6 H), 1.32 (s, 18 H). IR (KBr, cm^{-1}): 2963, 1777, 1724, 1518, 1376, 1253, 1123, 745. HR-FAB-MS (MNBA matrix) for $C_{46}H_{41}N_2O_6$ ($M + H^+$): calcd, 717.2965; found, 717.2978.

***endo*-Peroxide 9.** **3c** (0.5 mg) was dissolved in 0.5 mL of $CDCl_3$ in a standard ^1H NMR tube, giving an orange/red solution. The solution was exposed to room light for 15 h during which time the color changed to pale yellow (λ (nm): 324, 267, 224). The ^1H NMR was examined before and after purification by chromatography (silica gel, CH_2Cl_2 eluent), showing that **9** was formed quantitatively. ^1H NMR ($CDCl_3$): δ 8.06 (s, 4 H), 2.80 (m, 4 H), 1.60 (m, 4 H), 1.42 (m, 8 H), 0.95 (m, 6 H). IR (KBr, cm^{-1}): 2956, 2924, 2855, 1660, 1611, 1305, 722. HR-FAB-MS (ONPOE matrix) for $C_{38}H_{43}O_6$ ($M + H^+$): calcd, 595.3060; found, 595.3074.

ESR Spectra. ESR spectra were recorded on an IBM-Bruker ESP 300 X-band spectrometer equipped with a variable-temperature control unit. The spectroelectrochemical cell consisted of a 8.5-cm-length quartz tube that was constricted to 1.2-mm internal diameter at the bottom. A 7-mm Pt wire was encased with insulation tubing except for an exposed end (ca. 1 cm) which was placed in the constricted portion of the cell. A Pt wire counter electrode and a Ag wire reference electrode were positioned away from the working compartment. Following addition of the sample solution, the cell was sealed with a modified septum and degassed with argon. Reductions were performed by setting the potential slightly positive of the corresponding $E^{\circ\prime}$ values and gradually changing the potential to more negative values. The same ESR tube was used without electrodes present for anion radicals prepared by bulk electrolysis. Temperatures are in Kelvin.

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Uncatalyzed and Chorismate Mutase Catalyzed Claisen Rearrangements of 5,6-Dihydrochorismate and 6-Oxa-5,6-dihydrochorismate

John J. Delany, III, Robert E. Padykula, and Glenn A. Berchtold*

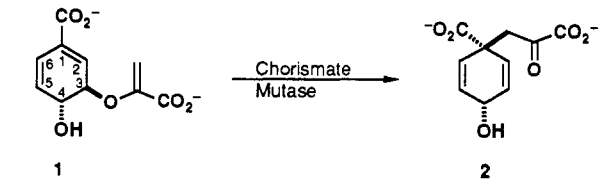
Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. *Received April 5, 1991. Revised Manuscript Received September 27, 1991*

Abstract: The synthesis of 6-oxa-5,6-dihydrochorismic acid (**4**) from D-xylose is described. The half-lives for the uncatalyzed Claisen rearrangements of 5,6-dihydrochorismic acid (**3**) and **4** in D_2O at 30 °C were 49 000 and 1200 h, respectively, compared to a half-life of 15.6 h for chorismic acid (**1**) under similar conditions. Both **3** and **4** were processed by the mutase activity of chorismate mutase-prephenate dehydrogenase from *Escherichia coli* with $k_{cat}/k_{uncat} = 1 \times 10^6$ and 4×10^5, respectively, compared to $k_{cat}/k_{uncat} = 2 \times 10^6$ for **1**.

The rearrangement of chorismate (**1**) to prephenate (**2**) is catalyzed by chorismate mutase. It is the first step in the biosynthesis of phenylalanine and tyrosine from **1**, by what is formally a [3,3] sigmatropic rearrangement, and is one of the most intriguing transformations found in nature (Scheme I).[1] The details of the mechanism of the enzymatic process are not understood irrespective of the extensive investigations from numerous laboratories. In a recent publication, we described studies that defined the structural requirements for catalysis by the mutase site of the biofunctional enzyme chorismate mutase-prephenate dehydrogenase from *Escherichia coli*.[2] Crucial to the study was the demonstration that 5,6-dihydrochorismic acid (**3**, Chart I) was a substrate for chorismate mutase. Described herein are the detailed studies of the thermal and enzyme-catalyzed rearrangement of **3** and the related dihydropyran analogue **4**, which proved to be another effective substrate for chorismate mutase.

Haslam and co-workers reported that **3** did not display any tendency to rearrange with chorismate mutase, but it was a modest inhibitor.[3] The dihydro analogue **3** 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 compared to the uncatalyzed rearrangement of **1**. The dihydro analogue **3** and a 1,2-dihydrochorismic acid of undetermined stereochemistry were prepared by Haslam and co-workers by diimide reduction of (-)-**1**.[3] Selective preparation of the two isomers was accomplished by a clever modification of reaction temperature followed by fractional crystallization. Since we had difficulty with the recrystallization

Scheme I

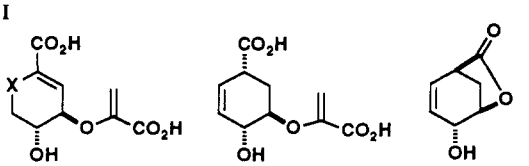


1 2

Scheme II

8 9, X = -NC; 10, X = -CN

Chart I



3, X = CH_2; 4, X = O 5 6

(1) For reviews, see: (a) Weiss, U.; Edwards, J. M. *The Biosynthesis of Aromatic Compounds*; Wiley: New York, 1980. (b) Haslam, E. *The Shikimate Pathway*; Halstead Press, Wiley: New York, 1974. (c) Ganem, B. *Tetrahedron* **1978**, *34*, 3353-3383.

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(3) Ife, R. J.; Ball, L. F.; Lowe, P.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1776-1783.

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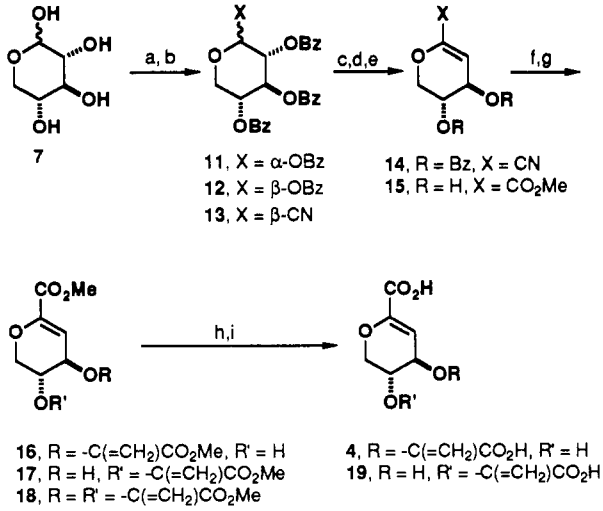
Scheme III[a]



[a] (a) Bz_2O, Et_3N, DMAP. (b) Me_3SiCN, $BF_3{\cdot}Et_2O$, $MeNO_2$. (c) DBU, CH_2Cl_2. (d) NaOH, H_2O/MeOH, 80 °C. (e) Me_2SO_4. (f) $N_2C(CO_2Me)PO(OMe)_2$, $Rh_2(n\text{-}C_7H_{15}CO_2)_4$, C_6H_6, reflux. (g) LiHMDS, H_2CO. (h) NaOH, H_2O/MeOH. (i) Aqueous HCl.

procedure, the mixture from the diimide reduction of (−)-**1** was esterified (dimethyl sulfate), and the mixture was separated by HPLC. The pure dihydro isomers were obtained by ester hydrolysis and acidification. That 1,2-dihydro isomer **5** possessed the stereochemistry indicated (Chart I) was established from the observation that the ^1H NMR spectrum of the dimethyl ester of **5** differed from the ^1H NMR spectrum of the epimeric diester obtained from **6**[4] by silylation of the hydroxyl group, methanolysis of the lactone group, and preparation of the enol pyruvate derivative by established methods.[2]

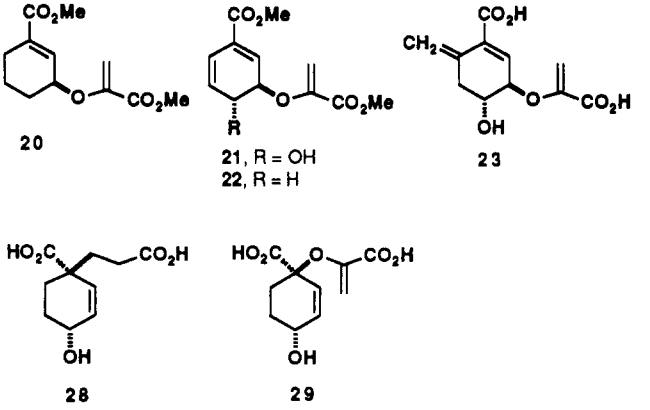
Our approach to the synthesis of **4** is based on the fact that the absolute stereochemistry at C-3 and C-4 of D-xylose (**7**) corresponds to the absolute stereochemistry at C-3 and C-4 of natural (−)-**1**, and consequently, the tactics required a procedure to convert the 1,2-diol moiety of the xylopyranose derivative to a vinyl ester. There are precedents in the literature which suggest that this is a reasonable strategy. Ribofuranose tetraacetate is converted to the corresponding 1-cyano derivative by treatment with $Me_3SiCN/BF_3{\cdot}Et_2O$,[5] and 2-deoxyglucose tetraacetate can be converted to the 1-cyano analogue by treatment with the same reagents in nitromethane.[6] Initially, we explored the reaction of β-D-xylose tetraacetate (**8**) with $Me_3SiCN/ZnCl_2$ in CH_2Cl_2. The sole product obtained was assigned the isonitrile structure **9** (Scheme II) since it decomposed at room temperature. Characterization was not pursued further.

Reaction of **8** with $Me_3SiCN/BF_3{\cdot}Et_2O$ in nitromethane appeared to give the desired product (**10**), but the reaction was sluggish and difficult to reproduce. We chose to use a more active leaving group for the ionization reaction. Treatment of **7** with benzoic anhydride/triethylamine/DMAP gave the tetrabenzoate anomer **12** in 93% yield after recrystallization, with no indication that any of the α anomer (**11**) was formed (Scheme III). Benzoate **12** reacted smoothly under the same conditions ($Me_3SiCN/BF_3{\cdot}Et_2O$ in nitromethane) to give a mixture of **11** and **13**. The α anomer (**11**) apparently was formed during the cyanation reaction by Lewis acid-catalyzed epimerization of **12** but was unreactive under the reaction conditions,[5] and attempts to convert **11** to **13** failed. The stereochemistry of **13** at C-1 was not rigorously assigned. The structure shown is the expected anomer based upon the fact that anchimeric assistance is commonly seen in these systems.[5] The stereochemistry of **13** was not crucial since **13** was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the vinyl nitrile **14** by elimination of benzoic acid. In practice, the conversion of **12** to **14** is performed as a one-pot procedure to give **14** in 62% overall yield. Nitrile **14** was treated with NaOH in aqueous methanol for 3 days at 80 °C. The acid was not isolated; the reaction mixture was neutralized to pH 8 with $NaHCO_3$ was treated with dimethyl sulfate. After 18 h at room temperature, ester **15** was obtained in 59% yield after chromatography.

Table I. Data for the Claisen Rearrangements of **1**, **3**, and **4** at 30 °C in D_2O

compd	$\Delta G^{\ddagger}$, kcal/mol	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, eu	k, s^{-1}
1	24.7	20.7	−12.8	1.22×10^{-5}
3	29	26	−12	3.9×10^{-9}
4	27	27	−1.0	1.7×10^{-7}

Chart II



What remained was a regiochemical problem of how to differentiate between the two hydroxyl groups in order to assemble the enol pyruvate side chain at C-3. Fortunately, reaction of diol **15** with trimethyl diazophosphonoacetate with Rh(II) catalysis gave a mixture of phosphonates which, when treated with lithium hexamethyldisilazide and formaldehyde, gave a 4:1:2.5 mixture of **16**, **17**, and **18**. The regioselectivity observed is surprising for a trans diol **15** in which the hydroxyl groups are expected to be in a diequatorial disposition. Product **18** was removed from **16** and **17** by flash chromatography on silica gel, and **16** and **17** were separated by HPLC on C_{18} silica gel. Hydrolysis of each pure isomer provided acids **4** and **19**. Confirmation of the assigned regiochemistry for **4** and **19** was provided by the observation that **4** undergoes a thermal Claisen rearrangement as described below.

The uncatalyzed Claisen rearrangement of **4** at 30 °C in D_2O (pD 7.4) occurred ~75 times slower than the Claisen rearrangement of chorismate (**1**) under the same conditions. Claisen rearrangement of the dihydrochorismate analogue **3** was not observed at 30 °C, but it could be followed by ^1H NMR spectroscopy at higher temperatures. Arrhenius plots were obtained for the Claisen rearrangements of **3** and **4**, and the parameters calculated from the data for rearrangement at 30 °C are provided in Table I along with the corresponding data for **1** at 30 °C.[7]

Knowles and co-workers[8] have concluded from secondary tritium isotope effects at C-1 and C-3 of **1** that the Claisen rearrangement proceeds with an unsymmetrical transition state, with little, if any, C–C bond formation while the C–O bond is substantially broken. Additional evidence for a dipolar transition state in the Claisen rearrangement has been presented by Coates, Curran, and co-workers[9] and by Carpenter, Gajewski, Ganem,

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(5) Utimoto, K.; Wakabayashi, Y.; Horiie, T.; Inoue, M.; Shishiyama, Y.; Obayashi, M.; Nozaki, H. *Tetrahedron* **1983**, *39*, 967–973.
(6) Widlanski, T. S.; Bender, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 1873–1875.
(7) The rate of thermal reactions of **1** at various temperatures in H_2O (pH 7.5) have been reported previously: Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492–3498.
(8) Addadi, L.; Jaffe, E. K.; Knowles, J. R. *Biochemistry* **1983**, *22*, 4494–4501.
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Table II. Uncatalyzed and Chorismate Mutase Catalyzed Data for **1**, **3**, and **4** at 30 °C

substrate	$t_{1/2}$, h	k_{uncat}, s^{-1}	K_m, mM	k_{cat},[a] s^{-1}	k_{cat}/k_{uncat}
1[b]	15.6	1.2×10^{-5}	0.14	25	2×10^6
3	49000	3.9×10^{-9}	0.55	0.004	1×10^6
4	1200	1.7×10^{-7}	0.28	0.070	4×10^5

[a] Per dimer of enzyme. [b] Copley and Knowles[12] find $k_{cat} = 51\ s^{-1}$ and $K_m = 41\ \mu M$ with highly purified enzyme in a different buffer system.

and co-workers.[10] Of particular interest to us were the results of the former group, which showed that 4- or 6-alkoxy substitution on the allyl vinyl ether and hydrogen-bonding solvents increased the rate of the Claisen rearrangement, and the rate acceleration was attributed to the increased dipolar character of the transition state.[9,11]

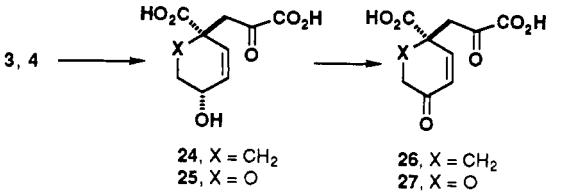
Possible mechanisms for the uncatalyzed and enzyme-catalyzed Claisen rearrangement of **1** and related structures have been discussed in detail.[10,13] Our observation that the rate of the uncatalyzed rearrangement of dihydrochorismate analogue **3** in D_2O is ~3000 times slower than the rate of rearrangement of **1** is striking. In related work, Carpenter and Ganem observed that **20** (Chart II) rearranged ~390 times slower than dimethyl chorismate (**21**) and ~38 000 times slower than dimethyl deshydroxychorismate (**22**) in aqueous methanol.[10] These investigators point out that the faster rates of **21** and **22** can be rationalized on the basis of stabilization by delocalization of the cyclohexadienyl cation fragment by cleavage of the C–O bond, but caution should be exercised before concluding how much of the rate difference is due to an unusually slow rearrangement of **20** as opposed to an unusually fast rearrangement of **22**.[10] It should be pointed out that **23** rearranges at approximately one-fourth the rate of rearrangement of **3** in DMSO-d_6.[14] A similar rate retardation (or lack of rate enhancement) has been observed with the trans arrangement of the additional bond in acyclic model systems.[10]

Pyran **4**, in which the CH_2 group at the six position of the carbocyclic ring of **3** is replaced by an oxygen atom, provides an analogue in which an alkoxy substituent is introduced at the C-6 position of the allyl vinyl ether moiety. In line with the observations of Coates, Curran, and co-workers,[9] the rate of rearrangement of **4** was ~40 times the rate of rearrangement of **3**. Although **1** and **3** show similar $\Delta S^{\ddagger}$ values for rearrangement, pyran **4** has a substantially less negative $\Delta S^{\ddagger}$ value.

Both dihydro analogue **3** and pyran analogue **4** were metabolized slowly by the mutase activity of chorismate mutase–prephenate dehydrogenase from *E. coli*. Kinetic parameters are given in Table II. Remarkably, the mutase accelerates the rearrangements of **3** and **4** by factors of 10^5–10^6. In view of the unusually long half-life for the Claisen rearrangement of **3** (~5.5 years!) compared to the half-life for rearrangement of **1** (15.6 h), it is not surprising that Haslam and co-workers[3] did not detect any enzymic catalysis with the quantity of enzyme used and the time period over which the reaction was observed. The K_m values of **1**, **3**, and **4** correlate well with the steric bulk of the substrates at C-5 and C-6. Gratifyingly, $K_m(\mathbf{3})/K_m(\mathbf{1}) \approx 4$, which agrees with the value of $K_i(\mathbf{3})/K_m(\mathbf{1})$ reported by Haslam and co-workers.[3]

An independent confirmation of the metabolism of **3** and **4** reported here was sought. In practice, a coupled assay with the prephenate dehydrogenase activity of the enzyme was a convenient method. Prephenate dehydrogenase is fairly selective in that both the C-1 carboxylate group and the C-4 hydroxyl group are necessary for activity.[15] Both **24** and **25**, generated in situ from **3** and **4** in the presence of chorismate mutase–prephenate dehydrogenase and NAD^+, reacted to give NADH and presumably the corresponding ketones, **26** and **27** (Scheme IV). The rate of NADH formation was compared to the rate of disappearance of substrates **3** and **4**, and analysis of the results indicated that, indeed, the expected turnover was occurring. These results are based on the appearance of an absorption in the ultraviolet spectrum of NADH at 340 nm. That **24** and **25** should be expected to be processed by prephenate dehydrogenase to **26** and **27**, respectively, appears eminently reasonable since it has been demonstrated that both enantiomers of deoxydihydroprephenate **28**[15] and **29**[16] (Chart II) are processed by prephenate dehydrogenase to the corresponding ketone.

Scheme IV



The experimental results described above confirm our earlier report that unsaturation at the C-5, C-6 positions is not required for active site binding and catalysis. In fact, k_{cat}/k_{uncat} for **3** and **4**, within experimental error, is essentially the same as for **1**.

Experimental Section

General. All ^1H NMR spectra were obtained at 250 or 300 MHz, and ^{13}C NMR spectra were obtained at 75.45 MHz with chemical shift values reported in parts per million downfield from tetramethylsilane. Unless otherwise indicated, NMR spectra were recorded in $CDCl_3$. IR spectra were recorded using thin films (oils) or KBr pellets (solids). Unless otherwise indicated, solutions were dried over $MgSO_4$. Melting points are uncorrected. Mass spectra were recorded on a Finnegan MAT8200 using an 70 eV ionization potential. Microanalyses were performed by Robertson Laboratories, Madison, NJ. Flash chromatography refers to the procedure developed by Still and co-workers.[17]

Preparation and Purification of the Dimethyl Esters of 3 and 5.[3] To a solution of (−)-**1** (0.662 g, 2.9 mmol) in MeOH (25 mL) at room temperature was added potassium azodiformate (1.85 g, 9.5 mmol) followed by the addition of HOAc (1.3 mL, 23 mmol) with rapid stirring. After 3.5 h, the yellow color had faded and gas evolution had ceased. After removal of the solvent, H_2O (7 mL) was added, and the pH was adjusted to 2.8 with concentrated HCl. The suspension was extracted with Et_2O (4 × 50 mL), the combined extracts were dried, and the solvent was removed on a rotary evaporator. The oil was flash chromatographed on a silica gel column (7 × 3 cm, elution with 1% HCOOH in EtOAc). Fractions with material of $R_f = 0.25$ (UV, streaks) in this solvent system were combined and evaporated to give an oil (0.40 g, 1.8 mmol) which consisted of approximately 55% of **3** and 45% of **5**. The stereochemistry of **5** was established as indicated since the ^1H NMR spectrum of **2** differed from the ^1H NMR spectrum of the epimeric diacid obtained from **6**.[4]

Since attempts to purify **3** by crystallization failed, the mixture of **3** and **5** was esterified and separated by HPLC. The mixture of **3** and **5** was dissolved in H_2O, the pH was adjusted to 7.0 with NaOH, and the solvent was removed on a rotary evaporator. To the residue were added MeOH (11 mL), $NaHCO_3$ (0.15 g), and dimethyl sulfate (1.4 mL, 14.8 mmol, 8 equiv), and the mixture was stirred at room temperature for 18 h. After evaporation of the solvent, H_2O (5 mL) was added, and the solution was extracted with Et_2O (2 × 25 mL). The combined ether extracts were dried, evaporated, and flash chromatographed on silica gel (3 × 8 cm, elution with 60% EtOAc/40% hexane). Fractions which contained material of $R_f = 0.35$ (UV) in this solvent system were combined and evaporated to give 0.279 g of a mixture of the dimethyl esters of **3** and **5**. Separation of the isomers was achieved with semipreparative HPLC (Alltech silica 10 m, internal diameter 10 mm, length 25 cm, flow rate 4 mL/min) using 40% EtOAc/60% hexane to give 74 mg of the dimethyl ester of **5** and 120 mg of the dimethyl ester of **3** as oils. ^1H NMR ($CDCl_3$) of the dimethyl ester of **5**: δ 1.68–1.80 (1 H, m),

(10) Gajewski, J. J.; Jurayj, J.; Kimbrough, D. R.; Gande, M. E.; Ganem, B.; Carpenter, B. K. *J. Am. Chem. Soc.* **1987**, *109*, 1170–1186.

(11) The effect of solvent on the Claisen rearrangement of chorismic acid, chorismate dianion, and related structures has been reported.[12]

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2.42–2.54 (1 H, m), 3.34 (1 H, br), 3.73 (3 H, s), 3.80 (3 H, s), 4.18–4.26 (1 H, m), 4.36–4.41 (1 H, br), 5.22 (1 H, d, J = 3 Hz), 5.49 (1 H, d, J = 3 Hz), 5.75–5.90 (2 H, m). ^{1}H NMR ($CDCl_3$) of the dimethyl ester of **3**: δ 1.65–1.85 (1 H, m), 2.05–2.20 (1 H, m), 2.30–2.60 (2 H, m), 3.74 (3 H, s), 3.82 (3 H, s), 3.98 (1 H, m), 4.50 (1 H, m), 4.87 (1 H, d, J = 3 Hz), 5.54 (1 H, d, J = 3 Hz), 6.75 (1 H, br t).

Preparation of 3 by Hydrolysis of the Dimethyl Ester of 3. A solution of 1 M NaOH (1.1 mL, 1.1 mmol) was added dropwise with stirring at room temperature to a solution of the dimethyl ester of **3** (93 mg, 0.36 mmol) in THF (6 mL) and H_2O (6 mL). After 3 h, most of the solvent was removed on a rotary evaporator. Water (8 mL) was added, and the pH was adjusted to 2.5 with HCl. The solution was saturated with NaCl and extracted with Et_2O (4 × 10 mL). The combined extracts were dried, and the solvent was removed on a rotary evaporator to give 78 mg of **3** as an oil. Precipitation from EtOAc/hexane gave 47 mg (7% from chorismic acid) of pure **3** (mp 151–152 °C). The ^{1}H NMR spectrum matches that reported.[3]

β-D-Xylopyranose Tetrabenzoate (12). To a vigorously stirred suspension of D-xylose (30.0 g, 200 mmol) (Sigma Chem., Grade II) in 800 mL of CH_2Cl_2 were added DMAP (20 mg), triethylamine (170 mL, 1.20 mol), and benzoic anhydride (227 g, 1.0 mol). The resulting suspension was stirred at room temperature for 60 h, during which time the D-xylose completely dissolved. The reaction mixture was washed with 300-mL portions of saturated NH_4Cl solution and water. The organic layer was dried, filtered, and evaporated to give a golden oil. Rapid trituration with 500 mL of ether, followed by cooling to −20 °C gave 97.5 g of a white crystalline powder after filtration. A second crop of crystals from ether (7.5 g) was combined to give 105 g (93%) of **12**: mp 176–177.5 °C; IR 3065, 1724, 1601, 1261, 1100, 708 cm^{-1}; ^{1}H NMR (300 MHz) δ 8.05 (8 H, m), 7.57 (4 H, m), 7.38 (8 H, m), 6.38 (1 H, d, J = 4.5 Hz), 5.85 (1 H, t, J = 6 Hz), 5.64 (1 H, t, J = 5 Hz), 5.41 (1 H, q, J = 5 Hz), 4.58 (1 H, dd, J = 13, 3.3 Hz), 4.03 (1 H, dd, J = 13, 5 Hz); ^{13}C NMR δ 165.7, 165.1, 164.6, 133.7, 133.5, 133.4, 130.1, 130.0, 128.9, 128.5, 128.4, 92.2, 69.1, 68.5, 68.1, 61.8; mass spectrum (rel intensity) 445 (1.7), 924 (63), 106 (53), 105 (100), 77 (86); $[\alpha]_D$ = −49.5° (c = 1, acetone).

(3*R*,4*R*)-6-Cyano-3,4,5-tris(benzoyloxy)-3,4-dihydro-2*H*-pyran (13). To a suspension of **12** (20.0 g, 35.3 mmol) in 350 mL of anhydrous nitromethane were added TMSCN (7.06 mL, 53.0 mmol) and freshly distilled BF_3·Et_2O (400 mL, 3.5 mmol). The reaction mixture was stirred at room temperature for 3 h and evaporated under reduced pressure. The resulting oil was dissolved in 400 mL of ether and washed with two 200-mL portions of saturated $NaHCO_3$. The ether layer was dried, filtered, and evaporated to give crude **13** as a brown oil. The resulting oil was dissolved in 200 mL of CH_2Cl_2, and DBU (10.4 mL, 70 mmol) was added. The resulting dark solution was stirred at room temperature for 3.5 h. The reaction mixture was washed with 500 mL of saturated NH_4Cl solution, dried, filtered, and evaporated. The residue was filtered through a short column of silica gel (1:2 ethyl acetate/petroleum ether) to give 11.5 g of a 3:1 mixtrue of **14** and **11** (62%), which was used directly in the next step. Pure **14** was obtained by chromatography on silica gel (1:2 ethyl acetate/petroleum ether). **13**: ^{1}H NMR δ 7.62 (6 H, m), 7.00 (9 H, m), 5.34 (1 H, t, J = 4.5 Hz), 5.07 (1 H, t, J = 4.5 Hz), 4.88 (1 H, m), 4.61 (1 H, d, J = 4 Hz), 4.14 (1 H, dd, J = 13, 2.5 Hz), 3.70 (1 H, dd, J = 13, 3.5 Hz). **14**: IR (neat) 3098, 2247, 1721, 1647, 1450, 1259, 1105, 706 cm^{-1}; ^{1}H NMR δ 8.05 (4 H, d, J = 7 Hz), 7.63 (2 H, t, J = 8 Hz), 7.45 (4 H, t, J = 7 Hz), 6.04 (1 H, dd, J = 5.5, 1.5 Hz), 5.48 (1 H, m), 5.44 (1 H, s), 4.59 (1 H, dt, J = 13, 2 Hz), 4.32 (1 H, dd, J = 13, 5 Hz); ^{13}C NMR δ 165.1, 164.9, 133.8, 133.7, 133.2, 129.9, 129.8, 128.9, 128.8, 128.6, 113.4, 110.8, 66.2, 65.5, 62.6; mass spectrum (rel intensity) 348 (0.7), 228 (11), 227 (51), 105 (100), 77 (100); HRMS calcd for $C_{13}H_9NO_3$ 227.0582, found 227.0583; $[\alpha]_D$ = −241.6° (c = 1, acetone).

Methyl (3*R*,4*R*)-3,4-Dihydroxy-3,4-dihydro-2*H*-pyran-6-carboxylate (15). To a solution of crude **14** (10.6 g, contained 27.3 mmol **14** by ^{1}H NMR) in 400 mL of methanol was added 340 mL of 1 N aqueous NaOH. The reaction mixture was heated to 85 °C for 50 h. The reaction mixture was cooled to room temperature, and 1 N aqueous HCl was added to neutralize the solution (pH 6). The solution was evaporated under reduced pressure, and the solid residue was diluted with 400 mL of methanol. $NaHCO_3$ (10 g) and Me_2SO_4 (30.0 mL) were added, and the resulting suspension was stirred at room temperature for 48 h. The reaction mixture was evaporated, and the residue was filtered through a 1-in. silica column (ethyl acetate) to give 2.80 g (59%) of pure **15**: IR (neat) 3390, 2955, 1719, 1265, 1126 cm^{-1}; ^{1}H NMR δ 6.08 (1 H, d, J = 4.5 Hz), 4.16 (1 H, t, J = 4.5 Hz), 4.11 (2 H, m), 3.83 (3 H, s), 3.80 (1 H, q, J = 4 Hz); ^{13}C NMR δ 162.9, 142.9, 109.6, 68.0, 67.2, 65.8, 52.7; mass spectrum (rel intensity) 174 (3), 143 (3), 131 (23), 122 (55), 71 (100); HRMS calcd for $C_7H_{10}O_5$ 174.0527, found 174.0528; $[\alpha]_D$ = −113.2°.

Methyl (3*R*,4*R*)-3-Hydroxy-4-[[1-(methoxycarbonyl)ethenyl]oxy]-3,4-dihydro-2*H*-pyran-6-carboxylate (16). To a solution of **15** (437 mg, 2.51 mmol) in 15 mL of anhydrous benzene were added trimethyl diazophosphonoacetate (630 mg, 3.00 mmol) and $Rh_2(n\text{-}C_7H_{15}CO_2)_4$ (20 mg). The solution was heated at reflux for 4 h. The reaction mixture was concentrated under reduced pressure, diluted with 15 mL of anhydrous THF, and cooled to −70 °C. Lithium hexamethyldisilazide (3.00 mL of a 1 N solution in THF) was added, and formaldehyde, generated thermally from paraformaldehyde (800 mg), was bubbled through the solution. The reaction mixture was warmed to room temperature and stirred for 15 min. The reaction mixture was evaporated, and the residue was chromatographed on silica gel (1:2 ethyl acetate/petroleum ether) to give 190 mg (22%) of pure **18** and 292 mg (45%) of a 4:1 mixture of **16** and **17**. Ester **16** was purified by HPLC chromatography on C_{18} silica gel (μBondapak, 19 mm × 15 cm, 10 mL/min, 92:8 water/2-propanol; t_R: **16** = 12 min, **17** = 18 min). For **16**: IR (neat) 3470, 2955, 1736, 1267, 1203 cm^{-1}; ^{1}H NMR δ 6.14 (1 H, dd, J = 4, 2 Hz), 5.57 (1 H, d, J = 3 Hz), 4.90 (1 H, d, J = 3 Hz), 4.45 (1 H, t, J = 4 Hz), 4.20 (2 H, m), 4.10 (1 H, q, J = 4 Hz), 3.83 (3 H, s), 3.81 (3 H, s), 2.60 (1 H, br s); ^{13}C NMR δ 163.8, 162.5, 149.2, 146.4, 105.0, 98.1, 71.8, 67.8, 65.2, 53.6, 52.8; mass spectrum (rel intensity) 258 (0.6), 240 (3), 181 (10), 157 (100), 127 (39); HRMS calcd for $C_{11}H_{14}O_7$ 240.0637, found 240.0637. For **17**: IR (neat) 3495, 2965, 1740, 1632, 1273, 1209 cm^{-1}; ^{1}H NMR δ 6.12 (1 H, d, J = 4 Hz), 5.58 (1 H, d, J = 3 Hz), 4.93 (1 H, d, J = 3 Hz), 4.45 (1 H, t, J = 4 Hz), 4.20 (3 H, m), 3.84 (3 H, s), 3.80 (3 H, s); ^{13}C NMR δ 163.5, 162.4, 149.4, 145.0, 109.2, 99.3, 74.2, 63.7, 63.2, 52.5, 52.4. For **18**: ^{1}H NMR δ 6.22 (1 H, d, J = 4 Hz), 5.62 (1 H, d, J = 3 Hz), 5.59 (1 H, d, J = 3 Hz), 4.94 (1 H, d, J = 3 Hz), 4.90 (1 H, d, J = 3 Hz), 4.64 (1 H, br s), 4.46 (1 H, dm, J = 13 Hz), 4.42 (1 H, m), 4.16 (1 H, d, J = 13 Hz), 3.84 (3 H, s), 3.80 (3 H, s), 3.78 (3 H, s).

(3*R*,4*R*)-4-Hydroxy-3-[(1-carboxyethenyl)oxy]-3,4-dihydro-2*H*-pyran-6-carboxylic Acid (4). To a solution of **16** (8.0 mg, 31 mmol) in 0.7 mL of methanol was added NaOH (93 mL of 1 N solution, 93 mmol). The solution was stirred at room temperature for 1 h. The solution was diluted with water (5 mL), and HCl (90 mL of 1 N solution) was added. The solution was extracted with ether (6 × 10 mL). The combined ether extracts were dried, filtered, and evaporated to give 5.9 mg (83%) of pure **4** as a colorless oil: ^{1}H NMR δ 6.08 (1 H, d, J = 4 Hz), 5.47 (1 H, d, J = 3 Hz), 4.98 (1 H, d, J = 3 Hz), 4.50 (1 H, t, J = 3.5 Hz), 4.16 (1 H, dd, J = 13, 2 Hz), 4.02 (2 H, m); ^{13}C NMR δ 163.8, 163.0, 150.1, 147.1, 104.7, 96.5, 71.5, 67.8, 65.0; mass spectrum (rel intensity) 212 (4.6), 185 (7.5), 142 (97), 125 (55), 112 (84), 97 (100), 85 (57), 69 (60).

(3*R*,4*R*)-4-[(1-Carboxyethenyl)oxy]-3-hydroxy-3,4-dihydro-2*H*-pyran-6-carboxylic Acid (19). To a solution of **17** (9.0 mg, 35 mmol) in 0.7 mL of methanol was added NaOH (105 mL of 1 N solution, 105 mmol). The solution was stirred at room temperature for 1 h. The solution was diluted with water (5 mL), and HCl (90 mL of 1 N solution) was added. The solution was extracted with ether (6 × 10 mL). The combined ether extracts were dried, filtered, and evaporated to give 6.2 mg (78%) of pure **19** as a colorless oil: ^{1}H NMR δ 6.07 (1 H, dd, J = 4, 1 Hz), 5.47 (1 H, d, J = 3 Hz), 4.98 (1 H, d, J = 3 Hz), 4.34 (1 H, dm, J = 13 Hz), 4.23 (1 H, d, J = 13 Hz), 4.04 (2 H, m).

General Assay Procedures for Chorismate Mutase–Prephenate Dehydrogenase from *E. coli* JFM30. For studies with chorismate mutase, reaction was monitored by disappearance of substrate (chorismate (**1**) λ = 273 nm, ϵ = 2630; pyran **4** λ = 239 nm, ϵ = 5000; dihydro **3** λ = 230, ϵ = 3700). All assays were performed at 30 °C in a buffer of 100 mM Tris·HCl (pH 7.5), 1 mM EDTA, 1 mM DTT, and 0.1 mg/mL bovine serum albumin. K_m and V_{max} values were obtained by monitoring the reaction rate at varied substrate concentrations. The quantity of enzyme used for each substrate was as follows: 0.01 mg for **1**, 0.10 mg for **4**, and 0.20 mg for **3**. For studies with prephenate dehydrogenase, reaction was monitored by the appearance of NADH (λ = 340 nm, ϵ = 6400). All kinetic studies were performed at 30 °C in a buffer of 100 mM Tris·HCl (pH 7.5), 1 mM EDTA, 1 mM DTT, and 0.1 mg/mL bovine serum albumin. The V_{max} and K_m values of prephenate were determined in the presence of 0.1 mM NAD^+. For determination of V_{max} of alternate substrates, 2.0 mM NAD^+ (final concentration) was added to the assay mixture.

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