Carbohydrate Research, 228 (1992) 399–414
Elsevier Science Publishers B.V., Amsterdam

Convergent synthesis of higher-order oligosaccharides corresponding to the cell-wall polysaccharide of the β-hemolytic *Streptococci* Group A. A branched hexasaccharide hapten

Kerry B. Reimer, Shannon L. Harris, Vikram Varma, and B. Mario Pinto*
Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6 (Canada)

(Received August 12th, 1991; accepted November 1st, 1991)

ABSTRACT

A convergent synthesis of a hexasaccharide corresponding to the cell-wall polysaccharide of the β-hemolytic *Streptococci* Group A is described. The strategy relies on the preparation of a key branched trisaccharide unit α-L-Rha*p*-(1→2)-[β-D-Glc*p*NAc-(1→3)]-α-L-Rha*p* which functions both as a glycosyl acceptor and donor. The hexasaccharide is obtained after only three glycosylation reactions. This fully functionalized unit can serve, in turn, as a glycosyl acceptor or donor for the synthesis of higher-order structures. Deprotection gives a hexasaccharide for use as a hapten in immunochemical studies. The characterization of all compounds by high resolution ^{1}H- and ^{13}C-n.m.r. spectroscopy is also described.

INTRODUCTION

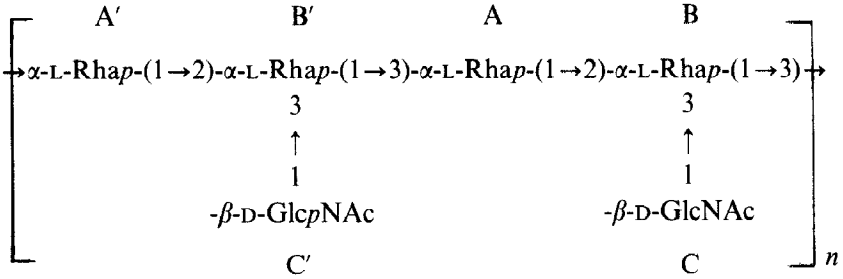
The Gram-positive β-hemolytic *Streptococci* Group A is one of the primary infective agents in humans, causing streptococcal pharyngitis, commonly known as strep throat[1]. In a small number of cases the initial streptococcal infection can develop into the more serious condition of rheumatic fever[2]. Streptococcal infections are also implicated in the development of other disease conditions such as heart-valve disease, glomerulonephritis, rheumatoid arthritis, and other rheumatic disorders[3]. The detection of streptococcal diseases and their rapid treatment is, therefore, important. As part of a program designed to furnish increasingly more complex oligosaccharides corresponding to the cell-wall polysaccharide of the β-hemolytic *Streptococci* Group A, we have reported[4–6] the synthesis of di- up penta-saccharide units. These structures represent different epitopes, and serve to map the combining sites of complementary antibodies that could be used as immunodiagnostic reagents. We now report an efficient, convergent synthesis of a hexasaccharide unit that is suitably functionalized to serve as a precursor of higher-order structures.

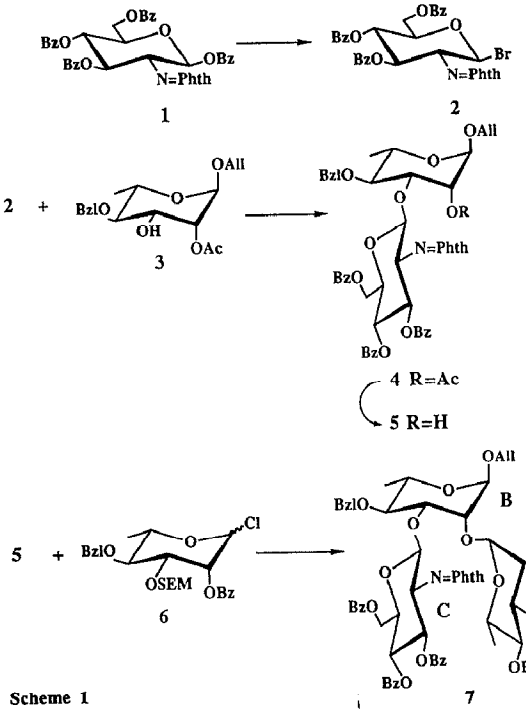
* To whom correspondence should be addressed.

0008-6215/92/$ 05.00

RESULTS AND DISCUSSION

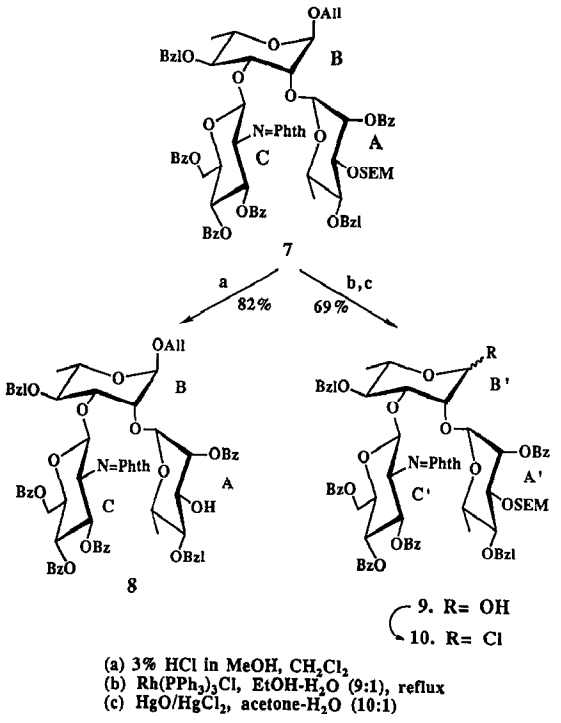
The cell-wall carbohydrates of the Group A *Streptococci* are comprised of a rhamnose backbone consisting of alternating α-L-(1→2) and α-L-(1→3) linkages, with *N*-acetyl-β-D-glucosamine residues attached to the 3-positions of the rhamnose backbone[7].





Scheme 1

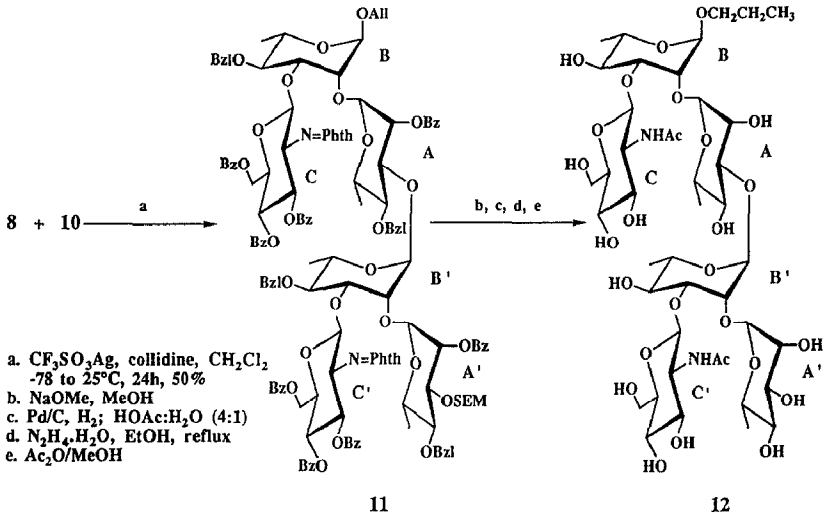
Retrosynthetic analysis indicated that disconnections based on key linear (AB′C′) or branched B(C)A trisaccharide sequences would be desirable. We report here a synthesis based on the latter strategy. Thus, 1,3,4,6-tetra-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**1**), prepared in analogous fashion to the corresponding tetraacetate[8], was converted into the glycosyl bromide **2** (Scheme 1). Glycosylation of allyl 2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside (**3**), prepared in analogous fashion



Scheme 2

to the 2-*O*-benzoyl derivative[9], with the donor **2** under silver trifluoromethanesulfonate promotion in the presence of collidine[8] afforded the disaccharide **4** in 57% yield (Scheme 1). The crucial test of this synthetic sequence was whether one could successfully remove the 2-*O*-acetyl group of the disaccharide **4** without removing any of the benzoyl groups on the *N*-acetylglucosamine moiety. This selective deprotection was achieved by mild transesterification conditions. The disaccharide **4** was treated with 3% methanolic HCl to give the desired acceptor **5** in 76% yield.

The key [B-(C)-A] branched trisaccharide intermediate was then synthesized from acceptor **5** and glycosyl chloride[10] **6** (Scheme 2) with silver trifluoromethanesulfonate promotion. The reaction proceeded with α-stereoselectivity to give trisaccharide **7** in 61% yield. This trisaccharide could serve, in principle, as both a glycosyl donor and acceptor in future glycosylation reactions. Previous work in our laboratory had indicated that the (2-trimethylsilyl)ethoxymethyl (SEM) acetal could be selectively removed in the presence of benzoate esters[10]. Indeed, the 3'-position of **7** was deblocked by treatment with methanolic HCl[8], to give the trisaccharide acceptor **8** in 82% yield (Scheme 3). The key question remaining was whether the glycosyl chloride derived from the trisaccharide **7** would function adequately as a glycosyl donor. The concern was the absence of a participating group at the 2-position of the proposed glycosyl donor; this being replaced by a rhamnosyl unit. There were precedents in the literature for this type



Scheme 3

of reaction. For example, Bundle *et al.*[11] had prepared several homopolymers of α-(1→2)-linked perosamine (4,6-dideoxy-4-formamido-D-mannose) units. In these glycosylation reactions, there were no participating groups at the 2-positions of the glycosyl donors but rather, other glycosyl residues, and good α-stereoselectivity was observed. In addition, Ogawa *et al.* have used both α-D-mannosyl bromides[12], and α-D-mannosyl trichloroacetimidates[13] to prepare oligosaccharides with α-stereoselectivity; in both cases the mannosyl donors had other sugar residues at the 2-positions. In contrast, Srivastava and Hindsgaul[14] found extensive β-glycoside formation with mannosyl donors that were glycosylated at C-2.

The trisaccharide **7** was converd into the hemiacetals **9** by treatment with Wilkinson's catalyst[15], followed by hydrolysis of the enol ethers with mercuric oxide–mercuric chloride[16]. The glycosyl chlorides **10** were then prepared by treatment with *N,N*-dimethyl(chloromethylene)ammonium chloride[17].

Glycosylation of the trisaccharide acceptor **8** with the trisaccharide donor **10** (Scheme 4) was performed in an analogous fashion to the synthesis of trisaccharide **7**, and proceeded with exclusive α-stereoselectivity to give **11** in 50% yield. The hexasaccharide **11** thus prepared had the same features as the parent trisaccharide **7**, namely, an allyl group as the aglycon which could be removed to generate a glycosyl donor, and the SEM group which could be selectively removed to generate a glycosyl acceptor. This result confirms the utility of the key trisaccharide **7** as a common intermediate in the synthesis of higher-order structures of the *Streptococci* Group A cell-wall polysaccharide and defines an efficient, convergent synthetic route. The hexasaccharide **11** results from only three glycosylation reactions and a nonasaccharide could, in principle, be derived from a fourth glycosylation reaction (namely, removal of the SEM group of hexasaccharide **11** and glycosylation with the trisaccharide **10**). The synthesis of higher-order structures that span two or more branch points will allow better definition of the

extended binding sites displayed by monoclonal antibodies raised against a streptococcal vaccine[18] and should permit the design of improved immunodiagnostic reagents.

The deblocked hexasaccharide **12** was obtained from **11** by successive treatment with (*1*) 3% methanolic HCl to remove the SEM group, (*2*) sodium methoxide in methanol to remove the benzoate esters, (*3*) hydrazinolysis of the phthalimido group and *N*-acetylation of the resultant amine, and (*4*) hydrogenolysis of the benzyl and allyl ethers.

Compounds were fully characterized by high resolution ^{1}H- and ^{13}C-n.m.r. spectroscopy (Tables I, II, III). ^{1}H-Homonuclear chemical-shift correlated (COSY) experiments[19] and ^{13}C–^{1}H chemical-shift correlated experiments[20] were performed as necessary in order to facilitate assignments. In the case of the hexasaccharide **12**, TOCSY[21] and ROESY[22] experiments were also performed. The ^{13}C–^{1}H chemical-shift correlated experiments were carried out in the inverse mode,[23–25] thereby taking advantage of the sensitivity of the ^{1}H nucleus. Experiments that were performed without carbon decoupling during acquisition permitted the measurement of the one-bond ^{13}C–^{1}H coupling constants ($^1J_{13C-1H}$) for the anomeric carbons. The stereochemical integrity of the hexasaccharides (**11** and **12**) was confirmed by examination of the one-bond ^{13}C–^{1}H coupling constants, $^1J_{13C-1H}$, for the anomeric carbons[26] (see, for example, Fig. 1) and the vicinal coupling constants, $^3J_{1H-1H}$, of the ring-protons in the monosaccharide units.

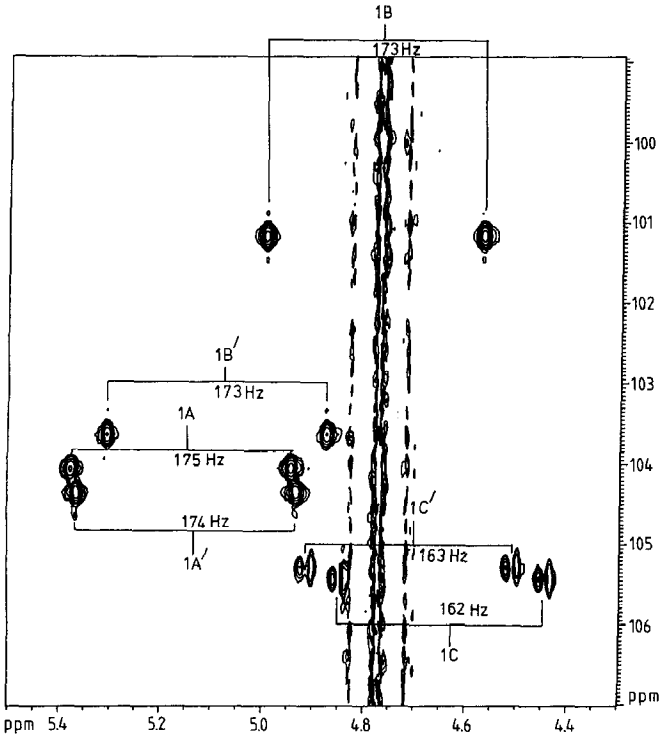


Fig. 1. Anomeric-signal region of the ^{1}H-detected ^{13}C–^{1}H chemical-shift correlated 2D spectrum of hexasaccharide **12**.

TABLE I

^{1}H- and ^{13}C-n.m.r. data[a] for compounds **1**, **2**, **4**, and **5**

Ring	*^{1}H-n.m.r.*				*^{13}C-n.m.r.*		
	1	**2**	**4**	**5**	**1**	**4**	**5**
1B[b]			4.71 (1.8)	4.55 (1.7)		96.3	98.2
2B			5.35 (1.8,3.5)	4.06		74.6	70.0
3B			4.15 (3.5,9.5)	3.95 (3.2,9.2)		79.3	78.7
4B			3.38 (19.0)[c]	3.39 (18.7)[c]		79.8	83.6
5B			3.65 (6.4,9.5)	3.58 (6.2,9.5)		67.4	67.1
6B			1.11 (6.4)	1.10 (6.2)		17.7	17.6
1C	6.94 (8.9)	6.61 (9.5)	5.85 (8.5)	5.82 (8.5)	90.5	99.0	98.5
2C	4.95 (8.9,10.6)	4.90 (9.5,10.5)	4.67 (8.5,10.6)	4.70 (8.5,10.7)	53.9	55.2	54.6
3C	6.48 (9.5,10.6)	6.25 (10.5,9.5)	6.22 (9.5,10.6)	6.25 (9.2,10.7)	69.6	71.4	71.1
4C	5.84 (19.0)[c]	5.81 (19.0)[c]	5.75 (19.5)[c]	5.64 (9.2,10.0)	70.9	69.7	69.6
5C	4.50 (2.0,4.5,9.50)	4.35 (3.0,5.0,10.4)	4.26	4.34 (2.7,7.0,10.7)	72.9	71.4	72.6
6C	4.66 (2.0,9.0)	4.66 (3.0,12.4)	4.59 (2.8,12.2)	4.74 (2.7,12.2)			
6′C	4.52 (4.5,9.0)	4.52 (5.0,12.4)	4.45 (4.0,12.2)	4.49 (7.0,12.2)	62.7	62.8	62.8

[a] In $CDCl_3$. The numbers in parentheses denote coupling constants in Hz. [b] Indicates the ring to which the aglycon is attached. [c] The values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$.

Compounds were also characterized by microanalysis. In the case of **12**, however, owing to its hygroscopic nature, a plasma desorption mass spectrum[27] was obtained as a confirmation of composition. The peak appearing at *m/z* 1074 was assigned to the M^+ ion of the sodium salt. $M + Na^+$ ions are commonly observed in plasma-desorption mass spectra, particularly of compounds containing labile hydrogens or anionic moieties[5,6,28].

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. The ^{1}H and ^{13}C-n.m.r. spectra were recorded with a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz, for proton and carbon, respectively. All spectra were recorded in CDCl3 unless otherwise stated, and chemical shifts are given in p.p.m. downfield from Me_4Si. For those spectra measured in

TABLE II

^{1}H- and ^{13}C-n.m.r. data[a] for compounds **7**, **8**, and **9**

Ring	*^{1}H-n.m.r.*			*^{13}C-n.m.r.*	
	7	**8**	**9**	**7**	**8**
1B[b]	4.65 (1.5)	4.66 (1.5)	5.01	97.9	97.8
2B	4.33	4.23	4.31	72.2	72.2
3B	4.06 (3.2,9.5)	4.08 (3.2,9.5)	4.12 (3.2,9.3)	81.6	81.7
4B	3.36 (19.0)[c]	3.28 (19.0)[c]	3.34 (19.0)[c]	78.9	79.3
5B	3.59	3.59 (6.2,9.5)	3.81	67.5	67.5
6B	1.06 (6.3)	1.07 (6.2)	1.03 (6.2)	17.8	17.8
1C	5.85 (8.5)	5.82 (8.5)	5.85 (8.5)	99.9	99.8
2C	5.11 (8.5,10.6)	5.08 (8.5,10.8)	5.13 (8.5,10.6)	54.9	55.0
3C	6.31 (9.4,10.6)	6.33 (9.4,10.8)	6.32 (9.4,10.6)	71.4	71.1
4C	5.79 (19.0)[c]	5.85 (19.0)[c]	5.81 (19.0)[c]	70.2	70.1
5C	4.25	4.32	4.31	75.4	75.1
6C	4.64	4.65 (2.5,12.0)	4.61 (2.5,11.5)		
6'C	4.35	4.43 (6.8,12.0)	4.36 (7.0,11.5)	63.5	63.4
1A	5.45 (1.8)	5.34 (1.8)	5.42 (1.8)	99.7	99.7
2A	5.71 (1.8,3.2)	5.66 (1.8,3.2)	5.68 (1.8,3.2)	71.6	70.4
3A	4.26	4.34	4.25	79.4	79.6
4A	3.60 (19.0)[c]	3.53 (19.0)[c]	3.58 (19.0)[c]	80.2	80.9
5A	3.94 (6.2,9.5)	3.94 (6.2,9.5)	3.92 (6.2,9.5)	67.8	67.9
6A	1.24 (6.2)	1.30 (6.2)	1.22 (6.2)	18.1	18.2

[a] In $CDCl_3$. The numbers in parentheses denote coupling constants in Hz. [b] Indicates the ring to which the aglycon is attached. [c] The values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$.

D_2O, chemical shifts are given in p.p.m. 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.

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.

TABLE III

^{1}H- and ^{13}C-n.m.r. data[a] for compounds **11** and **12**

Ring	*^{1}H-n.m.r.*		*^{13}C-n.m.r.*	
	11	**12**	**11**	**12**
1B[b]	4.61	4.77	97.9 (171)[d]	101.1 (173)[d]
2B	4.29	4.13 (1.8,3.1)	78.3	79.3
3B	4.06 (3.2,9.5)	3.81 (3.1,9.7)	81.7	82.7
4B	3.35 (19.0)[c]	3.45	78.9	74.0
5B	3.59	3.68	67.7	71.6
6B	1.06 (6.2)	1.25 (6.2)	17.6	19.3
1C	5.69 (8.5)	4.63 (8.3)	100.1 (165)[d]	105.3 (162)[d]
2C	5.14	3.69	54.7	58.5
3C	6.22 (9.4,10.5)	3.50	71.4	76.5
4C	5.77 (19.5)[c]	3.44	70.1	72.5
5C	3.68	3.38	71.8	78.4
6C	4.25	3.84 (4.2,12.2)		
6′C	4.18	3.72	62.5	63.5
1A	5.58	5.15 (1.8)	98.9 (175)[d]	104.0 (175)[d]
2A	5.58	4.03 (1.8,3.2)	73.2	72.5
3A	4.23	3.81 (3.2,9.7)	77.6	79.3
4A	3.61 (19.0)[c]	3.52	80.3	74.4
5A	3.83	3.71	67.8	72.0
6A	1.10 (6.2)	1.24 (6.1)	17.8	19.2
1B′	5.14	5.08 (1.6	100.9 (171)[d]	103.6 (173)[d]
2B′	4.34	4.25 (1.6,3.1)	80.1	78.9
3B′	4.10 (3.2,9.5)	3.95 (3.1,9.7)	81.3	82.4
4B′	3.36 (19.0)[c]	3.50	78.8	73.8
5B′	3.87 (6.2,9.5)	3.77	68.7	72.0
6B′	1.06 (6.2)	1.27 (6.2)	17.9	19.5

Ring	*^{1}H-n.m.r.*		*^{13}C-n.m.r.*	
	11	**12**	**11**	**12**
1C′	5.84 (8.5)	4.70 (8.4)	99.9 (168)[d]	105.2 (163)[d]
2C′	5.11	3.71	55.0	58.5
3C′	6.28 (9.0,10.5)	3.51	71.5	76.6
4C′	5.78 (19.0)[c]	3.44	69.7	72.5
5C′	4.32	3.38	72.1	78.4
6C′	4.61	3.90 (4.2,12.4)		
6′C′	4.38	3.73	63.4	63.5
1A′	5.40 (1.6)	5.14 (1.8)	99.6 (174)[d]	104.3 (174)[d]
2A′	5.64	4.01 (1.8,3.4)	71.5	72.6
3A′	4.25	3.74	75.2	72.7
4A′	3.54 (19.0)[c]	3.39	80.1	74.7
5A′	4.00	3.63	68.0	71.8
6A′	1.12 (6.2)	1.21 (6.2)	18.1	19.3

[a] **10** in $CDCl_3$; **11** in D_2O. The numbers in parentheses denote coupling constants in Hz. [b] Indicates the ring to which the aglycon is attached. [c] The values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$. [d] These values are the one-bond ^{13}C–^1H coupling constants ($J_{^{13}C-^1H}$) in Hz.

A TOCSY spectrum of compound **12** was recorded by use of the pulse sequence d1–90°–d0–MLEV spinlock–FID, with a solvent presaturation pulse of 2 s during d1. The power level used for the spinlock gave a 25 μs 90° pulse. The spinlock (MLEV-17) was applied for a period of 250 ms. Experiments (512) of 24 scans each were acquired to give an initital 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 **12** was acquired by use of the pulse sequence d1–90°–d0–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 watts of power at the frequency of the HDO peak. Experiments (512) 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 $^1H(^{13}C)$–^{13}C correlation[24]; the same sequence, incorporating a BIRD pulse in the preparation period, was used for the ^1H–^{13}C correlation[23]. In both cases, time proportional phase increments were used[25] in F_1. 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 Hz/point and 1.0 Hz/point in the F_1- and the F_2-directions, respectively.

The CF-252 plasma-desorption mass spectrum was obtained on a BIN-10K instrument from BIO-ION Nordic (Uppsala, Sweden). The sample was prepared in a solution of MeOH–water and electrosprayed onto Al foils. The spectrum was acquired and the mass was assigned by use of the BIO-ION data system, based upon the PDP 11/73 processor. The experimental masses were obtained by determination of the time centroid of each peak above the baseline and by comparison of these with the times of flight of the H^+ and Na^+ peaks appearing in the spectrum. Mass accuracy is approximately ± 1 a.m.u. in the mass range of 500–1000 a.m.u.

Analytical thin-layer chromatography (t.l.c.) was performed on aluminum plates precoated with Merck Silica Gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to u.v. light and/or sprayed with 5% H_2SO_4 in EtOH, and heated at 150°. All compounds were purified by medium-pressure column chromatography on Kieselgel 60 (230–400 mesh) according to a published procedure[29]. 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°.

Reactions performed under N_2 were also carried out in deoxygenated solvents. Transfers under N_2 were effected by means of standard Schlenk-tube techniques.

*1,3,4,6-Tetra-*O*-benzoyl-2-deoxy-2-phthalimido-β-*D*-glucopyranose* (**1**). — To a solution of 2-carboxybenzamido-2-deoxy-D-glucopyranose[8] (9.69 g, 29.6 mmol), in pyridine (80 mL), was added BzCl (22 mL) during 10 min. The mixture was stirred for a further 3 h at room temperature. The semi-solid mixture was then poured into ice (400 mL), and the ice mixture extracted with CH_2Cl_2 (3 × 150 mL). The combined extracts were dried (Na_2SO_4), and evaporated to dryness to give a pink solid. The solid was taken up in CH_2Cl_2 (150 mL), and washed with M HCl, until neutral, and then washed with water. The organic layer was dried (Na_2SO_4), and evaporated to dryness to give a solid. The solid was suspended in diethyl ether, and collected by filtration. It was identified as 1,3,4,6-tetra-*O*-benzoyl-2-(2-benzoyloxycarbonylbenzamido)-2-deoxy-β-D-glucopyranose (12.1 g, 55%); m.p. 218–220°, $[\alpha]_D^{23}$ −44.4° (*c* 2.4, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 6.33 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 6.00 (t, 1 H, $J_{2,3}$ + $J_{3,4}$ 19.5 Hz, H-3), 5.80 (dd, 1 H, $J_{3,4}$ + $J_{4,5}$ 19.5 Hz, H-4), 4.91 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.0 Hz, H-2), 4.65 (dd, 1 H, $J_{6,6'}$ 12.2, $J_{5,6}$ 3.0 Hz, H-6), 4.50 (dd, 1 H, $J_{5,6'}$ 4.9, $J_{6,6'}$ 12.2 Hz, H-6'), and 4.43 (ddd, 1 H, $J_{5,6}$ 3.0, $J_{5,6'}$ 4.9, $J_{4,5}$ 10.0 Hz, H-5); ^{13}C(^{1}H)-n.m.r. (100.6 MHz): δ 166.3, 165.5, 165.4, 164 (benzoyl CO), 94.1 (C-1), 74.1 (C-5), 73.3 (C-3), 69.4 (C-4), 63.0 (C-6), and 62.0 (C-2).

Anal. Calc. for $C_{49}H_{37}NO_{13}$: C, 69.42; H, 4.40; N, 1.65. Found: C, 69.73; H, 4.28; N, 1.77.

To a portion of the sample (6.58 g, 8.85 mmol) in pyridine (112 mL) was added Ac_2O (56 mL). The solution was refluxed 25 h under N_2. Following reflux, the solutiion was poured into ice (250 mL), and the resulting mixture extracted with CH_2Cl_2 (2 × 75 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The resulting syrup was taken up in CH_2Cl_2, and washed successively with 2M HCl, and aq. $NaHCO_3$. The organic layer was dried (Na_2SO_4) and the solvent evaporated to give a yellow foam. The foam was then triturated with EtOH (100%) to give **1** as a cream-colored solid (5.85

g, 91%); m.p. 170–172°, $[\alpha]_D^{23}$ + 24.8° (*c* 5.2, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz) see Table I; $^{13}C(^1H)$-n.m.r. (100.6 MHz): δ 167.5 (2 C, phthalimido CO) 166.0, 165.5, 165.1, and 164.1 (benzoyl CO) see Table II also.

Anal. Calc. for $C_{42}H_{31}NO_{11}$: C, 69.51; H, 4.31; N, 1.93. Found: C, 69.34; H, 4.41; N, 1.96.

*3,4,6-Tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl bromide* (**2**). — To a sample of compound **1** (7.31 g, 10.1 mmol) in anhydr. CH_2Cl_2 (50 mL), was added a solution of 48% HBr in HOAc (15 mL). The mixture was stirred under N_2 for 2 h at room temperature. The mixture was worked-up by washing successively (by use of ice-cold solutions) with distilled water, and aqueous $NaHCO_3$. The CH_2Cl_2 fraction was dried (Na_2SO_4), and the solvent removed by evaporation to give a white foam. The reaction proceeded quantitatively, and the product was used in the subsequent glycosylation reaction without further purification; ^{1}H-n.m.r. (400.13 MHz): see Table I.

*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* (**4**). — Compound **2** (6.84 g, 10.0 mmol) in anhydr. CH_2Cl_2 (12.0 mL) was stirred with 4A molecular sieves, under N_2, for 30 min at room temperature. To this solution was added silver trifluoromethanesulfonate (2.60 g, 10.1 mmol). The solution was stirred for a further 10 min, and then collidine (1.34 mL, 10.2 mmol) was added. The solution of **2** was then cooled to − 30°, and a solution of allyl 2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside (**3**, 2.26 g, 6.72 mmol) in anhydr. CH_2Cl_2 (11.0 mL), previously stirred with 4A molecular sieves, was added dropwise under N_2. The dropping funnel was rinsed with additional portions of anhydr. CH_2Cl_2 and the washings added to the mixture. The mixture was removed from the cooling bath, and stirred in the dark under N_2 for 8 h. It was then filtered to remove the solids, and the filtrate was washed successively with M HCl, aq. $NaHCO_3$, and distilled water. The organic layer was dried (Na_2SO_4), and the solvent removed by evaporation. The resulting syrup was purified by chromatography with 15:1 toluene–EtOAc as eluant. Compound **4** was obtained as a clear, colorless syrup (3.82 g, 57%); $[\alpha]_D^{23}$ + 16.3° (*c* 2.6, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 5.82 (m, 1 H, $CH_2CH=CH_2$), 5.25 (m, 1 H, J_{trans} 15.5 Hz, $CH_2CH=CHH$ *trans*), 5.17 (m, 1 H, J_{cis} 10.5 Hz, $CH_2CH=CHH$ *cis*), 4.41 and 4.26 (ABq, 2 × 1 H, $J_{A,B}$ 12.0 Hz, OCH_2Ph), 4.07 and 3.90 (ABq, 2 × 1 H, 12.5 Hz, $CH_2CH=CH_2$), and 2.01 (s, 3 H, $OCOCH_3$), see Table I also; $^{13}C(^1H)$-n.m.r. (100.6 MHz): δ 170.1 (acetyl CO), 166.1, 165.7, 165.1 (benzoyl CO), 117.7 ($OCH_2CH=CH_2$), 72.1 (OCH_2Ph), 68.3 ($OCH_2CH=CH_2$), and 20.9 ($OCOCH_3$), see Table I also.

Anal. Calc. for $C_{53}H_{49}NO_{15}$: C, 67.72; H, 5.25; N, 1.49. Found: C, 67.94; H, 5.33; N, 1.42.

*Allyl 3-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-4-*O*-benzyl-α-*L*-rhamnopyranoside* (**5**). — To the disaccharide (**4**) (3.59 g, 3.82 mmol) was added methanolic HCl (35 mL) [prepared by treating anhydr. MeOH (100 mL) with acetyl chloride (4 mL)]. Anhydrous CH_2Cl_2 (25 mL) was added to the solution to completely dissolve the sample. The reaction mixture was stirred under N_2 for 72 h at room temperature. Some starting material still remained; however, since some breakdown products were forming, the reaction was worked-up at this point. The reaction

mixture was washed with aq. $NaHCO_3$ until neutral. The organic layer was dried (Na_2SO_4), and then evaporated to dryness. The resulting syrup was purified by chromatography with 3:1 hexane–EtOAc as eluant. *Compound* **5** was obtained as a clear, colorless syrup which was dried *in vacuo* to give a white foam (2.61 g, 76%); $[\alpha]_D^{23}$ + 1.43° (*c* 2.1, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 5.77 (m, 1 H, $CH_2CH=CH_2$), 5.21 (m, 1 H, J_{trans} 15.5 Hz, $CH_2CH=CHH$ *trans*), 5.15 (m, 1 H, J_{cis} 10.5 Hz, $CH_2CH=CHH$ *cis*), 4.39 and 4.26 (ABq, 2 × 1 H, $J_{A,B}$ 12.0 Hz, OCH_2Ph), 4.02 and 3.79 (ABq, 2 × 1 H, $J_{A,B}$ 12.5 Hz, $CH_2CH=CH_2$); see Table I also; ^{13}C(^{1}H)-n.m.r. (100.6 MHz): δ 166.1, 165.5, 165.2 (benzoyl CO), 117.4 ($OCH_2CH=CH_2$), 74.5 (OCH_2Ph), 67.8 ($OCH_2CH=CH_2$); see Table I also.

Allyl 2-O-(2-O-benzoyl-4-O-benzyl-3-O-[[2-(trimethylsilyl)ethoxy]methyl]-α-L-rhamnopyranosyl)-3-O-(3,4,6-tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranoside (**7**). — A sample of the disaccharide alcohol **5** (2.61 g, 2.91 mmol) in anhydr. CH_2Cl_2 (15 mL) with silver trifluoromethanesulfonate (2.24 g, 8.72 mmol) and 1,1,3,3-tetramethylurea (1.0 mL, 8.36 mmol), was stirred with 4A molecular sieves for 30 min, and then cooled to − 78°. A solution of the monosaccharide chloride[10] **6** (2.21 g, 4.36 mmol) in anhydr. CH_2Cl_2 (7.0 mL), previously stirred with 4A molecular sieves for 30 min and then cooled to − 78°, was added dropwise to the stirred solution of **5**. The dropping funnel containing **6** was rinsed with additional portions of CH_2Cl_2 (3 × 3.0 mL) and the washings were added to the mixture. The mixture was stirred under N_2, in the dark, and allowed to warm to room temperature. After 17 h of reaction, the mixture was filtered through a pad of Celite, and the filtrate was washed successively with M HCl, and sat. aq. NaCl. The organic phase was dried (Na_2SO_4) and the solvent was evaporated. The remaining syrup was purified by chromatography, with 3:1 hexane–EtOAc as eluant. *Compound* **7** was obtained as a clear, light-yellow syrup which was dried *in vacuo* (2.24 g, 61%); $[\alpha]_D^{23}$ − 19.8° (*c* 4.8, $CHCl_3$); ^{1}H-n.m.r. 400.13 MHz): δ 5.68 (m, 1 H, $OCH_2CH=CH_2$), 5.17 (m, 1 H, J_{trans} 15.5 Hz, $OCH_2CH=CHH$ *trans*), 5.10 (m, 1 H, J_{cis} 10.5 Hz, $OCH_2CH=CHH$ *cis*), 5.03 and 4.88 (ABq, 2 × 1 H, $J_{A,B}$ 7.0 Hz, $OCH_2OCH_2CH_2SiMe_3$), 4.97, and 4.71 (ABq, 2 × 1 H, $J_{A,B}$ 11.0 Hz, OCH_2Ph), 4.38 and 4.21 (ABq, 2 × 1 H, $J_{A,B}$ 12.5 Hz, OCH_2Ph), 4.00, and 3.80 (ABq, 2 × 1 H, $J_{A,B}$ 12.5 Hz, $OCH_2CH=CH_2$), 3.71 (m, 2 H, $OCH_2OCH_2CH_2SiMe_3$), 1.00 (m, 2 H, $OCH_2OCH_2CH_2SiMe_3$), − 0.075 (s, 9 H, $OCH_2OCH_2CH_2SiMe_3$); see Table II also: ^{13}C(^{1}H)-n.m.r. (100.6 MHz): δ 168.3, 166.9, 166.0, 165.7, 165.6, and 165.2 (CO), 117.3 ($OCH_2CH=CH_2$), 94.1 ($OCH_2OCH_2CH_2SiMe_3$), 75.1, and 74.4 (OCH_2Ph), 67.7 ($OCH_2CH=CH_2$), 65.6 ($OCH_2OCH_2CH_2SiMe_3$), 19.9 ($OCH_2OCH_2CH_2SiMe_3$), − 1.4 ($OCH_2OCH_2CH_2SiMe_3$); see Table II also.

Anal. Calc. for $C_{77}H_{81}NO_{20}$: C, 67.58; H, 5.96; N, 1.02. Found: C, 67.81; H, 6.01; N, 0.98.

Allyl 2-O-(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-rhamnopyranoside (**8**). — A sample of the trisaccharide **7** (0.906 g, 0.662 mmol) was dissolved in methanolic HCl (20 mL) [prepared by treating anhydr. MeOH (100 mL) with acetyl chloride (4.0 mL)]. This solution was diluted by the addition of anhydr. MeOH (20 mL), and anhydr.

CH_2Cl_2 (5.0 mL). The mixture was stirred for 3 h at room temperature, under N_2. The reaction was quenched by adding aq. $NaHCO_3$. This solution was extracted with CH_2Cl_2, the organic layer dried (Na_2SO_4), and the solvent removed by evaporation. The remaining syrup was purified by chromatography with 2:1 hexane–EtOAc as eluant. *Compound* **8** was obtained as a clear colorless syrup which was dried *in vacuo* to give a white foam (0.675 g, 82%); $[\alpha]_D^{23}$ −22.7° (*c* 2.7, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 5.68 (m, 1 H, $OCH_2CH=CH_2$), 5.18 (m, 1 H, J_{trans} 15.5 Hz, $OCH_2CH=CHH$ *trans*), 5.09 (m, 1 H, 10.05 Hz, $OCH_2CH=CHH$ *cis*), 4.94 and 4.77 (ABq, 2 × 1 H, $J_{A,B}$ 11.0 Hz, OCH_2Ph), 4.24, and 4.14 (ABq, 2 × 1 H, $J_{A,B}$ 12.0 Hz, OCH_2Ph), 3.99 and 3.80 (ABq, 2 × 1 H, $J_{A,B}$ 12.5 Hz, $OCH_2CH=CH_2$), 2.62 (d, 1 H, $J_{OH,3A}$ 5.3 Hz, O*H*), see Table II also; ^{13}C(^{1}H)-n.m.r. (100.6 MHz): δ 166.2, 166.0, 165.7, and 165.1 (benzoyl CO), 117.3 ($OCH_2CH=CH_2$), 74.5 and 73.8 (OCH_2Ph), 67.8 ($OCH_2CH=CH_2$), see Table II also.

Anal. Calc. for $C_{71}H_{67}NO_{19}$: C, 68.87; H, 5.45; N, 1.13. Found: C, 68.64; H, 5.45; N, 1.13.

2-O-(2-O-Benzoyl-4-O-benzyl-3-O-[[2-trimethylsilyl]ethoxy]methyl)-α-L-rhamnopyranosyl)-3-O-(3,4,6-tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranose (**9**). — To a sample of the trisaccharide **7** (1.36 g, 0.994 mmol) in 9:1 EtOH–water (60 mL) was added tris(triphenylphosphine)rhodium (I) chloride (0.265 g). The reaction 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 10:1 acetone–water (40 mL). To this solution was added yellow mercury(II) oxide (0.323 g, 1.49 mmol), followed by the addition of mercury(II) chloride (0.405 g, 1.49 mmol). The mixture was stirred for 40 h at room temperature. The solvent was then removed by evaporation and the residue was taken up in EtOAc and filtered through a pad of Celite. The filtrate was washed successively with sat. KI (2 ×), aq. sodium thiosulfate (2 ×), and water (2 ×). The organic layer was dried (Na_2SO_4) and the solvent was removed by evaporation. The remaining syrup was purified by chromatography with 2:1 hexane–EtOAc as eluant. *Compound* **9** was obtained as a clear, light yellow syrup, which was dried *in vacuo* to give a foam (0.909 g, 69%); ^{1}H-n.m.r. (400.13 MHz): δ 5.01 and 4.87 (ABq, 2 × 1 H, $J_{A,B}$ 7.0 Hz, $OCH_2OCH_2CH_2SiMe_3$), 4.96 and 4.70 (ABq, 2 × 1 H, $J_{A,B}$ 11.2 Hz, OCH_2Ph), 4.25 and 4.18 (ABq, 2 × 1 H, $J_{A,B}$ 12.0 Hz, OCH_2Ph), 3.70 (m, 2 H, $OCH_2OCH_2CH_2SiMe_3$), 2.75 (d, 1 H, $J_{OH,1B}$ 3.3 Hz, O*H*), 0.98 (m, 2 H, $OCH_2OCH_2CH_2SiMe_3$), −0.10 (s, 9 H, $OCH_2OCH_2CH_2SiMe_3$); see Table II for other signals.

Anal. Calc. for $C_{74}H_{77}NO_{20}Si$: C, 66.90; H, 5.84; N, 1.05. Found: C, 66.94; H, 6.03; N, 1.01.

2-O-(2-O-Benzoyl-4-O-benzyl-[[2.trimethylsilyl]ethoxy]methyl)-α-L-rhamnopyranosyl-3-O-(3,4,6-tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-L-rhamnopyranosyl chloride (**10**). — To a solution of DMF (0.23 mL, 2.97 mmol) in anhydr. CH_2Cl_2 (5.0 mL) was added oxalyl chloride (0.26 mL, 2.98 mmol). The mixture was stirred under N_2 for 5 min, then the solvent was evaporated under reduced pressure, and the resulting white salt dried *in vacuo* with gentle heating for 25 min. To

the *N,N*-dimethyl(chloromethylene)ammonium chloride salt was added a solution of the trisaccharide hemiacetal **9** (0.774 g, 0.582 mmol) in anhydr. CH_2Cl_2 (5.0 mL); the flask was rinsed with additional portions of CH_2Cl_2 (3 × 1.0 mL). The mixture was stirred under N_2 for 30 min, at room temperature, after which the reaction was quenched by the addition of ice-cold aq. $NaHCO_3$. This mixture was diluted with CH_2Cl_2 and the organic layer was then washed with sat. aq. NaCl, and dried (K_2CO_3), and the solvent was removed by evaporation. The reaction proceeded nearly quantitatively, as determined by t.l.c., and the product **10** (0.775 g, 98%) was used immediately, without further purification, in the subsequent glycosylation reaction.

Allyl 2-O-(3-O-(2-O-(2-O-benzoyl-4-O-benzyl-3-O-[[2-trimethylsilyl]ethoxy]methyl)-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-rhamnopyranosyl)-3-O-(3,4,6-tri-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranoside (**11**). — A sample of the trisaccharide alcohol **8** (0.198 g, 0.149 mmol) in anhydr. CH_2Cl_2 (3.0 mL) with silver trifluoromethanesulfonate (0.102 g, 0.397 mmol) and collidine (0.6 mL), was stirred with 4A molecular sieves for 30 min, and then cooled to −78°. A solution of the trisaccharide chloride **10** (0.345 g, 0.256 mmol) in anhydr. CH_2Cl_2 (4.0 mL), previously stirred with 4A molecular sieves for 30 min and then cooled to −78°, was added dropwise to the stirred solution of **8**. The dropping funnel containing **10** was rinsed with additional portions of CH_2Cl_2 (3 × 0.75 mL) which were added to the mixture. The mixture was stirred under N_2, in the dark, and allowed to warm to room temperature. After 24 h of reaction, the mixture was filtered, and the filtrate was washed successively with M HCl, aq. $NaHCO_3$, and water. The organic phase was dried (Na_2SO_4) and the solvent was evaporated to dryness. The remaining syrup was purified by chromatography, with 2:1 hexane–EtOAc as eluant. *Compound* **11** was obtained as a clear, light yellow syrup which was dried *in vacuo* (0.190 g, 50%); $[\alpha]_D^{23}$ −14.6° (*c* 2.8, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 5.92 (m, 1 H, $OCH_2CH=CH_2$), 5.18 (m, 1 H, J_{trans} 15.5 Hz, $OCH_2CH=CHH$ *trans*), 5.10 (m, 1 H, J_{cis} 10.5 Hz, $OCH_2CH=CHH$ *cis*), 4.99, and 4.62 (ABq, 2 × 1 H, $J_{A,B}$ 11.0 Hz, OCH_2Ph), 4.97, and 4.81 (ABq, 2 × 1 H, $J_{A,B}$ 7.0 Hz, $OCH_2OCH_2CH_2SiMe_3$), 4.94, and 4.69 (ABq, 2 × 1 H, $J_{A,B}$ 11.5 Hz, OCH_2Ph), 0.94 and 0.91 (m, 2 H, $OCH_2OCH_2CH_2SiMe_3$), −0.13 (s, 9 H, $OCH_2OCH_2CH_2SiMe_3$) see Table III also; ^{13}C(^{1}H)-n.m.r. (100.6 MHz): δ 168.3, 166.0, 165.7, (2 C), 165.5, 165.4, 165.1, and 164.9 (benzoyl CO), 117.2 ($OCH_2CH=CH_2$), 93.8 ($OCH_2OCH_2CH_2SiMe_3$), 75.2, 74.8, 74.4, and 73.7 (OCH_2Ph), 67.7 ($OCH_2CH=CH_2$), 65.5 ($OCH_2OCH_2CH_2SiMe_3$), 17.9 ($OCH_2OCH_2CH_2SiMe_3$), −1.5 ($OCH_2OCH_2CH_2SIMe_3$), see Table III also.

Anal. Calc. for $C_{145}H_{142}N_2O_{38}Si$: C, 68.33; H, 5.61; N, 1.09. Found: C, 68.12; H, 5.58; N, 1.10.

Propyl 2-O-(3-O-(2-O-(α-L-rhamnopyranosyl)-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-rhamnopyranoside (**12**). — The hexasaccharide **11** (190 mg; 0.074 mmol) was dissolved in a mixture of methanolic HCl (3.0 mL) [prepared by treating anhydr. MeOH (100 mL) with acetyl chloride (4.0 mL)], MeOH (3.0 mL), and

CH_2Cl_2 (1.0 mL) in order to remove the SEM group. The solution was stirred for 24 h at room temperature, under N_2. The mixture was then neutralized by the addition of aq. $NaHCO_3$. This solution was then extracted with CH_2Cl_2 (3 ×), the combined extracts were dried (Na_2SO_4), and the solvent was removed by evaporation to give a syrup which was then chromatographed with 5:4 hexane–EtOAc as eluant. The compound lacking the SEM group was obtained as a clear, colorless syrup which was dried *in vacuo* to give a white foam (0.136 g; 75.4%); $[\alpha]_D^{23}$ − 17.1° (*c* 0.6, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 5.82 (H-$1_{C'}$), 5.67 (H-1_C), 5.54 (H-1_A), 5.19 (H-$1_{A'}$), 5.17 (H-$1_{B'}$), 4.59 (H-1_B); ^{13}C(^{1}H)-n.m.r. (100.6 MHz): δ 166.5, 166.5, 166.0, 165.9, 165.7, 165.65, 165.59, 165.5, 165.1, and 164.8 (benzoyl CO), 117.2 ($OCH_2CH = CH_2$), 100.7 (C-$1_{B'}$), 100.0 (C-1_C), 99.9 (C-$1_{C'}$), 99.8 (C-$1_{A'}$), 98.9 (C-1_A), 97.8 (C-1_B).

Anal. Calc. for $C_{139}H_{128}O_{37}N_2$: C, 69.03; H, 5.33, N, 1.16. Found: C, 68.76; H, 5.35; N, 1.17.

A portion of this sample (86.2 mg; 0.0356 mmol) was dissolved in sodium methoxide (0.26M in MeOH). The mixture was stirred for 18 h at room temperature and then neutralized by stirring with Rexyn 101 H^+ resin beads. 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 (130 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 the residue co-evaporated several times with EtOH to remove traces of HOAc. The residue was then taken up in EtOH (20 mL) to which was added hydrazine hydrate (100%) (0.4 mL), and the solution was refluxed under N_2 for 18 h. The solution was then filtered to remove a fine grey precipitate and the filtrate co-evaporated several times with MeOH. The residue was taken up in MeOH (10 mL) to which was added acetic anhydride (1 mL). The solution was left to stand for 30 min at room temperature and then evaporated to dryness. The residue was co-evaporated several times with MeOH and the residue was chromatographed with 6:3:1 EtOAc–MeOH–water as eluant. The solvent was removed by evaporation and the residue was further purified by passing the sample through a column of Sephadex LH20 with MeOH as eluant. *Compound* **12** was obtained as a white amorphous solid (11.1 mg; 30%); ^{1}H-n.m.r. (400.13 MHz, D_2O): δ 3.67 and 3.53 (2 H, $OCH_2CH_2CH_3$), 1.99 and 1.98 (s, 2 × 3 H, $NHCOCH_3$), 1.63 (2 H, $OCH_2CH_2CH_3$), 0.93 (3 H, $OCH_2CH_2CH_3$), see also Table III; ^{13}C(^{1}H)-n.m.r. (100.6 MHz, D_2O): δ 177.4 and 177.2 ($NHCOCH_3$), 72.4 ($OCH_2CH_2CH_3$), 24.9 ($NHCOCH_3$), 24.6 ($OCH_2CH_2CH_3$), 12.5 ($OCH_2CH_2CH_3$), see also Table III.

Plasma-desorption m.s. Calc. for $C_{43}H_{74}N_2O_{27}Na$: *m/z* 1074; Found: *m/z* 1074 $[M + Na]^+$.

ACKNOWLEDGMENTS

We are grateful to the Natural Sciences and Engineering Research Council of Canada and the Heart and Stroke Foundation of B.C. and the Yukon for financial support. We also thank Dr. L. Chen for recording the plasma-desorption mass spectrum and Applied Biosystems, California, U.S.A., for providing the facilities.

REFERENCES

1 S. E. Read and J. B. Zabriskie (Eds.), *Streptococcal Diseases and the Immune Response*, 1980, Academic Press, New York.
2 A. L. Bisno, in G. L. Mandell, R. G. Douglas, J. E. Benett (Eds.), *Principles and Practice of Infectious Diseases*, 2nd edn., 1985, Wiley, New York, pp. 1133–1142.
3 J. B. Zabriskie, *Circulation*, 71 (1985) 1077–1086.
4 K. B. Reimer and B. M. Pinto, *J. Chem. Soc., Perkin Trans. 1*, (1988) 2103–2111.
5 J. S. Andrews and B. M. Pinto, *J. Chem. Soc., Perkin Trans. 1*, (1990) 1785–1792.
6 B. M. Pinto, K. B. Reimer, and A. Tixidre, *Carbohydr. Res.*, 210 (1991) 199–219.
7 (a) J. E. Coligan, T. J. Kindt, and R. M. Krause, *Immunochemistry*, 15 (1978) 755–760; (b) D. H. Huang, N. R. Krishna, and D. G. Pritchard, *Carbohydr. Res.*, 155 (1986) 193–199.
8 R. U. Lemieux, T. Takeda, and B. Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90–115.
9 B. M. Pinto, D. G. Morissette, and D. R. Bundle, *J. Chem. Soc., Perkin Trans. 1*, (1987) 9–14.
10 B. M. Pinto, M. M. W. Buiting, and K. B. Reimer, *J. Org. Chem.*, 55 (1990) 2177–2181.
11 (a) T. Peters and D. R. Bundle, *Can. J. Chem.*, 67 (1989) 491–496; 497–502; (b) J. Kihlberg, E. Eichler, and D. R. Bundle, *Carbohydr. Res.*, 211 (1991) 59–75.
12 T. Ogawa, T. Kitajma, and T. Nukada, *Carbohydr. Res.*, 123 (1983) c5–c7.
13 T. Ogawa, M. Sugimoto, T. Kitajma, K. K. Sadozai, and T. Nukada, *Tetrahedron Lett.*, 27 (1986) 5739–5742.
14 O. P. Srivastava and O. Hindsgaul, *Can. J. Chem.*, 64 (1986) 2324–2330.
15 (a) E. J. Corey and J. W. Suggs, *J. Org. Chem.*, 38 (1973) 3224; (b) A. Emery, A. C. Oehlschlager, and A. M. Unrau, *Tetrahedron Lett.*, (1970) 4401–4403.
16 R. Gigg and C. D. Warren, *J. Chem. Soc. C*, (1968) 1903–1911.
17 (a) H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, 42 (1959) 1653–1658; (b) T. Iversen and D. R. Bundle, *Carbohydr. Res.*, 103 (1982) 29–40; (c) R. U. Lemieux, S. Z. Abbas, and B. Y. Chung, *Can. J. Chem.*, 60 (1982) 58–62.
18 H. Herbst, D. Lavanchy, and D. G. Braun, *Ann. Immunol. (Paris)*, 134D (1973) 349–372.
19 A. Bax and R. Freeman, *J. Magn. Res.*, 44 (1981) 542–561.
20 P. H. Bolton and F. A. Wilde, *J. Magn. Res.*, 59 (1984) 343–346.
21 L. Braunschweiler and R. R. Ernst, *J. Magn. Res.*, 53 (1983) 121–122.
22 (a) A. Bax and D. G. Davis, *J. Magn. Res.*, 63 (1985) 207–213; (b) C. Griesinger and R. R. Ernst, *J. Magn. Res.*, 75 (1987) 261—271.
23 A. Bax and S. Subramanian, *J. Magn. Res.*, 67 (1986) 565–569.
24 A. Bax, R. H. Griffey, and B. L. Hawkins, *J. Magn. Res.*, 55 (1983) 301–315.
25 D. Marion and K. Wutrich, *Biochem. Biophys. Res. Commun.*, 117 (1983) 967–974.
26 (a) K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2* (1974) 293–297; (b) K. Kock and C. Pedersen, *Acta Chem. Scand. Ser. B*, 29 (1975) 258–264; (c) A. S. Perlin and B. Casu, *Tetrahedron Lett.*, (1969) 2921–2924.
27 R. J. Cotter, *Anal. Chem.*, 60 (1988) 781A–793A.
28 R. R. Townsend, M. Alai-Safar, M. R. Hardy, and C. C. Fenselau, *Anal. Biochem.*, 171 (1988) 180–191.
29 D. R. Bundle, T. Iversen, and S. Josephson, *Am. Lab.*, 12 (1980) 93–98.