 67.4 (C-5_C), 62.2 (C-6_D), 56.9 (C-2_D), 54.5 (OCH_3), 29.1 (CCH_3), 23.2, 21.1 (C(O)CH_3), 19.1 (CCH_3), 17.9 (C-6_C), and 17.8 (C-6_A).

Crude **3** (990 mg, 1.07 mmol) and donor **2** (1.54 g, 1.5 mmol) were dissolved in anhydrous Et_2O (10 mL) and the solution was cooled to −78°C. TMSOTf (20 μL, 100 μmol) was added, and the reaction mixture was stirred overnight while the bath temperature was slowly coming back to rt. TLC (solvent A, 97:3) showed that only little acceptor remained, Et_3N was added and volatiles were evaporated. Chromatography (solvent A, 12:1) provided the fully protected pentasaccharide **25** (1.28 g, 67%) as a white foam; $[\alpha]_D=+24°$ (*c* 1.0); NMR: ^1H, δ 8.13–7.05 (m, 45H, Ph), 5.70 (bs, 1H, H-2_B), 5.60 (d, 1H, $J_{NH,2}$=7.7 Hz, NH), 5.21 (d, 1H, $J_{1,2}$=3.5 Hz, H-1_E), 5.20 (bs, 1H, H-1_B), 4.99 (bd, 1H, $J_{1,2}$=1.7 Hz, H-2_C), 4.98–4.36 (m, 15H, OCH_2), 4.82 (d, overlapped, 1H, H-1_D), 4.69 (bs, 1H, H-1_C), 4.66 (bs, 1H, H-1_A), 4.34 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{1,2}$=9.5 Hz, H-3_B), 4.24 (d, 1H, *J*=12.1 Hz, OCH_2), 4.13 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=9.5 Hz, H-3_C), 4.06–3.85 (m, 8H, H-3_E, 5_E, 5_C, 3_D, $6a_D$, 2_A, 5_B, 3_A), 3.75–3.41 (m, 10H, H-$6b_D$, 4_E, 4_B, 5_A, $6a_E$, 2_E, 4_D, $6b_E$, 4_C, 2_D), 3.38 (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), 2.09, 1.82 (2s, 6H, C(O)CH_3), 1.46 (s, 3H, $C(CH_3)_2$), 1.41 (d, 3H, H-6_B), 1.40 (s, 3H, $C(CH_3)_2$), 1.32 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A), and 1.20 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C); ^{13}C, δ 171.1, 170.3, 165.8 (3C, C=O), 138.7–127.3 (Ph), 102.2 (C-1_D, $J_{C,H}$=161 Hz), 100.1 (C-1_A, $J_{C,H}$=170 Hz), 99.4 ($C(CH_3)_2$), 99.3 (C-1_B, $J_{C,H}$=172 Hz), 97.8 (C-1_C, $J_{C,H}$=170 Hz), 92.3 (C-1_E, $J_{C,H}$=169 Hz), 81.9 (C-3_E), 80.8 (C-4_A), 79.8 (C-4_C), 79.6 (C-$4_E{}^*$), 79.5 (C-3_A), 79.0 (C-2_E), 77.9 (C-3_C), 77.8 (C-3_D), 77.5 (C-$4_B{}^*$), 77.3 (C-2_A), 76.2, 75.4, 75.3, 74.9, 73.1, 72.8 (7C, OCH_2), 72.7 (C-2_C), 72.5 (C-4_D), 72.2 (C-3_B), 72.1 (OCH_2), 70.1 (C-5_E), 68.9 (C-5_B), 68.4 (C-2_B), 68.3 (C-6_E), 67.6 (C-$5_A{}^*$), 67.5 (C-$5_C{}^*$), 67.4 (C-5_D), 62.2 (C-6_D), 57.8 (C-2_D), 54.5 (OCH_3), 29.0 (CCH_3), 23.2, 21.0 (C(O)CH_3), 19.0 (CCH_3), 17.8 (C-6_B), 17.7 (C-6_A), and 17.6 (C-6_C). FABMS for $C_{101}H_{115}NO_{25}$ (M, 1741.78) *m/z* 1764.8 ($[M+Na]^+$). Anal. Calcd for $C_{101}H_{115}NO_{25}$: C, 69.60; H, 6.65; N, 0.80%. Found: C, 69.48; H, 6.81; N, 0.96%.

3.3.12. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 26. 50% aq. TFA (12 mL) was added, at 0°C, to a solution of pentasaccharide **25** (1.19 g, 683 μmol) in CH_2Cl_2 (20 mL), and the biphasic mixture was stirred vigorously at rt for 1 h. Repeated coevaporation with toluene and chromatography of the residue (solvent A, 193:7) provided diol **26** (1.09 g, 94%) as a white foam; $[\alpha]_D=+29°$ (*c* 1.0); NMR: ^1H, δ 8.00–7.06 (m, 45H, Ph), 5.69 (bs, 1H, H-2_B), 5.62 (d, 1H, $J_{NH,2}$= 7.3 Hz, NH), 5.20 (bs, 2H, H-1_B, 1_E), 5.00 (bs, 1H, H-2_C), 4.97–4.34 (m, 18H, OCH_2, H-1_D, 1_A, 1_C), 4.31 (dd, 1H, $J_{2,3}$=2.9 Hz, $J_{1,2}$=9.4 Hz, H-3_B), 4.22 (d, 1H, *J*=12.0 Hz, OCH_2), 4.15 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=9.4 Hz, H-3_C), 4.01 (m, 2H, H-3_E, 5_E), 3.94–3.73 (m, 6H, H-5_C, 2_A, $6a_D$, 3_A, 5_B, $6b_D$), 3.67 (dd, 2H, H-4_E, 4_B), 3.59–3.40 (m, 8H, H-5_A, $6a_E$, 3_D, 2_E, 2_D, 4_C, $6b_E$, 4_D), 3.37 (pt, 1H, $J_{3,4}$= $J_{4,5}$=9.4 Hz, H-4_A), 3.30 (s, 3H, OCH_3), 3.28 (m, 1H,

H-5_D), 2.10 (s, 3H, OC(O)CH_3), 2.07 (m, 1H, OH-6_D), 1.78 (s, 3H, NC(O)CH_3), 1.36 (d, 3H, H-6_B), 1.31 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_A), and 1.27 (d, 3H, $J_{5,6}$=6.2 Hz, H-6_C); ^{13}C, δ 170.5, 170.2, 165.8 (3C, C=O), 138.7–127.4 (Ph), 102.0 (C-1_D), 100.0 (C-1_A*), 99.6 (C-1_C*), 99.4 (C-1_B), 92.4 (C-1_E), 86.2 (C-3_D), 81.9 (C-3_E), 80.8 (C-4_A), 79.8 (C-3_A), 79.5 (2C, C-4_B*, 4_C), 79.0 (C-2_E), 77.5 (C-4_E*), 77.2 (C-2_A), 76.9 (C-3_C), 76.0, 75.6, 75.5 (3C, OCH_2), 75.4 (2C, OCH_2, C-5_D), 74.9, 73.2, 72.9 (3C, OCH_2), 72.2 (2C, OCH_2, C-3_B), 72.1 (C-2_C), 70.3 (C-4_D), 70.1 (C-5_E), 69.1 (C-5_C), 69.0 (C-5_B), 68.3 (C-6_E), 68.2 (C-2_B), 67.6 (C-5_A), 62.6 (C-6_D), 55.6 (C-2_D), 54.6 (OCH_3), 23.3, 21.1 (2C, C(O)CH_3), and 18.0, 17.9 (3C, C-6_B, 6_A, 6_C); CIMS for $C_{98}H_{111}NO_{25}$ (M, 1701.74) *m/z* 1719.8 ([M+NH_4]$^+$). Anal. Calcd for $C_{98}H_{111}NO_{25}$·H_2O: C, 68.40; H, 6.62; N, 0.81%. Found: C, 68.20; H, 6.67; N, 1.15%.

3.3.13. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 27. 1N methanolic sodium methoxide was added dropwise to a solution of diol **26** (1.09 g, 640 μmol) in methanol (5 mL) until pH 10 was reached. The mixture was stirred at rt overnight, and neutralized with resin IR 120 (H^+). The crude material was purified by chromatography (solvent A, 49:1) to give the tetraol **27** (963 mg, 97%) as a white foam; $[\alpha]_D$=+20° (*c* 1.0); NMR: ^{1}H, δ 7.36–7.08 (m, 40H, Ph), 5.62 (d, 1H, $J_{NH,2}$=7.0 Hz, NH), 5.13 (bs, 1H, H-1_B), 4.96–4.19 (m, 16H, OCH_2), 4.85 (d, overlapped, 1H, H-1_E), 4.68 (m, overlapped, 3H, H-1_D, 1_A, 1_C), 4.07–3.62 (m, 14H, H-3_E, 3_C, 3_B, 5_C, 5_E, 2_A, 2_B, 2_C, 5_B, 3_A, $6a_D$, $6b_D$, 4_E, 5_A), 3.60–3.29 (m, 13H, H-2_D, 2_E, 4_B, 4_D, 3_D, $6a_E$, $6b_E$, 4_C, 4_A, 5_D, OCH_3), 2.50 (d, 1H, *J*=2.4 Hz, OH), 2.12 (m, 3H, OH), 1.72 (s, 3H, C(O)CH_3), 1.39 (d, 3H, $J_{5,6}$=6.1 Hz, H-6_B), 1.33 (d, 3H, H-6_A), and 1.30 (d, 3H, H-6_C); ^{13}C, δ 170.3 (C=O), 138.4–127.5 (Ph), 102.3 (C-1_D), 101.5 (C-1_A*), 100.3 (C-1_B), 100.0 (C-1_C*), 94.2 (C-1_E), 86.3 (C-3_D), 82.3 (C-3_E), 80.9 (C-4_A), 79.9 (C-3_A), 79.7 (C-4_C), 79.1 (C-4_B), 78.8 (C-2_E), 77.9 (C-3_C), 77.7 (C-4_E), 77.4 (C-2_A), 76.7 (C-3_B), 75.7, 75.6 (2C, OCH_2), 75.5 (2C, OCH_2, C-5_D), 75.0, 74.5, 74.4, 73.4, 73.1 (5C, OCH_2), 70.8 (C-5_E), 70.5 (C-2_C), 70.4 (C-4_D), 69.3 (C-5_C), 68.0 (C-5_B), 67.9 (C-6_E), 67.6 (2C, C-5_A, 2_B), 62.8 (C-6_D), 55.4 (C-2_D), 54.6 (OCH_3), 23.4 (C(O)CH_3), and 18.2, 18.0 (3C, C-6_A, 6_B, 6_C). FABMS for $C_{89}H_{105}NO_{23}$ (M, 1555.71) *m/z* 1578.5 ([M+Na]$^+$). Anal. Calcd for $C_{89}H_{105}NO_{23}$: C, 68.66; H, 6.80; N, 0.90%. Found: C, 68.51; H, 6.90; N, 0.93%.

3.3.14. Methyl α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-α-L-rhamnopyranoside 1. The benzylated pentasaccharide **27** (411 mg, 264 μmol) was dissolved in a 9:1 methanol/acetic acid mixture (20 mL), treated with 10% Pd–C catalyst (200 mg), and the suspension was stirred at rt for 4 days, under a hydrogen atmosphere. At this time, TLC (solvent E, 7:1:2) showed that the starting material had been transformed into a more major polar product. The suspension was filtered on a bed of Celite, and the filtrate was concentrated. Reverse phase chromatography (solvent F, gradient) of the residue, followed by lyophilization, gave the target pentasaccharide **1** (284 mg, 67%) as a white powder; $[\alpha]_D$=0° (*c* 1.0, water). The ^{1}H and ^{13}C NMR data are reported in Tables 1–3. FABMS for $C_{33}H_{57}NO_{23}$ (M, 835.3) *m/z* 858.4 ([M+Na]$^+$). Anal. Calcd for $C_{33}H_{57}NO_{23}$–$3H_2O$: C, 44.54; H, 7.14; N, 1.57%. Found: C, 44.45; H, 7.03; N, 1.58%.

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