Fructose 1,6-Diphosphate Aldolase-catalysed Stereoselective C–C Bond Formation†

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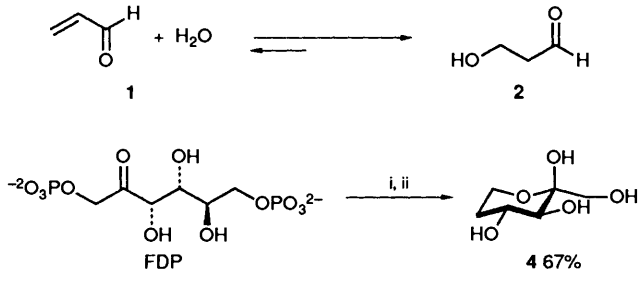
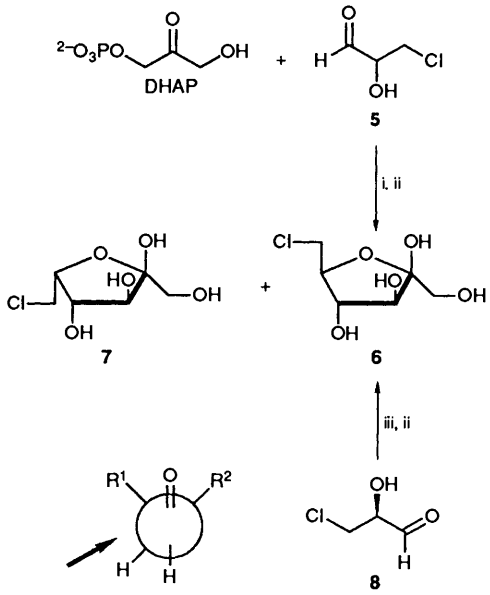
Fructose 1,6-diphosphate aldolase has been used as a catalyst for the synthesis (1—15 mmol scales) of several unusual sugar derivatives, including 5-deoxy-D-fructose, 6-deoxy-6-chloro-D-fructose, 6-deoxy-6-vinyl-D-fructose, 5,7-dideoxy-7-azido-L-*xylo*-heptulose, 5-deoxy-L-*xylo*-heptulose, 5,7-dideoxy-7-amino-L-*xylo*-heptulose and 5-deoxy-5-acetamido-L-*xylo*-hexulose.

Fructose 1,6-diphosphate (FDP) aldolase catalyses a reversible cleavage of FDP. The enzyme from rabbit muscle[1–5] or *E. coli*[6] has been used as a catalyst for a number of aldol condensation reactions. It accepts a broad range of aldehydes as substrates except hindered and α,β-unsaturated aldehydes.[1] With all substrates reported, the enzyme-catalysed aldol condensation is stereospecific, forming two new stereogenic centres with 3*S*,4*R* stereochemistry as shown in Scheme 1. Enantiomerically pure aldehydes with stereochemistry related to D-glyceraldehyde react faster than the L-isomers,[1,4] and negatively charged aldehydes are better substrates than their neutral or positively charged counterparts.[1] The Zn^{2+}-containing FDP aldolase from *E. coli* has been cloned and overexpressed in *E. coli*.[6,7] The metal-containing enzyme is more stable ($t_{\frac{1}{2}}$ *ca.* 60 d, 25 °C) than the Schiff base-forming enzyme ($t_{\frac{1}{2}}$ *ca.* 2 d) from rabbit muscle.[6] To extend the synthetic utility of FDP aldolase-catalysed condensation reactions, we report here the enzymatic synthesis of a number of new sugar-related compounds. We also report a detailed synthesis of 5-deoxy-D-fructose.[5]



Scheme 1 Synthesis of 5-deoxy-D-fructose: *Reagents:* i, **2** (4 equiv.), aldolase–triose-P isomerase; ii, Pase



equiv. of DHAP	equiv. of 5	6–7	yield (%)
1	1.2 (*RS*)	1.3:1	78
1	2.6 (*RS*)	6.5:1	70
1	1.2 (*S*), *i.e.* **8**	100:0	91

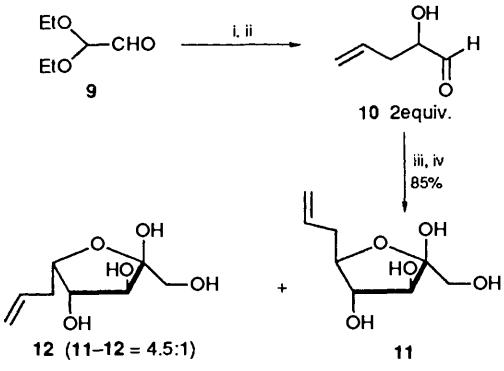
Scheme 2 Kinetically controlled aldol condensation: *Reagents:* i, aldolase; ii, Pase; iii, DHAP–aldolase

Results and Discussion

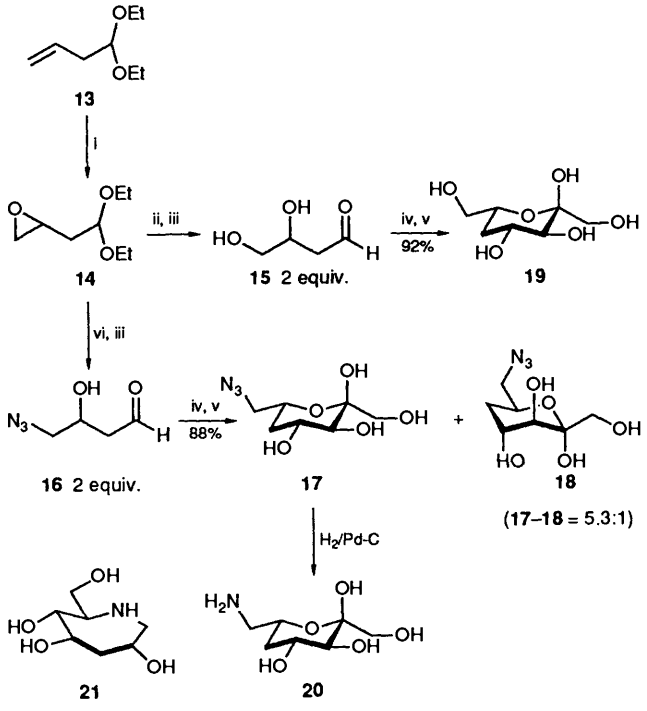
Schemes 1–6 illustrate the use of FDP aldolase in the synthesis of several fructose-related compounds, all based on reactions with modified aldol acceptors. When α-hydroxy aldehydes were used as acceptors (Schemes 2–3), the D-isomer always reacted faster than the L-isomer to form preferentially a kinetic product of the D-fructofuranose type of structure. The L-sorbose type of product was obtained as a minor product. It was observed that the product ratio increased with increase of the aldehyde component. When 2-deoxy-3-hydroxy aldehyde derivatives were used as acceptors (Scheme 4), however, thermodynamic products were preferentially obtained. The aldol product from the L-aldehyde forms a pyranose with an equatorial substitution at position 6, which is more stable than the product from the D-aldehyde (*e.g.*, **17** *vs.* **18**). The kinetically and thermodynamically controlled approaches to the FDP aldolase reactions thus provide a useful route to uncommon sugars starting from racemic aldehyde substrates. The results were consistent with previous observations in FDP aldolase-catalysed reactions with other substrates.[1,4,5] It should be noticed, however, that a single enzyme product can be obtained with the use of enantiomerically pure aldehyde substrate (Schemes 5—6),‡ albeit that preparation of the aldehyde is not trivial. The source of the enzymes seems not to affect the specificity as illustrated in the synthesis of azidoketose **26** (Scheme 6), in which the FDP aldolase from three different species gives the same result.

The stereochemistry of the aldol products was determined with NMR spectroscopy based on the adjacent proton–proton

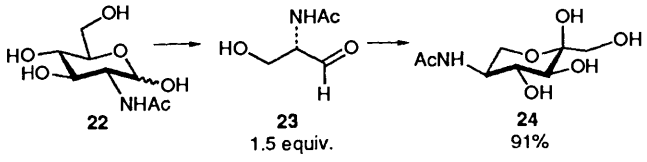
† Submitted to mark the 150th Anniversary of the Chemical Society/Royal Society of Chemistry.
‡ FDP aldolase from rabbit muscle, *E. coli* or staphylococcus aureus type XII.



Scheme 3 Kinetically controlled aldol condensation: *Reagents:* i, $H_2C{=}CHCH_2MgBr$; ii, H^+; iii DHAP–aldolase; iv, Pase



Scheme 4 Thermodynamically controlled aldol condensations: *Reagents:* i, H_2O_2; ii, KOH; iii, H^+; iv, DHAP–aldolase; v, Pase; vi, NaN_3

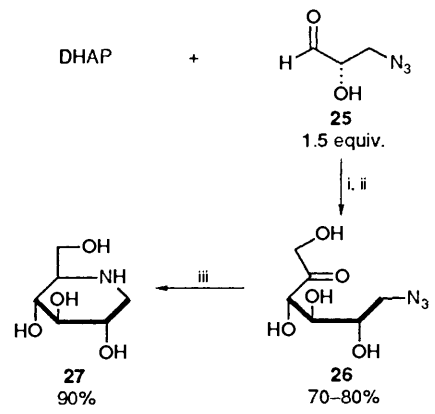


Scheme 5 *Reagents:* i, $NaBH_4$; ii, $NaIO_4$; iii, DHAP–aldolase; iv, Pase

coupling, and further confirmed by a separate synthesis starting from enantiomerically pure aldehyde (*e.g.*, Scheme 2, **8**→**6**).

5-Deoxy-D-fructose.—The enzymatic procedure illustrated here provides a simple and efficient route to a preparative synthesis of pure 5-deoxy-D-fructose (67–72% yield). A six step chemical synthesis of 5-deoxy-D-fructose starting from D-fructose was reported[8] with an overall yield of 13%.

The substrate DHAP used in this work was generated *in situ* from D-fructose 1,6-diphosphate *via* the combined catalysis of FDP aldolase and triosephosphate isomerase.[1,3,4] It can also be prepared chemically[9] or enzymatically.[10] Alternatively, it can be replaced with a mixture of dihydroxyacetone and a catalytic amount of arsenate.[11] This enzymatic approach has the advantage of being carried out in aqueous media at room temperature and protecting groups are not needed.



Scheme 6 *Reagents:* i, aldolase;[a] ii, Pase; iii, H_2–Pd/C
[a] FDP aldolase from rabbit muscle, *E. coli* or staphylococcus aureus type XII.

FDP aldolase (immobilized or not) will completely lose all activity in the presence of acrolein, presumably due to a Michael addition reaction with some essential nucleophile in the active site of the enzyme. One noteworthy aspect was the absence of any evidence that acrolein was incorporated at any measurable level in the aldol condensation. Both proton and carbon NMR spectroscopy indicated only the 5-deoxy sugar and no vinylic functionality was observed.

The compounds prepared in this study are either known or new carbohydrate synthons. For example, compounds **6**, **7**, **11**, **12**, **17** and **18** all have a functional group (olefin, Cl or N_3) and can be further converted to other sugar derivatives. In an attempt to prepare the seven-membered azasugar **21** from **17** *via* a Pd-mediated reductive amination, compound **20** was obtained as the sole product. Further hydrogenolysis of **20** gave a mixture of products which were not separated. As described previously,[2,5,6] reductive amination of azidoketose **26**, however, gave deoxynojirimycin **27** in very high yield (90%).

Experimental

Materials and Methods.—Fructose 1,6-diphosphate (FDP) aldolase (EC 4.1.2.13) from rabbit muscle type IV was from Sigma and was used without further purification. It was a crystalline suspension in 2.5 mol dm^{-3} $(NH_4)_2SO_4$, 0.01 mol dm^{-3} TRIS, 0.001 mol dm^{-3} EDTA, buffer (pH 7.5). One unit (U) of enzyme activity is defined as the formation of 1 μmol of product per minute. 300 U is equivalent to approximately 0.85 cm^3 of the enzyme suspension. Triosephosphate isomerase (TPI; EC 5.3.1.1) from rabbit muscle type III-S was purchased from Sigma as a crystalline suspension in 3.2 mol dm^{-3} $(NH_4)_2SO_4$ solution, pH 6.0. 1000 U is equivalent to about 0.036 cm^3 of the suspension. Acid phosphatase (Pase, EC 3.1.3.2) from sweet potato type X was also purchased from Sigma and used without further purification. Fructose 1,6-diphosphate was purchased from Sigma as the monocalcium salt. It was converted into the trisodium salt by dissolving the sample in 50 cm^3 of water, adjusting the pH to 1.5 with Dowex 50 (H^+) resin, filtering and readjusting the pH back to 7.0 with NaOH (2 mol dm^{-3}). The cloudy solution was filtered through a glass fritted funnel containing Celite 545 and the filtrate was lyophilized. The white powder was assayed enzymatically (see below) for FDP and was shown to be 86% pure. Dihydroxyacetone phosphate (DHAP) was prepared chemically according to the improved procedure.[9]

All J values are in Hz. All $[\alpha]_D$ values are in 10^{-1} deg cm^2 g^{-1}.

Enzymatic Assay for FDP.—The assay for FDP was described

by Bergmeyer[12] or Boehringer Mannheim.[13] The following procedure provides a modified detail. FDP is split into the C_3 compounds DHAP and (*R*)-glyceraldehyde 3-phosphate (G3P) by aldolase; both compounds are interconverted by the TPI reaction. DHAP is reduced to (*R*)-glycerol 1-phosphate by NADH in the α-glycerophosphate dehydrogenase (GDH, EC 1.1.1.8, from rabbit muscle type III) reaction. The enzyme GDH used for the enzymatic analysis contained TPI and was used without further purification.

$$\text{FDP} \xleftrightarrow{\text{FDP aldolase}} \text{DHAP} + \text{G3P}$$

$$\text{G3P} \xleftrightarrow{\text{TPI}} \text{DHAP}$$

$$2\,\text{DHAP} + 2\,\text{NADH} + 2\,\text{H}+ \xrightarrow{\text{GDH}} 2\,(R)\text{-Glycerol 1-phosphate} + 2\,\text{NAD}$$

The cuvettes used were disposable semimicro polystyrene cuvettes, with 1.6 cm^3 capacity and 1 cm length. In a representative assay, into a cuvette was pipetted: (i), triethanolamine (TEA) buffer (0.1 mol dm^{-3}; 940 mm^3) at pH 7.5; (ii), NADH solution (12 mmol dm^{-3}; 20 mm^3) in TEA buffer (0.1 mol dm^{-3}); (iii), FDP aldolase suspension (10 mm^3); (iv), GDH/TPI suspension (10 mm^3). The suspension was mixed and the initial absorbance (A_0) at 340 nm was read. Then 20 mm^3 of an FDP sample was added, the suspension was mixed and the final absorbance (A_t) was read after about 2 min. The best results were obtained when A_0 was below 2.2 absorbance units and A_t was above 0.4 absorbance units. If A_t fell below 0.4, the sample was diluted and assayed again. From Beer's Law, $\Delta A = A_0 - A_t = \varepsilon bc$, the concentration of FDP in the cuvette (c) can be calculated as shown below, where ε_{340} for NADH = 6.22 $mmol^{-1}$ dm^3 cm^{-1}, b = 1.0 cm, and c = concentration of FDP (mmol dm^{-3}).

$$c = \frac{\Delta A}{2\varepsilon b}$$

The factor 2 is included because 1 mmol of FDP requires 2 mmol of NADH. The concentration of FDP in the sample (c_1) was determined by multiplying by the dilution factor.

$$c_1 = c \times \frac{\text{total volume in cuvette (1 cm}^3\text{)}}{\text{volume of FDP sample (0.02 cm}^3\text{)}}$$

The Ames test[14] was used to determine the concentrations of inorganic and organic phosphate. The test is based on the principle that inorganic phosphate will complex with ammonium molybdate and is reduced by ascorbic acid to produce a blue phosphomolybdate complex (ε_{820} = 25.25 $mmol^{-1}$ cm^3 cm^{-1}). All glassware was scrupulously clean (free of phosphates) because of the sensitivity of this method.

To determine the phosphate concentration, the phosphate solution was diluted to 0.5–1.0 mmol dm^{-3}. The total phosphate concentration was determined by pipetting 300 mm^3 of the phosphate solution into a 10 × 13 mm Pyrex test tube and adding $Mg(NO_3)_2 \cdot 6H_2O$ solution (40 mmol dm^{-3}; 30 mm^3) in 95% ethyl alcohol. This solution was evaporated and ashed by a strong flame until the brown fumes disappeared. The test tube was allowed to cool, HCl (0.5 mol dm^{-3}; 1.2 cm^3) was added, and the tube capped with a marble and heated in a 100 °C water-bath for 15 min. The top of the test tube can be cooled with a stream of air, which helps minimize evaporation. The test tube was allowed to cool, ammonium molybdate·$4H_2O$ (3.4 mmol dm^{-3} 3.0 cm^3) in H_2SO_4 (1 mol dm^{-3}) and ascorbic acid (56 mmol dm^{-3}; 0.5 cm^3) was added. This mixture was warmed at 45 °C for 20 min. The absorbance was taken at 820 nm and is defined as A_t.

The inorganic phosphate concentration in solution was determined by the procedure described above except that the $Mg(NO_3)_2 \cdot 6H_2O$ and ashing steps were omitted. This absorbance is defined as A_p. A blank was run on the water used in the assay. The blank was determined as described above except that the $Mg(NO_3)_2 \cdot 6H_2O$ and ashing steps were omitted. This absorbance is defined as A_b.

$$\Delta A_1 = (A_t - A_b) \text{ and } \Delta A_2 = (A_p - A_b)$$

From Beer's Law, $\Delta A = \varepsilon bc$, the concentration of inorganic phosphate can be calculated as shown below, where ε_{820} = 25.25 $mmol^{-1}$ dm^3 cm^{-1}, b = 1.0 cm, and c = concentration of inorganic phosphate (mmol dm^{-3}).

$$C = (\Delta A)\left(\frac{\text{mmol dm}^{-3}}{25.25}\right)\left(\frac{5.0\text{ cm}^3}{0.3\text{ cm}^3}\right)$$

When ΔA_1 equals ΔA_2, the reaction is complete.

Preparation of Dowex 50 (Ba^{+2}) 100–200 *Mesh Resin Column.*—To a 3 dm^3 beaker fitted with an overhead stirrer was added 500 g of Dowex 50 (H^+) 100–200 mesh resin (Sigma) and 1.0 dm^3 of purified water. The mixture was cooled at 0 °C in an ice-bath. Barium hydroxide [$Ba(OH)_2 \cdot 2H_2O$, 254.5 g, 1.04 mol) was added in small portions over a period of 2 h. The mixture was adjusted to pH 7.0 with $Ba(OH)_2$ (0.1 mol dm^{-3}; *ca.* 200 cm^3) and stirred at room temperature for 30 min. The resin was filtered, rinsed with purified water and added to 400 cm^3 of EtOH–H_2O (1:1). This slurry was poured into a (3.0 × 100 cm) column and rinsed with EtOH–H_2O (1:1) overnight. The product was loaded onto the column and EtOH–H_2O (1:1) was used as the mobile phase.

Regeneration of the Dowex 50 (Ba^{+2}) 100–200 *Mesh Column.*—The resin was rinsed with purified water (2 dm^3) and added to a beaker containing $BaCl_2$ (1.0 mol dm^{-3}; 100 cm^3) and stirred with a mechanical stirrer for 30 min at room temperature. The resin was rinsed with EtOH–H_2O (1:1) and loaded onto the column as stated above. The column should be regenerated after each run.

5-*Deoxy*-D-*fructose.*—The procedure was essentially the same as previously reported[3] with some modifications. To a 1 dm^{-3} round-bottomed flask equipped with a magnetic stirrer bar, was added water (350 cm^3) containing trisodium FDP (5.78 g, 13.1 mmol; 86% pure) and 3-hydroxypropanal[3] (1.0 mol dm^{-3}; 52 cm^3, 52 mmol) in H_2SO_4 (0.1 mol dm^{-3}). The aldehyde solution was first washed with chloroform (2 × 50 cm^3) to remove residual acrolein as it would completely inactivate the aldolase.[3] The pH was adjusted to 6.9 with NaOH (2 mol dm^{-3}) and the solution was purged with argon. FDP aldolase (300 U) and TPI (1000 U) were added and the solution was stirred slowly. After 8 h, the reaction was assayed enzymatically for FDP which indicated that 70% of the FDP had been consumed. An additional 300 U of aldolase was added, and after an additional 5 h, enzymatic determination indicated that 100% of the FDP had been consumed; also the reaction mixture had become a faint yellow colour. The pH of the solution was adjusted to 4.7 with HCl (2 mol dm^{-3}). Acid phosphatase (Pase, 480 U) was added and the solution was incubated at 37 °C with stirring for 24 h. The Ames test[14] for phosphates indicated 95% hydrolysis of the organic phosphate. The solution was re-adjusted to pH 7.0, filtered through Celite 545 and lyophilized.

The golden coloured syrup was treated with methanol (3 × 150 cm^3) and filtered to remove the insoluble material. To the combined methanol fractions was added activated carbon (250 mg; Fisher brand 200 mesh) and the mixture was stirred at room temperature for 30 min. The carbon was removed by filtration and the methanol was removed under reduced pressure (the water-bath temperature was kept below 40 °C to prevent the sugar from decomposing). Methanol (10 cm^3) and water (4 cm^3) were added to the product and the solution was loaded on a flash chromatography column (37 mm × 61 cm, J. T. Baker) packed with 130 g of flash silica gel (40 μm, J. T. Baker). The solution was adsorbed, eluted with 100 cm^3 ethyl acetate and the product was separated using ethyl acetate–methanol (7:1) as the mobile phase. Fractions (50 cm^3) were collected, the fractions containing the product (500–1900 cm^3) were combined and the solvent was evaporated. The product was recrystallized by dissolving the crystals into a minimal amount of methanol (45 cm^3) saturated with ethyl acetate (*ca.* 200 cm^3). The solution was placed in a −20 °C freezer for 8 h. The crystals were filtered off and dried under reduced pressure. The mother liquor was evaporated under reduced pressure until crystals formed. Ethyl acetate (50 cm^3) was added and the solution was placed in a −20 °C freezer. The crystals were filtered off and dried as described above. 5-Deoxy-D-fructose (2.96 g, 69%) was obtained as white crystals. These crystals were shown to be pure by TLC [R_f 0.21, silica gel on glass using ethyl acetate–methanol (7:1) as the mobile phase and 10% sulphuric acid in ethanol as the developing agent[15]]. HPLC analysis showed an R_t of 6.8 min, using a Waters Carbohydrate Analysis column (3.9 mm × 30 cm, 10 μ). The mobile phase was MeCN–H_2O (85:15), with a flow rate of 1.20 cm^3 min^{-1}. Samples were diluted with an equal volume of MeCN and centrifuged prior to HPLC analysis. $[\alpha]_D^{25}$ −67.5 (*c* 0.154, H_2O), δ_H(400 MHz; D_2O) 1.50 [1 H, $J_{4ax.5ax}$ 11.5, $J_{5ax.6x}$ 13.0, $J_{5ax.6eq}$ 5.0, $J_{5ax.5eq}$ 13.0, C(5)-H_{ax}], 1.83 [1 H, $J_{4ax.5eq}$ 5.0, $J_{5eq.6eq}$ 1.8, $J_{5eq.6ax}$ 2.25, $J_{5eq.5ax}$ 13.0, C(5)-H_{eq}], 3.29 [1 H, d, $J_{3ax.4ax}$ 9.5, C(3)-H_{ax}], 3.32, 3.56 [1 H, d, $J_{\alpha.\beta}$ 11.5, C(1)-α or -β], 3.56 [m, $J_{5eq.6eq}$ 1.8, C(3)-H_{ax}], 3.32, 3.56 [1 H, d, $J_{\alpha.\beta}$ 11.5, C(1)-α or -β], 3.56 [1 H, m, $J_{5eq.6eq}$ 1.8, $J_{5ax.6eq}$ 5.0, $J_{6ax.6eq}$ 12.0 C(6)-H_{eq}], 3.74 [1 H, m, $J_{5ax.6ax}$ 13.0, $J_{5eq.6ax}$ 2.25, $J_{6ax.6eq}$ 12.0, C(6)-H_{ax}] and 3.76 [1 H, m, $J_{3ax.4ax}$ 9.5, $J_{4ax.5ax}$ 11.5, $J_{4ax.5eq}$ 5.0, C(4)-H_{ax}]; δ_C(50 MHz; D_2O) 98.1 (C-2), 72.2, 68.3, 63.6, 58.7 (C-1, C-3, C-4, C-6) and 32.8 (C-5).

The compound exists in solution predominantly in the β-D-$^2C_5$ pyranose form.[3,8] The physical data are consistent with those reported.[3,8]

An alternative purification procedure was to divide the product into two equal parts, each part then being passed through a Dowex 50 (Ba^{+2}) 100–200 mesh resin column (3.0 × 76 cm) using a mixture of ethanol and water (1:1) as the mobile phase, with a flow rate of 20 drops per minute. The fractions 305–565 cm^3 were combined, concentrated under reduced pressure to remove the ethanol, and the remaining solution was lyophilized to yield 5-deoxy-D-fructose (3.05 g, 72%) as an off-white solid. 5-Deoxy-D-fructose was recrystallized from methanol and ethyl acetate as described.

6-Deoxy-6-chloro-D-fructose **6** *and 6-Deoxy-6-chloro-L-sorbose* **7**.—To a flask containing 3-chloro-2-hydroxypropanal diethyl acetal[6,16] (293 mg, 1.6 mmol) was added buffer solution (10 cm^3; pH 1) and the mixture was heated at 50 °C overnight. TLC indicated complete hydrolysis of the starting material. DHAP (1.3 mmol) was added and the solution was adjusted to pH 7. FDP aldolase (300 U) was added and the mixture was stirred at room temperature. After 36 h, the pH was adjusted to 4.7 with HCl (1 mol dm^{-3}) and acid phosphatase (300 U) was added and the mixture was incubated at 37 °C. After 36 h, the solution was adjusted to pH 7 and lyophilized. The solid obtained was triturated with methanol (3 × 20 cm^3). The methanol fractions were combined and concentrated under reduced pressure. The product was purified with silica gel chromatography ($CHCl_3$–MeOH, 6:1).

Compound **6**: R_f 0.33 ($CHCl_3$–MeOH, 4:1); $[\alpha]_D^{23}$ +1.32 (*c*, 2.28, MeOH); δ_H(500 MHz; D_2O) 3.36 (1 H, d, *J* 12, 1-H), 3.42 (1 H, d, *J* 12, 1-H), 3.56 (1 H, dd, *J* 12, 6, 6-H), 3.66 (1 H, dd, *J* 12, 4.5, 6-H), 3.82 (1 H, m, 5-H), 3.95 (1 H, d, *J* 8, 3-H) and 4.03 (1 H, t, *J* 8, 4-H); δ_C(CD_3OD) 47.16 (CH_2Cl), 64.54 (CH_2OH), 77.43, 79.00, 82.72 and 103.82 (C-2) (Found: M, 330.9349. Calc. for $C_6H_{11}ClO_5$: $M + C_s^+$, 330.9349).

Compound **7**: R_f 0.47 ($CHCl_3$–MeOH, 4:1); $[\alpha]_D^{23}$ −3.2 (*c*, 1.55, CH_3OH); δ_H(D_2O) 3.44 (1 H, d, *J* 5), 3.46 (1 H, m), 3.61 (1 H, dd, *J* 4, 12), 3.95 (1 H, d, *J* 5.5) and 4.23–4.24 (2 H, br) and 4.27 (1 H, m); δ_C(D_2O) 44.1 (CH_2Cl), 64.1 (CH_2OH), 76.8, 78.9, 82.6, 103.3 (C-2); (Found: M, 330.9350. Calc. for $C_6H_{11}ClO_5$: $M + C_s^+$, 330.9349).

6-Deoxy-6-vinyl-D-fructose **11** *and 6-Deoxy-6-vinyl-L-sorbose* **12**.—Allylmagnesium bromide (8.6 cm^3; 1 mol dm^{-3} solution in ether) was slowly added to a stirred solution of compound **9**[17] (1.1 g, 8.3 mmol) in dry ether (20 cm^3) at −78 °C. The reaction mixture was warmed to room temperature for 1 h. Ice–water was added and the reaction mixture was extracted with ether. The ethereal extract was washed with water and dried over Na_2SO_4. Silica gel column chromatography (EtOAc–hexane, 1:9) of the extract gave 3-vinyl-2-hydroxypropanal diethyl acetal (1 g, 72%); R_f 0.21 (EtOAc–hexane, 1:9); δ_H($CDCl_3$) 1.21 (6 H, m), 2.14–2.48 (2 H, m), 3.50–3.78 (5 H, m), 4.29 (1 H, d, *J* 7), 5.08–5.12 (2 H, m) and 5.82–5.95 (1 H, m); δ_C($CDCl_3$) 15.6, 36.5, 63.7, 71.3, 104.6, 117.5 and 134.9.

A solution containing 3-vinyl-2-hydroxypropanal diethyl acetal in pH 1 buffer solution (0.2 mol dm^{-3}) was stirred at room temperature for 20 h to obtain compound **10** for aldol reactions.

Compound **11** and compound **12** were obtained similarly *via* the FDP aldolase-catalysed aldol condensation with **10**. The aldehyde used was 1 equiv. in excess of DHAP (total yield 85%).

Compound **11**: R_f 0.15 ($CHCl_3$–MeOH, 6:1); $[\alpha]_D^{23}$ +8 (*c*, 2.5, CH_3OH); δ_H(D_2O) 2.19–2.36 (2 H, m, 2 × 6-H), 3.32 (1 H, d, *J* 12, 1-H), 3.39 (1 H, d, *J* 12, 1-H), 3.61–3.66 (1 H, m), 3.86 (1 H, d, *J* 7.5, 3-H), 3.85–3.92 (1 H, m), 4.96–5.06 (2 H, m, 2 × 8-H) and 5.56–5.74 (1 H, m, 7-H); δ_C(CD_3OD) 40.3 (C-6), 64.8 (C-1), 77.3, 80.2, 81.7 (3 × CH), 102.9 (C-2), 117.4 (C-8) and 136.1 (C-7) (Found: M, 322.9900. Calc. for $C_8H_{14}O_5$: $M + C_s^+$, 322.9895).

Compound **12**: R_f 0.25 ($CHCl_3$–MeOH, 6:1); $[\alpha]_D^{23}$ −5.6 (*c*, 1.25, CH_3OH); δ_H(D_2O) 2.09–2.26 (2 H, m, 2 × 6-H), 3.37 (1 H, d, *J* 12, 1-H), 3.42 (1 H, d, *J* 12), 3.89 (1 H, d, *J* 4.5, 3-H), 4.04–4.14 (2 H, m, 4-H and 5-H), 4.93–5.06 (2 H, m, 2 × 8-H) and 5.63–5.77 (1 H, m, 7-H); δ_C(CD_3OD) 34.7 (C-6), 66.0 (C-1), 78.6, 79.0, 80.5 (3 × CH), 103.2 (C-2), 117.2 (C-8) and 136.4 (C-7); (Found: M, 322.9908. Calc. for $C_8H_{14}O_5$: $M + C_s^+$, 322.9895).

5,7-Dideoxy-7-azido-L-xylo-heptulose **17** *and 5,7-Dideoxy-7-azido-D-arabino-heptulose* **18**.—To a stirred suspension of $KHCO_3$ (500 mg, 5 mmol) in methanol (50 cm^3) was added compound **13** (4.78 g, 33.1 mmol), benzonitrile (3.4 cm^3) and H_2O_2 (50%; 1 cm^3). The solution was warmed to 40 °C in a water-bath. After 12 h, H_2O_2 (50%; 1 cm^3) was added and after an additional 24 h, H_2O_2 (1 cm^3) and benzonitrile (1 cm^3) were added. The solution was allowed to react for an additional 24 h then NaN_3 (4.3 g, 66.2 mmol) was added. The pH was adjusted to 7 with H_2SO_4 (1 mol dm^{-3}) and the mixture was kept at that pH by adding H_2SO_4 (1 mol dm^{-3}) with a peristaltic pump. The solution was warmed to 30 °C for 36 h, and the methanol was removed under reduced pressure. Water (50 cm^3) was added and the solution was extracted with CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. Hexane

was added to precipitate benzamide. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography (EtOAc–hexane, 1:3) to obtain 4-azido-3-hydroxybutanal diethyl acetal (4.14 g, 62% from **13**); R_f 0.36 (EtOAc–hexane, 1:3); $\delta_H(CDCl_3)$ 1.20–1.25 (6 H, m), 1.75–1.85 (2 H, m), 3.27–3.31 (1 H, m), 2.52–3.77 (4 H, m), 3.95–4.05 (1 H, m) and 4.72 (1 H, t, *J* 7.5); $\delta_C(CDCl_3)$ 15.4, 15.5, 37.8, 55.8, 62.1, 62.9, 68.1 and 102.1; (Found: M, 203.1262. Calc. for $C_8H_{18}N_3O_3$: $M + H^+$, 203.1262).

A solution containing 4-azido-3-hydroxybutanal diethyl acetal in pH 1 buffer solution (0.2 mol dm^{-3}) was stirred at 45 °C for 12 h to obtain compound **16**. Compounds **17** and **18** were prepared *via* the general procedure for enzymatic aldol condensation (aldehyde–DHAP, 2:1, total yield 88%) and dephosphorylation.

Compound **17**; R_f 0.38 ($CHCl_3$–MeOH, 4:1); $[\alpha]_D^{23}$ −33.72 (*c*, 5.22, CH_3OH); $\delta_H(D_2O)$ 1.30 (1 H, ddd, *J* 12.5, 5a-H), 1.84 (1 H, ddd, *J* 2, 5, 12.5, 5e-H), 3.18–3.23 (2 H, m, 2 × 7-H), 3.29 (1 H, d, *J* 12, 3-H), 3.32 (1 H, d, *J* 12, 1-H), 3.53 (1 H, d, *J* 12, 1-H), 3.78 (1 H, ddd, *J* 5, 9.5, 12.5, 4-H) and 3.98 (1 H, m, 6-H); $\delta_C(CD_3OD)$ 37.63 (C-5), 55.74 (C-7), 66.05 (C-1), 69.22, 69.77, 74.30 (3 × CH) and 99.29 (C-2) (Found: M, 220.0098. Calc. for $C_7H_{14}N_3O_5$: $M + H^+$, 220.0812).

Compound **18**; R_f 0.5 ($CHCl_3$–MeOH, 4:1); $[\alpha]_D^{23}$ +14 (*c*, 2.35, CH_3OH), $\delta_H(D_2O)$ 1.47 (1 H, d, *J* 13.5, 5e-H), 1.75 (1 H, td, *J* 3, 13.5, 5a-H), 3.20 (1 H, dd, *J* 7.5, 13, 7-H), 3.28 (1 H, d, *J* 11.5, 1-H), 3.27–3.30 (1 H, m, 7-H), 3.53 (1 H, d, *J* 11.5, 1-H), 3.52 (1 H, d, *J* 3, 3-H), 3.95 (1 H, m, 4-H) and 4.20 (1 H, m, 6-H); $\delta_C(CD_3OD)$ 31.20 (C-5), 56.53 (C-7), 65.01 (C-1), 66.25, 67.57, 70.21 (3 × CH) and 99.48 (C-2) (Found: M, 220.0805. Calc. for $C_7H_{14}N_3O_5$: $M + H^+$, 220.0812).

5-*Deoxy*-L-xylo-*heptulose* **19**.—The procedure for **15** was similar to that for **16** except that to the solution containing compound **14** was added KOH (2 equiv.) instead of NaN_3. The reaction was finished after 48 h at 30 °C. The crude product was subjected to silica gel column chromatography (EtOAc, 100%) to obtain 3,4-dihydroxybutanal diethyl acetal (45% from **13**); R_f 0.26 (EtOAc 100%); $\delta_H(CDCl_3)$ 1.18–1.26 (6 H, m), 1.74–1.84 (2 H, m), 3.46–3.75 (6 H, m), 3.90–3.98 (1 H, m) and 4.73 (1 H, t, *J* 7.5); $\delta_C(CDCl_3)$ 15.4, 43.1, 63.5, 71.9, 73.9 and 103.9 (Found: M, 311.0254. Calc. for $C_8H_{18}O_4$: $M + Cs^+$, 311.0259). This compound in pH 1 buffer solution (0.2 mol dm^{-3}) was stirred at room temperature for 3.5 h to obtain compound **15** for the enzymatic synthesis of **19** (aldehyde–DHAP, 2:1; yield 92%).

Compound **19**; R_f 0.5 (EtOAc–MeOH–H_2O, 6:3:1); $[\alpha]_D^{23}$ −17.8 (*c*, 4.61, CH_3OH); $\delta_H(D_2O)$ 1.30 (1 H, ddd, *J* 12.1, 5a-H), 1.81 (1 H, ddd, *J* 1.9, 4.9, 12.1, 5e-H) and 3.27 (1 H, d, *J* 9.5, 3-H) (1 H, m, 6-H); $\delta_C(D_2O)$ 35.66 (C-5), 65.32, 65.65 (C-1, C-7), 69.60, 70.61, 73.55 (3 × CH) and 99.43 (C-2) (Found: M, 194.1104. Calc. for $C_7H_{15}O_6$: $M + H^+$, 194.0790).

5,7-*Dideoxy*-7-*amino*-L-xylo-*heptulose* **20**.—A solution containing compound **17** (80 mg, 0.36 mmol) in H_2O (10 cm^3) was hydrogenated with 12 mg Pd/C under 50 psi of hydrogen for 1 d. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to remove the solvent. The residue was purified with Bio-gel (200–400 mesh) column to yield compound **20** (50 mg, 72% yield); R_f 0.45 (isopropyl alcohol–NH_4OH–H_2O, 6:3:2); $[\alpha]_D^{23}$ −28.75 (*c*, 1.6, H_2O); $\delta_H(D_2O)$ 1.27 (1 H, ddd, *J* 12, 5a-H), 1.91 (1 H, ddd, *J* 2, 5, 12, 5e-H), 2.84 (1 H, dd, *J* 8.8, 13.3, 7-H), 3.00 (1 H, dd, *J* 13.3, 2.9, 7-H), 3.27 (1 H, d, *J* 9.5, 3-H), 3.38 (1 H, d, *J* 11.6, 1-H), 3.57 (1 H, d, *J* 11.6, 1-H), 3.81 (1 H, m, 4-H) and 4.02 (1 H, m, 6-H); $\delta_C(D_2O)$ 36.76 (C-5), 44.32 (C-7), 65.12 (C-1), 66.92, 69.05, 73.28 (3 × CH) and 99.36 (C-2).

5-*Deoxy*-5-*acetamido*-L-xylo-*hexulose* **24**.—Compound **23** was prepared according to the method developed by Giannis *et al.*[18] Sodium borohydride (347 mg, 9.2 mmol) was slowly added to a stirred solution of compound **22** (2 g, 9 mmol) in water (25 cm^3) at room temperature. The solution until gas evolution had stopped. Sodium periodate (3.8 g, 17.8 mmol) was added to the stirred solution at 0 °C for 10 min. The solution was lyophilized and the residue was subjected to silica gel column chromatography ($CHCl_3$–MeOH, 7:1) to obtain compound **23** and its hydrate form (85%) (Found: M, 150.0765 and 132.0670. Calc. for $C_5H_{11}NO_4$ and $C_5H_{10}NO_3$: $M + H_2O + H^+$ and $M + H$, 150.0766 and 132.0760).

Compound **24** was obtained from **23** *via* the enzymatic aldol condensation (aldehyde–DHAP, 1.5:1; yield 91%); R_f 0.83 (MeOH); $[\alpha]_H^{23}$ −25.6 (*c*, 2.7, CH_3OH), $\delta_H(CD_3OD)$ 1.82 (3 H, s, *Me*CONH) and 3.15–3.8 (7 H, complex multiplet); δ_C (CD_3OD) 22.66 (Me), 49.84 (C-6), 53.06 (C-5), 61.65 (C-1), 65.63, 73.09 (2 × CH), 98.93 (C-2) and 173.83 (CO); (Found: M, 221.0912. Calc. for $C_8H_{16}NO_6$: $M + H^+$, 221.0899).

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