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Review

Diversifying Arena of Drug Synthesis: In the Realm of Lipase Mediated Waves of Biocatalysis

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Abstract: Hydrolases, being most prominent enzymes used in industrial processes have left no stone unturned in fascinating the pharmaceutical industry. Lipases, being a part of acyl hydrolases are the ones that function similarly to esterases (except an interfacial action) wherein they generally catalyze the hydrolysis of ester bonds. Be it in terms of stereoselectivity or regioselectivity, lipases have manifested their promiscuous proficiency in rendering biocatalytic drug synthesis and intermediates thereof. Industrial utilization of lipases is prevalent since decades ago, but their distinctive catalytic competencies have rendered them suitable for maneuverability in various tides of biocatalytic industrial process development. Numbers of exquisite catalysts have been fabricated out of lipases using nanobiotechnology whereby enzyme reusability and robustness have been conferred to many of the organic synthesis procedures. This marks a considerable achievement of lipases in the second wave of biocatalysis. Furthermore, in the third wave an advent of genetic engineering has fostered an era of customized lipases for suitable needs. Be it stability or an enhanced efficacy, genetic engineering techniques have ushered an avenue for biocatalytic development of drugs and drug intermediates through greener processes using lipases. Even in the forthcoming concept of co-modular catalytic systems, lipases may be the frontiers because of their astonishing capability to act along with other enzymes. The concept may render feasibility in the development of cascade reactions in organic synthesis. An upcoming wave demands fulfilling the vision of tailored lipase whilst a far-flung exploration needs to be unveiled for various research impediments in rendering lipase as a custom fit biocatalyst in pharmaceutical industry.

Keywords: hydrolases; stereoselectivity; regioselectivity; biocatalysis; nanobiotechnology

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1. Introduction

Biocatalysis–a denotation that pertains to the utilization of enzymes in chemical syntheses or conversions. These enzymes are well curated by Mother Nature for their discrete role in the biological machinery of an array of creatures (ranging from prokaryotes to eukaryotes). Enzymes like chemical catalysts render the conversions of chemical moieties in the biochemical pathways and harnessing them for the purpose (diversifying the scope of conversions) for which they are not evolved is where an essence of biocatalysis lies [1]. Enzymes being greener alternatives to the conventional chemical procedures exhibit unique characteristics such as high catalytic efficiency and selectivity under mild reaction conditions. This broadens the outlook of applicability in fine chemical synthesis, pharmaceutical drug manufacturing and food industries. Despite most of the enzymes in their free state being less stable with limitations in industrial use, biocatalysis have reached its industrially proven level through recurring waves of technological analysis and innovations [2,3]. Lipases (EC 3.1.1.3) belong to the class of hydrolases carrying out the dual role of hydrolyzing as well as synthesizing acylglycerols with long acyl chains (number of

carbon atoms >10) [4]. Lipases differ from esterases in a way they function by acting on interface in contrast to a homogenous solution. This distinctive competence befits them for industrial and biotechnological applications ranging from detergent industry to chiral organic synthesis of fine chemicals and drugs. Remarkable endeavor has been put forth in optimizing activity, specificity and stability of lipases for their appropriate functioning in varied environmental conditions. There has been a consistent rising demand for the production of lipases which matches with a pace in their utility in industrially relevant processes [5]. Their capability of being acting in harsh conditions with thorough stability in organic solvents along with broad substrate specificity as well as high regioselectivity and/or stereoselectivity in catalytic processes potentiates them as one of the most widely used group of biocatalysts for biotechnological processes [6]. The past decade has witnessed an extensive phase of biocatalytic innovation wherein the immobilization of enzymes onto nanostructured supports has furnished biocatalysts with their proven applicability. This has facilitated easy handling, operational stability, facile recovery and reusability thereby leading to better streamlined and economical bioprocesses [7]. Immobilization on nanomaterials imparts significant characteristics to enzymes in regard to thermal or pH stability as well as easy downstream processing which otherwise would have been a challenging issue. This in turn amplifies the scope of immobilized enzyme in various processes ranging from bioconversion to bioremediation to biosensing and so on [8]. Sustained efforts have been put into existence to explore new immobilization strategies for enzymes with high stability and specificity. Moreover, protein engineering has laid an avenue for the design of new catalysts for organic reactions. The field encompasses deciphering the unforeseen prospects of the structural properties of the proteins thus paving way for the tailored enzyme for industry. Protein engineering has focused on such enzymes as bamase, lysozyme, subtilisin and lipase, etc. [9]. Improving enzyme activity and thermo stability is a boon for industrial applications. To render the goal successful, suitable and efficient structure-based rational design and random or "irrational" mutagenesis design are employed. The past decade has witnessed extensive research into the development of novel perspectives of random mutagenesis and subsequent screening or selection for novel desired properties of the enzymes [10]. The adventitious progression of lipases with an emphasis on drug synthesis in the past decade has been discussed. The readers are being directed towards contemporary cognizance on the progressive era of lipase mediated biocatalysis.

Drug synthesis has been an exquisite tradition in the organic synthesis since decades ago. With better understanding of the human biological machinery, novel targets for various diseases show up with a possibility of curing a disease. Hence, novel therapeutic targets are required at almost every stage of drug development and so are the novel strategies to synthesize them. Enzymes have emerged as bliss for Medicinal Chemists with their excellent capability of transforming complex chemical moieties, thus rolling out the possibilities of better targets at every juncture. With consistent upgradation in scientific understandings of the novel molecular mechanisms in the human pathophysiology, drug development continues to be an evolutionary phenomenon. The future medicine relies on deciphering the processes involved in so called complex molecules. Enzymes do come into the play when complicated anomalies are involved. Enzymes have the distinctive feature of "**promiscuity**" which expands their horizon in functional group tolerance. The said, the feature of promiscuity is considered on the basis of serendipity, be it in terms of unpredictable reactions, substrates or reaction conditions. Lipases have displayed a remarkable promiscuity and can be foreseen from their conventional catalyzing hydrolysis, esterification, and transesterification reactions to their catalyzing ability in organic reactions (such as Mannich reaction, Michael addition, Knoevenagel condensation, Baylis-Hillman reaction, Canizzaro reaction, Aldol condensation and much more). The structural properties facilitate the stereoselectivity, chemoselectivity and regioselectivity in lipase action [11,12].

2. Lipases: A Versatile Manifesto

With a general impression for lipases falling under the class of enzymes known as Hydrolases, their further sub-categorization by IUBMB under EC 3.1 family of hydrolyzing enzymes typically make them suitable for acting on ester bonds. The UniProt database harbors enormous number of entries for different types of hydrolases, primarily acting on ester bonds [13]. The typical function of lipases involves the hydrolysis of glycerides (mono-, di- and tri-) into the corresponding fatty acids and alcohols but their tendency to adept to the different chemical environments have been utilized in carrying out an array of biotransformations ranging from regio- and enatio- selective conversions to asymmetric synthesis. These in turn are known to avert the number of side reactions or side product formations which otherwise pose hurdles in conventional organic synthesis [14–17]. The number of hosts with a likelihood presence of lipases involves different species of plants, animals and microorganisms. However, the microbial ones are favored over other sources possibly because of an (a) easy maneuverability in terms of genetic manipulation, (b) economical perspective of faster growth with an onus to lesser doubling time, (c) high yielding fermentative production, (d) robustness and convenience in terms of uninterrupted supply (d) higher performance in range of pH and temperature conditions, etc. [18–21]. The widely accepted norm of environmental-friendly conditions in enzyme catalysis involves use of aqueous media (mainly water) as a solvent. But a limited scope of water as a solvent esp. in case of hydrophobic reagents poses several challenges in utilization of enzymes. Thankfully, the problem has been overcome using various much greener alternatives such as co-solvents, ionic liquids, supercritical fluids, etc. Considering the biocompatibility of enzymes, hydrolases show an utmost level of tolerance in low water media thereby emanating as superior class of catalysts in organic synthesis. Lipases being remarkably adaptable to wide range of complex chemical environments have evolved as a stellar enzyme among the various classes of enzymes [22–25]. With a plausible notion of "*Enzymes in Organic Synthesis*" to "*Enzymes in Medicinal Chemistry*", lipases have left no stone unturned in emerging as tenacious catalysts in industrial settings [26–28]. An evolving notion of "*Enzymes in Process Chemistry*" is no more to be envisaged enigmatic as the forthcoming years glorifies biocatalysis with remarkable performance of lipases.

However, lipases being proteins are not far away from the conventional demerits associated with the protein structures. First of all, being a source from natural origin such as microbes or animals, there is a different level of selectivity, stability and of course the suitability towards varied reaction environments among various types of lipases. Generally, lipases are more favorable towards water immiscible solvents than the water miscible ones and also show inconsistencies in different organic solvents. Closed lid conformation of the lipase active site in the aqueous media has been the major reason behind enzyme inactivation. Also, downstream processing in regard to the concerns to separating and reusing lipase has always been a drawback in industrial usage. In addition to that, thermostability has always been a desire for the production chemists to achieve feasibility of the industrial scale process. Enzymes being protein in nature suffer from the drawback of catalyzing the reactions at elevated temperatures and so are the lipases. To address these major challenges in lipase-mediated biocatalysis, different waves have been emerged with an endeavor to solve the said anomalies (as depicted in Figure 1) [29–39]. Following sections describe the different waves of lipases which have come across in the recent times with an aim of sewing up the said complications (with a distinction in the drugs and drug intermediate synthesis).

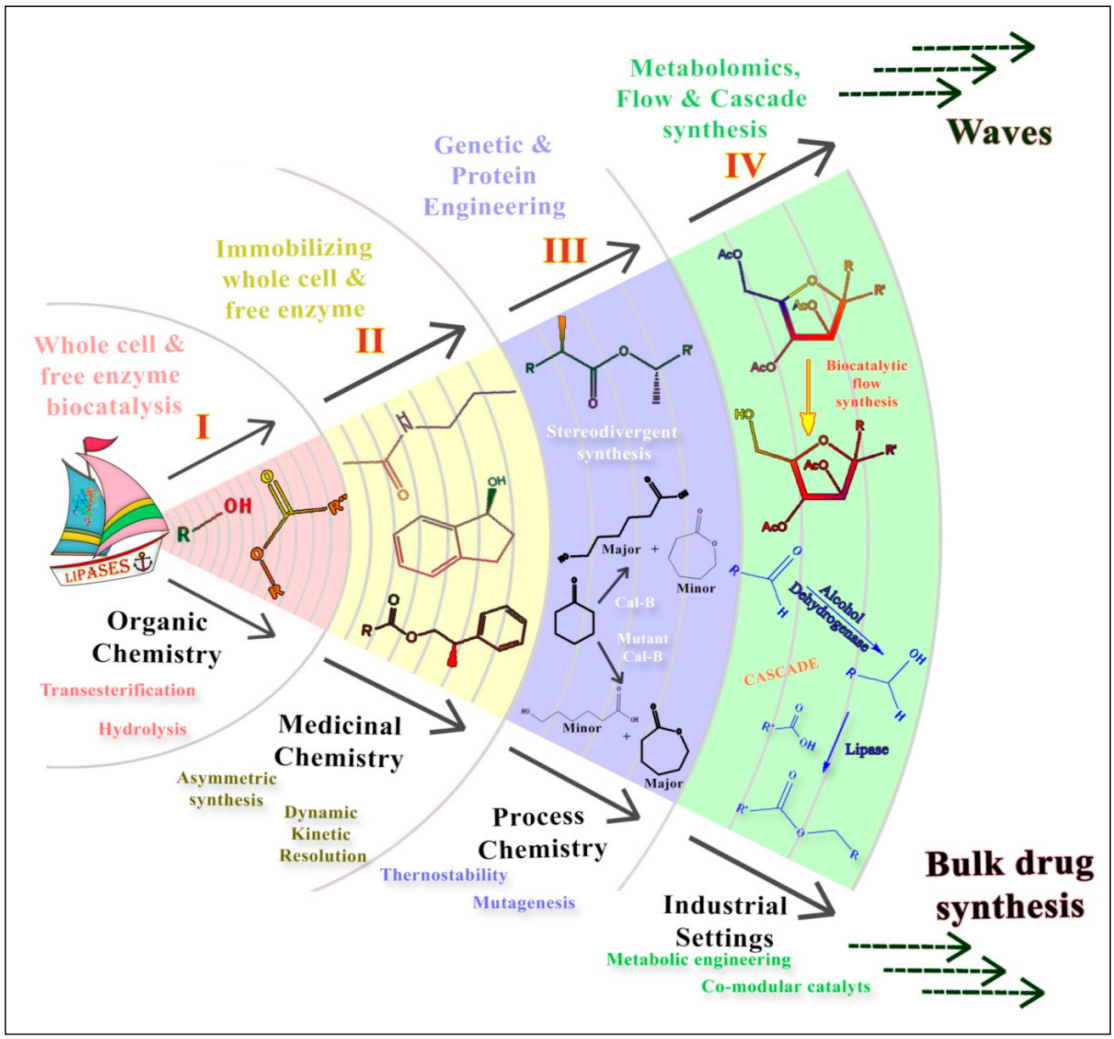


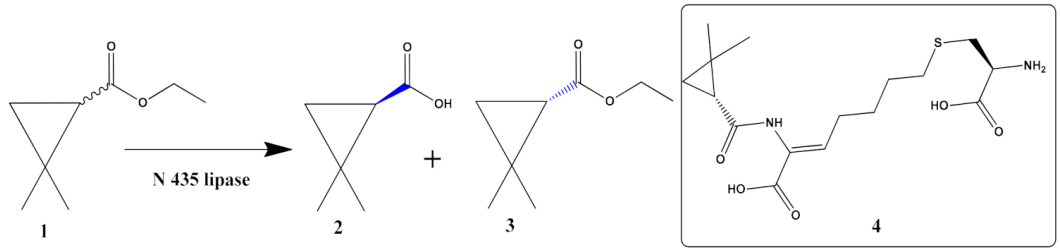
Figure 1. Evolution of Lipases through different waves of biocatalysis [33–39].

3. Second Wave of Lipase Mediated Drug Synthesis

Emergence of enzymes some 100 years ago as useful agents in rendering successful chemical transformations earmarked the course of action for biocatalytic waves. The waves through subsequent progression have made technologies serviceable to the mankind. According to *Bornscheuer*, the second wave of biocatalysis commenced with a focus on stabilizing the enzymes through immobilization as well as emerging protein engineering technologies that focused on structural aspects of proteins. This in turn aided in extending the substrate scope for enzymes along with reusability. The evolution earmarked the widened scope for plausible extraordinary synthetic intermediates thus setting apart the odds to process pharmaceutical intermediates and fine chemicals [1]. Immobilization has been an extensive endeavor of the researchers around the world. Nevertheless to say, the onus is on the specifically designed spatial structures (1D or 3D) which generally through their peculiar characteristics in regard to the shape and size render the possible accumulation of enzymes on their surfaces. These hosts aid in the fixation of enzymes on their surfaces in active form and the resultant enzyme stability is exhibited along with the restricted access to the inhibitors. Lipases are known to exist in two conformational forms viz. open (active) and closed (inactive) form. The open and closed conformation pertains to the lid type structure on the active site of the enzyme which is responsible for binding to the substrate. Lipases are known to get immobilized with high efficiency on the supports. The hydrophobic areas around the active site participate in the immobilization

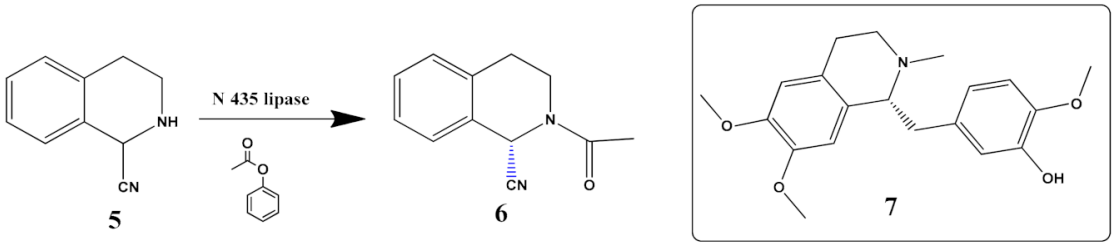
process and aid in keeping the lipases in open configuration. This in turn potentiates the favorable enhancement in the activity of immobilized lipases. Furthermore, the affixed enzymes show higher accumulation of host surfaces (so is the higher activity per unit mass) as compared to their free form [40–42]. Following are the few examples where the utility of lipases in drug synthesis have been employed using the immobilized lipases.

Novozyme 435 (N435), is a commercially available immobilized lipase from Novozymes company. It is based on immobilizing lipase B enzyme from *Candida antarctica* (CALB) on a Lewatit VP OC 1600 anionic resin via interfacial activation. The resin is a macro-porous support with spherical morphology of beads formed by poly (methyl methacrylate) cross linked with divinylbenzene [43]. Numbers of useful biotransformations related to drug intermediates have been reported using N 435. *Liang* et al. in their study utilizing modified N 435 version of lipase has reported the chiral synthesis of (S)-(+)-2,2-dimethylcyclopropane carboxylic acid **2**, a key intermediate in the synthesis of Cilastatin **4**. The drug is a renal dehydropeptidase inhibitor used in combination therapy to prevent the hydrolysis of β-lactam antibiotics such as imipenem. Albeit moderate yields were obtained using the 2, 2-dimethylcyclopropanecarboxylate **1** as a racemic substrate, yet the enatiomeric excess (ee) value obtained was high for the chiral products (Scheme 1). Moreover, Glutaraldehyde modification of the N435 has provided better insights into the reusability characteristics of the immobilized enzyme [44].



Scheme 1. N 435 lipase mediated chiral synthesis of S-(+)-2,2-dimethylcyclopropane carboxylic acid.

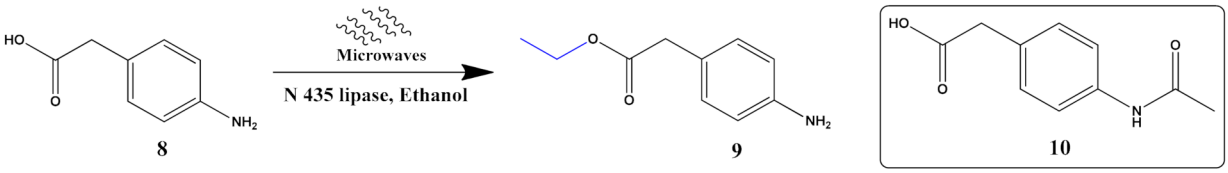
In an interesting work by *Sakulsombat* et al., investigation using different types of lipases has shown a dual capability of simultaneous racemization and amidation of N-methyl α-aminonitriles **5**. Among different types of lipases, N 435 has shown better conversion efficacy when the scope of substrate was extended in the asymmetric synthesis of 1-cyano-1,2,3,4-tetrahydroisoquinoline 6 and derivatives. The said synthesized products (as shown in Scheme 2) find their utility as important class of alkaloid natural products as well as biogenetic precursors of further complex alkaloid families. These ultimately possess a vast scope in synthetic chemistry concerned to natural products. The said alkaloid scaffolds relate to benzylisoquinolines such as (+)-laudanidine **7**, (+)-armepavine, (+)-laudanosine, etc. [45,46].



Scheme 2. N 435 lipase catalyzed dual role of racemization & amidation.

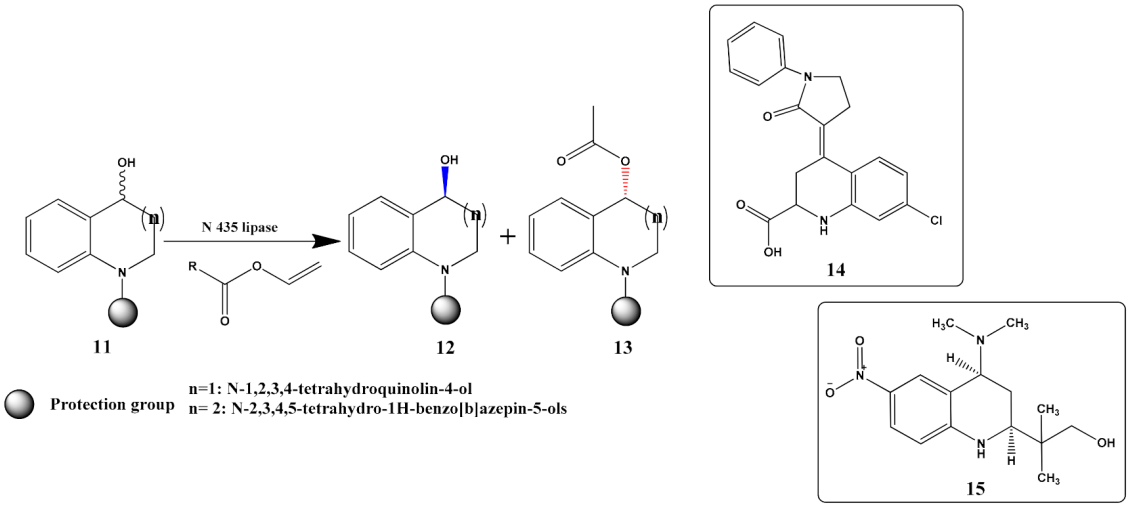
Sontakke et al. has reported the successful synthesis of ethyl-2-(4-aminophenyl) acetate **9** (an intermediate for the disease-modifying antirheumatic drug (DMARD), Actarit **10**) from 4-aminphenylacetic acid **8** using N435 has been achieved using microwave irradiation

(Scheme 3). Lipases have shown an excellent esterification capability in the organic solvent toluene for the said conversion [47].



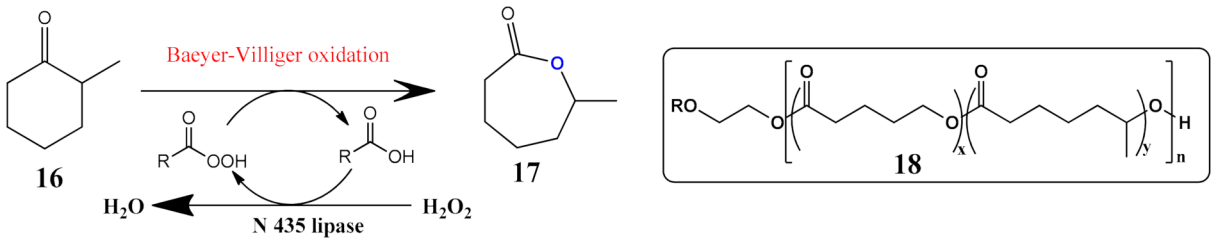
Scheme 3. Microwave assisted esterification using N 435 lipase.

Another study by *Zhou* et al. has evaluated the efficiency of N 435 in rendering enantioselective kinetic resolution of racemic N-protected 1,2,3,4-tetrahydroquinolin-4-ol **11** and 2,3,4,5-tetrahydro-1H-benzo[b]azepin-5-ols **11** to the corresponding enantioselective chiral alcohol **12** as well as acylated derivatives **13** (Scheme 4). The products were obtained with high ee using vinyl 2-chloroacetate as an acyl donor [48]. The chiral precursors N-1,2,3,4-tetrahydroquinolin-4-ol and N-2,3,4,5-tetrahydro-1H-benzo[b] azepin-5-ols finds number of applications in medicinal chemistry like NMDA Glycine site antagonists for prevention of relapsing of smoking (e.g., GW-468816, **14** investigational); selective androgen receptor modulator (SARM) developed for the treatment of osteoporosis (e.g., S-40503, **15** investigational), etc. [49,50].



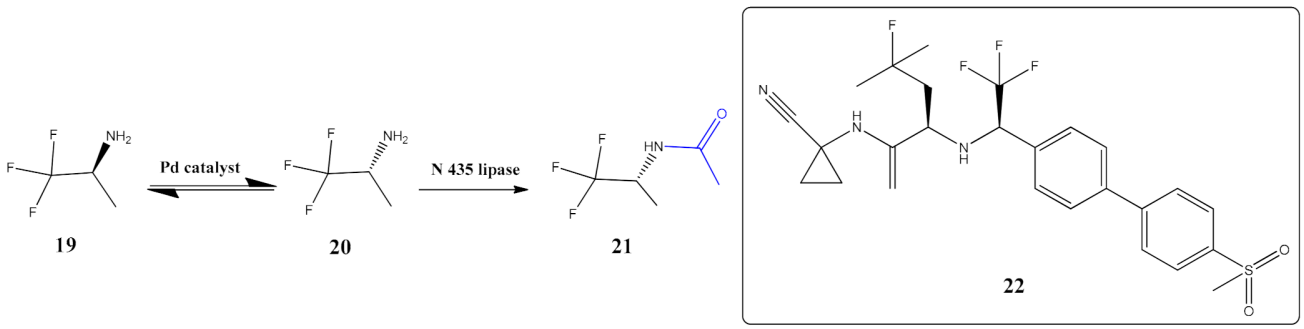
Scheme 4. N435 lipase mediated kinetic resolution to chiral alcohols.

To the heights of promiscuity, lipases have been used in extraordinary circumstances to attain the new levels of chemoenzymatic reactions in organic synthesis. One such study by *Drożdż* et al. has shown an unproven utility of N 435 lipase in rendering clean Baeyer-Villiger oxidation process with a catalyst separation and reusability by using hydrogen peroxide and ionic liquids. A kind of a cyclic cascade in which the oxidation of cyclic ketone **16** to the subsequent ester/lactone **17** proceeds via a peracid which in turn is generated through the carboxylic acid using H_2O_2 as an oxidant and N 435 lipase as a catalyst (Scheme 5). The findings have laid the path to the synthetic chemistry involved in biopolymer synthesis of Oxetane-2-ones (β-lactones) and related compounds. One such study has demonstrated the utility of 6-methyl-ε-caprolactone **17** to generate a co-polymer (poly (δ-valerolactone-co-6-methyl-ε-caprolactone) **18**) with δ-valerolactone via ring opening polymerization process [51,52].



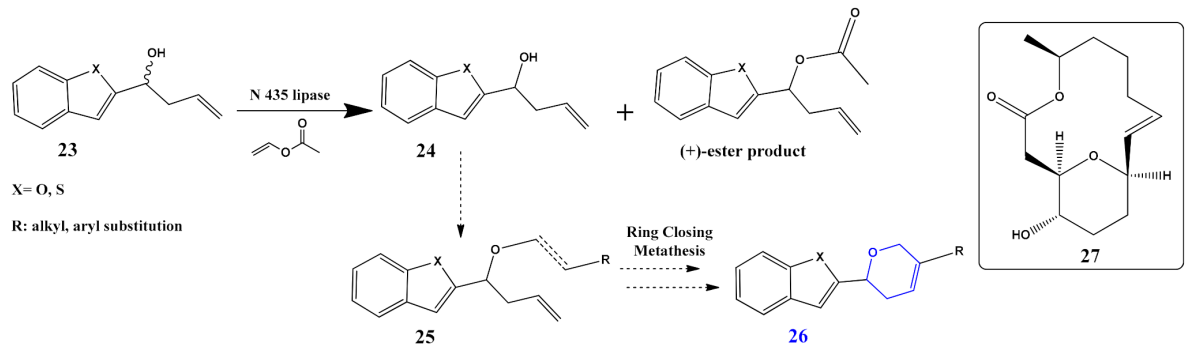
Scheme 5. Baeyer-Villiger oxidation catalyzed by N 435 lipase.

In another study published by *Jiang* et.al., co-operation of Pd catalyst along with N 435 has demonstrated an excellent dynamic kinetic resolution of 1,1,1-trifluoroisopropylamine (TFPA) **19** into the corresponding chiral N-acetyl-1,1,1-trifluoroisopropyl-amine **20** and amide **21** (Scheme 6). Capability of Pd catalysts to render racemizations has prompted the authors to investigate the co-operative compatibilities of the said catalyst with enzymes (N 435 in this case). Response surface method (RSM) – (a modeling technique based on mathematical and statistical analysis to optimize the outcome) has been applied to fine tune various reaction parameters in harmony, which in turn has provided an enhanced response (DKR in this case) for the synthesis of enantiomerically pure amines. On evaluating an effect of Response Surface Methodology (RSM), conversions as good as 95.7% has been obtained for TFPA [53]. Enantiopure α-trifluoromethyl amines find applications in number of biologically active drug motifs bearing a range of potential applications from anti-cancer agents to anti-rheumatic ones and so on. Consider for example, Odanacatib 22, an investigational drug candidate with benzylic α-trifluoromethyl amine functionalization has shown cathepsin K inhibition activity which is desired for the treatment of osteoporosis [54].



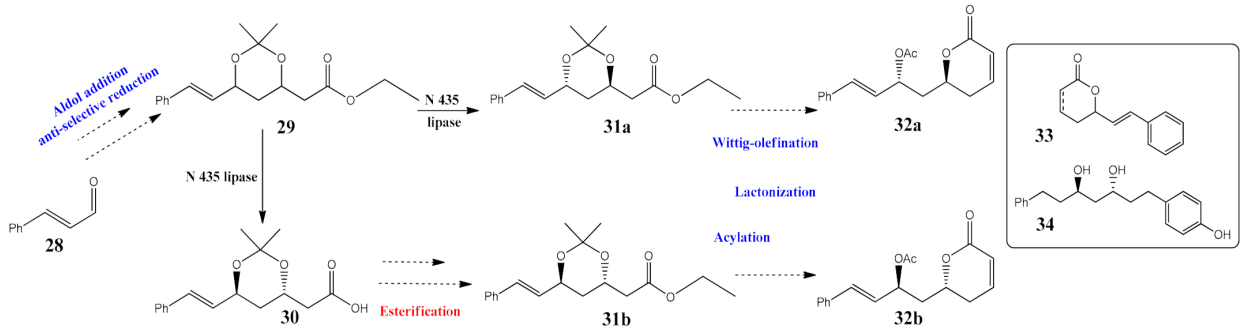
Scheme 6. N 435/Pd conjugation system for dynamic kinetic resolution of amines.

In one of the interesting syntheses of dihydropyran rings using ring closing metathesis (Scheme 7), lipases (N 435) have shown a tremendous potential in stereoselective synthesis of benzofuran and benzothiophene substituted homoallyl alcohol structures from the corresponding racemic homoallyl alcohols **23**. The obtained enantio-enriched homoallyl alcohol products **24** were further rendered chiral diene/enyne structures **25** which were then subsequently subjected to the ring closing metathesis using one of the reported protocols in the literature to achieve the benzofuran and benzothiophene substituted dihydropyran derivatives **26** thereof [55]. Dihydropyran derivatives find their utility in the number of applications of total synthesis of natural products in medicinal chemistry because of their potential pharmacological importance. Macrocycles such as aspergillides (e.g. (−)-aspergillide A, **27**) are known to produce antiproliferative activities thus enabling them as antineoplastic agents in medicinal chemistry [56].



Scheme 7. N 435 lipase catalyzed chiral homoallylic alcohol synthesis.

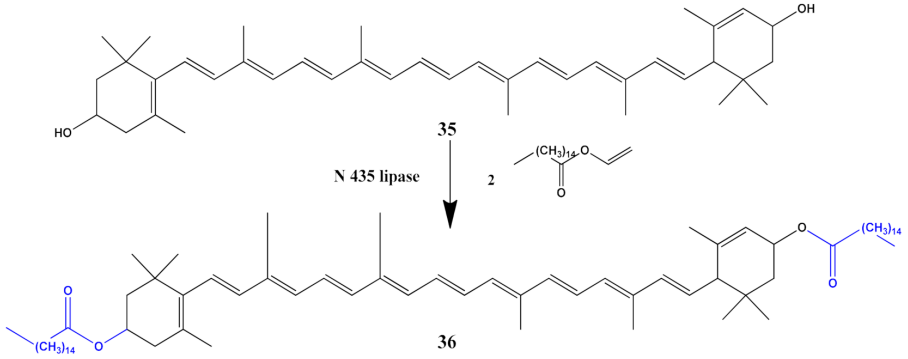
Vast majorities of natural products possess an array of biological activities for which the onus is generally given to the optical/stereo centers present inside the complex chemical molecules. Thus, the total synthesis of complex chemical moieties has always been an intriguing fascination for the medicinal chemists. Chemoenzymatic synthesis has always been a prime choice in the stereochemical synthesis of various scaffolds pertaining to the natural products. One such study using N 435 lipase has been carried out in the synthesis of optically active anti-1,3-diol with styryl unit which have a presence in a number of bioactive natural products such as styryllactones **33** and diarylheptanoids **34** (Scheme 8). Total synthesis of stereoselective crytptolactones **32** have been reported with the chemoenzymatic protocol which involves the conversion of trans-cinnamaldehyde **28** to racemic anti-ethyl-(E)-2-(2,2-dimethyl-6-styryl-1,3-dioxan-4-yl) acetate **29** by aldol addition and anti-selective reduction. Then the Dynamic Kinetic Resolution of **29** to anti-(4R,6R)-ethyl-(E)-2 -(2,2-dimethyl-6-styryl-1,3-dioxan-4-yl) acetate **31a** and corresponding anti-(4S,6S) hydrolyzed acid **30** takes place using N 435 lipase. **30** on subsequent esterification has rendered the anti-(4S,6S)-ethyl-(E)-2-(2,2-dimethyl-6-styryl-1,3-dioxan-4-yl) acetate **31b**. The two chiral products obtained with high ee values were further subjected to chemical reactions to render the synthesis of chiral (+)-(6R,2′S) cryptocaryalactone **32a** and (−)-(6S,2′R) cryptocaryalactone **32b** scaffolds. This kind of natural products scaffolds possess a number of pharmacological activities ranging from anticancer to anti-emetic ones [57].



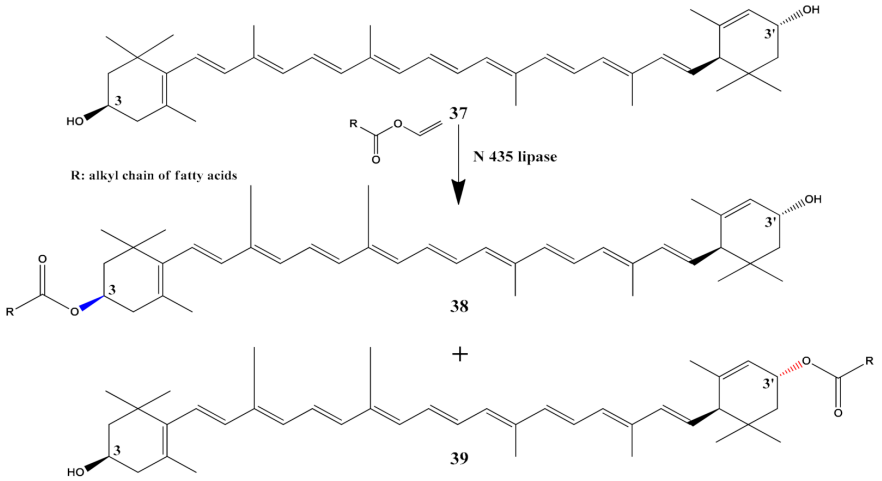
Scheme 8. N 435 lipase mediated optically active anti-1,3-diol with styryl unit.

Not only in drugs, but the utility of N 435 has also been a well-to-do endeavor in synthesizing dietary supplements and derivatives thereof. One such investigation by the *R. Wang* et al. has depicted a transesterification of lutein **35** to the subsequent dipalmitate derivative **36** using N 435 as a biocatalyst (Scheme 9). The reported benefits of diesterified derivatives of lutien have prompted the authors to synthesize the said products with biocatalytic ability thus achieving the conversion rates of >80% in organic solvents [58]. In addition, another study carried out by *J.A. Lujan-Montelongo* et al. has shown regioselective mono-esterification of chiral lutein **37** to render novel 3-O (β-ionone) derivatives using N

435 lipase (Scheme 10). Enzyme mediated esterification has shown higher ratio of lutien-3-O ester, **38**: lutien-3′-O-ester, **39** product thus demarcating a regioselective synthesis of derivatives in natural product synthesis. The study has shown an enhanced action of N 435 over lipases from other sources thus proving its robustness in rendering mono-esterification of the lutein and concerned xanthophylls. The mono-esterified derivatives of xanthophylls possess better bioavailability along with pharmacological properties as antineoplastic agents as well as antioxidants in various eye related disorders [59].



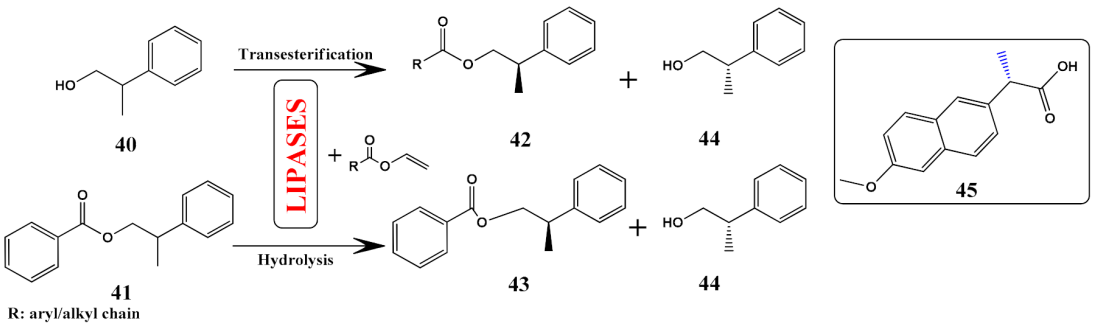
Scheme 9. Diesterification of natural product using N 435 lipase.



Scheme 10. Regioselective mono-esterification of natural products using N 435 lipase.

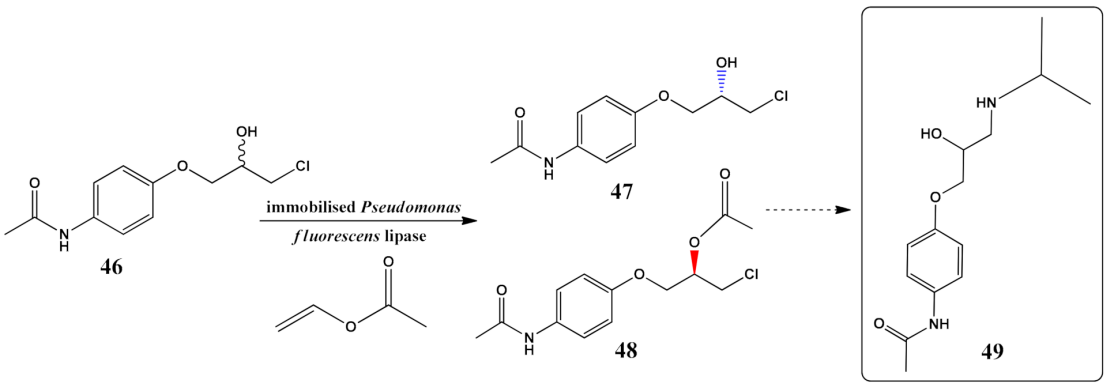
Besides commercially available N 435 lipase, number of other nanostructures has been designed for immobilization of lipases and their utility in organic synthesis of drugs and drug intermediates has been described. Delving into the bottom of promiscuity, *Foley A. M.* and coworkers of lipases have decrypted the paradox for lipases in catalyzing the reactions for varied acylated substrates (Scheme 11). The acylated substrates used for lipase mediated reactions generally involve α-alkyl carboxylic acids or secondary alcohols, however, here the authors have shown the effect of lipase on the β and γ- alkyl carboxylic acids as well as primary alcohols with an aim of resolving remote stereocenters in the chemical moieties. Lipases from different sources (such as *B. cepacia*, *A. niger*, *Alcaligenes* sp., etc., in free form and *Candida antarctica* lipase B; *Pseudomonas fluorescens* lipase in immobilized form) were screened for an effective transesterification of primary alcohols **40** to the corresponding (R)-specific acylated product **42** as well as hydrolysis of β-aryl/alkyl acetates **41** to corresponding enantioselective acetate **43**. (S)-2- Phenyl-1-propanol **44** was obtained as a product in both of the transesterification as well as hydrolysis. Mild to moderate conversions were obtained with satisfactory ee values, but this opens the door for vast scope of substrates for enzyme catalyzed reactions. The research finds an application

in the synthesis of chiral primary alcohols with benzylic stereocenters. The compounds act as intermediaries in the asymmetric synthesis of drug candidates such as those belonging to the class of 2-arylpropionic acids which act as nonsteroidal anti-inflammatory drugs such as Naproxen **45**, ibuprofen, etc. [33].



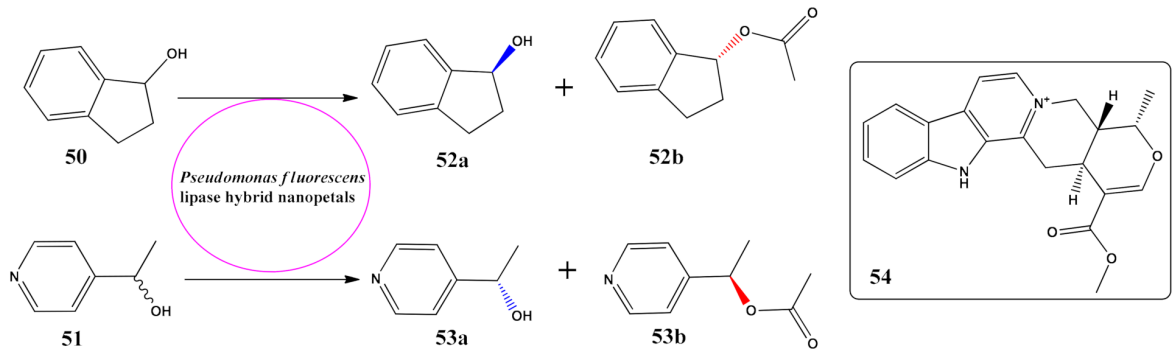
Scheme 11. Lipase mediated biotransformations of atypical substrates.

Physical adsorption of enzyme also plays important roles in developing a robust nanobiocatalyst with easy process viability. One of the findings by *Dwivedee* et al. has demonstrated a physical adsorption efficiency of *Pseudomonas fluorescens* lipase onto the polyaniline nanofibers synthesized by oxidative polymerization of aniline. The proclaimed nanobiocatalyst exhibited an enhanced enzyme efficacy, reusability and thermal stability in biotransformation reactions. Resolution of (RS)-N-(4-(3-chloro-2-hydroxy-propoxy) phenyl) acetamide **46** to subsequent stereoselective acetamide **47** and acetate product **48** has been successfully carried out using the immobilized lipase in the presence of vinyl acetate (Scheme 12). The said acetamide analogues find their potential role in the synthesis of Practolol **49**, a selective β-blocker which is used to treat hypertension [60].



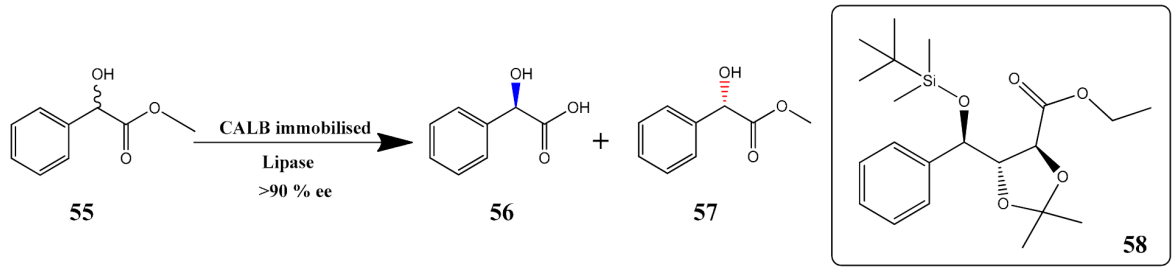
Scheme 12. Immobilized lipase mediated synthesis of carboetomide analogues.

In a subsequent study, authors have reported a synthesis of lipase metal nanohybrids (nanopetals) using *Pseudomonas fluorescens* lipase with a combination of metal salts out of which Cobalt (II) Phosphate [$(CO)_3(PO_4)_2$] showed interesting results. Further effect of sonication and chemical modifiers such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide was also evaluated which has shown an enhanced activity and recyclability profiling of an enzyme in biotransformation reactions of (RS)-1-indanol **50** as well as (RS)-α-methyl-4-pyridinemethanol **51** to subsequent stereoselective alcohols **52a**, **53a** and acetate products **52b**, **53b** (Scheme 13). The former finds an application in the asymmetric synthesis of hetero-yohimbine scaffolds which fall under the subclass of monoterpene indole alkaloids (Ajmalicine, **54**) while the latter in case of steroid analogues thus advancing the prospects in medicinal chemistry of natural products [34].



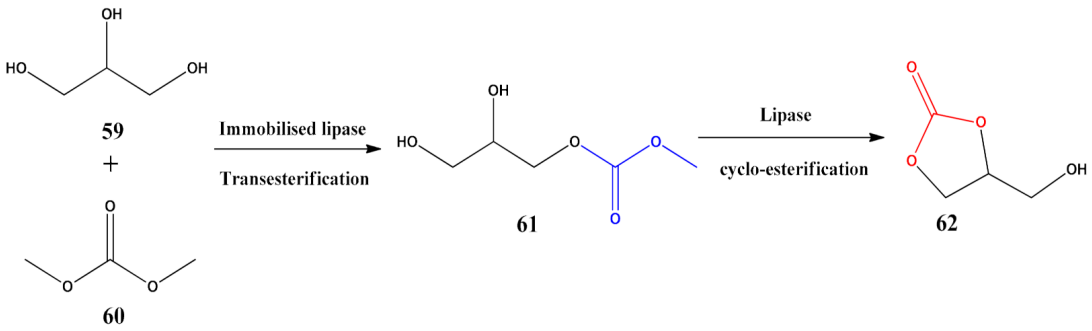
Scheme 13. Immobilized lipase mediated synthesis of stereo analogues required in natural product and steroid synthesis.

In another study by *Viñambres M.* et al., surface engineering of nanocarriers have been done to modulate the catalytic properties of the lipase B from *Candida Antarctica.* Firstly, magnetic iron oxide nanoparticles were made by co-precipitation method and then fabricated with carboxylic acid groups such as citric acid, succinic anhydride and oleic acid. Subsequently, the aminated lipase was covalently immobilized onto the citric acid and succinic anhydride modified surfaces whereas the hydrophobic adsorption was done in case of oleic acid modified ones. Though the maximum enzyme activity was observed in case of physical immobilization, but the covalently modified ones showed enhanced thermostability as well as enantioselectivity towards the kinetic resolution of (RS)-methyl mandelate **55** with >90% ee towards (R)-Mandelic acid **56** than (S)- enatiomer **57** (Scheme 14) [61]. The (R)-Mandelic acid finds applications as a chiral precursor in the synthesis of number of drugs belonging to class of antibiotics (cephalosporin); antineoplastics, antiobesity and anti-thrombotic agents [62]. One such application of (R)-Mandelic acid has been found in synthesizing ethyl (2S, 3S, 4R) -4-(t-butyldimethylsilyloxy) -2,3-isopropylidenedioxy -4-phenylbutanoate **58**, an intermediate in the synthesis of styryllactones which show anti-tumor activity [63].



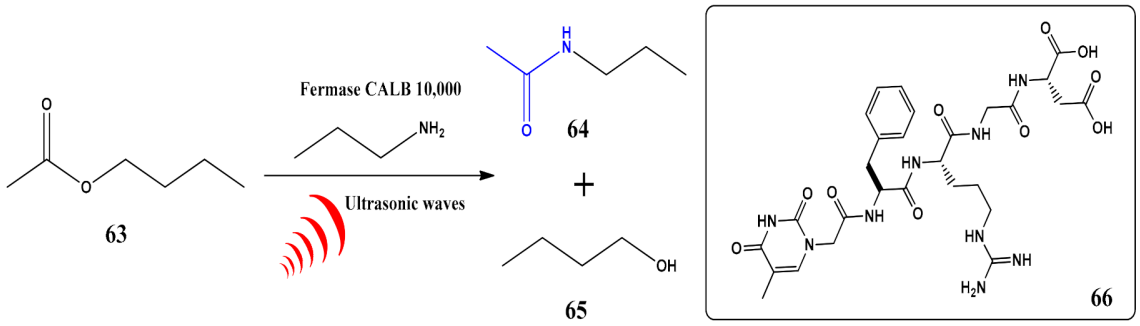
Scheme 14. Immobilized lipase mediated resolution of racemic mandelic acid.

Du Y et al. has demonstrated an eco-friendly conversion of dimethyl carbonate to glycerol carbonate with a yield of more than 88% using immobilized CALB on magnetic organosilica nanoflowers in a solvent free system. The said nanocarrier system with covalent bound lipase was utilized for the simultaneous transesterification reactions which involved the transesterification of glycerol **59** with dimethyl carbonate **60** to a short term intermediate **61** which undergoes cyclo-esterification to yield glycerol carbonate **62** and other side products (Scheme 15). On comparing the efficacy of this immobilized lipase with that of the commercial one, satisfactory conversion rates were obtained for glycerol carbonate in a solvent free system. Glycerol carbonate finds its utility as a bio-based solvent in several organic synthesis protocols, thus in turn emerging as a green solvent. The utility of glycerol carbonate has itself been implied in the lipase mediated biocatalysis as a biosolvent [64,65].



Scheme 15. Lipase nanoflowers mediated synthesis of glycerol carbonate.

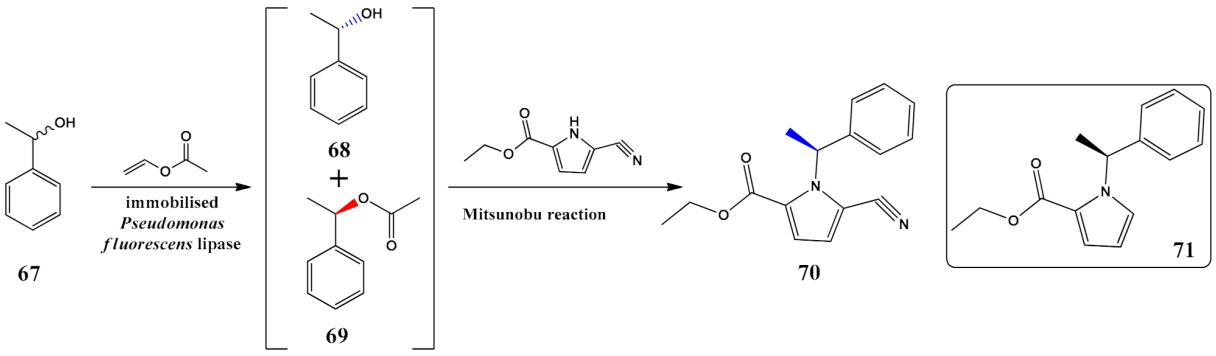
Sono-chemical synthesis is gaining attention in the field of organic synthesis, but its applicability in the biocatalysis has provided insights into the novel mechanisms enhancing enzyme activity alongside reaction process. Change in the enzymatic structure on exposure to ultrasonic waves has perceived an attention of the chemists in exploring novel enzyme mechanisms for rendering the feasibility of complex chemical reactions. One such study by *Bansode* and *Rathod* has proved the effect of ultrasonic waves on the structural aspects of lipase due to which an enhanced activity in rendering stereo selective amide synthesis was made. With the utilization of commercial Fermase CALB 10,000 (*Candida antarctica* lipase B immobilized on glycidyl methacrylate terdivinylbenzene-ter-ethylene glycol dimethacrylate nanosupports), authors have achieved more than 95% conversion of butyl acetate **63** to the corresponding propyl acetamide **64** in lesser time and at higher temperature with the use of ultrasonic waves. Butanol **65** was obtained as a side product in the reaction (Scheme 16). In addition, immobilized enzyme showed reusability under the similar conditions with retention of at least 50% enzyme activity after 5 cycles, thus holding a promising strategy for the lipase mediated biotransformation in ultrasonic waves [35]. Stereo selective amides play an important role in number of organic processes such as the synthesis of D-amino acid oligopeptides which are of valuable interest to the medicinal chemists in altering the pharmacological properties of the drug molecules (such as in D-amino acid glycosides, **66**). D-amino acids have gained a considerable attention because it renders the drug binding to the target receptors protecting it from proteolysis as compared to the L-amino acids. The concept opens up the platform for enhanced bioavailability of the drug molecules [66].



Scheme 16. Commercial immobilized lipase catalyzed amide synthesis.

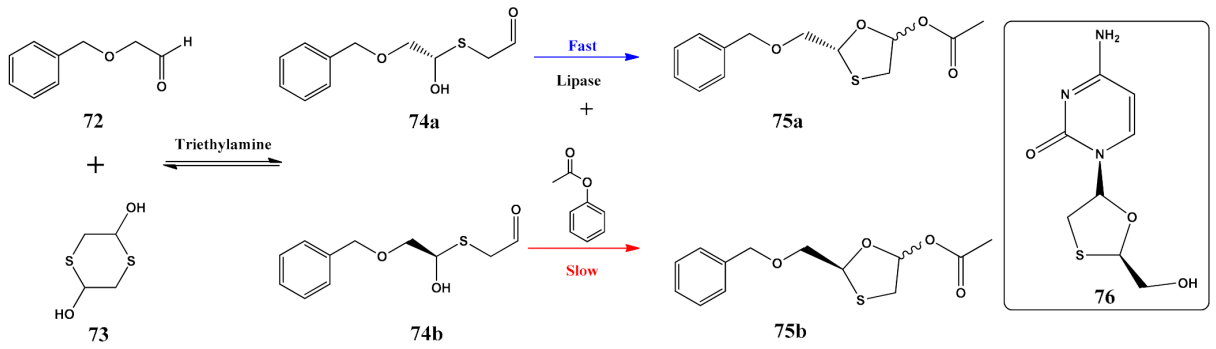
A robust and reusable biocatalyst of *Pseudomonas fluorescens* lipase has been successfully developed by *Dwivedee* et al. in which the immobilization was carried out on the surface functionalized carbon nanofibers with modified with 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC). The applicability of this catalyst on nano supports have been found in the asymmetric synthesis of (R)-ethyl 5 cyano 1 (1 phenylethyl) 1H pyrrole 2- carboxylate **70**, a carboetomidate analogue which finds applications as anesthetic drugs with lower

potency then that of etomide analogues (for e.g., Carboetomide **71**). Lipase mediated kinetic resolution of (RS)-1-phenylethanol **67** to racemic (R)- 1-phenylethanol **68** and (S)-Phenylethyl acetate **69** affords a biocatalytic intermediate which on the subsequent Mitsunobu reaction with ethyl-5-cyano-1H-pyrrole carboxylate affords a racemic derivative of carboetomidate which owes the pharmacological properties to the (R)-stereoisomers and analogues thereof (Scheme 17) [67].



Scheme 17. Immobilized lipase mediated synthesis of chiral analogues of carboetomidate.

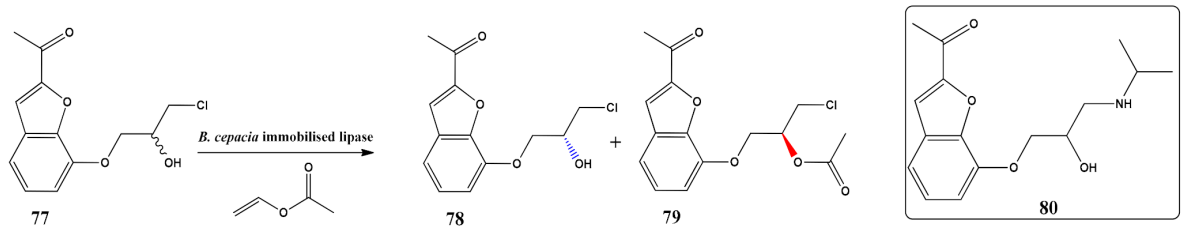
Asymmetric synthesis of enantiopure 1,3-oxathiolanes is required in processes for the synthesis of series of bioactive scaffolds such as lamivudine **76** and derivatives which acts as nucleoside reverse transcriptase inhibitors thus finding their applications as antiviral agents. *Zhang* and coworkers have developed a one pot dynamic kinetic resolution protocol for the synthesis of the enantiopure ((R)-5-acetoxy-1,3-oxathiolan-2-yl) ethyl benzoate **75a** from a combination of three different substrates namely 2-(phenylmethoxy) acetaldehyde **72**, 1,4-dithiane-2,5-diol **73**, and phenyl acetate. *Trichosporon laibachii* CBS5791-immobilized lipase (in situ immobilization by adding diatomites as carrier with activity of 1280 $U \cdot g^{-1}$) was employed as a catalyst for the biotransformation which proceeds through dual mechanism of reversible hemithioacetal **74a** and **74b** transformation and enantiomer-selective lactonization (Scheme 18). The immobilized lipase has provided a conversion of 99.6% of substrates to the product with a yield of 97.3% and 96.5% ee [68].



Scheme 18. Immobilized lipase mediated asymmetric synthesis of enantiopure 1,3-oxathiolanes.

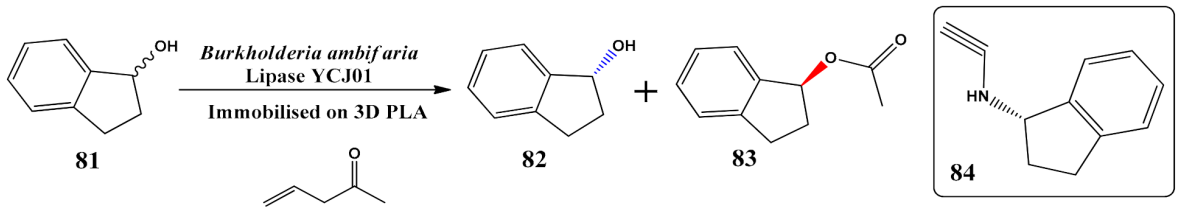
In one of the studies by *Soni* et al., surfactant treated lipase from *Burkholderia cepacia* was immobilized on polyaniline nanofibers. Comparative studies on non-treated, surfactant treated and immobilized lipase has shown an enhanced activity of immobilized one along with increased stability and reusability in carrying out the biocatalytic reactions. The immobilized lipase found to have a utility in kinetic resolution of racemic (RS)-1-(7-(3-chloro-2- hydroxypropoxy) benzofuran-2-yl) ethenone **77**. This is an intermediate in the racemic synthesis of befunolol **80** which is used as antihypertensive agents falling

under the class of β-blockers (Scheme 19). Conversions up to 50% with >95% ee have been reported for the (S)-intermediate **78** [69].



Scheme 19. Immobilized lipase mediated kinetic resolution of Benzofuran derivatives.

In another fascinating study on lipase immobilization, lipase YCJ01 from *Burkholderia ambifaria* has been immobilized on novel 3D printed eco-friendly polylactic acid scaffolds with programmable particle as well as aperture size. Further chain length functionalization of the scaffolds with different modifying groups has provided an enhanced utility of the scaffolds which provide promising surface designing in the enzyme immobilization processes. The authors have utilized the said scaffolds for lipase immobilization and an excellent conversion of racemic 1-Indanol **81** has been achieved with high ee as well as operational stability up to 10 cycles in a biphasic solvent system (Scheme 20). The said application finds an importance in the chiral synthesis of anti-parkinsonism drug named Rasagiline **84** [70].



Scheme 20. Immobilized lipase catalyzed kinetic resolution of 1-Indanol.

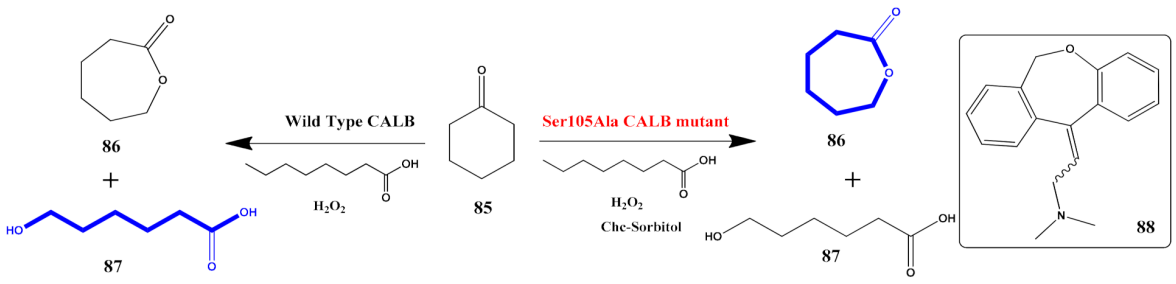
One of the interesting concepts for enhancing enzyme activity is the use of ultrasonic waves in enzyme activation which renders homogenous phase biotransformations possible. Mild frequency ultrasound waves cause increase in the rate of reaction during reaction kinetics by various factors such as: (a) activating the enzyme's active site by increasing the entropy of the system, (b) enhancing mass transfer thus minimizing the need for heterogeneous phases, (c) preventing product accumulation, etc. In contrast high frequency ultrasound waves along with prolonged exposure are known to deactivate the enzymes. Number of ultrasound mediated lipase biotransformations have been extensively reviewed in the literature and readers are suggested to wade through the same [71].

The second wave of biocatalysis has earmarked an important achievement in the field of drug synthesis esp. using lipases, which are on the verge of turning the tide in the present scenario. Be it in terms of research potential or process utility, Novozyme 435 has been a trendsetter depicting peculiar characteristics in regard to stability and activity. The trend towards immobilization is increasing because of number of processes shifting towards green chemistry and so the development of novel matrices/materials (nano) as efficient immobilization supports. The footprints of the second wave are being evident in the fourth wave of biocatalysis esp. in the flow biocatalysis (discussed in Section 5). However, due to different process utilities and variable reaction conditions the suitability of any single immobilized support is questionable. The development of the so called "versatile" support is yet to be seen in industrial settings. Moreover, with lesser number of commercially viable immobilized supports, much of the research needs to be focused towards the same.

4. Third Wave of Lipase Mediated Drug Synthesis

The fundamental goal is to "*revamp the enzyme suitable for the biocatalytic process*" in contrast to an earlier notion of "*readjusting the processes according to the catalyst*" [72]. The first thing that strikes in the mind pertains to the modification/alteration of proteins those suits the process, be it in terms of protein stability or in terms of substrate adjustability. The thing that only bodes well for the purpose is the "Protein Engineering". Not to say much, but the term is in prevalence from past few decades which has marked an onset of the third wave of biocatalysis. Advent in DNA and protein engineering technologies for biocatalysis has further supported the cause. Bioinformatics tools have emerged as biggest promoters in the advancement of the concepts of redesigned biocatalysts with operational suitability at commercial levels [1]. Typical protein engineering process involves changing the structural aspects of protein which is generally conducted through mutations at different amino acid sites making up the folded structure of proteins. The said is achieved through substitution, insertion, shuffling or omission of the amino acids involved in making up the active binding sites of the protein structure. As a result, a number of properties may be imparted to the protein ranging from its thermostability to substrate specificity [73]. The protein engineering revolves around two basic strategies of namely (a) directed evolution and (b) rational design. The former works on the principle of generating a random library of mutants and subsequently screening all of them via suitable screening method, whereas the latter focuses on the prerequisites of the structural, functional and other aspects of the proteins so as to make a better prediction of amino acid targets suitable for mutation [74,75]. Rational design as such is considered as a better aspect because of lesser screening of variants involved. However, in modern engineering methodologies, the combination of both the strategies is being applied to cut short the tedious task of creating and screening large number of libraries. The concept is heading towards the creation of ultra-small library of mutants by predicting the better targets at the active site with an aid of bioinformatics tools and then creating randomized small libraries of the predicted targets [76].

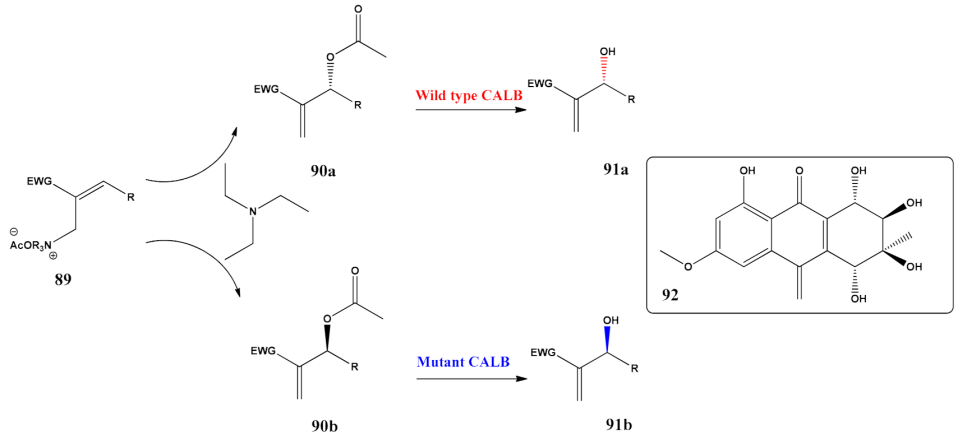
In an interesting work of *Wang* et al., a Ser105Ala mutant of CALB has shown excellent ability towards the per-hydrolase-only in type reactions. A kind of single point mutation at the catalytic triad of active site of CALB has been carried out by replacing Ser105 with Ala which cosseted an enzyme's hydrolysis capability. Almost complete absence of hydrolase type activity (6-hydroxyhexanoic acid **87**) was observed during Baeyer–Villiger type oxidation of cyclohexanone **85** to caprolactone **86** in deep eutectic solvents (Scheme 21). Choline chloride D-Sorbitol based deep eutectic solvents have provided enhanced conversion efficiencies among various screened ones. Due to susceptible hydrolysis of esters in aqueous medium, the implications of the mutant in deep eutectic solvents have provided an insight into plausible promiscuity of lipases in carrying out the oxidation reactions [36]. Lactone based moieties find applications in number of drugs molecules such as Doxepin **88** (an antidepressant), and other related scaffolds such as 5, 6, 5-tricyclic lactone of natural product based bioactive agents [77,78].



Scheme 21. Lipase mutants with reduced hydrolytic activity.

Xia Bo et al. have shown a tremendous utilization of engineering CALB as a co-catalyst in mediating dynamic kinetic resolution of racemic Morita-Baylis-Hillman Acetates **90a**

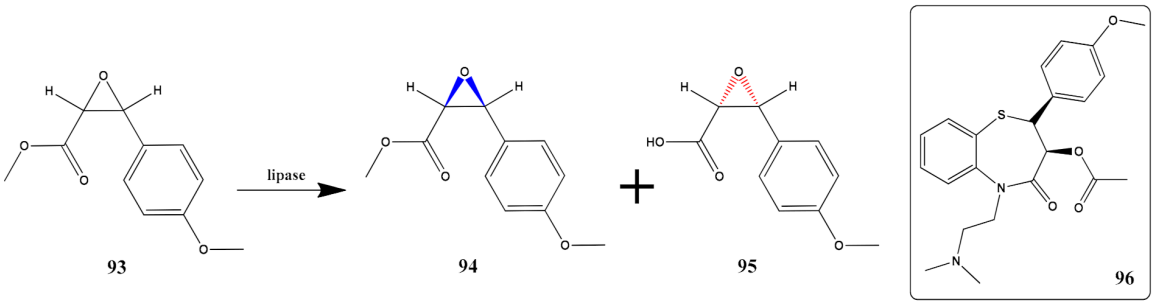
and **90b** (Scheme 22). After initial successful attempts of deploying a wild type CALB as a co-catalyst in mediating (R)- selective reactions, the (S)-selective reactions posed a considerable challenge for which the mutants of CALB were designed.



Scheme 22. Engineered lipase mediated co catalysis for dynamic kinetic resolution.

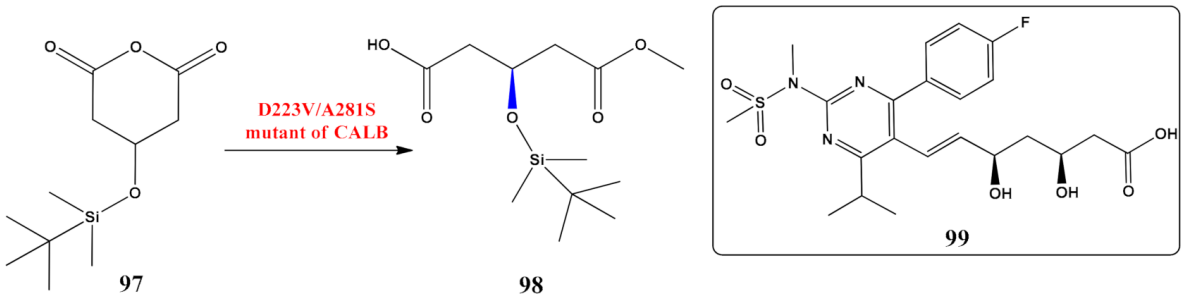
From the constructed library of mutants, four important residues namely W104, A281, A282, L278 were found to have a significant effect on the substrate binding. Iterative saturation mutagenesis (ISM) at Combinatorial Active Site Saturation Test (CAST) sites was used for creating a library of 16 mutants. The process involves the iterative substitution of amino acid residues to generate a small library of possible mutants with best hits. The best fit mutant WB13 with amino acid substitutions pertaining to W104V/A281L/A282K sites was found to have almost 80% ee for (R)- selectivity. Along with the racemization capability of triethylamine, the utilization of both the wild type CALB as well as mutant CALB has been shown to render both (S) **91a** and (R) **91b** stereoselectivity for Morita–Baylis–Hillman alcohols. In this way, a tunable switch in enantioselectivity has been rendered for the suitable product needs [79]. The said Baylis-Hillman adducts find an application in the synthesis of number of pharmacologically active scaffolds with antimicrobial and anticancer activities. One such scaffold is altersolanol A **92**, which is an enantiopure triol possessing tertiary hydroxyl group between two secondary hydroxyl groups. *Zuckerman* and *Woerpel* has shown the efficient synthesis of enantiopure triols from racemic Baylis−Hillman adducts. In this way, the said adducts may pose an advantage in the chemoenzymatic synthesis of biologically active scaffolds [80].

Sometimes, an insoluble expression of enzyme of interest poses hurdles while using heterologous expression hosts such as *Escherichia coli.* Homologues expressions are desired for the same which works in enriching biological machinery of the similar host to produce more of the protein and that too without any pretreatment. One such kind of homologous expression for lipase A from *Serratia marcescens* using pUC18 expression vector has been carried out by *Chen* et al. in which two best selected mutants ($LipA_{L315S}$ and $LipA_{S271F}$) screened through combinatorial saturation mutation library of 14 amino acid residues lying within an 8 A° distance at the active site of enzyme were identified. The mutants (individual and combinatorial) have shown an enhanced biocatalytic performance (esp. $LipA_{S271F}$) towards an optically pure (−)-3-(4′-methoxyphenyl) glycidic acid methyl ester **94** which is a key intermediate in the chiral synthesis of a drug Diltiazem **96**, an antihypertensive agent (Scheme 23). Up to 3.5 folds stereoselectivity has been achieved by the authors by carrying out the said engineering and homologous expression of lipase A for the desired substrate. Moreover, the mutant $LipA_{S271F}$ was found showing relatively higher activities at alkaline pH range of 8.0–10.0 and at optimum temperatures of 40 °C which makes them promising candidates for industrial process utility [81].



Scheme 23. Homologues host expressing lipase with enhanced activity towards optically pure compound.

In an attempt to enhance the enantioselectivity of CALB, *Yang* and coworkers have developed the D223V, A281S and D223V/A281S mutants targeting the amino acid residues D223 and A281 in the active site of lipase pockets. Based on the conformational dynamics of the lipase pocket for rendering biotransformation at 30 °C, the said residues were finally selected out of the thirteen screened residues with potential effect on enantioselectivity. The mutant D223V/A281S showed an almost 12-fold increase in the ee value for the esterification of 3-t-butyl-dimethyl-silyloxy glutaric anhydride **97** to (R)-3-t-butyl-dimethyl-silyloxy glutaric acid methyl monoester **98** (Scheme 24), thus potentiating an application in the synthesis of antihyperlipidemic agents such as Rosuvastatin **99** and related statins. The said mutation using site directed mutagenesis has solved an issue of using earlier mutants at lower temperatures, thereby pertaining to the issues of industrial process development [82,83].

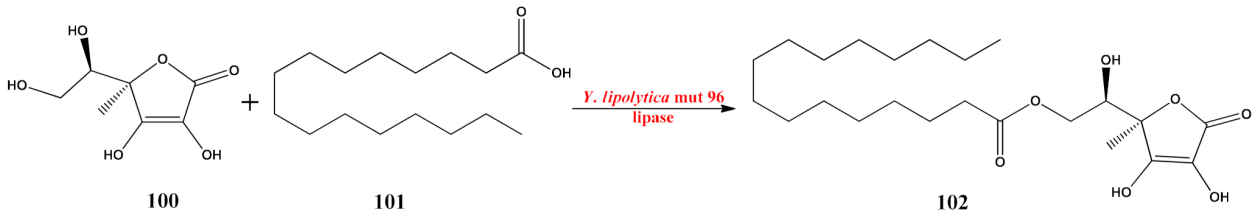


Scheme 24. CALB mutant with enantioselectivity towards (R)-isomer of monoester.

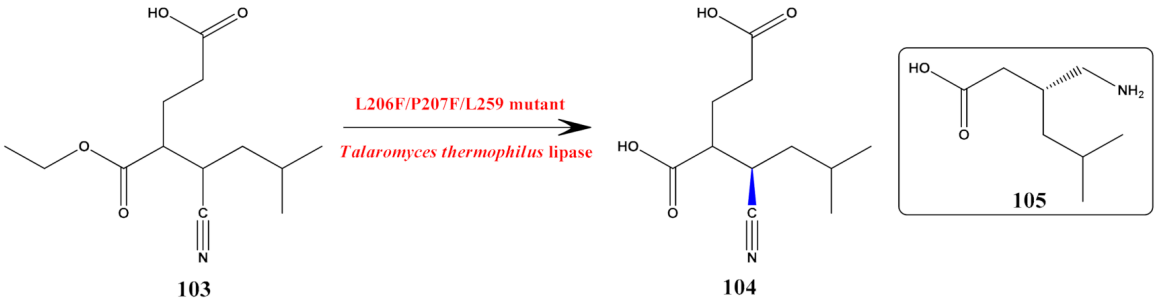
In a work published by *Ping* et al., enhancement in extracellular lipase production from *Yarrowia lipolytica* has been achieved using low-energy ion beam implantation technique for inducing mutation. Using the above described method, authors have achieved near to 5300 mutants in a library out of which only 19 were selected for further studies showing higher lipase activity. The best mutant among them has showed the highest lipase production of about 30 U/mL which was almost >5% enhancement as compared to the wild type strain. For the model reaction, synthesis of L-ascorbyl palmitate **102** was carried out using lipase mediated esterification of L-ascorbic acid **100** and palmitic acid **101** (Scheme 25). The mutant version of the microorganism was further adsorbed on the AB-8, a macroporous acrylic resin and was utilized for the biosynthesis of **102**. An almost two fold increase in production of **102** has been reported by the mutant vesion as compared to the wild type strain. L-ascorbyl fatty acid esters are known to have pharmacological actions in suppressing tumors by their inhibitory action on tumor promotion [84].

In a research work of *Ding* et al., the scissile fatty acid binding site of lipase from *Talaromyces thermophilus* was engineered by targeting particularly the crevice-like binding site to improve the catalytic performance. The substitution of residues L206F, P207F and L259F at the said site were replaced with Phe resulted in a mutant with higher stereoselec-

tivity towards (3S)-2-carboxyethyl-3-cyano-5-methylhexanoic acid **104** by the hydrolysis of (RS)-2-carboxyethyl-3-cyano-5-methylhexanoic acid ethyl ester **103** (Scheme 26). It was observed that the single mutant P207F did not show much improvement in catalytic performance as compared to the variant P207F/L259F, thus indicating a synergistic interaction between two residues at the crevice binding site. Moreover, authors have also tried out heterologous expression of the said mutants in *E. coli* as well as *P. pastoris*, in which the former has given a higher enantioselective enzyme but with lower soluble expression, whereas the latter has provided better insights. The said compound is a chiral intermediate in the synthesis of dug Pregabalin **105**, an antiepileptic agent [85].



Scheme 25. Mutant lipase from *Yarrowia lipolytica* mediating esterification.

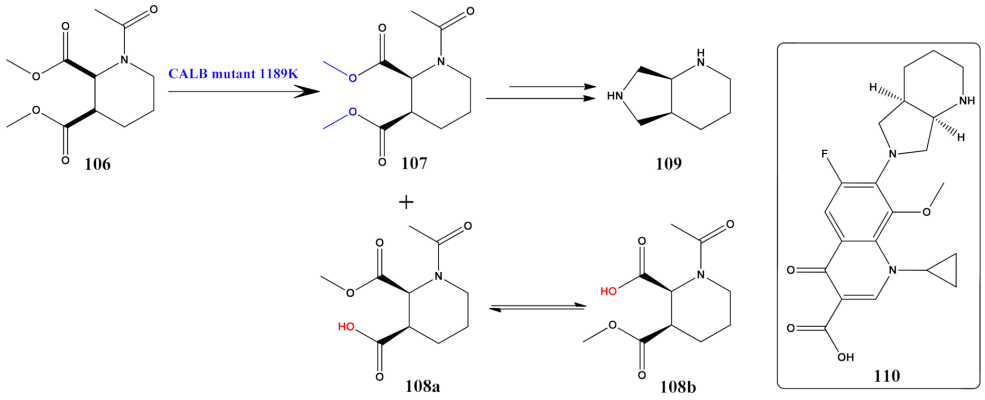


Scheme 26. Lipase mutant with high enantioselectivity towards chiral intermediate for Pregabalin.

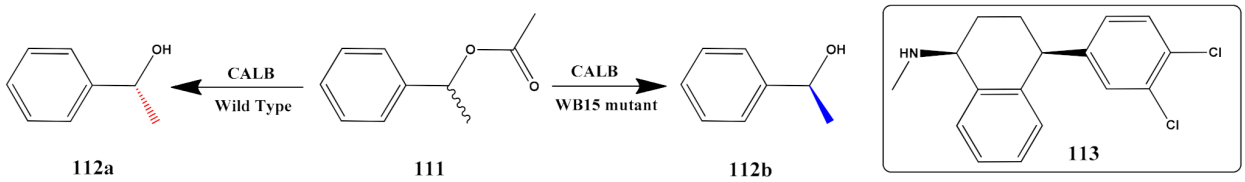
In a study carried out by *Shen* et al., engineered CALB has been utilized for solving an practical anomaly of lower enzyme yields associated with kinetic resolution of *cis*-(±)-dimethyl 1-acetylpiperidine-2,3-dicarboxylate **106** to subsequent chiral ester (2S, 3R)-dimethyl 1-acetylpiperidine-2,3-dicarboxylate **107** as well as hydrolysed products **108a** and **108b**. **103** is required for generating (S, S)-2,8-diazobicyclo [4.3.0] nonane **109**, a most important intermediate in the chemical synthesis of moxifloxacin **106**, a fluoroquinolone antibiotic (Scheme 27). Authors have employed a semi-rational design approach involving Combinatorial Active Site Saturation Test (CAST) as well as site directed mutagenesis for screening various mutants with an enantioselectivity towards **107**. The best screened mutant with effective modifications at amino acid residues 189 and 190 was found to have a 193 times more efficiency as compared to the wild type CALB. Indeed, an industrial challenge has been tried to overcome using the third wave of biocatalysis [86].

In another attempt, a favorably distinct mutant library for CALB has been created by the *Cen* and co-workers with an aim of stereo inversion for the desired product. An instinct of wild type CALB has been maneuvered from (R)-selectivity towards (S)-selectivity for secondary alcohols. Precise for its kind, the said directed evolutions strategy implying Iterative Saturation Mutagenesis (ISM) based on Combinatorial Active Site Saturation Test (CAST) has focused on creating a very small library of (~20) mutants considering only four amino acids namely alanine, leucine, lysine and tryptophan into play. More than 90% ee has been obtained for reversed stereoselectivity towards (S)-selective secondary alcohols with three best mutants W104V/A281L/A282K among the screened ones. Point mutation at W104 position has shown an enhancement of alcohol- binding pocket whereas

mutations at A281/A282 positions are supposed to modify the entrance of active site which in turn allows (S)-selectivity for sec-alcohol. The synergistic effect among the three amino acids has been suggested by the authors due to lesser selectivity being observed in single mutants. Moreover, the mutants have also widened the substrate specificity for an enzyme. The engineered lipases find applications in the synthesis of (S)-1-phenylethanol **112b** (Scheme 28) which is a chiral intermediate in the synthesis of Sertraline **113**, an antidepressant agent [87,88].

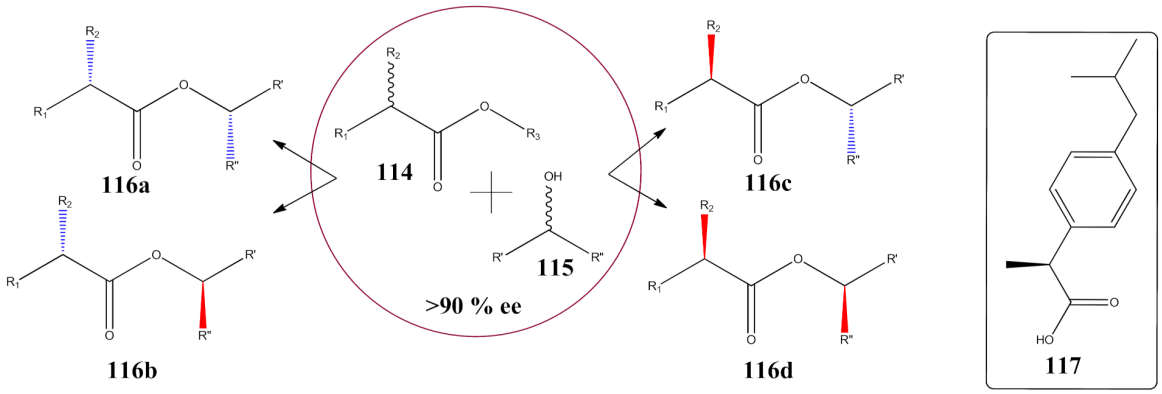


Scheme 27. Engineered CALB mediated kinetic resolution.



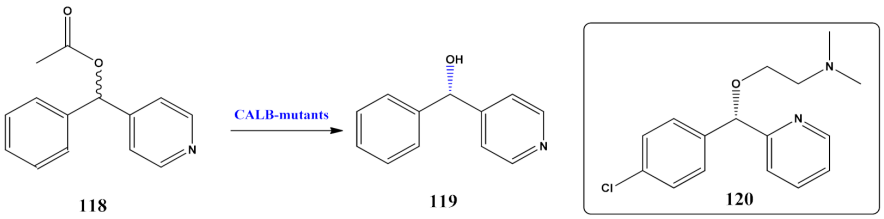
Scheme 28. Enantioselectivity of engineered lipase towards (S)-1-Phenylethanol.

In a novel type of mutagenesis, stereodivergent protein engineering has been done to get an enhanced promiscuity of lipase towards highly stereocompetent transesterified products in organic solvents. The said alteration was achieved in the non-aqueous medium with lesser screening of variants which otherwise would have been a burdensome task for directed mutagenesis to cope up with. Thanks to the implication of plausible protein engineering approach known as "**Focused Rational Iterative Site-specific Mutagenesis (FRISM)**"! The strategy bodes well with rational decisions which are based on choosing highly concise set of amino acids to be modified at desired sites with site specific mutagenesis in an iterative manner. In this case site- specific mutagenesis was applied by mainly targeting steric properties of the amino acids which in turn modified the selected domains in the CALB binding sites iteratively for different substrates. With the screening of less than 100 mutants, authors have been able to get a conversion of >90% in each of the four stereiosomers involved, thereby opening the doors for the novel prospects in medicinal chemistry. The so-called creation of minute/ultra-small library of mutants with minimal screening for enantioselectivity finds applicability in the complex chemical moieties with two stereogenic centers where the mutants are desired for both absolute and relative configuration. This lays advantage in providing access to all possible stereiosomers **116a**–**116d** of a chemical moiety which are of high value in screening drug variants with higher therapeutic action as well as lower toxicity such as in case of phenylpropanoic acid class of drugs (e.g., Ibuprofen **117**) (Scheme 29) [37].



Scheme 29. Lipase engineered through FRISM showing switchable enantioselectivity.

In a study carried out by *Li* et al., CALB has been tailored to carry out the kinetic resolution of racemic phenyl(pyridin-4-yl) methyl acetate **118** for the synthesis of enantioselective diarylmethanols **119** which otherwise are difficult to resolve by the wild type CALB because of steric hindrance. The said research by the authors has focused on the electronic as well as the solvation effects for an efficient enzyme-substrate affinity/binding. The above parameters have been outweighed over the steric effects for efficient substrate selectivity. Rational engineering has been employed to maneuver the amino acid residues' polarity as well as cavity volume. W104 site was chosen for an efficient modification using three of polar amino acids namely cysteine (C), Serine (S), and Threonine (T) as compared to the previously reported W104A mutant, W104C and W104T. Mutants were found to have better *S*-selectivity. Using the said mutant, almost 99% ee has been obtained for the **119** as compared to the 91% ee using W104A (Scheme 30). Based on the results, the authors further elaborated the substrate scope for pyrindinyl ring position as well as effect of substitution and to their surprise the said mutants were found to be efficient in these conversions too. The study finds an application in the synthesis chiral antihistaminic drugs such as (S)-Carbinoxamine **120** [89].

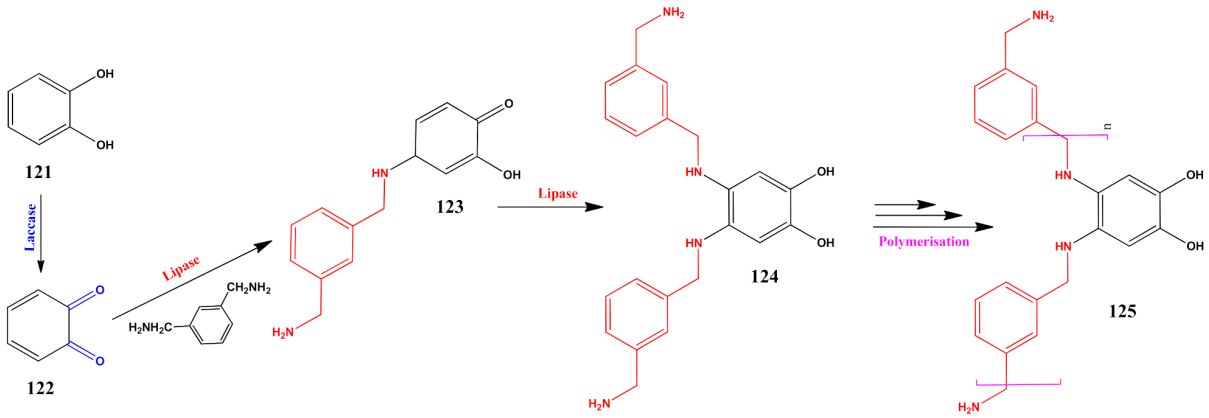


Scheme 30. Synthesis of enantioselective diarylmethanols using mutant CALB.

The third wave of biocatalysis has rendered important achievements for lipases. To the charm of Medicinal Chemist's paradise, genetically engineered lipases have achieved milestones in drug designing by aiding in creating an array of libraries for the potential drug molecules. With step-by-step realization of the goals, the third wave of biocatalysis has emerged as a boon in designing tailored fit lipases for the suitable research needs. From initial classical strategies of directed evolution and rational design involving cumbersome library creation to the more refined techniques of ISM, CAST, FRISM, etc., the concept has come up with an eminent feasibility in transfiguring the lipase according to the variable process conditions. Among the discussed techniques, FRISM seems befitting in providing an appropriate utility in engineering lipases as a host for the number of suitable processes and so to the substrates. Similar techniques requiring rational decisions using ultra small library of mutants are desired for utilization in Medicinal Chemistry. Anyhow, the concept is in full pace of being apt to the drug synthesis, but the impediments in regard to scale-up and process utility are not to be disregarded.

5. Burgeoning Fourth Wave of Biocatalysis

Well in pursuit of upgradation, the fourth wave of biocatalysis is nonetheless afar with emerging biocatalytic trends of multi-step cascade synthesis, co-modular catalytic processes, flow synthesis, etc. being emerging stakeholders in industrial settings. The compliance with green chemistry matrices along with the economically driven biocatalytic process desires an incorporation of various metabolic and protein engineering tools. This initiates the trend of currently flourishing fourth wave of biocatalysis [90–92]. One such instance of lipase as a co-modular catalyst alongside laccase has been efficiently utilized in the work of *Schiebel* and *Gitsov*, where the synthesis of number of variable structured copolymers with alternating monomeric units has been carried out (Scheme 31). Promiscuity of laccases and lipases have been exploited to render the synthesis of novel alternating copolymer with a co-catalytic cascade reaction involving the oxidizing potential of laccase to oxidize o- catechol **121** to o-quinone **122** and subsequent Michael addition of m-xylylenediamine/p-xylyleneamine to **122**, thus leading to the formation of alternating co-polymer system of o-quinone-m-xylylenediamine **125** and o-quinone-p-xylylenediamine. Copolymers find number of applications in biomedical imaging and thus potentiate as imaging agents in the medical field with further derivatization potential of the scaffolds [93].

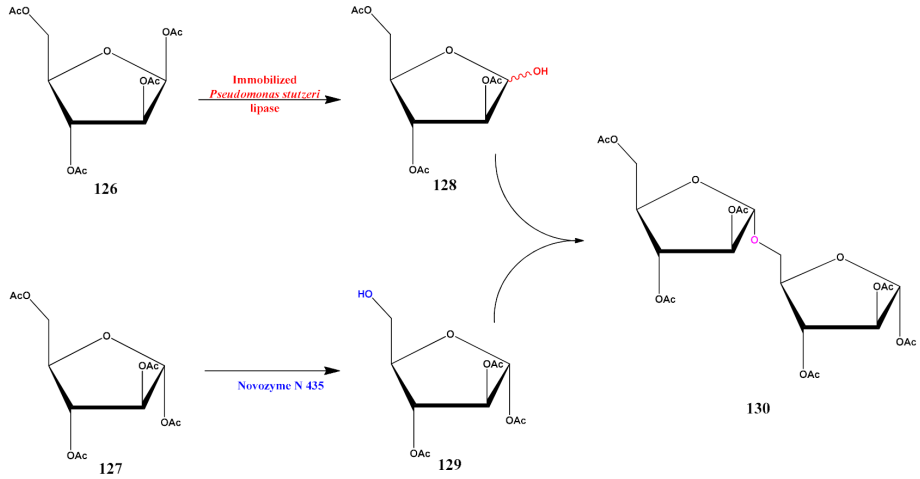


Scheme 31. Laccase and lipase catalyzed polymerization.

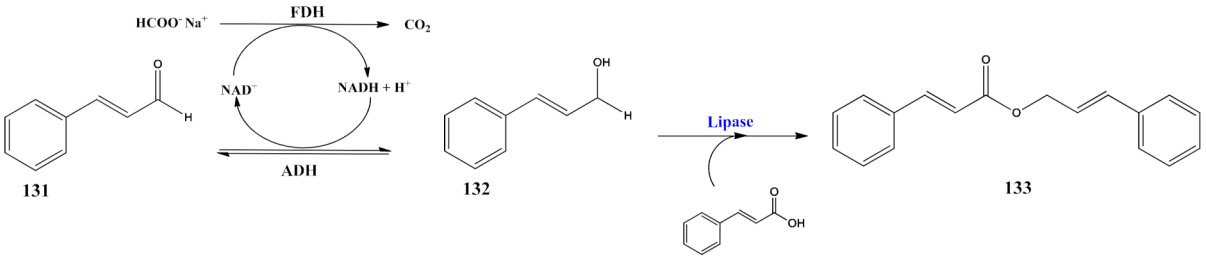
In another concept of flow synthesis, immobilized lipase has been used for the synthesis of per-acetylated arabinofuranosyl-1, 5-arabinofuranose **130** which finds an application as glycoconjugates (glycoproteins and glycolipids) in vaccines responsible for eliciting an immune response. Authors have tried the hydrolysis of per-acetylated arabinose in a packed bed flow reactor which has provided better specific reaction rates in gram synthesis of analogues. Using different immobilized lipases namely N435 and *Pseudomonas stutzeri* lipase (Scheme 32), authors have been able to modulate the C1, **126** and C5, **127** selectivity for the substrate in a flow bed reactor along with faster reaction time, reusability and downstream processing as compared to the traditional batch processes [38].

Cascade syntheses is yet another interesting concept in which an artificial cascade comprising of two or more number of enzymes is deployed for the linear transformation of the substrates and the formation of intermediate products. In one such example (Scheme 33), lipase has been used in cascade synthesis of cinnamic acid esters which finds an application as high value aromatic compounds in flavors and fragrances. In a typical reaction, cinnamaldehyde **131** has been converted into cinnamyl alcohol **132** using alcohol dehydrogenase (ADH) and then subsequent esterification of cinnamic acid with the formed cinnamyl alcohol in a cascade yield of cinnamyl cinnamate ester **133**. The typical reaction by ADH proceeds with an aid of formate dehydrogenase (FDH), another enzyme from *Candida boidinii* which replenishes the nicotinamide adenine dinucleotide (NADH) cofactor during catalytic cycle of ADH by transferring H^+ ions from formic acid. The

combined utility of three enzyme cascade has shown good amount of conversion yield of >50%. Moreover, immobilization of enzymes have solved the problem of stability of FDH during reaction, thereby providing successful conversion of >35% on scaling up the reaction conditions [39]. Besides flavors and fragrances, the cinnamic acid esters also finds utility as bioactive agents with numerous pharmacological applications ranging from antineoplastics and antibiotics to treat diabetes and neurological problems [94]. The said potential of cascade syntheses may have better role to play in the medicinal chemistry of cinnamic acid esters and derivatives thereof. Although the novel concepts related to fourth wave of biocatalysis are flourishing, yet their far-flung potential in medicinal chemistry remained challengeable.



Scheme 32. Flow synthesis of per-acetylated arabinofuranosyl-1, 5-arabinofuranose.



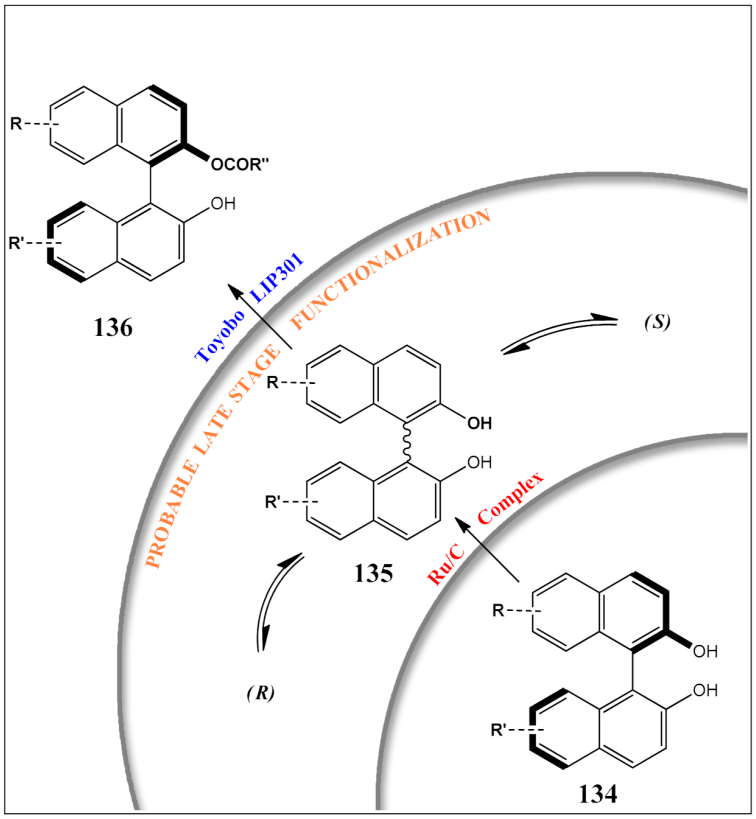
Scheme 33. Lipase mediated cascade synthesis of cinnamyl acid esters.

6. Lipase Mediated Late-Stage Functionalization (LSF)

Late-Stage Functionalization (LSF) arises from the concept of transforming complex chemical moieties in Medicinal Chemistry wherein an array of modifications on the moieties (be it in terms of functional group or scaffolds) are accomplished through number of methods deemed fit for the purpose. The past few years have witnessed an intensified effort towards the progression in the functionalization of complex chemical molecules. This has created an array of opportunities in medicinal chemistry [95,96]. An enigmatic feature of enzymatic promiscuity towards better functional group tolerance also adds on to the merits of LSF. In addition, their capability to act on indigenous functional groups of the chemical molecules further knocks out the necessity of adding several functionally modified groups which otherwise have been a general practice in chemical catalysis. Genetically engineered enzymes acting on non-natural substrates also define their capability of rendering LSF reactions. Considering this prerogative, the third and fourth wave of biocatalysis may be defined on a par with this concept of LSF in chemistry. So, the question here arises, "*Do lipases render late-stage functionalization of chemical moieties?*" Well, the previous sections

have outlined an extensive effort put forth in fine tuning the lipase mediated reactions (esp. during the third wave of catalysis) for a wide number of prevailing chemical scaffolds. The competency as LSF contenders may be interpreted from the same. Consider, for an example, the work of *Xu* et al. (Scheme 29), tunable switching of the enantioselectivity among different chiral intermediates by genetically engineered lipases provides the best illustration of enzyme mediated late stage functionalization. Utilization of the methodology for creation of library of molecular derivatives of the lead molecule is the major implication [37].

In another interesting work of *Moustafa* et al., lipase has been utilized as a catalyst alongside ruthenium complex in rendering the dynamic kinetic resolution of racemic homochiral 2, 2′-dihydroxy-1, 1′-biaryls. A kind of a novel development in the asymmetric synthesis, simultaneous racemization as well as dynamic kinetic resolution of the C1- and C2- symmetric biaryl diols to the corresponding enantio-enriched derivatives have clearly depicted the ability of lipases in mediating late-stage functionalization on complex motifs. As shown in Scheme 34, the one pot reaction involves the conversion of (R)-1,1′-bi-2-naphthol **134** into the racemic precursor **135** by the Ruthenium metal catalyst. **135** is then further acted upon by the immobilized *Pseudomonas* sp. lipoprotein lipase (Toyobo LIP301) which renders the dynamic kinetic resolution to the corresponding racemic ester (R)-**136**. By the virtue of lipases, authors have been able to achieve an array of compounds with different functionalities in regard to the functional group modifications on the said motifs [97]. Though not envisaged, the work by the authors is at par with the said definition of the late-stage functionalization as promulgated by *Börgel* and *Ritter* [95]. Nevertheless, to say, an inclination towards the concept is burgeoning for complex molecules. Enzyme mediated LSF is regardless to be stated at the nascent juncture with an optimistic fate of turning the tide.



Scheme 34. Lipase mediated late stage functionalisation of axially chiral 2, 2′-dihydroxy-1, 1′-biaryls.

7. Conclusions and Perspectives

On a concluding note, we aimed to make the readers of this review aware about the evolution of lipases in the field of drug synthesis. The notable aspect of enzyme mediated biocatalysis has put forth a quest among the medicinal chemists across the globe to pave out the way for greener drug synthesis esp. in times when green chemistry prospects are of due diligence in rising global demand. Lipase-mediated promiscuous reactions in the organic synthesis have laid an exploratory path for the novel findings in the field. Lipases have come up across the various waves of biocatalysis in a successful manner. Successful evolution of genetic as well as protein engineering has curated interest among researchers in providing custom-fit lipases for the desired purposes. These engineered lipases have left no stone unturned in mediating late-stage functionalization of complex molecules which otherwise often becomes cumbersome with chemical catalysts.

Some of the eminent researchers have substantially reviewed the successful lipase mediated biotransformation and most of the findings are mentioned in this review. The present review has significance in the field of Medicinal and Process Chemistry where the chemo enzymatic reactions find a worthwhile weightage. From mediators of traditional esterification reactions to the enantioselective catalysts, lipases have come across as one of the most successful biocatalysts and their succession through various waves of biocatalysis have been markedly reviewed with an emphasis on drugs and drug intermediates' syntheses. With an aim of enlightening readers into the prospects of greener drug synthesis, the review summarizes few of present-day noteworthy achievements of lipases in the field of Medicinal Chemistry. The trend of lipase-mediated biocatalysis is never ending as the number of natural sources (microorganisms or plants) with an enzymatic pool is yet to be unearthed and (of course) the trend will never cease. We aspire that the imminent researchers in the domain shall pursue to the advancement concerning broader prospects of utilizing lipases in the further waves of catalysis. Till then Happy Catalyzing!

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