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Isomerization of deoxyhexoses: green bioproduction of 1-deoxy-D-tagatose from L-fucose and of 6-deoxy-D-tagatose from D-fucose using *Enterobacter agglomerans* strain 221e

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Abstract—1-Deoxy-D-tagatose was produced by the hydrogenation of 6-deoxy-L-galactose (L-fucose) to L-fucitol followed by oxidation with *Enterobacter agglomerans* 221e; a similar sequence on D-fucose afforded 6-deoxy-D-tagatose. Thus, the polylol dehydrogenase recognizes the D-*galacto*-configuration of both D-fucitol and L-fucitol. The procedures were conducted in water and show the power of green, environmentally friendly biotechnology in the preparation of new monosaccharides with a potential for novel bioactive properties. 6-Deoxy-D-tagatose was also synthesized from D-tagatose via the efficient formation of 1,2:3,4-di-*O*-isopropylidene-α-D-tagatofuranose; a difficult final removal of protecting groups by acid makes the biotechnological route more attractive.

1. Introduction

Although Izumoring has provided the biotechnology for the large scale production of any hexose,[1] very few reports of the isomerization of deoxy hexoses have appeared.[2] Recently, the efficient isomerization of L-rhamnose (the only cheaply available deoxy hexose) to 1-deoxy-L-fructose has been reported on a multigram scale.[3]

Herein, we extend the isomerization of deoxyhexoses by the conversion of L-fucose **1L** to 1-deoxy-D-tagatose **3** by initial hydrogenation to L-fucitol **2L** followed by microbial oxidation using *Enterobacter agglomerans* 221e; a similar isomerization of D-fucose **1D** involved sequential hydrogenation to D-fucitol **2D** and oxidation by the same microbe to afford 6-deoxy-D-tagatose **4** (Scheme 1). L-Fucitol **1L** can be viewed as either 6-deoxy-L-galactitol or as 1-deoxy-D-galactitol; both enantiomers of fucitol are oxidized to form D-tagatoses **3** and **4** so that the microbe selectively recognizes the D-*galacto*—rather than the L-*galacto*—structural motif. This is consistent with the selectivity in the oxidation of achiral galactitol **6** by *E. agglomerans* 221e, which exclusively affords D-tagatose **5D** as the sole product with none of the enantiomeric L-tagatose **5L** being formed (Scheme 2).[4] An authentic sample of 6-deoxy-D-tagatose **4** was prepared from D-tagatose **5D** via the efficient formation of diacetone tagatose **7**.

2. Results and discussion

2.1. Synthesis of 1-deoxy-D-tagatose 3 and of 6-deoxy-D-tagatose 4

1-Deoxy-D-tagatose **3** was prepared by using a combined biotechnological and chemical route from L-fucose **1L** (Scheme 3). The hydrogenation of L-fucose **1L** in water in the presence of a nickel catalyst at 1.2 MPa, 50 °C gave L-fucitol **2L** in virtually quantitative yield when 1.0% (w/v) of the substrate was used. The NMR of L-fucitol **2L** (Table 1) is unusual in that there is no averaging of the coupling constants for C2H–C3H–C4H–C5H.

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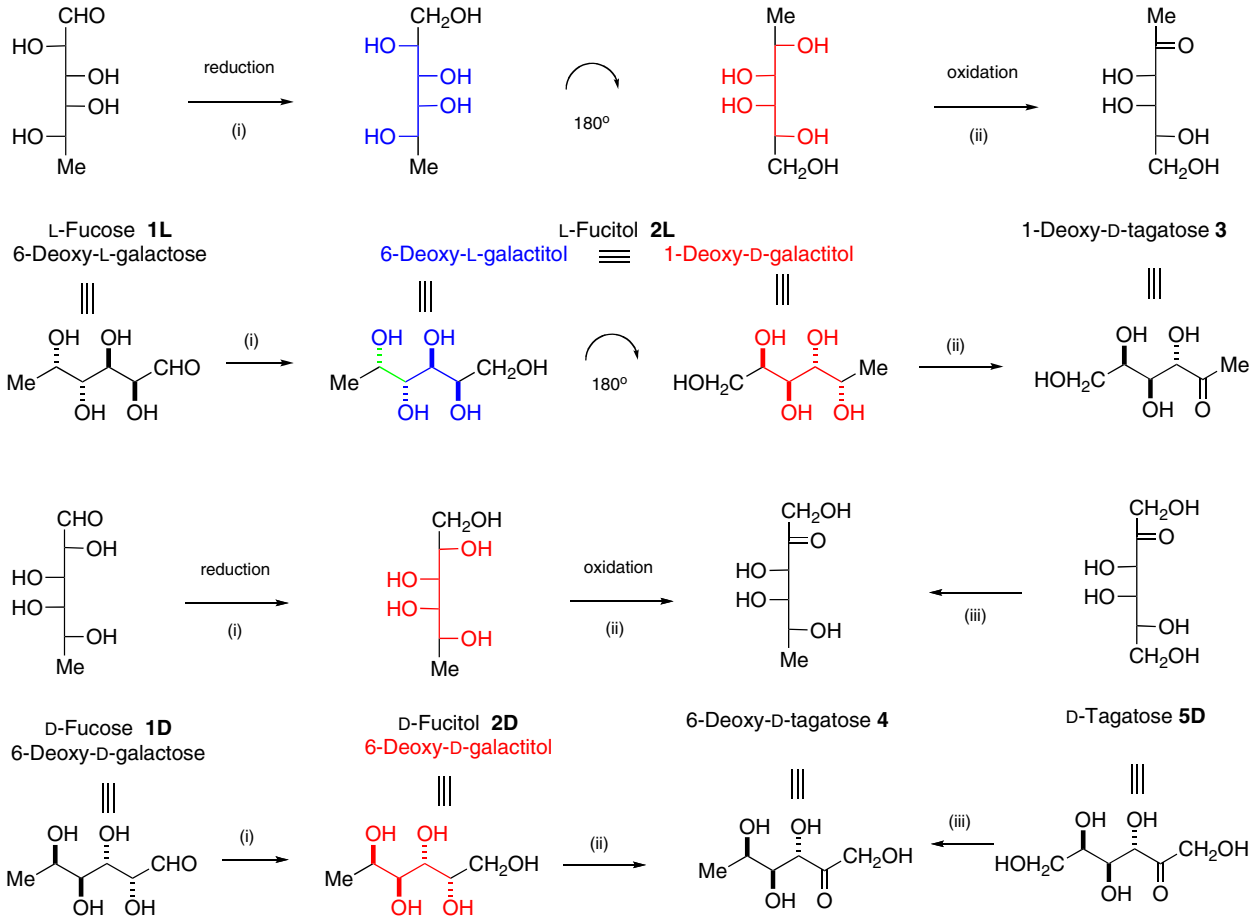
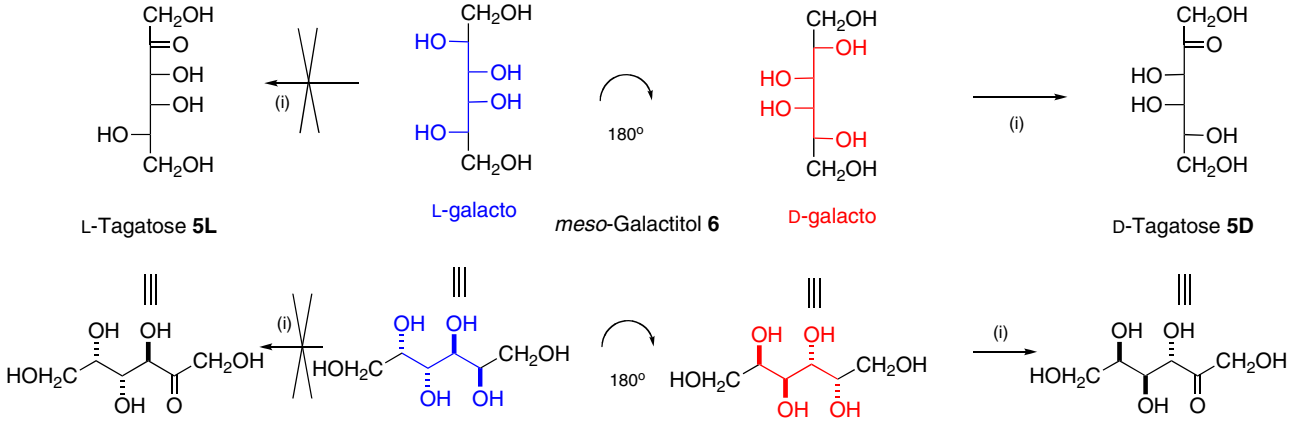
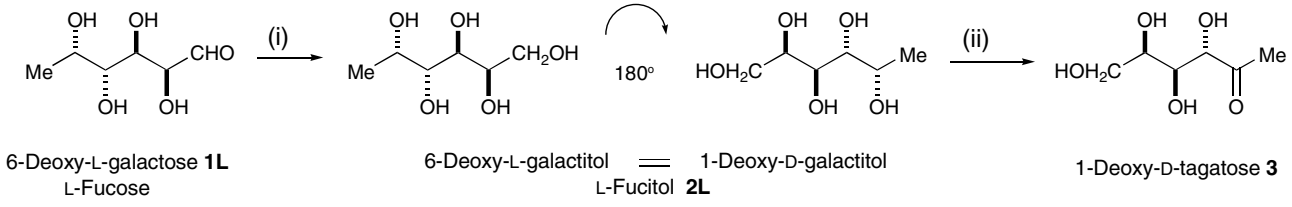


Scheme 1. Reagents: (i) H_2, Ni, H_2O; (ii) *Enterobacter agglomerans* 221e, H_2O; (iii) see text.



Scheme 2. Reagents: (i) *Enterobacter agglomerans* 221e, H_2O.



Scheme 3. Reagents and conditions: (i) Nickel, H_2, H_2O, 1.2 MPa pressure, 50 °C, 96%; (ii) *Enterobacter agglomerans* strain 221e, 86%.

Table 1. NMR analysis of 1D (^{1}H and ^{13}C) and HSQC of L-fucitol **2L** (chemical shift referenced to acetone at 2.22 ppm for ^{1}H and 30.9 ppm for ^{13}C), pH 5.0, ^2H_2O

	Label	^{1}H			^{13}C
		δ (ppm)	Mult	$^3J_{HH}$ (Hz)	δ (ppm)
HOH_2C^1	1	3.676	m		63.9
OH	2	3.955	m		71.2
HO	3	3.633	dd	1.5/9.0	70.5
OH	4	3.475	dd	9.0/1.8	73.6
HO	5	4.082	dq	1.8/6.6	66.7
$_6CH_3$	6	1.237	d	6.6	19.3

Microbial oxidation of L-fucitol **2L** to afford 1-deoxy-D-tagatose **3** was carried out using *E. agglomerans* 221e, a well-established catalyst for oxidation–reduction reactions between various polyols and ketoses.[5] The highest activity for the oxidation of L-fucitol **2L** was observed when this microbe was grown on a medium containing 0.5% polypepton, 0.5% yeast extract, 0.5% NaCl, 1.0% erythritol and 1.0% glycerol. A study of a range of different buffers between pH 4.0 and 11.0 (50 mM sodium acetate pH 4.0–6.0, 50 mM sodium–phosphate pH 6.0–8.0, 50 mM Tris–HCl pH 7.0–9.0 and 50 mM glycine–NaOH pH 9.0–11.0) showed that the optimum conditions for the oxidation occurred at a pH of 8.0 in a Tris–HCl buffer. Under these optimum conditions, 1-deoxy-D-tagatose **3** was isolated from L-fucitol **2L** in 86% yield; a very small quantity of a by-product was also formed but was removed by crystallization. The purity of both substrate **2L** and of product **3** was established by HPLC (Fig. 1).

1-Deoxy-D-tagatose **3** crystallizes as two different polymorphs of 1-deoxy-α-D-tagatopyranose,[6] which is also the major form present in the solution; the β-pyranose form is present at about one quarter the concentration together with minor amounts of three other forms, giving a complex NMR spectrum in solution. The ^{13}C NMR of 1-deoxy-D-tagatose **3** in water was identical to that of a synthetic sample.[7] Although **3** has been synthesized in small amounts by chemical reactions before,[8] the present biotechnological procedure allows sufficient quantities to be readily prepared for full biological evaluation.

A similar procedure was used for the conversion of D-fucose **1D** to 6-deoxy-D-tagatose **4** (Scheme 4). Hydrogenation of D-fucose **1D** in water in the presence of a nickel catalyst proceeded to give D-fucitol **2D** in nearly quantitative yield. The optimum conditions for the oxidation of D-fucitol **2D** by *E. agglomerans* 221e to 6-deoxy-D-tagatose **4** were the same as those for the conversion of L-fucitol **2D** to 1-deoxy-D-tagatose **2** (80% yield). Separation and purification of 6-deoxy-D-tagatose **4** was achieved by column chromatography on Dowex 50W-X2 (Ca^{2+}) at 60 °C.

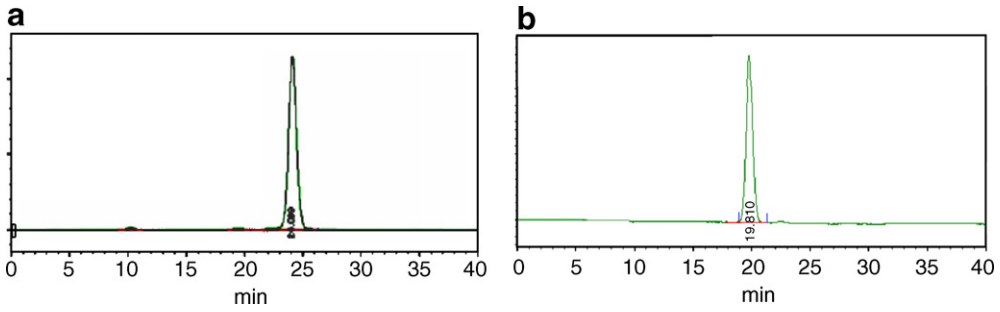
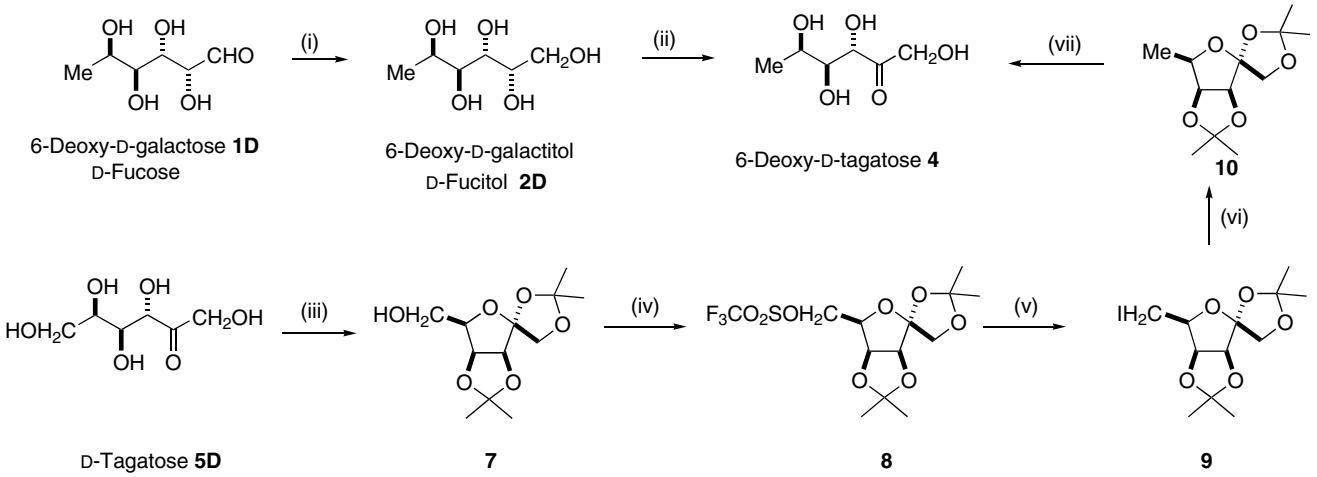


Figure 1. HPLC analysis of (a) L-fucitol **2L** and (b) purified 1-deoxy-D tagatose **3**.



Scheme 4. Reagents and conditions: (i) Nickel, H_2, H_2O, 1.2 MPa pressure, 50 °C, 86%; (ii) *Enterobacter agglomerans* strain 221e, 74%; (iii) Me_2CO, $CuSO_4$, concd H_2SO_4, 82%; (iv) $(CF_3SO_2)_2O$, CH_2Cl_2, pyridine, 98%; (v) $Bu_4N^+I^-$, THF, 96%; (vi) H_2, 10% Pd/C, Et_3N, EtOH, 96%—see text; (vii) Dowex (50W-X8, H^+), H_2O, 90%.

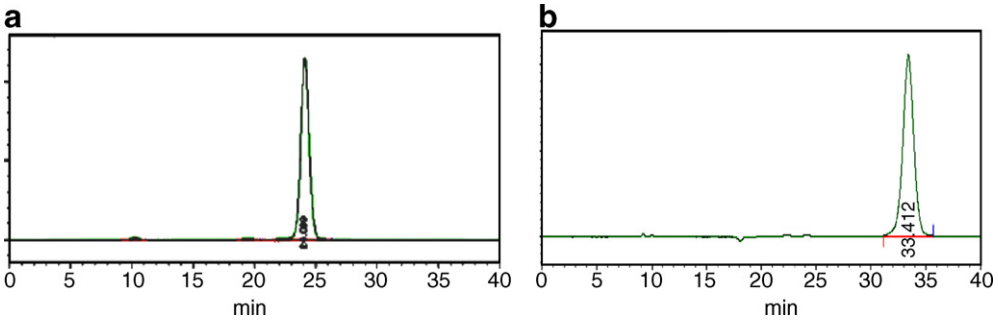


Figure 2. HPLC analysis of (a) D-fucitol **2D** and (b) purified 6-deoxy-D tagatose **4**.

6-Deoxy-D-tagatose **4**, was identified by HPLC (Fig. 2). This is the first report of the bioproduction of 6-deoxy-D-tagatose **4** from 6-deoxy-D-galactose (D-fucose) **1D**.

An authentic sample of 6-deoxy-D-tagatose **4** was also prepared by a chemical route from D-tagatose **5D**. D-Tagatose **5D**, a possible food substitute,[9] has only recently become readily available at a reasonable cost[10] for the use as a member of the chiral pool.[11] In the past, the acetonides of tagatose have usually been made by a multi-step processes from fructose[12] or sorbose.[13] However, treatment of D-tagatose **5D** with acetone in the presence of copper sulfate and sulfuric acid gave the easily crystallized diacetonide **7**[14] in 82% yield. The free primary alcohol in **7** was esterified with trifluoromethanesulfonic (triflic) anhydride in dichloromethane in the presence of pyridine to afford triflate **8** (98% yield), which on reaction with tetrabutylammonium iodide in THF gave the corresponding iodide **9** in 96% yield. Hydrogenolysis of iodide **9** with 10% palladium on carbon in ethanol in the presence of triethylamine formed the protected deoxy tagatose **10** in 96% yield; the diacetonide **10** is sensitive to acid and partially decomposed on silica gel chromatography. Careful hydrolysis of **10** to remove the isopropylidene protecting groups by Dowex ion exchange resin (50W-X8, H^+) gave an authentic sample of 6-deoxy-D-tagatose **4** in 90% yield; the hydrolysis of ketose diacetonides is frequently experimentally difficult, probably due to the sensitivity of the monoacetonides to acid.[15] Although the synthetic sequence is efficient (70% overall yield from D-tagatose **5D**), the difficulties of acid hydrolysis in the final step make this an unattractive method in comparison to the biotechnological route.

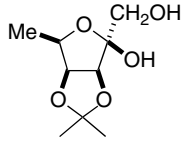
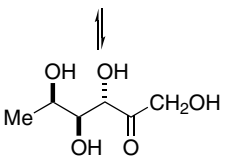
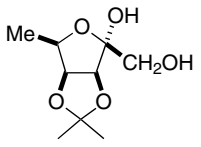
The NMR of 6-deoxy-D-tagatose **4** in solution is complex but it mainly exists as the two furanoses, together with other minor forms. The ^{13}C NMR of the sample of 6-deoxy-D-tagatose **4** prepared from D-fucitol **2D** was identical to that of the sample prepared from D-tagatose **5D**.

The detailed NMR analysis of the solution spectrum of 6-deoxy-D-tagatose **4** is shown in Table 2. There are two major spin systems, with relative intensities of approximately 2 to 1 which probably correspond to the β **4**β and α **4**α anomers of the furanose form, respectively; both tagatose **5** and 1-deoxy-D-tagatose **3** exist mainly as pyranose forms with only minor amounts of the respective furanoses present. As both spin-systems are in the furanose form, it is difficult to assign relative configurations based on coupling constants. There are very few useful NOEs; a medium enhancement of **4**β-1 to **4**β-3 is consistent with the β-furanose form and the weak enhancement of **4**α-1 to **4**α-3 is consistent with the α-furanose For both tagatose **5** and 6-deoxy-tagatose **4**, the α-furanose forms have a C3H–C4H coupling of 5.0 and 5.3 Hz, respectively, and the β-furanose a coupling of 4.9 and 4.5 Hz, consistent with spin-system 2 being the α-anomer. In the furanose forms of tagatose **5** and 1-deoxy-tagatose **3**, the ratios of α/β anomer are 0.35/1 and 1/1, respectively, the former again being consistent with spin system 2 being the α-anomer.

3. Conclusion

In conclusion, we have reported the overall isomerizations of the enantiomers of fucose **2L** and **2D** to 1-deoxy-D-tagatose **3** and 6-deoxy-D-tagatose **4**, respectively. These

Table 2. NMR analysis of 1D (^1H and ^{13}C) and HSQC of 6-deoxy-D-tagatose **4** (chemical shift referenced to acetone at 2.22 ppm for ^1H and 30.9 ppm for ^{13}C), pH 6.9, ^2H_2O

	Label	^1H			^{13}C
		δ (ppm)	Mult	$^3J_{HH}$ (Hz)	δ (ppm)
 **4β**	**4**β-1	3.570	d	−12.0	63.39
		3.504	d	−12.0	
	4β-2	—	—	—	102.86
	4β-3	4.233	d	4.7	71.80
	4β-4	4.122	dd	4.7/?	73.17
	4β-5	4.115	dq	?/6.2	77.09
	4β-6	1.277	d	6.2	15.21
 **4**	**4**α-1	3.650	d	−11.8	63.10
		3.576	d	−11.8	
	4α-2	—	—	—	105.24
	4α-3	4.287	d	5.0	79.21
	4α-4	4.13	dd	5.0/3.5	73.54
	4α-5	4.338	dq	3.5/6.5	76.10
	4α-6	1.216	d	6.5	14.22
 **4α**					

syntheses are further examples of environmentally friendly access to very rare deoxy sugars to allow evaluation of their biological properties; the biotechnological synthesis of such sugars gives much cleaner materials due to the sensitivity of the product ketoses to the acid conditions needed at the final deprotection in chemical synthesis.

4. Experimental

L-Fucose and D-fucose and all other biochemicals were purchased from Sigma Chemical Co. (MO, USA) and Wako Pure Chemicals (Osaka, Japan). D-Tagatose was obtained as a generous gift from Arla Foods. 50 mM Tris–HCl buffer, used for L- and D-fucitol oxidations, was prepared from tris(hydroxymethy)aminoethane $H_2NC(CH_2OH)_3$ by adjusting the pH to 8.0 with 1.0 N HCl. D- and L-Fucose hydrogenation was performed in TEM-1000M hydrogenation apparatus (Taiatsu Techno Co. Ltd, Japan). Microbe growth and oxidation reactions were carried in bioreactors (TS-M-15L fermentor and TS-M-5L fermentor) from Takasugi Seisakusho Co. Ltd and oxidation reactions were carried out in Erlenmeyer flasks (500 mL). Polyol oxidation and ketose accumulation in the reaction mixture was determined by Nelson–Somogyi method[16] by UV by a visible spectrophotometer (UV-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. 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 (^1H: 500 MHz and ^{13}C: 125.7 MHz) and Bruker DPX 400 and DQX 400 spectrometers (^1H: 400 MHz and ^{13}C: 100.6 MHz) in the deuterated solvent stated. For spectra recorded in D_2O methanol was used as an internal reference. Residual signals from other solvents were used as an internal reference. NMR spectra for **2L** and **4** were recorded on a Varian Unity INOVA 500 (^1H: 500 MHz and ^{13}C: 125 MHz) spectrometer, in D_2O, with a probe temperature of 30 °C. Chemical shifts were measured relative to internal standards (^1H: acetone at 2.220 ppm and ^{13}C: acetone at 30.9 ppm). Two-dimensional gradient COSY, HSQC, HMBC and HSQC-TOCSY spectra were used to aid assignment of ^1H and ^{13}C spectra. NOESY spectra were recorded with a 400 ms mixing time. 1D ^1H NMR spectral simulations were performed using the program gNMR (Cherwell Scientific Publishing). All chemical shifts (δ) are quoted in ppm and coupling constants (J) in Hertz.

4.1. L-Fucitol 2L and D-fucitol 2D

4.1.1. Preparation of catalyst. Raney nickel (30 g) was dissolved in 300 mL of 20% aqueous sodium hydroxide (300 mL). The catalyst was heated at 80 °C for 6 h and then washed several times with distilled water to obtain pH 9.2. The resultant precipitate was used for hydrogenation of both enantiomers of fucose.

4.1.2. L-Fucitol 2L. The prepared catalyst was added to 1% solution of L-fucose **1L** (5 g) in water; the hydrogenation reaction mixture was adjusted to a total volume of 500 mL. The reaction was carried out at 1.2 MPa hydrogen pressure at 50 °C for 24 h to afford L-fucitol **2L**, (4.8 g, 96%), as an oil, $[\alpha]_D^{20} = +1.9$ (c 1.0, water).

D-Fucitol **2D** was prepared in an identical procedure from D-fucose **1D** (5 g) in water to give D-fucitol **2D** (4.8 g, 96%), oil, $[\alpha]_D^{20} = -1.9$ (c 1.0, water).

4.2. 1-Deoxy-D-tagatose 3 and 6 by microbial oxidation of 2L and 2D

4.2.1. Optimization of conditions. *E. agglomerans* 221e, isolated in our laboratory,[17] was used for the oxidations of L-fucitol **2L** to 1-deoxy-D-tagatose **3** and of D-fucitol **2L** to 6-deoxy-D-tagatose **4**. The strain was maintained at 4 °C on 2% TSB agar slant. This bacteria was grown aerobically in Erlenmeyer flasks (500 mL) containing 200 mL medium (0.5% polypepton, 0.5% yeast extract, 0.5% NaCl, 1.0% erythritol and 1.0% glycerol) at 30 °C for 24 h. The cells were cultivated using the above medium for 24 h and harvested by centrifugation at 9000 rpm for 10 min. The collected cells were washed twice with deionized water and centrifuged at 9000 rpm for 10 min. The washed cells were suspended in various buffers for carrying out the biotransformations. The effect of pH from 6.0 to 11.0 on both oxidations was studied using buffers (50 mM sodium acetate pH 4.0–6.0, 50 mM sodium phosphate pH 6.0–8.0, 50 mM Tris–HCl pH 7.5–9.0, 50 mM glycine–NaOH pH 9.0–11.0). The reaction mixtures were analyzed by HPLC after

2 h for the transformations of L-fucitol **2L** to 1-deoxy-D-tagatose **3** and of D-fucitol **2D** to 6-deoxy-D-tagatose **4**.

4.2.2. 1-Deoxy-D-tagatose 3. The *E. agglomerans* 221e cells were cultivated, harvested and washed as described above. The collected cells were suspended in 50 mM Tris–HCl buffer (pH 8.0) and the transformation reaction was carried out in a 500 mL Erlenmeyer flasks containing 1% L-fucitol **2L** (4.8 g, 0.03 mol) at 30 °C with continuous shaking at 180 rpm. The formation of 1-deoxy-D-tagatose **3** was monitored by HPLC. At the end of the reaction, the cells were removed by centrifugation at 10,000 rpm for 10 min. The supernatant was decolorized using activated charcoal which was then removed using centrifugation and filtration. The decolorized supernatant was deionized using ion exchange resin diaion (SKIB, H^+) and amberlite (IRA-411, CO_3^{2-}). This deionized solution was concentrated using a rotary evaporator; the product **3** (3.8 g, 0.02 mol 80%) purified by crystallization mp 132 °C $[\alpha]_D^{20} = -14.7$ (*c* 1.0, water) with identical ^1H and ^{13}C NMR to an authentic sample. 1-Deoxy-D-tagatose **4** was recrystallized from a mixture of EtOAc and MeOH to give colorless crystals as two polymorphs;[17] mp 136–138 °C and 143–145 °C (lit.[23a] mp 121–123 °C, lit.[23b] mp 130 °C); $[\alpha]_D^{22} = -13$ (*c* 2.0, H_2O) {lit.[23a] $[\alpha]_D^{23} = -14$ (*c* 2.0, H_2O)}.

4.2.3. 6-Deoxy-D-tagatose 4. The oxidation of D-fucitol **2D** (4.8 g, 0.03 mol) by *E. agglomerans* 221 to 6-deoxy-D-tagatose **4** was performed in an identical manner to that of L-fucitol **2D** above. After removal of the animal charcoal, the supernatant was deionized using ion exchange resin diaion (SKIB, H^+) and amberlite (IRA-411, CO_3^{2-}). The deionized supernatant was concentrated and the product separated using Dowex 50W-X2 resin (Ca^{2+}). The purity of the product **4** was established by HPLC (Fig. 2); 6-deoxy-D-tagatose **4** (3.5 g, 0.02 mol 73%) was isolated as an oil, $[\alpha]_D^{20} = -2.2$ (*c* 1.0, water) {lit. an oil, except for enantiomer mp 68–69 °C[21b]}; $[\alpha]_D^{20} = -1.6$ (*c* 1.4, water) {$[\alpha]_D^{20} = -3$ (*c* 2, water),[18] $[\alpha]_D^{18} = -2$ (*c* 2, water),[19] $[\alpha]_D^{23} = 0$ (*c* 1.9, water),[20] for L enantiomer $[\alpha]_D^{18} = +2.7$ (*c* 12.8, water) $[\alpha]_D^{18} = -2$ (*c* 2, water).[21] HRMS (ESI −ve) found: 163.0608 ($M-H^+$); $C_6H_{11}O_5$ requires: 163.0601; *m/z* (ESI −ve): 163 ($M-H^+$, 100%). For NMR data, which is the same as the chemical sample prepared below, see text above and Table 2.

4.3. 6-Deoxy-D-tagatofuranose 4 from D-tagatose 5D

4.3.1. 1,2:3,4-Di-*O*-isopropylidene-α-D-tagatofuranose 7. Anhydrous copper sulfate (35.8 g, 0.22 mol) and concentrated sulfuric acid (0.4 mL) were added to a solution of D-tagatose **5D** (10.1 g, 0.06 mol) in acetone (200 mL). The reaction mixture was stirred at room temperature for 18 h, after which time TLC (EtOAc/cyclohexane, 1:1) showed the formation of one major product (R_f 0.55). The reaction mixture was neutralized with sodium carbonate (solid), filtered through Celite and concentrated in vacuo. The crude residue was purified by column chromatography (EtOAc/cyclohexane, 1:4) to give the diacetonide **7** (12 g, 82%) as a white crystalline solid, mp 55–58 °C $[\alpha]_D^{22} = +66.3$ (*c* 1.04, $CHCl_3$), {lit.[22] mp 63–65 °C; $[\alpha]_D = +81.5$ (*c* 1.7, Me_2CO)} for enantiomer:[13] mp 65–66 °C; $[\alpha]_D = -63$ (*c* 1.0, $CHCl_3$)}; ν_{max} (thin film): 3491 (m, br, OH); δ_H ($CDCl_3$, 400 MHz): 1.31 (3H, s, Me), 1.40 (3H, s, Me), 1.43 (3H, s, Me), 1.47 (3H, s, Me), 2.21–2.24 (1H, dd, OH, *J* 4.8, 7.7), 3.85–3.97 (2H, m, H6, H6′), 4.04–4.08 (1H, m, H5), 4.08 (1H, a-d, H1, *J* 9.7), 4.27 (1H, d, H1′, *J* 9.7), 4.62 (1H, d, H3, *J* 5.9), 4.83–4.85 (1H, dd, H4, *J* 5.8, 3.9); δ_C ($CDCl_3$, 100.6 MHz): 24.6, 25.9, 26.4 (4 × CH_3CO), 61.1 (C6), 69.2 (C1), 78.8 (C5), 80.5 (C4), 85.4 (C3), 111.7 (×2), 112.9 (2 × CH_3CO and C2).

4.3.2. 1,2:3,4-Di-*O*-isopropylidene-6-trifluoromethanesulfonyl-α-D-tagatofuranose 8. A solution of the diacetonide **7** (1.56 g, 6.0 mmol) in dichloromethane (8 mL) and pyridine (1.45 mL, 18 mmol) was cooled to −30 °C and treated with triflic anhydride (1.61 mL, 9.6 mmol). The reaction mixture was stirred at −30 to −10 °C for 3 h after which time TLC (EtOAc/cyclohexane, 1:1) showed the complete consumption of the starting material (R_f 0.55) and the formation of one major product (R_f 0.83). The reaction mixture was diluted with dichloromethane (30 mL) and washed with aqueous hydrochloric acid (2 M, 20 mL), and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with brine (30 mL), dried over magnesium sulfate and concentrated under reduced pressure to give the crude triflate **8** (2.307 g, 98%) as a crystalline solid which was used without further purification, mp 44–46 °C; $[\alpha]_D^{22} = +43.6$ (*c* 0.89, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 1.30 (3H, s, Me), 1.41 (3H, s, Me), 1.42 (3H, s, Me), 1.47 (3H, s, Me), 4.08 (1H, d, H1, *J* 9.9), 4.24–4.28 (1H, a-dt, H5, *J* 3.8, 7.5), 4.29 (1H, d, H1′, *J* 9.8), 4.60–4.64 (1H, dd, H6, *J* 7.5, 10.9), 4.64 (1H, d, H3, *J* 5.7), 4.75–4.78 (1H, dd, H6′, *J* 3.9, 10.9), 4.83–4.85 (1H, dd, H4, *J* 3.8, 5.7); δ_C ($CDCl_3$, 100.6 MHz): 24.7, 25.8, 26.1, 26.4 (4 × CH_3CO), 69.2 (C1), 74.1 (C6), 76.2 (C5), 79.5 (C4), 85.0 (C3), 112.1, 112.2, 113.5 (2 × CH_3CO and C2), 118.6 (CF_3, d, *J* 320).

4.3.3. 6-Deoxy-1,2:3,4-di-*O*-isopropylidene-6-iodo-α-D-tagatofuranose 9. Tetrabutylammonium iodide (1.4 g, 3.78 mmol) was added to a solution of triflate **8** (987 mg, 2.52 mmol) in THF (10 mL). The reaction mixture was stirred in the dark at room temperature for 18 h after which time TLC analysis (EtOAc/cyclohexane, 1:1) showed the consumption of the starting material (R_f 0.83) and the formation of one major product (R_f 0.88). The reaction was concentrated in vacuo and the residue dissolved in dichloromethane (50 mL) after which it was washed with sodium thiosulfate (satd, aq, 30 mL). The aqueous layer was re-extracted with dichloromethane (2 × 15 mL) and the combined organics were dried over magnesium sulfate and concentrated in vacuo. The resulting residue was purified by column chromatography (EtOAc/cyclohexane, 1:6) to give the iodide **9** (896 mg, 96%) as a pale yellow oil which crystallized on standing, HRMS (ESI +ve) found: 393.0168 ($M+Na^+$); $C_{12}H_{19}INaO_5$ requires: 393.0169; mp 37–39 °C; $[\alpha]_D^{22} = +44.5$ (*c* 0.92, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 1.33 (3H, s, Me), 1.40 (3H, s, Me), 1.42 (3H, s, Me), 1.48 (3H, s, Me), 3.24–3.28 (1H, dd, H6, *J* 6.1, 9.6), 3.31–3.35 (1H, dd, H6′, *J* 8.2, 9.5), 3.89 (1H, d, H1, *J* 9.8), 4.18–4.23 (1H, ddd, H5, *J* 3.5, 6.1, 8.2), 4.25 (1H, d, H1′, *J* 9.8), 4.64 (1H, d, H3, *J* 5.8), 4.82–4.85 (1H, dd, H4, *J* 3.5, 5.8); δ_C ($CDCl_3$, 100.6 MHz): −1.0 (C6), 25.0

(Me), 26.1 (Me), 26.4 (×2, Me), 69.4 (C1), 79.8 (×2, C4, C5), 85.4 (C3), 111.9 (×2), 112.9 (C2, 2 × CH_3CO); *m/z* (ESI +ve): 393 ($M+H^+$, 100%).

4.3.4. 6-Deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-tagatofuranose 10. A solution of the iodide (850 mg, 2.3 mmol) and triethylamine (0.96 mL, 6.9 mmol) in ethanol (23 mL) in the presence of 10% Pd/C (85 mg, 10 wt %.) was flushed with argon and then hydrogen and stirred in the dark under hydrogen for 18 h. After this time TLC showed the conversion of the starting material (R_f 0.88) to one major product (R_f 0.80). The reaction was flushed with argon, filtered through Celite and concentrated in vacuo. The residue was dissolved in dichloromethane (50 mL) and washed with sodium thiosulfate (satd, aq, 30 mL), dried over magnesium sulfate and evaporated under reduced pressure. The crude product was purified by column chromatography (EtOAc/cyclohexane, 1:6) to give the 6-deoxy compound **10** as a colorless oil (538 mg, 96%). (The compound appeared to be unstable on silica as on re-columning only 109 mg, 19%, was isolated). HRMS (ESI +ve) found: 267.1201 ($M+Na^+$); $C_{12}H_{20}NaO_5$ requires: 267.1203; $[\alpha]_D^{22} = +64.3$ (*c* 0.96, $CHCl_3$); δ_H ($CDCl_3$, 400 MHz): 1.31 (3H, d, Me, *J* 6.4), 1.33 (3H, s, CH_3CO), 1.40 (3H, s, CH_3CO), 1.43 (3H, s, CH_3CO), 1.47 (3H, s, CH_3CO), 4.03 (1H, d, H1, *J* 9.7), 4.06–4.11 (1H, dq, H5, *J* 3.5, 6.4), 4.26 (1H, d, H1′, *J* 9.7), 4.61 (1H, d, H3, *J* 5.9), 4.63–4.66 (1H, dd, H4 *J* 3.5, 5.8); δ_C ($CDCl_3$, 100.6 MHz): 13.5 (Me), 25.1 (CH_3CO), 26.1 (CH_3CO), 26.5 (×2) (CH_3CO), 69.2 (C1), 75.2 (C5), 81.3 (C4), 85.8 (C3), 111.4, 111.5, 112.5 (C2, 2 × CH_3CO); *m/z* (ESI +ve): 267 ($M+H^+$, 100%).

4.3.5. 6-Deoxy-D-tagatofuranose 4. Dowex (50W-X8, H^+) was added to a suspension of diacetonide **4** (76 mg, 0.31 mmol) in water (1 mL). The reaction was stirred at room temperature for 6 days after which time TLC analysis (EtOAc) showed almost complete conversion of the starting material to one major product (R_f 0.0). The reaction was filtered and diluted with water (20 mL) and washed with EtOAc (2 × 20 mL). The aqueous layer was concentrated under reduced pressure to give 6-deoxy-D-tagatofuranose **4** (46 mg, 90%) as a colorless oil and approximately 5:2 mixture of anomers; for data for **4**, see bioproduction of **4** above.

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References

1. (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.
2. Leang, K.; Takada, G.; Ishimura, A.; Okita, O.; Izumori, K. *Appl. Environ. Microbiol.* **2004**, *70*, 3298–3304.
3. Gullapalli, P.; Shiji, T.; Rao, D.; Yoshihara, A.; Morimoto, K.; Takata, G.; Fleet, G. W. J.; Izumori, K. *Tetrahedron: Asymmetry* **2007**, *18*, 1995–2000.
4. Muniruzzaman, S.; Tokunaga, H.; Izumori, K. *J. Ferment. Bioeng.* **1994**, *78*, 145–148.
5. (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.
6. Jones, N. A.; Jenkinson, S. F.; Soengas, R.; Izumori, K.; Fleet, G. W. J.; Watkin, D. J. *Acta Crystallogr., Sect. C* **2007**, *63*, o7–o10.
7. 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.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007**, *18*, 774–786.
8. (a) Wolfrom, M. L.; Waisbrot, S. W.; Brown, R. L. *J. Am. Chem. Soc.* **1942**, *64*, 1701; (b) Dills, W. L.; Covey, T. R. *Carbohydr. Res.* **1981**, *89*, 338–341.
9. Skytte, U. P. *Cereal Foods World* **2002**, *47*, 224–227.
10. Beadle, J. R.; Saunders, J. P.; Wajda, T. J. U.S. Patent WO5078796, 1992.
11. Soengas, R.; Izumori, K.; Simone, M. I.; Watkin, D. J.; Skytte, U. P.; Soetaert, W.; Fleet, G. W. J. *Tetrahedron Lett.* **2005**, *46*, 5755–5759.
12. Cubero, I.; Lopez-Espinosa, M. T. P.; Osorio, P. L. T. *An. Quim.* **1988**, *84*, 340–343.
13. Furneaux, R. H.; Tyler, P. C.; Whitehouse, L. A. *Tetrahedron Lett.* **1993**, *34*, 3609–3612.
14. Watkin, D. J.; Glawar, A. F. G.; Soengas, R.; Skytte, U. P.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J. *Acta Crystallogr., Sect. E* **2005**, *61*, o2891–o2893.
15. Behling, J.; Farid, P.; Medich, J. R.; Scaros, M. G.; Prunier, M.; Weier, R. M.; Khanna, I. *Synth. Commun.* **1991**, *21*, 1383–1386.
16. (a) Nelson, N. *J. Biol. Chem.* **1944**, *153*, 375–380; (b) Somogyi, M. *J. Biol. Chem.* **1952**, *195*, 19–23.
17. Muniruzzaman, S.; Kunihisa, Y.; Ichiraku, K.; Izumori, K. *J. Ferment. Bioeng.* **1995**, *79*, 496–498.
18. Ekeberg, D.; Svein Morgenlie, S.; Stenstrøm, Y. *Carbohydr. Res.* **2002**, *337*, 779–786.
19. (a) Barnett, J.; Reichstein, T. *Helv. Chim. Acta* **1937**, *20*, 1529–1536; (b) Barnett, J.; Reichstein, T. *Helv. Chim. Acta* **1938**, *21*, 913–914.
20. Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280–6289.
21. (a) Barnett, J.; Reichstein, T. *Helv. Chim. Acta* **1937**, *20*, 1529–1536; (b) Barnett, J.; Reichstein, T. *Helv. Chim. Acta* **1938**, *21*, 913–914.
22. Reichstein, T.; Bosshard, W. *Helv. Chim. Acta* **1934**, *17*, 753–761.
23. (a) Cerny, M.; Kollmann, M.; Pacak, J.; Budesinsky, M. *Collect. Czech. Chem. Commun.* **1974**, *39*, 2507–2519; (b) Cubero, I.; Olea, M. D. P.; Poza, D. G. *Carbohydr. Res.* **1985**, *134*, 327–331.