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Convergent synthesis of an elusive hexasaccharide corresponding to the cell-wall polysaccharide of the β-hemolytic *Streptococcus* Group A

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ABSTRACT

A convergent synthesis of a hexasaccharide corresponding to the cell-wall polysaccharide of the β-hemolytic *Streptococcus* Group A is described. The strategy relies on the preparation of a key linear trisaccharide unit β-D-Glc*p*NAc-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p* which has previously resisted our efforts. The trisaccharide functions both as a glycosyl acceptor and donor to give an elusive hexasaccharide. This fully functionalized unit can serve, in turn, as a glycosyl acceptor or donor for the synthesis of higher-order structures. Deprotection gives a hitherto unknown hexasaccharide for use as a hapten in immunochemical studies. The characterization of all compounds by high-resolution ^{1}H and ^{13}C NMR spectroscopy is also described.

INTRODUCTION

In recent years, we have actively pursued a program to develop and characterize antibody-based immunodiagnostic reagents with specificity for various carbohydrate epitopes present on the cell-wall polysaccharide of *Streptococcus* Group A with which to probe the relationship between *Streptococcus* Group A infections and autoimmune diseases such as acute rheumatic carditis[1]. As part of this effort, we have focused on the synthesis of increasingly complex oligosaccharides that constitute different epitopes of the cell-wall polysaccharide, and on the development of convergent synthetic routes that would readily furnish higher-order structures[2–5]. Although we have enjoyed considerable success in this endeavour, an efficient route based on one particular disconnection has eluded us thus far. The disconnection of interest yields a key linear trisaccharide, β-D-Glc*p*NAc-(1 →

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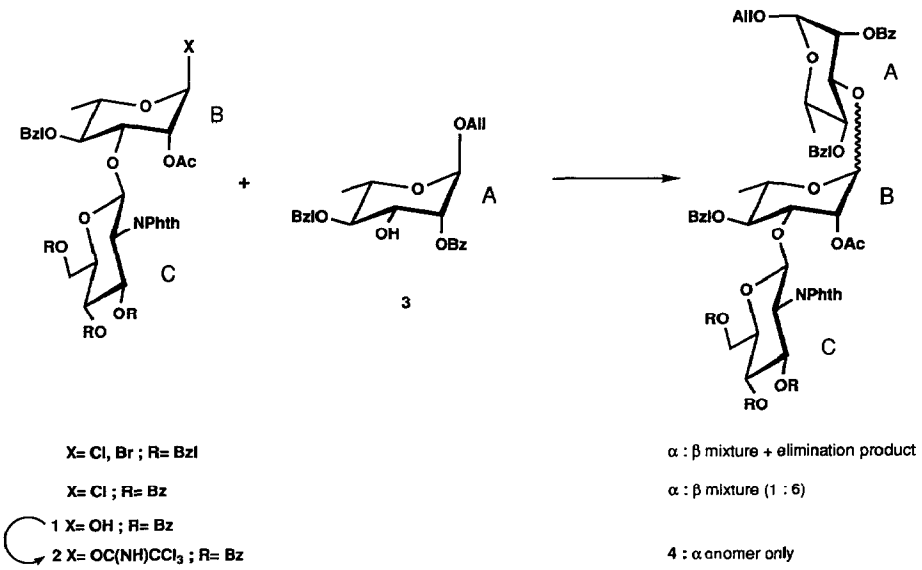
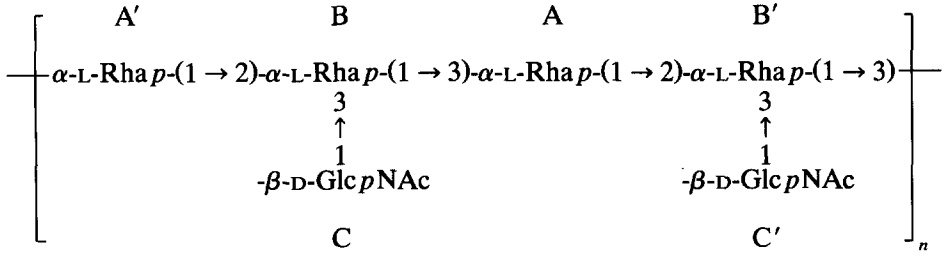


Scheme 1.

3)-α-L-Rh*p*-(1 → 3)-α-L-Rh*p* synthon which would function both as a glycosyl donor and acceptor in future glycosylation reactions. The proposed syntheses of two suitable trisaccharides, starting from the disaccharide halides, are illustrated in Scheme 1. However, despite numerous attempts, using various combinations of promoters, bases, and reaction media, we have been unable to achieve a satisfactory outcome for these reactions[1]. Thus, the reactions have proceeded with poor stereoselectivity and have, surprisingly, yielded the unwanted β anomer as the major product (Scheme 1). A further complication was the formation of a significant amount of the 1,2-elimination product (Scheme 1). The results suggested that the disaccharide donors were too reactive to permit good stereoselectivity. Accordingly, we chose to examine other disaccharide donors which could be activated under milder reaction conditions. We now report the use of a disaccharide, as its glycosyl trichloroacetimidate[6], to give the key linear trisaccharide unit, and the successful implementation of our original strategy to yield an elusive hexasaccharide. This convergent synthetic route also affords a fully functionalized hexasaccharide unit that can readily serve as a precursor of even higher-order structures.

RESULTS AND DISCUSSION

The cell-wall polysaccharide of the *Streptococcus* Group A is comprised of a rhamnose backbone consisting of alternating α-L-(1 → 2) and α-L-(1 → 3) linkages, with *N*-acetyl-β-D-glucosamine residues attached to the 3-positions of the rhamnose backbone[7].

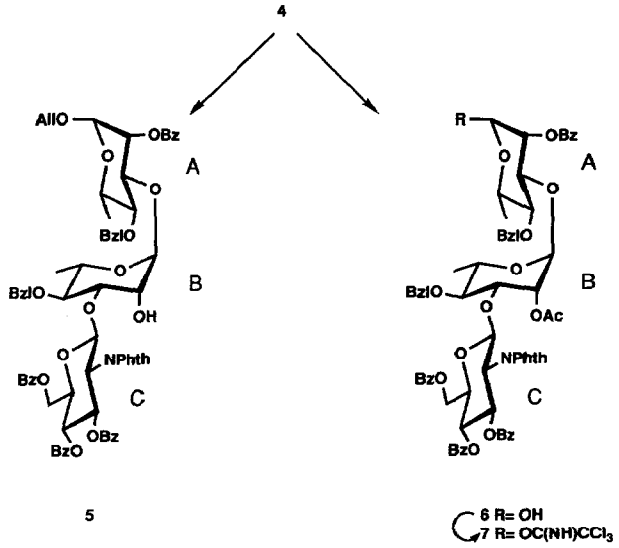


Retrosynthetic analysis indicated that disconnections based on key linear ABC or branched B(C)A' trisaccharide sequences would be desirable for the synthesis of higher-order structures. We have previously reported the synthesis of a hexasaccharide based on the latter strategy[5]. However, all our attempts to implement the former strategy have been thwarted by our inability to achieve an efficient synthesis of the key trisaccharide unit (see Introduction). We now report the resolution of the problem and the successful application of the strategy to the synthesis of a frame-shifted hexasaccharide.

The hemiacetal **1** was obtained from the corresponding allyl glycoside[5] by treatment with Wilkinson's catalyst[8], followed by hydrolysis of the resulting propenyl glycosides[9]. The glycosyl trichloroacetimidate **2** was then obtained from **1** by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and trichloroacetonitrile[10]. With the disaccharide donor **2** in hand, we were now in a position to attempt the critical glycosidation reaction. Unlike the outcome of the previous reactions with the glycosyl halides as donors[1], glycosidation of allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside[11] (**3**), with the trichloroacetimidate donor **2** proceeded without incident. Indeed, the reaction in dichloromethane with triethylsilyl trifluoromethanesulfonate as catalyst (0.05 equiv), at −78°C, was complete in 10 min, and afforded the desired trisaccharide **4** in 89% yield (Scheme 1).

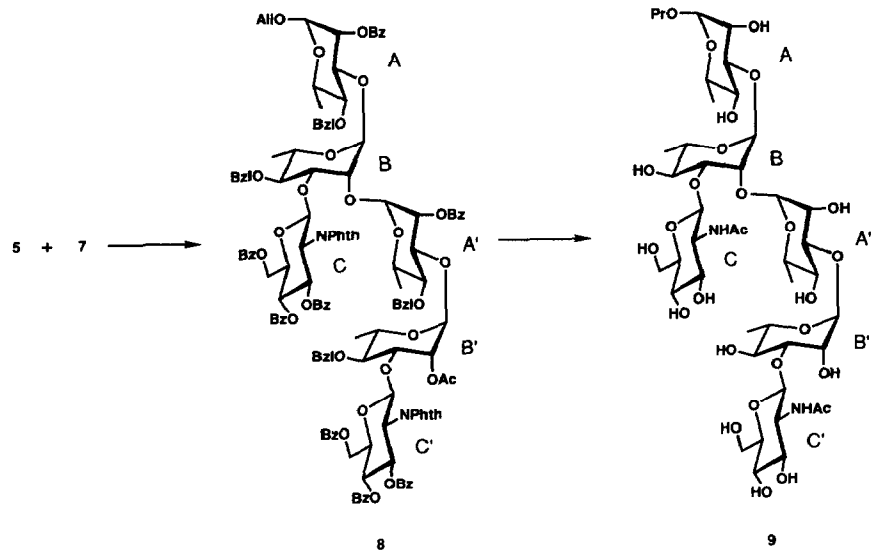
The key ABC trisaccharide **4** could function, in principle, as both a glycosyl donor and acceptor in future glycosidation reactions. Thus, selective removal of the acetate ester in the presence of the benzoate esters by treatment with methanolic HCl[12] gave the trisaccharide acceptor **5** in 82% yield (Scheme 2). The encouraging results obtained in the glycosidation of the disaccharide donor **2** prompted us to examine the use of a trisaccharide donor as its glycosyl trichloroacetimidate. Accordingly, successive conversion of **4** to the hemiacetal **6** and the trichloroacetimidate **7**, as described for the case of the disaccharides **1** and **2**, afforded the corresponding donor (Scheme 2).

Glycosidation of the trisaccharide acceptor **5** with the trisaccharide donor **7** (Scheme 3) was performed in an analogous fashion to the synthesis of trisaccharide **4**, and proceeded with exclusive α-stereoselectivity to give **8** in 68% yield. The hexasaccharide **8** thus prepared has the same features as the parent trisaccharide **4**, namely, an allyl group as the aglycon which could be removed to generate a glycosyl donor, and the acetate group which could be selectively removed to generate a glycosyl acceptor. This result confirms the utility of the key trisaccharide **4** as a common intermediate in the synthesis of higher-order structures of the



Scheme 2.

Streptococcus Group A cell-wall polysaccharide and defines a second efficient, convergent synthetic route[5]. The hexasaccharide **8** results from only three glycosidation reactions and a nonasaccharide or dodecasaccharide could, in principle, be derived from a fourth glycosidation reaction. The synthesis of higher-order struc-



Scheme 3.

tures that represent different epitopes will allow better definition of the extended binding sites displayed by monoclonal antibodies raised against a streptococcal vaccine[1,13,14] and should permit the design of improved immunodiagnostic reagents and vaccines.

The deblocked hexasaccharide **9** was obtained from **8** by successive treatment with (*1*) sodium methoxide in methanol to remove the esters, (*2*) hydrogenolysis of the benzyl ethers and hydrogenation of the allyl group with palladium on carbon, and (*3*) hydrazinolysis of the phthalimido group and *N*-acetylation of the resultant amine.

Compounds were fully characterized by high resolution ^1H and ^{13}C NMR spectroscopy (Tables I, II and III). ^1H-Homonuclear chemical-shift correlated (COSY) experiments[15] and ^{13}C–^1H chemical-shift correlated experiments[16] were performed, as necessary, in order to facilitate assignments. In the case of the hexasaccharide (**9**), TOCSY[17] and ROESY[18] experiments were also performed. The ^{13}C–^1H chemical-shift correlated experiments were carried out in the inverse mode[19–21], thereby taking advantage of the sensitivity of the ^1H nucleus. Experi-

TABLE I

^1H and ^{13}C NMR data [a] for compounds **1** and **2**

Ring	^1H NMR		^{13}C NMR
	1 [b]	**2**	**1** [b]
1B	5.07	6.14	91.7
		(2.0)	
2B	5.46	5.52	72.0
	(1.8, 3.5)	(2.0, 3.0)	
3B	4.27	4.20	79.1
	(3.5, 9.5)	(3.0, 9.0)	
4B	[c]	3.46	79.2
		(9.0, 9.0)	
5B	3.87	3.82	67.4
6B	1.09	1.12	17.7
1C	5.95	5.85	98.9
	(8.5)	(8.0)	
2C	[c]	4.68	55.1
		(8.0, 11.0)	
3C	6.26	6.24	71.3
	(9.5, 10.6)	(11.0, 9.0)	
4C	5.77	5.75	69.5
	(19.0) [d]	(9.0, 9.0)	
5C	4.35	4.25	72.0
6C	4.65	4.59	
	(2.8, 12.2)	(3.0, 12.0)	
6'C	4.46	4.42	62.6
	(4.0, 12.2)	(4.0, 12.0)	

[a] In $CDCl_3$. The numbers in parentheses denote coupling constants in Hz. [b] The data are given for the α anomer only. [c] The resonances are obscured. [d] The values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$.

TABLE II

^1H and ^{13}C NMR data [a] for compounds **4**, **5** and **6**

Ring	^1H NMR			^{13}C NMR		
	4	**5**	**6**	**4**	**5**	**6**
1A	4.91 (2.0)	4.81 (1.8)	5.29 (1.8)	95.9	101.3	92.0
2A	5.37 (2.0, 3.0)	5.39 (1.8, 3.4)	5.40 (1.8, 3.3)	72.1	72.6	72.9
3A	4.28 (3.0, 9.5)	4.09 (3.4, 9.4)	4.33 (3.3, 9.4)	77.9	79.0	78.5
4A	3.62 (19.0) [b]	3.60 (18.8) [b]	3.63 (18.9) [b]	80.0	79.9	80.2
5A	3.85 (9.5, 6.0)	3.86 (9.4, 6.1)	4.07 (9.5, 6.3)	67.2	67.9	67.8
6A	1.35 (6.0)	1.38 (6.1)	1.35 (6.3)	18.0	18.0	18.0
1B	5.02 (2.0)	4.93 (1.6)	5.03 (1.8)	98.6	96.5	98.7
2B	5.27 (2.0, 3.2)	3.99 (1.6, 3.1)	5.29 (1.8, 3.4)	71.0	70.0	71.6
3B	4.02 (3.2, 10.0)	3.85 (3.1, 9.2)	4.03 (3.4, 9.2)	68.3	83.3	79.2
4B	3.26 (20.0) [b]	3.29 (18.5) [b]	3.27 (18.5) [b]	78.6	78.5	79.0
5B	3.64 (10.0, 6.2)	3.58 (9.3, 6.1)	3.66 (9.3, 6.3)	68.3	68.2	68.4
6B	0.89 (6.2)	0.85 (6.1)	0.90 (6.3)	17.0	17.4	17.5
1C	5.51 (8.0)	5.48 (8.5)	5.51 (8.4)	98.6	98.6	98.9
2C	4.55 (8.0, 10.0)	4.60 (8.5, 10.7)	4.56 (8.4, 10.7)	55.0	54.8	55.1
3C	6.03 (10.0, 9.0)	6.12 (10.7, 9.3)	6.04 (10.7, 9.3)	71.0	71.1	71.3
4C	5.63 (9.0, 9.0)	5.55 (9.3, 9.9)	5.65 (9.3, 9.7)	69.3	70.0	69.6
5C	3.36 (9.0, 2.5, 3.5)	3.40 (9.9, 5.8, 2.5)	3.35 (9.7, 2.5, 2.8)	71.0	72.3	71.7
6C	4.18 (2.5, 12.0)	4.41 (2.5, 12.4)	4.14 (2.8, 12.2)			
6'C	4.25 (3.5, 12.0)	4.28 (5.8, 12.4)	4.26 (2.5, 12.2)	61.7	62.3	62.2

[a] In $CDCl_3$. The numbers in parentheses denote coupling constants in Hz. [b] The values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$.

ments that were performed without carbon-decoupling during acquisition, permitted the measurement of the one-bond $^{13}C-^1H$ coupling constants ($^1J_{^{13}C-^1H}$) for the anomeric carbons. The stereochemical integrity of the hexasaccharide **9** was confirmed by examination of the one-bond $^{13}C-^1H$ coupling constants, $^1J_{^{13}C-^1H}$, for the anomeric carbons[22] and the vicinal coupling constants, $^3J_{^1H-^1H}$, of the ring-protons in the monosaccharide units.

TABLE III

^{1}H and ^{13}C NMR data [a] for compounds **8** and **9**

Ring	^{1}H NMR		^{13}C NMR	
	8	**9**	**8**	**9**
1A	4.91 (1.5)	4.74 (1.8)	96.4	102.3 (174) [b]
2A	5.38	3.97 (1.8, 3.3)	72.7	72.8
3A	4.20	3.77 (3.3, 9.4)	78.0	80.4
4A	3.69	3.51 (19.4) [c]	80.4	74.6
5A	3.80	3.72 (10.0, 6.5)	68.4 [d]	71.5
6A	1.38	1.25 (6.5)	17.9	19.3
1B	4.98 (1.0)	5.07 (1.5)	100.4	103.8 (173) [b]
2B	4.26 (1.0, 3.0)	4.26 (1.5, 3.1)	79.2	78.9
3B	3.95 (3.0, 9.5)	3.94 (3.1, 9.5)	81.2	82.5
4B	3.23 (19.0) [c]	3.49 (19.4) [c]	78.4	74.1
5B	3.61 (9.5)	3.78 (9.8, 6.1)	68.6	72.2
6B	0.86	1.26 (6.1)	17.6	19.5
1C	5.59 (9.0)	4.68 (8.4)	98.8	105.2 (163) [b]
2C	4.57 (9.0, 11.0)	3.70	55.2	58.7
3C	6.08 (11.0, 9.0)	3.51	71.6	76.7
4C	5.67 (19.5) [c]	3.41	69.5	72.6
5C	3.50	3.42	71.6	76.5 [e]
6C	4.29 (12.0, 2.5)	3.73		
6'C	4.18	3.90	62.1	63.7
1A'	5.37 (1.8)	5.15 (1.8)	98.8	104.1 (174) [b]
2A'	5.47 (1.8, 3.1)	4.05 (1.8, 3.2)	73.0	72.6
3A'	4.21	3.81 (3.2, 9.5)	78.0	70.9
4A'	3.58	3.50 (19.5) [c]	80.2	74.3
5A'	3.87 (9.5)	3.67 (10.0, 6.2)	67.6	72.4
6A'	1.10	1.23 (6.2)	17.9	19.4

TABLE III (continued)

Ring	^{1}H NMR		^{13}C NMR	
	8	**9**	**8**	**9**
1B′	5.10 (1.2)	5.00 (1.5)	98.8	104.5 (172) [b]
2B′	5.37 (1.2, 3.1)	4.24 (1.5, 3.3)	71.5	72.8
3B′	4.12 (3.1, 9.5)	3.89 (3.1, 9.8)	79.6	82.8
4B′	3.30 (19.0) [c]	3.48 (19.5) [c]	79.2	73.7
5B′	3.80 (9.5)	3.79 (9.7, 6.32)	67.6 [d]	72.1
6B′	1.00	1.26 (6.2)	17.4	19.6
1C′	5.57 (8.0)	4.68 (8.7)	100.0	105.4 (163) [b]
2C′	5.00 (8.0, 11.0)	3.72	54.8	58.6
3C′	6.12 (11.0, 9.0)	3.53	71.6	76.5
4C′	5.67 (19.5) [c]	3.42	69.5	72.6
5C′	3.50	3.42	71.6	76.7 [e]
6C′	4.09	3.72		
6′C′	4.18	3.88 (10.5)	62.7	63.5

[a] **8** in $CDCl_3$ and **9** in D_2O. The numbers in parentheses denote coupling constants in Hz. [b] These values are the one-bond ^{13}C–^1H coupling constants ($J_{^{13}C-^1H}$) in Hz. [c] The values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$. [d] Assignments may be interchanged. [e] Assignments may be interchanged.

Compounds were also characterized by microanalysis. In the case of **9**, however, owing to its hygroscopic nature, a fast-atom bombardment (FAB) mass spectrum was obtained as a confirmation of composition. The peak appearing at m/z 1051.4 was assigned to the $[M + H]^+$ ion.

EXPERIMENTAL

General methods.—Melting points were determined on a Fisher–Johns apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz, for proton and carbon, respectively. All spectra were recorded in $CDCl_3$ unless otherwise stated, and chemical shifts are given in ppm downfield from Me_4Si. For those spectra measured in D_2O, chemical shifts are given in ppm downfield from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. For the sake of brevity, only the

chemical shifts of the ring protons and carbons have been reported (see Tables), and signals resulting from the protecting groups have been omitted.

The ^{1}H-homonuclear chemical-shift correlated (COSY) spectra were acquired with initial data sets of 512 × 2048 data points which were zero-filled once in the F_1 direction to give a final data set of 1024 × 1024 real data points.

A TOCSY spectrum of compound **9** was recorded by use of the pulse sequence d1–90°–d0–[MLEV spinlock]–FID, with a solvent presaturation pulse of 2 s during dl. The power level used for the spinlock gave a 25μs 90° pulse. The spinlock (MLEV-17) was applied for a period of 250 ms. 512 Experiments of 24 scans each were acquired to give an initial data set of 512 × 2048 data points that was zero-filled once in the F_1-direction to give a final data set of 1024 × 1024 real data points.

A ROESY spectrum of **9** was acquired by use of the pulse sequence d1–90°–d0–[CW spin lock]–FID, with a presaturation pulse of 2 s during the relaxation delay d1. The CW spin-lock was applied for 250 ms at 0.5 W at the frequency of the HDO peak. 512 Experiments of 24 scans each were recorded by use of phase-sensitive detection.

For the inverse detection experiments a 4-pulse sequence was used for the ^{1}H{^{13}C}–^{13}C correlation[20]; the same sequence, incorporating a BIRD pulse in the preparation period, was used for the ^{1}H–^{13}C correlation[19]. In both cases, time proportional phase increments were used in F_1 (ref. 21). The data sets of 512 × 2048 data points were zero-filled once in both the F_1- and the F_2-directions, to give a final data set of 1024 × 2048 real data points, with a digital resolution of 10.3 and 1.0 Hz/pt in the F_1- and the F_2-directions, respectively.

The fast atom bombardment (FAB) mass spectrum was obtained with a Kratos Concept-H mass spectrometer. The sample was dissolved directly in glycerol and used in a glycerol matrix. Mass accuracy is $\sim \pm 0.1$ amu.

Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck Silica Gel $60F_{254}$ as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with 5% H_2SO_4 in EtOH, and heated at 150°C. All compounds were purified by medium pressure column chromatography on Kieselgel 60 (230–400 mesh) according to a published procedure[23]. Purification at each stage was crucial to the success of subsequent glycosylation reactions.

Solvents were distilled before use and were dried, as necessary, by literature procedures. Solvents were evaporated under diminished pressure and below 40°C.

Reactions performed under nitrogen were also carried out in deoxygenated solvents. Transfers under nitrogen were effected by means of standard Schlenk-tube techniques.

3-O-(3,4,6-Tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-O-acetyl-4-O-benzyl-α,β-L-rhamnopyranose (**1**).—To a sample of allyl 3-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside[5] (1.12 g, 1.19 mmol) in 9:1 EtOH–H_2O (157 mL) was added

tris(triphenylphosphine)rhodium(I) chloride (0.27 g, 0.28 mmol) and 1,4-diazabicyclo[2.2.2]octane (0.078 g, 0.67 mmol). The mixture was refluxed under N_2 for 14 h. Following reflux, the solvent was removed by evaporation and the residue was taken up in EtOAc and filtered through a short column of silica gel. The filtrate was evaporated to dryness and the resulting syrup was dissolved in 90% aq acetone (40 mL). To this solution was added yellow HgO (387 mg, 1.79 mmol), followed by the dropwise addition of $HgCl_2$ (486 mg, 1.79 mmol) in 90% acetone (9 mL). The mixture was stirred for 12 h at room temperature. The solvent was removed by evaporation. The residue was taken up in EtOAc and filtered through a pad of Celite. The filtrate was washed successively with satd KI (2 ×), aq $Na_2S_2O_3$ (2 ×), and water (2 ×). The organic layer was dried (Na_2SO_4) and the solvent was removed by evaporation. The residue was purified by column chromatography with 2:1 hexane–EtOAc as eluant to give **1** as a light-yellow foam (0.864 g, 80%). Anal. Calcd for $C_{50}H_{45}NO_{15}$: C, 66.74; H, 5.04; N, 1.56. Found: C, 66.52; H, 5.26; N, 1.47.

O-*(3-O-(3,4,6-Tri-O-benzoyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-2-O-acetyl-4-O-benzyl-α-*L*-rhamnopyranosyl) trichloroacetimidate* (**2**).—To a mixture of **1** (2.24 g, 2.49 mmol) and trichloroacetonitrile (286 μL, 2.84 mmol) in anhyd CH_2Cl_2 (12 mL) at 0°C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (95 μL, 0.63 mmol). The mixture was stirred at room temperature under N_2 overnight. The solvent was removed by evaporation and the residue was purified by column chromatography with 2:1 hexane–EtOAc as eluant to yield **2** as a solid (2.25 g, 87%), $[\alpha]_D^{23}$ +0.18° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.64 (s, 1 H, OC(N*H*)CCl_3), see Table I also. The compound was used immediately in the next reaction.

*Allyl 3-O-(3-O-(3,4,6-tri-O-benzoyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-2-O-acetyl-4-O-benzyl-α-*L*-rhamnopyranosyl)-2-O-benzoyl-4-O-benzyl-α-*L*-rhamnopyranoside* (**4**).—A mixture of compound **2** (1.0 g, 0.95 mmol) and allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside[11] (**3**) (491 mg, 1.2 mmol) in anhyd CH_2Cl_2 (10 mL), under N_2, was stirred with 4A molecular sieves, for 20 min at room temperature. The mixture was cooled to −78°C and triethylsilyl trifluoromethanesulfonate (TESTfl) (12 μL, 0.05 mmol) was added dropwise. The mixture was stirred for 10 min after which TLC indicated that the reaction was complete. The Lewis acid was neutralized at room temperature with Et_3N (20 μL). The mixture was filtered through Celite and the filtrate was evaporated in vacuo. The residue was purified by column chromatography with 2:1 hexane–EtOAc as eluant to yield **4** as an amorphous solid (1.14 g, 89%), $[\alpha]_D^{23}$ +0.31° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{73}H_{69}NO_{20}$: C, 68.48; H, 5.43; N, 1.09. Found: C, 68.18; H 5.61; N, 1.05.

*Allyl 3-O-(3-O-(3,4,6-Tri-O-benzoyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-4-O-benzyl-α-*L*-rhamnopyranosyl)-2-O-benzoyl-4-O-benzyl-α-*L*-rhamnopyranoside* (**5**).—Trisaccharide **4** (1.2 g, 0.92 mmol) was dissolved in anhyd CH_2Cl_2 (11.5 mL) and methanolic HCl (28 mL) [prepared by treating anhyd MeOH (100 mL) with

acetyl chloride (40 mL)] was added. The mixture was stirred for 72 h at room temperature, under N_2. The mixture was diluted with CH_2Cl_2 (80 mL) and washed successively with aq $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by chromatography with 2:1 hexane–EtOAc as eluant to afford **5** as an amorphous solid (0.95 g, 82%), $[\alpha]_D^{23}$ +0.55° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{71}H_{67}NO_{19}$: C, 68.86; H, 5.45; N, 1.13. Found: C, 68.45; H, 5.57; N, 1.10.

3-O-(3-O-(3,4,6-Tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranose (**6**).—To a solution of the trisaccharide **4** (1.2 g, 0.92 mmol) in 9:1 EtOH–H_2O (43 mL) was added tris(triphenylphosphine)rhodium(I) chloride (50.6 mg, 0.053 mmol) and 1,4-diazabicyclo[2.2.2]octane (60.9 mg, 0.54 mmol). The mixture was refluxed under N_2 for 14 h. Following reflux, the solvent was removed by evaporation and the residue was taken up in EtOAc and filtered through a short column of silica gel. The filtrate was evaporated to dryness and the resulting syrup was dissolved in 90% aq acetone (65 mL). To this solution was added yellow HgO (213 mg, 0.97 mmol), followed by the dropwise addition of $HgCl_2$ (214 mg, 0.97 mmol) in 90% acetone (3 mL). The mixture was stirred for 12 h at room temperature. The solvent was removed by evaporation. The residue was taken up in EtOAc and filtered through a pad of Celite. The filtrate was washed successively with satd KI (2×), aq $Na_2S_2O_3$ (2×), and water (2×). The organic layer was dried (Na_2SO_4) and the solvent removed by evaporation. The residue was purified by column chromatography with 2:1 hexane–EtOAc as eluant to give **6** as a syrup (0.86 g, 74%), $[\alpha]_D^{23}$ +0.46° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{70}H_{65}NO_{20}$: C, 67.78; H, 5.28; N, 1.12. Found: C, 67.60; H, 5.35; N, 1.11.

Allyl 3-O-(2-O-(3-O-(3,4,6-tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-3-O-(3,4,6-tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranosyl)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**8**).—The trichloroacetimidate **7** (obtained from **6** following the same procedure described for **1**) (520 mg, 0.37 mmol) and acceptor **5** (311 mg, 0.25 mmol) were dissolved in anhyd CH_2Cl_2 (12 mL) and the mixture was stirred with 4A molecular sieves under N_2 for 20 min at room temperature. The solution was cooled to −78°C and TESTfl (4.2 μL, 0.017 mmol) was added dropwise. The mixture was stirred for 10 min after which TLC indicated that the reaction was complete. The Lewis acid was neutralized at room temperature with Et_3N, the mixture was filtered through Celite, and the filtrate was evaporated in vacuo. The residue was purified by column chromatography with 2:1 hexane–EtOAc as eluant to yield **8** as a solid (418 mg, 68%), $[\alpha]_D^{23}$ +0.1° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{141}H_{130}N_2O_{38}$: C, 68.82; H, 5.32; N, 1.13. Found: C, 69.05; H, 5.54; N, 1.11.

Propyl 3-O-(2-O-(3-O-(3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**9**).—The hexasaccharide **8** (34

mg) was dissolved in methanolic NaOMe (0.26 M, 4 mL). The mixture was stirred for 18 h at room temperature and then neutralized by stirring with Rexyn 101 H^+ resin. The resin was removed by filtration and the filtrate evaporated to dryness. The resulting syrup was taken up in 4:1 HOAc–water (10 mL) and stirred with Pd–C (50 mg) under H_2 (52 psi). After 20 h the mixture was filtered through a pad of Celite, and the Celite was rinsed with EtOH. The combined filtrates were evaporated to dryness and EtOH was evaporated several times from the residue to remove traces of HOAc. The residue was then taken up in EtOH (10 mL) to which was added hydrazine hydrate (100%, 150 μL), and the solution was refluxed under N_2 for 18 h. The solution was then filtered to remove a fine grey precipitate and MeOH was evaporated several times from the filtrate. The residue was taken up in MeOH (10 mL) to which was added Ac_2O (400 μL). The solution was kept for 30 min at room temperature and then evaporated to dryness and MeOH was evaporated several times from the residue. The solvent was removed by evaporation and the residue was purified by column chromatography with 6:3:1 EtOAc–MeOH–H_2O. The fractions containing compound **9** were collected, concentrated and further purified by passing the sample through a column of Sephadex LH20 with MeOH as eluant. Compound **9** was obtained as a white amorphous solid (8.7 mg, 61%); FABMS Calcd for $C_{43}H_{74}N_2O_{27}$: m/z 1050.4; Found: m/z 1051.4 $[M + H]^+$.

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