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Synthesis of the Branchpoint Tetrasaccharide of the *O*-Specific Determinant of *Salmonella* Serogroup B

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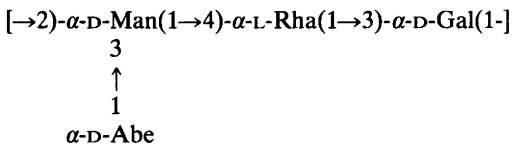
The tetrasaccharide 4-*O*-(3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-2-*O*-(α-D-galactopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranose, the repeating unit of the *O*-specific determinant of *Salmonella* serogroup B, has been synthesised using silver trifluoromethanesulfonate (triflate) as the promoter in all glycosylation reactions. The reducing end of the tetrasaccharide was substituted with a linking arm for attachment to a protein carrier. Reaction of 8-methoxycarbonyloct-1-yl 2,3-*O*-cyclohexylidene-α-L-rhamnopyranoside (*2*) with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (*1*) in the presence of silver triflate gave 8-methoxycarbonyloct-1-yl 4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,3-*O*-cyclohexylidene-α-L-rhamnopyranoside (*3*) (79 % yield). Deacetylation of *3* followed by reaction with 6-*O*-acetyl-2-*O*-allyl-3,4-di-*O*-benzoyl-α-D-galactopyranosyl bromide (*5*) gave the trisaccharide (*6*) in 88 % yield. Reprotection of (*6*) gave the trisaccharide (*8*), ready for glycosylation at the 3-position of the mannose unit. Reaction of (*8*) with 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl chloride (*9*) gave the tetrasaccharide (*10*) in 62 % yield. Finally deprotection of *10* afforded the desired tetrasaccharide (*11*) in 71 % yield.

The repeating units of the *O*-specific chains of *Salmonella* serogroups A, B and D, are characterised by a common linear backbone trisaccharide substituted by 3,6-dideoxy-α-D-*ribo*-hexopyranose (paratose), 3,6-dideoxy-α-D-*xylo*-hexopyranose (abequose) or 3,6-dideoxy-α-D-*arabino*-hexopyranose (tyvelose), respectively, with the 3,6-dideoxyhexoses as the immunodominant part.[1] The *O*-specific repeating unit of *S. typhimurium*, belonging to serogroup B, has the structure:



Many attempts to synthesise antigenic determinants possessing the partial structure of this polysaccharide have drawn attention to the problems encountered in such syntheses [1–7] and have, at the same time, provided valuable insight into the immunological specificities of antibodies directed toward the antigens.[1]

Recently Lindberg *et al.*[8] have isolated the branchpoint tetrasaccharide from *S. typhimurium* by phage degradation of the polysaccharide and compared the immunological activity of this tetrasaccharide with that of the dimeric octasaccharide and the trimeric dodecasaccharide in an inhibition study. The activity of the tetrasaccharide was shown to be approximately half the activity of the octasaccharide. However, in the inhibition study the mutarotated mixture of the free oligosaccharides was used; a test, however, with the glycoside (*11*) might give different results. Precipitation studies with the same oligosaccharides linked to a protein carrier[9] confirmed the above results but in the linking process the rhamnopyranose unit is modified to an open-chain aldonic acid amide; conceivably, this rupture of the integrity of the antigen might be

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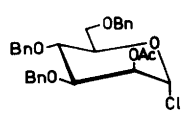

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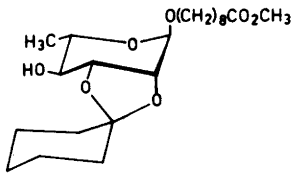

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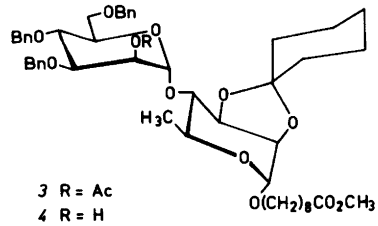
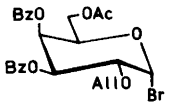


3 R = Ac
4 R = H



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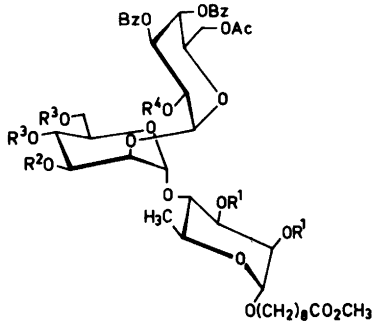

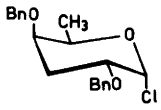

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6 R^1 = Cyclohexylidene, $R^2 = R^3$ = Bn, R^4 = All
7 $R^1 = R^4$ = Ac, $R^2 = R^3$ = Bn
8 $R^1 = R^4$ = Ac, R^2 = H, R^3 = Cyclohexylidene

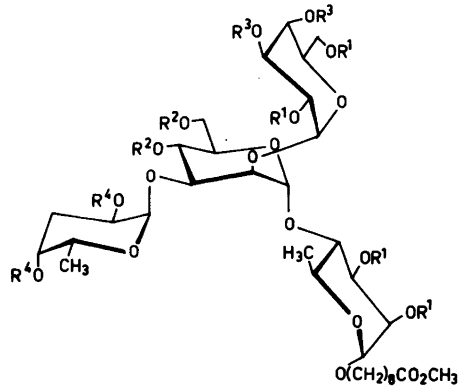
crucial to the interpretation of the results obtained. The study of a glycosidic antigen such as (*11*) might indicate if such problems are to be considered.

In order to study this potential branchpoint antigen we have developed a synthetic route to the tetrasaccharide (*11*) involving a suitably protected backbone trisaccharide which by glycosylation with other deoxyhexoses can yield modified antigens or antigens of *Salmonella* serogroups A and D_1.

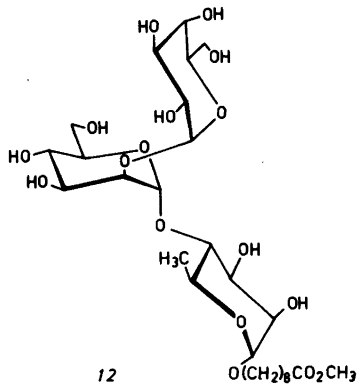
RESULTS AND DISCUSSION

In the present synthesis recent methodology by Garegg[3–7] and by Bock and Meldal[2,10] was utilized in order to prepare a *Salmonella* serogroup B tetrasaccharide antigen.

Reaction of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[5] (*1*) with 8-methoxycarbonyloct-1-yl 2,3-*O*-cyclohexylidene-α-L-rhamnopyranoside[10] (*2*), promoted by silver trifluoromethanesulfonate gave a 79 % yield of the α-linked disaccharide (*3*). Transesterification produced the disaccharide (*4*) in 95 % yield, ready for glycosylation in the 2-position of the mannose unit. Reaction of *4* with 6-*O*-acetyl-2-*O*-allyl-3,4-di-*O*-benzoyl-α-D-galactopyranosyl bromide[10] (*5*), again promoted by silver trifluoromethanesulfonate, gave an 88 % yield of the trisaccharide (*6*). Mercury(II) iodide catalysis[10] at ambient temperature was attempted but con-



10 R^1 = Ac, R^2 = Cyclohexylidene, R^3 = Bz, R^4 = Bn
11 $R^1 = R^2 = R^3 = R^4 = H$



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siderable amounts of trisaccharides containing a β-linked galactopyranosyl unit could be isolated together with the corresponding α-anomer. Furthermore, by-products were observed and the total yield of trisaccharide did not exceed 30 %.

The allyl group of the trisaccharide (*6*) was isomerized by tris(triphenylphosphine)rhodium chloride as described by Gigg and Gent.[11] The reaction was accompanied by the formation of 15 % of trisaccharide containing a propyl group. Furthermore, cleavage of the l-propenyl ether by the conventional reaction with mercury chloride and mercury oxide,[12] usually an instantaneous reaction, took 7 h and required a large excess of reagent to give the cleavage product in 78 % yield.

The cyclohexylidene group of the rhamnose unit in *6* was removed with acetic acid and water and the product was acetylated with acetic anhydride in pyridine containing a small amount of 4-dimethylaminopyridine giving 70 % of the fully protected trisaccharide (*7*).

Hydrogenolysis of *7* over palladium on charcoal in methanol and acetic acid gave the debenzylated product quantitatively. This was reacted with 2 equivalents of l-ethoxycyclohexene[13] in acetonitrile containing a catalytic amount of *p*-toluenesulfonic acid and the trisaccharide (*8*), ready for glycosylation at the 3-position of the mannose unit, was obtained in 92 % yield.

The reaction of *8* with 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl chloride[2] (*9*), catalysed by silver trifluoromethanesulfonate and *N,N'*-tetramethylurea gave a tetrasaccharide mixture containing 82 % of (*10*) and 18 % of the β-D-*xylo*-hexopyranosyl isomer according to a ^{13}C NMR spectrum.

The mixture could be separated chromatographically giving 62 % of *10* and 14 % of the β-D-*xylo*-hexopyranosyl isomer.

Deprotection of *10* by conventional methods involving hydrogenolysis over palladium on charcoal, hydrolysis with aqueous acetic acid and transesterification gave the tetrasaccharide (*11*) in 71 % yield. Transesterification of *7* gave the corresponding deprotected trisaccharide (*12*), useful as a model compound in ^1H and ^{13}C NMR spectroscopy.

The assignment of ^1H and ^{13}C NMR spectra was done using a combination of selective proton decoupled ^{13}C NMR spectra, coupled ^{13}C NMR spectra, proton decoupled ^1H NMR spectra, partially relaxed ^1H NMR spectra and ^1H NMR nuclear Overhauser enhancement (NOE) experiments.

The synthesis described above concerns the branchpoint tetrasaccharide of the *O*-specific chain of *Salmonella* serogroup B. Similarly, the trisaccharide *8* should provide access to serogroups A and D_1 antigens as well as antigens containing modified deoxyhexoses which may be important in serological studies. The synthesis of these compunds will be described in a forthcoming publication.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141

polarimeter. NMR spectra were obtained on Bruker WH-90, HX-270 and WM-400 NMR instruments. The spectra of protected compound were measured in $CDCl_3$ and non-protected products in D_2O. Acetone (δ 2.12) was used as internal reference for ^1H NMR spectra and dioxane (67.4 ppm) for ^{13}C NMR spectra in D_2O. Microanalyses were performed by NOVO microanalytical laboratory. TLC was performed on silica gel-coated plates (Merck F-254) and detection was done by charring with sulfuric acid.

8-Methoxycarbonyloct-1-yl 4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-O-cyclohexylidene-α-L-rhamnopyranoside (3). 8-Methoxycarbonyloct-1-yl 2,3-*O*-cyclohexylidene-α-L-rhamnopyranoside[10] (*2*) (5.9 g, 14 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[5] (*1*) (9.2 g, 18 mmol) were dissolved in toluene–nitromethane (1:1, 80 ml) and the solution was stirred with molecular sieves (4Å, 6 g) under an atmosphere of nitrogen at −50 °C for 2 h. Anhydrous silver trifluoromethanesulfonate (5.5 g, 20 mmol) was dissolved in the same solvent (30 ml) and was added with stirring at −50 °C. The mixture was stirred for 30 min at −40 °C and neutralised by the addition of collidine (3.5 ml). Stirring was continued for 5 min and the mixture was diluted with ethyl acetate (200 ml), filtered through celite and washed with sodium thiosulfate solution (10 %, 100 ml), water (100 ml), cold sulfuric acid (1 M, 50 ml) and saturated sodium hydrogencarbonate solution. Drying (sodium sulfate) and evaporation gave a syrup which was purified by chromatography on a silica gel column (ethyl acetate–pentane: 23:77). The main fraction gave 9.76 g (79 %) of *3* $[\alpha]_D^{20}$ +16° (*c* 0.5, $CHCl_3$). Analysis for $C_{51}H_{58}O_{13}$: C,H. ^1H NMR: δ 4.98 (H-1,1); 5.28 (H-2.1); 3.98 (H-3.1); 4.08 (H-4.1); 4.11 (H-5.1); 3.90 (H-6.1); 3.71 (H-6′.1); 4.92 (H-1.2); 4.04 (H-2.2); 4.02 (H-3.2); 3.34 (H-4.2); 3.64 (H-5.2); 1.25 (H-6.2). $J_{12.1}$ 1.8 Hz; $J_{23.1}$ 3.1; $J_{34.1}$ 9.0; $J_{45.1}$ 9.0; $J_{56.1}$ 2.3; $J_{56'.1}$ 1.2; $J_{66'.1}$ 10.9; $J_{12.2}$ 0; $J_{23.2}$ 6.5; $J_{34.2}$ 6.5; $J_{45.2}$ 9.5; $J_{56.2}$ 6.5.

8-Methoxycarbonyloct-1-yl 4-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-O-cyclohexylidene-α-L-rhamnopyranoside (4). Compound (*3*) (9.47 g, 10.4 mmol) was dissolved in methanolic sodium methoxide (0.1 M, 100 ml) and the solution was stirred for 3 h at 30 °C. Sodium ions were removed from the solution by stirring with ion-exchange resin (Amberlite IRC 50) for 1 h. Filtration, evaporation and purification on a silica gel column (ethyl acetate–pentane: 1:2) gave 8.70 g (95 % yield) of *4*. $[\alpha]_D^{20}$ +35 ° (*c* 0.3, $CHCl_3$). ^1H NMR: δ 5.01 (H-1.1); 3.64 (H-2.1); 3.85 (H-3.1); 4.03 (H-4.1); 4.01 (H-5.1); 3.82 (H-6.1); 3.68 (H-6′.1); 4.91 (H-1.2); 4.01 (H-2.2); 4.00 (H-3.2); 3.33 (H-4.2); 3.59 (H-5.2); 1.23 (H-6.2). $J_{12.1}$ 1.8 Hz; $J_{23.1}$ 3.2; $J_{34.1}$ 8.5; $J_{45.1}$ 8.5; $J_{56.1}$ 1.9; $J_{56'.1}$ 1.0; $J_{66'.1}$ 10.5; $J_{12.2}$ 0; $J_{23.2}$ 6.0; $J_{34.2}$ 6.0; $J_{45.2}$ 10.0; $J_{56.2}$ 6.5.

8-Methoxycarbonyloct-1-yl 4-O-(2-O-(6-O-acetyl-2-O-allyl-3,4-di-O-benzoyl-α-D-galactopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-O-cyclohexylidene-α-L-rhamnopyranoside (6). Compound (*4*) (8.64 g, 10.2 mmol) and 6-*O*-acetyl-2-*O*-allyl-3,4-di-*O*-benzoyl-α-D-galactopyranosyl bromide[10] (*5*) (8.0 g, 15 mmol) was dissolved in 20 ml toluene–nitromethane (1:1) and the solution was stirred with molecular sieves (4Å, 5 g) for 2.5 h at −30 °C under an atmosphere of nitrogen. Anhydrous silver trifluoromethanesulfonate (4.36 g, 17 mmol) dissolved in the same solvent (35 ml) was added at −40 °C and the mixture was stirred at −30 °C for 45 min. Collidine (2.8 ml) was added and stirring was continued for 10 min. The mixture was diluted with ethyl acetate (200 ml) and filtered. After evaporation, silver carbonate (2 g) was added and the mixture was suspended in water–acetone (1:20, 100 ml) and stirred for 10 min at 20 °C. Filtration, evaporation, addition of ethyl acetate (100 ml) and work-up, as described for *3* gave 15.5 g crude product. Purification by gradient silica gel column chromatography (ethyl acetate–pentane: 1:3→1:2) gave 11.65 g (88 % yield) of *6*. $[\alpha]_D^{20}$ +63 ° (*c* 1.3, $CHCl_3$). Analysis for $C_{74}H_{90}O_{20}$: C,H. ^1H NMR: δ 5.56 (H-1.1); 4.03 (H-2.1); 5.65 (H-3.1); 5.81 (H-4.1); 4.48 (H-5.1); 4.15 (H-6.1); 4.14 (H-6′.1); 5.09 (H-1.2); 4.05 (H-2.2); 3.97 (H-3.2); 4.29 (H-4.2); 4.04 (H-5.2); 3.89 (H-6.2); 3.72 (H-6′.2); 4.94 (H-1.3); 4.04 (H-2.3); 4.03 (H-3.3); 3.38 (H-4.3); 3.62 (H-5.3); 1.27 (H-6.3). $J_{12.1}$ 3.6 Hz; $J_{23.1}$ 10; $J_{34.1}$ 3.2; $J_{45.1}$ 0; $J_{56.1}$ 6.8; $J_{56'.1}$ 6.8; $J_{12.2}$ 1.5; $J_{23.2}$ 2.4; $J_{34.2}$ 9.3; $J_{45.2}$ 9.3; $J_{56.2}$ 3.2; $J_{56'.2}$ 1.7; $J_{66'.2}$ 10.7; $J_{12.3}$ 0; $J_{23.3}$ 6.2; $J_{34.3}$ 6.2; $J_{45.3}$ 9.3; $J_{56.3}$ 6.5.

8-Methoxycarbonyloct-1-yl 2,3-di-O-acetyl-4-O-(2-O-(2,6-di-O-acetyl-3,4-di-O-benzoyl-α-D-galactopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (7). The trisaccharide (*6*) (11.4 g, 8.8 mmol) was dissolved in a mixture of ethanol (100 ml), toluene (50 ml), and water (12 ml) and the solution was heated to reflux with stirring for 10 min. 1,8-Diaza-[2.2.2.]-bicyclooctane (300 mg) and tris (triphenylphosphine)rhodium chloride (3.0 g, 1.6 mmol) were added and the mixture was stirred at reflux for 3.5 h. The mixture was cooled, filtered through celite and evaporated. The residue was dissolved in acetone (100 ml) and water (10 ml), red mercury(II) oxide (4.8 g 22 mmol) followed by mercury(II) chloride (4.8, 17.6 mmol) were added and the mixture stirred at room temperature for 7 h. The reaction mixture was filtered

through celite, evaporated and the residue was dissolved in dichloromethane. After washing twice with a solution of sodium hydrogencarbonate, potassium iodide solution (10 %) and with water, drying (magnesium sulfate), filtering and evaporation, 12.3 g of a syrup containing two components were isolated. Separation on a silica gel column (ethyl acetate–pentane: 3:8) gave a minor fraction (1.57 g 15 %) consisting of a compound which according to a ^{1}H NMR spectrum contained a propyl group. The major fraction, (8.7 g, 78 %) was dissolved in acetic acid (100 ml) and water (10 ml) and stirred at 60 °C for 27 h and then evaporated twice with toluene. Pyridine (50 ml), acetic anhydride (20 ml) and 4-dimethylaminopyridine (200 mg) were added and the mixture was stirred overinight at room temperature. The product was evaporated and the evaporation was repeated twice with toluene. The residue was purified on a silica gel column (ethyl acetate–pentane: 1:2) giving 5.92 g (overall 54 % yield) of *7*, $[\alpha]_D^{20}$ +76.2 ° (*c* 1.6, $CHCl_3$). Analysis for C_{71},H_{84},O_{23}: C,H. ^{1}H NMR: δ 5.54 (H-1.1); 5.37 (H-2.1); 5.74 (H-3.1); 5.83 (H-4.1); 4.55 (H-5.1); 4.15 (H-6.1); 4.09 (H-6′.1); 5.04 (H-1.2); 3.85 (H-2.2); 3.81 (H-3.2); 4.04 (H-4.2); 3.84 (H-5.2); 3.76 (H-6.2); 3.69 (H-6′.2); 4.62 (H-1.3); 5.19 (H-2.3); 5.14 (H-3.3); 3.65 (H-4.3); 3.61 (H-5.3); 1.26 (H-6.3); $J_{12.1}$ 3.9 Hz; $J_{23.1}$ 10.5; $J_{34.1}$ 3.3; $J_{45.1}$ 0; $J_{56.1}$ 6.0; $J_{56'.1}$ 6.0; $J_{66'.1}$ 11.5; $J_{12.2}$ 0; $J_{34.2}$ 9.2; $J_{45.2}$ 9.2; $J_{56.2}$ 3.9; $J_{56'.2}$ 0; $J_{66'.2}$ 10.5; $J_{12.3}$ 1.8; $J_{23.3}$ 3.2; $J_{34.3}$ 9.0; $J_{45.3}$ 9.0; $J_{56.3}$ 6.5.

8-Methoxycarbonyloct-1-yl 4-O-(4,6-O-cyclohexylidene-2-O-(2,6-di-O-acetyl-3,4-di-O-benzoyl-α-D-galactopyranosyl)-α-D-mannopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (8). The trisaccharide (*7*) (5.60 g, 4.45 mmol) was dissolved in methanol (100 ml) and acetic acid 25 ml and hydrogenolysed over palladium on charcoal (5 %, lg) at 1 atmosphere of hydrogen for 24 h. TLC showed the reaction to be complete and the mixture was filtered and evaporated giving 4.65 g (yield 100 %) of a syrup. A sample of this material (663 mg, 0.641 mmol) was dissolved in anhydrous acetonitrile (10 ml) and cooled to 0 °C by stirring. 1-Ethoxycyclohexene (162 μl, 1.29 mmol) and *p*-toluenesulfonic acid (2 mg) were added and the mixture was stirred at 0 °C for 7 min. Pyridine 60 μl was added and the mixture was evaporated. Separation by preparative TLC on silica gel (ethyl acetate–pentane: 2:3) gave 655 mg (yield 92 %) of *8*. $[\alpha]_D^{20}$ +77° (*c* 0.4, $CHCl_3$). Analysis for $C_{56}H_{74}O_{23}$: C,H. ^{1}H NMR: δ 5.42 (H-1.1); 5.50 (H-2.1); 5.68 (H-3.1); 5.92 (H-4.1); 4.48 (H-5.1); 4.22 (H-6.1); 4.10 (H-6′.1); 5.08 (H-1.2); 3.89 (H-2.2); 3.72 (H-3.2); 3.82 (H-4.2); 3.92 (H-5.2); 3.92 (H-6.2); 3.82 (H-6′.2); 4.64 (H-1.3); 5.23 (H-2.3); 5.20 (H-3.3); 3.70 (H-4.3); 3.82 (H-5.3); 1.35 (H-6.3). $J_{12.1}$ 3.8 Hz; $J_{23.1}$ 10.6; $J_{34.1}$ 3.7; $J_{45.1}$ 0; $J_{56.1}$ 6.0; $J_{56'.1}$ 6.4; $J_{66'.1}$ 11.4; $J_{12.2}$ 0; $J_{23.2}$ 1.8; $J_{34.2}$ 9.4; $J_{45.2}$ 9.4; $J_{56'.2}$ 2.8; $J_{66'.2}$ 9.4; $J_{12.3}$ 1.5; $J_{23.3}$ 3.5; $J_{34.3}$ 9.7; $J_{45.3}$ 9.7; $J_{56.3}$ 6.5.

*8-Methoxycarbonyloct-l-yl 4-O-(4-O-(4,6-O-cyclohexylidene-2-O(2,6-di-O-acetyl-3,4-di-O-benzoyl-α-D-galactopyranosyl)-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl)α-D-mannopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (10).* Compound (*8*) (180 mg, 0.164 mmol) and 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl chloride[2] (*9*) (125 mg, 0.36 mmol) was dissolved in toluene (2 ml), acetonitrile (0.5 ml) and N,N′-tetramethyl urea (100 μl) and the solution was stirred with molecular sieves (4Å, 0.5 g) for 2.5 h at −30 °C under an atmosphere of nitrogen. Silver trifluoromethanesulfonate (108 mg 0.39 mmol) dissolved in anhydrous acetonitrile was added and the mixture was stirred at −30 °C for 2 h. The temperature was allowed to reach room temperature overnight and TLC (toluene–ethyl acetate: 25:7) showed that all the aglycone had been consumed and two new components had been formed. Collidine (150 μl) was added and the mixture was diluted with toluene (15 ml). The mixture was filtered and worked up as described for *3*. Purification of the resultant syrup by silica gel column chromatography (toluene–ethyl acetate: 25:7) gave *10* as a major fraction (142 mg, yield 62 %), $[\alpha]_D^{20}$ +82° (*c* 0.9 $CHCl_3$). Analysis for $C_{76}H_{96}O_{26}$: C,H. ^{13}C NMR: δ 101.7, 97.4, 97.3, 97.1 (C-1.1, C-1.2, C-1.3, C-1.4). ^{1}H NMR: δ 5.58 (H-1.1); 5.60 (H-2.1); 5.73 (H-3.1); 5.93 (H-4.1); 4.41 (H-5.1); 4.21 (H-6.1); 4.12 (H-6′.1); 5.32 (H-1.2); 1.76 (H-3a.2); 3.46 (H-4.2); 1.19 (H-6.2); 4.89 (H-1.3); 3.94 (H-2.3); 4.23 (H-4.3); 4.19 (H-5.3); 4.67 (H-1.4); 5.24 (H-2.4); 5.27 (H-3.4); 3.69 (H-4.4); 1.34 (H-6.4); 3.73–3.89 (H-2.2, H-5.2, H-3.3, H-6.3, H-6′.3, H-5.4). $J_{12.1}$ 4.0 Hz; $J_{23.1}$ 10.5; $J_{34.1}$ 3.5; $J_{45.1}$ 1.0; $J_{56.1}$ 6.0; $J_{56'.1}$ 6.8; $J_{66'.1}$ 11.5; $J_{12.2}$ 3.3; $J_{23a.2}$ 2.6; $J_{3a3e.2}$ 13.5; $J_{56.2}$ 6.5; $J_{12.3}$ 1.6; $J_{23.3}$ 3.0; $J_{34.3}$ 9.4; $J_{45.3}$ 9.4; $J_{56.3}$ 2.5; $J_{12.4}$ 1.9; $J_{23.4}$ 3.7; $J_{34.4}$ 9.3; $J_{45.4}$ 9.3; $J_{56.4}$ 6.2.

A second minor fraction (33 mg, yield 14 %) was characterised as the product of β-glycosylation according to the ^{13}C and ^{1}H NMR spectra. ^{13}C NMR: δ 105.2, 100.8, 98.1, 96.7 (C-1.1, C-1.2, C-1.3, C-1.4). ^{1}H NMR: δ 5.60 (H-1.1); 5.35 (H-2.1); 5.70 (H-3.1); 5.89 (H-4.1); 4.39 (H-5.1); 4.10 (H-6.1); 4.10 (H-6′.1); 4.47 (H-1.2); 2.40 (H-3e.2); 3.34 (H-4.2); 1.20 (H-6.2); 4.84 (H-1.3); 4.12 (H-4.3); 3.83 (H-6.3); 3.77 (H-6′.3); 4.62 (H-1.4); 5.23 (H-2.4); 5.17 (H-3.4); 3.70 (H-4.4); 1.21 (H-6.4). $J_{12.1}$ 4.1 Hz; $J_{23.1}$

10.8; $J_{34.1}$ 3.4; $J_{45.1}$ 1.0; $J_{56.1}$ 6.4; $J_{56'.1}$ 6.4; $J_{12.2}$ 7.8; $J_{23e.2}$ 4.5; $J_{3a3e.2}$ 13.5; $J_{3a4.2}$ 2.5; $J_{3e4.2}$ 3.0; $J_{45.2}$ 1.0; $J_{56.2}$ 6.2; $J_{12.3}$ 1.0; $J_{34.3}$ 9.6 $J_{45.3}$ 9.6; $J_{56.3}$ 0; $J_{56'.3}$ 4.6; $J_{66'.3}$ 10.4; $J_{12.4}$ 1.8; $J_{23.4}$ 3.4; $J_{34.4}$ 9.3; $J_{45.4}$ 9.3; $J_{56.4}$ 6.5.

*8-Methoxycarbonyloct-1-yl 4-O-(3-O-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-2-O-(α-D-*ga*lactopyranosyl)-α-D-*mannopyranosyl)-α-L-rhamnopyranoside* (11). The tetrasaccharide (*10*) (104 mg, 0.073 mmol) was dissolved in methanol (10 ml) and acetic acid (2 ml) and hydrogenolysed over palladium on charcoal (5 %, 150 mg) at one atmosphere of hydrogen pressure at 20 °C for 20 h. The mixture was filtered through celite and evaporated. The residue was dissolved in acetic acid (8.5 ml) and water (1.5 ml) and stirred at 40 °C for 4 h. The mixture was evaporated twice with toluene and the residue (80 mg) was dissolved in methanolic sodium methoxide (0.1 M 10 ml) and left overnight with stirring. Sodium ions were removed by stirring with ion exchange resin (Amberlite IRC 50) and filtration. Methanol was removed by evaporation and the residue was purified by successive chromatography on silica gel (ethyl acetate–methanol–acetic acid–water: 6:2:1:1) and gel filtration (sephadex (G 15), methanol–water: 1:1). The collected material was lyophilised giving 41 mg (71 %) of *11*. $[\alpha]_D^{20}$ +64° (*c* 0.2, $CHCl_3$). ^{13}C NMR: δ 101.4 (C-1.1); 64.6 (C-2.1); 34.1 (C-3.1); 69.4 (C-4.1); 67.8 (C-5.1); 16.7 (C-6.1); 102.2 (C-1.2); 69.8 (C-2.2); 70.6 (C-3.2); 70.3 (C-4.2); 72.4 (C-5.2); 62.1 (C-6.2); 101.1 (C-1.3); 80.1 (C-2.3); 78.4 (C-3.3); 67.5 (C-4.3); 74.6 (C-5.3); 61.7 (C-6.3); 100.7 (C-1.4); 71.6 (C-2.4); 70.5 (C-3.4); 82.6 (C-4.4); 68.5 (C-5.4); 18.1 (C-6.4); 69.1 ($-CH_2-O-$); 53.2 (CH_3-O-). $^1J_{CH-1.1}$ 171 Hz (kva, 4 Hz), $^1J_{CH-1.2}$ 170 (tri, 3 Hz) $^1J_{CH-1.3}$ 171 (d, 5Hz) $^1J_{CH-1.4}$ 168 (S).

^1H NMR: δ 4.98 (H-1.1); 3.92 (H-2.1); 1.88 (H-3a.1); 1.88 (H-3e.1); 3.77 (H-4.1); 3.97 (H-5.1); 1.08 (H-6.1); 5.04 (H-1.2); 3.67 (H-2.2); 3.79 (H-3.2); 3.88 (H-4.2); 3.92 (H-5.2); 3.63 (H-6.2); 3.58 (H-6'.2); 5.17 (H-1.3); 3.87 (H-2.3); 3.91 (H-3.3); 3.68 (H-4.3); 3.87 (H-5.3); 3.74 (H-6.3); 3.69 (H-6'.3); 4.67 (H-1.4); 3.81 (H-2.4); 3.73 (H-3.4); 3.41 (H-4.4); 3.68 (H-5.4); 1.22 (H-6.4). $J_{12.1}$ 3.5 Hz; $J_{23a.1}$ 8.8; $J_{23e.1}$ 2.5; $J_{45.1}$ 1.2; $J_{56.1}$ 6.6; $J_{12.2}$ 3.7; $J_{23.2}$ 10.2; $J_{34.2}$ 3.1; $J_{45.2}$ 1.0; $J_{56.2}$ 6.0; $J_{56'.2}$ 6.0; $J_{66'.2}$ 12.0; $J_{12.3}$ 2.0; $J_{23.3}$ 2.5; $J_{34.3}$ 9.8; $J_{45.3}$ 9.8; $J_{56.3}$ 2.2; $J_{56'.3}$ 4.5; $J_{66'.3}$ 12.1; $J_{12.4}$ 1.9; $J_{23.4}$ 3.2; $J_{34.4}$ 9.4; $J_{45.4}$ 9.4; $J_{56.4}$ 6.5.

8-Methoxycarbonyloct-1-yl 4-O-(-2-O-(α-D-galactopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranoside (12). Compound (*8*) (65 mg, 0.058 mmol) was dissolved in methanolic sodium methoxide (0.1 M, 5 ml) and left for 3 d. Sodium ions were removed with ion exchange resin (Amberlite IRC 50) and the mixture was filtered and evaporated. The residue was dissolved in acetic acid (8 ml) and water (2 ml) and was left at room temperature for 2 d. Evaporation and separation on a silica gel plate (ethyl acetate–methanol–water–acetic acid: 6:2:1:1) gave 35 mg (98 % yield) of *12*. ^{13}C NMR: δ 102.3 (C-1.1); 69.8 (C-2.1); 70.3 (C-3.1); 70.4 (C-4.1); 72.3 (C-5.1); 62.1 (C-6.1); 100.8 (C-1.2); 80.7 (C-2.2); 71.5 (C-3.2); 68.0 (C-4.2); 74.1 (C-5.2); 61.9 (C-6.2); 100.5 (C-1.3); 71.5 (C-2.3); 70.4 (C-3.3); 82.5 (C-4.3); 68.5 (C-5.3); 18.0 (C-6.3). $^1J_{CH-1.1}$ 170 Hz (d, 3.5 Hz); $^1J_{CH-1.2}$ 171 (d, 5.0 Hz); $J_{CH-1.3}$ 169 (S).

^1H NMR: δ 5.03 (H-1.1); 3.69 (H-2.1); 3.73 (H-3.1); 3.88 (H-4.1); 3.98 (H-5.1); 3.63 (H-6.1); 3.59 (H-6'.1); 5.19 (H-1.2); 3.88 (H-2.2); 3.80 (H-3.2); 3.61 (H-4.2); 3.83 (H-5.2); 3.75 (H-6.2); 3.68 (H-6'.2); 4.67 (H-1.3); 3.79 (H-2.3); 3.78 (H-3.3); 3.41 (H-4.3); 3.62 (H-5.3); 1.23 (H-6.3). $J_{12.1}$ 3.8 Hz; $J_{23.1}$ 8.0; $J_{34.1}$ 3.0; $J_{45.1}$ 0; $J_{56.1}$ 6.0; $J_{56'.1}$ 6.0; $J_{66'.1}$ 12.0; $J_{12.2}$ 0; $J_{23.2}$ 2.4; $J_{34.2}$ 10.0; $J_{45.2}$ 10.0; $J_{56.2}$ 2.5; $J_{56'.2}$ 5.2; $J_{66'.2}$ 11.6; $J_{12.3}$ 2.0; $J_{23.3}$ 3.4; $J_{34.3}$ 10.0; $J_{45.3}$ 10.0; $J_{56.3}$ 6.0.

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