Synthesis of 4-deoxyquinic, 4-deoxyshikimic and 4-deoxy-3-dehydroshikimic acids

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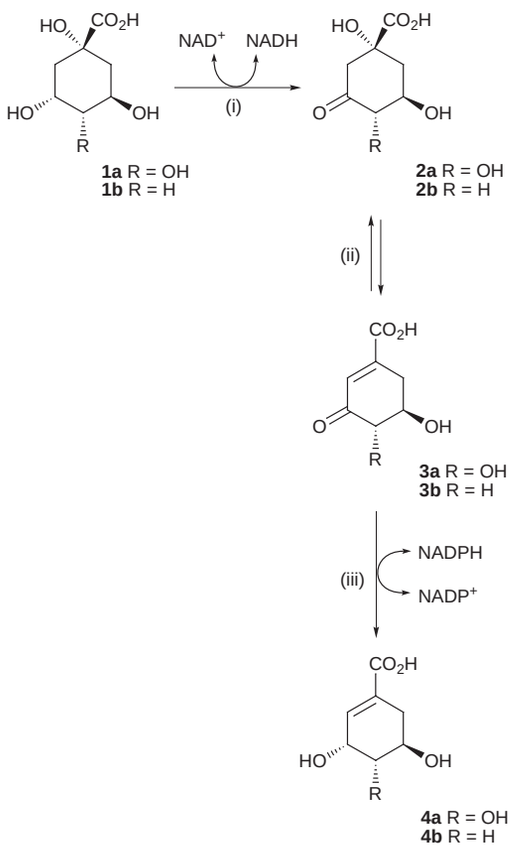
Received (in Cambridge) 14th December 1998, Accepted 28th January 1999

The synthesis of 4-deoxyquinic acid (**1b**) from (−)-quinic acid (**1a**) and 4-deoxyshikimic acid (**4b**) from shikimic acid (**4a**) respectively are described. 4-Deoxyshikimic acid (**4b**) was converted enzymatically into 4-deoxy-3-dehydroshikimic acid (**3b**) using shikimate dehydrogenase. The C(4) hydroxy group is shown not to contribute significantly to substrate specificity for shikimate dehydrogenase. Attempts to synthesise 4-deoxy-3-dehydroquinic acid (**2b**) are described.

The shikimic acid pathway[1] to the aromatic amino acids continues to attract the attention of synthetic and biological chemists, almost forty years after Professor Raphael's elegant synthesis of shikimic acid.[2] The pathway is the target for the herbicide glyphosate,[3] and recent results suggest that inhibitors of the pathway may potentially be antiparasitic agents.[4] As part of our studies of the pathway we are determining the important interactions involved in substrate recognition for several of the enzymes. We have previously reported studies on the substrate specificity of dehydroquinase[5] and shikimate dehydrogenase[6] using substrate analogues lacking either the C(5), or the C(4) and C(5) hydroxy groups.

In this paper we describe the first syntheses of three novel substrate analogues lacking only the C(4) hydroxy group. 4-Deoxyquinic acid‡ (**1b**) has been synthesised from quinic acid (**1a**), and 4-deoxyshikimic acid§ (**4b**) from shikimic acid (**4a**). Although quinic acid is not on the shikimic acid pathway it is the precursor to 3-dehydroquinic acid (**2a**), which is common to the shikimic acid and quinic acid pathways (Scheme 1). 4-Deoxyshikimic acid (**4b**) was converted enzymatically using shikimate dehydrogenase to 4-deoxy-3-dehydroshikimic acid¶ (**3b**). Synthetic approaches to 4-deoxy-3-dehydroquinic acid (**2b**) proved unsuccessful due to the propensity of the β-hydroxy ketone to eliminate and aromatise.



Scheme 1 *Enzymes*: (i) quinate dehydrogenase; (ii) dehydroquinase; (iii) shikimate dehydrogenase.

Synthesis of 4-deoxyquinic acid

The synthetic route to 4-deoxyquinic acid (**1b**) is shown in Scheme 2. The key to the approach is the ability to differentially functionalise the three secondary hydroxy groups in (−)-quinic acid (**1a**). We have previously shown that the lactone **5**, formed from **1a** under acid catalysis (*p*-TSOH), can be silylated selectively using *tert*-butyldimethylsilyl chloride at 0 °C on C(5) to form **6**.[7]

The secondary alcohol in **6** was selectively reacted with phenyl chlorothionoformate to form the phenyl thiocarbonate ester in **7**. Slow addition of the phenyl chlorothionoformate was necessary to avoid reaction at the tertiary hydroxy group. The phenyl thiocarbonate ester was then removed by radical reduction with tri-*n*-butyltin hydride to afford the lactone **8**. This reaction also needed careful control of the reaction conditions. Slow addition of a dilute solution of **7** in toluene to a solution of tri-*n*-butyltin hydride and AIBN in toluene at reflux minimised decomposition of the product, which was formed in 88% yield.

Based on our previous studies of related compounds,[7] we expected to be able to remove the silyl group and open the

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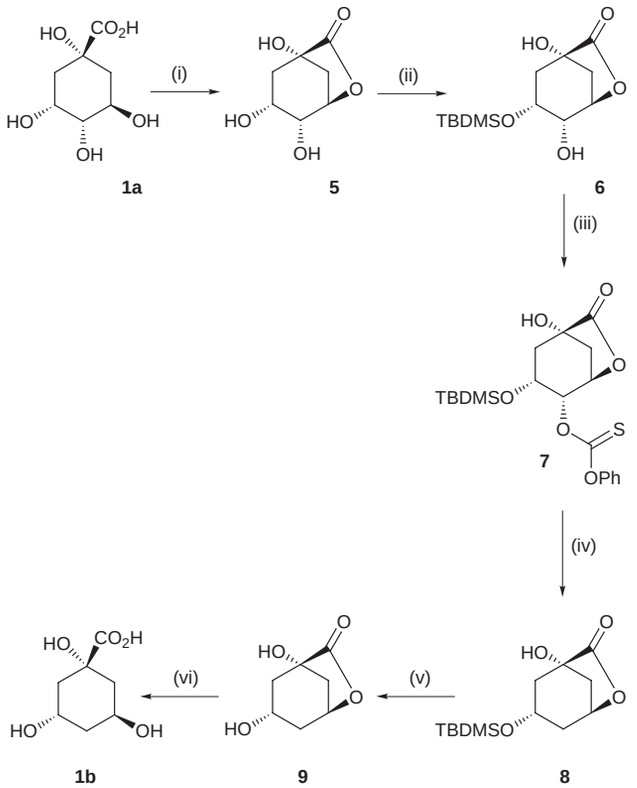
‡ IUPAC name: (1*S*,3*S*,5*S*)-1,3,5-trihydroxycyclohexanecarboxylic acid.
§ IUPAC name: (3*S*,5*S*)-3,5-dihydroxycyclohex-1-enecarboxylic acid.
¶ IUPAC name: (5*S*)-5-hydroxy-3-oxocyclohex-1-enecarboxylic acid.



Scheme 2 *Reagents, conditions and yields*: (i) ref. 10 (95%); (ii) ref. 10 (79%); (iii) ClCSOPh, DMAP, acetonitrile, RT (62%, from recovered starting material 84%); (iv) $^{n}Bu_3SnH$, AIBN, toluene, reflux (88%); (v) TBAF, AcOH, THF, RT (99%); (vi) NaOH, H_2O, RT (99%).

lactone in **8** in a one pot reaction by mild acid treatment (AcOH–THF–H_2O). However these conditions gave unidentified degradation products, prompting us to use a stepwise approach. Removal of the silyl protecting group with TBAF gave the alcohol **9**, in a low yield (25%). Suspecting that the problem was due to the basicity of TBAF, we carried out the reaction in the presence of acetic acid. Under these conditions the alcohol **9** was obtained in 99% yield. Hydrolysis of the lactone with sodium hydroxide gave the desired 4-deoxyquinic acid (**1b**) quantitatively.

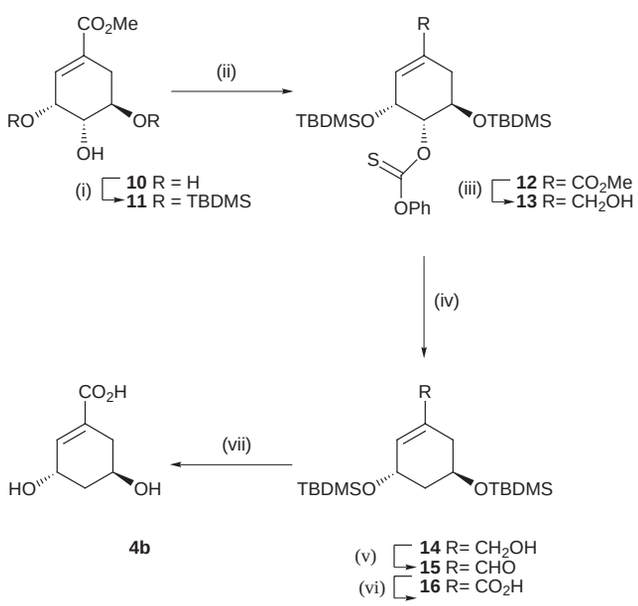
Synthesis of 4-deoxyshikimic acid

The synthesis of 4-deoxyshikimic acid (**4b**) is shown in Scheme 3. The synthesis of the protected 4-deoxyaldehyde **15** from shikimic acid (**4a**) followed a previously published sequence, albeit one for which not all of the intermediates had been significantly characterised.[8,9] Methyl shikimate **10** was first diprotected on the C(3) and C(5) hydroxys using TBDMSCl. This left the C(4) hydroxy in **11** free to react with phenyl chlorothionoformate, to form the phenyl thiocarbonate **12**. Reduction of the ester using DIBAL-H at −78 °C produced the alcohol **13**, from which the C(4) substituent was removed by radical elimination using tri-*n*-butyltin hydride and AIBN in toluene. The 4-deoxy alcohol **14** was finally oxidised to the aldehyde **15**. The yield over six steps was 31%. To complete the synthesis of **4a**, the aldehyde was oxidised with sodium chlorite[10] to the protected acid **16** in 98% yield. The hydroxy groups were then deprotected by treatment with HCl in ethanol for two hours, which gave 4-deoxyshikimic acid (**4b**) in 99% yield. Longer reaction times resulted in progressive epimerization at C(3).

Synthesis of 4-deoxy-3-dehydroshikimic acid

The regiospecific oxidation of 4-deoxyshikimic acid (**4b**) to form 4-deoxy-3-dehydroshikimic acid (**3b**) was achieved by incubation with shikimate dehydrogenase in sodium bicarbonate buffer at pH 10.6 and 25 °C and in the presence of $NADP^+$ (Scheme 1). The reaction was monitored by ^1H NMR spectroscopy. Formation of **3b** was followed by the appearance of a singlet at δ 6.33 due to the C(2) proton, and the disappearance of the corresponding singlet at δ 6.69 for 4-deoxyshikimic acid (**4b**). After chromatographic purification, 4-deoxy-3-dehydroshikimic acid (**3b**) was obtained in 85% yield. This compound decomposed to form 3-hydroxybenzoic acid on storage in less than 24 hours.

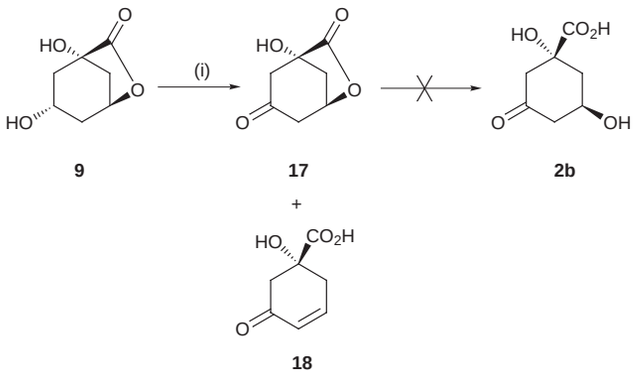
The enzymatic conversion of **4b** into **3b** was also studied spectrophotometrically at 340 nm, monitoring the formation of NADPH, to determine the steady state kinetic constants. The K_M was 2.75 mM, k_{cat} 152 s^{-1} and k_{cat}/K_M 0.55×10^5 M^{-1} s^{-1}. This compares with a K_M of 0.44 mM, k_{cat} 232 s^{-1} and k_{cat}/K_M 5.28×10^5 M^{-1} s^{-1} for shikimic acid (**4a**). We had previously shown that the 5-deoxy-, and 4,5-dideoxy-3-dehydroshikimic acids are substrates for shikimate dehydrogenase.[6] This study showed removal of the C(5) hydroxy group alone resulted in only a two-fold drop in k_{cat}/K_M, but lack of both C(4) and C(5) hydroxy groups caused a 10^6-fold drop in substrate specificity. A naive interpretation of that result suggested that removal of the C(4) hydroxy group alone should have a large effect on substrate specificity. Consequently the finding that removal of this group in 4-deoxyshikimic acid (**4b**) only results in a 10-fold loss of specificity relative to shikimic acid (**4a**) is surprising. Taken together these observations suggest that it is important to have a single hydroxy group at either C(4) or C(5), and that the presence of the second hydroxy group [at C(5) or C(4)] makes only a small additional contribution to the substrate specificity.



Scheme 3 *Reagents and conditions:* (i) TBDMSCl, DMAP, Bu_4NI, Et_3N, DMF, RT (87%); (ii) ClCSOPh, DMAP, acetonitrile, RT (82%); (iii) DIBAL-H, toluene, −78 °C (93%); (iv) $^{n}Bu_3SnH$, AIBN, toluene, reflux (62%); (v) PCC, dichloromethane, 4 Å molecular sieves, RT (83%); (vi) $NaClO_2$, NaH_2PO_4, $^{t}BuOH$, 2-methylbut-2-ene, RT (98%); (vii) HCl, ethanol (1%), 0 °C→RT (99%).

Attempted formation of 4-deoxy-3-dehydroquinic acid

The remaining 4-deoxy analogue in Scheme 1, 4-deoxy-3-dehydroquinic acid (**2b**) proved to be an elusive target, due to its propensity to aromatise. Oxidation of the secondary hydroxy group in **9** using pyridinium chlorochromate (PCC) in the presence of 4 Å activated molecular sieves at neutral pH gave the ketolactone **17** in 52% yield and also 17% of enone **18** (Scheme 4), which was incompletely characterised because of its instability. Attempts to open the lactone **17** under either



Scheme 4 *Reagents*, *conditions and yields*: (i) PCC, dichloromethane, 4 Å molecular sieves, RT (52%).

mildly basic or acidic conditions led to formation of 3-hydroxybenzoic acid. This compound was also formed from **17** slowly on storage.

Having failed to open the lactone **17**, selective oxidation of the axial C(3) hydroxy group of the 4-deoxyquinic acid (**1b**) was attempted. Several approaches were tried by analogy with the formation of 3-dehydroquinic acid (**2a**) from quinic acid (**1a**). No reaction was observed on treatment with oxygen on platinium,[11] whereas attempted oxidation with nitric acid,[12] led to decomposition.

Finally we attempted to synthesise 4-deoxy-3-dehydroquinic acid (**2b**) enzymatically. 4-Deoxy-3-dehydroshikimic acid (**3b**) was generated from 4-deoxyshikimic acid (**4b**), using shikimate dehydrogenase with $NADP^+$ as cofactor. This was then incubated with dehydroquinase (Scheme 1) (either type I from *E. coli* or type II from *M. tuberculosis* or *A. nidulans*) in potassium phosphate buffer at pD 7.0 and 26 °C. The incubation was followed by ^1H NMR spectroscopy. In no case was formation of 4-deoxy-3-dehydroquinic acid (**2b**) observed. There was no decrease in the intensity of the singlet at δ 6.33 due to the proton at C(2) of **3b**, although deuterium exchange of the protons at C(4) was observed (after 48 h: 85% deuteriation in 4_{eq}-H and 40% deuteriation in 4_{ax}-H). Analysis of the reaction mixture by HPLC also provided no evidence for formation of 4-deoxy-3-dehydroquinic acid (**2b**). The failure to detect the formation of **2b** from **3b** may be due to an unfavourable equilibrium between these species. The corresponding equilibrium between **2a** and **3a** is 15 in favour of the 3-dehydroshikimic acid (**3a**).[13] Preliminary studies using quinate dehydrogenase and NAD^+ to oxidise 4-deoxyquinic acid (**1b**) showed slow formation of NADH and suggested that some **2b** was being formed.[14] However, problems of enzyme stability, the slow turnover and an apparently unfavourable equilibrium meant that this approach was not pursued further.

Experimental

General

All organic solvents were purified and freshly distilled prior to use according to the methods of Armarego *et al*.[15] Analytical thin layer chromatography was carried out on commercial Kieselgel 60 0.25 mm silica plates using either UV absorption, iodine staining or potassium permanganate(VII) spray for visualisation. Flash chromatography was carried out using 230–400 mesh Kieselgel 60 silica. Where quoted, carboxylic acids were analysed or purified by HPLC which was carried out on either a semi-preparative (300 × 8 mm), or preparative (300 × 16 mm) Bio-Rad Aminex Ion Exclusion HPX-87H Organic Acids column and on a LKB HPLC system. The eluent used for these columns was 50 mM aqueous formic acid, at a flow rate of 0.6 cm^3 min^{-1} (semi-preparative column) or 1.2 cm^3 min^{-1} (preparative column). FPLC was carried out on a Pharmacia FPLC system equipped with a GP-250 plus gradient programer, a UV-M monitor and a Frac-100 fraction collector, using a Mono Q HR 10/10 column and eluting with a gradient of ammonium bicarbonate at 1.0 cm^3 min^{-1} with the UV detector set at 254 nm.

Melting points were determined on either a Buchi 510 or Reichert melting points apparatus and are uncorrected. IR spectra were recorded on a 1710 Fourier Transform spectrometer. Mass spectra were recorded on a Kratos MS890 double-focussing magnetic sector apparatus (for EI and FAB) or on a Bruker Bio-Apex 4.7E for ESI. Electrospray mass spectra were recorded on a VG BioQ quadrupole mass spectrometer. Optical rotations were measured on a AA-10 automatic polarimeter (Optical Activity Ltd.), $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra and enzyme assays were recorded on a Varian Cary 1E UV/Visible spectrophotometer at 25 °C (using either a temperature control thermostat or a thermostatted water bath), using 1 cm path quartz cells. NMR spectra were recorded on either a Bruker WM-250, WM-400, DPX-250 or DPX-500 NMR spectrometer in deuterated solvents. *J* values are given in Hz.

Type II dehydroquinase from *M. tuberculosis* and *A. nidulans* were purified as described previously,[16] a concentrated solution (1.5 and 0.08 mg cm^{-3}, respectively) in potassium phosphate buffer (50 mM, pH 7.0), DTT (1 mM) was filter-sterilised 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,[17] and the concentrated solution (2.3 mg cm^{-3}) was 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. Deuteriated buffers were 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 change in absorbance at 234 nm in the UV spectrum due to the absorbance of the enone carboxylate chromophore of 3-dehydroshikimic acid (**3a**) (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 3-dehydroquinic acid (**2a**) and 0.7 U type II dehydroquinase. Each assay was initiated by addition of the enzyme. Solutions of 3-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.

Assay of shikimate dehydrogenase

Shikimate dehydrogenase was assayed in its forward or reverse direction by monitoring the change in absorbance at 340 nm in the UV spectrum due to the absorbance of NADPH (ε/M^{-1} cm^{-1} 6200). Standard assay conditions for shikimate dehydrogenase in the forward direction were pH 7.0 at 25 °C with 200 μM NADPH in potassium phosphate buffer (50 mM).[18] For assays in the reverse direction, the conditions were pH 10.6 and 25 °C with 170 μM $NADP^+$ in sodium bicarbonate buffer (50 mM). Each assay was initiated by addition of the enzyme

(typically 1 U). 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 changes in the concentration of NADPH.

(1*R*,3*R*,4*S*,5*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-hydroxy-4-phenoxythiocarbonyloxycyclohexane-1,5-carbolactone 7

To a stirred solution of the alcohol[7] **6** (190 mg, 0.66 mmol), DMAP (121 mg, 0.99 mmol) in dry acetonitrile (6 cm^3) under argon was added, slowly during 1 h, phenyl chlorothionoformate (100 mm^{-3}, 0.73 mmol). The yellow solution was stirred at room temperature for 24 h. The solvent was evaporated, the crude redissolved in diethyl ether (20 cm^3) and washed with HCl (1 M, 20 cm^3), brine (2 × 20 cm^3), dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash chromatography eluting with diethyl ether–hexane (50:50), to yield starting material (50 mg, 26%) and the *thiol* **7** (175 mg, 62%) as a colourless foam, ν_{max} (KBr)/cm^{-1} 3464 (OH) and 1805 (C=O); δ_H (400 MHz, $CDCl_3$) 7.42 (2 H, m, ArH), 7.30 (1 H, m, ArH), 7.08 (2 H, m, ArH), 5.81 (1 H, t, *J* 4.7, 4-H), 4.98 (1 H, m, 5-H), 4.13–4.08 (1 H, m, 3-H), 2.46 (2 H, m, 8-CHH), 2.11 (2 H, m, 2-CHH), 0.90 (9 H, s, ^{t}Bu) and 0.09 (6 H, s, $SiCH_3$); δ_C (100 MHz, APT, $CDCl_3$) 194.2 (C), 177.2 (C), 153.2 (C), 130.0 (CH), 126.8 (CH), 121.6 (CH), 75.7 (CH), 73.1 (CH), 72.0 (C), 65.9 (CH), 40.8 (CH_2), 37.5 (CH_2), 25.6 ($C(CH_3)_3$), 17.9 ($C(CH_3)_3$) and −5.0 (2 × $SiCH_3$); *m*/*z* (FAB + ve) 425 (MH^+) (Found MH^+, 425.1465. $C_{20}H_{29}SiSO_6$ requires *M*H^+, 425.1454).

(1*R*,3*R*,5*R*)-3-(*tert*-Butyldimethylsilyloxy)-1-hydroxycyclohexane-1,5-carbolactone 8

A solution of the thiol **7** (1.16 g, 2.72 mmol) in dry toluene (50 cm^3), was added dropwise over 1 h to a solution of the ^{n}Bn_3SnH (5 cm^3, 19.07 mmol) and AIBN (313 mg, 1.91 mmol) in dry toluene (190 cm^3) heated at reflux under argon. The mixture was heated at reflux for 1 h. After cooling at room temperature the solvent was evaporated and the crude product was purified by flash chromatography, eluting with a gradient of diethyl ether–hexane (1:3 to 3:1) to yield the *deoxylactone* **8** (649 mg, 88%) as white needles, mp 79–80 °C (from hexane) (Found C, 57.4; H, 8.9. $C_{13}H_{24}SiO_4$ requires C, 57.3; H, 8.9%); $[\alpha]_D^{20}$ −49 (*c* 0.7 in MeOH); ν_{max} (KBr)/cm^{-1} 3357 (OH) and 1778 and 1747 (C=O); δ_H (400 MHz; $CDCl_3$) 4.82 (1 H, t, *J* 11.2, 5-H), 4.02–3.93 (1 H, m, 3-H), 2.72 (1 H, br s, OH), 2.52–2.46 (1 H, m, 8-C*H*H), 2.41–2.35 (1 H, m, 8-CH*H*), 2.21–2.16 (1 H, m, 2-C*H*H), 2.04 (1 H, d, *J* 5.2, 2-CH*H*), 1.87 (1 H, dd, *J* 10.4 and 12.1, 4-C*H*H), 1.46 (1 H, dd, *J* 7.9 and 13.7, 4-CH*H*), 0.85 (9 H, s, ^{t}Bu) and 0.03 (6 H, s, $SiCH_3$); δ_C (100 MHz; APT; $CDCl_3$) 178.0 (C), 74.9 (CH), 73.1 (C), 65.2 (CH), 44.8 (CH_2), 43.0 (CH_2), 36.9 (CH_2), 25.7 ($C(CH_3)_3$), 17.9 ($C(CH_3)_3$), −4.8 ($SiCH_3$) and −4.9 ($SiCH_3$); *m*/*z* (FAB + ve) 273 (MH^+) (Found MH^+, 273.1522. $C_{13}H_{25}SiO_4$ requires *M*H^+, 273.1509).

(1*R*,3*R*,5*R*)-1,3-Dihydroxycyclohexane-1,5-carbolactone 9

To a stirred solution of the silyl ether **8** (187 mg, 0.67 mmol) in dry THF (14 cm^3) and glacial acetic acid (0.16 cm^3, 2.75 mmol) was added a 1.0 M solution of TBAF in THF (*ca.* 2 cm^3, 2.06 mmol). The resultant solution was stirred at room temperature for 36 h. The solvent was removed under reduced pressure to give a pink residue which was purified by flash chromatography eluting with ethyl acetate–methanol (95:5), to afford the *alcohol* **9** (108 mg, 99%) as white needles, mp 110–111 °C (from ethyl acetate), $[\alpha]_D^{20}$ −59 (*c* 0.8 in MeOH); ν_{max} (KBr)/cm^{-1} 3284 (O–H) and 1777 and 1756 (C=O); δ_H (400 MHz; CD_3OD) 4.79 (1 H, t, *J* 5.4, 5-H), 3.84 (1 H, m, 3-H), 2.44–2.33 (2 H, m, 8-CHH), 2.24–2.18 (1 H, m, 2-C*H*H), 1.96 (1 H, d, *J* 11.2, 2-CH*H*), 1.70 (1 H, t, *J* 11.4, 4-C*H*H) and 1.43 (1 H, dd, *J* 10.2 and 13.4, 4-CH*H*); δ_C (100 MHz; APT; CD_3OD) 179.7 (C), 76.0 (CH), 74.1 (C), 65.4 (CH), 44.5 (CH_2), 44.4 (CH_2) and 37.3 (CH_2); *m*/*z* (ESI) 181 (MNa^+) (Found MNa^+, 181.0479. $C_7H_{10}O_4Na$ requires *M*Na^+, 181.0477).

(1*S*,3*S*,5*S*)-1,3,5-Trihydroxycyclohexanecarboxylic acid [4-deoxyquinic acid] 1b

A solution of the lactone **9** (321 mg, 2.03 mmol) in 10 cm^3 of a 2.5 M solution of NaOH was stirred at 0 °C for 1 h. The resultant mixture was diluted with water (10 cm^3) and Dowex 5WX8-400 (H^+) was added until the pH dropped from 13.1 to 5. Filtration and lyophilisation yielded the *acid* **1b** as a hygroscopic white amorphous solid (352 mg, 99%), $[\alpha]_D^{20}$ −3 (*c* 0.6 in MeOH); ν_{max} (KBr)/cm^{-1} 3408 (O–H) and 1718 (C=O); λ_{max} (H_2O)/nm 193; δ_H (400 MHz; CD_3OD) 4.21–4.12 (2 H, m, 3-H and 5-H), 2.03 (1 H, dd, *J* 13.7 and 3.3, 6-C*H*H), 1.98–1.82 (3 H, m, 6-CH*H* and 2-CHH) and 1.69–1.64 (2 H, m, 4-CHH); δ_C (100 MHz; APT; CD_3OD) 180.9 (C), 77.2 (C), 67.9 (CH), 64.9 (CH), 43.4 (CH_2), 42.7 (CH_2) and 42.0 (CH_2); *m*/*z* (ESI) 199 (MNa^+) (Found MNa^+, 199.0584. $C_7H_{12}O_5Na$ requires *M*Na^+, 199.0582).

Methyl (3*S*,4*S*,5*S*)-3,5-bis(*tert*-butyldimethylsilyloxy)-4-hydroxycyclohex-1-enecarboxylate 11[8]

To a stirred solution of the methyl shikimate **10** (2.52 g, 13.40 mmol), DMAP (459 mg, 3.75 mmol) and Bu_4NI (495 mg, 1.34 mmol) in dry DMF (22 cm^3) and dry triethylamine (4.1 cm^3, 29.48 mmol) at 0 °C under argon was added 4.34 g (28.81 mmol) of *tert*-butyldimethylsilyl chloride. The solution was stirred at this temperature for 1 h and then 3 h at room temperature. The resultant suspension was eluted with ethyl acetate (100 cm^3) and filtered over Celite. The solution was washed successively with 1 M HCl (100 cm^3) and brine (3 × 100 cm^3), dried ($MgSO_4$), filtered and evaporated. The yellow residue was purified by flash chromatography, eluting with diethyl ether–hexane (25:75) to yield disilyl ether **11**[8] (4.83 g, 87%). It crystallises from hexane as white needles, mp 44–45 °C (from hexane), ν_{max} (NaCl)/cm^{-1} 3364 (OH), 1722 (C=O) and 1656 (C=C); δ_H (400 MHz; $CDCl_3$) 6.61 (1 H, br s, 2-H), 4.51 (1 H, br s, 3-H), 4.18 (1 H, t, *J* 4.4 and 3.8, 4-H), 3.74 (3 H, s, CH_3O), 3.70 (1 H, dt, *J* 11.5 and 4.2, 5-H), 2.60 (1 H, dt, *J* 18.2 and 2.9, C*H*H), 2.22 (1 H, d, *J* 18.2, C*H*H), 0.91 (9 H, s, ^{t}Bu), 0.91 (9 H, s, ^{t}Bu), 0.84 (6 H, s, $SiCH_3$) and 0.84 (6 H, s, $SiCH_3$); δ_C (100 MHz; APT; $CDCl_3$) 167.2 (C), 137.3 (CH), 126.4 (C), 70.7 (CH), 68.1 (CH), 67.5 (CH), 51.6 (CH_3), 29.6 (CH_2), 25.8 ($C(CH_3)_3$), 25.7 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$) and 17.9 ($C(CH_3)_3$), −4.7, −4.8, −4.9 and −5.0 (each $SiCH_3$); *m*/*z* (FAB + ve) 417 (MH^+) and 399 ($M^+ - OH$) (Found MH^+, 417.2503. $C_{20}H_{29}SiSO_6$ requires *M*H^+, 417.2493.

Methyl (3*S*,4*S*,5*S*)-3,5-bis(*tert*-butyldimethylsilyloxy)-4-phenoxythiocarbonyloxycyclohex-1-enecarboxylate 12[8]

To a stirred solution of the alcohol **11** (3.75 g, 9.04 mmol) and DMAP (1.66 g, 13.56 mmol) in dry acetonitrile (90 cm^3) under argon was added, slowly during 1 h, 1.5 cm^3 (10.85 mmol) of phenyl chlorothionoformate. The yellow solution was stirred at room temperature for 24 h. The solvent was evaporated, the crude redissolved in diethyl ether (100 cm^3) and washed with HCl (1 M, 2 × 100 cm^3), brine (2 × 100 cm^3), dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash chromatography eluting with diethyl ether–hexane (50:50), to yield thiol **12** (4.08 g, 82%) as a colourless foam; ν_{max} (NaCl)/cm^{-1} 1724 and 1655 (C=O) and 1593 (C=C); δ_H (400 MHz; $CDCl_3$) 7.40 (2 H, t, *J* 7.8, ArH), 7.18 (1 H, m, ArH), 7.05 (2 H, d, *J* 7.7, ArH), 6.76 (1 H, d, *J* 3.7, 2-H), 5.37 (1 H, dd, *J* 7.6 and 3.7, 4-H), 4.79 (1 H, t, *J* 3.7, 3-H), 4.42 (1 H, m, 5-H), 3.76 (3 H, s, CH_3O), 2.75 (1 H, dd, *J* 18.4 and 5.1, 6_{eq}-H), 2.34 (1 H, dd, *J* 18.4 and 5.6, 6_{ax}-H), 0.93

(9 H, s, ^{t}Bu), 0.93 (9 H, s, ^{t}Bu), 0.16, 0.15, 0.14 (3 H, s, $SiCH_3$) and 0.12 (3 H, s, $SiCH_3$); δ_C (100 MHz; APT; $CDCl_3$) 195.2 (C), 166.8 (C), 153.3 (C), 137.1 (CH), 129.5 (CH), 129.1 (C), 126.5 (CH), 121.9 (CH), 83.2 (CH), 65.0 (CH), 52.0 (MeO), 32.2 ($C(CH_3)_3$), 25.7 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), −4.7 ($SiCH_3$), −4.8 ($SiCH_3$), −4.9 ($SiCH_3$) and −4.9 ($SiCH_3$); *m/z* (FAB + ve) 552 (MH^+) and 537 ($M^+ - Me$) (Found MH^+, 552.2397. $C_{27}H_{44}O_6SSi_2$ requires MH^+, 552.2383).

(3*S*,4*S*,5*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-1-hydroxymethyl-4-phenoxythiocarbonyloxycyclohex-1-ene 13[9]

A solution of *ca*. 1.5 M diisobutylaluminium hydride in toluene (0.63 cm^3; 0.95 mmol) was added to a stirred solution of the ester **12** (249 mg, 0.45 mmol) in dry toluene (5 cm^3) under argon at −78 °C. The mixture was stirred for 15 min and then quenched with water. The organic layer was extracted with diethyl ether (3 × 30 cm^3). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by flash chromatography, eluting with diethyl ether–hexane (1:3), to yield alcohol **13** (219 mg, 93%) as a foam. ν_{max} (NaCl)/cm^{-1} 3364 (O–H) and 1592 (C=C); δ_H (400 MHz; $CDCl_3$) 7.41 (2 H, t, *J* 7.9, ArH), 7.28 (1 H, t, *J* 7.4, ArH), 7.07 (2 H, d, *J* 7.7, ArH), 5.68 (1 H, d, *J* 4.2, 2-H), 5.32 (1 H, dd, *J* 8.9 and 3.7, 4-H), 4.67 (1 H, dd, *J* 4.2 and 3.7, 3-H), 4.46 (1 H, q, *J* 7.3, 5-H), 4.04 (2 H, s, CH_2OH), 2.49 (1 H, dd, *J* 17.6 and 5.7, 6_{eq}-H), 2.15 (1 H, dd, *J* 17.6 and 7.7, 6_{ax}-H), 0.94 (9 H, s, ^{t}Bu), 0.93 (9 H, s, ^{t}Bu), 0.18 (6 H, s, $SiCH_3$), 0.16 (6 H, s, $SiCH_3$), 0.14 (6 H, s, Si CH_3) and 0.12 (6 H, s, $SiCH_3$); δ_C (100 MHz; APT; $CDCl_3$) 195.2 (C), 153.4 (C), 138.4 (C), 129.4 (CH), 126.4 (CH), 122.0 (CH), 121.6 (CH), 85.3 (CH), 65.7 (CH), 65.1 (CH), 65.0 (CH), 34.7 (CH_2), 25.8 ($C(CH_3)_3$), 25.7 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), −4.8 (2 × $SiCH_3$) and −4.9 (2 × $SiCH_3$); *m/z* (FAB + ve) 524 (MH^+), 509 ($M^+ - Me$); MH^+ 524.2465; $C_{26}H_{44}O_5SSi_2$ requires 524.2448.

(3*S*,5*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-1-hydroxymethylcyclohex-1-ene 14[9]

To a refluxed solution of the ^{n}Bn_3SnH (4.8 cm^3, 18.20 mmol) and AIBN (300 mg, 1.82 mmol) in dry toluene (180 cm^3) under argon was added, dropwise during 1 h, a solution of the thiol **13** (1.36 g, 2.60 mmol) in dry toluene (50 cm^3), and then the solution was refluxed for 1 h. After cooling at room temperature the solvent was evaporated and the crude was purified by flash chromatography eluting with dichloromethane to yield deoxyalcohol **14** (595 mg, 62%) as an oil, ν_{max} (NaCl)/cm^{-1} 3350 (O–H); δ_H (400 MHz; $CDCl_3$) 5.64 (1 H, br s, 2-H), 4.40 (1 H, br m, 3-H), 4.16 (1 H, m, 5-H), 3.99 (2 H, s, CH_2OH), 2.23 (1 H, dd, *J* 17.2 and 4.7, 6_{eq}-H), 1.91 (1 H, dd, *J* 17.2 and 7.1, 6_{ax}-H), 1.72 (2 H, m, 4-CHH), 0.88 (9 H, s, ^{t}Bu), 0.87 (9 H, s, ^{t}Bu), 0.06 (6 H, s, $SiCH_3$), 0.06 (6 H, s, $SiCH_3$), 0.05 (6 H, s, $SiCH_3$) and 0.05 (6 H, s, $SiCH_3$); δ_C (100 MHz; APT; $CDCl_3$) 137.7 (C), 124.1 (C), 66.4 (CH), 65.9 (CH), 65.1 (CH), 41.0 (CH_2), 35.4 (CH_2), 25.9 ($C(CH_3)_3$), 25.8 ($C(CH_3)_3$), 18.2 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), −4.8 (2 × $SiCH_3$) and −4.8 (2 × $SiCH_3$); *m/z* (FAB + ve) 371 (MH^+); MH^+ 371.2430; $C_{19}H_{39}O_3Si_2$ requires 371.2438.

(3*S*,5*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)cyclohex-1-enecarbaldehyde 15[9]

To a stirred suspension of the alcohol **14** (589 mg, 1.58 mmol) and activated 4 Å molecular sieves (360 mg) in dry dichloromethane (16 cm^3) was added pyridinium dichromate (715 mg, 1.90 mmol). The resultant suspension was stirred at room temperature for 2 h and then diluted with diethyl ether (60 cm^3) and filtered over Celite. The solution was washed successively with HCl (5%, 2 × 50 cm^3) and brine (2 × 50 cm^3), dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash chromatography, eluting with diethyl ether–hexane (1:4) to afford aldehyde **15** (485 mg, 83%) as an oil, ν_{max} (NaCl)/cm^{-1} 1712 (C=O); δ_H (400 MHz; $CDCl_3$) 6.61 (1 H, m, 2-H), 4.69 (1 H, m, 3-H), 4.22 (1 H, m, 5-H), 2.40 (1 H, m, 6_{eq}-H), 2.14 (1 H, ddd, *J* 18.0, 4.7 and 1.0, 6_{ax}-H), 1.94 (1 H, dt, *J* 12.9 and 6.3, 6_{ax}-H), 1.69 (1 H, dddd, *J* 12.9 and 2.4, 4_{eq}-H), 0.90 (9 H, s, ^{t}Bu), 0.85 (9 H, s, ^{t}Bu), 0.11 (3 H, s, $SiCH_3$), 0.10 (3 H, s, $SiCH_3$), 0.05 (3 H, s, $SiCH_3$) and 0.03 (3 H, s, $SiCH_3$); δ_C (100 MHz; APT; $CDCl_3$) 194.4 (CHO), 150.7 (CH), 138.7 (C), 65.5 (CH), 64.9 (CH), 40.0 (CH_2), 30.6 (CH_2), 25.8 and 25.7 (each $C(CH_3)_3$), 18.1 ($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), −4.7 ($SiCH_3$), −4.8 ($SiCH_3$), −4.9 ($SiCH_3$) and −4.9 ($SiCH_3$); *m/z* (FAB + ve) 371 (MH^+); MH^+ 371.2465; $C_{19}H_{39}O_3Si_2$ requires 371.2438.

(3*S*,5*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)cyclohex-1-enecarboxylic acid 16

A solution containing sodium chlorite (288 mg, 3.18 mmol) and sodium dihydrogen phosphate (300 mg, 2.50 mmol) in water (1.2 cm^3) was added dropwise to a stirred solution containing aldehyde **15** (134 mg, 0.36 mmol) and 2-methylbut-2-ene (*ca*. 8.2 cm^3, *ca*. 2 M in THF) in *tert*-butyl alcohol (4 cm^3). The mixture was stirred at room temperature for 18 h and the solvents and volatile components were evaporated. The residue was poured into water (10 cm^3) and was extracted with diethyl ether (3 × 30 cm^3). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow residue which was purified by flash chromatography, eluting with diethyl ether–hexane (1:1) to afford the *acid* **16** (193 mg, 98%). The acid crystallised from hexane as white needles mp 120–121 °C (Found: C, 58.3; H, 10.0. $C_{19}H_{38}Si_2O_4 \cdot 1/4\ H_2O$ requires C, 58.3; H, 9.9%); $[a]_D^{20}$ −80 (*c* 0.5 in MeOH); ν_{max} (KBr)/cm^{-1} 3196 (O–H) and 1715 (C=O); δ_H (400 MHz; $CDCl_3$) 6.93 (1 H, d, *J* 3.1, 2-H), 4.56 (1 H, m, 3-H), 4.20 (1 H, m, 5-H), 2.52 (1 H, dd, *J* 17.9 and 4.3, 6-C*H*H), 2.16 (1 H, dd, *J* 17.9 and 5.5, 6-C*H*H), 1.63 (1 H, m, 4-C*H*H), 1.69 (1 H, ddd, *J* 12.8, 10.3 and 2.5, 4-C*H*H), 0.89 and 0.87 (each 9 H, s, ^{t}Bu), 0.09, 0.08, 0.06 and 0.06 (each 3 H, s, $SiCH_3$); δ_C (100 MHz; APT; $CDCl_3$) 172.3 (C), 142.6 (CH), 128.0 (C), 65.4 (CH), 65.0 (CH), 39.6 (CH_2), 33.3 (CH_2), 25.8 ($C(CH_3)_3$), 25.8 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), −4.7 ($SiCH_3$), −4.8 ($SiCH_3$), −4.8 ($SiCH_3$) and −4.9 ($SiCH_3$). *m/z* (FAB + ve) 387 (MH^+) (Found MH^+, 387.2378. $C_{19}H_{39}O_4Si_2$ requires MH^+, 387.2387).

(3*S*,5*S*)-3,5-Dihydroxycyclohex-1-enecarboxylic acid [4-deoxyshikimic acid] 4b

A solution of the acid **16** (205 mg, 0.53 mmol) in a 1% solution of HCl in ethanol (11 cm^3) was stirred for 2 h. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography eluting with ethyl acetate–ethanol–acetic acid (90:9:1) to yield *4-deoxyshikimic acid* **4b** (83 mg, 99%) as white needles, $[a]_D^{20}$ −9 (*c* 0.1 in MeOH); λ_{max} (H_2O)/nm 206; ν_{max} (KBr)/cm^{-1} 3288 (O–H) and 1687 (C=O); δ_H (500 MHz; D_2O) 6.69 (1 H, br s, 2-H), 4.48 (1 H, m, 3-H), 4.14 (1 H, m, 5-H), 2.56 (1 H, d m, *J* 17.8, 6_{eq}-H), 2.11 (1 H, dd, *J* 17.8 and 6.0, 6_{ax}-H), 1.90 (1 H, m, 4_{ax}-H) and 1.74 (1 H, ddd, *J* 16.8, 6.0 and 2.7, 4_{eq}-H); δ_C (100 MHz; APT; D_2O) 175.0 (C), 139.4 (CH), 134.1 (C), 66.6 (CH), 66.6 (CH), 39.3 (CH_2) and 34.9 (CH_2); *m/z* (ESI) 181 (MNa^+) (Found, MNa^+ 181.0496. $C_7H_{10}O_4Na$ requires, MNa^+, 181.0477).

Data for *(3R,5S)-3,5-dihydroxycyclohex-1-enecarboxylic acid [3-epi-4-deoxyshikimic acid]:* δ_H (500 MHz; D_2O) 6.87 (1 H, br s, 2-H), 4.46 (1 H, m, 3-H), 4.12 (1 H, m, 5-H), 2.60 (1 H, dt, *J* 17.9 and 2.2, 6_{eq}-H), 2.16 (1 H, dd, *J* 17.9 and 6.0, 6_{ax}-H), 1.89 (1 H, m, 4_{eq}-H) and 1.78 (1 H, ddd, *J* 12.9, 6.0 and 2.8, 4_{eq}-H); δ_C (62 MHz; APT; D_2O) 167.1 (C), 139.0 (CH), 129.0 (C), 63.7 (CH), 63.5 (CH), 37.9 (CH_2) and 32.5 (CH_2); *m/z* (ESMS +ve) 158 (M^+).

(1*R*,5*R*)-1-Hydroxy-3-oxocyclohexane-1,5-carbolactone 17 and (1*R*)-1-hydroxy-3-oxocyclohex-4-enecarboxylic acid 18

To a stirred suspension of the alcohol **9** (58 mg, 0.37 mmol) and 4 Å activated molecular sieves (300 mg) in dry dichloromethane (3.7 cm^3) was added pyridinium chlorochromate (158 mg, 0.73 mmol). The resultant suspension was stirred at room temperature for 2 h and then diluted with ethyl acetate (10 cm^3) and filtered over Celite to afford the *ketone* **17** (30 mg, 52%) and the *enone* **18** (10 mg, 17%).

Data for the ketone **17** [obtained by flash chromatography eluting with ethyl acetate–ethanol–acetic acid (90:9:1)]: ν_{max} (KBr)/cm^{-1} 3408 (O–H), 1762 and 1734 (C=O); δ_H (500 MHz; CD_3OD) 5.00 (1 H, m, 5-H), 2.85 (1 H, d, *J* 17.3, 2-C*H*H), 2.74–2.64 (4 H, m, 2-C*H*H, 6-C*H*H and 4-CHH) and 2.42 (1 H, d, *J* 11.6, 6-C*H*H); δ_C (62 MHz; APT; CD_3OD) 204.9 (C), 177.5 (C), 72.5 (CH), 72.5 (CH), 72.0 (C), 51.2 (CH_2), 43.8 (CH_2) and 40.5 (CH_2); *m*/*z* (ESMS + ve) 179 (MNa^+) and 156 (M^+).

Data for the enone **18** [obtained by FPLC on a Mono Q HR 10/10 column eluting with a gradient of 0–0.3 M ammonium bicarbonate over 140 cm^3 and 0.3–1 M over 110 cm^3 at 1.0 cm^3 min^{-1} at 254 nm; fractions eluting at 60–100 mM ammonium bicarbonate were lyophilised to give enone as a white powder]: ν_{max} (KBr)/cm^{-1} 3420 (O–H), 1718 (C=O) and 1602 (C=C); δ_H (500 MHz; D_2O) 7.03 (1 H, m, 5-H), 6.02 (1 H, d, *J* 10.2, 4-H), and 2.48 (4 H, m, 2-CHH and 6-CHH).

(3*S*,5*S*)-3,5-Dihydroxy-3-oxocyclohex-1-enecarboxylic acid [4-deoxy-3-dehydroshikimic acid] 3b

To a solution of the 4-deoxyshikimic acid **4b** (7.6 mg, 48.10 μmol) and $NADP^+$ (74 mg, 96.20 μmol) in 0.6 cm^3 Na_2CO_3–$NaHCO_3$ buffer (600 mM) at pH 10.6 and 25 °C was added shikimate dehydrogenase (20 mm^3, 20 U). The reaction was followed by ^1H NMR spectroscopy for 15 min, by which time total conversion to product had occurred. The enzyme was removed by centrifugation on an Amicon Centricon-10 microconcentrator and the resultant mixture was purified by FPLC in a Mono Q HR 10/10 column (eluting with a gradient of 0–0.3 M ammonium bicarbonate over 140 cm^3 and 0.3–1 M over 110 cm^3 at 1.0 cm^3 min^{-1} at 254 nm). Fractions eluting at 50–60 mM amonium bicarbonate were lyophilised to give *4-deoxydehydroshikimic acid* **3b** as a white powder (6.4 mg, 85%), ν_{max} (KBr)/cm^{-1} 3074 (O–H), 1685 (C=O) and 1598 (C=C); δ_H (500 MHz; D_2O) 6.33 (1 H, s, 2-H), 4.29 (1 H, br s, 4_{eq}-H), 3.88 (1 H, q, *J* 7.1, 5-H), 3.60 (1 H, dd, *J* 17.7 and 5.1, 4_{ax}-H), 2.65 (1 H, dd, *J* 17.7 and 5.1, 6_{eq}-H) and 2.09 (1 H, dd, *J* 17.7 and 7.4, 6_{ax}-H). δ_C (100 MHz; APT; D_2O) 190.0 (3-C), 162.9 (CO_2H), 142.4 (1-C), 129.8 (2-CH), 68.7 (5-CH), 47.5 (4-CH_2) and 36.8 (6-CH_2); *m*/*z* (ESMS + ve) 173 (MNH_4^+).

Acknowledgements

We thank the BBSRC for postdoctoral funding for C. G. B. and John Greene for enzyme preparation. We thank Professor Raphael for the kindness, guidance and enthusiasm which both inspired and facilitated our studies in shikimate chemistry.

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Paper 8/09809C