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Synthesis of Tetrasaccharides Related to the *O*-Specific Determinants of *Salmonella* Serogroups A, B and D_1

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The branched tetrasaccharides, 4-*O*-(3-*O*-(deoxy-α-D-hexopyranosyl)-2-*O*-(α-D-galactopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranose, with the deoxy-α-D-hexopyranosyl equal to 3,6-dideoxy-α-D-*ribo*-hexopyranose, 3,6-dideoxy-α-D-*arabino*-hexopyranose, 3,6-dideoxy-α-L-*arabino*-hexopyranose, 2,3,6-trideoxy-α-D-*threo*-hexopyranose, and 3,4,6-trideoxy-α-D-*erythro*-hexopyranose, respectively, have been synthesized using silver trifluoromethane sulfonate (triflate) as a promotor in all glycosylation reactions. The reducing end of the oligosaccharides was substituted with a linking arm for attachment to a protein carrier. The hexopyranosyl chlorides: 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hexopyranosyl chloride (*2*), 2,4-di-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranosyl chloride (*4*), and 2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl chloride (*7*) were prepared in almost quantitative yield by modification of published procedures. Methyl 2,3,6-trideoxy-4-*O*-benzoyl-β-D-*threo*-hexopyranoside (*13*) was prepared in 27 % yield from methyl 4,6-*O*-benzylidene-β-D-galactopyranoside and transformed into the chloride (*14*). 2-*O*-Benzyl-3,4,6-trideoxy-α-D-*erythro*-hexopyranosyl chloride (*20*) was prepared from methyl 3,4-dideoxy-α-D-*erythro*-hexopyranoside in 60 % yield. The trisaccharide 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, (*21*) was glycosylated with the hexopyranosyl chlorides (*2*), (*4*), (*7*), (*14*) and (*20*) to give the corresponding α-linked tetrasaccharides in 36, 100, 94, 30 and 58 % yield, respectively. Deprotection gave the desired tetrasaccharides, (*27*), (*28*), (*29*), (*30*) and (*31*) in good yields.

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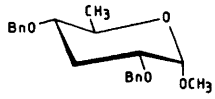
The repeating units of the *O*-specific chains of *Salmonella* serogroups A, B and D_1, are of the general structure:[1,2]

$$[\rightarrow 2)\text{-}\alpha\text{-D-Man}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}(1-]$$
$$\begin{matrix} & & & & 3 & & \\ & & & & \uparrow & & \\ & & & & 1 & & \\ & & & & \alpha\text{-D-Ddh} & & \end{matrix}$$

where α-D-Ddh stands for 3,6-dideoxy-α-D-*ribo*-hexopyranose (paratose), 3,6-dideoxy-α-D-*xylo*-hexopyranose (abequose) and 3,6-dideoxy-α-D-*arabino*-hexopyranose (tyvelose), respectively.

We have recently published the preparation of the branched tetrasaccharide of *Salmonella* Serogroup B with the α-D-Ddh equal to 3,6-dideoxy-α-D-*xylo*-hexopyranose.[1] In the present report we describe the preparation of the tetrasaccharide determinants of *Salmonella* Serogroup A and D_1 together with some artificial antigens with related structures. These compounds can then be used in a conformational analysis of the lipopolysaccharides of the *Salmonella* strain and in more detailed serological studies.[3]

The linear structures, α-D-Ddh(1→3)-α-D-Man(1→4)-α-L-Rha(1→3)-α-D-Gal which presents the terminal of the lipopolysaccharide, have been prepared recently by Garegg and Norberg.[4] Inhibition experiments with the linear and the branched antigens, can hopefully reveal the importance of the interior parts of the *O*-specific chains in the immune response in comparison to the terminal part of the *O*-chain. Inhibition studies with the artificial antigens *29*, *30* and *31*, may on the other hand provide information about



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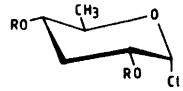
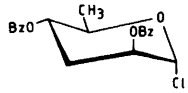


2 R = Bn
3 R = CHO



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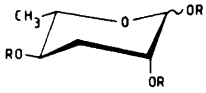
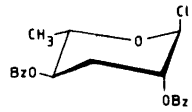


5 R = H
6 R = Bz



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the binding specificities in the antigen–antibody recognition process of the immunodominant 3,6-dideoxy-hexoses.

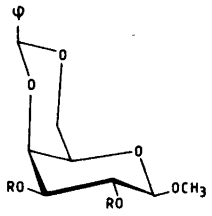
RESULTS AND DISCUSSION

The present synthesis rests on methodology developed by Bock and Meldal,[1,5,6] Garegg and co-workers[4,7–9] and by Bundle and Iversen.[2] The reaction of methyl 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hexopyranoside[4,9] (*1*) with anhydrous hydrogen chloride in diethyl ether and trimethyl silyl chloride gave quantitatively the hexopyranosyl chloride[2] (*2*). Omission of trimethyl silyl chloride led to considerable amounts of decomposition products. Attempts to prepare (*2*) by reaction of (*1*) with 1,1-dichloromethyl methyl ether and anhydrous zinc chloride, as described by Bundle and Iversen[2] in the case of the β-anomer, gave in our hands exclusively 3,6-dideoxy-2,4-di-*O*-formyl-α-D-*ribo*-hexopyranosyl chloride (*3*), the structure of which followed unequivocally from its spectroscopic characteristics.

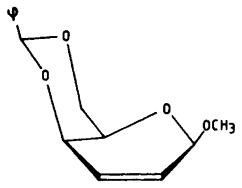
Reaction of methyl 2,4-di-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside[2,10–12] under the same conditions gave the chloride (*4*).[2,4]

The enantioner (*7*) was prepared from 3,6-dideoxy-L-*arabino*-hexose[13] (*5*) by low-temperature benzoylation to give (*6*) in 34 % yield. It was converted into the chloride (*7*) in 96 % yield upon reaction with 1,1-dichloromethyl methyl ether and zinc chloride.

Reaction of methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (*8*) with sulfuryl chloride and imidazole as described by Hanessian and Vatéle[14] gave methyl 4,6-*O*-benzylidene-2,3-di-*O*-imidazolesulfonyl-β-D-galactopyranoside (*9*) in 73 % yield. Elimination conducted by a modification of the procedure described by Tipson and Cohen,[15] gave a 66 % yield of the unsaturated compound (*10*), hydrogenation of which, over palladium on charcoal, gave *11* in 98 % yield. Reaction of *11* with *N*-bromosuccinimide[16] afforded the 6-bromo-6-deoxy compound *12* in quantitative yield. The hydrogenolysis of *12* over palladium on charcoal was very slow and only a 57 % yield of *13* could be isolated after 72 h of reaction. Reaction of *13* with anhydrous hydrogen chloride in diethyl ether afforded the unstable hexopyranosyl chloride (*14*) in 60 % yield.



8 R=H
9 R= Imidazoylsulfonyl



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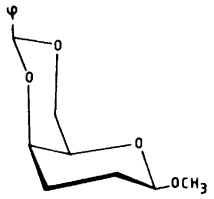

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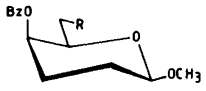
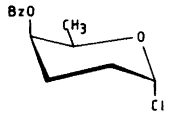


12 R=Br
13 R=H



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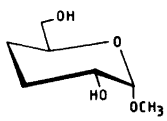
Methyl 3,4-dideoxy-α-D-*erythro*-hexopyranoside [17] (*15*) was treated with tribromoimidazole [18] and triphenylphosphine as described by Classon *et al.*[9] and after benzoylation with benzoyl chloride and pyridine the benzoate (*16*) was obtained in 82 % yield. Hydrogenolysis over palladium on charcoal afforded the 3,4,6-trideoxy-derivative (*17*) in 93 % yield. Transesterification of *17* gave *18* in 82 % yield. Benzylation of *18* with sodium hydride and benzyl bromide gave a 96 % yield of *19* which was converted quantitatively into the hexopyranosyl chloride (*20*) on reaction with anhydrous hydrogen chloride in diethyl ether.

The hexopyranosyl chlorides (*2, 4, 7, 14,* and *20*) were reacted with the trisaccharide (*21*) in the presence of silver triflate to give *22, 23, 24, 25* and *26* respectively. Reaction conditions and results are given in Table 1.

As seen from the table it is necessary to use a large excess of the highly unstable hexopyranosyl chlorides (*2, 14,* and *20*) in order to obtain a resonable yield. The low reactivity of the hydroxyl group of *21* results in competitive decay of the alkylating species to 2-*O*-benzyloxy glycal (entry 2 and 6) or glycal (entry 5). The 2-*O*-



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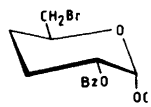

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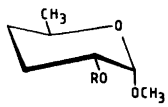

17 R=Bz
18 R=H
19 R=Bn

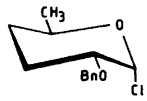

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Table 1. Product distribution and yield of tetrasaccharides.

	Reactants	Equivalents of chloride	Reaction conditions[a]	Time/h	Temperature/°C[b]	Anomer distribution of isolated products *a/b*	Total yield of tetrasaccharides/%
1	*2+21*	3.5	A	18	−35→20	0.64/0.36	57[c]
2	*3+21*	3.0	B	18	−35→20	Orthoformate	68
3	*4+21*	3.5	C	18	−35→20	1.00/0.00	94
4	*7+21*	3.5	C	18	−35→20	1.00/0.00	100
5	*14+21*	1.0	C	18	−50→20	1.00/0.00	30[c]
6	*20+21*	3.8	A	18	−35→20	0.67/0.33	86[c]

[a] A: AgTfl (silver triflate), Toluene–CH_3CN–$[(CH_3)_2N]_2CO$ 20:5:2, Molecular sieves 4A. B: AgTfl, CH_3CN–$[(CH_3)_2N]_2CO$ 20:1, Molecular sieves 4A. C: AgTfl, Toluene–$[(CH_3)_2N]_2CO$ 10:1, Molecular sieves 4A. [b] The mixture was kept at the low temperature for 2 h and then slowly allowed to reach room temperature. [c] Main by-product was 2-*O*-benzyloxy-glycal (entry 1 and 6) or glycal (entry 5).

benzyloxyglycal derived from the chloride (*2*, entry 1) was isolated from the reaction mixture, the only other product observed being the unreacted aglyone (*21*). Acetonitrile was added to stabilize the carbonium ions and to enhance the selectivity of α-glycosylation as described by Schmidt and Rücker.[19] Glycosylations with the more stable chlorides, (*4*) and (*7*), were quantitative.

The exclusive formation of an α-glucoside (entry 5) from the 2,3-dideoxy-glycosyl chloride (*14*) is most likely attributable to an intermediate benzoxonium ion.

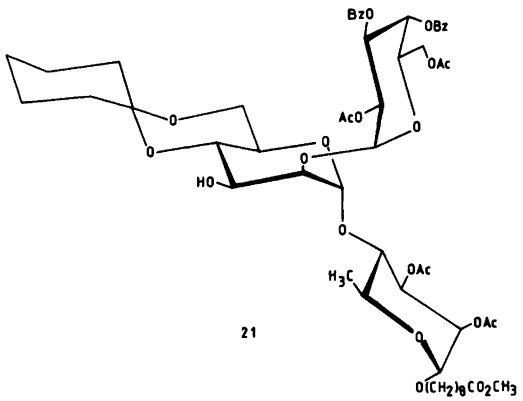
The reaction of the hexopyranosyl chloride (*3*) with *21* gave an orthoformate as the only product. Attempts to rearrange this with boron trifluoride etherate in anhydrous acetonitrile were unsuccessful, probably due to the low reactivity of the aglycone. Deprotection of the tetrasaccharides (*22, 23, 24* and *26*) was accomplished by conventional procedures. Benzyl groups were removed by hydrogenolysis over palladium. Transesterification and hydrolysis in acetic acid and water gave the tetrasaccharides (*27, 28, 29* and *31*) in 89, 85, 80 and 87 % yield respectively. The hydrolysis of the cyclohexylidene group in *25* with acetic acid and water led to considerable cleavage of the labile glycosidic linkage of the trideoxy-hexopyranosyl moiety. This problem was partly solved by the use of anhydrous ethylene glycol and acetic acid in the hydrolysis. Transesterification of the reaction product gave *30* in 32 % yield.

The assignment of ^1H and ^{13}C NMR spectra was accomplished by 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 (n.O.e.) experiments.

The tetrasaccharides (*27, 28, 29, 30* and *31*) can be attached to a protein carrier *via* the substituent at the anomeric position of the α-L-rhamnopyranose unit, or alternatively, used directly in inhibition experiments.

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-



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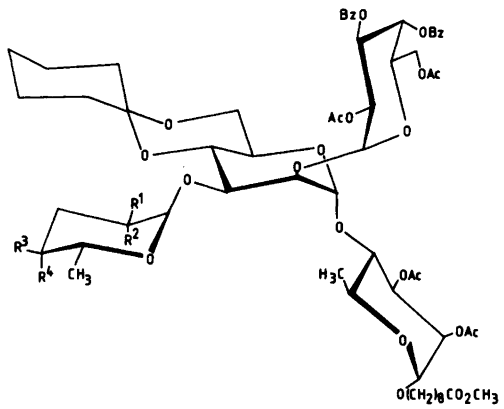


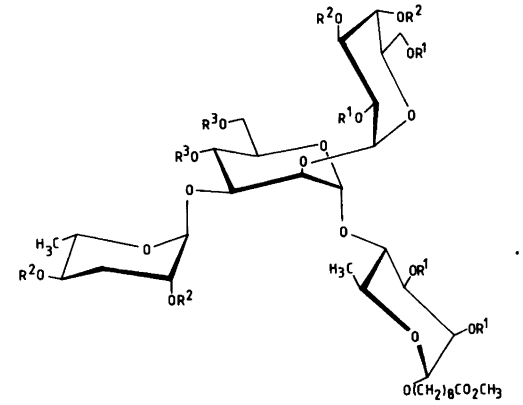
22 $R^1 = R^3 = OBn$, $R^2 = R^4 = H$
23 $R^2 = R^3 = OBz$, $R^1 = R^4 = H$
25 $R^1 = R^2 = R^3 = H$, $R^4 = OBz$
26 $R^1 = OBn$, $R^2 = R^3 = R^4 = H$

instruments. The spectra of protected compounds were measured in $CDCl_3$. Non-protected products were measured in D_2O relative to the internal reference, acetone (δ 2.12) for ^1H NMR spectra, and dioxane (67.4 ppm) for ^{13}C NMR spectra. Microanalyses were performed by Novo Microanalytical Laboratory. TLC was performed on silica gel coated plates (Merck F-254) and the components were visualized upon charring with sulphuric acid.

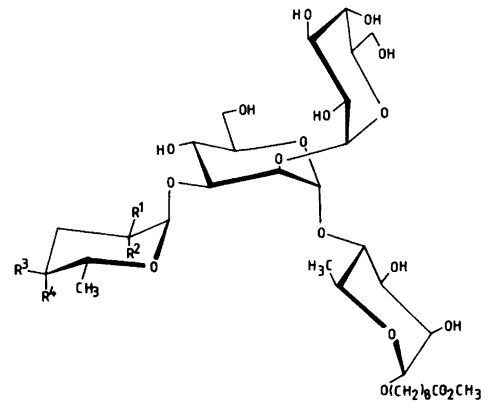
*2,4-Di-*O*-benzyl-3,6-dideoxy-α-*D*-ribo-hexopyranosyl chloride* (2). Methyl 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hexopyranoside[5,10] (*1*) (325 mg 0.95 mmol) was dissolved in dry diethyl ether (20 ml), which had been saturated with hydrogen chloride at 0 °C, and trimethylsilyl chloride (200 µl) was added. The mixture was kept at 4 °C for 15 h and then co-evaporated at 10 °C with toluene.

Drying for 1 h at 0.5 mmHg over phosphorus pentoxide gave 325 mg (99 %) of *2*. ^1H NMR δ 6.08 (H-1); 3.63 (H-2); 1.92 (H-3a); 2.33 (H-3e); 3.10 (H-4): 3.95 (H-5); 1.26 (H-6). J_{12} 3.8 Hz; J_{23a} 11.6; J_{23e} 4.5; J_{3a3e} 11.4; J_{3a4} 11.1; J_{3e4} 4.2; J_{45} 8.9; J_{56} 6.5. ^{13}C NMR; 93.3 ppm (C-1); 70.7 (C-2); 30.0 (C-3); 70.7 (C-4); 70.6 (C-5); 17.4 (C-6); 76.6 and 74.1 (benzyl methylene carbon atom). Reported[2] 93.8 (C-1); 30.3 (C-3); 17.8 (C-6).

*3,6-Dideoxy-2,4-di-*O*-formyl-α-*D*-ribo-hexo-*



24 R^1 = Ac, R^2 = Bz, R^3 = cyclohexylidene
29 $R^1 = R^2 = R^3 = H$



27 $R^1 = R^3 = OH$, $R^2 = R^4 = H$
28 $R^2 = R^3 = OH$, $R^1 = R^4 = H$
30 $R^1 = R^2 = R^3 = H$, $R^4 = OH$
31 $R^1 = OH$, $R^2 = R^3 = R^4 = H$

pyranosyl chloride (3). The methyl glycoside (*1*) (300 mg, 0.87 mmol) was dissolved in 1,1-dichloromethyl methylether (2.5 ml) and anhydrous zinc chloride (50 mg) was added. The mixture was stirred at 20 °C for 1 h and diluted with toluene (5 ml); evaporation at 20 °C gave a black residue which was extracted with toluene (10 ml). The extract was evaporated and the residue was dried at 1 mmHg for 2 h. to give 270 mg of the chloride (*3*) mixed with dibenzyl ether. ^{1}H NMR; δ 6.23 (H-1); 5.12 (H-2); 2.35 (H-3a); 2.17 (H-3e); 4.76 (H-4); 4.11 (H-5); 1.27 (H-6); 8.02 (H-2-O-formyl); 8.05 (H-4-O-formyl). J_{12} 4.6 Hz; J_{13e} 0.9; J_{23a} 12.3; J_{23e} 4.0; $J_{2\text{-formyl}}$ 1.0; J_{3a3e} 11.7; J_{3a4} 11.4; J_{3e4} 5.0; J_{45} 10.1; $J_{4\text{-formyl}}$ 0.8; J_{56} 6.5. ^{13}C NMR: δ 90.7 ppm (C-1); 69.8, 68.3, 67.8 (C-2, C-4, C-5); 28.6 (C-3); 16.8 (C-6).

3,6-Dideoxy-1,2,4-tri-O-benzoyl-α-L-arabino-hexopyranose (6). 3,6-Dideoxy-L-*arabino*-hexose[13] (*5*) (444 mg, 3 mmol) was dissolved in pyridine (5 ml) and cooled to −10 °C. Benzoyl chloride (1.56 ml, 13.5 mmol) in pyridine (5 ml) was added at this temperature with stirring over a period of 3 h and the mixture was stirred for 16 h at 20 °C. Water (0.5 ml) was added and the mixture was stirred for 3 h at 20 °C. The mixture was dissolved in dichloromethane (20 ml) and washed successively with water (20 ml), cold sulfuric acid (3M, 30 ml), water (20 ml), saturated sodium hydrogencarbonate (20 ml) and water (10 ml). Drying (magnesium sulfate), filtration and evaporation gave 1.08 g of a syrup. A ^{1}H NMR-spectrum showed that it was a mixture of α-pyranose, β-pyranose- and α-furanose in a ratio of (2.2:1:1). Separation on silica gel plates (toluene–ethyl acetate: 12/1) gave 460 mg (34 %) of the α-pyranose-form (*6*) containing 5 % of the β-pyranose-form. ^{1}H NMR (α-pyranose) δ 6.36 (H-1); 5.36 (H-2): 2.31 (H-3a); 2.65 (H-3e); 5.24 (H-4); 4.20 (H-5); 1.35 (H-6). J_{23a} 3.6 Hz, J_{23e} 4.5; J_{3a3e} 14.3; J_{3a4} 11.0; J_{3e4} 3.0; J_{45} 9.5; J_{56} 6.3.

2,4-Di-O-benzoyl-3,6-dideoxy-α-L-arabino-hexopyransyl chloride (7). The tribenzoate (6) (460 mg, 1.0 mmol) was dissolved in 1,1-dichloromethyl methylether (2.5 ml) and anhydrous zinc chloride (50 mg) was added. The mixture was stirred for 2 h at 20 °C and then co-evaporated with toluene (5 ml). The residue was dissolved in dichloromethane (15 ml), washed twice with sulfuric acid (1M, 15 ml), and with a saturated solution of sodium hydrogencarbonate (15 ml) and water (15 ml). The solution was filtered through a layer of sodium sulfate and the filtrate was stirred with molecular sieves (4A, 0.3 g) for 1 h. Filtration and evaporation gave 360 mg (96 %) of (*7*) as a syrup. ^{1}H NMR: δ 6.12 (H-1); 5.33 (H-2); 2.48 (H-3a; H-3e); 5.21 (H-4); 4.31 (H-5): 1.34 (H-6). J_{12} 0.0 Hz; J_{23a} 3.3; J_{23e} 4.7; J_{3a4} 7.6; J_{3e4} 7.6; J_{45} 9.9; J_{56} 6.5.

Methyl 4,6-O-benzylidene-2,3-di-O-(N-imidzoylsulfonyl)-β-D-galactopyranoside (9). Methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (*8*) (2.0 g, 7.1 mmol) was dissolved in dry N,N-dimethylformamide (DMF, 25 ml) and cooled to −40 °C with stirring. Sulfuryl chloride (2.3 ml, 28 mmol) was added and the temperature was allowed to reach −30 °C within 20 min and then 25 °C within 15 min. Imidazole (11.6 g, 142 mmol) was added with cooling at this temperature over a period of 3 min. After stirring for 3 h at 25 °C the mixture was poured into chloroform (100 ml) and ice–water (200 ml) was added. The mixture was stirred for 5 min and the phases were separated. Successive washings with water (2×100 ml) and with saturated sodium hydrogencarbonate (100 ml), drying (magnesium sulfate), filtration and repeated evaporation with toluene (30 ml) gave 3.9 g of a syrup which crystallized on standing. The material was stirred twice with diethyl ether, dried and 2.8 g (73 %) of (*9*) was isolated. The material could not be recrystallized from ethyl acetate or diethyl ether but addition of diethyl ether and pentane to a syrup obtained by dissolving the compound in ethyl acetate and evaporating, gave the semi-crystalline compound. $[\alpha]_D^{20}$ 0.0° (*c* 0.3, $CHCl_3$). Analysis for $C_{20}H_{22}N_4O_{10}S$: C,H. ^{1}H-NMR: δ 4.26 (H-1); 4.88 (H-2); 4.60 (H-3); 4.20 (H-4); 3.42 (H-5); 4.31 (H-6); 3.99 (H-6'). J_{12} 7.2 Hz; J_{23} 9.6; J_{34} 3.8; J_{45} 0.5; J_{56} 1.4; $J_{56'}$ 1.4; $J_{66'}$ 12.8.

Methyl 4,6-O-benzylidene-2,3-dideoxy-β-D-threo-hex-2-enopyranoside (10). Compound (*9*) (2.6 g, 4.6 mmol) was dissolved in dry DMF (20 ml) and zinc powder (1.5 g) and sodium iodide (4 g) was added. The mixture was stirred at 70 °C for 20 h and poured into chloroform (50 ml) and water (50 ml). The mixture was stirred for 15 min and the phases were separated. The organic phase was washed with aqueous sodium thiosulfate and with saturated sodium hydrogencarbonate. Drying (magnesium sulfate) and evaporation gave a crystalline residue which was recrystallized from ethanol (10 ml) to give 785 mg (66 %) of (*10*). Mp. 118–119 °C. $[\alpha]_D^{20}$ −176° (*c* 0.4, $CHCl_3$) (Reported[20] mp. 122–123 °C. $[\alpha]_D^{25}$ −172° (*c* 1.1 $CHCl_3$)). Analysis for $C_{14}H_{16}O_4$: C,H. ^{1}H NMR: δ 5.17 (H-1); 5.92 (H-2): 6.04 (H-3): 4.18 (H-4): 3.47 (H-5): 4.40 (H-6); 4.14 (H-6'). J_{12} 0.8 Hz; J_{13} 0.8; J_{23} 10.1; J_{34} 4.6; J_{45} 1.5; J_{56} 1.2; $J_{56'}$ 2.7; $J_{66'}$ 12.8.

Methyl 4,6-O-benzylidene-2,3-dideoxy-β-D-threo-hexopyranoside (11). The unsaturated derivative (*10*) (690 mg 2.78 mmol) was dissolved in ethyl acetate and hydrogenated by stirring at atmospheric hydrogen pressure over palladium

on charcoal (5 %, 100 mg) for 2 h. The mixture was filtered through celite and evaporated. The crystalline residue was recrystallized from ethanol and water giving 680 mg of needles (98 %). Mp. 108.0 °C $[\alpha]_D^{20}$+90° (*c* 0.3, $CHCl_3$). Analysis for $C_{14}H_{18}O_4$: C,H. ^{1}H NMR: δ 4.40 (H-1): 1.52–2.23 (H-2a, H-2e, H-3a, H-3e); 3.85 (H-4); 3.42 (H-5); 4.17 (H-6); 4.04 (H-6′). J_{12a} 6.5 Hz; J_{12e} 1.8; J_{45} 1.6; J_{56} 1.3; $J_{56'}$ 2.0; $J_{66'}$ 11.8.

*Methyl 4-O-benzoyl-6-bromo-2,3,6-trideoxy-β-*D-threo-*hexopyranoside* (12). The methyl glycoside (*11*) (670 mg, 2.68 mmol) was dissolved in tetrachloromethane (50 ml) and barium carbonate (1.6, 8.1 mmol) was added. Tetrachloromethane (20 ml) was removed by distillation and N-bromosuccinimide (623 mg, 3.5 mmol) was added. The mixture was stirred at reflux temperature for 2.5 h and filtered hot. The filter was washed with hot tetrachloromethane (15 ml). The combined filtrates were evaporated and the residue was dissolved in diethyl ether. The solution was washed 3 times with water (3.5 ml), dried (sodium sulfate) and filtered. Evaporation gave 0.88 g of (*12*) (100 %) as a syrup. $[\alpha]_D^{20}$ +22° (*c* 1.4, $CHCl_3$). ^{1}H NMR: δ 4.47 (H-1); 1.55–2.33 (H-2a, H-2e, H-3a, H-3e); 5.25 (H-4); 3.90 (H-5); 3.47 (H-6); 3.47 (H-6′). J_{45} 1.0 Hz; J_{56} 6.6; $J_{56'}$ 6.6.

*Methyl 4-O-benzoyl-2,3,6-trideoxy-β-*D-threo-*hexopyranoside* (13). Compound (*12*) (813 mg, 2.47 mmol) was dissolved in methanol (12 ml) and triethylamine (0.30 ml) and the mixture was hydrogenolyzed by stirring with palladium on charcoal (5 %, 200 mg) under an atmospheric pressure of hydrogen for 72 h. The mixture was filtered and evaporated and the residue was dissolved in dichloromethane (20 ml) and washed twice with water (10 ml). The organic phase was dried (magnesium sulfate), filtered and evaporated. Purification by preparative TLC (ethyl acetate/pentane: 1/5) gave a minor fraction of (*12*) and 350 mg (57 %) of (*13*). $[\alpha]_D^{20}$ −73° (*c* 0.2, $CHCl_3$). Analysis for $C_{14}H_{18}O_4$: C,H. ^{1}H NMR: δ 4.40 (H-1); 1.51–2.30 (H-2a, H-2e, H-3a, H-3e); 4.96 (H-4); 3.81 (H-5); 1.27 (H-6). J_{45} 1.3 Hz; J_{56} 6.3.

*4-O-benzoyl-2,3,6-trideoxy-α-*D-threo-*hexopyranosyl chloride* (14). The methyl glycoside (*13*) (140 mg, 0.56 mmol) was dissolved in dry diethyl ether (10 ml) saturated with hydrogen chloride and stirred for 2 h at room temperature. Evaporation with toluene (2 ml) gave 140 mg of syrup containing 40 % of the α-methyl glycoside and 60 % of (*14*) as seen from a ^{1}H NMR spectrum. ^{1}H NMR: δ 6.37 (H-1); 1.5–2.5 (H-2a, H-2e, H-3a, H-3e); 5.03 (H-4); 4.38 (H-5); 1.23 (H-6). J_{12} 2.3 Hz; J_{45} 1.3; J_{56} 6.3. The unstable chloride was used directly for glycosylation without purification.

*Methyl 2-O-benzoyl-6-bromo-3,4,6-trideoxy-α-*D-erythro-*hexopyranoside* (16). Methyl 3,4-dideoxy-α-D-*erythro*-hexopyranoside[17] (*15*) (560 mg, 3.46 mmol) was dissolved in toluene (50 ml) and 2 ml of toluene was removed by distillation to remove traces of methanol. Triphenylphosphine (1.82 g, 6.9 mmol) and tribromoimidazole (1.07 g, 3.5 mmol) were added at 85 °C and the mixture was stirred at 85 °C for 12 h and then at 60 °C overnight. Pyridine (1.70 ml) and benzoyl chloride (1.23 ml) were added with stirring at 20 °C and after 26 h at room temperature the mixture was evaporated. The residue was dissolved in pyridine (3 ml) and water (1 ml) was added. The mixture was stirred for 16 h and water (20 ml) and dichloromethane (25 ml) were added. The organic phase was washed successively with 20 ml portions of cold sulfuric acid (3M), saturated sodium hydrogencarbonate solution, sodium thiosulfate solution, and water. Drying (magnesium sulfate), filtration and evaporation gave a semi-crystalline residue, which was purified by preparative TLC on silica gel (ethyl acetate–pentane 3:20) to give 930 mg (82 %) of (*16*). $[\alpha]_D^{20}$ +79° (*c* 1.2, $CHCl_3$). Analysis for $C_{14}H_{17}O_4Br$: C,H. ^{1}H NMR: δ 4.92 (H-1); 5.01 (H-2); 1.48–2.26 (H-3a, H-3e, H-4a, H-4e); 3.97 (H-5); 3.39 (H-6); 3.39 (H-6′). J_{12} 3.4 Hz; J_{23a} 8.3; J_{23e} 6.4; J_{4a5} 10.7; J_{4e5} 2.9; J_{56} 5.6.

*Methyl 2-O-benzoyl-3,4,6-trideoxy-α-*D-erythro-*hexopyranoside* (17). Compound (*16*) (890 mg, 2.71 mmol) was dissolved in methanol (10 ml) and triethylamine (0.50 ml) and hydrogenolyzed by stirring with palladium on charcoal (5 %, 400 mg) under 1 atmosphere of hydrogen for 90 h at 20 °C. The mixture was filtered through celite and evaporated. The residue was dissolved in dichloromethane (10 ml) and was washed twice with water (10 ml). Drying (magnesium sulfate) filtration and evaporation gave 626 mg (93 %) of *17*. $[\alpha]_D^{20}$ +80° (*c* 0.5, $CHCl_3$). Analysis for $C_{14}H_{18}O_4$: C,H. ^{1}H NMR: δ 4.84 (H-1); 4.99 (H-2) 1.25–2.34 (H-3a, H-3e, H-4a, H-4e); 3.88 (H-5); 1.18 (H-6). J_{12} 3.4 Hz; J_{23a} 10.2; J_{23e} 5.4; J_{4a5} 10.5; J_{4e5} 2.9; J_{56} 6.2.

*Methyl 3,4,6-trideoxyα-*D-erythro-*hexopyranoside* (18). The methyl glycoside (*17*) (550 mg, 2.2 mmol) was dissolved in methanol (5 ml) and sodium methoxide in methanol (1M, 1 ml) was added. The mixture was left for 5 h and solid carbon dioxide was added. The mixture was evaporated at 10 °C and 10 ml of ethyl acetate was added to the residue and it was filtered. The filtrate was evaporated at 10 °C and the resulting oil was purified by preparative TLC on silica gel plate (ethyl acetate–pentane 6:1) to give 263 mg (82 %) of *18*. $[\alpha]_D^{20}$ +171° (*c* 0.3, $CHCl_3$). ^{1}H

NMR: δ 4.60 (H-1); 3.63 (H-2); 1.23–2.02 (H-3a, H-3e, H-4a, H-4e); 3.75 (H-5); 1.15 (H-6). J_{12} 3.6 Hz; J_{56} 6.2.

Methyl 2-O-benzyl-3,4,6-trideoxy-α-D-erythro-hexopyranoside (19). Compound (*18*) (245 mg, 168 mmol) dissolved in dry DMF (2 ml) was added to a stirred suspension of sodium hydride (100 mg, 4.2 mmol) in DMF (6 ml) at 0 °C. The mixture was stirred for 20 min. Benzyl bromide (0.41 ml, 3.4 mmol) was added at 0 °C and stirring was continued for 3 h. at 20 °C. Methanol (1 ml) was added and after 45 min the mixture was diluted with dichloromethane (15 ml) and washed with sodium hydrogencarbonate solution (20 ml) and water (10 ml). Drying (magnesium sulfate), filtration and evaporation gave an oil which was purified by preparative TLC on silica gel plates (ethyl acetate–pentane 1:8) to give 380 mg (96 %) of *19* $[\alpha]_D^{20}$ +68° (*c* 1.4, $CHCl_3$). Analysis for $C_{14}H_{20}O_3$: C,H. ^{1}H NMR: δ 4.63 (H-1); 3.43 (H-2); 1.11–2.05 (H-3a, H-3e, H-4a, H-4e); 3.78 (H-5); 1.12 (H-6). J_{12} 3.5 Hz; J_{23a} 8.5; J_{23e} 6.5; J_{4a5} 10.5; J_{4e5} 3.0; J_{56} 6.3.

2-O-benzyl-3,4,6-trideoxy-α-D-erythro-hexopyranosyl chloride (20). The methyl glycoside (*19*) (50 mg, 0.21 mmol) was dissolved in dry diethyl ether (15 ml) saturated with hydrogen chloride. The mixture was stirred for 2.5 h and was evaporated with toluene (1 ml) at 20 °C. The unstable chloride (*20*) thus obtained, was pure according to a ^{1}H NMR spectrum and was used directly in the glycosylation reaction. ^{1}H NMR: δ 6.19 (H-1); 3.60 (H-2); 1.15–2.17 (H-3a, H-3e, H-4a, H-4e); 4.11 (H-5); 1.17 (H-6). J_{12} 3.1 Hz; J_{23a} 8.6; J_{23e} 5.1; J_{4a5} 10.7; J_{4e5} 2.1; J_{56} 6.1.

General procedure for glycosylation reactions. The trisaccharide (*21*)[1] was dissolved in 10 volumes of solvent and molecular sieves (4Å, 0.5–1.0 g) was added in an atmosphere of nitrogen. The solvents used were A: toluene–acetonitrile–*N,N'*-tetramethyl urea 20:5:2, B: acetonitrile–*N,N'*-tetramethyl urea 20:1, C: toluene–*N,N'*-tetramethyl urea 10:1. The hexopyranosyl chlorides, (*2, 3, 4, 7, 14* or *20*) were dissolved in 10 volumes of solvent and added to the reaction mixture at −35 °C. The mixture was stirred at −35 °C for 2.5 h and anhydrous silver trifluoromethane sulfonate (1.2 equivalent to the chloride) was added. The mixture was stirred for 2 h at −35 °C and then slowly allowed to reach room temperature. After 16 h the mixture was diluted with dichloromethane (30 ml) and filtered through celite. The filtrate was washed twice with a saturated sodium hydrogencarbonate solution (20 ml) and with water (20 ml). The organic phase was dried (magnesium sulfate), filtered and evaporated. The residue was separated by preparative chromatography on silica gel plate (toluene–ethyl acetate 18:7).

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)-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-ribo-hexopyranosyl)-α-D-mannopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (22). The trisaccharide (*21*)[1] (325 mg, 0.29 mmol) was glycosylated with (*2*) (325 mg, 0.93 mmol) in solvent A as described above. Work up and purification by preparation TLC gave 150 mg (36 %) of (*22*). $[\alpha]_D^{20}$ +100° (*c* 0.3, $CHCl_3$). Analysis for $C_{76}H_{96}O_{26}$: C,H ^{1}H NMR: δ 5.60 (H-1.1); 5.67 (H-2.1); 5.70 (H-3.1); 5.91 (H-4.1); 4.41 (H-5.1); 4.19 (H-6.1); 4.14 (H6'.1); 5.26 (H-1.2); 3.43 (H-2.2); 1.76 (H-3a.2); 2.26 (H-3e.2); 3.06 (H-4.2); 3.62 (H-5.2); 1.24 (H-6.2); 4.92 (H-1.3); 3.98 (H-2.3); 4.19 (H-3.3); 4.28 (H-4.3); 3.81 (H-5.3); 3.84 (H-6.3); 3.77 (H-6'.3); 4.65 (H-1.4); 5.22 (H-2.4); 5.25 (H-3.4); 3.69 (H-4.4); 3.85 (H-5.4): 1.35 (H-6.4). $J_{12.1}$ 4.0 Hz; $J_{23.1}$ 9.9; $J_{34.1}$ 3.2; $J_{45.1}$ 1.0; $J_{56.1}$ 5.5; $J_{56'.1}$ 6.7; $J_{66'.1}$ 11.5; $J_{12.2}$ 3.5; $J_{23a.2}$ 11.9; $J_{23e.2}$ 5.0; $J_{3a3e.2}$ 11.9; $J_{3a4.2}$ 11.9; $J_{3e4.2}$ 3.5; $J_{45.2}$ 9.5; $J_{56.2}$ 6.5; $J_{12.3}$ 1.8; $J_{23.3}$ 2.9; $J_{34.3}$ 9.2; $J_{45.3}$ 9.0; $J_{56.3}$ 0.0; $J_{56'.3}$ 5.5; $J_{12.4}$ 1.5; $J_{23.4}$ 3.0; $J_{34.4}$ 9.0; $J_{45.4}$ 9.0; $J_{56.4}$ 6.5. A second fraction of 85 mg (20 %) was characterized as the product of β-glycosylation. ^{1}H NMR; δ 5.62 (H-1.1); 5.37 (H-2.1); 5.72 (H-3.1); 5.90 (H-4.1); 4.39 (H-5.1); 4.12 (H-6.1); 4.11 (H-6'.1); 4.49 (H-1.2); 3.25 (H-2.2): 2.61 (H-3a.2); 1.44 (H-3e.2); 3.09 (H-4.2); 3.42 (H-5.2); 1.19 (H-6.2); 4.83 (H-1.3); 3.97 (H-2.3); 3.98 (H-3.3); 4.09 (H-4.3); 3.65 (H-5.3); 3.84 (H-6.3); 3.77 (H-6'.3); 4.64 (H-1.4); 5.25 (H-2.4); 5.17 (H-3.4); 3.66 (H-4.4); 3.69 (H-5.4); 1.25 (H-6.4). $J_{12.1}$ 3.9 Hz; $J_{23.1}$ 10.9; $J_{34.1}$ 3.4; $J_{45.1}$ 1.0; $J_{56.1}$ 5.5; $J_{56'.1}$ 7.5; $J_{12.2}$ 7.8; $J_{23a.2}$ 11.9; $J_{23e.2}$ 5.3; $J_{3a3e.2}$ 11.9; $J_{3a4.2}$ 11.6; $J_{3e4.2}$ 4.8; $J_{45.2}$ 9.2; $J_{56.2}$ 5.5; $J_{12.3}$ 0.5; $J_{23.3}$ 4.2; $J_{34.3}$ 9.5; $J_{45.3}$ 9.5; $J_{56'.3}$ 4.8; $J_{66'.3}$ 10.5; $J_{12.4}$ 1.6; $J_{23.4}$ 3.4; $J_{34.4}$ 9.6; $J_{45.4}$ 9.6; $J_{56.4}$ 6.2. Only two other byproducts cold be isolated, a minor fraction (80 mg, 25 %) of unreacted aglycon (*21*) and 160 mg of a compound characterized by a ^{1}H NMR spectrum to be the 2-benzyloxy glycal derived from *2*. ^{1}H NMR; δ 6.18 (H-1); 2.31 (H-3a); 2.61 (H-3e); 3.42 (H-4); 3.69 (H-5); 1.30 (H-6). J_{13} 0.7 Hz; $J_{13'}$ 1.5; $J_{33'}$ 16.5; J_{34} 7.5; $J_{3'4}$ 6.0; J_{45} 7.5; J_{56} 6.0.

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)-3-O-(2,4-di-O-benzoyl-3,6-di-deoxy-α-D-arabino-hexopyranosyl)-α-D-mannopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (23). Glycosylation of *21*[1] (480 mg, 0.43 mmol) with (*4*)[2] in solvent C as described above gave after work up 575 mg (94 %) of (*23*). $[\alpha]_D^{20}$ +44° (*c* 0.9, $CHCl_3$). Analysis for $C_{76}H_{92}O_{28}$: C,H. ^{1}H NMR: δ 5.75 (H-1.1); 5.44

(H-2.1); 5.77 (H-3.1); 5.94 (H-4.1); 4.45 (H-5.1); 4.27 (H-6.1); 4.17 (H-6′.1); 5.35 (H-1.2); 5.26 (H-2.2); 2.16 (H-3a.2); 2.45 (H-3e.2); 5.20 (H-4.2); 3.96 (H-5.2); 1.39 (H-6.2); 4.96 (H-1.3); 4.07 (H-2.3); 4.20 (H-3.3); 4.22 (H-4.3); 3.79 (H-5.3); 3.87 (H-6.3); 3.80 (H-6′.3); 4.66 (H-1.4); 5.23 (H-2.4); 5.27 (H-3.4); 3.74 (H-4.4); 3.87 (H-5.4); 1.35 (H-6.4). $J_{12.1}$ 3.9 Hz; $J_{23.1}$ 11.0; $J_{34.1}$ 3.6; $J_{45.1}$ 1.1; $J_{56.1}$ 6.3; $J_{56'.1}$ 7.0; $J_{66'.1}$ 11.5; $J_{12.2}$ 1.3; $J_{23a.2}$ 3.2; $J_{23e.2}$ 13.5; $J_{3a4.2}$ 10.6; $J_{3e4.2}$ 5.2; $J_{45.2}$ 9.5; $J_{56.2}$ 6.2; $J_{12.3}$ 1.6; $J_{23.3}$ 2.5; $J_{45.3}$ 9.1; $J_{56.3}$ 0.8; $J_{66'.3}$ 11.4; $J_{12.4}$ 1.6; $J_{23.4}$ 3.6; $J_{34.4}$ 9.4; $J_{45.4}$ 9.4; $J_{56.4}$ 6.3.

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)-3-O-(2,4-di-O-benzoyl-3,6-dideoxy-α-L-arabino-hexopyranosyl)-α-D-mannopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (24). The trisaccharide (*21*)[1] (279 mg, 0.25 mmol) was glycosylated with *7* (340 mg, 0.90 mmol) in solvent C and worked up as described above to give 365 mg (100 %) of *24* $[\alpha]_D^{20}$ +54° (*c* 0.7, $CHCl_3$). Analysis for $C_{76}H_{92}O_{28}$: C,H. ^{1}H NMR: δ 5.59 (H-1.1): 5.52 (H-2.1); 5.81 (H-3.1); 5.99 (H-4.1); 4.48 (H-5.1); 4.23 (H-6.1); 4.19 (H-6′.1); 5.02 (H-1.2); 5.23 (H-2.2); 2.12 (H-3a.2); 2.54 (H-3e.2); 5.24 (H-4,2); 4.61 (H-5.2); 1.31 (H-6.2); 5.07 (H-1.3); 4.12 (H-2.3); 4.89 (H-3.3); 3.99 (H-4.3); 3.84 (H-5.3); 3.90 (H-6.3); 3.85 (H-6′.3); 4.70 (H-1.4); 5.32 (H-2.4); 5.29 (H-3.4); 3.77 (H-4.4); 4.19 (H-5.4); 1.37 (H-6.4). $J_{12.1}$ 3.9 Hz; $J_{23.1}$ 11.2; $J_{34.1}$ 3.7; $J_{45.1}$ 0.9; $J_{56.1}$ 5.9; $J_{56'.1}$ 6.9; $J_{66'.1}$ 11.2; $J_{12.2}$ 0.0; $J_{23a.2}$ 3.0; $J_{23e.2}$ 3.3; $J_{3a3e.2}$ 13.2; $J_{3a4.2}$ 10.5; $J_{3e4.2}$ 4.0; $J_{45.2}$ 10.5; $J_{56.2}$ 6.2; $J_{12.3}$ 1.7; $J_{23.3}$ 2.3; $J_{34.3}$ 10.4; $J_{45.3}$ 10.4; $J_{56.3}$ 4.8; $J_{56'.3}$ 3.0; $J_{66'.3}$ 11.2; $J_{12.4}$ 1.8; $J_{23.4}$ 3.9; $J_{34.4}$ 9.6; $J_{45.4}$ 9.6; $J_{56.4}$ 6.3.

*8-Methoxycarbonyloct-1-yl 4-O-(3-O-(4-O-benzoyl-2,3,6-trideoxy-α-D-*threo*-hexopyranosyl)-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* (25). Glycosylation of the trisaccharide (*21*)[1] (270 mg, 0.24 mmol) with *14* (0.25 mmol prepared from 105 mg of *13*) in solvent C as described above, but with the initial temperature at −50 °C, and work up gave 95 mg (30 %) of *25*. $[\alpha]_D^{20}$ +64° (*c* 1.1, $CHCl_3$). Analysis for $C_{69}H_{88}O_{26}$: C,H. ^{1}H NMR: δ 5.12 (H-1.1); 5.32 (H-4.1); 4.06 (H-5.1); 1.23 (H-6.1); 5.70 (H-1.2); 5.46 (H-2.2); 5.77 (H-3.2); 5.94 (H-4.2); 4.43 (H-5.2); 4.25 (H-6.2); 4.15 (H-6′.2); 4.91 (H-1.3); 4.01 (H-2.3): 4.11 (H-4.3); 4.16 (H-5.3); 4.68 (H-1.4); 5.25 (H-2.4); 5.28 (H-3.4); 4.71 (H-4.4); 1.39 (H-6.4); 3.75−3.91 (H-3.3, H-6.3, H-6′.3, H-5.4). $J_{12.1}$ 2.0 Hz, $J_{12'.1}$ 3.0; $J_{45.1}$ 1.0; $J_{56.1}$ 6.6; $J_{12.2}$ 4.0; $J_{23.2}$ 11.0; $J_{34.2}$ 3.1; $J_{45.2}$ 0.5; $J_{56.2}$ 6.0; $J_{56'.2}$ 6.4; $J_{66'.2}$ 11.4; $J_{12.3}$ 1.9; $J_{23.3}$ 2.4; $J_{34.3}$ 9.4; $J_{45.3}$ 9.4; $J_{56.3}$ 2.5; $J_{12.4}$ 1.0; $J_{34.4}$ 9.2; $J_{45.4}$ 9.2; $J_{56.4}$ 6.0. ^{13}C NMR: 101.2 ppm (C-1.1); 38.0 (C-2.1); 34.0 (C-3.1); 17.2 (C-6.1): 97.4 (C-1.2); 61.9 (C-6.2); 97.4 (C-1.3); 76.3 (C-2.3); 61.3 (C-6.3); 97.9 (C-1.4); 78.4 (C-4.4); 18.0 (C-6.4); 72.0, 71.0, 70.4, 70.1, 69.8, 68.4, 68.2, 68.0, 67.8, 67.0, 66.9, 66.0, 65.8 (residual oxygenated secondary ring carbon atoms and C-1 of linking arm). The main by-products were unreacted aglycon (105 mg) and an unpolar component considered to be the glycal derived from *14*.

*8-Methoxycarbonyloct-1-yl 4-O-(2-O-benzyl-3,4,6-trideoxy-α-D-*erythro*-hexopyranosyl)-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* (26). The trisaccharide (*21*)[1] (190 mg, 0.17 mmol) was glycosylated with *20* (153 mg, 0.65 mmol) in solvent A as described above. Work up and separation by preparative TLC gave 130 mg (58 %) of *26*. $[\alpha]_D^{20}$ +95° (*c* 0.4, $CHCl_3$). Analysis for $C_{69}H_{90}O_{25}$: C,H. ^{1}H NMR: δ 5.22 (H-1.1): 3.34 (H-2.1); 1.46−1.81 (H-3.1, H-4.1); 3.71 (H-5.1); 1.13 (H-6.1); 5.61 (H-1.2); 5.55 (H-2.2); 5.69 (H-3.2); 5.90 (H-4.2); 4.37 (H-5.2); 4.16 (H-6.2); 4.09 (H-6′.2); 4.86 (H-1.3); 3.94 (H-2.3); 4.14 (H-3.3); 4.19 (H-4.3); 3.73 (H-5.3); 3.76 (H-6.3); 3.74 (H-6′.3); 4.64 (H-1.4); 5.21 (H-2,4); 5.24 (H-3.4); 3.64 (H-4.4); 3.79 (H-5.4); 1.33 (H-6.4). $J_{12.1}$ 3.5 Hz; $J_{56.1}$ 6.5; $J_{12.2}$ 3.8; $J_{23.2}$ 10.8; $J_{34.2}$ 3.4; $J_{45.2}$ 0.8; $J_{56.2}$ 5.8; $J_{56'.2}$ 6.8; $J_{66'.2}$ 11.4; $J_{12.3}$ 1.5; $J_{23.3}$ 2.8; $J_{34.3}$ 9.6; $J_{45.3}$ 9.6; $J_{12.4}$ 1.6; $J_{23.4}$ 3.7; $J_{34.3}$ 9.3; $J_{45.3}$ 9.3; $J_{56.3}$ 6.0.

The main byproduct (64 mg, 28 %) was characterized as the product of β-glycosylation by ^{1}H NMR: δ 4.41 (H-1.1); 3.16 (H-2.1); 3.52 (H-5.1); 1.18 (H-6.1); 5.60 (H-1.2); 5.35 (H-2.2), 5.69 (H-3.2); 5.86 (H-4.2); 4.36 (H-5.2); 4.10 (H-6.2; 4.06 (H-6′.2); 4.79 (H-1.3); 3.95 (H-2.3); 3.92 (H-3.3); 4.05 (H-4.3); 3.64 (H-5.3); 3.75 (H-6.3); 3.67 (H-6′.3); 4.61 (H-1.4); 5.22 (H-2.4); 5.13 (H-3.4); 3.84 (H-4.4); 3.64 (H-5.4); 1.14 (H-6.4). $J_{12.1}$ 7.5 Hz; $J_{23a.1}$ 10.8; $J_{23e.1}$ 5.0; $J_{4a5.1}$ 10.7; $J_{4e5.1}$ 1.8; $J_{56.1}$ 6.0; $J_{12.2}$ 4.0; $J_{23.2}$ 10.9 $J_{34.2}$ 3.5; $J_{56.2}$ 5.9; $J_{56'.2}$ 7.4; $J_{12.3}$ 1.1; $J_{23.3}$ 2.9; $J_{34.3}$ 9.6; $J_{45.3}$ 9.6; $J_{56.3}$ 4.8; $J_{56'.3}$ 4.3; $J_{66'.3}$ 10.7; $J_{12.4}$ 1.6; $J_{23.4}$ 3.8; $J_{34.4}$ 9.4; $J_{45.4}$ 9.8; $J_{56.4}$ 6.1.

General procedure to remove protecting groups. Tetrasaccharides containing benzyl groups were dissolved in methanol (10 ml) and acetic acid (1 ml) and hydrogenolyzed by stirring at 20 °C with palladium on charcoal (5 %, 100 mg) under 1 atmosphere of hydrogen pressure for 20 h. The mixture was filtered and evaporated.

The residue was dissolved in methanol (10 ml) containing sodium methoxide (1 M, 10.3 mmol) and stirred for 48 h at 20 °C. Acetic acid (1 ml) and water (5 ml) were added and the mixture was

evaporated. The residue was dissolved in water (5 ml) and acetic acid (5 ml) and the mixture was stirred at 40 °C for 16 h and evaporated.

The residue was purified by successive chromatography on silica gel (ethyl acetate–methanol–acetic acid–water 8:2:1:1) and gel filtration on Sephadex G15 (methanol–water 1:1) and freeze dried from water.

*8-Methoxycarbonyloct-1-yl 4-O-(3-O-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-2-O-(α-D-galactopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranoside* (27). The tetrasaccharide (*22*) (122 mg 0.085 mmol) was deprotected as described above to give 60 mg (89 %) of *27*. $[\alpha]_D^{20}$ +80° (*c* 0.3, H_2O) ^1H NMR: δ 4.95 (H-1.1); 3.71 (H-2.1); 1.60 (H-3a.1); 2.03 (H-3e.1); 3.26 (H-4.1); 3.59 (H-5.1);1.13 (H-6.1); 5.12 (H-1.2); 3.68 (H-2.2); 3.80 (H-3.2); 3.90 (H-4.2); 3.93 (H-5.2); 3.64 (H-6.2); 3.58 (H-6'.2); 5.15 (H-1.3); 3.89 (H-2.3): 3.90 (H-3.3); 3.69 (H-4.3); 3.82 (H-5.3); 3.75 (H-6.3); 3.69 (H-6'.3); 4.67 (H-1.4); 3.81 (H-2.4); 3.71 (H-3.4); 3.41 (H-4.4); 3.66 (H-5.4); 1.22 (H-6.4). $J_{12.1}$ 3.8 Hz; $J_{23a.1}$ 12.0; $J_{23e.1}$ 5.5; $J_{3a3e.1}$ 11.5; $J_{3a4.1}$ 11.5; $J_{3e4.1}$ 4.3; $J_{45.1}$ 9.5; $J_{56.1}$ 6.4; $J_{12.2}$ 3.8; $J_{23.2}$ 10.1; $J_{34.2}$ 3.3; $J_{56.2}$ 5.0; $J_{56'.2}$ 6.5; $J_{66'.2}$ 11.3; $J_{12.3}$ 1.6; $J_{23.3}$ 2.9; $J_{34.3}$ 9.4; $J_{45.3}$ 9.4; $J_{56.3}$ 2.8; $J_{56'.3}$ 4.5; $J_{66'.3}$ 12.0; $J_{12.4}$ 1.9; $J_{23.4}$ 3.4; $J_{34.4}$ 9.4; $J_{45.4}$ 9.4; $J_{56.4}$ 6.4; ^{13}C NMR: 100.7 ppm (C-1.1); 68.2 (C-2.1); 35.9 (C-3.1); 70.2 (C-4.1); 71.0 (C-5.1); 17.8 (C-6.1); 102.0 (C-1.2); 69.8 (C-2.2); 70.8 (C-3.2); 70.4 (C-4.2); 72.4 (C-5.2); 62.1 (C-6.2); 101.4 (C-1.3); 79.7 (C-2.3); 78.3 (C-3.3); 67.5 (C-4.3); 74.7 (C-5.3); 61.7 (C-6.3); 100.8 (C-1.4); 71.7 (C-2.4); 70.5 (C-3.4); 83.2 (C-4.4); 68.5 (C-5.4); 18.2 (C-6.4); 69.0 ($-O-CH_2-$); 53.2 ($-O-CH_3$).

*8-Methoxycarbonyloct-1-yl 4-O-(3-O-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-2-O-(α-D-galactopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranoside* (28). The tetrasaccharide (*23*) (522 mg, 0.36 mmol) was deprotected as described above with omission of the hydrogenolysis to give 242 mg (85 %) of *28*. $[\alpha]_D^{20}$ +75° (*c* 0.3, H_2O). ^1H NMR: δ 4.78 (H-1.1); 3.95 (H-2.1); 1.69 (H-3a.1); 1.94 (H-3e.1); 3.50 (H-4.1); 3.65 (H-5.1); 1.15 (H-6.1); 5.09 (H-1.2); 3.69 (H-2.2) 3.79 (H-3.2); 3.88 (H-4.2); 3.93 (H-5.2); 3.64 (H-6.2); 3.58 (H-6'.2); 5.18 (H-1.3); 3.88 (H-2.3); 3.93 (H-3.3); 3.84 (H-4.3); 3.83 (H-5.3) 3.75 (H-6.3); 3.68 (H-6'.3); 4.67 (H-1.4); 3.81 (H-2.4); 3.71 (H-3.4); 3.41 (H-4.4); 3.68 (H-5.4); 1.21 (H-6.4). $J_{12.1}$ 1.3 Hz; $J_{23a.1}$ 3.1; $J_{23e.1}$ 3.2; $J_{3a3e.1}$ 13.7; $J_{3a4.1}$ 11.3; $J_{3e4.1}$ 4.2; $J_{45.1}$ 9.7; $J_{56.1}$ 6.3; $J_{12.2}$ 3.9; $J_{23.2}$ 10.4; $J_{34.2}$ 3.2; $J_{45.2}$ 0.8; $J_{56.2}$ 4.1; $J_{56'.2}$ 6.3; $J_{66'.2}$ 11.0; $J_{12.3}$ 1.8; $J_{23.3}$ 3.0; $J_{34.3}$ 9.3; $J_{56.3}$ 1.4; $J_{56'.3}$ 3.8; $J_{66'.3}$ 11.7; $J_{12.4}$ 1.9; $J_{23.4}$ 3.6; $J_{34.4}$ 9.4; $J_{45.4}$ 9.4; $J_{56.4}$ 6.5. ^{13}C NMR: 102.1 ppm (C-1.1); 70.4 (C-2.1); 34.6 (C-3.1); 68.0 (C-4.1); 71.5 (C-5.1); 17.9 (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); 100.9 (C-1.3); 79.7 (C-2.3); 78.0 (C-3.3); 67.4 (C-4.3); 74.6 (C-5.3); 61.6 (C-6.3); 100.6 (C-1.4); 71.4 (C-2.4) 70.4 (C-3.4); 82.8 (C-4.4); 68.4 (C-5.4); 18.1 (C-6.4); 69.1 ($-CH_2-O-$); 53.2 ($-O-CH_3$).

*8-Methoxycarbonyloct-1-yl 4-O-(3-O-(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)-2-O-(α-D-galactopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranoside* (29). The tetrasaccharide (*24*) (290 mg 0.2 mmol) was deprotected as described above with omission of the hydrogenolysis to give 125 mg (80 %) of *29*.$[\alpha]_D^{20}$ +9° (*c* 0.2, H_2O). ^1H NMR; δ 4.67 (H-1.1); 3.85 (H-2.1); 1.76 (H-3a.1); 1.94 (H-3e.1); 3.51 (H-4.1); 3.79 (H-5.1); 1.13 (H-6.1); 5.00 (H-1.2); 3.66 (H-2.2); 3.78 (H-3.2); 3.84 (H-4.2); 3.92 (H-5.2); 3.63 (H-6.2); 3.58 (H-6'.2); 5.17 (H-1.3); 4.05 (H-2.3); 3.87 (H-3.3); 3.80 (H-4.3); 3.86 (H-5.3); 3.75 (H-6.3); 3.71 (H-6'.3); 4.67 (H-1.4); 3.8 (H-2.4); 3.74 (H-3.4); 3.40 (H-4.4); 3.66 (H-5.4); 1.21 (H-6.4). $J_{12.1}$ 1.9 Hz; $J_{23a.1}$ 3.1; $J_{23e.1}$ 5.8; $J_{3a3e.1}$ 13.4; $J_{3a4.1}$ 11.5; $J_{3e4.1}$ 3.7; $J_{45.1}$ 9.2; $J_{56.1}$ 6.3; $J_{12.2}$ 3.9; $J_{23.2}$ 10.2; $J_{34.2}$ 3.3; $J_{56.2}$ 5.1; $J_{56'.2}$ 6.5; $J_{66'.2}$ 12.5; $J_{12.3}$ 1.8; $J_{23.3}$ 2.9; $J_{34.3}$ 9.4; $J_{45.3}$ 9.4; $J_{56.3}$ 1.8; $J_{56'.3}$ 6.0; $J_{66'.3}$ 11.7; $J_{12.4}$ 2.0; $J_{23.4}$ 3.8; $J_{34.4}$ 9.6; $J_{45.4}$ 9.6; $J_{56.4}$ 6.4. ^{13}C NMR: 96.6 ppm (C-1.1); 68.7 (C-2.1); 34.6 (C-3.1); 71.0 (C-4.1); 66.1 (C-5.1); 17.9 (C-6.1); 102.1 (C-1.2); 69.8 (C-2.2); 70.5 (C-3.2); 70.3 (C-4.2); 72.5 (C-5.2); 62.1 (C-6.2); 101.0 (C-1.3); 76.1 (C-2.3); 75.7 (C-3.3); 68.0 (C-4.3); 74.2 (C-5.3); 61.7 (C-6.3); 100.7 (C-1.4); 71.6 (C-2.4); 70.4 (C-3.4); 82.7 (C-4.4); 68.5 (C-5.4); 18.1 (C-6.4); 69.1 ($-CH_2-O-$); 53.1 ($-O-CH_3$). $^1J_{CH-1.1}$ 167.0 Hz (m); $^1J_{CH-1.2}$ 170.5 (d, 4.9 Hz); $^1J_{CH-1.3}$ 171.8 (d, 5 Hz); $^1J_{CH-1.4}$ 169.7 (s).

*8-Methoxycarbonyloct-1-yl 4-O-(2-O-(α-D-galactopyranosyl)-3-O-(2,3,6-trideoxy-α-D-*threo*-hexopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranoside* (30). The tetrasaccharide (*25*) (72 mg, 0.055 mmol) was dissolved in anhydrous acetic acid (5 ml) and 1,2-dihydroxyethane (2 ml) and the mixture was stirred for 26 h at 60 °C. The mixture was diluted with toluene (10 ml) and washed twice with water (10 ml) and with a saturated sodium hydrogencarbonate solution (10 ml). Drying (magnesium sulfate), filtration and evaporation gave a syrup, which was purified on a silica gel plate (ethyl acetate–pentane 7:3) to give 35 mg of material. This was dissolved in methanolic sodium methoxide (0.1 M; 3.3 ml) and stirred for 27 h. The solution was neutralized with acetic acid (45 μl) and evaporated. Purification as described above gave 13 mg (32 %) of *30* $[\alpha]_D^{20}$ +62° (*c* 0.2, H_2O), ^1H NMR: δ 5.02 (H-1.1); 3.67 (H-2.1); 3.81 (H-3.1); 3.88 (H-4.1);

3.98 (H-5.1); 3.63 (H-6.1); 3.58 (H-6'.1); 4.98 (H-1.2); 1.90 (H-2a.2); 1.61 (H-2e.2); 1.87 (H-3a.2); 1.66 (H-3e.2); 3.73 (H-4.2); 3.97 (H-5.2); 1.06 (H-6.2); 5.23 (H-1.3); 3.82 (H-2.3); 3.90 (H-3.3); 3.79 (H-4.3); 3.83 (H-5.3); 3.74 (H-6.3); 3.69 (H-6'.3); 4.66 (H-1.4); 3.81 (H-2.4); 3.71 (H-3.4); 3.40 (H-4.4); 3.67 (H-5.4); 1.24 (H-6.4); $J_{12.1}$ 3.9 Hz; $J_{23.1}$ 10.8; $J_{34.1}$ 3.4; $J_{56.1}$ 4.8; $J_{56'.1}$ 8.2; $J_{66'.1}$ 12.1; $(J_{12a.2}+J_{12e.2}+J_{13e.2})$=4.5; $J_{56.2}$ 6.5; $J_{12.3}$ 1.2; $J_{23.3}$ 2.9; $J_{34.3}$ 9.3; $J_{45.3}$ 9.3; $J_{56.3}$ 2.3; $J_{56'.3}$ 4.6; $J_{66'.3}$ 12.5; $J_{12.4}$ 4.7 $J_{23.4}$ 3.8; $J_{34.4}$ 9.4; $J_{45.4}$ 9.4; $J_{56.4}$ 6.4. ^{13}C NMR; 102.6 ppm (C-1.1); 69.9 (C-2.1); 70.5 (C-3.1); 70.3 (C-4.1); 72.4 (C-5.1); 62.1 (C-6.1); 100.9 (C-1.2); 25.5 (C-2.2); 23.7 (C-3.2); 68.3 (C-4.2); 67.2 (C-5.2); 17.5 (C-6.2); 101.1 (C-1.3); 80.9 (C-2.3); 78.0 (C-3.3); 67.5 (C-4.3); 74.5 (C-5.3); 61.7 (C-6.3); 100.7 (C-1.4); 71.6 (C-2.4); 70.4 (C-3.4); 82.9 (C-4.4); 68.5 (C-5.4); 18.1 (C-6.4); 69.1 (−O−CH_2−); 53.2 (−O−CH_3).

8-Methoxycarbonyloct-1-yl 4-O-(2-O-(α-D-galactopyranosyl)-3-O-(3,4,6-trideoxy-α-D-erythro-hexopyranosyl)-α-D-mannopyranosyl)-α-L-rhamnopyranoside (31). The tetrasaccharide (*26*) (120 mg, 0.091 mmol) was deprotected by the general procedure described above to give 61 mg (87 %) of *31*. $[\alpha]_D^{20}$ +84° (*c* 0.3 H_2O). ^{1}H NMR; δ 5.08 (H-1.1); 3.68 (H-2.1); 3.80 (H-3.1); 3.89 (H-4.1); 3.94 (H-5.1); 3.64 (H-6.1); 3.56 (H-6'.1); 4.94 (H-1.2); 3.62 (H-2.2); 1.42−1.73 (H-3.2, H-4.2); 3.89 (H-5.2); 1.04 (H-6.2); 5.16 (H-1.3); 3.89 (H-2.3); 3.91 (H-3.3); 3.67 (H-4.3); 3.83 (H-5.3); 3.75 (H-6.3); 3.69 (H-6'.3); 4.66 (H-1.4); 3.81 (H-2.4); 3.70 (H-3.4); 3.40 (H-4.4); 3.62 (H-5.4); 1.21 (H-6.4). $J_{12.1}$ 4.1 Hz; $J_{23.1}$ 10.5; $J_{34.1}$ 3.2; $J_{45.1}$ 1.0; $J_{56.1}$ 4.5; $J_{56'.1}$ 6.3; $J_{66'.1}$ 11.5; $J_{12.2}$ 3.6; $J_{4a5.2}$ 11.2; $J_{4e5.2}$ 2.1; $J_{56.2}$ 6.1; $J_{12.3}$ 1.5; $J_{23.3}$ 3.2; $J_{34.3}$ 9.6; $J_{45.3}$ 9.6; $J_{56.3}$ 2.3; $J_{56'.3}$ 4.9; $J_{66'.3}$ 12.0; $J_{12.4}$ 1.8; $J_{23.4}$ 3.3; $J_{34.4}$ 9.4; $J_{45.4}$ 9.4; $J_{56.4}$ 6.4. ^{13}C NMR: 102.3 ppm (C-1.1); 69.9 (C-2.1); 70.8 (C-3.1); 70.3 (C-4.1); 72.4 (C-5.1); 62.1 (C-6.1); 101.8 (C-1.2); 66.5 (C-2.2); 32.7 (C-3.2); 27.0 (C-4.2); 69.3 (C-5.2); 21.3 (C-6.2); 101.4 (C-1.3); 80.3 (C-2.3); 78.2 (C-3.3); 67.5 (C-4.3); 74.6 (C-5.3); 61.7 (C-6.3); 100.9 (C-1.4); 71.7 (C-2.4); 70.6 (C-3.4); 83.2 (C-4.4); 68.5 (C-5.4); 18.2 (C-6.4), 69.0 (−O−CH_2−); 53.0 (−O−CH_3).

8-Methoxycarbonyloct-1-yl 4-O-(4,6-O-cyclohexylidene-2-O-(1,6-di-O-acetyl-3,4-di-O-benzoyl-α-D-galactopyranosyl)-3-O-exo-(3,6-dideoxy-4-O-formyl-α-D-ribo-hexopyranose-1,2-O-methylidenyl)-α-D-mannopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (32). The trisaccharide (*21*) (260 mg 0.190 mmol) was treated with the hexopyranosyl chloride (*3*) (prepared from (*1*) (250 mg 0.73 mmol) in solvent B under the glycosylation conditions described above. Work up and separation by preparative TLC gave 217 mg (68 %) of *32*. ^{1}H NMR: δ 5.44 (H-1.1); 5.28 (H-2.1); 5.74 (H-3.1); 5.91 (H-4.1); 4.41 (H-5.1); 4.17 (H-6.1); 4.09 (H-6'.1); 5.76 (H-1.2); 4.34 (H-2.2); 2.20 (H-3.2); 2.20 (H-3'.2); 4.93 (H-4.2); 3.73 (H-5.2); 1.26 (H-6.2); 4.88 (H-1.3); 3.89 (H-2.3); 3.91 (H-3.3); 4.06 (H-4.3); 3.71 (H-5.3); 3.83 (H-6.3); 3.80 (H-6'.3); 4.66 (H-1.4); 5.26 (H-2.4); 5.22 (H-3,4); 3.72 (H-4.4); 3.86 (H-5.4); 1.36 (H-6.4); 6.26 (*H*-orthoformate). $J_{12.1}$ 3.9 Hz; $J_{23.1}$ 11.0; $J_{34.1}$ 3.6; $J_{56.1}$ 5.6; $J_{56'.1}$ 7.5; $J_{66'.1}$ 11.5; $J_{12.2}$ 5.2; $J_{23.2}$ 2.8; $J_{23'.2}$ 2.8; $J_{34.2}$ 4.0; $J_{3'4.2}$ 5.1; $J_{45.2}$ 8.5; $J_{56.2}$ 6.6; $J_{12.3}$ 0; $J_{23.3}$ 3.1; $J_{34.3}$ 9.5; $J_{45.3}$ 9.5; $J_{56.3}$ 0; $J_{56'.3}$ 5.0; $J_{66'.3}$ 10.5; $J_{12.4}$ 1.9; $J_{23.4}$ 3.4; $J_{34.4}$ 9.6; $J_{45.4}$ 9.6; $J_{56.4}$ 6.2. ^{13}C-NMR: 97.9 ppm (C-1.1); 61.4 (C-6.1); 100.6 (C-1.2); 33.9 (C-3.2); 17.9 (C-6.2); 97.2 (C-1.3); 77.1 (C-2.3); 77.1 (C-3.2); 64.1 (C-6.3); 97.2 (C-1.4); 77.1 (C-4.4); 19.1 (C-6.4); 160.3 (*C*-formyl) 115.4 (*C*-orthoformate); 51.2 (−O−CH_3).

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