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Diastereoselectivity (Enantioselectivity) of Aldol Condensations Catalyzed by Rabbit Muscle Aldolase at C-2 of RCHOHCHO if R Has an Appropriately Placed Negatively Charged Group[1]

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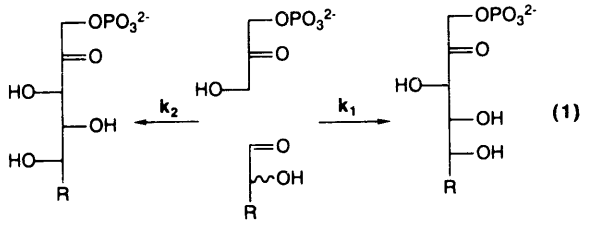
D-Fructose 1,6-bis(phosphate) aldolase from rabbit muscle (RAMA, E.C. 4.1.2.13) catalyzes the aldol condensation between dihydroxyacetone phosphate (DHAP) and various aldehydes; the products are ketosugars with 3(*S*),4(*R*) stereochemistry. When racemic α-hydroxy aldehydes are condensed with DHAP, two diastereomeric products are formed. This paper demonstrates that RAMA can kinetically resolve α-hydroxy aldehydes with a negative charge removed four or five atoms from the aldehydic center. Kinetic resolution of either uncharged α-hydroxy aldehydes, or of α-hydroxy aldehydes with a negative charge removed three or seven atoms from the aldehydic carbon, is generally not as successful.

Introduction

The stereoselective formation of carbon–carbon bonds using aldol condensations is important in organic synthesis.[3] We[4–7] and others[8,9] have used the enzyme fructose 1,6-bis(phosphate) aldolase (E.C. 4.1.2.13; from rabbit muscle, rabbit muscle aldolase, RAMA)[10–12] to synthesize carbohydrates. In nature, RAMA catalyses the aldol interconversion between dihydroxyacetone 1-phosphate (DHAP), glyceraldehyde 3-phosphate (G3P), and D-fructose 1,6-bis(phosphate). The product of a RAMA-catalyzed aldol condensation between DHAP and an aldehyde that is chiral at C-2 (C-5 in the adduct with DHAP) can, in principle, produce either of the two epimeric products corresponding to the two enantiomers of the aldehyde (eq 1) or a mixture of the two. The ratio of these two products



depends upon three factors: the kinetic selectivity of the enzyme, the relative thermodynamic stability of the two products, and the extent to which the mixture of products approaches thermodynamic equilibrium. In this paper, we address the kinetic resolution of aldehydes (eq 1) in aldol condensations catalyzed by RAMA. The objective of this work is to establish the practicality of RAMA in setting the stereochemistry at C-5 (and thereby fixing three contiguous chiral centers) starting from a mixture of enantiomeric aldehydes and (achiral) DHAP.[13]

For reactions involving two enantiomers of the aldehyde, the kinetic selectivity of RAMA is given by the ratio of initial rate constants k_1/k_2 (eq 1). Lardy and co-workers have reported that RAMA shows a high kinetic selectivity for D- over L-glyceraldehyde 3-phosphate ($k_1/k_2 \gg 1$).[14] In contrast, our preliminary survey established that RAMA showed no kinetic selectivity for D-glyceraldehyde over L-glyceraldehyde ($k_1/k_2 = 1$).[15] The contrast between these results suggested that a negative charge remote from the aldehyde group might be important in obtaining kinetic selectivity. Independent evidence has suggested that a negative charge can increase the rate of aldolase-catalyzed reactions: Rutter[16] reported that both fructose 1,6-bis(phosphate) (K_m = 15 μM, V_{max} = 6250) and sorbose 1,6-bis(phosphate) (K_m = 44 μM, V_{max} = 360) are better substrates than their monophosphate analogues fructose 1-phosphate (K_m = 6300 μM, V_{max} = 70) and sorbose 1-phosphate (K_m = 600 μM, V_{max} = 95). We have reported previously that aldose phosphates react more rapidly with DHAP than with the corresponding nonphosphorylated aldose in aldol condensations catalyzed by RAMA.[7] These results suggest that an appropriately placed negative charge enhances the *rate* of RAMA-catalyzed aldol condensations. Barker[17] has reported that polyol diphosphates—mimics of the product obtained by an aldol condensation between a negatively charged aldehyde and DHAP—are stronger inhibitors of RAMA than analogous monophosphates; specifically, arabitol, xylitol, and ribitol 1,5-bis(phosphate) (K_i = 40–3 μM) were better inhibitors

(1) Supported by the NIH, Grant GM30367.
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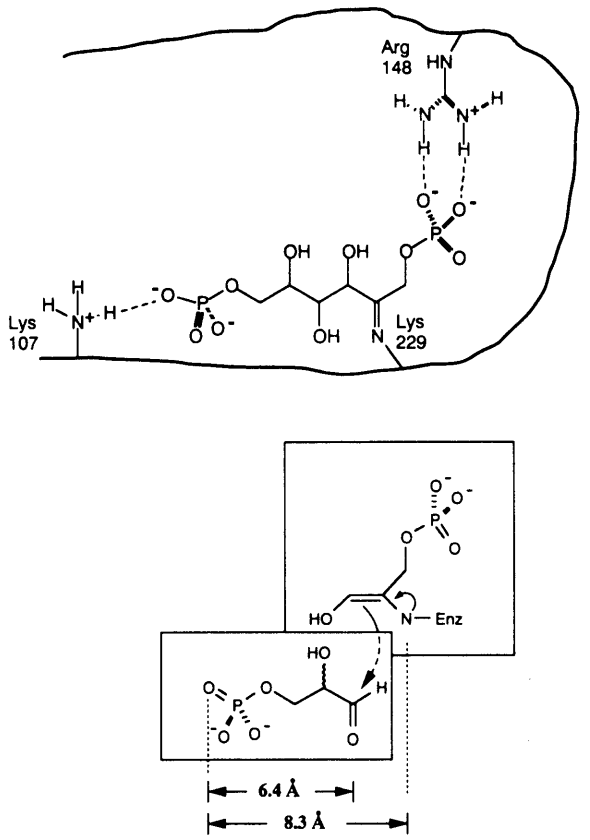

Figure 1. Important residues in the active site of RAMA and the proposed transition state for the enzymatic formation of fructose 1,6-bis(phosphate) from dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P). The ionization states of the phosphates, arginine, and lysines in the active site are unknown. The G3P is presumed to be trans-extended in the transition state.

than the corresponding 1,4-anhydro 5-phosphates (K_i = 4000–1000 μM). These results suggest that an appropriately placed negative charge enhances the *binding* of substrate to RAMA.

Several groups have reported that the binding of fructose 1,6-bis(phosphate) to RAMA involves important interactions between charges.[18-24] The active site of the enzyme contains three residues that have been proposed to interact with fructose 1,6-bis(phosphate): Lys-229 may form a Schiff's base with C-2,[18,19] and Arg-148[20] and Lys-107[21-23] may interact with the 1- and 6-phosphate groups, respectively. The crystal structure of RAMA has been resolved to 2.7 Å[24] and is consistent with this model (Figure 1).

The proposed transition state for the C–C bond formation in the active site of RAMA is depicted in Figure 1.[27] In this transition state, glyceraldehyde 3-phosphate is trans-extended. When this transition state is viewed perpendicularly to the trans-extended glyceraldehyde 3-phosphate, the C-1 of glyceraldehyde 3-phosphate overlaps with the C-3 of the eneamine formed between Lys-229 and the ketone of DHAP. Molecular modeling using the MM2 parameter set[28] showed that the in-plane distance between an oxygen on the 3-phosphate of glyceraldehyde 3-phosphate and the N^{ϵ} of Lys-229 in the proposed transition state is 8.3 Å. The distance between the N^{ϵ} of Lys-229 and Lys-107 in the unoccupied crystal structure (using data kindly supplied by Prof. Sygusch and co-workers) was 8.9 Å.[24] The similarity of these two distances suggests that group-specific interactions (hydrogen bonds and/or charge–charge interactions) make important contributions to the binding energy of FDP to the enzyme and subsequent conversion to product. The unoccupied crystal structures of human muscle[29] and drosophila melanogaster[30] aldolase have also been solved and shown to be similar to that of RAMA.

To investigate the hypothesis that a negative charge is important in obtaining kinetic selectivity with RAMA, we examined the kinetic selectivity of α-hydroxy aldehydes of general structure $R(CH_2)_nCHOHCHO$, where R was CH_3, OH, or CO_2H and $0 < n < 6$. We chose substrates containing an α-hydroxy group in order to mimic the natural substrate. We chose a carboxyl group rather than other negatively charged groups (sulfate, sulfonate, phosphate, or phosphonate) because the carbonyl group was conveniently accessible by synthesis and because the carboxy-terminated products from the aldol condensation are potentially useful in carbohydrate synthesis.

Results

Synthesis of Aldehydes. The aldehydic substrates (1–7, Table I) were usually prepared by the hydrolysis of the dialkyl acetal (see supplementary material for the synthesis of the dialkyl acetals). To show that the product of the hydrolysis was predominantly an aldehyde (in its various forms: hydrate, aldehyde, dimer, and polymer),[31] the dimethyl acetals of the five- and six-carbon carboxy-terminated aldehydes were regenerated in acidic methanol and compared to the original acetal. The five-carbon carboxy-terminated aldehyde formed the dimethyl acetal of the methyl ester and the dimethyl acetal of the lactone in acidic methanol.

Aldol Condensations. The α-hydroxy aldehyde and DHAP were mixed together in two NMR tubes (50–150 mM of each). One tube contained more aldehyde than DHAP (approx. 3:2); the other tube contained less aldehyde than DHAP (approx. 2:3). The initial concentration of DHAP was determined using an enzymatic assay.[12] The initial concentration of α-hydroxy aldehyde

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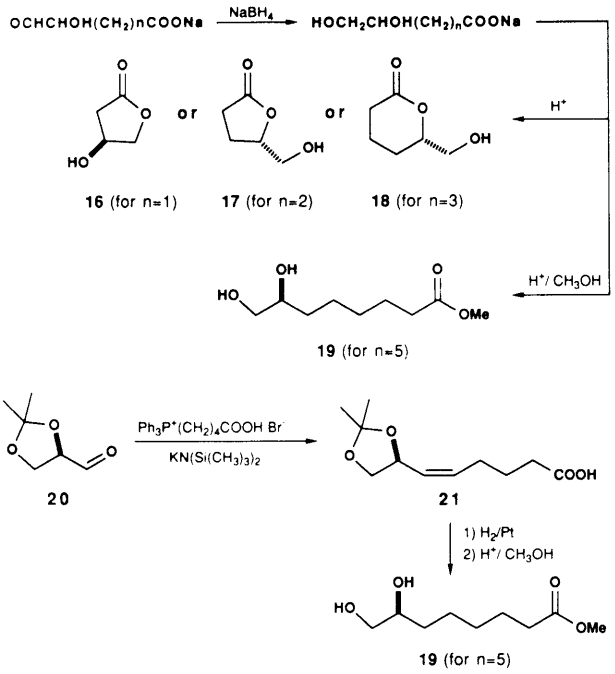
(31) By ^1H NMR spectroscopy it was determined that the aldehyde exists as a 40–100% monomer depending on the aldehyde.

Scheme I. Reaction Sequences Used To Determine the Absolute Stereochemistry of the Products



was determined relative to the concentration of DHAP by integration of the ^{1}H NMR spectrum. RAMA was then added to each of these NMR tubes, and the relative concentrations of the monomeric aldehyde, nonmonomeric aldehyde (dimeric and polymeric), and each of the two diastereomeric products were determined as a function of time by integration of ^{1}H NMR spectra taken at different times.

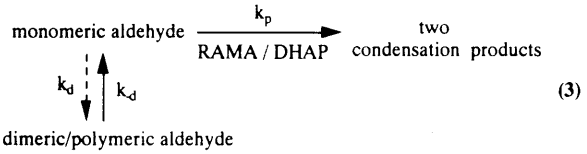
We measured the relative concentrations of the various components in solution using two different procedures. In the first procedure, we were interested in the relative concentrations of monomeric aldehyde and each of the diastereomeric products (vide infra). We integrated the resonances corresponding to the following: the α *CH*OH group of the monomeric aldehyde, the H-4 proton of the diastereomeric product having the fructose stereochemistry; and the H-4 and/or H-5 proton (in some cases the resonances overlapped) of the diastereomeric product having the sorbose stereochemistry. In the second procedure, we were interested in the sum of the concentrations of aldehyde (monomer, dimer, and polymer) and products relative to the concentration of products ([total aldehyde + products]/[products]). The sum of the concentration of total aldehyde and products ([total aldehyde + products]) was determined by integrating the ^{1}H NMR resion of the following (1) a CH_2 group α to a carboxylic acid (for carboxy-terminated aldehydes); (2) an alkyl CH_3 group (for alkyl-terminated aldehydes); and (3) the $(CH_2)_n$ chain (for hydroxy-terminated aldehydes). We determined the relative concentrations of the diastereomeric products as described in the first procedure.

Determination of Absolute Stereochemistry. In the RAMA-catalyzed condensation of 3-hydroxy-4-oxobutanoate (**1**, n = 1, Scheme I), 4-hydroxy-5-oxopentanoate (**2**, n = 2), 5-hydroxy-6-oxohexanoate (**3**, n = 3), and 7-hydroxy-8-oxooctanoate (**4**, n = 5) with DHAP, the stereochemistry of the residual aldehyde at approximately 50% conversion was determined by reduction of the reaction mixture followed by lactonization (n = 1, 2, or 3) or esterification (n = 5) and comparison with a compound of known absolute stereochemistry and optical rotation.[32-34] For 7-hydroxy-8-oxooctanoate (**4**, n = 5), the esterification product, methyl 7,8-dihydroxyoctanoate, was synthesized from 2,3-isopropylidene-D-glyceraldehyde. In all four cases, the lactone or ester and, by inference, the residual aldehyde had the *S* configuration. We conclude, therefore, that RAMA reacts preferentially with the aldehyde having the *R* configuration.

Calculation of Diastereoselectivity. The diastereoselectivity of the reaction between an aldehyde and DHAP was estimated by the integration of the ^{1}H NMR spectra of the enzyme-catalyzed reaction mixtures. We calculated the enantiomeric ratio, E, using eq 2, where c is the percent

$$\frac{\ln [1 - c(1 + \mathrm{ee(P)})]}{\ln [1 - c(1 - \mathrm{ee(P)})]} = \frac{(k_{cat}/K_m)_A}{(k_{cat}/K_m)_B} = E \qquad (2)$$

conversion divided by 100, ee(P) is the diastereomeric excess of the product, K_m is the Michaelis constant, k_{cat} is the turnover number, and k_{cat}/K_m is the specificity constant for enantiomer A or B.[35] The value of E is the ratio of initial rates, k_1/k_2 in eq 1, in the presence of racemic aldehyde; it is independent of the starting concentration of aldehyde or DHAP. Since the error in the calculation of the percent conversion is probably ±5% absolute, the relative error in values of E is larger for large values of E. As a consequence, we report values of E greater than 10 as simply >10. Values of E were calculated using two separate definitions of percent conversion: [products]/[total aldehyde + product], and [products]/[monomeric aldehyde + product]. The former calculation is valid when the rate of formation of monomeric aldehyde (hydrate) from oligomeric forms is fast relative to the rate of formation of product (eq 3, $k_{-d} > k_p$[DHAP]). The latter



(3)

calculation is valid when the rate of formation of monomeric aldehyde (hydrate) from oligomeric forms is slow relative to the rate of formation of product (eq 3, $k_{-d} < k_p$[DHAP]), that is, in essence, when the nonmonomeric aldehyde does not participate in the condensation reaction. The former calculation provides the minimum value of E and the latter calculation provides the maximum value of E. The value of E is therefore reported as a range spanning these two estimates (Table I).

The discrepancy between the two methods of calculation will be larger at higher conversion. In order to minimize this discrepancy, the ^{1}H NMR spectrum taken at approximately 50% conversion of monomeric aldehyde was

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Table I. Values of E for Various α-Hydroxy Aldehydes (RCHOHCHO)

R	hydroxy aldehyde no.	condensation product no.	E^a
$HOCH_2$			1
$Na_2O_3POCH_2$		8	>10
NaO_2CCH_2	1	9	3–4
$NaO_2C(CH_2)_2$	2	10	>10
$NaO_2C(CH_2)_3$	3	11	>10
$NaO_2C(CH_2)_5$	4	12	4–6
$HOCH_2(CH_2)_2$	5	13	2–3
$HOCH_2(CH_2)_3$	6	14	2–3
$H_3C(CH_2)_2$	7	15	1

[a] Those values of E given as ranges represent different assumptions concerning the extent to which the aldehyde is in thermodynamic equilibrium with equivalent forms (hydrate, dimer, other) under the reaction conditions. See the test for discussion.

used in the calculations. In some cases with conversions lower than 50%, resonances corresponding to the minor product could not be detected. The values of E provided in Table I do not, in most cases, describe the diastereomeric excess in the usual form as a function of conversion from 0 to 100%. We are unable to report standard values of E because the aldehyde exists in various forms: The ee of the monomeric aldehyde (hydrate) in solution at a given conversion, therefore, may not be the same as the ee's of the aldehyde in its oligomeric forms. Nevertheless, these values of E provide useful qualitative descriptions of the kinetic selectivity of RAMA toward the aldehydes studied in this work.

Synthesis of Sugar Phosphates. The aldehyde (2.5–3.0 equiv), DHAP (1 equiv, in most cases 2 mmol), and RAMA were stirred together until the reaction reached approximately 80% conversion. At this point the reaction mixture was purified by ion-exchange chromatography. For 4-hydroxy-5-oxopentanoate (**2**) and 5-hydroxy-6-oxohexanoate (**3**), the ratio of the fructose product (reaction with *R*-aldehyde) to sorbose product (reaction with *S*-aldehyde) in the isolated material was greater than 10:1; this result confirmed the inferences from the NMR experiments. For 3-hydroxy-4-oxobutanoate (**1**) and 7-hydroxy-8-oxooctanoate (**4**) the ratios of the fructose product to sorbose product in the isolated material were 3:1 and 7:1, respectively, again confirming the inferences from the NMR experiments.

Discussion

RAMA shows high diastereoselectivity—as measured by E—between enantiomeric α-hydroxy aldehydes with negative charges five or six bonds removed from the aldehydic carbon. RAMA exhibits lower diastereoselectivity between α-hydroxy aldehydes with negative charges four or eight atoms removed from the aldehydic center and between α-hydroxy aldehydes with no negative charge. These results are tabulated in Table I. Analysis of the crystal structure of RAMA[24] helps to rationalize these observations. As discussed above, the active site of RAMA contains a number of charged residues that may interact with the ligands[18–23] (Figure 1). We believe that it is important that the product of the aldol condensation spans the distance between the lysine groups in order to obtain high diastereoselectivity. This conformation is more easily established when a charged group in the aldehyde can interact with Lys-107.

In order to make comparisons between the transition-state structures formed from a series of aldehydes, we

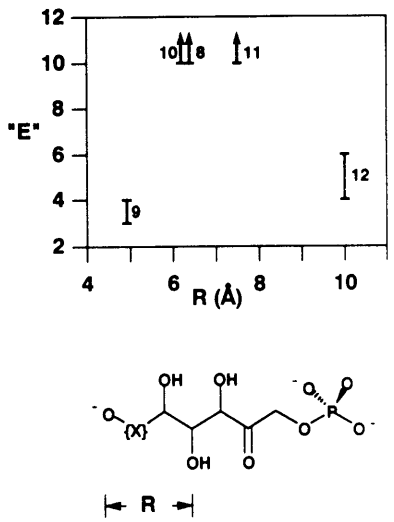
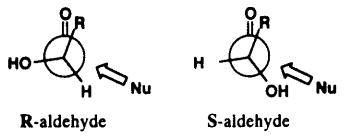


Figure 2. Stereoselectivity of the aldolase-catalyzed reaction (E) plotted against the C-4 to terminal oxygen distance of various trans-extended aldol condensation products (**8**–**12**) as determined by force-field calculations.



R-aldehyde S-aldehyde

Nu = enzyme bound DHAP

Figure 3. End-on view of the attack of DHAP (Nu) on each of the enantiomers of the aldehyde.

compared the distance between C-4 and the furthest in-plane oxygen of the trans-extended products from an aldol condensation using force field calculations (Figure 2). We chose C-4 because this is the first carbon atom derived from the aldehyde in the chain of the condensation product. The distance between C-4 and an oxygen on the carboxylate of 6-deoxy-6-carboxyfructose 1-phosphate (**9**; the product of the aldol condensation between DHAP and 3-hydroxy-4-oxobutanoic acid, **1**) is 4.9 Å. This distance is 1.5 Å shorter than the distance between C-4 and an oxygen on the 6-phosphate of trans extended fructose 1,6-bis(phosphate) (**8**). This conformation of FDP spans (vide supra) the distance between the two lysine residues in the unoccupied active site; consequently, compound **9** may be too short to have an effective interaction between its terminal carbonyl group and Lys-107. The observation that RAMA shows low kinetic selectivity in the formation of this product (**9**) but high kinetic selectivity in the formation of compounds **10** (6.2 Å), **11** (6.4 Å), and **12** (7.5 Å) is consistent with the hypothesis that formation of an ion pair is necessary for high kinetic selectivity.

We hypothesize that the orientation and the location of the carbonyl group of the aldehyde are fixed in the active site of the enzyme. The two-point binding of the carbonyl group and the carboxylate in the five- and six-carbon carboxy-terminated aldehydes probably limits the rotational freedom of the α-hydroxy group in the bound aldehyde. This limited rotation causes the enzyme-bound form of one enantiomer of the aldehyde to present the α-alkyl chain or the α-hydroxy group on its *si* face; the other enantiomer of the aldehyde presents the sterically smaller α-hydrogen on its *si* face. In Figure 3, we assume that the aldehydic oxygen and the alkyl group are syn since this orientation will maximize the distance between the C-1 phosphate and the terminal group. This constraint

restricts the transition state for each enantiomer to that shown in Figure 3. DHAP always attacks the *si* face of the aldehyde; any group blocking this attack should slow the rate of the catalytic reaction. These constraints predict that the *R* enantiomer of the aldehyde (the faster reacting enantiomer) presents a hydrogen on its *si* face while the *S* enantiomer presents a bulkier group on its *si* face. Diastereoselectivity results from this difference.

The eight-carbon carboxy-terminated aldehyde **4** may or may not be able to form an ion pair with the lysine residue. If **4** forms an ion pair with the lysine residue, its alkyl chain should still be flexible because the distance between C-1 and the terminal oxygen is 3.6 Å greater than the corresponding distance in fructose 1,6-bis(phosphate). This added flexibility should allow rotation about the α-hydroxy carbon, in a fashion similar to noncharged aldehydes, removing the constraint that the aldehyde and alkyl group must be syn to each other. If **4** does not form an ion pair then the constraint that the molecule is fixed at two points is removed. This added flexibility or loss of the ion pair may explain the lower kinetic selectivity of the enzyme toward **4** relative to the other charged aldehydes (**2**, **3**).

We conclude that RAMA can provide diastereoselectivity in the reaction of DHAP with α-hydroxy aldehydes, provided that the aldehydes possess a negatively charged atom removed at least five bonds from the aldehydic carbon (Table I). Analysis of the unoccupied crystal structure suggests that an ion pair formed between the substrate and Lys-107 is required to produce diastereoselectivity. If this ion pair cannot be formed—either because there is no negative charge on the substrate or because the substrate is unable to reach Lys-107 with its negative charge—then little or no diastereoselection occurs.

Experimental Section

Materials and Methods. Chemicals were purchased from Aldrich and were reagent grade. Enzymes and biochemicals were obtained from Sigma. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded using tetramethylsilane (TMS) or chloroform ($CHCl_3$) as an internal standard or dioxane as an external standard.

Condensation Reaction. General Procedure. A freshly prepared solution of DHAP (pH 7.0) in D_2O (0.5 mL) was added to a solution of aldehyde in D_2O (pH 7.0, 0.5 mL). The relative concentration of these two components was determined by integration of the ^1H NMR spectrum. Two 5-mm NMR tubes were prepared: one containing a slight excess of aldehyde, the other one containing a slight excess of DHAP. A ^1H NMR spectrum was then acquired (t = 0). Aldolase was added, and the reactions were followed by ^1H NMR analysis.

Condensation Reaction for 4-Hydroxy-5-oxopentanoic Acid (2). The relative concentration of the two stock solutions (DHAP and the lactone of 4-hydroxy-5-oxopentanoic acid) were determined as in the general procedure. A solution containing a slight excess of aldehyde relative to DHAP was made up. Sodium deuteride was added slowly until the solution maintained a pH value of 12 (opening of the lactone). 4-Hydroxy-5-oxopentanoic acid (resonances attributed to hydrate): ^1H NMR (400 MHz, D_2O) δ 4.59 (d, J = 4.9 Hz, 1 H), 3.22 (ddd, J = 9.4, 4.9, 3.4 Hz, 1 H), 2.10 (ddd, J = 15.0, 9.6, 5.9 Hz, 1 H), 2.00 (ddd, J = 15.0, 9.2, 6.7 Hz, 1 H), 1.61 (dddd, J = 14.0, 9.8, 6.7, 3.3 Hz, 1 H), 1.39 (dtd, J = 14.3, 9.3, 5.9 Hz, 1 H); ^{13}C NMR (100 MHz, D_2O) δ 182.94, 91.56, 73.39, 33.53, 27.91; HRMS-FAB [M − H]$^-$ calcd for $C_5H_8O_4$ 131.0344, obsd 131.0346. The pH was adjusted to pH 7.0 with DCl. The solution was then placed in two 5-mm NMR tubes (0.9 mL). One of the tubes was diluted with the stock DHAP solution (0.1 mL, excess DHAP tube), the other tube was diluted with D_2O (excess aldehyde tube). A ^1H NMR spectrum was then acquired (t = 0) of each tube. Aldolase was added, and the reactions were followed by ^1H NMR analysis.

6-Carboxy-6-deoxyfructose 1-Phosphate (9) and 6-Carboxy-6-deoxysorbose 1-Phosphate. Sodium 3-hydroxy-4-oxobutanoate diethyl acetal (1.31 g, 5.7 mmol), water (30 mL), and ion-exchange resin (Dowex 50W-X8, H^+ form, 13.9 g) were stirred together for 16 h. The reaction mixture was filtered, partially concentrated at 1 Torr, and adjusted to pH 7 with 1 N NaOH. To this solution was added a solution of DHAP (20 mL of a 100 mM solution, 2 mmol). The pH was readjusted to pH 7. Aldolase (50 U) was added and the reaction followed by monitoring the consumption of DHAP. After 2.5 h (80% completion), the reaction mixture was purified by anion exchange chromatography (AG1-X8 bicarbonate form/eluant: triethylammonium bicarbonate 0–600 mM) to provide 6-deoxy-6-carboxyfructose 1-phosphate and 6-deoxy-6-carboxysorbose 1-phosphate as their triethylammonium salts. Due to a contamination with 10 mol % of the starting aldehyde, the sample was repurified by ion-exchange chromatography on the above column. The triethylammonium salt solution was concentrated at 1 Torr to dryness, dissolved in water (100 mL), acidified with ion-exchange resin (Dowex 50W-X8, H^+ form), filtered, and adjusted to pH 7 with 1 N NaOH to form the sodium salt. The sodium salt solution was lyophilized to provide a mixture of 6-deoxy-6-carboxyfructose 1-phosphate and 6-deoxy-6-carboxysorbose 1-phosphate (386 mg, 3:1 ratio) each of which exists as a mixture of two anomers (approximate ratio 3:1 for each). The resonances corresponding to 6-deoxy-6-carboxyfructose 1-phosphate are labeled "f", and the resonances corresponding to 6-deoxy-6-carboxysorbose 1-phosphate are labeled "s". The resonances corresponding to the minor anomers are labeled "a" and the resonances corresponding to the major anomers are labeled "b". Not all the resonances corresponding to the minor anomers are reported: ^1H NMR (D_2O, 500 MHz) δ 4.40 (m, 5sa), 4.36 (dt, J = 7.9, 5.9 Hz, 1 H, 5sb), 4.09–4.05 (m, 4sb, 5fa), 4.03 (dd, J = 4.8, 2.3 Hz, 4sa), 3.94 (d, J = 2.0 Hz, 3sa), 3.91 (d, J = 8.0 Hz, 1 H, 3fb), 3.88 (d, J = 5.0 Hz, 1 H, 3sb), 3.86–3.81 (m, 5fb, 3fa), 3.80 (t, J = 7.8 Hz, 4fb), 3.71 (t, J = 5.5 Hz, 4fa), 3.68–3.53 (m, 1f, 1s), 2.43 (dd, J = 15.3, 4.3 Hz, 6fb′), 2.39 (dd, J = 15.4, 5.2 Hz, 6fa′), 2.30 (dd, J = 15.1, 6.0 Hz, 6sb′), 2.32–2.27 (m, 6fa″), 2.28 (dd, J = 15.6, 8.4 Hz, 6fb″), 2.20 (dd, J = 15.3, 8.0 Hz, 6sb″). The resonances are labeled large (l, probably the major anomer of 6-deoxy-6-carboxyfructose 1-phosphate), medium (m, probably the minor anomer of 6-deoxy-6-carboxyfructose 1-phosphate, or the major anomer of 6-deoxy-6-carboxysorbose 1-phosphate), or small (s, probably the minor anomer of 6-deoxy-6-carboxyfructose 1-phosphate), depending on their intensities: ^{13}C NMR (D_2O, 100 MHz) 179.37m, 179.151, 104.16m (d, J = 7.7 Hz), 100.94m (d, J = 8.5 Hz), 100.60l (d, J = 8.7 Hz), 81.71m, 79.90s, 79.40m, 77.63l, 77.07m, 76.08m, 75.90m, 75.68l, 66.48m (d, J = 5.9 Hz), 65.78l (d, J = 4.2 Hz), 65.02m (d, J = 4.3 Hz), 42.33l, 41.35m, 38.43s, 37.63m [Some of the signals in the ^{13}C NMR spectrum were assigned after the pH had been adjusted to reduce signal overlap]; HRMS-FAB [M − H]$^-$ calcd for $C_7H_{13}O_{10}P$ 287.0168, obsd 287.0149.

6-Deoxy-6-methylenecarboxyfructose 1-Phosphate (10) and 6-Deoxy-6-methylenecarboxysorbose 1-Phosphate. 4-Hydroxy-5-oxopentanoic acid lactone (0.736 g, 6.4 mmol) in water (20 mL) was stirred for 90 min while the solution was slowly evaporated at aspirator pressure. Monosodium phosphate (68 mg, 0.5 mmol) was added, and the solution was maintained between pH 10.5 and 9.0 with 1 N NaOH (6.0 mL). The solution was then adjusted to pH 7.0 with 1 N HCl. To this solution was added a solution of DHAP (sodium salt, 20 mL of a 100 mM solution, 2 mmol) at pH 7.0. Aldolase (25 U) was added and the reaction followed by monitoring the consumption of DHAP. After 2.5 h (85% completion), the reaction mixture was purified by anion-exchange chromatography (AG1-X8 bicarbonate form/eluant: triethylammonium bicarbonate 0–600 mM) to provide 6-deoxy-6-methylenecarboxyfructose 1-phosphate as the triethylammonium salt. The triethylammonium salt solution was concentrated at 1 Torr to dryness, dissolved in water (100 mL), acidified with ion-exchange resin (Dowex 50W-X8, H^+ form), filtered, and adjusted to pH 7 with 1 N NaOH to form the sodium salt. The sodium salt solution was lyophilized to provide a mixture of two components: sodium 6-deoxy-6-methylenecarboxyfructose 1-phosphate (713 mg) which exists as a mixture of

two anomers (ratio 4:1) and sodium 6-deoxy-6-methylenecarboxysorbose 1-phosphate (ratio of diastereomers (14:1)). The minor anomer of sodium 6-deoxy-6-methylenecarboxyfructose 1-phosphate is labeled "a" and the major anomer is labeled "b": ^{1}H NMR (D_2O, 500 MHz) δ 3.89 (d, J = 8.2 Hz, 3b), 3.81 (d, J = 4.4 Hz, 3a), 3.76 (t, J = 7.9 Hz, 4b), 3.76–3.72 (m, 5a), 3.67 (dd, J = 10.5, 8.7 Hz, 1a'), 3.67–3.65 (m, 4a), 3.61–3.51 (m, 1a'', 5b, 1b), 2.11 (ddd, J = 14.9, 10.1, 6.0 Hz, 7a', 7b'), 2.03 (ddd, J = 14.9, 10.1, 5.9 Hz, 7a'', 7b''), 1.97–1.61 (m, 6a, 6b); ^{13}C NMR (D_2O, 100 MHz) 182.54a, 182.37b, 104.15a (d, J = 6.7 Hz), 100.54b (d, J = 8.4 Hz), 81.91a, 81.51a, 79.66b, 79.42a, 77.59b, 75.90b, 65.77b (d, J = 4.2 Hz), 64.81a (d, J = 4.4 Hz), 33.34a, 33.15b, 30.62b, 29.38a; HRMS-FAB [M - H]$^-$ calcd for $C_8H_{15}O_{10}P$ 301.0324, obsd 301.0318. The fractions from the AG1-X8 ion-exchange column containing aldehyde were combined, and 1.8 mmol of DHAP and 950 units of RAMA were added. After 16 h, the reaction mixture was purified by chromatography as above to provide 606 mg of a mixture of sodium 6-deoxy-6-methylenecarboxyfructose 1-phosphate and sodium 6-deoxy-6-methylenecarboxysorbose 1-phosphate (ratio 1:1.2). After subtracting the NMR spectrum of sodium 6-deoxy-6-methylenecarboxyfructose 1-phosphate from the NMR spectrum of the mixture, the resonances corresponding to sodium 6-deoxy-6-methylenecarboxysorbose 1-phosphate were determined. The minor anomer of sodium 6-deoxy-6-methylenecarboxysorbose 1-phosphate is labeled "a" and the major is labeled "b" (ratio 1:3): ^{1}H NMR (D_2O, 500 MHz) δ 4.02–3.94 (m, 4b, 5b, 3a, 4a, 5a), 3.90 (d, J = 3.7 Hz, 3b), 3.77 (dd, J = 11.8, 8.0 Hz, 1a'), 3.61 (d, J = 6.0 Hz, 1b'), 3.61 (d, J = 5.9 Hz, 1b''), 3.55 (dd, J = 11.7, 7.0 Hz, 1a''), 2.15–2.00 (m, 7a, 7b), 1.77–1.61 (m, 6a, 6b'), 1.58–1.51 (m, 6b''); ^{13}C NMR (D_2O, 100 MHz) 182.39 (a + b), 105.29a (d, J = 7.3 Hz), 101.29b (d, J = 8.6 Hz), 82.35a, 79.74a, 78.46b, 77.27b, 76.03b, 75.40a, 66.72b (d, J = 4.6 Hz), 64.65a (d, J = 4.7 Hz), 33.66a, 33.47b, 26.33a, 25.23b; HRMS-FAB [M - H]$^-$ calcd for $C_8H_{15}O_{10}P$ 301.0324, obsd 301.0353; [M - 2H + Na]$^-$ calcd 323.0144, obsd 323.0157.

6-Deoxy-6-ethylenecarboxyfructose 1-Phosphate (11). Methyl 5-hydroxy-6-oxohexanoate dimethyl acetal (1.18 g, 5.7 mmol), water (30 mL), and 9 mL of 1 N NaOH were mixed together. After being stirred for 30 min, the reaction mixture was acidified with ion-exchange resin (Dowex 50W-X8, H$^+$ form, 13.2 g). After being stirred for 18 h, the reaction mixture was filtered, partially concentrated at 1 Torr, and adjusted to pH 7 with 1 N NaOH. To this solution was added a solution of DHAP (20 mL of a 100 mM solution, 2 mmol). The pH was readjusted to pH 7. Aldolase (25 U) was added and the reaction followed by monitoring the consumpton of DHAP. After 2.5 h (80% completion), the reaction mixture was purified by anion-exchange chromatography (AG1-X8 bicarbonate form/eluant: triethylammonium bicarbonate (0–500 mM) to provide 6-deoxy-6-ethylenecarboxyfructose 1-phosphate as the triethylammonium salt. The triethylammonium salt solution was concentrated at 1 Torr to dryness, dissolved in water (100 mL), acidified with ion-exchange resin (Dowex 50W-X8, H$^+$ form), filtered, and adjusted to pH 7 with 1 N NaOH to form the sodium salt. The sodium salt solution was lyophilized to provide sodium 6-deoxy-6-ethylenecarboxyfructose 1-phosphate (789 mg) which exists as a mixture of two anomers (ratio 3:1). Due to a contamination with 10 mol % of the starting aldehyde, the sample was repurified by ion-exchange chromatography on the above column to yield 661 mg of product. The minor anomer is labeled "a" and the major anomer is labeled "b": ^{1}H NMR (D_2O, 500 MHz) δ 3.88 (d, J = 8.5 Hz, 3b), 3.80 (d, J = 4.0 Hz, 3a), 3.77–3.74 (m, 5a), 3.74 (t, J = 8.0 Hz, 4b), 3.68 (dd, J = 11.6, 8.4 Hz, 1a'), 3.64 (dd, J = 5.6, 4.4 Hz, 4a), 3.59–3.52 (m, 1a''), 3.57–3.48 (m, 5b, 1b), 2.02–1.96 (m, 8a, 8b), 1.50–1.36 (m, 7a, 7b, 6a, 6b); ^{13}C NMR (D_2O, 100 MHz) 183.22 (a and b), 104.15a (d, J = 7.1 Hz), 100.49b (d, J = 8.4 Hz), 81.88a, 81.77a, 79.83b, 79.63a, 77.73b, 75.94b, 65.86b (d, J = 4.3 Hz), 64.81a (d, J = 4.3 Hz), 37.20b, 37.10a, 33.58b, 32.32a, 21.91a, 21.60b; HRMS-FAB [M - H]$^-$ calcd for $C_9H_{17}O_{10}P$ 315.0481, obsd 315.0486.

6-Deoxy-6-butylenecarboxyfructose 1-Phosphate (12). Methyl 7-hydroxy-8-oxohexanoate dimethyl acetal (0.713 g, 3.0 mmol), water (15 mL), and 4 mL of 1 N NaOH were mixed together. After being stirred for 2.5 h, the reaction mixture was lyophilized and dissolved in 300 mL water. The solution was acidified with ion-exchange resin (Dowex 50W-X8, H$^+$ form, 14 g). After being stirred for 48 h, the reaction mixture was neutralized with 1 N NaOH, filtered, and partially concentrated at 1 Torr to a total volume of about 30 mL. To this solution was added a solution of DHAP (10 mL of a 100 mM solution, 1 mmol). The pH was readjusted to pH 7. Aldolase (100 U) was added and the reaction followed by monitoring the consumption of DHAP. After 1.0 h (85% completion), the reaction mixture was purified by anion-exchange chromatography (AG1-X8 bicarbonate form/eluant: triethylammonium bicarbonate 0–600 mM) to provide 6-deoxy-6-butylenecarboxyfructose 1-phosphate as the triethylammonium salt. The triethylammonium salt solution was concentrated at 1 Torr to dryness, dissolved in water (100 mL), acidified with ion-exchange resin (Dowex 50W-X8, H$^+$ form), filtered, and adjusted to pH 7 with 1 N NaOH to form the sodium salt. The sodium salt solution was lyophilized to provide a mixture of sodium 6-deoxy-6-ethylenecarboxyfructose 1-phosphate which exists as a mixture of two anomers (ratio 3:1) and sodium 6-deoxy-6-ethylenecarboxysorbose 1-phosphate (ratio 7:1 fructose/sorbose). Due to a contamination with 10 mol % of the starting aldehyde, the sample was repurified by ion-exchange chromatography on the above column to yield 350 mg of product. The minor anomer is labeled "a" and the major anomer is labeled "b": ^{1}H NMR (D_2O, 500 MHz) δ 3.87 (d, J = 8.2 Hz, 3b), 3.81 (d, J = 4.0 Hz, 3a), 3.77–3.73 (m, 5a), 3.73 (t, J = 8.1 Hz, 4b), 3.69 (dd, J = 11.4, 7.7 Hz, 1a'), 3.63 (dd, J = 6.0, 4.2 Hz, 4a), 3.59–3.48 (m, 1a'', 5b, 1b), 1.96 (t, J = 7.5 Hz, 10a, 10b), 1.51–1.36 (m, 6a, 6b), 1.34 (pentet, J = 7.4 Hz, 9a, 9b), 1.28–1.06 (m, 8a, 8b, 7a, 7b); ^{13}C NMR (D_2O, 100 MHz) 183.94 (a and b), 104.18a (d, J = 7.2 Hz), 100.46b (d, J = 8.7 Hz), 82.14a, 81.89a, 80.01b, 79.79a, 77.82b, 76.08b, 65.91b (d, J = 4.6 Hz), 64.84a (d, J = 5.4 Hz), 37.36 (a and b), 33.65b, 32.44a, 28.54 (a and b), 25.57 (a and b), 24.55a, 24.18b; HRMS-FAB [M - H]$^-$ calcd for $C_{11}H_{22}O_{10}P$ 343.0794, obsd 343.0812. The fractions from the second AG1-X8 ion-exchange column containing aldehyde were combined, and 0.4 mmol of DHAP and 400 units of RAMA were added. After 24 h, the reaction mixture was purified by chromatography as above to provide a mixture of sodium 6-deoxy-6-butylenecarboxyfructose 1-phosphate, sodium 6-deoxy-6-butylenecarboxysorbose 1-phosphate (ratio 1:1), and citrate (buffer salt from the added aldolase). Resonances corresponding to the minor anomer and major anomer of sodium 6-deoxy-6-butylenecarboxysorbose 1-phosphate are labeled "sa" and "sb", respectively. Resonances corresponding to the anomers of sodium 6-deoxy-6-butylenecarboxyfructose 1-phosphate are labeled "f". Resonances corresponding to citrate are labeled "c": ^{1}H NMR (D_2O, 500 MHz) δ 4.05–3.95 (m, 5sb, 3sa, 4sa, 5sa), 3.94 (dd, J = 4.7, 3.4 Hz, 4sb), 3.88 (d, J = 3.5 Hz, 3sb), 3.77 (dd, J = 11.4, 8.7 Hz, 1sa'), 3.59 (d, J = 6.4 Hz, 1sb'), 3.59 (d, J = 6.3 Hz, 1sb''), 1.95 (t, J = 7.5 Hz, 10s), 1.50–1.10 (m, 9s, 8s, 7s, 6s); ^{13}C NMR (D_2O, 100 MHz) 184.02 (sa, sb, f), 181.76 (c), 179.05 (c), 101.45 (d, J = 7.7 Hz, sb), 82.89 (sa), 82.18 (f), 81.94 (f), 80.04 (f), 79.87 (f), 79.75 (sa), 79.04 (sb), 77.84 (sb), 76.37 (sb), 76.09 (f), 75.55 (c), 75.11 (sa), 66.85 (d, J = 4.7 Hz, sb), 65.89 (f), 45.51 (c), 37.44 (sa, sb, f), 33.71 (f), 32.49 (f), 29.32 (sa), 28.60 (sa, sb, f), 27.96 (sb), 25.65 (sa, sb, f), 24.98 (sa), 24.81 (sb), 24.60 (f), 24.22 (f); HRMS-FAB [M - H]$^-$ calcd for $C_{11}H_{21}O_{10}P$ 343.0794, obsd 343.0812.

6-Deoxy-6-ethylfructose 1-Phosphate (15) and 6-Deoxy-6-ethylsorbose 1-Phosphate. After a mixture of 2-hydroxypentanal dimethyl acetal (560 mg, 3.78 mmol), ion-exchange resin (Dowex 50W X-8 H$^+$ form, 4.4 g), and water (10 mL) was stirred for 14 h, the mixture was filtered. The solution was mixed with dihydroxyacetone phosphate (20 mL of a 100 mM solution, 2 mmol), neutralized to pH 7.0 with 1 N NaOH, and diluted to 100 mL. RAMA (100 U) was then added to the mixture. After 20 h (100% completion), the reaction mixture was purified by anion-exchange chromatography (AG1-X8 bicarbonate form/eluant: triethylammonium bicarbonate 0–300 mM) to provide a mixture of 6-deoxy-6-ethylfructose 1-phosphate and 6-deoxy-6-ethylsorbose 1-phosphate as their triethylammonium salts. The triethylammonium salt solution was concentrated at 1 Torr to dryness, dissolved in water (100 mL), acidifed with ion-exchange resin (Dowex 50 W-X8, H$^+$ form), filtered, and adjusted to pH 7 with 1 N NaOH to form the sodium salt. The sodium salt solution was lyophilized to provide 587 mg of a mixture of 6-deoxy-6-ethylfructose 1-phosphate and 6-deoxy-6-ethylsorbose 1-phos-

phate (1:1 ratio). The resonances corresponding to 6-deoxy-6-carboxyfructose 1-phosphate are labeled "f", and the resonances corresponding to 6-deoxy-6-carboxysorbose 1-phosphate are labeled "s". The resonances corresponding to the major anomers are labeled "a", and the resonances corresponding to the minor anomers are labeled "b". Not all the resonances corresponding to the minor anomers are reported: ^{1}H NMR (D_2O, 500 MHz) δ 4.06–4.02 (m, 5sb), 4.00 (dt, J = 7.5, 5.4 Hz, 5sa), 3.95–3.92 (m, 4sa, 3sb, 4sb), 3.87 (d, J = 3.9 Hz, 3sa), 3.87 (d, J = 6.0 Hz, 3fa), 3.80 (d, J = 4.2 Hz, 3fb), 3.76 (q, J = 6.5 Hz, 5fb), 3.71 (t, J = 8.1 Hz, 4fa), 3.67 (dd, J = 11.5, 7.8 Hz, 1fb′), 3.63 (dd, J = 6.1, 4.4 Hz, 4fb), 3.59–3.51 (m, 5fa, 1fa, 1sa, 1fb″, 1sb″), 1.46–1.08 (m, 6f, 6s, 7f, 7s), 0.72–0.66 (m, 8s, 8f). The resonances are labeled large (l, probably the major anomer of 6-deoxy-6-ethylfructose 1-phosphate or 6-deoxy-6-ethylsorbose 1-phosphate) or small (s, probably the minor anomer of 6-deoxy-6-ethylfructose 1-phosphate or 6-deoxy-6-ethylsorbose 1-phosphate), depending on their intensities: ^{13}C NMR (D_2O, 100 MHz) 101.39l (d, J = 7.6 Hz), 100.47l (d, J = 6.1 Hz), 82.50s, 81.79s, 79.63l, 78.68l, 77.80l, 77.68l, 76.27l, 76.12l, 75.47s, 66.78l, 65.94l, 64.69s, 64.38s, 35.97l, 34.69s, 31.37s, 30.23l, 18.60s, 18.45l, 18.20s, 17.92l, 13.23l, 13.19l; HRMS-FAB [M − H]$^-$ calcd for $C_8H_{17}O_8P$ 271.0583, obsd 271.0591.

6-Deoxy-6-ethylfructose and 6-Deoxy-6-ethylsorbose. Water (200 mL), acid phosphatase (200 U), and a mixture of 6-deoxy-6-ethylfructose 1-phosphate (**15**) and 6-deoxy-6-ethylsorbose 1-phosphate (1:1 ratio, sodium salt, 491 mg) were mixed together and left at room temperature. After 48 h, the solution was concentrated in vacuo and purified by silica gel chromatography (eluent: acetone/CH_2Cl_2 (1:5 going to 1:1)) to provide 138 mg of a mixture of 6-deoxy-6-ethylfructose and 6-deoxy-6-ethylsorbose. The mixture was purified by silica gel chromatography (methanol/CH_2Cl_2 (1:10 going to 1:5)). The early fractions were used to characterize 6-deoxy-6-ethylsorbose, and the later fractions were used to characterize 6-deoxy-6-ethylfructose. 6-Deoxy-6-ethylsorbose: The resonances corresponding to the major anomers are labeled "a", and the resonances corresponding to the minor anomers are labeled "b" (ratio 5:1): ^{1}H NMR (D_2O, 500 MHz) δ 4.04–3.92 (m, 3b, 4b, 5b), 4.00 (dt, J = 7.6, 5.2 Hz, 5a), 3.96 (dd, J = 4.8, 3.7 Hz, 4a), 3.84 (d, J = 3.6 Hz, 3a), 3.48 (d, J = 11.9 Hz, 1b′), 3.38 (d, J = 12.0 Hz, 1a′, 1b″), 3.32 (d, J = 11.9 Hz, 1a″), 1.43–1.06 (m, 6, 7), 0.73–0.70 (m, 8b), 0.70 (t, J = 7.3 Hz, 8a). The resonances are labeled large (l, probably the major anomer of the compound) or small (s, probably the minor anomer of the compound), depending on their intensities. ^{13}C NMR of mixture (D_2O, 100 MHz) 105.11s, 101.91l, 81.95s, 79.84s, 78.61l, 76.72l, 76.26l, 76.02s, 63.60l, 62.32s, 31.43s, 30.32l, 18.59s, 18.43l, 13.17 (s +1); HRMS-FAB [M + Na]$^+$ calcd for $C_8H_{16}O_5$ 215.0895, obsd 215.0884. 6-Deoxy-6-ethylfructose: The resonances corresponding to the major anomers are labeled "a", and the resonances corresponding to the minor anomers are labeled "b" (ratio 4:1): ^{1}H NMR (D_2O, 400 MHz) δ 3.84 (d, J = 8.3 Hz, 3a), 4.83–4.82 (m, 3b), 3.75 (t, J = 7.8 Hz, 4a), 3.75–3.72 (m, 5b), 3.58 (dd, J = 7.0, 5.2 Hz, 4b), 3.51 (td, J = 7.6, 5.2 Hz, 5a), 3.39 (s, 1b), 3.35 (d, J = 12.0 Hz, 1a′), 3.28 (d, J = 12.0 Hz, 1a″), 1.46–1.35 (m, 6), 1.27–1.10 (m, 7), 0.70 (t, J = 7.4 Hz, 8). The ^{13}C NMR resonances corresponding to the second compound were determined after the resonances corresponding to the first compound had been subtracted from the ^{13}C NMR spectrum of the mixture. The resonances are labeled large (l, probably the major anomer of the second compound) or small (s, probably the minor anomer of the second compound), depending on their intensities: ^{13}C NMR of mixture (D_2O, 100 MHz), 103.82s, 101.89, 100.93l, 82.15s, 80.40s, 79.80l, 78.60, 77.94l, 76.69, 76.23, 75.18l, 63.58, 62.69s, 62.60l, 62.29, 35.94l, 34.40s, 31.42, 30.31, 18.59, 18.42, 18.10s, 17.89l, 13.14 (s + 1) ppm; HRMS-FAB [M + Na]$^+$ calcd for $C_8H_{16}O_5$ 215.0895, obsd 215.0898.

3-Hydroxybutyrolactone (16). The reaction of sodium 3-hydroxy-4-oxobutanoate (**1**, 1.74 mmol) with DHAP in D_2O (about 20 mL) was run as described in the condensation procedure. At 40% conversion the reaction was quenched with sodium borohydride (305 mg, 8.06 mmol). The reaction mixture was acidified to pH 1.0 with 1 N HCl, concentrated in vacuo to 4 mL, and extracted with ethyl acetate (2 × 100 mL). The ethyl acetate solution was then dried ($MgSO_4$) and concentrated at aspirator pressure. The residue was purified by silica gel chromatography (ethyl acetate/hexane (1:1–5:1)) to give 3-hydroxybutyrolactone (**16**, 24 mg, 0.24 mmol): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.65 (ddt, J = 6.0, 4.4, 1.7 Hz, 1 H), 4.39 (dd, J = 10.3, 4.4 Hz, 1 H), 4.28 (dt, J = 10.3, 1.3 Hz, 1 H), 3.25 (br s, 1 H), 2.72 (dd, J = 18.0, 6.0 Hz, 1 H), 2.49 (dt, J = 18.0, 1.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) 176.73, 76.18, 67.40, 37.75 ppm; HRMS-CI [M + NH_4]$^+$ calcd for $C_4H_6O_3$ 120.0661, obsd 120.0659; $[\alpha]^{25}_D$ = −31.5° (c = 1.3, EtOH) [lit.[32] $[\alpha]^{25}_D$ = +88.9° (R, c = 1.36, EtOH).

4-(Hydroxymethyl)butyrolactone (17). The reaction of 4-hydroxy-5-oxopentanoic acid (**2**, 162 mg, 1.42 mmol) with DHAP in D_2O (about 20 mL) was run as described in the condensation procedure to 40% conversion as determined by ^{1}H NMR whereupon sodium borohydride (111 mg, 3.0 mmol) was added. After the pH was adjusted to 1.0 with 1 N HCl, the reaction mixture was continuously extracted with ethyl acetate (200 mL) for 36 h. The ethyl acetate was concentrated at aspirator pressure and the resulting residue purified by silica gel chromatography (ethyl acetate/hexane (1:1 to 5:1)) to provide 4-(hydroxymethyl)butyrolactone (**17**, 34 mg, 0.29 mmol): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.59 (tdd, J = 7.4, 4.5, 2.9 Hz, 1 H), 3.85 (dd, J = 12.5, 2.8 Hz, 1 H), 3.64 (dd, J = 12.5, 4.5 Hz, 1 H), 3.04 (s, 1 H), 2.59 (ddd, J = 17.9, 10.0, 5.9 Hz, 1 H), 2.50 (ddd, J = 17.9, 9.7, 8.1 Hz, 1 H), 2.23 (dddd, J = 18.9, 9.8, 7.6, 5.9 Hz, 1 H), 2.12 (dddd, J = 18.7, 10.0, 8.1, 6.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) 177.87, 80.85, 63.96, 28.62, 23.07 ppm; HRMS-CI [M + NH_4]$^+$ calcd for $C_5H_8O_3$ 134.0817, obsd 134.0805; $[\alpha]^{25}_D$ = +19.2° (c = 1.8, EtOH) [lit.[33] $[\alpha]^{25}_D$ = +31.5° (S, c = 2.66, EtOH)].

6-(Hydroxymethyl)caprolactone (18). Methyl 5-hydroxy-6-hexanoate dimethyl acetal (416 mg, 2.0 mmol) in water (7.0 mL) was mixed with an aqueous solution of 1 N NaOH (3.0 mL). After 45 min the solution was acidified with Dowex 50-X-8 ion-exchange resin (H^+ form, 4.0 g). After being stirred for 16 h, the solution was filtered, partially concentrated at aspirator pressure, and neutralized with 1 N NaOH. DHAP (20 mL of a 0.1 M solution, 2 mmol) was added and the solution neutralized with 1 N NaOH. Aldolase was added (50 U), and the reaction was followed by enzymatic assay of DHAP concentration. At approximately 40% consumption of DHAP (2 h), the reaction was quenched with $NaBH_4$ (52 mg, 1.4 mmol). After 1 h, the solution was acidified with concentrated HCl to pH 1.0 and partially concentrated at 1 Torr. The aqueous solution (30 mL) was then continuously extracted with ethyl acetate (150 mL). After 18 h, the organic layer was dried ($MgSO_4$), filtered, concentrated at aspirator pressure, and purified by silica gel chromatography (eluent: methanol/CH_2Cl_2 (1:10)) to provide 6-(hydroxymethyl)caprolactone (12 mg) and ethyl 5,6-dihydroxyhexanoate. This ethyl ester, Dowex 50-X-8 ion-exchange resin (H^+ form, 0.5 g), and acetonitrile (40 mL) were stirred together. After 16 h, the solution was filtered, concentrated at aspirator pressure, and purified by silica gel chromatography (eluent: CH_3CN/CH_2Cl_2 (1:1)) to provide 14.4 mg of 6-(hydroxymethyl)caprolactone: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.40 (ddt, J = 11.3, 5.6, 3.3 Hz, 1 H), 3.78 (dd, J = 12.3, 3.2 Hz, 1 H), 3.65 (dd, J = 12.3, 5.6 Hz, 1 H), 2.61 (dddd, J = 17.7, 6.5, 4.9, 1.4 Hz, 1 H), 2.45 (ddd, J = 17.7, 9.3, 7.0 Hz, 1 H), 2.0–1.81 (m, 4 H (O*H*)), 1.75–1.65 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.43, 81.04, 64.87, 29.56, 23.59, 18.33 ppm; HRMS-CI [M + H]$^+$ calcd for $C_6H_{10}O_3$ 131.0708, obsd 131.0699; $[\alpha]^{25}_D$ = +25.92° (c = 1.2, $CHCl_3$) [lit.[34] $[\alpha]^{25}_D$ = +34.68° (S, c = 1.3, $CHCl_3$)].

Methyl 7,8-Dihydroxyoctanoate (19). Methyl 7-hydroxy-8-hexanoate dimethyl acetal (387 mg, 1.65 mmol) in water (8.0 mL) was mixed with an aqueous solution of 1 N NaOH (2.2 mL). After 2 h, the solution was lyophilized, dissolved in 150 mL of H_2O, and acidified with Dowex 50-X-8 ion-exchange resin (H^+ form, 4.0 g). After being stirred for 48 h, the solution was neutralized with 1 N NaOH to pH 6.3, filtered, and partially concentrated at aspirator pressure to 15 mL. DHAP (10 mL of a 0.1 M solution, 1 mmol) was added and the solution neutralized with 1 N NaOH. Aldolase was added (100 U), and the reaction was followed by enzymatic assay of DHAP concentration. At approximately 25% consumption of DHAP (2 h), the reaction was quenched with $NaBH_4$ (100 mg, 3.4 mmol). After 1 h, the solution was lyophilized, dissolved in CH_3OH (50 mL), and acidified with Dowex 50-X-8 ion-exchange resin (H^+ form, 5.0 g). After being stirred for 48 h, the solution was filtered through Celite, concentrated at aspirator pressure, and purified by silica gel chromatography (eluent: ethyl acetate/methanol (100:0 going

to 20:1)) to provide 155 mg of methyl 7,8-dihydroxyoctanoate. Spectral properties were identical to those of methyl 7(*S*),8-dihydroxyoctanoate (vide infra) with the exception of the magnitude of the optical rotation: $[\alpha]^{25}_D = -0.73°$ (c = 1.81).

7,8-*O*-Isopropylidene-7(*S*),8-dihydroxyoct-5-enoic Acid (21). (4-Carboxybutyl)triphenylphosphonium bromide (1.356 g, 3.0 mmol) in dry THF (80 mL) was sonicated for 10 min. Potassium bis(trimethylsilyl)amide (12 mL of a 0.5 M solution in toluene, 6.0 mmol) was then added over a 10-min period. After 45 min, the reaction mixture was cooled to −20 °C, and *R*-solketal (**20**, 392 mg, 3.0 mmol) in THF (5 mL) was added over a 10-min period. After 30 min, the solution was warmed to 21 °C. After 1 h the solution was partially concentrated at aspirator pressure and partitioned between ether (200 mL) and pH 3.0 water (0.1 M phosphate). The organic phase was dried ($MgSO_4$), filtered, concentrated at aspirator pressure, and purified by silica gel chromatography (eluent: hexane/ethyl acetate/acetic acid (3:1:0 going to 5:1:0.25)) to provide 446 mg (2.1 mmol, 70%) of 7,8-*O*-isopropylidene-7(*R*),8-dihydroxyoct-5-enoic acid (9:1 mixture cis/trans alkene). Only the distinct proton resonances corresponding to the trans alkene are reported. The other proton resonances corresponding to the trans alkene are presumed to occur at approximately the same chemical shift as the corresponding proton resonances in the cis compound: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (dt, J = 15.3, 6.8 Hz, 1 H trans), 5.58 (dt, J = 10.9, 7.5 Hz, 1 H cis), 5.44 (br t, J = 9.8 Hz, 1 H cis), 4.79 (br q, J = 7.6 Hz, 1 H cis), 4.44 (br q, J = 7.3 Hz, 1 H trans), 4.04 (dd, J = 8.0, 6.2 Hz, 1 H cis), 3.53 (t, J = 8.1 Hz, 1 H trans), 3.49 (t, J = 8.0 Hz, 1 H cis), 2.33 (t, J = 7.3 Hz, 1 H cis), 2.32 (t, J = 7.2 Hz, 1 H trans), 2.21–2.06 (m, 2 H), 1.74–1.67 (m, 2 H), 1.39 (s, 3 H), 1.36 (s, 3 H); ^{13}C NMR (100 MHz $CDCl_3$) 179.43 (cis), 134.27 (trans), 133.55 (cis), 128.38 (trans), 128.27 (cis), 109.15 (cis), 77.10 (trans), 71.74 (cis), 69.34 (trans), 69.30 (cis), 33.23 (trans), 33.14 (cis), 31.37 (trans), 26.84 (cis), 26.71 (trans), 26.65 (cis), 25.88 (cis), 24.34 (cis), 23.68 (trans) ppm; HRMS-FAB [M + H]$^+$ calcd for $C_{11}H_{18}O_4$ 215.1283, obsd 215.1298.

7,8-*O*-Isopropylidene-7(*S*),8-dihydroxyoctanoic Acid. Platinum (10% on carbon, 45 mg) and 7,8-*O*-isopropylidene-7(*R*),8-dihydroxyoct-5-enoic acid (214 mg, 1.0 mmol) were added to hexane (10 mL). The reaction mixture was then put under a hydrogen atmosphere. After 30 min, the solution was filtered through Celite and concentrated at aspirator pressure to provide 208 mg (0.96 mmol, 96%) of 7,8-*O*-isopropylidene-7(*R*),8-dihydroxyoctanoic acid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.07–3.98 (m, H-8a, H-7), 3.47 (t, J = 7.3 Hz, H-8b), 2.32 (t, J = 7.4 Hz, H-2), 1.65–1.56 (m, 3 H), 1.51–1.25 (m, 5 H), 1.37 (s, 3 H), 1.32 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) 179.82, 108.68, 75.93, 69.36, 33.87, 33.28, 28.98, 26.88, 25.68, 25.39, 24.46 ppm; HRMS-FAB [M + H]$^+$ calcd 217.1440, obsd 217.1449; $[\alpha]^{25}_D = +13.6°$ (c = 1.4). Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 61.19; H, 9.40.

Methyl 7(*S*),8-Dihydroxyoctanoate (19). Dowex 50W-X8 ion-exchange resin (H^+ form, 100 mg), 7,8-*O*-isopropylidene-7(*R*),8-dihydroxyoctanoic acid (45 mg, 0.21 mmol), and methanol (5.0 mL) were stirred for 24 h, filtered, concentrated at aspirator pressure, and purified by silica gel chromatography (eluent: ethyl acetate/methanol (100:0 going to 20:1)) to provide 34 mg (0.18 mmol, 85%) of methyl 7(*S*),8-dihydroxyoctanoate: ^{1}H NMR ($CDCl_3$ with a drop of D_2O, 400 MHz) δ 3.68–3.60 (m, 1 H), 3.63 (s, 3 H), 3.57 (dd, J = 11.2, 2.8 Hz, 1 H), 3.36 (dd, J = 11.2, 7.7 Hz, 1 H), 2.27 (t, J = 7.5 Hz, 2 H), 1.59 (pentet, J = 7.4 Hz, 2 H), 1.46–1.28 (m, 6 H); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.68–3.55 (m, 2 H), 3.62 (s, 3 H), 3.40–3.34 (br m, 1 H), 3.05–2.65 (m, 2 H, O*H*), 2.27 (t, J = 7.5 Hz, 2 H), 1.59 (pentet, J = 7.4 Hz, 2 H), 1.46–1.28 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) 174.34, 72.09, 66.70, 51.51, 33.88, 33.78, 28.99, 25.12, 24.70 ppm; HRMS-FAB [M + H]$^+$ calcd 191.1283, obsd 191.1284; $[\alpha]^{25}_D = -0.79°$ (c = 1.78, $CHCl_3$). Anal. Calcd for $C_9H_{18}O_4$: C, 56.82; H, 9.54. Found: C, 56.94; H, 9.42.

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Supplementary Material Available: Experimental procedures and characterization data for the synthesis of aldehydes 1–7 and characterization data for sugar phosphates **9**–**12** (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.