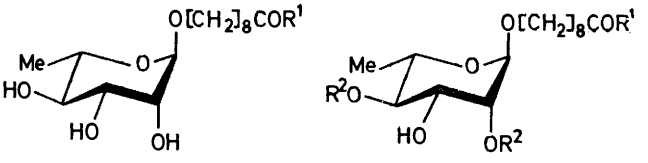
Artificial Carbohydrate Antigens: Synthesis of Rhamnose Trisaccharide and Disaccharide Haptens common to *Shigella flexneri* O-Antigens

By **Staffan Josephson** and **David R. Bundle,*** Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6

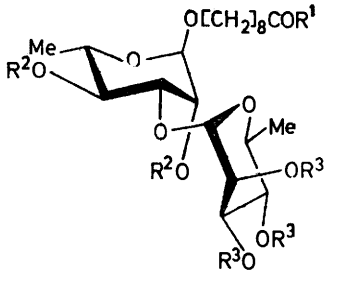
The rhamnose di- and tri-saccharide glycosides, α-L-Rham(*p*)-(1 ⟶ 3)-α-L-Rham(*p*) and α-L-Rham(*p*)-(1 ⟶ 2)-α-L-Rham(*p*)-(1 ⟶ 3)-α-L-Rham(*p*), representing portions of the *Shigella flexneri* serogroup Y lipopolysaccharide repeating unit, have been synthesised in high yield by a series of Königs–Knorr reactions. The synthetic methods used are designed to provide di- and tri-saccharide artificial antigens, after complete deblocking and covalent attachment to protein. 8-Methoxycarbonyloctyl α-L-rhamnopyranoside was converted into the corresponding 2,4-di-*O*-benzoate *via* a 2,3-orthobenzoate. Königs–Knorr reaction between this partially blocked rhamnoside and either 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide or 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride gave respectively the fully blocked disaccharide and the disaccharide precursor of the trisaccharide. Selective transesterification of the 2′-*O*-acetate group in the presence of 2,4-di-*O*-benzoates provides the disaccharide selectively deblocked at the C-2′ position. Reaction with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide gave the fully blocked trisaccharide. Removal of the blocking groups yielded the trisaccharide hapten. The ^{13}C and ^{1}H n.m.r. spectra together with the T_1 parameters for the haptens are consistent with a conformation about the aglyconic-oxygen-to-aglyconic-carbon bond (torsional angle ψ) that approaches the eclipsed rather than the staggered arrangement.

PREVIOUSLY [1,2] we have reported the synthesis of three disaccharides and one trisaccharide which constitute portions of the tetrasaccharide repeating unit of the *Shigella flexneri* serogroup Y polysaccharide.[3] The manner in which these oligosaccharides are synthesised allows covalent attachment to protein after the deblocking sequence thereby providing artificial antigens for the examination of serogroup factors and immunodeterminant groupings.[4] In conjunction with somatic cell fusion,[5] as a source of homogeneous antibody, these antigens should provide interesting probes for antigen–antibody interactions. ^{1}H N.m.r. data from several of the oligosaccharides previously synthesised [1,2] have provided information towards an appreciation of the conformation of the tetrasaccharide repeating unit, an essential prerequsite for studies of antigen–antibody interactions. We now report the synthesis of the trisaccharide antigenic determinant *O*-α-L-rhamnopyranosyl-(1 ⟶ 2)-*O*-α-L-rhamnopyranosyl-(1 ⟶ 3)-*O*-α-L-rhamnopyranoside (11) and the disaccharide *O*-α-L-rhamnopyranosyl-(1 ⟶ 3)-*O*-α-L-rhamnopyranoside (4). The ^{1}H n.m.r. spectra of both compounds suggest that the *exo*-anomeric effect exerts a profound influence on the conformation about the glycosidic linkages. By extrapolation of these findings to the polysaccharide antigen a more precise knowledge of the conformation of the immunodominant groupings should emerge.

As with earlier work,[1,2] the antigenic determinant is elaborated on the alcohol, 8-methoxycarbonyloctanol.[6] After removal of blocking groups, covalent attachment to protein is effected through a two-step sequence utilising the ester function of the nine-carbon bridging arm.[6,7] The choice of 8-methoxycarbonyloctanol as the bridging arm is based both upon synthetic convenience[6] and on well documented immunochemical factors which accrue from the choice of the nine-carbon aliphatic aglycon.[6-8] Reaction of tri-*O*-acetyl-α-L-rhamnopyranosyl bromide with 8-methoxycarbonyloctanol under Helferich[9] conditions gave the acetylated form of the α-L-rhamnopyranoside (1). After de-*O*-acetylation and chromatographic purification, the pure rhamnoside (1) was obtained in 61% yield. The rhamnoside (1) was converted into a 2,3-orthobenzoate which after benzoylation and hydrolysis provided the 2,4-di-*O*-benzoate (2) in



(1) R^1 = OMe (2) R^1 = OMe, R^2 = Bz

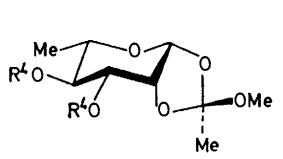


(3) R^1 = OMe, R^2 = Bz, R^3 = Ac
(4) R^1 = OMe, R^2 = R^3 = H
(5) R^1 = $NHNH_2$ R^2 = R^3 = H

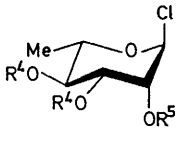
77% yield. This scheme is essentially that of Garegg and Hultberg[10] and does not require isolation of the intermediates. The selectively blocked rhamnopyranoside (2) gave the fully blocked disaccharide (3) following a silver trifluoromethanesulphonate (triflate) promoted Königs–Knorr reaction[11] with tri-*O*-acetyl-α-L-rhamnopyranosyl bromide. Purification by preparative high-pressure chromatography provided the disaccharide (3) in 68% yield. Transesterification in methanol gave the deblocked disaccharide (4), which was converted into the hydrazide derivative (5) for subsequent antigen synthesis.[6,7]

In order to provide a route to the trisaccharide (11), the selectively blocked 2,4-di-*O*-benzoyl-α-L-rhamno-

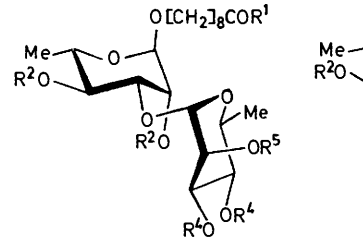
pyranoside (2) was used, but to gain access to a 2-substituted rhamnose unit a glycosyl halide other than tri-*O*-acetyl-α-L-rhamnopyranosyl bromide was required. In earlier work [1,2] 3-4-di-*O*-benzyl-β-L-rhamnopyranose 1,2-(methyl orthoacetate) (6) was used in a typical orthoester glycosylation [12] to provide a rhamnopyranoside with persistent blocking groups at ring positions 3



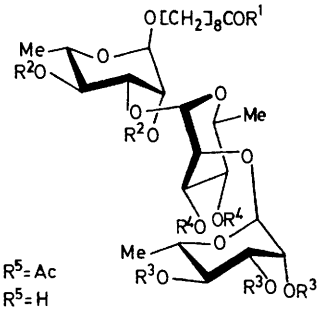
(6) R = CH_2Ph



(7) $R^4 = CH_2Ph$, R^5 = Ac



(8) R^1 = OMe, R^2 = Bz, $R^4 = CH_2Ph$, R^5 = Ac
(9) R^1 = OMe, R^2 = Bz, $R^4 = CH_2Ph$, R^5 = H



(10) R^1 = OMe, R^2 = Bz, R^3 = Ac, $R^4 = CH_2Ph$
(11) R^1 = OMe, $R^2 = R^3 = R^4$ = H
(12) $R^1 = NHNH_2$, $R^2 = R^3 = R^4$ = H

and 4. However, in our hands it has proved difficult, using molar stoicheiometry, to raise yields of glycosides by such reactions above 60%. Reaction of the orthoester (6) in dichloromethane with trimethylchlorosilane under reflux for 2 hours provides an essentially quantitative yield of the glycosyl chloride (7). This general route to glycosyl chlorides was recently used with a mannose 1,2-orthoester [13] and has considerable potential for oligosaccharide syntheses, which use monosaccharide building blocks tailored for subsequent steps. The requirement for such mild conditions during reactions leading to glycosyl halides is well demonstrated by this example. Benzyl ethers, in other respects most useful blocking groups, are particularly labile to hydrogen bromide–acetic acid mixtures [14] and even a slight excess of hydrogen bromide in dichloromethane caused significant de-*O*-benzylation of 1,2-di-*O*-acetyl-3,4-di-*O*-benzyl-β-L-rhamnopyranose when it was used in earlier, abortive attempts to synthesise the bromo-analogue of (7).[15] Glycosyl chlorides are sufficiently reactive to give excellent yields of glycosides [reactions of the glycosyl chloride (7) with the partially blocked monosaccharide (2) gives an 87% yield of disaccharide (8) after chromatography] when a powerful promoter such as silver triflate is employed. Access to glycosyl bromides under similarly mild conditions is possible using trimethylsilyl bromide [16] if the enhanced reactivity of a glycosyl bromide is necessary. We have also employed αα-dichloromethyl methyl ether [17] to yield the chloride (7) from the 1,2-orthoester (6). This and the variant using αα-dibromomethyl methyl ether [18,19] provide alternative routes from glycosides, monosaccharide 1-*O*-acetates, and 1,2-orthoesters to labile halogeno-sugars, which remain the most valuable intermediates for glycoside synthesis.

In order to convert the fully blocked disaccharide (8) into the partially blocked derivative (9) it was necessary to remove selectively the 2′-*O*-acetate group without affecting the benzoate esters. Catalytic amounts of sodium in methanol gave the hydroxy-derivative (9) but this initially-formed product, which could be isolated in low yield, reacted further to give the deacylated disaccharide. Triethylamine in aqueous methanol or one molar equivalent of sodium hydroxide in ethanol gave even lower yields of the disaccharide (9). Magnesium methoxide has been used [20] as a particularly mild base for the catalytic de-*O*-acetylation of 2-acetamido-2-deoxy-D-glucopyranose derivatives. These conditions avoid the formation of elimination products (which are related to the Morgan–Elson chromogen [21]), observed when conventional Zemplen [22] transesterification was employed. Catalytic quantities of magnesium methoxide converted the blocked disaccharide (8) to the selectively protected derivative (9), which was recovered in 67% yield after chromatography. When the disaccharide (9) was treated with tri-*O*-acetyl-α-L-rhamnopyranosyl bromide, the trisaccharide (10) was obtained as a pure syrup in 60% yield. Removal of blocking groups was achieved as normal; catalytic hydrogenolysis of the benzyl groups preceded the de-*O*-acylation step. The hydrazide (12) was prepared from (11) and was used for the synthesis of artificial antigens as previously described.[6,7]

Anomeric purity and configuration of the di- and trisaccharides were confirmed by ^1H and ^{13}C n.m.r. The proton spectra of both the disaccharide (5) and the trisaccharide (12) show well-separated signals indicating high anomeric purity and, in addition, the two resonances at δ 5.18 and 4.98 exhibit chemical shifts typical of α-L-rhamnopyranosides. However, proton $J_{1,2}$ coupling constants are not of assistance in assigning the configuration of rhamnopyranosides since the *eq–eq* couplings are similar in magnitude to *ax–ax* couplings. Carbon-13 shifts of the anomeric carbon atoms, with the exception of that at 103.5 p.p.m. are typical of α-linked rhamnopyranosyl residues. That all three resonances are in fact due to α-linked C-1 atoms is confirmed by single bond $J_{^{13}C_1,^1H_1}$ coupling constants.[23,24] All coupled C-1 resonances have ^1J values in the range 171—176 Hz, typical of α-pyranosides rather than β-pyranoside, which exhibit ^1J values *ca.* 10 Hz lower. The residue providing the highest field C-1 resonance was assigned to the middle rhamnose residue by comparison of the shifts of disaccharide (5) and trisaccharide (12). In both cases the shift of C-1′ is 103.5 p.p.m.

The origin of the high-shift value derives in part from conformational features concerning the *exo*-anomeric

effect.[25] Lemieux and Koto [26] have shown that conformations approaching the eclipsed form about the aglyconic oxygen to carbon bond (torsional angle ψ) satisfy the requirement of minimised non-bonded interactions, when the ϕ angle is fixed at *ca.* 60°. The shifts of the C-1″ and C-1′ resonances are consistent with eclipsed conformers [26] and we have previously reported [2] a similar C-1 shift for an α-L-rhamnopyranoside, which on ^{1}H n.m.r. evidence alone must closely approach the eclipsed conformation. Analysis of accumulated *X*-ray data for oligosaccharides supports the existence of such a conformational bias in the solid state,[27] and furthermore, recent molecular orbital calculations [28] provide a theoretical basis for the *exo*-anomeric effect. For these reasons we propose that the solution conformations of the oligosaccharides (5) and (12) are dictated by the *exo*-anomeric effect.[25] Proton data in support of this were obtained for the blocked trisaccharide (10). The proposed conformation would dispose the C–Me of the terminal rhamnose unit towards the first rhamnose residue, which for compound (10) carried a benzoate at C-4. The shielding of H-6″ which results from such a juxtaposition is demonstrated by the upfield shift of this resonance from the usual 1.20 to 0.88 p.p.m.

Longitudinal relaxation times, T_1, could also be expected to provide information concerning these anomeric conformers, since the van der Waals radii of the anomeric and the aglyconic bound protons are in virtual contact in such situations. Proton T_1 values are inversely proportional to the inverse sixth power of inter-proton distances (for a discussion and more detailed bibliography see references 29 and 30). Hall and his coworkers,[29,30] have shown that proton T_1 values of anomeric protons receive a substantial contribution to their relaxation process from protons bound to the adjacent sugar ring. For β-glycosides, *syn*-axial contributions from H-5 and H-3 protons occur, but for α-glycosides with an equatorial anomeric proton the contribution to relaxation by other ring protons is minimised. Thus, for α-L-rhamnopyranosides in which the eclipsed conformer is adopted, T_1 values would be predominantly determined by the interaction between the anomeric proton and that proton bound to the aglyconic carbon. Space-filling molecular models for the trisaccharide (12) show the H-1′ proton to be close to the H-3 proton in the eclipsed conformer, but in addition, when the terminal rhamnose unit is also in a similar eclipsed arrangement with H-2′, then H-5″ is spacially very close to H-1′. The T_1 values for H-1″ and H-1′ are respectively 1.15 and 0.64 s, which qualitatively, at least, support the envisaged conformations about the anomeric bonds. Further studies to determine and correlate T_1 values with conformations (of *S. flexneri* antigens) about the anomeric linkage are in progress.

EXPERIMENTAL

Thin-layer chromatography was performed with Merck precoated silica gel 60F-254 plates, and compounds were detected by spraying with 5% sulphuric acid in ethanol and then heating. Column chromatography was performed on silica gel G60 (70—230 mesh) with redistilled solvents. The loading on all columns was 1 : 100. Separations were also performed on a Prep 500 (Water Associates) high-pressure liquid chromatograph. Skellysolve B refers to hexane supplied by Getty Refining and Marketing Company, Tulsa, Oklahoma. 10% Palladium–charcoal was purchased from Engelhard Industries, Newark, New Jersey. Solvents were purified and dried according to standard procedures.[31] Processed solutions were dried over anhydrous sodium sulphate and removal of solvents was achieved with bath temperatures of 40 °C or lower, unless otherwise stated. Melting points were determined on a Fisher–Johns apparatus. Optical rotations were measured at 589 nm in a 1-dm cell at room temperature (20—23 °C). Carbon-13 and ^{1}H n.m.r. spectra were recorded at 20 and 79.9 MHz, respectively, in the pulsed Fourier-transform mode on a Varian CFT-20 spectrometer. Proton chemical shifts are expressed relative to 1% tetramethylsilane (Me_4Si) for solutions in deuteriochloroform and [$^2H_4$]-methanol, and to external Me_4Si for solutions in deuterium oxide. Carbon-13 shifts are expressed relative to internal Me_4Si for solutions in deuteriochloroform and [$^2H_4$]-methanol, and to external Me_4Si for solutions in deuterium oxide. Carbon-13 assignments are tentative for all compounds except compound (2), for which selective irradiation was employed. Proton T_1 measurements were performed on degassed samples utilising the two-pulse inversion recovery method.[32]

8-Methoxycarbonyloctyl α-L-Rhamnopyranoside (1).—A solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide [33] (63.5 g, 0.18 mol) in dichloromethane (100 cm^3) was added to a stirred solution of 8-ethoxycarbonyloctanol (37.6 g, 0.18 mol) in dichloromethane (300 cm^3) containing mercuric cyanide (45.6 g, 0.18 mol) and Drierite (20 g). The mixture was stirred overnight and then filtered and extracted with water, saturated sodium hydrogencarbonate, and water. After drying and evaporation, the residue was dissolved in methanol (200 cm^3) containing a catalytic amount of sodium and left overnight. Sodium ions were removed with Rexyn 101 (H^+) resin. Evaporation gave a syrup which was chromatographed with ethyl acetate–methanol (17 : 3) on silica gel to give pure (1) (38.5 g, 61.5%), $[\alpha]_{589}$ −42.6° (*c* 1.1 in methanol), R_F 0.73 (ethyl acetate–methanol, 85 : 15), m.p. 49—51 °C (from ethanol); δ ($CDCl_3$) 1.10—1.90 (15 H, m, 6-H and $-[CH_2]_6-$), 2.28 (2 H, t, CH_2CO), 3.65 (3 H, s, OMe), and 4.71br (1 H, s, 1-H); δ_C ($CDCl_3$) 99.7 (C-1), 72.7 (C-4), 71.8 (C-2), 71.0 (C-3), 68.0 (C-5), 67.6 (OCH_2), and 17.4 (C-6) (Found: C, 57.5; H, 9.2. $C_{16}H_{30}O_7$ requires C, 57.45; H, 9.05%).

8-Methoxycarbonyloctyl 2,4-Di-O-benzoyl-α-L-rhamnopyranoside (2).—The rhamnoside (1) (7.0 g, 21.0 mmol), trimethyl orthobenzoate (4.55 g, 25.0 mmol), and toluene-*p*-sulphonic acid (50 mg) in dry dimethylformamide (DMF) (50 cm^3) were stirred at room temperature for 6 h and then the solution was evaporated under reduced pressure. The residue was dissolved in pyridine (30 cm^3), benzoyl chloride (3.5 g, 25 mmol) was added dropwise, and the solution was stirred overnight.

Dichloromethane (200 cm^3) was added and the mixture washed with water (100 cm^3), 1M-hydrochloric acid (50 cm^3), saturated aqueous sodium hydrogencarbonate (100 cm^3), and water. The dried solution was evaporated to a syrup that showed on t.l.c. two spots with R_F 0.45 (orthoester) and 0.36 [2,4-di-*O*-benzoate (2)]. (Skellysolve B-ethyl

acetate 2 : 1). The syrup was then dissolved in acetic acid (50 cm³) and the solution stirred for 30 min. Evaporation again gave a syrup showing only one spot on t.l.c., R_F 0.36. H.p.l.c. using Skellysolve B–ethyl acetate (2 : 1) gave the pure 2,4-*di*-O-*benzoate* (2) (8.8 g, 77%) which crystallised on standing, $[\alpha]_{589}$ 26.2° (*c* 1.3 in $CHCl_3$); R_F 0.36 (solvent as above); δ ($CDCl_3$) 1.10—1.70 (15 H, m, 6-H and $-[CH_2]_6-$), 2.32 (2 H, t, CH_2CO), 3.65 (3 H, s, OMe), 4.30 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.7 Hz, 3-H), 4.95 (1 H, d, $J_{1,2}$ 1.5 Hz, 1-H), 5.27 (1 H, t, $J_{3,4}$ 9.7 Hz, 4-H), 5.38 (1 H, dd, $J_{1,2}$ 1.7, $J_{2,3}$ 3.5 Hz, 2-H), 7.30—7.65 (6 H, m, ArH), and 8.00—8.20 (4 H, m, ArH); δ_C ($CDCl_3$) 97.4 (C-1), 75.6 (C-2), 73.5 (C-4), 68.9 (C-3), 68.3 (C-5), 66.3 (OCH_2), and 17.6 (C-6) (Found: C, 66.6; H, 7.0. $C_{30}H_{38}O_9$ requires C, 66.4; H, 7.05%).

8-*Methoxycarbonyloctyl* 3-O-(2′,3′,4′-*Tri*-O-*acetyl*-α-L-*rhamnopyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (3).—The partially protected glycoside (2) (2.3 g, 4.1 mmol) was dissolved in dichloromethane (30 cm³) containing silver trifluoromethanesulphonate (silver triflate) (1.8 g, 6.8 mmol) and tetramethylurea (3.0 cm³, 25 mmol). The solution was cooled to −70 °C and 2,3,4-tri-*O*-rhamnopyranosyl bromide [33] (2.2 g, 6.2 mmol), dissolved in dichloromethane (15 cm³), was added dropwise with stirring. The reaction was allowed to warm to room temperature overnight and then filtered. Following washing with saturated aqueous sodium hydrogencarbonate (25 cm³) and water, the concentrated syrup was purified by h.p.l.c. with Skellysolve B–ethyl acetate (2 : 1) as solvent. The pure *disaccharide* (3) (2.3 g) was obtained in 68% yield, $[\alpha]_{589}$ 24.1° (*c* 1.3 in $CHCl_3$), R_F 0.25 (Skellysolve B–ethyl acetate, 2 : 1); δ ($CDCl_3$) 1.01 (3 H, d, $J_{5,6}$ 6.3 Hz, 6-H), 1.10—2.00 (15 H, m, 6-H and $-[CH_2]_6-$), 1.80 (3 H, s, MeCO), 1.86 (6 H, s, MeCO), 2.27 (2 H, t, $-CH_2CO-$), 3.63 (3 H, s, OMe), 7.10—8.10 (6 H, m, ArH), 8.25—8.70 (4 H, m, ArH), and 3.20—5.60 (m, ring protons); δ_C ($CDCl_3$) 99.1 (C-1′), 97.2 (C-1), 75.7 (C-3), 73.3 (C-2), 72.3 (C-4), 70.9 (C-4′), 69.7 (C-3′), 68.4 (C-2′), 68.2 (C-5), 67.1 (C-5′), 66.6 (OCH_2), 17.6 (C-6), and 17.1 (C-6′).

8-*Methoxycarbonyloctyl* 3-*O*-(α-L-*Rhamnopyranosyl*)-α-L-*rhamnopyranoside* (4).—Compound (3) (0.83 g, 1.0 mmol) in methanol (50 cm³) containing a catalytic amount of sodium was left for 48 h at room temperature. The syrup, obtained after removal of sodium ions with Rexyn 101 (H^+) resin, filtration, and evaporation, was purified on a silica gel column with ethyl acetate–methanol–water (7 : 2 : 1) as eluant. The *disaccharide* (4) (0.35 g, 70%) had $[\alpha]_{589}$ −74.8° (*c* 1.0 in methanol), R_F 0.72 (solvent 7 : 2 : 1 as above); δ (D_2O, 85 °C) 1.16—1.64 (18 H, m, 6-H, 6′-H, and $-[CH_2]_6-$), 2.35 (2 H, t, $-CH_2CO-$), 3.68 (3 H, s, OMe), 4.73 (1 H, d, $J_{1,2}$ 1.6 Hz, 1-H), 5.05 (1 H, d, $J_{1,2}$ 1.6 Hz, 1′-H), and 3.35—4.15 (remaining protons); δ_C (CD_3OD) 103.8 (C-1′), 101.4 (C-1), 79.5 (C-3), 73.9 (C-4), 73.1 (C-4′), 72.0 (3 C, C-2, C-2′, and C-3′), 69.9 (2 C, C-5 and C-5′), 68.5 (OCH_3), and 17.9 (2 C, C-6 and C-6′) (Found: C, 54.65; H, 8.35. $C_{22}H_{40}O_{11}$ requires C, 55.0; H, 8.4%).

8-*Hydrazinocarbonyloctyl* 3-O-(α-L-*Rhamnopyranosyl*)-α-L-*rhamnopyranoside* (5).—The deblocked disaccharide (4) (200 mg, 0.4 mmol) was dissolved in ethanol (5 cm³) to which an 85% solution of hydrazine hydrate (0.5 g) was added. The solution was stirred for 48 h, then evaporated and dried under high vacuum. Chromatography on silica gel with ethyl acetate–methanol–water (6 : 3 : 1) as eluant gave the pure *hydrazide* (5) (180 mg, 90%), $[\alpha]_{589}$ −63.5° (*c* 1.1 in water); R_F 0.57 (solvent as above); δ (D_2O, 85 °C) 1.05—1.80 (18 H, m, 6-H, 6′-H and $-[CH_2]_6-$), 2.20 (2 H, t, $-CH_2CO$), 4.73 (1 H, d, $J_{1,2}$ 1.3 Hz, 1-H), 5.04 (1 H, d, $J_{1,2}$ 1.4 Hz, 1′-H), and 3.30—4.10 (ring protons); δ_C (D_2O) 103.5 (C-1′), 101.0 (C-1), 79.2 (C-3), 73.2 (C-4), 72.6 (C-4′), 71.4 (3 C, C-2, C-2′, and C-3′), 70.1 (2 C, C-5 and C-5′), 69.0 (OCH_2), and 18.1 (2 C, C-6 and C-6′) (Found: C, 52.35; H, 8.55; N, 5.95. $C_{21}H_{40}N_2O_{10}$ requires C, 52.5; H, 8.4; N, 5.85%).

2-O-*Acetyl*-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranosyl Chloride* (7).—Trimethylchlorisilane (3 cm³) was added to the ortho-ester (6) (2.0 g, 5 mmol) in dichloromethane (40 cm³) and the solution refluxed for 2 h. Evaporation and drying under vacuum left (7) as a slightly yellow syrup (2.0 g), purity >95% (^{13}C and ^1H n.m.r.). Due to the lability of this compound no attempts were made to crystallise it; δ ($CDCl_3$) 1.35 (3 H, d, $J_{5,6}$ 6.2 Hz, 6-H), 2.15 (3 H, s, MeCO), 3.48 (1 H, t, $J_{3,4}$ 9.5 Hz, 4-H), 3.78—4.32 (2 H, m, 3- and 5-H), 4.57 and 4.65 (AB, J_{AB} 10.6 Hz, CH_2Ph), 4.62 and 4.88 (AB, J_{AB} 10.2 Hz, CH_2Ph), 5.46 (1 H, dd, $J_{1,2}$ 1.7 $J_{2,3}$ 2.8 Hz, 2-H), 5.96 (1 H, d, $J_{1,2}$ 1.7 Hz, 1-H), and 7.20—7.60 (10 H, m, ArH); δ_C ($CDCl_3$) 90.3 (C-1), 79.4 and 76.6 (2 C, CH_2Ph), 75.5 (C-4), 72.1 (C-3), 71.4 (C-2), 70.9 (C-5), and 17.7 (C-6).

8-*Methoxycarbonyloctyl* 3-O-(2′-O-*Acetyl*-3′,4′-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (8).—The glycoside (2) (2.5 g, 4.6 mmol) was dissolved in dichloromethane (40 cm³) containing silver triflate (1.7 g, 6.6 mmol) and tetramethylurea (3.0 cm³, 25 mmol). The solution was cooled to −70 °C and the rhamnosyl chloride (7) (2.5 g, 6.2 mmol), dissolved in dichloromethane (15 cm³), was added dropwise with stirring. The reaction was allowed to warm to room temperature overnight and was then filtered. Following extraction of the filtrate with saturated aqueous sodium hydrogencarbonate and water, the concentrated syrup was purified by h.p.l.c. on silica gel columns with Skellysolve B–ethyl acetate (3 : 1) as solvent. The pure *disaccharide* (8) (3.6 g, 87%) had $[\alpha]_{589}$ 37.2° (*c* 1.1 in $CHCl_3$); R_F 0.32 (solvent as above); δ ($CDCl_3$) 1.06 (3 H, d, $J_{5,6}$ 6.2 Hz, 6-H′), 1.10—1.85 (15 H, m, 6-H and $-[CH_2]_6-$), 1.91 (3 H, s, MeCO), 2.29 (2 H, t, $-CH_2CO$), 3.66 (3 H, s, OMe), 6.90—7.75 (16 H, m, ArH), 8.00—8.25 (4 H, m, ArH), and 3.50—5.65 (ring protons); δ_C ($CDCl_3$) 99.4 (C-1′), 97.3 (C-1), 79.5 and 77.4 (2 C, CH_2Ph), 76.2 (C-3), 74.3 (C-4′), 73.4 (C-2), 72.3 (C-4), 71.3 (C-3′), 68.9 (C-2′), 68.7 (C-5′), 68.3 (C-5), 66.6 (OCH_2), and 17.7 (2 C, C-6 and C-6′) (Found: C, 68.45; H, 6.95. $C_{52}H_{62}O_{14}$ requires C, 68.55; H, 6.85%).

8-*Methoxycarbonyloctyl* 3-O-(3′-4′-*Di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (9).—Compound (8) (3.0 g, 3.3 mmol) in methanol (30 cm³) was cooled to 0 °C and a freshly prepared solution of magnesium methoxide in methanol (10 cm³ of a 1% solution) was added. The reaction mixture was then stirred for 19 h at 0 °C. After removal of magnesium ions with Rexyn 101 (H^+) resin, filtration, and evaporation, the residual syrup was purified by h.p.l.c. on silica gel columns with Skellysolve B–ethyl acetate (3 : 1) as solvent. The selectively deblocked *disaccharide* (9) (1.9 g, 67%) (pure by t.l.c. and n.m.r.) had $[\alpha]_{589}$ 43.1° (*c* in $CHCl_3$); R_F 0.20 (solvent as above); δ ($CDCl_3$) 1.13 (3 H, d, $J_{5,6}$ 6.0 Hz, 6′-H), 1.00—1.90 (15 H, m, 6-H and $-[CH_2]_6-$), 2.28 (2 H, t, CH_2CO), 3.66 (3 H, s, OMe), 7.10—7.75 (16 H, m, ArH), 7.95—8.28 (4 H, m, ArH), and 3.00—5.65 (ring protons); δ_C ($CDCl_3$) 101.1 (C-1′), 97.3 (C-1), 79.4 (2 C, CH_2Ph), 75.6 (C-3), 74.3 (C-4′), 73.6 (C-2), 72.5 (C-4), 71.7 (C-3′), 68.5 (C-2′), 68.3 (2 C, C-5 and C-5′), 66.6 (OCH_2), and 17.7 (2 C, C-6 and

C-6′) (Found: C, 68.75; H, 7.0. $C_{50}H_{60}O_{13}$ requires C, 69.1; H, 6.95%).

8-*Methoxycarbonyloctyl* 3-O-[2′-O-(2″,3″,4″-*Tri*-O-*acetyl*-α-L-*rhamnopyranosyl*)-3′,4′-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*]-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (10).—The partially protected glycoside (9) (870 mg, 1.0 mmol) was dissolved in dichloromethane (30 cm³) containing silver triflate (540 mg, 2.1 mmol) and tetramethylurea (1.0 cm³, 8 mmol). The solution was cooled to −70 °C and 2,3,4-tri-*O*-rhamnopyranosyl bromide [33] (750 mg, 2.1 mmol), dissolved in dichloromethane (10 cm³), was added dropwise with stirring. The reaction was allowed to warm to room temperature overnight and then filtered. Following extraction with saturated aqueous sodium hydrogencarbonate (20 cm³) and water, the concentrated syrup was chromatographed on silica gel with Skellysolve B–ethyl acetate (2 : 1) as eluant to give the pure *trisaccharide* (10) (680 mg, 60%) $[\alpha]_{589}$ 16.1° (*c* 1.0 in $CHCl_3$); R_F 0.29 (solvent as above); δ ($CDCl_3$) 0.88 (3 H, d, $J_{5,6}$ 6.1 Hz, 6-H″), 1.13 (3 H, d, $J_{5,6}$ 5.7 Hz, 6′-H), 1.21 (3 H, d, $J_{5,6}$ 5.4 Hz, 6-H), 0.85—1.90 (12 H, m, $-[CH_2]_6-$), 1.93 (3 H, s, MeCO), 2.00 (3 H, s, MeCO), 2.01 (3 H, s, MeCO), 2.28 (2 H, t, $-CH_2CO$), 3.64 (3 H, s, OMe), 6.75—7.65 (16 H, m, ArH), 7.85—8.20 (4 H, m, ArH), and 3.15—5.65 (ring protons); δ_C ($CDCl_3$) 100.5 (C-1′), 98.9 (C-1″), 97.3 (C-1), 79.4 and 78.6 (2 C, CH_2Ph), 77.0 (C-2′), 75.8 (C-3), 75.2 (C-4′), 74.5 (C-3′), 73.7 (C-2), 72.4 (C-4), 72.1 (C-4″), 71.0 (C-3″), 69.5 (C-2″), 69.2 (C-5′), 69.0 (C-5″), 68.3 (C-5), 66.6 (OCH_2), 17.6 (C-6), 17.4 (C-6′), and 17.2 (C-6″) (Found: C, 65.0; H, 6.65. $C_{62}H_{76}O_{20}$ requires C, 65.25; H, 6.7%).

8-*Methoxycarbonyloctyl* 3-O-[2′-O-(α-L-*Rhamnopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranoside* (11).—Compound (10) (250 mg, 0.31 mmol) was dissolved in acetic acid (40 cm³) and hydrogenated with 10% palladium–charcoal (0.3 g) at 505 KPa for 2 h. Filtration and co-evaporation with toluene (3 × 50 cm³) gave a syrup that gave only one spot on t.l.c. [R_F = 0.60 (Skellysolve B–ethyl acetate, 1 : 5)]. The syrup was dissolved in methanol (50 cm³) containing a catalytic amount of sodium and the solution was left for 48 h at room temperature. The syrup, obtained after removal of sodium ions with Rexyn 101 (H^+) resin, filtration, and evaporation, was chromatographed on silica gel with ethyl acetate–methanol–water (7 : 2 : 1) as eluant to give the pure *trisaccharide* (11) (150 mg, 77%), $[\alpha]_{589}$ −77.9° (*c* 1.0 in methanol); R_F 0.48 (solvent as above); δ (D_2O, 85 °C) 1.10—1.80 (21 H, m, 6-H, 6′-H, 6″-H, and $-[CH_2]_6-$), 2.36 (2 H, t, $-CH_2CO$), 3.70 (3 H, s, OMe), 4.74 (1 H, d, $J_{1,2}$ 1.2 Hz, 1-H), 4.98 (1 H, d, $J_{1,2}$ 1.7 Hz, 1″-H), 5.19 (1 H, d, $J_{1,2}$ 0.8 Hz, 1′-H), and 3.30—4.20 (remaining protons); δ_C (CD_3OD) 103.8 (C-1′), 102.4 (C-1″), 101.5 (C-1), 79.9 (C-2′), 79.4 (C-3), 74.4 (C-4), 74.0 (C-4′), 73.3 (C-4″), 72.0 (4 C, C-2, C-2″, C-3′, and C-3″), 70.1 (3 C, C-5, C-5′, and C-5″), 68.5 (OCH_2), and 17.9 (3 C, C-6, C-6″, and C-6″) (Found: C, 53.5; H, 8.25. $C_{28}H_{50}O_{15}$ requires C, 53.65; H, 8.05%)

8-*Hydrazinocarbonyloctyl* 3-O-[2′-O-(α-L-*Rhamnopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranoside* (12).—The deblocked trisaccharide (11) (90 mg, 0.14 mmol) was dissolved in ethanol (5 cm³) to which an 85% solution of hydrazine hydrate (0.5 g) was added. The solution was stirred for 48 h, then evaporated and dried under high vacuum. Chromatography on silica gel with ethyl acetate–methanol–water (6 : 3 : 1) as eluant gave the pure *hydrazide* (12) (70 mg, 77% yield), $[\alpha]_{589}$ −67.7° (*c* 0.8 in water); R_F 0.40 (solvent as above); δ (D_2O, 85 °C) 1.10—1.80 (21 H, m, 6-H, 6′-H, 6″-H, and $-[CH_2]_2-$), 2.21 (2 H, t, $-CH_2CO$), 4.76 (1 H, d, $J_{1,2}$ 1.7 Hz, 1-H), 4.98 (1 H, d, $J_{1,2}$ 1.7 Hz, 1″-H), 5.18 (1 H, d, $J_{1,2}$ 0.7 Hz, 1′-H), and 3.30—4.15 (ring protons); δ_C (D_2O) 103.5 ($^1J_{^{13}C_1,^1H_1}$ 174.2 Hz, C-1′), 102.0 ($^1J_{^{13}C_1,^1H_1}$ 175.3 Hz, C-1″), 101.0 ($^1J_{^{13}C_1,^1H_1}$ 171.8 Hz, C-1), 79.4 (C-2′), 78.7 (C-3), 73.3 (2 C, C-4, and C-4′), 73.0 (C-4), 71.3 (4 C, C-2, C-2″, C-3′, and C-3″), 70.4 (2 C, C-5, and C-5″), 70.0 (C-5′), 69.2 (OCH_2), 17.8 (3 C, C-6, C-6′, and C-6″) (Found: C, 51.8; H, 8.15; N, 4.4. $C_{27}H_{50}N_2O_{14}$ requires C, 51.75; H, 8.05; N, 4.45%).

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