Tetrahedron: *Asymmetry* 19 (2008) 1904–1918

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Green syntheses of new 2-*C*-methyl aldohexoses and 5-*C*-methyl ketohexoses: D-tagatose-3-epimerase (DTE)—a promiscuous enzyme

Nigel A. Jones [b,d], Devendar Rao [a], Akihide Yoshihara [a], Pushpakiran Gullapalli [a], Kenji Morimoto [a], Goro Takata [a], Stuart J. Hunter [b], Mark R. Wormald [c], Raymond A. Dwek [c], Ken Izumori [a,*], George W. J. Fleet [b,*]

[a] *Rare Sugar Research Center, Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0795, Japan*
[b] *Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, OX1 3TA, UK*
[c] *Glycobiology Institute, Department of Biochemistry, Oxford University, South Parks Road, Oxford OX1 3QU, UK*
[d] *Dextra Laboratories Limited, The Science and Technology Centre, Whiteknights Road, Reading RG6 6BZ, UK*

ARTICLE INFO

Article history:
Received 16 June 2008
Accepted 29 July 2008
Available online 28 August 2008

This paper marks the 80th birthday of E. J. Corey

ABSTRACT

The Kiliani synthesis on the 4 readily accessible ketohexoses (D-fructose, D-tagatose, L-sorbose, D-psicose) allows access to 4 diastereomeric 2-*C*-methyl-aldohexoses (2-*C*-methyl-D-mannose, 2-*C*-methyl-D-talose, 2-*C*-methyl-L-gulose, 2-*C*-methyl-D-allose) and 4 diastereomeric 2-*C*-methyl-alditols (2-*C*-methyl-D-mannitol, 2-*C*-methyl-D-talitol, 2-*C*-methyl-L-gulitol, 2-*C*-methyl-D-allitol). Microbial oxidation of 2-*C*-methyl-D-mannitol and 2-*C*-methyl-L-gulitol gave 5-*C*-methyl-D-fructose; microbial oxidation of 2-*C*-methyl-D-talitol afforded 5-*C*-methyl-D-psicose, whereas 2-*C*-methyl-D-allitol formed 5-*C*-methyl-L-psicose. Both enantiomers of 5-*C*-methyl-fructose were equilibrated by D-tagatose-3-epimerase (DTE) with both enantiomers of 5-*C*-methyl-psicose. These transformations demonstrate that polyol dehydrogenases and DTE act on branched synthetic sugars. Full NMR analyses show that 5-*C*-methyl-D-fructose is present as the β-pyranose and β-furanose forms in a ratio of 90:10; all pyranose and furanose forms of 5-*C*-methyl-D-psicose are present in solution. The combination of chemical and biological procedures allows the environmentally friendly generation of a new family of branched monosaccharides.

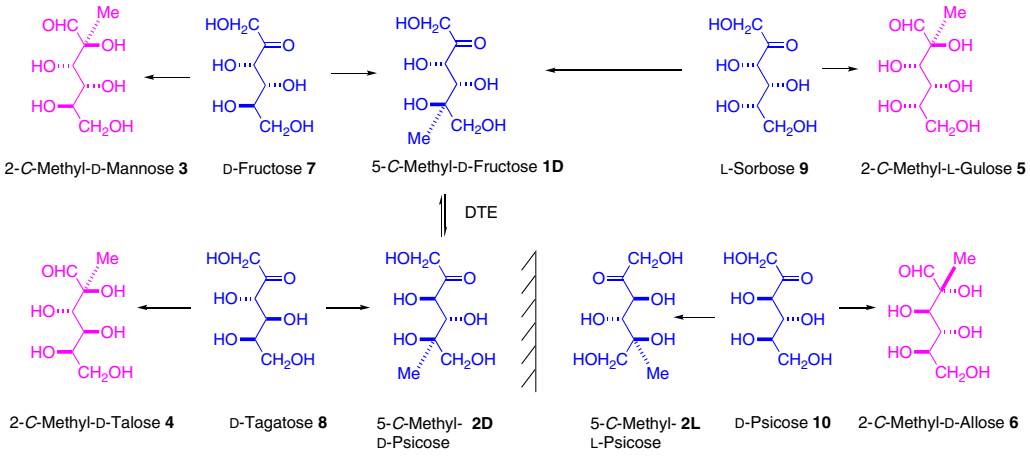
1. Introduction

Due to the multitude of biological pathways controlled by carbohydrates and sugar mimics, much effort has been directed toward de novo syntheses of sugars.[1] Unprotected monosaccharides, soluble only in water and existing as complex mixtures, which are present in up to five open chain, pyranose and furanose forms, are challenging targets for synthetic organic chemists. Development of the synthesis of rare sugars for use as healthy alternative foodstuffs[2] has also demonstrated that rare and new monosaccharides which interact with a number of biological receptors have a wide range of potential chemotherapeutic uses;[3] a 2-*C*-methyl-branched mannose has provided the first example of a small molecule that binds to a DC-SIGN receptor.[4] D-Psicose has been identified as the first anthelmintic sugar.[5] Although the use of enzymes to perform specific transformations in organic synthesis[6] is generally limited by their substrate specificity, directed evolution and the discovery of new enzymes has shown the potential of such biotechnological procedures.[7] Izumoring[8] allows the isomerization of all of the 16 aldohexoses and 8 ketohexoses in water under environmentally friendly conditions; this concept depends on (i) polyol dehydrogenases, which allow the specific oxidation of alditols to ketoses;[9] (ii) D-tagatose-3-epimerase (DTE), which equilibrates C-3 in each of 4 pairs of ketoses;[10] and (iii) aldose isomerases, which equilibrate ketoses and aldoses. The strategy of Izumoring has been extended to 1- and 6-deoxy hexoses[11,12] and 4-*C*-methyl-branched pentoses.[13]

This paper describes the preparation of both enantiomers of 5-*C*-methyl-ketohexoses **1** and **2**, and of four 2-*C*-methyl-aldohexoses **3**–**6** from the four readily available diastereomeric ketohexoses **7**–**10** (Scheme 1). The syntheses depend on the efficient Kiliani reactions of D-fructose and L-sorbose,[14,15] and of D-psicose and D-tagatose.[16,17] Thus, D-fructose **7** may efficiently be converted by chemical synthesis to 2-*C*-methyl-D-mannose **3**, and by a combination of chemical and biotechnological steps to 5-*C*-methyl-D-fructose **1D**. The other three readily available ketohexoses (D-tagatose **8**, L-sorbose **9**, D-psicose **10**) can be converted by similar pathways to 2-*C*-methyl-aldohexoses (2-*C*-methyl-D-talose **4**, 2-*C*-methyl-L-gulose **5**, 2-*C*-methyl-D-allose **6**) and to 5-*C*-methyl-aldoketoses (5-*C*-methyl-D-psicose **2D**, 5-*C*-methyl-D-fructose **1D**, 5-*C*-methyl-L-psicose **2L**). The paper provides further evidence of the promiscuity of D-tagatose-3-epimerase (DTE), which equilibrates 5-*C*-methyl-D-psicose **2D** and 5-*C*-methyl-L-psicose **2L**

* Corresponding authors.
E-mail address: george.fleet@chem.ox.ac.uk (G. W. J. Fleet).

0957-4166/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2008.07.034



Scheme 1. Summary of branched sugars synthesized. (For the clarity of different classes of monosaccharides, all ketoses in the paper are shown in blue, alditols in red in red and aldoses in purple.)

with 5-*C*-methyl-D-fructose **1D** and 5-*C*-methyl-L-fructose **1L**, respectively. Such procedures allow the availability of new monosaccharides with carbon branches for the evaluation of their potential biological activity.

The general scheme is illustrated by the transformations of D-fructose **7** to 2-*C*-methyl-D-mannose **3** and to 5-*C*-methyl-D-fructose **1D** and 5-*C*-methyl-D-psicose **2D** (Scheme 2). Fructose **7** underwent an efficient Kiliani reaction in a Felkin-Ahn controlled cyanide addition to give **11** as the major hydroxy acid formed. Treatment of the crude reaction mixture with acetone and acid allowed the isolation under thermodynamic conditions of the diacetonide **12** with only the branched hydroxymethyl group unprotected. Reduction of the primary alcohol **12** gave the 2-*C*-methyl-branched lactone **13**. DIBAL reduction of **13** followed by deprotection of the resulting lactol afforded 2-*C*-methyl-D-mannose **3**. Alternative hydride reduction of **13** to the corresponding diol, followed by deprotection gave the branched alditol **14**, 2-*C*-methyl-D-mannitol, which is identically related by 180° rotation to 5-*C*-methyl-D-mannitol; microbial oxidation of **14** afforded 5-*C*-methyl-D-fructose **1D**; DTE efficiently epimerized the C-3 position to 5-*C*-methyl-D-psicose **2D**.

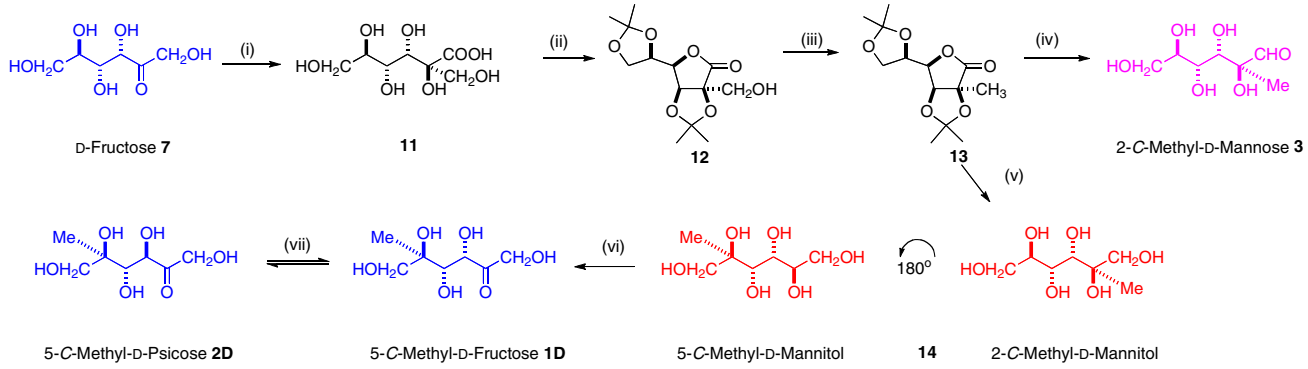
Four of the eight ketohexoses are readily available (Scheme 3). D-Tagatose **8**, epimeric at C-4 with D-fructose **7**, is readily available from the isomerization of galactose, effectively a waste product from milk;[18] the Kiliani-acetonation procedure on **8** affords ready access to the 2-*C*-methyl-branched lactone **15**, which may be chemically transformed to 2-*C*-methyl-D-talose **4** or to the branched alditol, 2-*C*-methyl-D-talitol **16**. Microbial oxidation of **16**, which may also be viewed as 5-*C*-methyl-D-altritol, gives 5-*C*-methyl-D-psicose **2D**, which can be equilibrated by DTE with 5-*C*-methyl-D-fructose **1D**. L-Sorbose **9**, epimeric at C-5 with D-fructose, is available from the industrial synthesis of vitamin C and may be converted to the 2-*C*-methyl-branched lactone **17**. Again, efficient hydride reductions allow the efficient synthesis of 2-*C*-methyl-L-gulose **5** and of 2-*C*-methyl-L-sorbitol **18**. Specific microbial oxidation of **18**, which may also be viewed as 5-*C*-methyl-D-glucitol, gives 5-*C*-methyl-D-fructose **1D**, the same branched ketose as obtained by microbial oxidation of 5-*C*-methyl-D-mannitol **14**.

D-Psicose **10**, conveniently obtained in large quantities by epimerization at C-3 of D-fructose by DTE[10] or from microbial oxidation of allitol,[19] provides access to the enantiomeric 5-*C*-methyl-L-ketoses **2L** and **1L**. Thus, the Kiliani-acetonation sequence on **10** allows access to 2-*C*-methyl-D-allose **6** and to 2-*C*-methyl-D-allitol **20**. 2-*C*-Methyl-D-allitol **20** is identical to 5-*C*-methyl-L-allitol, so that microbial oxidation of **20** affords 5-*C*-methyl-L-psicose **2L**, which can be equilibrated by DTE with 5-*C*-methyl-L-fructose **1L**.

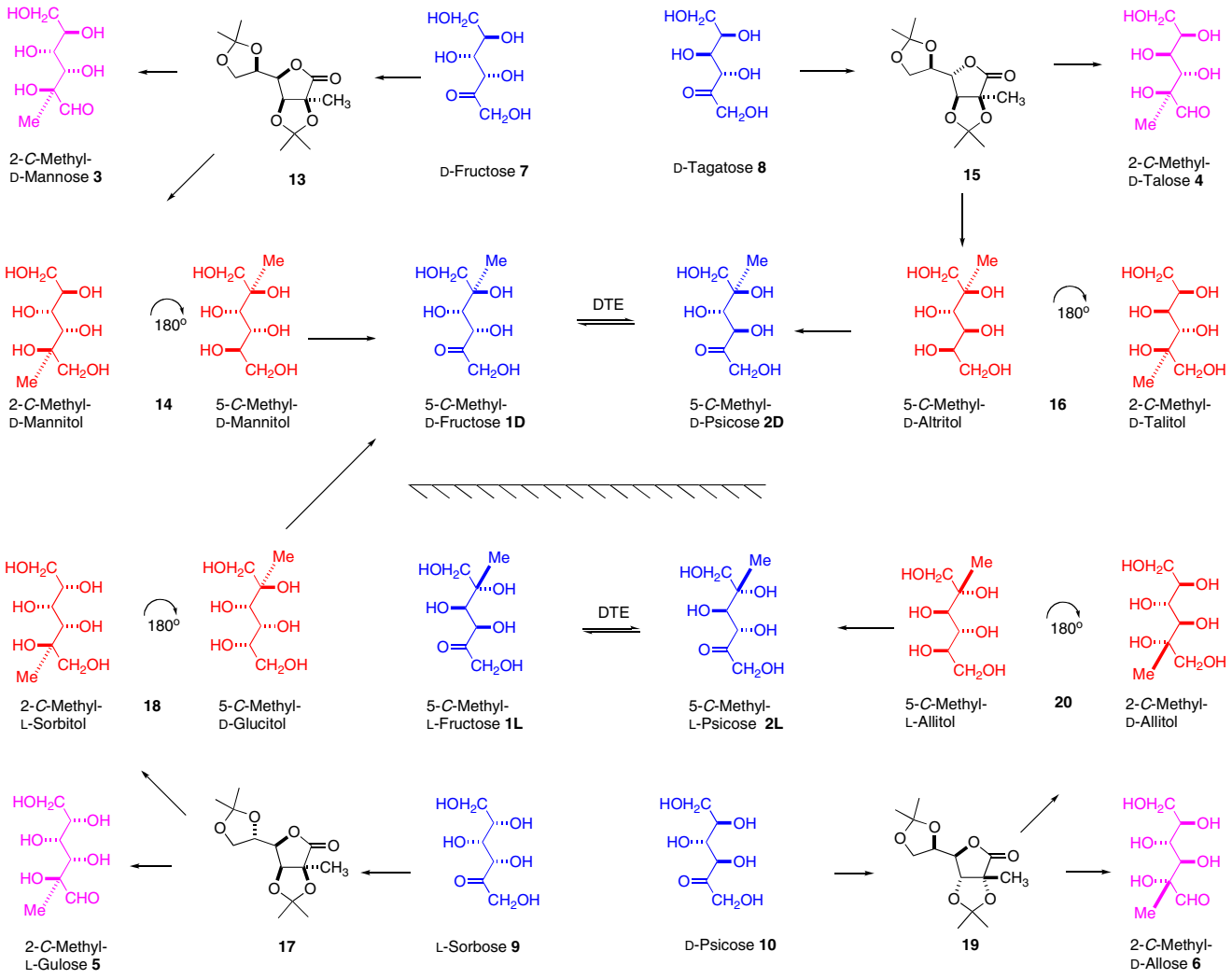
2. Conversion of ketohexoses to 2-*C*-methyl-aldohexoses and 5-*C*-methyl-ketohexoses

2.1. D-Fructose 7 to 2-*C*-methyl-D-mannose 3 and 2-*C*-methyl-D-mannitol (5-*C*-methyl-D-mannitol) 14

Reaction of D-fructose **7** with sodium cyanide in water, followed by treatment of the crude product with acetone and sulfuric acid afforded the protected lactone **12** in 51% yield, as previously



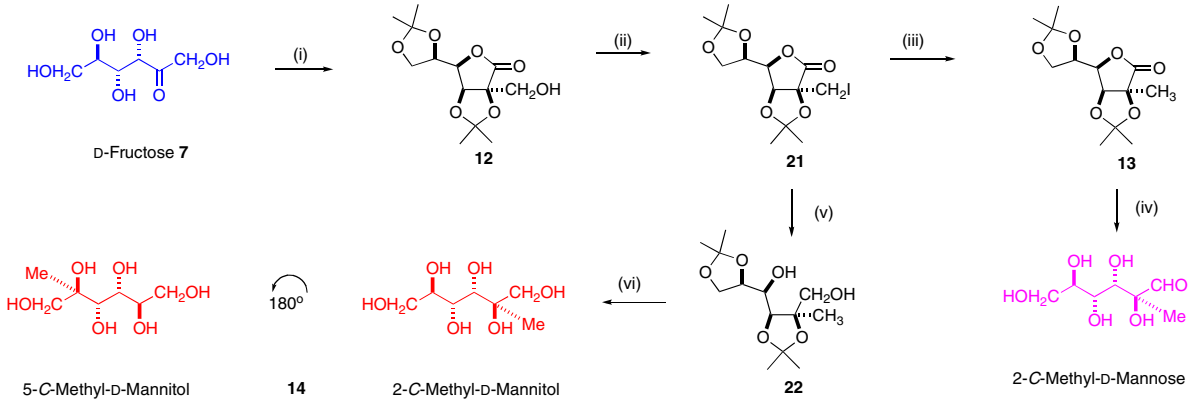
Scheme 2. (i) Kiliani reaction; (ii) acetonation; (iii) conversion of CH_2OH to CH_3; (iv) reduction to lactol and deprotection; (v) reduction to diol and deprotection; (vi) microbial oxidation; (vii) DTE equilibration.



Scheme 3.

described,[14] which on reaction with triphenylphosphine and iodine in toluene in the presence of imidazole underwent an efficient Appel reaction[20] to form the iodide **21** in 85% yield (Scheme 4).

Reduction of both the iodide and lactone functionalities in **21** by lithium aluminum hydride afforded diacetonide **22** (65% yield). Removal of the isopropylidene-protecting groups by treatment with Dowex resin in aqueous dioxane gave 2-*C*-methyl-D-mannitol **14** (90% yield), related by a 180° rotation to 5-*C*-methyl-D-mannitol, as a substrate for microbial oxidation to 5-*C*-methyl ketoses. The hydrogenation of **21** to the methyl lactone **13** (87% yield), followed by diisobutylaluminum (DIBALH) reduction and deprotection to 2-*C*-methyl-D-mannose **3** have been reported previously.[4]



Scheme 4. Reagents and conditions: (i) NaCN, H_2O; then Me_2CO, H_2SO_4, 51%; (ii) Ph_3P, I_2, imidazole, PhMe, 85%; (iii) H_2, Pd/C, Et_3N, EtOH, 87%; (iv) DIBAL-H, CH_2Cl_2 then Dowex® 50WX8-100 (H^+), dioxane/H_2O, 83% over 2 steps; (v) $LiAlH_4$, THF, 65%; (vi) Dowex® 50WX8-100 (H^+), dioxane/H_2O, 90%.

2.2. D-Tagatose 8 to 2-*C*-methyl-D-talose 4 and 2-*C*-methyl-D-talitol (5-*C*-methyl-D-altritol) 16

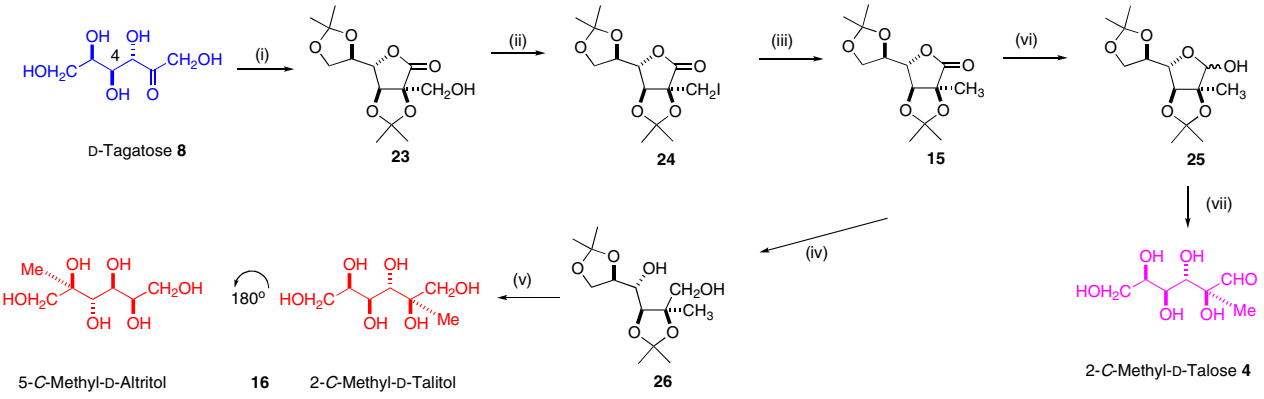
D-Tagatose **8** on successive treatment with sodium cyanide and acetonation of the crude reaction mixture gave the readily crystallized acetonide **23** as the major product in 51% yield.[16] Reaction of **23** with triphenylphosphine, iodine, and imidazole in toluene afforded the iodide **24** in 76% yield. Hydrogenation of **24** in the presence of palladium on carbon gave the branched methyl lactone **15** (99% yield). Reduction of the lactone **15** with lithium borohydride in THF formed the protected diol **26** (99% yield), which on deprotection with ion exchange resin produced 2-*C*-methyl-D-talitol **16** (100% yield)—equivalent to 2-*C*-methyl-D-altritol. Alternatively, DIBALH reduction of the lactone **15** yielded the corresponding lactols **25** (94% yield), which with ion exchange resin gave the unprotected 2-*C*-methyl-D-talose **4** (93% yield) (Scheme 5).

2.3. L-Sorbose 9 to 2-*C*-methyl-L-gulose 5 and 2-*C*-methyl-L-gulitol (5-*C*-methyl-D-glucitol) 18

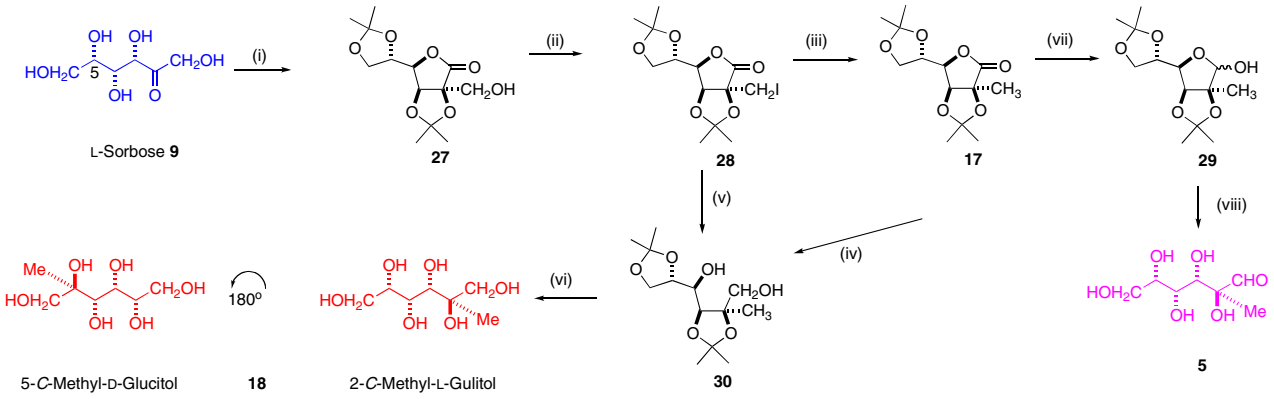
The Kiliani reaction on L-sorbose **9** proceeds very efficiently, but it has proved relatively difficult to crystallize out the major diacetonide **27** formed in 17% overall yield by acetonation of the crude reaction mixture;[14] in spite of this, because of the low cost of **9**, it is possible to isolate large amounts of **28** as a synthetic intermediate. Conversion to the iodide **28** with triphenylphosphine, imidazole, and iodine in toluene (89% yield) allowed subsequent reduction by hydrogenation in the presence of palladium to the branched methyl lactone **17** (98% yield) or by lithium aluminum hydride to the protected diol **30** (72% yield). The methyl lactone **17** could also be reduced to the diol **30** by sodium borohydride (70% yield). Deprotection of lactols **29** (obtained in 89% yield by DIBALH reduction of **17**) by acid ion-exchange resin afforded 2-*C*-methyl-L-gulose **5** in quantitative yield; removal of the acetonides in **30** by aqueous trifluoroacetic acid gave 2-*C*-methyl-L-gulitol **18** (93% yield), related by 180° rotation to 5-*C*-methyl-D-glucitol (Scheme 6).

2.4. D-Psicose 10 to 2-*C*-methyl-D-allose 6 and 2-*C*-methyl-D-allitol (5-*C*-methyl-L-allitol) 20

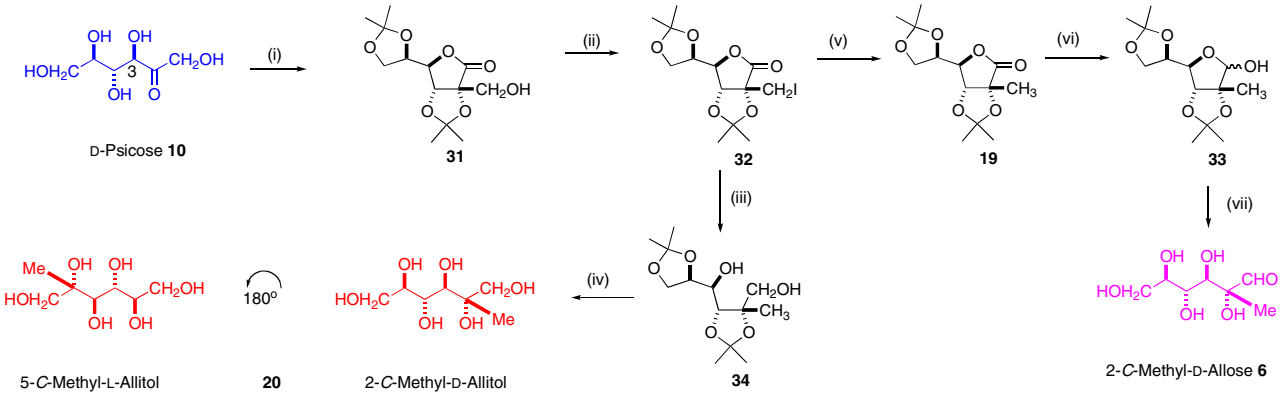
D-Psicose **10** synthesis by the Kiliani-acetonation sequence allows the easy isolation of the crystalline thermodynamic diacetonide **31** in an overall yield of 41%; a higher yield can be isolated by chromatography.[16] Appel conversion of the hindered alcohol **31** (89% yield) to the iodide **32** allowed reduction to either the methyl lactone **19** (98% yield) by catalytic hydrogenation or to the methyl-branched diol **34** (97% yield) by lithium aluminum hydride; subsequent DIBALH reduction of **19** afforded the lactols **33** in quantitative yield. Finally, removal of the acetonide-protecting groups from the lactols **33** by acid ion-exchange resin afforded 2-*C*-methyl-D-allose **6** in quantitative yield, and from **34** by acid ion exchange resin gave 2-*C*-methyl-D-allitol **18** (93% yield), related by 180° rotation to 5-*C*-methyl-L-allitol (Scheme 7).



Scheme 5. Reagents and conditions: (i) NaCN, H_2O; then Me_2CO, H_2SO_4, 51%; (ii) Ph_3P, I_2, imidazole, PhMe, 76%; (iii) H_2, Pd/C, Et_3N, EtOH, 99%; (iv) $LiBH_4$, THF, 99%; (v) Dowex® 50WX8-100 (H^+), dioxane/H_2O, 100%; (vi) DIBAL-H, PhMe, 94%; (vii) Dowex® 50WX8-100 (H^+), dioxane/H_2O, 93%.



Scheme 6. Reagents and conditions: (i) NaCN, H_2O; then Me_2CO, H_2SO_4, 17%; (ii) Ph_3P, I_2, imidazole, PhMe, 89%; (iii) H_2, Pd/C, Et_3N, dioxane, 98%; (iv) $NaBH_4$, EtOH, 70%; (v) $LiAlH_4$, THF, 72%; (vi) CF_3COOH/H_2O, dioxane/H_2O, 100%; (vii) DIBALH, PhMe, 89%; (viii) Dowex® 50WX8-100 (H^+), dioxane/H_2O, 93%.



Scheme 7. Reagents and conditions: (i) NaCN, H_2O; then Me_2CO, H_2SO_4, 41%; (ii) Ph_3P, I_2, imidazole, PhMe, 89%; (iii) $LiAlH_4$, THF, 97%; (iv) Dowex® 50WX8-100 (H^+), dioxane/H_2O, 100%; (v) H_2, Pd/C, Et_3N, dioxane, 98%; (vi) DIBALH, PhMe, 100%; (vii) Dowex® 50WX8-100 (H^+), dioxane/H_2O, 100%.

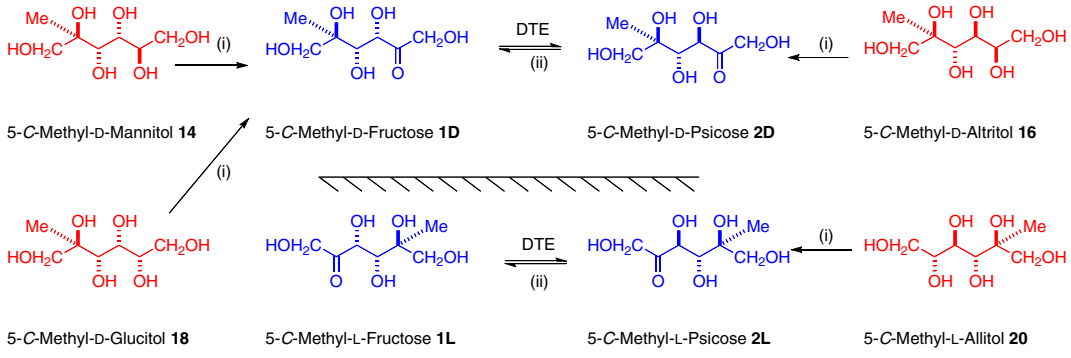
3. Microbial oxidation of 2-*C*-methyl-alditols to 5-*C*-methyl-ketohexoses; D-tagatose-3-epimerase (DTE)-catalyzed equilibration of 5-*C*-methyl-D-fructose 1D with 5-*C*-methyl-D-psicose 2D, and of 5-*C*-methyl-L-fructose 1L with 5-*C*-methyl-L-psicose 2L

The microbial oxidations (Scheme 8) of the 5-*C*-methyl-branched polyols were studied. Both 5-*C*-methyl-D-mannitol **14** and 5-*C*-methyl-D-glucitol **18** were oxidized in high yield to 5-*C*-methyl-D-fructose **1D**. 5-*C*-Methyl-D-altritol **16** was oxidized to 5-*C*-methyl-D-psicose **2D**, whereas 5-*C*-methyl-L-allitol **20** gave the enantiomer 5-*C*-methyl-L-psicose **2L**. Both enantiomers of 5-*C*-methyl-fructose **1** were equilibrated with both enantiomers of 5-*C*-methyl-psicose **2** by D-tagatose-3-epimerase (DTE).

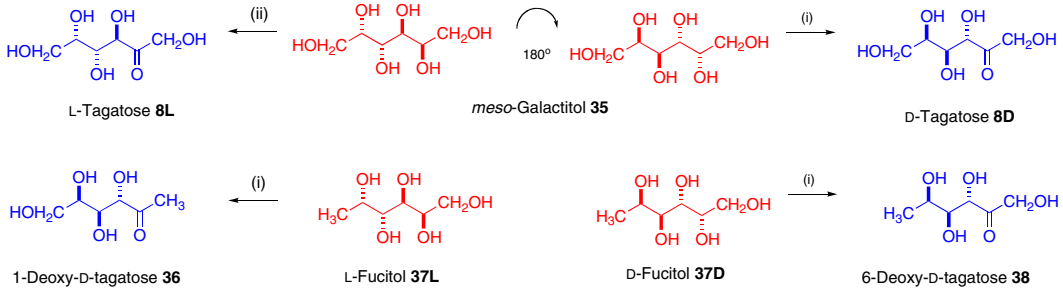
3.1. Microbial oxidation of 5-*C*-methyl-D-altritol 16 to 5-*C*-methyl-D-psicose 2D and 5-*C*-methyl-L-allitol 20 to 5-*C*-methyl-L-psicose 2L

Microbial oxidations of polyols are highly specific and highly efficient.[9,21] For example (Scheme 9), *Enterobacter agglomerans* 221e oxidizes achiral galactitol **35** stereospecifically to D-tagatose **8D**, whereas *Klebsiella pneumoniae* 40b stereospecifically affords L-tagatose **8L**.[9] *E. agglomerans* 221e recognizes the D-*galacto*—rather than the L-*galacto*—structural motif, and thus oxidizes L-fucitol **37L** to 1-deoxy-D-tagatose **36**, but transforms the enantiomeric D-fucitol **37D** to 6-deoxy-D-tagatose **38**.[12]

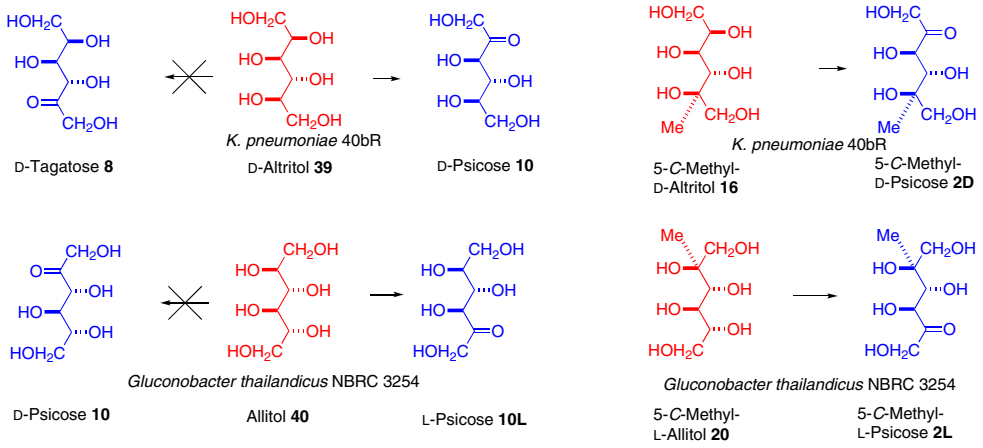
K. pneumoniae 40bR, a newly isolated mutant of *K. pneumoniae* 40b, efficiently oxidizes D-altritol **39** to D-psicose **10** with no



Scheme 8. (i) Polyol dehydrogenases (see Section 3); (ii) D-tagatose-3-epimerase.



Scheme 9. (i) *Enterobacter agglomerans* 221e and (ii) *Klebsiella pneumoniae* 40b.



Scheme 10. Microbial oxidation to both enantiomers of 5-*C*-methyl-D-psicose **2**.

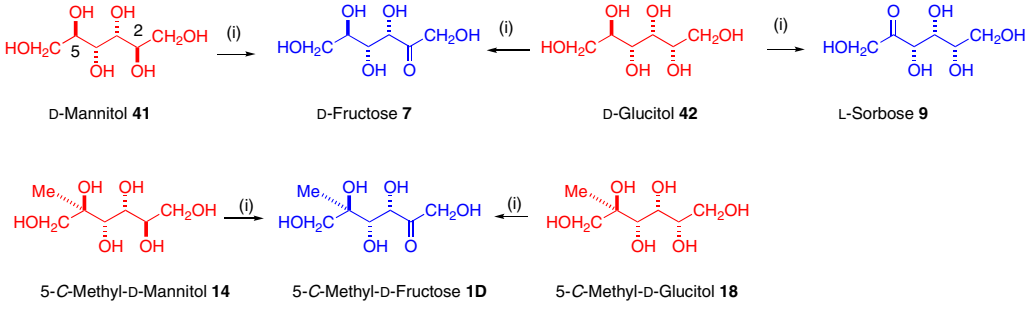
oxidation to D-tagatose **8D** (Scheme 10); similarly, the branched hexitol 5-*C*-methyl-D-altritol **16** was oxidized at C-2 by using resting cells of *K. pneumoniae* 40bR to afford 5-*C*-methyl-D-psicose **2D** {oil, $[\alpha]_D^{20} = -25.2$ (*c* 1.0, water)} in 65% yield.

Gluconobacter thailandicus NBRC 3254 has been shown to oxidize allitol **40** to L-psicose **10L** with no oxidation to the enantiomer D-psicose **10**;[9] similarly, the branched hexitol 5-*C*-methyl-L-allitol **20** was oxidized at C-2 by using the resting cell of *G. thailandicus* NBRC 3254 to afford 5-*C*-methyl-L-psciose **2L** {oil, $[\alpha]_D^{20} = +24.5$ (*c* 1.0, water)} in 70% yield.

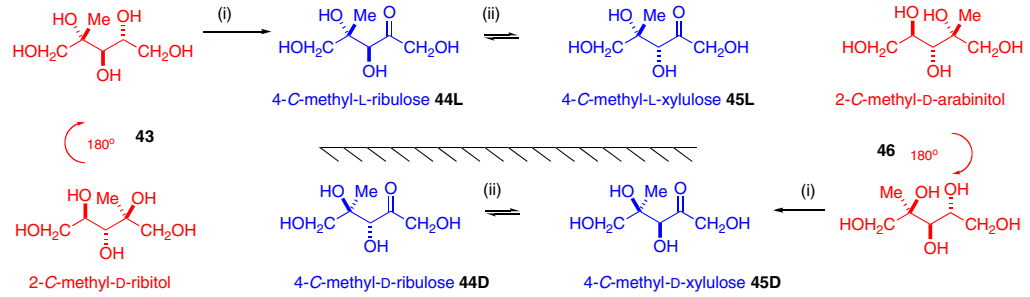
3.2. Microbial oxidation of 5-*C*-methyl-D-mannitol 5 and 5-*C*-methyl-D-glucitol 17 to 5-*C*-methyl-D-fructose 1D

G. thailandicus NBRC 3254 also oxidizes C-2 or C-5 of C_2 symmetric D-mannitol **41** to D-fructose **7**;[9] similarly, the branched hexitol 5-*C*-methyl-D-mannitol **14** was oxidized at C-2 by using the resting cell of *G. thailandicus* NBRC 3254 to afford 5-*C*-methyl-D-fructose **1D** {oil, $[\alpha]_D^{20} = -85.2$ (*c* 1.0, water)} in 70% yield. *G. thailandicus* NBRC 3254 also oxidizes D-glucitol **42** at C-2 and C-5 to produce D-fructose **1D** and L-sorbose **9**, respectively. Similarly, 5-*C*-methyl-D-glucitol **18** was oxidized at C-2 to 5-*C*-methyl-D-fructose **1D** with no oxidation to the enantiomer probably due to structural difference of 5-*C*-methyl-D-glucitol with D-glucitol at C-5. The branched hexitol 5-*C*-methyl-D-glucitol **18** was oxidized at C-2 by using the resting cell of *G. thailandicus* NBRC 3254 *to* afford 5-*C*-methyl-D-fructose **1D** {oil, $[\alpha]_{20}^{D} = -84.6$ (*c* 1.0, water)} in 70% yield (see Scheme 11).

Recently, *G. thailandicus* NBRC 3254 has been demonstrated to oxidize branched 4-*C*-methyl pentitols to 4-*C*-methyl pentuloses; thus 2-*C*-methyl-D-ribitol **43** can be oxidized to 4-*C*-methyl-L-ribulose **44L** and 2-*C*-methyl-D-arabinitol **46** to 4-*C*-methyl-D-xylulose **45D** (Scheme 12).[13] 5-*C*-Methyl-branched carbohydrates are thus also substrates handled by microbes in specific oxidations.



Scheme 11. (i) *Gluconobacter thailandicus* NBRC 3254.



Scheme 12. (i) *Gluconobacter thailandicus* NBRC 3254; (ii) DTE.

It appears that specific transformations of synthetic branched and deoxy monosaccharides may be achieved efficiently by microbial oxidation; this allows access to a wide range of new sugars without the need for any protection or chemical manipulation.

3.3. Equilibration of both enantiomers of 5-*C*-methyl-psicose 2 with 5-*C*-methyl-fructose 1 by D-tagatose-3-epimerase (DTE)

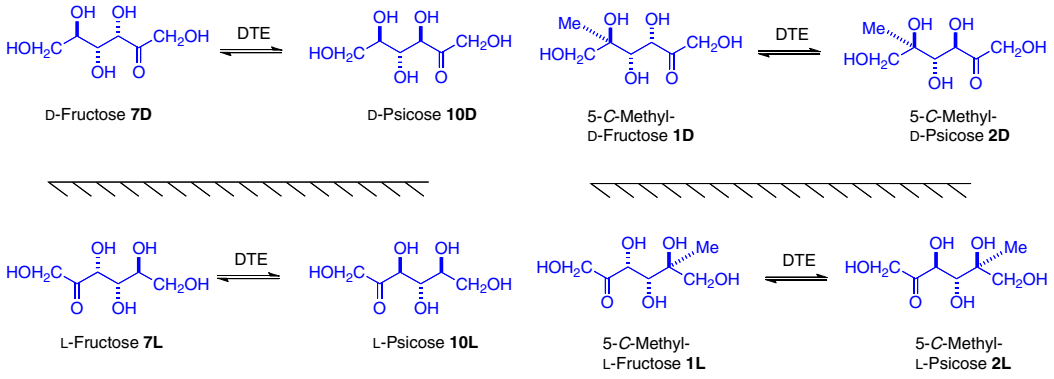
DTE epimerizes the C-3 position of many ketoses and, in particular, equilibrates D-fructose **7D** with D-psicose **10D** (Scheme 13).[10] Epimerization of 5-*C*-methyl-D-psicose **2D** by DTE gave an equilibrium mixture of **2D** and 5-*C*-methyl-D-fructose **1D** in a ratio of 20:80; this allowed the isolation of 5-*C*-methyl-D-fructose **1D** {oil, $[\alpha]_D^{20} = -85.8$ (*c* 1.0, water)} in 30% yield. DTE also epimerizes L-psicose **10L** at C-3 position to produce L-fructose **7L**.[10] Reaction of 5-*C*-methyl-L-psicose **2L** with DTE afforded a mixture of the two epimers **2L** and **1L** in a ratio of 20:80, allowing the isolation of 5-*C*-methyl-L-fructose **1L** {oil, $[\alpha]_D^{20} = +83.2$ (*c* 1.0, water)} in 30% yield.

Thus, as well as equilibration of all the pairs of ketohexoses and ketopentose, DTE can also cause epimerization at C-3 of 5-*C*-methyl-branched ketohexoses; the equilibration of 4-*C*-methyl-L-ribulose **44L** with 4-*C*-methyl-L-xylulose **45L** and of 4-*C*-methyl-D-xylulose **45D** with 4-*C*-methyl-D-ribulose **44D** has been described (Scheme 12).[13] Substrate limitation for C-3-epimerization by DTE has yet to be determined.

4. NMR studies on 5-*C*-methyl-fructose 1 and 5-*C*-methyl-psicose 2

NMR analyses were carried out on 5-*C*-methyl-D-fructose **1D** and 5-*C*-methyl-D-psicose **2D** in ^2H_2O solution, pD 7.1, at a temperature of 30 °C. In both cases, the corresponding L-enantiomers had identical spectra with slightly higher levels of impurities. The 1D ^1H spectra of **1D** and **2D** are shown in Figure 1, and the ^1H and ^{13}C assignments are given in Tables 1–3. The pyranose forms could be identified by the HMBC peak between C2 and H6/H6′. There are no characteristic HMBC peaks in the furanose forms, and so these could only be identified by elimination and by the high chemical shifts of the H3 and H4 protons (>4 ppm). There was no evidence of the open chain *keto*-form within the detection limit in either sample (<2%).

5-*C*-Methyl-D-fructose **1D** showed two major components in the ^1H spectrum, with a ratio of intensities 90:10. The major component is in the pyranose form, while the minor component is in the furanose form. For the major pyranose component, there is a



Scheme 13. DTE equilibrations.

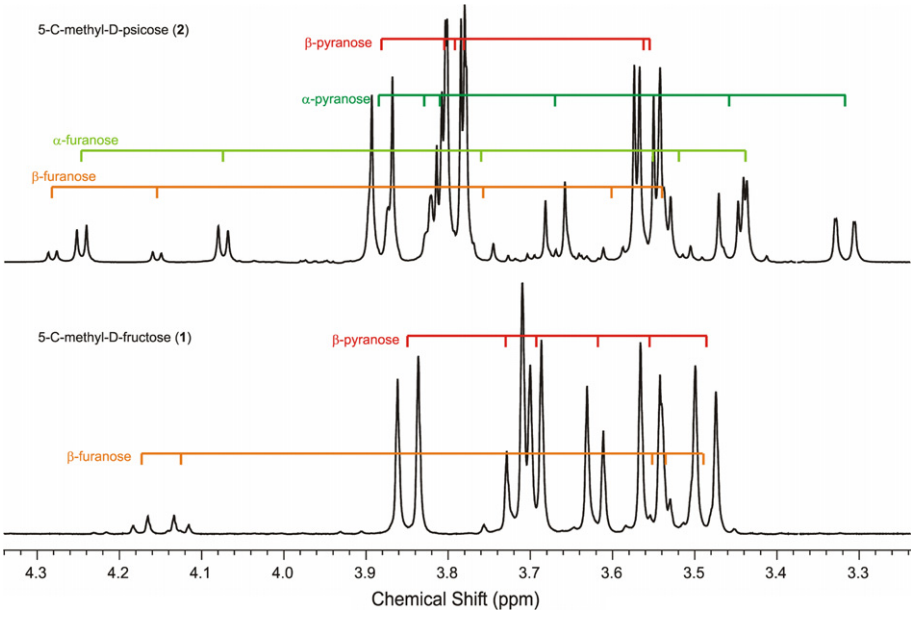


Figure 1. 1D ^1H NMR spectra of **1** and **2**, showing the spin-systems for the α-pyranose, β-pyranose, α-furanose and β-furanose.

Table 1
^{13}C chemical shifts of 5-*C*-methyl-ketoses (referenced to acetone at 30.90 ppm)

	%age	^{13}C chemical shift (ppm)						
		C1	C2	C3	C4	C5	C6	C5C
5-C-Methyl-D-fructose **1**								
β-Pyranose	90	64.66	98.77	69.07	74.00	72.84	67.89	21.13
β-Furanose	10	63.20	100.50	75.06	76.21	82.81	67.60	18.67
5-C-Methyl-D-psicose **2**								
β-Pyranose	55	64.71	99.36	70.95	68.83	72.98	68.77	20.78
α-Pyranose	23	63.75	98.05	65.59	75.95	70.18	63.49	22.36
α-Furanose	17	63.6[a]	103.57	71.29	73.13	86.35	67.35	19.57
β-Furanose	5	63.46	104.86[a]	77.01	73.13	86.35	68.35[a]	19.07

Percentages were estimated from the peak area in the ^{1}H 1D spectrum.
[a] Can only be resolved in the HMBC spectrum.

Table 2
^{1}H chemical shifts of 5-*C*-methyl-ketoses (referenced to acetone at 2.220 ppm)

	%age	^{1}H chemical shift (ppm)						
		H1	H1′	H3	H4	H6	H6'	C5CH$_3$
5-C-Methyl-D-fructose **1**								
β-Pyranose	90	3.697	3.553	3.718	3.620	3.848	3.486	1.178
β-Furanose	10	3.541	3.491	4.124	4.173	3.538	3.538	1.133
5-C-Methyl-D-psicose **2**								
β-Pyranose	55	3.792	3.561	3.805	3.781	3.880	3.554	1.166
α-Pyranose	23	3.670	3.458	3.81	3.83	3.885	3.318	1.288
α-Furanose	17	3.55[a]	3.52[a]	4.246	4.074	3.44	3.44	1.283
β-Furanose	5	3.756	3.600	4.154	4.281	3.54[a]	3.54[a]	1.206

Percentages were estimated from peak area in the ^{1}H 1D spectrum.
[a] Can only be resolved in the HMBC spectrum.

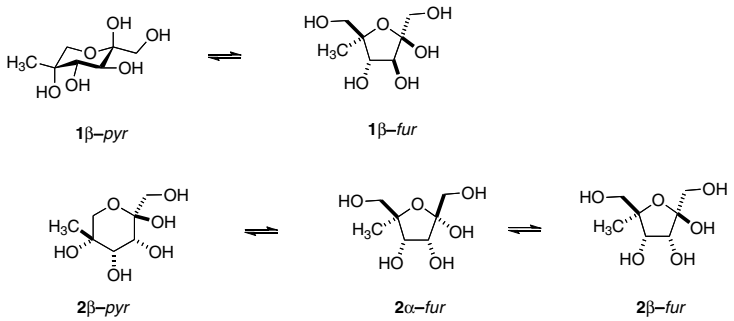
Table 3
Two and three-bond J_{HH} values of 5-*C*-methyl-ketoses

	%age	J_{HH} (Hz)		
		H1–H1′	H3–H4	H6–H6′
5-C-Methyl-D-fructose **1**				
β-Pyranose	90	−11.8	9.8	−12.6
β-Furanose	10	−12.1	8.9	nd
5-C-Methyl-D-psicose **2**				
β-Pyranose	55	−11.7	3.3	−12.5
α-Pyranose	23	−11.8	nd	−11.3
α-Furanose	17	nd	5.9	nd
β-Furanose	5	−11.8	5.2	nd

Percentages were estimated from the peak area in the ^{1}H 1D spectrum.

strong NOE from the C5CH$_3$ group to H4 and a much weaker one to H3, consistent with a *cis* arrangement of the OH groups at C4 and C5 in the six-membered ring. The large *J*-coupling between H3 and H4 indicates that these two protons are *trans* di-axial with the ring in the $^{2}C_5$ conformation. The α-anomer would be expected to show an NOE between H1/H1′ and H4, which was not observed; a tentative assignment may thus be made as the β-anomer. For the minor furanose component, there is a strong NOE from the C5CH$_3$ group to H3 and a much weaker one to H4, again consistent with the same relative stereochemistry for these two centers. There is also a weak NOE between H1/H1′ and H3, suggesting the β-anomer. Neither the α-pyranose nor α-furanose forms are seen within the detection limit (<2%). In comparison, fructose exists in aqueous solution as a 60:30:10 mixture of the β-pyranose/β-furanose/α-furanose, with only trace α-pyranose,[22] and 1-deoxy-fructose exists as a 69:14:7:3:7 mixture of β-pyranose/β-furanose/α-furanose/α-pyranose/open chain.[23] Thus, the presence of the 5-*C*-methyl group appears to stabilize the β-pyranose form relative to all other forms.

5-*C*-Methyl-D-psicose **2D** showed four major components in the ^{1}H spectrum, with a ratio of intensities 55:23:17:5. The two major components are in the pyranose form, while the two minor components are in the furanose form. For the minor, furanose, components, both give an NOE between H6/H6′ and H3, and neither give an NOE from C5CH$_3$ to either H3 or H4, consistent with C3OH, C4OH, and C5CH$_3$, all being cis in the five-membered ring. The larger of these two components (17%) also shows an NOE between H3 and H1/H1′, indicating that this is the α-anomer, and thus the smaller (5%) is the β-anomer. For the major component, pyranose, components, there are no useful NOEs to determine the anomericity. Comparing the pattern of C2, C3, and C4 ^{13}C chemical shifts with those of 1-deoxy-psicose,[23] particularly for C3 and C4, it is tentatively suggested that the larger of the two components is the β-anomer. Psicose exists in aqueous solution as an approximately 33:25:25:15:2 mixture of β-pyranose/α-pyranose/α-furanose/β-furanose/open chain;[24] 1-deoxy-psicose exists as a 27:25:21:20:7 mixture of β-pyranose/α-pyranose/α-furanose/β-furanose/open chain.[23] Thus, again the presence of the 5-*C*-methyl group appears to stabilize the β-pyranose form relative to all other forms.



5. Conclusion

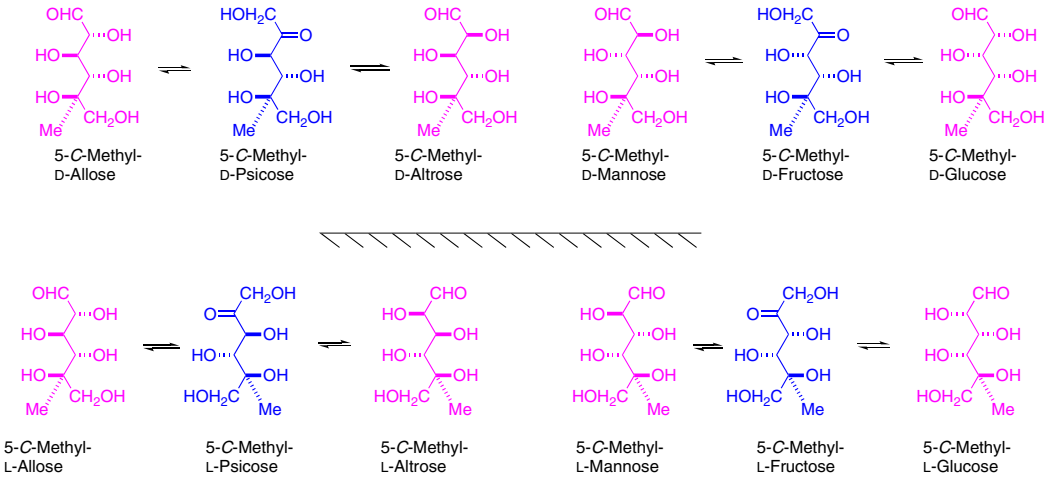
In conclusion, we have reported the efficient chemical and biotechnological syntheses of 2-*C*-methyl- and 5-*C*-methyl-hexoses for biological evaluation. Conversion of 5-*C*-methyl-ketohexoses to 5-*C*-methyl-aldoses by aldose isomerases (Scheme 14) will further extend this family of new sugars for investigation of their biological activity. These transformations demonstrate that polyol dehydrogenases and DTE act on branched synthetic sugars. Full NMR analyses show that 5-*C*-methyl-D-fructose is present as the β-pyranose and β-furanose forms in a ratio of 90:10; all pyranose and furanose forms of 5-*C*-methyl-D-psicose are present in solution.

Ketoses can also be equilibrated by aldose isomerases to the corresponding aldose.[25] Thus, as well as the four 2-*C*-methyl aldoses reported in this paper, the 5-*C*-methyl-ketoses **1** and **2** should allow the synthesis of eight 5-C-methyl hexoses (Scheme 14). The combination of chemical and biological procedures allows the environmentally friendly generation of a new family of branched monosaccharides and it may be that the microbes and DTE will accommodate a wider range of substrates allowing the synthesis of a new generation of sugars under environmentally friendly conditions.

We have also reported the synthesis of novel monosaccharides by a combination of both chemical and biochemical methods; it precisely illustrates that chemistry and biotechnology are complementary in that the synthesis of 2-*C*-methyl sugars is easier by chemical methods, but that 5-*C*-methyl monosaccharides are most easily accessed by biochemical routes. Particularly noteworthy is the ability of a single enzyme DTE to accept so many unnatural substrates for the equilibration of the C-3 epimers of ketoses. Unnatural amino acids have long been the targets of chemists for incorporation into peptides for their ability to modify secondary structures and for the bioactive properties of simple analogues of natural products. Nature uses many rare mono- and di-*C*-methyl-branched sugars as conjugates in highly active chemotherapeutic agents; a recent example is the four sugars conjugated to the macrocycle of apoptolidin,[26] (one has a mono *C*-methyl-branched and two have di-*C*-methyl-branched monosaccharides). Ready access to new monosaccharides—including those with carbon branches may well provide useful building blocks for incorporation into analogues of bioactive materials as well having chemotherapeutic potential in the own right.

6. Experimental

Tetrahydrofuran was purchased dry from the Aldrich chemical company in sure-seal bottles. Pyridine was purchased dry from the Fluka chemical company in sure-seal bottles over molecular sieves. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reactions were performed under an atmosphere of nitrogen or argon, unless stated otherwise. Thin layer chromatography (TLC) was performed on aluminum sheets coated with 60 F_{254} Silica. Sheets were visualized using a spray of 0.2% w/v cerium (IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid. Flash chromatography was performed on Sorbsil C60 40/60 silica. Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g 100 mL^{-1}. Infrared spectra were recorded on a Perkin–Elmer 1750 IR Fourier Transform spectrophotometer using thin films on NaCl plates (thin film). Only the characteristic peaks are quoted. Low resolution mass spectra (m/z) were recorded on VG MassLab 20–250, Micromass BIOQ-II, Micromass Platform 1, Micromass TofSpec 2E, or Micromass Autospec 500 OAT spectrometers and high resolution mass spectra (HRMS m/z) on a Micromass Autospec 500 OAT spectrometer. The technique used was electrospray ionization (ESI). Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AMX 500 (^{1}H: 500 MHz and ^{13}C: 125.7 MHz) and Bruker DPX 400 and DQX 400 spectrometers (^{1}H: 400 MHz and ^{13}C: 100.6 MHz) in the deuterated solvent. For spectra recorded in D_2O, methanol was used as an internal reference. Residual signals from other solvents were used as an internal reference. All chemicals were purchased from Sigma Chemical Co. (MO, USA) and Wako Pure Chemicals (Osaka, Japan). Microbe growth and oxidation reactions were carried out in Erlenmeyer flasks. Polyol oxidation and ketose accumulation in the reaction mixture was determined by colorimetric method by using visible spectrophotometer (U.V-1700 pharmaspec, Shimadzu, Kyoto). ^{13}C NMR spectra (Bruker AMX 500, 126 MHz) were recorded in D_2O using acetone as internal standard. Optical rotations were recorded on a Jasco R1030 polarimeter, Na^+ lamp, (Jasco, Tokyo, Japan) at 20 °C in deionized H_2O polarimeter with a path length of 1 dm. Concentrations are quoted in g 100 mL^{-1}. The product was analyzed by high-performance liquid chromatography (Hitachi GL-611 column, Tokyo, Japan and Shimadzu RID-6A refractive index detector, Kyoto, Japan) at 60 °C, eluted with 10^{-4} M NaOH at a flow rate of 1.0 mL/min. NMR spectra for **1D** and **2D** were recorded on a



Scheme 14. 5-*C*-Methyl aldoses by aldose isomerases on ketoses.

Varian UnityINOVA 500 (^{1}H—500 MHz: ^{13}C—125 MHz) spectrometer, in ^2H_2O, pD 7.1 ± 0.1, with a probe temperature of 30 °C. Chemical shifts were measured relative to internal standards (^{1}H—acetone at 2.220 ppm: ^{13}C—acetone at 30.9 ppm). Two-dimensional gradient COSY, HSQC, HMBC, and HSQC-TOCSY spectra were used to aid assignment of ^{1}H and ^{13}C spectra. NOESY spectra were recorded with a 500 ms mixing time. All chemical shifts (δ) are quoted in ppm and coupling constants (J) in hertz.

7. Chemical syntheses

7.1. From D-fructose 7

7.1.1. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-iodomethyl-D-mannono-1,4-lactone 21

Imidazole (630 mg, 9.24 mmol), triphenylphosphine (1.75 g, 6.67 mmol), and iodine (1.69 g, 6.67 mmol) were added to a stirred solution of diacetonide **12** (740 mg, 2.57 mmol) in toluene (12 mL) and stirred at 85 °C for 1.5 h. The reaction mixture was allowed to cool to room temperature, concentrated in vacuo and then partitioned between CH_2Cl_2 (30 mL) and saturated sodium hydrogen carbonate solution (30 mL), and the resulting solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with water (2 × 30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (cyclohexane/EtOAc 6:1→3:1) to afford the iodide **21**, a colorless oil, (863 mg, 85%); $[\alpha]_D^{22} = +31.8$ (*c* 1.09, $CHCl_3$); ν_{max} (NaCl): 1791 cm^{-1} (CO); δ_H (400 MHz, $CDCl_3$): 1.39 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.48 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.38 (1H, d, $J_{2'a,2'b}$ = 10.4 Hz, H-2′a), 3.50 (1H, d, $J_{2'a,2'b}$ = 10.4 Hz, H-2′b), 4.07–4.17 (2H, m, H-6a, H-6b), 4.43–4.49 (2H, m, H-4, H-5), 4.07 (1H, d, $J_{3,4}$ = 3.2 Hz, H-3); δ_C (100 MHz, $CDCl_3$): 0.4 (–*C*H$_2$I), 24.8 (CH_3), 26.3 (CH_3), 26.6 (2 × CH_3), 65.9 (C-6), 72.1, 78.3, 80.4 (C-3, C-4, C-5), 84.7 (C-2), 109.5 (*C*Me$_2$), 114.1 (*C*Me$_2$), 172.1 (C-1); HR ESI-MS: found *m*/*z* 421.0123 [M+Na]$^+$, calcd for $C_{13}H_{19}IO_6Na$ 421.0119.

7.1.2. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-D-mannitol 22

Lithium aluminum hydride (2.0 M in THF, 2.16 mL, 4.32 mmol) was added to a stirred solution of the iodide **21** (860 mg, 2.16 mmol) in THF (10 mL) at 0 °C. After stirring for 16 h at room temperature, methanol (10 mL) was slowly added followed by saturated potassium sodium tartrate solution (20 mL) and the reaction mixture was stirred for 1 h. The reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL), the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (cyclohexane/EtOAc 2:1→0:1) to afford compound **22**, a colorless oil (390 mg, 65%); $[\alpha]_D^{22} = -2.8$ (*c* 1.02, $CHCl_3$); ν_{max} (NaCl): 3384 cm^{-1} (OH); δ_H (400 MHz, $CDCl_3$): 1.31 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.41 (6H, s, 2 × CH_3), 1.48 (3H, s, CH_3), 3.45 (1H, d, $J_{1a,1b}$ = 11.8 Hz, H-1a), 3.62 (1H, d, $J_{1a,1b}$ = 11.8 Hz, H-1b), 3.68–3.69 (1H, m, H-5), 4.02 (1H, s, H-3), 4.07–4.10 (3H, H-4, H-6a, H-6b); δ_C (100 MHz, $CDCl_3$): 22.1 (CH_3), 25.3 (CH_3), 26.7 (CH_3), 26.9 (CH_3), 27.9 (CH_3), 65.6 (C-1), 67.0 (C-6), 68.7 (C-5), 76.4 (C-4), 80.8 (C-3), 81.7 (C-2), 107.9 (*C*Me$_2$), 109.5 (*C*Me$_2$); HR ESI-MS: found *m*/*z* 299.1465 [M+Na]$^+$, calcd for $C_{13}H_{24}O_6Na$ 299.1465.

7.1.3. 2-*C*-Methyl-D-mannitol 14

A mixture of the protected diol **22** (420 mg, 1.52 mmol) and Dowex® 50WX8-100 (H^+) ion-exchange resin (400 mg) in dioxane/water (1:1, 5 mL) was stirred for 16 h at 45 °C, filtered, and concentrated in vacuo, affording free branched methyl polyol **14** as a white solid (270 mg, 90%); mp 126–128 °C; $[\alpha]_D^{21} = -8.0$ (*c* 1.02, MeOH); δ_H (400 MHz, D_2O): 1.07 (3H, s, CH_3), 3.44 (1H, d, $J_{1a,1b}$ = 11.7 Hz, H-1a), 3.49 (1H, d, $J_{1a,1b}$ = 11.7 Hz, H-1b), 3.50 (1H, dd, $J_{5,6a}$ = 6.3 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6a), 3.55–3.59 (1H, m, H-5), 3.66–3.68 (2H, m, H-3, H-4), 3.70 (1H, dd, $J_{5,6b}$ = 2.5 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6b); δ_C (100 MHz, D_2O): 19.8 (CH_3), 63.2 (C-6), 66.6 (C-1), 69.7, 71.8, 72.0 (C-3, C-4, C-5), 75.3 (C-2); HR ESI-MS: found *m*/*z* 219.0836 [M+Na]$^+$, calcd for $C_7H_{16}O_6Na$ 219.0839.

7.2. From D-tagatose 8

7.2.1. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-iodomethyl-D-talono-1,4-lactone 24

Imidazole (1.70 g, 25.0 mmol), triphenylphosphine (4.70 g, 18.0 mmol), and iodine (4.60 g, 18.0 mmol) were added to a stirred solution of alcohol **23** (2.0 g, 6.94 mmol) in toluene (25 mL) and stirred at 85 °C for 2 h. The reaction mixture was allowed to cool to room temperature, concentrated in vacuo and then partitioned between CH_2Cl_2 (30 mL) and saturated sodium hydrogen carbonate solution (30 mL), and the resulting solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with water (2 × 30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (cyclohexane/EtOAc 6:1→3:1) to afford the iodide **24**, (2.10 g, 76%); mp 90–92 °C; $[\alpha]_D^{22} = -44.5$ (*c* 1.0, $CHCl_3$); ν_{max} (NaCl): 1784 cm^{-1} (CO); δ_H (400 MHz, $CDCl_3$): 1.35 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.68 (1H, d, $J_{2'a,2'b}$ = 11.4 Hz, H-2′a), 3.78 (1H, d, $J_{2'a,2'b}$ = 11.4 Hz, H-2′b), 3.99 (1H, dd, $J_{5,6a}$ = 7.4 Hz, $J_{6a,6b}$ = 8.4 Hz, H-6a), 4.15 (1H, dd, $J_{5,6b}$ = 6.9 Hz, $J_{6a,6b}$ = 8.4 Hz, H-6b), 4.32–4.36 (1H, m, H-5), 4.51 (1H, s, H-4), 4.62 (1H, s, H-3); δ_C (100 MHz, $CDCl_3$): 5.0 (–*C*H$_2$I), 25.5 (CH_3), 26.9 (CH_3), 27.4 (CH_3), 27.5 (CH_3), 65.4 (C-6), 75.2 (C-5), 81.0 (C-4), 83.7 (C-3), 83.9 (C-2), 110.7 (*C*Me$_2$), 114.4 (*C*Me$_2$), 171.62 (C-1); HR ESI-MS: found *m*/*z* 421.0120 [M+Na]$^+$, calcd for $C_{13}H_{19}O_6Na$ 421.0119.

7.2.2. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-D-talono-1,4-lactone 15

Iodolactone **24** (2.1 g, 5.28 mmol) in ethanol (20 mL) was stirred under hydrogen for 2 days in the presence of 10% palladium on carbon (200 mg) and triethylamine (0.9 mL, 6.33 mmol); the reaction mixture was filtered through Celite and the filtrate was evaporated in vacuo. The residue was dissolved in dichloromethane (30 mL), washed with saturated aqueous sodium thiosulfate solution (20 mL), water (2 × 20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (cyclohexane/EtOAc 5:1→2:1) to afford the protected methyl lactone **1**, oil, (1.39 g, 99%); $[\alpha]_D^{22} = +15.1$ (*c* 1.0, $CHCl_3$); ν_{max} (NaCl), 1783 cm^{-1} (CO); δ_H (400 MHz, $CDCl_3$): 1.34 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.68 (3H, s, CH_3), 3.99 (1H, dd, $J_{5,6a}$ = 7.5 Hz, $J_{6a,6b}$ = 8.4 Hz, H-6a), 4.13 (1H, dd, $J_{5,6b}$ = 6.8 Hz, $J_{6a,6b}$ = 8.4 Hz, H-6b), 4.29–4.33 (1H, m, H-5), 4.45 (1H, s, H-4), 4.54 (1H, s, H-3); δ_C (100 MHz, $CDCl_3$): 19.7 (CH_3), 25.5 (CH_3), 25.7 (CH_3), 26.7 (CH_3), 26.8 (CH_3), 65.4 (C-6), 75.4 (C-5), 80.6 (C-4), 82.4 (C-2), 83.1 (C-3), 110.6 (*C*Me$_2$), 112.9 (*C*Me$_2$), 176.1 (C-1); HR ESI-MS: found *m*/*z* 295.1153 [M+Na]$^+$, calcd for $C_{13}H_{20}O_6Na$ 295.1152.

7.2.3. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-D-talitol 26

Lithium borohydride (2.0 M in THF, 2.16 mL, 4.32 mmol) was added to a stirred solution of the lactone **15** (1.39 g, 5.11 mmol) in THF (14 mL) at 0 °C. After stirring for 30 min at 0 °C, the reaction mixture was warmed to room temperature and stirred for a further 16 h. Dowex® 50WX8-100 (H^+) ion-exchange resin was carefully added until the reaction mixture was neutral, the mixture was then filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (cyclohexane/EtOAc 2:1→0:1) to afford the protected diol **26** as a amorphous solid

(1.40 g, 99%); $[\alpha]_D^{21} = +11.1$ (*c* 1.0, $CHCl_3$); ν_{max} (NaCl): 3387 cm^{-1} (OH); δ_H (400 MHz, $CDCl_3$): 1.39 (9H, s, 3 × CH_3), 1.43 (3H, s, CH_3), 1.46 (3H, s, CH_3), 3.54 (1H, d, $J_{1a,1b}$ = 11.1 Hz, H-1a), 3.69 (1H, d, $J_{1a,1b}$ = 11.1 Hz, H-1b), 3.72–3.75 (1H, m, H-4), 3.79 (1H, d, $J_{3,4}$ = 9.4 Hz, H-3), 3.91 (1H, dd, $J_{5,6a}$ = 6.9 Hz, $J_{6a,6b}$ = 8.5 Hz, H-6a), 4.07 (1H, dd, $J_{5,6b}$ = 6.8 Hz, $J_{6a,6b}$ = 8.5 Hz, H-6b), 4.23–4.17 (1H, m, H-5); δ_C (100 MHz, $CDCl_3$): 23.7 (CH_3), 25.2 (CH_3), 26.5 (2 × CH_3), 28.3 (CH_3), 65.2 (C-1), 66.3 (C-6), 69.9 (C-4), 76.8 (C-5), 82.1 (C-3), 82.9 (C-2); HR ESI-MS: found *m*/*z* 299.1465 $[M+Na]^+$, calcd for $C_{13}H_{24}O_6Na$ 299.1465.

7.2.4. 2-*C*-Methyl-D-talitol 16

A mixture of the protected diol **26** (1.40 g, 5.07 mmol) and Dowex® 50WX8-100 (H^+) ion-exchange resin (900 mg) in dioxane/water (1:1, 14 mL) was stirred for 16 h at 45 °C, filtered, and concentrated in vacuo, affording 2-*C*-methyl-D-talitol **16** as a white solid (1.0 g, quant.); mp 102–104 °C; $[\alpha]_D^{21} = -17.2$ (*c* 1.0, H_2O); δ_H (400 MHz, D_2O): 1.07 (3H, s, CH_3), 3.36 (1H, d, $J_{1a,1b}$ = 11.8 Hz, H-1a), 3.51–3.54 (3H, H-1b, H-6a, H-6b), 3.60 (1H, d, $J_{3,4}$ = 9.3 Hz, H-3), 3.66 (1H, d, $J_{3,4}$ = 9.3 Hz, H-4), 3.83 (1H, t, $J_{5,6a} = J_{5,6b}$ = 6.6 Hz, H-5); δ_C (100 MHz, D_2O): 18.2 (CH_3), 63.3 (C-6), 67.1 (C-1), 69.8 (C-3), 70.8 (C-5), 71.2 (C-4), 75.7 (C-2); HR ESI-MS: found *m*/*z* 219.0844 $[M+Na]^+$, calcd for $C_7H_{16}O_6Na$ 219.0839.

7.2.5. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-D-talofuranose 25

Diisobutylaluminum hydride (1.0 M in toluene, 1.30 mL, 1.30 mmol) was added dropwise to a stirred solution of the branched methyl lactone **15** (320 mg, 1.18 mmol) in dry dichloromethane (3.0 mL) at −78 °C. After stirring for 30 min at −78 °C, TLC analysis (cyclohexane/EtOAc 3:1) revealed conversion of the starting material (R_f 0.70) into one major product (R_f 0.40). The reaction mixture was quenched with methanol (3 mL) and allowed to warm to room temperature. Saturated aqueous potassium sodium tartrate solution (5 mL) was added and the reaction mixture was stirred for 1 h at room temperature. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (cyclohexane/EtOAc 3:1→1:1) to afford the protected methyl lactols **25** as a colorless oil (300 mg, 94%); A/B = 1:0.65 (from integration of ^1H NMR signals); $[\alpha]_D^{22} = -15.8$ (*c* 1.0, $CHCl_3$); ν_{max} (NaCl), 3442 cm^{-1} (OH); δ_H (400 MHz, $CDCl_3$): 1.33 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.52 (3H, s, CH_3), 1.57 (6H, s, 2 × CH_3), 3.83–3.86 (1H, m, H-6a B), 3.92 (1H, d, $J_{1,OH}$ = 11.0 Hz, 1-OH A), 3.96–4.05 (4H, m, H-6a A, H-6b A, 1-OH B, H-6b B), 4.04–4.07 (1H, m, H-4 A), 4.13–4.19 (2H, m, H-5 A, H-5 B), 4.22–4.24 (1H, m, H-4 B), 4.40 (1H, s, H-3 B), 4.49 (1H, s, H-3 A), 5.13 (1H, d, $J_{1,OH}$ = 11.0 Hz, H-1 A), 5.21 (1H, d, $J_{1,OH}$ = 11.1 Hz, H-1 B); δ_C (100 MHz, $CDCl_3$): 20.1 (CH_3), 21.4 (CH_3), 25.5 (CH_3), 25.6 (CH_3), 26.0 (CH_3), 26.1 (CH_3), 26.9 (CH_3), 27.2 (CH_3), 27.8 (CH_3), 28.3 (CH_3), 65.5 (C-6 A), 66.0 (C-6 B), 76.2, 77.1 (C-5 A and C-5 B), 80.0 (C-4 A), 84.1 (C-4 B), 87.7 (C-3 A), 87.7 (C-2 B), 88.3 (C-3 B), 91.9 (C-2 A), 102.6 (C-1 A), 105.2 (C-1 B), 109.7 (*C*Me_2 A), 110.1 (*C*Me_2 B), 112.8 (*C*Me_2 B), 113.3 (*C*Me_2 A); HR ESI-MS: found *m*/*z* 297.1302 $[M+Na]^+$, calcd for $C_{13}H_{22}O_6Na$ 297.1309.

7.2.6. 2-*C*-Methyl-D-talose 4

A mixture of the protected **25** (295 mg, 1.08 mmol) and Dowex® 50WX8-100 (H^+) ion-exchange resin (300 mg) in dioxane/water (1:1, 3 mL) was stirred for 16 h at 45 °C, filtered, and concentrated in vacuo, affording 2-*C*-methyl-D-talose **4** as an amorphous solid (196 mg, 93%); A/B/C/D = 5:6:8:8 (from integration of ^1H NMR signals); $[\alpha]_D^{22} = +11.8$ (*c* 2.0, H_2O); δ_H (400 MHz, D_2O) [Partial NMR data]: 1.09 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.15 (3H, s, CH_3), 4.48 (1H, s, H-1), 4.82 (1H, s, H-1), 4.91 (1H, s, H-1), 4.95 (1H, s, H-1); δ_C (100 MHz, D_2O): 18.5 (CH_3), 19.0 (CH_3), 20.5 (2 × CH_3), 61.5 (C-6), 61.7 (C-6), 63.1 (C-6), 63.4 (C-6), 68.5, 69.4, 70.1, 71.1, 71.5, 71.5, 72.3, 74.4, 74.4 (C-2), 74.6, 74.9 (C-2), 75.6, 75.7 (C-2), 79.0 (C-2), 80.6, 81.5, 97.5 (C-1), 98.1 (C-1), 101.0 (C-1), 102.6 (C-1); HR ESI-MS: found *m*/*z* 217.0682 $[M+Na]^+$, calcd for $C_7H_{14}O_6Na$ 217.0683.

7.3. From L-sorbose 9

7.3.1. 2-*C*-Iodomethyl-2,3:5,6-di-*O*-isopropylidene-L-gulono-1,4-lactone 28

Imidazole (2.33 g, 34.3 mmol), triphenylphosphine (4.61 g, 17.6 mmol), and iodine (4.46 g, 17.6 mmol) were added to a solution of 2-*C*-hydroxymethyl-2,3:5,6-di-*O*-isopropylidene-L-gulono-1,4-lactone **27** (2.41 g, 8.36 mmol) in toluene (42 mL). The reaction mixture was stirred at 85 °C for 1 h, after which time TLC analysis (cyclohexane/EtOAc, 1:1) revealed complete conversion of starting material (R_f 0.34) to a UV-active product (R_f 0.69). The solvent was removed in vacuo, and the residue was partitioned between EtOAc (90 mL) and saturated aqueous sodium bicarbonate (90 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting residue was purified by flash column chromatography (cyclohexane/EtOAc, 4:1) to yield iodide **28** as a crystalline solid (2.73 g, 82%); mp 157–159 °C; $[\alpha]_D^{23.5} = +27.8$ (*c* 1.07, $CHCl_3$); ν_{max} (NaCl): 1788 cm^{-1} (CO); δ_H (400 MHz, $CDCl_3$): 1.40 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.49 (3H, s, CH_3), 3.41 (1H, d, $J_{2'a,2'b}$ = 10.8 Hz, H-2′a), 3.50 (1H, d, $J_{2'a,2'b}$ = 10.8 Hz, H-2′b), 3.83 (1H, dd, $J_{5,6a}$ = 5.9 Hz $J_{6a,6b}$ = 8.9 Hz, H-6a), 4.21 (1H, dd, $J_{5,6b}$ = 6.3 Hz, $J_{6a,6b}$ = 8.9 Hz, H-6b), 4.45 (1H, m, H-5), 4.49 (1H, dd, $J_{3,4}$ = 3.7 Hz, $J_{4,5}$ = 8.3 Hz, H-4), 4.58 (1H, d, $J_{3,4}$ = 3.7 Hz, H-3); δ_C (100 MHz, $CDCl_3$): 1.0 (–CH_2I), 25.2 (CH_3), 26.8 (CH_3), 26.8 (CH_3), 27.0 (CH_3), 65.2 (C-6), 75.0 (C-5), 80.9 (C-3), 81.2 (C-4), 84.9 (C-2), 110.6 (*C*Me_2), 115.0 (*C*Me_2), 172.0 (C-1); *m*/*z* (ES+): HR ESI-MS: found *m*/*z* 399.0300 $[M+H]^+$, calcd for $C_{13}H_{20}IO_6$ 399.0305; $C_{13}H_{19}IO_6$ requires C, 39.21; H, 4.81. Found: C, 39.30; H, 4.79.

7.3.2. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-L-gulono-1,4-lactone 17

Iodolactone **28** (291 mg, 0.73 mmol) in dioxane (1.5 mL) was stirred under hydrogen for 17 h in the presence of 10% palladium on carbon (78 mg) and triethylamine (204 μL, 1.46 mmol); the reaction mixture was filtered through Celite, eluting with 1,4-dioxane, and the filtrate was evaporated in vacuo. The residue was dissolved in EtOAc (10 mL), washed with saturated aqueous sodium thiosulfate solution (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (cyclohexane/EtOAc 3:2) to afford the protected methyl lactone **17** as a crystalline solid (195 mg, 98%); mp 117–118 °C; $[\alpha]_D^{23.5} = +59.0$ (*c* 1.09, $CHCl_3$); ν_{max} (NaCl): 1789 cm^{-1} (CO); δ_H (400 MHz, $CDCl_3$): 1.38 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.56 (3H, s, CH_3), 3.80 (1H, dd, $J_{5,6a}$ = 6.4 Hz, $J_{6a,6b}$ = 8.7 Hz, H-6a), 4.20 (1H, dd, $J_{5,6b}$ = 6.7 Hz, $J_{6a,6b}$ = 8.7 Hz, H-6b), 4.32–4.35 (2H, m, H-3, H-4), 4.45 (1H, m, H-5); δ_C (100 MHz, $CDCl_3$): 18.3 (CH_3), 25.2 (CH_3), 26.8 (3 × CH_3), 65.1 (C-6), 75.2 (C-5), 79.7 (C-4), 80.5 (C-3), 83.0 (C-2), 110.5 (*C*Me_2), 113.7 (*C*Me_2), 175.6 (C-1); HR ESI-MS: found *m*/*z* 273.1332 $[M+H]^+$, calcd for $C_{13}H_{21}O_6$ 273.1338; $C_{13}H_{20}O_6$ requires C, 57.34; H, 7.40. Found: C, 57.36; H, 7.41.

7.3.3. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-L-gulitol 30

Method A: The methyl lactone **17** (971 mg, 3.57 mmol) and sodium borohydride (270 mg, 7.13 mmol) were stirred in ethanol (14 mL) at room temperature. After 19 h, TLC (EtOAc) indicated complete consumption of starting material (R_f 0.85) and formation of a major product (R_f 0.44). Saturated aqueous ammonium

chloride solution was added dropwise until the excess hydride had been destroyed, indicated by evolution of hydrogen gas. The solution was diluted with EtOAc (35 mL), the mixture shaken with brine (35 mL), and the aqueous layer re-extracted with EtOAc (2 × 35 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was purified by flash chromatography (cyclohexane/EtOAc 2:1→1:0) to give the diacetonide **30** as a colorless oil (694 mg, 70%); $[\alpha]_D^{22} = -7.3$ (*c* 1.52, $CHCl_3$); ν_{max} (NaCl): 3379 cm^{-1} (OH); δ_H (400 MHz, $CDCl_3$): 1.30 (CH_3), 1.37 (CH_3), 1.37 (CH_3), 1.45 (CH_3), 1.48 (CH_3), 3.18 (1H, a-br-s, 1-OH), 3.36 (1H, d, $J_{4,OH}$ = 5.2 Hz, 4-OH), 3.42 (1H, dd, $J_{1a,1b}$ = 11.8 Hz, $J_{1a,OH}$ = 5.2 Hz, H-1a,), 3.64 (1H, dd, $J_{1a,1b}$ = 11.8 Hz, $J_{1b,OH}$ = 5.2 Hz, H-1b), 3.69 (1H, d, $J_{3,4}$ = 2.0 Hz, H-3), 3.81 (1H, br t, H-4), 3.85 (1H, dd, $J_{5,6a}$ = 6.7 Hz, $J_{6a,6b}$ = 8.3 Hz, H-6a), 4.06 (1H, dd, $J_{5,6b}$ = 6.5 Hz, $J_{6a,6b}$ = 8.3 Hz, H-6b,), 4.30 (1H, q, *J* = 6.4 Hz, H-5); δ_C (100 MHz, $CDCl_3$): 22.0 (CH_3), 25.3 (CH_3), 26.5 (CH_3), 26.6 (CH_3), 27.9 (CH_3), 65.5 (C-1), 65.9 (C-6), 68.6 (C-4), 76.8 (C-5), 81.2 (C-3), 81.9 (C-2), 107.8 (*C*Me_2), 109.8 (*C*Me_2); HR ESI-MS: found *m/z* 299.1465 $[M+Na]^+$, calcd for $C_{13}H_{24}O_6Na$ 299.1466.

Method B: Lithium aluminum hydride (2.0 M in THF, 7.24 mL, 14.4 mmol) was added slowly to a stirred solution of the iodomethyl lactone **28** (1.44 g, 3.62 mmol) in THF (14.5 mL) at 0 °C. The solution was allowed to warm to room temperature, and after 14 h, TLC analysis indicated complete conversion of starting material (R_f 0.60) to one major product (R_f 0.17). The solution was cooled to 0 °C and methanol was added dropwise until evolution of gas ceased. The reaction mixture was diluted with saturated aqueous sodium potassium tartrate solution (40 mL) and stirred for 1.5 h, after which the solution was extracted with EtOAc (40 mL, then 2 × 20 mL). The combined organic fractions were dried over $MgSO_4$, filtered, evaporated to dryness, and the crude residue was purified by flash chromatography (cyclohexane/EtOAc, 1:3) to yield the protected diol **30** as a colorless oil (717 mg, 72%), identical to the material prepared in method A.

7.3.4. 2-*C*-Methyl-L-gulitol 18

Diacetonide **30** (1.21 g, 4.36 mmol) was stirred in trifluoroacetic acid/water (1:1, 9 mL). After stirring for one night, TLC analysis (EtOAc/methanol, 3:1) indicated complete consumption of starting material (R_f 0.60) and formation of a major product (R_f 0.00). The solvent was removed in vacuo, co-evaporated with water and toluene to yield 2-*C*-methyl-L-gulitol **18** as a colorless oil (900 mg, quantitative); $[\alpha]_D^{22} = -9.7$ (*c* 0.76, H_2O); δ_H (400 MHz, D_2O): 1.06 (3H, s, CH_3), 3.42 (1H, d, $J_{1a,1b}$ = 11.7 Hz, H-1a), 3.45–3.50 (1H, m, H-6a), 3.48 (1H, d, $J_{1a,1b}$ = 11.7 Hz, H-1b), 3.52 (1H, d, $J_{3,4}$ = 1.6 Hz, H-3), 3.60 (1H, dd, $J_{5,6b}$ = 4.1 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6b), 3.66–3.70 (1H, m, H-5), 3.77 (1H, dd, $J_{3,4}$ = 1.6 Hz, $J_{4,5}$ = 5.4 Hz, H-4); δ_C (100 MHz, D_2O): 19.3 (CH_3), 62.7 (C-6), 66.7 (C-1), 69.4 (C-4), 73.2 (C-3), 74.2 (C-5), 75.3 (C-2); HR ESI-MS: found *m/z* 219.0840 $[M+Na]^+$, calcd for $C_7H_{16}O_6Na$ 219.0839.

7.3.5. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-L-gulofuranose 29

Diisobutylaluminum hydride (1.0 M in toluene, 0.88 mL, 0.88 mmol) was added dropwise to a stirred solution of the branched methyl lactone **17** (200 mg, 0.882 mmol) in dry dichloromethane (2 mL) at −78 °C. The reaction mixture was stirred for 1 h at −78 °C under an atmosphere of nitrogen, after which time infra red spectroscopy indicated starting material was still present. Further diisobutylaluminum hydride (1.0 M in toluene, 0.44 mL, 0.44 mmol) was added to the flask and the reaction mixture was stirred for 1 h. The reaction mixture was quenched with methanol (0.5 mL) and allowed to warm to room temperature. Saturated aqueous potassium sodium tartrate solution (5 mL) was added and the reaction mixture was stirred for 1 h at room temperature. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (cyclohexane/EtOAc 5:1→2:1) to afford lactols **29** (177 mg, 89%) as a colorless oil; A/B = 3:1 (from integration of ^1H NMR signals); $[\alpha]_D^{22} = -1.0$ (*c* 1.32, H_2O); ν_{max} (NaCl): 3440 cm^{-1} (OH); δ_H (400 MHz, $CDCl_3$): 1.37 (3H, s, CH_3 A), 1.38 (3H, s, CH_3 A), 1.38 (3H, s, CH_3 B), 1.40 (3H, s, CH_3 B), 1.44 (9H, s, 2 × CH_3 A, CH_3B), 1.45 (3H, s, CH_3 B), 1.48 (3H, s, CH_3 A), 1.51 (3H, s, CH_3 B), 3.17 (1H, s, 1-OH A), 3.56 (1H, dd, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 8.0 Hz, H-4 B), 3.65–3.71 (1H, m, H-6a B), 3.69 (1H, dd, $J_{5,6a}$ = 7.6 Hz, $J_{6a,6b}$ = 8.2 Hz, H-6a A), 3.83 (1H, d, $J_{1,OH}$ = 12.0 Hz, 1-OH B), 4.11 (1H, dd, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 8.6 Hz, H-4 A), 4.15 (1H, dd, $J_{5,6b}$ = 6.7 Hz, $J_{6a,6b}$ = 8.2 Hz, H-6b B), 4.18 (1H, dd, $J_{5,6b}$ = 6.5 Hz, $J_{6a,6b}$ = 8.2 Hz, H-6b A), 4.22 (1H, d, $J_{3,4}$ = 3.0 Hz, H-3 B), 4.24 (1H, d, $J_{3,4}$ = 3.3 Hz, H-3 A), 4.35–4.42 (2H, m, H-5 A, H-5 B), 4.70 (1H, d, $J_{1,OH}$ = 12.0 Hz, H-1 B), 5.28 (1H, s, H-1 A); δ_C (100 MHz, $CDCl_3$): 20.0 (CH_3 A), 21.5 (CH_3 B), 25.3 (CH_3 B), 25.5 (CH_3 A), 26.7 (CH_3 A), 26.8 (CH_3 B), 26.9 (CH_3 B), 27.4 (CH_3 B), 27.4 (CH_3 A), 27.8 (CH_3 A), 65.8 (C-6 B), 65.9 (C-6 A), 75.2 (C-5 A), 75.3 (C-5 B), 77.4 (C-4 B), 81.7 (C-4 A), 85.1 (C-3 B), 86.4 (C-3 A), 86.9 (C-2 B), 92.5 (C-2 A), 101.3 (C-1 B), 103.2 (C-1 A), 109.8 (*C*Me_2 B), 109.9 (*C*Me_2 A), 113.3 (*C*Me_2 A), 113.6 (*C*Me_2 B); HR ESI-MS: found *m/z* 297.1303 $[M+Na]^+$, calcd for $C_{13}H_{22}O_6Na$ 297.1309.

7.3.6. 2-*C*-Methyl-L-gulose 5

A mixture of lactols **29** (151 mg, 0.551 mmol) and Dowex® 50WX8-100 (H^+) ion-exchange resin (100 mg) in dioxane/water (1:1, 1 mL) was stirred for 48 h at 45 °C, filtered, and concentrated in vacuo, affording 2-*C*-methyl-L-gulose **5** as a colorless solid (100 mg, 93%); A/B/C/D = 1:2:2:3 (from integration of ^1H NMR signals); $[\alpha]_D^{22} = +12.0$ (*c* 1.0, H_2O); δ_H (400 MHz, D_2O) [Partial NMR data]: 1.09 (3H, s, CH_3), 1.11 (9H, s, 3 × CH_3), 4.76 (1H, s, 2 × H-1), 4.88 (1H, s, H-1), 5.04 (1H, s, H-1); δ_C (100 MHz, D_2O): 17.5 (CH_3), 17.9 (CH_3), 21.3 (CH_3), 21.6 (CH_3), 59.1 (C-6), 61.4 (C-6), 62.9 (C-6), 64.0 (CH_3), 69.4, 69.9, 70.2, 71.5, 73.3, 74.2, 75.0, 75.1, 75.2, 75.3, 75.8, 78.3, 79.9, 95.6 (C-1), 96.0 (C-1), 100.1 (C-1), 101.9 (C-1); HR ESI-MS: found *m/z* 217.0682 $[M+Na]^+$, calcd for $C_7H_{14}O_6Na$ 217.0683.

7.4. From D-psicose 10

7.4.1. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-iodomethyl-D-allono-1,4-lactone 32

Imidazole (1.06 g, 15.6 mmol), triphenylphosphine (2.97 g, 11.3 mmol), and iodine (2.87 g, 11.3 mmol) were added to a stirred solution of alcohol **31** (1.25 g, 4.34 mmol) in toluene (20 mL) and stirred at 85 °C for 2 h. The reaction mixture was allowed to cool to room temperature, concentrated in vacuo, and then partitioned between CH_2Cl_2 (30 mL) and saturated sodium hydrogen carbonate solution (30 mL) and the resulting solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with water (2 × 30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (cyclohexane/EtOAc 6:1→3:1) to afford the iodide **32** as a crystalline solid (1.54 g, 89%); mp 104–106 °C; $[\alpha]_D^{22} = +32.3$ (*c* 1.0, $CHCl_3$); ν_{max} (NaCl): 1784 cm^{-1} (CO); δ_H (400 MHz, $CDCl_3$): 1.26 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.58 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.59 (1H, d, $J_{2'a,2'b}$ = 11.3 Hz, H-2′a), 3.64 (1H, d, $J_{2'a,2'b}$ = 11.3 Hz, H-2′b), 3.85 (1H, dd, $J_{5,6a}$ = 6.2 Hz, $J_{6a,6b}$ = 8.8 Hz, H-6a), 4.18 (1H, dd, $J_{5,6b}$ = 7.3 Hz, $J_{6a,6b}$ = 8.8 Hz, H-6b), 4.40–4.44 (1H, m, H-5), 4.48 (1H, d, $J_{4,5}$ = 3.9 Hz, H-4), 4.61 (1H, s, H-3); δ_C (100 MHz, $CDCl_3$): 5.1 (–CH_2I), 24.3 (CH_3), 26.5 (CH_3), 27.2 (CH_3), 27.4 (CH_3), 65.4 (C-6), 74.2 (C-5), 80.9 (C-3), 83.5 (C-4), 84.2 (C-2), 110.7 (*C*Me_2), 114.6 (*C*Me_2), 171.4 (C-1); HR ESI-MS: found *m/z* 399.0305 $[M+H]^+$, calcd for $C_{13}H_{20}IO_6$ 399.0305.

7.4.2. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-D-allitol 34

Lithium aluminum hydride (2.0 M in THF, 3.5 mL, 7.09 mmol) was added to a stirred solution of compound **32** (1.41 g, 3.54 mmol) in THF (14 mL) at 0 °C. After stirring for 16 h at room temperature, methanol (10 mL) was slowly added followed by saturated potassium sodium tartrate solution (30 mL), and the reaction mixture was stirred for 1 h. The reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (cyclohexane/EtOAc 2:1→0:1) to afford diol **34** as a colorless oil (950 mg, 97%); $[\alpha]_D^{25} = +7.7$ (*c* 1.0, $CHCl_3$); ν_{max} (NaCl), 3419 cm^{-1} (OH); δ_H (400 MHz, $CDCl_3$): 1.35 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.45 (3H, s, CH_3), 2.79 (1H, br s, 1-OH), 3.08 (1H, br s, 4-OH), 3.50 (1H, d, $J_{1a,1b}$ = 10.9 Hz, H-1a), 3.62 (1H, d, $J_{3,4}$ = 9.2 Hz, H-3), 3.70 (1H, d, $J_{1a,1b}$ = 10.9 Hz, H-1b), 4.01 (2H, d, $J_{5,6a} = J_{5,6b}$ = 7.0 Hz, H-6a, H-6b), 4.13 (1H, dd, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 3.0 Hz, H-4), 4.37 (1H, td, $J_{4,5}$ = 3.0 Hz, $J_{5,6a} = J_{5,6b}$ = 7.0 Hz, H-5); δ_C (100 MHz, $CDCl_3$): 23.4 (CH_3), 25.2 (CH_3), 26.3 (CH_3), 26.6 (CH_3), 28.3 (CH_3), 63.4 (C-6), 65.1 (C-1), 68.3 (C-4), 76.4 (C-5), 81.6 (C-3), 82.8 (C-2), 108.1 (*C*Me_2), 109.0 (*C*Me_2); HR ESI-MS: found *m*/*z* 299.1465 $[M+Na]^+$, calcd for $C_{13}H_{24}O_6Na$ 299.1465.

7.4.3. 2-*C*-Methyl-D-allitol 20

A mixture of the protected diol **34** (950 mg, 3.44 mmol) and Dowex® 50WX8-100 (H^+) ion-exchange resin (1.0 g) in dioxane/water (1:1, 10 mL) was stirred for 16 h at 45 °C, filtered, and concentrated in vacuo, affording free branched methyl polyol **20** as a colorless oil (670 mg, quant.); $[\alpha]_D^{25} = +12.5$ (*c* 0.99, MeOH); δ_H (400 MHz, D_2O): 1.12 (3H, s, CH_3), 3.36 (1H, d, $J_{1a,1b}$ = 11.8 Hz, H-1a), 3.50 (1H, d, $J_{1a,1b}$ = 11.8 Hz, H-1b), 3.53–3.58 (2H, m, H-3, H-6a), 3.68 (1H, dd, $J_{5,6b}$ = 3.0 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6b), 3.75–3.82 (2H, m, H-4, H-5); δ_C (100 MHz, D_2O): 18.9 (CH_3), 62.3 (C-6), 66.9 (C-1), 72.5, 72.7, 73.3 (C-3, C-4, C-5), 75.7 (C-2); HR ESI-MS: found *m*/*z* 219.084 $[M+Na]^+$, calcd for $C_7H_{16}O_6Na$ 219.0839.

7.4.4. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-D-allono-1,4-lactone 19

Iodolactone **32** (655 mg, 1.65 mmol) in ethanol (10 mL) was stirred under hydrogen for 3 days in the presence of 10% palladium on carbon (70 mg) and triethylamine (0.28 mL, 1.97 mmol); the reaction mixture was filtered through Celite and the filtrate was evaporated in vacuo. The residue was dissolved in dichloromethane (10 mL), washed with saturated aqueous sodium thiosulfate solution (10 mL), water (2 × 10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (cyclohexane/EtOAc 5:1→2:1) to afford the protected methyl lactone **19** as a crystalline solid (440 mg, 98%); mp 52–54 °C (cyclohexane/Et_2O); mp 52–54 °C (EtOAc/cyclohexane); $[\alpha]_D^{20} = -50$ (*c* 1.02, $CHCl_3$); ν_{max} (film), 1775 cm^{-1} (C=O); δ_H (400 MHz, $CDCl_3$): 1.34 (3H, s, CH_3) 1.42 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.63 (3H, s, CH_3), 3.89–3.92 (1H, m, H-6a), 4.14–4.21 (2H, m, H-4, H-6b), 4.36–4.38 (1H, m, H-5), 4.50 (1H, s, H-3); δ_C (100 MHz, $CDCl_3$): 20.4 (CH_3), 24.5 (CH_3), 26.4 (CH_3), 26.9 (CH_3), 27.0 (CH_3), 66.1 (C-6), 74.2 (C-4), 80.8 (C-3), 82.0 (C-2), 82.6 (C-5), 110.7 (*C*Me_2), 113.1 (*C*Me_2), 175.8 (C-1); HR ESI-MS: found *m*/*z* 295.1149 $[M+Na]^+$, calcd for $C_{13}H_{20}O_6Na$ 295.1152;

7.4.5. 2,3:5,6-Di-*O*-isopropylidene-2-*C*-methyl-D-allose 33

Diisobutylaluminum hydride (1.0 M in toluene, 0.32 mL, 0.32 mmol) was added dropwise to a stirred solution of the branched methyl lactone **19** (80 mg, 0.324 mmol) in dry dichloromethane (1 mL) at −78 °C. The reaction mixture was stirred for 30 min at −78 °C under an atmosphere of nitrogen. The reaction mixture was quenched with methanol (0.5 mL) and allowed to warm to room temperature. Saturated aqueous potassium sodium tartrate solution (5 mL) was added and the reaction mixture was stirred for 1 h at room temperature. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (cyclohexane/EtOAc 4:1→2:1) to afford the lactols **33** (80 mg, quant.) as a colorless oil; A/B = 1.9:1 (from integration of ^1H NMR signals); $[\alpha]_D^{22} = -1.0$ (*c* 1.0, $CHCl_3$); ν_{max} (NaCl), 3442 cm^{-1} (OH); δ_H (400 MHz, $CDCl_3$): 1.35 (3H, s, CH_3 A), 1.37 (3H, s, CH_3 B), 1.44 (3H, s, CH_3 B), 1.45 (3H, s, CH_3 A), 1.47 (6H, s, CH_3 A, CH_3 B), 1.48 (3H, s, CH_3 B), 1.49 (3H, s, CH_3 B), 1.52 (3H, s, CH_3 A), 1.55 (3H, s, CH_3 A), 3.79–3.83 (3H, m, H-6a A, H-6a B, OH A), 3.90 (1H, d, $J_{1,OH}$ = 10.5 Hz, OH B), 4.02 (1H, dd, $J_{3,4}$ = 1.5 Hz, $J_{4,5}$ = 5.4 Hz, H-4 A), 4.10–4.18 (4H, m, H-5 A, H-6b A, H-4 B, H-6b B), 4.35 (1H, td, *J* = 3.9 Hz, *J* = 6.7 Hz, H-5 B), 4.44 (1H, s, H-3 B), 4.46 (1H, d, $J_{3,4}$ = 1.5 Hz, H-3 A), 4.96 (1H, d, $J_{1,OH}$ = 10.1 Hz, H-1 A), 5.20 (1H, d, $J_{1,OH}$ = 10.0 Hz, H-1 B); δ_C (100 MHz, $CDCl_3$): 20.1, 22.0, 24.5, 24.7, 26.2, 26.5, 27.0, 27.2, 27.7, 28.3 (10 × CH_3), 66.0 (C-6 B), 66.5 (C-6 A), 75.6, 77.0, 82.1 (C-4 A), 85.9, 86.2, 86.5, 87.7 (C-2 A), 92.3 (C-2 B), 101.6 (C-1 A), 105.1 (C-1 B), 110.1, 110.4, 112.7, 113.8 (4 × *C*Me_2); HR ESI-MS: found *m*/*z* 297.1296 $[M+Na]^+$, calcd for $C_{13}H_{22}O_6Na$ 297.1309.

7.4.6. 2-*C*-Methyl-D-allose 6

A mixture of the lactols **33** (80 mg, 0.292 mmol) and Dowex® 50WX8-100 (H^+) ion-exchange resin (80 mg) in dioxane/water (1:1, 1 mL) was stirred for 16 h at room temperature, filtered, and concentrated in vacuo, affording 2-*C*-methyl-D-allose **6** as a colorless oil (54 mg, 95%); $[\alpha]_D^{22} = -3.5$ (*c* 1.0, H_2O): A/B/C/D = 18:12:4:3 (from integration of ^1H NMR signals); δ_H (400 MHz, D_2O) [Partial NMR data]: 1.05 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.16 (3H, s, CH_3), 4.69 (1H, s, H-1), 4.79 (3H, s, H-1), 4.90 (1H, s, H-1), 4.95 (1H, s, H-1); δ_C (100 MHz, D_2O): 16.6 (CH_3), 18.5 (CH_3), 20.9 (CH_3), 22.2 (CH_3), 61.0 (C-6), 61.6 (C-6), 62.5 (C-6), 62.7 (C-6), 65.4, 66.0, 68.1, 70.9 (C-2), 72.1, 72.9 (C-2), 73.0, 74.0, 74.6, 75.0, 75.5, 76.1 (C-2), 79.3 (C-2), 81.5, 94.7 (C-1), 96.8 (C-1), 100.7 (C-1), 102.5 (C-1); HR ESI-MS: found *m*/*z* 217.0684 $[M+Na]^+$, calcd for $C_7H_{14}O_6Na$ 217.0683.

8. Biotechnological syntheses

K. pneumoniae 40bR, a mutant of *K. pneumoniae* 40b,[9] an isolate of the Rare Sugar Research Center, was maintained at 4 °C on agar plate containing 2% agar, 0.5% polypepton, 0.5% yeast extract, 0.5% NaCl, and 1% xylitol. After various optimizations, it was found that cells grown aerobically with continuous shaking (200 rpm) for 18 h at 30 °C in 2 L Erlenmeyer flasks with broth volume 800 mL containing medium of the following composition: 0.5% polypepton, 0.5% yeast extract, 0.5% NaCl, and 1% xylitol were best for carrying out biotransformation. The cells were harvested by centrifugation at 12,000 rpm for 10 min and washed twice with distilled water and re-suspended in appropriate buffer for carrying out the biotransformation. The effect of pH, from 7.0 to 10, was studied using buffers (50 mM sodium phosphate pH 7.0–8.0, 50 mM Tris–HCl pH 7.0–9.0, 50 mM glycine–NaOH pH 9.0–10.0). It was found that 50 mm Tris–HCl (pH 7) was best for the oxidation of 5-*C*-methyl-D-altritol **16** to 5-*C*-methyl-D-psicose **2D**.

G. thailandicus NBRC 3254 was obtained from the Institute for Fermentation, Osaka (IFO). The strain was maintained at 4 °C on agar plate containing 2% agar, 0.5% polypepton, 0.5% yeast extract, 0.5% NaCl, and 1% glucose. After various optimizations, it was found that cells grown aerobically with continuous shaking (200 rpm) for 24 h at 30 °C in 2 L Erlenmeyer flasks with broth volume 800 mL containing medium of the following composition:

0.5% polypepton, 0.5% yeast extract, 0.5% NaCl, and 1% glucose were best for carrying out biotransformations. The cells were harvested by centrifugation at 12,000 rpm for 10 min and washed twice with distilled water and re-suspended in appropriate buffer. The effect of pH, from 7.0 to 10, was studied using buffers (50 mM sodium phosphate pH 7.0–8.0, 50 mM Tris–HCl pH 7.0–9.0, 50 mM glycine–NaOH pH 9.0–10.0). It was found that 50 mm Tris–HCl, pH 9, was best for oxidations of 5-*C*-methyl-L-allitol **20** to 5-*C*-methyl-L-psicose **2L**, of 5-*C*-methyl-D-mannitol **14** to 5-*C*-methyl-D-fructose **1D**, and of 5-*C*-methyl-D-glucitol **18** to 5-*C*-methyl-D-fructose **1D**.

For the preparation of DTE, Recombinant *Escherichia coli* JM105 was grown in LB medium (1% polypepton, 0.5% yeast extract and 1% NaCl) in a 3 L jar fermentor and cells were harvested by centrifugation at 10,000*g* for 10 min at 4 °C and washed twice with 50 mM Tris–HCl buffer (pH 7.5). The enzyme was partially purified by polyethylene glycol fractionation and immobilized on Chitopearl beads according to the method described previously. D-Tagatose was used as a substrate for the DTE assay; the enzyme activity of DTE was calculated by measuring the amount of D-sorbose formed using HPLC as described previously.[10] Finally, DTE (10,000 U) was immobilized on 100 g (wet weight) of Chitopearl BCW 2510 (Fuji Spinning, Tokyo), which had been previously equilibrated with 50 mM Tris–HCl (pH 7.5).

8.1. From 5-C-methyl-D-altritol 16

8.1.1. 5-*C*-Methyl-D-psicose 2D

The *K. pnemoniae* 40bR cells were grown and collected as described earlier and the transformation reaction was carried out in a 200 mL Erlenmeyer flask for 12 h at 30 °C with shaking (200 rpm). The composition of the reaction mixture was as follows: 1 g 5-*C*-methyl-D-altritol **16** in 50 mL of cell suspension in Tris–HCl buffer, pH 7, 0.05 M adjusted to give Cell OD = 30 at 600 nm. The conversion of substrate to product was around 97% with negligible amount of any by-product or substrate left in reaction mixture. After the transformation, the cells were removed by centrifugation at 12,000*g* for 10 min. The supernatant was then treated with activated charcoal and filtered after centrifugation at 12,000*g* for 30 min to remove the charcoal. The filtrate was deionized with a mixture of SKIB (H^+; Mitsubishi Chemical, Tokyo) and Amberlite IRA-41 I (CO_3^{2-}; Organo, Tokyo) ion-exchange resins. The deionized content was then evaporated and concentrated under reduced pressure at 39 °C. After concentration, the content was applied to a column of Dowex 5OW-X2 (The Dow Chemical, MI, USA) in the Ca^{2+} form. The column was eluted with deionized water and 3 mL fractions were collected. The fractions containing only 5-*C*-methyl-D-psicose **2D** were pooled and concentrated by evaporation under vacuum at 39 °C to afford pure 5-*C*-methyl-D-psicose (0.65 g, 20% as oil) showing $[\alpha]_D^{20} = -25.2$ (*c* 1.0, water).

8.1.2. 5-*C*-Methyl-D-fructose 1D

Equilibration of 5-*C*-methyl-D-psicose **2D** to 5-*C*-methyl-D-fructose **1D** was carried out with shaking at 42 °C in a 50-mL Erlenmeyer flask containing 1 g of immobilized DTE (100 U) prepared as described previously, 10 mL of 50 mM Tris–HCl buffer (pH 7.5), 5-*C*-methyl-D-psicose **2D** (final concentration: 4% w/v), and $MnCl_2$ (final concentration: 1 mM). 5-*C*-Methyl-D-psicose **2D** and 5-*C*-methyl-D-fructose **1D** reached an equilibrium state after 6 h with a ratio of 20% substrate **2D** and 80% product **1D**. The accumulation of product was analyzed by HPLC. After the equilibrium state was achieved, the Chitopearl beads were removed from the reaction mixture by filtration. The filtrate was deionized as described previously, and then evaporated and concentrated under reduced pressure at 39 °C. After concentration, the content was applied to a column of Dowex 5OW-X2 in the Ca^{2+} form. The column was eluted with deionized water and 3 mL fractions were collected. The fractions containing only **1D** were pooled and concentrated by evaporation under vacuum at 39 °C to give pure 5-*C*-methyl-D-fructose **1D** (0.12 g, 20% as oil), $[\alpha]_D^{20} = -85.8$ (*c* 1.0, water).

8.2. From 5-*C*-Methyl-L-allitol 20

8.2.1. 5-*C*-Methyl-L-psicose 2L

The *G. thailandicus* NBRC 3254 cells were grown and collected as described above and re-suspended in 50 mm Tris–HCl (pH 9) buffer. The biotransformation was carried out in a 200 mL Erlenmeyer flask for 10 h at 30 °C with shaking (200 rpm). The composition of the reaction mixture was as follows: 0.75 g 5-*C*-methyl-L-allitol **20** in 50 mL of cell suspension in Tris–HCl buffer, pH 9, 0.05 M adjusted to give Cell OD = 30 at 600 nm. The conversion of substrate to product was around 95% with negligible amount of any by-product or substrate left in the reaction mixture. The purification steps are same as described above for production of 5-*C*-methyl-D-psicose **2L** from 5-*C*-methyl-D-altritol **16** to allow isolation of pure 5-*C*-methyl-L-psicose **2L** (0.52 g, 20% as oil), $[\alpha]_D^{20} = +24.5$ (*c* 1.0, water).

8.2.2. 5-*C*-Methyl-L-fructose 1L

The equilibration of 5-*C*-methyl-L-psicose **2L** with 5-*C*-methyl-L-fructose **1L** was carried out with shaking at 42 °C in a 50 mL Erlenmeyer flask containing 1 g of immobilized DTE (100 U) prepared as described previously, 10 mL of 50 mM Tris–HCl buffer (pH 7.5), 5-*C*-methyl-L-psicose (final concentration: 3% w/v), and $MnCl_2$ (final concentration: 1 mM). 5-*C*-Methyl-L-fructose and 5-*C*-methyl L-psicose reached an equilibrium state after 7 h with ratios of 20% substrate and 80% product. To purify the product, same step as described above for production of the enantiomers **1D** and **1L** to allow the isolation of pure **5**-*C*-methyl-L-fructose (0.1 g, 20% as oil), $[\alpha]_D^{20} = +83.2$ (*c* 1.0, water) was followed.

8.3. From 5-*C*-methyl-D-mannitol 14

8.3.1. 5-*C*-Methyl-D-fructose 1D

The *G. thailandicus* NBRC 3254 cells were grown and collected as described earlier and re-suspended in 50 mm Tris–HCl (pH 9) buffer. The oxidation was carried out in a 500 mL Erlenmeyer flask for 8 h at 30 °C with shaking (200 rpm). The composition of the reaction mixture was as follows: 1 g 5-*C*-methyl-D-mannitol **14** in 100 mL of cell suspension in Tris–HCl buffer, pH 9, 0.05 M adjusted to give Cell OD = 30 at 600 nm. The conversion of substrate to product was around 95% with negligible amount of any by-product or substrate left in the reaction mixture. The purification steps are same as described earlier for production of 5-*C*-methyl-D-psicose **2D** from 5-*C*-methyl-D-altritol **16** to obtain pure 5-*C*-methyl-D-fructose **1D** (0.7 g, 20% as oil), $[\alpha]_D^{20} = -85.2$ (*c* 1.0, water).

8.4. From 5-*C*-methyl-D-glucitol 18

8.4.1. 5-*C*-Methyl-D-fructose 1D

The *G. thailandicus* NBRC 3254 cells were grown and collected as described earlier and re-suspended in 50 mm Tris–HCl (pH 9) buffer. The reaction was carried out in a 200 mL Erlenmeyer flask for 18 h at 30 °C with shaking (200 rpm). The composition of the reaction mixture was as follows: 0.5 g 5-*C*-methyl-D-glucitol **18** in 50 mL of cell suspension in Tris–HCl buffer, pH 9, 0.05 M adjusted to give Cell OD = 30 at 600 nm. The conversion of substrate to product was around 80%. The purification steps are the same as those described above for the formation **2D** from **16** to give pure 5-*C*-methyl-D-fructose **1D** (0.35 g, 20% as oil), $[\alpha]_D^{20} = -84.6$ (*c* 1.0, water).

Acknowledgment

This work was supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

References

1. (a) Zhou, M.; O'Doherty, G. *Curr. Top. Med. Chem.* **2008**, *8*, 114–125; (b) Ahmed, M. M.; O'Doherty, G. A. *Carbohydr. Res.* **2006**, *341*, 1505–1521; (c) Davies, S. G.; Nicholson, R. L.; Smith, A. D. *Org. Biomol. Chem.* **2005**, *3*, 348–359; (d) Markert, M.; Mahrwald, R. *Chem. Eur. J.* **2008**, *14*, 40–48; (e) Enders, D.; Chow, S. *Eur. J. Org. Chem.* **2006**, 4578–4584; (f) Grondal, C.; Enders, D. *Adv. Synth. Catal.* **2007**, *349*, 694–702; (g) Zhang, H.; Ramasastry, S. S. V.; Tanaka, F.; Barbas, C. F. *Adv. Synth. Catal.* **2008**, *350*, 791–796; (h) Stallforth, P.; Adibekian, A.; Seeberger, P. H. *Org. Lett.* **2008**, *10*, 1573–1576; (i) Luo, S. Z.; Xu, H.; Zhang, L.; Li, J. Y.; Cheng, J. P. *Org. Lett.* **2008**, *10*, 653–656; (j) Jiang, L. J.; Zhang, Z. G. *Chin. J. Org. Chem.* **2006**, *26*, 618–626; (k) Ley, S. V.; Sheppard, T. D.; Myers, R. M.; Chorghade, M. S. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 1451–1472; (l) Suri, J. T.; Mitsumori, S.; Albertshofer, K.; Tanaka, F.; Barbas, C. F. *J. Org. Chem.* **2006**, *71*, 3822–3828; (m) Northrup, A. B.; Mangion, I. K.; Hettche, F.; MacMillan, D. W. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 2152–2154; (n) Zhao, G. L.; Liao, W. W.; Cordova, A. *Tetrahedron Lett.* **2006**, *47*, 4929–4932.
2. (a) Levin, G. V. *J. Med. Food* **2002**, *5*, 23–36; (b) Sun, Y. X.; Hayakawa, S.; Ogawa, M.; Izumori, K. *Food Control* **2007**, *18*, 220–227; (c) Matsuo, T.; Shirai, Y.; Izumori, K. *Faseb J.* **2006**, *20*, A594; (d) Sun, Y. X.; Hayakawa, S.; Jiang, H. H.; Ogawa, M.; Izumori, K. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 2859–2867.
3. (a) Nakajima, Y.; Gotanda, T.; Uchimiya, H.; Furukawa, T.; Haraguchi, M.; Ikeda, R.; Sumizawa, T.; Yoshida, H.; Akiyama, S. *Cancer Res.* **2004**, *64*, 1794–1801; (b) Feng, L.; Senchenkova, S. N.; Yang, J. H.; Shashkov, A. S.; Tao, J.; Guo, H. J.; Zhao, G.; Knirel, Y. A.; Reeves, P.; Wang, L. *J. Bacteriol.* **2004**, *186*, 383–392; (c) Hossain, M. A.; Wakabayashi, H.; Izuishi, K.; Okano, K.; Yachida, S.; Tokuda, M.; Izumori, K.; Maeta, H. *J. Biosci. Bioeng.* **2006**, *101*, 369–371; (d) Sui, L.; Dong, Y. Y.; Watanabe, Y.; Yamaguchi, F.; Hatano, N.; Tsukamoto, I.; Izumori, K.; Tokuda, M. *Int. J. Oncol.* **2005**, *27*, 907–912; (e) Sui, L.; Dong, Y. Y.; Watanabe, Y.; Yamaguchi, F.; Hatano, N.; Izumori, K.; Tokuda, M. *Anticancer Res.* **2005**, *25*, 2639–2644; (f) Hossain, M. A.; Izuishi, K.; Tokuda, M.; Izumori, K.; Maeta, H. *J. Hepatobil. Pancreatic Surg.* **2004**, *11*, 181–189; (g) Sui, L.; Nomura, R.; Dong, Y.; Yamaguchi, F.; Izumori, K.; Tokuda, M. *Cryobiology* **2007**, *55*, 87–92.
4. Mitchell, D. A.; Jones, N. A.; Hunter, S. J.; Cook, J. M. D.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007**, *18*, 1502–1510.
5. Sato, M.; Kurose, H.; Yamasaki, T.; Izumori, K. *J. Nat. Med.* **2008**, *62*, 244–246.
6. (a) Tao, J.; Zhao, L.; Ran, N. *Org. Process Res. Dev.* **2007**, *11*, 259–267; (b) Panke, S.; Held, M.; Wubbolts, M. *Curr. Opin. Biotechnol.* **2004**, *15*, 272–279.
7. (a) Sugiyama, M.; Hong, Z. Y.; Liang, P. H.; Dean, S. M.; Whalen, L. J.; Greenberg, W. A.; Wong, C. H. *J. Am. Chem. Soc.* **2007**, *129*, 14811–14817; (b) Sugiyama, M.; Hong, Z. Y.; Whalen, L. J.; Greenberg, W. A.; Wong, C. H. *Adv. Synth. Catal.* **2007**, *349*, 1308–1320.
8. (a) Izumori, K. *J. Biotechnol.* **2006**, *124*, 717–722; (b) Granstrom, T. B.; Takata, G.; Tokuda, M.; Izumori, K. *J. Biosci. Bioeng.* **2004**, *97*, 89–94; (c) Izumori, K. *Naturwissenschaften* **2002**, *89*, 120–124; (d) Morimoto, K.; Park, C. S.; Ozaki, M.; Takeshita, K.; Shimonishi, T.; Granstrom, T. B.; Takata, G.; Tokuda, M.; Izumori, K. *Enzyme Microb. Technol.* **2006**, *38*, 855–859.
9. (a) Izumori, K.; Tsuzaki, K. *J. Ferment. Technol.* **1988**, *66*, 225–227; (b) Izumori, K.; Yamakita, M.; Tsumura, T.; Kobayashi, H. *J. Ferment. Bioeng.* **1990**, *70*, 26–29; (c) Rahman, A. K.; Tokunaga, H.; Yoshida, K.; Izumori, K. *J. Ferment. Bioeng.* **1991**, *72*, 488–490; (d) Muniruzzaman, S.; Tokunaga, H.; Izumori, K. *J. Ferment. Bioeng.* **1995**, *9*, 323–327; (e) Shimonishi, T.; Okumura, Y.; Izumori, K. *J. Ferment. Bioeng.* **1995**, *79*, 620–622; (f) Takeshita, K.; Shimonishi, T.; Izumori, K. *J. Ferment. Bioeng.* **1996**, *81*, 212–215; (g) Sasahara, H.; Mine, M.; Izumori, K. *J. Ferment. Bioeng.* **1998**, *85*, 84–88; (h) Bhuiyan, S. H.; Ahmed, Z.; Utamura, M.; Izumori, K. *J. Ferment. Bioeng.* **1998**, *86*, 513–516; (i) Sasahara, H.; Izumori, K. *J. Biosci. Bioeng.* **1999**, *87*, 548–550; (j) Ahmed, Z.; Shimonishi, T.; Bhuiyan, S. H.; Utamura, M.; Takata, G.; Izumori, K. *J. Biosci. Bioeng.* **1999**, *88*, 444–448; (k) Mizanur, R. M.; Takeshita, K.; Moshino, H.; Takata, G.; Izumori, K. *J. Biosci. Bioeng.* **2001**, *92*, 237–241; (l) Ahmed, Z.; Sasahara, H.; Bhuiyan, S. H.; Saiki, T.; Shimonishi, T.; Takada, G.; Izumori, K. *J. Biosci. Bioeng.* **1999**, *88*, 676–678; (m) Sultana, I.; Mizanur, R. M.; Takeshita, K.; Takada, G.; Izumori, K. *J. Biosci. Bioeng.* **2003**, *95*, 342–347.
10. (a) Yoshida, H.; Yamada, M.; Nishitani, T.; Takada, G.; Izumori, K.; Karnitori, S. *J. Mol. Biol.* **2007**, *374*, 443–453; (b) Takeshita, K.; Suga, A.; Takada, G.; Izumori, K. *J. Biosci. Bioeng.* **2000**, *90*, 453–455; (c) Izumori, K.; Khan, A. R.; Okaya, H.; Tsumura, T. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 1037–1039; (d) Itoh, H.; Sato, T.; Izumori, K. *J. Ferment. Bioeng.* **1995**, *80*, 101–103; (e) Itoh, H.; Izumori, K. *J. Ferment. Bioeng.* **1996**, *81*, 351–353; (f) Itoh, H.; Sato, T.; Takuchi, T.; Khan, A. R.; Izumori, K. *J. Ferment. Bioeng.* **1995**, *79*, 184–185; (g) Ishida, Y.; Kamiya, T.; Izumori, K. *J. Ferment. Bioeng.* **1997**, *84*, 348–350.
11. Gullapalli, P.; Shiji, T.; Rao, D.; Yoshihara, A.; Morimoto, K.; Takata, G.; Fleet, G. W. J.; Izumori, K. *Tetrahedron: Asymmetry* **2007**, *18*, 1995–2000.
12. Yoshihara, A.; Haraguchi, S.; Gullapalli, P.; Rao, D.; Morimoto, K.; Takata, G.; Jones, N.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J.; Izumori, K. *Tetrahedron: Asymmetry* **2008**, *19*, 739–745.
13. Rao, D.; Yoshihara, A.; Gullapalli, P.; Morimoto, K.; Takata, G.; da Cruz, F. P.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J.; Izumori, K. *Tetrahedron Lett.* **2008**, *49*, 3316–3321.
14. Hotchkiss, D. J.; Soengas, R.; Simone, M. I.; van Ameijde, J.; Hunter, S.; Cowley, A. R.; Fleet, G. W. J. *Tetrahedron Lett.* **2004**, *45*, 9461–9464.
15. (a) Cowley, A. R.; Fleet, G. W. J.; Simone, M. I.; Soengas, R. *Acta Crystallogr., Sect. E* **2004**, *60*, o2142–o2143; (b) van Ameijde, J.; Cowley, A. R.; Fleet, G. W. J.; Nash, R. J.; Simone, M. I.; Soengas, R. *Acta Crystallogr., Sect. E* **2004**, *60*, o2140–o2141; (c) Harding, C. C.; Shallard-Brown, H.; Watkin, D. J.; Soengas, R.; Fleet, G. W. J. *Acta Crystallogr., Sect. E* **2004**, *60*, o2334–o2336.
16. Soengas, R.; Izumori, K.; Simone, M. I.; Watkin, D. J.; Skytte, U. P.; Soetart, W.; Fleet, G. W. J. *Tetrahedron Lett.* **2005**, *46*, 5755–5759.
17. (a) Harding, C. C.; Watkin, D. J.; Cowley, A. R.; Soengas, R.; Skytte, U. P.; Fleet, G. W. J. *Acta Crystallogr., Sect. E* **2005**, *61*, o250–o252; (b) Shallard-Brown, H. A.; Harding, C. C.; Watkin, D. J.; Soengas, R.; Skytte, U. P.; Fleet, G. W. J. *Acta Crystallogr., Sect. E* **2004**, *60*, o2163–o2164; (c) Watkin, D. J.; Parry, L. L.; Soengas, R.; Izumori, K.; Fleet, G. W. J. *Acta Crystallogr., Sect. E* **2005**, *61*, o2955–o2957.
18. (a) Skytte, U. P. *Cereal Foods World* **2002**, *47*, 224; (b) Beadle, J. R.; Saunders, J. P.; Wajda, T. J. US Patent 5078796, 1992.
19. (a) Poonperm, W.; Takata, G.; Ando, Y.; Verasak, S.; Pipob, L.; Saisamorn, L.; Izumori, K. *J. Biosci. Bioeng.* **2007**, *103*, 282–285; (b) Gullapalli, P.; Takata, G.; Poonperm, W.; Rao, D.; Morimoto, K.; Akimitsu, K.; Tajima, S.; Izumori, K. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 3048–3054.
20. Appel, R. *Angew. Chem., Int. Ed.* **1975**, *14*, 801–811.
21. (a) Poonperm, W.; Takata, G.; Izumori, K. *Biosci., Biotechnol., Biochem.* **2008**, *72*, 231–235; (b) Poonperm, W.; Takata, G.; Morimoto, K.; Granstrom, T. B.; Izumori, K. *Enzyme Microb. Technol.* **2007**, *40*, 1206–1212.
22. De Bruyn, A.; Anteunis, M.; Verhegge, G. *Carbohydr. Res.* **1975**, *41*, 295–297.
23. Jones, N. A.; Jenkinson, S. F.; Soengas, R.; Fanefjord, M.; Wormald, M. R.; Dwek, R. A.; Kiran, G. P.; Devendar, R.; Takata, G.; Morimoto, K.; Izumori, K.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007**, *18*, 774–786.
24. Wormald, M. R. Personal communication.
25. (a) Yoshida, H.; Yamaji, M.; Yamada, M.; Takada, G.; Izumori, K.; Ishii, T.; Kamitori, S. *FEBS J.* **2007**, *274*, 271; (b) Takata, G.; Poonperm, W.; Rao, D.; Souda, A.; Nishizaki, T.; Morimoto, K.; Izumori, K. *Biosci., Biotechnol., Biochem.* **2008**, *71*, 2876–2885 and references cited therein.
26. Daniel, P. T.; Koert, U.; Schuppan, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 872–893; Handa, M.; Smith, W. J.; Roush, W. R. *J. Org. Chem.* **2008**, *73*, 1036–1039.