

SYNTHESIS OF 3,6-DIDEOXY-3-(METHYLAMINO)HEXOSES FOR G.L.C.–M.S. IDENTIFICATION OF *Rhizobium* LIPOPOLYSACCHARIDE COMPONENTS*

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ABSTRACT

A direct synthetic route from methyl α -D-glucopyranoside to 3,6-dideoxy-3-(methylamino)hexoses having the D-*gluco*, D-*galacto*, and D-*manno* configurations has been developed. Methyl α -D-glucoside was converted into the 4,6-O-benzylidene-2,3-di-O-tosyl derivative, which was then transformed into the 4-O-benzyl-6-deoxy 2,3-ditosylate (**5**) by successive reductive cleavage of the acetal ring, iodination, and reduction. The intermediate **5** was readily converted into the *allo* 2,3-epoxide, which yielded the pivotal intermediate methyl 4-O-benzyl-3,6-dideoxy-3-(methylamino)- α -D-glucopyranoside (**7**) by cleavage of the oxirane ring with methylamine. The amino compound **7** can be directly converted into the derivatized *galacto* and *manno* derivatives for mass-spectrometric identification by selective inversion at C-4 and C-2, respectively, followed by hydrolysis, reduction, and acetylation.

INTRODUCTION

The glycosyl components of lipopolysaccharides (LPS) from *Rhizobium* are complex and consist of common hexoses (mannose, galactose, and glucose) and deoxyhexoses (rhamnose and fucose), as well as such unusual, substituted saccharides as a 6-deoxy-2-O-methylhexose, a 6-deoxy-3-O-methylhexose, a 3-O-methylhexose, a 3,6-dideoxy-3-(methylamino)hexose, and 2-amino-2,6-dideoxy-D-glucose (quinovosamine)^{1–3}. Quinovosamine was recently synthesized⁴, and identified as an important component³ of the LPS of *R. trifolii* 0403. However, determination of the configuration of many of the other unusual saccharides in rhizobial LPS is hindered by the lack of standards for comparison by g.l.c.–m.s.

The ability of *R. trifolii* LPS to bind a clover lectin, trifoliin A, changes with culture age³. The relative proportion of 3,6-dideoxy-3-(methylamino)hexose, a

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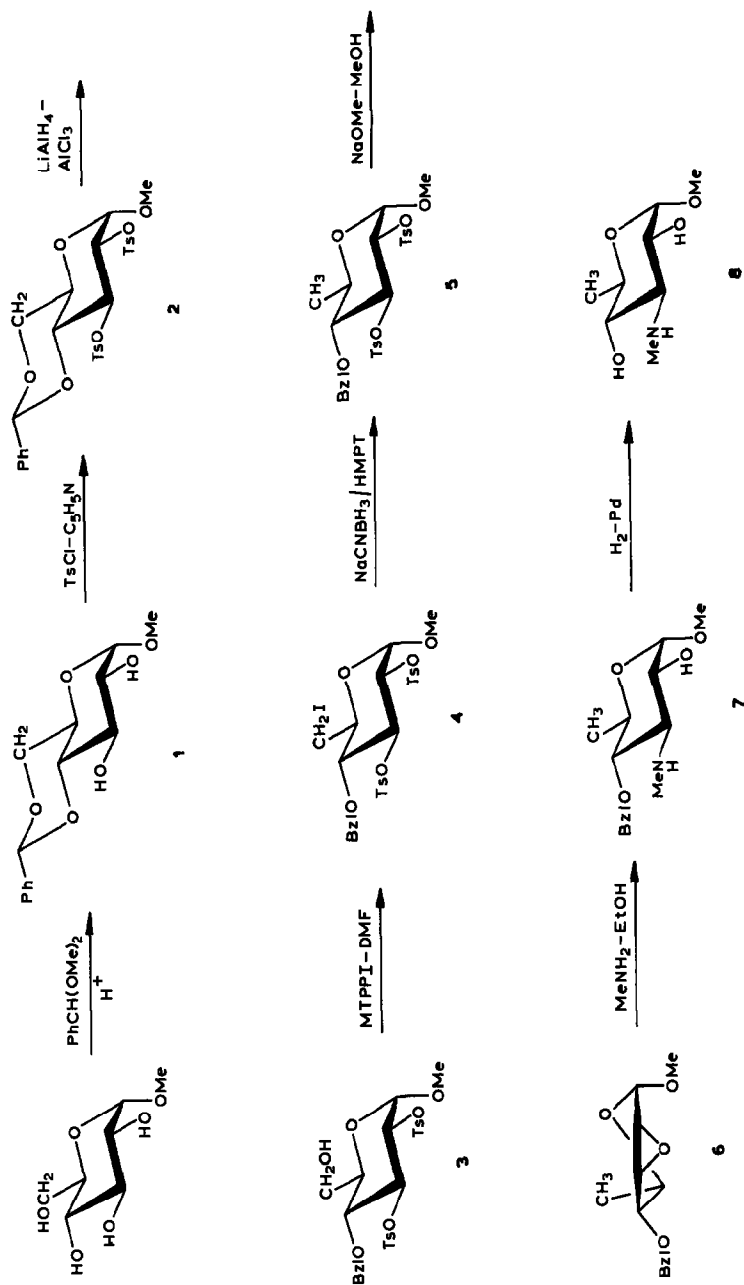
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major component of *R. trifolii* 0403 LPS, also changes with culture age³. A viable synthetic route to the most probable configurations of this isomeric series was, therefore, necessary in order to achieve definitive identification of LPS components and to obtain authentic samples for use in hapten-inhibition studies. This could ultimately define the sugar-binding specificity of trifoliin A. The synthetic route we chose was designed to generate a product of known configuration and a well-defined intermediate that could be converted, unequivocally, into derivatives of other configurations for g.l.c.-m.s. studies, without necessarily isolating and characterizing the intermediates. The partially protected *gluco* analog **7** possessed the required synthetic flexibility to be readily converted into the *manno* (**12**) and *galacto* (**16**) derivatives.

RESULTS AND DISCUSSION

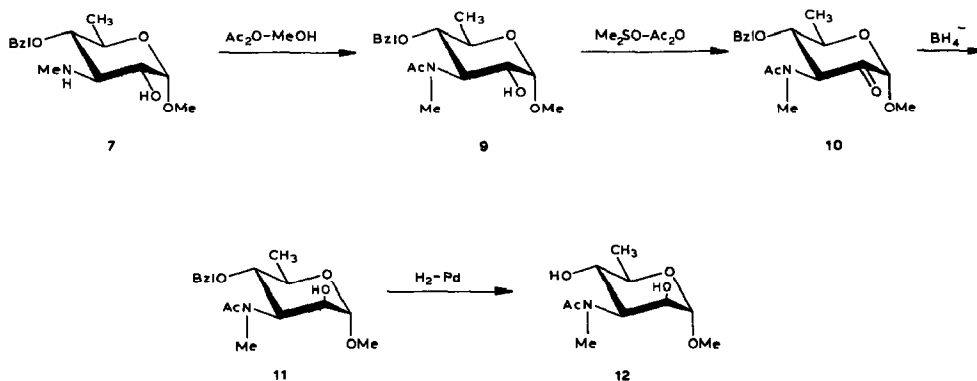
Methyl α -D-glucopyranoside was converted into the 4,6-benzylidene acetal **1** in high yield by a one-step modification of an earlier acetal-exchange reaction⁵. The acetal **1** was converted into the ditosylate **2**. Reductive cleavage of the dioxane ring of **2** with lithium aluminum hydride-aluminum chloride yielded exclusively the 4-benzyl ether **3**; this is consistent with the observation that the specificity of this reagent is determined by steric effects⁶. The bulky tosyl groups in the 2- and 3-positions hindered attack of the dioxane ring at O-4, leading to preferential attack at O-6. The 4-benzyl ether **3** was converted into the 6-iodo compound **4** with methyltriphenoxyphosphonium iodide (MTPPI) in *N,N*-dimethylformamide (DMF). The iodo compound underwent facile reduction with cyanoborohydride to the 6-deoxy compound **5**. Treatment of **5** with sodium methoxide in methanol resulted in ready conversion into the *allo* 2,3-epoxide **6**. Cleavage of the oxirane ring in **6** with methylamine yielded the 3-methylamino *gluco* compound **7** as the major product. The alternative, 2-methylamino *altro* compound is the "normal" cleavage product expected to be generated from the half-chair conformer **6**. The 3-substituted *gluco* product is formed from the alternative half-chair conformer. The 1,3-*trans* interaction between the glycosidic methoxyl group and H-5 (present in **6**) is absent for the alternative half-chair conformation. The absence of a severe, steric interaction between the would-be axial benzyl group and the pyran-ring oxygen atom in the conformer alternative to **6** more than offset the destabilizing influence of the axial methyl group. The cleavage product **7** was then converted into the desired *gluco* compound **8** by hydrogenolysis with palladium-on-carbon as the catalyst.

The ¹H-n.m.r. spectrum of **8** showed a 3-proton singlet signal at δ 2.70, readily assignable to the methyl protons of the methylamino group. The *gluco* configuration was confirmed by the presence of two triplets, at δ 3.27 and 3.40, each with splittings of ~ 10 Hz, indicating that each proton had two *trans*-diaxial neighbors. These signals were assigned to H-3 and H-4, respectively. The signal for the proton on the ring-carbon atom to which the methylamino group is attached is



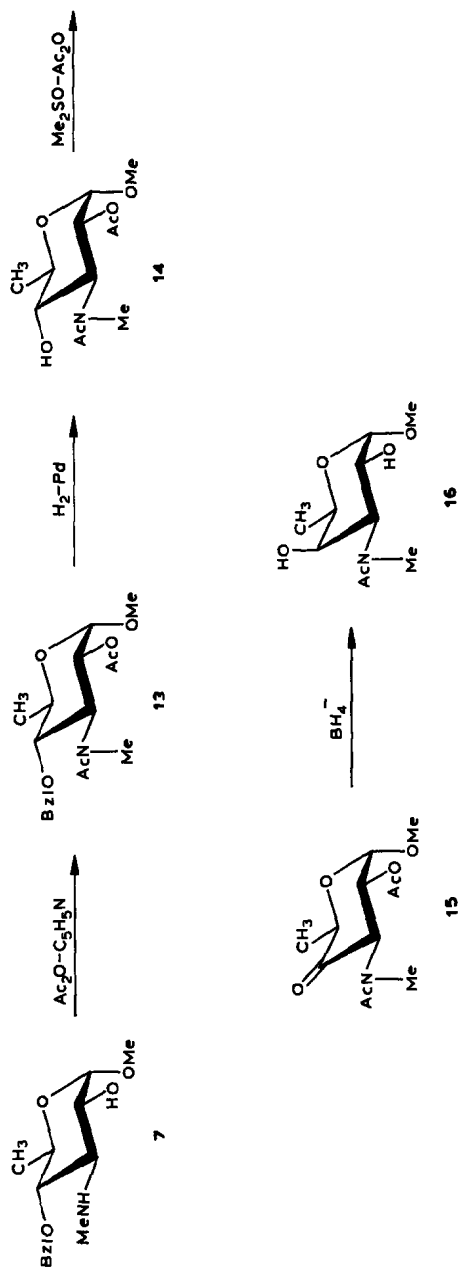
expected to be farthest upfield; the splitting and multiplicity indicated that the proton was, indeed, H-3. As expected, H-2 gave a doublet of doublets (10.0 and 2.40 Hz), the smaller splitting being due to H-1. The protons of the 6-deoxy function were readily recognizable as a 3-proton doublet (J 6 Hz) at δ 1.20. The H-5 signal was a symmetrical multiplet centered at δ 3.68.

The partially protected *gluco* product **7** was *N*-acetylated to give **9**, and this was converted into the 2-keto product **10** by oxidation with dimethyl sulfoxide-acetic anhydride; then **10** was reduced to an epimeric mixture of *manno* and *gluco* alcohols. The mixture was debenzylated by hydrogenolysis, and the *manno* compound **12** identified by g.l.c.-m.s. after acid hydrolysis of the mixture in 2M trifluoroacetic acid followed by borohydride reduction to yield a trihydric alcohol which was converted into its triacetate with acetic anhydride and pyridine.



Intermediate **7** was also converted into the *galacto* derivative; first, the *N*-acetyl-*O*-acetyl intermediate **13** was debenzylated to give the alcohol **14**, which was oxidized to the 4-keto compound **15**. Reduction of **15** gave the *galacto* diol **16** along with the *gluco* isomer. The *galacto* product **16** was also identified by conversion into the peracetylated alditol derivative, followed by g.l.c.-m.s. The *gluco* compound predominated in preparations of both the *galacto* and *manno* peracetates but no attempt was made to optimize the yield of these isomers as the synthetic schemes were designed for analytical preparations.

The *gluco* compound **8** was hydrolyzed in 2M trifluoroacetic acid, and the resulting aldehyde group was reduced with sodium borohydride. The resulting alcohol was peracetylated, and the electron-impact mass spectrum of the peracetate (**17**) was recorded (see Fig. 1). The major fragments were derived by cleavage of the peracetylated derivative on either side of the carbon atom bearing the *N*-methyl group. The primary fragments **18** and **22** (m/z 230 and 244, respectively) generated from this process then lost additional neutral species *via* parallel pathways. Loss of acetic acid from **18** gave **19** (m/z 170), which readily lost ketene to generate **20** (m/z 128). It is also possible for **18** to lose acetic anhydride in a single step to give a fragment at m/z 128. The fragment **20** then eliminated ketene again, to give **21** (m/z 86). The primary fragment **22** generated **25** *via* a similar process.



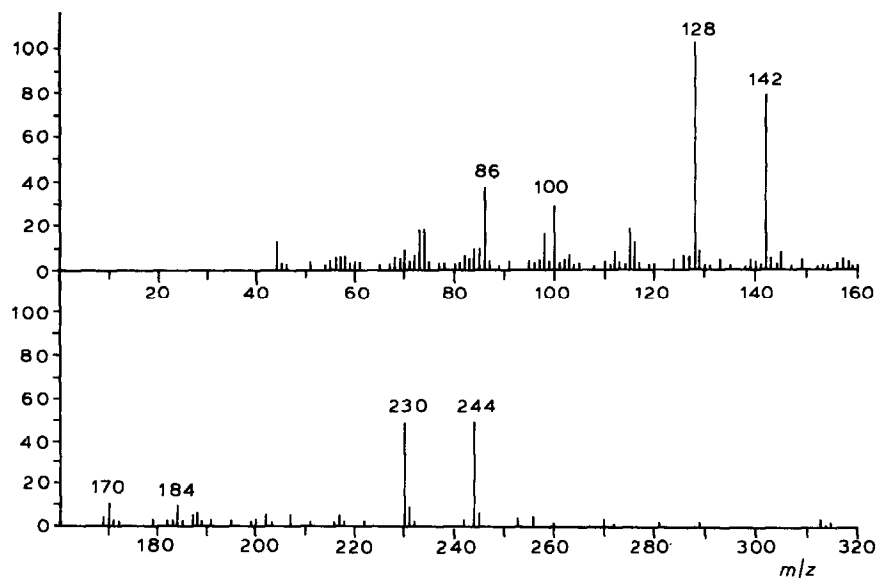
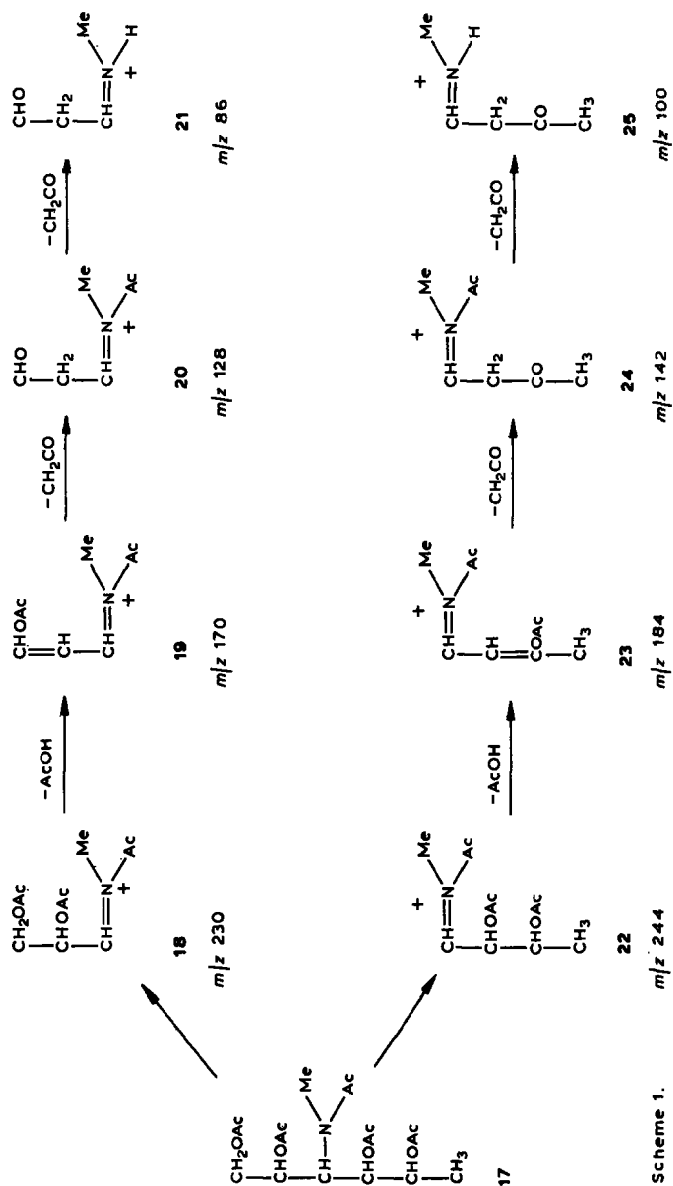


Fig. 1. The 70-eV, electron-impact mass spectrum of the alditol acetate derivative from methyl 3,6-di-deoxy-3-(methylamino)- α -D-glucopyranoside (**8**).

The mass spectra of the alditol acetate derivatives of the three isomeric compounds were indistinguishable. The method of conversion of intermediate **7** into the *galacto* and *manno* derivatives was found especially suited for g.l.c.-m.s. identification. All of the reactions could be performed on a microscale in a vial having a Teflon-lined stopper.

EXPERIMENTAL

General methods. — Melting points were obtained on a Thomas-Hoover capillary melting-point apparatus, and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Thin-layer chromatography was performed on pre-made Merck Kieselgel-60 layers containing a 254-nm fluorescent indicator. Column chromatography was conducted on silica (60–200 mesh). *N,N*-Dimethylformamide was dried by distillation, and stored over molecular sieves (4A). Pyridine was distilled from anhydrous barium oxide, and stored over potassium hydroxide pellets. Dimethyl sulfoxide was distilled *in vacuo*, and stored over molecular sieves (4A). Infrared spectra were obtained on a Perkin-Elmer 700 spectrometer using chloroform solutions. N.m.r. spectra were recorded with a Bruker WM-250 spectrometer for chloroform solutions, unless otherwise stated; chemical shifts are given relative to internal tetramethylsilane. G.l.c.-m.s. was performed on a Hewlett-Packard 5840 gas chromatograph interfaced with a Hewlett-



Scheme 1.

Packard 5985 mass spectrometer operated at 70 eV. Samples were analyzed on 3% of OV-225 on 80–100 Supelcoport in glass columns (2 m \times 0.3 cm), using helium as the carrier.

Samples were peracetylated by dissolving in the minimal volume of pyridine and adding an equal volume of acetic anhydride; the mixture was kept for 18 h at 25°, and then evaporated to dryness. A solution of the residue in chloroform (0.5 mL) was successively washed with 5% sodium hydrogencarbonate (3 \times 1 mL) and water (2 \times 1 mL) and then dried (anhydrous sodium sulfate). All concentrations were carried out in a Büchi rotary evaporator fitted with a water aspirator.

Methyl 4-O-benzyl-2,3-di-O-p-tolylsulfonyl- α -D-glucopyranoside (3). — Methyl α -D-glucopyranoside was converted into the 4,6-benzylidene acetal **1** by acetal exchange between the sugar and benzaldehyde dimethyl acetal, the reagent being generated from benzaldehyde and trimethyl orthoformate, in methanol containing *p*-toluenesulfonic acid as the catalyst, as described⁵. The sugar was added to the reaction mixture in *N,N*-dimethylformamide without isolation of the dimethyl acetal. The 4,6-acetal **1** was converted into the ditosylate **2** by tosylation in pyridine with *p*-toluenesulfonyl chloride. Lithium aluminum hydride (0.8 g, 20 mmol) was suspended in anhydrous diethyl ether (40 mL) and the mixture cooled to -5° . Anhydrous aluminum chloride (2.6 g, 20 mmol) in dry diethyl ether (15 mL) was slowly added from a constant-pressure dropping-funnel. Ditosylate **2** (5.92 g, 10.0 mmol) in dry dichloromethane (20 mL) was then added, with the temperature maintained at -5° . When addition was complete, the temperature was allowed to rise to 25° during 4 h and then the mixture was boiled under reflux for 12 h. The excess of reagents was decomposed by careful, dropwise addition of water-saturated ethyl acetate while the flask was cooled in an ice bath. Ethanol (10 mL) was then added, followed by 5% sodium carbonate solution (50 mL). The white precipitate of aluminum hydroxide was vigorously stirred with ethyl acetate (75 mL), the mixture filtered, and the solid washed with ethanol (20 mL) and ethyl acetate (150 mL). The filtrate and washings were combined, backwashed with cold water (2 \times 75 mL), and dried (sodium sulfate). Thin-layer chromatography in 6:1 chloroform–hexane indicated that the product was largely one component; it was purified by column chromatography using the same solvent-system, to yield a white foam (4.85 g, 82.0%) of **3**; $[\alpha]_D^{+60.7^\circ}$ (*c* 0.34, CHCl₃); ν_{\max} 3575, 3360, and 1600 cm⁻¹; ¹H-n.m.r.: δ 2.35 (s, 3 H, ArCH₃), 2.45 (s, 3 H, ArCH₃), 3.26 (s, 3 H, CH₃O), 3.53–3.80 (m, 4 H, H-4,5,6,6'), 4.27 (dd, 1 H, *J* 8.9 and 3.7 Hz, H-2), 4.40 (d, 1 H, *J* 10.1 Hz, benzylic H), 4.65 (d, 1 H, *J* 10.1 Hz, benzylic H), 4.82 (d, 1 H, *J* 3.7 Hz, H-1), 5.18 (t, 1 H, *J* 8.9 Hz, H-3), and 7.15–7.85 (m, 13 H, aromatic protons).

Anal. Calc. for C₂₈H₃₂O₁₀S₂: C, 56.74; H, 5.44; S, 10.82. Found: C, 56.64; H, 5.55; S, 10.84.

Methyl 4-O-benzyl-6-deoxy-6-iodo-2,3-di-O-p-tolylsulfonyl- α -D-glucopyranoside (4). — To a solution of compound **3** (4.0 g, 6.8 mmol) in anhydrous *N,N*-dimethylformamide (15 mL) was added methyltriphenoxyphosphonium iodide

(6.2 g, 14 mmol). The mixture was flushed with dry nitrogen, and kept, with stirring, for 24 h at room temperature. The deep-orange solution was evaporated, and a solution of the resulting syrup in chloroform (100 mL) was successively washed with cold, 5% sodium thiosulfate solution (2×25 mL) and cold water (4×50 mL), dried (anhydrous sodium sulfate), and evaporated to a syrup which was purified by column chromatography in 3:1 chloroform-hexane to yield a straw-colored gum (3.5 g, 72.0%) of **4**; $[\alpha]_D +67.6^\circ$ (c 0.66, CHCl_3); ν_{\max} 3050, 2955, and 1600 cm^{-1} ; $^1\text{H-n.m.r.}$: δ 2.37 (s, 3 H, ArCH_3), 2.46 (s, 3 H, ArCH_3), 3.15–3.30 (m, 2 H, H-6,6'), 3.28 (s, 3 H, CH_3O), 3.32–3.55 (m, 2 H, H-4,5), 4.33 (dd, 1 H, J 8.5 and 2.9 Hz, H-2), 4.58 (d, 1 H, J 10.0 Hz, benzylic H), 4.77 (d, 1 H, J 2.9 Hz, H-1), 4.82 (d, 1 H, J 10.0 Hz, benzylic H), 5.20 (t, 1 H, J 8.5 Hz, H-3), and 7.20–7.92 (m, 13 H, aromatic protons).

Anal. Calc. for $\text{C}_{28}\text{H}_{31}\text{IO}_9\text{S}_2$: C, 47.87; H, 4.45; I, 18.06; S, 9.13. Found: C, 48.16; H, 4.69; I, 18.26; S, 9.32.

Methyl 4-O-benzyl-6-deoxy-2,3-di-O-p-tolylsulfonfyl- α -D-glucopyranoside (5). — To a solution of compound **4** (3.0 g, 4.2 mmol) in dry *N,N*-dimethylformamide (5 mL) were successively added hexamethylphosphoric triamide (15 mL) and sodium cyanoborohydride (0.53 g, 8.4 mmol), and the mixture was stirred for 5 h at 60° and then for 10 h at 25° . The solution was poured into cold water (50 mL), and the mixture extracted with toluene (5×50 mL). The extracts were combined, backwashed with water (3×50 mL), dried (anhydrous sodium sulfate), and evaporated to a syrup which was chromatographed on a column of silica in 3:1 chloroform-hexane to yield compound **5** as a colorless gum (1.94 g, 80.0%); $[\alpha]_D +48.9^\circ$ (c 0.60, CHCl_3); ν_{\max} 3050, 2955, and 1600 cm^{-1} ; $^1\text{H-n.m.r.}$: δ 1.17 (d, 3 H, J 7.1 Hz, H-6 \times 3), 2.36 (s, 3 H, ArCH_3), 2.44 (s, 3 H, ArCH_3), 3.15 (t, 1 H, J 10.0 Hz, H-4), 3.25 (s, 3 H, CH_3O), 3.77 (m, 1 H, $W/2$ 19.8 Hz, H-5), 4.30 (dd, 1 H, J 10.0 and 3.9 Hz, H-2), 4.33 (d, 1 H, J 3.5 Hz, benzylic H), 4.69 (d, 1 H, J 3.5 Hz, benzylic H), 4.71 (d, 1 H, J 3.9 Hz, H-1), 5.13 (t, 1 H, J 10.1 Hz, H-3), and 7.18–7.97 (m, 13 H, aromatic protons).

Anal. Calc. for $\text{C}_{28}\text{H}_{32}\text{O}_9\text{S}_2$: C, 58.32; H, 5.59; S, 11.12. Found: C, 58.13; H, 5.55; S, 11.33.

Methyl 2,3-anhydro-4-O-benzyl-6-deoxy- α -D-allopyranoside (6). — Sodium hydride (0.4 g, 17 mmol) was added to anhydrous methanol (20 mL), with cooling at 5° . Then, compound **5** (1.9 g, 3.4 mmol) in dry chloroform (20 mL) was added, and the mixture was stirred for 48 h at 5° and for 12 h at 25° , poured into a slurry of crushed ice and water, and extracted with chloroform (4×50 mL). The extracts were combined, washed to neutrality with cold brine, dried (anhydrous sodium sulfate), and evaporated to dryness, giving **6** (0.68 g, 75.0%); $[\alpha]_D +143.0^\circ$ (c 0.07, CHCl_3); ν_{\max} 3575, 3360, and 3025 cm^{-1} ; $^1\text{H-n.m.r.}$: δ 1.27 (d, 3 H, J 7.3 Hz, H-6 \times 3), 3.42 (d, 1 H, J 2.8 Hz, H-2), 3.46 (s, 3 H, CH_3O), 3.50 (dd, 1 H, J 9.8 and 1.5 Hz, H-4), 3.68 (d, 1 H, J 1.5 Hz, H-3), 3.95 (m, 1 H, H-5), 4.68–4.82 (m, 2 H, benzylic protons), 4.83 (d, 1 H, J 2.8 Hz, H-1), and 7.25–7.43 (m, 5 H, aromatic protons).

Anal. Calc. for $\text{C}_{14}\text{H}_{18}\text{O}_4$: C, 67.18; H, 7.27. Found: C, 67.33; H, 7.08.

Methyl 3,6-dideoxy-3-(methylamino)- α -D-glucopyranoside (8). — Epoxide **6** (0.4 g, 1.5 mmol) was heated with 40% methylamine in ethanol (10 mL) in a heavy-walled, sealed glass ampoule for 20 h at 155°. The excess of the reagent was removed in a rotary evaporator, and a solution of the resulting syrup in ethyl acetate (50 mL) was washed with ice-cold brine (4 \times 10 mL), and dried (anhydrous sodium sulfate). Thin-layer chromatography in 3:1 ethyl acetate–chloroform indicated two components of mobility substantially lower than that of the starting epoxide. The component having the lower mobility was the major product. The syrup was chromatographed on a silica column, using 2:1 ethyl acetate–chloroform. This yielded 0.081 g (18.2%) of the more mobile [2-(methylamino) *altro*] product and 0.24 g (55%) of the 3-(methylamino) *gluco* compound **7** as a pale-yellow syrup; $[\alpha]_D +64.6^\circ$ (*c* 0.13, CHCl₃); ν_{\max} 2955 and 1600 cm⁻¹; ¹H-n.m.r.: δ 1.31 (d, 3 H, *J* 7.2 Hz, H-6 \times 3), 2.50 (s, 3 H, CH₃N), 2.80 (t, 1 H, *J* 9.4 Hz, H-3), 3.09 (t, 1 H, *J* 9.4 Hz, H-4), 3.44 (s, 3 H, CH₃O), 3.50 (dd, 1 H, *J* 9.4 and 3.0 Hz, H-2), 3.78 (m, 1 H, H-5), 4.65–4.80 (m, 3 H, H-1, benzylic protons), and 7.26–7.44 (m, 5 H, aromatic protons).

To a solution of compound **7** (50 mg, 0.18 mmol) in ethanol (10 mL) was added acetic acid (0.1 mL). Palladium-on-carbon (5%; 1 mg) was added, and the mixture hydrogenated for 14 h at room temperature and atmospheric pressure. The catalyst was removed by centrifugation, and the benzyl alcohol by repeated dissolution in water and evaporation in a rotary evaporator at 60°. T.l.c. in 5:1 acetone–ethyl acetate indicated complete conversion to a single product, namely, **8**; yield: 32 mg, 95.0%; m.p. 77.0–79.0° (prisms from acetone–water), $[\alpha]_D +57.2^\circ$ (*c* 0.1, H₂O); $\nu_{\max}^{\text{Nujol}}$ 3575 and 3360 cm⁻¹; ¹H-n.m.r. (D₂O): δ 1.20 (d, 3 H, *J* 6.0 Hz, H-6), 2.70 (s, 3 H, CH₃N), 3.27 (t, 1 H, *J* 10.0 Hz, H-3), 3.34 (s, 3 H, CH₃O), 3.40 (t, 1 H, *J* 10.0 Hz, H-4), 3.69 (m, 1 H, H-5), 3.85 (dd, 1 H, *J* 10.0 and 2.4 Hz, H-2), and 4.70 (d, 1 H, *J* 2.4 Hz, H-1).

Anal. Calc. for C₈H₁₇NO₄: C, 50.24; H, 8.96; N, 7.32. Found: C, 50.13; H, 8.89; N, 7.19.

Alditol acetate derivative from 3,6-dideoxy-3-(methylamino)-D-mannose. — Benzylated *gluco* compound **7** (50 mg) was dissolved in anhydrous dimethyl sulfoxide (0.2 mL), and acetic anhydride (0.1 mL) was added. The mixture was kept for 20 h at room temperature and then the reagents were removed under a stream of nitrogen. T.l.c. analysis using 3:1 ethyl acetate–hexane indicated almost complete conversion into a material with higher *R_F* and infrared absorptions at 1740 and 1640 cm⁻¹. The residue was dissolved in ethanol (1 mL), sodium borohydride (5 mg) was added, the mixture was kept for 2 h at room temperature, and then the excess of the reagent was decomposed by dropwise addition of 20% acetic acid in methanol. Water (0.5 mL) was added, the aqueous mixture extracted with chloroform (3 \times 1 mL), and the extracts were combined, washed once with water, dried (anhydrous sodium sulfate), and evaporated to a syrupy compound which was debenzylated by hydrogenolysis under atmospheric pressure for 18 h at room temperature in ethanol (0.5 mL) containing a trace of acetic acid, using 5%

palladium-on-carbon as the catalyst. The catalyst was removed by centrifugation, and the solvent evaporated under a stream of nitrogen. The residue was hydrolyzed in 2M trifluoroacetic acid for 1.5 h at 120°, the solvent removed under a stream of nitrogen, the residue dissolved in ethanol (0.5 mL), and sodium borohydride (3 mg) added. After 3 h, the excess of borohydride was decomposed by dropwise addition of 20% acetic acid in methanol, acetic acid in methanol being added in five 1-mL aliquots, with evaporation after each addition. The mixture was peracetylated and analyzed by g.l.c.-m.s.

Alditol acetate derivatives from 3,6-dideoxy-3-(methylamino)-D-galactose. — Benzylated gluco compound **7** was dissolved in pyridine (0.5 mL), and acetic anhydride (0.5 mL) was added. The mixture was kept for 14 h at room temperature, and then the excess of reagents was removed under a stream of nitrogen. The last traces of pyridine were removed by dissolving the residue in toluene (0.5 mL) and evaporating to dryness. The residue was dissolved in ethanol (1 mL) and acetic acid (10 μ L) was added. Palladium-on-carbon (5%; 20 μ g) was added, and the mixture hydrogenolyzed for 18 h at room temperature and atmospheric pressure. The catalyst was removed by centrifugation, and the solution evaporated to dryness under a stream of nitrogen. The residue was then oxidized, the α -keto-aldoside reduced, the product hydrolyzed, and the materials derivatized as described for the *manno* compound. The resulting mixture of peracetates was analyzed by g.l.c.-m.s.

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