Synthesis and evaluation of 2,5-dihydrochorismate analogues as inhibitors of the chorismate-utilising enzymes†

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A library of 2,5-dihydrochorismate analogues were designed as inhibitors of the chorismate-utilising enzymes including anthranilate synthase, isochorismate synthase, salicylate synthase and 4-amino-4-deoxychorismate synthase. The inhibitors were synthesised in seven or eight steps from shikimic acid, sourced from star anise. The compounds exhibited moderate but differential inhibition against the four chorismate-utilising enzymes.

Introduction

The product of the shikimate pathway, chorismate (**1**) serves as the branchpoint for a number of biosynthetic pathways leading to the formation of a diverse array of aromatic metabolites including the amino acids tyrosine, tryptophan and phenylalanine.[1] Essential vitamins such as the folate coenzymes, benzoid and naphthenoid quinones are also produced through these pathways.[1] Four of the branchpoint enzymes; anthranilate synthase, isochorismate synthase, salicylate synthase and 4-amino-4-deoxychorismate synthase (ADC synthase), appear to share a number of mechanistic and structural features.[3] A pairwise sequence alignment of the four enzymes (from a variety of organisms) shows sequence identities ranging from 21–27% (see ESI†). Perhaps more striking is the similarity in the active sites of these enzymes which contain a number of conserved amino acid residues (see ESI†). This has led to the hypothesis that these enzymes may have diverged from a common ancestor.[4]

Anthranilate synthase catalyses the conversion of chorismate (**1**) to anthranilate (**2**) and represents the first committed step in tryptophan biosynthesis (Fig. 1).[1,5] Isochorismate synthase catalyses the addition of water to C-2 of chorismate to give isochorismate (**3**).[1,2b,6] Similarly, salicylate synthase catalyses the reversible formation of isochorismate. However, like anthranilate synthase, this enzyme has a second activity which aromatises the isochorismate intermediate to afford salicylate (**4**).[7] Isochorismate synthase and salicylate synthase represent the first committed steps en route to the iron chelating siderophores in bacteria.[2,7] Finally, ADC synthase is responsible for the addition of an amino functionality (with retention of stereochemical configuration) to the C-4 position of chorismate to give 4-amino-4-deoxychorismate (ADC, **5**).[1,8] This is the first step in folate biosynthesis in a number of organisms.[1,8]

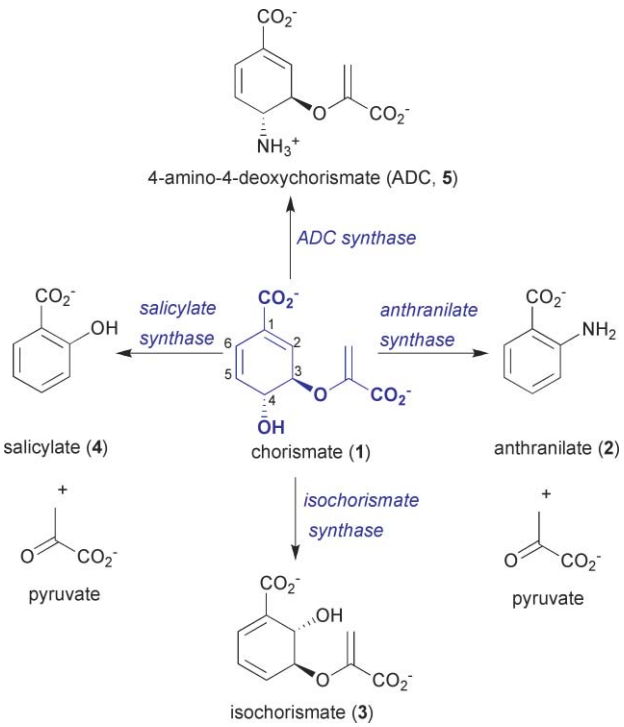

Fig. 1 Four chorismate-utilising enzymes.

There have been several proposed mechanisms for this series of enzymes.[3] Recent reports are consistent with a unified mechanism for all four enzymes, whereby the first step involves nucleophilic attack at the C-2 position of chorismate resulting in concomitant loss of the C-4 hydroxyl group. This step is thought to involve general acid catalysis by a conserved glutamic acid residue (Scheme 1).[3a,b] Depending on the enzyme, the nucleophile at C-2 is either ammonia (anthranilate synthase) or water (isochorismate synthase and salicylate synthase) leading to the formation of ADIC, or isochorismate, respectively. The mechanism of aromatisation of anthranilate synthase and salicylate synthase is largely unknown, but recent evidence suggests that it may proceed *via* a rare pericyclic rearrangement.[7d,9] The mechanism of ADC formation by ADC synthase is slightly different in that the nucleophile which attacks the C-2 position of chorismate is the amino side chain of

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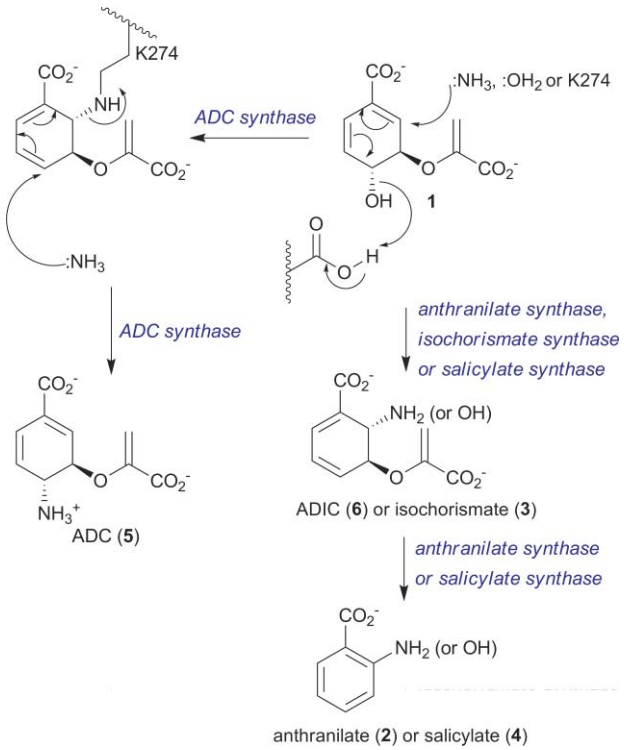
† Electronic supplementary information (ESI) available: Docking figures, sequence alignments, and additional experimental procedures. See DOI: 10.1039/b901694e



Scheme 1 Putative unified mechanism for anthranilate synthase, isochorismate synthase, salicylate synthase and ADC synthase.

an active site lysine residue (K274 in the *E. coli* enzyme).[3a,b,10] This generates an enzyme-bound intermediate which is then released by attack of ammonia at the original C-4 position of chorismate to afford ADC. The chorismate-utilising enzymes are only found in plants, bacteria and fungi. Inhibitors of this enzyme family may be considered as potential herbicidal, antibacterial and antifungal agents.

Over a decade ago Kozlowski *et al.* reported the synthesis and evaluation of three potent inhibitors (**7–9**) of chorismate-utilising enzymes that were designed based on a putative transition state for anthranilate synthase and isochorismate synthase (Fig. 2).[3c] These compounds represent the most potent inhibitors of the chorismate-utilising enzymes reported to date.

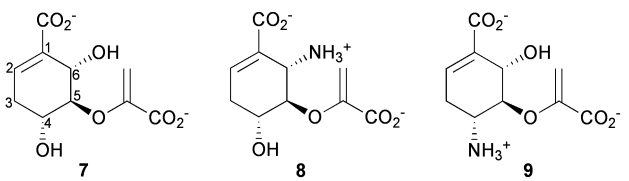


Fig. 2 Inhibition constants for chorismate-utilising enzyme inhibitors **7–9**; AS = *S. marcescens* anthranilate synthase, IS = *E. coli* isochorismate synthase and ADCS = *E. coli* ADC synthase.

Compound **8** containing a C-6 amino group was the most potent inhibitor of anthranilate synthase (K_I = 620 nM) and isochorismate synthase (K_I = 53 nM), despite the fact that the latter enzyme normally catalyses the attack of water at this position. Isomeric amino alcohol **9** showed comparable affinities for isochorismate synthase and anthranilate synthase, which suggested that these enzymes operate through a pseudo-symmetric transition state.[3c] In contrast, **7–9** exhibited only micromolar inhibiton of ADC synthase.

As part of our long term goal to develop potent inhibitors against the chorismate-utilising enzymes, we chose to re-examine the 2,5-dihydrochorismate scaffold for the design of more potent inhibitors. We were also interested in gaining an understanding of the binding modes of these compounds into the active sites of the enzymes to aid in the design of other families of inhibitors active against the chorismate-utilising enzymes.

Results and discussion

Molecular docking

The initial phase of the research program involved the use of molecular docking studies to help predict the binding mode of chorismate and other enzyme intermediates and products of the chorismate-utilising enzymes. Docking studies were conducted using GOLD.[11] Anthranilate synthase from *S. marcescens* was used as a representative model for all of the chorismate-utilising enzymes studied here. Fig. 3a shows chorismate docked into the active site of anthranilate synthase. The C-4 hydroxyl interacts with E309 and the side chain carboxylate of the enol-pyruvyl side chain. The C-1 carboxylate appears to make an electrostatic interaction with the magnesium ion in the active site. ADIC, the intermediate of the anthranilate synthase reaction, docked into the active site in a similar binding mode to chorismate (Fig. 3b, see ESI† for isochorismate and ADC docking solutions). The obvious difference is the interactions of the C-6 substituents of ADIC and isochorismate (C-2 of chorismate) which are situated in close proximity to a lysine residue (K502). For ADC synthase, the recently proposed mechanisms[3a,b] and crystal structures of *E. coli* PabB[12] suggest that a side chain amino group of the catalytically important lysine residue (K274) would be positioned in close proximity to C-2 of chorismate (i.e. the 6-position of **7–9**). The lower binding affinity of **7–9** against this enzyme may therefore be a result of steric crowding at this position. Additionally, an unfavourable charge–charge interaction with the C-6 amino group of compound **8** may further reduce potency (K_I = 635 μM).

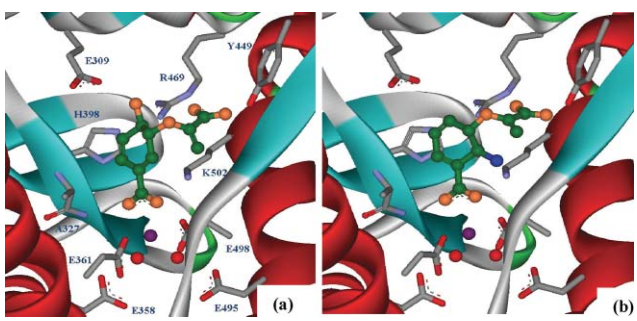


Fig. 3 Docking of (a) chorismate and (b) ADIC into the active site of *S. marcescens* anthranilate synthase.

Based on these docking studies and the previous inhibition work described by Kozlowski *et al.* we chose to embark on the synthesis of 2,5-dihydrochorismate **10**, lacking a C-6 substituent altogether

(Fig. 4). Our aims were to understand the importance of the C-6 substituent in **7–9** and to probe the specificity of the side-chain binding interactions. Previous studies on aromatic inhibitors of chorismate-utilising enzymes suggested that a lactyl side chain is a good mimic for the native enol-pyruvyl side chain.[13] Docking studies into the active site of *S. marcescens* anthranilate synthase suggested that this may also be the case for its incorporation into the proposed 2,5-dihydrochorismate-based inhibitors (see ESI†). To this end compounds were synthesised with glycol (**11**), lactate (**12** and **13**) and acrylate (**14**) side chains at C-5.

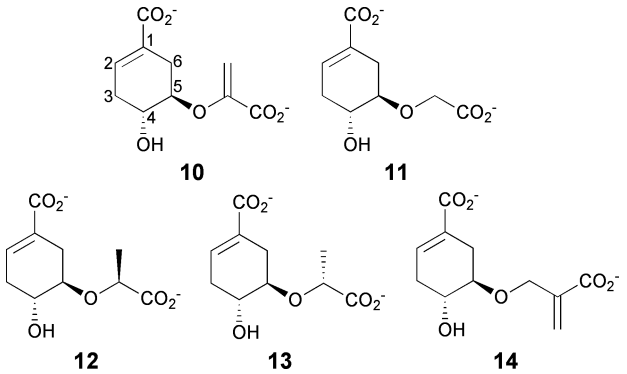


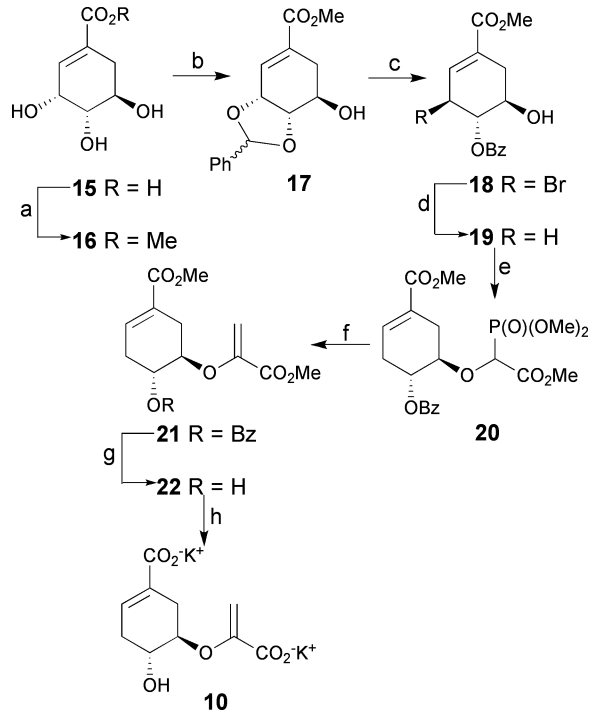
Fig. 4 Proposed inhibitors of chorismate-utilising enzymes.

Synthesis

The synthesis of inhibitors **10–14** was initiated from shikimic acid (**15**). This metabolite was isolated from star anise using the method of Adams *et al.*[14] Methyl ester formation, protection of the 3,4-diol as the benzylidene acetal and radical-induced bromination was carried out as described previously to afford C-3 bromide **18** in 68% yield (Scheme 2).[14] Palladium-catalysed hydrodebromination of **18** was achieved by treatment with tributyltin hydride in the presence of tetrakistriphenylphosphine palladium to afford the desired alcohol **19** in 62% yield.

Incorporation of the enol-pyruvate side chain of 2,5-dihydrochorismate **10** was carried out by first reacting alcohol **19** with trimethyl diazophosphonoacetate and rhodium(II) acetate dimer to give the desired phosphonate **20** in 64% yield. Introduction of the enol-pyruvyl functionality was achieved *via* a Horner–Wadsworth–Emmons reaction using lithium bis(trimethylsilyl)amide and formaldehyde to afford **21** in quantitative yield. Methanolysis of the C-4 benzoyl group, followed by hydrolysis of the methyl esters with aqueous potassium hydroxide gave the desired 2,5-dihydrochorismate inhibitor **10** as the di-potassium salt.

Inhibitors **11–14** were synthesised from benzylidene alcohol **17** (Scheme 3). Deprotonation of **17** with sodium hydride followed by treatment with methylbromoacetate, methyl-2-bromopropionate or methyl-2-(bromomethyl)acrylate gave the corresponding C-5 alkylated adducts in good yields. Radical bromination of these products proceeded in moderate yields for the glycol and the lactate side chains, however, yields were poorer for the compound containing the acrylate side chain, due to the preferential bromination of the acrylate double bond. Diastereomeric bromides **24** and **25** were separable by silica gel column chromatography at this stage. Palladium-catalysed hydrodebromination of **23–26** furnished **27–30** in 35–64% yields. Methanolysis of the benzoyl ester protecting groups at C-4, followed by hydrolysis of the methyl esters with aqueous potassium hydroxide then furnished the desired inhibitors **11–14** as their di-potassium salts.



Scheme 2 Synthesis of 2,5-dihydrochorismate (**10**) from shikimic acid: a) dowex $50H^+$, methanol, reflux, quant.; b) PhCHO, *p*-TsOH, THF, reflux, 79%; c) NBS, AIBN, benzene, reflux, 68%; d) Bu_3SnH, $Pd(Ph_3)_4$, THF, 62%; e) trimethyl diazophosphonoacetate, $Rh(OAc)_2$ dimer, benzene, reflux, 64%; f) (i) $LiN(SiMe_3)_2$, THF, −78 °C, (ii) H_2CO, −78 °C, quant.; g) NaOMe, MeOH, 0 °C, 84%; h) KOH, THF/H_2O, 87%.

Evaluation of inhibitors

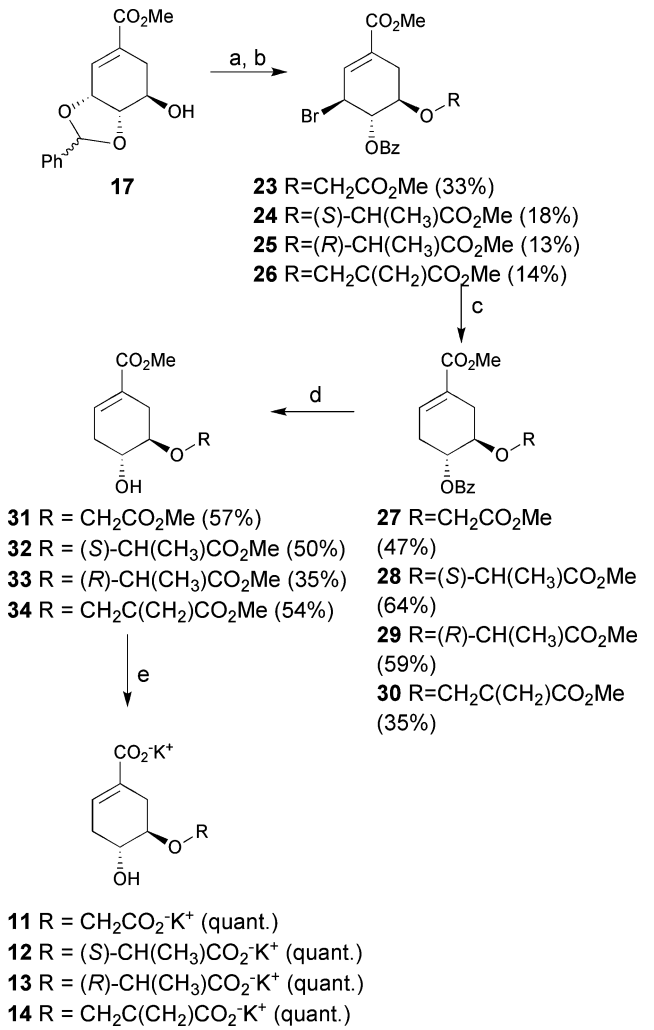
The 2,5-dihydrochorismate inhibitors **10–14** were tested against four different chorismate-utilising enzymes: *S. marcescens* anthranilate synthase, *E. coli* isochorismate synthase, *Y. enterocolitica* salicylate synthase and *E. coli* ADC synthase. The assays for anthranilate synthase, isochorismate synthase and salicylate synthase were fluorescence-based and were carried out in 96 well plates at 25 °C. Anthranilate synthase was assayed by detecting the formation of anthranilate (ex. 313 nm, em. 390 nm).[6,15] Isochorismate synthase and salicylate synthase were assayed by detecting the formation of salicylate (ex. 305 nm, em. 440 nm).[2b,6,13c] Isochorismate synthase was assayed using a coupling enzyme, *Pseudomonas aeroginosa* isochorismate pyruvate lyase in order to convert isochorismate into salicylate, which was detected fluorimetrically. Compounds were screened against *E. coli* ADC synthase using a coupled assay with *E. coli* ADC lyase and lactate dehydrogenase, by measuring the consumption of NADH at 340 nm.[8b,3c] The inhibition constants of **10–14** against the four chorismate-utilising enzymes are shown in Table 1. All compounds proved to be competitive reversible inhibitors of the four enzymes with respect to the substrate chorismate.

Analogue **10** containing the enol-pyruvyl side chain, found on chorismate, was the most potent inhibitor of three of the four

Table 1 Inhibition constants of 2,5-dihydrochorismate analogues **10–14** against *S. marcescens* anthranilate synthase (AS), *E. coli* isochorismate synthase (IS), *Y. enterocolitica* salicylate synthase (SS) and *E. coli* ADC synthase/ADC lyase

	AS[a] K_I (μM)	IS[a] K_I (μM)	SS[a] K_I (μM)	ADC synthase[a] K_I (μM)
10	290 ± 40	30 ± 3	160 ± 10	1060 ± 220
11	1280 ± 150	>2000	>2000	930 ± 160
12	>2000	>2000	>2000	410 ± 84
13	>2000	>2000	>2000	840 ± 180
14	980 ± 130	>2000	440 ± 65	>2000

[a] Kinetic parameters of the chorismate-utilising enzymes: AS: $K_M = 3.7 \pm 0.5$ μM, $k_{cat} = 5.6$ s^{-1}; IS: $K_M = 15 \pm 2.4$ μM, $k_{cat} = 13$ s^{-1}; SS: $K_M = 4.2 \pm 0.9$ μM, $k_{cat} = 0.1$ s^{-1}; ADC synthase: $K_M = 5.4 \pm 0.7$ μM, $k_{cat} = 0.23$ s^{-1}.



Scheme 3 Synthesis of 2,5-dihydrochorismate inhibitors **11–14**: a) Br-R, NaH, MeCN; b) NBS, AIBN, benzene; c) $Pd(PPh_3)_4$, Bu_3SnH, THF; d) NaOMe, MeOH, 0 °C–22 °C; e) KOH, 1:1 v/v THF/H_2O.

enzyme systems. The compound exhibited inhibition constants of 290 μM, 30 μM and 160 μM against anthranilate synthase, isochorismate synthase and salicylate synthase respectively. Compounds that contained a C-5 glycol or lactyl side chain were poor inhibitors of all three of these enzymes, with most exhibiting inhibition constants greater than 2 mM. This shows the importance of the C-3 side chain for inhibition of the chorismate-utilising enzymes, and contrasts somewhat with the trend observed for a series of previously reported aromatic analogues where the incorporation of a lactate side chain proved to be a good replacement for the enol-pyruvyl side chain.[13] Compounds **10–13** were moderate inhibitors of ADC synthase, with inhibition constants ranging from 410–1060 μM. Surprisingly, analogues **12** and **13** containing (*R*)- and (*S*)-lactate side chains at C-5 were more potent inhibitors than compound **10** against this enzyme. ADC synthase showed some selectivity in binding compound **12** with an (*S*)-lactate side chain ($K_I = 410$ μM) over the corresponding (*R*)-analogue ($K_I = 840$ μM). It appears that there is a requirement for a double bond in the C-5 side chain for activity against salicylate synthase. Compounds **10** and **14** exhibited inhibition constants of 160 μM and 440 μM respectively, whilst compounds **11–13** showed no measurable inhibition at a concentration of 2 mM. Compounds **12** and **13** containing lactate side chains appear to selectively inhibit ADC synthase over the remainder of the chorismate-utilising enzymes suggesting that inhibitors may be made specific for individual enzymes of the family. The inhibition of isochorismate synthase and salicylate synthase was broadly similar reflecting their common chemistry.

Conclusions

Compounds **10–14** proved to be significantly less potent as inhibitors of the chorismate-utilising enzymes when compared to those synthesised by Kozlowski *et al.* (**7–9**).[3c] Specifically, removal of the C-6 amino substituent in **8** (the most potent inhibitor in the series) to generate **10** caused a 500-fold decrease in potency against anthranilate synthase and isochorismate synthase and a two-fold reduction against ADC synthase. This suggests that the presence of hydrogen-bonding amino or hydroxy substituents at C-6 is important for tight binding of these compounds to the chorismate-utilising enzymes. It is not clear from our docking studies why the introduction of this substituent has such a marked effect on the inhibition constants. It is possible that **8** may have a different binding mode to that observed in the dockings of chorismate, ADIC, isochorismate and ADC.

The inhibitors synthesised in this study give some insight into the features required for inhibition of the chorismate-utilising enzymes and should aid in the design of more potent inhibitors. Current efforts of our laboratory are focused on obtaining structural information on the binding of compound **10** with the chorismate-utilising enzymes.

Experimental

(4*R*,5*R*)-Methyl-4-benzoyloxy-5-hydroxy-1-cyclohexene-1-carboxylate 19

$Pd(PPh_3)_4$ (10 mol%, 0.16 g, 0.14 mmol) was added to a solution of **18**[14] (0.50 g, 1.41 mmol) in THF (15 ml). The entire solution was degassed (by three freeze-pump-thaw cycles) and allowed to warm to 22 °C before the dropwise addition of tributyltin hydride (0.42 ml, 1.55 mmol). The reaction was stirred at 50 °C for 2 h. The reaction was allowed to cool to 22 °C before the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (20 ml) and washed with 10% aqueous potassium fluoride solution

(3 × 20 ml). The ethyl acetate fraction was filtered, washed with water (20 ml), brine (20 ml) and the solvent was removed *in vacuo.* The residue was dissolved in acetonitrile (20 ml) and washed with hexane (2 × 20 ml). The acetonitrile fraction was dried ($MgSO_4$) and the solvent removed *in vacuo* to afford the crude product as an orange oil. The product was purified by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) to afford the desired alcohol **19** as a pale yellow solid (0.24 g, 62%). R_F [2:1 v/v petroleum ether:ethyl acetate] = 0.50; ^{1}H NMR (400 MHz, $CDCl_3$) δ, 2.36–2.58 (2H, m, H-6_{ax}, H-3_{ax}), 2.88 (1H, m, H-3_{eq}), 2.93 (1H, m, H-6_{eq}), 3.76 (3H, s, CO_2Me), 4.15 (1H, m, H-5), 5.17 (1H, ddd, *J* 6.8, 8.1, 14.7 Hz, H-4), 6.89 (1H, m, H-2), 7.42 (2H, m, 2 × *m*-ArH), 7.55 (1H, m *p*-ArH), 8.01 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 31.3, 52.2, 67.8, 72.7, 128.0, 128.8, 130.0, 130.2, 133.7, 136.0, 166.7, 167.3; LC-MS [M + H]$^+$ 277.1, Rt = 3.7 min; HRMS calcd for $C_{15}H_{16}O_5Na$: *M*Na$^+$, 299.0895. Found: MNa$^+$, 299.0894.

(4*R*,5*R*)-Methyl 4-*O*-benzoyl 5-[1′-(dimethoxyphosphinyl)-2′-methoxy-2′-oxo-ethoxy] cyclohex-1-ene-carboxylate 20

Trimethyl diazophosphonoacetate[16] (221 mg, 1.04 mmol) was added dropwise to a stirred solution of **19** (210 mg, 0.74 mmol) and rhodium acetate dimer (6.7 mg, 0.01 mmol) in dry benzene (5 ml). The mixture was heated at 85 °C for 24 h. The reaction was allowed to cool to 22 °C before the solvent was removed *in vacuo.* The product was purified by column chromatography (eluent: 5:1 v/v ethyl acetate/petroleum ether) to afford the desired phosphonate **20** as a pale green oil (230 mg, 69%). R_F [5:1 v/v ethyl acetate/petroleum ether] = 0.18; $\nu_{max.}$ (ATR): 1753, 1713 (C=O, str), 1655 (C=C), 1451, 1436 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ, 2.35–2.69 (2H, m, H-3_{ax} and H-6_{ax}), 2.78–2.95 (2H, m, H-3_{eq} and H-6_{eq}), 3.72 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 3.80 (3H, d, *J* 5.7 Hz, P-OMe), 3.83 (3H, d, *J* 5.7 Hz, P-OMe), 3.95 (1H, m, H-5), 4.65 (1H, d, J_{HP} 18.4 Hz, C-H), 5.37 (1H, ddd, *J* 5.1, 10.0, 12.0 Hz, H-4), 6.88 (1H, ddd, *J* 2.2, 3.9, 5.1 Hz), 7.42 (2H, m, 2 × ArH), 7.55 (1H, m, ArH), 7.98 (2H, m, 2 × ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 31.7, 53.4, 54.5, 55.6, 56.9, 72.2 (d, $J_{C,P}$ 169 Hz), 78.2, 79.2, 129.4, 131.1, 132.2, 135.9 × 2, 137.8, 138.4, 168.4, 169.4; HRMS calcd for $C_{20}H_{25}O_{10}PNa$: *M*Na$^+$, 479.1083. Found: MNa$^+$, 479.1072.

(4*R*,5*R*)-Methyl 4-*O*-benzoyl [5-(methoxycarbonyl)ethenyl] cyclohex-1-ene-carboxylate 21

Lithium bis(trimethylsilyl)amide (0.49 ml, 1.0 M solution in THF, 0.49 mmol) was added over a period of 5 min to a stirred solution of phosphonate **20** (202 mg, 0.44 mmol) in THF (3 ml) at −78 °C. The solution was stirred for a further 5 min before a formaldehyde solution, produced by cracking paraformaldehyde (approximately 4.4 mmol in 30 ml THF) was added dropwise and the solution stirred for a further 30 min. The reaction was quenched by the dropwise addition of saturated aqueous ammonium chloride solution (10 ml) followed by extraction of the aqueous layer with ethyl acetate (4 × 20 ml). The combined organic fractions were washed with water (50 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. The product was purified by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) to afford the desired enol ether **21** as a white solid (160 mg, quant.). $\nu_{max.}$ (ATR): 2949, (Ar C-H stretch), 1729, 1710 (C=O, str), 1655, 1622 (C=C), 1449, 1436 (C=C, Ar) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45–2.90 (4H, m, H-6_{ax}, H-6_{eq}, H-3_{ax}, H-3_{eq}), 3.71 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 4.57 (1H, m, H-5), 4.93 (1H, d, J 2.8 Hz, C=CHH), 5.43 (1H, m, H-4), 5.51 (1H, d, J 2.8 Hz, C=CHH), 6.96 (1H, m, H-2), 7.41 (2H, m, 2 × *m*-ArH), 7.53 (1H, m, *p*-ArH), 7.94 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 28.0, 51.7, 52.2, 67.2, 71.4, 97.5, 126.5, 128.4 × 2, 129.6, 133.2, 135.3, 149.6, 163.4, 165.7, 166.8; HRMS calcd for $C_{19}H_{21}O_7$: MH$^+$, 361.1287. Found: MH$^+$, 361.1287.

(4*R*,5*R*)-Methyl 4-hydroxy [5-(methoxycarbonyl)ethenyl] cyclohex-1-ene-carboxylate 22

A solution of sodium methoxide (12 mg, 0.22 mmol) in methanol (0.4 ml) was added dropwise to a solution of benzoyl ester **21** (71 mg, 0.20 mmol) in methanol (2.0 ml) at 0 °C. The reaction was stirred at 22 °C for 4.5 h, before the addition of saturated aqueous ammonium chloride solution (1 ml). The reaction was diluted with ethyl acetate (15 ml) and the organic fraction washed with water (15 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to afford the crude product. Purification by column chromatography (eluent: 1:1 v/v petroleum ether/ethyl acetate) gave the desired alcohol **22** as a colourless oil (47 mg, 84%). $\nu_{max.}$ (ATR): 3429 (br O-H str.), 1712 (C=O, str), 1654, 1621 (C=C), 1437 (C=C, Ar) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26–3.03 (4H, m, H-6_{ax}, H-6_{eq}, H-3_{ax}, H-3_{eq}), 3.31 (1H, br s, OH), 3.71 (3H, s, CO_2Me), 3.79 (3H, s, CO_2Me), 4.02 (2H, m, H-5 and H-4), 4.88 (1H, d, *J* 2.6 Hz, C=C*H*H), 5.51 (1H, d, *J* 2.6 Hz, C=CH*H*), 6.85 (1H, m, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 32.3, 51.8, 52.5, 68.2, 79.2, 99.0, 127.2, 136.6, 149.9, 164.0, 166.5; HRMS calcd for $C_{12}H_{16}O_6Na$: *M*Na$^+$, 279.0839. Found: MNa$^+$, 279.0832.

(4*R*,5*R*)-4-Hydroxy-5-(1-carboxyvinyloxy)-cyclohex-1-ene carboxylate 10

Potassium hydroxide (13 mg, 0.23 mmol) in milli-Q water (1 ml) was added dropwise to a solution of **22** (20 mg, 0.08 mmol) in THF (1 ml). The resulting solution was stirred at 22 °C for 3 h. The reaction was diluted with milli-Q water (4 ml) and washed with dichloromethane (10 ml) before the aqueous fraction was adjusted to pH 8.0 with 0.1 M HCl. The solution was lyophilised to give the desired di-carboxylate **10** as a white solid (0.20 mmol, 87%, as determined by ^{1}H NMR spectroscopic studies using TSP as an internal standard). $\nu_{max.}$ (ATR): 3304 (br acid O-H str.), 1657, 1559 (C=O, str), 1553 (C=C, str.) cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 2.06–2.95 (4H, m, H-6_{ax}, H-6_{eq}, H-3_{ax}, H-3_{eq}), 3.99 (1H, m, CH), 4.12 (1H, m, CH), 4.63 (1H, d, *J* 2.4 Hz, C=C*H*H), 5.12 (1H, d, *J* 2.4 Hz, C=CH*H*), 6.43 (1H, m, H-2). ^{13}C NMR (125 MHz, D_2O) δ 28.5, 31.5, 67.9, 76.9, 92.4, 131.2, 132.1, 153.6, 171.0, 175.6; HRMS calcd for $C_{10}H_{10}O_6K_3$: *M*K$^+$, 342.9383. Found: MK$^+$, 342.9380.

(1*S*,2*S*,6*R*)-Benzoic acid-2-bromo-4-methoxycarbonyl-6-methoxycarbonylmethoxy-cyclohex-3-enyl ester 23

Alcohol **17**[14] (400 mg, 1.45 mmol) was dissolved in acetonitrile (20 ml) and the reaction cooled to 0 °C. Methylbromoacetate (178 µl, 1.88 mmol) was added dropwise at 0 °C before sodium hydride (65 mg, 1.59 mmol, 60% dispersion in oil) was added

in three separate portions over a 30 min period. The reaction was stirred at 22 °C overnight at which point the solvent was removed *in vacuo* at 30 °C and the product was purified by column chromatography (eluent: 3:2 v/v petroleum ether/ethyl acetate) to afford the desired product as a separable mixture of diastereoisomeric benzylidene acetals as a pale yellow oil (440 mg, 87%). *Major Diastereoisomer*: R_F [3:2 v/v petroleum ether:ethyl acetate] = 0.53; $\nu_{max.}$ (NaCl plate): 3066, 3034, 3000, 2952, 2913 2850 (Ar C-H stretch), 1753, 1718 (C=O, str), 1655 (C=C), 1437, 1403 (C=C, Ar) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.39 (1H, br dd, *J* 6.2, 17.6 Hz, H-6_{ax}), 2.87 (1H, dd, *J* 4.3, 17.6 Hz, H-6_{eq}), 3.70 (3H, s, CO_2Me), 3.77 (3H, s, CO_2Me), 3.78 (1H, dt, *J* 4.3, 6.2 Hz, H-5), 4.27 (2H, s, CH_2), 4.37 (1H, t, *J* 6.2 Hz, H-4), 4.83 (1H, m, H-3), 5.89 (1H, s, CHPh), 6.91 (1H, m, H-2) 7.36–7.42 (5H, m, 5 × ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.3, 51.0, 51.4, 66.6, 73.1, 75.9, 76.1, 103.4, 126.0, 127.6, 128.8, 129.7, 132.4, 135.7, 165.6, 170.0; HRMS calcd for $C_{18}H_{20}O_7Na$: *M*Na^+, 371.1107. Found: MNa^+, 371.1091. *Minor Diastereoisomer*: R_F [3:2 v/v petroleum ether:ethyl acetate] = 0.43; $\nu_{max.}$ (NaCl plate): 3060, 2915 2849 (Ar C-H stretch), 1750, 1720 (C=O, str), 1653 (C=C), 1434, 1407 (C=C, Ar) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.48 (1H, br dd, *J* 6.2, 17.6 Hz, H-6_{ax}), 2.80 (1H, dd, *J* 4.3, 17.6 Hz, H-6_{eq}), 3.74 (3H, s, CO_2Me), 3.79 (3H, s, CO_2Me), 3.94 (1H, dt, *J* 4.3, 6.2 Hz, H-5), 4.27 (2H, s, CH_2), 4.50 (1H, t, *J* 6.2 Hz, H-4), 4.93 (1H, m, H-3), 5.92 (1H, s, CHPh), 6.94 (1H, m, H-2), 7.37–7.44 (5H, m, 5 × ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.4, 52.3, 52.6, 67.7, 73.2, 76.1, 76.3, 103.1, 126.7, 128.8, 129.7, 131.2, 134.0, 138.1, 166.8, 171.1; HRMS calcd for $C_{18}H_{20}O_7Na$: *M*Na^+, 371.1107. Found: MNa^+, 371.1100.

A solution of the above mixture of benzylidene acetals (440 mg, 1.265 mmol), *N*-bromosuccinimide (248 mg, 1.39 mmol) and AIBN (1.66 mg, 0.01 mmol) in anhydrous benzene (50 ml) was heated at reflux (85 °C) for 2 h. The reaction was allowed to cool to 22 °C before diluting with dichloromethane (40 ml). The reaction mixture was washed with saturated sodium carbonate solution (60 ml), water (60 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to afford the crude product as a pale yellow oil. Purification by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) afforded the desired bromide **23** as a milky oil (207 mg, 38%, 33% yield over the two steps). R_F [3:1 v/v petroleum ether/ethyl acetate] = 0.47; $\nu_{max.}$ (NaCl plate): 3064, 2952, 2915, 2853 (Ar C-H stretch), 1740, 1722 (C=O, str), 1650 (C=C), 1602, 1583, 1492 (C=C, Ar) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.65 (1H, m, H-5_{ax}), 3.01 (1H, dd, *J* 5.3, 16.7 Hz, H-5_{eq}), 3.62 (3H, s, CO_2Me), 3.78 (3H, s, CO_2Me), 3.88 (1H, m, H-6), 4.17 (2H, s, CH_2), 4.80 (1H, m, H-2), 5.70 (1H, dd, *J* 6.5 Hz, 8.5 Hz, H-1), 6.94 (1H, m, H-3), 7.45 (2H, t, *J* 1.5 Hz, 2 × *m*-ArH), 7.58 (1H, t, *J* 1.5 Hz, *p*-ArH), 8.05 (2H, d, *J* 1.5 Hz, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.5, 45.0, 52.2, 52.7, 67.6, 75.7, 76.0, 128.9, 129.2, 129.4, 130.2, 133.8, 136.1, 165.7, 166.2, 171.6; HRMS calcd for $C_{18}H_{19}O_7BrNa$: *M*Na^+, 449.0212. Found: MNa^+, 449.0202.

(1*R*,6*R*)-Benzoic acid-4-methoxycarbonyl-6-methoxycarbonylmethoxy-cyclohex-3-enyl ester 27

Hydrodebromination of **23** (207 mg, 0.49 mmol) was conducted in an identical manner to that described for the synthesis of **19**. The product was purified by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) to afford the desired triester **27** as a pale yellow oil (80 mg, 47%). R_F [3:1 v/v petroleum ether:ethyl acetate] = 0.26; $\nu_{max.}$ (NaCl plate): 2999, 2952, 2915, 2849 (Ar C-H stretch), 1755, 1715 (C=O, str), 1657 (C=C), 1602, 1492 (C=C, Ar) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.92–2.38 (4H, m, H-2_{ax}, H-2_{eq}, H-6_{ax}, H-6_{eq}), 3.70 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 3.94 (1H, td, *J* 5.0, 6.4 Hz, H-6), 4.21 (1H, d, *J* 19.0 Hz C*H*H), 4.26 (1H, d, *J* 19.0 Hz CH*H*), 5.37 (1H, dt, *J* 5.0, 11.4 Hz, H-1), 6.91 (1H, m, H-3), 7.42 (2H, t, *J* 7.8 Hz, 2 × *m*-ArH), 7.50 (1H, t, *J* 7.8 Hz, *p*-ArH), 7.98 (2H, d, *J* 7.8 Hz, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.7, 29.2, 52.2, 52.3, 67.7, 69.6, 75.2, 127.4, 128.8, 130.0, 130.3, 133.6, 136.0, 166.1, 167.3, 171.1; HRMS calcd for $C_{18}H_{20}O_7Na$: *M*Na^+, 371.1107. Found: MNa^+, 371.1108.

(4*R*,5*R*)-4-Hydroxy-5-methoxycarbonylmethoxy-cyclohex-1-enecarboxylic acid methyl ester 31

Benzoyl ester deprotection of **27** (75 mg, 0.22 mmol) was conducted in an identical manner to that described for the synthesis of **22**. Purification by column chromatography (eluent: 1:1 v/v petroleum ether/ethyl acetate) gave the desired alcohol **31** as a colourless oil (30 mg, 57%). R_F (1:1 v/v petroleum ether/ethyl acetate) = 0.26; $\nu_{max.}$ (ATR): 2954 (br OH str.), 1750, 1714 (C=O, str), 1651 (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.19–2.92 (4H, m, H-3_{ax}, H-3_{eq}, H-6_{ax}, H-6_{eq}), 3.36 (1H, m, H-5), 3.70 (3H, s, CO_2Me), 3.76 (3H, s, CO_2Me), 3.81 (1H, m, H-4), 3.90 (1H, br s, OH), 4.09 (1H, d, *J* 16.9 Hz, C*H*H), 4.33 (1H, d, *J* 16.9 Hz, CH*H*), 6.80 (1H, m, H-2); HRMS calcd for $C_{11}H_{16}O_6Na$: *M*Na^+, 267.0839. Found: MNa^+, 267.0840; HRMS calcd for $C_{11}H_{17}O_6$: *M*H^+, 245.1020. Found: MH^+, 245.1021.

(4*R*,5*R*)-4-Hydroxy-5-carboxymethoxy-cyclohex-1-ene-carboxylate 11

Diester **31** (20 mg, 0.08 mmol) was deprotected using an identical procedure to that described for the synthesis of **10** to afford desired di-carboxylate **11** as a white solid. (0.25 mmol, quant., as determined by ^1H NMR spectroscopic studies using TSP as an internal standard). $\nu_{max.}$ (ATR): 3270 (br acid O-H str), 1659, (C=O, str) 1558 (C=C) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 2.12–2.80 (4H, m, H-3_{ax}, H-3_{eq}, H-6_{ax}, H-6_{eq}), 3.54 (1H, m, H-4), 3.87 (1H, m, H-5), 3.96 (1H, d, *J* 15.8 Hz, C*H*H), 4.12 (1H, d, *J* 15.8 Hz, CH*H*), 6.41 (1H, m, H-2); ^{13}C NMR (125 MHz, D_2O) δ 29.8, 31.9, 68.4, 68.5, 79.7, 131.3, 132.3, 175.8, 178.5. HRMS calcd for $C_9H_{10}O_6K_3$: *M*K^+, 330.9383. Found: MK^+, 330.9384.

(1*S*,2*S*,6*R*,7*S*)-Benzoic acid-2-bromo-4-methoxycarbonyl-6-(1-methoxycarbonyl-ethoxy)-cyclohex-3-enyl ester 24 and (1*S*, 2*S*, 6*R*, 7*R*)-benzoic acid-2-bromo-4-methoxycarbonyl-6-(1-methoxycarbonyl-ethoxy)-cyclohex-3-enyl ester 25

Alcohol **17**[14] (446 mg, 1.62 mmol) was dissolved in acetonitrile (20 ml) and the reaction cooled to 0 °C. Methyl-2-bromopropionate (235 μl, 2.10 mmol) was added dropwise at 0 °C before sodium hydride (64 mg, 1.62 mmol, 60% dispersion in oil) was added in three separate portions over a 30 min period. The reaction was stirred at 22 °C for 16 h at which point the solvent was removed *in vacuo* at 30 °C. The product was purified by column chromatography (eluent: 3:2 v/v petroleum ether/ethyl acetate) to afford the desired diester (as a mixture of four diastereoisomers) as

a yellow oil (440 mg, 87%). R_F [3:2 petroleum ether:ethyl acetate] = 0.53–0.43; $\nu_{max.}$ (NaCl plate): 3034, 2988, 2952, 2910, 2848 (Ar C-H stretch), 1730 (C=O, str), 1657 (C=C), 1454 (C=C, Ar) cm^{-1}; HRMS calcd for $C_{19}H_{22}O_7Na$: MNa$^+$, 385.1263. Found: MNa$^+$, 385.1266.

The mixture of the above diastereoisomers (101 mg, 0.28 mmol), *N*-bromosuccinimide (55 mg, 0.31 mmol) and AIBN (0.37 mg, 0.01 mmol) in anhydrous benzene (12 ml) was heated at reflux (85 °C) for 2 h. The reaction was allowed to cool to 22 °C before dilution with dichloromethane (15 ml). The reaction mixture was washed with saturated sodium carbonate solution (25 ml), water (25 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to afford the crude product as a pale yellow oil. Purification by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) furnished the diastereomeric bromides **24** and **25** as colourless oils. (44 mg, 36%, 18% (1*S*,2*S*,6*R*,7*S*)-diastereoisomer (**24**); 13% (1*S*,2*S*,6*R*,7*S*)- diastereoisomer (**25**); 5% of an inseparable mixture of **24** and **25**, 31% yield over two steps).

24. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (3H, d, *J* 6.9 Hz, CH_3), 2.68 (1H, dddd, *J* 2.7, 5.1, 7.8, 15.4 Hz, H-5_{ax}), 3.02 (1H, dd, *J* 5.1 Hz, 18.2 Hz, H-5_{eq}), 3.71 (3H, s CO_2Me), 3.79 (3H, s, CO_2Me), 3.79 (1H, m, H-6), 4.19 (1H, q, *J* 6.9 Hz, CH), 4.78 (1H, m, H-2), 5.68 (1H, dd, J 6.7, 8.4 Hz, H-1), 6.92 (1H, m, H-3), 7.46 (2H, m, 2 × *m*-ArH), 7.59 (1H, m, *p*-ArH), 8.03 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.9, 30.4, 45.2, 52.4, 52.7, 75.6, 76.0, 76.3, 129.0, 129.7, 130.0, 130.1, 133.9, 136.7, 165.5, 166.2, 173.9.

25. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, d, *J* 6.8 Hz, CH_3), 2.52 (1H, dddd, *J* 2.9, 5.2, 11.4, 17.6 Hz, H-5_{ax}), 2.95 (1H, dd, *J* 5.2, 17.6 Hz, H-5_{eq}), 3.41 (3H, s CO_2Me), 3.79 (3H, s, CO_2Me), 3.84 (1H, ddd, *J* 5.2, 8.8, 17.5 Hz, H-6), 4.15 (1H, q, *J* 6.8 Hz, CH), 4.80 (1H, m, H-2), 5.68 (1H, dd, *J* 7.2, 9.1 Hz, H-1), 6.93 (1H, m, H-3), 7.46 (2H, m, 2 × *m*-ArH), 7.57 (1H, m, *p*-ArH), 8.07 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.3, 29.5, 45.7, 52.1, 52.7, 74.2, 75.0, 76.3, 128.7, 129.1, 130.1, 130.2, 133.9, 136.7, 165.7, 166.2, 172.9.

(1*R*,6*R*,7*S*)-Benzoic acid 4-methoxycarbonyl-6-(1-methoxycarbonyl-ethoxy)-cyclohex-3-enyl ester 28. Hydrodebromination of **24** (0.20 g, 0.45 mmol) was conducted in an identical manner to that described for the synthesis of **19**. The product was purified by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) to afford the desired triester **28** as a yellow oil (0.11 g, 64%). $\nu_{max.}$ (ATR): 1723, 1718 (C=O, str), 1451 (C=C Ar.) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, d, *J* 6.9 Hz, CH_3), 2.38–2.90 (4H, m, H-2_{ax}, H-2_{eq}, H-5_{ax}, H-5_{eq}), 3.70 (3H, s, CO_2Me), 3.74 (3H, s, CO_2Me), 3.87 (1H, m, H-6), 4.28 (1H, q, *J* 6.8 Hz, CH), 5.29 (1H, ddd, *J* 5.3, 6.9, 12.3 Hz, H-1), 6.88 (1H, m, H-3), 7.42 (2H, m, 2 × *m*-ArH), 7.57 (1H, m, *p*-ArH), 7.98 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.1, 28.1, 29.4, 51.9, 52.2, 70.0, 74.5, 75.3, 127.7, 128.6, 129.7, 130.0, 133.4, 135.3, 165.9, 167.0, 173.9; HRMS calcd for $C_{19}H_{22}O_7Na$: MNa$^+$, 385.1263. Found: MNa$^+$, 385.1274.

(4*R*,5*R*,7*S*)-4-Hydroxy-5-(1-methoxycarbonyl-ethoxy)-cyclohex-1-enecarboxylic acid methyl ester 32. Benzoyl ester deprotection of **28** (95 mg, 0.26 mmol) was conducted in an identical manner to that described for the synthesis of **22**. Purification by column chromatography (eluent: 1:1 v/v petroleum ether/ethyl acetate) gave the desired alcohol **32** as a colourless oil (34 mg, 50%). R_F [1:1 v/v petroleum ether/ethyl acetate] = 0.31; $\nu_{max.}$ (ATR): 3477 (br acid O-H str), 1715, 1712 (C=O, str), 1652 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, d, *J* 6.8 Hz, CH_3), 2.19–2.84 (4H, m, H-3_{ax}, H-3_{eq}, H-6_{ax}, H-6_{eq}), 2.97 (1H, d, *J* 1.8 Hz, OH), 3.47 (1H, m, CH), 3.70 (3H, s, CO_2Me), 3.73 (3H, s, CO_2Me), 3.81 (1H, m, CH), 4.21 (1H, q, *J* 6.8 Hz, CH), 6.79 (1H, m, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.6, 29.5, 32.7, 51.7, 52.1, 68.9, 73.9, 79.2, 127.8, 136.3, 166.7, 173.9.

(4*R*,5*R*,7*S*)-5-(1-Carboxy-ethoxy)-4-hydroxy-cyclohex-1-enecarboxylate 12. Diester **32** (26 mg, 0.10 mmol) was deprotected using an identical procedure to that described for the synthesis of **10** to afford the desired di-carboxylate **12** as a white solid (0.30 mmol, quant., as determined by ^{1}H NMR spectroscopic studies using TSP as an internal standard). ^{1}H NMR (400 MHz, D_2O) δ 1.28 (3H, d, *J* 6.8 Hz, CH_3), 2.09–2.70 (4H, m, H-3_{ax}, H-3_{eq}, H-6_{ax}, H-6_{eq}), 3.42 (1H, ddd, *J* 5.6, 7.8, 16.1 Hz, CH), 3.74 (1H, ddd, *J* 5.8, 8.0, 16.0 Hz, CH), 4.09 (1H, q, *J* 6.8 Hz, CH), 6.39 (1H, m, H-2); ^{13}C NMR (125 MHz, D_2O) δ 18.3, 29.4, 31.5, 68.1, 76.1, 77.8, 131.2, 132.4, 175.8, 182.1; HRMS calcd for $C_{10}H_{13}O_6K_2$: MH$^+$, 306.9981. Found: MH$^+$, 306.9975.

(1*R*,6*R*,7*R*)-Benzoic acid 4-methoxycarbonyl-6-(1-methoxycarbonyl-ethoxy)-cyclohex-3-enyl ester 29. Hydrodebromination of **25** (0.21 g, 0.47 mmol) was conducted in an identical manner to that described for the synthesis of **19**. The product was purified by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) to afford the desired triester **29** as a pale yellow oil (0.10 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, d, *J* 6.8 Hz, CH_3), 2.39–2.92 (4H, m, H-2_{ax}, H-2_{eq}, H-5_{ax}, H-5_{eq}), 3.64 (3H, s, CO_2Me), 3.74 (3H, s, CO_2Me), 3.92 (1H, dd, J H-6), 4.21 (1H, q, *J* 6.8 Hz, CH), 5.35 (1H, dd, *J* 4.7, 10.6 Hz, H-1), 6.92 (1H, m, H-3), 7.40 (2H, m, 2 × *m*-ArH), 7.59 (1H, m, *p*-ArH), 8.03 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.1, 27.0, 28.4, 51.7, 51.9, 68.8, 73.1, 73.8, 126.7, 128.3, 129.6, 130.0, 133.0, 136.0, 165.6, 167.0, 173.2.

(4*R*,5*R*,7*R*)-4-Hydroxy-5-(1-methoxycarbonyl-ethoxy)-cyclohex-1-enecarboxylic acid methyl ester 33. Benzoyl ester deprotection of **29** (80 mg, 0.22 mmol) was conducted in an identical manner to that described for the synthesis of **22**. Purification by column chromatography (eluent: 1:1 v/v petroleum ether/ethyl acetate) gave the desired alcohol **33** as a colourless oil (20 mg, 35%). R_F [1:1 v/v petroleum ether/ethyl acetate] = 0.36; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, d, *J* 6.9 Hz, CH_3), 2.12–2.92 (4H, m, H-3_{ax}, H-3_{eq}, H-6_{ax}, H-6_{eq}), 3.32 (1H, ddd, J 4.0, 9.5, 18.9 Hz, CH), 3.71 (3H, s, CO_2Me), 3.76 (3H, s, CO_2Me), 3.79 (1H, m, CH), 4.10 (1H, s, OH), 4.18 (1H, q, *J* 6.9 Hz, CH), 6.81 (1H, m, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.4, 29.6, 33.2, 51.8, 52.4, 68.9, 73.6, 80.5, 127.3, 137.0, 166.7, 174.7; HRMS calcd for $C_{12}H_{19}O_6$: MH$^+$, 259.1182. Found: MH$^+$, 259.1183.

(4*R*,5*R*,7*R*)-5-(1-Carboxy-ethoxy)-4-hydroxy-cyclohex-1-enecarboxylate 13. Diester **33** (14 mg, 0.05 mmol) was deprotected using an identical procedure to that described for the synthesis of **10** to afford the desired di-carboxylate **13** as a white solid (0.05 mmol, quant., as determined by ^{1}H NMR spectroscopic studies using TSP as an internal standard). $\nu_{max.}$ (ATR): 3329 (br acid O-H str), 1657, 1604 (C=O, str), 1553 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 1.29 (3H, d, *J* 6.9 Hz, CH_3), 2.03–2.75 (4H, m, H-3_{ax}, H-3_{eq}, H-6_{ax}, H-6_{eq}), 3.47 (1H, ddd, *J* 5.6, 8.3, 17.1 Hz,

CH), 3.77 (1H, ddd, *J* 6.0, 8.3, 17.0 Hz CH), 4.05 (1H, q, *J* 6.9 Hz, CH), 6.38 (1H, m, H-2); ^{13}C NMR (125 MHz, D_2O) δ 19.0, 30.1, 32.0, 68.6, 76.2, 79.0, 131.3, 132.3, 175.7, 182.0; HRMS calcd for $C_{10}H_{13}O_6K_2$: *M*H$^+$, 306.9981. Found: MH$^+$, 306.9978.

(1*S*,2*S*,6*R*)-Benzoic acid-2-bromo-4-methoxycarbonyl-6-(2-methoxycarbonyl-allyloxy)-cyclohex-3-enyl ester 26. Alcohol **17**[14] (446 mg, 1.62 mmol) was dissolved in acetonitrile (20 ml) and the reaction cooled to 0 °C. Methyl-2-(bromomethyl)acrylate (250 μl, 2.10 mmol) was added dropwise at 0 °C before sodium hydride (64 mg, 1.62 mmol, 60% dispersion in oil) was added in three separate portions over a 30 min period. The reaction was stirred at 22 °C for 16 h at which point the solvent was removed *in vacuo* at 30 °C. The product was purified by column chromatography (eluent: 3:2 v/v petroleum ether/ethyl acetate) to afford the desired benzylidene acetal 26 as a 3:1 mixture of inseparable diastereoisomers as a colourless oil (543 mg, 89%). $\nu_{max.}$ (NaCl plate): 2952, 2908 (Ar C-H stretch), 1738 (C=O, str), 1655 (C=C), 1442, 1437 (C=C, Ar) cm^{-1}; LCMS [M + H]$^+$ 375.2, R_t 4.2 min (br); HRMS calcd for $C_{20}H_{22}O_7Na$: *M*Na$^+$, 397.1263. Found: MNa$^+$, 397.1252.

Major Diastereoisomer. (67% based on NMR integrals); R_F [3:2 v/v petroleum ether:ethyl acetate] = 0.76; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45–2.52 (1H, m, H-6$_a$), 2.72–2.77 (1H, m, H-6$_b$), 3.74 (3H, s, CO_2Me), 3.78 (3H, s, CO_2Me), 3.95 (1H, ddd, *J* 4.2, 5.7, 11.4 Hz, H-5), 4.30 (2H, s, CH_2), 4.43 (1H, t, *J* 6.0 Hz, H-4), 4.92 (1H, m, H-3), 5.87 (1H, m, C=C*H*H) 5.90 (1H, s, CHPh), 6.28 (1H, dd, *J* 1.2, 2.6 Hz, C=CH*H*), 6.90 (1H, m, H-2), 7.33–7.46 (5H, m, 5 × ArH).

Minor diastereoisomer. (22% based on NMR integrals); R_F [3:2 v/v petroleum ether:ethyl acetate] = 0.66; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32–2.41 (1H, m, H-6$_a$), 2.77–2.82 (1H, m, H-6$_b$), 3.73 (3H, s, CO_2Me), 3.76 (3H, s, CO_2Me), 3.75 (1H, m, H-5), 4.30 (2H, s, CH_2), 4.30 (1H, t, *J* 6.0 Hz, H-4), 4.81 (1H, m, H-3), 5.87 (1H, m, C=C*H*H), 5.91 (1H, s, CHPh), 6.23 (1H, dd, *J* 1.2, 2.6, C=CH*H*), 6.97 (1H, m, H-2), 7.33–7.46 (5H, m, 5 × ArH).

A solution of the above diastereomeric mixture (436 mg, 1.17 mmol), *N*-bromosuccinimide (228 mg, 1.28 mmol) and AIBN (2 mg, 0.01 mmol) in benzene (30 ml) was heated at reflux (85 °C) for 1.5 h. The reaction was allowed to cool to 22 °C before dilution with dichloromethane (20 ml). The reaction mixture was washed with saturated sodium carbonate solution (40 ml), water (40 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to afford the crude product as a pale yellow oil. Purification by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) gave the desired bromide **26** as a colourless oil (87 mg, 16%). R_F [3:1 v/v petroleum ether:ethyl acetate] = 0.59; $\nu_{max.}$ (NaCl plate): 3063, 2952, 2875 (Ar C-H stretch), 1730 (C=O, str), 1643 (C=C), 1602, 1583, 1435 (C=C, Ar) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 (1H, m, H-5$_a$), 2.94 (1H, m, H-5$_b$), 3.61 (3H, s, CO_2Me), 3.77 (3H, s, CO_2Me), 3.83 (1H, m, H-2), 4.20 (1H, td, *J* 1.5, 13.9 Hz, C*H*H), 4.35 (1H, td, *J* 1.5, 13.9 Hz, CH*H*), 4.81 (1H, ddd, *J* 1.8, 3.2, 6.5 Hz, H-6), 5.69 (1H, dd, *J* 6.5, 8.4 Hz, H-1), 5.71 (1H, d, *J* 1.6 Hz, C=C*H*H), 6.14 (1H, d, *J* 1.6 Hz, C=CH*H*), 6.94 (1H, ddd, *J* 1.1, 2.4, 3.3 Hz, H-3), 7.42 (2H, m, 2 × *m*-ArH), 7.56 (1H, m, *p*-ArH), 8.01 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.3, 45.0, 52.1, 52.5, 68.5, 74.1, 75.4, 126.7, 128.9, 129.4, 129.9, 130.2, 133.7, 136.2, 136.9, 165.7, 166.3, 166.4; HRMS calcd for $C_{20}H_{22}O_7Br$: *M*H$^+$, 453.0543. Found: MH$^+$, 453.0537.

(1*R*,6*R*)-Benzoic acid-4-methoxycarbonyl-6-(2-methoxycarbonyl-allyloxy)-cyclohex-3-enyl ester 30

Hydrodebromination of **26** (410 mg, 0.90 mmol) was conducted in an identical manner to that described for the synthesis of **19**. The product was purified by column chromatography (eluent: 3:1 v/v petroleum ether/ethyl acetate) to afford the desired triester **30** as a colourless yellow oil (120 mg, 35%). R_F [4:1 petroleum ether:ethyl acetate] = 0.29; $\nu_{max.}$ (NaCl plate): 2952, (Ar C-H stretch), 1715 (C=O, str), 1655 (C=C), 1601, 1437 (C=C, Ar) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39–2.84 (4H, m, H-5$_{ax}$, H-5$_{eq}$, H-2$_{ax}$, H-2$_{eq}$), 3.69 (3H, s, CO_2Me), 3.74 (3H, s, CO_2Me), 3.92 (1H, dd, *J* 5.1, 11.5 Hz, H-6), 4.29 (1H, d, *J* 13.9 Hz, C*H*H), 4.37 (1H, d, *J* 13.9 Hz, CH*H*), 5.35 (1H, dd, *J* 4.9, 11.5 Hz, H-1), 5.82 (1H, d, *J* 1.2 Hz, C=C*H*H), 6.22 (1H, d, *J* 1.2 Hz, C=CH*H*), 6.90 (1H, m, H-3), 7.41 (2H, m, 2 × *m*-ArH), 7.52 (1H, m, *p*-ArH), 7.97 (2H, m, 2 × *o*-ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 28.3, 51.1 (x 2), 67.2, 68.5, 73.2, 125.5, 126.5, 127.8, 129.0, 129.4, 132.5, 135.0, 136.5, 165.2, 165.5, 166.4; HRMS calcd for $C_{20}H_{22}O_7Na$: *M*Na$^+$, 397.1263. Found: MNa$^+$, 397.1266.

(4*R*,5*R*)-4-Hydroxy-5-(2-methoxycarbonyl-allyloxy)-cyclohex-1-enecarboxylic acid methyl ester 34

Benzoyl ester deprotection of **30** (70 mg, 0.19 mmol) was conducted in an identical manner to that described for the synthesis of **22**. Purification by column chromatography (eluent: 1:1 v/v petroleum ether/ethyl acetate) gave the desired alcohol **34** as a colourless oil (30 mg, 54%). R_F (1:1 v/v petroleum ether/ethyl acetate) = 0.15; $\nu_{max.}$ (ATR): 3348 (br acid O-H str), 1713 (C=O, str), 1653 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20–2.96 (4H, m, H-3$_{ax}$, H-3$_{eq}$, H-6$_{ax}$, H-6$_{eq}$), 3.28 (1H, d, *J* 1.2 Hz OH), 3.47 (1H, m, CH), 3.73 (3H, s, CO_2Me), 3.79 (3H, s, CO_2Me), 3.80 (1H, m, CH), 4.29 (1H, d, *J* 12.2 Hz, C*H*H), 4.38 (1H, d, *J* 12.2 Hz, CH*H*), 5.88 (1H, dd, *J* 1.3, 9.8 Hz, C=C*H*H), 6.31 (1H, d, *J* 1.3 Hz, C=CH*H*), 6.83 (1H, m, H-2); HRMS calcd for $C_{13}H_{18}O_6Na$: *M*Na$^+$, 293.0996. Found: MNa$^+$, 293.0993; HRMS calcd for $C_{13}H_{19}O_6$: *M*H$^+$, 271.1176. Found: MH$^+$, 271.1177.

(4*R*,5*R*)-5-(2-Carboxy-allyloxy)-4-hydroxy-cyclohex-1-enecarboxylate 14

Diester **34** (11 mg, 0.04 mmol) was deprotected using an identical procedure to that described for the synthesis of **10** to afford the desired di-carboxylate **14** as a white solid (0.04 mmol, quant., as determined by ^{1}H NMR spectroscopic studies using TSP as an internal standard). $\nu_{max.}$ (ATR): 3331 (br acid O-H str), 1579 (C=O, str), 1400 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 2.56–2.79 (4H, m, H-3$_{ax}$, H-3$_{eq}$, H-6$_{ax}$, H-6$_{eq}$), 3.67 (1H, ddd, *J* 5.6, 7.7, 15.8 Hz, H-4), 3.92 (1H, ddd, *J* 5.9, 7.7, 14.0 Hz, H-5), 4.33 (1H, d, *J* 12.7 Hz, C*H*H), 4.38 (1H, d, *J* 12.7 Hz, CH*H*), 5.63 (1H, d, *J* 1.1 Hz, C=C*H*H), 5.91 (1H, d, *J* 1.1 Hz, C=CH*H*), 6.42 (1H, m, H-2); ^{13}C NMR (125 MHz, D_2O) δ 29.4, 31.4, 68.2, 69.5, 78.0, 122.8, 131.3, 132.2, 142.6, 174.9, 175.8; HRMS calcd for $C_{11}H_{12}O_6K_3$: *M*K$^+$, 356.9540. Found: MK$^+$, 356.9536.

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