DOI: 10.1002/adsc.200606057

Candida antarctica Lipase B: An Ideal Biocatalyst for the Preparation of Nitrogenated Organic Compounds

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Received: February 16, 2006; Accepted: March 24, 2006

Abstract: *Candida antarctica* lipase B (CAL-B) is a very effective catalyst for the production of amines and amides using different enzymatic procedures. Simplicity of use, low cost, commercial availability and recycling possibility make this lipase an ideal tool for the synthesis and resolution of a wide range of nitrogenated compounds that can be used for the production of pharmaceuticals and interesting manufactures in the industrial sector.

Keywords: amides; amines; aminolysis; ammonolysis; *Candida antarctica* lipase B; enzymatic resolution

1 Introduction

Hydrolytic enzymes are widely used in organic synthesis as environmentally friendly catalysts that possess broad substrate specificities, display high stereoselectivity, work under mild reaction conditions, are commercially available and do not require the use of cofactors.[1] From the hydrolases group, lipases (EC 3.1.1.3) are the most popular biocatalysts and the most useful in asymmetric synthesis.[2] Applications of lipases include chemo-, regio- and stereoselective processes, like the kinetic resolution of racemic alcohols, acids, esters or amines,[3] as well as the desymmetrization of prochiral or *meso* compounds.[4] In addition, other non-conventional processes such as aldol reactions or Michael additions can be catalyzed by some lipases.[5] The role of lipases in organic synthesis has dramatically increased in the last decade, finding interesting uses for the production of pharmaceuticals, fine chemicals or agrochemicals, and especially for the pharmaceutical sector.[6]

Candida antarctica lipase B (CAL-B) is one of the most effective catalysts in the resolution of alcohols and especially amines, allowing the preparation of an important variety of optically active hydroxy and amino compounds. The high stereoselectivity and simplicity of these processes make this strategy the most adequate for the resolution of primary amines bearing the amino group in the stereocenter. Another type of *Candida antarctica* lipase, the *Candida antarctica* lipase A (CAL-A), proves to be more active than CAL-B in the acylation of secondary amines or tertiary alcohols, representing an ideal catalyst in the bioresolution of sterically hindered compounds, however this biocatalyst has been much less employed in organic synthesis than CAL-B.[7]

CAL-B is constituted of 317 amino acids and has a molecular mass of 33 kDa (Figure 1). This lipase has an α/β-hydrolase-like fold, and it was firstly obtained from the *Candida antarctica* yeast, meanwhile lipases with broad ranges of pH optima and thermostability were screened from different microbial sources.[8] The X-ray structure of CAL-B was elucidated in 1994,[9] and it was also crystallized, forming different structures.[10] Viewed with the catalytic triad Asp187-His224-Ser105 oriented from left to right, it contains a large hydrophobic pocket above the Asp-His-Ser triad and a medium-sized pocket below it. In the catalytic pathway, the acyl moiety of the substrate lies in the large subsite, while the leaving group/nucleophile moiety lies in the medium pocket, but may extend into the solvent and/or into the large pocket since it has little space for substituents larger than propyl. The large hydrophobic subsite in CAL-B is lined by Ile189 and Val190 on the left, Val154 on the far right, as well

Vicente Gotor (1947) received his Ph.D. from the University of Zaragoza in 1974. After leaving Zaragoza, Dr. Gotor carried out two years of postdoctoral studies at Max Planck Institut für Kohlenforschung (Mülheim/Ruhr, Germany) in the area of organometallic chemistry. He joined the Chemistry Faculty at the University of Oviedo as Assistant Professor in 1977; he assumed his current position as Professor of Organic Chemistry at the same Institution in 1982. His research fields include the areas of heterocyclic and bioorganic chemistry. He worked in heterocyclic chemistry until 1988. In this year, he started his work in the field of biotransformations. Specific areas of his research interest are enzymatic amidation reactions with hydrolases, enzymatic chemoselective transformations of natural products, biotransformations with oxynitrilases and oxidoreductases, and chiral recognition with azamacrocycles. He was Vice-chancellor of Research at the University of Oviedo for four years until June 2000. At present, he is the leader of Bioorganic group in the Chemistry Faculty at the University of Oviedo and Head of Department of Organic and Inorganic Chemistry. He has published more than 240 papers and supervised over 40 Doctoral Thesis as well as being coauthor of 10 patents.

Vicente Gotor-Fernández (1974) studied organic chemistry at the University of Oviedo, where he received his Ph.D. in 2001 working under the guidance of Professor Vicente Gotor. In June 2002 he moved to Edinburgh with a Marie Curie fellowship for post-doctoral stay under the supervision of Professor Nicholas J. Turner. Two years later he moved back to Oviedo with a Juan de la Cierva contract where he is currently working. His research interests include the use of enzymes in non-conventional reactions and the development of chemoenzymatic routes for the production of novel chiral nucleophilic catalysts.

Eduardo Busto (1982) studied organic chemistry at the University of Oviedo, where he graduated with honors in June 2004. He joined Prof. Gotor's group and is currently completing his Ph. D. working on the development of new chiral catalysts employing chemoenzymatic strategies.

as Leu140 and Leu144 at the top of the pocket. Deep in this subsite, Asp134 is on the left and Gln157 on the right. The medium pocket is below the catalytic Ser105 and is crowded by Trp104 below it and the Leu278-Ala287 helix to the right.

The restricted active site of CAL-B can explain the excellent enantiopreference shown towards secondary alcohols and other nucleophiles such as primary amines.[11] Recently this biocatalyst has been object of several structural modifications using genetic engineering to improve its selectivity[12] and to create novel synthetic applications.[13]

CAL-B has allowed the preparation of both non-chiral and chiral interesting amides and amines over the years. Here we have summarized some of the most representative results obtained, using this biocatalyst, mainly by aminolysis and ammonolysis reactions, including different procedures for the resolution of valuable intermediates such as kinetic and dynamic kinetic resolutions of racemates, and desymmetrization of prochiral compounds. Esterification, transesterification or hydrolysis processes can also be catalyzed by this biocatalyst, however for these reactions other hydrolytic enzymes can also be successfully employed. In addition, other non-conventional processes catalyzed by CAL-B have recently appeared in the literature such as Michael additions or aldol reactions, and will be briefly commented.

2 Enzymatic Preparation of Amides

Lipase-catalyzed aminolysis of carboxylic esters or condensation of carboxylic acids and amines are among the most common strategies for the preparation of amides and many examples have been described.[14] The syn-

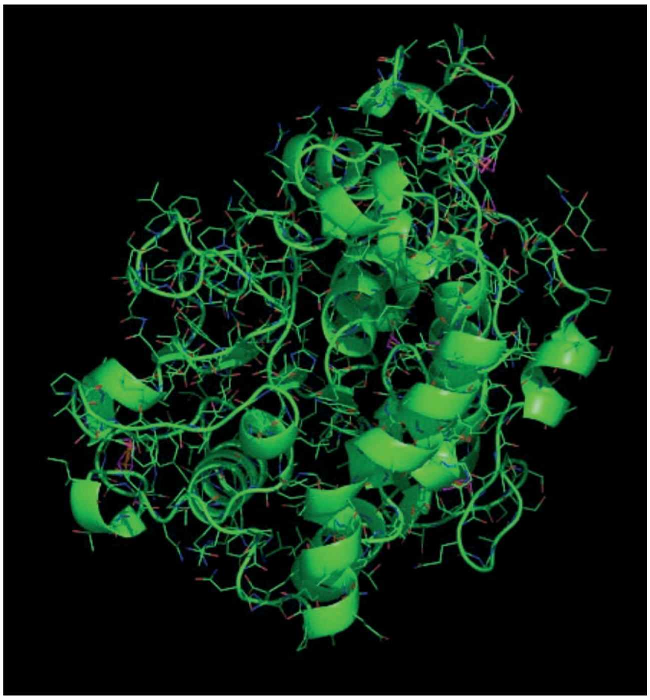
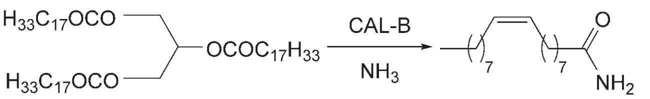


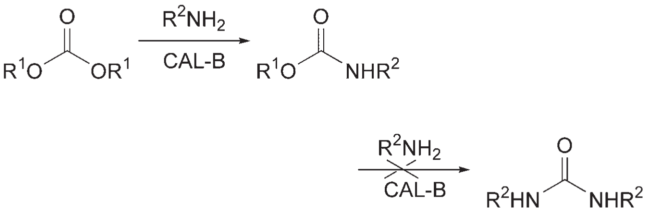
Figure 1. Structure of *Candida antarctica* lipase B.

thesis of oleamide, used as slip and anti-blocking agent in plastic processing, was carried out through an enzymatic ammonolysis reaction of triolein and ammonia in the presence of CAL-B (Scheme 1), some of the advantages of this procedure are that no purification is needed and it has been possible to scale-up the process to an industrial scale.[15] Years later, direct enzymatic amidation processes of different carboxylic acids with ammonia[16] or ammonium salts[17] were also studied using CAL-B and different organic solvents.[18]

Sheldon and co-workers used different nucleophiles like hydroxylamine, hydrazine and their derivatives in the reaction with octanoic acid and also ethyl octanoate.[19] However, the first examples of CAL-B-catalyzed ammonolysis of esters were described in 1993 with the preparation of β-keto amides from β-keto esters,[20] and the production of octanamide from ethyl octanoate in 95% yield.[21] In the aminolysis and ammonolysis reactions of different carbonates with CAL-B, the formation of carbamates was observed (Scheme 2), however the reaction do not proceed beyond the carbamate stage as these compounds are known to be slow-reacting substrates or even reversible inhibitors of lipases.[22]



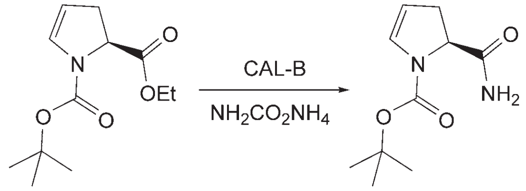
Scheme 1. Preparation of oleamide by enzymatic ammonolysis.



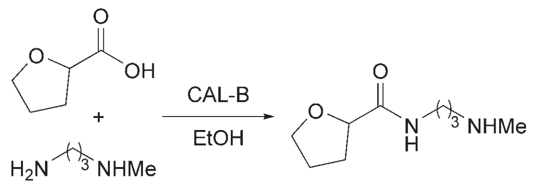
Scheme 2. CAL-B-catalyzed aminolysis and ammonolysis of carbonates.

The synthesis of capsaicin analogues, the pungent principle in *Capsicum fruits*, was carried out with low to moderate yields by amidation of vanillylamine with fatty acid derivatives in a two-phase system.[23] Other interesting examples have been recently described about the production of important goods. For instance, an efficient biocatalytic method on a multi-gram scale has been developed for the synthesis of the pyrrole-amide that is an intermediate for the synthesis of the dipeptidyl peptidase IV that regulates plasma levels of the insulinotropic pro-glucagon. CAL-B catalyzes the ammonolysis of the ester with ammonium carbamate as the source of ammonia (Scheme 3).[24] The use of Ascarite and calcium chloride as adsorbents for carbon dioxide and ethanol by-products, respectively, increases the yield to 98% far surpassing traditional chemical routes, which yield 64% of the desired product.

A one-pot, biocatalyzed preparation of *N*-substituted amides from the corresponding carboxylic acid was reported, providing a valuable intermediate for the synthesis of Alfuzosin, a reducing agent of symptoms associated with benign prostatic hypertrophy. CAL-B catalyzes the esterification of the corresponding carboxylic acid and ethanol followed by an aminolysis reaction (Scheme 4).[25]

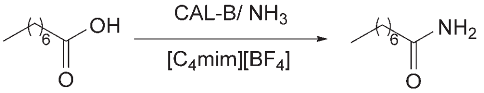


Scheme 3. Enzymatic ammonolysis using ammonium carbamate as the source of ammonia.



Scheme 4. Enzymatic one-pot preparation of amides from carboxylic acids.

Although some examples have been described, the enzymatic aminolysis of carboxylic acids in conventional solvents is less common than when esters are used as starting materials, due to the tendency of the reactants to form unreactive salts.[26] Recently, the use of ionic liquids has emerged in organic synthesis and in some cases it has represented an important advance in the biocatalytic field.[27] Ionic liquids represent a clean alternative to carry out processes that present serious difficulties in organic solvents or water. In addition, chiral ionic liquids have appeared in the last decade with new possibilities in asymmetric organic synthesis.[28] One of the first examples of the application of ionic liquids in biocatalysis was the synthesis of octanamide by reaction of octanoic acid and ammonia using CAL-B as biocatalyst (Scheme 5).[29]



Scheme 5. Enzymatic ammonolysis of octanoic acid in an ionic liquid.

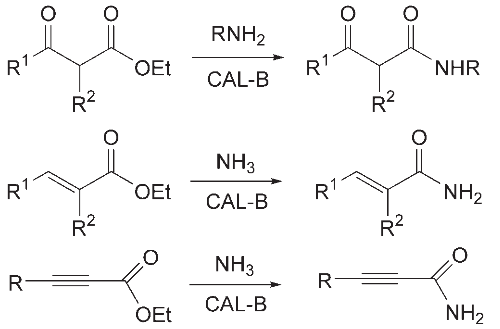
3 Chemoselective and Regioselective Procedures

The selective modification of a unique functional group in a polyfunctional molecule avoiding side-reactions and tedious protection/de-protection steps, is one of the most difficult tasks in organic synthesis, and is not an easy goal using conventional reactants, especially when the target molecule is not stable under drastic conditions (temperature, pressure, or others). Biocatalytic methods represent an attractive alternative for these chemoselective and regioselective transformations, and here are shown a few representative examples.

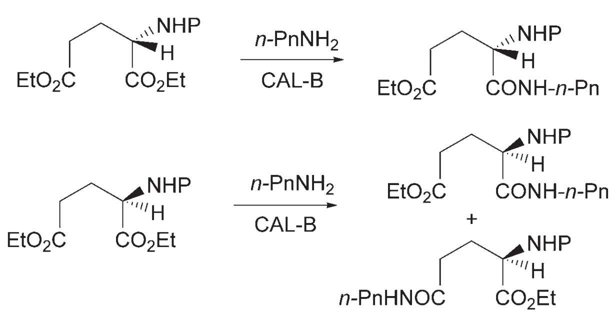
In Scheme 6 typical examples of useful enzymatic chemoselective processes are shown. Thus, the aminolysis and ammonolysis of β-keto esters yielded β-keto amides with high yields.[30] In the absence of enzyme only the enamino ester was obtained. In addition, a practical synthesis of acryl and propargyl amides was possible by enzymatic ammonolysis of acrylic and propargyl esters using CAL-B as biocatalyst.[31]

Sometimes the same functional group is present at different positions of a polyfunctionalized molecule, so the selective transformation of only one of these groups is not an easy task. Regioselective amidation has been described as an interesting solution to this problem. For instance, reaction of *N*-blocked glutamic acid diesters with *n*-pentylamine depended on the absolute configuration of the stereocenter and of the *N*-protecting group of the amino diester, in fact different molecular ratios of γ:α monoamides were obtained (Scheme 7).[32] In this manner, L-glutamic derivatives produced exclusively α-regioisomers, however D-glutamic substrates that react with lower reaction rates, mainly led to γ-derivatives, demonstrating that regioselectivity is dependent on the nature of the *N*-protecting group of the diester. New acetylcholinesterase inhibitors were also synthesized *via* a CAL-B-mediated regioselective amidation of *N*-substituted (*S*)-glutamic acid diesters using 4-(2-aminoethyl)-*N*-benzylpiperidine.[33] The regioselective amidation of different *N*-protected aspartic acid diesters was determined by the enantiomer used as substrate: L-aspartic derivatives preferentially undergo α-substitution, while D-substrate compounds showed different results influenced by the *N*-protecting group.[34]

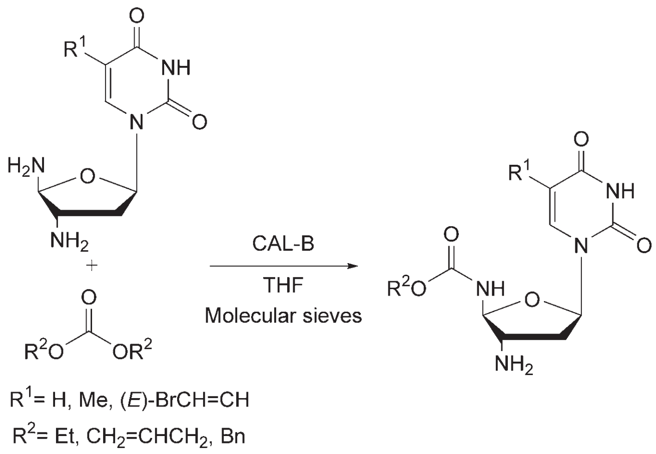
The regioselective enzymatic acylation of pyrimidine 3′,5′-diaminonucleoside derivatives was possible depending of the biocatalyst employed in the process.[35] *N*-5′-Acylated products were obtained using CAL-B as biocatalyst, while *Pseudomonas cepacia* lipase (PSL-C) was selective towards the *N*-3′-position. Molecular modeling studies were developed to explain the behavior of CAL-B in these enzymatic processes.[36] The alkoxycarbonylation of amines yields carbamates, which are valuable intermediates in medicinal chemistry because this functionality is part of various therapeutic agents since it allows, for example, a better permeability across the cellular membranes. Moreover, these processes also allow the protection of the amino group under mild conditions. The first regioselective enzymatic



Scheme 6. Chemoselective enzymatic processes catalyzed by CAL-B.



Scheme 7. Examples of regioselective reactions mediated by CAL-B.



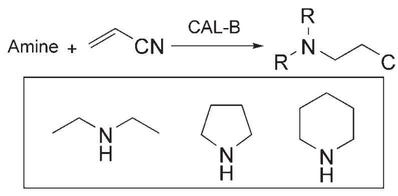
Scheme 8. Enzymatic alkoxycarbonylation of 3′,5′-diaminonucleosides.

alkoxycarbonylation of a primary amino group was achieved with pyrimidine 3′,5′-diaminonucleosides using CAL-B and non-activated homocarbonates allowing the synthesis of several *N*-5′-carbamates with moderate and high yields (Scheme 8).[37]

4 Non-Conventional Reactions

Generally, wild-type enzymes or mutants obtained by directed evolution methods are able to catalyze more than one specific reaction. Nowadays, the aim of many research groups is to use mechanistic reasoning to discover new enzymatic applications, or modify by directed evolution methods the wild-type enzyme to obtain an improved version of the original enzyme in terms of reactivity and selectivity. Among the lipases, CAL-B is the biocatalyst that has shown a major promiscuity.[13a] For instance, the reaction of acrylonitrile with different secondary amines in the presence of CAL-B led to the corresponding Michael adducts faster than in the absence of the biocatalyst (Scheme 9).[38]

The CAL-B mutant Ser105Ala was generated by site-directed mutagenesis and it has demonstrated an outstanding catalytic activity in aldol reactions.[39] Molecular modeling studies have provided a basis about the role of the oxyanion hole in CAL-B with regard to activating the aldehyde for addition, the histidine residue acting as a base, whereas the active site serine is not involved in this process.[40] In addition, three recent papers have quoted the potential of this mutant to catalyze Michael-type additions. This lipase, obtained by directed evolution methods, catalyzed the addition of acetylacetone to acrolein[13b] and the addition of thiols and diethylamine to α,β-unsaturated aldehydes (Scheme 10).[41]



Scheme 9. Michael-type addition of secondary amines to acrylonitrile catalyzed by CAL-B.

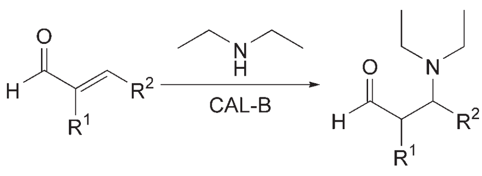
An example of competition between Michael addition and aminolysis processes is represented in Scheme 11 in the reaction of methyl acrylate with 1-(*N*,*N*-dimethyl)-3-propanediamine. The enzymatic aminolysis reaction was optimized using CAL-B wild-type as biocatalyst, focusing on a study of the influence of reactant concentrations, organic solvent, temperature, amount of enzyme and biocatalyst form to minimize the formation of the Michael addition products. The economic efficiency of this process was also investigated by reusing the enzyme in the best reaction conditions, and observing no significant loss of activity after three reaction cycles.[42]

5 Stereoselective Conventional Processes

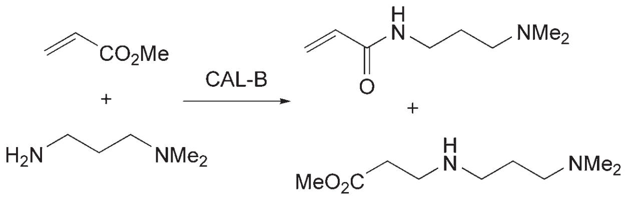
Traditionally, stereoselective biotransformations have been divided into two main groups: kinetic resolutions (KR) of racemates, which also include dynamic kinetic resolutions (DKR) and parallel kinetic resolutions (PKR) of racemic mixtures (Scheme 12), and on the other hand enantioselective enzymatic desymmetrization (EED) of prochiral or *meso* compounds.

5.1 Kinetic Resolutions

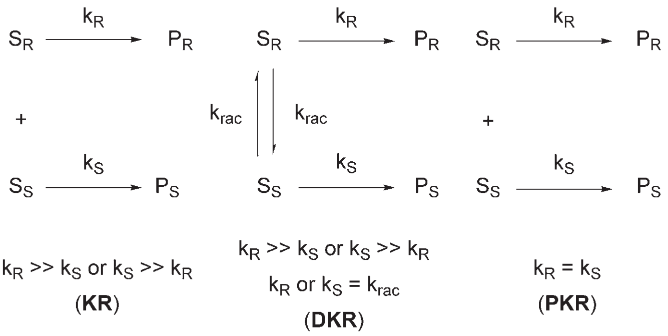
KR is based on a transformation, which subsequently makes easier the separation of the two enantiomers of the racemic substrate. It is the most common methodol-



Scheme 10. Enzymatic Michael addition of diethylamine to α,β-unsaturated aldehydes.



Scheme 11. Enzymatic amidation *versus* Michael addition.



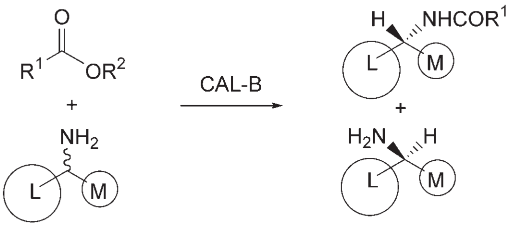
Scheme 12. Kinetic resolution (KR), dynamic kinetic resolution (DKR) and parallel kinetic resolution (PKR).

ogy used in biocatalysis in spite of the fact that the isolated yields of substrate and products are limited to 50%. There are different examples of this type of reactions and we have divided the application of CAL-B into three types depending of the reaction that it catalyzes.

5.1.1 Enantioselective Acylation and Alkoxycarbonylation of Amines

Chiral amines and amides are important classes of organic compounds because of their utility for the preparation of pharmaceutical and industrial products of relevant interest. In the last few years a great number of racemic monoamines have been resolved with CAL-B in different organic solvents, and some companies even use lipases for the production of enantiopure amines on a multi-kilogram scale.[43] The use of lipases in aminolysis reactions for the synthesis of nitrogenated organic compounds has been reviewed over the years,[44] and CAL-B has been identified as the best biocatalyst in most of the processes.

The schematic representation of a KR between racemic amines and non-activated esters using CAL-B as biocatalyst is shown in Scheme 13. In the same manner as in the transesterification of secondary alcohols, this process fits Kazlauskas' rule and as a consequence of this rule and some molecular modeling studies, an empirical model has been established for the active site of this enzyme.[45]

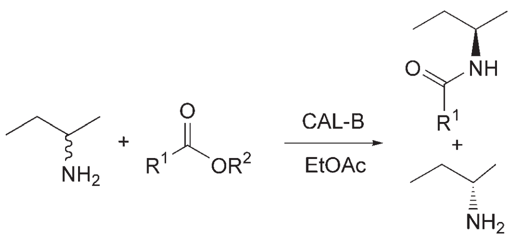


Scheme 13. Schematic representation of Kazlauskas' rule.

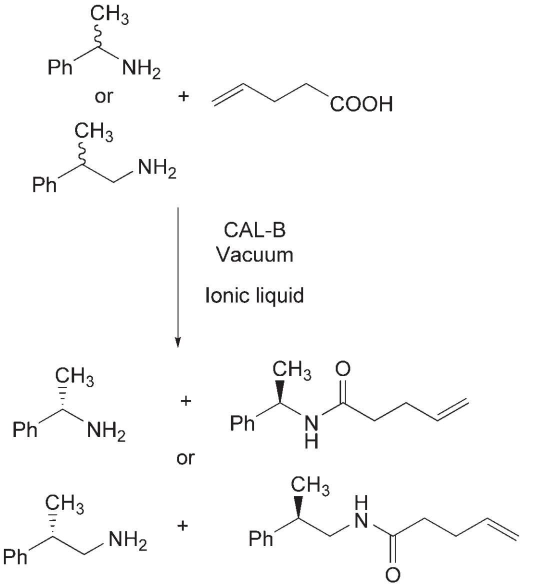
Normally, ethyl acetate is used in these processes as solvent and acyl donor, however, other acylating agents such alkyl methoxyacetates are also of utility, while vinyl esters, the best reagents for the resolution of alcohols, are inadequate reagents for the resolution of primary amines due to their high reactivity. In some cases the enantioselectivity can be increased using ethyl esters of long-chain fatty acids, for instance, in the acylation of *sec*-butylamine mediated by CAL-B (Scheme 14).[46]

Amines and carboxylic acids usually present solubility problems because they are high polar substrates but also the formation of salts represents an important inconvenience. To overcome these limitations Irimescu and Kato have recently described KR in ionic liquids instead of organic solvents (Scheme 15). The resolution with CAL-B is based in the fact that the reaction equilibrium was shifted toward amide synthesis by the removal of water under reduced pressure.[47]

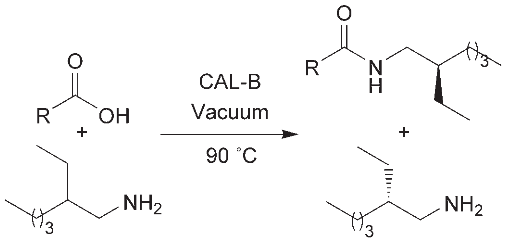
Non-solvent systems have also been employed in enantioselective amidation processes, reacting racemic amines with aliphatic acids (Scheme 16). The best conversion of acids to amides was observed using CAL-B at 90 °C under vacuum, no amidation occurred in the ab-



Scheme 14. Bioresolution of *sec*-butylamine.



Scheme 15. KR of 1-phenylethylamine and 2-phenyl-1-propylamine using ionic liquids.



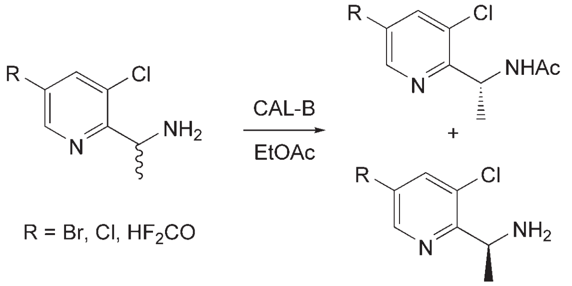
Scheme 16. Biocatalytic amidation of carboxylic acids in a non-solvent system.

sence of enzyme or in the presence of organic solvents.[48]

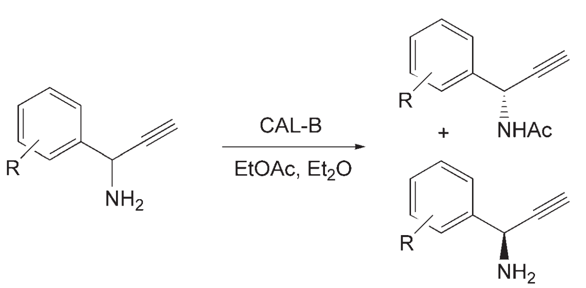
In the last few years many examples of KRs of primary amines have been published, for instance, 1-(heteroaryl)-ethylamines can thus be prepared in high optical purity.[49] Also, a great number of bicyclic 1-heteroaryl primary amines has been obtained with excellent enantiomeric excesses using this methodology.[50] Enzymatic resolution of (±)-2-(1-aminoethyl)-3-chloro-5-substituted-pyridines led to the (*R*)-amide according to Kazlauskas' rule in the CAL-B-catalyzed reaction with ethyl acetate (Scheme 17), however the *E* value was only moderate ($E=27$).[51]

Chiral 1-aryl-2-propynylamines are important synthons for the preparation of numerous biologically active compounds.[52] Resolution of these derivatives can be carried out employing CAL-B as biocatalyst and ethyl acetate as acyl donor in diethyl ether, obtaining in general the (*R*)-acetamides and the (*S*)-amines with high enantioselectivities and moderate to high yields (Scheme 18).[53] The same approach was successfully developed for the resolution of a variety of substituted 1-aryl-2-propenylamines.[54]

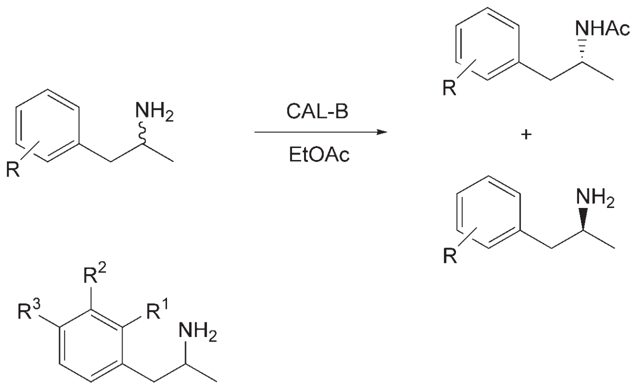
Enzymatic aminolysis has also provided the resolution of amphetamine and related derivatives, like *o*-, *m*-, and *p*-methoxyamphetamines using CAL-B as enzyme, and ethyl acetate as acyl donor and solvent (Scheme 19).[55] These compounds present different physiological activities depending of the type and position of the substitution in the phenyl ring or on the nitrogen atom. For example, the presence of methoxy groups on the aromatic ring or methyl groups on the nitrogen atom increase the activity of the drug, and the stereochemistry of these amines has a great influence on their pharmacological properties.[56]



Scheme 17. Kinetic resolution of 2-(1-aminoethyl)-3-chloro-5-substituted-pyridines.



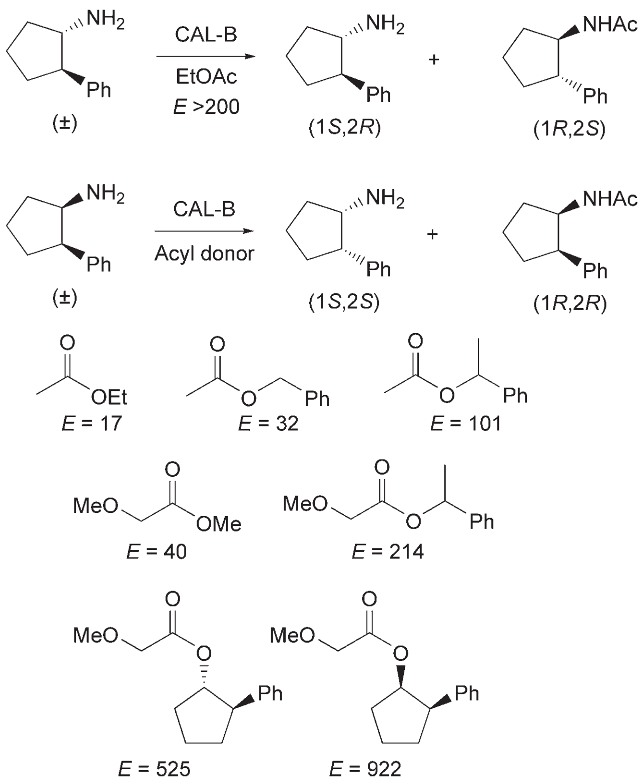
Scheme 18. Kinetic resolution of 1-aryl-2-propynylamines.



$R^1 = R^2 = R^3 = H$, $E = 37$
$R^1 = OMe$, $R^2 = R^3 = H$, $E = 79$
$R^1 = R^3 = H$, $R^2 = OMe$, $E = 70$
$R^1 = R^2 = H$, $R^3 = OMe$, $E = 52$

Scheme 19. Enzymatic resolution of amphetamine derivatives by aminolysis processes.

2-Phenylcyclopentanamines are precursors of semicyclic amidines with potent hypoglycemic activity,[57] and *trans*-2-phenylcyclopentanamine (cypenamine) is used as an antidepressant.[58] The KR of (±)-*trans* and (±)-*cis*-2-phenylcyclopentanamines has been effectively performed by CAL-B-catalyzed aminolysis reaction (Scheme 20). Although the reaction between (±)-2-*trans*-phenylcyclopentanamine and ethyl acetate proceeded with high enantioselectivity, the corresponding acylation of the *cis*-isomer under the same conditions occurred with low enantiopreference. To improve the *E* value of the *cis*-isomer several acyl donors were tested, achieving the best results when the leaving group more closely resembles the amine.[59] The KR of compounds which differ in the ring size has also been studied, observing good enantioselectivities with CAL-B in the reaction with *trans*-phenylcyclohexanamine, however, this biocatalyst is not efficient for the *cis*-isomer or *trans*-phenylcyclopropanamine, where CAL-A showed a better behavior.[60]

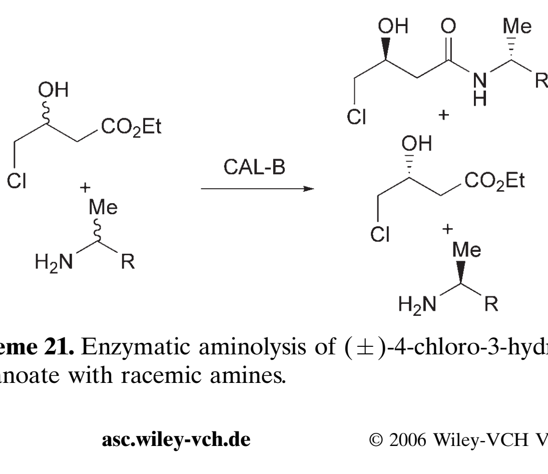


Scheme 20. Kinetic resolution of (±)-*trans*- and (±)-*cis*-2-phenylcyclopentanamines.

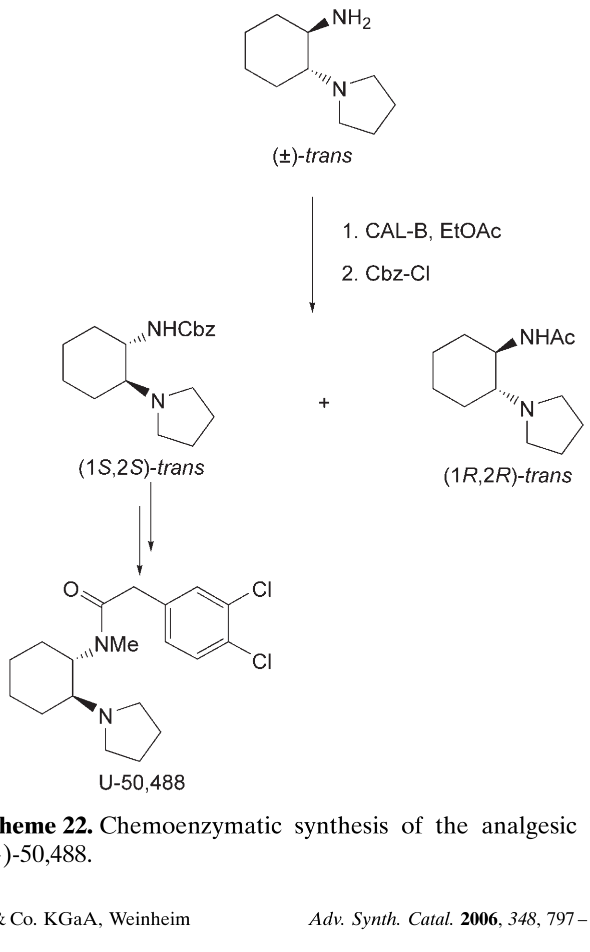
A number of 1- and 2-aminoalkanephosphonates were resolved with high enantioselectivity by CAL-B-catalyzed acetylation using ethyl acetate, obtaining precursors of the corresponding aminoalkanephosphonic acids.[61] CAL-B also catalyzes the amidation between ethyl (±)-4-chloro-3-hydroxybutanoate and racemic amines, leading to the corresponding optically active amides with very high enantiomeric and diastereomeric excesses (Scheme 21).[62] Besides, remaining ester and amine are recovered with good enantiomeric excesses. In this way, three enantioenriched compounds are obtained from an easy one-step reaction.

Enantiomers or diasteroisomers often show opposite behaviors in their pharmacological properties, for this reason the selective preparation of chiral drugs is of outstanding importance. Recently, the chemoenzymatic synthesis of the analgesic U-(−)-50,488 has been described. In this case the (1*S*,2*S*)-isomer exhibits a higher activity than the counterpart, and also than the *cis* isomers. The key step was the stereospecific formation of (±)-*trans*-2-(pyrrolidin-1-yl)cyclohexanamine and subsequent enzymatic resolution using CAL-B and ethyl acetate, with the acetylation of the (1*R*,2*R*)-diamine occurring with high enantioselectivity ($E=170$). To facilitate the isolation of the (1*R*,2*R*)-amide (94% enantiomeric excess) and the unreacted (1*S*,2*S*)-diamine (99% enantiomeric excess), the reaction mixture was treated with benzyl chloroformate. After conventional chemical processes through the carbamate of the diamine, it is possible to obtain the drug with very high yield and without racemization (Scheme 22).[63]

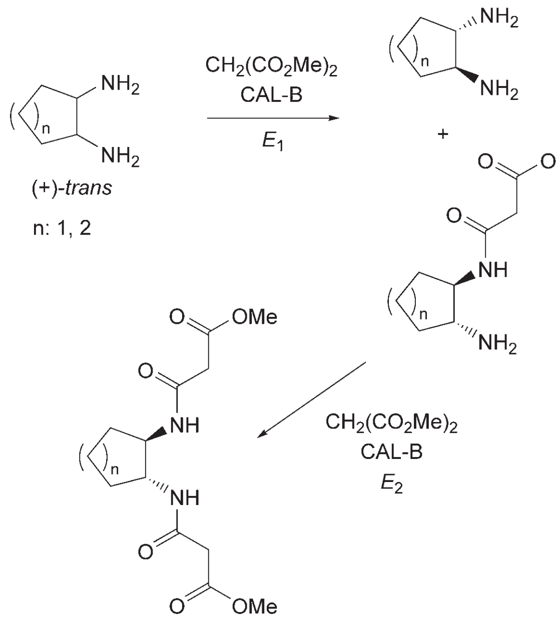
In some cases sequential biocatalytic resolutions by one-pot double enzymatic reaction are of great utility because, with moderate enantioselectivity in both processes, it is possible to achieve a high enantiomeric excess of both substrate and product. An example is the resolution of *trans*-cyclohexane-1,2-diamine and *trans*-cyclopentane-1,2-diamine with dimethyl malonate and using CAL-B as biocatalyst (Scheme 23).[64] The formation of the (*R*,*R*)-bisamido ester involves two biocatalytic steps, and the enzyme shows the same stereochemical prefer-



Scheme 21. Enzymatic aminolysis of (±)-4-chloro-3-hydroxybutanoate with racemic amines.



Scheme 22. Chemoenzymatic synthesis of the analgesic U-(−)-50,488.



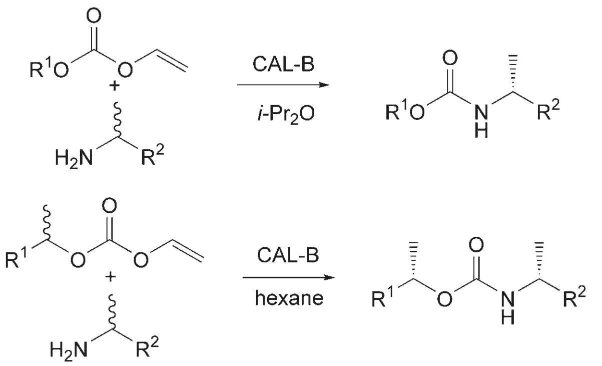
Scheme 23. Sequential kinetic resolution of cyclic 1,2-diamines.

ence towards the (*R*,*R*)-enantiomer of the substrate in both reactions, however, the enantioselectivity of the second step is always higher which is in good agreement with Kazlauskas' rule, because the monoacylated compound has a bigger steric difference in the substituents of the stereocenter than the corresponding free diamine. This bisamido ester is an excellent synthon for the preparation of aza-macrocycles.

CAL-B has also shown its potential in the enantioselective alkoxycarbonylation of amines. Alkyl carbonates are much less reactive than the corresponding esters in enzymatic processes, so in this type of reactions benzyl or allyl carbonates together with oximes or vinyl carbonates are the most efficient alkoxycarbonylating reagents and very useful for alkoxycarbonylating alcohols or amines.[65] In Scheme 24 the kinetic resolutions of some racemic amines with vinyl alkyl carbonates catalyzed by CAL-B are shown.[66] In addition, the double enantioselective lipase-catalyzed alkoxycarbonylation of racemic amines with racemic vinyl carbonates has been carried out using CAL-B, yielding carbamates with two stereogenic centers. This process shows that the combination of an enzyme with an adequate vinyl carbonate and amine can yield urethanes with two stereogenic centers in one step and with high diastereo- and enantioselectivity.[67]

5.1.2 Resolution of Esters

The enzymatic resolution of esters *via* aminolysis or ammonolysis processes represents an efficient alternative to the resolution of substrates by transesterification

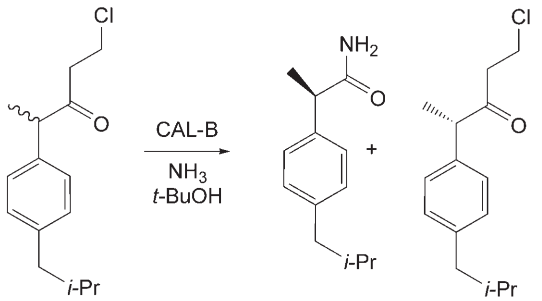


Scheme 24. Kinetic resolution of amines with vinyl alkyl carbonates.

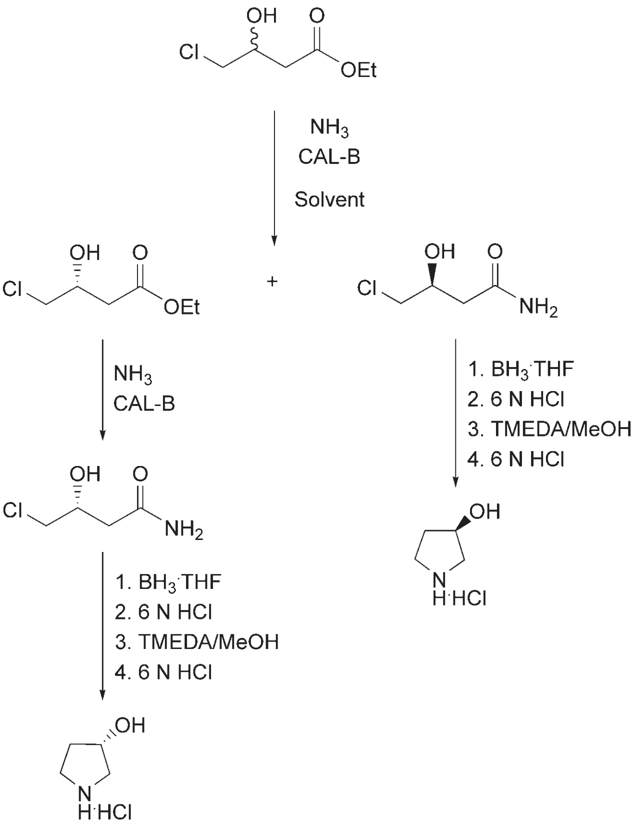
and hydrolysis processes. For instance, the ammonolysis of ibuprofen 2-chloroethyl ester reached higher enantioselectivities than the enzymatic hydrolysis (Scheme 25).[21] Application of enzymatic procedures for the synthesis of interesting drugs has also been demonstrated through enantioselective aminolysis of racemic isobutyl 2-[3-(4-cyanophenyl)-4,5-dihydro-5-isoxazolyl]acetate, the precursor of Roxifiban, a non-peptide platelet glycoprotein IIb/IIIa antagonist with antithrombotic activity, catalyzed by CAL-B.[68]

Enantiomerically enriched pyrrolidin-3-ol derivatives are precursors of alkaloids and other interesting compounds in medicinal chemistry, and can be prepared *via* enzymatic ammonolysis of ethyl (±)-4-chloro-3-hydroxybutanoate. The optically pure amides obtained are starting materials for the preparation of both isomers of pyrrolidin-3-ol (Scheme 26).[69] The enantiomerically enriched ester (98% enantiomeric excess at 60% conversion) was better converted in the corresponding (*R*)-amide by biocatalytic reaction than by chemical reaction because, in the absence of the enzyme, other side products were formed.

Interesting aminolysis processes of resolution by chiral axes, instead of a stereogenic carbon atom, have been recently reported by Aoyagi et al.[70] The side chain of the binaphthyl moiety played an important role in the lipase-catalyzed amidation of 1,1′-binaphthylamines.[70a]



Scheme 25. Ammonolysis of ibuprofen 2-chloroethyl ester.



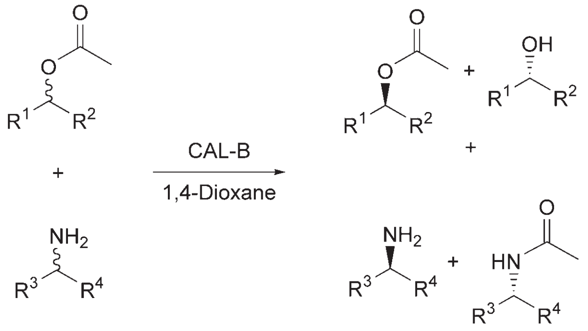
Scheme 26. Chemoenzymatic synthesis of pyrrolidin-3-ol.

In the study of the different recognition of (*E*)- and (*Z*)-1,1′-binaphthyl ketoximes using lipase-catalyzed reactions, this group has also investigated different possibilities for the resolution of binaphthyl esters and binaphthylamines.[70b] Thus, two forms of *Candida antarctica* lipase B (Novozyme 435 and Chirazyme L-2) catalyzed the resolution of a binaphthyl ester, which has an ethylene spacer between the naphthyl ring and the ester group (Scheme 27).

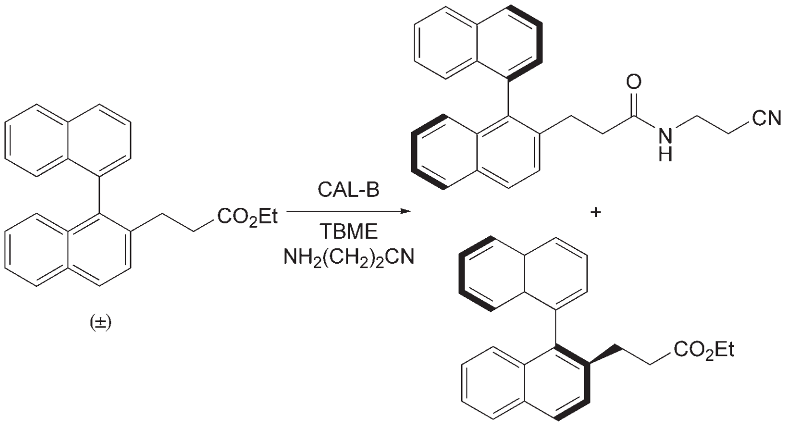
An elegant and effective procedure based on the one-pot resolution of racemic secondary alcohol ester derivatives with racemic amines catalyzed by CAL-B allowed the formation of four products (ester, amine, alcohol and amide) with excellent enantiomeric excesses (Scheme 28).[71] In all cases the corresponding ester and the amine were in an equimolar ratio and anhydrous conditions were absolutely necessary in order to avoid the competitive enzymatic hydrolysis of the ester.

5.1.3 *Resolution of* β-*Amino Esters and* β-*Amino Nitriles*

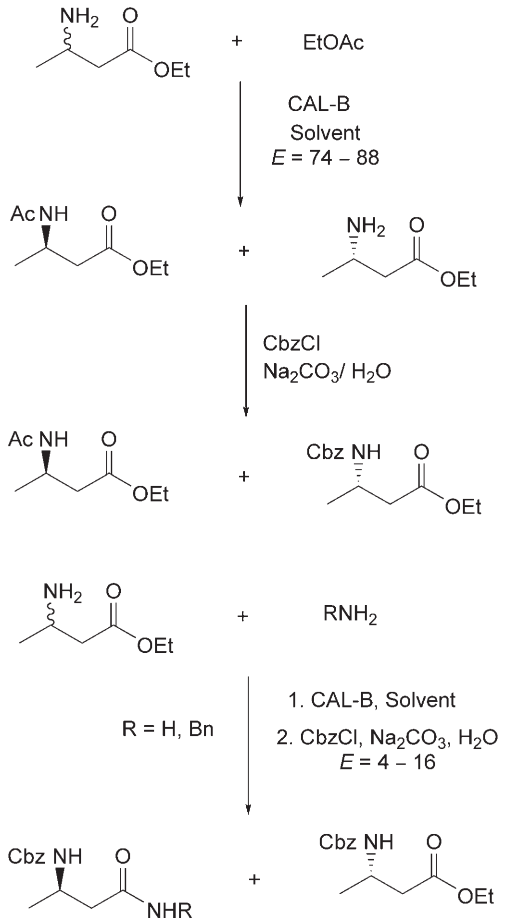
The preparation of optically active β-amino esters and the corresponding amino acids is an area of special relevance in medicinal chemistry. These compounds can be easily prepared in racemic form, and it is possible to carry out a biocatalytic resolution by acylation of the amino group or by hydrolysis, transesterification or aminolysis through the ester function. Racemic β-aminobutyrate esters having the amino function protected have been recently resolved by enantioespecific transesterification using *iso*-butyl alcohol and CAL-B as biocatalyst.[72] However, it is also possible to efficiently resolve ethyl (±)-3-aminobutyrate (Scheme 29), in this case the resolution by acylation of the amino group ($E = 74 - 88$) be-



Scheme 28. Enzymatic one-pot resolution of alcohol and amines.



Scheme 27. Kinetic resolution of a binaphthyl ester.

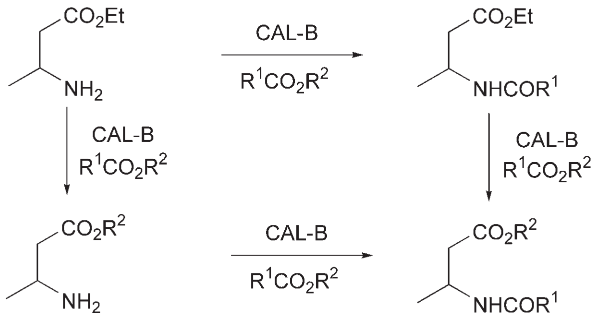


Scheme 29. Enzymatic resolution of β-amino esters.

ing more selective than the aminolysis of the ester (E = 4–16).[73]

In some cases there is no suitable enzyme or reaction conditions for the efficient resolution of a given substrate. In these situations some substrate engineering can be undertaken. In this context, maybe one of the most useful ways of increasing the enantiomeric excess of the final product is to perform a sequential biocatalytic process, which involves several enzymatic reactions. The enantioenriched product of the first step undergoes another process that increases even more the enantiomeric excess of the final product. Kanerva et al. applied this methodology for the resolution of ethyl 3-aminobutanoate (Scheme 30).[74] Several enzymes and acyl donors were tested, and a preparation of lipase B from *Candida antarctica* (Chirazyme L2) has shown the best results. Years later the same methodology was applied for the resolution of other five β-amino esters, which showed that the possibility of the sequential resolution became less likely with increasing the size of the substrate from ethyl 3-aminobutanoate to pentanoate or larger.[75]

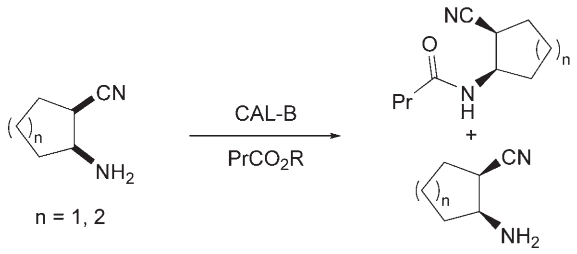
CAL-B catalyzed the resolution of racemic *cis*-2-aminocyclopentane- and cyclohexanecarbonitriles affording satisfactory results, however, PSL was the most efficient biocatalyst for these processes and also when *trans*-isomers were used (Scheme 31).[76] For the resolution of other difunctional compounds such as 1,2- or 1,3-amino alcohols the *O*-acylation resolution is recommended over the *N*-protected amino alcohol.[77] α-Amino nitriles are important intermediates in the synthesis of amino acids and amino acid amides, and three different substrates were resolved by enzymatic acylation in the presence of CAL-B, thereby observing an excellent preference for the (*S*)-enantiomer with ethyl phenylacetate while the reaction with ethyl acetate was accompanied by an unexpected turnover-related racemization of the product.[78]



Scheme 30. Resolution of ethyl 3-aminobutanoate by a transesterification-aminolysis sequence.

5.2 Dynamic Kinetic Resolution of Amines

The fact that sometimes only one enantiomer of a racemic mixture is required constitutes a disadvantage of KRs and different approaches have been developed to overcome this limitation. The one to which more attention has been recently paid is the dynamic kinetic resolution (DKR), which involves the combination of an enantioselective transformation with an *in situ* racemization process affording enantiomerically pure compounds in yields approaching 100%. Nowadays, the most common methodology involves a lipase as biocatalyst and a metal-organic complex as chemical catalyst responsible of the substrate racemization.



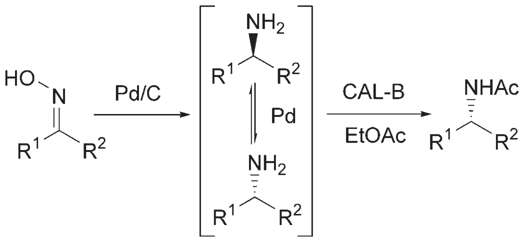
Scheme 31. Resolution of β-amino nitriles by enzymatic acylation.

A new concept has been added in the resolution of racemic mixtures: this is the case when both enantiomers of a substrate react with similar rates to give two different products with high enantiomeric excess; this strategy is called parallel kinetic resolution (PKR). Although, CAL-B can catalyze a wide range of KR and some DKR, usually oxidoreductases are more versatile biocatalysts for PKR processes.[79]

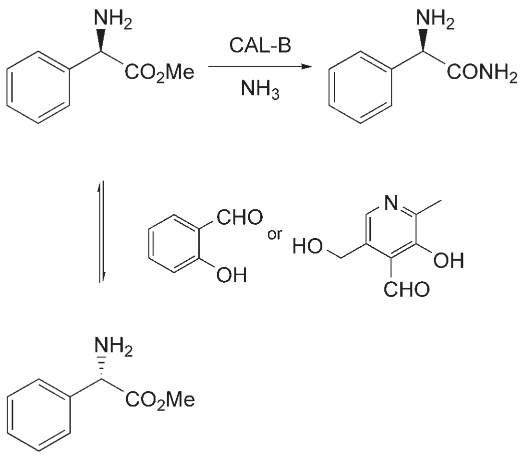
Examples of DKR through enzymatic aminolysis reactions are scarcely described in the literature. Kim and co-workers described the DKR of prochiral ketoximes that were transformed to optically active amines in the acetylated forms by coupled lipase-palladium catalysis in the presence of an acyl donor under 1 atm of hydrogen, and using *N*,*N*-diisopropylethylamine to suppress the reductive elimination of the amino group (Scheme 32).[80] This procedure improved the results and versatility of the first DKR catalyzed by CAL-B as reported by Reetz.[81]

D-Phenylglycine and its 4-hydroxy derivative are present in the side-chain of β-lactams. DKR of phenylglycine esters was reported *via* lipase-catalyzed ammonolysis combining the use of CAL-B with chemical reactants like pyridoxal or salicylaldehyde, the reagents being responsible for the *in situ* racemization at low temperature of the unconverted ester, affording D-phenylglycine amide with 88% enantiomeric excess and 85% conversion and observing a slower racemization of the amide in comparison with the ester (Scheme 33).[82] A synthesis of optically active α-amino acid derivatives was developed by Turner and co-workers through CAL-B-catalyzed DKR of racemic 2-benzyl-4-substituted-5(4*H*)-oxazolones in the presence of alcohol and optimized solvent conditions and amount of NEt_3.[83]

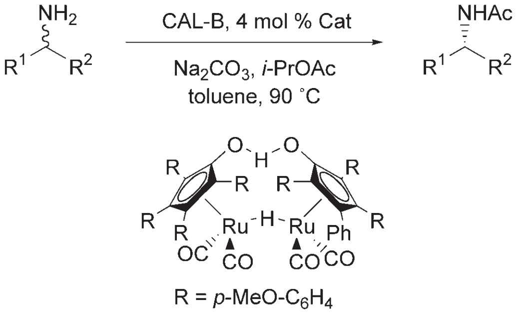
Bäckvall and co-workers described the use of a ruthenium hydrogen-transfer catalyst for the racemization process in combination with CAL-B-mediated acetylation of 1-arylethylamines, obtaining the corresponding acetamides in good yields and almost in enantiopure form.[84] Recently, the same research group has reported a more versatile DKR of several primary amines using a ruthenium catalyst, CAL-B, isopropyl acetate and sodium carbonate (Scheme 34).[85] An efficient DKR process takes place affording the corresponding amides with high yields and enantiomeric excesses, however, a high temperature (90 °C) is still needed.



Scheme 32. DKR of racemic amines from ketoximes.



Scheme 33. DKR of phenylglycine methyl ester.

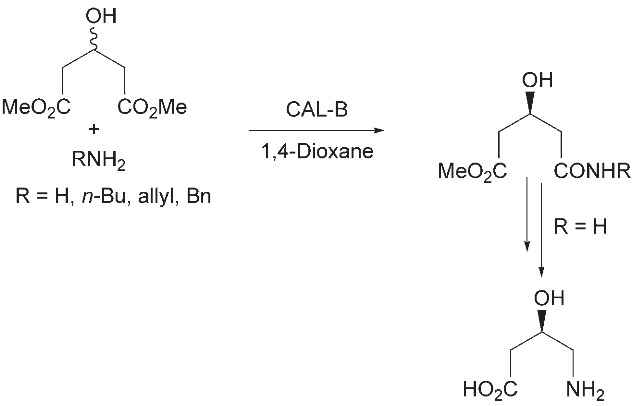


Scheme 34. DKR of primary amines with sodium carbonate as additive.

5.3 Desymmetrization of Prochiral Glutarates

EEDs imply the formation of one or more chirality elements in a substrate, so allowing a maximum yield of 100%, and for this reason they constitute a very interesting alternative to KRs for the preparation of optically active compounds.[4] Although many examples of enzymatic hydrolysis or transesterification have been published, only ammonolysis and aminolysis processes have been described for the desymmetrization of prochiral glutarates. The first example consisted in the enzymatic aminolysis and ammonolysis of dimethyl 3-hydroxyglutarate with different amines and ammonia catalyzed by CAL-B that exclusively led to the corresponding (*S*)-monoamide (Scheme 35).[86] The same reaction has been carried out for the synthesis of chiral amido esters.[87] In this manner, the enantiopure monoamides obtained in the reaction with ammonia have been used in the preparation of (*R*)-GABOB (3-hydroxy-4-aminobutanoic acid), a biologically interesting γ-amino acid precursor of (*R*)-carnitine.[86]

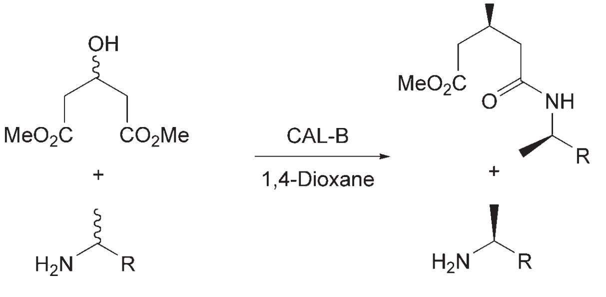
A double process of desymmetrization-resolution has been carried out in the reaction of dimethyl 3-hydroxy-



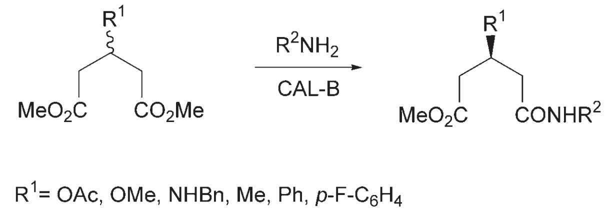
Scheme 35. Desymmetrization of dimethyl 3-hydroxyglutarate for the preparation of (*R*)-GABOB.

glutarate with 1-phenylethylamine or 2-furylethylamine. The process takes place under similar conditions to those given for the resolution of 4-chloro-3-hydroxybutanoate using CAL-B, which showed a total enantioselectivity toward the (*R*)-isomer of the amines, hence (3*S*,1*R*′)-amido esters were obtained with high selectivity (Scheme 36).[62]

3-Aminoglutarate derivatives are valuable synthons for the preparation of 3,4-diamino acids. For instance, dimethyl 3-benzylaminoglutarate can be prepared by a Michael-type addition of benzylamine to dimethyl glutaconate and later desymmetrized through enzymatic amonolysis and aminolysis reactions.[88] The enzymatic aminolysis and ammonolysis desymmetrization of alkyl and aryl glutarates were also studied, showing that conversions and enantiomeric excesses clearly depended on the substrate structure.[89] Thus, when the substituent in the 3 position had a heteroatom (O, N) the process was very efficient, leading quantitatively to the enantiopure monoamide. However both enantiomeric excess and yield dropped with aliphatic or aromatic groups (Scheme 37).



Scheme 36. Desymmetrization-resolution of dimethyl 3-hydroxyglutarate.



Scheme 37. Desymmetrization of 3-substituted glutarates.

6 Actual Trends

Lipases have been established as valuable catalysts in organic synthesis in order to perform chemo-, regio- and stereoselective processes. However natural enzymes do not always show high activity and enantioselectivity. For that reason many improvements have been made in order to enhance the selectivity and reactivity of the biocatalyst during the enzymatic reaction. Among them the use of ionic liquids,[90] additives like triethylamine or crown ethers, and inhibitors that involves the participation of the slow-reacting enantiomer of a racemate have attracted great attention.[91]

Advances in structural biology have revealed the three-dimensional structures of many enzymes which has permitted, through molecular modeling techniques, the explanation of mechanistic aspects and experimental results. These studies have provided a detailed basis for predictions of how to increase selectivity by modifying the amino acid sequence of the enzyme or through substrate modifications.[92]

Nowadays, rational strategies for directed evolution of enzymes have been applied to CAL-B for increasing thermostability properties,[93] discovering applications in non-conventional processes for lipases like aldol reactions or Michael additions,[5,13,39–41] expanding the substrate specificity[12] and also for altering enantioselectivity properties.[94] One of the most important keys to success is the availability of suitable high-throughput screening systems, methods that allow the rapid and reliable identification of active lipase mutants.[95]

7 Summary and Outlook

Biocatalysis represents a well-established methodology for the chemo- and regioselective modification of non-chiral compounds, resolution of racemates and desymmetrization of prochiral substrates. Simplicity of use and their friendly environmental properties make lipases powerful tools for the production of important intermediates, and among them CAL-B is so far one of the best biocatalysts. In this review we have shown how CAL-B-catalyzed processes in different solvent systems have provided potential applications for the preparation of amines and amides, compounds which play an important role in the production of fine chemicals and pharmaceuticals. Nowadays, it is clear that for the resolution of primary amines this lipase is the best enzyme, however CAL-A proves to be more appropriate for the resolution of secondary amines. In addition, the combination of genetic engineering and molecular modeling techniques are playing a major role in the development of enzymes that will show in the future better results than those currently achieved, and will offer new pathways and possibilities for the synthesis and resolution of interesting products for the industrial sector.

Acknowledgements

Financial support of this work by the Spanish Ministerio de Educación y Cultura (Project CTQ-2004-04185) and European Union (EU-04-LSHB-2003-503017) is gratefully acknowledged. VGF and EB thank also the MEC for personal grants (Program Juan de la Cierva and predoctoral fellowship).

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