Synthesis of Streptococcal Groups A, C and Variant-A Antigenic Determinants

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The antigenic determinants of the cell wall polysaccharides belonging to the β-haemolytic *Streptococci* Groups A, A-variant, and C have been synthesized as the glycosides of 8-methoxycarbonyloctanol. In this form they may be used to generate artificial antigens and immunoabsorbents. The terminal disaccharide, 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside, of the Group A polysaccharide was synthesized by a Königs–Knorr reaction between 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (1) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (2) which gave the antigenic determinant (6) after removal of the blocking groups. Similarly, addition of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride (3) to (1) gave a disaccharide (7). The suitably blocked benzylidene acetal (11) was treated with the glactopyranosyl chloride (3) to yield the trisaccharide (12). The deblocked trisaccharide, *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-α-L-rhamnopyranoside (14), is similar to the determinant of the Group C streptococcal cell wall and is also related to the Forssman antigen. The previously synthesized disaccharide glycoside, 8-methoxycarbonyloctyl 3,4-di-*O*-benzyl-2*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (15) was subjected to sequential chain-extension reactions with 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride (4) to give the trisaccharide (16) and from this the slectively blocked precursor (17) from which the tetrasaccharide (18) was formed by reaction with (4). The tetrasaccharide glycoside *O*-(α-L-rhamnopyranosyl)-(1→2)-*O*-(α-L-rhamnopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→2)-α-L-rhamnopyranoside (19) mimics the core structure of Groups A and C streptococcal polysaccharides and is identical to the sequence of the variant-A cell-wall polysaccharide.

BACTERIAL polysaccharides confer immunological protection [1,2] and serve as markers for the detection of bacterial infection.[3,4] With respect to the latter, the synthesis of disaccharide determinants of *Salmonella O*-antigens has provided artificial antigens which improve the methodology for serogrouping these organisms.[5,6] The antibody raised to these antigens is more specific for the immunodominant 3,6-dideoxyhexose-containing determinants than those elicited by whole cell vaccines. The synthesis of antigenic determinants,[7-10] therefore, constitutes an area of growing interest and application. Although advances in glycoside syntheses provide a repertoire of methods for efficient and controlled syntheses of oligosaccharides, relatively few bacterial determinants excluding disaccharides of *Salmonella* [11-14] have been synthesized in a manner which permits covalent attachment to proteins. Several tri-,[7] tetra-,[8,9] and penta-saccharides [10] have been synthesized but none incorporate a ' bridging arm.'

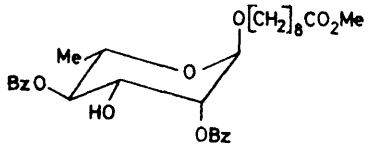
During the synthesis of oligosaccharides [15-17] related to *Shigella flexneri O*-antigens, selectively blocked oligosaccharide units were employed which serve as convenient precursors to various streptococcal determinants. These determinants [18,19] are of importance since whole cell vaccines of the β-haemolytic *Streptococci* were the first to be used to induce a rabbit antibody displaying restricted heterogeneity.[20] In particular, recent work has revealed the occurrence of both high- and low-affinity antibodies with requirements for A and variant-A determinants.[21] Furthermore, Group C determinants also possess potential as tumour related antigens.[22-24]

We now report the synthesis of the terminal disaccharide and trisaccharide determinants of Groups A and C *Streptococci* and also the tetrasaccharide sequence thought to constitute the variant-A polysaccharide. The syntheses described for the three structures provide compounds capable of being converted into artificial antigens after covalent linkage to protein.[25]

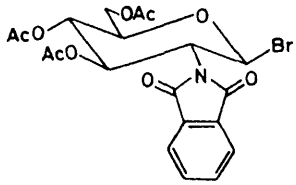
RESULTS AND DISCUSSION

In previous syntheses [15,16] of the repeating unit of *Shigella flexneri*, serogroup Y lipopolysaccharide, we used a 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside derivative (1) which provides a route to 3-*O*-substituted rhamnopyranosides. Reaction of this derivative with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide [26] (2) under silver trifluoromethanesulphonate promotion in the presence of 2,4,6-trimethylpyridine (collidine) gave the fully blocked disaccharide (5) in 90% yield, when the reaction was conducted at room temperature in the presence of molecular sieves. In the absence of molecular sieves the yield of (5) was reduced to 60% and purification was complicated by the presence of a component, thought to be a ' trehalose ' type disaccharide, with chromatographic mobility very close to that of (5). This impurity arises from hydrolysis of the glycosyl bromide (2) and reaction of the hydrolysis product, 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranose, with (2). The resolution of (5) from this disaccharide impurity required the use of preparative h.p.l.c. as previously reported.[27] Transesterification followed by hydrazinolysis of the phthalimido-function gave the deblocked disaccharide which was *N*-acetylated in methanol to yield the amide (6). The hydrazinolysis leaves intact [28] the ester function of the bridging arm which is essential for efficient synthesis of artificial antigens.[25]

The Streptococcal Group C determinant, which resembles the Forssman antigen in its terminal disac-



(1)



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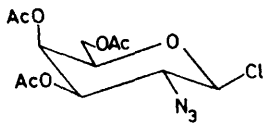

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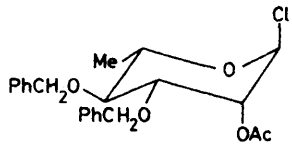

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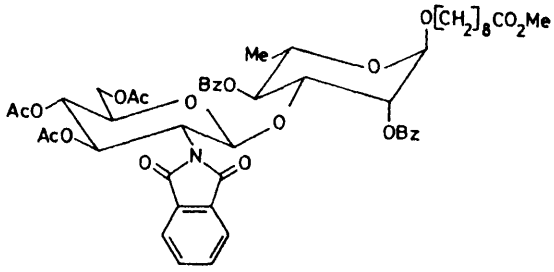
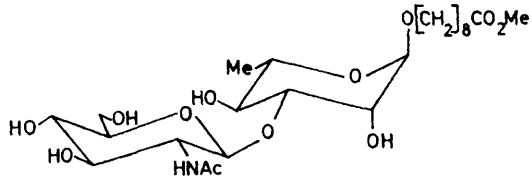
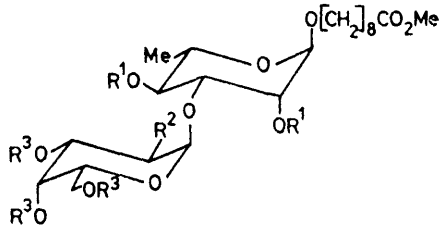


(5)

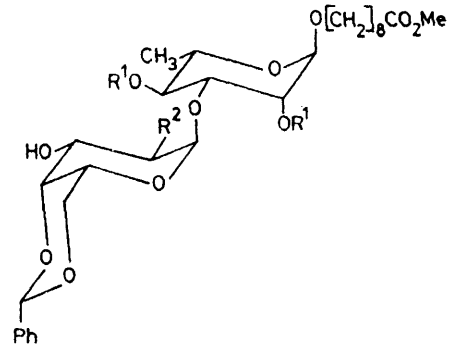


(6)

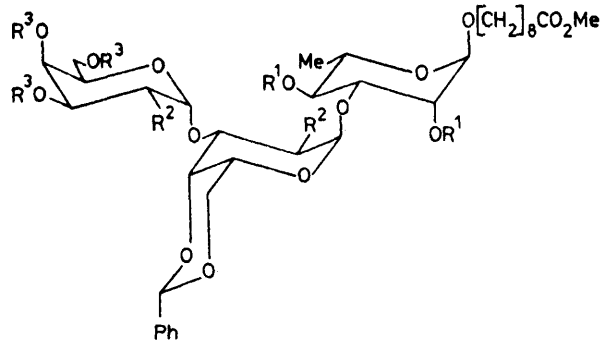
charide, was synthesized as a trisaccharide. The 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (1) was glycosylated by 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride (3), prepared *via* azidonitration of tri-*O*-acetyl-D-galactal.[29] Under silver salt activation, 2-azido-2-deoxyglycosyl halides [30] yield the appropriate α-glycosides and accordingly reaction of (1) with (3) provided the disaccharide (7). Hydrogenation, *N*-acetylation, and de-*O*-acetylation by a solution of sodium methoxide gave the disaccharide (9) by way of (8). Magnesium methoxide solution by comparison de-*O*-acetylates compound (7) to give 8-methoxycarbonyloctyl 2,4,-di-*O*-benzoyl-3-*O*-(2-azido-2-deoxy-α-D-galactopyranosyl)-α-L-rhamnopyranoside (10). The ability to retain the benzoate groups [15,16] thus permits the preparation of the selectively blocked disaccharide (11) *via* acid-catalysed



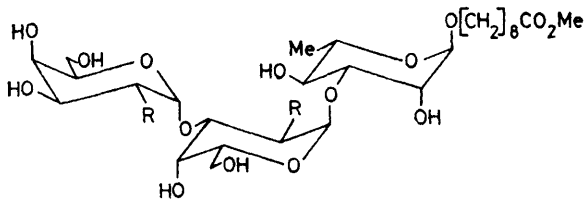
(7) R^1 = Bz, R^2 = N_3, R^3 = Ac
(8) R^1 = Bz, R^2 = NHAc, R^3 = Ac
(9) R^1 = H, R^2 = NHAc, R^3 = H
(10) R^1 = Bz, R^2 = N_3, R^3 = H



(11) R^1 = Bz, R^2 = N_3

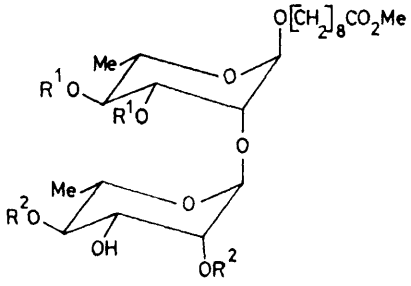


(12) R^1 = Bz, R^2 = N_3, R^3 = Ac

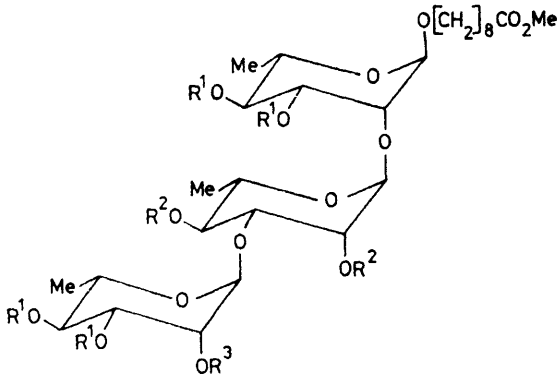


(13) R = N_3
(14) R = NHAc

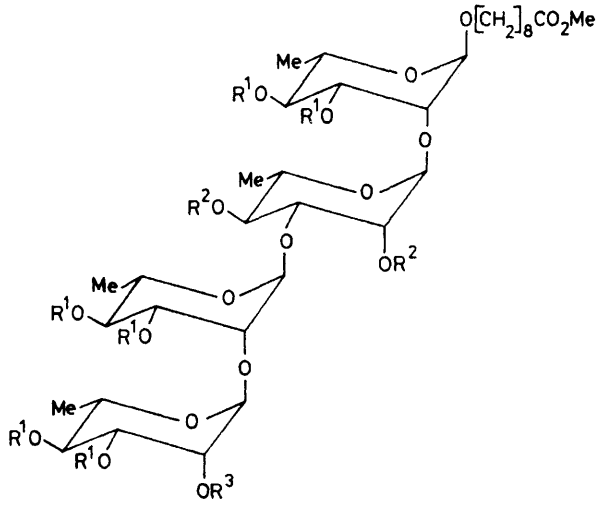
acetal exchange using dimethoxytoluene in acetonitrile. Glycosylation of (11) by (3) in the manner described above gave the target trisaccharide as its fully protected derivative (12). Removal of the benzylidene acetal was accomplished by hydrolysis with trifluoroacetic acid, followed by de-*O*-acetylation. Hydrogenation of (13) followed by acetylation gave the trisaccharide (14). The acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride (4) using silver trifluoromethanesulphonate and *NN*-tetramethylurea [32] gave the trisaccharide (16) in 81% yield. Selective transesterification of the single *O*-acetyl group was achieved with magnesium methoxide solution [15-17] and yielded the selectively blocked trisaccharide (17). Employing identical silver salt activation [32] the rhamno-



(15) $R^1 = CH_2Ph$, $R^2 = Bz$



(16) $R^1 = CH_2Ph$, $R^2 = Bz$, $R^3 = Ac$
(17) $R^1 = CH_2Ph$, $R^2 = Bz$, $R^3 = H$



(18) $R^1 = CH_2Ph$, $R^2 = Bz$, $R^3 = Ac$
(19) $R^1 = R^2 = R^3 = H$

synthesis of Forssman structures has been reported [30,31] but not in a form suitable for artificial antigen preparation.

The core structure upon which the Groups A and C are synthesized is believed [19] to be a rhamnose backbone of alternating α-1,2- and α-1,3-linkages. The synthesis of a tetrasaccharide sequence representing a determinant of significant size was envisaged using 8-methoxycarbonyloctyl 3,4-di-*O*-benzyl-2-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranoside as the starting material. This compound was available from other work [28] and selective benzoylation as previously described [15] gave the required disaccharide (15). Reaction of this compound with 2-*O*-pyranosyl chloride (4) reacts with (17) to give the protected tetrasaccharide (18) in 85% yield. The tetrasaccharide (18) was deblocked by hydrogenolysis with palladium–acetic acid followed by transesterification which yielded the analytically pure tetrasaccharide (19).

The chemical synthesis of the Streptococcal Group C terminal trisaccharide is of particular interest since the unambiguous determination of structure has yet to be published in full [18,19] and because of the cross-serological reactivity [22,23] of this bacterial antigen and the important mammalian Forssman antigen.[24] The ^{13}C n.m.r. data for the trisaccharide (14) and the disaccharide (9) should prove valuable for comparison with similar data for the

Group C polysaccharide. It is as yet uncertain whether the configuration of the 2-acetamido-2-deoxy-D-galactopyranosyl residue linked to L-rhamnose is either α or β. In separate work [33] we have also synthesized the β-linked analogue of (9). The potential of the Group C *Streptococcus* as an immunotherapeutic agent has been mentioned [19] in view of the implication of the Forssman antigen (with which the Group C polysaccharide is serologically cross-reactive) as a tumour antigen.[24] In this respect the availability of chemically defined antigens and immunoabsorbents which result from the synthetic approach described here is of considerable biological potential. Rapid identification of antigen-specific hybridoma clones produced by somatic cell fusion of mouse myeloma with spleen cells [34] facilitates the production of monoclonal antibody specific for defined antigenic determinants. The use of antigens prepared from the A, A-variant and C-haptenic structure synthesized here for the simplification of such screening procedures enhances the ability to generate and immortalize antibodies of precise specificity.

EXPERIMENTAL

The materials and general methods employed are similar to those described in earlier work.[15-17] ^{1}H N.m.r. shifts are expressed relative to 1% tetramethylsilane for solutions in deuteriochloroform and [$^2H_4$]methanol, and to sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate for solutions in deuterium oxide. Carbon-13 shifts are referenced to internal tetramethylsilane for solutions in deuteriochloroform and [$^2H_4$]-methanol, and to external tetramethylsilane for solutions in deuterium oxide.

8-*Methoxycarbonyloctyl* 3-O-(3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (5).—The rhamnopyranoside (1) (0.9 g, 1.7 mmol) was dissolved in dichloromethane (20 cm^3) together with collidine (0.35 g, 2.9 mmol), 4A molecular sieve (2 g), and silver triflate (0.7 g, 2.7 mmol). Addition of the glycosyl bromide (2) (1.2 g, 2.4 mmol) at room temperature followed by stirring for 30 min gave, after column chromatography with Skellysolve B–ethyl acetate (1 : 1 v/v) as solvent, pure (5) (1.3 g, 90%), $[\alpha]_D$ 27.0° (*c*, 1.0 in chloroform); R_F 0.28 (solvent as above); δ ($CDCl_3$) 1.11 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6), 1.22—1.70 (12 H, m, $[CH_2]_6$), 1.71 (3 H, s, MeCO), 1.91 (3 H, s, MeCO), 1.96 (3 H, s, MeCO), 2.30 (2 H, t, CH_2CO), 3.67 (3 H, s, OMe), 4.92 (1 H, d, $J_{1,2}$ 1.6 Hz, H-1), 5.00 (1 H, t, H-4′), 5.35 (3 H, t, H-4), 5.40 (1 H, dd, H-2), 5.51 (1 H, d, H-1′), 5.63 (1 H, t, H-3′), 7.00—8.20 (14 H, m, ArH), and 3.25—4.40 (ring protons); δ_C ($CDCl_3$) 98.5 (C-1′), 97.1 (C-1), 76.6 (C-3), 72.5 (C-2), 72.2 (C-5′), 71.4 (C-3′), 70.5 (C-4), 68.6 (C-4′), 68.2 (C-5), 66.0 (OCH_2), 61.8 (C-6′), 54.5 (C-2′), and 17.4 (C-6) (Found: C, 62.45; H, 6.05; N, 1.6. $C_{50}H_{57}NO_{18}$ requires C, 62.56; H, 5.99; N, 1.46%).

8-*Methoxycarbonyloctyl* 3-O-(2-*Acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (6).—The fully protected disaccharide (5) (400 mg, 4.2 mmol) was dissolved in methanol (50 cm^3) containing a catalytic amount of sodium *methoxide*. The reaction was stirred for 3 days at room temperature, sodium ions were removed with Rexyn 101 (H^+) resin, and filtration followed by evaporation gave a syrupy residue, that was washed with Skellysolve B–toluene (1 : 1 v/v; 10 cm^3) to remove methyl benzoate. The syrup was then dissolved in ethanol (25 cm^3) containing hydrazine hydrate (0.20 g, of an 85% solution, 34 mmol) and refluxed for 3 h. The solution was evaporated and thoroughly dried to remove traces of hydrazine. The resulting product was dissolved in methanol (25 cm^3), and acetic anhydride (2.5 cm^3) was added. The solution was then stirred at room temperature overnight. Concentration followed by chromatography on silica gel with ethyl acetate–methanol–water (85 : 10 : 5 v/v) gave the pure *amide* (6) (180 mg, 80%), $[\alpha]_D$ −35.1 (*c*, 1.1 in water); R_F 0.27 (solvent as above); δ (D_2O) 1.10—1.80 (15 H, m, $[CH_2]_6$ and H-6), 1.97 (3 H, s, *Me*CONH), 2.31 (2 H, t, CH_2CO), 3.63 (3 H, s, OMe), 4.65 (1 H, d, $J_{1,2}$ 8.2 Hz H-1′), and 4.69 (1H, d, $J_{1,2}$ 1.5 Hz, H-1); δ_C (D_2O) 103.9, (C-1′), 100.4 (C-1), 81.3 (C-3), 76.9 (C-5′), 74.8 (C-3′), 72.0 (C-4), 71.2 (C-2), 70.9 (C-5), 70.0 (C-4′), 69.1 (OCH_2), 61.8 (C-6′), 56.9 (C-2′), and 17.7 (C-6) (Found: C, 53.5; H, 8.1; N, 2.6. $C_{24}H_{43}NO_{12}$ requires C, 53.62; H, 8.06; N, 2.61%).

8-*Methoxycarbonyloctyl* 3-O-(3,4,6-*Tri*-O-*acetyl*-2-*azido*-2-*deoxy*-α-D-*galactopyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (7).—A solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride [29] (3) (2.5 g, 7.4 mmol) in dichloromethane (10 cm^3) was added dropwise to compound (1) (2.2 g, 4.0 mmol) in dichloromethane (40 cm^3) containing silver trifluoromethanesulphonate (0.18 g, 0.7 mmol), silver carbonate (9.1 g, 33.2 mmol), and 3A molecular sieves (5 g) under nitrogen. The reaction was stirred for 4 h and then filtered through Celite. Extraction of the reaction mixture with water followed by evaporation gave a syrup which was purified on a silica-gel column with Skellysolve B–ethyl acetate (2 : 1 v/v) as eluant to afford the *disaccharide* (7) (2.2 g, 64%) $[\alpha]_D$ 131.2° (*c*, 1.0 in $CHCl_3$); R_F 0.19 (solvent as above); δ ($CDCl_3$) 1.10—1.80 (15 H, m, $[CH_2]_6$ and H-6), 1.83 (3 H, s, MeCO), 1.87 (3 H, s, MeCO), 1.96 (3 H, s, MeCO), 2.25 (2 H, t, CH_2CO), 3.59 (3 H, s, OMe), and 7.10—8.30 (10 H, m, ArH); δ_C ($CDCl_3$) 97.6 (C-1), 94.4 (C-1′), 72.3 (2 C, C-3 and C-2), 68.3 (C-4), 68.1 (2 C, C-5 and C-5′), 66.9 (C-3′), 66.5 (C-4′), 66.4 (OCH_2), 60.9 (C-6′), 57.1 (C-2′), and 17.6 (C-6) (Found: C, 58.7; H, 6.1; N, 4.8. $C_{42}H_{53}N_3O_{16}$ requires C, 58.94; H, 6.24; N, 4.91%).

8-*Methoxycarbonyloctyl* 3-O-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-α-D-*galactopyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (8).—Compound (7) (0.5 g, 0.6 mmol) was dissolved in ethanol (30 cm^3) and hydrogenated for 3 h in the presence of Adams catalyst (PtO_2) (50 mg). The resulting solution was filtered, and the filtrate was evaporated and dissolved in methanol (10 cm^3) to which solution acetic anhydride (0.5 cm^3) was then added. This solution was stirred overnight, evaporated and chromatographed on silica gel with ethyl acetate–Skellysolve B (2 : 1 v/v) as eluant to give the pure *product* (8) (0.45 g, 86%), $[\alpha]_D$ 97.2° (*c*, 1.0 in $CHCl_3$); R_F 0.31 (solvent as above); δ ($CDCl_3$) 1.10—1.78 (m, $[CH_2]_6$ and H-6), 1.54 (3 H, s, MeCO), 1.88 (3 H, s, MeCO), 1.91 (3 H, s, MeCO), 2.03 (3 H, s, *Me*CONH), 2.29 (2 H, t, CH_2CO), 3.66 (s, OMe), 4.91 (1 H, d, $J_{1,2}$ 1.2 Hz, H-1), 5.05 (1 H, d, $J_{1',2'}$ 3.4 Hz, H-1′), 5.42 (1 H, dd, H-3), 5.42 (1 H, dd, H-2), 5.92 (1 H, d, $J_{NH,2'}$ 9.3 Hz, MeCONH), 4.32—4.62 (m, ring protons), and 7.28—8.24 (10 H, m, ArH); δ_C ($CDCl_3$) 97.5 (C-1), 96.5 (C-1′), 73.9 (C-3), 72.7 (C-2), 69.1 (C-4), 68.4 (C-5′), 67.8 (C-5), 66.7 (C-3′), 66.5 (C-4′), 60.7 (C-6′), 47.2 (C-2′), and 17.6 (C-6) (Found: C, 60.55; H, 6.7; N, 1.7. $C_{44}H_{57}NO_{17}$ requires C, 60.61; H, 6.59; N, 1.61%).

8-*Methoxycarbonyloctyl* 3-O-(2-*Acetamido*-2-*deoxy*-α-D-*galactopyranosyl*)-α-L-*rhamnopyranoside* (9).—Compound (8) (0.2 g, 0.23 mmol) was dissolved in methanol (50 cm^3) and

a catalytic amount of freshly prepared sodium methoxide solution was added. The reaction was left for 48 h at room temperature, sodium ions were removed with Rexyn 101 (H^+) resin and the solvent was then evaporated off. The syrup was chromatographed on silica gel with ethyl acetate–methanol–water (7 : 2 : 1 v/v) as eluant to give the pure *disaccharide* (9) (100 mg, 81%), $[\alpha]_D$ 77.3° (*c*, 1.0 in water); R_F 0.48 (solvent as above); δ (D_2O) 1.10—1.80 (15 H, m, $[CH_2]_6$ and H-6), 2.07 (3 H, s, MeCO), 2.40 (2 H, t, CH_2CO), 3.72 (3 H, s, OMe), 4.82 (1 H, d, $J_{1,2}$ 2.0 Hz, H-1), and 5.08 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1′); δ_C (D_2O) 100.8 (C-1), 95.6 (C-1′), 76.8 (C-3), 71.9 (C-4), 71.6 (C-2), 69.5 (2 C, C-5′ and C-3′), 68.9 (2 C, C-5 and C-4′), 68.1 (OCH_2), 62.0 (C-6′), 50.9 (C-2′), and 18.1 (C-6) (Found: C, 53.6; H, 8.0; N, 2.6. $C_{24}H_{43}NO_{12}$ requires C, 53.62; H, 8.06; N, 2.61%).

8-*Methoxycarbonyloctyl* 3-O-(2-*Azido*-2-*deoxy*-α-D-*galacto*-*pyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (10).—Compound (7) (2.8 g, 3.3 mmol) in methanol (90 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 left for 16 h at 0 °C. After removal of magnesium ions with Rexyn 101 (H^+) resin, filtration, and evaporation, the crude syrup was chromatographed on silica gel with chloroform–methanol (7 : 1 v/v) as solvent to give the pure *deacetylated product* (10) (2.1 g, 88%), $[\alpha]_D$ 127.7 (*c*, 1.0 in methanol); R_F 0.50 (solvent as above); δ (CD_3OD) 1.15—1.85 (15 H, m, $[CH_2]_6$, H-6), 2.31 (2 H, t, CH_2CO), 3.63 (3 H, s, OMe), 4.97br (1 H, s, H-1), 5.15 (1 H, d, $J_{1',2'}$ 3.1 Hz, H-1′), 5.51 (1 H, dd, $J_{4,5} \sim J_{3,4}$ 10.0 Hz, H-4), 5.66 (1 H, m, H-2), 7.30—8.20 (10 H, m, ArH), and 3.20—4.70 (remaining ring protons); δ_C (CD_3OD) 98.9 (C-1), 95.5 (C-1′), 74.1 (C-3), 72.3 (C-2), 71.9 (C-4), 70.7 (C-5′), 69.3 (2 C, C-3′ and C-4′), 68.7 (C-5), 67.7 (OCH_2), 62.7 (C-6′), 60.8 (C-2′), and 18.2 (C-6) (Found: C, 58.95; H, 6.45; N, 5.7. $C_{36}H_{47}N_3O_{13}$ requires C, 59.25; H, 6.49; N, 5.76%).

8-*Methoxycarbonyloctyl* 3-O-(2-*Azido*-4,6-O-*benzylidene*-2-*deoxy*-α-D-*galactopyranosyl*)-2,4-*di*-O-*benzoyl*-α-L-*rhamno*-*pyranoside* (11).—Compound (10) (1.9 g, 2.6 mmol) in acetonitrile (20 cm³) was added to a solution of dimethoxytoluene (0.66 g, 4.3 mmol) and toluene-*p*-sulphonic acid (30 mg) in acetonitrile (20 cm³). The solution was stirred overnight, neutralized with triethylamine, and evaporated to a syrup which was then chromatographed on silica gel with Skellysolve B–ethyl acetate (2 : 1 v/v) as solvent to give the *blocked disaccharide* (11) (1.8 g, 85%), $[\alpha]_D$ 156.8° (*c*, 1.1 in $CHCl_3$); R_F 0.24 (solvent as above); δ ($CDCl_3$) 1.10—1.80 (15 H, m, $[CH_2]_6$ and H-6), 3.65 (3 H, s, OMe), 4.94 (1 H, d, $J_{1,2}$ 1.7 Hz, H-1), 5.12 (1 H, d, $J_{1,2}$ 3.2 Hz, H-1′), 5.27 (1 H, s, acetal H), 5.51 (1 H, t, $J_{3,4}$ 10.0 Hz, H-4), 5.56 (1 H, dd, $J_{1,2}$ 1.7, $J_{2,3}$ 3.5 Hz, H-2), and 7.20—8.20 (15 H, m, ArH); δ_C ($CDCl_3$) 101.0 (CHPh), 97.6 (C-1), 96.3 (C-1′), 75.2 (C-3), 73.3 (C-2), 72.9 (C-4), 69.0 (C-4′), 68.4 (2 C, C-5 and C-5′), 67.2 (C-3′), 66.4 (OCH_2), 62.9 (C-6′), 60.3 (C-2′), and 17.7 (C-6) (Found: C, 63.35; H, 6.2; N, 5.0. $C_{43}H_{51}N_3O_{13}$ requires C, 63.15; H, 6.29; N, 5.14%).

8-*Methoxycarbonyloctyl* O-(3,4,6-*Tri*-O-*acetyl*-2-*azido*-2-*deoxy*-α-D-*galactopyranosyl*)-(1 ⟶ 3)-O-(2-*azido*-4,6-O-*benzylidene*-2-*deoxy*-α-D-*galactopyranosyl*)-(1 ⟶ 3)-2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (12).—Compound (11) (1.4 g, 1.7 mmol), silver trifluoromethanesulphonate (0.1 g, 0.4 mmol), silver carbonate (4.5 g, 16.4 mmol), and molecular sieves (3A) were suspended in dichloromethane (40 cm³) and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride [29] (3) (1.3 g, 3.9 mmol) in dichloromethane (10 cm³) was added dropwise. The reaction was stirred overnight, then filtered and evaporated to give a syrup, which was chromatographed on silica gel with Skellysolve B–ethyl acetate (3 : 2 v/v) as eluant to give the *protected trisaccharide* (12) (1.2 g, 62%), $[\alpha]_D$ 190.5° (*c*, 1.0 in $CHCl_3$); R_F 0.29 (solvent as above); δ ($CDCl_3$) 1.10—1.90 (15 H, m, $[CH_2]_6$ and H-6), 1.95 (3 H, s, MeCO), 1.98 (3 H, s, MeCO), 2.07 (3 H, s, MeCO), 2.32 (2 H, t, C_2HCO), 3.62 (3 H, s, OMe), and 7.00—8.20 (15 H, m, ArH); δ_C ($CDCl_3$) 101.5 (*C*HPh), 97.7 (C-1), 95.9 (C-1″), 94.6 (C-1′), 73.2 (C-3), 72.9 (C-3′), 71.6 (C-2), 71.1 (C-4), 68.8 (C-4′), 68.4 (C-5), 67.5 (4 C, C-3″, C-4″, C-5′, and C-5″), 66.4 (OCH_2), 62.7 (C-6′), 61.2 (C-6″), 58.4 (C-2′), 56.9 (C-2″), and 17.7 (C-6) (Found: C, 58.25; H, 6.0; N, 7.4. $C_{55}H_{66}N_6O_{20}$ requires C, 58.40; H, 5.88; N, 7.43%).

8-*Methoxycarbonyloctyl* O-(2-*Azido*-2-*deoxy*-α-D-*galacto*-*pyranosyl*)-(1 ⟶ 3)-O-(2-*azido*-2-*deoxy*-α-D-*galactopyrano*-*syl*)-(1 ⟶ 3)-α-L-*rhamnopyranoside* (13).—Compound (12) (1.0 g, 0.9 mmol) in methanol (100 cm³) containing a catalytic amount of sodium methoxide was left for 2 days at room temperature. The syrup, obtained after removal of sodium ions with Rexyn 101 (H^+) resin, filtration and evaporation, was dissolved in 90% aqueous trifluoroacetic acid (10 cm³) at 0 °C and kept for 1 h. Evaporation and co-distillation with water gave a syrup that was chromatographed on silica gel with ethyl acetate–methanol–water (85 : 10 : 5 v/v) as eluant to give the *deacetylated derivative* (13) (0.42 g, 67%), $[\alpha]_D$ 149.8° (*c*, 0.9 in water); R_F (solvent as above) 0.56; δ (D_2O, 80 °C) 1.15—1.85 (15 H, m, $[CH_2]_6$ and H-6), 2.37 (2 H, t, CH_2CO), 3.45—4.35 (ring H), 3.68 (3 H, s, OMe), 4.81 (1 H, d, $J_{1,2}$ 1.7 Hz, H-1), 5.26 (1 H, d, $J_{1,2}$ 3.3 Hz, H-1′), and 5.33 (1 H, d, $J_{1,2}$ 3.6 Hz, H-1″); δ_C (D_2O) 101.2 (C-1), 96.5 (C-1′), 95.4 (C-1″), 78.8 (C-3′), 74.2 (C-3), 73.0 (C-4), 72.1 (2 C, C-2 and C-4′), 70.7 (C-5′), 69.8 (C-5″), 69.5 (C-3″), 68.9 (C-5), 68.6 (C-4), 65.9 (OCH_2), 62.4 (2 C, C-6′ and C-6″), 61.7 (C-2′), 60.8 (C-2″), and 18.1 (C-6).

8-*Methoxycarbonyloctyl* O-(2-*Acetamido*-2-*deoxy*-α-D-*galactopyranosyl*)-(1 ⟶ 3)-O-(2-*acetamido*-2-*deoxy*-α-D-*galactopyranosyl*)-(1 ⟶ 3)-α-L-*rhamnopyranoside* (14).—Compound (13) (180 mg, 0.25 mmol) was dissolved in ethanol (50 cm³) containing Adams catalyst (PtO_2, 50 mg) and hydrogenated overnight at 505 kPa. After filtration and evaporation the syrup was dissolved in methanol (10 cm³) to which acetic anhydride (0.5 cm³) was added. The reaction mixture was stirred overnight, evaporated, and chromatographed on silica gel with ethyl acetate–methanol–water (7 : 2 : 1 v/v) as eluant to give the *trisaccharide* (14) (110 mg, 59%), $[\alpha]_D$ 146.5 (*c*, 0.5 in water); R_F (solvent as above) 0.55; δ (D_2O, 80 °C) 1.10—1.80 (15 H, m, $[CH_2]_6$ and H-6), 2.07 (6 H, s, MeCO), 2.47 (2 H, t, CH_2CO), 3.71 (3 H, s, OMe), 4.82 (1 H, d, H-1), and 5.54 (2 H, dd, $J_{1',2'} \sim J_{1'',2''}$ 3.6 Hz, H-1′, H-1″); δ_C (D_2O) 100.8 (C-1), 95.6 (C-1′), 94.6 (C-1″), 76.6 (C-3′), 73.3 (C-3), 72.4 (C-4), 71.8 (C-2), 71.5 (C-5″), 69.5 (2 C, C-5′ and C-4″), 69.0 (2 C, C-3″ and C-4′), 68.0 (C-5), 65.5 (OCH_2), 61.9 (2 C, C-6′ and C-6″), 50.6 (C-2″), 48.9 (C-2′), and 18.1 (C-6) (Found: C, 51.7; H, 7.7; N, 3.65. $C_{32}H_{56}N_2O_{17}$ requires C, 51.9; H, 7.62; N, 3.78%).

8-*Methoxycarbonyloctyl* 2-O-(2,4-*Di*-O-*benzoyl*-α-L-*rhamno*-*pyranosyl*)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (15).—8-Methoxycarbonyloctyl 2-*O*-(α-L-rhamnopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside [28] (2.5 g, 3.8 mmol), trimethyl orthobenzoate (1.1 g, 6.0 mmol), and toluene-*p*-sulphonic acid (50 mg) in dry dimethylformamide (50 cm³) were stirred at room temperature overnight, the solution was neutralized with triethylamine, concentrated and puri-

fied by h.p.l.c. with Skellysolve B–ethyl acetate (2 : 1 v/v) as solvent (R_F 0.37). This gave the corresponding sugar orthoester (2.5 g, 3.2 mmol) (85%) which was dissolved directly in chloroform (30 cm³) containing pyridine (5 cm³). The reaction was cooled to 0 °C, benzoyl chloride (2 cm³) was added dropwise, and the mixture was stirred for 1 h at 0 °C. The cooling-bath was removed and the reaction was stirred for an additional 5 h. The chloroform solution was then extracted with water, dried, and evaporated. The syrup obtained was co-evaporated twice with toluene to remove traces of pyridine. The reaction mixture was then dissolved in 75% aqueous acetic acid (50 cm³), stirred at 50 °C for 1 h and evaporated. The resultant syrup was again evaporated twice with toluene to remove traces of acetic acid. Chromatography on silica gel with Skellysolve B–ethyl acetate (2 : 1 v/v) as eluant gave the pure *disaccharide* (15) (2.3 g, 81%), $[\alpha]_D$ 13.2° (*c*, 1.0 in $CHCl_3$); R_F 0.50 (solvent as above); δ ($CDCl_3$) 1.15—1.75 (18 H, m, $[CH_2]_6$, H-6 and H-6′), 2.32 (2 H, t, CH_2CO), 3.68 (3 H, s, OMe), 4.60—5.02 (4 H, m, OCH_2Ph), 5.63 (1 H, dd, H-2′), 7.01—8.20 (20 H, m, ArH), and 3.35—5.40 (OCH_2 and ring protons); δ_C ($CDCl_3$) 99.2 (C-1), 98.8 (C-1′), 80.3 (CH_2Ph), 79.7 (CH_2Ph), 75.9 (C-3), 75.5 (2 C, C-4 and C-2′), 73.2 (C-3), 72.4 (C-4′), 68.8 (C-3′), 68.1 (C-5), 67.7 (C-5′), 66.9 (OCH_2), 18.1 (C-6), and 17.7 (C-6′) (Found: C, 68.95; H, 6.95. $C_{50}H_{60}O_{13}$ requires C, 69.11; H, 6.96%).

8-*Methoxycarbonyloctyl* O-(3,4-*Di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-(1 ⟶ 3)-O-(2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1 ⟶ 2)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (17).—The selectively blocked disaccharide (15) (2.0 g, 2.3 mmol) was dissolved in dichloromethane (40 cm³) containing silver trifluoromethanesulphonate (1.0 g, 3.7 mmol) and tetramethylurea (2.0 cm³, 32 mmol). This solution was cooled to −70 °C and 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride (4) (1.5 g, 3.7 mmol) dissolved in dichloromethane (15 cm³) was added dropwise with stirring. The reaction was allowed to warm to room temperature overnight, and the mixture was filtered. Following extraction with saturated sodium hydrogen carbonate and water, the syrupy product was chromatographed on silica gel with Skellysolve B–ethyl acetate (2 : 1 v/v) as solvent to give compound (16) (2.5 g), having the same R_F (0.50) as the starting disaccharide. This product was dissolved in methanol–tetrahydrofuran (3 : 1 v/v; 40 cm³) and cooled to 0 °C and a freshly prepared solution of magnesium methoxide in methanol (15 cm³ of a 1% solution) was added and the reaction was left for 48 h at 0 °C. After removal of magnesium ions with Rexyn 101 (H^+) resin, followed by filtration and evaporation, the syrupy product was chromatographed on a silica gel column using Skellysolve B–ethyl acetate (2 : 1 v/v) as eluant to give the *trisaccharide* (17) (1.9 g, 69%), $[\alpha]_D$ 24.6° (*c*, 1.0 in $CHCl_3$); R_F 0.43 (solvent as above); δ_C ($CDCl_3$) 100.9 (C-1″), 99.2 (C-1), 98.9 (C-1′), 80.3 (CH_2Ph), 79.5 (3 C, CH_2Ph), 76.0 (C-2), 75.5 (C-3), 74.5 (2 C, C-4 and C-4′), 73.8 (C-3″), 72.3 (2 C, C-3 and C-2′), 71.9 (C-4″), 68.6 (C-2″), 68.4 (C-5″), 68.1 (C-5), 67.7 (C-5′), 67.3 (OCH_2), 18.2 (C-6″), and 17.8 (2 C, C-6 and C-6′) (Found: C, 70.2; H, 7.0. $C_{70}H_{82}O_{17}$ requires C, 70.33; H, 6.91%).

8-*Methoxycarbonyloctyl* O-(2-O-*Acetyl*-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-(1 ⟶ 2)-O-(3,4-di-O-*benzyl*-α-L-*rhamnopyranosyl*)-(1 ⟶ 3)-O-(2,4-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1 ⟶ 2)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (18).—The partially blocked trisaccharide (17) (1.1 g, 0.9 mmol) was dissolved in dichloromethane (30 cm³) containing silver trifluoromethanesulphonate (0.7 g, 2.7 mmol) and tetramethylurea (2.0 cm³, 32 mmol). The solution was cooled to −70 °C and 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride (4) (1.0 g, 2.4 mmol) dissolved in dichloromethane (10 cm³) was added dropwise with stirring. The mixture was allowed to warm to room temperature overnight and then filtered. Following extraction with saturated aqueous sodium hydrogen carbonate and water the concentrated residue was chromatographed on silica gel with Skellysolve B–ethyl acetate (3 : 1 v/v) to give the *tetrasaccharide* (18) (1.2 g, 85%), $[\alpha]_D$ 18.9° (*c*, 1.0 in $CHCl_3$); R_F 0.25 (solvent as above); δ ($CDCl_3$) 0.94 (3 H, d, $J_{5,6}$ 5.8 Hz, H-6″), 1.11 (3 H, d, $J_{5,6}$ 6.0 Hz, H-6‴), 0.90—1.80 (18 H, m, $[CH_2]_6$, H-6 and H-6′), 2.05 (3 H, s, MeCO), 2.28 (2 H, t, CH_2CO), 3.66 (3 H, s, MeO), and 7.00—8.30 (40 H, m, ArH); δ_C ($CDCl_3$) 100.5 (C-1″), 99.2 (C-1), 99.0 (2 C, C-1′ and C-1‴), 80.1 (CH_2Ph), 79.5 (2 C, CH_2Ph), 79.3 (2 C, CH_2Ph), 77.6 (CH_2Ph), 76.0 (C-2), 75.6 (C-2″), 75.3 (C-3), 74.7 (C-4″), 74.3 (2 C, C-4 and C-4‴), 73.8 (C-2), 72.3 (2 C, C-3 and C-3″), 71.8 (2 C, C-4 and C-3‴), 69.1 (C-2‴), 68.9 (C-5″), 68.2 (2 C, C-5 and C-5‴), 67.7 (C-5′), 67.3 (OCH_2), 18.2 (C-6″), and 17.9 (3 C, C-6, C-6′ and C-6‴) (Found: C, 70.4; H, 6.75. $C_{92}H_{106}O_{22}$ requires C, 70.66; H, 6.83%).

8-*Methoxycarbonyloctyl* O-(α-L-*Rhamnopyranosyl*)-(1 ⟶ 2)-O-(α-L-*rhamnopyranosyl*)-(1 ⟶ 3)-O-(α-L-*rhamnopyranosyl*)-(1 ⟶ 2)-α-L-*rhamnopyranoside* (19).—Compound (18) (0.8 g, 0.5 mmol) was dissolved in acetic acid (40 cm³) and hydrogenated with 10% palladium–charcoal (0.5 g) at 505 kPa for 2 h. Filtration and co-evaporation with toluene (3 × 50 cm³) gave a syrup, which was dissolved in methanol (50 cm³) containing a catalytic amount of sodium methoxide and the solution was left for 48 h at room temperature. The syrup, obtained after removal of sodium ions with Rexyn 101 (H^+) resin, followed by filtration and evaporation, was chromatographed on silica gel with ethyl acetate–methanol–water (7 : 2 : 1 v/v) as eluant to give the pure *tetrasaccharide* (19) (250 mg, 63%), $[\alpha]_D$ −68.2° (*c*, 0.5 in water); R_F 0.35 (solvent as above); δ (D_2O, 75 °C) 1.17—1.82 (24 H, m, $[CH_2]_6$, H-6, H-6′, H-6″, and H-6‴), 2.39 (2 H, t, CH_2CO), 3.71 (3 H, s, OMe), 4.85br (1 H, s, H-1), 4.99br (2 H, dd, H-1‴ and H-1′), 5.20br (1 H, s, H-1″), and 3.25—4.17 (ring protons); δ_C (D_2O) 103.3 (2 C, C-1′ and C-1‴), 102.0 (C-1″), 99.6 (C-1), 79.4 (2 C, C-2 and C-2″), 78.6 (C-3′), 73.2—68.7 (other ring carbons and OCH_2), and 17.9 (4 C, C-6, C-6′, C-6″, and C-6‴) (Found: C, 52.4; H, 7.65. $C_{34}H_{60}O_{19}$ requires C, 52.84; H, 7.83%).

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