Multi-Step Enzyme Catalysis

Biotransformations and Chemoenzymatic Synthesis

Edited by
Eduardo Garcia-Junceda

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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.

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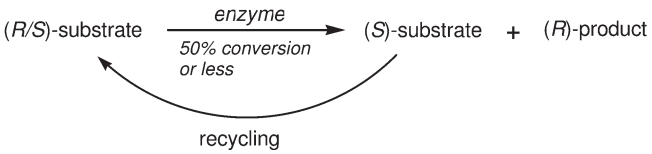
1
Asymmetric Transformations by Coupled Enzyme and Metal Catalysis: Dynamic Kinetic Resolution

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

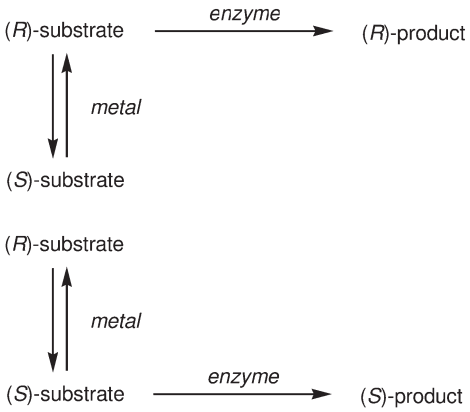
1.1
Introduction

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.



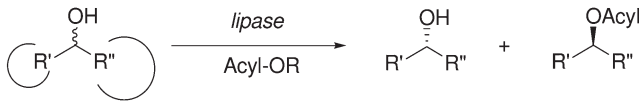
Scheme 1.2 (*R*)- and (*S*)-Selective enzymo-metallic DKR.

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.

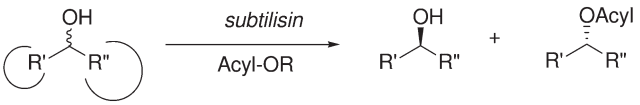
The lipase-catalyzed resolutions usually are performed with racemic secondary alcohols in the presence of an acyl donor in hydrophobic organic solvents such as toluene and *tert*-butyl methyl ether (Scheme 1.3). In case the enzyme is highly enantioselective (E = 200 or greater), the resolution reaction 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.

(*R*)-selectivity toward simple secondary alcohols carrying one small and one relatively larger substituent at the hydroxyl methane center, and the selectivity in general increases with an increase in the size difference between two substituents. The size of the small substituent limits the reactivity of substrate toward lipase. If it exceeds a three-carbon unit, the substrate reacts very slowly or does not react at a synthetically useful rate. Accordingly, the Kazlauskas rule is useful as a guideline for predicting substrates that can be efficiently resolved by lipase as well as the stereochemistry of resolved substrates.

Lipase, which is highly useful for kinetic resolution, however, has a limitation for use in DKR in that it cannot be used for (*S*)-configuration products. For this purpose, subtilisin, a protease from *Bacillus licheniformis*, can replace lipase since it provides complementary enantioselectivity (Scheme 1.4). Subtilisin, however, has been much less frequently employed in resolution compared to lipase because it displays poor catalytic performance in organic media. Subtilisin is inferior to lipase in several properties such as activity, enantioselectivity and stability. Accordingly, the use of the enzyme usually requires some special treatments for activation and stabilization before use. For example, the treatment of subtilisin with surfactants has enhanced substantially its activity and stability up to a synthetically useful level.



Scheme 1.4 Subtilisin-catalyzed resolution of secondary alcohols.

1.2.2
Metal Catalysts for Racemization

Many different metal catalysts have been explored for racemization of secondary alcohols. Among them, ruthenium-based organometallic complexes have been most intensively tested as the racemization catalyst (Figure 1.1).

These ruthenium catalysts catalyze the racemization of secondary alcohol through a dehydrogenation/hydrogenation cycle with or without releasing ketone as a byproduct (Scheme 1.5). Catalysts **6–9** display good activities at room temperature, while others show satisfactory activities at elevated temperatures. Catalyst **1**, for example, requires a high temperature (70 °C) for dissociation into two monomeric species (**1a** and **1b**) acting as racemization catalysts (Scheme 1.6).

Most ruthenium catalysts except **8** and **9** are highly sensitive to oxygen or air and must be used under anaerobic conditions. The latter can be used under aerobic conditions. Currently, no rationale is available for explaining the difference in stability between these ruthenium catalysts. In general, racemizations by these catalysts take place more rapidly with benzylic alcohols compared to non-benzylic or aliphatic alcohols.

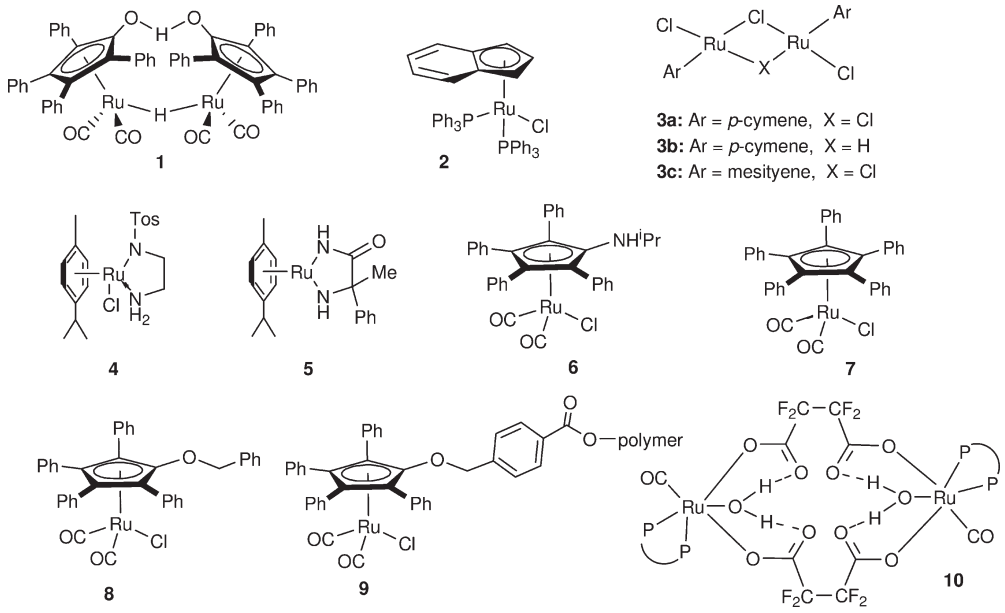
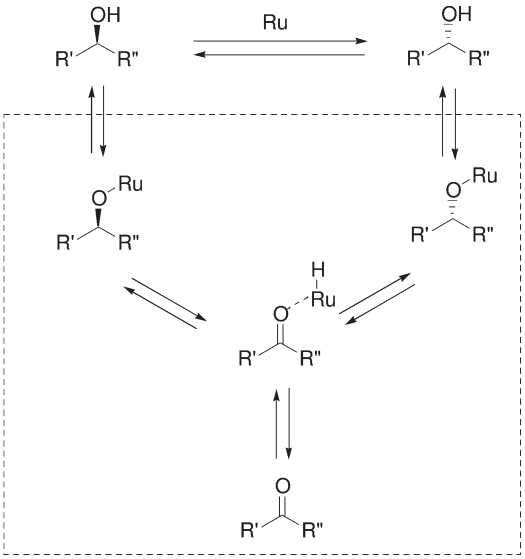
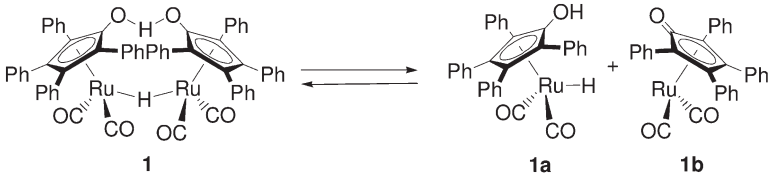


Figure 1.1 Ruthenium catalysts.



Scheme 1.5 A simplified mechanism for ruthenium-catalyzed racemization of *sec*-alcohol.



Scheme 1.6 Dissociation of catalyst **1**.

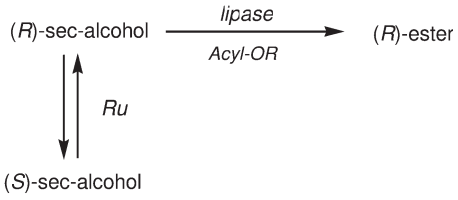
1.2.3
Enzyme–Metal Combination for DKR

DKR of secondary alcohol is achieved by coupling enzyme-catalyzed resolution with metal-catalyzed racemization. For efficient DKR, these catalytic reactions must be compatible with each other. In the case of DKR of secondary alcohol with the lipase–ruthenium combination, the use of a proper acyl donor (required for enzymatic reaction) is particularly crucial because metal catalyst can react with the acyl donor or its deacylated form. Popular vinyl acetate is incompatible with all the ruthenium complexes, while isopropenyl acetate can be used with most monomeric ruthenium complexes. *p*-Chlorophenyl acetate (PCPA) is the best acyl donor for use with dimeric ruthenium complex **1**. On the other hand, reaction temperature is another crucial factor. Many enzymes lose their activities at elevated temperatures. Thus, the racemization catalyst should show good catalytic efficiency at room temperature to be combined with these enzymes. One representative example is subtilisin. This enzyme rapidly loses catalytic activities at elevated temperatures and gradually even at ambient temperature. It therefore is compatible with the racemization catalysts **6–9**, showing good activities at ambient temperature. In case the racemization catalyst requires an elevated temperature, CALB is the best counterpart.

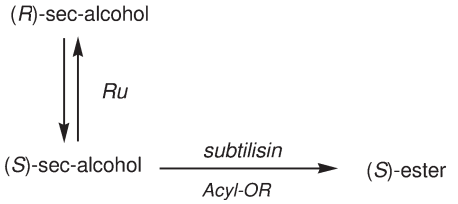
1.2.4
(*R*)- and (*S*)-Selective DKR

Thanks to two complementary enzymes, lipase and subtilisin, both (*R*)- and (*S*)-selective DKR can be performed to obtain the corresponding enantiomeric products.

DKR by the lipase–ruthenium combination provides (*R*)-products, while DKR by the subtilisin–ruthenium combination gives products of the opposite configuration (Schemes 1.7 and 1.8).



Scheme 1.7 (*R*)-Selective DKR with the lipase–ruthenium combination.



Scheme 1.8 (*S*)-Selective DKR with the subtilisin–ruthenium combination.

1.3 Examples of DKR

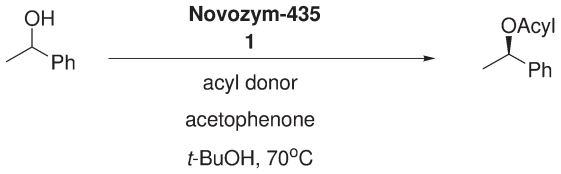
1.3.1 First DKR of Secondary Alcohols

The first use of a metal catalyst in the DKR of secondary alcohols was reported by Williams *et al.* [7]. In this work, various rhodium, iridium, ruthenium and aluminum complexes were tested. Among them, only $Rh_2(OAc)_4$ and $[Rh(cod)Cl]_2$ showed reasonable activity as the racemization catalyst in the DKR of 1-phenylethanol. The racemization occurred through transfer-hydrogenation reactions and required stoichiometric amounts of ketone as hydrogen acceptor. The DKR of 1-phenylethanol performed with $Rh_2(OAc)_4$ and *Pseudomonas fluorescens* lipase gave (*R*)-1-phenylethyl acetate of 98% e.e. at 60% conversion after 72 h.

1.3.2 DKR of Secondary Alcohols with Racemization Catalyst 1

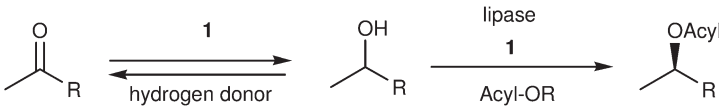
Significantly improved DKR was reported by Bäckvall *et al.* who used diruthenium complex **1** together with CALB [8]. This work demonstrated for the first time the superiority of PCPA as the acyl donor over popular acyl donors such as vinyl and isopropenyl acetate. The DKR of 1-phenylethanol by this procedure afforded optically pure (*R*)-1-phenylethyl acetate in a high yield (Scheme 1.9) [8b].

However, the procedure has some drawbacks to overcome. First, it requires an elevated temperature (70 °C) for the activation of the racemization catalyst. Such a high temperature is unacceptable for thermally less-stable enzymes. Second, the racemization proceeds through a mechanism including the release of ketone as a byproduct and thus the lowering of yield is inevitable. Third, PCPA used in an



acyl donor	ee (%)	yield (%)
vinyl acetate	>99	50
Isopropenyl acetate	>99	72
p-chlorophenyl acetate	>99	100

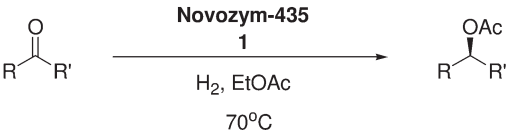
Scheme 1.9 DKR of 1-phenylethanol with ruthenium catalyst **1**.



Scheme 1.10 Hydrogenation and DKR of ketones.

excess amount is often difficult to remove from the acylated products during work-up. In spite of these limitations, the procedure with ruthenium catalyst **1** has been successfully applied in the DKR of a variety of simple and functionalized alcohols, including diols [9], hydroxy acid esters [9b, 10], hydroxyl aldehydes [9b], β-azido alcohols [11], β-hydroxyl nitriles [12], β-halo alcohols [13] and hydroxyalkanephosphonates [14].

An interesting application of **1** is the use in the asymmetric reductive acetylation of ketones via DKR of alcohol intermediates. In this transformation, ruthenium-catalyzed hydrogenation of ketone takes place in a concerted fashion with DKR of alcohol to produce the corresponding acyl products (Scheme 1.10). The idea of this process was to take advantage of ketone formation, which is a problem observed in the DKR of secondary alcohols with **1**. A key to this process was the selection of hydrogen donors compatible with the DKR conditions. 2,6-Dimethyl-4-heptanol, which cannot be acylated by lipases, and hydrogen molecules were effective hydrogen donors [15]. Asymmetric reductive acetylation of ketones under 1 atm hydrogen in ethyl acetate gave products in good yields and high optical purities (Scheme 1.11) [15b]. Here, ethyl acetate was used as both acyl donor and solvent.



R	R'	ee (%)	yield (%)
Me	Ph	96	81
Me	4-MeO-Ph	99	85
Me	4-Cl-Ph	97	72

Scheme 1.11 Asymmetric reductive acetylation of ketones.

Asymmetric reductive acetylation was also applicable to acetoxyphenyl ketones. In this case the substrate itself acts as an acyl donor. For example, *m*-acetoxyacetophenone was transformed to (*R*)-1-(3-hydroxyphenyl)ethyl acetate under 1 atm H_2 in 95% yield [16] (Scheme 1.12). The pathway of this reaction is rather complex. It was confirmed that nine catalytic steps are involved: two steps for ruthenium-catalyzed reductions, two steps for ruthenium-catalyzed racemizations, two steps