2nd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-2), http://www.mdpi.org/ecsoc/, September 1-30, 1998

[al001]

BIOCATALYTIC STRATEGIES FOR THE PREPARATION OF CHIRAL BUILDING BLOCKS IN 100% CHEMICAL AND OPTICAL YIELD FROM RACEMATES

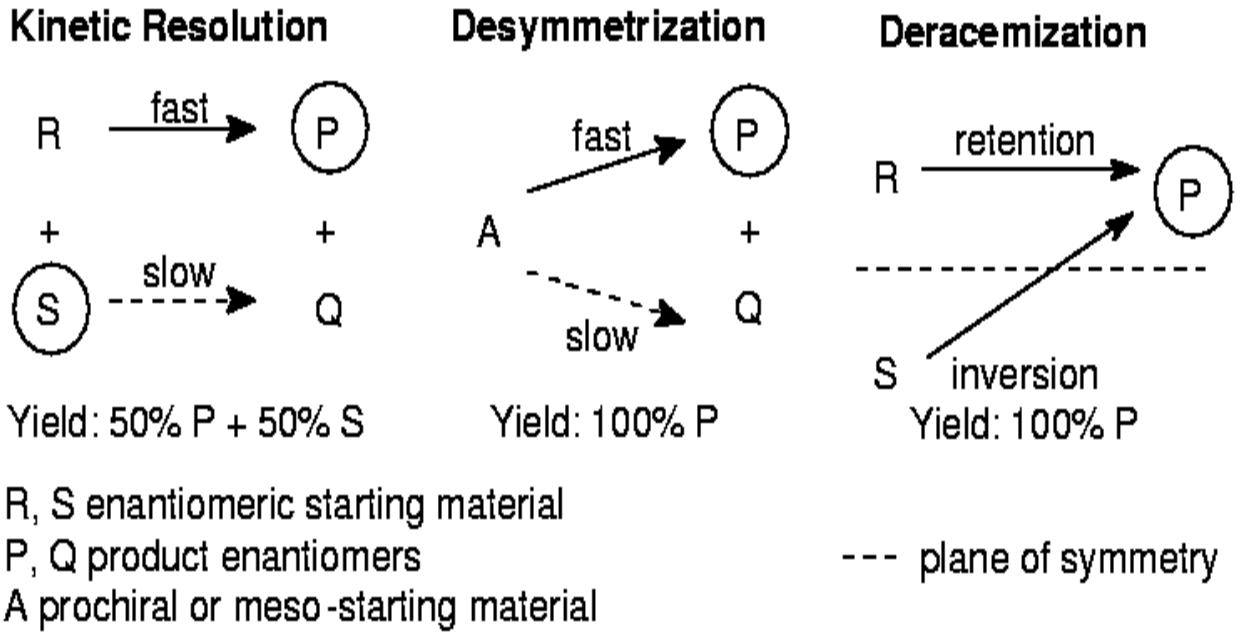
U. Felfer, W. Kroutil, U. T. Strauss, K. Faber*

Institute of Organic Chemistry, University of Graz, Heinrichstraße 28,
A-8010 Graz, Austria. <FABER@ORGC.TU-GRAZ.AC.AT>
http://www-orgc.tu-graz.ac.at/fabgroup

1 INTRODUCTION

Driven by the increased demand for chiral drugs in enantiomerically pure form following the release of new FDA´s marketing guidelines, the search for novel methods for EPC-syntheses is 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], the latter is astonishingly dominant in number of applications (~1:4) [6], which is probably due to the fact that meso- and prochiral substrates are less easily synthesized than racemates. 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 an ideal resolution process should provide a single enantiomeric product in 100 % yield. The most obvious drawbacks are as follows:
(i) The theoretical yield of each enantiomer can never exceed a 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 pathway in an enantio-convergent fashion, but this requires additional labour and 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 standpoint - i.e. 50 % conversion [9].

Scheme 1. Principles of Kinetic Resolution and Desymetrization



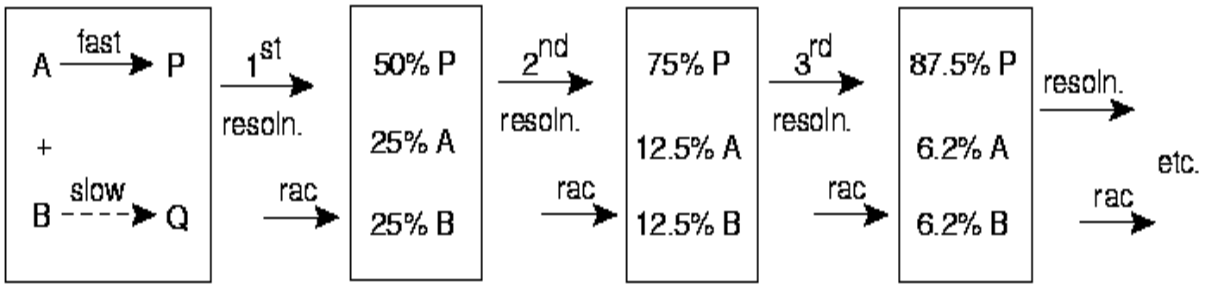
As a consequence, alternatives to resolution techniques that can deliver a single isomer from a racemate are highly advantageous. The latter are generally denoted as 'deracemization' [10]. All of these processes are dealing with a common stereochemical phenomenon, i.e. both of the substrate enantiomers 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 deviding 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 paper, biocatalytic strategies which lead to the formation of a single enantiomeric product in 100 % theoretical yield from a racemate are reviewed.

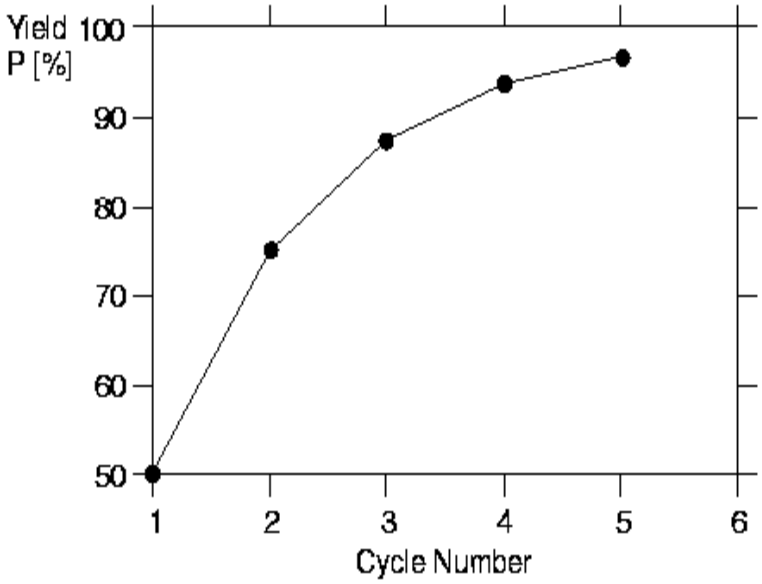
2 IMPROVING KINETIC RESOLUTION

2.1 RE-RACEMIZATION AND REPEATED RESOLUTION

In order to avoid the loss of half of the material, it has been a common practice in kinetic resolutions, 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]. For obvious reasons, this laborious procedure is not justified on laboratory-scale reactions, but it is a viable option for resolutions on an industrial scale, in particular for continuous processes, where the re-racemized material is simply fed back into the subsequent batch of the resolution process. On a quick 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, certain merits are discovered which makes it a viable option: It can be seen from the graph in Scheme 2, 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 racemization are essentially 'clean' without loss of materials.

Scheme 2. Overall Yield in a Repeated Resolution Process via Re-Racemisation



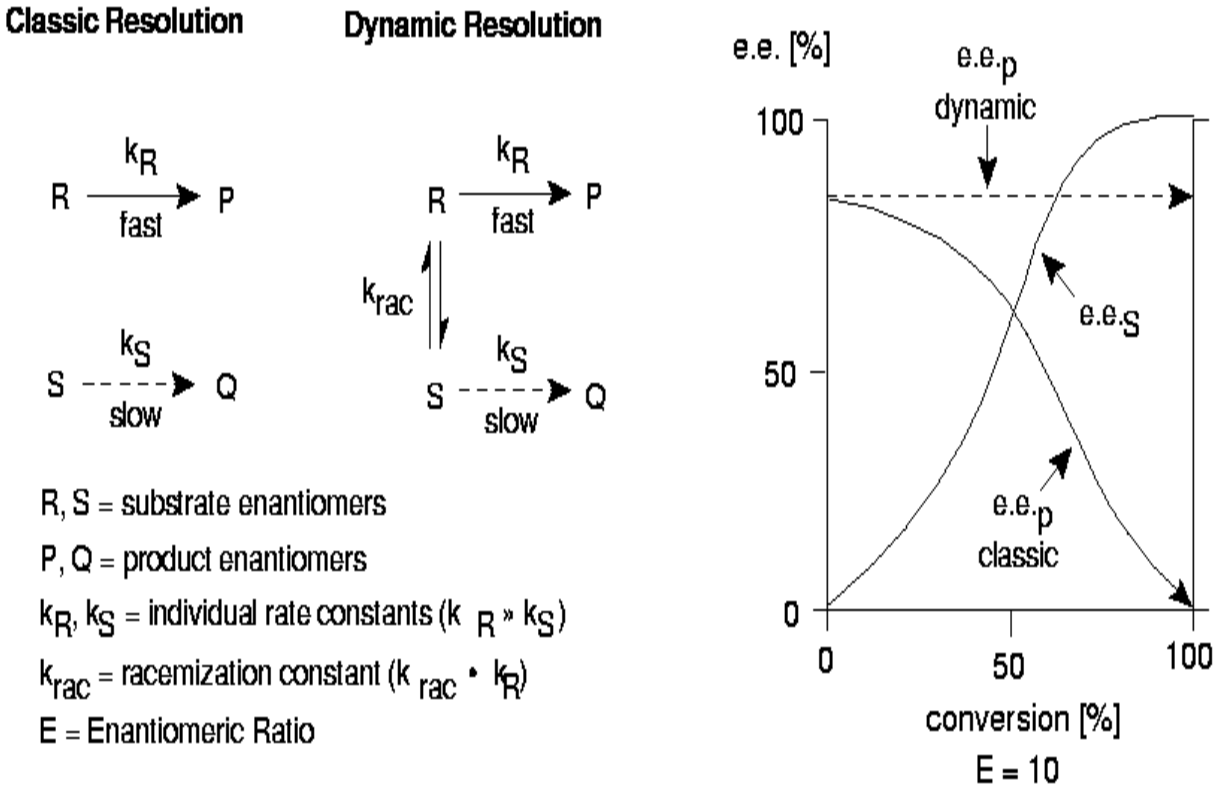


The key to successful repeated resolution are mild conditions for re-racemization which cause a minimum loss of materials and thus avoid the need for an additional purification step. Unfortunately, controlled racemization is often impeded by the neccessity of strongly acidic or basic reaction conditions, which lead to undesired side-reactions [12]. In this context, the use of enzymes - racemases - holds great potential, as they operate under mild physiological reaction conditions and thus are completely devoid of side reactions [13].

2.2 DYNAMIC RESOLUTION

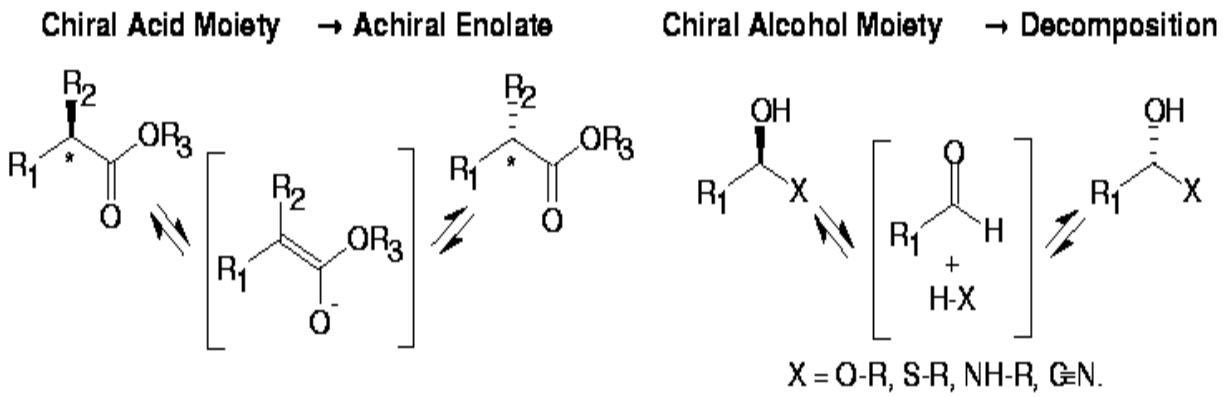
The disadvantages of kinetic resolution can largely be avoided by employing a so-called 'dynamic resolution' [14] (Scheme 3). Such a process comprises kinetic resolution with an additional feature, i.e. in-situ racemization of the starting material, which is usually achieved via chemo-catalysis. As a consequence, 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 (or comes even to a standstill) at 50 % conversion, when the fast reacting enantiomer R is consumed and the slow reacting counterpart remains, substrate racemization ensures the formation of R from S during the course of the reaction and thus avoids the depletion of R. As a consequence, the reaction does not come to a standstill and can be run to completion by converting all of the 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 [15]: From Scheme 3 it can be seen that the e.e. of the substrate (e.e.S) is at its maximum at the onset of the reaction and gradually begins to decline as the faster reacting enantiomer is depleted from the reaction mixture, in particular at around half-way during the reaction. On the other hand, this depletion does not occur if the substrate is constantly racemized during the resolution process and, as a consequence, 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] = [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 3. Principles of Kinetic and Dynamic Resolution



In order to design a successful dynamic resolution, both of the parallel reactions - i.e. kinetic resolution and in-situ racemization - have to be carefully tuned:
(i) The kinetic resolution should be irreversible in order to ensure high enantioselectivity,
(ii) the Enantiomeric Ratio (E-value, E = k_R/k_S) [16] should be at least greater than ~20,
(iii) to avoid depletion of R, racemization (k_{rac}) should be at least equal or greater than the reaction rate of the fast enantiomer (k_R),
(iv) in case the selectivities are only moderate, k_{rac} should be greater than k_R by a factor of ~10,
(v) for obvious reasons, any spontaneous reaction involving the substrate enantiomers as well as racemization of the product should be absent.
(vi) Dynamic resolution is generally limited to compounds possessing one single stereocenter.
All of the dynamic resolution processes reported to date based on biocatalysis, made use of a combination of an enzyme-catalyzed kinetic resolution coupled to in-situ racemization through chemo-catalysis. Two general principles can be drawn as follows (Scheme 4):
(i) For compounds possessing a chirality center bearing an acidic proton (e.g. adjacent to an activating carbonyl group, such as an ester or ketone), racemization is usually facilitated through the formation of an achiral enolate species via base-catalyzed H-abstraction. (ii) For those cases, where this is impossible, e.g. a secondary alcohol, racemization is achieved via a decomposition reaction, such as the cleavage of hemi(thio)acetals and cyanohydrins.

Scheme 4. Principles of In-Situ Racemization of Substrate in Dynamic Resolution Processes

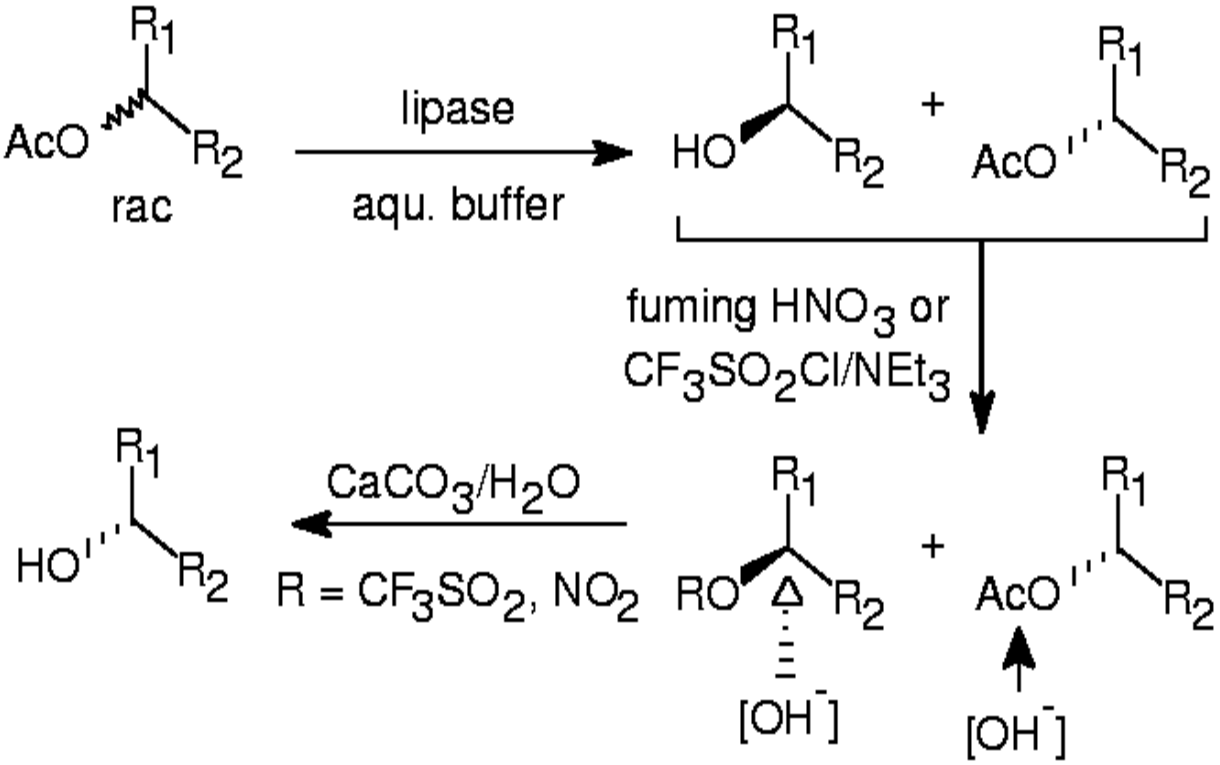


Since enzymes and chemical catalysts, such as acid and base, are usually working in quite different environments, their combination in a single reactor to form dynamic resolution processes is often difficult to meet in practice. On the other hand, enzymes are easily compatible with each other, as they generally work under the same (physiological) reaction conditions. As a consequence, the use of racemases for in-situ substrate racemization combined with biocatalyzed kinetic resolution holds great potential for dynamic resolution processes.

2.3 STEREO-INVERSION

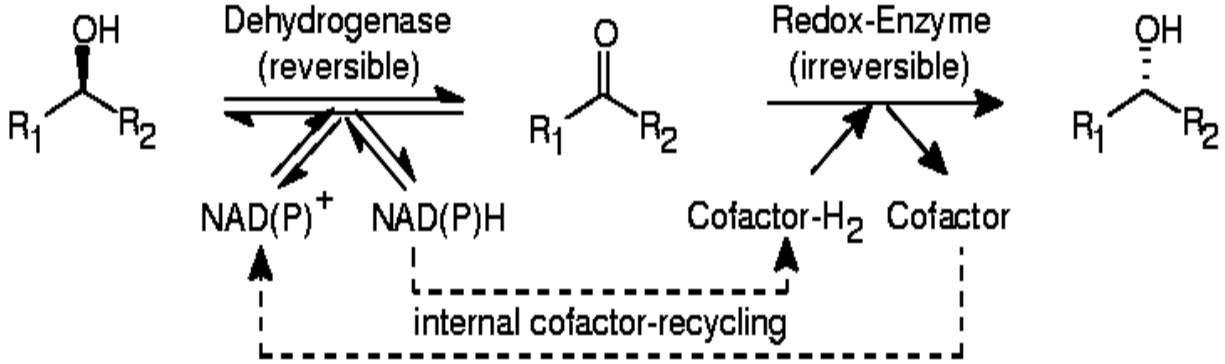
The difficulty to achieve in-situ racemization with compounds possessing a configurationally stable chirality center, such as secondary alcohols, may be overcome by employing a so-called stereo-inversion [17]. The latter may either be achieved through chemical or biocatalytic methods.
The principle of deracemization coupled to chemical stereo-inversion is outlined in Scheme 5 [18]. Thus, in a first step, kinetic resolution of a secondary alcohol was achieved using lipase-catalyzed ester hydrolysis furnishing a mixture of sec-alcohol and the corresponding enantiomeric ester. Without separation, the mixture was subjected to chemical inversion of the alcohol by treatment with mesyl chloride or (for large-scale reactions) with fuming nitric acid under carefully controlled reaction conditions, which gave a mixture of enantiomeric activated and non-activated esters [19]. The latter were hydrolyzed by strong base with inversion and retention of configuration, respectively. As a consequence, a single enantiomeric sec-alcohol was formed as the sole product.

Scheme5. Resolution Coupled to Chemical Stereo-Inversion



Due to the fact that in step 1 (i.e. kinetic resolution) the enantiomeric excess of product (e.e.P) and substrate (e.e.S) are a function of the conversion, the point of stopping the lipase-catalysed reaction and the switch to the chemical inversion process has to be carefully chosen, in order to obtain a maximum e.e. of the product. The latter can be calculated as a function of the selectivity of the reaction and is usually at or somewhat beyond a conversion of 50 % [20].

Scheme 6. Biocatalytic Stereo-Inversion of sec-Alcohols via an Oxidation-Reduction Sequence



Alternatively, stereo-inversion of sec-alcohols may be achieved by biocatalytic methods via an oxidation-reduction sequence Scheme 6 [21]. Thus, one enantiomer out of a racemic mixture is selectively oxidized to the corresponding ketone under catalysis of a dehydrogenase, while the mirror-image counterpart remains unaffected. Then, the ketone is reduced again in a subsequent step by a different enzyme displaying opposite stereochemical preferences. Overall, this two-step oxidation-reduction sequence constitutes a deracemization process. Due to the involvement of two consecutive oxidation-reduction reactions, the net redox balance of the process is zero and in an ideal case no external cofactor-recycling is neccessary, since between both steps, the redox-equivalents, such as NAD(P)H, may be recycled internally, e.g. by using whole-cell systems.
The success of a biocatalytic stereo-inversion via a redox-process is determined by the following crucial point: In

order to pay the entropy balance of the process, which is required to achieve a high optical purity of the product, at least one of both redox-reactions has to be irreversible [22].

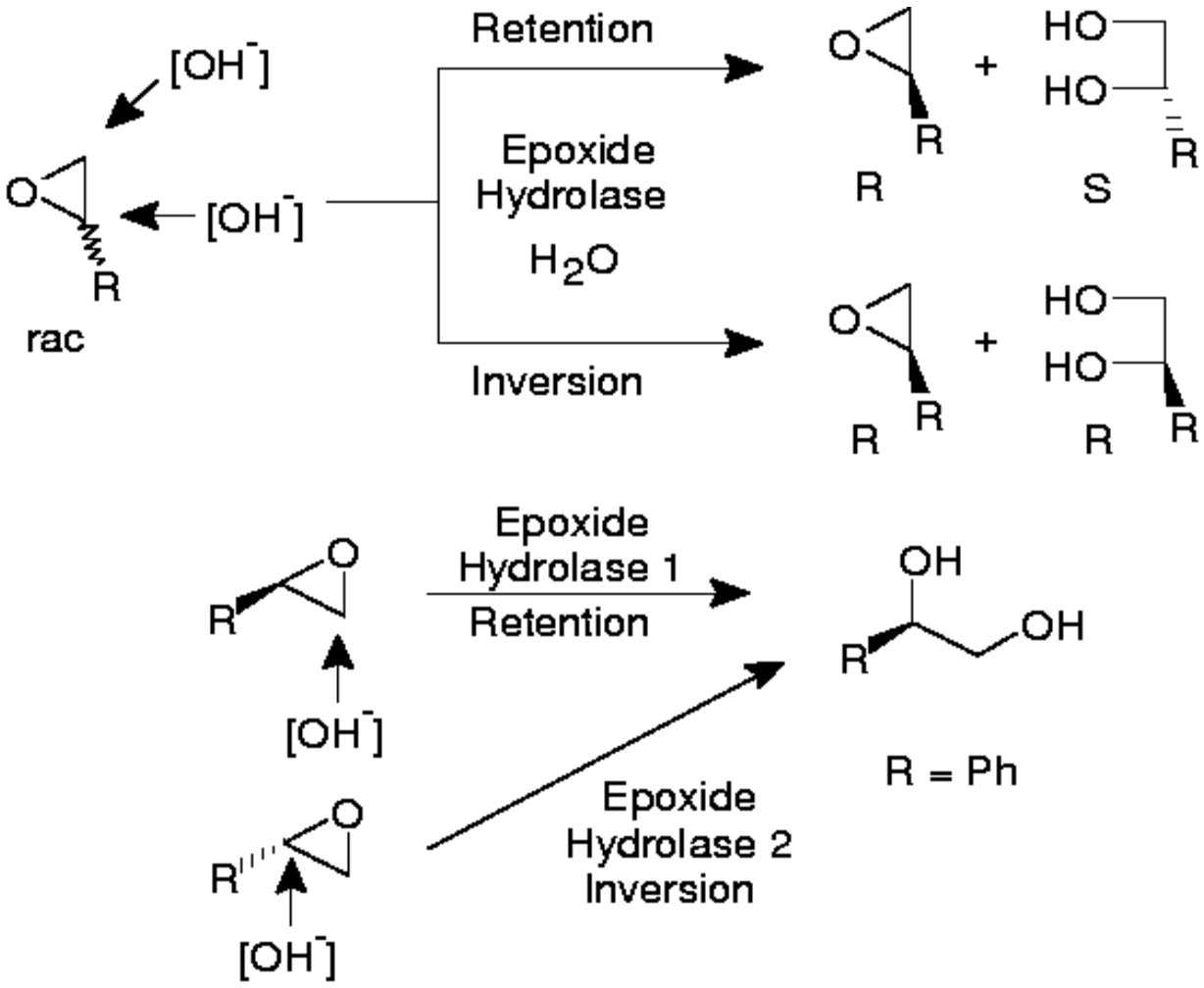
3 ENANTIO-CONVERGENT PROCESSES

Deracemization may be achieved through so-called enantioconvergent processes in such way, that each of the enantiomers is converted into the same product enantiomer P via two independent pathways (Scheme 1). Thus, whereas enantiomer R is reacted to product P through retention of configuration, its counterpart S is transformed with inversion. In general, both of the reactions are conducted in a step-wise fashion. The crucial prerequisites for the proper function of such systems are as follows:
(i) The first step has to show combined excellent enantiospecificity and stereospecificity with respect to retention/inversion, in other words, the demands with respect to the chiral recognition and stereochemistry of the transformation are high and it is not surprising that these specificities are usually only achieved by enzymes.
(ii) Since the starting material for the second step (i.e. S) is enantiomerically enriched (or even pure), only high stereospecificity is required with respect to inversion/retention of configuration. As a consequence, this step may also be performed by using a chemo-catalyst.
(iii) An important factor with respect to the economics of the whole process is the compatibility of the reaction conditions of both steps with each other. If, for instance, the conditions are incompatible, separation of product P (formed during step 1 from R) from remaining enantiomeric starting material S is required, which is usually going in hand with loss of material. After all, it appears anachronistic, to separate materials from each other, which are to be combined at the end of the process. Thus, successful enantioconvergent processes should always be performed in a one-pot fashion.

3.1 ENANTIO-CONVERGENCE THROUGH TWO BIOCATALYSTS

To date, the only enzymes which may transform non-natural compounds by acting with inversion of configuration during catalysis are (i) glycosidases [23], (ii) dehalogenases [24], (iii) sulfatases [25] and (iv) epoxide hydrolases [26]. Whereas glycosidases cannot be employed for deracemization since their substrates represent diastereomers rather than enantiomers, dehalogenases are not widely distributed in Nature and they exhibit a limited substrate tolerance. Similarly, the number of applications of sulfatases in preparative biotransformations is extremely limited and they are restricted to aryl-sulfatases, where no chirality is involved [27]. On the other hand, epoxide hydrolases from microbial sources such as bacteria and fungi have recently been shown to possess a great potential for the stereoselective hydrolysis of epoxides to furnish the corresponding vicinal diols [28]. In contrast to ester hydrolysis catalyzed by lipases, esterases or proteases, where the absolute configuration at the stereogenic center(s) always remains the same throughout the reaction, enzymatic hydrolysis of epoxides may take place via attack on either carbon atom of the oxirane ring (Scheme 7) and it is the structure of the substrate and of the enzyme involved which determine the regioselectivity of the attack [29]. This is exemplified as follows (Scheme 7): If the (S)-enantiomer is preferentially hydrolysed from the racemate with retention of configuration, kinetic resolution furnishes a mixture of (S)-diol and unreacted (R)-epoxide. On the contrary, the corresponding (R)-diol is produced from the (S)-oxirane, if the enzyme acts with inversion of configuration. As a consequence, enantioconvergent hydrolysis of epoxides was shown to be feasible through the availability of appropriate enzymes.

Scheme 7. Enzymatic Hydrolysis of Epoxides Proceeding with Retention or Inversion of Configuration

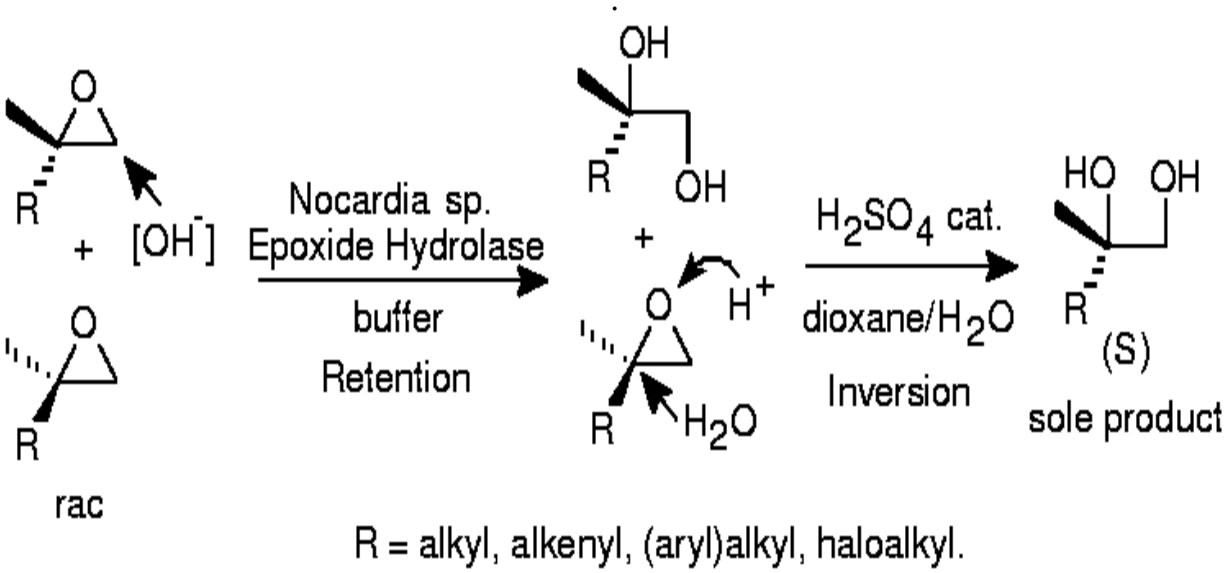


An elegant deracemization of (±)-styrene oxide was developed by making use of two epoxide hydrolase activites from fungal sources [30]. Whereas Aspergillus niger preferentially hydrolyzed the (R)-enantiomer with retention of configuration by producing (R)-phenylethan-1,2-diol (Scheme 7 , epoxide hydrolase 1), Beauveria bassiana showed opposite enantiopreference [i.e. (S)] with matching opposite regioselectivity causing inversion of configuration (epoxide hydrolase 2). Combination of both bicoatalysts in a single reactor lead to almost complete deracemization.

3.2 ENANTIO-CONVERGENCE THROUGH BIO- AND CHEMO-CATALYSIS

Bacterial epoxide hydrolases have been shown to be the biocatalysts of choice for the enantioselective hydrolysis of 2,2-disubstituted oxiranes, by showing virtually absolute enantioselectivities (E >200) [31]. In this case, the reaction proved to proceed invariably through retention of configuration. Since the existence of enzymes being able to attack a quarternary carbon atom with inversion of configuration seems rather unlikely, deracemization via a two-enzyme system as described above was impossible. However, the combination of bio- and chemo-catalysis proved to be very efficient (Scheme 8) [32]. Thus, kinetic resolution of 2,2-disubstituted epoxides by using a Nocardia sp. epoxide hydrolase proceeded with excellent enantio- and regioselectivity by furnishing the corresponding (S)-diol and (R)-epoxide in a first step. Then, the remaining epoxide was transformed through acid-catalysis with inversion of configuration in a second step under carefully controlled reaction conditions to yield the corresponding (S)-diols in virtually enantiopure form and in high chemical yields (>90 %). This technique proved to be highly flexible and was also applicable to styrene-oxide type substrates [33].

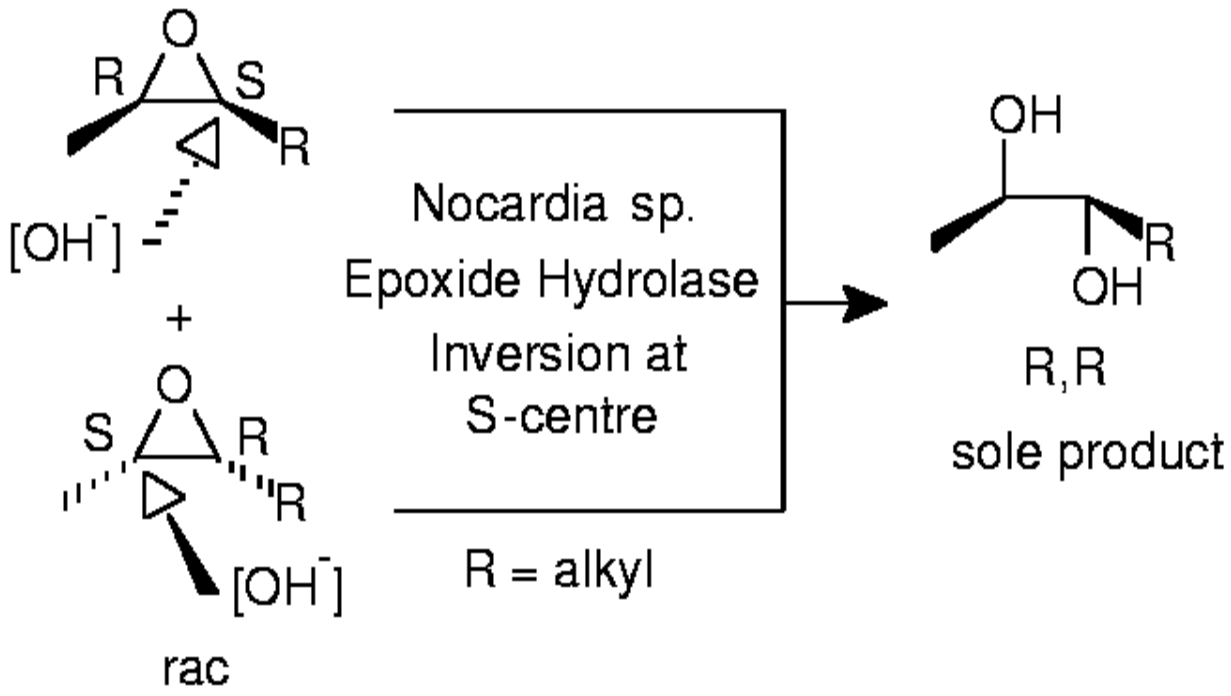
Scheme 8. Enantioconvergent Hydrolysis of 2,2-Disubstituted Epoxides through Combination of Bio- and Chemo-Catalysis



3.3 ENANTIO-CONVERGENCE THROUGH SINGLE BIOCATALYST

Processes depending on more than one catalyst are generally sensitive with respect to the tuning of both reactions and it can be expected that enantioconvergent reactions which depend on a single catalyst are more reliable in practice. However, the requirements to this single catalyst are extremely high, i.e. it has to exhibit not only high enantioselectivity but also matching opposite regioselectivity for the transformation of each enantiomer at the same time in order to make the process enantio-convergent. As a consequence, such processes catalysed by a single (bio)catalyst are very rare (Scheme 9) [34]. For instance, an epoxide hydrolyse from Nocardia sp. hydrolyzed both enantiomers of cis-2,3-disubstituted epoxyalkanes through attack at their respective (S)-configurated oxirane carbon atom with inversion of configuration yielding the corresponding (2R,3R)-diol as the sole product in up to 92 % e.e. and 85 % chemical yield [35].

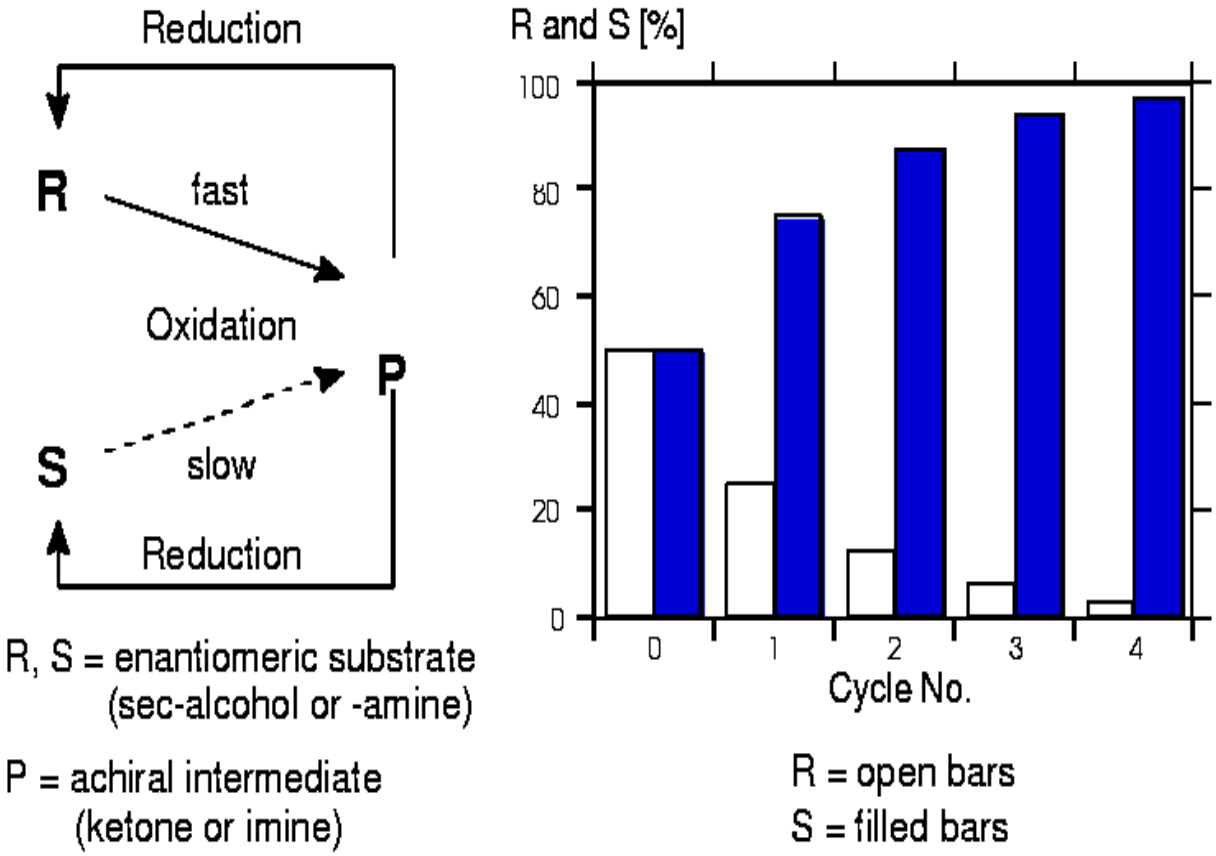
Scheme 9. Enantioconvergent Hydrolysis of 2,3-Disubstituted Epoxides Using a Single Biocatalyst



4 DERACEMIZATION THROUGH CYCLIC OXIDATION-REDUCTION SEQUENCE

Deracemization of compounds bearing a chiral sec-hydroxy or -amino group can be achieved via a novel process consisting of a cyclic oxidation-reduction sequence [36 , 37]. The system consists of two independent reactions outlined in Scheme 10. First one enantiomer of the secondary alcohol or amine (R) is selectively oxidized from the starting racemate (R+S) to yield the achiral intermediate P, i.e. the corresponding ketone or imine, respectively. Then, the latter is chemically reduced in a non-selective fashion to yield again a mixture of R + S in racemic form. Both reactions alone are of limited use for the preparation of enantiopure material, since step 1 (i.e. a kinetic resolution through enantioselective oxidation) is limited to a 50 % theoretical yield of chiral non-reacting S and achiral P, and step 2 does not show any chiral induction at all. However, combination of both steps in a cyclic mode leads to a highly versatile deracemization technique. The practical feasibility of cyclic deracemization based on oxidation-reduction was verified for the deracemization of (±)-a-amino acids through combination of an amino acid oxidase coupled to $NaBH_4$-reduction of the corresponding intermediate imino acid [38].
The functioning of this system is explained along the following example: If (for reasons of clarity) the selectivity of step 1 is assumed to be absolute, only R is selectively oxidized 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 after a single full cycle is now equal 25/75. It can be seen from the graph in Scheme 10, that further cycles lead to a gradual increase of enantiomer S at the expense of R, and that the enantiomeric excess of the starting material is already well above 90 % after only four cycles, assuming absolute enantioselectivity. Overall, if the cyclic process is driven in the forward direction, enantiomer S represents the 'sink' of material in the whole system.

Scheme 10. Deracemisation of sec-Alcohols and -Amines through Cyclic Oxidation-Reduction



For practical applications, however, selectivities are ranging often below E-values of 100. For these cases, the enantioselectivity determines two crucial factors of the system, i.e. (i) the maximum obtainable e.e. at equilibrium and (ii) the number of cycles which are required to reach this value. The merits and limits of cyclic deracemization systems have been recently described based on the underlying kinetics [36].

5 SUMMARY

The development of methods for the preparation of chiral compounds in 100 % chemical and optical yields from racemates is one of the current challenges in asymmetric synthesis. Several principles have been described to far, which are either based on modifications of classic kinetic resolution or on the transformation of enantiomers via enantioconvergent pathways, which is usually achieved by combination of chemo- and/or biocatalysts in seqential reactions or - most elegantly - even by a single (bio)catalyst. It has to be emphasized, however, that each of the above described principles offers a solution only to certain types of stereochemical problems and the corresponding substrate classes, but none of the methods can be employed as a general solution.

Acknowledgements: This work was performed within the Spezialforschungsbereich 'Biokatalyse' and financial support by the Fonds zur Förderung der wissenschaftlichen Forschung (project F115) and the Austrian Federal Ministry of Science and Transport is gratefully acknowledged.

6 REFERENCES AND NOTES

1. a) The enantiopure drug market has been estimated as US $ 18 billion worldwide. See: S. C. Stinson, *Chem. Eng. News* **1992**, Sept. 28, 46-78; b) R. A. Sheldon, Chirotechnology, New York, Marcel Dekker, 1993; c) A. N. Collins, G. N. Sheldrake, J. Crosby, (eds), Chirality in Industry, Chichester, Wiley, vol. I, 1992; vol. II, 1997.
2. K. Faber, Biotransformations in Organic Chemistry, 3rd edn., Springer, Heidelberg, 1997.
3. E. Schoffers, A. Golebiowski, C. R. Johnson, *Tetrahedron* **1996**, *52*, 3769-3826.
4. H. B. Kagan, J. C. Fiaud, *Topics Stereochem.* **1988**, *18*, 249-330.
5. a) A. Horeau, *Tetrahedron* **1975**, *31*, 1307-1309; b) For biocatalyzed reactions: C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294-7299; c) For non-biocatalyzed reactions: V. S. Martin, S. S. Woodard, T. Katsuki, Y. Yamada, M. Ikeda, K. B. Sharpless, *J. Am. Chem. Soc.* **1981**, *103*, 6237-6240. d) For prebiotic reactions: G. Balavoine, A. Moradpour, H. B. Kagan, *J. Am. Chem. Soc.* **1974**, *96*, 5152-5158.
6. For biocatalyzed reactions, data from database Faber, ~9000 entries, July 1998.
7. K. Faber, *Indian J. Chem., Sect. B*, **1992**, *31B*, 921-924.
8. I. C. Cotterill, R. Jaouhari, G. Dorman, S. M. Roberts, F. Scheinmann, B. J. Wakefield, *J. Chem. Soc., Perkin Trans. 1*, **1991**, 2505-2512.
9. C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294-7299.
10. H. Stecher, K. Faber, *Synthesis* **1997**, 1-16.
11. For a typical example see: a) Y.-C. Xie, H.-Z. Liu, J.-Y. Chen, *Biotechnol. Lett.* **1998**, *20*, 455-458; b) J. Kamphuis, W. H. J. Boesten, B. Kaptein, H. F. M. Hermes, T. Sonke, Q. B. Broxterman, W. J. J. van den Tweel, H. E. Schoemaker, in: Chirality in Industry, A. N. Collins, G. N. Sheldrake, J. Crosby, eds., pp. 187-208, Wiley, New York, 1992.
12. E. J. Ebbers, G. J. A. Ariaans, J. P. M. Houbiers, A. Bruggink, B. Zwanenburg, *Tetrahedron* **1997**, *53*, 9417-9476.
13. E. Adams, *Adv. Enzymol. Relat. Areas Mol. Biol.* **1976**, *44*, 69-138.
14. a) R. S. Ward, *Tetrahedron: Asymmetry* **1995**, *6*, 1475-1490; b) S. Caddick, K. Jenkins, *Chem. Soc. Rev.* **1996**, *25*, 447-456; c) R. Noyori, M. Tokunaga, M. Kitamura, *Bull. Chem. Soc. Jpn.* **1995**, *68*, 36-56.
15.For the mathematical treatment of dynamic kinetic resolutions see: M. Kitamura, M. Tokunaga, R. Noyori, *J. Am. Chem. Soc.* **1993**, *115*, 144-152; M. Kitamura, M. Tokunaga, R. Noyori, *Tetrahedron* **1993**, *49*, 1853-1860.
18. For biocatalyzed reactions, the 'binding' of the substrate enantiomers (which can be neglected with chemical

catalysts) usually plays an important role in the chiral selection process and E-values of enzyme-catalyzed reactions are therefore defined through Michaelis-Menten kinetics: E = (kcat/KM)R/(kcat/KM)S.
17.A. J. Carnell, *Adv. Biochem. Eng. Biotechnol.* **1998**, *63*, 57-72.
18.a) H. Danda, T. Nagatomi, A. Maehara, T. Umemura, *Tetrahedron* **1991**, *47*, 8701-8716; b) K. Lemke, S. Ballschuh, A. Kunath, F. Theil, *Tetrahedron: Asymmetry* **1997**, *8*, 2051-2055; c) E. Vänttinen, L. T. Kanerva, *Tetrahedron: Asymmetry* **1995**, *6*, 1779-1786; d) S. Takano, M. Suzuki, K. Ogasawara, *Tetrahedron: Asymmetry* **1993**, *4*, 1043-1046; e) S. Mitsuda, T. Umemura, H. Hirohara, *Appl. Microbiol. Biotechnol.* **1988**, *29*, 310-315.
19. For small-scale reactions, Mitsunobu-conditions may be likewise employed.
20.a) L. T. Kanerva, *Acta Chem. Scand.* **1996**, *50*, 234-242; b) S. Pedragosa-Moreau, C. Morisseau, J. Baratti, J. Zylber, A. Archelas, R. Furstoss, *Tetrahedron* **1997**, *53*, 9707-9714.
21. a) D. Buisson, R. Azerad, C. Sanner, M. Larcheveque, *Biocatalysis* **1992**, *5*, 249-265; b) K. Nakamura, Y. Inoue, T. Matsuda, A. Ohno, *Tetrahedron Lett.* **1995**, *36*, 6263-6266; c) G. Fantin, M. Fogagnolo, P. P. Giovannini, A. Medici, P. Pedrini, *Tetrahedron: Asymmetry* **1995**, *6*, 3047-3053; d) M. Takemoto, K. Achiwa, *Tetrahedron: Asymmetry* **1995**, *6*, 2925-2928; e) S. Tsuchiya, K. Miyamoto, H. Ohta, *Biotechnol. Lett.* **1992**, *14*, 1137-1142; f) E. Takahashi, K. Nakamichi, M. Furui, *J. Ferment. Bioeng.* **1995**, *80*, 247-250; g) A. J. Carnell, G. Iacazio, S. M. Roberts, A. J. Willetts, *Tetrahedron Lett.* **1994**, *35*, 331-334; h) S. Matsumura, Y. Kawai, Y. Takahashi, K. Toshima, *Biotechnol. Lett.* **1994**, *16*, 485-490; i) S. Shimizu, S. Hattori, H. Hata, H. Yamada, *Enzyme Microb. Technol.* **1987**, *9*, 411-416.
22. The origin of the irreversibility of microbial/enzymatic deracemization of *sec*-alcohols via an oxidation-reduction sequence is currently under investigation and the data available to date reveal a rather puzzling picture: For instance, deracemization of various terminal (±)-1,2-diols by the yeast *Candida parapsilosis* has been claimed to operate via a (*R*)-specific NAD+-linked dehydrogenase and a (*S*)-specific NADPH-dependent reductase. Although no detailed data were given, the latter step was claimed to be irreversible: J. Hasegawa, M. Ogura, S. Tsuda, S. Maemoto, H. Kutsuki, T. Ohashi, *Agric. Biol. Chem.* **1990**, *54*, 1819-1827. On the other hand, observations on the fungus *Geotrichum candidum* prove the requirement of molecular oxygen, which would suggest the involvement of an alcohol oxidase rather than an alcohol dehydrogenase: R. Azerad, D. Buisson, in: Microbial Reagents in Organic Synthesis; S. Servi, ed., NATO ASI Series C, vol. 381, pp. 421-440, Dordrecht, Kluwer, 1992.
23. M. L. Sinnott, *Chem. Rev.* **1990**, *90*, 1171-1202.
24. T. Leisinger, R. Bader, *Chimia* **1993**, *47*, 116-121.
25.A. B. Roy, *The Enzymes* **1971**, *5*, 1-19.
26. R. V. A. Orru, A. Archelas, R. Furstoss, K. Faber, *Adv. Biochem. Eng. Biotechnol.* **1998**, *63*, 145-167.
27.G. Pelsy, A. M. Klibanov, *Biotechnol. Bioeng.* **1983**, *25*, 919-928.
28.a) K. Faber, M. Mischitz, W. Kroutil, *Acta Chem. Scand.* **1996**, *50*, 249-258; b) A. Archelas, R. Furstoss, *Annu. Rev. Microbiol.* **1997**, *51*, 491-525; c) Archer, I. V. J., *Tetrahedron* **1997**, *53*, 15617-15662.
29. M. Mischitz, C. Mirtl, R. Saf, K. Faber, *Tetrahedron: Asymmetry* **1996**, *7*, 2041-2046.
30. S. Pedragosa-Moreau, A. Archelas, R. Furstoss, *J. Org. Chem.* **1993**, *58*, 5533-5536.
31. M. Mischitz, W. Kroutil, U. Wandel, K. Faber, *Tetrahedron: Asymmetry* **1995**, *6*, 1261-1272.
32. R. V. A. Orru, S. F. Mayer, W. Kroutil, K. Faber, *Tetrahedron* **1998**, *54*, 859-874.
33. S. Pedragosa-Moreau, C. Morisseau, J. Baratti, J. Zylber, A. Archelas, R. Furstoss, *Tetrahedron* **1997**, *53*, 9707-9714.
34.a) S. Pedragosa-Moreau, A. Archelas, R. Furstoss, *Tetrahedron* **1996**, *52*, 4593-4606; b) G. Bellucci, C. Chiappe, A. Cordoni, *Tetrahedron: Asymmetry* **1996**, *7*, 197-202.
35.W. Kroutil, M. Mischitz, K. Faber, *J. Chem. Soc., Perkin Trans. 1* **1997**, 3629-3636.
36. W. Kroutil, K. Faber, *Tetrahedron: Asymmetry*, **1998**, submitted.
37. A free shareware program ('Cyclo') running under Windows is available via the Internet at <http://www-orgc.tu-graz.ac.at/products/software.htm> or directly from the authors (W. Kroutil, K. Faber, © 1998). A description how to use the program is given in the help-file which accompanies the program.
38.a) J. W. Huh, K. Yokoigawa, N. Esaki, K. Soda, *J. Ferment. Bioeng.* **1992**, *74*, 189-190; b) J. W. Huh, K. Yokoigawa, N. Esaki, K. Soda, *Biosci. Biotechnol. Biochem.* **1992**, *56*, 2081-2082.

Comments

During 1-30 September 1998, all comments on this poster should be sent by e-mail to ecsoc@listserv.arizona.edu with al001 as the message subject of your e-mail. After the conference, please send all the comments and reprints requests to the author.
