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Synthesis of Methyl 3-*O*-(3,6-Dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside

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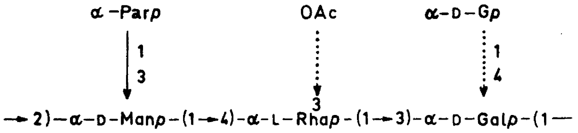
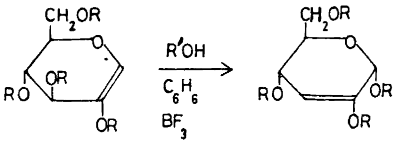
The synthesis of methyl 3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside, required for immunological studies, is described. The key step in the synthesis consists in the boron trifluoride catalyzed reaction of 2,3,4,6-tetra-*O*-benzoyl-D-*arabino*-hex-1-enopyranose (I) with methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (II).

The repeating unit of the O-specific side-chains of the *Salmonella* serogroup A cell wall lipopolysaccharide may be formulated as follows.[1] α-Par*p* denotes a 3,6-dideoxy-α-D-*ribo*-hexopyranosyl (α-paratosyl) unit. The serological O-factor 2 is thought to be associated with the α-paratosyl unit linked to the 3-position of the α-D-mannose residue. It is of immunological interest to have available di- and oligosaccharides corresponding to the various postulated O-factors. Syntheses of methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-α-D- and -β-D-mannopyranosides corresponding to the *Salmonella* O-factors 9 and 46 have previously been communicated from this laboratory.[2,3] In these syntheses, advantage was taken of the orthoester glycosylation method,[4] which produces the required 1,2-*trans* geometry at the 1- and 2-positions in the 3,6-dideoxyhexose moiety. In the corresponding D-*ribo* isomer (paratose), however, this relationship is 1,2-*cis*. Two recent methods of potential value for the synthesis of α-D-glycosides and disaccharides with *cis*-geometry at positions 1 and 2 have been described. One of these, devised by Lemieux and co-workers, uses nitrosyl chloride adducts of glycals.[5] In the other, devised by Ferrier and co-workers, an acylated 2-hydroxyglycal is treated with an alcohol, which may be a suitably protected monosaccharide unit, in benzene



$$\begin{array}{ccccc} \alpha\text{-Par}p & & \text{OAc} & & \alpha\text{-D-G}p \\ \downarrow{\scriptstyle 1 \atop 3} & & \vdots & & \vdots{\scriptstyle 1 \atop 4} \\ & & 3 & & \\ \to 2)\text{-}\alpha\text{-D-Man}p\text{-}(1\to 4)\text{-} & & \alpha\text{-L-Rha}p\text{-}(1\to 3)\text{-} & & \alpha\text{-D-Gal}p\text{-}(1\text{—} \end{array}$$

solution, with boron trifluoride as catalyst. A displacement reaction takes place in which the double bond migrates to the 2,3-positions and the acyloxy group at C-3 is expelled.

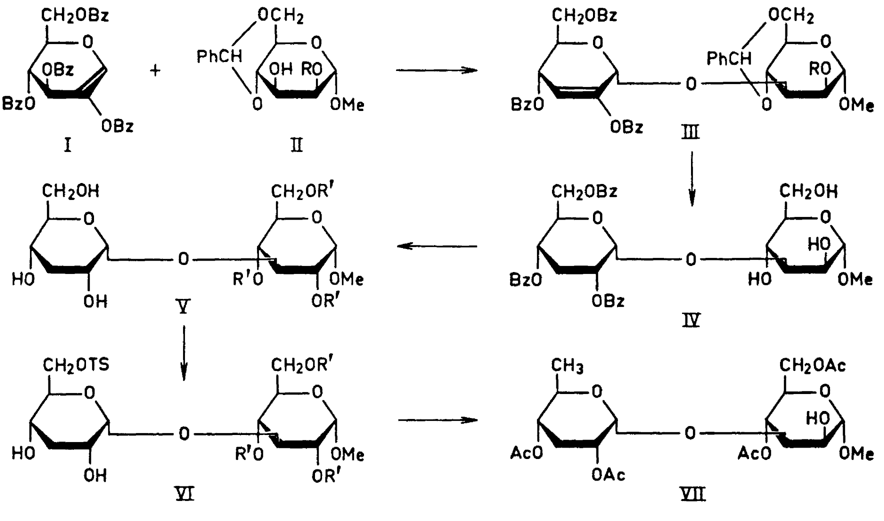


R = COCH$_3$ or COC$_6$H$_5$

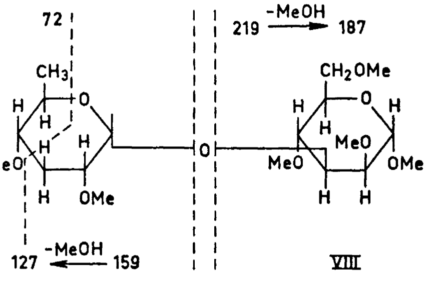
In 3,6-dideoxyglycoside synthesis, the latter method has the attractive feature of glycosidation occurring with the simultaneous introduction of the 3-deoxy function. This and the high yields generally obtained in the reaction offset the fact that the glycosidation is not stereospecific.

The present paper describes the synthesis, by the Ferrier method[6] of methyl 3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside (VII), required for immunological studies which will be reported elsewhere.

2,3,4,6-Tetra-*O*-benzoyl-1-deoxy-D-*arabino*-hex-1-enopyranose (I) was allowed to react with methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (II) in benzene containing a catalytic amount of boron trifluoride. The yield of syrupy III, purified by silica gel column chromatography, was 51 %. A slower-moving fraction which probably contained the impure β-anomer of III was also obtained. The olefinic disaccharide III was dissolved in the



Bz = C$_6$H$_5$CO- R = C$_6$H$_5$CH$_2$- R' = CH$_3$CHOEt minimum amount of tetrahydrofuran, the solution was diluted with ethanol and hydrogenated with 10 % palladium on charcoal at atmosphere pressure. The choice of solvent was important for the success of the reaction, *i.e.* simultaneous removal of benzyl and benzylidene as well as hydrogenation of the pyranosidic double bond. Chromatographic purification of the product afforded pure, syrupy IV in a 74 % yield. The epimeric isomer of IV containing a 3-deoxy-D-*arabino*-hexosyl unit instead of the 3-deoxy-D-*ribo*-hexosyl unit of IV was present in a minor fraction isolated by chromatography. The 3-deoxy-hexosyl unit of IV was converted into the corresponding 3,6-dideoxy-hexosyl unit by the following route: Acetalization of the three free hydroxyl groups in the mannosyl unit of IV by the acid-catalyzed reaction of IV with ethyl vinyl ether, followed by debenzoylation afforded syrupy V in a 48 % yield. Monotosylation of V at low temperature afforded a 39 % yield of VI in addition to unreacted starting material. Reduction of VI with lithium aluminium hydride introduced the required 6-deoxy group. Careful hydrolysis of the product with 50 % aqueous acetic acid at room temperature removed the 1′-etoxyethyl residues from the disaccharide. Acetylation of the resulting disaccharide and chromatographic purification of the final product afforded pure VII in a yield of 68 % from VI. The product VII gave a correct elemental analysis. The optical rotation is in agreement with the expected anomeric configuration. Further confirmation of the structure of VII was obtained as follows. Deacetylation yielded methyl 3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside. Mild acid hydrolysis of an aliquot of this material, followed by borohydride reduction and acetylation gave methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside and 1,2,4,5-tetra-*O*-acetyl-3,6-dideoxy-α-D-*ribo*-hexitol in the expected proportion. Another aliquot was fully methylated to yield VIII which was examined by GLC, in which a single component was observed, and by MS. The mass spectrum was that expected for the postulated structure. The origin of some of the pertinent fragment is indicated in VIII.



VIII

The various fragments obtained and their relative intensities are given in the experimental part. The mass spectrum is consistent with the structure VIII.[7,8] An identical spectrum, apart from minor differences in relative peak intensities, was observed for the corresponding pentamethyl ether of the previously synthesized methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl-β-D-mannopyranoside.

EXPERIMENTAL

General methods. Concentrations were performed at reduced pressure. Optical rotations were determined at room temperature (20 – 22°) using a Perkin-Elmer 141 polarimeter. NMR spectra (in deuteriochloroform) were recorded with a Varian A-60 A spectrometer with a V 6058 A unit for decoupling experiments. Tetramethylsilane was used as internal reference and chemical shifts (δ) are given in ppm downfield from this reference. Pertinent parts of the various NMR spectra are given in the appropriate sections below, the remainder of the spectra were invariably in accordance with the presumed structures. GLC-MS was run on a Perkin-Elmer 270 instrument at a manifold temperature of 200°, an ionization potential of 70 eV, ionization current of 80 μA and a temperature at the ion source chamber of 80°. TLC was performed on silica gel F_{254} (Merck). Sulphuric acid was used as spray reagent. GLC analyses were performed on a Perkin-Elmer F 11 instrument fitted with glass columns. The carrier gas was nitrogen and the flow rate 30 ml/min. The column packing was 3 % ECNSS-M on Gas Chrom Q unless otherwise stated.

*Methyl 3-O-(2,4,6-tri-O-benzoyl-3-deoxy-α-D-*glycero*-hex-2-enopyranosyl)-2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside* (III). 2,3,4,6-Tetra-*O*-benzoyl-D-*arabino*-hex-1-enopyranose, I,[9] (1.50 g) and methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside, II[2], (0.98 g) were dissolved in dry benzene (25 ml). Boron trifluoride etherate (45 %, 1.5 ml) was added and the solution was allowed to stand at room temperature for 25 min. The reaction mixture was neutralized with sodium carbonate, poured into water and the aqueous solution extracted with chloroform. The combined chloroform phases were dried over sodium sulphate, filtered and concentrated. The resulting syrupy product was purified by silica gel column chromatography (solvent, toluene – ethyl acetate 4:1). The yield of syrupy III was 1.11 g. The product III which was chromatographically pure was used directly in the next step. NMR: δ 5.79 (1 H), doublet, $J = 2$ Hz, H-3; δ 5.29 (1 H), singlet, benzylidene methine, δ 4.92 (1 H), singlet, H-1; δ 4.42 (2 H), singlet, benzyl methylene; δ 2.82 (3 H), singlet, methoxyl proton. A slower fraction containing the aglycone II together with another disaccharide component, possibly the β-anomer of III, was also obtained.

*Methyl 3-O-(2,4,6-tri-O-benzoyl-3-deoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside* (IV). The above product III (3.18 g) was dissolved in the minimum quantity of tetrahydrofuran, diluted with ethanol and hydrogenated with 10 % palladium on charcoal (1.0 g) at atmospheric pressure. The theoretical amount of hydrogen was absorbed after 36 h. The product was purified by TLC (solvent, ethyl acetate) to yield a chromatographically pure syrup (1.71 g). A minor fraction was obtained which upon debenzoylation gave a disaccharide glycoside which on sugar analysis (see below) was shown to contain mannose and 3-deoxy-D-*arabino*-hexopyranose. NMR of IV: δ 7.2 – 8.2 (15 H) aromatic; no olefinic, methine or methylene protons present (*cf.* III above); δ 3.26 (3 H), singlet, methoxyl. NMR on debenzoylated (see below) and per-trimethylsilylated IV: Two anomeric signals were observed, δ 5.11, $J = 3.5$ Hz; δ 4.57, $J = 2$ Hz, showing α-configuration for the 3-deoxy-D-*ribo*-hexosyl moiety. An aliquot of IV was debenzoylated as described below, hydrolyzed with 0.12 M aqueous sulphuric acid, neutralized with barium carbonate, filtered, concentrated and converted into the corresponding alditol acetate mixture. GLC[10] showed the presence of two components in a ratio 1:1 with retention times indistinguishable from 1,2,4,5,6-penta-*O*-acetyl-3-deoxy-D-glucitol (which has a higher retention time than the corresponding D-mannitol derivative) and 1,2,3,4,5,6-hexa-*O*-acetyl-D-mannitol.

*Methyl 3-O-(3-deoxy-6-O-p-tolylsulphonyl-α-D-*ribo*-hexopyranosyl)-2,4,6-tri-O-(1′-ethoxyethyl)-α-D-mannopyranoside* (VI). The above product IV (1.71 g) dissolved in dichloromethane (40 ml) containing 0.4 % hydrogen chloride, was treated with ethyl vinyl ether (4.0 ml) for 96 h, neutralized with sodium carbonate, filtered and concentrated. TLC (solvent, ethyl ether – light petroleum (40 – 60°) 3:1) showed a major component contaminated by small amounts of faster- and slower-moving materials. The major product was obtained in a chromatographically pure state as a syrup (1.19 g) by TLC. This, without further characterization, was used directly in the next step. The syrup (510 mg) in methanol (50 ml) was treated with barium oxide (100 mg) at reflux temperature for 1 h. The mixture was cooled, filtered and concentrated to yield a syrup which after purification by TLC (solvent, ethyl ether – acetone 1:1) yielded chromatographically pure material (301 mg). This was dissolved in pyridine (20 ml). *p*-Toluenesulphonyl chloride (134 mg) was added at −30°. After 24 h at this temperature further *p*-toluenesulphonyl chloride (134 mg) was added at the same temperature. After a further 48 h at −30° the solution was poured into ice-water. The mixture was extracted with chloroform. The combined chloroform phases were dried over magnesium sulphate, filtered and concentrated to yield an impure syrup (275 mg) from which the major component (145 mg) was isolated by TLC (solvent, ethyl ether−acetone 1:1). NMR: δ 7.97 (2 H), doublet $J=8$ Hz, aromatic, δ 7.50 (2 H), doublet, $J=8$ Hz, aromatic, $J=8$ Hz; δ 3.45 (3 H), singlet, methoxyl; δ 2.50 (3 H), singlet, toluene methyl protons.

*Methyl 3-O-(3,6-dideoxy-α-*D-ribo*-hexopyranosyl)-α-*D*-mannopyranoside pentaacetate* (VII). The above monotosylate VI (145 mg) was dissolved in tetrahydrofuran (10 ml). Lithium aluminium hydride (excess) was added and the mixture refluxed for 3 h. The reaction mixture was neutralized with aqueous phosphoric acid, filtered and concentrated to a syrup (140 mg). The latter was hydrolyzed in 50 % aqueous acetic acid at room temperature for 15 min and concentrated to a syrup which was acetylated with acetic anhydride (1 ml) in pyridine (1 ml) at room temperature overnight. The impure product VII was poured onto ice-water and the mixture extracted with chloroform. The combined chloroform phases were dried over magnesium sulphate and concentrated to yield a syrup (119 mg) which was purified by TLC (toluene−ethyl acetate 3:2) to yield chromatographically pure VII (80 mg), $[\alpha]_D$ +85°. (Found: C 51.7; H 6.53. $C_{23}H_{34}O_{14}$ requires: C 51.7; H 6.41.) An aliquot of VII was deacetylated with 1.67 % ammoniacal methanol. Part of the deacetylated VII was subjected to mild acid hydrolysis (0.125 M aqueous sulphuric acid, for 1 h at 100°), the hydrolysate was neutralized with barium carbonate and worked up. The mixture was reduced with sodium borohydride and per-acetylated. Examination by GLC-MS [11,12] revealed the presence of methyl α-D-mannoside tetraacetate, indistinguishable from authentic material and also 1,2,4,5-tetra-*O*-acetyl-3,6-dideoxy-α-D-*ribo*-hexitol with a higher retention time than 1,2,4,5-tetra-*O*-acetyl-3,6-dideoxy-α-D-*arabino*-hexitol, but with the same mass spectrum, apart from minor differences in peak intensities. Another part of the deacetylated VII was methylated [13] to yield the methyl 3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside pentamethyl ether VIII which was pure by GLC on an XE-60 (3 % on Gas chrom Q) column. The MS showed, *inter alia*, the following peaks (relative intensities in brackets): 45 (16), 71 (18), 72 (100), 73 (9), 85 (12), 88 (5), 95 (5), 99 (8), 101 (12), 127 (12), 145 (24), 159 (17), 219 (5), 233 (9), 265 (8).

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REFERENCES

1. Hellerqvist, C. G., Lindberg, B., Samuelsson, K. and Lindberg, A. A. *Acta Chem. Scand.* **25** (1971) 955.
2. Borén, H., Garegg, P. J. and Wallin, N. H. *Acta Chem. Scand.* **26** (1972) 1082.
3. Garegg, P. J. and Wallin, N. H. *Acta Chem. Scand.* **26** (1972) 3892.
4. Kochetkov, N. K., Khorlin, A. J. and Bochkov, A. F. *Tetrahedron* **23** (1967) 693.
5. Lemieux, R. U., Suemitsu, R. and Gunner, S. W. *Can. J. Chem.* **46** (1968) 1040.
6. Ferrier, R. J., Prasad, N. and Sankey, G. H. *J. Chem. Soc.* **C 1969** 587.
7. Kochetkov, N. K. and Chizhov, O. S. *Advan. Carbohydrate Chem.* **21** (1966) 39.
8. Johnson, G. S., Ruliffson, W. S. and Cooks, R. G. *Chem. Commun.* **1970** 587.
9. Ferrier, R. J. and Sankey, G. H. *J. Chem. Soc.* **C 1966** 2339.
10. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. *Anal. Chem.* **37** (1965) 1602.
11. Björndal, H., Lindberg, B. and Svensson, S. *Acta Chem. Scand.* **21** (1967) 1801.
12. Björndal, H., Lindberg, B. and Svensson, S. *Carbohyd. Res.* **5** (1967) 433.
13. Hakomori, S. *J. Biochem. (Tokyo)* **55** (1964) 205.

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