Elucidation of the catalytic mechanisms of the non-haem iron-dependent catechol dioxygenases: synthesis of carba-analogues for hydroperoxide reaction intermediates

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The catalytic mechanisms of the non-haem iron-dependent intradiol and extradiol catechol dioxygenases are thought to involve transient hydroperoxide reaction intermediates, formed by reaction of a catechol substrate with dioxygen. The synthesis of carba-analogues of these intermediates is described in which the hydroperoxide functional group (–OOH) is replaced by a hydroxymethyl group ($–CH_2OH$), and the cyclohexadienone skeleton simplified to a cyclohexanone. Analogues of the "proximal" hydroperoxide in which the hydroxymethyl group was positioned axially with respect to the ring were found to act as reversible competitive inhibitors (K_i 0.7–7.6 mM) for the extradiol enzyme 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB) from *Escherichia coli*, whereas analogues in which the hydroxymethyl group was positioned equatorially showed no inhibition. In contrast, assays *versus* the intradiol-cleaving protocatechuate 3,4-dioxygenase from *Pseudomonas* sp. showed inhibition only by an analogue containing an equatorial hydroxymethyl group (IC_{50} 9.5 mM). These data support the existence of a proximal hydroperoxide intermediate in the extradiol catechol dioxygenase mechanism, and suggest that the conformation adopted by the hydroperoxide reaction intermediate may be an important determinant in the reaction specificity of the extradiol and intradiol dioxygenases.

Introduction

The catechol dioxygenase family of enzymes catalyses the oxidative cleavage of catechol and substituted catechols which lie on catabolic pathways for the bacterial degradation of aromatic compounds.[1] The intradiol dioxygenases catalyse the cleavage of the bond situated between the two catechol hydroxy groups, utilising non-haem iron(III) as cofactor, whereas the extradiol dioxygenases catalyse the cleavage of the bond adjacent to the two hydroxy groups, utilising non-haem iron(II) as cofactor. The three-dimensional structures of three extradiol catechol dioxygenases, 2,3-dihydroxybiphenyl 1,2-dioxygenase (BphC) from *Pseudomonas* LB400,[2] catechol 2,3-dioxygenase (XylE) from *Pseudomonas putida* mt-2,[3] and protocatechuate 4,5-dioxygenase (LigAB) from *Sphingomonas paucimobilis*[4] have been determined, and in each case the mononuclear iron(II) centre is ligated by two histidine ligands and one glutamic acid ligand. In contrast the mononuclear iron(III) centre of the intradiol-cleaving protocatechuate 3,4-dioxygenase from *Pseudomonas aeruginosa* is ligated by two histidine ligands and two tyrosine ligands.[5] The choice of reaction pathway, intradiol *versus* extradiol, and its relationship to the oxidation state and coordination state of the metal centre, is therefore an intriguing mechanistic problem.

Previous studies in our laboratory have focussed on the reaction mechanism of the extradiol enzyme 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB) from *Escherichia coli*.[6] Mechanistic studies using substrates containing cyclopropyl radical traps have established that, following the ligation of the catechol and dioxygen by the iron(II) centre, the catalytic mechanism proceeds *via* single electron transfers to give a semiquinone–iron(II)–superoxide intermediate.[7] C–O bond formation could then take place to give two possible hydroperoxide intermediates, either a C-1 hydroperoxide (distal to the catechol oxygens) or a C-2 hydroperoxide (proximal to the catechol oxygens). ^{18}O-Labelling studies have established the existence of a seven-membered lactone intermediate, formed by Criegee rearrangement of the hydroperoxide: either *via* acyl migration of the distal hydroperoxide, or *via* alkenyl migration of the proximal hydroperoxide.[8] The reaction is completed by attack of iron(II) hydroxide upon the lactone to give the extradiol ring fission product.[8]

The present study concerns the choice of proximal *vs.* distal hydroperoxide in the catalytic mechanism of MhpB (Fig. 1). The prior observation that substrate analogues containing either $–OCH_2CO_2H$ or $–CH{=}CHCO_2H$ sidechains at C-1 were processed at comparable rates by MhpB disfavoured the distal hydroperoxide,[7] however since there are literature examples of both acyl migration and alkenyl migration in hydroperoxide rearrangements,[1] a more direct probe for the hydroperoxide intermediate was required.

The approach described in this paper is the synthesis of carba-analogues of each hydroperoxide in which the peroxide functional group (–OOH) is replaced by a hydroxymethyl group ($–CH_2OH$), and the cyclohexadienone skeleton simplified to a cyclohexanone. Although the latter skeleton is more puckered in conformation than a cyclohexadienone, the incorporation of suitable substituents on the cyclohexane ring would allow an investigation of the preferred conformation of the hydroperoxide in the active site: axial with respect to the ring, or equatorial. It was anticipated that the optimal conformation of

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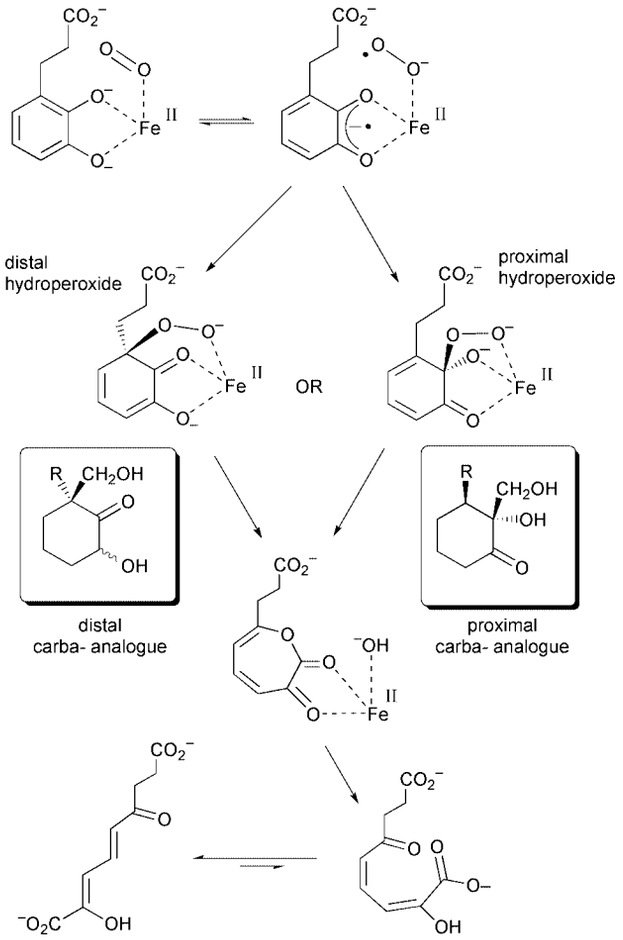


Fig. 1 Proposed catalytic mechanisms for 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB), illustrating the proximal and distal hydroperoxide intermediates and their respective carba-analogues.

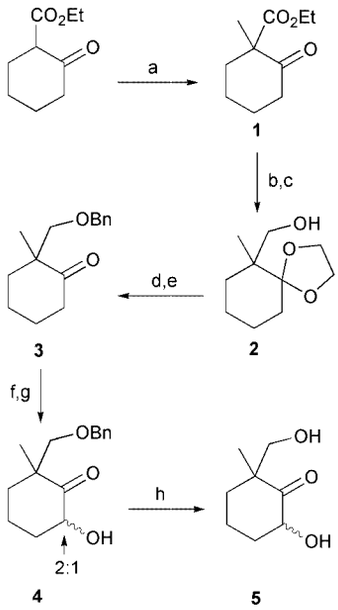
the proximal hydroperoxide for alkenyl migration might be one in which the hydroperoxide group was positioned axially with respect to the ring, thus aligning the migrating C–C bond antiperiplanar to the O–O bond being broken, a stereoelectronic effect which has recently been supported experimentally.[9] We describe the synthesis of carba-analogues of proximal and distal hydroperoxides, an analysis of their conformational preference, and their interaction with extradiol and intradiol dioxygenase enzymes.

Results

Synthesis of carba-analogue of distal hydroperoxide

A carba-analogue for the distal hydroperoxide, containing a methyl sidechain, was synthesised as shown in Scheme 1. 2-Ethoxycarbonylcyclohexanone was alkylated at C-2 by methyl iodide, using potassium *tert*-butoxide and phase transfer conditions,[10] to give (**1**) in 97% yield. The ketone group was protected as its ethylene ketal, in 63% yield, and the ester group reduced to alcohol (**2**) using lithium aluminium hydride, in 90% yield. Protection of the primary alcohol as its benzyl ether and deprotection of the ketal proceeded in 53% overall yield to give ketone (**3**). The Davis oxaziridine method[11] was used to carry out the α-hydroxylation of ketone (**3**), giving the α-hydroxy ketone (**4**) as a 2:1 mixture of diastereoisomers, in 62% yield. Attempts to separate the diastereoisomers by chromatography were unsuccessful. Deprotection of the benzyl ether proceeded in 85% yield to give analogue (**5**) as a 2:1 mixture of diastereoisomers.

Analogue (**5**) was assayed against dioxygenase MhpB, as described in the Experimental section, but showed no observable enzyme inhibition at 10 mM concentration.



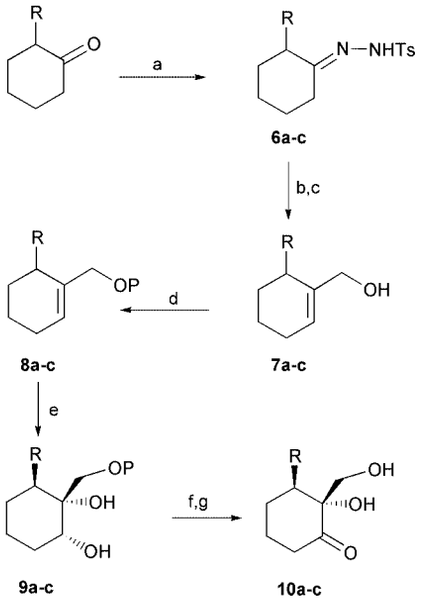
Scheme 1 Synthetic route for distal carba-analogue (**5**). *Reagents and yields*: a, *t*-BuOK–MeI, Aliquat 336, 97%; b, $HO(CH_2)_2OH$, H^+, 63%; c, $LiAlH_4$, THF, 90%; d, NaH, BnBr, THF, 61%; e, H^+–acetone–H_2O, 87%; f, KHMDS, THF, −78 °C; g, PhCH(O)NTs, 62% overall; h, H_2–Pd–C, 85%.

Synthesis of carba-analogues of proximal hydroperoxide

A series of carba-analogues for the proximal hydroperoxide, containing methyl, *tert*-butyl or phenyl sidechains, were synthesised using the route shown in Scheme 2. The commercially available 2-substituted cyclohexanones were converted to the corresponding tosylhydrazones (**6a**–**c**), in 90–99% yield. The tosylhydrazones were then subjected to a Shapiro lithiation procedure:[12] treatment with *n*-butyllithium at −78 °C generated in each case the intermediate vinyllithium species, which was reacted with paraformaldehyde to give the allylic alcohols (**7a**–**c**) regioselectively in 31–55% yield. The modest yields are attributed to the sluggish reaction of paraformaldehyde, since other carbonyl reagents were found to react in higher yield (data not shown).

The primary alcohol functional group was then protected, either with a *tert*-butyldimethylsilyl group (to give **8a** in 93% yield) or with a methoxymethyl protecting group (to give **8b**,**c** in 81–85% yield). When bulky sidechains were present the MOM group was found to be superior in this sequence to the TBDMS group, the latter giving low yields in the subsequent dihydroxylation step, presumably due to increased steric hindrance. Dihydroxylation of the alkene functional group was then carried out using the conditions of Minato *et al.*,[13] employing 1–5 mol% K_2OsO_4 in the presence of $K_3Fe(CN)_6$ and $MeSO_2NH_2$. Reaction occurred selectively on the face opposite the R substituent, although as mentioned above the reaction of this alkene was sluggish and highly sensitive to steric bulk. The methyl analogue (**9a**) was isolated in 96% yield as a 7:3 diastereomeric mixture after 24 hours at room temperature, whereas only a 40% yield of the *tert*-butyl analogue (**9b**) was obtained after 7 days, and a 25% yield of the phenyl analogue (**9c**) was obtained, in each case as a single diastereoisomer. Synthesis of the desired analogues (**10a**–**c**) was completed by oxidation of the secondary alcohol, using either the Dess–Martin oxidation[14] or the Swern oxidation[15] conditions, followed by deprotection of the primary hydroxy group (yields given in Scheme 2).

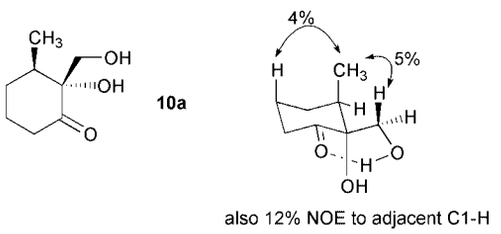
Analysis of analogue **10a** (R = Me) by NMR spectroscopy in $CDCl_3$ revealed an unexpected conformational preference. The methyl sidechain was found to adopt an axial position, as shown by analysis of coupling constants and by measurement of NOE's from the $–CH_3$ group to the $–CH_2OH$ sidechain (5%)



Scheme 2 Synthetic route for proximal carba-analogues **10a** (R = CH_3), **10b** (R = *t*-Bu), and **10c** (R = Ph). P = protecting group: either TBDMS or MOM. *Reagents and yields* shown below:

	R = CH_3	R = *t*-Bu	R = Ph
a, $TsNHNH_2$, MeOH, H^+	**6a** 99%	**6b** 92%	**6c** 73%
b, *n*-BuLi, THF, −78 °C; c, CH_2O	**7a** 53%	**7b** 31%	**7c** 43%
P = TBDMS: d, TBDMSCl, DMAP, DMF	**8a** 93%	—	—
P = MOM: d, $CH_2(OMe)_2$, LiBr–TsOH	—	**8b** 85%	**8c** 81%
e, K_2OsO_4–$K_3Fe(CN)_6$, $MeSO_2NH_2$, *t*-BuOH–H_2O	**9a** 96%	**9b** 40%	**9c** 25%
P = TBDMS: f, Swern ox.; g, TBAF, THF	**10a** 30%	—	—
P = MOM: f, Dess–Martin ox.; g, HCl–MeOH	—	**10b** 35%	**10c** 43%

and to a C-5 axial ring hydrogen (4%). This conformation is stabilised by the formation of an intramolecular hydrogen bond between the primary alcohol of the sidechain and the C-3 ketone group. In contrast, analogues **10b** (R = *t*-Bu) and **10c** (R = Ph) were found by NMR spectroscopy to adopt the expected conformation in which the R sidechain is situated equatorially, and the hydroxymethyl group axially. This assignment was confirmed by determination of X-ray crystal structures for single crystals of **10b** and **10c**, which are shown in Fig. 2.



also 12% NOE to adjacent C1-H

Assay of analogues **10a**–**c** *versus* dioxygenase MhpB revealed that **10a** showed no inhibition at 10 mM concentration, whereas **10b** and **10c** both acted as reversible inhibitors, with K_i values of 4.9 mM and 0.7 mM respectively (see Table 1). Kinetic analysis using a Dixon plot[16] showed in each case that inhibition was competitive with respect to the natural substrate 2,3-dihydroxyphenylpropionic acid (see Fig. 3). Although the K_i values are significantly higher than the K_m for its natural substrate (K_m 26 μM for 2,3-dihydroxyphenylpropionic acid[6]), the MhpB active site does appear to recognise the carba-analogues of the proximal hydroperoxide in which the hydroxymethyl group is positioned axially with respect to the ring.

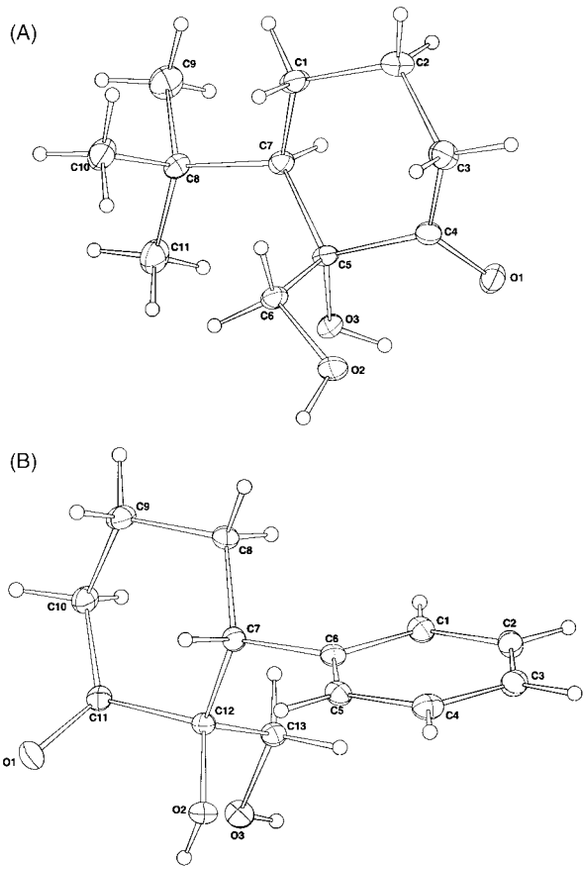


Fig. 2 X-Ray crystal structures of carba-analogues **10b** (A) and **10c** (B).

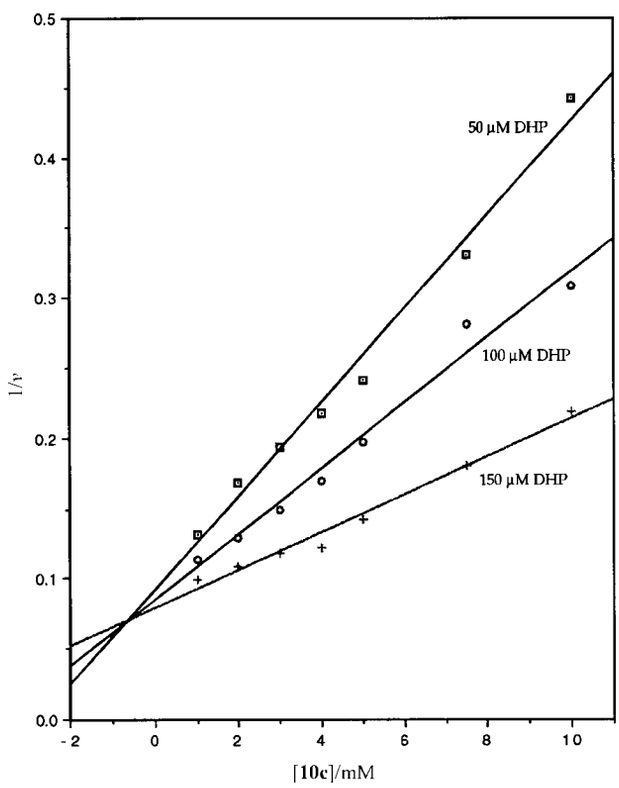
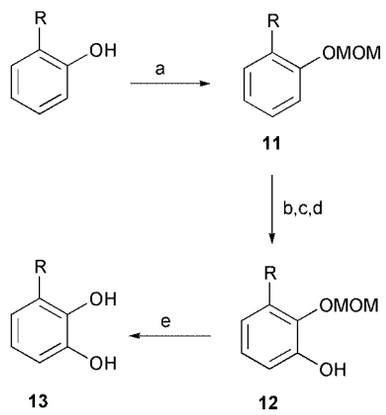


Fig. 3 Dixon plot[16] showing inhibition of *E. coli* 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB) by carba-analogue **10c**. Assays were carried out at 50, 100, and 150 μM concentrations of 2,3-dihydroxyphenylpropionic acid (DHP), as indicated. The point of intersection of the lines gives a K_i value of 0.7 mM.

Analogues **10a–c** were incubated for extended periods of time with MhpB, in order to examine whether any enzyme-catalysed reactions might take place (*e.g.* pinacol rearrangement reactions), however no new characterisable peaks were found by HPLC analysis.

The effect of the steric bulk of the *tert*-butyl and phenyl sidechains on substrate binding was investigated by synthesis of the corresponding catechol substrates, 3-*tert*-butylcatechol and 3-phenylcatechol. This was carried out by an ortho-lithiation strategy reported concurrently to this work by Snieckus *et al.*,[17] as shown in Scheme 3. Ortho-lithiation of MOM-protected



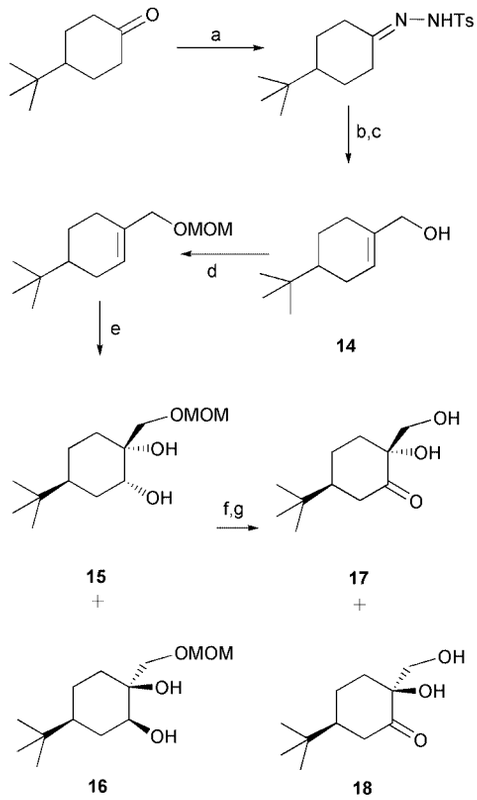
Scheme 3 Synthetic route of 3-substituted catechols **13a** (R = Ph) and **13b** (R = *t*-Bu). *Reagents and yields* (for **13b**): a, CH_3OCH_2Cl, NaH, THF, 89%; b, *n*-BuLi, THF, −78 °C; c, $B(OMe)_3$; d, Oxone, 65% overall; e, H^+–H_2O, 82%.

phenols (**11a/b**) was found to proceed smoothly using *n*-butyllithium as base, and the resulting alkyllithiums were trapped by trimethyl borate. Oxidation of the boronic acids to phenols (**12a/b**) was achieved best using Oxone in aqueous acetone–$NaHCO_3$, a reagent which in our hands was superior to aqueous hydrogen peroxide. The catechols (**13a/b**) were assayed against MhpB, and 3-phenylcatechol (**13a**) was found to be a good substrate (K_m 78 μM), indicating that a phenyl substituent can be accommodated at the enzyme active site. The 3-*tert*-butylcatechol (**13b**) was found to be very unstable towards air oxidation at pH 7.5–8.0 in aqueous solution, thus a reliable K_m determination was not possible.

Analogues were also synthesised in which the alkyl substituent was situated *para* with respect to the hydroxymethyl substituent, instead of *ortho*. The synthetic strategy was the same as that used for synthesis of analogues **10a–c**, but the starting material in this case was 4-*tert*-butylcyclohexanone. Synthetic steps and yields are shown in Scheme 4. Shapiro reaction with formaldehyde proceeded in 26% yield to give alcohol **14**, and after MOM protection the dihydroxylation procedure as above gave a mixture of two diastereoisomers **15** and **16**, in a 1:1 ratio, which were separated by careful silica column chromatography. Oxidation separately gave the *syn* (**17**) and *anti* (**18**) carba-analogues with the hydroxymethyl group axial and equatorial respectively. When assayed *versus* MhpB, analogue **17** showed competitive reversible inhibition (K_i 7.6 mM), whereas analogue **18** showed no enzyme inhibition at 10 mM concentration (see Table 1). These data are entirely consistent with the results obtained for **10a–c**, in that only those analogues containing an axial hydroxymethyl group showed inhibition of MhpB.

Synthesis and assay of a hydroxyethyl carba-analogue

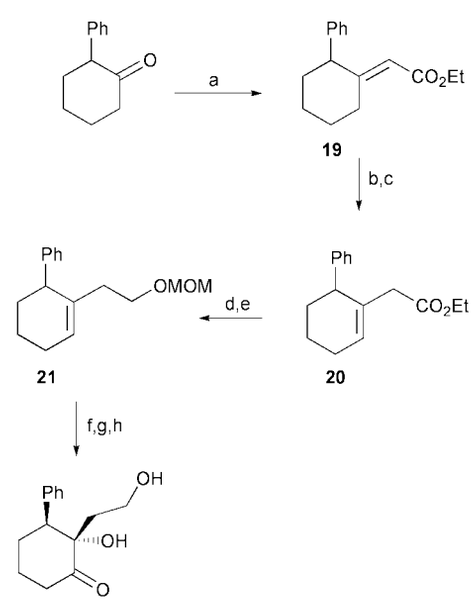
One possible explanation for the relatively low affinity of the above carba-analogues is that the hydroxymethyl substituent (–CH_2OH) is shorter in length than the putative hydroperoxide intermediate (–OOH). Consequently, it is conceivable that the hydroxymethyl group is not of sufficient length to form an



Scheme 4 Synthetic route for carba-analogues **17** and **18**. *Reagents and yields*: a, $TsNHNH_2$, MeOH, H^+, 99%; b, *n*-BuLi, THF, −78 °C; c, CH_2O, 26% overall; d, $CH_2(OMe)_2$, LiBr–TsOH, 81%; e, K_2OsO_4–$K_3Fe(CN)_6$, $MeSO_2NH_2$, *t*-BuOH–H_2O, 97%; f, Dess–Martin ox.; g, HCl–MeOH, 74–79% overall.

effective bridge to the non-haem iron centre at the dioxygenase active site. In order to investigate this hypothesis, a further analogue was synthesised, containing an extended sidechain (–CH_2CH_2OH).

The synthetic strategy used for this compound was a Horner–Wadsworth–Emmons reaction on the 2-substituted cyclohexanone, as shown in Scheme 5. This reaction failed with 2-*tert*-butylcyclohexanone, but proceeded in 75% yield



Scheme 5 Synthetic route for extended carba-analogue **22**. Reagents and yields: a, $(EtO)_2POCH_2CO_2Et$, NaH, THF, 75%; b, LDA, THF, −78 °C; c, NH_4Cl–H_2O, 85% overall; d, $LiAlH_4$, 96%; e, MOMCl, i-Pr_2NEt, CH_2Cl_2, 94%; f, K_2OsO_4–$K_3Fe(CN)_6$, $MeSO_2NH_2$, *t*-BuOH–H_2O, 98%; f, Dess–Martin ox.; g, HCl–MeOH, 42% overall.

Table 1 Inhibition of *E. coli* 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB) and *Pseudomonas* sp. protocatechuate 3,4-dioxygenase (3,4-PCD) by carba-analogues. Assays were carried out as described in the Experimental section. K_i values were determined using a Dixon plot. K_m values: MhpB 26 μM (2,3-dihydroxyphenylpropionic acid); 3,4-PCD 20 μM (protocatechuic acid). NA = not assayed

Compound	Hydroperoxide mimic	Sidechain	MhpB K_i (mM)	3,4-PCD IC_{50} (mM)
5	Distal $-CH_2OH$	2-CH_3	No inhibition	NA
10a	Proximal $-CH_2OH_{eq}$	2-CH_3	No inhibition	No inhibition
10b	Proximal $-CH_2OH_{ax}$	2-*t*-Bu	4.9	No inhibition
10c	Proximal $-CH_2OH_{ax}$	2-Ph	0.7	NA
17	Proximal $-CH_2OH_{ax}$	4-*t*-Bu	7.6	No inhibition
18	Proximal $-CH_2OH_{eq}$	4-*t*-Bu	No inhibition	9.5
22	Proximal $-CH_2CH_2OH_{ax}$	2-Ph	1.4	NA

with 2-phenylcyclohexanone to give the unsaturated ester **19**. Treatment with LDA at −78 °C effected the isomerisation to endocyclic 2,3-alkene (**20**). Only a small amount of the 1,2-alkene was observed in the reaction product (3–4% by NMR spectroscopy) when this reaction was carried out at −78 °C, which could be removed by careful column chromatography. Reduction of the ester by lithium aluminium hydride, followed by MOM protection of the primary alcohol, gave the MOM ether **21**. Dihydroxylation as before, followed by Dess–Martin oxidation and deprotection, gave the extended analogue **22**. Assays *versus* MhpB revealed that **22** was a reversible, competitive inhibitor with K_i 1.4 mM, of comparable affinity to the earlier series (see Table 1).

Assay of carba-analogues against an intradiol dioxygenase

Having observed some selectivity for binding by extradiol dioxygenase MhpB, it was of interest to examine whether the same, or different, selectivity was exhibited by an intradiol dioxygenase enzyme. Accordingly, a selection of analogues were tested as inhibitors of commercially available *Pseudomonas* sp. protocatechuate 3,4-dioxygenase. No inhibition was observed by the 2-methyl analogue **10a**, the 2-*tert*-butyl analogue **10b**, or the *syn* 4-*tert*-butyl analogue **17**, however inhibition was observed by the *anti tert*-butyl analogue **18** (IC_{50} 9.5 mM). Again the observed IC_{50} value is higher than the substrate K_m (K_m 20 μM determined for protocatechuic acid), however the selectivity for these analogues is markedly different to the selectivity found for MhpB (see Table 1).

Discussion

A series of carba-analogues for the putative proximal and distal hydroperoxide reaction intermediates have been synthesised and tested as inhibitors for extradiol and intradiol catechol dioxygenases. The observation of a selective interaction of these analogues with the respective enzymes gives experimental support and insight into the existence of hydroperoxide intermediates which to date have been presumed but unverified.

Although the binding affinity of these analogues is approximately 100-fold weaker than the observed K_m values for their natural substrates (see below), a clear pattern of enzyme inhibition is observed. Carba-analogues **10b**, **10c** and **17** of the proximal hydroperoxide in which the hydroxymethyl group was positioned axially with respect to the cyclohexane ring acted as reversible competitive inhibitors for MhpB, whereas analogue **5** of the distal hydroperoxide, and analogues **10a** and **18** of the proximal hydroperoxide in which the hydroxymethyl group is positioned equatorially show no inhibition. These observations provide experimental support for a proximal hydroperoxide intermediate in the MhpB catalytic mechanism, rather than a distal hydroperoxide, a conclusion which is consistent with earlier observations of the substrate specificity of MhpB.[7] The proximal hydroperoxide structure has also been observed in a model transition metal catechol–dioxygen adduct.[18]

The iron(III)-dependent intradiol dioxygenases also presumably access the same type of proximal hydroperoxide reaction

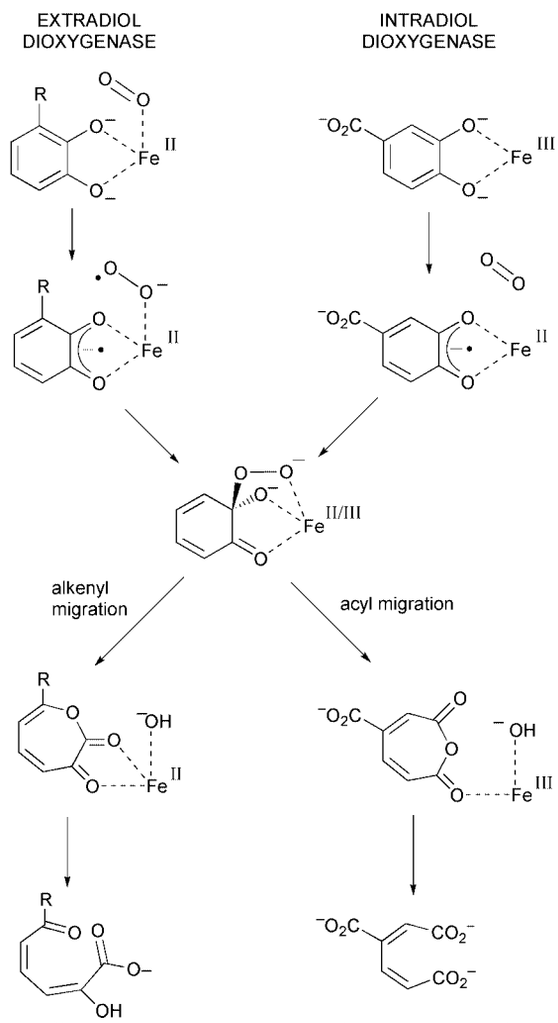


Fig. 4 Illustration of the convergence of the reaction mechanisms of the extradiol and intradiol catechol dioxygenases onto a similar proximal hydroperoxide intermediate, followed by divergence *via* alkenyl *vs.* acyl migration.

intermediate, but catalyse intradiol C–C cleavage to give an anhydride species.[1] This work therefore leads to the conclusion that the catalytic mechanisms of these two families of dioxygenase enzymes converge on a similar hydroperoxide intermediate, and subsequently diverge to give regiospecific oxidative cleavage (see Fig. 4). How then is the site of bond cleavage dictated in intradiol *vs.* extradiol enzymes?

The observed inhibition data suggest that there is a conformational preference of the MhpB active site, since **10b**, **10c** and **17** are recognised by the enzymes whereas **10a** and **18** are not. If one considers the Criegee rearrangement of the presumed proximal hydroperoxide intermediate on stereoelectronic grounds (Fig. 5), then one might predict that this species would be optimally aligned for O–O bond cleavage if the hydroperoxide functional group is positioned axially with respect to the cyclohexadienone ring. In this conformation the O–O bond is

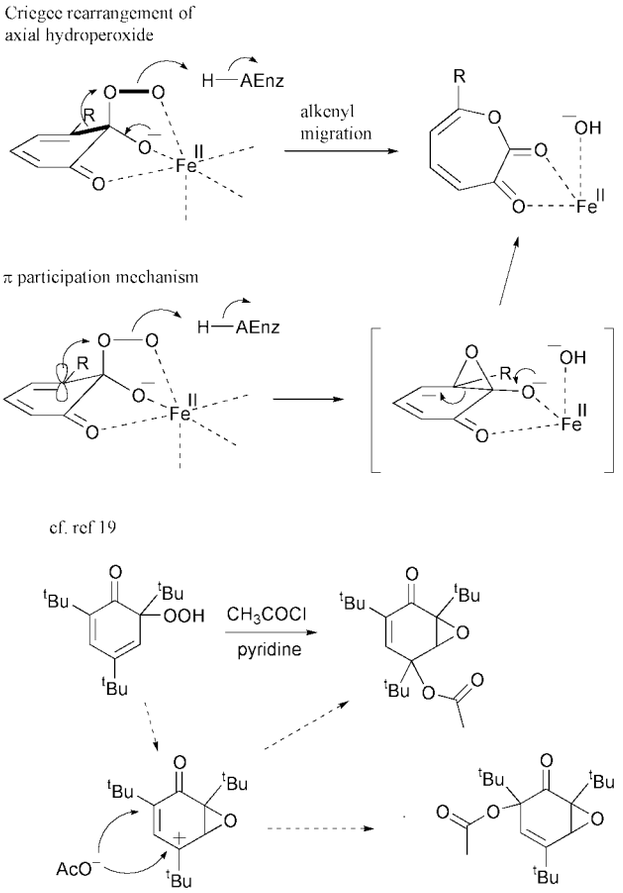


Fig. 5 Mechanisms for alkenyl migration of an axial proximal hydroperoxide intermediate, either *via* Criegee rearrangement (σ bond migration) or participation of diene π system. Literature precedent for the latter mechanism (see ref. 19) is illustrated.

aligned antiperiplanar with respect to the migrating C–C bond, an arrangement which is known to be favoured experimentally for the Criegee rearrangement.[9] It is therefore significant that only the carba-analogues containing an axial hydroxymethyl group inhibit MhpB, which provides some experimental support for the existence of this active conformation for extradiol cleavage.

In this conformation an alternative mechanism is also possible: the participation of the π electrons of the diene system, which would overlap with the σ* orbital of the O–O bond, to give a transient 1,2-epoxide containing an allylic carbocation, which could fragment with C–C cleavage to give the desired lactone (see Fig. 5). The latter π participation mechanism has some precedent in the rearrangement of a known cyclohexadienyl hydroperoxide,[19] and the rearrangements of lipid hydroperoxides,[20] to give epoxide products. Analysis of the X-ray crystal structure of the iridium(III) catechol model complex mentioned above, which contains a proximal hydroperoxide species, reveals that in this case the hydroperoxide functional group is also positioned axially with respect to the ring system.[18]

Assays of the carba-analogues *versus* the intradiol enzyme protocatechuate 3,4-dioxygenase from *Pseudomonas* sp. reveal a different selectivity, this enzyme being inhibited only by analogue **18** containing an equatorial hydroxymethyl group. Assuming that both families of enzyme access a proximal hydroperoxide intermediate, mimicked by the carba-analogues, these data suggest that the conformation adopted by the hydroperoxide intermediate may be an important determinant in the choice of reaction pathway. We speculate that the reaction specificity of the intradiol *vs.* extradiol dioxygenases may be controlled by the precise conformation in which the hydroperoxide intermediate is bound: an axial hydroperoxide leading to extradiol cleavage, and an equatorial hydroperoxide leading to intradiol cleavage.

Preliminary computational studies indicate that for a 6,6-disubstituted cyclohexa-2,4-dienone the energy barrier between these two extreme conformations is small (<2 kJ mol^{-1}), however if bound to an active site iron cofactor then the choice of conformation could be dictated by the coordination state of the metal centre. Thus, the extradiol iron(II) centre bound facially by three protein ligands would have three vacant coordination sites with which it could stabilise an axial hydroperoxide conformation. The intradiol iron(III) centre bound initially by four protein ligands might have only two vacant coordination sites, which might favour an equatorial hydroperoxide. Nearby active site amino acid sidechains also presumably contribute to the choice of extradiol *vs.* intradiol reaction pathways.

There are several possible explanations for the relatively weak binding observed for the carba-analogues. First is that the cyclohexane ring of the analogues is not as planar as the cyclohexadienone ring of the presumed reaction intermediate. The axial substituents on the cyclohexane ring may give rise to unfavourable binding interactions, although the observation that the 4-*tert*-butyl analogue **17** also inhibits MhpB suggests that the enzyme active site tolerates considerable substitution in this position at least. Second is that the length of the hydroxymethyl substituent may not effectively mimic the length of the hydroperoxide functional group. However, analogue **22** containing a hydroxyethyl group exhibited a very similar K_i value to hydroxymethyl analogue **10c** containing a hydroxymethyl group, suggesting that the length of the sidechain is not a major factor. Third (and perhaps most likely) is that the acidity of the alcohol functional groups of the carba-analogues (pK_a 15–16) does not match the acidity of the phenolic hydroxy groups of the natural substrate (pK_a 9–10) and the acidity of the hydroperoxide group (pK_a 12.8 for *t*-BuOOH[21]). In order to effectively ligate the active site iron(II) centre the ligand atoms should be deprotonated, for which active site histidine bases have been identified in the X-ray crystal structure of BphC.[2] The lower acidity of the alcohol functional groups in the analogues may dictate their relatively weak binding efficacy. Nevertheless, we note that these analogues are the first non-aromatic compounds found to inhibit the catechol dioxygenases.

In conclusion, these data support the existence of a proximal hydroperoxide reaction intermediate in the reaction mechanisms of both the extradiol and intradiol dioxygenases, raising the intriguing question of how the extradiol *vs.* intradiol selectivity is controlled by the two families of enzyme. This work provides experimental evidence that the MhpB active site shows a conformational preference for the binding of reaction intermediate analogues, hence providing a clue that an important factor in the reaction specificity of the extradiol *vs.* intradiol dioxygenases may be the conformation in which the hydroperoxide intermediate is bound at the respective enzyme active sites.

Experimental

General

Nuclear magnetic resonance spectra were recorded on a Bruker AM300 Fourier transform spectrometer (300 MHz). Ultraviolet–visible spectra were recorded on a Cary-1 UV–visible spectrophotometer. Infrared spectra were recorded on a 1600 series Perkin-Elmer FTIR spectrometer. Mass spectra were recorded on a VG-70-250 mass spectrometer in electron impact (EI) or chemical ionisation (CI) mode, or a VG Platform Quadrupole Electrospray Ionisation mass spectrometer (ES). 3-(2,3-Dihydroxyphenyl)propionic acid was prepared from 2,3-dimethoxycinnamic acid using a published procedure.[22] 2-(*p*-Tolylsulfonyl)-3-phenyl-1,2-oxaziridine was prepared by

the method of Davis.[11] Dess–Martin periodinane was prepared according to reference 14. All other chemicals were supplied by Sigma-Aldrich Chemical Co.

Ethyl 1-methyl-2-oxocyclohexan-1-carboxylate 1

To ethyl 2-oxocyclohexane-1-carboxylate (5.00 g, 29.38 mmol) was added Aliquat 336 (0.72 g, 1.76 mmol, 6 mol%). The mixture was then cooled (ice bath) and solid potassium *tert*-butoxide (3.30 g, 29.40 mmol, 1 eq.) added portionwise over 10 minutes with magnetic stirring. Methyl iodide (4.17 g, 29.40 mmol, 1 eq.) was then added slowly, and stirring was continued for a further 30 minutes. The mixture was then diluted with ethyl acetate (50 ml), filtered through a pad of Celite and the solvent removed *in vacuo*. The resultant yellow oil was further purified by short path distillation to afford **1** as a colourless oil (5.15 g, 28.0 mmol, 97%). R_f (40% EtOAc–petroleum ether) 0.57; IR (liquid film) 2938 (m), 2867 (m), 1711 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.20 (2H, q, $J = 7$ Hz, OCH_2CH_3), 2.46 (3H, m), 2.00 (1H, m), 1.70 (3H, m), 1.45 (1H, m), 1.29 (3H, s, CH_3), 1.26 (3H, t, $J = 7$ Hz, OCH_2CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 208.5, 173.2, 61.4, 57.3, 40.8, 38.4, 27.7, 22.8, 21.4, 14.2 ppm; *m/z* (EI) 184.0 (62%, M^+), 155.9 (82%, $[M - C_2H_5]^+$).

(6-Methyl-1,4-dioxaspiro[4.5]decan-6-yl)methanol 2

Ethylene glycol (158 g, 25.50 mmol) and *p*-TsOH (0.22 g, 1.16 mmol) were added to a solution of **1** (4.27 g, 23.20 mmol) in toluene (150 ml). The mixture was heated under reflux with azeotropic removal of water (Dean–Stark apparatus) for 16 hours. The mixture was washed with sat. sodium bicarbonate solution (20 ml), brine (20 ml), dried over anhydrous K_2CO_3 and the solvent removed *in vacuo*. The resultant oil was then purified by flash silica chromatography (EtOAc–hexane, 20 : 80) to afford the ethylene ketal as a colourless oil (3.36 g, 14.70 mmol, 63%). R_f (20% EtOAc–hexane) 0.32; IR (liquid film) 2935 (s), 2867 (s), 1721 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.18 (2H, q, $J = 7$ Hz, OCH_2CH_3), 3.95 (4H, m, OCH_2CH_2O), 1.35–2.15 (8H, m), 1.25 (3H, s, CH_3 and 3H, t, $J = 7$ Hz, OCH_2CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 175.0, 110.8, 65.5, 64.7, 60.4, 51.2, 34.8, 32.0, 23.5, 21.7, 19.2, 14.3 ppm; *m/z* (EI) 228.0 (38%, M^+), 182.9 (23%, $[M - C_2H_5O]^+$).

To a cooled (−60 °C acetone–dry ice) solution of $LiAlH_4$ (1.0 M in THF, 30 ml) in dry THF (50 ml) was added ethyl 6-methyl-1,4-dioxaspiro[4.5]decane-6-carboxylate (3.66 g, 16.09 mmol) in dry THF (50 ml) dropwise over 20 minutes with stirring. The solution was then stirred for a further 3 hours at −30 °C then diluted with ethyl acetate (50 ml). Saturated NH_4Cl solution (150 ml) was added and the resultant viscous solution warmed to room temperature. The solution was filtered through Celite and the filter cake washed with ethyl acetate (50 ml). The aqueous phase was then extracted with ethyl acetate (3 × 20 ml), and the combined organic phase washed with sat. $NaHCO_{3(aq)}$ (50 ml), brine (50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. The resultant oil was then purified by flash silica chromatography (40% EtOAc–hexane) to afford the product alcohol **2** as a colourless oil (2.68 g, 14.43 mmol, 90%). R_f (40% EtOAc–hexane) 0.31; IR (liquid film) 3495 (m, br), 2935 (s), 2864 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.98 (4H, m, OCH_2CH_2O), 3.62 (1H, d, $J = 11$ Hz, $CHOH$), 3.48 (1H, d, $J = 11$ Hz, $CHOH$), 2.80 (1H, s, OH), 1.30–1.80 (8H, m), 1.02 (3H, s, CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 113.9, 68.9, 64.7, 64.4, 41.9, 33.3, 30.6, 23.6, 20.6, 18.8 ppm; *m/z* (EI) 186.1 (4%, M^+), 159.9 (20%, $[M - CH_2O]^+$), 112.8 (82%, $[M - C_3H_5O_2]^+$).

2-Methyl-2-[(phenylmethoxy)methyl]cyclohexan-1-one 3

To a solution of NaH (0.78 g, 16.31 mmol, 1.2 eq.) in THF (20 ml) cooled to 0 °C was added a solution of **2** (2.52 g, 13.59 mmol) in THF (5 ml) dropwise over 10 minutes under N_2. Once the evolution of H_2 had ceased, benzyl bromide (4.65 g, 27.19 mmol, 2.0 eq.) and tetra-*n*-butylammonium iodide (0.15 g, 0.41 mmol, 3 mol%) in THF (10 ml) was added dropwise over 5 minutes. The solution was stirred for a further hour under N_2 then quenched with saturated NH_4Cl solution (30 ml), extracted with ethyl acetate (3 × 20 ml), washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The resultant oil was purified by flash silica chromatography (10% EtOAc–hexane) to afford the benzyl ether as a colourless oil (2.30 g, 8.34 mmol, 61%). R_f (10% EtOAc–hexane) 0.24; IR (liquid film) 2936 (s), 2868 (s), 1601 (w), 1583 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.20–7.40 (5H, m, Ar), 4.52 (2 H, 2 × d, $J = 12$ Hz, OCH_2Ph), 3.90 (4H, m, OCH_2CH_2O), 3.55 (1H, d, $J = 9$ Hz, $CHOBn$), 3.40 (1H, d, $J = 9$ Hz, $CHOBn$), 1.40–1.77 (8H, m), 1.09 (3H, s, CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 139.3, 128.4, 127.6, 127.4, 111.9, 74.1, 73.6, 65.1, 64.8, 43.4, 33.0, 31.2, 23.8, 20.9, 18.5 ppm; *m/z* (EI) 276.2 (8%, M^+), 185.0 (96%, $[M - C_7H_7]^+$).

To a solution of (6-methyl-1,4-dioxaspiro[4.5]decan-6-yl)-(phenylmethoxy)methane (2.15 g, 7.80 mmol) in acetone–water (90 : 10, 50 ml) was added a catalytic amount of toluene-*p*-sulfonic acid (10 mg, 0.04 mmol) and the mixture heated under reflux for 15 hours. The mixture was then diluted with diethyl ether (50 ml), washed with brine (20 ml), dried ($MgSO_4$), then filtered through a short pad of silica and the solvent removed *in vacuo* to afford the product ketone **3** as a colourless oil (1.57 g, 6.77 mmol, 87%). R_f (10% EtOAc–hexane) 0.31; IR (liquid film) 2938 (m), 2867 (m), 1701 (s), 1602 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.35 (5H, m, Ar), 4.55 (2H, 2 × d, $J = 12$ Hz, OCH_2Ph), 3.52 (2H, s, CH_2OBn), 2.40 (2H, m, CH_2CO), 1.65–1.95 (6H, m), 1.18 (3H, s, CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 214.6, 138.6, 128.5, 127.6, 75.5, 73.5, 49.8, 39.2, 36.5, 27.2, 21.3, 21.2 ppm; *m/z* (CI) 250 ($[M + NH_4]^+$); HRMS (CI) found $[M + NH_4]^+$, 250.1816. $C_{15}H_{24}O_2N$ requires 250.1807.

6-Hydroxy-2-(hydroxymethyl)-2-methylcyclohexan-1-one 5

Potassium hexamethyldisilazanide (0.5 M in THF, 12.93 ml, 6.46 mmol) was diluted with dry THF (100 ml) and purged with N_2. The solution was cooled to −78 °C (acetone–dry ice) and a solution of **3** (1.00 g, 4.31 mmol) in dry THF (10 ml) was added slowly. The reaction mixture was stirred for 30 minutes followed by dropwise addition of 2-(*p*-tolylsulfonyl)-3-phenyl-1,2-oxaziridine (1.77 g, 6.46 mmol, prepared by the method of Davis[11]) in dry THF (10 ml) over 10 minutes. The mixture was stirred for a further 2 hours then quenched with sat. $NH_4Cl_{(aq)}$ and allowed to warm to room temperature. The mixture was then extracted with ethyl acetate (50 ml), washed with sat. $NaHCO_3$ solution (50 ml), brine (50 ml), dried ($MgSO_4$) and concentrated to *ca.* 10 ml *in vacuo*. Addition of hexane (10 ml) precipitated by-product as a white crystalline solid which was removed by filtration. Removal of the remaining solvent and purification by flash silica chromatography (EtOAc–hexane, 10 : 90) afforded the crude product **4** as a pale yellow oil (0.66 g, 2.67 mmol, 62%). R_f 0.17 (EtOAc–petroleum ether, 10 : 90), which was used directly in the next experiment.

To a solution of 5% palladium on charcoal (20 mg) in degassed ethyl acetate (10 ml) under N_2 was added a solution of **4** (0.50 g, 2.01 mmol) in ethyl acetate (5 ml), and the solution was thoroughly degassed. The solution was then placed under a hydrogen atmosphere and stirred for 15 hours. The solution was then filtered through a short pad of silica and the solvent removed *in vacuo*. The resultant oil was then purified by flash silica chromatography (EtOAc) to afford **5** as a colourless oil (0.27 g, 1.71 mmol, 85%, 3 : 1 mixture of diastereomers). R_f (EtOAc) 0.46; IR (liquid film) 3391 (s, br), 2939 (s), 2873 (s), 1703 (s) cm^{-1}; δ_H (300 MHz, d_6-DMSO) 4.82 (0.25H, t, $J = 6$ Hz, minor CH_2OH), 4.75 (0.75H, d, $J = 5$ Hz, major $CHOH$), 4.61 (0.25H, d, $J = 5$ Hz, minor $CHOH$), 4.42 (0.75H, t, $J = 6$ Hz, major CH_2OH), 4.31 (1H, m, $CHOH$ both diastereomers), 3.68 (0.25H, dd, $J = 6$, 11 Hz, minor $CHOH$), 3.52 (0.75H,

dd, $J = 6$, 11 Hz, major CH*H*OH), 3.33 (0.25H, dd, $J = 6$, 11 Hz, minor C*H*HOH), 3.21 (0.75H, dd, $J = 6$, 11 Hz, major C*H*HOH), 1.30–2.15 (6H, m, CH_2, both diastereomers), 1.08 (2.25H, s, major CH_3), 0.95 (0.75H, s, minor CH_3) ppm; δ_C (75 MHz, d_6-DMSO) 212.7, 211.9, 70.9, 70.3, 65.1, 64.6, 49.6, 48.0, 35.2, 35.0, 34.6, 33.5, 19.2, 18.5, 17.7, 17.2 ppm; *m/z* (CI) 176 (80%, $[M + NH_4]^+$), 159 (21%, $[M + H]^+$). HRMS (CI) found $[M + NH_4]^+$, 176.1290. $C_8H_{18}O_3N$ requires 176.1287.

Preparation of tosylhydrazones 6a–c

Procedure for 6a. To a stirred suspension of tosylhydrazine (5.0 g, 26.85 mmol) in MeOH (20 ml) was added 2-methylcyclohexanone (3.0 g, 26.75 mmol) in MeOH (10 ml). Addition of HCl (0.5 ml) caused the mixture to clear rapidly. After stirring overnight crystals had formed which were filtered off, washed with cold MeOH and dried yielding tosylhydrazone **6a** as a white crystalline solid, 6.14 g (26.44 mmol, 99%). Mp 122 °C [lit. 121–122 °C].[23] The same procedure was used to prepare the tosylhydrazones of 2-*tert*-butylcyclohexanone (to give **6b** in 92% yield), 2-phenylcyclohexanone (to give **6c** in 73% yield), and 4-*tert*-butylcyclohexanone (in 99% yield).

Data for **6a**: IR (Nujol mull) 3201 (m), 2956 (s), 2853 (s), 1632 (w), 1596 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.85 (2H, d, $J = 7.9$ Hz, Ar), 7.30 (2H, d, $J = 7.9$ Hz, Ar), 2.57 (1H, m, C*H*Me), 2.42 (3 H, s, C*H*$_3$Ph), 2.24 (1H, m), 1.87 (2H, m), 1.72 (2H, m), 1.42 (2H, m), 1.22 (1H, m), 1.02 (3H, d, $J = 6.6$ Hz, CHC*H*$_3$) ppm; δ_C (75 MHz, $CDCl_3$) 165.4, 143.9, 135.4, 129.4, 128.4, 39.3, 35.5, 26.6, 26.3, 24.6, 21.8, 17.1 ppm; *m/z* (ES^+) 281.2 (100%, $[M + H]^+$).

Preparation of 6-substituted cyclohex-1-ene-1-methanols 7a–c

Procedure for 7a. 2-Methylcyclohexanone tosylhydrazone **6a** (2.0 g, 7.14 mmol) was placed in a flame dried 100 ml 3-necked RBQF flask fitted with a solids addition adapter, charged with paraformaldehyde (1.5 g, 50 mmol). THF (50 ml) and TMEDA (10 ml) were added and the solvent degassed with dry nitrogen. The solution was then cooled to −78 °C (acetone–dry ice) and *n*-BuLi (10 ml, 2.5 M in hexane) added dropwise *via* syringe. The solution was then stirred at −78 °C for 30 minutes until a bright orange colour was observed. The solution was then warmed to RT slowly with the evolution of N_2 gas, until a yellow solution was obtained. After the evolution of gas had ceased the solution was cooled once more to −78 °C and the paraformaldehyde was added, giving rise to an exothermic reaction. After stirring for 1 hour the solution was poured onto crushed ice (20 g) and acidified to ~ pH 6.0 (c. HCl) and the water layer extracted with diethyl ether (4 × 50 ml). The combined organic layers were washed with 10% aqueous citric acid (50 ml), saturated brine (2 × 50 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The resulting oil was purified by flash silica chromatography (20% EtOAc–hexane) to give alcohol **7a** as a colourless oil (476 mg, 3.78 mmol, 53%). The same procedure was used for the preparation of the 6-*tert*-butyl alcohol **7b** (31% yield) and 6-phenyl alcohol **7c** (43% yield).

Data for **7a**: R_f (CH_2Cl_2) 0.23; IR (liquid film) 3331 (s, br), 2926 (s), 2870 (s), 1635 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.67 (1H, s, C*H*=C), 4.11 (1H, d, $J = 12.5$ Hz, C*H*$_2$OH), 3.98 (1H, d, $J = 12.5$ Hz, C*H*$_2$OH), 2.31 (1H, m), 2.00 (2H, m), 1.34–1.71 (4H, m), 1.04 (3H, d, $J = 6.9$ Hz, CH*Me*) ppm; δ_C (75 MHz, $CDCl_3$) 141.8, 123.7, 65.9, 31.3, 29.6, 25.5, 19.7, 19.5 ppm; *m/z* (APCI) 126 ($[M]^+$, 70%). HRMS (CI) found $[M + NH_4 - H_2O]^+$, 126.1287. $C_8H_{16}N$ requires 126.1283.

Data for **7b**: R_f (CH_2Cl_2) 0.38; IR (liquid film) 3387 (br), 2953 (s), 2867 (s) 1582 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.90 (m, 1H, C*H*=C), 4.15 (2 × d, 2H, $J = 12$ Hz, C*H*$_2$OH), 2.10 (m, 2H), 1.75 (m, 2H), 1.52 (m, 3H), 1.00 (s, 9H, *t*-Bu) ppm; δ_H (75 MHz, $CDCl_3$) 140.5, 126.8, 68.5, 43.8, 34.2, 30.0, 26.4, 24.7, 20.2 ppm; *m/z* (EI) 168 (5%, M^+), 150 (12%, $[M - H_2O]^+$); HRMS (CI) $[M + NH_4]^+$ found 186.1860. $C_{11}H_{24}ON$ requires 186.1858.

Data for **7c**: R_f (20% EtOAc–hexane) 0.17; IR (liquid film) 3316 (br), 2927 (s), 2861 (s), 1599 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.15–7.48 (5H, m, Ar), 6.04 (1H, m, C*H*=C), 3.82 (1H, d, $J = 9$ Hz, C*H*HOH), 3.67 (1H, d, $J = 9$ Hz, CH*H*OH), 3.54 (1H, m, benzylic H), 1.40–2.53 (6H, m) ppm; δ_C (75 MHz, $CDCl_3$), 145.2, 138.5, 130.7, 128.6, 127.5, 126.3, 71.1, 41.7, 32.7, 25.6, 18.6 ppm; *m/z* (EI) 188 (5%, M^+), 170 (30%, $[M - H_2O]^+$); HRMS (CI) $[M + NH_4]^+$ found 206.1554. $C_{13}H_{20}ON$ requires 206.1545.

tert-Butyl(1,1-dimethyl)silyl [(6-methylcyclohex-1-enyl)methyl] ether 8a

To a solution of **7a** (0.578 g, 4.58 mmol) in DMF (5 ml) were added imidazole (0.469 g, 6.88 mmol, 1.5 eq.) and DMAP (0.005 g, catalytic amount). The solution was cooled to −10 °C and TBDMSCl (0.761 g, 5.05 mmol, 1.1 eq.) was added and the reaction mixture stirred overnight. The mixture was then extracted with diethyl ether (3 × 10 ml), washed with water (5 × 10 ml) and washed with brine (2 × 10 ml). The resulting pale yellow oil was purified by silica column chromatography (CH_2Cl_2) to yield **8a** as a colourless oil (1.02 g, 4.25 mmol, 93%). R_f (CH_2Cl_2) 0.80; IR (liquid film) 2928 (s), 2856 (s), 1640 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.65 (m, 1H, C*H*=C), 4.12 (d, 1H, $J = 13$ Hz, C*H*HOSi), 4.03 (d, 1H, $J = 13$ Hz, CH*H*OSi), 2.36 (m, 1H), 2.00 (m, 2H), 1.50–1.80 (m, 3H), 1.41 (m, 1H), 1.05 (d, 3H, $J = 7$ Hz, C*H*$_3$CH), 0.92 (s, 9H, *t*-Bu), 0.08 (s, 6H, C*H*$_3$Si) ppm; δ_C (75 MHz, $CDCl_3$) 141.2, 121.6, 65.7, 31.1, 29.2, 26.0, 25.3, 19.5, 19.4, 18.4, −5.2 ppm; *m/z* (EI) 240 (100%, M^+).

6-*tert*-Butyl-1-[(methoxymethoxy)methyl]cyclohex-1-ene 8b

To a stirred solution of **7b** (0.601 g, 3.58 mmol) in anhydrous dimethoxymethane (10 ml) were added LiBr (62 mg, 0.72 mmol) and *p*-TsOH·H_2O (68 mg, 0.36 mmol) and the solution stirred for 2 hours at which time a further portion of LiBr (62 mg, 0.72 mmol) was added and stirring continued for 4 hours at room temperature. The mixture was then treated with saturated $NaCl_{(aq)}$ and the mixture extracted with diethyl ether (2 × 20 ml). The combined organic layers were dried (Na_2SO_4), and concentrated *in vacuo*. The resulting oil was purified by silica column chromatography (EtOAc–petroleum ether, 10 : 90) to give the product as a colourless oil (0.642 g, 3.03 mmol, 85%). R_f (EtOAc–petroleum ether, 10 : 90) 0.55; IR (liquid film) 2942 (s), 2875 (s), 1061 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.90 (m, 1H, C*H*=C), 4.64 (2 × d, 2H, $J = 6$ Hz, C*H*$_2$OCH$_3$), 4.15 (d, 1H, $J = 12$ Hz, C*H*HOMOM), 4.01 (d, 1H, $J = 12$ Hz, CH*H*OMOM), 3.40 (s, 3H, C*H*$_3$O), 2.10 (m, 2H), 1.78 (m, 2H), 1.55 (m, 3H), 1.02 (s, 9H, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 136.7, 129.0, 95.4, 72.9, 55.4, 43.6, 34.2, 30.2, 26.3, 24.9, 19.9 ppm; *m/z* (CI) 230 (13%, $[M + NH_4]^+$), 151 (100%, $[M - OCH_2OCH_3]^+$).

6-Phenyl-1-[(methoxymethoxy)methyl]cyclohex-1-ene 8c

To a solution of **7c** (1.34 g, 7.10 mmol) in CH_2Cl_2 (20 ml) was added diisopropylethylamine (1.86 ml, 1.38 g, 10.65 mmol, 1.5 eq.) and chloromethyl methyl ether (0.81 m, 0.86 g, 10.65 mmol, 1.5 eq.) under nitrogen at 0 °C and the solution stirred overnight at room temperature. Aqueous KOH (2 M, 20 ml) was then added and stirring continued for 30 minutes. The aqueous phase was then extracted with diethyl ether, and the organic phase dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by silica column chromatography (5% EtOAc–hexane) to afford **8c** as a colourless oil (1.33 g, 5.74 mmol, 81%). R_f (5% EtOAc–hexane) 0.25; IR (liquid film) 2929 (s), 2865 (s), 1599 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.30 (5H, m, Ar), 6.02 (1H, C*H*=C), 4.60 (2H, m, OCH$_2$O), 3.79 (1H, d, $J = 9$ Hz, C*H*HOMOM), 3.42 (1H, d, $J = 9$ Hz, CH*H*OMOM), 3.54

(1H, m, benzylic H), 3.28 (3H, s, OCH_3), 1.50–2.30 (6H, M) ppm; δ_C (75 MHz, $CDCl_3$) 146.0, 135.6, 131.3, 128.5, 128.1, 125.9, 96.6, 75.7, 55.2, 41.6, 33.1, 25.3, 18.3 ppm; *m/z* (CI) 250 (60%, $[M + NH_4]^+$). HRMS (CI) $[M + NH_4]^+$ found 250.1816. $C_{15}H_{24}O_2N$ requires 250.1807.

Dihydroxylation of protected 6-substituted hydroxymethylcyclohex-1-ene (preparation of 9a–c)

Preparation of 9a. To a 50% aqueous solution of *t*-BuOH (20 ml) were added K_2CO_3 (1.65 g, 11.94 mmol, 3.7 eq.), $K_3Fe(CN)_6$ (3.92 g, 11.94 mmol, 3.7 eq.), $MeSO_2NH_2$ (0.340 g, 3.57 mmol, 1.1 eq.) and $K_2OsO_4 \cdot 2H_2O$ (11 mg, 0.03 mmol, 1 mol%). The solution was cooled to 0 °C (ice bath) and a solution of **8a** (0.78 g, 3.24 mmol) in *t*-BuOH (2 ml) was added dropwise, and the solution stirred vigorously for 24 hours at 0 °C. After the reaction was judged to be complete by thin layer chromatography, excess Na_2SO_3 (4.0 g) was added with ice cooling and the mixture was stirred for 1 hour. The mixture was then extracted with CH_2Cl_2 (20 ml). The aqueous portion was then further extracted with CH_2Cl_2 (3 × 10 ml) and the combined organic fractions washed with aqueous KOH (1 M), then dried ($MgSO_4$) and evaporated *in vacuo*. Silica column chromatography (3% EtOAc–CH_2Cl_2) afforded the product diol **9a** as a colourless oil (0.754 g, 3.11 mmol, 96%), as a mixture of two diastereoisomers (7:3 ratio). R_f (CH_2Cl_2) 0.21; IR (liquid film) 3456 (br), 2926 (s), 2856 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.84 (2 × d, 1H, J = 10 Hz, major and minor CHHOH), 3.64 (2 × d, 1H, J = 10 Hz, major and minor CHHOH), 1.10–2.05 (m, 8H), 0.9–1.0 (m, 12H, CHCH_3 and *t*-Bu), 0.10 (s, 6H, CH_3Si) ppm; δ_C (75 MHz, $CDCl_3$) 74.5, 74.3, 73.2, 70.4, 69.2, 65.7, 35.9, 34.3, 29.7, 29.5, 25.8, 23.4, 18.9, 18.2, 14.9, −5.5 ppm; *m/z* (ES^-) 273 (100%, [M − H]).

Diol **9b** was prepared from 6-*tert*-butyl MOM ether **8b** in 40% yield by the same procedure, except that the reaction was increased to 7 days at room temperature. Data for **9b**: R_f (EtOAc–petroleum ether, 10:90) 0.55; IR (liquid film) 3446 (br), 2940 (s), 2875 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.66 (2H, 2 × d, J = 7 Hz, OCH_2OCH$_3$), 3.97 (1H, m, CHOH), 3.84 (2H, s, CH_2OMOM), 3.39 (3H, s, OCH_3), 2.53 (2H, br s, OH), 1.10–1.90 (7H, m), 1.02 (9H, s, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 97.3, 78.1, 71.2, 68.7, 55.7, 47.2, 33.9, 30.7, 29.0, 25.9, 19.9 ppm; *m/z* (CI) 246 (8%, M^+).

Diol **9c** was prepared from 6-phenyl MOM ether **8c** in 25% yield by the same procedure, using a reaction time of 4 days at room temperature. Data for **9c**: R_f (30% EtOAc–hexane) 0.13; IR (liquid film) 3415 (br), 2932 (s), 1600 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.20 (5H, m, Ar), 4.51 (2H, two d, J = 6 Hz, OCH_2OCH$_3$), 4.14 (1H, m, CH(OH)), 3.93 (1H, d, J = 11 Hz, OCHHOMOM), 3.34 (1H, m, benzylic H), 3.30 (3H, s, OMe), 3.23 (1H, d, J = 11 Hz, OCHHOMOM), 2.50 (2H, br s, OH), 1.94 (1H, m), 1.75 (3H, m), 1.60 (2H, m) ppm; δ_C (75 MHz, $CDCl_3$), 141.0, 129.6, 128.0, 126.7, 97.2, 75.1, 69.8, 68.9, 55.7, 45.3, 28.8, 28.3, 19.6 ppm; *m/z* (CI) 284 (24%, $[M + NH_4]^+$), 252 (82%, $[M + NH_4 - MeOH]^+$), 207 [58%, $[M + NH_4 - Ph]^+$).

2-(Hydroxymethyl)-2-hydroxy-3-methylcyclohexan-1-one 10a

A solution of oxalyl chloride (0.32 ml, 0.425 g, 3.35 mmol, 1.1 eq.) in anhydrous CH_2Cl_2 (20 ml) was cooled to −78 °C (acetone–dry ice bath) and anhydrous (DMSO (0.57 ml, 0.571 g, 7.31 mmol, 2.4 eq.) in CH_2Cl_2 (10 ml) added dropwise. The solution was stirred for 5 minutes until gas evolution ceased. A solution of **9a** (0.836 g, 3.05 mmol) in CH_2Cl_2 (10 ml) was added dropwise over 5 minutes. Stirring was continued for 15 minutes at −60 °C after which time anhydrous triethlamine (1.541 g, 15.23 mmol, 5.0 eq.) was added, and stirring was continued for a further 10 minutes. The solution was then warmed to room temperature and quenched with water (20 ml). The aqueous layer was then separated and extracted with CH_2Cl_2 (3 × 10 ml). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Flash column chromatography afforded the product ketone as a colourless oil (0.610 g, 2.24 mmol, 74%). R_f (CH_2Cl_2) 0.51.

To a solution of 2-{*tert*-butyl(dimethyl)silyloxymethyl}-2-hydroxy-3-methylcyclohexan-1-one (0.5668 g, 2.09 mmol) in anhydrous THF (5 ml) was added excess tetra-*n*-butylammonium fluoride (1.0 M in 95% THF–H_2O, 4.0 ml) and glacial acetic acid (0.25 ml, 0.263 g, 4.38 mmol), and the reaction mixture stirred for 12 hours at room temperature. Water (5 ml) was then added and the mixture extracted with ethyl acetate (3 × 10 ml). The combined organic layers were washed with 10% citric acid (10 ml) and brine (10 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Silica column chromatography (60% EtOAc–hexane) afforded **10a** as a colourless oil (0.130 g, 0.83 mmol, 40%). R_f (60% EtOAc–hexane) 0.27; IR (liquid film) 3426 (br), 2980 (s), 2871 (s), 1713 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.05 (d, 0.3H, J = 11 Hz, minor CHH$_2$OH), 4.00 (d, 0.7H, J = 12 Hz, major CHH$_2$OH), 3.87 (d, 0.7H, J = 12 Hz, major CHH_2OH), 3.74 (d, 0.3H, J = 11 Hz, minor CHH_2OH), 3.21–3.45 (br s, 2H, OH), 1.50–2.75 (m, 7H), 1.09 (d, 0.7H, J = 7 Hz, major CH_3), 0.81 (d, 0.3H, J = 7 Hz, minor CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 213.2, 212.8, 82.9, 82.7, 67.1, 63.4, 44.5, 38.9, 37.7, 30.7, 28.8, 26.3, 22.7, 15.2, 13.2 ppm; *m/z* (CI) 176 (15%, $[M + NH_4]^+$); HRMS (CI) found $[M + NH_4]^+$ 176.1295. $C_8H_{18}O_3N$ requires 176.1287.

3-(*tert*-Butyl)-2-hydroxy-2-(hydroxymethyl)cyclohexan-1-one 10b

To a solution of Dess–Martin periodinane [14] (0.68 g, 1.62 mmol) in CH_2Cl_2 (5 ml) was added a solution of **9b** (0.20 g, 0.81 mmol) in CH_2Cl_2 (5 ml) with stirring which was continued overnight at room temperature. Once complete (15 hours) the reaction mixture was diluted with diethyl ether (30 ml). Aqueous 1.0 M NaOH (10 ml) was added, and stirring was continued for a further 20 minutes. The organic phase was then separated, and the aqueous phase extracted with Et_2O (3 × 10 ml). The combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was then purified by flash silica chromatography (40% EtOAc–hexane) to afford the product ketone as a colourless oil (0.170 g, 0.70 mmol, 86%). R_f (40% EtOAc–hexane) 0.53; IR (liquid film) 3446 (w), 2950 (s), 2869 (s), 1712 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.60 (2H, 2 × d, J = 7 Hz, OCH_2OCH$_3$), 4.52 (1H, s, OH), 4.16 (1H, d, J = 10 Hz, CHH$_2$ORMOM), 3.86 (1H, d, J = 10 Hz, CHH_2ORMOM), 3.32 (3H, s, OMe), 2.59 (2H, m, CH_2CO), 2.18 (1H, m), 1.95 (1H, m), 1.30–1.80 (3H, m), 1.05 (9H, s, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 211.6, 97.1, 84.4, 70.1, 58.5, 55.8, 38.4, 35.2, 30.6, 26.5, 25.7 ppm; *m/z* (CI) 244 (100%, M^+) 213 (24%, $[M - OCH_3]^+$), 183 (63%, $[M - OCH_2OCH_3]^+$).

To a stirred solution of 3-*tert*-butyl-2-hydroxy-2-[(methoxymethoxy)methyl]cyclohexan-1-one (94 mg, 0.30 mmol) in methanol (5 ml) was added 40% c. HCl in methanol (5 ml) to give a solution containing 20% HCl. Stirring was continued for 1 hour and the reaction monitored by thin layer chromatography. Once complete, water (5 ml) was added and the mixture extracted with ethyl acetate (5 × 10 ml). The organic phase was washed once with saturated $NaHCO_3$ solution (5 ml) and dried ($MgSO_4$). Flash silica chromatography (40% EtOAc–petroleum ether) afforded **10b** as a colourless oil (48 mg, 0.18 mmol, 60%). R_f (40% EtOAC–petroleum ether) 0.36; IR (liquid film) 3416 (s), 3371 (s), 2951 (s), 2865 (s), 1712 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.60 (1H, s, OH), 4.05 (2H, two d, J = 10 Hz, CH_2OH), 2.60 (2H, m), 2.20 (2H, m), 1.95 (1H, m), 1.35–1.75 (3H, m), 1.04 (s, 9H, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 212.7, 85.8, 64.3, 58.3, 38.1, 35.1, 30.5, 26.5, 25.6 ppm; *m/z* (EI) 200 (25%, M^+), 182 (20%, $[M - H_2O]^+$); HRMS (CI) $[M + NH_4]^+$ found 218.1760. $C_{11}H_{24}O_3N$ requires 218.1756.

The same procedures were used to oxidise **9c** to give 3-phenyl-2-hydroxy-2-[(methoxymethoxy)methyl]cyclohexan-1-

one in 52% yield, which was deprotected in 83% yield to give **10c**. Data for **10c**: R_f (30% EtOAc–hexane) 0.22; IR (liquid film) 3481 (br), 2930 (m), 2886 (m), 1718 (s), 1595 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.30 (5H, m, Ar-H), 4.50 (2H, two d, $J = 7$ Hz, OCH_2OCH_3), 4.20 (1H, d, $J = 10$ Hz, CHHOMOM), 3.29 (3H, s, OCH_3), 3.24 (1H, d, $J = 10$ Hz, CHHOMOM), 2.94 (1H, dd, $J = 4$, 13 Hz, CHPh), 2.72 (2H, m), 2.38 (2H, m), 2.02 (1H, m), 1.78 (1H, m) ppm; δ_C (75 MHz, $CDCl_3$), 211.1, 138.8, 128.9, 128.3, 127.3, 97.1, 81.5, 70.4, 55.7, 54.6, 38.4, 28.3, 26.3 ppm; *m/z* (CI) 282 (29%, $[M + NH_4]^+$), 250 (32%, $[M + NH_4 - MeOH]^+$), 205 (74%, $[M + NH_4 - Ph]^+$). Data for **10c**: R_f (30% EtOAc–hexane) 0.12; IR (liquid film) 3453 (br), 2925 (w), 2870 (w), 1703 (s), 1593 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.16–7.28 (5H, m, Ar), 4.26 (1H, br s, OH), 4.05 (1H, d, $J = 12$ Hz, CHHOH), 3.37 (1H, d, $J = 12$ Hz, CHHOH), 2.85 (1H, dd, $J = 4$, 13 Hz, benzylic H), 2.73 (1H, dt, $J = 6$, 14 Hz, $CH_{ax}H_{eq}C{=}O$), 2.59 (1H, m, $CH_{ax}H_{eq}C{=}O$), 2.25 (2H, m), 1.94 (1H, m), 1.71 (1H, m) ppm; δ_C (75 MHz, $CDCl_3$) 211.9, 138.5, 128.8, 128.1, 127.2, 82.4, 64.3, 54.5, 37.7, 27.8, 26.2 ppm; *m/z* (EI) 220 (20%, M^+), 190 (12%, $[M - CH_2O]^+$); HRMS (CI) $[M + NH_4]^+$ found 238.1448. $C_{13}H_{20}O_3N$ requires 238.1443.

1-*tert*-Butyl-2-(methoxymethoxy)benzene 11b

NaH (1.69 g, 35.2 mmol, 2 eq., dispersion in mineral oil) was washed twice with dry hexane 5 ml) and re-suspended in dry THF (50 ml). 2-*tert*-Butylphenol (2.65 g, 17.6 mmol, 2.7 ml) was added dropwise with ice cooling and stirring continued for 30 minutes until H_2 evolution had cased. Chloromethyl methyl ether (**CARE**! known carcinogen, 4.38 g, 54.4 mmol, 3.1 eq.) was then added dropwise and the solution stirred for a further 12 hours. Aqueous KOH (2 M, 50 ml) was then added and stirring continued for 30 minutes. The aqueous phase was then extracted with diethyl ether, and the organic phase dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by column chromatography (hexane) to afford **11b** as a colourless oil (3.03 g, 15.6 mmol, 89%). R_f (hexane) 0.40; IR (liquid film) 2954 (s), 1598 (w), 1580 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.90–7.35 (4H, m, Ar-H), 5.26 (2H, s, OCH_2OCH_3), 3.53 (3H, s, OCH_2OCH_3), 1.43 (9H, s, *t*-Bu) ppm; *m/z* (CI) 212 (20%, $[M + NH_4]^+$), 195 (20%, $[M + H]^+$), 194 (18%, M^+). The same method was used to prepare 1-phenyl-2-(methoxymethoxy)benzene (**11a**) from 2-phenylphenol, in 65% yield.

1-*tert*-Butyl-2-(methoxymethoxy)phenol 12b

To a solution of **11b** (3.01 g, 15.5 mmol) in dry THF (50 ml) was added *n*-butyllithium (10.1 ml, 1.7 M solution in hexane, 17.1 mmol, 1.1 eq.) with ice cooling under N_2. The solution was warmed to room temperature and stirred for 1 hour (yellow colour observed), then cooled to 0 °C (ice bath) and quenched with trimethyl borate (1.94 g, 18.1 mmol, 1.2 eq.). Stirring was continued for a further 30 minutes, then the solution was concentrated *in vacuo* to afford a solid. The solid was resuspended in aqueous acetone (20%, 50 ml) containing $NaHCO_3$ (5 g). Oxone (9.54 g, 15.5 mmol, 1 eq.) was added, and stirring continued. After 5 minutes solid $NaHSO_3$ (2 g) was added. The solution was then extracted with ethyl acetate (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The resulting oil was purified by flash silica chromatography (EtOAc–hexane, 1:19) to afford **12b** as a yellow oil (2.12 g, 10.1 mmol, 65%). R_f (EtOAc–hexane, 1:19) 0.39; δ_H (300 MHz, $CDCl_3$) 6.70–7.10 (3H, m, Ar-H), 6.12 (1H, s, ArOH), 5.73 (2H, s, $ArOCH_2OMe$), 3.53 (3H, s, OCH_2CH_3), 1.43 (9H, s, *t*-Bu) ppm. The same method was used to prepare 1-phenyl-2-(methoxymethoxy)phenol (**12a**) from **11a**, in 81% yield.

3-*tert*-Butylcatechol 13b

To a solution of **12b** (1.50 g, 7.14 mmol) in methanol (20 ml) was added 20% c. HCl in methanol (10 ml), and the reaction stirred at room temperature for 1.5 h. The solution was then neutralised with solid $NaHCO_3$ portionwise until effervescence ceased, followed by addition of solid $MgSO_4$. The solution was filtered and the residue washed twice with methanol. The material was then purified by flash silica chromatography (30% EtOAc–hexane) to afford **13b** as a yellow oil (0.97 g, 5.86 mmol, 82%). IR (liquid film) 3466 (br), 2954 (s), 2870 (m), 1617 (w), 1587 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.89 (1H, m), 6.73 (2H, m), 5.73 (1H, s, OH), 5.41 (1H, s, OH), 1.45 (9H, s, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 143.4, 142.9, 136.6, 119.2, 119.1, 112.9, 34.6, 29.5 ppm; *m/z* (EI) 166 (50%, M^+), 151 (100%, $[M - CH_3]^+$); HRMS (CI) $[M + NH_4]^+$ found 184.1345, $C_{10}H_{18}O_2N$ requires 184.1338. The same method was used to prepare 3-phenylcatechol (**13a**) from **12a**, in 44% yield. Data: R_f (30% EtOAc–petroleum ether) 0.05; IR (liquid film) 3542 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.50 (5H, s), 7.42 (1H, m), 6.93 (1H, t, $J = 7$ Hz), 6.84 (1H, dd, $J = 2.7$ Hz), 5.48 (1H, br s), 5.33 (1H, br s) ppm.

(4-*tert*-Butylcyclohex-1-enyl)methanol 14

N′-(4-*tert*-Butylcyclohexylidene)-4-methylbenzene-1-sulfonohydrazide (prepared from 4-*tert*-butylcyclohexanone by the above method in 99% yield, 2.00 g, 6.20 mmol) was placed in a flame dried 100 ml 3-necked flash fitted with a solids addition adapter charged with paraformaldehyde (0.65 g, 21.7 mmol). THF (30 ml) and TMEDA (10 ml) were added, the solution was then cooled to −78 °C (acetone–dry ice) and *n*-butyllithium (2.2 ml, 10.0 M in hexane, 3.5 eq., 21.71 mmol) added dropwise *via* syringe. The solution was stirred at −78 °C for 30 minutes, giving an orange precipitate. The solution was warmed to room temperature slowly, with the evolution of N_2 gas, giving a dark green solution. After the evolution of gas had ceased the solution was cooled to −78 °C and paraformaldehyde added (exothermic). After stirring for 1 hour, the colourless solution was poured onto crushed ice and acidified to ~ pH 6.0 (c. HCl), and the product extracted with diethyl ether (4 × 20 ml). The combined organic layers were washed with 10% aqueous citric acid (20 ml), saturated brine (2 × 20 ml), dried ($MgSO_4$), then concentrated *in vacuo*. The resulting yellow oil was purified by flash silica chromatography (EtOAc–hexane, 15:85) to give the product **14** as a colourless oil (0.269 g, 1.60 mmol, 26%). R_f (EtOAc–hexane, 15:85) 0.19; IR (liquid film) 3330 (br), 2943 (s), 2865 (s), 1632 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.68 (m, 1H, CH=C), 4.00 (2 × d, 2H, $J = 12$ Hz, CH_2OH), 2.11 (m, 2H), 1.84 (m, 2H), 1.55 (m, 1H), 1.24 (2H, m), 0.89 (s, 9H, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 137.4, 123.3, 67.3, 44.2, 32.2, 27.2, 27.0, 26.5, 23.8 ppm; *m/z* (EI) 168 (11%, M^+), 150 (19%, $[M - H_2O]^+$); HRMS (CI) $[M + NH_4]^+$ found 186.1865. $C_{11}H_{24}ON$ requires 186.1858.

4-*tert*-Butyl-1-[(methoxymethoxy)methyl]cyclohexane-1,2-diol 15, 16

Alcohol **14** was converted to its MOM ether using the same procedure employed for preparation of **8b** above, in 81% yield. Data: R_f (EtOAc–hexane, 10:90) 0.27; IR (liquid film) 2945 (s), 2867 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.72 (m, 1H, CH=C), 4.64 (s, 2H, OCH_2OCH_3), 3.96 (s, 2H, CH_2OMOM), 3.39 (s, 3H, OCH_2OCH_3), 2.10 (m, 3H), 1.85 (m, 2H), 1.29 (m, 2H), 0.90 (s, 9H, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 134.2, 125.7, 95.3, 71.6, 55.1, 44.0, 32.2, 27.5, 27.2, 26.6, 23.8 ppm; *m/z* (CI) 230 (44%, $[M + NH_4]^+$), 151 (100%, $[M - OCH_2OMe]^+$).

To a 50% aqueous solution of *t*-BuOH (10 ml) were added K_2CO_3 (589 mg, 4.26 mmol, 3.7 eq.), $K_3Fe(CN)_6$ (1.41 g, 4.26 mmol, 3.7 eq.), $MeSO_2NH_2$ (123 mg, 1.29 mmol, 1.1 eq.) and $K_2OsO_4 \cdot 2H_2O$ (5 mg, 0.01 mmol, 1 mol%). The solution was cooled to 0 °C (ice bath) and a solution of 4-*tert*-butyl-1-[(methoxymethoxy)methyl]cyclohex-1-ene (247 mg, 1.17 mmol) in *t*-BuOH (2 ml) added dropwise, then the solution was stirred vigorously for 24 hours. Solid Na_2SO_3 (3 g) was added with ice

cooling and the mixture was stirred for 1 hour. The mixture was then extracted with CH_2Cl_2 (10 ml). The aqueous portion was then further extracted with CH_2Cl_2 (3 × 10 ml). The combined organic fractions were washed with aqueous KOH (1 M, 10 ml), dried ($MgSO_4$) and evaporated *in vacuo* to give an oil, found to contain 1:1 mixture of diastereomers. Careful silica column chromatography (petroleum ether–EtOAc, 40:60) allowed separation of the products to give compounds **15** and **16**.

Compound **15** (axial–CH_2OMOM) as a colourless oil (0.13 g, 0.53 mmol, 45%). R_f (EtOAc–petroleum ether, 60:40) 0.28; IR (liquid film) 3419 (br), 2951 (s), 2867 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.68 (s, 2H, OCH_2OCH_3), 3.89 (t, 1H, $J = 3$ Hz, equatorial C*H*(OH)), 3.66 (d, 1H, $J = 11$ Hz, CH_2OMOM), 3.56 (d, 1H, $J = 11$ Hz, CH_2OMOM), 3.40 (s, 3H, OCH_2OCH_3), 2.97 (s, 1H, OH), 2.65 (br s, 1H, OH), 1.97 (m, 1H), 1.50–1.85 (m, 4H), 1.25 (m, 1H), 1.02 (m, 1H), 0.87 (s, 9H, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 97.2, 74.2, 71.4, 55.5, 46.1, 39.5, 32.5, 30.9, 27.5, 21.0 ppm; *m*/*z* (CI) 264 (8%, $[M + NH_4]^+$), 232 (58%, $[M + NH_4 - MeOH]^+$).

Compound **16** (equatorial–CH_2OMOM) as a white crystalline solid (0.12 g, 0.50 mmol, 42%). Mp 75–76 °C; R_f (EtOAc–petroleum ether, 60:40) 0.34; IR (liquid film) 3459 (br), 2953 (s), 2865 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.67 (2 × d, 2H, $J = 7$ Hz, OCH_2OCH_3), 3.66 (dd, 1H, $J = 4$ and 11 Hz, axial C*H*(OH)), 3.58 (2 × d, 2H, $J = 10$ Hz, CH_2OMOM), 3.40 (s, 3H, OCH_2OCH_3), 2.78 (br s, 2H, OH), 1.80 (m, 2H), 1.52 (m, 1H), 1.31 (m, 4H), 0.90 (s, 9H, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 97.1, 74.2, 71.2, 55.5, 46.1, 32.5, 32.3, 30.9, 27.5, 21.0 ppm; *m*/*z* (CI) 264 (14%, $[M + NH_4]^+$), 232 (100%, $[M + NH_4 - MeOH]^+$).

(2*R*,5*S*)-5-*tert*-Butyl-2-hydroxy-2-(hydroxymethyl)cyclohexan-1-one 17

To a solution of Dess–Martin periodinane (3.24 g, 7.65 mmol) in CH_2Cl_2 (10 ml) was added a solution of diol **15** (0.62 g, 2.55 mmol) in CH_2Cl_2 (10 ml), and the reaction was stirred overnight at room temperature. The reaction mixture was diluted with diethyl ether (30 ml) and aqueous 1.0 M NaOH (30 ml) was added, and the mixture was stirred for a further 20 minutes. The organic phase was then separated, and the aqueous phase extracted with diethyl ether (3 × 20 ml). The combined organic phase was then dried (Na_2SO_4), and concentrated *in vacuo*. The resulting oil was then purified by flash silica chromatography (EtOAc–hexane, 30:70) to afford the product as a colourless oil (0.55 g, 2.24 mmol, 88%). R_f (EtOAc–hexane, 30:70) 0.36; IR (liquid film) 3406 (br), 2952 (s), 2870 (s), 1712 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.68 (2H, 2 × d, $J = 6$, 7 Hz, OCH_2OCH_3), 3.90 (1H, d, $J = 10$ Hz, CH*H*OR), 3.45 (1H, d, $J = 10$ Hz, C*H*HOR), 3.40 (3H, s, OCH_2OCH_3), 2.75 (1H, t, $J = 12$ Hz, OH), 2.32 (1H, m), 2.05 (1H, m), 1.40–1.80 (5H, m), 0.92 (9H, s, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 212.7, 97.2, 76.1, 71.5, 55.4, 50.5, 39.7, 35.7, 33.0, 27.2, 21.3 ppm; *m*/*z* (CI) 262 (18%, $[M + NH_4]^+$), 230 (19%, $[M + NH_4 - MeOH]^+$), 213 (100%, $[M - OCH_3]^+$).

To a solution of 5-*tert*-butyl-2-hydroxy-2-[(methoxymethoxy)methyl]cyclohexan-1-one (330 mg, 1.35 mmol) in methanol (5 ml) was added 40% c. HCl in methanol (5 ml) to give a solution containing 20% HCl. Stirring was continued for 1 hour and the reaction monitored by thin layer chromatography. Once complete, water (5 ml) was added and the mixture extracted with ethyl acetate (5 × 10 ml). The organic phase was washed with saturated $NaHCO_3$ solution (5 ml), dried ($MgSO_4$) and evaporated *in vacuo*. Flash silica chromatography (40% EtOAC–petroleum ether) afforded **17** as a colourless oil (199 mg, 1.00 mmol, 74%). R_f (40% EtOAc–petroleum ether) 0.20; IR (liquid film) 3448 (s), 3238 (s), 2953 (s), 2865 (s), 1705 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.85 (1H, d, $J = 12$ Hz, CH_2OH), 3.30 (1H, d, $J = 12$ Hz, CH_2OH), 2.90 (2H, br s, OH), 2.72 (1H, t, $J = 12$ Hz), 2.30 (1H, m), 1.89 (1H, m), 1.73 (2H, m), 1.45 (2H, m), 0.91 (9H, s, *t*-Bu) ppm; δ_H (75 MHz, $CDCl_3$) 215.5, 75.8, 66.7, 50.2, 39.9, 34.5, 33.0, 27.6, 21.2 ppm; *m*/*z* (CI) 218 (17%, $[M + NH_4]^+$); HRMS (CI) $[M + NH_4]^+$ found 218.1760. $C_{11}H_{24}O_3N$ requires 218.1756.

The same procedures were used to oxidise diol **16** in 83% yield to the corresponding ketone, which was deprotected in 79% yield to give the (2*S*,5*S*)-isomer **18**. Data for protected ketone: R_f (EtOAc–hexane, 30:70) 0.30; IR (liquid film) 3461 (br), 2949 (s), 2869 (s), 1715 (s) cm^{-1}; δ_H (300 MHz, CDCl) 4.62 (2H, 2 × d, $J = 6$, 7 Hz, OCH_2OCH_3), 4.03 (1H, d, $J = 10$ Hz, C*H*HOR), 3.62 (1H, d, $J = 10$ Hz, CH*H*OR), 3.40 (1H, s, OH), 3.35 (3H, s, OCH_3), 2.60 (1H, m), 2.18–2.40 (2H, m), 1.89 (1H, m), 1.63–1.40 (3H, m), 0.93 (9H, s, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$), 212.1, 96.8, 78.7, 72.1, 55.5, 50.4, 39.9, 36.6, 32.8, 27.2, 24.0 ppm; *m*/*z* (CI) 262 (19%, $[M + NH_4]^+$), 230 (18%, $[M + NH_4 - MeOH]^+$). Data for **18**: R_f (40% EtOAc–petroleum ether) 0.18; IR (liquid film) 3326 (br), 2954 (s), 2865 (m), 1716 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.94 (1H, d, $J = 12$ Hz, CH_2OH), 3.70 (1H, d, $J = 12$ Hz, CH_2OH), 2.60 (1H, m), 2.35 (1H, t, $J = 12$ Hz), 2.25 (1H, m), 1.85 (1H, m), 1.40–1.60 (3H, m), 0.91 (9H, s, *t*-Bu) ppm; δ_C (75 MHz, $CDCl_3$) 213.0, 80.0, 66.9, 50.8, 39.8, 36.5, 33.0, 27.3, 23.9 ppm; *m*/*z* (CI) 218 (15%, $[M + NH_4]^+$); HRMS (CI) $[M + NH_4]^+$ found 218.1762. $C_{11}H_{24}O_3N$ requires 218.1756.

Ethyl 2-(2-phenylcyclohexylidene)acetate 19

To a suspension of NaH (50% dispersion in mineral oil, 2.19 g, 45.69 mmol) in THF (50 ml) was added triethyl phosphonoacetate (9.0 ml, 10.24 g, 45.69 mmol) dropwise at 0 °C (ice bath). Once effervescence had ceased, a solution of 2-phenylcyclohexanone (5.31 g, 30.46 mmol) in dry THF (10 ml) was added dropwise over 10 minutes, and the reaction stirred for 1 hour at room temperature. Water (50 ml) and ethyl acetate (50 ml) were added, and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 20 ml), and the combined organic phase dried (Na_2SO_4). The crude product was then purified by flash silica chromatography (5% EtOAc–hexane) to afford **19** as a colourless oil (5.55 g, 22.74 mmol, 75%). R_f (5% EtOAc–hexane) 0.34; IR (liquid film) 2930 (s), 2857 (s), 1711 (s), 1640 (s) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.17–7.40 (5H, m, Ar-H), 5.13 (1H, s, C*H*=C), 4.10 (2H, q, $J = 7$ Hz, OCH_2CH_3), 3.75 (1H, m), 3.43 (1H, dd, $J = 4$, 11 Hz, C*H*Ph), 2.25 (1H, m), 2.00 (4H, m), 1.65 (2H, m), 1.25 (3H, t, $J = 7$ Hz, OCH_2CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 167.1, 165.4, 141.7, 128.5, 126.6, 114.4, 59.6, 51.8, 34.3, 30.1, 28.1, 25.9, 14.2 ppm; *m*/*z* (EI) 244 (84%, M^+), 199 (51%, $[M - C_2H_5O]^+$).

Ethyl 2-(6-phenylcyclohex-1-enyl)acetate 20

To a solution of diisopropylamine (3.16 ml, 2.28 g, 22.55 mmol) in dry THF (50 ml) was added *n*-butyllithium (2.38 M solution in hexane, 9.47 ml, 22.55 mmol) dropwise *via* syringe at −78 °C under nitrogen. The solution was warmed to room temperature for 10 minutes, then cooled to −78 °C prior to the addition of a solution of ethyl 2-(2-phenylcyclohexylidene)acetate (5.00 g, 20.50 mmol) in dry THF (10 ml) dropwise over 5 minutes. The solution was maintained at −78 °C for a further 30 minutes before the addition of aqueous NH_4Cl (1 M, 50 ml). The solution was warmed to room temperature, diluted with ethyl acetate (50 ml), and the phases separated. The aqueous phase was extracted with ethyl acetate (3 × 20 ml) and the combined organic phase dried (Na_2SO_4) and evaporated *in vacuo*. The resulting oil was then purified by careful flash silica chromatography (2% EtOAc–hexane) to afford **20** as a colourless oil (4.27 g, 17.51 mmol, 85%). R_f (2% EtOAc–hexane) 0.11; IR (liquid film) 2931 (m), 2861 (m), 1731 (s), 1559 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.15–7.35 (5H, m, Ar-H), 5.88 (1H, m, C*H*=C), 4.05 (2H, 2 × q, $J = 7$ Hz, OCH_2CH_3), 3.54 (1H, br m, C*H*Ph), 2.88 (1H, d, $J = 15$ Hz, CH*H*CO_2Et), 2.75 (1H, d, $J = 15$ Hz, C*H*HCO_2Et), 2.18 (2H, m), 2.02 (1H, m), 1.50–1.75 (3H, m),

1.22 (3H, t, $J = 7$ Hz, OCH_2CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 172.1, 144.6, 132.3, 128.5, 128.4, 128.3, 126.1, 60.3, 44.1, 41.6, 32.5, 25.5, 18.6, 14.1 ppm; *m/z* (EI) 244 (40%, M^+), 198 (100%, $[M - EtOH]^+$).

[2-(6-Phenylcyclohex-1-enyl)ethoxy]methoxymethane 21

To a suspension of lithium aluminium hydride (1.21 g, 31.98 mmol) in dry THF (50 ml) was added **20** (3.12 g, 12.79 mmol) under nitrogen. The solution was then heated under gentle reflux for 12 hours, then cooled to 0 °C (ice bath), then carefully quenched by the slow addition of water (50 ml). The solution was then acidified to ~ pH 7 (1 M HCl), then diluted with ethyl acetate (50 ml), and the phases separated. The aqueous phase was extracted with ethyl acetate (3 × 20 ml). The combined organic phases were washed with aqueous $NaHCO_3$ (30 ml), brine (30 ml), dried (Na_2SO_4), and evaporated *in vacuo*. The resulting oil was then purified by flash silica chromatography (20% EtOAc–hexane) to afford the product alcohol as a colourless oil (2.49 g, 12.35 mmol, 96%). R_f (20% EtOAc–hexane) 0.20; IR (liquid film) 3301 (br), 2928 (s), 2858 (s), 1599 (m) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.15–7.35 (5H, m, Ar-H), 5.85 (1H, s, C*H*=C), 3.58 (2H, m, CH_2OH), 3.39 (1H, m, C*H*Ph), 1.90–2.25 (4H, m), 1.40–1.74 (4H, m) ppm; δ_C (75 MHz, $CDCl_3$) 145.2, 135.2, 128.6, 128.4, 127.0, 126.2, 60.6, 43.9, 39.3, 32.9, 25.6, 18.9 ppm; *m/z* (EI) 202 (35%, M^+), 184 (36%, $[M - H_2O]^+$), 170 (30%, $[M - MeOH]^+$); HRMS (CI) $[M + NH_4]^+$ found 220.1701. $C_{14}H_{22}ON$ requires 220.1701.

The alcohol was then converted to the corresponding MOM ether **21** in 94% yield, using the same procedure used above for preparation of **8c**. Data for **21**: R_f (5% EtOAc–hexane) 0.25; IR (liquid film) 2928 (s), 2879 (s), 1599 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.17–7.34 (5H, m, Ar-H), 5.80 (1H, m, C*H*=C), 4.56 (2H, s, OCH_2OCH_3), 3.54 (2H, t, $J = 7$ Hz, CH_2CH_2OMOM), 3.38 (1H, m, C*H*Ph), 3.33 (3H, s, OCH_3), 1.93–2.24 (5H, m), 1.45–1.73 (3H, m) ppm; δ_C (75 MHz, $CDCl_3$) 145.2, 135.5, 128.5, 128.1, 125.9, 125.5, 96.2, 66.2, 55.1, 44.2, 35.9, 32.7, 25.5, 18.8 ppm; *m/z* (CI) 264 (35%, $[M + NH_4]^+$).

2-Hydroxy-2-(2-hydroxyethyl)-3-phenylcyclohexan-1-one 22

Dihydroxylation of **21** was carried out using the procedure given above for preparation of **9a**, to give after chromatography a 4 : 1 mixture of diastereomeric diols as a colourless oil (1.37 g, 4.92 mmol, 98%). R_f (30% EtOAc–hexane) 0.15; IR (liquid film) 3456 (br), 2931 (s), 2875 (s), 1660 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.14–7.31 (5H, m, Ar-H), 4.57 (0.2H, 2 × d, $J = 8$ Hz, minor OCH_2OCH_3), 4.49 (0.8H, 2 × d, $J = 7$ Hz, major OCH_2OCH_3), 4.00 (1H, m, C*H*Ph), 3.43–3.69 (2H, m, CH_2OR), 3.35 (0.6H, s, minor OCH_3), 3.28 (2.4H, s, major OCH_3), 3.22 (0.8H, dd, $J = 5$, 9 Hz, major C*H*OH), 2.60–3.05 (2H, br s, OH), 2.50 (0.2H, dd, $J = 4$, 14 Hz, minor C*H*OH), 1.45–2.00 (6H, m, CH_2) ppm; δ_C (75 MHz, $CDCl_3$) 142.6, 141.2, 129.9, 129.5, 128.3, 127.9, 126.6, 96.6, 75.5, 74.9, 74.2, 71.0, 64.3, 64.0, 55.8, 55.7, 51.1, 46.8, 37.5, 30.9, 29.5, 29.1, 28.8, 28.4, 24.1, 19.7 ppm; *m/z* (CI) 298 (5%, $[M + NH_4]^+$), 266 (25%, $[M + NH_4 - MeOH]^+$).

Using the methods given above for preparation of **10b**, the mixture of diols was then oxidised using the Dess–Martin periodinane in 52% yield to give the corresponding ketone, which was deprotected under acidic conditions to give **22** in 80% yield. Data for ketone: R_f (30% EtOAc–hexane) 0.35; IR (liquid film) 3448 (br), 2934 (s), 2871 (s), 1703 (s), 1599 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$), 7.20–7.40 (5H, m, Ar-H), 4.41 (2H, 2 × d, $J = 7$ Hz, OCH_2OCH_3), 3.43 (2H, m, CH_2OMOM), 3.28 (3H, s, OCH_3), 2.92 (2H, m), 2.61 (1H, m), 2.40 (2H, m), 2.25 (1H, m), 1.97 (1H, m), 1.65 (1H, m), 1.55 (1H, m) ppm; δ_C (75 MHz, $CDCl_3$) 213.7, 139.2, 129.5, 128.0, 127.2, 96.8, 79.9, 62.8, 56.9, 55.5, 38.3, 33.5, 28.1, 26.9 ppm; *m/z* (CI) 296 (5%, $[M + NH_4]^+$), 264 (15%, $[M + NH_4 - MeOH]^+$). Data for **22**: R_f (30% EtOAc–hexane) 0.14; IR (CH_2Cl_2 solution) 3366 (br), 2937 (m), 2859 (m), 1702 (s), 1598 (w) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.15–7.30 (5H, m, Ar-H), 4.34 (1H, br s, OH), 3.96 (2H, q, $J = 8$ Hz, CH_2CH_2OH), 3.80 (1H, t, $J = 8$ Hz, CH_2CH_2OH), 3.48 (1H, m, C*H*Ph), 2.80 (2H, t, $J = 8$ Hz, CH_2CH_2OH), 1.80–2.60 (4H, m), 1.36 (2H, m) ppm; δ_C (75 MHz, $CDCl_3$) 206.4, 140.9, 129.4, 128.5, 127.9, 127.2, 104.7, 62.8, 49.7, 34.9, 32.6, 29.0, 22.7 ppm; *m/z* (CI) 234 (60%, M^+), 216 (30%, $[M - H_2O]^+$); HRMS (CI) $[M + NH_4]^+$ found 252.1605. $C_{14}H_{22}O_3N$ requires 252.1605.

Inhibition of 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB)

Escherichia coli 2,3-dihydroxyphenylpropionate 1,2-dioxygenase was purified to near homogeneity by the method described previously.[6,7] Enzyme was re-activated by treatment with iron(II) ammonium sulfate and sodium ascorbate prior to assay.[6] Stock solutions of inhibitors were made in water at 50–100 mM concentration. MhpB was assayed by UV spectroscopy, monitoring the appearance of the extradiol ring fission product at 394 nm ($\varepsilon = 15\,600$ M^{-1} cm^{-1}) at pH 8.0 in 50 mM potassium phosphate buffer. Assays (1.0 ml) contained 50 mM potassium phosphate buffer, 50–150 μM 2,3-dihydroxyphenylpropionic acid, 1–10 mM inhibitor, and 0.1 unit of re-activated enzyme. Assays were carried out at 20 °C, in duplicate, at a fixed concentration of substrate, and variable concentrations of inhibitor. K_i values and type of inhibition were then determined by Dixon plot (1/*v versus* [I]).[16] In each case linear plots were obtained, and competitive inhibition was observed. Data are shown in Table 1.

Inhibition of protocatechuate 3,4-dioxygenase

Pseudomonas sp. protocatechuate 3,4-dioxygenase was purchased from Sigma Chemical Co. The enzyme was assayed by UV spectroscopy, monitoring the appearance of the intradiol ring fission product at 290 nm ($\varepsilon = 3890$ M^{-1} cm^{-1}).[24] Assays (1.0 ml) contained 50 mM Tris buffer pH 8.5, 50 μM protocatechuic acid, 1–20 mM inhibitor, and 0.05 unit enzyme. Assays were carried out at 20 °C, in duplicate, at a fixed concentration of substrate, and variable concentrations of inhibitor. The IC_{50} value for **18** was determined by pot of *v vs.* [I] (see Table 1). Under these conditions a K_m value of 20 μM was determined for protocatechuic acid.

Crystal structure determinations

Crystal data for 10b. $C_{11}H_{20}O_3$, $M = 200.27$, triclinic, $a = 6.5434(13)$, $b = 6.8746(14)$, $c = 13.017(3)$ Å, $U = 562.2(2)$ Å^3, $T = 150(2)$ K, space group $P\bar{1}$, $Z = 2$, $\mu = 0.084$ mm^{-1}, 5176 reflections measured, 1954 unique reflections ($R_{int} = 0.0659$), R indices (all data) $R1 = 0.0851$, $wR2 = 0.2018$. CCDC reference number 207/470.

Crystal data for 10c. $C_{13}H_{16}O_3$, $M = 220.26$, monoclinic, $a = 14.783(3)$, $b = 5.9121(12)$, $c = 12.150(2)$ Å, $U = 1059.6(4)$ Å^3, $T = 150(2)$ K, space group $P2_1/c$, $Z = 4$, $\mu = 0.097$ mm^{-1}, 9544 reflections measured, 2400 unique reflections ($R_{int} = 0.0496$), R indices (all data) $R1 = 0.0599$, $wR2 = 0.1586$. CCDC reference number 207/470.

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