Synthesis of 2-Bromo- and 2-Fluoro-3-dehydroshikimic Acids and 2-Bromo- and 2-Fluoroshikimic Acids Using Synthetic and Enzymatic Approaches

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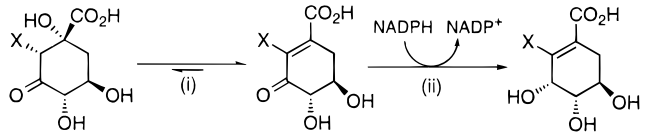
The syntheses of 2-bromo-3-dehydroshikimic acid[1] (**5**) and 2-bromoshikimic acid[2] (**8**), are described. Their formation from (2*R*)-2-bromo-3-dehydroquinic acid (**2**) using dehydroquinase and shikimate dehydrogenase is also reported. The corresponding enzymatic formation of 2-fluoro-3-dehydroshikimic acid[1] (**6**) and 2-fluoroshikimic acid[2] (**9**) from (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) are reported.

Introduction

The shikimate pathway is the biosynthetic pathway utilized by plants, fungi, and microorganisms[3] for the synthesis of the amino acids L-phenylalanine, L-tryptophan, and L-tyrosine, as well as precursors to the folate coenzymes, alkaloids, and vitamins. Analogues of shikimate pathway intermediates are of interest as potential enzyme inhibitors, and consequently a lot of synthetic effort has been directed to this area.[4] However, it is only recently that much attention has been focused on analogues substituted at the 2-position with the publication of syntheses of 2-chloro-[5] and 2-fluoroshikimic acids,[6] and (2*R*)-bromo- and (2*R*)-2-fluoro-3-dehydroquinic acids.[7] Syntheses of (2*R*)- and (2*S*)-2-hydroxyquinic acids[8] and (2*R*)-2-bromoquinic acid had been published previously.[9] In this paper we report the first syntheses of the 2-bromo-3-dehydroshikimic acid (**5**), 2-fluoro-3-dehydroshikimic acid (**6**), 2-bromoshikimic acid (**8**), and a novel synthesis of 2-fluoroshikimic acid (**9**), using a combination of synthetic and enzymatic approaches (Scheme 1).

Scheme 1[a]



1 X= H
2 X= Br
3 X= F

4 X= H
5 X= Br
6 X= F

7 X= H
8 X= Br
9 X= F

[a] Enzymes: (i) dehydroquinase; (ii) shikimate dehydrogenase.

Synthesis of 2-Bromo-3-Dehydroshikimic Acid. We have previously published a synthesis of (2*R*)-2-bromo-3-dehydroquinic acid (**2**).[7] The bromolactone **11** was a key intermediate in that synthesis, formed in six steps and 29% from quinic acid (**10**) (Scheme 2). This compound is the starting point for the synthesis of 2-bromo-3-dehydroshikimic acid (**5**).

Methyl dehydroquinate has been reported to undergo quantitative conversion to methyl dehydroshikimate upon treatment with triethylamine in dichloromethane.[10] We therefore initially attempted to prepare 2-bromo-3-dehydroshikimic acid in the same way, after first converting the lactone **11** into the methyl ester **12** (Scheme 2). Opening the lactone **11** with a catalytic quantity of sodium methoxide in methanol afforded the expected methyl ester **12** in 68% yield, along with a small quantity of the epoxide **13** (6%). This reaction could not easily be optimized further as use of more sodium methoxide increased the proportion of epoxide formed, and using greater than a stoichiometric amount of sodium methoxide or extended reaction times led to aromatization.

Treatment of **12** with triethylamine in dichloromethane did not result in the expected formation of **16** (Scheme 3), but rather the rapid formation of **14** (84%). An alternative two-step procedure was therefore devised, involving initial elimination of water from **12** using TFAA/pyridine. This proceeded well in 90% yield but resulted in unwanted trifluoracetylation at C-5. The

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(1) IUPAC names: (4*S*,5*R*)-2-bromo-4,5-dihydroxy-3-oxocyclohex-1-enecarboxylic acid, (4*S*,5*R*)-2-fluoro-4,5-dihydroxy-3-oxocyclohex-1-enecarboxylic acid, respectively.

(2) IUPAC names: (3*R*,4*S*,5*R*)-2-bromo-3,4,5-trihydroxycyclohex-1-enecarboxylic acid, (3*R*,4*S*,5*R*)-2-fluoro-3,4,5-trihydroxycyclohex-1-enecarboxylic acid, respectively.

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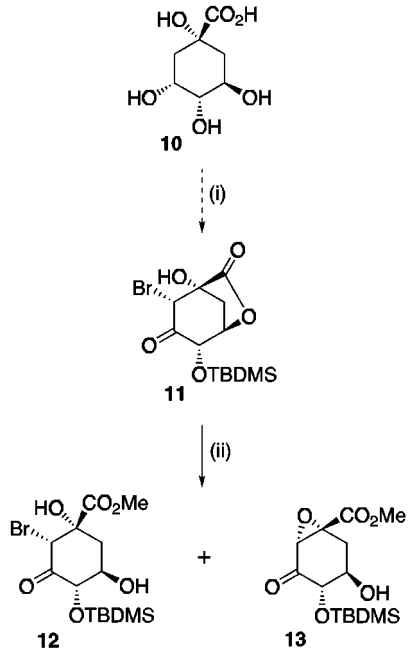
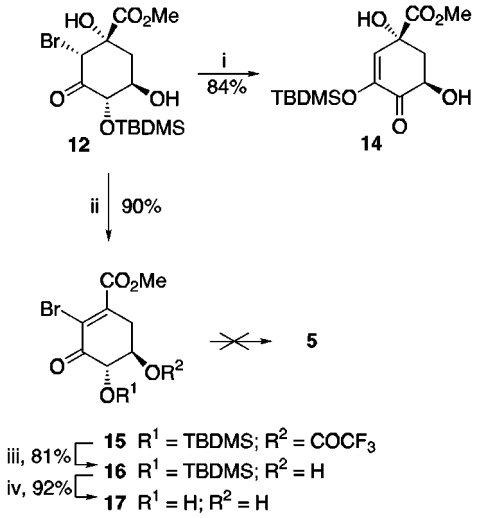
Scheme 2[a]



[a] Reagents, conditions, and yields: (i) ref 7; (ii) CH_3ONa, methanol (74%).

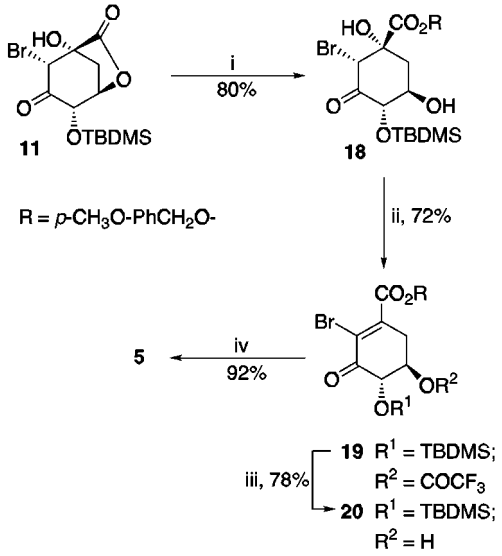
Scheme 3[a]



[a] Reagents and conditions: (i) Et_3N, dichloromethane, room temp; (ii) TFAA, pyridine, room temp; (iii) K_2CO_3, H_2O, THF, room temp.; (iv) AcOH, THF, H_2O, (4:1:1), 40 °C.

trifluoracetyl group was removed from **15** by using potasssium carbonate in water−THF, affording **16** in 81% yield, which upon desilylation (THF/HOAc/H_2O) gave a 92% yield of the methyl 2-bromo-3-dehydroshikimate (**17**). Unfortunately conditions could not be found for the removal of the methyl ester despite a number of attempts, by using inter alia K_2CO_3/H_2O/THF, BBr_3,[11] LiI/pyridine,[12] pig liver esterase,[13] rabbit liver esterase[14] and

Scheme 4[a]



[a] Reagents and conditions: (i) *p*-CH_3OPhCH_2OH, *n*-BuLi, THF, room temp; (ii) TFAA, pyridine, room temp; (iii) K_2CO_3, H_2O, THF, room temp; (iv) AcOH, THF, H_2O (4:1:1), 40 °C.

H_2SO_4/H_2O. In these reactions either no reaction was observed or the starting material decomposed with none of the required product formed.

Because of the problems of removing the methyl ester, the strategy was revised so that the final step involved removal of the more labile, acid-sensitive *p*-methoxybenzyl ester. The ester **18** was formed easily in 80% yield by opening the lactone **11** with the anion of *p*-methoxybenzyl alcohol (Scheme 4). The subsequent elimination and trifluoracetylation proceeded in 72% yield to give **19**, from which the trifluoracetyl group was smoothly removed to form *p*-methoxybenzyl 2-bromo-3-dehydroshikimate (**20**) (78%). Final removal of both the TBDMS and *p*-methoxybenzyl protecting groups from **20** was readily achieved employing HOAc/H_2O/THF at 40 °C to give the required 2-bromo-3-dehydroshikimic acid **5** in 92% yield.

Enzymatic Conversion of (*2R*)-Bromo- and (*2R*)-2-Fluorodehydroquinic Acids. An alternative approach to the synthesis of 2-bromo-3-dehydroshikimic acid (**5**) is by enzyme-catalyzed elimination of water from (*2R*)-2-bromo-3-dehydroquinic acid (**2**). This would be analogous to the conversion of 3-dehydroquinic acid (**1**) to 3-dehydroshikimic acid (**4**) catalyzed by the third enzyme on the shikimate pathway, dehydroquinase (3-dehydroquinate dehydratase).[3]

There is a subtlety in the enzymology of dehydroquinase that we needed to take into account before proceding. The most studied form of dehydroquinase is the type I enzyme from *E. coli*. This enzyme catalyses the conversion of **1** to **4** (Scheme 1) by a mechanism involving Schiff base formation between the substrate and an active site lysine and involves loss of the equatorial *pro-R* hydrogen from C-2, corresponding to an overall syn elimination.[15] The structurally distinct type II dehydroquinases, which are involved in both the biosynthetic shikimate pathway and the catabolic quinate pathway,[16] catalyze the same conversion but with the opposite

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Table 1. Kinetic Parameters for the Conversion of Dehydroquinic Acid (1), (2*R*)-2-Bromo-3-dehydroquinic Acid (2) and (2*R*)-2-Fluoro-3-dehydroquinic Acid (3) with Type II Dehydroquinases from *M. tuberculosis* and *A. nidulans*[a]

source	substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}\ s^{-1}$) $\times 10^5$
M. tuberculosis	**1**	13	3.3	2.5
	2	15	5.5	3.67
	3	8	0.7	0.86
A. nidulans	**1**	122	290	23.9
	2	26	70	26.9
	3	86	80	9.3

[a] Enzyme assays were performed at a range of substrate concentrations, typically 0.2-5 K_m, measuring the initial rate of the reaction at 234 nm for **1**, 264 nm for **2**, and 249 nm for **3**. Reactions were performed at 25 °C in Tris/HCl buffer (50 mM, pH 7.0).

stereochemistry,[17] by a mechanism which is thought to involve initial proton abstraction followed by stabilization of the carbanion intermediate as an enolate.[18]

As the conversion of (2*R*)-2-bromo-3-dehydroquinic acid (**2**) into 2-bromo-3-dehydroshikimic acid (**5**) involves an anti elimination of water, the type II dehydroquinase was used as a catalyst. In fact the *M. tuberculosis* enzyme from the biosynthetic shikimate pathway and the *A. nidulans* enzyme from the catabolic quinic acid pathway were both tested as they are known to have quite distinct kinetic properties.[18] Parallel studies were also carried out using (2*R*)-2-fluoro-3-dehydroquinic acid **3** as a substrate.[7] The steady-state kinetic parameters for both **2** and **3** together with the normal substrate 3-dehydroquinic acid (**1**) for reaction with both type II dehydroquinases are shown in Table 1.

The conversion of **2** was followed by UV spectrometry, monitoring the increase in absorbance at 264 nm (ϵ/M^{-1} cm^{-1} 6 300) due to formation of the extended chromophore in **5**. The transformation could also be followed by ^{1}H NMR spectroscopy (Figure 1) by monitoring the decrease of the singlet at δ 5.55 due to the proton at C-2 of **2**. Similarly, the conversion of (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) was followed by UV spectrometry at 249 nm (ϵ/M^{-1} cm^{-1} 9 600), or by the decrease of the doublet at δ 5.64 due to the C-2 proton of **3** in the ^{1}H NMR spectrum (Figure 2). The reaction could additionally be followed by ^{19}F NMR spectroscopy, monitoring the appearance of the broad singlet at δ −133 due to the vinylic fluorine at C-2 of **6** (Figure 3).

The equilibrium constant between (2*R*)-2-bromo-3-dehydroquinic acid (**2**) and 2-bromo-3-dehydroshikimic acid (**5**) was measured by ^{1}H NMR spectroscopy, by letting the enzymatic conversion run to equilibrium in the NMR tube (Figure 1). A value of 7.3 in favor of **5** was determined. The corresponding equilibrium betweeen (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) and 2-fluoro-3-dehydroshikimic acid (**6**) is 4.0 (Figure 2). These values compare with an equilibrium constant between dehydroquinic acid (**1**) and 3-dehydroshikimic acid (**4**) of 15.[19] The decrease in the equilibrium constant may reflect increasing destabilization of the 3-dehydroshikimic acid form by the progressively increasing inductive effects in moving from a hydrogen to bromine and then a fluorine substitutent at C-2.

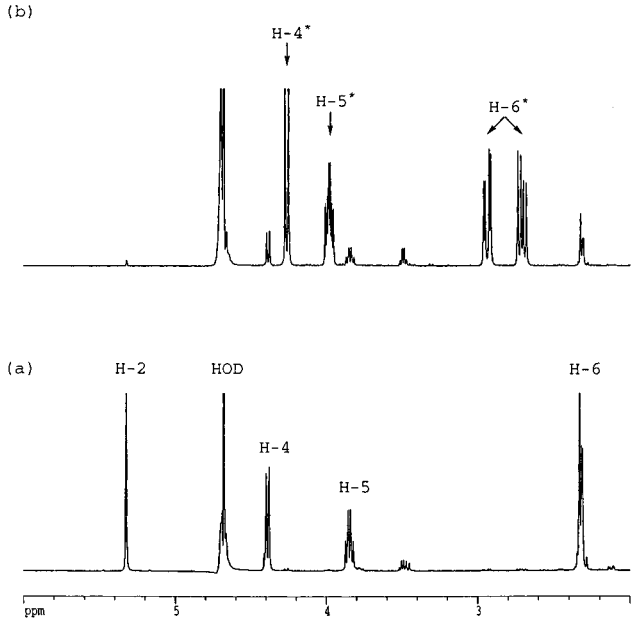

Figure 1. ^{1}H NMR spectrum (D_2O, 500 MHz) of (a) (2*R*)-2-bromo-3-dehydroquinic acid (**2**) and (b) an equilibrium mixture of (2*R*)-2-bromo-3-dehydroquinic acid (**2**) and 2-bromo-3-dehydroshikimic acid (**5**) formed in potassium phosphate buffer (50 mM, pH 7.0, 26 °C), by incubation over 48 h with type II dehydroquinase from *M. tuberculosis* (15 μL, 2.6 U) in a total volume of 0.6 mL. Peaks labeled with (*) belong to 2-bromo-3-dehydroshikimic acid (**5**).

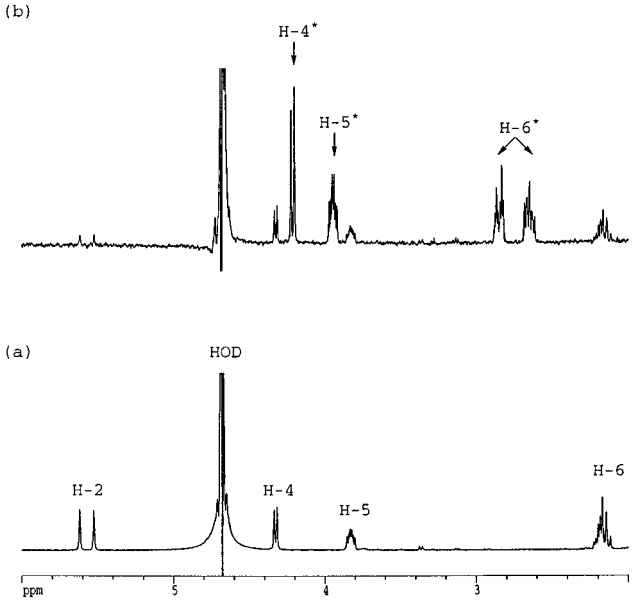

Figure 2. ^{1}H NMR spectrum (D_2O, 500 MHz) of (a) (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) and (b) an equilibrium mixture of (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) and 2-fluoro-3-dehydroshikimic acid (**6**) formed in potassium phosphate buffer (100 mM, pH 7.0, 26 °C), by incubation over 21 h with type II dehydroquinase from *M. tuberculosis* (15 μL, 2.6 U) in a total volume of 0.6 mL. Peaks labeled with (*) belong to 2-fluoro-3-dehydroshikimic acid **6**.

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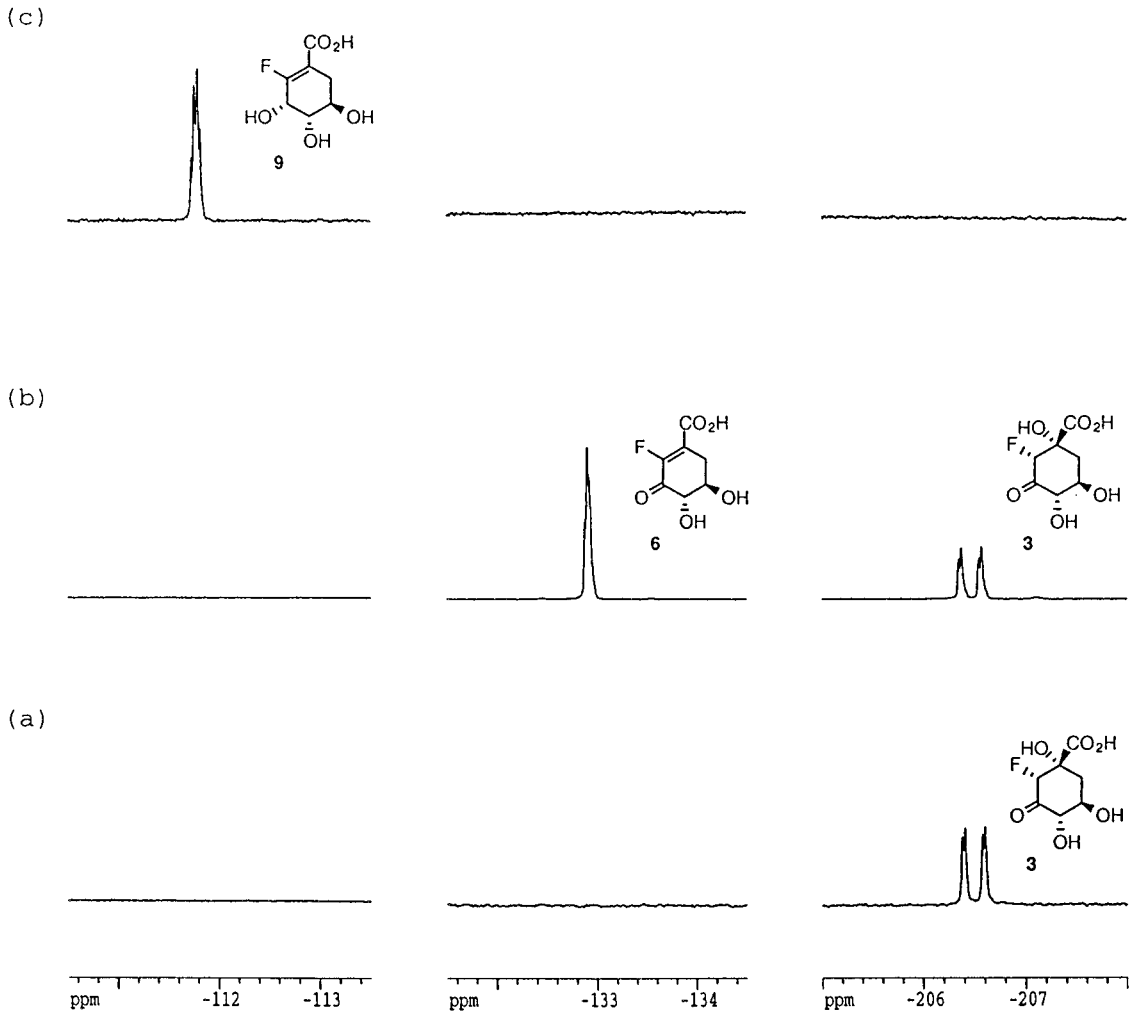

Figure 3. ^{19}F NMR spectra (D_2O, 235 MHz) showing the conversion of (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) into 2-fluoroshikimic acid (**9**) in potassium phosphate buffer (100 mM, pH 7.0, 26 °C). Type II dehydroquinase from *M. tuberculosis* (15 μL, 2.6 U) in a total volume of 0.6 mL was added inmediately after spectrum (a). NADPH and shikimate dehydrogenase (10 μL, 10 U) was added inmediately after spectrum (b): (a) $t = 0$, (b) $t = 25$ h. (c) $t = 48$ h.

Time-dependent irreversible inhibition of the type II dehydroquinases from *M. tuberculosis* and *A. nidulans* by (2*R*)-2-bromo-3-dehydroquinic acid (**2**) and (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) was also investigated. In each case two parallel incubations were set up with type II dehydroquinase in Tris/HCl buffer 50 mM at pH 7.0. The first contained **2** (or **3**), and the second was a control that lacked potential inhibitor. Both enzyme mixtures were identically incubated at 25 °C, and the enzyme activity was periodically monitored by assaying an aliquot with 3-dehydroquinic acid (**1**). There was no time dependent inhibition of either type II dehydroquinase with **2**. Likewise, the *M. tuberculosis* enzyme was stable in the presence of **3**. It was therefore somewhat surprising that incubation with **3** resulted in slow irreversible inhibition of the *A. nidulans* enzyme (50% over 4 h).

The above enzymatic studies were a prelude to using the type II dehydroquinases to synthesize 2-halo-3-dehydroshikimates. 2-Bromo-3-dehydroshikimic acid (**5**) was synthesized enzymatically in 88% yield using either the type II dehydroquinase from *A. nidulans* or *M. tuberculosis*. Because of the problems of inhibition of the *A. nidulans* enzyme by **3**, the type II dehydroquinase from *M. tuberculosis* was used for the conversion of (2*R*)-2-fluoro-3-dehydroquinic acid into 2-fluoro-3-dehydroshikimic acid (**6**) in 88% yield. The incubations were carried out in 100 mM ammonium bicarbonate buffer at pH 7.8 and 25 °C. After the reactions had reached equilibrium, the products were purifed by HPLC.

In conclusion, 2-bromo-3-dehydroshikimic acid (**5**) has been synthesized in nine steps in 11% overall yield from quinic acid. This improves to an overall yield of 25% if (2*R*)-2-bromo-3-dehydroquinic acid (**2**) is first synthesized and then converted enzymatically to (**5**). The corresponding synthesis of **6** via **3** gives 2-fluoro-3-dehydroshikimic acid **6** in an overall yield of 23% from quinic acid.

Synthesis of 2-Bromoshikimic Acid 8. The availability of the 2-halo-3-dehydroshikimates **5** and **6** opened up the possibility of a route to the 2-haloshikimic acids by enzymatic reduction using shikimate dehydrogenase (Scheme 1), albeit no C-2 substituted analogues of dehydroshikimic **4** or shikimic acid **7** had previously been reported as substrates for this enzyme. Shikimate dehydrogenase catalyzes the stereospecific reduction of 3-dehydroshikimic acid **4** to shikimic acid **7** and requires NADPH as a cofactor (Scheme 1).[20] The gene for the *E. coli* enzyme has been cloned and overexpressed.[21] Pre-

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liminary UV assays of shikimate dehydrogenase with 2-bromodehydroshikimic acid (**5**) and NADPH in potassium phosphate buffer at pH 7.0 indicated that **5** was a substrate. Detailed kinetic studies were performed to determine a k_{cat} of 2.7 s^{-1} and a K_m of 4.0 mM. Although the specificity constant ($k_{cat}/K_m = 6.8 \times 10^2$ M^{-1} s^{-1}) is 10^3 lower than for 3-dehydroshikimic acid **4**, **5** is still a good enough substrate for shikimate dehydrogenase to be used preparatively to make 2-bromoshikimic acid (**8**). This transformation was followed by ^{1}H NMR spectroscopy, monitoring the upfield shift of the proton at C-5 from δ 4.06 in **5** to δ 3.94 in **8**, and the appearance of a double doublet at δ 2.26 due to the axial proton at C-6 in **8**. The other peaks were obscured by the signals due to NADPH and $NADP^+$. After HPLC, an 83% yield of 2-bromoshikimic acid (**8**) was obtained.

An analogous study was carried out using 2-fluoro-3-dehydroshikimic acid (**6**), which proved an even better substrate for shikimate dehydrogenase than **5**. Kinetic studies in potassium phosphate buffer at pH 7.0 and 25 °C, were used to determine a K_m of 235 μM, and a k_{cat} of 103 s^{-1}. This gives a specificity constant ($k_{cat}/K_m = 4.4 \times 10^5$ M^{-1} s^{-1}) only five times lower than that for the natural substrate.

A transformation from (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) to 2-fluoroshikimic acid (**9**) was carried out by sequential use of type II dehydroquinase and shikimate dehydrogenase. The transformation was monitored by ^{19}F NMR spectroscopy (Figure 3). In the ^{19}F NMR spectrum the fluorine in (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) gives rise to a double doublet at δ −206. Addition of type II dehydroquinase results in a reduction of this signal and appearance of a broad singlet at δ −133 due to **6** as the equilibrium mixture of **3** and **6** is formed. At this point, NADPH and shikimate dehydrogenase were added. After several hours the only signal in the ^{19}F NMR spectrum is a quartet at δ −112 due to 2-fluoroshikimic acid (**9**). Under the conditions of the incubation, the equilibrium is completely pulled over toward product. 2-Fluoroshikimic acid (**9**) was isolated in 91% yield from this transformation.

A synthesis of 2-fluoroshikimic acid (**7**) has recently been published.[6] The synthesis required 10 steps from shikimic acid and proceeded in an overall 4% yield. Our approach, using a combination of synthetic chemistry and enzymatic transformations, proceeds in eight steps from quinic acid in an overall 21% yield.

Experimental Section

General. Where quoted, carboxylic acids were analyzed or purified by HPLC which was carried out on either a semipreparative (300 mm × 8 mm), or preparative (300 × 16 mm) Bio-Rad Aminex Ion Exclusion HPX-87H Organic Acids column. The eluent used for these columns was 50 mM aqueous formic acid, at a flow rate of 0.6 mL min^{-1} (semipreparative column) or 1.2 mL min^{-1} (preparative column). FPLC was carried out using a Mono Q HR 10/10 column and eluting with a gradient of ammonium bicarbonate at 1.0 mL min^{-1} with the UV detector set at 254 nm. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra and enzyme assays were performed at 25 °C (using either a temperature control thermostat or a thermostated water bath), using 1 cm path quartz cells.

Type II dehydroquinases from *M. tuberculosis* and *A. nidulans* were purified as described previously:[22] a concentrated solution (1.5 and 0.08 mg mL^{-1}, respectively) in potassium phosphate buffer (50 mM, pH 7.0), DTT (1 mM) was filter-sterilized through a 0.2 μm filter and stored at 4 °C under which conditions it was stable for at least 9 months. When required for assays, aliquots of the enzyme stocks were diluted into water and stored on ice. Shikimate dehydrogenase was purified as described previously[23] and the concentrated solution (2.3 mg mL^{-1}) stored in Tris/HCl buffer (50 mM, pH 7.5), DTT (0.4 mM), KCl (50 mM) and 50% (v/v) glycerol at −20 °C. When required for assays aliquots of the enzyme stocks were diluted into water and stored on ice.

One unit (1 U) of enzyme is defined as the amount of enzyme required to convert 1 μmol of substrate to product in 1 min. Buffer reagents were purchased from Sigma Chemical Company, and pH values of prepared buffers were adjusted using HOAc or HCl (c). All pH measurement were made at 25 °C. Deuterated buffer was made up in 99.9% D_2O and pD adjusted with DCl or DOAc. pD values have been quoted where pH = meter reading + 0.4.

Assays of Dehydroquinase. Dehydroquinase was assayed in the forward or reverse direction by monitoring the increase or decrease in absorbance at 234 nm in the UV spectrum due to the absorbance of the enone-carboxylate chromophore of 3-dehydroshikimic acid (**4**) (ϵ/M^{-1} cm^{-1} 12 000). Standard assay conditions for type II dehydroquinase were pH 7.0 at 25 °C in Tris/HCl (50 mM) unless otherwise indicated. A typical assay of type II dehydroquinase contained 50 mM Tris/HCl at pH 7.0, 0.5 mM dehydroquinic acid (**1**) and 0.7 U type II dehydroquinase. Each assay was initiated by addition of the enzyme. Solutions of dehydroquinic acid (and analogues) were calibrated by equilibration with type II dehydroquinase and measurement of the change in the UV absorbance at 234 nm due to the formation of the enone-carboxylate chromophore of dehydroshikimic acid.[24]

Assay of Shikimate Dehydrogenase. Shikimate dehydrogenase was assayed in its forward or reverse direction by monitoring the increase or decrease in absorbance at 340 nm in the UV spectrum due to the absorbance of NADPH (ϵ/M^{-1} cm^{-1} 6 200). Standard assay conditions for shikimate dehydrogenase were in the forward direction pH 7.0 at 25 °C with 200 μM NADPH in potassium phosphate buffer (50 mM) unless otherwise indicated.[25] A typical assay of shikimate dehydrogenase contained 50 mM potassium phosphate buffer at pH 7.0, 50 μM dehydroshikimic acid and 1 U of shikimate dehydrogenase. Each assay was initiated by addition of the enzyme. Solutions of shikimic acid (and analogues) were calibrated by equilibration with shikimate dehydrogenase and measurement of the change in the UV absorbance at 340 nm due to the disappearance of NADPH.

Methyl (1*S*,2*R*,4*S*,5*R*)-2-Bromo-4-[(*tert*-butyldimethylsilyl)oxy]-1,5-dihydroxy-3-oxocyclohexanecarboxylate (12). To a solution of **11** (100 mg, 0.27 mmol) in methanol (3 mL) was added sodium methoxide (0.03 mmol as a 10% solution in methanol), and the resultant solution was stirred for 15 min. The reaction mixture was diluted with diethyl ether (20 mL) and then washed successively with 5% HCl (20 mL), water (20 mL), and brine (20 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography eluting with ethyl acetate−hexane (1:2) to yield the *epoxide* **13** (5 mg, 6%), followed by the *bromide* **12** (74 mg, 68%), both as amorphous solids.

Data for **12**: mp 111−112 °C (from diethyl ether−hexane); ^{1}H NMR (250 MHz; $CDCl_3$) δ 5.03 (1 H, d, J = 1.1, 2-H), 4.23 (1 H, dd, J = 1.1 and 9.0, 4-H), 4.08 (1 H, ddd, J = 5.0, 9.0

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(24) 264 nm for (2*R*)-2-bromo- (**2**) and 249 nm for (2*R*)-2-fluoro-3-dehydroquinic acid (**3**).

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and 11.4, 5-H), 3.87 (3 H, s, OCH_3), 3.79 (1 H, s, OH), 3.47 (1 H, s, OH), 2.52 (1 H, dd, J = 5.0 and 13.7, 6_{eq}-H), 2.34 (1 H, dd, J = 11.4 and 13.7, 6_{ax}-H), 0.92 (9 H, s, ^{t}Bu), 0.16 and 0.04 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 194.4 (C), 171.7 (C), 82.9 (CH), 77.1 (C), 71.5 (CH), 60.7 (CH), 54.0 (OCH_3), 38.3 (CH_2), 25.8 (C(CH_3)$_3$), 18.4 (*C*(CH_3)$_3$), −4.4 and −5.4 (each, $SiCH_3$); υ_{max} (Nujol)/cm^{-1} 3640 (OH), 3590 (OH), 1740 (C=O). Anal. Calcd for $C_{14}H_{25}BrSiO_6$: C, 42.32; H, 6.30. Found: C, 42.23; H, 6.40.

Data for *methyl (1S,2R,4S,5R)-4-[(tert*-butyldimethylsilyl)-oxy]-1,2-epoxy-1,5-dihydroxy-3-oxocyclohexanecarboxylate (**13**): mp 121−122 °C (from hexane); ^{1}H NMR (250 MHz; $CDCl_3$) δ 3.87 (1 H, ddd, J = 4.9, 9.6 and 10.2, 5-H), 3.79 (3 H, s, OCH_3), 3.74 (1 H, d, J = 9.6, 4-H), 3.56 (1 H, d, J = 0.8, 2-H), 2.89 (1 H, ddd, J = 0.8, 4.9 and 15.5, 6_{eq}-H), 2.46 (1 H, dd, J = 10.2 and 15.5, 6_{ax}-H), 2.38 (1 H, br s, OH), 0.90 (9 H, s, ^{t}Bu), 0.17 and 0.07 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 198.2 (C), 167.8 (C), 80.8 (CH), 66.3 (CH), 57.3 (C + CH), 53.2 (OCH_3), 29.7 (CH_2), 25.8 (C(CH_3)$_3$), 18.4 (*C*(CH_3)$_3$), −4.3 and −5.4 (each, $SiCH_3$); υ_{max} (CCl_4)/cm^{-1} 3680 (OH), 2920 and 1730 (C=O). Anal. Calcd for $C_{14}H_{25}BrSiO_6$: C, 53.16; H, 7.59. Found: C, 52.86; H, 7.55.

Employing greater quantities of sodium methoxide (>0.2 equiv) and extended reaction times (>30 min) resulted in the formation of a white precipitate (purified as described above) of *methyl 3-[(tert*-butyldimethylsilyl)oxy]gallate (40−80%): mp 116−117 °C (from ethyl acetate−hexane); ^{1}H NMR (250 MHz; $CDCl_3$) δ 7.30 (1 H, d, J = 1.9, ArH), 7.14 (1 H, d, J = 1.9, ArH), 5.65 (1 H, s, OH), 5.52 (1 H, br.s., OH), 3.85 (3 H, s, OCH_3), 1.00 (9 H, s, ^{t}Bu) and 0.28 (6 H, s, 2 × $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 166.8 (C), 143.7 (C), 142.3 (C), 138.9 (C), 121.6 (C), 112.0 (CH), 111.0 (CH), 52.1 (OCH_3), 25.7 (C(*C*H_3)$_3$), 18.2 (*C*(CH_3)$_3$) and −4.3 (2 × $SiCH_3$). Anal. Calcd for $C_{14}H_{22}SiO_4$: C, 56.38; H, 7.38. Found: C, 56.16; H, 7.22.

Methyl (1*S*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1,5-dihydroxy-4-oxocyclohex-2-enecarboxylate (14). To a solution of the alcohol **12** (10 mg, 24.94 μmol) in dichloromethane (2 mL) was added triethylamine (15 μL, 99.75 μmol). The resultant mixture was stirred at room temperature for 8 h and then was diluted with dichloromethane (10 mL) and washed with 1 M HCl (2 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography eluting with ethyl acetate−hexane (1:2) to yield the *enone* **14** (7 mg, 84%), an amorphous white solid: mp 94−95 °C (from hexane); ^{1}H NMR (250 MHz; $CDCl_3$) δ 5.86 (1 H, d, J = 1.8, 2-H), 4.71 (1 H, ddd, J = 5.7, 2.1 and 12.1, 5-H), 3.85 (3 H, s, OCH_3), 3.41 (1 H, s, OH), 3.36 (1 H, d, J = 2.1, OH), 2.45 (1 H, ddd, J = 1.8, 5.7 and 12.8, 6_{eq}-H), 2.32 (1 H, dd, J = 12.1 and 12.8, 6_{ax}-H), 0.93 (9 H, s, ^{t}Bu), 0.18 and 0.15 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 196.6 (C), 174.7 (C), 147.8 (C), 123.3 (CH), 71.4 (C), 69.4 (CH), 53.8 (OCH_3), 41.2 (CH_2), 25.5 (C(*C*H_3)$_3$), 18.3 (*C*(CH_3)$_3$), −4.7 and −4.8 (each, $SiCH_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3600−3500, 1740, 1700, 1630. Anal. Calcd for $C_{14}H_{24}SiO_6$: C, 53.16; H, 7.59. Found: C, 52.93; H, 7.70.

Methyl (4*S*,5*R*)-2-Bromo-4-[(*tert*-butyldimethylsilyl)-oxy]-5-(trifluoroacetyl)-3-oxocyclohex-1-enecarboxylate (15). To a solution of the alcohol **12** (15 mg, 38 μmol) in dichloromethane (2 mL) and pyridine (100 μL, 1.26 mmol) was added trifluoroacetic anhydride (12 μL, 83.3 μmol). The resultant mixture was stirred for 2 h after which time it was diluted with dichloromethane (15 mL). The mixture was washed with 1 M HCl (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash chromatography eluting with ethyl acetate−hexane (1:9) to afford the *trifluoroester* **15** (16 mg, 90%) as an amorphous solid: mp 74−75 °C (from petroleum ether bp 30−40 °C); ^{1}H NMR (250 MHz; $CDCl_3$) δ 5.38 (1 H, ddd, J = 5.4, 9.1 and 10.3, 5-H), 4.42 (1 H, d, J = 10.3, 4-H), 3.90 (3 H, s, OCH_3), 3.24 (1 H, dd, J = 5.4 and 18.0, 6_{eq}-H), 2.84 (1 H, dd, J = 9.1 and 18.0, 6_{ax}-H), 0.88 (9 H, s, ^{t}Bu), 0.16 and 0.09 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 188.9 (C), 165.4 (C), 156.4 (*C*OCF_3, q, J = 43), 144.0 (C), 122.5 (C), 114.2 (CF_3, q, J = 285), 75.9 (CH), 74.6 (CH), 53.1 (OCH_3), 32.3 (CH_2), 25.4 (C(*C*H_3)$_3$), 18.1 (*C*(CH_3)$_3$), −4.6 and −5.8 (each, $SiCH_3$); υ_{max} (CCl_4)/cm^{-1} 2900, 1765, 1698. Anal. Calcd for $C_{16}H_{22}BrF_3SiO_6$: C, 40.42; H, 4.63. Found: C, 40.25; H, 4.60.

Methyl (4*S*,5*R*)-2-Bromo-4-[(*tert*-butyldimethylsilyl)-oxy]-5-hydroxy-3-oxocyclohex-1-enecarboxylate (16). To a solution of the ester **15** (60 mg, 0.13 mmol) in tetrahydrofuran (2 mL) was added 1 mL of an aqueous solution of K_2CO_3 (17 mg, 0.13 mmol). The resultant mixture was stirred for 2 h, diluted with diethyl ether (25 mL), and washed successively with 1 M HCl (10 mL), water (10 mL), and brine (10 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to afford the *alcohol* **16** (39 mg, 81%) as an amorphous solid: mp 65−66 °C (from petroleum ether bp 40−60 °C); ^{1}H NMR (250 MHz; $CDCl_3$) δ 4.15 (1 H, d, J = 10.4, 4-H), 4.02 (1 H, ddd, J = 5.1, 9.4 and 10.4, 5-H), 3.88 (3 H, s, OCH_3), 3.05 (1 H, dd, J = 5.1 and 18.3, 6_{eq}-H), 2.66 (1 H, dd, J = 9.4 and 18.3, 6_{ax}-H), 2.52 (1 H, br s, OH), 0.91 (9 H, s, ^{t}Bu), 0.20 and 0.08 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 190.4 (C), 166.3 (C), 146.0 (C), 121.6 (C), 80.2 (CH), 70.3 (CH), 53.0 (OCH_3), 35.0 (CH_2), 25.8 (C(*C*H_3)$_3$), 18.4 (*C*(CH_3)$_3$), −5.5 and −4.4 (each, $SiCH_3$); υ_{max} (Nujol)/cm^{-1} 3550 (OH), 1710 (C=O). Anal. Calcd for $C_{14}H_{23}BrSiO_5$: C, 44.33; H, 6.07. Found: C, 44.30; H, 6.06.

Methyl (4*S*,5*R*)-2-Bromo-4,5-dihydroxy-3-oxocyclohex-1-enecarboxylate (17). A solution of the silyl ether **16** (45 mg, 0.12 mmol) in tetrahydrofuran (0.3 mL), acetic acid (0.4 mL), and water (0.4 mL) was stirred at 35 °C for 72 h. It was then lypholized to afford **17** (29 mg, 92%) as a fine yellow powder. Recrystallization afforded pure *diol* **17** as clear light yellow plates: mp 125−126 °C (from dichloromethane−hexane); ^{1}H NMR (250 MHz; d_6-acetone) δ 5.0−4.6 (2 H, br s, 2 × OH), 4.26 (1 H, d, J = 10.0, 4-H), 4.06 (1 H, ddd, J = 5.1, 8.7 and 10.0, 5-H), 3.86 (3 H, s, OCH_3), 3.01 (1 H, dd, J = 5.1 and 18.1, 6_{eq}-H), and 2.71 (1 H, dd, J = 8.7 and 18.1, 6_{ax}-H); ^{13}C NMR (63 MHz; APT; d_6-acetone) δ 192.4 (C), 167.0 (C), 148.7 (C), 120.6 (C), 79.1 (CH), 70.6 (CH), 53.0 (OCH_3), 36.4 (CH_2); υ_{max} (Nujol)/cm^{-1} 3500−3200 (OH), 1740, 1710 (each C=O). Anal. Calcd for $C_8H_9BrO_5$: C, 36.23; H, 3.40. Found: C, 36.33; H, 3.42.

***p*-Methoxybenzyl (1*S*,2*R*,4*S*,5*R*)-2-Bromo-4-[(*tert*-butyldimethylsilyl)oxy]-1,5-dihydroxy-3-oxocyclohexanecarboxylate (18).** To a stirred solution of the lactone **11** (760 mg, 2.08 mmol) in tetrahydrofuran (30 mL) was added *p*-methoxybenzyl alcohol (218 mg, 1.58 mmol). To this was added 10 mmol aliquots of lithium *p*-methoxybenzyloxide (prepared by addition of *n*-BuLi to a solution of *p*-methoxybenzyl alcohol in tetrahydrofuran) in 15 min intervals until reaction was complete. The reaction mixture was diluted with ethyl acetate (150 mL) and washed with 1 M HCl (20 mL) and water (20 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a residue which was purified by flash chromatography eluting with ethyl acetate−hexane (4:1) to yield the *ester* **18** as an oil (660 mg, 80%): ^{1}H NMR (250 MHz; $CDCl_3$) δ 7.31 (2 H, d, J = 8.7, ArH), 6.90 (2 H, d, J = 8.7, ArH), 5.23 and 5.17 (each 1 H, d, J = 11.7, C*H*HAr), 4.99 (1 H, d, J = 1.1, 2-H), 4.20 (1 H, dd, J = 1.1 and 9.0, 4-H), 4.07 (1 H, ddd, J = 5.0, 13.8 and 11.5, 5-H), 3.81 (3 H, s, OCH_3), 3.63 (1 H, br s, OH), 2.58 (1 H, br s, OH), 2.47 (1 H, dd, J = 5.0 and 13.8, 6_{eq}-H), 2.31 (1 H, dd, J = 11.5 and 13.8, 6_{ax}-H), 0.91 (9 H, s, ^{t}Bu), 0.14 and 0.02 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 194.5 (C), 171.2 (C), 160.1 (C), 130.7 (CH), 126.3 (C), 114.1 (CH), 82.6 (CH), 77.0 (C), 71.7 (CH), 68.9 (CH_2), 60.6 (CH), 55.3 (OCH_3), 38.4 (CH_2), 25.8 (C(*C*H_3)$_3$), 18.4 (*C*(CH_3)$_3$), −4.5 and −5.4 (each $SiCH_3$); υ_{max} (CCl_4)/cm^{-1} 3600, 2800−3000, 1720, 1590.

***p*-Methoxybenzyl (4*S*,5*R*)-2-Bromo-4-[(*tert*-butyldimethylsilyl)oxy]-5-(trifluoroacetyl)-3-oxocyclohex-1-enecarboxylate (19).** To a solution of the alcohol **18** (500 mg, 1.26 mmol) in dichloromethane (40 mL) and pyridine (300 μL, 3.78 mmol) was added trifluoroacetic anhydride (375 μL, 2.65 mmol), and the resultant mixture was stirred at room temperature for 4 h. The reaction mixture was washed successively with 1 M HCl (2 × 40 mL), water (40 mL), and then brine (40 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated to leave a light yellow oil. Purification

by flash chromatography (ethyl acetate−hexane, 1:4) afforded the desired *ester* **19** (416 mg, 72%) as an oil: ^{1}H NMR (250 MHz; $CDCl_3$) δ 7.34 (2 H, d, J = 8.7, ArH), 6.90 (2 H, d, J = 8.7, ArH), 5.37 (1 H, ddd, J = 5.5, 9.4 and 10.5, 5-H), 5.24 (2 H, s, CH_2Ar), 4.41 (1 H, d, J = 10.5, 4-H), 3.81 (3 H, s, OCH_3), 3.19 (1 H, dd, J = 5.5 and 18.0, 6_{eq}-H), 2.82 (1 H, dd, J = 9.4 and 18.0, 6_{ax}-H), 0.87 (9 H, s, ^{t}Bu), 0.17 and 0.05 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 188.7 (C), 165.0 (C), 160.1 (C), 156.4 ($COCF_3$, q, J = 43), 144.7 (C), 130.7 (CH), 126.2 (C), 122.4 (C), 114.2 (CF_3, q, J = 285), 114.1 (C), 76.0 (CH), 74.6 (CH), 68.3 (CH_2), 32.4 (CH_2), 25.4 ($C(CH_3)_3$), 18.2 ($C(CH_3)_3$), −4.6 and −5.8 (each $SiCH_3$); υ_{max} (CCl_4)/cm^{-1} 2880, 1770 and 1698; MS (CI$^+$) m/z (%) 485 and 483 ([M + NH_4]$^+$ − $COCF_3$); HRMS calcd for $C_{21}H_{28}BrSiO_6$: MNH_4^+, 485.0818. Found: MNH_4^+, 485.0813.

***p*-Methoxybenzyl (4*S*,5*R*)-2-Bromo-4-[(*tert*-butyldimethylsilyl)oxy]-5-hydroxy-3-oxocyclohex-1-enecarboxylate (20).** To a stirred solution of the ester **19** (340 mg, 0.6 mmol) in tetrahydrofuran (15 mL) and water (15 mL) was added 10 mL of an aqueous solution of K_2CO_3 (84 mg, 0.61 mmol) over a 30 min period. After stirring for a further 15 min, dichloromethane (50 mL) was added and washed successively with 1 M HCl (20 mL), water (20 mL), followed by brine (20 mL). The organic phase was dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure to afford the *alcohol* **20** as an oil, which was purified by flash chromatography (ethyl acetate−hexane, 1:4) to yield **20** as an oil (220 mg, 78%): ^{1}H NMR (250 MHz; $CDCl_3$) δ 7.34 (2 H, d, J = 8.6, ArH), 6.89 (2 H, d, J = 8.6, ArH), 5.24 (2 H, s, CH_2Ar), 4.13 (1 H, d, J = 10.5, 4-H), 4.00 (1 H, dddd, J = 1.5, 5.2, 9.5 and 10.5, 5-H), 3.81 (3 H, s, OCH_3), 3.04 (1 H, dd, J = 5.2 and 18.3, 6_{eq}-H), 2.65 (1 H, dd, J = 9.5 and 18.3, 6_{ax}-H), 2.54 (1 H, br d, J = 1.5, OH), 0.92 (9 H, s, ^{t}Bu), 0.21 and 0.08 (each 3 H, s, $SiCH_3$); ^{13}C NMR (63 MHz; APT; $CDCl_3$) δ 190.5 (C), 165.8 (C), 160.1 (C), 146.2 (C), 130.6 (C), 126.5 (C), 121.5 (C), 114.1 (CH), 80.2 (CH), 70.3 (CH), 68.0 (CH_2Ar), 55.3 (CH_3), 35.1 (CH_2), 25.9 ($C(CH_3)_3$), 18.4 ($C(CH_3)_3$), −4.3, and −5.5 (each $SiCH_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3560 and 1710.

(4*S*,5*R*)-2-Bromo-4,5-dihydroxy-3-oxocyclohex-1-enecarboxylic acid [2-bromoshikimic acid] (5). A solution of **20** (220 mg, 0.43 mmol) in tetrahydrofuran (0.5 mL), acetic acid (2 mL), and water (0.5 mL) was stirred at 50 °C for 72 h. The solvent was removed under reduced pressure, and the residue was partitioned between water (30 mL) and diethyl ether (30 mL). The aqueous fraction was washed further with diethyl ether (30 mL) and lyophilized to afford **5** (100 mg, 92%). Recrystallization gave pure *acid* **5** as white needles: mp 176−178 °C (from ethyl acetate−hexane); ^{1}H NMR (250 MHz; D_2O) δ 4.33 (1 H, d, J = 11.2, 4-H), 4.06 (1 H, ddd, J = 5.5, 9.9 and 11.2, 5-H), 3.03 (1 H, dd, J = 5.5 and 18.0, 6_{eq}-H) and 2.76 (1 H, dd, J = 9.9 and 18.0, 6_{ax}-H); ^{13}C NMR (63 MHz; APT; D_2O) δ 196.8 (C), 174.2 (C), 156.1 (C), 116.4 (C), 80.6 (CH), 72.4 (CH), 38.3 (CH_2); υ_{max} (Nujol)/cm^{-1} 3100−3650 (br), 1720, 1690; λ_{max} (H_2O)/nm 264 (ϵ/M^{-1} cm^{-1} 6 300). Anal. Calcd for $C_7H_7BrO_5 \cdot H_2O$: C, 31.23; H, 3.34. Found: C, 31.52; H, 3.26.

(4*S*,5*R*)-2-Fluoro-4,5-dihydroxy-3-oxocyclohex-1-enecarboxylic Acid (6). To a solution of the (2*R*)-2-fluoro-3-dehydroquinic acid (**3**) (1 mg, 4.2 nmol) in 0.5 mL ammonium bicarbonate (100 mM) at pH 7.8 and 25 °C was added type II dehydroquinase from *M. tuberculosis* (10 μL, 1.7 U). After 24 h, approximately 88% conversion had occurred by reaching the equilibrium. The enzyme was removed by centrifugation on Amicon Centricon-10 microconcentrator,and lyophilized, and the resultant mixture was purified using a preparative organic acids HPLC column (eluting with 50 mM formic acid at 1.2 mL min^{-1} detection at 277 nm). Fractions eluting with retention time of 17 min were lyophilized to give the *acid* **6** as an oil: ^{1}H NMR (500 MHz; D_2O) δ 4.20 (1 H, d, J = 11.1, 4-H), 3.94 (1 H, m, 5-H), 2.84 (1 H, dt, J = 5.0 and 17.4, 6_{eq}-H), 2.64 (1 H, m, 6_{ax}-H); ^{19}F NMR (235 MHz; D_2O) δ −133 (1 F, br s); λ_{max} (H_2O)/nm 249 (ϵ/M^{-1} cm^{-1} 9 600); MS (FAB +ve) m/z (%) 191 (MH^+); HRMS calcd for $C_7H_8FO_5$: MH^+, 191.0320. Found: MH^+, 191.0314.

(3*S*,4*S*,5*R*)-2-Fluoro-3,4,5-trihydroxycyclohex-1-enecarboxylic Acid [2-fluoroshikimic acid] (9).[6] To a solution of (2*R*)-2-fluorodehydroquinic acid (**3**) (6 mg, 29 μmol) in potassium phosphate buffer at pH 7.0, 26 °C (0.6 mL, includes 10% D_2O, 100 mM) was added type II form *M. tuberculosis* (15 μL, 2.6 U). The reaction was monitored by ^{19}F NMR spectroscopy. After 24 h, the reaction had reached equilibrium, and NADPH (29 mg, 35 μmol) and shikimate dehydrogenase (10 U, 10 μL) were added and after 24 h it was observed that all (2*R*)-2-fluorodehydroquinic acid (**3**) had been transformed into 2-fluoroshikimic acid (**9**). The crude mixture was purified by FPLC using a Mono Q HR 10/10 column. Eluting with a gradient of 0−0.3 M ammonium bicarbonate over 140 mL and then 0.3−1 M over 110 mL, at 1.0 mL min^{-1} at 254 nm. Fractions eluting at 220 mM ammonium bicarbonate contained 2-fluoroshikimic acid (**9**) (5 mg, 91%), ^{1}H NMR (500 MHz; D_2O) δ 4.34 (1 H, dd, J = 8.7 and 4.5, 3-H), 3.82 (1 H, m, 5-H), 3.63 (1 H, dd, J = 9.9 and 4.5, 4-H), 2.61 (1 H, dt, J = 16.7 and 5.6, 6_{eq}-H), 2.12 (1 H, m, 6_{ax}-H); ^{19}F NMR (235 MHz; D_2O) δ −112 (1 F, q, J = 7).

(3*S*,4*S*,5*R*)-2-Bromo-3,4,5-trihydroxycyclohex-1-enecarboxylic Acid [2-bromoshikimic acid] (8). To a solution of 2-bromodehydroshikimic acid (**5**) (6 mg, 22 μmol) in deuterated potassium phosphate buffer at pD 7.0, 26 °C (0.5 mL, 100 mM) were added NADPH (30 mg, 36 μmol) and shikimate dehydrogenase (10 U, 10 μL). The reaction was followed by ^{1}H NMR spectroscopy. After 24 h, approximately 78% conversion had occurred, and the reaction was assumed to have reached equilibrium. NADPH/$NADP^+$ were removed by anion exchange chromatography using a SAX HPLC column (eluting with 50 mM NH_4OAc, pH 6.9 at 4.00 mL min^{-1}, UV detection at 260 nm). Fractions eluting between 4 and 6 min were collected and further purified using a semipreparative organic acids HPLC column (eluting with 50 mM formic acid at 1.2 mL min^{-1}, UV detection at 240 nm). Fractions eluting with a retention time between 16 and 18 min were combined and lyophilized to give *2-bromoshikimic acid* **8** (5 mg, 90%) as an oil: ^{1}H NMR (400 MHz; D_2O) δ 4.39 (1 H, d, J = 4.4, 3-H), 3.94 (1 H, ddd, J = 10.2, 9.0 and 5.8, 5-H), 3.75 (1 H, dd, J = 10.2 and 4.4, 4-H), 2.76 (1 H, dd, J = 17.2 and 5.8, 6_{eq}-H), 2.27 (1 H, dd, J = 17.2 and 9.0, 6_{ax}-H); ^{13}C NMR (100 MHz; APT; D_2O) δ 175.5 (C), 137.9 (C), 128.6 (C), 67.7 (3 × CH) and 37.5 (CH_2); υ_{max} (thin film)/cm^{-1} 3368 (OH), 2921 (CH), 1725 (C=O) and 1640 (C=C); λ_{max} (H_2O)/nm 196; MS (CI$^+$) m/z (%) 272 and 270 ([M + NH_4]$^+$); HRMS calcd for $C_7H_{13}BrNO_5$: MNH_4^+, 269.9977. Found: MNH_4^+, 269.9977.

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