cases gave 2.0 g (55%) of 4-chlorobenzoin phenylacetate (**10f**), mp 64–65 °C: IR ν_{max} (KBr) 3090, 3060, 3030 and 2920 (CH), 1735 and 1695 (C=O), 1590 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.83 (2 H, s, methylene), 6.80 (1 H, s, methine), 7.55 (14 H, m, Ar). Anal. Calcd for $C_{22}H_{17}O_3Cl$: C, 72.53; H, 4.67. Found: C, 72.26; H, 4.41.

To a solution of **10f** (728 mg, 2 mmol) in dry dimethyl sulfoxide (50 mL) was added sodium hydride (48 mg, 2 mmol), and the mixture was stirred at 20 °C for 2 h and later at 60–70 °C for 10 min. Workup as in earlier cases gave 350 mg (50%) of **3e**, mp 173–174 °C (mixture melting point).

Preparation of the Phenanthrofuranone 5e. A solution of **3e** (50 mg, 0.14 mmol) in benzene (150 mL) was irradiated for 4 h (RPR, 3000 Å) and removal of the solvent under vacuum gave 35 mg (71%) of **5e**, mp 262–264 °C (mixture melting point).

Low-Conversion Photolysis of 1e. Low-conversion photolysis experiments were carried out with **1e** as a representative case. The general procedure was to dissolve 35 mg (0.01 mmol) of **1e** in benzene (20 mL) containing acetophenone (25 mg, 0.21 mmol) and irradiate the resulting solution at 3500 Å (filtered through a 2 mM solution of naphthalene in benzene). The photolysis was conducted for three different time periods, viz. 10, 20, and 40 min. In each case the solvent was removed under vacuum and the residue was dissolved completely in $CDCl_3$ for ^{1}H NMR. The relative concentrations (%) of the starting material and the photoproducts as obtained from ^{1}H NMR are given below. Within the limit of our detection, no evidence could be obtained for the formation of the isomer, 3-(4-chlorophenyl)-4,5-diphenyl-2-(5*H*)-furanone (<5%).

irradn time, in	startg matl (1e)	3e	5e	7e
10	66	22	not observed	11
20	20	43	8	29
40	12	35	12	41

Laser Flash Photolysis. For laser flash photolysis, use was made of a computer-controlled set-up described elsewhere.[23] The pulsed laser sources were Lambda-Physik EMG 101 excimer (248 and 308 nm, ~20 ns), Molectron UV-400 nitrogen (337.1 nm, ~8 ns), and Quanta-Ray DCR-1 Nd-YAG (third harmonic, 355 nm, ~6 ns). The laser intensities were attenuated and kept in the range 2–10 mJ/pulse. For transient spectra, a flow cell was used. Deoxygenation of solutions was effected by saturation with argon.

Acknowledgment. We thank the Department of Science and Technology, Government of India, Indian Institute of Technology, Kanpur, and the Office of Basic Energy Sciences of the U. S. Department of Energy for financial support of this work.

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Fumarase-Catalyzed Synthesis of L-*threo*-Chloromalic Acid and Its Conversion to 2-Deoxy-D-ribose and D-*erythro*-Sphingosine

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Received February 2, 1987

This paper describes the use of pig heart fumarase (E.C. 4.2.1.2) as a catalyst in the multigram synthesis of L-*threo*-chloromalic acid (1) (≥99.5% enantiomeric excess) on 50-g scale. L-*threo*-Fluoromalic acid has been synthesized in a coupled enzymic system from difluorofumaric acid. Compound 1 serves as starting material for syntheses of 2-deoxy-D-ribose and D-*erythro*-sphingosine.

Introduction

Much of the current effort in developing applications of enzymes to enantioselective organic synthesis has focused on classes of enzymes with broad substrate specificity: lipases, esterases, amidases, and related hydrolases.[1-3] These enzymes are indisputably valuable but have certain disadvantages, among which is often imperfect enantioselectivity. The standards in enantioselective synthesis are now very high: for most applications, an enantiomeric excess (ee) in an enantioselective step of less than 90% is essentially useless, and values of ee >99% are very desirable.[4] It is often difficult to achieve broad substrate selectivity and high enantioselectivity simultaneously. This difficulty poses a strategic question for the synthetic chemist: Is it better to use a structurally "efficient" intermediate with modest enantiomeric purity in a synthetic scheme—that is, an intermediate that can be generated easily and converted to the desired product by using an efficient synthetic sequence (but with imperfect overall control of enantiomeric purity)—or is it better to use a less "efficient" intermediate having higher enantiomeric purity? There is no general answer to this question, but it does pose a related question in enzyme-catalyzed synthesis: What are the relative values as catalysts of enzymes with broad substrate specificity and moderate enantioselectivity relative to enzymes with narrow substrate specificity and high enantioselectivity? This paper explores the utility in organic synthesis of a representative enzyme—fumarase—in the second catagory.

Fumarase (E.C. 4.2.1.2) generates a chiral center(s) from an achiral substrate by stereospecific hydration of a carbon–carbon double bond.[5] In vivo, fumarase catalyzes the reversible hydration of fumarate to L-malate; it is used in a commercial synthesis of this substance (eq 1).[3] We demonstrate here that it also is a useful catalyst for conversion of chlorofumaric acid to L-*threo*-chloromalic acid (1) (eq 2); its broader application in organic synthesis is, however, limited, because only a few analogs of fumaric

(1) Whitesides, G. M.; Wong, C. H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617–638.
(2) Findeis, M. A.; Whitesides, G. M. *Annu. Rep. Med. Chem.* **1984**, *19*, 263–272.
(3) Chibata, I. *Immobilized Enzymes, Research and Development*; Halsted: New York, 1978.
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(5) Stryer, L. *Biochemistry*, 2nd ed.; W. H. Freeman: San Francisco, 1981; p 288.

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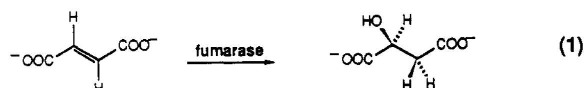
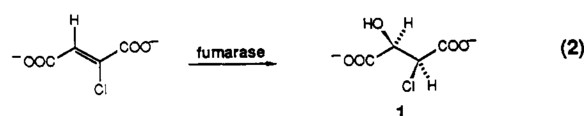
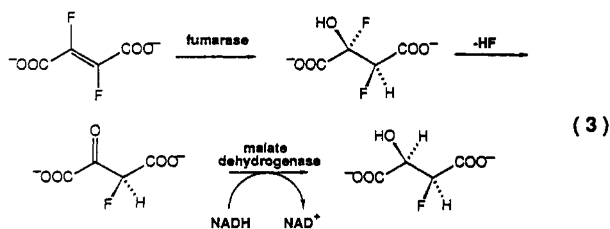




acid other than chlorofumaric acid are substrates (vide infra).

Fumarase is a component of the citrate cycle and is found in a wide variety of organisms.[6] The pig heart enzyme is a tetramer composed of four identical subunits with an aggregate MW of 194 000. The enzyme has a variable isoelectric point from 5 to 8 depending on the buffer in which the determination is made, a reflection of differential binding of various ions. There is no cofactor required for catalysis. There are no disulfide bonds between subunits; these are associated noncovalently. Thiols in the subunits appear to be buried in the folded protein.[6]

Although fumarase from pig heart has been described as absolutely specific for fumarate and L-malate,[7] it actually accepts several dicarboxylic acids other than fumarate or L-malate although generally with low reaction velocities.[8,9] Fluorofumarate is the only reported unnatural substrate to have a V_{max} greater than that for fumarate.[9] Limitations in fumarase's applicability are apparent, however, from the small number of substrates that will allow synthetically useful reaction velocities. The cost and specific activity of fumarase presently limits the practically useable substrates to those with a value of $V_{max} > 1\%$ that of fumarate.[10] The only substrate that fits these criteria and is also an economically practical starting material is chlorofumarate. While mono- and difluorofumarate are both excellent substrates they also both suffer from drawbacks for use as substrates for fumarase. Fluorofumarate is of no practical use since it is hydrated by fumarase predominantly in an orientation that results in the formation of oxalacetate from the decomposition of 2-fluoromalate.[8] 1,2-Difluorofumarate is hydrated to 1,2-difluoromalate. This substance spontaneously eliminates HF and forms 3-fluorooxalacetate. 3-Fluorooxalacetate is sufficiently stable in solution that it can be trapped by in situ reduction with malate dehydrogenase (eq 3).[11] Difluorofumarate is, however, expensive.[12]



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(9) Nigh, W. G.; Richards, J. H. *J. Am. Chem. Soc.* **1969**, *91*, 5847–5848.

(10) Fumarase purchased from Sigma costs ~\$45/5000 U (L-malate as substrate. 1 U = 1 μmol of substrate converted to product per minute). To produce 1 mol of product per day requires ~700 U constant activity.

(11) Marletta, M. A.; Cheung, Y.-F.; Walsh, C. *Biochemistry* **1982**, *21*, 2637–2644. Walsh, C. In *Advances in Enzymology and Related Areas of Molecular Biology*; Meister, A., Ed.; Wiley: New York, 1983; pp 197–289.

Table I. Compounds Tested as Substrates for Hydration by Fumarase (Equation 4)

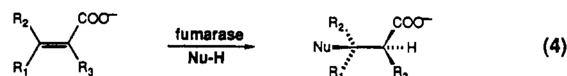
R_1	R_2	R_3	Nu-H	V_{rel}[a]	ref
CO_2^-	H	H	HO-H	100	7
	F	F		330, 394	7, 8
	H	H		2.7	7
		Br		0.35	7
		I		0.005	7
		CH_3		0.003	7
	(C≡C)			0.26	7
	CH_3	CH_3		<0.00002	7
	F	F		130[b] (83)	(8)
$CONH_2$	H	H		<1	
CH_2OH				<1	
CH_2Cl				<1	
NO_2				<1	
CO_2^-			NC-H	<1[c,d]	
			HS-H	<1[c,e]	
			HOO-H	<1[d,e]	

[a] Reaction velocities were determined in this work in 10 mM HEPES buffer, pH 7.6, in a 1-mL volume containing ≤0.1 mg/mL of fumarase. [b] In reactors, the reaction velocity is reduced (see text). [c] Assayed in the presence of 1 M nucleophile. [d] Cyanide inhibits fumarase. [e] No change in the equilibrium of the assay mixture in the presence of $^-$OOH or HS$^-$ was observed.

The objectives of this paper are to define the breadth of synthetically useful substrates accepted by fumarase, to develop a practical synthesis of 1 from chlorofumaric acid, and to convert 1 to representative natural products—2-deoxy-D-ribose and D-*erythro*-sphingosine—by using routes that establish the utility of 1 as a new chiral synthon.

Results and Discussion

Substrate Specificity. Table I lists compounds that have been tested as substrates for hydration by fumarase (eq 4), together with estimates of reaction rates. In the



case of 3-nitroacrylate a slow loss of UV absorbance of the olefin was observed, but this reaction was independent of the presence of enzyme and probably reflects nonspecific hydration. We observed no evidence for alternative nucleophile participation in enzymic reaction mixtures including HS$^-$, $^-$CN, and HOO$^-$. Cyanide is a potent inhibitor of the enzyme, and peroxide would be expected to eventually inactivate the enzyme by oxidation. From these results and the literature data we concluded that fumarase would not be broadly useful as a catalyst and turned to the exploitation of two good substrates of fumarase—chlorofumarate and difluorofumarate—for the synthesis of L-*threo*-chloromalic acid and L-*threo*-fluoromalic acid.

Fumarase: Stability. To be useful in organic synthesis, an enzyme must be stable for a sufficiently long period under reactor conditions to allow the accumulation of useful quantities of product. Fumarase contains thiols and is sensitive to oxidation.[13] In dilute buffer solution (10 mM TRIS acetate, pH 7.3) the enzyme losses all activity within 16 h. With 5 mM dithiothreitol (DTT) present, one-third of the original activity remained in the same period of time. In reactors producing 1 from chlorofumarate, DTT cannot be used as an antioxidant, however, because the presence of DTT in this system leads to inactivation of the enzyme and no accumulation of prod-

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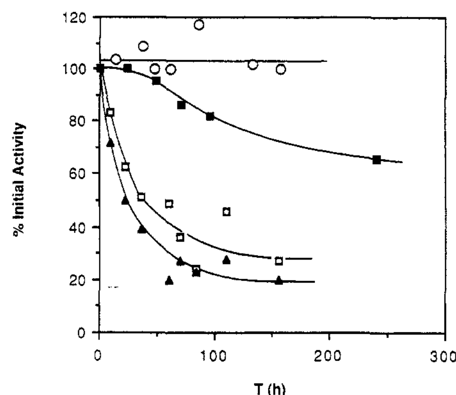


Figure 1. Stability of fumarase under varying conditions: (■) soluble enzyme in 32 mM $(NH_4)_2SO_4$, under nitrogen; (□) in 10 mM TRIS acetate, under nitrogen; (▲) in 10 mM TRIS acetate, exposed to air; (○) immobilized in PAN gel suspended in 10 mM TRIS acetate. All solutions: pH 7.3–7.5.

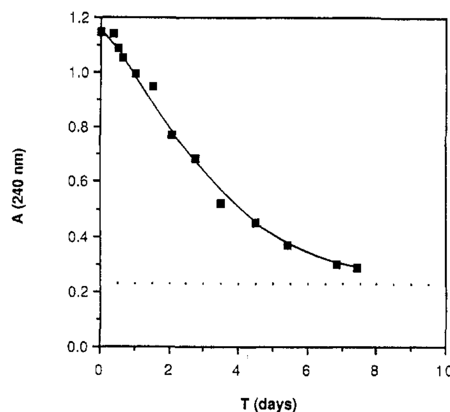


Figure 2. Progress of a reactor containing PAN gel immobilized fumarase (45 U, chlorofumarate as substrate) and chlorofumarate (64 g), pH 7.5.

uct.[14] All solutions used in reactors with fumarase were thoroughly deoxygenated. Fumarase is more stable when immobilized than when free in solution and more stable when at high ionic strength than at low (Figure 1). Immobilization yields, however, have been only moderate when PAN gel[15] is used (≤25%). It is thus problematic whether to immobilize and gain stability at the cost of initial loss in activity or to use soluble enzyme. For small-scale reactions (up to 5 g of chlorofumarate) it is more convenient to use soluble enzyme. For synthesis of larger quantities, it is worthwhile to immobilize the enzyme to improve its stability in reactions requiring extended operating time (1–2 weeks).

L-*threo*-Chloromalic Acid. Small-scale synthesis of L-*threo*-chloromalic acid is straightforward using soluble enzyme; the procedure is described in the Experimental Section. Equilibrium (K_{eq} = 6.2 toward hydration, chlorofumarate as substrate)[8] limits the maximum theoretical yield to 86% of starting material; with recycling, yields can in principle be nearly quantitative. In practice, losses in handling and the slow hydration of chlorofumarate to 2-chloromalate and thence to oxalacetate preclude quantitative yield.[8]

Large-scale reactions have been more difficult to carry out using soluble enzyme. The long reaction times (~1 week) employed to minimize the quantity of enzyme used resulted in sufficient deactivation of the enzyme (Figure 1) that it was necessary to add additional enzyme. A reaction using the more stable immobilized enzyme was more practical. Figure 2 shows the progress of a reaction initially containing 64 g of chlorofumaric acid and 45 U (chlorofumarate as substrate) of fumarase immobilized in PAN[15] gel in 0.7 L of solution. After 7.5 days the reactor was stopped and the enzyme-containing gel collected by centrifugation. After isolation of product the recovered starting material was recombined with the immobilized enzyme. From these two reactors a total of 54.5 g of crystalline L-*threo*-chloromalic acid was isolated. The recovered enzyme gel still retained most (>60%) of its activity after use in both reactions.

(14) We believe the inactivation of fumarase in the presence of chlorofumarate and DTT results from reaction of the enzyme with a reactive intermediate caused by the reaction of DTT with chlorofumarate. We do not know if the enzyme promotes the formation of the reactive species.

(15) Pollak, M.; Blumenfeld, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324–6336.

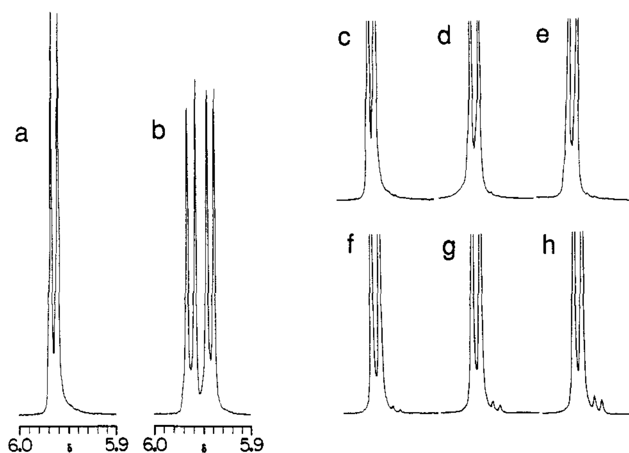


Figure 3. ^{1}H NMR spectra (250 MHz) at δ 5.9–6.0 of the methine proton at C-2 of chloromalate of dimethyl (+)-MTPA esters of (a) enzymically produced L-*threo*-chloromalic acids, (b) DL-*threo*-chloromalic acid, (c) base line detail of a, (d) c, with 0.5% of b, (e) with 1% of b, (f) with 2% of b, (g) with 5% of b, (h) with 10% of b.

Enantiomeric Purity of 1. The L-*threo*-chloromalic acid obtained from these enzymic reactors was of comparable melting point and optical rotation to that synthesized chemically from D-tartaric acid.[16] We determined the enantiomeric purity of the enzymically prepared material using the MTPA ester[17,18] of the dimethyl chloromalate. Racemic *threo*-chloromalic acid prepared from *cis*-epoxysuccinic acid was used for comparison (Figure 3). Addition of as little as 0.5% of the MTPA ester of racemic dimethyl *threo*-chloromalate to the MTPA ester of the enzymically derived dimethyl chloromalate resulted in an observable signal in the ^{1}H NMR spectrum. We conclude that enzymically sythesized L-*threo*-chloromalic acid has ee ≥99.5%.

2-Deoxy-D-ribose. We confirmed the stereochemistry of 1 and illustrated its use as a chiral synthon in organic synthesis by synthesis of two natural products. 2-Deoxy-D-ribose was prepared following the sequence of reactions summarized in Scheme I. Compound 1 was reduced to chloro triol 2 with borane in THF.[19] Triol 2 was converted to acetonide 3 and then treated with base to form epoxide 4 as has been previously done with racemic 2.[20] Opening of 4 with a carbon nucleophile proved troublesome. Re-

(16) Kuhn, R.; Zell, R. *Chem. Ber.* **1926**, *59*, 2514–2521.

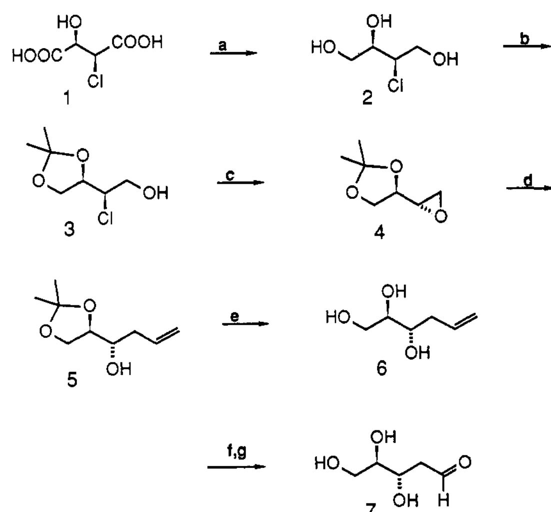
(17) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.

(18) The use of strongly basic conditions in the workup of the MTPA esters of halomalates reported here resulted in decomposition of the product and was therefore avoided.

(19) Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38*, 2786–2792.

(20) Niedballa, U.; Gries, H. Ger. Offen. DE 3150917, 1983 [to Schering A.-G., p 17].

Scheme I. Synthesis of 2-Deoxy-D-ribose from (−)-*threo*-Chloromalic Acid[a]



[a] (a) BH_3, THF; (b) MeCOMe, $MeC(OMe)_2Me$, TsOH; (c) KOH, Et_2O; (d) $(CH_2CH)_2Cu(CN)Li_2$, THF, −78 °C; (e) H_2O, MeOH, TFA; (f) O_3, MeOH; (g) Me_2S, MeOH.

action with 2-lithio-1,3-dithiane[21] was slow and incomplete. Vinylmagnesium bromide resulted in an unidentified rearrangement product without addition of the vinyl group. Lipshutz's higher order cuprate method[22] yielded olefin **5** in a fast, efficient reaction. Olefin **5** is the same intermediate used in other syntheses of 2-deoxy-D-ribose that use 2,3-*O*-isopropylidene-D-glyceraldehyde as starting material.[23,24] Olefin **5** was hydrolyzed to the olefin triol **6** and ozonized as previously described[23] followed by reduction with dimethyl sulfide[24] to yield 2-deoxy-D-ribose **7**.

D-*erythro*-Sphingosine. The synthesis of D-*erythro*-sphingosine **18** was achieved as outlined in Scheme II. Hydroxy acetonide **3** was allowed to react with benzyl isocyanate and give the acyclic carbamate **8**, which was cyclized with displacement of chloride by treatment with potassium *tert*-butoxide.[25] The acetonide **9** was hydrolyzed[26] to the diol **10**, which was selectively silylated[27] to afford cleanly the secondary alcohol **11**. When benzylated[28] the fully protected amino triol **12** was obtained mixed with ~30% of the 1-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl isomer resulting from silyl group migration under the benzylation conditions. Desilylation allowed the isolation of the monobenzyl diol **13**, which was cleanly oxidized by the method of Swern to afford the aldehyde **14**.[29] This aldehyde was stable to rapid chromatography on silica gel but epimerized (as observed by ^1H NMR spectroscopy) if exposed to acid during workup. Introduction of the desired alkenyl group using the Schlosser–Wittig procedure proceeded in low yield.[30] Reaction of aldehyde **14** with

(21) Seebach, D.; Corey, E. J. *J. Org. Chem.* **1975**, *40*, 231.
(22) Lipshutz, B. H.; Wilhelm, R. S.; Kozlowski, J. A.; Parker, D. *J. Org. Chem.* **1984**, *49*, 3928–3938.
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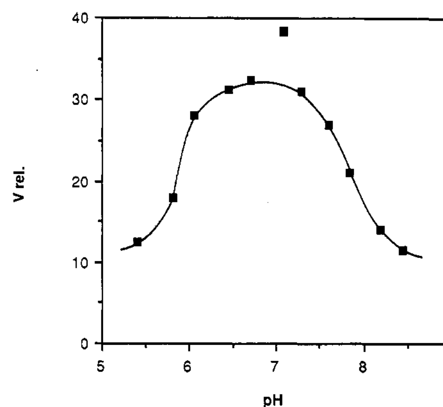
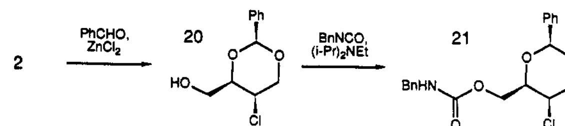


Figure 4. Curve of velocity vs. pH for fumarase acting on difluorofumarate.

lithiated phenyl *n*-tetradecyl sulfone followed by acetylation yielded the acetoxy sulfone **15** as a mixture of diastereomers which on Na–Hg reduction produced predominantly the *trans*-olefin **16**.[31] Debenzylation gave the hydroxy carbamate **17**, an intermediate in a published synthesis of **18**.[32] Hydrolysis of **17** afforded crude sphingosine indistinguishable from an authentic sample by ^1H and ^{13}C NMR spectroscopy. The triacetylated derivative of this material was also indistinguishable from triacetylated authentic **18**.[33-36]

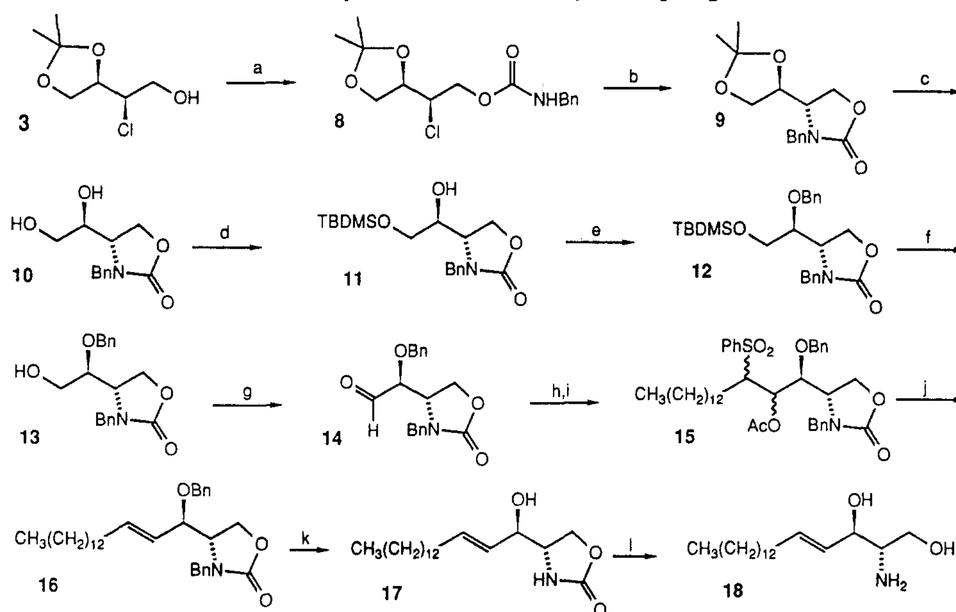
L-*threo*-Fluoromalic Acid. Use of difluorofumarate for the enzymic synthesis of L-*threo*-fluoromalate requires a more complicated catalytic system than that used for the one-step hydration of chlorofumarate since malate dehydrogenase catalyzed reduction of intermediate fluorooxalacetate with NADH is necessary.[11] On a scale of <1 g of difluorofumarate, a stoichiometric quantity of NADH can be used, but this procedure increases the quantity of organic material present in the reactor and complicates isolation of product. The inclusion of an NADH recycling scheme increases the complexity of the reactor by adding another enzyme but has the advantage of introducing additional driving force for the rapid reduction of fluorooxalacetate and reduces the organic content of the reactor. We used the formate/formate dehydrogenase method of regeneration of NADH.[1] While this enzyme is more costly than others that can be used for NADH regeneration, the

(30) Schlosser, M.; Christmann, K. F. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 126.
(31) Kocienski, P. J.; Lythogoe, B.; Ruston, S. *J. Chem. Soc., Perkin Trans. 1* **1978**, 829–834.
(32) Julina, R.; Herzig, T.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 368–373.
(33) In an alternative strategy to obtain **18**, triol **2** was protected as the benzylidene **20** in moderate yield.[34-36] Carbamate **21** was readily prepared, but all attempts to cyclize it with displacement of chloride to give **21** were unsuccessful. The failure of this reaction appeared to result from a preference for trans-diaxial elimination of HCl from **20**:



(34) De Belder, A. N. *Adv. Carbohydr. Chem.* **1965**, *20*, 219–302.
(35) The stereochemistry of **20** has been assigned on the basis of ^1H NMR spectroscopy. Coupling constants indicate that the chlorine is an axial position in a benzylidene ring with a boat conformation. The exocyclic hydroxymethyl is hydrogen-bound to the adjacent ring oxygen in a relatively rigid conformation. The phenyl group is assigned to the equatorial position. If it were in the pseudoaxial position we would expect the ring to flip into the alternative boat resulting in equatorial chlorine.
(36) The synthesis of compound **20** was carried out by Jaesang Kim.

Scheme II. Synthesis of *trans*-D-*erythro*-Sphingosine[a]



[a](a) BnNCO, (*i*-Pr)$_2$NEt, PhH; (b) KO-*t*-Bu, THF, DMF; (c) 1 N HCl, THF; (d) TBDMSCl, NEt_3, DMAP, pyridine; (e) NaH, BnBr, TBAI, THF; (f) TBAF, THF; (g) ClCOCOCl, Me_2SO, CH_2Cl_2, NEt_3; (h) $C_{14}H_{29}SO_2Ph$, BuLi, THF; (i) Ac_2O; (j) Na–Hg, MeOH/EtOAc; (k) Na, NH_3, Et_2O; (l) 1 N NaOH, EtOH.

product from formate is CO_2, and the workup is simple.

Previously reported kinetic data for the fumarase-catalyzed hydration of difluorofumarate were obtained in phosphate buffer.[9] Phosphate, however, is a known inhibitor of fumarase at the concentration used,[6,37] and values of the Michaelis constant K_m will be raised in the presence of this ion. We redetermined K_m for difluorofumarate in 10 mM TRIS acetate buffer (pH 7.3) and obtained a value of 4.4×10^{-5} M, almost an order of magnitude lower than the previously reported value.[9] Determination of V_{max} under these conditions resulted in a value 1.3 times greater than V_{max} for fumarate under the same conditions. The previously reported values[9] gave difluorofumarate as a slightly less active substrate than fumarate in 10 mM phosphate buffer [V_{max}(difluorofumarate)/V_{max}(fumarate) = 0.83]. In assays with high concentrations of difluorofumarate, however, the rate of reaction is only about 7% of that with fumarate after an initial period of high activity. We have not investigated the cause of this reduced activity under reactor conditions. To further characterize the hydration of difluorofumarate by fumarase we determined the profile of reaction velocity vs. pH to find the pH optimum for this process (Figure 4). With difluorofumarate as substrate, fumarase displays a broad range of activity from pH 6 to 8 with the optimum at pH 7.0. This compares with pH optima for chlorofumarate and bromofumarate of 7.6 and 7.8,[7] respectively, and an optimum pH for the hydration of fumarate at pH 6.8–7.0 depending on concentration and buffer conditions.[37]

L-*threo*-Fluoromalic acid was prepared as shown in eq 3 and isolated as the dimethyl ester. The pH of a reaction producing fluoromalate from difluorofumarate depends on competing factors: release of HF tends to lower the pH; formation of alkoxide by the reduction of fluorooxalacetate by NADH tends to raise it. Depending on the relative amounts of substrates and enzymic activities, the pH may move in either direction. To avoid damaging enzymes and decomposing NAD (at high pH) or NADH (at low pH),[38] the pH was controlled by the addition of 1 N HCl or 1 N NaOH.

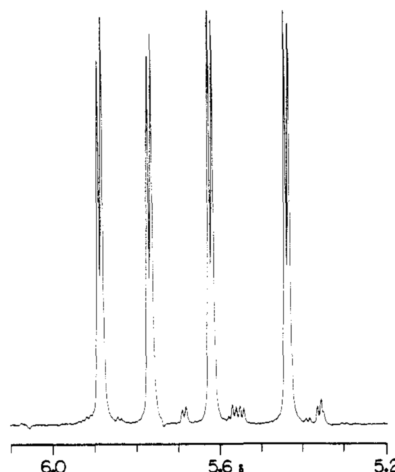


Figure 5. ^{1}H NMR spectrum (250 MHz) at δ 5.2–6.1 of the (+)-MTPA ester of dimethyl L-*threo*-fluoromalate produced enzymically from difluorofumaric acid. The major peaks shown correspond to the methine protons of fluoromalate at C-2 [δ 5.83 (*J* = 30 , 2.3 Hz) and C-3 [δ 5.52 (*J* = 46, 2.3 Hz).

The crude material from the enzymatic reaction contains primarily a single compound with the same ^{1}H NMR spectrum previously reported for *threo*-fluoromalic acid.[39] After chromatography, the stereochemical purity of the enzymically synthesized fluoromalate was determined by preparing the (+)-MTPA ester of the isolated dimethyl fluoromalate. Figure 5 indicates that there is a minor product present in the isolated dimethyl fluoromalate representing <5% of the total. We have not positively identified this material but believe that it is the MTPA dimethyl ester of L-*erythro*-fluoromalic acid resulting from epimerization at C-3 of 3-fluorooxalacetate before it is

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enzymically reduced with NADH in the reaction mixture or resulting from epimerization during the preparation of the MTPA ester.

Conclusions

Fumarase has narrow substrate specificity. Although it will accept a modest number of substrates with low activity, the only useful substrates at current costs for the enzyme are fumaric acid, chlorofumaric acid, and 1,2-difluorofumaric acid. If the cost of the enzyme were to be reduced (for example, through the development of an efficient recombinant DNA-based fermentation) it might be possible to include bromo-, iodo-, and 2-methylfumaric acids as useful substrates.[7] We have focused our attention on fumarase from pig heart since it is commercially available at reasonable cost. It is possible that a fumarase from some other source (especially a microbial source) might have different and broader specificity. In the longer term, it may be possible to broaden the specificity of the enzyme by site-specific mutagenesis. The first requirement for an effort of this sort—an X-ray crystal structure of the enzyme—is not available.

Within its limited range of application, fumarase is a useful catalyst for the preparation of L-*threo*-chloromalic (1) and L-*threo*-fluoromalic acids. The fumarase-based route to 1 is superior to presently available chemical syntheses from tartaric or fumaric acids: these procedures proceed in low to moderate yield and require resolutions.[16,40] There is presently no competing route to L-*threo*-fluoromalic acid, although tartaric acid might also provide a starting material for this compound. Readily available asymmetric natural products have received much attention as sources of chiral synthons.[41-44] Compound 1 is equivalent to selectively functionalized *meso*-tartaric acid in which the *R* configuration hydroxyl group has been replaced with inversion by chloride. No procedure for the direct selective modification of *meso*-tartrate to obtain 1 is currently available. The D and L isomers of tartrate cannot be manipulated to provide 1 in a straightforward manner either.

The syntheses demonstrating conversion of 1 to 2-deoxy-D-ribose and D-*erythro*-sphingosine are not the most direct or efficient routes to these compounds, but they demonstrate methods for conversion of 1 into a number of potentially useful chiral synthons. Moreover they provide routes that may be useful for specialized applications such as the preparation of labeled compounds. The best preparation of 2-deoxy-D-ribose is certainly by isolation from DNA. Recent chemical syntheses of 7 have used D-glyceraldehyde as a starting material[23,24,45-49] or have relied on degradation of more complex carbohydrate precursors to obtain 7.[50-52] The syntheses starting from D-glyceraldehyde are, however, stereoselective rather than stereospecific and afford diasteromeric intermediates.[53] The synthesis of 7 that we present here is stereospecific starting from 1. While it does not allow the possibility of substitution at C-2 of 7 that other syntheses do, our synthesis represents the best available chemical route to 7 that does not involve a degradative strategy.

Sphingosine is costly to isolate from natural sources.[54] Several syntheses of 18 and derivatives have been published;[32,55-63] the procedures of Vasella[32] and Schmidt[58] being among the best developed. In comparison, our synthesis is longer and less efficient. The intermediates 8–14 do, however, serve to expand the demonstrated synthetic utility of 1. The syntheses of 7 and 18 from 1 and its derivatives demonstrate the utility of 1 in asymmetric synthesis now that it is readily available by fumarase-catalyzed hydration of chlorofumaric acid.

Experimental Section

General. Pig heart fumarase was purchased as a crystalline suspension in 3.2 M ammonium sulfate from Sigma or Boehringer. Ultrafiltration devices used for desalting and concentrating enzymes solutions were from Amicon and fitted with PM 10 or YM 10 membranes. Dialysis tubing was from Spectrapor. PAN-850 was prepared previously in this laboratory.[15] NADH disodium salt, 94% purity, was from Sigma. *cis*-Epoxysuccinic acid was from Fluka. Diazald was from Aldrich. Ficoll, TRIS, and HEPES were from Sigma. 1,1,2-Trichloro-2,3,3-trifluorocyclobutane was from SCM Speciality Chemicals. Vinyllithium was from Organometallics. Methylene chloride, pyridine, diisopropylethylamine, and triethylamine was distilled from calcium hydride. Carbon tetrachloride was distilled from phosphorus pentoxide. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Anhydrous dimethylformamide (DMF) and dimethyl sulfoxide (Me_2SO) were from Aldrich. Potassium *tert*-butoxide was sublimed. *m*-Chloroperbenzoic acid was purified by washing with pH 7.5 phosphate buffer.[64] Oxalyl chloride was redistilled. Water was deionized and then distilled in a Corning AG-1b glass still. Water was deoxygenated by refluxing and distilling under nitrogen. Fumaric acid was recrystallized from water. DL-*threo*-Chloromalic acid,[16] chlorofumaric acid,[65] difluorofumaric acid,[12] fumaric acid monoamide,[66] *trans*-4-chloro-2-butenoic acid,[67] nitroacrylic acid[68] and *trans*-4-hydroxy-2-butenoic acid[69] were prepared by the literature methods. (+)-MTPA chloride was prepared from (+)-methoxy[(trifluoromethyl)phenyl]acetic acid by the published method.[17] All other chemicals were reagent grade. TRIS acetate buffers were 10 mM in TRIS base and acetic acid was added to the desired pH. NMR spectra were obtained by using solvent peaks as reference when possible; otherwise

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tetramethylsilane was the reference. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared spectra were taken from thin films unless otherwise specified. All thin-layer chromatography (TLC) was with silica gel 60 thin-layer chromatography plates from Merck. Melting points were determined on a Thomas-Hoover apparatus equipped with a calibrated thermometer. Elemental analyses were done by Galbraith Laboratories. K_m and V_{max} values were calculated by using the method of Scatchard.[70,71]

Desalting Fumarase. (a) Fumarase ammonium sulfate suspension (0.1 mL, 10 mg protein/mL) was dissolved in 10 mL of pH 7.3 buffer previously deoxygenated with nitrogen gas. In a 25 mm diameter Amicon stirred ultrafiltration cell under argon pressure, this solution was concentrated to 1 mL. Buffer was added to a total volume of 10 mL, followed by concentration to 1 mL. The enzyme solution was diluted and concentrated once more. This procedure reduces the ammonium sulfate concentration from 3.2 M to less than 0.5 mM. These solutions of fumarase in deoxygenated buffer were stable for several days at 4 °C.

(b) Fumarase ammonium sulfate suspension (1.5 mL) was transferred to 10-mm dialysis tubing. The tubing was then sealed with plastic clips. The sealed tube was placed in 200 mL of HEPES buffer (50 mM, pH 7.5, with 5 mM DTT). After 8 h the buffer was changed. At this point the contents of the tube were still cloudy and had absorbed buffer, resulting in a tightly filled tube. After sitting overnight, the solution was unchanged in appearance. The tube was removed from the buffer and dry Ficoll (MW 400 000) was applied to the outside of the dialysis tubing and allowed to stand for 20–30 min, at which time the resulting gel was gently scraped from the dialysis tubing and dry Ficoll was reapplied to the tubing. In 2 h the volume in the tubing was reduced to less than 1 mL, and the solution became clear. The solution of enzyme was recovered and the dialysis tubing rinsed with buffer. The combined solution and washes had a volume of 1.2 mL. Half of the starting fumarase activity was recovered.

Assays of Potential Substrates for Activity with Fumarase. Assays were based on standard procedures.[7–9] A typical assay used 0.01–0.1 mg/mL desalted fumarase in 10 mM HEPES buffer (pH 7.6). Test substrates were added to a concentration of ~1 mM. The enzyme activity present was sufficient to reach equilibrium with 1 mM fumarate in 1–10 s. Thus, activity on the order of a fraction of 1% of that of fumarate should have been detectable. UV measurements of olefinic absorbance were made in the region around 250 nm.

Fumarase Stability in 10 mM TRIS Acetate (pH 7.3): With and without 32 mM Sulfate. (a) TRIS acetate buffer (10 mM, pH 7.3) was degassed with nitrogen and 1.0-mL portions were added to a pair of 1.5-mL plastic microtubes. Desalted fumarase solution (80 μL) prepared as described was added to each tube. One tube was purged with nitrogen, sealed, and placed under nitrogen in a glovebag. The other tube was left open to the atmosphere. Periodic aliquots (10 μL) were removed, diluted to 1.0 mL with TRIS acetate buffer, and vortexed to mix. Fumarase activity was assayed in quartz curvettes containing 1.0 mL of TRIS acetate (10 mM, pH 7.3), 50 μL of 1 mM fumarate solution, and 100 μL of the diluted enzyme solution. Loss of absorbance at 230 nm was monitored in the UV spectrometer.

(b) Ammonium sulfate suspension of fumarase (10 μL, 3.2 M in ammonium sulfate) was added directly to 1.0 mL of 10 mM TRIS acetate, resulting in a solution 32 mM in ammonium sulfate. Assay for enzymic activity was with 50 μL of the enzyme solution diluted 100-fold in buffer added to 1.0 mL of buffer plus 0.1 mL of 1 mM fumarate. Absorbance at 230 nm was monitored.

(c) Sodium sulfate was dissolved in 10 mM TRIS acetate to a concentration of 32 mm (pH 7.5). Fumarase (2 μL) was added directly to 10 mL of the sodium sulfate solution. Aliquots (25 μL) were removed for assay in 1 mL of TRIS acetate buffer and 0.1 mL of 1 mL fumarate.

Immobilization of Fumarase in PAN Gel.[15] PAN was assayed at a content of 883 μmol/g of active ester. HEPES buffer (0.3 M, pH 7.5, 24 mL), containing 10 mM fumarate and 5 mM DTT, was added to 6.0 g of PAN, and the mixture was stirred with a glass rod for 2 min to dissolve the PAN. The thick solution was stirred magnetically for another minute, at which time 6 mL of desalted fumarase solution (20 000 U, fumarate as substrate) was added, and this mixture was stirred for 90 s. Triethylenetetramine (4.5 mL, 0.5 M solution) was added. The rapidly stirred mixture gelled in 40 s. The gel was allowed to stand at room temperature for 1 h under nitrogen and then transferred to a blender containing 200 mL of 50 mM HEPES buffer with 50 mM ammonium sulfate. This mixture was blended at low speed for 2 min followed by high speed for 30 s. The resulting suspension of immobilized fumarase was stirred for 15 min and centrifuged (3000 rpm, 10 min). The supernatant was decanted and 175 mL of 50 mM HEPES buffer (no ammonium sulfate) was added to the swollen gel. This mixture was stirred for 15 min and collected by centrifugation as before. The volume of the gel was 68 mL. It assayed for 1200 U of activity (fumarate as substrate). On the basis of the rate of conversion of chlorofumarate to chloromalate in a reaction, the gel contained 1675 U (fumarate as substrate, calculated from an initial activity of 45.2 U with chlorofumarate as substrate)[8] for an immobilization yield of 8%.

Small-Scale Synthesis of L-*threo*-Chloromalate (1). Chlorofumaric acid (1.00 g) and ammonium sulfate (63 mg) were dissolved in deoxygenated water, and deoxygenated 1 N NaOH was added to bring the pH up to 7.6. The solution volume was made up to 20 mL with water, and 50 μL of a suspension of fumarase was added. The mixture was stirred gently under nitrogen. Absorbance at 240 nm was monitored over 4 days. On the fourth day the solution was concentrated by evaporation to 10 mL and acidified to pH 1.5 with concentrated HCl. The acidified solution was extracted continuously with ether. The ether extract was concentrated to a syrup that was dried to a white solid under vacuum, 1.0 g. The crude product was dissolved in wet ether with warming, and petroleum ether (20–40 °C fraction) was added until the solution was cloudy. On standing clear colorless crystals were obtained. Continued additions of petroleum ether resulted in further crystallization, until a grainy white precipitate of chlorofumaric acid began to appear, at which point a small amount of ether was added to redissolve this material. The precipitate was collected and dried in vacuo to white crystals; 0.69 g (62%); mp 166–168 °C (lit. mp 168–170 °C);[40] TLC [hexane/ether/95% formic acid (10:10:1)] R_f 0.71 (detection with basic bromphenol blue in methanol); $[\alpha]^{22}_D$ −12.0° (*c* 2.0, 96% ethanol) [lit.[16] $[\alpha]^{22}_D$ −11.2° (*c* = 7.4, 96% ethanol], ^1H NMR (CD_3COCD_3) δ 4.8 (br s, 1 H), 4.93 (d, *J* = 2.9 Hz, 1 H), 5.01 (d, *J* = 2.9 Hz, 1 H), 11.0 (br s, 2 H); ^{13}C NMR δ 60.6, 72.0, 167.9, 171.7.

Large-Scale Synthesis of L-*threo*-Chloromalic Acid (1) with Soluble Fumarase. Chlorofumaric acid (50 g, 332 mmol) and ammonium sulfate (2.22 g) were dissolved in water. NaOH was added to pH 7.5 in a volume of 500 mL. The solution was filtered through a medium-porosity glass frit. The frit was washed with 150 mL of water. The water was combined with the chlorofumarate solution and degassed by stirring under reduced pressure. Fumarase (5000 U) was added, and the reaction mixture was stirred under nitrogen. After 6 days ~30% of the chlorofumarate had been consumed and the residual enzymic activity was low. More fumarase (~4000 U) in 100 mL of deoxygenated water was added and the mixture stirred momentarily and allowed to stand under nitrogen. After a further 7 days the mixture was ultrafiltered through a YM 10 membrane. The filtrate was concentrated at reduced pressure (<40 °C) to 160 mL and acidified with concentrated HCl to pH 1.3. The solution was extracted continuously with ether for 16 h. The ethereal solution was concentrated to a damp solid and taken up in ether. Complete dissolution of the solid was aided by the addition of a small amount of water. Petroleum ether was added with warming until the solution was cloudy. Vigorous stirring of this solution at 0 °C resulted in 25 g of a white precipitate. Starting material (8 g) was recovered from the supernatant. Further extraction and precipitation as described above yielded a total of 34 g (60%) of chloromalic acid with the same properties as that obtained from the small-scale reaction. Recovered starting material totaled 10 g (21%).

Large-Scale Synthesis of L-*threo*-Chloromalic Acid (1) with PAN-Immobilized Fumarase. Chlorofumaric acid (63.7 g, 423 mmol) and ammonium sulfate (3.57 g) were dissolved in water. NaOH was used to adjust the pH to 7.5. This solution

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(700 mL) was filtered and degassed as above. PAN gel containing 45 U of fumarase (chlorofumarate as substrate) was added, and the resulting mixture was stirred gently under nitrogen. After 7.5 days the enzyme-containing gel was collected by centrifugation (3000 rpm × 10 min), and the supernatant was concentrated under reduced pressure at 40 °C to a volume of 500 mL. The gel was stirred with 100 mL of water and centrifuged and the wash combined with the solution from the reactor. The pH of the solution was adjusted to pH 1.5 by slow addition of concentrated HCl. The crude product was collected by continuous extraction with ether as above. From this first reaction 42.5 g of crystalline chloromalic acid was isolated and 17.7 g of crude chlorofumaric acid. The recovered starting material was recombined with the recovered immobilized fumarase for a further 7 days. The initial fumarase activity in this reactor was 80% that of the first reactor. The second reactor was worked up in the same manner as the first. The enzyme-containing gel was stored at 4 °C. Chloromalic acid (12.0 g) was obtained from the second reactor for a total yield of 54.5 g (76%) of isolated crystalline material with the same properties as described above. Crude starting material (2.3 g) was also recovered.

Dimethyl L-*threo*-Chloromalate. Diazomethane was generated from Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide).[72] To L-*threo*-chloromalic acid (337 mg, 2 mmol) in a minimum amount of MeOH at 0 °C was added dropwise diazomethane in ether until a yellow color persisted. A minimum of methanolic HCl was added to decompose the excess diazomethane, and the solution was concentrated to a colorless oil to yield dimethyl L-*threo*-chloromalate quantitatively: TLC [ether/20–40 °C petroleum ether (55:45)] R_f 0.39; $[\alpha]^{25}_D$ –0.15° (*c* 2, $CHCl_3$); IR 3480, 2960, 1745, 1435, 1270, 1115, 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.78 (s, 3 H), 3.79 (s, 3 H), 3.95 (br s, 1 H), 4.76 (d, *J* = 2.5 Hz, 1 H), 4.84 (d, *J* = 2.5 Hz, 1 H); ^{13}C NMR δ 53.1, 53.4, 59.5, 71.8, 166.8, 170.5. Anal. Calcd for $C_6H_9ClO_5$: C, 36.66; H, 4.61. Found: C, 36.53; H, 4.68.

Dimethyl DL-*threo*-chloromalate was prepared in the same manner as above from DL-*threo*-chloromalic acid. The racemic material had the same chromatographic and spectral properties as dimethyl L-*threo*-chloromalate.

(+)-MTPA Esters of Dimethyl Chloromalates. Pyridine (0.30 mL) was transferred via syringe to an oven-dried 10 mm × 75 mm test tube sealed with a serum cap. (+)-MTPA chloride (26 μL) was added and the mixture swirled to mix. Carbon tetrachloride (0.30 mL) was added via syringe followed by dimethyl L-*threo*-chloromalate (15 μL, 0.1 mmol), and the mixture was shaken. Reaction began at once as evidenced by the precipitation of pyridinium hydrochloride. The reaction appeared to be complete within 15 min but was allowed to stand for 1 h to ensure complete reaction. The reaction mixture was diluted with 1 mL of ether and transferred to a small separatory funnel and washed with 2 × 1 mL of 3 N HCl, 2 × 1 mL 10% $NaHCO_3$, and 1 mL of saturated NaCl. The organic layer was passed through a Pasteur pipet containing a short column of $MgSO_4$ over Celite which was washed with 1 mL of ether. The combined organic phases were evaporated, and the residue was dried in vacuo for several minutes to ensure removal of organic solvents. The residue was taken up in $CDCl_3$ for NMR experiments: TLC [silica gel, ether/hexanes (90:10)] R_f 0.75; ^1H NMR ($CDCl_3$) δ 3.54 (d, *J* = 1.0 Hz, 3 H), 3.79 (s, 3 H), 3.81 (s, 3 H), 4.99 (d, *J* = 3.9 Hz, 1 H), 6.00 (d, *J* = 3.9 Hz, 1 H), 7.35–7.45 (m, 3 H), 7.55–7.65 (m, 2 H).

The (+)-MTPA ester of dimethyl DL-*threo*-chloromalate was prepared in the same manner: ^1H NMR ($CDCl_3$) δ 3.53 (d, *J* = 1.0 Hz, 1.5 H), 3.58 (s, 1.5 H), 3.62 (d, *J* = 1.2 Hz, 1.5 H), 3.76 (s, 1.5 H), 3.77 (s, 1.5 H), 3.82 (s, 1.5 H), 4.95 (d, *J* = 3.4 Hz, 0.5 H), 4.98 (d, *J* = 3.9 Hz, 0.5 H), 5.95 (d, *J* = 3.4 Hz, 0.5 H), 5.99 (d, *J* = 3.9 Hz, 0.5 H), 7.35–7.45 (m, 3 H), 7.55–7.65 (m, 2 H).

(2*R*,3*R*)-3-Chlorobutane-1,2,4-triol (2). L-*threo*-Chloromalic acid (16.9 g, 100 mmol) was dissolved in 300 mL of THF and stirred at 0 °C. Borane (1 M in THF, 300 mL, 300 mmol) was added dropwise. The reaction was stirred at room temperature for 5 h, during which time a white precipitate developed. The reaction was quenched at 0 °C with MeOH (12.5 mL), resulting in a clear solution. The reaction mixture was concentrated to a foaming syrup, stirred in 100 mL of MeOH overnight, and concentrated to a clear colorless oil. A further 100 mL of MeOH was removed from the crude product by evaporation at reduced pressure and drying in vacuo to an oil, 15.9 g. Column chromatography on silica gel with ethyl acetate and 10% EtOH in ethyl acetate gave a clear colorless oil, 12.5 g (89%). On standing, slow crystallization to long thick needles was observed: mp 36–40 °C; TLC [ethyl acetate] R_f 0.28, with trailing; $[\alpha]^{22}_D$ –0.24° (*c* 2.7, H_2O); ^1H NMR (CD_3COCD_3 with D_2O) δ 3.55–3.58 (m, 2 H), 3.66–3.88 (m, 2 H), 3.91–3.98 (m, 1 H), 4.05–4.13 (m, 1 H); ^{13}C NMR (D_2O; reference, MeOH peak at δ 49.0) δ 62.9, 63.4, 63.6, 70.7. Anal. Calcd for $C_4H_9ClO_3$: C, 34.18; H, 6.45. Found: C, 34.02; H, 6.59.

1,2-*O*-Isopropylidene-(2*R*,3*R*)-3-chlorobutane-1,2,4-triol (3). Crude triol (a light brown oil) from the above procedure (4.96 g, 35.3 mmol) was combined with 20 mL of acetone, dimethoxypropane (5.21 mL, 42.4 mmol), and TsOH (69 mg, 0.35 mmol). After 17 h, NaOMe (20 mg, 0.35 mmol) was added and the mixture concentrated to a volume of 10 mL. EtOAc (50 mL) was added and evaporated at reduced pressure. Ether (100 mL) was added and the resulting mixture filtered and concentrated to a pale brown oil: 5.8 g (92%); TLC [ethyl acetate/hexane (1:1)] R_f 0.50; $[\alpha]^{22}_D$ –16.0° (*c* 7.2, EtOAc); IR 3430, 2980, 2940, 2890, 1710, 1455, 1385, 1255, 1215, 1155, 1060, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (s, 3 H), 1.44 (s, 3 H), 2.47 (br s, 1 H), 3.82–4.12 (m, 5 H), 4.38 (dt, *J* = 6.4, 4.5 Hz, 1 H); ^{13}C NMR δ 25.1, 26.0, 62.2, 64.0, 66.2, 76.0, 110.1; MS (CI, *i*-BuH), exact mass calcd for $C_7H_{14}ClO_3^+$ 181.0631, found 181.0634.

3,4-*O*-Isopropylidene-(2*S*,3*R*)-3,4-dihydroxybut-1-ene Oxide (4). To 2.34 g (13 mmol) of acetonide **3** in 15 mL of ether was added KOH (836 mg, 13 mmol) in small portions and the mixture stirred at gentle reflux for 2 h. Ether (15 mL) was added, and the mixture was filtered through Celite and concentrated to a light brown oil (1.72 g, 92%): TLC [ethyl acetate/hexane (1:1)] R_f 0.70, [ethyl acetate/hexane (1:2)] R_f 0.46; $[\alpha]^{22}_D$ +16.4° (*c* 11.6, EtOAc); IR ($CHCl_3$) 2990, 2940, 2890, 1380, 1150, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (s, 3 H), 1.42 (s, 3 H), 2.62 (dd, *J* = 4.9, 2.6 Hz, 1 H), 2.81 (ddd, *J* = 4.9, 4.0, 0.8 Hz, 1 H), 2.96–3.01 (m, 1 H), 3.76–3.92 (m, 2 H), 4.06–4.12 (m, 1 H); ^{13}C NMR: δ 25.3, 26.5, 45.6, 52.0, 66.9, 76.4, 109.8; MS (CI, *i*-BuH), exact mass calcd for $C_7H_{13}ClO_3^+$ 145.0865, found 145.0862.

5,6-*O*-Isopropylidene-(4*S*,5*R*)-hex-1-ene-4,5,6-triol (5). To CuCN (1.60 g, 17.9 mmol) stirred in suspension in 10 mL of THF at –78 °C was added dropwise vinyllithium (0.9 M in THF, 40 mL, 35.8 mmol). The mixture was stirred at 0 °C for 1 h and cooled to –78 °C. Epoxide **4** (1.72 g, 11.9 mmol) was added dropwise. The mixture was allowed to warm to 0 °C, by which time the reaction was complete by TLC. The reaction was quenched with 60 mL of saturated NH_4Cl/NH_4OH (9:1), stirred for 30 min, and extracted with ether. The combined organic phase (100 mL) was dried over $MgSO_4$, filtered, and concentrated to a yellow oil, which was purified by column chromatography on silica gel [EtOAc/hexane (1:2.5)]: yield, 948 mg (46%); TLC [ethyl acetate/hexane (1:2)] R_f 0.40; $[\alpha]^{22}_D$ +4.7° (*c* 0.6, EtOAc); IR 3470, 3080, 2990, 2900, 1640, 1375, 1250, 1215, 1160, 1065, 915, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (s, 3 H), 1.43 (s, 3 H), 2.12–2.40 (m, 3 H), 3.77 (dt, *J* = 8, 4 Hz, 1 H), 3.89–4.06 (m, 3 H), 5.12 (br s, 1 H), 5.19 (m, 1 H), 5.76–5.94 (m, 1 H); ^{13}C NMR δ 25.3, 26.5, 37.6, 65.2, 70.4, 78.1, 109.0, 118.1, 133.9; MS (CI, *i*-BuH), exact mass calcd for $C_9H_{17}O_3^+$ 173.1178, found 173.1178.

(4*S*,5*R*)-Hex-1-ene-4,5,6-triol (6). A mixture of acetonide **5** (431 mg, 2.5 mmol), 0.3 mL of trifluoroacetic acid, 3 mL of water, and 3 mL of MeOH was allowed to stand for 12 h. The mixture was concentrated to an oil, and 5 mL of water was added. The water was removed from the residue by evaporation. Addition and evaporation of 5 mL of EtOAc left a brownish precipitate, which was recrystallized from EtOAc/petroleum ether (40–60 °C) as a white solid: mp 55.5–56.5 °C (lit.[30] mp 54–55 °C); yield, 249 mg (75%); $[\alpha]^{22}_D$ +7.0° (c 5.0, D_2O) [lit.[23] $[\alpha]^{20}_D$ +9.2 (*c* = 5.7, D_2O)]; ^1H NMR (D_2O; reference, HOD peak at δ 4.80) δ 2.40 (m, 1 H), 2.55 (m, 1 H), 3.65–4.10 (m, 4 H), 5.31 (m, 2 H), 6.05 (m, 1 H); ^{13}C NMR (D_2O; reference, MeOH peak at δ 49.0) δ 36.6, 62.6, 71.3, 74.2, 117.6, 135.1.

2-Deoxy-D-ribose (7). Olefin triol **6** (231 mg) was dissolved in 50 mL of MeOH and stirred at –78 °C. Ozone was passed into

(72) Black, T. H. *Aldrichimica Acta* **1983**, *16*, 3–10.

this solution until a blue color persisted (5 min), and after 20 min nitrogen was passed through the solution for 0.5 h to eliminate excess ozone. Dimethyl sulfide (5.5 mL) was added and the mixture allowed to warm to room temperature. The solution was concentrated at reduced pressure and dried in vacuo to a clear colorless syrup: 283 mg; $[\alpha]^{22}_D$ -45° (c 3.2, H_2O) [lit.[73] $[\alpha]_D$ -58° (c 1.65, H_2O)]; ^{13}C NMR (D_2O; reference, MeOH peak at δ 49.0) δ 34.0, 35.2, 41.2, 61.6, 62.6, 62.8, 64.7, 65.5, 66.5, 67.2, 67.4, 71.0, 71.2, 85.2, 85.8, 91.6, 93.7, 98.0. Authentic 7:[74] ^{13}C NMR δ 33.9, 35.2, 41.2, 61.6, 62.7, 62.8, 64.7, 65.7, 66.5, 67.3, 67.4, 71.0, 71.2, 85.2, 85.8, 91.7, 93.8, 98.1.

(2*R*,3*R*)-1,2-*O*-Isopropylidene-4-(*N*-benzylcarbamyl)-3-chlorobutane-1,2,4-triol (8). Acetonide 3 (9.75 g, 54 mmol), benzyl isocyanate (7.91 g, 59.4 mmol), and diisopropylethylamine (14.1 mL, 81 mmol) were combined with benzene (100 mL) and stirred at 50–60 °C for 6 h. The mixture was concentrated on a rotary evaporator and then in vacuo to a light brown oil, 19.1 g. Chromatography on silica gel (4 × 20 cm) with ethyl acetate/hexane (1:2.5) afforded acyclic carbamate 8 as a light yellow oil: 16.4 g (97%); TLC [ethyl acetate/hexane (1:2)] R_f 0.35; $[\alpha]^{25}_D$ -2.1° (c 1.4, $CHCl_3$); IR 3340, 2985, 2930, 2880, 1720, 1520, 1370, 1245, 1065, 845 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (s, 3 H), 1.46 (s, 3 H), 3.93 (dd, J = 8, 6 Hz, 1 H), 4.04–4.12 (m, 2 H), 4.27–4.41 (m, 5 H), 5.27 (br s, 1 H), 7.24–7.36 (m, 5 H); ^{13}C NMR δ 25.2, 26.1, 45.1, 58.9, 65.4, 66.2, 75.4, 110.1, 127.5, 128.6, 138.0, 155.6. Anal. Calcd for $C_{15}H_{20}ClNO_4$: C, 57.42; H, 6.42; N, 4.46. Found: C, 57.45; H, 6.62; N, 4.31.

(4*S*,1′*S*)-3-Benzyl-4-[1,2-(isopropylidenedioxy)ethyl]-1,3-oxazolidin-2-one (9). Acyclic carbamate 8 (13.6 g, 43.2 mmol) was dissolved in THF/DMF (4:1, 50 mL). Potassium *tert*-butoxide (5.34 g, 47.6 mmol) was added in several portions with stirring. The resulting mixture became warm. After 2 h solvent was removed in vacuo, the residue dissolved in ether and filtered, and solvent evaporated to afford a light orange residue, which was recrystallized from methylene chloride/hexane, affording slightly colored 9, 5.70 g (48%). Recrystallization from ethyl acetate/hexane gave flat colorless needles: mp 133–135 °C; TLC [ethyl acetate/hexane (1:1)] R_f 0.36; $[\alpha]^{22}_D$ -0.9° (c 1.0, $CHCl_3$); IR 2990, 2890, 1735, 1430, 1200, 1010, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (s, 3 H), 1.43 (s, 3 H), 3.53 (dd, J = 8.6, 6.1 Hz, 1 H), 3.67 (m, 1 H), 3.97 (dd, J = 8.6, 6.9 Hz, 1 H), 4.16 (d, J = 15.5 Hz, 1 H), 4.21–4.38 (m, 3 H), 4.86 (d, J = 15.5 Hz, 1 H), 7.26–7.38 (m, 5 H); ^{13}C NMR δ 24.4, 26.0, 46.6, 55.8, 63.3, 65.6, 74.0, 109.6, 127.7, 128.7, 135.8, 158.4. Anal. Calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 65.06; H, 7.05; N, 4.96.

(4*S*,1′*S*)-3-Benzyl-4-(1,2-dihydroxyethyl)-1,3-oxazolidin-2-one (10). Acetonide 9 (3.73, g, 13.5 mmol) was dissolved in 1 N HCl/THF (1:1, 140 mL) and stirred for 6 h. THF was evaporated at reduced pressure, the reaction mixture was neutralized with NaOH (2.8 g, 70 mmol), and the mixture was evaporated to dryness in vacuo at <40 °C. The resulting white solid was taken up in hot ethyl acetate and filtered to remove NaCl. On cooling, clusters of colorless needles were obtained (2.26 g). Concentration of the supernatant and further crystallization yielded further 10 (0.72 g): total yield, 2.99 g (94%); mp 106.5 °C; $[\alpha]^{22}_D$ -9.9° (c 1.0, CH_3CN); IR 3400, 2900, 1720, 1440, 1240, 1030 cm^{-1}; ^1H NMR (CD_3CN) δ 3.00 (t, J = 5.6 Hz, 1 H, OH), 3.38 (dd, J = 5.6, 5.6 Hz, 2 H) (with D_2O collapse to d), 3.46 (d, J = 4.4 Hz, 1 H, OH), 3.72 (ddd, J = 9, 6, 2 Hz, 1 H), 3.88 (tdd, J = 5.6, 5, 2 Hz, 1 H) (with D_2O collapse to td), 4.17 (d, J = 15.7 Hz, 1 H), 4.18 (dd, J = 9, 9 Hz, 1 H), 4.32 (dd, J = 9, 6 Hz, 1 H), 4.69 (d, J = 15.7 Hz, 1 H), 7.26–7.40 (m, 5 H); ^{13}C NMR δ 46.6, 57.5, 63.3, 63.6, 68.9, 128.6, 128.8, 129.7, 137.8, 159.9. Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.72; H, 6.51; N, 5.94.

(4*S*,1′*S*)-3-Benzyl-4-[2-((*tert*-butyldimethylsilyl)oxy)-1-hydroxyethyl]-1,3-oxazolidin-2-one (11). Diol 10 (2.73 g, 11.5 mmol) was stirred with triethylamine (2.0 mL, 14.4 mmol) and (dimethylamino)pyridine (14 mg, 0.115 mmol) in DMF (20 mL) under nitrogen. *tert*-Butyldimethylsilyl chloride (1.91 g, 12.65 mmol) in DMF (26 mL) was added dropwise with stirring. After 30 min, solvent was removed in vacuo at <35 °C. The residue was taken up in 150 mL of ethyl acetate, filtered from triethylamine hydrochloride, and then concentrated to a pale yellow oil, which crystallized to a white solid on drying in vacuo, 4.36 g. This material was dissolved in a minimum of hot ethyl acetate and diluted with several volumes of hexane. On standing at room temperature fine colorless needles separated from solution and were collected by filtration: 3.76 g (93%); mp 94.5–95 °C; TLC [ethyl acetate/hexane (1:2)] R_f 0.28; $[\alpha]^{22}_D$ +9.3° (c 1.1, $CHCl_3$); IR 3450, 2925, 2860, 1750, 1725, 1425, 1255, 1060, 840, 770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.00 (s, 6 H), 0.81 (s, 3 H), 0.82 (s, 6 H), 3.13 (m, 1 H), 3.53 (m, 2 H), 3.77 (ddd, J = 9, 7, 2 Hz, 1 H), 3.91 (m, 1 H), 4.14–4.23 (m, 1 H), 4.20 (d, J = 15.3 Hz, 1 H), 4.43 (dd, J = 9, 7 Hz, 1 H), 4.79 (d, J = 15.3 Hz, 1 H); ^{13}C NMR δ -5.5, 25.8, 46.4, 56.6, 62.5, 63.6, 67.9, 127.8, 128.0, 128.7, 135.9, 159.0. Anal. Calcd for $C_{18}H_{29}NO_4Si$: C, 61.50; H, 8.32; N, 3.98. Found: C, 61.59; H, 8.25; N, 4.14.

(4*S*,1′*S*)-3-Benzyl-4-[1-(benzyloxy)-2-((*tert*-butyldimethylsilyl)oxy)ethyl]-1,3-oxazolidin-2-one (12). Alcohol 11 (3.52 g, 10 mmol), benzyl bromide (1.31 g, 11 mmol), and tetrabutylammonium iodide (185 mg, 0.5 mmol) were stirred in THF (50 mL) at 0 °C. Sodium hydride (60% dispersion in oil, 0.40 g, 10 mmol) was added in small portions over 10 min. The reaction was stirred a further 10 min at 0 °C and then at room temperature for 1.5 h. The mixture was concentrated at reduced pressure to a volume of 10 mL, ether (100 mL) was added, and precipitated salts were removed by filtration, followed by concentration to a yellow oil, 4.99 g. Chromatography on silica gel (4 × 18 cm) with ethyl acetate/hexane (1:5) afforded 12 mixed with its 1-*O*-benzyl-2-*O*-TBDMS isomer as a pale yellow oil: 3.77 g (85%); TLC [ethyl acetate/hexane (1:2)] R_f 0.47; $[\alpha]^{22}_D$ -31.3° (c 1.3, $CHCl_3$); IR 2930, 2860, 1750, 1420, 1250, 1090, 830, 775 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.0 (s, 4.3 H), 0.08 (s, 0.9 H), 0.10 (s, 0.9 H), 0.83 (s, 6.8 H), 0.89 (s, 2.2 H), 3.25–3.48 (m, 0.7 H), 3.59 (s, 2 H), 3.67 (d, J = 15.7 Hz, 0.7 H), 3.78 (dd, J = 9.3, 6.1 Hz, 1 H), 3.95 (d, J = 15.2 Hz, 0.2 H), 4.05–4.12 (m, 0.7 H), 4.17 (dd, J = 9.3, 8.6 Hz, 0.7 H), 4.35 (d, J = 12.0 Hz, 0.7 H), 4.35–4.47 (m, 0.8 H), 4.51 (dd, J = 8.6, 5.9 Hz, 0.8 H), 4.61 (d, J = 11.8 Hz, 0.7 H), 4.63 (d, J = 15.3 Hz, 0.7 H), 4.95 (d, J = 15.2 Hz, 0.2 H), 7.15–7.40 (m, 10 H); ^{13}C NMR δ -5.6, 18.0, 25.7, 45.9, 55.9, 61.4, 62.6, 71.7, 74.3, 127.2, 127.6, 127.7, 127.9, 128.2, 128.5, 135.7, 137.5, 158.5, [minor isomer peaks] 17.8, 25.6, 45.7, 56.2, 62.2, 67.6, 71.1, 73.3. Anal. Calcd for $C_{25}H_{35}NO_4Si$: C, 67.99; H, 7.99; N, 3.17. Found: C, 67.83; H, 8.09; N, 3.24.

(4*S*,1′*S*)-3-Benzyl-4-[1-(benzyloxy)-2-hydroxyethyl]-1,3-oxazolidin-2-one (13). Protected diol 12 (3.99 g, 7.67 mmol) was dissolved in THF (50 mL). Tetrabutylammonium fluoride (1 M in THF, 7.7 mL, 7.7 mmol) was added and the mixture stirred for 15 min and then concentrated under reduced pressure to an oil that was chromatographed on silica gel (4 × 16 cm). The column was washed with ethyl acetate/hexane (1:1, 500 mL; 6:5, 500 mL) and ethyl acetate (300 mL). Fractions containing the main component were pooled and concentrated to a pale yellow oil that slowly crystallized to a white solid, 13, 1.66 g (66%). An analytical sample was obtained by recrystallization from ethyl acetate/hexane: mp 83.5–84 °C; TLC [ethyl acetate] R_f 0.60; $[\alpha]^{22}_D$ -25.7° (c 1.0, $CHCl_3$); IR 3420, 3030, 2920, 2870, 1725, 1435, 1345, 1055, 735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.52 (dd, J = 11.5, 3.8 Hz, 1 H), 3.63 (dddd, J = 4.2, 3.8, 1.9, 1 H), 3.69 (dd, J = 11.5 4.2 Hz, 1 H), 3.73 (m, 1 H), 4.23 (t, J = 9 Hz, 1 H), 4.43 (br s, 1 H), 4.40 (d, J = 12 Hz, 1 H), 4.46 (dd, J = 8.8, 5.8 Hz, 1 H), 4.58 (d, J = 12 Hz, 1 H), 4.66 (d, J = 15.3 Hz, 1 H), 7.15–7.40 (m, 10 H); ^{13}C NMR δ 46.4, 56.3, 61.3, 63.3, 72.3, 75.5, 127.9, 128.1, 128.5, 135.8, 137.4, 158.7. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 70.00; H, 6.69; N, 4.24.

A less polar minor component, the 2-*O*-benzyl isomer of 13, was also isolated: 0.45 g (18%); ^1H NMR ($CDCl_3$) δ 3.42 (d, J = 5.2 Hz, 2 H), 3.74 (ddd, J = 8.7, 6.5, 1.8 Hz, 1 H), 4.03 (m, 1 H), 4.16 (dd, J = 9, 9 Hz, 1 H), 4.20 (d, J = 15.3 Hz, 1 H), 4.40 (dd, J = 8.7, 6.5 Hz, 1 H), 4.45 (s, 2 H), 4.77 (d, J = 15.3 Hz, 1 H), 7.2–7.4 (m, 10 H); ^{13}C NMR δ 46.5, 56.9, 62.7, 70.4, 73.6, 127.6, 127.8, 127.9, 128.4, 128.8, 136.0, 137.3, 158.9.

(4*S*,1′*S*)-3-Benzyl-4-[1-(benzyloxy)-2-oxoethyl]-1,3-oxazolidin-2-one (14). Oxalyl chloride (366 μL, 4.2 mmol) in methylene chloride (9.2 mL) was cooled to -50 to -60 °C under nitrogen. Dimethyl sulfoxide (593 μL, 8.4 mmol) in methylene chloride was added dropwise with stirring. After 5 min, alcohol 13 (917 mg, 2.8 mmol) in methylene chloride (4 mL) was added

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dropwise. After 20 min, triethylamine (1.95 mL, 14 mmol) was added and the mixture stirred at -60 °C for 5 min and then at room temperature for 10 min. Water (25 mL) was added to the reaction mixture. The aqueous phase was extracted with methylene chloride (2 × 20 mL), and the pooled organic phases were dried over $MgSO_4$ and concentrated to an oil that was chromatographed on silica gel with ethyl acetate/hexane (5:2) to afford **14** as a clear colorless oil that was dried by evaporation of toluene from the oil (3 × 10 mL): yield, 843 mg (93%); TLC [ethyl acetate] R_f 0.67; $[\alpha]^{22}{}_D$ -53.6° (c 0.7, $CHCl_3$); IR 3380, 3030, 2920, 2875, 1725, 1430, 1230, 1065, 745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.73 (d, J = 15.3 Hz, 1 H), 3.83 (dd, J = 2.3, 0.9 Hz, 1 H), 3.92 (qd, J = 9.0, 5.2, 2.3 Hz, 1 H), 4.13 (dd, J = 9.0, 8.8 Hz, 1 H), 4.23 (dd, J = 8.8, 5.2 Hz, 1 H), 4.38 (d, J = 11.8 Hz, 1 H), 4.55 (d, J = 15.3 Hz, 1 H), 4.57 (d, J = 11.8 Hz, 1 H), 7.1–7.4 (m, 10 H), 9.57 (d, J = 0.9 Hz, 1 H); ^{13}C NMR δ 46.6, 55.5, 62.5, 73.5, 80.1, 128.05, 128.1, 128.4, 128.6, 128.8, 135.5, 136.1, 158.2, 201.2; MS (CI, *i*-BuH), exact mass calcd for $C_{19}H_{20}NO_4{}^+$ 326.1392, found 326.1394.

Phenyl *n*-Tetradecyl Sulfide. Tetradecyl bromide (2.77 g, 10 mmol), thiophenol (1.03 mL, 10 mmol), triethylamine (1.53 mL, 11 mmol), and tetrabutylammonium iodide (40 mg) were dissolved and stirred in THF (50 mL). A white precipitate soon began to form. After 6 h, solvent was evaporated and the residue taken up in ether, filtered, and concentrated to a white solid, which was recrystallized from ethyl acetate/methanol to yield colorless flat needles: 2.75 g (90%); mp 41 °C; TLC [hexane] R_f 0.56; IR 2920, 2850, 1460, 1440, 730, 685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 7 Hz, 3 H), 1.2–1.5 (m, 22 H), 1.64 (p, J = 7 Hz, 2 H), 2.91 (t, J = 7 Hz, 2 H), 7.1–7.4 (m, 5 H); ^{13}C NMR δ 14.1, 22.8, 28.9, 29.3, 29.7, 31.0, 32.0, 33.9, 125.6, 128.7, 129.0, 137.1. Anal. Calcd for $C_{20}H_{34}S$: C, 78.27; H, 11.16. Found: C, 78.36; H, 11.29.

Phenyl *n*-Tetradecyl Sulfone. Phenyl tetradecyl sulfide (4.39 g, 14.3 mmol) was dissolved in methylene chloride (50 mL) and stirred at room temperature. A solution of *m*-chloroperbenzoic acid (6.18 g, 35.8 mmol) in methylene chloride (70 mL) was added dropwise. After 1 h, the solution was filtered, washed with 10% Na_2SO_3 (15 mL) and saturated $NaHCO_3$ (2 × 25 mL), dried over Na_2SO_4, filtered, and evaporated to a white solid 4.86 g (100%). Recrystallization from methanol (4 °C) yielded fine colorless needles: 4.47 g (92%); mp 43–44 °C; IR 3060, 2920, 2850, 1770, 1450, 1310, 1215, 1150, 1090, 725, 690, 600, 570 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 7 Hz, 3 H), 1.1–1.4 (m, 22 H), 1.70 (m, 2 H), 3.07 (m, 2 H), 7.5–7.7 (m, 3 H), 7.85–7.95 (m, 2 H); ^{13}C NMR δ 14.1, 22.7, 28.4, 29.0, 29.3, 29.4, 29.5, 29.7, 32.0, 56.5, 128.0, 129.1, 133.3, 139.7. Anal. Calcd for $C_{20}H_{34}SO_2$: C, 70.87; H, 10.11. Found: C, 71.02; H, 10.11.

(4*S*,1′*S*)-3-Benzyl-4-[2-acetoxy-1-(benzyloxy)-3-(phenylsulfonyl)hexadecyl]-1,3-oxazolidin-2-one (15). Phenyl tetradecyl sulfone (67.7 mg, 0.2 mmol) was stirred in THF (4 mL) at -70 °C. *n*-Butyllithium (2.5 M in hexanes, 20 μL) was slowly added until a yellow color persisted. Additional butyllithium (80 μL, 0.2 mmol) was then added dropwise, resulting in a clear yellow solution. Aldehyde **14** (30 mg, 0.09 mmol) in THF (0.5 mL) was added dropwise to the cooled sulfone solution. After 30 min, acetic anhydride (75 μL, 0.8 mmol) was added and the reaction stirred at -70 °C for 1 h. The reaction was quenched by the addition of saturated NH_4Cl (0.5 mL). The aqueous phase was extracted with ether (3 × 2 mL). The combined organic phases were dried over $MgSO_4$ and concentrated to a colorless oil, which was chromatographed on silica gel with ethyl acetate/hexane (1:2.5), affording **15** as a clear colorless oil: 39.1 mg (55%); TLC [ethyl acetate/hexane (1:3)] R_f 0.28 (intermediate hydroxy sulfone, R_f 0.11); ^1H NMR ($CDCl_3$) δ 0.88 (m, 3 H), 1.0–1.5 (m, 24 H), 1.86–1.89 (m, 3 H), 2.8–5.3 (m, 10 H), 7.1–7.5 (m, 5 H); ^{13}C NMR (major component) δ 14.2, 22.7, 29–30 (m), 31.9, 46.4, 54.6, 62.3, 68.0, 73.6, 128–130 (m), 134.0, 135.2, 158.2. Anal. Calcd for $C_{41}H_{55}NO_7S$: C, 69.76; H, 7.85; N, 1.98. Found: C, 69.27; H, 7.92; N, 1.99.

(2′*E*,4*S*,1′*R*)-3-Benzyl-4-[1-(benzyloxy)hexadec-2-enyl]-1,3-oxazolidin-2-one (16). Sulfone acetate **15** (627 mg, 0.85 mmol) was stirred in methanol/ethyl acetate (2:1, 18 mL) at 0 °C with 10 g of 2% sodium amalgam for 6 h. The solution was decanted from the amalgam and extracted with 5 mL of 50% saturated NH_4Cl. The aqueous phase was extracted with 50 mL of ether. The combined organic solutions were dried over $MgSO_4$, concentrated, and chromatographed on silica gel [ethyl acetate/hexane (1:5)] to yield **16** as a clear colorless oil: 231 mg (51%); TLC [ethyl acetate/hexane (1:2)] R_f 0.66; $[\alpha]^{22}{}_D$ -48.2° (c 1.2, $CHCl_3$); IR 2920, 2855, 1755, 1420, 1205, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 7 Hz, 3 H), 1.15–1.4 (m, 22 H), 2.09 (q, J = 7 Hz, 2 H), 3.65 (ddd, J = 9, 6, 3 Hz, 1 H), 3.79 (dd, J = 8, 3 Hz, 1 H), 3.95 (d, J = 15.1 Hz, 1 H), 4.17–4.24 (m, 2 H), 4.21 (d, J = 11.9 Hz, 1 H), 4.55 (d, J = 11.9 Hz, 1 H), 4.76 (d, J = 15.1 Hz, 1 H), 5.24 (dd, J = 15.4, 8.0 Hz, 1 H), 5.75 (dt, J = 15.4, 7 Hz, 1 H), 7.1–7.4 (m, 10 H); ^{13}C NMR δ 14.1, 22.7, 28.1–29.7 (m), 31.9, 32.4, 46.5, 57.4, 63.4, 69.8, 77.9, 124.4, 127.6–128.5 (m), 137.5, 137.6, 138.4, 158.7. Anal. Calcd for $C_{33}H_{47}NO_3$: C, 78.37; H, 9.37; N, 2.77. Found: C, 78.01; H, 9.66; N, 2.79.

(2′*E*,4*S*,1′*R*)-4-(1-Hydroxyhexadec-2-enyl)-1,3-oxazolidin-2-one (17). Dibenzyl olefin **16** (233 mg, 0.46 mmol) was dissolved in ether (5 mL) and added to a solution of sodium metal (25 mmol) in ammonia (10 mL) at -70 °C. The reaction mixture was stirred at -70 °C for 2.5 h. The reaction was quenched by the addition of solid NH_4Cl, and the ammonia was allowed to evaporate at room temperature. The ethereal solution was filtered and evaporated to a white solid, which was chromatographed on silica gel [ethyl acetate/hexane (2:1)] to yield **17**: 100 mg (67%); TLC [ethyl acetate/hexane (1:2)] R_f 0.07; mp 63–64 °C (lit.[32] mp 73–74 °C); ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.6 Hz, 3 H), 1.26 (m, 22 H), 2.07 (q, J = 7 Hz, 2 H), 3.83 (dtd, J = 8.9, 5.0, 0.9 Hz, 1 H), 4.05 (t, J = 6 Hz, 1 H), 4.32 (dd, J = 8.9, 5.0 Hz, 1 H), 4.46 (t, J = 8.8 Hz, 1 H), 5.07 (br s, 1 H), 5.41 (dd, J = 15.4, 7.6 Hz, 1 H), 5.86 (dt, J = 15.4, 6.8 Hz, 1 H); ^{13}C NMR δ 14.1, 22.7, 29.1, 29.3, 29.7, 31.9, 32.4, 56.5, 65.9, 72.2, 126.2, 135.4, 160.7 (lit.[30] δ 14.1, 22.6, 28.9–29.6, 31.9, 32.4, 56.4, 66.1, 72.7, 126.4, 136.0, 160.5).

D-*erythro*-Sphingosine (18). Hydroxy carbamate **17** (100 mg, 0.31 mmol) was hydrolyzed as described[32] in 1 N NaOH/ethanol (1:1) at 80 °C for 2.5 h. The hydrolysis mixture was diluted with ether (25 mL) and extracted with 1 N NaOH (2 × 5 mL). The combined ethereal solutions were dried over $MgSO_4$ and concentrated to crude **18**: 92 mg (100%); ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 7 Hz, 3 H), 1.15–1.4 (m, 22 H), 2.06 (q, J = 7 Hz, 2 H), 2.89 (m, 1 H), 3.67 (m, 2 H), 4.04 (t, J = 6.3 Hz, 1 H), 5.48 (dd, J = 15.3, 7.1 Hz, 1 H), 5.76 (dt, J = 15.3, 6.7 Hz, 1 H); ^{13}C NMR δ 14.1, 22.7, 29.4, 29.7, 32.0, 32.4, 56.2, 63.5, 74.9, 129.2, 134.3. Authentic **18**: ^1H NMR ($CDCl_3$) δ 0.87 (t, J = 7 Hz, 3 H), 1.15–1.4 (m, 22 H), 2.05 (q, J = 6.8 Hz, 2 H), 2.33 (br s, 3 H), 2.84 (t, J = 5.2 Hz, 1 H), 3.65 (m, 2 H), 4.04 (t, J = 6.3 Hz, 1 H), 5.46 (dd, J = 15.4, 7.1 Hz, 1 H), 5.75 (dt, J = 15.4, 7 Hz, 1 H); ^{13}C NMR δ 14.1, 22.7, 29.3, 29.3, 29.5, 29.7, 32.0, 32.4, 56.3, 64.2, 75.5, 129.3, 134.6.

***N*,*O*,*O*-Triacetyl-D-*erythro*-sphingosine (19).** Crude **18** (92 mg, 0.30 mmol) was acetylated as described[32] in methylene chloride (4 mL) with acetic anhydride (0.18 mL, 1.95 mmol), triethylamine (0.75 mL, 5.4 mmol), and catalytic (dimethylamino)pyridine (1.5 mg) for 1.5 h. Methanol (1 mL) was added and the mixture stirred for 10 min; the mixture was concentrated at reduced pressure and diluted with ether (25 mL). The ethereal solution was washed with saturated NaCl (3 × 3 mL), dried over $MgSO_4$, and concentrated to afford crude **19** (123 mg, 96%), which was chromatographed on silica gel [ethyl acetate/hexane (1:1)] and recrystallized from hexane: yield, 50 mg (39%); mp 101–102 °C (lit.[32] for synthetic **19** mp 103.5–104 °C; for natural **19** mp 101–102 °C); $[\alpha]^{25}{}_D$ -13.3° (c 1.4, $CHCl_3$) [lit.[32] $[\alpha]^{25}{}_D$ -12.8° (c 1, $CHCl_3$)]; ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 7 Hz, 3 H), 1.15–1.4 (m, 22 H), 1.95–2.1 (m, 11 H; Me peaks at δ 1.98, 2.06, 2.07), 4.04 (dd, J = 11.5, 3.8 Hz, 1 H), 4.30 (dd, J = 11.5, 6.0 Hz, 1 H), 4.38–4.48 (m, 1 H), 5.28 (t, J = 7 Hz, 1 H), 5.38 (dd, J = 15.0, 7.4 Hz, 1 H), 5.68 (d, J = 9 Hz, 1 H, NH), 5.79 (dt, J = 15.0, 7 Hz, 1 H); ^{13}C NMR δ 14.1, 20.8, 21.1, 22.7, 23.3, 28.9, 29.1, 29.3, 29.4, 29.6, 31.9, 32.3, 50.6, 62.5, 73.7, 124.0, 137.2, 169.6, 169.8, 170.7. This product was indistinguishable from authentic triacetyl **19** prepared as above from authentic **18**.

(2*S*,4*R*,5*R*)-2-4-*O*-Benzylidene-3-chloro-1,2,4-butanetriol (20). Triol **2** (440 mg, 3.1 mmol), fused zinc chloride (947 mg, 9.4 mmol), and freshly distilled benzaldehyde (1.48 mL, 14.6 mmol) were stirred in benzene (5 mL) at 25 °C for 48 h. Ethyl acetate (10 mL) was added to dissolve the resulting slurry, and the solution was extracted with water (10 mL, 2 × 2 mL) and dried over $MgSO_4$. Solvent was evaporated and **20** was isolated by chromatography on silica gel [ethyl acetate/hexane (1:1)], R_f 0.28. Crystalline **20**: yield, 310 mg (41%); mp 113–114 °C; $[\alpha]^{25}{}_D$ -16.7°

(c 1.0, $CHCl_3$); IR 3300, 2940, 2870, 1450, 1390, 1365, 1140, 1080, 1015, 745 cm^{-1}; ^1H NMR ($CDCl_3$) 1.96 (br s, 1 H), 3.73 (dd, J = 11.6, 5.2 Hz, 1 H), 3.92 (dd, J = 11.6, 7.2 Hz, 1 H), 4.03 (ddd, J = 1.7, 1.6, 1.4, 1 H), 4.22 (ddd, J = 7.0, 5.2, 1.6 Hz, 1 H), 4.31 (dd, J = 12.7, 1.7 Hz, 1 H), 4.37 (dd, J = 12.7, 1.4 Hz, 1 H), 5.57 (s, 1 H), 7.3–7.6 (m, 5 H); ^{13}C NMR δ 53.7, 63.4, 72.3, 78.7, 101.8, 126.2, 128.3, 129.3, 137.3. Anal. Calcd for $C_{11}H_{13}ClO_3$: C, 57.78; H, 5.73. Found: C, 57.74; H, 5.63.

(2*S*,4*R*,5*R*)-5-Chloro-4-[(*N*-benzylcarbamyl)hydroxymethyl]-2-phenyl-1,3-dioxane (21). Benzylidene **20** (229 mg, 1.0 mmol), benzyl isocyanate (148 μL, 1.2 mmol), and diisopropylethylamine (260 μL, 1.5 mmol) were stirred in benzene (5 mL) at 55 °C for 5 h. The solution was concentrated and chromatographed on silica gel [ethyl acetate/hexane (1:1.75)]: R_f 0.35; mp 171–172 °C; $[\alpha]^{25}_D$ +23.1° (c 0.6, $CHCl_3$); IR 3340, 2980, 2870, 1700, 1540, 1270, 1140, 1080, 755, 740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.01 (s, 1 H), 4.32–4.43 (m, 7 H), 5.56 (s, 1 H), 7.2–7.4 (m, 8 H), 7.55–7.7 (m, 2 H); ^{13}C NMR δ 45.2, 53.7, 65.2, 72.2, 76.5, 101.8, 126.3, 127.6, 128.3, 128.7, 129.2, 137.5, 139.0, 156.1. Anal. Calcd for $C_{19}H_{20}ClNO_4$: C, 63.07; H, 5.57; N, 3.87. Found: C, 63.30; H, 5.61; N, 4.06.

L-*threo*-Fluoromalic Acid. Difluorofumaric acid (1.22 g, 8 mmol) was dissolved in water (50 mL), and NaOH (0.64 g, 16 mmol) was added to neutralize the solution. Sodium formate (0.58 g, 8.5 mmol) was added, and the solution was deoxygenated. The pH was adjusted to 7.2, and NADH (125 mg, 0.16 mmol) was added, followed by formate dehydrogenase (375 mg, 250 U), malate dehydrogenase (43 μL, 500 U), and fumarase (0.8 mL, 1250 U, fumarate as substrate). The solution was stirred under nitrogen with the pH maintained at 7.1–7.2 by using a pH controller operating a peristaltic pump adding 1 N HCl or 1 N NaOH to the reaction mixture. Progress of the reaction was monitored at 230 nm. After 24 h the mixture was acidified to pH 1 with concentrated HCl and continuously extracted with ether. The ether extracts were evaporated and dried in vacuo to a white solid; 1.35 g; ^1H NMR (D_2O) δ 4.85 (dd, J = 33.0, 1.7 Hz, 1 H), 5.56 (dd, J = 46.4, 1.7 Hz, 1 H) [lit.[61] δ 4.88 (dd, J = 33, 2 Hz, 1 H), 5.55 (dd, J = 45, 2 Hz, 1 H)]; ^{13}C NMR (D_2O; reference, MeOH peak at δ 49.0) δ 70.6 (d, J = 20.2 Hz), 89.6 (d, J = 186.8 Hz), 170.3 (d, J = 24.7 Hz), 172.8.

Dimethyl L-*threo*-Fluoromalate. The crude product from the above reaction was dissolved in ether, filtered, and treated with excess diazomethane at 0 °C. The solution was evaporated to a clear oil, which was chromatographed on silica gel [ethyl acetate/hexane (2:3)] to yield crude dimethyl L-*threo*-fluoromalate as a clear colorless oil (1.12 g, 78% from difluorofumarate): TLC [ethyl acetate/hexane (2:3)] R_f 0.23. This material was rechromatographed on silica gel [ether/hexane (1.5:1)] with partial separation of the main product from a less polar minor product, yielding crystalline dimethyl L-*threo*-fluoromalate: 703 mg (49%); mp 53.5–54 °C; IR 3480, 2960, 1745, 1435, 1270, 1215, 1130, 1075, 970, 595 cm^{-1}; $[\alpha]^{22}_D$ −14.1° (c 2.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.25 (d, J = 7.5 Hz, 1 H, OH), 3.84 (s, 3 H), 3.86 (s, 3 H), 4.66 (ddd, J = 30.5, 7.4, 1.7 Hz, 1 H), 5.23 (dd, J = 47.0, 1.7 Hz, 1 H); ^{13}C NMR δ 52.7, 53.2, 71.1 (d, J = 20.8 Hz), 88.7 (d, J = 193.0 Hz), 166.7 (d, J = 24.4 Hz), 170.2. Anal. Calcd for $C_6H_9FO_5$: C, 40.11; H, 5.04. Found: C, 39.87; H, 5.02. A mixed fraction of dimethyl fluoromalates was also obtained, 169 mg (12%).

(+)-MTPA ester of dimethyl L-*threo*-fluoromalate was prepared in the same manner as for chloromalic acids above: ^1H NMR ($CDCl_3$) δ 3.51 (d, J = 0.9 Hz, 3 H), 3.80 (s, 3 H), 3.83 (s, 3 H), 5.45 (dd, J = 46.2, 2.3 Hz, 0.05 H), 5.52 (dd, J = 45.9, 2.3 Hz, 1 H), 5.62 (dd, J = 30.5, 2.3 Hz, 0.05 H), 5.83 (dd, J = 29.7, 2.3 Hz, 1 H), 7.26–7.59 (m, 5 H).

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Isolation and Structure of Bryostatins 10 and 11[1]

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The first two members of a new class of bryostatins designated 10 and 11 have been isolated from Gulf of California and Gulf of Mexico specimens of the marine animal *Bugula neritina* (Bryozoa phylum). Both bryostatins were found to closely resemble bryostatin 4 (**1b**) during isolation (~10^{-7}% yields), and that relationship proved useful in initial structure studies. Definitive structural assignments for bryostatins 10 (**2a**) and 11 (**2d**) were based on analysis of ^{13}C NMR and 400-MHz ^1H NMR spectral data combined with results of selective acetylation (**2a** → **2b**), dehydration, and oxidation (**2a** → **4b** and **2a** → **4d**) experiments. The two new 20-desoxybryostatins 10 (**2a**) and 11 (**2d**) displayed substantial cell growth inhibitory (**2a**, PS ED_{50} 7.6 × 10^{-4} μg/mL, and **2d**, 1.8 × 10^{-5} μg/mL) and antineoplastic activity against the P388 lymphocytic leukemia (PS) with bryostatin 11 leading to a 64% life extension at 92.5 μg/kg. The antineoplastic properties of bryostatins 10 and 11 eliminate an oxygen substituent at C-20 as a prerequisite for such important biological properties.

Fossil records suggest many cataclysmic events in evolution of the phylum Bryozoa and a great number of ancient members have become extinct. The 4000 plus species that presently exist represent a very competitive group of animals with highly developed survival mechanisms.[2] Perhaps due to their generally pedestrian appearance and likelihood of being mistaken for seaweeds, hydroids, or corals, these otherwise fascinating "moss animals" have

(1) Antineoplastic Agents. 119. For the preceding contribution in this series, see: ref 6f.

(2) For example, refer to: Harvell, C. D. *Science* (Washington D.C.) **1984**, *224*, 1357–1359.

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