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Note

Synthesis and 2D-n.m.r. analysis of a pentasaccharide glycoside of the biological repeating units of *Shigella flexneri* variant Y polysaccharide and the preparation of a synthetic antigen*

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As part of a program designed to probe molecular recognition processes between complex carbohydrate antigens and their complementary monoclonal antibodies, we have recently reported[1–3] the synthesis and n.m.r. analysis of higher-order oligosaccharide haptens of the O-specific chain of *Shigella flexneri* variant Y[4,5]. These oligosaccharides were intended to map the extended combining sites in some of the monoclonal antibodies generated against this organism[6]. During the course of these studies, it became apparent that a synthetic antigen containing a higher-order oligosaccharide would be useful as an immunogen in the hybrid myeloma protocol and would also assist in the selection of antibodies from the somatic cell-fusion experiments[7,8]. We now report the synthesis of a pentasaccharide glycoside corresponding to the biological repeating unit of *S. flexneri* variant Y, suitably derivatized to permit covalent attachment to proteins, cell surfaces, and immunoadsorbent supports. We report also the preparation of a synthetic antigen.

The synthetic route employed here is patterned after our previous work[1] on the synthesis of a pentasaccharide as its methyl glycoside. The modification involves the incorporation of the 8-methoxycarbonyloctyl linking arm of Lemieux and co-workers[9]. We describe herein two approaches to this target molecule.

Synthesis. — The biological repeating unit[4] of the lipopolysaccharide *O*-antigen of the bacterium *S. flexneri* variant Y[5] has the following structure:

*Part IV of the Series "Oligosaccharides corresponding to Biological Repeating Units of *Shigella flexneri* Variant Y Polysaccharide".

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

A B C D

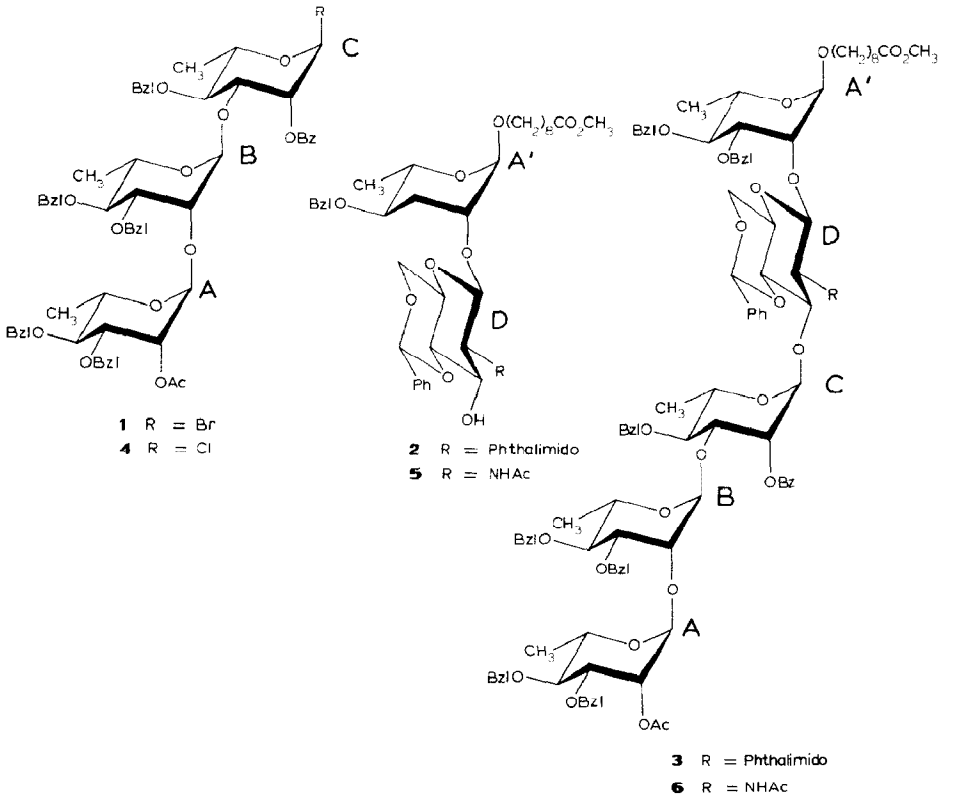
The first approach to the desired pentasaccharide comprising the ABCDA′ sequence employed the key rhamnose trisaccharide unit, α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p* (ABC), as a glycosyl donor. Thus, the trisaccharide bromide[2] **1**, in conjunction with the selectively protected β-D-Glc*p*NPhth-(1→2)-α-L-Rha*p* (DA′) acceptor **2**, afforded the pentasaccharide **3** in only 40% yield. The salient features of this reaction are the use of the phthalimido protecting group on the glucosamine moiety, the presence of the acceptor as its 8-methoxycarbonyloctyl glycoside, and the use of mercury(II) cyanide as promoter. It is noteworthy that the corresponding reaction with the less-reactive glycosyl chloride[1] **4** was not very satisfactory and proceeded in low yield, as did the reactions of **2** with **1** or **4** when silver trifluoromethanesulfonate was used as promoter in the presence of 1,1,3,3-tetramethylurea[10].

The alternative approach to the pentasaccharide employed the DA′ acceptor as its *N*-acetyl derivative **5** and was based on the premise that the poor yields encountered in the reaction of **1** with **2** might arise because of the influence of the phthalimido group. The optimum conditions for formation of the pentasaccharide **6** were now found to be when **5** was glycosylated with the ABC trisaccharide as its glycosyl chloride[1] **4**, the bromide **1** being too reactive and resulting in significant decomposition. In this case, best results were obtained when silver trifluoromethanesulfonate was used as a promoter in the presence of 1,1,3,3-tetramethylurea[10], the pentasaccharide **6** being formed in 73% yield.

Compound **3** was deprotected in the following manner. Transesterification, followed by hydrogenolysis of the product in the presence of palladium-on-charcoal in aqueous acetic acid, hydrazinolysis of the phthalimido group, and finally, selective *N*-acetylation of the resultant amine, yielded the deprotected pentasaccharide **7**. Successive chromatography on silica gel and Sephadex LH20 then afforded the analytically pure compound **7** in 46% yield. Removal of the blocking groups in compound **6** was accomplished by successive transesterification and hydrogenolysis of the product in the presence of palladium-on-charcoal in aqueous acetic acid. Chromatography, as before, then yielded the analytically pure **7** in 54% yield.

It would appear then, for the case at hand, that there is a significant advantage to converting the phthalimido group into an acetamido group at the disaccharide stage, and that this transformation should not await the final deprotection sequence.

N.m.r. analysis. — The ^1H and ^{13}C-n.m.r. spectra of the compounds were in agreement with the assigned structures. Routine ^1H, $^{13}C(^1H)$ n.m.r. spectra were acquired for all the compounds. Coupling constants ($^1J_{^{13}C-^1H}$) were obtained from a ^{13}C spectrum of **7**. ^1H-Homonuclear chemical-shift correlated (COSY) experi-



α-L-Rhap-(1→2)-α-L-Rhap-(1→3)-α-L-Rhap-(1→3)-β-D-GlcpNAc-(1→2)-α-L-Rhap-O(CH$_2$)$_8$CO$_2$CH$_3$

7

ments[11] were performed with compound **3**, **6**, and **7**, and a ^{13}C–^{1}H chemical-shift correlated experiment[12] with compound **6**.

The vicinal coupling-constants of the ring-protons in the individual monosaccharide units were consistent with the $^4C_1$(D) conformation for the glucopyranosyl residues and the $^1C_4$(L) conformation for the rhamnopyranosyl units. The stereochemical integrity of the glycosidic linkages of **7** were confirmed by the magnitude of the one-bond ^{13}C–^{1}H coupling constants ($^1J_{^{13}C,^1H}$) for the anomeric carbon atoms. The rhamnosyl anomeric carbon atoms had coupling constants of 170–173 Hz, whereas this value was 164 Hz for the glucosamine unit. These values are consistent with the presence of the α-L configuration for the rhamnosyl units and the β-D configuration for the glucosamine unit[13].

Assignment of the ^1H-n.m.r. spectra of **3**, **6**, and **7** was facilitated by the examination of their COSY spectra. The chemical-shift values for individual ring-proton signals within overlapped multiplets were determined from the cross-peaks in the COSY spectra. Individual vicinal coupling constants were determined from separated signals in the one-dimensional ^1H-n.m.r. spectra. The COSY spectra per-

mitted the identification of sets of ^{1}H-n.m.r. signals belonging to protons of the same ring. The assignment of the ^{1}H-n.m.r. spectrum of **3** was made as follows. The rhamnosyl H-2 signal at δ 5.50 showed COSY cross-peaks at δ 4.94 and at δ 3.96, indicating the chemical-shifts of the H-1 and H-3 signals, respectively, of the same ring. By following the COSY cross-peak patterns, the remaining ^{1}H-n.m.r. signals from the same ring could be identified. In this fashion all of the signals were assigned to a given spin-system, corresponding to the ^{1}H-n.m.r. signals of a single ring. All that remained was the assignment of the sets of signals to a given ring in the oligosaccharide sequence.

The glucosamine ring was readily distinguished from the rhamnosyl rings on the basis of its characteristic vicinal coupling constants in the ^{1}H-n.m.r. spectrum. The sets of signals due to the rhamnosyl rings were distinguished from each other based on chemical-shift arguments. Thus, the rhamnosyl H-2 signal at δ 5.50 was assigned to the A-ring based on the expected deshielding of H-2 due to the 2-*O*-acetyl group. Similarly, the H-2 signal at δ 4.91 was assigned to the C-ring (H-2 deshielded by the 2-*O*-benzoyl group). The most-shielded rhamnosyl H-1 was assigned to the unit bearing the 8-methoxycarbonyloctyl aglycon (the A′-ring). The remaining set of rhamnosyl ^{1}H-n.m.r. signals was assigned to the B-ring.

The ^{1}H-n.m.r. spectrum of **6** was assigned similarly. The ^{13}C(^{1}H)-n.m.r. spectrum of **6** was then assigned directly following examination of the ^{13}C–^{1}H chemical-shift correlated spectrum[14]. The attribution of signals in the ^{1}H-n.m.r. spectrum of deblocked pentasaccharide **7** was made by first examining the COSY spectrum (Fig. 1 and 2) to identify sets of signals attributable to individual rings, followed by assignment of these sets of signals to specific rings in the oligosaccharide sequence. The latter assignment was based on chemical-shift correlations with the shifts in the spectra of the homologous synthetic hexasaccharide[2] and heptasaccharide[3], made up of an ABCDA′B′ and an ABCDA′B′C′ sequence, respectively.

The ^{13}C(^{1}H)-n.m.r. spectrum of **7** was assigned based on chemical-shift correlations of the ring-carbon signals (after correction for the different references used in the various studies) with those in the spectra of the natural polymer and various key synthetic sequences[15,16].

Preparation of a synthetic antigen. — The glycoconjugate of the pentasaccharide **7** and bovine serum albumin (BSA) was prepared by the modified acyl azide methodology of Pinto and Bundle[17]. Thus, the ester **7** was converted into its hydrazide, and the latter was then treated with dinitrogen tetraoxide as the nitrosating agent. The resultant acyl azide was then treated immediately with BSA in buffer solution to provide the glycoconjugate, [α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-*O*-$(CH_2)_8CO]_n$NH-BSA. A level of hapten incorporation of 22% was achieved, and afforded a glycoconjugate possessing 13 haptens per molecule of protein[18]. This value refers, of course, to an average level of incorporation and no attempt has been made to evaluate the uniformity of the preparation. In addition, we assume that the oligosaccharides

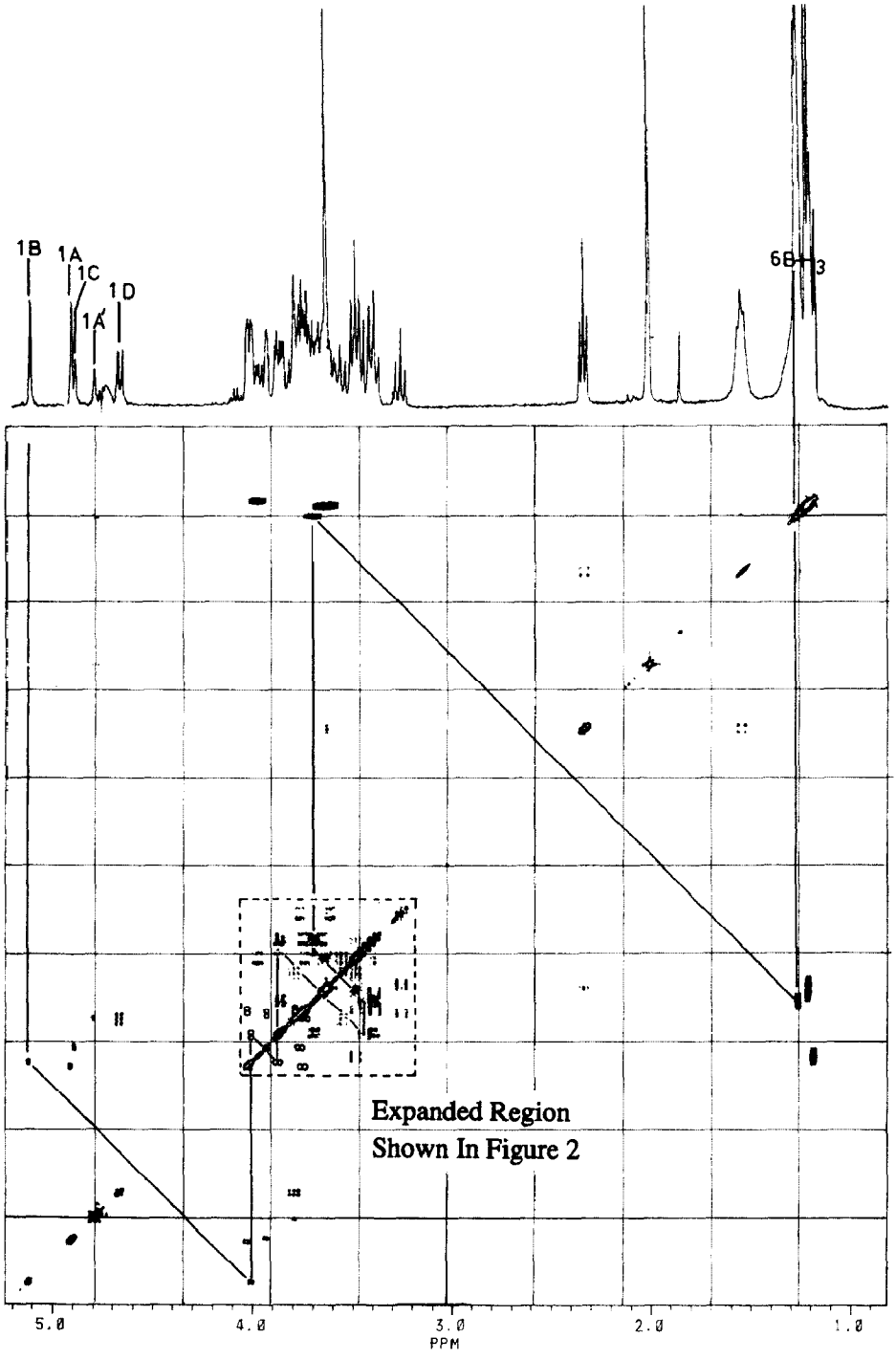


Fig. 1. 400 MHz Two-dimensional ^{1}H-n.m.r. COSY spectrum of the deprotected pentasaccharide **7**.

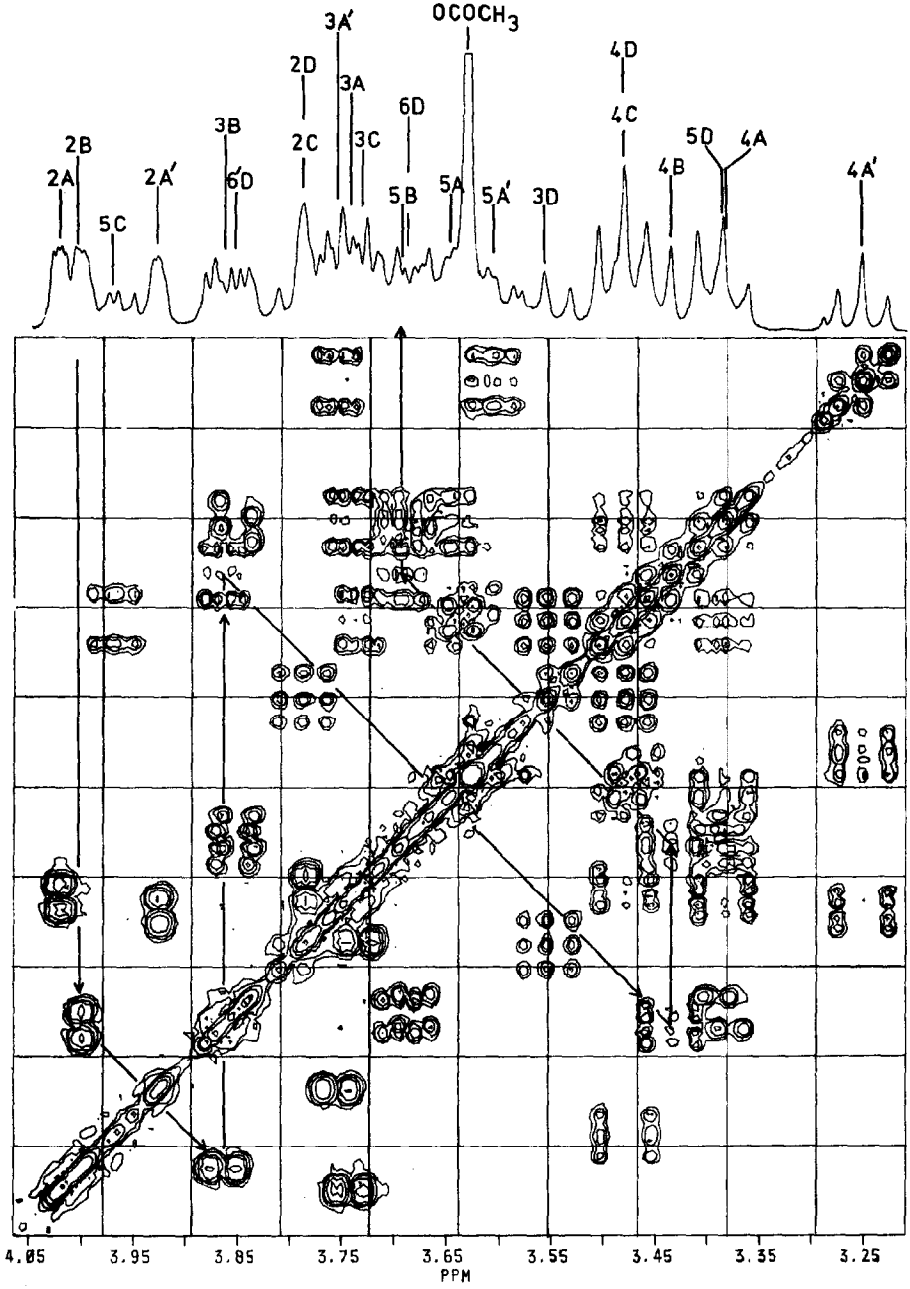


Fig. 2. Expanded region of the two-dimensional ^{1}H-n.m.r. COSY spectrum of the deprotected pentasaccharide **7**.

have been incorporated into the protein as intact moieties without significant fragmentation. Support for the latter contention comes from the observed t.l.c. behaviour in the acyl azide-generation reaction, the conditions of the reaction being sufficiently mild that no significant decomposition is observed[17]. The synthetic antigen may be used in the hybrid-myeloma protocol as an immunizing antigen and also as a screening agent for monoclonal antibodies.

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. (400.13 MHz) and ^{13}C-n.m.r. (100.6 MHz) spectra were recorded with a Bruker WM-400 n.m.r. spectrometer. 1D N.m.r. spectra were acquired with 32 K data sets for both ^{1}H-n.m.r. and ^{13}C-n.m.r. spectra. Spectra were measured in $CDCl_3$ (40 mg mL^{-1}) for the protected compounds and in D_2O (20 mg/mL) for the deprotected compound; the chemical shifts are given in p.p.m. downfield from Me_4Si and 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), respectively. 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 2K × 1K data sets with 512 experiments. The ^{13}C–^{1}H chemical-shift spectra were acquired with 2K × 1K data sets and 128 experiments.

Optical rotations were recorded with a Perkin–Elmer P22 spectropolarimeter. Analytical thin-layer chromatography (t.l.c.) was performed on precoated aluminum plates with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to u.v. light and/or sprayed with 10% 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[19]. 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.

8-Methoxycarbonyloctyl 4,6-di-O-benzyl-2-O-(4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**2**). — The title compound **2** was prepared in a manner analogous to that described[20] for the 2′-deoxy-2′-acetamido disaccharide **5**, and was obtained as a clear, colourless syrup, $[\alpha]_D^{24}$ −25.3° (*c* 2.1, CH_2Cl_2); ^{1}H-n.m.r. (400.13 MHz, $CDCl_3$): δ 5.58 (s, 1 H, PhC*H*O_2), 5.27 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1_D), 4.87 (m, 1 H, H-3_D), 4.68 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-$1_{A'}$), 4.37 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.5 Hz, H-2_D), 4.32 and 4.14 (ABq, 2 × 1 H, $J_{A,B}$ 12.5 Hz, PhCH_2O), 4.01 and 3.86 (ABq, 2 × 1 H, $J_{A,B}$ 10.5 Hz, PhCH_2O), 3.65 (s, 3 H, OCOCH_3), 3.27 [m, 1 H, OCH_aH_b $(CH_2)_7$COCH_3], 3.03 (t, 1 H, $J_{2,4}$ + $J_{4,5}$ = 19.3 Hz, H-$4_{A'}$), 2.27 [t, 2 H, $J_{A,B}$ + $J_{B,C}$ = 15.0 Hz,

$O(CH_2)_7CH_2CO_2CH_3$], 1.60, 1.45 and 1.25 (m's, aglycon CH_2's), and 1.20 (d, 3 H, $J_{5,6}$ 6.3 Hz, H_3-$6_{A'}$; $^{13}C\{^1H\}$-n.m.r. (100.6 MHz, $CDCl_3$): δ 174.2 (carbonyl), 101.9 ($PhCHO_2$), 100.7 (C-$1_{A'}$), 98.9 (C-1_D), 65.9 (C-6_D), 56.7 (C-2_D), 51.3 ($O(CH_2)_8CH_3$), 34.0, 29.4, 29.14, 29.07, 29.0, 25.9, 24.9, [$OCH_2(CH_2)_7CO_2CH_3$], and 17.7 (C-$6_{A'}$).

Anal. Calc. for $C_{51}H_{59}NO_{13}$: C, 68.53; H, 6.61; N, 1.57. Found: C, 68.25; H, 6.77; N, 1.55%.

*8-Methoxycarbonyloctyl 2-O-{3-O-{3-O-[2-O-(2-O-acetyl-3,4-di-O-benzyl-α-*L*-rhamnopyranosyl)-3,4-di-O-benzyl-α-*L*-rhamnopyranosyl]-2-O-benzoyl-4-O-benzyl-α-*L*-rhamnopyranosyl}-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl}-2,4-di-O-benzyl-α-*L*-rhamnopyranoside* (**3**). — A mixture of disaccharide **2** (81.5 mg, 0.110 mmol) and $(HgCN)_2$ (82.9 mg, 0.328 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was stirred with 4Å molecular sieves, in a Schlenk tube fitted with a dropping funnel, for 20 min under nitrogen. A solution of the trisaccharide[2] **1** (0.274 g, 0.246 mmol) in CH_2Cl_2 (2.0 mL) was stirred under nitrogen with 4Å molecular sieves and transferred to the dropping funnel via a cannula. The flask was rinsed with additional portions (2 × 2.0 mL) of CH_2Cl_2 and transferred as before. The cooled (−78°) glycosyl bromide solution was added dropwise (over 10 min) to the cooled alcohol solution, rinsing the dropping funnel with additional portions of CH_2Cl_2 (2 × 1.0 mL). After 64 h, the solids were removed by filtration and the filtrate washed successively with saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried (Na_2SO_4) and evaporated to a syrup that was chromatographed using 2:1 hexane–EtOAc as eluant. Compound **3** (R_F 0.47) was obtained as a colourless glass (86 mg, 40%), $[\alpha]_D^{25}$ −10.6° (*c* 0.79, CH_2Cl_2); ^1H-n.m.r. (400.13 MHz, $CDCl_3$): δ 5.61 (s, 1 H, $PhCHO_2$), 5.50 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, H-2_A), 5.29 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1_D), 4.91 (overlapped by ABq, 1 H, H-2_C), 4.86 (dd, 1 H, $J_{3,4}$ 9.0, $J_{2,3}$ 10.4 Hz, H-3_D), 4.97, 4.94, 4.76, and 4.70 (d's, 4 × 1 H, J_{AB} ≈1.8 Hz, H-1_B, H-1_A, H-1_C, and H-$1_{A'}$), 4.92 and 4.61 (ABq, 2 × 1 H, J_{AB} 11.0 Hz, OCH_2Ph), 4.74 and 4.55 (ABq, 2 × 1 H, J_{AB} 11.0 Hz, OCH_2Ph), 4.73 and 4.51 (ABq, 2 × 1 H, J_{AB} 11.5 Hz, OCH_2Ph), 4.47 (overlapped by ABq's, 1 H, H-2_D), 4.67 and 4.46 (ABq, 2 × 1 H, J_{AB} 11.0 Hz, OCH_2Ph), 4.50 and 4.42 (ABq, 2 × 1 H, J_{AB} 11.0 Hz, OCH_2Ph), 4.38 (dd, 1 H, $J_{5,6eq}$ 5.0, $J_{6eq,6ax}$ 10 Hz, H_{eq}-6_D), 4.35 and 4.18 (ABq, 2 × 1 H, J_{AB} 12.3 Hz, OCH_2Ph), 4.10 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.5 Hz, H-3_C), 3.96 (overlapped by H-3_A, 1 H, H-5_C), 3.96 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3_A), 4.01 and 3.87 (ABq, 2 × 1 H, J_{AB} 10.5 Hz, OCH_2Ph), 3.92–3.75 (3 × 1 H, m containing: 3.87, H-2_B; 3.82, H_{ax}-6_D; 3.80, H-5_A), 3.73 (t, 1 H, $J_{3,4}$ + $J_{4,5}$ = 18.5 Hz, H-4_D), 3.67 [s, 3 H, $O(CH_2)_8CO_2CH_3$], 3.57–3.70 (4 × 1 H, m containing: 3.67, H-5_D; 3.63, H-3_B; 3.60, H-$2_{A'}$; 3.59, H-$3_{A'}$], 3.48–3.56 [2 × 1 H, m containing: 3.54, $OCH_aH_b(CH_2)_7CO_2CH_3$; 3.51, H-$5_{A'}$] 3.43 (dq, 1 H, $J_{5,6}$ 6.2, $J_{4,5}$ 9.3 Hz, H-5_B), 3.40 (t, 1 H, $J_{3,4}$ + $J_{4,5}$ = 19 Hz, H-4_A), 3.32 (t, 2 × 1 H, $J_{3,4}$ +$J_{4,5}$ = 19 Hz, H-4_B and H-4_C), 3.29 [m, 1 H, $OCH_aH_b(CH_2)_7CO_2CH_3$], 3.04 (t, 1 H, $J_{3,4}$ + $J_{4,5}$ 19 Hz, H-$4_{A'}$), 1.22, 1.21, 0.99, and 0.72 (d's, 4 × 3 H, *J*'s ≈6.2 Hz, H_3-6_A, H_3-$6_{A'}$, H_3-6_B and H_3-6_C); $^{13}C\{^1H\}$-n.m.r. (100.6 MHz, $CDCl_3$): δ 174.1, 171.0, 169.9 and 164.9 (carbonyl), 101.9 ($PhCHO_2$), 100.9, 100.6, 99.0, 98.9 and 97.3

(anomeric C's), 51.3 [$O(CH_2)_8CO_2CH_3$], 34.0, 29.4, 29.1, 25.9 and 24.8 [$OCH_2(CH_2)_7CO_2CH_3$], 20.9 ($NHCOCH_3$ and $OCOCH_3$), 17.9, 17.7, and 17.2 (C-$6_{A'}$, C-6_C, C-6_B amd C-6_A).

Anal. Calc. for $C_{113}H_{125}NO_{27}$: C, 70.35; H, 6.53; N, 0.73. Found: C, 70.24; H, 6.55; N, 0.61%.

8-Methoxycarbonyloctyl 2-O-{2-acetamido-2-deoxy-4,6-O-benzylidene-3-O-{3-O-[2-O-(2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl)-3,4-di-O-benzyl-α-L-rhamnopyranosyl]-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl}-β-D-gluco-pyranosyl}-3,4-di-O-benzyl-α-L-rhamnopyranoside (**6**). — Addition of the trisaccharide[1] **4** (0.277 g, 0.259 mmol) to the disaccharide[20] **5** (71.3 mg, 0.0885 mmol) was carried out analogously to the preparation of **3**, using silver trifluoromethanesulfonate (80.5 mg, 0.313 mmol) as promoter with 1,1,3,3-tetramethylurea (36 μL, 0.3 mmol). After 40 h of reaction, work-up, and column chromatography (using 5:2 hexane–EtOAc as eluant) afforded **6** (R_F 0.29) as a colourless glass (0.118 g, 72.5%), $[\alpha]_D^{25}$ −9.1° (*c* 1.26, CH_2Cl_2); ^{1}H-n.m.r. (400.13 MHz, $CDCl_3$): δ 5.49 (s, 1 H, $PhCHO_2$), 5.48 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, H-2_A), 5.22 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, H-2_C), 5.03 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1_D), 4.95, 4.92, 4.85 and 4.76 (d's, 4 × 1 H, $J_{1,2}$ ≈1.8 Hz, H-1_B, H-1_A and H-1_C, H-$1_{A'}$), 4.89 and 4.61 (ABq, 2 × 1 H, J_{AB} 10.7 Hz, OCH_2Ph), 4.89 and 4.57 (ABq, 2 × 1 H, J_{AB} 10.7 Hz, OCH_2Ph), 4.71 and 4.51 (ABq, 2 × 1 H, J_{AB} 11.0 Hz, OCH_2Ph), 4.74 and 4.50 (ABq, 2 × 1 H, J_{AB} 11.4 Hz, OCH_2Ph), 4.68 and 4.47 (ABq, 2 × 1 H, J_{AB} 11.0 Hz, OCH_2Ph), 4.49 and 4.42 (ABq, 2 × 1 H, J_{AB} 11.6 Hz, OCH_2Ph), 4.36–4.26 (2 × 1 H, m containing: 4.32, H-3_D; 4.29, H_{eq}-6_D), 4.11 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.5 Hz, H-3_C), 4.00–3.88 (3 × 1 H, m containing: 3.95, H-5_C; 3.91, H-3_A; 3.90, H-2_B), 3.87–3.82 (2 × 1 H, m containing: 3.85, H-$2_{A'}$; 3.84, H-$3_{A'}$), 3.78 (dq, 1 H, $J_{5,6}$ 6.1, $J_{4,5}$ 9.5 Hz, H-5_A), 3.74–3.69 (2 × 1 H, m containing: 3.72, H_{ax}-6_D; 3.71, H-3_B), 3.65 (m, 4 H, H-$5_{A'}$ overlapped by $COCH_3$ singlet), 3.58 [m, 2 × 1 H, H-5_B and $OCH_aCH_b(CH_2)_7CO_2CH_3$], 3.55–3.45 (2 × 1 H, m containing: 3.51, H-4_d; 3.49, H-5_D), 3.44–3.32 (5 × 1 H m containing: 3.40, H-$4_{A'}$; 3.39, H-2_D; 3.38, H-4_A; 3.37, H-4_C; 3.33, $[OCH_aCH_b(CH_2)_7CO_2CH_3]$, 3.31 (t, 1 H, $J_{3,4}$ + $J_{4,5}$ 19.0 Hz, H-4_B), 2.12 [s, 3 H, $OCOCH_3$), 1.86 (s, 3 H, $NHCOCH_3$), 1.33 (d, 3 H, $J_{5,6}$ 6.2 Hz, H_3-$6_{A'}$), 1.23 (d, 3 H, $J_{5,6}$ 6.1 Hz, H_3-6_A), 1.02 (d, 3 H, $J_{5,6}$ 6.1 Hz, H_3-6_B), and 0.70 (d, 3 H, $J_{5,6}$ 6.0 Hz, H_3-6_C); ^{13}C{^{1}H}-n.m.r. (100.6 MHz, $CDCl_3$): δ 174.2, 171.3, 170.0 and 165.7 (carbonyl), 101.9 ($PhCHO_2$), 100.8 (C-1_B), 99.1 (C-$1_{A'}$), 99.0 (C-1_B), 97.6 (C-1_C), 58.6 (C-6_D), 51.4 (C-2_D), 31.4, 29.5, 29.2, 29.11, 29.06, 26.0 and 24.9 [$OCH_2(CH_2)_7CO_2CH_3$], 23.4 ($NHCOCH_3$), 21.0 ($OCOCH_3$), 17.99 (C-6_A), 17.94 (C-$6_{A'}$), 17.7 (C-6_B), and 17.3 (C-6_C).

Anal. Calc. for $C_{107}H_{125}NO_{26}$: C, 69.80; H, 6.84; N, 0.76. Found: C, 69.98; H, 6.77; N, 0.94%.

8-Methoxycarbonyloctyl 2-O-{2-acetamido-2-deoxy-3-O-{3-O-[2-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranosyl}-β-D-gluco-pyranosyl}-α-L-rhamnopyranoside (**7**). — A solution of pentasaccharide **6** (0.101 g, 0.054 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was treated with NaOMe (M) in MeOH

(5 mL). After stirring for 24 h under nitrogen the mixture was made neutral by the addition of methanolic HCl (3%). The solids were removed by filtration and the filtrate concentrated to a syrup. The syrup was taken up in 80% aq. AcOH (11 mL) and hydrogenolyzed over 10% palladium-on-carbon (20.2 mg) at a hydrogen pressure of 52 lb.in^{-2} for 5 days. Work-up afforded a syrup which was chromatographed on silica gel using (7:2:1) EtOAc–MeOH–H_2O as eluant (R_F 0.27), followed by Sephadex LH-20 gel filtration with MeOH as solvent. An analytically pure sample was obtained as an amorphous white powder (28.3 mg, 54%). Pentasaccharide **3** (96.7 mg, 0.05 mmol) was deblocked by NaOMe-catalyzed transesterification, followed by palladium-catalyzed hydrogenolysis, removal of the phthalimido group by hydrazinolysis, and finally, *N*-acetylation by treatment with Ac_2O in MeOH. After chromatography as before, a compound was obtained whose ^{1}H-n.m.r. spectrum was identical to that of the deblocked compound **6** (22.6 mg, 46%), $[\alpha]_D^{26}$ −57° (*c* 1.4, H_2O); ^{1}H-n.m.r. (400.13 MHz, D_2O): δ 5.12, 4.91, 4.89, and 4.80 (d's, 4 × 1 H, $J_{1,2}$ ≈1.8 Hz, H-1_B, H-1_A, H-$1_{A'}$ and H-1_C), 4.67 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1_D), 4.03 and 4.01 (dd's, 2 × 1 H, $J_{1,2}$ + $J_{2,3}$ = 4.5 Hz, H-2_A and H-2_B), 3.98 (dq, 1 H, $J_{5,6}$ 6.0, $J_{4,5}$ 9.5 Hz, H-5_C), 3.93 (dd, 1 H, $J_{1,2}$ + $J_{2,3}$ ≈4.5 Hz, H-$2_{A'}$), 3.91–3.83 (2 × 1 H, m containing: 3.87, H-3_B and 3.85 H_b-6_D), 3.82–3.59 [10 × 1 H, m containing: 3.79, H-2_C and H-2_D; 3.76, H-$3_{A'}$; 3.74, H-3_A; 3.73 H-3_C; 3.70, H-5_B; 3.69, H_a-6_D; 3.65, H-5_A; 3.64, $OCH_aCH_b(CH_2)_7CO_2CH_3$ and 3.62, H-$5_{A'}$], 3.56 (t, 1 H, $J_{2,3}$ + $J_{3,4}$ = 18.5 Hz, H-3_D), 3.52–3.43 [4 × 1 H, m containing: 3.48, H-4_C, $OCH_aH_b(CH_2)_7CO_2CH_3$ and H-4_D; 3.44 H-4_B], 3.39 (m, 2 × 1 H, H-4_A and H-5_D), 3.26 (t, 1 H, $J_{3,4}$ + $J_{4,5}$ = 19.0 Hz, H-$4_{A'}$), 2.02 (s, 3 H, $NHCOCH_3$), 1.26 (3 H, H_3-6_B), 1.21 (d, 2 × 3 H, $J_{5,6}$ ≈6.0 Hz, H_3-$6_{A'}$ and H_3-6_A), and 1.89 (d, 3 H, $J_{5,6}$ 6.2 Hz, H_3-6_C); ^{13}C(^{1}H)-n.m.r. (100.6 MHz, D_2O): δ 180.6 [$O(CH_2)_8CO_2CH_3$], 177.0 ($NHCOCH_3$), 105.0 ($^1J_{^{13}C-^1H}$, 172, C-1_A), 104.8 ($^1J_{^{13}C-^1H}$, 164, C-1_D), 103.9 ($^1J_{^{13}C-^1H}$, 170, C-1_C), 103.5 ($^1J_{^{13}C-^1H}$, 173, C-1_B), 101.2 ($^1J_{^{13}C-^1H}$, 173, C-$1_{A'}$), 84.2 (C-3_D), 81.5 (C-$2_{A'}$), 80.7 (C-2_B), 79.9 (C-3_C), 78.6 (C-5_D), 75.0 (C-$4_{A'}$), 74.7 (C-4_B and C-4_A), 74.3 (C-4_C), 73.3 (C-3_A), 72.7 [$OCH_2(CH_2)_7CO_2CH_3$, C-3_B, C-2_C and C-2_A], 71.8 (C-5_C and C-5_A), 71.7 (C-4_D), 71.4 (C-5_B), 71.0 (C-$3_{A'}$), 70.7 (C-$5_{A'}$), 63.4 (C-6_D), 58.3 (C-2_D), 54.8 [$O(CH_2)_8CO_2CH_3$], 36.4, 31.1, 30.8 (3 carbons), 27.9 and 27.0 [$OCH_2(CH_2)_7CO_2CH_3$], 25.0 ($NHCOCH_3$), 19.3 (3 carbons), and 19.1 (C-6_A, C-6_B, C-6_C and C-$6_{A'}$).

Anal. Calc. for $C_{42}H_{73}NO_{24}$: C, 51.68; H, 7.54; N, 1.43. Found: C, 51.47; H, 7.62; N, 1.68%.

Preparation of a synthetic antigen. — The 8-(methoxycarbonyl)octyl glycoside **7** (28.3 mg, 29.0 μmol) was dissolved in abs. EtOH (0.3 mL) and hydrazine hydrate (0.15 mL). After 12 h at room temperature, the solution was evaporated to a syrup, from which water (1 mL) was evaporated three times. T.l.c. on silica gel, using 7:2:1 (v/v/v) EtOAc–MeOH–water, indicated that all of the ester had been converted into a single, more-polar component. The product was dissolved in distilled water (1 mL), the solution lyophilized, and the compound used directly in the subsequent reaction. A stirred solution of the hydrazide in freshly distilled *N,N*-di-

methylformamide (0.4 mL) was cooled to −40°, and a solution of N_2O_4 in CH_2Cl_2 (200 μL; 72 μmol, 0.36M) was added by means of a microlitre syringe. The temperature was maintained for 35 min at −20 to −10° and the mixture was then added to a stirred solution of bovine serum albumin in buffer (1.16 μmol; 10.0 mg mL^{-1}) (0.08M in $Na_2B_4O_7$ and 0.35M in $KHCO_3$) at 0°. After being kept overnight at 0°, the mixture was dialysed against six changes of de-ionized water in an Amicon ultrafiltration cell equipped with a PM-10 membrane, and then lyophilized, to provide the glyconjugate as a white powder. The incorporation level was established on the basis of carbohydrate content, determined by the method of Dubois *et al.*[18].

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