× 0.5 mm × 0.9 mm was mounted along the elongated *b* axis in a thin-walled glass capillary tube with a small amount of mother liquor.

Diffraction data to 1.78 Å resolution were collected at 19 °C on a modified Nonius precession camera using the rotation mode, flat-film cassette, with a crystal-to-film distance of 48.4 mm. A Rigaku Denki rotating-anode generator operating at 3.6 kW and a monochromatized (pyrolytic graphite) beam were used with a 0.5-mm collimator. By use of Kodak DEF-2 film, data were collected over 3° rotation intervals, the usual rate being 3°/3 h.

The intensity of the reflections was measured by means of an Optronics rotating-drum densitometer and evaluated by program FILME.[47] After completion of the 90° scan about the *b* axis, the crystal was mounted about the *c* axis and an additional 21° was scanned.

Data processing was performed by the PROTEIN program system of Steigemann.[17] Separate films were scaled and multiple measurements of the same reflection including those related by symmetry were merged. The internal consistency checking feature of the PROTEIN program was used to delete reflections exhibiting gross discrepancies. This method corrects for slight crystal decay. Based upon similar studies,[14] radiation damage is typically less than 5% over 7 days. Here, 5 days was required for complete data collection; no correction for crystal decay nor absorption was made. R_{merge} measures the agreement of intensity measurements from each source with mean values obtained from several sources and is defined as $\sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the intensity value of individual measurements and $\langle I\rangle$ the corresponding mean values, the summation being over all measurements common to two or more films. R_{merge} values ranged from 0.068 to 0.123 with a mean R_{merge} value of 0.087. R_{symm} (defined as $\sum|I_i - \langle I\rangle|/\sum I_i$, where $\langle I\rangle$ is the average intensity and I_i the intensity of individual measurements with symmetrical correspondence) was in the range of 0.043–0.078. Of 36 367 reflections above FILME's 1σ significance level, 16 151 unique reflections, comprising 71% of the possible reflections to 1.78 Å resolution, were obtained. As a separate evaluation of the effective resolution of the data, Sparrow's resolution criterion was employed,[23] giving 1.98 Å for this data set. Both reflection data and coordinates are deposited with Protein Data Bank.[48,49]

Acknowledgment. We wish to acknowledge the assistance of Anne Strimpler with biochemical measurements. Diffraction facilities were provided, thanks to the hospitality of Prof. Robert Huber. Computational facilities were provided by Dr. John Dinkel, Provost for Computing, Teaxs A&M University.

Registry No. 1a, 95924-70-2; **1b**, 109522-66-9; **1c**, 109522-75-0; **1d**, 109522-76-1; **1f**, 119720-82-0; **1g**, 119720-81-9; PPE, 9004-06-2; $BrCF_2COOEt$, 667-27-6; $PhCH_2CH_2NH_2$, 64-04-0; Ac-L-Ala, 97-69-8; Cbz-proline, 1148-11-4; L-valinol, 2026-48-4.

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(49) Diffraction data and coordinates have been deposited in the Protein Data Bank[48] and have been assigned the access codes R4ESTSF and 4EST.

Structural Requirements for Catalysis by Chorismate Mutase

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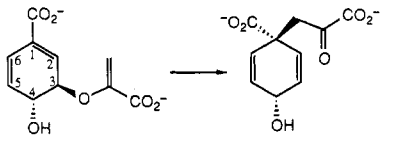
Abstract: The structural requirements for mutase-catalyzed Claisen rearrangement by chorismate mutase–prephenate dehydrogenase from *Escherichia coli* have been established. The chorismate analogue lacking the carboxyl group at C_1 (**5**) was not a substrate for chorismate mutase. The methyl ether of chorismate [(±)-**6**] was a good substrate for chorismate mutase ($k_{cat}/k_{uncat} = 2.0 \times 10^4$). The half-lives for Claisen rearrangement and aromatization of 4-deshydroxychorismate (**19**) in D_2O at 30 °C, pD 7.2, were 3.5 and 8 min, respectively. In the presence of large amounts of enzyme, it was demonstrated that the Claisen rearrangement of enantiomerically pure **19** was accelerated at least 100-fold by chorismate mutase. Data available from other studies have demonstrated that ester **3** is not a substrate for chorismate mutase, and the k_{cat}/k_{uncat} for dihydrochorismate analogue **4** is similar to that for chorismate. These results establish that the only functional groups required on the allyl vinyl ether moiety of chorismate for mutase-catalyzed rearrangement are the two carboxylate groups.

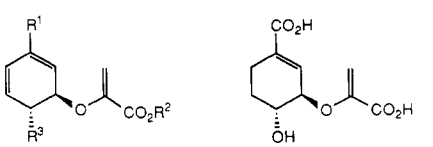
Chorismate (**1**) is the branch-point intermediate in the biosynthesis of aromatic amino acids and growth factors in bacteria, fungi, and higher plants.[1] The first step in the biosynthesis of phenylalanine and tyrosine from **1**, the intramolecular rearrangement to prephenate (**2**), is catalyzed by the enzyme chorismate mutase (Scheme I). The uncatalyzed rearrangement of **1** to **2** occurs readily in aqueous solution with a half-life of 15.7 h at 30 °C, pH 7.5.[2] Chorismate mutase accelerates the rearrangement by a factor of 2×10^6 at 37 °C, pH 7.5.[2] Both the uncatalyzed[3] and the enzyme-catalyzed[4,5] reactions proceed through a chairlike transition state.

Scheme I



1 2

Chart I



3, R^1=CO_2H, R^2=Me, R^3=OH
5, R^1= R^2=H, R^3=OH
6, R^1=CO_2H, R^2=H, R^3=OMe
7, R^1=CO_2H, R^2=R^3=H

4

The chorismate mutase catalyzed Claisen rearrangement of **1** to **2** appears to be the only established example of an enzyme-catalyzed pericyclic reaction in primary metabolism.[6] This unique

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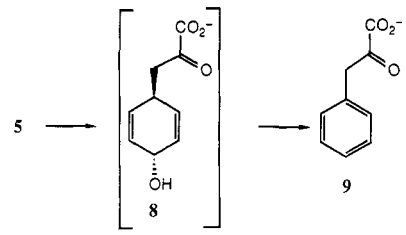
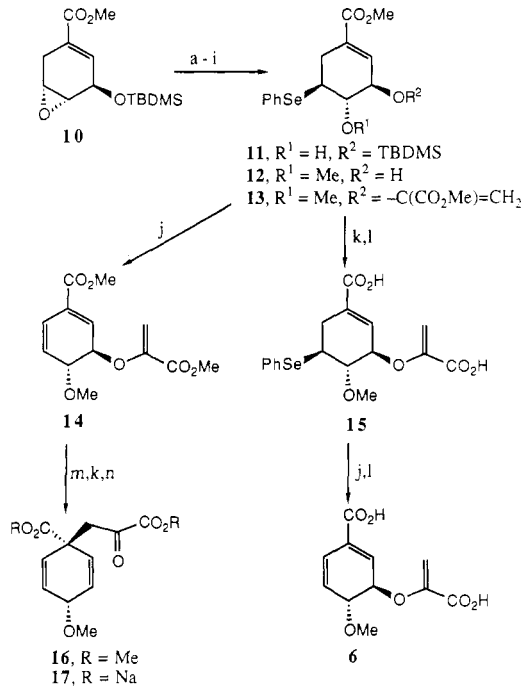
0002-7863/89/1511-3374$01.50/0

Scheme II



Scheme III[a]



[a] Key: (a) $(PhSe)_2$, $NaBH_4$, MeOH; (b) *n*-BuLi, $(MeO)_2SO_2$; (c) 37% aqueous HCl, MeOH; (d) MeO_2CCOCO_2Me; (e) $SOCl_2$, pyridine; (f) Zn, HOAc; (g) $CH_2{=}N^+Me_2\ I^-$, Et_3N, CH_2Cl_2; (h) $(MeO)_2SO_2$, MeOH; (i) Na_2CO_3, H_2O; (j) H_2O_2, acetone; (k) NaOH, THF, H_2O; (l) 1 M HCl; (m) benzene (reflux); (n) Amberlite IR-120.

situation has stimulated considerable effort to understand the details of the enzyme-catalyzed process, and a variety of enzyme-catalyzed mechanisms have been considered.[7,8] Of central importance is establishment of which structural features of **1** are essential for enzyme catalysis. Haslam and co-workers have reported investigations with **3** and **4** (Chart I).[9] Ester **3** was not a substrate for chorismate mutase, and it did not inhibit enzyme processing of **1**. These observations establish that the side-chain carboxylate group is essential for enzymatic activity. These workers reported also that **4** did not display any tendency to rearrange with chorismate mutase, but it was a modest inhibitor. Dihydro analogue **4** is, in fact, an excellent substrate for chorismate mutase, but observation of enzymatic catalysis requires special experimental conditions since the uncatalyzed reaction is so slow.[10] Therefore, the C_5–C_6 olefinic group is not important for mutase catalysis. Unexplained thus far is the importance of the C_1 carboxylate group and the C_4 hydroxyl group. To answer these questions, we have investigated analogues **5**–**7** (Chart I).

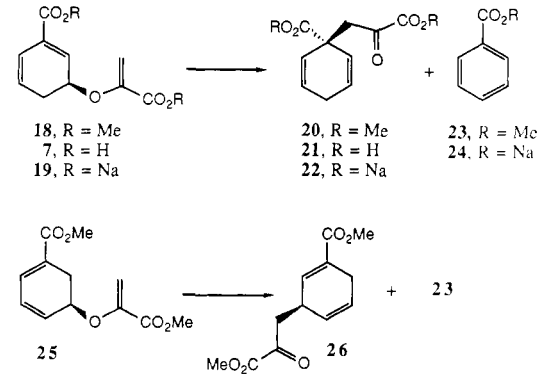
The methyl ester of **5** was prepared as described previously.[8] Base-catalyzed hydrolysis and acidification provided **5** (hygroscopic solid). Acid **5** underwent facile Claisen rearrangement to **8** with subsequent dehydration to phenylpyruvate (**9**) (Scheme II). When the reaction was monitored by ^1H NMR spectroscopy, prephenate analogue **8** was not detected. The half-life for rearrangement of **5** in D_2O at 30 °C, pD 7.4, was 1.7 h. The rearrangement of **5** was not catalyzed by the mutase activity of chorismate mutase–prephenate dehydrogenase, and **5** was a modest competitive inhibitor of mutase activity for the processing of chorismate (K_i = 0.4 mM with K_m = 0.16 mM for **1**). The C_1 carboxyl group is essential for mutase activity.

Table I. Data for the Uncatalyzed and Enzyme-Catalyzed Rearrangement of (−)-**1** and (±)-**6** at 30 °C, pH 7.5[a]

	(−)-**1**	(±)-**6**
k_{uncat} (s^{-1})	1.24×10^{-5}	2.8×10^{-5}
K_m (mM)	0.14	1.9
k_{cat} (s^{-1})	29	0.56
enzymic rate acceleration (k_{cat}/k_{uncat})	2.3×10^6	2.0×10^4

[a] Error limits: enzyme-catalyzed reactions, ±10%; uncatalyzed reactions, ±5%.

Scheme IV



The methyl ether of chorismic acid (**6**) and the corresponding dimethyl ester (**14**) were prepared as outlined in Scheme III. Reaction of oxirane **10**[11] with $PhSe^-$ gave **11** (78%). Alkylation of the alkoxide salt of **11** with dimethyl sulfate followed by cleavage of the *tert*-butyldimethylsilyl (TBDMS) ether group provided alcohol **12** (60%). Conversion of **12** to the malonate derivative (54%, steps d–f) and subsequent transformation to enolpyruvate ester **13** (steps g–i, 62%) followed procedures developed earlier for the synthesis of **1**.[12] Elimination of the selenoxide derivative of **13** gave ester **14** (73%), and hydrolysis of **13** followed by selenoxide elimination gave racemic **6** (20%). Thermolysis of **14** in benzene effected Claisen rearrangement to **16**, and hydrolysis of **16** provided **17**.

In $CDCl_3$ at 30 °C **14** underwent Claisen rearrangement to **16** (77%) and aromatization to methyl *p*-methoxybenzoate (13%). The half-life for the formation of **16** under these conditions was 185 h. In D_2O at 30 °C, pD 7.4, the dianion of **6** formed *p*-methoxybenzoate (4%) and a mixture (96%) of **17** and **9** from aromatization of **17**. The half-life for the Claisen rearrangement was 6.8 h. Analogue **6** was a reasonable substrate for the mutase activity of chorismate mutase–prephenate dehydrogenase. A comparison of the uncatalyzed rate of Claisen rearrangement and the enzyme-catalyzed data for (−)-**1** and (±)-**6** is provided in Table I. Clearly the enzyme does not require the free hydroxyl group at C_4 for catalysis. It is reasonable to assume that the less effective binding and turnover of **6** compared to **1** are due to the steric requirement of the 4-*O*-methyl group.

To answer the question whether any oxygen function was needed at C_4 for catalysis by chorismate mutase required the preparation of 4-deshydroxychorismate (**7**, Scheme IV). Investigations by other workers showed that at 75 °C (2:1 methanol/water) ester **18** underwent Claisen rearrangement to **20** at a rate 97 times faster

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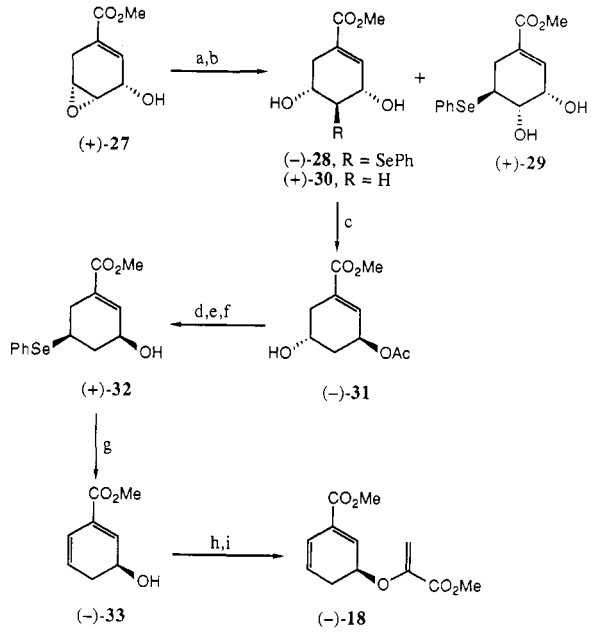
Scheme V[a]

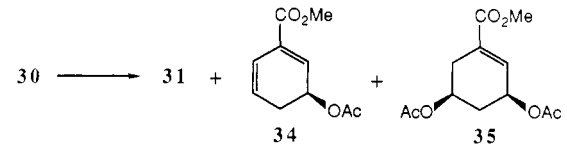


[a] Key: (a) PhSeLi, THF, −20 °C; (b) n-Bu_3SnH, benzene (reflux); (c) DIAD, Ph_3P, HOAc, THF; (d) MsCl, DMAP, CH_2Cl_2; (e) NaOMe, MeOH, 0–5 °C; (f) PhSeLi, THF; (g) n-Bu_4NIO_4, Na_2HPO_4, $CDCl_3$; (h) $MeO_2CC(N_2)PO(OMe)_2$, $Rh_2(OAc)_4$, benzene, 75 °C; (i) $LiN(SiMe_3)_2$, H_2CO, THF, −78 °C.

than Claisen rearrangement of dimethyl chorismate; in addition, substantial aromatization to **23** was observed.[8] Ester **18** was prepared by the literature procedure,[8] and for comparison, dimethyl 6-deshydroxyisochorismate (**25**) was prepared in similar fashion from the corresponding cyclohexadienol. In $CDCl_3$ at 30 °C, the half-life for rearrangement of **18** to **20** was 7.3 h, and the half-life for aromatization to **23** was 7.3 h. Under the same conditions, **25** rearranged to **26** with a half-life of 2.3 h, and the half-life for aromatization to **23** was 7.4 h. (For comparison, dimethyl isochorismate undergoes Claisen rearrangement with a half-life of 40 h.[13]) These results are consistent with solvent effects and the effects of conformational equilibria on the rate of Claisen rearrangement of chorismate-type structures and other related systems that have been described in detail elsewhere,[7,8,14,15] and from the data it was clear that the Claisen rearrangement of **7** would be a very fast reaction. Initial experiments of the low-temperature, base-catalyzed hydrolysis of (±)-**18** gave mixtures of **19**, **22**, and **24**; and at 30 °C in D_2O (pD 7.2), the half-lives for Claisen rearrangement and aromatization of **19** were approximately 3 and 8 min, respectively. These observations made it clear that meaningful results with chorismate mutase would require enantiomerically pure material.

Epoxy alcohol (+)-**27** (Scheme V) was selected as the starting material for synthesis of (−)-**18**, since it was available in >98% ee from enzyme-catalyzed kinetic resolution of the butyrate ester.[11] The regioselectivity of oxirane ring opening of **27** by $PhSe^-$ was quite sensitive to the choice of experimental conditions. After a survey of various conditions (variation of temperature and concentration and the presence or absence of mild Lewis acids with (±)-**27**,[16]) the optimum conditions found (−20 °C and no catalyst) gave desired regioisomer (−)-**28** (77%) which could be separated from regioisomer (+)-**29** (9%) by fractional recrystallization followed by flash chromatography. Optimum conditions for the preparation of (±)-**29** from (±)-**27** are provided under Experimental Section. Reductive removal of the phenylselenide group was accomplished with n-Bu_3SnH in refluxing benzene to provide (+)-**30**. Diol (+)-**30** was subjected to Mitsunobu conditions[17] with diisopropyl azodicarboxylate (DIAD), Ph_3P, and HOAc to provide (−)-**31** (70%).

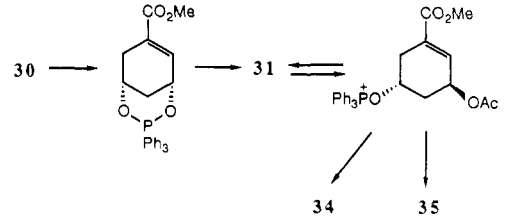
Table II. Mitsunobu Reaction of **31** under Various Conditions



entry	DIAD (equiv)	Ph_3P (equiv)	HOAc (equiv)	% **31**	% **34**	% **35**
1	1.0	1.4	2.8	100	nd[a]	nd
2	2.5	2.5	4	nd	20	80
3	2.6	2.6	21	nd	12	88
4	2.6	2.6	100	100	nd	nd

[a] Not detected by ^1H NMR.

Scheme VI



Product distribution in the Mitsunobu reaction was dependent on the ratio of reagents used. A survey of the conditions investigated is presented in Table II. The reagent ratio indicated by entry 1 gave exclusively **31**. When 2.5 equiv of DIAD and Ph_3P and 4.0 equiv of HOAc were used (entry 2), a 1:4 mixture of diene **34**[18] and diacetate **35** resulted; and the ratio increased to 1:7 with 21 equiv of HOAc (entry 3). Surprisingly, when 100 equiv of HOAc was used, the only product observed was **31**.

A rationale for these transformations is depicted in Scheme VI. In the presence of excess Ph_3P and HOAc, diol **30** would react with the first equivalent of DIAD to give a triphenylphosphorane intermediate.[19] Displacement by acetic acid could be expected to occur exclusively at the allylic position to give **31**. Compound **31** would react with a second equivalent of DIAD to form an alkoxyphosphonium salt. Elimination of triphenylphosphine oxide would give diene **34**, while displacement with HOAc would lead to **35**. Excess acetic acid would tend to suppress the elimination pathway; however, in the presence of a 100 equiv of HOAc, solvolysis to give back **31** would predominate.

Either enantiomer of epoxy alcohol **27** was therefore suitable for the synthesis. Diol (−)-**30**, obtained from (−)-**27** as described for the conversion of (+)-**27** to (+)-**30**, was converted to **35** by double Mitsunobu reaction in 58% isolated yield (conditions of entry 3, Table II). Diacetate **35** was subjected to methoxide-catalyzed ester interchange in MeOH to give (+)-**30** in 78% yield. Thus, the yield from (−)-**30** to (+)-**30** was 45%.

Mesylation of (−)-**31** (Scheme V), followed by methoxide-catalyzed ester interchange to convert the C_3 acetoxy group to a hydroxyl group, and subsequent displacement of the mesylate group with PhSeLi in THF gave (+)-**32** in 51% yield from (−)-**31**.

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Table III. Data for the Enzyme-Catalyzed Rearrangement of Enantiomerically Pure **19**[a]

conditions	expt 1 fraction	expt 1 % **19**	expt 1 % **22**	expt 1 % **24**	expt 1 [S]/[E][b]	expt 2 fraction	expt 2 % **19**	expt 2 % **22**	expt 2 % **24**	expt 2 [S]/[E][b]
initial	1	67	22	11		1	51	23	26	
buffer only						2	0	64	36	
inactivated enzyme	2	0	70	30	4700	3	0	61	39	2600
active enzyme	3	0	79	21	90	4	0	76	24	30
	4	0	82	18	45					
theory[c]		0	89	11			0	74	26	

[a] Initial product distributions were determined by ^{1}H NMR in CD_3OD; all other determinations were by ^{1}H NMR in D_2O. [b] The molar ratio of substrate to enzyme was calculated by assuming an enzyme molecular weight of 88 000 for dimeric chorismate mutase–prephenate dehydrogenase from *E. coli*. [c] Theory refers to the expected product distribution if all **19** present is rearranged to **22**.

The optical purity of (+)-**32** was checked by esterification with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(+)-MTPA-Cl].[20] The ^{1}H NMR spectrum revealed the presence of only one diastereomer. Oxidation of (+)-**32** to the selenoxide with n-Bu_4NIO_4 followed by elimination gave very sensitive dienol (−)-**33** in 75% yield after flash chromatography. Coupling of (−)-**33** and trimethyl diazophosphonoacetate with rhodium acetate catalysis and quenching the monoanion of the phosphonate with gaseous formaldehyde[11,21] at −78 °C gave (−)-**18** in ∼34% yield from (−)-**33**. The overall yield from (+)-**27** to (−)-**18** was 7%.

Saponification of (−)-**18** with 1.0 M NaOH in THF/H_2O at 0 °C gave a mixture of **19**, **22**, and **24** (Scheme IV). Disodium 4-deshydroxychorismate (**19**) proved to be extremely labile, and it could not be isolated in pure form. Usually, a ∼2:1:1 mixture of **19**/**22**/**24** was obtained after saponification, adjustment of the pH to 6.6 with acidic resin, and concentration at 0 °C under high vacuum. This material was used in all subsequent thermal and enzymatic studies without any additional purification.

In D_2O (phosphate buffer, pD 7.2) at 30 °C the half-lives for Claisen rearrangement and aromatization of **19** to **22** and **24** were 3.5 and 8 min, respectively; and in the same solvent (Tris-DCl buffer, pD 7.5) the values were 3.3 and 9 min (product ratio: 73% **22**; 27% **24**). Thus, the half-life for disappearance of **19** under these conditions is 2.4 min. Under similar conditions the half-lives for Claisen rearrangement and aromatization of chorismate (**1**) are 935 and 8400 min, respectively.[2] In CD_3OD **19** was substantially more stable; the Claisen rearrangement and aromatization half-lives were 220 and 70 min, respectively (product ratio: 23% **22**; 77% **24**).

Dimethyl 4-deshydroxyprephenate (**20**) was prepared by heating (±)-**18** at 60 °C overnight in $CDCl_3$ to effect complete reaction. This gave a ∼1:1 mixture of **20**/**23**, which was purified by flash chromatography to give **20** (23%). Saponification of **20** with NaOH in THF/H_2O, followed by adjustment of the pH to 6.5 with acidic resin, gave **22** (91%) as an off-white solid. Attempts to prepare pure 4-deshydroxyprephenic acid (**21**) failed due to product instability. Salt **22** was tested as an inhibitor of the dehydrogenase activity of chorismate mutase–prephenate dehydrogenase from *E. coli*.[22] With a modification of the procedure of Heyde and Morrison,[23] **22** was found to be a weak competitive inhibitor with K_i = 0.82 mM ([NAD] = 0.1 mM, [**22**] = 1.0 mM). Prephenate was found to have a V_{max} = 3.8 μmol min^{-1} mg^{-1} and a K_m = 0.099 mM under the conditions of the assay.

A detailed kinetic investigation of the mutase-catalyzed rearrangement of enantiomerically pure **19** was not possible under normal conditions with catalytic quantities of enzyme due to the thermal instability of **19**. However, since 27% benzoate was formed in the thermal reaction (Tris buffer), it was possible to do experiments with excess enzyme to determine whether **19** was metabolized by chorismate mutase. The premise of the experiment was that if **19** were a viable substrate, then an increased amount of Claisen rearrangement product relative to aromatization product would be observed.

The results of two separate experiments with excess enzyme are listed in Table III. Diester (−)-**18** was saponified with NaOH as described earlier. After acidification, the filtrate was separated into four fractions of known volume, and each fraction was concentrated under high vacuum at 0 °C; the fractions were handled in as identical a manner as possible. Fractions 2–4 were stored briefly[24] at −55 °C while the ^{1}H NMR spectrum of fraction 1 was obtained. It was assumed that the other fractions had the same product ratios. For both experiments, incubation solutions were made up to 2.0-mL volume by combining appropriate amounts of Tris-HCl buffer with either inactivated chorismate mutase–prephenate dehydrogenase[25] or active chorismate mutase–prephenate dehydrogenase[26] (see Experimental Section). The appropriate incubation solution was then added to fraction 2, 3, or 4 at −55 °C, and the resulting mixtures were kept at 30 °C for 1 h. After removal of the enzyme, as needed, each fraction was concentrated. Some fractions were exchanged with D_2O to reduce interference caused by H_2O and glycerol. ^{1}H NMR spectra in D_2O were then obtained to determine the final product distribution for each fraction (Table III).

In both experiments, the ratio of **22**:**24** that was formed in the presence of inactivated enzyme was found to be ∼73:27, after correction for the amount of **22** and **24** present at the start of the reaction. A similar ratio was obtained with buffer alone. However, when active enzyme was used, an enhanced amount of Claisen rearrangement relative to aromatization was observed. Thus, when the substrate to enzyme ratio was ∼90, an enhancement of ∼47% over the thermal reaction was noted (experiment 1, fraction 3). When the ratio was ∼45, an enhancement of ∼63% was obtained (experiment 1, fraction 4). When the molar ratio was reduced further to ∼30, a ∼100% enhancement was observed (experiment 2, fraction 4). In other words, under these conditions **19** was completely metabolized by the enzyme.

From the data in Table III, it is possible to estimate the enzymic turnover number (k_{cat}) and therefore the enzymic rate acceleration. In experiment 1, the data from fractions 3 and 4 both give a k_{cat} = 0.35 s^{-1} (per dimer of enzyme). The enzyme was found to have a turnover number of ∼11 s^{-1} (per dimer of enzyme) with chorismate; therefore, chorismate mutase–prephenate dehydrogenase catalyzes the rearrangement of **19** at ∼3% of the rate of the natural substrate. Additionally, since the rate constant for the uncatalyzed rearrangement is 3.5 × 10^{-3} s^{-1}, the enzymic rate acceleration is ∼100-fold under the conditions of the assay. This is considered a minimum value since the high concentrations of enzyme used are not conditions where maximum velocity would be expected. In comparison, the rate of rearrangement of chorismate is accelerated by a factor of ∼2 × 10^6 by chorismate mutase–prephenate dehydrogenase at 37 °C.[2]

From these results we conclude that the C_4 hydroxyl group is neither essential for binding of the substrate to the active site nor needed for catalysis of the Claisen rearrangement. Any mecha-

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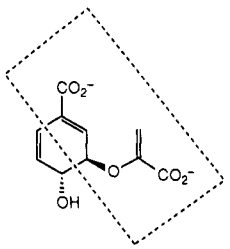
(21) Lesuisse, D.; Berchtold, G. A. *J. Org. Chem.* **1988**, *53*, 4992–4997.
(22) Chorismate mutase–prephenate dehydrogenase from *E. coli* JFM30 was obtained from Dr. J. F. Morrison: (a) SampathKumar, P.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *702*, 204–211. (b) Bhosale, S. B.; Rood, J. I.; Sneddon, M. K.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *717*, 6–11.
(23) Heyde, E.; Morrison, J. F. *Biochemistry* **1978**, *17*, 1573–1580.
(24) In general, the samples were used within 1 h.
(25) The enzyme (0.76 mg/mL)[22] was denatured by heating on a steam bath for 30 min.
(26) The enzyme had a mutase specific activity of 7.2 μmol min^{-1} mg^{-1} and a K_m = 0.22 mM. It was used as a 6.0 mg/mL solution.

Chart II



nism for the enzymic rearrangement of chorismate to prephenate which utilizes the hydroxyl moiety as a crucial element can therefore be eliminated. However, hydrogen-bond formation involving the C_4 hydroxyl group still could be important for enhanced stabilization of the transition state and to provide the rate acceleration observed with the natural substrate.

In summary, the structural requirements for catalysis of the Claisen rearrangement by chorismate mutase are indicated in the dotted box for the chorismate structure in Chart II. In addition to the allyl vinyl ether moiety, the enzyme requires only the two carboxylate groups for active site binding and catalysis. The C_4 hydroxy group, although not required, may increase catalytic efficiency. Whether a cyclic substrate is required for catalytic activity is yet to be tested. In light of the conformational restraints imposed by a cyclic structure, it is expected that noncyclic analogues of chorismate would be metabolized by chorismate mutase with only poor catalytic efficiency, if at all.

Experimental Section[27]

***trans*-1-[(1-Carboxyethenyl)oxy]-2-hydroxy-3,5-cyclohexadiene (5).** The dimethyl ester[8] of **5** (37 mg, 0.19 mmol) was dissolved in 1:1 H_2O/THF and cooled in an ice bath. Aqueous 1 N NaOH (190 μL) was added dropwise, and the solution was stirred for 0.5 h. After acidification to pH 4 with Amberlite IR-120 resin, the solution was filtered and concentrated under reduced pressure to give a quantitative yield (34 mg) of **5** as an oil. Trituration with pentane gave a low-melting, hygroscopic solid: IR (KBr) 3500, 1690, 1615 cm^{-1}; ^1H NMR (acetone-d_6) δ 6.02–5.88 (4 H, m), 5.40 (1 H, s), 4.82 (1 H, d, J = 10.7 Hz), 4.76 (1 H, s), 4.65 (1 H, d, J = 10.8 Hz); high-resolution mass spectrum, calcd for $C_9H_{10}O_4$ 182.0579, found 182.0579.

Methyl (3β,4α,5β)-3-[[(Dimethylethyl)dimethylsilyl]oxy]-4-hydroxy-5-(phenylseleno)-1-cyclohexene-1-carboxylate (11). To a suspension of diphenyl diselenide (4.12 g, 13.2 mmol) in dry MeOH (100 mL) at 10 °C under N_2, $NaBH_4$ was added in small portions until the yellow color had been discharged. Epoxide **10**[11] (5.00 g, 17.6 mmol) in MeOH (15 mL) was added, and the mixture was allowed to warm to room temperature. After 34 h, HOAc (10 mL) was added, and most of the solvent was removed under reduced pressure at 30 °C. The residue was cooled in an ice bath. Ether (150 mL) was added followed by the slow addition of 5% Na_2CO_3 (100 mL). After CO_2 evolution had ceased, the organic layer was separated, and the aqueous portion was extracted with additional ether (50 mL). The combined ether extracts were dried and concentrated. Flash chromatography on silica gel with ether/hexane (15:85) until the yellow-orange band of diphenyl diselenide had eluted and then with ether/hexane (1:3) gave **11** (R_f = 0.38, 6.09 g, 78%) as a pale yellow oil: IR (thin film) 3520, 1719 cm^{-1}; ^1H NMR δ 7.62 (2 H, m), 7.32 (3 H, m), 6.56 (1 H, m), 4.32 (1 H, m), 3.71 (3 H, s), 3.47 (1 H, m), 3.23 (1 H, sextet), 2.97 (1 H, br s), 2.88 (1 H, dd, J = 18 and 5 Hz), 2.41 (1 H, m), 0.92 (9 H, s), 0.16 (6 H, d).

Methyl (3β,4α,5β)-3-Hydroxy-4-methoxy-5-(phenylseleno)-1-cyclohexene-1-carboxylate (12). Selenide **11** (5.00 g, 11.3 mmol) was dissolved in THF (150 mL) under N_2 and was cooled to −75 °C with stirring. A solution of *n*-BuLi in hexane (4.8 mL of 2.35 M, 11.3 mmol) was added over a 10-min period. After 5 min, dimethyl sulfate (2.1 mL, 22 mmol) was added in one portion. The solution was kept at −75 °C for 20 min, warmed to room temperature over a 1-h period, and allowed to stir for 3 h. The mixture was concentrated under reduced pressure, and to the residue was added ether (100 mL), H_2O (25 mL), and NaCl (1.0 g). The organic extract was separated, and the aqueous phase was extracted with additional ether (50 mL). The combined extracts were dried and concentrated. The residue was flash chromatographed on silica gel with EtOAc/hexane (1:19) as the initial eluent. When the methoxy compound began to elute [R_f = 0.33 in EtOAc/hexane (1:9)], the solvent was changed to EtOAc/hexane (1:9) to give 3.68 g (71%) of the methoxy derivative as an oil: ^1H NMR δ 7.61 (2 H, m), 7.28 (3 H, m), 6.62 (1 H, m), 4.30 (1 H, m), 3.70 (3 H, s), 3.59 (3 H, s), 3.57–3.35 (2 H, m), 2.70 (1 H, dm), 2.54 (1 H, ddt), 0.94 (9 H, s), 0.16 (6 H, d).

To a solution of the methoxy derivative (1.55 g, 3.4 mmol) in MeOH (50 mL) at room temperature, 37% HCl (1 mL) was added with stirring. After 2 h solid $NaHCO_3$ (1.0 g) was added, and the mixture was concentrated without heating. Ether (40 mL) and H_2O (20 mL) were added, the organic phase was separated, and the aqueous portion was extracted with ether (40 mL). The combined organic extracts were dried, concentrated, and flash chromatographed on silica gel (1:1 EtOAc/hexane) to give 0.987 g (85%) of **12** (R_f = 0.35) as an oil which crystallized on standing. Recrystallization from EtOAc/hexane gave pure **12**: mp 106–107.5 °C; IR (KBr) 3505, 1706 cm^{-1}; ^1H NMR δ 7.62 (2 H, m), 7.29 (3 H, m), 6.81 (1 H, m), 4.32 (1 H, br s), 3.72 (3 H, s), 3.61–3.54 (4 H, m with s at 3.57), 3.47 (1 H, m), 2.83–2.54 (3 H, m).

Methyl (3β,4α,5β)-3-[[1-(Methoxycarbonyl)ethenyl]oxy]-4-methoxy-5-(phenylseleno)-1-cyclohexene-1-carboxylate (13). A solution of **12** (2.04 g, 5.98 mmol) and dimethyl oxomalonate[28] (2.12 g, 14.5 mmol) in benzene (4.0 mL) was heated under N_2 at 60 °C for 14 h. The solution was diluted with THF (25 mL) and cooled to −70 °C. Pyridine (0.630 mL, 7.8 mmol) was added followed by the dropwise addition of $SOCl_2$ (0.445 mL, 6.10 mmol). The mixture was slowly warmed to 0 °C over a period of 1.5 h and left overnight at 0 °C. The mixture was filtered, concentrated, and flash chromatographed on silica gel with initial elution with 3:7 EtOAc/hexane. Further elution with EtOAc/hexane (1:1) gave 3.17 g of chloromalonate derivative that was mixed with NaOAc (2 g), HOAc (4.5 mL), EtOAc (25 mL), and MeOH (100 mL). Zinc powder (1.65 g, 4 equiv) was added, and after 10 h the mixture was concentrated at 30 °C. The residue was mixed with ether (100 mL) and H_2O (50 mL). $NaHCO_3$ (3.0 g) was added in small portions with stirring. The organic layer was separated, and the aqueous phase was extracted with additional ether (100 mL). The combined organic extracts were dried and evaporated to a semisolid that was dissolved in a mixture of warm EtOAc (13 mL) and hexane (30 mL). The mixture was filtered, and the filtrate was cooled to −20 °C overnight. The precipitate was collected by filtration and air-dried to give 1.52 g (54%) of methyl (3β,4α,5β)-3-[bis(methoxycarbonyl)methoxy]-4-methoxy-5-(phenylseleno)-1-cyclohexene-1-carboxylate: mp 87–88 °C (after recrystallization from EtOAc/hexane).

A solution of the malonate (287 mg, 0.610 mmol), Eschenmoser's salt (150 mg, 0.81 mmol), and Et_3N (110 μL, 0.79 mmol) in CH_2Cl_2 (9 mL) was stirred under N_2 at 0 °C for 1 h, warmed to room temperature, and left overnight. The solution was washed with 5% $NaHCO_3$ (5 mL), the organic phase was separated, and the aqueous portion was extracted with ether (10 mL). The organic portions were concentrated, the residue was dissolved in MeOH (3 mL), and $NaHCO_3$ (40 mg) and dimethyl sulfate (75 μL, 0.79 mmol) were added. The mixture was stirred for 9 h, concentrated, and treated with ether (10 mL), H_2O (4 mL), and Na_2CO_3 (130 mg). After being stirred for 12 h, the organic phase was separated. The aqueous phase was extracted with ether (10 mL), and the combined organic extracts were dried and concentrated. Flash chromatography on silica gel (1:1 EtOAc/hexane) gave 160 mg (62%) of **13** (R_f = 0.45) as an oil which crystallized on standing at 0 °C: mp 64–65 °C (after recrystallization from EtOAc/hexane); IR (KBr) 1729, 1714, 1623 cm^{-1}; ^1H NMR δ 7.64 (2 H, m), 7.30 (3 H, m), 6.74 (1 H, m), 5.54 (1 H, d, J = 3 Hz), 4.81 (1 H, d, J = 3 Hz), 4.69 (1 H, m), 3.82 (3 H, s), 3.70 (3 H, s), 3.65–3.47 (5 H, m), 3.60 (3 H, s), 2.77 (1 H, dd, J = 18 and 6 Hz), 2.55 (1 H, ddt, J = 18, 10, and 3 Hz).

Methyl *trans*-3-[[1-(Methoxycarbonyl)ethenyl]oxy]-4-methoxy-1,5-cyclohexadiene-1-carboxylate (14). A solution of H_2O_2 (30%, 0.090 mL, 0.50 mmol) was added with stirring to a solution of **13** (107 mg, 0.252 mmol) in acetone (3.6 mL) at 0 °C. After 15 min, diisopropylamine (0.070 mL, 0.50 mmol) was added, and the solution was warmed to room temperature. After 1 h the solution was concentrated, and ether (5.0 mL) and a saturated solution of NaCl (1 mL) were added. After vigorous stirring, the organic layer was separated, and the aqueous phase was extracted with additional ether (5.0 mL). The combined ether extracts were dried and concentrated. Flash chromatography on silica gel (1:3 EtOAc/hexane) gave 49.3 mg (73%) of **14** as an oil which eventually solidified: mp 78–80 °C (after recrystallization from EtOAc/hexane); IR (KBr) 1735, 1719, 1627 cm^{-1}; ^1H NMR δ 6.87 (1 H,

(27) ^1H NMR spectra were obtained at 250, 270, or 300 MHz. ^{13}C NMR spectra were obtained at 67.9 or 75.4 MHz. Unless otherwise indicated, NMR spectra were obtained in $CDCl_3$. Solutions were dried over $MgSO_4$. Flash chromatography refers to the procedure developed by Still and co-workers: Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(28) Prepared from dimethyl malonate by the literature procedure for the diethyl ester: Pardo, S. N.; Salomon, R. G. *J. Org. Chem.* **1981**, *46*, 2598–2599.

br s), 6.42 (1 H, dt, J = 9, 2 Hz), 6.05 (1 H, dd, J = 9 and 3 Hz) 5.54 (1 H, d, J = 3 Hz), 5.04 (1 H, dd, J = 10 and 3 Hz), 4.78 (1 H, d, J = 3 Hz), 4.42 (1 H, dt, J = 10 and 3 Hz), 3.82 (3 H, s), 3.78 (3 H, s), 3.46 (3 H, s); ^{13}C NMR (75.4 MHz) δ 164.8, 163.4, 149.2, 133.3, 129.0, 128.9, 122.1, 97.2, 78.9, 78.8, 57.3, 52.4, 52.1; high-resolution mass spectrum, calcd for $C_{13}H_{16}O_6$ 268.0947, found 268.0945.

(3β,4α,5β)-3-[(1-Carboxyethenyl)oxy]-4-methoxy-5-(phenylseleno)-1-cyclohexene-1-carboxylate (15). A solution of NaOH (1.0 M, 1.75 mL, 1.75 mmol) was added dropwise to a solution of **13** (0.2154 g, 0.597 mmol) in THF (10 mL) and H_2O (10 mL). The solution was stirred for 1.5 h and then concentrated, and H_2O (5 mL) was added. The pH was adjusted to 3.0 (1 M HCl), and the solution was saturated with NaCl and extracted with ether (2 × 25 mL). The ether extracts were dried and concentrated to give **15** as a clear oil that was used without further purification: ^{1}H NMR δ 7.62 (2 H, m), 7.30 (3 H, m), 6.85 (1 H, br m), 5.67 (1 H, d, J = 3 Hz), 4.92 (1 H, d, J = 3 Hz), 4.72 (1 H, br m), 3.66–3.49 [5 H, m with s (3 H) at 3.59], 2.75 (1 H, dd, J = 18 Hz), 2.52 (1 H, m).

***trans*-3-[(1-Carboxyethenyl)oxy]-4-methoxy-1,5-cyclohexadiene-1-carboxylic Acid (6).** A solution of H_2O_2 (30%, 0.230 mL, 2.2 mmol) was added with stirring to a solution of **15** (prepared above) in MeOH (10 mL) at 0 °C. After 15 min NH_4OH (30%, 0.40 mL) was added, and the solution was warmed to room temperature. After 1 h the mixture was concentrated at 0 °C, and cold H_2O (5 mL) was added. The pH was adjusted to 7.0 (1 M NaOH), and the mixture was extracted with CH_2Cl_2 (2 × 5 mL). The combined CH_2Cl_2 extracts were back-extracted with cold H_2O (2 mL), the pH of the combined aqueous portions was adjusted to 3.0 (1 M HCl), NaCl (2 g) was added, and the mixture was extracted with cold ether (3 × 8 mL). The ether extracts were dried and concentrated. Flash chromatography on silica gel (10 mL of 1:1 EtOAc/hexane followed by pure EtOAc) gave fractions containing material of R_f = 0.15 (99:1 EtOAc/HOAc, streaks) that were concentrated. The residue was dissolved in CH_2Cl_2 (5.0 M) and cooled to −15 °C for 1.5 days. The white solid was collected by filtration, washed with a small portion of cold CH_2Cl_2, and dried under high vacuum to give 29 mg (20% from **13**) of **6**: mp 117–119 °C (dec.); IR (KBr) 3700–2300, 1704, 1621 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.87 (1 H, m), 6.39 (1 H, dt, J = 9 and 2 Hz), 6.10 (1 H, dd, J = 9 and 3 Hz), 5.50 (1 H, d, J = 2 Hz), 5.12 (1 H, dd, J = 10 and 3 Hz), 4.97 (1 H, d, J = 2 Hz), 4.36 (1 H, dt, J = 10 and 2 Hz), 3.42 (3 H, s); high-resolution mass spectrum, calcd for $C_{11}H_{12}O_6$ 240.0634, found 240.0645.

Dimethyl 4-*O*-Methylprephenate (16). A solution of **14** (64.2 mg, 0.239 mmol) was heated in benzene (5 mL) to gentle reflux under N_2 for 10 h, and the benzene was removed under reduced pressure. Flash chromatography on silica gel (1:19 EtOAc/CH_2Cl_2) gave 27.8 mg (43%, R_f = 0.32) of **16** as a clear oil: IR (thin film) 1732 cm^{-1}; ^{1}H NMR δ 6.04 (4 H, m), 4.38 (1 H, m), 3.87 (3 H, s), 3.71 (3 H, s), 3.28 (3 H, s), 3.25 (2 H, s); high-resolution mass spectrum, calcd for $C_{12}H_{12}O_5$ (M^+ − CH_3OH) 236.0685, found 236.0687.

Disodium 4-*O*-Methylprephenate (17). A solution of **16** (11.1 mg, 0.414 mmol) in 1:1 THF/H_2O (2 mL) was cooled to 0 °C with stirring, and NaOH (1 M, 0.125 mL) was added dropwise. After 30 min at 0 °C, the solution was kept at room temperature for 30 min. The solvent was removed under vacuum at room temperature. The residue was dissolved in H_2O (4 mL), and the pH was adjusted to 7.3 by the addition of small amounts of Amberlite IR-120 resin (careful, delayed response). The solution was filtered, and the filtrate was evaporated under high vacuum. Several portions of absolute EtOH (1 mL) were added to the residue followed by evaporation to induce solidification. The resulting solid was dried under high vacuum (0.5 mm) to give 14 mg of **17**: ^{1}H NMR (D_2O) δ 6.02 (2 H, d, J = 10 Hz), 5.79 (2 H, dm, J = 10 Hz), 4.32 (1 H, br s), 3.19 (3 H, s), 3.00 (s, partially exchanged with deuterium).

(±)-Methyl 3-[[1-(Methoxycarbonyl)ethenyl]oxy]-1,5-cyclohexadiene-1-carboxylate (18). Ester **18** was prepared by the literature procedure.[8]

Methyl 5-[[1-(Methoxycarbonyl)ethenyl]oxy]-1,3-cyclohexadiene-1-carboxylate (25). Ester **25** was made from methyl 5-hydroxy-1,3-cyclohexadiene-1-carboxylate[12,29] by the same procedure used to prepare **18**. For **25**: IR (neat) 1728 cm^{-1}; ^{1}H NMR (250 MHz) δ 7.09 (1 H, d, J = 5.4 Hz), 6.31 (1 H, dd, J = 9.6 and 5.4 Hz), 6.22 (1 H, dd, J = 9.6 and 3.9 Hz), 5.49 (1 H, d, J = 2.6 Hz), 4.83 (1 H, dt, J = 7.6 and 3.9 Hz), 4.72 (1 H, d, J = 2.6 Hz), 3.78 (6 H, s), 2.98 (1 H, dd, J = 18.8 and 7.2 Hz), 2.75 (1 H, ddd, J = 18.8, 8.3, and 1.6 Hz).

(−)-Methyl (3α,4β,5α)-3,5-Dihydroxy-4-(phenylseleno)-1-cyclohexene-1-carboxylate [(−)-28]. Selenophenol[30] (2.4 mL, 22.4 mmol, 1.1 equiv) was dissolved in dry THF (700 mL, from Na) in a flame-dried flask under N_2 and then cooled to 0–5 °C. Addition of 2.32 M *n*-BuLi (9.5 mL, 22.0 mmol, 1.05 equiv, in hexanes) over several minutes via syringe gave a colorless solution, which was warmed to room temperature for 5 min and then cooled to −20 °C (dry ice/CCl_4). To the selenide solution was added (+)-**27** (3.564 g, 20.9 mmol) in dry THF (100 mL) and cooled to −20 °C, over 15 min via cannula. After 2 h, the ^{1}H NMR spectrum of an aliquot indicated complete reaction. The mixture was concentrated to ~10-mL volume, saturated aqueous KH_2PO_4 (50 mL) was added, and the aqueous solution was extracted with ethyl acetate (6 × 100 mL). The combined extracts were dried, filtered, and concentrated to give 7.37 g of a pale yellow solid. The ^{1}H NMR spectrum indicated a 6:1 mixture of (−)-**28**/(+)-**29** which was fractionally recrystallized from ethyl acetate to give 1.294 g (19%) of pure (−)-**28**. The residue from the mother liquor was recrystallized twice from ethyl acetate/petroleum ether to give 2.142 g (31%) of (−)-**28**. The combined mother liquors were flash chromatographed on silica gel (ethyl acetate/petroleum ether: 1:100, 0.5 L; 1:10, 0.5 L; 1:5, 3.0 L; 1:2, 2.7 L; 1:1, 1.0 L; 2:1, 1.0 L. 7 × 15 cm column) to give another 1.85 g (27%) of solid, for a total yield of 77% of (−)-**28**: mp 119–120 °C; $[\alpha]^{22}_D$ −39.1° (c 0.501, $CHCl_3$); IR (solid deposit) 3400–3359, 1709, 1657, 1435, 1246 cm^{-1}; ^{1}H NMR δ 7.62 (2 H, m), 7.31 (3 H, m), 6.88 (1 H, s), 4.20 (1 H, m), 3.78 (1 H, m), 3.75 (3 H, s), 3.21 (1 H, dd, J = 9.5 and 7.9 Hz), 2.98 (2 H, m), 2.94 (1 H, dd, J = 18 and 6.2 Hz), 2.41 (1 H, ddt, J = 18, 7.9, and 2.7 Hz).

In addition, 0.64 g (9%) of (+)-**29** was obtained: mp 142–144 °C (softened at 136 °C); $[\alpha]^{22}_D$ +118° (c 0.500, $CHCl_3$); IR (KBr) 3272–3241, 1723, 1655, 1242, 1088 cm^{-1}; ^{1}H NMR δ 7.61 (2 H, m), 7.34 (3 H, m), 6.86 (1 H, s), 4.51 (1 H, d, J = 3.9 Hz), 3.75 (3 H, s), 3.56 (2 H, m), 3.18 (1 H, d, J = 2.0 Hz), 3.03 (1 H, dd, J = 19 and 4.9 Hz), 2.75 (1 H, d, J = 3.9 Hz), 2.41 (1 H, dd, J = 19 and 9.0 Hz).

Methyl (3α,4α,5β)-3,4-Dihydroxy-5-(phenylseleno)-1-cyclohexene-1-carboxylate [(±)-29]. Selenophenol (0.74 mL, 6.92 mmol, 2.1 equiv) was dissolved in dry THF (10 mL, from Na) in a flame-dried flask under N_2 and then was cooled to 0–5 °C. Addition of 2.35 M *n*-BuLi (1.67 mL, 3.92 mmol, 1.1 equiv, in hexanes) over several minutes via syringe gave a slightly yellow solution, which was warmed to room temperature for 10 min and then recooled to 0–5 °C. To epoxy alcohol (±)-**27** (0.558 g, 3.28 mmol) at 0–5 °C in dry THF (5 mL) was added $ZnBr_2$ (1.56 g, 6.93 mmol, 2.1 equiv) in dry THF (5 mL) over 15 min via cannula. The resulting solution was added to the selenide solution at 0–5 °C over 30 min via cannula. The mixture was warmed to room temperature and stirred overnight. After 21 h, the reaction was quenched by addition of saturated aqueous NH_4Cl (10 mL) followed by extraction with ethyl acetate (4 × 15 mL). The combined organic layers were dried, filtered, and concentrated to give 2.048 g of a yellow foam. The ^{1}H NMR spectrum showed a 5:20:1 ratio of (±)-**28**/(±)-**29**/(±)-bromohydrin (**29** with Br instead of PhSe) which was purified by flash chromatography on silica gel [ethyl acetate/petroleum ether (2:3); 3.5 × 25 cm column] to give 0.662 g (57%, corrected for impurities) of (±)-**29** which was contaminated with 4% (by weight) of (±)-**28** and 3% (by weight) of (±)-bromohydrin. Recrystallization from ethyl acetate/petroleum ether gave pure (±)-**29**: mp 122.5–123 °C. In addition, 0.184 g (17%) of (±)-**28** was obtained.

(+)-Methyl (3α,5α)-3,5-Dihydroxy-1-cyclohexene-1-carboxylate [(+)-30]. To (−)-**28** (5.719 g, 17.5 mmol), dissolved in dry benzene (150 mL, from Na) under N_2 and heated to a gentle reflux, was added *n*-Bu_3SnH (9.5 mL, 35.3 mmol, 2.0 equiv) and a spatula tip full of AIBN. The mixture was kept at a gentle reflux for 12 h, at which time TLC analysis indicated complete reaction. The solution was cooled to room temperature, the solvent was removed in vacuo, and the residue was partitioned between CH_3CN (100 mL) and hexanes (100 mL). The CH_3CN layer was extracted with hexanes (4 × 50 mL) and concentrated to give 3.31 g of a slightly yellow oil. The crude mixture was used in succeeding experiments. In another experiment pure (+)-**30** was obtained by flash chromatography on silica gel: $[\alpha]^{22}_D$ +65.9° (c 0.534, $CHCl_3$); IR (neat) 3403–3337, 1717, 1649, 1439, 1262 cm^{-1}; ^{1}H NMR δ 6.98 (1 H, m), 4.83 (1 H, br s), 4.21 (1 H, br s), 3.77 (4 H, s), 3.48 (1 H, br s), 2.48 (2 H, m), 1.99 (2 H, m).

(−)-Methyl (3β,5α)-3-Acetoxy-5-hydroxy-1-cyclohexene-1-carboxylate [(−)-31]. To crude diol (+)-**30** (3.311 g, ~16 mmol, purity ~88%) dissolved in dry THF (80 mL, from CaH_2/Na) under N_2 were added Ph_3P (6.055 g, 23.1 mmol, ~1.4 equiv) and acetic acid (2.60 mL, 45.5 mmol, 2.8 equiv). The mixture was cooled to 0–5 °C (ice/H_2O), and diisopropyl azodicarboxylate (3.30 mL, 16.8 mmol, 1.04 equiv) was added over 30 min via syringe pump.[31] The reaction was warmed to room temperature, and after 20 h the ^{1}H NMR spectrum showed complete conversion to (−)-**31**. The mixture was concentrated to an orange oil which was flash chromatographed on silica gel (ethyl acetate/petro-

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(31) Syringe pump: Sage 341A, VWR Scientific.

leum ether: 1:5, 1.2 L; 1:2, 4.5 L; 1:1, 1.5 L. 7 × 15 cm column) to give 2.393 g [64% from (−)-**28**] of (−)-**31**. An additional 0.82 g of (−)-**31** contaminated with triphenylphosphine oxide was obtained. Flash chromatography as described above gave 0.240 g [6% from (−)-**28**] of pure (−)-**31**, for a combined yield of 70% of (−)-**31**: $[\alpha]^{22}_D$ −187° (*c* 0.766, $CHCl_3$); IR (neat) 3486–3434, 2953, 1721, 1655, 1439 cm^{-1}; ^1H NMR δ 6.86 (1 H, m), 5.61 (1 H, m), 4.21 (1 H, m), 3.77 (3 H, s), 3.27 (1 H, br s), 2.72 (2 H, dm, *J* = 18 Hz), 2.27 (1 H, ddm, *J* = 18 and 5.7 Hz), 2.09 (3 H, s), 1.95 (2 H, complex m).

(+)-Methyl (3α,5α)-3,5-Dihydroxy-1-cyclohexene-1-carboxylate [(+)-30 from (−)-30]. To (−)-**30** (46.5 mg, 0.26 mmol) dissolved in dry THF (5 mL, from Na) under N_2 were added Ph_3P (177 mg, 0.67 mmol, 2.6 equiv) and acetic acid (310 μL, 5.42 mmol, 21 equiv). DIAD (133 μL, 0.68 mmol, 2.6 equiv) was added over 5 min via syringe. After overnight stirring, the ^1H NMR spectrum showed remaining **31**, so additional Ph_3P (10 mg, 0.04 mmol, 0.15 equiv) and DIAD (6 μL, 0.03 mmol, 0.1 equiv) were added. After a total of 30 h, the ^1H NMR spectrum showed a 1:7 ratio of **34**/**35**. The reaction mixture was concentrated and flash chromatographed on silica gel (ethyl acetate/petroleum ether: 1:10, 400 mL; 1:5, 200 mL. 2 × 15 cm column) to give 38.2 mg (58%) of **35**: IR (neat) 2955, 1738, 1655, 1437 cm^{-1}; ^1H NMR δ 6.87 (1 H, m), 5.60 (1 H, m), 5.11 (1 H, m), 3.81 (3 H, s), 2.77 (1 H, dd, *J* = 18 and 5.4 Hz), 2.44 (1 H, ddt, *J* = 18, 7.5, 2.3 Hz), 2.30 (1 H, dm, *J* = 13 Hz), 2.12 (3 H, s), 2.10 (3 H, s), 1.95 (1 H, m). In a separate experiment, previously reported[18] acetate **34** was obtained.

To a solution of diacetate **35** (195 mg, 0.76 mmol, purity ~92%) in CH_3OH (4 mL), under argon and cooled to 0–5 °C, was added $NaOCH_3$ (41 mg, 0.76 mmol, 1.0 equiv) in CH_3OH (3 mL) via syringe. After 5 h, TLC analysis indicated complete reaction. Acetic acid (45 μL, 0.79 mmol, 1.0 equiv) was added, and the mixture was concentrated. The residue was taken up in ethyl acetate (20 mL) and washed with 5% KH_2PO_4 (10 mL), and the aqueous layer was extracted with additional ethyl acetate (5 × 10 mL). The combined organic layers were dried, filtered, and concentrated to give 141 mg of an oil. The oil was flash chromatographed on silica gel [ethyl acetate/petroleum ether (2:1), 500 mL; 2.5 × 15 cm column] to give 94 mg (78%) of (+)-**30** which was identical with (+)-**30** obtained previously.

(+)-Methyl (3β,5β)-3-Hydroxy-5-(phenylseleno)-1-cyclohexene-1-carboxylate [(+)-32]. To a solution of (−)-**31** (2.22 g, 10.4 mmol) in CH_2Cl_2 (40 mL) under a N_2 atmosphere was added DMAP (1.53 g, 12.5 mmol, 1.2 equiv), and the mixture was cooled to 0–5 °C. Mesyl chloride (0.960 mL, 12.4 mmol, 1.2 equiv) was added dropwise via syringe pump,[31] which gave a copious precipitate. The mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched by washing the reaction mixture was saturated aqueous KH_2PO_4 (2 × 20 mL), followed by extraction of the aqueous layers with CH_2Cl_2 (5 × 15 mL). The combined organic layers were dried, filtered, and concentrated to give 3.024 g of crude mesylate as an oil which was used directly in the next reaction: ^1H NMR δ 6.91 (1 H, m), 5.61 (1 H, m), 5.18 (1 H, m), 3.78 (3 H, s), 3.07 (3 H, s), 2.88 (1 H, dd, *J* = 18 and 4.8 Hz), 2.61 (1 H, dd, *J* = 18 and 6.1 Hz), 2.27 (1 H, m), 2.09 (3 H, s), 2.06 (1 H, m).

To a solution of the mesylate (3.024 g, 10.4 mmol) in dry CH_3OH (30 mL, from CaH_2) at 0–5 °C was added 0.5 M $NaOCH_3$ (4.1 mL, 2.1 mmol, 0.2 equiv) over 15 min via syringe pump.[31] TLC analysis after 40 min showed no remaining starting material. The reaction was quenched by addition of acetic acid (120 μL, 2.1 mmol, 0.2 equiv) followed by concentration to ~5-mL volume. The concentrate was taken up in ethyl acetate (50 mL) and washed with 5% KH_2PO_4 (50 mL). The aqueous portion was extracted with ethyl acetate (6 × 50 mL), and the combined organic layers were dried, filtered, and concentrated. This gave 2.640 g of crude alcohol which was used directly in the next reaction: ^1H NMR δ 6.98 (1 H, m), 5.20 (1 H, m), 4.65 (1 H, m), 3.79 (3 H, s), 3.05 (3 H, s), 2.81 (1 H, dm, *J* = 19 Hz), 2.58 (1 H, dm, *J* = 19 Hz), 2.31 (1 H, m), 2.25–2.00 (1 H, br), 1.98 (1 H, ddd, *J* = 14, 6.8, and 3.4 Hz).

To a solution of selenophenol[30] (1.16 mL, 10.8 mmol, 1.05 equiv) in dry THF (10 mL) under N_2 at 0–5 °C was added dropwise 2.32 M *n*-BuLi (4.6 mL, 10.7 mmol, 1.03 equiv) via syringe pump.[31] The resulting solution was added via cannula to the mesylate (2.640 g, 10.4 mmol) in dry THF (10 mL). The mixture was warmed to room temperature and stirred overnight. After 13 h, TLC analysis showed no starting material, and the reaction was quenched by addition of H_2O (5 mL). The mixture was concentrated to ~10-mL volume, taken up in ethyl acetate (100 mL), and washed withh 5% KH_2PO_4 (50 mL). The aqueous portion was extracted with ethyl acetate (3 × 50 mL, 2 × 25 mL); the combined organic layers were dried, filtered, and concentrated to give 3.36 g of a viscous oil. The oil was flash chromatographed on silica gel (ethyl acetate/petroleum ether: 1:5, 1800 mL; 1:2, 500 mL. 4 × 15 cm column) to give 1.640 g [51% from (−)-**31**] of crystalline (+)-**32**: mp 78–80 °C (sublimes); $[\alpha]^{22}_D$ +11.7° (*c* 0.316, $CHCl_3$);[32] IR (neat) 3443–3397, 3056, 2950, 1717, 1651, 1578, 1478, 1437 cm^{-1}; ^1H NMR δ 7.61 (2 H, m), 7.31 (3 H, m), 6.88 (1 H, m), 4.14 (1 H, br s), 3.75 (3 H, s), 3.38 (1 H, m), 2.75 (1 H, dm, *J* = 19 Hz), 2.45 (3 H, m), 1.71 (1 H, m).

(−)-Methyl 3-Hydroxy-1,5-cyclohexadiene-1-carboxylate [(−)-33]. To a solution of selenide (+)-**32** (104 mg, 0.33 mmol) in $CDCl_3$ (2 mL) were added Na_2HPO_4 (95 mg, 0.66 mmol, 2.0 equiv) and *n*-Bu_4NIO_4 (289 mg, 0.66 mmol, 2.0 equiv), and the mixture was stirred at room temperature. After 105 min, ^1H NMR analysis indicated complete reaction. The orange solution was diluted with CH_2Cl_2 (20 mL) and washed with 5% $NaHCO_3$ (10 mL). The aqueous portion was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were dried, filtered, and concentrated to give 296 mg of a tacky solid. The solid was triturated with ethyl acetate/petroleum ether (1:1, 10 mL) and suction filtered, and the filtrate cake was washed with additional ethyl acetate/petroleum ether (1:1, 5 × 2 mL). The combined filtrates were concentrated to ~1-mL volume and flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5, 300 mL) + 1% (v/v) Et_3N, 1.2 × 15 cm column] to give 40.7 mg (75%, corrected for aromatic impurity) of (−)-**33**[18] which was contaminated with 6% methyl *m*-hydroxybenzoate: $[\alpha]^{22}_D$ −184° (*c* 0.383, $CHCl_3$, corrected for aromatic impurity); ^1H NMR δ 6.91 (1 H, d, *J* = 4.3 Hz), 6.44 (1 H, dm, *J* = 9.7 Hz), 5.99 (1 H, dt, *J* = 9.7 and 3.7 Hz), 4.49 (1 H, m), 3.80 (3 H, s), 2.72 (1 H, br s), 2.49 (2 H, m).

(−)-Dimethyl 4-Deshydroxychorismate [(−)-18]. To a solution of alcohol (−)-**33** (245 mg, 1.59 mmol) in dry benzene (8 mL, from Na) under N_2 was added $Rh_2(OAc)_4$ (23 mg, 0.054 mmol, 3 mol %). A solution of trimethyl diazophosphonoacetate (455 mg, 2.19 mmol, 1.4 equiv) in dry benzene (2 mL) was added, and the green mixture was heated to 75 °C (N_2 evolution). After 3 h, the ^1H NMR spectrum indicated complete reaction. The mixture was cooled to room temperature and concentrated to an oil. The oil was flash chromatographed on silica gel (ethyl acetate/petroleum ether: 1:1, 800 mL; 2:1, 800 mL. 3 × 15 cm column) to give 307 mg (~47%, estimated purity ~82%) of phosphonate as an oil: ^1H NMR ($CDCl_3$) δ 6.91–6.88 (1 H, d, *J* = 5.0 Hz), 6.46 (1 H, dm, *J* = 9.7 Hz), 6.00 (1 H, m), 4.53, 4.50 (1 H, d, *J* = 20 Hz), 4.45 (1 H, m), 3.90–3.77 (12 H, complex), 2.75–2.37 (2 H, m).

The phosphonate (307 mg, 0.75 mmol, ~82% purity) was dissolved in dry THF (20 mL, from Na) under a N_2 atmosphere and cooled to −78 °C (dry ice/acetone). A 1.0 M solution of lithium bis(trimethylsilyl)-amide (1.0 mL, 1.0 mmol, 1.3 equiv) in hexanes was added over 10 min via syringe. Gaseous formaldehyde (generated from paraformaldehyde, 129 mg, 9.2 mmol, 12 equiv)[33] was bubbled into the cold solution over 20 min. TLC analysis indicated complete reaction, so the mixture was quenched at −78 °C by addition of saturated aqueous NH_4Cl (5 mL) and H_2O (5 mL), and the solution was extracted with cold ethyl acetate (5 × 15 mL). The extracts were kept cold (~4 °C) during this operation. The combined extracts were dried and suction filtered. The filtrate was again dried and gravity filtered, and the filtrate was split in four fractions. Each fraction was concentrated under high vacuum to give a total of 174 mg [~34% from (−)-**33**, estimated purity ~73%] of (−)-**18** as an oil.[34] The ^1H NMR spectrum of this material was identical with that of racemic **18** and was used in further studies without additional purification. Since (−)-**18** was impure, only an estimate of the optical rotation can be given: $[\alpha]^{22}_D$ −168° (*c* 0.409 at ~73% purity, $CHCl_3$); IR (neat) 2955, 1725, 1622, 1439, 1258, 1202, 1169 cm^{-1}; ^1H NMR δ 6.90 (1 H, d, *J* = 3.8 Hz), 6.46 (1 H, dq, *J* = 9.8 and 1.7 Hz), 6.03 (1 H, dt, *J* = 9.8 and 4.3 Hz), 5.50 (1 H, d, *J* = 2.7 Hz), 4.94 (1 H, td, *J* = 9.3 and 3.8 Hz), 4.70 (1 H, d, *J* = 2.7 Hz), 3.80 (3 H, s), 3.79 (3 H, s), 2.65–2.57 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 165.6 (s), 163.6 (s), 149.3 (s), 131.7 (d), 130.3 (s), 126.8 (d), 121.5 (d), 96.7 (t), 71.1 (d), 52.5 (q), 52.0 (q), 27.9 (t).

The thermal half-life for disappearance of (±)-**18** at 30 °C in $CDCl_3$ was determined by ^1H NMR to be 220 min, with equal amounts of **20** and **23** formed. The individual half-life for formation of both **20** and **23** was therefore 440 min.

Disodium 4-Deshydroxychorismate (19). To (−)-**18** (12.6 mg, 0.039 mmol)[34] in THF/H_2O (2:1, 1.5 mL) at 0 °C was added 1.0 M NaOH (0.16 mL, 0.16 mmol, 4.1 equiv), with just enough THF (~0.5 mL) added to maintain homogeneity. After 50 min, TLC analysis indicated complete conversion. Amberlite IR-120 acidic resin was added, and the pH was adjusted to 6.6. The resin was removed by suction filtration and rinsed with additional THF (3 × 0.5 mL), and the filtrate was partitioned into four fractions of known volume which were kept at 0 °C. THF was

(32) See footnote 20 of ref 11 for the procedure for the preparation of the Mosherester.

(33) Paraformaldehyde was heated at an oil bath temperature of ~175 °C with a constant stream of N_2 passing over it and through $^1/_4$-in. Teflon tubing into the reaction mixture.

(34) This was calculated by assuming 73% purity by weight of (−)-**18**, the remainder consisting of methyl benzoate (2% by weight) and a trimethyl diazophosphonoacetate byproduct (25% by weight).

removed on a high-vacuum rotary evaporator; the remaining aqueous solution was snap-frozen in liquid N_2, and the H_2O was removed under high vacuum at 0 °C. After removal of the H_2O (~1 h), three of the fractions were stored at −55 °C until used in further experiments. The ^1H NMR spectrum of the last sample in CD_3OD was obtained quickly, and this showed a 2:1:1 ratio of **19**/**22**/**24**. In subsequent experiments a similar ratio of products was obtained, and it was assumed that the conversion of (−)-**18** to **19**, **22**, and **24** was quantitative: ^1H NMR of **19** (CD_3OD) δ 6.63 (1 H, d, J = 3.6 Hz), 6.45 (1 H, dd, J = 9.8, 1.3 Hz), 5.91 (1 H, dt, J = 9.8 and 4.9 Hz), 5.18 (1 H, d, J = 1.5 Hz), ~4.9 (carbinol proton, obscured by HOD), 4.42 (1 H, d, J = 1.5 Hz), 2.55 (2 H, m).

Diacid **7** was produced as described above, except that the pH was adjusted to 3.5 prior to isolation: ^1H NMR (acetone-d_6) δ 6.92 (1 H, d, J = 3.7 Hz), 6.43 (1 H, dd, J = 9.4 and 1.5 Hz), 6.06 (1 H, dt, J = 9.4 and 4.7 Hz), 5.43 (1 H, d, J = 2.6 Hz), 5.06 (1 H, dt, J = 3.7 and 7.7 Hz), 4.90 (1 H, d, J = 2.6 Hz), 4.72 (acid protons + H_2O), 2.61 (2 H, m).

The thermal half-life for disappearance of **19** at 30 °C was measured in CD_3OD, phosphate buffer (prepared by evaporation of pH 7.2 phosphate buffer, followed by dissolution in D_2O), and Tris-DCl buffer (prepared by evaporation of pH 7.5 Tris-HCl buffer, followed by dissolution in D_2O). The half-life was found to be 51 min in CD_3OD and only 2.4 min in both phosphate and Tris-DCl buffer. The ratio of **22**/**24** formed in CD_3OD was 23:77, and therefore, the individual half-life for [3,3] rearrangement was 220 min, while the half-life for aromatization was 70 min. The ratio of **22**/**24** formed in phosphate buffer was 73:27, and therefore, the individual half-life for Claisen rearrangement was 3.5 min, while the half-life for aromatization was 8.0 min. Finally, the ratio of **22**/**24** formed in Tris-DCl buffer was 70:30, and therefore, the individual half-life for Claisen rearrangement was 3.3 min, while the half-life for aromatization was 9.0 min.

Disodium 4-Deshydroxyprephenate (22). To a solution of quaternary ammonium salt[8] for fragmentation to **18** (267 mg, 0.61 mmol) in THF/H_2O (1:1, 2 mL) at 0–5 °C was added 1.0 M NaOH (0.91 mL, 0.91 mmol, 1.5 equiv) over 5 min. The mixture was stirred for another 10 min, diluted with saturated aqueous NaCl (10 mL) and H_2O (2 mL), and extracted with CH_2Cl_2 (4 × 10 mL). The combined extracts were dried, filtered, and concentrated to give 90 mg of crude (±)-**18** as an oil. The oil was dissolved in $CDCl_3$ (5 mL) and was kept 60 °C overnight to give a 1:1 ratio of **20**/**23**. The solvent was removed in vacuo, and the oily residue was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5), 1.2 × 15 cm column] to give 33 mg (23%) of **20**: IR (neat) 3040, 2965, 1735, 1437 cm^{-1}; ^1H NMR δ 5.93 (2 H, dt, J = 10.4 and 3.2 Hz), 5.78 (2 H, dt, J = 10.4 and 1.8 Hz), 3.86 (3 H, s), 3.71 (3 H, s), 3.28 (2 H, s), 2.69 (2 H, m).[1] To a solution of **20** (33 mg, 0.14 mmol) in THF/H_2O (2.5:1, 3.5 mL) at 0 °C was added 1.0 M NaOH (0.35 mL, 0.35 mmol, 2.5 equiv) via syringe. The mixture was warmed to room temperature and stirred for 6 h. At this time TLC analysis indicated complete reaction. Amberlite IR-120 acidic resin was added, and the pH was adjusted to 6.5. The resin was removed by suction filtration, and the filtrate was concentrated to give 32 mg (91%) of **22** as an off-white solid. An analytical sample was prepared by precipitation from acetone: ^1H NMR (CD_3OD) δ 5.88 (2 H, dt, J = 10.4 and 1.8 Hz), 5.69 (2 H, dt, J = 10.4 and 3.2 Hz), 3.14 (2 H, s, exchangable), 2.60 (2 H, s); ^1H NMR (D_2O) δ 5.69 (2 H, dt, J = 10 and 2.4 Hz), 5.58 (2 H, d, J = 10 Hz), 2.94 (2 H, s, exchangable), 2.45 (2 H, s). Disodium salt **22** did not melt at temperatures below 310 °C. Attempts to isolate pure 4-deshydroxyprephenic acid (**21**) failed due to instability of the product under acidic conditions.

Enzyme Experiments with (−)-19. Experiment 1. A sample of (−)-**18** (12.5 mg, 0.038 mmol) was saponified and split in four fractions of known volume as described above:[35] fraction 1 (2.7 mg, 0.011 mmol), fraction 2 (2.7 mg, 0.011 mmol), fraction 3 (0.5 mg, 0.002 mmol), and fraction 4 (0.5 mg, 0.002 mmol). The ^1H NMR spectrum of fraction 1 in CD_3OD showed a 67:22:11 ratio of **19**/**22**/**24**. Incubation solutions were made up to 2.0-mL volume by combining pH 7.5 Tris-HCl buffer (A)[36] with inactivated chorismate mutase (B)[25] or active chorismate mutase (C).[26] These solutions were added to fractions 2–4 at −55 °C, and the resulting mixtures were immediately immersed in a constant-temperature bath at 30 °C: fraction 2 (1.868 mL of A + 0.132 mL of B), fraction 3 (1.833 mL of A + 0.167 mL of C), and fraction 4 (1.667 mL of A + 0.333 mL of C). After 1 h, fractions 3 and 4 were heated briefly at 100 °C to denature the enzyme. Each fraction was filtered through a 0.45-μm Millex-HV (Millipore) disposable HPLC filter, and each filter was rinsed with D_2O (2 × 1 mL) to ensure complete transfer. The filtrate of each fraction was concentrated on a high-vacuum rotary evaporator and exchanged several times with more D_2O (3 × 2 mL). The ^1H NMR spectrum of each fraction was obtained in D_2O. Thus, fraction 2 showed a 70:30 ratio, fraction 3[37] showed a 79:21 ratio, and fraction 4[37] showed an 82:18 ratio of **22**/**24**.

Experiment 2. A sample of (−)-**18** (12.6 mg, 0.039 mmol) was saponified and split in four fractions of known volume as described above:[35] fraction 1 (1.5 mg, 0.006 mmol), fraction 2 (1.5 mg, 0.006 mmol), fraction 3 (1.5 mg, 0.006 mmol), and fraction 4 (0.5 mg, 0.002 mmol). The ^1H NMR spectrum of fraction 1 in CD_3OD showed a 51:23:26 ratio of **19**/**22**/**24**. Incubation solutions were made up to 2.0-mL volume by combining pH 7.5 Tris-HCl buffer (A)[36] with inactivated chorismate mutase (B)[25] or active chorismate mutase (C).[26] These solutions were added to fractions 2–4 at −55 °C, and the resulting mixtures were immediately immersed in a constant-temperature bath at 30 °C: fraction 2 (2.0 mL of A), fraction 3 (1.868 mL of A + 0.132 mL of B), and fraction 4 (1.5 mL of A + 0.5 mL of C). After 1 h, each fraction was concentrated on a high-vacuum rotary evaporator. ^1H NMR spectra of fractions 2 and 3 were obtained in D_2O. Fraction 2 showed a 64:36 ratio of **22**/**24**, and fraction 3 showed a 61:39 ratio of **22**/**24**. The exchangeable protons of fraction 4 were removed by dissolution in D_2O (5 mL), followed by concentration in vacuo. This procedure was repeated twice. The residue was dissolved in D_2O (5 mL) and passed through a 0.45-μm Spartan-25 (Schleicher & Schuell) disposable HPLC filter to remove denatured enzyme. The filtrate was concentrated, and the ^1H NMR spectrum of the residue in D_2O showed a 76:24 ratio of **22**/**24**.[37]

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Supplementary Material Available: Analytical data, ^1H NMR, ^{13}C NMR, and/or mass spectral data of **11**, **12**, the intermediate to **13**, **13**, **18**, **22**, **25**, (−)-**28**, (+)-**29**, (+)-**30**, (−)-**31**, (+)-**32**, the Mosher ester of (+)-**32** and (−)-**32**, **34**, and **35** (4 pages). Ordering information is given on any current masthead page.

(35) The amount of **19** in each fraction was estimated by assuming a quantitative conversion from (−)-**18** to **19** and was corrected for the amount of **22** and **24** present at the start for the experiment.

(36) The buffer was 100 mM tris(hydroxymethyl)aminomethane (Tris), which was adjusted to pH 7.5 by addition of HCl. It also contained 1.0 mM EDTA and 1.0 mM dithiothreitol. Bovine serum albumin (0.1 mg/mL) was added just prior to use.[23]

(37) The enzyme was stored in buffer that contained glycerol,[22a] which obscured a large portion of the ^1H NMR spectrum. This problem could be alleviated by removal of exchangeable protons as described and also by decoupling at ~3.2 ppm. These actions removed the majority of the signals caused by glycerol.