Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 2901–2913

TETRAHEDRON: *ASYMMETRY*

# Deracemization of compounds possessing a *sec*-alcohol or -amino group through a cyclic oxidation–reduction sequence: a kinetic treatment

Wolfgang Kroutil and Kurt Faber *

*Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria*

Received 3 July 1998; accepted 30 July 1998

**Abstract**

Deracemization of compounds bearing a chiral *sec*-hydroxy or -amino group can be achieved in a one-pot reaction via a novel process consisting of a cyclic oxidation–reduction sequence. Thus, in the first step, one enantiomer from the racemic starting material (**R+S**) is enantioselectively oxidized forming an achiral intermediate product (**P**, a ketone or imine, respectively). In the second step, the latter is non-selectively reduced to give again **R+S** in racemic form. Cyclic repetition of this oxidation–reduction sequence leads to an overall chiral inversion of the faster reacting enantiomer from the racemic starting material to yield the slower reacting enantiomer as the final product in a theoretical 100% chemical and enantiomeric yield. Mathematical treatment of the kinetics of this 'cyclo-process' allowed the estimation of the practical feasibility at hand of two crucial parameters: (i) the maximal obtainable enantiomeric excess and (ii) the number of cycles required (expressed as theoretical turnover) to reach equilibrium. A computer program for analysis and optimization of such processes was developed, which is available via the Internet. © 1998 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

Driven by the increased demand for chiral bioactive compounds in enantiomerically pure form, the preparation of enantiopure compounds has become a major area of interest for contemporary organic chemistry.[1] From the two principles of asymmetric catalytic processes, i.e. (i) asymmetrisation of *meso*- or prochiral compounds[2,3] and (ii) kinetic resolution of racemates,[4] the latter is dominant in by far the greater number of applications,[5] although it comprises of several serious disadvantages, bearing in mind that the ideal process should lead to a single enantiomer in 100% yield. Thus, the theoretical yield of each enantiomer is limited to 50%, and separation of product formed from the remaining substrate may be laborious, particularly on an industrial scale. Furthermore, in general only one stereoisomer

* Corresponding author. E-mail: faber@orgc.tu-graz.ac.at

0957-4166/98/$ - see front matter © 1998 Elsevier Science Ltd. All rights reserved.
*PII:* S0957-4166(98)00306-1

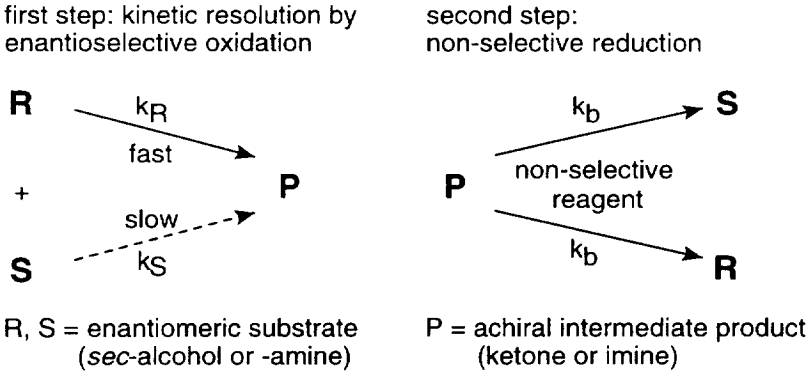
is desired and there is little or no use for the other. As a consequence, processes which lead to the formation of a single enantiomer from a racemic mixture — so-called deracemization techniques[6] — have gained increased interest. A highly versatile approach to circumvent the disadvantages of classic kinetic resolution makes use of *in situ* racemization[7] of the starting material to furnish a so-called dynamic resolution[6,8–10]. The latter is particularly applicable to chirally labile compounds, such as α-substituted carboxylic acid derivatives, which can be racemized through an enol-intermediate by abstraction of an acidic proton. However, this technique fails for compounds bearing chirally stable centers, such as *sec*-alcohol or -amino groups. For secondary alcohols, biocatalytic deracemization has been achieved via a so-called stereoinversion[6] involving an oxidation–reduction sequence. The basis of this technique operates along the following mechanism: First, one enantiomer of a secondary alcohol out of a racemic mixture is selectively oxidized to the corresponding ketone under catalysis of a dehydrogenase, while the other remains untouched. Then, the ketone is reduced in a second step, by another enzyme system displaying the opposite stereochemical preference. Although the microbial stereoinversion of secondary alcohols has been reported,[11–19] these systems suffer from several drawbacks:

(i) the requirement for two (bio)catalysts exhibiting opposite stereo-preference is difficult to meet;
(ii) at least one of the steps has to be irreversible, in order to achieve high enantiomeric purity of the final product;
(iii) furthermore, all of the studies published to date made use of whole-cell systems with an unknown number of active enzymes and cofactor(s), which renders the true kinetics rather obscure; and
(iv) furthermore, severe discrepancies on the nature of the cofactor(s) and the types of enzyme(s) exist.[20]

We wish to present a deracemization technique for secondary alcohols and amines which relies on (i) enantioselective oxidation coupled to (ii) non-specific reduction. The kinetics of this process as well as the merits and limits of this method are presented in this study.

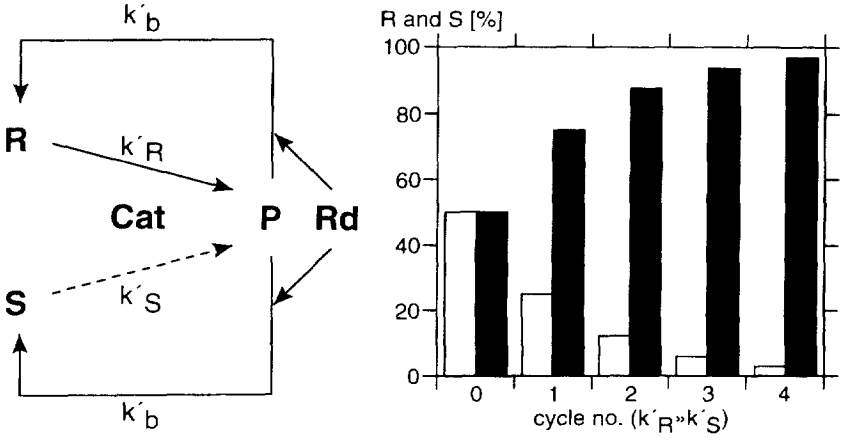
## 2. Deracemization through cyclic oxidation–reduction

The system consists of two independent reactions, which are outlined in Scheme 1. First, one enantiomer of the secondary alcohol or amine (**R**) is selectively oxidized from the racemate (**R+S**) to yield the achiral intermediate **P**, i.e. the corresponding ketone or imine, respectively. The selectivity of the system is determined by the ratio of the rate constants of the enantiomers ($E=k_R/k_S$) and is generally described by the enantiomeric ratio (E) for biocatalyzed reations.[4b] In the second step, **P** is chemically reduced in a non-selective fashion (determined by $k_b$) to yield a mixture of **R+S** in racemic form.




Scheme 1. Components of the cyclo-system: (i) kinetic resolution by enantioselective oxidation and (ii) non-selective reduction

Both reactions alone are of limited use for the preparation of enantiopure material, since step one (i.e. kinetic resolution by enantioselective oxidation) is limited to a 50% theoretical yield of chiral non-reacting **S** and achiral product **P**. Step two does not show any chiral induction at all. However, combination of both steps in a single reactor in a cyclic mode leads to a highly versatile deracemization technique (Scheme 2).




Scheme 2. Deracemization via cyclic oxidation–reduction sequence. **R**, **S**=enantiomeric starting material (*sec*-alcohol or -amine); **P**=achiral intermediate product (ketone or imine); **Cat**=asymmetric (bio)catalyst; **Rd**=non-selective reducing agent; $k_R$, $k_S$=second-order rate constants of enantiomers during oxidation; $k_b$=second-order rate constant of reduction

The characteristics of this system are explained along the following example: If (for reasons of clarity) the selectivity of step one is assumed to be absolute (i.e. $k_R \gg k_S$, E>200), enantiomer **R** is selectively oxidized from the racemic starting material to form achiral **P** in 50% yield by leaving **S** untouched. In the second step, **P** is non-selectively reduced to furnish **R+S** in equal amounts of 25% each. As a consequence, the enantiomeric composition of **R/S** is equal to 25/75 after a single cycle. It can be easily seen from the graph in Scheme 2, that further cycles lead to a gradual increase of enantiomer **S** (solid columns) at the expense of **R** (open columns), and that the enantiomeric excess of the starting material is already well above 90% after only four cycles,[21] assuming absolute enantioselectivity. Overall, if this cyclic process is driven in the forward direction, enantiomer **S** represents the 'sink' of material in the whole process.

In practical applications, however, enantioselectivities are usually incomplete with E-values ranging below 100. For such cases, two crucial dependencies are encountered: the selectivity does not only determine (i) the maximum obtainable e.e. at equilibrium but also (ii) the number of cycles which are necessary to reach this value. In order to calculate the feasibility of a cyclic deracemization reaction, the kinetics of this process have been elucidated as described below.

## 3. Kinetics

### *3.1. General remarks*

The following assumptions were made:

(i) the activity of the asymmetric (bio)catalytic oxidation reaction remains constant throughout the whole process ($k'_R$, $k'_S$, [Cat]=const.) and any types of inhibition phenomena are absent;

(ii) the reverse reduction reaction is non-selective, i.e. $k_b$ is equal for both pathways forming enantiomers **R** and **S** in equal amounts;
(iii) the reverse reaction is constant ($k'_b$, [Rd]=const., e.g. **Rd** is in excess or continuously added); and
(iv) any spontaneous undesired side reactions are absent.

### 3.2. Calculation of concentrations

The cyclic system depicted in Scheme 2 can be described by the following four differential equations, with **R**, **S**, **Cat**, **P** and **Rd** standing for the concentrations of the respective (re)agents. $\mathbf{A}_0$ stands for the start concentration of the racemate ($\mathbf{A}_0$=**R**+**S**).

$$\frac{d\mathbf{R}}{dt} = -k'_R \cdot \mathbf{R} \cdot \mathbf{Cat} + k'_b \cdot \mathbf{P} \cdot \mathbf{Rd} \tag{1}$$

$$\frac{d\mathbf{S}}{dt} = -k'_S \cdot \mathbf{S} \cdot \mathbf{Cat} + k'_b \cdot \mathbf{P} \cdot \mathbf{Rd} \tag{2}$$

$$\frac{d\mathbf{Rd}}{dt} = -2 \cdot \mathbf{P} \cdot k'_b \cdot \mathbf{Rd} \tag{3}$$

$$\mathbf{R} + \mathbf{P} + \mathbf{S} = \mathbf{A}_0 \tag{4}$$

In order to solve the system, the equations above had to be simplified as follows: If one assumes that the concentration and/or specific activity of the chiral (bio)catalyst (**Cat**) remains constant, one can summarize the products **Cat**·$k'_R$ and **Cat**·$k'_S$ to give constants $k_R$ and $k_S$, respectively. Furthermore, the concentration of reducing agent (**Rd**) is maintained at a constant level, which can be achieved by using it in excess or through continuous addition. Thus, $k'_b$·**Rd** is constant too, and becomes $k_b$. Then, Eqs. 1 and 2 are simplified as follows:

$$\frac{d\mathbf{R}}{dt} = -k_R \cdot \mathbf{R} + k_b \cdot \mathbf{P} \tag{5}$$

$$\frac{d\mathbf{S}}{dt} = -k_S \cdot \mathbf{S} + k_b \cdot \mathbf{P} \tag{6}$$

Substitution of **R** in Eq. 5 by Eq. 4 yields

$$-\left(\frac{d\mathbf{P} + d\mathbf{S}}{dt}\right) = -k_R \cdot (\mathbf{A}_0 - \mathbf{P} - \mathbf{S}) + k_b \cdot \mathbf{P} \tag{7}$$

which is equivalent to:

$$-\left(\frac{d\mathbf{P} + d\mathbf{S}}{dt}\right) = -k_R \cdot \mathbf{A}_0 + k_R \cdot \mathbf{S} + \mathbf{P} \cdot (k_R + k_b) \tag{8}$$

Eq. 6 is multiplied by $\frac{k_R + k_b}{k_b}$, and from the result, Eq. 8 is subtracted to give:

$$\frac{d\mathbf{S}}{dt} + \frac{k_R + k_b}{k_b} \cdot \frac{d\mathbf{S}}{dt} + \frac{d\mathbf{P}}{dt} = -k_S \cdot \mathbf{S} \cdot \frac{k_R + k_b}{k_b} + k_R \cdot \mathbf{A}_0 - k_R \cdot \mathbf{S} \tag{9}$$

The concentration of **P** is explicitly expressed out of Eq. 6 to give Eq. 10:

$$\mathbf{P} = \frac{1}{k_b}\left(\frac{d\mathbf{S}}{dt} + k_S \cdot \mathbf{S}\right) \tag{10}$$

If the concentration of **P** in $\frac{d\mathbf{P}}{dt}$ of Eq. 9 was substituted by Eq. 10, the second-order differential Eq. 11 was obtained:

$$\frac{d^2\mathbf{S}}{dt^2} + W \cdot \frac{d\mathbf{S}}{dt} + N \cdot \mathbf{S} = k_R \cdot k_b \cdot \mathbf{A}_0 \tag{11}$$

with $W=k_R+k_S+2k_b$ and $N=k_R \cdot k_b+k_R \cdot k_S+k_S \cdot k_b$. The term $W^2-4N$ is greater than nil, which is equivalent to $(k_R-k_S)^2+4k_b^2>0$, since every single term is greater than nil. Solutions of the homogeneous differential equation (Eq. 11) are the two following exponential functions:

$$\mathbf{S}_1(t) = e^{r_1 \cdot t} \tag{12}$$

$$\mathbf{S}_2(t) = e^{r_2 \cdot t} \tag{13}$$

with

$$r_1 = -\frac{W}{2} + \tfrac{1}{2} \cdot \sqrt{W^2 - 4N} \tag{14}$$

$$r_2 = -\frac{W}{2} - \tfrac{1}{2} \cdot \sqrt{W^2 - 4N} \tag{15}$$

Now, a special solution for Eq. 11 is obtained by:

$$\tilde{\mathbf{S}}(t) = \frac{-e^{r_1 \cdot t}}{r_2 - r_1} \cdot \int_0^t e^{-r_1 \cdot x} \cdot k_R \cdot k_b \cdot \mathbf{A}_0 \cdot dx + \frac{e^{r_2 \cdot t}}{r_2 - r_1} \cdot \int_0^t e^{-r_2 \cdot x} \cdot k_R \cdot k_b \cdot \mathbf{A}_0 \cdot dx \tag{16}$$

Solving the integrals above one obtains Eq. 17:

$$\tilde{\mathbf{S}}(t) = \frac{k_R \cdot k_b \cdot \mathbf{A}_0}{(r_2 - r_1)} \cdot \left( \frac{1}{r_1} - \frac{e^{r_1 \cdot t}}{r_1} - \frac{1}{r_2} + \frac{e^{r_2 \cdot t}}{r_2} \right) \tag{17}$$

The general solution of differential Eq. 11 is defined as:

$$\mathbf{S}(t) = c_1 \cdot \mathbf{S}_1(t) + c_2 \cdot \mathbf{S}_2(t) + \tilde{\mathbf{S}}(t) \tag{18}$$

with the constants $c_1$ and $c_2$.

The first constraint for this system is the fact that the starting material represents a racemate ($\mathbf{S}_0=\frac{\mathbf{A}_0}{2}$) at the beginning of the reaction (t=0) with no product being present ($\mathbf{P}_0=0$). If these conditions are combined with Eq. 18, one obtains:

$$\frac{\mathbf{A}_0}{2} = \mathbf{S} = c_1 + c_2 \tag{19}$$

Since the whole process is symmetrical, one obtains $c_1=c_2$, thus $c_1 = c_2 = \frac{\mathbf{A}_0}{4}$. Using these preconditions, Eq. 19 can be transformed into Eq. 20:

$$\mathbf{S}(t) = \frac{\mathbf{A}_0}{4} \cdot \mathbf{S}_1(t) + \frac{\mathbf{A}_0}{4} \cdot \mathbf{S}_2 + \tilde{\mathbf{S}}(t) \tag{20}$$

Analogous equations are obtained for the concentration of **R**(t), except that in Eq. 17 $k_S$ is replaced by $k_R$. The concentration of **P** can be calculated from Eq. 4.

## 4. Characteristics of the system

*E.e.$_{max}$*: If the system reaches equilibrium, Eqs. 4 and 5 have to be zero ($d\frac{\mathbf{R}}{dt}$ and $d\frac{\mathbf{S}}{dt}=0$). After substraction of Eq. 5 from Eq. 4 one obtains the concentrations of **R** and **S** at equilibrium:

$$\frac{\mathbf{S}_{equ.}}{\mathbf{R}_{equ.}} = \frac{k_R}{k_S} \tag{21}$$

Since the enantiomeric excess is defined as the absolute value of $\frac{(\mathbf{R}-\mathbf{S})}{(\mathbf{R}+\mathbf{S})}$, and the selectivity (E-value) can be expressed as $\frac{k_R}{k_S}$, the maximum e.e. is obtainable at equilibrium:

$$\text{E.e.}_{max} = \frac{E-1}{E+1} \tag{22}$$

It is interesting to note that the latter expression is exactly the same as derived for dynamic kinetic resolutions.[22]

*Concentrations at equilibrium*: The concentrations of **R**, **S** and **P** at equilibrium are calculated by Eqs. 4 and 21 and Eqs. 5 and 6 being set to zero. Thus, $\mathbf{R}_{equ.}$, $\mathbf{S}_{equ.}$ and $\mathbf{P}_{equ.}$ are obtained as follows:

$$\mathbf{R}_{equ.} = \frac{\mathbf{A}_0}{\left(1 + \frac{k_R}{k_S} + \frac{k_R}{k_b}\right)} \tag{23}$$

$$\mathbf{S}_{equ.} = \frac{\mathbf{A}_0}{\left(1 + \frac{k_S}{k_R} + \frac{k_S}{k_b}\right)} \tag{24}$$

$$\mathbf{P}_{equ.} = \frac{\mathbf{A}_0}{\left(1 + \frac{k_b}{k_R} + \frac{k_b}{k_S}\right)} \tag{25}$$

As can be seen from Eqs. 23–25, the equilibrium concentrations depend not only on the selectivity of the system ($k_R$ and $k_S$) as does the e.e.$_{max}$, but also on the reverse reaction characteristics ($k_b$).

*Conversion*: Since it is impossible to apply the term 'conversion' in the conventional way for a cyclic reaction, another term had to be defined in order to describe the progress of the process. It was chosen to define it as 'percent of equilibrium' (Poe) and it describes how far the deracemization has proceeded towards the ultimate obtainable goal—the equilibrium. The Poe at a given point of the reaction is obtained by using the corresponding e.e. value in the following way: Since the e.e.$_{max}$ represents a parameter which indicates the end of the process, the latter can be used as standard for the progress of the process corresponding to 100%. If the e.e. value at any given point of the reaction is then compared with the e.e.$_{max}$ and expressed as % value, the Poe is obtained. In practice, the course of the reaction can be easily monitored (expressed as Poe) by measuring the e.e. at a given point and by comparing it with the e.e.$_{max}$.

$$\text{Poe} = \text{e.e.}\frac{E+1}{E-1} \tag{26}$$

Poe=percent of equilibrium.

*Turnover*: It is a crucial point that the selectivity of the reaction (as determined by the ratio of $k_R/k_S$), determines the number of cycles, which have to be passed in order to reach equilibrium. Thus, for highly selective processes only few cycles are required, since enantiomers **R** and **S** are sorted out with high efficiency, whereas for low E-values more cycles are needed to reach the end point.[23] A cycle is defined

as a single reaction sequence (out of the whole process) consisting of a consecutive oxidation–reduction reaction.

In theory, equilibrium may be reached also for processes showing low E-values, as long as the process is driven forward towards the end point through a large number of cycles. For practical applications, however, such cases are likely to be impeded with low overall yields, since an enhanced throughput of material via a large number of forward and reverse reaction sequences inherits the danger of undesired side-reactions, causing loss of material. As a consequence, the practical feasibility of a cyclic deracemization has to be estimated according to the throughput of material required to reach equilibrium. In addition, the flux of material determines the amount of oxidizing and reducing reagents which are needed. For this purpose, the parameter 'theoretical turnover' (Tht) was introduced.[24] The Tht is dimensionless and is expressed as equivalents with respect to starting material.

The function of the Tht is explained along the following example by assuming two preconditions for simplicity: (i) the selectivity of the oxidation is absolute (i.e. **R** is quickly oxidized and **S** is not converted at all, $k_R \gg k_S$) and (ii) the reverse reaction is considerably faster than the forward reaction, leading to a negligable concentration of **P** at any point of the process. At the onset of the reaction, the concentration of each **R** and **S** is 50%. During the first cycle, all of **R** is selectively oxidized to give **P**, which in turn yields **R** and **S** in equal amounts of 25% each during reduction. Thus, the amount of oxidation and reduction reagents needed in the first cycle are 0.5 equiv. For cycle two, the starting concentrations of **R** and **S** are 25 and 75%, respectively. As a consequence, transformation of **R** through the cycle requires an additional 0.25 equiv. of reagents, and so on for further cycles. If the amounts of reagents for each cycle are summed up (0.5+0.25+0.125+...) until equilibrium is reached (through an infinite number of cycles in theory), the theoretical turnover is obtained. For the simplified case described above (i.e. absolute selectivity) the Tht is exactly 1 equiv. as derived from Eq. 27.

$$\mathrm{Tht_{abs.}} = \lim_{n \to \infty} \sum_{i=1}^{n} \left(\frac{1}{2}\right)^i = 1 \tag{27}$$

$\mathrm{Tht_{abs.}}$=theoretical turnover for absolute selectivity; $n$=number of cycles.

In case the selectivity is not absolute, the selection of enantiomers is less accurate during oxidation and more cycles are required to reach equilibrium. As a consequence, the throughput of material and the equivalents of reagents needed is larger. For these cases, the theoretical turnover of the whole process is obtained by summing up the equivalents of each single cycle ($\mathrm{Equ._{cycle}}$). The latter value is expressed in the following way as a function of the selectivity of the reaction [$\frac{k_R}{k_S}$, Eq. 28]:

$$\mathrm{Equ._{cycle}} = \mathbf{R} \cdot \frac{k_R}{k_R + k_S} + \mathbf{S} \cdot \frac{k_S}{k_R + k_S} = \mathbf{R} \cdot \frac{E}{E+1} + \mathbf{S} \cdot \frac{1}{E+1} \tag{28}$$

$$\mathrm{Tht} = \frac{1}{\mathbf{A}_0} \sum \mathrm{Equ._{cycle}} \tag{29}$$

For practical reasons, the Tht was calculated for 100 successive cycles.

Depending on the rate constants $k_b$, $k_R$ and $k_S$, the concentration of any of the species involved (**R**, **S** or **P**) may proceed through a maximum or minimum during the course of the process. The points of interest — i.e. the location of the maximum concentration of **R**, **S** or **P** during the course of the deracemization (calculated for the time-scale,[25] with non-limiting reverse reaction) — are characterized as follows: whereas the faster reacting enantiomer of the starting material has its maximum concentration at equilibrium, the slower reacting counterpart is enriched at the start. On the contrary, the concentration of the intermediate **P** may proceed through a maximum during the reaction. The latter is determined

through Eq. 30. The latter value represents the most unfavourable point of the reaction for the harvest of the enantiomerically enriched material **R+S**.

$$t_{Pmax} = \frac{1}{r_2 - r_1} \ln \left[ \frac{2(k_R + k_S) \cdot k_b - r_1(r_2 - r_1)}{r_2(r_2 - r_1) + 2(k_R + k_S) \cdot k_b} \right] \quad (30)$$

For $r_1$ and $r_2$ see Eqs. 14 and 15.

## 5. Computer program features

In order to facilitate the applicability of cyclic deracemization and to allow simulation and optimization of such processes, a computer program ('Cyclo') was developed based on the kinetics described above[26] The main features of the program are as follows:

### *5.1. Analysis*

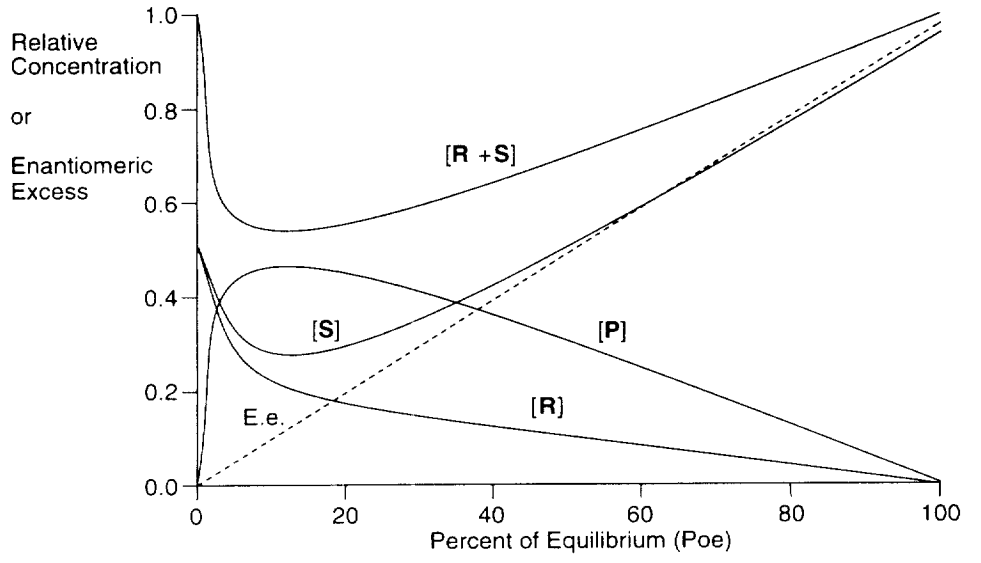
A cyclic deracemization process can be analyzed and estimated for its practical feasibility. Thus, based on the second-order rate constants of the forward and reverse reaction ($k'_R$, $k'_S$, $k'_b$) and the concentrations of the catalyst [**Cat**], the reducing agent [**Rd**] and the starting material [$\mathbf{R}_0$+$\mathbf{S}_0$], the parameters which determine the quality of the process — i.e. the $e.e._{max}$ at equilibrium and the theoretical turnover (Tht) needed to reach the end-point — are calculated and the concentrations for [**R**], [**S**] and [**P**] at this point are given. Furthermore, maximum concentrations ([$\mathbf{R}_{max}$], [$\mathbf{S}_{max}$], [$\mathbf{P}_{max}$]) are given on the time-scale.

### *5.2. Simulation and optimization*

Based on assumed second-order rate constants ($k'_R$, $k'_S$, $k'_b$) and concentrations for the starting material [**R+S**], the catalyst [**Cat**] and the reducing agent [**Rd**], cyclic deracemization processes can be simulated. The parameters of interest — either concentrations {[**R**], [**S**], [**R+S**] or [**P**]} or the e.e. — can be followed along the reaction coordinate by choosing either the time or percent of equilibrium-scale (Poe). Plots can be printed or transferred to other application programs via the clipboard. This feature facilitates the optimization of deracemization processes through variation of concentrations and selectivities. Single sets of full matching data (consisting of time, Poe, [**R**], [**S**], [**R+S**], [**P**], and e.e.) can be obtained from each single value out of the set by using the 'single-value' option.

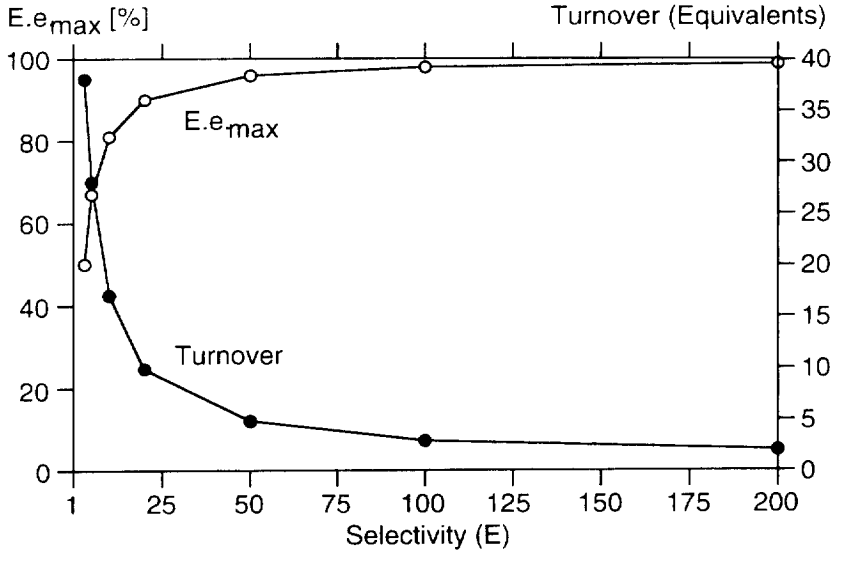
The characteristics of the kinetic behavior of a cyclic deracemization are explained along a representative example depicted in Scheme 3. The following parameters were chosen for the simulation of a highly successful process: the selectivity is high (E=100, $k'_R$=100, $k'_S$=1), and the reverse reaction is significantly faster than the forward reaction [$k'_b/(k'_R+k'_S) \sim 10$]. Starting material (**R+S**) and reducing agent (**Rd**) are applied in equimolar amounts and the catalyst (**Cat**) is used in $10^{-3}$ mol% of the reducing agent. Upon the onset of the reaction — when only **R+S** are present — the oxidation reaction is predominant, by producing achiral **P** at the expense of **R+S**. As a consequence, the concentration of **P** reaches a maximum at about 10% Poe, which is then followed by a gradual decline towards the end of the process.[27] The e.e. increases in a linear way during the course of the reaction and reaches an $e.e._{max}$ of 98.0% at equilibrium. In order to reach it, only 2.9 equiv. of reagents (Tht) are needed in theory.[28]

The merits and limits of cyclic deracemization can be estimated along two crucial parameters:




Scheme 3. Plot of concentrations of [**R**], [**S**], [**R**+**S**], [**P**] (full lines) and enantiomeric excess (dashed line) versus the reaction coordinate (Poe-scale)

(i) The selectivity of the oxidation ($E=k'_R/k'_S$) determines the maximum e.e. at equilibrium and the number of cycles (Tht) required to reach it (Scheme 4). A remarkably low E-value of 9 is sufficient to reach an $e.e._{max}$ of 80%, albeit at a large number of cycles (Tht=19). Good selectivity (E=18) yields an $e.e._{max}$ of 90% (Tht=11), whereas an E-value of 40 is required to obtain the starting material in 95% e.e. (Tht=5.7). However, only absolute specificities (E=100, 200) lead to virtually enantiopure material (98 and 99%, respectively, Tht=2–3).




Scheme 4. $E.e._{max}$ (open symbols) and theoretical turnover (filled symbols) as a function of the selectivity (E-value)

(ii) On the contrary, the chemical yield of enantiomerically enriched material is a function of the ratio of forward versus the reverse reaction, which determines the amount of residual **P** at equilibrium.

As a rule of thumb, it is recommended that the reverse reaction should be (at least) twice as fast as the forward reaction in order to ensure high yields ([**R**+**S**]≥90% results in 10% residual **P**).

## 6. Applicability

Due to the fact that the cyclic deracemization process described above is based on a sequence representing a repeated destruction and (re)formation of a chiral center, the chemical basis of such reactions is linked to oxidation and reduction.[29,30] With pure chemical methods alone, the presence of an oxidation and reduction reaction acting on the same starting material and product, respectively, is hard to imagine due to (almost in principle) incompatibilities. However, given the high specificity of biocatalysts, several efficient cyclic deracemization systems based on the kinetics derived above have been reported (see below). No attempts, however, have been reported to optimize such processes based on the kinetics. In all of the studies, a highly compatible combination of bio- and chemo-catalysts was employed. The applicability of cyclic deracemization is discussed below.

### 6.1. *Substrates possessing* sec-*alcohol groups*

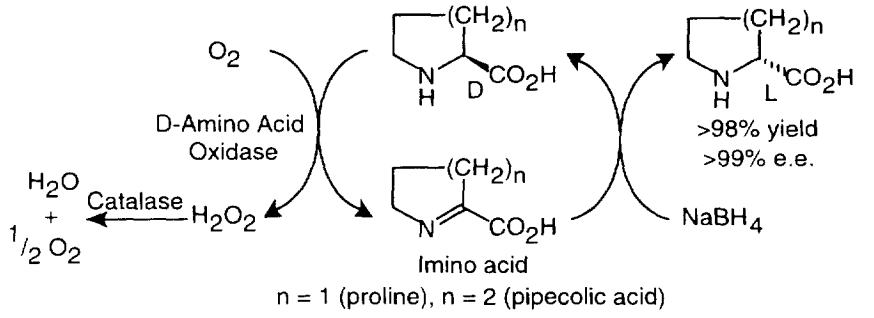
Enantioselective biocatalytic oxidation of *sec*-alcohols is possible by using several types of redox-enzymes. The use of alcohol dehydrogenases [EC 1.1.1.X] is linked to sensitive cofactors, such as $NAD(P)^+$. Whereas these systems have been proven to be highly useful in reduction, oxidation reactions have not found widespread application due to several drawbacks: (i) oxidation of alcohols is thermodynamically unfavorable, which impedes the recycling of oxidized nicotinamide-cofactors; (ii) product inhibition is common and (iii) oxidation is favored at elevated pH, where NAD(P)-cofactors are unstable. Since cyclic deracemization requires a driving force to pump up the racemic substrate to a single enantiomer against entropy, these facts exclude alcohol dehydrogenases from being promising candidates.

On the contrary, the use of oxidases [EC 1.1.3.X] is advantageous. These enzymes possess tightly bound cofactors,[31] which do not require external recycling. Thus, *sec*-alcohol functionalities are oxidized to the corresponding carbonyl group at the expense of molecular oxygen with concomitant formation of hydrogen peroxide. The latter is destroyed by catalase, which is usually present in systems showing oxidase activity. By this means, the process is driven towards completion.

Among oxidases, two subgroups warrant detailed investigation: (i) *sec*-alcohol oxidase[32,33] [EC 1.1.3.18] and (ii) α-hydroxyacid oxidase[34,35] [EC 1.1.3.15].

### 6.2. *Substrates possessing* sec-*amino groups*

In an analogous fashion, enantioselective biocatalytic oxidation of compounds bearing *sec*-amino groups with formation of the corresponding imino-derivatives[36] can be achieved by using (i) amine oxidases[37] [EC 1.4.3.6 and 1.4.3.7] and (ii) amino acid oxidases[38] [EC 1.4.3.2 and 1.4.3.3]. To date, only two cases for a successful cyclic deracemization have been reported (Scheme 5). Thus, (±)-α-amino acids have been successfully transformed into the L-isomers by using a combination of D-amino acid oxidase coupled to non-specific *in situ* reduction of the formed α-iminoacid at the expense of $NaBH_4$. The process proved to be highly successful by providing enantiomerically pure L-pipecolic acid[39] and L-proline[40] in >98% chemical yield from the racemate.




Scheme 5. Cyclic deracemization of amino acids using D-amino acid oxidase coupled to $NaBH_4$-reduction

Cyclic deracemization of *sec*-alcohols by using novel *sec*-alcohol oxidases from bacterial origin is currently under study in our laboratory.

## 7. Summary

The kinetics of a novel deracemization method based on a one-pot cyclic oxidation–reduction sequence ('cyclo-process') is described. The latter allows the transformation of (±)-*sec*-alcohols and -amines into a single enantiomer in 100% theoretical yield. Thus, in a first step, one enantiomer from the racemic starting material (**R+S**) is enantioselectively oxidized forming an achiral intermediate product (**P**, a ketone or imine, respectively). In a second step, the latter is non-selectively reduced to give again **R+S** in racemic form. Cyclic repetition of this oxidation–reduction sequence leads to an overall chiral inversion of the reacting enantiomer from the racemic starting material to yield the non-reacting enantiomer as the sole product.

A free shareware computer program which was developed for analysis and optimization of 'cyclo-processes' allowed the estimation of the feasibility at hand of two crucial parameters: (i) the selectivity of enantioselective oxidation determines the maximal obtainable enantiomeric excess at equilibrium and the number of cycles required to reach it; (ii) the chemical yield of **R+S** is determined by the ratio of the forward- and reverse-reaction rate.

## Acknowledgements

This work was performed within the Spezialforschungsbereich 'Biokatalyse' and financial support from the Fonds zur Förderung der wissenschaftlichen Forschung (Project no. F115) and the Austrian Ministry of Transport and Science is gratefully acknowledged.

## References

1. (a) The enantiopure drug market has been estimated as US $18 billion worldwide. See: Stinson, S. C. *Chem. Eng. News* 1992, Sept. 28, 46–78; (b) Sheldon, R. A., *Chirotechnology*, Marcel Dekker: New York, 1993; (c) Collins, A. N.; Sheldrake, G. N.; Crosby, J., Eds, *Chirality in Industry*, Wiley: Chichester, Vol. I, 1992; Vol. II, 1997.
2. Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769–3826.
3. Kagan, H. B.; Fiaud, J. C. *Top. Stereochem.* **1988**, *18*, 249–330.
4. (a) Horeau, A. *Tetrahedron* **1975**, *31*, 1307–1309; (b) for biocatalyzed reactions: Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299; (c) for non-biocatalyzed reactions: Martin, V. S.; Woodard, S. S.;

Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237–6240; (d) for prebiotic reactions: Balavoine, G.; Moradpour, A.; Kagan, H. B. *J. Am. Chem. Soc.* **1974**, *96*, 5152–5158.

5. In biocatalyzed reactions, the ratio of asymmetrizations versus kinetic resolutions is ~1:4, database Faber, ~9000 entries.
6. Stecher, H.; Faber, K. *Synthesis* **1997**, 1–16.
7. Ebbers, E. J.; Ariaans, G. J. A.; Houbiers, J. P. M.; Bruggink, A.; Zwanenburg, B. *Tetrahedron* **1997**, *53*, 9417–9476.
8. Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475–1490.
9. Noyori, R.; Tokunaga, M.; Kitamura, M. *Bull. Chem. Soc. Jpn* **1995**, *68*, 36–56.
10. Caddick, S.; Jenkins, K. *Chem. Soc. Rev.* **1996**, *25*, 447–456.
11. Buisson, D.; Azerad, R.; Sanner, C.; Larcheveque, M. *Biocatalysis* **1992**, *5*, 249–265.
12. Nakamura, K.; Inoue, Y.; Matsuda, T.; Ohno, A. *Tetrahedron Lett.* **1995**, *36*, 6263–6266.
13. Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Medici, A.; Pedrini, P. *Tetrahedron: Asymmetry* **1995**, *6*, 3047–3053.
14. Takemoto, M.; Achiwa, K. *Tetrahedron: Asymmetry* **1995**, *6*, 2925–2928.
15. Tsuchiya, S.; Miyamoto, K.; Ohta, H. *Biotechnol. Lett.* **1992**, *14*, 1137–1142.
16. Takahashi, E.; Nakamichi, K.; Furui, M. *J. Ferment. Bioeng.* **1995**, *80*, 247–250.
17. Carnell, A. J.; Iacazio, G.; Roberts, S. M.; Willetts, A. J. *Tetrahedron Lett.* **1994**, *35*, 331–334.
18. Matsumura, S.; Kawai, Y.; Takahashi, Y.; Toshima, K. *Biotechnol. Lett.* **1994**, *16*, 485–490.
19. Shimizu, S.; Hattori, S.; Hata, H.; Yamada, H. *Enzyme Microb. Technol.* **1987**, *9*, 411–416.
20. Deracemization of various terminal (±)-1,2-diols by the yeast *Candida parapsilosis* has been claimed to operate via an (*R*)-specific $NAD^+$-linked dehydrogenase and an (*S*)-specific NADPH-dependent reductase: Hasegawa, J.; Ogura, M.; Tsuda, S.; Maemoto, S.; Kutsuki, H.; Ohashi, T. *Agric. Biol. Chem.* **1990**, *54*, 1819–1827. On the other hand, observations on the fungus *Geotrichum candidum* prove the need for molecular oxygen, which would suggest the involvement of an alcohol oxidase rather than an alcohol dehydrogenase: Azerad, R.; Buisson, D. Stereocontrolled reduction of β-ketoesters with *Geotrichum candidum*. Servi, S., Ed. In *Microbial Reagents in Organic Synthesis*, Vol. 381, NATO ASI Series C, Kluwer: Dordrecht, 1992; pp. 421–440.
21. E.e.=93.75% after four cycles; however, for an e.e. of 100%, an infinite number of cycles is required in theory.
22. For the mathematical treatment of dynamic kinetic resolutions see: Kitamura, M.; Tokunaga, M.; Noyori, R. *J. Am. Chem. Soc.* **1993**, *115*, 144–152; Kitamura, M.; Tokunaga, M.; Noyori, R. *Tetrahedron* **1993**, *49*, 1853–1860.
23. This can be conveniently explained by different paths of various gradient leading to a hilltop, the gradient determines the length of each path to reach the top.
24. The term 'theoretical turnover' indicates the theoretical equivalents of reagents needed if oxidation and reduction are considered as 'ideal' reactions. In practice, however, the amounts required will be larger because excess reagent(s) may be needed in order to drive the reaction towards completion and/or due to decomposition of reagents during undesired side reactions.
25. Alternatively, the Poe-scale can be used. The Poe-value corresponding to a time-value can be easily calculated through the e.e. at a given point of the reaction.
26. Kroutil, W.; Faber, K. (© 1998). 'Cyclo' is a free shareware program run through Windows. The program is available via the Internet at http://www-orgc.tu-graz.ac.at or directly from the authors. A description of how to use the program is given in the help-file which accompanies the program.
27. No maximum for **P** is observed if the amount of reducing agent is below the theoretical turnover. In this case, the process is disrupted by termination of the reverse reaction through lack of reducing agent.
28. In order to reduce the Tht down to the absolute minimum (Tht=1), absolute selectivity (E=∞) is required.
29. Since the destruction and formation of chiral centers is closely connected to a change in hybridization (e.g. $sp^3$–$sp^2$ carbon atoms), the process is linked to oxidation and reduction.
30. In principle, redox-systems comprising three types of chiral centers may be envisaged: (i) *sec*-alcohol/ketone [EC 1.1.3.X], (ii) *sec*-amine/imine [EC 1.4.3.X] and (iii) alkane/alkene [EC 1.3.3.X]. For the latter system, however, no feasible enantioselective biocatalytic oxidation system has been described to date.
31. Most alcohol oxidases are flavin-dependent (FAD or FMN), apart from few exceptions, such as glucose oxidase, which requires PQQ. Alternatively, metal ions, such as $Cu^{2+}$, $Mn^{2+}$ or non-heme iron are involved.
32. For the enantioselective oxidation of *sec*-alcohols by *sec*-alcohol oxidases see: Müller, H.-G.; Güntherberg, H.; Drechsler, H.; Mauersberger, S.; Kortus, K.; Oehme, G. *Tetrahedron Lett.* **1991**, *32*, 2009–2012; Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Poli, S.; Gardini, F. *Tetrahedron: Asymmetry* **1993**, *4*, 1607–1612.
33. For the enantioselective oxidation of *sec*-alcohols by cholesterol oxidase see: Dieth, S.; Tritsch, D.; Biellmann, J.-F. *Tetrahedron Lett.* **1995**, *36*, 2243–2246.
34. Belmouden, A.; Lederer, F. *Eur. J. Biochem.* **1966**, *238*, 790–798.

35. For the enantioselective oxidation of α-hydroxyacids by glycolate oxidase see: Gavagan, J. E.; Fager, S. K.; Seip, J. E.; Clark, D. S.; Payne, M. S.; Anton, D. L.; DiCosimo, R. *J. Org. Chem.* **1997**, *62*, 5419–5427; Eisenberg, A.; Seip, J. E.; Gavagan, J. E.; Payne, M. S.; Anton, D. L.; DiCosimo, R. *J. Mol. Catal. B* **1997**, *2*, 223–232; Adam, W.; Lazarus, M.; Boss, B.; Saha-Möller, C. R.; Humpf, H.-U.; Schreier, P. *J. Org. Chem.* **1997**, *62*, 7841–7843.
36. Hafner, E. W.; Wellner, D. *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 987–991.
37. Hartmann, C.; McIntire, W. S. *Methods Enzymol.* **1997**, *280*, 98–150; Frebort, I.; Adachi, O. *J. Ferment. Bioeng.* **1995**, *80*, 625–632.
38. From the large number of amino acid oxidases, those showing wide substrate tolerance are of particular interest, see: Rodwell, V. W. *Methods Enzymol.* **1971**, *17B*, 174–188; Wikström, P.; Szwajcer, E.; Brodelius, P.; Nilsson, K.; Mosbach, K. *Biotechnol. Lett.* **1982**, *4*, 153–158.
39. Huh, J. W.; Yokoigawa, K.; Esaki, N.; Soda, K. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 2081–2082.
40. Huh, J. W.; Yokoigawa, K.; Esaki, N.; Soda, K. *J. Ferment. Bioeng.* **1992**, *74*, 189–190.