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Recombinant 2-Deoxyribose-5-phosphate Aldolase in Organic Synthesis: Use of Sequential Two-Substrate and Three-Substrate Aldol Reactions

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Abstract: A new procedure has been developed for the large scale preparation of recombinant 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4) from *E. coli* strain DH5α (ATCC 86963). The enzyme was purified to homogeneity with an overall yield of 83% and in sufficient quantity to grow crystals suitable for X-ray diffraction studies. Using the sequential two- or three-substrate aldol reaction, DERA was applied to the synthesis of a variety of sugar analogs including deoxyriboses, 2-deoxyfucose analogs, dideoxyhexoses, trideoxyhexoses, deoxythiosugars, and ^{13}C-substituted 2-deoxyribose-5-phosphate.

Introduction

2-Deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4) is a class 1 aldolase which reversibly catalyzes the aldol reaction of acetaldehyde with D-glyceraldehyde-3-phosphate to form 2-deoxyribose-5-phosphate via a Schiff base intermediate between the active-site lysine and acetaldehyde.[1] DERA is the only aldolase reported to accept two or three aldehydes in a sequential and stereoselective manner in the aldol addition reaction, and in addition to acetaldehyde, the enzyme is also able to accept propanal, acetone, and fluoroacetone as donor substrates.[2] We describe here the large-scale preparation and purification of DERA from recombinant *E. coli* strain DH5α (ATCC 86963)[2] and the application of DERA in the synthesis of a variety of sugar analogs, including 2-deoxy-L-fucose and analogs, thiosugars, and glycolipid precursors. The sequential aldol reaction has also been utilized in the synthesis of a variety of 2,4-dideoxy- and 2,4,6-trideoxyhexoses. Of special interest is the synthesis of uniformly ^{13}C-substituted 2-deoxyribose-5-phosphate from commercially available ^{13}C-substituted glycerol and ^{13}C-substituted acetaldehyde, which may find use in the preparation of ^{13}C-substituted nucleic acids for three dimensional structural determination by NMR.[3]

Results and Discussion

E. coli cells from the strain DH5α, transformed with the plasmid pVH17 containing the DERA gene, were used to provide about 124 000 U of DERA per 6 L of culture. Using lysozyme provided a convenient method for obtaining cell free extracts and is comparable to the disruption of the cells in a French Press (~1600 U/g of cells).[2] The lysozyme method was especially useful when processing a large volume of cells. A net increase of 251% in the recovered activity was obtained after ammonium sulfate precipitation, salt removal and buffer exchange, compared to only about 30% recovered activity after overnight dialysis using tubing with a molecular weight cut off of 5000. Our new strategy for purification was based on the use of anion exchange chromatography and chromatofocusing. Scaleup of the anion exchange chromatography was relatively easy as DERA was eluted in the void volume. Further purification by chromatofocusing using a pH gradient from 6 to 4 gave a main peak corresponding to DERA which upon analysis by SDS-PAGE revealed a single band of 28 kD. However, further analysis by isoelectrofocusing (IEF) revealed the existence of two more proteins. A narrower pH range (5.5−4.5) was used, and the peak containing DERA activity was analyzed by SDS-PAGE and IEF (Figure 1); a single band was obtained in both cases. This is the first reported purification to homogeneity of 2-deoxyribose-5-phosphate aldolase which is especially noteworthy as the purification sequence produced an overall yield of 83% (Table 1). For crystallization, DERA was dialyzed against Tris-HCl and concentrated to 10 mg/mL; crystals were immediately obtained upon treatment with polyethylene glycol.[4]

For synthesis, we found that using DERA after the ammonium sulfate precipitation was a convenient method for using DERA in synthesis. However, the cell free extracts usually produced similar results. Using α-hydroxyaldehydes with different substitution at the β-position, a variety of novel sugars were produced. Due to DERA's selectivity for D-2-hydroxyaldehydes,[2b] a single diastereomer was isolated when racemic 2-hydroxy-3-butenal[5] (**1**) and 3-thioglyceraldehyde[6] (**7**) were used as acceptors. (*R*)-3-bromo-2-hydroxypropanal[7] (**3**) afforded 5-bromo-2-deoxyribose (**4**) when acetaldehyde was used as a donor and the 2-methylribose **6** was formed when propionaldehyde was

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1995.

(1) (a) Horecker, B. L.; Pontremoli, S.; Ricci, C.; Cheng, T. *Proc. Natl. Acad. Sci. U.S.A.* **1961,** *47,* 1942. (b) Valentin-Hansen, P.; Boëtius, F.; Hammer-Jespersen, K.; Svendsen, I. *Eur. J. Biochem.* **1982,** *125,* 561.

(2) (a) Barbas III, C. F.; Wang, Y-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1990,** *112,* 2013. (b) Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.* **1992,** *114,* 741. (c) Gijsen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1994,** *116,* 8422.

(3) (a) Nikonowicz, E. P.; Pardi, A. *Nature* **1992,** *355,* 184. (b) Nikonowicz, E. P.; Pardi, A. *J. Am. Chem. Soc.* **1992,** *114,* 1082. (c) Varani, G.; Tinoco, I. *Q. Rev. Biophys.* **1991,** *24,* 479. (d) Varani, G.; Tinoco, I. *J. Am. Chem. Soc.* **1991,** *113,* 9349. (e) Clore, G. M.; Gronenborn, A. M. *Science* **1991,** *252,* 1390. (f) Clore, G. M.; Kay, L. E.; Bax, A.; Gronenborn, A. M. *Biochemistry* **1991,** *30,* 12. (g) Clore, G. M.; Bax, A.; Driscoll, P. C.; Wingfield, P. T.; Gronenborn, A. M. *Biochemistry* **1990,** *29,* 8172. (h) Fesik, S. W.; Zuiderweg, E. R. P. *Q. Rev. Biophys.* **1990,** *23,* 97. (i) Fesik, S. W.; Eaton, H. L.; Olejniczak, E. T.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **1990,** *112,* 886. (j) Kay, L. E.; Ikura, M.; Bax, A. *J. Am. Chem. Soc.* **1990,** *112,* 888.

(4) For exact crystallization conditions see: Stura, E. A.; Ghosh, S.; Garcia-Junceda, E.; Chen, L.; Wong, C.-H.; Wilson, I. A. *Proteins,* in press.

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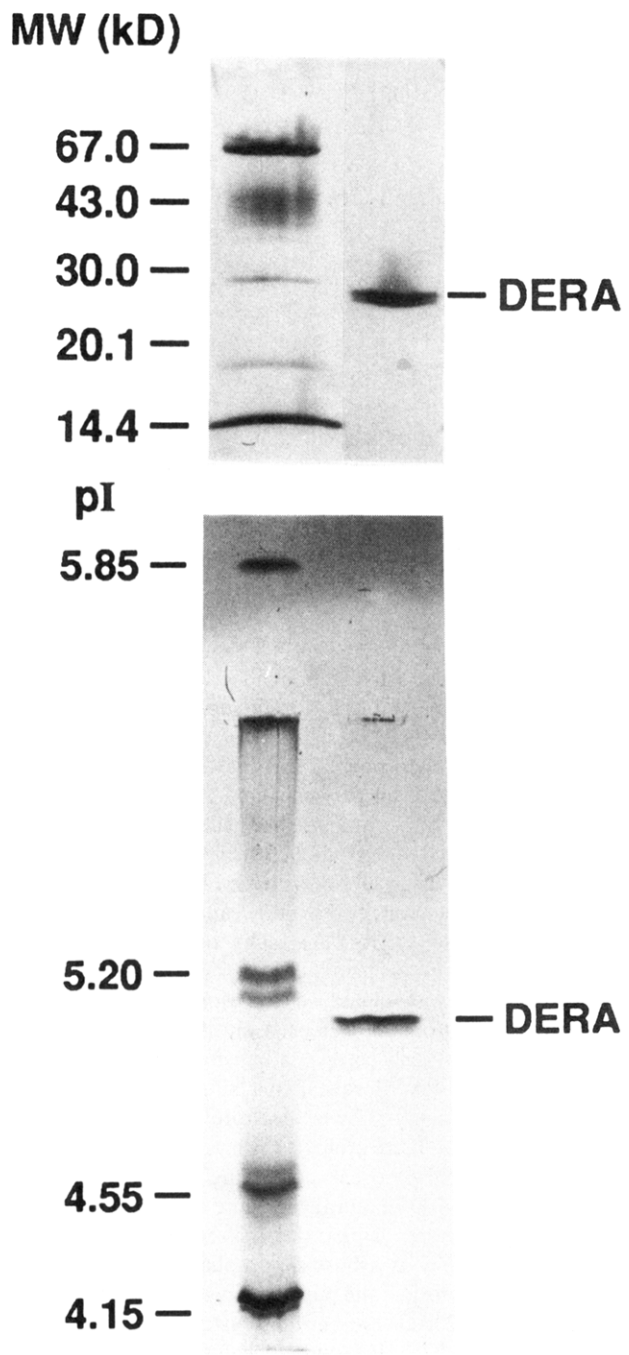


Figure 1. Electrophoretic analysis of DERA after chromatofocusing (pH 5.5−4.5): (A, top) SDS-PAGE and (B, bottom) IEF (pH 6.5−4).

Table 1. DERA Purification Table

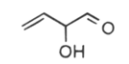
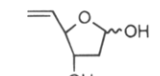
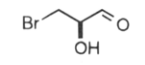
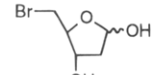
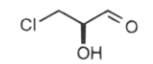
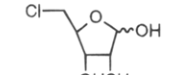
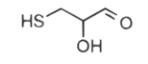
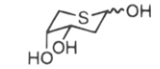
	prot (mg)	activ (U)	spec act (U/mg)	yield (%)	purification
cell free extract[a]	607.8	2297.6	3.8	100	1
Strept sulf	538.2	1993.9	3.7	87	1
am sulf 40−65%	468.4	5773.3	12.4	251	3.3
anion exchange	72.9	4061.1	55.7	177	14.7
chromatofocusing	32.6	1897.6	58.1	83	15.4

[a] 6 g of cells were used to obtain the extract.

used as donor and 3-chloro-2-hydroxypropanal[8] (**5**) was used as acceptor (Table 2). DERA proved to accept a wide variety of functionality as indicated by the high yields associated with acceptors containing azide, sulfide, or disulfide groups. Using

(8) Pederson, R. L.; Liu, K. K.-C.; Rutan, J. F.; Chen, L.; Wong, C.-H. *J. Org. Chem.* **1990**, *55*, 4897.

Table 2. Dera Catalyzed Aldol Reactions

Substrate	Donor	Product	Yield
 **1**	CH_3CHO	 **2**	12%
 **3**	CH_3CHO	 **4**	30%
 **5**	CH_3CH_2CHO	 **6**	8%
 **7**	CH_3CHO	 **8**	33%

racemic 3-azido-3-deoxyerythrose[9] (**9**) resulted in the formation the 5-azido-hexofuranose (**10**) and the C4, C5-diastereomer in a 6:1 ratio. This is the only example in this study where we found evidence for a minor reaction of DERA with the L-2-hydroxyaldehyde when presented with a racemic mixture. 2-Deoxy-L-fucose, which is a constituent of the rhodomycins, cinerubins A and B, the antibiotic azalomycins-B,[10] and the anthracyclines,[11] was synthesized from **11a**[12] which is available from the Sharpless asymmetric dihydroxylation. Dihydroxyaldehyde **11b**[12] afforded the unusual sugar **12b** in an analogous manner. The synthesis of thio-containing compounds **14** and **16** proceeded in good yields. Aldehyde **13**, synthesized by regioselective ring-opening of glycidaldehyde diethyl acetal with 3-mercapto-1,2-propanediol (Scheme 2), reacted with acetaldehyde to produce **14** as a mixture of diastereomers. Similarly, dialdehyde **15**, synthesized by regioselective opening of *R*-glycidaldehyde diethyl acetal with potassium thiolacetate (Scheme 3), reacted with acetaldehyde to afford the sulfide-linked disaccharide **16** (Table 3).

When acetaldehyde is used as the donor and acceptor, the resulting β-hydroxy aldehyde cannot form an internal hemiacetal which results in an aldehyde being available for a second aldol reaction with acetaldehyde. The aldehyde resulting from this second addition, 2,4,6-trideoxy-D-hexapyranoside (**17a**), exists as the hemiacetal and was isolated. When α-substituted acetaldehydes are used that contain functionality that will not cyclize after the first aldol reaction, the products from the sequential aldol reaction then cyclize in the pyranose form, stopping the polymerization after the addition of two acetaldehyde monomers. In this manner, 2,4-dideoxyhexoses with various substituents at the six position (compounds **17b−d**) were obtained.[2c] Succinic semialdehyde proved to be a very good acceptor with the octapyranuronic acid **18** being produced in an 80% yield. The high yield of this reaction is attributed to

(9) Henderson, I.; Laslo, K.; Wong, C.-H. *Tetrahedron Lett.* **1994,** *35,* 359.
(10) Horton, D.; Cheung, T.-M.; Weckerle, W. *Methods Carbohydr. Chem.* **1980**, *8*, 201 and references therein.
(11) Florent, J.-C.; Gaudel, G.; Monneret, C.; Hoffmann, D.; Kraemer, H.-P. *J. Med. Chem.* **1993**, *36*, 1364.
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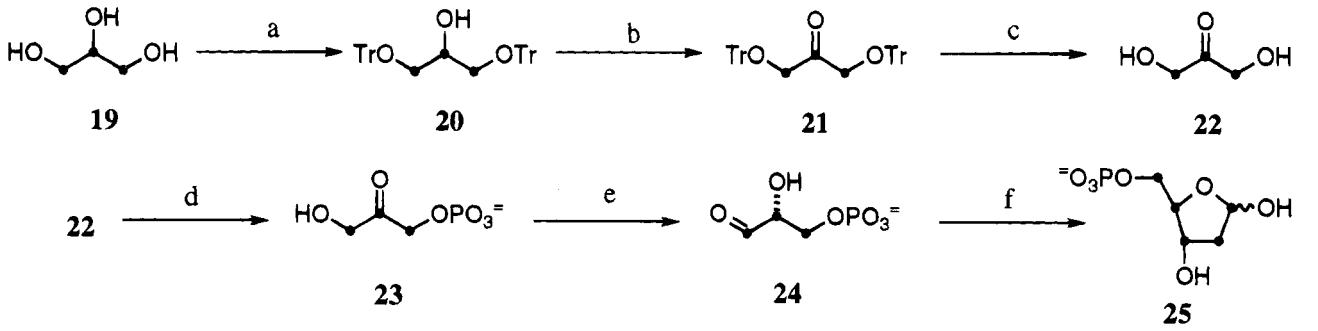
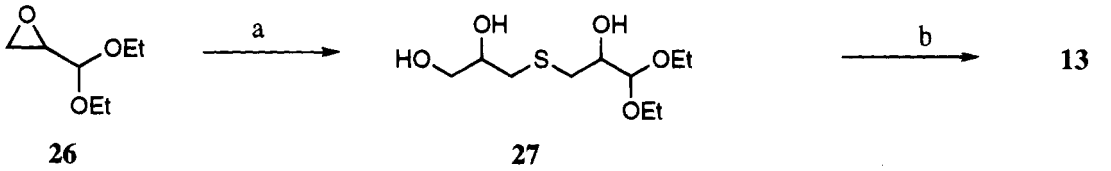
(13) Heukeshoven, J.; Dernick, R. *Electrophoresis.* **1988,** *9,* 28.
(14) Bradford, M. *Anal. Biochem.* **1976,** *72,* 248.
(15) De Bruyn, A.; Anteunis, M.; Garegg, P. J.; Norberg, T. *Acta Chem. Scand., Ser. B*, **1976**, *B 30(9)*, 820.

Scheme 1



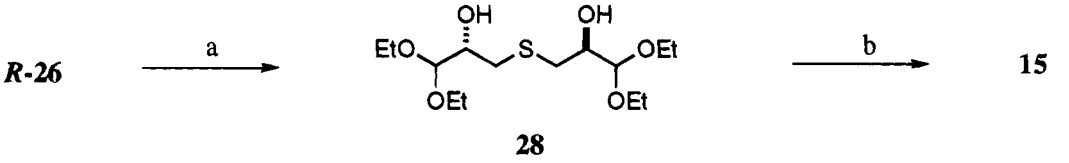
[a] (a) TrCl, pyr, DMAP, CH_2Cl_2, 91%; (b) PDC, 4 Å sieves, CH_2Cl_2, 75%; (e) TsOH, CH_2Cl_2, MeOH; (d) GK, ATP regeneration; (e) TPI; (f) [1,2-$^{13}C_2$]acetaldehyde, DERA, 79% from **21**.

Scheme 2



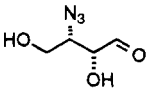
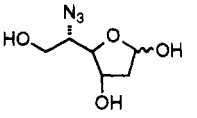
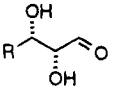
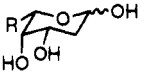
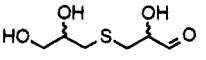
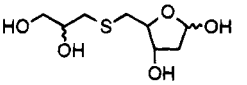
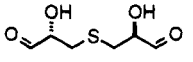
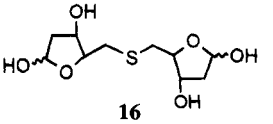
[a] (a) 3-Mercapto-1,2-propanediol, NaH, THF, 69%; (b) HCl, pH 1.

Scheme 3



[a] (a) $KSCOCH_3$, EtOH, 36%; (b) HCl, pH 1.

Table 3. Dera Catalyzed Reactions

Substrate	Donor	Product	Yield
 **DL-9**	CH_3CHO	 **10**	46%
 **11** **a**; R = CH_3 **b**; R = Ph	CH_3CHO	 **12** **a**; R = CH_3 **b**; R = Ph	51% 46%
 **13**	CH_3CHO	 **14**	27%
 **15**	CH_3CHO	 **16**	58%

the negative charge of the carboxylic acid at pH 7.3 being an effective mimic of the phosphate group of the natural substrate (Table 4).

Using DERA with its natural, but ^{13}C-substituted, substrates produced ^{13}C-substituted 2-deoxyribose-5-phosphate. D-(1,2,3-$^{13}C_3$)Glyceraldehyde-3-phosphate was synthesized by a combined chemical and enzymatic route. Selective protection of the primary alcohols of (1,2,3-$^{13}C_3$)glycerol (**19**) using trityl chloride in the presence of pyridine and DMAP gave 1,3-di-*O*-trityl(1,2,3-$^{13}C_3$)glycerol (**20**) in high yield. Oxidation of **20** using PDC furnished di-*O*-trityldihydroxy(1,2,3-$^{13}C_3$)acetone (**21**). Deprotection of the trityl groups provided dihydroxy-

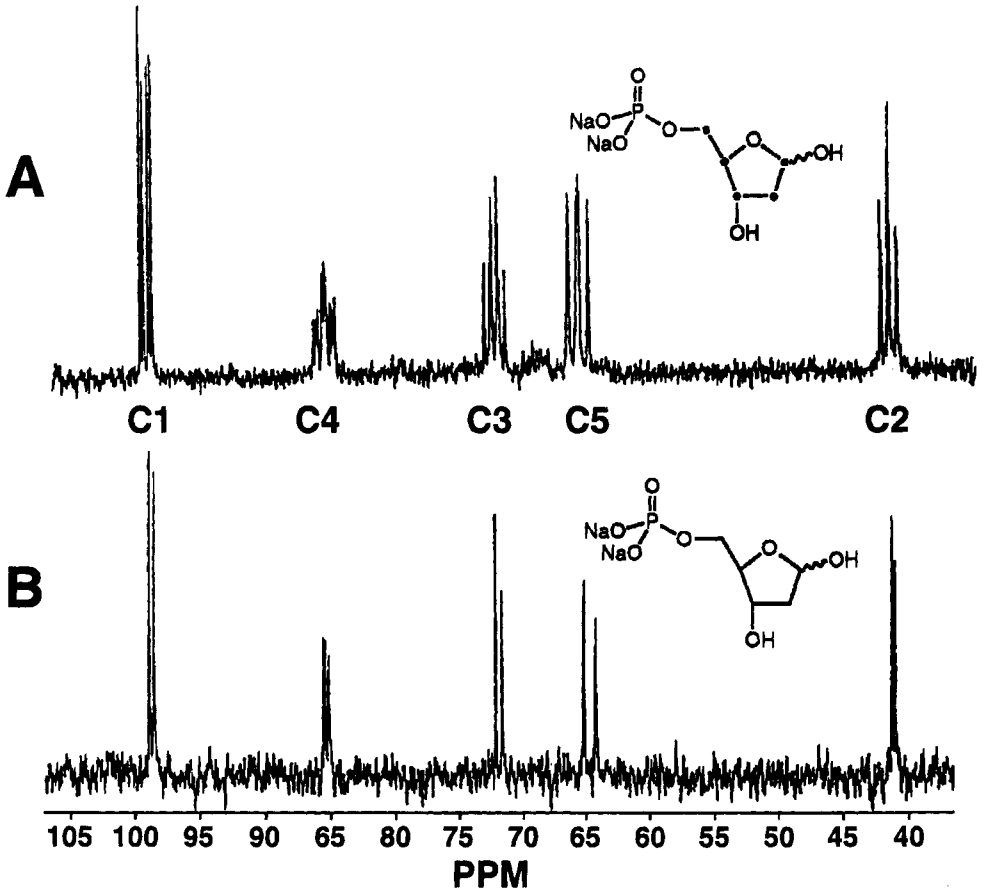
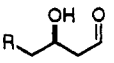
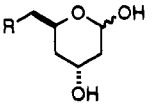
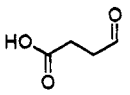
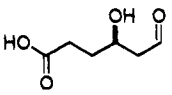
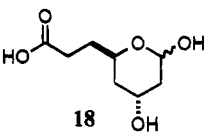


Figure 2. ^{13}C NMR spectra of (A) ^{13}C-substituted 2-deoxy-ribose-5-phosphate and (B) unsubstituted 2-deoxy-ribose-5-phosphate.

Table 4. Dera Catalyzed Polymerization

Substrate	Donor	Intermediate	Donor	Product	Yield
	CH_3CHO		CH_3CHO	 **17**	**a**; R = H 22% **b**; R = OMe 65% **c**; R = Cl 70% **d**; R = N_3 23%
	CH_3CHO		CH_3CHO	 **18**	80%

$(1,2,3\text{-}^{13}C_3)$acetone (**22**) which was then phosphorylated with ATP, catalyzed by glycerokinase, to give dihydroxy$(1,2,3\text{-}^{13}C_3)$-acetone phosphate (DHAP) (**23**) with the resulting ADP converted to ATP in a catalytic cycle. The ^{13}C-substituted DHAP was isomerized by triose phosphate isomerase (TPI) providing D-$(1,2,3\text{-}^{13}C_3)$glyceraldehyde-3-phosphate (**24**) which then reacted with $(1,2\text{-}^{13}C_2)$acetaldehyde, catalyzed by DERA, to give 2-deoxy$(1,2,3,4,5\text{-}^{13}C_5)$ribose-5-phosphate (**25**) (Scheme 1). The ^{13}C NMR spectra of the unsubstituted 2-deoxyribose-5-phosphate and the uniformly ^{13}C substituted compound are shown in Figure 2. This multiple enzyme catalyzed reaction was performed smoothly on >1 mmol scale and is amenable to further scale-up.

In summary, we have further exploited the synthetic utility of DERA and demonstrated that the enzyme is able to accept a variety of substrates in the aldol addition reaction. The sequential, two- and three-substrate, one-pot aldol reactions described here provide a rapid entry to a number of novel aldol structures useful in organic synthesis. The enzyme is highly stable and active and is easily prepared from the recombinant species and can be used in a crude form for synthesis. It is expected that DERA will be widely used as a catalyst in synthesis.

Experimental Section

General Methods. Fast protein liquid chromatography was performed on a Pharmacia FPLC system with columns purchased from Pharmacia. SDS-PAGE and IEF were performed with a Pharmacia PhastSystem instrument, using preformulated gels from the same company. UV and visible spectroscopy were obtained with a Beckman DU-70 spectrophotometer at 25 °C. NMR spectra were obtained on Bruker AMX-400 or AMX-500 spectrometers. High resolution mass

spectra (HRMS) were obtained on a VG ZAB-ZSE mass spectrometer in electron impact (EI), fast atom bombardment (FAB), or with solid probe. All chemicals and enzymes, except DERA, were purchased from Aldrich, Sigma, or Cambridge Isotope Laboratories.

Preparation of Cell Free Extract Using Lysozyme. To a suspension of cells, in Tris buffer (8 mL/g cells, 50 mM, pH 8.0), were added EDTA (50 mM, pH 8.2) and lysozyme (2 mg/g cells). The suspension was gently stirred at room temperature for 1 h, and the suspension was kept at 4 °C overnight. The preparation was gently sonicated for 20 min to decrease viscosity, DNase (10 μg/g cells) and $MgCl_2$ (0.95 μg per mL of preparation) were added, and the mixture was refrigerated for 20 min. The mixture was then centrifuged for 30 min at 16 000 × g, and the supernatant was used in the next purification steps.

In order to assess the efficiency of the above method, a CFE was prepared by disruption of the cells in a French Press. Five grams of cells were suspended in Tris buffer (45 mL, 50 mM, pH 8.0) and lysed twice in a French Press at 16 000 lb/in. After centrifugation for 30 min at 16 000 × g, total proteins and DERA activities were measured in the supernatant and found to be similar to the lysozyme method.

Purification of DERA. Streptomycin sulfate was added at 4 °C with stirring to the CFE obtained by digestion of the cells with lysozyme until a concentration of 1% was obtained, and stirring was continued for 20 min. The solution was then centrifuged for 30 min at 16 000 × g, the supernatant was collected, and ammonium sulfate was added at 4 °C with stirring until a concentration of 40% was obtained. The solution was then centrifuged for 30 min at 16 000 × g, the supernatant was collected, and ammonium sulfate was added at 4 °C with stirring until a concentration of 65% was obtained. The solution was then centrifuged for 30 min at 16 000 × g, and the resulting pellet was resuspended in Tris buffer (100 mM, pH 7.6), containing 2 mM EDTA (buffer A). This solution was desalted using Centriprep tubes (Amicon). Further purification was achieved by FPLC at room temperature.

Anion Exchange Chromatography. Anion exchange chromatography was performed on a Mono Q column 10/10 with about 150 mg of protein loaded on the column in each run. The sample was eluted with a gradient of 1 M NaCl in 200 mL of buffer A. Fractions (4 mL) containing protein were detected by absorbance at 280 nm, the active fractions were pooled, and the buffer was exchanged with the initial buffer of the chromatofocusing using Centriprep tubes.

Chromatofocusing. Chromatofocusing was performed on a Mono P column 5/20 using two different pH gradients: 6−4 and 5.5−4.5. In the first case, the initial buffer was Bis-Tris (25 mM, pH 6.3 with HCl). The elution buffer was polybuffer 7−4 (pH 4 with HCl), diluted by a factor of ten with distilled water. In the second case, the initial buffer was piperazine (25 mM, pH 6.3). The elution buffer was prepared as before with a final pH of 4.5. In both cases, before loading the sample, a pregradient was made by washing the column with 3 mL of the elution buffer. The separation was optimized by loading 100 μg of protein in a total volume of 100 μL. To scale up the process, 15 mg of protein were applied in each run, and the protein fractions (0.5 mL each) were monitored by absorbance at 280 nm.

Determination of Enzyme Purity. Fractions obtained from different columns were analyzed by SDS-PAGE (polyacrylamide gel electrophoresis) and isoelectrofocusing (IEF). For SDS-PAGE, preformulated gels were used with a gradient (8−25%) of polyacrylamide in the separating zone. Prior to electrophoresis, the samples were boiled at 100 °C for 3 min in a solution containing 0.5% sodium dodecyl sulfate and 5% 2-mercaptoethanol. The IEF was performed on preformulated gels with a pH range of 4.5−6.5. In both cases, the markers used were from Pharmacia. The gels were stained using the Pharmacia PhastSilver Kit, modified to provide higher sensitivity. This technique can detect proteins in the range of 0.1−0.05 ng of protein per band.[13]

Enzymatic Assay. DERA activity was assayed with a coupled enzymatic system where 0.5 mM of 2-deoxyribose-5-phosphate, 0.12 mM NADH, and a mixture of glycerophosphate dehydrogenase and triose phosphate isomerase was incubated in triethanolamine buffer (50 mM, pH 7.5) at 25 °C. The assay was initiated by addition of DERA, and the decrease in the absorbance at 340 nm was monitored. The extinction coefficient for NADH was taken as 6.22×10^3 M^{-1} cm^{-1}. Protein concentration was measured by the Bradford assay[14] using the Coomasie Plus Kit Reagent from Pierce Co., instead of the method described previously.[2a,b] The specific activity of the enzyme is, therefore, different.

General Procedure for DERA-Catalyzed Reactions. DERA (400 U) was added to a 10 mL solution containing 100 mM of acceptor aldehyde and 300 mM of donor aldehyde, 100 mM triethanolamine buffer (pH 7.3), and 1 mM EDTA. The resulting solution was stirred in the dark for 2 days under N_2. The reaction was quenched by addition of 2 volumes of acetone, then cooled to 0 °C for 20 min, and centrifuged to remove the precipitated protein. After removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography.

2,5,6-Trideoxy-D-*erythro*-5-hexenose (2). Using the general procedure, a 12% yield of compound **2** was obtained. Major anomer: ^{1}H NMR (500 MHz, D_2O) δ 2.03 (d, J = 13.8 Hz, 1H), 2.10 (dt, J = 4.9, 13.8 Hz, 1H), 4.13 (d, J = 5.1 Hz, 1H), 4.72 (br d, J = 5.7 Hz, 1H), 5.14 (dt, J = 1.4, 10.4 Hz, 1H), 5.29 (dt, J = 1.4, 17.1 Hz, 1H), 5.64 (d, J = 4.6 Hz, 1H), 5.75 (ddd, J = 5.4, 10.4, 17.0 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 39.8, 75.8, 88.1, 99.2, 115.9, 135.7; HRMS for $C_6H_9DO_3$ (M + Li^+) calcd 138.0853, found 138.0853.

5-Bromo-2,5-dideoxy-D-*erythro*-pentose (4). Using the general procedure, a 30% yield of compound **4** was obtained. β anomer: ^{1}H NMR (500 MHz, D_2O) δ 2.18 (m, 2H), 3.51 (dd, J = 4.5, 10.9 Hz, 1H), 3.56 (dd, J = 5.4, 10.9 Hz, 1H), 4.08 (dt, J = 3.9, 6.1 Hz, 1H), 4.44 (dt, J = 3.9, 5.8 Hz, 1H), 5.62 (t , J = 4.6 Hz, 1H); α anomer, ^{1}H NMR (500 MHz, D_2O) δ 1.89 (ddd, J = 2.6, 2.6, 14.5 Hz, 1H), 2.47 (ddd, J = 5.6, 6.7, 14.5 Hz, 1H), 3.5 (dd, J = 3.5, 10.9 Hz, 1H), 3.56 (dd, J = 5.4, 10.9 Hz, 1H), 4.31−4.27 (m, 2H), 5.57 (dd, J = 2.2, 5.5 Hz, 1H); α and β mixture, ^{13}C NMR (125 MHz, D_2O) δ 33.7, 34.0, 41.5, 41.6, 73.4, 73.5, 84.5, 85.4, 98.8, 99.0; HRMS for $C_5H_9O_3Br$ (M + Na^+) calcd 218.9633, found 218.9651.

5-Chloro-2,5-dideoxy-2(*R*)-methyl-D-*erythro*-pentose (6). Using the general procedure, an 8% yield of compound **6** was obtained (characterized as 5-chloro-1,3-di-*O*-acetyl-2,5-dideoxy-2(*R*)-methyl-D-ribose). Major anomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (d, J = 7.3 Hz, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 2.58−2.72 (dq, J = 3.0, 6.8 Hz, 1H), 3.5−3.8 (m, 2H), 4.28 (dt, J = 5.0, 6.1 Hz, 1H), 5.30 (dd, J = 4.7, 6.6 Hz, 1H), 5.96 (d, J = 2.8 Hz, 1H); HRMS for $C_{10}H_{15}O_5Cl$ (M + Cs^+) calcd 382.9662, found 382.9663.

2-Deoxy-5-thio-D-*eythro*-pentose (8). Using the general procedure, a 33% yield of compound **8** was obtained (characterized as 2-deoxy-5-thio-1,3,4-tri-*O*-acetyl-D-*eythro*-pentose). Purification by preparative TLC (R_f 0.27, methanol/chloroform/hexane, 1:90:10) afforded the α-anomer (41 mg, 15%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.06 (s, 3H), 2.09 (s, 3H), 2.13 (s, 3H), 2.29 (ddd, J = 2.8, 3.8, 15.4 Hz, 1H), 2.50 (dd, J = 3.8, 12.8 Hz, 1H), 2.63 (ddd, J = 3.2, 4.4, 15.4 Hz, 1H), 3.36 (dd, J = 11.1, 12.8 Hz, 1H), 5.12 (ddd, J = 2.6, 3.9, 11.1 Hz, 1H) 5.22−5.25 (m, 1H), 5.79 (t, J = 3.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.87, 20.97, 21.09, 22.73, 35.87, 67.39, 68.84, 70.18, 169.25, 169.83, 170.09; HRMS for $C_{11}H_{16}O_6S$ (M + Na^+) calcd 299.0565, found 299.0565. β-Anomer (50 mg, 18% R_f 0.36, methanol/chloroform/hexane, 1:90:10): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.12 (s, 3H), 2.08−2.15 (m, 1H), 2.16 (s, 3H), 2.44 (ddd, J = 2.9, 11.3, 13.6 Hz, 1H), 2.87 (dd, J = 1.6, 14.6 Hz, 1H), 3.26 (dd, J = 1.9, 14.6 Hz, 1H), 5.21 (ddd, J = 3.8, 3.8, 11.3 Hz, 1H), 5.34 (ddd, J = 1.6, 1.9, 3.8 Hz, 1H), 6.03 (br s, 1H); ^{13}C NMR (125 MHz, $CDCL_3$) δ 21.0, 21.1, 21.2, 28.4, 32.5, 66.1, 67.3, 72.4, 169.4, 170.1, 170.4; HRMS for $C_{11}H_{16}O_6S$ (M + Na^+) calcd 299.0565, found 299.0577.

5-Azido-2,5-dideoxy-L-*lyxo*-hexose (10). Using the general procedure, a 46% yield of compound **10** was obtained. α and β anomers of **10**: ^{1}H NMR (500 MHz, D_2O) δ 1.83 (ddd, J = 2.6, 3.7, 14.2, 1H), 2.1−2.2 (m, 2H), 2.41 (ddd, J = 5.6, 7.3, 13.3, 1H), 3.59 (dd, J = 4.1, 8.6, 2H), 3.66 (dd, J = 8.0, 11.8, 1H), 3.70 (dd, J = 8.4, 11.8, 1H), 3.80 (dd, J = 2.3, 11.9, 1H), 3.81 (dd, J = 2.4, 11.9, 1H), 3.80−3.82 (m, 1H), 4.06 (t, J = 4.3, 1H), 4.32 (dt, J = 4.0, 7.3, 1H), 4.46 (dd, J = 4.8, 6.4, 1H), 5.52 (dd, J = 2.4, 5.4, 1H), 5.55 (dd, J = 3.4, 5.0, 1H); ^{13}C NMR (125 MHz, D_2O) δ 41.63 41.69, 62.05, 62.44, 64.55, 65.52, 72.26, 72.69, 84.46, 85.07, 98.88, 99.00; HRMS for $C_6H_{11}N_3O_4$ (M + Na^+) calcd 212.0647, found 212.0649.

2,6-Dideoxy-L-*lyxo*-hexose (12a). Aldehyde **11a**[12] (41.3 mg, 0.40 mmol) and acetaldehyde (52.8 mg, 1.2 mmol) were dissolved in 1 mM

Tris and 0.01 mM EDTA buffer (4 mL, pH 7.3), and 160 units of DERA were added. The product, 2-deoxyfucose (**12a**) (36 mg, 51%), was purified by silica gel column chromatography (10:2, $CHCl_3$/MeOH). The ^1H NMR spectra was identical to that reported in the literature.[15]

2-Deoxy-5-phenyl-L-*lyxo*-pentose (12b). A solution of 3-(3-phenyl-1*R*,2*S*-dihydroxypropyl)-1,5-dihydro-3*H*-2,4-benzodioxepine[12] (200 mg, 0.70 mmol) in 0.1 N HCl (7 mL) was heated at 70 °C for 3 h. The solution was cooled to room temperature, and the pH was adjusted to 7.5. Acetaldehyde (0.12 mL, 2.1 mmol) and DERA (280 U) were added, and the solution was maintained at 25 °C in the dark for 2 days. Purification by column chromatography ($CHCl_3$/MeOH, 2:1) afforded **12b** (67 mg, 46%) as a thick oil: ^1H NMR (400 MHz, CD_3OD) δ 1.36 (dd, J = 4.9, 15.1 Hz, 1H), 1.45 (dd, J = 5.2, 15.1 Hz, 1H), 3.40 (m, 1H), 3.58 (d, J = 5.0 Hz, 1H), 3.71 (dd, J = 4.0, 5.2 Hz, 1H), 5.10 (d, J = 3.1 Hz, 1H), 7.27−7.44 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 40.35, 62.90, 73.50, 73.94, 106.65, 127.80, 128.17, 128.54, 128.63, 129.15, 140.81; HRMS for $C_{11}H_{14}O_4$ ($M + Na^+$) calcd 233.0790, found 233.0779.

***S*-(2,3-Dihydroxypropyl)-2-deoxy-5-thio-D-*erythro*-pentose (14).** Using the general procedure, compound **14** was obtained in 27% yield: ^1H NMR (500 MHz, D_2O) δ 1.80 (ddd, J = 2.3, 3.5, 14.5 Hz, 1H), 2.07−2.10 (m, 2H), 2.38 (dt, J = 5.5, 14.0 Hz, 1H), 2.58 (dd, J = 8.0, 14.0 Hz, 1H), 2.55 - 2.61 (m, 1H), 2.72 (dd, J = 2.5, 14.0 Hz, 1H), 2.73 (dd, J = 1.5, 14.0 Hz, 1H), 3.48 (dd, J = 6.0, 14.0 Hz, 1H), 3.46−3.50 (m, 1H), 3.57 (dd, J = 2.3, 13.8 Hz, 1H), 3.58 (dd, J = 2.3, 13.8 Hz, 1H), 3.75−3.80 (m, 2H), 3.88−3.92 (m, 1H), 4.12−4.17 (m, 2H), 4.30 (q, J = 5.5 Hz, 1H), 5.46 (dd, J = 2.0, 5.5 Hz, 1H), 5.52 (t, J = 4.0 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 35.1, 35.7, 35.8, 36.1, 41.3, 65.0, 71.4, 71.5, 74.1, 74.3, 84.5, 85.5, 98.5, 98.9; HRMS for $C_8H_{16}O_5S$ ($M + Na^+$) calcd 247.0616, found 247.0611.

Bis(2,5-dideoxy-5-D-*erythro*-pentofuranosyl)sulfide (16). Using the general procedure, compound **16** was obtained in 58% yield: ^1H NMR (400 MHz, D_2O) δ 1.86 (dt, J = 3.5, 14.3 Hz, 1H), 2.10−2.19 (m, 2H), 2.45 (dt J = 5.9, 14.2 Hz, 1H), 2.65−2.88 (m, 4H), 3.95−4.00 (m, 1H), 4.18−4.23 (m, 2H), 4.33−4.38 (m, 1H), 5.52 (br d, J = 5.0 Hz, 1H), 5.58 (t, J = 4.6 Hz, 1H); ^{13}C NMR (100 MHz, D_2O), δ 34.53, 34.62, 35.59, 35.74, 40.77, 73.46, 73.69, 84.03, 84.14, 84.84, 84.88, 98.00, 98.31; HRMS for $C_{10}H_{18}O_6S$ ($M + Na^+$) calcd 289.0722, found 289.0723.

2,4,6-Trideoxy-D-*erythro*-hexose (17a). The reaction was performed according to the general procedure on a 20 mL scale with 1000 units of DERA. After stirring for 6 days and workup, the crude product was purified by flash chromatography (silica, EtOAc) to give **17a** (60 mg, 22%) as a mixture of anomers (α:β ratio in D_2O 1:8): ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (d, J = 6.3 Hz, 3H, α), 1.23 (d, J = 6.3 Hz, 3H, β), 1.42−2.00 (m, 4H), 3.09 (d, J = 6.2 Hz, 1H, α), 3.43 (d, J = 5.1 Hz, 1H, β), 4.07 (ddq, J = 2.2, 6.3, 11.4 Hz, 1H, β), 4, 17 (s, 1H), 4.18 (s, 1H), 4.18−4.24 (m, 1H, α), 4.32 (dq, J = 2.7, 5.4 Hz, 1H, β), 4.42 (ddq, J = 2.3, 6.3, 11.8 Hz, 1H, α), 5.16 (br d, J = 10.2 Hz, 1H, β), 5.32 (t, J = 4.8 Hz, 1H, α); α-anomer, ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.43, 34.90, 39.86, 59.00, 65.02, 92.25; β-anomer, ^{13}C NMR ($CDCl_3$) δ 21.31, 39.42, 39.55, 65.59, 66.57, 92.97; HRMS for $C_6H_{12}O_3$ ($M + Na^+$) calcd 155.0684, found 155.0684.

6-*O*-Methyl-2,4-dideoxy-D-*erythro*-hexose (17b). The reaction was performed according to the general procedure on a 20 mL scale with 1000 units of DERA. After stirring for 6 days and workup, the crude product was purified by flash chromatography (silica, EtOAc to EtOAc/MeOH 12:1) to give **17b** (211 mg, 65%) as a mixture of anomers (α:β ratio in D_2O 1:7): ^1H NMR (400 MHz, $CDCl_3$) δ 1.45−1.97 (m, 4H), 3.35 (s, 3H, β), 3.36 (s, 3H, α), 3.35−3.46 (m, 2H), 4.08−4.15 (m, 1H, β), 4.17−4.20 (m, 1H, α), 4.28−4.32 (m, 1H, β), 4.42−4.49 (m, 1H, α), 5.14 (dd, J = 2.2, 9.8 Hz, 1H, β), 5.34 (d, J = 3.3 Hz, 1H, α); α-anomer, ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.11, 34.81, 59.08, 62.23, 64.35, 75.54, 92.14; β-anomer, ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.83, 39.30, 59.05, 64.88, 69.51, 75.76, 92.70; HRMS for $C_7H_{14}O_4$ ($M + Na^+$) calcd 185.0790, found 185.0796.

6-Chloro-2,4,6-trideoxy-D-*erythro*-hexose (17c). The reaction was performed according to the general procedure on a 20 mL scale with 1000 units of DERA. After stirring for 6 days and workup, the crude product was purified by flash chromatography (silica, EtOAc/hexane 2:1 to 3:1) to give **17c** (235 mg, 70%) as a mixture of anomers (α:β ratio in D_2O 1:6): ^1H NMR (400 MHz, $CDCl_3$) δ 1.53−2.00 (m, 4H), 3.52−3.62 (m, 2H), 4.12−4.18 (m, 1H, β), 4.23−4.28 (m, 1H, α), 4.34−4.38 (m, 1H, β), 4.45−4.52 (m, 1H, α), 5.20 (dd, J = 2.1, 9.5 Hz, 1H, β), 5.37 (br t, J = 4.1 Hz, 1H, α); α-anomer, ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.07, 39.23, 47.03, 63.24, 64.89, 92.54; β-anomer, ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.74, 35.35, 47.75, 64.49, 70.50, 93.03; HRMS for $C_6H_{11}O_3Cl$ ($M + Na^+$) calcd 189.0294, found 189.0288.

6-Azido-2,4,6-trideoxy-D-*erythro*-hexose (17d). The reaction was performed according to the general procedure on a 20 mL scale with 1000 units of DERA. After stirring for 6 days and workup, the crude product was purified by flash chromatography (silica, EtOAc/hexane 1:1 to 2:1) to give **17d** (81 mg, 23%) as a mixture of anomers (α:β ratio 2:3): ^1H NMR (400 MHz, D_2O) δ 1.54−1.98 (m, 4H), 3.37−3.51 (m, 2H), 4.09−4.16 (m, 1H, β), 4.42−4.27 (m, 1H, α), 4.35−4.39 (m, 1H, β), 4.41−4.48 (m, 1H, α), 5.15 (dd, J = 2.0, 10.0 Hz, 1H, β), 5.31 (br t, J = 2.2 Hz, 1H, α); α-anomer, ^{13}C NMR (100 MHz, D_2O) δ 36.02, 37.64, 56.49, 65.61, 66.44, 94.01; β-anomer, ^{13}C NMR (100 MHz, D_2O) δ 35.94, 40.46, 56.69, 66.98, 72.55, 94.44; HRMS for $C_6H_{11}O_3N_3$ ($M + Na^+$) calcd 196.0698, found 196.0706.

2,4,6,7-Tetradeoxy-D-*erythro*-octapyranuronic Acid (18). The reaction was performed according to the general procedure on a 20 mL scale with 1000 units of DERA and in the absence of buffer to facilitate workup. After stirring for 4 days, the crude product was purified by flash chromatography (silica, EtOAc/MeOH, 6:1 to 1:1) to give the acid **18** (305 mg, 80%) as a mixture of anomers (α:β ratio 1:5): ^1H NMR (400 MHz, D_2O) δ 1.47−1.98 (m, 6H), 2.45−2.57 (m, 2H), 3.87−3.95 (m, 1H, β), 4.19−4.28 (m, 2H, α), 4.31−4.35 (m, 1H, β), 5.09 (dd, J = 2.1, 10.1 Hz, 1H, β), 5.23 (t, J = 3.4 Hz, 1H, α); α-anomer, ^{13}C NMR (100 MHz, D_2O) δ 31.62, 32.76, 38.44, 38.79, 65.87, 67.31, 93.58, 181.06; β-anomer, ^{13}C NMR (100 MHz, D_2O) δ 32.42, 32.66, 38.76, 40.60, 67.31, 72.65, 94.47, 181.06; HRMS for $C_8H_{14}O_5$ ($M + Na^+$) calcd 213.0739, found 213.0745.

1,3-Di-*O*-trityl(1,2,3-$^{13}C_3$)glycerol (20). To a solution of (1,2,3-$^{13}C_3$)glycerol (**22**) (400 mg, 4.21 mmol), DMAP (49.5 mg), and pyridine (2.2 mL) in CH_2Cl_2 (20 mL) was added trityl chloride (2.333 g), and the reaction mixture stirred for 18 h at room temperature. The mixture was washed with H_2O, 1 N HCl, $NaHCO_3$, and H_2O. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to yield a residue which was purified by column chromatography (SiO_2, hexane/EtOAc/CH_2Cl_2, 50:2:2 to 50:8:5) to give **20** (2.229 g, 92%) as a thick oil: ^1H NMR (400 MHz, $CDCl_3$) δ 2.26−2.30 (dm, J = 2.6 Hz, 1H), 3.25 (dm, J = 142.9 Hz, 1H), 3.30 (dm, J = 143.1 Hz, 2H), 3.95 (dm, J = 142.9 Hz, 1H), 7.20−7.40 (m, 30H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 64.40 (d, J = 42.4 Hz, 2C), 70.18 (t, J = 42.7 Hz, 1C), 127.00, 127.81, 128.65, 143.80; HRMS for $^{12}C_{38}{}^{13}C_3H_{36}O_3$ ($M + Cs^+$) calcd 712.1819, found 712.1819.

Di-*O*-trityldihydroxy(1,2,3-$^{13}C_3$)acetone (21). To a solution of 1,3-di-*O*-trityl(1,2,3-$^{13}C_3$)glycerol (1.77 g, 3.06 mmol) containing powdered molecular sieves (4 Å, 1.1 g) in anhydrous CH_2Cl_2 (50 mL) was added PDC (1.3 g) in portions, and the reaction mixture was stirred for 21 h at room temperature. Another portion of PDC (0.65 g) was added, and the solution was stirred for additional 19 h, passed through a short neutral Al_2O_3 column (activated, standard grade, 150 mesh), and eluted with CH_2Cl_2. A portion (7.5%, 133 mg) of the crude product was purified by preparative TLC (hexane/EtOAc/CH_2Cl_2, 50:6:5) to give **21** (99.0 mg, 75% yield) as a thick oil: ^1H NMR (400 MHz, $CDCl_3$) δ 3.97 (ddt, J = 4.6, 7.0, 143.0 Hz, 4H), 7.22−7.39 (m, 30H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.81 (d, J = 45.7 Hz, 2C), 127.24, 127.95, 128.43, 143.06, 204.54 (t, J = 45.3 Hz, 1C); HRMS for $^{12}C_{38}{}^{13}C_3H_{34}O_3$ ($M + Cs^+$) calcd 710.6016, found 710.6030.

Dihydroxy(1,2,3-$^{13}C_3$)acetone (22). The majority (63.7%, 1.13g) of the crude di-*O*-trityldihydroxy(1,2,3-$^{13}C_3$)acetone was washed consecutively with 1 N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$ and evaporated *in vacuo* to give a white solid which was redissolved in CH_2Cl_2 (90 mL) and methanol (16 mL). After the addition of $TsOH{\cdot}H_2O$ (50 mg) and stirring for 17 h at room temperature, the reaction mixture was extracted with water (3 × 16 mL). The aqueous layers were combined, neutralized, concentrated (to about 8 mL), and used in the enzymatic aldol reaction without further purification.

2-Deoxy(1,2,3,4,5-$^{13}C_5$)ribose-5-phosphate (25). To a 0.1 M HEPES solution (20 mL, pH 7.4) containing crude dihydroxy(1,2,3-$^{13}C_3$)acetone (**22**) (prepared as above), PEP (trisodium salt, 182 mg),

ATP (15 mg), acetaldehyde (1,2-$^{13}C_2$, 3.2 mmol), $MgCl_2$ (32.5 mg), KCl (121 mg), 2-deoxyribose-5-phosphate aldolase (54 U), glycerokinase (92 U, from *Cellulomonas sp.*), pyruvate kinase (392 U, type X, from chicken muscle), and triose phosphate isomerase (1590 U, type IIIS, from rabbit muscle) were added, and the mixture was stirred at room temperature. Another portion of PEP (179 mg) was added after 22 h, and the solution was stirred for an additional 27 h. The pH of the solution was adjusted to 8.0, $BaCl_2 \cdot 2H_2O$ (1.5 g) was added, and the solution was stirred for 40 min. Two volumes of ethanol were added, and the mixture was refrigerated for 1.5 days affording a white solid. The solid was centrifuged, washed with ethanol, dried *in vacuo*, and treated with Dowex 50-H^+ to give a clear solution. The overall yield of **25** from di-*O*-trityl-dihydroxy(1,2,3-$^{13}C_3$)acetone (**21**) was 79% (2 steps) as determined by enzymatic assay. For purification, 25 mg of the crude 2-deoxy(1,2,3,4,5-$^{13}C_5$)ribose-5-phosphate was dissolved in water (2 mL), Dowex 50-H^+ (100 mg) was added, and the reaction mixture was stirred for 3 h. The Dowex resin was then removed by filtration, and the solution was adjusted to pH 7.0 (NaOH) and concentrated to about 0.5 mL. This solution was then applied to a DEAE Sephadex A-25 column and eluted with a gradient of NH_4HCO_3 from 0−0.5 M. Each fraction (*ca.* 1 mL) was lyophilized, and the residue was examined by ^{13}C NMR: ^{13}C NMR (100 MHz, D_2O) δ 98.8 (d, J = 40.0 Hz), 98.5 (d, J = 40.0 Hz), 85.2 (ddd, J = 8.2, 30.4, 44.0 Hz), 84.8 (ddd, J = 7.9, 31.5, 44.2 Hz) 71.9 (dd $J_1 = J_2$ = 36.0 Hz) 71.5 (dd, $J_1 = J_2$ = 36.5 Hz) 65.4 (d, J = 42.5 Hz), 64.5 (d, J = 42.1 Hz), 41.3 (dd, J = 12.2, 39.1 Hz) 41.0 (dd, J = 12.7, 39.0 Hz).

3-(3,3-Diethoxy-2-(*R,S*)hydroxypropyl)-3-mercapto-1,2-propanediol (27). To a solution of glycidaldehyde diethyl acetal (309 mg, 2.1 mmol) and 3-mercapto-1,2-propanediol (235 mg, 2.1 mmol) in anhydrous THF (10 mL) was added NaH (0.48 mmol) at −40 °C under argon. After 3 days at room temperature, the reaction mixture was quenched with 1 N HCl at −40 °C and neutralized with 1 N NaOH. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography ($CHCl_3$ to methanol/$CHCl_3$, 1:17) to afford **27** (533 mg, 68.6%) as a syrup: ^1H NMR (500 MHz, D_2O) δ 1.16(t, J = 7.1 Hz, 6H), 1.16 (t, J = 7.0 Hz, 6H), 2.52 (dd, J = 7.5, 13.5 Hz, 1H), 2.56 (dd, J = 7.5, 14.0 Hz, 1H), 2.58 (dd, J = 7.5, 14.0 Hz, 1H), 2.62 (dd, J = 8.0, 14.0 Hz, 1H), 2.71 (d, J = 1.7, 14.0 Hz, 1H), 2.72 (d, J = 1.7, 14.0 Hz, 1H), 2.82 (dd, J = 3.3, 13.8 Hz, 2H), 3.49 (dd, J = 2.8, 13.3 Hz, 1H), 3.50 (dd, J = 2.8, 13.3 Hz, 1H), 3.57−3.64 (m, 6H), 3.66−3.80 (m, 8H), 4.47 (d, J = 5.5 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 15.2, 34.9, 35.0, 35.8, 35.9, 64.9, 65.1, 65.4, 71.3, 71.5, 104.4; HRMS for $C_{10}H_{22}O_5S$ ($M + Na^+$) calcd 277.1086, found 277.1072. Aldehyde **13** was revealed by stirring **27** in 0.1 N HCl until the starting material disappeared when monitored by TLC. This solution was then used in the enzymatic reaction.

Bis(3,3-diethoxy-(2*S*)-hydroxypropyl)sulfide (28). A solution of potassium thiolacetate (205 mg, 1.8 mmol) in ethanol (4 mL) was added to (*R*)-glycidaldehyde diethyl acetal (3.6 mmol) at −5 °C, and the reaction mixture was stirred at room temperature under the exclusion of oxygen (argon atmosphere). An additional portion of potassium thiolacetate (25 mg) was added after 1 h, and the solution was stirred for an additional 6 h. The solvent was evaporated, and the residue was partitioned between CH_2Cl_2 and water. The aqueous phase was extracted with dichloromethane, the combined organic layers were washed with brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. Purification of the residue by column chromatography (CH_2Cl_2/ether, 6:1) afforded **28** (211 mg, 36%) as a colorless oil: $[\alpha]^{21}_D$ +54° (c = 3.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.22 (t, J = 7.0 Hz, 6H), 1.24 (t, J = 7.0 Hz, 6H), 2.70 (dd J = 7.5, 14.0 Hz, 2H), 2.89 (br s, 2H), 2.92 (dd, J = 3.5, 14.0 Hz, 2H), 3.56−3.63 (m, 4H), 3.71−3.80 (m, 6H), 4.45 (d, J = 5.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.3, 35.1, 63.5, 63.8, 71.4, 103.7; HRMS for $C_{14}H_{30}O_6S$ ($M + Na^+$) calcd 349.1661, found 349.1661. Aldehyde **15** was revealed by stirring **28** in 0.1 N HCl until the starting material disappeared when monitored by TLC. This solution was then used in the enzymatic reaction.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for selected compounds **4**, **8** (α and β triacetates), **10**, **14**, **16**, **17a−d**, and **18** (19 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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