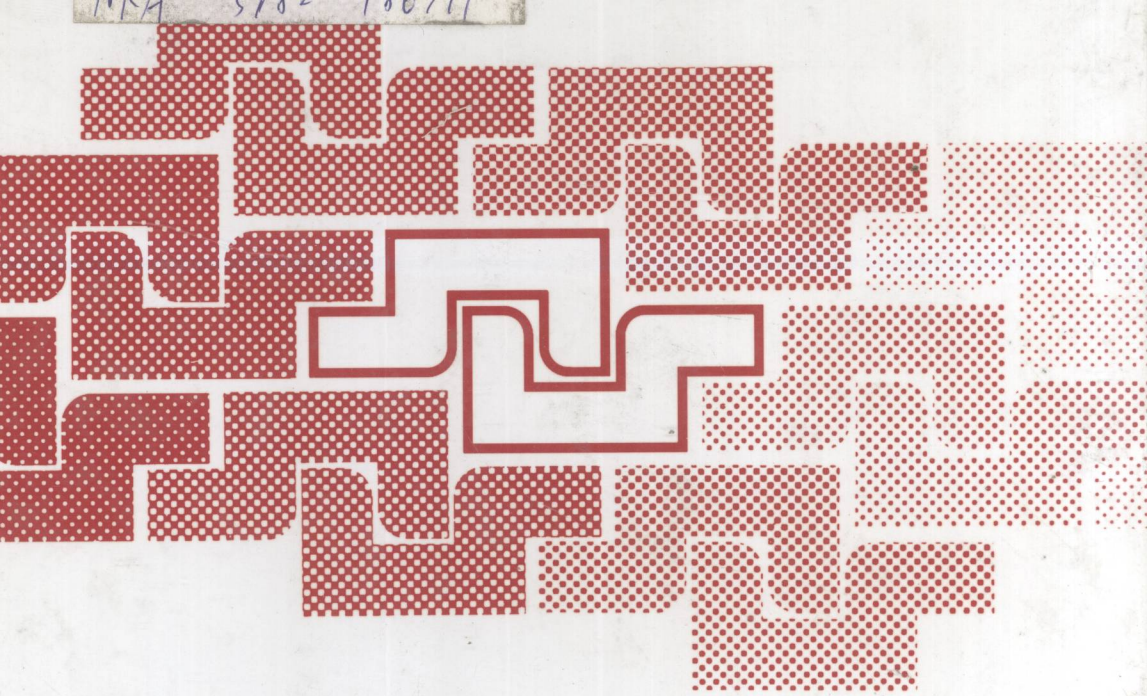

Enzymes in Action

Green Solutions for Chemical Problems

Edited by

Binne Zwanenburg, Marian Mikołajczyk
and Piotr Kiełbasiński

NATO Science Series

1. Disarmament Technologies – Vol. 33

Enzymes in Action
Green Solutions for Chemical Problems

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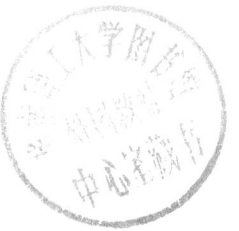

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PREFACE

The objective of the NATO Advanced Study Institute on enzymes in heteroatom chemistry was to discuss the current state of the art of organic enzyme chemistry, the general prospects of biocatalysis and the potential of practical application. The meeting was held in hotel Erika in Berg en Dal, near Nymegen, The Netherlands from June19-30, 1999. A group of 19 highly qualified speakers enthusiastically presented a series of 33 superb lectures, covering various aspects of enzyme chemistry. The ASI was attended by 65 scholars from 18 different countries.

In this book "Enzymes in Action" the presented chemistry is described in 22 chapters. In the section on methodologies and fundamentals of enzyme chemistry various aspects concerning the concepts of enzymes in molecular operations are covered. Heteroatom enzyme chemistry is treated in the second section. Considerable attention has been devoted to the use of enzymes in the detoxification of chemical warfare agents and the application of enzymes in solving environmental problems. In the final section the strategic use of enzymes in organic chemistry is highlighted by applications from different areas. Here the term green chemistry is appropriate, as enzyme mediated processes take place under mild environmentally benign conditions. Moreover, enzymes enable chemists to perform new chemical operations that otherwise are difficult to achieve at all.

The book gives a timely overview of a modern development in organic chemistry. It is clear that bioreagents require a different way of thinking of organic chemists. But by doing so many new avenues of exciting new chemistry open up, allowing to solve chemical problems in an elegant manner. It is clear that the impact of enzymes on organic chemistry just has started; much is to be expected in the coming years.

Prof. Dr. Binne Zwanenburg

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Part IV: Strategic Use of Enzymes in Molecular Synthesis

BIO- AND CHEMO-CATALYTIC DERACEMISATION TECHNIQUES

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Abstract: Methods for the preparation of chiral building blocks in 100% (theoretical) chemical and optical yield from racemates are reviewed and their specific merits and limitations are discussed. This so-called 'deracemisation' is achieved by employing a bio- or chemo-catalyst or a combination of both. Four general categories of processes can be characterized: (i) Improvement of classic kinetic resolution by re-racemisation and repeated resolution, (ii) dynamic resolution based on *in-situ* racemisation of the starting material, (iii) enantioselective stereoinversion, and (iv) enantioconvergent processes.

1. Introduction

The increased demand for chiral drugs in enantiomerically pure form, following the release of new FDA's marketing guidelines, turned the search for novel methods for the syntheses of enantiomerically pure compounds (EPC) into a major topic in contemporary organic synthesis [1]. In this context, the use of biocatalysts has found widespread application in preparative organic chemistry over the last decade [2]. From the two principles of biocatalytic reactions where chiral molecules are involved, *i.e.* (i) desymmetrization of *meso*- and prochiral compounds [3,4], and (ii) kinetic resolution of racemates [5] (Scheme 1), the latter is remarkably dominant in number of applications (~1:4) [6], which is probably due to the fact that racemates can probably more readily be synthesized than *meso*- and prochiral substrates. Despite its widespread application, kinetic resolution is impeded by several inherent disadvantages for practical applications, in particular on an industrial scale. After all, it should be kept in mind that

B. Zwanenburg et al. (eds.), Enzymes in Action, 1–23.

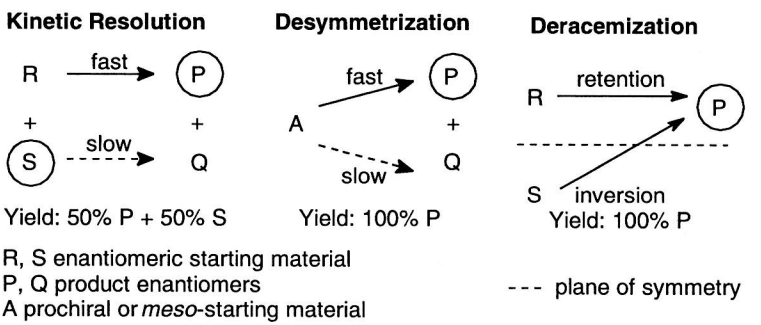
an ideal resolution process should provide a single enantiomeric product in 100% yield. The most obvious drawbacks are as the following:

(i) The theoretical yield of each enantiomer can never exceed the limit of 50%.

(ii) Separation of the formed product from the remaining substrate may be laborious in particular for those cases, where simple extraction or distillation fails and chromatographic methods are required [7].

(iii) In the majority of processes, only one stereoisomer is desired and there is little or no use for the other. In some rare cases, the unwanted isomer may be used through a separate synthetic pathway in an enantio-convergent fashion, but this requires additional effort put into a highly flexible synthetic strategy [8].

(iv) For kinetic reasons, the optical purity of substrate and/or product is depleted at the point, where separation of product and substrate is most desirable from a preparative point of view — *i.e.* 50% conversion [9].



Scheme 1: Principles of kinetic resolution and desymmetrisation.

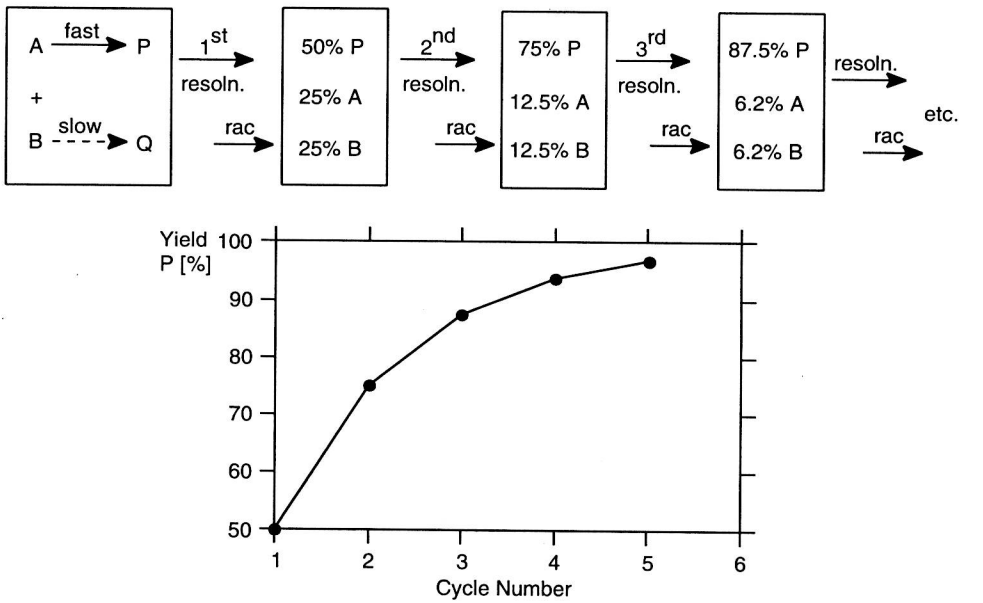
As a consequence, alternatives to kinetic resolution techniques that can deliver a single stereoisomer from a racemate are highly advantageous, such processes are generally denoted as 'deracemisation' [10] (Scheme 1). All of these techniques are dealing with a common stereochemical phenomenon, *i.e.* both substrate enantiomers have to be processed *via* two different stereochemical pathways: whereas the stereochemistry of R remains the same during its transformation to P, enantiomer S has to cross the symmetry plane (which is dividing R and S) in order to become P. As a consequence, S has to be reacted with *inversion* of configuration, whereas the stereochemistry of R is *retained* throughout the process.

In this chapter, general strategies which lead to the formation of a single enantiomeric product in 100% theoretical yield from a racemate are reviewed.

2. Improving Kinetic Resolution

2.1. RE-RACEMISATION AND REPEATED RESOLUTION

In order to avoid the loss of half of the material in kinetic resolution, it has been a common practice to racemize the unwanted isomer after separation from the desired product and to subject it again to kinetic resolution in a subsequent cycle, and so forth, until virtually all of the racemic material has been converted into a single stereoisomer [11].



Scheme 2: Principles of deracemisation *via* repeated resolution and re-racemisation.

On a first glance, repeated resolution appears to be less than optimal and certainly lacks synthetic elegance, bearing in mind that an infinite number of cycles are theoretically required to transform all of the racemic starting material into a single stereoisomer. Upon closer examination, however, it becomes a viable option. The graph in Scheme 2 reveals that the overall yield of product P reaches a value of >95% after only five cycles, provided that both reactions - *i.e.* kinetic resolution and racemisation are essentially 'clean' without loss of material. As a consequence, racemisation is the main challenge to

be met, which can be achieved by both ways, *via* chemo- or bio-catalysis. Each technique provides several merits and disadvantages [12].

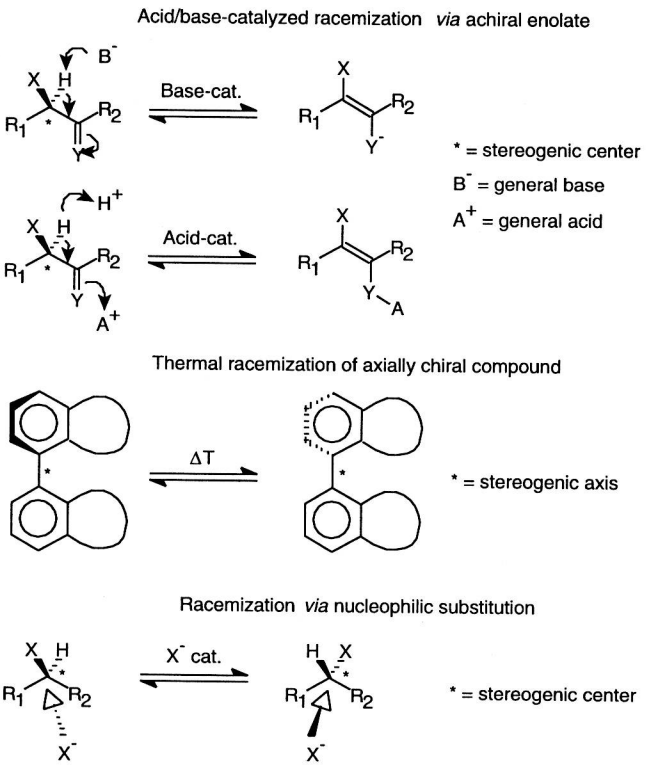
Chemical racemisation can be achieved by a number of concepts (Scheme 3). In industry, acid- or base-catalysed, as well as thermal racemisation are the most widespread techniques because they are cheap processes and they are relatively easy to handle. Base-catalyzed racemisation *via* an achiral enolate-type intermediate can be applied to almost all compounds bearing an acidic hydrogen at the chiral center, and, as a consequence, it became one of the most widespread techniques used. On the contrary, acid-catalyzed racemisation is usually applicable to substances subject to keto-enol tautomerism and because of this, it is less common. Compounds showing axial chirality can be racemized *via* rotation around a σ-bond and they are viable targets for thermal racemisation. Photochemical racemisation has been employed more rarely for specific types of compounds, such as chrysanthemic acid derivatives. Chiral organohalides and nitriles are racemized *via* nucleophilic substitution catalyzed by halide and cyanide, respectively. More recently, milder methods for racemisation have been brought to attention: For instance, chiral *sec*-alcohols can be racemized *via* a transition-metal catalyzed oxidation-reduction sequence or *via* Pd^{II}-catalyzed allylic rearrangement of the corresponding acetate esters (Scheme 6). In a related fashion, interconversion of enantiomers of *sec*-amines is accomplished by Pd/C-catalyzed dehydrogenation-hydrogenation (Scheme 7).

Compounds bearing a configurationally stable center cannot be racemized directly and they have to be (chemically) converted into a configurationally unstable intermediate. For instance, amino acid amides or -esters can be racemized *via* Schiff-base derivatives of aromatic aldehydes involving the α-amino group or, alternatively, for free amino acids, mixed acid anhydrides are used. However, after acid/base-catalyzed racemisation of the respective intermediates, the starting compound has to be liberated again, which makes this technique a rather tedious multi-step procedure.

The general disadvantage of most techniques based on chemo-catalysis is the fact that they require harsh reaction conditions causing side reactions, thus leading to product degradation, which results in loss of material.

On the contrary, the most striking merit of biocatalytic racemisation is the mild reaction conditions — e.g. room temperature, atmospheric pressure and neutral pH. Under these conditions, side reactions are largely suppressed [13]. Unfortunately, Nature does not rely on racemates and, as a consequence, biochemical racemisation is a rather scarce feature, which makes racemases a small group of enzymes, that are found in certain biological niches. One of the major targets for biochemical racemisation involves

stereogenic centers in carbohydrates, *i.e.* *sec*-alcohol groups. However, since both stereoisomers of these reactions represent diastereomers rather than enantiomers, 'epimerisation' would be the more correct term. Various enzymes — epimerases — are involved in the racemisation of *sec*- alcohol groups, these enzymes are usually NAD^+-dependent and of very little interest for practical applications.



Scheme 3: Concepts of chemical racemisation.

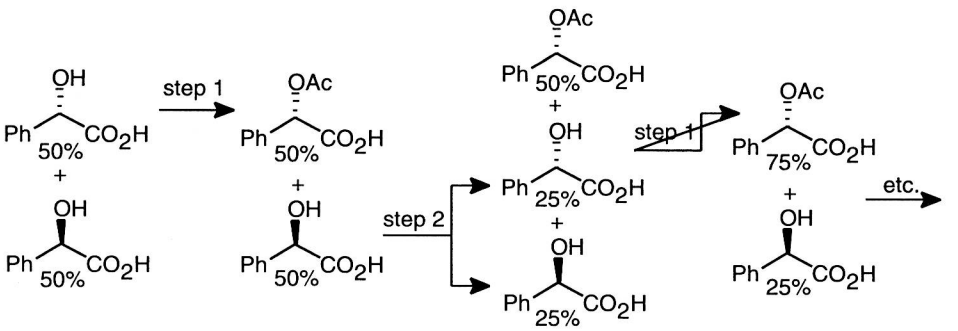
Among non-carbohydrate 'true' racemases, base-catalysis seems to be the general scheme of biochemical racemisation, and two major groups can be classified according to their reaction mechanism [14], *viz.* racemases employing a one-base mechanism — *i.e.* the proton at the chiral center is abstracted by the same base-functionality of the enzyme, that reads it — and those employing a two-base mechanism — *i.e.* one base is capable of *abstracting* the proton at the chiral center and another (conjugated) base *puts it back on* from the opposite side, thus resembling a ping-pong mechanism.

Mandelate racemase [EC 5.1.2.2] belongs to the latter group employing a two-base mechanism [15]. The Mg^{2+}-dependent enzyme is capable of racemising a remarkably broad substrate spectrum, which opens up large possibilities for deracemisation of various kinds of α-hydroxy acids. Substrates that meet the following constraints are accepted by mandelate racemase:

(i) The α-hydroxy acid moiety is (almost strictly) required, as the only exception to this rule seems to be an α-hydroxy carboxamide functionality [16].

(ii) A π-electron system has to be present in the β,γ-position. The π-electron system can be freely varied, including the corresponding α-hydroxy-β,γ-alkenoic and α-hydroxy-α-aryl carboxylic acids. Even heteroaromatic systems are accepted at reasonable rates [17]. In general, electron-withdrawing groups attached to the β,γ-unsaturated (or aromatic) system, which help to stabilize the (anionic) transition intermediate *via* resonance, enhance the reaction rates significantly [18]. Several cofactor-independent amino acid racemases, such as proline racemase, aspartic acid racemase and glutamate racemase, also belong to the two-base type racemases. Racemases employing the one-base mechanism all display a common feature, *i.e.* they require pyridoxal phosphate as an essential cofactor for catalytic activity. Examples for these enzymes are α-amino-ε-caprolactam racemase, alanine racemase and (probably) arginine, threonine and serine racemases. Amino acid racemases are widely used in industry because of the stong commercial importance of amino acids and -derivatives.

A novel deracemisation technique based on a two-enzyme system consisting of a (i) lipase-catalyzed enantioselective acylation, followed by (ii) mandelate racemase catalyzed racemisation of the remaining non-reacted substrate enantiomer is shown in Scheme 4 [19]. Thus, in a first step, (±)-mandelic acid is subjected to lipase catalyzed *O*-acylation in an organic solvent producing (*S*)-*O*-acetyl mandelate, by leaving the (*R*)-enantiomer behind. Due to the high selectivity (E >100), the reaction comes to a standstill at 50% conversion.



step 1: *Pseudomonas* sp. lipase, vinyl acetate, *i*-Pr_2O (E >200)
step 2: Mandelate racemase, aqu. buffer

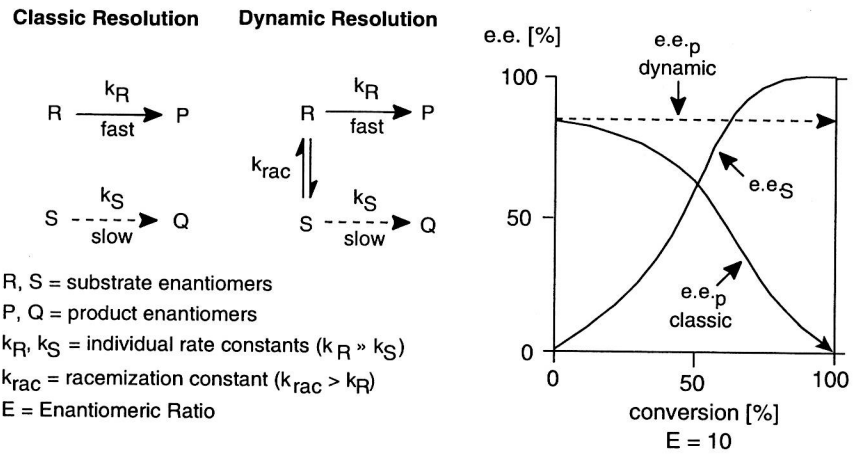
Scheme 4: Deracemisation of mandelate *via* repeated resolution employing a two-enzyme process.

Then, in a subsequent step, the organic solvent is changed to aqueous buffer [20], and the remaining unreacted (*R*)-mandelic acid is racemized in the presence of (*S*)-*O*-acetyl mandelate, which is non-substrate. When this two-step process was repeated four times, (*S*)-*O*-acetyl mandelate was obtained in >80% chemical yield and >99% enantiomeric excess as the sole product. It should be emphasized that separation of the formed product from the remaining starting material is not required due to the high specificity of the racemase employed.

2.2. DYNAMIC RESOLUTION

The disadvantages of kinetic resolution can largely be avoided by employing a so-called 'dynamic resolution' [21] (Scheme 5) Such a process comprises kinetic resolution with an additional feature, *i.e. in-situ* racemisation of the starting material, which is usually achieved using chemo-catalysis. Ultimately, all of the substrate R+S is transformed into a single product enantiomer P in 100% theoretical yield. In contrast to kinetic resolution, where the reaction slows down at 50% conversion (or comes even to a standstill, if the enantioselectivity is sufficiently high), when the fast-reacting enantiomer R is consumed and only the slow-reacting counterpart remains, substrate-racemisation ensures the continuous formation of R from S during the course of the reaction and thus avoids the depletion of R. The reaction does not come to a standstill and it therefore can be run to completion by gradually converting all racemic starting material into product P. In order to indicate the non-static behaviour of such a process, the term 'dynamic resolution' has

been aptly coined. The following properties are typical for dynamic resolution processes (Scheme 5) [22]. The e.e. of the substrate ($e.e._S$) is at its minimal at the onset of the reaction and gradually begins to increase as the faster reacting enantiomer is depleted from the reaction mixture, in particular around half-way of the reaction. On the other hand, this depletion does not occur if the substrate is constantly racemized during the resolution process and, thus, in a dynamic resolution the $e.e._P$ is *not* a function of the conversion but remains constant throughout the reaction. Since the catalyst always faces a racemic starting material (*i.e.* [R] always equals [S]), it is understandable, that the selection of the faster reacting enantiomer from the substrate remains a simple task, as opposed to kinetic resolution where depletion of R occurs.



Scheme 5: Principles of kinetic and dynamic resolution.

In order to design a successful dynamic resolution process, both parallel reactions — *i.e.* kinetic resolution and in-situ racemisation — have to be carefully tuned, by taking into account the following aspects:

(i) The kinetic resolution should be irreversible in order to ensure high enantioselectivity.

(ii) The enantiomeric ratio ('E-value', $E = k_R/k_S$) [23] should be greater than ~20.

(iii) In order to avoid depletion of R, racemisation (k_{rac}) should be at least equal or greater than the reaction rate of the fast enantiomer (k_R).

(iv) For moderate selectivities, k_{rac} should be greater than k_R by a factor of about 10,

(v) For obvious reasons, any spontaneous reactions involving the substrate enantiomers as well as racemisation of the product should be absent.