CHEMICAL REVIEWS

EDITORIAL

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Introduction to Enzymes in Synthesis

It is a great honor for me to serve as a Guest Editor for this special issue entitled "Enzymes in Synthesis". It is my hope that the publication of this collection of excellent articles from experts in enzymatic and biological chemistry will serve to narrow the gap in communication between biologists and chemists. But first, some background and a little bit of criticism . . . aimed primarily at my own community of synthetic organic chemists.

Much has been written on green chemistry, environmentally conscious manufacturing, renewable resources, alternative energy, and other politically correct topics. The establishment and the subsequent rise to fame of the Green Chemistry movement in the mid-1990s led many synthetic chemists to attempt redesign of known reactions so that they would proceed in aqueous medium to avoid the use of organic solvents. Furthermore, the past 15 years have witnessed a proliferation of publications in the field of [transition-metal] catalysis to respond to the directives associated with efficiency in manufacturing.

The 12 Principles of Green Chemistry (Table 1), put forth by Anastas and Warner,[1] advocate the use of "atom economy" and catalysis as the common sense approaches to efficiency; however, while noble, they fall short of actual accomplishments. First, the concept of atom economy[2] remains only a semantic descriptor and does not in any way define efficiency or "green" aspect of a process. Many other efficiency metrics, quantitatively far more useful and accurate, are available,[3] including a very detailed evaluation of synthetic efficiency by Andraos (refs 3g and 3h). Second, the Anastas principles completely ignore biological methods; the words "enzymatic catalysis" or "biological methods" are curiously absent, although, arguably, enzymes do belong into the broadly defined discipline of catalysis. Third, synthetic organic chemists frequently speak of the "interface of chemistry and biology", "chemical biology", or "biological and bioorganic chemistry" while exhibiting an almost pathological phobia with regard to actually using biological methods in preparation of organic compounds. Occasionally, synthetic practitioners will use baker's yeast or a lipase-mediated desymmetrization, but very rarely will they themselves use either isolated oxidoreductases or simple fermentation techniques. The belief that catalysis implies solely the use of transition metal complexes is ingrained in our community as well as among the membership of the various panels that control funding. Many outstanding practitioners of biocatalysis in the U.S. have had their careers terminated, or at least made difficult, by lack of funding once they submitted proposals advocating the use of enzymatic methods.

I have written extensively on the topic of biocatalysis in organic synthesis as well as on various reasons underlying the fact that, compared to the rest of the world, it is almost nonexistent in the U.S. academic community. I will not repeat my arguments here as there are more detailed sources for the interested reader.[4]

Clearly, the use of fermentation methods carried out in an aqueous medium is an obvious response to the Green Chemistry mantra advocated by Anastas and Warner and many others. Any process involving fermentation demands less energy than various "green" processes requiring supercritical carbon dioxide or water. Conveniently, authors almost never mention such energy requirements in claiming improvements in green manufacturing. Despite a few credible advances in environmentally conscious manufacturing, most of the so-called principles of green chemistry remain unfulfilled at this time, at least in the realm of academe. It would serve the Green Chemistry movement well if more researchers in academia became involved in biological methods. There are, of course, countless examples of the use of fermentation in industry; in production of erythromycin to insulin, enzymatic methods are more effective than those employed in traditional synthesis.

The lack of emphasis on biological methods of manufacturing is a cause for concern for several reasons. Biology will have to

Table 1. Twelve Principles of Green Chemistry[1]

1. Prevention: It is better to prevent waste than to treat or clean up waste after it has been created.

2. Atom Economy: Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

3. Less Hazardous Chemical Syntheses: Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4. Designing Safer Chemicals: Chemical products should be designed to effect their desired function while minimizing their toxicity.

5. Safer Solvents and Auxiliaries: The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

6. Design for Energy Efficiency: Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

7. Use of Renewable Feedstocks: A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

8. Reduce Derivatives: Unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

9. Catalysis: Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

10. Design for Degradation: Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. Real-Time Analysis for Pollution Prevention: Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

12. Inherently Safer Chemistry for Accident Prevention: Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

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provide solutions for small-molecule supply once chemical companies are not able to rely on oil-based sources of catalog items or commodity chemicals. It is therefore important to increase the dialogue between chemists and biologists so that solutions to significant problems may be attained in the very near future.

The first step toward this goal is for those chemists involved in synthesis of chemicals and/or materials to exhibit a bit more open mind than they have shown in the past. The publication of this special issue should constitute the first step toward such dialogue as it makes obvious that the combination of biological and synthetic methods is almost always more efficient than either method alone.

The history of biocatalysis (if one does not consider wine and beer making) can be traced to Brown's disclosure in 1866[5] of converting mannitol to fructose by fermentation. He provided a prophetic statement:

> ". . . I think that 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 . . . ."

Emil Fischer, just a few years later, became a strong advocate for the use of biological methods in the preparation of organic compounds. His interest in biology led him to investigate enzymatic cleavage of glucosides[6] and the formulation of the "lock-and-key" model for the binding of substrates in active sites.[7] Until the use of biocatalysis in industry became prevalent in the middle of the 20th century, it had been confined to biotransformations of carbohydrates, aliphatic and aromatic compounds, or steroids. A great source of information on the history and evolution of applications in this field is the 1976 compilation by Kieslich.[8] One of the early proponents of application of biocatalysis in total synthesis of natural products in the academic realm was Sih, who employed an enzymatic reduction as a first step in his asymmetric synthesis of compactin in 1981.[9] In the last 20 years the use of enzymatic and biotransformation methods has increased, especially in the European academic community.

This special issue was made possible by contributions from experts in all areas of biocatalysis. It is organized in such a way that the uninitiated reader will be first exposed to those methods in biocatalysis that do not require highly specialized equipment or experience and only then be guided to the more complex applications that involve the use of oxidoreductase or C−C bond-forming enzymes. The most technically and experimentally demanding application, the use of anaerobic organisms, is not covered in detail in this collection.

The first chapter, *Hydrolases in the Stereoselective Synthesis of N-Heterocyclic Amines and Amino Acid Derivatives* by Eduardo Busto, Vicente Gotor-Fernández, and Vicente Gotor, provides a great survey of the applications of hydrolase enzymes to the synthesis of enantiopure heterocyclic compounds as well as amino acids. Acylations and hydrolyses are among the most user-friendly applications of enzymatic reactions. The examples portrayed in this chapter should convince the reader of the utility of these simple processes that lead to enantiomeric enrichments without the need for additional cofactors or special equipment. This is one of the reasons why hydrolases are the most widely used enzymes by synthetic organic chemists.

The chapter entitled *Biocatalytic Methods for the Synthesis of Enantioenriched Odor Active Compounds* by Elisabetta Brenna, Claudio Fuganti, Francesco Gatti, and Stefano Serra provides an interesting overview of the preparation of various chiral odorants via biocatalysis. The synthesis of single enantiomers of various monoterpenes and irridoids of commercial significance is covered together with the discussion of biocatalytic methods of kinetic resolution via acylation or hydrolysis, as well as stereoselective terpene oxidations and reductions.

Nicholas Turner, in his contribution *Enantioselective Oxidation of C−O and C−N Bonds Using Oxidases*, provides examples of the use of oxidases in oxidation of alcohols (from aliphatic alcohols to carbohydrates) and amines (from amino acids to N-heterocyclic amines).

Mélanie Hall and Andreas Bommarius provide a concise chapter on *Enantioenriched Compounds via Enzyme-Catalyzed Redox Reactions* in which numerous examples of both enzymatic reductions and oxidations are provided with an eye for applications in providing optically pure compounds for the chiral pool as well as intermediates for total synthesis. From alcohol dehydrogenase to mono- and dioxygenase applications, examples are provided for processes such as chiral reduction, conversion of meso compounds to single enantiomers, and oxidation of achiral substrates.

Daniela Monti, Gianluca Ottolina, Giacomo Carrea, and Sergio Riva provide an overview of applications in their chapter on *Redox Reactions Catalyzed by Isolated Enzymes*. The discussion begins with various strategies of cofactor regeneration, substrate supply, including continuous flow systems, and reaction medium. The use of dehydrogenases, oxidases, peroxidases, and oxygenases is covered through selected applications.

The article entitled *Application of Designed Enzymes in Organic Synthesis* by Gernot Strohmeier, Harald Pichler, Oliver May, and Mandana Gruber-Khadjawi introduces the reader to various methods of enzyme modification for specific purposes via either rational design or mutagenesis. The introduction covers recombinant DNA techniques that are essential to this strategy. Several classes of designed enzymes are covered (oxidoreductases, hydrolases, lyases, and isomerases, among others) with illustrations of how directed evolution improved selectivity of transformations.

Hannes Leisch, Krista Morley, and Peter Lau in their contribution, *Baeyer−Villiger Monooxygenases: More Than Just Green Chemistry,* review the evolution and the applications of the enzymatic Baeyer−Villiger reaction in synthesis. The history of monooxygenases, as well as their applications and directed evolution, is covered first before a long section on the applications to asymmetric synthesis. Detailed discussion of the structures of enzymes, protein engineering, scale-up processes, and cofactor recycling strategies completes this chapter.

Jan Duchek, David Adams, and Tomas Hudlicky provide an overview of the *Chemoenzymatic Synthesis of Inositols, Conduritols, and Cyclitol Analogues* by methods involving various enzymes. This chapter focuses on the use of cyclohexadiene-*cis*-diols derived by enzymatic dihydroxylation of arenes as well as on the desymmetrization of meso compounds, resolution of racemates, or the use of C−C bond-forming enzymes. Inositols, conduritols, and their nitrogen- and sulfur-containing analogues are covered in the chapter.

Ryan Schmaltz, Sarah Hanson, and Chi-Huey Wong in their contribution entitled *Enzymes in the Synthesis of Glycoconjugates* provide a very detailed survey of enzymatic methods for the construction of glycoproteins, glycopeptides, and the use of glycosyl transferese, glycosidase, and glycosynthase enzymes in carbohydrate synthesis. A survey of glycosylation pathways

engineering in mammalian and nonmammalian organisms is also provided. The last two sections provide an overview of engineering of cellular processes as well as construction of recombinant bacteria for production of carbohydrates.

Precision Polysaccharide Synthesis Catalyzed by Enzymes by Junichi Kadokawa provides the reader with a survey of types of reactions and enzymes such as hydrolase, phosphorylase, and sucrase types, to accomplish the synthesis of complex oligo- and polysaccharides.

The chapter entitled *C−C Bond-Forming Lyases in Organic Synthesis* by Margarita Brovetto, Daniela Gamenara, Patricia Saenz Méndez, and Gustavo Seoane illustrates the applications of C−C bond-forming reactions catalyzed by enzymes. The review covers aldol reactions, acyloin reaction, and cyanohydrin formation, and provides relevant examples of syntheses of industrially important compounds.

All together, this special issue provides a very concise collection of articles in the field of biocatalysis. It remains for me to thank all of the authors who contributed their time to make this project possible. I also thank Robert Kuchta for the invitation to serve as the Guest Editor for this most worthwhile endeavor and to Saundra Richter for her invaluable help with this issue. I hope the contents serve as a guidebook for many newcomers to the discipline.

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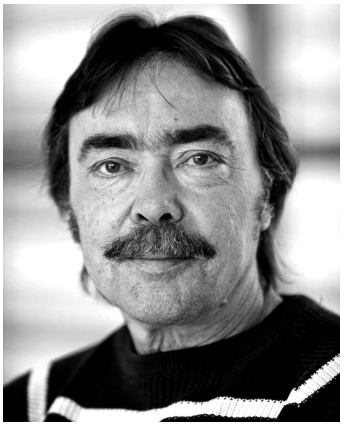
BIOGRAPHY



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 U.S. 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.S. in chemistry in 1973, and he 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 oligosaccharide conjugates with new molecular properties. His hobbies are skiing, hockey, martial arts, and music.

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