Edited by Eduardo Garcia-Junceda

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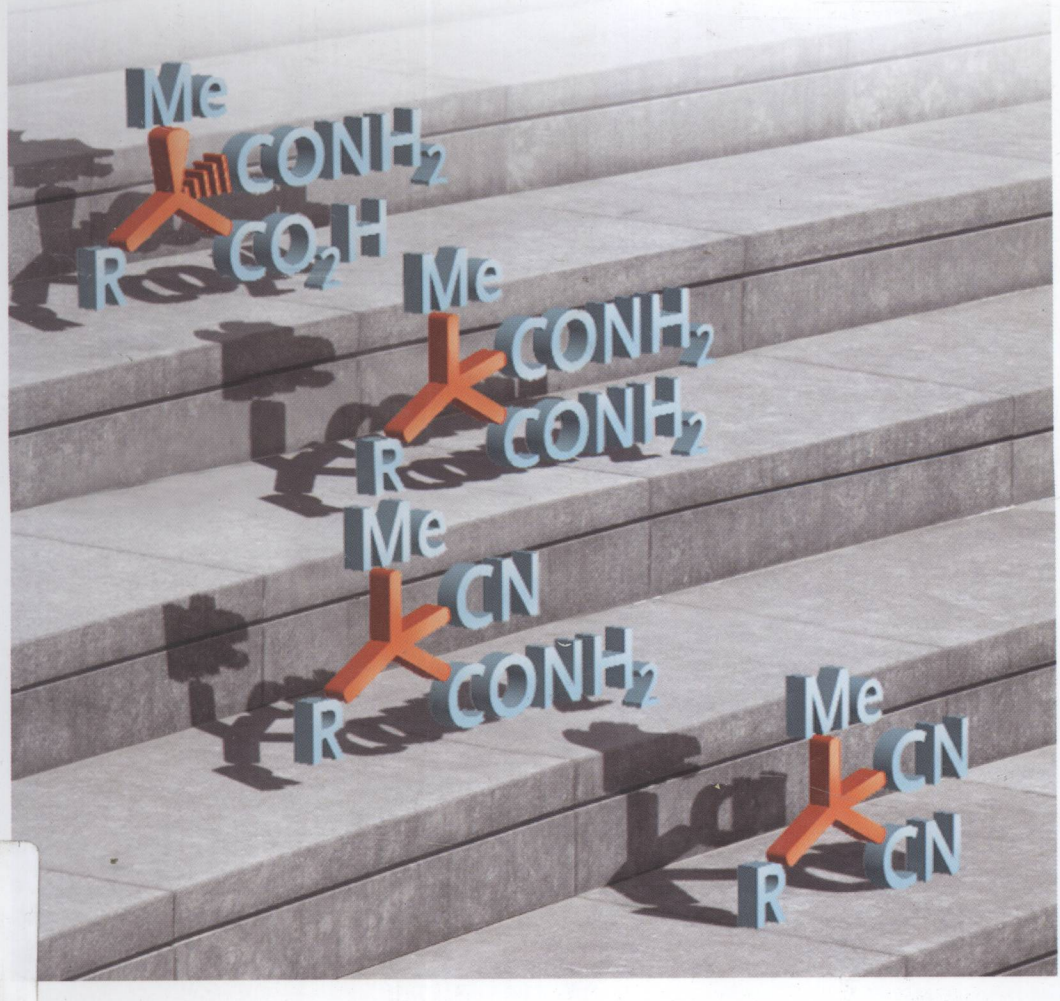
Multi-Step Enzyme Catalysis

Biotransformations and Chemoenzymatic Synthesis



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Further Reading

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Hydrolases in Organic Synthesis

Regio- and Stereoselective Biotransformations

2006
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Preface

> "The abundance of substances of which animals and plants are composed, the remarkable processes whereby they are formed and then broken down again have claimed the attention of mankind of old, and hence from the early days they also persistently captivated the interest of chemists".
>
> *Emil Fischer, Nobel Lecture, 1902*

> "The chemist who designs and completes an original and esthetically pleasing multistep synthesis is like the composer, artist or poet who, with great individuality, fashions new forms of beauty from the interplay of mind and spirit".
>
> *Elias James Corey, Nobel Lecture, 1990*

Nature has always been a permanent source of inspiration for chemists but, as Emil Fischer brightly indicated in his Nobel award acceptance lecture in 1902, it is not only the vast diversity of compounds that living beings are capable of creating, but also the extraordinary strategies of synthesis deployed. Evidently, the catalysts used by living beings – enzymes – are key to Nature's Synthesis Strategies. Emil Fischer himself foretold, a few paragraphs further into his lecture, that chemistry would employ enzymes at large and – to our greatest surprise, bearing in mind that these words were written as early as 1902 – that artificial enzymes would be tailor-made to serve its purposes.

The longing of biocatalysis to transfer to the laboratory the exquisite efficiency shown by enzymes in Nature has begun to become a reality since the late 1980s, with the invention of the polymerase chain reaction (PCR). The level of development and access brought about by the PCR to genetic material handling and transformation, has allowed the number of available enzymes to grow exponentially. Modifying the catalytic properties of enzymes to adapt them to their new environment in a test tube has become a reality. We have learned to imitate the strategies used by Nature to create new enzymes, and to adapt the existing ones to new synthetic needs. Eventually, Emil Fischer's prediction has come true.

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Living beings do not use enzymes in isolation, however. A large portion of the extraordinary synthetic effectiveness that enzymes display in Nature comes from the fact that living beings apply a multistep synthesis strategy, catalyzed by enzymes acting sequentially. It is the utilization of more- or less-complicated biosynthetic routes that allows living beings to build complex structures from simple elements; to obtain and to store energy; and to know and to communicate with their environment. The jointed action of a sequence of enzymes can make irreversible a reversible process, eliminate inhibition problems caused by product excess, or prevent the lack of substrate scattered on the bulk solution. Evidently, in order to develop, biocatalysis could not look away from these and other synthesis opportunities served by multistep reactions. The level of relevance attained by the development of this synthetic strategy in the field of biocatalysis and biotransformation is evidenced by the celebration in April 2006 of the first Symposium on *Multistep Enzyme-Catalyzed Processes*, organized jointly by the Applied Biocatalysis Research Centre at Gratz and the European Federation of Biotechnology Section of Applied Biocatalysis (ESAB), to which this book is indebted.

The aim of this handbook is to bring together various key aspects to cover the broad field of *multistep enzyme-catalyzed processes*, from the 'simplest' system in which one or a few isolated enzymes are used alone or in combination with non-enzyme-catalyzed steps, to the most 'complex' system in which artificial or natural pathways are created or even whole cells are modified to be used as synthetic factories.

I would like thank all those authors who have participated in this exciting project for their superb work, valuable time and remarkable efforts; and in particular, I thank Elke Maase and Stefanie Volk at Wiley-VCH for their patience, friendliness and precious help in editing.

I hope that you enjoy reading this book, and that it can serve as an inspirational source and stimulus to researchers of all levels – especially the youngest – who are working in the biocatalysis field.

Madrid, July 2008 *Eduardo García-Junceda*

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Contents

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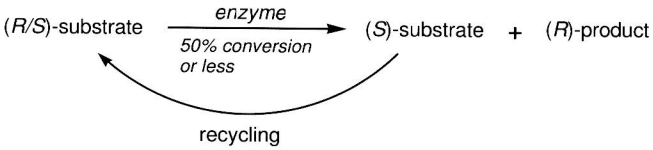
1
Asymmetric Transformations by Coupled Enzyme and Metal Catalysis: Dynamic Kinetic Resolution

Mahn-Joo Kim, Jaiwook Park, and Yoon Kyung Choi

1.1
Introduction

The enzymatic resolution of racemic substrates now is a well-established approach for the synthesis of single enantiomers [1, 2]. A representative example is the kinetic resolution of secondary alcohols via lipase-catalyzed transesterification for the preparation of enantiomerically enriched alcohols and esters [3]. The enzymatic resolution in general is straightforward and satisfactory in terms of optical purity, but it has an intrinsic limitation in that the theoretical maximum yield of a desirable enantiomer cannot exceed 50%. Accordingly, additional processes such as isolation, racemization and recycling of unwanted isomers are required to obtain the desirable isomer in a higher yield (Scheme 1.1).



Scheme 1.1 (*R*)-Selective enzymatic resolution with recycling of unreacted (*S*)-substrate.

The limitation of enzymatic resolution, however, can be overcome by introducing an efficient catalyst for racemization of substrate into the resolution, leading to the process called dynamic kinetic resolution (DKR) [4]. Theoretically, DKR can provide single enantiomeric products [99% enantiomeric excess (e.e.) or greater] in 100% yield in the case where a highly efficient racemization catalyst is combined with a highly enantioselective enzyme. In the last decade, several metal-based catalysts have been developed for the racemization and successfully incorporated into the resolution process [5]. Now a wide range of racemic substrates can be converted to enantiomeric products of high optical purity in good yields via the enzymometallic DKR (Scheme 1.2). This chapter covers these developments with detailed examples.

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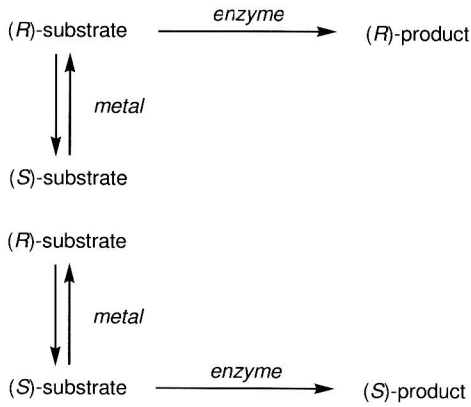


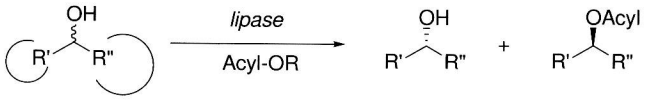
Scheme 1.2 (*R*)- and (*S*)-Selective enzymo-metallic DKR.

1.2
Some Fundamentals for DKR

1.2.1
Enzymes for Kinetic Resolution

The resolution of a racemic substrate can be achieved with a range of hydrolases including lipases and esterases. Among them, two commercially available lipases, *Candida antarctica* lipase B (CALB; trade name, Novozym-435) and *Pseudomonas cepacia* lipase (PCL; trade name, Lipase PS-C), are particularly useful because they have broad substrate specificity and high enantioselectivity. They display satisfactory activity and good stability in organic media. In particular, CALB is highly thermostable so that it can be used at elevated temperature up to 100 °C.

The lipase-catalyzed resolutions usually are performed with racemic secondary alcohols in the presence of an acyl donor in hydrophobic organic solvents such as toluene and *tert*-butyl methyl ether (Scheme 1.3). In case the enzyme is highly enantioselective ($E = 200$ or greater), the resolution reaction in general is stopped at nearly 50% conversion to obtain both unreacted enantiomers and acylated enantiomers in enantiomerically enriched forms. With a moderately enantioselective enzyme ($E = 20$–50), the reaction carries to well over 50% conversion to get unreacted enantiomer of high optical purity at the cost of acylated enantiomer of lower optical purity. The enantioselectivity of lipase is largely dependent on the structure of substrate as formulated by Kazlauskas [6]: most lipases show



Scheme 1.3 Lipase-catalyzed resolution of secondary alcohols.