# Syntheses of the Biological Repeating Units of Salmonella Serogroups A, B, and $D_1$ O-Antigenic Polysaccharides

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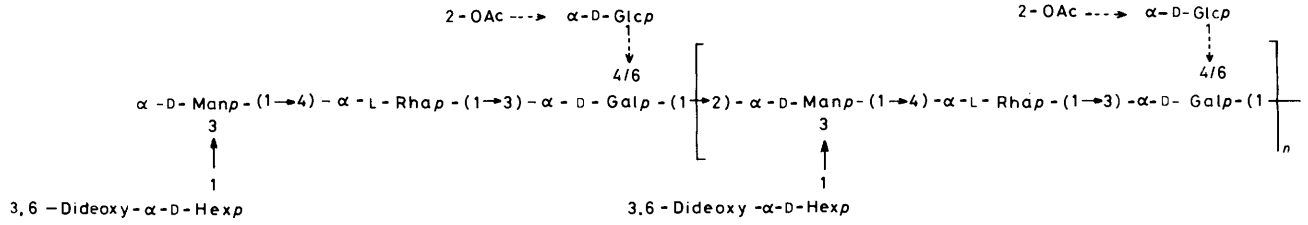
The immunogenic polysaccharide part of the Salmonella lipopolysaccharide of the cell walls of Salmonella bacteria belonging to serogroups A, B, and $D_1$ is depicted below.

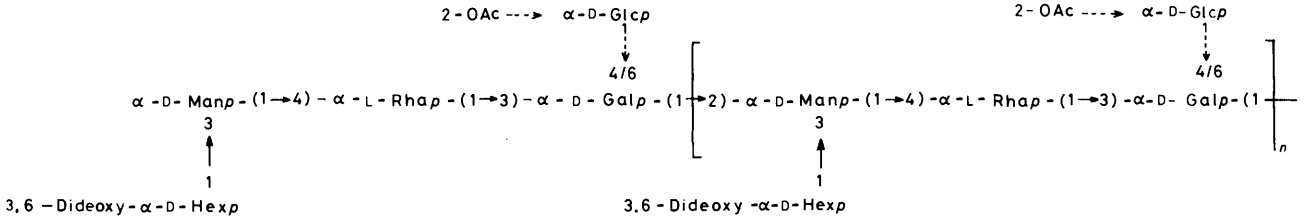



Syntheses of the following three tetrasaccharides are described: *p*-trifluoroacetamidophenyl *O*-(3,6-dideoxy-α-D-hexopyranosyl)-(1→3)-*O*-α-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-D-galactopyranoside, in which the 3,6-dideoxyhexopyranosyl group is abequosyl (28), paratosyl (29), and tyvelosyl (30), respectively. The three tetrasaccharides were made from a common trisaccharide precursor, a derivative (24) of *p*-trifluoroacetamidophenyl *O*-α-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-D-galactopyranoside with all hydroxy-groups protected but the one at C-3 in the mannosyl unit. In the various glycosylations, halide-ion catalysis or silver triflate-promotion was used in the construction of 1,2-*cis*-glycosidic bonds from glycosyl halides with a non-participating group in the 2-position. Silver triflate was used as promotor in the construction of 1,2-*trans*-glycosidic bonds from glycosyl halides carrying a participating acyl group in the 2-position.

In our programme aimed at the synthesis of oligosaccharides which are part of O-antigenic *Salmonella* polysaccharides, we have previously synthesized glycosides of the disaccharides 3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranose,[1,2] 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl-)-α-D-mannopyranose,[3] 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranose,[4] and 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl-)-α-L-rhamnopyranose.[5] Also synthesized were the disaccharides 4-*O*-(α-D-mannopyranosyl-)-α-L-rhamnopyranose[6] and 4-*O*-(β-D-mannopyranosyl-)-α-L-rhamnopyranose[6] and the trisaccharide *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→4)-α-L-rhamnopyranose,[7] the latter constituting the common backbone unit in polysaccharides from *Salmonella* serogroups A, B, and $D_1$ (see below).

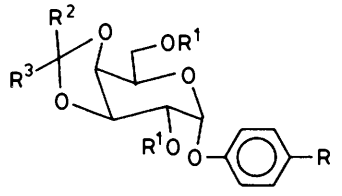
the antigens prepared from the first four disaccharides above have been successfully used as diagnostic reagents.[8]

For protective immunization experiments with animals, larger oligosaccharides corresponding to O-antigenic *Salmonella* polysaccharides were needed. Such oligosaccharides can be prepared by hydrolysis with phage enzymes, which specifically hydrolyze the α-L-rhamnosyl linkages.[9] The oligosaccharides obtained will therefore preponderantly contain terminal galactosyl groups. Oligosaccharides containing the terminal 3,6-dideoxyhexosyl-(1→3)-α-D-mannopyranosyl group are, however, of special interest as they correspond to the non-reducing terminals of the O-specific side-chains. It has been assumed that the outermost parts of these chains play a dominant role in determining the immune-response.[10] We therefore now report syntheses of the *p*-trifluoroacet-




All these oligosaccharides were synthesized as glycosides of either *p*-trifluoroacetamidophenol, *p*-nitrophenol, or 8-methoxycarbonyloctan-1-ol, thus making them suitable for attachment to proteins (to give artificial antigens) or to solid carriers (to give immunoadsorbents). Antisera raised against

amidophenyl tetrasaccharide glycosides (28), (29), and (30), corresponding to the biological repeating units of O-antigenic polysaccharides from *Salmonella* serogroups A, B, and $D_1$ respectively. In connection with this work, the *p*-nitrophenyl glycoside of 4-*O*-(α-D-mannopyranosyl)-α-L-rhamnopyranose

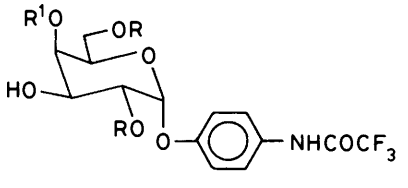


(1) R = $NO_2$, $R^1$ = H, $R^2$ = $R^3$ = Me

(2) R = $NO_2$, $R^1$ = Bz, $R^2$ = $R^3$ = Me

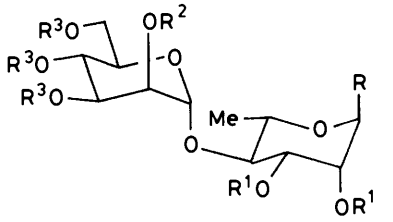
(3) R = $NHCOCF_3$, $R^1$ = Bz, $R^2$ = $R^3$ = Me

(5) R = $NHCOCF_3$, $R^1$ = Bz, $R^2$, $R^3$ = Ph, OEt



(4) R = Bz, $R^1$ = H

(6) R = $R^1$ = Bz



(8) R = OBz, $R^1$ = Bz, $R^2$ = Ac, $R^3$ = Bn

(9) R = OBz, $R^1$ = $R^3$ = Bz, $R^2$ = Ac

(10) R = Br, $R^1$ = $R^3$ = Bz, $R^2$ = Ac

(11) R = O·$C_6H_4$·$NO_2$-*p*, $R^1$ = $R^3$ = Bz, $R^2$ = Ac

(12) R = O·$C_6H_4$·$NO_2$-*p*, $R^1$ = $R^2$ = $R^3$ = H

(12) was synthesized by a novel route. Furthermore, the *p*-trifluoroacetamidophenyl glycoside (22) of *O*-β-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-α-D-galactopyranose,[11] corresponding to the backbone unit in the *Salmonella* serogroup E polysaccharides, was obtained as a by-product in the tetrasaccharide syntheses. Compounds similar to the deblocked intermediate oligosaccharides in this work have been synthesized before by other routes.[12] The synthesis of a pentasaccharide corresponding to the biological repeating unit of the *Salmonella* serogroup $D_2$ has been reported [12] using a conceptually different route to the one described here.

In the syntheses of (28), (29), and (30), the trisaccharide derivative (24), carrying protective groups on all hydroxy-groups but the mannosyl 3-OH, was first prepared. The 3,6-dideoxyhexosyl residues were then attached by using either halide-ion catalyzed [13] or silver triflate-promoted [14] glycosidation.

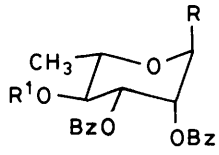
The trisaccharide (24) was constructed by two consecutive silver triflate-promoted 1,2-*trans*-glycosidations, starting from the galactosyl end. Benzoates and acetates were used for persistent blocking of hydroxy-groups, and benzyl ethers and chloroacetates for temporary blocking. The use of benzyl ethers for temporary blocking required the use of *p*-trifluoroacetamidophenyl instead of *p*-nitrophenyl as the 'handle aglycone', since the nitro-group is not inert towards hydrogenolysis conditions. The blocked *p*-trifluoroacetamidophenyl glycosides were found to crystallize well; in fact, all such derivatives in this work were crystalline compounds with relatively high melting points.

## Results and Discussion

The starting galactosyl compound chosen for the tetrasaccharide syntheses was *p*-nitrophenyl α-D-galactopyranoside.[15] This was treated with acetone and a catalytic amount of toluene-*p*-sulphonic acid to give two isomeric isopropylidene acetals which were separated by chromatography on silica gel. The 3,4-acetal (1) was obtained in a 64% yield. The compound was distinguished from its 4,6-isomer (isolated in 26% yield) by virtue of the $^1$H n.m.r. spectrum in $(CD_3)_2SO$.[16] One hydroxy-proton in the acetal (1) appeared as a triplet, showing attachment to primary carbon, whereas both hydroxy-protons in the 4,6-isomer appeared as doublets, showing attachment to secondary carbons. Subsequent benzoylation of the acetal (1) with benzoyl chloride in pyridine gave compound (2) in 93% yield. The nitro-group was then converted into a trifluoroacetamido-group by catalytic hydrogenation followed by treatment with trifluoroacetic anhydride in cold pyridine. Compound (3) was obtained in an 89% yield. Hydrolytic removal (80% aqueous acetic acid) of the 3,4-*O*-isopropylidene group in compound (3) gave the diol (4) in 82% yield. Treatment of (4) with triethyl orthobenzoate gave the orthoester (5) which was opened hydrolytically to give the expected [17] monohydroxy-derivative (6) [61% yield from (4)]. The substitution pattern of (6) was confirmed by methylation analysis,[18] using methyl triflate [19,20] for methylation.

The derivative considered as a possible rhamnose synthon was 1,2,3-tri-*O*-benzoyl-α-L-rhamnopyranose (7), a compound first synthesized by Ness, Fletcher, and Hudson [21] in a 30% yield by the partial benzoylation of L-rhamnose monohydrate. The compound was described as a rhamnose tribenzoate with unknown substitution pattern. We found that (7) could be obtained in at least 40% crystalline yield by following their procedure and we also established its structure by methylation analysis,[18] using methyl triflate [19,20] for methylation, and n.m.r. spectroscopy.

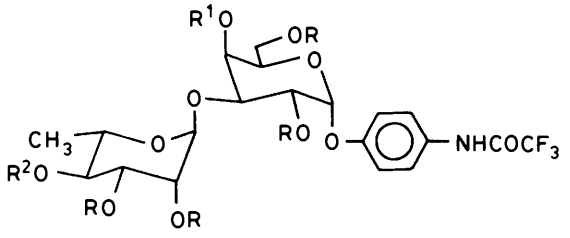
Our first attempt at synthesizing the trisaccharide (23) was based on a sequential build-up strategy, starting from the mannosyl end. Thus the rhamnosyl synthon (7) was mannosylated, using silver triflate as promotor, with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride [3] to give the disaccharide derivative (8) in 67% yield. Attempted preparation of a bromo-sugar from this compound by treatment with hydrogen bromide in acetic acid or dichloromethane failed, however. A t.l.c. analysis of the reaction mixture revealed the presence of several compounds, one of them having the same chromatographic mobility as compound (7). Apparently, cleavage of the inter-glycosidic linkage in (8) had occurred at a rate comparable to that of glycosyl bromide formation. When the benzyl groups in (8) were removed by catalytic hydrogenation and replaced by benzoyl groups, disaccharide bromide (10) formation could be achieved in good yield by treatment of (9) with hydrogen bromide in acetic acid. The higher sensitivity of the inter-glycosidic linkage in (8) towards an acidic reagent as compared to the benzoylated analogue (9) is not surprising in view of earlier observations[22] on the sensitivity of glycosides towards acids; it probably reflects the higher overall electron-withdrawing effects of *O*-acyloxy-groups compared



(7) R = OBz, $R^1$ = H

(13) R = OBz, $R^1$ = $ClCH_2CO$

(14) R = Br, $R^1$ = $ClCH_2CO$



(15) R = $R^1$ = Bz, $R^2$ = $ClCH_2CO$

(16) R = Bz, $R^1$ = H, $R^2$ = $ClCH_2CO$

(17) R = $R^1$ = Bz, $R^2$ = H

(18) R = $R^1$ = $R^2$ = H

with those of *O*-benzyl groups. The reactivity of glycosyl halides follows the same pattern.[23]

The bromide (10) was used in a silver triflate-promoted glycosidation of *p*-nitrophenol to give compound (11) in 62% yield. Deprotection gave compound (12), identical with that previously synthesized.[6]

The bromide (10) was, however, not suitable for the synthesis of the trisaccharide (24) since it lacked a temporary protecting group at 3-OH in the α-D-mannopyranosyl residue. The trisaccharide (24) was therefore constructed, not from the mannosyl end as initially envisaged, but, instead, from the galactosyl end. Temporary protection of the 4-OH in the rhamnosyl residue was obtained by chloroacetylation [24] of (7),[21] affording (13) in an 88% yield. Compound (13) was treated with hydrogen bromide in acetic acid to give the rhamnosyl bromide (14). This was used in a silver triflate-promoted glycosylation reaction with the galactosyl derivative (6). The disaccharide derivative (15) was obtained in an 86% yield. The bromide (14) was also treated with the galactosyl derivative (4) using similar glycosylation conditions. The yield of the (1→3)-linked disaccharide derivative (16) was 56%. The (1→4)-linked derivative and a dirhamnosylgalactoside were also formed, in 20 and 15% yields, respectively. Difficulties in preserving the chloroacetyl group during attempted acetylation of the product (16) made the latter route unsuitable. However, introduction of an α-(1→4)-linked glucosyl unit should be possible on (16), thus making this compound a potentially valuable intermediate for the synthesis of more complex structures representing *Salmonella* O-antigens. The chloroacetyl derivative (15) was treated with thiourea in methanol–ethyl acetate to give the monohydroxy-compound (17) in 87% yield. This compound was mannosylated with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride,[3] again using silver triflate as promotor. The trisaccharide derivative (19) was isolated in a 73% yield. A small amount of the corresponding β-mannoside derivative (20) was also isolated from the reaction mixture (2% yield). Deprotection of (19) and (20) by catalytic hydrogenation followed by treatment with methanolic sodium methoxide gave the unprotected glycosides (21) and (22), whose undecoupled $^{13}C$ n.m.r. spectra established [25] the anomeric configuration of the sugar residues as indicated in the formulae.

The triol (23), obtained in 91% yield by catalytic hydrogenation of (19), was treated with α,α-dimethoxytoluene in acidic *N*,*N*-dimethylformamide to give the key trisaccharide derivative (24) in a 73% yield.

In order to synthesize the tetrasaccharide (25), the monohydroxy-derivative (24) was engaged in a halide-ion catalyzed glycosidation reaction [13] with the 3,6-dideoxy-*xylo*-hexosyl bromide [3] (36). The yield of the desired product (25) was 61%. Deprotection by catalytic hydrogenation followed by de-*O*-acylation with methanolic sodium methoxide gave the target molecule (28) in a 55% yield from (25). The structure of (28) was confirmed by n.m.r. spectroscopy and methylation analysis.[18]

An attempt to prepare the epimeric tetrasaccharide (29) by treating (24) with the bromide (37) under analogous halide-ion catalysis conditions gave low yields of the desired product (26). Similar unsatisfactory results have been obtained before [26] with the corresponding chloride, but the reason for the difference in behaviour of (37) as compared with the bromide (36) in halide-ion catalyzed glycosidation reactions remains unclear.

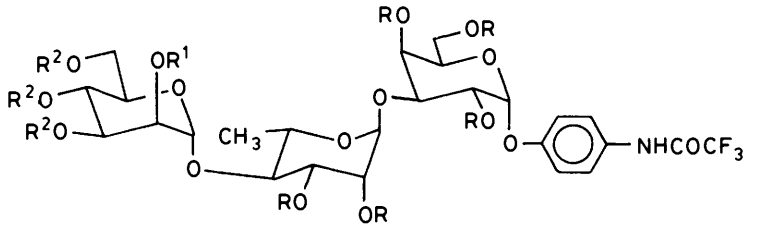
The preparation of (29) was instead performed using silver triflate-promoted glycosylation of the monohydroxy-derivative (24) with the bromide (37). Both anomeric tetrasaccharide derivatives were formed in an α/β ratio of 2.3 : 1; the yield of the desired α derivative (26) was 54%. Deprotection as above in the preparation of (28) gave the target molecule (29) [57% yield from (26)].

Finally, the tetrasaccharide glycoside (27) was prepared in a 66% yield by treating the monohydroxy-derivative (24) with the chloride (38) [26] using silver triflate as promotor. Deprotection of (27) by treatment with methanolic sodium methoxide followed by treatment with 70% aqueous trifluoroacetic acid gave the target molecule (30) in 51% yield from (27).

The preparations of the halides (36) and (37) require some further comments. Both were prepared starting from the corresponding methyl 3,6-dideoxy-α-D-hexopyranosides which were, in turn, prepared using known procedures.[27,28] Benzylation of these glycosides with benzyl bromide and sodium hydride in *N*,*N*-dimethylformamide gave the dibenzyl ethers (31) [3] and (32). Conversions of these glycosides into the glycosyl bromides (36) and (37) were effected either by direct treatment with bromotrimethylsilane [3] or by treatment of the corresponding 1-*O*-nitrobenzoates with hydrogen bromide in dichloromethane. The latter procedure gave glycosyl bromides less contaminated with by-products, and was therefore preferred. The 1-*O*-*p*-nitrobenzoates (33) and (35) were prepared from (31) and (32) by hydrolysis (80% aqueous trifluoroacetic acid) followed by treatment of the free sugars with *N*-*p*-nitrobenzoylimidazole in acetonitrile.

## Experimental

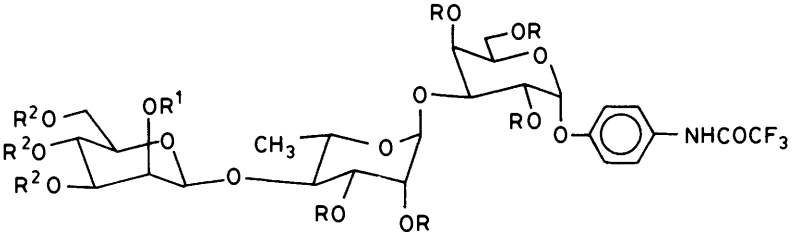
*General Methods.*—Melting points are corrected. Concentrations were performed under reduced pressure at a bath temperature below 40 °C unless otherwise stated. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter and 99.55 MHz $^1H$ and 25.02 MHz $^{13}C$ n.m.r. spectra were recorded in the Fourier-transform mode using a JEOL JNM FX 100 instrument. Chemical shifts are given in p.p.m. downfield from internal ($CDCl_3$ solutions) or external ($D_2O$ solutions) tetramethylsilane. N.m.r. spectra, recorded for all new compounds were invariably in agreement with the postulated structures; only selected n.m.r. parameters are reported below. T.l.c. was performed using pre-coated silica-gel plates ($F_{254}$,



(19) R = Bz, $R^1$ = Ac, $R^2$ = Bn

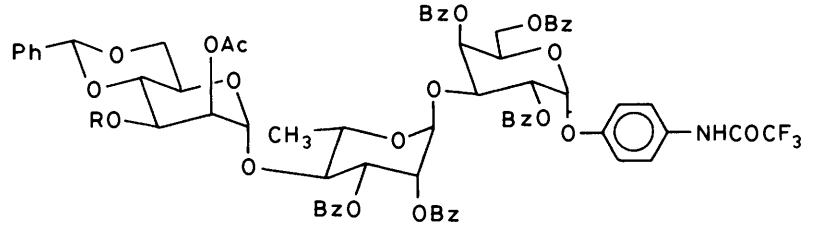
(21) R = $R^1$ = $R^2$ = H

(23) R = Bz, $R^1$ = Ac, $R^2$ = H



(20) R = Bz, $R^1$ = Ac, $R^2$ = Bn

(22) R = $R^1$ = $R^2$ = H



(24) R = H

(25) R = 2,4-Di-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl-

(26) R = 2,4-Di-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hexopyranosyl

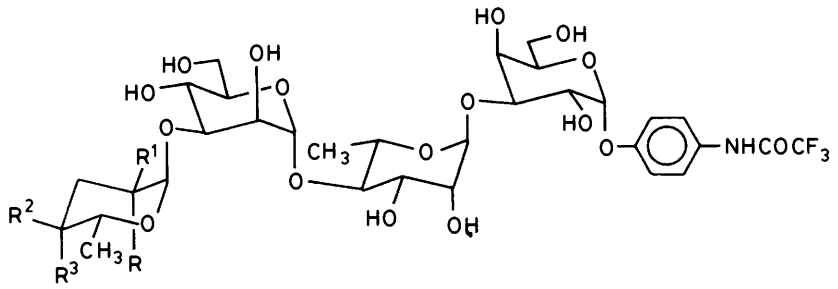
(27) R = 2,4-Di-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranosyl-

Merck) and the spots were detected with u.v. light when applicable and by charring with aqueous sulphuric acid. Column chromatography was performed on silica gel 60 (0.040—0.063 mm, Merck) in the flash mode [29] unless otherwise stated. Molecular sieves (4 Å, Union Carbide) were desiccated *in vacuo* at 300 °C overnight and ground immediately before use.

p-*Nitrophenyl* 3,4-O-*Isopropylidene*-α-D-*galactopyranoside* (1) *and* p-*Nitrophenyl* 4,6-O-*Isopropylidene*-α-D-*galactopyranoside*.—A mixture of *p*-nitrophenyl α-D-galactopyranoside [15] (10.0 g), dry acetone (200 ml), and toluene-*p*-sulphonic acid monohydrate (200 mg) was stirred at room temperature for 3 h. Addition of triethylamine (1 ml) followed by concentration left a residue, which was taken up in 1 : 1 acetone–ethyl acetate (50 ml) and applied to a column of silica gel (250 g), packed and eluted with ethyl acetate. The first fraction was the 3,4-acetal (1) (7.3 g, 64%) which was recrystallized from water, m.p. 175—177 °C, $[\alpha]_D$ +202° (*c* 0.5, $CHCl_3$) (Found: C, 52.8; H, 5.5. $C_{15}H_{19}NO_8$ requires C, 52.8; H, 5.61%). The $^1H$ n.m.r. spectrum in $(CD_3)_2SO$ showed, *inter alia*, an OH doublet at δ 5.2 and an OH triplet at 4.4. Further elution with ethyl acetate gave the 4,6-acetal (2.9 g, 26%) which was crystallized from ethyl acetate, m.p. 164—168 °C, $[\alpha]_D$ +212° (*c* 0.6, $CHCl_3$) (Found: C, 52.7; H, 5.45. $C_{15}H_{19}NO_8$ requires C, 52.8; H, 5.61%). The $^1H$ n.m.r. spectrum in $(CD_3)_2SO$ showed, *inter alia* two OH doublets at δ 4.3 and 4.8.

p-*Nitrophenyl* 2,6-*Di*-O-*benzoyl*-3,4-O-*isopropylidene*-α-D-*galactopyranoside* (2).—A solution of (1) (6.1 g, 11.1 mmol) in pyridine (60 ml) was stirred and cooled in ice while benzoyl chloride (3.5 ml, 30 mmol) was added dropwise. The mixture was left overnight at room temperature, then a piece of ice was added and stirring was continued for 30 min. Processing of the mixture by partitioning between dichloromethane and water, washing of the organic layer with 2M-sulphuric acid, and then aqueous sodium hydrogencarbonate, followed by drying ($MgSO_4$), filtration, and concentration gave a solid, which was recrystallized from ethanol. Pure compound (2) was obtained (9.1 g, 93%), m.p. 118—119 °C, $[\alpha]_D$ +141° (*c* 0.4, $CHCl_3$) (Found: C, 63.4; H, 4.9. $C_{29}H_{27}NO_{10}$ requires C, 63.4; H, 4.95%).

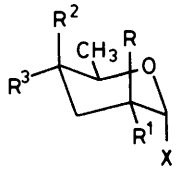
p-*Trifluoroacetamidophenyl* 2,6-*Di*-O-*benzoyl*-3,4-O-*isopropylidene*-α-D-*galactopyranoside* (3).—Hydrogenation at atmospheric pressure of (2) (7.9 g) in ethyl acetate (150 ml) using platinum(IV) oxide (Adams catalyst 0.4 g) yielded, after filtration and concentration, a syrupy amine which was directly taken up in pyridine (100 ml). Trifluoroacetic anhy-



(28) $R = R^2 = H, R^1 = R^3 = OH$

(29) $R = R^3 = H, R^1 = R^2 = OH$

(30) $R = R^2 = OH, R^1 = R^3 = H$



(31) $X = Me, R = R^3 = H, R^1 = R^2 = OBn$

(32) $X = Me, R = R^2 = H, R^1 = R^3 = OBn$

(33) $X = OOC \cdot C_6H_4 \cdot NO_2\text{-}p, R = R^3 = H, R^1 = R^2 = OBn$

(34) $X = OH, R = R^2 = H, R^1 = R^3 = OBn$

(35) $X = OOC \cdot C_6H_4 \cdot NO_2\text{-}p, R = R^2 = H, R^1 = R^3 = OBn$

(36) $X = Br, R = R^3 = H, R^1 = R^2 = OBn$

(37) $X = Br, R = R^2 = H, R^1 = R^3 = OBn$

(38) $X = Cl, R = R^3 = OBz, R^1 = R^2 = H$

dride (4.0 ml) was added dropwise while the mixture was stirred and cooled in ice. The mixture was stirred for a further 30 min at room temperature, and then processed as described in the preparation of compound (2). Recrystallization of the crude product from ethanol gave pure compound (3) (7.9 g, 89%), m.p. 180—181 °C, $[\alpha]_D$ +107° (*c* 0.5, $CHCl_3$) (Found: C, 60.4; H, 4.65; N, 2.25; F, 9.05. $C_{31}H_{28}F_3NO_9$ requires C, 60.5; H, 4.58; N, 2.28; F, 9.26%).

p-*Trifluoroacetamidophenyl* 2,6-*Di*-O-*benzoyl*-α-D-*galacto*-*pyranoside* (4).—Compound (3) (6.8 g) was treated with 80% aqueous acetic acid at 90 °C until t.l.c. showed complete conversion of (3) into a slower-migrating compound (1 h). Concentration and co-evaporations with ethanol gave a solid residue, which was recrystallized from ethanol. Pure compound (4) (5.2 g, 82%) was obtained, m.p. 116—118 °C, $[\alpha]_D$ +105° (*c* 0.4, acetone) (Found: C, 58.4; H, 4.25. $C_{28}H_{24}F_3NO_9$ requires C, 58.4; H, 4.20%).

p-*Trifluoroacetamidophenyl* 2,4,6-*Tri*-O-*benzoyl*-α-D-*galacto*-*pyranoside* (6).—The diol (4) (6.8 g) was dissolved in a mixture of triethyl orthobenzoate (40 ml) and *N*,*N*-dimethylformamide (10 ml) containing toluene-*p*-sulphonic acid monohydrate (300 mg). The mixture was then rotated on a Rotavapor (1.0 kPa) at 60 °C for 10 min. Further toluene-*p*-sulphonic acid (200 mg) was added, and rotation was resumed for another 10 min. After a third addition of acid (200 mg) and a further 10 min on the Rotavapor, the mixture was neutralized with pyridine (3 ml). Steam distillation of the reaction mixture removed most of the non-carbohydrate material (*ca.* 1.6 l of distillate was collected), and t.l.c. of the residue indicated that the initially formed orthoester had been completely converted into a new, slower-migrating compound. The residue was extracted with 2 : 1 dichloromethane–ethyl acetate, the extract was dried ($MgSO_4$), filtered, and concentrated to give a material which was subjected to chromatography on a column of silica gel (350 g), packed and first eluted with toluene–ethyl acetate (8 : 2). Further elution with toluene–ethyl acetate (7 : 3) gave fractions containing compound (6). These fractions were pooled, concentrated, and crystallized from ethyl acetate–diethyl ether–hexane to give pure compound (6) (4.9 g, 61%), m.p. 158—160 °C, $[\alpha]_D$ +92° (*c* 0.5, $CHCl_3$) (Found: C, 61.6; H, 4.2. $C_{35}H_{28}F_3NO_{10}$ requires C, 61.9; H, 4.15%), $\delta_C$ ($CDCl_3$, 25° C) 63.02 (C-6), 67.35, 68.38, 71.37, 71.57 (ring carbons except C-1) 95.65 (C-1), 117.75, 122.06, 128.32—133.71, and 154.25 (aromatic C), and 166.05, 166.46, and 166.75 p.p.m. (benzoyl CO). Methylation of (6) with methyl triflate,[19,20] debenzoylation, acid hydrolysis, sodium borohydride reduction, and acetylation gave, according to the mass spectrum,[18] 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methylgalactitol. Further elution of the column with ethyl acetate gave some starting material (4) (1.3 g, 19%).

4-O-(2-O-*Acetyl*-3,4,6-*tri*-O-*benzyl*-α-D-*mannopyranosyl*)-1,2,3-*tri*-O-*benzoyl*-α-L-*rhamnopyranose* (8).—A solution of silver triflate (3.1 g, 12.0 mmol) and *s*-collidine (0.79 ml, 6 mmol) in nitromethane–toluene (1 : 1; 10 ml) was added at −25 °C to a stirred solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride [3] (prepared from 5.1 g, 10 mmol, of orthoester precursor) and 1,2,3-tri-*O*-benzoyl-α-L-rhamnopyranose (3.7 g, 7.5 mmol) in nitromethane–toluene (1 : 1; 50 ml) containing molecular sieve. After 15 min at −25 °C more *s*-collidine (1 ml) was added, and the mixture was diluted with diethyl ether. Filtration and washing of the

filtrate with, successively, aqueous sodium thiosulphate, water, 2M-sulphuric acid, and aqueous sodium hydrogencarbonate gave, after drying ($MgSO_4$), filtration, and concentration, a syrup containing (8) as the major component. Chromatography on silica gel (350 g) using toluene–ethyl acetate (85 : 15) as eluant gave pure compound (8) (4.9 g, 67%) as a glass, $[\alpha]_D$ +40° (*c* 0.5, $CHCl_3$), $\delta_C$ ($CDCl_3$, 25 °C) 18.17 (Rha C-6), 20.91 (acetyl $CH_3$), 67.60—78.69 (ring carbons and Man C-6) 91.10 (Rha C-1), 99.22 (Man C-1), 127.28—138.39 (aromatic C), 163.79, 164.96, and 165.42 (benzoyl CO), and 170.45 p.p.m. (acetyl CO).

4-O-(2-O-*Acetyl*-3,4,6-*tri*-O-*benzoyl*-α-D-*mannopyranosyl*)-1,2,3-*tri*-O-*benzoyl*-α-L-*rhamnopyranose* (9).—Compound (8) (2.0 g) was hydrogenated overnight in ethyl acetate–ethanol (1 : 5, 120 ml) at 400 kPa using palladium on carbon (10%, 0.5 g) as catalyst. The product was filtered and concentrated and then taken up in pyridine (25 ml). Benzoyl chloride (2.0 ml) was added dropwise to the solution whilst it was stirred and cooled in ice. Processing of the reaction mixture in the usual way gave a syrup, containing (9) as the major component. Chromatography on silica gel (150 g) using toluene–ethyl acetate (40 : 1) gave pure compound (9) (1.6 g, 77%) as a glass, $[\alpha]_D$ +72° (*c* 0.5, $CHCl_3$), $\delta_C$ ($CDCl_3$, 25 °C) 18.23 (Rha C-6) 20.57 (acetyl $CH_3$), 62.09—80.00 (ring carbons and Man C-6), 91.28 (Rha C-1), 98.74 (Man C-1), 128.26—133.88 (aromatic C) 164.10—165.70 (benzoyl CO), and 169.94 p.p.m. (acetyl CO).

p-*Nitrophenyl* 4-O-(2-O-*Acetyl*-3,4,6-*tri*-O-*benzoyl*-α-D-*mannopyranosyl*)-2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranoside* (11).—Hydrogen bromide in acetic acid (30%, 5 ml) was added to a solution of (9) (1.2 g) in dichloromethane (5 ml) cooled in ice. After 2 h, t.l.c. indicated complete conversion into a slightly faster-migrating product. The mixture was diluted with dichloromethane and the solution was washed with ice-cold water and aqueous sodium hydrogencarbonate. Drying ($MgSO_4$), filtration, and concentration left a syrup, containing a major component, which was tentatively assigned the structure (10). The crude bromide (10) was taken up in 1 : 1 nitromethane–toluene (10 ml) containing *p*-nitrophenol (0.28 g) and molecular sieve. The mixture was cooled to −20 °C. A solution of silver triflate (0.39 g) in nitromethane–toluene (1 : 1, 5 ml) was added. After 10 min the mixture was processed as described for the preparation of (8). The crude product was subjected to chromatography on silica gel (150 g) using toluene–ethyl acetate (9 : 1) as eluant. Pure compound (11) (0.76 g, 62%) was obtained as a glass, $[\alpha]_D$ +45° (*c* 0.5, $CHCl_3$), $\delta_C$ ($CDCl_3$, 25 °C) 18.18 (Rha C-6), 20.57 (acetyl $CH_3$), 62.04—79.34 (ring carbons and Man C-6), 95.57 (Rha C-1), 98.69 (Man C-1) 116.43, 125.89, 128.23—133.89, 142.99, and 160.44 (aromatic C), 165.36—165.66 (benzoyl CO), and 169.99 p.p.m. (acetyl CO).

p-*Nitrophenyl* 4-O-(α-D-*Mannopyranosyl*-)-α-L-*rhamnopyranoside* (12).—Compound (11) (0.66 g) was taken up in methanolic sodium methoxide (0.1M; 25 ml). Heating to boiling point completed the reaction within a few minutes, as judged by t.l.c. Neutralization with Dowex-50 ($H^+$), filtration, and concentration gave a syrup, which was partitioned between diethyl ether and water. Lyophilization of the aqueous phase gave essentially pure compound (12) (215 mg, 75%). The $^{13}C$ and $^1H$ n.m.r. spectra were identical with the corresponding spectra of a previously synthesized [6] sample.

1,2,3-*Tri*-O-*benzoyl*-4-O-*chloroacetyl*-α-L-*rhamnopyranose* (13).—A solution of 1,2,3-tri-*O*-benzoyl-α-L-rhamnopyranose [21] (7) (7.4 g, 15 mmol) in pyridine (75 ml) was stirred and cooled to −10 °C while chloroacetyl chloride (3.4 ml, 45 mmol) was added dropwise. After 2 h a piece of ice was added and stirring was continued for 30 min. The mixture was diluted with dichloromethane and washed with water, 2M-sulphuric acid, and aqueous sodium hydrogencarbonate. Drying ($MgSO_4$), filtration, and concentration gave a syrup which was passed through a short column of silica gel (200 g) using toluene–ethyl acetate (85 : 15) as eluant to give compound (13) (7.5 g, 88%) as a syrup which crystallized. Recrystallization from methanol yielded pure compound (13), m.p. 116—118 °C, $[\alpha]_D$ +16° (*c* 0.5, $CHCl_3$) (Found: C, 62.9; H, 4.75; Cl, 6.25. $C_{29}H_{25}ClO_9$ requires C, 63.0; H, 4.56; Cl, 6.41), $\delta_C$ ($CDCl_3$, 25 °C) 17.59 (C-6), 40.40 ($CH_2Cl$), 68.66, 69.59, 69.73, and 72.53 (C-2—C-5), 91.10 (C-1) 128.48—133.91 (aromatic C), and 163.78, 165.12, 165.46, and 166.51 p.p.m. (benzoyl and chloroacetyl CO).

2,3-*Di*-O-*benzoyl*-4-*O*-*chloroacetyl*-α-L-*rhamnopyranosyl Bromide* (14).—Compound (13) (210 mg) was dissolved in dichloromethane (2.0 ml) and a solution of hydrogen bromide in acetic acid (30%, 2.0 ml) was added to it. After 2 h at room temperature, the mixture was diluted with dichloromethane and washed with, successively, ice-water and aqueous sodium hydrogencarbonate. The dried ($MgSO_4$) and filtered solution was concentrated to give syrupy (14) (190 mg, 97%), $\delta_H$ ($CDCl_3$, 25 °C) 1.41 (d, 3 H, *J* 7 Hz, 6-H), 4.01 (s, 2 H, $CH_2Cl$), 4.15—4.45 (m, 1 H, 5-H), 5.54 (t, 1 H, *J* 9 Hz, 4-H), 5.80 (dd, 1 H, *J* 1.5 and 4 Hz, 2-H), 6.02 (dd, 1 H, *J* 4 and 9 Hz, 3-H), and 6.47 (d, 1 H, *J* 1.5 Hz, 1-H). The compound was unstable and was therefore used directly in the next step.

p-*Trifluoroacetamidophenyl* 2,4,6-*Tri*-O-*benzoyl*-3-O-(2,3-*di*-O-*benzoyl*-4-O-*chloroacetyl*-α-L-*rhamnopyranosyl*)-α-D-*galactopyranoside* (15).—A mixture of compound (6) (7.5 g, 11.0 mmol) and the bromide (14) [prepared from (13) (9.1 g, 16.4 mmol)] in nitromethane–toluene (1 : 1; 100 ml) containing molecular sieve was cooled to −25 °C and stirred while a solution of silver triflate (4.5 g, 17.5 mmol) in 1 : 1 nitromethane–toluene (25 ml) was added. After 10 min, *s*-collidine (3 ml) was added, and the mixture was diluted with ethyl acetate–diethyl ether (1 : 1) and filtered. The filtrate was washed with, successively, aqueous sodium thiosulphate, water, 2M-sulphuric acid, and aqueous sodium hydrogencarbonate. Filtration, drying, and concentration left essentially pure compound (15). Application to a short column of silica gel (250 g) and elution with toluene–ethyl acetate (9 : 1) removed minor impurities. Crystallization from methanol gave pure compound (15) (10.6 g, 87%), m.p. 155—157 °C, $[\alpha]_D$ +109° (*c* 0.5, $CHCl_3$) (Found: C, 60.7; H, 4.3; Cl, 3.0. $C_{57}H_{47}ClF_3NO_{17}.H_2O$ requires C, 60.7; H, 4.38; Cl, 3.14%.) The water of crystallization appeared as a 2 H singlet at δ 1.61 in the $^1H$ n.m.r. spectrum ($CDCl_3$). $\delta_C$ ($CDCl_3$, 25 °C) 17.50 (Rha C-6), 40.53 ($CH_2Cl$), 63.09 (Gal C-6), 67.21—73.33 (ring carbons except C-1), 95.36 (Gal C-1), 99.45 (Rha C-1), 117.65, 121.97, 128.33—133.78, and 153.99 (aromatic C), and 164.86, 164.95, 166.02, 166.15, 166.27, and 166.90 p.p.m. (benzoyl and chloroacetyl CO).

*Glycosidation of the Diol* (4) *with the Bromide* (14).—The diol (4) (3.8 g, 6.61 mmol) and the bromide (14) [freshly prepared from 7.93 mmol of (13)] in nitromethane–ethyl acetate (5 : 9; 140 ml) containing molecular sieve was stirred and cooled to −30 °C while silver triflate (2.55 g, 9.92 mmol) in 5 : 1 nitromethane–toluene (18 ml) was added. After 10 min, pyridine (0.5 ml) was added and the mixture was diluted with ethyl acetate and filtered. The filtrate was washed with, successively, aqueous sodium thiosulphate, aqueous sodium chloride, 2M-sulphuric acid, and aqueous sodium hydrogen-

carbonate. Drying and filtration gave a syrup, which was subjected to chromatography on silica gel (500 g) using toluene–ethyl acetate (8 : 2) as eluant. The first fraction (1.43 g, 15%) was, according to the $^{13}C$ n.m.r. spectrum, a trisaccharide containing two rhamnosyl residues. The second fraction (1.3 g, 20%) was, according to spectral data, a disaccharide. It was assumed to be the 4-*O*-rhamnosyl isomer of (16), and was not investigated further. The third fraction (3.7 g, 56%) was pure (16) which was obtained as a glass, $\delta_C$ ($CDCl_3$, 25 °C) 17.52 (Rha C-6), 40.42 ($CH_2Cl$), 67.03—75.77 (ring carbons), 95.17 (Gal C-1), 99.43 (Rha C-1) 117.64, 121.99, 128.35—133.35, and 153.96 (aromatic C), and 164.97, 165.22, 166.29, 166.43, and 166.82 p.p.m. (benzoyl and chloroacetyl CO). A portion of (16) was deacylated with sodium methoxide in methanol to give a compound which was identical with compound (18). The (1→3) linkage in (16) is thus proved.

p-*Trifluoroacetamidophenyl* 2,4,6-*Tri*-O-*benzoyl*-3-O-(2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-α-D-*galactopyranoside* (17).—Compound (15) (9.8 g, 8.8 mmol) in ethyl acetate–methanol (1 : 1; 100 ml) was treated with thiourea (2.0 g) at room temperature overnight, followed by 1 h at 60 °C. The mixture was concentrated and partitioned between chloroform and aqueous sodium chloride. The organic layer was dried, concentrated and applied to a silica-gel column (350 g). Elution with ethyl acetate gave pure compound (17) (7.9 g, 87%), which crystallized from ethanol, had m.p. 217—220 °C, $[\alpha]_D$ +105° (*c* 0.4, $CHCl_3$) (Found: C, 63.0; H, 4.4. $C_{55}H_{46}F_3NO_{16}.H_2O$ requires C, 62.8; H, 4.60%). The water of crystallization appeared as a 2 H singlet in the $^1H$ n.m.r. spectrum of (16) in $CDCl_3$, $\delta_C$ ($CDCl_3$, 25 °C) 17.89 (Rha C-6), 63.06 (Gal C-6), 68.47—72.15 (ring carbons except C-1), 95.43 (Gal C-1, 99.25 (Rha C-1), 117.62, 122.06, 128.25—133.80, and 153.98 (aromatic C), and 165.00, 166.07, 166.14, 166.34, and 166.44 p.p.m. (benzoyl CO).

p-*Trifluoroacetamidophenyl* 3-O-(α-L-*Rhamnopyranosyl*)-α-D-*galactopyranoside* (18).—Compound (17) (0.40 g) was dissolved in 0.25M-methanolic sodium methoxide. After 2 h, the mixture was neutralized with Dowex-50 ($H^+$) and concentrated. Partitioning between water and diethyl ether and lyophilization of the aqueous phase gave pure (18) (0.16 g, 75%) which, crystallized from isopropyl alcohol, had m.p. 228—231 °C, $[\alpha]_D$ +84° (*c* 0.5, $H_2O$), $\delta_H$ ($D_2O$, 85 °C) 1.28 (d, 3 H, *J* 6.4 Hz, Rha 6-H), 3.36—4.15 (ring protons and Gal 6-H) 5.09 (broad s, Rha 1-H), 5.64 (broad s, Gal 1-H), and 7.15, 7.25, 7.43, and 7.52 (4 H, aromatic H); $\delta_C$ ($D_2O$, 25 °C) 17.93 (Rha C-6), 62.07 (Gal C-6), 68.57, 70.20, 70.37, 71.37, 72.93, 73.27, and 78.49 (ring carbons except C-1), 98.74 (Gal C-1), 103.56 (Rha C-1), and 118.89, 124.72, 130.81, and 155.66 p.p.m. (aromatic C). The CO and $CF_3$ carbons appeared as quartets centred around δ 157.78 and 117.11 p.p.m., respectively, with $J_{CF}$ spacings of 37.8 and 286.9 Hz, respectively.

p-*Trifluoroacetamidophenyl* O-(2-O-*Acetyl*-3,4,6-*tri*-O-*benzyl*-α-D-*mannopyranosyl*)-(1→4)-O-(2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1→3)-2,4,6-*tri*-O-*benzoyl*-α-D-*galactopyranoside* (19).—Compound (17) (7.2 g, 6.9 mmol) was glycosylated with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride [3] (prepared from 4.6 g of the corresponding methyl 1,2-orthoacetate by treatment with chlorotrimethylsilane for 15 min followed by evaporation) in nitromethane–toluene (1 : 1; 90 ml) at −20 °C using silver triflate (2.7 g) as promoter, essentially as described for the preparation of compound (15). The reaction time was 30 min. Column chromatography of the processed reaction mixture on silica gel (500 g), eluted in the gravity mode with toluene–ethyl acetate (8 : 2), gave first a minor fraction (0.18 g, 2%) which, according to its $^{13}C$ n.m.r. spectrum, was a trisaccharide. It was tentatively assigned the structure (20). Crystallized from chloroform–hexane the material had m.p. 105—109 °C, $[\alpha]_D$ +55° (*c* 0.5, $CHCl_3$) (Found: C, 66.7; H, 5.0. $C_{84}H_{76}F_3NO_{22}$ requires C, 66.9; H, 5.08). Further elution gave pure (19) (7.6 g, 73%). Crystallization from ethanol gave material with m.p. 97—98 °C, $[\alpha]_D$ +112° (*c* 0.5, $CHCl_3$) (Found: C, 66.6; H, 5.2. $C_{84}H_{76}F_3NO_{22}$ requires C, 66.9; H, 5.08%), $\delta_C$ ($CDCl_3$, 25 °C) 18.23 (Rha C-6) 20.96 (acetyl $CH_3$) 95.30 (Gal C-1), 99.01 and 99.47 (Man and Rha C-1) 117.53, 122.04, 128.00—138.65, and 153.91 (aromatic C), 164.83, 164.90, 166.00, 166.07, and 166.12 (benzoyl CO) and 170.72 (acetyl CO). Last to be eluted was starting material (17) (0.90 g, 13%).

p-*Trifluoroacetamidophenyl* O-(2-O-*Acetyl*-α-D-*mannopyranosyl*)-(1→4)-O-(2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1→3)-2,4,6-*tri*-O-*benzoyl*-α-D-*galactopyranoside* (23).—Compound (19) (7.2 g) in ethanol–ethyl acetate (1 : 1; 200 ml) was hydrogenated overnight at room temperature and 400 kPa using palladium on carbon (10%, 1.0 g) as catalyst. Filtration and concentration gave pure compound (23) (5.4 g, 91%) which crystallized from ethanol, m.p. 222—226 °C $[\alpha]_D$ +90° (*c* 0.5, $CHCl_3$) (Found: C, 59.7; H, 4.6. $C_{63}H_{58}F_3NO_{22}$ requires C, 61.1; H, 4.72%).

p-*Trifluoroacetamidophenyl* O-α-D-*Mannopyranosyl*-(1→4)-O-α-L-*rhamnopyranosyl*-(1→3)-α-D-*galactopyranoside* (21).—Compound (23) (310 mg) was deacylated as described for the preparation of (18) to give a material containing (21) and minor impurities. Chromatography on silica gel (35 g) using ethyl acetate–methanol–acetic acid–water (12 : 3 : 3 : 2) as eluant followed by gel filtration on a Sephadex G15 column (water elution) gave pure compound (21) (100 mg, 59%) as an amorphous powder, $[\alpha]_D$ +106° (*c* 0.5, $H_2O$), $\delta_H$ ($D_2O$, 85 °C) 1.26 (d, 3 H, $J_{5,6}$ 6.4 Hz, Rha 6-H), 4.94 (d, 1 H, $J_{1,2}$ 1.7 Hz, Man 1-H), 5.06 (d, 1 H, $J_{1,2}$ 2.0 Hz, Rha 1-H), 5.62 (broad s, 1 H, Gal 1-H) and 7.12, 7.22, 7.40, and 7.49 (4 H, aromatic H); $\delta_C$ ($D_2O$, 25 °C) 18.13 (Rha C-6), 61.94—82.51 (ring carbons and C-6), 98.74 (Gal C-1), 102.54 (Man C-1), 103.13 (Rha C-1), 118.87, and 124.62, 130.81, and 155.61 (aromatic C). The undecoupled $^{13}C$ n.m.r. spectrum showed $J_{CH}$ spacings for Gal, Man, and Rha C-1 carbons of 173, 168, and 170 Hz, respectively, indicative [25] of α configurations at all anomeric centres.

p-*Trifluoroacetamidophenyl* O-β-D-*Mannopyranosyl*-(1→4)-O-α-L-*rhamnopyranosyl*-(1→3)-α-D-*galactopyranoside* (22).—Compound (20) (150 mg) was hydrogenated with palladium on carbon (100 mg) in ethanol–ethyl acetate (1 : 1; 20 ml) at 400 kPa overnight; the filtered and concentrated residue was deacylated with 0.25M-methanolic sodium methoxide, as described in the preparation of compound (18). The residue after lyophilization (70 mg, 100%) was essentially pure compound (22) as an amorphous powder, $[\alpha]_D$ + 57° (*c* 0.7, $H_2O$), $\delta_H$ ($D_2O$, 85 °C) 1.34 (d, 3 H, $J_{5,6}$ 5.9 Hz, Rha 6-H), 4.87 (broad s, 1 H, Man 1-H), 5.10 (broad s, 1 H, Rha 1-H) 5.66 (broad s, 1 H, Gal 1-H), and 7.17, 7.27, 7.45, and 7.54 (4 H, aromatic H); $\delta_C$ ($D_2O$, 25 °C) 18.28 (Rha C-6), 61.94—80.98 (ring carbons and C-6), 98.74 (Gal C-1), 101.91 (Man C-1), 103.56 (Rha C-1), and 118.96, 124.86, 130.81, and 155.71 p.p.m. (aromatic C). The undecoupled $^{13}C$ n.m.r. spectrum showed $J_{CH}$ spacings for Gal, Man, and Rha C-1 carbons of 173, 161, and 171 Hz, respectively, indicative [25] of α-β-α-configurations.

p-*Trifluoroacetamidophenyl* O-(2-O-*Acetyl*-4,6-O-*benzylidene*-α-D-*mannopyranosyl*)-(1→4)-O-(2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1→3)-2,4,6-*tri*-O-*benzoyl*-α-D-*galacto*-

*pyranoside* (24).—Compound (23) (4.9 g) was treated with α,α-dimethoxytoluene (10 ml) in acetonitrile (50 ml) and *N,N*-dimethylformamide (50 ml) containing toluene-*p*-sulphonic acid monohydrate (100 mg) for 5 h at room temperature. The reaction mixture was neutralized with *s*-collidine (3 ml) and concentrated (0.5 kPa, 40 °C) to a residue which was subjected to chromatography on a silica gel column (450 g), eluted with toluene–ethyl acetate (7 : 3). The product (24) (3.8 g, 73%) crystallized from ethyl acetate–hexane, m.p. 139—141 °C, $[\alpha]_D$ +107° (*c* 0.4, $CHCl_3$) (Found: C, 63.5; H, 4.85. $C_{70}H_{62}F_3NO_{22}$ requires C, 63.4; H, 4.71%), $\delta_C$ ($CDCl_3$, 50 °C) 18.32 (Rha C-6), 20.81 (acetyl $CH_3$), 63.21 and 64.19 (Gal and Man C-6), 66.82—80.42 (ring carbons), 95.82 (Gal C-1), 99.47 and 99.81 (Man and Rha C-1), 102.10 (acetal C), 117.84, 122.23, 126.42—137.49, and 154.25 (aromatic C), 164.97—166.39 (benzoyl CO), and 170.63 p.p.m. (acetyl CO).

2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-1-O-p-*nitrobenzoyl*-β-D-xylo-*hexopyranose* (33).—A solution of methyl 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranoside (31) [3] (3.3 g) in 80% aqueous trifluoroacetic acid (20 ml) was kept at 60 °C for 2 h. Concentration followed by several co-evaporations with ethanol gave a syrupy residue which was subjected to chromatography on a silica gel column (130 g), using toluene–ethyl acetate (15 : 35) as eluant. The fractions containing the main product were concentrated to give a syrup (2.0 g), which was taken up in acetonitrile (100 ml). A solution of *N*-*p*-nitrobenzoylimidazole (prepared [30] from 2.0 g of imidazole and 2.41 g of *p*-nitrobenzoyl chloride) in dichloromethane (35 ml) was added, and the mixture was heated to 65 °C for 48 h. Dilution with diethyl ether, washing with water, 2M-sulphuric acid, and aqueous sodium hydrogencarbonate, drying ($MgSO_4$), filtration, and concentration left a solid, which was taken up in methanol. A total of 1.8 g (39%, calculated from the methyl glycoside) of (33) was obtained, m.p. 104—106 °C, $[\alpha]_D$ −57° (*c* 0.5, $CHCl_3$) (Found: C, 67.95; H, 5.7; N, 2.8. $C_{27}H_{27}NO_7$ requires C, 67.9; H, 5.70; N, 2.93%). The $^1H$ n.m.r. spectrum in $CDCl_3$ showed a doublet ($J_{1,2}$ 6.0 Hz) centred at δ 5.84, indicative of the β-configuration.

*Methyl* 2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-α-D-ribo-*hexopyranoside* (32).—A solution of methyl 3,6-dideoxy-α-D-*ribo*-hexopyranoside [28] (2.0 g) in *N,N*-dimethylformamide (25 ml) was benzylated with sodium hydride (50% in oil; 1.5 g) and benzyl bromide (3.0 ml), essentially as previously described.[3] Chromatography on a silica gel column (250 g) using toluene–ethyl acetate (85 : 15) as eluant gave pure syrupy compound (32) (3.2 g, 76%), $[\alpha]_D$ +93° (*c* 1.0, $CHCl_3$), $\delta_C$ ($CDCl_3$, 25 °C) 17.74 (C-6), 29.97 (C-3), 54.66 ($OCH_3$), 66.94, 70.57, 70.91, 74.08, and 77.78 (C-2, C-4, C-5, 2 benzyl $CH_2$), 96.96 (C-1), and 127.62, 127.74, 128.35, 138.17, and 138.24 p.p.m. (aromatic C).

2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-α-D-ribo-*hexopyranose* (34).—Compound (32) (2.0 g) was hydrolyzed with aqueous trifluoroacetic acid as described in the preparation of (33). Chromatography on a silica gel column (100 g) using toluene–ethyl acetate (8 : 2) as eluant gave a solid material (1.48 g, 77%) which was recrystallized from diethyl ether–hexane, m.p. 90—92 °C, $[\alpha]_D$ +56° ⟶ +55° (24 h, *c* 0.3, $CHCl_3$) (Found: C, 73.0; H, 7.55. $C_{20}H_{24}O_4$ requires C, 73.2; H, 7.37%.

2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-1-O-p-*nitrobenzoyl*-β-D-ribo-*hexopyranose* (35).—Compound (34) (1.28 g) in acetonitrile (70 ml) was mixed with a solution of *p*-nitrobenzoylimidazole [prepared [30] from imidazole (1.28 g) and *p*-nitrobenzoyl chloride (1.54 g)] in dichloromethane (15 ml). After being heated to 65 °C for 48 h, the mixture was processed as described for compound (33). Crystallization from methanol gave compound (35) (0.95 g, 56%), m.p. 115—117 °C, $[\alpha]_D$ −8° (*c* 0.5, $CHCl_3$) (Found: C, 67.9; H, 5.75; N, 2.85. $C_{27}H_{27}NO_7$ requires C, 67.9; H, 5.70; N, 2.93). The $^1H$ n.m.r. spectrum in $CDCl_3$ showed a doublet at δ 5.87 ($J_{1,2}$ 8.3 Hz), indicative of the β-configuration.

2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-α-D-xylo-*hexopyranosyl Bromide* (36) *and* 2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-α-D-ribo-*hexopyranosyl Bromide* (37).—The above bromides were highly unstable and were therefore prepared immediately before use in the glycosylation reaction. A solution of the corresponding 1-*O*-β-*p*-nitrobenzoates (33) or (35) in dichloromethane was saturated with dry hydrogen bromide at 0 °C. When t.l.c. indicated complete conversion (<10 min), the mixture was quickly filtered and the filtrate concentrated.

p-*Trifluoroacetamidophenyl* O-(2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-α-D-xylo-*hexopyranosyl*)-(1→3)-O-(2-O-*acetyl*-4,6-O-*benzylidene*-α-D-*mannopyranosyl*)-(1→4)-O-(2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1→3)-2,4,6-*tri*-O-*benzoyl*-α-D-*galacto-pyranoside* (25).—A mixture of compound (24) (900 mg, 0.68 mmol), tetraethylammonium bromide (200 mg), molecular sieve, and the bromide (36) [freshly prepared from 1.36 mmol of precursor (33)] in dichloromethane (10 ml) was stirred at room temperature for 48 h. Then pyridine (1 ml) was added and stirring was continued for a few hours. Filtration followed by washing with aqueous sodium hydrogencarbonate and concentration gave a syrup which was subjected to chromatography in the gravity mode on a silica gel column (150 g). Toluene–ethyl acetate (8 : 2) eluted pure compound (25) (680 mg, 61%) as the main band. Crystallization from ethyl acetate–hexane gave compound (25), m.p. 187—188 °C, $[\alpha]_D$ +103° (*c* 0.4, $CHCl_3$) (Found: C, 66.0; H, 5.2. $C_{90}H_{84}F_3NO_{25}$ requires C, 66.1; H, 5.17%), $\delta_C$ ($CDCl_3$, 50 °C) 16.42 (Abe C-6), 18.23 (Rha C-6), 20.91 (acetyl $CH_3$), 28.12 (Abe C-3), 63.16, 64.53 (Gal and Man C-6), 66.72—80.66 (ring carbons and benzyl $CH_2$), 95.72 (Gal C-1), 97.13 (Abe C-1), 99.52 and 100.20 (Man and Rha C-1), 102.01 (acetal C), 117.80, 122.08, 126.33—138.70, and 154.20 (aromatic C) 164.73—166.24 (benzoyl CO), and 170.43 (acetyl CO).

p-*Trifluoroacetamidophenyl* O-(2,4-*Di*-O-*benzyl*-3,6-*dideoxy*-α-D-ribo-*hexopyranosyl*)-(1→3)-O-(2-O-*acetyl*-4,6-O-*benzylidene*-α-D-*mannopyranosyl*)-(1→4)-O-(2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1→3)-2,4,6-*tri*-O-*benzoyl*-α-D-*galacto-pyranoside* (26).—A mixture of compound (24) (600 mg, 0.45 mmol), molecular sieve, and the bromide (37) [freshly prepared from the precursor (35) (0.62 mmol)] in acetonitrile–dichloromethane (3 : 1; 4 ml) was cooled to −40 °C and silver triflate (180 mg, 0.70 mmol) in acetonitrile (1 ml) was added. After 5 min, the mixture was processed as described for compound (15) to give, after concentration, a syrup which was subjected to column chromatography in the gravity mode on silica gel (150 g) using toluene–ethyl acetate (8 : 2) as eluant. The first fraction (170 mg, 23%) crystallized from ethyl acetate–hexane, and had m.p. 185—188 °C, $[\alpha]_D$ +95° (*c* 0.4, $CHCl_3$) (Found: C, 65.9; H, 5.25. $C_{90}H_{84}F_3NO_{25}$ requires C, 66.1; H, 5.17%), $\delta_C$ ($CDCl_3$, 50 °C) 17.96 (Par C-6), 18.28 (Rha C-6), 20.88 (acetyl $CH_3$), 34.90 (Par C-3), 63.19 and 64.72 (Gal and Man C-6), 67.65—80.54 (ring carbons and benzyl ($CH_2$), 95.74 (Gal C-1) 99.50 and 99.93 (Man and Rha C-1), 101.69 (acetal C), 103.22 (Par C-1), and 117.87, 122.13, 126.45—138.88, and 154.30 (aromatic C), 164.78—166.27 (benzoyl CO), and 170.41 (acetyl CO). According to the $^{13}C$ n.m.r. spectrum, this was the β-paratosyl analogue of compound (26). The second fraction (400 mg, 54%) was compound (26) which crystallized from ethyl acetate–hexane, had m.p.

193—196 °C, $[\alpha]_D$ +121° (*c* 0.5, $CHCl_3$) (Found: C, 65.9; H, 5.2. $C_{90}H_{84}F_3NO_{25}$ requires C, 66.1; H, 5.17%), $\delta_C$ ($CDCl_3$, 50 °C) 17.69 (Par C-6), 18.18 (Rha C-6), 20.91 (acetyl $CH_3$), 30.41 (Par C-3), 63.16 and 64.62 (Gal and Man C-6), 67.99—80.71 (ring carbons and benzyl $CH_2$), 95.72 (Gal C-1), 96.55 (Par C-1), 99.52 and 100.06 (Man and Rha C-1), 102.12 (acetal C), 117.80, 122.13, 126.42—138.56, and 154.20 (aromatic C), 164.78—166.19 (benzoyl CO), and 170.43 (acetyl CO).

p-*Trifluoroacetamidophenyl* O-(2,4-*Di*-O-*benzoyl*-3,6-*dideoxy*-α-D-arabino-*hexopyranosyl*)-(1→3)-O-(2-O-*acetyl*-4,6-O-*benzylidene*-α-D-*mannopyranosyl*)-(1→4)-O-(2,3-*di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1→3)-2,4,6-*tri*-O-*benzoyl*-α-D-*galactopyranoside* (27).—A mixture of compound (24) (900 mg, 0.68 mmol), molecular sieve, and the chloro-sugar (38) [freshly prepared [26] from the dibenzoyl α-methyl glycoside (1.0 mmol)] in nitromethane–toluene (1 : 1; 7 ml) was stirred and cooled to −25 °C. A solution of silver triflate (300 mg, 1.17 mmol) in nitromethane–toluene (1 : 1, 3 ml) was quickly added. After 5 min, the reaction mixture was neutralized with pyridine (1 ml) and the cooling bath was removed. The diluted (1 : 1, diethyl ether–ethyl acetate) reaction mixture was filtered and the filtrate was washed with, successively, aqueous sodium thiosulphate, water, 2M-sulphuric acid, and aqueous sodium hydrogencarbonate. Drying, filtration and concentration left a syrup, which was taken up in ethyl acetate. A mixture of diethyl ether and hexane was added to turbidity. Chromatographically homogeneous crystals of compound (27) (750 mg, 66%) were obtained. The pure compound had m.p. 238—240 °C, $[\alpha]_D$ +71° (*c* 0.4, $CHCl_3$) (Found: C, 64.8; H, 4.9. $C_{90}H_{80}NO_{27}F_3$ requires C, 64.9; H, 4.84%), $\delta_C$ ($CDCl_3$, 50 °C) 17.84 (Tyv C-6), 18.32 (Rha C-6), 20.91 (acetyl $CH_3$), 29.58 (Tyv C-3), 63.07 and 64.53 (Gal and Man C-6), 67.45—80.37 (ring carbons), 95.72 (Gal C-1), 97.47 (Tyv C-1), 99.57 and 99.86 (Man and Rha C-1), 101.13 (acetal C), 117.80, 122.13, 126.03, 127.93—137.49, and 154.20 (aromatic C), 164.78—166.29 (benzoyl CO), and 170.24 (acetyl CO).

p-*Trifluoroacetamidophenyl* O-(3,6-*Dideoxy*-α-D-xylo-*hexopyranosyl*)-(1→3)-O-α-D-*mannopyranosyl*-(1→4)-O-α-L-*rhamnopyranosyl*-(1→3)-α-D-*galactopyranoside* (28).—A solution of compound (25) (0.55 g) in ethyl acetate–ethanol (1 : 1; 30 ml) was hydrogenated overnight at 400 kPa using palladium on carbon (10%; 0.35 g) as catalyst. The filtrate was concentrated and taken up in methanolic sodium methoxide (0.25M; 20 ml). After 3 h, the mixture was neutralized with Dowex-50 ($H^+$) and concentrated. Chromatography on a silica gel column (30 g) using ethyl acetate–acetic acid–methanol–water (12 : 3 : 3 : 2) as eluant gave fractions containing pure compound (28). These were pooled and concentrated and the material so obtained was gel-filtered on a Sephadex G-15 column. Pure compound (28) (155 mg, 55%) was obtained, as an amorphous solid, $[\alpha]_D$ +100° (*c* 0.7, $H_2O$). $\delta_H$ ($D_2O$, 85 °C) 1.19 (d, 3 H, $J_{5,6}$ 6.8 Hz, Abe 6-H), 1.36 (d, 3 H, $J_{5,6}$ 6.4 Hz, Rha 6-H), 1.70—2.30 (m, 2 H, Abe 3-H, 3′-H), 5.04 (d, 1 H, $J_{1,2}$ 2.0 Hz, Man 1-H), 5.13 (d, 1 H, $J_{1,2}$ 4.4 Hz, Abe 1-H), 5.16 (d, 1 H, $J_{1,2}$ 2.0 Hz, Rha 1-H), 5.70 (broad s, 1 H, Gal 1-H), and 7.21, 7.31, 7.49, and 7.58 (aromatics, 4 H); $\delta_C$ ($D_2O$, 25 °C) 16.76 (Abe C-6), 18.30 (Rha C-6), 34.26 (Abe C-3), 62.02 and 62.11 (Man and Gal C-6), 64.84—82.90 (ring carbons except Abe C-3 and all C-1), 98.81 (Gal C-1), 101.61 (Abe C-1), 102.57 (Man C-1), 103.27 (Rha C-1), and 118.99, 124.81, 130.88, and 155.74 p.p.m. (aromatic C). The CO and $CF_3$ carbon signals appeared as quartets, centred around δ 157.83 and 117.18, respectively with $J_{CF}$ spacings of 37.2 and 286.9 Hz, respectively. Methylation analysis [18] yielded 1,5-di-*O*-acetyl-2,4-di-*O*-methylabequitol, 1,4,5-tri-*O*-acetyl-2,3,di-*O*-methylrhamnitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol in an approximately 1 : 1 : 1 : 1 ratio. The substitution pattern of (28) and also of its trisaccharide precursors (24), (19), and (23) is thus proved.

p-*Trifluoroacetamidophenyl* O-(3,6-*Dideoxy*-α-D-ribo-*hexopyranosyl*)-(1→3)-O-α-D-*mannopyranosyl*-(1→4)-O-α-L-*rhamnopyranosyl*-(1→3)-α-D-*galactopyranoside* (29).—Deprotection of compound (26) (200 mg), carried out essentially as described for (28), gave pure compound (29) (58 mg, 57%) as an amorphous solid, $[\alpha]_D$ +119° (*c* 0.5, $H_2O$), $\delta_H$ ($D_2O$, 85 °C) 1.16 (d, 3 H, $J_{5,6}$ 6.3 Hz, Par 6-H), 1.28 (d, 3 H, $J_{5,6}$ 6.3 Hz, Rha 6-H), 1.55—2.25 (m, 2 H, Par 3-H, 3′-H), 4.96 (d, 1 H, $J_{1,2}$ 2.0 Hz, Man 1-H), 5.00 (d, 1 H, $J_{1,2}$ 4.6 Hz, Par 1-H), 5.08 (d, 1 H, $J_{1,2}$ 1.4 Hz, Rha 1-H), 5.62 (broad s, Gal 1-H), 7.14, 7.23, 7.41, and 7.50 (4 H, aromatics); $\delta_C$ ($D_2O$, 25 °C) 17.64 (Par C-6), 18.18 (Rha C-6), 35.72 (Par C-3), 61.85, 62.09 (Man and Gal C-6), 66.96—82.75 (ring carbons except Par C-3 and all C-1), 98.74 (Gal C-1), 100.79 (Par C-1), 102.54 (Man C-1), 103.27 (Rha C-1), and 118.96, 125.01, 131.00, and 155.66 (aromatic C). The CO and $CF_3$ carbons appeared as quartets centred around 157.94 and 117.17, respectively, with $J_{C-F}$ spacings of 37.2 and 287.0 Hz, respectively.

p-*Trifluoroacetamidophenyl* O-(3,6-*Dideoxy*-α-D-arabino-*hexopyranosyl*)-(1→3)-O-α-D-*mannopyranosyl*-(1→4)-O-α-L-*rhamnopyranosyl*-(1→3)-α-D-*galactopyranoside* (30).—Compound (27) (0.50 g) was taken up in methanolic sodium methoxide (0.25M; 5 ml). After 4 h, the mixture was neutralized with Dowex-50 ($H^+$), concentrated and taken up in 70% aqueous trifluoroacetic acid (5 ml). After 5 min, the mixture was concentrated and the residue was subjected to column chromatography and gel-filtration as described for compound (28). Pure compound (30) (130 mg, 51%) was obtained, as an amorphous solid, $[\alpha]_D$ +107° (*c* 0.5, $H_2O$), $\delta_H$ ($D_2O$, 85 °C) 1.15 (d, 3 H, $J_{5,6}$ 6.1 Hz, Tyv 6-H), 1.23 (d, 3 H, $J_{5,6}$ 6.1 Hz, Rha 6-H), 1.50—2.20 (m, 2 H, Tyv 3-H, 3′-H), 4.78 (broad s, Tyv 1-H), 4.90 (broad s, Man 1-H), 5.02 (broad s, Rha 1-H), 5.58 (broad s, Gal 1-H), and 7.10, 7.19 7.37, and 7.46 (4 H, aromatics); $\delta_C$ ($D_2O$, 25 °C) 18.06 (Tyv C-6), 18.28 (Rha C-6), 34.70 (Tyv C-3), 61.94 and 62.11 (Gal and Man C-6), 67.06—82.85 (ring carbons except Tyv C-3 and all C-1), 98.74 (Gal C-1), 102.49 (Tyv C-1), 102.59 (Man C-1), 103.27 (Rha C-1), and 118.96, 124.94, 130.81, 155.71 p.p.m. (aromatic C). The CO and $CF_3$ carbons appeared as quartets, centred around δ 157.94 and 117.17 p.p.m., respectively, with $J_{C-F}$ spacings of 37.2 and 286.9 Hz, respectively.

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