Convergent Synthesis, NMR and Conformational Analysis of Tetra- and Pentasaccharide Haptens of the *Shigella flexneri* Serotype 5a *O*-Specific Polysaccharide[‡]

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Convergent syntheses of the methyl glycosides of the branched pentasaccharide 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* [**A(E)BCD**], featuring the biological repeating unit of the *O*-specific polysaccharide of *Shigella flexneri* serotype 5a, and of a related linear tetrasaccharide (**EBCD**) are described. The strategy, based on the trichloroacetimidate methodology, relied on the use of a key **EB** disaccharide donor and appropriate **CD** acceptors. The use of an isopropylidene acetal to block OH-4 and OH-6 of residue **D** was found to be a suitable alternative to the employment of the more commonly used benzylidene acetal. Conformational analysis of **EBCD-OMe** and **A(E)BCD-OMe** was based on analysis of ^{1}H and ^{13}C chemical shifts and inter-proton distances data obtained by NMR spectroscopy. The data showed that residue **A** had no influence on the conformational behaviour of residue **E**, although these two residues were involved in a 2,3-*cis* vicinal glycosylation pattern in **A(E)BCD-OMe**. Comparison of ^{1}H and ^{13}C chemical shifts of the two oligosaccharides with those of their corresponding sequences in the *O*-specific polysaccharide of *S. flexneri* 5a showed that the two oligosaccharides presented a distribution of solution conformations similar to that in the *O*-specific polysaccharide. The conformation of **A(E)BCD-OMe** was investigated by two approaches: (i) energy minimisation based on ROE-derived distances with the DISCOVER program and (ii) a conformational searching method (the CICADA algorithm interfaced with MM3 force-field). The minimised conformation obtained by the former approach was in total agreement with the average of the two major families of conformations resulting from the CICADA calculations.

Introduction

Carbohydrates naturally contain a large amount of structural information. Their location at cell surfaces make them ideal messengers in cell-cell recognition processes. In particular, they occur at the surface of Gram negative bacteria, either as capsular polysaccharides (CPs) or as lipopolysaccharides (LPSs). As such, they can be the basis for the many type-specificities of bacteria and often behave as important virulence factors. That carbohydrates could be used as vaccines to elicit type-specific protection was demonstrated early.[1] At present, vaccination with purified CPs is used to protect adults and older children from infections such as those caused by *Streptococcus pneumoniae*, *Neisseria meningitidis* or *Salmonella typhi*.[2] Since CPs are T-independent antigens, however, they cannot be used as vaccines in children less than two years old. In order to gain the T-dependent properties required to be useful as efficient vaccines in those populations most concerned by bacterial infections − namely children under two years of age and immunocompromised individuals − they have to be conjugated to a protein carrier.[2] The polysaccharide moieties of LPSs are much shorter than CPs and often behave as haptens. They are not immunogenic in humans as such, but it has been demonstrated for several human enterobacteria that the corresponding protein conjugates were safe and immunogenic in humans.[3] In the case of *Shigella sonnei*, such a conjugate resulted in 75% protective efficacy during a field trial.[4]

Improvement of the immunogenicity of bacterial surface polysaccharide:protein conjugates is of considerable interest, but may be impaired by poor knowledge of the critical

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parameters. As a possible alternative to such conjugates, the design of chemically defined vaccines, based on the use of synthetic mimics of the "protective carbohydrate epitopes", is under study in several laboratories. Common approaches involve the use either of oligosaccharides[5] or of peptides.[6] In both approaches, knowledge of the major structural features of the native protective epitopes should help in the design of appropriate mimics.

In the laboratory, such approaches are being developed on the model *Shigella flexneri*, a Gram negative bacterium and the major causative agent of the endemic form of shigellosis or bacillary dysentery.[7] There is as yet no licensed vaccine against this pathogen, which is resistant to the first line of available antibiotics in several countries.[7]

As part of this project, the complementarity between the *O*-specific polysaccharide portion (O-SP) of the *S. flexneri* 5a LPS and homologous protective antibodies[8] is being investigated. This study is based on the use of synthetic oligosaccharides representative of the native O-SP. The latter, the biological repeating unit of which is the branched pentasaccharide **I** − composed of α-L-rhamnoses, 2-acetamido-2-deoxy-β-D-glucose and a branched α-D-glucose[9,10] − has been shown to be the major protective antigen against infection caused by this pathogen.[8]

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↑3)

E α-D-Glc*p* **I**

The preparation of the required frame-shifted di-, tri-, tetra- and pentasaccharides, all bearing the characteristic **EB** motif, was undertaken.[11−14] In this paper we describe convergent chemical syntheses of the linear tetrasaccharide **EBCD** and the branched pentasaccharide **I**. The target compounds were synthesised as their methyl glycosides **1** and **2**, respectively, with the natural anomeric configuration at their reducing end terminus to allow both conformational analysis and binding studies in solution. Next we report on their conformational behaviour in solution, analysed by NMR spectroscopy, and on the conformational analysis of **2** by molecular modelling. This preliminary step was essential in order to ascertain whether **1** and **2** were able to mimic the conformation of the corresponding sequences in the native polysaccharide, since such mimicry is believed to be necessary for immunogenicity once these sequences are conjugated to an appropriate carrier.

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

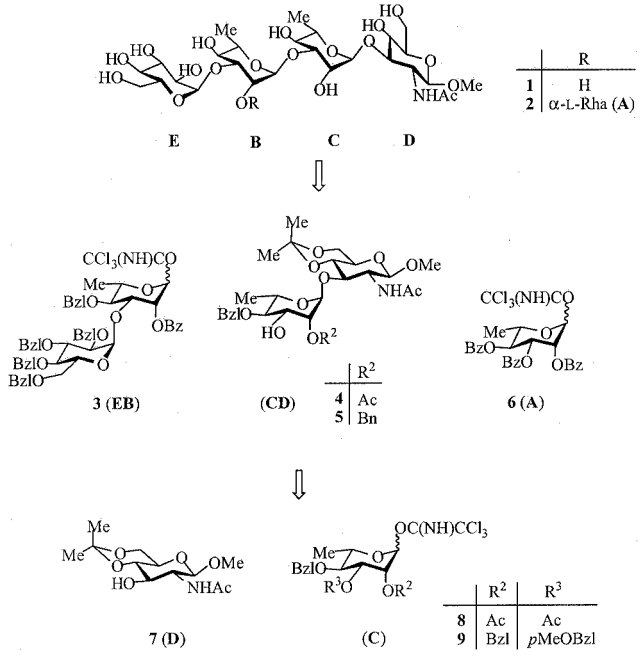
EBCD-OMe **1**

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*-OMe

A(E)BCD-OMe **2**

Results and Discussion

The syntheses of the targets **1** and **2** followed a common disconnection approach (Scheme 1) involving two dimeric building blocks − the known **EB** donor **3**[14] with a participating group at C-2_B and an appropriate **CD** acceptor such as **4** or **5** − and was based on the use of trichloroacetimidate chemistry.[15] Indeed, in the case of the branched pentasaccharide **2**, the donor **6**[16] was chosen as a readily available precursor to residue **A**. Although the benzylidene acetal is the commonly used choice to block the 4- and 6-OH groups of *N*-acetyl glucosamine, it may interfere somewhat with both the solubility and the reactivity of acceptor or derivatives, as observed previously.[17] This phenomenon appears to be less pronounced when an isopropylidene acetal is used instead of the benzylidene moiety. In particular, this transformation partially overcomes the problems of solubility usually encountered when the corresponding benzylidene derivative[18] is used in a condensation process. Compound **7**[17] was therefore chosen as an appropriate precursor to residue **D**.

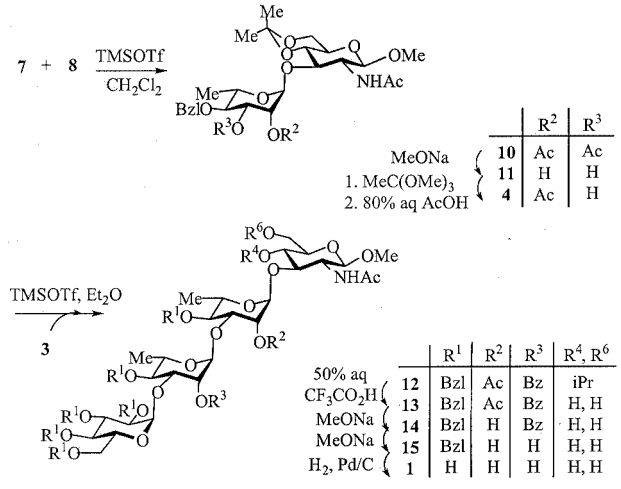


Scheme 1

Synthesis of the Linear Tetrasaccharide EBCD-OMe (1)

The rhamnopyranose derivative **8**,[13] with temporary protecting groups at positions 2 and 3, was used as a suitable precursor to residue **C**. Like the building blocks **3**, **6** and **7**, compound **8** was readily available by standard protecting group/activation strategies. Coupling of glycosyl donor **8** with glycosyl acceptor **7** was performed in CH_2Cl_2 in the presence of a catalytic amount of trimethylsilyl triflate (TMSOTf) to afford the α-linked disaccharide **10** in over 84% yield. Deacetylation of **10** under Zemplén conditions gave diol **11** (98%), which was treated with trimethyl orthoacetate in the presence of a catalytic amount of *p*-

toluenesulfonic acid. The acetyl protecting group was introduced at position 2_C by regioselective opening of the obtained intermediate orthoester with 80% aq. AcOH. Condensation of the resulting crude **4** with the **EB** trichloroacetimidate donor **3** was performed in CH_2Cl_2 containing a catalytic amount of TMSOTf, to give the fully protected tetrasaccharide **12** (48%) together with unchanged **4**. The stereochemistry of the linkages in **12** was ascertained from the $^1J_{C1,H1}$ heteronuclear coupling constants.[19,20] Conventional deblocking of the isopropylidene group gave **13** (91%). Transesterification of the benzoyl group at position 2_B of **13** was particularly slow, affording a mixture of two closely migrating compounds: the tetraol **15** and the assumed benzoylated intermediate **14**. As was to be expected, further heating in methanolic MeONa converted **14** into the tetraol **15** (95% yield from **12**). Analogously with previous observations,[11] steric hindrance conferred by the vicinal tetra-*O*-benzyl-α-D-glucopyranosyl residue at position 2_B of **13** was hypothesised. Finally, hydrogenolysis of **15** afforded the target tetrasaccharide **1** (82%).



		R^2	R^3
	10	Ac	Ac
	11	H	H
	4	Ac	H

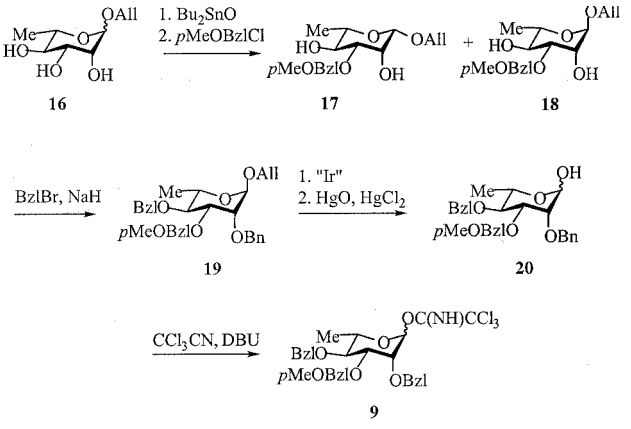
		R^1	R^2	R^3	R^4, R^6
	12	Bzl	Ac	Bz	iPr
	13	Bzl	Ac	Bz	H, H
	14	Bzl	H	Bz	H, H
	15	Bzl	H	H	H, H
	1	H	H	H	H, H

Scheme 2

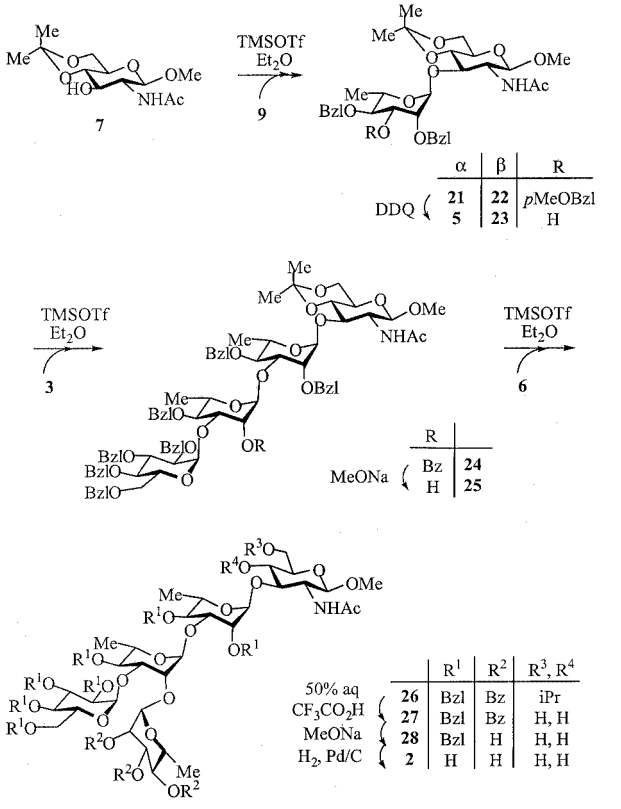
Synthesis of the Branched Pentasaccharide A(E)BCD-OMe (2)

As can be seen in the retrosynthetic scheme described for compound **2** (Scheme 1), a critical step in the synthesis is the selective deblocking of the hydroxyl group at position 2_B to allow the introduction of residue **A**. For that reason, the disaccharide acceptor **5**, possessing a 2_C-*O*-benzyl group instead of the 2_C-*O*-acetyl group present in the analogue **4**, was chosen as a suitable **CD** precursor to **2**. It was prepared by condensation of **7** with the trichloroacetimidate donor **9**. This in turn was obtained from allyl α/β-L-rhamnopyranoside (**16**).[21] Regioselective introduction of a *para*-methoxybenzyl group at the equatorial hydroxyl of the corresponding dibutylstannylene acetal intermediate, by use of *p*-methoxybenzyl chloride in the presence of caesium fluoride,[22] gave the two monoprotected anomers **17** (6.9%) and **18** (66%), as ascertained by the NMR spectroscopic data.[19,20,23] Benzylation of **18** gave **19** (81%), which was de-*O*-allylated to yield the hemiacetal **20** (82%) by a two-step process involving iridium(I)-catalysed isomerisation[24] of the allyl glycoside to the prop-1-enyl glycoside and subsequent hydrolysis.[25,26] The selected trichloroacetimidate anomeric leaving group was introduced by treatment of **20** with trichloroacetonitrile in the presence of DBU, which resulted in the formation of **9** (85%). TMSOTf-mediated glycosylation of **9** with **7** resulted in the isolation of disaccharide **21**, slightly contaminated with the β anomer **22** (α/β ≈ 9:1, 93%). Identification of the latter was based on that of the partially deprotected **23**. Although β-rhamnopyranosides are known to be highly disfavoured, a possible explanation for the formation of **22** was the absence of any participating group at position 2 of **9** and the high reactivity of the donor. Removal of the *p*-methoxybenzyl group from **21** was best performed by treatment with DDQ[27] to give the appropriate disaccharide acceptor **5** (95%). When run on a mixture of **21** and **22**, the deprotection step produced the partially deblocked α- and β-condensation products **5** and **23**, which could be separated at this stage. Full NMR characterisation of **23** confirmed that the **CD** linkage was β, as indicated by the downfield shifts[23] of C-1_C, C-3_C and C-5_C in **23** compared to those in **5**. TMSOTf-promoted condensation between the **EB** trichloroacetimidate donor **3** and **5** gave the fully protected tetrasaccharide **24** in an acceptable yield (79%). As mentioned for the deacylation of **13**, transesterification at position 2_B of **24** to give **25** (94%) proceeded extremely slowly at room temp. Again, this slow process was tentatively associated with steric hindrance and partial loss of conformational flexibility of the starting **24**, as suggested by the presence of several broad signals, such as those tentatively assigned to C-1_B, C-2_C, C-3_C and C-3_D in the corresponding ^{13}C NMR spectrum. Glycosylation of the appropriately protected **25** with the donor **6** was promoted by a small amount of TMSOTf (0.1 equiv.). In agreement with the apparent steric hindrance of OH-2_B, the reaction was rather slow. It was stopped before completion in order to avoid any degradation. The fully protected pentasaccharide **26** was thus isolated in 65% yield, while the unchanged acceptor **25** was partially recovered (33%). As above, the stereochemistry of new glycosidic linkages was unambiguously established by measurement of the $^1J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants. Steric hindrance was again apparent from the ^{13}C NMR spectrum. Sequential deprotection of **26** involved acidic hydrolysis of the acetal protecting group to give **27** (88%), followed by Zemplén debenzoylation, affording the pentaol **28** (96%). Subsequent hydrogenolysis of the benzyl groups with palladium on charcoal as the catalyst provided the branched pentasaccharide target **2** (75%).

It is worth mentioning that compound **24** could also serve as an appropriate intermediate to target **1**. Indeed, although the use of a residue **C** precursor with a non-participating group at position 2 did result in the formation of the β-**CD** condensation product (**22**), the route to **24** compared favourably with that to **12** in terms of yield and number of steps.



Scheme 3



Scheme 4

Conformational Analysis of Tetrasaccharide 1 and Pentasaccharide 2

The complete ^1H assignments of the methyl glycosides **1** and **2**, summarised in Table 1, were based on combined analysis of one- and two-dimensional ^1H NMR spectra including DQF-COSY[28] and TOCSY.[29] ^{13}C assignments (Table 2) were obtained from $^1H-^{13}C$ heteronuclear experiments such as gHSQC[30] and gHSQC-TOCSY.[30,31] The nature of the glycosidic linkages was confirmed from gHMBC[30] and off-resonance ROESY[32] experiments. The vicinal coupling constants (Table 1) of the ring protons in the monosaccharide units were found to be consistent with a $^1C_4$ (L) conformation for the rhamnopyranosyl residues and a $^4C_1$ (D) conformation for the glucose and the *N*-acetyl glucosamine residues. The anomeric configurations were confirmed by the values of heteronuclear one bond $^1J_{C1,H1}$ coupling constants[19] (Table 2) in the gHMBC spectrum. Data similarities for **1** and **2** suggested that the introduction of rhamnose **A** at position 2_B of the tetrasaccharide **1** did not induce any major conformational modifications. Interestingly, residue **E**, involved in a 2,3-*cis* vicinal glycosylation pattern with residue **A** in pentasaccharide **2**, showed the same conformational behaviour in **1** and **2**. ^1H and ^{13}C chemical shifts for the monosaccharides in **1** and **2** showed close similarities to those of

Table 1. ^1H NMR spectroscopic data for **EBCD-OMe** (**1**) and **A(E)BCD-OMe** (**2**)

H-atom	Chemical shifts[a][b][c] **1**		**2**		Coupling constants (Hz) $^3J_{H,H}$	**1**	**2**
H-1_A			5.10	(−0.04)	$1_A, 2_A$		1.7 [d]
H-2_A			4.04	(−0.10)	$2_A, 3_A$		3.3 [d]
H-3_A			3.78	(−0.11)	$3_A, 4_A$		9.7 [e]
H-4_A			3.42	(0.08)	$4_A, 5_A$		9.7 [e]
H-5_A			3.67	(−0.03)	$5_A, 6_A$		6.3 [d]
H-6_A			1.25	(0.00)			
H-1_B	5.05	(−0.17)	5.16	(−0.04)	$1_B, 2_B$	1.4 [d]	1.7 [d]
H-2_B	4.24	(−0.01)	4.25	(0.00)	$2_B, 3_B$	3.9 [e]	3.1 [d]
H-3_B	3.89	(−0.12)	3.97	(−0.04)	$3_B, 4_B$	9.7 [e]	9.7 [d]
H-4_B	3.56	(−0.05)	3.60	(−0.01)	$4_B, 5_B$	9.6 [e]	9.7 [e]
H-5_B	3.81	(0.02)	3.78	(−0.01)	$5_B, 6_B$	6.5 [d]	6.3 [d]
H-6_B	1.31	(−0.03)	1.32	(−0.02)			
H-1_C	4.81	(−0.07)	4.81	(−0.07)	$1_C, 2_C$	1.4 [e]	1.7 [d]
H-2_C	3.86	(−0.01)	3.82	(−0.05)	$2_C, 3_C$	3.2 [e]	3.2 [e]
H-3_C	3.77	(−0.03)	3.77	(−0.03)	$3_C, 4_C$	9.7 [e]	9.7 [e]
H-4_C	3.51	(−0.05)	3.52	(−0.04)	$4_C, 5_C$	9.7 [e]	9.8 [e]
H-5_C	4.02	(0.01)	4.01	(0.00)	$5_C, 6_C$	6.4 [e]	6.3 [d]
H-6_C	1.22	(−0.05)	1.22	(−0.05)			
H-1_D[c]	4.46	(−0.32)	4.45	(−0.33)	$1_D, 2_D$	8.6 [d]	8.6 [d]
H-2_D	3.80	(−0.03)	3.80	(−0.03)	$2_D, 3_D$	9.6 [e]	10.2 [e]
H-3_D	3.58	(−0.08)	3.58	(−0.08)	$3_D, 4_D$	9.7 [e]	9.2 [e]
H-4_D	3.49	(0.00)	3.51	(0.02)	$4_D, 5_D$	9.7 [e]	9.7 [e]
H-5_D	3.46	(−0.02)	3.45	(−0.03)	$5_D, 6_D$	3.2 [d]	3.2 [e]
H-$6a_D$	3.93	(−0.01)	3.92	(−0.02)	$5_D, 6b_D$	5.4 [e]	5.6 [e]
H-$6b_D$	3.74	(−0.02)	3.73	(−0.03)	$6_D, 6b_D$	11.8 [d]	12.4 [e]
H-1_E	5.08	(−0.01)	5.08	(−0.01)	$1_E, 2_E$	3.8 [d]	3.8 [d]
H-2_E	3.56	(−0.04)	3.59	(−0.01)	$2_E, 3_E$	9.6 [e]	9.7 [e]
H-3_E	3.78	(−0.02)	3.75	(−0.05)	$3_E, 4_E$	9.7 [e]	9.7 [e]
H-4_E	3.44	(−0.02)	3.44	(−0.02)	$4_E, 5_E$	9.7 [e]	9.7 [e]
H-5_E	3.97	(0.05)	3.92	(0.00)	$5_E, 6_E$	2.7 [e]	3.2 [e]
H-$6a_E$	3.80	(−0.06)	3.81	(−0.05)	$5_E, 6b_E$	5.6 [e]	5.6 [e]
H-$6b_E$	3.76	(−0.02)	3.75	(−0.03)	$6_E, 6b_E$	12.4 [e]	12.4 [e]

[a] Chemicals shifts measured in ppm with an accuracy of ± 0.01 ppm are referenced to external DSS ($\delta_H = 0.00$). [b] For a given proton, numbers in parentheses represent the difference in ppm between its chemical shifts in **1** or **2** and the corresponding value in the O-SP of *S. flexneri* 5a.[33] [c] The chemical shifts of the aglyconic methyl and *N*-acetyl groups were $\delta = 3.50$ and 2.03 ppm, respectively. [d] Directly measured on the 1D ^1H spectrum (Hz ± 0.1 Hz). [e] Measured on the DQF-COSY spectrum (Hz ± 0.5 Hz).

the respective residues in the native *S. flexneri* 5a O-SP (Tables 1 and 2).[33,34] This observation suggests that, in solution, similar distributions of conformations must exist for the three molecules.

Table 2. ^{13}C NMR spectroscopic data for **EBCD-OMe** (**1**) and **A(E)BCD-OMe** (**2**)

C-atom	Compound[a][b][c][d] 1			2		
$C\text{-}1_A$				104.3	(0.8)	*170.3*
$C\text{-}2_A$				72.7	(−8.5)	
$C\text{-}3_A$				72.7	(−0.2)	
$C\text{-}4_A$				74.7	(−0.5)	
$C\text{-}5_A$				71.8	(−0.2)	
$C\text{-}6_A$				19.3	(−0.1)	
$C\text{-}1_B$	104.6	(1.4)	*171.4*	103.4	(0.8)	*171.7*
$C\text{-}2_B$	69.5	(−7.8)		76.2	(−1.1)	
$C\text{-}3_B$	78.2	(1.3)		77.6	(0.7)	
$C\text{-}4_B$	72.9	(−0.6)		73.2	(−0.2)	
$C\text{-}5_B$	71.8	(0.0)		71.6	(−0.2)	
$C\text{-}6_B$	19.3	(−0.4)		19.5	(−0.1)	
$C\text{-}1_C$	104.1	(0.3)	*169.6*	104.0	(0.2)	*169.7*
$C\text{-}2_C$	73.1	(−0.3)		73.2	(−0.2)	
$C\text{-}3_C$	80.8	(0.7)		80.0	(−0.1)	
$C\text{-}4_C$	73.8	(−0.6)		74.3	(−0.2)	
$C\text{-}5_C$	71.7	(−0.1)		71.6	(−0.2)	
$C\text{-}6_C$	19.0	(−0.4)		19.0	(−0.4)	
$C\text{-}1_D$ [d]	104.1	(−0.5)	*161.4*	104.0	(−0.6)	*163.4*
$C\text{-}2_D$	57.6	(−0.8)		57.7	(−0.6)	
$C\text{-}3_D$	84.5	(0.3)		84.5	(0.3)	
$C\text{-}4_D$	71.2	(−0.3)		71.2	(0.1)	
$C\text{-}5_D$	78.7	(0.0)		78.6	(0.1)	
$C\text{-}6_D$	63.5	(−0.3)		63.5	(−0.3)	
$C\text{-}1_E$	98.3	(0.6)	*169.5*	98.0	(0.4)	*169.5*
$C\text{-}2_E$	74.0	(0.0)		73.7	(−0.4)	
$C\text{-}3_E$	75.6	(−0.3)		75.5	(−0.4)	
$C\text{-}4_E$	72.1	(−0.5)		72.2	(−0.3)	
$C\text{-}5_E$	74.5	(−0.1)		74.3	(−0.3)	
$C\text{-}6_E$	63.0	(−0.5)		63.1	(−0.4)	

[a] Chemicals shifts measured in ppm with an accuracy of ± 0.2 ppm are referenced to external DSS (δ_C = 0.0). [b] For a given carbon, numbers in parentheses represent the difference in ppm between its chemical shifts in **1** or **2** and that in the O-SP of *S. flexneri* 5a.[34] [c] Data in italic are $^1J_{C1\text{-}H1}$ coupling constants measured in Hz with a digital resolution of 0.5 Hz. [d] The chemical shifts of the aglyconic methyl, carboxyl and *N*-acetyl groups were 59.6, 176.8 and 24.7 ppm, respectively.

The conformation of pentasaccharide **2**, which represents the biological repeating unit of *S. flexneri* 5a O-SP, was investigated by two methods. A restrained energy minimisation was performed with the DISCOVER program. The (Φ, Ψ) torsion angles of the glycosidic linkages in the starting conformations were adjusted to the corresponding values in the minimum energy conformation of the equivalent disaccharide fragments in order to avoid van der Waals contacts. The constraints used in the energy minimisation consisted of a set of inter-residue distances (see Table 3) calculated from the volume of the inter-residue proton cross-peaks in the off-resonance ROESY spectrum. Several ROE interactions between protons located around each glycosidic linkage were observed. Moreover, non-sequential ROEs were observed between protons from residues **B** and **D** and also between protons from residues **A** and **D**, suggesting that the structure of **2** was not extended. Furthermore, protons from residues **A** and **E** in **2** did not appear to be involved in any ROE interactions, supporting the implication from the ^1H and ^{13}C chemical shift analysis that residue **A** had no influence on the conformational behaviour of residue **E**. Restrained energy minimisation with the DISCOVER program yielded the structure shown in Figure 1. This structure is characterised by the Φ and Ψ torsion angles listed in Table 4.

Table 3. Inter-residue $^1H-^1H$ distances for **A(E)BCD-OMe** (**2**)

Atom pairs[a]	Distances (Å)
$H\text{-}1_E/H\text{-}2_B$	2.4
$H\text{-}1_E/H\text{-}3_B$	2.5
$H\text{-}5_E/H\text{-}4_B$	3.9
$H\text{-}1_B/H\text{-}2_C$	3.7
$H\text{-}1_B/H\text{-}3_C$	2.3
$H\text{-}6_B/H\text{-}2_C$	4.5
$H\text{-}6_B/C(O)CH_{3D}$	5.4
$H\text{-}1_C/H\text{-}3_D$	2.3
$H\text{-}1_C/C(O)CH_{3D}$	4.4
$H\text{-}2_C/C(O)CH_{3D}$	4.5
$H\text{-}1_A/H\text{-}1_B$	3.3
$H\text{-}1_A/H\text{-}2_B$	2.3
$H\text{-}1_A/H\text{-}4_B$	4.8
$H\text{-}5_A/H\text{-}1_B$	2.5
$H\text{-}5_A/H\text{-}2_B$	4.1
$H\text{-}6_A/H\text{-}1_B$	4.1
$H\text{-}6_A/H\text{-}2_B$	6.1
$H\text{-}6_A/C(O)CH_{3D}$	6.1

[a] The distances were obtained from ROE intensity with errors of about 10%.

In order to assess whether this conformation corresponded to a true energy minimum or to a virtual conformation, a complete conformational search was also performed, with the aid of the CICADA algorithm[35] interfaced with the MM3 force-field. The CICADA program has proved to be very efficient for exploration of the conformational spaces of flexible molecules,[36] including oligosaccharides.[37,38] After 8457 energy minimisations, the CICADA calculations gave a total of 2969 energy minima on the potential energy surface. The complete ensemble of conformations resulting from the CICADA analysis was clustered into different conformational families within an energy window 5 kcal mol^{-1} above the global minimum. Those conformational families with populations of more than 1% are listed in Table 5. Families 1 and 2 are the most populated, accounting for more than 65% of the whole population. They differ in the orientation of the Ψ torsion angle at the C−D glycosidic linkage. Comparison of the $\Psi_{C\text{-}D}$ torsion angle of the conformation of **2** obtained by restrained energy minimisation with those of families 1 and 2 resulting from the CICADA calculations (Figure 1) shows that its value was in agreement with the average of the values obtained for the two conformational families. For the rest of

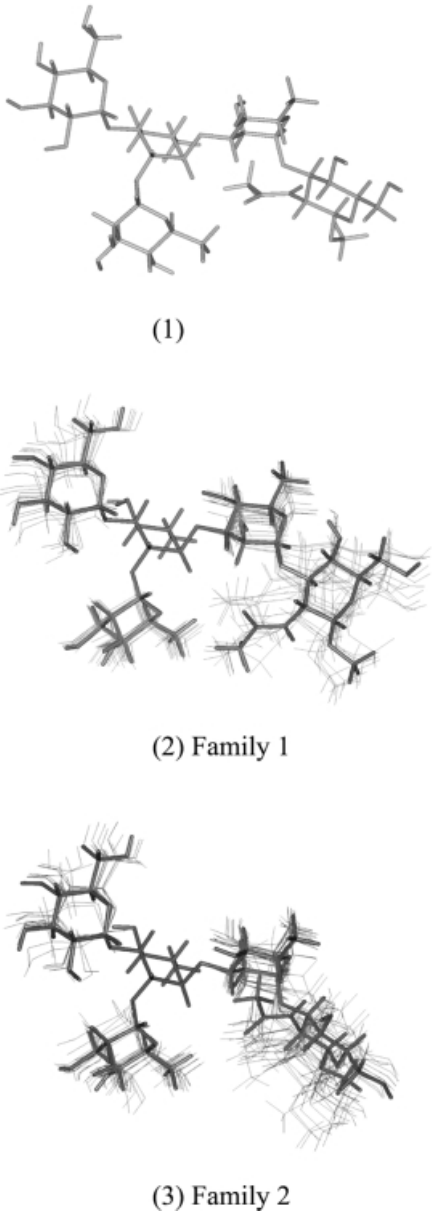


Figure 1. (1): Structure of the pentasaccharide **A(E)BCD-OMe** (**2**) obtained by energy minimisation with ROE-derived distance constraints; (2) and (3): the two major conformational families of **2** determined by the CICADA method; for each family, the lowest energy conformation has been drawn in black; the conformations with the largest difference in Φ and Ψ torsion angles within an energy window of 5 kcal mol^{-1} are drawn in grey; representations correspond to a fit of the ring atoms of residue **B** in order to give an idea of the global flexibility; the methyl aglycone is oriented towards the lower right

Table 4. Inter-residue dihedral angles (°) in the structure of **2** obtained by restrained minimisation

Dihedral angle[a]	Residue pair			
	A−B	**B−C**	**C−D**	**E−B**
Φ	286.7	285.3	276.4	69.8
Ψ	203.5	203.4	114.7	212.3

[a] Measured in the INSIGHT/DISCOVER program.

the molecule, there is almost a perfect agreement between the two methods. Thus, the CICADA procedure allowed the conformational behaviour of the pentasaccharide **2** to be investigated in solution in a complete way. From these results, it can be assumed that the conformation of **2** cannot be described in terms of a unique family of conformations, due to the great flexibility of its reducing end.

The results presented here, although not yet complete as they are based only on chemical shift analysis, suggest conformational similarities between **2** and the native bacterial O-SP. If these are confirmed by a full structural characterisation of the O-SP (work in progress in this laboratory), then the pentasaccharide **2** could be a good mimic candidate for *S. flexneri* 5a O-SP.

Experimental Section

NMR Spectroscopy of Compounds 1 and 2: The concentrations of the samples were 24 mM and 30 mM for the tetrasaccharide **1** and the pentasaccharide **2**, respectively. NMR experiments were carried out on a Varian Unity Inova 500 MHz spectrometer equipped with a triple resonance, pulsed field gradient probe with an actively shielded *z* gradient. All spectra were recorded at 35 °C in D_2O. Chemical shifts are given in ppm, with sodium 2,2-dimethyl-2-silapentane 5-sulfonate (DSS) used as external reference (δ_H, δ_C = 0.00). DQF-COSY, TOCSY and off-resonance ROESY spectra were recorded with a spectral width of 2.2 kHz in both dimensions, a 90° pulse of 4.3 μs, 2 K (F1) × 2 K (F2) points data sets, zero-filled to 4 K in F1 dimension and with 16, 8 and 32 scans per increment, respectively. The TOCSY experiment was acquired by use of 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 over 400 ms. To avoid Hartmann−Hahn artefacts, the offset of the spin lock carrier was shifted by approximately 8 kHz from the centre 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 with shifted sine-bell windows in both dimensions. Gradient-enhanced HSQC, gHSQC-TOCSY and gHMBC experiments were performed with spectral widths of 2.2 kHz and 24 kHz in the ^{1}H and ^{13}C dimensions, respectively, with ^{1}H and ^{13}C 90° pulse widths 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 4 K in the F1 dimension and 2 K in the *F*2 dimension. For the 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 to allow evolution of long-range couplings. For these heteronuclear experiments, shifted Gaussian windows were applied in both dimensions. All 2D data except for gHMBC data were collected in the phase-sensitive mode by the States−Haberkorn method.[39] The $^3J_{H,H}$ coupling constants were obtained from one-dimensional spectra with digital resolution of 0.1 Hz/point, or from the DQF-COSY experiments with digital resolution of 0.5 Hz/point. The $^1J_{C1,H1}$ coupling constants were measured from the gHMBC spectra with digital resolution of 0.5 Hz/point.

Inter-Proton Distances from Cross-Peak Volumes: The off-resonance ROESY cross-peak volumes were measured with the VNMR software. The intra-residue distance of 2.52 Å between H-

Table 5. Characteristics of the pentasaccharide **2** conformational families (only the families with an energy-weighted populations of more than 1% at 298 K have been listed. "Conf-min" indicates the glycosidic linkages torsion angles and relative energy of the lowest energy conformation, whereas "min." and "max." are the limit values for each torsion angle within an energy window of 5 kcal·mol^{-1})

Families	$\Phi_{A\text{-}B}$	$\Psi_{A\text{-}B}$	$\Phi_{B\text{-}C}$	$\Psi_{B\text{-}C}$	$\Phi_{C\text{-}D}$	$\Psi_{C\text{-}D}$	$\Phi_{E\text{-}B}$	$\Psi_{E\text{-}B}$	E_{rel}	%
1										
Conf-min	269.4	184.7	264.2	187.8	271.9	76.7	76.7	200.5	0.0	31.3
min.	262.0	180.7	255.9	182.3	254.5	63.9	68.3	183.5		
max.	280.6	215.2	291.2	224.5	276.2	109.1	95.9	211.2		
2										
Conf-min	269.4	188.6	278.4	200.0	272.9	140.5	78.7	199.5	0.02	34.3
min.	263.1	179.8	256.1	184.4	263.7	117.7	67.4	191.6		
max.	286.8	242.3	284.6	220.8	281.1	149.80	102.9	212.5		
3										
Conf-min	281.8	247.7	274.0	197.7	262.1	81.7	89.1	283.4	0.87	4.3
min.	267.7	222.9	260.5	186.0	259.6	71.4	83.8	275.9		
max.	290.2	260.5	282.6	212.6	274.1	100.1	94.0	291.8		
4										
Conf-min	285.5	235.1	276.4	212.6	263.0	89.4	75.0	201.0	0.89	3.7
min.	265.7	226.5	260.1	181.5	260.6	68.2	68.7	194.0		
max.	287.9	258.1	278.7	212.6	274.8	93.1	84.2	214.2		
5										
Conf-min	273.6	186.4	280.2	247.8	269.9	74.9	76.8	201.2	1.00	3.6
min.	265.2	185.4	256.0	238.6	258.0	66.6	63.7	189.8		
max.	278.4	202.0	298.1	279.3	275.7	82.7	91.9	207.5		
6										
Conf-min	270.9	187.3	217.0	196.5	270.8	77.4	83.5	201.0	1.00	3.2
min.	262.8	186.0	171.6	181.5	262.9	70.0	67.6	181.8		
max.	279.5	207.6	217.0	205.7	273.6	83.4	93.4	205.9		
7										
Conf-min	265.2	185.2	263.4	191.1	266.4	76.6	96.5	290.7	1.20	3.7
min.	261.6	181.3	259.1	184.7	257.8	63.7	84.2	277.3		
max.	271.7	197.4	289.2	227.3	274.7	103.4	96.5	298.1		
8										
Conf-min	281.8	209.1	285.6	262.1	276.0	136.3	74.7	198.1	1.21	1.6
min.	264.5	186.7	273.2	240.2	268.8	126.5	69.3	193.8		
max.	281.8	209.1	285.6	268.3	280.7	148.3	92.8	209.7		
9										
Conf-min	266.5	183.5	269.8	188.5	274.1	135.7	86.2	286.0	1.27	5.2
min.	262.7	179.2	262.5	185.7	263.5	129.3	83.1	276.0		
max.	283.1	235.6	287.4	218.2	281.9	148.9	99.5	303.4		
10										
Conf-min	269.3	194.5	264.4	193.2	201.6	88.6	81.6	203.7	1.65	1.2
min.	264.3	182.3	259.2	182.9	197.6	81.9	71.6	189.8		
max.	273.8	194.5	272.1	196.2	211.8	89.3	89.4	207.0		

1 and H-2 of rhamnose **B** was used as the reference for distance calibration. The proton-proton distances were calculated by use of the usual $1/r^6$ NOE/distance relationship.[40]

Molecular Modelling

Nomenclature: The two torsion angles describing a glycosidic linkage are defined as $\Phi = O_5-C_1-O_1-C'_X$ and $\Psi = C_1-O_1-C'_X-C'_{X+1}$, with the primed atoms belonging to the reducing side and the sign being in agreement with IUPAC nomenclature.[41]

Restrained Energy Minimisation of Tetrasaccharide 1 and Pentasaccharide 2: Energy minimisation was performed with the DISCOVER molecular modelling package, version 95.0 (Biosym Technologies Inc.), interfaced with the AMBER force-field by Homans extensions for the anomeric atoms.[42] The calculations were run on Silicon Graphics Octane workstations running under the IRIX 6.5 operating system with a distance-dependent dielectric constant of 80. A gradient conjugate energy minimisation was conducted for 5000 steps by use of the ROE-derived distance constraints.

CICADA Calculations: Exploration of the potential energy surface of pentasaccharide **2** was performed with the CICADA program.[35] The input for the CICADA program, which is an interface to the MM3 force-field, consisted of one or a few conformers in MM3 format and a file containing the list of torsion angles to be driven and/or monitored. During the CICADA calculations, the selected torsion angles are driven one after the other in each direction from the initial conformation at a given increment. For the pentasaccharide **2**, the driven torsion angles were Φ and Ψ at each linkage and the torsion angle of each hydroxymethyl group ($O_5-C_5-C_6-O_6$), resulting in a dimensionality of 10 for the potential energy surface to explore. The torsion angle of the *N*-acetyl group ($C_1-C_2-N-C_8$) and the torsion angles of all the secondary hydroxyl groups were monitored, but not driven. The increment step was set to 20°, and two conformations were considered to be different when one of the driven or monitored angles differed by at least 30°. A relative energy cut-off of 50 kcal/mol was applied for exploration of the potential energy surface. The search was stopped when no new conformer with an energy of less than 5 kcal/mol could be detected.

Analysis of the Potential Energy Surface: The conformations and transition states found by CICADA were analysed by use of the PANIC program,[43] which explores the paths along the potential energy surface. Conformations were clustered into families within an energy window of 5 kcal·mol^{-1} with the FAMILY program.[44] In this study, a conformer is regarded as belonging to a conformational family if at least one of its torsion angles differs from at least one of the conformers of the family by less than 10°. As for the relative importance of each family, their populations were calculated by use of a Boltzmann type of distribution.

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 60 F_{254} silica gel (Merck) was performed with solvent mixtures of appropriately adjusted polarity consisting of: *A*, dichloromethane/methanol; *B*, cyclohexane/ethyl acetate; *C*, toluene/acetone; *D*, toluene/EtOAc; *E*, 2-propanol/ammonia-water; *F*, water-acetonitrile. Detection was performed, 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). Reversed-phase column chromatography was performed by elution from columns of Lichoprep® RP-18 (25−40 μm). The NMR spectra of all compounds other than **1** and **2** were recorded at 25 °C for solutions in $CDCl_3$, unless stated otherwise, on a Bruker AC 300P spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C). TMS (δ = 0.00 ppm for both ^{1}H and ^{13}C) was used as an external reference for solutions in $CDCl_3$. Proton signal assignments were made by first-order analysis of the spectra, and also by analysis of two-dimensional ^{1}H−^{1}H correlation maps (COSY) and selective TOCSY experiments. Of the two magnetically nonequivalent geminal protons at C-6, the one resonating at lower field is denoted as H-6a and the one at higher field is denoted as H-6b. The ^{13}C NMR assignments were supported by two-dimensional ^{13}C−^{1}H correlation maps (HETCOR). Interchangeable assignments are marked with an asterisk in the listings of signal assignments. Sugar residues in oligosaccharides are lettered serially 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 ionisation mass spectra (CIMS) were obtained by using NH_3 as the ionising gas. Fast atom bombardment mass spectra (FABMS) were recorded in the positive ion mode with dithioerythritol/dithio-L-threitol (4:1, MB) as the matrix, in the presence of NaI and with 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.

Methyl (2,3-Di-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (10): TMSOTf (270 μL) was added at −78 °C to a stirred solution of the imidate **8**[13] (8.44 g, 15.9 mmol) and the alcohol **7**[17] (3.84 g, 13.9 mmol) in CH_2Cl_2 (50 mL). After 7 h, the bath temperature had reached 5 °C and TLC (solvent *A*, 19:1) showed that no alcohol **7** remained. The mixture was treated with Et_3N and was then concentrated. Chromatography of the residue (solvent *A*, 39:1) gave the fully protected disaccharide **10** as pure material (6.92 g, 84%), together with a slightly contaminated fraction (1.31 g) that could be used as such for the next step. An analytical sample of **10** was crystallised from a mixture of EtOAc and petroleum ether; m.p. 187−188 °C, $[\alpha]_D$ = −8.6 (*c* = 1.0). ^{1}H NMR: δ = 7.29−7.22 (m, 5 H, Ph), 5.85 (d, 1 H, $J_{NH,2}$ = 7.4 Hz, NH), 5.19 (dd, 1 H, $J_{3,4}$ = 9.8, $J_{2,3}$ = 3.4 Hz, H-3$_C$), 5.08 (dd, 1 H, $J_{1,2}$ = 1.8 Hz, H-2$_C$), 4.87 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1$_D$), 4.69 (d, 1 H, H-1$_C$), 4.60 (d, 1 H, *J* = 11.6 Hz, OCH_2), 4.56 (d, 1 H, OCH_2), 4.27 (pt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3$_D$), 4.02 (dq, 1 H, $J_{4,5}$ = 9.0 Hz, H-5$_C$), 3.86 (dd, 1 H, $J_{5,6a}$ = 5.4, $J_{6a,6b}$ = 10.7 Hz, H-6a$_D$), 3.69 (pt, 1 H, $J_{5,6b}$ = 10.5 Hz, H-6b$_D$), 3.47 (pt, 1 H, $J_{4,5}$ = 9.4 Hz, H-4$_D$), 3.42 (pt, 1 H, H-4$_C$), 3.40 (s, 3 H, OCH_3), 3.29 (m, 1 H, H-5$_D$), 3.00 (m, 1 H, H-2$_D$), 2.04, 1.91, 1.89 [3s, 9 H, C(O)CH_3], 1.40, 1.32 [2s, 6 H, $C(CH_3)_2$], 1.21 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6$_C$) ppm. ^{13}C NMR: δ = 171.4, 170.6, 170.5 [3C, C(O)], 138.6−125.7 (Ph), 101.0 (C-1$_D$), 99.9 [$C(CH_3)_2$], 98.3 (C-1$_C$), 79.2 (C-4$_C$), 76.6 (C-3$_D$), 75.2 (OCH_2), 73.7 (C-4$_D$), 71.8 (C-3$_C$), 71.2 (C-2$_C$), 68.1 (C-5$_C$), 67.4 (C-5$_D$), 62.6 (C-6$_D$), 59.5 (C-2$_D$), 57.5 (OCH_3), 29.5 [$C(CH_3)_2$], 23.9, 21.3 [3C, C(O)CH_3], 19.6 [$C(CH_3)_2$], 18.2 (C-6$_C$) ppm. CIMS for $C_{29}H_{41}NO_{12}$ (M, 595.26): *m*/*z* = 596 [M + H]$^+$. $C_{29}H_{41}O_{12}$: calcd. C 58.48, H 6.94, N 2.35; found C 58.18, H 7.37, N 2.10%.

Methyl (4-*O*-Benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (11): A solution of diacetate **10** (277 mg, 465 μmol) in a mixture of MeOH and CH_2Cl_2 (2 mL) was treated overnight with a catalytic amount of 1 N NaOMe. TLC (solvent *A*, 19:1) showed that no starting material remained. After neutralisation with Amberlite IR-120 (H^+), filtration and evaporation of the solvent, the crude product was chromatographed (solvent *A*, 191:9) to give the pure diol **11** (233 mg, 98%) as a white foam. $[\alpha]_D$ = −63.7 (*c* = 1.0). ^{1}H NMR ([D$_6$]DMSO): δ = 7.96 (d, 1 H, $J_{NH,2}$ = 9.2 Hz, NH), 7.34−7.23 (m, 5 H, Ph), 4.91 (d, 1 H, $J_{OH,2}$ = 4.4 Hz, OH-2$_C$), 4.91 (d, 1 H, *J* = 11.8 Hz, OCH_2), 4.77 (d, 1 H, $J_{OH,3}$ = 7.0 Hz, OH-3$_C$), 4.64 (br. s, 1 H, H-1$_C$), 4.56 (d, 1 H, OCH_2), 4.34 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1$_D$), 3.91−3.79 (m, 2 H, H-5$_C$, 6a$_D$), 4.74−3.65 (m, 2 H, H-2$_D$, 6b$_D$), 3.61−3.47 (m, 4 H, H-3$_C$, 3$_D$, 2$_C$, 4$_D$), 3.32 (s, 3 H, OCH_3), 3.26−3.18 (m, 2 H, H-5$_D$, 4$_C$), 1.84 [s, 3 H, C(O)CH_3], 1.41, 1.27 [2s, 6 H, $C(CH_3)_2$], 1.06 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6$_C$). ^{13}C NMR: δ = 171.7, 170.9 [2C, C(O)], 138.6−127.7 (Ph), 101.4 (C-1$_D$), 99.5 [$C(CH_3)_2$], 98.4 (C-1$_C$), 81.1 (C-4$_C$), 78.4 (br. s, C-3$_D$), 75.0 (OCH_2), 73.6 (C-2$_C$), 72.9 (C-4$_D$), 69.4 (C-3$_C$), 67.7 (C-5$_C$), 67.1 (C-5$_D$), 62.1 (C-6$_D$), 57.3 (br. s, C-2$_D$), 56.8 (OCH_3), 29.2 [$C(CH_3)_2$], 23.4, 21.1 [2C, C(O)CH_3], 19.1 [$C(CH_3)_2$], 17.9 (C-6$_C$). FABMS for $C_{25}H_{37}NO_{10}$ (M, 511.24): *m*/*z* = 534.3 [M + Na]$^+$. $C_{25}H_{37}NO_{10}$: calcd. C 58.70, H 7.29, N 2.74; found C 58.52, H 7.42, N 2.74%.

Methyl (2-*O*-Acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (4): *p*-Toluenesulfonic acid (5 mg) was added to a suspension of diol **11** (255 mg, 0.5 mmol) in MeCN (5 mL) containing methyl orthoacetate (200 μL, 1.57 mmol). The mixture was stirred for 1 h at room temp. TLC (solvent *A*, 19:1) showed that no starting material remained. The reaction mixture was cooled to 0 °C, and 80% aq. AcOH (800 μL) was added. After 30 min at room temp., 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, and dried. The solvents were evaporated to dryness to give **4** quantitatively as a white foam. $[\alpha]_D$ = −27.2 (*c* = 0.86). ^{1}H NMR: δ = 7.37−7.23 (m, 5 H, Ph), 5.75 (d, 1 H, $J_{NH,2}$ = 7.8 Hz, NH), 4.96 (br. s, 1 H, H-2$_C$), 4.81 (d, partially overlapped, 1 H, OCH_2), 4.80 (br. s, overlapped, 1 H, H-1$_C$), 4.76 (d, $J_{1,2}$ = 8.2 Hz, 1 H, H-1$_D$), 4.73 (d, 1 H, *J* = 11.2 Hz, OCH_2), 4.17 (m, 1 H, $J_{3,OH}$ = $J_{3,4}$ = 9.5 Hz, H-3$_C$), 4.08−3.98 (m, 2 H, H-5$_C$, 3$_D$), 3.94 (dd, 1 H, $J_{6a,6b}$ = 10.8, $J_{5,6a}$ = 5.4 Hz, H-6a$_D$), 3.77 (br. d, 1 H, H-6b$_D$), 3.52 (m, 1 H, $J_{2,3}$ = 9.4 Hz, H-2$_D$), 3.46 (s, 3 H, OCH_3), 3.99−3.23 (m,

3 H, H-5_D, 4_D, 4_C), 2.03, 1.98 [2s, 6 H, C(O)CH_3], 1.60 (br. s, 1 H, OH-3_C), 1.47, 1.41 [2s, 6 H, C(CH_3)$_2$], 1.29 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_C) ppm. ^{13}C NMR: δ = 171.7, 170.9 [2C, C(O)], 138.6−127.7 (Ph), 101.4 (C-1_D), 99.5 [C(CH_3)$_2$], 98.4 (C-1_C), 81.1 (C-4_C), 78.4 (br. s, C-3_D), 75.0 (OCH_2), 73.6 (C-2_C), 72.9 (C-4_D), 69.4 (C-3_C), 67.7 (C-5_C), 67.1 (C-5_D), 62.1 (C-6_D), 57.3 (br. s, C-2_D), 56.8 (OCH_3), 29.2 [C(CH_3)$_2$], 23.4, 21.1 [2C, C(O)CH_3], 19.1 [C(CH_3)$_2$], 17.9 (C-6_C) ppm. CIMS for $C_{27}H_{39}NO_{11}$ (M, 553.25): *m/z* = 554 [M + H]$^+$. $C_{27}H_{39}NO_{11}$: calcd. C 58.58, H 7.10, N 2.53; found C 58.44, H 7.29, N 2.61%.

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-glucopyranoside (12): TMSOTf (6 μL, 33 μmol) was added at −78 °C to a stirred solution of the crude acceptor **4** (382 mg, obtained from 0.66 mmol of **11**) and the imidate **3**[14] (1.03 g, 0.73 mmol) in anhydrous CH_2Cl_2 (5 mL). The mixture was stirred overnight and treated with Et_3N, and then concentrated. Chromatography of the residue (solvent *A*, 99:1 → 49:1) first gave the hydrolysed donor, then the fully protected tetrasaccharide **12** (452 mg, 48%) as the second eluting product, and lastly the slightly contaminated unchanged **4** (170 mg, 46%). Compound **12**, isolated as a white foam, had [α]$_D$ = +21.7 (*c* = 1.0). ^{1}H NMR: δ = 8.03−7.06 (m, 35 H, Ph), 5.75 (d, 1 H, $J_{NH,2}$ = 7.5 Hz, NH), 5.68 (dd, 1 H, H-2_B), 5.17 (m, 2 H, H-1_E, 1_B), 5.06 (dd, 1 H, $J_{1,2}$ = 1.6, $J_{2,3}$ = 3.2 Hz, H-2_C), 4.92 (d, 1 H, overlapped, $J_{1,2}$ = 8.4 Hz, H-1_D), 4.74 (br. s, overlapped, 1 H, H-1_C), 4.98−4.35 (m, 11 H, OCH_2), 4.34 (m, overlapped, 1 H, H-3_B), 4.27 (pt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3_D), 4.16 (d, 1 H, *J* = 12.1 Hz, OCH_2), 4.10 (dd, 1 H, $J_{3,4}$ = 9.5 Hz, H-3_C), 4.05−3.96 (m, 3 H, H-5_C, 3_E, 5_E), 3.93 (dd, 1 H, $J_{5,6a}$ = 5.1, $J_{6a,6b}$ = 10.7 Hz, H-$6a_D$), 3.83 (dq, 1 H, $J_{4,5}$ = 9.4 Hz, H-5_B), 3.73 (d, 1 H, H-$6b_D$), 3.72−3.68 (m, 2 H, H-4_B, 4_E), 3.60 (dd, 1 H, $J_{5,6a}$ = 3.0, $J_{6a,6b}$ = 11.0 Hz, H-$6a_E$), 3.54 (dd, 1 H, $J_{1,2}$ = 3.3, $J_{2,3}$ = 9.6 Hz, H-2_E), 3.49 (dd, 1 H, $J_{4,5}$ = 9.8 Hz, H-4_D), 3.47 (s, 3 H, OCH_3), 3.43 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4_C), 3.41 (dd, 1 H, $J_{5,6b}$ = 2.6 Hz, H-$6b_E$), 3.35 (m, 1 H, H-5_D), 3.06 (m, 1 H, H-2_D), 2.09, 2.01, 1.65 [3s, 9 H, C(O)CH_3], 1.47, 1.39 [2s, 6 H, C(CH_3)$_2$], 1.37 (d, 3 H, $J_{5,6}$ = 6.4 Hz, H-6_B), 1.29 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_C) ppm. ^{13}C NMR: δ = 171.3, 170.5, 165.8 [3C, C(O)], 138.5−127.2 (Ph), 100.7 (C-1_D, $^1J_{C,H}$ was not extractable), 99.4 [C(CH_3)$_2$], 99.3 (C-1_B, $^1J_{C,H}$ = 170 Hz), 97.6 (C-1_C, $^1J_{C,H}$ = 171 Hz), 92.3 (C-1_E, $^1J_{C,H}$ = 169 Hz), 81.9 (C-3_E), 79.6 (2C, C-4_B, 4_C), 79.0 (C-2_E), 78.5 (C-3_C), 77.5 (C-4_E), 76.2 (2C, C-3_D, OCH_2), 75.4, 74.9, 73.1 (4C, OCH_2), 73.0 (C-4_D), 72.5 (C-2_C), 72.1 (2C, C-3_B, OCH_2), 70.2 (C-5_E), 69.0 (C-5_B), 68.3 (2C, C-6_E, 2_B), 67.7 (C-5_C), 67.0 (C-5_D), 62.2 (C-6_D), 59.0 (C-2_D), 57.1 (OCH_3), 29.1 [C(CH_3)$_2$], 23.4, 21.0 [2C, C(O)CH_3], 19.1 [C(CH_3)$_2$], 17.9 (C-6_B), 17.9 (C-6_C) ppm. CIMS for $C_{81}H_{93}NO_{21}$ (M, 1415.62): *m/z* = 1433.7 [M + NH_4]$^+$. $C_{81}H_{93}NO_{21}$: calcd. C 68.68, H 6.62, N 0.99; found C 68.62, H 6.59, N 0.99%.

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-glucopyranoside (13): A solution of the fully protected tetrasaccharide **12** (210 mg, 148 μmol) in CH_2Cl_2 (5 mL), stirred at 0 °C, was treated with 50% aq. CF_3CO_2H (700 μL) for 45 min. TLC (solvent *A*, 97:3) showed that all starting material had been converted into a more polar product. Volatiles were eliminated by repeated coevaporation with toluene, and the residue was column chromatographed to give diol **13** (185 mg, 91%) as a white foam, together with a slightly contaminated fraction (20 mg), [α]$_D$ = +25.5 (*c* = 1.0). ^{1}H NMR: δ = 8.04−7.04 (m, 35 H, Ph), 5.87 (d, 1 H, $J_{NH,2}$ = 6.9 Hz, NH), 5.70 (br. s, 1 H, H-2_B), 5.20 (br. s, 2 H, H-1_E, 1_B), 5.10 (br. s, 1 H, H-2_C), 4.97 (d, 2 H, *J* = 10.9 Hz, OCH_2), 4.84 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1_D), 4.79 (br. s, 1 H, H-1_C), 4.85−4.36 (m, 9 H, OCH_2), 4.34 (dd, 1 H, $J_{2,3}$ = 2.6, $J_{3,4}$ = 9.6 Hz, H-3_B), 4.23 (d, 1 H, *J* = 12.1 Hz, OCH_2), 4.16−4.09 (m, 2 H, H-3_C, 3_D), 4.06−3.90 (m, 4 H, H-3_E, 5_E, 5_C, $6a_D$), 3.86−3.76 (m, 2 H, H-5_B, $6b_D$), 3.73−3.65 (m, 2 H, H-4_E, 4_B), 3.61 (m, 1 H, H-$6a_E$), 3.59 (m, 1 H, H-2_E), 3.50 (dd, 1 H, H-4_C), 3.49 (m, 4 H, H-$6b_E$, OCH_3), 3.40 (m, 2 H, H-4_D, 5_D), 3.14 (m, 1 H, H-2_D), 2.11, 2.03 [2s, 6 H, C(O)CH_3], 1.36 (d, 3 H, $J_{5,6}$ = 6.0 Hz, H-6_B), 1.28 (d, 3 H, $J_{5,6}$ = 6.1 Hz, H-6_C) ppm. ^{13}C NMR: δ = 171.1, 170.4, 165.8 [3C, C(O)], 138.6−127.3 (Ph), 100.5 (C-1_D), 99.4 (2C, C-1_B, 1_C), 92.5 (br. s, C-1_E), 84.2 (C-3_D), 81.9 (C-3_E), 79.4 (br. s, C-4_E*), 79.3 (C-4_C), 79.0 (C-2_E), 77.5 (C-4_B*), 77.4 (C-3_C), 76.0 (br. s, OCH_2), 75.6, 75.4 (2C, OCH_2), 75.2 (C-4_D), 74.9, 73.2 (2C, OCH_2), 72.2 (2C, C-3_B, OCH_2), 72.1 (C-2_C), 70.7 (C-5_D), 70.2 (C-5_E), 69.1 (C-5_C), 69.0 (C-5_B), 68.3 (2C, C-2_B, 6_E), 62.6 (C-6_D), 57.1 (C-2_D), 56.9 (OCH_3), 23.4, 21.0 [2C, C(O)CH_3], 18.0 (C-6_B), 17.8 (C-6_C) ppm. CIMS for $C_{81}H_{93}NO_{21}$ (M, 1375.59): *m/z* = 1393.7 [M + NH_4]$^+$. $C_{81}H_{93}NO_{21}$: calcd. C 68.06, H 6.52, N 1.02; found C 67.87, H 6.49, N 0.95%.

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-glucopyranoside (15): A solution of the fully protected tetrasaccharide **12** (300 mg, 212 μmol) in CH_2Cl_2 (2 mL), stirred at 0 °C, was treated with 50% aq. CF_3CO_2H (1.3 mL) for 45 min. TLC (solvent *A*, 19:1) showed that no starting material remained. Volatiles were eliminated by repeated coevaporation with toluene, and the residue was taken up in a mixture of CH_2Cl_2 (2 mL) and MeOH (3 mL). Excess MeONa (1 N, 500 μL) was added, and the mixture was stirred for 3 days at room temp. TLC (solvent *A*, 11.5:1) showed that the intermediate diol had been converted into two more polar products. Heating of the mixture at 55 °C for 7 h and then at 45 °C overnight resulted in the conversion of the less polar intermediate, most probably the still benzoylated **14**, into the more polar **15**. After neutralisation with Amberlite IR-120 (H^+), filtration and evaporation of the solvent, the crude product was chromatographed (solvent *A*, 24:1) to give the tetraol **15** (248 mg, 95%) as a white foam. [α]$_D$ = +20.7 (*c* = 0.9). ^{1}H NMR: δ = 7.34−7.11 (m, 30 H, Ph), 5.80 (d, 1 H, $J_{NH,2}$ = 7.6 Hz, NH), 5.21 (br. s, 1 H, H-1_B), 4.94 (d, 1 H, *J* = 11.0 Hz, OCH_2), 4.89 (d, 1 H, OCH_2), 4.84 (br. s, 1 H, H-1_C), 4.82 (d, 1 H, H-1_E), 4.80 (d, 1 H, H-1_D), 4.84−4.26 (m, 10 H, OCH_2), 4.08 (m, 1 H, H-3_D), 4.02−3.06 (m, 10 H, H-3_E, 3_B, 5_C, 3_C, 2_C, 2_B, 5_E, 5_B, $6a_D$, $6b_D$), 3.69 (pt, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4_E), 3.57 (dd, 1 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 9.6 Hz, H-2_E), 3.50 (m, 4 H, H-4_B, OCH_3), 3.43 (m, 4 H, H-4_C, 4_D, 5_D, $6a_E$), 3.35 (bd, 1 H, $J_{6a,6b}$ = 10.9 Hz, H-$6b_E$), 3.22 (m, 1 H, H-2_D), 2.51 (d, 1 H, *J* = 2.9 Hz, OH), 2.23 (m, 1 H, *J* = 6.6 Hz, OH-6_D), 1.97 [s, 3 H, C(O)CH_3], 1.73 (s, 2 H, OH), 1.36 (d, 3 H, $J_{5,6}$ = 6.0 Hz, H-6_B), 1.31 (d, 3 H, $J_{5,6}$ = 6.1 Hz, H-6_C) ppm. ^{13}C NMR: δ = 170.8 [C(O)], 138.6−127.7 (Ph), 101.4 (C-1_C), 100.7 (C-1_D), 100.6 (C-1_B), 94.1 (C-1_E), 84.3 (C-3_D), 82.3 (C-3_E), 79.6 (C-4_C), 79.0 (C-4_B), 78.8 (C-2_E), 78.4 (C-3_C), 77.7 (C-4_E), 76.4 (C-3_B), 75.7, 75.6 (2C, OCH_2), 75.2 (C-4_D), 75.0, 74.9, 74.3, 73.4 (4C, OCH_2), 70.7 (3C, C-2_C, 2_B, 5_D), 69.3 (C-5_C), 68.0 (C-5_B), 67.9 (C-6_E), 67.6 (C-5_E), 62.5 (C-6_D), 56.9 (OCH_3), 56.5 (C-2_D), 23.7 [C(O)CH_3], 18.2 (C-6_B), 18.1 (C-6_C) ppm. CIMS for $C_{69}H_{83}NO_{19}$ (M, 1229.56): *m/z* = 1247.7 [M + NH_4]$^+$. $C_{69}H_{83}NO_{19}$: calcd. C 67.36, H 6.80, N 1.14; found C 67.18, H 6.94, N 1.07%.

Methyl α-D-Glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1): The benzylated tetrasaccharide **15** (244 mg, 198

μmol) was dissolved in a mixture of methanol (19 mL) and acetic acid (1 mL), and treated with 10% Pd-C catalyst (100 mg). The suspension was stirred under a hydrogen atmosphere at room temp. for 2 days. At this point, TLC (solvent *E*, 7:1:2) showed that the starting material had been transformed into a major more polar product. The suspension was filtered through a bed of Celite, and the filtrate was concentrated. Reversed-phase chromatography (solvent *F*, gradient) of the residue, followed by lyophilisation, gave the target tetrasaccharide **1** as a colourless foam (112 mg, 82%). $[\alpha]_D = +1.3$ ($c = 1.0$, water). FABMS for $C_{27}H_{47}NO_{19}$ (M, 689.27): m/z = 690.5 $[M + H]^+$, 712.5 $[M + Na]^+$. ^1H and ^{13}C NMR spectroscopic data are listed in Tables 1 and 2, respectively. Because of the highly hygroscopic nature of the material, no correct elemental analysis could be obtained.

Allyl 3-*O*-*p*-Methoxybenzyl-β-L-rhamnopyranoside (17) and Allyl 3-*O*-*p*-Methoxybenzyl-α-L-rhamnopyranoside (18): Allyl L-rhamnopyranoside (**16**),[21] isolated as a 9:1 α/β mixture (7.8 g, 38.2 mmol), and dibutyl tin oxide (10.0 g, 40.2 mmol) were suspended in toluene (400 mL). The mixture was heated under reflux overnight in a Soxhlet apparatus containing activated molecular sieves (3 Å). Caesium fluoride (11.6 g, 76.4 mmol) was added to the cooled solution, and the suspension was concentrated to dryness. The residue was suspended in DMF (220 mL), *p*-methoxybenzyl chloride (11.1 mL, 76.4 mmol) was added, and the mixture was stirred at room temp. for 24 h. Evaporation of the volatiles yielded a residue that was taken up in CH_2Cl_2. The resulting suspension was filtered through a pad of Celite, and the organic phase was washed with satd. aq. $NaHCO_3$, water and satd. aq. NaCl, and dried. Evaporation of the volatiles and column chromatography of the residue (solvent *D*, 8:3) gave the α anomer **18** (8.22 g, 66%) as a colourless oil, $[\alpha]_D = -25$ ($c = 1.0$). ^1H NMR: δ = 7.31−6.90 (m, 4 H, Ph), 5.92 (m, 1 H, CH=CH_2), 5.29 (m, 1 H, CH=CH_2), 5.21 (m, 1 H, CH=CH_2), 4.87 (d, 1 H, $J_{1,2}$ = 1.2 Hz, H-1), 4.65 (d, 1 H, J = 11.2 Hz, OCH_2), 4.50 (d, 1 H, OCH_2), 4.18 (m, 1 H, OCH_2), 4.04 (m, partially overlapped, 1 H, H-2), 4.01 (m, 1 H, OCH_2), 3.82 (s, 3 H, *p*OCH_3), 3.71 (dq, 1 H, $J_{4,5}$ = 9.4 Hz, H-5), 3.67 (dd, $J_{2,3}$ = 3.2 Hz, 1 H, H-3), 3.53 (ddd, 1 H, $J_{3,4}$ = 9.3, $J_{OH,4}$ = 2.5 Hz, H-4), 2.37 (d, 1 H, $J_{OH,2}$ = 9.3 Hz, OH-2), 2.16 (d, 1 H, OH-4), 1.31 (d, 3 H, $J_{5,6}$ = 6.1 Hz, H-6) ppm. ^{13}C NMR: δ = 159.6, 133.8, 129.7, 117.4, 114.2 (*p*MeOPh, CH=CH_2), 98.4 (C-1, $^1J_{C,H}$ = 170 Hz), 79.5 (C-3), 71.5 (C-4), 71.3, 67.9 (2C, OCH_2), 67.8 (C-2), 67.7 (C-5), 55.3 (*p*OCH_3), 17.6 (C-6) ppm. FABMS for $C_{17}H_{24}O_6$ (M, 324.2): m/z = 347.4 $[M + Na]^+$. $C_{17}H_{24}O_6$: calcd. C 62.95, H 7.46; found C 62.85, H 7.58%.

Further elution of the column gave the more polar β anomer **17** (850 mg, 6.9%), which crystallised on standing, m.p. 99−101 °C (from EtOAc), $[\alpha]_D = +95.9$ ($c = 1.0$). ^1H NMR: δ = 7.39−6.88 (m, 4 H, Ph), 5.93 (m, 1 H, CH=CH_2), 5.29 (m, 1 H, CH=CH_2), 5.22 (m, 1 H, CH=CH_2), 4.73 (d, J = 11.4 Hz, 1 H, OCH_2), 4.49 (d, 1 H, OCH_2), 4.46 (br. s, 1 H, H-1), 4.41 (m, 1 H, OCH_2), 4.12 (m, 2 H, H-2, OCH_2), 3.81 (s, 3 H, *p*OCH_3), 3.61 (m, 1 H, H-4), 3.28 (m, 2 H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.2, $J_{4,5}$ = 9.6 Hz, H-3, 5), 2.35 (br. s, 1 H, OH), 2.26 (br. s, 1 H, OH), 1.36 (d, 3 H, $J_{5,6}$ = 6.1 Hz, H-6) ppm. ^{13}C NMR: δ = 159.6, 133.8, 129.6, 118.0, 114.1 (*p*MeOPh, CH=CH_2), 98.5 (C-1, $^1J_{C,H}$ = 156 Hz), 80.7 (C-3), 71.8 (C-5), 71.2 (C-4), 70.6, 69.8 (2C, OCH_2), 67.7 (C-2), 55.3 (*p*OCH_3), 17.7 (C-6) ppm. FABMS for $C_{17}H_{24}O_6$ (M, 324.2): m/z = 347.4 $[M + Na]^+$. $C_{17}H_{24}O_6$: calcd. C 62.95, H 7.46; found C 62.77, H 7.59%.

Allyl 2,4-Di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranoside (19): Sodium hydride (5.54 g of a 60% suspension in oil, 138 mmol) was added portionwise at 0 °C to a stirred solution of diol **18** (6.95 g, 21.5 mmol) in DMF (100 mL). At the end of the addition, the mixture was stirred for another hour at room temp. and then cooled to 0 °C. Benzyl bromide (12.2 mL, 103 mmol) was added dropwise. The mixture was stirred at room temp. overnight, and TLC (solvent *B*, 2:1) showed that all starting material had been transformed into a less polar product. The suspension was cooled to 0 °C, methanol was added slowly, and stirring was continued for 2 h at room temp. Volatiles were evaporated, and the residue was taken up in CH_2Cl_2, and washed with 5% aq. HCl, water, 5% aq. $NaHCO_3$, and satd. aq. NaCl, and dried. Evaporation of the volatile components and column chromatography of the residue (solvent *B*, 93:7) gave the fully protected **19** as a colourless oil (8.77 g, 81%). $[\alpha]_D = -36.7$ ($c = 1.0$). ^1H NMR: δ = 7.43−6.84 (m, 14 H, Ph), 5.83 (m, 1 H, CH=CH_2), 5.21 (m, 1 H, CH=CH_2), 5.16 (m, 1 H, CH=CH_2), 4.96 (d, 1 H, J = 10.8 Hz, OCH_2), 4.80 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.78 (d, 1 H, OCH_2), 4.73 (d, 1 H, J = 12.5 Hz, OCH_2), 4.64 (d, 1 H, OCH_2), 4.57 (s, 2 H, OCH_2), 4.13 (m, 1 H, OCH_2), 3.91 (m, 1 H, OCH_2), 3.88 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, H-3), 3.82 (s, 3 H, *p*OCH_3), 3.79 (dd, 1 H, H-2), 3.71 (dq, 1 H, $J_{4,5}$ = 9.4 Hz, H-5), 3.61 (pt, 1 H, $J_{3,4}$ = 9.3 Hz, H-4), 1.34 (d, 3 H, $J_{5,6}$ = 6.1 Hz, H-6) ppm. ^{13}C NMR: δ = 159.1 (*p*MeOPh), 138.7−126.2 (Ph), 117.1 (CH=CH_2), 113.7 (*p*MeOPh), 97.2 (C-1), 80.5 (C-4), 79.9 (C-3), 75.4 (OCH_2), 74.8 (C-2), 72.8, 71.9 (OCH_2), 68.1 (C-5), 67.6 (OCH_2), 55.3 (*p*OCH_3), 18.0 (C-6) ppm. FABMS for $C_{31}H_{36}O_6$ (M, 504.2): m/z = 527.4 $[M + Na]^+$. $C_{31}H_{36}O_6$: calcd. C 73.79, H 7.19; found C 73.79, H 7.32%.

2,4-Di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α/β-L-rhamnopyranose (20): A solution of 1,5-cyclooctadiene-bis(methyldiphenylphosphane)iridium hexafluorophosphate (50 mg, 59 μmol) in anhydrous THF (20 mL) was degassed, and the catalyst was activated by passage of a stream of hydrogen until the solution had turned yellow (ca. 3 min). The solution was degassed again, and a degassed solution of compound **19** (1.50 g, 2.98 mmol) in anhydrous THF (30 mL) was added. The reaction mixture was stirred overnight under Ar. TLC (solvent *C*, 97:3) showed that no starting material remained, and the mixture was concentrated to dryness. Mercuric oxide (1.5 g, 7.0 mmol) and mercuric chloride (4.6 g, 5.89 mmol) were added to a solution of the residue in a mixture of acetone and water (250 mL, 9:1). The suspension, protected from light, was stirred at room temp. 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 *C*, 47:3) to furnish the hemiacetal **20** (1.13 g, 82%) as a mixture of α and β anomers, which crystallised on standing; m.p. 95−97 °C (from isopropyl ether/petroleum ether), $[\alpha]_D = +6.1$ ($c = 1.0$). ^1H NMR: δ = 7.41−6.84 (m, 14 H, Ph), 5.16 (br. s, 1 H, H-1), 4.95 (d, 1 H, J = 10.9 Hz, OCH_2), 4.80 (d, 1 H, J = 12.5 Hz, OCH_2), 4.72 (d, 1 H, OCH_2), 4.65 (d, 1 H, OCH_2), 4.58 (s, 2 H, OCH_2), 3.94 (dq, 1 H, $J_{4,5}$ = 9.6 Hz, H-5), 3.93 (dd, 1 H, $J_{2,3}$ = 2.3, $J_{3,4}$ = 9.6 Hz, H-3), 3.82 (s, 3 H, *p*OCH_3), 3.79 (dd, 1 H, H-2), 3.62 (pt, 1 H, H-4), 2.73 (d, J = 3.2 Hz, 1 H, OH-1), 1.33 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6) ppm. ^{13}C NMR: δ = 159.1 (*p*MeOPh), 138.7−127.6 (Ph), 113.8 (*p*MeOPh), 93.0 (C-1), 80.4 (C-4), 79.3 (C-3), 75.3 (OCH_2), 75.0 (C-2), 72.9 (OCH_2), 72.0 (OCH_2), 68.2 (C-5), 55.3 (*p*OCH_3), 18.1 (C-6) ppm. FABMS for $C_{28}H_{32}O_6$ (M, 464.2): m/z = 487.4 $[M + Na]^+$. $C_{28}H_{32}O_6$: calcd. C 72.39, H 6.94; found C 72.27, H 7.09%.

2,4-Di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl Trichloroacetimidate (9): Trichloroacetonitrile (2.7 mL, 26.9 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 60 μL) were added to a solution of the hemiacetal **20** (995 mg, 2.14 mmol) in anhydrous

CH_2Cl_2 (1.4 mL). The solution was kept for 2 h at room temp., at which point TLC (solvent *D*, 47:3, containing Et_3N 5‰) showed that the reaction was over. Volatiles were evaporated and the residue was flash chromatographed (solvent *B*, 19:1, containing Et_3N 5‰) to give **9** (1.11 g, 85%) as a colourless oil. Compound **9** was isolated as a mixture of α and β anomers. The former showed ^1H NMR: δ = 8.52 (s, 1 H, NH), 7.45−6.81 (m, 14 H, Ph), 6.24 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.97 (d, 1 H, *J* = 10.7 Hz, OCH_2), 4.78 (s, 2 H, OCH_2), 4.66 (d, 1 H, OCH_2), 4.59 (d, 1 H, *J* = 11.5 Hz, OCH_2), 4.54 (d, 1 H, OCH_2), 3.89 (m, 2 H, $J_{2,3}$ = 3.2 Hz, H-3, 5), 3.82 (dd, 1 H, H-2), 3.81 (s, 3 H, *p*OCH_3), 3.68 (pt, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 1.36 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6) ppm. ^{13}C NMR: δ = 160.5, 159.2 (C=NH, *p*MeOPh), 138.3−127.8 (Ph), 113.8 (*p*MeOPh), 96.0 (C-1), 91.0 (CCl_3), 79.7 (C-4), 78.5 (C-3), 75.6 (OCH_2), 73.9 (C-2), 72.8, 72.0 (2C, OCH_2), 71.1 (C-5), 55.3 (*p*OCH_3), 18.0 (C-6) ppm. $C_{30}H_{32}Cl_3NO_6$: calcd. C 59.17, H 5.30, N 2.30; found C 59.25, H 5.46, N 2.19%.

Methyl (2,4-Di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (21): TMSOTf (135 μL) was added at −78 °C to a stirred solution of the acceptor **7**[17] (2.06 g, 7.47 mmol) and the donor **9** (5.91 g, 9.71 mmol) in Et_2O (250 mL). The mixture was stirred overnight while the bath temperature was slowly coming back to room temp. TLC (solvent *B*, 19:1, containing Et_3N 5%; solvent *D*, 47:3, solvent *A*, 19:1) showed that the reaction was complete, and Et_3N was added. Concentration to dryness and chromatography of the residue (solvent *A*, 49:1) gave the fully protected disaccharide **21** as a white solid (3.35 g, 62%), together with a fraction contaminated by the β-condensation product **22** (1.7 g, 31%, α/β ≈ 4:1). Compound **21** had $[\alpha]_D$ = −32.8 (*c* = 1.0). ^1H NMR: δ = 7.36−6.82 (m, 14 H, Ph), 5.42 (d, 1 H, $J_{NH,2}$ = 8.5 Hz, NH), 4.92 (d, 1 H, *J* = 10.8 Hz, OCH_2), 4.90 (br. s, 1 H, H-1_C), 4.74−4.52 (m, 5 H, OCH_2), 4.58 (d, 1 H, H-1_D), 4.05−3.91 (d, 3 H, H-5_C, 3_D, $6a_D$), 3.81 (s, 3 H, *p*OCH_3), 3.82−3.77 (m, 2 H, H-3_C, $6b_D$), 3.73 (dd, $J_{2,3}$ = 3.1 Hz, 1 H, H-2_C), 3.60−3.49 (m, 3 H, H-4_C, 4_D, 2_D), 3.46 (s, 3 H, OCH_3), 3.31 (m, 1 H, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 5.3 Hz, H-5_D), 1.84 [s, 3 H, C(O)CH_3], 1.46, 1.40 [(2s, 6 H, $C(CH_3)_2$], 1.27 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_C) ppm. ^{13}C NMR: δ = 170.2 [C(O)], 159.1 (*p*MeOPh), 138.9−127.5 (Ph), 113.7 (pMeOPh), 101.6 (C-1_D), 99.6 [$C(CH_3)_2$], 99.0 (C-1_C), 80.2 (C-4_C), 79.7 (C-3_C), 76.7 (C-3_D), 75.8 (C-2_C), 75.0, 72.8 (2C, OCH_2), 72.7 (C-4_D), 71.9 (OCH_2), 68.3 (C-5_C), 67.2 (C-3_D), 62.2 (C-6_D), 57.2 (C-2_D), 56.7 (OCH_3), 55.3 (*p*OCH_3), 29.1 [$C(CH_3)_2$], 23.5 [C(O)CH_3], 19.2 [$C(CH_3)_2$], 18.0 (C-6_C) ppm. FABMS for $C_{40}H_{51}O_{11}$ (M, 721.35): *m*/*z* = 744.4 $[M + Na]^+$. $C_{40}H_{51}O_{11}\cdot H_2O$: calcd. C 64.94, H 7.22, N 1.89; found C 64.88, H 6.91, N 2.09%.

Methyl (2,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (5): Water (2.25 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.72 g, 7.74 mmol) were added at 0 °C to a solution of the pure disaccharide **21** (3.65 g, 5.06 mmol) in CH_2Cl_2 (40 mL). After 1 h at this temperature, the mixture was treated with satd. aq. $NaHCO_3$ until complete dissolution of the precipitate, and then extracted with CH_2Cl_2. The organic phase was washed with water and satd. aq. NaCl, and then concentrated to an oily residue, which was chromatographed (solvent *A*, 49:1) to give **5** (2.91 g, 95%) as a white foam. $[\alpha]_D$ = −29 (*c* = 1.0). ^1H NMR: δ = 7.39−7.10 (m, 10 H, Ph), 5.66 (d, 1 H, $J_{NH,2}$ = 8.3 Hz, NH), 5.00 (d, 1 H, $J_{1,2}$ = 0.9 Hz, H-1_C), 4.84 (d, 1 H, *J* = 11.3 Hz, OCH_2), 4.68 (d, 1 H, *J* = 11.7 Hz, OCH_2), 4.66 (d, 1 H, OCH_2), 4.68 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.53 (d, 1 H, OCH_2), 4.09 (pt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 8.2 Hz, H-3_D), 4.00 (dq, 1 H, $J_{4,5}$ = 9.4 Hz, H-5_C), 3.97−3.87 (m, 2 H, H-3_C, $6a_D$), 3.79 (d, 1 H, $J_{6a,6b}$ = 10.4 Hz, H-$6b_D$), 3.74 (dd, 1 H, $J_{2,3}$ = 3.5 Hz, H-2_C), 3.58 (pt, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 4_D), 3.49 (m, 1 H, H-2_D), 3.46 (s, 3 H, OCH_3), 3.38−3.28 (m, 2 H, H-4_C, 5_D), 2.27 (d, 1 H, $J_{OH,3}$ = 8.8 Hz, OH-3_C), 1.84 [s, 3 H, C(O)CH_3], 1.47, 1.41 [2s, 6 H, $C(CH_3)_2$], 1.28 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_C) ppm. ^{13}C NMR: δ = 170.3 [C(O)], 138.6−127.6 (Ph), 101.4 (C-1_D, $^1J_{C,H}$ = 162 Hz), 99.5 [$C(CH_3)_2$], 97.7 (C-1_C, $^1J_{C,H}$ = 168 Hz), 81.9 (C-4_C), 79.2 (C-2_C), 76.3 (C-3_D), 74.8, 72.9 (2C, OCH_2), 72.8 (C-4_D), 71.4 (C-3_C), 67.5 (C-5_C), 67.2 (C-5_D), 62.2 (C-6_D), 57.6 (C-2_D), 56.8 (OCH_3), 29.2 [$C(CH_3)_2$], 23.7 [C(O)CH_3], 19.3 [$C(CH_3)_2$], 18.1 (C-6_C) ppm. FABMS for $C_{32}H_{43}NO_{10}$ (M, 601.29): *m*/*z* = 624.3 $[M + Na]^+$. $C_{32}H_{43}NO_{10}$: calcd. C 63.88, H 7.20, N 2.33; found C 63.76, H 7.26, N 2.35%.

When a contaminated fraction of **21** (2.22 g, 3.08 mmol) was treated as described above, TLC (solvent *A*, 19:1) showed that after 1 h, the reaction did not proceed any further, although some starting material appeared to remain. Chromatography of the residue afforded methyl (2,4-di-*O*-benzyl-β-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**23**) as the first eluting product (97 mg, 5%) together with the more polar **5** (1.61 g, 87%). Available data for compound **23** are ^1H NMR: δ = 7.37−7.15 (m, 10 H, Ph), 5.87 (d, 1 H, $J_{NH,2}$ = 7.3 Hz, NH), 4.95 (d, 1 H, *J* = 11.9 Hz, OCH_2), 4.91 (d, 1 H, *J* = 11.0 Hz, OCH_2), 4.78 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.64−4.54 (m, 3 H, H-1_C, OCH_2), 4.10 (pt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3_D), 3.94 (dd, 1 H, $J_{5,6}$ = 5.4, $J_{6a,6b}$ = 10.8 Hz, H-$6a_D$), 3.82−3.74 (m, 2 H, H-2_C, $6b_D$), 3.63−3.57 (m, 2 H, H-4_D, 3_C), 3.47 (s, 3 H, OCH_3), 3.41−3.18 (m, 4 H, H-2_D, 5_D, 5_C, 4_C), 2.50 (d, 1 H, *J* = 10.0 Hz, OH-3_C), 1.96 [s, 3 H, C(O)CH_3], 1.46, 1.37 (2s, 6 H, $C(CH_3)_2$], 1.35 (d, 3 H, $J_{5,6}$ = 5.7 Hz, H-6_C) ppm. ^{13}C NMR: δ = 171.3 [C(O)], 138.5−127.5 (Ph), 102.1 (C-1_D, $^1J_{C,H}$ not extractable due to overlapping), 102.0 (C-1_C, $^1J_{C,H}$ not extractable due to overlapping), 99.3 [$C(CH_3)_2$], 81.9 (C-4_C), 78.2 (C-2_C), 76.9 (C-3_D), 75.0 (2C, OCH_2), 74.5 (C-4_D*), 74.1 (C-3_C*), 71.4 (C-5_C), 66.8 (C-5_D), 62.2 (C-6_D), 57.0, 56.9 (2C, C-2_D, OCH_3), 29.0 [$C(CH_3)_2$], 23.7 [C(O)CH_3], 19.1 [$C(CH_3)_2$], 18.2 (C-6_C) ppm. CIMS for $C_{32}H_{43}NO_{10}$ (M, 601.29): *m*/*z* = 602 $[M + H]^+$, 619 $[M + NH_4]^+$.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (24): TMSOTf (80 μL) was added at −50 °C to a solution of donor **3**[14] (5.59 g, 5.45 mmol) and acceptor **5** (2.57 g, 4.27 mmol) in anhydrous CH_2Cl_2 (60 mL). The reaction mixture was stirred overnight in a cold room while the bath temperature was slowly coming back to 5 °C. TLC (solvent *A*, 24:1) showed that hardly any acceptor remained. Et_3N was added and volatiles were evaporated. Chromatography (solvent *A*, 199:1) provided the fully protected tetrasaccharide **24** (4.94 g, 79%) as a white foam. $[\alpha]_D$ = +26 (*c* = 1.0). ^1H NMR: δ = 8.05−7.05 (m, 40 H, Ph), 5.78 (br. s, 1 H, H-2_B), 5.51 (d, 1 H, $J_{NH,2}$ = 8.1 Hz, NH), 5.28 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1_E), 5.25 (br. s, 1 H, H-1_B), 4.98 (d, 1 H, *J* = 10.4 Hz, OCH_2), 4.94 (d, 1 H, *J* = 11.2 Hz, OCH_2), 4.90 (br. s, 1 H, H-1_C), 4.85−4.36 (m, 12 H, OCH_2), 4.63 (d, overlapped, 1 H, H-1_D), 4.43 (d, partially overlapped, 1 H, H-3_B), 4.18−3.90 (m, 7 H, H-3_D, 3_E, 3_C, 5_E, 5_B, $6a_D$, 5_C), 3.79−3.61 (m, 4 H, H-$6b_D$, 4_B, 2_C, 4_E), 3.57−3.46 (m, 5 H, H-4_C, 2_E, 4_D, $6a_E$, 2_D), 3.45 (s, 3 H, OCH_3), 3.41−3.27 (m, 2 H, H-$6b_E$, 5_D), 1.82 [s, 3 H, C(O)CH_3], 1.45 [s, 3 H, $C(CH_3)_2$], 1.41 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_B), 1.38 [s, 3 H, $C(CH_3)_2$], 1.20 (d, 3 H, $J_{5,6}$ = 6.1 Hz, H-6_C) ppm. ^{13}C NMR: δ = 170.1, 165.7 [C(O)], 138.7−127.2 (Ph), 101.3 (C-1_D, $^1J_{C,H}$ = 158 Hz), 99.5 [$C(CH_3)_2$], 99.2 (br. s, C-1_B, $^1J_{C,H}$ = 170 Hz), 98.2 (C-1_C, $^1J_{C,H}$ = 170 Hz), 92.5 (C-1_E, $^1J_{C,H}$ = 168 Hz), 82.0 (C-3_E), 80.5 (C-4_C), 79.9 (C-4_E), 79.1 (C-2_E), 78.3 (br. s, C-3_D), 78.1 (br. s, C-2_C), 77.5 (C-4_B), 76.1 (C-3_C, OCH_2), 75.5, 75.2,

74.9, 73.2 (4C, OCH_2), 72.9 (C-4_D), 72.7 (OCH_2), 72.4 (C-3_B), 72.2 (OCH_2), 70.3 (C-5_E), 68.7 (C-5_B*), 68.4 (C-2_B*), 68.3 (C-5_C*), 68.1 (C-6_E), 67.2 (C-5_D), 62.3 (C-6_D), 57.7 (C-2_D), 56.8 (OCH_3), 29.1 [$C(CH_3)_2$], 23.5 [C(O)CH_3], 19.2 [$C(CH_3)_2$], 18.3 (C-6_B), 18.0 (C-6_C) ppm. FABMS for $C_{86}H_{97}NO_{20}$ (M, 1463.66): *m*/*z* = 1486.5 [M + Na]$^+$. $C_{86}H_{97}NO_{20}$: calcd. C 70.52, H 6.68, N 0.96; found C 70.10, H 6.67, N 0.97%.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (25): A solution of the fully protected tetrasaccharide **24** (220 mg, 150 μmol) in a mixture of MeOH (650 μL) and CH_2Cl_2 (800 μL) was treated with 1 N NaOMe (460 μL) until TLC (solvent *A*, 49:1) showed that no starting material remained (several days). After neutralisation with Amberlite IR-120 (H^+), filtration and evaporation of the solvent, the crude product was chromatographed (solvent *A*, 197:3) to give the acceptor **25** (193 mg, 94%) as a white foam, $[\alpha]_D$ = +14.7 (*c* = 1.0). ^{1}H NMR: δ = 7.37−7.10 (m, 35 H, Ph), 5.59 (d, 1 H, $J_{NH,2}$ = 8.1 Hz, NH), 5.23 (br. s, 1 H, H-1_B), 4.95 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1_C), 4.92−4.45 (m, 13 H, OCH_2), 4.86 (d, 1 H, H-1_E), 4.70 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.26 (d, 1 H, *J* = 1 2.0 Hz, OCH_2), 4.14 (pt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3_D), 4.08−3.90 (m, 7 H, H-3_C, 3_B, 3_E, 2_B, 5_C, $6a_D$, 5_E), 3.87 (dq, 1 H, $J_{4,5}$ = 9.7 Hz, H-5_B), 3.77 (m, 1 H, $J_{6a,6b}$ = 10.3 Hz, H-$6b_D$), 3.72 (m, 2 H, H-4_E, 2_C), 3.63−3.28 (m, 11 H, H-4_D, 2_E, 4_C, 4_B, 2_D, OCH_3, $6a_E$, 5_D, $6b_E$), 1.88 [s, 3 H, C(O)CH_3], 1.72 (br. s, 1 H, OH-2_B), 1.49, 1.41 [s, 3 H, $C(CH_3)_2$], 1.33 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_B), 1.25 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_C) ppm. ^{13}C NMR: δ = 170.2 [C(O)], 138.6−127.5 (Ph), 101.3 (C-1_D), 101.0 (br. s, C-1_B), 99.5 [$C(CH_3)_2$], 97.9 (br. s, C-1_C), 93.8 (C-1_E), 82.4 (C-3_E), 80.8 (br. s, C-4_C*), 79.3 (C-4_B), 78.9 (C-2_E*), 78.3 (C-4_E*), 78.0 (br. s, C-3_B), 77.7 (C-2_C*), 76.6 (C-3_C), 75.9 (C-3_D), 75.6, 75.4, 75.0, 74.9, 74.1, 73.4 (6C, OCH_2), 73.0 (C-4_D), 72.4 (OCH_2), 70.7 (C-5_E), 68.3 (br. s, C-5_C), 67.9 (C-6_E), 67.8 (C-5_B), 67.7 (C-2_B), 67.2 (C-5_D), 62.3 (C-6_D), 57.9 (C-2_D), 56.8 (OCH_3), 29.1 [$C(CH_3)_2$], 23.5 [C(O)CH_3], 19.2 [$C(CH_3)_2$], 18.0 (C-6_B), 17.9 (C-6_C) ppm. FABMS for $C_{79}H_{93}NO_{19}$ (M, 1359.63): *m*/*z* = 1382.7 [M + Na]$^+$. $C_{79}H_{93}NO_{19}$: calcd. C 69.74, H 6.89, N 1.03; found C 69.56, H 6.98, N 1.09%.

Methyl (2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)]-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (26): TMSOTf (8 μL) was added at −60 °C to a solution of donor **6**[16] (456 mg, 725 μmol) and acceptor **25** (500 mg, 367 μmol) in anhydrous CH_2Cl_2 (10 mL). The reaction mixture was stirred overnight while the bath temperature was slowly coming back to 20 °C. TLC (solvent *A*, 97:3) showed that some acceptor still remained. However, in order to prevent any degradation, the reaction was stopped at this stage by addition of Et_3N. Volatiles were evaporated, and the residue was chromatographed (solvent *A*, 199:1) to give the fully protected pentasaccharide **26** (434 mg, 65%) as the first eluting product. The remaining acceptor **25** (165 mg, 33%) was eluted next. Compound **26**, isolated as a white foam, had $[\alpha]_D$ = +33.2 (*c* = 1.0). ^{1}H NMR: δ = 8.01−7.06 (m, 50 H, Ph), 6.07 (dd, 1 H, $J_{1,2}$ = 1.7 Hz, H-2_A), 5.92 (br. s, 1 H, H-1_A), 5.91 (dd, 1 H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 10.0 Hz, H-3_A), 5.63 (pt, 1 H, $J_{4,5}$ = 10.0 Hz, H-4_A), 5.50 (bd, 1 H, NH), 5.26 (br. s, 1 H, H-1_B), 4.98 (br. s, 1 H, H-1_C), 4.94−4.48 (m, 13 H, OCH_2), 4.83 (d, overlapped, 1 H, H-1_E), 4.64 (d, overlapped, 1 H, H-1_D), 4.30−3.93 (m, 11 H, H-5_A, 2_B, 3_B, 3_D, OCH_2, 3_C, 3_E, 5_C, 5_E, 5_B, $6a_D$), 3.86−3.71 (m, 3 H, H-4_B, $6b_D$, 2_C), 3.65−3.55 (m, 3 H, H-4_E, 4_D, 4_C), 3.48 (s, 3 H, OCH_3), 3.46−3.32 (m, 3 H, H-2_D, 2_E, 5_D), 3.26 (d, 1 H, H-$6a_E$), 3.15 (d, 1 H, $J_{6a,6b}$ = 10.0 Hz, H-$6b_E$), 1.90 [s, 3 H, C(O)CH_3], 1.51 [s, 3 H, $C(CH_3)_2$], 1.49 (d, overlapped, 3 H, H-6_B), 1.42 [s, 3 H, $C(CH_3)_2$], 1.24 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_A), 1.19 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_C) ppm. ^{13}C NMR: δ = 170.0, 165.8, 165.5, 165.0 (4C, C(O)], 138.8−127.2 (Ph), 101.2 (C-1_D, $^1J_{C,H}$ was not extractable), 101.1 (br. s, C-1_B, $^1J_{C,H}$ = 168 Hz), 99.5 [$C(CH_3)_2$], 98.4 (C-1_A, $^1J_{C,H}$ = 168 Hz), 97.9 (C-1_C, $^1J_{C,H}$ = 167 Hz), 96.0 (C-1_E, $^1J_{C,H}$ = 170 Hz), 82.1 (C-3_E), 80.4 (C-4_C), 79.5 (C-4_B), 78.8 (br. s, C-3_C), 78.3 (2C, C-2_E, 2_C), 77.6 (C-4_E), 76.9 (C-3_B), 76.2 (C-3_D), 75.6, 75.4, 75.0, 74.8 (4C, OCH_2), 74.3 (C-2_B), 73.4, 73.2 (2C, OCH_2), 73.0 (C-4_D), 72.3 (OCH_2), 72.0 (C-4_A), 70.4 (C-5_E*), 70.2 (2C, C-2_A, 3_A), 69.6 (C-5_B), 68.3 (C-5_C*), 67.8 (C-6_E), 67.2 (2C, C-5_A, 5_D), 62.3 (C-6_D), 58.1 (C-2_D), 56.9 (OCH_3), 29.1 [$C(CH_3)_2$], 23.5 [C(O)CH_3], 19.2 [$C(CH_3)_2$], 18.2 (C-6_B), 17.9 (C-6_C), 17.9 (C-6_A) ppm. FABMS for $C_{106}H_{116}NO_{26}$ (M, 1817.77): *m*/*z* = 1841.7 [M + Na]$^+$. $C_{106}H_{115}NO_{26}$: calcd. C 69.99, H 6.37, N 0.77; found C 69.85, H 6.35, N 0.89%.

Methyl (2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)]-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (27): A solution of the fully protected pentasaccharide **26** (287 mg, 158 μmol) in CH_2Cl_2 (5 mL), stirred at 0 °C, was treated with 50% aq. CF_3CO_2H (2 mL) for 45 min. TLC (solvent *A*, 97:3) showed that all starting material had been converted into a more polar product. Volatiles were eliminated by repeated coevaporation with toluene and the residue was column chromatographed to give diol **27** (247 mg, 88%) as a white foam, $[\alpha]_D$ = +38.5 (*c* = 0.84). ^{1}H NMR: δ = 7.99−7.06 (m, 50 H, Ph), 6.05 (br. s, 1 H, H-2_A), 5.94 (br. s, 1 H, H-1_A), 5.90 (dd, 1 H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 10.2 Hz, H-3_A), 5.66 (dd, 1 H, $J_{4,5}$ = 9.9 Hz, H-4_A), 5.52 (bd, 1 H, NH), 5.19 (br. s, 1 H, H-1_B), 4.91 (br. s, 1 H, H-1_C), 4.93−4.31 (m, 13 H, OCH_2), 4.82 (m, 2 H, H-1_E, 1_D), 4.29−4.08 (m, 6 H, H-2_B, 5_A, 3_B, 3_D, OCH_2, 3_C), 4.04−3.77 (m, 7 H, H-3_E, 5_E, $6a_D$, 5_C, 5_B, $6b_D$, 4_B), 3.69 (br. s, 1 H, H-2_C), 3.62 (m, 2 H, H-4_E, 4_C), 3.51 (s, 3 H, OCH_3), 3.46−3.43 (m, 3 H, H-2_E, 4_D, 5_D), 3.26 (d, 1 H, H-$6a_E$), 3.17 (d, 1 H, $J_{6a,6b}$ = 9.8 Hz, H-$6b_E$), 3.07 (m, 1 H, H-2_D), 2.14 (d, 1 H, OH-6_D), 1.81 [s, 3 H, C(O)CH_3], 1.38 (d, 3 H, $J_{5,6}$ = 5.2 Hz, H-6_B), 1.26 (d, partially overlapped, 3 H, $J_{5,6}$ = 5.6 Hz, H-6_A), 1.24 (d, partially overlapped, 3 H, $J_{5,6}$ = 6.2 Hz, H-6_C) ppm. ^{13}C NMR: δ = 170.2, 165.8, 165.6, 165.0 [4C, C(O)], 138.7−127.3 (Ph), 101.1 (br. s, C-1_B), 100.5 (C-1_D), 99.7 (C-1_C), 99.4 (C-1_A), 96.1 (C-1_E), 84.2 (C-3_D), 82.1 (C-3_E), 79.7 (C-4_C*), 79.3 (C-4_B), 78.2 (C-2_E), 77.6 (C-4_E*), 77.3 (br. s, C-3_C), 77.2 (C-2_C), 76.8 (C-3_B), 75.7, 75.4, 75.3 (3C, OCH_2), 75.1 (C-4_D), 74.8 (OCH_2), 73.9 (C-2_B), 73.5, 73.3, 72.2 (3C, OCH_2), 72.0 (C-4_A), 71.2 (C-5_D), 70.4 (C-5_E), 70.2 (2C, C-2_A, 3_A), 69.8 (C-5_C), 69.5 (C-5_B), 67.8 (C-6_E), 67.2 (C-5_A), 62.8 (C-6_D), 57.1 (C-2_D), 57.0 (OCH_3), 23.3 [C(O)CH_3], 17.8 (C-6_B), 17.7 (C-6_{A*}), 17.5 (C-6_{C*}) ppm. FABMS for $C_{103}H_{111}NO_{26}$ (M, 1777.74): *m*/*z* = 1800.8 [M + Na]$^+$. $C_{103}H_{111}NO_{26}$: calcd. C 69.54, H 6.29, N 0.79; found C 69.38, H 6.37, N 0.90%.

Methyl α-L-Rhamnopyranosyl-(1→2)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)]-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (28): A solution of diol **27** (368 mg, 207 μmol) in MeOH (5 mL) was treated overnight with a catalytic amount of 1 N NaOMe. TLC (solvent *A*, 47:3) showed that no starting material remained. After neutralisation with Amberlite IR-120 (H^+), filtration and evaporation of the solvent, the crude product was chromatographed (solvent *A*, 193:7 → 189:11) to give the pentaol **28** (291 mg, 96%) as a white foam, $[\alpha]_D$ = +9.3 (*c* = 1.0). ^{1}H NMR: δ = 7.36−7.05 (m, 35 H, Ph), 6.02 (br. s, 1 H, NH), 5.23 (br. s, 1 H, H-1_A), 5.05 (br. s, 1 H, H-1_B), 4.97−4.39 (m, 13

H, OCH_2), 4.93 (d, 1 H, H-1_E), 4.87 (br. s, 1 H, H-1_C), 4.75 (d, 1 H, H-1_D), 4.17−4.10 (m, 3 H, H-3_B, 2_B, OCH_2), 4.07−3.65 (m, 13 H, H-3_D, 3_E, 5_E, 3_C, 2_A, $6a_D$, 5_C, 5_B, $6b_D$, 3_A, 4_E, 5_A, 2_C), 3.62−3.48 (m, 3 H, H-4_C, 2_E, 4_B), 3.47 (s, 3 H, OCH_3), 3.43−3.30 (m, 4 H, H-$6a_E$, 4_D, 5_D, 4_A), 3.26 (d, 1 H, $J_{6a,6b}$ = 10.0 Hz, H-$6b_E$), 3.16 (m, 1 H, H-2_D), 2.95, 2.70, 2.35, 2.08 (m, 4 H, OH), 1.87 [s, 3 H, $C(O)CH_3$], 1.27 (d, 3 H, $J_{5,6}$ = 6.1 Hz, H-6_B), 1.23 (d, 6 H, H-6_A, 6_C) ppm. ^{13}C NMR: δ = 170.7 [C(O)], 138.6−127.5 (Ph), 101.6 (br. s, C-1_B), 100.7 (br. s, C-1_A), 100.6 (C-1_D), 99.1 (C-1_C), 94.8 (C-1_E), 83.6 (C-3_D), 82.1 (C-3_E), 79.7 (C-4_B), 79.4 (C-4_C), 78.9 (C-2_E), 77.9 (C-4_E), 77.8 (br. s, C-3_C), 77.4 (C-2_C), 75.7 (2C, C-3_B, OCH_2), 75.5, 75.3 (2C, OCH_2), 75.2 (C-4_D), 73.4 (C-4_A), 73.3 (OCH_2), 73.0 (br. s, C-2_B), 72.2 (OCH_2), 71.4 (C-3_A), 70.8 (C-2_A*), 70.7 (C-5_D), 70.3 (C-5_E*), 69.6 (C-5_C), 69.1 (C-5_B), 68.7 (C-5_A), 68.0 (C-6_E), 62.5 (C-6_D), 56.7 (OCH_3), 56.5 (C-2_D), 23.5 [$C(O)CH_3$], 18.1, 18.0, 17.8 (3C, C-6_B, 6_A, 6_C) ppm. FABMS for $C_{82}H_{99}NO_{23}$ (M, 1465.66): *m/z* = 1488.7 $[M + Na]^+$. $C_{82}H_{99}NO_{23}\cdot H_2O$: calcd. C 66.34, H 6.86, N 0.94; found C 66.46, H 7.05, N 1.06%.

Methyl α-L-Rhamnopyranosyl-(1→2)-[α-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (2): The benzylated pentasaccharide **28** (286 mg, 193 μmol) was dissolved in a 4:1 methanol/acetic acid mixture (10 mL) and treated with 10% Pd-C catalyst (200 mg). The suspension was stirred under a hydrogen atmosphere at room temp. for 4 days. At this point, TLC (solvent *E*, 7:1:2) showed that the starting material had been transformed into a more polar major product. The suspension was filtered through a bed of Celite, and the filtrate was concentrated. Reversed-phase chromatography (solvent *F*, gradient) of the residue, followed by lyophilisation, gave the target pentasaccharide **2** (121 mg, 75%) as a colourless foam. $[\alpha]_D$ = −14.4 (*c* = 1.0, water). FABMS for $C_{33}H_{57}NO_{23}$ (M, 835.3): *m/z* = 858.4 $[M + Na]^+$. ^{1}H and ^{13}C NMR spectroscopic data are listed in Tables 1 and 2, respectively. Because of the highly hygroscopic nature of the material, no correct elemental analysis could be obtained.

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