# Hydrolases in organic chemistry. Recent achievements in the synthesis of pharmaceuticals

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**Abstract:** Hydrolases are versatile enzymes for synthetic purposes as they are able to catalyze multiple transformations in both aqueous medium and organic solvents. Their easiness of use and lack of cofactor dependency have attracted the attention of many organic chemists, which have successfully applied this family of enzymes in hydrolytic and reverse synthetic reactions. In this review, the authors have focused in the recent application of hydrolases for the synthesis of active pharmaceutical ingredients and different families of compounds with exceptional biological profiles. Thus, their action has been classified in non-selective and selective transformations including regio- and stereoselective processes. Asymmetric transformations are presented as key issues for the production of drug enantiomers, so examples of hydrolase-catalyzed kinetic resolutions, dynamic kinetic resolutions and desymmetrizations will be extensively discussed, presenting hydrolases as very versatile enzymes for the development of scalable synthetic transformations towards pharmaceuticals.

## 1. Introduction.

The use of hydrolases (EC 3)[1] in synthetic chemistry is highly attractive because of their remarkable properties such as broad substrate specificity, commercial availability, lack of cofactor dependency, and ability to work at high substrate concentrations in aqueous but also organic and neoteric solvents.[2] These issues currently make hydrolases the favorite class of enzymes for the majority of organic chemists,[3-5] specially for the production of different families of compounds in enantiomerically pure form such as alcohols, amines, amides, carboxylic acids, carbonates and esters.[6,7] In this context, lipases are probably the most recurrent enzymes with multiple applications, including the synthesis of pharmaceuticals.[8,9]

In the last decades chemical industrial companies have implemented their facilities with research and production departments that make use of enzymes for selected transformations. Based on their low environmental impact, efficiency and selectivity, the chemoenzymatic syntheses of high added value compounds, agrochemicals and pharmaceuticals have been possible,[10-15] hydrolases playing sometimes a remarkable role.[16-22]

In this revision, we have mainly focused on recent works that include the conversion and isolated yield of the obtained products. The development of adequate immobilization techniques,[23-28] site-specific chemical modifications[29] and fermentation engineering[30] have highly contributed to the success of hydrolytic enzymes. Using these techniques, improvements in enzyme stability towards pH and temperature have been achieved, leading to more active and selective catalysts. In addition, the enzyme specificity can also be changed offering new synthetic possibilities for demanding products. Importantly, the use of supported enzymes allows their easy recovery at the end of the process through a simple filtration or centrifugation step, which facilitates the purification of the final products with high isolated yields. The application of continuous flow reactions has also attracted recent attention, finding an improvement of the productivity for some transformations.[31,32]

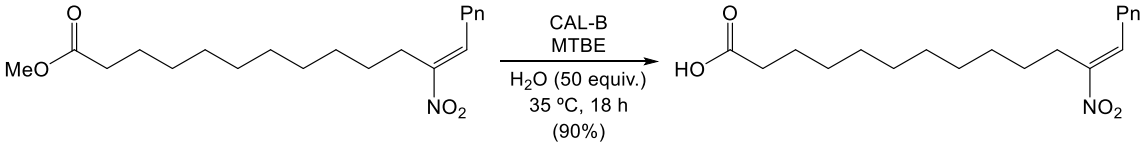
The aim of this review is to disclose the state of the art about the use of hydrolases in the synthesis of compounds with interesting biological profiles, including the chemoenzymatic synthesis of active pharmaceutical ingredients (APIs). With that purpose and focusing in the last six years, a classification has been made depending on the type of reaction and the enzyme used. Firstly, non-stereoselective transformations will be presented covering hydrolytic or synthetic procedures reactions using lipases, epoxide hydrolases or amidases in a non-selective fashion. Examples of regioselective reactions will be later discussed, showing the versatility of this class of enzymes for the modification of a desired target. Finally, the application of hydrolases in asymmetric transformations will be discussed in depth including asymmetric hydrolysis of esters, acylation of alcohols and amines, and other stereoselective transformations including kinetic resolutions and desymmetrization reactions.

**2.1. Hydrolase-catalyzed non asymmetric transformations.**

Hydrolases, mainly lipases, esterases and proteases inside this class, have allowed the development of extremely useful regio- and chemoselective transformations for the production of pharmaceuticals, agrochemicals and high-added value compounds.[33,34] The main efforts have been focused in the design of efficient hydrolytic procedures of esters in aqueous medium, the acylation of alcohol and amines or the esterification of carboxylic acids in organic solvents. Next, some recent achievements are discussed.

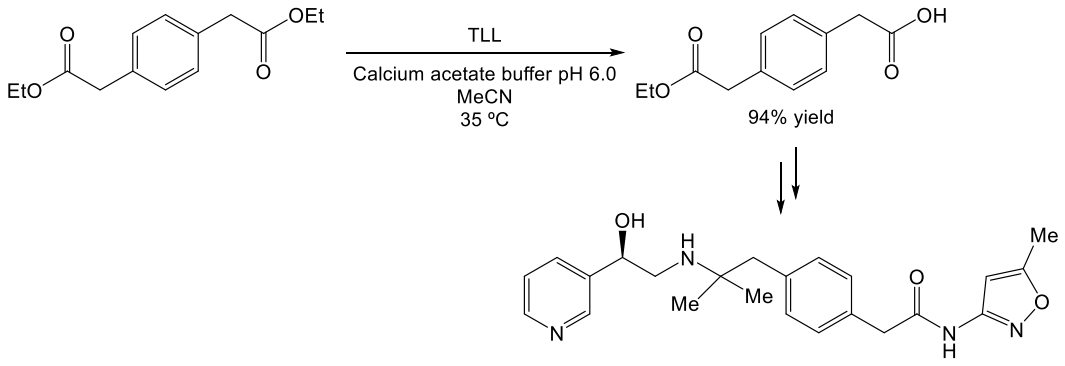
The efficient preparation of nitro-fatty acids, important signaling molecules related to inflammatory diseases, has been achieved by hydrolytic cleavage of methyl esters using a "buffer-free" system.[35] The reaction medium contains 50 equiv. of water and a series of organic solvents can be successfully used. Methyl *tert*-butyl ether (MTBE) was found as the best solvent in the combination with *Candida antarctica* lipase type B (CAL-B), leading to the 10-nitrolinoleic acid in 90% isolated yield (Scheme 1). This study improves previous enzymatic experiments in buffer for the synthesis of 10-nitrolinoleic

acid using Lipozyme® from *Thermomyces lanuginosus* (TL).[36] This enzymatic approach was successfully extended to the formation of other interesting products in high to excellent yields (87-98%) such as prostaglandin-$E_2$, isoprostane-$A_2$, preclavulone-A, isoprostane $E_{2t}$, phytoprostanes-$B_1$ type I and type II and prostaglandin-$F_{2\alpha}$.




**Scheme 1**. Lipase-catalyzed hydrolysis for the synthesis of 10-nitrolinoleic acid (Pn: pentyl group).

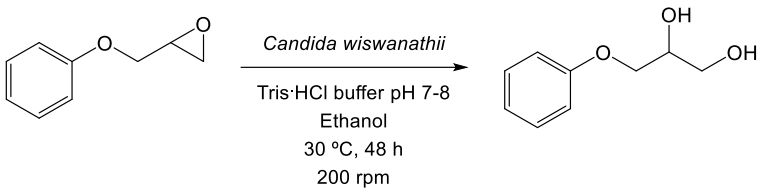
Carroll and co-workers developed the preparation of a β-3 receptor agonist intermediate using a chemoenzymatic approach involving the monohydrolysis of diethyl 1,4-phenylenediacetate.[37] Starting from 200 g of diester, the TL lipase catalyzed the formation of the monodiester in 94% yield under mild reaction conditions using a monophasic system composed by acetonitrile (MeCN) and an acetate buffer pH 6.0 (Scheme 2).




**Scheme 2**. Lipase-catalyzed monohydrolysis of 1,4-phenylenediacetate in the total synthesis of a potent β-3 receptor agonist.

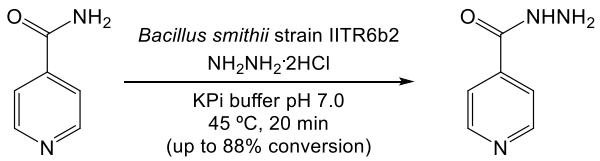
Epoxide hydrolases (EHs) are also versatile enzymes allowing the hydrolysis of epoxides under mild reaction conditions. The EH from *Candida wiswanathii* has efficiently hydrolyzed the phenyl glycidyl

ether to form the 3-phenoxy-1,2-propanediol that is an intermediate in the synthesis of centrally acting muscle relaxant, β-adrenoblockers, cardiovascular drugs and anti-bacterial agents, among others.[38] The reaction was exhaustively optimized using this microorganism in whole cell form, finding the best values after 48 h at 30 ºC and 200 rpm in a mixture of buffer at pH 7-8 and ethanol as cosolvent for the production in >90% conversion of 3-phenoxy-1,2-propanediol (Scheme 3).




**Scheme 3**. Hydrolysis of phenyl glycidyl ether using an epoxide hydrolase from *Candida wiswanathii*.

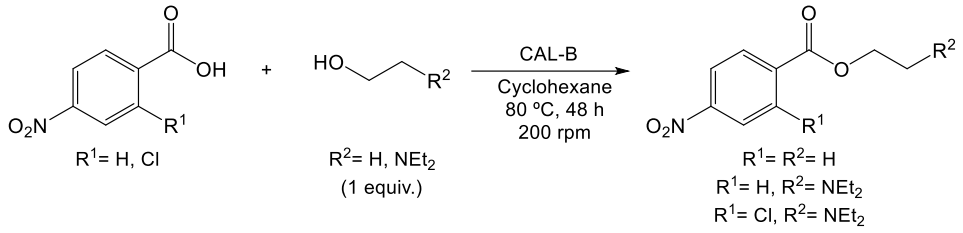
Nitrilases are powerful enzymes that allow the transformation of cyanides in carboxylic acids, while amidases allow the production of carboxylic acids from amides.[39] Both classes of hydrolases are usually employed as microorganisms in whole cell form, and in this context probably the most studied biotransformation is the conversion of 3-cyanopyridine into nicotinic acid, one of the main forms of vitamin $B_3$.[40] Other related transformation is, for instance, the one reported by Choudhury and co-workers about the conversion of isonicotinamide into Isoniazid, an important first-line anti-tubercular drug.[41] The acyltransferase activity of whole cells amidase of *Bacillus smithii* strain IITR6b2 was demonstrated in aqueous medium by using hydrazine dihydrochloride as nucleophile (Scheme 4).




**Scheme 4**. Formation of Isoniazid from isonicotinamide employing an amidase.

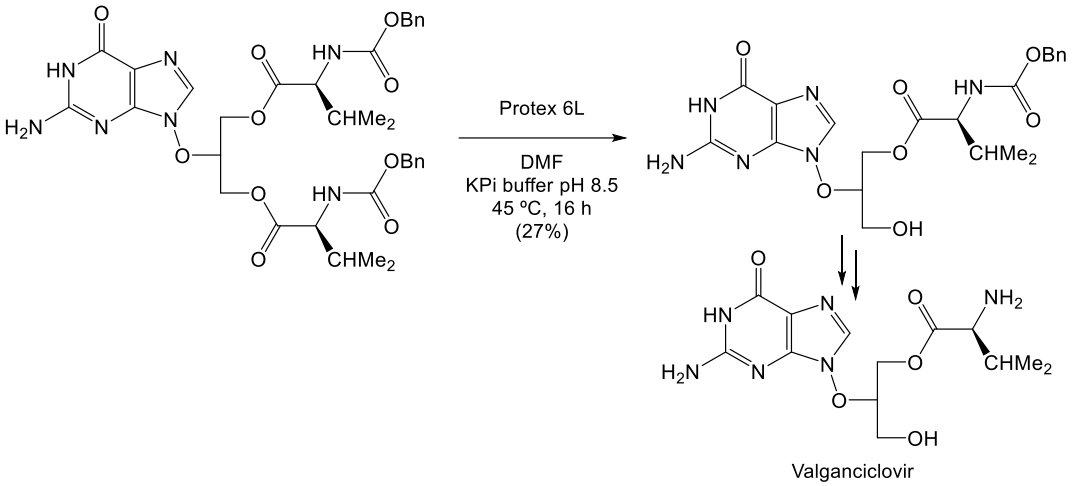
The opposite approach by using alcohols instead of water as nucleophiles in the reaction with substituted benzoic acids has allowed the production of interesting alkyl esters, which are key

intermediates in the synthesis of benzocaine, procaine and chloroprocaine anesthetics.[42] The reaction with CAL-B and equimolecular amounts of an alcohol led to the best values in cyclohexane as solvent, providing access to the desired esters in quantitative yields after a simple isolation by filtration and solvent evaporation (Scheme 5).



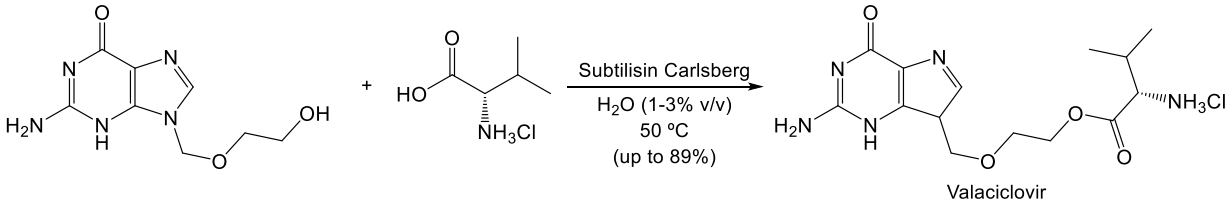

**Scheme 5**. CAL-B catalyzed esterification of benzoic acids as anesthetic precursors.

CAL-B has also shown an excellent activity in the alcoholysis of cephalosporin derivatives, which are potential antibiotics.[43] Because other functionalities are present such as carboxylic acids, amides or lactams, the reaction occurs in mild reaction conditions using *sec*-butanol instead of water as nucleophile in a mixture of hexane and tetrahydrofuran (THF), thus avoiding the formation of undesired lactones that rapidly occurs at acidic pHs. Valganciclovir hydrochloride is an anti-viral active pharmaceutical intermediate used in the treatment of cytomegalovirus infection, associated with AIDS disease. Structurally, it is a monoester prodrug of ganciclovir that exists as a mixture of diastereoisomers (Scheme 6), and its synthesis has been successfully achieved by selective hydrolytic deprotection catalyzed by Protex 6L, a bacterial alkaline protease derived from a selected strain of *Bacillus licheniformis*.[44] The 20-g hydrolysis reaction was carried out in a mixture of (*N*,*N*)-dimethylformamide (DMF) and a phosphate buffer pH 8.5 (3:2) at 45 ºC for 16 h, leading to the desired mono-L-valyl ester in 27% isolated yield.




**Scheme 6**. Chemoenzymatic synthesis of Valganciclovir involving a protease-catalyzed hydrolysis reaction.

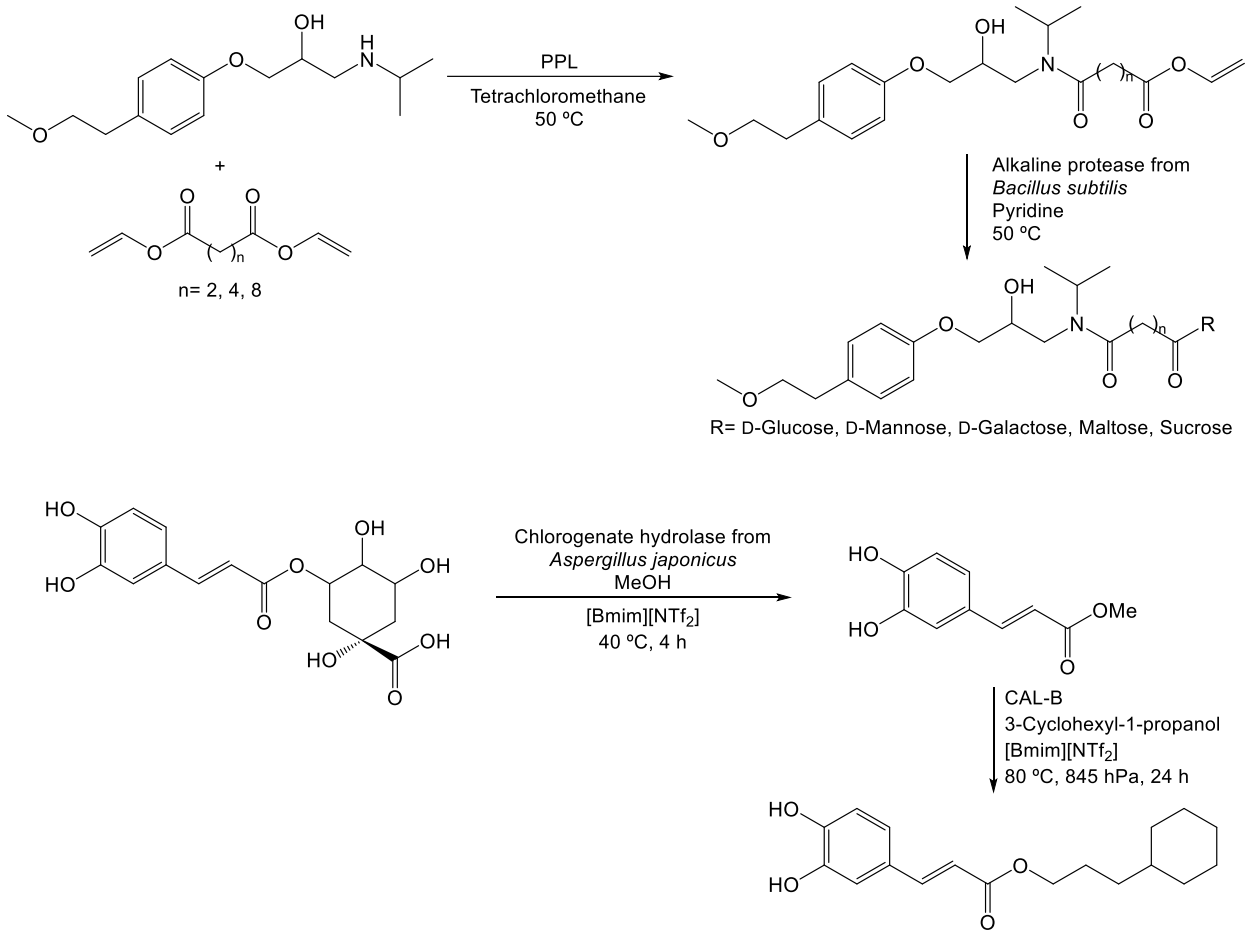
In some cases the poor solubility of both substrate and product lead to the development of solid-to-solid biotransformations as occurs in the reaction of the HIV reverse transcriptase inhibitor Aciclovir with L-valine methyl ester for the formation of its prodrug Valaciclovir, the active ingredient of *Valtrex*® (Scheme 7).[45] The reaction occurs in a large excess at 50 ºC and a little water content (1-3% v/v) to prevent the stability of Subtilisin protease from *Bacillus licheniformis* also known as Subtilisin Carlsberg, yielding the final product in up to 89%.




**Scheme 7**. Synthesis of Valaciclovir by Subtilisin-catalyzed acylation reaction.

In more complicated approaches, the combination of two hydrolytic enzymes can lead to more complex structures in consecutive transformations. A series of examples are presented here. On one hand, Lin and co-workers reported an efficient protocol to prepare metoprolol-saccharide conjugates.[46]

Metoprolol is a β-blocker with high efficiency in angina pectoris, hypertension, arrhythmias, migraine headaches and other disorders. The strategy occurs via the transesterification of metoprolol with a series of divinyl dicarboxylates (divinyl succinate, divinyl adipate and divinyl sebacate) to form the *N*-(viniloxycarbonyl)metoprolol, which served for the subsequent acylation of three monosaccharides (glucose, mannose and galactose) and two disaccharides (maltose and sucrose) using the alkaline protease from *Bacillus subtilis* (Scheme 8). Optimization of both individual processes allowed the recovery of metoprolol-saccharide conjugates in moderate yields. On the other hand, the combination of a chlorogenate hydrolase and a lipase provides useful access to caffeic acid esters, which present a broad spectrum of biological activities, including anti-microbial, anti-inflammatory, anti-oxidant and anti-tumor activities.[47] The system is based in the alcoholysis reaction with methanol using the chlorogenate hydrolase from *Aspergillus japonicus* and the subsequent transesterification with 3-cyclohexyl-1-propanol using CAL-B in a series of ionic liquids (Scheme 8). The best results were found in [Bmim][$NTf_2$] being possible the development of a one-pot process after vaporization of the methanol (MeOH), byproduct of the transesterification reaction, at 845 hectopascals. Optimization of the individual biotransformations allowed the recovery, among other esters, of 3-cyclohexylpropyl caffeate starting from 5-caffeoylquinic acid. Bommarius and co-workers have reported a cascade approach involving penicillin G acylase and an α-amino ester hydrolase for the production of Ampicillin in a maximum 47% conversion, combining a hydrolytic procedure and an aminolysis reaction.[48]

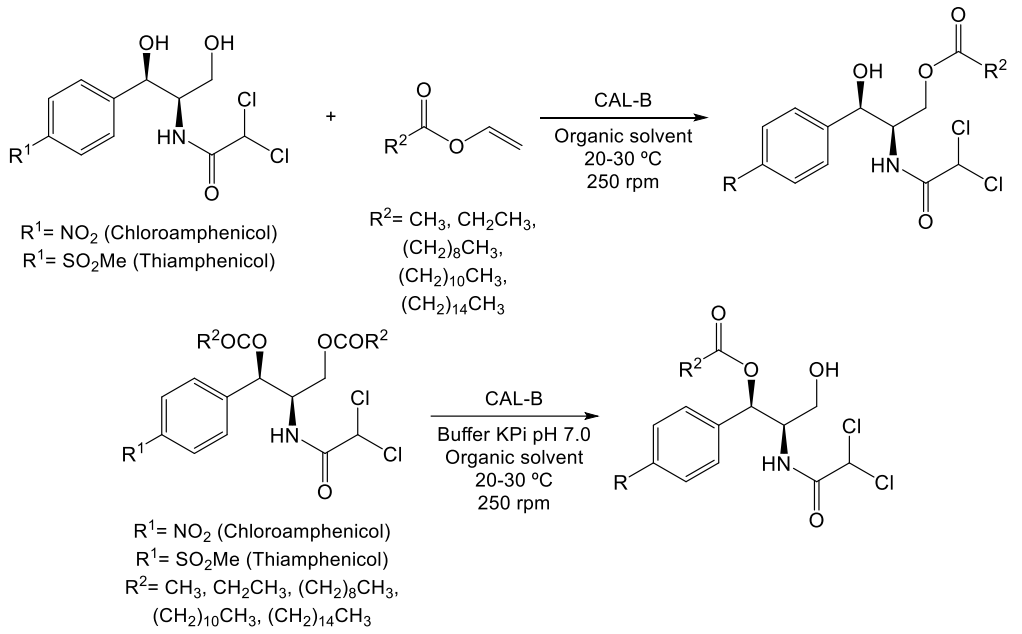



**Scheme 8.** Consecutive hydrolase-catalyzed reactions for the formation of metoprolol saccharide conjugates (top) and 3-cyclohexylpropyl caffeate (bottom).

## 2.2. Hydrolase-catalyzed regioselective transformations.

Hydrolytic and transesterification hydrolase-catalyzed reactions are complementary processes, which provide many advantages for the production of regioisomers. The development of regioselective enzymatic transformations has been successfully applied specially in natural product synthesis but also for the production of drugs. For instance, Chloroamphenicol is a bacteriostatic anti-microbial that acts as an effective agent against a broad spectrum of bacteria. For its acylation, the adequate selection of biocatalyst, solvent, acyl donor and temperature is crucial to obtain 3'-monoesters in high yields by modification of the primary hydroxyl group (Scheme 9).[49] Reactions proceeded smoothly at 150 and

250 mM with CAL-B, the enzyme reuse being possible in some case for 10 cycles due to the short reaction times needed. Alternatively, the complementary 1'-monoesters can be obtained by hydrolysis of the diesters using also CAL-B and a buffer:acetonitrile (80:20 v/v) system as reaction medium, although when longer ester moieties were employed, lower conversions were reached. Similarly, the production of thiamphenicol esters can be satisfactorily achieved using the same enzyme preparation and similar reaction conditions (Scheme 9).[50]




**Scheme 9**. CAL-B catalyzed production of Chloroamphenicol and Thiamphenicol monoesters by regioselective acylation or hydrolysis processes.

The development of regioselective processes is also highly appealing in natural product synthesis, acylation procedures providing outstanding solutions for the selective modification of very complex polyols, as occurs in the acylation of the 42-*O*-substituted molecules from the Rapamycin family. In this context, the selective acylation of 41-desmethoxy-rapamycin A to the biologically active Rapamycin derivative 41-desmethoxy *temsirolimus* B has been achieved by using two novel esters, butanedione monooxime ester and the *N*-acetylhydroxamate ester, which are more suitable for scale-up

in comparison with other more common vinyl esters.[51] Lipozyme TL seems to be a good biocatalyst for this process in a presence of less than two equivalents of acyl donor, yielding to the final acylated products in 94% or 75% yield, respectively, after 60 h at 42 ºC. Moris and co-workers have described the regioselective synthesis of chromomycin esters via CAL-B catalyzed acylation of the 4'-hydroxyl group located in the aglycone side chain.[52] These novel esters have displayed significant cytotoxicity values against four tumor cell lines, the chromomycin $A_3$ 4'-vinyladipate showing 3-5 times higher activity in comparison with its parent chromomycin $A_3$. The same authors used a similar approach for the production of mitramycin analogues finding also remarkable cytotoxicity values for the synthesized mono- and diesters using CAL-B or *Candida antarctica* lipase type A (CAL-A).[53]

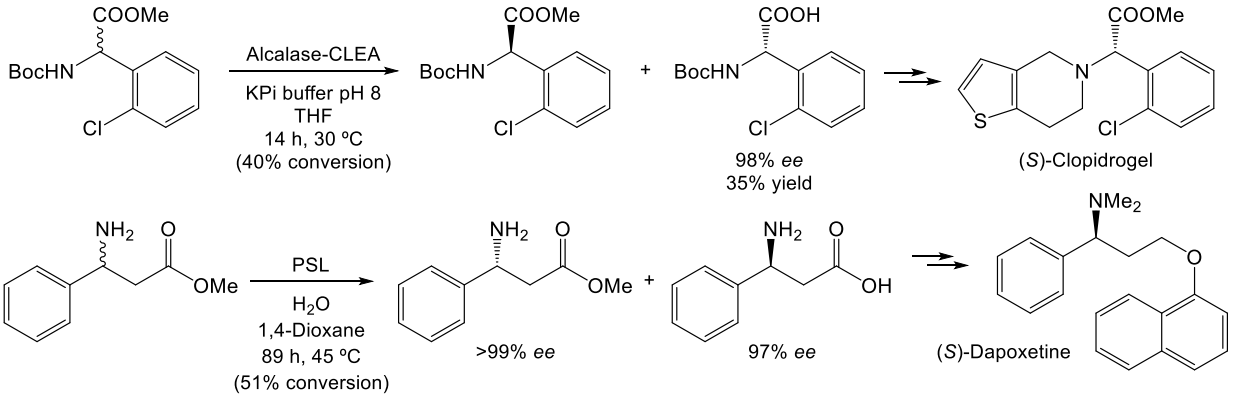
### 2.3. Hydrolase-catalyzed stereoselective transformations.

Asymmetric hydrolase-catalyzed processes have fuelled the synthesis of enantiopure drugs through selective modification of the own final target, or alternatively modifying an adequate intermediate.[54,55] We have classified this section depending on the reaction type so firstly, hydrolytic reactions of esters will be disclosed, focusing later in synthetic reactions such as acylations of alcohols and amines, aminolysis and alcoholysis of esters carried out in organic solvents.

#### 2.3.1. Asymmetric hydrolytic reactions.

The hydrolysis of esters to produce carboxylic acids is a recurrent strategy towards optically active drugs. With that purpose two approaches are usually attempted as they are the use of equivalents of water in organic medium, or the transformation in pure buffer, usually the latest being accomplished with the use of an organic cosolvent for substrate solubilization. Some examples are presented here focused in the synthesis of interesting pharmaceuticals.

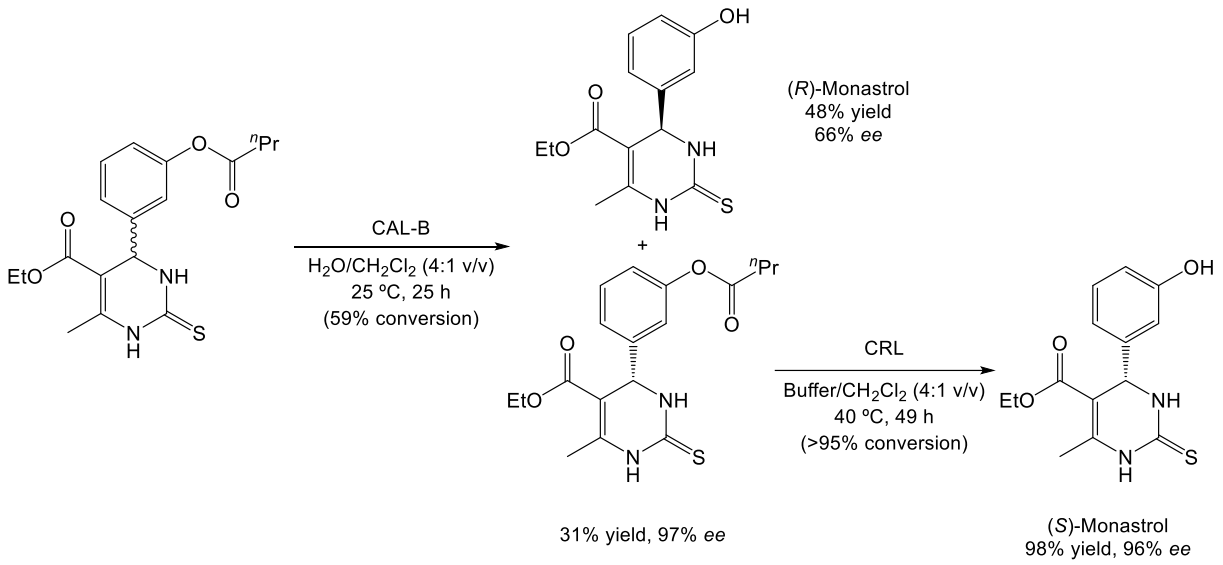
The different pharmacological activity of drug enantiomers has motivated the development of elegant enzymatic classical kinetic resolutions. Clopidogrel is an effective anti-aggregatory and anti-thrombotic drug commercialized as the brand name *Plavix*, the administration of the (*R*)-enantiomer being avoided due to demonstrated convulsion in animal experiments. The selective synthesis of (*S*)-Clopidogrel has been successfully achieved by a chemoenzymatic route where the resolution of a *N*-protected amino ester occurs with excellent selectivity using the subtilisin Alcalase-CLEA® in a system composed by a buffer and THF (10:1) after 14 h at 30 ºC (Scheme 10).[56] (*S*)-Dapoxetine is a potent serotonin re-uptake inhibitor used for the treatment of a variety of disorders as anxiety, bulimia, depression and premature ejaculation. The resolution of the commercially available 3-amino-3-phenylpropionic methyl ester was possible by *Pseudomonas cepacia* lipase (PSL or PCL depending on the authors and commercial sources, and also recently known as *Burkolderia cepacia* lipase) catalyzed hydrolysis using 5 equiv. of water and 1,4-dioxane as solvent at 45 ºC (Scheme 10).[57] This enzymatic asymmetric procedure was easily extended to a broad family of 3-amino-3-arylpropionic methyl esters, leading to the corresponding esters and carboxylic acids with high selectivity.




**Scheme 10.** Chemoenzymatic synthesis of (*S*)-Clopidogrel and (*S*)-Dapoxetine.

Racemic Monastrol is the first small molecule inhibitor of the mitotic motor Eg5 (kinesin spindle protein) with broad applicability in anti-cancer research, the (*S*)-enantiomer showing 15-times higher

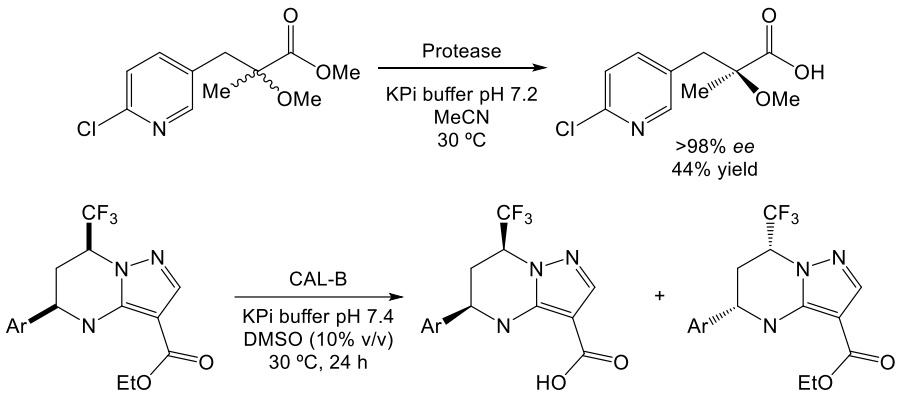
potency compared with its (*R*)-antipode. Gröger and co-workers reported the resolution of the *O*-butanoyl Monastrol, leading to the remaining (*S*)-ester in 97% *ee* (Scheme 11).[58] Subsequent hydrolysis of this ester catalyzed by *Candida rugosa* lipase (CRL) led to the (*S*)-Monastrol in 96% *ee*.




**Scheme 11.** Resolution of racemic Monastrol by lipase-catalyzed hydrolysis.

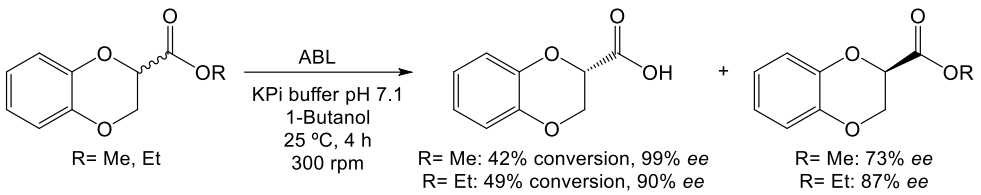
Peroxisome proliferator-activated receptors (PPARs) display wide-ranging effects on key transcriptional pathways for lipid handling, insulin sensitivity and inflammatory diseases, among others. A hydrolysis of a methyl ester derivative bearing a quaternary stereocenter was successfully achieved using the protease from bovine pancreas to produce the carboxylic acid intermediate showed in Scheme 12, which serves as precursor of the final target.[59] The enzymatic route was presented as an alternative of chemical chiral salt resolutions or a diastereomeric separation route, although no information about the other antipode was reported. Recently, Fernández-Álvaro and co-workers reported the stereoselective hydrolysis of an ethyl ester using CAL-B for the formation of enantiomerically pure tetrahydropyrazolo[1,5-α]pyrimidines, which are useful pharmacological agents in tuberculosis, osteoporosis and hepatitis C.[60] The lipase seems to accept a variety of cosolvents such as organic solvents or deep eutectic solvents, finding the best results with 10% DMSO and a phosphate

buffer pH 7.4 (Scheme 12). In all these examples the enzyme selectivity is high, but in some cases the use of two complementary hydrolases is necessary to improve the optical purity of the product. This fact occurs in the resolution of a Thallusin acetate intermediate that requires the action of PSL and lipase M Amano-10 in a mixture of buffer and dichloromethane to yield the corresponding alcohol in 92% *ee*.[61]



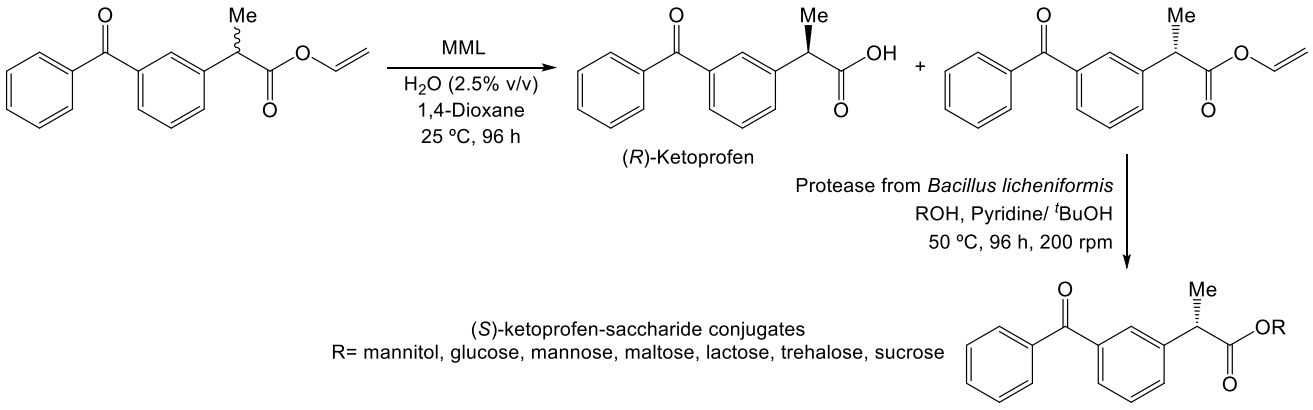

**Scheme 12.** Hydrolyase-catalyzed resolution of interesting alkyl esters through hydrolytic procedures.

The alkyl group in the ester functionality has a dramatic influence in an asymmetric process as occurs in the synthesis of both enantiomers of 1,4-benzodioxan-2-carboxylic acid, a key intermediate of therapeutic agents such as Piperoxan, Prosympal, Dibozane and Doxazosin.[62] The *Arthrobacter* species lipase (ABL) in phosphate buffer and using 1-butanol as cosolvent displayed a high selectivity in the resolution of the methyl ester, affording the corresponding carboxylic acid in enantiomerically pure form, while a loss of optical purity was observed in the resolution of the ethyl ester (Scheme 13). On the other hand, Wirz and coworkers reported the resolution of 7-oxabicyclo[2.2.1]heptan-2-*exo*-alkyl ester, which is a key precursor of an A2a receptor antagonist. CAL-A was found as a suitable enzyme that displayed the highest selectivities with longer chain alkyl esters (pentyl, hexyl and octyl).[63] Other hindered esters, such as a chloroacetate precursor of the inhibitor Sedanolide, have been successfully resolved using the lipase OF from Meito in phosphate buffer pH 7.0.[64]



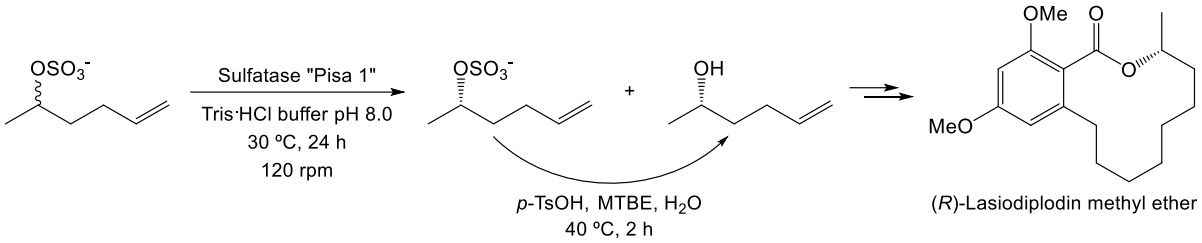

**Scheme 13.** Influence of the alkyl group in the resolution of benzodioxane esters.

The family of 2-arylpropionic acids possesses remarkable anti-inflammatory properties as is the case of Ibuprofen and Ketoprofen. However, their applications are normally limited because of their low solubility in water. In this context, Habibi and co-workers described the resolution of racemic ibuprofen alkyl esters using an enzymatic preparation of CRL and *Rhizopus oryzae* lipase (ROL) immobilized on octyl-sepharose via physical adsorption.[65] On the other hand, Yu and co-workers reported the synthesis of seven ketoprofen-saccharide conjugates through a two-step procedure based on the resolution of ketoprofen racemic vinyl ester by using Lipozyme® immobilized from *Mucor miehei* lipase (MML, Scheme 14).[66] Next the protease from *Bacillus licheniformis* catalyzed the transesterification of the (*S*)-ketoprofen vinyl ester with a series of saccharides (0.25 equiv.) in organic media. The so-obtained ketoprofen conjugates present better water solubility than parent ketoprofen, and thus they seem suitable for pharmaceutical application.




**Scheme 14.** Enzymatic two-step synthesis of ketoprofen-saccharide conjugates.

Sulfatases are a class of hydrolytic enzymes less explored for synthetic applications, but with interesting applications. Depending on their mode of action, they either cleave the S-O bond (leading to a retention of the configuration of the secondary alcohol), or the C-O bond (causing the stereoinversion of the stereogenic center). Faber and co-workers reported the chemoenzymatic asymmetric synthesis of (*R*)-Lasiodiplodin methyl ether, which is a precursor in the synthesis of the anti-leukemic agent Lasiodiplodin.[67] The sulfatase "Pisa 1" from *Pseudomonas* sp. DSM 6611 catalyzed the enantioconvergent hydrolysis of a racemic methyl sulfate ester precursor, accomplished with the acidic hydrolysis of the remaining sulfate ester for the formation of the desired alcohol in high conversion and optical purity as sole product (Scheme 15).




**Scheme 15.** Enantioconvergent sulfate ester hydrolysis using a sulfatase from *Pseudomonas* sp. DSM 6611 for the synthesis of a Lasiodiplodin key fragment.

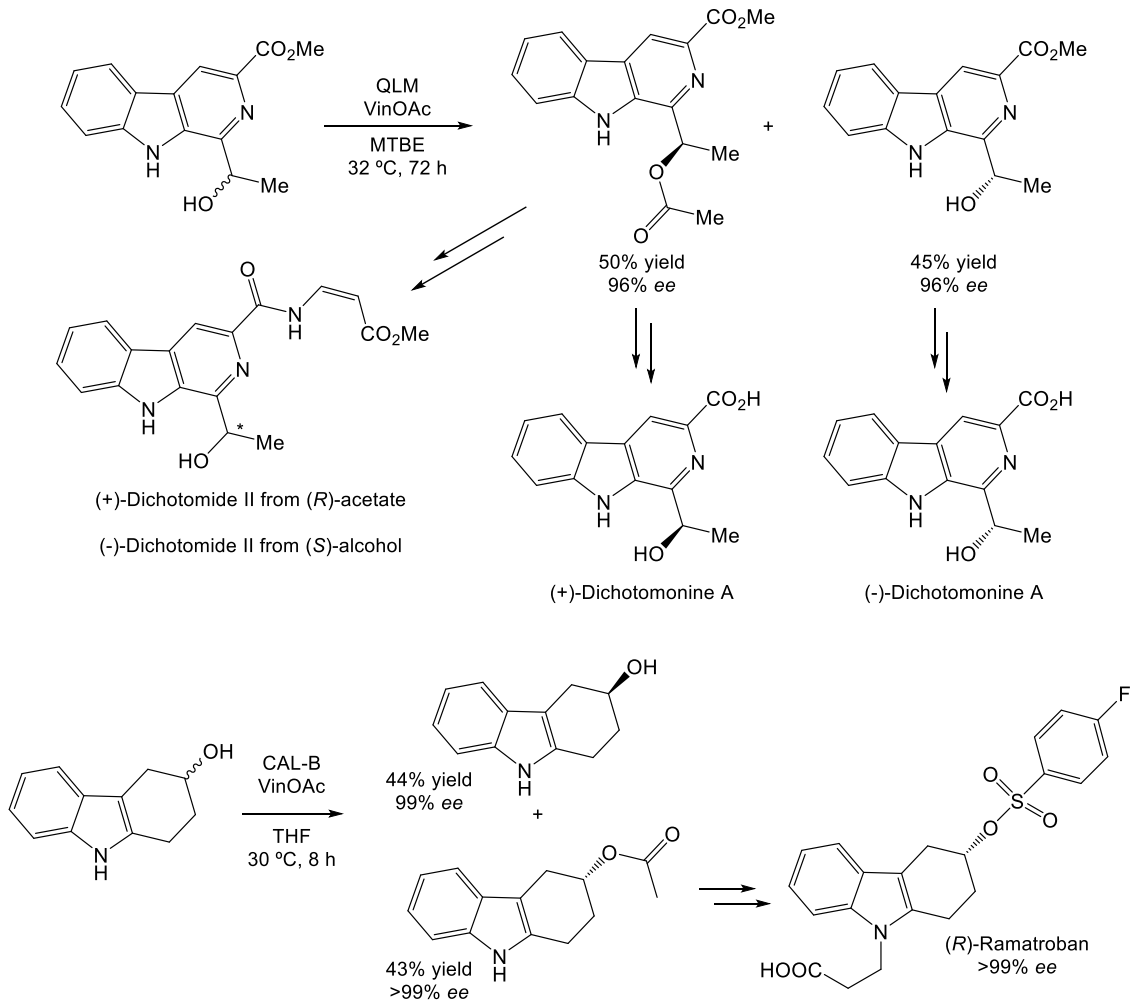
### 2.3.2. Asymmetric acylation reactions.

The resolution of alcohols and amines is probably nowadays the most recurrent strategy for the preparation of alcohol and amine derivatives in enantiomerically pure form. The efficiency of hydrolytic enzymes in organic media makes them attractive enzymes for some challenging transformations. In addition of classical kinetic resolutions, hydrolases can easily facilitate the development of dynamic kinetic resolutions (DKRs) that allow a theoretically 100% yield of an enantiopure compound starting from a racemic mixture.[68-71] This possibility is feasible when a perfect combination between the hydrolases and a (bio)chemical catalyst is achieved. In this context, ruthenium and palladium metal complexes have allowed the most effective DKRs. On the other hand,

the enantioselective desymmetrization of prochiral and *meso*-compounds is also possible.[72] Next, selected examples in these areas will be discussed, starting with the reactivity of the alcohol functionality.

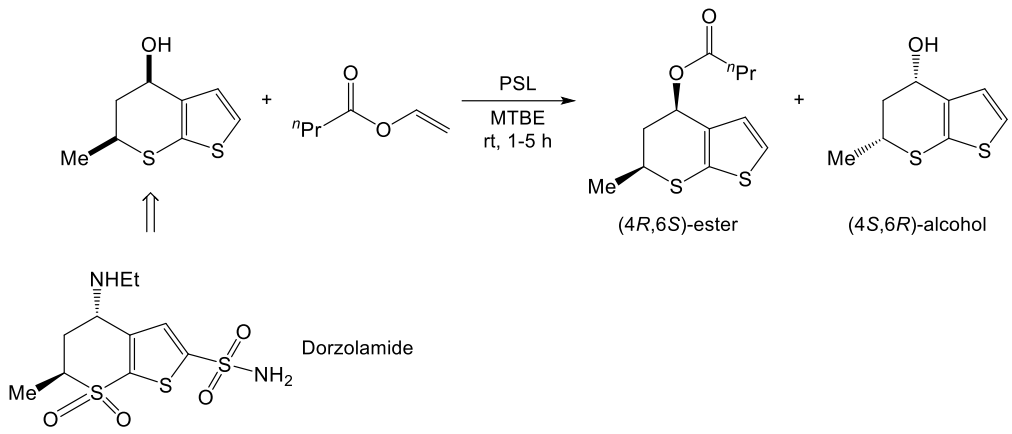
**2.3.2.1. Asymmetric acylation reactions of alcohols and polyols.**

Dichotomine A and Dichotomide II are β-carboline alkaloids, which posses remarkable biological profiles including anti-allergic properties. Their syntheses have been possible through enantioselective acetylation of a key alcohol intermediate namely methyl 1-(1-hydroxyethyl)pyrido[3,4-*b*]indole-3-carboxylate.[73] The lipase QLM in combination with vinyl acetate (VinOAc) in MTBE led to the (*S*)-(−)-alcohol and the (*R*)-(+)-acetate both in 96% *ee* after 72 h at 32 ºC (Scheme 16). Other indole structure is Ramatroban, which is commercialized in Japan with the trade name *Baynas*. The activity of this drug mainly resides in the (*R*)-enantiomer, being applicable in the treatment of asthma, rhinitis and coronary diseases. The selective acylation of its key precursor racemic 2,3,4,9-tetrahydro-1*H*-carbaz-3-ol was possible using CAL-B and 3 equiv. of vinyl acetate at 30 ºC in THF for 8 h, yielding both the (*R*)-acetate and the (*S*)-alcohol in enantiopure form (Scheme 16).[74] In similar conditions, the same research group reported the synthesis of Frovatriptan that is a second generation molecule with high affinity for human 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors.[75] CAL-B in combination with vinyl acetate allowed the production of the (*R*)-acetate and the (*S*)-alcohol in enantiopure form, studying also the possibility to obtain the desired alcohol by bioreduction processes. Alcohol dehydrogenase from *Rhodococcus ruber* provided an efficient synthetic alternative for the production of the desired (*S*)-alcohol in >99% *ee*.



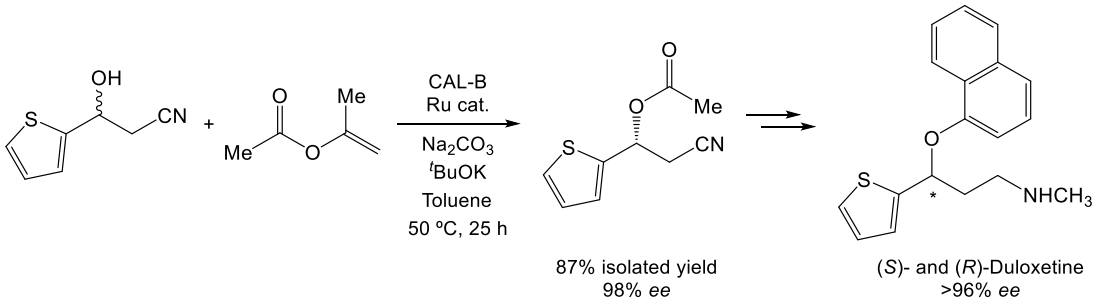

**Scheme 16.** Lipase-catalyzed acetylation of indole derivatives for the asymmetric synthesis of β-carboline alkaloids and (*R*)-Ramatroban.

Following with the resolution of cycloalkanols, Juhl and co-workers reported the efficiency of CAL-B in the resolution of *cis*-3-phenyl-1-indanol using diisopropyl ether ($^{i}Pr_2O$) as solvent and vinyl butyrate, obtaining the non acylated alcohol in enantiopure form and 45% yield.[76] This precursor was then applied in the synthesis of (+)-Indatraline, a potent re-uptake inhibitor of serotonin, noradrenalin and dopamine. Similarly, the optimized combination of PSL and vinyl butyrate in MTBE has led to the highly enantioselective synthesis of valuable intermediates of Dorzolamide, an anti-glaucoma agent used in ophthalmic solutions with the name of *Trusopt* (Scheme 17).[77]



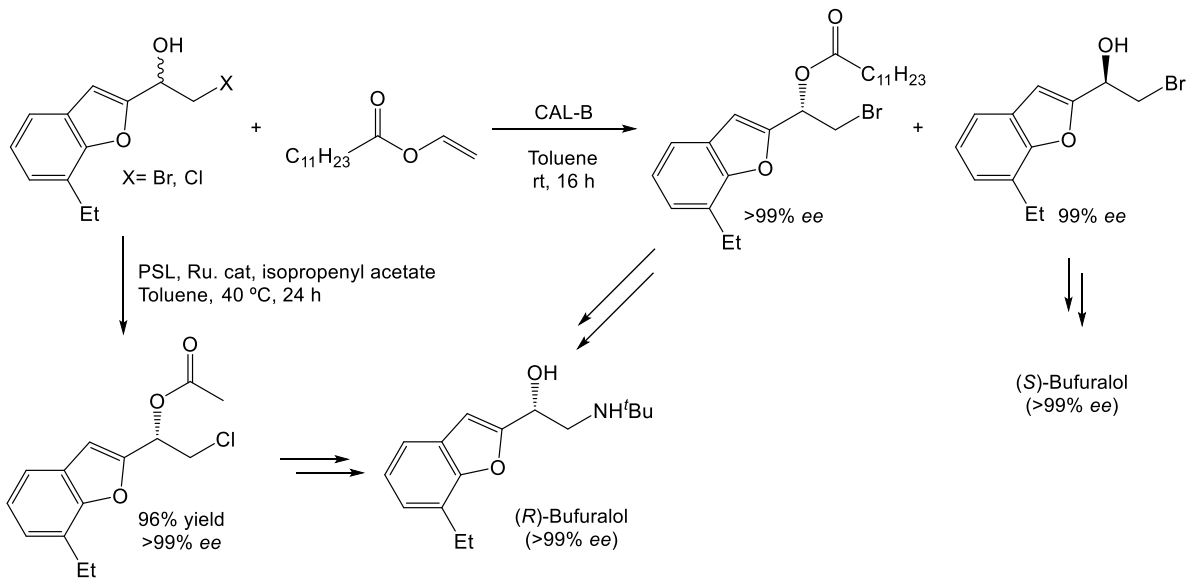

**Scheme 17.** Retrosynthetic analysis for the anti-glaucoma agent Dorzolamide, including a stereoselective lipase-catalyzed acylation reaction.

Fluoxetine, Tomoxetine and Nisoxetine are well-known anti-depressants, all of them containing the 3-aryloxy-3-phenylpropylamine core. Their enantiomers present different biological responses so the performance of asymmetric synthetic strategies is required in the pharmaceutical industry. Bäckvall and co-workers have reported the DKR of a β-hydroxynitrile precursor of Duloxetine using a ruthenium catalyst in combination with 3 equiv. of isopropenyl acetate, yielding the (*R*)-acetate in 87% isolated yield and 98% *ee* (Scheme 18).[78] Alternatively, the PSL-catalyzed resolution of γ-azido alcohol analogues using a large excess of isopropenyl acetate (6 equiv.) has allowed the global syntheses of Fluoxetine and Duloxetine enantiomers.[79]



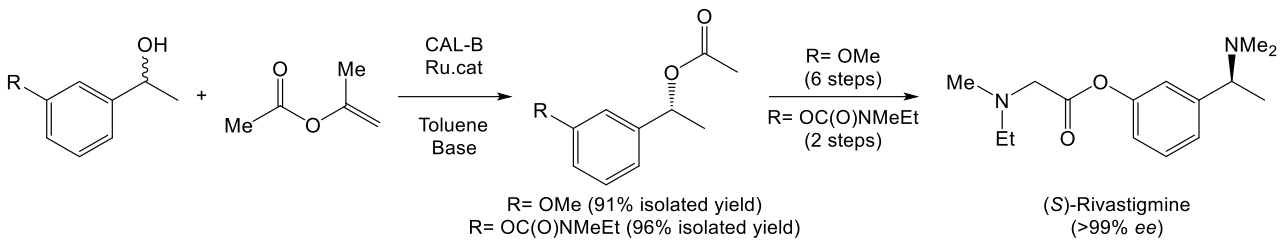

**Scheme 18.** Lipase-catalyzed acetylation of a β-hydroxynitrile for the synthesis of Duloxetine enantiomers.

Bufuralol is a potent β-adrenergic receptor antagonist with applications in the treatment of hypertension, the (*S*)-enantiomer being 100 times more active than its antipode. The enantioselective acylation of racemic 2-bromo-1-(7-ethylbenzofuran-2-yl)ethanol has provided access to the (*R*)-alcohol and a series of (*S*)-esters depending on the acyl donor used in the lipase-catalyzed reaction (Scheme 19). Thus, CAL-B has catalyzed the 1 gram scale resolution using 2 equivalents of vinyl dodecanoate in toluene after 16 h at room temperature, yielding both alcohol and ester in enantiopure form.[80] The DKR of 2-chloro-1-(7-ethylbenzofuran-2-yl)ethanol was also effectively achieved using the combination of PSL, isopropenyl acetate and chlorodicarbonyl(1,2,3,4,5-pentphenylcyclopentadienyl)ruthenium(II) at 40 ºC for 24 h, yielding the enantiopure (*R*)-acetate in 96% yield (Scheme 19).[81] Another halohydrydrin such as 1-chloro-3-(4-(2-methoxyphenyl)piperazin-1-yl)propan-2-ol has been stereoselectively acetylated using a large excess of vinyl acetate in combination with CRL as biocatalyst and toluene as solvent, obtaining the (*R*)-acetate in 98% *ee* and the (*S*)-alcohol in 96% *ee*, the latest compound being used in the synthesis of (*S*)-Encipracine that present diverse biological profiles including its action as cardiovascular, hypotensive and local anesthetic.[82]



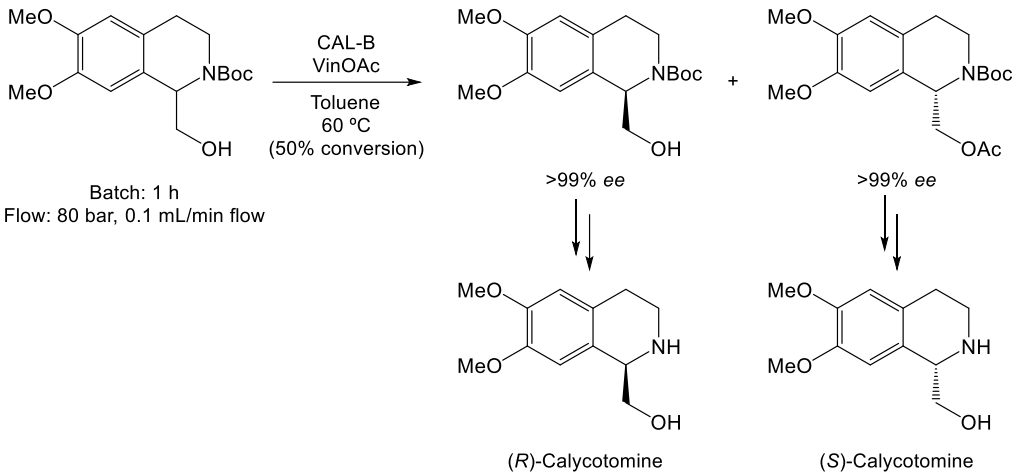

**Scheme 19.** Lipase-catalyzed resolution of 2-halo-1-(7-ethylbenzofuran-2-yl)ethanols for the synthesis of Bufuralol enantiomers.

Rivastigmine is an acetylcholinesterase inhibitor of the carbamate type, which is selective in the brain region, the (*S*)-enantiomer having applications in dementia caused by Parkinson's disease as the commercialized tartrate salt namely *Exelon*. Two independent DKRs based on lipase-catalyzed acetylation combined with metal catalysis have been described so far after exhaustive optimization of the easier KR procedure (Scheme 20). Firstly, racemic 1-(3-methoxyphenyl)ethanol was enantioselective acylated using CAL-B in combination with an excess of isopropenyl acetate (1.5 equiv.) and chlorodicarbonyl(1,2,3,4,5-pentaphenylcyclopentadienyl)ruthenium(II) as racemization agent (Scheme 20). Thus, the corresponding enantiopure (*R*)-acetate was isolated in 91% yield after 24 h at 50 ºC in toluene.[83] Alternatively, the acetylation of 3-(1-hydroxyethyl)phenyl ethyl(methyl)carbamate was successfully achieved at room temperature using a ruthenium polymer-bound catalyst, obtaining the desired acetate in 96% isolated yield (Scheme 20).[84]



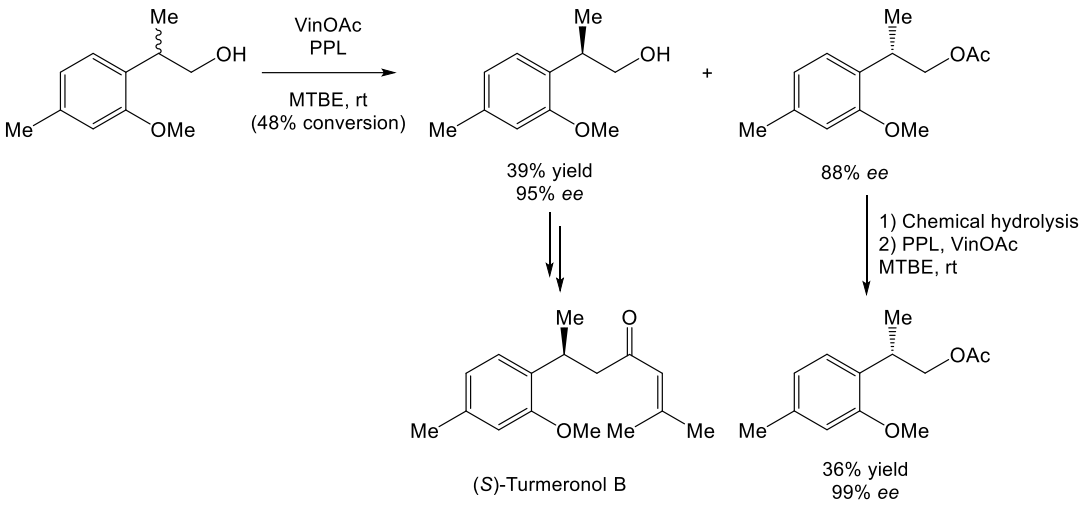

**Scheme 20.** DKRs of racemic 3-substituted-(1-phenyl)ethanols using CAL-B and isopropenyl acetate in combination with a ruthenium catalyst.

(*S*)-Propanolol is a β-adrenergic blocking agent widely used as anti-hypertensive drug and in the treatment of cardiovascular diseases. Its chemoenzymatic synthesis through selective *O*-acylation has been extensively explored in the literature during the past few years. Recently, Doerr and co-workers reported an experimental study to investigate the effect of the substrate, acyl donor ratio, enzyme purification and the use of methanol as cosolvent in the competition between *O*- and *N*-modification.[85] Other amino alcohols have also been efficiently resolved using lipases and vinyl acetate, although the previous chemical *N*-protection is required to avoid the formation of the amide or diacylated byproducts. This is the case of a series of tetrahydroisoquinoline derivatives, a common core in naturally-occurring alkaloids. Both enantiomers of Calycotomine have been obtained through CAL-B catalyzed *O*-acylation of *N*-Boc protected (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methanol in both batch and flow continuous processes (Scheme 21).[86] Unfortunately, when the alkanol functionality was elongated for the production of Crispine A enantiomers, lower selectivities were observed.[87]



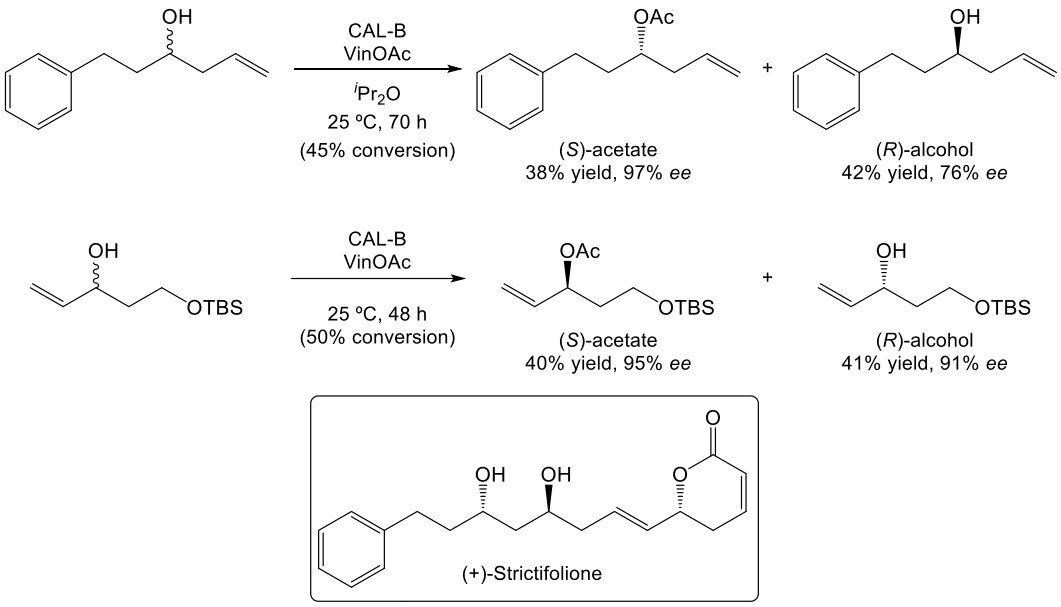

**Scheme 21.** Chemoenzymatic synthesis of Calycotomine enantiomers.

Lipase-catalyzed resolutions do not always provide excellent selectivities, but this hurdle can be overcome by including a second KR, this consists in a double KR. For instance, Serra reported the resolution of 2-aryl-propanols, which are common precursors of non steroidal anti-inflammatory drugs. After the first KR of 2-(2-methoxy-4-methylphenyl)propan-1-ol using porcine pancreatic lipase (PPL) the formation of the optically enriched (*S*)-acetate reached around 60% conversion, so the alcohol and the acetate were separated by flash chromatography, and then the enantioenriched acetate submitted to a chemical hydrolysis process and another lipase-catalyzed resolution, leading to the desired (*S*)-acetate in enantiomerically pure form.[88] The so-obtained (*R*)-alcohol has served as adequate intermediate in the synthesis of the phenolic sesquiterpene (*S*)-Turmenorol B, which is a strong anti-oxidant and lipoxygenase inhibitor. Similarly, the KR of other substituted 2-aryl-propan-1-ols with the same enzyme has led to the formation of interesting sesquiterpenes such as (*R*)-Curcumene, (*R*)-Curcuphenol, (*R*)-Xanthorrhizol and (*R*)-Curcuhydroquinone (Scheme 22). The structurally similar 2-(4-((4-methoxybenzyloxy)methyl)phenyl)propan-1-ol was resolved with high selectivity using PSL and 8 equiv. of vinyl acetate in $^{i}Pr_2O$, yielding with a 48% conversion the (*S*)-acetate and the (*R*)-alcohol in 98% and 94% *ee*, respectively, after 12 h at room temperature.[89] The optically active acetate served as precursor in the synthesis of the anti-inflammatory agent Loxoprofen.




**Scheme 22.** Double KR of 2-phenyl-propan-1-ol with vinyl acetate and PPL for the synthesis of (*S*)-Turmenorol B.

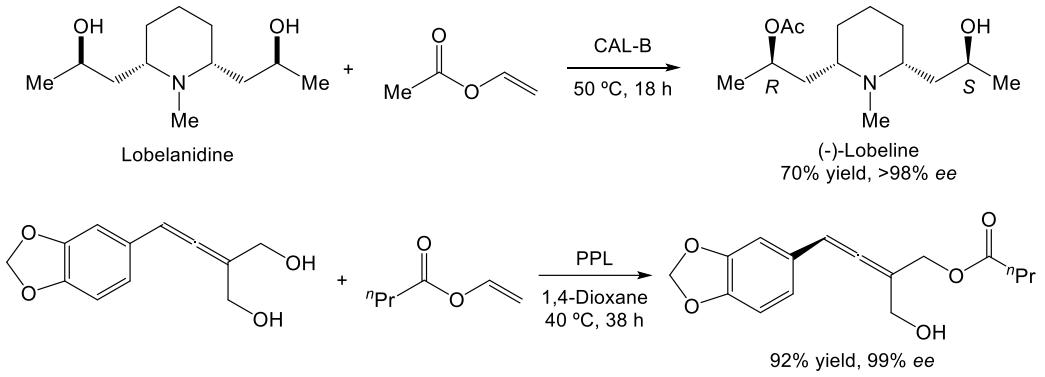
The asymmetric synthesis of (+)-Strictifolione bearing a lactone moiety has been possible through the fusion of two independent fragments obtained using chemoenzymatic approaches. Both of them involve the kinetic resolution of key fragments using lipase-catalyzed acetylation reactions (Scheme 23).[90] For the recovery of the 1-phenylhex-5-en-3-ol a second acetylation was neccesary to improve its enantiomeric excess from 76% to 94% *ee*. The same authors have recently reported the selective asymmetrization of different aliphatic fragments through acetylation procedures using CAL-B and vinyl acetate in organic solvent, which has made possible the chemoenzymatic synthesis of tetrahydropyran, macrolide and macrodiolide compounds.[91] Structurally similar to 1-phenylhex-5-en-3-ol are 4-(3-hydroxybutyl)phenols, a family of diols including Zingerol and Rhododendrol that possess interesting inhibition properties. Their lipase-catalyzed kinetic resolutions have been efficiently achieved using PSL and vinyl acetate in $^{i}Pr_2O$ at 35 ºC.[92]




**Scheme 23.** Structure of (+)-Strictifolione and lipase-catalyzed resolution of two independent fragments.

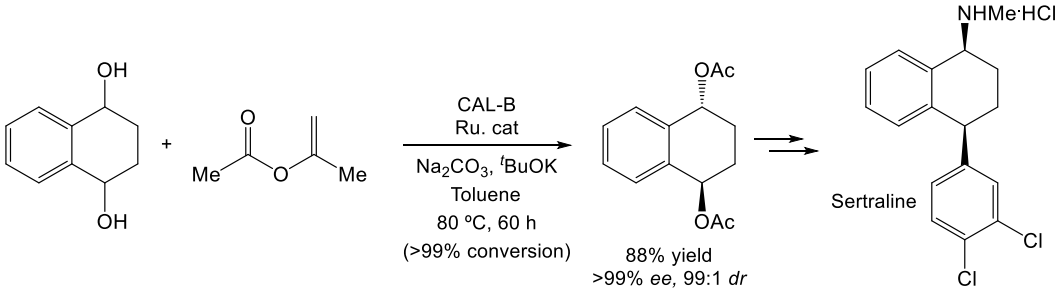
Enzymatic desymmetrization reactions allow the introduction of chirality breaking a symmetry element in prochiral and *meso*-compounds.[72] The examples in this area are less prominent than the resolution of racemates, nevertheless many different enzymes have been used with that purposes. For instance, Chênevert and Morin developed the desymmetrization of Lobelanidine to produce Lobeline that has a long tradition as therapeutic agent since the 19$^{th}$ century when it was prescribed as an emetic or a respiratory stimulant.[93] The desymmetrization occurs through an acylation procedure using CAL-B as biocatalyst and vinyl acetate as both solvent and acyl donor, yielding the enantiopure (*R*,*S*)-monoacetate called (–)-Lobeline in 70% yield and just a 3% of the diacetate byproduct at 50 ºC for 18 h (Scheme 24). The enzymatic acetylation of primary allenic alcohols provides a straightforward access to interesting building block in the synthesis of fungal metabolites. The PPL and 5 equiv. of vinyl butyrate in 1,4-dioxane has allowed the desymmetrization of a prochiral allenic diol, obtaining the monobutyrate precursor of hyperione A and B in 92% isolated yield and 99% *ee* (Scheme 24).[94] Alternatively, the same enzyme had previously catalyzed the kinetic resolution of racemic allenols with

5 equiv. of vinyl butyrate now in $^{i}Pr_2O$ at room temperature, yielding the remaining (*S*)-alcohols in variable yields.[95,96] One of these remaining optically active alcohols served as intermediate in the synthesis of (–)-Striatisporolide A.




**Scheme 24.** Enzymatic desymmetrization of diols for the synthesis of compounds with interest in medicinal chemistry.

Finally, in this section is worth mentioning the dynamic kinetic asymmetric transformations (DYKAT) by combining a lipase and a metal-catalyzed towards diols, allowing the production of diacetates in excellent yield and optical purity by coupling the resolution and the epimerization of a *meso*-compound *in situ*. In this context, Bäckvall and co-workers reported the synthesis of the anti-depressant Sertraline by selective acetylation of a bicyclic diol using CAL-B, a ruthenium catalyst and 4 equiv. of isopropenyl acetate at 50 ºC (Scheme 25).[97]

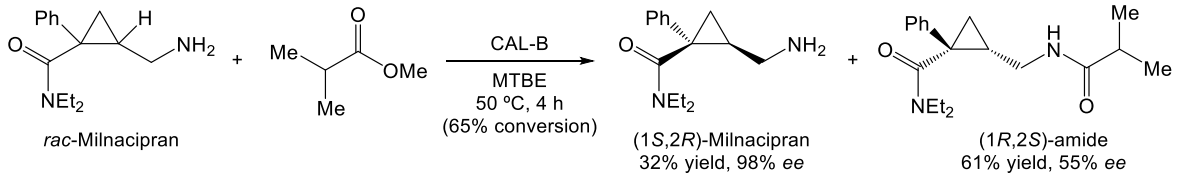



**Scheme 25.** DYKAT of a bicylic diol for the synthesis of Sertraline.

**2.3.2.2. Asymmetric acylation and alcoxycarbonylation reactions of amines.**

Chiral amines are important building blocks in the synthesis of APIs and agrochemicals.[98] In recent years, the development of successful KRs and DKRs of primary and secondary amines has led to the production of amine derivatives such as amides and carbamates in optically active form with application in medicinal chemistry.[99] Normally, the acylation reactions are preferred for primary amines while alkoxycarbonylation processes are often employed for the resolution of secondary amines. Because of the higher nucleophilicity of the amino group in comparison with the alcohol functionality, less activated esters or carbonates are required for the formation of the corresponding amides and carbamates, respectively.

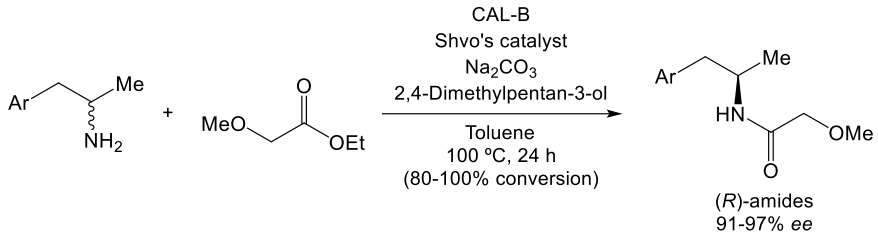
Milnacipran is a selective serotonin re-uptake inhibitor used as anti-depressant that also relieves chronic pain associated with fibromyalgia. Its classical kinetic resolution using CAL-B has been studied employing a wide panel of acyl donors, unfortunately low to moderate selectivities were usually attained (Scheme 26).[100] The best values were found using a large excess of methyl *iso*-butyrate in MTBE at 50 ºC for 4 h, isolating the (1*R*,2*S*)-amide and the remaining (1*S*,2*R*)-amine in 55% and 98% *ee*, respectively after a 65% conversion in 4 h.




**Scheme 26.** KR of Milnacipran using CAL-B and methyl *iso*-butyrate.

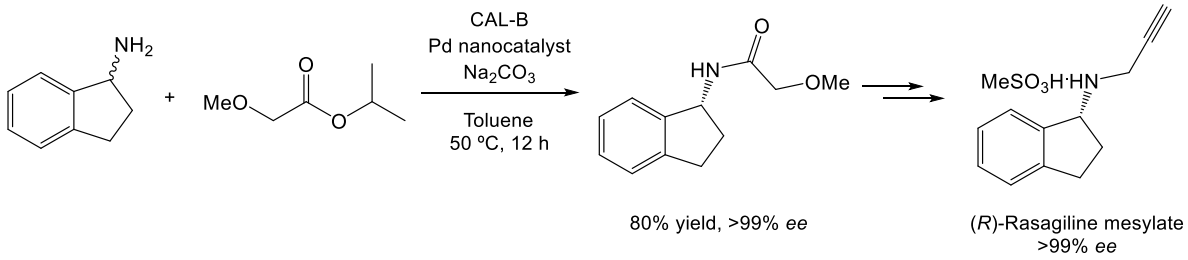
The KRs of pharmacologically active 1-aryl-[101] and 1-heteroarylpropan-2-amines[102] have been studied, based on the importance of these structures as stimulants. CAL-B has been found as an efficient biocatalyst for the selective acylation of the (*R*)-enantiomers using ethyl methoxyacetate as acyl donor in THF as solvent under mild reaction conditions. Remarkably, the DKR using the Shvo's

catalyst was possible for selected substrates, yielding the (*R*)-methoxyacetamides in high isolated yield at 100 ºC in toluene (Scheme 27).




**Scheme 27.** DKR of 1-aryl- and 1-heteroarylpropan-2-amines using CAL-B, ethyl methoxyacetate and the Shvo's catalyst.

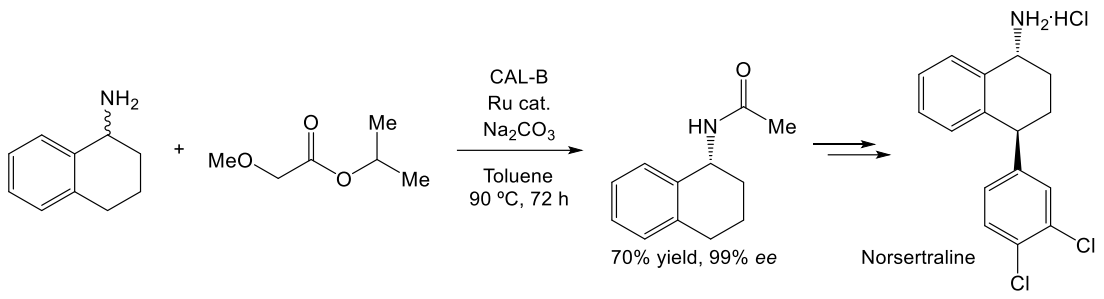
Rasagiline is an irreversible and selective monoamine oxidase inhibitor applied for the treatment of Parkinson's disease in the form of (*R*)-Rasagiline mesylate. Its synthesis has been recently reported identifying the DKR of racemic 1-indanamine as a key step (Scheme 28).[103] This chemoenzymatic step was performed on a 73 g scale at 200 g/L using a palladium nanocatalyst as racemizating agent in combination with CAL-B and isopropyl methoxyacetate as acyl donor. The desired amide was isolated in enantiopure form after a final recrystallization.




**Scheme 28.** DKR of racemic 1-indanamine for the synthesis of (*R*)-Rasagiline mesylate.

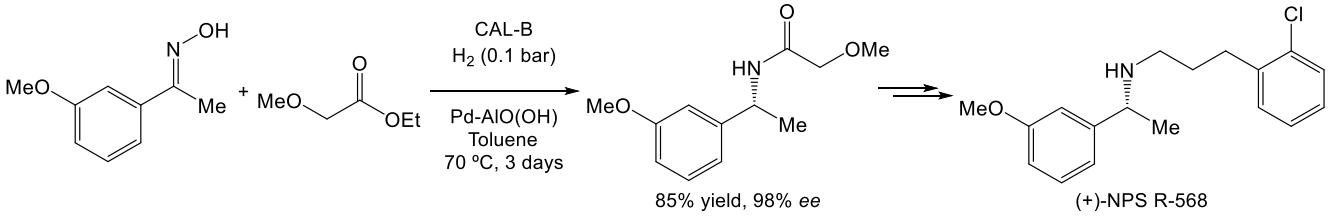
The DKR of the commercially available 1,2,3,4-tetrahydro-1-naphthylamine has allowed the synthesis of Norsertraline, which is the chief metabolite of sertraline, currently marketed by Pfizer as *Zoloft* for the treatment of depression as a selective serotonin reuptake inhibitor. The enzymatic step was catalyzed by CAL-B using isopropyl acetate and a ruthenium dimeric catalyst for amine racemization

(Scheme 29).[104] The corresponding (*R*)-acetamide was finally obtained in 70% yield and 99% *ee* after 72 h at 90 ºC.




**Scheme 29.** DKR of 1,2,3,4-tetrahydro-1-naphthylamine for the synthesis of Norsertraline.

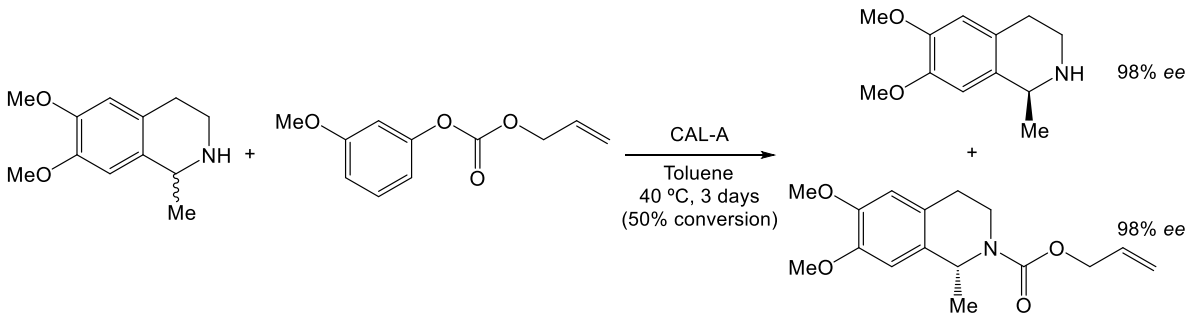
NPS R-568 is a potent calcimimetic for the treatment of primary and secondary hyperparathyroidism, the activity of the (*R*)-enantiomer being 10-100 times more active than its corresponding counterpart. Instead of using a racemic amine as starting material, a ketoxime directly obtained from 3-methoxyacetophenone was subjected to an asymmetric reductive acylation process using Pd-AlO(OH), CAL-B and 1.5 equiv. of ethyl methoxyacetate under hydrogen atmosphere, obtaining the (*R*)-methoxyacetamide in 85% isolated yield and 98% *ee* after 3 days at 70 ºC (Scheme 30).[105] Remarkably, the system was reused for 4 cycles without any loss of the catalytic activity. Applying the same methodology, the synthesis of optically active α-trifluoromethylated amides was accomplished, producing an (*S*)-inhibitor of phenylethanolamine *N*-methyltransferase without loss of the chiral information.[106]




**Scheme 30.** Synthesis of (+)-NPS R-568 via asymmetric reductive acylation of a ketoxime using CAL-B and Pd/AlO(OH) for the asymmetric process.

The lipase-catalyzed acylation of secondary amines often results in low selectivities because the occurrence of high background reactions, so the resolution of these nitrogenous compounds is usually performed by the proper combination of a lipase and a reactive carbonate to form the corresponding optically active amine and the resulting carbamate. Because of the established interactions between a drug candidate and a target, the carbamate group is currently considered a key structural fragment in many approved drugs and prodrugs.[107]

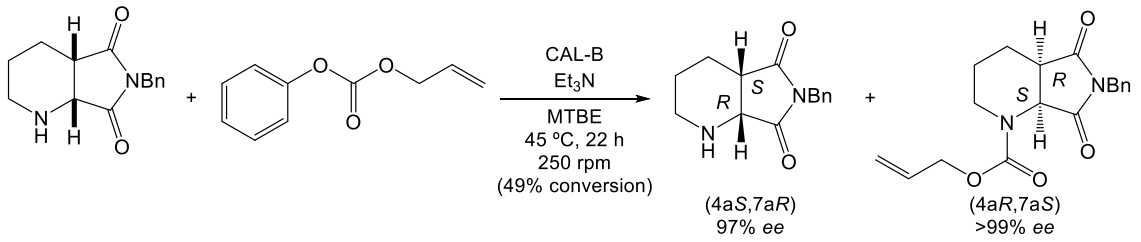
This strategy has been used by Deng and co-workers in the synthesis of the alkaloid (*R*)-Salsolinol, which is the 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, a compound detected in the urine of patients with Parkison's disease.[108] After an exhaustive screening of carbonates, allyl 3-methoxyphenyl carbonate was found to be the best reagent using CAL-A in toluene at 40 ºC. Thus, after 72 h the (*R*)-allyl carbamate and the remaining (*S*)-amine were obtained both in 98% *ee* (Scheme 31). Finally, the optically active carbamate was chemically hydrolyzed to afford the (*R*)-Salsolinol.




**Scheme 31.** Kinetic resolution of a Salsolinol intermediate using CAL-A and allyl 3-methophenyl carbonate.

Wang and co-workers reported the resolution of racemic *cis*-6-benzyltetrahydro-1H-pyrrolo[3,4-*b*]pyridine-5,7(6*H*,7a*H*)-dione for the preparation of an intermediate in the synthesis of Moxifloxacin, an anti-bacterial agent.[109] The combination of CAL-B as biocatalyst, MTBE as solvent, 2.5 equiv. of

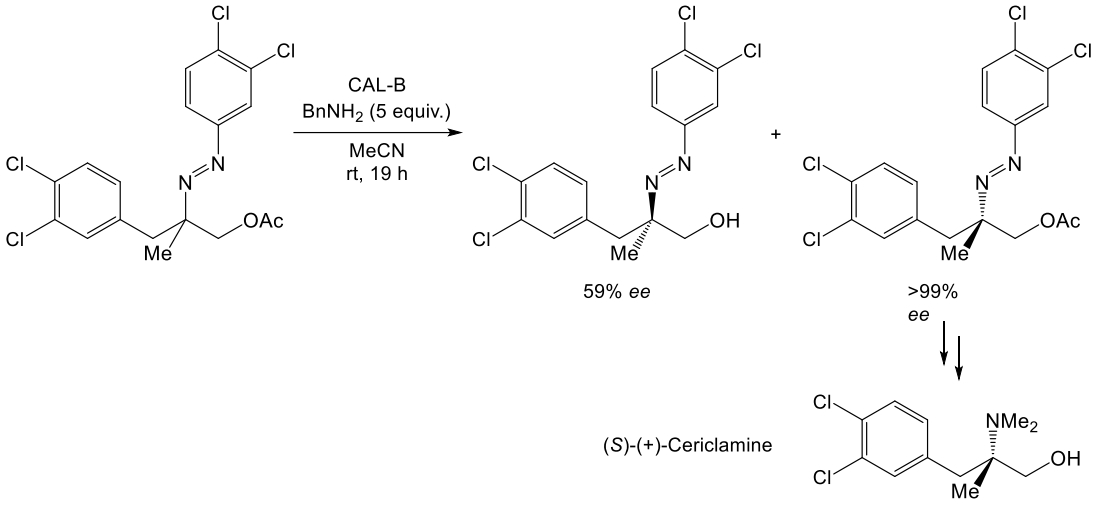
allyl phenyl carbonate and 5 equiv. of trietylamine ($Et_3N$) as additive, resulted in the formation of the (4a*R*,7a*S*)-carbamate and the (4a*S*,7a*R*)-amine with excellent selectivity (Scheme 32).




**Scheme 32.** Asymmetric alcoxycarbonylation of a Moxifloxacin intermediate using CAL-B and allyl phenyl carbonate.

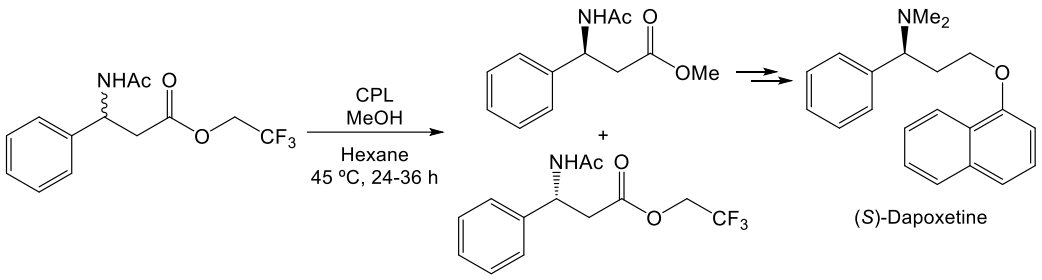
### 2.3.3. Other hydrolase-catalyzed asymmetric transformations.

Finally, inside the hydrolase-catalyzed asymmetric reactions, some less explored type of processes must be also mentioned. For instance, benzylamine has been used instead of water for the resolution of azo acetates, including a precursor of (*S*)-(+)-Cericlamine, a serotonin re-uptake inhibitor (Scheme 33).[110] Although overall the azo acetate is hydrolyzed to the corresponding alcohol, the reaction can also be considered as an aminolysis due to the ability of benzylamine to form *N*-benzylacetamide, which is generated as byproduct. The reaction occurred with low selectivitiy, yielding the (*S*)-acetate in enantiopure form and the (*R*)-alcohol in 59% *ee* after 19 h.



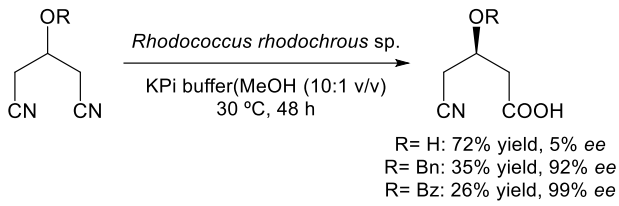

**Scheme 33**. Synthesis of (*S*)-(+)-Cericlamine including an aminolysis reaction of an azo acetate intermediate with benzylamine.

In a previous section the synthesis of (*S*)-Dapoxetine was achieved using a hydrolytic procedure over a methyl ester, providing access to a valuable carboxylic acid intermediate.[57] Alternatively, Wei and co-workers reported an alcoholysis reaction of a 2,2,2-trifluorethyl ester using 10 equiv. of methanol and the *Carica papaya* lipase (CPL) as biocatalyst (Scheme 34).[111] After an exhaustive optimization study, the resolution occurred with complete selectivity in hexane, yielding the enantiopure remaining (*R*)-trifluorethyl ester and the (*S*)-methyl ester in 50% conversion. The KR through esterification of carboxylic acids is also a versatile reaction that leads to the formation of esters and carboxylic acids in good optical purity. Ostazewski and co-workers reported the synthesis of 3-phenyl-γ-aminobutyric acid, a lipophilic analogue of GABA, the main neurotransmitter of the central nervous system.[112] To assure the irreversibility of the process orthoesters, acetals and ketals were tested as donors in the asymmetrization of 3-phenyl-4-pentenoic acid finding the best results with triethyl orthoacetate in toluene as solvent and using a broad panel of hydrolases at 40 ºC.




**Scheme 34**. Chemoenzymatic synthesis of (*S*)-Dapoxetine through asymmetric alcoholysis reaction.

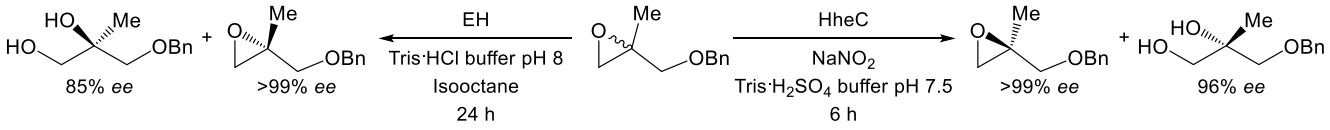
As mentioned in a previous section, the hydrolysis of nitriles into carboxylic is a very useful transformation catalyzed by nitrilases. Some examples dealing with the synthesis of interesting pharmaceutical fragments have been developed in recent years. For instance, the microorganism *Rhodococcus rhodochrous* SP 361 from Novozymes has catalyzed the formation of *O*-protected and unprotected (*R*)-3-hydroxy-4-cyanobutanoics (Scheme 35), an intermediate in the synthesis of Atorvastatin marketed by Pfizer as a calcium salt under the trade name *Lipitor*, drug used as HMG-CoA reductase inhibitor for lowering blood chlolesterol preventing cardiovascular diseases.[113] Another recurrent reaction is the formation of (*R*)-(–)-mandelic acid by hydrolysis of mandelonitrile, as described by Zheng and co-workers using the microorganism *Alcaligenes faecalis* ZJUTB10 immobilized in different supports.[114]




**Scheme 35**. Nitrilase-catalyzed hydrolysis of nitriles for the production of (*R*)-3-hydroxy-4-cyanobutanoic and *O*-protected derivatives.

Last but not least, haloalkane dehalogenases are presented as α/β-hydrolases that catalyzed the cleavage of carbon-halogen bonds.[115] The enantiocomplementary synthesis of both enantiomers of a

racemic epoxide has been achieved by using a halohydrin dehalogenase (HheC) from *Agrobacterium radiobacter* AD1 or complementary epoxide hydrolase from *Rhodococcus ruber* CBS 717.73 (Scheme 36).[116] The intermediates have been applied in the synthesis of both enantiomers of Chromanemethanol, related to Vitamin E chemistry.




**Scheme 36**. Enantiocomplementary synthesis of Chromanemethanol enantiomers using a halohydrin dehalogenase or an epoxide hydrolase.

**3. Conclusions.**

Enzymes play nowadays an outstanding synthetic role in organic chemistry, hydrolases providing useful solutions for the modification of a wide number of compounds for the synthesis of interesting final products. The natural role of hydrolytic enzymes (EC 3) is the hydrolysis of functional groups such as esters, amides, lactones, lactams, epoxides or nitriles. Nevertheless, in certain conditions hydrolases can catalyze the reverse reactions favoring the synthesis over the hydrolysis. The activity displayed by hydrolases has been here discussed, giving an insight about their broad substrate specificity, efficiency, robustness and scalability. Satisfactorily, their versatility has been demonstrated, providing access to active pharmaceutical ingredients and related compounds, in general with high isolated yields and good selectivities. Many different transformations have been presented, such as acylation, alkoxycarbonylation, aminolysis, hydrolysis, interesterification and transesterification, among others. Their commercial availability and simplicity of use have allowed the integration of hydrolases in industrial processes inclusively at an industrial level. However, there is still chance to improve these results by an appropriate modification of the enzymes in order to overcome some limitations regarding their catalytic activity, stability and specificity. Thus, immobilization, chemical modification and directed evolution techniques are bringing to the market more efficient biocatalysts,

which hopefully will cover the demand of the industrial sector in future years by means of the development of single or multienzymatic transformations.

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