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ENZYMATIC ALDOL REACTION/ISOMERIZATION AS A ROUTE TO UNUSUAL SUGARS

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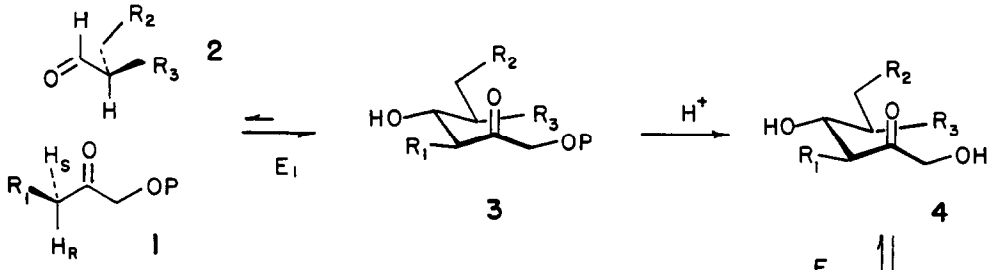
Summary: Utilizing fructose diphosphate aldolase and glucose isomerase as catalysts, 3-, 5, and 6-deoxy- and 6-O-methylhexoses have been synthesized from dihydroxyacetone phosphate or acetol phosphate and the corresponding hydroxypropionaldehydes.

Unnatural or unusual sugars are difficult to prepare using standard organic synthetic methods. Generally, sugar syntheses involve elaborate protection, deprotection schemes and have problems in regioselectivity. In addition, these methods are not realistic for large scale synthesis. These problems have led to the evolution of two elegant approaches based on asymmetric epoxidation[1] and asymmetric Diels-Alder reactions[2] to a wide range of unusual sugars. As part of our program to evaluate enzymes as enantioselective synthetic reagents, we have explored the use of fructose diphosphate aldolase (FDP aldolase) coupled with glucose isomerase as an alternative route to unusual sugars.

FDP aldolase, a key enzyme in sugar metabolism, catalyzes the stereospecific condensation of dihydroxyacetone phosphate **1a** and D-glyceraldehyde-3-phosphate to give fructose-1,6-diphosphate[3]. The reaction is freely reversible but lies in favor of the hexose phosphate. This enzyme is quite specific for **1a** as one substrate, but will accept a wide variety of aldehydes as the second[4,5]. The second enzyme, glucose isomerase, used industrially to generate high fructose corn syrups, catalyzes the interconversion of D-glucose and D-fructose. It has shown a wide substrate specificity[6]. We have further investigated the substrate specificity of these two enzymes and succeeded in developing practical methods to synthesize several unusual sugars as depicted in the Scheme. The following synthesis of 6-deoxy-D-fructose **4c** and 6-deoxy-D-glucose **5c** is a typical procedure. In a 3-neck 100 mL round bottom flask equipped with gas bubbling tube, gas exist and a stirring bar, 38 mL of water containing D-lactaldehyde **2c** (15.6 mmol)[7] and 2.75 g fructose-1,6-diphosphate ($Na_3 8H_2O$, 5 mmol, a source of **1a**) were combined. The pH of the solution was adjusted to 7.0 with NaOH, and argon was bubbled through the solution to degas. Triose phosphate isomerase (500 U) was added along with 263 U of FDP aldolase. After 22 h, the reaction assayed greater than 98% complete. The reaction mixture was placed in a 250 mL beaker, and 2.5 g $BaCl_2$ (12 mmol) was added, and the pH was adjusted to 8.2 with NaOH.

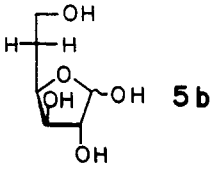
Acetone (150 mL) was added, and the solution was chilled overnight. The barium salt was centrifuged, washed with acetone and then redissolved in 50 mL water. Dowex 50 (H^+, 20 g) was added with stirring for 30 min to remove barium ions. Filtration followed by an additional deionization with 10 g Dowex 50 (H^+) and a final filtration yielded 95 mL of a pale yellow, clear filtrate of pH ~2. A 50 mL aliquot was removed and the pH adjusted to 7.5 with NaOH. The solution was liophilized to yield 0.94 g (62% overall) of 6-deoxy-D-fructose-1-phosphate disodium salt (**3c**). Hydrolysis of an aliquot of the remaining solution (80 °C for 8-10 h) yielded only **4c**[8]. HPLC analysis using a Waters carbohydrate column



cmpd	R_1	R_2	R_3
1a	OH		
1b	H		
2a		OH	OH
2b		OH	H
2c		H	OH
2d		OCH_3	OH
3a – 5a	H	OH	OH
3b – 5b	OH	OH	H
3c – 5c	OH	H	OH
3d – 5d	OH	OCH_3	OH

E_1: FDP Aldolase

E_2: Glucose isomerase



5b

detected no D-fructose. A 10 mL aliquot of the hydrolyzed sugar phosphate was used in a subsequent isomerase-catalyzed reaction. The pH was adjusted to 7.5 with NaOH, and Co^{+2} and Mg^{+2} were added to give a final concentration of 1 mM and Mn^{+2} was added to give a concentration of 0.5 mM. Takasweet (1 g, an immobilized form of glucose isomerase from Miles) was added, and the solution warmed at 40 ^{o}C for 4 h with intermittent swirling. HPLC analysis indicated significant (~50%) formation of **5c**. The mixture was passed through a Dowex 50-Ba^{+2} column (2 x 100 cm) and eluted with water to give **4c** and **5c** in about 45% yield for each compound[9].

Using a racemic aldehyde results in an epimeric mixture (example - use of DL-lactaldehyde will result in, after hydrolysis of the phosphate group, a 1:1 mixture of 6-deoxy-D-fructose and 6-deoxy-L-sorbose). These mixtures can be separated by using a Dowex 50-Ba^{+2} column with water as an eluent. In like manner, mixtures of the aldoses and ketoses resulting from glucose isomerase treatment can be separated.

In a similar manner, this procedure was used to prepare several other unusual aldose sugars such as 3-deoxy-D-glucose **5a** (42% yield from **1a**), 5-deoxy-D-fructose **4b** (80% yield from **1a**), 6-O-methyl-D-glucose **5d** (40% yield from **1a**) and others in approximately 40% yield as shown in the Scheme[10].

The enzymatic routes to sugars as illustrated here have the advantages over other chemical approaches that the reactions are stereospecific and operated at room temperature under mild conditions without protection of functional groups. A limitation of the aldol reactions is that there is no way of changing the stereochemistry of the two chiral centers formed in the condensations. We note, however, that more than twenty other aldolases which display different stereospecificities in C-C bond formation may also accept a wide variety of substrates[11], and their synthetic utility remains to be explored.

Acknowledgment

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8. ^{13}C-NMR(62.8 MHz, D_2O) for α form: δ 68.91 (C-1), 106.45 (C-2), 85.13, 78.27, 83.24 (C-3-5). 21.52 (C-6); ^{1}H-NMR δ 1.32 (CH_3); for β form: δ 65.62 (C-1), 103.65 (C-2), 78.92, 77.92, 82.04 (C-3-5), 21.61 (C-6); ^{1}H-NMR δ 1.30 (CH_3). The values are in agreement with those reported: Swaminathan, P.; Anderson, L.; Sundaralingam, M. Carbohydr. Res., **1979**, 75, 1.

9. Compound **5c**: m.p. 147-9 °C (EtOAc), $[\alpha]_D^{25}$ +32 (c1, H_2O)(lit. +30, Evans, M.E.; Parrish, F.W. Method. Carbohydr. Chem., VI, p177). ^{1}H-NMR (90 MHz, D_2O) δ 1.25 (3H, dd), 3.0-4.1 (4H, highly detailed peak cluster), 4.60 (βH, d, J = 8.5 Hz), 5.15 (αH, d, J = 3.6 Hz). ^{13}C-NMR (50 MHz, D_2O), α form δ 93.0 (C-1), 72.9 (C-2), 73.5 (C-3), 76.5 (C-4), 68.8 (C-5), 18.0 (C-6); β form δ 96.7 (C-1), 75.6 (C-2), 76.1-76.8 (C-3,4), 73.0 (C-5), 18.0 (C-6). Anal Calcd for $C_6H_{12}O_5$: C, 43.90; H, 7.32. Found: C, 43.97; H. 7.70.
10. Compound **2a** was from Sigma. Compound **2b** was prepared by hydration of acrolein: Pressman, D.; Lucas, H.J. J. Am. Chem. Soc. **1942**, 64, 1953. Compound **2d** (racemic) was prepared by methanolysis of glycidaldehyde diethyl acetal: Wright, J.B. J. Am. Chem. Soc. **1957**, 79, 1694. The following are physical constants of the representative compounds (specific rotations were measured at 25 °C with sodium D-line at 589 nm): **5a** +24.6 (c1.3, H_2O); ^{1}H-NMR (D_2O, 90 MHz) δ 1.33-2.56 (m, 2H). For chemical synthesis, see Hedgley, E.J.; Overend, W.G.; Rennie, R.A.C. J. Chem. Soc. (C)., **1967**, 888; Barton, D.H.R.; McCombie, S.W. J. Chem. Soc. Perk. I. **1975**, 1574. Compound **5b** +24.1 (c7.8, H_2O); ^{1}H-NMR (D_2O, 90 MHz) δ 1.65-2.04 (m, 2H). For chemical synthesis, see Hedgley, E.J.; Meresz, O.; Overend, W.G. J. Chem. Soc. (C), **1967**, 888; Klemer, A.; Hofmeister, H.; Lemmes, R. Carbohydr. Res., **1979**, 68, 391. Compound **3b** (barium salt) -28.3 (c4.7, H_2O); ^{1}H-NMR (90 MHz, D_2O) δ1.4-2.2 (2H, broad), 3.2-4.2 (6H, broad). Compound **4b** -30.5 (c4.9, H_2O); ^{1}H-NMR (90 MHz, D_2O) δ 1.2-2.2 (2H, highly detailed peak cluster), 3.3-4.1 (6H, highly detailed peak cluster); ^{13}C-NMR (50 MHz, D_2O) δ 98.1 (C-2), 72.2, 68.3, 63.6, 58.7 (C-1, C-3, C-4, C-6), 32.8 (C-5). Compound **3c** (barium salt) -4.68 (c3.4, H_2O); ^{1}H-NMR (90 MHz, D_2O) δ1.29 (3H, d, J = 7 Hz), 3.5-4.2 (5H, cluster). Compound **4c** -6.61 (c6.2, H_2O); ^{1}H-NMR (90 MHz, D_2O) δ 1.30 (3H, d, J = 7 Hz), 3.4-4.2 (5H, peak cluster). Compound **5d** +56 (c2, H_2O); m.p. 149-50 °C (EtOAc/EtOH); ^{1}H-NMR (90 MHz, CD_3OD) δ 5.10 (1H, d, J = 3.6 Hz, H-1), 4.47 (1H, d, J = 7.8 Hz, H-1), 3.38 (3H, s, OCH_3); ^{13}C-NMR (D_2O) α form: δ 92.8 (C-1), 72.4 (C-2), 73.6 (C-3), 70.6-70.7 (C-4,5), 72.1 (C-6), 59.5 (OCH_3); β form: δ 96.7 (C-1), 74.9 (C-2), 76.6 (C-3), 70.6-70.7 (C-4), 75.4 (C-5), 72.0 (C-6), 59.4 (6-OCH_3). Anal Calcd for $C_7H_{14}O_6$: C, 43.3; H, 7.3. Found: C, 43.7; H, 7.5. The NMR data are in agreement with those reported: Cleve, J.W.V.; Inglett, G.E.; Tjarks, L.W. Carbohydr. Res. **1985**, 137, 259.
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