Enzymatic Aldol Condensation/Isomerization as a Route to Unusual Sugar Derivatives

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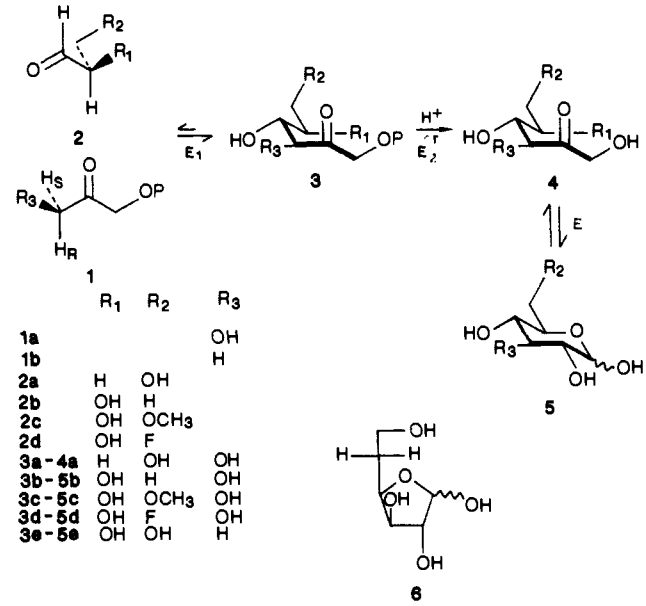
Abstract: Preparative enzymatic synthesis of several unusual hexoketose and hexoaldose derivatives using fructose bisphosphate aldolase and glucose isomerase as catalysts has been developed. Fructose bisphosphate aldolase catalyzes a stereospecific aldol condensation between dihydroxyacetone phosphate and a number of aldehydes to form ketose 1-phosphates which upon removal of the phosphate groups by hydrolysis are converted stereospecifically to aldose derivatives catalyzed by glucose isomerase. These combined enzymatic processes allow preparation of 5-deoxy, 6-deoxy, 6-deoxy-6-fluoro-, and 6-*O*-methylhexoketoses and -hexoaldoses on 4–20-mmol scales. Dihydroxyacetone in the presence of inorganic arsenate has been found to be an effective substrate for fructose bisphosphate aldolase.

Sugars are a ubiquitous class of compounds that play a broad range of roles in biochemistry. As such, they have found application as pharmaceuticals,[1] biological probes,[2] and chemical intermediates.[3] A recent review article reflects the importance of these compounds by enumerating hundreds of individual papers in the area of carbohydrate synthesis.[4] Traditional sources of rare or unnatural sugars have been chemical modification of natural sugars and total synthesis. The former was proven successful in many cases. However, total synthesis has evolved to a very elegant and sophisticated level, employing asymmetric epoxidations,[5] Diels–Alder condensations,[6] and other asymmetric condensations[7] as key transformations. Asymmetric carbon–carbon bond formations via aldol reactions are also quite useful in synthesizing complex compounds, including carbohydrates.[8]

Recently, an enzyme, fructose bisphosphate aldolase (fructose diphosphate aldolase, FDP aldolase, EC 4.1.2.13), has found synthetic utility in catalyzing stereospecific aldol condensations.[9] The FDP aldolase from rabbit muscle catalyzes the condensation of dihydroxyacetone phosphate (DHAP, **1a**) and D-glyceraldehyde 3-phosphate (G3P) to give D-fructose 1,6-bisphosphate (D-fructose 1,6-diphosphate, FDP). The reaction is freely reversible but lies in favor of the ketose phosphate. The enzyme is rather specific for DHAP although it will reluctantly accept some modifications.[9] Phosphate derivatives of aldehydes apparently are good substrates for the enzyme, which allows preparation of sugar phosphates with a different carbon number.[10] Conversely, it will accept a wide variety of unphosphorylated aldehydes.[9] The ketose 1-phosphates formed can be hydrolyzed easily to remove the phosphate moiety and subsequently converted to ketose 6-phosphates catalyzed by hexokinase and to aldose 6-phosphates catalyzed by phosphoglucose isomerase, if they are the substrates for the enyzmes.[9]

We recently have reported a preliminary result regarding a new isomerization procedure using glucose isomerase (GI, EC 5.3.1.5) as catalyst to convert ketoses (prepared from FDP aldolase reactions followed by phosphatase-catalyzed removal of the phosphate moiety) to aldoses without proceeding through the phosphorylation step which requires ATP.[12] The procedure also allows preparation of 6-deoxyaldoses from the corresponding 6-deoxyketoses prepared from FDP aldolase reactions. In this paper, we describe the detailed procedures for the improved preparation of these compounds and the new preparation of 6-fluorinated hexose derivatives and intermediates using the combined aldol condensation/isomerization reactions catalyzed by FDP aldolase and glucose isomerase (Scheme I). The substrate DHAP used in the aldol reactions can be replaced with a mixture of dihydroxyacetone and inorganic arsenate. We also report a detailed study on the substrate specificity of glucose isomerase. This combined enzymatic approach to sugars has the advantage of being carried out

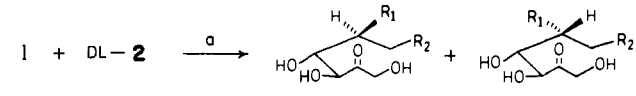
Scheme I. Synthesis of Unusual Sugars Using FDP Aldolase (E_1) and Glucose Isomerase (E_3) as Catalysts (E_2, Acid Phosphatase)



	R_1	R_2	R_3
1a			OH
1b			H
2a	H	OH	
2b	OH	H	
2c	OH	OCH_3	
2d	OH	F	
3a-4a	H	OH	OH
3b-5b	OH	H	OH
3c-5c	OH	OCH_3	OH
3d-5d	OH	F	OH
3e-5e	OH	OH	H

E_1, FDP aldolase
E_2, acid phosphatase
E_3, glucose isomerase

Scheme II



in aqueous media at room temperature. Protecting groups are not needed. The critical C3–C4 bond is formed stereospecifically,

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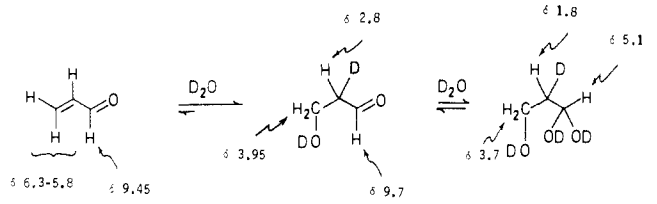

Figure 1. ^{1}H NMR assignments of the hydration products of acrolein.

and the subsequent isomerization gives only the glucoaldose. Ideally, this technique will be used in conjunction with other synthetic methodologies to further the synthesis of unusual carbohydrates.

Results and Discussion

Sugars. Generally, the sugars were synthesized in 4–20-mmol quantities by reacting DHAP (vide infra) and the appropriate aldehyde in buffered aqueous solutions. The substrate DHAP used in this work was generated in situ from fructose 1,6-diphosphate via the combined catalysis of FDP aldolase and triose phosphate isomerase (TPI, EC 5.3.1.1). Utilization of racemic aldehydes will result in a 1:1 mixture of fructose and sorbose derivatives, as shown in Scheme II. These sugars are diastereomers and can be easily separated by column chromatography after removal of the phosphate group.[13] Due to the chiral instability of α-hydroxy aldehydes in solution and the difficulty of preparing these compounds in optically pure forms, we prefer to use racemic mixtures of aldehydes for aldol condensations.

The 5-deoxy-D-fructose 1-phosphate (**3a**) and 5-deoxy-D-fructose (**4a**) were generated by using as a substrate 3-hydroxypropionaldehyde (**2a**) which was prepared from acrolein via acid-catalyzed hydration. In aqueous solution at room temperature, **2a** and acrolein are at an equilibrium ratio of ~30:1.[14] The formation of **2a** was observed by monitoring by a 1 M solution of acrolein in 0.1 N H_2SO_4 with D_2O as the solvent by ^{1}H NMR. After 5 days at room temperature, signals were clearly present at δ 1.85, 2.80, 3.70, 3.95, 5, and 9.75. These would correspond to the assignments shown in Figure 1. Precipitation of a polymer was not observed even after a month, at which time the signals for acrolein were quite faint and the spectrum was dominated by the aforementioned signals.

A problem in the described aldol condensations is the constant presence of acrolein. Being a strong Micheal acceptor, it readily alkylates nucleophilic species. This is evidently the case in that FDP aldolase (immobilized or not) completely loses all activity in the presence of acrolein. To remove the small amount of acrolein, the solution of **2a** should be concentrated under vacuum before use or washed with chloroform. 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 indicate only the 5-deoxy sugar and no vinylic functionality.

Scheme III

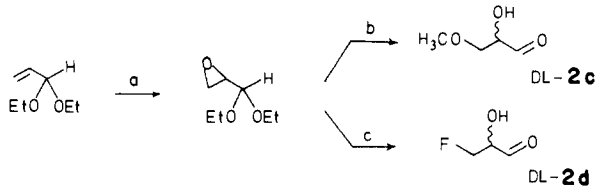

Table I. Unnatural Sugars and Isomerase

substrate	conditions[b]	equilibrium ratios[a] ketose, %	aldose, %
3-deoxy-D-glucose[c]	1	38	62
5-deoxy-D-fructose (**4a**)	2	100	0
6-deoxy-D-fructose (**4b**)	2	20	80
6-*O*-methyl-D-fructose (**4c**)	2	40	60
6-deoxy-6-fluoro-D-fructose (**4d**)	3	20	80

[a] The equilibrium ratios were calculated by comparing the peak heights of the compounds as analyzed by HPLC. Some sugars had double peaks. In these cases, the sum of the two peaks was used. [b] Condition 1: pH 8.5, 24 h at 65 °C. Condition 2: pH 7.5, 45–50 °C with millimolar concentrations of Mg^{2+}, Co^{2+}, and Mn^{2+}. Sugar concentration was 50–100 mM. Condition 3: Same as 2 except 37 °C. [c] See ref 12.

The condensation was successful in generating **3a** and **4a** with the addition of supplemental FDP aldolase. Subsequent treatment with isomerase, however, did not generate 5-deoxy-D-glucose (**6**) measurably. In fact, treatment of a chemically synthesized sample of **6** with isomerase proceeded quantitatively to **4a**. Treatment of the mixture of 6-deoxy-D-fructose (**4b**) and 6-deoxy-L-sorbose (6DS) with GI resulted in a mixture of **4b** (10%), 6DS (50%), and 6-deoxy-D-glucose (**5b**) (40%). HPLC analysis indicates that 6DS is not a substrate for GI. These compounds were readily separated by column chromatography. The substrate DL-lactaldehyde ((±)-**2b**) was derived from dihydroxyacetone[15] and could be prepared on a large scale.[16] D-Lactaldehyde (**2b**) was prepared from L-threonine.[17]

Condensation of 3-*O*-methyl-DL-glyceraldehyde ((±)-**2c**, prepared according to Scheme III) with DHAP gave 6-*O*-methyl-D-fructose 1-phosphate (**3c**) and 6-*O*-methyl-L-sorbose 1-phosphate. The 6-*O*-methyl sugars are of interest in that they are not easily derived from the natural sugars. Such compounds are of value in studies of the Maillard and Amadori reactions and in formation of reductones and their transformations.[19] Although the 6-*O*-methylketoses could not be distinguished by HPLC or TLC (see Experimental Section), treatment with isomerase yielded only a single new product. This aldose, 6-*O*-methyl-D-glucose (**5c**), was subsequently isolated and characterized. The 6-*O*-methylketoses were also generated on a 20-mmole scale by using the inexpensive dicalcium salt of FDP as the source of **1**.

The 6-deoxy-6-fluoro sugars were generated by condensing DL-3-fluoro-2-hydroxypropanal ((±)-**2d**) with DHAP. Acroein diethyl acetal was the source of **2d**, using a similar approach to the preparation of **2c**.[18,20] As in the case of the 6-*O*-methyl sugars,

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(11) GI is used industrially to convert glucose to a mixture of fructose and glucose (Chen, W.-P. *Process Biochem.* **1980**, *15*, 30–41). The enzyme is readily available and has a wide substrate specificity (Bock, K.; Meldal, M.; Meyer, B.; Wiebe, L. *Acta Chem. Scand., Ser. B.* **1983**, *37*, 101–108.
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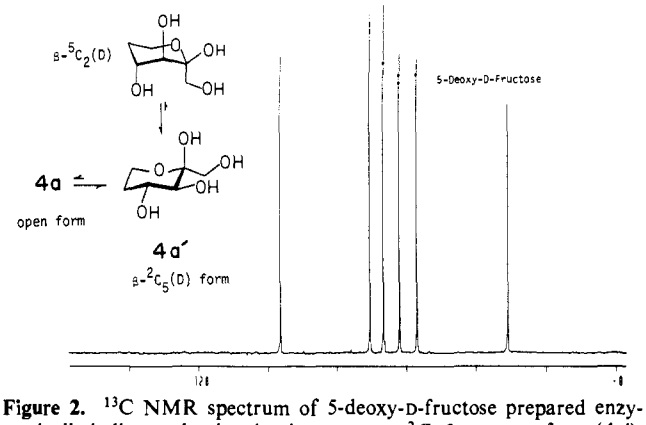

Figure 2. ^{13}C NMR spectrum of 5-deoxy-D-fructose prepared enzymatically indicates that it exists in water as a $^2C_5$ β-pyranose form (**4a'**), determination based on the shift of the anomeric carbon compared to the spectra of the corresponding 1,5-anhydroalditol and the empirical rules developed by Que and Gray (Que, L., Jr.; Gray, G. R. *Biochemistry* **1974**, *13*, 146). The conformation was also prepared by Martin et al. (ref 25b).

6-deoxy-6-fluoro-D-fructose (**4d**) and 6-deoxy-6-fluoro-L-sorbose were indistinguishable by HPLC or TLC. However, treatment with isomerase yielded a mixture from which 6-deoxy-6-fluoro-D-glucose (**5d**) was easily isolated by column chromatography and characterized.

Replacement of the OH group with F introduces relatively small changes in the polarity and van der Waals radii of the fluorinated carbohydrate analogues.[21] Such analogues have been utilized for studies of the active site of enzymes and of membrane transport systems;[21] of chemotherapeutic interest is the observation that compound **5d** was active against solid tumors.[22] We note that compound **5d** can also be prepared by a chemical method using (diethylamino)sulfur trifluoride (DAST).[23]

Glucose Isomerase. The practicality of using this enzyme is derived from the following criteria. First, it has a wide substrate specificity.[11,12] Modifications at the 3- or 6-position of a glucose (or fructose) are tolerated as well as 5-deoxy. Modifications at the 4-position, however, or epimerization at 3 or 5 (L sugars) is not. This last point leads to a second factor. The D and L sugars of a mixture of epimers formed from a racemic aldehyde can be differentiated. Subsequent purification is promoted by the relative ease of separating an aldose and ketose compared to epimeric ketoses. Finally, the enzyme is readily available as an immobilized form and is relatively inexpensive.

The mechanism of this reaction[24] is believed to involve the α anomers only and to proceed through a *cis*-enediol intermediate. At equilibrium, the ratio of ketose to aldose is thermodynamic in nature. Table I summarizes results of glucose isomerase treatment of the unnatural sugars of this study. Most favor the aldose, possibly since only the aldose can exist as a pyranose. The 5-deoxy case is quite unusual, totally favoring the pyranose form of ketose **4a**. This is consistent with the NMR analysis (Figure 2). To date, there has been significant work concerning altering the equilibrium to favor the ketose;[25a] however, there is no general method of favoring the aldose. We note that 5-deoxy-D-fructose (**4a**) has been prepared chemically in six-step reactions with an overall yield of 10% starting with 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose.[25b] Compound **4a** has several unusual properties which deserve further comments: First, in solution it exists exclusively as a $^2C_5$ β-pyranose form[25b] (**4a'**, Figure 2) with no other detectable isomers. Second, it fits as well as β-D-fructopyranose (the active sweet component of D-fructose) into the taste receptor model as shown in Figure 3 and thus tastes nearly as sweet as D-fructose, the sweetest natural sugar.[25b] Third, in the presence of glucose isomerase, compound **6** is completely converted to **4a'** while the reverse reaction does not take place at all. Energy calculation of the other tautomers and related sugars (Table II) indicates that **4a'** is about 1 kcal/mol more stable than the corresponding α form and about 4 kcal/mol more stable than **6**, which can only exist in a furanose form due to the absence of the 5-OH group. These calculated results are in agreement with those observed. Fourth, it is not a substrate for the enzymes hexokinase, glucose dehydrogenase, and sorbitol dehydrogenase, implying that it may have potential value as a low-calorie sweet substitute.

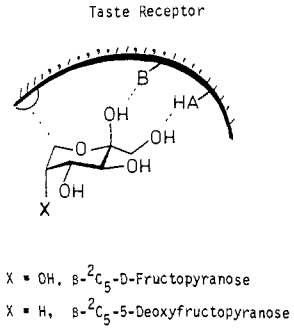

Figure 3. H-bonding and lipophilic interactions between substrate and the taste receptor. The receptor model was proposed by Schallenberger (Shallenberger, R. S.; Acree, T. E. *Nature* (*London*) **1977**, *216*, 480).

Table II. Calculated Energy (kcal/mol at 25 °C) and Observed Mole Fraction in Water of Various Sugar Tautomers[a]

sugar	conform	free energy[a]	mole fraction calcd	mole fraction obsd
5-deoxy-D-fructopyranose	α-$^2C_5$	3.50	0.07	<0.1
	α-$^5C_2$	3.30	0.10	<0.1
	β-$^2C_5$	2.05	0.83	>0.9
	β-$^5C_2$	5.65	0	<0.01
5-deoxy-D-glucofuranose	α	6.34[b]	0.48	0.48[c]
	β	6.29[b]	0.52	0.52[c]
D-glucopyranose	α-$^5C_2$	2.40	0.36	0.37[d]
	β-$^5C_2$	2.05	0.64	0.63[d]
D-fructopyranose	α-$^5C_2$	3.65	0.14	0[e]
	α-$^2C_5$	4.30	0.12	0[e]
	β-$^5C_2$	6.00	0	0[e]
	β-$^2C_5$	2.85	0.74	0.72[e]

[a] For energy calculation, see: Angyal, S. J. *Aust. J. Chem.* **1968**, *21*, 2731. [b] Back-calculated assuming that the equilibrium ratio of D-glucofuranose and D-glucopyranose represents an approximate conformational energy for **2** and that the equilibrium ratio of α and β anomers for **2** (see *c*) holds for D-glucofuranose as well. In water, D-glucose exists 0.14% as the β-D-furanose form (Williams, C.; Allerhand, A. *Carbohydr. Res.* **1977**, *56*, 173–180). [c] Based on the ^{13}C NMR spectra of the anomeric carbon. [d] Exists 0.2–0.8% as furanose forms (see *b*). [e] Contains 5% α-furanose, 23% β-furanose, and <2% α-pyranose form (Que and Gray in Figure 2).

A typical small-scale isomerase experiment would involve buffering the ketose at ~7.5, followed by addition of the metal cofactors (Mg^{2+}, Co^{2+}, and Mn^{2+}) and the immobilized glucose isomerase (Takasweet, from Miles Laboratories). The solution is warmed at ~50 °C in a water bath and intermittingly swirled during the course of the reaction. Alternatively, the apparatus as shown in Figure 4 was used. The desired solution (sugar, buffer, and metal cofactors at pH ~7.5) was passed through a stationary bed of glucose isomerase; the flow rate was controlled with a peristaltic pump. The bed is in a jacketed reactor maintained at 50 °C by a constant-temperature circulator.

Sugar Purification. Column chromatography using different salts of ion-exchange resins[13] as a stationary phase and water as

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Table III. Purification of Sugars

mixture	column	mobile phase	comments
6-deoxy-D-fructose and 6-deoxy-L-sorbase	Dowex 50, Ba^{2+}, 100–200 mesh[a]	water	some overlap with 6-deoxy-D-fructose first
6-deoxy-D-fructose and D-fructose	Dowex 50, Ba^{2+}, 100–200 mesh[a]	1:1 $EtOH/H_2O$	good separation with D-fructose first
6-deoxy-D-fructose and 6-deoxy-D-glucose	Dowex 50, Ba^{2+}, 100–200 mesh[a]	1:1 $EtOH/H_2O$	separation incomplete, ketose first
6-*O*-methyl ketoses and 6-*O*-methyl-D-glucose	Dowex 50, Ba^{2+},[b] 200–400 mesh	water	separation required two passages, aldose first
6-fluoro-ketoses and 6-fluoroglucose	Dowex 1 (HSO_3^-),[c] 50–100 mesh	water	ketoses unseparated, aldose well resolved ketoses first

[a] 3 × 76 cm. [b] 3 × 35 cm. [c] 3 × 63 cm.

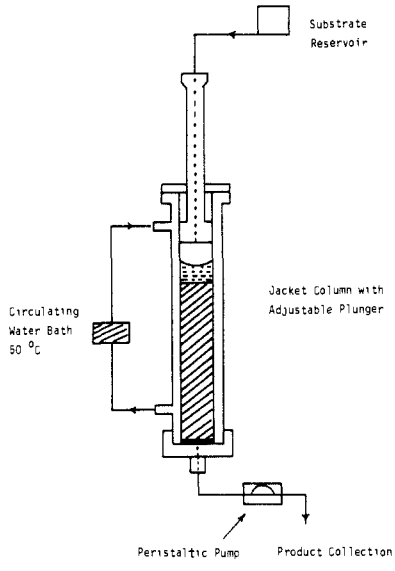

Figure 4. Schematic representation of a column reactor (1 × 10 cm).

a mobile phase was employed when necessary to purify sugars. Mixtures of sugars result from (1) use of a racemic aldehyde in the aldol condensation, (2) equilibrium mixtures of aldose and ketose produced by the action of isomerase, and (3) contamination with fructose or other compounds, especially when the calcium salt of FDP is used as a source of **1**.

Data pertaining to individual separations can be found in Table III. Generally, there is no one column and mobile phase suited for all sugar separations. The anion-exchange column (Dowex 1, HSO_3^-) seems to have good potential to separate aldoses and ketoses even though the 6-*O*-methylketoses were not well retained.

Dihydroxyacetone/Arsenate as a Substrate for DHAP. Dihydroxyacetone in the presence of inorganic arsenate was found to be very effective as a substrate for FDP aldolase. This eliminates the need for preparation of DHAP, which is less stable and more expensive than dihydroxyacetone. Several sugars including D-fructose, 6-deoxy-D-fructose, and 5-deoxy-D-fructose (**4a**) have been prepared on millimole scales. Further study with respect to the generality of this type of reaction in synthetic applications is in progress.

It has been reported that several enzymes whose natural substrates are the phosphorylated derivatives also act on the nonphosphorylated analogues if arsenate or vanadate is present, presumably due to the spontaneous formation in solution of the arsenate or vanadate ester, which is then accepted by the enzyme as a phosphate analogue.[27] Kinetic studies (Lagunas in ref 27) indicate that the formation of arsenate esters at pH 7 is ca. 10^5 times greater and that of vandate esters is ca. 10^{11} times greater than the formation of homologous glucose 6-phosphate esters (ca. 9×10^{-11} M^{-1} s^{-1}). We found that no appreciable reaction was observed in the aldol reactions when arsenate was replaced with vanadate.

Conclusion

Several examples of unnatural substrates have been explored in synthesizing unusual sugars. The reactions have been carried out from 4- to 20-mmol scales. Modifications of glucose or fructose at the 6-position can be readily achieved by using a glyceraldehyde appropriately substituted at the 3-position. Such aldehydes can be generated from acrolein diethyl acetal. A change at the 5-position has only been modestly explored (5-deoxy series and all L-sorbose derivatives), and indications are that the process will stop at the ketose stage. Altering the isomerase equilibria to favor the aldose and exploring ways to isomerize the sorboses to the corresponding idose are subjects for additional research. Thus, this approach offers an alternate route to 5- and 6-position-derived monosaccharides. The disadvantage of this process is that there is no way of changing the stereochemistry of the C–C bond formation in the aldol reactions. Although there have been more than 20 different aldolases isolated and each of them catalyzes a different type of aldol reaction using different substrates,[26] their synthetic utility remains to be explored.

Experimental Section

FDP aldolase (rabbit muscle, EC 4.1.2.13), TPI (EC 5.3.1.1), and acid phosphatase (EC 3.1.3.2) were purchased from Sigma. Glucose isomerase (EC 5.3.1.5) was obtained from Miles in its immobilized form, Takasweet. Fructose diphosphate (Na_3 and Ca_2, >98% and ≤70% pure, respectively) and dihydroxyacetone were obtainid from Sigma. Acrolein diethyl acetal, glycidol, and KHF_2 were obtained from Aldrich. Acrolein and benzonitrile were obtained from Kodak and distilled prior to use (acrolein must be stored cold and in the dark). Dowex resins were obtained from Bio-Rad Laboratories. All other reagents were reagent grade. UV spectra were taken with a Beckman DU-6 spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in either a 1-dm or a 1-cm cell. HPLC analyses were done on a Gilson chromatography system, which included a Model 302 pump (1–1.5 mL/min), a Model 131 refractive index detector, and N1 recorder, a Rheodyne injector (20-μL loop), and a Waters Carbohydrate Analysis column (3.9 mm × 30 cm, 10 μm). The mobile phase used 85% CH_3CN/15% H_2O. Samples were diluted with an equal volume of CH_3CN and centrifuged prior to HPLC analysis. Enzymatic assays and analyses were carried out by standard methods.[28] Aldolase was immobilized as described previously.[29] Phosphates were assayed by the method of Ames.[30] Proton-decoupled ^{13}C NMR (50 MHz) and ^1H NMR (200 MHz) were obtained with a Varian XL-200 spectrometer. Proton NMR (90 MHz) were obtained on a Varian EM 390 spectrometer. Proton standards were 3-(trimethylsilyl)-1-propanesulfonic acid, TMPS (δ 0), and acetonitrile (δ 1.95). Carbon standards were $CDCl_3$ (δ 77.0), acetonitrile (δ 1.3), methanol (δ 49.0), and acetone (v 30.6).

3-Deoxy-D-glucose. 3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose prepared according to the reported procedure[31] (0.21 g, 0.9 mmol) was dissolved in 5 mL of water to which 0.05 g of Dowex 50-X8 (H^+) was added. The mixture was refluxed for 2 h, evaporated, and

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lyophilized to yield 0.11 g of an amorphous solid: $[\alpha]^{24}_D$ +24.6° (*c* 1.3, H_2O) [lit.[31] $[\alpha]_D$ +29.0° (*c* 1, H_2O)]. ^1H NMR (90 MHz, D_2O) shows methylene absorption [δ 1.33–2.56 (m, 2 H)] and is in agreement with that reported.[32]

5-Deoxy-D-glucose (5a). 5-Deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose[33] (100 mg, 0.49 mmol) and 20 mg of Dowex (50-X8 (H^+) were refluxed in H_2O (2 mL) for 2 h. After filtration and freeze-drying, 78.8 mg (98%) of a pale-yellow oil was obtained: $[\alpha]^{18}_D$ +24.1° (*c* 7.8, H_2O) [lit.[34a] $[\alpha]_D$ +24° (*c* 2.1, H_2O), +17.3° (*c* 5.5, H_2O), +40° (*c* 1.7, H_2O)]; ^1H NMR (D_2O) δ 5.49 (d, 1 H, $H_{1\beta}$, *J* = 4 Hz), 5.22 (s, 1 H, H, $H_{1\alpha}$), 4.46–4.04 (m, 3 H), 3.90–3.61 (m, 2 H), 1.65–2.04 (m, 2 H, H_5); ^{13}C NMR (D_2O) δ α = 96.5 (C_1), 77.0 (C_2, C_3), 81.6 (C_4), 31.9 (C_5), 59.5 (C_6), β = 102.5 (C_1), 77.0 (c_2), 76.1 (C_3), 79.8 (C_4), 32.6 (C_5), 59.5 (C_6). The data are in agreement with those reported.[34b]

Fructose Diphosphate as a Source of DHAP (1). As mentioned earlier, FDP in the presence of aldolase and triose phosphate isomerase (TPI) acts as a source of DHAP (**1**). Two distinct sources of FDP were utilized: high purity (98–100%, Na_3, $8H_2O$, ~$5/g) and low purity (≤70%, Ca_2, ~$0.20/g). The high-purity phosphate gave quantitative results in the formation of the ketose phosphate **3**. The lower purity phosphate was also found to be quite useful, even though the resulting ketose phosphate may contain inorganic as well as other sugar phosphates in addition to the desired product. Subsequent hydrolysis followed by column chromatography purifies the sugars.

3-Hydroxypropionaldehyde (2a). Compound **2a** was generated by the approach of Hall and Stern.[14] To a solution of 1.2 mL of H_2SO_4 (43 mequiv) and 440 mL of H_2O was added 30 mL (25.2 g, 449 mmol) of freshly distilled acrolein. The solution was allowed to stand in the dark in a sealed bottle at room temperature for 3 days, after which it was stored in the refrigerator (0–4 °C). Subsequent analysis by titration of 1-mL aliquots (treat with excess KI_3, allow to stand 30 min, and back-titrate with $Na_2S_2O_3$ to a light-yellow end point) indicated very little (<5%) acrolein present, although the acrolein was present by olfactory detection. Freshly prepared solutions of ~1 M acrolein readily consumed the KI_3 in nearly quantitative results. ^1H NMR was also used to monitor the hydration of acrolein. A solution of acrolein (~1 M) in D_2O and H_2SO_4 (0.1 N) was observed over about a 1-month period. The sample was tightly capped and stored in a dark, cool cabinet between analyses. After ~5 days, signals were clearly present at ca. δ 1.8, 2.8, 3.7, 3.9, 5.1, and 9.7. These represent the $-CH_2-$ (hydrate, aldehyde), $-CH_2O-$ (hydrate, aldehyde), $CH(OH)_2$, and –COH protons, respectively. Even after a month, no clouding of the solution typical of polymerization was seen. The solution was used directly in aldolase reactions.

5-Deoxy-D-fructose 1-Phosphate (3a). To 100 mL of an aqueous solution containing 7 mmol of fructose diphosphate (from Ca_2FDP) was added 14 mL of 1 M **2a** (14 mmol) in 0.1 N H_2SO_4. The pH was adjusted to 6.9 with NaOH, and 50 mL of water was added. Aldolase (85 units) and triose phosphate isomerase (500 units) were added, and the solution was kept under argon in the dark. Four days later an additional 85 units of aldolase was added, and after 8 days the fructose diphosphate was consumed, as indicated by enzymatic analysis. $BaCl_2$ (5.5 g, 26 mmol) was dissolved in a minimal amount of water and added to the reaction mixture. The pH was adjusted to 7.8 with NaOH, this first precipitate was recovered, and an additional 3.0 g of $BaCl_2$ (14 mmol) dissolved in minimal water was added. The pH was again adjusted to 7.8 with NaOH, 750 mL of acetone was added, and the solution was chilled. Centrifugation followed by an acetone wash yielded the Ba^{2+} salt of **3a** (42% yield): $[\alpha]^{25}_D$ –16.2° (Ba^{2+} salt, *c* 0.93, H_2O); ^1H NMR (90 MHz, D_2O) δ 1.4–2.2 (m, 2 H, C5 CH_2), 3.2–4.2 (m, 6 H, C1, C3, C4, C6).

5-Deoxy-D-fructose (4a). The Ba^{2+} salt of **3a** (1.0 g, 2.6 mmol) was dissolved in 50 mL of water, and Dowex 50 (H^+) was added to adjust the pH to <2. The solution was heated in a 70 °C water bath for ~12 h to hydrolyze the phosphate. The solution was filtered, and the pH was adjusted to 7.5 with 0.1 M $Ba(OH)_2$. The solution was diluted with an equal volume of acetone, and the solution was chilled. After the solution was filtered through Celite 545, the acetone was removed under vacuum. The residue was then lyophilized to yield 0.52 g of **4a** (HPLC indicates this to be the only sugar; no fructose): $[A]^{25}_D$ –64.6° (*c* 4.3, H_2O); ^1H NMR (200 MHz, D_2O) δ 1.52 (1 H, q), 1.86 (1 H, m), 3.33 (2 H), 3.58 (2 H), 3.77 (2 H); ^{13}C NMR (50 MHz, D_2O) δ 98.1 (C2), 72.2, 68.3, 63.6, 58.7 (C1, C3, C4, C6), 32.8 (C5). Anal. Calcd for $C_6H_{12}O_5$: C, 43.9; H, 7.3. Found: C, 43.7; H, 7.1.

5-Deoxy-D-fructose (4a), 5-Deoxy-D-glucose (6), and Glucose Isomerase. The enzymatically prepared **4a** (~1 mmol) was dissolved in an aqueous solution of 5 mM $MgCl_2$ and $Co(NO_3)_2$, 2 mM $MnCl_2$, and 10 mM phosphate at pH 7.5 (total volume = 10 mL). The solution was circulated through a water-jacketed column, described earlier, containing 1.0 g of Takasweet at 50 °C for 60 h. No formation of 5-deoxy-D-glucose (**6**) was detectable by HPLC analysis using chemically synthesized **6**[33,34a] as a standard. However, treatment of the chemically synthesized **6** with isomerase (2-mL total volume in a vial with 0.65 g of Takasweet) quantitatively converted **6** to **4a** in ~2 h, as monitored by HPLC.

Pyruvaldehyde Dimethyl Acetal. In a 250-mL round-bottom flask, fitted with a reflux condenser, were combined 50 g of dihydroxyacetone (0.56 mol), 150 mL of dry MeOH, and 10 g of vacuum-dried Dowex 50 (H^+). An oil bath at 75–80 °C was used to warm the solution with stirring for 16 h. The dark-yellow, cloudy solution was cooled, filtered, and diluted with 200 mL of water. The aqueous solution was extracted 3 times with 200-mL portions of methylene chloride. The organic layers were combined, dried over Na_2SO_4, and concentrated on the rotovap. Vacuum distillation yielded 26 g (220 mmol, ~40%) of clear, light-yellow liquid boiling at 51–55 °C at 30 mmHg: ^1H NMR (90 MHz, neat) δ 2.15 (s, 3 H), 3.42 (s, 6 H), 4.45 (s, 1 H). Anal. Calcd for $C_5H_{10}O_3$: C, 50.85; H, 8.47. Found: C, 50.80; H, 8.50.

DL-Lactaldehyde Dimethyl Acetal.[16] Into a dry 250-mL three-neck flask containing 3.6 g of $LiAlH_4$ (95 mmol) in 75 mL of anhydrous ether under argon was added 10.2 g (86 mmol) of the dimethyl acetal of pyruvaldehyde (slowly moderated with an ice bath). After the addition was complete, an additional 75 mL of ether was added, and the reaction mixture was refluxed for 30 min, cooled, and quenched with dropwise addition of saturated aqueous NaCl. To the mixture was added Na_2SO_4 to absorb water, and the slurry was filtered. The solids were washed with ether, and all organics were combined and concentrated under vacuum. Distillation of the residue yielded 5.4 g (45 mmol) of DL-lactaldehyde dimethyl acetal: bp 62–67 °C (30 mmHg); 52% yield; ^1H NMR (90 MHz, CCl_4) δ 1.05 (d, *J* = 7 Hz, CH_3), 3.43 (s, OCH_3), 3.48 (s, OCH_3), 3.6 (m, CH), 3.95 (d, *J* = 5 Hz, $CH(OMe)_2$).

DL-Lactaldehyde (DL-2b, Racemic 2-Hydroxypropanal). An aqueous solution of DL-lactaldehyde dimethyl acetal (~0.7 M) was warmed with Dowex 50 (H^+) until TLC analysis (silica gel, $CHCl_3$/MeOH 8:2) indicated no starting material. This solution was used directly without further characterization in aldolase reactions.

D-Lactaldehyde (2b, (*R*)-2-Hydroxypropanal).[17] L-Threonine (6.01 g, 50 mmol) and citric acid monohydrate (12.64 g, 60 mmol) were dissolved in 1.2 L of H_2O. The pH was adjusted to 5.3 with 10 N NaOH. Ninhydrin (18.0 g, 101 mmol) was added, and the solution was heated to a boil and boiled for 15 min. The solution was allowed to stir on the hot plate for 2.5 h. The solution was cooled in an ice bath and filtered. The pH was adjusted to 6.5 with Dowex 1 (HCO_3^-) and stirred overnight. The solution was filtered and the pH was now 7.5. The pH was then adjusted to 4 with Dowex 50 (H^+), the solution was filtered, and the volume was reduced under vacuum to 250 mL. The pH was adjusted to 6.5 with Dowex 1 (HCO_3^-) and the solution was stirred overnight and filtered. The solution was filtered and the pH was now 7.5. The pH was then adjusted to 4 with Dowex 50 (H^+), the solution was filtered, and the volume was reduced under vacuum to 250 mL. The pH was adjusted to 6.5 with Dowex 1 (HCO_3^-) and the solution was stirred overnight and filtered. The pH was adjusted to 4 with Dowex 50 (H^+), the solution was filtered, and the volume was reduced under vacuum to ~80 mL. An assay of this solution with aldehyde dehydrogenase indicated 16.4 mmol of aldehyde (33% yield). This solution was used directly in aldolase reactions.

6-Deoxy-D-fructose 1-Phosphate (3b). In a three-neck, 100-mL round-bottom flask equipped with a gas-bubbling tube, a gas exit, and a stirring bar were combined 38 mL of water containing D-lactaldehyde (**2b**) (15.6 mmol) and 2.75 g of fructose 1,6-diphosphate (Na_3·$8H_2O$, 5 mmol, a source of DHAP). The pH of the solution was adjusted to 7.0 with NaOH, and argon was bubbled through the solution to degas it. Triose phosphate isomerase (500 units) was added along with 263 units of FDP adolase. After 22 h, the reaction assayed greater than 98% complete. The reaction mixture was placed in a 250-mL beaker, 2.5 g of $BaCl_2$ (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 wit acetone, and then redissolved in 50 mL of water. Dowex 50 (H^+, 20 g) was added with stiring for 30 min to remove barium ions. Filtration followed by an additional deionization with 10 g of Dowex 50 (H^+) and a final filtration yielded 95 mL of a pale-yellow, clear filtrate of pH ~2. A 50-mL aliquote was removed and the pH adjusted to 7.5 with NaOH. The

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solution was lyophilized to yield 0.94 g (62% overall) of the disodium salt of **3b**: $[\alpha]^{25}_D$ (−1.25° (*c* 0.72, H_2O, Ba^{2+} salt); ^1H NMR (200 mHZ, D_2O, Ba^{2+} salt) δ 1.20–122 (3 s, 3 H, CH_3), 3.6–3.9 (m, 4 H), 3.9–4.1 (m, 1 H).

6-Deoxy-D-fructose (4b). The Ba^{2+} salt of **3b** (2.0 g, 5.3 mmol) was suspended in 50 mL of H_2O, and Dowex 50 (H^+) was added with stirring to adjust the pH to <2. After it was filtered, the solution was warmed over a steam bath overnight to hydrolyze the phosphate (an example of phosphatase-catalyzed hydrolysis is given in the following experiment). The solution was filtered and the pH adjusted to 7.5 with 0.1 N $Ba(OH)_2$. An equal volume of acetone was added and the solution filtered through Celite 545. The acetone was removed under vacuum and the residue lyophilized to yield **4b** as a brown semisolid (1.11 g): $[\alpha]^{25}_D$ −6.61° (*c* 6.2, H_2O; ^1H NMR (200 MHz, D_2O) δ 1.31 (s, CH_3), 1.34 (s, CH_3), 3.52–4.12 (m, 5 H); ^{13}C NMR (50 MHz, D_2O) δ 20.1, 21.6 (C6), 65.5, 77.8, 78.8, 81.9, 83.7, 85.0 (C1, C3, C4, C5), 103.6 (C2).

6-Deoxy-D-glucose (5b). A sample of **3b** (Ba^{2+} salt, contaminated with some residue from Ca_2FDP, 975 mg) and Dowex 50 (4.3 g, H^+) were stirred together in 25 mL of water for ~30 min. The pH was adjusted to 7.0 with saturated $KHCO_3$ (aqueous) and the solution filtered. The pH was adjusted to 5.0 with Dowex 50 (H^+) and $KHCO_3$ (final volume ~40 mL). Phosphatase (70 mg, 50 units) was added, and the reaction mixture was placed in a 40 °C water bath. Phosphate (inorganic, organic) was monitored by using the method of Ames. After 33 h, the reaction was complete (HPLC and TLC indicated the presence of **4b**). Aliquots of 0.1 M $MgCl_2$, $Co(NO_3)_2$ (0.5 mL), and $MnCl_2$ (0.25 mL) were added to give final concentrations of ~1, 1, and 0.5 mM, respectively. The pH was adjusted to 7.5 with NaOH, and the solution was centrifuged. Takasweet (1 g, an immobilized form of glucose isomerase) was added. The solution was placed in a 50 °C water bath, and after 48 h the reaction mixture had reached the equilibrium ratio (4:1) of **5b**/**4b**. The solution was filtered and lyophilized. Column chromatography (Dowex 50, Ba^{2+} salt, 100–200 mesh, 50:50 ethanol/H_2O, 3 × 75 cm) readily separated the contaminant from the deoxy sugars. A second purification, utilizing the Dowex 1 (HSO_3^-) column (50–100 mesh, water, 2.5 × 65 cm, 0.5 mL/min), yielded **5b** (16 mg, 0.1 mmol): $[\alpha]^{25}_D$ +29.3° (*c* 0.16, H_2O) [lit.[36] $[\alpha]_D$ +30°]; ^1H NMR (200 MHz, D_2O) δ 1.15 (d, CH_3, *J* = 6 Hz), 1.16 (d, CH_3, *J* = 6 Hz), 2.95–3.22 (m), 3.22–3.47 (m), 3.47–3.58 (m), 3.58–3.98 (m), 3.98–4.08 (m), 4.48 (d, $H_{1\alpha}$, *J* = 7.4 Hz), 5.05 (d, $H_{1\beta}$, *J* = 4 Hz); ^{13}C NMR (50 MHz, D_2O) δ 17.21 (C6), 67.73, 72.12, 72.23, 72.85, 74.74, 75.26, 75.58, 75.84, 76.69 (C2 through C5), 92.31, 96.12 (C1). ^{13}C NMR data are consistent with thoose reported (see ref 35). Anal. Calcd for $C_6H_{12}O_5$: C, 43.9; H, 7.3. Found: C, 44.0; H, 7.2.

Glycidaldehyde Diethyl Acetal. To a suspension of $KHCO_3$ (1.5 g) in methanol (50 mL) was added acrolein diethyl acetal (12.84 g, 88 mmol). Benzonitrile (9.2 mL, 90 mmol) and 30% aqueous H_2O_2 (10.56 g, 93 mmol) were added, and the mixture was stired at room temperature for 18 h followed by 4 h at 45 °C. H_2O (50 mL) was added, and about 40 mL of methanol was removed under reduced pressure. The solution was extracted with $CHCl_3$ (4 × 30 mL). The organic layers were combined, dried over $MgSO_4$, and evaporated to near dryness. To the resulting residue was added hexane (35 mL) to precipitate benzamide. The benzamide was removed by filtration, and the hexane was removed under reduced pressure. The remaining liquid was distilled under reduced pressure to give glycidaldehyde diethyl acetal (7.52 g, 52 mmol, 58%): bp 86 °C (50 mmHg) [lit.[37] 62–63 °C (13 mmHg)]. ^1H NMR (90 MHz, $CDCl_3$) δ 1.1–1.4 (t, 6 H, CH_3), 2.70 (d, 2 H, H3), 3.1 (q, 1 H, H3), 3.1 (q, 1 H, H2), 3.4–3.8 (m, 4 H, OCH_2), 4.3 (d, 1 H, H1).

3-*O*-Methylglyceraldehyde Diethyl Acetal. To a stirred and gently refluxing solution of 0.1 g of sodium hydroxide in 20 mL of commercial anhydrous methanol was added dropwise 7.3 g (50 mmol) of glyceraldehyde diethyl acetal. The solution was heated under reflux for 4 h. The excess methanol was removed under reduced pressure and the residue distilled. There was obtained 6.7 g (70%) of a colorless liquid boiling at 120 °C (50 mmHg): ^1H NMR (90 MHz, $CDCl_3$) δ 1.1–1.4 (t, 6 H, CH_3), 2.60 (d, 1 H, OH), 3.3 (s, 3 H, OCH_3), 3.4–3.9 (m, m, 6 H, H1, CH_2, CH), 4.50 (d, 1 H, H2).

3-*O*-Methylglyceraldehyde (2c). A solution of 5.3 g (30 mmol) of the acetal in 50 mL of water was stirred with 1 g of Dowex 50 (H^+) resin at 40 °C for 4 h and monitored by TLC. After disappearance of the acetal spot, the resin was filtered off. The filtrate was concentrated under reduced pressure to give 3-*O*-methylglyceraldehyde (2.5 g) as an oil. This aldehyde was used directly without further purification in aldol reaction: ^1H NMR (90 mHz, D_2O) δ 3.36 (s, 3 H, OCH_3), 3.56 (m, 2 H, CH_2), 3.80 (m, 1 H, CH), 5.10–5.42 (m, m, 1 H, CH). The ^1H NMR spectrum indicates this aldehdye exists as a dimer.

6-*O*-Methylketose (3c, 4c) (4-mmol Scale). In a three-neck 100-mL round-bottom flask equipped with a gas-bubbling tube, a gas exist, and a stir bar were combined 80 mL of water containing 3-*O*-methylglyceraldehyde (860 mg, 8 mmol) and 1.1 g of fructose 1,6-diphosphate ($Na_3 \cdot 8H_2O$, 2 mmol). The pH of the solution was adjusted to 7.0 with NaOH, and argon was bubbled through the solution to degas it. TPI (500 units) was added along with 95 units of FDP aldolase. After 24 h, the reaction was complete as indicated in enzymatic analysis (100 μL of the reaction solution no longer lowered the absorbance at 340 nm when added to a solution (900 μL) containing aldolase, glycerophosphate dehydrogenase, TPI, and NADH). The reaction mixture was placed in a 500-mL beaker, 5.2 g of $BaCl_2$ (24 mmol) was added, and the pH was adjusted to 7.5 with NaOH. Acetone (250 mL) was added, and the barium salt was centrifuged, washed with acetone, and then redissolved in 50 mL of water. Dowex 50 (H^+) (10 g) was added with stirring for 30 min to remove Ba^{2+}. Filtration followed by an additional deionization with 10 g of Dowex 50 (H^+) and a final filtration yielded 95 mL of a pale-yellow, clear filtrate of pH 1.3. Hydrolysis of this filtrate solution at 80 °C for 7 h was monitored by TLC (1:1 EtOAc/MeOH, silica gel); it indicated one spot. The pH was adjusted to 7.1 with aqueous $NaHCO_3$, and the solution was lyophilized. The residue was purified by column chromatography (Dowex 50, Ba^{2+} salt) to yield 700 mg (3.6 mmol, 90% yield) of **4c**: $[\alpha]^{23}_D$ (−7.9° (*c* 1.59, H_2O); ^1H NMR (200 MHz, D_2O) δ 3.30 (s, 3 H, OCH_3), 3.50–3.70 (d, d, 4 H, CH_2), 3.86 (m, 1 Hh, CH), 4.00 (m, 1 H, CH), 4.30 (m, 1 H, CH).

6-*O*-Methyl-D-glucose (5c). A solution of 700 mg (3.6 mmol) of hexose **4c** in 8 mL of water was acidified with H_3PO_4 (50 μL). The pH was adjusted to 7.5 with NaOH, Co^{2+} and Mg^{2+} were added to give a final concentration of 1 mM (80 μL each of a 100 mM solution), and Mn^{2+} was added to give a concentration of 0.5 mM (40 μL of a 100 mM solution). Takasweet (1 g, an immobilized form of glucose isomerase) was added and the solution warmed at 45 °C for 8 h. TLC analysis (1:1 EtOAc/MeOH, silica gel) indicated two spots. The Takasweet was filtered off and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography (Dowex 50, Ba^{2+} salt) to give purified aldose **5c**: $[\alpha]_d$ +56.0° (*c* 1.07, H_2O) [lit.[38] $[\alpha]_D$ +55° (*c* 2, H_2O)]; isolated yield 120 mg (34%); ^{13}C NMR (50 MHz, D_2O) δ 96.8, 92.9, 76.6, 75.3, 74.9, 73.6 72.3, 72.0, 0, 70.7, 70.6, 59.4 (α,β form mixture) (C1, β) (C1, α) (C3, β) (C5, β) (C2, β) (C3, α) (C2, α) (C6, α, β) (C5, α) (C4, α, β) (6-OMe, α, β). The NMR data are consistent with those reported.[19] Anal. Calcd for $C_7H_{14}O_6$: C, 43.3; H, 7.2. Found: C, 43.1; H, 7.0.

6-*O*-Methylketose (20-mmol Scale). In a 125-mL erlenmeyer flask were combined 15.0 g of the dicalcium salt of FDP (36 mmol), 75 mL of water, and 30 g of Dowex 50 (H^+). The sample was stirred until complete dissolution (~0.5 h). The pH was adjusted to 7.0 with $K2CO_3$ (6 M). Activated carbon was added and the solution was filtered through Celite 545 The pH was adjusted to 2.0 with Dowex 50 (H^+), and the solution was assayed for FDP. The assay indicated 9.7 mmol of FDP (equivalent to 19.4 mmol of DHAP). The solution was transferred to a 250-mL three-neck flask equipped with a gas bubbler and a stir bar. Compound **2c** (3.15 g, 30 mmol) was added, and the solution was degassed with argon while the aldehyde dissolved. The pH was adjusted to 7.5 with NaOH (10 N) and PAN immobilized aldolase (100 units) and TPI (500 units) were added. The reaction was monitired by assaying for FDP enzymatically. After 5 days at room temperature with moderate stirring and under an argon atmosphere, the reaction assayed >90% complete. The aldolase was isolated by centrifugation. $BaCl_2$ (18.5, 76 mmol, dihydrate) dissolved in minimal water was added to the combined supernatant. The pH was adjusted to 7.5 with NaOH (10 N) and the precipitate recovered by filtration. To the filtrate was added two volumes of acetone, and this solution was chilled overnight in the freezer. Filtration yielded a white solid. Although both precipitates were treated separately, the second filtrate contained >90% of the product. The second precipitate was dissolved in 125 mL of water and stirred with ~20 g of Dowex 50 (H^+). Filtration yielded a clear-yellow solution. Acetic acid (0.5 mL, 9 mmol) was added, and the pH was adjusted to 4.3 with K_2CO_3 (6 M). Phosphatase (190 mg, 130 units) was added and the solution maintained at 37 °C. After 48 h, the solution assayed >95% complete. The solution was adjusted to pH 7 with K_2CO_3 (6 M), degassed under vacuum, filtered, and lyopholized. The residue was triturated with methanol (2×, 50 mL) and the methanol removed under reduced pressure. HPLC analysis indicated fructose plus other contaminants present. The syrupy residue was column chromatographed by using a Dowex 1 (HSO_3^-) column. The product came off first, and HPLC analysis indicated only the 6-*O*-methyl hexoses. Combining

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fractions and freeze-drying yielded 2.10 g (11.1 mmol, 57% yield) of a light-yellow solid. ^{1}H NMR and HPLC data were identical with those of the small-scale product.

3-Fluoro-2-hydroxypropanal Diethyl Acetal.[18,20] To a solution of glycidaldehyde diethyl acetal (9.81 g, 67.2 mmol) in DMF (10 mL) was added KHF_2 (5.6 g, 71.8 mmol), and the suspension was stirred under reflux. After 48 h, TLC (CH_2cl_2/ether 3:1) showed complete disappearance of starting material (R_f 0.62) and appearance of a product (R_f 0.49). The suspension was filtered to remove KF, and the solution was distilled under reduced pressure to give 3-fluoro-2-hydroxypropanal diethyl acetal (3.35 g, 20.2 mmol, 30.0%): bp 105 °C (40 mmHg); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.1–1.3 (overlapping triplets, 6 H, CH_3), 3.05 (s, 1 H, OH), 3.4–3.9 (m, 5 H, CH_2), 4.45 (d, 1 H, H1), 4.5 (doublet of multiplets, J_{HF} = 48 Hz, CH_2F). ^{1}H NMR results (200 MHz) in Me_2SO-d_6 were the same as in $CDCl_3$ except OH gives a doublet at δ 5.25, with no peak at δ 3.05.

3-Fluoro-2-hydroxypropanal ((±)-2d). A solution of 3-fluoro-2-hydroxypropanal acetal (2.7 g, 16.3 mmol) in water (50 mL) with Dowex 50 (H^+, 1.0 g) was heated at 60 °C for 2 h, after which TLC showed complete disappearance of starting material. The Dowex 50 was removed by filtration, and the solution was neutralized with aqueous NaOH. The solvent was removed under reduced pressure, with absolute ethanol being added to remove residual water to give (±)-**2d** as a white solid (1.27 g, 13.9 mmol, 85.1% yield).

6-Deoxy-6-fluoro-D-fructose 1-Phosphate (3d). In 80 mL of deionized water which had been deoxygenated with a stream of argon were dissolved (±)-**2d** (0.736 g, 8.0 mmol) and Ne_3FDP (1.10 g, 2.0 mmol). The pH of the resulting solution was adjusted to 7.3 with 2 N NaOH, after which FDP aldolase (100 units) and triose phosphate isomerase (100 units) were added. The solution was stirred under argon at room temperature for 12 h. Enzymatic assay indicated no fructose diphosphate remained; $BaCl_2$ (2.44 g, 10 mmol) was added followed by 350 mL of acetone to precipitate a mixture of 6-deoxy-6-fluoro-D-fructose 1-phosphate and 6-deoxy-6-fluoro-L-sorbose 1-phosphate as the barium salts. The precipitate was isolated by centrifugation and dried under vacuum to give the product as a white solid (1.50 g, 1.90 mmol, 95.0% yield). This product was used for further reactions without further purification.

6-Deoxy-6-fluoro-D-fructose (4d) and 6-Deoxy-6-fluoro-L-sorbose. A mixture of the barium salts of 6-deoxy-6-fluoro-D-fructose 1-phosphate and 6-deoxy-6-fluoro-L-sorbose 1-phosphate (1.50 g, 1.90 mmol) was dissolved in water (40 mL), and the solution was acidified to pH 1.5 with 2 N HCl. This solution was heated on a steam bath for 20 h, after which phosphate analysis indicated no organic phosphate remaining. The solution was neutralized with 4 N NaOH and filtered to remove precipitated barium phosphate. The filtrate was treated with activated charcoal and lyophilized to give a mixture of **4d** and 6-deoxy-6-fluoro-L-sorbose (0.32 g, 1.75 mmol, 92%). This mixture was used to in the next step without further purification.

6-Deoxy-6-fluoro-D-glucose (5d). To a solution of **4d** and 6-deoxy-6-fluoro-L-sorbose (0.32 g, 1.75 mmol) in water (15 mL) were added 50-μL aliquots of 100 mM solutions of $MnCl_2$, $MgCl_2$, and $Co(NO_3)_2$ and 50 μL of a 200 mM solution of sodium phosphate buffer (pH 7.4). The pH was adjusted to 7.4, and Takasweet (1.0 g) was added. The mixture was incubated at 37 °C. The reaction was monitored by HPLC. The D-fructose and L-sorbose analogues were not well separated, both having a retention time of 2.5 min, while the glucose analogue was well separated with a retention time of 2.8 min. After 24 h the reaction appeared to be at equilibrium. The mixture was filtered to remove Takasweet, and the solution was purified on a Dowex 1 (HSO_3^-) column (50–100 mesh, 2.5 cm × 65 cm) with water as the eluting solvent and a flow rate of 0.4 mL/min. The fructose and sorbose analogues eluted together with a retention volume of 175–275 mL. Compound **5d** eluted at 350–500 mL. The fractions containing **5d** were combined and lyophilizd to yield **5d** as a white solid (0.80 g): $[\alpha]^{25}_D$ +41.1° (*c* 3.44, H_2O) [lit.[39,40] $[\alpha]^{25}_D$ +46.8° (*c* 3.55, H_2O)]; ^{13}C NMR (D_2O) (α anomer) δ 92.52 (C1), 82.64 (C6, $J_{C6,F}$ = 167.88 Hz), 72.91 (C3), 71.68 (C2), 70.54 (C5, $J_{C5,F}$ = 16.95 Hz), 68.83 (C_4, $J_{C4,F}$ = 5.94 Hz) (β anomer) 96.31 (C1), 82.46 (C6, $J_{C6,F}$ = 167.88 Hz), 75.83 (C3), 74.68 (C5, $J_{C5,F}$ = 17.91 Hz), 74.29 (C2), 68.70 (C4, $J_{C4,F}$ = 6.74 Hz). All chemical shifts are reported relative to the methyl carbon of acetone which was assigned a shift of 30.60 ppm. Anal. Calcd for $C_6H_{11}O_5F$: C, 39.6; H, 6.0. Found: C, 39.5; H, 5.9.

Sugars from Dihydroxyacetone and Arsenate. In a general procedure, dihydroxyacetone (1 mmol) was added to a sodium arsenate solution (0.2 mmol of $Na_2HAsO_4 \cdot 7H_2O$ in 10 mL of water, ph 7.3). The enzyme FDP aldolase (10 units) and aldehyde (1.5–2 mmol) were added to the mixture. The solution was kept at room temperature. The progress of the reaction was monitored by HPLC. For the preparation of D-fructose, triose phosphate isomerase (100 units) was added in addition to the aldolase, and the aldehyde substrate was absent. The reaction was quantitative after 1–2 days, and the products were isolated with a Dowex column and characterized as described before (yield 60–80%).

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