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7-Carbon Mimics of D-Glucose and L-Fucose: Activation by 6R-, and Inactivation by 6S, -6C-Methylglucose of Glycogen Synthase: Inhibition of Glucokinase and/or Glucose-6-Phosphatase

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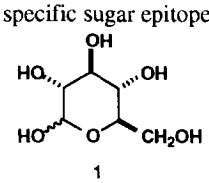
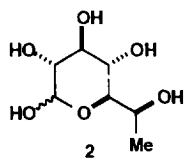
Abstract: Short efficient syntheses of epimeric C6-*C*-methyl glucoses are described. One C-6 epimer activates glycogen synthase while the other epimer inactivates the enzyme; C6*R*-C-methyl glucose **3** is the first example of a specific inhibitor of glucose-6-phosphatase and can increase the intracellular concentration of glucose-6-phosphate 20 times. C6*S*-C-methyl glucose **2** inhibits glucokinase and glucose-6-phosphatase, but also has the potential to give easy access to α-*C*-glycosides of L-fucose. *C*-6-Alkyl carbohydrates may provide a new range of sugar mimics that control enzymes associated with formation, hydrolysis and other fates of sugar-6-phosphates.

Sugar mimics that inhibit or stimulate specific metabolic processes provide potential for the treatment of a wide range of diseases and the investigation of many biochemical pathways. Ideally, the mimic should probably retain as many of the functional groups in the parent sugar as possible and, in particular, retain the stereochemistry at all the chiral centres of the carbohydrate. Thus for mimics of glucopyranose **1**, the sites available for such change are substitution of the ring oxygen [for example with nitrogen[1] to give nojirimycin and related systems] and the anomeric position principally by the formation of C-glycosides. In all these analogues it is possible to keep all of the four hydroxyl groups with the same stereochemistry as glucopyranos(id)es to provide a recognition site for the enzymes or receptors that handle glucose. *C*-Glycosides in particular offer attractive opportunities for the generation of combinatorial libraries which contain specific sugar epitopes.[2]



1



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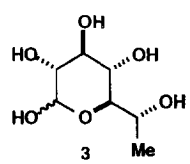

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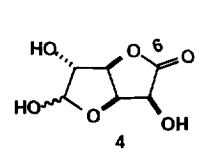
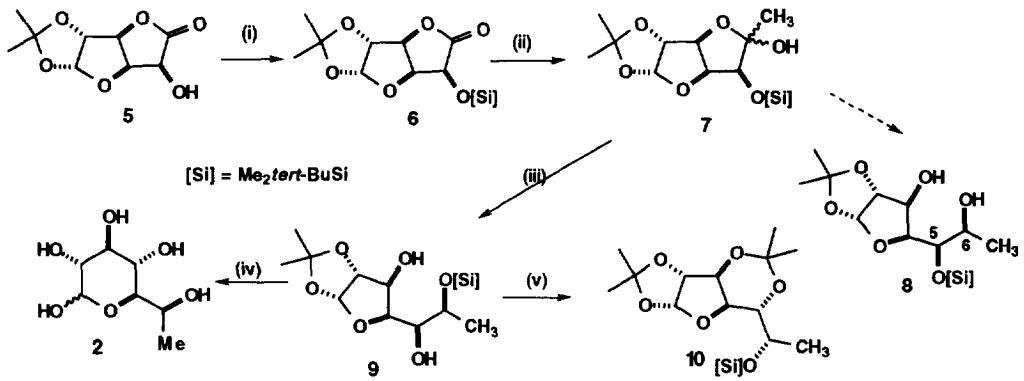


4

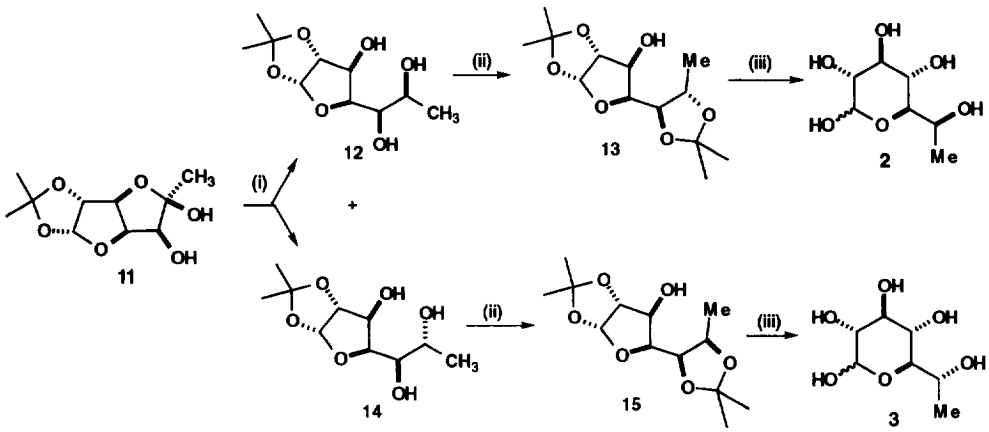
An additional site is the side chain primary hydroxyl group; substitution of one of the prochiral hydrogens by, for example a methyl group, would give the two diastereomers **2** and **3**. There are very few examples of such compounds[3] and the second isomer **3**, isolated from *Streptomyces setonensis*[4] and *S. purpeofuscus*[5] inhibits the greening of dark-grown *Scenedesmus obliquus*, probably by inhibition of carbon dioxide fixation. The two isomers **2** and **3** were considered potential inhibitors of glycogen phosphorylase (GP) as a possible strategy for the treatment of late-onset diabetes.[6] Both isomers may be derived from attack of methyl lithium on an aldehyde derived from C-6 of glucose[7] but this is a lengthy and non-selective procedure. This paper describes a short and efficient diastereoselective synthesis of **2** from glucuronolactone **4** in which C-6 is at a suitable oxidation level for attack by methyl lithium. A less stereoselective synthesis allows access to both **2** and **3**. The paper also reports the effects of **2** and **3** on GP, and on glucokinase and glucose-6-phosphatase (Glc-6-Pase).



Scheme 1 (i) Me_2*tert*-BuSiCl, DMF, imidazole (ii) MeLi, THF, -70°C (iii) $NaBH_4$, EtOH (iv) H_3O^+ dioxan (v) Me_2CO, $Me_2C(OMe)_2$, CSA

For the synthesis of **2**, the remaining hydroxyl group in the acetonide of glucuronolactone **5**[8] [Scheme 1] was protected as the silyl ether **6** m.p. 120-122°C, $[\alpha]_D^{22}$ +55.8 (c, 1.82 in $CHCl_3$) by treatment with *tert*-butyldimethylsilyl chloride in DMF in the presence of imidazole [92% yield]. Reaction of the fully protected lactone **6** with methyl lithium in THF at -70°C gave a mixture of the lactols **7** [80% yield]. Treatment of the anomeric mixture of **7** with sodium borohydride in ethanol induced a stereoselective Felkin-Ahn reduction to afford a single isolated silyl ether **9** [m.p. 97-99°C, $[\alpha]_D^{22}$ +2.9 (c, 0.95 in $CHCl_3$)] in 66% yield, with no other silyl ethers isolated. The stereochemistry at the C-6 in **9** was firmly established by treatment with tetrabutylammonium fluoride in THF to give **12** m.p. 135-137°C, $[\alpha]_D^{23}$ -16.0 (c, 0.5 in acetone), followed by isopropylidenation **13** [see below]. It was clear from the NMR that the anticipated product **8** had not been formed; D_2O exchange simplified the signal for the proton at C-5 [rather than C-6] from an apparent dt to a dd, indicating that the silyl protecting group had migrated during the reduction from C-5 oxygen to C-6 to give **9**; also reaction of **9** on treatment with acetone and 2,2-dimethoxypropane in the presence of camphor sulfonic acid afforded a diacetonide **10** [89% yield] with singlets for the $Me_2\underline{C}$ at δ 100.7 and 111.8, clearly demonstrating the presence of both a 5 and a 6 membered ring acetonide.[9] It is not clear whether this is a thermodynamic or kinetic phenomenon, or whether **8** is an initially formed product or if the silyl migration is concerted with the reduction; further studies on this are in progress. All the protecting groups were removed from **9** by treatment with ion exchange resin (Amberlyst IR-120, H+) in aqueous dioxan to give the homofucose **2** [10]in 92% yield [45% yield from **5**].

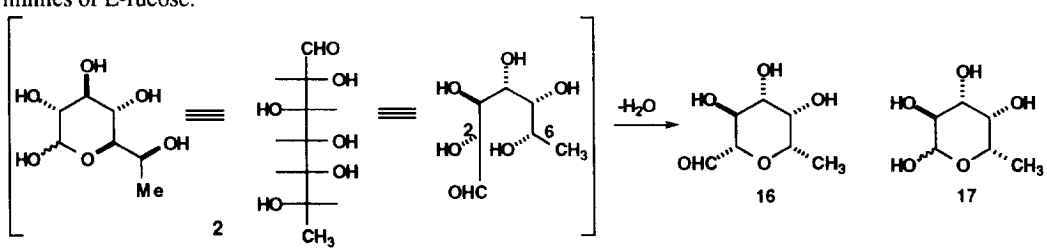
Removal of the silyl protecting group from **7** by treatment with tetrabutylammonium fluoride in THF gave the lactol **11**, m.p. 117-119°C, $[\alpha]_D^{22}$ +25.1 (c, 1.37 in $CHCl_3$), in 62% yield which crystallised as a single anomer, the structure of which was established by X-ray crystallographic analysis.[11] Although the reduction of the silyl ether **7** was highly diastereoselective, the reduction of deprotected lactol **11** by sodium borohydride in ethanol, in which the C-6 hydroxyl group was free, was relatively unselective and afforded an inseparable mixture of the diols **12** and **14** in 75% yield [Scheme 2]. Treatment of the diol mixture with acetone in the presence of camphor sulfonic acid gave the two separable acetonides **13** [m.p. 140-142° C, $[\alpha]_D^{22}$ -9.7 (c, 1.11 in $CHCl_3$)] and **15** [colourless oil, $[\alpha]_D^{22}$ -27.3 (c, 1.2 in $CHCl_3$)] in yields of 19% and 43% respectively. The singlets for the $Me_2\underline{C}$ in **13** were at δ 109.1 and 111.7 and in **14** at δ 108.3 and 111.7, showing only 5 ring acetonides were formed; the structure of **13** was firmly established by X-ray crystallographic analysis.



Scheme 2 (i) $NaBH_4$, EtOH (ii) Me_2CO, CSA (iii) CF_3COOH, H_2O

Treatment of the diacetonides **13** and **15** with aqueous trifluoroacetic acid gave the unprotected pyranoses **2** and **3**[12] in yields of 92 and 93%. Thus the reduction of either protected or unprotected lactols derived from treatment of protected uronic acids with a range of alkyl lithiums may provide general easy access to epimeric C-6-substituted hexoses with alkyl substituents.

It is also worth noting [Scheme 3] that while **2** may be viewed as an analogue of glucose, in an open Fischer projection, it is also a seven carbon analogue of L-fucose **17**. Elimination of water by activation of the hydroxyl group at C-2 of **2** and nucleophilic attack with inversion by the hydroxyl functionality at C-6 would give a short synthesis of α-C-glycosides **16** of L-fucose and such materials might usefully be developed into mimics of L-fucose.[13]



Scheme 3

Neither **2** nor **3** showed any inhibition of glycogen phosphorylase at six concentrations up to 30 mM.[14] However, the L-sugar homofucose **2** caused partial inactivation of glycogen synthase that had been activated previously by incubation of hepatocytes with 50mM glucose and also decreased the intracellular concentration of glucose-6-phosphate (Glc-6-P);[15] in complete contrast, the D-sugar homoaltrose **3** induced further activation of glycogen synthase and caused Glc-6-P levels to rise. Further studies showed that **2** inhibits both Glc-6-Pase and glucokinase, whereas **3** inhibits only the phosphatase causing intracellular Glc-6-P levels to rise by up to 20 fold. D-Homoaltrose **3** caused an accumulation of intracellular Glc-6-P, whether it originated from glucose or glycogen or from a glucose precursor (lactate). The inhibition of Glc-6-Pase was largely competitive - 6mM **3** increased the apparent K_m for Glc-6-P threefold while decreasing V_{max} by merely 20%. The specific inhibition of Glc-6-Pase by **3** provides Glc-6-P which promotes the dephosphorylation (activation) of glycogen synthase, and may furthermore enhance allosterically the catalytic activity of the

synthase. Enhancement of glycogen synthesis by **3** may provide a new strategy for the treatment of late-onset diabetes.

In summary this paper describes short and efficient syntheses of epimeric *C*6-methylglucoses, both by a diastereoselective reduction; the methodology should allow the easy formation of a wide range of *C*6-alkyl substituted carbohydrates from the corresponding uronic acids. The two different epimers of C6-methylglucose showed very different effects on glucokinase; there are no precedents for such control of the enzymes responsible for formation and hydrolysis of hexose-6-phosphates by diastereomers. It is clear that it will be worth exploring related derivatives of glucose, and also similar analogues of mannose and galactose, with the prospect of providing materials which will allow the investigation of enzymes responsible for the synthesis, hydrolysis, mutation and isomerisation of sugar phosphates. Full accounts of the enzymic studies on these compounds will appear in due course.[16]

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10. Data for homo-L-fucose **2**; m.p. 168-175°C; $[\alpha]_D^{23}$ +21.8 (c, 1.05 in H_2O, β anomer), +95.9 (c, 1.05 in H_2O, α anomer), (lit ref[5] $[\alpha]_D^{23}$ + 41.0 (*c*, 1 in H_2O)); δ_H (500 MHz, D_2O): 1.21 (3H, d, $J_{6,7}$ 6.6 Hz, Meα), 1.25 (3H, d, $J_{6,7}$ 6.6 Hz, Meβ), 3.18 (1H, dd, J 1.8, 9.7 Hz, H-5β), 3.20 (1H, app. t, J 8.6 Hz, H-2β), 3.41-3.54 (5H, m, H-2α, H-3β, H-4α, H-4β, H-5α), 3.66 (1H, app. t, J 9.4 Hz , H-3α), 4.08 (1H, dq, $J_{5,6}$ 1.8 Hz, $J_{6,7}$ 6.6 Hz, H-6β), 4.13 (1H, dq, $J_{5,6}$ 1.8 Hz, $J_{6,7}$ 6.6 Hz, H-6α), 4.57 (1H, d, $J_{1,2}$ 7.9 Hz, H-1β), 5.20 (1H, d, $J_{1,2}$ 3.8 Hz, H-1α); ^{13}C-NMR (125 MHz, D_2O + dioxan): 19.3 (q, Me), 19.5 (q, Me), 65.0, 65.1, 70.4, 70.6, 72.3, 73.8, 74.0, 75.1, 76.8, 78.5 (10 x d, C-2, C-3, C-4, C-5, C-6 α and β), 92.8 (d, C-1α), 96.9 (d, C-1β).
11. The details of the crystal structures of **11** and **13** will be presented in the full paper.
12. Data for homo-D-altrose **3**: foam, $[\alpha]_D^{21}$ + 41.5 (c, 0.8 in H_2O) (lit[7] $[\alpha]_D^{24}$ + 38 (c, 4 in H_2O)); δ_H (500 MHz; D_2O): 1.16 (3H, d, $J_{6,7}$ 6.2 Hz, Meα), 1.17 (3H, d, $J_{6,7}$ 6.4 Hz, Meβ), 3.17 (1H, H-2β), 3.30 (2H, H-4α, H-4β), 3.41 (2H, H-3β, H-5b), 3.47 (1H, H-2α), 3.64 (1H, H-3α), 3.81 (1H, H-5α), 4.09 (2H, H-6α, H-6β), 4.57 (1H, d, $J_{1,2}$ 7.9 Hz, H-1β), 5.17 (1H, d, $J_{1,2}$ 3.8 Hz, H-1α); ^{13}C-NMR (125 MHz, D_2O + dioxan): 14.7 (q, Me), 14.8 (q, Me), 66.1, 70.5, 70.7, 71.2, 72.8, 72.9, 74.0, 75.7, 77.7 (9 x d, C-2, C-3, C-4, C-5, C-6 α and β), 91.9 (d, C-1α), 96.0 (d, C-1β).
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