Arene *cis*-dihydrodiol formation: from biology to application

Derek R. Boyd*[a] and Timothy D. H. Bugg[b]

Received 19th September 2005, Accepted 10th October 2005
First published as an Advance Article on the web 9th December 2005
DOI: 10.1039/b513226f

The range of available arene dihydroxylating dioxygenase enzymes, their structure and mechanism, and recent examples of the application of arene *cis*-dihydrodiol bioproducts as chiral precursors in the synthesis of natural and unnatural products and chiral ligands are discussed.

1. Introduction and background

Addition reactions leading to dearomatisation of benzene rings are generally very slow, due to the significant resonance energy deficit involved and thermodynamic drive to preserve the aromatic sextet. This is evident from the current lack of general chemical methods for controlled dihydroxy addition to monocyclic arenes (*cis*-dihydroxylation) without further reaction occurring. Nature has, however, provided a remarkable family of biocatalysts that can regulate the activity of dioxygen and address this problem, *i.e.* the aromatic dihydroxylating dioxygenases (dioxygenases). These redox enzymes thus provide two electrons to the mild oxidant dioxygen (bearing two unpaired electrons), thereby activating and producing a very strong oxidizing agent linked to a mononuclear iron centre. Dioxygenases catalyse the incorporation of both activated dioxygen atoms during *cis*-dihydroxylation of benzenoid substrates, which is the initial step during arene biodegradation by bacteria. The first unequivocal identification of an arene *cis*-dihydrodiol metabolite was reported by Gibson *et al.* using the bacterium *Pseudomonas putida* F1 and benzene as substrate.[1] Following from this early study, several hundred different arene *cis*-dihydrodiol metabolites have since been isolated[2–11] and, according to biological activity or identity of nucleotide sequences, in excess of one hundred arene hydroxylating dioxygenases have been identified.[12] As the arene dihydroxylating dioxygenases were later found to catalyse both monooxygenase and dioxygenase reactions, they are more accurately identified as Rieske type non-heme iron oxygenases.[9] Although the term 'dioxygenase' is thus inappropriate in some cases, it is still widely used in the literature, and this practice is continued herein.

Environmental biodegradation of arenes often involves soil bacteria, *e.g. P. putida*, and the initial step is exemplified by the dioxygenase (DO)-catalysed dihydroxylation of the parent compound benzene **A** (R = H) to yield the benzene *cis*-diol **B** (R = H, Scheme 1).[1] *cis*-Diol dehydrogenase (DD) is also present in these wild-type bacterial cells and can catalyse the

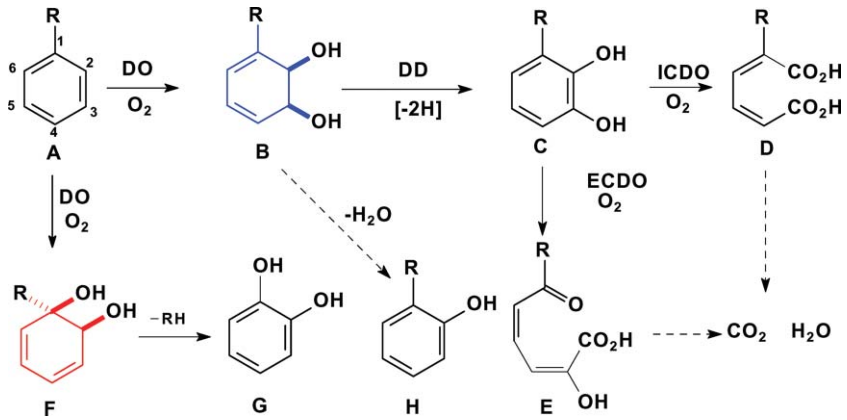
[a]*School of Chemistry and Centre for Theory and Application of Catalysis, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[b]*Department of Chemistry, University of Warwick, Coventry, UK CV4 7AL*

Derek Boyd is Professor of Organic Chemistry (Queen's University Belfast). Following his PhD and postdoctoral work at Queen's with Profs. H. B. Henbest and M. F. Grundon, he spent a year as a Visiting Scientist at the National Institutes of Health, Bethesda, USA working with Dr J. Daly and Dr D. M. Jerina. In 1969 he returned to Queen's as a lecturer in organic chemistry. Sabbatical years have been spent with Prof. G. A. Berchtold at the Massachusetts Institute of Technology (1977–78) and with the NIH and MIT groups (1980–81) on a Nuffield Foundation Science Research Fellowship. He was promoted to Professor in 1989 and to Head of Research in The School of Chemistry at Queen's (1996–2000). His research interests include catalytic chiral oxidations by chemical and enzyme-catalysed methods and the synthesis of enantiopure metabolites and related unnatural products.

Derek R. Boyd

Timothy D. H. Bugg

Tim Bugg is Professor of Biological Chemistry (University of Warwick). Following his PhD studies with Dr C. Abell at the University of Cambridge, he spent two years as a SERC/NATO postdoctoral research fellow with Prof. C. T. Walsh at Harvard Medical School. In 1991 he began his academic career as a lecturer in organic chemistry at the University of Southampton, before moving to Warwick in 1999. His research interests include study of enzyme mechanisms, principally enzymes involved in the bacterial degradation of aromatic compounds, and enzymes involved in bacterial peptidoglycan biosynthesis.



Scheme 1 Bacterial biodegradation pathways for monosubstituted benzene substrates.

second biodegradation step *i.e.* dehydrogenation of benzene *cis*-dihydrodiol **B** (R = H) to yield catechol **C** (R = H). Similarly the bacterial extradiol catechol dioxygenase (ECDO) or intradiol catechol dioxygenase (ICDO) enzymes catalyse the ring-opening reactions of catechol **C** (R = H) to yield *cis*,*cis*-muconic acid **D** (R = H, ICDO) or muconic semialdehyde **E** (R = H, ECDO) (Scheme 1).[13] The first three steps in the prokaryotic arene biodegradation pathway involve a dearomatisation (**A** → **B**), rearomatisation (**B** → **C**), dearomatisation (**C** → **D** or **E**) sequence, prior to mineralization.

Mono- and poly-cyclic arenes of both anthropogenic and xenobiotic origins are ubiquitous in the environment. They may originate from diverse sources including fuel and volatile solvent evaporation, automotive exhaust emissions, as well as partial combustion of tobacco and other plant materials. Polycyclic aromatic hydrocarbons (PAHs) containing a sterically hindered bay region, *e.g.* benzo[*a*]pyrene, can often form carcinogenic metabolites in mammalian systems. This eukaryotic pathway occurs *via* an oxidative dearomatisation step involving monooxygenase-catalysed epoxidation to yield arene oxides followed by epoxide hydrolase-catalysed hydrolysis to give the corresponding precarcinogenic *trans*-dihydrodiols.

The alternative oxidative dearomatisation routes used by soil bacteria (prokaryotic metabolism) to biodegrade mono- and poly-cyclic arenes, initially involving *cis*-dihydrodiol formation, do not appear to produce carcinogenic metabolites. As shown in Scheme 1, the initial step in the biodegradation of most mono-substituted monocyclic arene ring systems **A** by bacterial cells, generally involves facially selective *cis*-dihydroxylation exclusively at the 2,3-bond to form a *cis*-dihydrodiol metabolite **B**.[2–11] This is exemplified by toluene **A** (R = Me), the most abundant anthropogenic hydrocarbon present in the environment, forming *cis*-dihydrodiol **B** (R = Me), the first member of the chiral *cis*-dihydrodiol family to be isolated[14] (Scheme 1). Unfortunately type **B** *cis*-dihydrodiols (*e.g.* R = Me), while relatively stable, were difficult to isolate since they rearomatise rapidly *via* enzymatic dehydrogenation into substituted catechol intermediates **C** (*e.g.* R = Me) when wild-type strains are used. *cis*-Dihydrodiols, with strong electron-donating groups (*e.g.* **B**, R = OMe) are less stable, *i.e.* more susceptible to non-enzymatic dehydration under acidic conditions to give mainly *ortho*-monophenols **H** (*e.g.* R = OMe).[15]

cis-Dihydroxylation has also been found to occur exclusively at the 1,2-(*ipso*-) bond of nitrobenzene substrates to yield transient *cis*-dihydrodiols (*e.g.* **F**, R = NO_2). Although not isolated, the involvement of a *cis*-dihydrodiol intermediate has been inferred from the formation of the corresponding catechols (*e.g.* **G**, from **F**, R = NO_2), following the spontaneous elimination of nitrous acid (nitrite).[16,17]

Metabolite **B** (R = Me) is the forerunner of a large number (>300) of isolable chiral arene *cis*-dihydrodiols.[2–11] In the majority of cases these *cis*-dihydrodiols were enantiopure (>98% ee), but to date relatively few types have been used as chiral precursors in synthesis. This article is written from the perspective of: (i) providing selection guidelines related to different dioxygenase enzyme types, (ii) reviewing alternative mechanisms involved in arene dihydroxylation, (iii) demonstrating some synthetic applications of less commonly used arene *cis*-dihydrodiols, and (iv) predicting future trends in this area of biotransformation chemistry based on recent literature reports.

2. Types of dioxygenase enzymes and bacterial strains used in the production of arene *cis*-dihydrodiol metabolites

Dioxygenase enzymes (Rieske-type non-heme iron oxygenases) present in wild-type bacterial strains have been classified according to the arene originally used as a carbon source and many different types (*e.g.* benzene, toluene, biphenyl, chlorobenzene, benzoate, phthalate, nitrobenzene, naphthalene, *etc.*) have been identified from bacterial sources.[12,18] The more widely used types in biotransformations (and their abbreviations) include benzene (BDO), toluene (TDO), biphenyl (BPDO), chlorobenzene (CDO), benzoic acid (BZDO), nitrobenzene (NBDO) and naphthalene dioxygenase (NDO). Arene *cis*-dihydrodiol metabolites are generally present as stable intermediates but these are rapidly removed in wild-type bacterial strains due to further metabolism (*e.g.* **A** → **B** → **C** → **D** or **E** → CO_2 + H_2O, Scheme 1). A wild-type strain of *P. putida* (ML2, containing BDO) is exceptional in allowing several arene *cis*-dihydrodiols **B** (R = H, F, Cl, Br, I) to be accumulated and isolated. This is due to their being poor substrates for the corresponding benzene diol dehydrogenase (BDD).[19]

The first successful approaches to the accumulation and isolation of benzene *cis*-dihydrodiol metabolites used selected

whole-cell mutant strains containing TDO but with DD enzymes blocked (*e.g. P. putida* 39/D or UV4), NDO (*e.g. P. putida* 9816/11), BPDO (*e.g. Sphingomonas yanoikuyae* B8/36), or BZDO (*e.g. P. putida* U103 or *Alcaligenes eutrophus* B9).[11,12] The development of whole-cell *E. coli* recombinant strains expressing the appropriate encoded dioxygenase genes including TDO (*e.g. E. coli* JM109 [pDTG601] or JM109[pKST11]), NDO (*e.g. E. coli* JM109[DE3][pDTG141])[11] and BPDO (*e.g. E. coli* pKF2072)[20] and the possibility of using shuffled dioxygenase genes have become of increasing importance. This approach allows *cis*-dihydrodiol metabolites to be intercepted without catechol formation, and dioxygenases to be engineered to display modified selectivity and improved efficiency, particularly using site-directed mutation and directed-evolution methods.

Guidelines for the selection of a suitable dioxygenase to catalyse a particular arene biotransformation are of relevance to chemists wishing to utilise this type of biotechnology. Substrate size and type are obviously an important consideration due to the constraints imposed by the geometry of the dioxygenase active site. As a general rule, BDO and TDO enzymes can accommodate suitably substituted monocyclic arene substrates at the active site, yielding the corresponding benzene *cis*-dihydrodiols and do not accept polycyclic arene substrates larger than naphthalene. Conversely, NDO or BPDO can accommodate larger carbocyclic and heterocyclic arenes (di-, tri-, tetra- and penta-cyclic), but few monocyclic arenes (with the exception of biphenyl analogues)[20] have been dihydroxylated using NDO or BPDO. Other dioxygenases have been found to be more selective in specifically targeting benzene rings bearing carboxylate (BZDO)[21,22] or nitro groups (NBDO).[16,17]

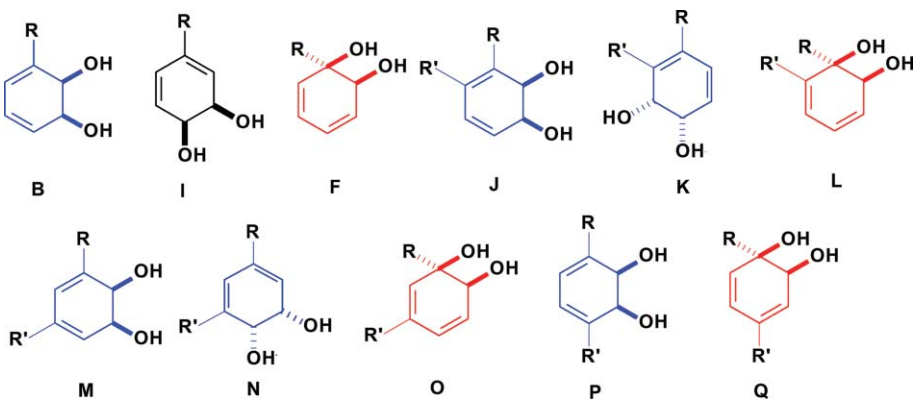
As discussed earlier, the term Rieske-type non-heme iron oxygenases has been proposed to account for their ability to catalyse different types of oxidation.[9] Recent examples reported using TDO (and in some cases NDO or BPDO) include: (i) dihydroxylation of cyclic or acyclic conjugated alkenes,[23,24] (ii) monohydroxylation at benzylic or allylic centres,[24,25] (iii) monosulfoxidation of alkylaryl or diaryl sulfides,[26,27] (iv) desaturation at allylic or benzylic positions[28,29], and (v) *N*- and *O*-dealkylation.[6] While the latter two types of dioxygenase-catalysed oxidation are uncommon, the possibility of any of the other five types of dioxgenase-catalysed oxidation (or a combination of either type) occurring during the biotransformation of substituted arenes, has to be considered. This Perspective Article aims to provide general guidelines for predicting the probable products from dioxygenase-catalysed oxidation and the preferred sequence when tandem oxidations occur using specific arene substrates and dioxygenases.

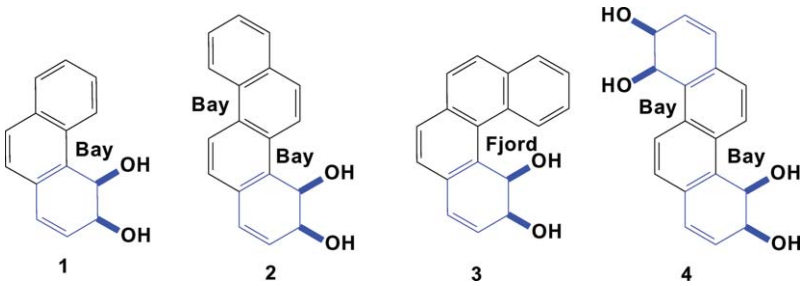
3. Types of regio- and chemo-selectivity from dioxygenase-catalysed oxidation of substituted arenes to yield arene *cis*-dihydrodiol metabolites

The most common *cis*-dihydrodiols are of type **B** (>50 known examples), resulting from TDO-catalysed *cis*-dihydroxylation of the 2,3-bond of monosubstituted benzene substrates **A** (Scheme 1).[2–11,30,31] The more polar substrates **A**, *e.g.* aniline (R = NH_2), benzoic acid (R = CO_2H) and benzene sulfonic acid (R = SO_3H), have not been reported as substrates for TDO. *cis*-Dihydroxylation at the 1,2- or 3,4-bonds of monosubstituted benzene substrates has not been found using TDO, but two types of dioxygenase-catalysed *cis*-dihydroxylation at the 1,2 (*ipso*)-bond of substrates **A** to yield type **F** *cis*-dihydrodiols, have been reported with other dioxygenases. Thus, benzoic acid **A** (R = CO_2H) yielded a stable *cis*-dihydrodiol of type **F** (BZDO, R = CO_2H),[21,22] while nitrobenzene was postulated to give a transient *cis*-dihydrodiol intermediate of type **F** (NBDO, R = NO_2).[16,17]

The sole example of a *cis*-dihydrodiol of type **I** resulting from *cis*-dihydroxylation at the 3,4-bond of a monosubstituted benzene was found using biphenyl as substrate **A** (R = Ph) and NDO. This type **I** *cis*-dihydrodiol (R = Ph) was isolated as a minor metabolite in the presence of the more abundant type **B** (R = Ph) metabolite.[32,33] The use of site-directed mutants of NDO caused a major change in regioselectivity and yielded *cis*-dihydrodiol **I** (NDO, R = Ph) as the major bioproduct.[32,33]

Regioselectivity observed during TDO-catalysed *cis*-dihydroxylation of 1,2- and 1,3-disubstituted benzenes was often found to be related to the relative size of the substituents (steric effect). Thus, the more sterically demanding substituent R had a dominant directing effect over the smaller substituent R′ (CF_3 > SMe > I > Br > Cl ≤ Me > F > H) with *cis*-dihydroxylation being regioselective for the proximate C=C bond yielding an excess of *cis*-diols of types **J** (over **K**) and **M** (over **N**). As TDO did not catalyse *cis*-dihydroxylation at an *ipso*-bond, only *cis*-diols of type **P** could be formed using 1,4-disubstituted benzene substrates.[27,34] An alternative type of directing effect was found using BZDO and NBDO enzymes where the R group (CO_2H or NO_2) was totally dominant over R′, possibly due to H-bonding to the R group at the active site,[17] and only *cis*-dihydrodiol types **L**, **O** and **Q** were formed.[16,17,21,22]





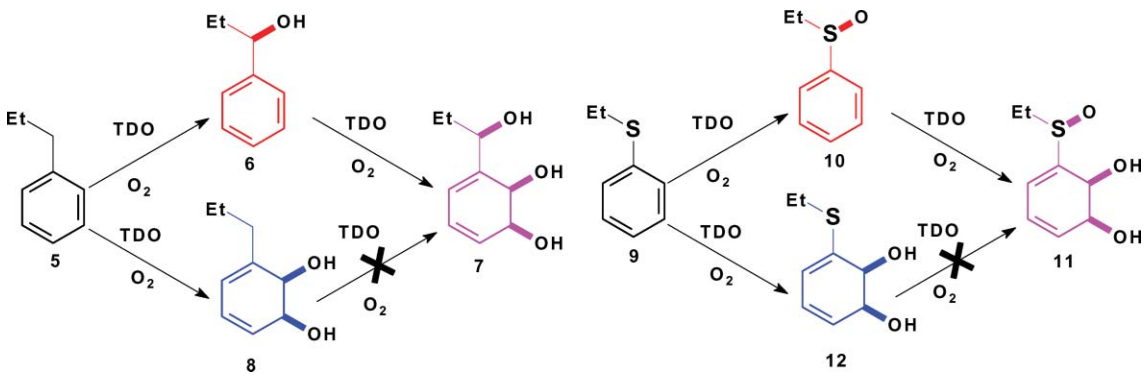
Regioselectivity was also observed in BPDO-catalysed *cis*-dihydroxylation of PAHs and their heteroarene analogues where a strong or exclusive preference was again shown for bonds proximate to 'bay' and 'fjord' regions as exemplified by the formation of *cis*-dihydrodiols of phenanthrene **1** (bay region),[32,33] chrysene **2** (two bay regions),[29,35] and benzo[*c*]phenanthrene **3** (fjord region).[36] Further biotransformation of the initial *cis*-dihydrodiol **2** using BPDO yielded *bis*-*cis*-dihydrodiol **4**, the first member of a new family of metabolites with four chiral centres resulting from sequential *cis*-dihydroxylation (tetraoxygenation).[29]

Sequential TDO-catalysed trioxygenation reactions involving benzylic hydroxylation then *cis*-dihydroxylation of alkyl benzene substrates to yield triols with three new chiral centres *e.g.* **5** → **6** → **7** (Scheme 2a) has recently been observed.[31,37]

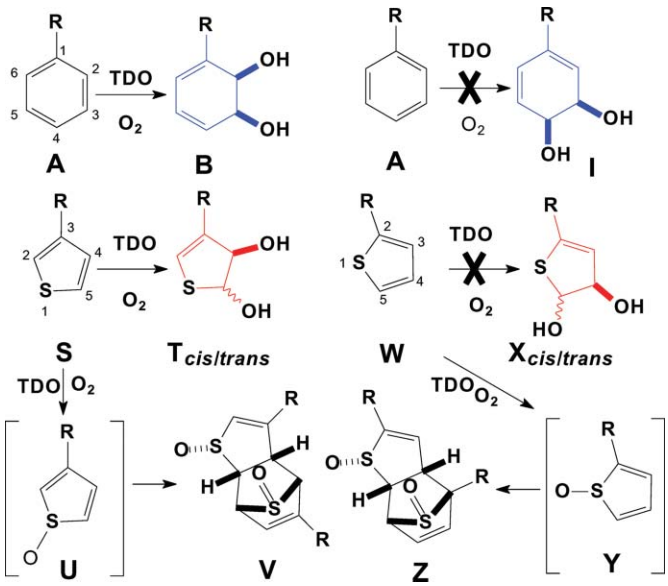
Similarly sequential sulfoxidation, then *cis*-dihydroxylation of alkylarylsulfide substrates to yield *cis*-diol sulfoxides containing three new chiral centres, *e.g.* **9** → **10** → **11** (Scheme 2b), has been observed.[37,38] Competing TDO-catalysed mono- and di-hydroxylation of alkylbenzene and alkylphenyl sulfide substrates were also found to occur during some biotransformations. However, while both benzylic alcohols and sulfoxides, *e.g.* **6** and **10**, were often good TDO substrates, the corresponding more water-soluble *cis*-dihydrodiol metabolites, *e.g.* **8** and **12**, were not. The ability of dioxygenases to catalyse alkane desaturation reactions yielding alkenes[6] and arenes,[24,28,29] has also resulted in a further type of tandem oxidation sequence involving alkane desaturation then *cis*-dihydroxylation, using both dihydro-(TDO)[24,28] and tetrahydro-arene substrates (BPDO).[29]

With a range of other dioxygenase-catalysed arene oxidation pathways available in addition to arene *cis*-dihydroxylation, the question of "Which dioxygenase-catalysed oxidation pathway is preferred?" is of relevance in the context of synthetic studies using whole cell systems. Attempts have recently been made to rationalise preferences for arene *cis*-dihydroxylation *vs.* benzylic hydroxylation,[31,37] arene *cis*-dihydroxylation *vs.* sulfoxidation[27,38] and arene *cis*-dihydroxylation *vs.* alkene dihydroxyation.[24] From these and other studies, a number of general trends were observed: (i) *cis*-dihydroxylation of monosubstituted monocyclic arenes occurs when using BDO, TDO or CDO but is less common when using NDO or BPDO, (ii) *cis*-dihydroxylation of disubstituted monocyclic arenes using TDO is generally slower relative to *cis*-dihydroxylation, benzylic hydroxylation, alkylaryl sulfoxidation or alkene dihydroxylation of monosubstituted arenes, (iii) arene *cis*-dihydroxylation of alkylbenzyl sulfides using TDO is much faster than sulfoxidation, while alkylaryl sulfides prefer the sulfoxidation pathway, (iv) TDO-catalysed arene *cis*-dihydroxylation of vinyl-benzene substrates is faster than alkene dihydroxylation when substituents are present on the alkene group but slower when they are present on the arene ring, (v) benzylic hydroxylation, sulfoxidation and alkene dihydroxylation using benzocyclo-alkanes, -alkenes, and -hetero analogues as substrates for TDO, are often observed while arene *cis*-dihydroxylation is rarely found, (vi) dialkylsulfide sulfoxidation, allylic hydroxylation and dealkylation are generally observed only when more facile TDO-catalysed oxidations, *e.g.* arene *cis*-dihydroxylation, are not possible, (vii) desaturation can be detected indirectly when dihydro- or tetrahydro-arene substrates are biotransformed *via* the arene bioproducts to the corresponding arene *cis*-dihydrodiols.

TDO-catalysed *cis*-dihydroxylation of electron-rich aromatic heterocyclic rings (*e.g.* thiophene, furan and pyrrole) has been observed[39–44] with the thiophene ring being more extensively studied. In common with monosubstituted benzene substrates **A**, thiophenes (general structures **S** and **W**, Scheme 3) are also planar aromatic rings with large resonance energies *i.e. ca.* 36 (**A**) and 29 kcal mol^{-1} (**S** or **W**), respectively. Similarly, substituted thiophene rings which are also prevalent in the environment,



Scheme 2 TDO-catalysed mono-,di- and tri-oxygenation pathways for propylbenzene **5** (Scheme 2a) and ethylphenylsulfide **9** (Scheme 2b)



Scheme 3 Comparison of TDO-catalysed *cis*-dihydroxylation of mono-substituted benzene and thiophene rings.

can again be biodegraded *via* TDO-catalysed dearomatisation to yield *cis*-dihydrodiols (Scheme 3). 3-Substituted thiophenes **S** yield the corresponding *cis*-dihydrodiol metabolites $\mathbf{T}_{cis}$ as a result of oxidation at the 4,5-bond (*cf.* *cis*-dihydroxylation of benzene substrates **A** at the 2,3-bond to give *cis*-dihydrodiols **B**). A major difference, however, was the ability of *cis*-dihydrodiols $\mathbf{S}_{cis}$ to isomerise spontaneously to the corresponding *trans*-dihydrodiols $\mathbf{T}_{trans}$ *via* an undetected acyclic aldehyde isomer.[43,44] However, 2-substituted thiophenes **W** were not converted into the corresponding *cis*/*trans*-dihydrodiols ($\mathbf{X}_{cis/trans}$ Scheme 3) (*cf.* the absence of *cis*-dihydroxylation of benzene substrates **A** at the 3,4-bond to yield *cis*-dihydrodiols **I**).

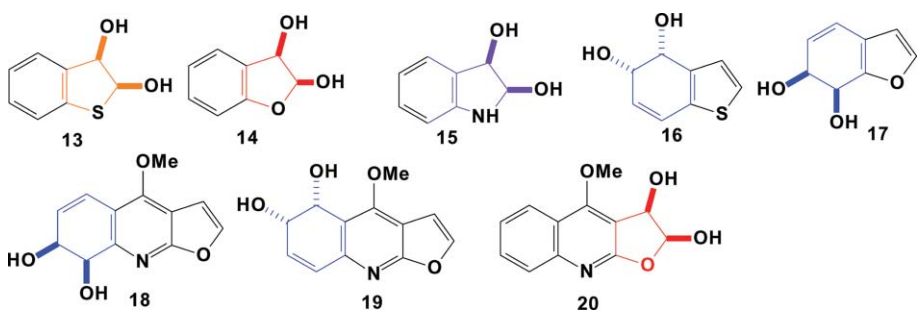
Alternative TDO-catalysed *S*-oxidation pathways for thiophenes also resulted in dearomatisation to yield transient thiophene sulfoxides (**U** and **Y**) which spontaneously racemise and dimerise to yield *bis*-sulfoxide cycloadducts (**V** and **Z**).[44] Biotransformation of 3-phenyl thiophene **S** (R = Ph) using TDO, indicated a regioselective preference (3 : 1) for *cis*-dihydroxylation of the carbocyclic over the heterocyclic ring.[44]

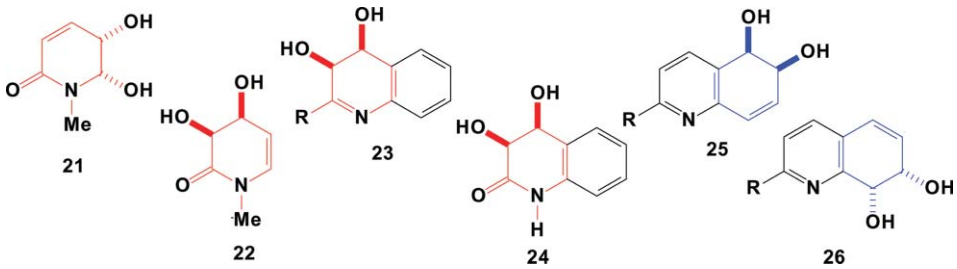
To date no *cis*-dihydrodiol metabolites of monocyclic furan or pyrrole substrates have been reported. It is highly probable that they are formed as transient intermediates which spontaneously open to yield the corresponding acyclic hydroxy-aldehydes or dehydrate to yield hydroxy-furan or -pyrrole products. Support for this view is provided by dioxygenase-catalysed oxidation of bicyclic benzothiophenes and benzofurans to yield isolable *cis*-dihydrodiols in both the heterocyclic (*e.g.* **13** and **14**) and carbocyclic rings (*e.g.* **16** and **17**).[37–44] The heterocyclic ring *cis*-dihydrodiols proved to be of variable stability with the benzofuran *cis*-dihydrodiol **14**[39,43] being less stable than the corresponding benzothiophene metabolite **13**.[41–44] The indole *cis*-dihydrodiol **15**, being too unstable to detect prior to spontaneous dehydration, formed indoxyl prior to autoxidation to give indigo dye.[45] Configurational instability (*cis*/*trans* isomerisation) was found in the heterocyclic *cis*-diols **13** and **14** (and presumably *cis*-diol **15**) but not in the carbocyclic analogues **16** and **17**. Control of regioselectivity during *cis*-dihydroxylation of benzothiophene was achieved by substitution of methyl groups at C-5 (exclusive *cis*-dihydroxylation in the heterocyclic ring), and C-3 (proportion of *cis*-dihydroxylation in the heterocyclic ring reduced but increased in the carbocyclic ring).[43]

It is surprising that, despite the well-established involvement of *cis*-dihydrodiol intermediates during dioxygenase-catalysed oxidations of arenes, with few exceptions (*e.g.* *p*-cymene[31]) virtually none have been isolated from aromatic natural products (*e.g.* alkaloids, coumarins, flavones, hemiterpenes, *etc.*). This could be the result of their transient nature and rapid conversion to phenols and catechols. The stable carbocyclic (**18**, **19**) *cis*-dihydrodiol metabolites, and transient heterocyclic analogue (**20**) recently obtained *via* BPDO-catalysed oxidation of the parent furoquinoline alkaloid dictamnine, may thus be forerunners of many other *cis*-dihydrodiol metabolites of natural products involved in both biosynthesis and biodegradation of natural products.[46]

Electron-poor azabenzene rings, *e.g.* pyridine, are generally unproductive substrates for dioxygenases and to date no *cis*-dihydrodiol metabolites of monocyclic pyridines have been isolated and characterised. Only alkyl monohydroxylation products were found when 2- and 3-alkylpyridine substrates were added to a mutant bacterial strain containing TDO.[47] As pyridine metabolites are likely to be highly unstable, spontaneous dehydration could occur and might account for the formation of monohydroxylated pyridines from biotransformations involving dioxygenase enzymes *e.g.* 3-hydroxy-4-methyl pyridine from 4-methyl pyridine[48] and 3-hydroxyquinoline from quinoline.[49] The monocyclic *cis*-dihydrodiols **21** and **22**, isolated as stable metabolites of 2-methylpyridone when using TDO, NDO or BPDO as biocatalysts, have similar structures to the elusive *cis*-dihydrodiols of substituted pyridines.[50,51]

Indirect evidence for the intermediacy of an unstable substituted pyridine *cis*-dihydrodiol **23** (R = Cl) was obtained when bioproduct **24** was isolated as a minor metabolite from TDO-catalysed oxidation of 2-chloroquinoline.[51] Spontaneous hydrolysis of the postulated pyridine *cis*-dihydrodiol **23** (R = Cl) could account for





the presence of compound **24** among the other major metabolites (**25**, **26**, R = Cl).[51] Azanaphthalene substrates in general appear to be acceptable substrates for dioxygenases to yield carbocyclic *cis*-dihydrodiols with heterocyclic phenols also being formed as minor bioproducts.[51–54]

In general terms, where polycyclic heteroarene substrates (bi-, tri- and tetra-cyclic) are biotransformed by dioxygenase enzymes (*e.g.* TDO, NDO and BPDO), *cis*-dihydroxylation will occur preferentially at a sterically hindered bay-region or fjord-region bond in a carbocyclic ring rather than at a more accessible bond. Both benzene rings and electron-rich five-membered heteroarene rings can be *cis*-dihydroxylated while electron-deficient azabenzene rings are generally resistant.

4. Conformations and configurations of arene *cis*-dihydrodiols

Although the parent benzene *cis*-dihydrodiol derivative **B** (R = H) appears to contain a plane of symmetry, in fact the diene moiety can adopt one of two enantiomeric helical conformations (enantiomeric *M* and *P* conformers) that rapidly interconvert *via* bond rotation. The chirality of *cis*-dihydrodiol derivatives of monosubstituted benzene substrates, *e.g.* **B** (R = Me), is thus due to both the *M*/*P* diene helicity and the presence of *R*/*S* stereogenic centres. Thus *cis*-dihydrodiols of this type can in principle exist as diastereoisomers (*e.g.* 1*S*, 2*R*, *M* or 1*S*, 2*R*, P, R = Me, Fig. 1). While the concept of *M*/*P* diene helicity in *cis*-dihydrodiol metabolites was recognised at an early stage through circular dichroism (CD) spectroscopy studies,[55] in the majority of subsequent papers this stereochemical feature has been overlooked.

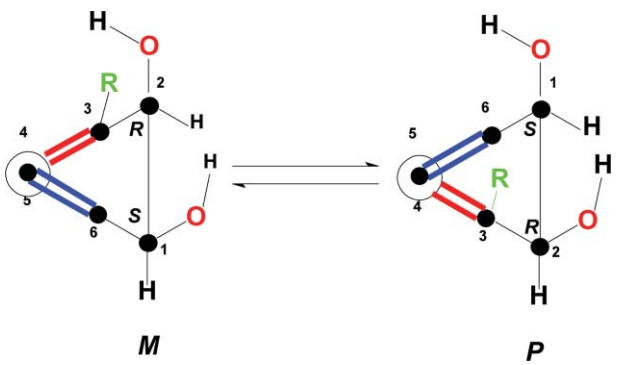


Fig. 1 Diastereoisomeric *M* and *P* conformations of *cis*-dihydrodiol **B**.

Recent studies[56] have shown that diastereoisomeric preferences for *cis*-dihydrodiol metabolites of both mono- and di-substituted benzene substrates exist in both the crystalline state (from X-ray crystallography), in solution (from experimental circular dichroism spectra, CD) and in the gas phase (from DFT calculated CD spectra).[56] Thus, X-ray crystallography has shown from six examples that there is a strong preference for the *M* conformation for most *cis*-dihydrodiols of type **B** (*e.g.* R = F or Br) in the crystalline phase[56] which could be due to steric repulsion between R group and the proximate OH group (on C-2) and/or hydrogen bonding interactions.

The equilibrium between the *M* and *P* diene conformers in *cis*-dihydrodiols of monosubstituted benzenes **B** in the solution or gaseous phase is however strongly dependent on the intramolecular OH–OH, OH–π and OH–F hydrogen bonding patterns. The OH group nearest to substituent R (on C-2) in the majority of similar type **B** *cis*-dihydrodiols (*e.g.* R = Me) appeared to prefer a pseudoequatorial conformation and a *P* diene conformation in solution (Fig. 1). *cis*-Dihydrodiol **B** (R = CF_3) was exceptional as the proximate OH group was pseudoaxial with an *M* diene configuration primarily due to a strong intramolecular OH–F hydrogen-bond (Fig. 1). It should be noted that *cis*-dihydrodiols **B** may show either a positive or a negative long-wavelength Cotton effect in the experimental CD spectra, depending on the conformer population and the nature of substituent R.[56]

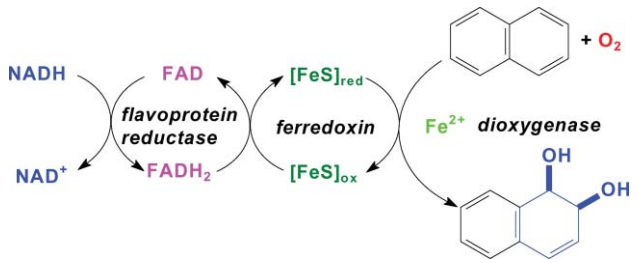
Methods used for the determination of absolute configuration of the *cis*-dihydrodiol metabolites also include X-ray crystallographic analysis of *cis*-dihydrodiols from substituted benzene substrates (*e.g.* from bromobenzene)[56] using the anomalous dispersion method. The ability to replace vinylic halogen substituents on the *cis*-dihydrodiol moiety (R or R′ *e.g.* Br or I) has also been used as a stereochemical correlation method for assignment of absolute configuration.[27,30,33]

Enantiopurity values for *cis*-dihydrodiol derivatives of both mono- and poly-cyclic arenes have been determined directly by chiral stationary phase HPLC, when both enantiomers are available.[27,34] The enantiomeric excess (ee) values of *cis*-dihydrodiols of monocyclic arenes have also been determined indirectly by: (i) GC-MS analysis of the corresponding *n*-butylboronate derivatives on a chiral stationary phase GC column (where both enantiomers are available),[57] (ii) NMR analysis following formation of cycloadducts using Cookson's dienophile (4-phenyl-1,2,4-triazoline-3,5-dione) followed by diesterification using (*R*)- and (*S*)-(α)-methoxy-α-(trifluoromethyl)phenylacetyl chloride (where one or both enantiomers are present),[30,58] and (iii) NMR analysis after formation of a boronate derivative by reaction with (*R*)- and (*S*)-2-(1-methoxyethyl)phenyl boronic acids (where one or both enantiomers are present).[27,29,59] Using a combination of these methods for enantiopurity determination of *cis*-dihydrodiols, the following trends were observed: (a) *cis*-dihydrodiols of all monosubstituted, 1,2-disubstituted and 1,3-disubstituted benzene substrates formed using TDO biocatalysis are enantiopure (>98% ee) except for those having only the

smallest substituents present *i.e.* F atoms, (b) *cis*-dihydrodiols of 1,4-disubstituted benzene substrates formed using TDO biocatalysis had ee values that varied depending on the relative sizes of substituents, (c) *cis*/*trans*-dihydrodiol metabolites isolated from TDO-catalysed dihydroxylation of thiophene or furan rings were found to have variable ee values (10–>98%) possibly due to partial racemisation *via* the acyclic aldehyde or ketone intermediates.

5. Dioxygenase active site structure and mechanism for dioxygenase-catalysed arene *cis*-dihydroxylation to yield arene *cis*-dihydrodiols

The dioxygenase system consists of three components, which form an electron transfer chain: an NADH-dependent flavoprotein reductase,[60] a ferredoxin containing two [2Fe2S] Rieske iron–sulfur clusters[61] and an $\alpha_3\beta_3$ Rieske oxygenase containing both a [2Fe2S] Rieske iron–sulfur cluster and a mononuclear iron(II) centre in the enzyme active site.[62–64] (Scheme 4).



Scheme 4 Electron transport chain in NDO.

On the basis of sequence alignments of the oxygenase α subunit, the Rieske dioxygenases have been grouped into four major clusters: (i) NDO, (ii) TDO/BPDO, (iii) BZDO and (iv) phthalate dioxygenase.[12,18]

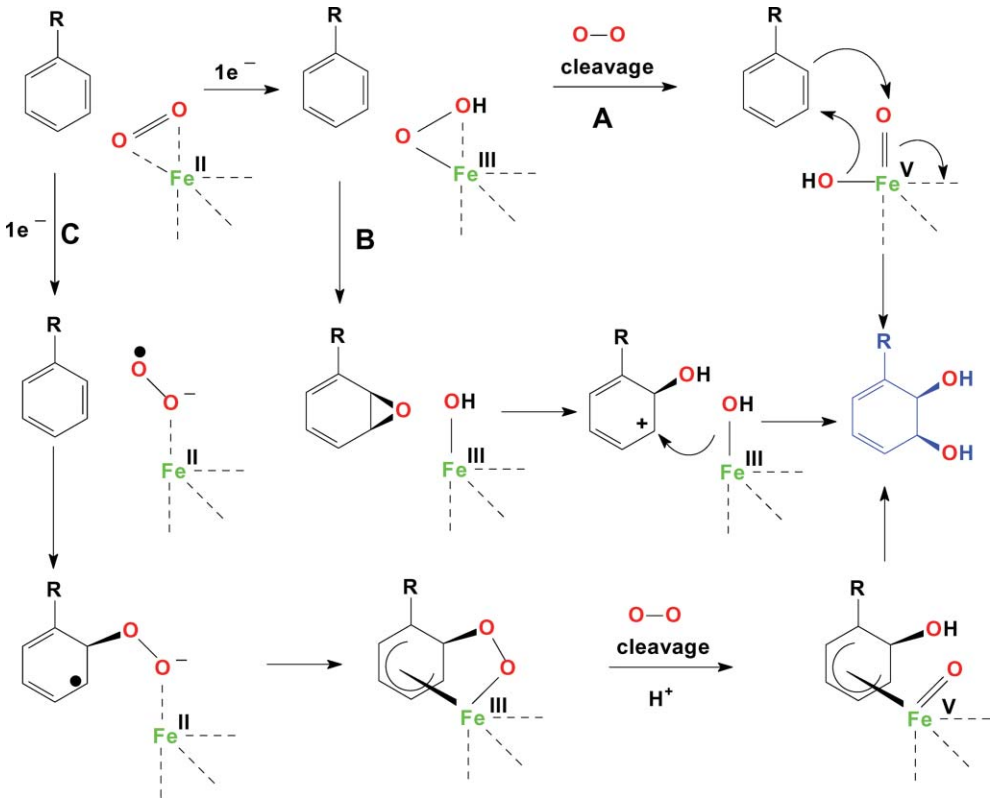
Recent crystallographic studies on Rieske dioxygenases have provided insights into possible catalytic mechanisms for *cis*-dihydrodiol formation. The active site of NDO contains a hydrophobic pocket with a mononuclear iron(II) centre, co-ordinated by His-208, His-213, and a bidentate Asp-362 (the 2-His-1-carboxylate motif).[65] The mononuclear iron(II) centre is positioned 12 Å from the [2Fe2S] cluster of another subunit in the $\alpha_3\beta_3$ oxygenase domain, which delivers two electrons to the dioxygenase active site during each turnover.[65] The original crystal structure contained an indole hydroperoxide ligand, ligated to the iron(II) centre, with the indole ring positioned at about 4 Å from the iron(II) centre,[66] possibly indicating the existence of a hydroperoxide intermediate in the catalytic mechanism. Structure determination of the ternary complexes with substrates (naphthalene or indole) and dioxygen have shown that dioxygen is bound side-on to the iron(II) centre, an unusual binding mode for dioxygen, and that the aryl substrate is positioned 4.3 Å from the iron(II) centre, beyond the bound oxygen.[67]

Single turnover studies of the NDO reaction have shown that turnover requires reduced enzyme and bound substrate, and that the mono-nuclear iron(II) centre and one Rieske [2Fe2S] cluster are oxidised during turnover, resulting in iron(III) at the end of the catalytic cycle.[68] Q-band ENDOR studies of NDO using ^{2}H-naphthalene have shown that the iron(II)-substrate distance shifts from 4.4 to 5.0 Å upon reduction of the [2Fe2S] cluster, implying that a conformational change occurs upon reduction, that may be significant during the catalytic cycle.[69] Structures of other Rieske dioxygenases have been determined recently.[12,64] The structure of a *Comamonas* NBDO is similar to that of the NDO enzyme,[65] with the exception of an active site Asn-258 residue, which forms a hydrogen bond to the nitro group of the substrate, and appears to be important in determining substrate specificity and regioselectivity.[17] The structure of a *Pseudomonas fluorescens* cumene dioxygenase shows an extended substrate binding site, into which a biphenyl substrate has been modelled.[70] The structure of 2-oxoquinoline 8-mono-oxygenase, which catalyses the hydroxylation of 2-oxoquinoline, is similar to that of NDO.[71] Structure determination of the oxidised and reduced enzyme showed a significant conformation change around the active site, suggesting that electron transfer from the [2Fe2S] cluster leads to this change, which in turn initiates the catalytic cycle.[71]

There is no clear consensus on the catalytic mechanism for *cis*-dihydroxylation. The involvement of [2Fe2S] iron-sulfur clusters implies that single electron transfer events are involved in the catalytic cycle. Scheme 5 shows that the catalytic cycle commences with a one electron reduction of Fe(III) to Fe(II) upon substrate binding and finishes with the iron cofactor in the + 3 oxidation state.[68] The observation of monooxygenase activity with certain substrates,[72] as noted elsewhere in this article, suggests that arene *cis*-dihydroxylation is not a concerted process, and that high-valent iron-oxo intermediates may be involved in mono-hydroxylation. It has been observed that hydrogen peroxide is released by NDO if benzene is used as substrate, which suggests that dioxygen is activated *via* reduction to superoxide, which in this case is uncoupled from substrate hydroxylation and is further reduced to peroxide.[73] Wolfe *et al.* have suggested that O–O bond cleavage occurs first, to give an O=Fe(V)–OH intermediate which could effect dihydroxylation (Scheme 5, path A) in a fashion similar to the dihydroxylation of alkenes by OsO_4.[68]

A recent theoretical study of possible mechanisms for the NDO-catalysed arene *cis*-dihydroxylation reaction has reported an activation energy barrier of 26.5 kcal mol^{-1} for the formation of an O=Fe(V)–OH intermediate, which is rather high for the enzyme-catalysed reaction.[74] A lower energy pathway (E_{act} 17.5 kcal mol^{-1}) involves the Fe(III)–OOH moiety acting as an electrophilic oxygen species, to form an epoxide (arene oxide) intermediate (Scheme 5, path B). Heterolytic C–O bond cleavage of this epoxide would form a carbocation, to which the second hydroxyl group is delivered suprafacially.

A third possible mechanism involves the formation of a hydroperoxide radical intermediate followed by O–O bond cleavage and delivery of a second oxygen atom from an iron(V)-oxo species (Scheme 5, path C). Calculations upon this pathway yielded an activation energy of about 18 kcal mol^{-1} for hydroperoxide formation, but the subsequent O–O cleavage of such an intermediate was found to proceed through a high energy barrier.[74] If mechanism B is operating, then one might expect to observe some arene oxide byproducts (obtainable by chemoenzymatic synthesis from the corresponding *cis*-dihydrodiols)[75] in *cis*-dihydrodiol formation which are not observed. One would also expect electron-withdrawing substrates, that would destabilise a carbocation, to be strongly disfavoured. The ability of Rieske dioxygenases to process electron-deficient aromatics (*e.g.* type **A** arenes, R = NO_2) would



Scheme 5 Possible catalytic mechanisms for dioxygenase-catalysed arene *cis*-dihydroxylation.

not support mechanism B. Recent ^{2}H-labelling studies on the hydroxylation of indene by NDO are consistent with a substrate radical intermediate.that can rotate prior to hydroxylation by an active oxygen species.[25] Stabilisation of a substrate radical intermediate by formation of an organometallic iron species (Scheme 5, path C) has precedent in the chemical literature,[76] but requires close contact of the substrate with the iron cofactor. Further mechanistic or fast kinetic studies are needed to evaluate the merits of these mechanistic possibilities.

Synthetic applications of arene *cis*-dihydrodiols

A rapid growth in the use of arene *cis*-dihydrodiols as chiral precursors for multi-step target synthesis has occurred since the pioneering work of Ley *et al.*[77] in 1987 and synthetic applications up to 2001 have been covered extensively in earlier reviews.[2–11] A major objective of this article is to highlight some of the more important recent applications and to identify relevant areas with potential for future development.

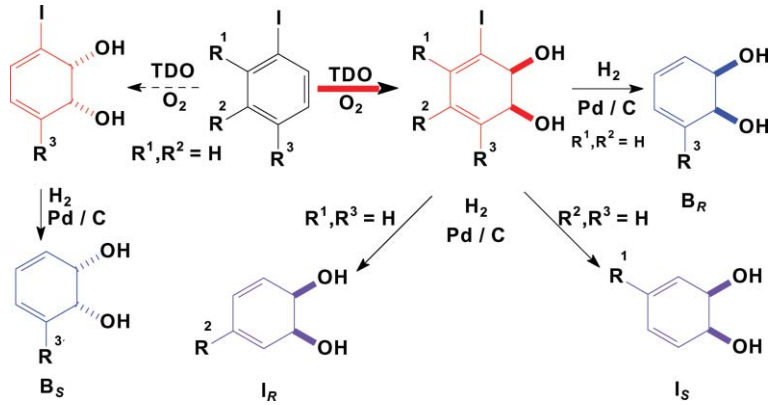
Despite the large numbers of *cis*-dihydrodiols of type $\mathbf{B}_S$ (Scheme 2) that have been reported (>50), only a small number have actually been used in synthesis (*e.g.* R = H, Cl, Br, I and Me). The earlier difficulty of accessing the majority of known benzene *cis*-dihydrodiols, and the relatively high cost of the small number of commercially available diols, has clearly been an inhibiting factor. Other limitations have been the unavailability of the unnatural *cis*-dihydrodiol regioisomers (*e.g.* **F** and **I**) and enantiomers of all regioisomers.

The earliest chemoenzymatic method to circumvent this limitation involved hydrogenolysis of an iodine atom from the *cis*-dihydrodiol metabolites of *ortho*-, *meta*- and *para*-substituted iodobenzene substrates to yield either the unnatural regioisomer $\mathbf{I}_R$ ($R^1 = R^3 = H$), its enantiomer $\mathbf{I}_S$ ($R^2 = R^3 = H$) or a mixture of enantiomers diol $\mathbf{B}_S/\mathbf{B}_R$ ($R^1 = R^2 = H$) of the natural *cis*-dihydrodiol (Scheme 6).[78]

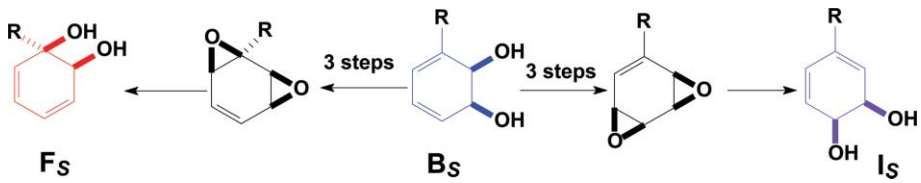
While this approach produced an unnatural regioisomer ($\mathbf{I}_R$) and two unnatural enantiomers ($\mathbf{I}_S$ and $\mathbf{B}_R$), in some cases the isolated yields from the substituted iodobenzene substrates were relatively low (*e.g.* from *meta*-iodobenzene), a mixture of enantiomers was produced ($\mathbf{B}_S/\mathbf{B}_R$, $R^2 = R^3 = H$, Scheme 6), and *cis*-dihydrodiols of type **F** were not produced (Scheme 1).

An alternative approach, based on a monosubstituted benzene *cis*-dihydrodiol $\mathbf{B}_S$, (R = alkyl) and involving a three-step synthesis and reduction of benzene dioxide intermediates, similar to that used to synthesise the corresponding *trans*-dihydrodiol analogues of compounds $\mathbf{B}_S$ and $\mathbf{I}_S$,[79] has recently been developed.[80] This provides an alternative route to *cis*-diol $\mathbf{I}_S$ and a new route to the previously unavailable regioisomer $\mathbf{F}_S$ (Scheme 7).

Earlier approaches to obtaining the reverse enantiomer $\mathbf{B}_R$ have involved the *cis*-diol dehydrogenase-catalysed kinetic resolution of enantiomeric mixtures $\mathbf{B}_R/\mathbf{B}_S$ obtained by direct biotransformation (*e.g.* of fluorobenzene) or by biotransformation/hydrogenolysis of *para*-substituted iodobenzene substrates.[78,81] The recent observation[57] that CBDO-catalysed *cis*-dihydroxylation of several monosubstituted benzene substrates bearing a nitrile group **A** (R = CN, CH_2CN, CH=CHCN) can yield both enantiomers $\mathbf{B}_R/\mathbf{B}_S$ (66–95% ee) is potentially very significant. While direct kinetic resolution of *cis*-dihydrodiols has been utilised using dehydrogenase enzymes, a tandem kinetic resolution process involving *cis*-dihydroxylation of such nitriles



Scheme 6 Chemoenzymatic synthesis of unnatural benzene *cis*-dihydrodiols $\mathbf{B}_R$, $\mathbf{I}_R$ and $\mathbf{I}_S$.



Scheme 7 Chemoenzymatic synthesis of unnatural benzene *cis*-dihydrodiols $\mathbf{F}_S$ and $\mathbf{I}_S$.

followed by stereoselective hydrolysis using nitrilase or amidase enzymes could in principle provide an alternative route to the unnatural *cis*-dihydrodiol enantiomers.

The recent study using CBDO[57] shows the potential of molecular biology methods to develop new recombinant strains containing a dioxygenase enzyme with the ability to yield both *cis*-dihydrodiol enantiomers. With the availability of *cis*-diol dehydrogenase enzymes having enantiocomplementary preferences *e.g.* benzene diol dehydrogenase, (BDD favouring $\mathbf{B}_R$)[19] and naphthalene diol dehydrogenase (NDD favouring $\mathbf{B}_S$),[37,81] it may also be possible to develop new recombinant strains containing (a) a dioxygenase that produces both enantiomers ($\mathbf{B}_R/\mathbf{B}_S$) and (b) an appropriate diol dehydrogenase to selectively remove either enantiomer. Ultimately it may also be possible to produce the unnatural enantiopure *cis*-dihydrodiols of types $\mathbf{B}_R$, $\mathbf{I}_S$, $\mathbf{I}_R$, $\mathbf{F}_S$ and $\mathbf{F}_R$ directly.

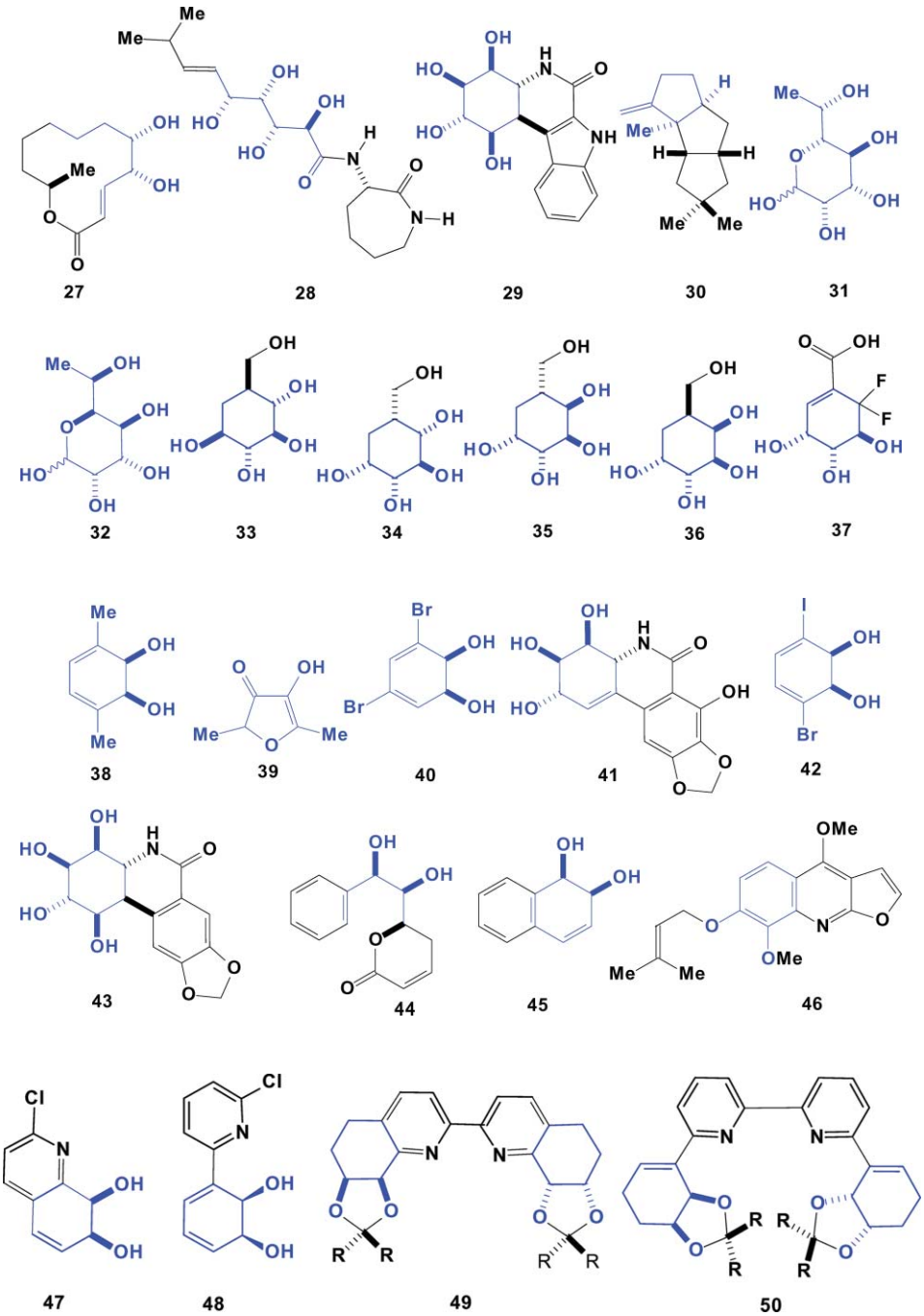
Benzene *cis*-dihydrodiol metabolites have been used to synthesise a wide range of natural and related unnatural products including alkaloids, carbohydrates, hemiterpenes, steroids and miscellaneous plant and marine metabolites. Within the limited scope of this article, it is impossible to do justice to the many elegant multi-step synthetic studies based on *cis*-dihydrodiol precursors. However, as the majority of these earlier multi-step syntheses have been discussed in reviews covering the literature up to 2001,[2–11] our focus will be on more recent studies. Some representative structures of recently synthesised natural products, unnatural analogues and the sections derived from their *cis*-dihydrodiol precursors are presented (blue).

The most widely used type $\mathbf{B}_S$ *cis*-dihydrodiol precursors for the synthesis of natural products have been those derived from chlorobenzene (R = Cl), bromobenzene (R = Br), and toluene (R = Me).[82] These chiral precursors continue to be extensively used and recent natural product target molecules synthesised include the macrolide (−)-cladospolide A, (**27**,11 steps from diol $\mathbf{B}_S$, R = Cl),[83] the marine metabolite (−)-*ent*-bengamide E (**28**,11 steps from diol $\mathbf{B}_S$, R = Br),[84] the *β*-carboline analogue of the *Amaryllidaceae* alkaloid 7-deoxypancratistatin (**29**,12 steps from diol $\mathbf{B}_S$, R = Br),[85] the sesquiterpene (−)-hirsutene (**30**,14 steps from diol $\mathbf{B}_S$, R = Br),[86] and the 6C-methyl-D-mannoses (**31** and **32**, 7 steps from diol $\mathbf{B}_S$, R = Me).[87]

On account of its easier halogen substitution reactions, the *cis*-dihydrodiol derived from iodobenzene ($\mathbf{B}_S$, R = I) is arguably the most versatile and useful of the four monohalogenated diols $\mathbf{B}_S$ (R = F, Cl, Br, I). Due to its lower stability and earlier commercial unavailability, its application in synthesis remains to be fully exploited. The reports on the synthesis of four carbasugars (**33**–**36**, 4–11 steps from diol $\mathbf{B}_S$, R = I)[88] and of 6,6-difluoroshikimic acid (**37**, 9 steps from diol $\mathbf{B}_S$, R = I)[89] is indicative of the increasing interest in this particularly useful bioproduct.

Recent studies also demonstrate the first synthetic applications of *cis*-dihydrodiols derived from disubstituted monocyclic arenes and polycyclic arenes or heteroarenes and of different dioxygenase types. These include the use of achiral diol **38** as precursor of a furanone strawberry flavour compound (**39**, 2 steps),[90] enantiopure diol **40** to yield alkaloid narciclasine (**41**, 11 steps),[91] and a scalemic mixture of diol **42** to give 7-deoxypancratistatin (**43**, 13 steps).[91] The bicyclic arene, naphthalene, has been used in the production of the styryllactone, (+)-goniodiol (**44**, 11 steps from diol **45**).[92]

Similarly, the parent furoquinoline alkaloid, dictamnine, a tricyclic heteroarene, yielded the enantiopure *cis*-dihydrodiol **18** which was in turn used as a synthetic precursor of several furoquinoline alkaloids including 7-isopentenyl-γ-fagarine (**46**, 3 steps from diol **18**).[46] The application of directed evolution methods in the production of compound **39** in high yield (*ca.* 20 g l^{-1}) from the *cis*-dihydrodiol **38**,[90] is a good example of the potential of this type of biotechnology in the manufacture of high-value compounds for the fine chemicals and food industry.



The application of enantiopure *cis*-dihydrodiols as a new type of chiral pool molecule remains to be developed in the context of catalytic asymmetric synthesis. Preliminary work from our laboratories[93] has already indicated that a new range of chiral bipyridine ligands can be synthesised from *cis*-dihydrodiols including compounds **47** and **48** derived from 2-chloroquinoline[51] and 2-chloro-6-phenyl pyridine respectively. Chiral bipyridines of general structures **49** and **50** were readily synthesised (3 steps) from *cis*-diols **47** and **48**, respectively. The potential of enantiopure bipyridine compounds **49** as chiral ligands has already been realised in asymmetric allylic hydroxylation, cyclopropanation and allylation reactions, to yield products often having high ee values (>98%).[93]

6. Conclusion

The primary objectives of this article are to provide information on the ever-increasing range of dioxygenase biocatalysts and *cis*-dihydrodiol bioproducts currently available for synthetic applications. While a universally acceptable mechanism for arene *cis*-dihydroxylation has not yet been agreed upon, alternative mechanistic proposals have been presented. With the major advances that have recently been made in molecular biology, the construction of new designer dioxygenases capable of producing *cis*-diols having unnatural configurations and regioisomers, and in improved yields, is now possible. Applications are expected to continue in the areas of new and improved synthetic routes

to increasingly complex biologically active natural products, *e.g.* morphine and vinblastine, using a wider range of monocyclic arene *cis*-dihydrodiol precursors. Further realisation of the full potential of the *cis*-dihydrodiols of polycyclic arenes and heteroarenes in the context of new chiral ligands of value in the catalytic production of single enantiomer pharmaceuticals *via* chemical asymmetric synthesis, is anticipated.

Acknowledgements

We wish to thank many friends and colleagues who have provided very helpful comments on both the biological and chemical aspects of our article and in some cases preprints of relevant articles currently in the press.

References

1 D. T. Gibson, J. R. Koch and R. E. Kallio, *Biochemistry*, 1968, **7**, 2653.
2 D. A. Widdowson and D. W. Ribbons, *Janssen Chim. Acta*, 1990, **8**, 3.
3 H. J. Carless, *Tetrahedron: Asymmetry*, 1992, **3**, 795.
4 G. N. Sheldrake, in *Chirality in Industry*, ed. A. N. Collins, G. N. Sheldrake and J. Crosby, J. Wiley, Chichester, 1992, ch. 6., p. 128.
5 S. M. Brown and T. Hudlicky, in *Organic Synthesis: Theory and Applications*, JAI Press, Inc., Greenwich, 1993, p. 113.
6 S. M. Resnick, K. Lee and D. T. Gibson, *J. Ind. Microbiol. Biotechnol.*, 1996, **17**, 438.
7 D. R. Boyd and G. N. Sheldrake, *Nat. Prod. Rep.*, 1998, **15**, 309.
8 T. Hudlicky, D. Gonzalez and D. T. Gibson, *Aldrichimica Acta*, 1999, **32**, 35.
9 D. T. Gibson and R. E. Parales, *Curr. Opin. Biotechnol.*, 2000, **11**, 236.
10 D. R. Boyd, N. D. Sharma and C. C. R. Allen, *Curr. Opin. Biotechnol.*, 2001, **12**, 564.
11 R. A. Johnson, *Org. React.*, 2004, **63**, 117.
12 R. E. Parales and S. M. Resnick, in *Pseudomomas Vol. 4, Molecular Biology of Emerging Issues*, ed. J.-L. Ramos and R. C. Levesque, Klewer Academic/Plenum Publishers, New York, 2005, in press.
13 T. Bugg, *Tetrahedron*, 2003, **59**, 7075.
14 D. T. Gibson, M. Hensley, H. Yoshioka and T. J. Mabry, *Biochemistry*, 1970, **9**, 1626.
15 D. R. Boyd, J. Blacker, B. Byrne, H. Dalton, M. V. Hand, S. C. Kelly, R. A. More O'Ferrall, S. N. Rao, N. D. Sharma and G. N. Sheldrake, *Chem. Commun.*, 1994, 313.
16 D. J. Lessner, G. R. Johnson, R. E. Parales, J. C. Spain and D. T. Gibson, *Appl. Environ. Microbiol.*, 2002, **68**, 634.
17 R. Friemann, M. M. Ivkovic-Jensen, D. J. Lessner, C.-L. Yu, D. T. Gibson, R. E. Parales, H. Eklund and S. Ramaswamy, *J. Mol. Biol.*, 2005, **348**, 1139.
18 L. P. Wackett, *Enzyme Microb. Technol.*, 2002, **31**, 577.
19 C. C. R. Allen, C. E. Walker, N. D. Sharma, N. A. Kerley, D. R. Boyd and H. Dalton, *Biocatal. Biotransform.*, 2002, **20**, 257.
20 N. Misawa, K. Shindo, H. Takahashi, H. Suenaga, K. Iguchi, H. Okazaki, S. Harayama and K. Fukawa, *Tetrahedron*, 2002, **58**, 9605.
21 G. N. Jenkins, D. W. Ribbons, D. A. Widdowson, A. M. Z. Slawin and D. J. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2647.
22 A. G. Myers, D. R. Siegel, D. J. Buzard and M. G. Charest, *Org. Lett.*, 2001, **3**, 2923.
23 D. R. Boyd, D. Clarke, M. C. Cleij, J. T. G. Hamilton and G. N. Sheldrake, *Monatsh. Chem.*, 2000, **131**, 673.
24 D. R. Boyd, N. D. Sharma, I. N. Brannigan, M. R. Groocock, J. F. Malone, G. McConville and C. C. R. Allen, *Adv. Synth. Catal.*, 2005, **347**, 1081.
25 D. R. Boyd, N. D. Sharma, N. I. Bowers, R. Boyle, J. S. Harrison, K. Lee, T. D. H. Bugg and D. T. Gibson, *Org. Biomol. Chem.*, 2003, **1**, 1298.
26 D. R. Boyd, N. D. Sharma, S. Haughey, M. A. Kennedy, J. F. Malone, S. Shepherd and C. C. R. Allen Howard Dalton, *Tetrahedron*, 2004, **60**, 549.
27 D. R. Boyd, N. D. Sharma, B. Byrne, S. A. Haughey, M. A. Kennedy and C. C. R. Allen, *Org. Biomol. Chem.*, 2004, **2**, 2530.
28 D. R. Boyd, N. D. Sharma, N. A. Kerley, R. A. S. McMordie, G. N. Sheldrake, P. Williams and H. Dalton, *J. Chem. Soc., Perkin Trans. 1*, 1996, 67.
29 D. R. Boyd, N. D. Sharma, F. Hempenstall, M. A. Kennedy, J. F. Malone, S. M. Resnick and D. T. Gibson, *J. Org. Chem.*, 1999, **64**, 4005.
30 D. R. Boyd, N. D. Sharma, B. Byrne, M. V. Hand, J. F. Malone, G. N. Sheldrake, J. Blacker and H. Dalton, *J. Chem. Soc., Perkin Trans. 1*, 1998, 1935.
31 D. R. Boyd, N. D. Sharma, N. I. Bowers, J. Duffy, J. S. Harrison and H. Dalton, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1345.
32 R. E. Parales, K. Lee, S. M. Resnick, H. Jiang, D. J. Lessner and D. T. Gibson, *J. Bacteriol.*, 2000, **182**, 1641.
33 R. E. Parales, S. M. Resnick, C. L. Yu, D. R. Boyd, N. D. Sharma and D. T. Gibson, *J. Bacteriol.*, 2000, **184**, 5495.
34 D. R. Boyd, N. D. Sharma, M. V. Hand, M. R. Groocock, N. A. Kerley, H. Dalton, J. Chima and G. N. Sheldrake, *Chem. Commun.*, 1993, 974.
35 D. R. Boyd, N. D. Sharma, R. Agarwal, S. M. Resnick, M. J. Schocken, D. T. Gibson, J. M. Sayer, H. Yagi and D. M. Jerina, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1715.
36 D. R. Boyd, N. D. Sharma, J. S. Harrison, M. A. Kennedy, C. C. R. Allen and D. T. Gibson, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1264.
37 D. R. Boyd, N. D. Sharma, B. E. Byrne, S. D. Shepherd, V. Ljubez, C. C. R. Allen, L. A. Kulakov, M. L. Larkin and H. Dalton, *Chem. Commun.*, 2002, 1914.
38 D. R. Boyd, N. D. Sharma, S. A. Haughey, J. F. Malone, A. King, B. T. McMurray, R. Holt and H. Dalton, *J. Chem. Soc., Perkin Trans. 1*, 2001, 3288.
39 D. R. Boyd, N. D. Sharma, R. Boyle, J. F. Malone, J. Chima and H. Dalton, *Tetrahedron: Asymmetry*, 1993, **4**, 1307.
40 D. R. Boyd, N. D. Sharma, M. A. Kennedy, S. Shepherd, J. F. Malone, A. Alves-Areias, R. Holt, S. G. Allenmark, M. A. Lemurell, H. Dalton and H. Luckarift, *Chem. Commun.*, 2002, 1452.
41 D. R. Boyd, N. D. Sharma, R. Boyle, B. T. McMurray, T. A. Evans, J. F. Malone, H. Dalton, J. Chima and G. N. Sheldrake, *Chem. Commun.*, 1993, 49.
42 R. W. Eaton and J. D. Nitterauer, *J. Bacteriol.*, 1994, **176**, 3992.
43 D. R. Boyd, N. D. Sharma, I. N. Brannigan, D. A. Clarke, H. Dalton, S. A. Haughey and J. F. Malone, *Chem. Commun.*, 1996, 2361.
44 D. R. Boyd, N. D. Sharma, N. Gunaratne, S. A. Haughey, M. A. Kennedy, J. F. Malone, C. C. R. Allen and H. Dalton, *Org. Biomol. Chem.*, 2003, **1**, 984.
45 B. D. Ensley, B. J. Ratzkin, T. D. Osslund, M. J. Simon, L. P. Wackett and D. T. Gibson, *Science*, 1983, **222**, 167.
46 D. R. Boyd, N. D. Sharma, C. O'Dowd, J. G. Carroll, P. L. Loke and C. C. R. Allen, *J. Chem. Soc., Chem. Commun*, 2005, 3989.
47 M. D. Garrett, R. Scott and G. N. Sheldrake, *Tetrahedron: Asymmetry*, 2002, **13**, 2201.
48 T. Sakamoto, J. M. Joern, A. Arisawa and F. H. Arnold, *Appl. Environ. Microbiol.*, 2001, **67**, 3882.
49 S. A. Barr, N. Bowers, D. R. Boyd, N. D. Sharma, L. Hamilton, R. A. S. McMordie and H. Dalton, *J. Chem. Soc., Perkin Trans. 1*, 1998, 3443.
50 L. Modyanova and R. Azerad, *Tetrahedron Lett.*, 2000, **41**, 3865.
51 D. R. Boyd, N. D. Sharma, L. V. Modyanova, J. G. Carroll, J. F. Malone, C. C. R. Allen, J. T. G. Hamilton, D. T. Gibson, R. E. Parales and H. Dalton, *Can. J. Chem.*, 2002, **80**, 589.
52 D. R. Boyd, R. A. S. McMordie, H. P. Porter, H. Dalton, R. O. Jenkins and O. W. Howarth, *Chem. Commun.*, 1987, 1722.
53 D. R. Boyd, N. D. Sharma, M. R. J. Dorrity, M. V. Hand, R. A. S. McMordie, J. F. Malone, H. P. Porter, J. Chima, H. Dalton and G. N. Sheldrake, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1065.
54 S. Fetzner, B. Vogler and F. Lingens, *Microbiol. Lett.*, 1993, **112**, 151.
55 H. Ziffer, K. Kabuto, D. T. Gibson, V. M. Kobal and D. M. Jerina, *Tetrahedron*, 1977, **33**, 2491.
56 J. Gawronski, M. Kwit, D. R. Boyd, N. D. Sharma, J. F. Malone and A. Drake, *J. Am. Chem. Soc.*, 2005, **127**, 4308.
57 S. Yildirim, T. T. Franko, R. Wohlgemuth, H.-P. E. Kohler, B. Witholt and A. Schmid, *Adv. Synth. Catal.*, 2005, **347**, 1060.
58 D. R. Boyd, M. R. J. Dorrity, M. V. Hand, J. F. Malone, N. D. Sharma, H. Dalton, D. J. Gray and G. N. Sheldrake, *J. Am. Chem. Soc.*, 1991, **113**, 666.
59 S. M. Resnick, D. S. Torok and D. T. Gibson, *J. Org. Chem.*, 1995, **60**, 3546.
60 B. E. Haigler and D. T. Gibson, *J. Bacteriol.*, 1990, **172**, 457.
61 B. E. Haigler and D. T. Gibson, *J. Bacteriol.*, 1990, **172**, 465.

62 B. D. Ensley and D. T. Gibson, *J. Bacteriol.*, 1983, **155**, 505.
63 W. C. Suen and D. T. Gibson, *J. Bacteriol.*, 1993, **175**, 5877.
64 D. J. Ferraro, L. Gukhar and S. Ramaswamy, *Biochem. Biophys. Res. Commun.*, 2005, **338**, 175.
65 B. Kauppi, K. Lee, E. Carredano, R. E. Parales, D. T. Gibson, H. Eklund and S. Ramaswamy, *Structure*, 1998, **6**, 571.
66 E. Carredano, A. Karlsson, B. Kauppi, D. Choudhury, R. E. Parales, J. V. Parales, K. Lee, D. T. Gibson, H. Eklund and S. Ramaswamy, *J. Mol. Biol.*, 2000, **296**, 701.
67 A. Karlsson, J. V. Parales, R. E. Parales, D. T. Gibson, H. Eklund and S. Ramaswamy, *Science*, 2003, **299**, 1039.
68 D. Wolfe, J. V. Parales, D. T. Gibson and J. D. Lipscomb, *J. Biol. Chem.*, 2001, **276**, 1945.
69 C. Yang, M. D. Wolfe, M. B. Neibergall, Y. Mekmouche, J. D. Lipscomb and B. M. Hoffmann, *J. Am. Chem. Soc.*, 2003, **125**, 2035.
70 Dong, S. Fushinobu, E. Fukuda, T. Terada, S. Nakamura, K. Shimizu, H. Nojiri, T. Omori, H. Shoun and T. Wakagi, *J. Bacteriol.*, 2005, **187**, 2483.
71 B. M. Martins, T. Svetlitchnaia and H. Dobbek, *Structure*, 2005, **13**, 817.
72 L. P. Wackett, L. D. Kwart and D. T. Gibson, *Biochemistry*, 1988, **27**, 1360.
73 K. Lee, *J. Bacteriol.*, 1999, **181**, 2719.
74 A. Bassan, M. R. A. Blomberg and P. E. M. Siegbahn, *JIBC, J. Biol. Inorg. Chem.*, 2004, **9**, 439.
75 D. R. Boyd, N. D. Sharma, H. Dalton and D. A. Clarke, *Chem. Commun.*, 1996, 45.
76 A. J. Pearson, *Acc. Chem. Res.*, 1980, **13**, 463.
77 S. Ley, F. Sternfield and S. Taylor, *Tetrahedron Lett.*, 1987, **28**, 225.
78 D. R. Boyd, N. D. Sharma, S. A. Barr, H. Dalton, J. Chima, G. M. Whited and R. Seemayer, *J. Am. Chem. Soc.*, 1994, **116**, 1147.
79 D. R. Boyd, N. D. Sharma, C. R. O'Dowd and F. Hempenstall, *Chem. Commun.*, 2000, 2151.
80 D. R. Boyd, N. D. Sharma, N. M. Llamas and C. C. R. Allen, manuscript submitted.
81 C. C. R. Allen, D. R. Boyd, N. D. Sharma, H. Dalton, I. N. Brannigan, N. A. Kerley, G. N. Sheldrake and S. C. Taylor, *Chem. Commun.*, 1995, 117.
82 M. Endoma, V. P. Bui, J. Hansen and T. Hudlicky, *Org. Process Res. Dev.*, 2002, **6**, 525.
83 M. G. Banwell, K. A. Jolliffe, D. T. J. Loong, K. J. McRae and F. Vounatsos, *J. Chem. Soc., Perkin Trans. 1*, 2002, 22.
84 M. G. Banwell and K. J. McRae, *J. Org. Chem.*, 2001, **66**, 6768.
85 U. Rinner, T. Hudlicky, H. Gordon and G. R. Petit, *Angew. Chem., Int. Ed.*, 2004, **43**, 5342.
86 M. G. Banwell, A. J. Edwards, G. J. Harfoot and K. A. Jolliffe, *J. Chem. Soc., Perkin Trans. 1*, 2002, 2439.
87 M. G. Banwell, A. M. Bray, A. J. Edwards and D. J. Wong, *New J. Chem.*, 2001, **25**, 3.
88 D. R. Boyd, N. D. Sharma, N. Llamas, J. F. Malone, C. R. O'Dowd and C. C. R. Allen, *Org. Biomol. Chem.*, 2005, **3**, 1953.
89 J. L. Humphreys, D. J. Lowes, K. A. Wesson and R. C. Whitehead, *Tetrahedron Lett.*, 2004, **45**, 3429.
90 L. M. Newman, H. Garcia, T. Hudlicky and S. A. Selifonov, *Tetrahedron*, 2004, **60**, 729.
91 T. Hudlicky, U. Rinner, D. Gonzalez, H. Akgun, S. Schilling, P. Siengalewicz, T. A. Martinot and G. R. Petit, *J. Org. Chem.*, 2002, **67**, 8726.
92 M. G. Banwell, M. J. Coster, O. P. Karunaratne and J. A. Smith, *J. Chem. Soc., Perkin Trans. 1*, 2002, 1622.
93 D. R. Boyd,N. D. Sharma,T. Belhocine,S. James,D. M. Murphy,E. Sbercia,C. C. R. Allen, manuscript in preparation.

