Lipase enzymes for sustainable synthesis of chiral active pharmaceutical ingredients (API) and key starting materials.

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Abstract

Biocatalysis is considered as a green and sustainable process for synthesis of chiral organic building blocks and active pharmaceutical ingredients (API). Lipase catalysed kinetic and kinetic dynamic resolution process is frequently used for in API or KSM synthesis. This review describes the reported synthesis of chiral API/KSM molecules using lipase enzyme, since last 10 years.

Introduction

The United Nations sustainable development goals are precious in pointing out critical areas of concern that must be addressed immediately to shift away from the unsustainable track we are currently on. No sustainable aim can be achieved unless the basic chemistry that forms our society's energy foundation and material is renewable instead of depleting, restorative instead of degrading, and remedial instead of toxic[1]. Green chemistry is an organized approach associated with sustainability used globally in industry and academia. It has produced sound knowledge and served as a crucial scientific basis for the adjustments to sustainability [2,3].

Concern over the massive volumes of waste materials generated by the chemical industry was on the rise. The conventional ideas for reaction selectivity and efficiency, which primarily emphasize chemical yield, clearly needed to give way to a fundamental change focusing more on maximizing the use of raw materials, cutting waste, and avoiding using hazardous materials and toxic substances. A cleaner, waste-free, more resource-efficient alternative was desperately required [4]. In the manufacture of chemical products, green chemistry effectively uses raw resources that are renewable, hazardous, and toxic substances, which are highly avoided, also minimizing waste [5–8].

Over the last few years, a significant number of big pharmaceutical industries have shifted to employing green chemistry techniques for drug manufacturing and development. The realization that more environmentally friendly and less expensive methods provide a competitive edge is causing this change [9,10]. Biocatalysis/chemoenzymatic reactions are key innovations in the pharmaceutical sector since it offers an effective, sustainable catalysis that leads to production of active pharmaceutical ingredients (APIs)[11–13]. These enzymes are prepared either in their inherent cells or can be genetically expressed proteins by recombinant technology in other host cells. These enzymes can be utilized in both whole-cell and isolated preparations [14,15]. The pharmaceutical sector finds biocatalysis to be a captivating technology for various reasons. Enzyme-mediated catalysis satisfies the growing need for sustainable industrial developments. Enzymes can catalyze reactions under mild reaction conditions. They can transform reactions deprived of protection-deprotection steps without changing the current functionality [16,17]. Enzymes meet the fundamental principles of green chemistry since they are prepared from inexpensive, renewable sources. As a result, their production costs are practically constant and easily suited for economic simulation. In contrast, the substantial environmental costs associated with unearthing rare precious metals and the unsettling price variations brought on challenging demand from other industries [18–21] APIs are difficult to synthesize as they involve inserting and modifying functional groups and stereogenic centers. In most cases, synthetic procedures need to follow protection-deprotection approaches and multiple steps. As a consequence of this, there is a significant amount of waste generated in APIs manufacturing (E factor>100) [22,23]. Biocatalysis drastically reduces the length of the multi-step synthetic approach; also, with optimized enzymes, this technology shows excellent regio, stereo-, and enantioselectivity [24,25]. The requirement of enantiopure drugs leads to significant growth in this sector. Primarily, esterases and lipases were used to synthesize chiral molecules, but later, the range of biocatalysts that were extended to include nitrilase, transaminases, ketoreductase, imine reductase, oxidoreductase, etc [26]. Since biocatalysis has been cost-effectively used for pharmaceutically active compounds and many bestseller drugs such as sitagliptin, boceprevir, montelukast, atorvastatin, pregabalin have been successfully synthesized at the industrial level [27–31]

Fig 1: Benefits of Biocatalysis

The most widely employed enzymes in biotechnological applications globally are lipases. Lipases (EC.3.1.1.3) are members of the hydrolase class of enzymes that perform the dual functions of synthesizing long acyl chains and hydrolyzing acylglycerols [32,33]. Lipases have diverse applications, including producing fine chemicals, detergents, biofuel, and polymers. It is also used in bioremediation, clinical diagnosis, the food industry, agriculture, etc [34,35]. Apart from these, lipases are used in the cosmetics and leather Industries [36,37]. Lipases are versatile in catalyzing different types of reactions like hydrolysis, esterification, transesterification, acidolysis, alcoholysis. Lipases are unique in kinetic resolution of racemic compounds to transform into their enantiopure forms [38–41]. This review will focus on implementing the chemoenzymatic approach catalyzed by lipase to synthesize several building blocks of APIs for the last ten years.

Chemoenzymatic synthesis of analgesics/non-sterodal anti-inflammatory drugs (NSAID)

Synthesis of (S)-Ibuprofen

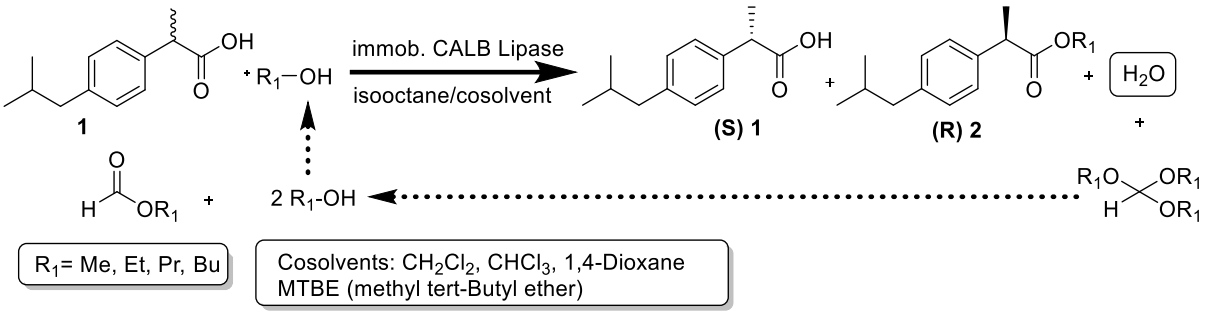
Research on preparing 2-aryl propionic acids (profens) is still advancing rapidly. It is commonly known to underpin the therapeutic efficacy of profens, which is the ability to inhibit cyclooxygenase to diminish prostaglandin synthesis. The eutomer exerts this is (S)-(+)-enantiomer. Remarkably, Ibuprofen is involved in the phenomenon known as chiral switch.

That involves substituting a chiral drug, used as a racemate, either partially or entirely with its eutomer. For this drug, the pharmaceutical market offers both its racemic and enantiopure forms[42]. Therefore, the necessity of the (S)-(+)-enantiomer's pharmacological applications and the fact that synthesizing enantiopure form is more expensive than producing racemate are the main reasons for the significant efforts made to produce enantiopure (S)-profens with biocatalysis approach being one of the recommended options.

In recent research on the synthesis of Ibuprofen, enzymatic kinetic resolution of Racemic Ibuprofen takes place using biocatalyst from immobilized lipase B of *candida Antarctica*. To significantly improve the enantioselectivity of (S)-2- (4-isobutyl phenyl) propanoic acid (ibuprofen eutomer), orthoesters were used where it plays a role as water trapper/ alcohol releaser molecule as well as removing extra water from the reaction medium that permits the reaction to be reversible which is one of the biggest problems with biocatalysis in organic media has resolved. Also, utilizing these acyl donors in the presence of an organic co-solvent improves enantiomeric excess and enantioselectivity after reaction conditions are optimized.

Since the esterification process is inherently reversible, it necessitates an extension of the conversion time (with a reduction in chemical yield) to achieve high optical purity of the non-converted (S)-enantiomer. Additionally, to encourage the hydrolysis of the generated ester, water retention on the media tends to produce a water layer on the biocatalyst, which assists enzyme inactivation and degrades enzyme performance. Using extremely hydrophobic supports for enzyme immobilization has lessened this adverse effect. Implementing irreversible procedures to prevent all the previously mentioned unwanted effects associated with kinetic resolution of racemic profens appears exceptionally reasonable. In this regard, using orthoesters is a wise move since they hold the water molecules created as esterification proceeds, which causes hydrolysis and constantly releases the alcohol needed for esterification. It is known that another method for enhancing the enantioselectivity of lipases in both hydrolysis and esterification processes with profens is the inclusion of a moderately polar cosolvent along with the primary solvent. In the acyl transfer process, it has been observed that apolar solvent is entirely compatible with lipases. Preliminary experiments in KR of racemic ibuprofen with triethyl orthoformate (TEOF) were conducted to determine the impact of adding cosolvents to the conventional isooctane. Different alkyl orthoformates, such as trimethyl orthoformate (TMOF), triethyl orthoformate (TEOF), and tripropyl orthoformate (TPOP), were tested with low concentrations of different alcohols, i.e., (MeOH, EtOH, n-propanol) for examining the effect of linear alcohols. Among them, TEOF is considered the best choice.

Different amounts of enzyme (CAL B) were administered at various increasing ratios (1/1, 1.5/1, 2/1: w/w enzyme/ibuprofen) with the racemic ibuprofen (concentration C_0 = 10.31 mg/ mL. Even though the reaction rate increased along with the amount of biocatalyst, there was an overall diminution of enantioselectivity.

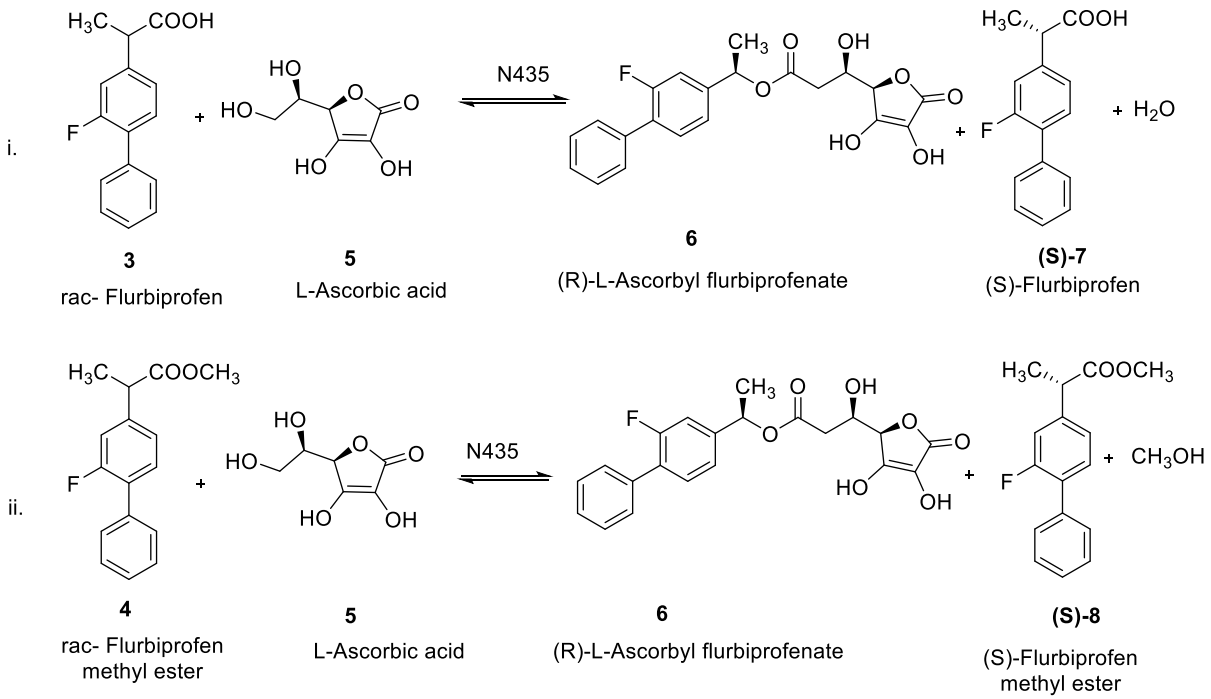


Scheme 1: Irreversible esterification of racemic Ibuprofen using Orthoformates by biocatalysis

A considerable improvement in selectivity was made possible by employing an apolar solvent and chlorinated polar cosolvent (80/20: v/v mixture) for dichloromethane (DCM) and chloroform. Additionally, 1,4-dioxane showed a sluggish reaction with a slight increase in enantioselectivity. Compared to the other cosolvents, tert-butyl methyl ether (MTBE) speeds up the process, albeit at the expense of enantioselectivity. The E-value attained with 20% DCM in isooctane is higher than any reported value (up to E = 31.8). The optimum reaction conditions were at 40 °C for 24 hrs at 250 rpm (scheme 1). The concentration of (R) and (S)-Ibuprofen were ascertained from the HPLC peak area of racemic ibuprofen. [43]

Synthesis of L-Ascorbyl Flurbiprofenate catalysed by lipase enzyme

In the search of a synthetic route for L-ascorbyl flurbiprofen, lipase-catalyzed transesterification and esterification both methods have been developed by Jia-ying Xin and his research team. Racemic flurbiprofen **(3)** or racemic flurbiprofen methyl ester **(4)** were used for lipase-catalyzed acylation of L-ascorbic acid **(5)** for esterification and transesterification, respectively.



Scheme 2: Lipase catalyzed esterification of racemic flurbiprofen and transesterification of racemic flurbiprofen methyl ester

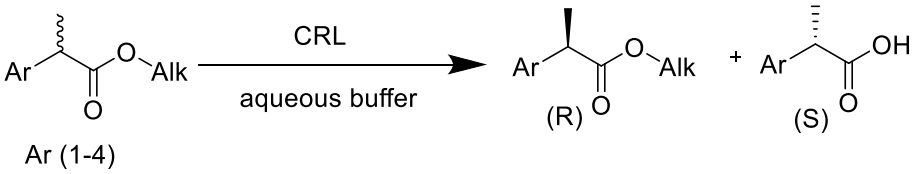
Only L-ascorbyl flurbiprofenate was produced by both reaction types according to HPLC analysis. From chiral HPLC analysis, it has been observed that lipases exhibit stereospecificity for the esterification and transesterification reaction. The (S)-isomer of flurbiprofenate interacted with lipase from *Candida rugosa* and PPL but could not react for catalysis. In contrast, Novozym 435 (N 435) lipase was reacted with (R)-isomer. different solvents evaluated for esterification and transesterification reaction such as acetone, ethyl acetate, tert-butanol, benzene, chloroform, tert-amyl alcohol, and tert-butanol gave the best result. The reaction was conducted at 50 °C for 72 hrs with 2.3 mmoles of flurbiprofen or flurbiprofen methyl ester; optimum amount of catalyst loading was 55 mg (scheme 2). For flurbiprofen, apparent Michaleis-Menten kinetics were observed as K_{rn} = 0.29 mol/L and apparent V_{max} = 0.563 mmol/L.h. For flurbiprofen methyl ester, apparent Michaleis-Menten kinetics was observed as K_{rn} = 0.14 mol/L and apparent V_{max} = 1.34 mmol/L. From these, it has been demonstrated that Novozym 435 had a decreased affinity for flurbiprofen and a higher affinity for flurbiprofen methyl ester. Compared to esterification, transesterification has a faster reaction rate.[44]

Lipase catalysed synthesis of (S)-enantiomer of naproxen.

One of the important NSAIDs is naproxen. The (S)-enantiomer of naproxen is more powerful than its (R)-enantiomer. Due to the hepatotoxicity of this enantiomer, it creates problems with renal clearance. That's why naproxen needs to be administered as a single enantiopure form[45]. As already mentioned, (R)-ibuprofen shows metabolic chiral switch as in the case of (R)-ketoprofen chiral switch is limited to 10% only[42]. Only (S)-flurbiprofen is a more potent inhibitor for the biosynthesis of prostaglandin.

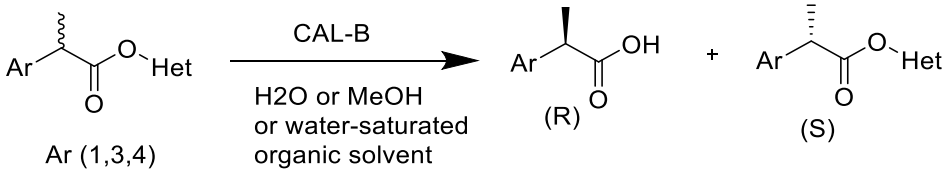
Biocatalytic techniques have been an essential and adaptable tool for the asymmetric synthesis of optically active NSAIDs over the past few decades. Here is a list of some remarkable instances among them:

i. The enantioselective hydrolytic kinetic resolution (KR) of the suitable racemic esters utilizing lipases from Candida rugosa (CRL, formerly Candida cylindracea (CLL)) and pig pancreas (PPL) or engineered Yarrowia lipolytica (Lip2p), respectively (scheme 3) [46,47].



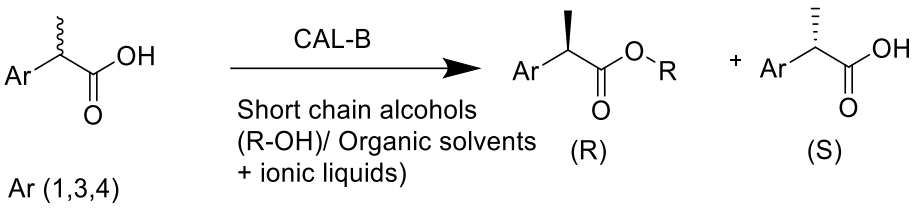
Scheme 3: Hydrolytic KR of racemic NSAIDs-esters.

ii. Hydrolytic KR was attempted with racemic NSAID-azolides; lipase B from Candida antarctica (CAL-B) outperformed other biocatalysts and catalyzed the reaction with an opposite (R)-stereo preference to the previously reported enzymes (scheme 4) [48].



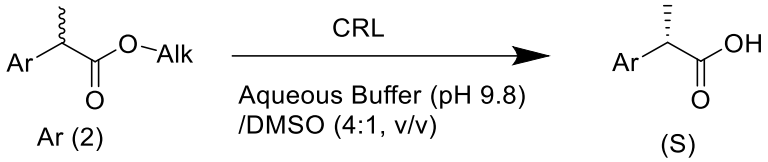
Scheme 4: Hydrolytic KR of racemic NSAIDs-azolides.

iii. Enantioselective esterification of racemic profens is a reversed enzymatic kinetic resolution process primarily catalyzed by CAL B and occasionally by CRL utilizing short-chain aliphatic alcohols (scheme 5) [49,50].

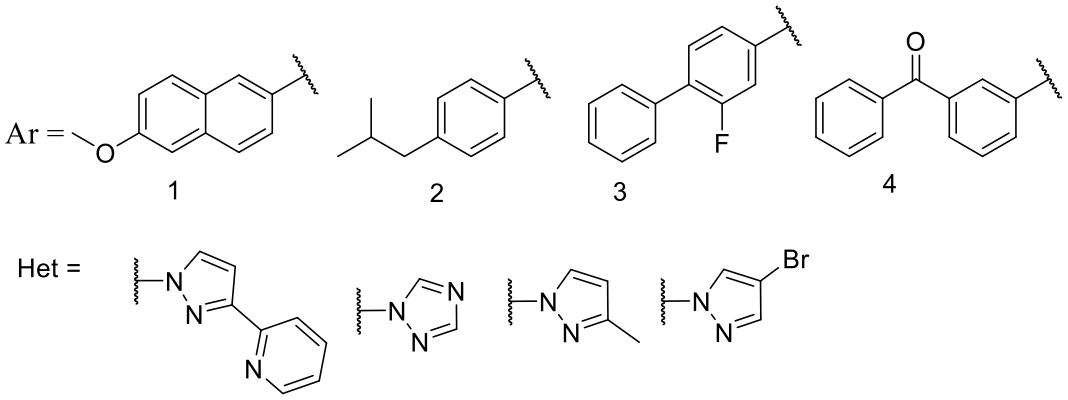


Scheme 5: KR of racemic NSAIDs via esterification.

iv. In an effort to find deracemization and/or desymmetrization methods that are more effective than conventional kinetic resolution, which is to 50% yield in theory. Candida rugosa lipase was used to create the hydrolytic dynamic kinetic resolution (DKR) of the methyl ibuprofen ester in a solution of an aqueous buffer (pH 9.8) and DMSO (4:1, v/v) suspension (scheme 6) [51].



Scheme 6: Hydrolytic DKR of methyl ibuprofen ester.



ENZYMES:

CRL- Lipase from Candida rugosa

CAL-B- Lipase B from Candida Antarctica

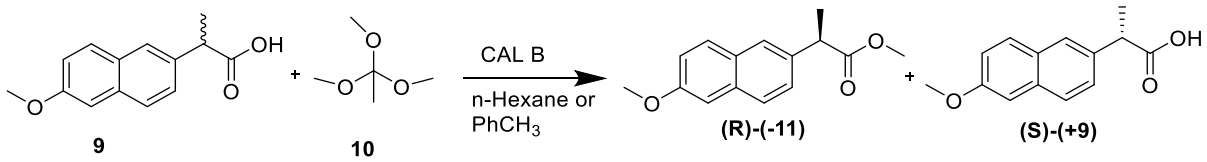
Substituents:

Alk- alkyl

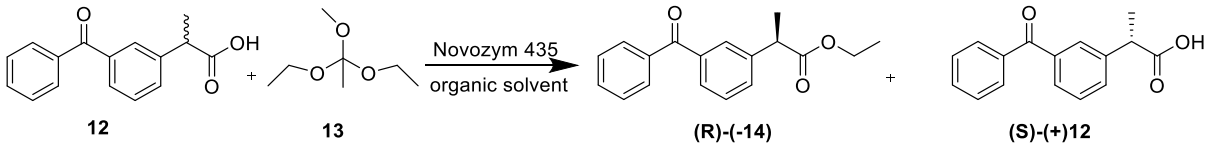
Ar- aryl, Het- hetero

The most frequent biocatalytic process involves enantioselective esterification of corresponding carboxylic acids using alkoxy donors, which are generally low molecular weight alcohols such as methanol or ethanol. Another biocatalytic process involves the hydrolytic kinetic resolution of racemic alkyl 2-arylpropanoates catalyzed by lipase in aqueous buffer.

However, transesterification utilizing short-chain alcohols reduces the stability of lipase and lowers the reaction rate [52,53]. In comparison, arduous extraction procedures hamper hydrolytic kinetic resolution. The extension of traditional lipase-catalyzed enantioselective esterification of the racemic profens utilizing kinetic resolution conditions and trialkyl orthoesters as irreversible alkoxy donors in organic solvents have been presented in this research work. In this present work, a chemoenzymatic pathway for synthesis of enantiopure dextro isomer of non-steroidal anti-inflammatory drug (NSAIDs) named naproxen, (S)-(+)-2-(6-methoxynapthalen-2-yl)propanoic acid **[(S)-(+9)]**, ketoprofen (*S*)-(+)-2-(3-benzoyl phenyl)propanoic acid, **[(*S*)-(+12)]**, has been developed.



Scheme 7: Lipase-catalyzed enantioselective resolution of racemic naproxen using trialkyl orthoesters in organic solvents.



Scheme 8: Lipase catalyzed enantioselective resolution of racemic ketoprofen

The chemoenzymatic step was carried out in 87 µmol scale at 40 °C, for 24 hours. 10 mg of enzyme (50% w/w referring to racemic substrate **9** and **12**) and three equivalents of trialkyl orthoacetate were reacted in solvent toluene (scheme 7 & 8). Screening of enzymes served as the first step in the enzymatic kinetic resolution of profens. There was no discernible reaction progress for the lipases from *Candida antarctica* type A (CAL-A, Chirazyme L-5), *Geotrichum candidum* (Chirazyme L-8), *Pseudomonas fluorescens* (Amano AK), *Burkholderia cepacia* (Amano PS-IM, PS-Immobead 150, Amano PS), *Aspergillus niger* (Amano A) and esterase from the porcine liver (PLE). Conversely, lipase type B from *Candida antarctica* gave the best result. HPLC analysis was used to measure the enantiomeric excess for the resolution product.

Furthermore, the most widely used heterogeneous biocatalyst lipase B from candida Antarctica adsorbed on polymethacrylate beads (Novozym 435®) for the enantioselective esterification

of racemic alcohols and acids, has undergone recent studies that confirm that short chain alcohols cause conformational changes followed by CAL B aggregation and alter the solid support's texture, encouraging the release of enzymes [54]. Long-chain fatty alcohols are used as an alternative to short-chain alcohols to overcome their limitations [54]. Since more large molecules diffuse slowly at the active site of the lipase, the enzymatic reaction rates are markedly reduced. Moreover, due to the increase in the length of the aliphatic chain, alcohol loses some of its nucleophilic properties. Consequently, another method for reducing the negative effect of short-chain alcohols is adding stepwise and solubilizing in tert-butanol [55,56].

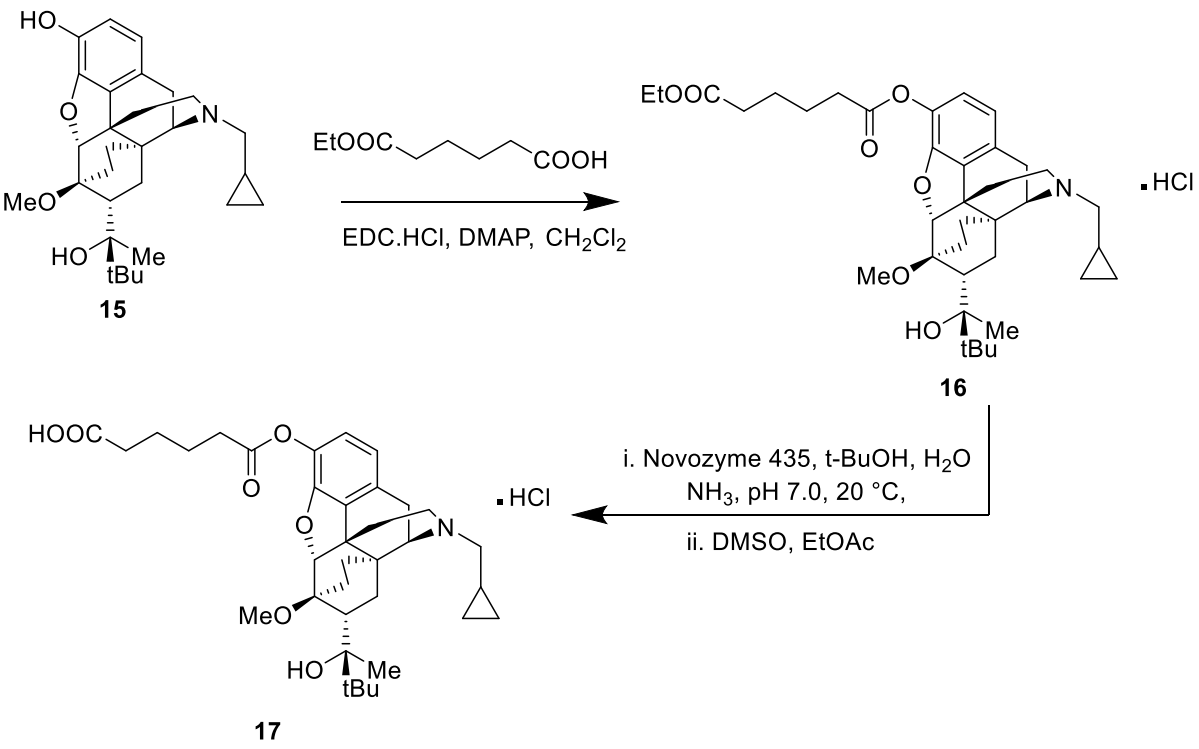
No matter the modifications applied, the problem of esterification employing alcohols is that there is no surety about the reaction's irreversibility because the forming water and the reaction's equilibrium are unfavorable.

Conversely, orthoesters are advantageous over conventional alkoxy donors. Lipase can catalyze esterification without enzyme inactivation, and this helps to preserve the irreversibility of the reaction by consuming water formed in situ, which simultaneously assists in releasing alcohol for the esterification[57].

The use of trialkyl orthoesters as irreversible alkoxy group donors in lipase-catalyzed kinetic resolution to get required eutomers with moderate- to high enantiomeric purity. Enzymatic kinetic resolution of racemic naproxen **(9)** gave **(S)-(+9)** in 63% ee. The measured specific rotation value observed for **(S)-(+9)** was $[\alpha]^{D}_{29}$ = +16.0 (c 0.50, $CHCl_3$). Whereas in the case of (S)-ketoprofen, (S)-(+12) was obtained in 69%ee. The specific optical rotation value for **(S)-(+12)** was $[\alpha]^{D}_{23}$ = +7.4 (c 0.61, CH_3OH). [58]

Buprenorphine pro-drug synthesis by biocatalysis

A method has been developed with a different pathway leading to a prodrug of buprenorphine containing hemiadipic acid. First, acetylation of buprenorphine was done using adipic acid monoethyl ester, followed by enzyme-catalyzed regioselective ester hydrolysis. Despite the presence of phenolic ester in the structure, which is more prone to hydrolysis, Enzymes selectively hydrolyzed less hindered alkyl ester. The previously reported method of synthesis for this drug is used to produce byproducts in 2:1 ratio and elimination of the excess byproduct and adipic acid at the completion of the reaction was presented a substantial hurdle.



Scheme 9: Synthetic route for prodrug of buprenorphine

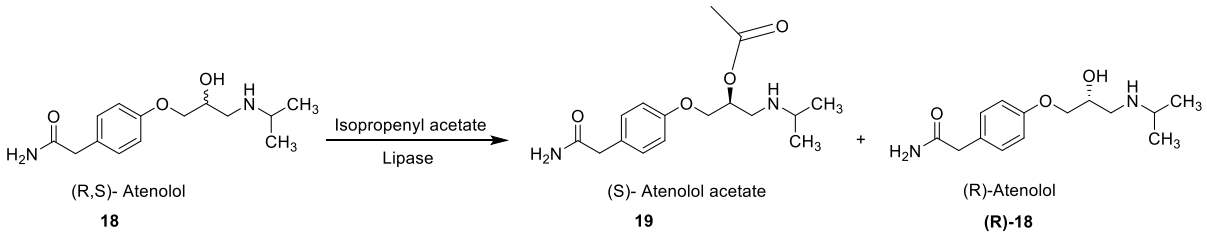
For conversion of ester to acid, a screening of enzymes for hydrolysis was conducted. Without significantly hydrolysing the phenolic ester, Candida Antarctica lipase B was found to be an appropriate enzyme capable of carrying out hydrolysis of ethyl ester selectively. Based on past experience, Novozyme 435 was used as a supported version of C.antartica lipase B. The reaction was performed at 20°C, for 24 hrs at pH 7.0 in aqueous t-BuOH (scheme 9). pH of the reaction was maintained by constant addition of aqueous NH_3. The intermediate **(17)** was isolated in 84% yield and HPLC purity of the intermediate was high, also 2:1 adduct was less than 0.1%. The overall yield from buprenorphine **(15)** to intermediate **(17)** was 75%. [59]

Synthesis of antihypertensive and cardiovascular drugs

Chemoenzymatic synthesis of β-blocker drug Atenolol

The β-blockers are important in management of a ranges of diseases in people. Since all β-blockers have chiral carbon in their structure, they all are enantiomers. Despite numerous research studies describing the (S)-enantiomers of these APIs monitor the intended therapeutic effects, β-blockers are nonetheless commercially available as racemates. However, substituting a racemic form of a drug into an optically pure enantiomeric form results in fewer side effects [60]. Numerous methods can be used to prepare enantiomerically pure β-blockers to accomplish this goal, together with chemical synthesis.

Adam Sikora *et.* Coworkers have investigated the kinetic resolution of atenolol by chemo-enzymatic method. The enantioselective acylation of racemic atenolol has been done by using lipase from *candida Rugosa* (MY and OF) in presence of ionic liquids. Due to (R, S)-atenolol's severely constrained solubility in a variety of organic solvents, the experiments were carried out in a two-phase system containing toluene and various ionic liquids, which provides an opportunity for the substrates and products to be separated from the catalytic system easily. This is plausible because the acetylated form of atenolol and its deacetylated counterpart only existed in the ionic liquid phase. Only [EMIM][BF_4] was competent for the enantioselective transesterification of racemic atenolol among all studied ionic liquids, including [EMIM][$EtSO_4$], [EMIM][OTf]. Using biotransformation, a 'trojan horse' strategy can be implemented, which involves repurposing the enzyme in a different catalytic system by easily substituting particular substrates and products for the ionic liquids. The catalyst has been reused up to 5th reaction cycle.



Scheme 10: Enantioselective transesterification of racemic atenolol

Biocatalyst (10 mg), racemic atenolol (3 mg), acetylating agent isopropenyl acetate (2µL) were taken for the reaction. Ionic liquid (500 µL), and toluene (10 mL) served as reaction media for the experiment (scheme 10). The biotransformation reaction was continued for 240 hrs of incubation at 30 C. Enantiopure (S)-atenolol acetate was produced with ee_p = 95.23% and E value 56.07. The chiral resolution of Racemic atenolol **(18)** and its acetylated form **(19)** has been analyzed by chiral HPLC.[61]

Enantioselective synthesis of (*S*)-Bisoprolol

Another β-blocker drug that is being used for heart failure and hypertension treatment is Bisoprolol (fig 2). This is one of the top ten medicine prescribed in the USA and mostly it is available as hemifumarate salt. Synthesis of β-blocker, (S)-Bisoprolol hemifumarate, has been done in six steps with 96% enantiomeric excess and 19% total yield by Egholm Jacobseny's research team. Kinetic resolution of racemic chlorohydrin by using CAL B as the chiral catalyst

allows for the production of chiral chlorohydrin building blocks in high enantiomeric excess to synthesize (S)-Bisoprolol.

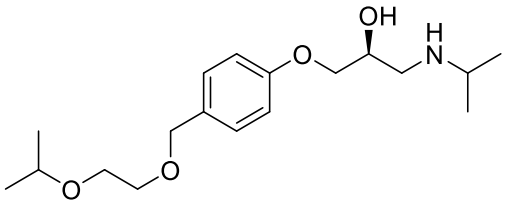
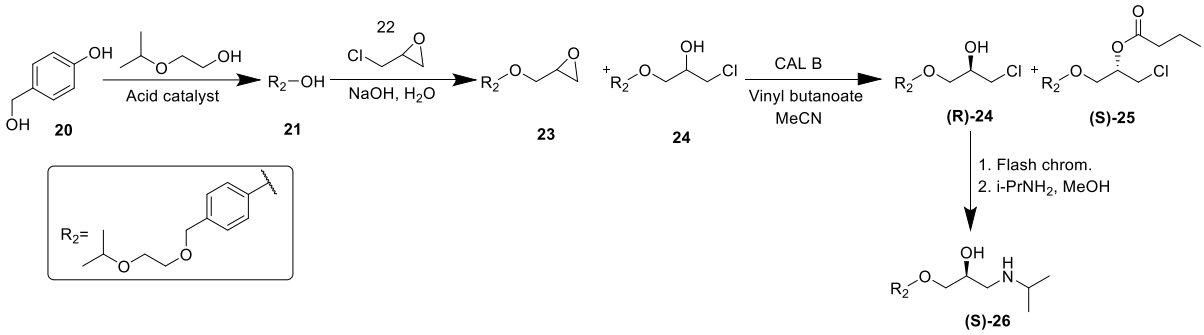


Fig 2: Structure of (S)-bisoprolol



Scheme 11: Chemoenzymatic Synthesis of (S)-Bisoprolol

The kinetic resolution of chlorohydrin **24** was catalyzed by Lipase B from Candida Antarctica (CAL B) in dry acetonitrile (scheme 11); as the acyl donor, vinyl butanoate was used where they got an E value of 52 (determined by E&K calculator, 2.1 b0 PPC) with 99% enantiomeric excess for **(R)-(24).** These kinetic resolutions using acetonitrile, are becoming more environmentally friendly and making greener synthesis than earlier processes where the reaction was done using toluene for maintaining high enantio-selectivity in the production of **(R)-24** [62]. Further, (S)-Bisoprolol was synthesized from (R)-Chlorohydrin **(R)-(24)** by using isopropylamine in methanol with 91% yield. Specific rotation for Chlorohydrin (R)**-(24)** in 99% ee observed was $[\alpha]_D^{20}$ = -17.0 (C 1.0 in methanol). Specific rotation for (S) bisoprolol hemifumarate **S-(25)** observed was $[\alpha]_D^{20}$ = -20.6 (C 1.0 in methanol).[63]

Synthesis of enantiopure (*S*)-Esmolol and (*S*)-Penbutolol

Hansen *et al.* coworkers have reported the synthesis of β-blocker drugs (*S*)-Esmolol and (*S*)-Penbutolol (fig 3) by chemo-enzymatic route following green synthesis protocols.

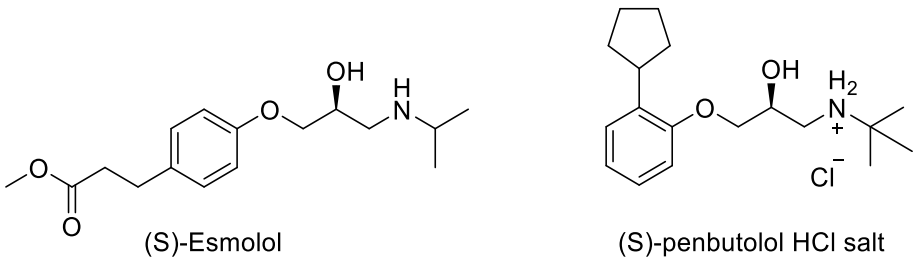
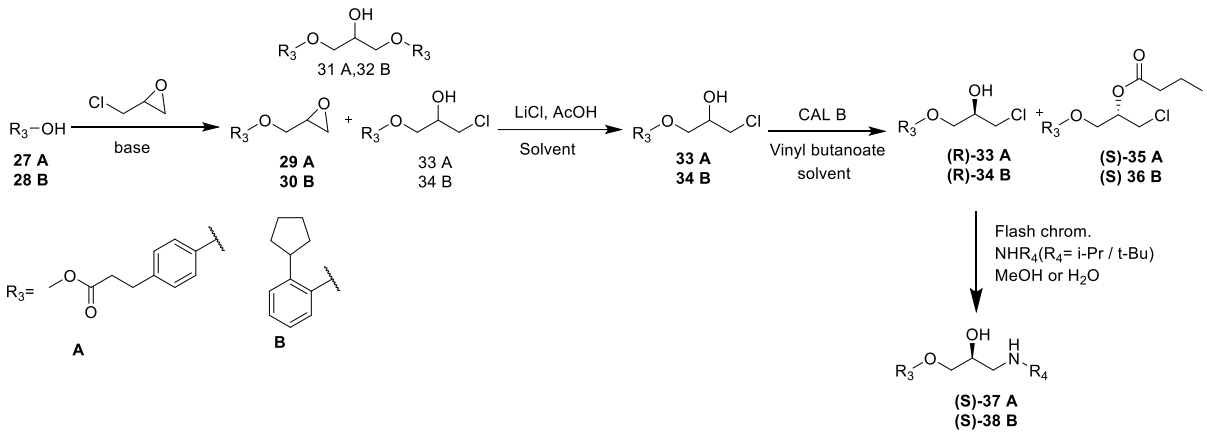


Fig: 3 Structure of (S)-Esmolol and (S)-penbutolol HCl salt

The synthesis of β-blocker (*S*)-esmolol has been reported using 4 steps process. The key step involves the transesterification of the racemic chlorohydrin, methyl 3-(4-(3-chloro-2-hydroxypropoxy)phenyl)propanoate **(33 A)** catalyzed by lipase B from *Candida Antarctica.* The synthesis resulted in 97% enantiomeric excess (ee), indicating a high level of enantiopurity of the desired enantiomer. The total yield of the synthesis was 26%.

The β-blocker (*S*)-Penbutolol was synthesized in 22% yield and in 99% enantiomeric excess. The synthesis involves the transesterification step of racemic chlorohydrin 1-chloro-3-(2-cyclopentylphenoxy)propan-2-ol **(34 B)** catalyzed by same lipase as used for esmolol.

Vinyl butanoate was used in the transesterification step as an acyl donor and dry acetonitrile as solvent. The lipase-catalyzed kinetic resolution was carried out for 23-48 hrs, and the temperature for this reaction was maintained at 30-38 °C (scheme 12). Kinetic resolution using acetonitrile as a solvent result in greener synthesis, while earlier publications used toluene.



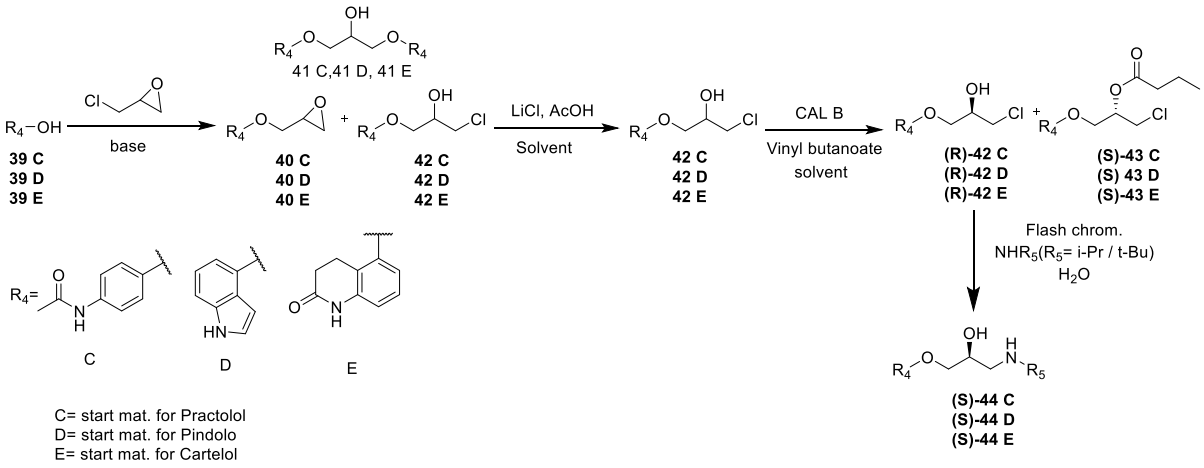
Scheme 12: Chemoenzymatic synthesis of (S)-esmolol (37 A) and (S)-penbutolol (38 B) with their chiral building block

These compounds have 50% yield limit because of the kinetic resolution step. However, by employing dynamic kinetic resolution, yield can be improved by reducing the waste

production. Specific rotation value observed for chlorohydrin **(*R*)-33 A** was $[\alpha]_D^{20}$ = -5.33 (C 1.6, i-PrOH) and **(*S*)- esmolol (S)-37 A** was $[\alpha]_D^{20}$ = -6.80 (C 1.03, $CHCl_3$). The specific rotation value for chlorohydrin **(*R*)-34 B** was found to be $[\alpha]_D^{20}$ = -14 (C 1.6, MeOH) and (S)-Penbutolol **(*S*)-38 B** $[\alpha]_D^{20}$ = -14 (C 1.0, MeOH).[64]

Lipase Catalyzed Synthesis of Practolol, Pindolol and Carteolol

Several β-blocker drugs have chlorohydrins in structure as building block. Synthesis of chlorohydrins for several β-blockers by chemoenzymatic route have discussed earlier. Now another sustainable chemoenzymatic method for β-blocker drugs like Practolol, Pindolol, and carteolol haven been developed with high enantiopurity. By kinetic resolution in transesterification reaction using CAL B, enantiopure chlorohydrins were synthesized. The synthetic approach for enantiomer of β-blocker drug practolol **(S)-43 C**, Pindolol **(S)-43 D**, and Carteolol **(S)-43 E** have been reported with shorter reaction duration, using less amount of reactant resulted in 92-97% ee (scheme 13).



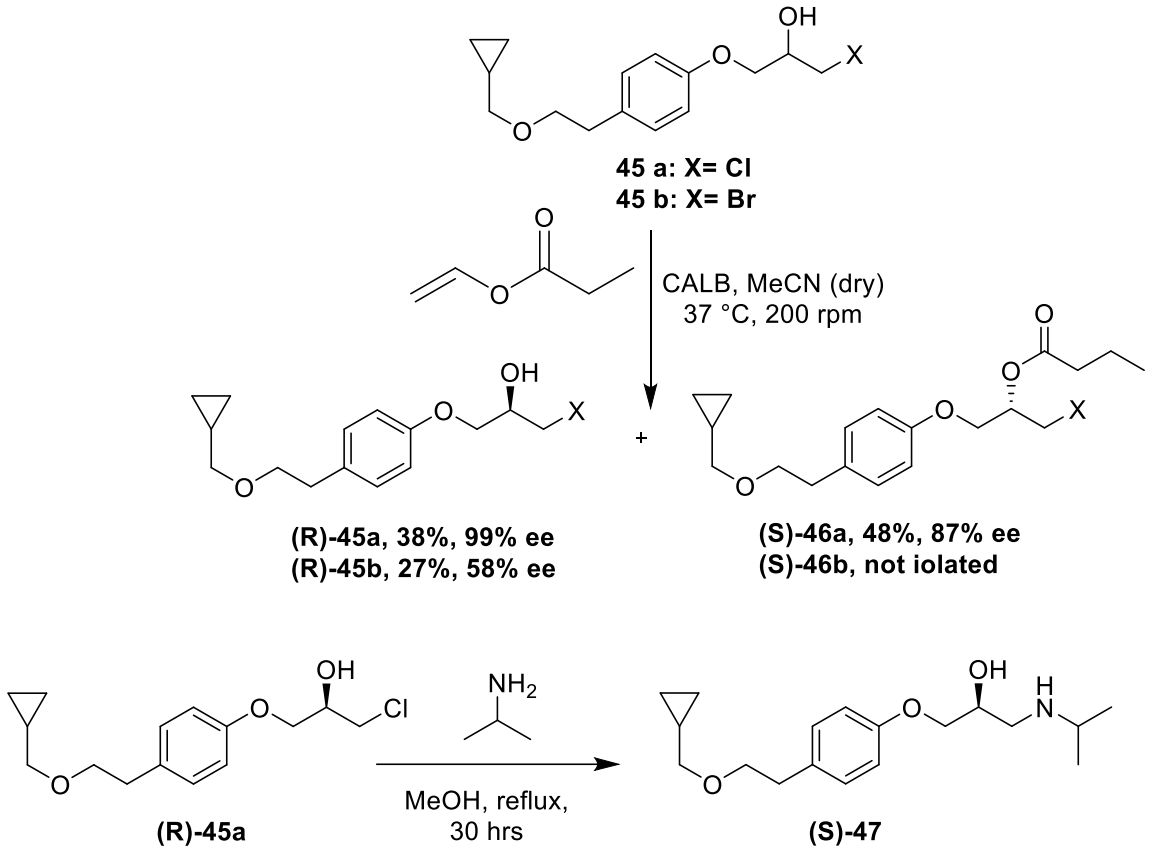
Scheme 13: Chemoenzymatic synthesis of (S)-enantiomers of Pracolol, Pindolol, and carteolol

The biocatalytic step have been performed by using CALB with vinyl butanoate which is a common acyl donor in biocatalysis in presence of various solvents. Kinetic resolution of **42 C** and **42 E** was carried out in dry acetonitrile (MeCN), whereas kinetic resolution of **42 D** was performed in dry DCM. The reaction condition for **42 C** and **42 D** was at 30°C for 24-26 hours. For **42 E**, the reaction was continued for 74 hours at 37°C. The (R)-enantiomer **(R)-42 C** was obtained in 97% ee, 38% yield, and the specific rotation value observed was $[\alpha]_D^{23}$ = -1.0 (C 1.0, i-PrOH). Further amination of **42 C** produced **(S)-44 C** [(S)-Practolol], in 96% ee and 16%

yield. Optical rotation for (S)-practolol **[(S)-44 C]** observed was $[\alpha]_D^{20} = -3.998°$ (C 1.0, EtOH). From the kinetic resolution step, **(R)-42 D** was obtained in 92% ee with E value = 66. Further, it was not taken for the amination step for (S)-Pindolol enantiomer **(S)-44 D**. **(R)-42 E** was obtained in 96% ee, 38% yield from kinetic resolution. Specific rotation observed for **(R)-42 E** was $[\alpha]_D^{20} = -9.9$ (C 1.0, DMSO). **(R)-42 E** was further reacted with tert-butyl amine to form (S)-enantiomer of Cartelol **(S)-44 E** with 96% ee and 70% yield [65]

Enantioselective synthesis of (*S*)-Betaxolol

Another β1-receptor antagonist drug, Betaxolol has been synthesized by chemoenzymatic route. The (S)-enantiomer of betaxolol is the most active in showing anti-hypertensive effect. Synthetic precursors of betaxolol are halohydrins 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol **(45a)** and 1-bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol **(45b).** First these synthesis precursors have been synthesized, later theses have been taken for enzymatic catalysis. For enantioselective transesterification of **45a** and **45b** enzyme used is CAL B in solvent dry acetonitrile with acyl donor vinyl butanoate. The reaction was continued at 37 °C in an incubator for 14 hrs at 200 rpm (scheme 14). Enantiopure **(R)-45a** obtained in 99% ee with 38% conversion. The counterpart butanoic ester **(S)-46a** was obtained in 87% ee, with 48% conversion. The bromohydrin with CAL B gave much lower result than **45a.** Further enantiopure **(R)-45a** was treated with isopropyl amine for amination in methanol to produce enantiopure (S)-betaxolol **[(S)-47]** with 99% ee and 95% yield.



Scheme 14: Enzymatic transesterification of 45a

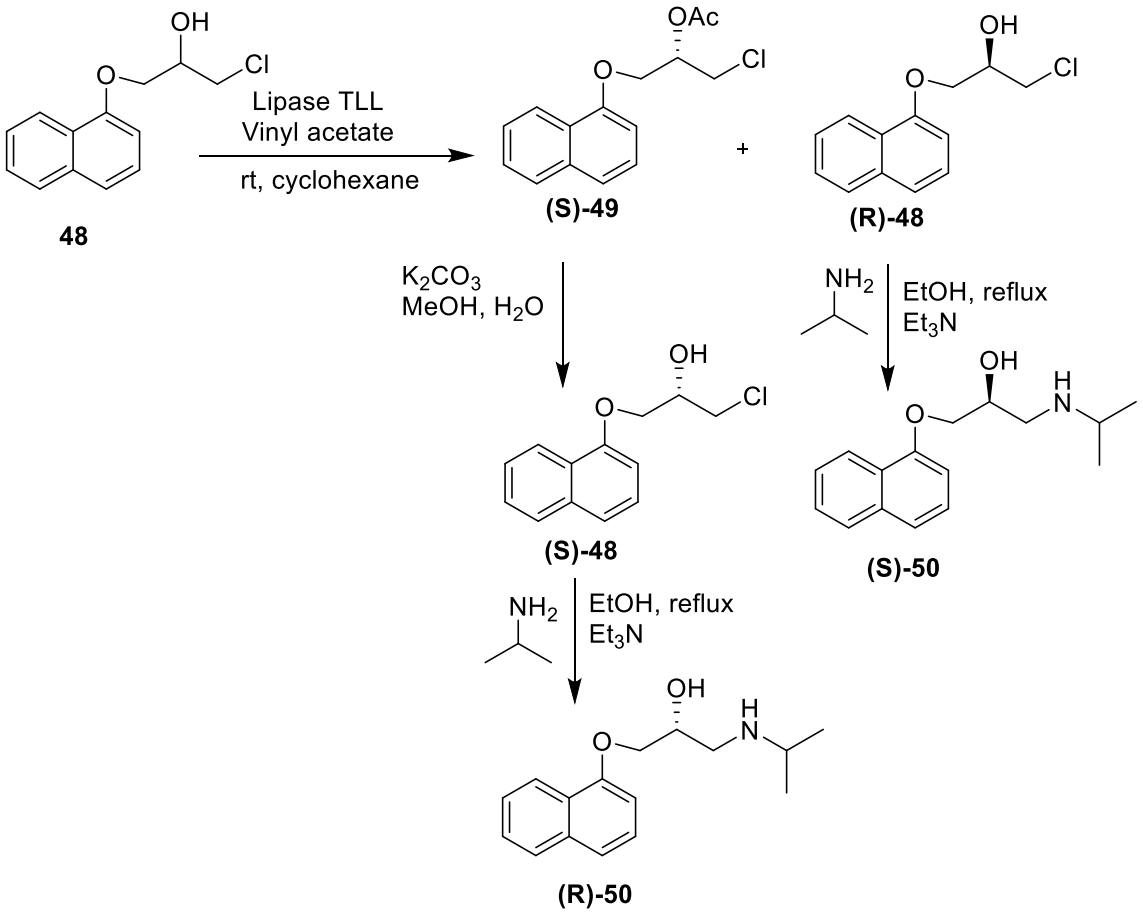
Specific rotation value observed for **(R)-45a** was $[\alpha]_D^{22}$ = -1.92 (c 1.04, $CHCl_3$) and specific rotation for (S)-betaxolol **(S)-47** was observed that $[\alpha]_D^{22}$ = -7.21 (c 0.97, $CHCl_3$).[66]

Lipase-catalysed synthesis of propranolol

Biocatalytic methods are becoming more environment-friendly and safer for the manufacture of drugs and drug intermediates. Most compounds' biological activity is directed by their stereochemistry. The biological activity of β- adrenergic receptor blocker 3-(aryloxy)-1-(alkylamino)-2-propanolol resides on the (S)-isomer. (S)-propranolol is 98 times more potent than (R)-Propranolol. (R)-Propranolol serves as contraceptive [67].

Santhosh Nayak Kethavath *et.* coworkers reported the synthesis of β- adrenergic receptor blocker drug, propranolol, by the chemoenzymatic method. Utilizing enzymatic kinetic resolution, a green synthetic pathway has been considered for this synthesis. **Racemic 48** (1-chloro-3-napthalen-1-yloxy) propan-2-ol) was prepared in a two-step reaction and has been taken for further enzymatic step. Transesterification of racemic alcohol **(48)** for kinetic resolution has been done by utilizing lipase addzyme 001 and vinyl acetate as an acyl donor. Various commercially available enzymes, such as sigma L4447, Addzym 002, Amano CES L-

7, Addzyme 001 were evaluated for the experiment. Among them best result has been obtained by Addzyme 001. To improve the enantiopurity and reaction rate a number of reaction parameters were optimized including solvent, temperature, reaction time. Several solvents have been studied for the reaction such as hexane, pentane, acetonitrile, DCM, chloroform cyclohexane. The highest enantiopurity was observed with cyclohexane. The optimum temperature for the lipase catalyzed kinetic resolution was found to be 40 °C and the reaction time was 48h (scheme 15). The highest conversion rate of the reaction was observed to be 49%. Enantioselectivity was observed as ee_p =98% **[(S)-49]**and ee_s = [97% **(R)-48].**

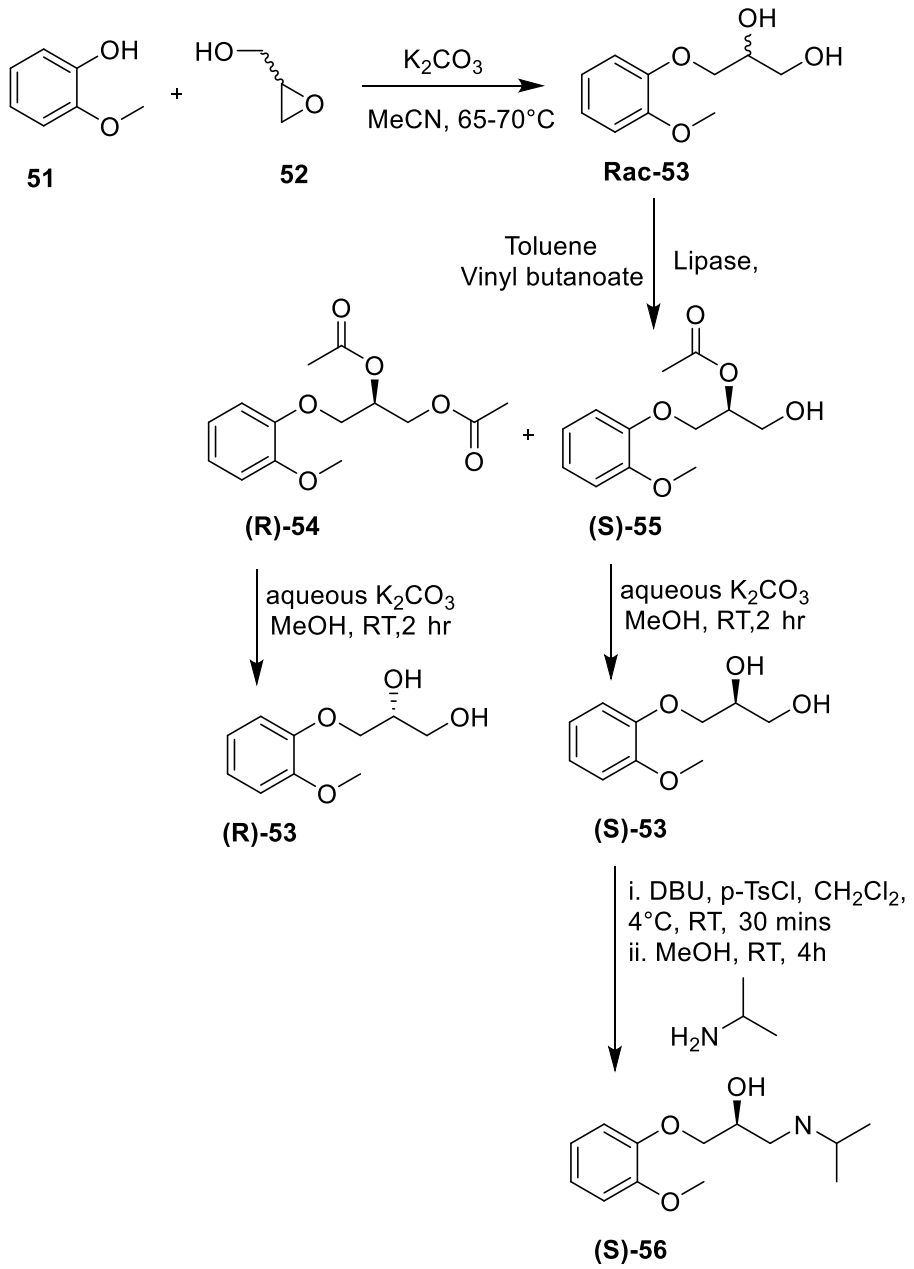


Scheme 15: Chemo enzymatic synthesis of (R)- and (S)-Propranolol

Further **(R)-48** was treated with isopropyl amine to produce (S)-propranolol **[(S)-48]** with 98% ee and 29% overall yield. **(R)-50** or (R)-propranolol was synthesized by deacetylation by chemical hydrolysis of **(S)-49** acetate followed by reaction with isopropyl amine.[68]

Enantioselective synthesis of (S)-Moprolol

The non selective β-blocker moprolol (1-(isopropylamino)-3-(o-methoxyphenoxy)-2-propanol) is having two enantiomers. (S)-enantiomer is a eutomer, while (R)-enantiomer is distomer. (S)-moprolol is more potent β-blocker than its racemic form. A new chemoenzymatic synthetic route for (S)-moprolol have been developed with high enantiopurity, greater yield, use of less hazardous and expensive chemicals. The key step in this method involves enantioselective transesterification of racemic 3-(2-methoxyphenoxy)-Propane-1,2-diol **(rac-53).**



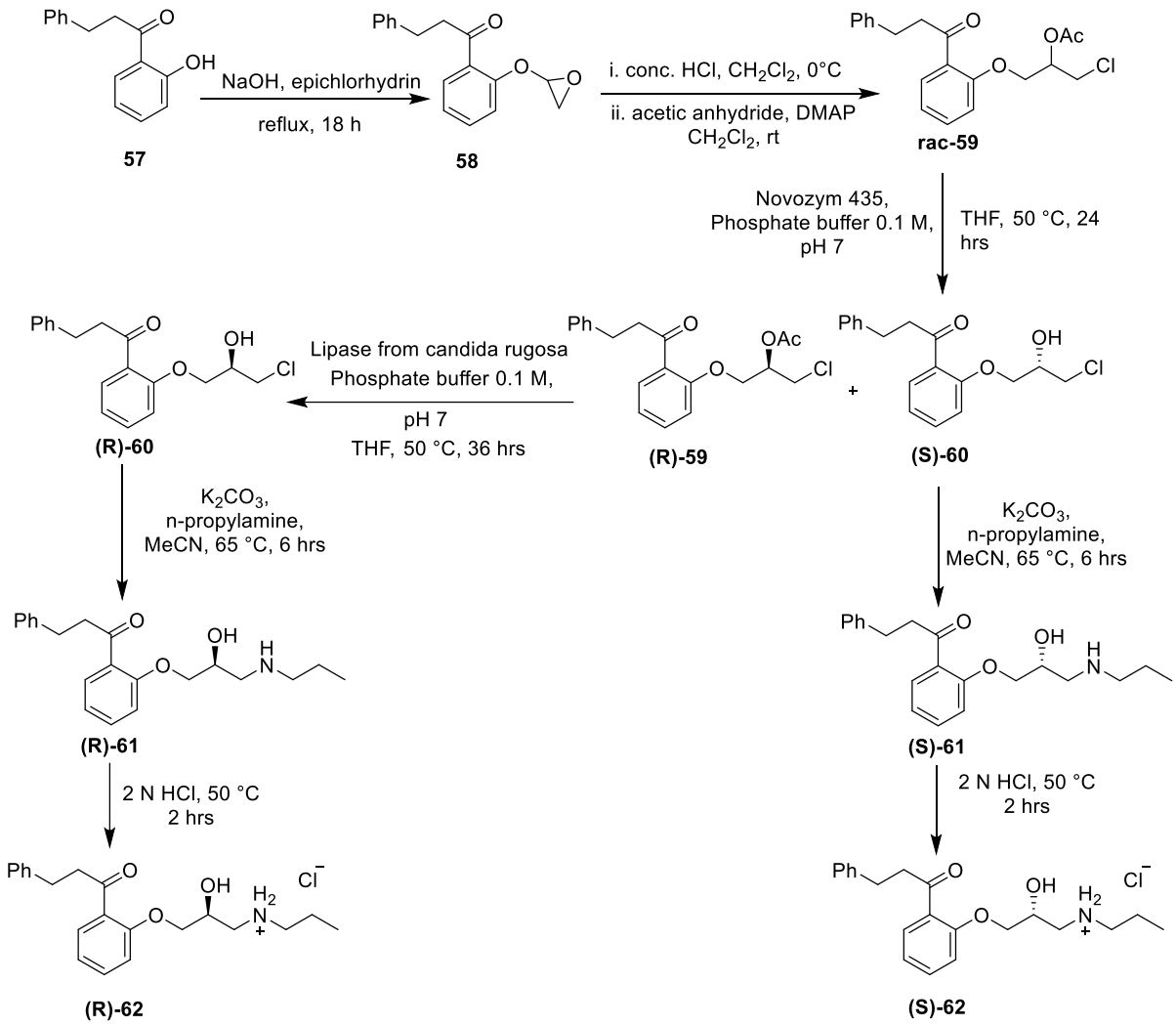
Scheme 16: Chemoenzymatic synthesis of both enantiomers of 3-(2-methoxyphenoxy)-Propane-1,2-diol

For racemization of racemic diol several lipases are screened from various sources i.e. *Candida antarctica, C. rugosa 62316*, *C. cylindracea*, AY "Amano"30, *Aspergillus niger* and other lipases. Among them *Aspergillus niger* showed the best result. The transesterification step was carried out with 20 mmol substrate **(rac-53)** and lipase from *Aspergillus niger* (ANL), in presence of acyl donor (vinyl acetate) and toluene at 30 °C. After 18 hrs of reaction, **(R)-53** observed with 52.4% conversion and **(S)-54** observed with 50% conversion and both obtained with >99% ee (scheme 16). The enantiomeric purity was calculated by chiral HPLC analysis.

Further deacetylation of both isomers gave the enantiopure diol which are the key chiral intermediate for moprolol. Later (S)-moprolol was synthesized from (S)-diol **(S)-53**. It is less expensive to synthesize the enantiopure isomers of diol by approaching enzymatic resolution than by chemical means, making a cost-effective procedure, a great example of green synthesis for (S)-moprolol preparation. [69]

Enantioselective synthesis of both enantiomers of propafenone hydrochloride

Propafenone hydrochloride or Rhythmol®, that commercially marketed by GlaxoSmithKline is used to treat arrythmia. Despite being taken in racemic form of 1-{o-(2-hydroxy-3-(propylamino)propoxy]phenyl}3-phenyl-propan-1-one hydrochloride or propafenone hydrochloride, both (R) and (S)- enantiomers have equal efficiency to treat arrythmia. Since the (S)-enantiomer can show β-blocking activity nearly 100-fold higher and patients who are blockade-intolerant may avail from the (R)- enantiomer. Therefore, it is advantageous to administer both isomers of propafenone in pure enantiomers . Francisco de Aquino Bezerra *et.* Coworkers have produced both enantiomers of propafenone hydrochloride using a simple chemoenzymatic pathway. The key step of the present invention is utilizing biocatalysis for the kinetic resolution of rac-1-(chloromethyl)-2-[*o*-(3-phenylpropionyl)phenoxy]ethyl acetate **(rac-59)** in hydrolytic pathway to produce both β-halodrins intermediates 1-{*o*-[(2*S*)-3-chloro-2-hydroxypropoxy]phenyl}- 3-phenyl-propan-1-one **[(S)-60 and (R)-60]** (Novozym 435 has been taken for the enzymatic hydrolysis in 2:1 (m/m) (enzyme and rac-72 ratio), in presence of phosphate buffer (0.1 M, pH 7). The reaction has been continued for 48 hrs at 35 °C (scheme 17). The solvent used in this reaction is THF. (Buffer: THF- 8:2). Several biocatalysts have been evaluated for the kinetic resolution of **rac-59**. Among *P. fluorescens*, Amano lipase, *Candida rugosa*, and Novozym 435, Novozym gave the best result with max 50% conversion in 48 hrs with E value 171. THF shown to be most effective co-solvent in the kinetic resolution of **rac-59.** Whether in presence of methanol, ethanol, isopropyl alcohol, MTBE as co-solvent very less conversion has been observed. Halohydrin **(S)-60** and corresponding acetate **(R)-59** was prepared with 47% yield, 95% ee and 46% yield, 96% ee respectively.

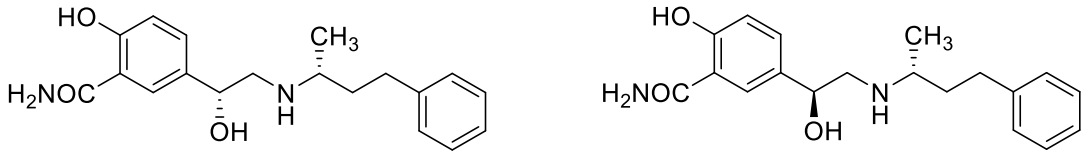


Scheme 17: Synthesis of (R)- and (S)-propafenone via biocatalytic pathway

Lastly, lipase from *Candida rugosa* successfully maintained 96% ee while catalyzing the hydrolysis of the residual acetate **(R)-59** in quantifiable yield. Later both halohydrins **(R)-60** and **(S)-60** were reacted with n-propylamine subsequently treated with and 2 N HCl to produce (R)-and (S)-propafenone hydrochloride **(R)-62** and **(S)-62** with 95% ee and 96% ee respectively. Specific Optical rotation value observed for (R)-propafenone hydrochloride $[\alpha]_D^{22}$ = +6.2° (c = 1, MeOH) for 95% ee and (S)-propafenone hydrochloride $[\alpha]_D^{22}$ = -6.3° (c = 1, MeOH) for 96% ee.[70]

Synthesis of the isomers of labetalol

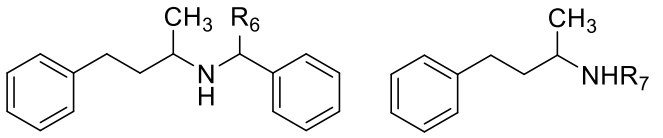
An antihypertensive drug Labetalol, shows both α_1 and β_1- adrenoreceptor antagonist characteristics, having four possible isomers. Among the isomers (S, R)-isomer (fig 4) is responsible for blocking of α_1 receptor and (R, R)-isomer shows β_1-blocking.



(R,R)-63 **(S,R)-63**

Fig 4: active isomer of labetalol

Since the documented pathways for the synthesis of single enantiomers of labetalol begin with N-benzyl-protected amine, **(64a, 64b)** (fig 5).

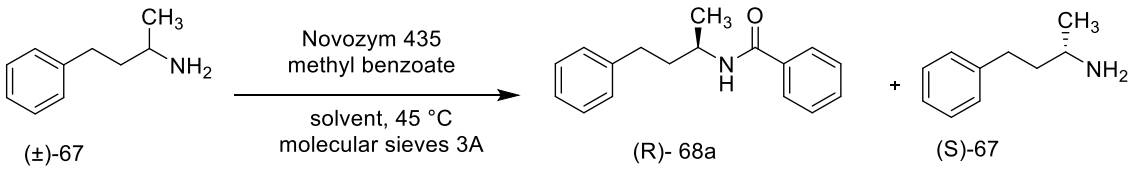


R_6= H, CH_3 R_7= H, -COPh, -COEt

64a, 64b **65, 66a, 66b**

Fig 5: synthetic precursors of labetalol

Claudia Sanfilippo *et.* Coworkers hypothesised an alternative an alternative synthetic strategy from 1-methyl-3-phenylpropylamine **(±) 67**, by kinetic resolution via enzymatic benzoylation without any chiral modification. This research experiment involved in the benzoylation of amine by enzymatic catalysis in anhydrous toluene with CAL-B immobilized on acrylic resin (Novozym 435) for acyl transfer in non-aqueous media at 55 °C (scheme 18). For acyl donor methyl benzoate have been used. A competitive enzymatic hydrolysis of benzoate ester was avoided by using molecular sieves to the reaction mixture. The reaction has been carried out for 5 days to give enantiopure benzamide **(R)-68a** together with unreacted **(S)-67**. Simple extraction separated both isomers to give desired benzamide in 42% yield and excellent enantiopurity (E >200).



(±)-67 (R)- 68a (S)-67

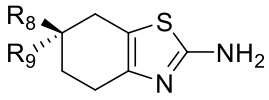
Scheme 18: enzyme catalyzed kinetic resolution of **(±) 67**

The synthesis of labetalol diastereoisomers **(R, R)-63**, and **(S, R)-63** was then completed using the enantiopure **(R)-68a**. The enantiomeric excess of the isomers has been assessed by chiral

HPLC analysis. The optical rotation observed for **(R)-68a** was $[\alpha]_{25}^{D} = -6.3$ (c 0.9, CH_2Cl_2). [71]

Chemoenzymatic synthesis of (S)-Pramipexole Dexpramipexole:

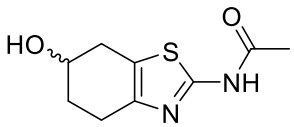
A dopamine agonist drug Pramipexole, sold under the brand name Mirapex, has been recognized as effective in treating Parkinson's disease and restless leg syndrome. Pramipexole possesses a stereo-genic centre on C6 and the isomer with anti-Parkinson effect is **(S)-69**. In contrast, another isomer **(R)-69** known as Dexpramipexole, may be used to treat eosinophil-related disorders (fig 6).



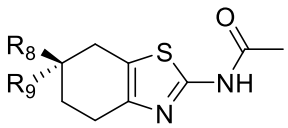
(S)-69 $R_8 = NHCH_2CH_2CH_3$, $R_9 = H$
(R)-69 $R_9 = H$, $R_8 = NHCH_2CH_2CH_3$

Fig 6: Structure of Pramipexole (S) and Dexpramipexole (R)

Due to Differing activity of the two enantiomers, optical purity is a critical factor in the synthesis of this compound. Samuele *Et*. Coworkers have investigated the resolution of the alcohol rac-3 through an enzymatic catalysis process. For synthesis of **(S)-69** dihydrochloride monohydrate, enantiopure alcohol **(R)-70** is an important synthon (fig 7).



rac-1

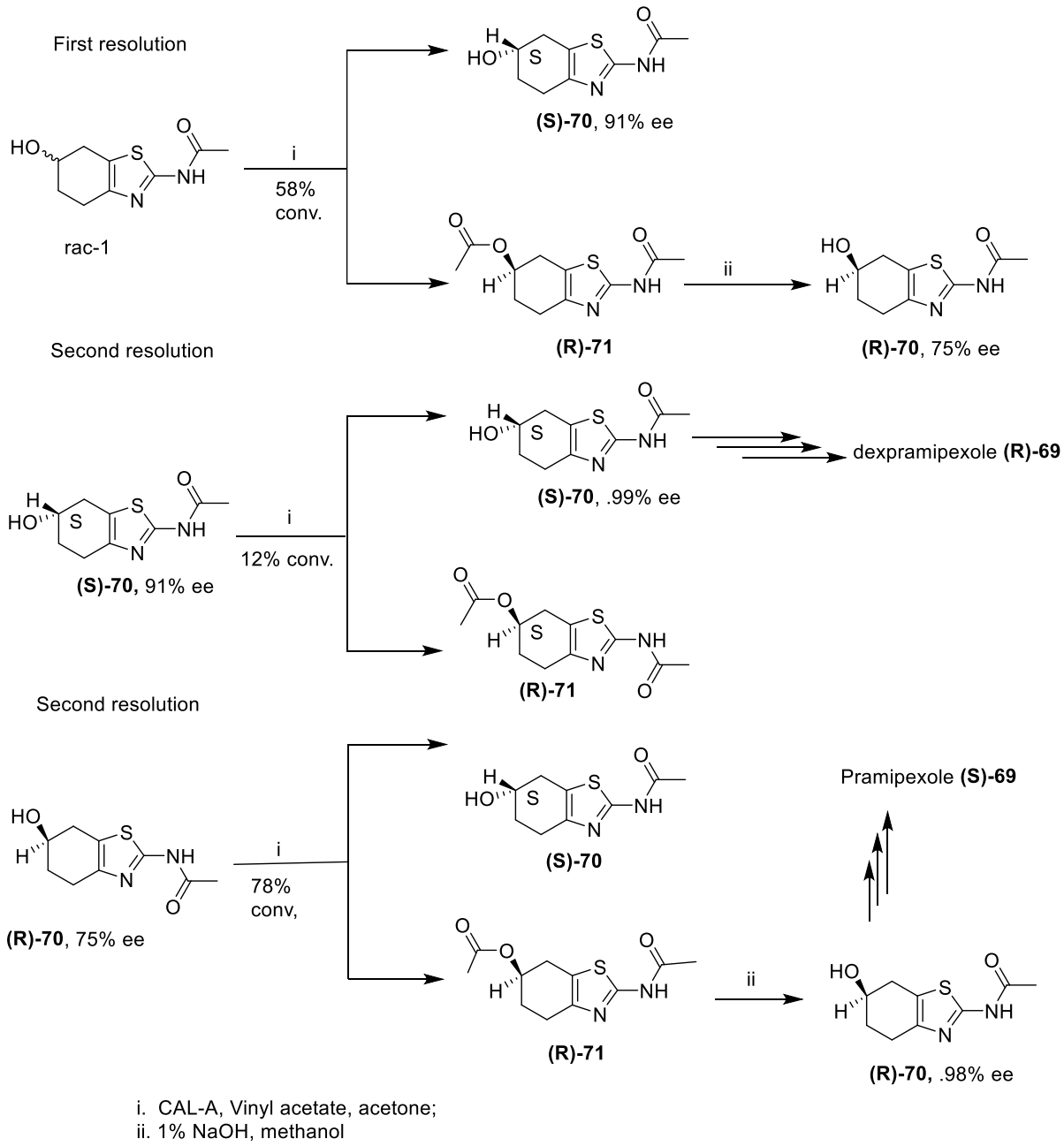


(R)-70. $R_8 = OH$, $R_9 = H$
(S)-70. $R_8 = H$, $R_9 = OH$

Fig 7: structure of rac-1, alcohol **(R)-70**, and its isomer **(S)-70**

The reaction was conducted through double kinetic resolution. CAL-A has been evaluated as best biocatalyst for this reaction among different tested biocatalysts such as lipase from porcine pancreas (PPL), *Candida cylindracea* (CCL), *Pseudomonas fluorescens* (PFL) or CAL-A. The reaction conditions were optimized while considering the solvent choice. For synthesizing enantiopure alcohol **(R) & (S)-70**, the catalytic system used the mixture of acetone/n-hexane (4:1) as a solvent which deemed a good balance between reactivity and enantioselectivity. Starting with racemic alcohol rac-1, the first transesterification was performed catalyzed by

CAL A. After chromatographic purification, 91% ee **(S)-70** and 75% ee **(R)-70** were produced (E value of 22) at 58% conversion (scheme 19).



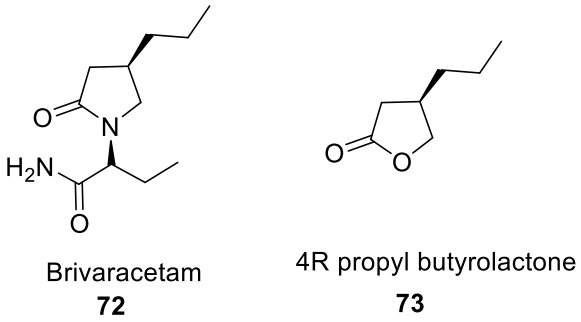
Scheme 19: Kinetic resolution of **(S) and (R)-55**

A second transesterification was performed using **(S)-70**, 91% ee as substrate. **(S)-70** produced in >99% ee at 12% conversion. The 75% ee acetate **(R)-71** was hydrolyzed by NaOH to produce enantiopure **(R)-70** which was the substrate for a second transesterification catalyzed by CAL-A. At 78% conversion, second enzymatic kinetic resolution came to an end. By using chromatography**(R)-70** >98% ee was produced after hydrolysis. The isolated enantiopure

alcohols **(S)-70** and **(R)-70** demonstrate the required configurations for the synthesis of Dexpramipexole **(R)-69** and pramipexole **(S)-69**.[72]

Enantioselective synthesis of the key Intermediate of brivaracetam

Brivaracetam (77), an antiepileptic drug, structurally contains two stereo genic centres at C-4 of pyrrolidinone ring and at C-2 of butanamide represents (R) and (S)- configuration respectively. One of the important chiral synthons for the preparation of Brivaracetam is (4R)-propylbutyrolactone **(73)** (fig 9). Samuele *et.* Coworkers have developed a chemoenzymatic pathway for synthesizing the chiral synthon (4R)-propylbutyrolactone, a key intermediate for Brivaracetam synthesis.

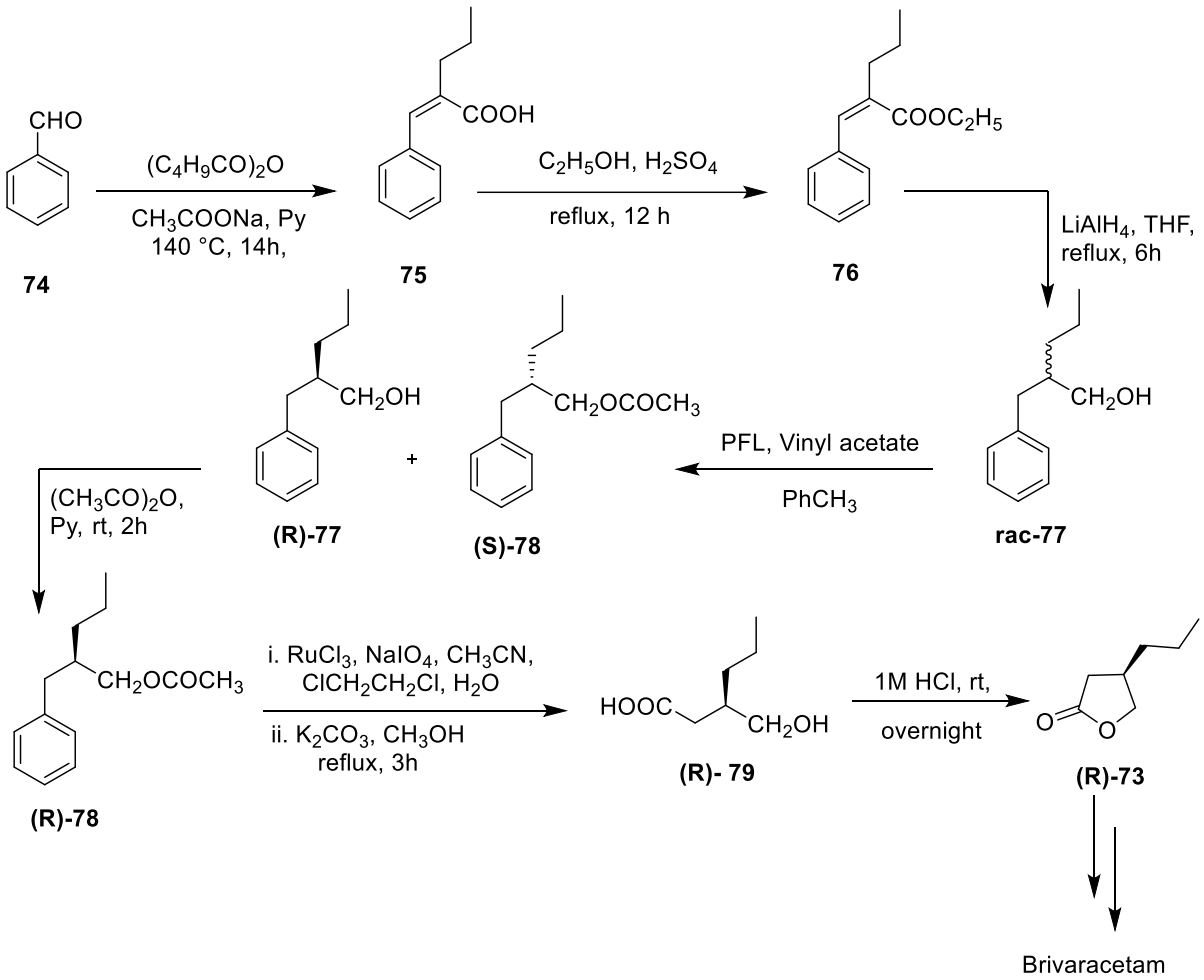


Brivaracetam
72

4R propyl butyrolactone
73

Fig 8: Structure of Brivaracetam and (4R)-propylbutyrolactone

The synthesis was started from benzaldehyde, reacted with valeric anhydride to form (E)-2-benzylidepentanoate acid **(75)**. Which further converted to corresponding ethyl ester **(76).** Then the desired alcohol **(77)** was prepared by reduction using $LiAlH_4$ in THF which has further taken for biocatalytic step. The resolution of racemic alcohol **(77)** has performed with lipase from *Pseudomonas fluorescens* (PFL), in toluene and time taken for the resolution was 48 hrs. Optically pure alcohol **(R)-77** was obtained with 62% conversion and 99% ee. Further enantiopure (R)- alcohol **(R-77)** was taken for reaction and obtained (R)-lactone **(R)-73** with 77% yield and >98% ee (scheme 20). Further this (R)-lactone has been utilized in Brivaracetam synthesis.



Scheme 20: Synthetic approach for Brivaracetam

The goal of the chiral stationary phase optimisation of the analytical chromatographic techniques (GC and HPLC) was to assess the ee of the chiral substances and offer a helpful supplement to the synthesis. [73]

Lipase catalysed synthesis of a key starting material of ozanimod

In the fight against multiple sclerosis, a newly discovered, potential active pharmaceutical ingredient (API) molecule is ozanimod. A novel synthetic strategy for (S)-4-cyano-1-aminoindane (fig 9), which is chiral key intermediate of ozanimod has been established in order to achieve scalability, more economically beneficial for manufacturing of this drug.

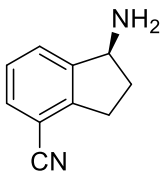
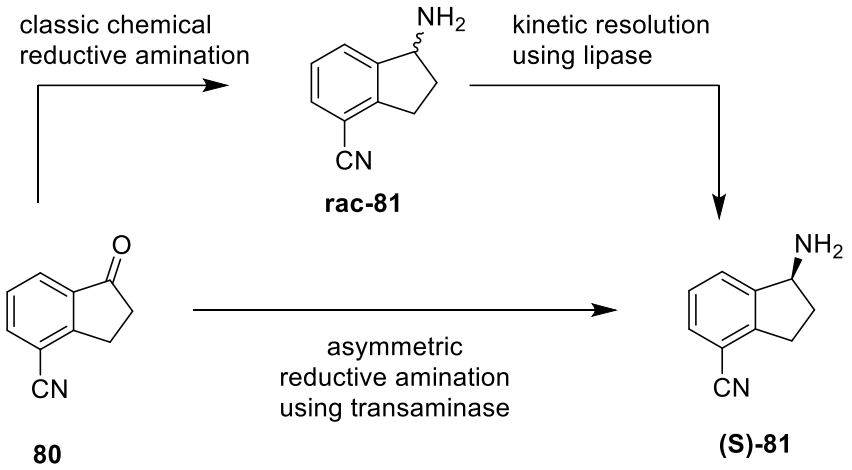


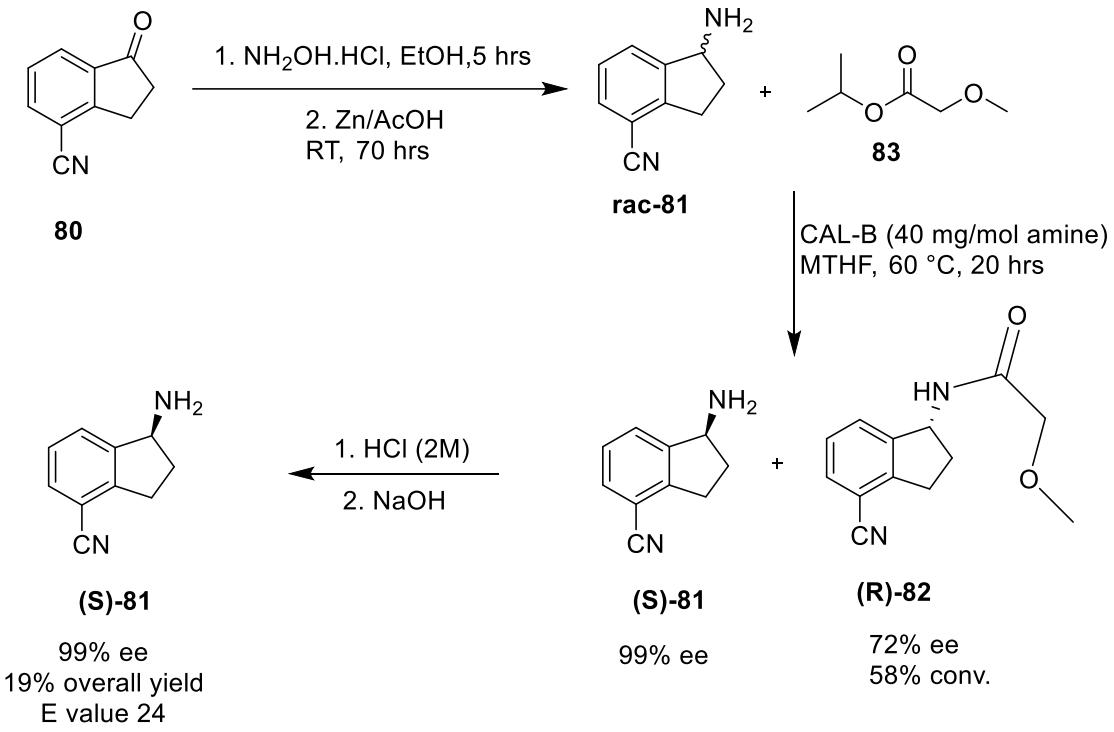
Fig 9: structure of (S)-4-cyano-1-aminoindane

Use of Naphthalene as starting material for synthesis of this intermediate make the synthesis more economically beneficial and advantageous as it is readily available and cost-effective. To introduce the chirality and the necessary absolute configuration, 4-carboxy indanone must be converted into (S)-4-cyano-1-aminoindane. A number of synthetic solutions based on biocatalysis with good ee can be considered as prospective method towards key building block **(S)-81** (scheme 21). Chemical reductive amination of 4-carboxy indanone subsequently resolution catalyzed by lipase, both in combination, proved to be the most effective synthetic strategy when evaluating chemo-enzymatic approaches using lipase and transaminase. This approach produced the desirable key intermediate with an attractive ee of 99% and adequate yield.



Scheme 21: Biocatalytic synthesis opportunities for production of enantiopure (S)-4-cyano-1-aminoindanes.

Florian Uthoff *et.* Coworkers have concentrated on assessing a lipase-catalyzed amine resolution for synthesizing the crucial intermediate **(S)-81.** Various acyl donors, CAL-B, and solvents are investigated in optimizing reaction parameters for kinetic resolution. Various Solvents i.e., MTBE, Toluene, methylcyclohexane (MCH), 2-Methyltetrahydrofuran(2-MTHF), n-hexane were examined as solvents and as acyl donors' ethyl methoxy acetate and diethyl malonate were used.



Scheme 22: Biocatalytic synthesis of enantiopure (S)-4cyano-aminoindane **(S)-49**

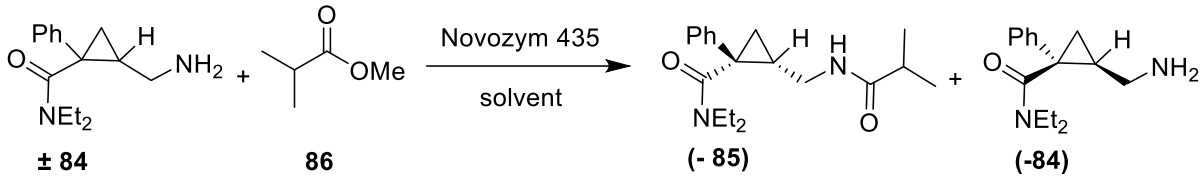
Before the biocatalytic step, first rac-81 has been synthesized started from prochiral 1-indanone **(80).** By reacting it with hydroxylamine hydrochloride followed by subsequent reduction with zinc produce racemic **81**. In the next step, acylation was done with isopropyl methoxy acetate **(83)** in MTHF solvent in the presence of biocatalyst CAL-B at 60 °C for 20 hrs (scheme 22). The amine **(S)-81** was produced with outstanding ee 99%, and 19% overall yield. (Based on ketone 80). [74]

Chiral synthesis of milnacipran

Kinetic resolution of racemic amines in organic solvent or in aqueous media can be carried out by various enzymes such as hydrolase, amineoxidase or transaminase. Lipase mediated reactions of racemic amine with acyl donor in organic solvent results in irreversible N-acylation of substrate due to non-reactivity of amides to enzymes. Chiral amines with aminomethyl group are difficult substrates for biocatalytic resolution. Lipase mediated resolution of milnacipran (1R,2S)-2-(aminomethyl)-N,N-diethyl-1-phenylcyarboxclopropane-carboxamide **(± 84)** has been studied. This is an example of distant stereogenic centre carrying primary amine. Milnacipran is a selective serotonin reuptake inhibitors class of drug, is used in treatment of depression. Among both enantiomers of milnacipran, **(1S,2R)-(-)-84** which is known as levomilnacipran shown greater activity in pharmacokinetic studies, and there is considerable

interest in the synthesis of enantioenriched **(-)-84** for innovative therapeutic formulations. Synthesis of optically pure milnacipran has been reported using biocatalytic approach by Claudia Sanfilippo *et.* Co-workers.

For the chemoenzymatic step, (± 84) was taken with methyl isobutyrate **(86)** as an acyl donor in tert-butyl methyl ether solvent. After addition of Novozym 435 enzyme, the reaction was continued for 4hrs at 50 °C (Scheme 23). 65% of substrate conversion was observed after 4hrs of reaction but, after acid -base work up with 1N HCl and 2N NaOH and MTBE (organic solvent for extraction) the enantioenriched **(-)-84** was obtained with 98% ee and 32% yield.



Scheme 23: Chemoenzymatic kinetic resolution of (±)-milnacipran

Among several lipases screened out for the reaction such as Lipozyme, Novozyme 435, Chirazyme L-9, PPL (crude lipase from *Porcine pancreas*), AK (from *Pseudomonas fluorescens*), the expected result observed with Novozyme 435. Aminomethyl group in milnacipran structure made this work challenging for lipase catalysed aminolysis and moderate enantiomeric purity observed in the experiment. Later optimization of acyl donor, reaction solvent, temperature helped to get satisfactory result. [75]

Kinetic resolution resolution of 3-hydroxy-3- phenylpropanonitrile

As the significance of synthesizing active pharmaceutical Ingredients in an enantiomerically pure form is gradually increasing, researchers are searching for new asymmetric synthesis techniques, and a possible method that can produce an enantiomerically pure compound. Which can exclusively be achieved by biocatalysis. Despite the numerous advantages of enzyme catalysis, some limitations exist to using free forms of natural enzymes due to low stability under unpleasant reaction conditions. Enzyme Immobilization, or the binding of an enzyme with solid support, is widely employed to enhance the characteristics of free enzymes. Solid support on free enzymes helps to protect enzymes, improve the separation of biocatalysts from the product, reusability, and decrease enzyme loss, which is advantageous from an economic standpoint.

Fluoxetine (fig 10) is an important drug, widely used as an anti-depressant and in anxiety relief. Due to the comparable effects of both enantiomers of fluoxetine on serotonin reuptake inhibition, it is marketed as a racemic mixture of (R) and (S)-fluoxetine enantiomers.

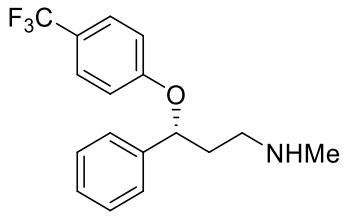
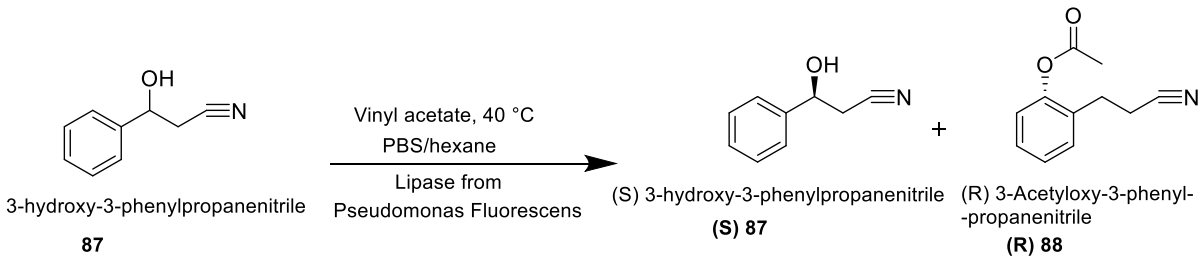


Fig 10: Structure of Fluoxetine

Oliwia Degórska *et.al* has performed the kinetic resolution (via transesterification) of a racemic mixture of 3-hydroxy-3 phenylpropionitrile (3H3P) to obtain pure (s) enantiomer of 3H3P which is an essential intermediate in fluoxetine synthesis pathway. The chemoenzymatic step has been carried out using lipase from Pseudomonas fluorescens immobilized on modified silica nanoparticles.



Scheme 24: Kinetic resolution of 3H3P by enzymatic catalysis

In order to Synthesize enantiopure 3H3P from racemic 3H3P by kinetic resolution, *Pseudomonas fluorescens* lipase immobilized on modified silica has been taken. An innovative method for transesterification was used, involving the utilization of imidazolium ionic liquids (IL) containing Cl^- and NTf_2 anions to stabilize the enzymes and boost their stereoselectivity. It was demonstrated that [BMIM]Cl was the most effective IL: using 1% (w/v) of IL in organic solvent resulted in efficiency and enantiomeric excess of 97.4% and 79.5%, respectively. In the kinetic resolution step, 3H3P **(87)** was reacted with two distinct ionic liquids [BMIM]NTf_2 and [BMIM]Cl. To the mixture containing racemic **(87),** immobilized lipase which was prepared freshly with APTES-modified support, was added. Vinyl acetate, phosphate buffer (pH 7) or hexane, and 10% W/V of ionic liquids were also added to the reaction mixture. The reaction continued for 7 days at 40 °C (scheme 24). ILs enhance enzyme activity and stability due to the protective properties on enzyme structure. Reaction conducted in buffer medium resulted

in transesterification with low efficiency (31.6%). It resulted in 97.4% efficiency and 79.5% ee in hexane. Chiral High-Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) was used to analyse each sample. [76]

Chemoenzymatic kinetic resolution of (+) and (-) 4-acetoxy-azetidin-2-one

Several biologically active β-lactam compounds can be synthesized using an important intermediary 4-acetoxy-azetidin-2-one **(rac-89).** It is, however only commercially available as a racemic mixture, which restricts its use for synthesizing enantiopure compounds.

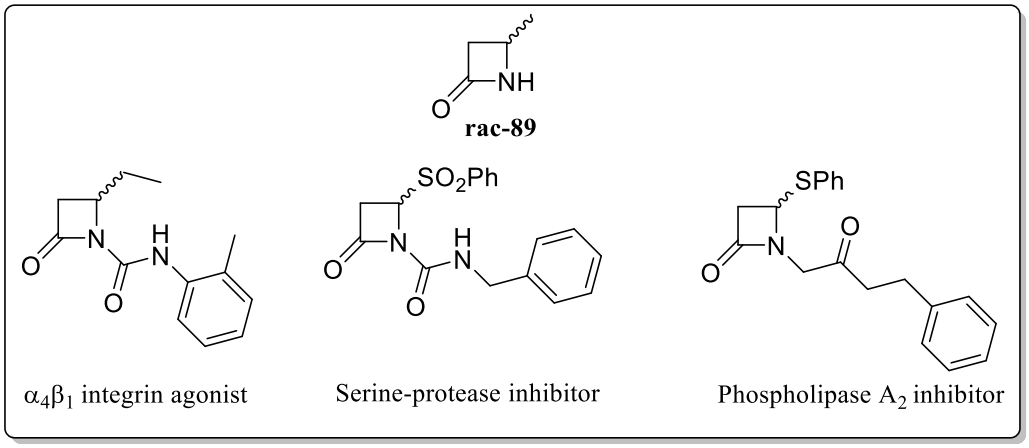
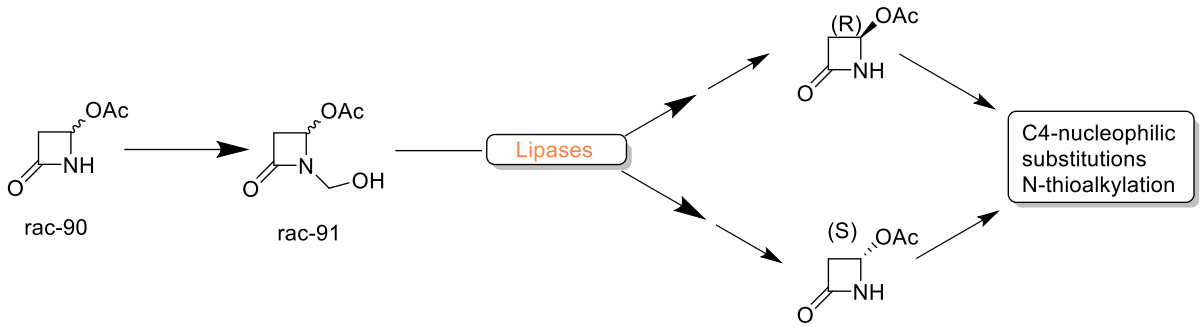


Fig 11: Some bioactive β-lactam compounds obtained from rac-89

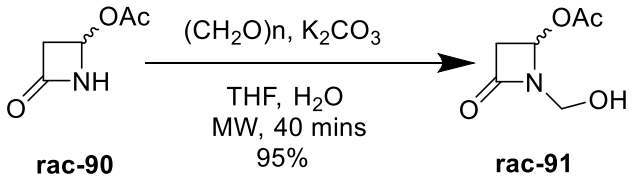
It has been well documented that two different enantiomeric drugs could be having different therapeutics effects, different pharmacological effects, and even they could have different pharmacokinetics and pharmacodynamics profile. So, it is essential to get an enantiomeric pure form of intermediary 4-acetoxy-azetidin-2-one **(rac-89)** for synthesis of bioactive β-lactam with specified stereochemistry (fig 11).

For conversion of racemates into single enantiomers, Enzymatic kinetic resolution is a popular biocatalytic technique and lipases are most potent tool for synthesis of enantiopure pharmaceuticals. Giulia *et.* coworkers have established a chemoenzymatic pathway by utilizing lipase in a kinetic resolution (KR) procedure to acquire the pure enantiomeric form of 4-acetoxy-azetidin-2-one from racemic **rac-91**, starting from commercially available **rac-90** and in order to assess the stereochemical integrity of the related products, preliminary test on single enantiomer has been conducted in some common reactions such as C-4 substitution and N-thioalkylation (scheme 25).



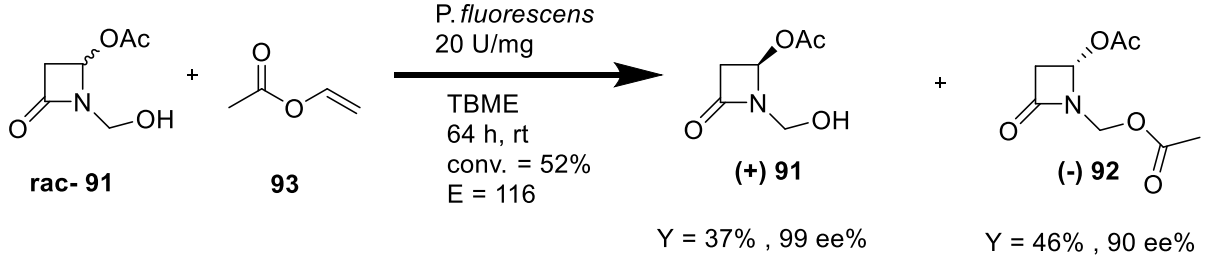
Scheme 25: Kinetic resolution of functionalized 4-acetoxy azetidinone **(rac-91)** by chemo-enzymatic route and assessment of stereochemical consequences during C-4 and N-4 functionalized reaction.

Racemic 4-acetoxy-azetidinone **(rac-90)** was subjected to a reaction involving paraformaldehyde and a catalytic amount of potassium carbonate under microwave irradiation, resulting in the efficient formation of the N-methylene hydroxy derivative **(rac-91)** with high yields (scheme 26).



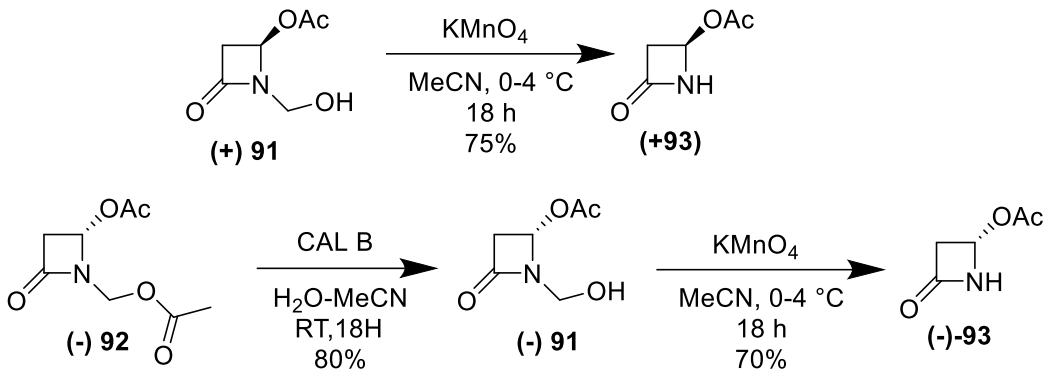
Scheme 26: Synthesis of N-methylene-hydroxy-azetidinone **(rac-91)**

During the performance of kinetic resolution, different lipases were evaluated and among them *Pseudomonas fluorescens* gave the best result. For kinetic resolution enzyme concentration used is 20 U/mg which gives good conversion and (+) **91** isomer obtained in 99% ee and (–) **92** isomer 90% ee (scheme 27). The best solvent to obtain good ee is TBME, and the optimal temperature is room temperature. The excellent enzymatic selectivity obtained is 116. Specific optical rotation observed for **(+) 91** isomers was $[\alpha]_D^{20}$ = + 35 (c= 1.3, MeOH) and **(–) 92** isomer $[\alpha]_D^{20}$= 17 (c= 1.0, MeOH)



Scheme 27: enzyme-catalyzed kinetic resolution of rac-91

The final separated enantiomers **(+)-93** and **(-)-93** were obtained with 95 ee% and 94 ee% respectively by reacting alcohol **(+) 91** and ester **(–) 92** further to remove the β-lactam nitrogen substitution) (scheme 28).

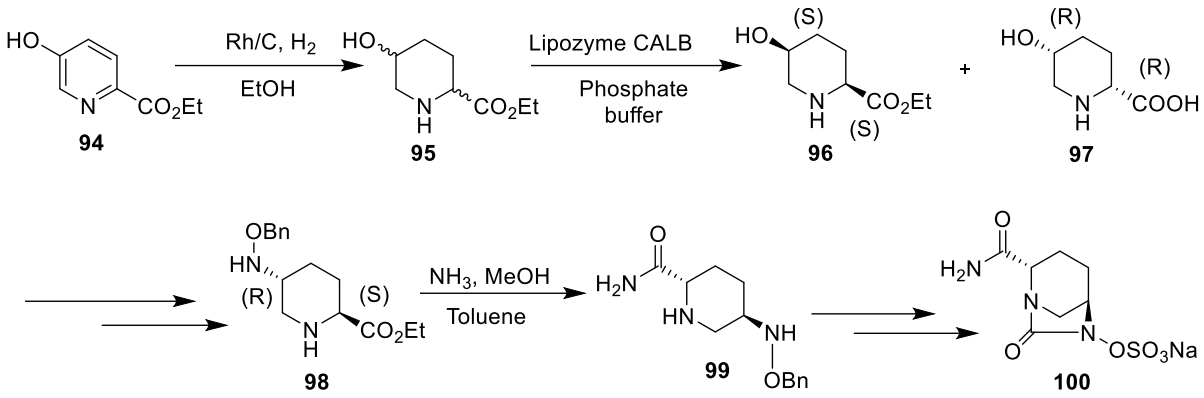


Scheme 28: Synthetic procedure for β-lactam enantiomers.

Specific optical rotation observed for **(+) 93** isomer was $[\alpha]_D^{25}$ = + 70 (c= 1.3, MeOH) and **(-) 93** isomer $[\alpha]_D^{25}$= -52 (c= 1.0, MeOH). After that, the pure enantiomers were subjected to nucleophilic substitution reaction to assess the stereochemical integrity.[77]

Synthesis of Avibactam

Sodium (2S,5R)-2-carbamoyl-7-oxo-1,6-diazabicicyclo [3.2.1]octane-6-yl sulfonate (**100**) or Avibactam is known as non-βlactamase inhibitor. A newer chemoenzymatic synthetic process have been developed. First from ethyl-5-hydroxypinacolinate (**94**) by catalytic hydrogenation (**96)** was produced with a stereoisomer ratio 97:3(cis/trans). Instead of using the ring opening and carbonized cyclization steps employed in previously described procedures, the starting material utilized in this process was readily accessible and reasonably priced ethyl-5-hydroxypicolinate. This has been taken as key material for further biocatalytic process. **(95)** was taken in potassium phosphate buffer solution (pH 8.0). The pH maintained to 7.5 with dipotassium hydrogen phosphate. Lipozyme CAL B has been taken as biocatalyst and the reaction continued for 12 hrs at room temperature (scheme 29).

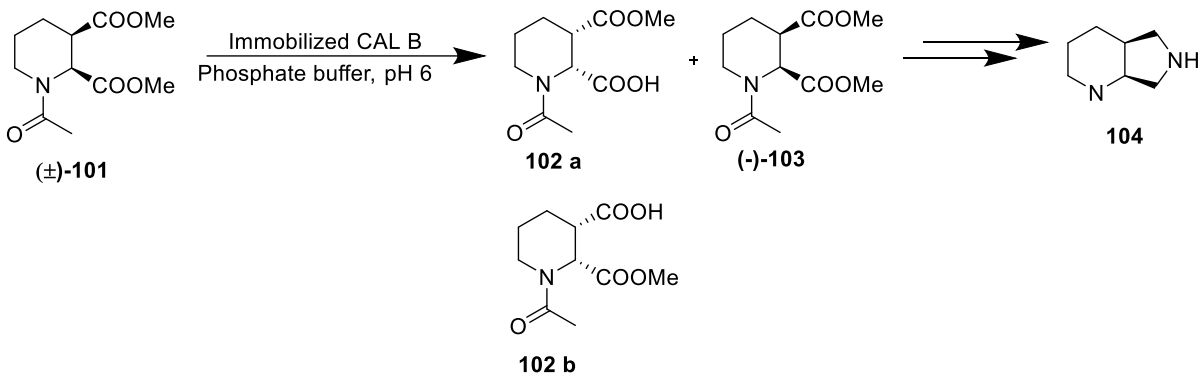


Scheme 29: Chemoenzymatic synthetic route for Avibactam

(2S,5S)-5-hydroxy-piperidine-2-carboxylate **(96)** the key intermediate for avibactam synthesis was obtained in high enantiopurity (d.r.≥99:1) by lipase catalyzed kinetic resolution with the counterpart **(97).** Later one-pot debenzylation/sulfation and cation exchange produced Avibactam sodium salt **(100).** The specific rotation observed for final Avibactam sodium salt was $[\alpha]_D^{22} = -46.40$ (c = 0.79, MeOH/H_2O = 1/1). [78]

Efficient Resolution of (-)-Dimethyl 1-Acetylpiperidine-2,3-dicarboxylate

A useful intermediate for a fluoroquinolone antibacterial drug moxifloxacin (2S,3R)-Dimethyl-1-acetylpiperidine-2,3-dicarboxylate, was synthesized by kinetic resolution using enzyme catalysis of racemic diester **(101).** Moxifloxacin is useful in respiratory infection. The need for this antibiotic on the market is growing yearly, hence it's important to develop more environmentally friendly and effective synthesis methods. (S,S)-2,8-Diazobicyclo [4.3.0] nonane **(104)** is an important chiral intermediate for moxifloxacin synthesis. Enzymatic biocatalysis approach for synthesis of nonane **(104)** using chiral intermediate **(-)-103** by kinetic resolution of racemic dimethyl 1-acetylpiperidine-2,3-dicarboxylate **(±)-(101).** N-acetyl dimethyl ester was **(±) -(101)** first synthesized then it was subjected to hydrolysis catalyzed by enzyme. The ideal temperature for the reaction was 45-50 °C and pH was maintained to 7.5 by continuous addition of 2N NaOH (scheme 30).



Scheme 30: Resolution of (±) dimethyl 1-acetylpiperidine-2,3-dicarboxylate by Lipase

At 45 °C, $V_{max,obsd}$ = 0.061 ± 0.008 M/h/g, $K_{m,obsd}$ = 0.2 ± 0.045 M which corresponds to standard Michaelis-Menten kinetics. In the optimum reaction conditions, the enantioselectivity obtained was good, with an E value of 80. Further preparative scale enzymatic resolution was also performed at 45 °C, using substrate loading 8%(W/W), 2:1 (W/W) Substrate-enzyme ratio in 0.01 M sodium phosphate buffer at pH 7.5 It took 16 hours to finish the reaction with high enantiopurity (>99% ee) and 47.5% yield. Enantiopurity of isomers has been analysed by chiral HPLC analysis. Specific optical rotation observed for the **(-)-103** was $[\alpha]^{D}_{25}$ = -114.8 (c = 1, CH_3OH). [79]

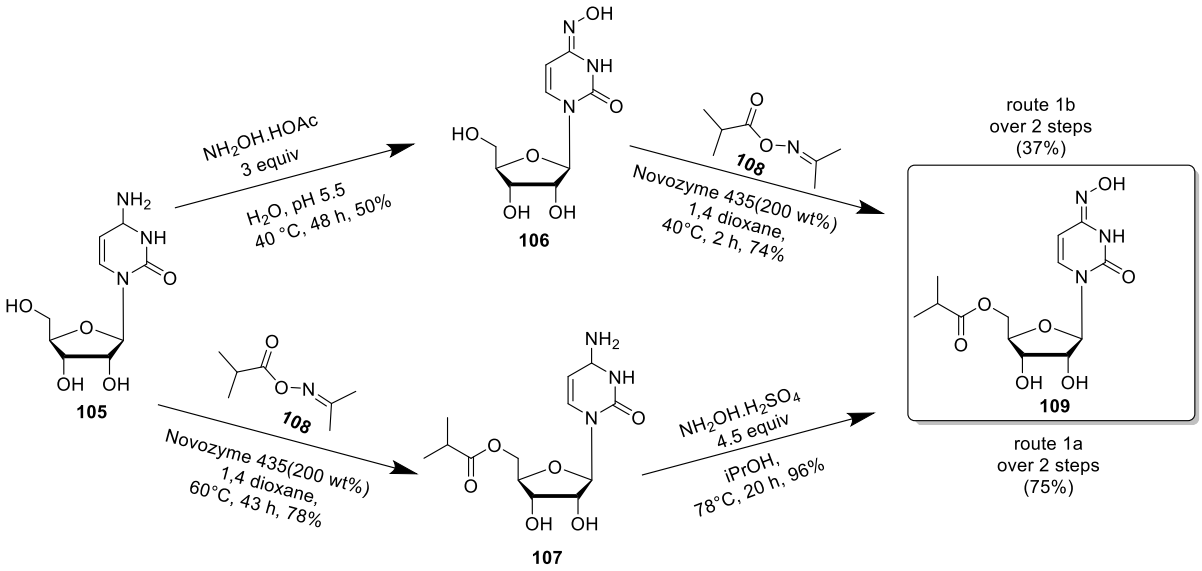
Synthesis of molnupiravir

One further application for biocatalysis in the pharmaceutical Industry is the chemoenzymatic synthesis of Molnupiravir. This is a nucleoside analogue used for the treatment of cancers and also a therapeutic agent for COVID-19. Enzyme-catalyzed reactions are outpacing to avoid conventional synthetic strategy using hazardous and expensive chemicals like uridine, multistep synthesis with less product yield, several protection-deprotection steps, and unwanted racemic mixtures.

One of the most significant disadvantages of using the chemical method for synthesizing molnupiravir is the formation of by-products from the acylation and hydroxy amination steps.

Vasudevan *et* co-workers have developed a chemo-enzymatic synthetic pathway consisting of two steps involving esterification and hydroxy amination of cytidine with an overall yield of 75%. Replacing expensive Uridine with easily available cytidine decreases the raw material cost, eliminates the need for protecting and activating groups, and simplifies the synthesis to a two-step process. In this research work the transamination of cytidine isobutyryl ester **(107)** was investigated, and interestingly, using $NH_2OH.H_2SO_4$ in iPrOH fully prevented

dihydroxamination. The key technical difficulty in the synthesis of a shorter and protecting group-free approach is selective acylation. N(4)-hydroxy cytidine (NHC) **(106)** required selective esterification for one of four hydroxyl groups. This objective has been accomplished via the esterification of cytidine catalyzed by enzyme using vinyl esters and anhydride acyl donors. The required selectivity for both cytidine **(105)** and NHC was given by CAL B (*Candida Antartica* Lipase B) (Scheme 31).

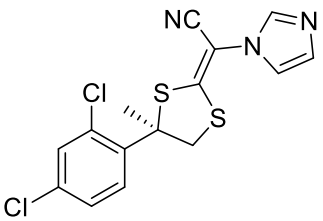


Scheme 31: Enzymatic synthetic route for synthesis of Molnupiravir

Acyl transfer agent isobutyric oxime ester **(108)** was utilized with solid-supported enzyme (200 wt%, 1.5 mol%). The best result observed with 1,4-dioxane and molnupiravir **(109)** recovered from **(107)** in 75% overall yield (route 1a). While hydroxyamination carried out first (route 1b), (**109**) was produced in 37% yield. [80]

Synthesis of Luliconazole

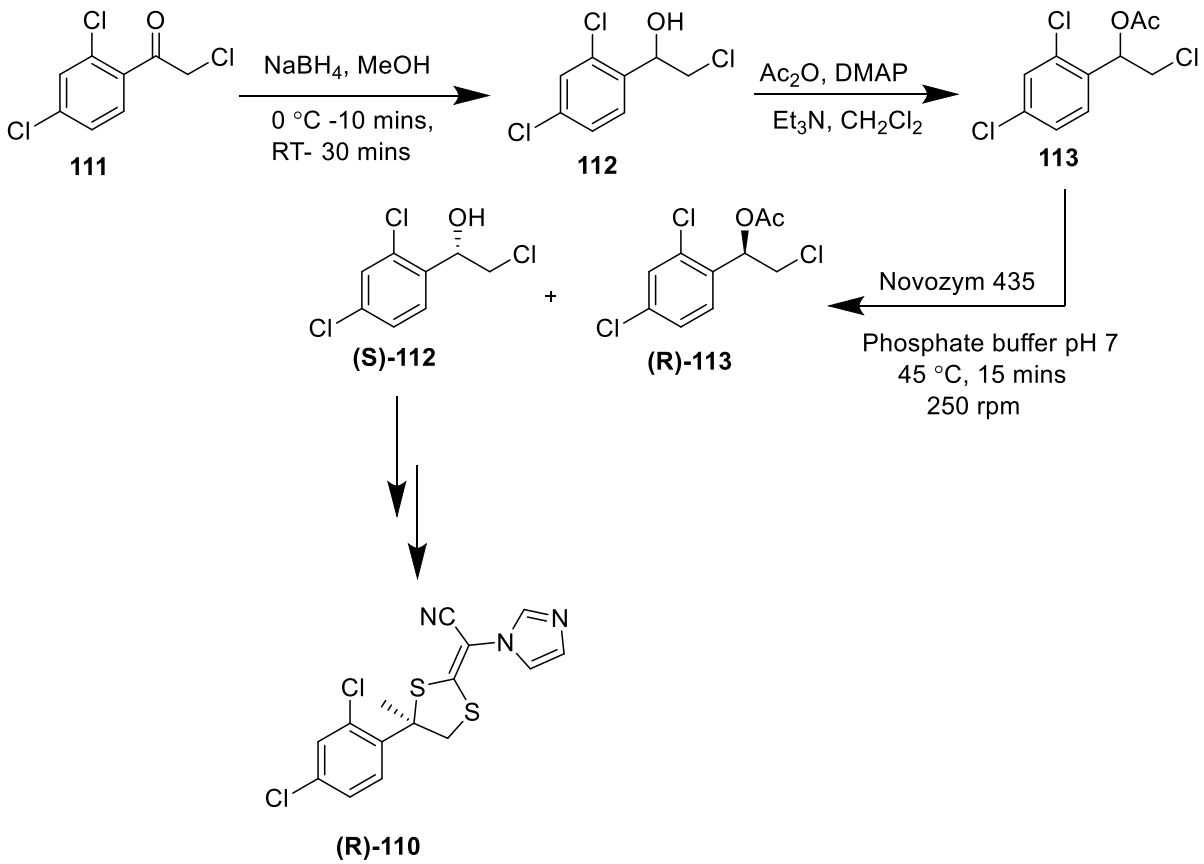
The synthesis of Luliconazole **(R)-110** or (-)-2E-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazole-1-yl)acetonitrile (fig 12) has been achieved by using a simple chemoenzymatic process.



(R)-110

Fig 12: Structure of Luliconazole

The (S)-E and /or Z-isomers of luliconazole are regarded as impurities and should be eliminated in order to produce the enantiopure API **(R)-110**. Thiago de S. Fonseca *et.* Coworkers have developed a novel method for obtaining luliconazole. The key step in the process is the synthesis of chiral-halohydrin intermediate by chemoenzymatic approach catalyzed by lipase. Chiral-halohydrin synthesis is significant because these compounds serve as bridges in the synthesis of many valuable compounds. Kinetic resolution of racemic acetate was the key step for synthesizing enantiopure β-halohydrin (1S)-2-Chloro-1-(2,4-dichlorophenyl)-1-ethanol. For chemoenzymatic step, reaction has been carried out 45 °C for 15 mins, lipase/**rac-(113)** has taken in 0.5:1 ratio (scheme 32). Among several lipases studied for hydrolysis of **rac-(113),** CAL B (Novozym 435®) and *Thermomyces lanuginosus* immobilized on immobead-150 (TLL) produced the greatest result. After 15 mins of reaction, these enzymes shown 50% conversion with E values >200, product and residual substrate both with >99% ee. Later **(S)-112** was subjected to further reaction for synthesis of **(R)-110** or (R)-luliconazole. In the final step (R)-luliconazole was obtained with >99% ee and in 43% yield. Specific optical rotation observed for (R)-acetate, **(R)-113** was $[\alpha]^{D}_{23}$ =–52.3 (*c* = 1.0 in EtOAc) and $[\alpha]^{D}_{20}$ = +51.1 (*c* = 2.50 in $CHCl_3$).



Scheme 32: Chemoenzymatic synthesis of Luliconazole

Also in recyclability studies, for Novozym 435® 50% conversion were noted during 10 reuse cycles and >90% ee upto 5 reuse cycle. The advantages of Novozym 435® over TLL were demonstrated by the outcomes of the enzyme loading and reuse assays. As opposed to 1.5:1 for TLL, Novozym 435® might actually be employed with a lower enzyme/substrate ratio of 0.5:1. Additionally, Novozym 435® could be recycled up to five times while still retaining good conversion and selectivity levels. Conversely, in the presence of TLL, the conversion already demonstrated a significant decline in the second reaction cycle, and selectivity continued to decline progressively in the following reaction cycles. In conclusion, this novel method of obtaining luliconazole can be deemed environmentally benign due to the use of highly enantioselective, stable, affordable, reusable, commercially accessible biocatalyst. [81]

Kinetic resolution of racemic 1,2,3,4 Tetrahydroquinoline

Chiral tetrahydroquinoline-based derivatives (fig 13) are crucial building blocks for numerous natural products, pharmaceuticals and potential prospects for drugs it is imperative to develop new synthetic methods to produce these substances in enantiomeric pure form.

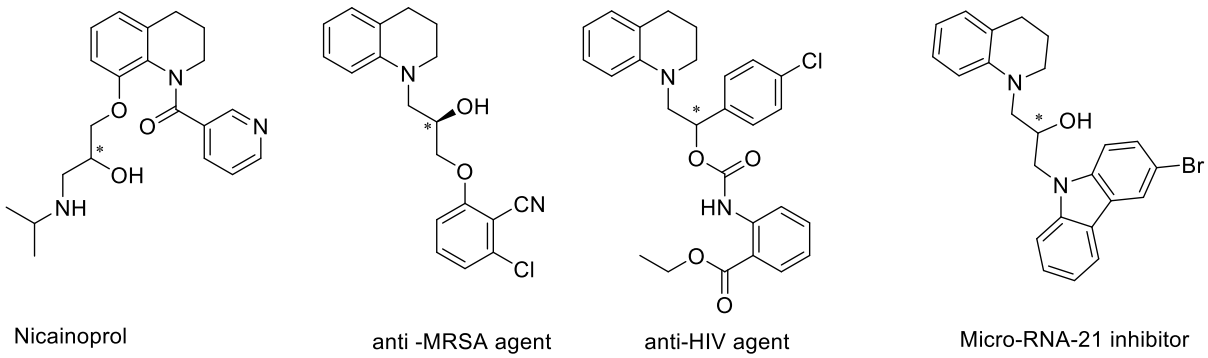
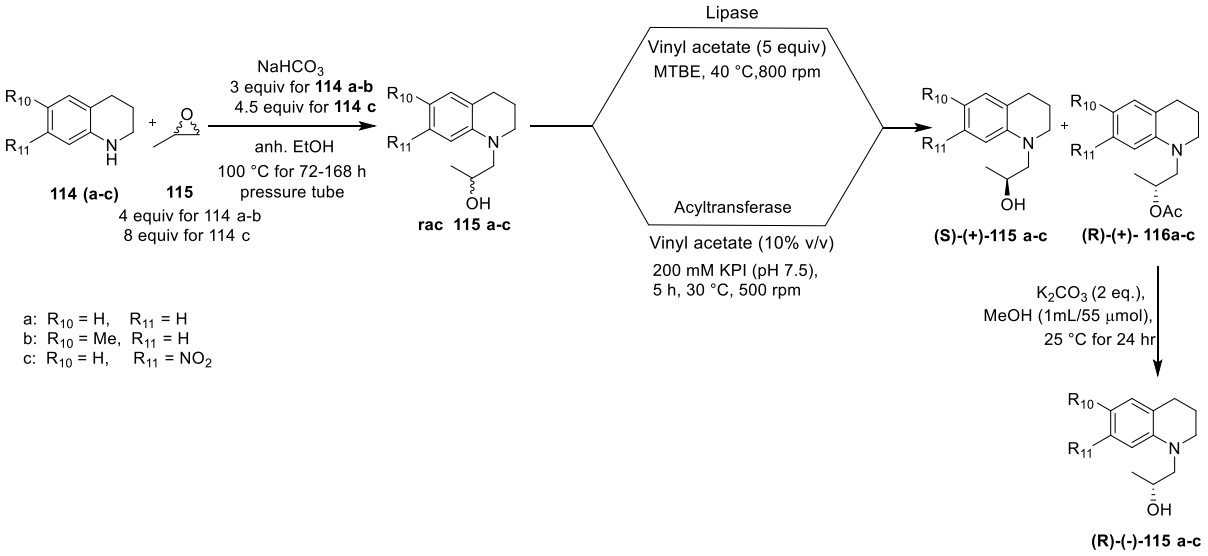


Fig 13: Chiral 1,2,3,4-tetrahydroquinoline scaffold

Beta Zdun et. Coworkers have developed a new biocatalytic method for the kinetic resolution of a group of racemic 1,2,3,4-tetrahydroquinoline-propan-2-ols in neat organic solvents (for CAL-B and BCL) or in water (for MsAcT) utilizing immobilized lipases obtained from *Burkholderia cepacia* (BCL), *Candida antartica* type B (CALB), engineered variations of acyltransferases from *Mycobacterium smegmatis* (MsAcT) and irreversible acyl donors (vinyl acetate) which provides enantiopure (S) alcohols and (R) acetates (scheme 33). The enzymatic selectivity is obtained up to 328 with highest optical purity (> 99% ee).



Scheme 33: Kinetic resolution of racemic alcohols containing 1,2,3,4-tetrahydroquinoline derivatives

The biocatalytic method for the synthesis of optically active alcohol consisting of 1,2,3,4-tetrahydro- quinoline (S)-(+) and (R)-(-) isomers has been reported in two different chemoenzymatic methods. Enantioselective transesterification of racemic 1-(3,4-dihydroquinolin-1(2H)-yl) propan-2-ols **(rac-115 a-c)** were carried out under kinetically

controlled catalyzed by lipase. In one strategy kinetic resolution performed in neat organic solvents. The second approach was accomplished, however, by using acyltransferase from engineered variants obtained from *Mycobacterium smegmatis* (MsAcT) in water rich environment. According to the reported procedure previously, in one biocatalytic approach, transesterification of racemic 1-(3,4-dihydroquinoline-1(2H)-yl)propan-2-ols **(rac-115 a-c)** were optimized. This research article has assessed how the acylation of alcohols by the enzymatic route was affected by temperature, different lipases, co-solvents, and time course in Kinetic resolution. For the resolution of **rac 115a** the relevant biocatalyst was suspended in a solution containing MTBE as a standard solvent and vinyl acetate as an irreversible acyl donor. The temperature maintained for the reaction was at 40 °C for 24 hrs. Concerning the investigated enzymes, i.e., Novozym 435, Lipozyme 435, *candida Antarctica* lipase type B (CAL B), *Thermomyces lanuginosus* lipase (TLL), Burkholderia cepacian lipase (BCL) immobilized on diatomaceous earth (Amano PS-IM), *Rhizomucor miehei* lipase (RML), CAL B demonstrated the highest catalytic activity, produced optically active alcohol **(S)-(+)- 115a** in >99% ee and acetate **(R)-(+)-116a** in 34-87% ee with 54-75% conversion. In contrast, BCL produced **(S)-(+)- 115a** in 79% ee and acetate **(R)-(+)-116a** in 98% ee with 45% conversion. RML and TML preparations had modest activity, produced **(S)-(+)- 115a** in 25-48% ee and acetate **(R)-(+)-116a** in 89-98% ee with very low conversion (20-30)%. MTBE was the most effective solvent evaluated in terms of enantioselectivity and reaction rate. To optimize reaction temperature, the reaction was carried out in a range of temperatures at 40-60°C. The enantioselectivity and rate of kinetic resolution did not appear to benefit from high temperatures. Thus, further optimization attempts were made at 40 °C.

Two further derivatives, **racemic-115b-c**, were put to preparative-scale kinetic resolution by enzymatic catalysis to explore the impact of various substituents' effects on the benzene ring of the tetrahydroquinoline. Evaluated lipases showed less enantioselectivity (E=21-140) towards substituted derivatives than rac- 115a. Additionally, the racemic substrate with strong electron-withdrawing group (nitro group) **rac-115c** proved to be harder for lipases to discriminate between enantiomers. The reaction catalyzed by Novozym 435 produced optically pure alcohol **(S)-(+)-115c** in >99% ee with 35% isolated yield. It was considered the ideal reaction condition. With novozym 435, racemic substrates **rac-115-b** and **rac-115c** proceeded at greater reaction rate than **rac-115a** with 60-62% conversion. Amano PS-IM changed the nitro derivative **rac-115c** more quickly, resulting 46% conversion in 24 hrs. The enantiomeric excess of the generated acetate **(R)-(+)-116c** decreased due to an increased reaction rate in **rac-**

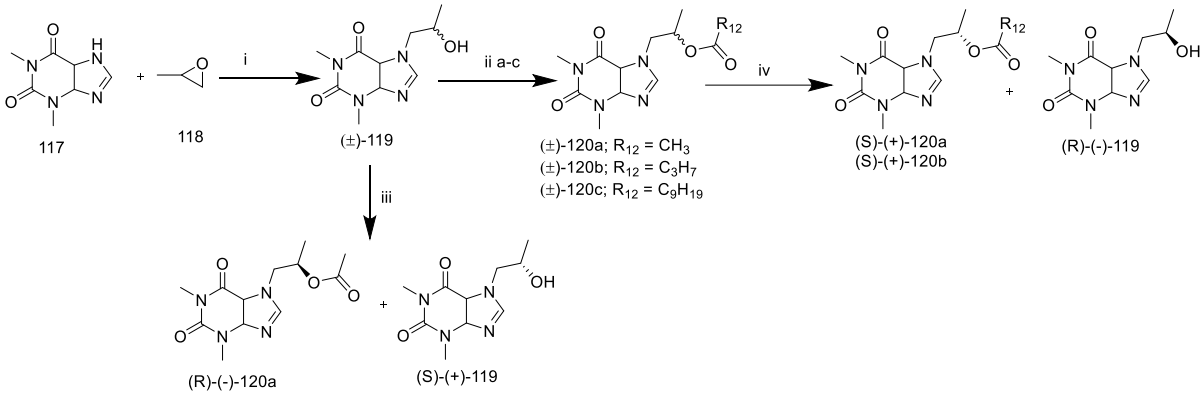
115c. The corresponding acetates **(R)-(+)-116a-c** were further reacted for methanolysis catalyzed by K_2CO_3 to produce **(R)-(-)-115a-c.** The reaction was conducted at 25 °C for 24 hrs resulting **(R)-(-)-115a** with 99% ee and quantifiable yield, **(R)-(-)-115b** with 98% ee and 27% yield, **(R)-(-)-115c** with 99% ee and 69% yield. In this research, also MsAcT variant catalyzed kinetic resolution of racemic **115 a-c** has been investigated in an 200mM potassium phosphate buffer (pH 7.5) with 1M, 10% v/v vinyl acetate as acyl donor. The reaction condition maintained was at 30 °C for 5hrs. For **rac-115a**, F150A/F154A variants gave better result than engineered MsAcTs (E=149). **(S)-(+)-115a** produced with 98% ee and **(R)-(+)-116a** with 51% conversion and 94% ee. For **rac-115b**, F154A and F150A/F154A variants gave E=328 and 198 respectively. F154A Produced **(S)-(+)-115b** with 50% ee, 34% conv and **(R)-(+)-116b** with 34% conversion and 99% ee. F150A/F154A variant produced **(S)-(+)-115b** with 89% ee, and **(R)-(+)-116b** with 48% conversion and 89% ee. All of the MsAcT variants showed moderate enantiomeric purity for rac-115c (E=1-27). **(R)-(+)-116c** was produced with E value 27 an 91% ee catalyzed by F154A. whereas F150A/F154A variant produced **(R)-(+)-116c** with 55%ee (E= 8).

Overall, Lipase catalyzed reactions showed greater enantiopurity. Surprisingly, MsAcT variant showed better enantioselectivity towards the **rac- 115b** (E=328) than lipase (E=140). It also mentioned that the electronic characteristics of the substituents in benzene ring of the tetrahydroquinoline scaffold had a major impact on the MsAcT variants. So, both approaches can be considered complimentary depending on racemic substrate being used. Specific optical rotation observed for **(S)-(+)-115 a** $[\alpha]_D^{26}$ = +22.75 (c 1.06, $CHCl_3$), **(S)-(+)-115 b** $[\alpha]_D^{28}$ = +19.42 (c 1.03, $CHCl_3$), **(S)-(+)-115 c** $[\alpha]_D^{26}$ = +75.12 (c 1.02, $CHCl_3$), **(R)-(+)-116 a** $[\alpha]_D^{26}$ = +4.90 (c 1.02, $CHCl_3$), **(R)-(+)-116 b** $[\alpha]_D^{26}$ = +4.85 (c 1.03, $CHCl_3$), **(R)-(+)-116 c** $[\alpha]_D^{26}$ = +23.90 (c 1.02, $CHCl_3$).[82]

Synthesis of proxyphylline enantiomers

Another renowned Pharmaceutically useful molecule Proxyphylline, having great vasodilator, cardiac stimulant, and bronchodilator actions have been synthesized by easy and effective chemoenzymatic method by using lipase. In spite of the fact that lipases are widely used biocatalysts for esterification of carboxylic acids, enantioselective hydrolysis of esters, enantioselective transesterification, such hydrolytic enzymes have not been applied previously for 1,3-dimethylxanthines. The synthesis of proxyphylline **(±)-119** was started from commercially available theophylline **(117)** and propylene oxide **(118)** to obtain racemic **(±)-**

119. which was further carried out for biocatalysis studies. O-butyryl ester **(±)-105b** was obtained with remarkable yield (94%) When racemic alcohol **(±)-119** was reacted with vinyl butanoate and Novozym 435 in MTBE as solvent (scheme 31). Surprisingly, Substrate **(±)-119** was not completely soluble in MTBE, but the solubility significantly increased upon adding vinyl butyrate, which allowed to complete the esterification more successfully with a better outcome than the conventional approach. In chloroform, the enzymatic kinetic resolution of **(±)-119** was studied using the conventional methodology, and an excess amount of vinyl acetate was added as acetyl transfer reagent. Whereas less polar solvents like hexane, cyclohexane, pentane tert-amyl alcohol and more polar solvents like DCM, MTBE, THF, diisopropyl ether, acetonitrile were appropriate for acetylation of **(±)-119**.



i. propylene oxide (95), Et_3N, MeOH iia. Ac_2O, DMAP, dry DCM, iib. C_3H_7COCl or C_9H_9COCl, NEt_3, DMAP, dry DCM iic. vinyl butanoate, novozyme 435 (5% w/w), MTBE iii. vinyl ester, lipase (20% w/w), iv. H_2O or Tris-HCl Buffer/ MeOH, Lipase

Scheme 31: Synthesis of both enantiomers of proxyphylline by chemoenzymatic approach

Another way of enzymatic catalysis has also been investigated which is lipase catalyzed alcoholysis of **(±)-120a**. Despite the fact that low molecular weight alcohols specially methanol and ethanol inactivate enzymes, enzymatic alcoholysis was carried out in methanol as acetyl-acceptor agent with high equivalent. Tendency of polar solvents are to take off the layer of water molecule from protein which is very essential in protein structure so as a result it alters the native structure of protein and finally denatures the enzyme. But in this case, it has been observed that the methanol was useful for the solubility of substrate **(±)-120a** yet, enantioselectivity (E= 16-22), enantiomeric excess of the produced alcohol **(R)-(-)-119** was observed in 72-73% ee. Even though alcoholysis produced slightly higher enantioselectivity than transesterification, the process took excessive time. For lipase catalyzed hydrolysis, lipophilicity of proxyphylline **(±) 119** has been increased by including longer fatty acyl chain in place of acetate moiety. Mimicking natural substrates, decanoate **(±)-120c** and butyrate **(±)-120b** has been introduced for better enantiopurity. But, unfortunately reaction monitoring for

(±)-120c was difficult because non-volatility of **(±)-120c** has made failed HPLC and GC analysis. Where as in case of **(±)-120a** and **(±)-120b**, kinetic resolution showed better result of enantiomeric purity. **(S)-(+)-120b** obtained with 96-98% ee and **(R)-119** obtained with 94-97% ee. So, among various enzymatic strategy applied for the reaction, methanolysis and hydrolysis was observed to be efficient method than transesterification approach. The methodology presented in this research work has demonstrated a promising potential for industrial application due good yield, high enantiopurity, non-chromatographic work-ups. [83]

Conclusion

Lipase enzymes was found to be a suitable enzyme for synthesis of pharmaceuticals products. A plenty of literature is reported for kinetic resolution of racemic alcohols to get desired chiral alcohols. These enzymes are recycled for further reactions, which makes chemoenzymatic reactions a green and sustainable process for manufacturing of pharmaceuticals.

REFERENCES

[1] P.T. Anastas, J.B. Zimmerman, The United Nations sustainability goals: How can sustainable chemistry contribute?, Curr Opin Green Sustain Chem 13 (2018) 150–153. https://doi.org/10.1016/j.cogsc.2018.04.017.

[2] R.A. Sheldon, Fundamentals of green chemistry: Efficiency in reaction design, Chem Soc Rev 41 (2012) 1437–1451. https://doi.org/10.1039/c1cs15219j.

[3] D.J.C. Constable, Green and sustainable chemistry – The case for a systems-based, interdisciplinary approach, IScience 24 (2021) 103489. https://doi.org/10.1016/J.ISCI.2021.103489.

[4] R.A. Sheldon, J.M. Woodley, Role of Biocatalysis in Sustainable Chemistry, Chem Rev 118 (2018) 801–838. https://doi.org/10.1021/acs.chemrev.7b00203.

[5] K.N. Ganesh, D. Zhang, S.J. Miller, K. Rossen, P.J. Chirik, M.C. Kozlowski, J.B. Zimmerman, B.W. Brooks, P.E. Savage, D.T. Allen, A.M. Voutchkova-Kostal, Green Chemistry: A Framework for a Sustainable Future, Org Process Res Dev 25 (2021) 1455–1459. https://doi.org/10.1021/acs.oprd.1c00216.

[6] D.M. Heard, A.J.J. Lennox, Dichloromeldrum's acid (DiCMA): A practical and green amine dichloroacetylation reagent, Org Lett 23 (2021) 3368–3372. https://doi.org/10.1021/acs.orglett.1c00850.

[7] Highly Enantioselective Epoxidation of α,β-Unsaturated Ketones Using Amide-Based Cinchona Alkaloids as Hybrid Phase-Transfer Catalysts, (n.d.). https://pubs.acs.org/doi/epdf/10.1021/acs.orglett.0c03272 (accessed January 8, 2024).

[8] A. Nitti, P. Osw, G. Calcagno, C. Botta, S.I. Etkind, G. Bianchi, R. Po, T.M. Swager, D. Pasini, One-Pot Regiodirected Annulations for the Rapid Synthesis of Ï-Extended Oligomers, Org Lett 22 (2020) 3263–3267. https://doi.org/10.1021/ACS.ORGLETT.0C01043/SUPPL_FILE/OL0C01043_SI_002.ZIP.

[9] J.L. Tucker, M.M. Faul, Industrial research: Drug companies must adopt green chemistry, Nature 2016 534:7605 534 (2016) 27–29. https://doi.org/10.1038/534027a.

[10] V.R. Veleva, B.W. Cue, S. Todorova, H. Thakor, N.H. Mehta, K.B. Padia, Benchmarking green chemistry adoption by the Indian pharmaceutical supply chain, Green Chem Lett Rev 11 (2018) 439–456. https://doi.org/10.1080/17518253.2018.1530802.

[11] R.A. Sheldon, D. Brady, Broadening the Scope of Biocatalysis in Sustainable Organic Synthesis, ChemSusChem 12 (2019) 2859–2881. https://doi.org/10.1002/cssc.201900351.

[12] A.M. Foley, A.R. Maguire, The Impact of Recent Developments in Technologies which Enable the Increased Use of Biocatalysts, European J Org Chem 2019 (2019) 3713–3734. https://doi.org/10.1002/EJOC.201900208.

[13] A. Kinner, P. Nerke, R. Siedentop, T. Steinmetz, T. Classen, K. Rosenthal, M. Nett, J. Pietruszka, S. Lütz, Recent Advances in Biocatalysis for Drug Synthesis, Biomedicines 2022, Vol. 10, Page 964 10 (2022) 964. https://doi.org/10.3390/BIOMEDICINES10050964.

[14] A. Fryszkowska, P.N. Devine, Biocatalysis in drug discovery and development, Curr Opin Chem Biol 55 (2020) 151–160. https://doi.org/10.1016/j.cbpa.2020.01.012.

[15] N.J. Turner, M.D. Truppo, Biocatalysis enters a new era, Curr Opin Chem Biol 17 (2013) 212–214. https://doi.org/10.1016/J.CBPA.2013.02.026.

[16] M.T. Reetz, Biocatalysis in organic chemistry and biotechnology: Past, present, and future, J Am Chem Soc 135 (2013) 12480–12496. https://doi.org/10.1021/ja405051f.

[17] G.W. Huisman, S.J. Collier, On the development of new biocatalytic processes for practical pharmaceutical synthesis, Curr Opin Chem Biol 17 (2013) 284–292. https://doi.org/10.1016/j.cbpa.2013.01.017.

[18] Thomas E. Gradel, James Clark, Duncan Macquarrie, Green Chemistry and Sustainable Development, weily, 2002.

[19] B. Hauer, Embracing Nature's Catalysts: A Viewpoint on the Future of Biocatalysis, ACS Catal 10 (2020) 8418–8427. https://doi.org/10.1021/acscatal.0c01708.

[20] R.A. Sheldon, D. Brady, M.L. Bode, The Hitchhiker's guide to biocatalysis: Recent advances in the use of enzymes in organic synthesis, Chem Sci 11 (2020) 2587–2605. https://doi.org/10.1039/c9sc05746c.

[21] M.A. Rao, R. Scelza, F. Acevedo, M.C. Diez, L. Gianfreda, Enzymes as useful tools for environmental purposes, Chemosphere 107 (2014) 145–162. https://doi.org/10.1016/j.chemosphere.2013.12.059.

[22] R.A. Sheldon, The E Factor: Fifteen years on, Green Chemistry 9 (2007) 1273–1283. https://doi.org/10.1039/b713736m.

[23] E.S. Okeke, T.P.C. Ezeorba, C.O. Okoye, Y. Chen, G. Mao, W. Feng, X. Wu, Environmental and health impact of unrecovered API from pharmaceutical manufacturing wastes: A review of contemporary treatment, recycling and management strategies, Sustain Chem Pharm 30 (2022) 100865. https://doi.org/10.1016/J.SCP.2022.100865.

[24] D. Klein-Marcuschamer, P. Oleskowicz-Popiel, B.A. Simmons, H.W. Blanch, The challenge of enzyme cost in the production of lignocellulosic biofuels, Biotechnol Bioeng 109 (2012) 1083–1087. https://doi.org/10.1002/bit.24370.

[25] S. Simić, E. Zukić, L. Schmermund, K. Faber, C.K. Winkler, W. Kroutil, Shortening Synthetic Routes to Small Molecule Active Pharmaceutical Ingredients Employing Biocatalytic Methods, Chem Rev 122 (2022) 1052–1126. https://doi.org/10.1021/acs.chemrev.1c00574.

[26] B.M. Nestl, S.C. Hammer, B.A. Nebel, B. Hauer, New generation of biocatalysts for organic synthesis, Angewandte Chemie - International Edition 53 (2014) 3070–3095. https://doi.org/10.1002/anie.201302195.

[27] T.P. Khobragade, S. Sarak, A.D. Pagar, H. Jeon, P. Giri, H. Yun, Synthesis of Sitagliptin Intermediate by a Multi-Enzymatic Cascade System Using Lipase and Transaminase With Benzylamine as an Amino Donor, Front Bioeng Biotechnol 9 (2021) 757062. https://doi.org/10.3389/FBIOE.2021.757062/BIBTEX.

[28] T. Li, J. Liang, A. Ambrogelly, T. Brennan, G. Gloor, G. Huisman, J. Lalonde, A. Lekhal, B. Mijts, S. Muley, L. Newman, M. Tobin, G. Wong, A. Zaks, X. Zhang, Efficient, chemoenzymatic process for manufacture of the boceprevir bicyclic [3.1.0]proline intermediate based on amine oxidase-catalyzed desymmetrization, J Am Chem Soc 134 (2012) 6467–6472. https://doi.org/10.1021/JA3010495/SUPPL_FILE/JA3010495_SI_001.PDF.

[29] (M Bhupathy, D.R. Sidler, J.M. Mcnamara, R.P. Volante, J.J. Bergan, Synthesis of Natural Products and Potential Drugs Development of a Biocatalytic Process as an Alternative to the (-)-DIP-Cl-Mediated Asymmetric Reduction of a Key Intermediate of Montelukast Synthesis of Montelukast The four-step conversion of B into montelukast sodium is described in the Merck process patent, Org. Process Res. Dev 14 (2010) 193–198. https://doi.org/10.1055/s-0029-1219648.

[30] S.-F. Li, W. Zhang, W. Zhang, A. Huang, J.-Q. Zhu, Y.-J. Wang, Y.-G. Zheng, Recent Advances in the Synthesis and Analysis of Atorvastatin and its Intermediates, Curr Med Chem 31 (2023). https://doi.org/10.2174/0109298673263728231004053704.

[31] C.K. Winkler, D. Clay, S. Davies, P. O'Neill, P. McDaid, S. Debarge, J. Steflik, M. Karmilowicz, J.W. Wong, K. Faber, Chemoenzymatic asymmetric synthesis of pregabalin precursors via asymmetric bioreduction of β-cyanoacrylate esters using ene-reductases, Journal of Organic Chemistry 78 (2013) 1525–1533. https://doi.org/10.1021/JO302484P/SUPPL_FILE/JO302484P_SI_001.PDF.

[32] S. Javed, F. Azeem, S. Hussain, I. Rasul, M.H. Siddique, M. Riaz, M. Afzal, A. Kouser, H. Nadeem, Bacterial lipases: A review on purification and characterization, Prog Biophys Mol Biol 132 (2018) 23–34. https://doi.org/10.1016/j.pbiomolbio.2017.07.014.

[33] N. Sarmah, D. Revathi, G. Sheelu, K. Yamuna Rani, S. Sridhar, V. Mehtab, C. Sumana, Recent advances on sources and industrial applications of lipases, Biotechnol Prog 34 (2018) 5–28. https://doi.org/10.1002/BTPR.2581.

[34] P. Chandra, Enespa, R. Singh, P.K. Arora, Microbial lipases and their industrial applications: A comprehensive review, Microb Cell Fact 19 (2020). https://doi.org/10.1186/s12934-020-01428-8.

[35] B.H. Kim, J. Hwang, C.C. Akoh, Liquid microbial lipase — recent applications and expanded use through immobilization, Curr Opin Food Sci 50 (2023) 100987. https://doi.org/10.1016/J.COFS.2023.100987.

[36] F. Yvergnaux, Lipases: Particularly effective biocatalysts for cosmetic active ingredients, in: OCL - Oilseeds and Fats, Crops and Lipids, EDP Sciences, 2017. https://doi.org/10.1051/ocl/2017013.

[37] Y. Khambhaty, Applications of enzymes in leather processing, Environmental Chemistry Letters 2020 18:3 18 (2020) 747–769. https://doi.org/10.1007/S10311-020-00971-5.

[38] K.K. Bhardwaj, R. Gupta, Synthesis of chirally pure enantiomers by lipase, J Oleo Sci 66 (2017) 1073–1084. https://doi.org/10.5650/jos.ess17114.

[39] A.S. de Miranda, L.S.M. Miranda, R.O.M.A. de Souza, Lipases: Valuable catalysts for dynamic kinetic resolutions, Biotechnol Adv 33 (2015) 372–393. https://doi.org/10.1016/J.BIOTECHADV.2015.02.015.

[40] D.A. Mitchell, N. Krieger, Kinetics of lipase-catalyzed kinetic resolutions of racemic compounds: Reparameterization in terms of specificity constants, Biochem Eng J 181 (2022) 108397. https://doi.org/10.1016/J.BEJ.2022.108397.

[41] A. Ghanem, H.Y. Aboul-Enein, Application of lipases in kinetic resolution of racemates, Chirality 17 (2005) 1–15. https://doi.org/10.1002/CHIR.20089.

[42] J. Vanden, M. Emília De Sousa, G. Wang, K. Kopka, A. Mayence, J. Jose, G. Hancu, A. Modroiu, Chiral Switch: Between Therapeutical Benefit and Marketing Strategy, Pharmaceuticals 2022, Vol. 15, Page 240 15 (2022) 240. https://doi.org/10.3390/PH15020240.

[43] O. Khiari, N. Bouzemi, J.M. Sánchez-Montero, A.R. Alcántara, On the Use of Orthoformates as an Efficient Approach to Enhance the Enzymatic Enantioselective Synthesis of (S)-Ibuprofen, Catalysts 13 (2023). https://doi.org/10.3390/catal13020251.

[44] J.Y. Xin, L.R. Sun, S.M. Chen, Y. Wang, C.G. Xia, Synthesis of L-Ascorbyl Flurbiprofenate by Lipase-Catalyzed Esterification and Transesterification Reactions, Biomed Res Int 2017 (2017). https://doi.org/10.1155/2017/5751262.

[45] W.F. Kean, C.J.L. Lock, J. Rischke, R. Butt, W.W. Buchanan, H. Howard-Lock, Effect of R and S enantiomers of naproxen on aggregation and thromboxane production in

human platelets, J Pharm Sci 78 (1989) 324–327. https://doi.org/10.1002/JPS.2600780413.

[46] E. Cernia, M. Delfini, E. Di Cocco, C. Palocci, S. Soro, Investigation of lipase-catalysed hydrolysis of naproxen methyl ester: use of NMR spectroscopy methods to study substrate–enzyme interaction, Bioorg Chem 30 (2002) 276–284. https://doi.org/10.1016/S0045-2068(02)00014-7.

[47] J.J. Lalonde, C. Govardhan, N. Khalaf, A.G. Martinez, K. Visuri, A.L. Margolin, Cross-Linked Crystals of Candida rugosa Lipase: Highly Efficient Catalysts for the Resolution of Chiral Esters, J Am Chem Soc 117 (1995) 6845–6852. https://doi.org/10.1021/JA00131A006/ASSET/JA00131A006.FP.PNG_V03.

[48] P.Y. Wang, Y.J. Chen, A.C. Wu, Y.S. Lin, M.F. Kao, J.R. Chen, J.F. Ciou, S.W. Tsai, (R,S)-Azolides as Novel Substrates for Lipase-Catalyzed Hydrolytic Resolution in Organic Solvents, Adv Synth Catal 351 (2009) 2333–2341. https://doi.org/10.1002/ADSC.200900391.

[49] M. Mohammadi, S. Gandomkar, Z. Habibi, M. Yousefi, One pot three-component reaction for covalent immobilization of enzymes: application of immobilized lipases for kinetic resolution of rac-ibuprofen, RSC Adv 6 (2016) 52838–52849. https://doi.org/10.1039/C6RA11284F.

[50] A. Ghanem, Direct enantioselective HPLC monitoring of lipase-catalyzed kinetic resolution of flurbiprofen, Chirality 22 (2010) 597–603. https://doi.org/10.1002/CHIR.20798.

[51] D. Chavez-Flores, J.M. Salvador, Facile conversion of racemic ibuprofen to (S)-ibuprofen, Tetrahedron Asymmetry 23 (2012) 237–239. https://doi.org/10.1016/J.TETASY.2012.02.014.

[52] C. José, R.D. Bonetto, L.A. Gambaro, M.D.P. Guauque Torres, M.L. Foresti, M.L. Ferreira, L.E. Briand, Investigation of the causes of deactivation–degradation of the commercial biocatalyst Novozym® 435 in ethanol and ethanol–aqueous media, J Mol Catal B Enzym 71 (2011) 95–107. https://doi.org/10.1016/J.MOLCATB.2011.04.004.

[53] M. Lotti, J. Pleiss, F. Valero, P. Ferrer, Effects of methanol on lipases: Molecular, kinetic and process issues in the production of biodiesel, Biotechnol J 10 (2015) 22–30. https://doi.org/10.1002/BIOT.201400158.

[54] M. Mangiagalli, D. Ami, M. de Divitiis, S. Brocca, T. Catelani, A. Natalello, M. Lotti, Short-chain alcohols inactivate an immobilized industrial lipase through two different mechanisms, Biotechnol J 17 (2022) 2100712. https://doi.org/10.1002/BIOT.202100712.

[55] L. Wang, W. Du, D. Liu, L. Li, N. Dai, Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in tert-butanol system, J Mol Catal B Enzym 43 (2006) 29–32. https://doi.org/10.1016/J.MOLCATB.2006.03.005.

[56] Y. Shimada, Y. Watanabe, T. Samukawa, A. Sugihara, H. Noda, H. Fukuda, Y. Tominaga, Conversion of vegetable oil to biodiesel using immobilized Candida antarctica lipase, J Am Oil Chem Soc 76 (1999) 789–793. https://doi.org/10.1007/S11746-999-0067-6.

[57] A.M. Panico, V. Cardile, F. Vittorio, G. Ronsisvalle, G.M. Scoto, C. Parenti, B. Gentile, R. Morrone, G. Nicolosi, Different in vitro activity of flurbiprofen and its enantiomers on human articular cartilage, Farmaco 58 (2003) 1339–1344. https://doi.org/10.1016/S0014-827X(03)00196-4.

[58] B. Zdun, P. Cieśla, J. Kutner, P. Borowiecki, Expanding Access to Optically Active Non-Steroidal Anti-Inflammatory Drugs via Lipase-Catalyzed KR of Racemic Acids Using Trialkyl Orthoesters as Irreversible Alkoxy Group Donors, Catalysts 12 (2022). https://doi.org/10.3390/catal12050546.

[59] J.S. Carey, E. McCann, Lipase-Catalyzed Regioselective Ester Hydrolysis as a Key Step in an Alternative Synthesis of a Buprenorphine Pro-Drug, Org Process Res Dev 23 (2019) 771–774. https://doi.org/10.1021/acs.oprd.9b00026.

[60] V.K. Vashistha, A. Kumar, Stereochemical facets of clinical β-blockers: An overview, Chirality 32 (2020) 722–735. https://doi.org/10.1002/CHIR.23200.

[61] A. Sikora, J. Chałupka, M.P. Marszałł, The use of ion liquids as a trojan horse strategy in enzyme-catalyzed biotransformation of (R,S)-atenolol, Catalysts 10 (2020). https://doi.org/10.3390/catal10070787.

[62] L. Banoth, U.C. Banerjee, New chemical and chemo-enzymatic synthesis of (RS)-, (R)-, and (S)-esmolol, Arabian Journal of Chemistry 10 (2017) S3603–S3613. https://doi.org/10.1016/J.ARABJC.2014.03.011.

[63] L. Bocquin, E.E. Jacobsen, Chemoenzymatic Protocol for the Synthesis of Enantiopure β-Blocker (S)-Bisoprolol, Catalysts 13 (2023). https://doi.org/10.3390/catal13010054.

[64] S.H. Troøyen, L. Bocquin, A.L. Tennfjord, K. Klungseth, E.E. Jacobsen, Green Chemo-Enzymatic Protocols for the Synthesis of Enantiopure β-Blockers (S)-Esmolol and (S)-Penbutolol, Catalysts 12 (2022). https://doi.org/10.3390/catal12090980.

[65] M.A. Gundersen, G.B. Austli, E.E. Jacobsen, S.S. Løvland, M.B. Hansen, M. Rødseth, Lipase catalyzed synthesis of enantiopure precursors and derivatives for β-blockers practolol, pindolol and carteolol, Catalysts 11 (2021). https://doi.org/10.3390/catal11040503.

[66] S.H. Troøyen, E.E. Jacobsen, Chemo-Enzymatic Synthesis of Enantiopure β-Antagonist (S)-Betaxolol, Catalysts 12 (2022). https://doi.org/10.3390/catal12121645.

[67] C.T. Seebauer, M.S. Graus, L. Huang, A. McCann, J. Wylie-Sears, F. Fontaine, T. Karnezis, D. Zurakowski, S.J. Staffa, F. Meunier, J.B. Mulliken, J. Bischoff, M. Francois, Non–beta blocker enantiomers of propranolol and atenolol inhibit vasculogenesis in infantile hemangioma, Journal of Clinical Investigation 132 (2022). https://doi.org/10.1172/JCI151109.

[68] S.N. Kethavath, R.R. Patlolla, K. Rosangzuala, A. Polumati, M. Nemali, S. V. Pawar, L. Banoth, Lipase catalyzed chemo-enzymatic synthesis of propranolol: A newer enzymatic approach, Journal of the Indian Chemical Society 100 (2023). https://doi.org/10.1016/j.jics.2023.101037.

[69] S. Ghosh, J. Bhaumik, L. Banoth, S. Banesh, U.C. Banerjee, Chemoenzymatic Route for the Synthesis of (S)-Moprolol, a Potential β-Blocker, Chirality 28 (2016) 313–318. https://doi.org/10.1002/chir.22574.

[70] F. de A. Bezerra, G. de C. Lima, A.C.L. de M. Carvalho, K.B. Vega, M.C.F. Oliveira, T.L.G. de Lemos, J.C. Sousa dos Santos, L.R.B. Gonçalves, N.S. Rios, R. Fernandez-Lafuente, F. Molinari, G. Zanatta, M.C. de Mattos, Chemoenzymatic synthesis of both enantiomers of propafenone hydrochloride through lipase-catalyzed process, Molecular Catalysis 529 (2022). https://doi.org/10.1016/j.mcat.2022.112540.

[71] C. Sanfilippo, A.A. Paternò, A. Patti, Resolution of racemic amines via lipase-catalyzed benzoylation: Chemoenzymatic synthesis of the pharmacologically active isomers of labetalol, Molecular Catalysis 449 (2018) 79–84. https://doi.org/10.1016/j.mcat.2018.02.017.

[72] S. Ciceri, P. Ferraboschi, P. Grisenti, S.R. Elahi, C. Castellano, M. Mori, F. Meneghetti, (S)-pramipexole and its enantiomer, dexpramipexole: A new chemoenzymatic synthesis and crystallographic investigation of key enantiomeric intermediates, Catalysts 10 (2020). https://doi.org/10.3390/catal10080941.

[73] S. Ciceri, P. Grisenti, S.R. Elahi, P. Ferraboschi, A new chemoenzymatic synthesis of the chiral Key intermediate of the antiepileptic Brivaracetam, Molecules 23 (2018). https://doi.org/10.3390/molecules23092206.

[74] F. Uthoff, J. Löwe, C. Harms, K. Donsbach, H. Gröger, Chemoenzymatic Synthesis of a Chiral Ozanimod Key Intermediate Starting from Naphthalene as Cheap Petrochemical Feedstock, Journal of Organic Chemistry 84 (2019) 4856–4866. https://doi.org/10.1021/acs.joc.8b03290.

[75] C. Sanfilippo, G. Nicolosi, A. Patti, Milnacipran as a challenging example of aminomethyl substrate for lipase-catalyzed kinetic resolution, J Mol Catal B Enzym 104 (2014) 82–86. https://doi.org/10.1016/j.molcatb.2014.03.015.

[76] O. Degórska, D. Szada, T. Jesionowski, J. Zdarta, A biocatalytic approach for resolution of 3-hydroxy-3-phenylpropanonitrile with the use of immobilized enzymes stabilized with ionic liquids, Comput Struct Biotechnol J 21 (2023) 1593–1597. https://doi.org/10.1016/j.csbj.2023.02.026.

[77] G. Martelli, M. Cirillo, V. Giraldi, D. Giacomini, Chemoenzymatic enantioselective route to get (+) and (−) 4-acetoxy-azetidin-2-one by lipase-catalysed kinetic resolution and their applications, Bioorg Chem 120 (2022). https://doi.org/10.1016/j.bioorg.2021.105580.

[78] T. Wang, L.D. Du, D.J. Wan, X. Li, X.Z. Chen, G.F. Wu, Retraction of: A new synthetic route to avibactam: Lipase catalytic resolution and the simultaneous debenzylation/sulfation (Organic Process Research and Development (2018) 22 (267-272) DOI: 10.1021/acs.oprd.7b00290), Org Process Res Dev 22 (2018) 655. https://doi.org/10.1021/acs.oprd.8b00137.

[79] P. Ramesh, T. Harini, N.W. Fadnavis, Efficient resolution of cis -(±)-dimethyl 1-acetylpiperidine-2,3-dicarboxylate with soluble Candida antarctica lipase B (CAL B), Org Process Res Dev 19 (2015) 296–301. https://doi.org/10.1021/op5003424.

[80] N. Vasudevan, G.P. Ahlqvist, C.P. McGeough, D.J. Paymode, F.S.P. Cardoso, T. Lucas, J.P. Dietz, T. Opatz, T.F. Jamison, F.B. Gupton, D.R. Snead, A concise route to MK-4482 (EIDD-2801) from cytidine, Chemical Communications 56 (2020) 13363–13364. https://doi.org/10.1039/d0cc05944g.

[81] T. de S. Fonseca, L.D. Lima, M. da C.F. de Oliveira, T.L.G. de Lemos, D. Zampieri, F. Molinari, M.C. de Mattos, Chemoenzymatic Synthesis of Luliconazole Mediated by Lipases, European J Org Chem 2018 (2018) 2110–2116. https://doi.org/10.1002/ejoc.201800250.

[82] B. Zdun, I. Kopińska, M. Dranka, T. Reiter, W. Kroutil, P. Borowiecki, Chemoenzymatic Synthesis of Optically Active Alcohols Possessing 1,2,3,4-Tetrahydroquinoline Moiety Employing Lipases or Variants of the Acyltransferase from Mycobacterium smegmatis †, Catalysts 12 (2022). https://doi.org/10.3390/catal12121610.

[83] P. Borowiecki, D. Paprocki, A. Dudzik, J. Plenkiewicz, Chemoenzymatic Synthesis of Proxyphylline Enantiomers, Journal of Organic Chemistry 81 (2016) 380–395. https://doi.org/10.1021/acs.joc.5b01840.