Applications of biocatalytic arene ipso,ortho cis-dihydroxylation in synthesis

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The dearomatising dihydroxylation of aromatic molecules mediated by arene dioxygenase enzymes can provide cyclohexadiene-diols that are versatile starting materials for organic synthesis. Whereas oxidation of a substituted arene to give its ortho,meta-dihydriodiol has been demonstrated for numerous substrates and dioxygenases, formation of ipso,ortho-dihydriodiol has historically been underutilised in comparison. This feature article presents a chronological account of reported uses of such diols.

Introduction

The first example of the dearomatising dihydroxylation of an aromatic ring by a microorganism was reported by Gibson in 1968.1 Some two decades later, it was recognised that the cyclohexadiene diols formed in this manner were useful starting materials for synthesis by virtue of their densely-packed, differentiated functionality. Large-scale production and use of these arene-derived diols has become established methodology and many are now commercially available; the field at large has been the subject of several excellent reviews.2

If the aromatic substrate is substituted (i.e. all cases other than benzene), multiple isomeric diol products can be envisaged. In fact, however, such bio-oxidations are highly selective and a widely-applicable predictive model has been developed by Boyd and co-workers.3 As shown in Scheme 1, the diol 2 derived from oxidation at the ortho- and meta-positions of substrate 1 is the sole product. Furthermore, in most cases, 2 is formed as a single enantiomer.4

The selectivity shown above is conserved across a large number of substrates and dioxygenases, for example toluene dioxygenase (TDO), naphthalene dioxygenase (NDO) and biphenyl dioxygenase (BPDO). However, exceptions to this predictive model are known. Organisms expressing benzoate dioxygenase (BZDO) enzymes dihydroxylate benzoic acids in a process that proceeds not only with different regioselectivity but also the opposite absolute sense of enantioinduction to that shown in Scheme 1. In these cases, the diol 4 derived from oxidation at the ipso- and ortho-positions of substrate 3 is isolated.

Initial isolation and substrate scope

The parent ipso,ortho benzoate dihydrodiol (4, R = H) was first reported in 1971 by Reiner and Hegeman.5 The prokaryote Alcaligenes eutrophus (now known as Ralstonia eutropha) was known to be able to metabolise benzoate via catechol and the β-ketoadipate pathway. Benzoate dihydrodiol 4 is the first
intermediate in this pathway, but in the wild-type organism it is only a fleeting metabolic intermediate and never accumulates to a synthetically useful concentration. The B9 mutant strain of \textit{R. eutropha} expresses BZDO able to mediate the formation of 4, but it possesses a lesion which renders the second enzyme in the pathway (DHB dehydrogenase) inactive. Accordingly, 4 was prepared by fermentation of benzoate with the B9 strain. Isotopic labelling studies demonstrated that both hydroxyl oxygens in 4 are incorporated from the same oxygen molecule (Scheme 3).

Reiner and Hegeman characterised 4 spectroscopically and inferred the cis relationship of the vicinal diol by means of chemical correlation, although the absolute configuration remained undetermined. They also deduced the positions of hydroxylation to be ipso and ortho by considering the products formed when 4 undergoes decomposition by re aromatisation at 45 °C or above, i.e. phenol and salicylic acid.

**Early uses in synthesis – cycloadditions and other diene functionalisations.**

In 1986, Ribbons et al. demonstrated that \textit{Pseudomonas putida} U103 was capable of accumulating cis diol 4 (R = H) in the same fashion as \textit{R. eutropha} B9. It was not until 1995, however, that cis diol 4 produced in this way was exploited for synthetic ends, by Widdowson and co-workers in collaboration with Ribbons. Comparison of [α] values showed that the material produced by the two organisms was the same enantiomer, but the absolute configuration remained undetermined. Widdowson and Ribbons addressed this through the synthesis of a derivative 14 containing a heavy atom and its crystallographic analysis, establishing the (1S,2R) configuration for the first time (Figure 3).

Widdowson’s report further described a variety of [4+2] cycloadditions employing derivatives of 4 as the dienes. For the more common arene ortho,meta diols of type 2, many such reactions had already been reported and a trend in the regioselectivity had been discerned: substrates with an unprotected diol undergo cycloaddition at the diene face syn to the diol, whereas when the diol is protected as a ketal, cycloaddition occurs at the anti face. In such dienes of type 2, the substituent R is in the plane of the diene and would not be expected to influence the regioselectivity of cycloaddation. In contrast, dienes derived from 4 possess a substituent at the C1 quaternary centre which is oriented over the diene face anto to the diol. The effect of this on the regioselectivity of various cycloadditions was determined (Scheme 4).

With acetonide protected substrates 15, singlet oxygen cycloaddition was found to proceed at the face anti to the acetonide, giving 16 as the sole product, even when the quaternary centre bears a bulky –CH3OTBDPS group. Similarly, treatment of acetonide 17 with N-phenyl urazole 18 gave adduct 19 only. In contrast, free diol 20 gave adduct 21 (in which dienophile and diol are syn) as the major product. Treatment of acetonide 17 with a non-symmetrical heterodienophile, nitrosobenzene, gave addition exclusively anti to the acetonide, but a mixture of regioisomers 23 and 24 was obtained; the adduct 23 in which the N-phenyl substituent is distal to the ester was the major product. Reaction of free diol 20 with nitrosobenzene gave all four possible regioisomers (not...
shown). Interestingly, when an acetonide with a reduced side chain, 25, was used in place of 17 in the nitrosobenzene cycloaddition, regioselectivity was reversed, with the major product 27 being the one in which the N-phenyl substituent and the ester are proximal.

In 2001, Myers and co-workers reported the synthesis of a library of derivatives of 4, demonstrating that each position on the ring may be functionalised selectively (Figure 4). While selective protection of the secondary alcohol in 4 is straightforward (e.g. 28 and 29) protection of the tertiary alcohol necessitated an indirect route via 30, which upon desilylation gave 31. This in turn underwent oxidation to cyclohexadienone 32 (a transformation which is low-yielding for the unprotected diol 20). Selective epoxidations to 33 and 34 were demonstrated, along with ring openings to diastereomeric trans diols 35 and 36; cis diols were accessible by OsO₄ catalysed dihydroxylation, e.g. 37. Intramolecular epoxide opening was shown to afford lactone 38 and acetalisation of the diol in 33 (a highly acid-sensitive substrate) was achieved under neutral conditions to give 39 (R/S 2:3). Bromolactonisation of 20 gave β-lactone 40, which underwent attack by methoxide to give 41. Attempts to access enones such as 42 through rearrangement of C3,C4 epoxides such as 33 were unsuccessful. Instead, an unexpected vinylogous Payne rearrangement was discovered: methylation of 33 followed by treatment with tert-butylimethylsilyl triflate led to formation of trisubstituted epoxide 43 in good yield.

Scheme 5. Johnson & Johnson synthesis of topiramate analogues.

Also in 2004, Mihovilovic and co-workers reported on intramolecular cycloadditions of derivatives of 4 bearing a tethered dienophile (Scheme 6). Cyclisation of 49 to 50 was high yielding, but introduction of an sp² centre in the tether retarded reaction: 51 gave 52 only under forcing conditions of microwave acceleration and 53 did not undergo intramolecular cyclisation at all. Interestingly, disubstituted dienophile 54 gave a mixture of diastereomeric products 55 and 56. Finally, in contrast to ester 53, amide 57 was able to cyclise to 58, albeit only under forcing conditions and in low yield; the structure of the cycloadduct 58 was secured by x-ray crystallography. This work demonstrated the possibility of rapidly accessing complex polycyclic architectures from 4.

Figure 4. Chirons derived from 4 by Myers et al.

The Myers group also reported a modified protocol for the fermentation of benzoate to produce 4 which was amenable to large-scale application. By this method, 270g of 4 was produced in a single batch. The authors speculated that the novelty of the building blocks shown above in conjunction with large-scale access to the starting material would enhance interest in the use of 4. Indeed, in 2004, Parker and co-workers at Johnson & Johnson reported using 37 to access carbocyclic analogues of the anticonvulsant agent topiramate 46, as well as carba-β-L-fructopyranose 47 and a C-substituted conduritol 48 (Scheme 5.).

First total synthesis

In 2005, Myers and co-workers reported the first use of 4 in complex natural product total synthesis. From their previously reported building block 43, tricyclic diketone 59 was accessible in a further 7 steps (10% overall yield from benzoate, Scheme 7). Diketone 59 serves as a common precursor to the
tetracycline AB-ring system and may be coupled with D-ring precursors such as 60 by a Michael–Dieckmann cascade cyclisation that forms the C-ring. Thus, after deprotection, the natural product (−)-6-deoxytetracycline 61 is accessible in 14 steps and 7.0% overall yield from benzoate. Several points about the synthesis are noteworthy. The yield represents an improvement of orders of magnitude over the yields for all previously reported total syntheses of tetracyclines. Thus, for the first time, novel tetracycline analogues became accessible in useful quantities; union of 62 with 59 to access 63 is a representative example. Secondly, previous total syntheses of tetracyclines had been bedevilled by the difficulty of installing the C12a tertiary alcohol at a late stage.\textsuperscript{14c} The Myers approach is conceptually distinct in that the C12a hydroxy group is installed in the very first step, i.e. it is the hydroxy group deriving from the microbial ipso hydroxylation. Finally, apart from the C12a stereocentre, all other stereocentres in the final tetracyclines are set under substrate control. Thus, all the stereochemical information in the final products may be considered ultimately to be of enzymatic origin.

Production with a recombinant organism

In 2008, Chen and co-workers reported the production of a recombinant strain of Pseudomonas putida, KTSY01 (pSYM01) expressing benzoate dioxygenase and able to effect formation of 4 on a 59g scale.\textsuperscript{17} This organism is engineered to overexpress the benABC genes from Pseudomonas putida KT2442 that encode benzoate dioxygenase, but lacks the benD gene that encodes the DHB dehydrogenase responsible for the further metabolism of 4 (c.f. Scheme 3). A potential advantage of using this organism for production of 4 is that it is not susceptible to the unwanted formation of revertants. In contrast, use of organisms such as Ralstonia eutropha B9 where inactivation of DHB dehydrogenase was achieved through random mutagenesis entails a risk of spontaneous reactivation, and hence consumption of 4 in the fermentation medium.

Organometallic chemistry

The formation of tricarbonyliron(0) complexes of arene ortho,meta diols of type 2 was known methodology\textsuperscript{18} and in 2010 we extended this to an arene ipso,ortho diol for the first time (Scheme 9). Formation of these \[\eta^1\] complexes from dienes of type 2 was known to be selective for the isomers in which the metal is endo with respect to the diol, i.e. 72 and 74. This had been rationalised in terms of an incoming 16 valence-electron Fe(CO)\textsubscript{4} fragment coordinating to the Lewis basic oxygen functionality before migrating to the diene. By this argument, it was not clear at the outset which face of diene 20 would be favoured in the complexation, since due to the quaternary centre 20 presents Lewis basic functionality on both sides of the ring. In the event, the isomer 75 with the metal endo to the diol was formed exclusively and the structure was secured by crystallography. The impetus for introducing the
The tricarbonyliron group had been to protect the diene functionality in 20. That tricarbonyliron was effective in this regard was demonstrated by clean oxidation of 75 to complexed cyclohexadienone 76. This transformation is low-yielding for unprotected 20 and necessitated benzyloxymethyl protection of the tertiary hydroxyl as 31 (Figure 4) when this transformation was examined by Myers. Dimerisation of cyclohexadienones is preceded and in the case of 32 reportedly proceeded with t1/2 ≈ 4 h (0.6 M, 23 °C, CDCl3). In contrast 76 was stable indefinitely.

In 2011, in an attempt to access a complex of 4 bearing the metal on the upper face, we subjected acetonide ester 17 to the same reaction conditions (Scheme 10), in the expectation that protection of the diol as an acetonide would attenuate its directing ability. In the event, a product was isolated in which the iron was indeed coordinated to the upper diene face, but in which a rearrangement had occurred, conjugating the ester to the diene and giving 77.20 We rationalise this result through initial formation of the expected product 78 and its subsequent co-ordination of an unknown Lewis acidic species to give 79. C–O bond scission would then afford cationic cyclohexadienyl complex 80. It is known that [η3] complexes such as 80 undergo nucleophilic attack at the termini of the dienyl system and the acetonide in 80 constitutes a tethered nucleophile. Its recombination at the position ω- to the ester21 and decomplexation of the Lewis acid would afford the observed product. 77. In support of this mechanism, we observed that selectively deuterated ipso,ortho diol 81 gave rise to 83 and not the isomeric 82, thereby demonstrating that the rearrangement involves “clockwise” acetonide migration and not “anticlockwise” ester migration. Decomplexation of 77 gave 84, which is significant insofar as it is the opposite enantiomer of the arene ortho,meta derivative that would be obtained by direct arene biotransformation (c.f. Scheme 1). Interestingly, formation of an isolobal cobalt cyclopentadienyl complex from 17 did not result in any such acetonide migration and instead gave 85.22
The organoiron chemistry described above has found application in total synthesis. Also in 2011, we described a route to (+)-grandifloracin (+)-94, a polyoxygenated cyclohexene derivative isolated from species in the genus Uvaria. The structure is a dimer deriving from Diels–Alder reaction of cyclohexadienone 95 in a remarkably regio- and stereoselective reaction.24 Accordingly, it proved possible to adapt the methodology shown in Scheme 9 for a concise synthesis of (+)-94.25 Complex 75 may be reduced to triol 96, which is then benzyolated to give 97. Chemoselective oxidations were then required: manganese dioxide had been established as the oxidant of choice for oxidising the secondary alcohol without cleaving the metal fragment, in this case giving 98. A different oxidant, in this case cerium ammonium nitrate, deprotected the diene to give 95, which underwent facile dimerisation to give (+)-94. At the time this work was carried out, grandifloracin had been isolated from Nature as a single enantiomer of unknown absolute configuration.26 Our work described the synthesis of material of known absolute configuration, but when its optical rotation was compared with that reported for the natural material, we found that our material (of structure (+)-94) had a positive rotation, whereas the natural material had a negative rotation and hence structure (−)-94. On this basis we stated that we had synthesised the “non-natural enantiomer of grandifloracin”. However, a year after our report, Awale and co-workers reported the isolation of (+)-94 from a different species of the same genus.27 Thus, both enantiomers of grandifloracin are in fact found in Nature and our statement that we had synthesised “the non-natural enantiomer” was in fact incorrect. Furthermore, Awale reported that the (+) enantiomer is an “anti-austerity” agent, i.e. shows preferential antiproliferative activity against pancreatic cancer cell lines in a nutrient-deprived condition.

Oxygenation

In the same year, we described the synthesis of “inosamino acids”, amino acid-inositol hybrid structures accessed from 4.28 An acynitroso dienophile (generated in situ by periodate oxidation of 100) reacted with benzyl ester 99 to give separable isomeric adducts 101 and 102 (Scheme 13). The major product (101) was that in which the benzyl ester was distal to the Cbz group, analogous with formation of 23 over 24 (Scheme 4). Additional oxygenation was introduced by stereoselective dihydroxylation of the residual olefin in 101 and 102, giving 103 and 104 respectively. Hydrogenolysis of these diols effected multiple reductive operations in one pot; this was followed by deprotection with aqueous acid to give inosaminoacids 107 and 108. These inosaminoacids possess six contiguous stereocentres, including the quaternary centre, and were accessed in just seven steps from benzoate, highlighting the usefulness of 4 for the rapid introduction of complexity.
Another example in 2011 of the synthesis of a highly oxygenated material from 4 was the total synthesis of (-)-idesolide 112 reported by Hudlický et al. This is another dimeric natural product, formed from a ketone, but in this instance the diene motif was reduced beforehand (Scheme 14). Reduction of one of the two olefins in 20 was effected with potassium azodicarboxylate, giving a 2:1 ratio favouring the desired isomer 109. This in turn was oxidised to ketone 111, which underwent base-mediated dimerisation to (-)-idesolide 112. Another noteworthy transformation reported by the group was the direct isomerisation of 20 to enone 113 using Grubbs’ 1st generation Ru metathesis catalyst.

In 2012, we returned to the polyoxygenated cyclohexene family of natural products, of which there are numerous monomeric members in both enantiomeric series in addition to the dimer grandifloracin 94. The key transformation to access both the zeylenol and zeylenone families of natural product was singlet oxygen cycloaddition, which transformed diene 114 into endoperoxide 115. From this key intermediate many members of these families were accessed. A representative example is shown in Scheme 15, whereby reductive O–O bond cleavage with thiourea gave diol 116. Straightforward benzylation and global deprotection gave uvaribonol A 118 as well as the parent (+)-zeylenol 119 by benzoyl migration.

Whereas access to the zeylenols required a reductive transformation of endoperoxide 115, access to the more highly oxygenated zeylenones ought to be possible from 115 by a redox-neutral process. The γ-hydroxy-α,β-unsaturated ketone motif in the zeylenoles could conceivably be accessed from the endoperoxide by a Kornblum–DeLaMare rearrangement. A variety of endoperoxides of general structure 16 underwent regioselective Kornblum–DeLaMare fragmentation with Hünig’s base to give the corresponding γ-hydroxy enones 120 (Scheme 16a). This regioselectivity is rationalised in terms of the base abstracting the less sterically hindered of the bridgehead protons in endoperoxide 16. Unfortunately the desired zeylenone skeleton has the opposite regiochemistry, exemplified by 121 (Scheme 16b). Such a γ-hydroxy enone isomer did eventually prove to be accessible by means of an intramolecular deprotonation: treatment of endoperoxide 115 with TBAF gave γ-hydroxy enone 123 with the correct zeylenone skeleton (Scheme 16c). We propose that the alkoxide 122 formed in situ by desilylation is able to effect the desired deprotonation leading to 123.

In 2012 we also reported further organoiron chemistry which exploits the oxygenation in complex 75 to access a range of cyclohexadienes bearing diverse substituents and with different substitution patterns. A tricarbonyliron [η⁴] diene complex bearing a leaving group adjacent to the diene is able to extrude this leaving group to form the corresponding [η³]⁺ complex (c.f. Scheme 10). In the case of 75, either of the two hydroxyl functionalities is capable of acting as the leaving group upon protonation with a Brønsted acid comprising a non-nucleophilic anion (Scheme 17a). This reaction was found to work best in acetic anhydride as solvent and proceeds with concomitant hydroxyl acetylation. Thus, if the tertiary hydroxy in 75 is the leaving group, [η³]⁺ complex 124 may be formed, whereas if the secondary hydroxy in 75 is the leaving group, [η³]⁺ complex 125 would form instead. Realtime NMR monitoring of this reaction revealed that in fact both cations are formed, with 125 being the major product (≈3:1 ratio). Once formed, both of these cations are susceptible to nucleophilic attack at their diene termini. For [η³]⁺ complex 124, attack α- or ipso- to the ester would afford regioisomeric products 126 or 127 respectively. As discussed above, regioselectivity in such nucleophilic additions is preceded and only products of type 126 are formed (Scheme 17b). For [η³]⁺ complex 125, loss of the secondary hydroxyl in fact introduces a plane of symmetry, i.e. 125 is achiral. Thus, the two diene termini are enantiotopic and addition of any achiral nucleophile to 124 will give a product (±)-128 as a racemic mixture.
The cationic complexes 124 and 125 were treated with a variety of nucleophiles and the resulting adducts were demetallated to give a library of novel cyclohexadienes (Figure 5). The possibility was considered that equilibration between cations 124 and 125 (by acetoxy migration) might lead to erosion of the e.e. of 125 and hence of dienes such as 129-132 derived from it. However, comparison of a derivative of 132 with racemic material showed it still to be enantiopure. The chemistry outlined in Scheme 17 was also applied to formal syntheses of oseltamivir and gabaculine.

In 2013 we revisited the inosaminocacid chemistry we had described two years previously, this time targeting structures bearing a side chain in a lower oxidation state; these were anticipated to have differing solubilities to the zwitterionic inosaminocacids 107-108 shown in Scheme 13. The chemistry bears many similarities to the previous report, but one notable difference is the reversal in regioselectivity for the acyl nitroso cycloaddition when the side chain is in a lower oxidation state (Scheme 18). The isomer in which the side chain and the NCBz group are proximal, 137, predominates over the isomer in which they are distal, 136. This is the opposite of the selectivity depicted in Scheme 13, but in fact this regiochemical switch is preceded in Widdowson’s early work (c.f. Scheme 4). An unexpected result during the course of this work was the formation of cyclic carbonate 142 upon exposure of diol 140 to desilylation conditions.

Most recently, in 2014, we have reported an additional selection of chironas derived from 4, this time focusing on structures possessing a saturated ring, e.g. 143-146 (Figure 6). This report also details routes to C-hydroxymethyl-mucoinositol 147 and bis(epoxide) 148.
removal by methanolysis gives the free hemiacetal whose open-chain form is able to undergo cyclic imine formation to give 157. After hydrogenolysis and removal of the ketal, the final product is isolated as its hydrochloride salt, 158.

![Scheme 19. Hudlický’s synthesis of a polyhydroxylated pyrrolidine from 4.](image)

**Future directions**

The chemistry reviewed above underscores the broad range of applications in synthesis for aren e ipso,ortho diols such as 4. Nevertheless, there is undoubtedly great scope for further novel applications of 4 – for example, not all the transformations outlined in Figure 1 have yet been realised. In addition to transformations of the parent unsubstituted 4, the metabolism of substituted benzoates to their ipso,ortho diols and their subsequent synthetic use is an obvious area ripe for exploitation, given the comparatively dearth of examples (see Schemes 8 and 11). Reiner & Hegeman’s initial report7 on *Ralstonia eutropha* B9 in fact also examined the susceptibility of substituted benzoates to dihydroxylation and in the ensuing decade, Knackmuss and co-workers studied this in more detail.8 It was determined that the meta-position was the most tolerant of substitution, followed by the para-position. The ortho-position was the least tolerant of substitution, with only 2-fluorobenzoate being turned over (Scheme 20). The first study to quantify product formation from meta-substituted benzoates stated that 5-substituted diols were formed more rapidly than the corresponding 3-substituted regioisomers.24 However, later studies determined this statement to be incorrect for meta-methyl28 and meta-bromo25 benzoates, with the 3-substituted products in fact predominating. (Note that a large number of aromatic substrates which are not substrates for BZDO from *Ralstonia eutropha* B9 have been identified.29)

![Scheme 20. Substituted benzoates as substrates (substrates in parentheses give especially slow turnover).](image)

Formation of ipso,ortho diols from multiply fluorinated benzoates has been described using *Pseudomonas putida* JT103.40 Furthermore, 2-trifluoromethylbenzoate is metabolised to its ipso,ortho diol by *Pseudomonas aeruginosa* 14241 and metabolism of both 1-naphthoate and 2-naphthoate to their ipso,ortho diols is also known.42 Dihydroxylation of terephthalic acid and isolation of the ipso,ortho diol has been disclosed in a patent.43 Save for simple derivatisation to aid in their structural elucidation, or their deliberate dehydrative rearomatisation, no synthetic uses of these diols (Figure 7) have been reported. When the possibility of expanding the substrate scope of BZDO and other dioxygenases by directed evolution is considered, it is clear that there is great potential for arene ipso,ortho diols to continue to provide rapid access to uncharted chemical space.

![Figure 7. Arenes ipso,ortho diols that have not been exploited synthetically.](image)

**Biography**

Simon Lewis was appointed as a lecturer at the University of Bath in 2008. Since then he has pursued a research programme that encompasses microbial arene oxidation and other biotransformations. In addition, he has research interests in dye-sensitized solar cells, C-H functionalization with base metal catalysts, asymmetric catalysis and the chemistry of azulene. His work on the iron-mediated acetamide migration in an ipso,ortho diol derivative (Scheme 10) was published in the 2011 *Chem. Commun.* “Emerging Investigators” special issue.20

**Notes and references**

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