Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 3316–3321
Tetrahedron Letters

Towards the biotechnological isomerization of branched sugars: D-tagatose-3-epimerase equilibrates both enantiomers of 4-*C*-methyl-ribulose with both enantiomers of 4-*C*-methyl-xylulose

Devendar Rao [a], Akihide Yoshihara [a], Pushpakiran Gullapalli [a], Kenji Morimoto [a], Goro Takata [a], Filipa P. da Cruz [b], Sarah F. Jenkinson [b], Mark R. Wormald [c], Raymond A. Dwek [c], George W. J. Fleet [b,*], Ken Izumori [a,*]

[a] *Rare Sugar Research Center, Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Mik-choi, 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*

Received 4 February 2008; revised 26 February 2008; accepted 7 March 2008
Available online 12 March 2008

Abstract

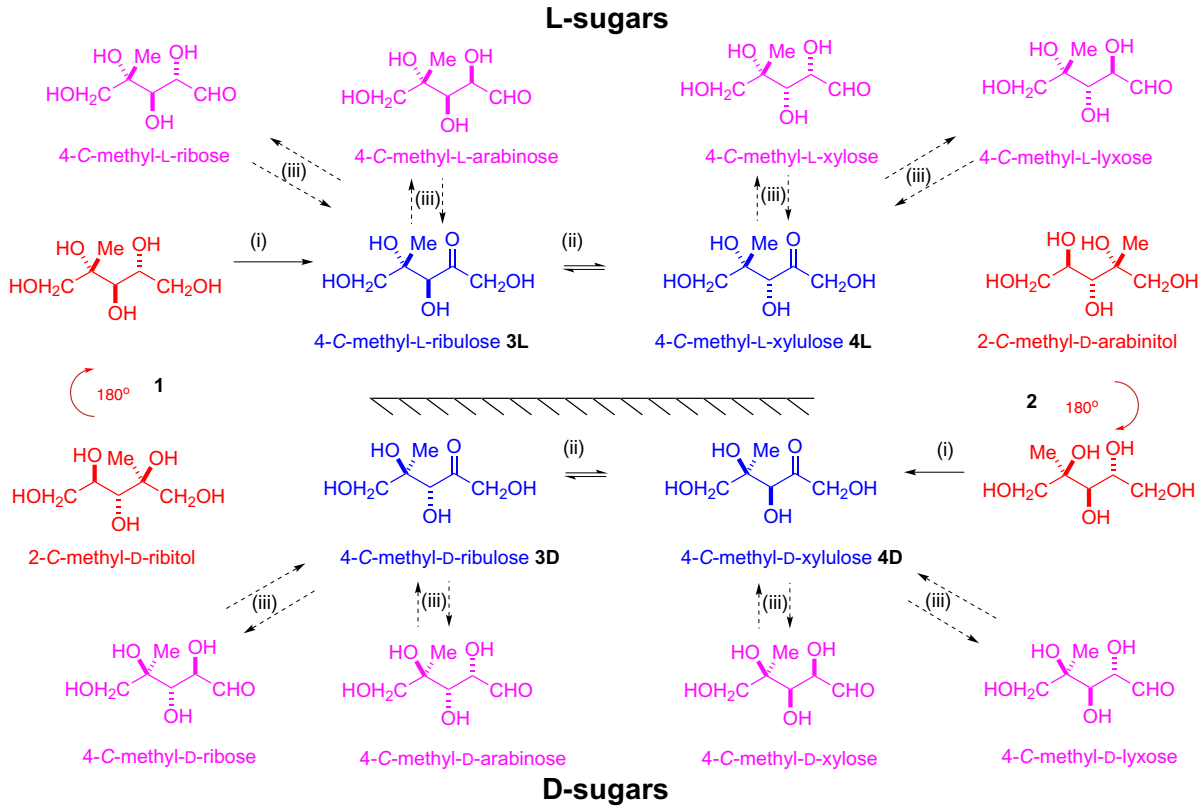
Microbial oxidation of 2-*C*-methyl-D-ribitol and 2-*C*-methyl-D-arabinitol by *Gluconobacter thailandicus NBRC* 3254 produces 4-*C*-methyl-L-ribulose and 4-*C*-methyl-D-ribulose, respectively. Further, 4-*C*-methyl-L-ribulose and 4-*C*-methyl-D-ribulose were equilibrated by D-tagatose-3-epimerase (DTE) with 4-*C*-methyl-L-xylulose and 4-*C*-methyl-D-xylulose, respectively. These transformations demonstrate that polyol dehydrogenase and DTE act on branched synthetic sugars. The green preparation of all of the stereoisomers of 4-*C*-methyl pentuloses illustrates the ability of biotechnology to generate novel branched monosaccharides.

Although the driving force for the initial preparation of rare sugars has been their potential as alternative foodstuffs,[1] it has become clear that rare and new monosaccharides interact with a number of biological receptors and have a wide range of potential chemotherapeutic uses.[2] Application of enzymes to organic synthesis[3] to perform specific transformations is generally limited by their substrate specificity but the directed evolution and the discovery of new enzymes have shown the potential of such biotechnological procedures.[4] The technique of Izumoring[5] has been developed under environmentally friendly conditions to allow the isomerization of all of the 16 aldohexoses and 8 ketohexoses in large amounts in water; this depends on (i) polyol dehydrogenases which allow the specific oxidation of alditols to ketoses,[6] (ii) D-tagatose-3-epimerase (DTE) which equilibrates C-3 in each of the four pairs of ketoses,[7] and (iii) aldose isomerases which equilibrate ketoses and aldoses; the isomerism on a multi-gram scale of L-rhamnose to 1-deoxy-L-fructose indicates that the technology may be extended to deoxy sugars.[8]

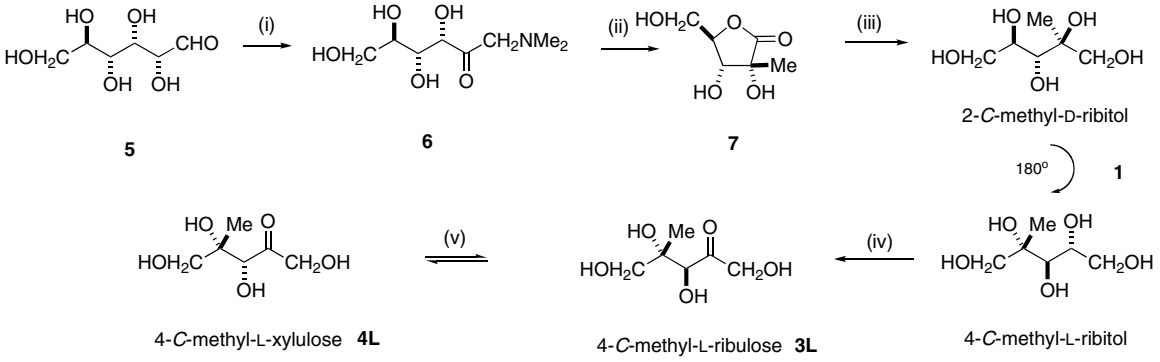
It may be that the concept of Izumoring can be applied generally to the synthesis of unnatural carbohydrates. As part of a project to investigate the synthesis of new branched sugars by green procedures (Scheme 1), this Letter establishes that 2-*C*-methyl-alditols **1** and **2** [in red] are selectively oxidized at C-2 to afford the corresponding ketoses **3L** and **4D** [in blue], respectively; DTE equilibrates **3L** with **4L**, and **4D** with **3D**—allowing for the first time, the preparation of all of the 4-*C*-methyl-pentuloses. There have been no reports of such *C*-methyl branched pentuloses although both enantiomers of ribulose and xylulose have wide ranging biological roles. L-Xylulose may be used as an inhibitor of glycosidases and also as an indicator of patients suffering from acute or chronic hepatitis and liver

* Corresponding authors.
E-mail addresses: george.fleet@chem.ox.ac.uk (G. W. J. Fleet), izumori@kagawa-u.ac.jp (K. Izumori).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.03.047



Scheme 1. Reagents: (i) Polyol dehydrogenases; (ii) D-tagatose-3-epimerase (DTE); (iii) aldose isomerases.



Scheme 2. Reagents and conditions: (i) Me_2NH, AcOH, EtOH, 80 °C; (ii) $Ca(OH)_2$, H_2O, 70 °C ~20% over two steps; (iii) $NaBH_4$, MeOH, 100%; (iv) *G. thailandicus NBRC 3254*, H_2O, 65%; (v) DTE, H_2O, ratio of 68:32, isolated 25% yield.

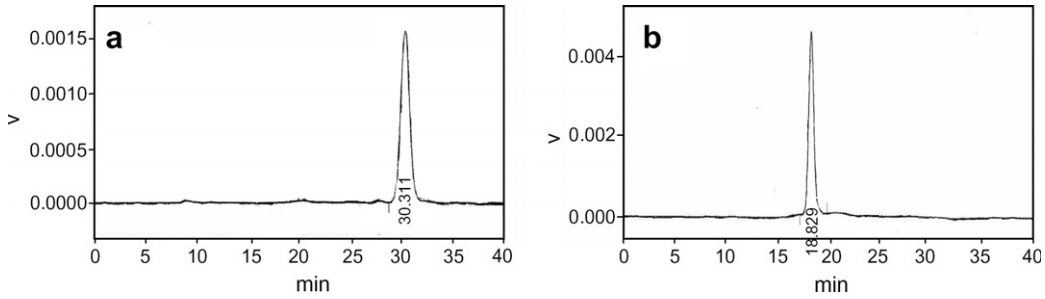
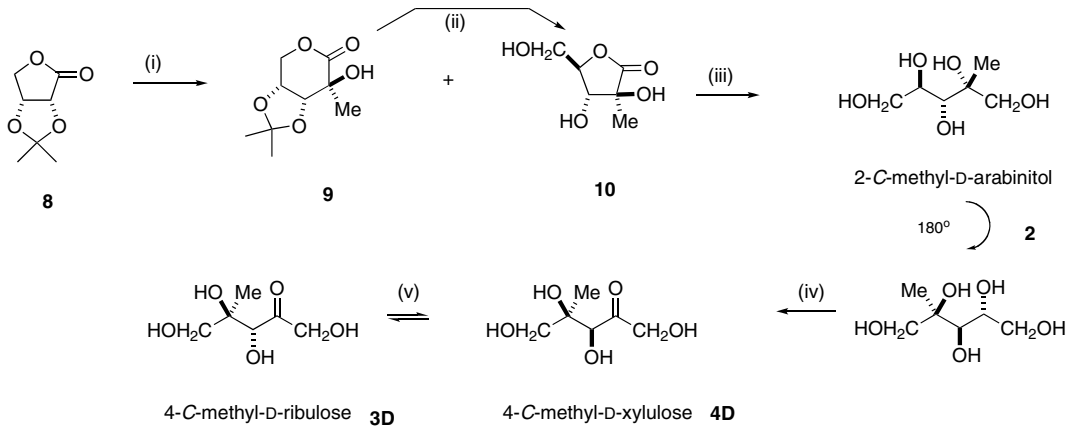


Fig. 1. HPLC profiles of purified products (a) 4-*C*-methyl-L-ribulose **3L**, (b) 4-*C*-methyl-L-xylulose **4L**.



Scheme 3. Reagents and conditions: (i) MeMgBr, THF; then NaCN, H_2O, ~65%; (ii) CF_3COOH, H_2O; (iii) $NaBH_4$, MeOH, 99%; (iv) *G. thailandicus NBRC 3254*, H_2O, 70%; (v) DTE, H_2O, ratio of 70:30, isolated 50% yield.

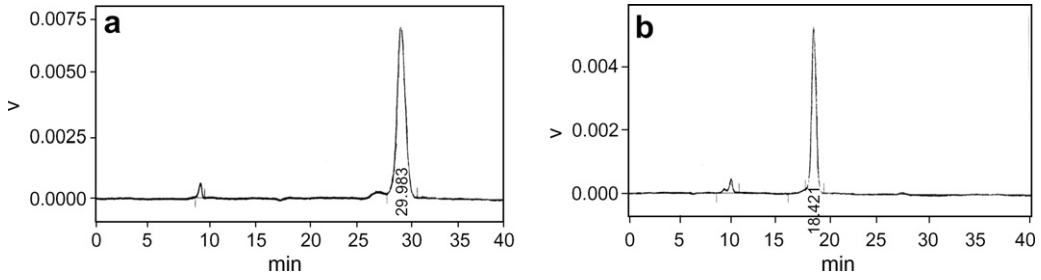


Fig. 2. HPLC profiles of purified products (a) 4-*C*-methyl-D-ribulose **3D**, (b) 4-*C*-methyl-D-xylulose **4D**.

cirrhosis. D-Xylulose can induce depletion of ATP and P_i in isolated rat hepatocytes. L-Ribulose is a promising precursor for the production of various biologically important compounds that show antiviral properties against HIV and the hepatitis virus and also plays an important role in human body sugar metabolism. D-Ribulose derivatives occur extensively in mammals such as in D-ribulose-peptides in human semen;[9] the syntheses described in this Letter will allow the evaluation of the effect of carbon branching on such sugars.

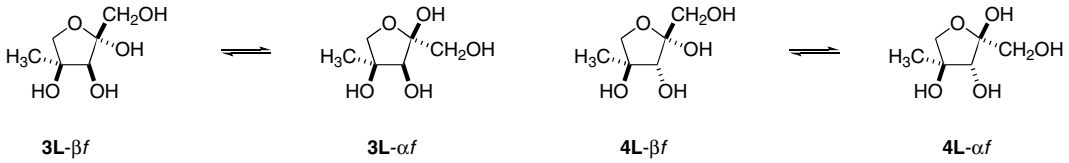
No 4-*C*-methyl or 2-*C*-methyl sugars occur as natural products, so the chemical synthesis of the 2-*C*-methyl pentitols is necessary. For the synthesis of the L-sugars **3L** and **4L**, 2-*C*-methyl-D-ribitol **1** [which is related to 4-*C*-methyl-L-ribitol by a 180° rotation] was prepared from D-glucose **5** (Scheme 2).

Treatment of glucose **5** with dimethylamine and acetic acid in ethanol afforded the Amadori ketose **6**, which on treatment with calcium hydroxide in water gave the branched ribonolactone **7** in around 20% yield on a large scale; lactone **7** is the most readily available *C*-branched carbohydrate chiron and may be prepared in substantial amounts by this method.[10] Reaction of lactone **7** with sodium borohydride in methanol afforded the corresponding *C*-methyl ribitol **1**[11] (quantitative yield).

Table 1
^1H and ^{13}C assignments in ^2H_2O, pH 6.6, referenced to acetone at 2.220 ppm (^1H) and 30.90 ppm (^{13}C), were made based on the 1D, 2D COSY and 2D HSQC spectra

	4-*C*-Methyl-ribulose **3**		4-*C*-Methyl-xylulose **4**	
	3αf 78%	**3**βf 22%	**4**αf 24%	**4**βf 76%
^1H δ (ppm)				
C-1 H	3.584	3.701	3.774	3.642
C-1H′	3.542	3.610	3.613	3.611
C-3H	3.883	3.953	3.913	3.929
C-5H	3.981	3.952	4.018	3.862
C-5H′	3.768	3.831	3.858	3.734
C-4CH_3	1.334	1.326	1.337	1.327
J_{HH} (Hz)				
H-1-H-1′	−12.1	−11.8	−11.9	−12.0
H-5-H-5′	−9.9	−10.1	−9.8	−9.7
^{13}C δ (ppm)				
C-1	63.33	63.33	63.21	64.87
C-2	103.68	106.04	107.80	104.73
C-3	74.62	83.22	81.77	78.55
C-4	76.91	77.60	80.43	79.55
C-5	76.72	76.10	78.07	76.30
C-4CH_3	21.65	20.81	18.99	19.65

The ring forms were confirmed from the 2D HMBC spectra. Proportions of the anomers were determined from the peak intensities in the 1D ^1H spectra.



Scheme 4. Furanose forms of 4-*C*-methyl-L-ribulose **3** and 4-*C*-methyl-L-xylulose **4**.

Gluconobacter thailandicus NBRC 3254 has been shown to oxidize *meso*-ribitol to L-ribulose with no oxidation to the enantiomer;[12] similarly, the branched pentitol 4-*C*-methyl-L-ribitol **1** was oxidized at C-2 by using the resting cell of *Gluconobacter thailandicus NBRC 3254* to afford 4-*C*-methyl-L-ribulose **3L** [oil, $[\alpha]_D^{20}$ −2.9 (*c* 1.0, water)] in 65% yield.

DTE epimerizes the C-3 position of many ketoses and, in particular, equilibrates L-ribulose with L-xylulose.[13] Epimerization of 4-*C*-methyl-L-ribulose **3L** by DTE gave a mixture of **3L** and **4L** in a ratio of 68:32 and allowed the isolation of 4-*C*-methyl-L-xylulose **4L** [oil, $[\alpha]_D^{20}$ +7.7 (*c* 1.0, water)] in 25% yield. The HPLC profiles of purified **3L** and **4L** are shown in Figure 1.

For the synthesis of the D-pentuloses, 2-*C*-methyl-D-arabinitol **2**, again related to 4-*C*-methyl-D-lyxitol by a 180° rotation was required. In comparison, with the short environmentally friendly preparation of ribitol **1**, from glucose, 2-*C*-methyl-D-arabinitol **2** required several steps for the synthesis (Scheme 3). Thus, the protected D-erythrono-lactone **8** was treated first with methyl magnesium bromide followed by a Kiliani reaction to give the protected δ-lactone **9** and the γ-lactone **10** in a combined yield of approximately 65%;[14] a small amount of the epimeric ribonolactone **7** [<10%] was formed during the reaction. Acetonide **9** was removed by the treatment with aqueous trifluoroacetic acid to form **10**, which on treatment with sodium borohydride in methanol afforded 2-*C*-methyl-D-arabinitol **2**[15] in 99% yield as a substrate for the microbial oxidation.

Gluconobacter thailandicus NBRC 3254 also oxidizes D-arabinitol at C-4 to produce D-xylulose with no other relevant by-products;[12] under the same conditions, the resting cells of *Gluconobacter thailandicus NBRC 3254* were used to oxidize 2-*C*-methyl-D-arabinitol **2** at C-4 to produce 4-*C*-methyl-D-xylulose, **4D** [oil, $[\alpha]_D^{20}$ −7.2 (*c* 1.0, water)] in

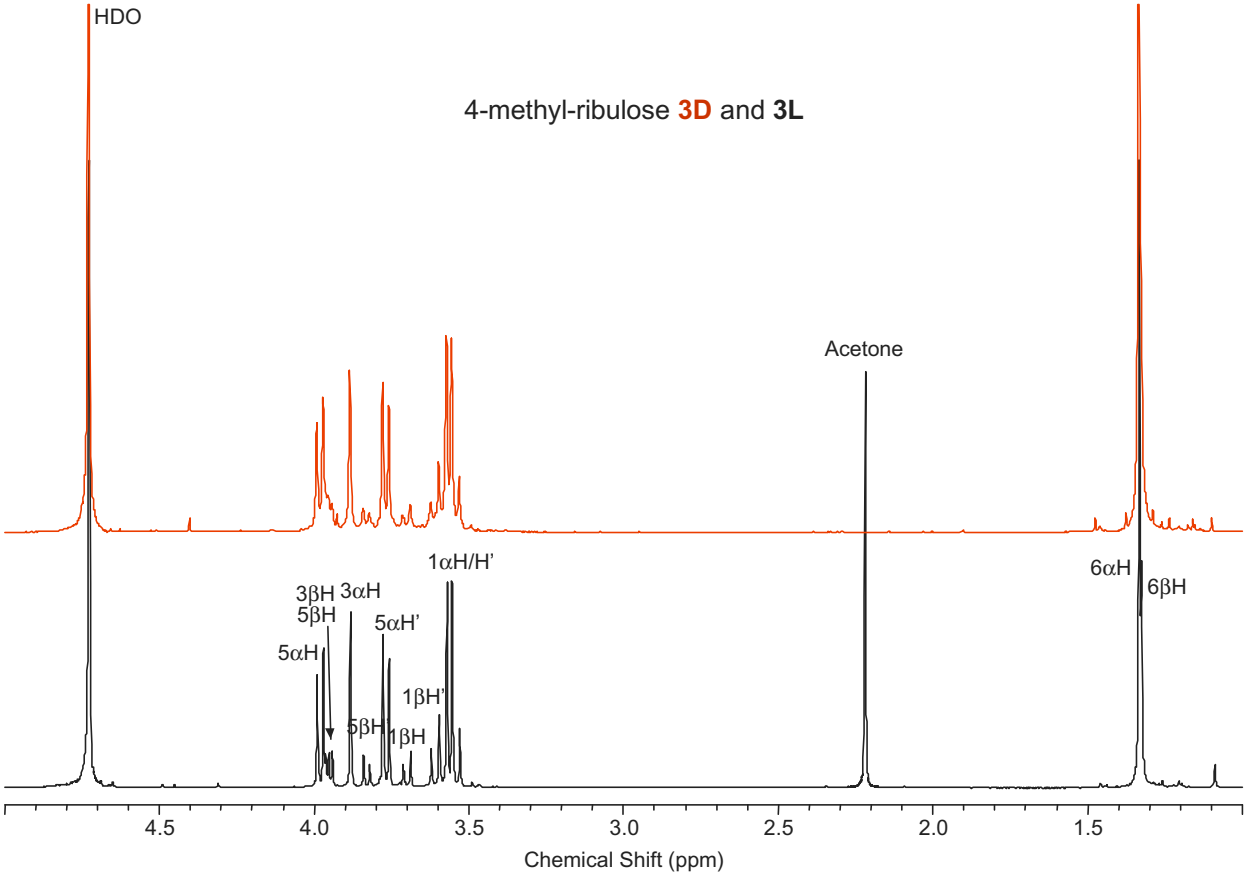


Fig. 3. ^{1}H NMR of 4-*C*-methyl-ribulose **3** L-enantiomer in black, D-enantiomer in red.

70% yield. DTE also epimerizes D-xylulose at C-3 to produce D-ribulose.[13] Reaction of 4-*C*-methyl-D-xylulose **4D** with DTE afforded a mixture of the two epimers **4D** and **3D** in a ratio of 30:70, allowing the isolation of 4-*C*-methyl-D-ribulose **3D** [oil, $[\alpha]_D^{20}$ +2.7 (*c* 1.0, water)] in 50% yield. The purity of the two epimers was established by HPLC (Fig. 2).

A detailed NMR study of pentuloses **3** and **4** was undertaken in order to establish their structures in solution. The ^{1}H NMR spectra of **3L** and **3D** are identical, as are the spectra of **4L** and **4D**. All the four samples are greater than 95% pure as judged by the NMR spectra.

Full ^{1}H and ^{13}C NMR assignments for 4-*C*-methyl ribulose **3** and the 4-*C*-methyl xylulose **4** are given in Table 1. Both **3** and **4** are present in solution in the two anomeric furanose forms (Scheme 4). If the keto-form is present in either, it is below the detection limit. In **3**, the C-4CH_3 resonances give an NOE to the C-3H resonances of similar magnitude to the stronger of the two NOEs to the C-5Hs, indicating that the methyl group and C-3H are on the same side of the ring (consistent with a ribulose configuration). In the major anomer of **4**, the C-4CH_3 resonance gives an NOE to the C-3H resonance of similar magnitude to the weaker of the two NOEs to the C-5Hs, indicating that the methyl group and C3H are on the opposite sides of the ring (consistent with a xylulose configuration). In **3**, and less certainly in **4**, the major isomer shows an NOE between C-1H/H′ and C-3H, indicating that the α anomer is the major isomer present in **3** and the β anomer is the major isomer present in **4**.

NMR studies of D-ribulose and D-xylulose have been previously reported.[16] The general pattern of ^{13}C chemical shifts is similar for the pentuloses and the 4-*C*-methyl pentuloses. The major anomers reported for the pentuloses are α-ribulose (61%) and β-xylulose (62%), as is found for the 4-*C*-methyl pentuloses. However, a significant proportion of the open-chain keto-form was observed for both pentuloses (~19%), whereas these forms are below the detection limit for the 4-*C*-methyl pentuloses (Figs. 3 and 4).

In summary, this Letter reports the first syntheses of both enantiomers of the hitherto unknown 4-*C*-methyl pentuloses. Although the isomerization of the ketoses with the corresponding aldoses [in purple in Scheme 1] by aldose isomerases has yet to be confirmed, this work indicates that the technique of Izumoring may be applied to a wide range of both naturally occurring and synthetic carbohydrates and provides practical amounts of the new monosaccharides to evaluate their biological potential. The combination of chemistry with biotechnology in this Letter illustrates the synergistic potential in the synthesis of novel sugars.

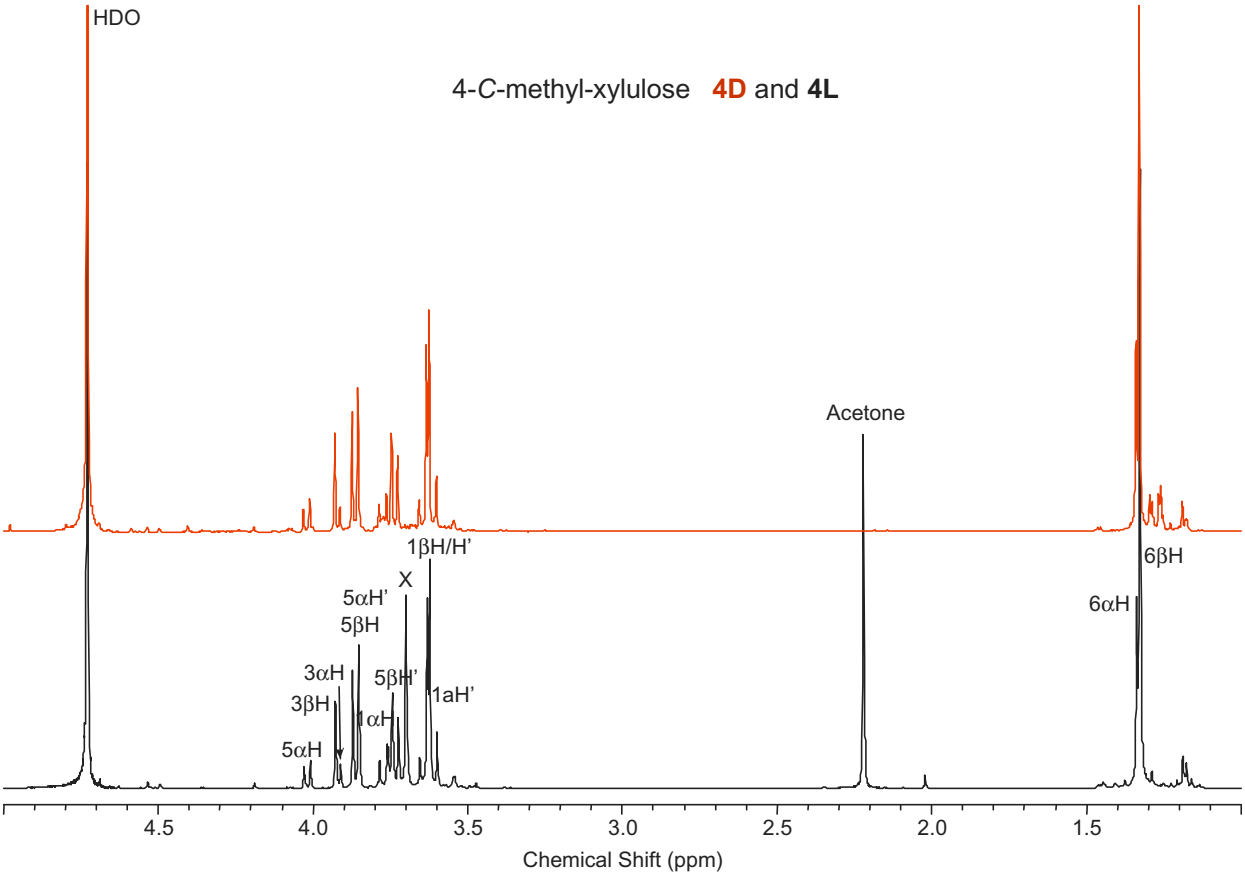


Fig. 4. ^{1}H NMR of 4-*C*-methyl-xylulose **4** L-enantiomer in black, D-enantiomer in red.

Acknowledgement

This work was supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN), by Summit plc and by Fundação para a Ciência e a Tecnologia, Portugal for a doctoral fellowship (F.P. da C., grant BD/17572/2004). We acknowledge a generous gift of 2-*C*-methyl-ribonolactone from Dr. T. Heinz of Novartis, Basel.

References and notes

1. (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) Bertelsen, H.; Jensen, B. B.; Buemann, B. *World Rev. Nutritional Dietetics* **1999**, *85*, 98–109; (e) Skytte, U. P. *Cereal Food World* **2002**, *47*, 224.
2. (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) 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.
3. (a) Tao, J.; Zhao, L.; Ran, N. *Org. Process Res. Dev.* **2007**, *11*, 259–267; (b) Panke, S.; Held, M.; Wubbolts *Curr. Opin. Biotechnol.* **2004**, *15*, 272–279.
4. (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.
5. (a) Izumori, K. *J. Biotechnol.* **2006**, *124*, 717–722; (b) Izumori, K. *Naturwissenschaften* **2002**, *89*, 120–124.
6. (a) Rahman, A. K.; Tokunaga, H.; Yoshida, K.; Izumori, K. *J. Ferment. Bioeng.* **1991**, *72*, 488–490; (b) Muniruzzaman, S.; Tokunaga, H.; Izumori, K. *J. Ferment. Bioeng.* **1995**, *9*, 323–327; (c) Bhuiyan, S. H.; Ahmed, Z.; Utamura, M.; Izumori, K. *J. Ferment. Bioeng.* **1998**, *86*, 513–516.
7. (a) Yoshida, H.; Yamada, M.; Nishitani, T.; Takada, G.; Izumori, K.; Karnitori, S. *J. Mol. Biol.* **2007**, *374*, 443–453; (b) Ishida, Y.; Kamiya, T.; Izumori, K. *J. Ferment. Bioeng.* **1997**, *84*, 348–350.
8. (a) Gullapalli, P.; Shiji, T.; Rao, D.; Yoshihara, A.; Morimoto, K.; Takata, G.; Fleet, G. W. J.; Izumori, K. *Tetrahedron: Asymmetry* **2007**, *18*, 1995–2000; (b) Yoshihara, A.; Haraguchi, S.; Gullapalli, P.; Rao, D.; Morimoto, K.; Takata, G.; Jones, N. A.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J.; Izumori, K. *Tetrahedron: Asymmetry* **2008**, *19*.
9. (a) Muniruzzaman, S.; Pan, Y. T.; Zeng, Y.; Atkins, B.; Izumori, K.; Elbein, A. D. *Glycobiology* **1996**, *6*, 795–803; (b) Oka, H.; Suzuki, S.; Suzuki, H.; Oda, T. *Clin. Chim. Acta* **1976**, *67*, 131–136; (c) Vincent, M. F.; Berghe, V. G.; Hers, H. G. *FASEB J.* **1989**, *3*, 1855–1861; (d) Muynck, C. D.; Pereira, C.; Soetaert, W.; Vandamme, E. *J. Biotechnol.* **2006**, *125*, 408–415; (e) Ito, M.; Amano, H.; Yanagisawa, I. *Syst. Biol. Reprod. Med.* **1978**, *1*, 77–82; (f) Huck, J. H. J.; Roos, B.; Jakobs, C.; Knaap, M. S. V.; Verhoeven, N. M. *Mol. Gen. Metab.* **2004**, *82*, 231–237; (g) Cho-Vega, J. H.; Tsavachidis, S.; Kim-Anh Do, K.-A.; Nakagawa, J.; Medeiros, L. J.; McDonnell, T. J. *Cancer Epidem. Biomarkers Prevent* **2007**, *16*, 2615–2622.
10. (a) Hotchkiss, D. J.; Soengas, R.; Booth, K. V.; Weymouth-Wilson, A. C.; Eastwick-Field, V.; Fleet, G. W. J. *Tetrahedron Lett.* **2007**, *48*, 517–520; (b) Hotchkiss, D. J.; Jenkinson, S. F.; Storer, R.; Heinz, T.; Fleet, G. W. J. *Tetrahedron Lett.* **2006**, *47*, 315–318.
11. *Data for 2-C-methyl-*D*-ribitol* **1**: HRMS (ESI+ve) Found: 189.0733 ($M+Na^+$); $C_6H_{14}NaO_5$ requires: 189.0733; $[\alpha]_D^{22}$ +15.8 (*c* 0.88, MeOH); ν_{max} (thin film): 3356 (br s, OH); δ_H (D_2O, 400 MHz): 1.20 (3H, s, Me), 3.49 (1H, d, H-1, *J* 11.8), 3.62 (1H, d, H-3, *J* 7.9), 3.63 (1H, d, H-1′, *J* 11.7), 3.66 (1H, dd, H-5, *J* 6.0, 11.9), 3.81 (1H, dd, H-5′, *J* 3.0, 11.9), 3.86 (1H, ddd, H-4, *J* 2.9, 6.0, 7.9); δ_C (D_2O, 100.6 MHz): 19.1 (Me), 63.8 (C-5), 67.2 (C-1), 72.3 (C-3), 72.5 (C-4), 75.8 (C-2); *m*/*z* (ESI−ve): 331 ($2M-H^+$, 8%), 225 ($M+OAc^-$, 28%), 165 ($M-H^+$, 100%).
12. (a) Mizanur, R. M.; Takeshita, K.; Moshino, H.; Takada, G.; Izumori, K. . *J. Biosci. Bioeng.* **2001**, *92*, 237–241; (b) Takeshita, K.; Shimonishi, T.; Izumori, K. *J. Ferment. Bioeng.* **1996**, *81*, 212–215; (c) Bhuiyan, S. H.; Ahmed, Z.; Utamura, M.; Izumori, K. *J. Ferment. Bioeng.* **1998**, *86*, 513–516; (d) Ahmed, Z.; Sasahara, H.; Bhuiyan, S. H.; Saiki, T.; Shimonishi, T.; Takada, G.; Izumori, K. . *J. Biosci. Bioeng.* **1999**, *88*, 676–678; (e) Sultana, I.; Mizanur, R. M.; Takeshita, K.; Takada, G.; Izumori, K. *J. Biosci. Bioeng.* **2003**, *95*, 342–347.
13. (a) Izumori, K.; Khan, A. R.; Okaya, H.; Tsumura, T. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1037–1039; (b) Itoh, H.; Sato, T.; Izumori, K. *J. Ferment. Bioeng.* **1995**, *80*, 101–103; (c) Itoh, H.; Izumori, K. *J. Ferment. Bioeng.* **1996**, *81*, 351–353; (d) Itoh, H.; Sato, T.; Takuchi, T.; Khan, A. R.; Izumori, K. . *J. Ferment. Bioeng.* **1995**, *79*, 184–185; (e) Ishida, Y.; Kamiya, T.; Izumori, K. *J. Ferment. Bioeng.* **1997**, *84*, 348–350.
14. The ratio of the protected lactone to the unprotected lactone varies in regard to the work-up of the Kiliani reaction. See Ref. 10 and: Jenkinson, S. F.; Jones, N. A.; Moussa, A.; Stewart, A. J.; Heinz, T.; Fleet, G. W. J. *Tetrahedron Lett.* **2007**, *48*, 4441–4445.
15. *Data for 2-C-methyl-*D*-arabinitol* **2**: HRMS (ESI+ve): 189.0734 ($M+Na^+$); $C_6H_{14}NaO_5$ requires: 189.0733; $[\alpha]_D^{21}$ +13.5 (*c* 1.0 in MeOH); ν_{max} (thin film): 3356 (s, br, OH); δ_H (D_2O, 400 MHz): 1.20 (3H, s, Me), 3.58–3.67 (4H, m, 3 × CH_2, 1 × CH), 3.80–3.85 (2H, m, 1 × CH_2, 1 × CH); δ_H (MeOD, 400 MHz): 1.23 (3H, s, Me), 3.52 (1H, d, H-3, *J* 7.1), 3.56–3.66 (3H, m, 3 × CH_2), 3.75–3.84 (2H, m, H-4, CH_2); δ_C (D_2O, 100.6 MHz): 20.1 (Me), 63.7 (CH_2), 67.0 (CH_2), 72.2 (CH), 74.8 (CH), 75.4 (C-2); δ_C (MeOD, 100.6): 21.7 (Me), 65.0 (CH_2), 68.2 (CH_2), 73.7 (C-4), 75.6 (C-2), 76.2 (C-3); *m*/*z* (ESI−ve): 165 ($M-H^+$, 100%).
16. Vuorinen, T.; Serianni, A. S. *Carbohydr. Res.* **1991**, *209*, 13–31.