Carbohydrate Research, 210 (1991) 199–219
Elsevier Science Publishers B.V., Amsterdam

Synthesis and n.m.r. analysis of branched trisaccharide and pentasaccharide haptens of the β-hemolytic Streptococci Group A and the preparation of synthetic antigens*

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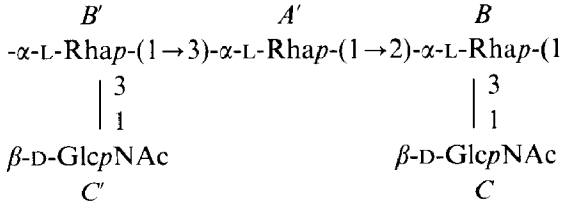
(Received May 18th, 1990; accepted for publication August 3rd, 1990)

ABSTRACT

The synthesis of branched trisaccharide and pentasaccharide portions of the cell-wall polysaccharide of the β-hemolytic Streptococci Group A is described. The key dissaccharide acceptors, allyl or 8-(methoxycarbonyl)octyl 3-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-*O*-benzyl-α-L-rhamnopyranoside, in conjunction with a selectively blocked α-L-rhamnopyranosyl chloride under Koenigs–Knorr conditions, afforded the branched trisaccharides in 81 and 62% yield, respectively. Analogously, glycosylation of the 8-(methoxycarbonyl)octyl disaccharide with a protected β-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p* chloride gave the pentasaccharide in 43% yield. The key disaccharide acceptors were obtained, in turn, from the allyl or 8-(methoxycarbonyl)octyl rhamnoside acceptors and 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride under Koenigs–Knorr conditions. The latter glycosyl donor has not been described previously. Removal of the protecting groups afforded the trisaccharide haptens as their 1-propyl and 8-(methoxycarbonyl)octyl glycosides and the pentasaccharide as its 8-(methoxycarbonyl)octyl glycoside. The compounds have been subjected to detailed analysis by two-dimensional n.m.r. methods. Preparation of the synthetic antigens followed coupling of the 8-(methoxycarbonyl)octyl glycosides to bovine serum albumin *via* the acyl azide intermediates.

INTRODUCTION

The repeating unit of the β-hemolytic Streptococci Group A cell-wall polysaccharide consists of a poly(α-L-rhamnopyranosyl) backbone composed of alternating (1→2) and (1→3) linkages to which β-D-*N*-acetylglucosamine residues are attached at the 3-position of the rhamnose residue.



* Part 3 of the series Oligosaccharides Corresponding to the Antigenic Determinants of the β-Hemolytic *Streptococci* Group A.

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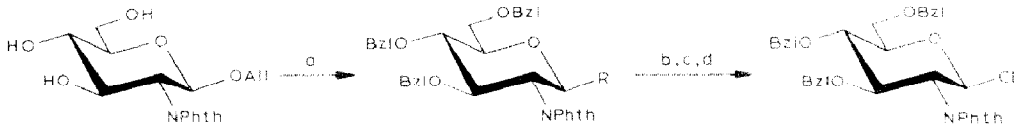
0008-6215/91/$ 03.50

As part of a program to develop immunodiagnostic reagents based on this structure, we have recently reported[1,2] the synthesis of disaccharide (BC), linear trisaccharide (ABC), and branched tetrasaccharide [AB(C)A′] haptens. The purpose of this program was to provide a variety of oligosaccharides and their corresponding glycoconjugates which could be used in inhibition studies, in conjunction with n.m.r. and molecular modelling studies, to identify a surface unique to the Streptococci Group A organism. The selected oligosaccharides and/or their complementary monoclonal antibodies could then be used in the development of immunodiagnostic kits[3] for the detection of Streptococci Group A bacteria. As a further extension of our synthetic program, we now report a route to the branched trisaccharide [B(C)A′] and pentasaccharide [B(C)A′B′C′] haptens as well as the preparation of the desired glycoconjugates.

RESULTS AND DISCUSSION

Retrosynthetic analysis indicated that a suitably functionalized β-D-Glc*p*NAc-(1→3)-α-L-Rha*p* (CB) disaccharide unit could serve as an acceptor for the synthesis of both tri- and penta-saccharides. The required acceptor would have to contain persistent blocking groups at the 3,4, and 6-positons of the β-D-Glc*p* unit and at the 4-position of the rhamnose unit, leaving the 2-position of the rhamnose unit accessible for glycosylation. We envisaged the use of benzyl ethers as the persistent blocking groups and either an acetate or benzoate ester as the latent blocking group on the 2-position of rhamnose. Analogous glycosylation reactions with a similar unit containing 2-trimethylsilylethoxymethyl (SEM) acetals as blocking groups proceeded in poor yield[3], presumably due to steric hindrance. Accordingly allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside[4] **1** was converted into its tri-*O*-benzyl derivative **2** with sodium hydride and benzyl bromide in 64% yield (Scheme 1). The reaction requires rigorously anhydrous conditions and the processing of the reaction mixture requires some care. Conversion of the allyl group in **2** into the 1-propenyl group by using Wilkinson's catalyst[5] and subsequent hydrolysis[6] of the vinyl ether then afforded the hemiacetals **3**. Treatment of the hemiacetals **3** with *N*,*N*-dimethyl(chloromethylene)ammonium chloride[7] then gave the glycosyl chloride **4** (68% yield from **2**) (Scheme 1). To the best of our knowledge, compound **4** has never



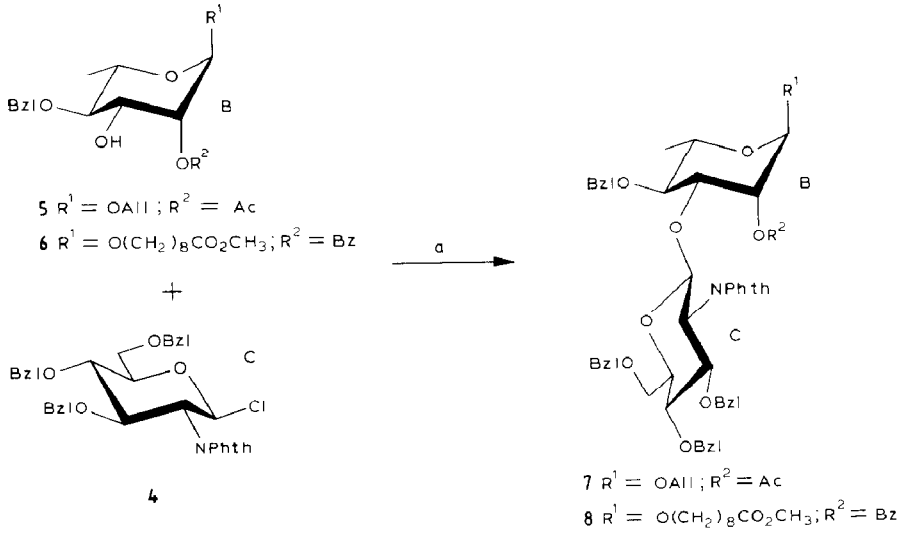
1 **2** R = OAll **4**

3 R = OH

Scheme 1

(a) NaH, $C_6H_5CH_2Br$, THF, 36 h;
(b) $Rh(PPh_3)_3Cl$, EtOH–H_2O (9:1), reflux 18 h;
(c) HgO, $HgCl_2$, $(CH_3)_2CO$–H_2O (10:1), 18 h;
(d) CO_2Cl_2, DMF, CH_2Cl_2, 2 h

been used as a glycosyl donor. We note, however, that a related donor, namely 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride has been used in glycosylation reactions[8]. Glycosylation of allyl 2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside **5** or 8-(methoxycarbonyl)octyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside **6** with the donor **4** under silver trifluoromethanesulfonate promotion in the presence of collidine[9] afforded the disaccharides **7** and **8** in 61 and 57% yield, respectively (Scheme 2). Transesterification of **7** and **8** then yielded the required disaccharide acceptors, **9** and **10**, respectively. The 8-(methoxycarbonyl)octyl chain in **10** were to be used for covalent attachment to protein[10,11] in the subsequent preparation of the glycoconjugates.

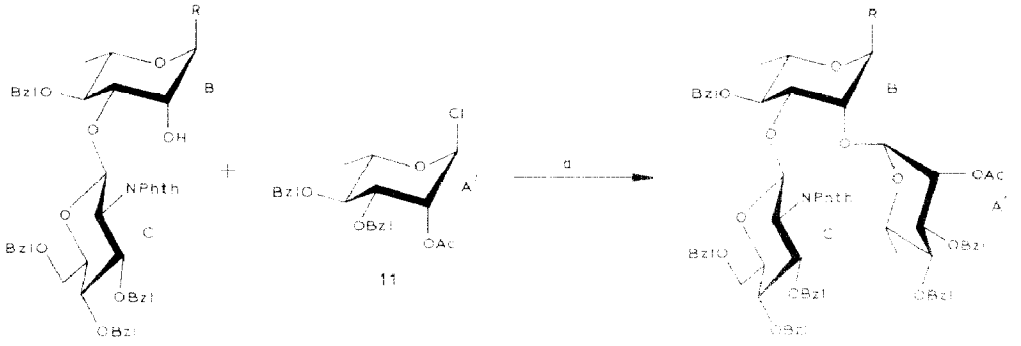


Scheme 2

(a) CF_3SO_3Ag, collidine, CH_2Cl_2, −78 to 25°

Treatment of the glycosyl acceptors **9** or **10** with 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride[12] **11** under silver trifluoromethanesulfonate promotion in the presence of 1,1,3,3-tetramethylurea[13] afforded the desired branched trisaccharides **12** and **13** in 81 and 62% yield, respectively (Scheme 3). Elaboration of the pentasaccharide unit now required a suitable trisaccharide donor. Thus, deallylation of trisaccharide[1] **14**, and conversion of the resultant hemiacetals **15** into the glycosyl chloride **16**, as already described, furnished the desired compound (Scheme 4). Glycosylation of the acceptor **10** with the trisaccharide chloride **16** under analogous conditions to those described for the preparation of **12** and **13** then gave the pentasaccharide **17** in 43% yield (Scheme 4).

Deprotection of compounds **12**, **13**, and **17** was accomplished by (*a*) base-catalyzed methanolysis of the ester functions, (*b*) hydrogenation of the benzyl ethers (and allyl ether in the case of **12**, (*c*) hydrazinolysis of the phthalimido group, and (*d*)



9 R = OAll
10 R = $O(CH_2)_8CO_2CH_3$

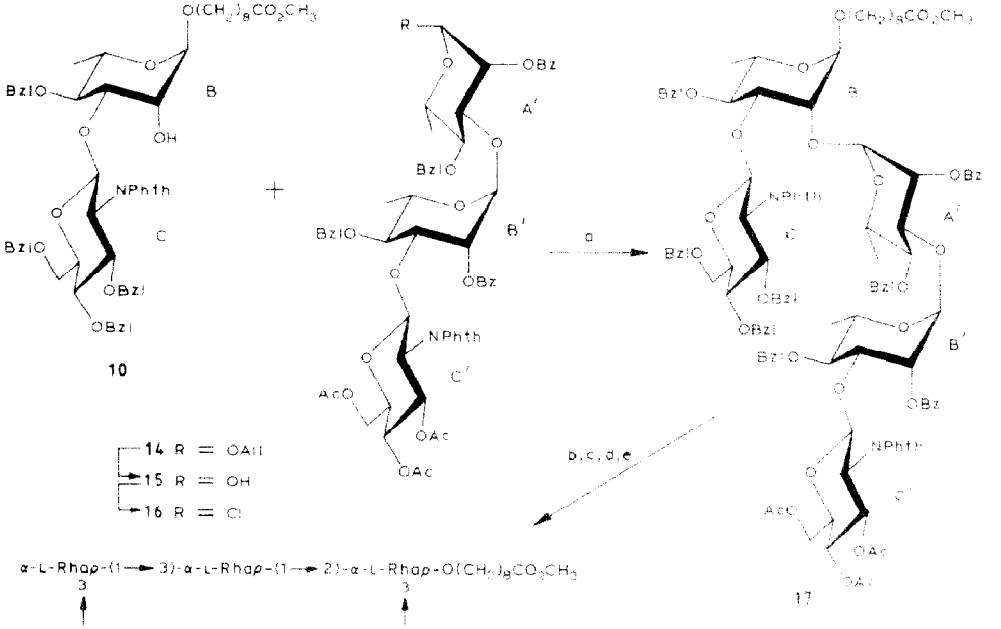
(a) CF_3SO_3Ag, TMU, CH_2Cl_2, −78 to 25°.
(b) NaOMe, MeOH, 10 h.
(c) Pd/C, H_2, (4 : 1 HOAc–H_2O).
(d) $N_2H_4 \cdot H_2O$, EtOH, reflux.
(e) Ac_2O, MeOH.

b, c, d, e

α-L-Rhap-(1→2)-α-L-Rhap-X
3
β-D-GlcpNAc

18 X = $OCH_2CH_2CH_3$
19 X = $O(CH_2)_8CO_2CH_3$

Scheme 3



(a) CF_3SO_3Ag, TMU, CH_2Cl_2, −78 to 25°.
(b) NaOMe, MeOH, 10 h.
(c) Pd/C, H_2, (4 : 1 HOAc–H_2O).
(d) $N_2H_4 \cdot H_2O$, EtOH, reflux.
(e) Ac_2O, MeOH.

Scheme 4

selective *N*-acetylation of the resultant amine (Scheme 4). Analytically pure compounds **18**, and **20** were obtained following successive chromatography on silica gel and Sephadex LH20. Owing to the hygroscopic nature of the compound, a satisfactory combustion microanalysis result was not obtained for compound **19**, despite several attempts. Therefore, a plasma-desorption mass spectrum[14] was obtained as a confirmation of composition. The peak appearing at *m/z* 708 was assigned to the M^+ ion of the sodium salt of compound **19**. $M+Na^+$ ions are commonly observed in plasma-desorption mass spectra, particularly of compounds containing labile hydrogens or anionic moieties[15].

The assigned structures were in accord with their ^{1}H- and ^{13}C-n.m.r. spectral data (Tables I-III). Compounds were characterized by use of routine ^{1}H, ^{13}C and $^{13}C\{^1H\}$ n.m.r. spectra. ^{1}H-Homonuclear chemical shift correlated (COSY) experiments[16] were performed on compounds **8**, **13**, **17**, **18**, **19**, and **20** in order to facilitate assignments, and ^{13}C–^{1}H chemical-shift correlated experiments[17] were performed on compounds **13**, **17** and **20**. The ^{13}C–^{1}H chemical-shift correlated experiments performed on compounds **17** and **20** were carried out in the inverse mode[18], thereby taking advantage of the sensitivity of the ^{1}H nucleus. In fact, the spectra were measured using 10-mg samples. Thus, the proton chemical-shifts are displayed along the F_2-axis and the carbon chemical-shifts are displayed along the F_1-axis. The experiments were carried out without carbon-decoupling during acquisition, permitting the measurement of the one-bond ^{13}C–^{1}H coupling constants ($^1J_{^{13}C-^1H}$) for the anomeric carbons along the F_2-axis, with a digital resolution of 1.5Hz/pt (see for example, Fig. 1).

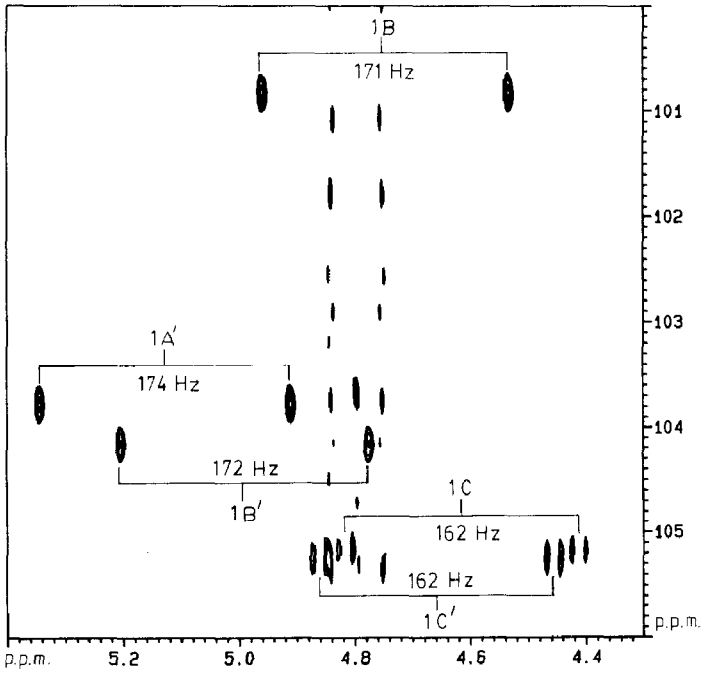


Fig. 1. Anomeric-signal region of the ^{1}H-detected ^{13}C–^{1}H chemical-shift correlated 2D spectrum of penta-saccharide **20**.

TABLE I

^{1}H-N.m.r. data[a] for the ring protons

Ring protons	**7**	**8**	**9**	**10**	**12**	**13**	**18**	**19**
1B[b]	4.81 (1.75)	4.84 (1.8)	4.75	4.69 (1.7)	4.64 (1.2)	4.58	4.78 (1.8)	4.78
2B	5.29 (1.75, 3.75)	5.43 (1.8, 3.5)	4.08 (1.5, 3.5)	4.03	4.23 (1.2, 3.5)	4.19 (1.2, 3.5)	4.10 (1.8, 3.0)	4.11
3B	4.05 (3.75, 9.25)	4.11 (3.5, 9.5)	3.84 (3.5, 9.0)	3.81 (3.2, 9.0)	3.96 (3.5, 9.0)	3.93 (3.1, 9.5)	3.80 (3.0, 9.5)	3.80 (3.2, 9.5)
4B	3.37 (19.0)[c]	3.44 (19.0)[c]	3.47 (18.0)[c]	3.33 (19.0)[c]	3.15 (18.0)[c]	3.13 (19.0)[c]	3.46	3.47
5B	3.67	3.64	3.56 (6.0, 9.0)	3.53	3.53 (9.0, 6.2)	3.50	3.67	3.67 (6.2)
6B	1.12 (6.0)	1.08 (6.2)	1.08 (6.0)	1.07 (6.2)	1.01 (6.2)	0.99 (6.2)	1.22 (6.1)	1.16
1C	5.44 (8.1)	5.44 (8.0)	5.39 (8.0)	5.41 (8.0)	5.37 (8.0)	5.375 (8.0)	4.64 (8.3)	4.66 (8.5)
2C	4.30 (8.1, 10.5)	4.23	4.26 ↑ multiplet	4.37 ↑ multiplet	4.45 (8.0, 10.5)	4.456 (8.0, 10.9)	3.69	3.70
3C	4.36 (8.5, 10.5)	4.28	↓ 4.37	↓ 4.28	4.38 (8.1, 10.5)	4.388 (8.0, 10.9)	3.50	3.52

4C	3.97 (8.0, 8.5)	3.80 (18.2)[c]	3.81 ↑	3.78 ↑	3.80 ↑	3.77 (17.5)[c]	3.41	3.43
5C	3.67	3.62	multiplet	multiplet	multiplet	3.64 ↑	3.40	3.41
6C	3.91 (3.5, 11.0)	3.82 (3.5, 11.0)	↓	↓	↓	multiplet ↓	3.87 (11.5)	3.88 (12.0)
6'C	3.82 (1.5, 11.0)	3.68	3.64	3.64	3.60	3.80	3.71	3.73
1A'					5.26 (1.8)	5.27 (1.8)	5.11 (1.8)	5.13
2A'					5.61 (1.8, 3.5)	5.625 (1.8, 3.2)	3.99 (1.8, 3.2)	4.01 (1.6, 3.2)
3A'					3.94 (3.5, 9.0)	3.96 (3.1, 9.5)	3.73 (3.2, 9.5)	3.74 (3.2, 9.5)
4A'					3.45 (18.0)[c]	3.46 (19.0)[c]	3.39	3.40
5A'					3.83 (9.0, 6.2)	3.86 (6.2, 9.5)	3.67	3.68
6A'					1.29 (6.2)	1.30 (6.2)	1.22 (6.1)	1.17 (6.2)

[a] In $CDCl_3$ for **7**, **8**, **9**, **10**, **12**, and **13**, and in D_2O for **18**, and **19**. The numbers in parentheses denote coupling constants, in Hz. [b] Indicates the ring to which the aglycon is attached. [c] These values are the sums of the individual coupling constants $J_{AX} + J_{BX}$.

TABLE II

^{13}C-N.m.r. data[a] for the ring carbons

Ring carbons	**7**	**8**	**9**	**10**	**12**	**13**	**18**	**19**
1B[b]	96.4	97.0	98.3	98.2	98.1	98.7	101.2 (171)	101.2 (172)
2B	72.0	72.9	67.0	66.8	78.4	78.3		
3B	79.43	79.7	82.8	82.9	80.5	80.4	79.6	79.5
4B	79.37	79.33	79.6	79.6	79.2	79.0	74.8	74.8
5B	68.2	68.1	68.9	68.9	67.7	67.3	71.7	
6B	17.6	17.8	17.5	17.7	17.7	17.6		19.4
1C	99.2	99.2	98.4	99.1	99.7	99.5	105.3 (162)	105.3 (163)
2C	56.2	56.2	55.9	55.9	56.13	55.9	58.7	58.7
3C	79.17	79.1	78.9	78.9	79.1	78.8	76.6	76.6
4C	79.24	79.28	79.3	79.2	79.6	79.4		
5C	74.8	74.7	74.7	74.7	74.8	74.6	78.6	78.6
6C	68.7	68.8	69.6	69.6	69.3	69.1	63.7	63.7
1A'					99.6	99.4	104.4 (169)	104.3 (175)
2A'					68.8	68.6		
3A'					77.6	77.4	82.7	82.7
4A'					80.3	80.1	73.9	73.9
5A'					67.9	67.7	71.9	
6A'					17.9	17.9		19.4

[a] In $CDCl_3$ for **7**, **8**, **9**, **10**, **12**, and **13**, and in D_2O for **18**, and **19**. The numbers in parentheses denote the one-bond ^{13}C–^1H coupling constants ($^1J_{^{13}C,^1H}$) in Hz. [b] Indicates the ring to which the aglycon is attached.

The vicinal coupling constants of the ring-protons in the monosaccharide units within oligosaccharides were consistent with a $^4C_1$ (D) conformation for the *N*-acetylglucosamine ring and with a $^1C_4$ (L) conformation for the rhamnopyranosyl units.

The stereochemical integrity of the trisaccharides **18** and **19** and the pentasaccharide **20** was confirmed by examination of the one-bond ^{13}C–^1H coupling constants, $^1J_{^{13}C,^1H}$ for the anomeric carbons[19].

TABLE III

^{1}H and ^{13}C n.m.r. data[a] for **15**, **17**, and **20**

Ring	*^{1}H-N.m.r.*			*^{13}C-N.m.r.*		
	15	**17**	**20**	**15**	**17**	**20**
1B[b]		4.58	4.76		98.8 (170)[d]	100.9 (171)[d]
2B		4.21	4.12		78.7	79.2
3B		3.85 (3.2, 9.5)	3.78		81.3	82.7
4B		3.22 (19.0)[c]	3.45		78.7	73.6
5B		3.47	3.66		67.2	71.4
6B		.095 (6.2)	1.24		17.7	19.0
1C		5.37 (8.4)	4.62 (8.5)		99.6 (163)[d]	105.2 (162)[d]
2C		4.49 (8.4, 10.5)	3.68		55.9	58.3
3C		4.30	3.55 ↑		79.0	76.3
4C		3.70	multiplet ↓		79.7	72.4
5C		3.66	3.40		74.2	78.3
6C		3.59 (10.9)	3.88		69.1	63.1
6'C		3.50	3.72			
1A'	5.27 (1.6)	5.43	5.13 (1.8)	91.9 (171)[d]	99.1 (177)[d]	103.8 (174)[d]
2A'	5.39	5.62	4.04 (1.8, 3.0)	72.9	72.7	72.4
3A'	4.34	4.31	3.80	77.5	77.1	79.6
4A'	3.65 (19.2)[c]	3.68	3.49	80.1	80.7	73.9
5A'	4.07	3.93	3.69	67.8	67.9	71.7
6A'	1.35 (6.2)	1.31 (6.2)	1.23 (6.2)	18.1	18.1	18.2
1B'	5.18 (1.8)	5.25 (1.8)	5.00 (1.8)	98.7 (173)[d]	98.8 (173)[d]	104.2 (172)[d]
2B'	5.39	5.48 (1.8, 3.2)	4.23 (1.8, 3.1)	72.1	72.3	72.5
3B'	4.06 (3.4, 9.5)	4.17 (3.2, 9.5)	3.88	79.7	79.6	82.5
4B'	3.38 (18.0)[c]	3.49	3.46	78.7	78.7	73.3
5B'	3.70	3.94	3.77	68.3	68.2	71.7
6B'	0.92 (6.2)	1.18 (6.2)	1.26	17.6	17.7	19.2
1C'	5.36 (8.5)	5.46 (8.5)	4.68 (8.5)	98.5 (173)[d]	98.5 (166)[d]	105.3 (162)[d]
2C'	4.26 (8.5, 10.5)	4.30	3.72	54.7	54.7	58.3

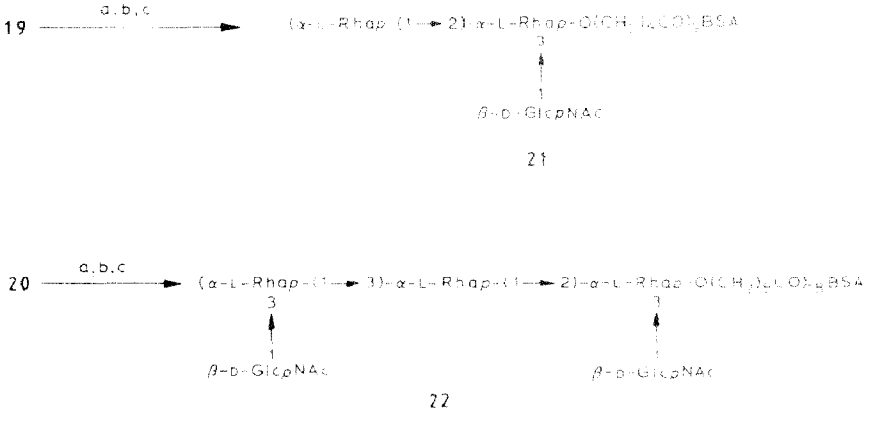
TABLE III (*continued*)

^{1}H and ^{13}C n.m.r. data[a] for **15**, **17**, and **20**

Ring[b]	^{1}H-N.m.r.			^{13}C-N.m.r.		
	15	**17**	**20**	**15**	**17**	**20**
3C′	5.54 (9.0, 10.5)	5.60 (9.3, 10.7)	3.55 [c]	70.8	70.8	76.3
4C′	4.97 (9.0, 10.0)	5.01 (19.0)[d]	multiplet [c]	68.2	68.2	72.4
5C′	3.06	3.17	3.40	71.2	71.2	78.3
6C′	3.88 (3.4, 12.5)	3.92	3.88	61.1	61.1	63.1
6′C′	3.75 (2.0, 12.5)	3.79 (2.0, 10.5)	3.72			

[a] In $CDCl_3$ for **15** and **17**, and in D_2O for **20**. 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–^{1}H coupling constants ($J_{C,H}$) in Hz.

Preparation of synthetic antigens. — The glycoconjugates of the trisaccharide **19** and pentasaccharide **20** with bovine serum albumin (BSA) were prepared by the modified acyl azide methodology of Pinto and Bundle[11]. Thus, the esters **19** and **20** were converted into their hydrazides, and the latter were then treated with dinitrogen tetraoxide as the nitrosating agent. The resultant acyl azides were then treated immediately with BSA in buffer solution to provide the corresponding glycoconjugates, **21** and **22** (Scheme 5). Levels of hapten incoroporation of 15 and 30%, respectively were achieved, and afforded glycoconjugates possessing 9 and 18 haptens, respectively per molecule of protein[20]. These synthetic antigens are currently being used in the hybrid-



Scheme 5

(a) $N_2H_4 \cdot H_2O$, EtOH, 20°, 12 h.
(b) N_2O_4, CH_2Cl_2, DMF.
(c) BSA (buffer solution).

mycloma protocol as immunizing antigens and also as screening agents for monoclonal antibodies.

EXPERIMENTAL

General. — Melting points are uncorrected. ^{1}H-N.m.r. and ^{13}C-n.m.r. spectra were recorded on either a Bruker WM-400 or a Bruker AMX-400 n.m.r. spectrometer in deuteriochloroform unless otherwise stated. For those spectra measured in deuterium oxide, chemical shifts are given in p.p.m. downfield from 4,4-dimethyl-4-silapentane-1-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 aquired using quadrature detection in both dimensions. The initial data sets of 512 × 2048 data points were zero-filled once in the F_1 direction to give a final data set of 1024 × 1024 real data points. A non-shifted sine bell function was applied prior to Fourier transformation. The magnitude spectra were symmetrized about the diagonal before analysis. For the inverse detection experiments a 4-pulse sequence incorporating a BIRD pulse in the preparation period, with phase-sensitive detection was used. The data set of 512 × 2048 data points was 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 9.3 Hz/pt and 1.5 Hz/pt in the F_1- and the F_2-directions respectively. The data set was phase corrected, and a base-line correction applied prior to analysis.

The n.m.r. data for the ring protons and carbons are recorded in Tables I–III. The remaining signals are recorded in the Experimental.

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 using 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 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 precoated Al plates with Merck Silica Gel 60F-254 as the absorbant. 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[21]. Purification at each stage was crucial to the outcome 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 nitrogen were also carried out in deoxygenated solvents. Transfers under nitrogen were effected by means of standard Schlenk-tube techniques.

Experimental procedures. — (*a*) *Allyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**2**). A solution of allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside[4] **1** (4.80 g, 13.7 mmol) in anhydrous tetrahydrofuran (30 mL) was added by means of a cannula under nitrogen to a cold suspension of NaH (2.0 g, 42 mmol) in tetrahydrofuran (20 mL). The flask was rinsed with additional portions of tetrahydrofuran (2 × 3 mL) and the contents were transferred as before. The mixture was stirred for 15 min and then benzyl bromide (5.1 mL, 42 mmol) was added dropwise during 45 min to the cold solution. The mixture was stirred under N_2 for 24 h at room temperature after which time an additional portion of NaH (0.20 g, 4.1 mmol) was added followed by the dropwise addition of benzyl bromide (0.51 mL, 4.6 mmol). After 12 h the mixture was poured, with vigorous stirring, into a cold solution of 2M HCl and extracted with EtOAc. The organic layer was washed with water until the pH was neutral, and dried (Na_2SO_4). The filtrate was concentrated and the resulting syrup was chromatographed using 2:1 hexane–EtOAc as eluant. Compound **2** was obtained as a clear colorless syrup (5.49 g, 64%); $[\alpha]_D^{25} + 1.13°$ (*c* 1.9 in CH_2Cl_2); ^{1}H-n.m.r. (400.13 MHz): δ 7.8–6.85 (19 H, m, ArH); 5.18 (1 H, d, $J_{1,2}$ 8.5 Hz, 1-H), 4.37 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 9 Hz, 3-H), 4.22 (1 H, dd, $J_{1,2}$ 8.5, $J_{2,3}$ 10.2 Hz, 2-H), 3.78 (3 H, m, 4-H, 6-Ha and 6-Hb), and 3.65 (1 H, dt, $J_{5,6a}$ 3.0, $J_{5,6b}$ 2.7 Hz, 5-H); ^{13}C{^{1}H}-n.m.r. (100.6 MHz): δ 133.9 ($CH_2CH=CH_2$), 117.1 ($CH_2CH=CH_2$), 97.5 (C-1), 79.8, 79.4 and 75.1 ($PhCH_2$), 74.8 and 74.7 (C-5 and C-4), 73.5 (C-3), 69.6 ($CH_2CH=CH_2$), 68.8 (C-6), and 55.9 (C-2).

Anal. Calc. for $C_{38}H_{38}NO_6$: C, 73.64; H, 6.18; N, 2.26. Found: C, 73.76; H, 6.15; N, 2.10.

(*b*) *3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranose* (**3**). Tris(triphenylphosphine)rhodium (I) chloride (0.80 g, 0.87 mmol) was added to a solution of the allyl glycoside **2** (5.4 g, 8.7 mmol) in 9:1 EtOH–water (300 mL) and the mixture was heated at reflux for 18 h under N_2. Removal of the solvent left a light-brown residue which was taken up in EtOAc and filtered through a column of silica gel. Evaporation of the solvent gave a syrup which was dissolved in 10:1 acetone–water (250 mL) containing yellow HgO (1.88 g, 8.68 mmol). To this solution $HgCl_2$ (2.36 g, 8.69 mmol) in 10:1 acetone–water (50 mL) was added dropwise, followed by dropwise addition of 10:1 acetone–water (20 mL). The mixture was stirred for 18 h following which the solvent was evaporated, and the resulting syrup was taken up in EtOAc. The solution was filtered through Celite, and the filtrate was washed successively with saturated aq. 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 resulting syrup was purified by chromatography using 2:1 hexane–EtOAc as eluant to give compound **3** as a clear colorless syrup (3.53 g, 70%); ^{1}H-n.m.r. (400.13 MHz): δ 7.67–6.84 (19 H, m, Ar*H*), 5.36 (1 H, br t, $J_{1,2} + J_{1,OH}$ 16 Hz, 1-H), 4.42 (1 H, dd, $J_{1,2}$ 8, $J_{2,3}$ 11 Hz, 2-H), 4.11 (1 H, dd, $J_{2,3}$ 11, $J_{3,4}$ 8.5 Hz, 3-H), 3.56 (4 H, m, 4-H, 5-H, 6-Ha and 6-Hb), and 3.46 (1 H, br d, $J_{1,OH}$ 8 Hz, D_2O exchangeable, O*H*); ^{13}C{^{1}H}-n.m.r. (100.6 MHz): δ 168.2 (carbonyl), 92.9 (C-1), 79.6, 79.1 and 75.0 ($PhCH_2$), 74.8 and 74.6 (C-4 and C-5), 73.4 (C-3), 68.7 (C-6), and 57.6 (C-2).

(*c*) *3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl chloride* (**4**). Oxalyl chloride (2.1 mL, 24 mmol) was added to a stirred solution of DMF (1.7 mL, 24 mmol) in anhydrous CH_2Cl_2 (15 mL) and the mixture was kept under N_2 for 5 min. The solvent was evaporated under diminished pressure and the white salt was dried *in vacuo* for 0.75 h. The *N,N*-dimethyl(chloromethylene)ammonium chloride was then dissolved in anhydrous CH_2Cl_2 and the solution was cooled to 0°. A solution of the hemiacetals **3** (3.5 g, 6.0 mmol) in anhydrous CH_2Cl_2 (15 mL) was transferred by means of a double-tipped needle. The flask was rinsed with an additional portion (5 mL) of solvent and the contents were transferred as before. The mixture was stirred under N_2 for 2 h after which time the reaction was quenched by the addition of cold aq. $NaHCO_3$ (15 mL). The organic layer was diluted with CH_2Cl_2, then washed with $NaHCO_3$ and water, and dried (Na_2SO_4). Evaporation of the solvent gave the glycosyl chloride **4** as a yellow syrup (3.48 g, 97%). The syrup was dried *in vacuo* and used directly in subsequent glycosylation reactions; ^{1}H-n.m.r. (400.13 MHz): δ 6.25 (0.4 H, d, $J_{1,2}$ 3 Hz, 1α-H), and 5.98 (0.6 H, d, $J_{1,2}$ 8 Hz, 1β-H).

(*d*) *Allyl 2-*O*-acetyl-4-*O*-benzyl-α-*L*-rhamnopyranoside* (**5**). Allyl 4-*O*-benzyl-α-L-rhamnopyranoside[22] (5.18 g, 17.6 mmol) was dissolved in MeCN (25 mL) containing methyl orthoacetate (3 mL). *p*-Toluenesulphonic acid (0.125 g) was added, the mixture was partially concentrated under vacuum at 50° on a rotary evaporator, and then stirred for 12 h at room temperature. Triethylamine (2.2 mL) was added and the mixture was concentrated to give a syrup. The syrup was dissolved in 80% aq. HOAc (20 mL) and after 5 min the solution was evaporated to dryness. The residue was chromatographed on silica gel using 3:1 hexane–EtOAc as eluant to yield the title compound **5** as a yellow syrup (5.02 g, 85%); $[\alpha]_D^{25}$ +5.5° (*c* 2.8 in CH_2Cl_2); ^{1}H-n.m.r. (400.13 MHz): δ 7.39–7.27 (5 H, m, Ar*H*), 5.11 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.8 Hz, 2-H), 4.77 (1 H, d, $J_{1,2}$ 1.8 Hz, 1-H), 4.12 (1 H, dd, $J_{2,3}$ 3.8, $J_{3,4}$ 9.5 Hz, 3-H), 3.75 (1 H, m, 5-H), 3.36 (1 H, t, $J_{3,4}$ + $J_{4,5}$ 19 Hz, 4-H), 2.15 (3 H, s, OCOCH_3), and 1.36 (3 H, d, $J_{5,6}$ 6.5 Hz, 6-H_3); ^{13}C{^{1}H}-n.m.r.(100.6 MHz): δ 133.5 (*C*H=CH_2), 117.4 (CH=*C*H_2), 96.6 (C-1), 81.7 (C-4), 75.1 (*C*H_2Ph), 72.9 (C-2), 70.2 (C-3), 68.1 (*C*H_2CH=CH_2), 67.5 (C-5), 20.9 (OCO*C*H_3), and 17.9 (C-6).

Anal. Calc. for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.33; H, 7.11.

(*e*) *8-(Methoxycarbonyl)octyl 2-*O*-benzoyl-4-*O*-benzyl-α-*L*-rhamnopyranoside* (**6**). 8-(Methoxycarbonyl)octyl-α-L-rhamnopyranoside[12] (10.0 g; 0.030 mol) in acetone (200 mL), *p*-toluenesulphonic acid (0.050 g), and 2,2-dimethoxypropane were stirred at room temperature for 1 h. Triethylamine (4.0 mL) was added and the mixture concentrated to a syrup that was dried over P_2O_5 for 1 h. A solution of the syrup in THF (50 mL) was added to a suspension of NaH (1.33 g, 60.0 mmol). After stirring for 10 min, benzyl bromide (5.0 g 30.0 mmol) was added dropwise and the stirring was continued overnight at 20°. Additional NaH (0.8 g; 8 mmol) was added to the stirred solution. The mixture was added rapidly to stirred 0.1M HCl at 0°. Ethyl acetate (150 mL) was added and the organic layer removed. The aqueous phase was extracted with EtOAc (3 × 150 mL). The combined organic layer was washed with $NaHCO_3$, dried ($MgSO_4$), and then concentrated to a syrup. Chromatography on silica gel, with 6:1 hexane–EtOAc as eluant, gave a colorless syrup (11.2 g, 80.6%). A portion of this syrup (10.0 g, 21.5

mmol) was taken up in HCl (0.5 M, 100 mL), and MeOH (100 mL) and refluxed for 1 h. T.l.c. 3:1 hexane–EtOAc indicated that the reaction was essentially quantitative. The cooled mixture was quenched with solid K_2CO_3, filtered, and the filtrate extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried ($MgSO_4$), and concentrated to a residue that was purified using a short silica gel column with 3:1 hexane–EtOAc as eluant. The solvent was removed and part of the syrup obtained (3.0 g, 7.0 mmol), was mixed with MeCN (80 mL) and trimethyl orthobenzoate (1.5 g, 8.0 mmol). *p*-Toluenesulphonic acid (0.25 g) was added and the mixture partially concentrated at 50° on a rotary evaporator and then stirred for 18 h at 20°. Triethylamine was added and the mixture concentrated to give a syrup. The syrup was dissolved in AcOH (80%, 50 mL), and after 5 min, the solution was evaporated to dryness. The residue was chromatographed on silica gel with 3:1 hexane–EtOAc as eluant. Pure **6** was obtained as a light-yellow syrup (2.57 g, 70%), $[\alpha]_D^{25}$ −13.6° (*c* 3.4 in CH_2Cl_2); ^{1}H-n.m.r. (400.13 MHz): δ 5.17 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, H-2), 4.83 (1 H, d, $J_{1,2}$ 1.8 Hz, H-1), 4.86 and 4.78 (2 H, ABq, $J_{A,B}$ 11.0 Hz, OC*H*$_2$Ph), 4.23 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3), 3.65 (1 H, dq, $J_{4,5}$ 9.5 Hz, H-5), 3.45 (1 H, t, $J_{3,4}$ + $J_{4,5}$ 19.0 Hz, H-4), 3.24 [2 H, m, OC*H*$_2$(CH$_2$)$_5$CO$_2$CH$_3$], 2.22 (1 H, d, O*H*), 1.58 [4 H, m, OCH$_2$C*H*$_2$(CH$_2$)$_4$C*H*$_2$CH$_2$CO$_2$CH$_3$], 1.38 (3 H, d, *J* 6.0 Hz, H-6), and 1.3 [8 H, m, OCH$_2$CH$_2$(C*H*$_2$)$_4$CH$_2$CH$_2$CO$_2$CH$_3$]; ^{13}C{^{1}H}-n.m.r. (100.6 MHz): δ 174 and 165 (carbonyl), 97.4 (C-1), 81.4 (C-4), 76.6 (OCH$_2$Ph), 75.2 (C-3), 70.6 (C-2), 67.9 [OCH$_2$(CH$_2$)$_5$CO$_2$CH$_3$], 67.4 (C-5), 51.4 [OCH$_2$(CH$_2$)$_5$CO$_2$*C*H$_3$], 34.0, 29.4, 29.1, 28.98, 28.9, 26.1 and 24.9 [OCH$_2$(*C*H$_2$)$_5$CO$_2$CH$_3$], and 18.2 (C-6).

Anal. Calc. for $C_{30}H_{30}O_8$: C, 68.16; H, 7.63. Found: C, 67.98; H, 7.48.

(*f*) *Allyl 3-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-2-*O*-acetyl-4-*O*-benzyl-α-*L*-rhamnopyranoside* (**7**). A mixture of allyl 2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside **5** (1.27 g, 3.79 mmol), silver trifluoromethanesulfonate (1.46 g, 5.68 mmol), collidine (0.75 mL, 5.7 mmol) and 4 Å molecular sieves in anhydrous CH_2Cl_2 (5 mL) was stirred under N_2 and cooled to −78°. A solution of 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride (**4**, 3.40 g, 5.68 mmol) in anhydrous CH_2CL_2 (8 mL), previously stirred with 4 Å molecular sieves for 0.5 h under N_2 and cooled to −78°, was added dropwise over 0.5 h. The flask was rinsed with additional portions of CH_2Cl_2 (2 × 2 mL) and the contents were transferred as before. The mixture was allowed to warm to room temperature and was stirred for 36 h. The solids were removed by filtration and the filtrate was washed successively with aq. $NaHCO_3$ and aq. NaCl. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup which was purified by chromatography using 2:1 hexane–EtOAc as eluant. Compound **7** was obtained as a clear colorless syrup (2.07 g, 61%). An analytically pure sample was obtained as white prisms from MeOH, m.p. 106–107°, $[\alpha]_D^{25}$ −4.04° (*c* 1.09 in CH_2Cl_2); ^{1}H-n.m.r. (400.13 MHz): δ 7.52–6.85 (24 H, m, Ar*H*), and 2.13 (3 H, s, OC*H*$_3$); ^{13}C{^{1}H}-n.m.r. (100.6 MHz): δ 170.7 (carbonyl), 117.6 (CH=CH$_2$), 75.0, 74.8, 74.7 and 74.6 (PhCH$_2$), 67.2 (CH$_2$-CH=CH$_2$), and 20.9 (OCOCH$_3$).

Anal. Calc. for $C_{53}H_{55}NO_{12}$: C, 70.88; H, 6.17; N, 1.56. Found: C, 70.65; H, 6.15; N, 1.48.

(*g*) *8-(Methoxycarbonyl)octyl 2-O-benzoyl-4-O-benzyl-3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-L-rhamnopyranoside* (**8**). A mixture of the monosaccharide alcohol **6** (0.703 g, 1.33 mmol), silver trifluoromethansulfonate 0.606 g, 2.36 mmol), and collidine (0.32 mL; 2.4 mmol) in CH_2Cl_2 (3 mL) was stirred under N_2 with 4 Å molecular sieves and cooled to $-78°$. To this mixture was added dropwise a cooled solution ($-78°$) of the glycosyl chloride **4** (1.41 g; 2.36 mmol) in dichloromethane (3 mL), previously stirred with 4 Å molecular sieves. The mixture was stirred under N_2 in the dark and allowed to warm to room temperature. After 72 h the mixture was worked up as for **7** and the resulting syrup chromatographed on a silica gel column using 3:1 hexane–EtOAc as eluant. The title compound **8** (R_F 0.38) was obtained as a syrup (0.826 g, 56.9%); $[\alpha]_D^{22}$ $-12.8°$ (*c* 0.73 in $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 4.72 and 4.61 (2 × 1 H, AB_q, J_{AB} 10.8 Hz, OCH_2Ph), 4.73 and 4.38 (2 × 1 H, AB_q, J_{AB} 12.0 Hz, OCH_2Ph), 4.36 and 4.26 (2 × 1 H, AB_q, J_{AB} 11.5 Hz, OCH_2Ph), 4.39 and 4.24 (2 × 1 H, AB_q, J_{AB} 12.0 Hz, OCH_2Ph), 3.67 [3 H, s, $O(CH_2)_8CO_2CH_3$]; 3.57 and 3.59 [2 × 1 H, overlaped multi, $OCH_2(CH_2)_7CO_2CH_3$]; $^{13}C\{^1H\}$-n.m.r. (100.6 MHz): δ 174.2 [$O(CH_2)_8CO_2CH_3$], 171.0 ($OCOCH_3$), 75.2, 74.6, 74.5 and 73.4 (OCH_2Ph), 67.2 [$OCH_2(CH_2)_7CO_2CH_3$], 51.3 [$O(CH_2)_8CO_2CH_3$], 34.1, 29.4, 29.3, 29.1 (2 carbons), 26.0 and 24.9 [$OCH_2(CH_2)_7CO_2CH_3$].

Anal. Calc. for $C_{65}H_{71}NO_{14}$: C, 71.61; H, 6.56; N, 1.28. Found: C, 71.54; H, 6.76; N, 1.11.

(*h*) *Allyl 3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranoside* (**9**). A solution of the disaccharide **7** (1.00 g, 1.11 mmol) in methanolic HCl (25 mL) [prepared by treating anhydrous MeOH (25 mL) with AcCl (1.45 mL)] was kept for 4 h at room temperature. The mixture was made neutral by the addition of triethylamine. Removal of the solvent afforded a residue which was dissolved in EtOAc. The resulting precipitate was removed by filtration. Evaporation of the filtrate gave a syrup which was chromatographed using 1:1 hexane–EtOAc as eluant. Compound **9** was obtained as a clear, colorless syrup (0.855 g, 90%); ^{1}H-n.m.r. (400.13 MHz): δ 7.51–6.81 (24 H, m, Ar*H*), and 3.18 (1 H, D_2O exchangeable, O*H*); $^{13}C\{^1H\}$-n.m.r. (100.6 MHz): δ 167.9 (carbonyl), 117.3 ($CH=CH_2$), 74.80, 74.78, 74.4 and 73.5 ($PhCH_2$), and 67.7 (CH_2-$CH=CH_2$).

Anal. Calc. for $C_{51}H_{53}NO_{11}$: C, 71.56; H, 6.24; N, 1.64. Found: C, 71.40; H, 6.24; N, 1.52.

(*i*) *8-(Methoxycarbonyl)octyl 4-O-benzyl-3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-L-rhamnopyranoside* (**10**). A sample of the disaccharide **8** (0.051 g, 0.047 mmol) was taken up in 0.1M NaOMe (5.0 mL). The solution was kept for 36 h at room temperature under N_2. The mixture was poured into HCl (0.15M), and the solution extracted with CH_2Cl_2 (5 × 10 mL). The combined organic fractions were washed with aq. $NaHCO_3$ followed by aq. NaCl. The organic layer was then dried (Na_2SO_4), and filtered. Evaporation of the filtrate gave a syrup which was chromatographed using 2:1 hexane–EtOAc as eluant. The title compound **10** (R_F 0.36) was obtained as a clear colorless syrup (27 mg, 58%); $[\alpha]_D^{22}$ $-12.8°$ (*c* 0.39, $CHCl_3$); ^{1}H-n.m.r. (400.13 MHz): δ 4.84 and 4.64 (2 × 1 H, AB_q, J_{AB} 11.0 Hz, OCH_2Ph), 4.77 and 4.41 (2 ×

1 H, AB_q, J_{AB} 11.9 Hz, OCH_2Ph), 4.59 and 4.52 (2 × 1 H, AB_q, J_{AB} 12.0 Hz, OCH_2Ph), 4.33 and 4.21 (2 × 1 H, AB_q, J_{AB} 11.7 Hz, OCH_2Ph), 3.66 [3 H, s, $O(CH_2)_8CO_2CH_3$], 3.53 and 3.30 [2 × 1 H, dt, *J*' s 7.1 and 10.0 Hz, $OCH_2(CH_2)_6CO_2CH_3$], and 3.18 (1 H, br s, exchangeable O*H*); $^{13}C\{^1H\}$-n.m.r. (100.6 MHz): δ 174.2 [$O(CH_2)_8CO_2CH_3$], 173.6 ($OCOCH_3$), 74.9, 74.8, 74.63 and 74.59 (OCH_2Ph), and 67.5 [$OCH_2(CH_2)_6CO_2CH_3$].

Anal. Calc. for $C_{58}H_{67}NO_{13}$: C, 70.64; H, 6.85; N, 1.42. Found: C, 70.40; H, 7.10; N, 1.22.

(*j*) *Allyl 2-O-(2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl)-3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranoside* (**12**). A mixture of the disaccharide **9** (0.85 g, 0.99 mmol), silver trifluoromethanesulfonate (0.763 g, 2.97 mmol), 1,1,3,3-tetramethylurea (0.35 mL, 3.0 mmol) and 4 Å molecular sieves in anhydrous CH_2Cl_2 (5 mL) was stirred under N_2 for 0.5 h. The mixture was cooled to −78° and a solution of 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride[12] **11** (1.20 g, 2.97 mmol) in anhydrous CH_2Cl_2 (3 mL), previously stirred with 4 Å molecular sieves for 0.5 h under N_2 and cooled to −78°, was added by means of a cannula under N_2. The flask was rinsed with additional portions of CH_2Cl_2 (2 × 2 mL) and the contents were transferred as before. The mixture was allowed to warm gradually to room temperature and was stirred for 36 h. The solids were removed by filtration and the filtrate was washed successively with $NaHCO_3$ and NaCl solutions. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup that was chromatographed using 3:1 hexane–EtOAc as eluant. Compound **12** was obtained as a white foam (0.957 g, 81%); $[\alpha]_D^{24}$ −3.4° (*c* 0.95, CH_2Cl_2); ^1H-n.m.r. (400.13 MHz): δ 7.44–6.71 (34 H, m, Ar*H*), and 2.12 (3 H, s, $OCOCH_3$); $^{13}C\{^1H\}$-n.m.r. (100.6 MHz): δ 169.9 (carbonyl), 117.1 ($CH_2CH=CH_2$), 74.7, 74.5, 74.2, 73.6 and 71.3 (OCH_2Ph), 67.6 (CH_2-$CH=CH_2$), and 21.0 ($OCOCH_3$).

Anal. Calc. for $C_{73}H_{75}NO_{16}$: C, 71.60; H, 6.34; N, 1.14. Found: C, 71.63; H, 6.40; N, 1.04.

(*k*) *8-(Methoxycarbonyl)octyl 2-O-(2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl)-3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranoside* (**13**). A mixture of the disaccharide **10** (0.109 g, 0.110 mmol), silver trifluoromethanesulfate (0.16 g, 0.62 mmol), 1,1,3,3-tetramethylurea (80 μL, 0.67 mmol), and 4 Å molecular sieves in anhydrous dichloromethane (2.0 mL) was stirred under nitrogen for 30 min and coolled to −78°. A cooled (−78°) solution of the rhamnopyranosyl chloride[12] **11** (0.177 g, 0.436 mmol) in anhydrous CH_2Cl_2 (2.0 mL), previously stirred with 4 Å molecular sieves, was added dropwise to the alcohol solution. The mixture was stirred in the dark under N_2, and allowed to reach room temperature. After 34 h the mixture was filtered, and the filtrate evaporated to dryness to give a syrup which was chromatographed using 3:1 hexane–EtOAc. The title compound **13** (R_F 0.18) was obtained as a clear colorless syrup (0.93 g, 62%); ^1H-n.m.r. (400.13 MHz): δ 4.95 and 4.61 (2 × 1 H, AB_q, J_{AB} 11.0 Hz, OCH_2Ph), 4.87 and 4.60 (2 × 1 H, AB_q, J_{AB} 11.5 Hz, OCH_2Ph), 4.85 and 4.65 (2 × 1 H, AB_q, J_{AB} 11.0 Hz, OCH_2Ph), 4.78 and 4.45 (2 × 1 H, AB_q, J_{AB} 12.2 Hz, OCH_2Ph), 4.59 and 4.52 (2 × 1 H, AB_q, J_{AB} 11.5 Hz, OCH_2Ph), 4.16 and 4.05 (2 × 1 H, AB_q, J_{AB} 11.9 Hz, OCH_2Ph), 3.66 [3H, s,

$O(CH_2)_8CO_2CH_3$], 3.50 and 3.26 [2 × 1 H, dt, *J*'s 7.1 and 10.0 Hz, $OCH_2(CH_2)_7CO_2CH_3$], and 2.11 (3 H, s $OCOCH_3$); $^{13}C(^1H)$-n.m.r. (100.6 MHz): δ 174.0 [$O(CH_2)_8CO_2CH_3$], 169.6 ($OCOCH_3$), 75.0, 74.5, 74.3, 74.1, 73.4 and 71.1 (OCH_2Ph), 67.2 ($OCH_2(CH_2)_7CO_2CH_3$), 51.1 [$O(CH_2)_8CO_2CH_3$], 33.8, 29.1, 28.95, 28.88 (2 carbons), 25.8 and 24.7 [$OCH_2(CH_2)_7CO_2CH_3$], and 20.9 ($OCOCH_3$).

Anal. Calc. for $C_{80}H_{91}NO_{18}$: C, 70.93; H, 6.77; N, 1.03. Found: C, 71.51; H, 6.87; N, 0.72.

(*l*) *3-O-[3-O-(3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-*D-glucopyranosyl)-2-O-*benzoyl-4-O-benzyl-α-*L-*rhamnopyranosyl]-2-O-benzoyl-4-O-benzyl-α-*L-*rhamnopyranoside* (**15**). A sample of trisaccharide **14**[1] (1.96 g, 1.70 mmol) was taken up in abs. 9:1 EtOH–water (55 mL). Tris(triphenylphosphine)rhodium(I) chloride (0.085 g, 0.092 mmol) was added and the solution was stirred at reflux under N_2 for 12 h. The solvent was removed by evaporation and the resulting dark-brown syrup was taken up in EtOAc and filtered through a short column of silica gel. The filtrate was evaporated to dryness and the resulting foam was dissolved in 90% aq. acetone (70 mL). To the solution was added yellow HgO (0.543 g, 2.51 mmol), followed by the dropwise addition of a solution of $HgCl_2$ (0.676 g, 2.49 mmol) in 90% aq. acetone (10 mL). The mixture was stirred for 12 h and the solvent evaporated, and the residue taken up in EtOAc and the mixture filtered through Celite. The filtrate was washed successively with saturated aq. KI (2 ×), aqueous $Na_2S_2O_3$ (2 ×), and water (2 ×). The organic layer was dried (Na_2SO_4), the solvent was evaporated, and the yellow syrup was chromatographed using 1:1 hexane–EtOAc as eluant. The title compound **15**, (R_F 0.36) was obtained as a clear, light-yellow syrup (1.37 g, 72.4%); ^1H-n.m.r. (400.13 MHz): δ 5.11 and 4.78 (2 × 1 H, AB_q, J_{AB} 11.2 Hz, OCH_2Ph), 4.34 and 4.21 (2 × 1 H, $AB_{q'}$, J_{AB} = 12.5 Hz, OCH_2Ph), 2.79 (1 H, s, exchangeable *OH*), 1.95, 1.76 and 1.71 (3 × 3 H, s's, $OCOCH_3$); $^{13}C(^1H)$-n.m.r. (100.6 MHz): δ 170.7, 170.1, 169.2 and 165.7 (2 carbons) (carbonyls), 75.1 and 73.8 (OCH_2Ph), 20.5 and 20.3 (2 carbons) ($OCOCH_3$).

Anal. Calc. for $C_{60}H_{61}NO_{20}$: C, 64.57; H, 5.51; N, 1.25. Found: C, 64.54; H, 5.52; N, 1.26.

(*m*) *3-O-[-O-(3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl)-2-O-benzoyl-4-O-benzyl-α-*L-*rhamnopyranosyl]-2-O-benzoyl-4-O-benzyl-α-*L-*rhamnopyranosyl chloride* (**16**). Compound **16** was prepared as already described for **4**. Oxalyl chloride (0.10 mL, 1.1 mmol) was added to a stirred solution of DMF (0.090 mL, 1.1 mmol) in anhydrous CH_2Cl_2 (3.0 mL), and the mixture was stirred under N_2 for 5 min. The solvent was removed by evaporation and the white salt dried *in vacuo* for 1.5 h. The *N,N*-dimethyl (chloromethylene)ammonium chloride was then taken up in CH_2Cl_2 (1.5 mL) and a solution of **15** (0.248 g, 0.223 mmol in 2.0 mL of CH_2Cl_2) was transferred to the salt solution, rinsing with additional portions of CH_2Cl_2 (3 × 0.75 mL). The mixture was stirred under N_2 for 2 h, and then quenched by the addition of cold aq. $NaHCO_3$. The organic layer was diluted with CH_2Cl_2 and washed successively with aq. $NaHCO_3$ and aq. NaCl. The organic layer was dried over $KHCO_3$ and the solvent removed by evaporation to give a clear light-yellow syrup (0.248 g, 98%) which was dried *in vacuo*, and used directly, without chromatography, in subsequent glycosylations.

(*n*) *8-(Methoxycarbonyl)octyl 2-O-[3-O-[3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl]-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl]-3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-4-O-benzyl-α-L-rhamnopyranoside* (**17**). A sample of trisaccharide chloride **16** (0.248 g, 0.218 mmol) and 1,1,3,3-tetramethylurea (0.060 mL, 0.50 mmol) in anhydrous CH_2Cl_2 (1.5 mL) was stirred with 4 Å molecular sieves for 30 min, and then cooled to −78°. This solution was added dropwise, under N_2, to a cooled solution (−78°) of disaccharide alcohol **10** (0.135 g, 0.137 mmol), silver trifluoromethanesulfonate, and 4 Å molecular sieves in CH_2Cl_2 (2.0 mL). The flask was rinsed with additional portions of CH_2Cl_2 (3 × 1.0 mL) and transferred as before. The mixture was stirred in the dark under N_2 and allowed to warm to room temperature. After 48 h the mixture was filtered and the filtrate evaporated to dryness. The resulting syrup was purified by chromatography using 1:1 hexane–EtOAc followed by chromatography using 3:1 toluene–EtOAc as eluants. The title compound **17** was obtained as a clear, colorless syrup (0.124 g, 43.4%); $[\alpha]_D^{22}$ −20.8° (*c* 0.83, $CHCl_3$).

Anal. Calc. for $C_{118}H_{126}N_2O_{32}$: C, 68.00; H, 6.09; N, 1.34. Found: C, 67.85; H, 6.16; N, 1.39.

(*o*) *Propyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranoside* (**18**). The fully blocked trisaccharide **12** (0.180 g; 0.147 mmol) was dissolved in methanolic HCl (6 mL) [prepared by treating anhydrous MeOH (100 mL) with AcCl (5.7 mL)] and kept under N_2 for 16 h. Triethylamine was added dropwise to the stirred solution until the pH became neutral. The solution was concentrated and the precipitated salt was removed by filtration with the aid of EtOAc. The filtrate was evaporated to dryness and the resulting syrup was purified by column chromatography using 5:2 hexane–EtOAc as eluant, (R_f 0.30). The syrup was then taken up in 80% aq. HOAc (14 mL) and hydrogenolyzed over 10% palladium-on-carbon (0.120 g) at a hydrogen pressure of 52 lb.in^{-2} for 5 d. The solids were removed by filtration through Celite and the filtrate was evaporated to dryness. Abs. EtOH (3 × 75 mL) was evaporated from the residue to remove the excess of AcOH and the residue was dried *in vacuo* for 16 h. The resulting light-brown syrup was taken up in abs. EtOH (20 mL) to which was added $N_2H_4 \cdot H_2O$ (100%, 0.3 mL). The solution was heated at reflux under N_2 for 24 h. Filtration of a fine grey precipitate, followed by evaporation of the filtrate, gave a clear, colorless syrup which was taken up in MeOH (16 mL) and then treated with Ac_2O (4 mL). The solution was kept under N_2 for 12 h at room temperature, after which time t.l.c. indicated that the reaction was complete. Removal of the solvent, followed by silica gel chromatography of the residue, using 7:2:1 EtOAc–MeOH–water as eluant, gave a white solid (R_f 0.34) which was passed through a column of Sephadex LH20 using MeOH as eluant. Compound **18** was obtained as a white, amorphous powder (0.0403 g, 49.3%); $[\alpha]_D^{25}$ +8.1° (*c* 1.78, H_2O); ^{1}H-n.m.r. (400.13 MHz, D_2O): δ 3.60 (1 H, $J_{a,b}$ 10.0 Hz, J_{b,CH_2} 6.3 Hz, $OCH_aH_bCH_2CH_3$), 3.46 ($OCH_aH_bCH_2CH_3$), 1.98 (3 H, s, $NHCOCH_3$), 1.56 (2 H, m, $OCH_2CH_2CH_3$), and 0.87 (3 H, t, *J* 7.5 Hz, $OCH_2CH_2CH_3$); ^{13}C{^{1}H}-n.m.r. (100.6 MHz, D_2O): δ 177.5 ($NHCOCH_3$), 72.9, 72.8, 72.7 and 72.5 ($OCH_2CH_2CH_3$, C-4, C-2$_\gamma$, and C-2$_\beta$), 25.0 ($NHCOCH_3$), 24.7 ($OCH_2CH_2CH_3$), 19.4 (C-6$_\beta$ and C-6$_\alpha$), and 12.6 ($OCH_2CH_2CH_3$).

Anal. Calc. for $C_{23}H_{41}NO_{14}$: C, 49.72; H, 7.43; N, 2.52. Found: C, 49.44; H, 7.20; N, 2.37.

(*p*) *8-(Methoxycarbonyl)octyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranoside* (**19**). A sample of the blocked trisaccharide **13** (0.121 g, 0.0893 mmol) was taken up in NaOMe (14 mL, 0.13M). The mixture was stirred for 10 h at room temperature under N_2 and then made neutral by stirring with Amberlyst 15 resin beads. The resin was removed by filtration and the filtrate evaporated to dryness. The resulting syrup was chromatographed using 2:1 hexane–EtOAc as eluant (R_F 0.45). The purified syrup (0.974 g) was then taken up in 80% aq. AcOH (15 mL), and abs. EtOH (2 mL) was added to completely dissolve the sample. To this solution was added 10% palladium-on-carbon (0.125 g). The mixture was stirred under hydrogen (52 lb.in^{-2}), for 14 h at room temperature. The mixture was filtered through a pad of Celite, the filtrate was concentrated, and abs. EtOH was evaporated several times from the syrup to remove traces of AcOH. The dried syrup was then taken up in abs. EtOH (20 mL) and $N_2H_4{\cdot}H_2O$ (100%; 0.15 mL) was added. The clear, light-brown solution was heated at reflux under N_2 for 18 h. The mixture was then filtered to remove a fine grey precipitate and the filtrate evaporated to dryness. The resulting syrup was then dried *in vacuo* for 2 h to remove traces of hydrazine. The dried syrup was then taken up in MeOH (12 mL) and Ac_2O was added (3.0 mL). The solution was stirred under N_2 for 24 h. The solvent was evaporated to 1/2 volume, additional MeOH was added, and the volume again decreased to 1/2. This was repeated several times in order to remove the Ac_2O without allowing the solution to become concentrated, thus avoiding the formation of the corresponding hydrazide from the methyl ester of the aglycon. Finally, the solvent was evaporated to dryness and the residue chromatographed using 7:2:1 EtOAc–MeOH–water as eluant (R_F 0.54). Further purification was carried out by passing the sample through a column of Sephadex LH20 using MeOH as eluant. The title compound **19** was obtained as a white, amorphous powder (20 mg, 33%); $[\alpha]_D^{22}$ −13.8° (*c* 0.16, H_2O); ^{1}H-n.m.r. (400.13 MHz, D_2O): δ 3.62 [$O(CH_2)_8CO_2CH_3$], and 2.00 ($NHCOCH_3$); ^{13}C(^{1}H)-n.m.r. (100.6 MHz, D_2O): δ 180.6 [$O(CH_2)_8CO_2CH_3$], 177.5 ($NHCOCH_3$), 72.8, 72.7 and 72.6 (C-2_B C-2_A, and C-4_C), 71.9 and 71.7 (C-5_B and C-5_A), 70.8 [$OCH_2(CH_2)_7CO_2CH_3$], 54.8 [$O(CH_2)_8CO_2CH_3$], 36.5, 31.1, 30.94, 30.88 (2 carbons), 28.0 and 27.0 [$OCH_2(CH_2)_7CO_2CH_3$], and 25.0 ($NHCOCH_3$).

Plasma-desorption m.s. Calc. for $C_{30}H_{53}NO_{16}Na$: *m/z* 707; Found: *m/z* 708 $(MNa)^+$.

(*q*) *8-(Methoxycarbonyl)octyl 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-rhamnopyranoside* (**20**). A sample of the fully blocked pentasaccharide **17** (0.141 g, 0.0677 mmol) was deblocked as already described for **19**. Following chromatography using 7:2:1 EtOAc–MeOH-water(R_F0.18) the title compound **20** was obtained as a white, amorphous powder (18.2 mg, 26%); $[\alpha]_D^{22}$ −52.2° (*c* 0.21 in H_2O).

Anal. Calc. for $C_{44}H_{76}N_2O_{25}$: C, 51.16; H, 7.42; N, 2.71. Found: C, 50.74; H, 7.39; N, 2.80.

Preparation of BSA-glycoconjugate (**21**). — The 8-(methoxycarbonyl)octyl glycoside **19** (11.7 mg, 17.1 μmol) was dissolved in abs. EtOH (200 μL), and $N_2H_4 \cdot H_2O$ (100 μL) was added. The mixture was kept at room temperature and, after 12 h, t.l.c. using 7:2:1 EtOAc–MeOH–water indicated that all of the starting ester had been consumed, giving a more-polar component. The solvent was removed by evaporation, water (3 × 1 mL) was distilled from the residue and then the product was taken up in water and the solution lyophilized. The white powder was used directly in the next reaction. The lyophilized hydrazide was taken up in freshly distilled *N*,*N*-dimethylformamide (0.4 mL) and the solution was cooled to −40°. A solution of N_2O_4 in CH_2Cl_2 (95 μL, 34 μmol, 0.36M) was added by means of a pre-cooled syringe. The solution was stirred for 15 min and an additional portion of N_2O_4 in CH_2Cl_2 (45 μL, 0.36M) was added. After a further 20 min the mixture was poured into a stirred solution of bovine serum albumin (BSA) (4.7 mL, 10 mg.mL^{-1}) in buffer (0.08M in $Na_2B_4O_7$ and 0.35M in $KHCO_3$) at 0°C. The BSA solution was stirred at 0° for 12 h and then dialyzed against distilled water (6 × 6 mL) using an Amicon ultrafiltration cell equipped with a PM-10 membrane. The residue was taken up in water and lyophilized to give **21** as a white powder (36.2 mg) having a level of hapten incorporation of 15% (assuming 60 lysine residues per BSA molecule), or 9 haptens per molecule of BSA. The incorporation level was established on the basis of carbohydrate content, determined by the method of Dubois *et al.*[20].

Preparation of BSA-glycoconjugate (**22**). — Pentasaccharide **20** (7.0 mg, 6.8 μmol) was dissolved in abs. EtOH (200 μL) and hydrazine hydrate (100 μL). After 12 h the mixture was worked up as with **21** and the lyophilized powder was dissolved in freshly distilled *N*,*N*-dimethylformamide (0.4 mL). The solution was cooled to −40° and a solution of N_2O_4 in CH_2Cl_2 (70 μL, 0.36M) was added. After 30 min this mixture was poured into a stirred solution of BSA (1.8 mL, 10 mg.mL^{-1}) in buffer (0.08M in $Na_2B_4O_7$ and 0.35M in $KHCO_3$) at 0°. After 12 h of stirring at 0° the mixture was dialyzed against distilled water (6 × 6 mL), using a Amicon ultrafiltration cell equipped with a PM-10 membrane, to remove any unbound hapten. The residue was taken up in distilled water and lyophilized to give a white powder (18.7 mg) with a hapten incorporation level of 30%, or 18 haptens per molecule of BSA.

ACKNOWLEDGMENTS

We are grateful to J. N. Wandu for technical assistance and the Natural Sciences and Engineering Research Council of Canada for administration of a Strategic Research Grant. We also thank Dr. R. J. Cotter for running the plasma-desorption mass spectrum and the Middle Atlantic Mass Spectrometry Facility, Johns Hopkins School of Medicine, Baltimore, MD, U.S.A., for providing the facilities.

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