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SYNTHESIS OF THE PENTASACCHARIDE REPEATING UNIT OF THE O-SPECIFIC POLYSACCHARIDE FROM *SALMONELLA STRASBOURG*

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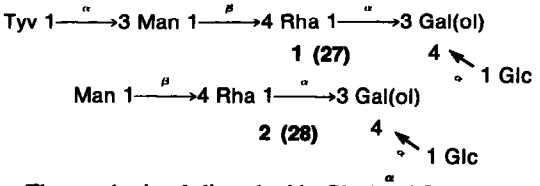
Abstract—The pentasaccharide α - Tyv - (1→3) - β - D - Man - (1→4) - α - L - Rha - (1→3) - D - Gal - (4←1) - α - D - Glc **1**, the repeating unit of the O-specific polysaccharide chain of the lipopolysaccharide from *S. strasbourg*, was obtained by glycosylation of benzyl - 2,6 - di - O - benzyl - 4 - O - (2,3,4 - tri - O - benzyl - 6 - O - benzoyl - α - D - glucopyranosyl) - β - D - galactopyranoside with 1,2 - methylorthoacetyl - 3 - O - acetyl - 4 - O - [3 - O - (2,4 - di - O - acetyl - 3,6 - dideoxy - α - D - arabino - hexopyranosyl) - 2,4,6 - tri - O - acetyl - β - D - mannopyranosyl] - β - L - rhamnopyranose **3** followed by removal of protecting groups. The structure of the synthetic pentasaccharide was proved by methylation analysis and ^{13}C NMR.

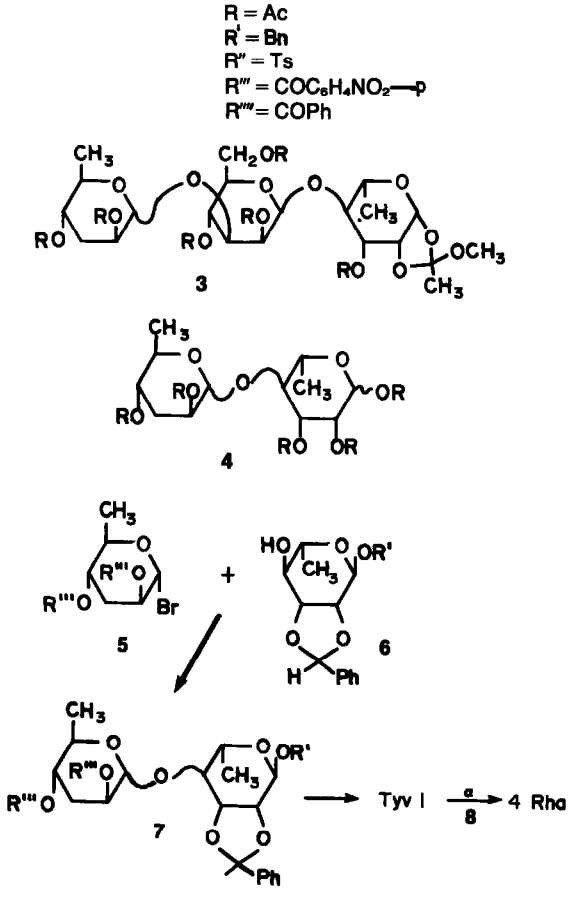
According to our program on synthesis of oligosaccharide repeating units of O-specific polysaccharides from Salmonella species[1] we report now the synthesis of branched pentasaccharide α - Tyv - (1→3) - β - D - Man - (1→4) - α - L - Rha - (1→3) - D - Gal - (4←1) - α - D - Glc **1** which is the repeating unit of *S. strasbourg* O-antigen (serological group D_2).[2] Specific difficulties in this synthesis were connected with the high lability of tyveloside linkage.†

The most rational strategy for the pentasaccharide synthesis seemed to be a 3+2 scheme. The scheme included separate preparations of protected trisaccharide derivative α - D - Tyv - (1→3) - β - D - Man - (1→4) - L - Rha and protected disaccharide α-D-Glc-(1→4)-D-Gal and condensation of these two fragments into the pentasaccharide at the final stage of the synthesis. The limiting point of the scheme was the preparation of activated derivative of the trisaccharide for final condensation, due to the high instability of tyveloside linkage in the trisaccharide under acid conditions, which seems to be unavoidable for conversion of the trisaccharide to acetogalogenose or orthoester derivative **3**. The synthesis of trisaccharide component comprised the introduction of tyvelose unit into the disaccharide β-D - Man-(1→4)-L-Rha, used as a starting material.

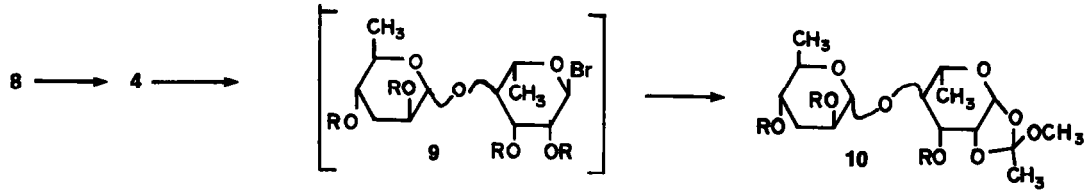


The synthesis of disaccharide Glc 1→4 Gal was described in the previous paper.[1] To find the best conditions for preparation of the trisaccharide orthoester, a simpler disaccharide α-Tyv-(1→4)-Rha has been synthesised and used as a model compound.

Glycosylation of benzyl 2,3-O-benzylidene-α-L-rhamnopyranoside **6**[4] with 3,6-dideoxy-2,4-di-O-p-nitrobenzoyl-α-D-arabino-hexopyranosyl bromide **5**[5] by a Helferich reaction, gave the disaccharide derivative **7** in 50% yield. PMR data of **7** were in accordance with the expected structure. After removal of protecting groups by Zemplen saponification and hydrogenolysis, the disaccharide **8** was obtained. Mild hydrolysis of **8** produced tyvelose and rhamnose, identified as acetates of derived alditols in 1:1 molar ratio by glc. Ion-exchange chromatography in borate buffer[6] showed **8** to be homogeneous. Acetylation of **8** with pyridine-acetic anhydride gave the pentaacetate of **8** (**4**), PMR data of which agreed with the expected structure.



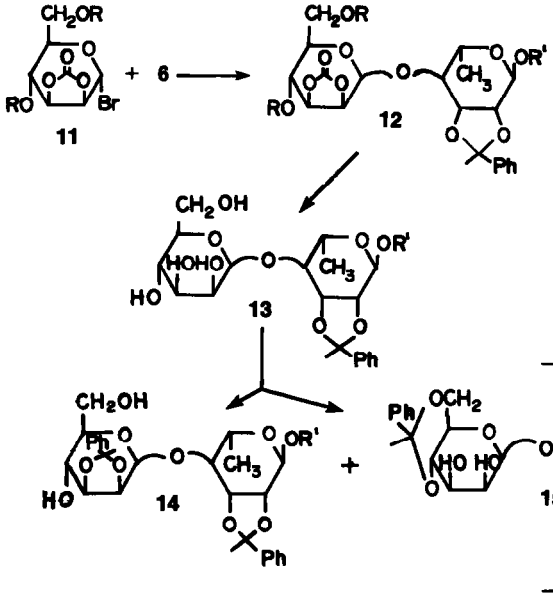
†For synthesis of disaccharide fragments containing 3,6-dideoxyhexoses see Ref. 3.



It was found, that optimal conditions for the conversion of the acetate **4** into orthoester, in which the splitting of the tyvelosyl-rhamnose glycosidic bond would be minimised, were the treatment of acetate **4** with saturated hydrogen bromide in methylene chloride at 0° followed by standard conversion of the bromide into the orthoester through treatment with methanol and collidine in nitromethane.[7]

The disaccharide orthoester **10** was obtained in 70% yield, the structure of **10** was confirmed by PMR. Taking into account this experience the synthesis of trisaccharide orthoester **3** was then realized; the synthesis of starting derivative of β-D-Man-(1→4)-L-Rha **18** has been accomplished according to ref. 8 as follows.

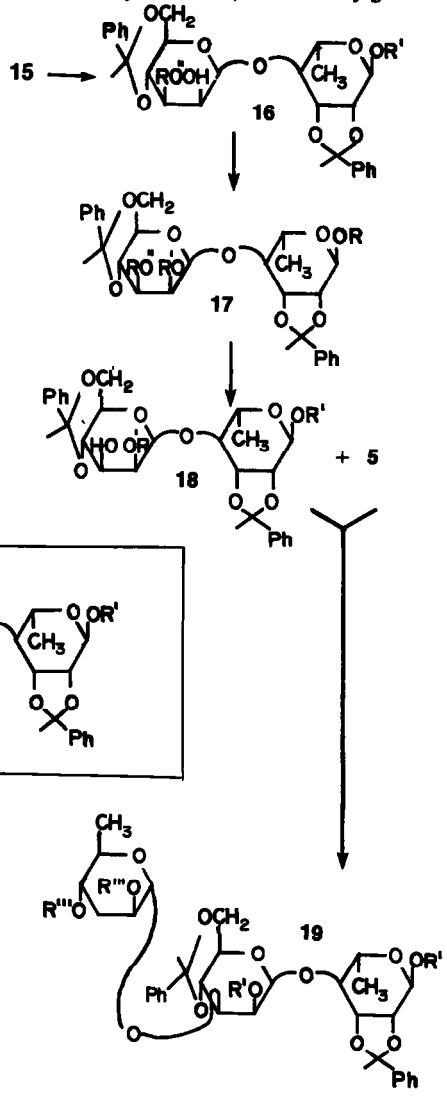
Glycosylation of benzyl rhamnoside **6** with mannosyl bromide **11**[8] gave disaccharide **12** in 90% yield. The structure of **12** was proved by conversion into the known crystalline heptaacetate of β-D-Man-(1→4)-α-L-Rha. Zemplen saponification of **12** led to the disaccharide derivative **13** in high yield. The coupling of **13** with



1 mole of benzylidene bromide in boiling pyridine[9] gave a mixture of dibenzylidene derivatives **14** and **15**; both compounds were isolated after chromatography on silicagel in 30% yield. The structures of **14** and **15** were established by methylation, methanolysis and glc identification of α-methyl-4,6-di-O-methylmannopyranoside for **14** and α-methyl-2,3-di-O-methylmannopyranoside for **15** correspondingly.

Selective tosylation of **15** with 1 mole of tosylchloride in pyridine[10] gave 3-O-tosylate **16** in 78% yield. Benzylation of **16** by benzyl bromide with Ag_2O in DMFA gave rise to completely protected derivative **17**, which was detosylated by boiling with methanolic sodium methoxide. Detosylated product **18** was obtained in 80% yield, and the presence of the only free OH-group at C_3 of mannose residue was supported by methylation analysis.

The coupling of tyvelosyl bromide **5** with disaccharide derivative **18** under conditions analogous to those used for synthesis of model disaccharide **7** have been accomplished for preparation of protected derivative **19** of the trisaccharide. PMR data of **19** were in agreement with the proposed structure. Deacylation of **19** followed by hydrogenolysis gave the free trisaccharide **20**. Ion-exchange chromatography in borate buffer showed **20** to be homogeneous. Methylation analysis of glycosyl-rhamnitol **21** obtained from **20** by $NaBH_4$ reduction, demonstrated the formation of acetates of 2,4-di-O-methyltyvelitol, 2,4,6-tri-O-methylmannitol and 1,2,3,5-tetra-O-methylrhamnitol, identified by glc-MS.



Comparison of ^{13}C NMR spectra of trisaccharide **20**, disaccharide **22**, and α-methyltyveloside **23**, finally confirmed the structure of **20** (see Table 1). The α-

Table 1.

	23	22			20				2					1					
	α-Tyv	β-Man	α-Rha	β-Rha	α-Tyv	β-Man	α-Rha	β-Rha	β-Man	α-Rha	α-Glc	α-Gal	β-Gal	α-Tyv	β-Man	α-Rha	α-Glc	β-Gal	α-Gal
C1	101.0	101.75	95.05	94.5	102.4	101.75	95.05	94.7	101.8	102.85* 102.7	101.3	93.6	97.81	102.28	101.68	102.77	101.3	97.73	93.6
C2	68.05	71.8	72.2	71.8	68.2	71.3	72.4	71.8	71.9	71.5	73.3	69.9	73.3	68.15	71.42	71.42	73.18	73.18	
C3	34.7	74.25	71.2	74.0	34.6	81.7	71.3	74.1	74.4	71.5	78.3	78.3	80.75	34.6	81.67	71.42	73.79	80.58	
C4	70.85	67.95	80.8	80.4	71.8	67.4	81.1	80.6	68.2	79.35	70.5	76.5	76.5	71.85	67.7	79.3	70.39	76.3	
C5	68.5	77.3	68.2	72.8	68.6	77.25	68.2	72.9	77.5	68.2	73.3	72.8	76.1	68.6	77.24	68.15	73.18	76.09	
C6	18.0	62.2	18.3	18.3	18.0	62.2	18.35	18.35	62.35	18.2	61.5	61.75	61.6	18.0	62.21	18.26	61.75	61.75	

*Splitting of C1 signal ... Rha $1 \xrightarrow{\alpha} 3$ α-D-Gal 102.7 ppm, ... Rha $1 \xrightarrow{\alpha} 3$ β-D-Gal 102.85 ppm.

configuration of the tyvelosyl-mannose glycosydic bond was determined unequivocally by comparison of chemical shifts of C-3 and C-5 carbon atom signals in ^{13}C NMR spectra of trisaccharide **20** and glycoside **23**.[11,12]

Acetylation of trisaccharide **20** with pyridine-acetic anhydride gave the octaacetate **24**, its structure was proved by PMR data.

The critical stage of the synthesis (conversion of octaacetate **20** into orthoester **3**, which is the glycosylating agent in final condensation) has been accomplished taking into consideration the optimal conditions selected from formation of the model orthoester **10**. Also the key trisaccharide orthoester **3** was obtained under these conditions (see above) in 70% yield; PMR data of a chromatographically homogeneous sample of **3** were in general accordance with the expected structure, but the integral intensity ratio of tyvelose C-methyl group to rhamnose C-methyl group was 0.8:1. This is an indication that approximately 20% of tyvelosyl residues were split off during the synthesis of the bromide. After glycosylation of the disaccharide **25**[1] with the orthoester **3** in the presence of 4 Å molecular sieves[1] the pentasaccharide derivative **26** was isolated in 17.5% yield. PMR data of the chromatographically homogeneous product agreed with the structure **26**, but the integral intensity of tyvelose C-methyl group was again 20% lower than that of rhamnose C-methyl group.

The removal of blocking groups from **26** under usual conditions gave rise to the mixture of pentasaccharide **1** and tetrasaccharide **2**, which were identified by ion-exchange chromatography in borate buffer and PC.

Sodium-borohydride reduction of the mixture of **1** and **2** in borate buffer[13] gave rise to the mixture of the corresponding glycosyl-galactitols **27** and **28**. Ion-exchange chromatography in borate buffer of the mixture of **27** and **28** (obtained directly from **26**) permitted the identification of glycosyl-galactitol **28** with the authentic sample[1] and to show 5:1 ratio of **1** and **2** in the reaction mixture (i.e. 20% of tetrasaccharide admixture after condensation). The formation of 20% admixture of tetrasaccharide **2** could be explained by partial splitting of tyvelosyl-mannose linkage during the synthesis of orthoester **3** from acetate **24**. As a result of this process we obtained the mixture of the disaccharide and the trisaccharide orthoesters unseparable by TLC, in 1:5 ratio (PMR data confirms this conclusion). Preparative importance of the synthesis is not influenced very much by this fact, as the final pentasaccharide **1** could be easily purified by PC.

Pure pentasaccharide **1** was easily isolated by preparative PC. Acid hydrolysis of the pentasaccharide followed by $NaBH_4$ reduction and acetylation gave acetates of tyvelitol, rhamnitol, mannitol, glucitol and galactitol, in 0,4:1:1:1:1 molar ratios as identified by glc (tyvelose is probably partially degraded through acid hydrolysis).

Methylation analysis of **27** gave rise to acetates of 2,4-di-O-methyltyvelitol, 2,3-di-O-methylrhamnitol, 1,2,5,6-tetra-O-methylgalactitol and 2,4,6-tri-O-methylmannitol identified by glc-MS.

Analysis of ^{13}C NMR spectrum of pentasaccharide **1** was unequivocal because trisaccharide **20** and tetrasaccharide **2** serve as a good reference compounds.

$$\underbrace{\text{Tyv } 1\xrightarrow{\alpha}3 \text{ Man } 1\xrightarrow{\beta}}_{A}\underbrace{4 \text{ Rha } 1\xrightarrow{\alpha}3 \text{ Gal } 4\xleftarrow{\alpha}1 \text{ Glc}}_{B} \quad \mathbf{1}$$

$$\underbrace{\text{Tyv } 1\xrightarrow{\alpha}3 \text{ Man } 1\xrightarrow{\beta}}_{A}4 \text{ Rha} \quad \mathbf{20}$$

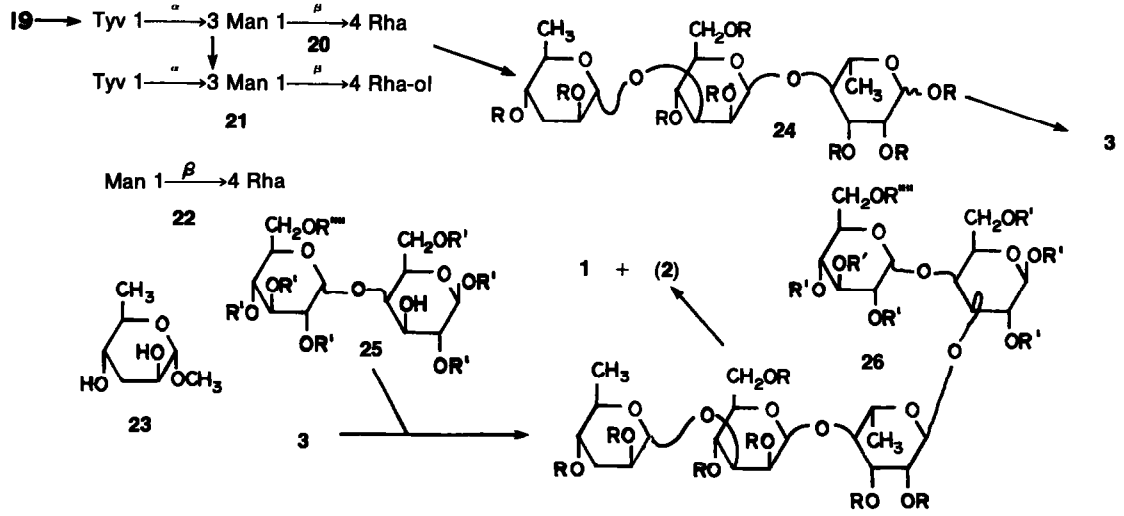
$$\text{Man } 1\xrightarrow{\beta}\underbrace{4 \text{ Rha } 1\xrightarrow{\alpha}3 \text{ Gal } 4\xleftarrow{\alpha}1 \text{ Glc}}_{B} \quad \mathbf{2}$$

The signals of carbon atoms in ^{13}C NMR spectra have good coincidence for the region A in spectra of **1** and **20**, and for region B in spectra of **1** and **2**. Specifically, the complete correlation of rhamnose signals in ^{13}C NMR spectra of **1** and **2** unequivocally confirmed the α-configuration of this residue in **1**.

In conclusion it should be noted, that synthetic scheme developed for synthesis of **1** could be considered as general pathway for the synthesis of biological repeating units of other O-specific polysaccharide from Salmonella A, B and D_1 serological groups.

EXPERIMENTAL

Melting points were determined with a Kofler apparatus and are uncorrected. PMR spectra were recorded on a Varian DA-60-IL spectrometer with Me_4Si as the internal standard. ^{13}C NMR spectra were obtained with a Bruker WP-60 spectrometer (15.08 MHz) having an 8 K memory. The spectral width used was 3750 Hz, the acquisition time 1.1 sec, the pulse width 3 μsec (30°) and the number of transients was ~10,000 for disaccharides



(concentration ~80 mg/ml) and ~90,000 for tetra- and pentasaccharides (concentration ~30 mg/ml). Substances were dissolved in D_2O with CH_3OH as the internal standard (50.15 ppm). All chemical shifts are expressed in δ. Glc-MS was carried out on a Varian MAT 111 (GNOM) instrument. Optical rotations were determined with a Perkin–Elmer 141 polarimeter. Solutions were concentrated *in vacuo* at 40°. Ion-exchange chromatography of neutral carbohydrates[6] was carried out with 71-100 A carbohydrate analyzer (ČSSR) on the Durrum DA X4 resin at 55° under following conditions: column A: 13×0.5 cm; 0.5 M sodium-borate buffer, pH 8.54; 20 ml/h; column B: 25×0.5 cm, 0.5 M sodium-borate buffer, pH 8.54, 60 ml/h; column C: 30×0.5 cm, 0.5 M sodium-borate buffer, pH 8.54, 20 ml/h; column D: 13×0.5 cm, 0.2 M sodium-borate buffer, pH 8.54, 20 ml/h. The orcinol-sulphuric acid reagent was used to monitor separations. Tlc was performed on silica gel LSL 5/40 μ (Chemapol), plc on silica gel containing 5% of gypsum; column chromatography on silica gel L 100/160 μ (Chemapol). Analytical PC was carried out by ascending method on Filtrak FN 11 paper, preparative PC on Filtrak FN 18 paper. The following solvent systems were used for chromatography: toluene–ethyl acetate 9:1 (1), chloroform–acetone 95:5 (2), ether-toluene 2:1 (3); ether-toluene 9:1 (4), benzene–ether 10:2 (5), toluene–ethyl acetate 94:6 (6), BuOH–pyridine–H_2O 6:4:3 (7), chloroform–acetone 9:1 (8), toluene–ethyl acetate 6:4 (9). Methylation analysis of oligosaccharides was performed by standard methods.[14]

Benzyl 2,3 - O - *benzylidene* - 4 - O - (2,4 - *di* - O - *p* - *nitrobenzoyl* - 3,6 - *dideoxy* - α - D - *arabino* - *hexopyranosyl*) - α - L - *rhamnopyranoside* **7**

Hydrogen bromide saturated solution of methyl 2,4 - di - O - p - nitrobenzoyl - 3,6 - dideoxy - α - D - arabino - hexopyranoside {500 mg, m.p. 138–141°, $[\alpha]_D^{20}-59°$ ($CHCl_3$)}[5] in CH_2Cl_2 (15 ml) was kept at 0° for 2 h, then evaporated as quickly as possible (HBr being removed by co-evaporation with CH_2Cl_2). The syrupy residue was dissolved in 10 ml of dry CH_3NO_2 and the solution was added dropwise (30 min) to a stirred solution of benzyl 2,3-O-(exo)-benzylidene-α-L-rhamnoside {400 mg, m.p. 132–133°, $[\alpha]_D^{20}-67°$ (c 2, $CHCl_3$)}[4] and $Hg(CN)_2$ (280 mg) in CH_3NO_2 (20 ml). Solution was stirred for 1 h at r.t., washed with H_2O and evaporated. Column chromatography (elution with benzene-ether) gave 7 (440 mg), 50%, $[\alpha]_D^{20}-25°$ (c 2, $CHCl_3$), R_f 0.8 (system 1). Calc. for $C_{40}H_{38}O_{14}N_2$: C, 62.4; H, 4.9; N, 3.6; Found: C, 62.3, H, 5.0; N, 3.7%. PMR data ($CDCl_3$): 8.0 (d, 8 H p-nitrobenzoate); 7.2 (10 H, aromatic); 1.35 (d, 3 H, $J_{5,6}=6$ Hz, C–Me of rhamnose), 1.1 (d, 3 H, $J_{5,6}=6$ Hz, C–Me of tyvelose).

4 - O - α - 3,6 - *dideoxy* - D - *arabino* - *hexopyranosyl* - L - *rhamnopyranose* **8**

A methanolic solution of 7 (240 mg) was deacetylated with 0.5 M methanolic sodium methoxide and then deionized with KU-2 (H^+) resin, filtered, and concentrated. The product was hydrogenated over 10% palladium–charcoal in 20 ml of ethanol to give 8 (100 mg), 35%, $[\alpha]_D^{20}+68.5°$ (c 2, H_2O). Ion-exchange chromatography in borate buffer showed 8 to be homogeneous with R_t 32 min (column D). Acid hydrolysis (0.1 M HCl, 100°, 2 h) of 8 followed by $NaBH_4$ reduction and acetylation with pyridine-acetic anhydride gave rise to acetate of tyvelitol and rhamnitol in 1:1 molar ratio, identified by glc.

1,2,3 - *Tri* - O - *acetyl* - 4 - O - (2,3 - *di* - O - *acetyl* - 3,6 - *di* - *deoxy* - α - D - *arabino* - *hexopyranosyl*) - L - *rhamnopyranose* **4**

Disaccharide 8 (20 mg) was acetylated by acetic anhydride-pyridine. Solution was evaporated with addition of toluene to give 4 (27 mg), $[\alpha]_D^{20}+28°$ (c 2.45, $CHCl_3$), R_f 0.7 (system 2). PMR data (CCl_4): 2.0 (6 H, OAc); 1.35 (d, 3 H, $J_{5,6}=6$ Hz, C–Me of rhamnose); 1.1 (d, 3H, $J_{5,6}=6$ Hz, C–Me of tyvelose).

3 - O - *Acetyl* - 1,2 - O - *methylorthoacetyl* - 4 - O - (2,4 - *di* - O - *acetyl* - 3,6 - *dideoxy* - α - D - *arabino* - *hexopyranosyl*) - β - L - *rhamnopyranose* **10**

A solution of HBr in CH_2Cl_2 (10 ml; saturated at 0°) was quickly added to a stirred solution of 4 (100 mg) in CH_2Cl_2 (10 ml, 0°, protected against moisture), stirring was continued for 5 min (0°). Solution was evaporated as quickly as possible and HBr was removed by co-evaporation with CH_2Cl_2. The syrupy residue was dissolved in CH_3NO_2 (10 ml), 2,4,6-collidine (0.055 ml) and methanol (0.03 ml) were added, and solution was kept at 37° for 48 h. The standard procedure[15] gave chromatographically pure 10 (70 mg), 70%, $[\alpha]_D^{20}+57°$ (c 7, $CHCl_3$). PMR data (CCl_4): 3.15 (3 H, OMe); 2.1 (6 H, OAc); 1.95 (3 H, OAc); 1.6 (3 H, C–Me of orthoester); 1.35 (d, 3 H, $J_{5,6}=6$ Hz, C–Me of rhamnose); 1.1 (d, 3 H, $J_{5,6}=6$ Hz, C–Me of tyvelose).

Benzyl 2,3 - O - *benzylidene* - 4 - O - (4,6 - *di* - O - *acetyl* - 2,3 - O - *carbonyl* - β - D - *mannopyranosyl*) - α - L - *rhamnopyranoside* **12**

Solution of 4,6 - di - O - acetyl - 2,3 - O - carbonyl - α - D - mannopyranosyl bromide {11, 5 g, m.p. 79–80°, $[\alpha]_D^{20}+89.8°$ ($CHCl_3$)}[8] in $CHCl_3$ (50 ml) was added dropwise in the darkness to a stirred soln of 6 (2.62 g), Ag_2O (5 g), 4 Å molecular sieve (3 g) in $CHCl_3$ (50 ml), stirring was continued for 30 min, followed by filtration and evaporation. Column chromatography of the residue (elution with benzene–ether) gave 12 (4.45 g), 91%, R_f 0.5 (system 3), $[\alpha]_D^{20}-67.9°$ (c 2, $CHCl_3$). Calc. for $C_{31}H_{34}O_{13}$: C, 60.1; H, 5.5. Found: C, 59.9; H, 5.9%. PMR data ($CDCl_3$): 7.0 (10 H, aromatic), 2.0 (6 H, OAc), 1.32 (d, 3 H, $J_{5,6}=6$ Hz, C–Me of rhamnose). A methanolic soln of 12 was deacylated with 0.5 M methanolic sodium methoxide, and then deionized with KU-2 (H^+) resin, filtered and concentrated. The product was hydrogenated over 10% palladium–charcoal in 20 ml of ethanol, soln was filtered and evaporated. The residue was acetylated with pyridine–acetic anhydride, the acetate formed was subjected to acetolysis with acetic anhydride–zinc chloride as described in Ref. 8 to give the heptaacetate of 4-O-β-D-mannopyranosyl-α-L-rhamnopyranose with m.p. 164–165° (EtOH) {lit.[8] m.p. 164–165°, $[\alpha]_D^{20}-67.8°$ ($CHCl_3$)}.

Benzyl 2,3 - O - *benzylidene* - 4 - O - (β - D - *mannopyranosyl*) - α - L - *rhamnopyranoside* **13**

A methanolic soln of 12 (2.7 g) was deacylated with 2 M methanolic sodium methoxide, and then deionized with KU-2 (H^+) resin, filtered and concentrated, to give 13 (2.12 g), 95%, $[\alpha]_D^{20}-91.4°$ (c 2, $CHCl_3$).

Benzyl - 2,3 - O - *benzylidene* - 4 - O - (4,6 - O - *benzylidene* - β - D - *mannopyranosyl*) - α - L - *rhamnopyranoside* **15**

A soln of 13 (2 g) and benzylidene bromide (0.68 ml) in pyridine (100 ml) was refluxed for 1 h, cooled and evaporated. Residue was dissolved in chloroform, washed with H_2O, evaporated. Column chromatography of the residue (elution with toluene–ether) gave 15 (540 mg), 30%, R_f 0.7 (system 4), m.p. 186–187° (ether), $[\alpha]_D^{20}-88.8°$ (c 2, $CHCl_3$). Calc. for $C_{33}H_{36}O_{10}$: C, 66.9; H, 61. Found: C, 66.8; H, 6.1% and 14 (510 mg) with R_f 0.6 (system 4). Methylation of 15 and 14 followed by methanolysis (4% HCl in MeOH, 16 h, 100°) gave 2,3-di-O-methyl-α-methyl-D-mannoside for 15 and 4,6-di-O-methyl-α-methyl-D-mannoside for 14, identified by glc with authentic samples.

Benzyl 2,3 - O - *benzylidene* - 4 - O - (3 - O - *tosyl* - 4,6 - O - *benzylidene* - β - D - *mannopyranosyl*) - α - L - *rhamnopyranoside* **16**

A solution of tosyl chloride (230 mg) in pyridine (5 ml) was added dropwise to a stirred cooled (−40°) soln of 15 (600 mg) in pyridine (12 ml). Stirring was continued for 1 h (−40°), the soln was kept at +5° overnight and evaporated; the residue was dissolved in chloroform (50 ml), washed with H_2O and concentrated. Column chromatography of the residue (elution with benzene–ether) gave 16 (470 mg), 78%, m.p. 203–204°, $[\alpha]_D^{20}-98.2°$ (c 2, $CHCl_3$), R_f 0.4 (system 2). Calc. for $C_{40}H_{42}O_{12}S$: C, 64.0; H, 5.7. Found: C, 63.8; H, 5.7%. PMR data ($(CD_3)_2CO$): 7.0 (19 H, aromatic), 2.3 (3 H, CH_3 of tosylate); 1.32 (d, 3 H, $J_{5,6}=4$ H_z, C–Me of rhamnose).

Benzyl 2,3 - O - *benzylidene* - 4 - O - (2 - O - *benzyl* - 3 - O - *tosyl* - 4,6 - O - *benzylidene* - β - D - *mannopyranosyl*) - α - L - *rhamnopyranoside* **17**

The mixture of **16** (1.3 g), benzyl bromide (3.8 ml), DMFA (1.5 ml) and Ag_2O (2.3 g) was stirred in the dark for 20 h at r.t., then the precipitate was filtered off and washed with chloroform; combined filtrates were concentrated. The residue was dissolved in chloroform, washed with H_2O, and chloroform layer was concentrated. Column chromatography of the residue (elution with benzene–ether) gave **17** (1.39 g), 95%, $[\alpha]_D^{20} - 77.6°$ (c 1, $CHCl_3$). Calc. for $C_{47}H_{48}O_{12}S$: C, 69.1; 5.3. Found: C, 69.9; H, 5.3%. PMR data (CCl_4): 7.0 (24 H, aromatic), 2.3 (3 H, CH_3 of tosylate), 1.32 (d, 3 H, $J_{5,6} = 5$ Hz, C–Me of rhamnose).

Benzyl 2,3 - O - *benzylidene* - 4 - O - (2 - O - *benzyl* - 4,6 - O - *benzylidene* - β - D - *mannopyranosyl*) - α - L - *rhamnopyranoside* **18**

A soln of **17** (1.46 g) in 50 ml of methanolic sodium methoxide (2.5 g of Na in 50 ml of absolute methanol) was boiled for 6 h, cooled, H_2O was added, and the soln was evaporated. The residue was extracted with chloroform, washed with H_2O, and evaporated. Column chromatography of the residue (elution with benzene–ether) gave **18** (1 g; 79%), R_f 0.5 (system 5), $[\alpha]_D^{20} -$ 102.5° (c 2, $CHCl_3$). Calc. for $C_{40}H_{42}O_{10}$: C, 70.5; H, 6.1. Found: C, 70.0; H, 6.2%. PMR data (CCl_4); 7.0 (20 H, aromatic), 1.32 (d, 3 H, $J_{5,6} = 5$ Hz, C–Me of rhamnose). Methylation of **18** followed by hydrogenolysis, hydrolysis, $NaBH_4$ reduction and acetylation gave acetates of rhamnitol and 3-O-methylmannitol, identified by glc-MS.

Benzyl 2,3 - O - *benzylidene* - 4 - O - [2 - O - *benzyl* - 4,6 - O - *benzylidene* - 3 - O - (2,4 - *di* - O - *p* - *nitrobenzoyl* - 3,6 - *di* - *deoxy* - α - D - *arabino* - *hexopyranosyl*) - β - D - *mannopyranosyl*] - α - L - *rhamnopyranoside* **19**

Solution of **5** (obtained from 120 mg methyl 2,4 - di - O - p - nitrobenzoyl - α - D - arabino - hexopyranoside, analogous to **7**) in CH_3NO_2 (5 ml) was added dropwise (0.5 h) to a stirred solution of **18** (90 mg) and Hg $(CN)_2$ (50 mg) in CH_3NO_2 (10 ml). Stirring was continued for 30 min, then solution was diluted with chloroform and washed with H_2O, chloroformic layer was concentrated. Column chromatography of the residue (elution with benzene–ether) gave **19** (50 mg), 35%, R_f 0.5 (system 6). PMR data (CCl_4): 8.0 (8 H, p-nitrobenzoate). 7.0 (20 H, aromatic), 1.32 (d, 3 H, $J_{5,6} = 5$ Hz, C–Me of rhamnose); 1.15 (d, 3 H, $J_{5,6} = 5$ Hz, C–Me of tyvelose).

1,2,3 - *Tri* - O - *acetyl* - 4 - O - [2,4,6 - *tri* - O - *acetyl* - 3 - O - (2,4 - *di* - O - *acetyl* - 3,6 - *dideoxy* - α - D - *arabino* - *hexopyranosyl*) - β - D - *mannopyranosyl*] - L - *rhamnopyranose* **24**

A methanolic soln of **19** (250 mg) was deacylated with 0.2 M methanolic sodium methoxide, and then deionized with KU-2 (H^+) resin, filtered, and concentrated. The product was debenzylated over 10% palladium–charcoal in ethanol (20 ml) to give **20** (90 mg), 90%, $R_{Glc} = 0.96$ (PC system 7). Ion-exchange chromatography in borate buffer showed **20** to be homogeneous with R_f 30 min (column D). ^{13}C NMR data are shown in Table 1. Reduction of **20** (2 mg) in H_2O (10 ml) with $NaBH_4$ (10 mg) followed by deionisation with KU-2 (H^+) resin, filtration and evaporation gave glycosyl-rhamnitol **21**. Methylation analysis of **21** gave rise to acetates of 2,4-di-O-methyltyvelitol, 1,2,3,5-tetra-O-methylrhamnitol and 2,4,6-tri-O-methylmannitol.

Acetylation of trisaccharide **20** (90 mg) with pyridine-acetic anhydride gave **24** (180 mg), 100%, $[\alpha]_D^{20} - 17°$ (C 1, $CHCl_3$), R_f 0.5 (system 8). PMR data (CCl_4): 2.2–1.9 (24 H, OAc); 1.32 (d, 3 H, $J_{5,6} = 6$ Hz, C–Me of rhamnose); 1.1 (d, 3 H, $J_{5,6} = 6$ Hz, C–Me of tyvelose).

3 - O - *Acetyl* - 1,2 - O - *methylorthoacetyl* - 4 - O - [2,4,6 - *tri* - O - *acetyl* - 3 - O - (2,4 - *di* - O - *acetyl* - 3,6 - *dideoxy* - α - D - *arabino* - *hexopyranosyl*) - β - D - *mannopyranosyl*] - β - L - *rhamnopyranose* **3**

A cooled (0°) saturated soln of hydrogen bromide in CH_2Cl_2 (30 ml) was added as quickly as possible to a cooled (0°) stirred solution of **24** (250 mg) in CH_2Cl_2 (5 ml). The mixture was kept for 20 min at 0°, then evaporated, hydrogen bromide was removed by co-evaporation with CH_2Cl_2, and the residue was dried *in vacuo*, and dissolved in CH_3NO_2 (10 ml), 2,4,6-collidine (0.3 ml) and MeOH (0.2 ml). The solution was kept at 37° for 72 h. After the treatment similar to that described for **10**, the solution was concentrated to give **3** (170 mg), 70%, R_f 0.5 (system 8), $[\alpha]_D^{20} + 11°$ (c 1, $CHCl_3$). PMR data (CCl_4): 3.15 (3 H, O–Me); 2.2–1.9 (18 H, OAc); 1.6 (3 H, C–Me of orthoester), 1.3 (d, 3 H, $J_{5,6} = 4$ Hz, C–Me of rhamnose), 1.1 (d, 2.4 H, $J_{5,6} = 4$ Hz, C–Me of tyvelose).

Benzyl 2,6 - *di* - O - *benzyl* - 3 - O - [2,3 - *di* - O - *acetyl* - 4 - O - {2,4,6 - *tri* - O - *acetyl* - 3 - O - (2,4 - *di* - O - *acetyl* - 3,6 - *di* - *deoxy* - α - D - *arabino* - *hexopyranosyl*) - β - D - *mannopyranosyl*} - α - L - *rhamnopyranosyl*] - 4 - O - (2,3,4 - *tri* - O - *benzyl* - 6 - O - *benzoyl* - α - D - *glucopyranosyl*) - β - D - *galactopyranoside* **26**

Disaccharide derivative **25** (230 mg)[1] was glycosylated as described in Ref. 16 with orthoester **3** (170 mg) to give **26** (50 mg; 17.5%) $[\alpha]_D^{20} + 21°$ (c 2.5, $CHCl_3$) after PLC (system 9, R_f 0.5). PMR data (CCl_4): 8.0–7.0 (34 H, aromatic), 2.0 (21 H, OAc), 1.32 (d, 3 H, $J_{5,6} = 5$ Hz, C–Me of rhamnose). 1.1 (d, 2.4 H, $J_{5,6} = 5$ Hz, C–Me of tyvelose).

3 - O - [4 - O - {3 - O - (3,6 - *dideoxy* - α - D - *arabino* - *hexopyranosyl*) - β - D - *mannopyranosyl*} - α - L - *rhamnopyranosyl*] - 4 - O - (α - D - *glucopyranosyl*) - D - *galactopyranose* **1**

A methanolic soln of **26** (50 mg) was deacylated with 0.2 M methanolic sodium methoxide, and then deionized with KU-2 (H^+) resin, filtered and concentrated. The product was debenzylated over 10% palladium–charcoal in EtOH (20 ml) to give the syrup (20 mg). PC of the syrup (system 7) showed the presence of two compounds: **1** (major, $R_{Glc} = 0.42$) and **2** (minor, $R_{Glc} = 0.28$, see Ref. 1). The reduction of mixture (2 mg) with $NaBH_4$ (10 mg) in water (2 ml) with H_3BO_3 (20 mg), followed by deionization with KU-2 (H^+) resin and concentration gave a mixture of glycosyl-galactitols (**27** and **28**) in the ratio 5:1 as showed by ion-exchange chromatography in borate buffer (**27**, R_f 97 min, **28** R_f 207 min, column C, see Ref. 1). The mixture of **1** and **2** (20 mg) was separated by PC to give **1** (15 mg), $[\alpha]_D^{20} +$ 42.5° (c 1, H_2O), $R_{Glc} = 0.42$ (PC system 7), ^{13}C NMR spectrum see Table 1 and **2** (4 mg), $R_{Glc} = 0.28$ (PC system 7, see Ref. 1). Acid hydrolysis of **1** (1 mg, 0.2 M HCl, 100°, 1 h) followed by $NaBH_4$ reduction and acetylation gave the acetates of tyvelitol, rhamnitol, glucitol, mannitol and galactitol in molar ratio 0.4:1:1:1:1 identified by glc. Reduction of pentasaccharide **1** (3 mg, as described for the mixture of **1** and **2**) gave glycosyl-galactitol **27**, methylation analysis of which gave rise to acetates of 2,4-di-O-methyltyvelitol, 2,3-di-O-methyl-rhamnitol, 2,3,4,6-tetra-O-methylglucitol, 1,2,5,6-tetra-O-methylgalactitol and 2,4,6-tri-O-methylmannitol.

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