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6*R*- and 6*S*-6*C*-Methylmannose from D-mannuronolactone. Inhibition of phosphoglucomutase and phosphomannomutase: agents for the study of the primary metabolism of mannose

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Abstract

The syntheses of 6*S*-**3** and 6*R*-**6** 6*C*-methylmannoses rely on opposite and highly stereoselective reductions of fully and partially protected ketones derived from D-mannuronolactone, respectively. Reduction of the silylated ketone **2** by sodium borohydride was accompanied by complete migration of the silyl protecting group to the new stereogenic centre; the silyl migration was suppressed when the reduction was conducted in the presence of cerium(III) chloride. Both epimers were good inhibitors of phosphoglucomutase and phosphomannomutase, and are specific inhibitors of phosphohexomutases. This work confirms that 6*C*-alkylhexoses provide a valuable set of compounds with good bioavailability for the study of enzymes involved in the primary metabolism of sugar phosphates. The X-ray crystallographic analysis of 7-deoxy-2,3:5,6-di-*O*-isopropylidene-α-L-*glycero*-D-*manno*-heptofuranose **16** is reported.

1. Introduction

This paper reports the synthesis from D-mannuronolactone **1** of the diastereomeric 6*C*-methylmannoses **3** and **6** by highly stereoselective reductions of silylated **2** and unsilylated **5** intermediate ketones, respectively; **3** and **6** are specific inhibitors of phosphoglucomutase and of phosphomannomutase (PMM). Diastereomeric 6-alkylglucoses **4** and **7**, prepared efficiently from glucuronolactone, differentially inhibit various enzymes involved in the primary metabolism of glucose

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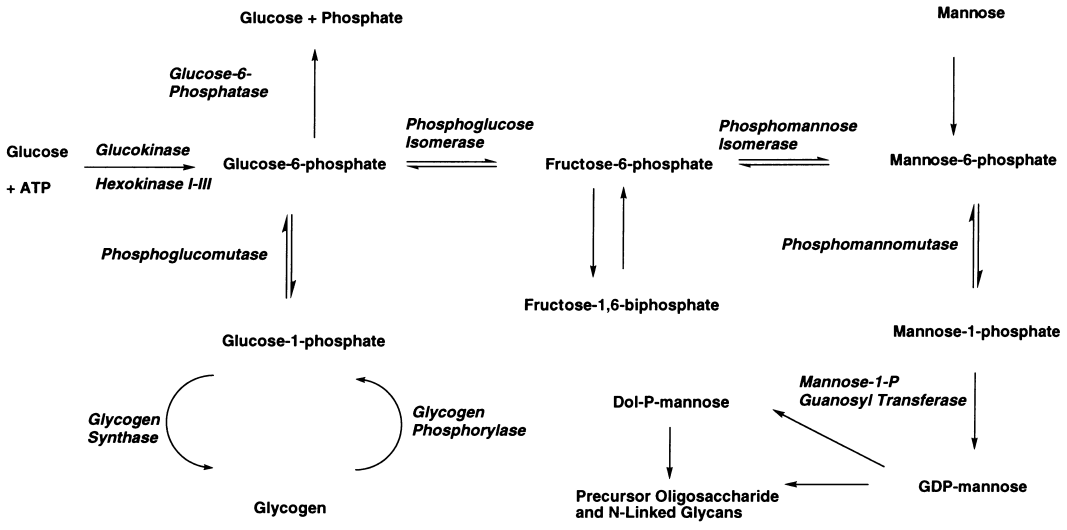
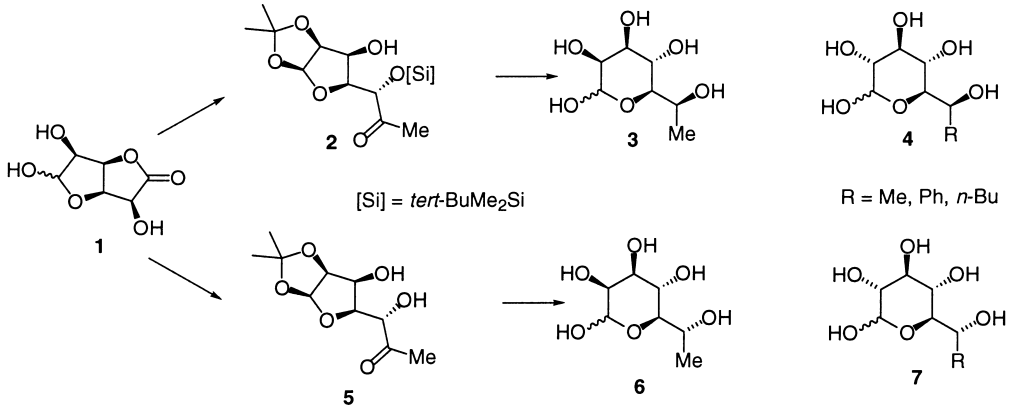


Figure 1. Relationship between primary metabolism of glucose and mannose

phosphates, including glucokinase, glucose-6-phosphatase, glucomutase[1] and glucose-6-phosphate dehydrogenase.[2–4]



A number of genetic diseases arise from enzyme deficiency in the primary metabolism of D-mannose (Fig. 1). Carbohydrate-deficient glycoprotein syndrome (CDGS) is a group of genetic disorders characterised by abnormal glycosylation of serum proteins, other extracellular proteins and possibly some cell-associated proteins. Five types of CDGS have been described on the basis of clinical symptoms and biochemical changes. The majority of patients have Type 1 in which there is a decreased capacity for *N*-glycosylation of proteins; most Type 1 patients have a deficiency of PMM, a key enzyme in the assembly of the common oligosaccharide precursor of N-linked glycans. Deficiency of PMM in CDGS Type 1 leads to lower levels of GDP-mannose, mannose-1-phosphate (man-1-P) and mannose-6-phosphate (man-6-P) but not of dolichol-P-mannose. The deficiency of phosphomannose isomerase (PMI) in CDGS Type 1 B also blocks the supply of man-6-P and it has been postulated that in hereditary fructose intolerance the accumulation of fructose-1-phosphate mimics this situation by inhibiting PMI. Selective inhibitors of PMI and phosphomannomutase (PMM) would provide useful biochemical tools for the study of such diseases.

The methylmannoses **3** and **6** in this paper provide permeant inhibitors and substrate analogues to block specifically phosphohexomutases. Further studies of other 6-alkylmannose derivatives may provide other

more potent and/or specific inhibitors of enzymes involved in the mannose utilisation pathway in normal and different CDGS Type cells and thus may allow the elucidation of factors controlling the supply of precursors for N-linked glycan biosynthesis.

2. Synthesis

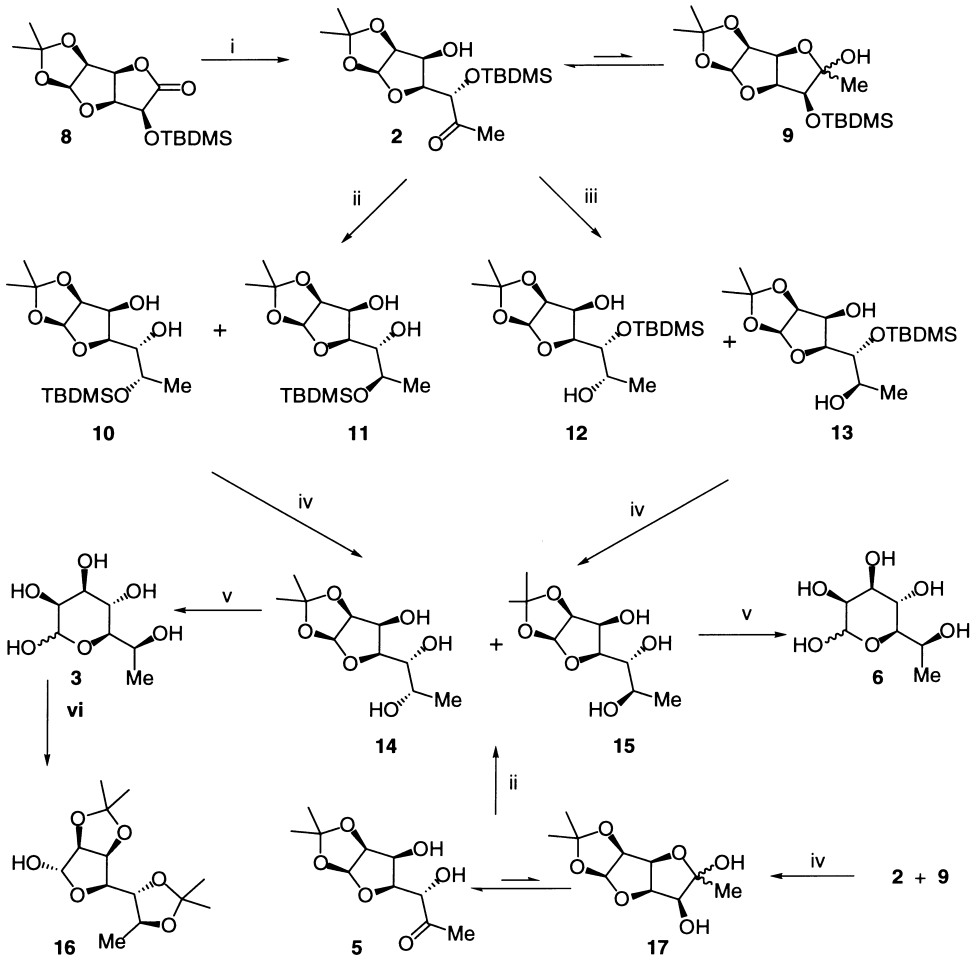
Even though the chemical behaviour of simple derivatives of D-mannuronolactone **1** is rather different from that of the corresponding analogues of D-glucuronolactone, the target 6*C*-methylmannoses **3** and **6** can be prepared by essentially analogous chemistry. D-Mannuronolactone **1** is readily available from the hydrolysis of alginic acid[5] and may be converted to the fully protected silylated lactone acetonide **8** in yields of up to 59%.[6] Treatment of the silylated lactone **8** with methyllithium gave a mixture of the ketone **2** and the lactols **9** in a combined yield of 65%; the ratio of **2** to **9** was approximately 2:1 and the ratio of the two lactols **9** was 6:1. The product mixture showed a strong carbonyl peak in the infra-red at 1721 cm^{-1}. This observation is in contrast to the product from methyllithium addition to the analogously protected glucuronolactone where there was no evidence for any open chain ketone and presumably reflects the more crowded nature of the tricyclic lactols **9**.

Reduction of the mixture of **2** and **9** with sodium borohydride in ethanol gave an inseparable mixture of alcohols **10** and **11** (Scheme 1) in a quantitative yield and a ratio of 7:1; in both products the silyl protecting group had completely migrated to the alcohol at the new stereogenic centre. When the reduction was performed in the presence of cerium(III) chloride in methanol, a more stereoselective reaction occurred to give an inseparable mixture of the epimeric alcohols **12** and **13** in a ratio of 15:1 and a combined yield of 97%; addition of cerium(III) chloride completely suppressed the migration of the silyl ether protecting group during the reduction.

Treatment of the mixture of silyl ethers **12** and **13** with tetrabutylammonium fluoride (TBAF) in THF afforded the readily separated triols **14** and **15** in isolated yields of 80% and 6%, respectively. The major **14** and minor **15** alcohols were also obtained by treatment of the migrated silyl ethers **10** and **11**. Reduction in both the presence and absence of cerium(III) showed the same diastereofacial selectivity. The acetonide protecting group in **14** was removed by treatment with aqueous trifluoroacetic acid to give the deprotected 6*S*-6*C*-methylmannose **3** in quantitative yield. The relative stereochemistry at C-6 in **3** was determined by the conversion of **3** to the crystalline diacetonide **16** (66% yield after crystallisation) by reaction with acetone in the presence of camphorsulfonic acid. The structure of the diacetonide **16** was unequivocally determined by X-ray crystallographic analysis (Fig. 2), thereby establishing the *S* configuration at C-6 of the unprotected 6*S*-6*C*-methylmannose **3**.

Removal of the silyl protecting group in the mixture of **2** and **9** prior to the treatment of the ketone moiety with sodium borohydride resulted in a marked change in the stereoselectivity of the reduction. Reaction of **2** and **9** with TBAF gave a mixture of the ketone **5** and the lactols **17** in an equilibrium ratio of 2:3 and a combined yield of 94%; the ratio of the two lactols **17** was approximately 2:1 as judged by NMR. Thus although the proportion of the open chain ketone **5** in the mixture of **5** and **17** is not as great as that of the silylated ketone **2** in the mixture of **2** and **5**, the ketone form is still a major species present. This is in marked contrast to the case of the related glucose system where there is no evidence for any open chain ketone **18** and the mixture is completely in the lactol form **19** (Scheme 2). Reduction of the mixture of **5** and **17** with sodium borohydride in ethanol gave the two separable epimeric alcohols **14** and **15** in a combined yield of 66% and a ratio of 1:4. Removal of the acetonide in **15** by acid hydrolysis afforded the unprotected 6*R*-6*C*-methylmannose **6**.

Thus the stereoselectivity observed in the reduction of the silylated mixture of **2** and **9** is reversed for



Scheme 1. (i) 1.1 equiv. MeLi, THF, −78°C; (ii) $NaBH_4$, EtOH, rt; (iii) $NaBH_4$, $CeCl_3$, MeOH, −78°C; (iv) TBAF, THF, rt; (v) TFA/H_2O, rt; (vi) acetone, CSA, rt

the reduction of the deprotected mixture of **5** and **17**. This is in marked contrast to the reduction of the unprotected glucose system **19** where there is essentially no stereoselectivity and the epimeric alcohols **20** and **21** are formed in a 1:1 mixture.

3. Enzyme inhibition studies

Preliminary experiments showed that 6*S*-6*C*-**3** and 6*R*-6*C*-**6** methylmannose were good inhibitors of both phosphoglucomutase (PGM) and phosphomannomutase (PMM). As the procedures used to assay these enzymes involved coupled enzymic reactions, the effect of **3** and **6** on the coupling enzymes was also investigated. Phosphomannose isomerase, phosphoglucose isomerase and glucose 6-phosphate dehydrogenase were not inhibited, indicating that the two compounds are specific inhibitors of the phosphohexose mutase activities. The inhibition of PMM by 6*S*-6*C*-**3** and 6*R*-6*C*-**6** methylmannose was very similar, with I_{50} values of 0.25 and 0.35 mM, respectively. We have shown previously that the 6*R*-6*C*-methylglucose [**7**: R=Me] is a better inhibitor of phosphoglucomutase than the 6*S*-6*C* epimer (**4**: R=Me). The I_{50} values for the inhibition of PGM and PMM by 6*R*-6*C*-methylmannose **6** were the same,

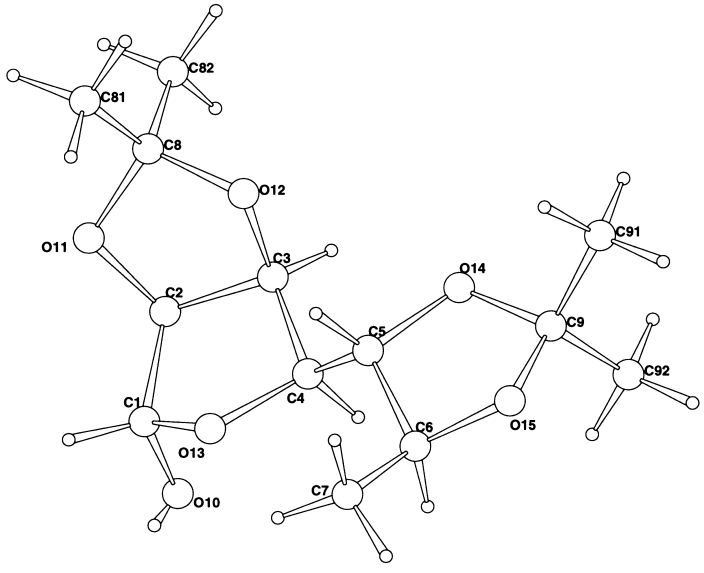
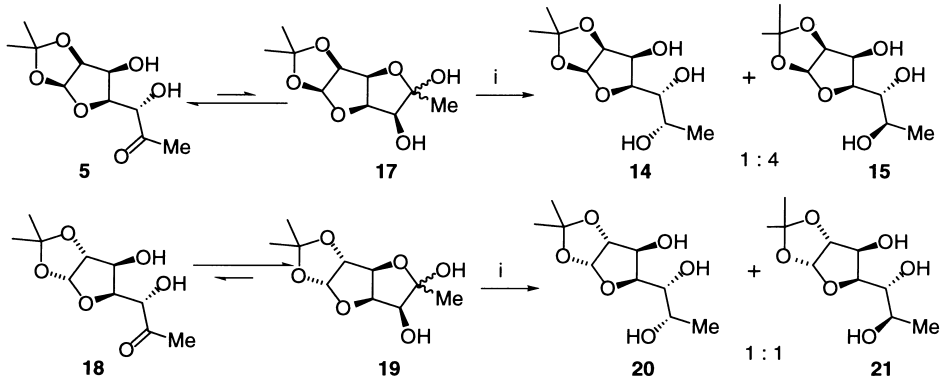


Figure 2. X-Ray structure of 7-deoxy-2,3:5,6-di-*O*-isopropylidene-α-L-*glycero*-D-*manno*-heptofuranose **16** showing crystallographic numbering scheme



Scheme 2. (i) $NaBH_4$, EtOH, rt

~0.4 mM. However, as the value of K_m (0.09 mM) and the substrate concentration used in the standard assay (0.5 mM) of PGM are much higher than those for PMM (K_m 0.013 mM and 0.1 mM, respectively), both **3** and **6** must inhibit PGM more strongly than PMM. Full details of the inhibition studies will be published elsewhere.[7]

In summary, this paper illustrates the use of D-mannuronolactone **1** as a valuable starting material in the synthesis of 6*C*-alkylmannoses. The epimeric 6*C*-methylmannoses **3** and **6** are specific inhibitors of phosphohexomutases; this work further indicates that carbohydrates containing an alkyl substituent may be useful biochemical tools for the study of enzymes that produce and process sugar phosphates.

4. Experimental

Melting points were recorded on a Kofler hot block and are corrected. ^1H NMR (δ_H) spectra were recorded on a Varian Gemini 200 (200 MHz), Bruker AC 200 (200 MHz) or a Bruker AM 500 (500 MHz) spectrometer. ^{13}C NMR (δ_C) spectra were recorded on a Varian Gemini 200 (50 MHz), a Bruker

AC 200 (50 MHz) or a Bruker AM 500 (125 MHz) spectrometer and multiplicities were assigned using the DEPT sequence. All chemical shifts are quoted on the δ-scale. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; dd, double-doublet; ddd, double-double-doublet; t, triplet; q, quartet; dq, double-quartet; m, multiplet; br, broad; app, apparent. Infra-red spectra were recorded on a Perkin–Elmer 1750 FT IR spectrophotometer. Mass spectra were recorded on a VG PLATFORM (APCI, positive or negative as stated) or a VG Autospec spectrometer or a VG 20-250 spectrometer (chemical ionisation [NH_3, CI] as stated). Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis service of the Dyson Perrins laboratory. Thin layer chromatography (TLC) was carried out on plastic or aluminium sheets coated with $60F_{254}$ silica, and plates were developed using a spray of 0.2% w/v cerium(IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; hexane was distilled at 68°C before use to remove less volatile fractions. The fully protected mannuronolactone **8** was prepared as previously described.[6] Cerium(III) chloride·$7H_20$ was warmed to 70°C over 1 h and heated for a further 4 h at 140°C, at 0.3 mmHg before use.

4.1. 5-O-tert-*Butyldimethylsilyl-7-deoxy-1,2*-O-*isopropylidene-β*-D-manno-*hept-6-ulofuranose* ***2*** *and* *5*-O-tert-*butyldimethylsilyl-7-deoxy-1,2*-O-*isopropylidene-β*-D/L-glycero-D-manno-*hept-6-ulo-keto-furanofuranose* ***9***

Methyllithium (3.0 ml, 4.8 mmol, 1.6 M in diethyl ether) was added dropwise to a stirred solution of the protected lactone **8** (1.40 g, 4.24 mmol) in dry THF (20 ml) at −78°C. TLC (hexane:ethyl acetate, 2:1) after 5 min indicated the absence of starting material (R_f 0.3) and the formation of a major product (R_f 0.4). The mixture was quenched with a saturated solution of ammonium chloride (2 ml) and concentrated in vacuo. The residue was diluted with pH 7 buffer solution (100 ml) and extracted with ethyl acetate (3×30 ml). The combined organic phases were dried over $MgSO_4$, filtered, concentrated in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 85:15) to afford a mixture of the ketone **2** and the lactols **9** [947 mg, 65%, ratio 14:(6:1 lactol components) in ^{1}H NMR spectrum (500 MHz, $CDCl_3$)] as a colourless oil; $[\alpha]_D^{21}$ +29.5 (*c*, 1.00 in $CHCl_3$, after 5 min); ν_{max} (film) 3510 (br, OH), 1721 (s, C=O) cm^{-1}; δ_H (500 MHz; $CDCl_3$) ketone **2**: 0.08, 0.12 (6H, 2×s, Si(C*H*$_3$)$_2$), 0.91 (9H, s, SiC(C*H*$_3$)$_3$), 1.41, 1.62 (6H, 2×s, C(C*H*$_3$)$_2$), 2.25 (3H, s, 7-C*H*$_3$), 3.07 (1H, d, $J_{OH,3}$ 6.0 Hz, exchanges with D_2O, OH-3), 3.85 (1H, dd, $J_{4,3}$ 4.9, $J_{4,5}$ 7.6 Hz, H-4), 4.27 (1H, app q, *J* 5.5 Hz, simplifies to dd with D_2O, H-3), 4.56 (1H, d, $J_{5,4}$ 7.6 Hz, H-5), 4.65 (1H, dd, $J_{2,1}$ 4.1, $J_{2,3}$ 6.0 Hz, H-2), 5.63 (1H, d, $J_{1,2}$ 4.1, H-1); major lactol **9**: 0.14, 0.15 (6H, 2×s, Si(C*H*$_3$)$_2$), 0.95 (9H, s, SiC(C*H*$_3$)$_3$), 1.38–1.72 (9H, 3×s, C(C*H*$_3$)$_2$, 7-C*H*$_3$), 3.95 (1H, d, $J_{5,4}$ 4.7 Hz, H-5), 4.39 (1H, s, exchanges with D_2O, OH-6), 4.45 (1H, dd, $J_{3,2}$ 5.5, $J_{3,4}$ 4.6 Hz, H-3), 4.51 (1H, app t, *J* 4.7 Hz, H-4), 4.70 (1H, dd, $J_{2,1}$ 4.4, $J_{2,3}$ 5.5 Hz, H-2), 5.82 (1H, d, $J_{1,2}$ 4.4 Hz, H-1); δ_C (50.3 MHz, $CDCl_3$) the following resonances were assigned; ketone: 69.0, 75.5, 80.1, 81.4 (4×d, C-2, C-3, C-4, C-5), 105.0 (d, C-1), 115.5 (s, *C*Me$_2$), 208.9 (s, C-6); major lactol: 76.8, 77.0, 81.4, 82.5 (4×d, C-2, C-3, C-4, C-5), 115.8 (s, *C*Me$_2$), 107.1 (d, C-1); minor lactol: 77.7, 78.7, 80.5, 85.0 (4×d, C-2, C-3, C-4, C-5), 108.8 (d, C-1); the following were also observed: −5.2, −5.0, −4.7 (3×q), 18.0, 18.2 (2×s), 25.0, 25.6, 26.5, 27.0, 27.1 (5×q), 104.4 (s), 109.9 (s); *m/z* (APCI+) 369 (M+Na$^+$, 100%). (Found: C, 55.50; H, 8.98; $C_{16}H_{30}O_6Si$ requires C, 55.46; H, 8.73%.)

4.2. 6-O-tert-Butyldimethylsilyl-7-deoxy-1,2-O-isopropylidene-β-L-glycero-D-manno-heptofuranose **10** and 6-O-tert-butyldimethylsilyl-7-deoxy-1,2-O-isopropylidene-β-D-glycero-D-manno-heptofuranose **11**

Sodium borohydride (19 mg, 0.50 mmol) was added to a stirred solution of the mixture of **2** and **9** (100 mg, 0.29 mmol) in ethanol (2 ml) at room temperature. TLC (hexane:ethyl acetate, 2:1) after 2 h indicated complete conversion of starting material (R_f 0.4) to a single product (R_f 0.3). The reaction mixture was stirred with an excess of solid ammonium chloride for 1 h and concentrated in vacuo. The residue was dissolved in ethyl acetate (75 ml) and washed with pH 7 buffer solution (25 ml). The organic phase was dried over $MgSO_4$, filtered, concentrated in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 2:1) to afford an inseparable mixture of the migrated silyl ethers **10** and **11** (101 mg, quantitative, ratio 7:1 in 500 MHz ^{1}H NMR spectrum) as a colourless oil; ν_{max} (film) 3468 (br, OH) cm^{-1}; δ_H (500 MHz; $CDCl_3$) major product **10**: 0.08, 0.10 (6H, 2×s, Si(CH_3)$_2$), 0.89 (9H, s, SiC(CH_3)$_3$), 1.23 (3H, d, $J_{7,6}$ 6.4 Hz, 7-CH_3), 1.38, 1.59 (6H, 2×s, C(CH_3)$_2$), 2.64 (1H, d, $J_{OH,5}$ 9.5 Hz, exchanges with D_2O, OH-5), 3.72 (1H, d, $J_{OH,3}$ 4.8 Hz, OH-3), 3.78 (1H, dd, $J_{4,3}$ 5.9, $J_{4,5}$ 9.8 Hz, H-4), 3.97 (1H, app t, J 9.7 Hz, simplifies to d with D_2O, H-5), 4.21 (1H, m, H-6), 4.40 (1H, app q, J 5.5 Hz, simplifies to app t with D_2O, H-3), 4.67 (1H, dd, $J_{2,1}$ 4.1, $J_{2,3}$ 5.7 Hz, H-2), 5.70 (1H, d, $J_{1,2}$ 4.1 Hz, H-1); δ_C (125 MHz, $CDCl_3$) major product **10**: −5.0, −4.4 (2×q, Si(*C*H$_3$)$_2$), 17.9 (s, Si*C*Me$_3$), 20.2 (q, C-7), 25.7 (q, SiC(*C*H$_3$)$_3$), 26.3, 26.7 (2×q, C(*C*H$_3$)$_2$), 66.6, 71.2, 73.0, 78.9, 79.9 (5×d, C-2, C-3, C-4, C-5, C-6), 104.7 (d, C-1), 114.0 (s, *C*Me$_2$); minor product **11**: −5.0, −4.4 (2×q, Si(*C*H$_3$)$_2$), 16.5 (q, C-7), 17.9 (s, Si*C*Me$_3$), 25.7 (q, SiC(*C*H$_3$)$_3$), 26.5, 26.9 (2×q, C(*C*H$_3$)$_2$), 68.8, 70.8, 72.9, 78.9, 79.9 (5×d, C-2, C-3, C-4, C-5, C-6), 105.0 (d, C-1), 114.3 (s, *C*Me$_2$); *m/z* (NH_3, probe CI) 366 (M+NH_4^+, 7%), 349 (M+H^+, 22%), 291 (100%). (Found for the mixture: C, 55.08; H, 9.46; $C_{16}H_{32}O_6Si$ requires C, 55.14; H, 9.25%.)

4.3. 5-O-tert-Butyldimethylsilyl-7-deoxy-1,2-O-isopropylidene-β-L-glycero-D-manno-heptofuranose **12** and 5-O-tert-butyldimethylsilyl-7-deoxy-1,2-O-isopropylidene-β-D-glycero-D-manno-heptofuranose **13**

Sodium borohydride (165 mg, 4.36 mmol) was added to a stirred solution of the mixture of **2** and **9** (754 mg, 2.18 mmol) together with anhydrous cerium(III) chloride (1.08 g, 4.38 mmol) in methanol (20 ml) at −78°C. TLC (hexane:ethyl acetate, 2:1) after 30 min indicated complete conversion of starting material (R_f 0.4) to a single product (R_f 0.5). The mixture was allowed to warm to room temperature, stirred with an excess of solid ammonium chloride for 1 h and concentrated in vacuo. The residue was dissolved in ethyl acetate, (200 ml) and washed with pH 7 buffer solution (70 ml). The organic phase was dried over $MgSO_4$, filtered, concentrated in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 7:3) to afford an inseparable mixture of the unmigrated silyl ethers **12** and **13** (733 mg, 97%, ratio 15:1 in 500 MHz ^{1}H NMR spectrum) as a white crystalline solid; ν_{max} (KBr) 3539 (m, OH), 3403 (s, OH) cm^{-1}; δ_H (500 MHz; $CDCl_3$) major product **12**: 1.14, 0.18 (6H, 2×s, Si(CH_3)$_2$), 0.90 (9H, s, SiC(CH_3)$_3$), 1.23 (3H, d, $J_{7,6}$ 6.6 Hz, 7-CH_3), 1.44, 1.61 (6H, 2×s, C(CH_3)$_2$), 2.48 (1H, d, J 10.6 Hz, exchanges with D_2O, OH), 2.68 (1H, d, J 4.0 Hz, exchanges with D_2O, OH), 3.73 (1H, dd, $J_{4,3}$ 3.6, $J_{4,5}$ 8.8 Hz, H-4), 3.89 (1H, m, simplifies to dq with D_2O, $J_{6,5}$ 2.3, $J_{6,7}$ 6.6 Hz, H-6), 4.11 (1H, m, simplifies to dd with D_2O, $J_{3,2}$ 5.9, $J_{3,4}$ 3.6 Hz, H-3), 4.17 (1H, dd, $J_{5,4}$ 8.8, $J_{5,6}$ 2.3 Hz, H-5), 4.70 (1H, dd, $J_{2,1}$ 4.4, $J_{2,3}$ 5.9 Hz, H-2), 5.68 (1H, d, $J_{1,2}$ 4.4 Hz, H-1); δ_C (50.3 MHz, $CDCl_3$); major product **12**: −4.8, −4.7 (2×q, Si(*C*H$_3$)$_2$), 18.3 (s, Si*C*Me$_3$), 18.9 (q, C-7), 26.0 (q, SiC(*C*H$_3$)$_3$), 27.1, 27.5 (2×q, C(*C*H$_3$)$_2$), 67.9, 68.7, 70.8, 80.9, 82.1 (5×d, C-2, C-3, C-4, C-5, C-6), 104.9 (d, C-1), 116.0 (s, *C*Me$_2$); *m/z* (APCI−) 347 (M−H^+, 100%). (Found: C, 55.11; H, 9.44; $C_{16}H_{32}O_6Si$ requires C, 55.14; H, 9.25%.)

4.4. 7-Deoxy-1,2-O-isopropylidene-β-L-glycero-D-manno-heptofuranose **14** and 7-deoxy-1,2-O-isopropylidene-β-D-glycero-D-manno-heptofuranose **15**

Method 1: Tetrabutylammonium fluoride (0.35 ml, 0.35 mmol, 1.0 M in THF) was added to a stirred solution of the 5-*O*-silyl ethers **12** and **13** (100 mg, 0.29 mmol, ratio 15:1) in dry THF (2 ml) at room temperature. TLC (ethyl acetate) after 30 min indicated complete conversion of starting material (R_f 0.7) to a single product (R_f 0.1). The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (ethyl acetate) to afford the triol **14** (54 mg, 80%) as white crystalline solid; mp 112–114°C; $[\alpha]_D^{21}$ −4.7 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr) 3419 (br, OH) cm^{-1}; δ_H (500 MHz; $CDCl_3$) 1.30 (3H, d, $J_{7,6}$ 6.5 Hz, 7-CH_3), 1.42, 1.61 (6H, 2×s, C(CH_3)$_2$), 2.18, 3.11, 3.25 (3H, 3×br s, OH-3, OH-5, OH-6), 3.91 (1H, dd, $J_{5,4}$ 8.8, $J_{5,6}$ 2.5 Hz, H-5), 4.03 (1H, dd, $J_{4,3}$ 6.0, $J_{4,5}$ 8.8 Hz, H-4), 4.05 (1H, dq, $J_{6,5}$ 2.5, $J_{6,7}$ 6.5 Hz, H-6), 4.42 (1H, app t, *J* 6.1 Hz, H-3), 4.72 (1H, dd, $J_{2,1}$ 4.2, $J_{2,3}$ 6.2 Hz, H-2), 5.76 (1H, d, $J_{1,2}$ 4.2 Hz, H-1); δ_C (50.3 MHz, $CDCl_3$) 19.4 (q, C-7), 26.5, 26.8 (2×q, C(*C*H_3)$_2$), 67.1, 70.6, 73.0, 79.6, 80.3 (5×d, C-2, C-3, C-4, C-5, C-6), 105.1 (d, C-1), 114.6 (s, *C*Me_2); *m/z* (NH_3, probe CI) 252 ($M+H^+$, 37%), 194 (100%). (Found: C, 51.38; H, 7.87; $C_{10}H_{18}O_6$ requires C, 51.27; H, 7.74%.)

Further elution afforded the isomeric triol **15** as the minor component (4 mg, 6%) as a colourless oil; $[\alpha]_D^{21}$ −6.2 (*c*, 1.2 in MeOH); ν_{max} (film) 3432 (br OH) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.24 (3H, d, $J_{7,6}$ 6.3 Hz, 7-CH_3), 1.40, 1.63 (6H, 2×s, C(CH_3)$_2$), 2.65 (1H, d, *J* 3.8 Hz, OH), 3.22 (1H, d, *J* 3.0 Hz, OH), 3.35 (1H, d, $J_{OH,3}$ 6.1 Hz, OH-3), 3.92 (1H, dd, $J_{4,3}$ 6.1, $J_{4,5}$ 8.9 Hz, H-4), 4.01–4.06 (2H, m, H-5, H-6), 4.43 (1H, app q, *J* 6.1 Hz, H-3), 4.66 (1H, dd, $J_{2,1}$ 4.1, $J_{2,3}$ 6.1 Hz, H-2), 5.75 (1H, d, $J_{1,2}$ 4.1 Hz, H-1); δ_C (125 MHz, $CDCl_3$) 17.3 (q, C-7), 26.7, 26.9 (2×q, C(*C*H_3)$_2$), 68.9, 70.7, 72.8, 79.4, 81.5 (5×d, C-2, C-3, C-4, C-5, C-6), 105.1 (d, C-1), 114.7 (s, *C*(CH_3)$_2$); *m/z* (APCI+) 257 ($M+Na^+$, 42%), 177 (100%). HRMS *m/z* found 235.1176 ($M+H^+$); $C_{10}H_{19}O_6$ requires 235.1182.

Method 2: Tetrabutylammonium fluoride (0.15 ml, 0.15 mmol, 1.0 M in THF) was added to a stirred solution of the 5-*O*-silyl ethers **12** and **13** (45 mg, 0.13 mmol, ratio 7:1) in dry THF (2 ml) at room temperature. TLC (ethyl acetate) after 30 min indicated complete conversion of starting material (R_f 0.8) to a single product (R_f 0.1). The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (ethyl acetate) to afford the triol **14** (13 mg, 44%) as a white crystalline solid and the triol **15** (2 mg, 6%) as a colourless oil, both compounds being identical to the materials described above.

4.5. 7-Deoxy-L-glycero-D-manno-heptopyranose [6S-6C-methylmannose] **3**

A solution of the acetonide **14** (32 mg, 0.135 mmol) in trifluoroacetic acid (0.6 ml) and water (0.4 ml) was stirred at room temperature. TLC (ethyl acetate:methanol, 9:1) after 1 h indicated complete conversion of starting material (R_f 0.5) to a single product (R_f 0.2). The reaction mixture was concentrated in vacuo to yield 7-deoxy-D-*glycero*-D-*manno*-heptopyranose **3** [26 mg, quantitative, 2:1 ratio of anomers α:β in ^1H NMR spectrum (500 MHz, D_2O)] as a foam; $[\alpha]_D^{21}$ +14.2 (*c*, 0.92 in D_2O, after 10 minutes); ν_{max} (KBr) 3421 (s, OH) cm^{-1}; δ_H (500 MHz; D_2O) α-anomer: 1.14 (3H, d, $J_{7,6}$ 6.6 Hz, 7-CH_3), 3.44 (1H, dd, $J_{5,4}$ 9.2, $J_{5,6}$ 1.8 Hz, H-5), 3.67 (1H, app t, *J* 9.4 Hz, H-4), 3.71 (1H, dd, $J_{3,2}$ 3.1, $J_{3,4}$ 9.4 Hz, H-3), 3.80 (1H, dd, $J_{2,1}$ 1.7, $J_{2,3}$ 3.1 Hz, H-2), 4.06 (1H, dq, $J_{6,5}$ 1.8, $J_{6,7}$ 6.6 Hz, H-6), 5.07 (1H, d, $J_{1,2}$ 1.7 Hz, H-1); β-anomer: 1.17 (3H, d, $J_{7,6}$ 6.6 Hz, 7-CH_3), 3.01 (1H, dd, $J_{5,4}$ 9.7, $J_{5,6}$ 2.0 Hz, H-5), 3.51 (1H, dd, $J_{3,2}$ 3.2, $J_{3,4}$ 9.7 Hz, H-3), 3.61 (1H, app t, *J* 9.7 Hz, H-4), 3.81 (1H, dd, $J_{2,1}$ 1.0, $J_{2,3}$ 3.2 Hz, H-2), 4.01 (1H, dq, $J_{6,5}$ 2.0, $J_{6,7}$ 6.6 Hz, H-6), 4.76 (1H, d, $J_{1,2}$ 1.0 Hz, H-1); δ_C (125 MHz, D_2O) α-anomer: 19.2 (q, C-7), 65.0, 67.2, 71.0, 74.6 (5×d, C-2, C-3, C-4, C-5, C-6), 94.5 (d, C-1); β-anomer:

19.1 (q, C-7), 65.0, 66.9, 71.6, 73.7, 78.3 (5×d, C-2, C-3, C-4, C-5, C-6), 94.3 (d, C-1); *m/z* (APCI−) 193 ($M-H^+$, 100%). HRMS *m/z* found 212.1135 ($M+NH_4^+$); $C_7H_{18}NO_6$ requires 212.1134.

4.6. 7-Deoxy-2,3:5,6-di-O-isopropylidene-α-L-glycero-D-manno-heptofuranose **16**

Camphorsulfonic acid (250 mg, 0.10 mmol) was added to a stirred solution of the free sugar **3** (140 mg, 0.72 mmol, ratio 15:1) in acetone (AR grade, 5 ml) at room temperature. TLC (hexane:ethyl acetate, 2:1) after 6 h indicated the absence of starting material (baseline) and the formation of a major product (R_f 0.3). The mixture was stirred with an excess of solid sodium hydrogen carbonate for 1 h, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 4:1) to afford the diacetonide **16** (171 mg, 86%) as a white crystalline solid which was subsequently recrystallised from hexane (130 mg, 66%, after first recrystallisation); mp 121–122°C (hexane); $[\alpha]_D^{21}$ +0.1 to −7.7 (*c*, 1.00 in $CHCl_3$, over 169 h); ν_{max} (KBr) 3508 (m, OH) cm^{-1}; δ_H (500 MHz; $CDCl_3$) 1.36 (3H, s, $C(CH_3)_2$), 1.39 (3H, d, $J_{7,6}$ 6.5 Hz, 7-CH_3), 1.42, 1.49 (9H, 3×s, $C(CH_3)_2$), 2.60 (1H, d, $J_{OH,1}$ 2.0, exchanges with D_2O, OH-1), 3.90 (1H, dd, $J_{5,4}$ 8.7, $J_{5,6}$ 7.3 Hz, H-5), 4.10 (1H, dd, $J_{4,3}$ 3.6, $J_{4,5}$ 8.7 Hz, H-4), 4.20 (1H, app q, *J* 6.4 Hz, H-6), 4.63 (1H, d, $J_{2,3}$ 5.9 Hz, H-2), 4.88 (1H, dd, $J_{3,2}$ 5.9, $J_{3,4}$ 3.6 Hz, H-3), 5.40 (1H, d, $J_{2,1}$ 2.1 Hz, simplifies to s with D_2O, H-1); δ_C (125 MHz, $CDCl_3$) 18.9 (q, C-7), 24.8, 26.0, 26.9, 27.6 (4×q, 2×$C(CH_3)_2$), 76.7, 78.5, 80.0, 81.7, 85.4 (5×d, C-2, C-3, C-4, C-5, C-6), 101.5 (d, C-1), 109.1 (s, 5,6-CMe_2), 112.7 (s, 2,3-CMe_2); *m/z* (APCI+) 273 ($M-H^+$, 25%), 215 (68%), 125 (100%). (Found: C, 56.97; H, 8.08; $C_{13}H_{22}O_6$ requires C, 56.92; H, 8.34%.)

4.7. 7-Deoxy-1,2-O-isopropylidene-β-D-manno-hept-6-ulofuranose **5** and 7-deoxy-1,2-O-isopropylidene-β-D/L-glycero-D-manno-hept-6-ulo-ketofuranofuranose **17**

Tetrabutylammonium fluoride (2.1 ml, 2.1 mmol, 1.0 M in THF) was added to a stirred solution of the mixture of silyl ethers **2** and **9** (600 mg, 1.72 mmol) in dry THF (12.3 ml) at room temperature. TLC (ethyl acetate) after 45 min indicated complete conversion of starting material (R_f 0.7) to a single product (R_f 0.4). The reaction mixture was concentrated in vacuo by co-evaporation with toluene and the residue purified by flash chromatography (hexane:ethyl acetate, 1:4) to afford a mixture of the ketone **5** and the lactols **17** [380 mg, 94%, ratio 2:(2:1 lactol components) in ^1H NMR spectrum (500 MHz, $CDCl_3$)] as a colourless oil; $[\alpha]_D^{21}$ +47.7 (*c*, 0.65 in $CHCl_3$, after 5 min); ν_{max} (film) 3447 (s, OH), 1718 (s, C=O) cm^{-1}; δ_H (500 MHz; C_6D_6) 1.03, 1.09, 1.38, 1.45, 1.59 (15H, 5×s, $C(CH_3)_2$, ketone, major, 7-CH_3, major), 1.06, 1.54, 1.56 (9H, 3×s, $C(CH_3)_2$, 7-CH_3, minor); ketone **5**: 2.09 (3H, s, 7-CH_3), 3.20 (1H, d, $J_{OH,3}$ 5.9 Hz, OH-3), 3.59 (1H, dd, $J_{4,3}$ 5.6, $J_{4,5}$ 7.5 Hz, H-4), 3.90 (1H, dd, $J_{2,1}$ 4.1, $J_{2,3}$ 6.1 Hz, H-2), 3.73 (1H, d, $J_{OH,5}$ 5.5 Hz, OH-5), 3.92–3.97 (1H, m, H-3), 4.54 (1H, dd, $J_{5,OH}$ 5.5, $J_{5,4}$ 7.5 Hz, H-5), 5.19 (1H, d, $J_{1,2}$ 4.1 Hz, H-1); major lactol: 3.15 (1H, d, $J_{OH,5}$ 11.0 Hz, OH-5), 3.54 (1H, dd, $J_{5,OH}$ 10.5, $J_{5,4}$ 5.1 Hz, H-5), 3.69 (1H, app t, *J* 5.2 Hz, H-3), 3.85 (1H, dd, $J_{2,1}$ 4.2, $J_{2,3}$ 5.5 Hz, H-2), 4.02 (1H, app t, *J* 5.0 Hz, H-4), 5.29 (1H, d, $J_{1,2}$ 4.1 Hz, H-1); minor lactol: 3.89–3.92 (1H, obscured, H-5), 3.92–3.97 (1H, obscured, H-2), 4.18 (1H, app t, *J* 6.7 Hz, H-3), 4.93 (1H, app t, *J* 6.5 Hz, H-4), 5.50 (1H, d, $J_{1,2}$ 4.1 Hz, H-1); δ_C (125 MHz, $CDCl_3$) 22.0, 24.3, 25.7, 25.8, 26.4, 26.6, 26.7, 26.8, 27.7 (9×q, $C(CH_3)_2$, C-7, ketone, major, minor), 70.8, 75.4, 75.8, 77.7, 79.3, 80.9, 81.2, 85.0 (8×d, C-2, C-3, C-4, C-5, ketone, major), 73.7, 78.0, 78.8, 87.5 (4×d, C-2, C-3, C-4, C-5, minor), 104.7 (s, C-6, major), 105.3, 106.7 (d, C-1, ketone, major), 108.3 (d, C-1, minor), 110.4 (s, C-6, minor), 113.7 (s, CMe_2, minor), 114.6, 115.0 (2×s, CMe_2, ketone, major), 209.6 (s, C-7, ketone); *m/z* (NH_3, probe CI) 250 ($M+NH_4^+$, 5%), 157 (100%). (Found: C, 51.53; H, 7.19; $C_{10}H_{16}O_6$ requires C, 51.72; H, 6.94%.)

*4.8. 7-Deoxy-1,2-*O*-isopropylidene-β-*D*-glycero-*D*-manno-heptofuranose **15** and 7-deoxy-1,2-*O*-isopropylidene-β-*L*-glycero-*D*-manno-heptofuranose **14***

Sodium borohydride (111 mg, 2.92 mmol) was added to a stirred solution of the mixture of non-silylated compounds **5** and **17** (380 mg, 1.624 mmol) in ethanol (21 ml) at room temperature. TLC (ethyl acetate) after 35 minutes indicated complete conversion of starting material (R_f 0.4) to a single compound (baseline). The reaction mixture was stirred with an excess of solid ammonium chloride for 1 h, then filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate) to afford 7-deoxy-1,2-*O*-isopropylidene-β-D-*glycero*-D-*manno*-heptofuranose **15** (233 mg, 61%) as a colourless oil and 7-deoxy-1,2-*O*-isopropylidene-β-L-*glycero*-D-*manno*-heptofuranose **14** (53 mg, 14%) as a white crystalline solid, both compounds being identical to the materials described above.

4.9. 7-Deoxy-D-glycero-D-manno-*heptopyranose [6*R-*6*C-*methylmannose] **6***

A solution of 7-deoxy-1,2-*O*-isopropylidene-β-D-*glycero*-D-*manno*-heptofuranose **15** (200 mg, 0.847 mmol) in trifluoroacetic acid (3.6 ml) and water (2.4 ml) was stirred at room temperature. TLC (ethyl acetate:methanol, 9:1) after 1 h indicated complete conversion of starting material (R_f 0.5) to a single product (R_f 0.2). The reaction mixture was concentrated in vacuo to yield 7-deoxy-D-*glycero*-D-*manno*-heptopyranose **6** [164 mg, quantitative, 2:1 ratio of anomers α:β in ^{1}H NMR spectrum (500 MHz, D_2O)] as a foam; $[\alpha]_D^{21}$ +7.1 (*c*, 1.5 in H_2O); ν_{max} (film) 3396 (br OH) cm^{-1}; δ_H (500 MHz, D_2O) α-anomer: 1.04 (3H, d, $J_{7.6}$ 6.6 Hz, 7-CH_3), 3.44 (1H, t, *J* 9.8 Hz, H-4), 3.63 (1H, dd, $J_{3,2}$ 3.4, $J_{3,4}$ 9.7 Hz, H-3), 3.64 (1H, dd, $J_{5,6}$ 2.8, $J_{5,4}$ 9.7 Hz, H-5), 3.73 (1H, dd, $J_{2,1}$ 1.8, $J_{2,3}$ 3.4 Hz, H-2), 3.98 (1H, dq, $J_{6,5}$ 2.8, $J_{6,7}$ 6.5 Hz, H-6), 4.98 (1H, d, $J_{1,2}$ 1.6 Hz, H-1); β-anomer: 1.05 (3H, d, $J_{7.6}$ 6.6 Hz, 7-CH_3), 3.17 (1H, dd, $J_{5,6}$ 2.7, $J_{5,4}$ 9.8 Hz, H-5), 3.36 (1H, t, *J* 9.7 Hz, H-4), 3.45 (1H, dd, $J_{3,2}$ 3.6, $J_{3,4}$ 9.6 Hz, H-3), 3.75 (1H, dd, $J_{2,3}$ 3.3, $J_{2,1}$ 9.7 Hz, H-2), 3.97 (1H, dq, $J_{6,5}$ 2.8, $J_{6,7}$ 6.5 Hz, H-6), 4.70 (1H, overlapped with D_2O, H-1); δ_C (125 MHz, D_2O); α-anomer: 16.2 (q, C-7), 67.3, 68.8, 71.3, 71.3, 74.8 (5×d, C-2, C-3, C-4, C-5, C-6), 94.8 (d, C-1); β-anomer: 16.2 (q, C-7), 67.3, 68.4, 71.8, 74.0, 78.8 (5×d, C-2, C-3, C-4, C-5, C-6), 94.6 (d, C-1); *m/z* (APCI−) 193 (M−H$^+$, 100%). HRMS *m/z* found 212.1142 (M+NH_4^+); $C_7H_{18}NO_6$ requires 212.1134.

5. Enzyme assays

Phosphoglucomutase (EC 5.4.2.2), phosphomannomutase (EC 5.4.2.28), phosphoglucose isomerase (EC 5.31.9) and phosphomannose isomerase (EC 5.3.1.8) were assayed as described by Van Schaftingen and Jaeken[8] and glucose-6-phosphate dehydrogenase was assayed as described previously[4] except for the following modifications. Fibroblasts were harvested by trypsinization and stored frozen at −20°C until required. Cell pellets were thawed, sonicated for 10 s (8 microns) on a Soniprep 150 and spun for 3 min in an Eppendorf microcentrifuge. The resulting supernatant was used for the enzyme assays. The rate of production of NADPH was measured fluorimetrically using an excitation wavelength of 340 nm and emission wavelength of 460 nm.

5.1. Assay of phosphomannomutase (PMM)

Cell extracts (25 μl; protein concentration 0.5–1.0 mg/ml) were assayed for PMM activity in duplicate by incubation with 0.25 mM NADP, 1 μM mannose-1,6-bisphosphate, 10 μg/ml phosphoglucose

Table 1
Atomic co-ordinates and equivalent isotropic temperature factors U(iso) with standard deviations in parentheses for 7-deoxy-2,3:5,6-di-*O*-isopropylidene-α-L-*glycero*-D-*manno*-heptofuranose **16**

Atom	*x/a*	*y/b*	*z/c*	*U(iso)*
C(1)	1.1513(3)	0.0779(2)	0.0953(1)	0.0497
C(2)	0.9988(3)	0.0591(2)	0.0409(1)	0.0495
C(3)	0.8262(3)	0.1388(2)	0.0650(1)	0.0437
C(4)	0.9017(3)	0.2041(2)	0.12817(9)	0.0397
C(5)	0.7537(3)	0.2250(2)	0.18350(9)	0.0398
C(6)	0.8205(3)	0.3039(2)	0.2435(1)	0.0458
C(7)	0.8969(4)	0.2315(3)	0.3031(1)	0.0617
C(8)	0.7219(4)	-0.0621(2)	0.0430(1)	0.0543
C(9)	0.5447(3)	0.3945(2)	0.2021(1)	0.0457
C(81)	0.6487(5)	-0.1774(3)	0.0794(2)	0.0767
C(82)	0.6408(6)	-0.0478(4)	-0.0276(1)	0.0819
C(91)	0.3311(3)	0.3826(2)	0.2157(1)	0.0533
C(92)	0.5994(4)	0.5216(3)	0.1732(2)	0.0682
O(10)	1.2785(2)	0.1711(2)	0.0709(1)	0.0598
O(11)	0.9295(3)	-0.0677(2)	0.0410(1)	0.0639
O(12)	0.6836(2)	0.0466(2)	0.08239(8)	0.0477
O(13)	1.0496(2)	0.1215(1)	0.15344(7)	0.0451
O(14)	0.6002(2)	0.2973(2)	0.15465(8)	0.0496
O(15)	0.6445(2)	0.3664(2)	0.26262(8)	0.0522

isomerase, 3.5 μg/ml phosphomannose isomerase, 10 μg/ml glucose-6-phosphate dehydrogenase, 5 mM $MgCl_2$ in 50 mM HEPES pH 7.1 in a total volume of 500 μl with and without 0.1 mM mannose-1-phosphate (added after a 20 min pre-incubation). The reaction was followed for 1 h at 30°C by measuring the production of NADPH fluorimetrically.

5.2. Assay of phosphoglucomutase (PGM)

Cell extracts (5 μl) were assayed in duplicate for PGM activity by incubation with 0.25 mM NADP, 1 μM glucose-1,6-bisphosphate, 10 μg/ml glucose-6-phosphate dehydrogenase, 5 mM $MgCl_2$ in 50 mM HEPES pH 7.1 in a total volume of 500 μl, with and without 0.5 mM glucose-1-phosphate. The reaction was followed for 20 min at 30°C by measuring the production of NADPH fluorimetrically.

6. X-Ray crystal structure analysis

The relative configurations of the stereogenic centres in the diacetonide **16** were established by X-ray single crystal structure analysis. Cell dimensions and intensity data were measured with an Enraf–Nonius Mach3 Diffractometer, and Lorentz, polarisation and psi scan absorption corrections were applied. All calculations were carried out on a 486PC computer. All non-hydrogen atoms were located by SIR92[9] and refined using CRYSTALS.[10] Illustrations were produced using CAMERON.[11] Hydrogen atoms were seen in the difference density map but placed geometrically. Non-hydrogen atoms (Table 1) were refined anisotropically using atomic scattering factors from International Tables.[12] Structural data for **16** have been deposited at the Cambridge Crystallographic Data Centre.[13]

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