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ANTIGENIC DETERMINANTS OF *Salmonella* SEROGROUPS A AND D_1. SYNTHESIS OF TRISACCHARIDE GLYCOSIDES FOR USE AS ARTIFICIAL ANTIGENS*

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ABSTRACT

The disaccharide glycoside 8-methoxycarbonyloctyl 4,6-*O*-cyclohexylidene-2-*O*-(tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-mannopyranoside (**7**) was used as a common intermediate to the trisaccharide determinants of both *Salmonella* serogroups A and D_1. Acetalation of 8-methoxycarbonyloctyl α-D-mannopyranoside provided the 4,6-acetal derivative, which was selectively benzoylated to give the partially protected mannoside **4**. Reaction of **4** with tetra-*O*-benzyl-α-D-galactopyranosyl chloride afforded the fully protected disaccharide, which, after transesterification, gave the selectively blocked, disaccharide glycoside (**7**). Addition of tyvelose by way of its 2,4-di-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranosyl chloride derivative gave the blocked trisaccharide determinant of *Salmonella* serogroup D_1. 2,4-Di-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hexopyranosyl chloride reacted with **7** to provide, after removal of blocking groups, the paratose-containing determinant of Serogroup A.

INTRODUCTION

Since the early work of Robbins and Uchida[1], and Staub and colleagues[2,3] established the immunodominance of 3,6-dideoxyhexoses in certain *Salmonella* O-antigens, considerable effort has been devoted to the synthesis of antigenic determinants incorporating these sugars[4–9]. In this regard, comprehensive studies by Garegg and co-workers[10–13] have helped to establish the important role that synthetic, defined, carbohydrate antigens now play in serodiagnosis[13–17]. As methods for glycoside synthesis have improved, increasingly complex and elaborate saccharide sequences of bacterial origin have been synthesized[18–20]. Together with repeating units derived from phage degradations[21,22], synthetic oligosaccharides have provided considerable insight into the antibody specificity of immune rabbit sera[13–16,21,22].

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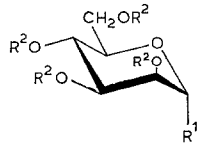
It is to be expected that a more complete understanding of such recognition will result from sound assessment of oligosaccharide conformation, which determines antigen topography. Semi-empirical methods[23,24] are now available for this purpose, and experimental support for the conclusions of computer calculations of molecular geometry have been obtained from n.m.r. measurements[23-25]. This approach has been pioneered and applied to human blood-group determinants by Lemieux and his collaborators[23-25], and we have utilized it in order to assess the conformation of *Shigella flexneri* O-antigens[26]. In order to apply this approach to those O-antigens of *Salmonella* serogroups A, B, and D_1 that contain 3,6-dideoxyhexoses, it was necessary to examine branched structures, the syntheses of which have as yet to be reported.

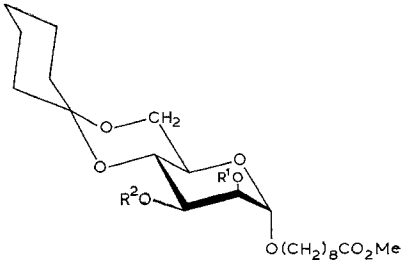
Because of the juxtaposition of pyranosyl substituents at vicinal oxygen atoms (O-2 and O-3), the trisaccharide structure α-D-Gal-(1→2)-[3,6-dideoxy-α-D-hexopyranosyl-(1→3)]-α-D-Man would be expected to possess considerable conformational rigidity; this results from steric[27] and electronic (*exo*-anomeric effect) factors[23,28], and is reminiscent of terminal, blood-group structures[29]. For these reasons, it is expected that this trisaccharide sequence should be intimately related to the dominant role that 3,6-dideoxyhexoses play in *Salmonella* serology. Consequently, we planned the synthesis of three trisaccharides, each containing a 3,6-dideoxyhexose, namely, abequose (3,6-dideoxy-D-*xylo*-hexopyranose), paratose (3,6-dideoxy-D-*ribo*-hexopyranose), and tyvelose (3,6-dideoxy-D-*arabino*-hexopyranose). The scheme employs a synthetic route common to all three trisaccharides, with the single exception of the final glycosylation step used to introduce the unique 3,6-dideoxyhexose. We now report the synthesis of two branched trisaccharide structures, those of *Salmonella* serogroups A and D_1 (ref. 3).

RESULTS

Tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[30] (**1**) was used to prepare 8-methoxycarbonyloctyl α-D-mannopyranoside (**2**). Acetalation of mannopyranosides under thermodynamic control readily leads to 2,3:4,6 diacetals.[31,32]. Vinyl ethers have been used to generate acetals under kinetic control[33,34]. For this reason, we



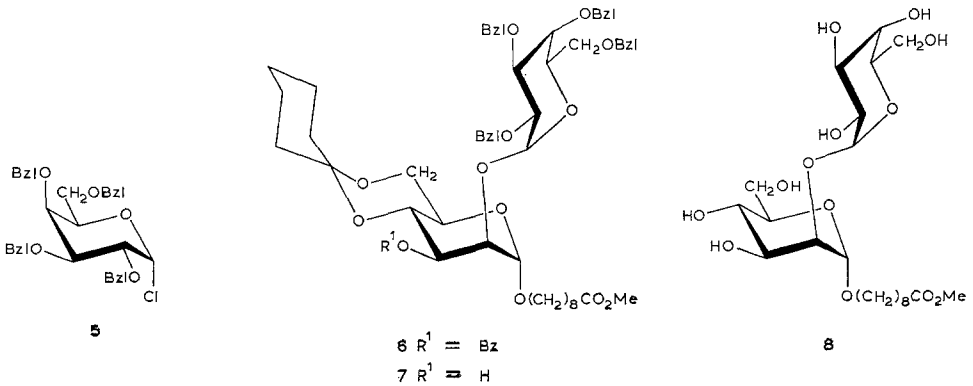
1 R^1 = Br, R^2 = Bz
2 R^1 = $O(CH_2)_8CO_2Me$, R^2 = H



3 R^1 = R^2 = H
4 R^1 = H, R^2 = Bz

chose to employ 1-ethoxycyclohexene[34] for the preparation of the 4,6-cyclohexylidene acetal **3**. In this way, kinetic and stoichiometric control provide a good yield of the 4,6-acetal **3**. Selective acylation of **3** gave 8-methoxycarbonyloctyl 3-*O*-benzoyl-4,6-*O*-cyclohexylidene-α-D-mannopyranoside (**4**).

Reaction of tetra-*O*-benzyl-D-galactopyranose with the Vilsmeier reagent[35] gave tetra-*O*-benzyl-α-D-galactopyranosyl chloride[36,37] (**5**), generation of the Vilsmeier reagent being performed *in situ* by use of a molar equivalent of oxalyl chloride[35] in the presence of a catalytic amount of *N*,*N*-dimethylformamide in dichloromethane. Performed in this way, tedious isolation of (chloromethylene)dimethyliminium chloride under rigorously anhydrous conditions is avoided, and the processing is simplified as compared to that used in other methods[35-37]. Glycosylation of the selectively blocked α-D-mannopyranoside **4** with **5**, with silver trifluoromethanesulfonate (triflate) as the promoter, gave the protected disaccharide **6**. Trans-esterification of the benzoic ester gave the selectively blocked disaccharide glycoside **7**, which served as the common precursor to the trisaccharide determinants of *Salmonella* serogroups A, B, and D_1. Hydrogenolysis of compound **7** in aqueous acetic acid gave the deblocked disaccharide glycoside **8**.

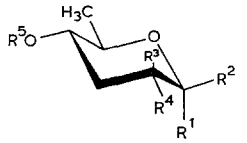


The configuration of the 3,6-dideoxy-D-hexose residue to be introduced at O-3 of the mannose unit is α in all instances. Paratose and tyvelose are C-2 epimers and, whereas the α-glycoside of 3,6-dideoxy-D-*ribo*-hexopyranose is an example of a 1,2-*cis*-glycoside, 3,6-dideoxy-α-D-*arabino*-hexopyranosides are 1,2-*trans*-glycosides. Thus, for glycosylation reactions, it may be anticipated that the glycosyl halide of tyvelose should possess a participating group at O-2 for efficient α-glycoside formation, whereas, in the case of paratose, its glycosyl halide should carry a nonparticipating blocking group at O-2.

Methyl 4-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside was prepared from methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside according to a published procedure[10]. However, specific improvements made in several steps leading to this derivative should be noted. Hicks and Fraser-Reid[38] described an efficient, one-step conversion of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside into

methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside. Reduction with lithium aluminum hydride then provides methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside[39], from which the dideoxy derivative is obtained by opening of the acetal with *N*-bromosuccinimide, followed by catalytic reduction of the 6-bromo-6-deoxy function. Benzoylation of methyl 4-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside gave the dibenzoate **9** as semi-crystalline material. As each reaction proceeds in good yield, and is well suited to a 10–20-g scale, synthesis of **9** in relatively large quantities presents little difficulty. The synthesis of methyl 3,6-dideoxy-β-D-*ribo*-hexopyranoside was also readily accomplished on a 10-g scale[40]. Benzylation then gave the dibenzyl ether **11** as a crystalline solid.

A major obstacle to efficient utilization of 3,6-dideoxyhexoses in glycosylation reactions is the difficulty of preparation, and the lability, of the corresponding glycosyl halides. For the conversion of **9** or **11** into the glycosyl chlorides **10** and **12**, strongly acidic reagents, such as hydrogen chloride in acetic acid or dichloromethane, should be avoided, in order to maintain the integrity of the benzyl ether protecting groups and to avoid the aqueous conditions essential for efficient removal of the acid reagent. The latter process undoubtedly causes significant hydrolysis of the labile glycosyl halides.



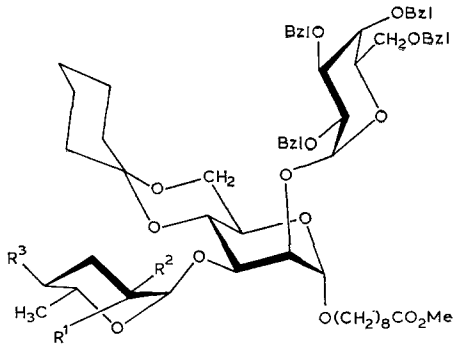
9 R^1 = OMe, R^2 = R^4 = H, R^3 = OBz, R^5 = Bz
10 R^1 = Cl, R^2 = R^4 = H, R^3 = OBz, R^5 = Bz
11 R^1 = R^3 = H, R^2 = OMe, R^4 = OBzl, R^5 = Bzl
12 R^1 = Cl, R^2 = R^3 = H, R^4 = OBzl, R^5 = Bzl

To circumvent this, the 3,6-dideoxyhexosides may be synthesized, and protected as allyl glycosides, and these converted, *via* intermediate 1-hydroxy derivatives, into glycosyl halides by Vilsmeier reagents. Alternatively, methyl glycosides **9** and **11** can be directly converted into glycosyl halides[41]. In this and other work, we have used dichloromethyl methyl ether or its dibromo analog to generate glycosyl chlorides[42] or bromides[43]. Conversion of methyl glycosides into glycosyl chlorides was accomplished in 1–2 h at room temperature, and the processing merely involved filtration of the insoluble Lewis acid, followed by concentration. The products **10** and **12**, obtained in quantitative yield, were checked for purity by ^{13}C- and ^{1}H-n.m.r. spectroscopy. A potential hazard associated with dihalogenomethyl methyl ethers relates to their toxicity and potential as carcinogens[44]. Consequently these compounds should be handled with care in the fumehood, with protection against skin contact.

Coupling of tyvelose to yield the trisaccharide **13** was accomplished with the glycosyl chloride **10**. Freshly prepared **10** reacted with the selectively protected disaccharide glycoside **7**, using silver triflate and 1,1,3,3-tetramethylurea in dichloro-

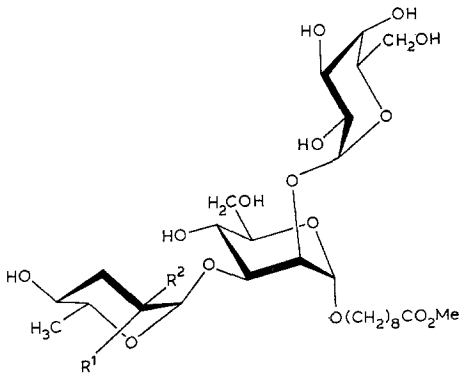
methane solution[45], the yield of the α-linked trisaccharide **13** being 67% after chromatography. Promotion of the glycosylation reaction with silver triflate was also used in order to effect the synthesis of the paratose trisaccharide **14**. A complication here was the formation of 10% of the β anomer, which was removed by chromatography. Halide-ion catalysis failed to provide recognizable products when the paratose compound **12** was subjected to this type of reaction with disaccharide **7**; indeed, the reaction mixture contained none of the starting disaccharide **7** and no trisaccharide products.

The protected trisaccharide derivatives were deblocked without isolation of intermediates. The tyvelose-containing trisaccharide **13** was first trans-esterified, and the product hydrogenolyzed in the presence of palladium-on-charcoal in aqueous acetic acid solution. The crude trisaccharide glycoside **15** thus obtained was chromatographed on Sephadex LH20 to remove traces of silica. The trisaccharide was the sole product obtained from the column, as judged by a refractive-index monitor; this product was analytically pure, and had ^{13}C-n.m.r. data consistent with the anticipated structure **15**. In line with mechanistic considerations of the final, glycosylation step,



13 $R^1 = R^3 = OBz, R^2 = H$
14 $R^1 = H, R^2 = R^3 = OBzl$

no β-linked tyvelose was evident. The paratose trisaccharide **14** was deblocked in a single hydrogenolysis step, to give crude **16**. Chromatography on silica gel failed to remove a contaminant present in the ratio of ~10:1; this component was detected by the ^{13}C-n.m.r. spectrum, where its appearance was readily discerned by low-intensity peaks in the anomeric and C-6 regions. The chemical shift of the low-intensity, anomeric resonance was consistent with the presence of a β-linked, paratose residue. Chromatography of crude **16** on Sephadex-LH20 resolved the mixture of the β-linked impurity and **16**; the ^{13}C-n.m.r. spectrum of thus purified **16** then showed only three peaks in the anomeric region, and one C-6 paratose resonance. For both trisaccharides, the ^{13}C- and ^{1}H-n.m.r. chemical shifts of anomeric carbon atoms and protons were consistent with the presence of α-linkages for each pyranoid residue.



15 R^1 = OH, R^2 = H
16 R^1 = H, R^2 = OH

DISCUSSION

The synthetic route employed herein provides the disaccharide common to *Salmonella* serogroups A, B, and D_1. The crucial, synthetic step is then the addition of the appropriate 3,6-dideoxy-D-hexose. We here describe the synthesis of the tyvelose- (serogroup D_1) and paratose- (serogroup A) containing trisaccharides **15** and **16**.

The glycosyl halides of 3,6-dideoxyhexoses are significantly less stable than those of simple hexoses. For this reason, we chose to employ the glycosyl chlorides **10** and **12**. The glycosylation of the disaccharide **7** by these derivatives was promoted by silver triflate. In the case of tyvelose, the conditions were standard for the synthesis of 1,2-*trans*-glycosides. In order to accomplish the synthesis of the paratose-containing trisaccharide **14**, it was necessary to employ somewhat unusual conditions. Glycosylation with silver triflate[45], or by halide-ion assistance under standard conditions[46], yielded complex reaction-mixtures from which neither the starting disaccharide nor any trisaccharide products could be isolated. A possible explanation of this observation is that electrophilic attack occurs at the acetal function[47]. In order to moderate the carbonium-ion character of the glycosyl intermediate, the reaction of **12** with **7** was performed in acetonitrile, a strongly solvating solvent. In this way, a good yield of the trisaccharide **15** was secured. As is often the case for glycosylation with *ribo*-hexopyranosyl chlorides[46], a small proportion of the β-glycoside was also formed, but this was readily removed by chromatography.

Recent immunochemical studies have shown that the tetrasaccharide repeating-unit derived from a *Salmonella* O-antigen by phage degradation is a potent inhibitor of O-4 serogroup specific antibody[22]. This compound contains a terminal, branched-trisaccharide sequence, the synthesis of which was an objective of these studies. As the most active of the constituent disaccharides, α-D-Abe-(1→3)-α-D-Man, was approximately one-fifth as active as the branched structure, the role of the α-linked galactose residue in the recognition is of some interest. In terms of the structure

of the branched trisaccharide, this role may relate to the conformational rigidity imposed upon pyranosyl residues juxtaposed at the O-2, O-3, vicinal-diol system of α-D-mannopyranoside; this situation bears some resemblance to that encountered with blood-group determinants[23,29]. The objective of our synthetic studies is to provide compounds suitable for n.m.r.-spectral and serological studies. The former approach in the manner demonstrated by Lemieux and his co-workers[23-25] provides an insight into oligosaccharide conformation in solution, and, as we have shown for the *S. flexneri* serogroup Y antigen, such results may be extrapolated to a model of the polymeric O-antigen conformation[26]. Conformational data may then be used to rationalize antigen–antibody recognition.

EXPERIMENTAL

General. — The general methods and materials employed in this work were similar to those described in recent publications from this laboratory[40,48]. Medium-pressure, column chromatography performed according to a published procedure[49] was used for most separations. N.m.r. spectra (^{13}C and ^{1}H) were recorded at 20 and 79.9 MHz, respectively. Proton chemical-shifts are expressed relative to internal 1% tetramethylsilane for solutions in chloroform-*d*, and relative to sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. Carbon-13 shifts are expressed relative to internal and external Me_4Si for solutions in chloroform-*d* and deuterium oxide.

8-Methoxycarbonyloctyl α-D-mannopyranoside (**2**). — A solution of tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[30] (**1**; 37.6 g, 57 mmol) in dichloromethane (50 mL) was added to an ice-cold mixture of 8-methoxycarbonyloctanol[50] (11.6 g, 62 mmol), mercuric cyanide (15 g, 59 mmol), molecular sieve 4A (10 g), and dichloromethane (200 mL). The mixture was allowed to warm to room temperature, and was then stirred overnight, and filtered; the filtrate was successively washed with saturated, aqueous sodium hydrogencarbonate and water, and evaporated to a syrup which was dissolved in methanol (300 mL) containing a catalytic amount of sodium methoxide, and the solution kept overnight. The base was neutralized with Rexyn 101 (H^+), resin, the suspension filtered, and the filtrate evaporated, to yield a syrupy residue which was purified on a column of silica gel with 17:2:1 (v/v/v) ethyl acetate–methanol–water, to give pure **2** (14.2 g, 71%); m.p. 81–82°, $[\alpha]_D^{20}$ +48.0° (*c* 0.9, water); ^{13}C-n.m.r. (D_2O): 100.9 p.p.m. (C-1).

Anal. Calc. for $C_{16}H_{30}O_8$: C, 54.86; H, 8.57. Found: 54.97; H, 8.65.

8-Methoxycarbonyloctyl 4,6-O-cyclohexylidene-α-D-mannopyranoside (**3**). — 1-Ethoxycyclohexene[34] (4.0 g, 32 mmol) was added dropwise to a stirred solution of **2** (5.5 g, 16 mmol) and *p*-toluenesulfonic acid (50 mg) in *N*,*N*-dimethylformamide (75 mL), and the mixture was kept for 2 h at room temperature. Addition of triethylamine (10 mL), followed by evaporation, gave a syrup which was purified on a column of silica gel with 2:1 (v/v) Skellysolve B–ethyl acetate, to give pure **3** (5.4 g, 80%); $[\alpha]_D^{20}$ +50.5° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.77 (bs, 1 H, H-1), 3.64 (s, 3 H, OMe), 2.28 (t, 2 H, $-CH_2-CO$), and 2.2–1.0 [m, 22 H, cyclohexylidene,

$(CH_2)_6$]; ^{13}C-n.m.r. ($CDCl_3$): δ_C 100.4 and 100.0 p.p.m. (2 C, acetal C and C-1).

8-Methoxycarbonyloctyl 3-O-benzoyl-4,6-O-cyclohexylidene-α-D-mannopyranoside (**4**). — A solution of benzoyl chloride (2.8 g, 20 mmol) in pyridine (25 mL) was added dropwise to a solution of **3** (8.0 g, 19 mmol) in pyridine (225 mL) at −40°, and the mixture was allowed to warm to room temperature overnight, and evaporated to a syrup which was purified on a column of silica gel with 3:1 (v/v) Skellysolve B–ethyl acetate, to give pure **4** (7.4 g, 74%); $[\alpha]_D^{20}$ +26.9° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.39 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.8 Hz, H-3), and 4.82 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1); ^{13}C-n.m.r. ($CDCl_3$): δ_C 174.4 (-CO_2Me), 165.7 (-COPh), and 100.8, 100.0 (2 C, acetal C and C-1).

Anal. Calc. for $C_{29}H_{42}O_9$: C, 65.15; H, 7.92. Found: C, 65.04; H, 7.99.

Tetra-O-benzyl-α-D-galactopyranosyl chloride (**5**). — Tetra-*O*-benzyl-α-D-galactopyranose[47] (4.4 g, 8.1 mmol) and *N*,*N*-dimethylformamide (0.2 mL) were dissolved in dichloromethane (25 mL), and then a solution of oxalyl chloride (2 mL, 23 mmol) in dichloromethane (10 mL) was added dropwise with stirring. The mixture was kept for 1 h at room temperature, and evaporated. The residue was taken up in 1:1 (v/v) Skellysolve B–ethyl acetate, and the suspension filtered through silica gel, to give, after evaporation of the solvents, **5** (4.2 g, 97%), which was used in the glycosylation step without purification.

8-Methoxycarbonyloctyl 3-O-benzoyl-4,6-O-cyclohexylidene-2-O-(tetra-O-benzyl-α-D-galactopyranosyl)-α-D-mannopyranoside (**6**). — A solution of **5** (4.2 g, 7.5 mmol) in dichloromethane (10 mL) was added to a mixture of **4** (2.0 g, 3.7 mmol), silver triflate (2.0 g, 7.8 mmol), and 1,1,3,3-tetramethylurea (1.5 g, 13 mmol) in dichloromethane (20 mL) at −40°. The mixture was allowed to warm to room temperature, and stirred overnight. Filtration, and evaporation of the filtrate, gave a syrup which was chromatographed on a column of silica gel with 9:1 (v/v) toluene–ethyl acetate, to give **6** (2.6 g, 65%); $[\alpha]_D^{20}$ +16.9° (*c* 1.0, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ_C 100.0 (2 C, acetal C and C-1) and 99.7 p.p.m. (C-1′).

8-Methoxycarbonyloctyl 4,6-O-cyclohexylidene-2-O-(tetra-O-benzyl-α-D-galactopyranosyl)-α-D-mannopyranoside (**7**). — A solution of **6** (1.7 g, 1.6 mmol) in methanol (75 mL) containing a catalytic amount of sodium methoxide was stirred overnight at room temperature. The base was neutralized with Rexyn 101 (H^+) resin, the suspension filtered, and the filtrate evaporated to a syrup which was purified on a column of silica gel with 2:1 (v/v) Skellysolve B–ethyl acetate, to give pure **7** (1.3 g, 85%); $[\alpha]_D^{20}$ +38.8° (*c* 1.2, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ_C 101.9, 99.9, and 99.8 p.p.m. (3 C, acetal C, C-1, and C-1′).

Anal. Calc. for $C_{56}H_{72}O_{13}$: C, 70.57; H, 7.61. Found: C, 70.61; H, 7.72.

8-Methoxycarbonyloctyl 2-O-α-D-galactopyranosyl-α-D-mannopyranoside (**8**). — A solution of **7** (0.20 g, 0.2 mmol) in aqueous acetic acid (30 mL, 80%) was hydrogenolyzed in the presence of 10% palladium-on-charcoal at 70 lb.in.$^{-2}$ overnight. Filtration, and evaporation of the filtrate, gave a residue which was purified on a column of silica gel with 7:2:1 (v/v) ethyl acetate–methanol–water, to give **8** (0.10 g, 93%); $[\alpha]_D^{20}$ +94.2° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O): δ 5.06 (bs, 2 H, H-1,1′), 2.28

(t, 2 H, -CH_2-CO), and 2.0–1.0 [m, 12 H, $(CH_2)_6$]; ^{13}C-n.m.r. (D_2O): δ_C 102.5 (C-1′) and 99.4 p.p.m. (C-1).

*Methyl 2,4-di-*O*-benzoyl-3,6-dideoxy-*α*-*D*-*arabino*-hexopyranoside* (**9**). — A solution of methyl 4-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside[10,38,39] (16.1 g, 60.5 mmol) in chloroform (60 mL) and pyridine (15 mL) was cooled in ice–water, and benzoyl chloride (13.8 mL, 104 mmol) was added dropwise with stirring. After 18 h, the solution was poured into 10% potassium hydrogencarbonate solution (200 mL), the mixture was extracted with dichloromethane (2 × 300 mL), and the extracts were processed in the usual way. The crude syrup (20 g) resulting was purified on a column of silica gel (500 g) with 3:1 (v/v) Skellysolve B–ethyl acetate, to give **9** as a homogeneous syrup (17.8 g) that crystallized on standing; m.p. 122–123° (recrystallized from Skellysolve B), $[\alpha]_D^{20}$ −53.0° (*c* 1.6, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ_C 97.5 (C-1), 55.0 (OCH_3), 29.7 (C-3), and 17.9 p.p.m. (C-6).

Anal. Calc. for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99. Found: C, 68.37; H, 6.11.

*2,4-Di-*O*-benzoyl-3,6-dideoxy-*α*-*D*-arabino-hexopyranosyl chloride* (**10**). — To a solution of **9** (1.0 g, 2.7 mmol) in dichloromethyl methyl ether (20 mL) was added anhydrous $ZnCl_2$ (~50 mg). The mixture was stirred for 2 h at room temperature, filtered through glass wool, the filtrate evaporated, and the syrup dried under high vacuum, to give syrupy chloride **10** (1.0 g, ~100%), which was immediately used in the glycosylation step: ^{13}C-n.m.r. ($CDCl_3$): δ_C 90.4 (C-1), 28.6 (C-3), and 17.6 p.p.m. (C-6).

*Methyl 2,4-di-*O*-benzyl-3,6-dideoxy-*β*-*D*-ribo-hexopyranoside* (**11**). — A solution of methyl 3,6-dideoxy-β-D-*ribo*-hexopyranoside[40] (41 g, 25 mmol) in $HCONMe_2$ (50 mL) containing suspended sodium hydride (4.0 g, 84 mmol) was stirred for 30 min at room temperature. Benzyl bromide (9 mL, 74 mmol) was added dropwise to this stirred mixture, and then it was kept for 18 h at room temperature. The excess of benzyl bromide was decomposed by addition of methanol (20 mL). The mixture was poured into water (200 mL), and extracted with ethyl acetate (3 × 100 mL). The extracts were combined, and processed in the usual way, to yield 8.5 g of **11** as a syrup. This was purified by chromatography on silica gel (400 g) with 5:1 Skellysolve B–ethyl acetate. The purified material (6.6 g) solidified on standing; $[\alpha]_D^{20}$ +25.2° (*c* 1.0, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ_C 106.1 (C-1), 56.7 (OCH_3), 35.1 (C-3), and 18.0 p.p.m. (C-6).

Anal. Calc. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.80; H, 7.67.

*2,4-Di-*O*-benzyl-3,6-dideoxy-*α*-*D*-ribo-hexopyranosyl chloride* (**12**). — To a solution of **11** (0.53 g, 1.5 mmol) in dichloromethyl methyl ether (5 mL) was added anhydrous $ZnCl_2$ (~50 mg). The mixture was stirred for 1 h at room temperature, filtered through glass wool, the filtrate evaporated, and the syrup dried under high vacuum, to give syrupy chloride **12** (0.53 g, ~100%), which was immediately used in the glycosylation step: ^{13}C-n.m.r. ($CDCl_3$): δ_C 93.8 (C-1), 30.3 (C-3), and 17.8 p.p.m. (C-6).

*8-Methoxycarbonyloctyl 4,6-*O*-cyclohexylidene-3-*O*-(2,4-di-*O*-benzoyl-3,6-dideoxy-*α*-*D*-arabino-hexopyranosyl)-2-*O*-(tetra-*O*-benzyl-*α*-*D*-galactopyranosyl)-*α*-*D*-man-*

nopyranoside (**13**). — The freshly prepared chloride **10** (1.0 g, 2.7 mmol) in dichloromethane (5 mL) was added to a stirred mixture of compound **7** (1.0 g, 1.0 mmol), silver triflate (1.0 g, 3.9 mmol), and 1,1,3,3-tetramethylurea (0.64 g, 5.5 mmol) in dichloromethane (10 mL) at −40°. The mixture was allowed to reach room temperature slowly, and was stirred overnight. Filtration, and evaporation of the filtrate, gave a syrup which was separated on a column of silica gel with 9:1 (v/v) toluene–ethyl acetate, to give trisaccharide **13** (0.90 g, 67%); $[\alpha]_D^{20}$ +49.9° (*c* 1.1, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ_C 100.1, (2 C, acetal and C-1), 98.3, and 97.6 (C-1′,1″).

8-Methoxycarbonyloctyl 4,6-O-cyclohexylidene-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-ribo-hexopyranosyl)-2-O-(tetra-O-benzyl-α-D-galactopyranosyl)-α-D-mannopyranoside (**14**). — A solution of the freshly prepared chloride **12** (0.9 g, 2.5 mmol) in acetonitrile (5 mL) was added to a stirred mixture of compound **7** (0.64 g, 0.7 mmol), silver triflate (0.65 g, 2.5 mmol), and 1,1,3,3-tetramethylurea (1.0 g, 9 mmol) in acetonitrile (10 mL) at −70°. The mixture was allowed to reach room temperature slowly, and was stirred overnight. Filtration, and evaporation of the filtrate, gave a syrup which was separated on a column of silica gel with 4:1 (v/v) Skellysolve B–ethyl acetate, to give trisaccharide **14** (0.53 g, 63%); $[\alpha]_D^{20}$ +69.9° (*c* 1.0, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ_C 100.4 (acetal C), 99.9 (C-1), 97.1, and 96.1 p.p.m. (C-1′,1″).

8-Methoxycarbonyloctyl 3-O-(3,6-dideoxy-α-D-arabino-hexopyranosyl)-2-O-α-D-galactopyranosyl-α-D-mannopyranoside (**15**). — A solution of trisaccharide **13** (0.90 g, 0.7 mmol) in methanol (100 mL) containing a catalytic amount of sodium methoxide was kept overnight, the base neutralized with Rexyn 101 (H^+) resin, the suspension filtered, and the filtrate evaporated to a syrup which was dissolved in aqueous acetic acid (30 mL, 80%) and hydrogenolyzed at 70 lb.in.$^{-2}$ in the presence of 10% palladium-on-charcoal for 4 h. Filtration of the suspension, and evaporation of the filtrate, gave a syrup which was purified on a column of silica gel with 7:2:1 (v/v) ethyl acetate–methanol–water, to give **15** (0.34 g, 76%); $[\alpha]_D^{20}$ +120.3° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O, 72°): δ 5.15 (d, 1 H, $J_{1',2'}$ 2.6 Hz, H-1′), 5.06 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.88 (d, 1 H, $J_{1'',2''}$ 1.4 Hz, H-1″), 3.67 (s, 3 H, OCH_3), 2.36 (t, 2 H, $-CH_2CO-$), 2.05–1.75 (bm, 2 H, H-3″*e*, H-3″*a*), and 1.25 (d, 3 H, $J_{5'',6''}$ 6.0 Hz, H-6″); ^{13}C-n.m.r. (D_2O): δ_C 102.3 (2 C, C-1′,1″), and 99.3 p.p.m. (C-1).

Anal. Calc. for $C_{28}H_{50}O_{16}$: C, 52.33; H, 7.84. Found: C, 52.18; H, 7.96.

8-Methoxycarbonyloctyl 3-O-(3,6-dideoxy-α-D-ribo-hexopyranosyl)-2-O-α-D-galactopyranosyl-α-D-mannopyranoside (**16**). — The trisaccharide **14** (0.14 g, 0.1 mmol) was dissolved in aqueous acetic acid (80%; 20 mL), and hydrogenolyzed at 70 lb.in.$^{-2}$ in the presence of 10% palladium-on-charcoal overnight. Filtration, and evaporation of the filtrate, gave a residue which was purified on a column of silica gel with 7:2:1 (v/v/v) ethyl acetate–methanol–water, to give **16** (57 mg, 81%). The ^{13}C-n.m.r. spectrum of **16** showed the presence of ~10% of the corresponding trisaccharide having the 3,6-dideoxy-*ribo*-hexopyranosyl group β-linked. This impurity was removed by chromatography on Sephadex LH-20 with 1:1 (v/v) methanol–water, to give analytically pure **16**; $[\alpha]_D^{20}$ +143.8° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O, 80°): δ 5.17 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″), 5.05 (d, 2 H, $J_{1,2} = J_{1',2'} = \sim 1.8$ Hz, H-1,1′),

2.35 (t, 2 H, -CH_2CO-), and 1.23 (d, 3 H, $J_{5'',6''}$ 6.1 Hz, H-6); ^{13}C-n.m.r. (D_2O): δ_C 102.2 (C-1′) 100.6 (C-1″), and 99.4 p.p.m. (C-1).

Anal. Calc. for $C_{28}H_{50}O_{16}$: C, 52.33; H, 7.84. Found: C, 52.10; H, 8.03.

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