CHEMICAL REVIEWS

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# Hydrolases in the Stereoselective Synthesis of *N*-Heterocyclic Amines and Amino Acid Derivatives

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## 1. INTRODUCTION

Chiral nitrogenated compounds are highly attractive compounds because of their importance in agrochemicals, fine chemicals, and pharmaceuticals but also due to their remarkable properties in organocatalytic reactions.[1] Among them, the synthesis of amines and amino acids is gaining increasing attention in recent years, as is concluded from their interesting applications in different research fields.[2] For example, α- and $\beta$-amino acids are pivotal structural elements in peptide synthesis, and other biologically active compounds, such as $\beta$-lactams, possess important antibiotic properties: facts that highlight the importance of developing asymmetric syntheses for these classes of compounds.[3−5] On the other hand, nonracemic chiral amines are highly demanding intermediates in drug syntheses, although they are not easily prepared.[6−9] From all the family of optically active nitrogenated compounds, probably prolines, pipecolic acid derivatives, indole alkaloids, or penicillins have attracted the major attention because of their applications in asymmetric organocatalysis and therapeutic uses (Chart 1).

Biocatalytic processes are used as very effective tools to perform a wide variety of chemical transformations allowing the preparation of very different families of organic compounds. Interestingly, the use of enzymatic sources allows the development of chemo-, regio-, and stereoselective processes under aqueous medium but also in organic or neoteric solvents.[10−16] Interesting revisions have appeared in recent years showing general strategies for the production of optically active amines,[17,18] amino acids,[19] or both.[20] This article focuses on the description of current existing methods for the preparation of optically active cyclic secondary amines and amino acids using hydrolases, probably the most studied class of enzymes, due to their advantages for the industrial sector.[21] Hydrolytic enzymes are considered ecofriendly and stable catalysts, which allow the performance of clean chemical processes under mild reaction conditions, including the use of atmospheric pressure or a wide range of temperatures and pH values, usually acting with a high degree of selectivity.[22] As mentioned

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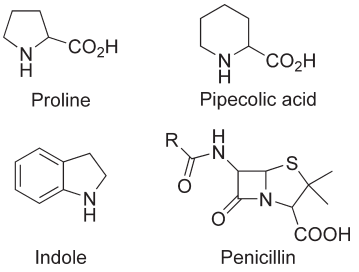
**Chart 1**




above, we will pay attention to the development of stereoselective processes taking advantage of the chiral composition of enzymes, made from L-amino acids in most of the cases, an intrinsic structural property that made them efficient chiral catalysts *via* the formation of the enzyme−substrate complex.[23]

The versatility of enzymes has also been demonstrated since the discovery that hydrolases and other enzymatic families can work not only in water, their "natural environment", but also in organic solvents.[11] Additionally and although their natural function is the hydrolysis of chemical bonds, they have also been applied to the synthesis of organic compounds through transesterification, interesterification, aminolysis, ammonolyis, hydrazynolisis perhydrolysis, or thiolysis reactions.[24−30] Recently, a wealth of studies has demonstrated the promiscuous behavior of hydrolytic enzymes, where mainly lipases and acylases have shown good levels of reactivity in C−C bond formation, C-heteroatom formation, oxidative processes, and novel hydrolytic reactions.[31,32]

In this manuscript we aim to review the asymmetric synthesis of three big families of cyclic nitrogenated organic compounds such as amines, amino acids, and $\beta$-lactams, with hydrolases being used in a crucial step for the production of these optically active products. Stereoselective transformations that affect the cyclic structure of the products will be covered. Thus, examples reported toward the synthesis of chiral amines will be first described, initially considering the modification of the amino group by acylation or alkoxycarbonylation reactions, and later the selective transformation suffered by any other positions of the nitrogenated cycle, mainly by hydrolytic or acylation processes, but also depending on the presence or not of a protecting group in the amine nitrogen. Next we will move forward to the description of stereoselective changes suffered by amino acid derivatives, which because of their intrinsic bifunctionality have allowed us to distribute this section in three main divisions depending on the functional group subjected to an enzymatic modification (amino group, carboxylic group, or other functionalities in the heterocyclic ring). Finally and because of the importance and medical implications of lactams, transformations carried out by hydrolytic enzymes will be exhaustively analyzed considering the modification at the amino or the carboxylic group. Special attention will be turned to the preparation of $\beta$-lactams, cyclic amides present in a wide series of antibiotics, for instance carbapenems, cephalosporins, monobactams, or penicillins.

## 2. STEREOSELECTIVE BIOTRANSFORMATIONS OF CYCLIC AMINES AND AMINE DERIVATIVES

Enantiomerically pure secondary amines are remarkable building blocks for the synthesis of biologically active compounds, as this motif is present in the structure of many pharmaceuticals and agrochemicals. Unfortunately, asymmetric chemical conventional methods usually employ drastic conditions or expensive catalysts in very different processes, such as resolutions using chiral salts, reductive aminations, hydrogenations, hydrosylilations, or alkylations of imines. Biocatalysis provides green alternatives for the production of this important class of compounds, traditionally using hydrolases, and more recently monoamine oxidases or transaminases.[17,18] Here, we have covered examples that appeared in the literature employing hydrolases for the stereoselective modification of cyclic secondary amines. Direct transformation of the cyclic amino group or alternatively the modification of functionalities directly linked to the heterocyclic structure will only be considered.

### 2.1. Modifications at the Amino Group

The enzymatic protection of amines is a process much less investigated than the corresponding reaction using alcohols, and this is because of two major drawbacks: (a) Amines are more nucleophilic than alcohols and usually present problems because of the formation of unwanted products. (b) Esters can be readily deprotected under basic or enzymatic conditions, whereas amides or carbamates require harsher reaction conditions to liberate the free amine. Although the applications of hydrolases have fulfilled the resolution of primary amines during the last two decades, the resolution of secondary amines *via* enzyme-catalyzed modifications of the amino group is a relatively rare process, which has attracted the attention of only a few researchers in recent years. Mainly two hydrolase-catalyzed strategies have been considered for the stereoselective modification of the amino functionality, acylation processes catalyzed by lipases using nonactivated esters, and lipase or protease mediated alkoxycarbonylation reactions using carbonates. Both of them will be next described in detail.

**2.1.1. *N*-Acylations.** Although with low stereoselectivity Asensio et al. studied the acetylation of 2-piperidinyl methanol (**1**), testing porcine pancreas lipase (PPL) and lipase Amano P from *Pseudomonas fluorescens* as biocatalysts (Scheme 1).[33] Although the starting material (+)-**1** was recovered with low enantiomeric excess and high yield when using PPL, the *N*-acetylated product (−)-**2a** was recovered in moderate to high optical purities depending on the conversion grade. On the other hand, lipase Amano P led to the formation of a complex mixture of optically active starting material, the *N*-acetylated product (−)-**2a**, and the *N*,*O*-diacetylated compound (−)-**3**.

Morgan et al. reported the chemoenzymatic synthesis of SCH66336, a nonsulfhydryl farnesyl protein transferase inhibitor. The racemic piperidine **4**, which exists as pairs of conformational enantiomers due to atropisomerism about the exocyclic double bond, was used as a key intermediate for the synthesis of the already mentioned drug. The enzymatic hydrolysis and acylation of adequate intermediates were studied using over 300 enzymatic preparations, and although none of them showed significant results in the deacylation processes, better activities were found in the acylation reaction. The experimental conditions were optimized in terms of acylating agent, solvent, and moisture content, identifying the combination of lipase Toyobo LIP-300 and trifluoroethyl isobutyrate as the best tandem for the enantioselective isobutyrylation of the (+)-enantiomer (Table 1).[34] Because of the high loading of enzyme (ratio 2:1 w/w enzyme/substrate) required for the achievement of 50% conversion with an excellent selectivity when using a bulky acylating agent, other acyl donors were tested, but the enantiodiscrimination showed by the lipase was considerably lower in shorter reaction times (entries 2 and 3). Finally, the authors successfully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**Scheme 1**

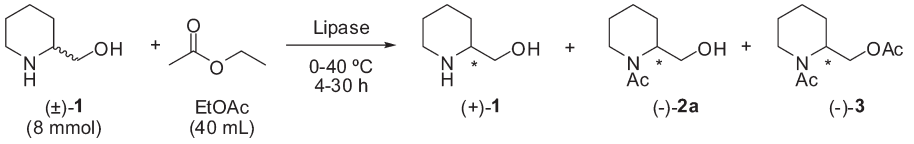
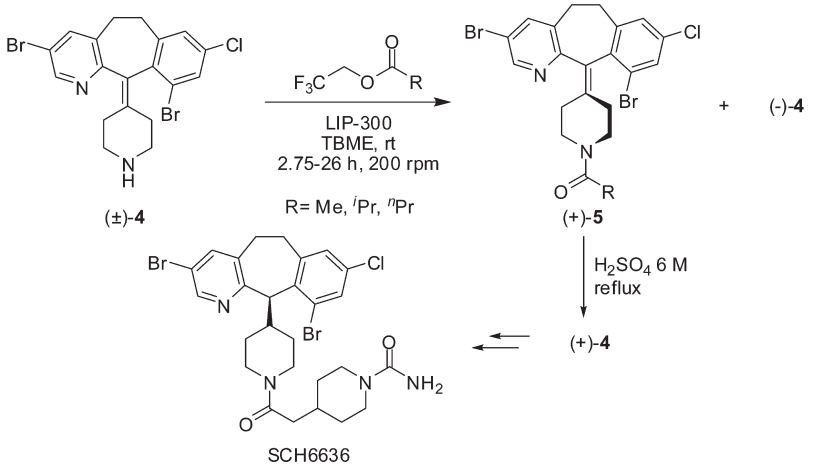



**Table 1. Acylation Reaction of Racemic Amine 4 with Trifluoroethyl Esters in TBME**



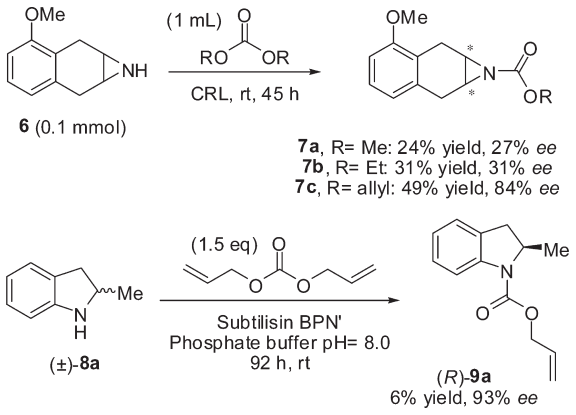

| entry | R | $t$ (h) | $ee_P$ (%) | $ee_S$ (%) | $c$ (%) | $E$ |
|---|---|---|---|---|---|---|
| 1 | $^{i}$Pr | 26 | 97 | 96 | 50 | >200 |
| 2 | Me | 2.75 | 89 | 81 | 48 | 44 |
| 3 | $^{n}$Pr | 5 | 96 | 71 | 43 | 95 |

achieved the recycling and reuse of the catalyst up to 15 cycles, in order to overcome the limitation for using a large amount of biocatalyst in the enzymatic reaction.

Several years later, Breen developed the enzymatic kinetic resolution of racemic 1-methyl tetrahydroisoquinoline, which is a precursor of YH1885 used in gastro-esophageal reflux disease and duodenal ulcers.[35] In a preliminary screening using ethyl acetate (EtOAc) as acyl donor, chiroCLEC-CR, a cross-linked enzyme crystal of *Candida rugosa* lipase (CRL, also known as *Candida cylindracea lipase*, CCL), was found as the only active biocatalyst, obtaining the racemic amide in 8% yield. The use of carbonates instead of EtOAc led to a successful enzymatic kinetic resolution that will be later discussed inside section 2.1.2.

**2.1.2. *N*-Alkoxycarbonylations.** Although the formation of amides through enzymatic acylations led to the first examples in the enzymatic kinetic resolution of cyclic secondary amines, the lack of general methods for the production of optically active amines has motivated the investigation of other possible resolving agents. In this sense, the replacement of nonactivated esters by carbonates has sometimes allowed the production of the corresponding amines and carbamates with excellent enantiomeric excesses. This fact occurs because stable carbamates are generated in the process, compounds that are not adequate substrates for hydrolytic undesired reactions mediated by serine-type esterases. Examples of hydrolase-catalyzed alkoxycarbonylation reactions will now be commented in depth. Related to this issue, the enzymatic and nonenzymatic production of enantiopure 2-substituted indolines has been recently revised by Anas and Kagan.[36] The first stereoselective enzymatic method for protecting amines as carbamates using lipases and proteases was described by Wong and co-workers.[37] Reaction of aziridine **6** with different homocarbonates catalyzed by subtilisin BPN′ or CRL led to the corresponding carbamates. The best results were found with CRL, which allowed the formation of the allyl carbamate **7c** in 49% yield and 84% *ee* after 45 h at room temperature (Scheme 2). In the same article, the authors also employed diallyl carbonate for the enzymatic kinetic resolution of racemic 2-methylindoline (**8a**), obtaining the (*R*)-carbamate **9a** in 93% *ee* but only with 6% yield when the reaction was carried out in a phosphate buffer. Disappointingly, no reaction was observed in diallyl carbonate as unique solvent.

**Scheme 2**





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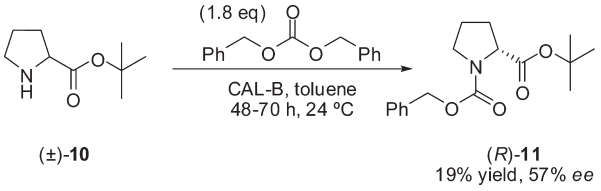
Wong and co-workers have tried to rationalize the action of different esters and carbonates for the adequate resolution of amino alcohols and primary or secondary amines. A clear correlation between the IR absorption maxima and the reactivity was probed.[38] Thus, (±)-**10** was stereoselectively alkoxycarbonylated using dibenzyl carbonate, obtaining the *tert*-butyl (2*R*)-1-benzylpyrrolidine-2-carboxylate (*R*)-**11** in 57% ee (Scheme 3).

Related to this correlation between IR and reactivity and as already commented, Breen reported the enzymatic kinetic resolution of 1-methyl tetrahydroisoquinoline (**12**),[35] a key intermediate for the preparation of YH1885, a drug used for the treatment of gastroesophageal reflux disease. The best results were obtained using diallyl carbonate and novel aryl allyl carbonates such as 3-methoxyphenyl allyl carbonate as alkoxycarbonylating agents (Scheme 4). The reaction rate was also enhanced using 0.05% w/w water, because some water is necessary to maintain the three-dimensional structure of the enzyme on organic media. Interestingly, the reaction was successfully performed in a multigram scale (5 g).

Recently, Page and co-workers have reported an efficient process for the dynamic kinetic resolution (DKR) of another tetrahydroisoquinoline [(±)-**14**] using a novel iridium-based amine racemization catalyst in combination with CRL, performing the preparation of carbamate (*R*)-**15** in 82% isolated yield and 96% ee using 3-methoxyphenyl propyl carbonate as alkoxycarbonylating agent (Scheme 4).[39] The development of the DKR process highly enhanced the value of the synthetic approach, as a 100% maximum theoretical yield can be achieved.

Our research group has also demonstrated the synthetic utility of the enzymatic kinetic resolution of secondary cyclic amines from the class of indolines, with the alkoxycarbonylation being more efficient than the acetylation process (Table 2).[40] Different lipases were tested in the alkoxycarbonylation of commercially available racemic 2-methylindoline (**8a**) and previously synthesized 2-phenylindoline (**8b**), 2-methyl-5-substituted-indolines (**8c**−**d**), and 3-methylindoline (**8e**). CAL-A was found to be the best biocatalyst for 2-substituted-indolines in combination with 3-methoxyphenyl allyl carbonate (entries 1, 2, 3, and 4), and CAL-B was found to be best in combination with diallyl carbonate for 3-methylindoline (entries 5 and 6). The correct choice of lipases, with a variety of allyl carbonates and *tert*-butyl methyl ether (TBME) as solvent, allowed the isolation of carbamates and amines in a high level of optical purity.

**Scheme 3**




## 2.2. Modification of Different Positions at the Heterocyclic Ring in Cyclic Amines

As we have seen in the previous section, the enzyme-catalyzed kinetic resolution of secondary amines is not an easy task, and only a few examples have appeared in the literature. The fact that only a few biocatalysts are useful for the enantioselective preparation of secondary amines led us also to explore other synthetic routes. Different research groups have attempted the hydrolase-catalyzed preparation of optically active secondary amines, considering other options rather the *N*-heterocyclic amino group. Selective modifications of functionalities directly linked to the heterocyclic frame have been extensively studied, with alcohol and esters groups being the most accessible functionalities. For that reason, this section has been divided into two main parts, considering amine or amide derivatives as the targeted compounds. For both of them, hydrolytic, solvolytic, and acylation reactions will be deeply examined.

**2.2.1. Ester Hydrolysis.** In order to develop an easy and mild alternative for the production of valuable optically active secondary amines, different research groups have explored the enzymatic hydrolysis of protected alcohols, which are directly linked to the heterocycle. Nomoto et al. developed a practical chemoenzymatic route for the production of (*R*)-quinuclidin-3-ol (**17**), a common pharmacophore that acts on muscarinic receptors and is widely employed as building block for the production of cognition enhancer Talsaclidine, antiasthma agent Revatropate, or the antirheumatic Evoxac.[41] To produce the enantioenriched compound (*R*)-**17**, the authors performed the *Aspergillus melleus* protease (AMP) catalyzed enantioselective hydrolysis of (±)-quinuclidin-3-yl butyrate (**16**, Scheme 5). Interestingly, the enzymatic hydrolysis was performed at an industrially accepted substrate concentration of 2 M (571 g/L), with the addition of

**Scheme 4**

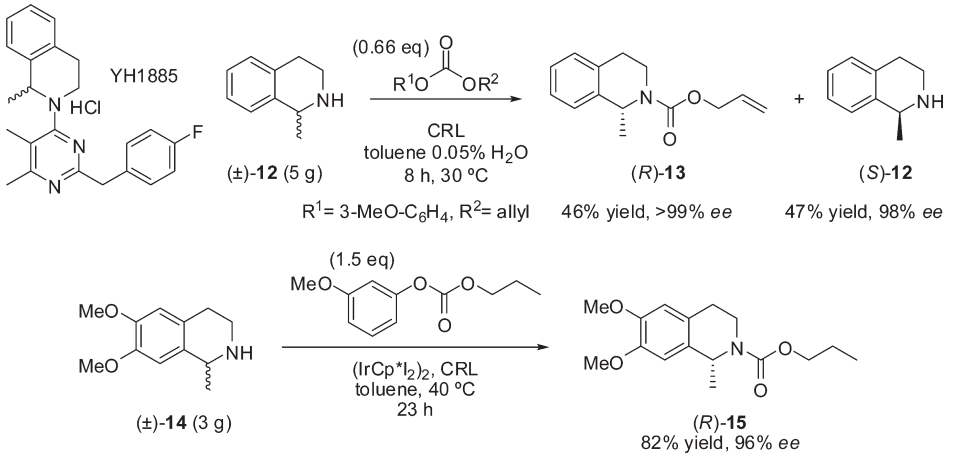




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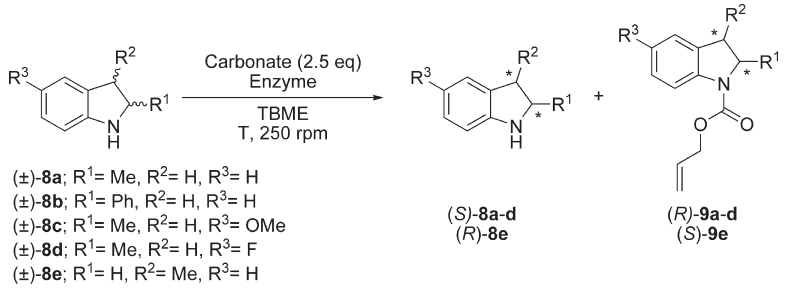
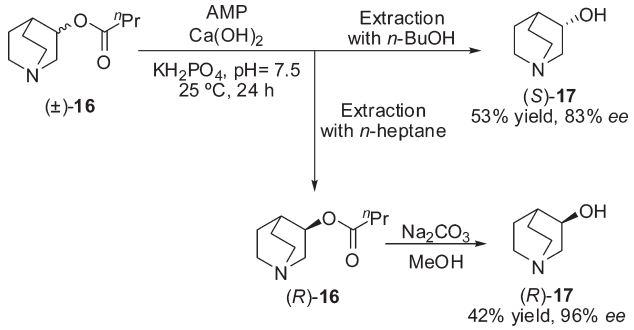
**Table 2. Enzymatic Kinetic Resolution of Different Indolines Using Allyl Carbonates**

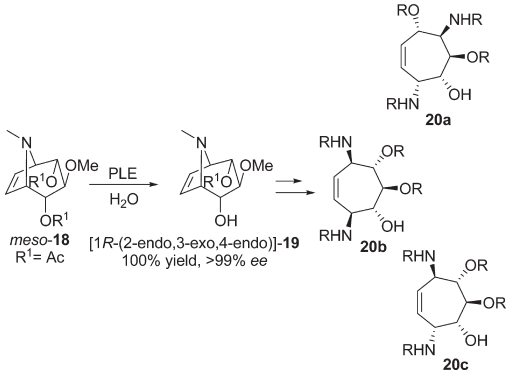



| entry | enzyme | carbonate | $R^1$ | $R^2$ | $R^3$ | $T$ (°C) | $t$ (h) | $ee_p$ (%) | $ee_s$ (%) | $c$ (%) | $E$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CAL-A | 3-methoxyphenyl allyl | Me | H | H | 45 | 66 | >99 | >99 | 50 | >200 |
| 2 | CAL-A | 3-methoxyphenyl allyl | Ph | H | H | 45 | 20 | >99 | 97 | 50 | >200 |
| 3 | CAL-A | 3-methoxyphenyl allyl | Me | H | OMe | 45 | 20 | 95 | >99 | 51 | >200 |
| 4 | CAL-A | 3-methoxyphenyl allyl | Me | H | F | 45 | 20 | >99 | >99 | 50 | >200 |
| 5 | CAL-B | 3-methoxyphenyl allyl | H | Me | H | 30 | 9 | 93 | >99 | 52 | 145 |
| 6 | CAL-B | diallyl | H | Me | H | 30 | 10 | 97 | >99 | 51 | >200 |

**Scheme 5**

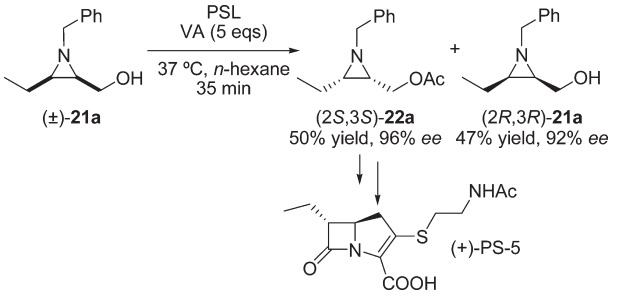



**Scheme 6**




$Ca(OH)_2$ being essential for the maintenance of the optimal pH for the enzymatic hydrolysis (7.5). Additionally, the products were isolated from the reaction media by simple extraction procedures, avoiding the drawbacks of chromatographic separation; in this manner, the remaining butyrate (*R*)-**16** was recovered in 53% yield and 83% ee by extraction with *n*-heptane, whereas (*S*)-**17** was isolated in 42% yield and 96% ee by extraction with *n*-butanol.

**Scheme 7**




Prinzbach and co-workers have performed the total synthesis of optically active *cis*- and *trans*-diaminotetradeoxycycloheptites **20a**−**c**, a class of biologically active compounds owing to the family of non-natural aminoglycosides (Scheme 6).[42] The optically active key precursor [(1*R*-2-endo,3-exo,4-endo)]-**19** was prepared in quantitative yield through the PLE catalyzed enzymatic desymmetrization of the diacetate *meso*-**18** that proceeded with total stereoselectivity.

**2.2.2. *O*-Acylations.** Lipase-catalyzed stereoselective acylation of hydroxyl groups in organic solvents has been employed as a very efficient alternative for the production of highly valuable optically active cyclic amines such as aziridines, azetidines, pyrrolidines, or piperidines, adequate precursors of biologically active compounds such as antibiotics, antihypertensive drugs, or alkaloids. The research group of Prati has described the enzymatic kinetic resolution of aziridine **21a** by PSL-catalyzed acetylation in *n*-hexane using vinyl acetate as acyl donor, affording both substrate and product in excellent isolated yields and enantiomeric excesses (Scheme 7).[43] Next, the optically enriched aziridine (2*S*,3*S*)-**22a** has been used as a key intermediate for the preparation of the $\beta$-lactam antibiotic (+)-PS-5 *via* cobalt carbonyl-catalyzed ring expansion of the optically active aziridine.

The same research group has extensively studied the enzymatic resolution or desymmetrization of a wide panel of 2-hydroxymethyl-3-substituted-aziridines **21a**−**i**, in order to evaluate the effect of ring substituents and the influence of their relative


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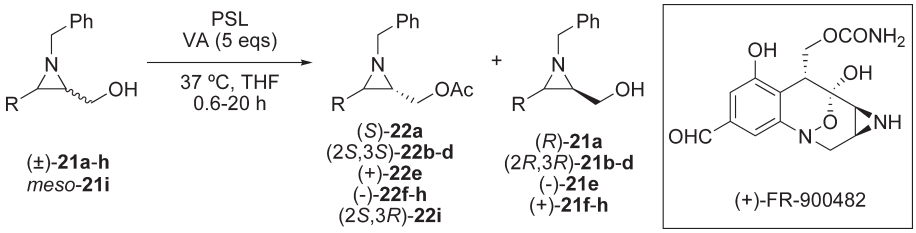
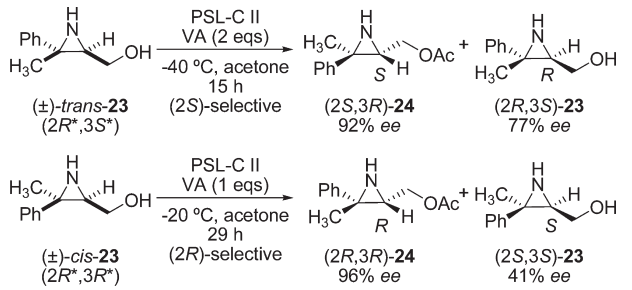
**Table 3. Stereoselective Modification of 2-Hydroxymethylaziridines 21a−h and *meso*-21i**

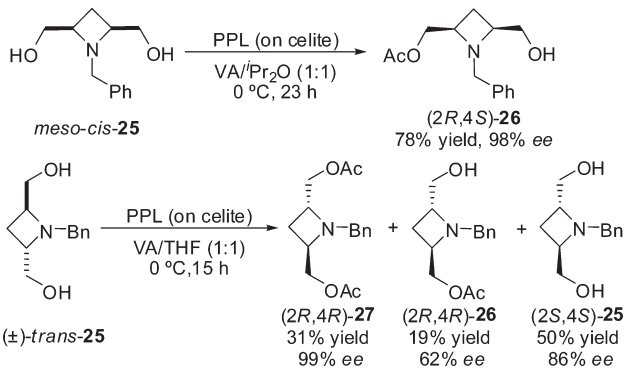



| entry | substrate | R | *t* (h) | $ee_P$ (%) | $ee_S$ (%) | *c* (%) | *E* |
|---|---|---|---|---|---|---|---|
| 1 | **21b** | H | 1.8 | 36 | >97 | 73 | 8 |
| 2 | **21c** | Me (*cis*) | 2.0 | 86 | >97 | 43 | 55 |
| 3 | **21a** | Et (*cis*) | 0.6 | 96 | 92 | 59 | 97 |
| 4 | **21d** | Ph (*cis*) | 20 | >98 | >97 | 50 | >200 |
| 5 | **21e** | Me (*trans*) | 1.0 | 24 | 31 | 56 | 2 |
| 6 | **21f** | Et (*trans*) | 1.3 | 38 | 37 | 49 | 3 |
| 7 | **21g** | Ph (*trans*) | 5.0 | n.d. | >30 | 55 | <2 |
| 8 | **21h** | $CF_3$ (*trans*) | 7.5 | 67 | 65 | 49 | 10 |
| 9 | **21i** | $CH_2OH$ (*cis*) | 4.5 | >97 | | | |

**Scheme 8**

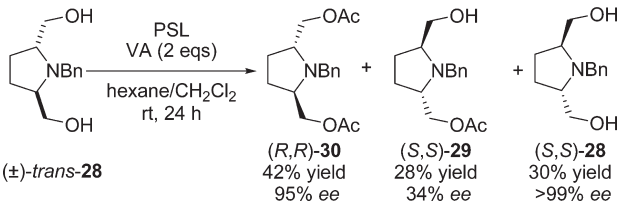



**Scheme 9**

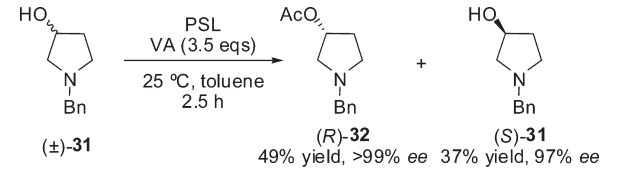



stereochemistry on the stereoselectivity displayed by the enzyme (Table 3).[44] A deep analysis of the enzymatic acetylation of racemic **21a−i** revealed that the enantioselectivity showed a strict dependence of the *cis*/*trans* relative stereochemistry of the aziridine ring substituents, since high *E* values were observed for *cis* derivatives **21a,c,d** (Table 3, entries 2−4) while low values of *E* were observed for the *trans* isomers **21e−h** (entries 5−8). For the *cis* derivatives, the enantioselectivity is strongly dependent on the steric hindrance in the 3-position, as higher enantiopreferences were

**Scheme 10**




**Scheme 11**




observed when the size of the R group was increased (R = Ph, entry 4). On the other hand, excellent results were achieved in the desymmetrization of the *meso* diol **21i**, bearing relative *cis*-stereochemistry, a key intermediate in the synthesis of the antitumor agent FR-900482. Additionally, the authors have observed that the *N*-benzyl protection is essential for the enzyme recognition, as very low enantiodiscrimination was observed when the enzymatic acetylation was carried out using unprotected aziridines (data not shown in the table).

In spite of the poor results reported by Prati for the kinetic resolution of unprotected aziridines, the group of Sakai examined the kinetic resolution of the racemic unprotected aziridines *trans*-**23** and *cis*-**23**, employing *Burkholderia cepacia* lipase immobilized on Toyonite (PSL-C II) in acetone.[45] In this case, low values of enantiodiscrimination were achieved when the kinetic resolution was performed at room temperature; however, little improvements were attained at low temperatures (Scheme 8). Interestingly, the biocatalyst showed opposite enantiopreference for the *cis*/*trans*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isomers, while the enzyme clearly acylated the (2*S*)-position at −40 °C of the *trans* isomer **23** and the *cis* isomer **23** was stereoselectively modified in the (2*R*)-position at −20 °C.

Riva and Guanti have reported the synthesis of enantiomerically enriched azetidines, promising chiral auxiliaries, by means of lipase catalyzed desymmetrization of *meso*-**25** or kinetic resolution of (±)-*trans*-**25**.[46] Vinyl acetate was used as irreversible acyl donor, and pig pancreatic lipase (PPL) immobilized on Celite was used as catalyst (Scheme 9). The desymmetrization of *meso*-*cis*-**25** using $^{i}Pr_2O$ as cosolvent allowed the recovery of the monoacetate (2*R*,4*S*)-**26** in high yield and near enantiopure form. On the other hand, the double kinetic resolution of the diol (±)-*trans*-**25** using THF as cosolvent led to the diacetate (2*R*,4*R*)-**27** in near enantiopure form, while the diol (2*S*,4*S*)-**25** was obtained in moderate enantiomeric excess. The optical purity of (2*S*,4*S*)-**25** can be increased to 95% ee by a simple recrystallization in acetone.

Similarly, the asymmetric enzymatic preparation of the homologous five-member ring derivatives, *trans*-2,5-bis(hydroxymethyl) pyrrolidines, was successfully achieved by Sibi and Lu through a PSL catalyzed sequential kinetic resolution of the diol (±)-*trans*-**28** (Scheme 10).[47] Both diacetate (*R*,*R*)-**30** and diol (*S*,*S*)-**28** were isolated in excellent optical purity, although with moderate yield due to the formation of the monoacetate (*S*,*S*)-**29** in low optical purity.

The optical resolution of racemic *N*-benzyl-3-pyrrolidinol (**31**), which is a useful intermediate for the synthesis of medicines as antibiotics, has been successfully performed by means of enzymatic acetylation, employing PSL as biocatalyst in toluene (Scheme 11).[48] The authors examined the possibility to carry out the stereoselective acetylation using a continuous column reactor, which offers many advantages in terms of easy automatization, safety, productivity, and reproducibility. When the steady state was reached, the column processed efficiently 625 mg/g of of enzyme/h, recovering the acetate (*R*)-**32** in 96% ee and the alcohol (*S*)-**31** in >99% ee. Interestingly, the activity of the reactor did not decrease at all after 13 h.

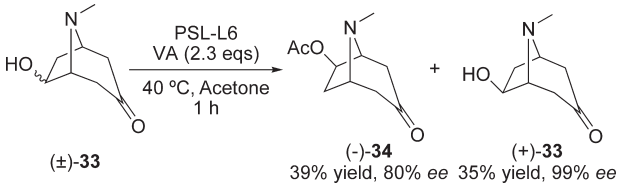
Laschat and co-workers studied the enzymatic kinetic resolution of the racemic tropane alkaloid 6-hydroxytropinone (**33**), employing Chirazyme L6 (PSL-L6), a PSL preparation as catalyst in acetone.[49] Moderate stereoselectivity was observed for the acetylation of (±)-**33**, isolating the acetate (−)-**34** in 39% yield and 80% ee, and the enantiopure alcohol (+)-**33** in 35% yield after a recrystallization purification step (Scheme 12).

Lesma and co-workers reported the synthesis of optically active *cis*-piperidine-3,5-dimethanol monoacetates **36a**−**b**, which are key intermediates for the production of Ibogan type alkaloids, by means of enzymatic asymmetrization using *Pseudomonas fluorescens* lipase (PFL) as biocatalyst.[50] The lipase catalyzed acetylation of **35a**−**b** in vinyl acetate afforded (3*S*,5*R*)-**36a**−**b** in high isolated yield and in enantiopure form after short reaction times (5−6 h, Scheme 13). On the other hand, PFL catalyzed the hydrolysis of diacetates **37a**−**b**, obtaining monoacetates (3*R*,5*S*)-**36a**−**b** in high isolated yield and enantiomerically pure form.

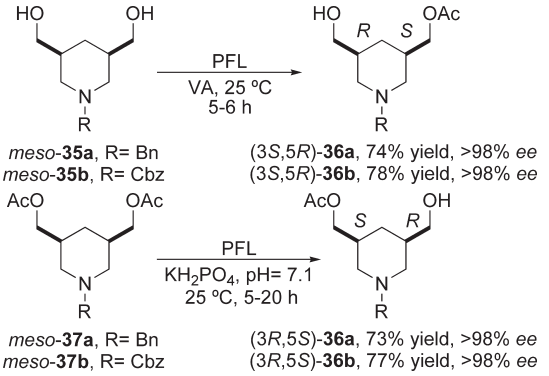
### 2.3. Transformation of Different Functional Groups in *N*-Heterocyclic Amides and Carbamates

The preparation of optically active secondary amines is not an easy task, mainly due to the problems associated with the manipulation of unprotected amines. High polarity, instability, and undesired reactivities are usual limitations that must be overcome. Different research groups have developed alternatives for the production of optically active secondary amines consisting in a two-step sequence: (a) chemical protection of the free

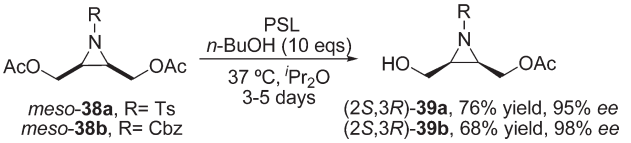
**Scheme 12**



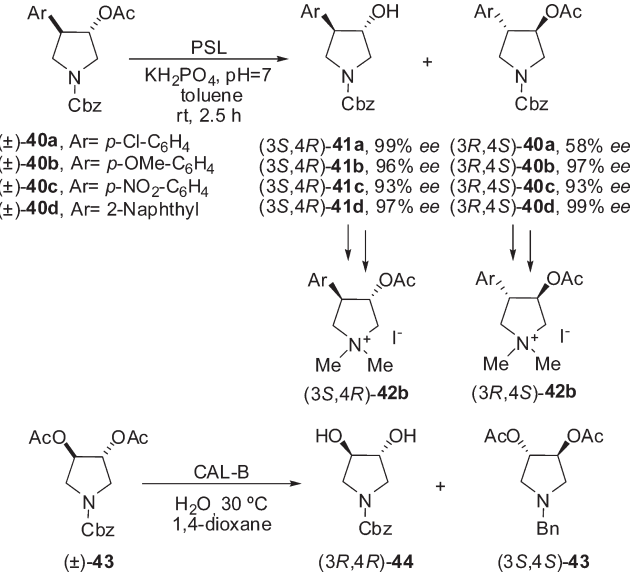

**Scheme 13**




**Scheme 14**




**Scheme 15**





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amino groups as highly stable amides or carbamates; (b) enzymatic modification of different functional groups directly linked to the heterocyclic frame such as alcohols, esters, or epoxides. Examples will be next mentioned considering mainly hydrolytic or transesterifications reactions.

**2.3.1. Hydrolysis and Solvolysis of Esters and Epoxides.** The lipase-catalyzed hydrolysis and alcoholysis of ester moieties directly linked to *N*-protected heterocycles have been widely used for the preparation of important classes of optically active compounds. For instance, Fuji and co-workers carried out the desymmetrization of the *cis*-*N*-protected-aziridines **38a**−**b** using *Burkholderia cepacia* lipase (formerly known as *Pseudomonas cepacia* lipase, PSL). Transesterification with *n*-butanol in diisopropyl ether ($^{i}Pr_2O$) led to the monoacetyl derivatives (2*S*,3*R*)-**39a**−**b** in good yields and very high enantiomeric excesses after long reaction times (3−5 days, Scheme 14). Both monoesters are key intermediates for the preparation of the already mentioned antitumor compound FR-900482 (see Table 3).[51]

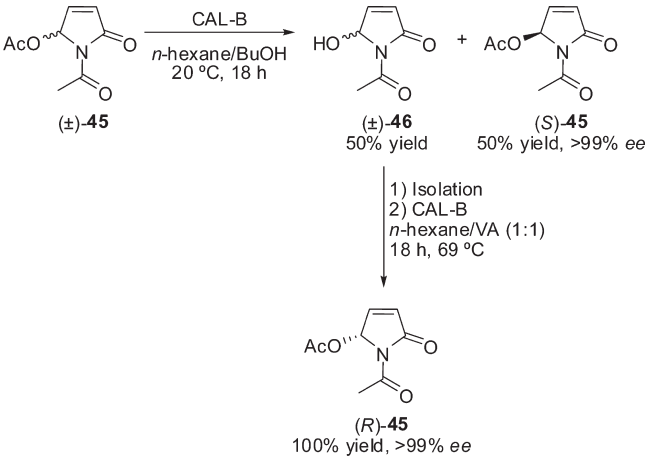
The enzymatic kinetic resolution of racemic *trans*-*N*-(benzyloxycarbonyl)-3-acetyloxy-4-arylpyrrolidines (**40a**−**d**) has been reported by Correia and co-workers through the hydrolysis of the ester rest (Scheme 15).[52] Lipase PSL from *Burkholderia cepacia* generally displayed excellent enantioselectivities ($E$ > 100), especially for (±)-**40d**, bearing the large $\beta$-naphthyl substituent. The so-obtained (3*S*,4*R*)-**40b** and (3*R*,4*S*)-**41b** were finally converted into novel enantioenriched and conformationally restricted acetylcholine analogues (3*R*,4*S*)-**42b** and (3*S*,4*R*)-**42b**, which can be used as acetylcholine esterase inhibitors. Recently, our research group has used a similar approach for the enantioselective preparation of optically active *trans*-1-benzyloxycarbonyl-3,4-dihydroxypyrrolidines. Optically active diol (3*R*,4*R*)-**44** was obtained by CAL-B catalyzed hydrolysis of the diester (±)-*trans*-**43**, using just 5 equiv of water in 1,4-dioxane at 30 °C (Scheme 15).[53]

Van der Deen et al. studied the enzymatic alcoholysis of the racemic pyrrolidinone **45** using CAL-B as biocatalyst in a *n*-hexane-butanol mixture (3:1) at 20 °C (Scheme 16).[54] The remaining (*S*)-**45** was isolated in quantitative yield and enantiopure form; however, the hydroxyl derivative **46** was recovered in quantitative yield but in racemic form due to racemization in the reaction medium. The spontaneous racemization of the alcohol **46** allowed the authors to perform its dynamic kinetic resolution by enzymatic esterification at 69 °C with vinyl acetate as acyl donor in *n*-hexane, recovering (*R*)-**45** in quantitative yield and enantiopure form.

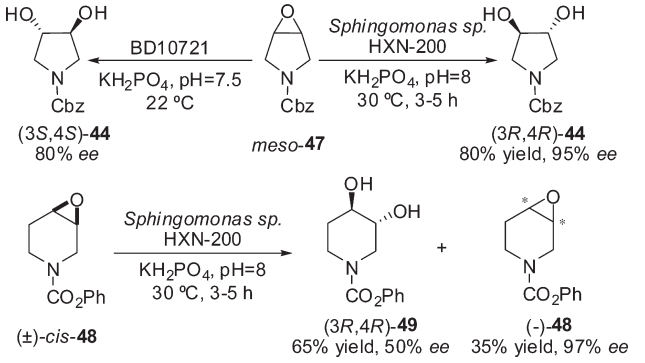
Whole cells of *Sphingomonas species* HXN-200 containing bacterial epoxide hydrolase (EH, EC 3.3.2.x), an enzyme that catalyzes the addition of water to an epoxide, forming the corresponding vicinal diol, were employed for the enantioselective hydrolysis of internal epoxides, such as *meso*-*N*-benzyloxycarbonyl-3,4-epoxypyrrolidine (**47**) and racemic *cis*-*N*-phenoxycarbonyl-3,4-epoxypiperidine (**48**, Scheme 17).[55,56] The asymmetrized *trans*-diol (3*R*,4*R*)-**44** was recovered with high optical purity and isolated yield. On the other hand, moderate stereoselectivity was observed in the enzymatic *trans*-hydrolysis of (±)-*cis*-**48**. More recently, Zhao et al. have employed a library of epoxide hydrolases discovered in nature for the hydrolysis of *meso*-**47**. Surprisingly, one of the EHs, named BD10721, provided access to the complementary diol (3*S*,4*S*)-**44** in good optical purity, although the reaction rates were 50−200 times lower relative to those for enzymes producing (*R*,*R*)-diols.[57]

The gram scale kinetic resolution of racemic diacylated 2-hydroxymethylpiperidine (**3**) has been performed with high enantioselectivity ($E$ > 100) by means of enzymatic alcoholysis in neat *n*-butanol employing lipase AK from *Pseudomonas fluorescens* (Scheme 18).[58] This method provides a clear advantage over that

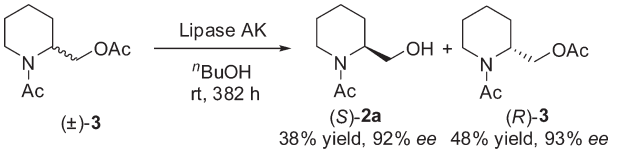
**Scheme 16**



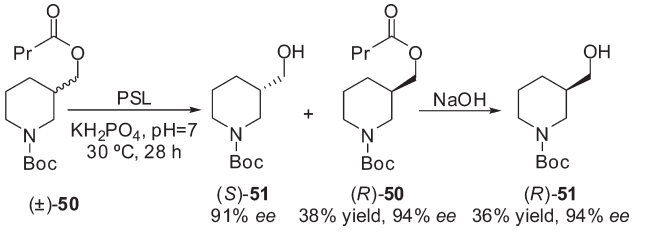

**Scheme 17**




**Scheme 18**




**Scheme 19**





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previously described for the preparation of optically active enantiomers of 2-hydroxymethylpiperidine,[33] since in a single enzymatic reaction both substrate (*R*)-**3** and product (*S*)-**2a** were recovered in high optical purity. Unfortunately, very long reaction times (382 h) were necessary to reach 50% conversion, a fact that limited its practical application.

The synthesis of optically active piperidine derivatives has traditionally attracted much attention, because the piperidine fragment is present in many secondary metabolites and biologically active compounds. For instance, optically active *tert*-butyl-(*R*)-3-hydromethyl-1-piperidinecarboxylate **51**, which is a key intermediate for the preparation of low-molecular weight thrombin inhibitors, has been prepared in 94% ee on a multikilogram scale by means of the PSL catalyzed enantioselective hydrolysis of the racemic propionate **50** (Scheme 19).[59]

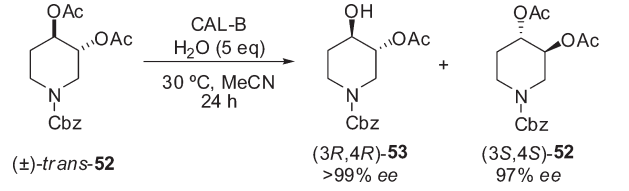
The preparations of enantiomerically enriched *trans*-3,4-dihydroxypiperidine derivatives, which are adequate precursors for the preparation of pharmacologically active compounds such as the antidepressant Ifoxetine and the gastroprokinetic agent Cisapride, have been successfully carried out in our research group.[60] In all cases, CAL-B exclusively catalyzed the hydrolysis of the acetyl group present at the *O*-4 position of the racemic *trans*-diacetate **52** (Scheme 20). After optimization of the reaction conditions, both substrate (3*S*,4*S*)-**52** and product (3*R*,4*R*)-**53** were recovered in high yield and in near enantiopure form. CAL-B was used as biocatalyst and MeCN as solvent containing 5 equiv of water. Then, the enzymatic hydrolysis of the *cis*-derivative was tried in order to extend the scope of this methodology; however, very low reaction rates and a poor regioselectivity were observed.

Chênevert and co-workers have studied the enzymatic desymmetrization of *meso cis*-2,6 and *cis,cis*-2,4,6-substituted-piperidines **54a**−**d**. These are interesting compounds, since *cis*-2,6-substituted-piperidines are present in many naturally occurring piperidine alkaloids (Scheme 21).[61,62] *Aspergillus niger* lipase (ANL) was found to be the ideal biocatalyst for the biotransformation in aqueous media, affording the corresponding enantiopure *cis*-monoacetates **55a**−**d** in high isolated yields. The so-obtained **55a**−**d** were used as adequate building blocks for the total synthesis of piperidine alkaloids such as (+)-Piperidine 241 D, which blocks the acetylcholine action, and (+)-Dihydropinidine, which is known to be a powerful teratogen.

Finally, Hu and co-workers reported an original work for the preparation of a variety of optically active secondary amines, where the access to both amine enantiomers was guaranteed in good yields after release of the oxalamic group.[63] 2-Ethylpiperidine was selected as model substrate, which after protection of the free amino group with ethyl chlorooxacetate led to the corresponding oxalamic amino ester **56**. Protease-catalyzed stereoselective hydrolysis reaction led to the (*R*)-acid **57** and (*S*)-amide **56** with an excellent selectivity, enabling the production of both amine **58** enantiomers by simple chemical hydrolysis in acidic media (Scheme 22). This approach was successfully extended to other secondary cyclic amines, testing also a set of proteases, allowing the preparation of both cyclic secondary amine single enantiomers in high enantiomeric excesses.

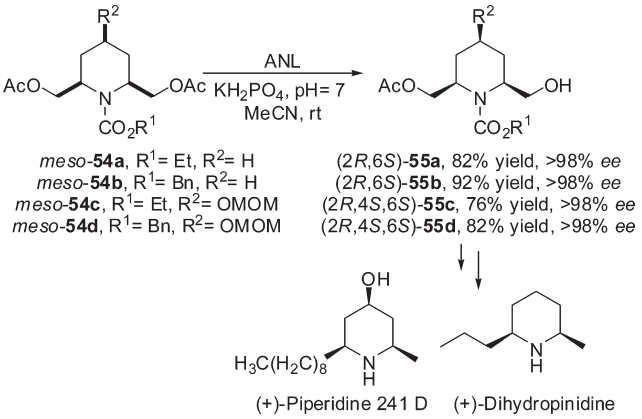
**2.3.2. *O*-Acylations.** Enzymatic acylation of hydroxyl groups directly linked to cyclic amines have been successfully achieved in dry organic solvents, for the production of chiral auxiliaries or pharmacologically active compounds. Optically active pantolactone is a common chiral auxiliary for the asymmetric production of α-arylpropionic acids. Unfortunately, its highly hydrophilic

**Scheme 20**




(±)-*trans*-**52** (3*R*,4*R*)-**53** >99% *ee* (3*S*,4*S*)-**52** 97% *ee*

**Scheme 21**




*meso*-**54a**, $R^1$= Et, $R^2$= H
*meso*-**54b**, $R^1$= Bn, $R^2$= H
*meso*-**54c**, $R^1$= Et, $R^2$= OMOM
*meso*-**54d**, $R^1$= Bn, $R^2$= OMOM

(2*R*,6*S*)-**55a**, 82% yield, >98% *ee*
(2*R*,6*S*)-**55b**, 92% yield, >98% *ee*
(2*R*,4*S*,6*S*)-**55c**, 76% yield, >98% *ee*
(2*R*,4*S*,6*S*)-**55d**, 82% yield, >98% *ee*

(+)-Piperidine 241 D (+)-Dihydropinidine

nature made difficult the recovery of the catalyst after the hydrolysis step, always necessary for the separation of the product from the auxiliary. Furthermore, the expensive L-pantonolactone is required for the production of the more active (*S*)-arylpropionic acids. To avoid these drawbacks, the group of Camps developed the synthesis of new pantolactone related chiral auxiliaries where the key step is a PSL-catalyzed kinetic resolution of the racemic alcohol **59** (Scheme 23).[64] After optimization of the reaction conditions, the biotransformation was performed in a 44 mmol scale (9 g), isolating (*R*)-**59** in enantiopure form and high yield. Next, hydrolysis of (*S*)-**60** and later crystallization allowed the isolation of (*S*)-**59** in enantiopure form and good yield. Interestingly, both enantiomers of the new chiral auxiliary are nonhygroscopic solids that can be easily purified by crystallization, facilitating their recovery from the reaction media.

Takabe and co-workers studied the lipase catalyzed kinetic resolution of 1-benzyl-5-hydroxypyrrolidin-2-one (**61**) and 5-hydroxy-1,5-dihydropyrrol-2-one derivatives (**63a**−**g**), which are valuable intermediates for the synthesis of natural products such as the antitumor alkaloid Jatropham.[65,66] Moderate stereoselectivity was observed for the PSL-catalyzed resolution of the succinimide derivative **61** in 1,4-dioxane (Table 4, entry 1). Next, the resolutions of the maleimide derivatives **63a**−**c** were considered (entries 2−4), observing that the enantioselectivity was highly dependent on the $R^3$ substituent, since low stereoselectivity and opposite enantiopreference were observed for the unprotected maleimide **63a** (entry 2), while high enantioselectivity was achieved with the medium-sized allyl group **63b** (entry 3) and complete selectivity was obtained with the bulky benzyl group **63c** (entry 4). Finally, the resolutions of citraconimide derivatives **63d**−**g** were analyzed (entries 5−8). As observed for the maleimide derivatives, the highest enantioselectivity values were obtained with the *N*-benzyl-protected derivatives **63f,g** (entries 7−8), while low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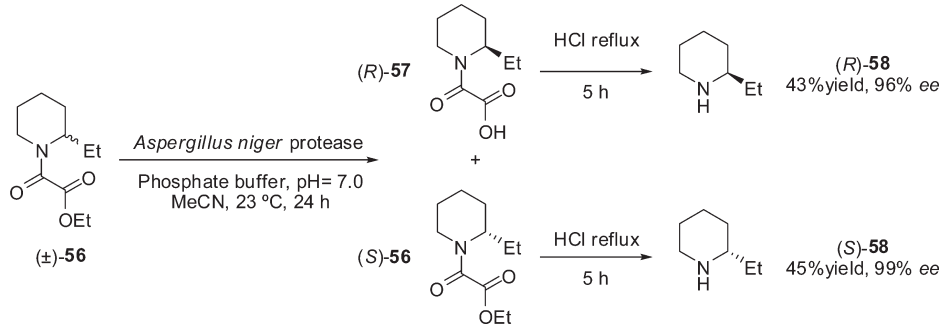
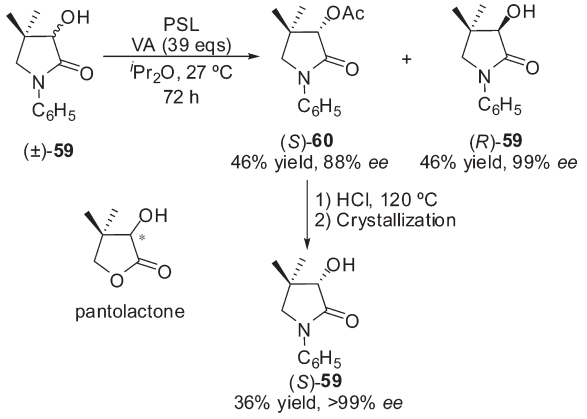
**Scheme 22**




**Scheme 23**




**Scheme 24**

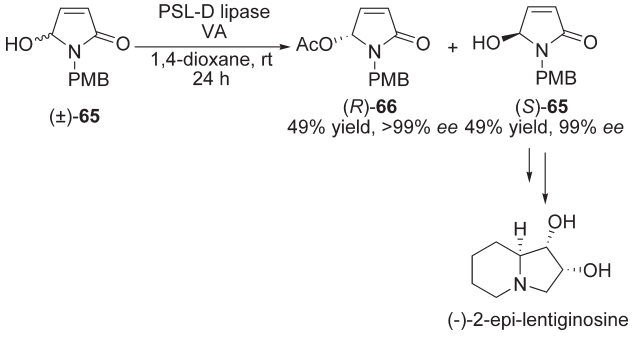
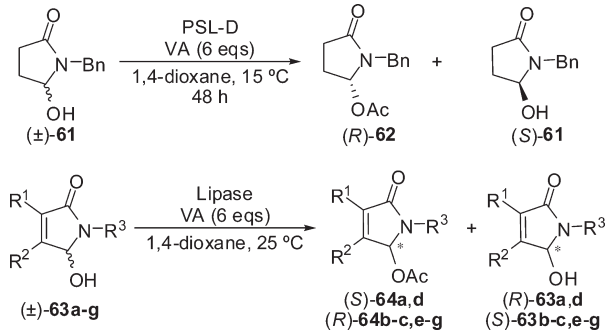



**Table 4. Kinetic Resolution of Racemic Pyrrol-2-one Derivatives 61 and 63a−g**




| entry | substrate | $R^1$ | $R^2$ | $R^3$ | lipase | $t$ (h) | $ee_P$ (%) | $ee_S$ (%) | $E$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **61** | H | H | Bn | PSL | 48 | 88 | >99 | 42 |
| 2 | **63a** | H | H | H | PSL | 5 | 75 | >99 | 15 |
| 3 | **63b** | H | H | allyl | PSL | 6 | 96 | 70 | 102 |
| 4 | **63c** | H | H | Bn | PSL | 24 | >99 | >99 | >200 |
| 5 | **63d** | Me | H | H | PL | 50 | 98 | 35 | 5 |
| 6 | **63e** | Me | H | allyl | CAL-B | 20 | 79 | 81 | 16 |
| 7 | **63f** | Me | H | Bn | CAL-B | 20 | >99 | >99 | >200 |
| 8 | **63g** | H | Me | Bn | PSL | 240 | 98 | 38 | 126 |

stereoselectivites were attained in the kinetic resolution of the unprotected derivative **63d** and *N*-allyl derivative **63e**.

Optically active (*S*)-5-hydroxy-1-(4-methoxybenzyl)pyrrolidin-2-one (**65**) has been obtained by means of a PSL-D catalyzed acetylation of the racemic alcohol in 1,4-dioxane. The 4-methoxybenzyl protecting group (PMB) was chosen because it can be easily removed under very mild reaction conditions. (*S*)-**66** has been employed as an adequate building block for the total synthesis of (−)-2-*epi*-lentiginosine, which is a potent inhibitor of glycosidases, and subsequently could be used in cancer chemotherapy (Scheme 24).[67]

Optically active *cis*- and *trans*-3-methoxy-4-methylaminopyrrolidines (**69**) are interesting compounds from a pharmaceutical point of view. For instance, (3*S*,4*S*)-**69** is a key compound for the preparation of the quinoline antitumor compound AG-7352; on the other hand, (3*R*,4*S*)-**69** has been employed for the synthesis of antibacterial and cytotoxic compounds. Taking advantage of the excellent properties displayed by lipases, Kamal and co-workers reported the chemoenzymatic synthesis of optically active 3-methoxy-4-methylaminopyrrolidines **69**, where the key step was the PSL catalyzed acetylation of *trans*- and *cis*-3-azido-1-benzyloxycarbonyl-4-hydroxypyrrolidine (**67**) in $^iPr_2O$ (Scheme 25).[68,69]

Herradón and Valverde extended the preliminary studies performed by Asensio and co-workers[33] to the resolution of racemic 3-piperidinyl methanol (**70**) using *Aspergillus niger* acylase I (AA-I) as biocatalyst in the presence of vinyl acetate (VA) as acyl donor (Table 5).[70] The reaction is known to occur in a two-step sequence. First of all, vinyl acetate might cause the chemical *N*-acetylation of **70**, giving the corresponding racemic amido ester **71**, which undergoes enzymatic acetylation to give the corresponding racemic amido ester **71** and the amido alcohol **72**, the latest with poor to low enantiomeric excess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**Scheme 25**

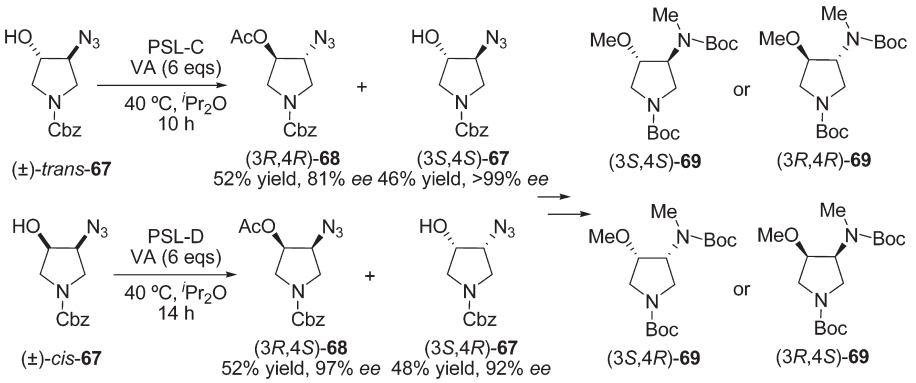
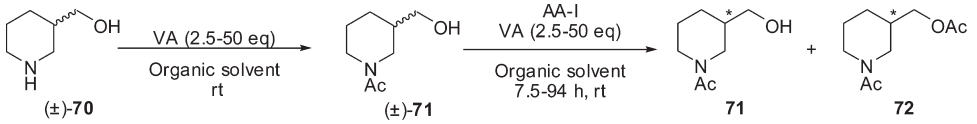



**Table 5. Acylation Reaction of Racemic 3-Piperidinyl Methanol (70) Catalyzed by AA-I**




| entry | AA-I (mg) | solvent | VA (equiv) | *t* (h) | **71** (%)[a] | **72** (%)[a] |
|---|---|---|---|---|---|---|
| 1 | 300 | VA | 50 | 91 | 69 (n.d.) | 29 (19) |
| 2 | 300 | MeCN | 5 | 94 | 42 (n.d.) | 37 (<2) |
| 3 | 100 | $CH_2Cl_2$ | 2.5 | 7.5 | 68 (0) | 0 |

[a] Enantiomeric excesses in parentheses.

when using vinyl acetate, acetonitrile, or dichloromethane as solvents (Table 5).

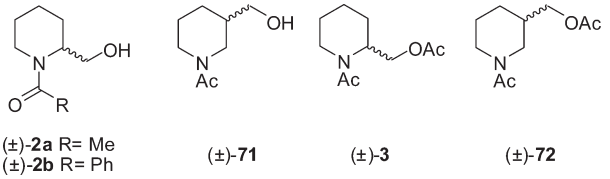
Different research groups have studied the enzyme-catalyzed acetylation of racemic *N*-acyl-2-hydroxymethylpiperidines **2a**−**b** and *N*-acetyl-3-hydroxymethylpiperidine **71** (Chart 2).[33,70] Disappointingly, in spite of the fact that a wide variety of biocatalysts were tested, in most cases, the authors have observed low stereoselectivities, isolating both substrates and products in moderate enantiomeric excesses. Toone and Jones have also explored the complementary hydrolysis of the acetyl derivatives (±)-**3** and (±)-**72** using pig liver esterase (PLE) as biocatalyst, observing for both substrates a very low enantiodiscrimination.[71]

Optically active *N*-protected-2-hydroxymethylpiperidine (**73**) has been synthesized through *Aspergillus niger* acylase type I (AA-I) catalyzed kinetic resolution of the racemic piperidine using vinyl butyrate (VB) as acyl donor (Scheme 26).[72] As only moderate enantioselectivity was displayed by the enzyme, a second kinetic resolution was necessary to isolate the alcohol (*S*)-**73** in enantiopure form, adequate precursor of a variety of optically active alkaloids such as (*S*)-pipecolic acid, (*R*)-coniine, and (*S*)-δ-coniceine.

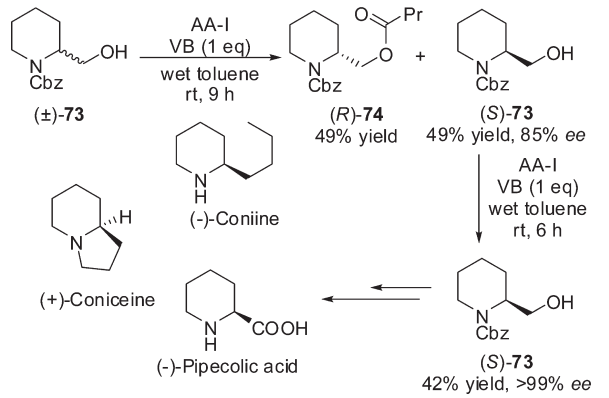
Chênevert and co-workers have performed the stereoselective acylation of *meso-cis*-2,6 and *cis-cis*-2,4,6-substituted-piperidines **75a,b** using vinyl acetate as both solvent and acyl donor and CAL-B as biocatalyst (Scheme 27). The corresponding monoesters **76a,b** were obtained in excellent enantiomeric purity and isolated yield in short reaction times.[73] Next, the total synthesis of the potent blocker for nicotinic acetylcholine receptors (+)-indolizidine 209 D was performed from enantiopure (2*S*,6*R*)-**76a** in eight steps.

The enzymatic acylation of *N*-phenyloxycarbonyl-*trans*-4-(4′-fluorophenyl)-3-hydroxymethylpiperidine (**77**), key intermediate

**Chart 2**




**Scheme 26**




for the synthesis of the antidepressant (−)-paroxetine hydrochloride, has been studied in our research group (Scheme 28).[74] An exhaustive optimization of the resolution was performed,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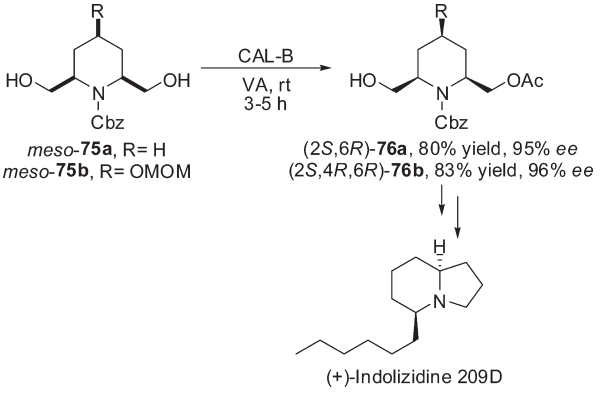
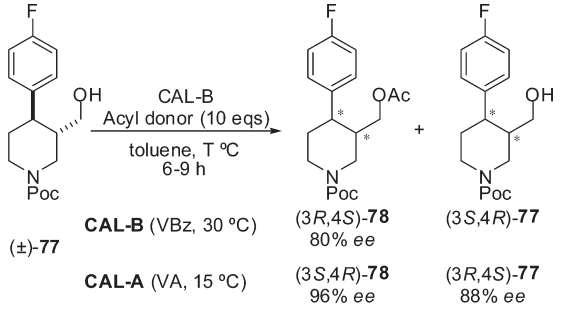
**Scheme 27**




**Scheme 28**

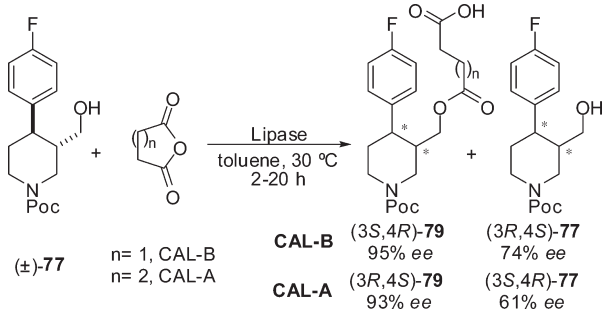



obtaining the best results when using CAL-A or CAL-B as biocatalysts in toluene. Vinyl acetate was identified as the ideal acyl donor for CAL-A, although better results were obtained with vinyl benzoate (VBz) for CAL-B. Interestingly, these two lipases displayed opposite stereochemical preference. CAL-A catalyzed the acylation of (3*S*,4*R*)-**77** with high enantioselectivity, whereas CAL-B preferred the acylation of (3*R*,4*S*)-**77**. Similar enantiodiscrimination values were observed for both processes; however, from a synthetic point of view, the CAL-B-catalyzed process is more interesting, since the remaining alcohol (3*S*,4*R*)-**77** has the required configuration to complete the synthesis of (−)-paroxetine.

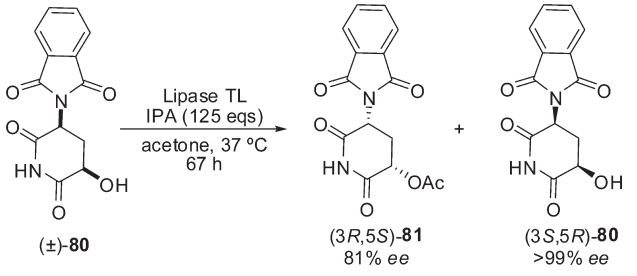
The major drawback for the large scale chemoenzymatic production of optically active (−)-paroxetine is the necessity of a chromatographic separation after the enzymatic acylation step. To overcome this limitation, cyclic anhydrides were used as acylating agents, in the reaction with (±)-**77**, obtaining the monoacid **79**, which can be separated from the unreacted alcohol **77** by a simple extraction procedure with base. In this manner, the preparation of (−)-paroxetine was successfully achieved in multigram scale (Scheme 29).[75] As occurred in the kinetic resolution of racemic **77** with vinyl esters, CAL-A and CAL-B displayed opposite enantiopreferences. Succinic anhydride was the optimal acyl donor for CAL-A, meanwhile glutaric anhydride led to the best results with CAL-B.

Shibata and co-workers performed the synthesis of both enantiomers of *cis*-5′-hydroxythalidomide **80**, the major metabolite of Thalidomide, using *Pseudomonas stutzeri* lipase (lipase TL) as biocatalyst and isopropenyl acetate (IPA) as acyl donor in acetone (Scheme 30).[76] A great excess of the acyl donor (125 equiv)

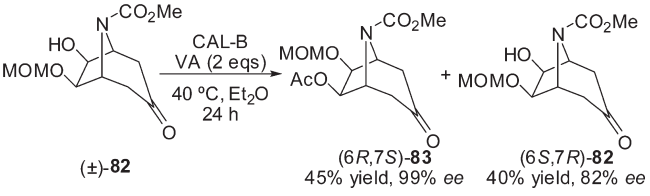
**Scheme 29**



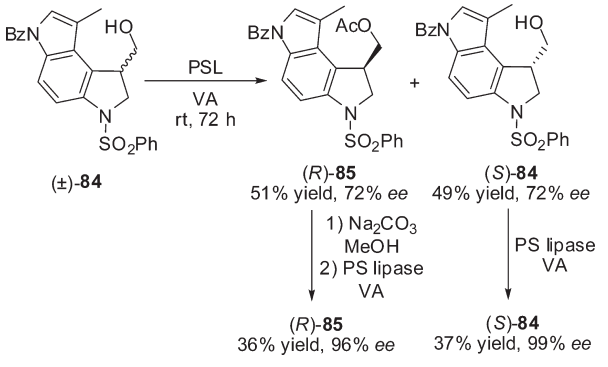

**Scheme 30**




**Scheme 31**




**Scheme 32**




was necessary for the formation of the enantiopure alcohol (3*S*,5*R*)-**80** after a reasonable reaction time (69 h). The biological evaluation of optically active (3*S*,5*R*)- and (3*R*,5*S*)-**80** showed that these derivatives failed to show the antiangiogenesis activity that is closely related with the teratogenicity of thalidomide. However, optically active **80** showed sedative and mild


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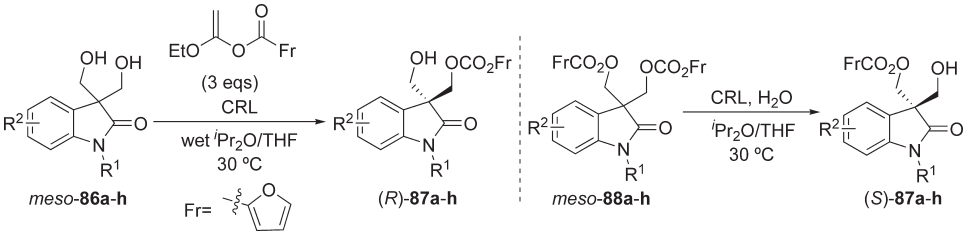
**Table 6. Enzymatic Desymmetrization of Oxindoles Catalyzed by CRL**




| entry | substrate | $R^1$ | $R^2$ | ratio (THF/$^i$Pr$_2$O) | time (h) | yield (%) | ee (%) |
|---|---|---|---|---|---|---|---|
| 1 | **86a** | Me | H | 1:10 | 7 | 53 | 87 |
| 2 | **86b** | Boc | H | 1:5 | 22 | 93 | 99 |
| 3 | **86c** | Boc | 5-OMe | 0:1 | 3 | 77 | 98 |
| 4 | **86d** | Boc | 6-OMe | 1:5 | 19 | 79 | 91 |
| 5 | **86e** | Cbz | H | 1:5 | 58 | 71 | 98 |
| 6 | **86f** | Ac | H | 1:5 | 48 | 90 | 97 |
| 7 | **86g** | MOM | H | 1:5 | 64 | 34 | 86 |
| 8 | **86h** | Bn | H | 1:5 | 144 | 59 | 68 |

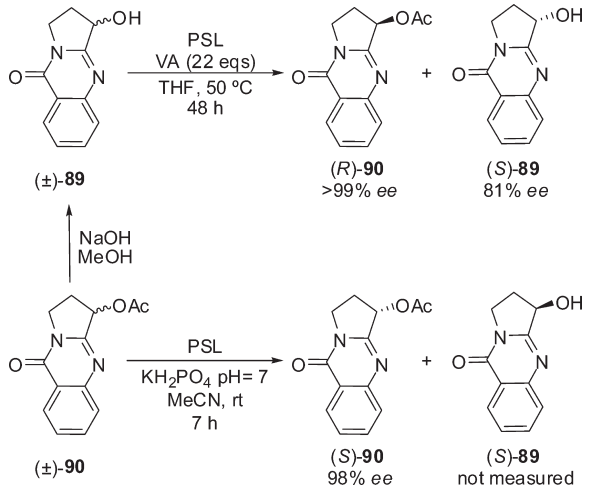
immunosuppressive activities and resistance to racemization and epimerization under physiological pH.

Affolter et al. performed the kinetic resolution of biologically active tropane-diols, which provide useful chiral scaffolds for asymmetric synthesis.[77] Unfortunately, when the authors carried out the enzymatic desymmetrization of the unprotected diol, the final product was isolated in racemic form due to intramolecular acyl migration. To overcome this limitation, the diol was mono-protected with the methoxymethyl group (MOM), with the racemic methoxymethylated derivative **82** being efficiently resolved by means of CAL-B catalyzed acetylation with vinyl acetate in $Et_2O$ (Scheme 31).

With the aim of preparing novel optically active antitumor compounds, Ling and Lown have reported the PSL lipase catalyzed kinetic resolution of the indole alkaloid (±)-**84**, which is an immediate precursor of cyclopropylindole (CPI), the DNA alkylation subunit of CC-1065, a potent antitumor antibiotic isolated from the culture cells of *Streptomyces zelensis* (Scheme 32).[78] Both substrate (*S*)-**84** and product (*R*)-**85** were recovered in moderate enantiomeric excess, so a second kinetic resolution step was necessary to isolate substrate and product in near enantiopure form.

The group of Kita performed the lipase-catalyzed enantiodivergent preparation of optically active oxindoles bearing a quaternary stereocenter at the C-3 position. This is a very interesting methodology, since the isolated products are present in many natural compounds but also possess many possibilities as chiral building blocks in indole synthesis. Complementary lipase-catalyzed acetylation and hydrolysis processes were jointly used to produce both enantiomers of the product.[79] *Candida rugosa* lipase (CRL, Meito OF) was found to be the ideal catalyst for the acylation process. In order to prevent racemization by nonenzymatic acyl migration, which is often observed in 1,3-propanediols, 1-ethoxyvinyl furoate was employed as acyl donor, as the monofuroate (Fr) rest is highly resistant to migration (Table 6). After the optimization of the reaction conditions, the use of THF/$^i$Pr$_2$O mixtures as solvent was found to be crucial, observing

**Scheme 33**




for most of the cases that the 1:5 was the optimal ratio. *N*-Acyl derivatives generally produced the corresponding (*R*)-monoalcohols **87b**−**f** in high optical purity and chemical yield (entries 2−6). On the contrary, the desymmetrization of *N*-alkyl derivatives **86a**,**g**−**h** resulted in lower optical purities and chemical yields (entries 1, 7, and 8). Nevertheless, recrystallization was effective to obtain enantiomerically pure alcohols from those with unsatisfactory optical purities. On the other hand, the CRL catalyzed hydrolysis of the difuroates **88a**−**h** opens up a new route for the preparation of the opposite enantiopure isomers (*S*)-**87a**−**h**; unfortunately, in this case, lower isolated yields (typically <50%) were obtained (data not shown in the table).

Vasicinone (**90**), a pyrrolo[2,1-*b*]quinazoline alkaloid with bronchodilatory activity, has been widely used for the treatment of asthma, cough, and bronchitis. Both enantiomers were conveniently prepared in enantiopure form *via* PSL catalyzed kinetic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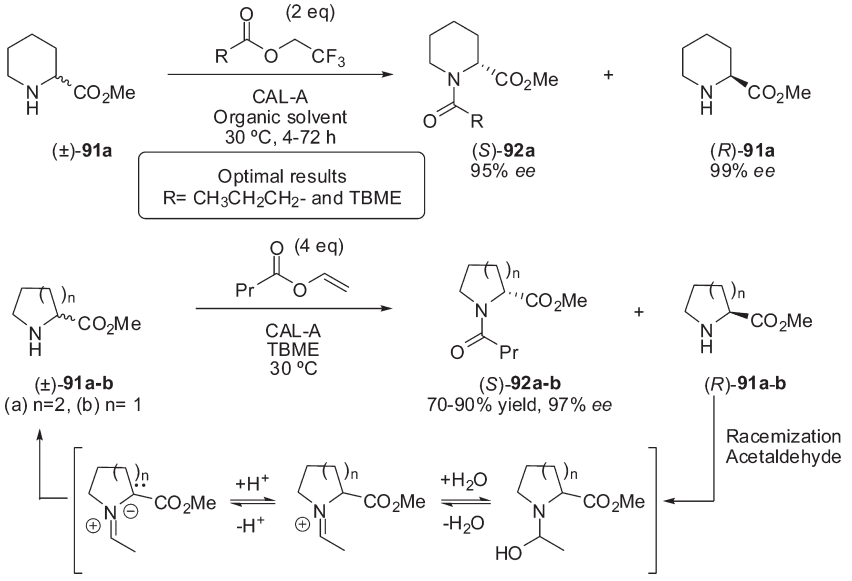
Scheme 34




resolution of the alcohol **89** or the acetate **90** (Scheme 33). The most active enantiomer (*S*)-**90** was prepared by means of the enzymatic hydrolysis of (±)-**90** in aqueous medium while (*R*)-**90** was prepared through the enzymatic acetylation of (±)-**89** in THF using a 22-fold excess of vinyl acetate.[80]

## 3. STEREOSELECTIVE BIOTRANSFORMATIONS OF CYCLIC AMINO ACID DERIVATIVES

From the chiral pool of *N*-heterocyclic amino acids, the chemical syntheses of proline analogues have been extensively investigated because proline is the catalyst of reference for many asymmetric organocatalytic reactions. Also in this section, examples of stereoselective methods for the preparation of the six-membered ring pipecolic acid will be discussed. With that in mind, we have classified this part in two main sections that include the modifications directly performed on the amino group, such as acylation, alkoxycarbonylation, and the less studied hydrolytic processes, and the ones performed on the carboxylic acid group, usually an ester functionality, which can be subjected to transesterification, interesterification, aminolysis, ammonolysis, or hydrolysis reactions.

### 3.1. Modifications at the Amino Group: *N*-Acylation and *N*-Alkoxycarbonylation Processes

The possibility to directly modify the nitrogen in an internal position of the cycle usually leads to excellent selectivities for the production of nitrogenated compounds. Some examples have already been described for the production of amides or carbonates using racemic amines as starting materials. We next discuss the use of the same synthetic approach for the production of optically enriched amino acid derivatives. Kanerva and co-workers reported the acylation of pipecolic acid derivatives catalyzed by *Candida antarctica* lipase type A (CAL-A), an enzyme with a larger pocket than CAL-B in the active site that accepts bulkier substrates such as secondary cyclic amines in its active site (Scheme 34).[81] The analysis and fractionation of the protein revealed two main protein species in roughly the same abundance.[82] The largest one was identified as mature CAL-A, while the smaller one was a truncated form of the enzyme that lacked the terminal C-region of the protein. Both fractions were able to catalyze the *N*-acylation of **91a** in a stereoselective manner, not observing significant differences in terms of activity. From ten organic solvents and eight acyl donors, the combination of *tert*-butyl methyl ether (TBME) as solvent and 2 equiv of trifluoroethyl butanoate was found to be the best system, yielding (*S*)-amido ester **92a** in 95% ee and (*R*)-amino ester **91a** in 99% ee after 49% conversion at 9 h. The gram-scale resolution of (±)-**91a** (1.5 g) was also successfully achieved, yielding the product in 98% ee and substrate in 90% ee after 48% conversion. The same authors later extended these excellent results toward the dynamic kinetic resolution of proline and pipecolic acid methyl esters, testing different strategies to efficiently perform the DKR processes, such as the use of (i) 2,2,2-trifluoroethyl butanoate as acyl donor with acetic acid and acetaldehyde to produce racemization; (ii) vinyl butanoate as acyl donor and acetic acid in order to enhance racemization; or (iii) vinyl butanoate as acyl donor and triethyl amine (or ammonium acetate) in order to enhance racemization and also binding the liberated butanoic acid.[83] Under optimized conditions using the combination of CAL-A and vinyl butanoate, 70% of the racemic pipecolic acid methyl ester (**91a**) and 90% of the racemic proline methyl ester (**91b**) were transformed into the (*S*)-butanamides **92a** and **92b** in 97% ee using a 1.5 g scale.

As an alternative to the acylation process, the alkoxycarbonylation of cyclic racemic amino esters has been investigated by Alatorre-Santamaría et al., exploring the possibility to resolve a series of racemic α-amino acid derivatives containing the 2,3-dihydroindole or octahydroindole core with excellent selectivities when using CAL-A as biocatalyst.[84] The appropriate choice of the alkoxycarbonylating agent allowed the isolation in enantiopure form of (*S*)-methyl-(*N*-allyloxycarbonyl)indoline-2-carboxylate **94** when using 3-methoxyphenyl allyl carbonate or (2*S*,3a*S*,7a*S*)-benzyl(*N*-allyloxycarbonyl)octahydroindole-2-carboxylate (**96**) with diallyl carbonate (Scheme 35). Very recently, our research group has tested the enzymatic alkoxycarbonylation of methyl-3-indoline carboxylate (**97**) using CAL-B as biocatalyst.[85] Unfortunately, a lipase-mediated transesterification process also occurred, giving considerable amounts of the allylic ester **99**. The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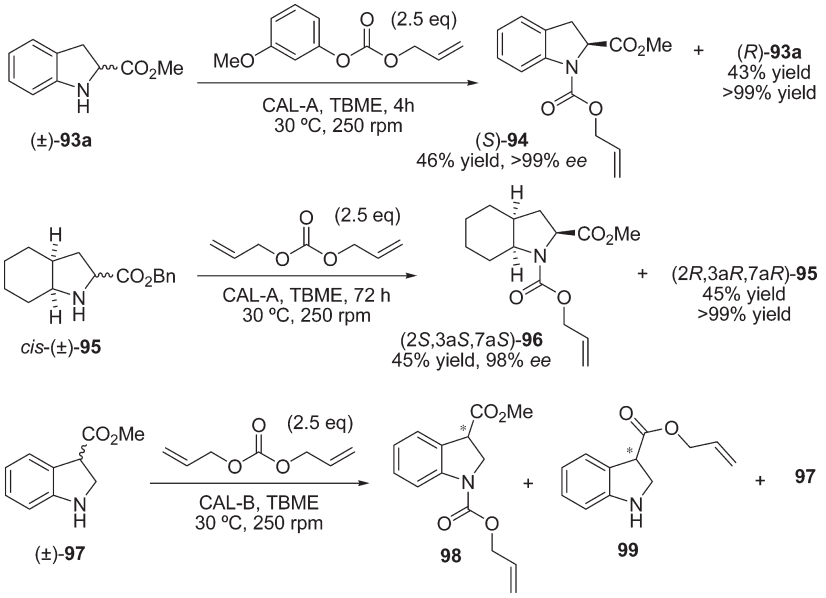
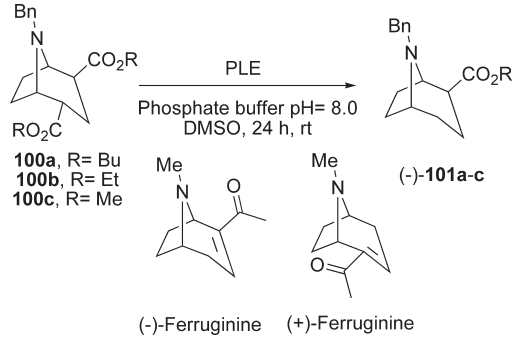
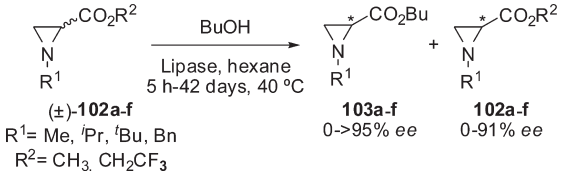
**Scheme 35**




**Scheme 36**




**Scheme 37**

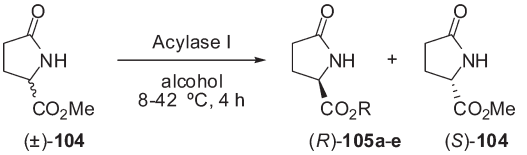



appearance of both amino esters **98** and **99** led to serious purification problems in the purification step; hence, we decided to study other enzymatic approaches that will be discussed below.

## 3.2. Modifications at the Carboxylic Group

Because of the bifunctionality shown by amino acid derivatives, enzymes have taken advantage of their abilities to perform chemo- and stereoselective processes. In this manner, modification of the amino or the carboxylic group is possible by simply adequate selection of the reaction parameters. Once we have reviewed the examples related to the modification of the amino group, we next show the examples related to the transformation

**Table 7. Acylase I-Catalyzed Alcoholysis of Racemic Methyl Pyroglutamate**




| entry | alcohol | $T$ (°C) | $c$ (%) | $E$ |
|---|---|---|---|---|
| 1 | ethanol (**a**) | 25 | 16 | 19 ± 8 |
| 2 | *n*-propanol (**b**) | 25 | 44 | 34 ± 9 |
| 3 | *n*-butanol (**c**) | 8 | 36 | 63 ± 5 |
| 4 | *n*-butanol (**c**) | 25 | 49 | 34 ± 4 |
| 5 | *n*-butanol (**c**) | 42 | 25 | 20 ± 2 |
| 6 | 1-pentanol (**d**) | 25 | 33 | 40 ± 3 |
| 7 | 1-hexanol (**e**) | 25 | 28 | 35 ± 7 |

of the carboxylic rest, generally an ester group, which can undergo transesterification, interesterification, aminolysis, ammonolysis, or hydrolysis reactions by reaction with alcohols, esters, amines, ammonia, or water, respectively. Additionally, the dealkoxycarbonylation of tropinone-type diesters **100a**−**c** have also been reported by action of pig liver esterase (PLE), obtaining the corresponding monoesters **101a**−**c** (38% yield and 96% ee for the ethyl ester **101b**), precursors of the tropane-type alkaloid (+) and (−)-ferruginine, nicotine acetylcholine receptor agonist (Scheme 36).[86,87]

**3.2.1. Transesterification and Interesterification of Racemic Esters and Carboxylic Acids.** The use of alcohols to selectively modify ester functionalities is commonly known as alcoholysis or transesterification. In most cases, a large excess of alcohol is required to drive the reaction to completion and avoid the reversible character of this process. Similarly, when a different ester is used as the source of nucleophile, the process is currently known as an interesterification reaction. Aziridines are a class of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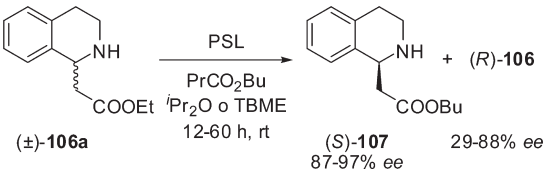
molecules possessing a large synthetic potential as chiral auxiliaries or intermediates in the stereoselective synthesis of amino acids, lactams, and others. Martres et al. reported the lipase-catalyzed transesterification of aziridine carboxylates **102a**−**f** using *n*-butanol (BuOH). The reaction was very sensitive to the size of the *N*-alkyl substituent, and the best results were obtained with *N*-methylated aziridines (Scheme 37).[88] Only PPL and CCL were tested as biocatalysts, obtaining a variety of esters in moderate enantiomeric excess, starting from methyl or 2,2,2-trifluoroethyl carboxylates. Absolute configurations were not reported.

Kanerva and co-workers performed the enzymatic kinetic resolution of 16 amino acid derivatives through alcoholysis with acylase I immobilized on Eupergit C.[89] The influence of different alcohols in the stereoselectivity of the process was studied in the kinetic resolution of racemic methyl pyroglutamate (**104**), finding the best enantioselectivity at 25 °C when using 1-pentanol ($E$ = 40, entry 6 of Table 7). A clear dependence of the temperature was also observed with *n*-butanol, achieving the best selectivities at lower temperatures, as usually occurs in biocatalysis (entries 3, 4, and 5). A compromise between temperature, reaction time, and amount of reagents is always crucial in order to obtain good enantioselectivities and at the same time conversions close to 50% in enzymatic kinetic resolutions. In a similar manner but starting from the ethyl ester, CAL-B catalyzed the transesterification reaction with a series of fatty acids (octanol, decanol, and dodecanol) in 2-methyl-2-butanol as solvent, yielding esters with interesting emulsifying properties or moisturizing or skin penetration activities.[90] No data of stereoselectivity were reported.

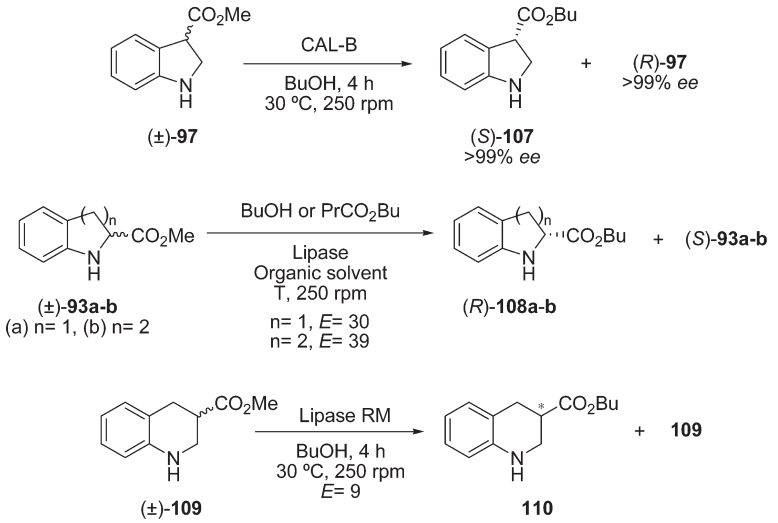
In a previous section, we have seen how Kanerva and co-workers explored the resolution of pipecolic acid methyl ester through an elegant CAL-A catalyzed alkoxycarbonylation reaction.[81] In the same work, the transesterification of (±)-**91a** was studied using CAL-B as biocatalyst, finding low stereoselectivities when butanol or isopropropanol were used as the reaction medium. Unfortunately, but expected, also very poor selectivities were found when using esters in interesterification processes. The same authors reported the lipase-catalyzed kinetic resolution of structurally similar compounds such as 1,2,3,4-tetrahydroisoquinoline-1-acetic acid derivatives (Scheme 38).[91] Moderate to good enantioselectivities ($E$ = 36−87) were found in both TBME and diisopropyl ether ($^i$Pr$_2$O) as organic solvents using butyl butanoate in the presence of *Burkholderia cepacia* lipase (also known as *Pseudomonas cepacia* lipase, PSL) for the production of the (*S*)-butyl ester **107**. The best results were obtained using hydrolytic processes, reactions that were extended to other related compounds, as will be discussed later in this review.

Very recently we have developed a stereoselective synthesis of optically active cyclic α and β-amino esters through lipase catalyzed transesterification and interesterification processes (Scheme 39).[85] Excellent enantiopreferences were observed in the CAL-B catalyzed butanolysis of **97**; however, lower enantiopreferences were observed in the transesterification or interesterification of the homologous 2-substituted ester **93a**. On the other hand, low stereoselectivities were obtained in the transesterification of the

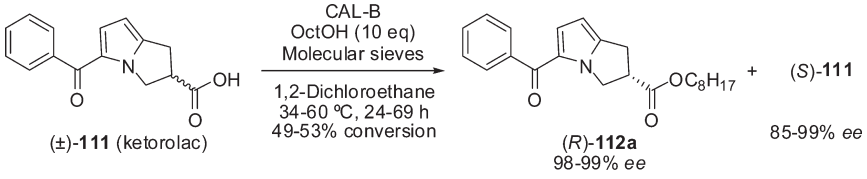
**Scheme 38**




**Scheme 39**




**Scheme 40**





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1,2,3,4-tetrahydroquinoline derivatives bearing an ester group at the 2-position (**93b**) or at the 3-position (**109**).

Starting from carboxylic acids instead of esters, this methodology has also been applied to other bicyclic structures such as ketorolac (**111**), a nonsteroidal anti-inflammatory drug with cyclooxygenase inhibitory and analgesic activities, with the *S*-enantiomer being more potent than its counterpart. Straight chain alcohols (ethanol, 1-butanol, 1-hexanol, 1-octanol, 1-decanol, and 1-tetradodecaol) reacted with this carboxylic acid in a very enantioselective manner using CAL-B as biocatalyst, affording the corresponding (*R*)-esters in nearly enantiopure form; meanwhile, the best enantiomeric excess for the remaining (*S*)-**111** was achieved with 1-butanol in 90% ee after 53% conversion (Scheme 40).[92] An optimization of the experimental conditions was done in terms of solvent and temperature, identifying the combination of 1,2-dichloroethane and 60 °C as the optimal reaction conditions, recovering substrate (*S*)-**111** and product (*R*)-**112a** in almost enantiopure form after 24 h.

**3.2.2. Aminolysis and Ammonolysis of Racemic Esters.** Biocatalytic processes using amines or ammonia as nucleophiles are less explored that the corresponding reactions with other nucleophiles, such as alcohols and especially water. Interestingly, and in order to avoid competitive unwanted reactions such as hydrolytic processes, solvents are used always in anhydrous form.

**Scheme 41**

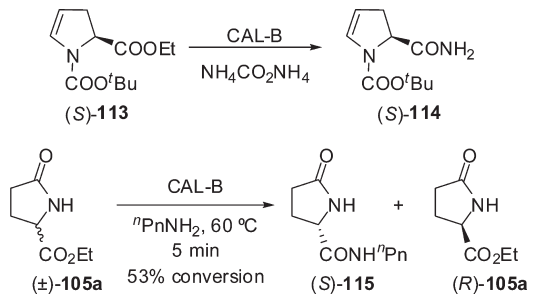
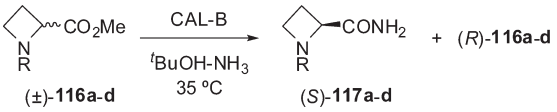



**Table 8. CAL-B-Mediated Ammonolysis of *N*-Alkylazetidine-2-carboxylates**




| entry | R | $ee_S$ (%) | $ee_P$ (%) | *c* (%) |
|---|---|---|---|---|
| 1 | Bn | >99 | 80 | 56 |
| 2 | 4-MeOBn | >99 | 84 | 54 |
| 3 | Allyl | >99 | 97 | 50 |
| 4 | $CHPh_2$ | 0 | – | 0 |

In this review we cover hydrolase-catalyzed stereoselective processes, although some nonasymmetric examples, such as the one reported by Patel and Gill, must also be mentioned because of its clinical application. A CAL-B-mediated reaction was reported for the ammonolysis of a (*S*)-*N*-substituted amino ester **113** to produce the corresponding (*S*)-amide **114**, precursor of a critical intermediate in the synthesis of the dipeptidyl peptidase IV inhibitor Saxagliptin (Scheme 41).[93] Conde and co-workers reported the aminolysis reaction of diethyl glutamate with *n*-pentylamine ($^n$PnNH$_2$) catalyzed by CAL-B in anhydrous diisopropyl ether ($^i$Pr$_2$O) and in the presence of molecular sieves, observing that (*S*)-**105a** was the fast reacting enantiomer, although the amidation proceeded with moderate enantioselectivity (*E* = 25, Scheme 41).[94]

Substituted azetidines are an important class of four-member ring derivatives employed in the synthesis of peptides and non-natural products. They also afford applications as chiral auxiliaries in organocatalysis. Azetidine esters were successfully resolved using CAL-B in *tert*-butanol saturated with ammonia at 35 °C, yielding the unaltered esters (*R*)-**116a**−**c** in enantiomerically pure form, unless the benzhydryl rest was present as *N*-substitution (**116d**, entry 4), where no reaction was observed (Table 8).[95]

**3.2.3. Hydrolysis of Amino Ester Derivatives.** Nucleophilic substitutions involving water are the most common processes involving hydrolases because this is the natural function of this class of enzymes. We have divided this section attending to the size of the cyclic amine subjected to stereoselective modifications, mainly kinetic resolution of racemates or desymmetrization processes of *meso* compounds. For that reason, we will explain the enzymatic results obtained in the preparation of aziridine carboxylates, azetidine carboxylates, proline derivatives, pipecolic acid derivatives, or aliphatic cyclic structures possessing a benzo-fused ring. In spite of its simplicity and although generally hydrolytic processes occur with excellent selectivities, the difficulties to isolate the final optically enriched carboxylic acids have made these type of reactions not always recommended as synthetically useful. Aziridine carboxylates are precursors of α- and β-amino acids and β-lactams but also have remarkable applications as chiral auxiliaries. Depending on the pattern substitution of the amino group and the 2 and/or 3-position, very different stereoselectivities have been observed. For example, Moretti and co-workers have prepared optically active *N*-chloro-2,2-bismethoxycarbonylaziridines in low to moderate chemical and optical yields after testing several hydrolases, such as lipases, esterases, or peptidases.[96] Hydrolysis reactions were carried out on a 1 g scale using phosphate buffer of pH 7.5 at room temperature. Three years later, the same authors extended these promising results to the kinetic resolution of methyl aziridine-2-carboxylates and 2,3-dicarboxylates using CCL in phosphate buffer of pH 7.5 at room temperature

**Scheme 42**[a]


[a] **118a**: $R^1$ = H and $R^2$ = H; **118b**: $R^1$ = Cl and $R^2$ = H; **118c**: $R^1$ = Ac and $R^2$ = H; **118d**: $R^1$ = COPr and $R^2$ = H; **118e**: $R^1$ = $SO_2Me$ and $R^2$ = H; **118f**: $R^1$ = 4-$C_6H_4$-$SO_2$ and $R^2$ = H; **118g**: $R^1$ = H and $R^2$ = $CO_2Me$; **118h**: $R^1$ = Cl and $R^2$ = $CO_2Me$; **118i**: $R^1$ = Ac and $R^2$ = $CO_2Me$; **118j**: $R^1$ = COPr and $R^2$ = $CO_2Me$; **118k**: $R^1$ = $SO_2Me$ and $R^2$ = $CO_2Me$.


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

(Scheme 42). Depending on the pattern substitution, final products were isolated with low to excellent stereochemical purity, observing inclusively the chemoselective and stereoselective hydrolysis of the corresponding amides for substrates **118c,d** within shorter reaction times.[97] Renold and Tamm also found PLE as an asymmetric catalyst for the hydrolysis of *cis*- and *trans*-dimethylaziridine-2,3-dicarboxylates.[98]

The regio- and enantioselective syntheses of fluorinated *anti*-α-functionalized-*β*-amino acids and *trans*-trifluoromethyl-*β*-lactams have been possible through the CAL-B mediated kinetic resolution of racemic *trans*-*N*-benzyl-3-trifluoromethylaziridine-2-carboxylate **120** (Scheme 43).[99] The enzymatic hydrolysis reaction was carried out in phosphate buffer pH 7.5 at 37 °C with a very low enzyme loading (1:50 w/w enzyme/aziridine). Interestingly, in only 30 min, 45% conversion was attained, obtaining finally optically enriched esters (+)-**120** and (−)-**122** with high enantiomeric excesses.

Kumar and co-workers have also described the kinetic resolution of different *N*-arylaziridine-2-carboxylates **123a**−**g** *via* CRL catalyzed enantioselective hydrolysis in phosphate buffer pH 7.5, isolating with low to high enantiomeric purity the unreacted esters (*S*)-**123a**−**g** depending on the substitution present on the phenyl ring (Table 9).[100] No data from the carboxylic acids **124a**−**g** were given, probably due to their instability that will be discused in the following sections.

(*S*)-Azetidine-2-carboxylic acid (**127**) is a nonproteogenic cyclic amino acid, a key component of deoxymugineic acid and nicotianamine, which are potent plant-origin promoters for the uptake of iron from soil. Its synthesis has been possible through a chemoenzymatic route involving the lipase-catalyzed hydrolysis of a (2.7:1) mixture of (2*S*,1′*S*)- and (2*R*,1′*S*)-monoester **125** isomers using CAL-B as biocatalyst (Scheme 44). Final hydrogenolysis and recrystallization allowed the isolation of (*S*)-**127** in enantiomerically pure form.[101]

Proline derivatives constitute probably the most employed family of organocatalysts in asymmetric synthesis but also present remarkable applications in medicinal chemistry or as building blocks for more complex structures. The research group of Kazlauskas studied the hydrolysis of protonated proline methyl ester (±)-**128** (Scheme 45) using different preparations of lipase from *Aspergillus niger* (ANL).[102] Both crude and partially purified lipase were tested as biocatalysts, finding higher enantioselectivities ($E$ = 100) with the purified lipase in comparison with the crude enzyme ($E$ = 20). The authors studied the influence of the pH on the enantioselectivity shown by partially purified ANL, identifying pH = 5 as the optimum condition.

Sugai and co-workers examined the enzyme-catalyzed kinetic resolution of *N*-substituted-proline derivatives (±)-**130a**−**c** using a wide a set of enzymes, with CAL-B being found as the most efficient biocatalyst for the hydrolysis of the carboxylic ester group.[103] The influence of the substitution present in the nitrogen atom of the cycle was compared (Table 10), observing no reaction with the *N*-carbamoyl substrate (entry 1, **130a**); meanwhile, an excellent stereoselection was achieved with the *N*-Boc (entry 2, **130b**) and the *N*-Cbz (entry 3, **130c**) derivatives. Both enantiomers of *N*-carbamoyl-2-methoxymethylpyrrolidine, precursors of Ender's chiral auxiliaries, were later chemically prepared.

DiCosimo and co-workers reported the CAL-B catalyzed hydrolysis of racemic 4-oxo-1,2-pyrrolidinecarboxylic acid dimethyl ester (**132**) with an excellent enantioselectivity. The compound was later chemically converted to optically active (2*R*,4*R*)-*cis*-4-hydroxy-D-proline, one of the four possible diasteromers of 4-hydroxyproline,

**Scheme 43**

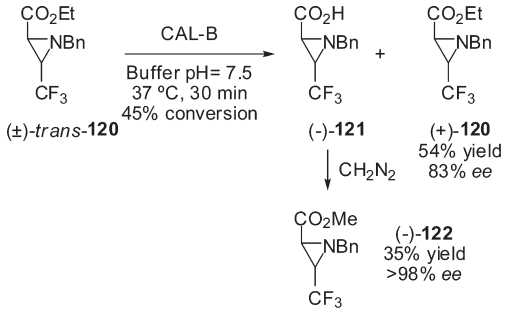
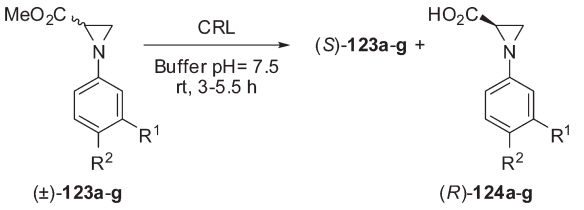


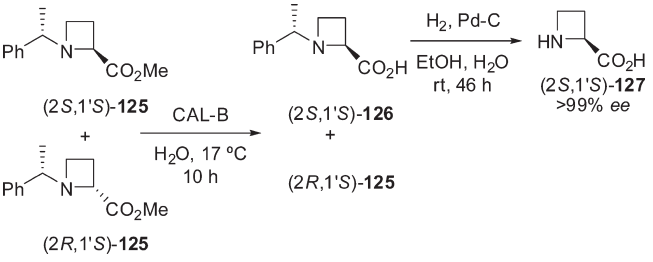

**Table 9. Enantioselective Hydrolysis of Racemic Aziridine-2-carboxylate Methyl Esters 123a−g by CRL**



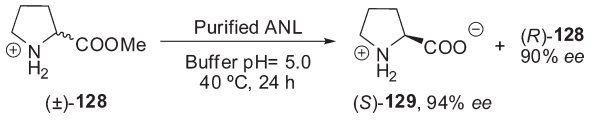

| entry | substrate | $R^1$ | $R^2$ | $t$ (h) | ratio[a] | $c$ (%) | $ee_{ester}$ (%) |
|---|---|---|---|---|---|---|---|
| 1 | **123a** | H | H | 4 | 1:2 | 48 | 84 |
| 2 | **123b** | H | OMe | 5 | 1:2 | 46 | 79 |
| 3 | **123c** | H | F | 5.5 | 1:4 | 44 | 7 |
| 4 | **123d** | H | Br | 5 | 1:2 | 50 | 99 |
| 4 | **123e** | H | Me | 4.5 | 1:2 | 48 | 70 |
| 3 | **123f** | H | $NO_2$ | 3 | 1:2 | 45 | 12 |
| 6 | **123g** | Br | Me | 5.5 | 1:4 | 46 | 15 |

[a] Ratio enzyme/substrate in weight.

**Scheme 44**




**Scheme 45**




which is a precursor of pharmaceuticals and agrochemicals (Scheme 46).[104] Additionally, diasteromeric mixtures of 4-hydroxy-1,2-pyrrolidinedicarboxylic acid dimethyl esters (2*R*,4*R*)-, (2*S*,4*R*)-, (2*R*,4*S*)-, and (2*S*,4*S*)-**135** were prepared and screened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against a collection of enzymes. CAL-B was found again as the best biocatalyst for the stereoselective hydrolysis of five out of six pairs of diasteromers of **135**. The ability to resolve several combinations of diasteromers with CAL-B was attributed to the large differences in the relative rates of methyl ester hydrolysis for each of the four diasteromers.

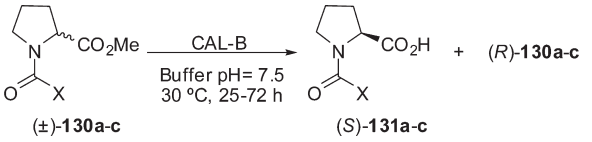
The separation of the *cis*/*trans* diasteromeric pyrrolidine mixture **136** was achieved through the enzymatic selective hydrolysis of the corresponding *trans*-diastereomer (Scheme 47).[105] Several hydrolases were tested, most of them acting with high activity but low selectivity. Fortunately, two selective enzymes were found: an esterase from *Candida lypolytica* catalyzed exclusively the hydrolysis of the *trans* diasteromer, while *Rhizomucor miehei* lipase (RM Lipase) selectively hydrolyzed only the *cis* diasteromer.

The first example of a kinetic resolution of α-*exo*-methylene-γ-lactams **138a**−**c**, the key structural unit in many natural bioactive compounds, was achieved through their hydrolysis reaction catalyzed by α-chymotrypsin.[106] The presence of a benzyl protecting group in the nitrogen atom was required for the stereoselective preparation of the corresponding (*R*)-acids **139a**−**c** and (*S*)-esters **138a**−**c** (Table 11).

Probably one of the most interesting challenges achieved by enzymatic sources is the desymmetrization of prochiral or *meso* compounds. Through the years, hydrolases have effectively allowed the development of this type of processes; for example, PLE catalyzed the asymmetrization of the diester **140** using 10% of acetone as cosolvent, isolating in high yield and with an 80% ee the monoester (*S*,*R*)-**141**, a useful intermediate for the synthesis of carbapenem antibiotics (Table 12, entry 1).[107] The use of dimethyl sulfoxide (DMSO) as cosolvent has played a crucial role in the enzymatic reaction, allowing complete stereoselectivity with 25% DMSO (entry 4) and isolation of the final product in enantiomerically pure form,[108] while a significant decrease was noticed in the absence of cosolvent (entry 2).

An exhaustive screening of enzymes was developed for the enzymatic kinetic resolution of methyl *N*-substituted pyrrolidine-3-carboxylate, finding lipase AS from *Aspergillus niger* as an excellent biocatalyst for the production of both enantiomers of

**Table 10. CAL-B Catalyzed Hydrolysis of *N*-Protected Proline Derivatives**




| entry | X | *t* (h) | ee$_p$ (%) | ee$_s$ (%) | *c* (%) | *E* |
|---|---|---|---|---|---|---|
| 1 | $NH_2$ | 72 | – | 0 | 0 | – |
| 2 | O$^t$Bu | 25 | >99.9 | 98.7 | 49.7 | >200 |
| 3 | OBn | 38 | 99.8 | 97.8 | 49.5 | >200 |

**Scheme 47**

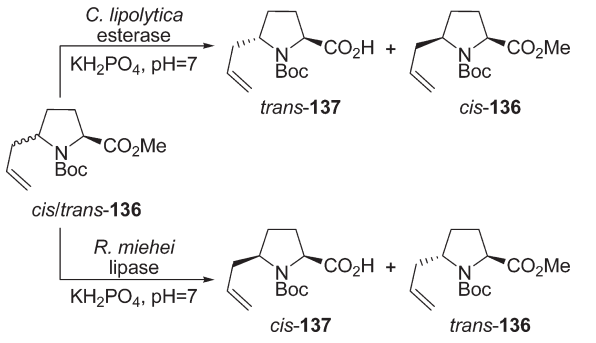
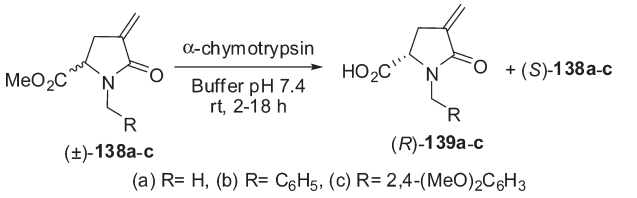



**Table 11. Enzymatic Hydrolysis of Racemic α-*exo*-Methylene-γ-lactams**




| entry | 138 | *c* (%)[a] | ee$_P$ (%)[b] | *c* (%) | ee$_S$ (%)[b] | *E* |
|---|---|---|---|---|---|---|
| 1 | **a** | 57 (3 h) | 55 (16) | 57 (3 h) | 72 (28) | 7 |
| 2 | **b** | 35 (6 h) | 99 (25) | 64 (18 h) | >99 (30) | >200 |
| 3 | **b** | 52 (8 h) | 97 (38) | 52 (8 h) | 98 (39) | >200 |
| 4 | **c** | 28 (2 h) | 98 (22) | 65 (14 h) | >99 (27) | 140 |
| 5 | **c** | 51 (4 h) | 95 (37) | 54 (4 h) | 96 (41) | 140 |

[a] Reaction times in parentheses. [b] Isolated yields in parentheses.

**Scheme 46**

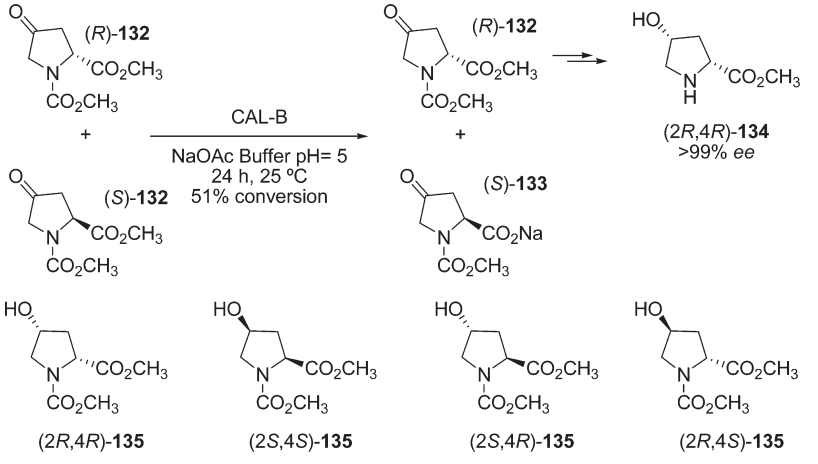




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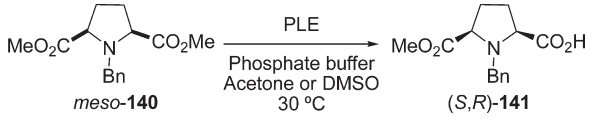
**Table 12. Enzymatic Hydrolysis of Diester 140**




| entry | cosolvent[a] | $ee_P$ (%) |
|---|---|---|
| 1 | acetone (10) | 80 |
| 2 | DMSO (0) | 17 |
| 3 | DMSO (10) | 61 |
| 4 | DMSO (25) | 100 |
| 5 | DMSO (50) | 97 |

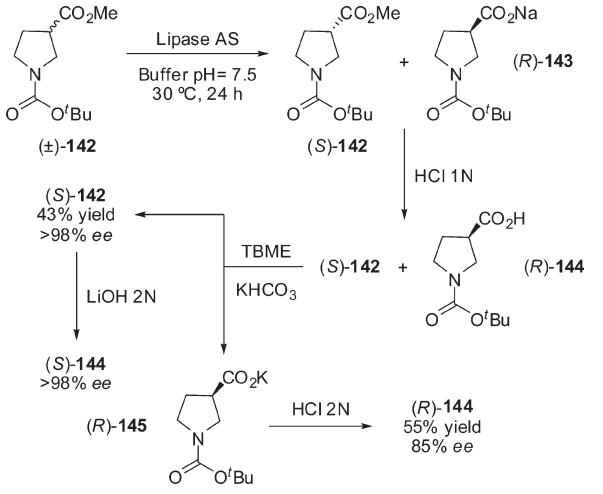
[a] Percentage of cosolvent in parentheses.

1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (**144**), starting from racemic 1-*tert*-butyl-3-methylpyrrolidine-1,3-dicarboxylate **142** (Scheme 48).[109] Scale-up reactions were performed on 1 g, 10 g, 100 g, and 1 kg of substrate using 20% w/w of lipase AS (substrate/enzyme at 5:1), developing a combination of chemical hydrolysis reactions and an extraction procedure to afford (*S*)-**144** in enantiopure form and (*R*)-**144** in 85% ee.

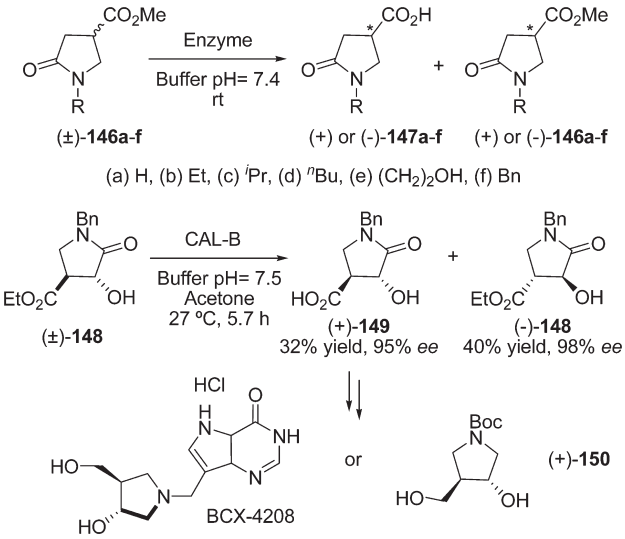
Valentin and co-workers described the synthesis of methyl *N*-substituted-5-oxo-pyrrolidine-3-carboxylates **146a**−**f** and their subsequent enzymatic kinetic resolution in order to prepare enantiomerically pure proline derivatives (Scheme 49).[110] Among a series of hydrolytic enzymes, pig liver acetone powder (PLAP), porcine pancreas lipase (PPL), and α-chymotrypsin gave the most significant results. The enantiodiscrimination was excellent only when the nitrogen atom was protected with a benzyl group (**146f**) and using α-chymotrypsin. Efforts to explain the results were done performing molecular mechanistic studies, attributing the mentioned good selectivity to the particularly favorable nonbonding interactions established in the enzyme−substrate complex. Similarly, Mason and co-workers reported a practical synthesis of (3*R*,4*R*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylpyrrolidin-3-ol (**150**), intermediate in the synthesis of a variety of inhibitors, developing a successful multigram scale (345 g) for the CAL-B catalyzed kinetic resolution of the *N*-benzyl precursor **148** using a phosphate buffer pH 7.5 (Scheme 49) and a very low loading of enzyme, only 10% w/w enzyme/substrate ratio.[111] This chemoenzymatic approach overcomes the limitations when attempting the enzymatic resolution of **150**, which proceeds with very low selectivity.[112] Precursor (+)-**150** has been also used for the synthesis of a purine nucleoside phosphorylase inhibitor BCX-4208.[113]

Pipecolic acid derivatives contain the piperdine subunit, which is present in a number of bioactive compounds. A wide set of enzymatic studies have been performed toward the preparation of this important chiral synthon employing biocatalytic methods. Using PLE as biocatalyst, Toone and Jones investigated the resolution of racemic *N*-protected unnatural amino acid derivatives **151**, **153**, and **155** by means of hydrolysis reactions at pH 7 in a 1 g scale (Scheme 50), obtaining the corresponding acids and methyl esters in low to moderate optical purity (6−45% ee).[71] Years later, Kazlauskas and co-workers selected octyl pipecolate as starting material because of its higher hydrophobicity, which simplifies the separation of acid and ester products, developing the efficient kinetic resolution of (±)-**157** (Scheme 43).[114] Reaction of the *N*-acetyl octyl pipecolate occurred with very low selectivity using lipase from *Aspergillus niger*; however, when

**Scheme 48**




**Scheme 49**




the amino group was protonated, much better selectivities were attained, showing a great influence due to the charge of the substituent and not their size. Finally, the purification of the enzyme led to the best results, obtaining in a 10 g scale reaction (*S*)-pipecolic acid **158** in 93% ee (19% yield referred to 50% conversion) and (*R*)-octyl pipecolate **157** in 73% ee ($E$ = 60), which can be transformed into (*R*)-**158** in enantiopure form after subsequent chemical transformations.

The synthesis of alkaloid (+)-cytisine, whose opposite enantiomer is a potent and α4β2 subtype-selective partial agonist at nicotinic acetycholine receptors, was possible by the resolution of the monoester (±)-**159** by means of hydrolytic reaction catalyzed by α-chymotrypsin (Scheme 51),[115] to give the isomer (*R*)-**160** in 42% yield and 98% ee. Also with synthetic medicinal purposes, the enzymatic kinetic resolution of (±)-*cis*-**161** was successfully performed using PLE in order to obtain complex macroheterocyclic peptidomimetic inhibitors of the aspartic protease β-site amyloid precursor protein cleaving enzymes (BACE).[116] After reaction in a buffer solution of pH 7.2,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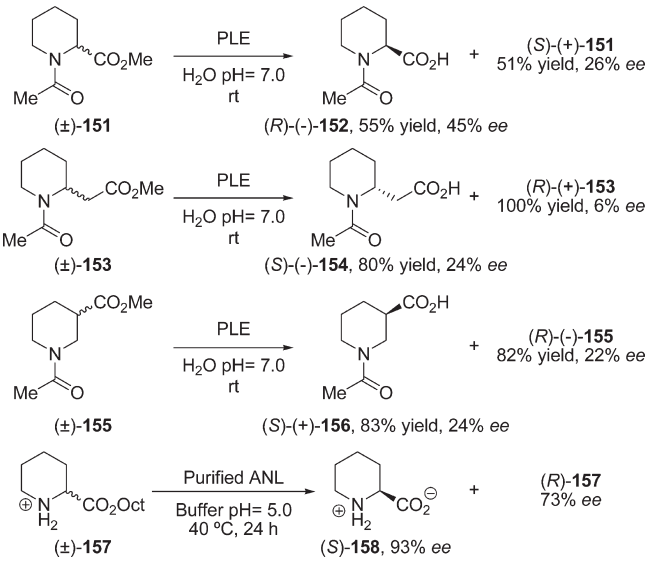
**Scheme 50**

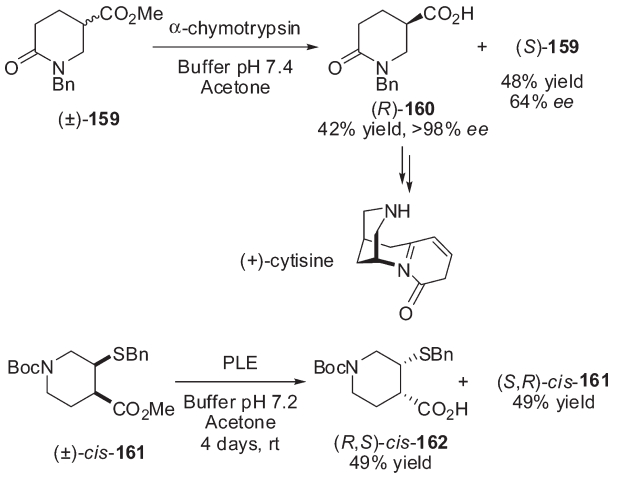



*cis*-ester **161** and *cis*-acid **162** were isolated in quantitative yield and possibly in enantiomerically pure form.

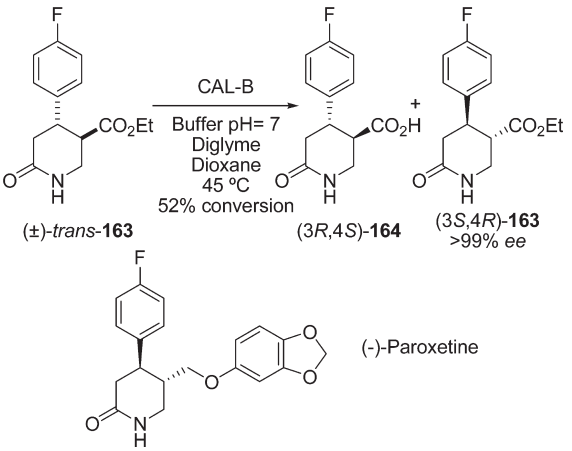
As previously mentioned in section 2.3.2, (−)-paroxetine is one of the most common commercially available drugs for the treatment of depression and obsessive compulsive and panic disorders, so many synthetic methods have been developed for its preparation in enantiopure form. Palomo and co-workers have done an extensive work in the enzymatic kinetic resolution of the intermediate (±)-*trans*-4-(4′-fluorphenyl)-6-oxopiperidin-3-ethyl carboxylate (**163**), finding the highest activities for CAL-B (Scheme 52).[117] This enzyme was purified through adsorption or immobilization protocols, obtaining the ester (3*S*,4*R*)-**163** in enantiomerically pure form after 50% conversion when using the immobilized glyoxyl-CAL-B preparation. Remarkably, other preparations led to completely nonselective reactions, highlighting the importance of a correct immobilization system for enzymatic sources. Also, the appropriate choice of cosolvents was highlighted, finding the best results for a mixture of diglyme (15%) and dioxane (5%), which allow a good solubility of the starting material but also the existence of low enzymatic inhibition. No significant loss of activity or enantioselectivity was found after reusing ten times the enzyme under the same experimental conditions. The same authors reported in the same year the use of CAL-A for the enzymatic production of ester (3*S*,4*R*)-**163**, observing that meanwhile the activity of different commercially available preparations was negligible, the purification of the enzyme led to the discovery of a concomitant esterase with a high activity.[118] This esterase was purified and immobilized, and this new enzymatic preparation was found to be highly enantioselective in the hydrolysis of (±)-*trans*-**163**. The enzyme was reused, and no loss of activity was found after three cycles.

Iding and co-workers reported the synthesis of nonracemic *N*-Boc-piperidine-3,5-dicarboxylic acid 3-methyl esters from a *cis*/*trans*-mixture of diester **165a** obtained by several consecutive enzymatic reactions (Scheme 53).[119] Each of the reactions were individually analyzed using a well plate based array based on the color of a pH indicator: (a) isolation of racemic *trans*-**165a** in 84% yield (12 g scale) by selective hydrolysis of the *cis*-diester **165a**; (b) Chirazyme L-2, a CAL-B preparation, hydrolysis of

**Scheme 51**




**Scheme 52**




the (*S*,*S*)-diester to afford the monoacid (*S*,*S*)-**166a** with a moderate selectivity (*E* = 39); (c) chemical esterification of the monoacid (*S*,*S*)-**166a**; (d) hydrolase ESP-ESL-1064 from Diversa Corporation of opposite enantiopreference than CAL-B allowed the isolation of the (*S*,*S*)-diester in 97% ee; (e) finally, PLE monohydrolyzed smoothly both optically active monoesters (*S*,*S*)- and (*R*,*R*)-**166a**. The overall yield with respect to the *trans*-isomer for the whole enzymatic sequence on the gram-scale was 23% for (*S*,*S*)-**166a** and 31% for (*R*,*R*)-**166a**.

At this point, we have shown the potential of hydrolases for the kinetic resolution of pipecolic acid derivatives possessing one or two stereogenic centers, and also how hydrolytic enzymes allow the separation of *cis*/*trans* mixtures. Now we will discuss the main achievements in the desymmetrization processes of *meso* or prochiral compounds for the production of optically active piperidines, reactions that lead to the final products in a maximum of 100% yield. Lesma and co-workers described the desymmetrization of dimethyl *meso*-piperidine-3,5-dicarboxylates **165b**−**d**, suitable precursors of the nontryptamine portion of some *pseudo*-aspidospermidine and takamine alkaloids.[120] After testing PLE, PPL, and CCL (Scheme 54), the latter gave the best results, obtaining **166b**,**d** with the best enantiomeric excesses (78−80% ee), although with a low degree of conversion (20−25%).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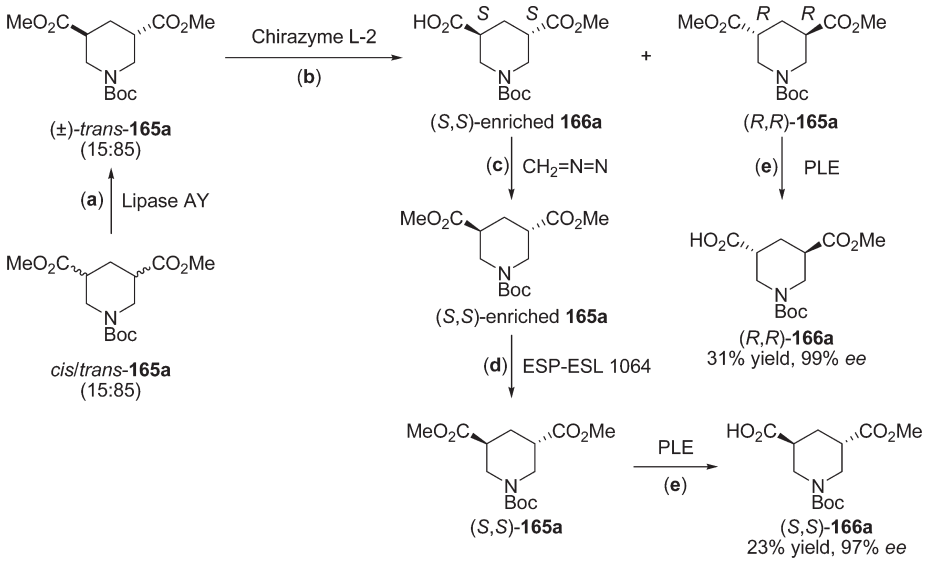
**Scheme 53**

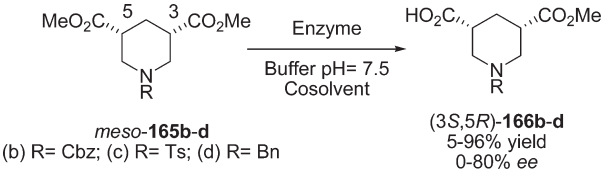



Isogalactofagomine, a galactosidase inhibitor, was prepared in a stereoselective manner involving the desymmetrization of *meso*-diester **167** using a pH 7.5 phosphate buffer.[121] After a screening of enzymes, lipase *Mucor javanicus* was found as the best biocatalyst, obtaining the monoacid (3*R*,4*S*,5*S*)-**168** in 95% yield and 99% ee (Scheme 55). Interestingly, the reaction was performed successfully on a multigram scale (8.46 g).

4-Aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylates have been widely used for the treatment of cardiovascular diseases. Enzymatic modification in any of the positions of the cyclic ring leads to optically active derivatives possessing an asymmetric carbon at the 4-position that can show different biological activities. For example, hydrolytic reactions were carried out over prochiral diesters **169a**−**e** by Hirose et al. using lipase AH or *Burkholderia cepacia* lipase (PSL).[122] By employing PSL, (*R*)-monoacids **170a**−**e** were obtained in general excellent enantiomeric excesses in $^{i}Pr_2O$ or cyclohexane (73−99% ee); surprisingly, lipase AH led to the corresponding (*S*)-monoacids in $^{i}Pr_2O$ (42−99% ee) or the (*R*)-monoacids when the reaction was carried out in cyclohexane (88−91% ee, Scheme 55). Years later, 16 different mutants of PSL substituted with each amino acid corresponding to AH lipase were prepared by site-directed mutagenesis.[123] Only a slight decrease of the enantioselectivity was observed for three mutants (V266L, L287I, and F221L). With these results in hand, the authors prepared a new mutant containing the three positive mutations (V266L, L287I, and F221L). Interestingly, this enzymatic variant showed reversed enantioselectivity compared with the native protein.

Finally in this section, examples of stereoselective hydrolytic processes starting from bicyclic amino acid derivatives will be discussed. Kurokawa and Sugai described the enantioselective hydrolysis of racemic *N*-Boc-indoline-2-carboxylic acid methyl ester (**171**) catalyzed by CAL-B, yielding the (*S*)-acid **172** and the remaining (*R*)-ester **171**, both in enantiopure form (Scheme 56).[124] Similarly, the unprotected indoline-3-carboxylic acid methyl ester (**97**) has been recently efficiently resolved by Pietruszka and Simon using the same enzymes but under much milder reaction conditions (0 °C instead of 60 °C), with shorter

**Scheme 54**



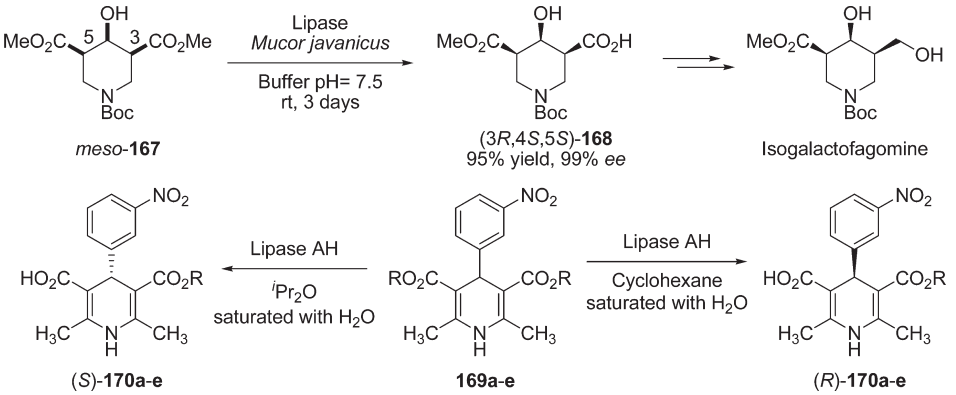

reaction times (3−4 h instead of 30 h), and with a really impressive low loading of catalyst (1:500 w/w enzyme to substrate).[125]

The enzymatic kinetic resolution of 2-substituted-1,2,3,4-tetrahydroisoquinolines **93b** and **175** has also been reported,[126] substrates that are interesting intermediates for the synthesis of tricyclic quinoxalinediones **177a**−**b**, a potent *N*-methyl-D-aspartate-glycine antagonist applied for the control of the memory function. Meanwhile, the (*R*)-enantiomer of racemic ester **93b** was stereoselectively hydrolyzed by CAL-B (*E* = 67, Scheme 57), and more than 30 hydrolases showed very little enantiodiscrimination in the hydrolysis of (±)-**175** in phosphate buffer (*E* < 9). Changing in this case to an organic solvent as a reaction medium, better selectivities were found, identifying polar solvents such as acetonitrile, THF, acetone, or dioxane as the ones that provided better results (*E* = 78−146 with CAL-B). In fact, the reaction in THF on a 9 g scale of substrate (1:5 w/w enzyme to substrate) at 30 °C and 23 equiv of water led after 72 h to a 50% conversion yielding the (*S*)-ester **175** in 93% ee with an optical purity that was increased to 97.5% ee after a recrystallization process.

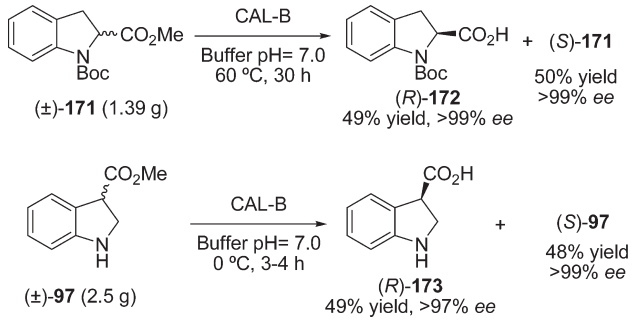
The kinetic resolution of structurally similar 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid has been exhaustively studied by Füllöp and co-workers using CAL-B and organic solvents in the presence of 0.5 equiv of water (Scheme 58).[127] $^{i}Pr_2O$ was found as the best solvent ($ee_S$ = 38% and $ee_P$ = 98%), whereas other parameters, such as the water content (0−5 equiv) or the temperature (3−25 °C), were not important to the enantioselectivity of the process. Finally, the authors developed an elegant dynamic kinetic resolution process, studying the behavior of the enzyme with different amines as additives (diisopropyl


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

Scheme 55




Scheme 56




ethylamine, dipropylamine, or piperidine) or toluene/acetonitrile mixtures as solvent (9:1 to 1:1), showing an efficient gram scale DKR process to obtain after 6 days the (*R*)-amino acid **179** in 96% ee, with it being necessary to add the enzyme in two portions, the second after the second day of reaction. In a similar approach but using an aqueous system at 3 °C after 24 h, the DKR of (±)-**178** with CAL-B proceeded in 98% ee and 85% yield for the isolation of (*R*)-**179**.[128] Also, the synthesis of both enantiomers of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (**179**) was accomplished through DKR processes based on CAL-B or subtilisin Carlsberg-catalyzed enantioselective hydrolysis of the corresponding ethyl ester enantiomers of **180** in an ammonium acetate buffer pH = 8.5. (*R*)-Amino acid **181** for CAL-B and (*S*)-**181** for subtilisin Carlsberg were obtained with high enantiopurity (92−93% ee) in good yields (85−92% ee).

Enantiomers of 1,2,3,4-tetrahydroisoquinoline- and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-acetic acids (**182a**−**b**) were prepared by the same authors through a kinetic resolution process using different PSL preparations as biocatalysts in a hydrolysis reaction in diisopropyl ether as solvent (Table 13). (*S*)-Acids **182a**−**b** were obtained enzymatically, whereas the (*R*)-acids were isolated after chemical hydrolysis of the (*R*)-esters **106a**−**d** obtained by biohyphen-qj;catalytic methods. PSL-D preparation (supported on diatomite) showed a higher stereoselectivity than PSL-C II (immobilized on Toyonite) under optimized experimental conditions with respect to solvent, temperature, or equivalents of water.[91] Methoxy substitutions on the benzene ring clearly enhanced the reactivity of (±)-**106c** (entry 3) compared to the unsubstituted compound (±)-**106a** (entry 1), whereas alkyl-activated (±)-**106b**, **d** (entries 2 and 4) behaves in a similar manner.

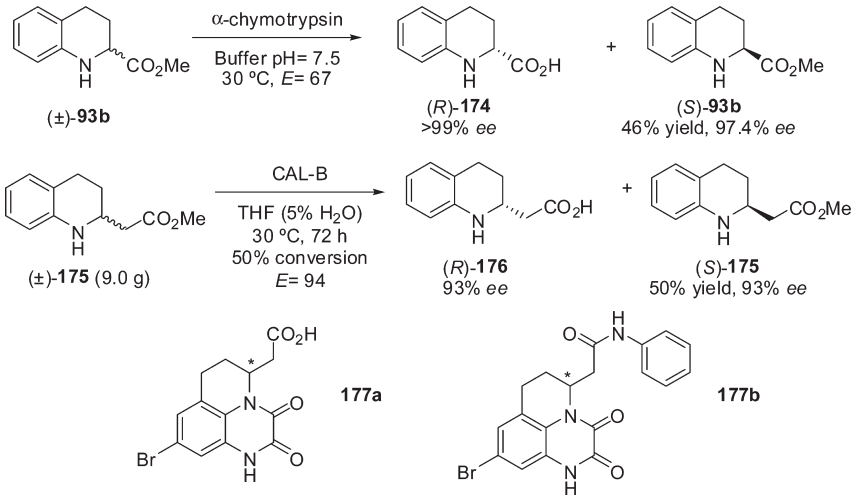
In a previous section we have described the enzymatic kinetic resolution of ketorolac (±)-**111** *via* a stereoselective esterification process;[92] however, the production of (*S*)-**111** can also be achieved through hydrolysis reactions. In the same contribution, the authors studied the behavior of the butyl ester (±)-**112b** with a set of hydrolases, finding CAL-B and *Streptomuces griseus* lipase as efficient catalysts for this transformation (Scheme 59). The conversion degree was carefully followed in order to isolate the ester or the acid in enantiomerically pure form. Fülling and Shi reported, before *Mucor miehei* lipase, *Streptomuces griseus* and *Aspergillus saitoi* as excellent biocatalysts for the kinetic resolution of (±)-**112c** ($E > 100$).[129] Interestingly, when the reaction with *Streptomuces griseus* lipase was carried out in phosphate buffer pH 9.7 during 24 h at 22 °C (Scheme 59), the (*S*)-**111** was isolated in 92% yield with 85% ee, optical purity that increased after a crystallization purification (94% ee). Finally, 140 native hydrolases were screened for the enantioselective hydrolysis of (1*RS*)-*N*-Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate (**183**), finding Seaprose S as the ideal catalyst for the production of enantiopure (*S*)-**184** and (*R*)-**183** in 43% and 46% yield, respectively (Scheme 59), where the major drawbacks for industrial implementation are the use of a high loading of catalyst (8:1 w/w enzyme to substrate), long reaction times (3 days), low substrate concentration (1 g/L), and the need of high-volume solvent extraction procedures for the recovery of optically active acid and ester.[130]

**3.2.4. Hydrolysis of Racemic Amides and Nitriles.** The enzyme catalyzed hydrolysis of nitriles is known to occur *via* two different pathways (Scheme 60).[131] The family of nitrilase enzymes (EC 3.5.5.1) catalyzes the direct conversion of nitriles to carboxylic acids and ammonia without the formation of intermediate amides. On the other hand, an alternative pathway involving the hydration of the nitrile to the amide by a nitrile hydratase (EC 4.2.1.84) followed by the hydrolysis of the amide by the action of an amidase (EC 3.5.1.4) is very common when whole microorganisms are employed as biocatalysts. In most cases, the nitrile hydration process occurs with low enantioselectivity, while the microbial amidases are generally very enantiospecific, producing in most cases the product in high optical purities.

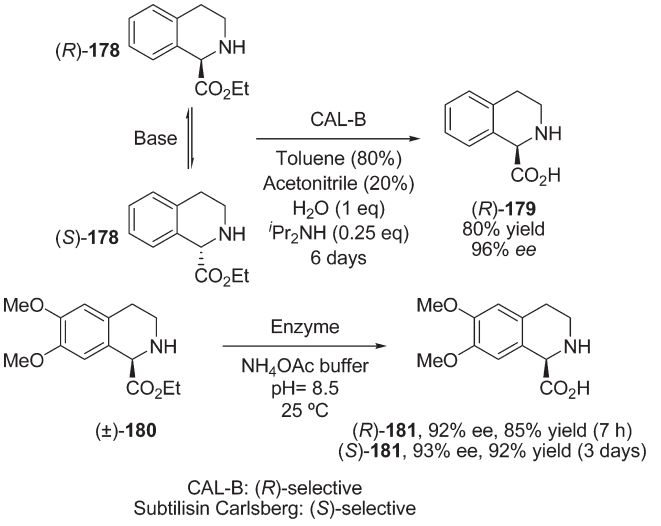
Wang and co-workers performed the hydrolysis of racemic 1-aryl-aziridine-2-carbonitriles **185a**−**e**,**h**−**i** using the bacteria *Rhodococous erythropolis* AJ270 as biocatalyst, a nitrile hydratase/amidase containing whole cell catalyst.[132] The authors found that the nitriles were readily converted into the racemic amides **186a**−**e**,**h**−**i** by the action of a nonselective nitrile hydratase.


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

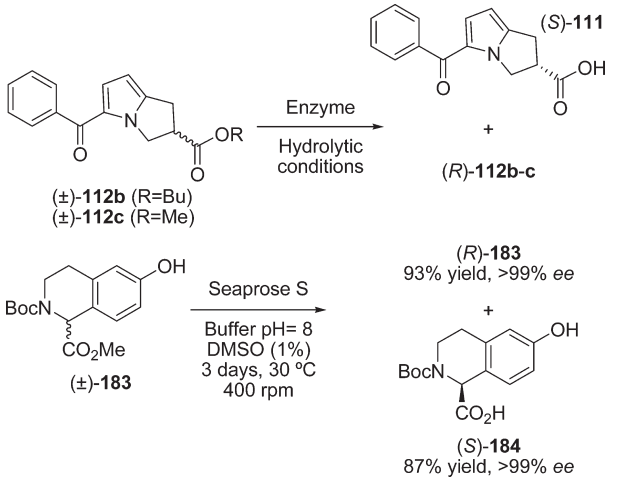
**Scheme 57**



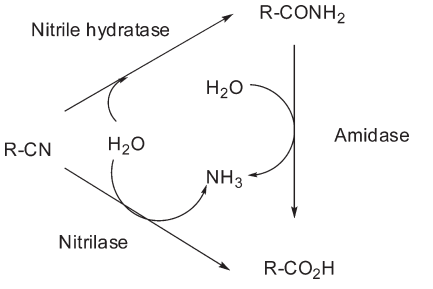

**Scheme 58**




**Scheme 59**




**Scheme 60**

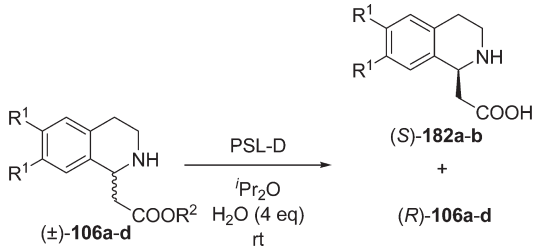



Then the action over **186a**−**e**,**h**−**i** of a (*R*)-selective amidase led to an efficient biotransformation in most cases, affording the corresponding optically active (*R*)-amides **186a**−**e**,**h**−**i** and (*S*)-amino acids **124a**−**e**,**h**−**i**, which were converted into the methyl esters **123a**−**e**,**h**−**i** in order to facilitate their isolation from the aqueous media. A concise analysis of the hydrolysis of **185a**−**e**, **h**−**i** revealed that the substitution pattern of the aromatic ring had a great influence on both enantioselectivity and reactivity (Table 14). A fast and stereoselective reaction was observed for the 4-substituted derivatives **185a**−**e** (entries 1−5). However, a lower reaction rate was observed for the 3-Me derivative **185h** (entry 6), and a very slow and nonselective hydrolysis was observed for 2-Me derivative **185i** (entry 7).

At the same time our research group has studied the biotransformation of different unactivated *N*-substituted-aziridine-2-carboxamides **186a**−**b**,**j**−**k** using whole cells of *Rhodococcus rhodochrous* IFO15664 as biocatalyst (Scheme 61).[133,134] The enantiomerically pure amides (*R*)-**186a**−**b**,**j**−**k** were obtained with excellent isolated yields, although, unfortunately, none of the amino acids **124a**−**b**,**j**−**k** could be isolated after the biotransformation. A great difference between the reaction rate of the *N*-aryl-derivatives **186a**−**b**,**j** (4.5−6.7 h) and the *N*-benzyl derivative **186k** (69 h) was observed, which is probably due to the stiffer character of the aryl substituents thus fitting more efficiently in the active site of the amidase. The biotransformation


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

**Table 13. Hydrolysis of 1,2,3,4-Tetrahydroisoquinoline Derivatives with PSL-D and 4 equiv of Water in $^{i}Pr_2O$ at Room Temperature**




| entry | substrate | $R^1$ | $R^2$ | $ee_S$ (%) | $ee_P$ (%) | $c$ (%) | $E$ |
|---|---|---|---|---|---|---|---|
| 1 | **106a** | H | Et | 94 | 97 | 49 | >200 |
| 2 | **106b** | H | $CH_2CH_2OMe$ | 97 | 98 | 50 | >200 |
| 3 | **106c** | OMe | Et | 89 | 98 | 48 | >200 |
| 4 | **106d** | OMe | $CH_2CH_2OMe$ | 97 | 96 | 50 | >200 |

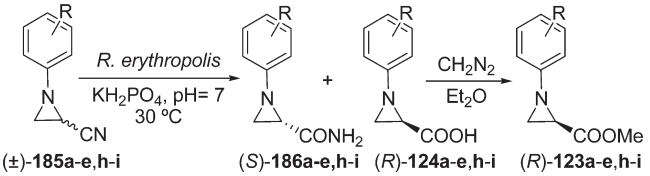
can also be performed starting from the nitriles **186a−b,j−k**, due to the presence of a concomitant nitrile hydratase in the microorganism. Optically active amide **186k** has been used to produce a series of high added value optically compounds such as the nonproteinogenic α-amino acids (*R*)-**187** and (*S*)-**188** and the vicinal diamine (*S*)-**189**.

Similarly, the group of Wang studied the enantioselective hydrolysis of racemic *trans*-3-arylaziridine-2-carboxamides **190a−h** using *Rhodococous erythropolis* whole cells as catalyst.[135] Excellent results were attained, with the *trans*-amides recovering, in most cases, the enantiomerically pure amides in excellent isolated yields (Table 15, entries 1−5). Interestingly, similar values of reactivity and stereoselectivity were observed for the *N*-unprotected aziridine **190h** (entry 8). Unfortunately, it was not possible to isolate the corresponding carboxylic acids **191a−h** because of their instability in the reaction media, as observed in our research group for similar substrates.[136] On the other hand, the relative stereochemistry of the aziridine ring seems to be essential for the enzyme recognition, since the *cis*-configured amides were not accepted by the biocatalyst.

Very recently, the same authors studied the enantioselective hydrolysis of racemic azetidine-2-carbonitriles using *Rhodococous erythropolis* whole cells as catalyst.[137] As observed with aziridines,[132−136] the hydration of the nitriles **192a−g** occurred extremely fast and with low enantioselectivity, due to the action of the bacterial nitrile hydratase. In contrast, the amidase catalyzed hydrolysis of the *N*-benzylazetidinecarboxamides **193a−f** acting with high to excellent stereoselectivities (Table 16, entries 1−6). Encouraged by the excellent results, the authors performed the hydrolysis of the 4-methyl-derivative, possessing a relative *trans* stereochemistry, **193g** (entry 7) and the 2-methyl-derivative, containing a quaternary carbon, **193h** (entry 8). Both substrates were accepted by the amidase; however, the reaction was much more enantioselective for **193g**.

The group of Kiener reported the preparation of optically active cyclic amino acids **196** and **158** by kinetic resolution of racemic piperazine-2-carboxamide (**195**) and piperidine-2-carboxamide (**197**), with selective amidases present in whole bacterial cells.[138] Interestingly, in the case of (±)-**195**, the use of two amidases with opposite stereoselectivity allowed the isolation of (*S*)-carboxylic acid **196** (*Klebsiella terrigena* DSM 9174, entry 1 in Table 17) or (*R*)-carboxylic acid **196** (*Burkholderia species*

**Table 14. *Rhodococous erythropolis* AJ270 Catalyzed Hydrolysis of the Nitriles 185a−e,h−i**




| entry | substrate | R | $t$ (h) | $ee_P$ (%)[a] | $ee_S$ (%)[a] | $E$ |
|---|---|---|---|---|---|---|
| 1 | **185a** | H | 0.9 | 91 (47) | 96 (48) | 83 |
| 2 | **185b** | 4-OMe | 3 | 96 (46) | >99 (47) | >200 |
| 3 | **185c** | 4-F | 0.7 | 94 (50) | >99 (48) | >200 |
| 4 | **185d** | 4-Br | 1.3 | 89 (48) | 89 (30) | 82 |
| 5 | **185e** | 4-Me | 1.2 | >99 (50) | >99 (50) | >200 |
| 6 | **185h** | 3-Me | 5 | 90 (50) | 97 (45) | 79 |
| 7 | **185i** | 2-Me | 96 | 0 (47) | 0 (45) | 1 |

[a] Isolated yields in parentheses.

**Scheme 61**

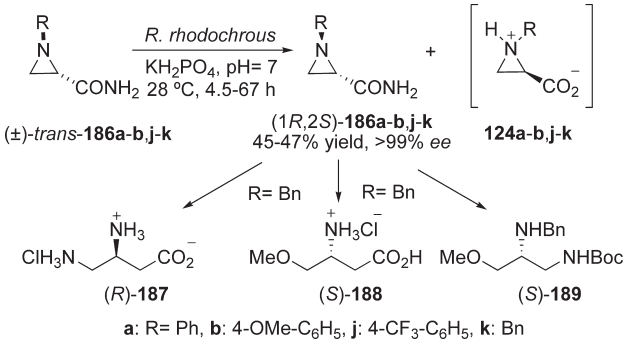
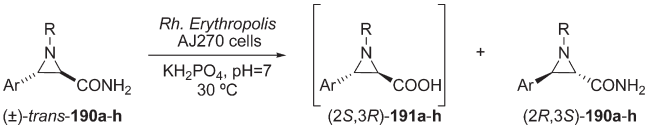



**Table 15. Synthesis of Enantiomerically Pure 3-Arylaziridine-2-Carboxamide Derivatives**




| entry | substrate | R | Ar | $t$ (h) | yield (%) | $ee_{amide}$ (%) |
|---|---|---|---|---|---|---|
| 1 | **190a** | Me | $C_6H_5$ | 0.33 | 48 | >99 |
| 2 | **190b** | Me | 4-Br-$C_6H_4$ | 2.0 | 48 | >99 |
| 3 | **190c** | Me | 4-F-$C_6H_4$ | 0.42 | 49 | >99 |
| 4 | **190d** | Me | 4-Cl-$C_6H_4$ | 1.0 | 48 | >99 |
| 5 | **190e** | Me | 3-Cl-$C_6H_4$ | 3 | 46 | >99 |
| 6 | **190f** | Me | 4-OMe-$C_6H_4$ | 1.0 | 40 | 5 |
| 7 | **190g** | Me | 2-Cl-$C_6H_4$ | 3.5 | 50 | 12 |
| 8 | **190h** | H | $C_6H_5$ | 3.0 | 47 | >99 |

DSM 9924, entry 2) in near enantiopure form. The synthesis of (*S*)-piperazine-2-carboxamide **195** starting from the 4-*N*-Boc-piperazine-2-carboxamide can also be achieved using L-leucine aminopeptidase.[139] In this manner, the corresponding unprotected (*S*)-carboxylic acid **196** was obtained in 71% yield through a hydrolytic process in a buffer of pH 8.6 and after 7 days, which


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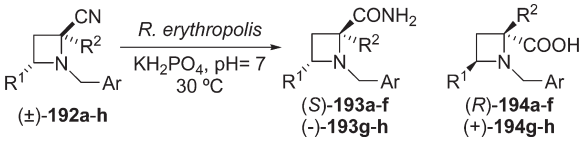
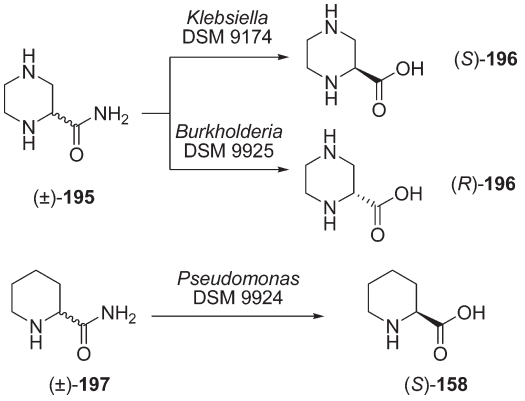
**Table 16. Biotransformation of Racemic Azetidine-2-carbonitriles**




| entry | substrate | Ar | $R^1$ | $R^2$ | $t$ (h) | $ee_P$ (%)[a] | $ee_S$ (%)[a] | $E$ |
|---|---|---|---|---|---|---|---|---|
| 1 | **192a** | $C_6H_5$ | H | H | 3.0 | >99 (41) | >99 (43) | >200 |
| 2 | **192b** | 4-Me-$C_6H_4$ | H | H | 4.7 | >99 (39) | >99 (45) | >200 |
| 3 | **192c** | 4-OMe-$C_6H_4$ | H | H | 3.0 | 91 (37) | >99 (45) | 111 |
| 4 | **192d** | 4-Br-$C_6H_4$ | H | H | 4.3 | 89 (43) | >99 (43) | 89 |
| 5 | **192e** | 3-Br-$C_6H_4$ | H | H | 4.8 | >99 (42) | >99 (42) | >200 |
| 6 | **192f** | 2-Br-$C_6H_4$ | H | H | 120 | >99 (44) | 97 (42) | >200 |
| 7 | **192g** | $C_6H_5$ | Me | H | 72 | 99 (39) | 99 (47) | >200 |
| 8 | **192h** | $C_6H_5$ | H | Me | 31 | 91 (30) | 80 (44) | 52 |

[a] Isolated yields in parentheses.

**Table 17. Enzymatic Hydrolysis of Racemic Amides 194 and 196**



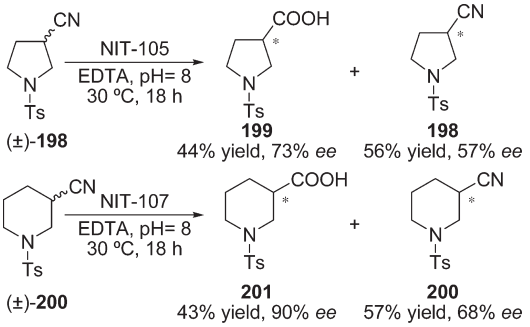

| entry | strain | pH | $T$ (°C) | product | $ee_P$ (%) | yield |
|---|---|---|---|---|---|---|
| 1 | *Klebsiella* | 8.0 | 40−47 | (*S*)-**196** | 99.4 | 41 |
| 2 | *Burkholderia* | 8.0 | 40−47 | (*R*)-**196** | 99.0 | 22 |
| 3 | *Pseudomonas* | 7.0 | 30 | (*S*)-**158** | 97.3 | 20 |

subsequently suffered a chemical ammonolysis reaction to obtain (*S*)-**195** in good overall yield.

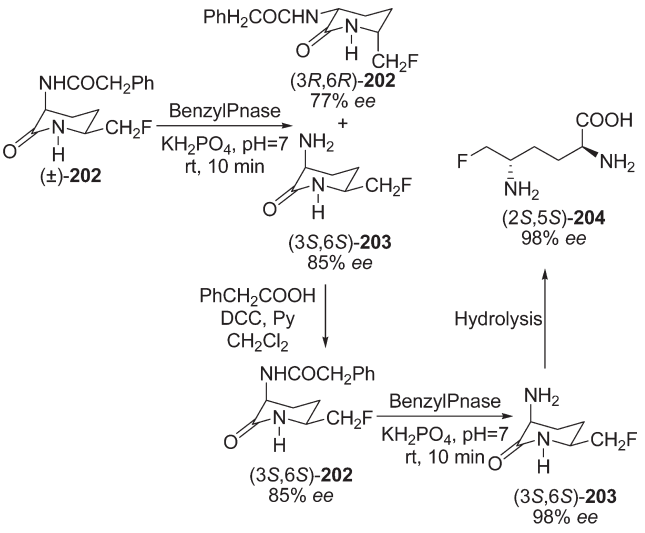
Klempier and co-workers studied the kinetic resolution of *N*-tosyl-3-cyanopiperidine (**198**) and *N*-tosyl-3-cyanopyrrolidine (**200**) using commercially available isolated nitrilases NIT-105 and NIT-107 (Scheme 62).[140] Nitrilases displayed moderate to high stereoselectivities in the hydrolysis of the nitrile groups, yielding the corresponding carboxylic acids (73−90% ee) under very mild reaction conditions.

(2*S*,5*S*)-5-Fluoromethylornithine (**204**), a potent irreversible inhibitor of ornitine aminotransferase, has been prepared through a multistep synthesis that involves the benzylpenicillase (BenzylPNase) catalyzed hydrolysis of the racemic amide **202**.[141] Both substrate (3*R*,6*R*)-**202** and product (3*S*,6*S*)-**203** were obtained in moderate optical purity, so it was necessary to perform a second kinetic resolution over amide (3*S*,6*S*)-**202** in

**Scheme 62**




**Scheme 63**




85% ee to finally obtain the amino acid (2*S*,5*S*)-**204** in near enantiopure form (Scheme 63).

### 3.3. Modifications in Different Functionalities of the Heterocyclic Ring

In this section we will describe different methodologies focused on the preparation of optically active amino acid derivatives by means of the hydrolase-mediated enantioselective modification of functionalities present at the cyclic ring, such as esters, carbonates, or alcohols. We have considered two different approaches for the stereoselective modification of functionalities in the cyclic ring: (1) hydrolysis of esters or carbonates and (2) acylation processes.

**3.3.1. Hydrolysis of Esters and Carbonates.** The enzymatic hydrolysis of esters and carbonates is a very attractive approach for the stereoselective modification of different functionalities directly bonded to *N*-heterocyclic amines, making possible the preparation of a great variety of optically active compounds under very mild reaction conditions. Lloyd et al. studied the lipase catalyzed diastereoselective hydrolysis of a 4-hydroxypipecolic acid derivative **205** present as a *cis*/*trans* mixture (Scheme 64).[142] Different lipases were tested, although only lipase AY30 from *Candida rugosa* led to the isolation of both substrate and product in high optical purity (80−91% de). A final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crystallization process allowed the isolation of (2*S*,4*S*)-**207** and (2*S*,4*R*)-**206** in up to 98% de.

The group of Kozikowski explored the enzymatic hydrolysis of tropane alkaloids **208a**−**e** with the goal of developing novel cocaine antagonists. Pig liver esterase (PLE) was selected as the optimal enzyme, since this biocatalyst has a particular affinity for benzoates.[143] A detailed analysis of the enzymatic hydrolysis of substrates **208a**−**e** showed that the enantioselectivity is strongly dependent on the relative stereochemistry of the $CO_2Me$ group (Table 18), since low or moderate *E* values were obtained for **208a**−**b** with the $CO_2Me$ group in relative *cis*-stereochemistry with the benzoate rest (entries 1−2), while, on the other hand, excellent enantiodiscriminations were attained for the pseudococaine derivatives **208c**−**e**, with the $CO_2Me$ group in relative *trans*-stereochemistry with the benzoate rest (entries 3−5).

The same authors studied the PLE catalyzed hydrolysis of *cis*- and *trans*-*N*-protected-4-benzyloxy-3-carbomethoxypiperidines **210a**−**b** and **210c**.[144] Excellent stereoselectivity was observed for the enzymatic hydrolysis of the *N*-methyl protected *trans*-**210a**; however, a dramatic drop of yield and stereoselectivity was observed for the hydrolysis of *trans*-**210b**, where the piperidine ring was protected with a bulkier group (Scheme 65). On the other hand, excellent results were obtained in the hydrolysis of the benzoate *cis*-**210c**, bearing a relative *cis*-stereochemistry, recovering both substrate (3*S*,4*R*)-**210c** and product (3*S*,4*R*)-**211c** in enantiopure form, although a lower kinetic rate was observed.

Ebiike et al. studied the enzymatic hydrolysis of 2-substituted-1,4-dihydropyridines **212a**−**b**, compounds that are known to behave as calcium antagonists and are widely employed as antihypertensive drugs (Scheme 66).[145] Lipase AH from *Pseudomonas species* was identified as the ideal biocatalyst for the hydrolysis of **212a** (Scheme 66), while for **212b** better results were attained with cholesterol esterase (CHE). For both substrates the hydrolysis was performed in a water saturated $^{i}Pr_2O$ using 10% of acetone as cosolvent. Unfortunately, the synthetic benefit of this methodology is quite limited due to the very long reaction times (3−4 days) required to reach conversions around 50%.

**3.3.2. *O*-Acylations.** Enzymatic acylation of hydroxyl groups directly linked to cyclic amines has been successfully achieved in dry organic solvents and efficiently used for the production of interesting enantioenriched compounds such as chiral auxiliaries and pharmacologically active compounds. Clinch et al. performed the total synthesis of both enantiomers of Inmucillin H (ImmH) and studied their bioactivities as transition state analogs of purine nucleoside phosphorylase (PNPases).[146] The key step for the preparation of both enantiomers was the CAL-B catalyzed acylation of the *trans*-hydroxyester **214**, which occurred with high stereoselectivity in short reaction times (2.5 h, Scheme 67). The kinetic studies of the interaction between (+)-ImmH and (−)-ImmH and different PNPases revealed that the (3*S*,4*S*)-isomer interaction is from 5 to 160 times higher than the interaction shown by the (3*R*,4*R*)-isomer.

Similarly, the group of Mason performed the CAL-B catalyzed kinetic resolution of the fluorinated alcohol **216** (Scheme 68).[147] In spite of the fact that the acetylation occurred with moderate stereoselectivity, this was not an important drawback, as the optical purity could be enhanced up to 95% ee by a simple recrystallization. Both optically active acetate (3*S*,4*R*)-**217** and alcohol (3*R*,4*S*)-**216** have been used as adequate starting materials for the total synthesis of novel fluorine containing inhibitors of PNPases. Accordingly with the previous kinetic studies performed by Clinch et al.,[146] the (3*S*,4*S*)-**218** enantiomer appeared as a potent inhibitor and the most active enantiomer.

Pyrrolizidine alkaloids are common constituents of plants possessing special medicinal properties. One of them, Hyacinthacine A1, has been chemically prepared by Laschat and co-workers through the lipase-catalyzed acetylation of racemic intermediate **219** using Chirazyme L-6 from *Burkholderia cepacia* (PSL-L6) (Scheme 68).[148] Acetate (−)-**220** was obtained in 42% yield with 99% ee, and the remaining alcohol (+)-**219** was obtained in 48% yield, albeit with only 75% ee (*E* = 22).

The PSL-catalyzed kinetic resolution of the protected *cis*-*β*-hydroxypipecolic acid (±)-**221**, which is an adequate building block for the preparation of the antitumor antibiotic Tetrazomine,

**Scheme 64**

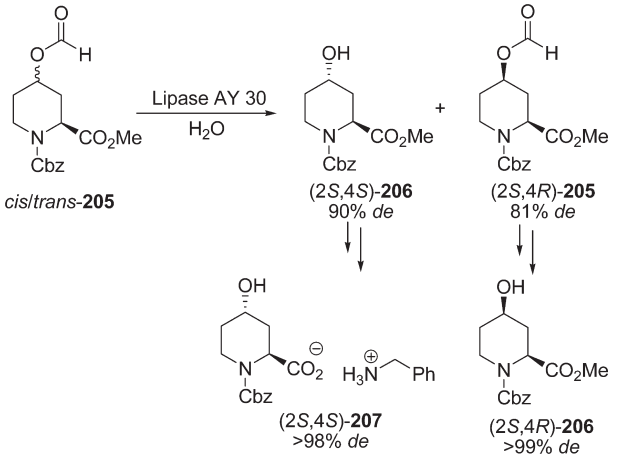
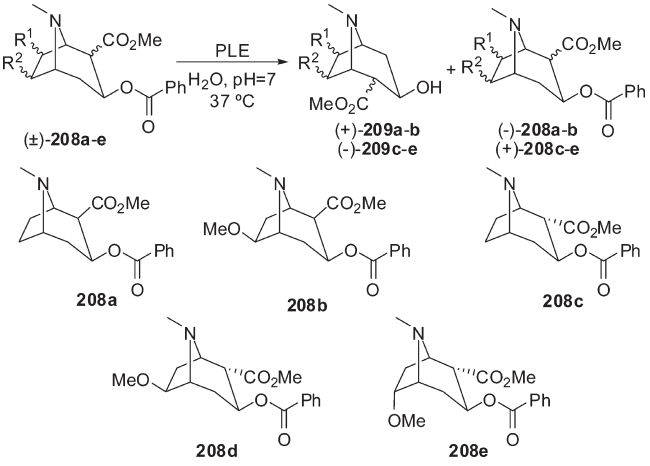



**Table 18. Enzymatic Kinetic Resolution of Cocaine Analogues 208a−e**




| entry | substrate | $ee_p$ (%)[a] | $ee_s$ (%)[a] | *E* |
|---|---|---|---|---|
| 1 | **208a** | 71 (17) | 82 (23) | 14 |
| 2 | **208b** | 95 (2) | 82 (17) | 99 |
| 3 | **208c** | 95 (43) | >99 (46) | >200 |
| 4 | **208d** | 99 (15) | 99 (30) | >200 |
| 5 | **208e** | 95 (13) | 97 (27) | 164 |

[a] Isolated yields in parentheses.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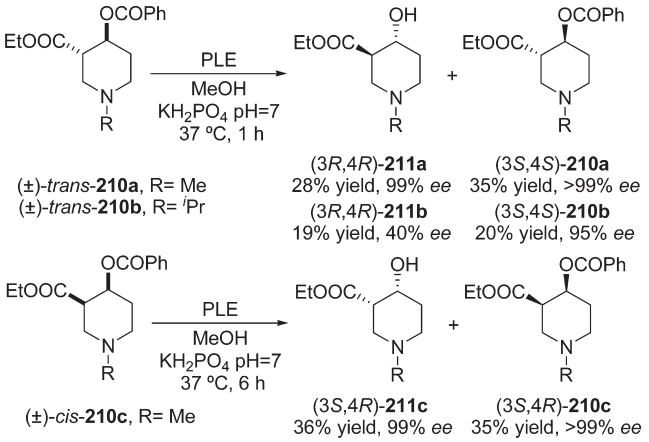
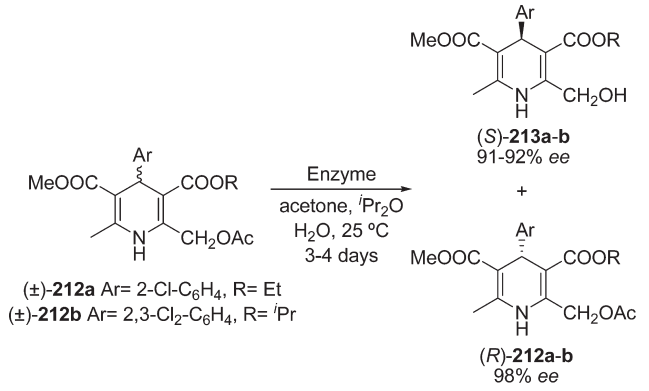
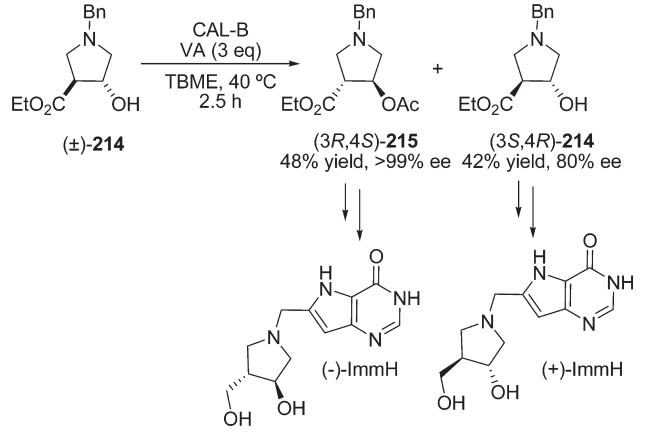
**Scheme 65**




**Scheme 66**



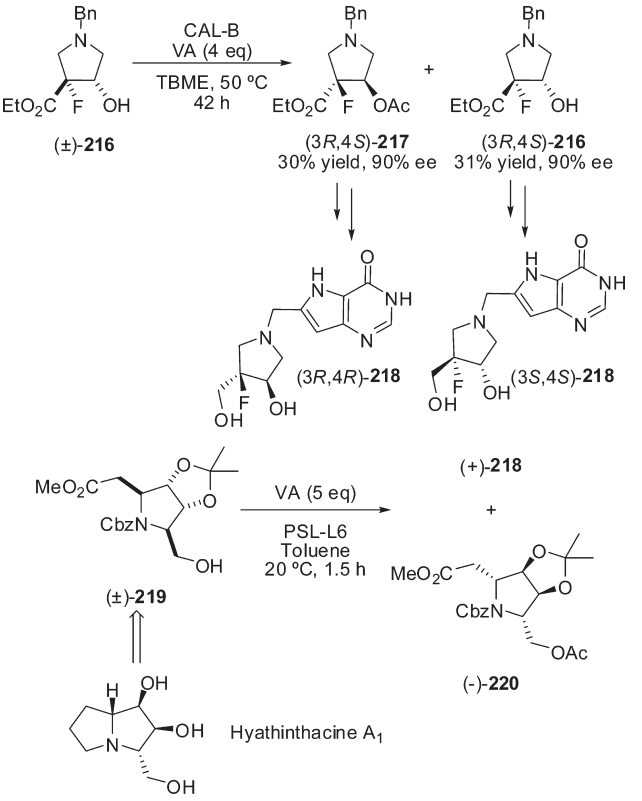

**Scheme 67**



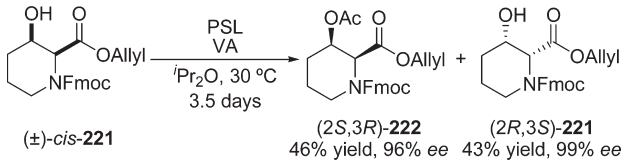

has been reported by Williams and co-workers (Scheme 69).[149] Both substrate (2*R*,3*S*)-**221** and product (2*S*,3*R*)-**222** were recovered in near enantiopure form, although a long reaction time (3.5 days) was necessary to reach conversions near 50%.

The enzyme-catalyzed kinetic resolution of highly valuable *N*-protected piperidine hydroxyl esters has been achieved by lipase

**Scheme 68**




**Scheme 69**




catalysis.[150] Both *cis*- and *trans*-1-(*tert*-butoxycarbonyl)-4-hydroxypiperidine-3-carboxylate (**223a** and **223b**) were efficiently resolved using immobilized lipase AK as biocatalyst and vinyl acetate as acyl donor in diisopropyl ether, affording both substrate and product in enantiopure form (Scheme 70). On the other hand, CAL-A was found as the unique biocatalyst able to promote the *O*-acetylation of *cis*-1-(*tert*-butoxycarbonyl)-3-hydroxypiperidine-4-carboxylate (**225**); unfortunately, moderate selectivity values were obtained even when performing the reaction at low temperature.

Achiwa and co-workers have reported the total synthesis of (*S*)-Nilvapidine and (*S*)-Manipidine, compounds that are clinically used for the treatment of hypertension and chronic major arterial occlusion.[151] The enzymatic hydrolysis and acylation of adequate intermediates were studied using different biocatalysts; however, optimal results were attained using acylation procedures with a large excess of vinyl acetate (270 equiv) and PSL in acetone. The authors observed higher enantiopreferences when the reaction was performed at low temperatures (0 °C), isolating (*S*)-**228** and (*R*)-**227** in high optical purity (Scheme 71). Finally, the acetate (*S*)-**228** was employed as adequate starting material for the chemical synthesis of enantioenriched (*S*)-Nilvapidine and (*S*)-Manipidine.


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

## 4. STEREOSELECTIVE BIOTRANSFORMATIONS OF LACTAMS

Optically active β-lactams are attractive compounds for medicinal chemistry because the β-lactam ring is part of several antibiotic structures, and they also are adequate building blocks in β-amino acid synthesis. Next, we will highlight the preparation of this class of compounds by means of classical kinetic resolutions catalyzed by whole cells containing lactamases or by isolated esterases or lipases. This part has been divided depending on the place for the modification as follows: (a) stereoselective cleavage of the β-lactam by reaction with the amino group; (b) transformations toward different functionalities attached to the lactam ring. In both cases, we will review the state of the art of lactam reactivity going from simple to more complex structures.

### 4.1. Modifications in the Amide Group: Stereoselective Cleavage of the β-Lactam Ring

Hydrolases have been widely used as catalysts for the stereoselective ring-opening of the β-lactam ring (azetidin-2-one) under very mild conditions, allowing the preparation of optically active high-added value goods such as β-amino-acids, taxoid antitumor agents, alkaloids, and amino acids. Earlier examples include the hydrolysis using microbial strains containing lactamases; however, in the last few years since the discovery of the promiscuous lipase-catalyzed cleavage of amide bonds, many examples have appeared in the literature regarding the stereoselective cleavage of lactams using lipases.[31] The lipase catalyzed cleavage of β-lactams in organic solvents has been successfully achieved with different 4-aryl-substituted-β-lactams **229a**−**g** (Scheme 72). The substituents of the aromatic ring did not affect significantly the enzymatic hydrolysis as the corresponding (*R*)-β-amino acids **230a**−**g**, and the unreacted (*S*)-β-lactams **229a**−**g** were obtained in all cases in good yields and excellent enantiomeric excesses after short reaction times.[152]

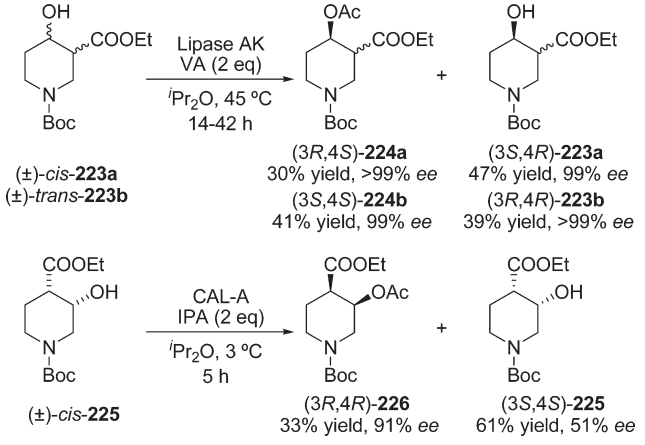
Kanerva and co-workers studied the kinetic resolution of fluorinated β-phenyl- β-lactams **231a**−**b** through a methanolysis reaction in anhydrous TBME catalyzed by *Burkholderia cepacia* lipase (*Pseudomonas cepacia* lipase immobilized over Celite, PSL-D).[153] The ring-opening proceeded with excellent enantioselectivity and exclusively by methanolysis in the case of **231a** (Table 19, entry 1); however, for **231b**, the authors observed a competitive hydrolysis phenomenon (9%, entry 2). The proportion of hydrolysis can be reduced by employing higher enzyme loading and methanol contents (entry 3). In the case of the unfluorinated derivative **229a**, no reaction was observed in the presence of methanol (entry 4). According to these results, the fluorine activation of the β-lactam ring seems to be essential for the PSL-D catalyzed ring-opening of the lactam.

The same research group discovered the ability of PSL-D lipase to catalyze the ring cleavage of the fluorine-activated β-lactam **231a** through aminolysis and ammonolysis processes in organic solvents. A highly selective ammonolysis process was observed in TBME saturated with ammonia, giving (*R*)-**231a** and (*S*)-**233a** in enantiopure form (Scheme 73).[154] Next, the aminolysis of **231a** with 1 equiv of $^{i}PrNH_2$ was considered, observing an excellent enantiodiscrimination in the formation of the amide (*S*)-**233b**; however, a competitive hydrolysis due to the water present in the enzymatic system was observed. The relative ratio of hydrolysis can be reduced by increasing the lipase content.

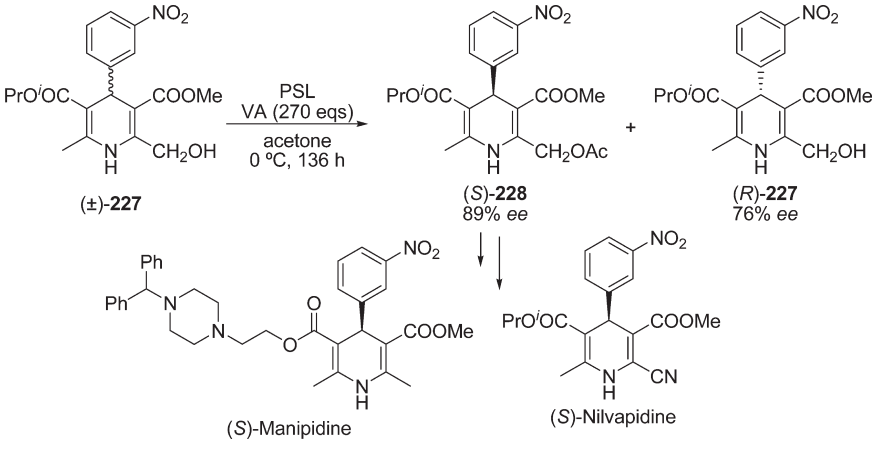
Very recently, Forró and Fülöp developed an enzymatic two-step cascade for the production of the C-13 side chain of Taxol by enzymatic hydrolysis in organic media, based on the CAL-B catalyzed deacylation followed by the β-lactam opening (Scheme 74).[155] The cascade reaction afforded two enantiomerically pure products in high yield; one of them, (2*R*,3*S*)-**235**, is an adequate precursor for the synthesis of the C-13 side chain of Taxol.

In 1991 Page and Jones reported the first example related to the promiscuous β-lactamase activity of a hydrolase.[156] In this

**Scheme 70**




**Scheme 71**





dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

**Scheme 72**

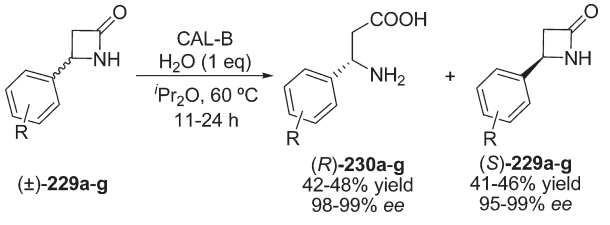
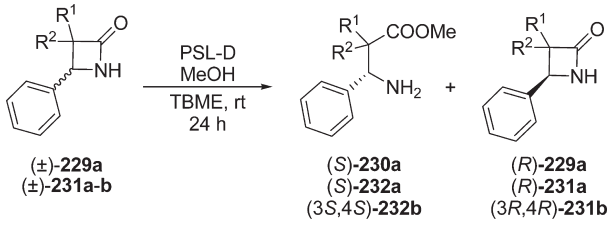



**Table 19. PSL-D Catalyzed Ring-Opening of Lactams 229a and 231a−b**



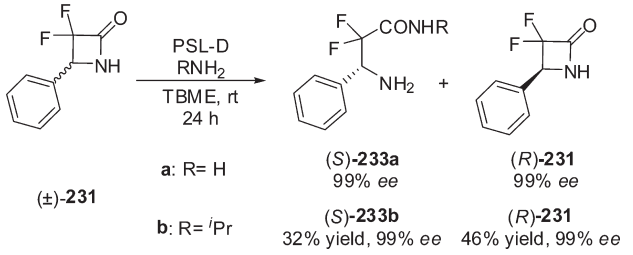

| entry | substrate | $R^1$ | $R^2$ | MeOH (equiv) | $c$ (%)[a] | $ee_p$ (%) | $ee_s$ (%) |
|---|---|---|---|---|---|---|---|
| 1 | **231a** | F | F | 5 | 50 | >99 | >99 |
| 2 | **231b** | F | H | 5 | 46 (9) | >99 | 59 |
| 3 | **231b**[b] | F | H | 10 | 40 (2) | >99 | 60 |
| 4 | **229a** | H | H | 5 | 0 | – | 0 |

[a] Percentage of the hydrolysis product in parentheses. [b] Double amount of enzyme was employed.

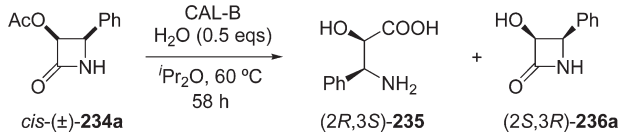
example, PLE catalyzed more efficiently the hydrolysis of the β-lactam ring than the hydrolysis of the methyl ester. Two years later and in the course of the investigations toward the preparation of the C-13 side chain of Taxol, the group of Sih reported for the first time the stereoselective ring cleavage of a β-lactam; *N*-benzoyl-β-lactam **237a** was cleaved in the presence of 5 equiv of MeOH as nucleophile and TBME as solvent at 50 °C (Scheme 75).[157] All the lipases showed the same stereochemical preference toward the cleavage of the (3*R*,4*S*)-**237a**, obtaining excellent results with lipase AK. Also requiring high temperatures (70 °C), but now using water as solvent, the stereoselective ring-opening reaction of unprotected hirtherto α-methylene-β-lactams **239a−c** allowed the formation of the corresponding optically active β-lactams and the β-amino acids in high enantiomeric excesses with CAL-B as biocatalyst (Scheme 75).[158]

Due to the excellent results obtained in the CAL-B catalyzed ring-opening of the 4-arylderivatives **229a−g**, the role of the distance between the chiral center and the aryl group was studied by the kinetic resolution of the 4-arylalkyl-substituted β-lactams **241a−b**.[159] Contrary to the excellent results achieved for **229a−g**, very low stereoselectivity ($E$ = 10) was observed for the ring-opening of the more conformationally flexible analogs **241a−b** (Chart 3). This behavior could be explained on the basis that the higher conformational flexibility of **241a−b** could favor the accommodation of the (*S*)-enantiomer in the active site of the lipase, leading to a decrease of the enantioselectivity.

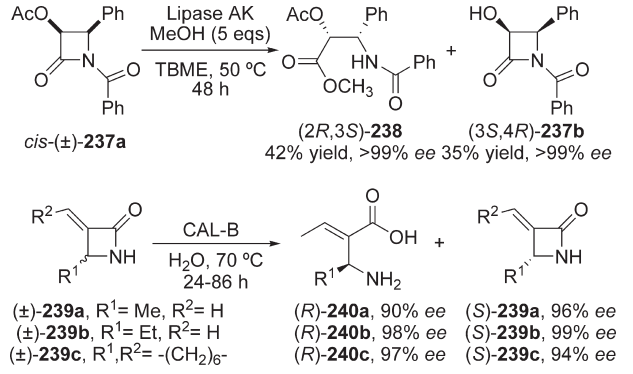
**Scheme 73**




**Scheme 74**




**Scheme 75**

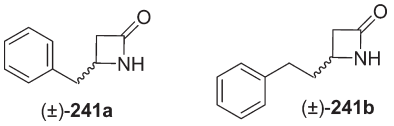



Forró and Fulöp described in 2003 the first lipase-catalyzed enantioselective ring-opening of unactivated alicyclic-fused β-lactams **242a−d** using an organic solvent (diisopropyl ether, $^i$Pr$_2$O) as reaction medium (Scheme 76).[160] Although a long reaction time and also a high temperature were needed (60 °C), just 1 equiv of water was used. An extensive screening of lipases was done, and Lipolase, a CAL-B preparation, was found as the best stereoselective enzymatic source for the gram-scale resolution of four different lactam structures, which are ideal intermediates in the synthesis of enantiopure β-amino acids **243a−d**.

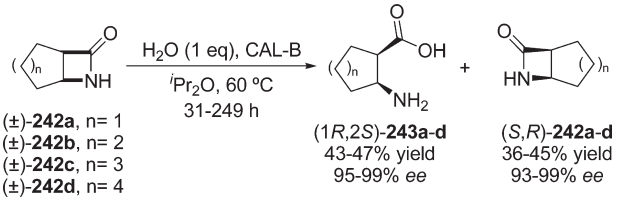
The first example that appeared in the literature related to enzymatic stereoselective ring cleavage of β-lactams was reported by Evans and co-workers.[161,162] In this example, whole cells of different microbial strains containing lactamases such as *Rhodococcus species* (Enza 1), *Pseudomonas solearum* (Enza 20), *Pseudomonas fluorescens* (Enza 22), and *Aureobacterium species* (Enza 25) were used as biocatalysts for the kinetic resolution of 2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-**244**]. All the bacterial strains showed enantioselective lactamase activity toward (±)-**244** (Scheme 77). Interestingly, the analysis of the reaction crudes indicated complementary specificities while the Enza 20 and Enza 22 strains catalyzed the hydrolysis of the (1*S*,4*R*)-**244**, and Enza 1 and Enza 25 showed hydrolytic activity toward the


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

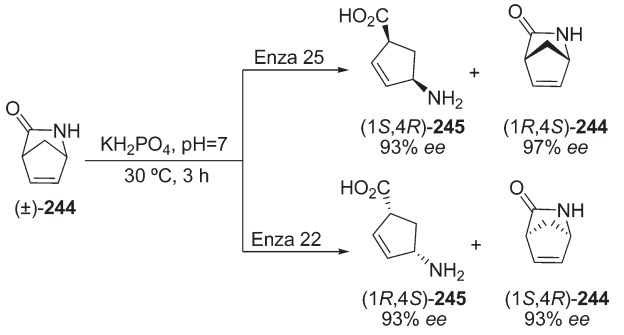
**Chart 3**




**Scheme 76**




**Scheme 77**



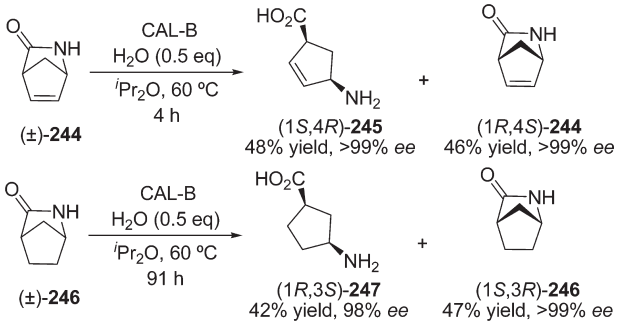

(1*R*,4*S*)-isomer. Years later, the same research group isolated an expressing $\gamma$-lactamase clone from a gene library, and once the enzyme had been isolated, a 500 L fermentation was carried out to resolve 5 t of lactam (±)-**244** with an excellent enantioselectivity (*E* > 400).[163] Optically active (1*R*,4*S*)-**245** has been used as adequate starting material for the preparation of the antiviral compound (−)-carbovir.

Forró and Fülöp developed an enzymatic method for the enantioselective ring cleavage of the unprotected $\gamma$-lactams (±)-**244** and (±)-**246** with only 0.5 equiv of $H_2O$, employing CAL-B as biocatalyst in $^iPr_2O$ (Scheme 78).[164] The CAL-B ring-opening reaction afforded the products in near enantiopure form and high isolated yields. In addition, the products can be easily recovered from the reaction media without chromatographic separation, as the optically active $\gamma$-amino acids **245** and **247** are insoluble in $^iPr_2O$ while the unreacted lactams **244** and **246** remain in solution.

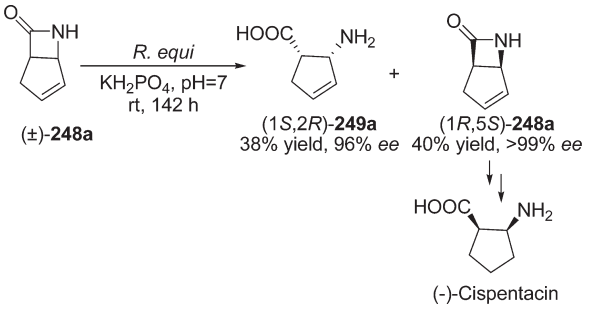
Whole cells from *Rhodococcus equi* NCIB 40213 containing lactamase enzymes have been responsible for the hydrolysis of the $\beta$-lactam **248a** by their use as biocatalysts (Scheme 79).[165] The remaining optically active lactam (1*S*,2*R*)-**248a** was isolated in enantiopure form and high yield, and then it was used as adequate starting material for the preparation of the antifungal antibiotic (−)-cispentacin (FR 109615).

Taking advantage of the excellent catalytic properties displayed by CAL-B in the enantioselective ring-opening of $\beta$-lactams, the same research group extended the CAL-B kinetic resolution in $^iPr_2O$ using just 1 equiv of water at 60 °C toward a wide variety

**Scheme 78**




**Scheme 79**



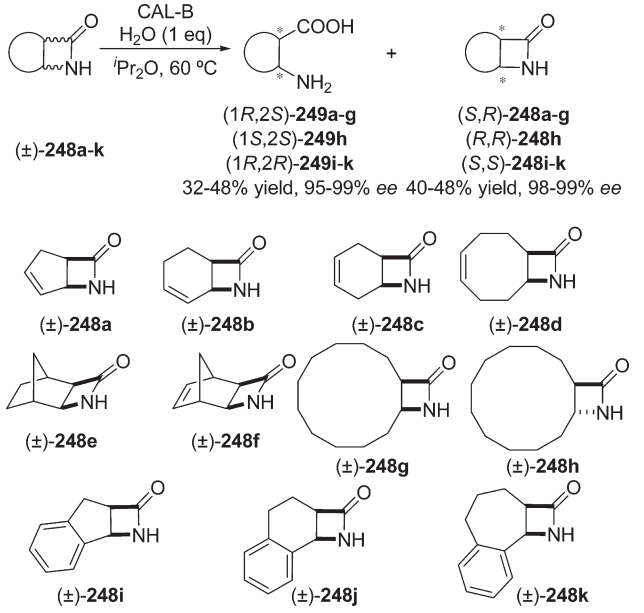

of $\beta$-lactams, such as the unsaturated alicyclic derivatives **248a**−**d**,[166−170] the tricyclic derivatives **248e**−**f**,[171] the inactivated cyclic $\beta$-lactams **248g**−**h**,[172] and the trycyclic benzofused lactams **248i**−**k**[173] (Scheme 80). Interestingly, CAL-B displayed a broad substrate tolerance producing the corresponding $\beta$-amino acids and $\beta$-lactams in high enantiomeric excesses and isolated yields. Lactams **248b**,**f** have also been efficiently resolved using a lactamase present in the whole cells of *Rhodococcus globerulus* (NCIMB 41042) after carrying out the reactions in phosphate buffer at pH 7 for 24 h, yielding (1*R*,6*S*)-**249b** in >95% ee and (−)-**248f** of >98% ee.[142] Computer modeling has also suggested that the molecular basis for the high enantioselectivity display by CAL-B in the ring-opening reaction of two bicyclic and two 4-aryl-substituted $\beta$-lactams is a severe steric clash between Ile189 and the phenyl substituent on the slow-reacting enantiomer of the $\beta$-lactam.[174] The same authors have also presented an enzymatic new solvent-free, vapor assisted method, for the ring-opening of a wide variety of $\beta$-lactams isolating a broad variety of optically active carbocyclic *cis*-$\beta$-amino acids on a preparative scale, using only 0.5 equiv of water at 70 °C.[175] Excellent results in terms of selectivity were achieved; disappointingly long reaction times are required to reach conversions around 50%.

### 4.2. Enantioselective Modification of Different Functionalities of the $\beta$-Lactam Ring

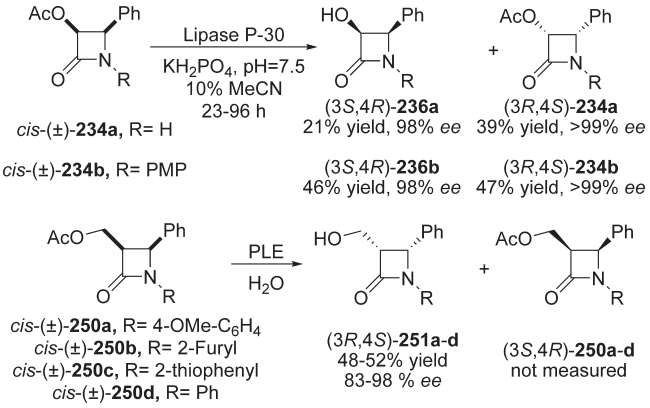
Because of the interest to isolate both enantiomers of the corresponding lactams, in many cases the final targeted products are not only the amino acids resulting from the stereoselective cleavage of the $\beta$-lactam ring. Different enzymatic nucleophilic substitutions will be next briefly discussed.


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

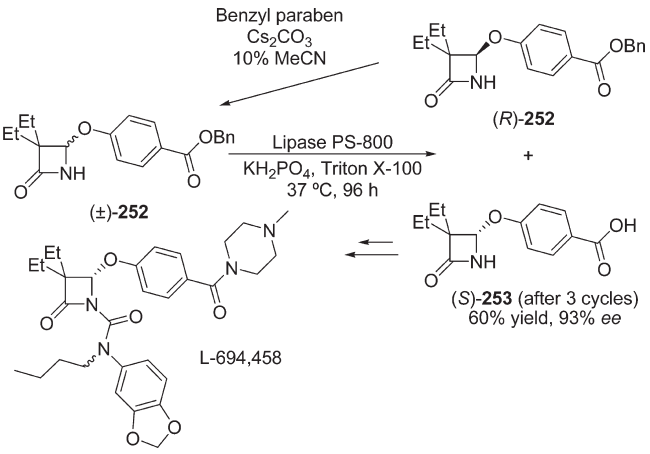
Scheme 80



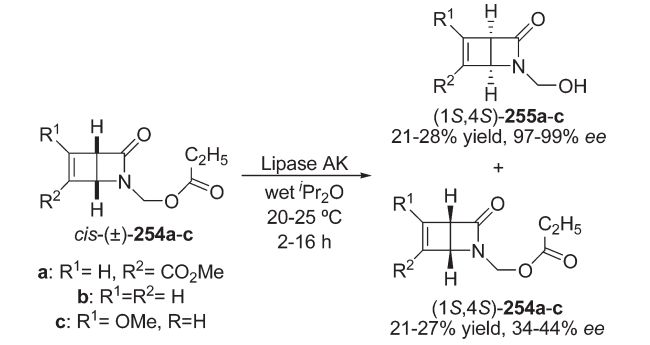

Scheme 81




Scheme 82




Scheme 83




**4.2.1. Enantioselective Hydrolysis and Alcoholysis of Ester Derivatives.** In this section we will describe the hydrolase catalyzed stereoselective modification of azetidin-2-ones by means of hydrolytic and solvolytic processes without altering the characteristic amide moiety of the lactams. Sih and co-workers studied the enzymatic hydrolysis of 3-acetoxy-4-aryl-$\beta$-lactam derivatives (±)-**234a**−**b**, which are key intermediates for the preparation of the C-13 side chain of Taxol.[157] Optimal reaction rates and enantioselectivity were obtained when lipase P-30 from *Burkholderia cepacia* was used as biocatalyst and 10% MeCN was employed as cosolvent, isolating both substrates in near enantiopure form. Excellent stereoselectivity was observed for both processes; however, higher reaction rates and isolated yields were observed for the *N*-protected derivative **234b** (Scheme 81). Similarly, Basak et al. performed the PLE catalyzed hydrolysis of *cis*-3-acetoxymethyl-4-substituted-lactams **250a**−**d** (Scheme 81).[176] The optically active alcohols (3*R*,4*S*)-**251a**−**d** were isolated in near quantitative yields and high to excellent optical purities. In contrast with the (3*S*,4*R*)-stereopreference observed by Sih[157] for the hydrolysis of the *cis*-3-acetoxy derivatives **234a**−**b**, the (3*R*,4*S*)-alcohols **251a**−**d** were attained in the hydrolysis of **250a**−**d**, where the acetoxy groups are replaced by acetoxymethyl rests.

Cvetovich and co-workers performed a convergent synthesis for the preparation of the potent leukocyte elastase inhibitor (L-694,458).[177,178] The asymmetric synthesis of lactam (*S*)-**253**, a key intermediate in the synthesis of the inhibitor, has been performed through lipase catalyzed hydrolysis of the ester **252** to produce (*S*)-**253** (60% yield, three cycles, 93% ee) with isolation, epimerization, and recycling of the undesired (*R*)-**252** (Scheme 82).

Years later Bisht and co-workers studied the effect of the 4-aryl ring substitution on the *Pseudomonas cepacia* lipase (PS-30)-catalyzed hydrolysis of 3-acetoxy-4-aryl-substituted-$\beta$-lactams.[179] Excellent stereoselectivity was observed for the hydrolysis of the (1*S*,4*S*) enantiomers of all the studied substrates. In general, lactams with electron withdrawing groups showed quantitative conversions (50%) in short reaction times, while compounds with electron donating groups needed longer reaction times to achieve reasonable conversion. The kinetic resolution of racemic *cis*-photopyridones **254a**−**c**, interesting synthons for the preparation of Carbapem antibiotics, has been achieved by lipase-catalyzed asymmetric hydrolysis in water-saturated diisopropyl ether.[180] In spite of the relatively long distance between the asymmetric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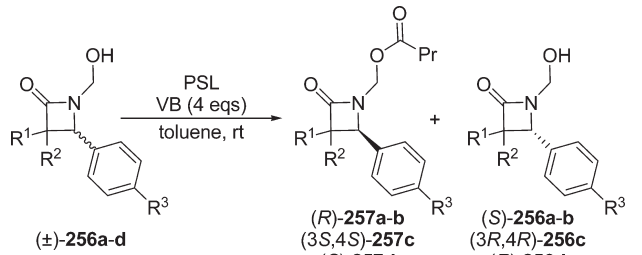
centers and the reaction site, a high degree of enantioselectivity was observed for the lipase AK catalyzed processes (Scheme 83). The complementary acylation process using vinyl acetate in TBME has also been tested; however, lower selectivities were observed.

**4.2.2. Stereoselective Acylation of Alcohols and Amines.** In this section we will highlight lipase-catalyzed enantioselective acylation processes of $\beta$-lactams. Most of the examples involve the kinetic resolution of *N*-hydroxymethylated-$\beta$-lactams, which has proven to be very useful for preparative purposes, allowing the preparation of both enantiomers of the $\beta$-lactam after the removal of the esterified or free hydroxymethyl group. The groups of Kanerva and Fülöp studied the PSL-catalyzed kinetic resolution of 4-aryl-substituted-$\beta$-lactams (±)-**256a**−**d**, which are interesting precursors for the preparation of unnatural $\beta$-amino acids, using vinyl butanoate as acyl donor in toluene.[181,182] Excellent stereoselectivity was observed for the acylation of **256a** (Table 20, entry 1); however, a significant decrease of the enantiodiscrimination was observed when the *p*-tolyl derivative **256b** was considered (entry 2). On the other hand, the authors studied the effect of the substitution of hydrogen atoms by isosteric fluorine. High stereoselectivity was observed for the difluorinated derivative **256c** (entry 3); however, a lower value was observed for **256d** (entry 4).

**Table 20. Enzymatic Kinetic Resolution of 4-Aryl-substituted-$\beta$-lactams 256a−d**




| entry | substrate | $R^1$ | $R^2$ | $R^3$ | $t$ (h) | $c$ (%) | $ee_P$ (%) | $ee_S$ (%) | $E$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **256a** | H | H | H | 1.5 | 49 | 97 | 94 | >200 |
| 2 | **256b** | H | H | Me | 1.5 | 52 | 88 | 95 | 57 |
| 3 | **256c** | F | F | H | 1.5 | 53 | 89 | 99 | 90 |
| 4 | **256d** | F | H | H | 2.3 | 59 | 70 | 99 | 18 |

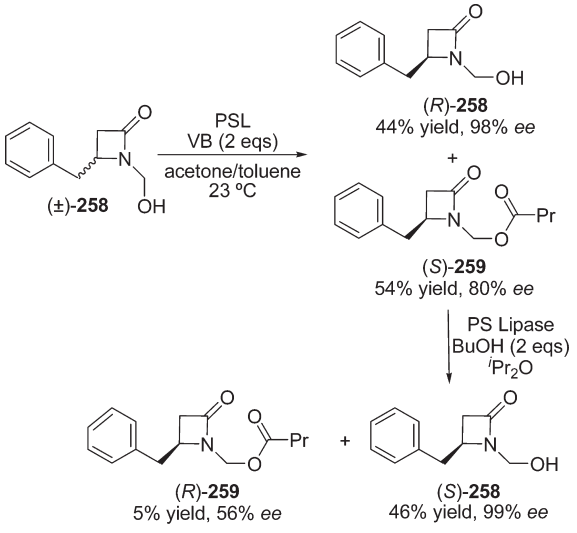
Kanerva and co-workers performed the PSL-catalyzed kinetic resolution of 1-hydroxymethyl-4-benzylazetidin-2-one (**258**), which is an adequate precursor for the production of both enantiomers of the unnatural amino acid $\beta$-phenylalanine (Scheme 84).[183] As only moderate enantioselectivity was displayed by the enzyme, optically active **258** has been prepared by employing a double kinetic resolution process. The first acylation step afforded (*R*)-**258** in high isolated yield and near enantiopure form; however, (*S*)-**259** was recovered in moderate enantiomeric excess (80%). Then, the PS-catalyzed butanolysis of (*S*)-**259** afforded (*S*)-**258** in high isolated yield and in enantiopure form.

A useful optical intermediate for the synthesis of Loracarbef, a carbacephalosporin antibiotic, has been prepared by the group of Zmijeski, through the Penicillin G acylase catalyzed enantioselective acylation of (±)-**260** using methyl phenoxyacetate (MPOA) as acyl donor in water (Scheme 85).[184] The optically active precursor (2*R*,3*S*)-**261** was obtained in high isolated yield and in enantiopure form.

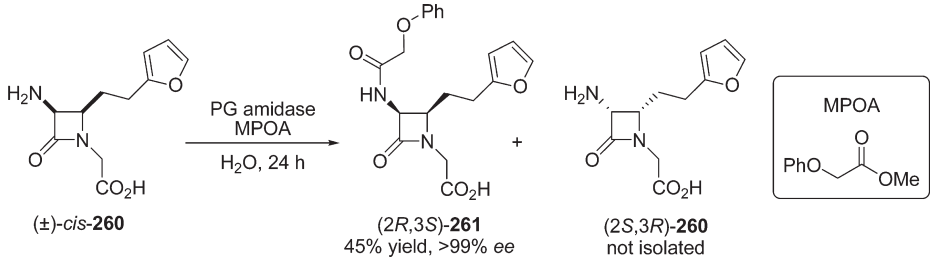
The kinetic resolution of the bicylic lactam (±)-**262**, which is a useful synthon for the preparation of (−)-carbovir, has been performed by means of a PSL-D catalyzed acetylation of (±)-**262** in TBME using vinyl acetate as acyl donor (Scheme 86). Both substrate and product were isolated in high optical purity after a very short reaction time.[185] The kinetic resolution of *N*-hydroxymethylated lactam (±)-**264** has been performed using lipase AK as biocatalyst and vinyl butyrate as acyl donor in acetone at 0 °C (Scheme 86).[186] The optically active lactam (1*R*,5*S*)-**265** was obtained in high isolated yield and in near enantiopure form, being an adequate precursor for t he preparation of the antifungal antibiotic (−)-cispentacin.

Similarly, the research group of Fülöp studied the kinetic resolution of the lactams (±)-**266a**−**c**, observing that an efficient kinetic resolution was achieved when PSL was used as biocatalyst and vinyl butyrate as acyl donor in acetone, isolating both substrates (1*S*,5*R*)-**266a**−**c** and products

**Scheme 84**



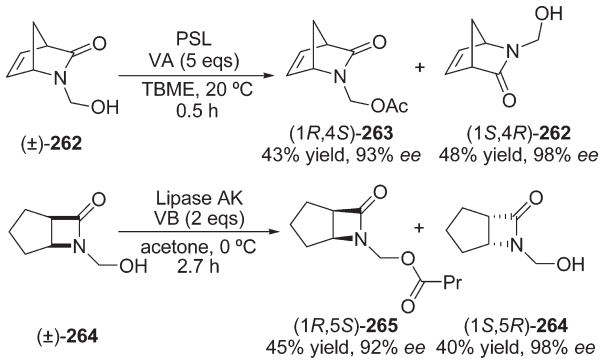

**Scheme 85**



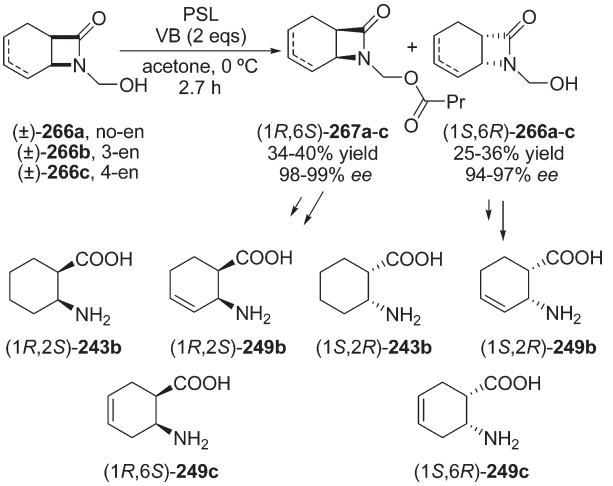


dx.doi.org/10.1021/cr100287w |*Chem. Rev.* 2011, 111, 3998–4035

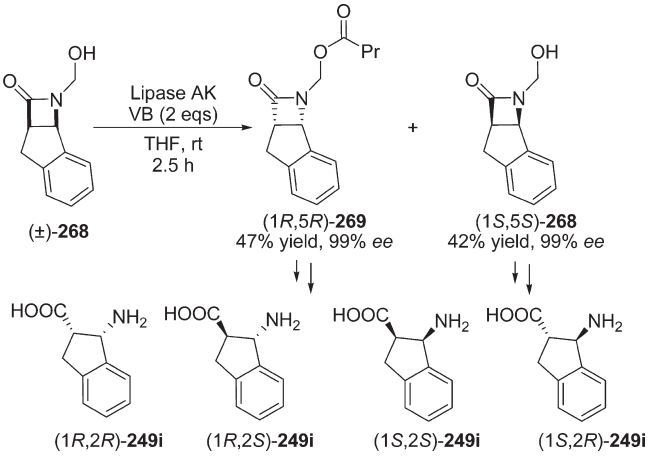
Scheme 86




Scheme 87




Scheme 88




(1*R*,5*S*)-**267a**−**c** in near enantiopure form (Scheme 87).[187] The optically active lactams were used for the preparation of the enantiomerically pure cyclic amino acids **249b**,**c** and **243b**. Excellent results were also obtained by Gyarmati et al. in the resolution of 7-, 8-, and 12-membered *N*-hydroxymethyl-*β*-lactam enantiomers.[188]

3,4-Benzo-6-hydroxymethyl-6-azabicyclo[3.2.0]heptan-7-one [(±)-**268**] has been efficiently resolved through lipase catalyzed asymmetric acylation of the primary hydroxyl group (Scheme 88).[189] Excellent stereoselectivity was observed when the enzymatic resolution was performed using lipase AK as biocatalyst in THF and using vinyl butyrate as acyl donor. Enantiomerically pure (1*R*,5*R*)-**269** and (1*S*,5*S*)-**268** were used as adequate starting materials for the preparation of the four stereoisomers of 1-aminoindane-2-carboxylic acids **249i**, compounds with antifungal activity.

## 5. SUMMARY AND OUTLOOK

Chemical access to optically active organic compounds, and especially to *N*-heterocyclic molecules, has attracted the attention of many synthetic researchers. Hydrolases have provided a wealth of opportunities for producing amine and amino acid derivatives by means of a wide spectrum of biocatalytic reactions that have been illustrated in this review. Certainly, the correct choice of the synthetic transformation and experimental conditions directly leads to the preparation of the desired cyclic structures in satisfactory chemical yields and excellent optical purities. Although commercially available enzymes permit quick access to the desired enantioenriched compounds, the combination of genetic engineering and molecular modeling techniques currently plays an understandable role in the development of new biocatalysts that should improve the results here reviewed.[190−195] Additionally, organic chemists and molecular biologists must take advantage of high-throughput screening methods, extremely useful in the search for enzyme activity, identifying appropriate enzymes in a short time span for the development of biotransformations with a high level of success.[196,197]

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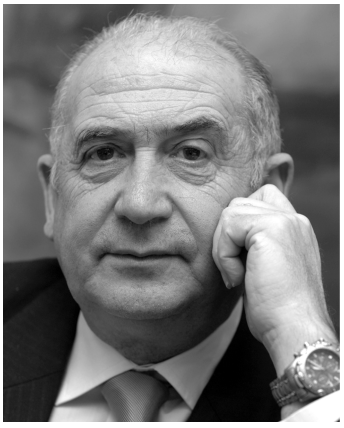
## BIOGRAPHIES

Eduardo Busto (1982) studied organic chemistry at the University of Oviedo, where he graduated with honors in June 2004. He then joined Professor Gotor's group, where he completed his Ph.D., working on the preparation of optically active organic compounds, such as nucleophiles, ionic liquids, and orthogonally protected diamines. During his doctoral studies, he spent a short period of time at the University of Stockholm

working, under the supervision of Jan Erling Bäckvall and Belén Martín-Matute. He is currently working at the University of Oviedo, where his main research interests include catalytic promiscuity, ionic liquids, and the use of chemoenzymatic approaches for the production of high added value compounds.

Vicente Gotor-Fernández (1974) studied organic chemistry at the University of Oviedo, where he received his Ph.D. in 2001, studying the chemoenzymatic synthesis of A-ring modified vitamin $D_3$ analogues. He moved to the University of Edinburgh for a postdoctoral stage (2002−2004) in the group of Professor Nicholas J. Turner, working in the deveoplment of deracemization processes by combination of evolved selective amine oxidase and chemical reducing agents. In July 2004 he moved back to Oviedo, where he was appointed as a senior scientist. His main research interest involves the design of novel synthetic methodologies using chemical and biocatalytic methods, mainly employing hydrolases and oxidoreductases for the preparation of organic compounds in optically active form.



Vicente Gotor received his Ph.D. from the University of Zaragoza in 1974, joining then the Max Planck Institut für Kohlenforschung (Mülhein/Rhur) in a two-year postdoctoral stage. In 1977 he moved to the University of Oviedo as Assistant Professor, assuming his current position as Professor of Organic Chemistry 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 on natural products, biotransformations with oxynitrilases and oxidoreductases, and chiral recognition with azamacrocycles in organic synthetic projects. He is coauthor of around 350 papers and 11 patents and has supervised 53 doctoral theses. He was Vice-chancellor of Research (1996−2000) and Head of the Organic and Inorganic Chemistry Department (2003−2008), and at present is Rector of Oviedo University, since May 2008.

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## ABBREVIATIONS

| | |
|---|---|
| AA-I | acylase from *Aspergillus niger* |
| AMP | *Aspergillus melleus* protease |
| ANL | *Aspergillus niger* lipase |
| Amano lipase P | lipase from *Pseudomonas fluorescens* |
| BACE | $\beta$-site amyloid protein cleaving enzymes |
| BenzylPNase | benzyl penicillase |
| Boc | *tert*-butyloxycarbonyl |
| BuOH | *n*-butanol |
| CAL-A | *Candida antarctica* lipase type A |
| CAL-B | *Candida antarctica* lipase type B |
| Cbz | benzyloxycarbonyl |
| CCL | *Candida cylindracea* lipase |
| CHE | cholesterol esterase |
| chiroCLEC-CR | cross-linked enzyme crystal of *Candida rugosa* lipase |
| CPI | cyclopropylindole |
| CRL | *Candida rugosa* lipase |
| de | diasteromeric excess |
| DKR | dynamic kinetic resolution |
| DMSO | dimethyl sulfoxide |
| *E* | enantioselectivity |
| $ee_p$ | enantiomeric excess of the product |
| $ee_s$ | enantiomeric excess of the substrate |
| EH | epoxide hydrolase |
| equiv | equivalents |
| EtOAc | ethyl acetate |
| Fr | furoate |
| ImmH | Immucilin H |
| IPA | isopropenyl acetate |
| MeCN | acetonitrile |
| MOM | methoxymethyl |
| MPOA | methyl phenoxyacetate |
| n.d. | not determined |
| OctOH | 1-octanol |
| PFL | *Pseudomonas fluorescens* lipase |
| lipase AK | commercially available preparation of *Pseudomonas fluorescens* lipase |
| lipase AH | commercially available preparation of *Pseudomonas species* lipase |
| lipase AS | commercially available preparation of *Aspergillus niger* lipase |
| lipase PL | commercially available preparation of *Alcaligenes species* lipase |
| lipase RM | commercially available preparation of *Rhizomucor miehei* lipase |

| | |
|---|---|
| lipase TL | commercially available preparation of *Pseudomonas stutzeri* lipase |
| MPOA | methyl phenoxyacetate |
| PLAP | pig liver acetone powder |
| PLE | pig liver esterase |
| PMB | 4-methoxybenzyl |
| $^{n}PnNH_2$ | *n*-pentyl amine |
| PNPase | purine nucleoside phosphorilase |
| Poc | phenyloxycarbonyl |
| PPL | porcine pancreatic lipase |
| $^{i}Pr_2O$ | diisopropyl ether |
| PSL | *Burkholderia cepacia* lipase (formerly known as *Pseudomonas cepacia* lipase) |
| PSL-D | *Burkholderia cepacia* lipase immobilized on diatomite |
| PSL-C II | *Burkholderia cepacia* lipase immobilized on Toyonite |
| PSL-L6 | *Burkholderia cepacia* lipase carried−fixed enzyme particles |
| TBME | *tert*-butyl methyl ether |
| VA | vinyl acetate |
| VB | vinyl butyrate |
| VBz | vinyl benzoate |
| w/w | weight/weight ratio |

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