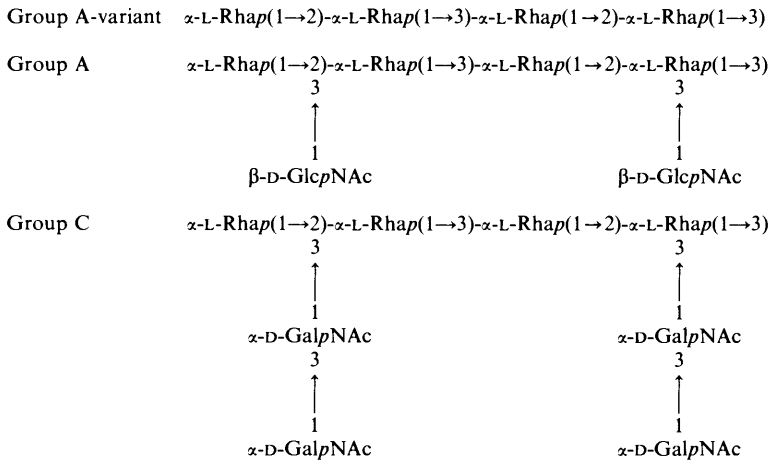
Synthesis of Oligosaccharides corresponding to the Antigenic Determinants of the β-Haemolytic *Streptococci* Group A. Part 1. Overall Strategy and Synthesis of a Linear Trisaccharide

Kerry B. Reimer and B. Mario Pinto *
Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

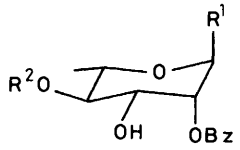
The overall strategy for the synthesis of higher-order oligosaccharides corresponding to the repeating unit of the cell-wall polysaccharide of the β-haemolytic *Streptococci* Group A is described. The trisaccharide, β-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p* has been synthesized by a series of Königs-Knorr reactions. The selectively protected rhamnose derivative, allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside, reacted with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide to give the blocked disaccharide. Deallylation, followed by treatment with *N*,*N*-dimethyl(chloromethylene)ammonium chloride then gave the corresponding disaccharide chloride. In conjunction with the same rhamnose monosaccharide unit or 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside, the synthesis of the blocked trisaccharide, as its allyl glycoside or its 8-methoxycarbonyloctyl glycoside, respectively, was accomplished. Transesterification, followed by hydrazinolysis, selective *N*-acetylation, and hydrogenolysis afforded the pure trisaccharide, as its propyl glycoside or 8-methoxycarbonyloctyl glycoside, for use as a hapten in binding studies and n.m.r. studies or for use in the preparation of glycoconjugates, respectively. Similar treatment of the blocked disaccharide afforded the hapten, β-D-Glc*p*NAc-(1→3)-α-LRha*p*, as its propyl glycoside.

The β-haemolytic *Streptococci* Group A are primary infective organisms in humans, causing streptococcal pharyngitis,[1] and are also implicated in the induction of rheumatic fever, acute glomerulonephritis, and joint and heart disease.[2] The immunological cross-reaction between the *Streptococci* Group A cell-wall polysaccharide and structural glycoproteins of heart valves has also been established.[3] Since standard culture methods take several days, treatment for streptococcal pharyngitis is often delayed or is begun without proper confirmation of infection by diagnostic testing. The development of a simple, rapid, and sensitive reagent for the diagnosis of the disease is, therefore, desirable, and a number of methods[4] have been investigated for laboratory diagnosis. Of these methods, two that are noteworthy for their speed, simplicity, and specificity are based on the direct extraction (with nitrous acid or enzymic) of the polysaccharide antigen of Group A *Streptococci* from throat swabs, followed by detection with antibody-linked latex particles. These methods have both been incorporated into test kits[5] and their clinical evaluation[6–8] has indicated good specificity as compared with the culture method; however, in two of these studies,[6,7] the sensitivity of the tests was found to be significantly lower than that obtained using the standard culture techniques, requiring the confirmation of negative results obtained with the test kits.

We propose that by synthesis of a highly defined streptococcal Group A oligosaccharide, it would be possible to prepare the corresponding glycoconjugates and complementary monoclonal antibodies to improve or replace existing diagnostic reagents. Structural studies[9] of the carbohydrate antigens of Group A, A-variant, and C of β-haemolytic *Streptococci* have indicated that they all possess a rhamnose backbone consisting of alternating α-L-(1→2) and α-L-(1→3) linkages. The A-variant structure consists solely of this backbone whereas the A structure contains β-D-*N*-acetylglucosamine residues attached to the 3-position of the rhamnose residue and the *C*-structure contains 3-*O*-α-D-*N*-acetylgalactosaminosyl-α-D-*N*-acetylgalactosamine disaccharide units linked to the 3-position of the rhamnose backbone (Scheme). Of these bacterial serogroups, only Group A is clinically significant. Thus far, the synthesis of the terminal

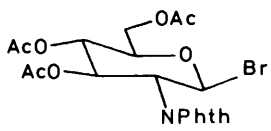


Scheme.

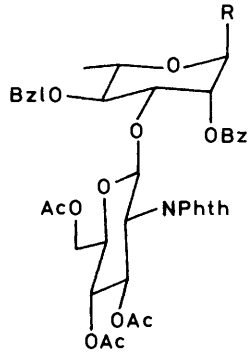


(1) $R^1 = OCH_2CH{=}CH_2, R^2 = Bzl$

(8) $R^1 = O[CH_2]_8CO_2Me$, $R^2 = Bz$



(2)



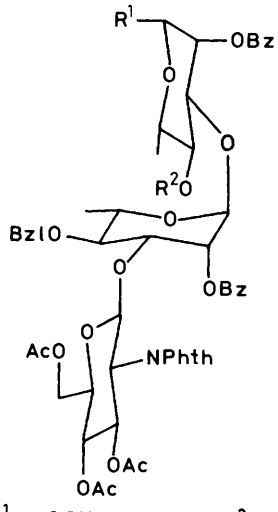
(3) $R = OCH_2CH{=}CH_2$

(4) $R = OCH{=}CHMe$

(5) $R = OH$

(6) $R = Cl$

NPhth = phthalimido



(7) $R^1 = OCH_2CH{=}CH_2, R^2 = Bzl$

(9) $R^1 = O[CH_2]_8CO_2Me$, $R^2 = Bz$

β-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-X

(10) X = OPr

(11) $X = O[CH_2]_8CO_2Me$

β-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-OPr

(12)

disaccharide and trisaccharide determinants of Groups A and C, respectively, and that of the tetrasaccharide core representing the variant-A structure have been reported.[10] The Group A and variant-A determinants were subsequently coupled to protein[11] and the antigens thus obtained have been used to investigate the fine specificities of two IgM myeloma proteins directed against streptococcal carbohydrate-associated antigens.[12]

Specifically, we propose to synthesize tri- and higher-order oligosaccharides corresponding to the antigenic determinants of the cell-wall polysaccharides of *Streptococci* A in order to determine the optimum requirements for a highly defined *Streptococci* A antigen. Computer modelling[13] of the three-dimensional structure of the antigen for 'infinite space' has indicated that the oligosaccharide adopts a helical structure, the *N*-acetylglucosamine residues being disposed around the

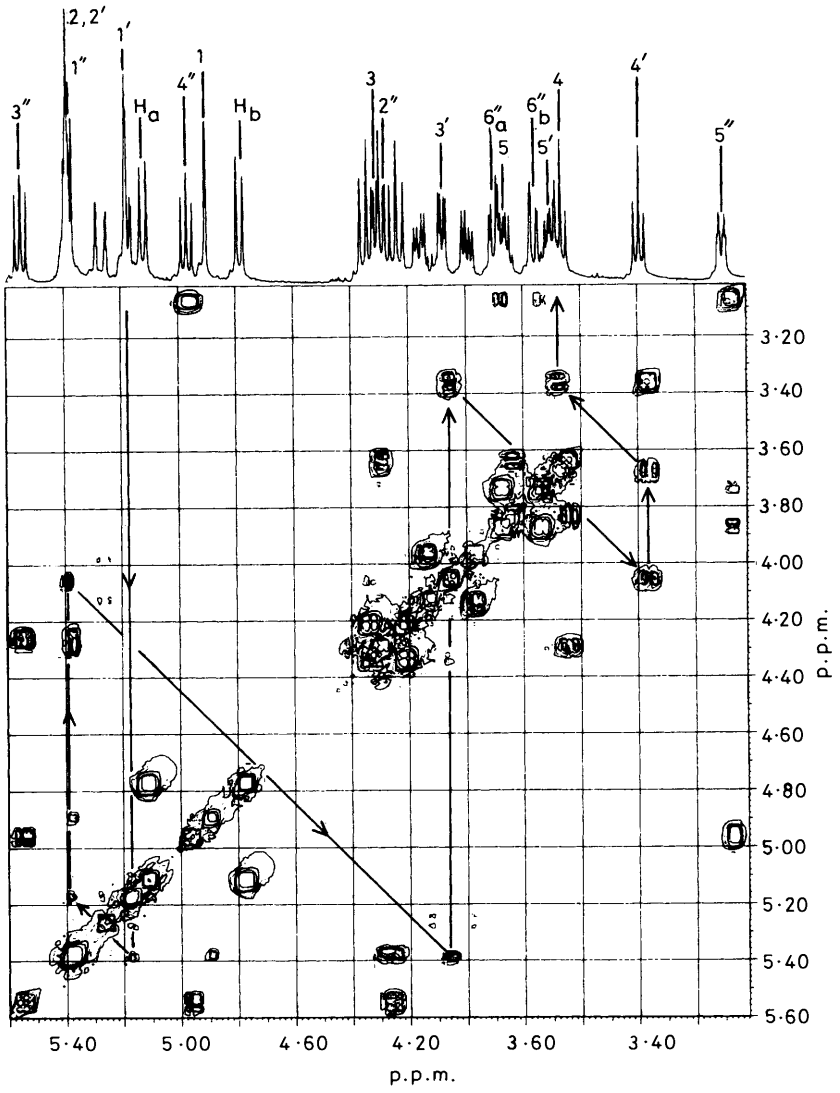


Figure 1. Partial 500 MHz two-dimensional ^{1}H n.m.r. COSY spectrum of the trisaccharide (**7**)

periphery. It is of interest, therefore, to determine the necessary features and the extent of the surface required for efficient and specific binding to antibody.

The block syntheses of tri- up to deca-saccharides have been designed, on the basis of a disconnection which ensures the use of the common linear trisaccharide β-D-GlcNAc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*, for the assembly of all the desired oligosaccharides. This key intermediate can function, with manipulation, as a donor or an acceptor in subsequent glycosylation reactions. Thus, selective protection, employing the 1-*O*-allyl group, in conjunction with benzoyl and acetyl protecting groups, allows either the selective removal[14] of the acetate to afford an acceptor for subsequent glycosylation, or the isomerisation of the allyl group to the prop-1-enyl group,[15] followed by hydrolysis[16] and subsequent conversion into the glycosyl halide (the donor molecule) by use of Vilsmeier-Haack reagents.[17,18] This overall scheme for generation of glycosyl donors and acceptors can be maintained throughout the synthetic plan for the higher-order oligosaccharides and is based on our recent work[18,19] on the synthesis of penta- up to octa-saccharides corresponding to the biological repeating unit of *Shigella flexneri* variant Y *O*-antigens. It is noteworthy that the same trisaccharide unit can be used sequentially to give the branched hexa- and nona-saccharides. The synthetic strategy has also been designed to yield both linear and branched chain-end sequences. Thus, the tri-, hexa-, and nona-saccharide could be 'capped' at the 2-position of the penultimate residue (α-L-Rha*p*) with an α-L-Rha*p* residue to give, respectively, the branched tetra-, hepta-, and deca-saccharide. This feature of the synthesis is of interest since the biological repeating unit and hence the structure at the 'non-reducing' terminus has not yet been established.

We report here the synthesis of a linear trisaccharide, as its propyl glycoside, for use as a hapten in binding and n.m.r. studies, and as its 8-methoxycarbonyloctyl glycoside, for use in the preparation of glycoconjugates. We report also the synthesis of a disaccharide, as its propyl glycoside.

The selectively blocked monosaccharide, allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (**1**), previously synthesized[18] for elaboration of oligosaccharides corresponding to the *O*-antigenic determinants of *Shigella flexneri* Y, was the starting point. Königs-Knorr reaction of compound (**1**) with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**2**)[20] using silver trifluoromethanesulphonate and 2,4,6-trimethylpyridine afforded the blocked disaccharide (**3**) in 90% yield. Rhodium(I)-catalysed isomerisation[15] (86%) of the allyl

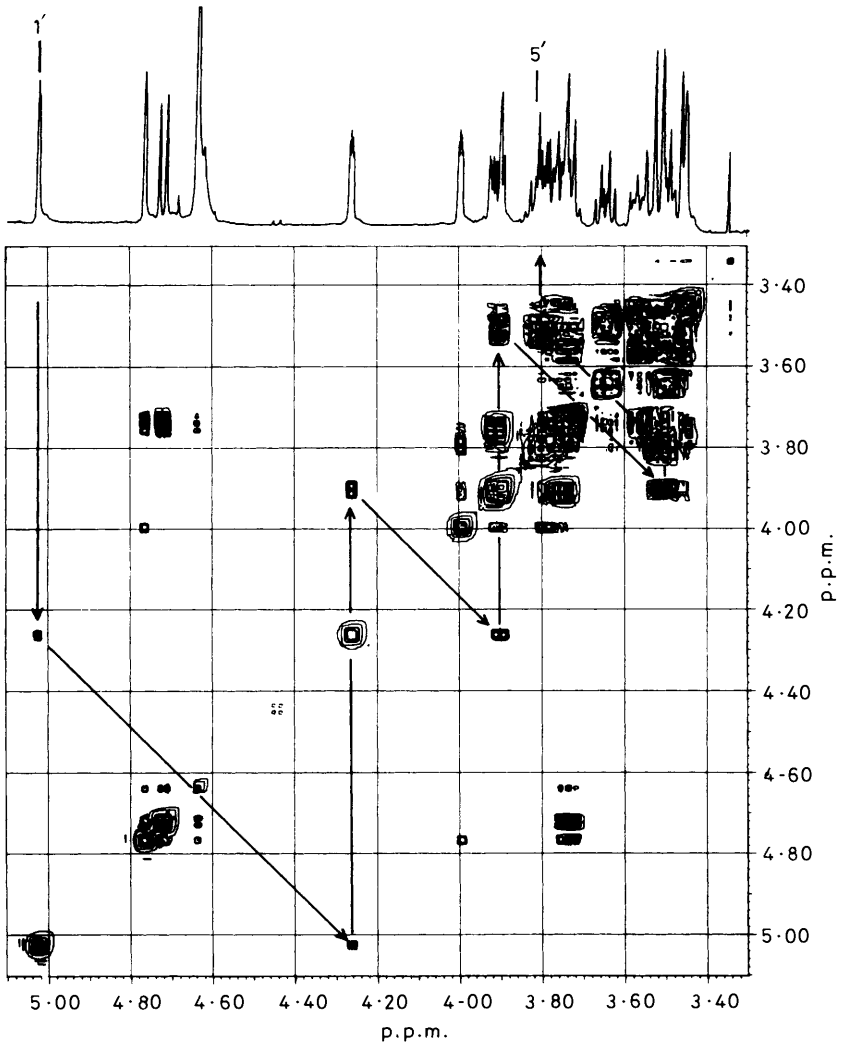


Figure 2. Partial 500 MHz two-dimensional ^{1}H n.m.r. COSY spectrum of the deprotected trisaccharide (**10**)

glycoside to the prop-1-enyl glycoside (**4**) and subsequent hydrolysis[16] (93%) gave the hemiacetals (**5**). The glycosyl donor, namely the disaccharide chloride (**6**), was then readily obtained by reaction of compound (**5**) with the Vilsmeier-Haack reagent *N*,*N*-dimethyl(chloromethylene)ammonium chloride.[17,18] In conjunction with the glycosyl acceptor (**1**) under silver trifluoromethanesulphonate promotion in the presence of 1,1,3,3-tetramethylurea,[21] the blocked trisaccharide (**7**) was obtained in 75% yield. Alternatively, the disaccharide chloride (**6**) was treated with 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (**8**)[22,23] as glycosyl acceptor under similar conditions to give the protected trisaccharide (**9**), as its 8-methoxycarbonyloctyl glycoside, in 53% yield. Transesterification, followed by hydrazinolysis of the phthalimido function, selective *N*-acetylation in methanol, and hydrogenolysis of compounds (**7**) and (**9**) then afforded the trisaccharide as its propyl glycoside (**10**) and its 8-methoxycarbonyloctyl glycoside (**11**), respectively. Finally, application of a similar deprotection sequence to the disaccharide (**3**) afforded β-D-Glc*p*NAc-(1→3)-α-L-Rha*p* as its propyl glycoside (**12**). The products were analytically pure.

The structures assigned were in accord with their ^{13}C and ^{1}H n.m.r. spectral data. Chemical shift assignments were made by comparison with data[10,18,23–30] for related compounds and also by comparison of data within the present series of compounds. ^{1}H N.m.r. assignments were confirmed, when possible, by means of homo-decoupling experiments.

The one-dimensional 400 MHz ^{1}H n.m.r. spectrum of the trisaccharide (**7**) revealed considerable overlap of the ring-proton signals in the region δ 3.5—5.5. Therefore, a 500 MHz COSY n.m.r. spectrum[31] (Figure 1) was obtained in order to establish the intra-ring H–H connectivities. For example, the signal at δ 5.18, assigned to an anomeric proton from a rhamnosyl unit, shows a cross-peak in the COSY spectrum at δ 5.39, indicating the position of the 2-H to which it is coupled. The 2-H in turn shows a cross-peak to a signal at δ 4.06, indicating the position of the 3-H of the same ring. The 4-, 5-, and 6-H signals of the same rhamnosyl ring were assigned in a similar fashion (Figure 1), as were all the remaining intra-ring connectivities. In order to distinguish one set of rhamnosyl ring proton resonances from the other, a nuclear Overhauser effect (n.O.e.) experiment[32] was performed in which irradiation at the frequency of one of the rhamnosyl anomeric protons was used to identify the proton across the interglycosidic linkage.[29,33,34] Thus, for example, irradiation at δ 4.9 (the anomeric proton resonance) caused a significant n.O.e. of the signal at δ 3.98, attributed to one of the diastereotopic protons of the aglycone $-CH_2CH{=}CH_2$. This enhancement indicated that the irradiated signal was in fact due to the 1-H; the signal at δ 5.18 was therefore assigned to 1′-H. The ring-proton signals of the *N*-

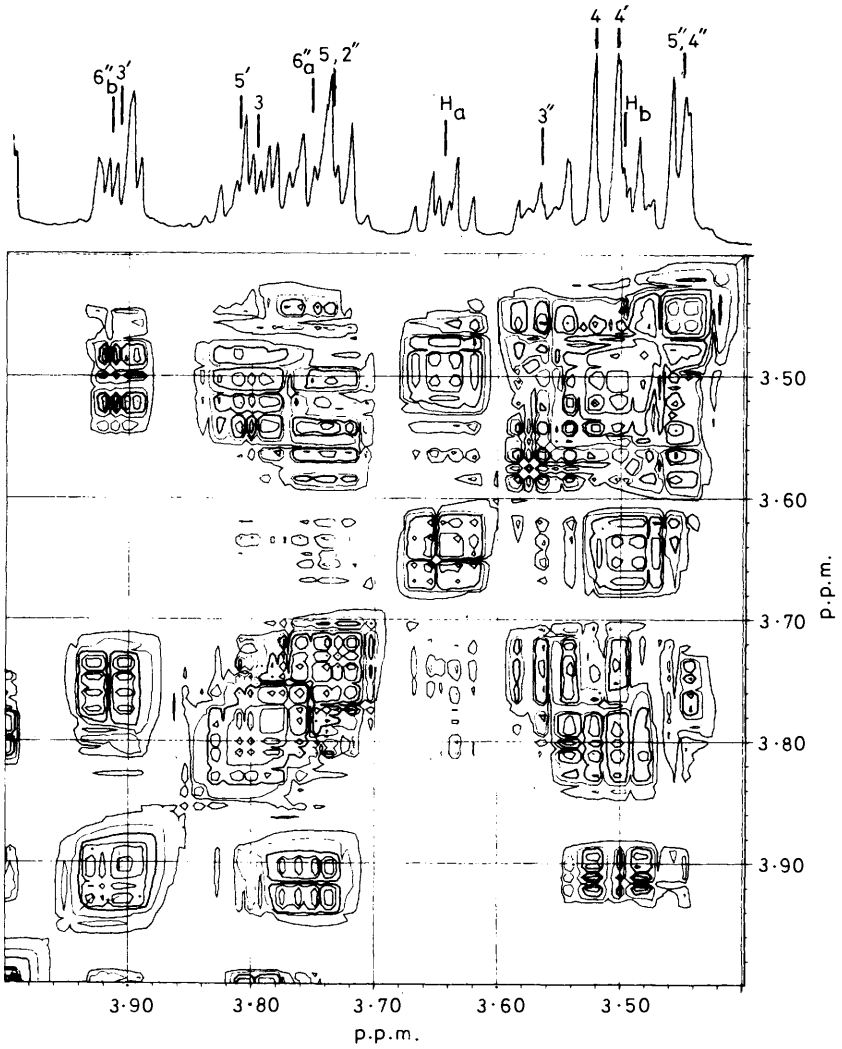


Figure 3. Expanded region of the two-dimensional ^{1}H n.m.r. COSY spectrum of the deprotected trisaccharide (**10**)

acetylglucosamine ring were readily distinguished from those of the rhamnosyl rings on the basis of vicinal coupling constants and hence assigned to the terminal ring. The assignment of the ^{1}H n.m.r. spectrum of the trisaccharide (**9**) was made by comparison with assignments made in the corresponding spectrum of the trisaccharide (**7**). The assignments were confirmed by spin-decoupling experiments, and n.O.e. experiments similar to those already described.

Analysis of the ^{13}C–^{1}H correlation (Chortle) spectrum[35] of (**7**), following the assignment of the ^{1}H n.m.r. spectrum from the COSY experiment, permitted the assignment of the ^{13}C{^{1}H} spectrum of the trisaccharide (**7**). Chemical shift assignments in the ^{13}C{^{1}H} spectra of compounds (**3**) and (**9**) were then made by comparison with those in the spectrum of compound (**7**).

Owing to the complex overlap of signals in the ^{1}H n.m.r. spectra of the deprotected compounds (**10**)—(**12**) in the region δ 3.5—4.0, a 500 MHz COSY n.m.r. spectrum of the trisaccharide (**10**) (Figures 2 and 3) proved invaluable in the assignment of some of the signals in that region. This spectrum, together with the ^{13}C–^{1}H correlation (Chortle) spectrum of the trisaccharide (**10**), and comparison with ^{1}H n.m.r. data of the natural polymer,[36] permitted the assignment of most of the carbon and proton signals in the spectra of (**10**). Most of the signals in the ^{13}C{^{1}H} and ^{1}H n.m.r. spectra of the deprotected structures (**11**) and (**12**) were then assigned by comparison with the data for (**10**).

Anomeric purity and configuration of the di- and trisaccharides were confirmed by means of ^{1}H and ^{13}C n.m.r. data. In particular, the one-bond ^1J (^{13}C, ^{1}H) coupling constants for the anomeric carbon atoms were diagnostic[37] of the expected α-L-linked rhamnose residues and the β-D-linked *N*-acetylglucosamine residue.

Experimental

M.p.s were determined with a Fisher–Johns apparatus. Routine ^{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. The ^{1}H homonuclear chemical shift-correlated (COSY) spectra and the ^{13}C–^{1}H correlated (Chortle) spectra were recorded with a Bruker AM-500 spectrometer operating at 500.13 MHz for ^{1}H and 125.7 MHz for ^{13}C. Spectra were measured for solutions in deuteriochloroform unless otherwise stated. Chemical shifts are given in p.p.m. downfield from Me_4Si. For those spectra measured for solution in deuterium oxide, chemical shifts are given in p.p.m. downfield from 2,2-dimethyl-2-silapentane-5-sulphonate (DSS). Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. N.O.e. experiments were performed at 400.13 MHz in the difference mode.

Analytical thin-layer chromatography (t.l.c.) was performed on pre-coated aluminium 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% sulphuric acid in ethanol, and heated at 150 °C. All compounds were purified by medium-pressure column chromatography on Kieselgel 60 (230—400 mesh) according to a published procedure.[38] 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 off under reduced pressure and below 40 °C.

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

Allyl 3-O-(3′,4′,6′-*Tri*-O-*acetyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-2-O-*benzoyl*-4-O-*benzyl*-α-L-*rhamnopyranoside** (**3**).—A mixture of allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (**1**)[18] (1.30 g, 3.26 mmol), silver trifluoromethanesulphonate (1.23 g, 4.77 mmol), and 4 Å molecular sieves in anhydrous dichloromethane (20 cm^3) was stirred for 0.5 h under nitrogen in a flask fitted with a dropping funnel, equipped with a cooling jacket. A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**2**)[20] (2.40 g, 4.82 mmol) and collidine (0.63 cm^3, 4.8 mmol) in anhydrous dichloromethane (5.0 cm^3), previously stirred under nitrogen for 0.5 h in the presence of 4 Å molecular sieves was transferred under nitrogen to the dropping funnel by means of a cannula. The flask was rinsed with additional portions of dichloromethane (2 × 2 cm^3). The solution of (**2**) was cooled to −78 °C and then added dropwise, over 10 min, to the mixture containing the allyl glycoside (**1**). The resulting mixture was allowed to warm gradually to room temperature and was stirred for 12 h in the dark under nitrogen. The solids were removed by filtration and the filtrate was washed successively with 1M hydrochloric acid, aqueous sodium hydrogen carbonate, and aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup which was purified by column chromatography using hexane–ethyl acetate (1:1) as eluant; R_F 0.43. *Compound* (**3**) was obtained as a syrup (2.43 g, 91%); $[\alpha]_D^{25}$ −15.5° (*c* 0.9 in CH_2Cl_2); δ_C(100.6 MHz) 17.7 (C-6), 20.2, 20.3, and 20.4 ($OCOCH_3$), 54.8 (C-2′), 61.7 (C-6′), 67.3 (C-5), 68.3 and 68.4 ($OCH_2CH{=}CH_2$, and C-4′), 70.9 (C-3′), 71.6 (C-5′), 72.1 (C-2), 74.6 (CH_2Ph), 79.2 (C-3), 80.2 (C-4), 96.2 (C-1), 98.5 (C-1′), 117.9 ($OCH_2CH{=}CH_2$), 133.7 ($OCH_2CH{=}CH_2$), and 166.1, 169.5, 170.2, and 170.8 (C=O); δ_H(400.13 MHz) 1.13 (3 H, d, $J_{5,6}$ 6.3 Hz, 6-H_3), 1.78, 1.80, and 1.97 (3 × 3 H, s, $OCOCH_3$), 3.48 (1 H, t, $J_{3,4} + J_{4,5}$ = 19 Hz, 4-H), 3.69 (1 H, m, 5-H), 3.85 (1 H, ddd, $J_{4',5'}$ 10.1, $J_{5',6a'}$ 4.0, $J_{5',6b'}$ 2.3 Hz, 5′-H), 4.04 (1 H, dd, $J_{5',6b'}$ 2.3, $J_{6a',6b'}$ 12.0 Hz, 6′-H_b), 4.13 (1 H, dd, $J_{5',6a'}$ 4.0, $J_{6a',6b'}$ 12.0 Hz, 6′-H_a), 4.18 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, 3-H), 4.37 (1 H, dd, $J_{2',3'}$ 10.8, $J_{1',2'}$ 8.5 Hz, 2′-H), 4.89 (1 H, d, $J_{1,2}$ 1.8 Hz, 1-H), 5.05 (1 H, dd, $J_{3',4'}$ 9.2, $J_{4',5'}$ 10.1 Hz, 4′-H), 5.44 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, 2-H), 5.67 (1 H, d, $J_{1',2'}$ 8.5 Hz, 1′-H), and 5.70 (1 H, dd, $J_{3',4'}$ 9.2, $J_{2',3'}$ 10.8 Hz, 3′-H) (Found: C, 63.3; H, 5.5; N, 1.7. $C_{43}H_{45}NO_{15}$ requires C, 63.3; H, 5.6; N, 1.7%).

Prop-1-*enyl* 3-O-(3′,4′,6′-*Tri*-O-*acetyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-2-O-*benzoyl*-4-O-*benzyl*-α-L-*rhamnopyranoside* (**4**).—Tris(triphenylphosphine)rhodium(I) chloride (85.7 mg, 0.093 mmol) was added to a solution of the allyl glycoside (**3**) (1.36 g, 1.66 mmol) in ethanol–water (9:1) (60 cm^3) and the mixture was heated at reflux for 16 h under nitrogen. The solvent was evaporated off to leave a dark brown residue, which was taken up in ethyl acetate and filtered through a short column of silica gel. Removal of the solvent left a light brown foam, which was further purified by column chromatography with hexane–ethyl acetate (1:1) as eluant (R_F 0.47). *Compound* (**4**) was obtained as a light brown syrup, as a mixture of *E*- and *Z*-isomers (1.17 g, 86%); δ_C(100.6 MHz) 9.2 and 12.1 ($CH{=}CHCH_3$ *E* and *Z*), 17.7 (C-6), 20.1, 20.2, and 20.4 ($OCOCH_3$), 96.4 and 96.5 (C-1 *E* and *Z*), 98.7 and 98.8 (C-1′, *E* and *Z*), 104.5 and 105.0 ($CH{=}CHCH_3$ *E* and *Z*), 166.0 ($OCOC_6H_5$), 167.8 (phthaloyl C=O), and 169.4, 170.1, and 170.7 ($OCOCH_3$); δ_H(400.13 MHz) 1.12 (3 H, m, 6-H_3, *E* and *Z*), 1.55 and 1.65 (3 H, 2 dd, *J* 1.9 and 7.0 Hz, $CH{=}CHCH_3$ *E* and *Z*), 1.79, 1.81, and 1.98 (3 × 3 H, s, $OCOCH_3$), 4.59 and 5.14 (1 H, 2 m, *J* 6.4 and 7.0 Hz, $CH{=}CHCH_3$ *Z*, and *J* 7.0 and 12.1 Hz, $CH{=}CHCH_3$ *E*), 5.45 and 5.53 (1 H, 2 dd, *J* 1.8 and 3.5 Hz, 2-H *E* and *Z*), and 6.06 and 6.13 (1 H, 2 m, *J* 1.9 and 6.4 Hz, $CH{=}CHCH_3$ *Z*, and *J* 1.9 and 12.1 Hz, $CH{=}CHCH_3$ *E*).

3-O-(3′,4′,6′-*Tri*-O-*acetyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-2-O-*benzoyl*-4-O-*benzyl*-L-*rhamnopyranose* (**5**).—The prop-1-enyl glycosides (**4**) (1.01 g, 1.23 mmol) were dissolved in 90% aqueous acetone (40 cm^3) and the solution was stirred. Yellow mercury(II) oxide (0.279 g, 1.29 mmol) was added, followed dropwise over 2 min, by a solution of mercury(II) chloride (0.336 g, 1.24 mmol) in 90% aqueous acetone (25 cm^3). The mixture was stirred for 5 h, then evaporated, and the residue was dissolved in ethyl acetate and filtered through Celite. The filtrate was washed successively with saturated aqueous potassium iodide (2 ×), aqueous sodium thiosulphate (2 ×), and water (2 ×). The organic layer was dried (Na_2SO_4) and evaporated, and the resulting yellow syrup chromatographed with hexane–ethyl acetate (3:2) as eluant. *Compound* (**5**) was obtained as a light yellow syrup (1.04 g, 93%); δ_C(100.6 MHz) (α-anomer) 17.8 (C-6), 20.2, 20.3, and 20.4 ($OCOCH_3$), 91.9 [$^1J(^{13}C, ^1H)$ 172 Hz, C-1], 98.6 [$^1J(^{13}C, ^1H)$ 167 Hz, C-1′], and 166.1, 169.5, 170.1, and 170.9 (C=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 Chloride* (**6**).—Oxalyl chloride (0.57 cm^3, 6.5 mmol) was added to a stirred solution of dimethylformamide (DMF) (0.5 cm^3, 6.5 mmol) in anhydrous dichloromethane (4.0 cm^3), and the mixture was stirred under nitrogen for 5 min. The solvent was evaporated off under reduced pressure and the white salt was dried *in vacuo* for 50 min. The *N*,*N*-dimethyl(chloromethylene)ammonium chloride was then dissolved in anhydrous dichloromethane (4 cm^3) and a solution of the hemiacetals (**5**) (1.04 g, 1.33 mmol) in anhydrous dichloromethane (2 cm^3) was transferred to the flask under nitrogen by means of a cannula. The flask was rinsed with additional portions of solvent and transferred as before. The mixture was stirred under nitrogen for 2 h, and then the reaction was quenched by the addition of cold aqueous sodium hydrogen carbonate (20 cm^3). The organic layer was diluted with dichloromethane and washed successively with aqueous sodium hydrogen carbonate and aqueous sodium chloride. The organic layer was dried (K_2CO_3) and evaporated to leave a light yellow syrup (**6**) (1.07 g, 99%), which was dried *in vacuo* and used directly in the subsequent glycosylation; δ_C(100.6 MHz) (α-anomer) 17.4 (C-6), 20.2, 20.3, and 20.4 ($OCOCH_3$), 54.8 (C-2′), 61.9 (C-6′), 68.6 (C-5), 74.7 (OCH_2Ph), 78.4 (C-3), 78.8 (C-4), 89.8 [$^1J(^{13}C, ^1H)$ 184 Hz, C-1], 98.9 [$^1J(^{13}C, ^1H)$ 158 Hz, C-1′], and 165.8, 169.5, 170.1, and 170.8 (C=O).

Allyl 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* (**7**).—A mixture of allyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (**1**)[34] (0.381 g, 0.957 mmol), silver trifluoromethanesulphonate (0.382 g, 1.49 mmol), and 4 Å molecular sieves in anhydrous dichloromethane (8 cm^3) was

* Primed locants are included in the names of new compounds in order to facilitate the description of n.m.r. assignments.

stirred under nitrogen for 0.5 h in a flask fitted with a dropping funnel, equipped with a cooling jacket. A solution of the glycosyl chloride (**6**) (1.07 g, 1.34 mmol), and 1,1,3,3-tetramethylurea (0.18 cm^3, 1.5 mmol) in anhydrous dichloromethane (4 cm^3), previously stirred with 4 Å molecular sieves for 0.5 h under nitrogen, was transferred under nitrogen to the dropping funnel by means of a cannula. The flask was rinsed with additional portions of anhydrous dichloromethane (2 × 2 cm^3) and transferred as before. The solution of the glycosyl chloride was cooled to −78 °C and added dropwise, during 20 min, to the cooled (−50 °C) solution of (**1**). The dropping funnel was rinsed with additional portions of anhydrous dichloromethane (2 × 2 cm^3). The mixture was allowed to warm to room temperature and stirred in the dark under nitrogen for 36 h. The solids were removed by filtration and the filtrate was diluted with dichloromethane (30 cm^3) and washed successively with aqueous sodium hydrogen carbonate and aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and evaporated to leave a syrup which was chromatographed [hexane–ethyl acetate (1:1) as eluant; R_F 0.51]. *Compound* (**7**) was obtained as a colourless syrup (0.835 g, 76%); $[\alpha]_D^{25}$ −4.5° (*c* 2.4 in CH_2Cl_2); δ_C(125.7 MHz) 17.5 (C-6′), 18.0 (C-6), 20.3 (2 × $OCOCH_3$), 20.5 ($OCOCH_3$), 54.6 (C-2″), 61.0 (C-6″), 67.7 (C-5), 68.0 (C-4″), 68.2 ($OCH_2CH{=}CH_2$), 68.3 (C-5′), 70.8 (C-3″), 71.2 (C-5″), 72.0 (C-2′), 72.6 (C-2), 73.7, and 75.2 (OCH_2Ph), 78.3 (C-3). 78.6 (C-4′), 79.7 (C-3′), 80.1 (C-4), 96.3 (C-1), 98.4 (C-1″), 98.8 (C-1′), 117.5 ($OCH_2CH{=}CH_2$), and 165.67, 165.73, 169.1, 170.0, and 170.6 (C=O); δ_H(400.13 MHz) 0.92 (3 H, d, $J_{5',6'}$ 6.2 Hz, 6′-H_3), 1.36 (3 H, d, $J_{5,6}$ 6.2 Hz, 6-H_3), 1.71, 1.76, and 1.96 (3 × 3 H, s, $OCOCH_3$), 3.06 (1 H, ddd, $J_{5'',6b''}$ 2.2, $J_{5'',6a''}$ 3.2, $J_{4'',5''}$ 10.2 Hz, 5″-H), 3.38 (1 H, t, $J_{3',4'} + J_{4',5'}$ 18.8 Hz, 4′-H), 3.65 (1 H, t, $J_{3,4} + J_{4,5}$ 18.8 Hz, 4-H), 3.68 (1 H, m, 5′-H), 3.73 (1 H, dd, $J_{5'',6b''}$ 2.2, $J_{6a'',6b''}$, 12.6 Hz, 6″-H_b), 3.85 (1 H, m, 5-H), 3.88 (1 H, dd, $J_{5'',6a''}$ 3.2, $J_{6a'',6b''}$ 12.6 Hz, 6″-H_a), 4.06 (1 H, dd, $J_{2',3'}$ 3.5, $J_{3',4'}$ 9.2 Hz, 3′-H), 4.27 (1 H, dd, $J_{1'',2''}$ 8.5, $J_{2'',3''}$ 10.5 Hz, 2″-H), 4.32 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.2 Hz, 3-H), 4.90 (1 H, d, $J_{1,2}$ 1.8 Hz, 1-H), 4.97 (1 H, dd, $J_{3'',4''}$ 9.3, $J_{4'',5''}$ 10.2 Hz, 4″-H), 5.18 (1 H, d, $J_{1',2'}$ 1.8 Hz, 1′-H), 5.37 (1 H, d, $J_{1'',2''}$ 8.5 Hz, 1″-H), 5.39 (2 × 1 H, dd, *J* 1.8 and 3.5 Hz, 2- and 2′-H), and 5.55 (1 H, dd, $J_{3'',4''}$ 9.3, $J_{2'',3''}$ 10.5 Hz, 3″-H) (Found: C, 65.4; H, 5.7; N, 1.4. $C_{63}H_{65}NO_{20}$ requires C, 65.45; H, 5.7; N, 1.2%).

Propyl 3-O-[3′-O-(2″-*Acetamido*-2″-*deoxy*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranoside* (**10**).—The fully protected trisaccharide (**7**) (0.755 g, 0.653 mmol) was dissolved in 1M sodium methoxide in methanol (10 cm^3). The mixture was kept under nitrogen for 20 h, then neutralised with Rexyn 101 (H^+) resin. The resin was removed by filtration and the filtrate was concentrated to give a syrup. The syrup was dissolved in ethanol (10 cm^3), containing hydrazine hydrate (100%; 2 cm^3), and the mixture was refluxed for 16 h. Removal of a white rubbery material by filtration, and evaporation, left a colourless syrup, which was chromatographed [ethyl acetate–methanol–water (85:10:5) as eluant; R_F 0.4]. The resulting colourless syrup was dissolved in methanol (25 cm^3) containing acetic anhydride (2.5 cm^3), and stirred overnight. Evaporation left a colourless syrup which was taken up in 80% aqueous acetic acid (20 cm^3) and hydrogenolysed over 10% palladium–carbon (0.20 g) at a hydrogen pressure of 52 lb in^{-2} for 48 h. The solids were removed by filtration through Celite and the solvent removed by evaporation to leave a light brown syrup, which was chromatographed [ethyl acetate–methanol–water (7:2:1) as eluant; R_F 0.41]. *Compound* (**10**) was obtained as a white amorphous solid (0.195 g, 53.9%); $[\alpha]_D^{29}$ −59.2° (*c* 1.0 in H_2O); δ_C(D_2O; 100.6 MHz) 12.8 ($OCH_2CH_2CH_3$), 19.6 (C-6 and -6′), 24.9 ($OCH_2CH_2CH_3$), 25.2 ($NHCOCH_3$), 58.8 (C-2″), 63.7 (C-6″), 71.7 (C-5), 72.2 (C-5′), 72.6, 72.8, 72.9, and 73.1 (C-2, C-2′, C-4″, and $OCH_2CH_2CH_3$), 74.0 (C-4′), 74.5 (C-4), 76.8 (C-3″), 78.7 (C-5″), 81.1 (C-3), 83.0 (C-3′), 102.6 [$^1J(^{13}C, ^1H)$ 170 Hz, C-1], 104.8 [$^1J(^{13}C, ^1H)$ 167 Hz, C-1′], 105.6 [$^1J(^{13}C, ^1H)$ 162 Hz, C-1″], and 178.0 ($NHCOCH_3$); δ_H(D_2O; 500.13 MHz) 1.27 and 1.29 (2 × 3 H, d, *J* 6.4 Hz, 6- and 6′-H_3), 2.04 (3 H, s, $NHCOCH_3$), 3.45 (2 H, m, 4″- and 5″-H), 3.46—3.60 (4 H, m, $OCH_2CH_2CH_3$, 4′-, 4-, and 3″-H), 3.64 (1 H, m, $OCH_2CH_2CH_3$), 3.70—3.77 (3 H, m, 2″- and 5-H, and 6″-H_a), 3.79 (1 H, dd, $J_{2,3}$ 3.2, $J_{3,4}$ 9.7 Hz, 3-H), 3.91 (1 H, br d, $J_{6a'',6b''}$ 12.3 Hz, 6″-H_b), 3.99 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, 2-H), 4.26 (1 H, dd, $J_{1',2'}$ 1.8, $J_{2',3'}$ 3.3 Hz, 2′-H), 4.71 (1 H, d, $J_{1'',2''}$ 8.6 Hz, 1″-H), 4.76 (1 H, d, $J_{1,2}$ 1.8 Hz, 1-H), and 5.01 (1 H, d, $J_{1',2'}$ 1.8 Hz, 1′-H), (Found: C, 49.5; H, 7.5; N, 2.3. $C_{23}H_{41}NO_{14}$ requires C, 49.7; H, 7.4; N, 2.5%).

Propyl 3-O-(2′-*Acetamido*-2′-*deoxy*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**12**).—A sample of the fully protected disaccharide (**3**) (0.784 g, 0.960 mmol) was dissolved in 1.0M sodium methoxide in methanol (10 cm^3). The solution was set aside at room temperature, under nitrogen. After 40 h the mixture was neutralised by stirring with Rexyn 101 (H^+) resin. The resin was removed by filtration and the solvent evaporated off to leave a syrup, which was then dissolved in ethanol (30 cm^3) containing hydrazine hydrate (100%; 0.08 cm^3, 1.65 mmol). The mixture was refluxed for 24 h under nitrogen. Filtration and evaporation left a colourless syrup which was dried *in vacuo* to remove traces of hydrazine. The white amorphous solid was dissolved in methanol (25 cm^3) containing acetic anhydride (2.5 cm^3) and kept under nitrogen. After 16 h the solvent was removed by evaporation and the resulting syrup was chromatographed [ethyl acetate–methanol–water as eluant (85:10:5); R_F 0.36]. The resulting colourless glass was then dissolved in 80% aqueous acetic acid (30 cm^3) and hydrogenolysed over 10% palladium–carbon (0.098 g) at a hydrogen pressure of 55 lb in^{-2} for 3 days. The solids were removed by filtration through a pad of Celite and the solvent was removed by evaporation. After chromatography [ethyl acetate–methanol–water (7:2:1) as eluant; R_F 0.47] *compound* (**12**) was obtained as a white amorphous solid (0.216 g, 55%); $[\alpha]_D^{29}$ −47.2° (*c* 0.9 in H_2O); δ_C(D_2O; 100.6 MHz) 12.6 ($OCH_2CH_2CH_3$), 19.4 (C-6), 24.8 ($CH_2CH_2CH_3$), 25.0 ($NHCOCH_3$), 58.7 (C-2′), 63.6 (C-6′), 71.7 (C-5), 72.6, 72.8, and 72.9 (C-2, C-4′, and $OCH_2CH_2CH_3$), 73.9 (C-4), 76.6 (C-3′), 78.6 (C-5′), 83.2 (C-3), 102.2 [$^1J(^{13}C, ^1H)$ 170 Hz, C-1), 105.7 [$^1J(^{13}C, ^1H)$ 163 Hz, C-1′], and 178.0 ($NHCOCH_3$); δ_H(D_2O; 400.13 MHz) 0.88 (3 H, t, *J* 7.5 Hz, $OCH_2CH_2CH_3$), 1.24 (3 H, d, $J_{5,6}$ 6.1 Hz, 6-H_3), 1.58 (2 H, m, $OCH_2CH_2CH_3$), 2.00 (3 H, s, $NHCOCH_3$), 3.40—3.82 (10 H, complex, ring hydrogens and $OCH_2CH_2CH_3$), 3.87 (1 H, br d, $J_{6a',6b'}$ 12.0 Hz, 6′-H_b), 4.11 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, 2-H), and 4.64 (1 H, d, $J_{1',2'}$ 8.3 Hz, 1′-H) (Found: C, 49.7; H, 7.5; N, 3.3. $C_{17}H_{31}NO_{10}$ requires C, 49.9; H, 7.6; N, 3.4%).

8-*Methoxycarbonyloctyl* 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*]-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (**9**).—A mixture of 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (**8**)[22,23] (0.146 g, 0.270 mmol), silver trifluoromethanesulphonate (0.12 g, 0.46 mmol) and 4 Å molecular sieves in anhydrous dichloromethane (2.0 cm^3) was stirred for 0.5 h under nitrogen in a Schlenk tube fitted with a dropping funnel, equipped with a cooling jacket. A solution of the disaccharide chloride (**6**) (0.368 g, 0.464 mmol) and 1,1,3,3-tetramethylurea (0.05 cm^3, 0.42 mmol) in anhydrous dichloromethane (2.0 cm^3), previously stirred with 4 Å molecular sieves for 0.5 h under nitrogen, was transferred *via* a cannula to the dropping funnel. The flask was rinsed with additional portions of anhydrous dichloromethane (2 × 1.0 cm^3) and transferred to the dropping funnel as before. The cooled (−78 °C) solution of the glycosyl chloride was added

dropwise over 30 min to the cooled (−35 °C) mixture containing the alcohol (**8**). The dropping funnel was rinsed with additional portions of anhydrous dichloromethane (2 × 1.0 cm^3). The mixture was stirred in the dark under nitrogen and allowed to warm to room temperature. After 72 h the solids were removed by filtration and the filtrate was diluted with dichloromethane (20 cm^3) and washed successively with aqueous sodium hydrogen carbonate and aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and evaporated to leave a syrup, which was chromatographed [hexane–ethyl acetate (1:1) as eluant; R_F 0.46]. *Compound* (**9**) was obtained as a light yellow syrup (0.186 g, 53%); $[\alpha]_D^{29}$ −7.6° (*c* 0.9 in CH_2Cl_2); δ_C(100.6 MHz) 17.2 (C-6′), 17.5 (C-6), 19.9, 20.0, and 20.3 ($OCOCH_3$), 24.7, 25.8, 28.86, 28.90, 28.94, 29.2, and 33.8 ($CH_3O_2C[CH_2]_7CH_2O$), 51.1 ($CH_3O_2C[CH_2]_8O$), 54.4 (C-2″), 60.7 (C-6″), 66.3 ($CH_3O_2C[CH_2]_7CH_2O$), 67.9, 68.1, 68.2, 70.6, 70.7, 71.8, 72.0, 72.9, 73.5, 76.7, 78.4, and 79.7 (other CH and CH_2Ph), 97.2 (C-1), 98.35 and 98.42 (C-1″, C-1′), and 165.59, 165.62, 165.9, 167.5, 169.0, 169.9, 170.5, and 174.1 (C=O); δ_H(400.13 MHz) 0.77 (3 H, d, $J_{5',6'}$ 6.3 Hz, 6′-H_3), 1.32 (3 H, d, $J_{5,6}$ 6.3 Hz, 6′-H_3), 1.59, 1.76, and 1.97 (3 × 3 H, s, $OCOCH_3$), 2.57 (1 H, m, 5″-H), 3.28 (1 H, t, $J_{3',4'} + J_{4',5'}$ 19 Hz, 4′-H), 3.41 (1 H, dd, $J_{5'',6b''}$ 1.8, $J_{6a'',6b''}$ 12.3 Hz, 6″-H_b), 3.63 (1 H, m, 5′-H), 3.65 (3 H, s, CH_3O), 3.74 (1 H, dd, $J_{5'',6a''}$ 3.0, $J_{6a'',6b''}$ 12.3 Hz, 6″-H_a), 3.95 (1 H, dd, $J_{2',3'}$ 3.4, $J_{3',4'}$ 9.5 Hz, 3′-H), 4.04 (1 H, m, 5-H), 4.16 and 4.30 (2 × 1 H, d, $J_{Ha,Hb}$ 12.4 Hz, CH_2Ph), 4.21 (1 H, dd, $J_{1'',2''}$ 8.4, $J_{2'',3''}$ 10.5 Hz, 2″-H), 4.33 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.8 Hz, 3-H), 4.87 (1 H, dd, $J_{3'',4''}$ 9.1, $J_{4'',5''}$ 10.0 Hz, 4″-H), 4.92 (1 H, d, $J_{1,2}$ 1.8 Hz, 1-H), 4.97 (1 H, dd, $J_{1',2'}$ 2.0, $J_{2',3'}$ 3.4 Hz, 2′-H), 5.01 (1 H, d, $J_{1',2'}$ 2.0 Hz, 1′-H), 5.10 (1 H, d, $J_{1'',2''}$ 8.4 Hz, 1″-H), 5.40 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, 2-H), 5.52 (1 H, dd, $J_{3'',4''}$ 9.1, $J_{2'',3''}$ 10.5 Hz, 3″-H), and 5.53 (1 H, t, $J_{3,4} + J_{4,5}$ 19.5 Hz, 4-H) (Found: C, 64.5; H, 5.9; N, 1.2. $C_{70}H_{77}NO_{23}$ requires C, 64.7; H, 6.0; N, 1.1%).

8-*Methoxycarbonyloctyl* 3-O-[3′-O-(2″-*Acetamido*-2″-*deoxy*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranoside* (**11**).—The fully protected trisaccharide (**9**) (0.250 g, 0.192 mmol) was dissolved in 1M sodium methoxide in methanol (14 cm^3) and set aside for 24 h. The mixture was neutralised by stirring with Rexyn 101 (H^+) resin, the resin was removed by filtration, and the solvent evaporated off to leave a light brown syrup. The syrup was dissolved in ethanol (25 cm^3) containing hydrazine hydrate (100%; 0.05 cm^3, 1.03 mmol) and the mixture was refluxed under nitrogen for 18 h. A fine white precipitate was removed by filtration and the filtrate was dried *in vacuo* to remove traces of hydrazine. The syrup was then dissolved in methanol (20 cm^3) containing acetic anhydride (2.0 cm^3), and set aside at room temperature for 18 h. The solvent was evaporated off to leave a light brown syrup, which was dissolved in 80% aqueous acetic acid (20 cm^3) and hydrogenolysed over 10% palladium–carbon (0.10 g) at a hydrogen pressure of 52 lb in^{-2} for 20 h. The mixture was filtered through Celite and the filtrate was evaporated to dryness. Chromatography [ethyl acetate–methanol–water (7:2:1) as eluant; R_F 0.49] gave *compound* (**11**) as a light brown syrup (0.099 g, 75.4%); $[\alpha]_D^{29}$ −48.3° (*c* 1.0 in H_2O); δ_C(D_2O; 100.6 MHz) 19.4 (C-6 and -6′), 25.0 ($NHCOCH_3$), 27.1, 28.1, 31.0, 31.1, 31.3, and 36.4 ($CH_3O_2C[CH_2]_7CH_2O$), 54.7 ($CH_3O_2C[CH_2]_8O$), 58.6 (C-2″), 63.5 (C-6″), 70.7 ($CH_3O_2C[CH_2]_7CH_2O$), 71.6 (C-5), 72.0 (C-5′), 72.6, 72.7, and 73.0 (C-2, 2′, and 4″), 73.7 (C-4′), 74.3 (C-4), 76.5 (C-3″), 78.5 (C-5″), 80.8 (C-3), 82.8 (C-3′), 102.6 [$^1J(^{13}C, ^1H)$ 169 Hz, C-1], 104.7 [$^1J(^{13}C, ^1H)$ 171 Hz, C-1′] 105.4 [$^1J(^{13}C, ^1H)$ 163 Hz, C-1″], 177.8 ($NHCOCH_3$), and 180.0 ($CH_3O_2C[CH_2]_8O$); δ_H(D_2O; 400.13 MHz) 1.20—1.35 (14 H, m), 1.51—1.61 (4 H, m), 1.99 (3 H, s, $NHCOCH_3$), 2.34 (2 H, t, $CH_3O_2CCH_2[CH_2]_7O$), 3.37—3.57 (6 H, complex m, ring protons), 3.61—3.81 (9 H, complex m, ring H protons), 3.87 (1 H, dd, $J_{2',3'}$ 3.2, $J_{3',4'}$ 9.8 Hz, 3′-H), 3.88 (1 H, d, $J_{6a'',6b''}$ 12.0 Hz, 6″-H_b), 3.95 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, 2-H), 4.24 (1 H, dd, $J_{1',2'}$ 1.8, $J_{2',3'}$ 3.2 Hz, 2′-H), 4.67 (1 H, d, $J_{1'',2''}$ 8.2 Hz, 1″-H), 4.71 (1 H, d, $J_{1,2}$ 1.8 Hz, 1-H), and 4.98 (1 H, d, $J_{1',2'}$ 1.8 Hz, 1′-H) (Found: C, 52.8; H, 8.1; N, 1.8. $C_{30}H_{53}NO_{16}$ requires C, 52.7; H, 7.8; N, 2.05%).

Acknowledgements

We thank Dr. D. R. Bundle, National Research Council of Canada, for recording the 500 MHz n.m.r. spectra, and the Natural Sciences and Engineering Research Council of Canada for administration of a strategic research grant.

References

1 'Streptococcal Diseases and the Immune Response,' eds. S. E. Read and J. B. Zabriskie, Academic Press, New York, 1980.
2 J. B. Zabriskie, *Circulation*, 1985, **71**, 1077.
3 I. Goldstein, L. Scebat, J. Renais, P. Hadjinsky, and J. Dutartre, *Isr. J. Med. Sci.*, 1983, **19**, 483; I. Goldstein, B. Halpern, and L. Robert, *Nature* (*London*), 1967, 44; I. Goldstein, P. Rebeyrotte, J. Parlebas, and B. Halpern, *ibid.*, 1968, 866.
4 *The Medical Letter*, 1985, **27**, 49.
5 See, for example, R. Rosenstein, K. P. Aspden, and P. Stopa, Eur. Pat. Appl. EP 153 477 1985 (*Chem. Abstr.*, 1986, **104**, 3136a); J. W. Holland, R. H. Walter, Eur. Pat. Appl., EP 0150 567 A2 1985.
6 R. A. Venezia, A. Ryan, S. Alward, and W. A. Kostun, *J. Clin. Microbiol.*, 1985, **21**, 395.
7 B. G. Miceika, A. S. Vitous, and K. D. Thompson, *J. Clin. Microbiol.*, 1985, **21**, 467.
8 R. M. Centor, F. A. Meier, and H. P. Dalton, *Ann. Int. Med.*, 1986, **105**, 892.
9 J. E. Coligan, T. J. Kindt, and R. M. Krause, *Immunochemistry*, 1978, **15**, 755.
10 T. Iversen, S. Josephson, and D. R. Bundle, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2379.
11 B. M. Pinto and D. R. Bundle, *Cardohydr. Res.*, 1983, **124**, 313.
12 F. Emmrich, D. Bundle, J. van der Zee, T. Out, G. Zenke, and K. Eichmann, *Scand. J. Immunol.*, 1985, **21**, 119.
13 D. R. Bundle, unpublished results.
14 N. E. Byramova, M. V. Ovchinnikiv, L. V. Backinowsky, and N. K. Kochetkov, *Carbohydr. Res.*, 1983, **124**, C8.
15 E. J. Corey and J. W. Suggs, *J. Org. Chem.*, 1973, **38**, 3224.
16 R. Gigg and C. D. Warren, *J. Chem. Soc. C*, 1968, 1903.
17 H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, 1959, **42**, 1653; T. Iversen and D. R. Bundle, *Carbohydr. Res.*, 1982, **103**, 29; R. U. Lemieux, S. Z. Abbas, and B. Y. Chung, *Can. J. Chem.*, 1982, **60**, 58.
18 B. M. Pinto, D. G. Morisette, and D. R. Bundle, *J. Chem. Soc., Perkin Trans. 1*, 1987, 9.
19 B. M. Pinto, D. G. Morissette, K. B. Reimer, and D. R. Bundle, in preparation.
20 R. U. Lemieux, T. Takeda, and B. Y. Chung, Am. Chem. Soc. Symposium Series, 1976, no. 39, p. 90.
21 S. Hanessian and J. Banoub, *Carbohydr. Res.*, 1977, **53**, 13.
22 S. Josephson and D. R. Bundle, *J. Chem. Soc., Perkin Trans. 1*, 1980, 297.
23 S. Josephson and D. R. Bundle, *Can. J. Chem.*, 1979, **57**, 3073.
24 D. R. Bundle and S. Josephson, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2736.
25 A. Liptak, P. Nanasi, and A. Nesmelyi, *Tetrahedron*, 1980, **36**, 1261.
26 V. Pozsgay and A. Neszmelyi, *Carbohydr. Res.*, 1980, **80**, 196.
27 H.-P. Wessel and D. R. Bundle, *Carbohydr. Res.*, 1983, **124**, 301.
28 D. R. Bundle and S. Josephson, *Can. J. Chem.*, 1979, **57**, 662.
29 K. Bock, S. Josephson, and D. R. Bundle, *J. Chem. Soc., Perkin Trans. 2*, 1982, 59.
30 D. R. Bundle and S. Josephson, *Carbohydr. Res.*, 1980, **80**, 75.
31 A. Bax and R. Freeman, *J. Magn. Res.*, 1981, **44**, 542.
32 J. H. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect,' Academic Press, New York, 1971.

33 H. Thogersen, R. U. Lemieux, K. Bock, and B. Meyer, *Can. J. Chem.*, 1982, **60**, 44.
34 K. Bock, *Pure Appl. Chem.*, 1983, **55**, 605.
35 G. A. Pearson, *J. Magn. Res.*, 1985, **64**, 487.
36 D. H. Huang, N. R. Krishna, and D. G. Pritchard, *Carbohydr. Res.*, 1986, **155**, 193.
37 K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, 1974, 293; K. Bock and C. Pedersen, *Acta Chem. Scand., Ser. B*, 1975, **29**, 258.
38 D. R. Bundle, T. Iversen, and S. Josephson, *Am. Laboratory*, 1980, **12**, 93.

Received 14*th September* 1987; *Paper* 7/1656