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Design, synthesis and evaluation of bifunctional inhibitors of type II dehydroquinase †

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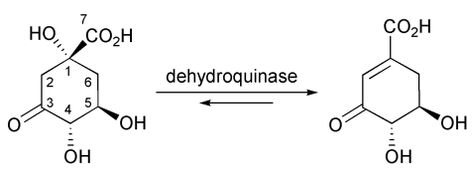
Inhibitors of type II dehydroquinase were designed to straddle the two distinct binding sites identified for the inhibitor (1*S*,3*R*,4*R*)-1,3,4-trihydroxy-5-cyclohexene-1-carboxylic acid and a glycerol molecule in a crystallographic study of the *Streptomyces coelicolor* enzyme. A number of compounds were designed to incorporate characteristics of both ligands. These analogues were synthesized from quinic acid, and were assayed against type I (*Salmonella typhi*) and type II (*S. coelicolor*) dehydroquinases. None of the analogues showed inhibition for type I dehydroquinase. Six of the analogues were shown to have inhibition constants in the micromolar to low millimolar range against the *S. coelicolor* type II dehydroquinase, while two showed no inhibition. The binding modes of the analogues in the active site of the *S. coelicolor* enzyme were studied by molecular docking with GOLD1.2. These studies suggest a binding mode where the ring is in a similar position to (1*S*,3*R*,4*R*)-1,3,4-trihydroxy-5-cyclohexene-1-carboxylic acid in the crystal structure and the side-chain occupies part of the glycerol binding-pocket.

Introduction

Dehydroquinase (EC 4.2.1.10, 3-dehydroquinate dehydratase) catalyses the conversion of 3-dehydroquinate (**1**) into 3-dehydroshikimate (**2**) (Scheme 1).[1] This is the third step in the shikimate pathway leading to chorismate, the precursor to the aromatic amino-acids L-phenylalanine, L-tryptophan and L-tyrosine, and other key metabolic intermediates (*e.g. p*-aminobenzoic acid).[2] The pathway is present in plants, fungi, bacteria and some parasites, and inhibitors of shikimate pathway enzymes are candidates for antibiotic, anti-parasitic and herbicidal agents (*e.g.* the herbicide glyphosate inhibits the sixth enzyme, 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthase).[3] The conversion of 3-dehydroquinate into 3-dehydroshikimate is also a step on the quinate pathway in fungi.[4]



1 **2**

Scheme 1 The reaction catalysed by dehydroquinase.

There are two structurally distinct forms (type I and type II) of dehydroquinase, which catalyse the same transformation by distinct mechanisms.[1] The type I enzyme catalyses the *syn* elimination of water from 3-dehydroquinate *via* imine intermediates that are covalently attached to a conserved lysine side chain.[5] The type II enzyme catalyses an *anti* elimination *via* an enol or enolate intermediate.[6] Inhibitors have been designed which specifically inhibit type I[7] and type II dehydroquinases.[8]

The crystal structures of both type I and type II dehydroquinases have been reported.[9] Recently, the structure of the *Streptomyces coelicolor* type II dehydroquinase has been determined at 1.8 Å resolution, with the potent reversible inhibitor **3** bound at the active site (PDB code: 1GU1, the crystals were obtained at pH 8.5 in Tris buffer in the presence of PEG8Kcrystallisation Na/K phosphate and sodium tartrate).[10] This complex identifies a number of key interactions involved in inhibitor binding, and sheds light on aspects of the catalytic mechanism of the enzyme. Also present in this structure was a molecule of glycerol bound 3.7 Å away from the inhibitor (Fig. 1). The glycerol originated from the enzyme storage buffer, but its adventitious appearance in the crystal structure suggests additional binding interactions which can be exploited in inhibitor design.

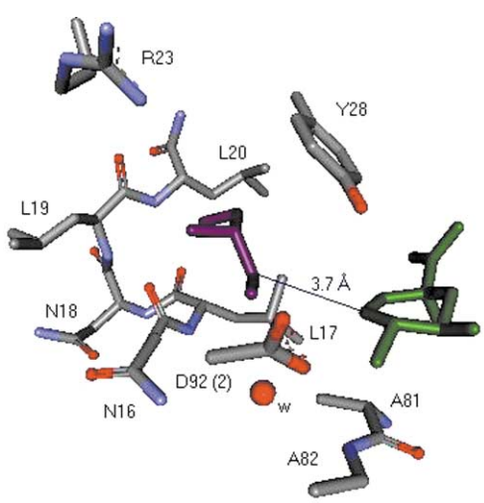


Fig. 1 Selected view of the active-site of type II dehydroquinase (*S. coelicolor*) from the crystal structure (1.8 Å resolution, PDB code 1GU1) showing the relative position of **3** (green) and the glycerol molecule (purple). The distance between the two closest carbon atoms in **3** and glycerol is 3.7 Å.

There are several examples of inhibitor design where two inhibitors which bind to distinct regions of an enzyme active site have been incorporated into a larger inhibitor with signifi-

† Electronic supplementary information (ESI) available: Dixon plots and results of docking experiments. See http://www.rsc.org/suppdata/ob/b3/b301731a/

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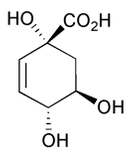
cant increases in potency.[11] In this paper we explore this idea by making compounds **5**–**12** which straddle the binding sites of both **3** and the glycerol molecule, and incorporate structural features from both. The results of inhibition studies with these compounds against type I (*Salmonella typhi*) and type II dehydroquinase (*S. coelicolor*), and of molecular docking studies into the active-site of *S. coelicolor* type II dehydroquinase using GOLD1.2[12] are described.



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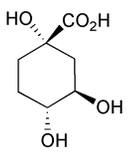

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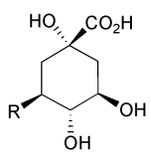
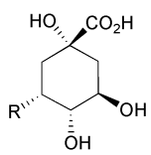


5 R = $CH_2CH{=}CH_2$
6 R = $CH_2CH(OH)CH_3$
7 R = $CH_2CH_2CH_2OH$
8 R = $CH_2CH(OH)CH_2OH$
9 R = $OCH_2CH{=}CH_2$
10 R = $OCH_2CH(OH)CH_2OH$



11 R = $OCH_2CH{=}CH_2$
12 R = $OCH_2CH(OH)CH_2OH$

Results

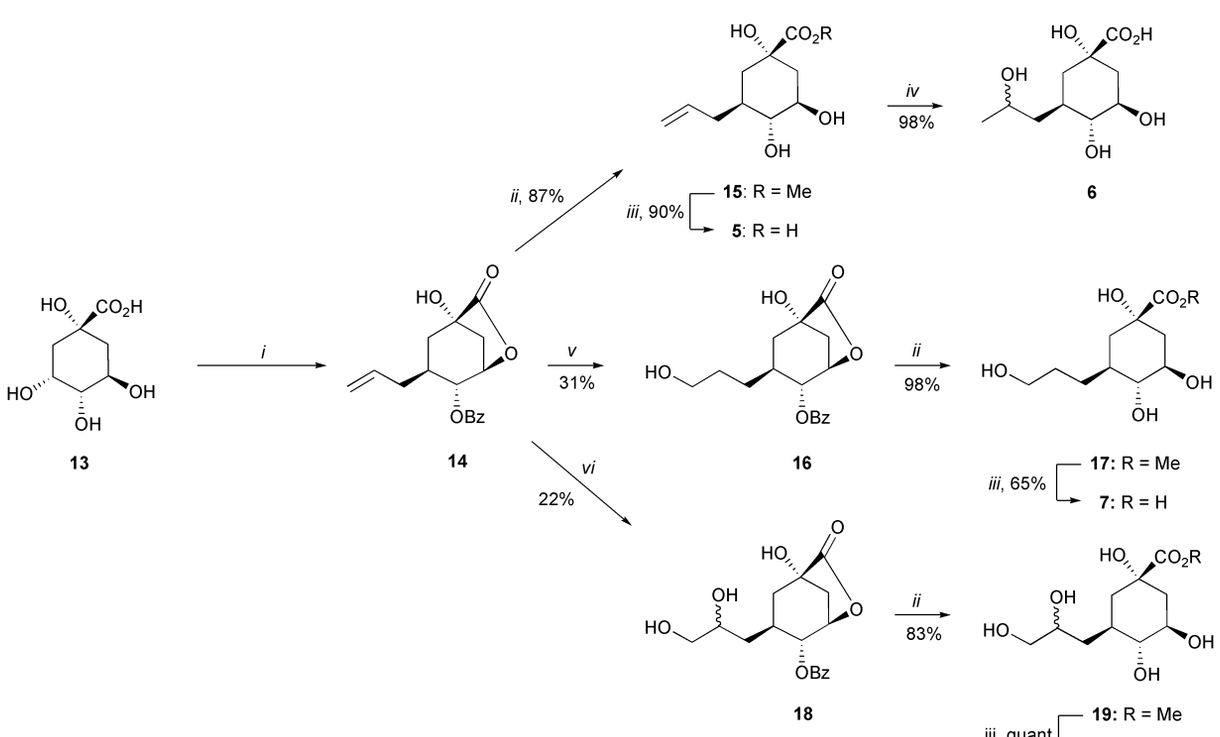
Synthesis

All of the target compounds were accessible from quinic acid (**13**). The first series **5**–**8** involved replacement of the C-3 hydroxy with an allyl group, which was subsequently elaborated to introduce hydroxy groups. The second series **11**–**12** involved attachment and modification of an allyl group attached to the C-3 hydroxy group of quinic acid (**13**). A similar approach using compounds epimeric at C-3 was used to make **9**–**10**.

The strategy used for making **5**–**8** (Scheme 2) involved the initial preparation of the protected *trans*-diaxial allylbenzoate analogue (**14**). This was made in 3 steps and 47% yield, following the methodology of Widlanski *et al.*[13] The synthesis of **5** was achieved by a two step deprotection of **14**. The lactone **14** was treated with a catalytic amount of sodium methoxide in methanol to remove the benzoate group and form the methyl ester **15** (87%). This was purified by extraction into water, washing with diethyl ether to remove the methyl benzoate, followed by lyophilization. Conversion of **15** to the 3-allyl quinic acid analogue **5** was achieved in 90% yield by ester hydrolysis, followed by treatment with Dowex 50 (H^+) ion-exchange resin and lyophilization. When deprotection of **14** was attempted in a single step using sodium hydroxide, the product was obtained in a mixture with benzoic acid which proved difficult to separate even by ion-exchange chromatography.

The introduction of the 2′-hydroxy group into the side chain olefin in **15** to form **6** was achieved in 98% yield by acid-catalysed hydroxylation with concentrated hydrochloric acid under reflux for 24 hours. The product was obtained as an approximately 1 : 1 mixture of diastereoisomers, epimeric at C-2′. The two diastereoisomers could be partially resolved by HPLC using a preparative organic acids column, but it was not possible to separate them cleanly. The analogue **6** could also be formed quantitatively by stirring **5** in concentrated hydrochloric acid for 4 days at room-temperature.

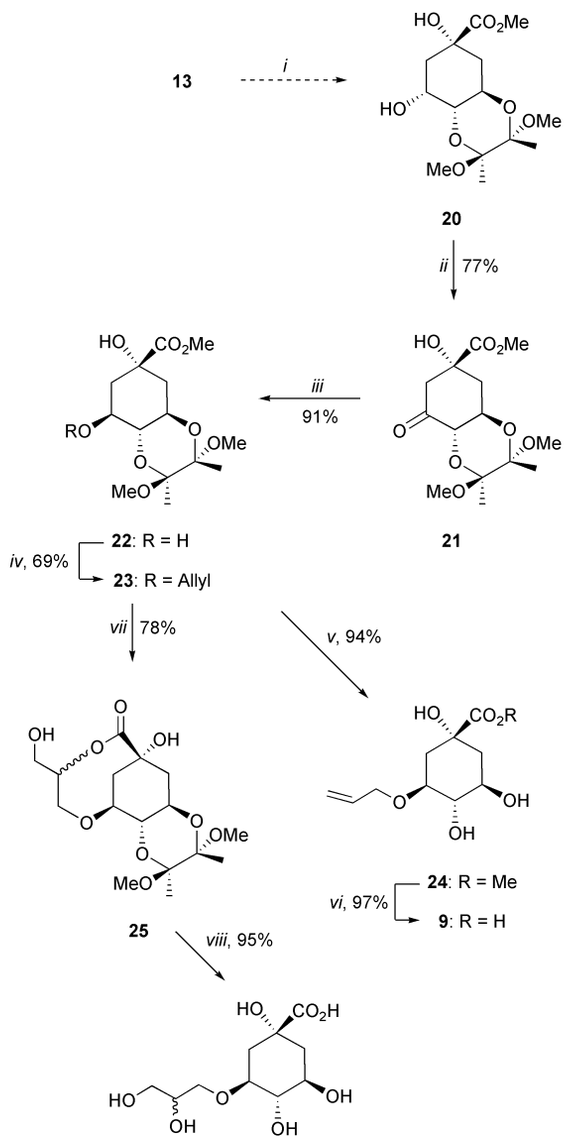
The anti-Markovnikov mono-hydroxylated compound **16** was obtained in 31% yield by hydroboration of **14**, using borane–THF complex followed by work-up with sodium perborate and water. The modest yield was due to the difficult purification of the product, which is partially deprotected during the work-up. Compound **16** was purified by column chromatography on silica gel and deprotected by treatment with sodium methoxide in methanol to give the methyl ester **17** (98%) which was then hydrolysed to the target compound **7** (65%). The hydroxylation reaction was also attempted using borane–dimethyl sulfoxide complex but a lower yield was obtained. Furthermore the standard work-up with the hydrogen peroxide–sodium hydroxide totally deprotected the hydroxy groups around the ring.



Scheme 2 *Reagents and conditions*: (i) ref. 13; (ii) MeOH, NaOMe, RT; (iii) 1. NaOH, H_2O, RT, 2. Dowex 50 (H^+); (iv) HCl, H_2O, Δ; (v) 1. BH_3–THF, THF, 0 °C, 2. $NaBO_3$, H_2O, 0 °C; (vi) OsO_4 (cat), NMO, acetone–H_2O (1 : 1), RT.

The first step towards the dihydroxylated analogue **8** required catalytic osmylation of **14**. This was achieved using *N*-methylmorpholine *N*-oxide to reoxidise the catalyst[14] to form **18** as a 1 : 1 mixture of diastereoisomers (epimeric at C-2′) in 22% yield. Attempts to separate the diastereoisomers by HPLC on an organic acids column and by acetylation and benzoylation of the free hydroxy groups were unsuccessful. The deprotection of **18** was carried out by initial treatment with sodium methoxide in methanol, to give the methyl ester **19** (83%). This was then hydrolysed with sodium hydroxide, followed by ion exchange to yield the acid **8** quantitatively. Osmylation of **14** using trimethylamine *N*-oxide as a reoxidant and dichloromethane as the only solvent[15] led to no reaction. Catalytic osmylation on the methyl ester **15** using *N*-methylmorpholine *N*-oxide was not successful.

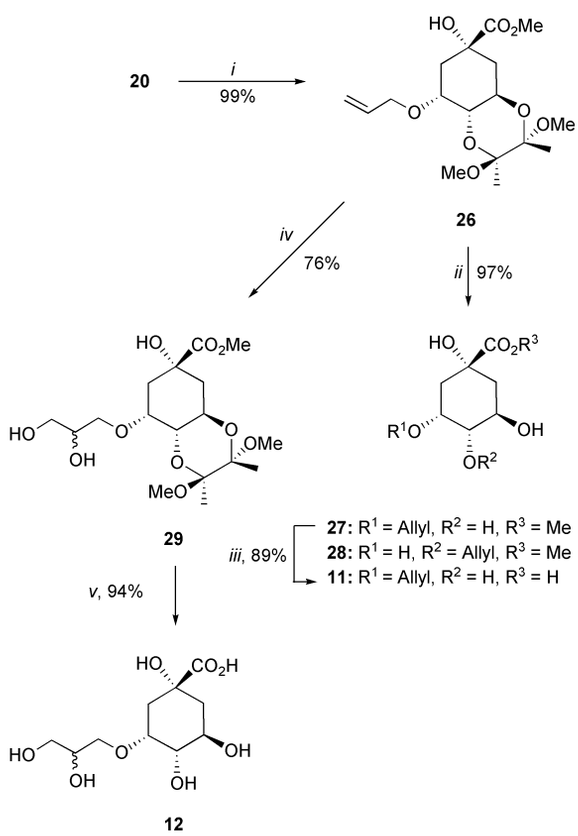
Compounds **9** and **10** were synthesized from the protected alcohol **20** prepared using a previously reported protocol (Scheme 3).[16] Ketone **21** was obtained *via* oxidation of **20** with pyridinium dichromate under dry conditions (77%), and was subsequently reduced to give alcohol **22**.[17] Allylation of **22** was achieved by treatment with allyl methyl carbonate and a catalytic amount of $Pd_2(dba)_2$/dppb to give **23** in 69% yield.[18]



Scheme 3 *Reagents and conditions*: (i) ref. 16; (ii) PDC, 4 Å MS, DCM, RT; (iii) ref. 17; (iv) CH_2=$CHCH_2OCO_2Me$, $Pd_2(dba)_2$, dppb, THF, Δ; (v) TFA–H_2O (20 : 1), RT; (vi) 1. LiOH, H_2O, RT, 2. Amberlite IR-120; (vii) OsO_4 cat, NMO, dioxane–H_2O, RT; (viii) 1. TFA–H_2O (20 : 1), RT, 2. LiOH, H_2O, RT, 3. Amberlite IR-120.

This step was particularly difficult because a competitive diallylation process was observed, which could be overcome by adding the solution of the carbonate slowly to the reaction mixture. The desired acid **9** was obtained by a two-step deprotection sequence. First, treatment with aqueous trifluoroacetic acid cleaved the bismethoxyacetal protecting group giving **24** in 94% yield, which upon basic hydrolysis followed by ion exchange gave the acid **9** (97%). Treatment of the allyloxy ether **23** with osmium tetraoxide (catalytic) resulted in hydroxylation with concomitant lactonization to afford **25** in 78% yield. Deprotection of **25** was achieved in the same manner as for **23**, to give the acid **10** as a 1.2 : 1 mixture of diastereoisomers in 95% yield.

Compounds **11** and **12** were synthesized from the protected alcohol **20** (Scheme 4). The allyl side-chain was introduced using palladium coupling with allyl methyl carbonate in quantitative yield to give **26**. Treatment of 3-allyloxy derivative **26** with aqueous trifluoroacetic acid led to deprotection of the bismethoxyacetal together with partial allyl migration to afford a chromatographically separable mixture of 3-allyloxy and 4-allyloxy derivatives **27** (72%) and **28** (25%). Finally, hydrolysis of the methyl ester **27** under basic conditions followed by ion exchange gave acid **11** in 89% yield. Hydroxylation of **26** with catalytic osmium tetraoxide gave **29** in 76% yield, which was deprotected using a combination of TFA, lithium hydroxide and Amberlite IR-120 to give **12** as a 1.2 : 1 mixture of diastereoisomers.



Scheme 4 *Reagents and conditions*: (i) CH_2=$CHCH_2OCO_2Me$, $Pd_2(dba)_2$, dppb, THF, Δ; (ii) TFA–H_2O (20 : 1); (iii) 1. LiOH, H_2O, RT, 2. Amberlite IR-120; (iv) OsO_4(cat), NMO, dioxane–H_2O, RT; (v) 1. TFA–H_2O (20 : 1), RT, 2. LiOH, H_2O, RT, 3. Amberlite IR-120.

Assay results

Inhibition studies against type I dehydroquinase (*S. typhi*) and type II dehydroquinase (*S. coelicolor*) were performed with compounds **5**, **7**, **9** and **11**, and compounds **6**, **8**, **10** and **12** as the prepared diastereomeric mixtures. The UV spectrophotometric assay[6c] was used to measure the initial rate of product formation, detecting the enone-carboxylate chromophore at

Table 1 Enzyme assay results (K_i values in μM)

Compound	Type I dehydroquinase *S. typhi*[a]	Type II dehydroquinase *S. coelicolor*[a]
3	3000 ± 1000[b]	30 ± 10[b]
4	4500 ± 500[b]	600 ± 200[b]
5	>20000	420 ± 50
6 (*R* + *S*)	>20000	>20000
7	>20000	180 ± 20
8 (*R* + *S*)	>20000	3000 ± 500
9	>20000	1200 ± 150
10 (*R* + *S*)	>20000	530 ± 50
11	>20000	>20000
12 (*R* + *S*)	>20000	3500 ± 400

[a] K_M values of 16 μM (type I dehydroquinase, *S. typhi*) and 250 μM (type II dehydroquinase, *S. coelicolor*) were obtained at the assay conditions. [b] Ref. 8.

234 nm in 3-dehydroshikimate (**2**). The K_i values (Table 1) were obtained from Dixon plots (1/*v* *vs.* [*I*]).[19]

The most striking observation is that none of the compounds **5**–**12** showed any measurable inhibition against the type I dehydroquinase. This result was not too surprising as substrate analogues where the carbonyl oxygen in 3-dehydroquinate (**1**) was replaced by either CH_2 or NOH had previously been shown not to inhibit type I dehydroquinase.[8]

The best inhibitor of type II dehydroquinase was **7** which had a K_i of 180 μM. This compared with a K_M for substrate of 250 μM under the same assay conditions. The related allyl compound **5** lacking the terminal hydroxy group had an increased K_i of 420 μM. A similar pattern was observed for the dihydroxylated ether **10** (K_i 530 μM) and corresponding allyl compound **9** (K_i 1200 μM). The diols **8** and **12** showed modest inhibition (3.0 and 3.5 mM, respectively), while no inhibition was observed for the secondary alcohol **6** and the C-3 epimeric allyl ether **11**.

Molecular modelling

The structures (ligands) of **4**, **5**, **7**, **9**, **11** and each stereoisomer of **6**, **8**, **10** and **12** were docked in the active-site of the structure of type II dehydroquinase from *S. coelicolor* (receptor) (Fig. 1), using the program GOLD (version 1.2).[12] The structures of the ligands were prepared using the program SYBYL6.5[20] and energy minimised using the Tripos force field. The receptor was also prepared using SYBYL6.5. The structures of **3**, the glycerol molecule, and all of the water molecules (except a key structural water molecule that is present in all the crystal structures, shown in Fig. 1) were removed from the crystal structure. No energy minimisation was performed on the receptor since the crystal structure used has the enzyme in the desired conformation, with bound ligands. All the ligands were docked as carboxylate anions and 25 independent GOLD runs were performed for each ligand.

Ligand **3** was initially docked as a control, and the result compared to the crystal structure of the enzyme inhibitor complex. All 25 GOLD runs gave results within 0.5 Å of each other, and the position of the docked ligand in the active-site coincided with the position in the crystal structure to within 0.3 Å RMSD. The reduced analogue **4** also docked consistently at the same site with an RMSD of 1.0 Å.

The docking of ligands **5**–**9** also gave highly consistent results. All 25 GOLD runs for each ligand showed the 6-membered ring in a similar position to **3**, with only small variations in the position of the side-chains. Compounds **10**, **11** and **12** also docked in this position in the large majority of the 25 experiments, but other binding modes were also found where the ring position was substantially changed. These were not considered further in this study.

Fig. 2 shows the docking results of ligands **7**, **8***R* and **10***S*, compared with the position of **3** and the glycerol molecule in the crystal structure. All the ligands have the ring in a similar position as **3** and the side-chain in the glycerol binding-pocket. For ligands **5**–**10** the ring adopts a chair conformation. However, in the dockings of ligands **11** and **12** the ring is flipped into a boat-conformation. This moves the side-chain onto an equatorial position so that it extends into the glycerol binding-pocket, and avoids steric clashes with protein side chains below the ring. From the ligand positions in the docking results no H-bond interactions are recognised between the hydroxy groups in the ligand side-chains and the enzyme.

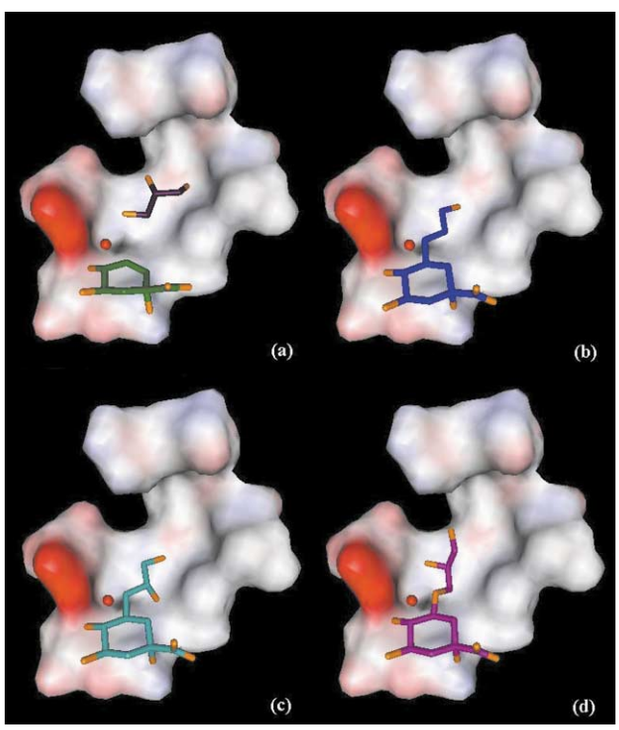


Fig. 2 Results from the docking experiments with GOLD1.2: positions of the ligands in the active-site of type II dehydroquinase (*S. coelicolor*), against a surface (coloured by electrostatic potential)[21] calculated for the protein residues shown in Fig. 1, omitting Y28 for clarity. Oxygen atoms are shown in orange. (a) **3** (green) and glycerol (purple), (b) **7** (blue), (c) **8***R* (light blue) and (d) **10***S* (magenta).

Discussion and conclusions

The observation of a glycerol molecule adventitiously bound at a distal site in the structure of the complex between *S. coelicolor* type II dehydroquinase and inhibitor **3** inspired the design of bifunctional inhibitors which would straddle the two sites and incorporate features of both **3** and glycerol. The target compounds **5**–**12** were all synthesized from quinic acid (**13**). Compounds with secondary hydroxy groups in their side chain were prepared and tested as diastereomeric mixtures.

None of the compounds **5**–**12** inhibited type I dehydroquinase. This was expected as the type I enzyme mechanism involves initial attack on the C-3 carbonyl of 3-dehydroquinate (**1**) by an active site lysine.[5a] Substrate analogues substituted at C-3 would be expected to suffer unfavourable steric clashes with this side chain.

Five compounds showed competitive inhibition against type II dehydroquinase, although all were less potent than the original inhibitor **3**. The most potent inhibitor **7** has a K_i below the K_M for substrate, and is more potent than **4**, the reduced analogue of **3** with sp^3 hybridisation at C-3. In analysing the inhibition data several trends were discerned:

(i) Introduction of a hydroxy group in the side chain three atoms remote from C-3 of quinic acid **13** is favorable. This is most clearly seen in the comparison of **7** *vs*. **5**.

(ii) Extending the side chain to four atoms appears to be less favourable, *e.g.* **5** *vs*. **9** and **7** *vs* **10**.

(iii) Introduction of a hydroxy group in the side chain two atoms remote from C-3 is unfavourable. This is clearly seen in the comparison of **6** with **5** and **7**, and also **8** *vs*. **7**.

(iv) There is a preference for the side chain to be on the β-face, *i.e*. the (*R*)- stereochemistry at C-3 is less favourable when comparing **10** *vs*. **12** and **9** *vs*. **11**.

These trends consistently account for the relative affinities of the compounds **5**–**12**. They are further supported by consideration of the structures of the ligands docked into the active site using GOLD, shown in Fig. 2.

Fig. 2a shows the position of **3** and the glycerol molecule in the active-site. Fig. 2b overlays of the docked structure of **7**, where the side-chain reaches into the glycerol binding-pocket and the terminal hydroxy overlaps the C-1 position of the glycerol molecule. Docking of **5** shows the ligand in a similar position to **7**.

The docking of the dihydroxy ligand **8***R* is shown on Fig. 2c. This suggests a position where the secondary hydroxy group at C-2′ abuts Leu17. This result is independent of whether or not the adjacent water molecule is removed. Ligands **6***R*, **6***S* and **8***S* dock in a similar position to **8***R*. The terminal primary hydroxy group on C-3′ of **8** reaches into the glycerol pocket and may account for its affinity relative to **6**. The extended diol **10***S* is shown in Fig. 2d. The diol **10** has the longest side chain and it docks so as to overlap both C-1 and C-2 of glycerol. This might have been expected to increase the affinity compared to **7**, but must be offset against the increased entropic cost of immobilizing the longer side chain. Finally, the docking results for ligands **11** and **12** (both with (*R*)-stereochemistry at C-3) suggest they bind in a similar position to **10***S*. However, to accommodate the side-chain in the glycerol pocket the six-membered ring is flipped into a boat conformation. This conformational switch, which would be forced by steric crowding below C-3 (see Fig. 1) preventing the side chain extending downwards, is likely to disrupt hydrogen bonding to the hydroxys at C-4 and C-5, contributing to their reduced affinity.

In conclusion, the strategy of making inhibitors combining structural features of both **3** and glycerol has resulted in the formation of new inhibitors. The docking experiments suggest that most of the compounds bind in a similar manner to **3**, with their side chains extending into the glycerol binding site. The modest affinity of the inhibitors is probably a consequence of the entropic cost of immobilising a flexible side chain, and the sensitivity of binding to sp^2 hybridisation at C-2 and C-3 of the six membered ring. Both of these issues are being addressed in the design of the next generation of inhibitors.

Experimental

General

All organic solvents were freshly distilled prior to use and Milli-Q deionised water was used for all biochemical work. Analytical thin layer chromatography was carried out on commercial silica gel 60 0.25 mm plates using either UV absorption, iodine staining, ceric molybdate solution or potassium manganate(VII) spray for visualisation. R_F values are quoted with respect to the solvent system used to develop the plate. Column chromatography was carried out using 230–400 mesh silica gel 60. Melting points are uncorrected. NMR spectra were recorded in deuterated solvents. In the spectra of diastereomeric mixtures, all resolvable peaks are given. Infrared spectra were recorded as NaCl plates, Nujol mulls or KBr discs. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Ultraviolet–visible spectra were recorded using black-walled quartz cuvettes. Carboxylic acids were analysed or purified by HPLC on a preparative (300 mm × 16 mm) Bio-Rad Aminex Ion Exclusion HPX-87H Organic Acids column. The eluant used for these columns was 0.05 M aqueous formic acid, at a flow rate of 1.0 ml min^{-1}. All procedures involving the use of ion-exchange resins were carried out at room temperature and used Milli-Q deionised water. Dowex 50W-X8 (H) (cation exchanger) and Amberlite IR-120 (H) (cation exchanger) were washed alternately with water, 10% HCl, water, 10% sodium hydroxide, water, 10% HCl and finally with water before use. Purified *S. typhi* type I dehydroquinase and *S. coelicolor* type II dehydroquinase were stored in aliquots as concentrated solutions in glycerol–water at −20 °C and 4 °C, respectively.

Dehydroquinase assay

The dehydroquinase enzymes were assayed by monitoring the increase in absorbance at 234 nm in the UV spectrum due to the absorbance of the enone-carboxylate chromophore of 3-dehydroshikimate (**2** $\varepsilon = 1.2 \times 10^4$ $M^{-1}cm^{-1}$). The assays were performed at 25 °C in potassium phosphate (0.05 M, pH 7) buffer for type I dehydroquinase and Tris-HCl (0.05 M, pH 7) buffer for type II dehydroquinase. The assay mixture was prepared *in situ* and the assay was initiated by addition of the enzyme solution to the mixture. Solutions of 3-dehydroquinate (**1**) were calibrated by equilibration with type I dehydroquinase and measurement from the change in UV absorbance at 234 nm. In these assays the concentration of inhibitor (I) was varied for a constant concentration of substrate and enzyme. The assays were repeated for a number of different concentrations of substrate and the K_i values were obtained through a Dixon plot ($1/v$ *vs*. $[I]$).

GOLD docking

All ligands and the receptor were prepared using SYBYL6.5 and used as MOL2 files. The ligands were designed as carboxylate anions and their structure was energy minimised using the Tripos force-field prior to docking. Each ligand was docked using GOLD1.2 in 25 independent genetic algorithm (GA) runs, and for each of these a maximum number of 100000 GA operations was performed on a single population of 50 individuals. Operator weights for crossover, mutation and migration in the entry box were used as default parameters (95, 95 and 10, respectively), as well as the hydrogen bonding (4.0 Å) and van der Waals (2.5 Å) parameters. The position of the active-site was introduced and the radius was set to 10 Å, with the automatic active-site detection on. The "flip ring corners" flag was switched on, while all the other flags were off.

Methyl (1*S*,3*R*,4*R*,5*S*)-5-allyl-1,3,4-trihydroxycyclohexane-1-carboxylate (15)

The allyl carbolactone **14**[13] (140 mg, 0.47 mmol) was dissolved in methanol (10 ml) and stirred in the presence of sodium methoxide (5 mg) for 24 hours at room temperature. The solvent was removed at reduced pressure, the crude product was taken in water (10 ml) and washed with diethyl ether (3 × 20 ml). The *ester* **15** was lyophilised and collected as an amorphous yellow solid (94 mg, 87%). δ_H (400 MHz, D_2O): 5.65

(1 H, m), 4.95 (2 H, m), 3.62 (3 H, s), 3.60 (1 H, ddd, J = 12.0, 9.4 and 5.1 Hz), 3.05 (1 H, t, J = 9.4 Hz), 2.35 (1 H, dddd, J = 14.2, 6.1, 3.2 and 1.5 Hz), 2.00–1.80 (2 H, m), 1.75–1.60 (3 H, m), 1.45 (1 H, t, J = 14.2 Hz); δ_C (100 MHz, DEPT, D_2O): 175.2 (C), 134.3 (CH), 115.1 (CH_2), 75.3 (CH), 72.6 (C), 68.7 (CH), 51.2 (OCH_3), 37.8 (CH_2), 35.3 (CH_2), 34.9 (CH), 33.2 (CH_2); HRMS calcd for $C_{11}H_{18}O_5Na$: MNa$^+$, 253.1052. Found: MNa$^+$, 253.1072.

(1*S*,3*R*,4*R*,5*S*)-5-Allyl-1,3,4-trihydroxycyclohexane-1-carboxylic acid (5)

The methyl ester **15** (94 mg, 0.41 mmol) was dissolved in water (10 ml) and taken to pH~12 with 10% NaOH, and stirred at room temperature overnight. The solution was acidified with Dowex 50 (H^+) ion-exchange resin, filtered and lyophilised to give the *allyl acid* **5** as an amorphous white solid (80 mg, 90%). HPLC retention time (organic acids column): 37 minutes; υ_{max} (Nujol)/cm^{-1} 3368b (OH) and 1712s (CO); δ_H (500 MHz, D_2O): 5.80 (1 H, m), 5.05 (2 H, m), 3.70 (1 H, ddd, J = 14.0, 9.3 and 4.9 Hz), 3.17 (1 H, dd, J = 10.1 and 9.3 Hz), 2.45 (1 H, dddd, J = 14.0, 6.3, 3.2 and 1.6 Hz), 2.00 (2 H, m), 1.80 (3 H, m), 1.58 (1 H, dd, J = 14.0 and 12.7 Hz); δ_C (100 MHz, DEPT, D_2O): 179.3 (C), 136.2 (CH), 116.8 (CH_2), 77.2 (CH), 74.4 (C), 70.7 (CH), 39.8 (CH_2), 37.2 (CH_2), 36.9 (CH), 34.0 (CH_2); HRMS calcd for $C_{10}H_{16}O_5$: $M^+ - H$, 215.0920. Found: $M^+ - H$, 215.0939.

(1*S*,3*R*,4*R*,5*S*)-5-(2′-Hydroxypropyl)-1,3,4-trihydroxycyclohexane-1-carboxylic acid (6)

The acid **5** (11 mg, 0.052 mmol) was stirred with concentrated hydrochloric acid (2 ml) under reflux for 24 hours. The acidic solution was diluted in water and the solvent and some of the acid removed at reduced pressure. The residue was redissolved in water (5 ml) and lyophilised to give a 1 : 1 mixture of diastereoisomers of the *secondary hydroxy acid* **6** as an amorphous white solid (12 mg, 98%). HPLC retention time (organic acids column): 36 and 37 minutes; υ_{max} (Nujol)/cm^{-1} 3381b (OH) and 1719s (CO); δ_H (500 MHz, D_2O): 4.27 (1 H, tq, J = 9.1 and 6.2 Hz), 3.81 (1 H, ddd, J = 11.0, 9.6 and 4.8 Hz), 3.30 (1 H, dd, J = 10.3 and 9.6 Hz), 2.20 (1 H, ddd, J = 12.0, 6.2 and 6.0 Hz), 1.98 (2 H, m), 1.85 (1 H, dd, J = 13.5 and 3.3 Hz), 1.75 (1 H, dd, J = 13.7 and 11.0 Hz), 1.69 (1 H, t, J = 13.5 Hz), 1.34 (1 H, td, J = 12.0 and 9.1 Hz), 1.25 (3 H, d, J = 6.2 Hz); δ_C (100 MHz, DEPT, D_2O): 179.7 (C), 86.3 (CH), 78.0 (CH), 77.3 (C), 70.4 (CH), 42.0 (CH_2), 40.2 (CH), 39.3 (CH_2), 37.5 (CH_2), 22.4 (CH_3); only ten peaks observed in ^{13}C spectrum of the mixture. HRMS calcd for $C_{10}H_{15}O_5$: $M(-H^+ - H_2O)$, 215.0920. Found: $M(-H^+ - H_2O)$, 215.0937.

(1*S*,3*S*,4*R*,5*S*)-5-(3′-Hydroxypropyl)-4-benzoyl-1-hydroxycyclohexane-1,3-carbolactone (16)

A solution of borane–THF complex (1.89 ml *ca.* 1.0 M in THF, 1.89 mmol, 2 equiv.) was added to a solution of the allyl carbolactone **14** (286 mg, 0.94 mmol) in THF (5 ml), at 0 °C in an ice-bath. The solution was stirred for 1 hour, and a solution of sodium perborate (286 mg, 1.89 mmol) in water (5 ml) was added and the ice-bath removed. The solution was stirred for a further 2 hour at room temperature. The THF was removed at reduced pressure, the product was dissolved in ethyl acetate (20 ml) and washed with water (3 × 20 ml). The organic layer was dried with anhydrous Na_2SO_4 and the solvent removed at reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with ethyl acetate–petroleum ether 40–60 (3 : 1). The *carbolactone* **16** (94 mg, 31%) was obtained as a colourless oil. R_F 0.22 [EtOAc–petroleum ether 40–60 (3 : 1)]; υ_{max} (NaCl)/cm^{-1} 3452b (OH) and 1790s (CO), 1723s (CO) and 1601s (Ar); δ_H (400 MHz, $CDCl_3$): 8.10 (2 H, d, J = 8.0 Hz), 7.60 (1 H, t, J = 8.0 Hz), 7.46 (2 H, t, J = 8.0 Hz), 5.20 (1 H, dd, J = 5.0 and 2.3 Hz), 4.89 (1 H, t, J = 5.0 Hz), 3.63 (2 H, m), 3.52 (1 H, s), 2.58–2.45 (2 H, m), 2.33 (1 H, dd, J = 13.3 and 9.1 Hz), 2.23 (1 H, m), 1.86 (1 H, m), 1.75–1.60 (3 H, m), 1.50 (1 H, m); δ_C (100 MHz, $CDCl_3$): 181.2, 167.5, 135.8, 131.8, 131.5, 130.8, 77.8, 74.2, 72.9, 64.4, 39.5, 39.3, 39.2, 33.0, 32.9; HRMS calcd for $C_{17}H_{20}O_6Na$: MNa$^+$, 343.1143. Found: MNa$^+$, 343.1158.

Methyl (1*S*,3*R*,4*R*,5*S*)-5-(3′-hydroxypropyl)-1,3,4-trihydroxycyclohexane-1-carboxylate (17)

The lactone **16** (87 mg, 0.27 mmol) was dissolved in methanol (5 ml) and stirred in the presence of sodium methoxide (5 mg) for 2 hours at room temperature. The solvent was removed at reduced pressure, the crude product was taken in water (10 ml) and washed with diethyl ether (3 × 10 ml). The product was lyophilised to give *ester* **17** as a colourless glass (66 mg, 98%). υ_{max} (Nujol)/cm^{-1} 3330b (OH) and 1730b (OH); δ_H (400 MHz, D_2O): 3.66 (3 H, s), 3.60 (1 H, m), 3.50 (2 H, t, J = 6.5 Hz), 3.08 (1 H, td, J = 9.0 and 7.2 Hz), 1.99 (1 H, ddd, J = 13.2, 4.0 and 3.1 Hz), 1.85−1.45 (6 H, m), 1.36 (1 H, m), 1.15 (1 H, m); δ_C (100 MHz, DEPT, D_2O): 178.7 (C), 79.6 (CH), 76.0 (C), 73.0 (CH), 63.3 (CH_2), 54.6 (OMe), 42.3 (CH_2), 39.7 (CH_2), 38.3 (CH), 29.7 (CH_2), 28.4 (CH_2).

(1*S*,3*R*,4*R*,5*S*)-5-(3′-Hydroxypropyl)-1,3,4-trihydroxycyclohexane-1-carboxylic acid (7)

The methyl ester **17** (10 mg, 0.040 mmol) was dissolved in water (2 ml), the pH adjusted to 12 with sodium hydroxide (10%) and the solution stirred at room temperature for 2 hours. The solution was acidified with Amberlite IR-120 (H^+) ion-exchange resin, filtered and lyophilised to give the *3′-hydroxy acid* **7** as a colourless glass (6 mg, 65%); HPLC retention time (organic acids column): 28 minutes; υ_{max} (Nujol)/cm^{-1} 3393b (OH) and 1711s (CO); δ_H (400 MHz, D_2O): 3.66 (1 H, ddd, J = 11.8, 9.5 and 4.6 Hz), 3.54 (2 H, t, J = 6.5 Hz), 3.12 (1 H, t, J = 9.5 Hz), 2.02 (1 H, ddd, J = 13.3, 4.6 and 3.1 Hz), 1.83–1.52 (6 H, m), 1.41 (1 H, ddd, J = 17.1, 11.2 and 6.5 Hz), 1.20 (1 H, m); δ_C (100 MHz, DEPT, D_2O): 179.4 (C), 77.6 (CH), 74.2 (C), 70.4 (CH), 61.7 (CH_2), 39.7 (CH_2), 37.0 (CH_2), 36.6 (CH), 27.8 (CH_2), 26.5 (CH_2); HRMS calcd for $C_{10}H_{18}O_6Na$: MNa$^+$, 257.1001. Found: MNa$^+$, 257.1001.

(1*S*,3*R*,4*R*,5*S*)-5-(2′,3′-Dihydroxypropyl)-4-benzoyl-1-hydroxycyclohexane-1,3-carbolactone (18)

To a solution of the carbolactone **14** (167 mg, 0.55 mmol) in acetone (2 ml) was added *N*-methylmorpholine oxide (76.7 mg, 0.65 mmol), water (2 ml) and osmium tetraoxide (1.1 mg, 55 µl of 2.5% wt. solution in *tert*-butanol, 5.5 µM). The mixture was degassed with nitrogen and stirred for 20 hours under argon, with additional osmium tetraoxide (1.1 mg, 55 µl of 2.5% solution in *tert*-butanol, 5.5 µM) added after 7 hours. A saturated solution of sodium bisulfite (10 ml) was added to destroy excess of osmium tetraoxide. The acetone was removed at reduced pressure and the product was extracted with ethyl acetate (2 × 20 ml). The extract was washed with water (4 × 20 ml). The solution was dried with $MgSO_4$ and evaporated at reduced pressure. The product was purified by column chromatography on silica gel eluting with ethyl acetate to give the *diol* (1 : 1 mixture of diastereomers) **18** as a colourless oil (40 mg, 22%). R_F 0.25 (ethyl acetate); δ_H (400 MHz, $CDCl_3$): 7.95 (2 H, d, J = 8.2 Hz), 7.55 (1 H, t, J = 8.2 Hz), 7.40 (2 H, t, J = 8.2 Hz), 5.10 (1 H, m), 4.82 (1 H, t, J = 4.5 Hz), 4.05–3.35 (5 H, m), 2.60–2.20 (4 H, m), 1.94 (1 H, dd, J = 10.2 and 9.0 Hz), 1.65 (1 H, m), 1.45 (1 H, m); δ_C (100 MHz, $CDCl_3$): 179.9, 179.8, 166.1, 166.0, 134.1, 134.0, 130.1 (2×), 129.7, 129.6, 129.0, 128.9, 76.1, 76. 0, 72.5, 72.2, 71.0 (2×), 70.5, 70.1, 67.0, 66.8, 38.4, 38.3, 37.8, 37.5, 37.2, 35.9, 34.2, 33.5; HRMS calcd for $C_{17}H_{20}O_7Na$: MNa$^+$, 359.1109. Found: MNa$^+$, 359.1158.

Methyl (1*S*,3*R*,4*R*,5*S*)-5-(2′,3′-dihydroxypropyl)-1,3,4-trihydroxycyclohexane-1-carboxylate (19)

The dihydroxy lactone **18** (40 mg, 0.12 mmol) was dissolved in methanol (10 ml) and stirred in the presence of sodium methoxide (1 mg) for 6 hours at room temperature. The solvent was removed at reduced pressure, the crude product was taken in water (10 ml) and washed with diethyl ether (3 × 20 ml). The solution was lyophilised to give the *ester* (1 : 1 mixture of diastereomers) **19** as a brown solid (27 mg, 83%). δ_H (400 MHz, D_2O): 3.68 (3 H, s), 3.67 (2 H, m) 3.48 (1 H, ddd, J = 17.9, 12.1 and 4.0 Hz), 3.36 (1 H, ddd, J = 17.9, 12.1 and 7.2 Hz), 3.08 (1 H, td, J = 9.0, 6.3 Hz), 2.02 (1 H, m), 1.90–1.73 (4 H, m), 1.60 (1 H, t, J = 12.0 Hz), 1.22 (1 H, m); δ_C (100 MHz, DEPT, D_2O): 179.5 (2 × C), 81.1 (CH), 80.4 (CH), 76.9 (CH_2), 76.8 (CH_2), 73.1 (CH), 72.9 (CH), 71.4 (2 × CH), 68.6 (CH_2), 67.7 (CH_2), 55.0 (2 × OCH_3), 42.0 (2 × CH_2), 40.8 (CH_2), 39.8 (CH_2), 37.3 (CH_2), 37.2 (CH), 36.9 (CH_2), 36.2 (CH).

(1*S*,3*R*,4*R*,5*S*)-5-(2′,3′-Dihydroxypropyl)-1,3,4-trihydroxycyclohexane-1-carboxylic acid (8)

The ester **19** (27 mg, 0.10 mmol) was dissolved in water (10 ml), the pH of the solution adjusted to 12 with sodium hydroxide (10%) and stirred at room temperature overnight. The solution was acidified with Dowex 50 (H^+) ion-exchange resin, filtered and lyophilised to give the two diastereisomers of the *dihydroxy acid* **8** (1 : 1 mixture) as a white solid (25 mg, quantitative). HPLC retention time (organic acids column): 22 and 24 minutes; υ_{max} (Nujol)/cm^{-1} 3401b (OH) and 1718s (CO); δ_H (400 MHz, D_2O): 3.73 (1 H, ddd, J = 14.0, 6.5 and 3.4 Hz), 3.64 (1 H, ddd, J = 14.1, 9.2 and 4.5 Hz), 3.53 (1 H, dd, J = 13.0 and 3.4 Hz), 3.36 (1 H, dd, J = 13.0 and 6.5 Hz), 3.10 (1 H, t, J = 9.2 Hz), 1.98 (1 H, ddd, J = 14.0, 4.5 and 3.1 Hz), 1.90–1.65 (4 H, m), 1.60 (1 H, t, J = 12.9 Hz), 1.20 (1 H, m); δ_C (100 MHz, DEPT, D_2O): 179.7 (C), 179.6 (C), 78.0 (CH), 77.8 (CH), 74.3 (CH_2), 74.2 (CH_2), 70.5 (CH), 70.4 (CH), 70.3 (CH), 68.6 (CH), 65.9 (CH_2), 64.9 (CH_2), 39.6 (2 × CH_2), 38.3 (CH_2), 37.3 (CH_2), 34.7 (CH), 34.6 (CH_2), 34.3 (CH_2), 33.7 (CH); HRMS calcd for $C_{10}H_{17}O_7$: M − H, 249.0974. Found: M − H, 249.0968.

Methyl (1*S*,3*S*,4*S*,6*S*,9*R*)-9-hydroxy-3,4-dimethoxy-3,4-dimethyl-7-oxo-2,5-dioxabicyclo[4.4.0]decane-9-carboxylate (21)

To a stirred suspension of the alcohol **20**[16] (2.69 g, 8.41 mmol) and activated molecular sieves 4 Å (3.4 g) in dry dichloromethane (80 ml) was added pyridinium dichromate (4.74 g, 12.61 mmol). The resultant suspension was stirred at room temperature for 2 hours and then diluted with diethyl ether (60 ml) and filtered over Celite. The solution was evaporated and the crude reaction was purified by flash chromatography, eluting with ethyl acetate, and then recrystallised from diethyl ether to afford the ketone **21** as white needles (2.06 g, 77%). Mp 194–195 °C (diethyl ether); δ_H (250 MHz, $CDCl_3$): 4.19 (1 H, dd, J = 10.3 and 0.9 Hz), 4.02 (1 H, td, J = 11.0 and 4.2 Hz), 3.60 (3 H, s), 3.02 (3 H, s), 2.99 (3 H, s), 2.69 (1 H, d, J = 14.3 Hz), 2.28 (1 H, dd, J = 14.3 and 2.8 Hz), 2.12 (1 H, t, J = 12.4 Hz), 1.87 (1 H, m), 1.15 (3 H, s), 1.06 (3 H, s); δ_C (63 MHz, DEPT, $CDCl_3$): 199.5 (C), 174.1 (C), 100.5 (C), 99.6 (C), 77.2 (CH), 74.0 (C), 67.0 (CH), 53.6 (OCH_3), 49.0 (CH_2), 48.3 (OCH_3), 48.0 (OCH_3), 37.8 (CH_2), 17.6 (CH_3), 17.5 (CH_3). MS (CI) *m*/*z* (%) 287 [MH^+ − CH_3OH]; HRMS calcd. For $C_{13}H_{19}O_7$: MH$^+$, 287.1130. Found MH$^+$, 287.1127.

Methyl (1*S*,3*S*,4*S*,6*S*,7*S*,9*R*)-7-allyloxy-9-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicyclo[4.4.0]dec-9-anecarboxylate (23)

To a stirred solution of $Pd_2(dba)_2$ (16 mg, 17 μmol) and dppb (29 mg, 68 μmol) in dry tetrahydrofuran (3 ml) under argon was added the *alcohol* **22** (217 mg, 0.68 mmol) and then a solution of allyl methyl carbonate in tetrahydrofuran (1.6 ml, 0.5 M). The resultant green suspension was heated under reflux for 20 hours. The solvent was removed under reduced pressure and the crude mixture was purified by flash chromatography eluting with diethyl ether–hexane (75%) to afford the *allyl ether* **23** as a colourless oil (170 mg, 69%). $[\alpha]_D^{20}$ +136 (*c* 1.40 in $CHCl_3$); υ_{max} (NaCl)/cm^{-1} 3700b (OH) and 1733s (CO); δ_H (250 MHz, $CDCl_3$): 5.91–5.76 (1 H, m), 5.24–5.06 (2 H, m), 3.92–3.75 (4 H, m), 3.67 (3 H, s), 3.40 (1 H, t, J = 9.6 Hz), 3.22 (3 H, s), 3.18 (3 H, s), 2.32 (1 H, ddd, J = 13.9, 4.6 and 2.8 Hz), 2.14 (1 H, ddd, J = 13.9, 4.2 and 2.8 Hz), 1.85–1.64 (2 H, m), 1.27 (3 H, s), 1.23 (3 H, s); δ_C (63 MHz,DEPT, $CDCl_3$): 172.5 (C), 134.0 (CH), 117.0 (CH_3), 99.5 (C), 99.5 (C), 79.0 (C), 76.2 (CH), 66.5 (CH), 65.7 (CH_3), 64.7 (CH), 52.3 (OCH_3), 47.8 (2 × OCH_3), 37.3 (CH_2), 34.9 (CH_2), 17.6 (2 × CH_3); MS (CI) *m*/*z* (%) 329 [(MH^+) − $HOCH_3$]; HRMS calcd for $C_{16}H_{25}O_7$: MH$^+$, 329.1600. Found: MH$^+$, 329.1601.

Methyl (1*S*,3*R*,4*R*,5*R*)-3-allyloxy-1,4,5-trihydroxycyclohexane-1-carboxylate (24)

A solution of acetal **23** (117 mg, 0.33 mmol) in 2 ml of a solution of 20 : 1 (v/v) of trifluoroacetic acid–water was stirred for 30 min and then the solvent was concentrated *in vacuo*. The crude mixture was purified by flash chromatography eluting with ethyl acetate–dichloromethane–methanol (7 : 2 : 1) to afford **24** as a colourless oil (78 mg, 94%). $[\alpha]_D^{20}$ −0.2 (*c* 2.6 in H_2O); υ_{max} (NaCl)/cm^{-1} 3399b (OH) and 1731s (CO); δ_H (300 MHz, D_2O): 6.01–5.98 (1 H, m), 5.46–5.32 (2 H, m), 4.08 (2 H, d, J = 5.9 Hz), 3.88 (3 H, s), 3.85 (1 H, m), 3.41 (1 H, t, J = 9.1 Hz), 2.42 (2 H, dd, J = 2.8 and 11.9 Hz), 1.94 (2 H, dd, J = 12.3 and 13.5 Hz); δ_C (125 MHz, DEPT, D_2O): 174.0 (C), 133.2 (CH), 118.2 (CH_2), 79.1 (C), 78.7 (CH), 68.6 (2 × CH), 65.8 (CH_2), 52.9 (CH_3), 37.1 (2 × CH_2); MS (CI) *m*/*z* (%) 247 (MH^+); HRMS calcd for $C_{11}H_{19}O_6$: MH$^+$, 247.1174. Found: MH$^+$, 247.1182.

(1*S*,3*R*,4*R*,5*R*)-3-Allyloxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid (9)

A solution of the methyl ester **24** (70 mg, 0.28 mmol) in aqueous lithium hydroxide (3 ml, 0.2M) was stirred for 1 hour. The reaction mixture was diluted with water and was washed with diethyl ether (3 × 5 ml). The aqueous layer was treated with Amberlite IR-120 until the pH was 6.0. The resin was filtered and washed with water. The filtrate was freeze-dried to afford the *acid* **9** as a colourless oil (67 mg, 97%). $[\alpha]_D^{20}$ −0.5 (*c* 2.2 in H_2O); υ_{max} (NaCl)/cm^{-1} 3399b (OH) and 1683s (CO); δ_H (300 MHz, D_2O): 6.11–6.00 (1H, m), 5.46–5.30 (2H,m), 4.05 (2 H, d, J = 5.9 Hz), 3.85–3.76 (2 H, m), 3.41 (1 H, t, J = 9.2 Hz), 2.39 (2 H, dd, J = 4.1 and 12.3 Hz), 1.88 (2 H, t, J = 12.8 Hz); δ_C (125 MHz, DEPT, D_2O): 177.3 (C), 133.8 (CH), 117.8 (CH_2), 79.9 (C), 79.0 (CH), 69.0 (2 × CH), 65.6 (CH_2), 37.6 (2 × CH_2); MS (CI) *m*/*z* (%) 233 (MH^+); HRMS calcd for $C_{10}H_{17}O_6$: MH$^+$, 233.1025. Found: MH$^+$, 233.1018.

Methyl (1*S*,3*S*,4*S*,6*S*,7*S*,9*R*)-7-(2′,3′-dihydroxy)propyloxy-9-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicyclo[4.4.0]-decane-9-carboxylate (25)

To a stirred solution of allyl ether **23** (87 mg, 0.24 mmol) and *N*-methylmorpholine oxide (34 mg, 0.29 mmol) in 50% aqueous dioxane (4 ml) was added a freshly made aqueous solution of sodium tetraoxide (0.3 ml, 0.12 M). After stirring for 1 hour ethyl acetate was added and then saturated sodium bisulfite. The reaction mixture was stirred for 20 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 10 ml). The combined organic layers were dried (anhydrous Na_2SO_4) and concentrated *in vacuo*. The crude residue was purified by flash chromatography eluting with 95% ethyl acetate–methanol to afford a 1.2 : 1 mixture of diastereoisomers of the *lactone* **25** (68 mg, 78%) as white

needles. υ_{max} (NaCl)/cm^{-1} 3425b (OH) and 1750s (CO); δ_H (250 MHz, $CDCl_3$): 4.58 (m, 1H), 3.94–3.66 (6 H, m), 3.48 (1 H, t, J = 9.6 Hz), 3.27 (3 H, s), 3.23 (3 H, s), 2.64 (br s), 2.45–2.16 (2 H, m), 2.08–1.77 (2 H, m), 1.30 (3 H, s), 1.28 (3 H, s); δ_C (63 MHz, DEPT, $CDCl_3$): 170.2 (C), 99.7 (C), 99.6 (C), 77.4 (C), 77.3 (C), 76.0 (CH), 66.4 (CH), 64.6 (CH), 61.4 (CH_2), 61.3 (CH_2), 59.2 (CH_2), 59.1 (CH_2), 48.0 (OCH_3), 47.9 (OCH_3), 39.4 (CH_2), 34.7 (CH_2), 17.7 (CH_3), 17.6 (CH_3); MS (CI) *m/z* (%) 331 [(MH^+) − $HOCH_3$]; HRMS calcd for $C_{15}H_{23}O_8$: MH^+, 331.1393. Found: MH^+, 331.1393.

(1*S*,3*R*,4*R*,5*R*)-1,4,5-Trihydroxy-3-(2′,3′-dihydroxy)propyloxycyclohexane-1-carboxylic acid (10)

A solution of acetal **25** (46 mg, 0.13 mmol) in 2 ml of a solution of trifluoroacetic acid–water (20 : 1 (v/v)) was stirred for 30 min and then concentrated *in vacuo*. The crude residue was redissolved in an aqueous lithium hydroxide (1.7 ml, 0.2 M) and stirred for 1 hour. The aqueous layer was washed with diethyl ether (3 × 5 ml) and then was treated with Amberlite IR-120 until the pH was 6.0. The resin was filtered and washed with water. The filtrate was freeze-dried to afford a 1.2 : 1 mixture of diastereoisomers of the *acid* **10** as a colourless oil (34 mg, 95%). υ_{max} (Nujol)/cm^{-1} 3390b (OH) and 1682s (CO); δ_H (300 MHz, D_2O): 3.70 (1 H, br s), 3.55–3.20 (5 H, m), 3.13 (1 H, t, J = 9.2 Hz), 2.10 (2 H, d, J = 11.5 Hz), 1.60 (2 H, t, J = 12.5 Hz); δ_C (75 MHz, DEPT, D_2O): 166.1 (C), 165.6 (C), 82.0 (C + CH), 73.6 (CH), 72.0 (2 × CH), 68.0 (CH_2), 65.6 (CH_2), 40.5 (CH_2), 40.3 (CH_2); MS (CI) *m/z* (%) 249 [(MH^+) − H_2O]; HRMS calcd for $C_{10}H_{17}O_7$: MH^+, 249.0974. Found: MH^+, 249.0970.

Methyl (1*S*,3*S*,4*S*,6*S*,7*R*,9*R*)-7-allyloxy-9-hydroxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicyclo[4.4.0]decane-9-carboxylate (26)

To a stirred solution of $Pd_2(dba)_2$ (14 mg, 15.8 μmol) and dppb (27 mg, 63 μmol) in dry tetrahydrofuran (2 ml) under argon was added the alcohol **20** (200 mg, 0.63 mmol) and then a solution of allyl methyl carbonate (85 μl, 0.75 mmol) in dry tetrahydrofuran (1.5 ml). The resultant green suspension was heated at 60 °C for 2 h. The solvent was removed under reduced pressure and the crude mixture was purified by flash chromatography eluting with 75% diethyl ether–hexane to afford the *allyl ether* **26** as a colourless oil (226 mg, 99%). $[\alpha]_D^{20}$ +125 (*c* 1.9 in $CHCl_3$); υ_{max} (NaCl)/cm^{-1} 3479b (OH) and 1737s (CO); δ_H (300 MHz, $CDCl_3$): 5.80 (1 H, m), 5.11 (2 H, m), 4.22 (2 H, m), 4.07–3.86 (2 H, m), 3.64 (3 H, s), 3.45 (1 H, ddd, J = 18.2, 10.2 and 2.5 Hz), 3.14 (3 H, s), 3.13 (3 H, s), 2.25 (2 H, m), 1.90–1.74 (2 H, m), 1.19 (3 H, s, CH_3), 1.16 (3 H, s, CH_3); δ_C (75 MHz, DEPT, $CDCl_3$): 173.4 (C), 134.3 (CH), 116.8 (CH_2), 99.6 (C), 99.1 (C), 83.9 (C), 75.8 (CH), 73.2 (CH), 72.1 (CH_2), 62.5 (CH), 52.3 (OCH_3), 47.6 (OCH_3), 47.5 (OCH_3), 39.0 (CH_2), 36.6 (CH_2), 17.5 (CH_3), 17.4 (CH_3); MS (CI) *m/z* (%) 329 [(MH^+) − $HOCH_3$]; HRMS calcd for $C_{16}H_{25}O_7$: MH^+, 329.1600. Found: MH^+, 329.1591.

Methyl (1*S*,3*S*,4*R*,5*R*)-3-allyloxy-1,4,5-trihydroxycyclohexane-1-carboxylate (27) and methyl (1*S*,3*S*,4*R*,5*R*)-4-allyloxy-1,3,5-trihydroxycyclohexane-1-carboxylate (28)

A solution of the acetal **26** (174 mg, 0.48 mmol) in a mixture 20 : 1 (v/v) of trifluoroacetic acid–water was stirred at room temperature for 15 min. The solvent was removed under reduced pressure and the crude mixture was purified by flash chromatography eluting with 50% acetone–dichloromethane to afford the *3-allyloxy ester* **27** (85 mg, 72%) and the *4-allyloxy ester* **28** (30 mg, 25%), both as colourless oils.

Data for methyl (1*S*,3*S*,4*R*,5*R*)-3-allyloxy-1,4,5-trihydroxycyclohexane-1-carboxylate (27). $[\alpha]_D^{20}$ −16 (*c* 2.05 in $(CH_3)_2CO$); υ_{max} (NaCl)/cm^{-1} 3423b (OH) and 1731s (CO); δ_H (300 MHz, CD_3OD): 6.05 (1 H, m), 5.43 (1 H, dq, J = 17.2 and 1.5 Hz), 5.35 (1 H, dq, J = 10.3 and 1.1 Hz), 4.28–4.08 (4 H, m), 3.86 (3 H, s), 3.77 (1 H, dd, J = 8.2 and 3.5 Hz), 2.24–2.06 (4 H, m); δ_C (75 MHz, DEPT, CD_3OD): 172.9 (C), 131.3 (CH), 115.5 (CH_2), 73.2 (CH), 72.2 (C), 70.0 (CH), 67.9 (CH_2), 64.2 (CH), 50.3 (OCH_3), 36.6 (CH_2), 31.0 (CH_2); MS (CI) *m/z* (%) 247 (MH^+); HRMS calcd for $C_{11}H_{19}O_6$: MH^+, 247.1182. Found: MH^+, 247.1185.

Data for methyl (1*S*,3*S*,4*R*,5*R*)-4-allyloxy-1,3,5-trihydroxycyclohexane-1-carboxylate (28). $[\alpha]_D^{20}$ −6 (*c* 2.95 in $(CH_3)_2CO$); υ_{max} (NaCl)/cm^{-1} 3419b (OH) and 1731s (CO); δ_H (300 MHz, $(CD_3)_3CO$): 5.79 (1 H, m), 5.13 (1 H, dq, J = 17.3 and 1.8 Hz), 4.97 (1 H, dq, J = 10.4 and 1.7 Hz), 3.94–3.70 (5 H, m), 3.65 (1 H, d, J = 5.4 Hz), 3.56 (3 H, s), 3.40 (1 H, d, J = 7.3 Hz), 2.10–2.00 (2 H, m), 1.95–1.79 (2 H, m); δ_C (75 MHz, DEPT, $(CD_3)_3CO$): 172.1 (C), 134.8 (CH), 115.2 (CH_2), 79.8 (C), 74.1 (CH), 67.9 (CH), 67.2 (CH), 64.8 (CH_2), 51.2 (OCH_3), 36.5 (CH_2), 35.1 (CH2); MS (CI) *m/z* (%) 247 (MH^+); HRMS calcd for $C_{11}H_{19}O_6$: MH^+, 247.1182. Found: MH^+, 247.1184.

(1*S*,3*S*,4*R*,5*R*)-3-Allyloxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid (11)

A solution of the ester **27** (34 mg, 0.14 mmol) in aqueous lithium hydroxide (1.7 ml, 0.2 M) was stirred at room temperature for 2 hours. The resultant solution was diluted with water and treated with Amberlite IR-120 until the pH was 6.0. The resin was filtered and washed with water. The filtrate was concentrated to afford the *acid* **11** (29 mg, 89%) as a colourless oil. $[\alpha]_D^{20}$ −25 (*c* 1.7 in H_2O); υ_{max} (NaCl)/cm^{-1} 3418b (OH) and 1713s (CO); δ_H (300 MHz, D_2O): 6.02 (1 H, m), 5.40 (1 H, d, J = 17.4 Hz), 5.31 (1 H, d, J = 10.2 Hz), 4.26–4.07 (3 H, m), 3.71 (1 H, dd, J = 11.2 and 2.6 Hz), 2.24–1.97 (4 H m); δ_C (75 MHz, DEPT, D_2O): 176.1 (C), 131.2 (CH), 115.4 (CH_2), 73.7 (CH), 73.0 (C), 70.9 (CH), 67.9 (CH_2), 64.2 (CH), 37.2 (CH_2), 31.2 (CH_2); MS (CI) *m/z* (%) 215 [(MH^+) − H_2O]; HRMS calcd for $C_{10}H_{15}O_5$: MH^+, 215.0916. Found: MH^+, 215.0919.

Methyl (1*S*,3*S*,4*S*,6*S*,7*R*,9*R*)-9-hydroxy-7-(2′,3′-dihydroxy)-propyloxy-3,4-dimethoxy-3,4-dimethyl-2,5-dioxabicyclo[4.4.0]-decane-9-carboxylate (29)

To a stirred solution of allyl ether **26** (629 mg, 1.75 mmol) and *N*-methylmorpholine oxide (246 mg, 2.10 mmol) in 50% aqueous dioxane (20 ml) was added a freshly made aqueous solution of sodium tetraoxide (2.2 ml, 0.12 M). After stirring for 1 hour, ethyl acetate was added followed by saturated sodium bisulfite. The reaction mixture was stirred for 20 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 20 ml). The combined organic layers were dried (anhydrous Na_2SO_4) and concentrated *in vacuo*. The crude residue was purified by flash chromatography eluting with 10% methanol–ethyl acetate, and crystallised from ethyl ether–hexane to afford a 1.2 : 1 mixture of diastereoisomers of the *glycerol* **29** (500 mg, 76%) as white needles. υ_{max} (Nujol)/cm^{-1} 3313b (OH) and 1735s (CO); δ_H (250 MHz, $CDCl_3$): 4.33–4.22 (1 H, m), 3.89–3.53 (5 H, m), 3.73 (3 H, s), 3.24 (3 H, s), 3.23 (3 H, s), 2.22–1.83 (4H, m), 1.30 (3 H, s), 1.25 (3 H, s); δ_C (63 MHz, DEPT, $CDCl_3$): 174.1 (C), 173.9 (C), 99.9 (C), 99.8 (C), 77.4 (CH), 77.3 (CH), 75.4 (C), 75.3 (C), 74.0 (CH_2), 73.4 (CH), 73.0 (CH), 72.8 (CH_2), 70.7 (CH), 69.9 (CH), 52.6 (OCH_3), 47.9 (OCH_3), 47.9 (OCH_3), 38.8 (CH_2), 38.7 (CH_2), 36.5 (CH_2), 36.4 (CH_2), 17.5 (CH_3), 17.4 (CH_3); MS (CI) *m/z* (%) 363 [(MH^+) − $HOCH_3$]; HRMS calcd for $C_{16}H_{27}O_9$: MH^+, 363.1655. Found: MH^+, 363.1650.

(1*S*,3*S*,4*R*,5*R*)-1,4,5-Trihydroxy-3-(2′,3′-dihydroxy)propyloxycyclohexane-1-carboxylic acid (12)

A solution of acetal **29** (95 mg, 0.24 mmol) in a solution of trifluoroacetic acid–water (2 ml, 20 : 1 (v/v)) was stirred for

30 min and then the solvent was removed *in vacuo*. The crude residue was dissolved in an aqueous lithium hydroxide (3 ml, 0.5 M) and stirred for 1 hour. The aqueous layer was washed with diethyl ether (3 × 5 ml) and then was treated with Amberlite IR-120 until the pH was 6.0. The resin was filtered and washed with water. The filtrate was freeze-dried to afford a 1.2 : 1 mixture of diastereoisomers of the *acid* **12** (60 mg, 94%) as an amorphous solid. υ_{max} (Nujol)/cm^{-1} 3430b (OH) and 1725s (CO); δ_H (300 MHz, D_2O): 4.64 (1 H, dt, J = 10.1 and 4.5 Hz), 4.53 (1 H, q, J = 3.4 Hz), 4.49–4.41 (1 H, m), 4.29–3.97 (5 H, m), 2.49–2.03 (4 H, m); δ_C (75 MHz, DEPT, D_2O): 181.3 (C), 81.6 (CH), 81.3 (CH), 78.4 (C), 77.3 (CH), 73.9 (CH_2), 73.7 (CH_2), 73.5 (CH), 73.4 (CH), 69.5 (CH), 65.5 (CH_2), 43.3 (CH_2), 43.2 (CH_2), 36.7 (CH_2), 36.5 (CH_2); MS (CI) *m*/*z* (%) 249 [(MH^+) − H_2O]; HRMS calcd for $C_{10}H_{17}O_7$: MH^+, 249.0974. Found: MH^+, 249.0967.

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