Five-Membered Ring Analogues of Shikimic Acid

Ming An, Tab Toochinda, and Paul A. Bartlett*

Center for New Directions in Organic Synthesis, †Department of Chemistry, University of California, Berkeley, California 94720-1460

paul@fire.cchem.berkeley.edu

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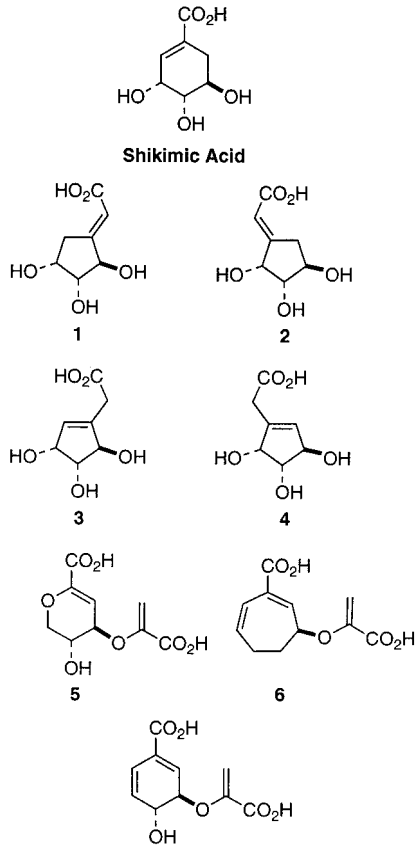
Shikimate and other intermediates of the shikimate−chorismate pathway are densely functionalized structures that seem to offer limited options for skeletal modification. We designed and synthesized cyclopentylidenes **1** and **2**, as well as cyclopentenes **3** and **4**, as novel ring-contracted analogues of shikimic acid. Enzymatic studies showed that analogues **1**−**3** are indeed processed by shikimate kinase to give phosphates **1-P**, **2-P**, and **3-P** as five-membered ring analogues of shikimate-3-phosphate. In particular, analogue **1** is converted by the enzyme at a rate only 3.5-fold slower than that of the native substrate, while analogue **3** binds to shikimate kinase with an apparent K_m of 1.7 mM, compared to 0.14 mM for shikimate.

Introduction

The shikimate−chorismate pathway constitutes the initial stage of the biosynthesis of aromatic compounds.[1] The enzymes in this pathway are unique to plants and some microorganisms, and thus they are important targets for herbicide and antibiotic development.[2] Shikimate and other substrates of these enzymes are small, densely functionalized molecules that pose unique synthetic challenges for developing enzyme inhibitors. One notable difficulty is the tendency of the native cyclohexene-based structures to aromatize.[3] Therefore, we became interested in altered ring templates that are less prone to aromatization yet are still accepted by the enzymes in the shikimate−chorismate pathway. Such novel skeletons may potentially provide a basis for the design of more synthetically accessible inhibitors. Furthermore, equally intriguing to us is the prospect of mimicking the cyclohexene-based intermediates with ring altered analogues.

Berchtold and co-workers have studied substrate analogues of chorismate in which the ring skeleton is altered electronically, as in dihydropyran **5**,[4] or structurally, as in cycloheptadiene **6**.[5] The ring-expanded analogue **6** is not processed by chorismate mutase, anthranilate synthase, or *p*-aminobenzoate synthase; however, it is a competitive inhibitor of the reactions they catalyze.[5]



We proposed cyclopentylideneacetates **1** and **2**, as well as the cyclopentenyl structures **3** and **4**, as ring-contracted analogues of shikimic acid. Replacing the cyclohexene template of shikimate with a five-membered ring unit represents a drastic but calculated change in structure. For example, the low energy conformers of shikimate and the cyclopentylidene analogues **1** and **2** can be superimposed by their hydroxyl oxygens and carboxyl carbon with rms differences of only 0.42 and 0.26 Å, respectively (Figure 1a).[6] The electrostatic surfaces of these three molecules also resemble each other closely

* To whom correspondence should be addressed.

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(1) Reviews: (a) Haslam, E. *Shikimic Acid Metabolism and Metabolites*; Wiley: New York, 1993. (b) Weiss, U.; Edwards, J. M. *The Biosynthesis of Aromatic Compounds*; Wiley-Interscience: New York, 1987; pp 260−270.

(2) (a) Sikorski, J. A.; Gruys, K. J. *Acc. Chem. Res.* **1997**, *30*, 2−8. (b) Roberts, F.; Roberts, C. W.; Johnson, J. J.; Kyle, D. E.; Krell, T.; Coggins, J. R.; Coombs, G. H.; Milhous, W. K.; Tzipori, S.; Ferguson, D. J. P.; Chakrabarti, D.; McLeod, R. *Nature* **1998**, *393*, 801−805.

(3) For a recent example, see: Kozlowski, M. C.; Tom, N. J.; Seto, C. T.; Sefler, A. M.; Bartlett, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2128−2140.

(4) Delany, J. J., III.; Padykula, R. E.; Berchtold, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 1394−1397.

(5) Pawlak, J. L.; Berchtold, G. A. *J. Org. Chem.* **1988**, *53*, 4063−4069.

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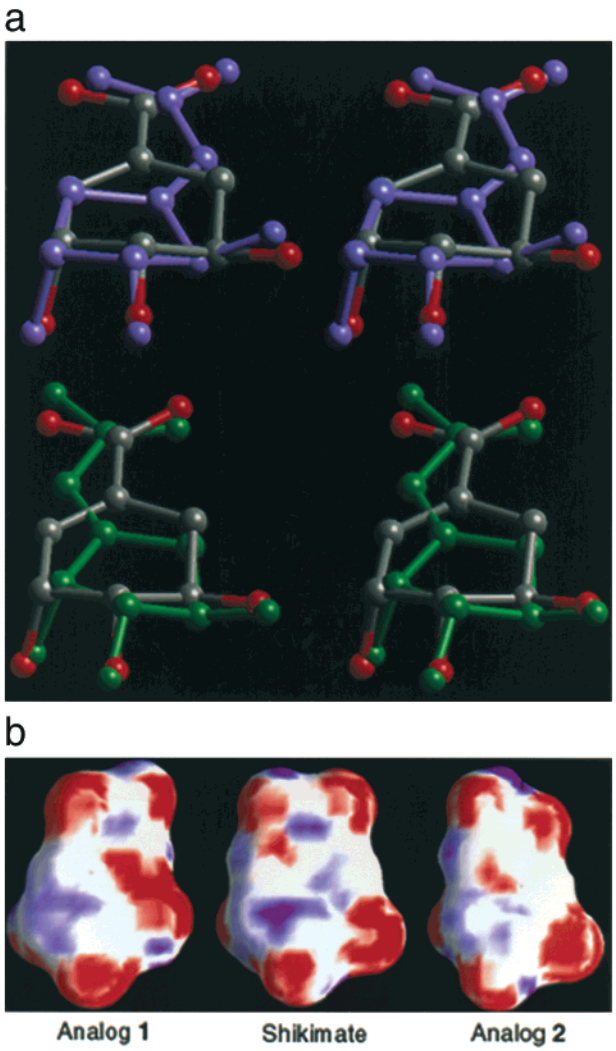


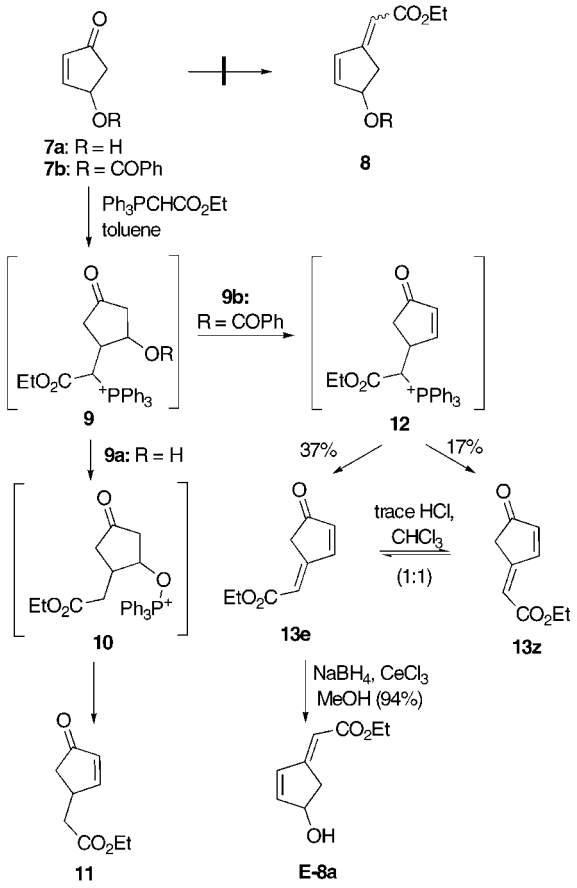
Figure 1. (a) Superpositions of shikimate (shown in gray and red) and analogues **1** (blue, top stereopair) and **2** (green); (b) comparison of electrostatic surfaces.

(Figure 1b).[7] We designed the two cyclopentylidenes **1** and **2** to best mimic the low energy conformation of shikimate while allowing cyclopentenes **3** and **4** to retain some degree of flexibility. In this study, we describe the syntheses of analogues **1**−**4** and their enzymatic evaluations as alternative substrates for shikimate kinase.

Results

Syntheses of Analogues 1−4. In the most straightforward fashion, we planned to build the cyclopentylideneacetate skeleton in the target structures **1** and **2** from a direct Wittig-type olefination of the known prostaglandin intermediate 4-hydroxycyclopent-2-en-1-one **7a**.[8] However, treatment of cyclopentenone **7a** with carbethoxytriphenylphosphorane[9] under various conditions gave the

Scheme 1



undesirable dihydro derivative **11** as the major product (20−30% yield), with the desired cyclopentenylidene **8a** isolated in low yield (5−13%) as an approximate 1:1 *E*/*Z* mixture (Scheme 1).[10] Similar results were obtained using trimethyl phosphonoacetate instead of the phosphorane ylide (not shown). Formation of the dihydro compound **11** presumably begins with 1,4-addition to the enone (**7a** → **9a**), which is precedented for reaction of both phosphonate and phosphorane ylides.[11] Intramolecular transfer of the phosphorus moiety (**9a** → **10**) then activates the hydroxyl group for *β*-elimination (**10** → **11**).[12]

However, when phosphorus transfer is prevented by protection of the hydroxyl group as the benzoate ester in **7b**, treatment with the phosphorane ylide affords another unexpected product: the fully conjugated 4-oxo-2-cyclopentenylidene ester **13**, as a 2:1 mixture of *E*/*Z* isomers

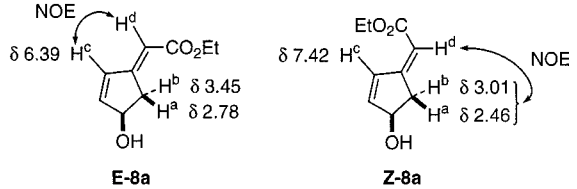
(6) Structures were minimized with MacroModel 7.0's MMFF94s forcefield (Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.) and superimposed using the program Cerius[2] 4.0.

(7) Charges were calculated with MacroModel 7.0's MMFF94s forcefield and displayed with the program GRASP (A. Nicholls and B. Honig, Columbia University).

(8) Racemic enone **7a** was prepared from 2-methylfuran in 39% yield over two steps (see Experimental Section for details).

(9) The ylide was prepared from triphenyl phosphine and ethyl bromoacetate in 84% yield over 2 steps, see: Denney, D. B.; Ross, S. T. *J. Org. Chem.* **1962**, *27*, 998−1000.

(10) The stereochemistry of the exocyclic double bond in **8a** was determined from ^{1}H NMR analysis and NOE difference NMR experiments. NOE's were observed from H^d to H^c in the *E* isomer and from H^d to H^a/H^b in the *Z* isomer. The chemical shift of the vinyl proton H^c in the *Z* isomer was found to be 1.03 ppm downfield from that in the *E* isomer as a consequence of the magnetic anisotropy of the cis ester carbonyl group. A similar anisotropic effect was observed for the ring methylene protons H^a and H^b in the *E* isomer.



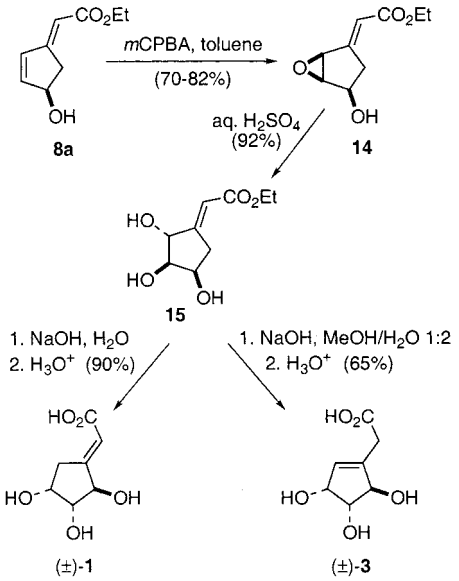
(11) (a) Freeman, J. P. *J. Org. Chem.* **1966**, *31*, 538−541. (b) Bergmann, E. D.; Sulomonovici, A. *Tetrahedron* **1971**, *27*, 2675−2678. (c) Wadsworth, W. S., Jr. *Org. React.* **1977**, *25*, 73.

in 54% yield.[13] In this instance, 1,4-addition (**7b** → **9b**) is followed by tandem β-eliminations (**9b** → **12** → **13**). Indeed, triphenylphosphine was isolated from reaction of benzoate **7b**, whereas triphenylphosphine oxide was the byproduct from reaction of alcohol **7a**.

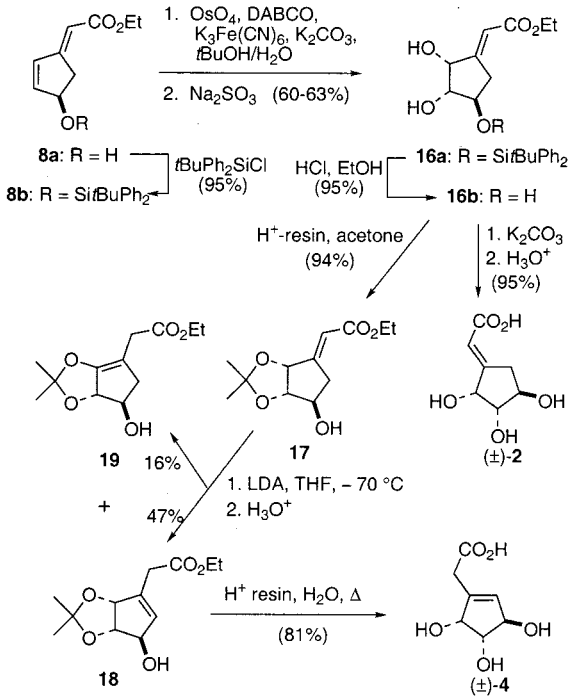
Although our original Wittig strategy was based on literature precedents,[14] we had not anticipated the roles that oxygen substituents at the 4-position would play. Nevertheless, enone **13** proved to be a useful product, affording the key intermediate **8a** via Luche reduction.[15] This alternative sequence proved to be advantageous in several ways: it affords **8a** in better overall yield than the Wittig reactions and in a more favorable *E*/*Z* ratio. Furthermore, the unwanted *Z* isomer **13z** can be isomerized to the desired *E* isomer in the presence of a trace amount of acid. Although this isomerization reaches equilibrium at 1:1 *E*/*Z* ratio, the *E* and *Z* isomers of enone **13** are readily separated by column chromatography, and nearly all of **13z** can be converted to **13e** by recycling the unwanted isomer. As a result, 4-hydroxycyclopentenone **7a** can be converted to the stereochemically pure *E* isomer of cyclopentenylidene **8a** in four steps in >40% overall yield on a gram scale. In contrast, no such isomerization is observed for the reduced product **8a** itself, and the separation of the *E*/*Z* isomers of compound **8a** proved to be difficult. Enone ester **13** can also be prepared from a direct olefination of 4-cyclopentene-1,3-dione using the phosphorane ylide, but only in poor yield (7−10%).

Hydroxy ester **8a** served as the key intermediate in synthesis of all the analogues. En route to **1** and **3**, epoxidation of the endocyclic double bond with *m*-CPBA gives the cis product **14** in 70−82% yield as a single diastereomer (Scheme 2). Treatment of **14** with aqueous sulfuric acid opens the epoxide at the allylic position to afford triol ester **15** in 92% yield. Both cyclopentylidene analogue **1** and cyclopentene analogue **3** can be obtained from triol ester **15** in a single step under basic conditions. When the saponification is carried out in water, analogue **(±)-1** is isolated as the only product in 90% yield. Alternatively, when a 2:1 water/methanol mixture is used as the solvent, isomerization competes with hydrolysis to give the cyclopentene analogue **(±)-3** as the major product. The extent of cyclopentylidene to cyclopentene isomerization seems to increase with the content of methanol in the solvent mixture up to the 2:1 ratio: a higher methanol content, and thus a higher proportion of methoxide, leads to side reactions that lower the combined yield of analogues **1** and **3**, while lower methanol content gives more of analogue **1**.

Scheme 2



Scheme 3



To obtain the stereoisomeric analogues **2** and **4**, allylic alcohol **8a** is first converted to the *tert*-butyldiphenylsilyl ether **8b**, then dihydroxylated selectively from the less hindered side using a catalytic amount of OsO_4 (Scheme 3). The desired diol **16a** is isolated as the predominant diastereomer in 60−63% yield. The diastereomeric excess for this substrate-directed dihydroxylation is >92%, since the all-cis diastereomer is isolated in only 1−3% yield. This reaction must be carried out under the Sharpless biphasic conditions if the desired regioselectivity (ring double bond vs the exocyclic unsaturation) is to be obtained and overoxidation avoided.[16] Subsequently,

(12) In fact, enolate β-addition to a 4-acyloxycyclopentenone followed by β'-elimination of the carboxylate group is a known method to generate 4-substituted cyclopentenones. (see: (a) Koksal, Y.; Osterthun, V.; Winterfeldt, E. *Liebigs Ann. Chem.* **1979**, 1300−1308. (b) Harre, M.; Raddatz, P.; Walenta, R.; Winterfeldt, E. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 480−492. (c) West, F. G.; Gunawardena, G. U. *J. Org. Chem.* **1993**, *58*, 5043−5044.) We are not aware of an instance in which the incoming nucleophile provides the activation for β'-elimination of a hydroxyl substituent, as occurs in the phosphorus transfer steps (cf. **9a** → **10**).

(13) Similar transformations from 4-acetoxycyclopentenone to 4-oxo-2-cyclopentenylidene products have been reported with sulfoxide and sulfone ylides, albeit with yields of 2% (see ref 12a) and 24% (see: Ito, M.; Kodama, A.; Hiroshima, T.; Tsukida, K. *J. Chem. Soc., Perkin Trans. 1.* **1986**, 905−907.), respectively. To our knowledge, the reaction of **7b** → **13** is the first example involving a phosphorane ylide.

(14) For examples involving phosphorous ylides, see: (a) Nakayama, M.; Ohira, S.; Shinke, S.; Matsushita, Y.; Matsuo, A.; Hayashi, S. *Chem. Lett.* **1979**, 1245−1246. (b) Tanaka, K.; Uchiyama, F.; Ikeda, T.; Inubushi, Y. *Chem. Pharm. Bull.* **1983**, *31*, 8−1971. (c) Novak, L.; Rohaly, J.; Galik, G.; Fekete, J.; Varjas, L.; Szantay, C. *Liebigs Ann. Chem.* **1986**, 509−524.

(15) Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.

(16) Kolb, H. C.; Vannieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.

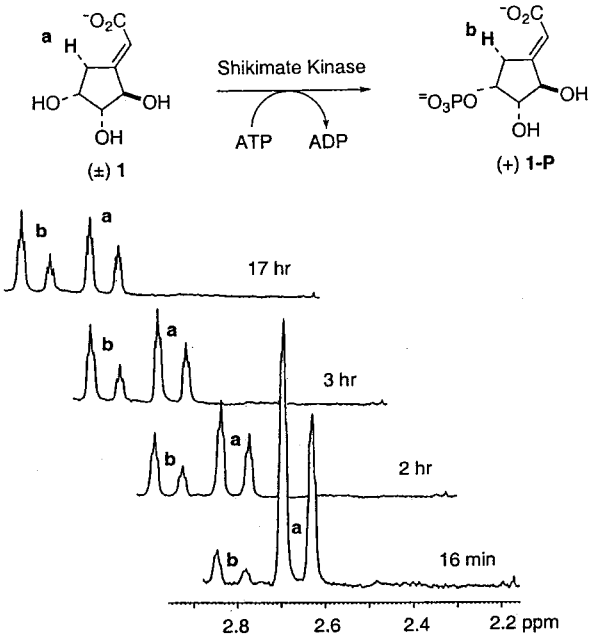


Figure 2. Enzymatic resolution of analogue **1**, as monitored by ^{1}H NMR.

when diol **16a** is stirred in 5 M ethanolic HCl, the silyl group is removed smoothly to afford the triol ester **16b**, which is then hydrolyzed in aqueous K_2CO_3 to give the cyclopentylidene analogue **(±)-2** in 90% yield over two steps.

Isomerizing the exocyclic double bond in ester **16b** into the thermodynamically favored[17] endocyclic position would provide analogue **4** upon hydrolysis (cf. **15** → **3**). However, in contrast to the ease with which this isomerization can be accomplished with the stereoisomer **15**, all attempts to do so with **16b** in various alkaline aqueous alcohol solutions were unsuccessful. In all cases, the cyclopentylidene **2** was isolated as the only product. These results were somewhat perplexing, since triol **16b** differs from triol **15** only in the configuration of the middle hydroxyl group.

An alternative route was devised to force the cyclopentylidene to cyclopentene isomerization (Scheme 3). The cis diol in compound **16b** is protected to give acetonide **17**, which is then deprotonated with excess LDA to generate the alkoxide/ester−enolate dianion. On acidification of the reaction mixture, α-protonation gives the desired cyclopentenyl acetonide **18** in 47% yield, along with enol ether **19** in 16% yield. Finally, heating a mixture of acetonide **18** and sulfonic acid resin in water removes all protecting groups to afford analogue **(±)-4** in 81% yield.

Enzymatic Evaluations of Racemic Analogues 1−4. Shikimate kinase (SK) phosphorylates the 3-hydroxyl group of shikimate selectively to give shikimate-3-phosphate (S-3-P) at the expenditure of one equivalent of ATP.[1] When racemic analogues **1**−**4** were treated with type II SK (EC 2.7.1.71) from *Escherichia coli* K12, monitoring of these enzymatic reactions by ^{1}H NMR immediately revealed three important results (for example, see Figure 2): first, cyclopentylidenes **1** and **2**, as well as cyclopentene **3**, are phosphorylated by SK to give phosphates **1-P**, **2-P**, and **3-P**, respectively, as ring contracted analogues of S-3-P; second, the reactions for racemates **1**−**3** are complete at 50% conversion, reflecting clean enzymatic resolution of these alternative substrates (Figure 3);[18] and third, no phosphorylation is detected for analogue **4** after 100 days of incubation with SK.

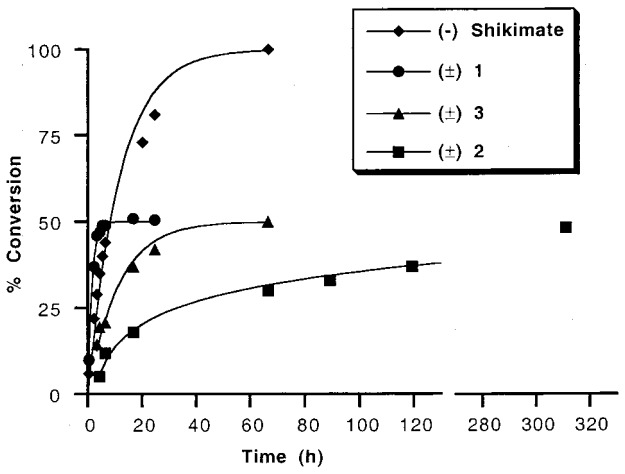
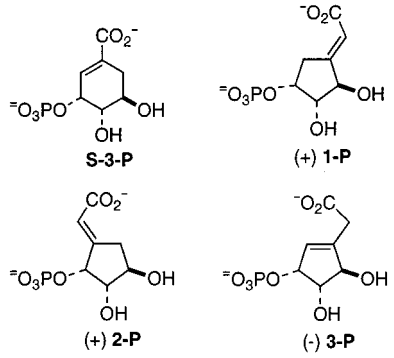


Figure 3. Conversion of shikimate and analogues **1**−**3** to phosphorylated products.



To isolate the phosphorylated products **1-P**, **2-P**, and **3-P**, the enzymatic reactions were carried out on 30−60 mg scale. After SK-catalyzed phosphorylation, reaction mixtures were treated with apyrase to degrade all residual ATP and byproduct ADP to AMP and inorganic phosphate. Subsequent anion exchange chromatography gave the phosphates as the triethylammonium salts, which were further purified via reverse phase HPLC to afford enantiomerically pure phosphates **1-P**, **2-P**, and **3-P** in 79%, 78%, and 88%[19] yields, respectively.

Steady-State Enzyme Kinetics Assays. Analogues **1**−**3** were further evaluated in steady-state kinetics assays. *E. coli* SK was assayed in the forward direction at 25 °C by coupling the release of ADP to the reactions catalyzed by pyruvate kinase (ADP + phosphoenolpyruvate → ATP + pyruvate) and lactate dehydrogenase (pyruvate + NADH → lactate + NAD^+).[20] This phospho-

(17) Allinger, N. L.; Dodziuk, H.; Rogers, D. W.; Naik, S. N. *Tetrahedron* **1982**, *38*, 1593.

(18) The data points in Figure 3 were obtained from SK-catalyzed phosphorylations carried out with 0.1 mmol of the racemic analogues **1**−**3** and 0.05 mmol of (−)-shikimate at 50 mM [substrate], 50 mM [ATP], and 16 mM [Mg^{2+}], using 2 units of SK (pH 7.1−7.4).

(19) The yield for phosphate **3-P** is that of the triethylammonium salt after anion exchange.

(20) Kinetics assays were performed according to the reported conditions except that the pH was changed to 7.8, see: Millar, G.; Lewendon, A.; Hunter, M. G.; Coggins, J. R. *Biochem. J.* **1986**, *237*, 427.

Table 1. Kinetic Parameters for Shikimate and Analogues 1 and 3

substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$) × 10^3
shikimic acid	0.14 ± 0.02	8.23	58.0
1	11 ± 1	2.41	0.214
3	1.7 ± 0.2	0.238	0.144

rylation-dependent oxidation of NADH (A_{340} = 6180 M^{-1} cm^{-1}) is monitored by following the decrease in absorbance at 340 nm. The apparent k_{cat} and K_m values for analogues **1** and **3** are compared with those of the native substrate shikimate in Table 1.[21] SK-catalyzed phosphorylation of cyclopentylidene **2** is too slow to be characterized kinetically even though, from the modeling point of view, it appeared to be the most faithful mimic of the low energy conformer of shikimate.

At 2.5 mM ATP and pH 7.8, the apparent K_m for shikimate was found to be 140 μM, compared to the reported value of 200 μM at 5 mM ATP.[22] Under the same conditions, cyclopentenyl analogue **3** binds to SK with an apparent K_m of 1.7 mM, versus 11 mM for cyclopentylidene analogue **1**. The k_{cat} values for analogues **1** and **3** are 2.41 s^{-1} and 0.238 s^{-1}, respectively, compared to that of 8.23 s^{-1} for the native substrate. Hence, the cyclopentenyl analogue **3** binds to SK with 6-fold higher affinity than does the cyclopentylidene analogue **1**, while the latter is turned over 10 times faster, resulting in similar k_{cat}/K_m values for both ring-contracted analogues.

Discussion

We designed these five-membered ring analogues of shikimate in the absence of any knowledge of the structure of substrate-bound SK.[23] Our design adopted two divergent approaches: first, constraint of cyclopentylidenes **1** and **2** to best mimic the low energy conformation of the native substrate; second, retention of flexibility in cyclopentenyl analogues **3** and **4**. In the event, we obtained two alternative substrates of equal efficacy (similar k_{cat}/K_m values for **1** and **3**) but with considerably different characteristics.

Cyclopentenyl analogue **3** binds to SK with the highest affinity among the ring-contracted analogues. This result is interesting considering the fact that the acetate sidechain in cyclopentene **3** would have to adopt a high energy, ring-eclipsed conformation to mimic the native substrate. However, tighter ground-state binding is not maintained at the transition state in this case, since k_{cat} for analogue **3** is relatively poor. It is the cyclopentylidene **1**, which has a lower ground-state affinity, that forms the most productive enzyme complex among all analogues. In fact, at 50 mM ATP and saturating substrate concentrations (50 mM), analogue **1** is turned over even faster than shikimate itself (see Figure 3).[24]

In addition to analogues **1** and **3**, cyclopentylidene **2** is also phosphorylated by SK, although it is bound more weakly and turned over more slowly. These analogues represent the first examples of alternative substrates for the shikimate−chorismate pathway in which the ring size has been altered. Moreover, their activity in the SK-catalyzed phosphorylation reaction demonstrates the first step in establishing an unnatural "biosynthetic" sequence based on these five-membered ring analogues. The next enzyme in the pathway, EPSP synthase, catalyzes the reaction of S-3-P with phosphoenolpyruvate to give 5-enolpyruvylshikimate-3-phosphate (EPSP).[1] It will be interesting to see if the ring-contracted S-3-P analogues **1-P**, **2-P**, and **3-P** can serve as alternative substrates of EPSP synthase as well.

Experimental Section

General. Unless otherwise noted, chemicals were obtained from commercial sources and were used without further purification. Chromatography employed 60-mesh silica gel from E. Merck & Co. and was performed according to the method of Still, Kahn, and Mitra.[25] Unless otherwise specified, $CDCl_3$ was the NMR solvent. NMR chemical shifts are referenced as follows, depending on solvent. ^{1}H: to internal TMS or residual $CHCl_3$ (7.27 ppm), H_2O (4.80 ppm), or CD_2-HOD (3.31 ppm). ^{13}C: $CDCl_3$ (77.23 ppm) or MeOH-d_4 (49.15 ppm). ^{31}P: internal trimethyl phosphate (3.086 ppm).

(±)-(*E*)-[2(R*),3(R*),4(R*)]-Trihydroxycyclopentylideneacetic Acid (1). A mixture of 71 mg (0.35 mmol) of triol ester **15**, described below, and 20 mL of 0.5 M aq NaOH (10 mmol, 29 equiv) was stirred at room temperature for 1 h, then acidified with 1 M aq HCl. After lyophilization to remove water, the residue was chromatographed (7:1:1:1 EtOAc/MeOH/dH_2O/acetone) to afford 55 mg (90%) of triol acid **1**. ^{1}H NMR (MeOH-d_4) δ 2.74−2.90 (m, 1), 2.97−3.13 (m, 1), 3.67 (dd, 1, J = 4.4, 8.8), 4.08 (t, 1, J = 4.3), 4.50 (td, 1, J = 2.3, 8.9), 5.93−5.97 (m, 1). ^{13}C NMR (MeOH-d_4) δ 37.8, 70.4, 78.5, 79.2, 116.2, 164.4, 170.3. IR (neat) 3361 (vbr), 2926, 1695, 1405, 1332, 1217, 1119, 1038, 875 cm^{-1}. HRMS (FAB+) MH^+ Calcd. for $C_7H_{11}O_5$: 175.0606. Found: 175.0605.

(±)-(*E*)-[2(R*),3(S*),4(R*)]-Trihydroxycyclopentylideneacetic Acid (2). A solution of 45 mg (0.22 mmol) of triol ester **16b** (described below) and 304 mg (2.2 mmol, 9.9 equiv) of K_2CO_3 in 5 mL of water was stirred at 60 °C for 25 h. The reaction mixture was acidified with 5 mL of 1 M aq HCl and lyophilized, and the residue was chromatographed (7:1:1:1 EtOAc/MeOH/dH_2O/acetone) to give 37 mg (95%) of triol acid **(±)-2**. ^{1}H NMR (MeOH-d_4) δ 2.62−2.71 (m, 1), 3.17−3.27 (m, 1), 3.83−3.88 (m, 1), 4.06−4.11 (m, 1), 4.62−4.66 (m, 1), 5.95−5.98 (m, 1). ^{13}C NMR (MeOH-d_4) δ 38.4, 74.3, 76.4, 77.9, 116.3, 165.9, 170.5. IR (neat) 3333 (vbr), 2929, 1699, 1408, 1218, 1112, 1046 cm^{-1}. HRMS (FAB−) $(M - H^+)^-$ Calcd. for $C_7H_9O_5$: 173.0450. Found: 173.0454.

(±)-[3(R*),4(R*),5(R*)]-Trihydroxy-1-cyclopenteneacetic Acid (3). To a solution of 25 mg (0.12 mmol) of triol ester **15** (described below) in 1 mL MeOH were added 2 mL of 1 M aq NaOH (2 mmol, 16 equiv). The reaction mixture was stirred at room temperature for 10 min, acidified with 1 M aq HCl, and lyophilized. The residue contained 14 mg (65%) of cyclopentene acid **3** and 5 mg (23%) of cyclopentylidene acid **1**, which were separated via chromatography (7:1:1:1 EtOAc/MeOH/dH_2O/acetone; spontaneous acetonide formation on the column was sometimes observed for compound **3**). ^{1}H NMR (MeOH-d_4) δ 3.08 (s, 2), 3.89 (t, 1, J = 5.3), 4.43−4.47 (m, 1), 4.57−4.60 (m, 1), 5.73−5.76 (m, 1). ^{13}C NMR (MeOH-d_4) δ 38.2, 73.2, 80.2, 81.6, 129.5, 146.5, 179.4. IR (neat) 3324 (vbr), 2922, 1574, 1391, 1091 cm^{-1}. HRMS (FAB−) $(M - H^+)^-$ Calcd. for $C_7H_9O_5$: 173.0450. Found: 173.0444.

(±)-[3(R*),4(S*),5(R*)]-Trihydroxy-1-cyclopenteneacetic Acid (4). A mixture of 12 mg (0.05 mmol) of protected triol ester **18** (described below), 200 mg of Bio-Rad AG 50W-X4

(21) All steady-state enzyme kinetics parameters were calculated using the EnzymeKinetics (v1.11) program and the reported molecular weight of 18 937[20] for *E. coli* K12 *aroL* SK II.

(22) Feyter, R. D.; Pittard, J. *J. Bacteriol.* **1986**, *165*, 331.

(23) A crystal structure of SK from *Erwinia chrysanthemi* in complex with shikimate was reported in 1998, see: Krell, T.; Coggins, J. R.; Lapthorn, A. J. *J. Mol. Biol.* **1998**, *278*, 983−997. This SK is also type II and shares 53% sequence analogy with *E. coli* SKII. However, there was not enough electron density to allow an unambiguous positioning of shikimate in the active site.

(24) It is known that SK activity is inhibited by high levels of shikimate, see: Feyter, R. D. *Methods Enzymol.* **1987**, *142*, 355−361.

(25) Still, W. C.; Kahn, M.; Mitra, S. *J. Org. Chem.* **1978**, *43*, 2923−2925.

resin, and 4 mL of water was stirred at 95 °C for 3 h. The reaction mixture was filtered, and the aqueous solution was lyophilized to give a colorless residue, which was chromatographed (7:1:1:1 → 6:2:1.25:1 EtOAc/MeOH/dH_2O/acetone) to afford 7 mg (81%) of the triol acid **(±)-4**. ^{1}H NMR (MeOH-d_4) δ 3.08 (d, 1, J = 15.8), 3.17 (d, 1, J = 15.8), 3.86 (dd, 1, J = 3.8, 5.5), 4.52 (d, 1, J = 5.5), 4.56 (s, 1), 5.62 (s, 1). ^{13}C NMR (MeOH-d_4) δ 40.2, 77.0, 80.8, 81.8, 132.7, 143.9, 179.3. IR (neat) 3297 (vbr), 2919, 1574, 1389, 1111 cm^{-1}. HRMS (FAB−) $(M - H^+)^-$ Calcd. for $C_7H_9O_5$: 173.0450. Found: 173.0449.

(+)-(*E*)-*O*-4-Phospho-[2(R),3(S),4(R)]-trihydroxycyclopentylideneacetic Acid (1-P). To a solution of 52 mg of racemic triol acid **1** (0.3 mmol, 2 equiv), 3.6 mg of $MgCl_2$ (0.038 mmol, 0.25 equiv), and 151 mg of ATP·Na_2·$3H_2O$ (0.25 mmol, 1.68 equiv) in deuterated triethanolamine·HCl/NaOH buffer (2.5 mL, 520 mM, pH = 9.8), was added 4 units of SK. After the reaction mixture was allowed to stand at room temperature for 187 h with occasional mixing, 12 units of apyrase was added. The resulting mixture was incubated at room temperature for 112 h, diluted with 500 mL of water, titrated with 1 M aq NaOH to pH 8.8, then subjected to anion exchange chromatography (DEAE Sephadex A-25, 0 → 1 M NEt_3H^+-HCO_3^- buffer, pH = 8.1). Fractions were lyophilized, and the desired product was detected by ^{1}H NMR. A total of 36 mg (95%) of the desired cyclopentylidene phosphate **1-P** anion was obtained in various forms of triethylammonium salts and as a mixture with AMP. The crude product was further purified by RP−HPLC with a water (0.1% TFA) to 99:1 water (0.1% TFA)/acetonitrile (0.1% TFA) gradient over 20 min at a flow rate of 10 mL/min (UV detection at 210 nm, product R_t = 6 min, AMP R_t = 12 min). All fractions corresponding to the product peak were combined and lyophilized to give 30 mg (79%) of the desired phosphate **1-P** in the acid form as a colorless residue. ^{1}H NMR (D_2O) δ 3.02−3.22 (m, 2), 3.86 (ddd, 1, J = 1.8, 4.3, 9.3), 4.51−4.68 (m, 2), 5.94−6.03 (m, 1). ^{13}C NMR (10:1 D_2O/MeOH-d_4) δ 36.6 (d, J = 1.5), 75.2 (d, J = 5.3), 77.58 (d, J = 5.3), 77.61, 115.8, 163.2, 170.9. ^{31}P NMR (D_2O) δ 0.96. $[\alpha]_D^{20}$ = +59.6° (c = 2.11, H_2O). HRMS (FAB−) $(M - H^+)^-$ Calcd. for $C_7H_{10}O_8P$: 253.0113. Found: 253.0121.

(+)-(*E*)-*O*-2-Phospho-[2(R),3(S),4(R)]-trihydroxycyclopentylideneacetic Acid (2-P). As described above for isomer **1**, 61 mg of racemic triol acid **2** (0.35 mmol) was phosphorylated and purified to give 34 mg (78%) of phosphate **2-P** in the acid form as a colorless residue. ^{1}H NMR (D_2O) δ 2.68 (d, 1, J = 20.5), 3.17−3.32 (m, 1), 4.12 (d, 1, J = 4.1), 4.21 (d, 1, J = 6.6), 5.04−5.16 (m, 1), 6.07 (d, 1, J = 2.3). ^{13}C NMR (10:1 D_2O/MeOH-d_4) δ 37.0, 73.4, 76.1 (d, J = 2.7), 79.3 (d, J = 5.2), 116.7, 162.4 (d, J = 6.0), 170.8. ^{31}P NMR (162 MHz, D_2O) δ 0.90. $[\alpha]_D^{20}$ = +26.5° (c = 1.02, dH_2O). HRMS (FAB+) MH^+ Calcd. for $C_7H_{12}O_8P$: 255.0270. Found: 255.0264.

(−)-*O*-3-Phospho-[3(R),4(S),5(R)]-trihydroxy-1-cyclopentenyl Acetate Trisodium Salt (3-P). As described above for isomer **1**, 30 mg of racemic triol acid **3** (0.172 mmol) was phosphorylated and purified to give 19 mg of crude product (88%) after anion exchange chromatography. This crude product was dissolved in water, titrated with 1 M aq NaOH to pH 12, and then purified by RP−HPLC using water as the solvent (UV detection at 191 nm, product R_t = 5.6 min, AMP R_t = 7 min). Fractions containing the product were combined and titrated with 0.1 M aq NaOH to pH 9, then lyophilized to give the desired phosphate **3-P** as the trisodium salt. ^{1}H NMR (D_2O) δ 3.01−3.14 (m, 2), 4.02 (t, 1, J = 5.3), 4.63 (d, 1, J = 4.4), 4.88−4.98 (m, 1), 5.81 (s, 1). ^{13}C NMR (10:1 D_2O/MeOH-d_4) δ 38.5, 76.8 (d, J = 5.2), 79.3 (d, J = 5.9), 81.3, 128.0 (d, J = 2.7), 146.9, 180.2. ^{31}P NMR (162 MHz, D_2O) δ 5.17. $[\alpha]_D^{20}$ = −84.8° (c = 0.28, dH_2O). HRMS (FAB−) $(M\text{-}3Na^+ + 2H^+)^-$ Calcd. for $C_7H_{10}O_8P$: 253.0113. Found: 253.0104.

(±)-4-Hydroxy-2-cyclopentenone (7a). Enone **7** was prepared from 2-methylfuran in two steps. For the first step, a modified version of Clauson-Kaas' procedure[26] was kindly provided by Lorin Thompson (UC Berkeley): To a mechanically stirred mixture of 18.2 g (222 mmol) of 2-methylfuran, 47.0 g (443 mmol, 2.02 equiv) of Na_2CO_3, and 170 mL of MeOH at −55 °C was added a solution of 35.4 g (222 mmol, 1.0 equiv) of bromine in 12 mL of CH_2Cl_2 over a 1-h period. The reaction mixture was allowed to warm to 10 °C over 3 h, then the mixture was filtered through Celite. The filtrate was mixed with 200 mL of brine and extracted with CH_2Cl_2 (one 200-mL portion followed by five 100-mL portions). The combined organic fractions were dried, filtered, and evaporated under reduced pressure to give 25.9 g (82%) of 2,5-dihydro-2,5-dimethoxy-2-methylfuran (mixture of stereoisomers) as a colorless oil, which was used in the subsequent reaction without further purification. ^{1}H NMR major isomer δ 1.49 (s, 3), 3.17 (s, 3), 3.48 (s, 3), 5.47 (s, 1), 5.9−6.0 (m, 2). ^{1}H NMR minor isomer 1.55 (s, 3), 3.10 (s, 3), 3.40 (s, 3), 5.76 (s, 1), 5.9−6.0 (m, 2). ^{13}C NMR δ 25.7, 26.1, 49.3, 49.9, 54.2, 55.5, 106.7, 107.7, 111.5, 112.7, 129.7, 130.1, 134.0, 134.4.

For the second step, a modified version of the patented procedure[27] was kindly provided by Ingrid Choong (UC Berkeley): To a stirred, deoxygenated solution of 10.0 g (69.4 mmol) of 2,5-dihydro-2,5-dimethoxy-2-methylfuran and 50 mg (0.45 mmol) of hydroquinone in 278 mL of water at 0 °C was added 0.84 g (14 mmol, 0.20 equiv) of acetic acid. After 1 h, a solution of 18.6 g (69.4 mmol, 1.0 equiv) of Na_2HPO_4·$7H_2O$ in 60 mL of water was added, and the mixture was heated to 60 °C for 2 h. Nonpolar side-products were removed by washing with 100 mL of hexanes, the aqueous layer was extracted with 1-butanol (seven 100-mL portions), and the combined 1-butanol fractions were concentrated under reduced pressure to give a crude brown oil, which was purified by chromatography (1:1 hexanes/EtOAc, R_f = 0.09) to afford 3.18 g (47%) of cyclopentenone **7** as a yellow oil. ^{1}H NMR δ 2.27 (dd, 1, J = 2.0, 18.6), 2.76 (dd, 1, J = 6.0, 18.5), 4.25 (d, 1, J = 6.2), 5.02−5.05 (m, 1), 6.21 (d, 1, J = 5.7), 7.63 (dd, 1, J = 2.3, 5.7). ^{13}C NMR δ 44.1, 70.0, 134.5, 164.4, 207.8. Spectra obtained were identical to those reported.[28]

Ethyl (*E*)- and (*Z*)-4-Oxo-2-cyclopentenylideneacetate (13e,z). Route 1: From (±)-4-Benzoyloxy-2-cyclopentenone (7b). To a solution of 1.90 g (9.4 mmol) of benzoyloxycyclopentenone **7b**[29] in 40 mL of toluene was added 3.37 g (9.7 mmol, 1.03 equiv) of carbethoxytriphenylphosphorane, and the reaction mixture was stirred at 60 °C for 8 h. After toluene was removed under reduced pressure, the residue was chromatographed (9:1 → 1:1 hexanes/EtOAc) to give 569 mg (37%) of cyclopentenone **13e** and 260 mg (17%) of cyclopentenone **13z** as individual isomers, as well as 154 mg (6%, 1:1 E/Z ratio) of the direct Wittig product (benzoate of **8a**); traces of triphenylphosphine oxide were removed by additional chromatography (19:1 CH_2Cl_2/acetone).

Less polar isomer **13z**: ^{1}H NMR δ 1.32 (t, 3, J = 7.2), 3.04 (d, 2, J = 1.2), 4.23 (q, 2, J = 7.2), 5.89−5.94 (m, 1), 6.54 (dd, 1, J = 1.7, 5,9), 8.86 (dd, 1, J = 0.8, 5.9). ^{13}C NMR δ 14.4, 39.9, 60.8, 115.9, 140.0, 151.5, 154.8, 165.4, 204.0. IR (neat) 3083, 2979, 2914, 1718, 1645, 1382, 1310, 1226, 1145 cm^{-1}. MS (EI+) m/z 166 (M^+). Anal. Calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 65.16; H, 6.17.

More polar isomer **13e**: For elemental analysis, **13e** was further purified to a white powder via sublimation; mp 49.5−50.0 °C. ^{1}H NMR δ 1.32 (t, 3, J = 7.2), 3.38 (d, 2, J = 1.7), 4.23 (q, 2, J = 7.2), 6.00−6.01 (m, 1), 6.55 (dd, 1, J = 0.6, 5.6), 7.79 (d, 1, J = 5.5). ^{13}C NMR δ 14.4, 39.4, 60.8, 116.8, 139.3, 152.8, 158.0, 165.8, 205.2. IR (KBr) 3069, 2988, 1708, 1649, 1239, 906 cm^{-1}. MS (EI+) m/z 166 (M^+). Anal. Calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 65.05; H, 6.32.

Route 2: By Isomerization of Ethyl (*Z*)-4-Oxo-2-cyclopentenylideneacetate (13z). To a solution of 40 mg (0.24 mmol) of cyclopentenone **13z** in 30 mL of chloroform was added 20 μL of concentrated HCl. The reaction mixture was stirred

(26) Clauson-Kaas, N. *Kgl. Danske Vidensk. Selsk. Math.-Fys. Medd.* **1947**, *22*, 6.

(27) Tanaka, T. Jpn. Pat. 54-163510; Japan Kokai 56-86128.

(28) Paquette, L. A.; Earle, M. J.; Smith, G. F. *Org. Synth.* **1995**, *73*, 39.

(29) Alcohol **7a** was treated with benzoyl chloride and pyridine in CH_2Cl_2 to give the known benzoate **7b** in 87% yield. (Dols, P. P. M. A.; Klunder, A. J. H.; Zwanenburg, B. *Tetrahedron* **1994**, *50*, 8515−8538.)

at room temperature for 3 d and washed with 20 mL of water. The aqueous portion was further extracted with CH_2Cl_2 (two 10-mL portions), and the combined organic fractions were dried and concentrated under reduced pressure to give 40 mg (100%) of **13e,z** in a 1:1 ratio. The *E*/*Z* isomers were separated by chromatography (4:1 hexanes/EtOAc).

(±)-Ethyl (*E*)-4-Hydroxy-2-cyclopentenylideneacetate (8a). To a solution of 0.31 g (1.87 mmol) of enone **13e** and 0.836 g (2.24 mmol, 1.2 equiv) of $CeCl_3 \cdot 7H_2O$ in 50 mL of MeOH at −3 °C was added slowly over 2 min 85 mg (2.25 mmol, 1.2 equiv) of $NaBH_4$. The reaction mixture was stirred at room temperature for 30 min, mixed with 10 mL of 0.5 M aq HCl (5 mmol, 2.7 equiv) and 25 mL of brine, and extracted with ether (five 30-mL portions). The combined ether fractions were dried and concentrated under reduced pressure, and the crude product was purified via chromatography (7:3 hexanes/EtOAc) to give 296 mg (94%) of allylic alcohol **8a** as a viscous, colorless oil. ^{1}H NMR δ 1.29 (t, 3, J = 7.1), 1.78−1.80 (m, 1), 2.78 (td, 1, J = 2.1, 19.4), 3.45 (ddd, 1, J = 2.2, 6.5, 19.3), 4.18 (q, 2, J = 7.1), 4.99−5.08 (m, 1), 5.80 (t, 1, J = 2.2), 6.39 (d, 1, J = 5.4), 6.52 (dd, 1, J = 2.3, 5.4). ^{13}C NMR δ 14.2, 39.9, 59.8, 75.2, 110.8, 135.7, 147.5, 162.9, 167.4. IR (neat) 3417 (br), 3060, 2981, 2935, 2903, 1698, 1633, 1580, 859 cm^{-1}. HRMS (EI+) M^+ Calcd. for $C_9H_{12}O_3$: 168.0786. Found: 168.0789.

(±)-Ethyl (*E*)-4-*t*-Butyldiphenylsiloxy-2-cyclopentenylideneacetate (8b). To a solution of 410 mg (2.44 mmol) of alcohol **8a** in 3 mL of DMF were added 581 mg (8.54 mmol, 3.5 equiv) of imidazole and 2 g (7.31 mmol, 3 equiv) of *tert*-butyldiphenylsilyl chloride. The reaction mixture was stirred at room temperature for 2 h, then diluted with 50 mL of brine and 50 mL of ether. The aqueous layer was extracted with ether (one 50 mL-portion followed by two 25-mL portions), the combined organic layers were washed with water, dried, and concentrated under reduced pressure, and the residue was chromatographed (9:1 hexanes/Et_2O) to give 939 mg (95%) of **8b** as a colorless, viscous oil. ^{1}H NMR δ 1.07 (s, 9), 1.26 (t, 3, J = 7.1), 2.90 (td, 1, J = 2.2, 19.0), 3.34 (ddd, 1, J = 2.0, 6.3, 19.0), 4.16 (q, 2, J = 7.2), 4.95−5.00 (m, 1), 5.72 (t, 1, J = 2.2), 6.23 (d, 1, J = 5.7), 6.26 (dd, 1, J = 1.9, 5.5) 7.30−7.50 (m, 6), 7.64−7.78 (m, 4). ^{13}C NMR δ 14.5, 19.2, 25.4, 40.5, 59.8, 77.0, 110.8, 127.8, 127.9, 129.9, 133.7, 134.2, 135.3, 135.8, 135.9, 147.6, 162.8, 167.4. IR (neat) 3070, 2958, 2931, 2893, 2857, 1703, 1634, 1214, 1110, 1064, 702 cm^{-1}. MS (EI+) m/z 406 (M^+). Anal. Calcd. for $C_{25}H_{30}O_3Si$: C, 73.85; H, 7.44. Found: C, 73.89; H, 7.66.

(±)-Ethyl (*E*)-[2(S*),3(R*)]-Epoxy-4(R*)-hydroxycyclopentylideneacetate (14). To a solution of 281 mg (1.67 mmol) of hydroxycyclopentenylidene **8a** in 50 mL of toluene at 0 °C was added slowly 1.08 g (3.57−5.44 mmol, 2.1−3.2 equiv) of 57−86% pure *m*-CPBA. The mixture was allowed to warm to room temperature over 1 h and then was stirred for 23 h. After toluene was removed under reduced pressure, the residue was chromatographed (1:1 hexanes/EtOAc, R_f = 0.26) to give 217 mg (70%) of epoxide **14** as a colorless oil. ^{1}H NMR δ 1.27 (t, 3, J = 7.2), 2.11 (ddd, 1, J = 3.0, 6.6, 18.4), 2.70−3.25 (br s, 1), 3.56 (ddd, 1, J = 1.8, 8.1, 18.3), 3.72 (d, 1, J = 2.7), 3.83−3.87 (m, 1), 4.11−4.22 (m, 2), 4.32−4.40 (m, 1), 5.99−6.02 (m, 1). ^{13}C NMR δ 14.5, 34.2, 59.3, 60.5, 60.7, 71.7, 117.7, 156.4, 165.7. IR (neat) 3413 (br), 2983, 1712, 1668, 1222, 857 cm^{-1}. MS (EI+) m/z 184 (M^+). Anal. Calcd. for $C_9H_{12}O_4$: C, 58.69; H, 6.57. Found: C, 58.38; H, 6.52.

(±)-Ethyl (*E*)-[2(R*),3(R*),4(R*)]-Trihydroxycyclopentylideneacetate (15). A mixture of 125 mg of epoxide **14** (0.68 mmol) and 5 mL of 0.75 M aq H_2SO_4 (3.75 mmol, 5.5 equiv) was stirred at room temperature for 20 min, then neutralized to pH 7 with 1 M $NaHCO_3$. After water was removed via lyophilization, the residue was chromatographed (EtOAc) to yield 126 mg (92%) of triol ester **15**. ^{1}H NMR (MeOH-d_4) δ 1.27 (t, 3, J = 7.2), 2.77−2.90 (m, 1), 2.98−3.12 (m, 1), 3.67 (dd, 1, J = 4.4, 8.9), 4.06−4.12 (m, 1), 4.15 (q, 2, J = 7.2), 4.51 (td, 1, J = 2.4, 9.0), 5.95 (q, 1, J = 2.4). ^{13}C NMR (MeOH-d_4) δ 14.8, 37.8, 61.1, 70.5, 78.5, 79.4, 115.6, 164.9, 168.3. IR (neat) 3366 (br), 2925, 1710, 1455, 1207, 1113 cm^{-1}. MS (FAB+) m/z 203 (MH^+). Anal. Calcd. for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.37; H, 7.28.

(±)-Ethyl (*E*)-4(R*)-*t*-Butyldiphenylsiloxy-[2(R*),3(R*)]-dihydroxycyclopentylideneacetate (16a). To a solution of 1.176 g (2.897 mmol) of silyl ether **8b** in 20 mL of *tert*-butyl alcohol was added 1.57 g of 2.5 wt % OsO_4 (39.3 mg, 0.155 mmol, 0.05 equiv) in *tert*-butyl alcohol, followed by 116 mg (1.04 mmol, 0.36 equiv) of DABCO and a solution of 3.05 g (9.27 mmol, 3.2 equiv) of $K_3Fe(CN)_6$ and 1.28 g (9.27 mmol, 3.2 equiv) of K_2CO_3 in 20 mL of water. The biphasic reaction mixture was stirred at room temperature for 50 h, and then 3.9 g of Na_2SO_3 was added. The resulting mixture was stirred at room temperature for 3 h, diluted with 100 mL of water, and extracted with EtOAc (six 100-mL portions). All EtOAc fractions were combined, dried, and concentrated under reduced pressure, and the residue was chromatographed (7:3 hexanes/EtOAc) to afford 805 mg (63%) of the desired triol **16a** and 35 mg (3%) of the all-cis triol. For **16a**: ^{1}H NMR δ 1.04 (s, 9), 1.26 (t, 3, J = 7.1), 2.05−2.09 (m, 1), 2.43 (d, 1, J = 8.7), 2.77−2.87 (m, 1), 3.09−3.21 (m, 1), 3.83−3.89 (m, 1), 4.14 (q, 2, J = 7.1), 4.16−4.21 (m, 1), 4.79−4.87 (m, 1), 6.04 (q, 1, J = 2.4), 7.33−7.48 (m, 6), 7.57−7.69 (m, 4). ^{13}C NMR δ 14.5, 19.3, 27.1, 38.2, 60.1, 74.9, 75.6, 76.7, 115.9, 128.0, 130.1, 133.5, 133.8, 135.9, 163.6, 166.7. IR (neat) 3434 (br), 2931, 2858, 1716, 1699, 1428, 1203, 1112, 822, 701 cm^{-1}. MS (FAB+) m/z 441 (MH^+). Anal. Calcd. for $C_{25}H_{32}O_5Si$: C, 68.15; H, 7.32. Found: C, 67.86; H, 7.55.

(±)-Ethyl (*E*)-[2(R*),3(S*),4(R*)]-Trihydroxycyclopentylideneacetate (16b). A solution of 46 mg (0.104 mmol) of silyl ether **16a** in 2 mL of 5 M ethanolic HCl was stirred at room temperature for 62 h. After ethanol was removed under reduced pressure, the crude product was chromatographed (EtOAc → 10:1 EtOAc/MeOH) to give 20 mg (95%) of triol **16b** as a colorless film. ^{1}H NMR (MeOH-d_4) δ 1.28 (t, 3, J = 7.1), 2.60−2.73 (m, 1), 3.21 (tdd, 1, J = 2.5, 6.2, 20.2), 3.84−3.88 (m, 1), 4.08 (d, 1, J = 6.3), 4.15 (q, 2, J = 7.1), 4.62−4.68 (m, 1), 5.94−6.00 (m, 1). ^{13}C NMR (MeOH-d_4) δ 14.8, 38.4, 61.1, 74.3, 76.5, 77.8, 115.5, 166.5, 168.5. IR (neat) 3370 (br), 2923, 1696, 1666, 1213, 1042 cm^{-1}. MS (EI+) m/z 202 (M^+). Anal. Calcd. for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.11; H, 7.29.

(±)-Ethyl (*E*)-[2(R*),3(S*)]-*O*-Isopropylidene-4(R*)-hydroxycyclopentylideneacetate (17). A mixture of 91 mg (0.45 mmol) of triol ester **16b** and 0.75 g of Bio-Rad AG 50W-X4 resin in 5 mL of acetone was stirred at room temperature for 20 h, then diluted with 10 mL of EtOAc and filtered. After solvent was removed under reduced pressure, the colorless residue was chromatographed (1:1 hexanes/EtOAc and 2:1 EtOAc/MeOH) to afford 78 mg of acetonide **17** (94% overall yield) as a colorless oil along with 22 mg of recovered starting material. ^{1}H NMR δ 1.22−1.32 (m, 3), 1.34 (s, 3), 1.41 (s, 3), 2.18−2.32 (br s, 1), 2.90−3.06 (m, 1), 3.25 (d, 1, J = 18.2), 4.17 (q, 2, J = 7.1), 4.37 (d, 1, J = 4.7), 4.47 (d, 1, J = 5.3), 4.95 (d, 1, J = 5.3), 6.09 (s, 1). ^{13}C NMR δ 14.3, 24.8, 26.8, 37.1, 60.4, 73.9, 82.3, 84.3, 111.9, 119.5, 160.5, 166.7. IR (neat) 3466 (br), 2986, 2933, 1716, 1374, 1212, 1158, 1039, 861 cm^{-1}. MS (EI+) m/z 242 (M^+). Anal. Calcd. for $C_{12}H_{18}O_5$: C, 59.49; H, 7.49. Found: C, 59.61; H, 7.84.

(±)-Ethyl [4(S*),5(R*)]-*O*-Isopropylidene-3(R*)-hydroxy-1-cyclopenteneacetate (18). To a solution of 300 μL (2.14 mmol, 10.6 equiv) of dry diisopropylamine in 2 mL of dry THF in a flame-dried flask under Ar at −70 °C was slowly added 600 μL of 1.6 M *n*-BuLi in hexanes (0.96 mmol, 4.7 equiv), and the mixture was stirred for 10 min. A solution of 49 mg (0.20 mmol) of acetonide **17** in 2 mL of dry THF was added slowly over 10 min. The reaction mixture was allowed to warm to −35 °C over 20 min, mixed with 5 mL of 1 M aq HCl, and partitioned between 30 mL of brine and 25 mL of ether. The aqueous portion was extracted with ether, and the combined organic layer was dried and concentrated under reduced pressure to give 45 mg of yellow residue, which was chromatographed (1:1 hexanes/EtOAc) to afford 23 mg (47%) of the desired isomerized acetonide **18** and 9 mg (16%) of the enol ether **19**. For **18**: ^{1}H NMR δ 1.28 (t, 3, J = 7.1), 1.35 (s, 3), 1.36 (s, 3), 2.09−2.17 (m, 1), 3.13−3.30 (m, 2), 4.17 (q, 2, J = 7.1), 4.53 (d, 1, J = 5.5), 4.70 (s, 1), 5.23 (d, 1, J = 5.5), 5.74 (s, 1). ^{13}C NMR δ 14.4, 26.3, 27.6, 34.3, 61.2, 80.0, 85.0, 86.3, 112.1, 130.9, 142.5, 170.7. IR (neat) 3414 (br), 2986, 2935, 1738,

1372, 1248, 1208, 1157, 1035, 872 cm^{-1}. HRMS (FAB+) MLi^+ Calcd. for $C_{12}H_{18}O_5Li$: 249.1314. Found: 249.1319.

SK Steady-State Enzyme Kinetics Assays for Shikimate, Analogue 1, and Analogue 3. *Escherichia coli* K12 *aroL* shikimate kinase II (EC 2.7.1.71) was kindly provided by Dr. Kenneth Gruys at Monsanto. The enzyme concentration was 7.6 mg/mL (280/205 UV absorption method)[30] with a specific activity of 26.1 units/mg (one unit of enzyme activity is defined as the amount of enzyme catalyzing the conversion of 1 μmol of substrate/min at 25 °C), estimated using Michaelis−Menten V_{max} values projected by the EnzymeKinetics (v1.11) program. The assay mixture comprised, in a final volume of 1 mL at 25 °C, the following: 50 mM triethanolamine·HCl/KOH buffer, pH 7.8; 50 mM KCl; 5 mM $MgCl_2$; 2.5 mM ATP (di-Na^+ salt); 1 mM phosphoenolpyruvate (mono-K^+ salt); 0.1 mM NADH (di-Na^+ salt); 1.75 units of pyruvate kinase; and 2.5 units of lactate dehydrogenase. Assays with shikimate as substrate were performed using 0.05 units of SK and substrate concentrations from 0.05 to 0.4 mM. For analogues **1** (0.1−4 mM) and **3** (0.1−1 mM), 0.4 units and 0.8 units of SK, respectively, were used for each assay. Assay reactions were initiated by the addition of SK. Observed activity was corrected against control runs in which the particular substrate was omitted.

(30) Scopes, R. K. *Protein Purification: Principles and Practice*, 3rd ed.; Springer-Verlag: New York, 1994; pp 46−48.

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Supporting Information Available: Detailed general experimental conditions, a figure depicting the enzymatic resolution of analogues **2** and **3**, as monitored by ^1H NMR, and additional characterization material (^1H, ^{13}C, and ^{31}P NMR spectra). This material is available free of charge via the Internet at http://pubs.acs.org.

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