Chemoenzymatic Synthesis of Isotopically Labeled Chorismic Acids

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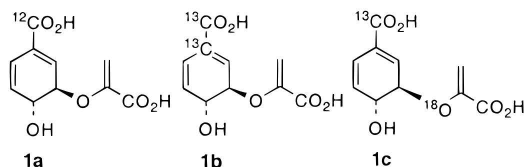
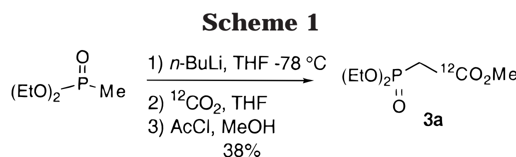
The rearrangement of chorismate to prephenate, catalyzed by chorismate mutase (CM), is a key step in the biosynthesis of aromatic amino acids in bacteria and plants.[1] Although CM has been much studied, details concerning its mechanism remain elusive.[2−5] Recently, as part of an investigation[6] to probe the structure of the enzymatic transition state with heavy-atom isotope effects,[7] we required a synthesis of the isotopically labeled chorismic acids **1a**−**c**.

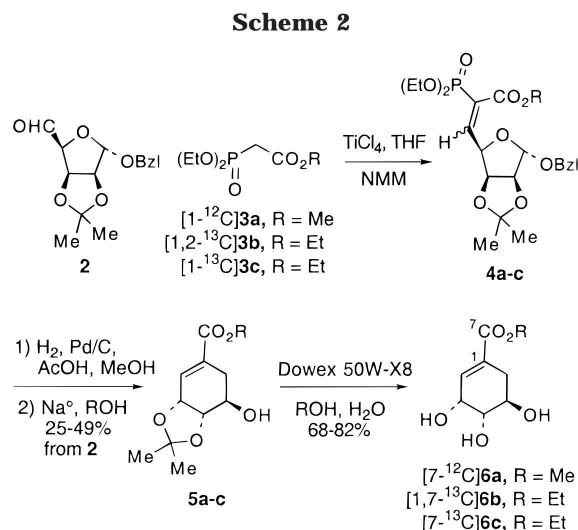


To date there have been two reported total syntheses of chorismic acid.[8,9] In principle, we could have used one of these routes to prepare **1a**−**c**, but incorporation of the labels would have necessitated cumbersome modifications unique to each derivative. A more flexible strategy for introducing the remote label[7] at the C(10) carboxyl group and the reporter groups at the site of C−C bond formation (**1b**) or the site of C−O bond cleavage (**1c**) was highly desirable. For this reason, we turned to a chemoenzymatic approach involving the synthesis of appropriately labeled shikimic acids, followed by enzymatic conversion to chorismic acid. Shikimic acid has been prepared in several ways.[10] Of these, we were particularly attracted to the synthesis described by Mirza[11] in which both C(1) and C(7) (corresponding, respectively, to the C(1) and C(10) positions in chorismate) are introduced by a Knoevenagel condensation on a relatively advanced intermediate, allowing preparation of each of the labeled chorismic acids from the same carbohydrate precursor.

Scheme 1



Scheme 2



Thus, starting with aldehyde **2**,[12] Knoevenagel condensation ($TiCl_4$, NMM, THF, −10 to 0 °C)[13] with the commercially available ^{13}C-labeled triethyl phosphonoacetates **3b** or **3c** allowed introduction of ^{13}C labels at either or both the C(1) and C(7) positions (Scheme 2). For the synthesis of [7-^{12}C]shikimate (**6a**), methyl [1-^{12}C]-diethylphosphonoacetate (**3a**) was prepared in 38% yield by carboxylation of diethyl methylphosphonate with $^{12}CO_2$, followed by Fisher esterification (Scheme 1). The moderate yield of the latter sequence is probably the result of in situ anion exchange. Addition of a solution of lithiated diethyl methylphosphonate to an excess of $^{12}CO_2$ in THF did not significantly improve the efficiency of the reaction, however.

Catalytic hydrogenation of vinyl phosphonate **4** with concomitant removal of the benzyl ether, followed by intramolecular Horner−Wadsworth−Emmons reaction, provided 3,4-(isopropylidene)shikimate esters **5a**−**c** in 25−49% from **2** without purification of the intermediates (Scheme 2). Subsequent acid-catalyzed hydrolysis of the isopropylidene acetals with Dowex 50W-X8 resin gave labeled shikimate esters **6a**−**c** in 68−82% yield. The ^{18}O label required for **1c** was introduced by a two-step procedure via 4,5-anhydroshikimate **7**[14] (Scheme 3). Acid-catalyzed epoxide opening with $^{18}OH_2$ is β-selective[14] and provides the doubly labeled shikimate ester **6d** in 74% overall yield.

With shikimates **6a**−**d** in hand, we turned to the enzymatic conversion of shikimate to chorismate. Although this transformation has been described previously using the potentially pathogenic chorismate mutase-

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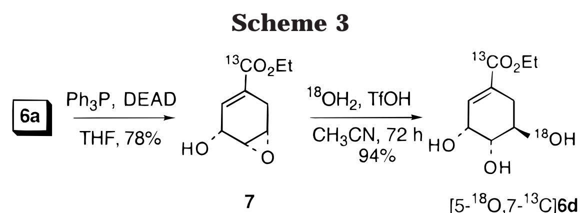
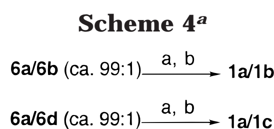
Scheme 3



Scheme 4[a]



[a] Key: (a) NaOH, THF, H_2O; (b) KA12/pKAD50 extract in 50 mM Tris-HCl (pH 8.1) containing PEP, ATP, FMN, KCl, $MgSO_4$, and $Na_2S_2O_4$. Yield over two steps: 49−54%.

deficient *Klebsiella pneumoniae* strain 62-1 (American *Type Culture* Collection no. 25306),[3,15] yields are typically low, and the experiments require a relatively large ratio of cell extract to shikimate for optimal results. We imagined that the yield of chorismate could be greatly improved by overexpressing the genes for the three enzymes on the biosynthetic pathway from shikimate to chorismate in KA12, a previously described chorismate mutase-deficient *Escherichia coli* strain.[16,17] The desired production strain was obtained by transforming KA12 with plasmid pKAD50,[18] which carries the *aroA*, *aroC* and *aroL* genes (encoding EPSP synthase, chorismate synthase, and shikimate kinase, respectively) under the control of the *lac* promoter. Dell and Frost showed previously that introduction of pKAD50 into *E. coli* increases the activities of EPSP synthase, chorismate synthase, and shikimate kinase 4- to 40-fold.[18]

Because the experimental protocol for measuring heavy-atom isotope effects via the remote label method involves a competition between unlabeled and doubly labeled substrates,[7] with the isotopic composition of the remote label [here the C(10) carboxylate of chorismate] set close to natural abundance (^{13}C = 1.1%), chorismates were prepared as isotopic mixtures. Thus, purified **6a** was combined with **6b** or **6d** at a ratio of ca. 99:1. These mixtures were saponified individually, and the crude sodium shikimates were converted to chorismate at 23 °C by incubation with phosphoenolpyruvate (PEP) and cell extracts from KA12/pKAD50 supplemented with ATP, flavin mononucleotide (FMN), magnesium sulfate, and sodium dithionite (Scheme 4).[18] Typical reaction times were 2 h. Under these conditions, labeled chorismic acid could be prepared on a several hundred milligram scale in 49−53% overall yield.

In summary, we have prepared the (doubly) labeled chorismic acids **1a**−**c** via a flexible chemoenzymatic route involving the chemical synthesis of shikimate esters **6a**−**d**, followed by enzymatic conversion to chorismic acid using an engineered chorismate mutase-deficient *E. coli* strain. This route has the advantage that each of the shikimate precursors are prepared from the readily available lyxose **2**, which allows complete control of the isotopic composition of the chorismate mixtures simply by mixing appropriately labeled shikimates prior to the enzymatic step. This is especially noteworthy given the instability of chorismate and in light of the fact that the remote label method for determination of heavy-atom isotope effects requires only trace amounts of the doubly labeled chorismate relative to "unlabeled" **1a** in which the indicator position is depleted in ^{13}C. The use of the engineered KA12/pKAD50 strain provides chorismate in approximately 50% yield from 500 mg of shikimate and represents a significant improvement over previously reported procedures.[3,15] Finally, this route can be easily adapted to the preparation of other doubly labeled chorismate derivatives for investigations of the enzymatic and nonenzymatic reactions. Studies along these lines will be reported in due course.

Experimental Section

All reactions were carried out in flame-dried glassware. THF and Et_2O were dried by distillation from sodium−benzophenone ketyl. CH_2Cl_2 was dried by distillation from CaH_2. All other commercially available reagents and solvents were used as received. High-resolution mass spectral data were recorded by the Scripps Research Institute mass spectrometry laboratory. Chromatographic purifications were performed with Kieselgel 60, 230−400 mesh silica gel. Eluent mixtures are reported as v:v percentages of the minor constituent in the major constituent.

Methyl [1-^{12}C]Diethylphosphonoacetate (3a). Methyl diethylphosphonate (10.0 mL, 68.4 mmol) was added to a −78 °C solution of *n*-BuLi (2.5 M in hexanes, 28 mL, 70 mmol) in THF (300 mL). After the addition was complete, the cooling bath was removed, and the solution was allowed to warm to −20 °C (internal temperature) over 30 min and then recooled to −78 °C. In a separate vessel, THF (75 mL) was degassed under vacuum and sparged with $^{12}CO_2$ (99.99% atom, Aldrich) at −78 °C, until approximately 10 g of $^{12}CO_2$ had been added (estimated gravimetrically). The $^{12}CO_2$ solution was added via cannula to the −78 °C solution of lithiated diethyl methylphosphonate. The resulting white slurry was allowed to warm to −10 °C over 2 h and then concentrated to dryness. The residue was dissolved in H_2O (15 mL) acidified to pH 1 using concentrated HCl and extracted with CH_2Cl_2 (5 × 40 mL). The combined extracts were washed with brine (15 mL), dried over Na_2SO_4, and concentrated. The concentrate was dissolved in MeOH (400 mL) and treated with AcCl (0.50 mL, 7.0 mmol). The mixture was stirred for 17 h, treated with concentrated NH_4OH (0.50 mL), and concentrated to near dryness. The concentrate was partitioned between CH_2Cl_2 (150 mL) and saturated aqueous $NaHCO_3$ (20 mL). The CH_2Cl_2 layer was washed with brine (20 mL), dried over Na_2SO_4, and concentrated. This gave a ca. 1:2.5 mixture of the desired methyl diethylphosphonoacetate and unreacted starting material. Purification of this mixture via careful distillation (45 °C at 0.15 mm Hg and then 92−96 °C at 0.15 mm Hg) gave **3a** (5.47 g, 38%): ^1H NMR (500 MHz, $CDCl_3$) δ 4.16 (apparent quintet, J = 7 Hz, 4 H), 3.73 (s, 3 H), 2.96 (d, J = 21.5 Hz, 2 H), 1.33 (t, J = 7 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 62.7, 52.6, 34.1 (d, J = 130 Hz), 16.3 (d, J = 6.3 Hz).

Methyl 3,4-[1-^{12}C](*O*-Isopropylidene)shikimate (5a). $TiCl_4$ (8.1 mL, 24.7 mmol) was added to THF (150 mL) at 0 °C. The solution was stirred for 5 min before adding a solution of **3a** (8.55 g, 40.7 mmol) and **2** (10.35 g, 37.0 mmol) in THF (75 mL) via cannula. The resulting mixture was stirred for 10 min. Then a solution of *N*-methylmorpholine (NMM) (16.2 mL, 147 mmol) in THF (30 mL) was added dropwise via cannula over a period of 30 min. After the addition was complete, the dark red-brown mixture was stirred for an additional 30 min before saturated aqueous $NaHCO_3$ (150 mL) was added. The resulting mixture was extracted with Et_2O (5 × 300 mL). The combined ethereal extracts were washed with H_2O (3 × 100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated. The crude product was filtered through a pad of silica using 80% EtOAc/hexanes as eluent, and the filtrate was concentrated. The material so

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obtained was dissolved in 0.5% AcOH/MeOH (120 mL) sparged with N_2 and treated with 10% Pd/C (1.96 g, 1.8 mmol). The mixture was shaken in a Parr apparatus under 50 atm of H_2 at 23 °C. After 17 h, an additional portion of 10% Pd/C (1.96 g, 1.85 mmol) was added, and shaking was continued for an additional 17 h. The reaction mixture was filtered through a pad of silica gel over Celite. The filter pad was washed with several portions of MeOH, and the filtrate was concentrated. The residue was dissolved in MeOH (100 mL) and added dropwise over 40 min to a 0 °C solution of Na metal (3.07 g, 134 mmol) dissolved in MeOH (200 mL). The mixture was stirred for an additional 2 h and then poured into saturated aqueous $NaHCO_3$ (350 mL). The aqueous mixture was extracted with Et_2O (5 × 400 mL). The combined extracts were divided into two portions, and each was washed with H_2O (3 × 100 mL) and brine (100 mL). The combined extracts were dried over Na_2SO_4 and concentrated. Purification of the crude product by silica gel chromatography (35% EtOAc/hexanes) gave shikimate **5a** (3.59 g, 43%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (s with fine splitting, 1 H), 4.75 (dd, J = 6.0, 5.0 Hz, 1 H), 4.08 (dd, J = 7.5, 6.5 Hz, 1 H), 3.88 (dt, J = 4.5, 8.5 Hz, 1 H), 3.73 (s, 3 H), 2.81 (dd, J = 17.5, 4.5 Hz, 1 H), 2.55 (s (br), 1 H), 2.23 (ddt, J = 17.5, 8.5, 2 Hz, 1 H), 1.46 (s, 3 H), 1.40 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.9, 130.6, 109.7, 77.9, 72.2, 68.7, 52.1, 29.3, 27.9, 25.7.

Ethyl 3,4-[1,7-^{13}C](*O*-Isopropylidene)shikimate (5b). Compounds **2** (1.35 g, 4.86 mmol) and **3b** (1.21 g, 5.35 mmol) were condensed in the presence of $TiCl_4$ (1.20 mL, 10.9 mmol) and NMM (2.4 mL, 21.8 mmol) as above. Hydrogenation of **4b** over 10% Pd/C (562 mg) in 0.5% AcOH/MeOH (55 mL), cyclization (508 mg Na^0 in 75 mL EtOH), and chromatographic purification gave **5b** (278 mg, 25%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (s (br), 1 H), 4.75 (d (br), J = 4.5 Hz, 1 H), 4.22 (qd, J = 7.0, 3.0 Hz, 2 H), 4.08 (dd, J = 7.5, 6.5 Hz, 1 H), 3.88 (m, 1 H), 2.82 (dt (br), J = 17.0, 3.0 Hz, 1 H), 2.23 (m, 1 H), 2.00 (s (br), 1 H), 1.46 (s, 3 H), 1.41 (s, 3 H), 1.30 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4 (d, J = 73 Hz, 100% ^{13}C), 133.9 (d, J = 66 Hz), 131.3 (d, J = 73 Hz, 100% ^{13}C), 109.9, 78.2 (d, J = 2.5 Hz), 72.4 (dd, J = 6.1, 2.5 Hz), 69.0 (d, J = 3.7 Hz), 61.1, 29.5 (dd, J = 44, 2.4 Hz), 28.0, 25.8, 14.2; HRMS (EI) calcd for $C_{10}{}^{13}C_2H_{18}O_5$ (M^+) 244.1222, found 244.1223.

Ethyl 3,4-[7-^{13}C](*O*-Isopropylidene)shikimate (5c). Compounds **2** (1.35 g, 4.85 mmol) and **3c** (1.20 g, 5.33 mmol) were condensed in the presence of $TiCl_4$ (1.20 mL, 10.9 mmol) and NMM (2.4 mL, 21.8 mmol) as above. Subsequent hydrogenation of **4c** (H_2, 505 mg Pd/C, 45 mL 0.5% AcOH/MeOH) and cyclization (508 mg Na^0 in 75 mL EtOH) yielded 578 mg of **5c** after purification (49%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (s with fine splitting, 1 H), 4.74 (dd, J = 5.0, 4.5 Hz, 1 H), 4.21 (qd, J = 7.5, 3.0 Hz, 2 H), 4.08 (dd, J = 7.5, 6.5 Hz, 1 H), 3.88 (td, J = 8.0, 4.5 Hz, 1 H), 2.80 (ddd, J = 17.0, 4.0, 3.0 Hz, 1H), 2.41 (s (br), 1 H), 2.23 (ddq, J = 17.0, 8.5, 1.5 Hz, 1 H), 1.45 (s, 3 H), 1.40 (s, 3 H), 1.29 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0 (100% ^{13}C), 133.5, 130.9 (d, J = 75 Hz), 109.6, 77.9, 72.2, (d, J = 6.3 Hz), 68.8 (d, J = 5 Hz), 61.0, 29.3, 27.9, 25.7, 14.1; HRMS (FAB, NBA) calcd for $C_{11}{}^{13}CH_{19}O_5$ (M^+ + H) 244.1266, found 244.1273.

Methyl [7-^{12}C]Shikimate (6a). Dowex 50W-X8 acidic ion-exchange resin (4.5 g) was added to a solution of **5a** (2.71 g, 12.0 mmol) in MeOH (300 mL) and H_2O (15 mL). The mixture was stirred at 23 °C for 36 h. The resin was then removed via filtration, and the filtrate was concentrated. Purification of the crude product by silica gel chromatography (10% MeOH/CH_2Cl_2) gave methyl shikimate **6a** (1.85 g, 82%).

Ethyl [1,7-^{13}C]Shikimate (6b). Hydrolysis of **5b** (198 mg, 1.66 mmol) with Dowex 50W-X8 (1.5 g) in 95% EtOH (50 mL) gave 113 mg of ethyl shikimate **6b** (68%) after chromatography: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88 (m, 1 H), 4.46 (s (br), 1 H), 4.21 (qd, J = 7.0, 3.0, 2 H), 3.97 (td, J = 9.0, 5.5 Hz, 1 H), 3.62 (dd, J = 9.0, 4.0 Hz, 1 H), 3.30 (s (br), 1 H), 2.94 (ddd, J = 18.0, 5.5, 2.5 Hz, 1 H), 2.74 (s (br), 1 H), 2.64 (s (br), 1 H), 2.21 (dd with fine splitting, J = 18.0, 8.5 Hz, 1 H), 1.30 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 168.2 (d, J = 75 Hz, 100% ^{13}C), 139.2 (d, J = 75 Hz), 130.4 (d, J = 75 Hz, 100% ^{13}C), 72.6, 68.4, 67.2 (d, J = 6.3 Hz), 61.8, 31.4 (d, J = 43 Hz), 14.5; HRMS (EI) calcd for $C_7{}^{13}C_2H_{14}O_5$ (M^+) 204.0909, found 204.0919.

Ethyl [7-^{13}C]Shikimate (6c). Hydrolysis of **5c** (403 mg, 1.66 mmol) with Dowex 50W-X8 (2.5 g) in 95% EtOH gave **6c** (275 mg, 82%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (s (br), 1 H), 4.47 (t (br), J = 5 Hz, 1 H), 4.22 (qd, J = 7.0, 3.0 Hz, 2 H), 3.98 (td, J = 9.0, 5.5 Hz, 1 H), 3.63 (dd, J = 9.0, 4.5 Hz, 1 H), 2.95 (d (br), J = 17.5 Hz, 1 H), 2.72 (s (br), 1 H), 2.40 (s (br), 1 H), 2.27 (s, (br), 1 H), 2.22 (dd, J = 17.5, 8.5 Hz, 1 H), 1.30 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5 (100% ^{13}C), 136.0, 130.5 (d, J = 75 Hz), 73.0, 66.6, 66.2 (d, J = 6.5 Hz), 61.1, 32.2, 14.1; HRMS (FAB, NBA) calcd for $C_8{}^{13}CH_{14}O_5$ (M^+ + H) 204.0953, found 204.0948.

Ethyl 4,5-[7-^{13}C]Anhydroshikimate (7).[14] DEAD (0.18 mL, 1.1 mmol) was added to a 0 °C solution of **6c** (187 mg, 0.92 mmol) and Ph_3P (286 mg, 1.1 mmol) in THF (7.5 mL). The solution was stirred for 2 h and concentrated, and the residue was heated to 120 °C in a Kugelrohr oven at 0.150 mmHg for 15 min. The volatile components were collected at −78 °C. The distillation flask was cooled, combined with the contents of the receiver flask, and concentrated. Purification of the residue by silica gel chromatography (20% Et_2O/hexanes) gave the epoxyalcohol **7** (132 mg, 78%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.71 (d (br), J = 6.5 Hz, 1 H), 4.56 (s, (br), 1 H), 4.20 (qd, J = 7.0, 3.0 Hz, 2 H), 3.57−3.54 (m, 2 H), 3.02 (d with fine splitting, J = 19.5 Hz, 1 H), 2.48 (dd with fine splitting, J = 19.5, 4.5 Hz, 1 H), 2.20 (s (br), 1 H), 1.29 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1 (100% ^{13}C), 135.7, 126.6 (d, J = 75 Hz), 65.4 (d, J = 6.3 Hz), 60.9, 54.6, 52.1, 24.2, 14.1; HRMS (FAB, NBA) calcd for $C_8{}^{13}CH_{12}O_4Na$ (M^+ + Na) 208.0667, found 208.0674.

Ethyl [5-^{18}O,7-^{13}C]Shikimate (6d). Trifluoromethanesulfonic acid (15 mL, 0.17 mmol) was added to a solution of **7** (52 mg, 0.28 mmol) in freshly distilled MeCN (0.80 mL) and ^{18}O-labeled H_2O (99% atom, Cambridge Isotopes) (0.30 mL). After stirring for 48 h, the resulting mixture was concentrated in vacuo. Purification of the residue by silica gel chromatography gave the doubly labeled ethyl shikimate **6d** (55 mg, 95%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (m, 1 H), 4.47 (t, J = 4.5 Hz, 1 H), 4.22 (qd, J = 7.0, 2.5 Hz, 2 H), 3.97 (dt, J = 9.0, 5.5 Hz, 1 H), 3.62 (dd, J = 9.0, 4.5 Hz, 1 H), 2.95 (dd with fine splitting, J = 18.0, 3.0 Hz, 1 H), 2.81 (s (br), 1 H), 2.52 (s (br), 1 H), 2.39 (s (br), 1 H), 2.20 (dd with fine splitting, J = 18.0, 8.5 Hz, 1 H), 1.30 (t, J = 7.0 Hz, 3 H); HRMS (FAB, NBA) calcd for $C_8{}^{13}CH_{14}O_4{}^{18}O$ (M^+ + H) 206.0996, found 206.0989.

Preparation of the Crude Cell Extract from *E. coli* Strain KA12/pKAD50. Miller's LB broth (100 mL) containing tryptophan (4 mg) was inoculated with a single colony of *E. coli* strain KA12 transformed with plasmid pKAD50.[18] The culture was shaken at 37 °C and 250 rpm for 24 h. Six 2-L Erlenmeyer flasks were each charged with Miller's LB broth (1 L) and Trp (20 mg/L) and sterilized by autoclaving. After cooling, each flask was provided with a solution of IPTG in EtOH (0.5 M, 0.4 mL) and a solution of chloramphenicol in EtOH (30 mg/mL, 2.0 mL). Finally, each flask was inoculated with 15 mL of the above prepared KA12/pKAD50 culture. The broth was shaken at 37 °C and 250 rpm for 24 h. After cooling to 23 °C, the cells were collected by centrifugation at 5000 rpm (4200 × *g*). The cell pellet was resuspended in Tris-HCl (50 mM, pH 8.2) containing dithiothreitol (DTT, 10 mM). The cells were again collected by centrifugation at 5000 rpm (4200 × *g*) to give 30 g of cells. The cell pellet so obtained was resuspended in Tris-HCl (100 mL, 50 mM, pH 8.2) containing DTT (10 mM), and the cells were ruptured by passing them through a French Press (three times). Cell debris was removed from the lysate by centrifugation at 14 500 rpm (30 000 × *g*). The supernatant was dialyzed against Tris-HCl (2 L, 50 mM, pH 8.2) containing DTT (10 mM) and phenylmethanesulfonyl fluoride (50 mg) for 8 h and then dialyzed 3 × 17 h against Tris-HCl (2 L, 50 mM, pH 8.2) containing DTT (10 mM). The crude cell extracts were stored at 4 °C prior to use. *Note: best results in the following preparations of chorismate were obtained with freshly prepared cell extract.*

Mixture of [10-^{12}C]Chorismic Acid (1a) and [5-^{18}O,10-^{13}C]-Chorismic Acid (1c). NaOH (1 M, 2.8 mL, 2.8 mmol) was added to a solution of **6a** (6.2 mg, 0.030 mmol) and **6d** (514 mg, 2.73 mmol) in a 1:1 mixture of THF and H_2O (41 mL). The mixture was stirred at 23 °C for 8 h. After the removal of THF by distillation in vacuo, the aqueous mixture was lyophilized. ATP (monosodium salt) (2.29 g, 4.14 mmol) was carefully dissolved in Tris-HCl (80 mL, 50 mM) while the pH of the solution was maintained between 6.5 and 9.0. To this solution was added FMN (142 mg, 0.276 mmol), phosphoenolpyruvate (PEP, tripo-

tassium salt) (1.95 g, 8.33 mmol), a solution of KCl (1.0 M aqueous, 6.9 mL), a solution of $MgSO_4$ (1.0 M aqueous, 0.69 mL), and a solution of the sodium shikimates (obtained above) in Tris-HCl (15 mL, 50 mM, pH 8.2). KA12/pKAD50 extract (35 mL, corresponding to the lysate of ca. 10 g of cells, estimated total protein concentration ca. 40 mg/mL) was added, and the pH of the resulting mixture was adjusted to 8.1 using HCl. Finally, solid sodium dithionite (272 mg, 1.56 mmol) was added. The resulting solution was sealed and gently stirred at 23 °C for 2.5 h. The reaction mixture was then filtered through an Amicon 10K cutoff ultrafiltration membrane at 4 °C. When nearly dry, the membrane was washed with Tris-HCl (100 mL, 50 mM, pH 8.2). The combined filtrate was acidified to pH 1.5 and extracted with EtOAc (6 × 150 mL). The combined extracts were dried over Na_2SO_4 and carefully concentrated in vacuo (while the bath for the rotary evaporator was maintained at ca. 20 °C). The residue was dissolved in a 1:1 mixture of H_2O and MeOH and filtered through a pad of charcoal. The filtrate was lyophilized to provide the mixture of chorismic acids **1a** and **1c** (310 mg, 49%), containing only minor impurities as judged by HPLC and ^1H and ^{13}C NMR (see Supporting Information for representative data). This material can be further purified by C18 reverse phase silica gel flash chromatography[17] as needed.

Mixture of [10-^{12}C]Chorismic Acid (1a) and [1,10-^{13}C]-Chorismic Acid (1b). A mixture of shikimate esters **6a** (620 mg, 3.29 mmol) and **6b** (10.8 mg, 0.052 mmol) were saponified in 1:1 THF/H_2O (41 mL) as described for the production of the chorismic acid mixture **1a** and **1c**. A solution of ATP (2.76 g, 5.01 mmol), FMN (162 mg, 0.334 mmol), PEP (2.35 g, 10.0 mmol), KCl (1.0 M aqueous, 8.4 mL), and $MgSO_4$ (1.0 M aqueous, 0.84 mL) was prepared in Tris-HCl (100 mL, 50 mM, pH 8.2) as described above. The solution of sodium shikimates in Tris-HCl (25 mL, 50 mM, pH 8.2) was then added. The crude cell extract of KA12/pKAD50 (35 mL) was added, and the pH was adjusted to 8.1. Finally, solid $Na_2S_2O_4$ (321 mg, 1.84 mmol) was added, and the reaction mixture was sealed and gently stirred at 23 °C for 2.5 h before being processed as described above to give the chorismic acid mixture **1a** and **1b** (403 mg, 53%).

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **3a**, **5c**, **6c**, **7**, and a representative **1a**/**1b** mixture. This information is free of charge via the Internet at http://pubs.acs.org.

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