and placed in a freezer overnight. Filtration afforded 0.27 g (56%) of keto lactam **47**. The filtrate was evaporated under reduced pressure, and the residue was recrystallized from acetone to afford an additional 0.11 g (24%) of keto lactam **47** (80% total): mp 217–218 °C dec; IR (KBr) 1690, 1660, 1550, 1370, 1335, 1310, 1240, 750, 710 $cm^{-1}$; $^{13}C$ NMR (acetone-$d_6$) δ 175.4, 159.0, 137.0, 134.3, 133.9, 133.5, 133.4, 131.0, 129.7, 129.2, 128.5, 126.8, 125.2, 123.6, 117.1, 116.3; MS *m/e* 247 ($M^+$, 100), 219, 190, 164, 149, 115, 95, 76; UV (95% EtOH) $\lambda_{max}$ 225 (sh), 240, 265, 360 nm. Anal. Calcd for $C_{16}H_9NO_2$: C, 77.72; H, 3.67; N, 5.67. Found: C, 77.69; H, 3.70; N, 5.65.

**6,11-Dimethyl-5*H*-benzo[*b*]carbazole (48).** To a solution of keto lactam **47** (0.289 g, 1.17 mmol) in dry THF (50 mL) at −78 °C was added slowly MeLi (3.38 mL of 0.76 M in ether, 2.57 mmol). The resulting dark green solution was stirred for 2 h at −78 °C and allowed to warm to room temperature over an additional 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in 95% EtOH (40 mL). Sodium borohydride (2 pellets) was added, and the mixture was refluxed for 24 h. The solvent was removed under reduced pressure, and the residue was taken up in $H_2O$ (15 mL), acidified with glacial HOAc, and then neutralized with 10% aqueous NaOH. Extraction with $CHCl_3$ (4 × 100 mL), drying ($Na_2SO_4$), and concentration in vacuo gave crude **48**. Recrystallization from $CCl_4$ gave 0.250 g (87%) of **48**: mp 211–213 °C (lit.[7a] mp 211–212 °C), which was identical (IR, UV, TLC) to a sample previously prepared in this laboratory:[28] IR (KBr) 3415, 1630, 1610, 1475, 1460, 1390, 1365, 1320, 1300, 1240, 745, 710 $cm^{-1}$; MS *m/e* 245 ($M^+$, 100), 230, 215, 202, 149, 115; UV (95% EtOH) $\lambda_{max}$ 234, 248 (sh), 271, 282 (sh), 297 nm.

(28) Gribble, G. W.; Saulnier, M. G.; Sibi, M. P.; Obaza-Nutaitis, J. A. *J. Org. Chem.* **1984**, *49*, 4518.

**Acknowledgment.** This investigation was supported by Grants CH-200 and CH-200A from the American Cancer Society, PHS Grant GM-30761 awarded by the National Institutes of Health, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and Merck Sharp and Dohme Research Laboratories. We thank the National Science Foundation for funds to purchase a Varian XL-300 NMR spectrometer, Dr. Catherine E. Costello (Massachusetts Institute of Technology) for the high-resolution mass spectra (NIH Resource Grant FR00317 from the Division of Research Facilities and Resources), and the Colorado State University Regional NMR Center, funded by NSF Grant No. CHE-8208821, for some high-resolution NMR spectra. G.W.G. is very grateful to American Cyanamid for an Academic Achievement Award and is especially indebted to Professor Marc Tius and his colleagues and students at the University of Hawaii at Manoa for their generous hospitality during a sabbatical year 1991–1992 when the manuscript was written.

# Substrate Specificity and Carbohydrate Synthesis Using Transketolase

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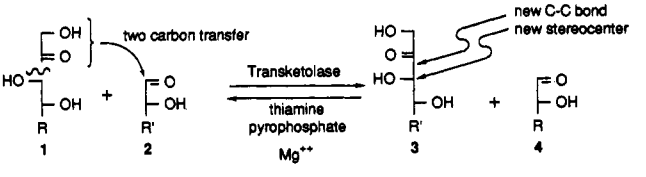
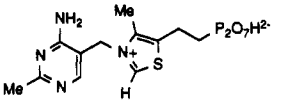
This paper describes the use of the enzyme transketolase as a catalyst in organic synthesis. The properties of transketolase from both yeast and spinach were investigated. The yeast enzyme was found to be more convenient for routine use. Examination of the substrate specificity of yeast transketolase 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

**Scheme I. Transketolase-Catalyzed Interconversion of Carbohydrates**



Thiamine pyrophosphate (TPP) **5**

of the anion of TPP (**7**) to HPA results in the formation of intermediate **8**. This adduct loses $CO_2$ 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**,

(1) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985; p 76.
(2) Datta, A.; Racker, E. *J. Biol. Chem.* **1961**, *236*, 624.
(3) Srere, P.; Cooper, J. R.; Tabachnick, M.; Racker, E. *Arch. Biochim. Biophys.* **1958**, *74*, 295. Compare: de la Haba, G.; Lafer, I. G.; Racher, E. *J. Biol. Chem.* **1955**, *214*, 409.
(4) For a review of thiamine chemistry see: Kluger, R. *Chem. Rev.* **1987**, *87*, 863.

0022-3263/92/1957-5899$03.00/0 

**Scheme II. Catalytic Cycle of Transketolase**

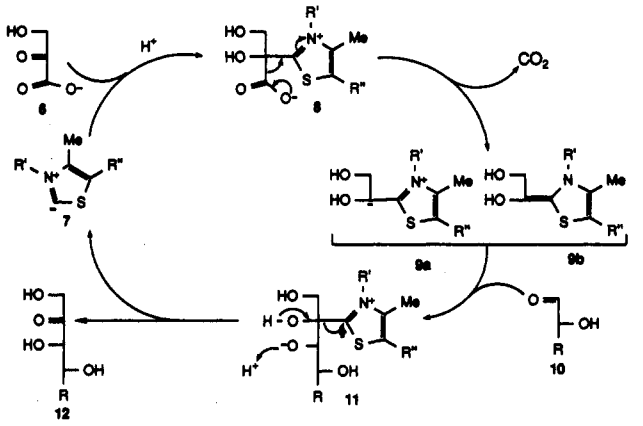


**Table I. Carbohydrates as Substrates for TK**

| substrate | $V_{substrate}/V_{glycolaldehyde}$ |
|---|---|
| D-glyceraldehyde 3-phosphate | 44% |
| D-erythrose 4-phosphate | 33 |
| D-arabinose 5-phosphate | 24 (ref 5) |
| D-glucose 6-phosphate | 9 |
| D-glyceraldehyde | 78 |
| L-glyceraldehyde | <0.01 |
| DL-glyceraldehyde | 56 |
| D-erythrose | 56 |
| D-glucose | 4 |

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 $CO_2$ (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.[5] 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 glycoaldeyde.[5,6] The new stereocenter formed in the TK-catalyzed addition of ketol to an aldehyde is set in a threo 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-threo 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.

**Table II. Properties of Transketolase**

| property | yeast (ref) | spinach leaves (ref) |
|---|---|---|
| no. of subunits | 2 | 4 (12) |
| MW of active form (kD) | 158–159 (13), 140 (14) | 150 kD (12), 110 ± 10 kD (9) |
| optimum pH | 7.6 (5) | |
| $K_m$ (HPA) (mM) | 7 (15), 33 (16) | |
| $K_m$ (TPP) (mM) | 0.032 (13) | <100 (17) |
| $K_m$ ($Mg^{2+}$) (mM) | ca. 0.4 (18) | |
| specific activity (U/mg) | 15–25 (19) | 7.8 (8), 50 (9) |
| cost ($/U) | 1.74 (20) | |

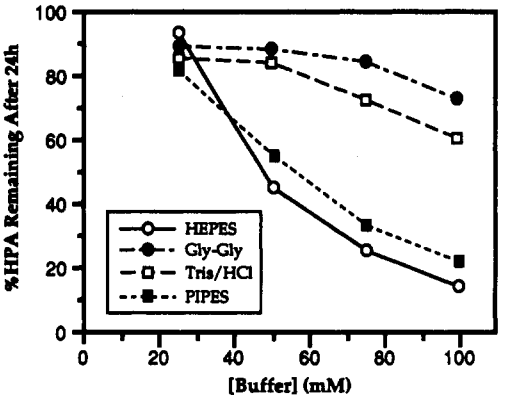



**Figure 1.** Percent HPA remaining in buffer solutions after 24 h. The initial concentration of HPA was 10 mM. See Experimental Section for assay conditions.

## 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-

(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.

(6) Bolte, J.; Demuynck, C.; Samaki, H. *Tetrahedron Lett.* **1987**, *28*, 5523.

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

(8) Villafranca, J. J.; Axelrod, B. *J. Biol. Chem.* **1971**, *246*, 3126.

(9) Demuynck, C.; Fisson, F.; Bennani-Baiti, I.; Samaki, H.; Mani, J. C. *Agric. Biol. Chem.* **1990**, *54*, 3073.

(10) Demuynck, C.; Bolte, J.; Hecquet, L.; Samaki, H. *Carbohydr. Res.* **1990**, *206*, 79.

(11) Schneider, G.; Sundstrom, M.; Lidqvist, Y. *J. Biol. Chem.* **1989**, *264*, 21619.

(12) Murphey, D. J.; Walker, D. A. *Planta* **1982**, *155*, 310.

(13) Kochetov, G. A. *Methods Enzymol.* **1982**, *90*, 209.

(14) Racker, E. *The Enzymes*, 2nd ed.; 1961; Vol. 5, pp 397–406.

(15) Bolte, J.; Demuynck, C.; Samaki, H. *Tetrahedron Lett.* **1987**, *28*, 5525.

(16) Wood, T. *Prep. Biochem.* **1973**, *3*, 509.

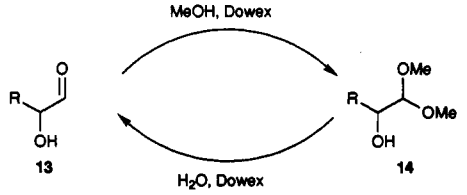
(17) Horecker, B. L.; Smyrniotis, P. Z. *J. Am. Chem. Soc.* **1953**, *75*, 1009.

(18) Heinrick, P. C.; Steffan, H.; Janser, P.; Wiss, O. *Eur. J. Biochem.* **1972**, *30*, 533.

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

(20) From Sigma Chemical Co.

**Scheme III. Interconversion of Aldehydes and Acetals**




bohydrate synthesis.[21,22] 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.[23]

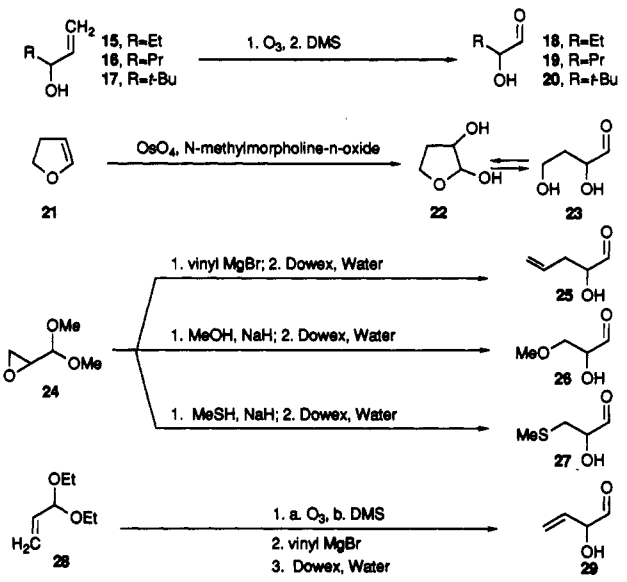
The assessment of TK as an enzyme for organic synthesis proceeded on two levels. First, we developed a practical protocol for the use of TK in preparative-scale synthesis. Second, we applied the enzyme to the synthesis of interesting target systems.

**Using TK as a Catalyst in Organic Synthesis.**[24] 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.

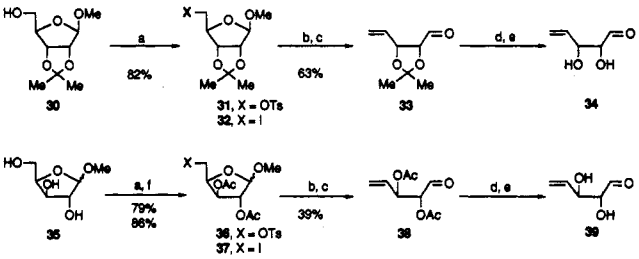
The traditional approach to pH control of the TK-mediated condensation of HPA and aldehydes has been the use of a buffered reaction medium.[15,24,25] Upon examining the stability of 10 mM HPA in several concentrations of four buffer systems, we found that, in all cases, significant decomposition of HPA 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 hydroxypyruvic 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).

**Scheme IV. Synthesis of Racemic α-Hydroxy Aldehydes**




**Scheme V. Synthesis of α-Hydroxy Aldehydes**[a]




[a] Key: (a) TsCl, pyridine; (b) NaI, 3-pentanone; (c) Zn, ether; (d) Dowex, methanol; (e) Dowex, water; (f) $Ac_2O$.

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 tetraoxide 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 glycidaldehyde dimethyl acetal (**24**). Epoxy acetal **24** was obtained by benzonitrile-mediated peroxide oxidation of acrolein dimethyl acetal.[26] Addition of vinylmagnesium 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-diethoxyacetaldehyde[27] 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

(21) Bednarski, M. D.; Simon, E. S.; Bischofberger, N.; Fessner, W. D.; Kim, M. J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 627.

(22) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365.

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

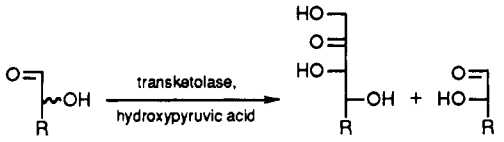
(24) A recent paper described the results of a kinetic study of substrates for TK and the synthesis of 4-deoxy-L-erythulose using TK. See Demuynck, C.; Bolte, J.; Heequet, L.; Dalmas, V. *Tetrahedron Lett.* **1991**, *32*, 5085.

(25) Mocali, A.; Alducci, D.; Paoletti, F. *Carbohydr. Res.* **1985**, *143*, 288.

(26) Durrwachter, J. R.; Druckhammer, D. G.; Nozaki, K.; Sweers, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812.

(27) 2,2-Diethoxyacetaldehyde was prepared by ozonolysis. See ref 33.

**Table III. Substrates for Transketolase**




| R | aldehyde | $V_R/V_{R=H}$ | adduct compd no. | adduct yield[a] (%) | recovered aldehyde yield[a] (%) | recovered aldehyde ee[b] (%) |
|---|---|---|---|---|---|---|
| H | **41** | 100 | **42** | 60 | n/a | n/a |
| $CH_3$ | **40** | 20 | **43** | 44 | 21 | >95 |
| $CH_3CH_2$ | **18** | 33 | **44** | 45 | 23 | 95 |
| $CH_3(CH_2)_2$ | **19** | 22 | **45** | 39 | 21 | 77 |
| $MeOCH_2$ | **26** | 27 | **46** | 38 | 9 | 94 |
| $MeSCH_2$ | **27** | 33 | | | | |
| $H_2C{=}CH$ | **29** | 56 | **47** | 30 | | |
| $H_2C{=}CHCH_2$ | **25** | 28 | **48** | 45 | 20 | 95 |
| (*S*)-$H_2C{=}CHCHOH$[c] | **34** | 36 | **49** | 60 | n/a | n/a |
| (*R*)-$H_2C{=}CHCHOH$[d] | **39** | 32 | **50** | 63 | n/a | n/a |
| $(CH_3)_3C$ | **20** | 11 | | | | |
| $HO(CH_2)_2$ | **23** | <10 | **51** | 7 | | |

[a] Isolated yield based on total quantity of starting aldehyde. [b] Enantiomeric excess determined on the correspondingdiol obtained by reduction of the aldehyde with sodium borohydride. [c] Optically active aldehyde derived from D-xylose. [d] Optically active aldehyde derived from D-ribose.

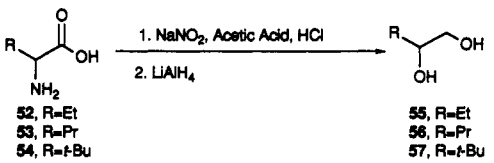
compound was the major product (see Experimental Section).

Dihydroxy aldehydes **34** and **39** (Scheme V) were prepared starting from D-xylose 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.[28] Methanolysis under acidic conditions of the crude products led sequentially to acetal formation and deprotection of the secondary hydroxy 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.[29] 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 $\beta$ 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 $\alpha$-Hydroxy Aldehydes.** We resolved several racemic $\alpha$-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 ($Eu(tfc)_3$).[30] The diols were purified by silica gel chromatography with 20% methyl alcohol 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.[31] 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.[26]




**52**, R=Et
**53**, R=Pr
**54**, R=*t*-Bu

**55**, R=Et
**56**, R=Pr
**57**, R=*t*-Bu

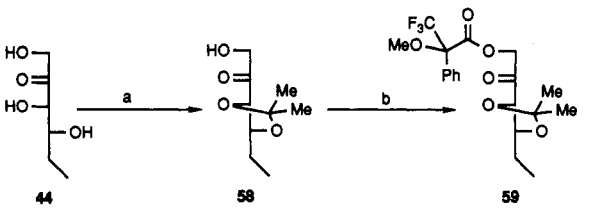
(1)

We were able to obtain the TK-resolved $C_2$-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

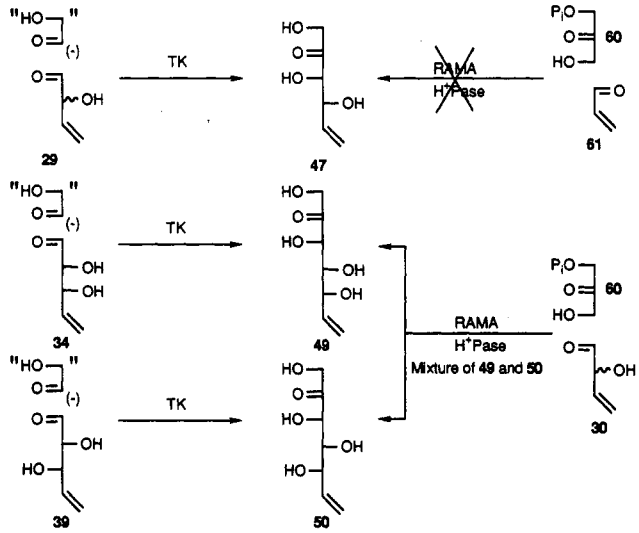
(28) Bernet, B. Vasella, A. *Helv. Chim. Acta* **1979**, *62*, 1990.
(29) Recently it has been shown that the spinach enzyme will accept some nonhydroxylated aldehydes. See ref 24.
(30) Sweeting, L. M.; Crans, D. C.; Whitesides, G. M. *J. Org. Chem.* **1983**, *52*, 2273.
(31) Winitz, M.; Bloch-Frankenthal, L.; Izumiya, N.; Birnbaum, S. M.; Baker, C. G.; Greenspan, J. P. *J. Am. Chem. Soc.*, **1956**, *78*, 2423.

**Scheme VI. Determination of Enantiomeric Excess[a]**



[a] Key: (a) $ZnI_2$, acetone; (b) *R* or *S* Mosher's acid chloride, triethylamine.

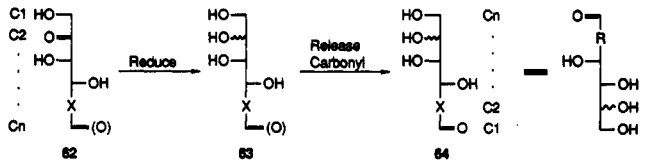
**Scheme VII. Comparison of TK and RAMA**



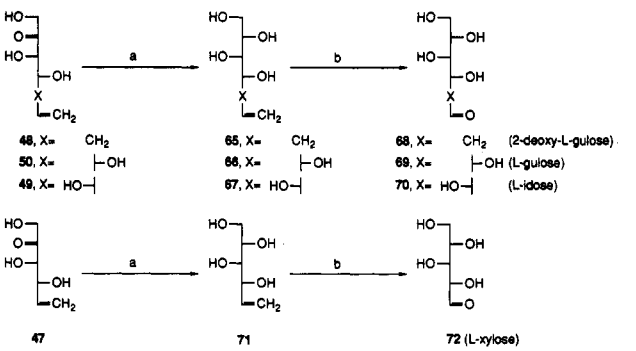
determined following their conversion to known carbohydrates (see below). Optical rotations obtained for our synthetic carbohydrates confirmed that we had correctly assigned the absolute configuration of the carbohydrates and suggested that the enantiomeric excess (ee) of these materials (and therefore the enzyme-catalyzed processes from which they came) was high. We determined the enantiomeric excesses of adduct **44** via acetonide **58** (Scheme VI). This material was converted to both its *R* and *S* Mosher's esters.[32,33] Proton NMR analysis of the esters showed that the ee was greater than 95%. In the cases where the products were not converted into known compounds, the stereochemical relationship was inferred based on the precedents established by the successful correlations. As before, for compounds that were not compared to authentic samples, the enantiomeric excesses were presumed to be high. No other products were isolated.

**TK-Catalyzed Synthesis of Carbohydrates.** Transketolase is a useful enzyme for the preparation of carbohydrates. The ketose products **42–51** (Table III) were obtained in good yields: 60–90% based on full utilization of the C2-R enantiomers. This range compares well with the range observed for RAMA-catalyzed aldols affording similar products. TK has several advantages over RAMA. The greatest of these is the ability of TK to resolve racemic aldehyde substrates. This ability makes it possible to obtain enantiomerically and diastereomerically homogeneous products from racemic starting materials. In addition, the products obtained directly from the enzyme-catalyzed experiment are not phosphorylated, greatly

**Scheme VIII. Inversion Strategy**



**Scheme IX. Aldose Synthesis[a]**



[a] Key: (a) IDH (EC 1.1.1.14), FDH (EC 1.1.1.14), NADH, $NaHCO_2$; (b) 1. $O_3$, 2. $Na_2SO_3$.

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 ketose **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 enantiospecific syntheses 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 ketoses 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 1.1.1.27) 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-xylose (**71**), respectively. The structures of L-gulose, L-idose, and L-xylose were firmly established

(32) Dale, J. A.; Duli, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(33) Myles, D. C.; Andrulis, P. J., III; Whitesides, G. M. *Tetrahedron Lett.* **1991**, *32*, 4835.
(34) Borysenko, C. W.; Spaltenstein, A.; Straub, J. A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 9275.
(35) Chakrovorty, M.; Viega, L. A.; Bacila, M.; Horecker, B. *J. Biol. Chem.* **1962**, *237*, 1014.

by comparison to authentic samples. The structure of 2-deoxy-L-gulose was confirmed by prepration 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 $\beta$-hydroxypyruvate 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 without further purification. 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 biochemicals were used as provided by the manufacturer. Proton and $^{13}C$ magnetic resonance experiments were conducted in $CDCl_3$ or $D_2O$. Spectra were referenced to $CHCl_3$ at $\delta$ 7.24 ppm, $CDCl_3$ at 77.5 ppm $\delta$, HOD at $\delta$ 4.80 ppm, or the carbon resonance of dioxane at $\delta$ 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. Trimethylacetaldehyde (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 $NH_4Cl$ 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 $MgSO_4$, successively. Filtration and concentration afforded an oily residue that was chromatographed (silica gel, 10% $CH_2Cl_2$ in pentane) to give 3.14 g of the product (31.3 mmol, 63%) as a colorless liquid: $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$ 5.85 (ddd, $J$ = 6.5, 10.5, 17.1 Hz, 1 H), 5.20 (ddd, $J$ = 1.2, 1.4, 17.1 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 Ozonolysis of 3-Hydroxyalkenes.** A solution of 3-hydroxyalkene (0.1–0.3 M) in $CH_2Cl_2$ was cooled to −78 °C in a dry ice/acetone bath. A stream of $O_3$ gas was bubbled through the solution until a light blue color persisted. The solution was then sparged with $N_2$ 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 acidic ion-exchange resin (Dowex AG-50W-H8, $H^+$ form, 1–3 g) and stirred at room temperature for 12 h. The resin beads were 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 acetal.

**1,1-Dimethoxy-2-hydroxybutane (dimethyl acetal of 18):** $^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 3.89 (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); $^{13}C$ NMR (62.5 MHz, $CDCl_3$) $\delta$ 107.15, 72.50, 54.97, 25.00, 10.01.

**1,1-Dimethoxy-2-hydroxypentane (dimethyl acetal of 19):** $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 4.09 (d, $J$ = 6.1 Hz, 1 H), 3.60–3.53 (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), 0.93 (t, $J$ = 6.2 Hz, 3 H); $^{13}C$ NMR (100.6 MHz, $CDCl_3$) $\delta$ 107.44, 71.34, 55.49, 34.31, 19.12, 14.53.

**1-Methoxy-2-hydroxytetrahydrofuran (Methyl Acetal of 22).** A solution of dihydrofuran (10.0 g, 143 mmol) and catalytic osmium tetraoxide in acetone/water (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 acidic ion-exchange resin (Dowex AG-50W-H8, $H^+$ 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, $CDCl_3$) $\delta$ 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), 3.27 (s, 3 H), 2.71 (br s, 1 H), 2.21–2.18 (m, 1 H), 1.81–1.73 (m, 1 H); $^{13}C$ NMR (100.6 MHz, $CDCl_3$) $\delta$ 109.41, 75.72, 66.73, 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 300 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 $MgSO_4$, 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 ($CDCl_3$, 300 MHz) $\delta$ 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); $^{13}C$ NMR (125.8 MHz, $CDCl_3$) $\delta$ 135.64, 116.71, 104.65, 72.78, 63.81, 63.55, 15.32, 15.29.

**3,3-Dimethoxy-1-(methylthio)propan-2-ol (Dimethyl Acetal of 27).** A solution of 2,3-epoxypropionaldehyde dimethyl acetal (1.01 g, 8.5 mmol) and sodium thiomethylate (0.90 g, 12.8 mmol) in MeOH (100 mL) was stirred at room temperature for 2 h. The solvent was evaporated, and the residue was chromatographed over silica gel (25% AcOH in hexane) to give 0.883 g of the acetal of 27 (5.3 mmol, 62%): $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$ 4.29 (d, $J$ = 5.7 Hz, 1 H), 3.76 (m, 1 H), 3.45 (s, 3 H), 3.43 (s, 3 H), 2.76, 2.57 (dABq, $J_{ax}$ = 3.7 Hz, $J_{bx}$ = 8.2 Hz, $J_{ab}$ = 13.8 Hz, $\Delta\nu$ = 47.5 Hz, 2 H), 2.52 (d, 3.1 Hz, 1 H), 2.13 (s, 3 H); $^{13}C$ NMR ($CDCl_3$, 125.8 MHz) $\delta$ 105.6, 69.9, 55.4, 54.9, 36.5, 16.0; FTIR (thin film) 3457, 2919, 2834, 1444, 1191, 1129, 1074, 974.

**4,4-Diethoxybut-1-en-3-ol (Diethyl Acetal of 29).** A solution of acrolein diethyl acetal (6.04 g, 46.4 mmol) in MeOH (300 mL) was treated with $O_3$ 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 $N_2$ flow until it became colorless. 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 sulfoxide and 2 (6.3 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 3 days at room temperature. The reaction mixture was treated with $NH_4Cl$ (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 $MgSO_4$, filtered, and concentrated in vacuo. Chromatography of the resulting residue over silica gel (25–67% AcOEt in hexanes) yielded 3.59 g of 3 (22.4 mmol, 48%): $^1H$ NMR ($CDCl_3$, 250 MHz)

δ 5.91 (ddd, $J$ = 6.5, 10.6, 17.3 Hz, 1 H), 5.39 (ddd, $J$ = 1.6, 1.6, 17.3 Hz, 1 H), 5.23 (ddd, $J$ = 1.6, 1.6, 10.6 Hz, 1 H), 4.27 (d, $J$ = 6.0 Hz, 1 H), 4.07 (m, 1 H), 3.7 (m, 2 H), 3.60 (m, 2 H), 2.28 (d, $J$ = 3.8 Hz, 1 H), 1.2 (m, 6 H); $^{13}$C NMR ($CDCl_3$, 125.8 MHz) δ 135.6, 116.7, 104.7, 72.8, 63.8, 63.6, 15.31, 15.28; FTIR 3431, 2978, 2931, 2899, 1741, 1374, 1243, 1120, 1065, 1000, 925 cm$^{-1}$.

**β-Methyl 5-Iodo-2,3-isopropylidineriboside (32).** A solution of tosylate **31** (5.10 g, 14.2 mmol, prepared according to ref 35) in acetone (60 mL) was treated with NaI (8.0 g, 53.5 mmol). The slurry was heated at reflux for 4 h. The mixture was then cooled to room temperature and diluted with a equal volume of ether and filtered through silica gel with an additional volume of ether. The solution was then concentrated in vacuo to afford a yellow oil. Silica gel chromatography (15% ethyl acetate in hexanes) afforded iodoriboside **32** as a colorless oil (4.58 g, 97%): $^1$H NMR (250 MHz, $CDCl_3$) δ 5.04 (s, 1 H), 4.75 (d, $J$ = 5.9 Hz, 1 H), 4.62 (d, $J$ = 5.9 Hz, 1 H), 4.43 (dd, $J$ = 10.1, 6.1 Hz, 1 H), 3.36 (s, 3 H), 3.31–3.10 (m, 2 H), 1.47 (s, 3 H), 1.31 (s, 3 H); $^{13}$C NMR (62.5 MHz, $CDCl_3$) δ 113.09, 110.13, 87.91, 85.83, 83.53, 55.75, 26.99, 25.57, 1.21; IR (cm$^{-1}$) 2995, 2945, 2805, 1365, 1105, 1020, 875.

**1,1-Dimethoxy-2(*R*),3(*R*)-dihydroxy-4-pentene (Dimethyl Acetal of 34).** Zinc powder (15 g) was washed sequentially with 6 N HCl (50 mL), water (3 × 50 mL), and ethanol (3 × 50 mL). The resulting paste was suspended in 2-propanol (100 mL) containing iodoriboside **32**. The mixture was stirred at reflux for 1 h. After being cooled to room temperature, the slurry was diluted with ether (200 mL), washed through a plug of silica gel, and concentrated in vacuo to afford a viscous yellow oil. This oil was taken up in methanol (100 mL) and treated with ion-exchange resin (Dowex AG-50W-H8, $H^+$ 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 vacuo 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%): $^1$H NMR (400 MHz, $CDCl_3$) δ 5.94 (ddd, $J$ = 17.1, 10.4, 6.4 Hz, 1 H), 5.35–5.20 (m, 2 H), 4.30 (d, $J$ = 5.9 Hz, 1 H), 4.22–4.18 (m, 1 H), 3.64 (dd, $J$ = 5.9, 5.0 Hz, 1 H), 3.42 (s 3 H), 3.40 (s 3 H), 2.65 (br s, 2 H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 136.84, 117.58, 105.11, 73.61, 73.27, 55.73, 55.22; IR (cm$^{-1}$) 3200, 2925, 2845, 1640, 1455, 1075; HRMS (CI, isobutane) for dimethyl ether (MeI, NaH, THF) of the dimethyl acetal of **34**, mass calcd for $C_9H_{19}O_4$ ($M^+$ + H), 191.1283; found 191.1272; $[\alpha]^{20}_D$ = +37° at $c$ = 1.2.

**Tosyl Xyloside 36.** A solution of α- and β-methyl xylofuranosides **35** (21.9 g, 133 mmol, from xylose, MeOH, HCl) in pyridine (200 mL) was treated with $p$-toluenesulfonyl chloride (27.9 g, 146 mmol). The mixture was stirred at room temperature for 24 h. Pyridine was then removed in vacuo to afford a yellow gum. The material was taken up in ethyl acetate (200 mL) and washed with saturated $CuSO_4$ (100 mL). The aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue could be used directly in the continuation of this procedure or purified by silica gel chromatography (5–15% ethyl acetate in hexanes to afford α- and β-methyl 5-$O$-($p$-toluenesulfonyl)xylosides (33.4 g, 79%). A solution of purified 5-$O$-tosylglycoside (17.0 g, 53.5 mmol) in $CH_2Cl_2$ (200 mL) was treated with acetic anhydride (12.0 g, 117.7 mmol), triethylamine (16.3 g, 160.4 mmol), and (dimethylamino)pyridine (ca. 100 mg, cat.) was stirred at room temperature for 36 h. Over the course of the reaction, a colorless precipitate formed. The precipitate was removed by filtration, and the filtrate was concentrated in vacuo. The residue was taken up in ethyl acetate (100 mL) and washed with saturated $NaHCO_3$ (50 mL). The aqueous phase was extracted with ethyl acetate (1 × 50 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, concentrated in vacuo, and chromatographed (silica gel, 15% ethyl acetate in hexanes) to afford tosyl xyloside **36** as a slightly yellow oil (18.46 g, 86%): $^1$H NMR ($CDCl_3$, 400 MHz) δ (7.78–7.65 (m, 2 H), 7.32–7.24 (m, 2 H), 5.40 (t, $J$ = 6.5 Hz, 0.4 H) 5.23 (dd, $J$ = 6.3, 4.6 Hz, 0.4 H), 4.98–4.92 (m, 1 H), 4.87 (dd, $J$ = 6.3, 4.6 Hz, 0.4 H), 4.75 (s, 0.6 H), 4.50 (q, $J$ = 6.6 Hz, 0.6 H), 4.39–4.34 (m, 0.4 H), 4.18–3.96 (m, 6 H), 3.25 (s, 1.2 H), 3.21 (s, 1.8 H), 2.37 (s, 3 H), 2.02 (s, 1.2 H), 2.01 (s 1.8 H), 1.99 (s, 1.2 H), 1.98 (s, 1.8 H); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 170.80, 170.76, 170.31, 170.23, 145.54, 133.10, 130.37, 128.42, 107.58, 100.33, 81.13, 78.07, 75.15, 74.85, 73.30, 68.41, 67.49, 56.10, 55.89, 22.13, 21.10, 21.06; IR (cm$^{-1}$) 2900, 1740, 1590, 1390, 1185, 1048; $[\alpha]^{20}_D$ = +52.0° at $c$ = 1.02 in $CHCl_3$; HRMS (FAB, sodium) calcd for $C_{17}H_{22}O_9SNa$ ($M^+$ + Na) 425.0883; found 425.0876.

**Iodoxyloside 37.** A solution of tosylxyloside **36** (8.90 g, 22.1 mmol) in 2-propanol (50 mL) was treated with NaI (10 g, 66.7 mmol). The suspension was stirred at reflux for 48 h. After it cooled to room temperature, the mixture was diluted with ether, filtered, and concentrated. The resulting yellow oil was dissolved in ether (100 mL) and washed with a 1:1 mixture of saturated $NaHSO_3$ and $NaHCO_3$ (50 mL). The aqueous phase was extracted with ether (2 × 50 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated. The resulting oil was purified by silica gel chromatography (20–40% ethyl acetate in hexanes) to afford iodoxyloside **37** as a yellow oil (5.63 g, 71%). Prolonged exposure of this material to room light led to decomposition: $^1$H NMR (400 MHz, $CDCl_3$) δ 5.45 (dd, $J$ = 5.9, 4.4 Hz, 0.4 H), 5.30 (dd, $J$ = 5.8, 1.4 Hz, 0.6 H), 5.12 (d, $J$ = 4.6 Hz, 0.4 H), 5.07 (s, 0.6 H), 4.99 (t, $J$ = 4.5 Hz, 0.4 H), 4.87 (s, 0.6 H), 4.63 (q, $J$ = 7.5 Hz, 0.6 H), 4.43 (q, $J$ = 6.3 Hz, 0.4 H), 3.39 (s, 1.8 H), 3.37 (s, 1.2 H), 3.31–3.13 (m, 6 H), 2.12 (s, 1.8 H), 2.11 (s, 1.2 H), 2.10 (s, 1.2 H), 2.08 (s, 1.8 H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.60, 170.50, 170.20, 169.95, 107.64, 100.64, 81.64, 81.39, 78.40, 76.48, 75.77, 75.27, 56.29, 56.20, 21.27, 21.22, 21.21, 21.06, 2.32, 0.23; IR (cm$^{-1}$) 2950, 1740, 1385, 1205; $[\alpha]^{20}_D$ = 10.8° ($c$ = 0.87 in $CHCl_3$).

**1,1-Dimethoxy-2(*R*),3(*S*)-dihydroxy-4-pentene (Dimethyl Acetal of 39).** Zinc powder (4.9 g) was washed sequentially with 6 N HCl (50 mL), water (3 × 50 mL), and ethanol (3 × 50 mL). The resulting paste was suspended in ether (50 mL) containing iodoxyloside **37**. The mixture was stirred at reflux for 40 min. After being cooled to room temperature, the slurry was washed through a plug of silica gel and concentrated in vacuo to afford a viscous yellow oil (crude **38**). This oil was taken up in methanol (50 mL) and treated with ion-exchange resin (Dowex AG-50W-H8, $H^+$ form, 1.0 g). The suspension was stirred at room temperature for 24 h. After removal of the resin by filtration, the solution was concentrated in vacuo 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 (876 mg, 37%): $^1$H NMR (500 MHz, $CDCl_3$) δ 5.90 (ddd, $J$ = 17.2, 10.6, 5.4 Hz, 1 H), 5.33 (ddd, $J$ = 17.2, 1.6, 1.6, 1 H), 5.19 (ddd, $J$ = 10.6, 1.4, 1.4 Hz, 1 H), 4.37 (d, $J$ = 5.8 Hz, 1 H), 4.27–4.24 (m, 1 H), 3.50 (dd, $J$ = 5.7, 2.5 Hz, 1 H), 3.43 (s, 3 H), 3.41 (s, 3 H), 2.63 (br s, 2 H); $^{13}$C NMR (120 MHz, $CDCl_3$) 138.07, 116.49, 105.54, 73.33, 71.76, 56.72, 55.37; IR (cm$^{-1}$) 3438, 2937, 1060; HRMS (CI, $CH_4$) for dimethyl ether calcd for $C_9H_{19}O_4$ ($M^+$ + H) 191.1208, found 191.1277; $[\alpha]^{20}_D$ −1.73° ($c$ = 1.85 in $CHCl_3$).

**Enzymatic Analysis.** β-Hydroxypyruvate concentration was assayed by measuring the decrease in absorbance at 340 nm in the presence of NADH and lactic dehydrogenase (from rabbit muscle, EC 1.1.1.27) at pH 7.5 (Tris). The concentrations of α-hydroxy aldehydes were assayed by reduction with alcohol dehydrogenase (from equine liver, EC 1.1.1.1) at pH 9.5 (Gly-Gly).

**Measurement of Relative Velocity of Aldehyde Substrates.** The velocity of the TK reactions was assayed by following the disappearance of β-hydroxypyruvate from the reaction mixture. The reaction was carried out at 30 °C in Gly–Gly buffer (60 mM, 500 mL, pH 7.6) with TPP (0.1 mM), of $MgCl_2$ (3 mM), β-hydroxypyruvate (20 mM), and α-hydroxy aldehyde (20 mM for achiral and scalemic aldehydes, 40 mM for racemic aldehydes). The reaction was started by the addition of TK (0.3–2.5 U). At times of 1, 2, 3, 5, 7, 10, 20, and 30 min, 50-mL aliquotes of solution were withdrawn from the reaction mixture and quenched by addition to 1 mL of 0.2% trichloroacetic acid solution.

The quantity of β-hydroxypyruvate in each sample was determined photometrically by measuring the change in absorbance of NADH at 340 nm due to reduction of residual HPA by NADH and lactate dehydrogenase (from rabbit muscle, EC 1.1.1.27, LDH). 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. From the decrease in absorbance at 340 nm, the amount of β-hydroxypyruvate in the sample was calculated. Apparent rates were calculated by plotting time versus consumption of β-hydroxypyruvate, 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-hydroxypyruvate 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 ($V_{sub}$). Relative rate ($V_{rel}$) was defined as $V_{rel} = V_{sub}/V_{glycolaldehyde}$, where $V_{glycolaldehyde}$ was 1.7 mM $min^{-1}$ $U^{-1}$.

**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 N NaOH solution. The product α-hydroxy aldehydes were used as substrates for TK without further purification.

**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 stirbar was placed 20–50 mL of water containing $MgCl_2$ (3 mM), TPP (0.1 mM), lithium β-hydroxypyruvate (10 mM), and α-hydroxy aldehyde (50–130 mM). The pH was adjusted to ca. 7.5 with a few drops of 0.1 N 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 β-hydroxypyruvic acid (40–130 mM at pH 4), which was obtained by ion-exchanging lithium β-hydroxypyruvate solution with cation-exchange resin (AG-50W-H8, $H^+$ form). The mixture was stirred slowly with a magnetic stirrer and kept under $N_2$ gas throughout the course 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 consumption of acid), the reaction mixture was concentrated in vacuo and the product purified by flash chromatography (silica gel, 20% methanol in dichloromethane). For the kinetic resolution of α-hydroxy aldehydes, the reaction was allowed to continue for a full day after the amount of hydroxypyruvate corresponding to half an equivalent of racemic aldehyde initially introduced was consumed.

**5-Deoxy-D-*threo*-pentulose (43)** (44%): $^1H$ NMR ($CD_3OD$, 300 MHz) δ 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 Hz, 1 H), 1.22 (d, $J$ = 2.7 Hz, 3 H).

**5,6-Dideoxy-D-*threo*-hexulose (44)** (45%): $^1H$ NMR ($CD_3OD$, 300 MHz) δ 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-Trideoxy-D-*threo*-heptulose (45)** (39%): $^1H$ NMR ($CD_3OD$, 300 MHz) δ 4.53 (d, $J$ = 19.2 Hz, 1 H), 4.43 (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), 1.6–1.3 (m, 4 H), 0.96 (t, $J$ = 7.3 Hz, 3 H).

**5-*O*-Methyl-D-*threo*-2-pentulose (46)** (30%): $^1H$ NMR ($D_2O$, 300 MHz) δ 4.58 ($AB_q$, $J$ = 19.4 Hz, Δν = 34.6 Hz, 2 H), 4.41 (d, $J$ = 2.2 Hz, 1 H), 4.21 (ddd, $J$ = 2.4, 5.1, 7.0 Hz, 1 H), 3.61 (dABq, $J_{ax}$ = 5.1 Hz, $J_{bx}$ = 3.0 Hz, $J_{ab}$ = 10.4 Hz, Δν = 16.8 Hz, 2 H), 3.40 (s, 3 H); $^{13}C$ NMR ($D_2O$, 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 acetal of **29** (3.10 g, 19.3 mmol) in acetone (130 mL) and $H_2O$ (4 mL) was stirred with 10 g of AG-50W-H8 (a cation-exchange resin) at room temperature for 2 days. After solids were filtered off, $H_2O$ (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-Dideoxy-D-*threo*-hex-5-en-2-ulose (47).** 2-Hydroxybut-3-en-1-al (**29**) is unstable at basic pH; therefore, a slightly acidic pH (6.5–6.8) was used. Aldehyde **29** was introduced into the reaction medium as a mixture with β-hydroxypyruvic acid solution. A small amount of aldehyde was placed in the reaction flask at the outset. This procedure was used to avoid prolonged exposure of the aldehyde to the reaction medium. The pH of the solution of **29** (1.15 mmol) and lithium β-hydroxypyruvate (100 mg, 0.78 mmol) in water (30 mL) containing $MgCl_2$ (3 mM) and TPP (0.1 mM) was adjusted to around 6.5 with a few drops of 0.1 N NaOH soln. Transketolase (25 U) was added, and pH was maintained between 6.6 and 6.8 by the addition of a mixture solution of free hydroxypyruvic acid (40 mM) and **29** (140 mM), and the reaction was continued for 4 days. During this time, a total of 4.76 mmol of **29** was added. Water was removed under reduced pressure, and residue was chromatographed over silica gel (1–10% MeOH in $CH_2Cl_2$) to give 0.309 mg (2.12 mmol) of **47** (30% yield): $^1H$ NMR ($CD_3OD$, 300 MHz) δ 5.97 (ddd, $J$ = 5.5, 10.0, 17.2 Hz, 1 H), 5.34 (ddd, $J$ = 1.5, 1.6, 17.3 Hz, 1 H), 5.19 (ddd, $J$ = 1.5, 1.5, 10.5 Hz, 1 H), 4.48 (ABq, $J_{ab}$ = 19.4 Hz, Δν = 25.6 Hz, 2 H), 4.42 (m, 1 H), 4.18 (d, $J$ = 2.9 Hz, 1 H); $^{13}C$ NMR ($CD_3OD$, 125.8 MHz) δ 214.5, 139.7, 117.8, 80.7, 75.7, 69.1.

**5,6,7-Trideoxy-D-*threo*-hept-5-en-2-ulose (48)** (45%): $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.88–5.75 (m, 1 H), 5.20–5.12 (m, 2 H), 4.47 (ABq, $J_{ab}$ = 19.4 Hz, Δν = 59.0 Hz, 2 H), 4.21 (d, $J$ = 2.0 Hz, 1 H), 3.95 (ddd, $J$ = 8.0, 6.0, 2.1 Hz, 1 H), 3.25–2.60 (br s, 3 H), 2.48 (m, 2 H); IR ($cm^{-1}$) 3198, 2920, 1750, 1675, 1100; HRMS (FAB) mass calcd for $C_7H_{11}O_4$ ($M^+$ – H) 159.0657, found 159.0656; $[\alpha]^{20}_D$ = 15.7° ($c$ = 1.6 in ethyl acetate).

**5,6-Dideoxy-L-*xylo*-hept-5-en-2-ulose (49)** (60%): $^1H$ NMR (400 MHz, $D_2O$) (major anomer) δ 5.90–5.78 (m, 1 H), 5.49–5.31 (m, 2 H), 4.69 (t, $J$ = 8.1 Hz, 1 H), 4.32 (t, $J$ = 6.6 Hz, 1 H), 4.07 (d, $J$ = 6.6 Hz, 1 H), 3.61 (ABq $J_{ab}$ = 11.2 Hz, Δν = 18.9 Hz, 2 H); $^{13}C$ NMR (100 MHz, $D_2O$ ext ref $CDCl_3$, 77.5 ppm) δ 133.78, 120.48, 102.76, 80.43, 77.09, 76.69, 64.10; $[\alpha]^{20}_D$ –30.2° ($c$ = 4.2 in $H_2O$).

**5,6-Dideoxy-D-*arabino*-hept-5-en-2-ulose (50)** (63%): $^1H$ NMR (400 MHz, $D_2O$, major anomer) δ 5.89 (ddd, $J$ = 17.3, 10.3, 7.4 Hz, 1 H), 5.42–5.25 (m, 2 H), 4.15–4.00 (m, 3 H), 3.55 (ABq, $J_{ab}$ = 9.3 Hz, Δν = 26.1 Hz, 2 H); $^{13}C$ NMR (100 MHz, $D_2O$, int ref C-6 = 65 ppm) δ 138.31, 121.61, 103.49, 83.77, 80.17, 77.00, 65.00; $[\alpha]^{20}_D$ = +8.7° ($c$ = 0.79 in $H_2O$).

**5-Deoxy-D-*threo*-hex-2-ulose (51)**: $^1H$ NMR (400 MHz, $D_2O$) δ 3.86–3.81 (m, 2 H), 3.66–3.62 (m, 1 H), 3.47 (Abq, $J_{ab}$ = 11.6 Hz, Δν = 93 Hz, 2 H), 3.41 (d, $J$ = 9.3 Hz, 1 H), 1.98–1.92 (m, 1 H), 1.67–1.56 (m, 1 H); $^{13}C$ NMR (100 MHz, $D_2O$/dioxane) δ 99.2, 73.19, 69.33, 64.65, 59.73, 33.83, 13.03.

**General Procedure for the Recovery and Reduction of Unreacted Aldehyde.** After completion of the enzymatic reaction (see above), water was removed under reduced pressure at room temperature. The residue was chromatographed over silica gel (1–5% MeOH in $CH_2Cl_2$) to afford the aldehydes in the yields given in Table III. The aldehydes were immediately dissolved in ethanol (ca. 5 mL), and the solutions were treated with $NaBH_4$ to afford the corresponding diols (80–100%).

**General Procedure for the Reduction of TK Adducts with Sorbitol Dehydrogenase.** To a solution of TK adduct (0.1 mM) in 100 mM phosphate buffer (pH 7.0) were added sodium formate (3 equiv), NADH sodium salt (0.05 equiv), sorbitol dehydrogenase from sheep liver (EC 1.1.1.14, 15 U), and formate dehydrogenase from yeast (EC 1.2.1.2, 15 U). The mixture was stirred for 1 h and then allowed to stand at room temperature for 3 days. The mixture was concentrated under reduced pressure and chromatographed over silica gel (5–20% methanol in dichloromethane) to give purified product.

**5,6,7-Trideoxy-D-*xylo*-hept-6-enitol (65)** (77%): $^1H$ NMR (400 MHz, $D_2O$ δ 5.91–5.82 (m, 1 H), 5.18–5.10 (m, 2 H), 3.86–3.78 (m, 2 H), 3.67 (dABq, $J_{ab}$ = 11.7 Hz, $J_{ax}$ = 4.4 Hz, $J_{bx}$ = 6.9 Hz, Δν = 30.1 Hz, 2 H), 3.51 (dd, $J$ = 4.6, 4.6 Hz, 1 H), 2.41–2.33 (m, 1 H), 2.29–2.23 (m, 1 H); $^{13}C$ NMR (100.6 MHz, $CDCl_3$) δ 137.64, 120.71, 75.77, 74.93, 74.06, 65.68, 40.20; HRMS (FAB) mass calcd for $C_7H_{15}O_4$ ($M^+$ + H) 163.0970, found 163.0974; $[\alpha]^{20}_D$ = 6.0 ($c$ = 2.6 in $H_2O$).

**6,7-Dideoxy-D-*gluco*-hept-6-enitol (66)** (68%): $^1H$ NMR (500 MHz, $D_2O$) δ 6.00 (ddd, $J$ = 17.4, 10.4, 7.0 Hz, 1 H), 5.42–5.33 (m, 2 H), 4.24 (t, $J$ = 6.9 Hz, 1 H), 3.81–6.64 (m, 5 H); $^{13}C$ NMR (125.8 MHz, $D_2O$) δ 137.65, 119.22, 74.41, 73.51, 72.64, 70.93, 63.53; $[\alpha]^{20}_D$ = 4.1° ($c$ = 0.86 in $H_2O$); mass spectral data were collected on the pentaacetate ($Ac_2O$, $N(Et)_3$, (dimethylamino)pyridine, $CH_2Cl_{12}$); HRMS (FAB) mass calcd for $C_{17}H_{24}O_{10}Na$ (M+Na)$^+$, 411.1267, found 411.1277.

**6,7-Dideoxy-L-*ido*-hept-6-enitol (67)** (92%): $^1H$ NMR (300 MHz, $D_2O$) δ 5.93 (ddd, $J$ = 17.2, 10.5, 7.7 Hz, 1 H), 5.44–5.30 (m, 2 H), 4.28 (t, $J$ = 6.2 Hz, 1 H), 3.90–3.83 (m, 1 H), 3.78–3.60 (m, 4 H); $^{13}C$ NMR (125 MHz, $D_2O$, ref C-6 = 65 ppm) δ 138.88, 120.30, 76.05, 75.60, 74.55, 72.85, 65.00; $[\alpha]^{20}_D$ = 4.5° ($c$ = 3.1 in MeOH); mass spectral data were collected on the pentaacetate ($Ac_2O$, $N(Et)_3$, (dimethylamino)pyridine, $CH_2Cl_{12}$); HRMS (FAB)

mass calcd for $C_{17}H_{24}O_{10}Na$ $(M + Na)^+$ 411.1267, found 411.1247.

**5,6-Dideoxy-D-*xylo*-hex-5-enitol (71)** (61%): $^1H$ NMR ($CD_3OD$, 500 MHz) $\delta$ 5.93 (ddd, $J$ = 6.5, 10.5, 17.1 Hz, 1 H), 5.34 (ddd, $J$ = 1.2, 1.8, 17.1 Hz, 1 H), 5.19 (ddd, $J$ = 1.3, 1.8, 10.5 Hz, 1 H), 4.20 (dddd, $J$ = 1.2, 1.3, 5.9, 6.5 Hz, 1 H), 3.69 (ddd, $J$ = 2.8, 5.4, 6.3 Hz, 1 H), 3.62 (dABq, $J_{ax}$ = 5.4 Hz, $J_{bx}$ = 6.5 Hz, $J_{ab}$ = 11.0 Hz, $\Delta\nu$ = 17.7 Hz, 2 H), 3.47 (dd, $J$ = 2.9, 5.9 Hz, 1 H); $^{13}C$ NMR ($CD_3OD$, 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 NaH/MeI in DMF: $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 5.77 (ddd, $J$ = 7.7, 10.5, 18.2 Hz, 1 H), 5.3–5.25 (m, 2 H), 3.76 (m, 1 H), 3.49 (dABq, $J_{bx}$ = 5.1 Hz, $J_{bx}$ = 5.5 Hz, $J_{ab}$ = 9.9 Hz, $\Delta\nu$ = 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 ($CD_3OD$, 125.8 MHz) $\delta$ 135.5, 118.5, 83.4, 83.2, 80.0, 71.8, 61.0, 59.1, 58.7, 56.6; HRMS (FAB) for $C_{10}H_{21}O_4$ $(M^+ + H)$ calcd 205.1440, found 205.1443.

**General Experimental Procedure for the Ozonolysis of Alkenepolyols.** Into a solution of alkene (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 $N_2$, $Na_2SO_3$ (ca. 2–4 g) was added. The mixture was vigorously stirred for 1 h at −78 °C and 15 h at room temperarture. After filtration and concentration, column chromatography over silica gel (20–50% MeOH in $CH_2Cl_2$) gave the desired aldose.

**2-Deoxy-L-xylohexose (67)** (84%). The product showed the same $^1H$ and $^{13}C$ NMR spectra as a sample of 2-deoxy-L-xylohexose prepared according to ref 33: $[\alpha]^{21}_D$ = −6.1 ($c$ = 1.5, $H_2O$) (authentic sample: $[\alpha]^{21}_D$ = −5.6 (c = 0.8, $H_2O$)).[37]

**L-Gulose (69)** (76%). The product showed the same $^1H$ and $^{13}C$ NMR spectra as authentic L-gulose: $[\alpha]^{21}_D$ = 17 ($c$ = 2.5, $H_2O$ (authentic sample: $[\alpha]^{20}_D$ = 20 ($c$ = 13.6, $H_2O$)).[37]

**L-Idose (70)** (68%). The product showed the same $^1H$ and $^{13}C$ NMR spectra as authentic L-idose: $[\alpha]^{21}_D$ = −14 ($c$ = 1.2, $H_2O$ (authentic sample: $[\alpha]^{21}_D$ = −17.4 ($c$ = 3.6, $H_2O$)).[37]

**L-Xylose (72)** (67%). The product showed the same $^1H$ and $^{13}C$ NMR spectra as authentic L-xylose: $[\alpha]^{21}_D$ = −17.4 ($c$ = 0.32, $H_2O$) (authentic sample: $[\alpha]^{21}_D$ = −18.8 ($c$ = 0.30, $H_2O$)).

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**Supplementary Material Available:** Spectrometric information ($^1H$ 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.

(36) Leonard, N. J.; Carraway, K. L. *J. Heterocycl. Chem.* **1960**, 485.
(37) *The Carbohydrates*; Collins, P. M., Ed.; Chapman and Hall: New York, 1987.

# Intermolecular Benzyne Cycloaddition (IBC), a Versatile Approach to Benzophenanthridine Antitumor Alkaloids. Formal Synthesis of Nitidine and Chelerythrine

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A new approach to the synthesis of benzophenanthridine alkaloids is described which is based on cycloaddition of arynes to pyrrolinediones, the pyrrolinediones behaving as aza diene equivalents. The synthesis of 2,3,8,9-substituted benzophenanthridines and the regioselective synthesis of a 2,3,7,8-substituted benzophenanthridine were performed. The formal synthesis of chelerythrine and the antitumor alkaloid nitidine is described.

Planar benzophenanthridinium salts 1 (Figure 1) have been recognized as being potentially useful as antitumor agents, having been shown to intercalate into the minor groove of DNA and bind to it covalently.[1] However, some 2,3,8,9-substituted derivatives, though among the most active in L1210 and P388 tests, have been reported to be toxic.[2] Efforts have accordingly been made to develop

(1) Ulrichová, J.; Walterová, D.; Simanek, V. *Acta Univ. Palacki. Olomuc., Fac. Med.* **1981**, *106*, 31.

(2) (a) Stermitz, F. R.; Gillespie, J. P.; Amoros, L. J.; Romero, R.; Stermitz, T. A. *J. Med. Chem.* **1975**, *18*, 708. (b) Zee-Cheng, R. K.-Y.; Cheng, C. C. *J. Med. Chem.* **1975**, *18*, 66.

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