Substrate Specificity and Carbohydrate Synthesis Using Transketolase

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This paper describes the use of the enzyme transketolase as a catalyst in organic synthesis. The properties of transketolase from both yeast and spinach were investigated. The yeast enzyme was found to be more convenient for routine use. Examination of the substrate specificity of yeast transketolase demonstrated that the enzyme accepts a wide variety of 2-hydroxy aldehydes as substrates. A practical protocol for transketolase-catalyzed condensation of hydroxypyruvic acid with these aldehydes has been developed and used for the synthesis of four carbohydrates: L-idose, L-gulose, 2-deoxy-L-xylohexose, and L-xylose.

This paper describes our studies of the use of transketolase (EC 2.2.1.1) (TK) in organic synthesis. As part of the oxidative pentose phosphate pathway, TK transfers a two-carbon ketol unit from a donor ketose (1) to an acceptor aldose (2) (Scheme I).1 The reaction is reversible, and the products of the reaction, a ketose homologated by two carbons (3) and an aldose shortened by two carbons (4), can also function as reaction partners. The TK-catalyzed two-carbon transfer reaction shown in Scheme I requires the presence of the cofactors thiamine pyrophosphate (TPP, 5) and magnesium(II).2

To drive the equilibrium established by TK, Srere et al. used β-hydroxypyruvic acid (HPA) (6) (Scheme II) as the ketol donor.3 This strategy coupled the formation of the glycolyl–TPP complex 9 with the decarboxylation of HPA and rendered the complete reaction irreversible. Scheme II shows the catalytic cycle for the TK-mediated condensation of HPA (6) and α-hydroxy aldehyde 10. Addition of the anion of TPP (7) to HPA results in the formation of intermediate 8. This adduct loses CO₂ and generates the glycolyl–thiamine pyrophosphate adduct 9. The adduct, represented by two canonical forms, 9a and 9b, is nucleophilic and adds to substrate 10 to afford 11.4 Fragmentation of 11 then regenerates the TPP anion 7.

Scheme I. Transketolase-Catalyzed Interconversion of Carbohydrates

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Scheme II. Catalytic Cycle of Transketolase

Table I. Carbohydrates as Substrates for TK

<table>
<thead>
<tr>
<th>Substrate</th>
<th>V_{substrate}/V_{glyceraldehyde}</th>
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<tbody>
<tr>
<td>d-glyceraldehyde 3-phosphate</td>
<td>44%</td>
</tr>
<tr>
<td>d-erythrose 4-phosphate</td>
<td>33</td>
</tr>
<tr>
<td>d-arabinose 5-phosphate</td>
<td>24 (ref 5)</td>
</tr>
<tr>
<td>d-glucose 6-phosphate</td>
<td>9</td>
</tr>
<tr>
<td>d-glyceraldehyde</td>
<td>78</td>
</tr>
<tr>
<td>L-glyceraldehyde</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>dL-glyceraldehyde</td>
<td>56</td>
</tr>
<tr>
<td>d-erythrose</td>
<td>56</td>
</tr>
<tr>
<td>d-glucose</td>
<td>4</td>
</tr>
</tbody>
</table>

Completing the catalytic cycle and affording the addition product 12.

The condensation of hydroxypyruvate anion and an aldehyde catalyzed by TK consumes one proton per cycle (Scheme II). The increase in pH is partially offset by reaction of CO₂ (from the decarboxylation of HPA) with hydroxide to form bicarbonate ion. In spite of this effect, the pH of the reaction will exceed 7.5, the optimum pH for TK.³ This pH can be maintained effectively either by using buffer or by adding acid slowly over the course of the reaction (see below).

Table I shows several carbohydrates that are substrates for TK and the reaction velocity of the carbohydrate relative to glyceraldehyde.⁵ The new stereocenter formed in the TK-catalyzed addition of ketol to an aldehyde is set in a three configuration with high diastereoselectivity. The absolute configuration of the product is also controlled by the enzyme. TK shows a kinetic preference for α-hydroxy aldehydes having C2-D stereochemistry as evidenced by the relative rates of D-glyceraldehyde (78%) and L-glyceraldehyde (ca. 0.00% (Table I)). The overall stereochemical result of the enzyme-catalyzed process is formation of a 1,2-diol in the D-three configuration. The preference shown by TK for C2-D stereochemistry in the aldehyde substrate suggests the possibility of synthesis of the L-enantiomer of α-hydroxy aldehydes by selective reaction of the C2-D enantiomer from a racemate. We demonstrate the practicality of this kinetic resolution in a following section.

Availability and Properties of TK. We have studied transketolase isolated from baker's yeast and from fresh spinach leaves.⁷-¹¹ The yeast-derived enzyme is commercially available at moderate cost (see Table II) and is stable for at least several months as the lyophilized powder (the form in which it is purchased) and for several weeks in pH 7.5 gly-gly buffer solution. The spinach enzyme is not currently available commercially, but can be isolated from fresh spinach leaves obtained from the local grocery. In our hands, the partially purified leaf extracts showed TK activity immediately after isolation, but activity decreased rapidly and was completely lost after a few days. The spinach enzyme may be purified to homogeneity, at which point it has been reported to be more stable than the crude extracts. Table II shows the properties of both yeast and, where the information is available, spinach transketolase.

Results and Discussion

Our objective was to evaluate TK as an enzyme for use in organic/carbohydrate synthesis. In examining the utility of the enzyme, our goal was to determine whether TK would be practical as a catalyst for preparative-scale synthesis. We were particularly interested in assessing how TK would complement fructose 1,6-diphosphate (rabbit muscle) aldolase (RAMA, EC 4.1.2.13) as a tool for car-

![Figure 1. Percent HPA remaining in buffer solutions after 24 h. The initial concentration of HPA was 10 mM. See Experimental Section for assay conditions.](image-url)

Table II. Properties of Transketolase

<table>
<thead>
<tr>
<th>Property</th>
<th>Yeast (ref)</th>
<th>Spinach leaves (ref)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subunits</td>
<td>2</td>
<td>4 (12)</td>
</tr>
<tr>
<td>MW of active form (kD)</td>
<td>158-159 (13), 160 (12), 140 (14), 110 ± 10 kD (9)</td>
<td></td>
</tr>
<tr>
<td>Optimum pH</td>
<td>7.6 (5)</td>
<td></td>
</tr>
<tr>
<td>Kᵢ (HPA) (mM)</td>
<td>7 (15), 33 (16)</td>
<td>0.032 (13)</td>
</tr>
<tr>
<td>Kᵢ (TPP) (mM)</td>
<td></td>
<td>&lt;100 (17)</td>
</tr>
<tr>
<td>Kᵢ (Mg⁺²) (mM)</td>
<td>ca. 0.4 (13)</td>
<td></td>
</tr>
<tr>
<td>Specific activity (U/mg)</td>
<td>15-25 (19)</td>
<td>7.8 (8), 50 (9)</td>
</tr>
</tbody>
</table>

(5) Although d-arabinose-5-P has the wrong stereochemistry at C-2, it has been reported as a substrate. Datta, A. G., Racker, E. J. Biol. Chem. 1961, 236, 617.


(7) de la Haba, G.; Leder, I. G.; Racker, G. J. Biol. Chem. 1961, 236, 617.


(12) Murphy, D. J.; Walker, D. A. Plant. J. 1982, 155, 310.


(19) As reported by Sigma Chemical Company. See also: Klein, H.; Karl, R. Z. Physiol. Chem. 1977, 358, 1325.

(20) From Sigma Chemical Co.
bohydride synthesis. Toward this end, we tested several α-hydroxy aldehydes to assess the range of substrates that would be accepted by TK. We also hoped to determine if TK would provide a useful route to optically enriched α-hydroxy aldehydes through kinetic resolution of racemic mixtures of substrates. We also examined several analogs to β-hydroxypyruvate and found that none was accepted by the enzyme.

The assessment of TK as an enzyme for organic synthesis proceeded on two levels. First, we developed a practical protocol for the use of TK in preparative-scale synthesis. Second, we applied the enzyme to the synthesis of interesting target systems.

Using TK as a Catalyst in Organic Synthesis. We examined both the yeast and spinach enzymes in the initial phases of this study. Reported substrate specificities and reactivities for both enzymes were similar. We found that the inconvenience of isolation and the marginal stability of the spinach enzyme more than outweighed the expense of the yeast enzyme. The results reported here are for experiments conducted using commercially available TK from yeast.

The traditional approach to pH control of the TK-mediated condensation of HPA and aldehydes has been the use of a buffered reaction medium. Upon examining the stability of 10 mM HPA in several concentrations of four buffer systems, we found that, in all cases, significant decomposition of HPA had occurred after 24 h (Figure 1). Interestingly, we found that HPA was stable in unbuffered water at pH 7.5 for 24 h. The decomposition of HPA thus appeared to be catalyzed by buffer. We have developed a system whereby the pH of the reaction medium is maintained at 7.5 by a pH controller through the addition of a solution of hydroxypropionic acid (ca. pH 4). The benefits of this procedure are 2-fold. First, separation of the reaction products from large quantities of buffer salts is avoided. Second, over the course of the reaction, the additional HPA introduced into the reaction mixture to control pH partially offsets the consumption of HPA by the reaction.

Preparation of α-Hydroxy Aldehyde Substrates. α-Hydroxy aldehydes are conveniently stored and characterized in the form of their corresponding dialkyl acetals. Acetals were synthesized by treating the aldehydes with anhydrous methyl alcohol and methanol-washed acidic ion-exchange resin (Dowex 50W-X8). Hydrolysis of the acetals was accomplished in a similar manner with water and acidic ion-exchange resin (Scheme III).

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Strategies used for the synthesis of the α-hydroxy aldehydes used in this study are shown in Schemes IV and V. The oxidation of alkenes was a useful method for the preparation of simple, racemic α-hydroxy aldehydes. Ozonolysis of commercially available allylic alcohols 15, 16, and 17 afforded, after reductive workup (DMS), aldehydes 18, 19, and 20. These aldehydes could be used directly from the ozonolysis procedure or stored as their acetals for later use. Oxidation of dihydrofuran (21) with catalytic osmium tetroxide and N-methylmorpholine N-oxide in water/acetone gave, after silica gel chromatography, aldehyde 23 in high yield. Aldehyde 23 was also stored as its methyl acetal.

Aldehyde 25 was synthesized from racemic glycic aldehyde dimethyl acetal (24). Epoxy acetal 24 was obtained by benzonitrile-mediated peroxide oxidation of acrolein dimethyl acetal. Addition of vinyl magnesium bromide to epoxide 24 gave the corresponding hydroxyacetal. Hydrolysis of this material furnished the desired aldehyde 25. Addition of methanol to epoxide 24 followed by hydrolysis of the acetal gave 26. Methylthio aldehyde 27 was prepared in a similar manner. Aldehyde 29 was prepared by addition of vinylmagnesium bromide to 2,2-dithiohydroacetaldelhyde followed by hydrolysis of the acetal. Aldehyde 29 displays a remarkable resistance to conjugation. Although some loss of aldehyde was seen during the hydrolysis step, the desired unconjugated


(23) Four analogs to β-hydroxyproprionate were examined: 2,3-dioxopropionic acid, 2,2-oxo-3-hydroxybutyric acid, 2-oxomalonic acid, and 3-ketoglucosonic acid showed no activity with TK.


(27) 2,2-Dithiohydroacetalddehyde was prepared by ozonolysis. See ref 33.
compound was the major product (see Experimental Section).

Dihydroxy aldehydes 34 and 39 (Scheme V) were prepared starting from D-xylene and D-ribose, respectively. The strategies follow essentially parallel lines—glycoside formation followed by protection of the secondary hydroxyl functions at C-2 and -3 and activation of C-5. The key step in these strategies was the Zn-promoted ring fragmentation of the 5-iodopentosides (32 to 33 and 37 to 38) which simultaneously liberated the aldehyde and introduced an alkene. Methanolation under acidic conditions of the crude products led sequentially to acetal formation and deprotection of the secondary hydroxyl moieties. Hydrolysis of the acetals was accomplished by treatment of the acetals with aqueous acidic ion-exchange resin as before.

Noncarbohydrate Substrates for TK. Table III shows compounds which were examined as substrates for TK. The yields given in Table III are for isolated products obtained after silica gel chromatography and are based on the quantity of acetal precursor prior to hydrolysis. The TK adducts (42-51) could be partially purified but were found to be somewhat unstable. The experiments were conducted on a 1-5 mmol scale (see Experimental Section). Reaction times were 3-5 days. Progress of the reactions was monitored by thin-layer chromatography (silica gel, 20% methanol in dichloromethane).

TK accepts a broad range of aldehydes, provided they have a C2-D hydroxyl group. Relative rate data shown in Table III were obtained under standard assay conditions at an aldehyde concentration of 40 mmol-L⁻¹ (see Experimental Section). Comparison of the relative rates of 18 (R = ethyl) and 20 (R = tert-butyl) suggests that the presence of carbon branching at C3 of the aldehyde adversely affects the ability of an aldehyde to function as a substrate for TK. The unexpectedly low rate of lactaldehyde 40 is an exception to this observation. Comparison of the relative rates of dihydroxy aldehydes 34 and 39 with monohydroxy compound 25 suggests a slight kinetic preference by TK for the more hydroxylated structures. Interestingly, the stereochemistry at the β carbon of the aldehyde seems not to exert a significant effect on the rate of reaction as evidenced by the similar rates of aldehydes 34 and 39.

Kinetic Resolution of Racemic α-Hydroxy Aldehydes. We resolved several racemic α-hydroxy aldehydes. For the aldehydes that were isolated, we observed excellent percent enantiomeric excesses (77-95% ee) in all but one case (Table III). Percent enantiomeric excesses were determined by reduction of the aldehydes with sodium borohydride in ethyl alcohol to the corresponding diols followed by ¹H NMR/chiral shift reagent analysis (Et(fio)₂). The diols were purified by silica gel chromatography with 20% methanol in dichloromethane as eluent, but we believe the purification had no effect on the observed ee. We prepared comparison samples of chiral diols derived from aldehydes 18, 19, and 20 (diols 55, 56, and 57, respectively) according to well-established procedures. Diazotization of the corresponding L-amino acids followed by reduction of the hydroxy acids with LAH afforded the expected dios (eq 1). The diol derived from 28 was prepared from L-glycidol by treatment of the epoxide with sodium methoxide in methanol.

We were able to obtain the TK-resolved C₂-L-hydroxy aldehydes in only modest yields. This inefficiency is likely due to the decomposition of the aldehyde during the reaction and during isolation. No other identifiable products were observed in the product mixture. We have not yet utilized this method to prepare 2-hydroxy aldehydes for use as substrates for other enzymes. We believe that TK-catalyzed resolution of racemic 2-hydroxy aldehydes is a potentially useful method to prepare small quantities of these compounds for use in enantiospecific synthesis.

Determination of Stereochemistry of Products. The stereochemistries of products 47-50 (Table III) were

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(29) Recently it has been shown that the spinach enzyme will accept some monohydroxylated aldehydes. See ref 24.
Carbohydrate Synthesis Using Transketolase

Scheme VI. Determination of Enantiomeric Excess

*Key: (a) ZnL2, acetone; (b) R or S Mosher’s acid chloride, triethylamine.

Scheme VII. Comparison of TK and RAMA

*Key: (a) IDH (EC 1.1.1.14), FDH (EC 1.1.1.14), NADH, NaHCO3; (b) 1. O3, 2. Na2SO4.

simplifying the procedures for monitoring the progress of the reaction as well as the isolation of the products. TK provides access to compounds that cannot be obtained via RAMA-catalyzed processes (Scheme VII). Alkene-containing ketone 47 is readily synthesized by the TK-catalyzed condensation of racemic aldehyde 29. TK adduct 47 would be the result of the RAMA-catalyzed aldol of dihydroxyacetone phosphate and acrolein followed by in situ dephosphorylation by acid phosphatase (H⁺ Pase, EC 3.1.3.2). Acrolein is, however, not a substrate for RAMA. Thus, it is not possible to prepare 47 using RAMA. Similarly, compounds 49 and 50 can be synthesized conveniently and independently via the TK-catalyzed condensation of HPA and aldehydes 34 and 39. Racemic aldehyde 29 would likely serve as a substrate for RAMA. This reaction would result, after dephosphorylation, in the formation of ketoses 49 and 50 as a mixture of diastereomers. To access 49 and 50 individually via a RAMA-based approach would require enantiomERIC synthesized of both antipodes of 29.

Alkene-containing aldehydes 25, 29, 34, and 39 offer the opportunity for further manipulation of the enzymatically-obtained products through functionalization of the unsaturation. We have previously used \(^{34}\) a strategy (Scheme VIII) in which reduction of the ketone followed by release of a protected distal aldehyde resulted in the conversion of enzymatically synthesized ketones to "inverted" aldoses.

The alkene, viewed as a latent aldehyde, suggests the possibility of TK-based aldose synthesis through the inversion strategy. Enzymatic reduction of TK adducts 47, 48, 49, and 50 with iditol dehydrogenase (EC 1.1.1.14) coupled with formate dehydrogenase (EC 1.1.27) and sodium formate to regenerate NADH furnished alkene-polyols 64, 65, 66, and 70.\(^ {35}\) Oxidative cleavage of the alkene with ozone followed by reductive workup with sodium sulfite afforded 2-deoxy-l-gulose (67), l-gulose (68), l-idose (69), and l-xylene (71), respectively. The structures of l-gulose, l-idose, and l-xylene were firmly established.


by comparison to authentic samples. The structure of 2-deoxy-l-gulose was confirmed by prepartion of an authentic sample (according to ref 34) and comparison of 1H NMR spectra.

Conclusions

TK has been shown to accept a wide variety of alpha-hydroxy aldehydes as substrates. The TK-catalyzed condensation of these substrates and beta-hydroxy pyruvate proceeds smoothly to afford ketoses. These carbohydrates were conveniently isolated using conventional silica gel chromatography. These easily handled materials can then be used as intermediates in the synthesis of a variety of structures. We have used intermediates from TK-catalyzed processes to synthesize enantiospecifically four carbohydrates (see above) and have previously described the preparation of noncarbohydrate structures using TK in the key step of the procedure.

Experimental Section

General. All enzymes were purchased from Sigma Chemical Co. except formate dehydrogenase (FDH), which was purchased from Boeringer-Mannheim. All enzymes were used further purified. All solvents were reagent grade and used without further purification except those purified according to the following: water was distilled from glass, THF and diethyl ether were distilled from deep blue solutions of sodium/benzophenone ketyl, and dichloromethane was distilled from a slurry of calcium hydride. All reagents and biochemistry were used as provided by the manufacturer. Proton and 13C magnetic resonance experiments were conducted in CDC13 or D2O. Spectra were referenced to CHCl3 at 8 7.24 ppm, CDC13 at 77.5 ppm, δ, FOD at 8 4.80 ppm, or the carbon resonance of dioxane at 8 66.7 ppm as indicated. Mass spectrometric analyses were conducted by Dr. A. Tyler of the Harvard University Department of Chemistry.

3-Hydroxy-4,4-dimethylpent-1-ene (17). Vinylmagnesium bromide (1 M solution in THF, 50 mL, 50 mmol) was placed in a round-bottomed flask and cooled to 0 °C. Trimethylacetaldelyde (3.60 g, 50 mmol) in 30 mL of THF was then added through a dropping funnel over 30 min. The temperature was allowed to rise to room temperature, and the reaction was stirred for 12 h. The reaction mixture was cooled to 0 °C, and saturated NH4Cl solution (40 mL) was added during which time a precipitate formed. The liquid phase was separated, and the organic phase was washed with saturated NaCl solution. After evaporation of solvent at reduced pressure, the residue was dissolved in 100 mL of ether and washed with water to remove any remaining THF. The solution was dried with saturated NaCl solution and MgSO4, successively. Filtration and concentration afforded an oily residue that was chromatographed (silica gel, 10% CH2Cl2 in pentane) to give 3.14 g of the product (31.3 mmol, 63%) as a colorless liquid: 1H NMR (CDCl3, 250 MHz) δ 5.85 (ddd, J = 6.5, 0.15, 7.17 Hz, 1 H), 5.20 (ddd, J = 1.2, 1.4, 17.11 Hz, 1 H), 5.14 (ddd, J = 1.2, 1.6, 10.5 Hz, 1 H), 3.84 (m, 1 H), 1.72 (m, 1 H), 0.90 (t, J = 7.0 Hz, 6 H).

General Procedure for Oxozonation of 3-Hydroxylakene.

A solution of 3-hydroxylakene (0.1–0.3 M) in CH2Cl2 was cooled to -78 °C in a dry ice/acetone bath. A stream of O2 gas was bubbled through the solution until a light blue color persisted. The solution was then sparged with N2 gas until colorless. The mixture was then treated with an excess of dimethyl sulfide and stirred at -78 °C for 1 h. The mixture was slowly warmed to room temperature and stirred for 12 h. Volatile components were then removed in vacuo, and the residue was dissolved in methanol. The methanolic solution was then treated with acetic ion-exchange resin (Dowex AG-50W-X8, H+ form, 1–4 g) and stirred at room temperature for 12 h. The resin beads were then removed by filtration. The filtrate was neutralized with sodium bicarbonate (1–5 g), filtered, and concentrated. The residue was chromatographed over silica gel (10–20% ethyl acetate in hexanes) to afford the desired acetel.

1,1-Dimethoxy-2-hydroxybutane (dimethyl acetal of 18): 1H NMR (250 MHz, CDCl3) δ 5.89 (d, J = 4.9 Hz, 1 H), 3.30–3.19 (m, 1 H), 3.20 (s, 3 H), 3.16 (s, 3 H), 2.72 (br s, 1 H), 1.49–1.27 (m, 1 H), 1.23–1.09 (m, 1 H), 0.73 (t, J = 6.5 Hz, 3 H); 13C NMR (62.5 MHz, CDCl3) δ 107.15, 72.50, 54.97, 25.00, 10.01.

1,1-Dimethoxy-2-hydroxypentane (dimethyl acetal of 19): 1H NMR (400 MHz, CDCl3) δ 4.09 (d, J = 6.1 Hz, 1 H), 3.69–3.53 (m, 1 H), 3.41 (s, 3 H), 3.38 (s, 3 H), 2.34 (d, J = 3.2 Hz, 1 H), 1.55–1.46 (m, 2 H), 1.39–1.31 (m, 2 H), 0.89 (s, J = 6.2 Hz, 3 H); 13C NMR (100.6 MHz, CDCl3) δ 107.44, 71.34, 55.49, 34.31, 10.12, 14.53.

1-Methoxy-2-hydroxytetrahydrofuran (Methyl Acetal of 22). A solution of dihydrofuran (10.0 g, 143 mmol) and catalytic osmium tetroxide in acetonewater (20 mL, 1:1) was cooled to 0 °C in an ice bath. Hydrogen peroxide solution (16 g, 30% in water) was added dropwise over 2 h via an addition funnel. The solution stirred at room temperature for 12 h. The mixture was then filtered through activated carbon and concentrated. The residue was dissolved in methanol, treated with acetic ion-exchange resin (Dowex AG-50W-H+, H+ form, 10 g), and stirred for 36 h at room temperature. The ion-exchange resin was removed by filtration, and the filtrate was concentrated in vacuo. The residue was chromatographed over silica gel (10% ethyl acetate in hexanes) to afford the desired product as a colorless oil (14.87 g, 88%): 1H NMR (400 MHz, CDCl3) δ 4.75 (s, 1 H), 4.21 (dd, J = 5.6, 1.5 Hz, 1 H), 4.07 (d, J = 7.2 Hz, 1 H), 3.92–3.86 (m, 1 H), 3.28 (s, 3 H), 0.97 (br s, 1 H), 2.30–2.13 (m, 1 H), 1.73 (s, 1 H); 13C NMR (100.6 MHz, CDCl3) δ 109.41, 75.72, 67.73, 60.92, 54.84, 32.66.

1,1-Dimethoxy-2-hydroxypent-4-ene (25). 2,3-Epoxypropionaldehyde dimethyl acetal (11.8 g, 100 mmol) was dissolved in THF (100 mL) and added to 1 M solution of vinylmagnesium bromide in THF at room temperature. The solution was heated at reflux for 6 h. The mixture was then cooled to room temperature and concentrated. The residue was added to 900 mL of ice-water mixture. The resulting aqueous phase was extracted with ether (3 × 100 mL). The combined organic phases were washed with saturated NaCl solution, treated with MgSO4, filtered, and concentrated. Distillation at reduced pressure (38 °C (0.5 mmHg)) afforded the desired product as a colorless oil (6.20 g, 40%): 1H NMR (CDCl3, 300 MHz) δ 5.86 (m, 1 H), 5.13 (d, J = 14.7 Hz, 1 H), 5.08 (d, J = 8.1 Hz, 1 H), 4.14 (d, J = 6.0 Hz, 1 H), 3.65 (m, 1 H), 3.43 (s, 3 H), 3.41 (s, 3 H), 2.36 (m, 1 H), 2.17 (m, 2 H); 13C NMR (125.8 MHz, CDCl3) δ 135.64, 116.71, 104.65, 72.78, 63.81, 63.55, 15.32, 15.29.

3,3-Dimethoxy-1-(methylthio)propan-2-ol (Diethyl Acetal of 27). A solution of 2,3-epoxypropionaldehyde dimethyl acetal (1.01 g, 8.5 mmol) and sodium thiomethyle (0.90 g, 12.8 mmol) in MeOH (100 mL) was treated with O3 at -78 °C. After a short time, the solution took on a light blue color, indicating the presence of excess ozone. The solution was flushed with N2, flow was stopped, and dimethyl sulfide (6 mL) was added, and the mixture was stirred at -78 °C for 1 h. The temperature was allowed to warm to room temperature, and the mixture was stirred for 15 h. The mixture was concentrated, and the oily residue was distilled under pressure (45 °C (0.2 mmHg)) to obtain a mixture of dimethyl sulfone and 2 (63 g). The mixture was dissolved in 130 mL of anhydrous THF and cooled to 0 °C in an ice bath. Vinylmagnesium bromide (45 mmol) in THF (150 mL) was added dropwise, and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was treated with NH4Cl (50 mL of saturated aqueous soln). The organic layer was separated, and the aqueous layer was extracted with ether (100 mL). The combined organic phases were washed with saturated NaCl aqueous solution, dried over MgSO4, filtered, and concentrated in vacuo. Chromatography of the resulting residue over silica gel (25–67% AcOEt in hexanes) yielded 3.69 g of 3 (22.4 mmol, 49%): 1H NMR (CDCl3, 250 MHz)
Carbohydrate Synthesis Using Transketolase

\[ \text{J. Org. Chem., Vol. 57, No. 22, 1992} \]

5905

δ 5.91 (dd, \( J = 6.5, 10.6, 17.3 \text{ Hz}, 1 \text{ H} \)), 5.39 (dd, \( J = 1.6, 1.6, 17.3 \text{ Hz}, 1 \text{ H} \)), 5.23 (dd, \( J = 1.6, 1.6, 10.6, 17.3 \text{ Hz}, 1 \text{ H} \)), 4.27 (d, \( J = 6.0 \text{ Hz}, 1 \text{ H} \)), 4.07 (m, 1 H), 3.7 (m, 2 H), 3.60 (m, 2 H), 2.28 (d, \( J = 3.8 \text{ Hz}, 1 \text{ H} \)), 1.2 (m, 6 H); \[ ^{13} \text{C} \text{NMR (CDCl₃, 125.8 MHz)} \]
\[ \delta 135.6, 116.7, 104.7, 72.8, 63.8, 63.6, 15.31, 15.28; \text{FTIR 3431, 2978, 2931, 2899, 1741, 1374, 1243, 1120, 1065, 1000, 925 \text{ cm}^{-1} \].

\( \text{β-Methyl-5-ido-2,3-isopropylideneriboside (32)} \). A solution of tosylate (51 (10.5 g, 14.2 mol) in methanol (50 mL) was treated with Na (50.0 g, 53.5 mmol). The slurry was heated at reflux for 4 h. The mixture was then cooled to room temperature and diluted with an equal volume of ether and filtered through silica gel with an additional volume of ether. The solution was then concentrated in vacuum to afford a viscous oil. Yellow gel chromatography (15% ethyl acetate in hexanes) afforded tosylide (32) as a colorless oil (4.58 g, 97%).

\( \text{H NMR (250 MHz, CDCl₃)} \) δ 5.04 (s, 1 H), 4.75 (d, \( J = 5.9 \text{ Hz}, 1 \text{ H} \)), 4.62 (d, \( J = 5.9 \text{ Hz}, 1 \text{ H} \)), 1.45 (s, 3 H), 1.31-1.34 (m, 2 H), 1.47 (s, 3 H), 3.13-3.14 (m, 2 H), 2.6 (br s, 2 H); \[ ^{13} \text{C} \text{NMR (62.5 MHz, CDCl₃)} \] δ 113.99, 110.13, 87.91, 85.83, 83.53, 55.75, 26.69, 25.57, 1.21; IR (cm\(^{-1}\)) 2995, 2945, 2805, 1365, 1105, 1020, 875.

1.1-Dimethoxy-2(3H)-dihydroxy-4-pentene (Dimethylenecacet of 34). Zinc powder (15 g) and ethanol (3 x 50 mL), and ethano (3 x 50 mL). The resulting paste was suspended in 2-propanol (100 mL) containing isodiiodeide 32. The mixture was stirred at reflux for 1 h. After being cooled to room temperature, the slurry was washed with ether (200 mL), washed through a plug of silica gel, and concentrated in vacuum to afford a viscous yellow oil. This oil was taken up in methanol (100 mL) and treated with ion-exchange resin (Dowex AG-50W-H₄, H⁺ form, 10 g). The suspension was stirred at room temperature for 12 h. After removal of the resin by filtration, the solution was concentrated in vacuum to afford a viscous brown oil. This oil was purified by silica gel chromatography (50% ethyl acetate in hexanes) to afford the dimethyl acetal of 34 as a pale yellow syrup (1.50 g, 63%): \[ \text{H NMR (400 MHz, CDCl₃)} \] δ 5.90 (m, 2 H), 5.30 (dd, \( J = 5.9, 1.8 \text{ Hz}, 0.6 \text{ H} \)), 5.12 (d, \( J = 4.6 \text{ Hz}, 0.4 \text{ H} \)), 5.07 (m, 0.7, 0.6, 0.4, 0.4, 0.4, 0.2 (m, 6 H), 2.12 (s, 1.8, 1.8, 1.2 (m, 6 H), 2.12 (s, 1.8, 1.8, 2.08 (s, 1.8 H); \[ ^{13} \text{C} \text{NMR (100 MHz, CDCl₃)} \] δ 170.60, 170.50, 170.20, 169.55, 107.64, 100.94, 81.64, 81.39, 78.40, 76.48, 75.77, 75.27, 56.29, 52.51, 23.92, 22.92 (br s, 2 H); IR (cm\(^{-1}\)) 2890, 1740, 1585, 1325; [\( \alpha \) \( _{D} \)] = 10.8° (c = 0.87 in CHCl₃).

1.1-Dimethoxy-2(3H)-dihydroxy-4-pentene (Dimethylenecacet of 39). Zinc powder (4.9 g) was washed sequentially with 6 N HCl (50 mL), water (3 x 50 mL), and ethanol (3 x 50 mL). The resulting paste was suspended in ether (50 mL) containing isodiiodeide 37. The mixture was stirred at reflux for 45 min. After being cooled to room temperature, the slurry was washed through a plug of silica gel and concentrated in vacuum to afford a viscous yellow oil (0.25 g, 97%). This oil was taken up in methanol (50 mL) and treated with ion-exchange resin (Dowex AG-50W-H₄, H⁺ form, 10 g). The suspension was stirred at room temperature for 2 h. After removal of the resin by filtration, the solution was concentrated in vacuum to afford a viscous brown oil. This oil was purified by silica gel chromatography (50% ethyl acetate in hexanes) to afford the acetal of 39 as a pale yellow syrup (0.76 g, 35%).

Measurement of Relative Velocity of Aldehyde Substrates. The velocity of the TK reaction was assayed by following the disappearance of 8-β-hydroxy-α-pyrone from the reaction mixture. The reaction was carried out at 30 °C in Gly-Gly buffer (80 mM, 500 mM, pH 7.6) with TTP (1.0 mM) of MgCl₂ (5 mM), α- and β-hydroxy-α-pyrone (500 mM), and α-acetoxy-β-hydroxy-α-pyrone (500 mM). The assay was performed in the presence of NBADH for 0.2 h. The reaction was started by the addition of TK (0.3–2.5 U). At times of 1, 2, 3, 4, 5, 10, 20, and 30 min, 50–500 aliquots of solution were withdrawn from the reaction mixture and quenched by addition to 1 mL of 0.2% trichloroacetic acid solution.

The quantity of 8-β-hydroxy-α-pyrone in each sample was determined photometrically by measuring the change in absorbance of NADH at 340 nm due to reduction of NAD⁺ by 8-β-hydroxy-α-pyrone (EC 1.1.1.27). A 3-mL plastic cuvette was charged with 3 mL of pH 7.5 Tris buffer (100 mM) containing 50 U of LDH and 0.2 mM of NADH. A 200-μL sample of the quenched solution was then added to the cuvette. The decrease in absorbance at 340 nm, the amount of 8-β-hydroxy-α-pyrone in the sample was calculated. Apparent rates were calculated by plotting time versus consumption of 8-β-hydroxy-α-pyrone, which usually ranged from 0.4 to 1.5 mM/min. Control reaction containing all of substrates but no enzyme were
also carried out. Nonenzymatic disappearance of β-hydroxy-
pyruvate was determined to be 0.03 mM/min. The apparent rate
was subtracted from the control rate and divided by the number
of units of TK used to give the specific rate for each substrate
(V_{\text{endo}}). Relative rate (V_{\text{endo}}) was defined as
V_{\text{endo}} = V_{\text{endo}} / V_{\text{endo}}^\star$
where $V_{\text{endo}}^\star$ is the maximum velocity of the reaction.

**General Procedure for Acetal Hydrolysis.** A solution (or emulsion)
of acetal (0.1–0.3 M) in water was treated with ion-
exchange resin (AG-50W-X8, H\(^+\) form, 1–3 g). The suspension
was stirred for 36 h after which time it was filtered to remove the
resin beads. The pH of the solution was then adjusted to ca. 7
by addition of 0.1 NaOH solution. The product α-hydroxy-
aldydes were used as substrates for TK without further pu-
rification.

**General Procedure for the TK-Catalyzed Condensation of HPA and α-Hydroxy Aldehydes.** In a three-necked
round-bottomed flask (100–300 mL) equipped with a pH electrode
and magnetic stirrer was placed 20–50 mL of water containing
MgCl\(_2\) (3 mM), TPP (0.1 mM), lithium β-hydroxy-
pyruvate (10 mM), and α-hydroxy aldehyde (50–130 mM). The pH was
adjusted to ca. 7.5 with a few drops of 0.1 NaOH solution. TK
(10–30 U) was then added. The pH of the reaction mixture was
maintained between 7.0 and 7.5 by addition of a solution of 0.1 M HCl (40–150 mM HCl), which was obtained by
ion-exchanging lithium β-hydroxy-
pyruvate solution with cation-exchange resin (AG-50W-HS, H\(^+\) form). The mixture was stirred
slowly with a magnetic stirrer and kept under N\(_2\) gas
throughout the period of the reaction. Reaction progress was
followed by TLC using 20% methanol in dichloromethane. When
the reaction was judged to be complete (by TLC and consump-
tion of acid), the reaction mixture was concentrated in vacuo and
the product purified by flash chromatography (silica gel, 20% meth-
anol in dichloromethane). For the kinetic resolution of
α-hydroxy aldehydes, the reaction was allowed to continue for a
day after the amount of hydroxypyruvate corresponding to
half an equivalent of racemic aldehyde initially introduced was
consumed.

5-Deoxy-δ-three-pentulose (43) (44%): \(^*\)HNMR (CD\(_2\)OD, 300 MHz) \(\delta\) 4.52 (d, \(J = 19.3\) Hz, 1 H), 4.44 (d, \(J = 19.3\) Hz, 1 H), 4.09 (dd, \(J = 2.7, 6.3\) Hz, 1 H), 4.04 (d, \(J = 2.7, 6.3\) Hz, 1 H), 1.22 (d, \(J = 2.7\) Hz, 3 H).

5,6-Dideoxy-δ-three-hexulose (44) (45%): \(^*\)HNMR (CD\(_2\)OD, 300 MHz) \(\delta\) 4.52 (d, \(J = 19.2\) Hz, 1 H), 4.49 (d, \(J = 19.2\) Hz, 1 H), 4.14 (d, \(J = 2.3\) Hz, 1 H), 3.78 (dt, \(J = 2.3, 6.5\) Hz, 1 H), 1.6 (m, 2 H), 0.96 (t, \(J = 7.4\) Hz, 3 H).

5,6,7-Tri-deoxy-δ-three-heptulose (45) (39%): \(^*\)HNMR (CD\(_2\)OD, 300 MHz) \(\delta\) 4.53 (d, \(J = 19.2\) Hz, 1 H), 4.49 (d, \(J = 19.2\) Hz, 1 H), 4.11 (d, \(J = 2.3\) Hz, 1 H), 3.89 (dt, \(J = 2.4, 6.6\) Hz, 1 H), 1.6–1.9 (m, 4 H), 0.96 (t, \(J = 7.3\) Hz, 3 H).

5-Methyl-γ-three-pentulose (40%): \(^*\)HNMR (D\(_2\)O, 300 MHz) \(\delta\) 5.48 (ABq, \(J = 19.4\) Hz, \(\Delta \delta = 54.6\) Hz, 2 H), 4.41 (d, \(J = 2.2\) Hz, 1 H), 4.21 (dd, \(J = 2.4, 5.1, 7.0\) Hz, 1 H), 3.61 (dt, \(J = 1.1, 4.3, 6.9\) Hz, 1 H), 3.03 (s, 3 H), 3.40 (s, 3 H). \(^*\) 13C NMR (D\(_2\)O, dioxane as internal reference; 67.6 ppm) δ 213.6, 76.5, 73.4, 71.0, 67.1, 59.5.

2-Hydroxybut-3-en-1-al (29). A solution of the dimethyl
acetel of 29 (3.10 g, 19.3 mol) in acetone (130 mL) and H\(_2\)O (4
mL) was stirred with 10 g of AG-50W-HS (a cation-exchange resin)
at room temperature for 2 days. After solids were filtered off, H\(_2\)O (20 mL) was added and acetone was evaporated under
reduced pressure. The content of aldehyde in the residue solution
was determined by enzymatic analysis (12 mmol, 62%). This
material was used without further purification in the following
procedure.

5,6,7-Tri-deoxy-δ-three-hexulose (46) (47). 2-Hydroxy-
but-3-en-1-al (29) is unstable at basic pH; therefore, a slightly
acidic pH (6.5–6.5) was used.

13C NMR (D\(_2\)O, δ 5.93 (d, \(J = 17.2, 7.7, 1\) Hz), 5.42–5.33
(2 H), 4.24 (t, \(J = 6.2\) Hz, 1 H), 3.81–3.64 (m, 5 H), 3.85 (d, \(J = 2.1\) Hz, 1 H), 2.29–2.23 (m, 1 H). \(^*\) 13C NMR (CD\(_3\)OD, δ 7.38, 75.4, 77.4, 74.0, 74.0, 66.3, 40.42; HRMS (FAB) mass calculated for C\(_3\)H\(_7\)O\(_4\)Na (M+H\(^+\)) = 163.0970, found 163.0975; \([\text{M} + \text{H}]^+ = 6.0 \text{ (e.c. = 2.6 in H}_2\text{O})].\)

6,7-Dideoxy-γ-glucol-6-enitol (66) (68%): \(^*\)HNMR (300 MHz, D\(_2\)O) δ 6.00 (ddd, \(J = 17.4, 10.4, 7.0\) Hz, 1 H), 5.42–5.33
(2 H), 4.24 (t, \(J = 6.2\) Hz, 1 H), 3.81–3.64 (m, 5 H), 3.85 (d, \(J = 2.1\) Hz, 1 H), 2.29–2.23 (m, 1 H). \(^*\)HNMR (100 MHz, CDCl\(_3\)) δ 137.64, 120.71, 75.77, 74.93, 74.06, 66.38, 40.20; HRMS (FAB) mass calculated for C\(_3\)H\(_7\)O\(_4\)Na (M+H\(^+\)) = 163.0970, found 163.0975; \([\text{M} + \text{H}]^+ = 6.0 \text{ (e. c. = 2.6 in H}_2\text{O).}]

mass calc'd for C$_7$H$_5$NO$_3$Na (M + Na$^+$) 411.1267, found 411.1247.

5,6-Dideoxy-β-D-xylene-5-enitol (71) (61%): $^1$H NMR (CD$_3$OD, 500 MHz) $\delta$ 5.93 (dd, $J = 6.5, 10.5, 17.1$ Hz, 1 H), 5.34 (dd, $J = 1.2, 1.8, 17.1$ Hz, 1 H), 5.19 (dd, $J = 1.3, 1.8, 10.5$ Hz, 1 H), 4.20 (dddd, $J = 1.2, 1.8, 5.9, 6.5$ Hz, 1 H), 3.69 (ddd, $J = 2.8, 5.4, 6.3$ Hz, 1 H), 3.62 (dABq, $J_{ab} = 5.4$ Hz, $J_{ba} = 6.5$ Hz, $J_{bb} = 11.0$ Hz, $\Delta_{ab} = 17.7$ Hz, 2 H), 3.47 (dd, $J = 2.9, 5.9, 1$ Hz, 1 H); $^{13}$C NMR (CD$_3$OD, 125.8 MHz) $\delta$ 140.5, 118.1, 76.2, 76.1, 74.1, 65.7.

For further confirmation of the structure, the O-methylated compound was synthesized by using NaN$_3$/MeI in DMP: $^1$H NMR (aceton-d$_6$, 500 MHz) $\delta$ 5.77 (dd, $J = 7.7, 10.5, 18.2$ Hz, 1 H), 5.3-5.25 (m, 2 H), 3.76 (m, 1 H), 3.49 (dABq, $J_{ab} = 5.1$ Hz, $J_{bb} = 5.5$ Hz, $J_{ba} = 9.9$ Hz, $\Delta_{ab} = 27.1$ Hz, 2 H), 3.42 (s, 3 H), 3.39 (m, 1 H), 3.33 (s, 3 H), 3.30 (s, 3 H), 3.23 (s, 3 H), 3.19 (dd, $J = 4.1, 6.1$ Hz, 1 H); $^{13}$C NMR (CD$_3$OD, 125.8 MHz) $\delta$ 135.5, 118.5, 83.4, 83.2, 80.4, 71.8, 61.0, 59.1, 55.7, 56.6: HRMS (FAB) for C$_7$H$_5$O$_2$Na (M$^+$ + H) calc'd 205.1440, found 205.1440.

General Experimental Procedure for the Ozonolysis of Alkenopolyols. Into a solution of alken (0.2-0.5 mM) in MeOH at -78°C was bubbled a stream of O$_3$ until a light blue color persisted, indicating the presence of excess O$_3$. After flushing out excess O$_3$ with N$_2$, Na$_2$SO$_4$ (ca. 2-4 g) was added. The mixture was vigorously stirred for 1 h at -78°C and 15 h at room temperature. After filtration and concentration, column chromatography over silica gel (20-50% MeOH in CH$_2$Cl$_2$) gave the desired aldoxide.

2-Deoxy-β-D-xylenehexose (67) (84%). The product showed the same $^1$H and $^{13}$C NMR spectra as a sample of 2-deoxy-β-D-xylenehexose prepared according to ref 33: $[\alpha]_{D}^{20} = -61$ (c 1.5, H$_2$O) (authentic sample: $[\alpha]_{D}^{20} = -5.6$ (c 0.8, H$_2$O)).$^{37}$

1-Gulose (69) (78%). The product showed the same $^1$H and $^{13}$C NMR spectra as authentic 1-gulose: $[\alpha]_{D}^{20} = -17$ (c 2.5, H$_2$O) (authentic sample: $[\alpha]_{D}^{20} = -17.4$ (c 2.3, H$_2$O)).$^{37}$

1-Idose (70) (68%). The product showed the same $^1$H and $^{13}$C NMR spectra as authentic 1-idose: $[\alpha]_{D}^{20} = -14$ (c 1.2, H$_2$O) (authentic sample: $[\alpha]_{D}^{20} = -17.4$ (c 2.3, H$_2$O)).$^{37}$

1-Xylose (72) (67%). The product showed the same $^1$H and $^{13}$C NMR spectra as authentic 1-xylose: $[\alpha]_{D}^{20} = -17.4$ (c 0.32, H$_2$O) (authentic sample: $[\alpha]_{D}^{20} = -18.8$ (c 0.30, H$_2$O)).

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Supplementary Material Available: Spectrometric information ($^1$H and $^{13}$C NMR) for new compounds (68 pages). This material is contained in many 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.