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Synthesis of Analogues of 1,3-Dihydroxyacetone Phosphate and Glyceraldehyde 3-Phosphate for Use in Studies of Fructose-1,6-diphosphate Aldolase[1]

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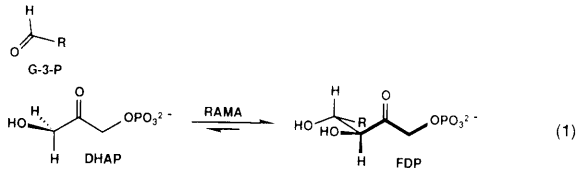
This paper describes the syntheses of five analogues of dihydroxyacetone phosphate (3-azidohydroxyacetone 1-phosphate (**5**), 3-(acetylamino)hydroxyacetone 1-phosphate (**12**), (*R*)-1,3-dihydroxy-2-butanone 1-phosphate (**18**), (±)-1,3-dihydroxy-2-butanone 3-phosphate (**26**), and phosphonomethyl glycolate (**31**)). The syntheses of **18** and **26** are based on a new reaction: that is, the introduction of the phosphate group by reaction of a diazo ketone with dibenzyl phosphate. These methods provide easy access to a number of compounds that are potential substrates for the synthetically useful enzyme aldolase (fructose-1,6-phosphate aldolase from rabbit muscle, EC 4.1.2.13, RAMA) and perhaps for other enzymes of glycolysis. This paper also describes syntheses of 14 aldehydes for examination as substrates for aldolase. When the precursor was available, ozonolysis of vinyl groups proved to be the best route to the corresponding aldehydes.

Introduction

Fructose-1,6-diphosphate aldolases catalyze the reaction of dihydroxyacetone phosphate (DHAP) with D-glyceraldehyde 3-phosphate (G-3-P) (eq 1).[7,8] The stereochemistry of the reaction is that indicated in eq 1; the enzyme removes the *pro-S* hydrogen of DHAP.[9] The enzyme from rabbit muscle (EC 4.1.2.13, RAMA) is commercially available and inexpensive (~$20/500 U, 1 U = 1 μmol of product formed per min with DHAP and G-3-P). It is useful as a catalyst in the synthesis of carbohydrates,[10-17] and its broader applicability in organic synthesis is now being actively developed.[18,19] The conclusions from these studies are (1) aldolase accepts a variety of aldehydes as substrates and (2) it will accept only a few close analogues of DHAP.



(1)

As part of a research program designed to explore the substrate specificity of aldolase and to evaluate the utility of its reactions in organic synthesis, we required a number of analogues of the natural substrates.[19] DHAP and G-3-P are relatively sensitive compounds, and syntheses of structural analogues of these substances are not trivial. Here we report synthetic routes to five new analogues of DHAP and 14 analogues of G-3-P. The best synthesis of DHAP analogues is based on a new method to generate

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(2) Postdoctoral Fellow 1984-1985; N.B. thanks the OeBWK, Austria and Ciba-Geigy, Basel, for generous support.
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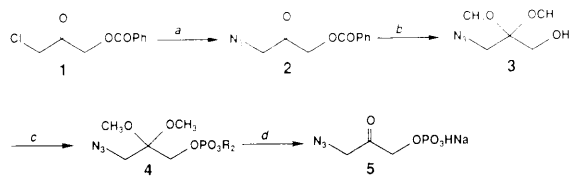
(4) DuPont Fellow, 1986-87.
(5) Natural Science and Engineering Research Council of Canada Predoctoral Trainee.
(6) American Cancer Society Postdoctoral Fellow, Grant No. PF-2762 1987-88.
(7) Morse, D. F.; Horecker, B. L. In *Advances in Enzymology*; Nord, F. F., Ed.; Interscience: New York, 1968; Vol 31, pp 126-175.
(8) Horecker, B. L.; Tsolas, O.; Lai, C. Y. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1972; Vol. VII, pp 213-258.
(9) Rose, I. A. *J. Am. Chem. Soc.* **1958**, *80*, 5835.
(10) Bednarski, M. D.; Waldmann, H. J.; Whitesides, G. M. *Tetrahedron Lett.* **1986**, *27*, 5807.
(11) Durrwachter, J. R.; Drueckhammer, D. G.; Nozaki, K.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812.
(12) Durrwachter, J. R.; Sweers, H. M.; Nozaki, K.; Wong, C.-H. *Tetrahedron Lett.* **1986**, *27*, 1261.
(13) Kapuscinski, M.; Franke, F. P.; Flanigan, J.; MacLeod, J. K.; Williams, J. F. *Carbohydr. Res.* **1985**, *140*69.
(14) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199.
(15) Wong, C.-H.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3493.
(16) Webster, D.; Jondorf, W. R.; Dixon, H. B. F. *Biochem. J.* **1976**, *155*, 433.
(17) Jones, J. K. N.; Sephton, H. H. *Can. J. Chem.* **1960**, *38*, 753.
(18) Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641.
(19) Bednarski, M. D.; Bischofberger, N.; Kim, M.-J.; Lees, W.; Saito, T.; Simon, E. S.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc.*, to be submitted.

α-hydroxy ketone phosphates by reaction of diazo ketones with dibenzyl phosphate. The best route for the synthesis of analogues of G-3-P is the ozonolysis of terminal olefins, when these olefins are accessible. These methods should be useful for the preparation of a number of other substances that are potential substrates of aldolase, triosephosphate isomerase, *sn*-glycerol-3-phosphate dehydrogenase, and perhaps of other enzymes of glycolysis.

We describe detailed studies of the reactivity of these compounds in reactions catalyzed by aldolase, and of their utility in synthesis, in a separate paper devoted to that subject.[19]

Results

Synthesis of Analogues of DHAP. 3-Azidohydroxyacetone 1-Phosphate (5). Following the established procedure for the preparation of halo hydroxy acetone phosphates,[20] chloromethyl ketone **1** (prepared in two steps from 1-chloropropane-2,3-diol[20] was converted to the azidomethyl ketone **2** (eq 2). Ketalization, ester

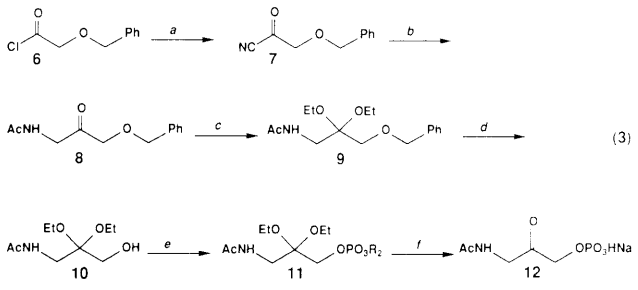


(2)

(a) NaN_3, CH_3COCH_3–H_2O, 12 h (93%); (b) $HC(OCH_3)_3$, H_2SO_4, MeOH, 2 days (73%); (c) $POCl_3$, pyridine, THF, 0 °C, 1 h; pyridine–water; ion exchange, Dowex 50W-X1 cyclohexylammonium form (26%), R = c-$C_6H_{11}NH_3^+$; (d) Dowex 50W-X1, H^+ form, 60 °C, 18 h; $NaHCO_3$ (71%).

hydrolysis, phosphorylation, and hydrolysis of the ketal yielded compound **5**. Efforts to reduce the azide group of **5** to an amino functionality resulted in reduction of the ketone group.

3-(Acetylamino)hydroxyacetone 1-phosphate (12) was synthesized by using a related procedure (eq 3).

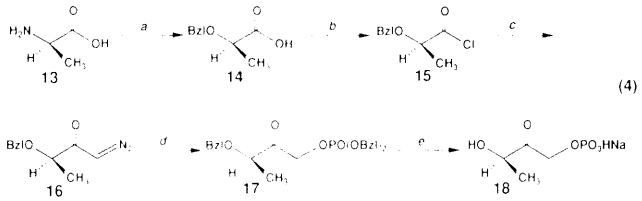


(3)

(a) TMSCN, 120 °C, 2 h (86%); (b) Zn, Ac_2O–AcOH, 1 h (71%); (c) $C(OEt_3)_3$, H_2SO_4, EtOH, 1 day (68%); (d) 10% Pd–C/H_2, EtOH, 3 days (91%); (e) $POCl_3$, pyridine, THF, 0 °C, 30 min; pyridine–water; ion exchange, Dowex 50W-X1, H^+ form, 80 °C, 5 h; $NaHCO_3$ (80%).

Treatment of the acid chloride **6**[21] with trimethylsilyl cyanide gave the acyl cyanide **7**. Reduction of **7** with zinc in acetic acid/acetic anhydride[22] occurred smoothly without affecting the α-alkoxymethyl ketone moiety and yielded **8**. Ketalization, deprotection, phosphorylation, and deketalization gave **12**.

The synthesis of **(*R*)-1,3-dihydroxy-2-butanone 1-phosphate (18)** illustrates the introduction of the phosphate group by a reaction of a diazo ketone with dibenzyl phosphate (eq 4). Aldolase selectively removes the *pro-S*



(4)

(a) Isoamyl nitrite, $MgSO_4$, $PhCH_2OH$, reflux, 2 h (27%); (b) $SOCl_2$, reflux; (c) CH_2N_2, Et_2O (79%); (d) $PhCH_2O)_2P(O)OH$, PhH, 60 °C, 12 h (89%); (e) 10% Pd–C/H_2, AcOH, 12 h; $NaHCO_3$ (37%).

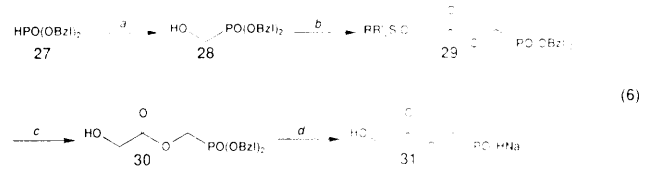
hydrogen of DHAP,[9] so we expected that only the *R* isomer **18** would be a substrate for aldolase. The starting material for our synthesis was the enantiomerically pure, benzyl-protected lactic acid chloride **15**. Previous preparations of this compound have started with α-bromopropionic acid and have required resolution.[23,24] We prepared both (*R*)- and (*S*)-*O*-benzyllactic acid from (*R*)- and (*S*)-alanine, respectively, with isoamyl nitrite using benzyl alcohol as a solvent.[25] The reaction proceeded with [illegible] retention of configuration (as judged from optical rotation), albeit in modest yield (27%). *O*-Benzyllactic acid was converted to its acid chloride[23] and subsequently treated with diazomethane to yield compound **16**. This diazomethyl ketone reacted with dibenzyl phosphate to give the protected phosphate **17**. Hydrogenation over palladium using acetic acid as solvent gave **18** in moderate yield; hydrogenation in methanol failed.

1,3-Dihydroxy-2-butanone 3-phosphate (26) was synthesized by using a strategy like that used in the preparation of **18** (eq 5). Due to the difficulties encoun-

(a) *t*-$Bu(CH_3)_2SiCl$, imidazole, 4 h (87%); (b) KOH, MeOH, H_2O, THF, −10 °C → 5 °C, 30 min, (90%); (c) $(COCl)_2$, PhH, reflux, 30 min; (d) CH_3CHN_2, Et_2O, (79%, 21 → 23); (e) $(PhCH_2O)_2P(O)OH$, PhH, 2 h (53%); (f) AcOH, H_2O, THF, reflux, 1 h (42%); 10% Pd–C/H_2, MeOH, 1 h (100%).

tered in the debenzylation of **17**, we employed a *tert*-butyldimethylsilyl protecting group.

Phosphonomethyl Glycolate (31). The phosphonate **29** was prepared in two steps from dibenzyl phosphonate (eq 6).[26] Cleavage of the silyl protecting group occurred



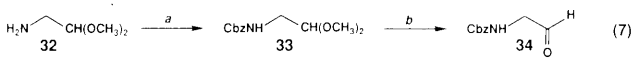
(6)

(a) Paraformaldehyde, Et_3N, PhH, reflux, 2 h (27%); (b) $(COCl)_2$, $PhCH_3$, **21** (58%); (c) AcOH, H_2O, THF, 24 h (89%); (d) 10% Pd–C/H_2, MeOH, 1 h (100%).

(20) Hartmann, F. C. *Biochemistry* **1970**, *9*, 1776.
(21) Benington, F.; Morin, R. D. *J. Org. Chem.* **1961**, *26*, 194.
(22) Pfaltz, A.; Anwar, S. *Tetrahedron Lett.* **1984**, *25*, 2977.
(23) Levene, P. A. *J. Biol. Chem.* **1936**, *113*, 153.
(24) Pinnen, F.; Zanotti, G.; Lucente, G. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1311.
(25) Hindley, N. C.; Low, J. A. U.S. Pat. 2480326, 1949; *Chem. Abstr.* **1949**, *43*, 9086i.
(26) Phillion, D. P.; Andrew, S. S. *Tetrahedron Lett.* **1986**, *27*, 1477.

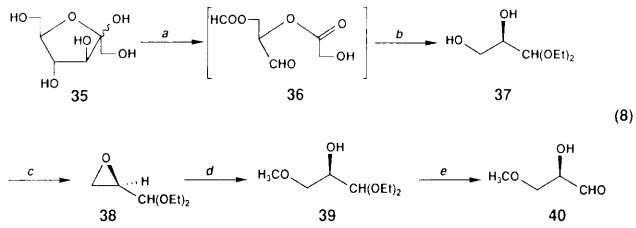
at room temperature by using a mixture of aqueous tetrahydrofuran and acetic acid as solvent;[27] hydrogenation removed the benzyl protecting group to give **31**.

Synthesis of Aldehydes. *N*-(Benzyloxycarbonyl)-aminoacetaldehyde (34) was prepared by two procedures: that shown in eq 7 and ozonolysis. The latter was the


(7)

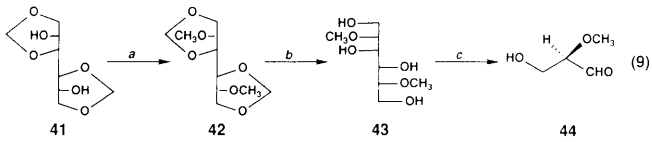
(a) $PhCH_2OCOCl$, EtOAc, pyridine (50%); (b) $(COOH)_2$, THF, H_2O, reflux, 4 days (72%).

preferred method (eq 14). The synthesis of **3-*O*-methyl-D-glyceraldehyde (40)** started with fructose (**35**);[28] the optical purity of **39** was established by measuring its optical rotation (eq 8). The preparation of **2-*O*-**


(8)

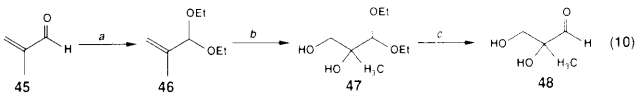
(a) AcOH, $Pb(OAc)_4$, $(COOH)_2$; (b) $HC(OEt)_3$, NH_4NO_3, EtOH (24%, **35** → **37**); (c) CH_3SO_2Cl, pyridine −10 °C → 0 °C; NaOH, H_2O (53%); (d) NaOMe, MeOH, reflux, 24 h (76%); (e) H_2SO_4, 40 °C, 12 h (91%).

methyl-D-glyceraldehyde (44) started from mannitol and followed the route leading to the analogous 2-*O*-benzyl derivative (eq 9).[29] To circumvent problems in isolation,


(9)

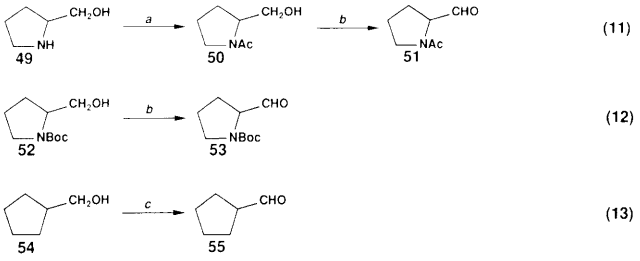
(a) NaH, MeI, $(CH_3OCH_2)_2$, 37–40 °C, 16 h (85%); (b) Ac_2O, AcOH, H_2SO_4 (60%); (c) Dowex 1-X8, IO_4^- form, H_2O (81%).

43 was oxidized with an anion exchange resin previously treated with periodate.[30] **(±)-2,3-Dihydroxy-2-methylpropanal (48)** was synthesized via oxidation and deketalization of **46**, prepared following the literature procedure for the preparation of acrolein diethyl acetal from acrolein (eq 10).[31]


(10)

(a) NH_4NO_3, EtOH, $HC(OCH_3)_3$, 40–45 °C, 3 h (68%); (b) $KMnO_4$, H_2O (45%); (c) Dowex 50W-X4, H^+ form (79%).

Oxidation of the corresponding N-protected alcohols by the method of Swern[32] led to ***N*-acetylprolinal (51)** and ***N*-(*tert*-butoxycarbonyl)prolinal (53)** (eq 11 and 12). Pyridinium dichromate oxidation of cyclopentane methanol **54** yielded **cyclopentanaldehyde (55)** (eq 13).[33]

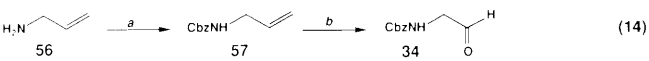

(11)

(12)

(13)

(a) Ac_2O, CH_2Cl_2 (95%); (b) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N; (c) PDC, CH_2Cl_2 (10%).

A general strategy based on ozonolysis[34] of an appropriately placed vinyl group followed by a reductive workup (zinc/acetic acid[34] or dimethyl sulfide[35]) provided aldehydes **34**, **59**, **61**, **64**, and **68** (eq 14–18). In the case of **34**, **59** and **61**, the corresponding protected allylamine derivative was the starting material. For the synthesis of aldehyde **68**, the addition of vinyl Grignard to benzaldehyde **65** followed by protection of the secondary alcohol with methyl iodide preceded ozonolysis.[36] In general, the approach based on ozonolysis gave cleaner reactions and higher yields of products than did other methods of forming aldehydes.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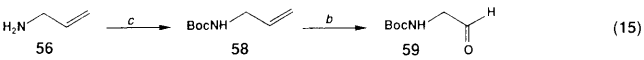
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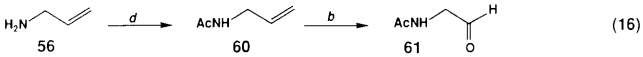
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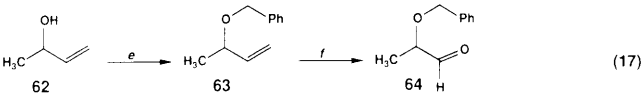
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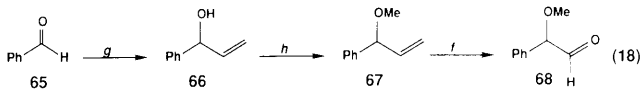
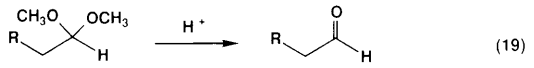

(18)

(a) $PhCH_2OCOCl$, K_2CO_3, EtOAc/H_2O (97%); (b) O_3, MeOH/CH_2Cl_2, Zn/AcOH (88–95%); (c) Et_3N, CH_2Cl_2, $(tBuOCO)_2O$, (91%); (d) Ac_2O (95%); (e) NaH, $PhCH_2Br$, THF (67%); (f) O_3, MeOH/CH_2Cl_2, $(CH_3)_2S$ (60–71%); (g) CH_2CH_2MgBr, THF (63%); (h) NaH, MeI, THF (71%).

Acid-catalyzed hydrolysis of the corresponding ketals lead to α-substituted aldehydes (eq 19). The concentra-


(19)

69, R = N_3
71, R = Cl
73, R = Br

70, R = N_3
72, R = Cl
74, R = Br

tion of these aldehydes was measured in aqueous solution by using the Willstaedter–Schudel alkaline iodide method.[37,38]

Discussion

Analogues of DHAP. Methods for the preparation of halohydroxyacetone phosphates have been described in the

(27) Corey, E. J.; Venkateswarlu, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(28) Racemic 3-*O*-methylglyceraldehyde has been reported as its dimer: Fischer, H. O. L.; Baer, E. *Ber.* **1932**, *65*, 345.
(29) Ballou, C. E.; Fischer, H. O. L. *J. Am. Chem. Soc.* **1955**, *77*, 3329.
(30) Harrison, C. R.; Hodge, P. *J. Chem. Soc., Perkin Trans. 1* **1982**, 509.
(31) Rabjohn, N. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 21.
(32) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.
(33) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.
(34) *Ozonation in Organic Chemistry*; Bailey, P. S., Ed.; Academic: New York, 1978; Vol. I.
(35) Pappas, J. J.; Keaveney, W. P.; Gancher, E.; Berger, M. *Tetrahedron Lett.* **1966**, 4273.
(36) Brown, C. A.; Barton, D. *Synthesis* **1974**, 434.
(37) Willstaetter, R.; Schudel, G. *Chem. Ber.* **1918**, *51*, 780.
(38) Baer, E.; Fischer, H. O. L. *J. Biol. Chem.* **1943**, *150*, 223.

literature,[20] but synthetic routes leading to more challenging analogues of dihydroxyacetone phosphate, especially the alkyl-branched compounds **18** and **26**, have not been reported. As demonstrated in eq 2–6, the former method can be extended to obtain additional heteroatom analogues of DHAP by nucleophilic displacement of halogens. The alkyl-branched derivatives were not available by using standard methods but could be synthesized by reaction of suitably functionalized acid chlorides, e.g., **15** and **22**, with diazoalkanes to form intermediate azo ketones, followed by introduction of the phosphate group with the aid of dibenzyl phosphate. This method should prove general for the synthesis of analogues of DHAP. We found the acid-labile *tert*-butyldimethylsilyl group to be superior to benzyl ether as a protecting group. The benzyl ethers exhibited an unexpected resistance to hydrogenolysis: we were not, for example, able to prepare **26** from the benzyl ether corresponding to **24**. The convenient synthesis of the protected epoxy aldehyde **38** from fructose provides another potential chiral building block for use in organic synthesis. Although the enantiomeric excess of **40** was not established independently, the method of synthesis suggests that it should be high.

DHAP is a substrate for three important enzymes: FDP aldolase, triosephosphate isomerase, and *sn*-glycerol-3-phosphate dehydrogenase.[39] Derivatives of DHAP have been suggested as medicinal agents.[40] The synthetic methods described in this paper provide routes to a variety of analogues of DHAP and should be useful in studying the enzymology and medicinal chemistry of this substance.

Analogues of G-3-P. The preparation of most aldehydes followed standard methods. Ozonolysis was especially effective because of its simplicity, high yields, and ease of product isolation. In addition, this method avoids introducing toxic metals into enzymatic reactions. In a reductive workup with dimethyl sulfide, for example, the side product (DMSO) is tolerated by aldolase. Time-consuming purification steps may thus be omitted.

Experimental Section

Materials and Methods. Chemicals were reagent grade. NMR spectra were recorded on a Bruker AM 300 (300-MHz ^{1}H NMR and 75-MHz ^{13}C NMR) or a Bruker AM 500 (500-MHz ^{1}H nMR and 125-MHz ^{13}C NMR) spectrophotometer with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standards. ^{31}P NMR spectra were recorded on a Bruker WM 300 machine (121.5 MHz). Optical rotations were measured with a Perkin-Elmer 241 polarimeter at room temperature unless otherwise noted. Elemental analyses have been performed by Galbraith Laboratories. High resolution mass spectra (HRMS) were obtained with a Kratos MS 50 spectrometer.

3-Azido-2-oxo-1-propyl Benzoate (2). To a solution of acetone (50 mL) and water (20 mL) was added a mixture of 3-chloro-2-oxo-1-propyl benzoate[20] (5 g, 23.5 mmol) and sodium azide (1.74 g, 26.8 mmol), and the solution was stirred at room temperature for 12 h. The reaction mixture was poured into methylene chloride (450 mL), extracted twice with water, and dried over $MgSO_4$. Concentration in vacuo yielded 4.8 g (93%) of a crystalline solid. An analytical sample was recrystallized from chloroform/cyclohexane (1:1): mp 61 °C; IR (KBr) 2100, 1735, 1605, 1270, 710 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.2–7.9 (m, 2 H), 7.7–7.25 (m, 3 H), 4.95 (s, 2 H), 4.1 (s, 2 H). Anal. Calcd for $C_{10}H_9N_3O_3$: C, 54.80; H, 4.14; N, 19.17. Found: C, 54.90; H, 4.32; N, 19.22.

3-Azido-2,2-dimethoxy-1-propanol (3). A mixture of the benzoate **2** (3.92 g, 17.9 mmol), trimethyl orthoformate (20 mL), methanol (10 mL), and concentrated sulfuric acid (two drops) was stirred at room temperature. After 2 days of analysis, TLC showed that all of the starting material had been consumed (cyclohexane/methylene chloride/ether 20:10:1). Potassium carbonate (2 g) was added and after stirring for an additional 24 h, the reaction mixture was poured into ethyl acetate (200 mL) and extracted twice with water and once with saturated aqueous NaCl. The organic layer was dried over $MgSO_4$ and the solvent was removed by evaporation in vacuo. The crude product (4.4 g) was chromatographed (silica gel, hexane/ethyl acetate 2:1 to 1:2) to yield **3** (2.1 g, 73%) as a colorless oil: IR (neat) 3620, 3500, 2120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.65 (d, J = 5 Hz, 2 H), 3.4 (s, 2 H), 3.3 (s, 6 H), 2.3 (s, 1 H, OH).

3-Azido-2,2-dimethoxy-1-propyl Phosphate, Bis(cyclohexylammonium salt) (4). A solution of 3-azido-2,2-dimethoxy-1-propanol (**3**) (2.5 g, 15.5 mmol) in tetrahydrofuran (50 mL) was added dropwise to a solution of phosphorus oxychloride (3 mL, 33 mmol) in pyridine (15 mL) and tetrahydrofuran (20 mL) at 0 °C. After stirring for 1 h at 0 °C, 40 mL of a solution of pyridine/water (1:3) was added. The solution was concentrated in vacuo and the residue was passed through an ion exchange column (Dowex 50W-X1, cyclohexylammonium form, 50–100 mesh, 3.5 × 15 cm). Addition of cyclohexylamine adjusted the solution to pH 8. The solution was evaporated repeatedly to dryness with continual addition of 2-propanol to remove water. The resulting oil was dissolved in a solution of 2-propanol/ethyl acetate/ether (1:1:1, 150 mL) and stored overnight at −10 °C. The resulting precipitate was separated by filtration and refluxed in 100 mL of ethanol. Filtration removed insoluble salts and the filtrate was diluted with ether (50 mL) and allowed to stand overnight. The precipitate was collected by filtration and crystallized from water/acetone (1:2.5). Concentration of the mother liquors and repeated crystallizations yielded **4** as a white powder (1.8 g, 26%): ^{13}C NMR (D_2O) δ 103.4, 63.9, 52.9, 51.7, 51.2, 32.9, 26.9, 26.4; ^{31}P NMR (D_2O, H_3PO_4) δ 4.1. Anal. Calcd for $C_{17}H_{38}N_5O_6P$: C, 46.46; H, 8.71; N, 15.93. Found: C, 46.63; H, 8.92; N, 15.63.

3-Azidohydroxyacetone 1-Phosphate (5). The cyclohexylammonium salt **4** (450 mg, 1 mmol) was dissolved in 2 mL of water and the solution was treated with ion exchange resin (Dowex 50W-X1, H^+ form). The resin was removed by filtration, the acidic eluant was heated on a steam bath at 60 °C for 18 h, and the solution was adjusted to pH 5.5 by addition of sodium bicarbonate. Water was added to bring the final volume to 5.0 mL and the concentration of compound **5** was determined by enzymatic assay[41] to 140 mM; 71% yield). The solution could be stored frozen for months without decomposing.

(Benzyloxy)acetyl Cyanide (7). A mixture of (benzyloxy)acetyl chloride (**6**)[21] (11 g, 59.4 mmol) and trimethylsilyl cyanide (TMSCN; 7.4 mL, 59.2 mmol)[42] was slowly heated to 120 °C over the course of 2 h. Trimethylsilyl chloride was removed by distillation and the residue remaining after cooling was distilled at reduced pressure to provide **7** as a colorless oil (9 g, 86%): bp (0.7 Torr) 95–105 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.0 (s, 5 H), 4.25 (s, 2 H), 3.85 (s, 2 H). Anal. Calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.52; H, 5.00; N, 8.32.

***N*-[2-Oxo-3-(benzyloxy)-1-propyl]acetamide (8).** To a slurry of 22 g of zinc (338 mg-atom) in a mixture of 35 mL of acetic acid and 35 mL of acetic anhydride was slowly added a solution of (benzyloxy)acetyl cyanide (**7**) (5.3 g, 30 mmol) in a solution of acetic acid (7 mL) and acetic anhydride (7 mL); the temperature of the solution was kept at 20 °C. After stirring the reaction mixture at room temperature for 1 h, the zinc was removed by filtration and washed with methylene chloride. The combined filtrate and washings were concentrated in vacuo and the resulting brown residue was chromatographed (silica gel, ethyl acetate) to provide **8** as a colorless oil (4.7 g, 71%): IR (neat) 3440, 3350, 1745, 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.3 (s, 5 H), 6.75 (s, 1 H, NH), 4.55 (s, 2 H), 4.25 (d, J = 5 Hz, 2 H), 4.15 (s, 2 H),

(39) Michal, G. In *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1984; Vol. VI, p 342.
(40) Rao, G. V.; Que, L.; Hall, L. D.; Fondy, T. P. *Carbohydr. Res.* **1975**, *40*, 311. Pero, R. W.; Babiarz-Tracy, P.; Fondy, T. P. *J. Med. Chem.* **1977**, *20*, 644.
(41) Lang, G. In *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1984; Vol. VI, pp 525–531.
(42) For the preparation of acyl cyanides, see: Huenig, S.; Schaller, R. *ACIEE* **1982**, *21*, 36. Herrmann, K.; Simchen, G. *Synthesis* **1979**, 204.

2.1 (s, 3 H). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 64.85; H, 6.60; N, 6.02.

***N*-[2,2-Diethoxy-3-(benzyloxy)-1-propyl]acetamide (9).** A mixture of the ketone **8** (2.0 g, 9 mmol), 10 mL of ethanol, 3 mL (18 mmol) of triethyl orthoformate, and two drops of sulfuric acid was stirred at room temperature for 1 day. Anhydrous potassium carbonate (200 mg) was added, and, after stirring overnight, the mixture was diluted with ether and extracted with a dilute solution of KOH and then with saturated aqueous NaCl. The organic layers were dried over $MgSO_4$ and concentrated in vacuo. Chromatography of the crude product (silica gel, ether/ethyl acetate 1:1 to ethyl acetate) yielded **9** as a yellowish oil (1.8 g, 68%): IR (neat) 3440, 3340, 2980, 2930, 2800, 1690, 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.30 (s, 5 H), 5.85 (s, 1 H, NH), 4.55 (s, 2 H), 3.8–3.3 (m, 8 H), 1.85 (s, 3 H), 1.15 (t, J = 7 Hz, 6 H). Anal. Calcd for $C_{16}H_{25}NO_4$: C, 65.05; H, 8.53; N, 4.76. Found: C, 65.23; H, 8.74; N, 4.41.

***N*-(2,2-Diethoxy-3-hydroxy-1-propyl)acetamide (10).** A solution of the ketal **9** (720 mg, 2.4 mmol) in 20 mL of ethanol was hydrogenated over 10% palladium on charcoal for 3 days. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to yield **10** as a colorless oil (450 mg, 91%): IR (neat) 1690, 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.3 (m, 1 H), 3.8–3.0 (m, 8 H), 2.05 (s, 3 H), 1.2 (t, J = 7 Hz, 6 H). Anal. Calcd for $C_9H_{19}NO_4$: C, 52.67; H, 9.33; N, 6.83. Found: C, 52.86; H, 9.04; N, 7.10.

3-(Acetylamino)-2,2-diethoxy-1-propyl Phosphate, Bis(cyclohexylammonium salt) (11). Following the procedure of Hartmann,[20] a solution of the diethyl ketal **10** (380 mg, 1.85 mmol) in 2.5 mL of tetrahydrofuran was added dropwise at 0 °C to a solution of phosphorus oxychloride (0.25 mL, 2.7 mmol), pyridine (0.5 mL, 6.2 mmol), and tetrahydrofuran (5 mL). After stirring at 0 °C for 30 min and quenching the reaction by slowly adding 3.5 mL of a solution of pyridine/water (1:6), the volatiles were removed in vacuo, and the remaining residue was passed through an ion exchange column (Dowex 50-X8, cyclohexylammonium form, 400 mesh, 8 × 1 cm). The eluant was adjusted to pH 8 by addition of cyclohexylamine, and the solution was evaporated to dryness in vacuo. The resulting white solid was treated with 10 mL of a solution of ethyl acetate/2-propanol (1:1), kept at 4 °C overnight, and then filtered. The precipitate was suspended in 20 mL of ethanol, and, after refluxing the suspension for 1 h, the insoluble portion (cyclohexylammonium phosphate) was removed by filtration. The filtrate was diluted with ether (10 mL) and kept below 0 °C for 24 h. The addition of 10 mL of ether caused a precipitate to form. The precipitate was isolated by filtration and recrystallized from water/acetone (1:1) to give **11** (170 mg, 19%): ^{31}P NMR (D_2O, H_3PO_4 as reference) δ 4.73. Anal. Calcd for $C_{21}H_{46}N_3O_7P$: C, 52.13; H, 9.58; N, 8.72. Found: C, 52.12, H, 9.26, N, 8.63.

3-(Acetylamino)hydroxyacetone 1-Phosphate (12). The bis(cyclohexylammonium salt) **11** (120 mg, 0.25 mmol) was dissolved in 1 mL of water, and the solution was passed through an ion exchange column (Dowex 50W-X1, H^+ form). The acidic eluant was heated at 80 °C for 5 h and the solution was then adjusted to pH 5.5 with sodium bicarbonate. Water was added to bring the final volume to 2.0 mL, and the content of **12** was determined by enzymatic assay[41] (c 100 mM, 80% yield).

(*S*)-2-(Benzyloxy)propionic Acid and (*R*)-2-(Benzyloxy)propionic Acid (14). A mixture of L-alanine (25.3 g, 284 mmol), benzyl alcohol (200 mL), isoamyl nitrite (40 mL, 299 mmol), and $MgSO_4$ (22 g) was heated under reflux for 2 h; gas evolution ceased after 1 h. The reaction mixture was allowed to cool to room temperature and was filtered. The filtrate was distilled under reduced pressure to yield the desired *S* acid (13.7 g, 27%): bp (0.05 Torr) 128–134 °C; $[\alpha]_D$ −53° (c 4.6, benzene) (lit.[23] $[\alpha]_D$ −62°). By the same procedure, starting from D-alanine, the corresponding (*R*)-(benzyloxy)propionic acid (**14**) (13.7 g, 36%) was obtained: $[\alpha]_D$ +56° (c 5.1, chloroform).

(*R*)-3-(Benzyloxy)-1-diazo-2-butanone (16). A solution of (*R*)-2-(benzyloxy)propionyl chloride (**15**) (2.31 g, 11.7 mmol; prepared from acid **14** by refluxing with $SOCl_2$ according to the literature[23]) in 5 mL of ether was added dropwise to a solution (36 mmol) of diazomethane (1.5 g) in ether (100 mL). Excess diazomethane was destroyed by the careful addition of acetic acid, and the solvent was removed by evaporation in vacuo. The product isolated after chromatography (silica gel, ether/hexane 1:1) was a yellow liquid (1.89 g, 79%): $[\alpha]_D$ +32° (c 4.8, chloroform); IR (neat) 2100, 1640, 1500, 1100, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4 (s, 5 H), 5.7 (s, 1 H), 4.5 (s, 2 H), 3.9 (q, J = 8 Hz, 1 H), 1.35 (d, J = 8 Hz, 3 H). The product decomposed on storing and was used immediately in the next step.

(*R*)-Dibenzyl 3-(Benzyloxy)-2-oxo-1-butyl Phosphate (17). A mixture of the diazo compound **16** (1.52 g, 7.4 mmol) and dibenzyl phosphate (2.41 g, 8.7 mmol) in benzene (40 mL) was heated at 60 °C for 12 h. The benzene was removed by evaporation in vacuo, and the crude product was chromatographed (silica gel, hexane/ethyl acetate/chloroform 2:1:1 to 1:1:1) to yield compound **17** as a colorless liquid (3.0 g, 89%): $[\alpha]_D$ +10.2° (c 5.0, chloroform); IR (neat) 1750, 1280, 1270, 1035, 1010, 1000, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.30 (s, 15 H), 5.10 (d, J = 8 Hz, 4 H), 5.00 (m, 1 H), 4.70 (m, 1 H), 4.50 (s, 2 H), 4.05 (q, J = 8 Hz, 1 H), 1.30 (d, J = 8 Hz, 3 H). Anal. Calcd for $C_{25}H_{27}O_6P$: C, 66.06; H, 5.99. Found: C, 66.15; H, 6.05.

(*R*)-3-Hydroxy-2-oxo-1-butyl Phosphate (18). A solution of the protected phosphate **17** (2.5 g, 5.5 mmol) in 50 mL of acetic acid was hydrogenated under 50 psi of hydrogen with 10% palladium on charcoal for 12 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in water, and the solution was adjusted to pH 7 with a solution of 1 N NaOH. A solution of 5 g of barium chloride in 20 mL of water was added, followed by 40 mL of ethanol. The mixture was chilled, and the precipitate was collected by centrifugation and redissolved in water by treatment with ion exchange resin (Dowex 50W-X1, H^+ form). The resin was removed by filtration, the solution was adjusted to pH 5.5, and the water was removed by distillation to yield a white powder (1.2 g, 35% pure as determined by enzymatic assay,[41] 37% yield): ^{13}C NMR (D_2O) δ 61.9, 58.2, 9.7.

***n*-Butyl [(*tert*-Butyldimethylsilyl)oxy]acetate (20).** To a mixture of *n*-butyl glycolate (**19**) (6.0 g, 45 mmol) and imidazole (9 g, 132 mmol) was added *tert*-butyldimethylsilyl chloride (8 g, 53 mmol) at 0 °C. After being stirred at room temperature for 4 h, distillation yielded **20** as a colorless liquid (9.7 g, 87%): bp (0.01 Torr) 59 °C; IR (neat) 1770, 1740, 1150, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.2 (s, 2 H), 4.15 (t, J = 7 Hz, 2 H), 1.9–0.8 (m, 7 H), 0.9 (s, 9 H), 0.1 (s, 6 H). Anal. Calcd for $C_{12}H_{26}O_3Si$: C, 58.49; H, 10.63. Found: C, 58.57; H, 10.40.

[(*tert*-Butyldimethylsilyl)oxy]acetic Acid (21). To a solution of the ester **20** (21.8 g, 88 mmol) in 50 mL of tetrahydrofuran was slowly added a solution of potassium hydroxide (5.0 g, 89 mmol) in methanol (10 mL) and water (20 mL) at −10 °C. The reaction mixture was allowed to warm to 5 °C over 30 min, diluted with 300 mL of water, and extracted with 200 mL of ether. The aqueous layer was acidified by addition of a solution of 9 mL (90 mmol) of concentrated HCl in 20 mL of water at 0 °C. This mixture was extracted twice with ether (200 mL), and the ether layer was then washed with water and with brine. After drying over $MgSO_4$ and removing the solvent by distillation, **21** was obtained as a colorless liquid which solidified upon cooling (15.2 g, 90%): ^1H NMR ($CDCl_3$, 300 MHz) δ 4.3 (s, 2 H), 0.95 (s, 9 H), 0.15 (s, 6 H). Anal. Calcd for $C_8H_{18}O_3Si$: C, 50.54; H, 9.54. Found: C, 50.80; H, 9.10.

3-Diazo-1-[(*tert*-butyldimethylsilyl)oxy]-2-butanone (23). The protected acid **21** (2.6 g, 13.7 mmol) was dissolved in benzene (20 mL) and a small amount of benzene (5 mL) was removed by distillation to remove water. Oxalyl chloride (2 mL, 23 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 30 min and then refluxed for 30 min. Excess oxalyl chloride and most of the benzene were removed by distillation at atmospheric pressure to give **22**. The remaining solution was added dropwise at 0 °C to a solution of diazoethane in ether (300 mL of a diazoethane solution prepared from 12 g of *N*-ethyl-*N*-nitroso-*N'*-nitroguanidine[43]). After the reaction mixture was stirred at room temperature overnight, the solvent was removed by evaporation in vacuo, and the remaining residue was chromatographed (silica gel, ether/hexane 1:5 to 2:5) to provide **23** as a yellow oil (2.1 g, 70%): IR (neat) 2080, 1740, 1635, 1260, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.35 (s, 2 H), 1.95

(43) McKay, A. F.; Ott, W. L.; Taylor, G. W.; Buchanan, N.; Crooker, J. F. *Can. J. Res.* **1950**, *28B*, 683.

(s, 3 H), 0.90 (s, 9 H), 0.05 (s, 6 H). The product was used directly in the next step.

(±)-Dibenzyl 4-[(*tert*-Butyldimethylsilyl)oxy]-3-oxo-2-butyl Phosphate (24). To a solution of dibenzyl phosphate (2.6 g, 9.3 mmol) in benzene (40 mL) was added a solution of diazo ketone **23** (2 g, 9.2 mmol) in 5 mL of benzene. The mixture was stirred at room temperature for 2 h, the solvent was removed by distillation, and the residue was chromatographed (silica gel, hexane/ethyl acetate/methylene chloride 3:1:1 to 2:1:1) to yield **24** as a colorless liquid (2.3 g, 53%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.3 (s, 10 H), 5.2–4.85 (m, 5 H), 4.35 (s, 2 H), 1.45 (d, J = 7 Hz, 3 H), 0.85 (s, 9 H), 0.05 (s, 6 H). Anal. Calcd for $C_{24}H_{35}O_6PSi$: C, 60.28; H, 7.38. Found: C, 60.50; H, 7.25.

(±)-Dibenzyl 4-Hydroxy-3-oxo-2-butyl Phosphate (25). A solution of **24** (1.7 g, 3.6 mmol) in a mixture of 30 mL of tetrahydrofuran, 5 mL of acetic acid, and 10 mL of water was refluxed for 1 h. The volatiles were evaporated in vacuo and the residue was chromatographed (silica gel, methylene chloride/ethyl acetate 2:1 to 1:3) to give **25** as a colorless liquid (550 mg, 42%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (s, 10 H), 5.2–4.75 (m, 5 H), 4.25 (s, 2 H), 3.1 (s, 1 H, OH), 1.35 (d, J = 7 Hz, 3 H). Anal. Calcd for $C_{18}H_{21}O_6P$: C, 59.38; H, 5.81. Found: C, 59.10; H, 5.79.

(±)-4-Hydroxy-3-oxo-2-butyl Phosphate (26). A solution of the dibenzyl phosphate **25** (180 mg, 0.5 mmol) in 5 mL of methanol was hydrogenated in the presence of 10% palladium on charcoal for 1 h.[27] The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in water, and the solution was adjusted to pH 5.5 with a solution of 1 N NaOH. The solvent was removed under reduced pressure to provide **26** in quantitative yield: ^{13}C NMR (D_2O) δ 72.8, 62.3, 17.1. Compound **26** could not be reduced by glycerol phosphate dehydrogenase (GPDH) in the presence of NADH in an enzymatic assay.[41]

Dibenzyl (Hydroxymethyl)phosphonate (28). A mixture of paraformaldehyde (1.2 g, 40 mmol), dibenzyl phosphonate (**27**) (5 g, 19.1 mmol), triethylamine (1 mL, 7 mmol), and 10 mL of benzene was refluxed for 2 h; paraformaldehyde slowly dissolved. Ethyl acetate (100 mL) was added, and the solution was extracted three times with a solution of 1 N HCl and once with water. After drying over $MgSO_4$ and concentration in vacuo, the residue was chromatographed (silica gel, methylene chloride/ethyl acetate 2:1 to methylene chloride/acetone 1:1) to yield the phosphonate **28** as a colorless oil (1.5 g, 27%): IR (neat) 1320, 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.05–4.98 (m, 4 H, 2 CH_2Ph), 4.35 (br, 1 H, OH), 3.84 (dd, J_1 = 5.8 Hz, J_2 = 5.9 Hz, 2 H, CH_2PO); ^{13}C NMR ($CDCl_3$) δ 136.1, 128.5 and 127.9 (3 s, aromatic C), 68.05 (CH_2PO), 58.8 and 56.25 (2 CH_2Ph). Anal. Calcd for $C_{15}H_{17}O_4P$: C, 61.64; H, 5.86. Found: C, 61.48; H, 6.04.

(Dibenzylphosphono)methyl 2-*O*-(*tert*-Butyldimethylsilyl)glycolate (29). Oxalyl chloride (2 mL, 23 mmol) was added dropwise to the protected acid **21** (1.33 g, 7 mmol) in 39 mL of toluene. The reaction mixture was stirred for 30 min at room temperature and was heated at 80 °C for 30 min. Approximately 15 mL of liquid was removed by distillation at atmospheric pressure, and the remaining mixture was slowly added to a solution of 1 g (3.4 mmol) of alcohol **28** in 30 mL of pyridine at 0 °C. After being stirred at room temperature for 24 h, the reaction mixture was concentrated in vacuo. The residue (in 100 mL of ether) was extracted three times with a 1 N solution of sodium bicarbonate and once with water. After drying over $MgSO_4$, the solvent was evaporated at reduced pressure and the material was chromatographed (silica gel, hexane/ethyl acetate 2:1) to yield 0.9 g (58%) of ester **29**: IR (neat) 1775, 1310, 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33 (m, 10 H), 5.08–5.04 (m, 4 H, 2 CH_2Ph), 4.38 (d, J = 8.6 Hz, 2 H, CH_2PO), 4.2 (s, 2 H, CH_2CO), 0.89 (s, 9 H, $((CH_3)_3C)$, 0.06 (s, 6 H, 2 CH_3). Anal. Calcd for $C_{23}H_{33}O_6PSi$: C, 59.46; H, 7.17. Found: C, 59.68; H, 6.99.

(Dibenzylphosphono)methyl Glycolate (30). The fully protected ester **29** (300 mg, 0.64 mmol) was dissolved in a mixture of 3 mL of acetic acid, 1 mL of tetrahydrofuran, and 1 mL of water and was stirred at room temperature for 24 h.[27] The solvents were evaporated at reduced pressure, and the residue was chromatographed (silica gel, methylene chloride/ethyl acetate 1:2) to yield 200 mg (89%) of **30** as a colorless liquid: IR (neat) 1750, 1320, 1130 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.23 (m, 10 H), 5.02–4.89 (m, 4 H, 2 CH_2Ph), 4.29 (d, J = 8.7 Hz, 2 H, CH_2PO), 4.03 (s, 2 H, CH_2CO), 3.67 (br, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 172.0 (CO), 135.5, 128.5 and 127.9 (3 s, aromatic C), 68.2 (CH_2PO), 60.25 (CH_2CO), 58.5 and 56.2 (2 CH_2Ph). Anal. Calcd for $C_{17}H_{19}O_6P$: C, 58.29; H, 5.46. Found: C, 58.60, H, 5.21.

Phosphonomethyl Glycolate (31). The dibenzyl phosphonate **30** (150 mg, 0.43 mmol) was treated with 10% palladium on charcoal as described for the phosphate **26** to yield 82 mg of **31**: ^{13}C NMR (D_2O) δ 174.9 (CO), 60.83 (CH_2PO), 50.0 (CH_2CO); MS(FAB), m/e calcd 191.979, m/e found 191.980 (M^+).

***N*-(Benzyloxycarbonyl)aminoacetaldehyde Dimethyl Acetal (33).** Benzyl chloroformate (30 mL, 210 mmol) was added dropwise to a solution of aminoacetaldehyde dimethyl acetal (**32**) (16.9 g, 161 mmol) in 50 mL of ethyl acetate and 50 mL of pyridine at 0 °C. The mixture was stirred at room temperature overnight, and the product was isolated by distillation as a colorless oil (20.2 g, 50%): bp 145 °C (0.03 Torr); IR (neat) 3465, 3350, 1730, 1305, 1220 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 156.1 (CO), 136.1, 127.9 and 127.5 (aromatic C), 102.2 ($CH(OCH_3)_2$), 69.1 (CH_2O), 53.4 (CH_2NH), 41.9 (CH_3). Anal. Calcd for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.55; H, 6.97; N, 5.80.

***N*-(Benzyloxycarbonyl)aminoacetaldehyde (34).** A mixture of the acetal **33** (2.4 g, 10 mmol), oxalic acid (200 mg), 20 mL of tetrahydrofuran, and 10 mL of water was refluxed for 4 days. After evaporation of the solvent, the mixture was chromatographed (silica gel, hexane/ethyl acetate 2:1 to 1:2) to yield **34** as a colorless liquid (1.4 g, 72%): IR (neat) 3460, 1730, 1710, 1320, 1105 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.3 (s, 1 H), 7.30 (m, 5 H), 5.65 (br, 1 H), 5.1 (s, 2 H), 4.05 (d, J = 5 Hz, 2 H). Anal. Calcd for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.57; H, 5.87; N, 6.81.

D-Glyceraldehyde Diethyl Acetal (37). D-Fructose (**35**) (20 g, 111 mmol) was dissolved in 20 mL of water and the solution was stirred with 1 L of glacial acetic acid. After cooling to 16 °C, lead tetraacetate (102 g, 230 mmol) was added with vigorous stirring during 10 min. After stirring for an additional 30 min, a solution of oxalic acid (20 g) in 200 mL of glacial acetic acid was added, and the resulting suspension was filtered through a layer of Celite. The solvent was removed in vacuo, the remaining oil was treated with ethyl acetate, and the insoluble matter was removed by filtration. The filtrate was concentrated in vacuo to give a colorless viscous oil **36** which was not characterized.[44] Triethyl orthoformate (50 mL) and a hot solution of 1 g (12.5 mmol) of ammonium nitrate in 20 mL of absolute ethanol was added to **36** with stirring, and the solution was kept at 30–32 °C for 9 h and then at room temperature for 15 h. Anhydrous sodium carbonate (7 g) was then added. After being stirred for 1.5 h, the mixture was diluted with 200 mL of ether and the insoluble matter was removed by filtration. Evaporation of the solvents in vacuo left an oil that was dissolved in 70 mL of methanol and added to a solution of 18 g of sodium methoxide in 300 mL of methanol. After stirring the reaction mixture at room temperature for 2 h, 5 mL of water was added and carbon dioxide was bubbled through the reaction mixture to neutralize the alkoxide. The resulting precipitate was separated by filtration, the filtrate was concentrated in vacuo, and 150 mL of ether was added to the residue. After filtration and concentration in vacuo, chromatography (silica gel, chloroform to chloroform/methanol 10:1) followed by distillation yielded **37** as a colorless oil (4.32 g, 24% yield based on fructose): bp 85 °C (0.5 Torr); $[\alpha]^{28}_D$ +30.3° (c 1.04, ethanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.49 (d, J = 5.7 Hz, 1 H, CH), 3.85–3.5 (m, 7 H), 2.48 (br, 1 H, OH), 2.2 (br, 1 H, OH), 1.23 (t, J = 7 Hz, 3 H, CH_3), 1.21 (t, J = 7 Hz, 3 H, CH_3). Anal. Calcd for $C_7H_{16}O_4$: C, 51.21; H, 9.82. Found: C, 51.17; H, 9.48. The product was further identified by conversion of the free aldehyde (obtained by treatment of **37** with 0.1 N H_2SO_4 at 35 °C over 20 h) to its dimedone derivative; mp 199 °C, $[\alpha]_D$ +216° (c 0.6, ethanol); lit.[44] mp 196–198 °C, $[\alpha]_D$ 210 (c 0.6, ethanol).

D-Glycidaldehyde Diethyl Acetal (38). A solution of 4.11 g (25 mmol) of D-glyceraldehyde diethyl acetal (**37**) in 15 mL of pyridine was cooled to −10 °C and 2.98 g (26 mmol) of methanesulfonyl chloride was added with stirring at −10 to −5 °C over 20 min. The reaction mixture was kept at 0 °C for 1.5 h and then evaporated to dryness. The resulting residue was partioned

(44) Perlin, A. S. In *Methods in Carbohydrate Chemistry*; Academic Press: New York, 1962; Vol. 1, p 61.

between methylene chloride (80 mL) and cold water (10 mL). The organic layer was washed three times each with saturated solutions of sodium bicarbonate and NaCl, dried over $MgSO_4$, and concentrated in vacuo to yield a colorless oil. Chromatography (silica gel, chloroform/ethanol 10:1) gave a colorless oil which was identified by its ^1H NMR spectrum to be the 3-*O*-mesylate of **37**: ^1H NMR ($CDCl_3$) δ 4.48 (d, J = 6 Hz, 1 H, CH), 4.42 (dd, J_{gem} = 11 Hz, J_{vic} = 4.4 Hz, 1 H, $CH_{2a}OSO_2$), 4.3 (dd, J_{gem} = 11 Hz, J_{vic} = 4.3 Hz, 1 H, $CH_{2b}OSO_2$), 3.83 (m, 1 H, CH), 3.85–3.7 (m, 4 H, 2 $CH_{2a}CH_3$), 3.7–3.5 (m, 2 H, 2 $CH_{2b}CH_3$), 3.06 (s, 3 H, SCH_3), 2.42 (d, J = 4 Hz, 1 H, CO*OH*), 1.23 and 1.21 (2 t, J = 7 Hz, 6 H, 2 CH_3). This material was used in the next step without further characterization.

A solution of 1 N NaOH was added to a solution of **37** in 20 mL of water at 30 °C containing phenolphthalein (1% in ethanol) as an indicator; 16.5 mL of NaOH solution was added during 60 min to keep the pH of the mixture slightly basic. The reaction mixture was then extracted three times with 20 mL of methylene chloride. The combined extracts were washed with brine, dried over $MgSO_4$, concentrated in vacuo, and distilled at reduced pressure to yield 1.94 g of **38** (53% based on D-glyceraldehyde diethyl acetal): bp 72 °C (24 Torr); $[\alpha]^{28}_D$ +7.2° (c 1, ethanol); ^1H NMR ($CDCl_3$) δ 4.32 (d, J = 4.3 Hz, 1 H, CH), 3.8–3.6 (m, 2 H, 2 $CH_{2a}CH_3$), 3.6–3.4 (m, 2 H, 2 $CH_{2b}CH_3$), 3.08 (m, 1 H, $CHCH_2$), 2.75 (m, 2 H, $CHCH_2$), 1.23 and 1.2 (2 t, J = 7 Hz, 2 CH_3). Anal. Calcd for $C_7H_{14}O_3$: C, 57.52; H, 9.65. Found: C, 57.09; H, 9.28.

3-*O*-Methyl-D-glyceraldehyde Diethyl Acetal (39). A solution of the epoxide **38** (1.46 g, 10 mmol) in 5 mL of methanol was added to a solution of sodium methoxide (100 mg, 1.85 mmol) in 45 mL of methanol and the resulting solution was refluxed for 24 h. Carbon dioxide then was bubbled through the solution, and the mixture was concentrated in vacuo and triturated with 20 mL of ether. The resulting precipitate was separated by filtration and the filtrate was evaporated to leave a colorless oil that was purified by distillation under reduced pressure to yield 1.35 g of **39** (76%); bp 111 °C (27 Torr); $[\alpha]_D$ +31.6 (c 1, ethanol); ^1H NMR ($CDCl_3$) δ 4.46 (d, J = 3.7 Hz, 1 H, CH), 3.8–3.4 (m, 7 H), 3.37 (s, 3 H, OCH_3), 2.37 (d, J = 3.5 Hz, 1 H, CHO*H*), 1.21 (2 t, J = 7 Hz, 2 CH_3). Anal. Calcd for $C_8H_{18}O_4$: C, 53.92; H, 10.18. Found: C, 53.71; H, 10.40.

3-*O*-Methyl-D-glyceraldehyde (40). A solution of 891 mg (5 mmol) of **39** in 5 mL of 0.1 N sulfuric acid was heated at 40–42 °C for 12 h. The solution was neutralized with 1 g of ion exchange resin (Dowex 1-X8, HCO_3^- form), the resin was removed by filtration, and the filtrate was concentrated in vacuo. The concentration of **40** in the aqueous solution was 4.58 M (91% yield) according to the Willstaetter–Schudel alkaline iodine method.[37,38] Identification of **40** as its (2,4-dinitrophenyl)hydrazone was as follows. An aliquot (0.1 mL, 0.458 mmol of aldehyde) of the aqueous solution was added to a mixture of 81 mg (0.41 mmol) of 2,4-dinitrophenylhydrazine, 2 mL of 2-methoxyethyl ether (diglyme), and 0.3 mL of acetic acid. After being stirred at room temperature, the solution was diluted with 5 mL water, and the precipitate that formed was separated by filtration, washed with water, and dried in vacuo to give a yellow solid (74 mg, 64%). Recrystallization from ethanol/water yielded **40** [(2,4-dinitrophenyl)hydrazone] as yellow needles: mp 122–123 °C; $[\alpha]_D$ –21.9° (c 0.33, chloroform/methanol 2:1); ^1H NMR ($CDCl_3$) δ 9.1 (d, J_{meta} = 2.5 Hz, 1 H, H-3′), 8.31 (dd, J_{ortho} = 9.5 Hz, 1 H, H-5′), 7.9 (d, 1 H, $CH_{2a}OCH_3$), 3.63 (dd, J_{vic} = 5.2 Hz, 1 H, $CH_{2b}OCH_3$), 3.43 (s, 3 H, OCH_3), 2.86 (d, J = 5.2 Hz, 1 H, OH), 1.55 (s, 1 H, NH); HRMS, m/e calcd 284.075, m/e found 284.075.

2,5-Di-*O*-methyl-1,3;4,6-di-*O*-methylene-D-mannitol (42). To a stirred suspension of 1,3;4,6-di-*O*-methylene-D-mannitol[45] (**41**) (11.96 g, 58 mmol) in 1,2-dimethoxyethane (200 mL) was added 16.8 g (0.35 mmol) of a 50% dispersion of sodium hydride in oil. After 15 min a solution of 34.1 g (0.24 mmol) of methyl iodide in 10 mL of 1,2-dimethoxyethane was added dropwise during 10 min. The mixture was stirred at room temperature for 3 h and then at 37–40 °C for 16 h. Methanol (20 mL) was added, the mixture was stirred for an additional 30 min, then water (10 mL) was added, and the reaction mixture was evaporated in vacuo. The residue was partitioned between water (100 mL) and chloroform (200 mL). The organic layer was washed three times with water, dried over Na_2SO_4, and concentrated in vacuo. Trituration with *n*-hexane yielded **42** as colorless needles after filtration (11.49 g, 85%). An analytical sample was recrystallized from hexane: mp 83–84 °C; $[\alpha]_D$ –69.6° (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.06 (d, J_{gem} = 5.9 Hz, 2 H, 2 $OCH_{2a}O$), 4.56 (d, 2 H, 2 $OCH_{2b}O$), 4.33 (dd, J_{gem} = 10.7 Hz, J_{vic} = 4.9 Hz, 2 H, 2 $OCH_{2a}CH$), 3.6 (dd, J_{vic} = ca. 0 Hz, 2 H, $CH_{2b}CH$), 3.55 (ddd, $J_{vic'}$ = 9.5 Hz, 2 H, 2 $CHOCH_3$), 3.37 (s, 6 H, 2 OCH_3), 3.31 (dd, $J_{vic'}$ = 10.2 Hz, 2 H, 2 CH(O)CH(O)). Anal. Calcd for $C_{10}H_{18}O_6$: C, 51.28; H, 7.74. Found: C, 51.20; H, 7.74.

2,5-Di-*O*-methyl-D-mannitol (43). A solution of **42** (10.78 g, 46 mmol) in 40 mL of glacial acetic acid and 90 mL of acetic anhydride was cooled to 0 °C and 0.2 mL of concentrated sulfuric acid was added. After the reaction mixture was stirred at room temperature for 80 min, the solution was poured into ice water (700 mL), and this mixture was stirred for 1 h and then was extracted four times with chloroform (200 mL). The combined organic layers were washed with a saturated solution of sodium bicarbonate and with water, dried over Na_2SO_4, and evaporated in vacuo to yield an oil that was not characterized but was dissolved in 200 mL of a solution of 1 N HCl in methanol and kept at room temperature for 24 h. After evaporation of the solution in vacuo, crystallization from chloroform yielded colorless needles of **43** (5.84 g, 60%): mp 55–57 °C; $[\alpha]_D$ –20.3° (c 1, CH_3OH); ^{13}C NMR (DMSO-d_6) δ 81.57, 68.61, 60.73, 58.02. Anal. Calcd for $C_8H_{18}O_6$: C, 45.72; H, 8.63. Found: C, 45.78; H, 8.66.

2-*O*-Methyl-D-glyceraldehyde (44). Ion exchange resin (Dowex 1-X8, IO_4^- form, prepared from the chloride form (2 g) according to the procedure of Harrison and Hodge[30]) was added to a solution of **43** (420 mg, 2 mmol) in 4 mL of water, and the mixture was stirred at room temperature for 3 h. The resin was removed by filtration and the filtrate was diluted with water to 10 mL. The concentration of aldehyde **44** in this solution was 325 mM (81% yield) according to the Willstaedter–Schudel alkaline iodine method.[37,38] An aliquot (1 mL) of this solution was added over a 10-min period to a solution of 2,4-dinitrophenylhydrazine (57 mg, 0.29 mmol) in 1.3 mL of 2-methoxyethyl ether (diglyme) and 0.2 mL of acetic acid. After being stirred for 17 h, the yellow solution was diluted with water (2 mL) and the resulting precipitate was removed by filtration, washed with water, and dried in vacuo (65 mg, 79%). Recrystallization from methanol gave yellow needles: mp 150–151 °C; $[\alpha]_D$ +48° (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.11 (d, J = 2.6 Hz, 1 H), 8.33 (dd, J_1 = 9.5 Hz, J_2 = 2.6 Hz, 1 H), 7.93 (d, J = 9.5 Hz, 1 H), 7.45 (d, J = 6.3 Hz, 1 H), 4.02 (m, 1 H), 3.95–3.7 (m, 2 H), 3.46 (s, 3 H), 2.07 (t, J = 6.5 Hz, 1 H); HRMS, m/e calcd 287.075, m/e found 287.075.

2-Methylpropenal Diethyl Acetal (46). A hot solution of 870 mg (11 mmol) of ammonium nitrate in 15 mL of ethanol was added to a stirred mixture of freshly distilled methacrolein (**45**) (16 g, 228 mmol) and triethyl orthoformate (30.7 g, 207 mmol), and the resulting suspension was heated at 40–45 °C for 3 h. A precipitate formed and was removed by filtration. Anhydrous sodium carbonate was added to the filtrate, and the mixture was stirred at room temperature overnight. The reaction mixture was filtered, concentrated in vacuo, and distilled to yield **46** as a colorless liquid (20.2 g, 68%), bp 142–143 °C. This compound was identified by converting it to its (2,4-dinitrophenyl)hydrazone as follows: To a solution of 179 mg (0.9 mmol) of 2,4-dinitrophenylhydrazine in a mixture of 5 mL of 2-methoxyethyl ether (diglyme) and 0.7 mL of acetic acid was added 144 mg (1 mmol) of acetal **46** in 0.5 mL of diglyme, followed by 2.5 mL of water. After stirring at 30–35 °C for 48 h, the precipitate that formed was removed by filtration, washed with water, and dried in vacuo. Crystallization from ethyl acetate furnished orange prisms (209 mg, 84%): mp 203–204 °C (lit.[46] mp 206–206.5 °C).

D,L-2,3-Dihydroxy-2-methylpropanal Diethyl Acetal (47). A suspension of acetal **46** (20 g, 139 mmol) in 170 mL of water was cooled to 0 °C and a solution of potassium permanganate (22 g, 139 mmol) in 420 mL of water was added at 5–8 °C during 3 h. After stirring at room temperature for 3 h and heating at 100 °C for 1 h, the manganese dioxide precipitate was separated and

(45) Haskins, W. T.; Hann, R. M.; Hudson, C. S. *J. Am. Chem. Soc.* **1943**, *65*, 67.

(46) Shriner, R. L.; Sharp, A. G. *J. Am. Chem. Soc.* **1940**, *62*, 2245.

washed thoroughly with water. The combined filtrates were cooled and 335 g of anhydrous potassium carbonate was added. After four extractions with ether, the combined organic layers were dried over $MgSO_4$, the solvents were removed in vacuo, and the product was distilled under reduced pressure to yield **47** (11 g, 45%) as a colorless liquid: bp 108 °C (17 Torr); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.35 (s, 1 H, CH), 3.9–3.8 (m, 2 H, 2 $CH_{2a}CH_3$), 3.72 (dd, J_{Hd-OH} = 5.3 Hz, J_{Hd-Hc} = 11.4 Hz, 1 H, $CH_{2d}OH$), 3.65–3.5 (m, 2 H, 2 $CH_{2a}CH_3$), 3.38 (dd, J_{Hc-OH} = 7.7 Hz, 1 H, $CH_{2c}OH$), 2.49 (dd, 1 H, CH_2OH), 1.22 (2 t, J = 7 Hz, 2 CH_3), 1.11 (s, 3 H, CH_3).

D,L-2,3-Dihydroxy-2-methylpropanal (48). A mixture of **47** (535 mg, 3 mmol) and ion exchange resin (Dowex 50W-X4, H^+ form) was stirred in water (6 mL) at 30 °C for 100 min. The resin was removed by filtration and the filtrate was concentrated in vacuo and then diluted with water to a volume of 10 mL. The concentration of **48** in this solution was 238 mM (79% yield).[37,38] The (dinitrophenyl)hydrazone (DNP) derivative was prepared as described for **44**. Yellow prisms of the DNP derivative were obtained from ethanol/water (1:2) in 76% yield: mp 177–178 °C; ^{1}H NMR ($CDCl_3$) δ 9.12 (d, J_{meta} = 2.5 Hz, 1 H, H-3'), 8.33 (dd, J_{ortho} = 9.5 Hz, 1 H, H-5'), 7.86 (d, 1 H, H-6'), 3.8 (dd, J_{gem} = 11.3 Hz, J_{vic} = 6.9 Hz, 1 H, $CH_{2a}OH$), 3.67 (dd, J_{vic} = 5.6 Hz, 1 H, $CH_{2b}OH$), 3.12 (s, 1 H, $HOC(CH_3)$), 2.04 (dd, 1 H, CH_2OH), 1.4 (s, 3 H, CH_3), 1.23 (s, 1 H, NH). Anal. Calcd for $C_{10}H_{12}N_4O_8$: C, 42.26; H, 4.25; N, 19.71. Found: C, 42.51; H, 4.41.

***N*-Acetylprolinol (50).** A 250-mL round-bottomed flask equipped with a magnetic stirring bar was charged with 4.0 g of L-prolinol (**49**) (40 mmol) in 100 mL of CH_2Cl_2. The flask was cooled in an ice bath, and acetic anhydride (6 mL, 60 mmol) was added during 30 min. The reaction was stirred for an additional 3 h and then was concentrated by evaporation at reduced pressure. Chromatography (silica gel, ethyl acetate) yielded 5.4 g (95%) of **62** whose ^{1}H NMR spectrum matched that of an authentic sample: ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 68.7, 66.1, 60.5, 28.0, 24.0, 22.4; IR (neat, NaCl) 3350 (br), 1740, 1720, 1615 cm^{-1}.

***N*-Acetylprolinal (51).** Oxidation of **50** to **51** followed the procedure of Swern[32] using freshly distilled reagents. To a dry, 500-mL round-bottomed flask was added 3.90 mL (55 mmol) of DMSO in 20 mL of methylene chloride. The solution was cooled to −78 °C and a cold solution of 2.46 mL of oxalyl chloride in 10 mL of methylene chloride was added. After stirring this solution for 1 h at −78 °C, a cold solution of alcohol **50** (3.58 g, 25 mmoles) in 20 mL of methylene chloride was added by syringe pump during 15 min. The solution was stirred for 3 h at −60 °C and then 14 mL of triethylamine was added. After being stirred for an additional 10 min, the reaction mixture was allowed to warm to room temperature, diluted with 50 mL of water, and filtered. The filtrate was extracted with three 50-mL portions of methylene chloride and the precipitate was washed three times with 20 mL of water; the aqueous layer was extracted with methylene chloride. The combined organic phases were dried over $MgSO_4$ and concentrated by rotary evaporation at reduced pressure to yield a mixture (3.6 g) containing predominantly hydrates of a compound identified as **51** by comparison of its ^{1}H NMR spectrum to that reported;[47] triethylamine and dimethyl sulfide were also present. This mixture was used without further purification in subsequent enzymatic reactions.

***N*-(*tert*-Butoxycarbonyl)prolinal (53).** Compound **52** (2.0 g, 10 mmol) in 20 mL of methylene chloride reacted with a solution of oxalyl chloride (0.96 mL, 11 mmol) and dimethyl sulfoxide (1.56 mL, 22 mmol) in 60 mL of cold methylene chloride according to the method of Swern.[32] After the addition of 3.5 mL of triethylamine, the reaction mixture was allowed to warm to room temperature and then 50 mL of water was added. Following extraction with three 50-mL portions of methylene chloride, the organic phases were washed with saturated aqueous NaCl, dried over $MgSO_4$, concentrated in vacuo, and passed through silica gel (eluant: hexane/ethyl acetate 1:1) to yield a pale yellow oil (1.8 g, 91%). The ^{1}H NMR spectrum was in agreement with the literature[48] and indicated that two isomers were present: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.50 (s) and 9.41 (d) (1 H, CHO), 4.14 and 3.07 (m, 1 H, methine), 3.55–3.36 (m, 2 H, NCH_2), 2.19–1.71 (m, 2 H, 2 CH_2), 1.43 and 1.35 (s, 9 H, Boc).

Cyclopentanaldehyde (55). A solution of 1.0 g (10 mmol) of cyclopentanemethanol (**54**) and 5.64 g (15 mmol) of pyridinium dichromate[33] in 25 mL of methylene chloride was stirred at room temperature for 1 day. Dilution with ether (25 mL), filtration, and purification on silica gel (eluant: hexane/ethyl acetate, 1:3) provided 103 mg (10%) of **55** whose ^{1}H NMR spectrum matched that reported.[49]

***N*-(Benzyloxycarbonyl)allylamine (57).**[50] A 1-L round-bottomed flask equipped with a magnetic stirring bar was charged with 5.70 g (100 mmol) of allylamine (**56**), 150 mL of water, 34.5 g (250 mmol) of potassium carbonate, and 150 mL of ethyl acetate. The flask was cooled in an ice bath and 15.7 mL (95%, 105 mmol) of benzyl chloroformate was added during 30 min by using a syringe pump. After stirring at room temperature for 2 h, the organic phase was separated and extracted twice with 100-mL portions of 1 N HCl, once with 100 mL of saturated aqueous NaCl, and dried over $MgSO_4$. The combined organic phases were concentrated in vacuo to yield 18.59 g (97.5 mmol, 97%) of **57** as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34 (m, 5 H, aromatic), 5.83 (m, 1 H, methine), 5.21–5.08 (m, 2 H, CH_2CH) (partially obscured by methylene peak), 5.10 (s, 2 H, CH_2Ph), 4.85 (br s, NH), 3.80 (t, 2 H, CH_2NH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.2, 136.4, 134.3, 128.3, 128.0, 115.8, 66.6, 43.3.

Alternative Synthesis of *N*-(Benzyloxycarbonyl)aminoacetaldehyde (34). A solution of 5.0 g (26 mmol) of **57** in 250 mL of methylene chloride and 25 mL of methanol was treated at −78 °C with ozone until a pale blue color persisted. Zinc (3 g) and acetic acid (5 mL) were then added and the reaction mixture was stirred overnight and allowed to warm to room temperature. Treatment of starch–iodine paper with the reaction mixture showed that peroxides were not present, and the reaction mixture was filtered and concentrated by rotary evaporation in vacuo and purified on silica gel (hexane/ethyl acetate, 1:1) to yield 4.46 g (88%) of **34** as an oil. The ^{1}H NMR spectrum matched that of material synthesized from **33**.

***N*-(*tert*-Butoxycarbonyl)allylamine (58).**[50,51] A 500-mL round-bottomed flask equipped with a magnetic stirring bar was charged with 2.28 g (40 mmol) of allylamine (**56**) and 10.5 mL (80 mmol) of triethylamine in 200 mL of methylene chloride. After cooling the flask in an ice bath, di-*tert*-butyl carbonate (9.6 g, 44 mmol) was slowly added.[52] After being stirred overnight at room temperature, the solution was concentrated by evaporation at reduced pressure and chromatographed on silica gel (hexane/ethyl acetate, 9:1) to yield **58** (5.88 g, 91%): ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.82 (m, 1 H, methine), 5.20–5.05 (m, 2 H, CH_2CH), 4.5 (br s, 1 H, N–H), 3.72 (t, 2 H, CH_2NH), 1.43 (s, 9 H, Boc).

***N*-(*tert*-Butoxycarbonyl)aminoacetaldehyde (59).**[53] Ozonolysis at −78 °C of a solution of 2.50 g (15.5 mmol) of **58** in 100 mL of methylene chloride/methanol (9:1) as described for compound **34** followed by reductive workup with zinc (2 g) and acetic acid (2 mL) and concentration in vacuo yielded a pale yellow oil. The oil was passed through a plug of silica gel (hexane/ethyl acetate, 1:1) to yield 2.40 g (95%) of **59** as a clear oil; the oil yellowed upon standing: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.61 (s, 1 H, CHO), 5.2 (br s, 1 H, NH), 4.04 (d, 2 H, CH_2), 1.42 (s, 9 H, Boc).

***N*-Acetylaminoacetaldehyde (61).** Acetylation of allylamine (**56**) to *N*-acetylallylamine (**60**) followed the literature.[54] Ozonolysis followed by reductive workup with zinc as described for compound **34** provided **61**, whose ^{1}H NMR spectrum was consistent with that reported in the literature.[55]

(47) Becker, Y.; Eisenstadt, A.; Stille, J. K. *J. Org. Chem.* **1980**, *45*, 2145.

(48) Evans, D. A.; Takacs, J. M.; Hurst, K. M. *J. Am. Chem. Soc.* **1979**, *101*, 371.

(49) Rodriguez, J. G.; Benito, Y.; Temprano, F. *J. Heterocycl. Chem.* **1985**, *22*, 1207.

(50) Compound **57** has also been made from the allylic selenide: Shea, R. G.; Fitzner, J. N.; Fankhauser, J. E.; Hopkins, P. B. *J. Org. Chem.* **1984**, *49*, 3647.

(51) Hanzlik, R. P.; Thompson, S. A. *J. Med. Chem.* **1984**, *27*, 711.

(52) For the use of di-*tert*-butyl dicarbonate: Moroder, L.; Hallett, A.; Wünsch, E.; Keller, O.; Wersin, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1976**, *357*, 1651. Commercially available material (Aldrich) was used in this work.

(53) Thompson, S. A.; Andrews, P. R.; Hanzlik, R. P. *J. Med. Chem.* **1986**, *29*, 104.

(54) Stille, J. K.; Becker, Y. *J. Org. Chem.* **1980**, *45*, 2139.

3-(Benzyloxy)-1-butene (63). A solution of sodium hydride (4.80 g of 50% dispersion, 100 mmol) in dry THF (60 mL) was warmed to 45 °C in an oil bath and benzyl bromide (6.0 mL, 50 mmol) was added.[36] 3-Hydroxybut-1-ene (**62**) (1.08 g, 15 mmol) in THF (20 mL) was added dropwise during 20 min. After being heated for an additional 45 min, the reaction mixture was allowed to cool to room temperature and water was added until evolution of hydrogen ceased. The aqueous layer was separated and extracted twice with ether; the combined organic solutions were washed with a saturated solution of NaCl, dried over $MgSO_4$, and evaporated in vacuo. Purification on silica gel (hexane/ethyl acetate, 20:1) gave 1.62 g (67%) of **63**:[56] ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 5 H), 5.83 (ddd, 1 H), 5.30–5.16 (m, 2 H), 4.61 (d, 1 H), 4.41 (d, 1 H), 3.95 (m, 1 H), 1.33 (d, 3 H).

2-(Benzyloxy)propanal (64). Ozone was bubbled through a solution of **63** (1.1 g, 7 mmol) in 100 mL of methylene chloride/methanol (9:1) at −78 °C until the solution remained blue. After purging with oxygen, 1.0 mL of dimethyl sulfide was added. When starch–iodide paper indicated that no peroxides were present, the solution was concentrated in vacuo and purified by chromatography (silica gel, hexane/ethyl acetate, 3:1) to give 0.82 g (71%) of **64**:[57] ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.65 (d, J = 2 Hz, 1 H), 7.40–7.25 (m, 5 H), 4.64 (d, J = 12 Hz, 1 H), 4.58 (d, J = 12 Hz, 1 H), 3.88 (qd, J = 7 Hz, 2 Hz, 1 H), 1.31 (d, 7 Hz, 3 H).

3-Phenyl-3-hydroxypropene (66). A solution of benzaldehyde (**65**) (2.55 mL, 25 mmol) in THF (60 mL) was cooled to −78 °C and 45 mL of 1.0 M solution of vinylmagnesium bromide in THF was added dropwise. The mixture was allowed to warm to room temperature and then 75 mL of a saturated solution of bicarbonate was added. The reaction mixture was filtered and the aqueous filtrate was extracted twice with 40-mL portions of ether. The combined organic phases were washed three times with 70 mL of saturated NaCl, dried over $MgSO_4$, and evaporated in vacuo to yield 4.3 g of a yellow oil. Chromatography (silica gel, hexane/ethyl acetate, 4:1) provided 2.1 g (63%) of **66**:[58] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.25 (m, 5 H), 6.07 (ddd, 1 H), 5.40–5.34 (m, 1 H), 5.24–5.19 (m, 2 H).

3-Phenyl-3-methoxypropene (67). A solution of sodium hydride (0.50 g of 50% dispersion in oil, 10 mmol) in dry THF (6 mL) was warmed to 45 °C in an oil bath and methyl iodide (0.60 mL, 9.7 mmol) was added.[36] Alcohol **66** (0.50 g) in THF (3.7 mL) was added dropwise during a 25-min period. After a further 45 min of heating, the reaction mixture was allowed to cool to room temperature and then water was added dropwise until evolution of hydrogen ceased. The aqueous layer was separated and extracted twice with ether; the combined organic solutions then were washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. Purification on silica gel (20:1 hexane/ethyl acetate) gave 0.38 g (71%) of **67**:[59] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.25 (m, 5 H), 6.00–5.90 (m, 1 H), 5.35–5.28 (m, 2 H), 4.65–4.61 (br d, 1 H), 3.35 (s, 3 H).

2-Methoxy-2-phenylethanal (68).[60] A solution of **67** (0.497 g, 3.4 mmol) in 10 mL of methylene chloride/methanol (9:1) was cooled to −78 °C, treated with ozone, and worked up as described for compound **64** to give 0.30 g (60%) of **68**: ^{1}H NMR (250 MHz) δ 9.58 (d, J = 1.7, 1 H), 7.41–7.32 (m, 5 H), 4.63 (d, J = 1.7, 1 H), 3.43 (s, 3 H).

2-Azidoacetaldehyde (70). A solution of acetal **69** (1 g, 7.6 mmol) in a solution of acetone (3 mL) and water (0.5 mL) containing approximately 20 mg of *p*-toluenesulfonic acid was allowed to stand at room temperature for 2 days. Analysis by gas chromatography indicated one product; the aldehyde concentration was determined to be 1.04 mmol by the Willstaedter–Schudel alkaline iodide method.[37,38] The material was used for enzymatic assay and was not further purified.

2-Chloroacetaldehyde (72). A mixture of acetal **71** (4 mL, 35 mmol), water (3.5 mL), and 1 N HCl (0.5 mL) was heated to 90 °C in an oil bath. The two-phase system became clear and homogenous after 1 h. The temperature of the oil bath was raised and a fraction distilling at 80–95 °C was removed. The residue was neutralized by the addition of 1 N NaOH. This material was used for enzymatic assay without further purification.

2-Bromoacetaldehyde (74). A stirred mixture of bromoacetaldehyde acetal **73** (24.8 g, 147 mmol) and concentrated sulfuric acid (0.5 mL) in 40 mL of water was heated to 100 °C on an oil bath for 2 h. Addition of 1 N NaOH to this solution raised the pH to near pH 5, and the volume was adjusted to 100.0 mL by the addition of water. Titration with $I_2/Na_2S_2O_3$[37,38] determined the concentration of the aldehyde to be 1.51 mmol.

Registry No. 1, 27933-26-2; 2, 114790-16-8; 3, 114790-17-9; 4, 114790-19-1; 5, 114790-20-4; 6, 19810-31-2; 7, 114790-21-5; 8, 114790-22-6; 9, 114790-23-7; 10, 114790-24-8; 11, 114790-26-0; 12, 114790-27-1; L-13, 56-41-7; D-13, 338-69-2; (*S*)-14, 33106-32-0; (*R*)-14, 100836-85-9; 15, 82977-93-3; 16, 114790-28-2; 17, 114790-29-3; 18, 114790-30-6; 19, 7397-62-8; 20, 114790-31-7; 21, 105459-05-0; 22, 78826-45-6; 23, 114790-32-8; 24, 114819-62-4; 25, 114790-33-9; 26, 114790-34-0; 27, 17176-77-1; 28, 114790-35-1; 29, 114790-36-2; 30, 114790-37-3; 31, 114790-38-4; 32, 22483-09-6; 33, 114790-39-5; 34, 67561-03-9; 35, 57-48-7; 36, 114790-40-8; 37, 114882-91-6; 37 (3-*O*-mesylate), 114790-47-5; 38, 114882-92-7; 39, 114790-41-9; 40, 105121-51-5; 40 [(2,4-dinitrophenyl)hydrazone], 114790-48-6; 41, 10300-97-7; 42, 114790-42-0; 43, 75828-97-6; 44, 114790-43-1; 44 [(2,4-dinitrophenyl)hydrazone], 114790-50-0; 45, 78-85-3; 45 [(2,4-dinitrophenyl)hydrazone], 5077-73-6; 46, 23553-27-7; 47, 114790-44-2; 48, 114790-45-3; 48 [(2,4-dinitrophenyl)hydrazone], 114790-49-7; 49, 23356-96-9; 50, 66158-68-7; 51, 73323-64-5; 52, 59378-81-3; 53, 59378-82-4; 54, 3637-61-4; 55, 872-53-7; 56, 107-11-9; 57, 5041-33-8; 58, 78888-18-3; 59, 89711-08-0; 60, 692-33-1; 61, 64790-08-5; 62, 598-32-3; 63, 53329-00-3; 64, 53346-05-7; 65, 100-52-7; 66, 4393-06-0; 67, 22665-13-0; 68, 19190-53-5; 69, 114790-46-4; 70, 67880-11-9; 73, 7252-83-7; 74, 17157-48-1; FDP aldolase, 9024-52-6; 71, 97-97-2; 72, 107-20-0.

(55) Lewis, C. A., Jr.; Wolfenden, R. *Biochemistry* **1977**, *16*, 4886.
(56) Beger, J.; Duschek, C.; Füellbier, H.; Gaube, W. *J. Prakt. Chem.* **1975**, *316*, 26.
(57) Takai, K.; Heathcock, C. H. *J. Org. Chem.* **1985**, *50*, 3247.
(58) Rautenstrauch, V.; Büchi, G.; Wüest, H. *J. Am. Chem. Soc.* **1974**, *96*, 2576.
(59) Hart, H.; Brewbaker, J. L. *J. Am. Chem. Soc.* **1969**, *91*, 716.
(60) Lodge, E. P.; Heathcock, C. H. *J. Am. Chem. Soc.* **1987**, *109*, 3353.