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Lipase-mediated chiral resolution of racemates in organic solvents

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Dedicated to Professor Volker Schurig on the occasion of his 65th birthday

Abstract—Lipase-catalyzed kinetic resolution of racemates is considered to be one of the most fascinating topics in asymmetric catalysis. This review focuses on some of the recent developments in this rapidly growing field demonstrating the versatility of the method in the resolution of racemates. The literature search is dated back to the last five years and covers some comprehensive examples. The main emphasis is on the use of lipases in organic solvents.

Contents

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1. Introduction

1.1. Enzymes in non-aqueous media: general aspects

Enzymatic catalysis in organic solvents significantly broadens conventional aqueous-based biocatalysis.[1–18] Water is a poor solvent for nearly all applications in industrial chemistry since most organic compounds of commercial interest are very sparingly soluble and are sometimes unstable in aqueous solutions. Furthermore, the removal of water is tedious and expensive due to its high boiling point and high heat of vaporization. In contrast, biocatalysis in organic solvents offer several advantages. Among these advantages are (i) the use of low boiling point organic solvents, which facilitates the recovery of the product with better overall yield, (ii) non-polar substrates are converted at a faster rate due to their increased solubility in the organic solvent,[15] (iii) deactivation and/or substrate or product inhibition is minimized, (iv) side reactions are largely suppressed, (v) immobilization of enzymes is not required, (vi) denaturation of enzymes (loss of the native structure and thus catalytic activity) is minimized and (vii) shifting thermodynamic equilibria to favor synthesis over hydrolysis.

1.2. Lipase-catalyzed reactions in organic synthesis

Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) have been well established as a valuable catalysts in organic synthesis.[12] They are usually distinguished from carboxyl esterases (EC 3.1.1.1) by their substrate spectra, that is, esterases prefer water soluble substrates and lipases show a significantly higher activity towards their natural substrates, triglycerides. Since the hydrolytic reaction is reversible in non-aqueous systems, these biocatalysts can also catalyze the formation of esters from acyl donors and alcohols.

Lipases do not require cofactors. A range of enzymes are commercially available in free and immobilized form. Most lipases accept a broad range of non-natural substrates and are thus very versatile for applications in organic synthesis. In many cases, they exhibit good to excellent stereoselectivity.

Lipases have been widely used in three main types of reactions yielding enantiomerically pure compounds. These are kinetic resolutions of racemic carboxylic acids or alcohols, enantioselective group differentiations of *meso* dicarboxylic acids or *meso* diols and enantiotopic group differentiation of prochiral dicarboxylic acid and diol derivatives.[3]

In kinetic resolutions of racemic alcohols via transesterification, the acyl donors of choice are enol esters such as vinyl acetate or isopropenyl acetate. The vinyl alcohol formed as a by-product when using vinyl acetate, undergoes keto–enol tautomerization to yield the corresponding carbonyl compound (acetaldehyde), while the isopropenyl alcohol, released when using isopropenyl acetate, tautomerizes to acetone thus making the reaction practically irreversible in both cases. Thus, these transesterifications are much faster compared to reactions using free carboxylic acids or simple esters such as ethyl acetate.

In contrast to asymmetric synthesis, a kinetic resolution yields at maximum 50% of the desired enantiomer.[19] To achieve higher yields, the non-wanted enantiomer can be separated and re-racemized in a second step. Alternatively, this can be achieved by a dynamic kinetic resolution (DKR).[20] Several methods have already been described,[21] and are reviewed below.

2. Routes to enantiomerically pure compounds

The methods used to access enantiomeric compounds can be divided into three categories depending on the type of starting material used[22] (Fig. 1).

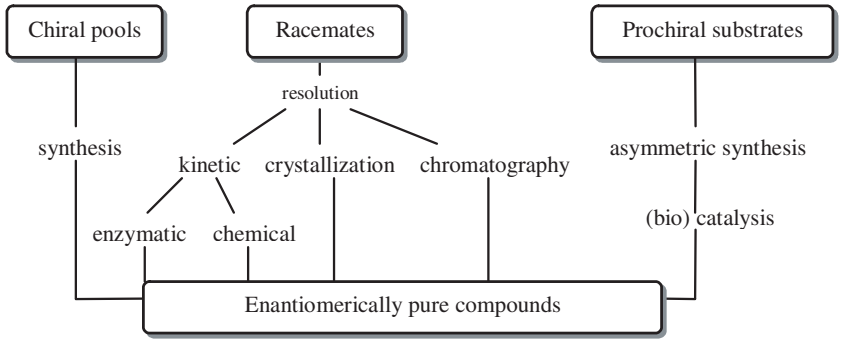
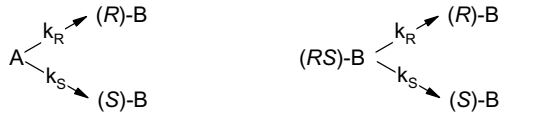


Figure 1. Methods to obtain enantiomerically pure compounds.

2.1. Stereoselective synthesis

Two methods are used for the preparation of enantiomers using enzymes:[23,24] (a) Stereoselective synthesis and (b) the resolution of the racemate.

Stereoselective synthesis is not covered in this article, however, a schematic representation of the stereoselective synthesis versus resolution of racemates is shown in Figure 2. The resolution of racemates is discussed below.



(a) Stereoselective synthesis (b) Resolution of the racemate

Figure 2. Stereoselective synthesis versus resolution of a racemate.

2.2. Resolution of racemates

Despite the impressive new progress in asymmetric synthesis, the dominant production method to obtain a single enantiomer in industrial synthesis consists of the resolution of racemates.[25–29] The resolution of enantiomers can be divided into four categories consisting of (i) direct preferential crystallization, (ii) crystallization of diastereomeric salts, (iii) chromatography and (iv) kinetic resolution. These methods are briefly discussed below with a particular focus on the kinetic resolution of racemates as a versatile method for the separation of enantiomers.

2.2.1. Preferential crystallization. (Also referred to as resolution by entrainment) is widely used on an industrial scale, for example, in the manufacture of chloramphenicol[30] and α-methyl-L-dopa.[31]

Haarmann and Reimer, the market leader in synthetic (−)-menthol, utilizes the preferential crystallization of menthyl benzoate enantiomers. This can be induced by seeding the bulk with one of the pure enantiomers and is used in the production of (−)-menthol.[32]

This process is technically feasible only with racemates that form conglomerates (ones that consist of mechanical mixtures of crystals of the two enantiomers in equal amounts). Unfortunately, less than 20% of all racemates are conglomerates, the rest comprising of true racemic compounds that cannot be separated by preferential crystallization. The success of the preferential crystallization is dependent on the fact that for a conglomerate, the racemic mixture is more soluble than either of the enantiomers.[22]

2.2.2. Diastereomer crystallization. If the racemate is a true racemic mixture, then it cannot be separated by preferential crystallization, but can be resolved using the diastereomer crystallization developed by Pasteur in 1848. A solution of the racemic mixture in water or methanol is allowed to react with a pure enantiomer (resolving agent), thereby forming a mixture of diastereomers that can be separated by crystallization.

2.2.3. Kinetic resolution catalyzed by lipases. The third method used in the resolution of racemates is the kinetic resolution. The success of this method is dependent on the fact that the two enantiomers react at different rates with a chiral entity. The chiral entity should be present in catalytic amounts; it may be a biocatalyst (enzyme or a microorganism) or a chemocatalyst (chiral acid or base or even a chiral metal complex). Kinetic resolution of racemic compounds is by far the most common transformation catalyzed by lipases, in which, the enzyme discriminate between the two enantiomers of racemic mixture, so that one enantiomer is readily transferred to the product faster than the other[1–18] (Fig. 3).

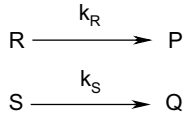


Figure 3. Catalytic kinetic resolution.

The kinetic resolution occurs when $k_R \neq k_S$ and the reaction is stopped somewhere between 0% and 100% conversion. Ideally one enantiomer reacts much faster than the other, for example, if the reactant (*R*) is the only reacting enantiomer ($k_S = 0$). In this case, (and at 50% conversion of the initial 50:50 mixture) it leads to a final mixture of 50% reactant (*S*) and 50% product (P). This route has the advantage of easy separation of both enantiomers by using a single enzyme.

2.2.4. Dynamic kinetic resolution. Such a conventional kinetic resolution as reported above often provides an effective route to access to the enantiomerically pure/enriched compounds. However, the limitation of such a process is that the resolution of two enantiomers will provide a maximum 50% yield of the enantiomerically pure materials. Such a limitation can be overcome in several ways. Among these ways are (i) the use of *meso* compounds or prochiral substrates,[33] (ii) inversion of the stereochemistry (stereoinversion) of the unwanted enantiomer (the remaining unreacted substrate),[34] (iii) the racemization and recycling of the unwanted enantiomer and (iv) dynamic kinetic resolution (DKR)[21] (Fig. 4).

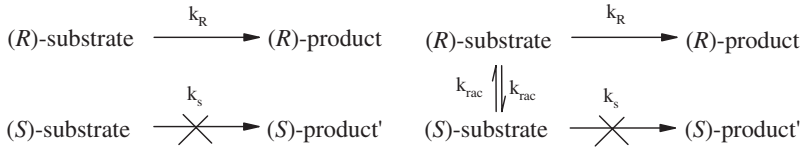


Figure 4. (a) Conventional kinetic resolution (max. 50% conv.), (b) dynamic kinetic resolution with theoretical 100% yield.

In conventional and dynamic kinetic resolution, the (*R*)-enantiomer substrate is transformed to the (*R*)-product faster than the (*S*)-enantiomer substrate ($k_R > k_S$) (Fig. 5).

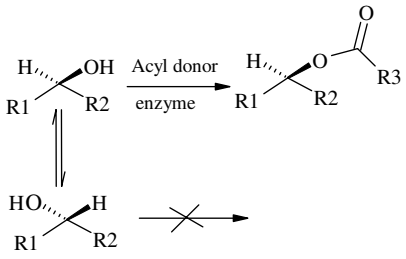


Figure 5. General strategy for the concept of dynamic kinetic resolution (DKR).

The only difference is that in conventional kinetic resolution the (*S*)-enantiomer substrate is left behind as an unreacted starting material while in the case of dynamic kinetic resolution, the substrate is continuously isomerized during the resolution process, thus the (*R*)- and (*S*)-substrates are in equilibrium, which allows for the possibility of converting all of the starting materials of the (*R*)-substrate into the (*R*)-product. Several conditions should be applied and are reviewed in literature.[21] For instance, Bäckvall and co-workers [20] developed an efficient system for the DKR based on the use of *p*-chlorophenyl acetate as an acyl donor and the robust ruthenium catalyst for the racemization. This methodology can be applied to a set of secondary alcohols. Depending on the substrate, the chemical yield ranges from 60% to 88% with more than 99% ee[20,35] (Fig. 6).

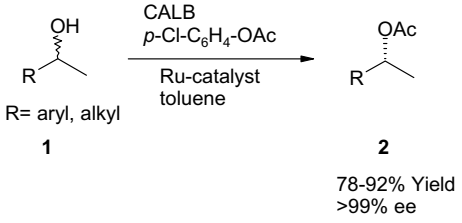


Figure 6. DKR of secondary alcohols using *p*-chlorophenyl acetate.

The innocuous and commercially available isopropenyl acetate can replace the acyl donor *p*-chlorophenyl acetate. However, an appropriate hydrogen source is required to prevent the drop in yield due to ketone formation (Fig. 7).

The combination of ruthenium and enzyme catalysis was also successfully applied to the DKR of diols. Thus, by using a ruthenium catalyst, immobilized lipase CALB and *p*-chlorophenyl acetate, as the acyl donor, in toluene afforded the diacetate in good yield and high enantioselectivity >99% (Fig. 8). However, for the 1,3- and 1,4-diols, low to moderate diastereoselectivity was observed.

Racemic α-hydroxy esters were also subjected to the chemoenzymatic DKR methodology. Thus, the trans-

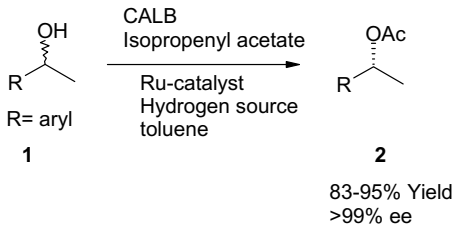


Figure 7. DKR of secondary alcohols using isopropenyl acetate and a hydrogen source.[20b]

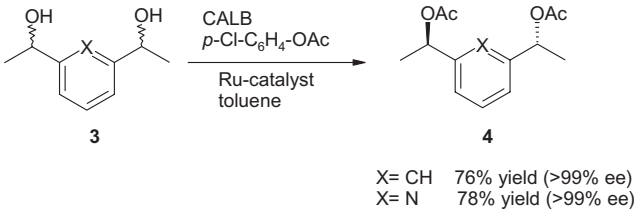


Figure 8. Example of DKR of diols.

esterification of α-hydroxy esters with *p*-chlorophenyl acetate in cyclohexane using immobilized PS-C and ruthenium catalyst afforded the acetate with 60–80% yield and 30–98% ee (Fig. 9).

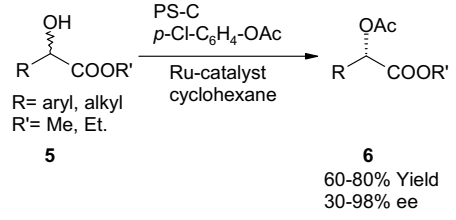


Figure 9. DKR of racemic α-hydroxy esters.

Other substrates have been subjected to DKR and are well documented[20] (Fig. 10).

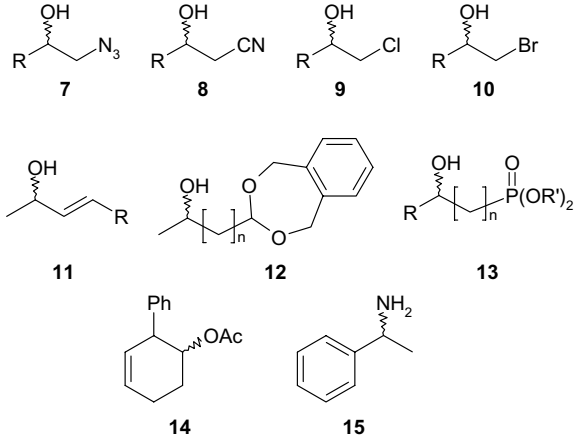


Figure 10. Some examples of substrates subjected to DKR.[20]

3. Enantioselectivity of lipases in organic solvents

Two important concepts should be understood in enzyme-catalyzed reactions; the enantiomeric excess (ee) and the enantiomeric ratio E.

The enantiomeric purity of any compound can be expressed in terms of its enantiomeric excess (ee) value defined as:

$$\%\ \mathrm{ee}_R = \frac{R-S}{R+S} \times 100 \quad \text{For } R > S \tag{1}$$

where R is the concentration of the (R)-enantiomer and S is the concentration of the (S)-enantiomer. Thus, for a racemic compound, the ee value is zero where as for an enantiomerically pure compound the ee value is 1 (or 100% ee).

Since lipases are chiral and possess the ability to distinguish between the two enantiomers of a racemic mixture. The parameter of choice to describe the stereoselectivity or the enantioselectivity of lipase-catalyzed reactions is the enantioselectivity, which is also called the enantiomeric ratio E. The E-value is defined as the ratio of the specificity constant for the two enantiomers.

$$E_{RS} = \frac{(k_{\mathrm{cat}}/k_{\mathrm{M}})_R}{(k_{\mathrm{cat}}/k_{\mathrm{M}})_S} \tag{2}$$

where k_{cat} is the rate constant or the turnover number and k_{M} is the Michaelis–Menten constant. Sih and coworkers[36,37] developed this equation in terms of the enantiomeric excess of the product ($\mathrm{ee_p}$), the unreacted substrate ($\mathrm{ee_s}$), and the conversion (c). Thus, for a reversible enzymatic reaction, the E value can be expressed by the following equation:

$$E = \frac{\mathrm{Ln}[1-(1+K)c(1+\mathrm{ee_p})]}{\mathrm{Ln}[1-(1+K)c(1-\mathrm{ee_p})]} = \frac{\mathrm{Ln}[1-(1+K)(c+\mathrm{ee_s}\{1-c\})]}{\mathrm{Ln}[1-(1+K)(c-\mathrm{ee_s}\{1-c\})]} \tag{3}$$

where K is the equilibrium constant. When the reaction is irreversible or the reverse reaction is negligible ($K = 0$), this equation can be reduced to the following:

$$E = \frac{\mathrm{Ln}[1-c(1+\mathrm{ee_p})]}{\mathrm{Ln}[1-c(1-\mathrm{ee_p})]} = \frac{\mathrm{Ln}[(1-c)(1-\mathrm{ee_s})]}{\mathrm{Ln}[(1-c)(1+\mathrm{ee_s})]} \tag{4}$$

where (c) is expressed by the following equation:

$$c = \frac{\mathrm{ee_s}}{\mathrm{ee_s}+\mathrm{ee_p}} \tag{5}$$

E can also be expressed in terms of the $\mathrm{ee_s}$ and $\mathrm{ee_p}$ only by the following equation:[12]

$$E = \frac{\mathrm{Ln}\left[\frac{1-\mathrm{ee_s}}{1+(\mathrm{ee_s}/\mathrm{ee_p})}\right]}{\mathrm{Ln}\left[\frac{1+\mathrm{ee_s}}{1+(\mathrm{ee_s}/\mathrm{ee_p})}\right]} \tag{6}$$

Thus, to calculate the E value, one can measure two of the three variables: $\mathrm{ee_s}$, $\mathrm{ee_p}$ and the extent of conversion (c). A non-selective reaction has an E-value of 1, while an E-value above 20 is the minimum for an acceptable resolution.[12]

3.1. Chiral recognition by lipases

An enzyme model always describes the mechanism of the enantioselectivity in an enzymatic reaction. The simplest models, more accurately referred to as rules, do not attempt to predict the degree of enantioselectivity, but only predict which enantiomer reacts faster. The earliest example of such a model is Prelog's rule,[38] which predicts the enantioselectivity of the reduction of ketones by yeast alcohol dehydrogenases based on the size of the two substituents on the carbonyl group. Other models are based on pockets, which give an indication of the size and shape of the molecules tolerated in the active site. Examples of such models are the model of Jones for pig liver esterase (PLE),[39] Subtilisin[40] and several lipases.[41–43] One example is the empirical rule of Kazlauskas for chiral recognition by lipases.[44] This rule predicts, as exemplified for lipase from *Pseudomonas cepacia*, enantiopreference towards a certain substrate, but cannot predict the degree of enantioselectivity. It is translated into an active site model for lipases consisting of two pockets of different size, a large one and a small one (Fig. 11).

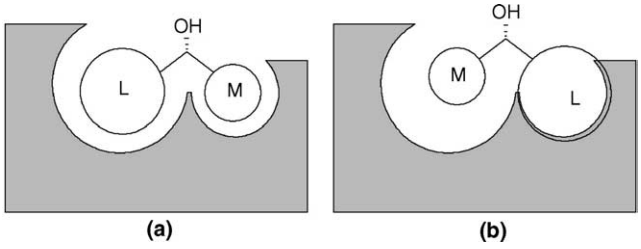


Figure 11. The fast reacting enantiomer (a) and the slow reacting one (b) in the active side model for lipases derived from Kazlauskas' rule.

The stereoselectivity for substrates bearing a small and a large substituent (e.g., a secondary alcohol as shown in Fig. 11) is explained by assuming that when the secondary alcohol is subjected to resolution by a lipase, the fast reacting enantiomer binds to the active side in the manner shown in Figure 11a. However, when the other enantiomer reacts with the lipase, it is forced to accommodate its large substituent into the smallest pocket (Fig. 11b). This rule works well for secondary alcohols. However, for primary alcohols, the rule is only applicable if an oxygen atom is attached to the stereocentre. A similar rule was also proposed for the resolution of carboxylic acids.

Additionally, a range of lipase structures have been solved by X-ray crystallography or are available by homology modelling. This information together with sequence data in public databases (e.g., www.led.uni-stuttgart.de) allows further insights into the structure–function relationships of lipases. Furthermore, rational protein design allowed the alteration of the enantioselectivity of lipases. Thus, the directed evolution

method combining random mutagenesis and high-throughput screening has been used as a versatile tool for tuning or engineering the enantioselectivity of lipases. In this particular point of research, Reetz et al. reported that the combination of error-prone PCR and DNA shuffling gave a lipase variant of *P. aeruginosa* having completely inverted enantioselectivity.[45,46] Recently, Koga et al. reported the inversion of the enantioselectivity of another thermostable lipase from *Burkhorderia cepacia* KWI-56 using a novel in vitro technique for construction and screening of a protein library by single-molecule DNA amplification by PCR followed by in vitro coupled transcription/translation system termed single-molecule-PCR-linked in vitro expression (SIMPLEX).[47]

4. Analytical methods: determination of the enantiomeric excesses (ee)

The development of accurate methods for the determination of enantiomeric purity, which began in the late 1960s, has been critical in the assessment of enantioselective synthesis. Thus a prerequisite in the enzyme-catalyzed kinetic resolution of racemates is a precise and reliable assessment of the degree of enantioselectivity (E), enantiomeric excess (ee) and conversion (c). Among these methods are: (1) polarimetric methods, (2) gas chromatographic methods, (3) liquid chromatographic methods and (4) NMR spectroscopy. The most convenient and sensitive methods used are chiral GC and HPLC.

4.1. Gas chromatographic methods

An attractive method for the determination of the enantiomeric excess of substrates and products resulting from the enzyme-catalyzed kinetic resolution of secondary alcohols is chiral gas chromatography (GC).[48,49] This sensitive method is quick, simple to carry out and unaffected by the presence of impurities in the analyzed sample. Therefore, isolation and purification of the analyzed sample is not required. A very small sample size is required for the analysis, hence, reactions can be done on small scale.

This method is based on the fact that molecular association may lead to an efficient chiral recognition leading to enantiomeric separation when a chiral stationary phase (e.g., cyclodextrins) is used in GC. The gas (mobile phase, e.g., hydrogen, helium, nitrogen) carries the chiral analyte through the stationary phase. The enantiomers to be analyzed undergo rapid and reversible diastereomeric interactions with the chiral stationary phase and hence may be eluted at different times. One of the limitations associated with this method is that the sample should be sufficiently volatile, thermally stable and resolvable on the chiral stationary phase used. The measurement of the enantiomeric excess using GC is linked with a high degree of precision (±0.05%) so that reliable data may be obtained.[50] It is noteworthy that high enantiomeric excesses (ee) up to 99% can be detected.[51–58]

4.2. HPLC methods

HPLC methods follow the same principles and advantages as GC-analysis. The major difference is that more polar and also non-volatile compounds can be analyzed.

5. Practical applications of lipases in the resolution of racemates

Resolution of racemates via lipase-catalyzed kinetic resolution is one of the most attractive methods used to access to enantiomerically pure compounds. Of all the methods used in kinetic resolution, transesterification in organic solvents catalyzed by lipase is the most dominant one. Thus, in the presence of a suitable acyl donor, an enzyme as well as the appropriate solvent, and at the optimum temperature, one enantiomer of the racemic mixture is selectively transferred to the corresponding ester leaving the second unreacted enantiomer in enantiomerically pure form.[51–58] If a good leaving group is present on the acyl donor, as is the case of trichloroethyl or trifluoroethyl esters (Fig. 12a), the reaction of the halogenated alcohol with the formed ester (the backward reaction) is minimized allowing the shift of the equilibrium to the product formation. Oxime esters have been proposed as acyl transfer agents (Fig. 12b) for irreversible process; however, this approach is limited due to some disadvantages incorporated to cosubstrate inhibition and reversibility of the reaction. The best method for the irreversible transesterification procedure is achieved when using enol esters (Fig. 12c) where the back reaction is suppressed due to the tautomerization of the resulting enol alcohol (to acetaldehyde or acetone depending on whether a vinyl or an isopropenyl ester serve as acyl donors), thereby shifting the equilibrium to the required product. However, acetaldehyde may have some detrimental effects on some enzymes.[8] Isopropenyl acetate was proposed as an innocuous and more suitable acyl donor in lipase-catalyzed irreversible transesterification in organic solvents (Fig. 12d). The use of different reagents in irreversible acylation catalyzed by lipase has been recently reviewed.[59]

5.1. Kinetic resolution of primary alcohols

Homochiral primary alcohols are useful building blocks for the synthesis of a wide range of biologically active compounds. While lipase-catalyzed enantioselective access to enantiomerically pure secondary alcohols are very efficient tools in organic synthesis, the kinetic resolution of racemates of primary alcohols by the same method is more difficult to achieve. This is due to lower enantioselectivities of lipases towards chiral primary alcohols. Lipase from *P. cepacia* (PSL) is the most efficient lipase, which shows high enantioselectivity towards a broad range of primary alcohol.[60] Nordin et al.[61] studied the enantioselectivities of lipases from *P. cepacia* towards a series of primary methyl-substituted alcohols using vinyl acetate as the acyl donor in transesterifications in organic solvents (Fig. 13).

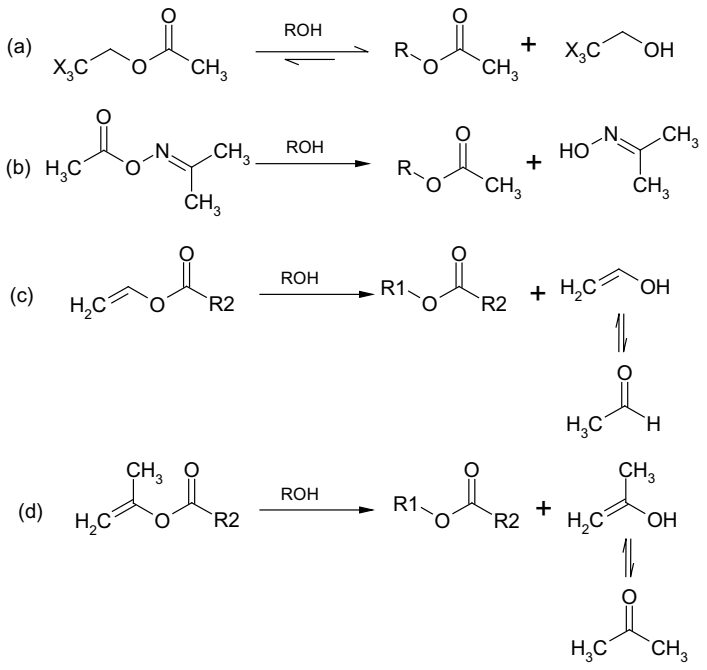


Figure 12. Lipase-catalyzed irreversible transesterification.

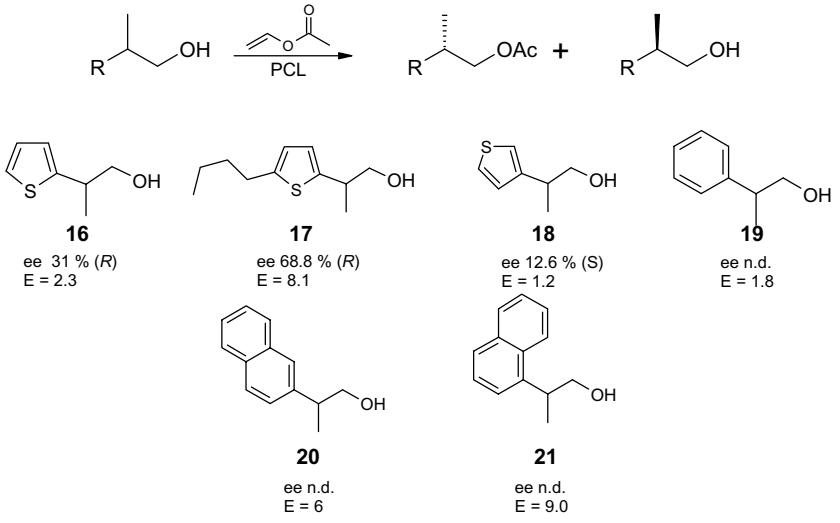


Figure 13. Lipase-catalyzed kinetic resolution of 2-substituted 2-methylethanols by transesterification with vinyl acetate.[61]

In terms of enantioselectivity, the best results were found for 3-aryl-2-methylpropan-1-ols with enantiomeric ratios (E) over 100 in most cases, whereas other 3-substituted primary 2-methylpropan-1-ols generally displayed lower enantioselectivities: 3-cycloalkyl-2-methylpropan-1-ols ($E = 20$) and 2-methylalkan-1-ols ($E = 10$) (Fig. 14).

Moving the aryl group closer or further away from the stereogenic center resulted in low enantioselectivities: 2-arylpropan-1-ols ($E < 10$), 2-methyl-4-(2-thienyl)-butan-1-ol ($E = 12$), 2-methyl-5-(2-thienyl)pentan-1-ol ($E = 3.2$) and 2-methyl-6-(2-thienyl)hexan-1-ol ($E = 3.8$). Other primary alcohols have also been successfully used as substrates for other lipases (Fig. 15).

5.2. Kinetic resolution of secondary alcohols

Secondary alcohols are by far the most frequently used targets in lipase-catalyzed resolutions. This is due to their importance in organic synthesis but also that lipases usually show much higher enantioselectivity in resolutions compared to primary and tertiary alcohols.

Numerous examples can be found in literatures and only a few selected examples are included in this survey. Schurig and co-workers reported a series of reports about the utility of isopropenyl acetate as an innocuous acyl donor in the lipase-catalyzed transesterification of secondary alcohols. The non-reacting alcohol enantiomers were obtained in >99% ee[51–57] (Fig. 16).

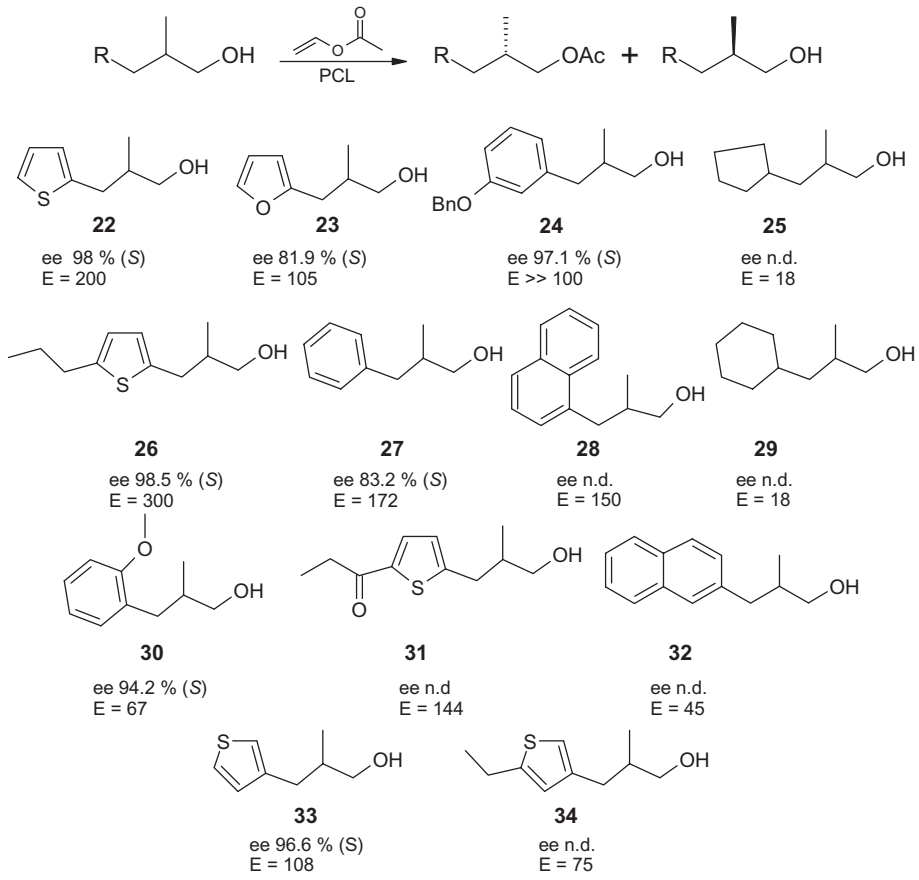


Figure 14. Lipase-catalyzed kinetic resolution of 3-substituted 2-methylpropan-1-ols by transesterification with vinyl acetate.[61]

In the transesterification of (*RS*)-secondary alcohols, the (*R*)-alcohol was the faster reacting enantiomer yielding the (*R*)-acetate in high *ee* and leaving (*S*)-alcohol as the enantiomerically pure unreacted enantiomer (Fig. 17).

trans-4-Phenyl-3-butene-2-ol **65**, another substrate possessing an allylic strain has been successfully resolved on a gram-scale via lipase-catalyzed enantioselective acylation of the alcohol **65** and hydrolysis of its corresponding acetate[57] (Fig. 18).

In order to reduce the time needed to perform a complete kinetic resolution, Lindner et al.[53] reported the use of the same allylic alcohol **65**, but in enantiomerically enriched form rather than a racemic mixture in kinetic resolution. Thus, the kinetic resolution of **65** was performed starting from the enantiomerically enriched alcohol (*R*) or (*S*)-**65** (45%) ee obtained by the ruthenium-catalyzed asymmetric reduction of **67** with the aim to reach ~100% ee in a consecutive approach (Fig. 19). Several lipases were screened in resolving the enantiomerically enriched **50** either in the enantioselective transesterification of (*S*)-**65** (45% ee) using isopropenyl acetate as an acyl donor in toluene in non-aqueous medium or in the enantioselective hydrolysis of the corresponding acetate (*R*)-**66**, (45% ee) using a phosphate buffer (pH = 6) in aqueous medium. An *E* value of 300 was observed and the reaction terminated after 3 h yielding (*S*)-**65** >99% ee and the ester (*R*)-**66** recovered with 86% ee determined by capillary GC after 50% conversion.

Instead of a two step reaction, Kamal et al. reported the one-pot lipase-catalyzed synthesis of enantiopure secondary alcohols starting from a carbonyl compounds. Thus, the reduction of acetophenones with sodium borohydride in the presence of neutral alumina in hexane followed by enantioselective acylation catalyzed by lipases was performed in one pot (Fig. 20).

Other secondary alcohols containing benzofuran **75**, azide **76**, alkylthio **77**, carboxylic acid ethyl ester **78**, α-methylene-β-hydroxy esters **79**, have successfully been resolved (Fig. 21).

Of particular note, the rapid screening of different hydrolases for the enantioselective hydrolysis of esters of the difficult to resolve substrates, such as pentalactone **84**, 1-methoxy-2-propanol **85**, 3-butyn-2-ol **86** and 3-hydroxy-tetrahydrofuarn **87** was performed in a pH-indicator-based format in microtiter plates.[81]

5.3. Kinetic resolution of tertiary alcohols

The kinetic resolution of tertiary alcohols is not well covered in literature. This is probably due to the difficulty associated with the accommodation of such sub-

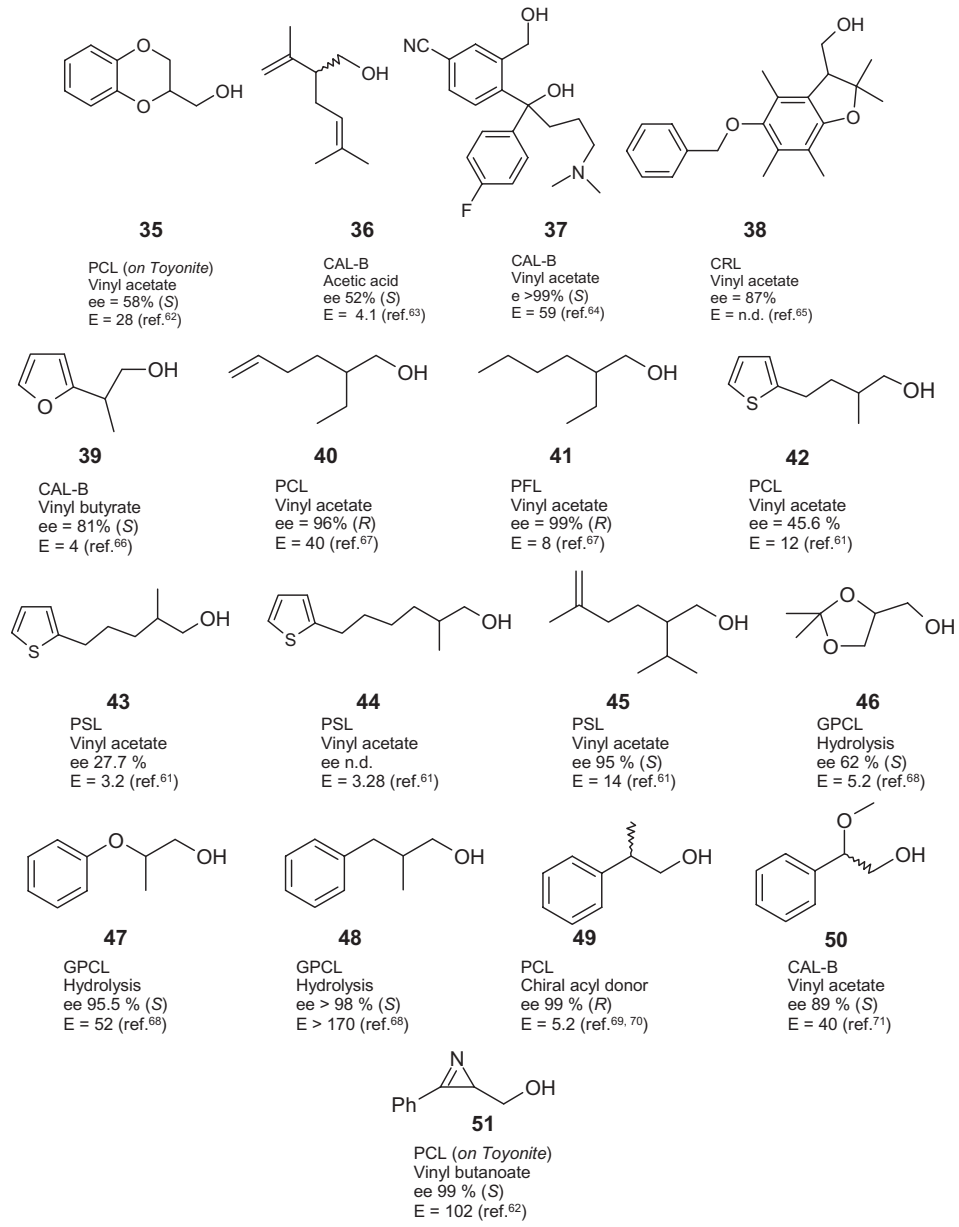


Figure 15. Selected examples showing the kinetic resolution of primary alcohols.

strates to the active site of lipases. Krishna et al.[82] reported the enantioselective transesterification of tertiary alcohol **88** using lipase A from *Candida antarctica* (CAL-A) and vinyl acetate as acyl donor in organic solvent. Attempts to resolve other tertiary alcohols (**89**,[83] **90**,[84] **91**[85]) are documented in the literature (Fig. 22).

5.4. Kinetic resolution of diols

5.4.1. Desymmetrization of prochiral substrates. The desymmetrization of prochiral substrates including *meso* and *P*-stereogenic substrates has become a powerful method in asymmetric synthesis.[86] The advantage of desymmetrization over conventional kinetic resolution is the potential ability to achieve high enantiomeric excess even at high conversion with a theoretical yield of 100%.[22] Among the widely used substrates in this approach, prochiral ketones and alcohols received much attention. For ketones, the known Baeyer–Villiger oxidation or deprotonation using chiral lithium amide bases serves to differentiate the two-prochiral groups attached to the carbonyl of the ketone. Apart from the chiral amide approach, Baeyer–Villiger mono-oxygenase enzymes have been used successfully in the desymmetrization of prochiral and *meso*-cyclohexanones.[27] In a complementary method, lipases have been used in the desymmetrization of enol esters derived from two synthetically important class of cyclic and bicyclic prochiral ketones[26] and in the desymmetrization of prochiral alcohols or acetate.[28]

5.4.2. Desymmetrization of racemic diols. Diols, such as optically active 1,1′-binaphthyl-2-2′-diol (BINOL), have been used as versatile templates and chiral auxiliaries in catalysts employed successfully in asymmetric synthesis.

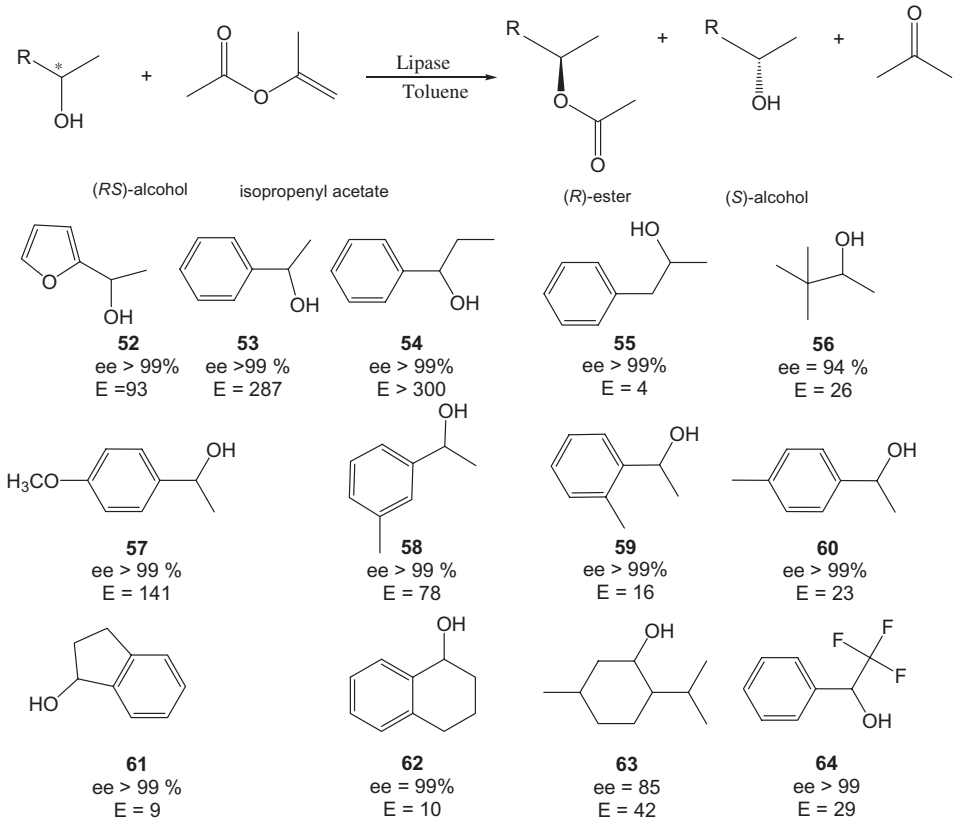


Figure 16. Lipase-catalyzed transesterification of secondary alcohols using isopropenyl acetate as acyl donor in toluene.[51–57]

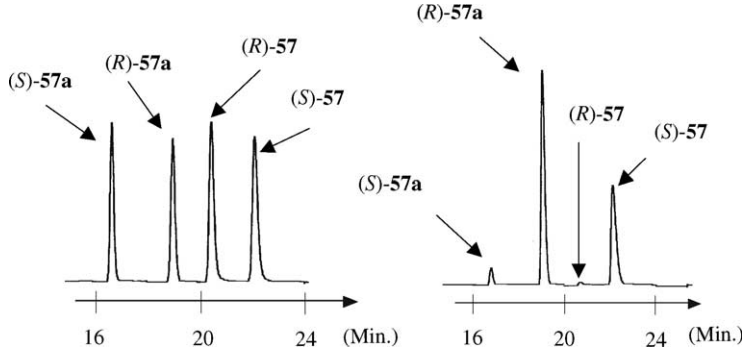


Figure 17. A representative chromatogram of the GC chiral separation of a secondary alcohol: (left) racemic 1-(4-methoxy-phenyl)ethanol **57** and its corresponding acetate **57a** (reference) and (right) lipase-catalyzed transesterification of 1-(4-methoxy-phenyl)ethanol **57** (4 h) using isopropenyl acetate as the acyl donor in toluene as the organic solvent: ee_s = 99.9%, ee_p = 87%, conv. = 53.4%, E = 141.

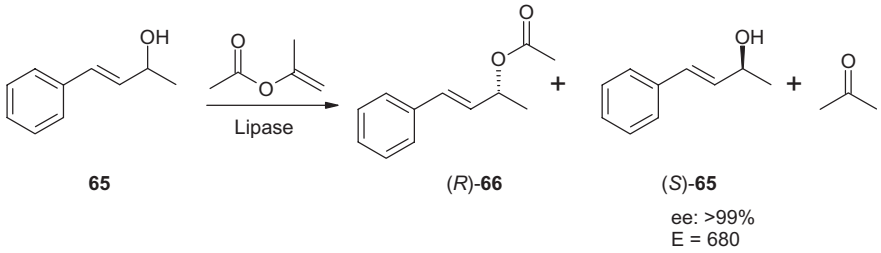
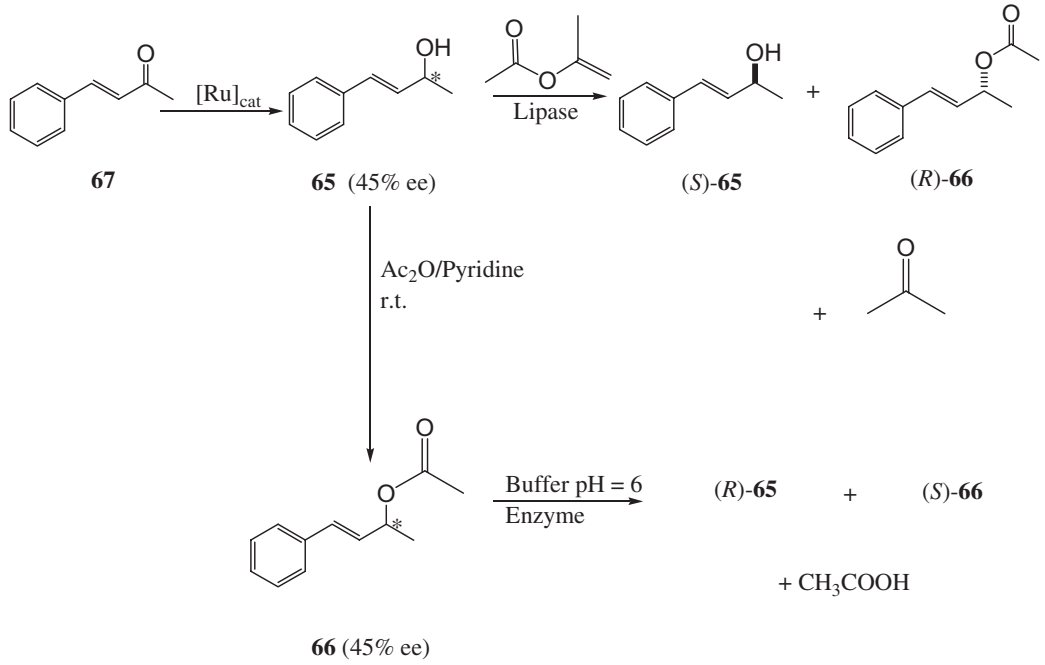


Figure 18. Lipase-catalyzed kinetic resolution of racemic **65** using isopropenylacetate as acyl donor in toluene as organic solvent.[57]

The application of enzymes in the enantioselective access to axially dissymmetric compounds was first reported by Fujimoto et al.[86] In aqueous media, the asymmetric hydrolysis of the racemic binaphthyl dibutyrate (the ester) using whole cells from bacteria species afforded the (*R*)-diol with 96% ee while the unreacted substrate (*S*)-ester was obtained with 94% ee at 50% conversion. Recently, in non-aqueous media, lipases



67 **65** (45% ee) (*S*)-**65** (*R*)-**66**

66 (45% ee) (*R*)-**65** + (*S*)-**66**

Figure 19. Ruthenium/lipase-catalyzed separation of enriched (*RS*)-**65**.[53]

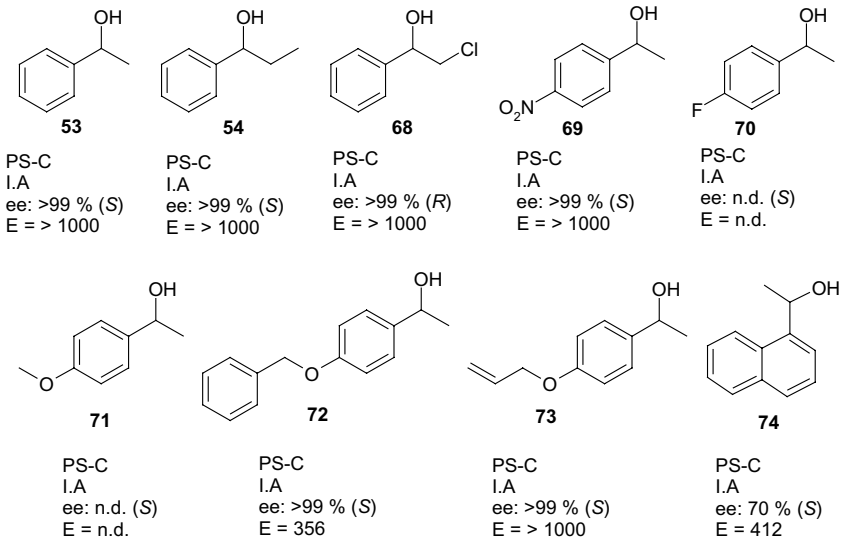


Figure 20. Selected examples of the one-pot lipase-catalyzed synthesis of enantiopure secondary alcohols.[72]

from *P. cepacia* and *Ps. fluorescens* have been employed in the enantioselective resolution and desymmetrization of racemic 6,6′-disubstituted BINOL derivatives using vinyl acetate.[87] Monoacetate (*R*)-**93** (product) was obtained in 32–44% chemical yields and 78–96% ee depending on the derivatives used. The unreacted BINOL (*S*)-**92** was obtained in 30–52% chemical yield and 55–80% ee (Fig. 23).

Biphenyls are recognized as stable analogues of BINOL and can be found in numerous natural products. Sanfilippo et al.[88] reported the *P. cepacia* lipase-catalyzed kinetic resolution of 2,2′-dihydroxy-6,6′-dimethoxy-1,1′-biphenyl **94** using vinyl acetate as an acyl donor in *tert*-butyl methyl ether as an organic solvent. (*R*)-**95** was obtained with an ee up to 98% while (*S*)-**94** was recovered with an ee up to 96% (Fig. 24).

Diols of different structures, such as *meso*-diol **96** (Fig. 25), the *C*2-symmetric diol rac-**99** (Fig. 26), the diol rac-**102** in which the primary hydroxy group is protected (Fig. 27) and the unprotected diol rac-**104** with a primary and secondary hydroxy group (Fig. 28) were used as substrates in the lipase-catalyzed transesterification using vinyl acetate as the acyl donor in organic solvents with the aim of preparing chiral buildings blocks of high enantiomeric purity.[89]

Apart from vinyl acetate, vinyl benzoate was used as the acylating agent in the *Mucor miehei* lipase (MML) and

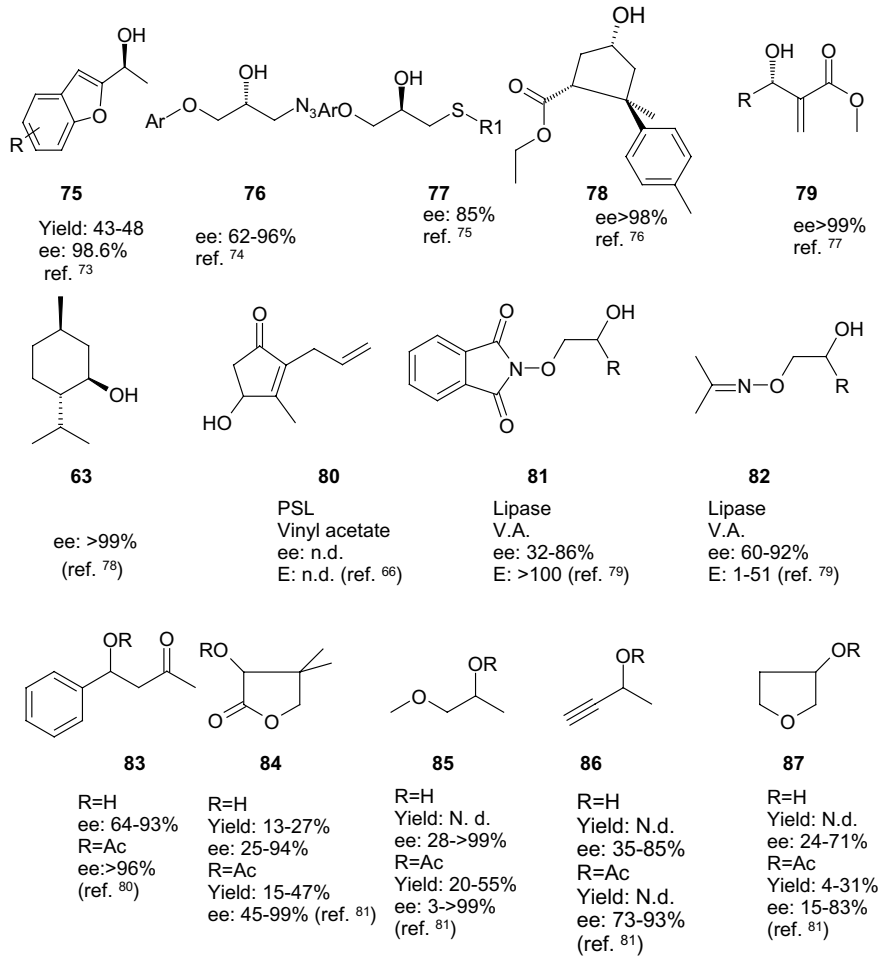


Figure 21. Selected examples of the kinetic resolution of secondary alcohols and difficult to resolve substrates (**84**–**87**).

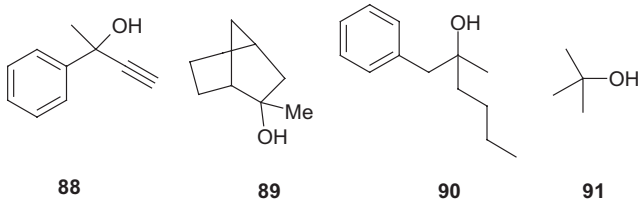


Figure 22. Examples of lipase-catalyzed kinetic resolution of tertiary alcohols.[82]

C. antarctica lipase (CAL)-catalyzed benzoylation of 1,2-diols **107** in organic solvents.[90] The reaction proceeded with high regioselectivity and moderate enantioselectivity (Fig. 29).

An efficient synthesis of (*R*)- and (*S*)-1-amino-2,2-difluorocyclopropanecarboxylic acids (DFACC) **111** via lipase-catalyzed desymmetrization of prochiral diols **109** and prochiral diacetates **112** has recently been reported.[28] Thus, the lipase-catalyzed transesterification of **109** using vinyl acetate as an acyl donor in benzene and di-*i*-propyl ether (20:1) as the organic solvent, afforded (*R*)-**110** with 91.3% ee and 96.5% chemical yield. The reverse enantioselective hydrolysis of **112** in a mixed solvent of acetone and phosphate buffer

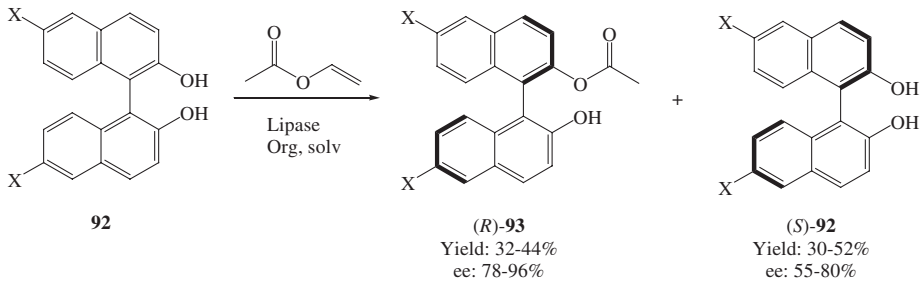


Figure 23. Lipase-catalyzed stereoselective resolution and desymmetrization of binaphthols **92**.[87]

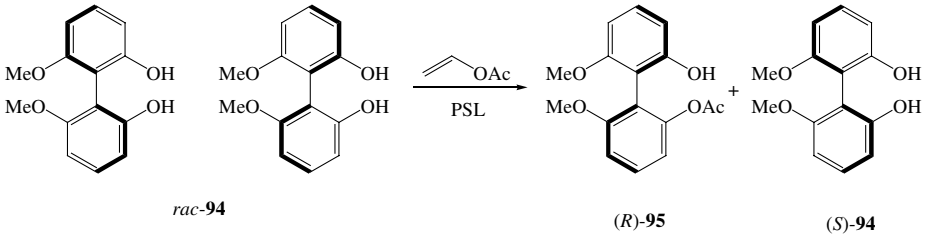


Figure 24. Lipase-catalyzed kinetic resolution of 2,2′-dihydroxy-6,6′-dimethoxy-1,1′-biphenyl **94**.[88]

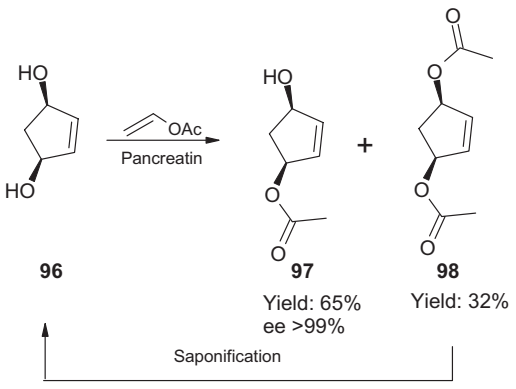


Figure 25. Lipase-catalyzed desymmetrization of *cis*-2-cyclopeptene-1,4-diol **96**.[89]

afforded (*S*)-**110** with 91.7% ee and 86.2% chemical yield (Fig. 30).

The first enzymatic desymmetrizations of prochiral phosphine oxides have recently been reported by Kielbasinski et al.[91] Thus, prochiral bis(methoxycarbonylmethyl)-phenylphosphine oxide **113** was subjected to the PLE-mediated hydrolysis in buffer to afford the chiral monoacetate (*R*)-**114** in 72% ee and 92% chemical yield. In turn, the prochiral bis(hydroxymethyl)phenylphosphine oxide **115** was desymmetrized using either lipase-catalyzed acetylation of **115** with vinyl acetate as an acyl donor in organic solvent or hydrolysis of **117** in phosphate buffer and solvent to afford the chiral monoacetate **116** with up to 79% ee and 76% chemical yield (Fig. 31).

Neri and Williams[92] reported the desymmetrization of *N*-Boc-serinol **118** by selective mono-acetylation using PPL (porcine pancreas lipase) and vinyl acetate as the acylating agent in organic solvent. The mono acetylated product (*R*)-**119** was obtained after 2 h with 99% ee and isolated in 69% chemical yield. Traces of diacetylated

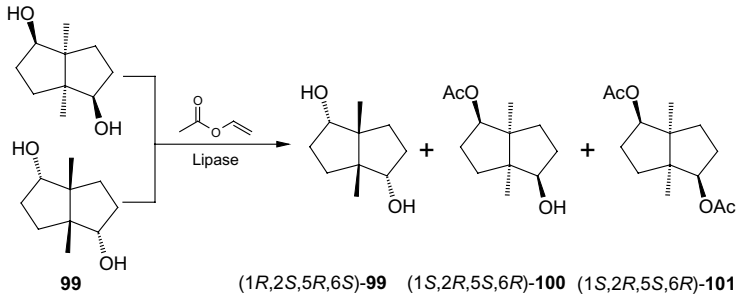


Figure 26. Lipase-catalyzed kinetic resolution of *endo-endo-cis*-bicyclo[3.3.0]octane-2,6-diol *rac*-**99**.[89]

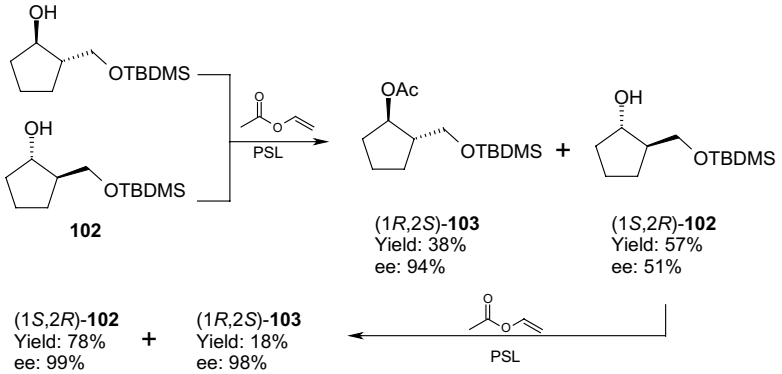


Figure 27. Lipase-catalyzed kinetic resolution of *trans*-2-(*tert*-butyldimethylsiloxymethyl) cyclopentanol *rac*-**102**.[89]

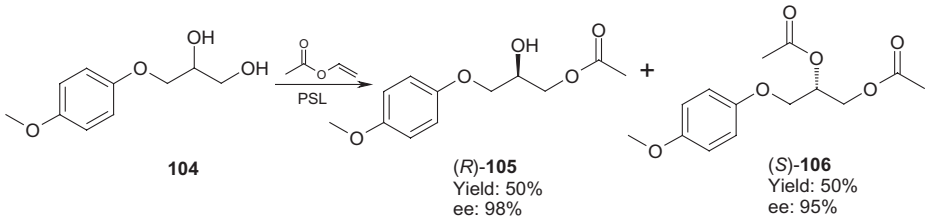


Figure 28. Lipase-catalyzed kinetic resolution of (*RS*)-3-(4-methoxyphenoxy)propane-1,2-diol *rac*-**96**.[89]

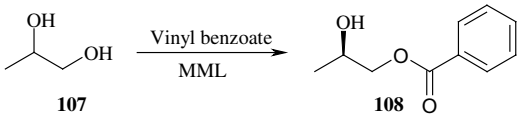


Figure 29. Lipase-catalyzed benzoylation of propane-1,2-diol **107**.[90]

product **120** were observed (Fig. 32). The cyclization of (*R*)-**119** in basic medium afforded racemic oxazolidinone **121**. The latter was subjected to enzymatic hydrolysis in phosphate buffer affording (*R*)-**122** in up to 93% ee and isolated in chemical yield up to 42%. To avoid basic conditions, (*S*)-**121** was also obtained in one step by cyclization of (*R*)-**119** with thionyl chloride. The reaction proceeded with >98% ee and 72% yield. The enzymatic hydrolysis of (*S*)-**121** afforded (*R*)-**122** in >98% ee and 77% yield (Fig. 33).

The kinetic resolutions of a series of racemic *trans*-cycloalkane-1,2-diol monoacetates *rac*-**123a–d** were reported using the enantioselective transesterification mode with vinyl acetate as acyl donor and commercial as well as self-prepared fungal lipases affording the diacetates (*R*,*R*)-**124a–d** and monoacetates (*S*,*S*)-**123a–d** in high enantiomeric purity (up to 99% ee). Monoacetates

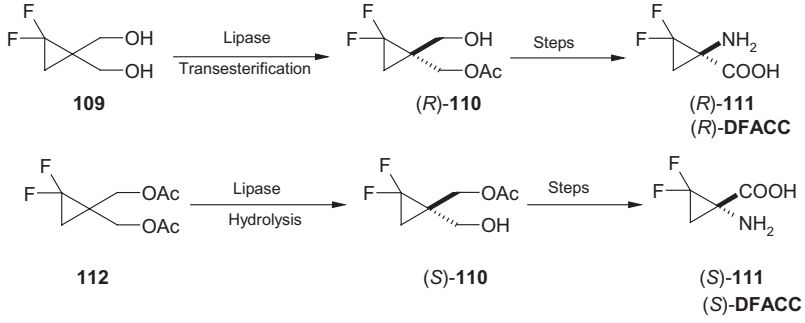


Figure 30. Lipase-catalyzed desymmetrization of prochiral diols **109** and diacetates **112**.[28]

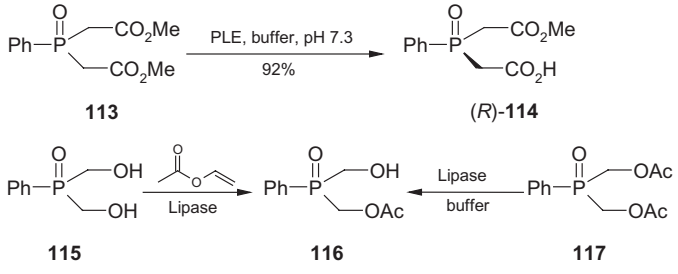


Figure 31. Lipase-catalyzed desymmetrizations of prochiral phosphine oxides.[91]

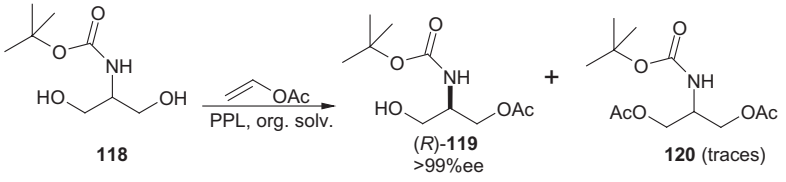


Figure 32. Desymmetrization of *N*-Boc-serinol **118** by PPL.[92]

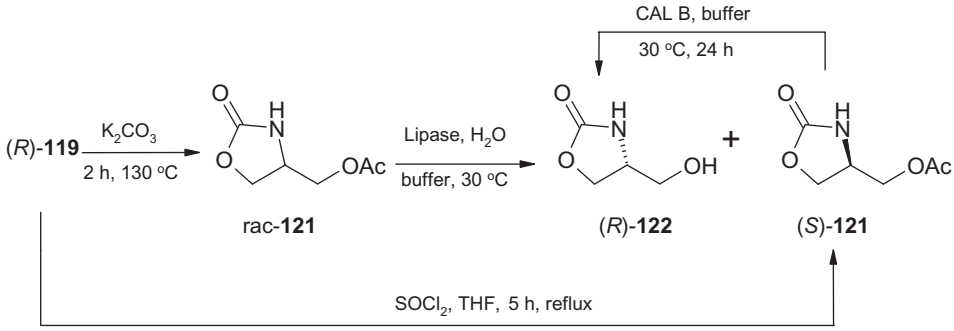


Figure 33. Synthesis of (*S*)-4-acetoxymethyl-2-oxazolidinone **121** and its enzymatic hydrolysis.[92]

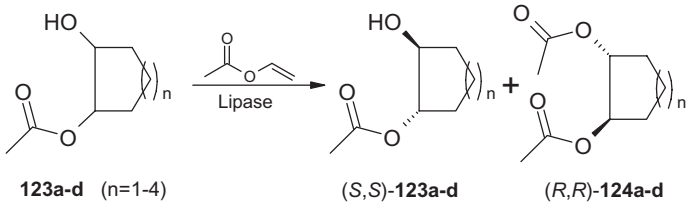


Figure 34. Enzymatic acylation of *trans*-2-acetoxycycloalkan-1-ols **115**.[93]

(*R*,*R*)-**123a**–**d** were also prepared starting from the racemic diacetates *rac*-**124a**–**d** by lipase-catalyzed hydrolysis[93] (Fig. 34).

The synthesis of (*E*)-3,7-dimethyl-2-octen-1,8-diol isolated from the hair pencils of male *Danaus chrysippus* (African Monarch) was investigated by Takabe et al. The key step of the sequence involves the asymmetric desymmetrization of 1,3-propanediol **125** with lipase. Monoacetate **126** was afforded with 90% ee and 75% chemical yield.[94] The diacetate and diol **125** were recovered in 20% and 2% yields, respectively (Fig. 35).

Using a chemoenzymatic synthesis strategy, Carr and Bisht reported the synthesis of the natural product (*S*)-imperanene and its (*R*)-enantiomer in nine steps starting from vanillin.[95] The key step in the synthesis involves the use of *P. cepacia* lipase (PS-30) and vinyl acetate as acyl donor to introduce asymmetrization of the intermediary prochiral 1,3-diol **127** in >97% ee (Fig. 36).

A simple enzymatic methodology for the selective monoacetylation of 1,*n*-diols ($n = 5$–8) using vinyl acetate has been reported by Framis et al.[96] The monoacetylation excesses of 81–87% at 74–90% 1,*n*-diol conversions were obtained in toluene and diisopropyl ether using *Thermomyces lanuginosus* lipase (TLL) immobilized on polypropylene powder as catalyst (Fig. 37).

A 40:60 mixture of *endo*,*endo*-9-oxabicyclo[4.2.1]nonane-2,5-diol *meso*-**132** and *endo*,*endo*-9-oxabicyclo[3.3.1]nonane-2,6-diol (±)-**133** were prepared and

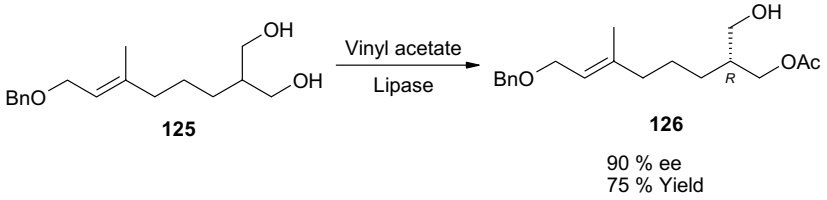


Figure 35. Asymmetric desymmetrization of 1,3-propanediol **125** catalyzed by lipase.[94]

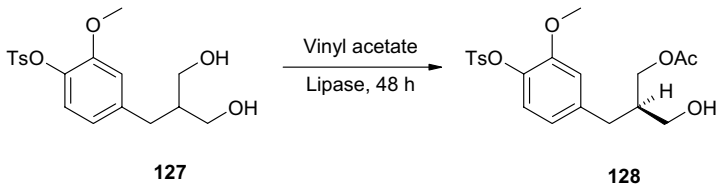


Figure 36. Asymmetric desymmetrization of the intermediary prochiral 1,3-diol catalyzed by lipase.[95]

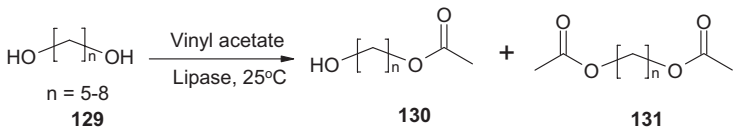


Figure 37. Lipase-catalyzed selective monoacetylation of 1,*n*-diols (*n* = 5–8).[96]

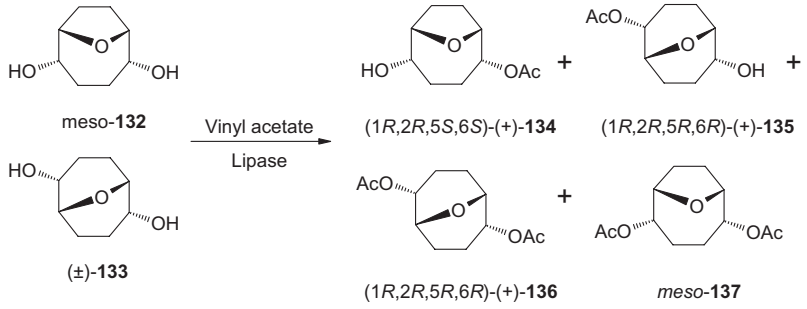


Figure 38. Lipase-catalyzed enantioselective acetylation of 9-oxabicyclononanediol.[97]

resolved by enantioselective acetylation with vinyl acetate catalyzed by lipase (Fig. 38). The optimal conversion was reached when the *meso*[4.2.1]isomer-**132** was quantitatively transformed to the monoacetate **134** (>98% ee) and one enantiomer of the diol (±)-**133** was completely acetylated to one enantiomer of **135** (80% ee). However, the latter monoacetate **135** was only an intermediate, and was further acetylated to the corresponding diacetate **136** (>98% ee). For the remaining diol (−)-**133** [14% isolated yield, 3% traces of *meso*-**132**] an enantiomeric excess of more than 98% was determined by GC. Adding to that, a 48:52 mixture of the monoacetates (+)-**134** (28% ee) and (+)-**135** (60% ee) was isolated with 56% yield while a 21:79 mixture of the *meso*-diacetate **137** and the diacetate (+)-**136** (82% ee) was isolated with 12% yield.[97]

5.5. Miscellaneous

Allenes, another class of compounds having interesting properties, have also been resolved by lipases. The kinetic resolution of a variety of racemic 1-ethenyl and ethynyl-substituted 2,3-allenols was reported using a lipase from *C. antarctica* type B (CAL-B) with vinyl acetate as acyl donors in organic solvent. The biocatalytic resolution afforded (*S*)-2,3-allenols (*S*)-**138** and (*R*)-2,3-allenyl acetates (*R*)-**139** in chemical yields up to 55% and an enantiomeric excess ee up to 99% for both enantiomers depending on the substituents[98] (Fig. 39).

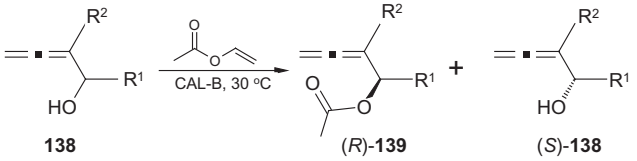


Figure 39. Lipase-catalyzed kinetic resolution of a variety of racemic 1-ethenyl and ethynyl-substituted 2,3-allenols **138**.[98]

Faure et al.[99] reported the first enzymatic resolution of phosphane–borane complex. Thus, the borane adduct of (2-hydroxypropyl)diphenylphosphane **140** was resolved using the lipase CAL-B and vinyl acetate as acyl donor in organic solvent. The remaining unreacted substrate (*S*)-**140** was recovered with 91% ee (Fig. 40).

The use of *C. antarctica* lipase B in the kinetic resolution of a series of bicyclic 1-heteroaryl primary amines **142** using ethyl acetate as an acyl donor in isopropyl ether as organic solvent was studied by Skupinska et al.[100] High yields and enantiomeric excesses of either enantiomer could be obtained. The undesired enantiomer can in some cases be recycled by thermal racemization (Fig. 41).

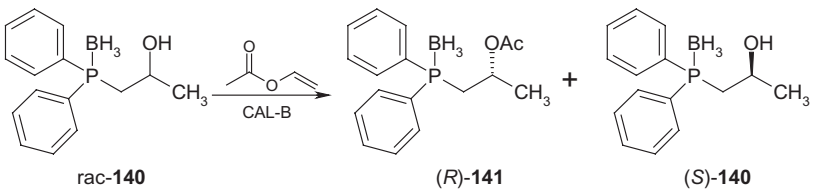


Figure 40. Lipase-catalyzed kinetic resolution of a phosphane–borane complex **140**.[99]

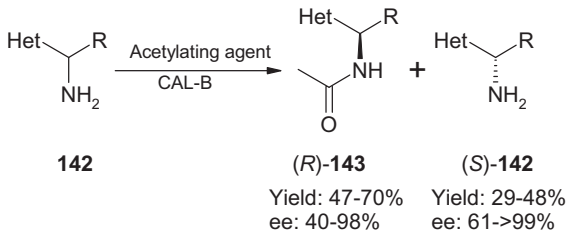


Figure 41. Lipase-catalyzed enantioselective acetylation of bicyclic 1-heteroaryl primary amines (rac-**142**).[100]

Irurre et al.[101] reported the enzymatic resolution of *trans*-10-azido-9-acetoxy-9,10-dihydrophenanthrene **144** on a gram-scale using *C. cyclindracea* lipase-catalyzed enantioselective hydrolysis in phosphate buffer. Substrate **144** (the ester) was obtained in 89% yield and 83% ee while product **145** (the alcohol) was obtained in 90% yield and 98% ee (Fig. 42).

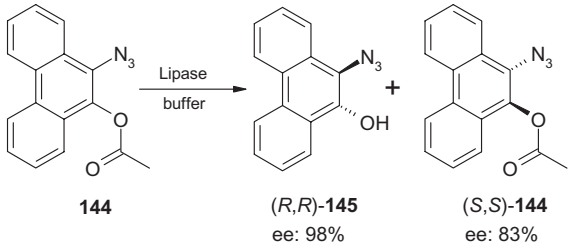


Figure 42. Kinetic resolution of azido acetate **144**.[101]

A practical method for the synthesis of chiral pyridazinone bearing a pyrazolopyridine ring via lipase-catalyzed resolution of 2-(acyloxymethyl)-4,5-dihydro-5-methylpyridazin-3(2H)-one derivatives **146** was reported by Yoshida et al.[102] (Fig. 43).

Forro and Fülöp[103] reported a very simple method for the synthesis of enantiopure β-amino acids **126a**–**129a** (e.g., cispentacin) and β-lactams **148b**–**151b** via the lipase-catalyzed enantioselective ring opening of unactivated alicyclic β-lactams **148**–**151** in organic media. High enantioselectivity ($E > 200$) was observed when using the CAL-B catalyzed reaction with H_2O (1 equiv) in diisopropyl ether at 60 °C. The products **148a**–**151a** and substrates **148b**–**151b** were obtained in up to 99% ee with chemical yields ranging from 36% to 47%. Other approaches for the resolution of compounds containing larger alicyclic rings were recently reported[104] (Fig. 44).

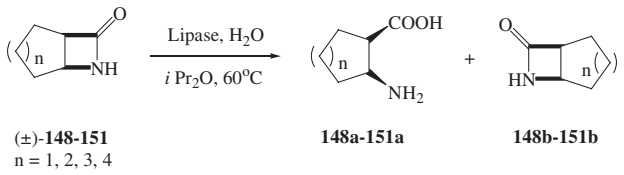


Figure 44. Lipase-catalyzed enantioselective ring opening of unactivated alicyclic β-lactams.[103]

5.6. Application of lipases in industry

As the use of lipase for industrial chemical synthesis becomes easier, several chemical companies have begun to increase significantly their biocatalytic process used in synthetic application. Among these companies is BASF, in which enantiomerically pure alcohols and amines are produced on an industrial scale[105] (Fig. 45).

The enantioselective hydrolysis of the racemic acetamide (*R,S*)-**155** was developed at Bayer in the middle of 1990s. The reaction was performed using *Candida antarctica* lipase B (CAL-B) to afford the free amine (*R*)-**156** in high enantiomeric excess (>99.5% ee).[106] However, the requirement of a high concentration of catalyst limits the exploitation of the process on an industrial scale[107] (Fig. 46).

Apart from amines and secondary alcohols, Ladner and Whitesides developed a procedure to resolve racemic glycidylbutyrate **157** with porcine pancreatic lipase (PPL) to afford (*R*)-**157** in 89% of the theoretical yield and with 92% ee.[108] This process was further developed and used by Andeno-DSM to produce the epoxy alcohol (*R*)-glycidol (*R*)-**158** and (*R*)-**157** on a multitone scale[109] (Fig. 47).

In the pharmaceutical industry, salt-activated biocatalysts have been used to synthesize a library of paclitaxel (taxol) derivatives. CAL was used in the hydrolysis of the terminal vinyl ester in taxol 2′-vinyladipate **159**. The resulting taxol 2′-adipic acid derivative **160** was

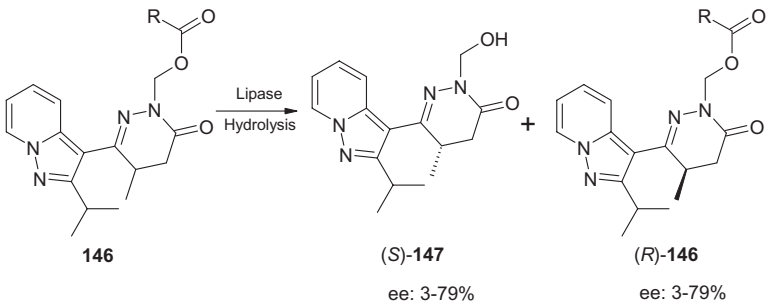


Figure 43. Lipase-catalyzed resolution of 2-(acyloxymethyl)-4,5-dihydro-5-methylpyridazin-3(2H)-one derivatives **146**.[102]

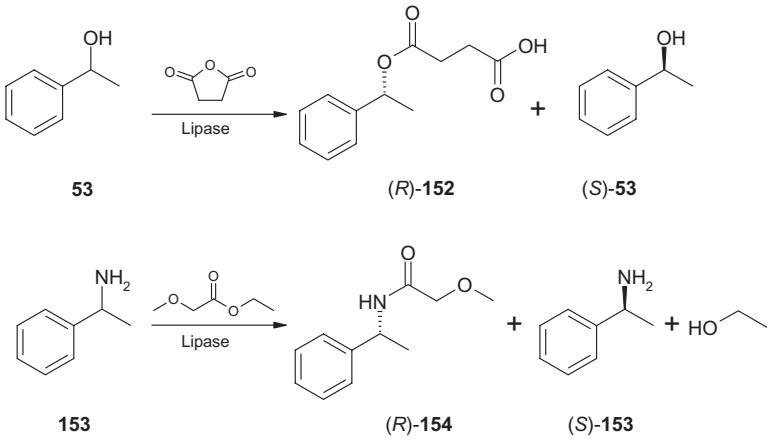


Figure 45. Some of the biocatalytic steps using lipase developed at BASF: lipase-catalyzed kinetic resolution of (a) phenyl ethanol **53** using succinic anhydride and (b) secondary amine **153** using ethyl methoxyacetate as acyl donor.[105]

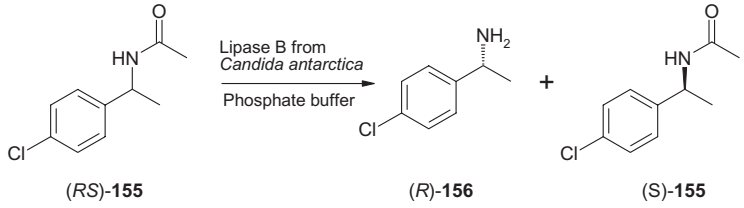


Figure 46. Enantioselective hydrolysis of racemic acetamide (*RS*)-**155** developed at Bayer.[106]

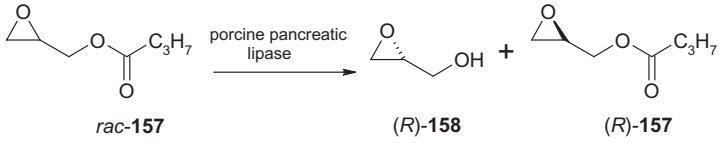


Figure 47. Enantioselective hydrolysis of racemic glycidylbutyrate **157** developed at Andeno-DSM.[104]

nearly 1700 times more soluble in water than the native taxol, a result in the design of taxol prodrugs with increased bioavailability[105] (Fig. 48).

A broader overview on the industrial methods used for the production of optically active intermediates has recently been reviewed by Breuer et al.[107]

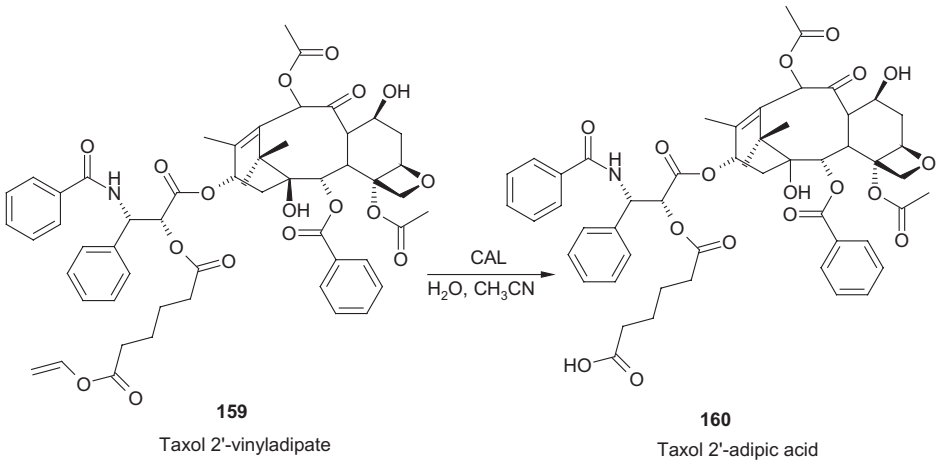


Figure 48. Lipase-catalyzed hydrolysis of the terminal vinyl ester in taxol 2′-vinyl adipate **159**.[105]

6. Conclusions and perspectives

The lipase-catalyzed access to enantiomerically pure compounds remains a versatile method for the separation of enantiomers. The selected examples shown in this survey demonstrate the broad applicability of lipases in terms of substrate structures and enantioselectivity. More recently, modern molecular biology methods, such as rational protein design and especially directed evolution,[110] will further boost the development of tailor-made lipases for future applications in the synthesis of enantiomerically pure compounds. It has already been shown that a virtually non-enantioselective lipase ($E = 1.1$ in the resolution of 2-methyldecanoate) can be evolved to become an effective biocatalyst ($E > 50$). Furthermore, variants were identified, which showed opposite enantiopreference.

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