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6*C*-Butylglucoses from Glucuronolactone: Suppression of Silyl Migration during Borohydride Reduction of Lactols by Cerium (III) Chloride: Inhibition of Phosphoglucomutase

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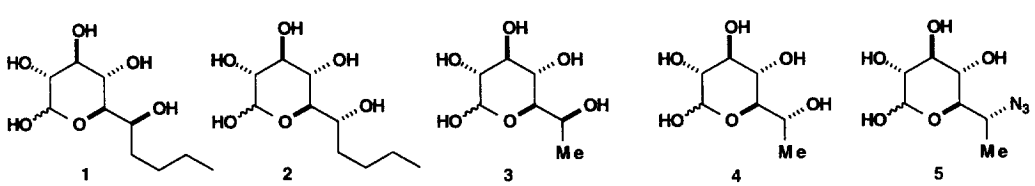
Abstract: The synthesis of the epimeric 6*C*-butylglucoses from D-glucuronolactone is reported. The sodium borohydride reduction of two fully protected lactols is highly stereoselective but is accompanied by migration of a silyl protecting group; in the presence of cerium(III) chloride, there is little change in the stereoselectivity but the migration of the silyl group is suppressed. 6*R*-6*C*-Methylglucose and 6*R*-6*C*-butylglucose are both better inhibitors of phosphoglucomutase than their 6*S* epimers.

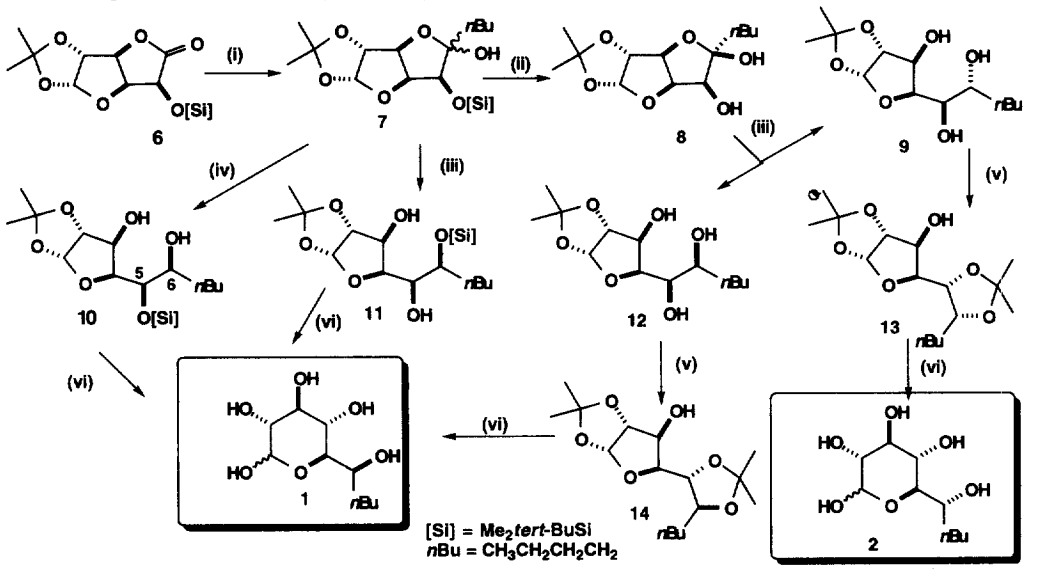
This paper reports the synthesis of 6*S*-6*C*-butylglucose **1** and 6*R*-6*C*-butylglucose **2** by two different routes from glucuronolactone. Two cases of highly stereoselective reductions of silyl lactols are reported in which the reduction is accompanied by migration of a silyl ether; it was found that addition of cerium(III) chloride to the reaction mixture with sodium borohydride gave the same stereoselectivity but completely suppressed the migration of the silyl ether. The preceding paper[1] reported details of the synthesis of the corresponding 6*S*-6*C*- **3** and 6*R*-6*C*- **4** methyl analogues, and of the 6*R*-azide **5**; the effect of these materials on phosphoglucomutase and glucose-6-phosphate dehydrogenase are reported. Such compounds[2] may allow the development of inhibitors of enzymes that are responsible for the synthesis, hydrolysis, mutation and isomerisation of sugar phosphates, not only of glucose but also of mannose and galactose.



1 2 3 4 5

The first synthesis followed a similar route to that used for the 6*C*-methyl glucose in the previous paper. The silyl ether of glucuronolactone acetonide **6**[3] was treated with *n*-butyl lithium to give a mixture of the lactols **7** in 68% yield [Scheme 1]. Removal of the silyl protecting group in **7** by tetrabutylammonium fluoride afforded a single lactol **8** in 57% yield; the stereochemistry proposed for **8** is by analogy with that proved by X-ray crystallographic analysis for the corresponding methyl and phenyl lactols. Reduction of **8** by sodium borohydride in ethanol gave an inseparable mixture of the epimeric alcohols **12** and **9** in 83% yield in approximately a 1:1 ratio. Reaction of the mixture of alcohols with acetone and 2,2-dimethoxypropane in the

presence of camphor sulfonic acid (CSA) gave two diacetonides **14** [33 %] and **13** [51%] which were readily separable by flash chromatography. The coupling constants between H-5 and H-6 in the side chain acetonide group were 7.8 Hz for the *trans*-substituted acetonide **14** and 5.2 Hz for the *cis*-acetonide **13**; this difference between the stereochemistry in such *cis*- and *trans*-substituted side chain acetonides has been found to be consistent in several cases where the outcome has been proved by X-ray crystallographic analysis.[4] Together with other chemistry reported in this synthesis, this provides strong evidence for the stereochemistry proposed. Deprotection of the diacetonides **14** and **13** with aqueous trifluoroacetic acid gave the targets L- **1** and D- **2** sugars in 88% and 81% yields, respectively.



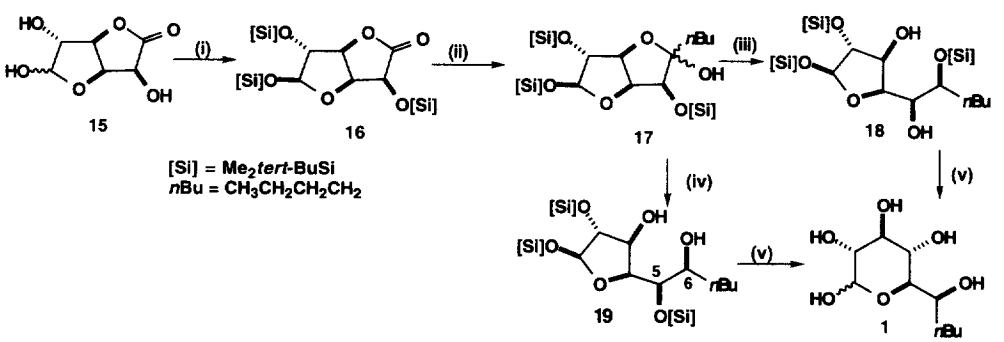
Scheme 1: (i) *n*BuLi, THF, -70°C (ii) Bu_4NF, THF (iii) $NaBH_4$, EtOH (iv) $CeCl_3$, $NaBH_4$, EtOH (v) Me_2CO, $Me_2C(OMe)_2$, CSA (vi) aq. CF_3COOH

Reduction of the silyl protected lactols **7** with sodium borohydride in ethanol gave, as in both the phenyl and methyl cases, only the silyl diol **11** in 95% isolated yield; **11** was the only reduction product isolated. The site of attachment of the silyl group in **11** is clearly evident from NMR studies in which the signals of the protons attached to C-3 and C-5 are coupled to OH protons, whereas there is no coupling of H-6 to any OH group. Acid hydrolysis of the silyl ether and acetonide in **11** gave the L-sugar **1**, free of any of the C-6 epimer **2**, in 84% yield; this route provides easy access to the butyl analogue of L-fucose **1** in three steps from **6** and an overall yield of 54%.

The highly stereoselective reduction of the silyl lactol **7**, accompanied by migration of the silyl group, is very similar to the reduction of methyl lactol analogue of **7** described in the preceding paper, and other lactols investigated. Among other reducing conditions studied in attempts to increase the amount of precursor to the D-sugar **2**, the effects of cerium(III) chloride[5] were investigated. Although it was anticipated that cerium(III) would affect the ratio of the diols produced[6] there was little - if any - change in the diastereoselectivity of the reductions of either the protected **7** or unprotected **8** lactols. However, reduction of **7** with sodium borohydride in the presence of cerium(III) chloride gave a *different* silyl ether **10** in 94% yield; the migration of the silyl group during the borohydride reduction was completely suppressed. Again NMR

studies of the protons attached to C-3, C-5 and C-6 clearly demonstrated that the silyl ether functionality was still clearly attached to C-5; exchange studies with D_2O showed, while there was no variation of the H-5 signal, the signal of the proton attached to C-3 was simplified from an apparent triplet to a doublet, and the multiplet for the protons attached to C-6 became an apparent quartet. The structure was unambiguously confirmed by a COSY experiment. Aqueous acid hydrolysis of **10** gave **1** in 85% yield.

An alternative protection strategy for the synthesis of **1** is shown in Scheme 2. All the free hydroxyl groups in glucuronolactone **15** were converted into silyl ethers by treatment with *tert*-butyldimethylsilyl chloride in dimethyl formamide in the presence of imidazole [71% yield]; a single compound **16** was formed and is ascribed as the β anomer since the coupling constant between H-1 and H-2 is 0 Hz, and the alternative α anomer would be very sterically crowded. Addition of *n*-butyl lithium to the fully silylated lactone **16** gave a mixture of lactols **17** in 70% yield. Reduction of **17** with sodium borohydride in ethanol gave a major product , the diol **18** [73%], in which the silyl group had migrated during the reduction; none of the isomer **19** was isolated. The lack of isolation of other silylated diols suggests that the silyl migration is concerted with the reduction of ketone, rather than formed in an equilibrium step after the reduction was complete; presumably in the absence of cerium(III), the silyl group is complexed to the developing anionic oxygen, whereas a different complexation is involved with cerium(III). As in the case of reduction of the acetonide **7**, reduction of **17** with sodium borohydride in the presence of cerium(III) chloride gave the 5-*O*-silyl ether **19** in 70% yield, showing that again cerium(III) chloride had suppressed the migration of the silyl ether during the reduction. The silyl ether protecting groups were removed from both **18** and **19** by treatment with tetrabutylammonium fluoride in tetrahydrofuran to give the L-sugar **1** in 95% and 78% yield, respectively.



Scheme 2: (i) Me_2*tert*-BuSiCl, DMF, imidazole (ii) *n*BuLi, THF, -70°C (iii) $NaBH_4$, EtOH (iv) $CeCl_3$, $NaBH_4$, EtOH (v) Bu_4NF, THF

Although the sequence through the persilylated lactone **16** is shorter than that *via* the acetonide **6**, the additional cost and molecular weight of the silyl protection makes this a less attractive route for the synthesis of **1**. However, there may be occasions with other lactones where the removal of all the protecting groups under non-acidic conditions would make the use of the silylated lactone attractive, relative to acid removal of an isopropylidene protecting group.

In any event, the suppression of silyl migration in the cerium(III)-borohydride reduction is noteworthy. Silyl ether migration during borohydride reduction of partially protected polyhydroxylated

materials frequently leads to unwanted mixtures of products; studies to confirm the generality of suppression of silyl migration in these reductions are currently in progress.

The effect of compounds **1-5** on phosphoglucomutase (PGM) was investigated as part of a project in the development of assays and studies on phosphomannomutase (PMM); PMM deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type 1,[7] and inhibitors of this enzyme may allow clarification of several mechanistic aspects of the disease. The effect of the inhibitors on glucose-6-phosphate dehydrogenase (G-6-PDH) was also investigated because it was used as a coupling enzyme in the assay of PGM. 6*R*,6*C*-methyl glucose **4** inhibited PGM and G-6-PDH by 34% and 1%, respectively, at a concentration of 1 mM, demonstrating that the inhibition was specific and not due to inhibition of the coupling enzyme. Inhibition was competitive with a K_i value of 0.5 mM (IC_{50} 4 mM). In contrast, the L-methyl analogue **3** only inhibited PGM and G-6-PDH by 10% and 0%, respectively, at the same concentration. The azide **5**, in which the hydroxyl group in **4** is replaced by an azide decreased the apparent inhibition of PGM to 28% at a concentration of 5 mM; however, it also inhibited the indicator enzyme, G-6-PDH, by 18%. The corresponding butyl analogues, 6*R*,6*C*-**2** and 6*S*,6*C*-**1** inhibited PGM by 49 and 30%, respectively at a concentration of 5 mM, compared with 80% by **4** at the same concentration; the increase in size of the alkyl chain only affected the inhibition of PGM slightly. Full details of the studies of the enzyme inhibition will be given elsewhere.[8] The diastereomeric 6-*C*-methyl- **3** and **4**[3] analogues of glucose have been shown to have differential effects on glucose 6-phosphatase and glucokinase, and both 6-*C*-phenylglucoses inhibit glucokinase.[2] This study confirms that there is considerable scope for substitution of the C-6 prochiral protons with alkyl and aryl groups, perhaps to provide specificity in regard to different enzymes that perform a variety of reactions on the same sugar. In particular all these materials are neutral carbohydrates, and may constitute a major class of biochemical tools for the study of several of the membrane -bound enzymes that participate in primary metabolism and in particular those involved in the control and metabolism of sugar phosphates.

In summary, this paper describes short and efficient syntheses of epimeric *C*6-butylglucoses from glucuronolactone. The different inhibition of various enzymes of primary metabolism by different C6-alkyl diastereomers of glucose provides the prospect of finding small molecules which will inhibit specifically the individual steps of sugar phosphate metabolism not only for glucose but also for other carbohydrates and, in particular, for mannose.

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Experimental: Melting points were recorded on a Kofler hot block and are corrected. Proton nuclear magnetic resonance (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 Nuclear magnetic resonance (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 DEPT sequence. All chemical shifts are quoted on the δ-scale. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; app, apparent. Infra-red spectra were recorded on a Perkin-Elmer 1750 IR FT spectrophotometer. Mass spectra were recorded on a VG Masslab 20-250, BIO-Q or using desorption chemical ionisation (DCI NH_3), chemical ionisation (CI NH_3), electrospray or thermospray, or atmospheric

pressure chemical ionisation ($APCI^+$ or $APCI^-$) 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 (t.l.c.) 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 2M 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. D-Glucuronolactone **15** was converted to the fully protected lactone **6**[2] as previously described.

(1S,3S/3R,4S,5S,7R,8R)-3-Butyl-4-O-tert-butyldimethylsilyl-7,8-O-isopropylidene-3,7,8-trihydroxy-2,6-dioxabicyclo[3,3,0]octane **7**. *n*-Butyl lithium (15.1 ml, 23.6 mmol, 1.57 M solution in THF) was added dropwise to a stirred solution of the fully protected lactone **6** (6.0 g, 18.18 mmol) in dry THF (60 ml) at -78°C under a nitrogen atmosphere. The reaction was stirred at -78°C for 1.5 hour when TLC (ethyl acetate/hexane 1:3) revealed no remaining starting material (Rf 0.3) and two major products (Rf 0.5, Rf 0.6). An excess of saturated aqueous ammonium chloride solution was added and the mixture stirred for 45 min. The reaction mixture was diluted with ethyl acetate (200 ml) and washed with water (200 ml). The aqueous phase was extracted with ethyl acetate (2 x 200 ml) and the combined organic extracts dried (magnesium sulphate), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (acetone/toluene 1:19) to yield *the lactols* **7** (4.84 g, 68%) as a colourless oil, $[\alpha]_D^{21}$ +12.8 (c, 1.10 in $CHCl_3$). ν_{max} (film/cm^{-1}): 3545 (OH), 2956, 2934 (C-H). δ_H (200 MHz, C_6D_6): 0.00, 0.06 (6H, 2 x s, $Si(C\underline{H}_3)_2$); 0.05-0.10 (3H, m, $CH_2C\underline{H}_3$), 0.90 (9H, s, $SiC(C\underline{H}_3)_3$), 1.07, 1.36 (6H, 2 x s, $C(C\underline{H}_3)_2$); 1.10-1.80 (6H, m, 3 x CH_2); 3.55 (1H, d, $J_{3,4}$ 4.6 Hz, H-3); 3.92 (1H, s, OH); 4.67, 4.44, 4.38 (3H, 3x d, H-2, H-3, H-4); 5.90 (1H, d, $J_{6,7}$ 3.7 Hz, H-6). δ_C (50 MHz, C_6D_6): -5.6, -5.0 (2 x q, $Si(\underline{C}H_3)_2$); 13.5 (q, $CH_2\underline{C}H_3$); 17.7 (s, $Si\underline{C}(CH_3)_3$); 25.2 (q, $SiC(\underline{C}H_3)_3$); 27.1, 26.4 (2 x q, $C(\underline{C}H_3)_2$); 22.8, 25.4, 37.9 (3 x q, 3 x CH_2); 75.9, 82.2, 83.4, 86.1 (4 x d, C-3, C-4, C-7, C-8); 104.5 (s, C-2); 107.4 (s, C-6); 112.7 (s, $\underline{C}Me_2$). *m/z* (CI, NH_3): 371 ($M-H_2O+H^+$, 100%); (Found: C, 58.36, H, 9.68; $C_{19}H_{36}O_6Si$ requires: C, 58.73; H, 9.34%.).

(1S,3R,4S,5S,7R,8R)-3-Butyl-7,8-O-isopropylidene-3,4,7,8-tetrahydroxy-2,6-dioxabicyclo[3,3,0]-octane **8**. Tetrabutyl ammonium fluoride (2.75 ml, 2.75 mmol, 1.0 M solution in THF) was added to a stirred solution of the silyl lactols **7** (875 mg, 2.25 mmol) in dry THF (25 ml) under nitrogen. After 10 min TLC (ethyl acetate/hexane 1:3) showed no starting material (Rf 0.5) and one product (Rf 0.2). The solvent was removed *in vacuo* and the residue purified by flash chromatography (ethyl acetate/hexane 1:3) to yield *the lactol* **8** (450 mg, 73%), m.p. 120-122°C (ethyl acetate/hexane). $[\alpha]_D^{21}$ +22.6 (c, 0.84 in $CHCl_3$). ν_{max} (film/cm^{-1}): 3297 (br, OH). δ_H (500 MHz, C_6D_6): 0.91 (3H, t, J 7.2 Hz, $C\underline{H}_3CH_2$); 1.32-1.49 (4H, m, 2 x CH_2); 1.69 (2H, t, J 8.0 Hz, CH_2); 2.83 (1H, s, OH-3); 2.95 (1H, d, $J_{OH,4}$ 10.2 Hz, OH-4); 3.81 (1H, dd, $J_{5,4}$ 5.4 $J_{4,OH}$ 10.2 Hz, H-4); 4.56 (1H, d, $J_{1,5}$ 4.7 Hz, H-1); 4.70 (1H d, $J_{7,8}$ 3.5 Hz, H-8); 4.83 (1H, t, J 5.0 Hz, H-5); 6.04 (1H, d, $J_{7,8}$ 3.5 Hz, H-7). δ_C (125 MHz, C_6D_6): 14.1 (q, $CH_2\underline{C}H_3$); 23.1 (t, $\underline{C}H_2CH_3$); 25.5 (t, $\underline{C}H_2CH_2CH_3$); 27.2, 27.7 (2 x q, $C(\underline{C}H_3)_2$); 38.2 (t, $\underline{C}H_2CH_2CH_2CH_3$); 87.1, 83.4, 81.7, 75.4 (4 x d, C-4, C-5, C-7, C-8); 104.0 (d, C-2); 107.7 (d, C-6); 112.9 (s, $\underline{C}Me_2$). *m/z* (CI, NH_3): 292 ($M+NH_4^+$, 14%), 274 ($M-H_2O+NH_4^+$, 7%), 257 ($M-H_2O+H^+$, 100%). (Found: C, 56.97; H, 8.19; $C_{13}H_{22}O_6$ requires C, 56.92; H, 8.08%).

6C-Butyl-1,2:5,6-di-O-isopropylidene-L-glycero-α-D-gluco-hexofuranose ***14*** *and 6C-Butyl-1,2:5,6-di-O-isopropylidene-D-glycero-α-D-gluco-hexofuranose* ***13***. Sodium borohydride (95 mg, 2.51 mmol) was added to a stirred solution of the desilylated lactol **8** (450 mg, 1.64 mmol) in ethanol (13 ml). After 3 hour, TLC (ethyl acetate/hexane 1:1) showed no starting material (Rf 0.5) and only baseline products. An excess of saturated aqueous ammonium chloride was added and the mixture stirred for 30 min. The reaction mixture was filtered through Celite (elution with ethanol), and the filtrate concentrated *in vacuo*. The residue was dissolved in ethyl acetate (50 ml) and washed with brine (50 ml). The aqueous phase was extracted with ethyl acetate (2 x 50 ml) and the combined organic extracts dried (magnesium sulphate), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (ethyl acetate/hexane 2:1) to yield a mixture of *the epimeric alcohols* **12** and **9** in approximately a 1:1 ratio (377 mg, 83%) as a white solid, *m/z* (APCI$^-$): 275 (M-H$^-$, 100%). (Found C, 56.35; H, 8.86; $C_{13}H_{24}O_6$ requires C, 56.51; H, 8.75%).

A mixture of triols **12** and **9** (350 mg, 1.26 mmol) and a catalytic amount of DL-camphor-10-sulfonic acid were suspended in acetone (18 ml) and 2,2-dimethoxypropane (2 ml) and the reaction mixture was stirred at room temperature under N_2. After 48 hour, the reaction was quenched by addition of an excess of $NaHCO_3$. After filtration of the solution through Celite, the solvent was removed *in vacuo* to give a pale yellow oil which was purified by flash chromatography (ethyl acetate/hexane 1:4) to afford *the trans-diacetonide* ***14*** (132 mg, 33%), m.p. 61-63°C. $[\alpha]_D^{22}$ -51.3 (c, 1.15 in CH_3OH). δ_H (500 MHz, $CDCl_3$): 0.91 (3H, t, J 7.1 Hz, H-10); 1.31-1.43 (3H, m, H-9, H-8); 1.32, 1.40, 1.41, 1.50 (12H, 4 s, 4 x CH_3); 1.46-1.60 (m, 2H, H-8, H-7); 1.67-1.73 (1H, m, H-7'); 3.05 (1H, s, OH); 3.95 (1H, app t., J 7.2 Hz, H-5); 3.98 (1H, dt, $J_{6,7}$ 3.4, $J_{5,6}$ 7.9 Hz, H-6); 4.05 (1H, dd, $J_{3,4}$ 2.6, $J_{4,5}$ 6.0 Hz, H-4); 4.32 (1H, d, $J_{3,4}$ 2.6 Hz, H-3); 4.53 (1H, d, $J_{1,2}$ 3.7 Hz, H-2); 5.97 (1H, d, $J_{1,2}$ 3.7 Hz, H-1). δ_C (125 MHz, $CDCl_3$): 13.9 ($\underline{C}H_3CH_2$); 22.7 ($CH_3\underline{C}H_2$); 26.1, 26.7, 26.9, 27.2 (4 x CH_3); 28.1 ($CH_3CH_2\underline{C}H_2$); 32.8 ($CH_3CH_2CH_2\underline{C}H_2$); 75.7, 78.6, 80.4, 80.4, 84.8 (C-2, C-3, C-4, C-5, C-6); 105.2 (C-1); 109.2, 111.6 (2 x $\underline{C}Me_2$). *m/z* (APCI$^+$): 317 (M+H$^+$, 5%), 201 (M-2 x MeCO+H$^+$, 100%). (Found C, 60.62; H, 8.94; $C_{16}H_{28}O_6$ requires C, 60.74; H, 8.92%).

Further elution afforded *the cis-diacetonide* ***13*** (205 mg, 51%) as a colourless oil, $[\alpha]_D^{22}$ -29.3 (c, 0.77 in CH_3OH). δ_H (500 MHz, C_6D_6): 0.86 (3H, t, J 7.3 Hz, $C\underline{H}_3CH_2$); 1.24-1.34 (2H, m, $CH_3C\underline{H}_2$); 1.07, 1.20, 1.32, 1.40 (12H, 4 x s, 2 x $(CH_3)_2C$); 1.36-1.44 (1H, m, $CH_3CH_2\underline{H}CH$); 1.52-1.57 (1H, m, $CH_3CH_2HC\underline{H}$); 1.78-1.89 (2H, m, $CH_3CH_2CH_2C\underline{H}_2$); 2.11 (1H, d, $J_{OH,3}$ 3.3 Hz, OH); 4.11 (1H, ddd, $J_{6,7}$ 4.5, $J_{5,6}$ 5.4, $J_{6,7'}$ 9.4 Hz, H-6); 4.25 (1H, dd, $J_{5,6}$ 5.5, $J_{5,4}$ 9.1 Hz, H-5); 4.39 (1H, app. d, J 2.9 Hz, H-3); 4.41 (1H, d, $J_{1,2}$ 3.5 Hz, H-2); 4.43 (1H, dd, $J_{3,4}$ 2.8, $J_{4,5}$ 9.1 Hz, H-4); 5.94 (1H, d, $J_{1,2}$ 3.5 Hz, H-1). δ_C (125 MHz, $CDCl_3$): 14.0 ($\underline{C}H_3CH_2$); 22.6 ($CH_3\underline{C}H_2$); 25.4, 26.3, 27.0, 28.1 (4 x CH_3); 28.5 ($CH_3CH_2\underline{C}H_2$); 28.7 ($CH_3CH_2CH_2\underline{C}H_2$); 75.9, 76.3, 77.6, 78.0, 84.2 (C-2, C-3, C-4, C-5, C-6); 105.7 (C-1); 108.1, 111.6 (2 x $\underline{C}Me_2$). *m/z* (APCI$^-$): 315 (M-H$^-$, 53%), 257 (315-butyl$^-$, 55%), 182 (100%). (Found C, 60.69; H, 9.17; $C_{16}H_{28}O_6$ requires C, 60.74; H, 8.92%).

6C-Butyl-L-glycero-D-gluco-hexopyranose, ***1***. Method 1: A solution of the diacetonide **14** (64 mg, 0.20 mmol) in a 1:1 mixture of trifluoroacetic acid/water (10 ml) was stirred for 18 hour at 60°C. After this time, TLC (ethyl acetate/hexane 1:4) showed no starting material (Rf 0.22) and a new baseline product. The solvent was removed under reduced pressure and coevaporated with toluene. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 5:1) to give *the butyl L-pyranose* ***1*** (42 mg, 88%) as a white solid, m.p. 165-171°C (Pr^iOH). $[\alpha]_D^{21}$ +14.9 (c, 0.23 in MeOH). ν_{max} (KBr/cm^{-1}): 3388, 3259 (OH). δ_H (500 MHz, D_2O): 0.75 (6H, t, J 6.7 Hz, $CH_2C\underline{H}_3$); 1.16-1.26 (8H, m, H-8α, H-8β, H-9α, H-9β); 1.40-1.56 (4H, m, H-7α, H-7β); 3.09 (1H, dd, $J_{2,1}$ 8.0 $J_{2,3}$ 9.3 Hz, H-2β); 3.15 (1H, dd, $J_{5,6}$ 1.5 $J_{5,4}$ 9.8 Hz, H-5β); 3.33 (1H, app. t, J 9.3 Hz, H-3β); 3.38 (1H, dd, $J_{2,3}$ 9.8, $J_{1,2}$ 3.8 Hz, H-2α); 3.40 (1H, ap. t, J 9.5 Hz, H-4α); 3.44 (1H, app. t, J 9.4 Hz, H-4β); 3.52-3.54 (1H, m, H-5α); 3.55 (1H, ap. t, J 9.4 Hz, H-3α); 3.75-3.78 (1H, m, H-6β); 3.82 (1H, app. t, J 7.1, H-6α); 4.46 (1H, d, $J_{1,2}$ 7.9 Hz, H-1β); 5.08 (1H, d, $J_{1,2}$ 3.8 Hz, H-1α).

δ_C (125 MHz, D_2O+dioxane) 14.0 ($\underline{C}H_3CH_2$); 22.6 ($CH_3\underline{C}H_2$); 28.0 ($CH_3CH_2\underline{C}H_2\alpha$); 28.1 ($CH_3CH_2\underline{C}H_2\beta$); 33.0 ($CH_3CH_2CH_2\underline{C}H_2\beta$); 33.0 ($CH_3CH_2CH_2\underline{C}H_2\alpha$); 68.6, 68.7, 70.1, 70.3, 72.2, 72.4, 73.9, 74.9, 76.8, 77.1 (C-2, C-3, C-4, C-5, C-6, α and β); 92.8 (C-1α); 96.9 (C-1β). *m/z* ($APCI^-$): 235 ($M-H^-$, 25%), 113 ($M-CHOH(CH_2)_3CH_3-2H_2O^-$, 100%). (Found C, 51.13; H, 8.71; $C_{10}H_{20}O_6$ requires C, 50.84; H, 8.53%).

Method 2: A solution of the 6-*O*-silyl ether **11** (41 mg, 0.10 mmol) in a 1:1 mixture of trifluoroacetic acid/water (6 ml) was stirred for 13 hour at 65°C. After this time, TLC (ethyl acetate/hexane 1:4) showed no starting material (Rf 0.24) and a new baseline product. The solvent was removed under reduced pressure and coevaporated with toluene. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 5:1) to give **1** (21 mg, 84%), identical to the material above. Method 3: Hydrolysis of the 5-*O*-silyl ether **10** (50 mg, 0.13 mmol) under the same conditions also afforded **1** (29 mg, 96%), again identical to the material above.

6C-Butyl-D-glycero-D-gluco-hexopyranose 2. The altrodiacetonide **13** (127 mg, 0.40 mmol) was dissolved in a 2:1 mixture of trifluoroacetic acid and water (10 ml), and stirred at 65°C. After 48 hour, TLC (ethyl acetate/hexane 2:1) showed no starting material (Rf 0.79) and a baseline compound. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 5:1) to give *the butyl D-pyranose 2* (77 mg, 81%), a colourless oil which slowly crystallised, m.p. 40-45°C, $[\alpha]_D^{21}$ +7.9 (c, 0.5 in H_2O). ν_{max} (KBr/cm^{-1}): 3417, 3296 (OH). δ_H (500 MHz, D_2O): 0.76 (6H, t, J 6.7 Hz, $CH_2C\underline{H}_3$); 1.15-1.26 (6H, m, 3 x CH_2); 1.30-1.48 (6H, m, 3 x CH_2); 3.08 (1H, app. t, J 8.3 Hz, H-2β); 3.28-3.35 (4H, m, H-3β, H-4α, H-4β, H-5β); 3.37 (1H, dd, $J_{2,3}$ 9.8, $J_{1,2}$ 3.8 Hz, H-2α); 3.55 (1H, ap. t, J 9.4 Hz, H-3α); 3.74 (1H, dd, J 10.1, J 2.8 Hz, H-5α); 3.76-3.80 (2H, m, H-6α, H-6β); 4.47 (1H, d, $J_{1,2}$ 7.9 Hz, H-1β); 5.07 (1H, d, $J_{1,2}$ 3.8 Hz, H-1α). δ_C (125 MHz, D_2O+dioxane): 14.0 ($\underline{C}H_3CH_2$), 22.6 ($CH_3\underline{C}H_2$); 28.3 ($CH_3CH_2\underline{C}H_2\beta$); 28.4 ($CH_3CH_2\underline{C}H_2\alpha$); 30.1 ($CH_3CH_2CH_2\underline{C}H_2\beta$); 30.5 ($CH_3CH_2$-$CH_2\underline{C}H_2\alpha$), 71.3, 71.4, 71.5, 71.7, 72.1, 73.8, 73.9, 74.8, 76.8, 78.7 (C-2, C-3, C-4, C-5, C-6, α and β); 92.7 (C-1α); 96.8 (C-1β). *m/z* ($APCI^-$): 235 ($M-H^-$, 100%). (Found C, 50.98; H, 8.82; $C_{10}H_{20}O_6$ requires C, 50.84; H, 8.53%).

6-O-tert-Butyldimethylsilyl-6C-butyl-1,2-O-isopropylidene-L-glycero-β-D-gluco-hexofuranose 11. Sodium borohydride (7.3 mg, 0.18 mmol) was added to a stirred solution of the silyl lactols **7** (50 mg, 0.13 mmol) in ethanol (5 ml). After 3 hour, TLC (ethyl acetate/hexane 1:4) showed no starting material (Rf 0.51) and one major product (Rf 0.24). An excess of ammonium chloride was added and the mixture stirred for 1 hour. The reaction mixture was filtered through Celite (elution with ethanol), and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography (ethyl acetate/hexane 1:4) to yield *the migrated silyl diol 11* (48 mg, 95%) as a colourless oil, $[\alpha]_D^{23}$ -5.1 (c, 0.92 in MeOH). ν_{max} (KBr/cm^{-1}): 3435 (OH). δ_H (500 MHz, $CDCl_3$): 0.12, 0.13 (6H, 2 x s, Me_2Si); 0.90 (3H, t, $J_{10,9}$ 7.0 Hz, H-10); 0.92 (9H, s, $SiC(C\underline{H}_3)_3$); 1.26-1.36 (4H, m, H-9, H-8); 1.32, 1.48 (6H, 2 x s, $C(C\underline{H}_3)_2$); 1.49-1.52 (1H, m, H-7); 1.69-1.73 (1H, m, H-7'); 2.63 (1H, d, exch. in D_2O, $J_{OH,3}$ 2.5 Hz, OH-3); 2.64 (1H, d, exch. in D_2O, $J_{OH,5}$ 9.5 Hz, OH-5); 3.73 (1H, m collapsed to t, J 8.8 Hz on exch. in D_2O, H-5); 3.88 (1H, dd, $J_{6,7}$ 4.4, $J_{5,6}$ 8.5 Hz, H-6); 4.07 (1H, dd, $J_{4,3}$ 2.8, $J_{4,5}$ 8.5 Hz, H-4); 4.34 (1H, s br collapsed to d, $J_{3,4}$ 2.9 Hz on exch. in D_2O, H-3), 4.55 (1H, d, $J_{2,1}$ 3.7 Hz, H-2), 5.94 (1H, d, $J_{1,2}$ 3.7 Hz, H-1). δ_C (125 MHz, $CDCl_3$): -4.8, -4.4 (2 x q, $Si(\underline{C}H_3)_2$); 13.8 (q, $\underline{C}H_3CH_2$); 17.9 (s, $Si\underline{C}(CH_3)_3$); 22.7 (t, $CH_3\underline{C}H_2$); 25.7 (q, $Si\ C(\underline{C}H_3)_3$); 25.7 (q, $C(\underline{C}H_3)_2$); 26.5 (q, $C(\underline{C}H_3)_2$); 27.3 (t, $CH_3CH_2\underline{C}H_2$); 33.7 (t, $CH_3CH_2CH_2\underline{C}H_2$); 69.7, 71.4, 75.3, 79.9, 85.1 (5 x d, C-2, C-3, C-4, C-5, C-6); 105.3 (d, C-1); 111.3 (s, $\underline{C}Me_2$). *m/z* ($APCI^-$): 425 ($M+Cl^-$, 100%). (Found C, 58.62; H, 9.97; $C_{19}H_{38}O_6Si$ requires C, 58.43; H, 9.81%).

5-O-tert-Butyldimethylsilyl-6C-butyl-1,2-O-isopropylidene-L-glycero-β-D-gluco-hexofuranose ***10***. Cerium(III) chloride (507 mg, 2.06 mmol) was added to a stirred solution of the silyl lactols **7** (400 mg, 1.03 mmol) in methanol (40 ml). After 5 min, sodium borohydride (58.4 mg, 1.54 mmol) was added in one portion. After 1 hour, TLC (ethyl acetate/hexane 1:4) showed no starting material (Rf 0.51) and one major product (Rf 0.21). Solid ammonium chloride was added and the mixture stirred for 2 hour, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (ethyl acetate/hexane 1:4) to give *the unmigrated silyl ether* ***10*** (380 mg, 94%) as a colourless oil, $[\alpha]_D^{22}$ -30.6 (c, 1.88 in MeOH). ν_{max} (film/cm^{-1}). 3464 (OH); δ_H (500 MHz, $CDCl_3$): 0.15, 0.19 (6H, 2 x s, Me_2Si); 0.91 (9H, s, $(CH_3)_3CSi$); 0.92 (3H, t, J 7.4 Hz, $C\underline{H}_3CH_2$); 1.33-1.53 (6H, m, $(C\underline{H}_2)_3$); 1.32 (3H, s, CH_3C); 1.49 (3H, s, CH_3C); 2.08 (1H, d, exch. in D_2O, $J_{OH,6}$ 6.8 Hz, OH-6); 3.66-3.68 (1H, m, collapsed to app. q, J 5.2 Hz on exch. in D_2O, H-6); 4.05 (1H, t, $J_{3,4}$ 2.8 Hz, H-4); 4.16 (1H, m, H-5); 4.27 (1H, d, exch. in D_2O, $J_{OH,3}$ 2.7 Hz, OH-3); 4.38 (1H, t, $J_{3,4}$ 2.6 Hz, collapsed to d, $J_{3,4}$ 2.6 Hz on exch. in D_2O, H-3); 4.51 (1H, d, $J_{1,2}$ 3.7 Hz, H-2); 5.92 (1H, d, $J_{1,2}$ 3.7 Hz, H-1). δ_C (125 MHz, $CDCl_3$): -5.5 (CH_3-Si); -4.9 (CH_3-Si); 13.5 ($\underline{C}H_3CH_2$); 17.7 ($Me_3\underline{C}$-Si); 22.1 ($CH_3\underline{C}H_2$); 25.4 (($\underline{C}H_3)_3C$); 25.6, 26.3 (($\underline{C}H_3)_2C$); 27.4 ($CH_3CH_2\underline{C}H_2$); 33.1 ($CH_3CH_2CH_2\underline{C}H_2$); 71.8, 75.4, 75.8, 78.5, 85.0 (C-2, C-3, C-4, C-5, C-6); 103.7 ($\underline{C}Me_2$); 111.0 (C-1). *m/z* (APCI$^+$): 413 (M+Na$^+$, 12%); 391 (M+H$^+$, 23%). (Found C, 58.54; H, 10.03; $C_{21}H_{34}O_6Si$ requires C, 58.43; H, 9.81%).

1,2,5-Tri-O-tert-butyldimethylsilyl-α-D-glucuronolactone ***16***. *tert*-Butyldimethylsilylchloride (1.50 g, 9.94 mmol) was added to a stirred solution of α-**D**-glucuronolactone **15** (500 mg, 2.84 mmol) and imidazole (1.45 g, 21.29 mmol) in dry DMF (15 ml) at 0°C. After stirring at room temperature for 15 hour, TLC (toluene) showed no starting material (baseline) and a major product (Rf 0.51). The solvent was removed *in vacuo* by co-evaporation with toluene; the residue was dissolved in hexane (50 ml) and washed with water (2 x 50 ml). The organic phase was dried (magnesium sulfate), filtered and concentrated under reduced pressure. The residue was crystallised from methanol/water to give *1,2,5-tri-O-tert-butyldimethylsilyl-α-**D**-glucuronolactone* ***16*** (1.05 g, 70%) as white needles, m.p. 133-135°C (MeOH/H_2O). $[\alpha]_D^{21}$ +14.5 (c, 1.02 in MeOH). ν_{max} (KBr/cm^{-1}) 1790 (C=O). δ_H (200 MHz, $CDCl_3$): 0.09, 0.11, 0.16, 0.17 (18H, 4 x s, 3 x $(CH_3)_2Si$); 0.87, 0.88, 0.95 (27H, 3 x s, 3 x $(CH_3)_3CSi$); 4.23 (1H, s, H-2); 4.37 (1H, d, $J_{5,4}$ 6.2 Hz, H-5); 4.60 (1H, d, $J_{3,4}$ 4.4 Hz, H-3); 4.81 (1H, dd, $J_{4,3}$ 4.5, $J_{4,5}$ 5.9 Hz, H-4); 5.29 (1H, s, H-1). δ_C (50 MHz, $CDCl_3$): -5.7, -5.2, -5.2, -5.1, -4.7 (3 x $(CH_3)_2Si$); 17.9, 18.2 (3 x $Me_3\underline{C}Si$); 25.5 (3 x $(\underline{C}H_3)_3CSi$); 71.1, 78.6, 80.2, 83.2 (C-2, C-3, C-4, C-5, C-6); 105.1 (C-1); 173.6 (CO). *m/z* (APCI$^+$): 536 (M+NH_4^+, 62%), 519 (M+H$^+$, 34%). (Found C, 55.61; H, 10.10; $C_{24}H_{50}O_6Si_3$ requires C, 55.55; H, 9.71%).

(1S,3S/3R,4S,5S,7S,8R)-3-Butyl-4,7,8-tri-O-tert-butyldimethylsilyl-3-hydroxy-2,6-dioxabicyclo[3,3,0]-octane ***17***. n-Butyl lithium (725 µl, 1.15 mmol, 1.6 M solution in hexane) was added dropwise to a stirred solution of the silylated lactone **16** (500 mg, 0.96 mmol) in dry THF (15 ml) at -75°C under a nitrogen atmosphere. The reaction was stirred at -78°C for 25 min. when TLC (4% ethyl acetate in hexane) revealed no remaining starting material (Rf 0.28) and two major products (Rf 0.45, Rf 0.58). An excess of saturated aqueous ammonium chloride solution was added and the mixture stirred for 30 min. The reaction mixture was allowed to warm to room temperature, diluted with ethyl acetate (50 ml) and washed with brine (50 ml). The aqueous phase was extracted with ethyl acetate (2 x 25 ml) and the combined organic extracts dried (magnesium sulfate), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (toluene) to afford *the lactols* ***17*** (390 mg, 70%) as a colourless oil. $[\alpha]_D^{23}$ +3.3 (c, 1.17 in MeOH). ν_{max} (film/cm^{-1}): 3537 (OH). *m/z* (APCI$^+$): 599 (M+Na$^+$, 21%), 427 (M-H_2O-TBDMSO$^+$, 100%). (Found C, 58.51; H, 10.70; $C_{28}H_{60}O_6Si_3$ requires C, 58.28; H, 10.48%). Major compound: δ_H (500 MHz, $CDCl_3$): 0.10, 0.11, 0.12, 0.14, 0.15, 0.15 (18H, 6 x s, 3 x $SiMe_2$); 0.90, 0.92, 0.94 (27H, 3 x s, $SiC(C\underline{H}_3)_3$); 0.93

(3H, t, J 6.9 Hz, $CH_2C\underline{H}_3$); 1.31-1.38 (4H, m, $CH_3(C\underline{H}_2)_2$); 1.43-1.49 (1H, m, $CH_3(CH_2)_2\underline{H}CH$); 1.58-1.63 (1H, m, $CH_3(CH_2)_2HC\underline{H}$); 3.92 (1H, d, $J_{1,5}$ 4.8 Hz, H-1); 4.19 (1H, d, $J_{4,5}$ 4.7 Hz, H-4); 4.20 (1H, d, $J_{7,8}$ 1.4 Hz, H-8); 4.53 (1H, t, $J_{5,1}$ 4.7 Hz, H-5); 4.66 (1H, s, OH); 5.25 (1H, d, $J_{7,8}$ 3.7 Hz, H-7). δ_C (125 MHz, $CDCl_3$): -5.2, -4.9, -4.8, -4.6, -4.5, -4.4 (3 x $Si(\underline{C}H_3)_2$); 14.0 ($CH_2\underline{C}H_3$); 18.0, 18.1, 18.1 (3 x $Me_3\underline{C}Si$); 23.0 ($\underline{C}H_2CH_3$); 25.6 ($\underline{C}H_2CH_2CH_3$); 25.7 (3 x $SiC(\underline{C}H_3)_3$); 37.8 ($\underline{C}H_2CH_2CH_2CH_3$); 75.5, 82.4, 84.5, 85.7 (C-1, C-4, C-5, C-8); 104.8 (s, C-3); 107.7 (s, C-7).

6C-Butyl-1,2,6-tri-O-tert-butyldimethylsilyl-L-glycero-β-D-gluco-hexofuranoside ***18***. Sodium borohydride (5 mg, 0.13 mmol) was added to a stirred solution of the lactols **17** (50 mg, 0.09 mmol) in ethanol (2 ml). After 12 hour, TLC (4% ethyl acetate in hexane) showed no starting material (Rf 0.5) and a new compound (Rf 0.25). Solid ammonium chloride was added and the mixture stirred for 1 hour, filtered through Celite (elution with ethanol), and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography (2% ethyl acetate in hexane) to give *the migrated 6-O-silyl ether* ***18*** (37 mg, 73%) as a colourless oil, $[\alpha]_D^{23}$ -24.8 (c, 1.22 in CH_3OH). ν_{max} (film/cm^{-1}): 3535 OH). δ_H (200 MHz, $CDCl_3$): 0.07, 0.09, 0.11 (18H, 3 x s, 3 x $(CH_3)_2Si$); 0.86-0.92 (30H, m, 3 x $(CH_3)_3CSi$, H-10); 1.19-1.48 (5H, m, H-7, H-8, H-9); 1.68-1.86 (1H, m, H-7'); 2.42 (1H, d, $J_{OH,3}$ 9.4 Hz, OH-3); 3.04 (1H, d, $J_{OH,5}$ 10.6 Hz, OH-5); 3.77 (1H, t, J 9.5 Hz, H-3); 3.90-4.08 (3H, m, H-4, H-5, H-6); 4.06 (1H, s, H-2); 5.07 (1H, s, H-1). δ_C (50 MHz, $CDCl_3$): -5.49, -5.21, -4.64, -4.52 (3 x $(\underline{C}H_3)_2Si$); 13.92 ($C\underline{H}_3CH_2$); 1766, 17.96 (3 x $Me_3\underline{C}Si$); 22.71 ($CH_3\underline{C}H_2$); 25.45, 25.78 (3 x $(\underline{C}H_3)_3CSi$); 27.64 ($CH_3CH_2\underline{C}H_2$); 33.73 ($CH_3CH_2CH_2\underline{C}H_2$); 59.72, 71.45, 76.48, 81.17, 82.17 (C-2, C-3, C-4, C-5, C-6); 103.37 (C-1). *m/z* ($APCI^-$): 613 ($M+Cl^-$, 100%). (Found C, 58.12; H, 11.16; $C_{28}H_{62}O_6Si_3$ requires C, 58.08; H, 10.79%).

6C-Butyl-1,2,5-tri-O-tert-butyldimethylsilyl-L-glycero-β-D-gluco-hexofuranoside ***19***. Cerium(III) chloride (64 mg, 0.26 mmol) was added to a stirred solution of the lactols **17** (75 mg, 0.13 mmol) in ethanol (5 ml). After 5 min, sodium borohydride (9.8 mg, 0.26 mmol) was added in one portion. After 7 hour, TLC (toluene) showed only traces of starting material (Rf 0.51) and one major product (Rf 0.31). Solid ammonium chloride was added and the mixture stirred for 1 hour, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (toluene) to yield *the 5-O-silyl ether* ***19*** (53 mg, 70%) as a colourless oil, $[\alpha]_D^{23}$ -46.8 (c, 1.30 in MeOH). ν_{max} (KBr/cm^{-1}): 3528 (OH). δ_H (500 MHz, $CDCl_3$): 0.13, 0.14, 0.16 (18H, 3 x s, 6 x Si-CH_3); 0.90-0.95 (30H, m, 3 x $SiC(C\underline{H}_3)_3$, $C\underline{H}_3CH_2$); 1.28-1.63 (6H, m, $CH_3(C\underline{H}_2)_3$); 2.47 (1H, d, exch. in D_2O, $J_{OH,6}$ 10.4 Hz, OH-6); 3.03 (1H, d, exch. in D_2O, $J_{OH,3}$ 11.6 Hz, OH-3); 3.70-3.74 (1H, m, H-6); 3.88 (1H, dd, $J_{3,OH}$ 11.5, $J_{3,4}$ 3.1 Hz, H-3); 4.04 (1H, s, H-2); 4.05 (1H, dd, $J_{5,6}$ 2.2, $J_{5,4}$ 9.1 Hz, H-5); 4.19 (1H, dd, $J_{4,3}$ 3.1, $J_{4,5}$ 9.1 Hz, H-4); 5.15 (1H, s, H-1). δ_C (125 MHz, $CDCl_3$): -5.4, -4.8, -4.8, -4.7, -4.7, -4.5 (3 x $Si(\underline{C}H_3)_2$); 14.1 ($CH_2\underline{C}H_3$); 17.7, 18.0, 18.3 (3 x $Si\underline{C}(CH_3)_3$); 22.7 ($CH_3\underline{C}H_2$); 25.5, 25.7, 26.0 (3 x $SiC(\underline{C}H_3)_3$), 28.4 ($CH_3CH_2\underline{C}H_2$); 32.9 ($CH_3CH_2CH_2\underline{C}H_2$); 70.9, 72.8, 75.8, 81.0, 83.6 (C-2, C-3, C-4, C-5, C-6); 103.4 (C-1). *m/z* ($APCI^-$): 613 ($M+Cl^-$, 100%). (Found C, 58.30; H, 11.01; $C_{19}H_{38}O_6Si$ requires C, 58.08; H, 10.79%).

6C-Butyl-L-glycero-D-gluco-hexopyranose ***1***. Method 1: To a solution of the *5-O-silyl ether* ***19*** (36 mg, 0.062 mmol) in THF (1 ml), tetrabutyl ammonium fluoride (1M solution in THF, 250 µl, 0.25 mmol) was added dropwise, and the mixture was stirred at room temperature under N_2. After 10 min, TLC (toluene) showed no starting material (Rf 0.35) and a new baseline product. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 5:1) to give *the butyl L-sugar* ***1*** (12 mg, 78%) as a white solid, identical to the material above. Method 2: Hydrolysis of the *6-O-silyl ether* ***18*** (37 mg, 0.064 mmol) under the same conditions also afforded **1** (15 mg, 95%), again identical to the material above.

Enzyme assays: *Assay of phosphoglucomutase (PGM):* PGM activity was measured in fibroblasts essentially as described by Van Schaftingen and Jaeken,[7] except that the rate of the reaction was followed fluorimetrically rather than spectrophotometrically. Extracts of fibroblasts (5 µl) were incubated 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, in the presence and absence of the inhibitor (1 or 5 mM). The reaction was followed for 10 min at 30°C by measuring the production of NADPH fluorimetrically, using an excitation wavelength of 340 nm and emission wavelength of 460 nm. *Assay of glucose-6-phosphate dehydrogenase (G-6-PDH):* G-6-PDH was obtained from Sigma (Cat. No.5885) and diluted 1000-fold in HEPES buffer, pH 7.1. 50 µl of the diluted enzyme was incubated with 0.5 mM glucose-6-phosphate, 0.25 mM NADP and 1 µM glucose-1,6-bisphosphate in HEPES buffer, pH 7.1, in a final volume of 500 µl. The reaction was followed fluorimetrically in the presence and absence of inhibitor for 10 min at 30°C using an excitation wavelength of 340 nm and emission wavelength of 460 nm.

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