

Celebrating 20 Years of *SYNLETT* – Special Account On the Merits of Biocatalysis and the Impact of Arene *cis*-Dihydrodiols on Enantioselective Synthesis

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Dedicated to David T. Gibson in honor of his discovery of enzymatic dihydroxylation of aromatic compounds 40 years ago, his subsequent work in the area, and the contributions he has made to chemoenzymatic synthesis.

Abstract: This account is a tribute to Professor David Gibson in recognition of his discovery of enzymatic dihydroxylation of aromatic compounds four decades ago. Here are highlighted some of the milestones in microbiology, biochemistry, molecular biology, and synthetic organic chemistry connected with this unique reaction. Gibson's discovery greatly contributed to advancing biocatalysis as a discipline with major impact on synthesis of optically pure compounds. Personal recollections of several chemists who have embraced this technology in their own work, along with the authors' recollections of the early days of research involving the *cis*-dihydrodiols, are provided as Notes at the end of the article.

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Key words: biocatalysis, enzymatic dihydroxylation of aromatics, arene *cis*-dihydrodiols, enantioselective synthesis, enantiopure metabolites

1 Introduction: History of Biocatalysis

For thousands of years, humans have exploited the process of fermentation for production of all manner of consumables such as wine and beer, bread and cheese, vinegar and soy sauce. Once Pasteur demonstrated in 1857 that alcoholic fermentation was effectuated by yeast,¹ others began studying its effect, then that of other microorganisms, on various substances. In 1874 Dumas reported the conversion of sulfur to hydrogen sulfide,² the first observation of the reducing nature of yeast. In 1886 Brown³ experimented with adding various substances to a culture of the organism involved in vinegar production, *Bacterium aceti* (now called *Acetobacter*). By this means, he successfully converted ethanol into acetic acid, propanol into propionic acid, and mannitol into fructose.

Brown's intentional conversion of one compound is considered by some to be onset of the discipline of biocatalysis, just as Sertürner's isolation of morphine in 1805,⁴ arguably, marked the beginning of natural products chemistry and that Wöhler's synthesis of urea⁵ 23 years later the inception of organic synthesis. Brown opined presciently: *"I think the experiments just described will be of interest to biologists as well as chemists, as they help to show that the vital functions of certain organized ferments are most intimately connected with the molecular constitution of bodies on which they act."*

During the same period, scientists continued the study of the nature of yeast. Fischer, credited with the 'lock and key' model of enzyme action,⁶ studied the consumption of various carbohydrates by yeasts.^{7,8} The first use of yeast to effect a carbonyl reduction was reported in 1911 by Lintner and von Liebig,⁹ who converted furfuraldehyde into furfuryl alcohol during an alcoholic fermentation. Since then yeast cultures have been often used for the reduction of carbonyl compounds.¹⁰

The study of enzymes goes back to 1833 when French chemists Payen and Persoz isolated a substance from malt extract that they called 'diastase' and observed that it saccharified starch.¹¹ This is the first documented example of an enzymatic reaction *in vitro*. The substance is now known to have been a mixture of lipases. (The convention of naming enzymes with the ending *-ase* originated here.) The use of isolated enzymes in chemistry dates to Dakin's 1903 description of the kinetic resolution of racemic ethyl mandelate by crude pig liver lipase.¹² Lipases have been used since for the acylation of alcohols or hydrolysis of esters (with concomitant resolution or desymmetrization of *meso* compounds and provision of enantiopure building blocks).¹³

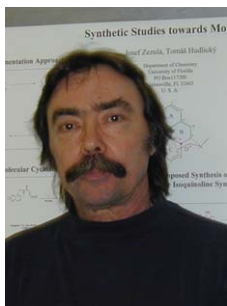
These days, whole-cell fermentation and enzyme-mediated transformations are widely used in the pharmaceutical industry,¹⁴ for example, in Abbott's process for erythromycin¹⁵ and that of Apotex for lovastatin.¹⁶ In general, the use of whole-cells as opposed to isolated enzymes is preferred for these transformations, especially those involving oxidoreductase enzymes, as the production of the necessary cofactors is built into the metabolism of the organism.

Advances in molecular biology and directed evolution make it possible to create recombinant and transgenic

organisms, both bacteria and yeast, for oxidative and reductive processes, for example the Baeyer–Villiger reaction.¹⁷ Recombinant strains of bacteria such as *Escherichia coli* can be constructed by plasmid engineering to overexpress the enzyme of interest for a given biotransformation. To design useful recombinant strains requires symbiosis between the synthetic practitioner and microbiologists.¹⁸

There is still some reluctance in academic circles to use these techniques for enantioselective (asymmetric)¹⁹ synthesis [Note 1]. The rather slow incorporation of biological methods into preparative organic chemistry in the academic sector may be due to the somewhat limited accessibility to chemists of published results within biological disciplines – this despite easy access to a number of databases, such as SciFinder and Medline, among others.

Biographical Sketches



Tomas Hudlicky was born in 1949 in Prague, Czechoslovakia, where he received his elementary and middle school education. After several years of working as a process chemist apprentice and in other odd jobs in pharmaceutical chemistry, it became apparent that higher education opportunities were closed to him. In 1968, he emigrated to the USA with his parents and sister. Hudlicky's educational experience continued at Blacksburg High School, from which he dropped out in the spring of 1969. Accepted as a probational student at Virginia Tech the following autumn, he received his B.Sc. in chemistry in 1973, and went on to pursue graduate studies at Rice University under the direction of Professor Ernest Wenkert in the field

of indole alkaloid total synthesis, earning his Ph.D. in 1977. He then spent a year at the University of Geneva working under the late Professor Wolfgang Oppolzer on the synthesis of isocomene. In 1978, he joined the faculty at the Illinois Institute of Technology as an Assistant Professor, and began the first phase of his research career in the field of general methods of synthesis for triquinane terpenes and other natural products containing five-membered rings by [4+1] cyclopentene, pyrroline, and dihydrofuran annulation methodologies. He returned to his alma mater, Virginia Tech, in 1982, and rose to the rank of Professor in 1988. One year later, at the 20-year class reunion of the Blacksburg High School class of 1969, he received his High

School Diploma. The next phase of his research involved the investigation of *cis*-cyclohexadienediols in enantioselective synthesis. In 1995, he moved to University of Florida in Gainesville. In 2003, Dr. Hudlicky accepted an offer from Brock University where he currently holds a position as Canada Research Chair. His current research interests include the development of enantioselective synthetic methods, bacterial dioxygenase-mediated degradation of aromatics, design and synthesis of fluorinated inhalation anesthetic agents, synthesis of morphine and Amaryllidaceae alkaloids, and design of unnatural oligo-saccharide conjugates with new molecular properties. His hobbies are skiing, hockey, martial arts, and music.



A native of North Carolina, **Josephine Reed** was educated at the University of North Carolina at Greensboro (B.A., English), Appalachian State University

(B.A., biology and chemistry), and Virginia Tech (Ph.D., chemistry, with David Kingston). She has been working with Tomas Hudlicky and his group as a

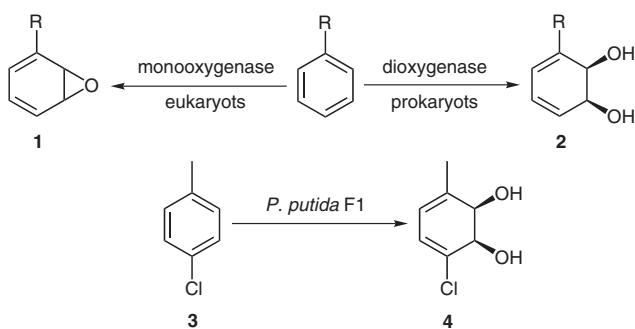
senior a research associate for the last two decades. She also works at the Office of Research Services at Brock University as a research grants facilitator.

steroidal compounds, published by Kieslich in 1976, to this date serves as a wonderful source of reactions, strains and metabolites. Our account traces the history, development, and applications of one remarkable enzymatic reaction – the dihydroxylation of aromatic compounds by prokaryotic enzymes.

2 The Discovery of Enzymatic Dihydroxylation

Meanwhile, by the early part of the 20th century, a number of scientists became interested in the metabolism of hydrocarbons by microorganisms,²² with the first reports addressing the attack of fungi on paraffin.²³ The first indication of microbial utilization of aromatic compounds was reported in 1908 when Störmer²⁴ described the isolation of *Bacillus hexacarovorum*, an organism that was able to consume toluene and xylene.

One may generally assume that there is little tendency of aromatic compounds to participate in reactions that lead to permanent disruption of aromaticity. There are exceptions, of course – processes such as Birch reduction, *para* alkylation of phenols, oxidation with or without alkylation to quinoid-type compounds, and some others. One of the most interesting of such reactions is the enzymatic oxidation of arenes by either cytochrome-type monooxygenases or by bacterial dioxygenases leading to compounds such the arene oxide **1** or *cis*-dihydrodiol **2**, respectively (Scheme 1). The latter process, which proceeds regio-, stereo-, and enantioselectively, has no equivalent in synthetic chemistry. Recently, some preliminary attempts at developing an equivalent chemical reagent have been made; these are discussed later in this account.



Scheme 1

The discovery of the process by which soil bacteria remediate contamination by aromatic compounds is credited to Gibson, who published the first example of enzymatic dihydroxylation of an aromatic compound (the *cis*-dihydrodiol derived from benzene) and the isolation of the first stable arene *cis*-dihydrodiol, the one derived from *p*-chlorotoluene (**3**) by the action of *Pseudomonas putida* F1, a mutant strain that he developed (Scheme 1).²⁵ The disclosure of the enantiomerically enriched diol **4**²⁶ to the chemical community in 1968 went unnoticed by synthetic chemists until ICI utilized benzene *cis*-dihydrodiol (a

meso compound) for the preparation of polyphenylene in 1983, considered to be the first application of these metabolites in synthesis.²⁷ Ley was the first academic researcher to recognize the power of the functional content of *cis*-dihydrodiols with his 1987 synthesis of pinitol from the metabolite derived from benzene.²⁸

Our own group was fortunate to have Larry Kwart join as a visiting researcher in 1985. Trained as a synthetic chemist by Wenkert, he had a penchant for biology that led him to continue his education as a postdoctoral associate with Gibson. It was Kwart who introduced our group to Gibson's mutant strain *Pseudomonas putida* 39D. We prepared our first metabolite, the *cis*-dihydrodiol derived from toluene, in a yield of several hundred milligrams per liter by means of a primitive fermentation system. Our first paper in this area, in 1988, described a formal synthesis of PGE_{2α} in four steps from toluene [Note 2],²⁹ an outcome that was a substantial improvement over all previous syntheses (12 steps and longer) reported in the literature at the time.³⁰

It was obvious to us that a combination of biology and traditional synthetic methods held great potential for increasing the overall efficiency of many syntheses. Thinking that we had discovered a gold mine of opportunity, we immediately submitted an NIH proposal describing a long-term program in enantioselective synthesis that would be built on the availability of arene *cis*-dihydrodiols and provide for efficient and exhaustive syntheses of many classes of compounds, from alkaloids and terpenes to carbohydrates and polymers. That proposal received the worst rating of all of the submissions of one of us, before or since. The idea was completely rejected by the NIH study section, as were all subsequent proposals submitted on this topic of chemoenzymatic synthesis [Note 3]. Although none was ever funded by the NIH, all were eventually supported by other agencies (in the United States by the National Science Foundation, the Environmental Protection Agency [Note 4], the Petroleum Research Fund, and in Canada by the Natural Sciences and Engineering Research Council), and we were able to expand the use of *P. putida* 39D metabolites to the synthesis of cyclitols, inositols, carbohydrates, terpenes, alkaloids, and even polymers.

That the original ideas had merit was demonstrated not only by our group but also by many other synthetic chemists who recognized the possibilities for using arene *cis*-dihydrodiols in synthesis of natural products. Research in this area now takes place on at least four continents [Notes 5–9]. As of this writing, several of the diols are commercially available,³¹ and the number of publications in this area has grown enormously since Ley's 1987 paper.²⁸ Worldwide, several groups are dedicated users of this technology in synthetic applications, exploiting the chirality and functionality of *cis*-dihydrodiols. A brief summary of historically relevant milestones both in biology and in synthesis follows.

3 Processing of Arenes by Oxidoreductase Enzymes

The processing of aromatic substrates by oxidoreductase enzymes proceeds by several diverse pathways. Eukaryotic organisms employ various cytochromes to oxidize arenes to the corresponding arene oxides, which either rearrange or are opened in a *trans* fashion by various nucleophiles. The first arene dihydrodiol metabolite isolated, by Boyland and Levi in 1935, was *trans*-1,2-dihydroxy-1,2-dihydroanthracene, a product of mammalian metabolism of anthracene via a cytochrome oxidation followed by hydrolysis of the arene oxide.³² However, a compound that may have been the *trans* diol derived from benzene was observed as early as the 1860s [Note 10]. In prokaryotic organisms, dioxygenase enzymes deliver the dioxygen molecule to the arene. In wild types of bacteria, the resulting diols are further oxidized to catechols, which then undergo *ortho* cleavage to the corresponding muconic acid.^{33,34} Hydration and retro-aldol reactions then provide acetate as an energy and carbon source (Scheme 2).

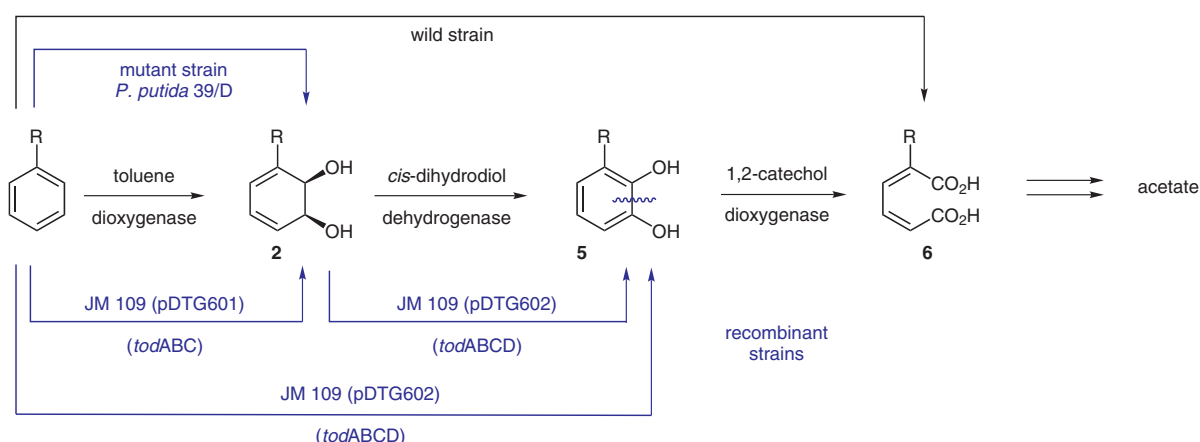
In Gibson's blocked mutant *Pseudomonas putida* 39D (Pp39D) the expression of catechol dehydrogenase is arrested so that the intermediate *cis*-dihydrodiol (**2**) accumulates in the fermentation medium.³⁵ Years of research have resulted in the nucleotide sequence of the genes encoding for the toluene dioxygenase (TDO) enzyme system. These genes have been overexpressed in *Escherichia coli* JM109 to form the clone JM109 (pDTG601), now used to produce *cis*-dihydrodiols.³⁶ The use of a related recombinant organism JM109 (pDTG 602)³⁶ leads to the direct synthesis of catechols from the corresponding arenes. The synthesis of various halocatechols from the corresponding haloarenes was accomplished by the whole-cell fermentation with the recombinant strain JM109 (pDTG602) expressing both toluene dioxygenase as well as catechol dehydrogenase.³⁷ The yields of halocatechols obtained by this method are lower (~1–2 g/L) than those for *cis*-dihydrodiols because the catechols are highly toxic to the organism. Nevertheless, the one-step fermentation procedure is superior to often multi-step chemical syntheses. It is, however, less efficient than the biocatalytic syn-

thesis of catechols from glucose reported by Frost³⁸ and Yoshida.³⁹

The major advantage of using whole-cell fermentation rather than isolated or immobilized dioxygenases in the preparation of the *cis*-dihydrodiols is the fact that no cofactors such as NADH are required for reductive steps in the cycle. Oxidoreductases in general perform their function along a complex electron-transport cycle and stoichiometric amounts of cofactors would have to be either added to the reaction involving isolated enzymes or various cofactor recycling loops established for each particular process. The whole-cell fermentation, especially one performed with recombinant organisms, avoids this issue because the living cell provides stoichiometric quantities of all 'reagents' required to complete the transformation. Furthermore, the dioxygenase enzymes are not very stable in pure form, complicating their use. A procedure for the medium-scale preparation of some of the more common *cis*-dihydrodiols and catechols by the whole-cell fermentation of arenes (15-L-fermentor scale) with recombinant strains has been published.⁴⁰ In addition, a very simple procedure for the synthesis of *cis*-dihydrodiols by fermentation with the blocked mutant *Pseudomonas putida* 39D has been published in *Organic Syntheses*.⁴¹ The use of a blocked mutant does not require any specialized or expensive equipment and can be performed with standard glassware available in any organic synthesis laboratory.

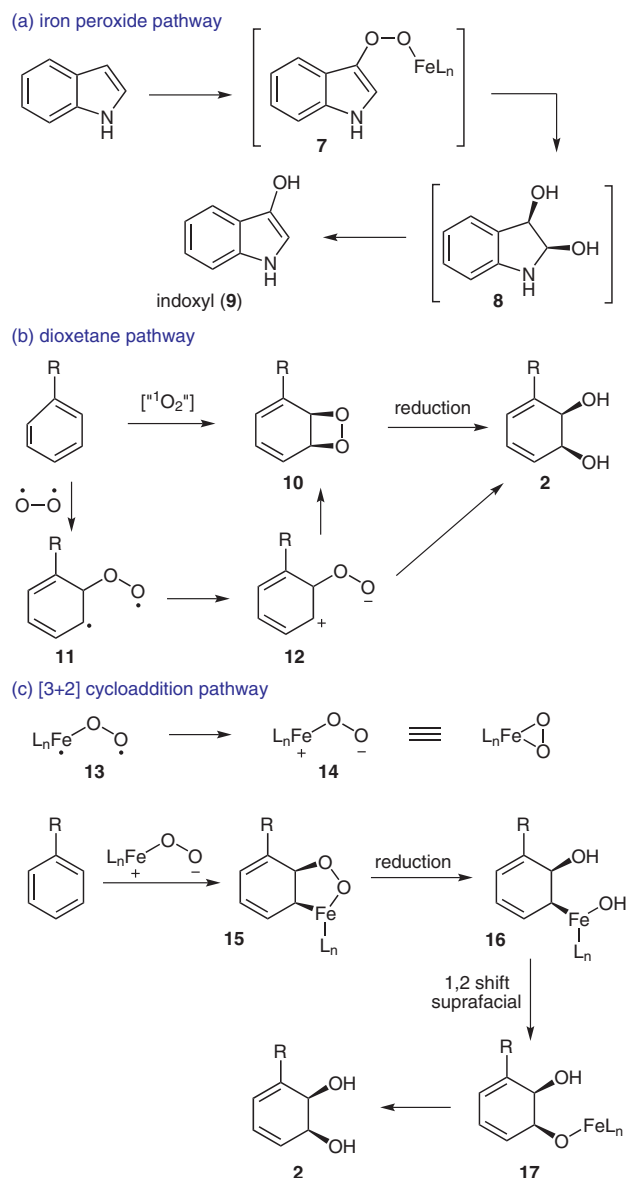
4 Considerations of the Mechanism

The X-ray structure of naphthalene dioxygenase, an enzyme that processes fused aromatics and that is topologically related to TDO, was solved in 2003.⁴² With all of the structural information available, still no rational mechanism has been proposed for the remarkable dihydroxylation. Initially, a dioxetane intermediate was suggested to account for the documented incorporation of both atoms of ¹⁸O₂ into the *cis*-dihydrodiol.⁴³ Such an intermediate, requiring a relatively high-energy cycloaddition of a singlet oxygen species (presumably generated from the triplet at the iron metal center), does not seem likely. The



Scheme 2 Metabolism of aromatic compounds by soil organisms

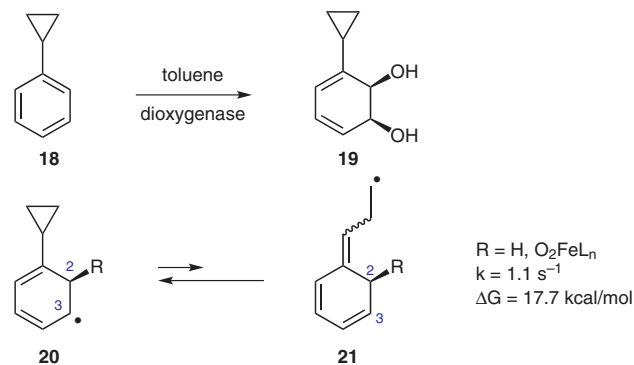
mechanism has not yet been solved; however, it has been suggested that a [3+2]-type cycloaddition of an iron(V) peroxide to the arene followed by reduction and a suprafacial migration of hydroxyl from the iron center (Scheme 3) is plausible [Note 11]. Evidence supporting the intermediacy of an iron-bound peroxide in the naphthalene dioxygenase-mediated dihydroxylation of indole was presented in 2000;⁴⁴ further investigations in 2003 revealed an iron(II)-bound dioxygen species **7** as a possible agent involved in the dihydroxylation.⁴²



Scheme 3 Mechanistic options for the enzymatic dihydroxylation of arenes

The possibility of the addition of a radical species that would generate C-3 dienyl radical of type **20** (Scheme 4) was investigated by subjecting cyclopropyl benzene to the fermentation with the JM109 (pDTG601) strain. It was thought that such a radical would lead to a cyclopropyl-carbinyl rearrangement of the dienyl-cyclopropane system. However, cyclopropylbenzene has been shown to be

an excellent substrate for TDO, and the corresponding diol was isolated in the yield of about 3 g/L. Calculations indicated that the cyclopropylcarbinyl rearrangement of **20** to **21** would be quite unfavorable both kinetically and thermodynamically compared to the parent system which rearranges with a $k = 10^8 \text{ s}^{-1}$.⁴⁵ The precise definition of the mechanism is yet to be formulated although the discourse continues in a speculative manner in the literature.^{45,46}



Scheme 4 The case of cyclopropylbenzene and the theoretical treatment of cyclopropylcarbinyl rearrangement

The structure, function, and constitution of the dioxygenase three-component protein, including cofactors and electron-transport chain, is relatively well understood from a biochemical perspective. Although the precise order and nature of chemical events is not yet known, the practical utility of the enzymatic dihydroxylation is firmly established.

5 Diversity of Metabolites

A great variety of substrates are processed by toluene dioxygenase and related enzymes. The dihydroxylation is remarkably regio-, stereo-, and enantiospecific while the enzyme tolerates a diversity of functionalities. Only a fraction of the more than 400 metabolites isolated to date have been employed so far in any synthetic ventures. Some of these products are shown in Figure 1. In addition to the compounds shown here a number of *cis*-dihydrodiols derived from fluorobenzene, phenylpyridines, *p*-xylene, perdeuteriochlorobenzene, phenanthrene, quinoline, and other heteroarenes have been reported. The listing of known metabolites and their use in synthetic ventures can be found in several compilations.⁴⁷ The most comprehensive and up-to-date list of known *cis*-dihydrodiols is included in Johnson's *Organic Reactions* chapter published in 2004.^{47a} The yields of *cis*-dihydrodiols vary greatly depending on the substrate structure and functionality as well as the strain that is used in the whole-cell fermentation. Among the best substrates are naphthalene, bromo- and chlorobenzene, β -bromoethylbenzene, styrene, and *m*-dibromobenzene. The fermentation of these compounds with the recombinant strains produces the *cis*-dihydrodiol metabolites in yields of up to 20 g/L.

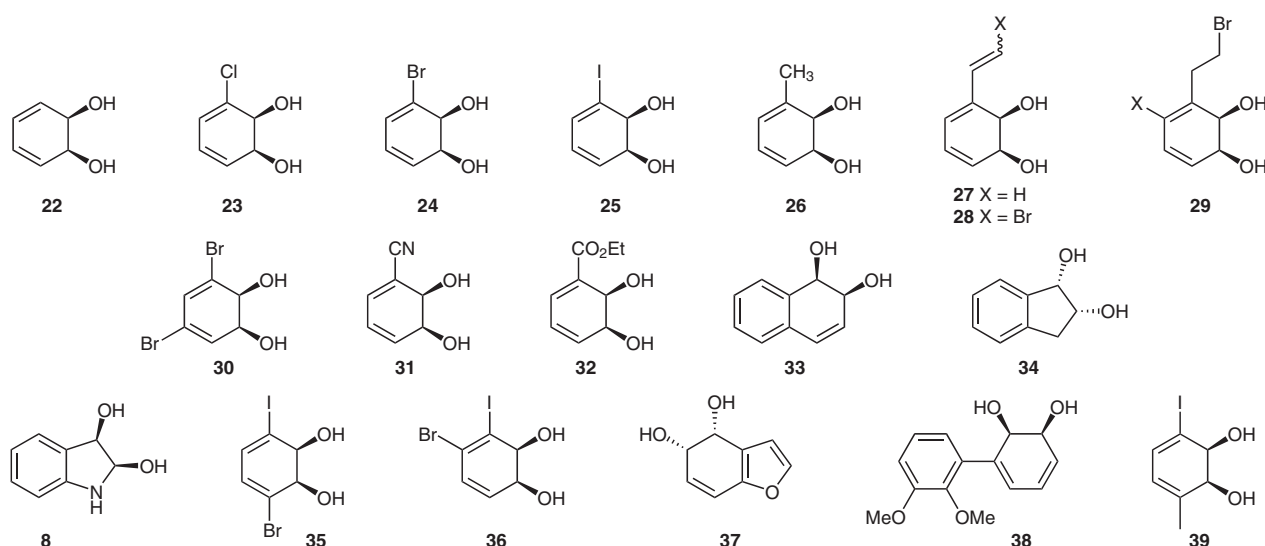


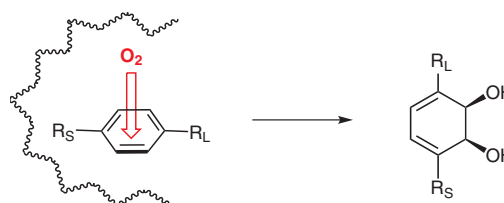
Figure 1 Some *cis*-dihydrodiol metabolites used in synthetic endeavors

In our preparations (15-L fermentor, with an 8–9-L working volume), batches of about 150 g are obtained from a single fermentation. In contrast, the use of the blocked mutant *P. putida* 39/D produces only about 200 mg/L. The protein synthesis must be induced in this organism by a ‘natural’ inducer (toluene, chlorobenzene), which necessitates the separation of the *cis*-dihydrodiol derived from the inducer from the one derived from the substrate of interest. The advantage of using the recombinant strains in which the protein synthesis is induced by isopropylthiogalactose (IPTG) alleviates the separation issues. On the other hand, the advantage of using the blocked mutant lies in the simplicity of operation with no requirements for specialized equipment.

The *cis*-dihydrodiols are easily extracted into base-washed ethyl acetate and are reasonably stable to further manipulation. They can be stored in solid form at $-78\text{ }^{\circ}\text{C}$ or as a suspension in phosphate pH 8 buffer either at $0\text{ }^{\circ}\text{C}$ or at room temperature. The *cis*-dihydrodiols must be kept free of any traces of the corresponding phenols. However, some *cis*-dihydrodiols [those derived from benzene and (β -bromoethyl)benzene] are not stable when stored in solid, dry form and may undergo exothermic (sometimes explosive!) aromatization. Care must be taken in handling these (or any other *cis*-dihydrodiols) at medium or large scales.⁴⁸

Based on the high number of metabolites and the relatively consistent production of similar regio- and stereoisomers from a wide variety of substrates, a model was developed⁴⁹ that can be effectively used to predict the outcome of the enzymatic hydroxylation for new and untried compounds, as illustrated in Scheme 5. Boyd’s model thus allows some security in planning to use certain *cis*-dihydrodiols as starting materials in anticipated synthetic schemes even before the actual metabolites are identified.

Gibson’s contributions were not limited to the investigation of biochemistry of the enzymatic process. His group reported the isolation of countless new metabolites over



Scheme 5 Model for predicting the regio- and stereochemical consequences for the dihydroxylation of single ring aromatics

the years. In the United Kingdom, several groups have contributed both to the understanding of the oxidation and to the production of new metabolites by a variety of organisms, all expressing enzymes related to toluene dioxygenase. To date, these researchers make up the largest single group devoted to the study and applications of the enzymatic dihydroxylation [Note 9]. One of the key figures was undoubtedly Douglas Ribbons, whose research closely overlapped Gibson’s program, especially with respect to oxidation of various benzoic acids [Note 12]. It is little known that Steven Ley and his group initiated research on the use of arene metabolites as early as 1978 [Note 13]. Like those of many researchers in the field who followed, his ideas were met with resistance by the organic community.

6 Arene Dihydrodiols as Synthetic Intermediates: Analysis of Reactivity and Symmetry Options

Dihydrodiol metabolites contain an amazing array of functionalities and reactive options as illustrated in Figure 2. The most obvious of such transformations involve the transfer of chirality from the biologically generated diol to the periphery of the cyclohexane ring; this aspect has been exhaustively exploited in the synthesis of cyclitols, conduramines, inositols and carbohydrates, both natural and unnatural. In the metabolite of bromobenzene,

the two allylic alcohol moieties are differentiated from one another by the proximity of the bromine atom and lend themselves to Claisen-type rearrangements, as has been demonstrated in an approach to morphine.⁵⁰ Cycloadditions to the diene, both intra- and intermolecular, lead to bicyclo[2.2.2]octanes, which can be further transformed into more complex systems through consecutive reaction sequences. An approach to morphine has benefited from a tandem Diels–Alder–oxy–Cope rearrangement to provide a truncated morphine skeleton in just a few steps.⁵¹ Other types of cycloadditions, as well as cyclopropanation and aziridination of the well-differentiated olefins have been exploited in many synthetic ventures and have been extensively covered in several reviews.⁴⁷

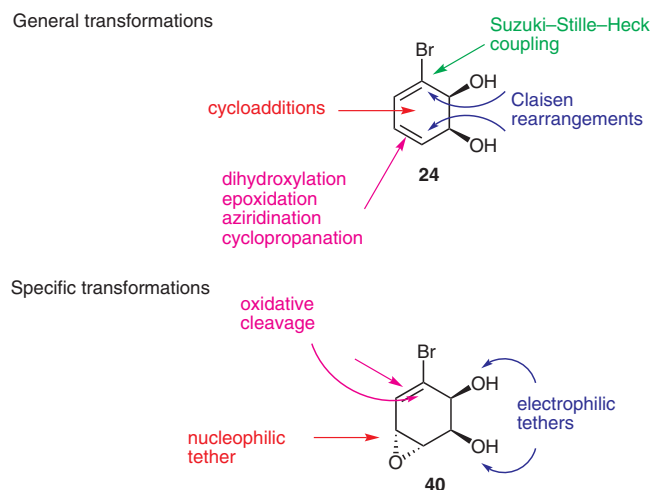


Figure 2 Reactive options for arene *cis*-dihydrodiols

In addition to their rich functional content, *cis*-cyclohexadienediols offer advantages for enantiodivergent synthesis. The provision of only one enantiomer of a target via enzymatic synthesis has been the focus of frequent criticism by traditional synthetic chemists; however, the diols lend themselves to symmetry-based design that can not only produce all possible diastereomers in the creation of the next stereogenic centers but also provide the target in either enantiomeric series. This concept, depicted in Figure 3, is based on the premise of ‘latent symmetry’, introduced to describe the strategy of enantiodivergent design from a single enantiomer of a starting diol.^{47c,52}

The diastereoselectivity parameters that operate in the introduction of any additional stereogenic centers are controlled by invoking a prodiastereotopic plane and creating either a *syn* or *anti* relationship of the moiety introduced next, either by directing effects of the free diol (*syn*) or by hindrance of the protected diol (*anti*), respectively. In this fashion the configuration of each stereogenic center introduced along the periphery of the diene system is controlled. In order to achieve enantiodivergence, a proenantiotopic plane bisecting the *cis*-diol is invoked to control the design of either the ‘plus’ or ‘minus’ nature of the target. Any operation above or below this plane with

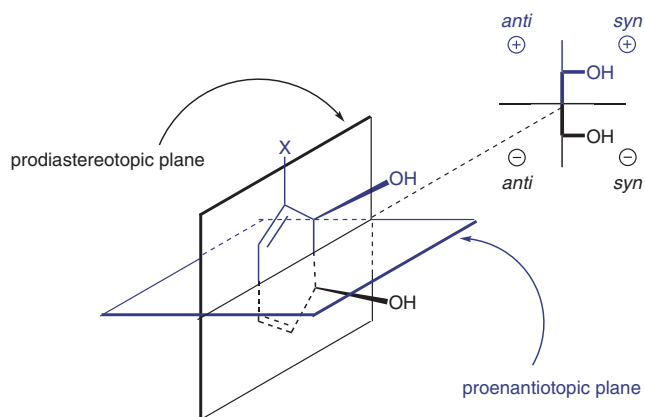


Figure 3 Symmetry analysis of arene *cis*-dihydrodiols. Four complementary spaces for incipient transformations of arene *cis*-diols (+ and – space assigned arbitrarily). [Hudlicky, T.; Reed, J. W. *The Way of Synthesis*, p 147, 2007. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.]

subsequent removal of a desymmetrizing element ‘X’ automatically leads to the ‘plus’ or ‘minus’ series of enantiomers. A hypothetical ‘1,4-switch’ of the X moiety in the cyclohexadienediol across the proenantiotopic plane would automatically generate the antipode; however, such an operation is impossible in practice without generating a *meso* compound. It is accomplished by introduction of the next stereogenic center (in either *syn* or *anti* fashion) followed by removal of X and continuing functionalization. The path to either enantiomer is identical in energy and identical in the number of steps, but it is *the order of execution of the individual steps* that leads to the enantiomeric switch. This concept has been exploited in exhaustive design for the synthesis of carbohydrates, inositols, and alkaloids in both enantiomeric series.⁵³

7 Historically Important Milestones in Applications to Synthesis

Shown in Table 1 are some of the historically important synthetic accomplishments emanating from the use of the *cis*-dihydrodiol metabolites. (For a more exhaustive listing of applications in syntheses the reader should consult recent reviews.⁴⁷) Taylor’s 1983 polyphenylene synthesis was followed the same year by Gibson’s disclosure of synthesis of indigo by dihydroxylation of indole by naphthalene dioxygenase⁵⁴ in a process that years later was optimized to an industrial-scale fermentation.⁵⁵ From late 1980s, reports on the use of these metabolites began to be quite numerous: a formal synthesis of PGE₂α in 1988,²⁹ total synthesis of zeylana in 1989⁵⁶ and pinitol in 1990,⁵² and a fully general method of synthesis for hexoses and other carbohydrates,^{47c,e,57} have been accomplished. The Amaryllidaceae alkaloids lycoricidine,⁵⁸ pancratistatin,⁵⁹ and narciclasine⁶⁰ have been synthesized, along with their enantiomers.⁶¹ Codeine⁶² and nangustine⁶³ were prepared. Dihydrodiols have served as starting materials for the synthesis of various cyclitols, inositols,⁶⁴ their oligomers,⁶⁵

and inositol phosphates.⁶⁶ In the terpene area, the attainment of specionin,⁶⁷ hirsutene,⁶⁸ hirsutic acid,⁶⁹ and approaches to taxanes⁷⁰ all benefited from the use of only a few of the known dihydrodiols. Many other targets have also been synthesized, among them pyrethroids,⁷¹ chiral polymers,⁷² macrocycles,⁷³ pseudo-^{57a,74} and azasugars,⁷⁵ hydroxylated pyrrolizidine alkaloids,⁷⁶ and sphingosines.⁷⁷ Recently, chiral 2,2'- and 4,4'-bipyridyl ligands,

to be exploited in transition-metal asymmetric catalysis, were synthesized from the *cis*-dihydrodiols derived from 4-chloroquinolines.⁷⁸ Amazingly, only six compounds – chlorobenzene, bromobenzene, iodobenzene, *m*-dibromobenzene, toluene, and β -bromoethylbenzene – were used as starting materials in most of the total syntheses. The vast majority of metabolites have yet to be exploited in synthetic ventures.

Table 1 Selected milestones in applications of dihydrodiols in synthesis

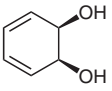
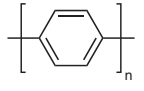
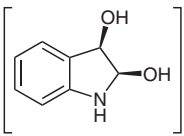
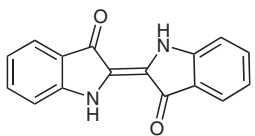
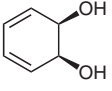
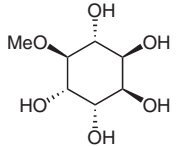
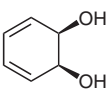
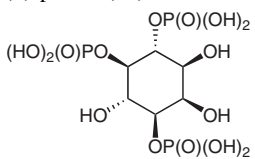
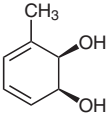
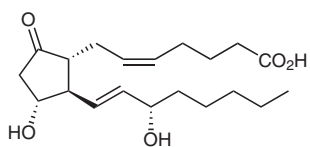
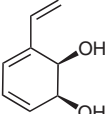
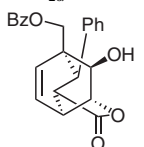
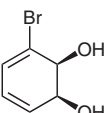
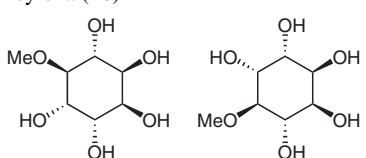
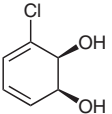
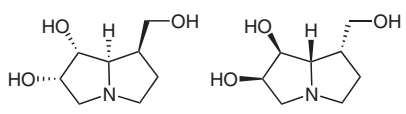
Starting material	Product	Author (year)	Reference
 22	 polyphenylene (41)	Taylor (1983)	27
 8	 indigo (42)	Gibson (1983)	54
 22	 (±)-pinitol (43)	Ley (1987)	28
 22	 inositol-1,4,5-triphosphate (44)	Ley (1988)	66
 26	 PGE _{2α} (45)	Hudlicky (1988)	29
 27	 zeylena (46)	Hudlicky (1989)	56
 24	 (+)- and (-)-pinitol (43)	Hudlicky (1990)	52
 23	 (+)- and (-)-trihydroxyheliotridane (47)	Hudlicky (1990)	76

Table 1 Selected milestones in applications of dihydrodiols in synthesis (continued)

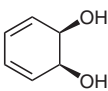
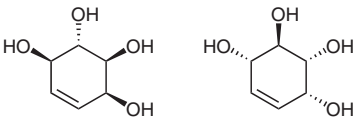
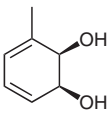
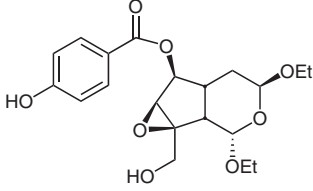
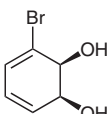
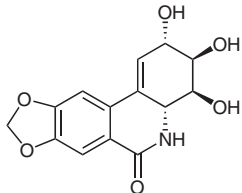
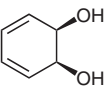
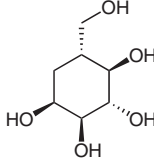
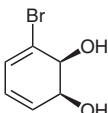
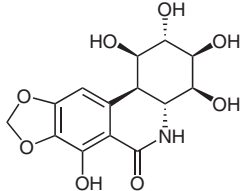
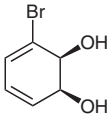
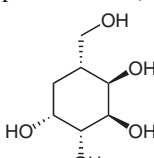
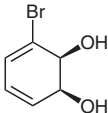
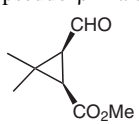
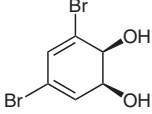
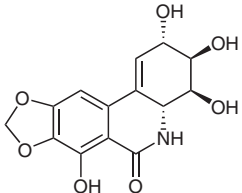
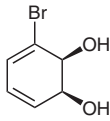
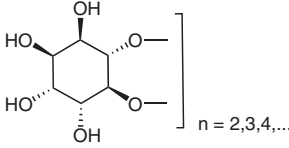
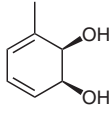
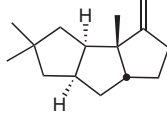
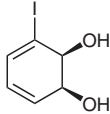
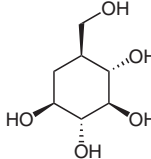
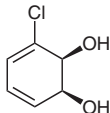
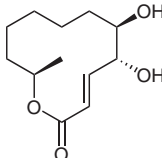
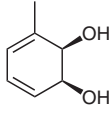
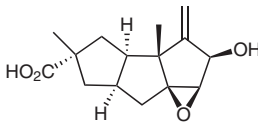
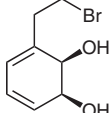
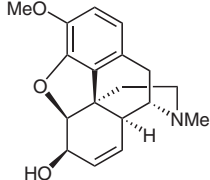
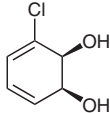
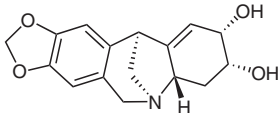
Starting material	Product	Author (year)	Reference
	 (+)- and (-)-conduritol F (48)	Ley (1990)	64a
	 specionin (49)	Hudlicky (1992)	67
	 lycoricidine (50)	Hudlicky (1992)	58
	 carba- α -D-glucopyranose (51)	Ley (1992)	74a
	 pancratistatin (52)	Hudlicky (1995)	59
	 pseudo- β -D-altropyranose (53)	Hudlicky (1995)	57a
	 54	Banwell (1996)	71
	 narciclasine (55)	Hudlicky (1999)	60

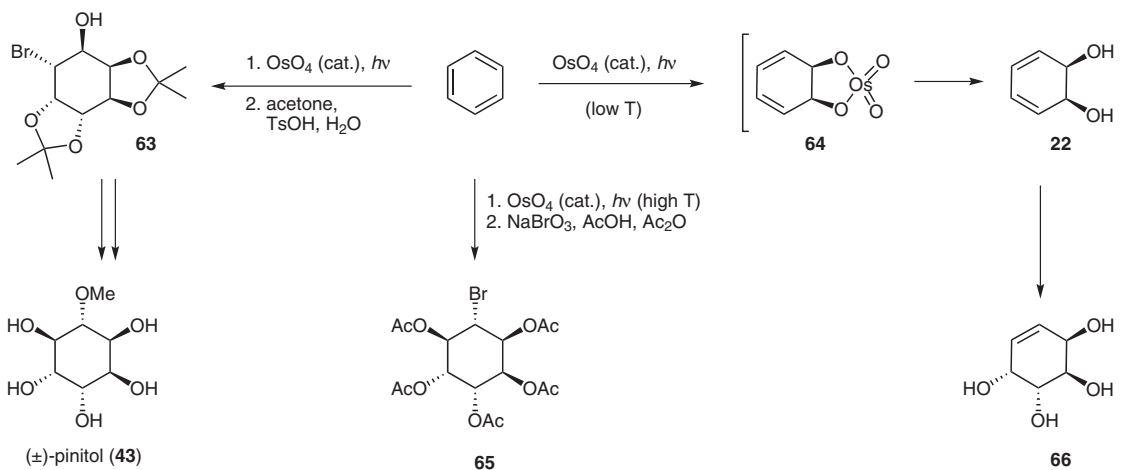
Table 1 Selected milestones in applications of dihydrodiols in synthesis (continued)

Starting material	Product	Author (year)	Reference
 24	 oligoinositols (56)	Hudlicky (2002)	64b–d, 65
 26	 (–)-hirsutine (57)	Banwell (2004)	68
 25	 carba-β-L-glucopyranose (58)	Boyd (2005)	78b
 23	 (–)-cladospolide (59)	Banwell (2005)	73
 26	 (+)-hirsutic acid (60)	Banwell (2007)	69
 29	 (+)-codeine (61)	Hudlicky (2007)	62
 23	 (+)-nangustine (62)	Banwell (2008)	63

In addition to efforts in total synthesis many methods have been developed to combine the power of the enzymatic dihydroxylation with traditional techniques in order to expand the repertoire. For example, Stille and related couplings are used to provide substituted dihydrodiols whose aromatic precursors may not be substrates for the dioxygenases.⁷⁹ The *ent*-metabolites have been prepared by taking advantage of the predictive model for the dihydroxylation and the rates of reduction of aryl halides: *p*-bromiodobenzene yields a diol whose installment is directed by the larger iodine.⁸⁰ Removal of the more reac-

tive iodine atom then furnishes the enantiomer of the diol derived from bromobenzene.

The most recent and quite exhaustive compilation of known *cis*-dihydrodiol metabolites was published in 2004.^{47a} Only few of these compounds have been incorporated into synthetic ventures, be it in either methodology or total synthesis. Despite the fact that several groups worldwide focused their research on synthetic applications of these remarkable compounds, a majority of these metabolites still await notice by creative synthetic practitioners. Given the diversity of applications of *cis*-dih-



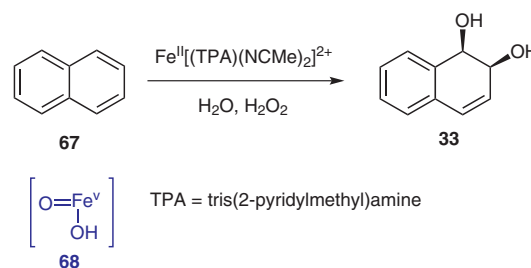
Scheme 6 Motherwell's photolytic conversion of benzene into a conduritol and into pinitol

driodols in synthesis, and given the continuing quest for chiral pool reagents and optically pure small-molecule starting materials required by the pharmaceutical companies, it is surprising that a greater appreciation for the utility of *cis*-dihydrodiols as intermediates for enantioselective synthesis had not yet surfaced [Note 14].

8 Outlook

Gibson's discovery of enzymatic dihydroxylation of aromatic compounds has had far-reaching consequences outside his own discipline, although the process of acceptance of this technology in synthesis has been remarkably slow. The impact his lifetime work produced in the fields of microbiology, biochemistry, and molecular biology is evident; the benefit to other disciplines, most notably that of enantioselective synthesis, is incalculable. The reaction itself constitutes a milestone comparable to such discoveries as Sharpless epoxidation, Grubbs metathesis, or Heck–Stille–Suzuki–Sonogashira coupling methodologies. The last few decades have witnessed explosive growth in the discovery of new catalytic methods for enantioselective synthesis. One would assume that the community would view the enzymatic dihydroxylation reaction – one of the few natural processes for which a fully asymmetric chemical equivalent has yet to be designed – as an enormous challenge for development of a chemical version of the catalyst, yet there have been few attempts to rise to the challenge. In 1995, Motherwell published on the synthesis of conduritol from benzene by means of a photolytic osmylation reaction⁸¹ (Scheme 6) [Note 15]. This disclosure was followed by a more detailed report of the stereochemical details and the conversion of benzene into pinitol.⁸² Very recently, Que reported the synthesis of *cis*-dihydrodiol **33** derived from naphthalene by way of iron(V)/ H_2O_2 oxidation (Scheme 7).⁸³ The iron(V) oxo species **68** was proposed as the agent responsible for the dihydroxylation.

These experiments certainly demonstrate that the chemical equivalent of dihydroxylation of aromatics is feasible,



Scheme 7 Que's dihydroxylation of naphthalene by an iron[V] complex

although at this time it does not compete with the biological process in terms of selectivity and efficiency. We hope that current and future generations of organic chemists are stimulated by the magnitude of this challenge and eventually succeed in the development of a catalytic process that would rival the enzymatic transformation and allow for this reaction to be removed from the list of unsolved problems in synthesis [Note 16].

9 Notes

(1) Biocatalysis is not recognized or respected by academicians in the United States to the extent that it is by those in Canada and Europe. At the biennial Gordon Research Conference on Biocatalysis, American participants are not a majority, as they are at other Gordon conferences. Its rosters indicate the following percentages: 1996, 48% US scientists; 1998, 41%; 2000, 42%; 2002, 38%. As of this writing there are fewer than ten US academic groups actively pursuing this field.

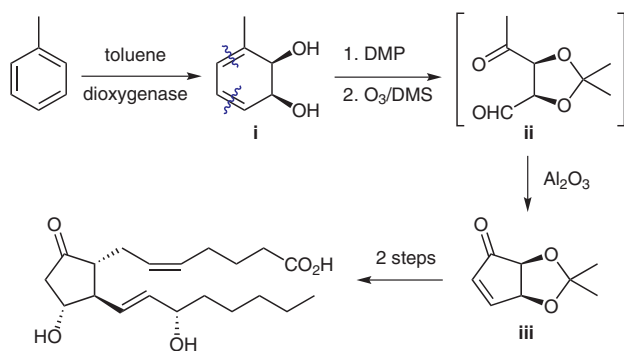
There are some in the synthetic community that openly reject enzymatic transformations as 'cheating' or the 'inability to design a catalyst properly' that would accomplish turnover rates similar to those of enzymes. Some views and discourse on the possible reasons for this resistance have been published.⁸⁴ Preparative biocatalysis, or the combination of enzymatic and chemical methods, has been accepted in the industrial world and in the academic

communities of Europe and Japan. By comparison, very few research groups in the United States actively pursue research in this area. The resistance to accept this field is also reflected in the reluctant funding of such programs. In spite of the demonstrated superiority that biocatalytic design has over traditional methods of synthesis of enantiomerically pure compounds, research proposals dealing with preparation of medicinally viable targets by such methods have not been well received by the NIH system (in our experience). On the other hand, when the review of such proposals was conducted in the format used by the NSF (or by industrial panels) the same proposals, rejected by the former system, were recommended by the latter. We do not believe that our own case is unique in this regard, but we accept the premise that any new technology requires a transition period before it is accorded a permanent status.

[Hudlicky, T.; Reed, J. W.: *The Way of Synthesis*, p 15, 2007. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.]

(2) [Personal recollection: T. Hudlicky, St. Catharines]

The first project in chemoenzymatic synthesis undertaken in our group was the synthesis of enone **iii** from toluene by employing *Pseudomonas putida* mutant 39D to generate diol **i**. That the formal synthesis of PGE_{2α} by this method was superior to all traditional syntheses was self-evident. We owe our initiation into the field of biocatalysis to Larry Kwart, (Ph.D. with E. Wenkert at Rice University and a postdoctoral fellow with D. Gibson at the University of Texas, Austin) who brought the technique as well as the Gibson organism to our attention and further use (Scheme 8).



Scheme 8

In the spirit of Note 1 above and the general resistance of the traditional sector to the use of biological methods it is interesting to note that the above synthesis was featured in the Preliminary Results section of our first proposal on the use of arene *cis*-diols, submitted to the NIH in 1987. This proposal received a rating of 96%, the worst rating I have ever received, and yet all the research proposed in that document was completed and published within five years of its submission.

[Hudlicky, T.; Reed, J. W.: *The Way of Synthesis*, p 16, 2007. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.]

(3) That the synthetic community has been reluctant to accept chemoenzymatic synthesis is evident from the following direct quotations from study section reviews of more than ten proposals submitted between 1988 and 1998 on the use of enzymatically derived arene *cis*-diols in enantioselective synthesis. None was funded by NIH; all were subsequently funded by NSF and other agencies. Following are some quotations from critiques of proposals for chemoenzymatic approaches to total synthesis:

- "The syntheses are poorly conceived and will not likely be successful." (Med. Chem. A, October 1987)
- "Manipulations of hydroxyls [in arene *cis*-diols] will probably result in elimination to aromatic substrates." (Med. Chem. A, June 1988)
- "The statement that fermentation chemistry might be used to turn halogenated arenes into useful synthetic intermediates is unfounded." (Med. Chem. A, February 1989)
- "No further significant advances in the use of *Pseudomonas putida* 39D can be expected from the proposed investigations." (Med. Chem. A, October 1989)
- "The overall strategies for applications of the *cis*-arene diols in synthesis are both simple and elegant." (Med. Chem. A, October 1990)
- "The synthetic work [involving arene *cis*-diols] is for the most part uninspiring." (Med. Chem. A, October 1991)

[Hudlicky, T.; Reed, J. W.: *The Way of Synthesis*, p 15–16, 2007. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.]

(4) The United States Environmental Protection Agency has invested generously in the support of 'green chemistry'. In 2005, it published a bibliometric analysis of citations of papers acknowledging their support.⁸⁵ Although the list of 'Twelve Principals of Green Chemistry',⁸⁶ articulated by Paul Anastas, never included biocatalysis per se, our 1999 *Aldrichimica Acta* article on dihydroxylation^{47b} was listed as being in the top 1% of the most highly cited pollution prevention papers in chemistry for the period 1995–2005. It also made the list of 'Hot Papers'.

(5) [Personal recollection: Martin Banwell, Canberra]

I first became aware of the 'diols' on seeing an advertisement for the parent system in *Chemistry in Britain* in the late 1980s. I contacted the advertiser, ICI PLC in the UK, and was rapidly provided with about 25 g of material, which we promptly learnt how to convert into the corresponding acetone without too much accompanying aromatization. We then started adding dihalocarbenes to the diene residue and using the ensuing bicyclo[4.1.0]heptenes as precursors to troponoids. However, just as our own chemistry was 'taking off' ICI advised that they could no longer supply the diol. Fortunately, at around the same time I found out (I can't recall precisely how) that Genencor International, then located in Rochester, NY, was producing various of the substituted and enantiomer-

ically pure diols. Since I happened to be attending an IUPAC-sponsored meeting on Organic Synthesis in Montréal in mid-1992, I took the opportunity to fly to Rochester and thus met Gregg Whited and Larry Kwart. I was very warmly welcomed and established what has proven to an enduring and delightful collaboration with Gregg who has continued to take a great interest in the area even as Genencor has moved away from it. I also met Tomas Hudlicky on the trip to Rochester and only then began to appreciate the real synthetic utility of the diols, particularly in regards to the hidden symmetry elements embedded within them. Even today, I don't think such features are fully appreciated by the community of synthesis chemists. This situation, coupled with the perceptions that the diols are 'hard to obtain', 'hard to handle' and/or 'difficult to make' certainly means that the mainstream has tended to shy away from using them. Yet, there is ample evidence that none of these perceptions is valid. Furthermore, the range of different diols now available is truly remarkable. One of the key groups that continues to demonstrate that this so is located at the Queen's University in Belfast and led by Derek Boyd.

As an Australian, giving lectures on our own work in the area, I often introduce the biotransformation leading to the diols as being something akin to a 'Birch oxidation'. That is to say, like the Birch reduction, the enzymatic dihydroxylation of arenes disrupts the aromaticity of the precursor and reveals new functionality that displays completely different patterns of reactivity. Of course, unlike the conventional form of the Birch reduction, the biotransformation is a highly enantioselective process. I would like to think that Arthur Birch might have enjoyed working with diols if he had had the opportunity to do so. Certainly, he would have recognized this area is a fertile one for synthetic chemists.

(6) [**Personal recollection: David Gonzalez, Montevideo**] **My first biocatalytic reaction:** When I first arrived to Professor Hudlicky's group ['T. Hud' hereafter] I was 'a biocatalytic virgin'. Before that I had obtained a Master's degree in organic chemistry working in a group where enzymatic reactions were basically considered to be at the level of cheating. In T. Hud's group I received a quick (one-week) training in how to grow *Pseudomonas putida*, add a substrate to the culture, and isolate a metabolite. So I grew my 'bugs' for a couple of days, add toluene to the culture and obtained two liters of a dark soup with plenty of insoluble matter (bacteria) that smelled very different from what my 'organic nose' was used to at the time. The person responsible for my training (Mary Ann Endoma) told me that I should extract the stuff with ethyl acetate and isolate a precious chiral compound that was supposed to be my starting material for a synthetic sequence. I am not a particularly pessimistic person, but at that moment I was expecting to obtain, in the best scenario, a complex mixture of compounds from which I would have to fish out my compound and pray to Lord that the yield would be in the 100-milligram range. I was absolutely amazed when the TLC of my ethyl acetate extract

showed a single spot that after removing the solvent yielded five grams of a chemically and optically pure material. That result, when compared with my previous experience in synthetic chemistry, was so incredible and rewarding that my attachment to biocatalysis has persisted until today. I have continued working with microbes, isolated enzymes and even carrot slices and have always been satisfied with the results. During my career I have met several chemists who are totally closed to biocatalysis and who still consider that enzymes should not be in the chemistry toolbox. I strongly encourage everyone to go beyond that feeling and consider biocatalysis as a valid alternative and a powerful chemical resource when attempting a synthesis.

(7) [**Personal recollection: Gustavo Seoane, Montevideo**] I usually think that my current work on chemoenzymatic synthesis using dioxygenases is the result of a thorough and elaborate decision, but I must acknowledge that there was also a big component of chance in it.

Ever since I was a graduate student, my main scientific interest has been related to enantioselectivity in organic synthesis. As a consequence, when I returned to Uruguay after finishing my doctorate, I wanted to work on the use of an enantioselective transformation to induce chirality in organic compounds. As a prospective young professor at a university in a small South American country, my options were heavily determined by 'logistic' issues such as infrastructure, reagent availability, costs, etc. At the end of the 1980s, at the time when I was planning to return to my country, the organic chemistry laboratory at our university was in really bad shape, lacking even the most basic equipment. Under those conditions, the use of expensive chiral reagents, which usually require dry conditions, very good solvents, etc., was out of the question. So the methodology of inducing chirality by means of 'biological' reagents seemed like a natural solution. By the time I finished my Ph.D., I had already decided that biotransformations were going to be the main theme of my own independent work once I went home. There was only a little detail left to decide: what type of biotransformation should I use?

At this point of the story, chance appeared. I was very lucky to be working with Professor Hudlicky when Dr. Larry Kwart joined the group. Larry had finished a post-doctoral fellowship in Dr. David Gibson's lab, where he knew first-hand the work on the mutant strain of *Pseudomonas putida* capable of oxidizing aromatics. He brought from Texas not only the organism but also a contagious excitement about its synthetic potential. After a few talks to Larry, I really wanted to work with that marvelous organism. I still remember the precise moment when I made that decision, during a talk to Larry next to the blackboard hung in the corridor outside the laboratory. This was twenty years ago!

So I told T. Hud I was eager to work on a biotransformation project. Finally, after completing my degree, I spent one year working with *Pseudomonas putida* 39D on the synthesis of zeylena. This was the beginning of my work

with dioxygenases, which is still the main topic of research in our group.

(8) [Personal recollection: Horacio F. Olivo, Iowa City]

I first learned about using microorganisms in the synthesis of natural products when I was taking a course on steroids. I was intrigued when I read in the literature on how only one carbon of a steroid skeleton could be hydroxylated regio- and stereoselectively by a microorganism! Interestingly, this microbial reaction is carried out today in industrial scale for the synthesis of steroidal anti-inflammatory agents. Were there other examples of this kind of transformation in the literature? That was when I first learned about Hudlicky's work on the total synthesis of natural products from a very interesting microbially generated non-racemic diol. Amazingly, a mutant strain of *Pseudomonas putida* was capable to oxidatively dearomatize cheap chlorobenzene and stop its metabolic process in non-racemic dienediol. A small molecule rich in powerful functionality, the halo-dihydrobenzenediol, was found to be an enormous gold mine. When I started my academic career, we applied a microbial oxidation of a 7-azanorbornane in the synthesis of a very unique alkaloid isolated in trace amount from Ecuadorian poison frogs. Epibatidine was found to be a very potent analgesic, several hundred times the potency of morphine, but acting on nicotinic receptors. Although our synthesis was only ten steps and showed the value of microorganisms in synthesis, it was not possible for me to get any funding on that project. More recently, we have used microorganisms to oxidize sulfur in an enantioselective fashion. Modafinil is a unique CNS stimulant which apparently lacks any addiction liability. It is approved by FDA to treat narcolepsy but NIH scientists have found it can be used to treat metamphetamine and cocaine addicts. It is also being used to replace Ritalin in children with ADHD. We prepared non-racemic modafinil utilizing one chemical and two microbial steps, and we prepared *rac*-modafinil in only two steps, one chemical and one microbial.

(9) [Personal recollection: Derek Boyd, Belfast]

A personal recollection of my journey with *cis*-dihydrodiols: During the 1960's it was common for PhD students from the UK to gain postdoctoral experience in the USA. Thus, forty years ago I travelled from Belfast to NIH Bethesda, having already gained some knowledge of fungal enzyme-catalysed aromatic hydroxylation and sulfoxidation of substituted benzenes while working on my PhD with Bernard Henbest. My one year leave of absence (1968–69) was granted to work with John Daly and Don Jerina on the *NIH Shift*. It was also hoped that I could help to establish the role of arene oxides as intermediates during mammalian metabolism to yield phenols and *trans*-dihydrodiols. It was a remarkable coincidence that this important work at NIH, and David Gibson's seminal discovery of *cis*-dihydrodiols as initial intermediates during dioxygenase-catalysed oxidation of aromatic substrates in prokaryotic systems, should occur independently but at virtually the same time. A collaborative link was then established between the Jerina (Bethesda, Maryland) and

Gibson (Austin, Texas) groups in 1971 that led to the stereochemical assignment of the enantiopure *cis*-dihydrodiol of naphthalene, the simplest member of the polycyclic aromatic hydrocarbon (PAH) series. In 1975 the Bethesda-Austin link was expanded to include Belfast and provided my first introduction to arene *cis*-dihydrodiols. Our role involved the identification of the structures and absolute configurations through synthesis of *cis*-dihydrodiol metabolites and derivatives from larger PAHs, including anthracene, phenanthrene, benz[*a*]anthracene, and chrysene. A remarkable renaissance of interest in arene *cis*-dihydrodiols occurred within the UK during the 1980's. I believe that this was catalysed by a report in 1983, that the *cis*-dihydrodiol of benzene produced by ICI could be used as a synthetic precursor of polyphenylene, and also by an advert from ICI Fine Chemicals appearing in *Chemistry in Britain* during 1986. This advert had the caption *A Major Breakthrough in Chiral Building Blocks* and showed the structures of the *cis*-dihydrodiols of benzene and toluene. This was soon followed by an ICI brochure offering several other *cis*-dihydrodiols for sale including the (1*S*,2*S*)-enantiomer from fluorobenzene. Many excited enquiries to ICI followed, including a rather unwelcome one from me. Unfortunately I felt compelled to point out to ICI that both their advert and brochure contained errors, including the fact that the *cis*-dihydrodiol from benzene was **achiral** and the *cis*-dihydrodiol from fluorobenzene was actually a **mixture** of (1*S*,2*S*)- and (1*R*,2*R*)-enantiomers!!! Despite this auspicious start to our relationship, a very friendly and productive collaboration was later formed with ICI staff including John Blacker and Bob Holt. The commercial availability of *cis*-dihydrodiols in 1986, soon allowed several groups in the UK to test their potential as synthetic precursors. While the Steve Ley group was the first to capitalise, and report, on the synthetic potential of monocyclic arene *cis*-dihydrodiols, other groups in England notably those of Stanley Roberts (Exeter), Howard Carless (London), David Widdowson and Doug Ribbons (London), David Crout (Warwick), and Richard Stephenson (East Anglia) were also soon active in the area and made early contributions to the *cis*-dihydrodiol literature. About the same time other groups worldwide, notably those of Tomas Hudlicky (USA) and Martin Banwell (Australia), made, and continue to make, major contributions to the literature on the synthetic applications of *cis*-dihydrodiols.

A further milestone in my journey with *cis*-dihydrodiols occurred in 1986 when I met the late Howard Dalton. By strengthening the earlier link with David Gibson, and forming this important new link, it became possible for us to produce a wider range of monocyclic *cis*-dihydrodiols and several new types of regio- and stereoisomeric *cis*-diols, heterocyclic *cis/trans*-diols, triols, sulfoxide-*cis*-diols and bis-*cis*-diols, in both the Warwick and Belfast laboratories and these have in turn provided new options for synthesis. Narain Sharma, my companion for most of this journey, and I, have now shared the excitement of discovering and utilising new *cis*-dihydrodiols for almost thirty years. However, following the untimely death of my good

friend and collaborator Sir Howard Dalton, FRS, during early 2008, and my recent transition to Emeritus status, it may soon be time to pass on the baton to our younger colleagues. These include the safe hands of Gary Sheldrake, a former ICI employee, and Chris Allen, one of Howard's former PhD students, who, to some degree, both owe their current academic positions at Queen's to earlier collaborative visits to Belfast, and to the 1968 discovery of arene-*cis*-dihydrodiols by David Gibson.

(10) The investigation of the metabolism of aromatic compounds by eukaryotes began in the mid-1800s. In 1867 Schultzen and Naunyn reported⁸⁷ the isolation of phenol from the urine of animals that had been administered benzene. In 1876, Munk⁸⁸ described the detection of phenol as well as 'phenol-forming substance' in the urine of animals that had ingested benzene. The 'phenol-forming substance' was observed prior to treatment of urine with mineral acid. It is quite possible that the 'phenol-forming substance' may have been a *trans* arene diol, the expected metabolite of benzene by cytochrome oxidation, which would have undergone aromatization by treatment with the acid. One fascinating aspect of this disclosure is that Munk, unencumbered by safety regulations in those days, ingested benzene himself and then analyzed his own urine in order to validate the results from the animal studies:

*"Schultzen and Naunyn having stated that benzene when administered is excreted as phenol, this statement appeared to Munk to be extraordinary, seeing that benzene has never been directly oxidised into phenol outside the living body. He finds that when he himself takes benzene neither this body nor phenol can be detected in his urine, but that the phenol-forming substance is thereby increased in proportion to the quantity of benzene ingested."*⁸⁹

The original article is delightful. The most relevant part dealing with the actual description of the experiments with benzene digestion, from pages 147–148, follows:

"Von Versuchen an Thieren haben wir wegen der Schwierigkeit, in heisser Sommerzeit unzersetzten Harn und ohne Verlust zu bekommen, Abstand genommen und die Versuche an uns selbst angestellt, indem wir mit 20 Tropfen Benzol beginnend, allmählig bis zu 50 Tropfen = c. 2.5 Gramm pro die aufstiegen. Der gesammte, in den nächsten 24 Stunden nach der Benzoleinnahme abgesonderten Harn wurde zur Untersuchung verwendet. Wegen des schlechten Geschmacks und der stark brennenden, fast ätzenden Eigenschaften des Benzol wurde das letztere mit Eigelb, und weiterhin, was wir noch vorteilhafter gefunden haben, mit Fleischbrühe emulgiert eingenommen. Der gesammte Tagesharn, ohne Säurezusatz auf dem Sandbade destilliert, gab ein kampherartig riechendes, klares Destillat, in gleicher Weise wie der normale Harn; die Klarheit des Destillats bewies schon die Abwesenheit freien unveränderten Benzols. Ebenso wenig war auch darin eine Spur freien Phenols durch Bromwasser nachweisbar. Was war denn aus dem eingenommen Benzol geworden? Ein Theil davon wird einfach ausgeschieden und zwar gasförmig. Unsere phe-

nolbildenden Bestimmungen der Substanz bei verschiedener Ernährung hatten es wahrscheinlich gemacht, dass ihre Entstehung auf in den Körper eingebrachte aromatische Verbindungen zurückzuführen ist; war nun das Benzol selbst im Stande diesen phenolbildenden Körper in reichlicher Quantität zu erzeugen? Es wurde zur Entscheidung dieser Frage der gesammte Harn des Benzolversuchstages mit verdünnter Schwefelsäure destilliert; in dem Destillate erzeugte Zusatz von Bromwasser eine sehr intensive Trübung, aus der sich ein reichlicher krystallinischer Niederschlag absetzte."

Translation: *"The idea of doing the experiments on animals' urine was abandoned, due to the difficulty of obtaining non-decomposed urine and without loss during the hot summer; we conducted the experiments on ourselves by taking initially 20 drops of benzene and gradually increasing to 50 drops. Urine collected over the ensuing 24 hours was used for the experiments. Because of the bad taste and the intense burning, almost caustic, properties of benzene, it was taken with egg yolk, and moreover – what we even found more advantageous – taken with meat broth.... The combined urine from the 24-hour period, distilled without the addition of acid, gave a clear distillate that smelled of camphor, similarly to the regular urine, the clearness of the distillate already indicated the absence of free unchanged benzene. No trace of the free phenol could be detected with bromine water. What happened to the benzene? Part of it is simply excreted as a gas.... The tests and assignments of our 'phenol-forming substance' at varying doses of benzene indicated that its formation is due to the uptake of aromatic substances. Would benzene be able to produce those phenol-forming compounds in substantial amounts? To answer this question the entire volume of urine from one benzene test day was distilled with dilute sulfuric acid; addition of bromine water to the distillate produced a very intense cloudiness, of which copious amounts of a crystalline substance was deposited."*

(11) **[Personal recollection: Tomas Hudlicky, St. Catharines]**

In 1991 I had an occasion to give a lecture at Oxford as part of a UK lecture tour. Our involvement in using the arene *cis*-dihydrodiols in synthesis just started to be the mainstay of our program and I had mentioned during the introductory part of the lecture that the mechanism of the enzymatic dihydroxylation was poorly understood. This statement led, at the end of the seminar, to an animated discussion with Professor Jack Baldwin as to 'why is it that organic chemists cannot figure out relatively simple things'. Because of his involvement with penicillin cyclase at the time he suggested that a high oxidation of iron (i.e., iron(V)), should be considered when proposing the mechanism of the dihydroxylation. Later that evening, seated at a local pub, Jack proposed the [3+2] cycloaddition possibility for the enzymatic process. Although this idea remains untested by experiment it seems the most reasonable of all other options, certainly on paper.

(12) [Personal recollection: Tomas Hudlicky, St. Catharines]

Douglas Ribbons was a prolific publisher in the area of aromatic metabolites during the last quarter of the 20th century. His research on the metabolites of benzoic acids and other arenes led to a number of papers and disclosures of new enantiopure diols. I had met Doug at various meetings connected with biotransformations and had the privilege of overlapping with him at the Technical University in Graz in 1998 when I taught a short course on synthesis there. A consummate scientist, he insisted on auditing the course despite the fact that synthesis was outside the domain of his research as a biochemist and a microbiologist. He always asked the very best questions, each beginning with ‘How do you know...?’.

In 2000, he worked for several months in my group at the University of Florida as a visiting scientist on a project dealing with the TDO-mediated oxidation of aromatics containing remote chiral centers on side chains and hence the discrimination of enantiomers by that enzyme.⁹⁰ (He had investigated, in 1991, the oxidation of 1-phenylethanol by various mutants expressing TDO.) It was a pleasure to share science with him. His untimely passing in 2002 left a serious void in the biocatalysis community.

(13) [Personal recollection: Steven Ley, Cambridge]

For the sake of brevity we first acquired the necessary bacterial strain on an agar slope from David Gibson in 1978 when we were struggling with a long and frustrating chemical route to the *cis* and *trans* diols. We (Chris Self, a Ph.D. student of mine at Imperial) needed those compounds in order to make an Fe(CO)₃ stabilized form of the arene oxide.

Our attempts to grow the bacterium in benzene were unimpressive as we had no skills with biotransformations at that time. Consequently, we dropped the project only to pick it up again in 1985 when I was given substantial amounts of diol this time by ICI (thanks to Steve Taylor) and I was working with a new Ph.D. student, Francine Sternfeld. The rest is history, apart from the fact that a total of three of our early proposals to establish the concept and strategic importance of these arene diols, based on ideas from our first paper, were crudely and cruelly turned down. Others in the UK, however, were later and somewhat remarkably well funded to work in the area. This left a bittersweet taste in my mouth. I hope I never find out who rejected these proposals as they were clearly misguided in their judgment!

(14) [Personal recollection: Theodore Martinot, Cambridge, MA]

Given the number of barely passable methods for selectively generating stereocenters, and our ever-increasing reliance on and demand for optically ‘pure’ products, I find any criticism for a sound synthetic methodology that reliably produces useful synthons completely unfounded. What better building block is there? An optically pure cyclohexadienediol, where each olefin, and each alcohol can be used differently to fit the construction of the target with only minimal manipulations! Experts in sugar chemistry have had to devise clever protection/deprotection

schemes to make glucose a useful synthon. And here, you can selectively alkylate, oxidize or reduce one olefin after the other with only one protection (the diol). It doesn’t get better than this.

The only objection that I have to the cyclohexadienediols is that they are not as mainstream as they should be. Whereas one will not hesitate to reach for typical ‘chiral pool’ building blocks like sugars or amino acids, the diols see significantly less use, in spite of their demonstrated versatility. Part of this may stem from their perceived inaccessibility that could be a result of reading the instructions for one such example in the Aldrich catalog [(1*S*-*cis*)-3-bromo-3,5-cyclohexadiene-1,2-diol, p. 455; 2007–2008 catalog] – one may be tempted to ask: ‘Is this a biological or organic compound?’ Well, both, and that’s exactly what’s brilliant about all of this. And it’s actually quite easy to use. Unfortunately, the misconception about the accessibility and ease of use of this diol has engendered a vicious cycle, whereby less exposure has produced less interest, and ultimately less fundamental research in the field. If the technology was improved such that the generation of these diol building blocks could be effected on significantly larger scale than I recall doing in the lab, then industry experts would know that they could rely on that starting material for pharmaceutical manufacturing (for example). So another vicious cycle is produced: Industry won’t touch it for pharmaceutical research because industry (the same?) won’t develop a fully scalable process for it.

Is the problem lack of creativity, lack of imagination, or simply misinformation?

(15) [Personal recollection: W. B. Motherwell, London]

On the invention of the photoinduced osmium-tetroxide-catalyzed dihydroxylation of arenes: The origin of the questions which any scientist asks, when then coupled with his ability to practice the Medawar ‘Art of the Soluble’, is, of course, the criterion by which he is judged, but is a topic which rarely surfaces in the scientific literature. The tale of our own foray into the invention of the photochemically induced osmium tetroxide catalysed dihydroxylation of arenes provides an opportunity to illustrate a somewhat less obvious thought process and the circumstances which can be involved in the invention and discovery of a new reaction.

In the first instance I was exceedingly fortunate as a young lecturer at Imperial College in the early eighties to have, both as a colleague and as a friend, Professor Steven Ley. We had in fact first met in the mid-seventies when we were both postdoctoral fellows in the group of Sir Derek Barton, and had always enjoyed the opportunity of discussing chemistry together. As a friend, the more mischievous side of my character would often surface in the form of teasing him as a way of exhorting him to even greater efforts. And so it was, when Steve was exploring the chemistry of the fragile *cis*-diene diol produced from benzene by the microorganism *Pseudomonas putida* and completing elegant syntheses of such targets as inositol phosphates and pinitol, that I would accuse him of being

lazy in allowing a 'bug' to do the first step of his synthetic sequence. A natural riposte to this statement was that this straightforward first step might provide a suitable opportunity for me to find a solution, and so I was effectively 'hoisted by my own petard'.

It was at this stage that my curiosity-driven approach to research came to my aid. I had always enjoyed reading the chemical literature very widely and even more so, under the tutelage of Sir Derek Barton, I had come to appreciate that any scientist should never cease to ask questions. As a consequence, I had always been fascinated by the less commonly used reactive intermediates of organic synthesis including free radicals and electron-transfer processes, arguing, quite simply, that there must be more to learn from such reactions. In particular, I was intrigued by a beautiful study by Kochi and Wallis who had demonstrated that actinic (UV) irradiation of the electron donor acceptor (EDA) complex between osmium tetroxide and benzene led, in the presence of an excess of osmium tetroxide, to a polymeric 2:1 adduct which could be isolated by the addition of pyridine, as the complex of the bisosmate ester derivative of conduritol E.

Thereafter, given such an insightful study by Kochi, the temptation to transform this observation into a catalytic process was irresistible. The only remaining problem was to find an oxygen atom transfer reagent which would not destroy the very weak EDA complex between the arene and osmium tetroxide, and this we eventually achieved by selecting a biphasic system and using barium chlorate, a reoxidant discovered by Hoffman in 1912. We were unable to prevent a second thermal *anti* osmylation, but further work established that we could favour the conduritol product over the inositol by operating at lower temperatures. Chance observations by a skilled experimentalist also feature in the invention and discovery of new reactions, and the isolation of a monochlorinated product by Alvin Williams led us to believe that hypochlorous acid, generated in situ during reductive work up with bisulfite, could also occur. In this way, through replacement of chlorate by bromate we were able to achieve stereocontrolled monobromopentahydroxylation of benzene, and this became the first step in our five-step, three-pot synthesis of racemic pinitol from benzene.⁸¹ When I told Steve about this reaction, his response was that a racemic mixture was unacceptable in modern day organic synthesis, and so we had failed again.

In the final analysis, I am certain that catalytic dihydroxylation of arenes will be achieved by the organic chemist at the bench. As always, Nature provides the inspiration, and as with the Gif system for the oxidation of hydrocarbons (yet another tale of discovery), iron will be the element of choice. The active site of the dioxygenase which delivers a *cis*-diol is now known, and an increasing number of papers on dihydroxylation of alkenes using iron catalysts are now appearing in the chemical literature. Clearly, the stage has been set and the challenge is now ripe for enantioselective arene dihydroxylation.

(16) One of the assignments for R. V. Stevens's 1974 graduate course in synthesis at Rice University was to

write a short paper on the development of new reactions for synthesis. As a guide Stevens provided a list of 'unsolved problems' that he perceived to be important.⁹¹ Prominent on this list figured the synthesis of unsymmetrical biphenyls (by means other than Ullman coupling), general methods for sp^2 - sp^2 and sp - sp^2 coupling, and a number of other problems for which solutions did not exist. We noted as recently as 2007 that of the 20 or so significant problems on this list, only one had been completely solved: the methods of Heck, Suzuki, Stille, and Sonogashira (and their variations) provide a nearly complete solution to olefinic and aromatic coupling. The other problems have not been solved or only partially so, with incomplete solutions being advanced. In spite of some claims to the contrary, synthesis can hardly be considered to be a mature science at the beginning of the 21st century.

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- (48) **Safety notice:** There are some hazards in handling and storage, especially for the *cis*-dihydrodiol derived from β -bromobenzene. This particular compound cannot be stored in solid form free of solvent. On several occasions it exploded on vacuum drying and on one occasion a bottle with about 25 g of crystalline solid exploded and burned in a freezer at $-78\text{ }^{\circ}\text{C}$. The aromatization of this material is highly exothermic and catalyzed by trace amounts of phenol; therefore, the isolation and handling of *cis*-dihydrodiols in

- amounts over 5–10 g must be attended to with caution. Ethyl acetate extracts must be washed with saturated carbonate solution to remove any trace amounts of the corresponding phenols, which may catalyze aromatization. In our own experience, we have encountered no problems with the *cis*-dihydrodiols derived from chloro- and bromobenzene; however, it is advisable to handle larger amounts in solution rather than in solid state.
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