Comparison of the substrate specificity of type I and type II dehydroquinases with 5-deoxy- and 4,5-dideoxy-dehydroquinic acid

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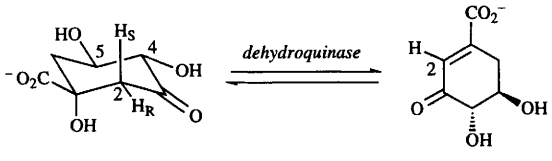
Syntheses of 5-deoxydehydroquinic acid and 4,5-dideoxydehydroquinic acid from quinic acid are described. These substrate analogues were tested against the mechanistically-distinct type I and type II dehydroquinases. The C-4 hydroxy group of the substrate is shown to be important for imine formation on the type I enzyme but appears not to contribute significantly to specificity on the type II dehydroquinase.

Introduction

The enzyme dehydroquinase (3-dehydroquinate dehydratase, E.C. 4.2.1.10) catalyses the reversible dehydration of dehydroquinic acid **1** to form dehydroshikimic acid **2** (Scheme 1).



1 **2**

Scheme 1 The 2-*pro-R* hydrogen of dehydroquinic acid is removed in the reaction catalysed by type I dehydroquinase and the 2-*pro-S* hydrogen is lost in the reaction catalysed by the type II dehydroquinase

This reaction is part of two metabolic pathways, the biosynthetic shikimate pathway and the catabolic quinate pathway.[1–3] Two classes of dehydroquinase exist, type I and type II, which possess different biochemical and biophysical properties and show no sequence similarity.[4] The type I enzymes are exclusively biosynthetic, whereas the type II enzymes have both biosynthetic and catabolic roles,[5,6] with a dual function enzyme also having been described.[7] The type I enzymes have a conserved lysine residue[8] and catalyse a *cis*-elimination *via* an imine intermediate with a conserved histidine as the general base.[9,10]

In contrast, both the non-enzymatic reaction and that catalysed by the type II enzymes have been shown to proceed by the *trans*-dehydration of dehydroquinic acid, with loss of the more acidic axial *pro-S* hydrogen from C-2.[11,12] There are no conserved lysine residues in the type II enzymes, which also show no inhibition on treatment with substrate and sodium borohydride.[4] Extensive studies of the type II dehydroquinase from *Aspergillus nidulans* have failed to show any involvement of a metal in catalysis.[13] Little is known about the mechanism of the reaction catalysed by type II dehydroquinase, although clearly it is distinct from that catalysed by type I dehydroquinase. The type II enzyme from *Mycobacterium tuberculosis* has recently been crystallised.[14] An essential arginine residue has been identified in the type II dehydroquinase of *A. nidulans*.[15]

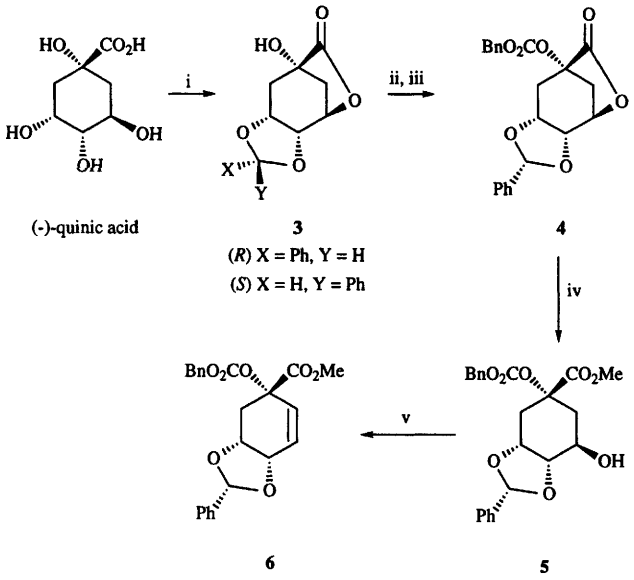
In this paper we describe the synthesis of two analogues of dehydroquinic acid **9** and **19** which lack the C-5, and C-4 and C-5 hydroxy groups respectively. We also characterise the interaction of these compounds with both type I and type II dehydroquinases. This is the first study of the substrate specificity of a type II dehydroquinase.

Results

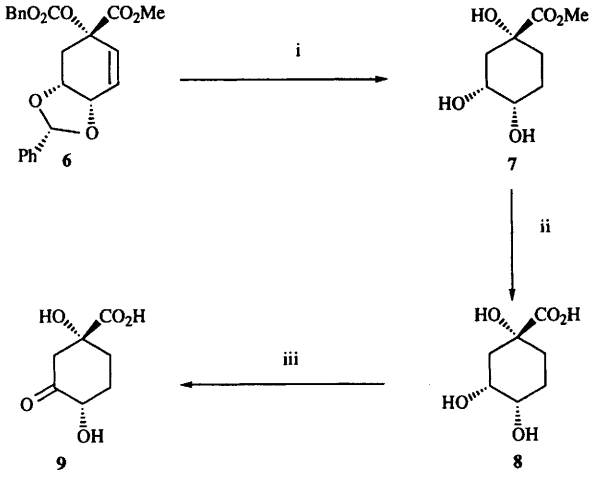
The target compounds **9** and **19** were synthesised from related acetal-protected alkenes **6** and **10**, which were derived from the naturally occurring (−)-quinic acid. For the synthesis of 5-deoxydehydroquinic acid **9** the benzylidene protected alkene **6** was used. Following the procedure of Lesuisse and Berchtold the C-3 and C-4 hydroxy groups of (−)-quinic acid were protected as the benzylidene acetal and the carboxy and C-5 hydroxy groups were lactonised to give **3** as a mixture of diastereomers (*R*:*S* 2.2:1).[16] The (*R*)-isomer was used in subsequent transformations. After protection of the C-1 hydroxy group as a benzyl carbonate **4**, the lactone was opened with sodium methoxide to give the ester **5** with a free hydroxy group at C-5. This was removed in a one-pot reaction involving formation of the trifluoromethanesulfonate followed by base-catalysed elimination, to give the alkene **6** (Scheme 2).[17]

The synthesis of 5-deoxydehydroquinic acid **9** was achieved from **6** in an additional three steps (Scheme 3). Hydrogenolysis of the benzylidene-protected alkene resulted in deprotection of the remaining three hydroxy groups and reduction of the double bond to give methyl 5-deoxyquinate **7**. Saponification of the methyl ester produced 5-deoxyquinic acid **8**, which was oxidised selectively at C-3 using concentrated nitric aid. This oxidation follows a procedure used for the synthesis of dehydroquinic acid from quinic acid.[18,19]

The synthesis of the 4,5-dideoxy analogue **19** (Scheme 4) proceeds from the known cyclohexylidene protected alkene **10** which has been synthesied from quinic acid.[17] Acid-catalysed deprotection of the cyclohexylidene moiety gave the alkenic diol **11**.[20] Selective hydroxy group functionalisation and regiospecific elimination of the allylic hydroxy group was achieved in two steps. The *cis*-diol **11** was converted to the allylic bromide **12** using 2-acetoxyisobutyryl bromide following a procedure developed by Greenberg and Moffat,[21] and used by Wood and Ganem in their synthesis of (−)-chorismic acid from (−)-shikimic acid.[22] Tributyltin hydride hydrodehalogen-



Scheme 2 *Reagents, conditions and yields:* i, PhCHO, TsOH, toluene, reflux (79%); ii, NaH, CH_2Cl_2; iii, BuOCOCl, Bu_4NI (82%); iv, NaOMe, MeOH (59%); v, Tf_2O, pyridine, CH_2Cl_2; vi, DBU, $CHCl_3$ (60%)

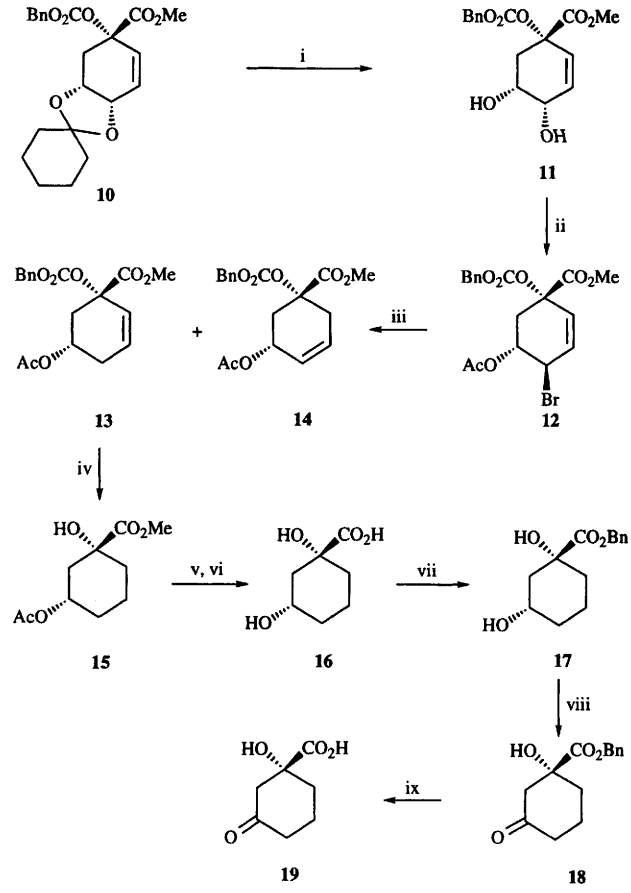


Scheme 3 *Reagents, conditions and yields:* i, H_2, 5% Pd–C, CH_2Cl_2 (quant.); ii, K_2CO_3, MeOH, H_2O (43%); iii, HNO_3, H_2O (20%)

ation of the allylic bromide gave a 2:1 mixture of the alkenes **13** and **14**, which could not be separated by chromatography.[23]

Reduction of the double bond in **13** and **14** required rather forcing conditions. It was necessary to carry out the hydrogenation of the mixture of alkenes in ethanol, with 30% palladium on charcoal catalyst, at 60 °C, under 5 atmospheres pressure (1 atm = 101 325 Pa) for 18 h. Under these conditions the protected 4,5-dideoxyquinic acid **15** was obtained in 86% yield. Saponification of the ester groups, followed by treatment with cation exchange resin gave 4,5-dideoxyquinic acid **16**. Unfortunately, it was not possible to oxidise **16** directly to **19** as had been intended, as all attempts resulted in significant decomposition of the product. It was therefore necessary to form the benzyl ester **17**. The C-3 alcohol could then be readily oxidised using pyridinium dichromate.[24] Mild hydrogenolysis of the benzyl ester **18** gave the desired 4,5-dideoxydehydroquinic acid **19**. The synthesis is long (twelve steps from quinic acid with an overall yield of 4%), but has the benefit of furnishing key intermediates for related studies.

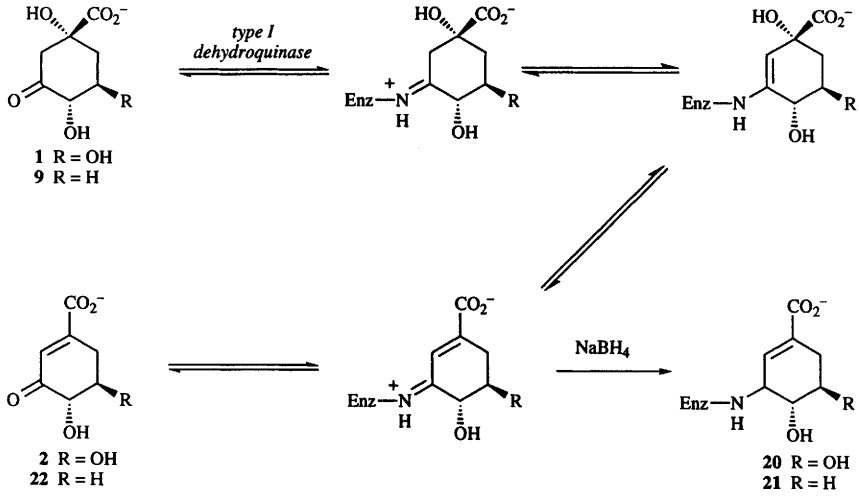
The reaction of 5-deoxydehydroquinic acid **9** with type I dehydroquinase was monitored by ^1H NMR spectroscopy. Turnover to 5-deoxydehydroshikimic acid **22** was observed, as characterised by the appearance of a singlet at δ 6.30 due to the vinylic proton at C-2 of 5-deoxydehydroshikimic acid **22**, and a



Scheme 4 *Reagents, conditions and yields:* i, TFA, H_2O (48%); ii, $Me_2C(OAc)COBr$, CH_3CN (91%); iii, Bu_3SnH, AIBN, toluene (93%); iv, H_2 (5 atm), 30% Pd–C, 60 °C, EtOH (86%); v, LiOH, H_2O, THF; vi, Amberlite IR-120 (quant); vii, BnBr, DMAP, NEt_3, DMF (70%); viii, PDC, CH_2Cl_2 (84%); ix, H_2, 5% Pd–C, MeOH (88%)

doublet of doublets at δ 4.40, due to the proton at C-4. From kinetic studies of 5-deoxydehydroquinic acid **9** with type I dehydroquinase K_M was determined to be 700 μmol dm^{-3}, k_{cat} 3 s^{-1} and k_{cat}/K_M 4.3 × 10^3 dm^3 mol^{-1} s^{-1}, which represents a reduction in specificity of 10^3 dm^3 mol^{-1} s^{-1} relative to the natural substrate.

An equilibrium mixture of **9** with type I dehydroquinase was treated with aliquots of sodium borohydride and the activity of dehydroquinase monitored for 30 min. After this time the incubation containing **9** had lost approximately 30% activity relative to a control incubation without substrate. A second control incubation, containing an equilibrium mixture of dehydroquinic acid and dehydroshikimic acid showed total loss of activity 5 min after addition of sodium borohydride. Electrospray mass spectrometric analysis of these samples showed the expected mass of 27 471 (±4) Da for the control enzyme solution with no substrate present, corresponding to unmodified type I dehydroquinase (predicted mass 27 467 Da). The control with dehydroquinic acid present showed a peak at 27 624 (±3) Da, corresponding to dehydroquinase modified by the reduced imine-bound dehydroshikimic acid **20**.[9] Finally, the spectrum of the sample from the incubation of type I dehydroquinase with **9** and sodium borohydride showed two species, the major peak with a mass of 27 471 (±4) Da corresponding to unmodified dehydroquinase, and the minor peak (*ca.* 20%) with a mass of 27 605 (±6) Da corresponding to modified dehydroquinase with an increase in mass of 134. This agrees, within the experimental error, with the addition of 5-deoxydehydroshikimic acid bound *via* a reduced imine **21**, which would give an expected increase in mass of 140. These results are consistent with 5-deoxydehydroquinic acid **9** being a substrate for type I dehydroquinase and binding *via* an imine linkage.



Scheme 5 Trapping the imine adduct formed on incubation of type I dehydroquinase with either dehydroquinate **1** (R = OH) or 5-deoxydehydroquinate **9** (R = H) by sodium borohydride

In contrast, assays of type I dehydroquinase with 4,5-dideoxydehydroquinic acid **19** showed no turnover to 4,5-dideoxydehydroshikimic acid. Treatment of an incubation of type I dehydroquinase with **19** and sodium borohydride resulted in no loss of activity, as determined in a subsequent assay with dehydroquinic acid. This implies that 4,5-dideoxydehydroquinic acid **19** does not form an imine linkage with the enzyme and consequently is not a substrate. This is consistent with preliminary findings that the dideoxy analogue of dehydroshikimic acid is not a substrate for the reverse reaction.[25] 4,5-Dideoxydehydroquinic acid **19** was shown to be a weak competitive reversible inhibitor of type I dehydroquinase, with a K_i value of 10 ± 1 mmol dm^{-3}.

The two substrate analogues **9** and **19** were also tested against the type II dehydroquinase from *M. tuberculosis*, a type II enzyme that operates on the shikimate (as opposed to quinate) pathway. 5-Deoxydehydroquinic acid **9** was found to be a substrate with a K_M value of 1.4 mmol dm^{-3}, k_{cat} 0.01 s^{-1} and k_{cat}/K_M 7 dm^3 mol^{-1} s^{-1}. Comparison with the corresponding values for the natural substrate of K_M 9 μmol, dm^{-3}, k_{cat} 4.3 s^{-1} and k_{cat}/K_M 4.6 × 10^5 dm^3 mol^{-1} s^{-1} show that 5-deoxydehydroquinic acid **9** is a very poor substrate for the type II dehydroquinase. Treatment with sodium borohydride, as above, resulted in no loss of activity. Electrospray mass spectrometric analysis showed no difference between the control sample with no substrate present [observed mass 15 660 (± 1)] and the sample with **9** present. This is not unexpected since all evidence to date suggests that the type II dehydroquinase-catalysed reaction does not proceed *via* a Schiff base mechanism.[4] 4,5-Dideoxydehydroquinic acid **19** was also found to be a poor substrate for the *M. tuberculosis* dehydroquinase, with K_M 30 mmol dm^{-3}, k_{cat} 0.16 s^{-1} and k_{cat}/K_M 5 dm^3 mol^{-1} s^{-1}. In addition, it proved to be a weak competitive inhibitor with a K_i value of 1.0 (± 0.2) mmol dm^{-3}.

Discussion

From the above results, it can be concluded that 5-deoxydehydroquinic acid **9** is a modest substrate for type I dehydroquinase, with the dehydration proceeding through an imine-bound species. Although the type I dehydroquinase will tolerate the absence of the C-5 hydroxy group, the reduction in specificity suggests that this group forms an important binding interaction with the enzyme. The apparent binding energy (ΔG_{app}) for the 5-hydroxy group, calculated from the ratio of the k_{cat}/K_M values for dehydroquinic acid and 5-deoxydehydroquinic acid, is 17.2 kJ mol^{-1} (4.1 kcal mol^{-1}). From experiments involving deletion of enzyme–substrate binding interactions by site-directed mutagenesis, it has been proposed that ΔG_{app} is a measure of specificity of binding and catalysis, and is a reasonable approximation of the true enzyme–substrate binding energy ΔG_{bind} in cases where mutation allows access of water to the region where deletion occurs.[26] Two classes of hydrogen-bonding interactions have been described: those formed to an uncharged donor or acceptor (where ΔG_{app} is in the range 2.1–7.5 kJ mol^{-1}) and those formed to a charged donor or acceptor (where ΔG_{app} is in the range 12.6–25.1 kJ mol^{-1}). The observed value of ΔG_{app} for the C-5 hydroxy group suggests that this group may be a hydrogen bonded to a charged acceptor such as a lysine or arginine sidechain.

The observation that 4,5-dideoxydehydroquinic acid **19** is not a substrate for type I dehydroquinase and does not form an imine-bound species is good evidence that the C-4 hydroxy group is in some way involved with imine formation. The fact that **19** acts as a poor competitive inhibitor of type I dehydroquinase indicates that the enzyme recognises the analogue and binds it at the active site, probably by hydrogen bonding with the ketone and acid, but is unable to form the catalytically important Schiff base linkage.

Both 5-deoxy- **9** and 4,5-dideoxy-dehydroquinic acid **19** are very poor substrates for type II dehydroquinase, with specificities of approximately 10^{-5} that of the natural substrate. The dideoxy analogue **19** also acts as a competitive inhibitor of type II dehydroquinase, with a K_i of 1 mmol dm^{-3}. The apparent binding energy for the C-5 hydroxy group was calculated as being 27.5 kJ mol^{-1} (6.6 kcal mol^{-1}). This suggests that the C-5 hydroxy group forms important enzyme–substrate binding interactions, which may be a hydrogen bond with a charged acceptor, but may be the sum of a number of hydrogen-bonding interactions which are deleted on removal of the C-5 hydroxy group. Significantly, removal of the C-4 hydroxy group does not appear to have any further effect on substrate binding. This suggests that either the C-4 hydroxy group does not form a significant enzyme-substrate binding interaction or, possibly, that removal of either C-4 or C-5 hydroxy group results in a breakdown of the important enzyme–substrate binding interactions with corresponding reduction in specificity.

These findings represent the first systematic study of the substrate specificity of the type I and type II dehydroquinases. They highlight differences in specificity which may have mechanistic significance and can be incorporated into inhibitor design, most notably the role of the C-4 hydroxy group. Removal of this group (in the dideoxy analogue **19**) has no additional effect on the type II catalysed reaction, but makes the compound not a substrate at all for the type I enzyme.

Studies are now in progress to synthesise 4-deoxydehydroquinic acid to complement this study and further characterise the role of the C-4 hydroxy group in binding and catalysis.

Experimental

General procedures

All reactions were carried out under dry argon where appropriate. All solvents were purified and distilled according to the methods of Perrin *et al.*[27] Progress of reactions was monitored by thin layer chromatography (TLC) on commercial Kieselgel 60 silica plates using either UV absorption, iodine staining or potassium manganate(VII) spray for visualization. Flash chromatography employed Kieselgel 60 silica, 230–400 mesh. Where quoted, carboxylic acids were analysed or purified by HPLC on either a semi-preparative (300 mm × 8 mm), or preparative (300 mm × 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} (semi-preparative column) or 1.2 ml min^{-1} (preparative column). Retention times are quoted in min, and were found to vary slightly (less than ±0.5 min) depending on the condition of the column and guard column. All HPLC separations were carried out on an LKB HPLC system. All procedures involving the use of ion exchange resins were carried out at room temperature and used Milli-Q deionised water. Amberlite CG-400 (100–200 mesh, anion exchanger, BDH) was washed several times in 10% HCl, and left in 10% HCl overnight then rinsed several times with water in a large beaker to remove fines prior to use. Dowex 50W-X8 (H) (50–100 mesh, cation exchanger) and Amberlite IR-120 (H) (cation exchanger) were generally washed alternately with water, 5% sodium hydroxide, water, 10% HCl, then finally with water before use.

Melting points were determined either on a Büchi 510 or Reichert melting point apparatus using open end capillary tubes and are uncorrected. Nuclear magnetic resonance spectra (NMR) were recorded on Bruker WM250, WM400 or AC250 instruments. The *J* values are given in Hz. Infrared spectra (IR) were determined with a Perkin-Elmer 1310 IR spectrometer, or a 1710 FT-IR spectrometer. Mass spectra (MS) were recorded on either a Kratos MS30 or MS50 instrument, in electron impact (EI) mode, unless otherwise stated. Fast atom bombardment (FAB) spectra were recorded on a Kratos MS80 spectrometer. Electrospray mass spectra were recorded on a VG BioQ quadrupole mass spectrometer. Ultraviolet–visible spectra and enzyme assays were recorded on a Varian Carey 1E UV–VIS spectrophotometer at 25 °C (controlled with a Grant LTD6 thermostatted water bath), using 1 ml quartz cells. All literature compounds had ^1H NMR, IR and mass spectra consistent with assigned structures. *E. coli* dehyroquinase was purified as reported previously,[28] and stored as concentrated solutions in 50% glycerol–water at −20 °C, under which conditions dehydroquinase is stable for at least 2 years. Type II dehydroquinase from *M. tuberculosis* was purified as described previously,[29] and the concentrated solution (3 mg ml^{-1}) 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. 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 the pH values of prepared buffers were adjusted using KOH or AcOH. All pH measurements were made at 25 °C using a Russell combined pH/reference cell electrode coupled to an alpha 200 pH and conductivity meter. Deuteriated buffers were made up in 99.9% D_2O and the pD adjusted with KOD or AcOD. pD values have been quoted where pD = meter reading +0.4.

Assays of dehydroquinase

Dehydroquinase was assayed by monitoring the increase in absorbance at 234 nm in the UV spectrum due to the absorbance of the enone carboxylate chromophore of dehydroshikimate ($\varepsilon = 1.2 \times 10^4$ dm^3 mol^{-1} cm^{-1}). Standard assay conditions for both type I and type II enzymes were pH 7.0 at 25 °C. Due to the inhibition of type I dehydroquinase by chloride and acetate, and the type II enzyme by phosphate, routine assays of each were carried out in different buffers: type I in potassium phosphate (50 mM) and type II in Tris–OAc (100 mM) unless otherwise indicated. A typical assay of type I dehydroquinase contained 50 mM potassium phosphate at pH 7.0, 0.5 mM dehydroquinate and 30 mU type I dehydroquinase. Each assay was initiated by addition of the enzyme. Solutions of dehydroquinate (and the deoxy analogues) were calibrated by equilibration with dehydroquinase and measurement of the change in the UV absorbance at 234 nm due to formation of the enone carboxylate chromophore of dehydroshikimate ($\varepsilon = 1.2 \times 10^4$ dm^3 mol^{-1} cm^{-1}).

Sodium borohydride inactivations

A stock solution of sodium borohydride (1 M) in aqueous sodium hydroxide (50 mM), which was stable for at least 45 min, was used for sodium borohydride inactivations. A 10 μl aliquot of this solution was added to a 1 ml incubation of enzyme and substrate, typically containing 50 mM potassium phosphate at pH 7.0, 0.5 mM dehydroquinate and 1 U type I dehydroquinase at 25 °C. A 10 μl aliquot of the incubation mix was assayed for dehydroquinase activity before and after addition of sodium borohydride. Loss of activity was indicative of imine formation *via* a Schiff base mechanism. A control experiment with no substrate present showed no loss of activity. For substrate analogues it was necessary to add sequential aliquots of the borohydride solution to obtain a high degree of inactivation. Typically 10 μl aliquots were added every 10 min for 30–40 min.

Preparation of enzyme samples for electrospray mass spectrometry

Following the irreversible inactivation of enzyme solutions, either by direct incubation with irreversible inhibitors or by borohydride reduction of an enzyme–substrate complex, the covalently modified enzyme was analysed by electrospray mass spectrometry. Typically enzyme solutions were prepared as follows. (i) Gel filtration through a NAP_{10} Sephadex G-25 column, eluting with Milli-Q water, to remove any excess small molecules, such as buffer salts and excess inhibitor, substrate or borohydride. (ii) Ultrafiltration using an Amicon Centricon-10 microconcentrator. This concentrates the protein into a volume of approximately 30 μl, by allowing molecules with a molecular weight of less than 10 000 to pass through an anisotropic membrane. (iii) Dialysis of the residual enzyme solution against water, at 4 °C overnight, using a Pierce Microdialysis kit with dialysis tubing cutoff *ca.* 12 000 Da (Sigma).

Solutions containing 100–200 pmol of enzyme in 20 μl of solvent are required for electrospray mass spectrometry. Therefore incubations of type I dehydroquinase in irreversible inactivation experiments typically contained 6 nmol of protein (0.17 mg). Dialysed samples were diluted to the required concentration with a 50% MeOH–H_2O mixture containing 1% AcOH, immediately prior to injection.

(1*S*,3*R*,4*R*,5*R*)-4,5-Benzylidenedioxy-1-hydroxycyclohexane-1,3-carbolactone 3

A mixture of (−)-quinic acid (4.94 g, 25.7 mmol), distilled benzaldehyde (3.9 ml, 38.6 mmol) and toluene-*p*-sulfonic acid (253 mg, 1.3 mmol) was heated at reflux in toluene (50 ml) in an apparatus fitted with a Dean–Stark trap. After 22 h most of the white suspension had dissolved (leaving a small amount of brown solid) and 0.85 ml (47 mmol) of water had collected. The solution was allowed to cool and then was concentrated under

reduced pressure. The oily residue was taken up in diethyl ether and decanted from the solid. The crude mixture was purified by flash chromatography [EtOAc–light petroleum (bp 40–60) (1:1)] to give *benzylidene lactone* **3** (5.31 g, 79%) as a mixture of diastereomers (2.2:1). On cooling, the viscous oil crystallised. Recrystallisation from diethyl ether gave the major diastereomer (with the *R* configuration at the benzylic carbon) as needles, mp 100–101 °C (lit.,[16] 95 °C) (Found: C, 63.9; H, 5.36%; M^+, 262.0820. $C_{14}H_{14}O_5$ requires C, 64.1; H, 5.38%; *M*, 262.0841); R_F 0.46 [EtOAc–light petroleum (1:1)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3540 (free OH), 3420 (H-bonded OH), 1800 (C=O) and 1470 (Ar C–C); λ_{max}(95% EtOH)/nm 211 and 258 (fine structure); δ_H(250 MHz, $CDCl_3$) 7.51–7.36 (5 H, m, Ph), 5.75 (1 H, s, $PhCHO_2$), 4.81 (1 H, dd, *J* 6.1, 2.1, 3-H), 4.52 (1 H, td, *J* 7.0, 2.7, 5-H), 4.37 (1 H, dt, *J* 7.0, 2.1, 4-H), 2.91 (1 H, br s, OH), 2.78 (1 H, d, *J* 11.9, 2-H^{ax}), 2.46 (1 H, ddd, *J* 15.1, 7.0, 2.1, 6-H^{eq}), 2.36 (1 H, dd, *J* 15.1, 2.7, 6-H^{ax}) and 2.34 (1 H, ddt, *J* 11.9, 6.1, 2.1, 2-H^{eq}); δ_C(100 MHz, $CDCl_3$) 178.9, 135.4, 129.9, 128.6, 126.6, 103.7, 75.5, 72.9, 72.7, 71.4, 37.7 and 34.4; *m/z* (EI) 262 (M^+), 261 ($M^+ - H$) and 105 ($PhCO^+$).

(1*S*,3*R*,4*R*,5*R*)-4,5-Benzylidenedioxy-1-(benzyloxycarbonyloxy)cyclohexane-1,3-carbolactone 4

The lactone **3** (4.00 g, 15.3 mmol) was dissolved in CH_2Cl_2 (160 ml) and cooled with stirring to 0 °C. Sodium hydride (800 mg of a 50% suspension in mineral oil, 0.16 mmol) was added as a suspension in CH_2Cl_2 (15 ml) to give a flocculent suspension. Benzyl chloroformate (3.26 ml, 22.8 mmol) was added dropwise over 5 min. Tetrabutylammonium iodide (75 mg) was added and the mixture stirred for 70 h, during which time the solution changed from colourless to pale yellow. Water (20 ml) was added and the mixture extracted with CH_2Cl_2 (3 × 30 ml). The combined extracts were washed with water (20 ml) and brine (20 ml), dried (Na_2SO_4), and concentrated under reduced pressure to give a yellow oil. Purification by flash chromatography [EtOA–light petroleum (3:7)] gave the *benzyl carbonate* **4** (4.89 g, 82%) as needles, mp 109–111 °C (from light petroleum–EtOAc) (Found: C, 67.1; H, 5.15%; M^+, 396.1172. $C_{22}H_{20}O_7$ requires C, 66.7; H, 5.09%; *M*, 396.1208); R_F 0.55 [EtOAc–light petroleum (1:1)]; $\nu_{max}(CH_2Cl_2$solution)/cm^{-1} 1810 (lactone C=O), 1740 (carbonate C=O) and 1470 (Ar C–C); λ_{max}(95% EtOH)/nm 209 and 256 (fine structure); δ_H(250 MHz, $CDCl_3$) 7.50–7.32 (10 H, m, 2 Ph), 5.76 (1 H, s, $PhCHO_2$), 5.22–5.12 (2 H, AB system, *J* 12.1, $PhCH_2OCO_2$), 4.88 (1 H, dd, *J* 6.3, 2.1, 3-H), 4.58 (1 H, td, *J* 7.0, 3.0, 5-H), 4.40 (1 H, ddd, *J* 7.0, 2.1, 1.9, 4-H), 3.13 (1 H, ddt, *J* 11.4, 6.3, 1.9, 2-H^{eq}), 2.71 (1 H, d, *J* 11.4, 2-H^{ax}), 2.61 (1 H, ddd, *J* 14.8, 7.0, 1.9, 6-H^{eq}) and 2.51 (1 H, dd, *J* 14.8, 3.0, 6-H^{ax}); δ_C(100 MHz, $CDCl_3$) 173.0, 152.5, 135.5, 134.5, 129.9, 128.8, 128.6, 128.4, 126.5, 125.9, 103.8, 75.1, 75.0, 73.0, 72.4, 70.3, 35.0 and 30.3; *m/z* (EI) 396 (M^+), 305 ($M^+ - PhCH_2$), 246 ($M^+ - PhCH_2OCO_2$) and 91 ($PhCH_2^+$).

Methyl (1*R*,3*R*,4*S*,5*R*)-3,4-benzylidenedioxy-1-(benzyloxycarbonyloxy)-5-hydroxycyclohexane-1-carboxylate 5

A solution of sodium methoxide was generated by addition of sodium metal (0.0803 g, 3.49 mmol) to methanol (25 ml) at room temp. under a dry nitrogen atmosphere. When the effervescence had subsided benzyl carbonate **4** (1.41 g, 3.49 mmol) was added and the solution stirred at room temp. for 1 h. Saturated aqueous NH_4Cl (6 ml) was added and the mixture extracted with EtOAc (5 × 50 ml). The combined organic extracts were washed with water (3 × 50 ml) and brine (50 ml), dried (Na_2SO_4), and concentrated under reduced pressure to give a brown oil. Purification by flash chromatography [EtOAc–hexane (1:1)] gave *alcohol* **5** (0.89 g, 59%) as a solid, mp 100–102 °C (from light petroleum–EtOAc) (Found: C, 63.7; H, 5.22%; M^+, 428.1449. $C_{23}H_{24}O_8$ requires C, 64.4; H, 5.65%; *M*, 428.1470); R_F 0.27 [EtOAc–hexane (1:1)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3598 (OH), 1747 (carbonate C=O), 1687 (ester C=O), 1605 (Ar C–C) and 1261 (C–O); λ_{max}(95% EtOH)/nm 212 and 234; δ_H(250 MHz, $CDCl_3$) 7.49–7.25 (10 H, m, Ph), 5.85 (1 H, s, $PhCHO_2$), 5.18–5.08 (2 H, AB system, *J* 12.3, $PhCH_2OCO_2$), 4.48 (1 H, ddd, *J* 6.0, 4.6, 2.9, 5-H), 4.08 (1 H, t, *J* 6.0, 4-H), 4.08 (1 H, ddd, *J* 10.0, 6.0, 3.9, 3-H), 3.75 (3 H, s, Me), 2.93 (1 H, ddd, *J* 16.1, 2.9, 2.0, 6-H^{eq}), 2.46 (1 H, dd, *J* 16.1, 4.6, 6-H^{ax}), 2.33 (1 H, ddd, *J* 14.0, 3.9, 2.0, 2-H^{eq}) and 2.03 (1 H, dd, *J* 14.0, 10.4, 2-H^{ax}); δ_C(100 MHz, $CDCl_3$) 171.9, 154.0, 137.0, 135.0, 129.4, 128.4, 128.3, 128.2, 126.7, 104.0, 80.5, 78.9, 74.8, 69.8, 68.0, 53.0, 36.7 and 30.6; *m/z* (EI) 428 (M^+), 396 ($M^+ - H - OMe$), 337 ($M^+ - PhCH_2$), 276 ($M^+ - H - PhCH_2OCO_2$) and 91 ($PhCH_2^+$).

Methyl (1*S*,4*S*,5*R*)-4,5-benzylidenedioxy-1-(benzyloxycarbonyloxy)cyclohex-2-ene-1-carboxylate 6

A solution of alcohol **5** (0.2061 g, 0.48 mmol) in pyridine (0.10 ml, 1.19 mmol) and CH_2Cl_2 (7 ml) was cooled to −15 °C (CCl_4–dry ice bath) under an atmosphere of dry nitrogen. Trifluoromethanesulfonic anhydride (0.126 ml, 0.76 mmol) was added dropwise over 5 min. The flask was allowed to warm to room temp. and after 1 h saturated aqueous NH_4Cl (2 ml) was added. The mixture was extracted with CH_2Cl_2 (3 × 30 ml), washed with water (3 × 20 ml) and brine (20 ml), and dried (Na_2SO_4). The pyridine was removed by evaporation under reduced pressure of a toluene solution. 1,8-Diazabicyclo-[5.4.0]undec-7-ene (DBU) (0.124 ml, 0.79 mmol) was added dropwise to a solution of the resulting yellow oil [R_F 0.49, EtOAc–light petroleum (1:1)] in $CHCl_3$ (5 ml). The mixture was heated at reflux for 3 h. After cooling, the solution was concentrated under reduced pressure and the residue purified by flash chromatography [EtOAc–light petroleum (1:4 to 1:1)] to give *alkene* **6** (0.1188 g, 60%) [Found: ($M^+ - H$), 409.1262. $C_{23}H_{21}O_7$ requires *m/z*, 409.1287]; R_F 0.53 [EtOAc–light petroleum (1:1)]; $\nu_{max}(CH_2Cl_2$ solution)/cm^{-1} 1747 (C=O), 1686 (C=O) and 1607 (Ar C–C); $\lambda_{max}(CH_2Cl_2)$/nm 228 and 258 (fine structure); δ_H(250 MHz, $CDCl_3$) 7.48–7.29 (10 H, m, Ph), 6.15 (2 H, s, 2-H and 3-H), 5.90 (1 H, s, $PhCHO_2$), 5.20–5.10 (2 H, AB system, *J* 12, $PhCH_2OCO_2$), 4.71 (1 H, ddd, *J* 7.8, 6.9, 5.4, 5-H), 4.61 (1 H, d, *J* 6.9, 4-H), 3.72 (3 H, s, Me), 2.75 (1 H, dd, *J* 13.4, 5.4, 6-H^{eq}) and 2.23 (1 H, dd, *J* 13.4, 7.8, 6-H^{ax}); *m/z* (EI) 410 (M^+), 409 ($M^+ - H$), 319 ($M^+ - PhCH_2$), 259 ($M^+ - PhCH_2OCO_2$) and 91 ($PhCH_2^+$, 100).

Methyl (1*R*,3*R*,4*S*)-1,3,4-trihydroxycyclohexane-1-carboxylate 7

To a solution of alkene **6** (0.100 g, 0.24 mmol) in CH_2Cl_2 (20 ml) was added 5% Pd–C (0.100 g). The flask was evacuated then the solution was stirred with hydrogen gas bubbling through for 3 h. The mixture was then stirred for 2 days under a hydrogen filled balloon. The catalyst was removed by filtration through Celite. The filtrate was concentrated under reduced pressure to give *triol* **7** (47 mg, quant.) (Found: MNH_4^+, 208.1185. $C_8H_{18}O_5N$ requires *m/z*, 208.1185); HPLC retention time 38 min (preparative Bio-Rad organic acids column); R_F 0.63 [AcOH–EtOH (1:9)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3684 (OH), 3598 (OH), 2985, 1729 (C=O) and 1605; δ_H(250 MHz, $CDCl_3$) 4.00 (1 H, br d, *J* 3.1, 3-H), 3.87–3.56 (2 H, m), 3.79 (3 H, s, Me), 2.13 (1 H, dm, *J* 14.6, 2-H^{eq}), 2.0 (1 H, dd, *J* 14.6, 3.1, 2-H^{ax}) and 1.9–1.8 (6 H, m); δ_C(100 MHz, $CDCl_3$) 175.9, 74.0, 70.3, 69.7, 53.0, 37.2, 33.2 and 24.1; *m/z* (EI) 191 (MH^+), 172 ($M^+ - H_2O$), 155 ($MH^+ - 2H_2O$), 146, 131 ($M^+ - CO_2Me$), 113 ($M^+ - CO_2Me - H_2O$), 103, 95, 86 and 71; *m/z* (CI–NH_3) 208 (MNH_4^+), 191 (MH^+) and 173 ($MH^+ - H_2O$).

(1*R*,3*R*,4*S*)-1,3,4-Trihydroxycyclohexane-1-carboxylic acid (5-deoxyquinic acid) 8

To a solution of triol ester **7** (40 mg, 0.21 mmol) in methanol (5 ml) was added a solution of potassium carbonate (0.120 g, 0.87 mmol) in water (5 ml). The mixture was stirred at room temp. for 24 h, then acidified to pH 1 [Dowex 50W-X8 (H^+) (50–100 mesh)]. The resin was removed by filtration and the solution

concentrated under reduced pressure to give 22 mg of a yellow oil which was purified by HPLC (preparative Bio-Rad organic acids column) to give *acid* **8** (16 mg, 43%) with a retention time of 25–26 min (Found: MH^+, 177.0761. $C_7H_{13}O_5$ requires *m/z*, 177.0763); R_F 0.48 [AcOH–EtOH (1:9)]; ν_{max}(thin film)/cm^{-1} 3600–2800 (OH), 2985, 1716 (C=O) and 1605; δ_H(400 MHz, D_2O) 3.96 (1 H, ddd, *J* 6.2, 3.5, 3.4, 3-H), 3.79 (1 H, dt, *J* 8.7, 3.4, 4-H), 2.07 (1 H, dd, *J* 14.1, 3.5, 2-H^{ax}), 1.95 (1 H, ddd, *J* 14.0, 6.2, 1.7, 2-H^{eq}), 1.92–1.79 (3 H, m, 6-H_2 and 5-H) and 1.69–1.64 (1 H, m, 5-H); δ_C(100 MHz, D_2O) 178.2, 75.2, 71.0, 38.5, 33.2 and 25.7; *m/z* (CI–NH_3) 194 (MNH_4^+) and 177 (MH^+); *m/z* (EI) 177 (MH^+), 158 ($M^+ - H_2O$), 140 ($M^+ - 2H_2O$), 131 ($M^+ - CO_2H$) and 113 ($M^+ - CO_2H - H_2O$).

(1*R*,4*S*)-1,4-Dihydroxy-3-oxocyclohexane-1-carboxylic acid (5-deoxydehydroquinatic acid) 9

To a solution of triol **8** (8 mg, 0.045 mmol) in H_2O (0.2 ml) at 0 °C was added concentrated nitric acid (0.5 ml). The solution was stirred for 30 min at room temp. and then at 0 °C for 60 h. The solution was diluted with H_2O (2 ml) and purified by HPLC (semi-preparative Bio-Rad organic acids column, UV detection at 277 nm) to give *ketone* **9** (1.5 mg, 20%) with a retention time of 12 min and unoxidised triol **8** (5.5 mg, 69%) with a retention time of 14 min. δ_H(400 MHz, D_2O) 4.50 (1 H, dd, *J* 12.0, 6.5, 4-H), 3.13 (1 H, d, *J* 14.0, 2-H^{ax}), 2.58 (1 H, dd, *J* 14.3, 2.7, 2-H^{eq}), 2.41–2.31 (2 H, m, 5-H and 6-H^{ax}) and 2.10–1.88 (2 H, m, 5-H and 6-H^{eq}); *m/z* (EI) 110 ($MH^+ - H_2O - CO_2H$) and 94 ($MH^+ - 2OH - CO_2H$).

(4*S*)-4-Hydroxy-3-oxocyclohex-1-ene-1-carboxylate (5-deoxydehydroshikimate) 22

5-Deoxydehydroquinic acid **9** (1 mg, 6 μmol) was dissolved in deuteriated potassium phosphate buffer (0.5 ml, 25 mmol). The pD was adjusted to 7.0 with KOD, then type I dehydroquinase (20 U) was added. The conversion to *5-deoxydehydroshikimate* **22** was monitored by ^1H NMR spectroscopy. The product was not isolated. δ_H(400 MHz, D_2O) 6.30 (1 H, s, 2-H), 4.40 (1 H, dd, *J* 13.9, 5.5, 4-H), 2.70 (2 H, m, 6-H_2), 2.33 (1 H, m, 5-H^{eq}) and 1.93 (1 H, m, 5-H^{ax}).

Methyl(1*S*,4*S*,5*R*)-1-Benzyloxycarbonyloxy-4,5-dihydroxycyclohex-2-ene-1-carboxylate 11

To a solution of methyl (1*S*,4*S*,5*R*)-1-benzyloxycarbonyloxy-4,5-(cyclohexylidenedioxy)cyclohex-2-ene-1-carboxylate **10** (6.5 g, 0.02 ml) in H_2O (15 ml) was added trifuoroacetic acid (TFA) (25 ml). The solution was stirred for 3 days. Water (200 ml) was added and the mixture extracted into CH_2Cl_2 (250 ml). The aqueous layer was extracted repeatedly with CH_2Cl_2 (6 × 50 ml). The organic extracts were combined and washed with saturated aqueous $NaHCO_3$ (50 ml), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography [EtOAc–hexane (3:7)] gave *diol* **11** (2.52 g, 48%) and cyclohexylidene starting material **10** (1.24 g, 19%) (Found: MNH_4^+, 340.1384. $C_{16}H_{22}O_7N$ requires *m/z*, 340.1396); R_F 0.17 [EtOAc–hexane (1:1)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3560 (OH), 2900 (CH) and 1740 (C=O); δ_H(200 MHz, $CDCl_3$) 7.36 (5 H, m, Ph), 6.13–6.08 (1 H, ABdd, *J* 10.2, 3.0, 3-H), 6.07–6.03 (1 H, ABd, *J* 10.3, 2-H), 5.15 (2 H, s, $PhCH_2$), 4.16–4.08 (2 H, m, 4-H and 5-H), 3.72 (3 H, s, CO_2Me), 2.60 (2 H, br s, 2 OH), 2.51–2.44 (1 H, dd, *J* 14.1, 3.4, 6-H^{eq}) and 2.37–2.28 (1 H, *J* 14.1, 8.1, 6-H^{ax}); δ_C(100 MHz, $CDCl_3$) 170.5, 153.5, 134.8, 133.7, 128.5, 128.2, 126.4, 79.8, 70.0, 65.7, 65.4, 52.9 and 33.7; *m/z* (CI–NH_3) 340 (MNH_4^+), 322 (M^+), 232 (M^+ – PhCH), 206, 190 and 188 ($MH^+ - PhCH_2OCO$).

Methyl (1*S*,4*R*,5*R*)-5-acetoxy-1-(benzyloxycarbonyloxy)-4-bromocyclohex-2-ene-1-carboxylate 12

To a solution of diol **11** (2.48 g, 7.7 mmol) in acetonitrile (60 ml) at 0 °C under dry N_2 was added 2-acetoxyisobutyryl bromide. The solution was stirred at 0 °C for 30 min. The solvent was evaporated under reduced pressure. The residue was taken up in EtOAc (200 ml) and washed with H_2O (3 × 50 ml) and brine (2 × 30 ml). The organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by flash chromatography [EtOAc–hexane (1:4)] gave *allylic bromide* **12** (3.0 g, 91%) (Found: MNH_4^+, 444.0698. $C_{18}H_{23}O_7NBr$ requires *m/z*, 444.0657); R_F 0.63 [EtOAc–hexane (1:1)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3000 (CH) and 1740 (C=O); δ_H(400 MHz, $CDCl_3$) 7.33 (5 H, s, Ph), 6.20 (1 H, dd, *J* 10.1, 3.8, 3-H), 6.07 (1 H, d, *J* 10.1, 2-H), 5.35 (1 H, dt, *J* 7.2, 3.6, 5-H), 5.15 (1 H, ABd, *J* 12.3, PhC*H*), 5.09 (1 H, ABd, *J* 12.2, PhC*H*), 4.55 (1 H, t, *J* 4.2, 4-H), 3.74 (3 H, s, CO_2Me), 2.79 (1 H, dd, *J* 14.7, 3.0, 6-H^{eq}), 2.38 (1 H, dd, *J* 14.7, 6.9, 6-H^{ax}) and 1.99 (3 H, s, Ac); *m/z* (CI–NH_3) 446, 444 (MNH_4^+), 366 (M^+ – Br), 277, 275 ($M^+ - CO_2Bn$ – Ac) and 213 ($M^+ - CO_2Bn$ – Ac – Br – Me).

Methyl (1*S*,5*S*)-5-acetoxy-1-(benzyloxycarbonyloxy)cyclohex-2-ene-1-carboxylate 13 and methyl (1*R*,5*R*)-5-acetoxy-1-(benzyloxycarbonyloxy)cyclohex-3-ene-1-carboxylate 14

To a stirred solution of Bu_3SnH (13.6 ml, 51 mmol) in toluene (50 ml) under N_2, was added a solution of allylic bromide **12** (2.75 g, 6.43 mmol) and azoisobutyronitrile (AIBN) (0.23 g, 14 mmol) in toluene (50 ml). The mixture was stirred at room temp. for 2 days, then was concentrated under reduced pressure and the residue partitioned between MeCN (75 ml) and hexane (75 ml). The tin residues were washed into the hexane. The MeCN was evaporated under reduced pressure and the residual oil purified by flash column chromatography [EtOAc–hexane (1:4)] to give *alkenes* **13** and **14** (2.07 g, 93%) as a 2:1 mixture (as determined by ^1H NMR spectroscopy) (Found: M^+, 348.1206; MH^+, 349.1280. $C_{18}H_{20}O_7$ requires *M*, 348.1209; $C_{18}H_{21}O_7$ requires *m/z*, 349.1287); R_F 0.47 [EtOAc–hexane (1:1)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2955, 1742 (C=O), 1655 (C=C), 1530 and 1498 (Ar); δ_H(200 MHz, $CDCl_3$) **13** 7.35 (5 H, m, Ph), 6.02 (1 H, ddd, *J* 10.0, 3.3, 3.0, 3-H), 5.90 (1 H, m, 2-H), 5.2 (1 H, m, 5-H), 5.15 (2 H, s, $PhCH_2$), 3.70 (3 H, s, CO_2Me), 2.63 (1 H, dd, *J* 13.3, 3.3, 4-H^{eq}), 2.55 (2 H, m, 6-H_2), 2.25 (1 H, dd, *J* 13.3, 9.7, 4-H^{ax}) and 2.00 (3 H, s, $OCOCH_3$); δ_H(200 MHz, $CDCl_3$) **14** 7.35 (5 H, m, Ph), 5.90 (2 H, m, 3-H and 4-H), 5.4 (1 H, m, 5-H), 5.15 (2 H, s, $PhCH_2$), 3.71 (3 H, s, CO_2Me), 2.78 (1 H, dt, *J* 16.0, 1.7, 6-H^{eq}), 2.55 (1 H, m, 2-H), 2.38 (1 H, dd, *J* 16.0, 6.3, 6-H^{ax}), 2.15 (1 H, m, 2-H) and 1.81 (3 H, s, $OCOCH_3$); δ_C(100 MHz, $CDCl_3$) **13** and **14** 171.6, 170.3, 153.6, 134.9, 131.1, 128.6, 128.5, 128.2, 126.6, 124.4, 123.8, 79.8, 78.6, 77.4, 77.1, 76.7, 69.8, 66.4, 64.8, 53.4, 52.9, 52.7, 34.8, 33.6, 31.6, 30.2, 23.3, 21.0 and 20.7; *m/z* (CI–NH_3) 366 (MNH_4^+), 289 (MH^+ – AcOH), 197 ($M^+ - PhCH_2OCO_2$, 100), 154 ($M^+ - PhCH_2OCO_2 - CH_3CO$), 137 ($M^+ - PhCH_2OCO_2$ – AcOH), 108, 91 ($PhCH_2^+$); *m/z* (FAB$^+$) 349 (MH^+), 289 (MH^+ – AcOH), 197, 154 and 137.

Methyl (1*R*,3*S*)-3-acetoxy-1-hydroxycyclohexane-1-carboxylate 15

To a solution of alkenes **13** and **14** (0.55 g, 1.58 mmol) in ethanol (50 ml) was added 30% Pd–C (0.12 g). The mixture was placed in a pressure hydrogenator and stirred at 60 °C under 5 atm pressure for 18 h. The mixture was filtered through Celite and concentrated under reduced pressure. Purification by flash chromatography [EtOAc–hexane (3:7)] gave *alcohol* **15** (0.293 g, 86%) (Found: MH^+, 217.1076. $C_{10}H_{17}O_5$ requires *m/z*, 217.1076); R_F 0.41 [EtOAc–hexane (1:1)], 0.65 [MeOH–EtOAc (1:9)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3543 (OH), 2953 (CH), 1732 (C=O) and 1245; δ_H(400 MHz, $CDCl_3$) 4.95 (1 H, m, *J* 3.6, 3-H), 3.67 (3 H, s, CO_2Me), 3.40 (1 H, s, OH), 2.12 (1 H, dd, *J* 13.9, 4.0, 2-H^{eq}), 1.94 (3 H, s, OAc), 1.84–1.66 (4 H, m) and 1.56–1.41 (3 H, m); δ_C(100 MHz, $CDCl_3$) 175.4, 170.1, 73.9, 69.3, 52.5, 38.5, 34.8, 29.4, 21.1 and 17.7; *m/z* (CI–NH_3) 234 (MNH_4^+), 217 (MH^+), 199 (M^+ – OH), 157 (M^+ – OAc) and 97

(M^+ − HOAc − CO_2Me); *m/z* (EI) 217 (MH^+), 199 (M^+ − OH) and 157 (M^+ − OAc).

(1*R*,3*S*)-1,3-Dihydroxycyclohexane-1-carboxylic acid (4,5-dideoxyquinic acid) 16

To a solution of alcohol **15** (0.28 g, 1.3 mmol) in THF (3 ml) and H_2O (4 ml) was added LiOH (0.22 g, 9.2 mmol). The mixture was stirred at room temp. for 30 min. The solution was concentrated under reduced pressure and the residue dissolved in H_2O. Amberlite IR-120 (14–52 mesh) ion exchange resin was added and stirred for 30 min. The resin was removed by filtration and the filtrate concentrated under reduced pressure to give *diol* **16** (0.20 g, quant.), which was used without purification [Found: (M^+ − OH), 143.0694. $C_7H_{11}O_3$ requires *m/z*, 143.0708]; R_F 0.67 [EtOAc–AcOH–H_2O (4:1:1)]; $\nu_{max}(CCl_4)/cm^{-1}$ 3543 (OH), 2953 (CH), 1732 (C=O) and 1245; δ_H(400 MHz, D_2O) 3.88 (1 H, m, 3-H), 2.2 (1 H, dd, *J* 13.0, 4.2, 2-H^{eq}), 1.92 (1 H, m), 1.73 (2 H, m), 1.48 (2 H, dd, *J* 13.0, 8.8, 2-H^{ax}) and 1.33 (2 H, m); δ_C(100 MHz, D_2O) 180.3, 77.3, 69.5, 43.5, 36.7, 34.5 and 20.2; *m/z* (CI–NH_3) 178 (MNH_4^+) and 161 (MH^+); *m/z* (EI) 161 (MH^+), 143 (M^+ − OH), 125 (M^+ − OH − H_2O), 115 (M^+ − CO_2H) and 97 (M^+ − CO_2H − H_2O).

Benzyl (1*R*,3*S*)-1,3-dihydroxycyclohexane-1-carboxylate 17

To a solution of diol **16** (0.133 g, 0.83 mmol) in *N*,*N*-dimethylformamide (DMF) (9 ml) was added NEt_3 (0.30 ml, 2.2 mmol), 4-dimethylaminopyridine (DMAP) (0.160 g, 1.3 mmol) and Bu_4NI (10 mg, 0.03 mmol). The mixture was stirred under nitrogen for 10 min. Benzyl bromide (0.24 ml, 1.9 mmol) was added and the reaction stirred for 2 days. HCl (10 ml), H_2O (10 ml) and CH_2Cl_2 (20 ml) were added. The organic phase was further washed with H_2O (3 × 10 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The product in DMF was taken up in diethyl ether (20 ml), washed with brine (10 ml), dried (Na_2SO_4) and concentrated under reduced pressure to give *benzyl ester* **17** (0.110 g, 70%) as needles, mp 94 °C (from hexane) (Found: C, 66.7; H, 7.26%; MH^+, 251.1286. $C_{14}H_{18}O_4$ requires C, 67.2; H, 7.25%; $C_{14}H_{19}O_4$ requires *m/z*, 251.1283); R_F 0.24 [EtOAc–hexane (1:1)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3500 (OH), 2900 (CH) and 1720 (C=O); δ_H(250 MHz, $CDCl_3$) 7.3 (5 H, m, Ph), 5.2 (2 H, s, CH_2Ph), 4.1 (1 H, m, 3-H), 3.6 (1 H, br s, OH), 2.1–1.8 (6 H, m) and 1.6–1.4 (2 H, m); δ_C(100 MHz, $CDCl_3$) 175.9, 135.1, 128.7, 128.6, 128.1, 74.9, 67.77, 66.4, 38.5, 34.4, 32.1 and 15.2; *m/z* (EI) 251 (MH^+), 233 (M^+ − OH), 215 (M^+ − OH − H_2O), 205 (M^+ − CO_2H), 161 (M^+ − CH_2Ph), 115 (M^+ − CO_2CH_2Ph) and 91 (CH_2Ph^+).

Benzyl (1*R*)-1-hydroxy-3-oxocyclohexane-1-carboxylate 18

To a solution of benzyl ester **17** (30 mg, 0.12 mmol) in CH_2Cl_2 (5 ml) was added pyridinium dichromate (PDC) (60 mg, 0.18 mmol). The mixture was stirred at room temp. for 48 h. Diethyl ether (5 ml) was added, causing precipitation of the PDC which was removed by filtration through a short column of Celite (in a Pasteur pipette). The product was extracted from the remaining traces of PDC by dissolving in boiling hexane and concentration under reduced pressure to give *ketone* **18** (25 mg, 84%) (Found: M^+, 248.1050; MH^+, 249.1119. $C_{14}H_{16}O_4$ requires *M*, 248.1048; $C_{14}H_{17}O_4$ requires *m/z*, 249.1126); R_F 0.44 [EtOAc–hexane (1:1)]; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3539 (OH), 2960 (CH), 1730 (C=O), 1606 and 1498 (Ph); δ_H(250 MHz, $CDCl_3$) 7.35 (5 H, m, Ph), 5.24 (2 H, ABqt, *J* 5.0, CH_2Ph), 3.08 (1 H, br s, OH), 2.79 (1 H, d, *J* 14.5, 2-H^{ax}), 2.45 (1 H, dd, *J* 14.6, 1.7, 2-H^{eq}), 2.45–2.30 (2 H, m), 2.27–2.1 (2 H, m) and 2.05–1.87 (2 H, m); δ_C(100 MHz, $CDCl_3$) 207.4, 174.6, 135.3, 128.9, 128.4, 68.2, 50.2, 40.6, 34.1 and 20.9; *m/z* (EI) 249 (MH^+), 248 (M^+), 231 (M^+ − OH), 124 (M^+ − OH − OBn), 113 (M^+ − CO_2Bn) and 91 (CH_2Ph^+).

(1*R*)-1-Hydroxy-3-oxocyclohexane-1-carboxylic acid (4,5-dideoxydehydroquinic acid) 19

To a solution of keto ester **18** (25 mg, 0.10 mmol) in methanol (2 ml) was added 5% Pd–C. The reaction vessel was evacuated and filled with H_2 three times. The mixture was stirred under a H_2-filled balloon for 30 min. The catalyst was removed by filtration through a plug of cotton wool and the solution concentrated under reduced pressure to give *keto acid* **19** (14 mg, 88%) as an oil which solidified on cooling at 4 °C (Found: M^+, 158.0579. $C_7H_{10}O_4$ requires *m/z*, 158.0579); R_F 0.50 [EtOAc–AcOH–H_2O (4:1:1)]; δ_H(250 MHz, CD_3OD) 2.79 (1 H, d, *J* 14.5, 2-H^{ax}), 2.32 (1 H, d, *J* 14.5, 2-H^{eq}), 2.34–2.20 (2 H, m, 4-H_2) and 2.15–1.75 (4 H, m, 5-H_2 and 6-H_2); δ_C(100 MHz, CD_3OD) 211.3, 177.6, 77.8, 50.9, 41.1, 34.6 and 21.9; *m/z* (EI) 158 (M^+), 140 (M^+ − H_2O) and 112 (M^+ − HCO_2H).

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