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review

Novel Biocatalytic Methodology: Low Temperature Enhanced Enantioselectivity of Enzyme Catalyzed Reactions in Organic Solvents

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Summary

After recent reports in 1997/98, the low-temperature enhancement of enantioselectivity of the lipase-catalyzed acylations in organic solvents, emerges as a new lab-scale methodology in biocatalysis. The examples of effective production of enantiomerically pure compounds (EPC) at low temperatures, either by kinetic resolution of racemates or by desymmetrization of prochiral substrates, are reviewed.

Key words: kinetic resolution, desymmetrization, free-energy of activation

Introduction

Temperature influences chemical reaction rate; usually elevating the temperature increases the reaction rate, the opposite examples are also known, (*1,2*). More importantly, any reaction rate has its specific temperature dependence and therefore the difference in the rates of two competitive and contemporaneous reactions can be enhanced or lowered by elevating or lowering the temperature. In other words, by proper choosing of the temperature, the optimal difference between the rates, and consequently high selectivity in obtaining the desired product can be achieved. When certain reaction is expected to produce one enantiomer of the two available, it is named enantioselective. Elementary descriptions of enantioselective reactions are given in the Figs. 1a and 1b (*3,4*). It is important to note that in the first case (Fig. 1a) prochiral molecule **I** with two *enantiotopic ligands* is converted into one enantiomer of the chiral molecule by substitution of one of them, whereas in the Fig. 1b. prochiral molecule **II**, with two *enantiotopic faces*, is transformed on the addition of reagent HL into analogous chiral molecule as presented in Fig. 1a.

As shown in the upper part of the Fig. 2, enantiotopic ligands and faces are mirror-related, *i.e.* prochiral molecule comprises mirror plane that bisects it in the two mirror-related halves. On the enzyme active site such molecules accommodate in the way that the enzyme bound reagent, either nucleophile X^- or enophile L-H, approach the substrate preferentially or exclusively from one direction, the bottom part of the Fig. 2. This approach results with enantioselective substitution of *pro-R* (or *Re*) group L in the first case, or by addition from the *Si*-face in the second (*5,6*).

Generally, enantioselectivity in a chemical reaction can be obtained only by intervention of some *chiral auxiliray agent* (C^{AA}). This can act on the stoichiometric level, or as the catalyst or, important for our considerations hereafter, it can be a biocatalyst, an enzyme. Common to all enantioselective processes is the kinetic control, rised by the difference in the free energy content between two transitions states that lead to either (*R*) or (*S*) enantiomer (Fig. 3).

Schematic presentation in the Fig. 3 underlines the effect of a C^{AA} on the selective lowering of the energy for the pathway leading to the (*R*) enantiomer relative to those for (*S*) enantiomer. The energy difference ($\Delta\Delta G^{\ominus}$) between two energy differences, *i.e.* those between pro-

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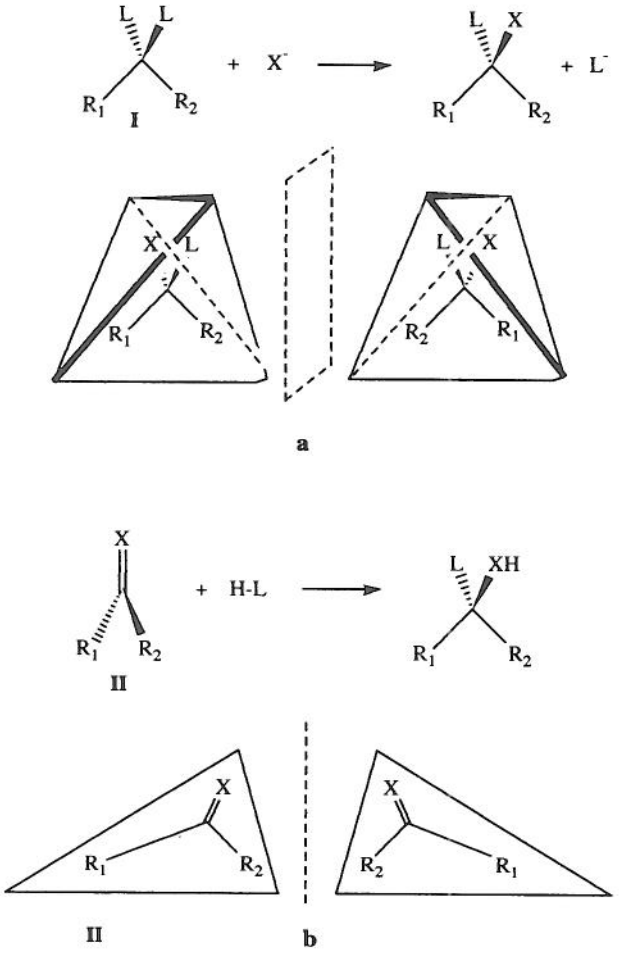


Fig. 1. Schematic presentation of enantioselective reactions on the two types of prochiral molecules; with enantiotopic ligands (1a, **I**), and with enantiotopic faces (1b, **II**)

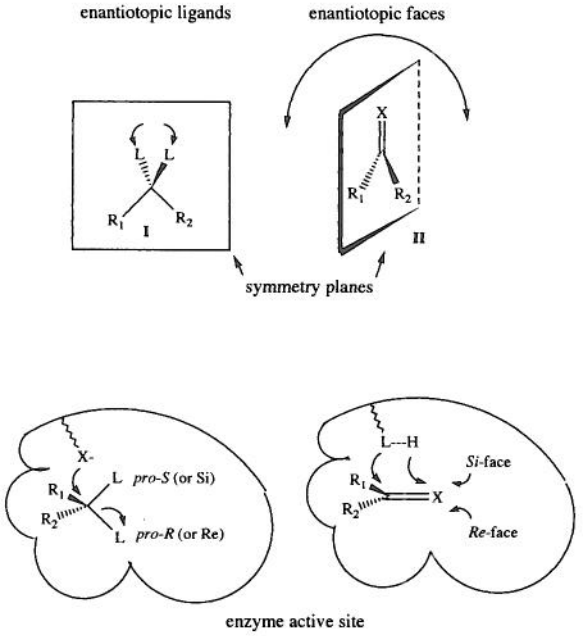


Fig. 2. Enantiotopic groups and faces, their recognition on the enzyme active site

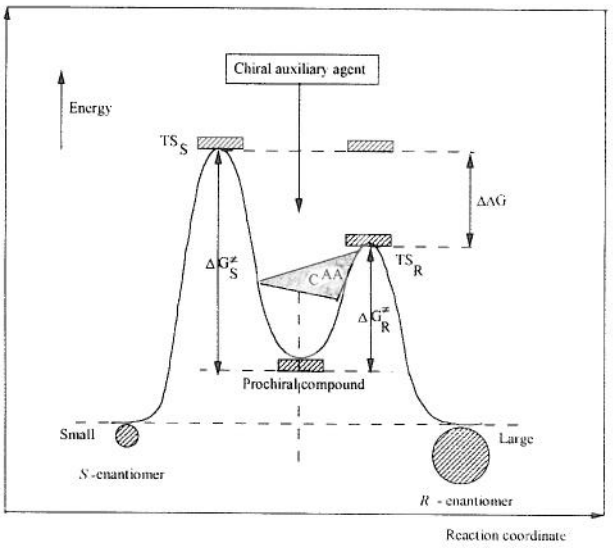


Fig. 3. Schematic presentation of the energy barriers on the reaction pathways leading prevalently to (*R*) over (*S*) enantiomer

chiral substrate and transition states leading to each enantiomer ($\Delta G_R^{\ominus}$ and $\Delta G_S^{\ominus}$), is the direct consequence of the stabilizing interactions along the reaction coordinate, exerted by C^{AA} on the enantiotopic units of the substrate. This interaction is represented by the dashed irregular triangle, and is larger on the pathway to (*R*) then to (*S*) enantiomer. It is important to note that both enatiomers, the products, are at the same energy level and »large« quantity of (*R*) enantiomer is formed exclusively under kinetic control, because of the lower energy of its transition state complex (TS_R) as compared to those (TS_S) leading to »small« quantity of (*S*) enantiomer. Furthermore, it is crucial that *two transition states* are in diastereomeric, *i.e.* in *non-mirror image relation*. In other words, schematic presentation in the Fig. 3 indicates by the symmetry plane (vertical dashed line in the middle) only internal symmetry of the prochiral substrate and mirror image relation of (*R*)- and (*S*)-enantiomer, not of the transition state complexes TS_R and TS_R, however, which comprise energetically unequal interactions with C^{AA}.

As to the quantitative aspect of enantioselective reactions, expressed by the enantiomeric excess (e.e.), it is correlated to $\Delta\Delta G^{\ominus}$ value as presented in the Fig. 3. One would expect that 90–100 % enantioselectivity (e.e.) requires huge difference between the energies of two transition states. On the contrary, as shown in the Fig. 4, this difference is very small.

It suffices to design enantioselective reaction such that $\Delta\Delta G^{\ominus}$ amounts 5.0 kJ mol^{-1} in order to get *ca.* 80 % e.e., whereas $\Delta\Delta G^{\ominus}$ of *ca.* 10 kJ mol^{-1} is enough to get ≥ 98 % e.e. The plot in the Fig. 4 is exponential, and among the scientists that are active in stereoselective chemocatalytic and biocatalytic reactions the energy difference of *ca.* 10 kJ mol^{-1} is commented as a thiny gap between excitation and depression in the laboratory!

By varying the temperatures one is able to effect also stereoselectivity, and in particular the enantioselec-

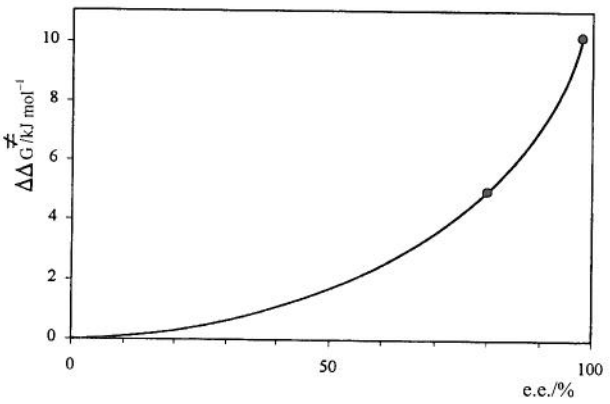


Fig. 4. Correlation between difference in energy of activation of two diastereomeric TS (Fig. 3) and enantiomeric excess (e.e.) for an enantioselective reaction at 25 °C

tivity of any stereoselective reaction. In order to elaborate this statement, some elementary terms that describe stereochemistry of chemical reaction will be considered. Most simple expression is given by the *Eq. 1*, which describes enantiomeric purity of the product of any enantioselective reaction as enantiomeric excess (e.e.). In this equation R and S represent molar ratio of (*R*) and (*S*) enantiomer in the mixture. In order to take into account the degree of conversion, expression in *Eq. 2* should be used, which allows calculation of the stereoselectivity factor as the *E* value; for its solution simple computer programs exist (*7,8*). Under kinetic control, the *E* value is related to the *difference* in the free energy of activation ($\Delta\Delta G^{\ominus}$) of the paths leading to the two enantiomers, *Eq. 3.*

$$\text{e.e./ \%} = \text{R–S/R+S} \cdot 100 \qquad /1/$$

$$E = \ln [1\text{–c } (1 + \text{e.e.}_p)]/\ln [1\text{–c } (1\text{– e.e.}_p)] \qquad /2/$$

$$\Delta\Delta G^{\ominus} = -RT \ln E. \qquad /3/$$

Since $\Delta\Delta G^{\ominus}$ comprises the Eyring activation parameters $\Delta\Delta H^{\ominus}$ and $\Delta\Delta S^{\ominus}$, it can be factored as in the *Eq. 4.* Specifically for the enzymatic reaction, *E* value can be correlated to the k_{cat} and K_m values as in the *Eq. 5.*

$$\Delta\Delta G^{\ominus} = \Delta\Delta H^{\ominus} - T\Delta\Delta S^{\ominus} \qquad /4/$$

$$E = (k_{cat}/K_M)_R/(k_{cat}/K_M)_S \qquad /5/$$

Combining *Eqs. 3* and *5* results in *Eq. 6.*, whereas combining *Eqs. 2* and *4* results in *Eq. 7.*

$$-RT \ln [(k_{cat}/K_M)_R/(k_{cat}/K_M)_S] = \Delta\Delta H^{\ominus} - T\Delta\Delta S^{\ominus} \qquad /6/$$

$$\ln E = \Delta\Delta S^{\ominus}/R - \Delta\Delta H^{\ominus}/RT \qquad /7/$$

The last equation correlates *E* value of an enzyme-catalyzed reaction with general thermodynamic terms of any kinetically controlled reaction, and thus allows their calculation from the experimentally determined *E* value.

It is important to note that for $E = 1$, the term $\Delta\Delta G^{\ominus} = 0$, and consequently the enthalpy and entropy contributions become equal, (*Eq. 8*).

$$\Delta\Delta H^{\ominus} = T\Delta\Delta S^{\ominus} \qquad /8/$$

The temperature at which these two terms become equal for an enzymatic enantioselective reaction is named *racemization temperature, Tr* (*9*).

Enantioselectivity in the Enzyme Catalyzed Reactions

An enzyme catalyzed reaction is traditionally regarded as not convenient for modulation by the temperature, in view of the well known sensitivity of any biological material, in particular the enzyme activity, to the enhancement of temperature. First progress in this direction was made by production of thermostable enzymes and strains of microorganisms, which allow chemical transformations at *higher* temperatures, therefore at higher rates, assuring high productivity of the biocatalytic process in terms of kg product/$m^3 \cdot h$ of reactor. Stereoselectivity usually is not the issue in the high-temperature conducted enzymatic reactions. When enhanced selectivity is needed for successful preparative application of a biocatalytic reaction, *lowering* the temperature is an obvious choice. This possibility was not considered until *the use of enzymes in organic solvents*, lipases and amidases in particular, which emerged as useful methodology in organic synthetic chemistry (*10–12*).

Acceptable optical purity of the commercial products is usually ≥ 99 % e.e., for the intermediates it ranges between 90–95 % e.e.; one crystallization usually affords optically pure material. If this e.e. cannot be achieved at ambient working temperature, or at those were the enzyme has the maximal turnover, lowering the temperature is an opportunity to enhance the enantioselectivity. For the most reactions that run at ambient temperature, to be effected by the temperature the difference should be at least 40–50 °C, *i.e.* the reaction temperatures between –30 and –70 °C should be considered. Obviously, such low temperatures are not available for the enzyme catalyzed reactions in aqueous solutions. Only after introduction of organic solvents as medium for biocatalytic transformations, contrary to the old dogma that organic solvent destroys the enzyme structure, such very low working temperatures became available for biocatalytic transformations.

Temperature-lowering Enhancement of Enantioselectivity in the Enzyme Catalyzed Reactions

Since pharmaceutical and agrochemical industries increasingly use homochiral (stereochemically homogeneous) substances also when biological product has more chiral centers (*13–15*), lipase catalysed resolution of such racemates became ever more important, as *e.g.* reported for the preparation of an enantiomericaly pure fungicide (*16*), for the anticancer agent taxol side-chain (*17*), and for a number of drugs in human therapy (*18*). Some lipase catalyzed enantioselective transformations in organic solvents have even been scaled up to the multi--kilogram and even to ton-production of some biologically active compounds or their commercially important intermediates in the optically pure form (*19,20*). Many examples of industrial relevance for lipase cata-

lyzed acylations, including data for optimized yields, enantioselectivity, and reaction conditions, are given in the recent review by Schmid and Verger (*21*). As already mentioned, the opportunity of using low temperatures in enzymatic reactions was for some time neglected, and only sporadic reports appeared. Ever growing pressure on the above industries for homochiral compounds prompted application of this method in the last few years.

The early papers report both an increase (*22*) and a decrease (*23*) of the selectivity with the lowering of temperature. First systematic investigation of the effect of temperature on the enantioselectivity in the lipase catalyzed reactions, hydrolysis and esterification, was performed in 1990/91 by Holmberg and Hult (*24*). These authors have found that *Eq. 11*, derived from the *Eq. 10*, correlates well with the experimental data for hydrolysis and transesterification at 37 and 6 °C of *rac* 1-phenylethyl butyrate (**1**) to (*S*)-1-phenylethanol (**2**) and **3a**,**b**, Scheme 1.

$$RT_1 \ln E_1 = RT_2 \ln E_2 \qquad /10/$$

$$(E_1)^{T_1} = (E_2)^{T_2} \qquad /11/$$

As can be seen from the *Eq. 10* the temperature is proportional to the logarithm of the *E* value or, as in *Eq. 11*, the *E* value is an exponential function of the temperature. With an *E* value of 10, a decrease of the temperature from 37 to 6 °C increases *E* value to 13, *i.e.* for a factor 1.3.

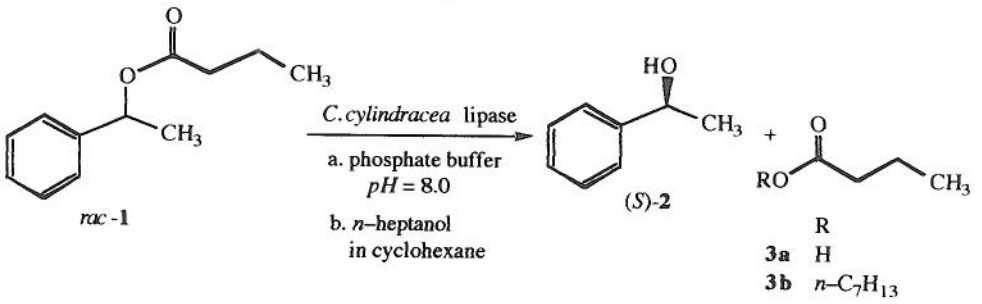
Phillips *et al.* have studied in detail temperature modulated stereoselectivity of the enzyme catalyzed reduction of ketones and oxidation of chiral *sec* alcohols (*25–28*). In some cases, reversal of enantioselectivity was observed; *rac* 2-butanol was oxidized by *Thermoanareorbacter ethanolicus* with (*S*)-2-butanol as a preferred substrate at temperatures below racemic temperature (*Tr*) of 26 °C, and with (*R*)-2-butanol preferred at temperatures greater than 26 °C. By contrast, for higher *sec* alcohols, 2-pentanol and 2-hexanol, the (*S*)-isomer is preferred at all temperatures, with the *E* value decreasing at higher temperatures. This result illustrates nicely the high sensitivity of the interactions at the active site of a lipase to the structural and conformational properties of the substrates.

Usually enhancement of the temperature in the lipase catalyzed reactions enhances the rate of non-stereoselective reactions, such as hydrolysis of triglycerides, the outcome of obvious technological importance. There are some cases, however, where enhancement of the temperature was paralleled by enhancement of enantioselectivity. Enhanced enantioselectivity of the lipase MY (*Candida rugosa*)-catalyzed esterification of 2-phenoxypropionic acids is explained by a general model for high temperature-induced high enantioselectivity (*29*). The optimum temperature of 37 °C was found for the *Candida cylindracea* resolution of menthol in organic media (*30*), the optimum temperature for enantioselective esterification of (*S*)-ibuprofen (**4**), a well-known antiinflammatory agent, in supercritical carbon dioxide by immobilized *Mucor miehei* lipase, (Scheme 2) (*31*), and the optimum temperature for enantioselective esterification of racemic naproxen, the only antiinflammatory agent commercialized as one enantiomer, in its *S*-form (**5**), is determined for *Candida cylindracea* lipase at 65 °C (Scheme 2) (*32*).

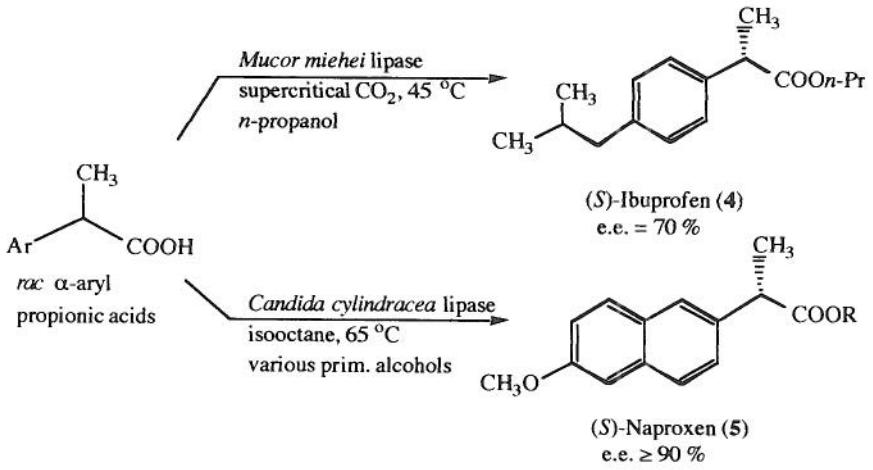
Enantioselectivity of the lipase catalyzed transesterification between tributylglycerol and 2-octanol in bulky organic solvents *increases* with temperature between 10–70 °C, it remains constant in heptane and decreases in the others (*33*). Enantioselective esterificaton of the afore mentioned antiinflammatory agent (*S*)-naproxen (**5**) in the racemic mixture by chemically modified *Candida rugosa* lipase was enhanced at 37 °C, whereas at temperatures higher than 50 °C modification of the enzyme has little effect on its activity and enantioselectivity (*34*). Large improvement of enantioselectivity, up to 5-fold, was reported for lipase catalyzed esterification of a bulky substrate 2-(2-methylphenoxy) propionic acid at 57 °C in organic solvent with a suitable amount of water added (*35*). For a similar substrate, 2-(4-ethylphenoxy)propionic acid, was found that temperature-dependent enantioselectivity depends also on the lipase-type; lipase MY (*Candida rugosa*) shows enhancement of enantioselectivity as the reaction temperature increases whereas the reverse trend of the temperature effect has been observed for lipase AY (*Candida rugosa*) (*36*). *Pseudomonas fluorescens* lipase exhibited temperature dependent enantioselectivity in acylation of butane-1,2,4-triol, which was sensitive also to some other reaction parameters (*37*).

A synthetically interesting reaction, enantioselective hydrolytic ring-opening of epoxides into 1,2-diols is catalyzed by the enzymes epoxide hydrolases (*38,39*). Racemic *para*-nitrostyrene oxide (**6**) is hydrolyzed to (*S*)-diol by epoxide hydrolase from *Aspergillus niger* (Scheme 3). This enzyme was shown to be temperature

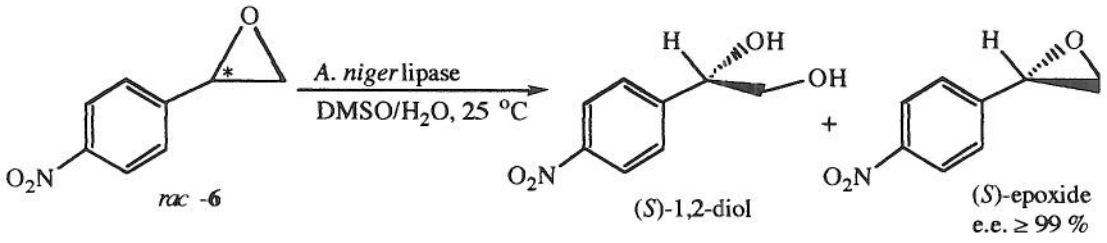
Scheme 1



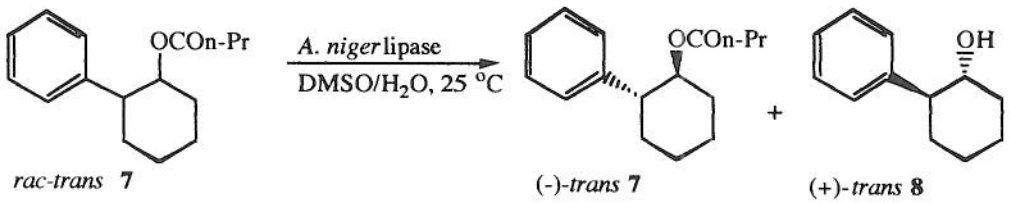
Scheme 2



Scheme 3



Scheme 4



dependent, and optimal temperature has been selected for bioconversion in a batch reactor (*40*).

Another peculiar behavior of the enzyme, depending on the preparation method and temperature, is reported (*41*). The enantioselectivity of liophilized subtilisin in esterification of *sec* phenylethyl alcohols increases when the temperature is rised from 7 to 45 °C; in contrast, the enantioselectivity decreas with temperature for the precipitated enzyme. Similarly, a systematic study of the enzymatic activity of immobilized lipase from *Rhizomucor miehei* (Lipozime IM) in the enantioselective esterification of 2-arylpropionic acids has been carried out (*42*), and effect of the temperature on the yield at constant reaction time is determined.

An instructive example represents hydrolytic resolution of racemic *trans*-1-acetoxy-2-phenylcyclohexanol (**7**), affording (+)-alcohol **8** and (-)-ester **7** (Scheme 4) (*43*).

This reaction was designed to reach the *E* value of > 200 at ambient temperature. The effect of the temperature-increase on the *E* value is presented in Table 1.

The results reveal that for each 10 °C temperature-increase the rate of hydrolysis was increased by only 2 %, but the *E* value decreased for *ca.* 25 %. Similar behaviour has been described for some other systems (*44,45*).

As already emphasized, lowering temperature has positive effect on the kinetic resolution. Thus, a number of *rac* 2-phenoxy-1-propanols was resolved by *Pseudomonas* sp. catalyzed transesterification with vinyl acetate as acyl donor (Scheme 5); resolution was improved with decreasing temperature, and linear correlation between $-RT \ln E$ and reaction temperature was observed for 2-phenoxy-1-propanol (Fig. 5.) (*46*).

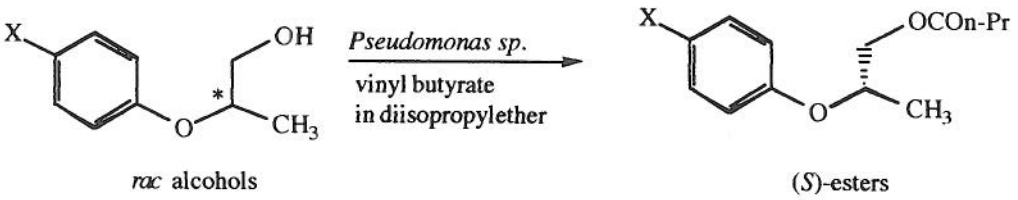
Positive correlation between temperature lowering and enantioselectivity enhancement was demonstrated for the lipase catalyzed aminolysis of some racemic amides (*47*), and for the hydrolysis of racemic methyl esters of antiinflammatory agent ibuprofen (*48*). Positive effect of temperature lowering is also reported for kinetic resolution performed in an enzyme membrane reactor (*49*).

Table 1. Temperature effect on enantioselectivity of the *Candida rugosa* lipase-catalyzed hydrolysis of *rac* **7** (*43*)

Entry	t	Time	Hydrolysis fraction	e.e. (product)	*E*
	°C	h	%	%	
1	15	66	63	98	≥ 200
2	25	52	88	95	~ 150
3	40	48	92	95	~ 125
4	50	50	93	93	~ 90

Sakai *et al.* have recently disclosed a strong and synthetically useful effect of lowering the temperature on enantioselectivity (*50*). They have studied kinetic resolution of racemic 3-phenyl-2H-azirine-2-methanol (**9**), Scheme 6, and observed that lowering the temperature to – 40 °C enhances e.e. of (*R*)-esters from 70 – 80 to 97 %. At the same time e.e. of the remaining alcohol was over 60 %, what resulted in an enhancement of the *E* value from 17 to 99. This result is shown in Fig. 6. as correlation between absolute temperature and ln *E*.

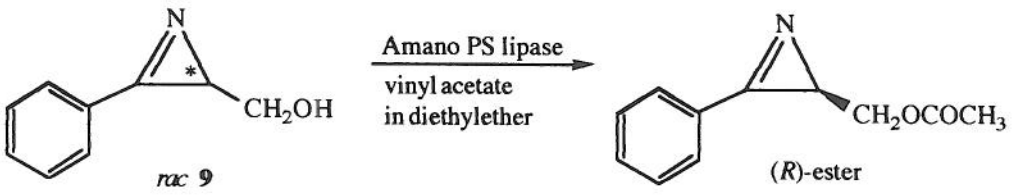
Scheme 5



rac alcohols (*S*)-esters

X	Conversion %	e.e. (*S*) %	*E*
H	51	89	35
F	50	86	34
Cl	50	90	58
Et	53	84	18
i–Pr	50	73	15

Scheme 6



rac **9** (*R*)-ester

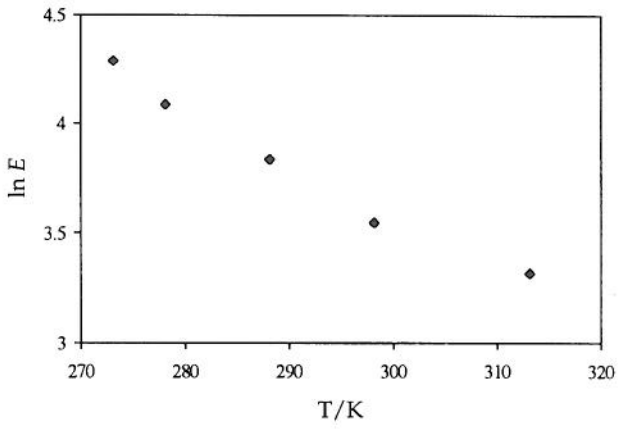


Fig. 5. Influence of temperature on the difference of the activation free energy for the reaction of 2-phenoxy-1-propanol presented in the Scheme 5 (X=H) (*46*)

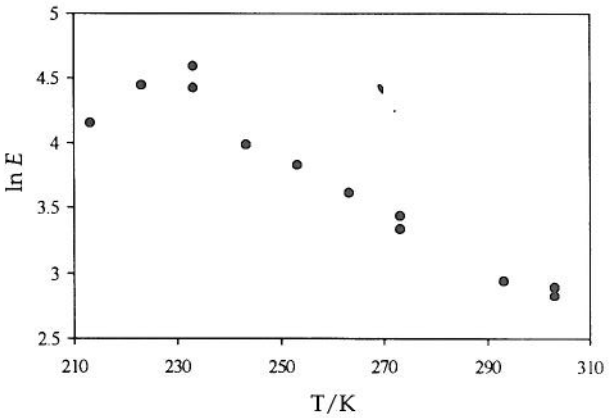


Fig. 6. Temperature effect on the enantioselectivity of acetylation of *rac* **9** according to Scheme 6 (*50*)

The authors made another important observation; when the reaction system was cooled, the reaction rate was decreased as expected, and therefore the amount of the enzyme was increased by *ca.* 40x to keep the reaction time within moderate limits, without significant influence on the *E* value. The results from the Fig. 6. allowed calculation of the activation parameters for this reaction according to the *Eq. 3.*; $\Delta\Delta H^{\ominus} = -12.6$ kJ mol^{-1} and $\Delta\Delta S^{\ominus} = -18.0$ J K^{-1} mol^{-1}.

From such study hypothetical temperature is available where no enantioselection occurs, defined by $E = 1$, and named by Philips as *racemization temperature* (*Tr*) (*9*). Recently we suggested as the more proper term *isoenantioselective temperature* (*51*). It is calculated to 425 K for the reaction in the Scheme 6. *Isoenantioselective temperature* reveals what thermodynamic analysis of the temperature effect on enantioselectivity predicts, *i.e.* that the temperature-dependent inversion of enantioselectivity, named by organic chemist also as enantioselective bias, can occur. It explaines why the optimization of enantioselectivity in an enzyme-catalyzed reaction may require either the raising or lowering of the reaction temperature.

Recently we have studied in detail lipase catalyzed kinetic resolution and desymmetrization of 3-substituted 1,4-benzodiazepin-2-ones **10-14** (Scheme 7) (*51,52*).

Racemic, optically stable compounds **10–13** are enantioselectively acetylated (kineticaly resolved) by Novozym 435, obtained by recombinant DNA technology; the gene coding has been transfered from a selected strain of *Candida antarctica* to the host organism, *Aspergillus oryzae*, and the isolated enzyme immobilized on a macroporous acrylic resin (*53*). The same enzyme has been used for acetylation of prochiral 3,3-bis-hydroxymethyl derivative **14.**

The results of enzymatic acetylation for three racemic substrates **10–12** with one methylene group in the side chain (n=1), for 3-β-hydroxyethyl derivative **13** (n=2) and for prochiral compound **14** are presented in Table 2.

First, one observes much lower efficacy of kinetic resolution for *rac* alcohol **13**, where the hydroxy group is distant (n=2) from the perturbing groups on the chiral center. Then, it is important to note that *M* and *P* conformers are diastereomorphic for C(3) chiral substrates, whereas for prochiral substrates they are enantiomorphic (*54,55*). This stereorelation is given in the Scheme 8. In the more stable *P*-conformation larger acetoxymethyl group occupies *pseudoequatorial* (ψ*e*) position, whereas in the less stable *M*-conformer it occupies *pseudoaxial* (ψ*a*) position, and falls into the anisotropy cone of the annelated aromatic ring.

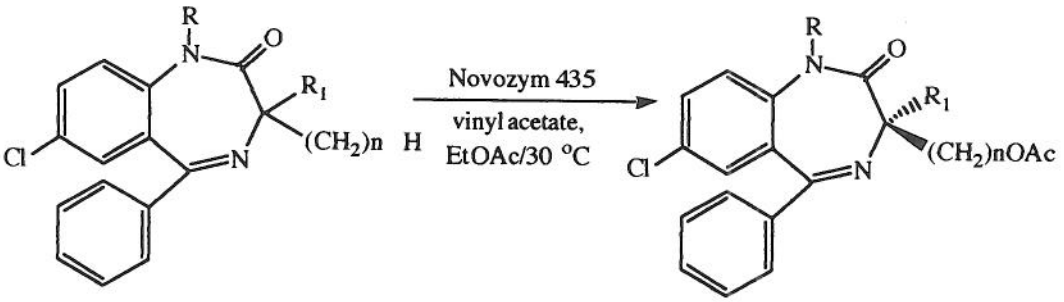
Table 2. Novozym 435 catalyzed acetylation of 3-hydroxymethyl-1,4-benzodiazepin-2-ones **10–14** (*51*)

Compound	t / °C	e.e. (ester) / %	e.e. (alcohol) / %	*E*
10	30	69.1	83.5	14.0
11	30	70.4	81.8	14.3
12	30	24.5	33.8	2.2
13	30	10.2	12.2	1.4
14	30	90.2	–	–

In view of the conformational mobility of both prochiral compound **14**, and high conformational stability of their chiral congeners **10–13**, enantioselectivity of acetylation was investigated at various temperatures for two representatives, **11** and **14**. The results of temperature dependent acetylation of *rac* **11** are presented in Table 3, and in Table 4 analogous data are collected for prochiral congener **14**.

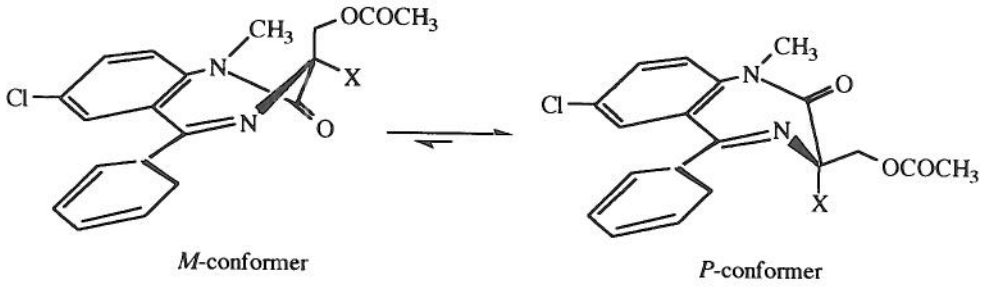
In the temperature interval from 40 to –15 °C enantioselectivity for *rac* **11**, expressed as *E*-value according to Sih *et al.* (*7*), enhances *ca.* 5 times. Since calculation of *E*-value for prochiral **14** would require exact data for initial rates of four different steps that include mono- and diacetylation (*8*), we express the enhanced enantioselectivity by e.e. at *ca.* 70–75 % conversion. As seen in Table 4, the e.e. enhances from *ca.* 70 % at 60 °C to 90 % at 0 °C.

Scheme 7



	R	R_1	n
10	H	H	1
11	CH_3	H	1
12	H	CH_3	1
13	H	H	2
14	CH_3	CH_2OH	1

Scheme 8



M-conformer *P*-conformer

X= H, CH_3, CH_2OH

Table 3. Temperature-dependent acetylation of racemic **11** (*51*)

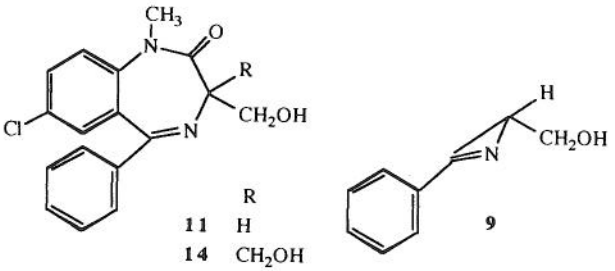
Entry	m (lipase)	e.e. (R-(-)-9)	e.e. (S-(+)-acetate)	Time	t	Conversion	*E*
	mg	%	%	min	°C	%	
1	40	58.1	58.8	35	40	49.7	6.80
2	40	64.3	68.5	50	30	48.4	10.2
3	40	76.6	67.4	60	20	53.2	11.6
4	40	51.0	81.5	90	10	38.5	16.2
5	80	67.5	83.8	135	0	44.6	22.8
6	80	89.7	80.2	195	–10	52.8	27.4
7	80	83.0	85.7	195	–15	49.2	33.6

Table 4. Temperature-dependent acetylation of prochiral **14** (*51*)

Entry	m (lipase)	Time	t	Conversion	e.e.
	mg	h	°C	%	%
1	1800	8.0	0	70.2	90.2
2	1800	6.0	10	74.7	87.7
3	900	7.0	20	73.8	85.2
4	900	5.0	30	74.8	80.6
5	900	3.0	40	77.2	77.2
6	450	4.5	50	74.5	73.5
7	450	3.0	60	73.6	68.6

Further insight into the origin of enantioselection for **11** and **14** offer thermodynamic parameters, $\Delta\Delta H^{\ominus}$ and $\Delta\Delta S^{\ominus}$, as well as racemic temperature *Tr*, Fig. 7. For the former substrate $\Delta\Delta H^{\ominus}$ =–18.7 kJ mol^{-1}, and $\Delta\Delta S^{\ominus}$ =–43.2 J K^{-1} mol^{-1} can be calculated. Comparing these data with those obtained for conformationally rigid racemic **9** (*50*), whose structure can be regarded as »truncated 1,4-benzodiazepin-2-one« **11**, one can observe large lowering of *Tr* to 160 °C, Table 5. Since the enthalpy therm $\Delta\Delta H^{\ominus}$ is more important for enantioselection, compared to the enthropy therm $\Delta\Delta S^{\ominus}$, even for more flexible **11**, correlation between enantioselection and temperature-lowering was observed. As presented in Table 5 for **11** and **14** there is large enhancement of the enthropic factor for **11** that lowers *Tr* .

Within our project of biocatalytic preparation of 2-ethylhexanol of extremely high optical purity (e.e. ≥ 99.8 %) for application in electroluminescent materials (*56*), preliminary attempts to enhance e.e. of acetylation of *rac* 2-ethylhexanol by vinylacetate catalyzed by various lipases, lowering the temperature to –30 °C, resulted with huge diminishing of the rate of acetylation and with only modest enhancement of e.e. (*57*).



R

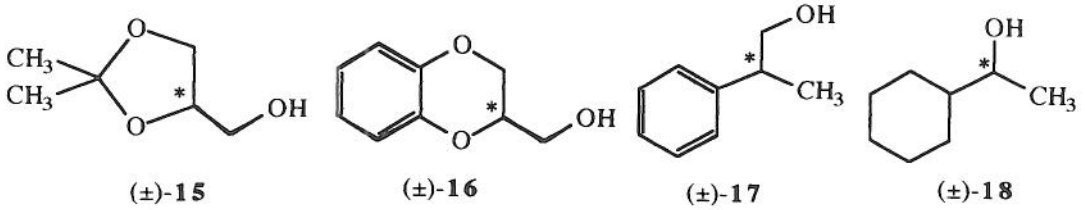
11 H

14 CH_2OH

9

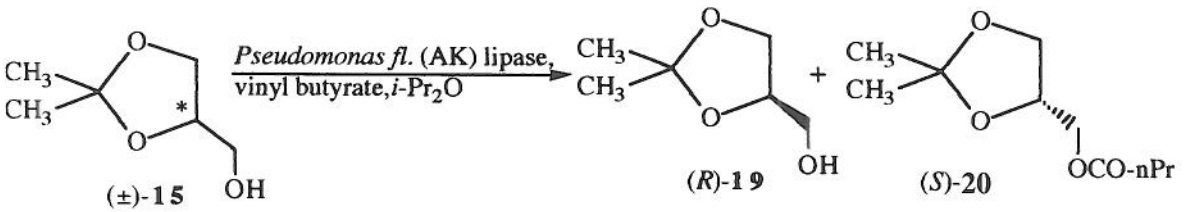
Table 5. Some thermodynamic parameters for the enantioselective acetylation of **9**, **11** and **14** (*51*)

Compound	E	$\Delta\Delta H^{\ominus}$	$\Delta\Delta S^{\ominus}$	*Tr*	Ref.
		kJ mol^{-1}	kJ K^{-1} mol^{-1}	°C	
9	86.0	–12.6	–18.0	425	*50*
11	33.6	–18.8	–43.1	160	*51*
14	–	–	–	352	*51*



(±)-**15** (±)-**16** (±)-**17** (±)-**18**

Scheme 9



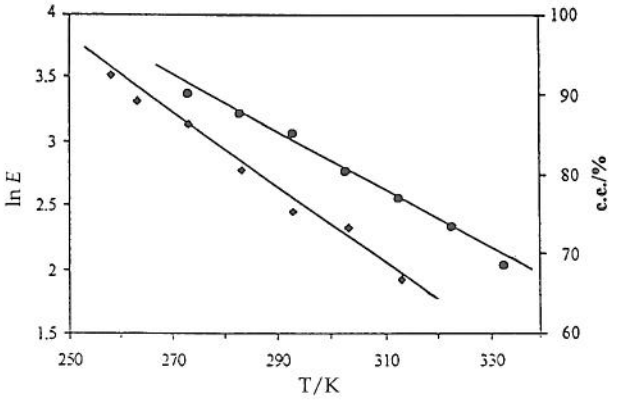


Fig. 7. Temperature dependence of the e.e. values (•-•-•) of **11**, and ln *E* values (◆-◆-◆) of **14**, for their acetylation by Novozym 435 (*51*)

Very recently Sakai *et al.* have reported continuation of the studies on the low-temperature method for enhancement of enantioselectivity, with *rac* solketal (**15**) and some other racemic chiral alcohols (*58*). They have proved that low-temperature method is widely applicable to primary and secondary alcohols, formulae **15–18**. As a typical example, the low-temperature method was applied to *rac* **15**, which is known as a usefull synthetic intermediate (C_3-synthon). It resulted with encrease of *E* value up to 55 by lowering the temperature to –40 °C in diisopropylether (Scheme 9 and Table 6).

Table 6. Temperature effect in the *Pseudomonas fluorescens* lipase-catalyzed kinetic resolution of Solketal (**15**) (*58*).

Entry	t / °C	m (lipase) / mg	Time / h	e.e. (ester) / %	e.e. (alcohol) / %	*E*
1	30	20	3	63	69	9
2	0	20	6	88	25	20
3	–20	60	11	92	32	26
4	–40	200	24	93	63	55
5	–60	200	48	93	51	44

Similar reactions using racemic alcohols **15–18** and selected lipases were also proved to obey *Eq. 7*, and some results are presented in the Table 7.

The authors concluded that thermodynamic parameters indicate activation enthalpy differences ($\Delta\Delta H^{\ominus}$) that govern the enantioselectivity, and reveal its origin in the difference of steric interactions between two enantiomers in the transition state.

Table 7. Thermodynamic parameters for the lipase-catalyzed resolutions of **15–18** (*i*–Pr_2O, vinyl acetate) (*58*)

Compound	Lipase	$\Delta\Delta H^{\ominus}$ / kJ mol^{-1}	$\Delta\Delta S^{\ominus}$ / J K^{-1} mol^{-1}	*Tr* / °C
15	AK	–14.8 ± 0.1	–30.0 ± 3.3	219
16	AK	–9.4 ± 0.5	–5.6 ± 1.7	1386
17	AK	–3.1 ± 0.2	–6.3 ± 0.8	217
18	PS	–15.6 ± 1.0	–12.6 ± 3.8	962

Concluding Remarks

In the recent asymmetric syntheses with artificial catalysts, the temperature effect has been revealed as an attractive method for fine-tuning of the enantioselectivity (*59*). As this review suggests, this method seems even more appropriate for natural biocatalysts, isolated or genetically modified enzymes (*60*). It can be expected that the latter can be genetically engeneered such as to gain high efficacy expressed as a turnover number, which will remain unchanged at low to very low temperatures, enabling such enzymes to act highly enantioselectivly even with substrates which are presently elusive to successful biocatalytic transformations.

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Nova biokatalitička metodologija: niskom temperaturom povećana enantioselektivnost lipazama kataliziranih reakcija u organskim otapalima

Sažetak

Nakon nedavnih izvještaja iz 1997./98. godine, niskotemperaturno povećanje enantioselektivnosti lipazama kataliziranih acilacija u organskim otapalima pojavljuje se kao nova biokatalitička metoda u laboratorijskom mjerilu. Dan je pregled primjera dobivanja enantiomerno čistih spojeva (engl. enantiomerically pure compounds, EPC) pri niskim temperaturama, bilo kinetičkom rezolucijom racemata, bilo desimetrizacijom prokiralnih supstrata.