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Research article

French Women in Chemistry in 2024

The need for Open Labs for fostering interdisciplinarities in Modern Chemistry. Biocatalysis: a necessary tool for synthetic chemists

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Abstract. Among the different fields of chemical approaches available to the synthetic chemists, biocatalysis is only being slowly adopted, especially due to a lack of know-how and practical experience. However, enzymatic catalysis is a mature technology, and this should encourage more chemists to take further ownership and expand applications in modern chemistry.

In the industrial sector, some pharmaceutical companies have been pioneers in the use and acceptance of biocatalysis for the unique properties that enzymes can deliver. In this article, the key concepts for biocatalysis applications will be addressed including some recommendations to new practitioners. Some industrial examples will be presented, such as polycyclization, regioselective acylation, alkene asymmetric bioreduction, *meso* desymmetrization, kinetic resolution and aldolization. Biocatalysis provides economic and environmental benefits, its awareness should be enhanced, especially in a *momentum* of conducting hybrid approaches and multicatalysis. Open your Labs!

Keywords. Biocatalysis, Enzymes, Catalysis, Asymmetric synthesis, Sustainability, Green chemistry.

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1. Introduction

For the past decade, the need to produce compounds with more complexity in terms of group functionality and chemical structure has expanded tremendously, including drug discovery in the pharmaceutical field and materials science. Moreover, the increasing pressure to reduce energy consumption, protect the environment and conserve natural resources challenge synthetic methods.

In response to this demand, the execution of complex chemical syntheses requires expert knowledge, usually acquired over many years of study and hands-on laboratory practice. However, when cross-fertilization of multi-competencies is achieved, the outcome may lead to incredible avenue of innovation and enlarge the vision on synthetic methods and strategies. Among the different tools available to synthetic chemists, enzymatic catalysis remains underused. Indeed, there is a lack of knowledge and training in this area which contribute mainly to the slow progress.

Considering the significant advances in biotechnologies over the past two decades, the emergence of plethora new enzymes has occurred. Contemporary tools are facilitating the access to new ways of conducting chemical transformations and should complement chemical intuition.

By examining the current key concepts, this article aims to demystify biocatalysis for bench chemists so that they may embrace it as a tool rather than fear it as a too complex technology. Spur the curiosity of

synthetic chemists who are willing to fulfill the gaps in knowledge and increase the number of successful stories in biocatalysis, especially within the trends of building multicatalysis approaches.

To familiarize yourself with biocatalysis, books by Grogan [1] and Faber [2], and comprehensive contemporary review which have been compiled by Faber *et al.* [3] and Whittall *et al.* [4], are worth reading. The recent review by Lin *et al.* [5] provides a convenient correlation of enzyme catalyzed reactions with named organic chemical reactions that organic chemists are familiar with.

2. Biocatalysis is in its Golden Age

The emergence of catalysis in the 18th century has had a transformative impact over the decades in many fields, including the production of chemicals, healthcare, materials, agriculture and in the environmental sector. A majority of all commercial chemicals are produced by methods involving at least one catalytic step. Still, research in catalysis remains one of the most dynamic areas of chemical synthesis research, and in addition, the quest for more sustainable methods encourages synthetic chemists to consider catalysis as a central science and as a key contributor.

Traditionally, catalysis has been subdivided into three fields: heterogeneous catalysis, homogeneous catalysis and biocatalysis. It can also be classified in terms of thermo-, electro-, and photocatalytic processes within the three fields. These disciplines have been developed mostly independently to address specific scientific and distinct catalytic challenges. Since the late 1990s, a series of fundamental studies on metal-free chiral catalysts, such as ketones, thioureas, amines and amino-acids for asymmetric catalysis have sparked a new craze for organocatalysis [6]. It is a promising alternative, generally exhibiting better moisture and air tolerance, as well as excellent compatibility with various functional groups. However, their main limitation is their low efficiency. In many cases, it is necessary to load a high quantity of catalyst and the selectivity is difficult to control. Besides, in some cases the molecular weight of the catalyst is superior to the weight of the desired product. The need to develop highly chemoselective and efficient catalysts remains a challenge in synthetic chemistry both in organocatalysis and in organometallic catalysis.

In recent years, cross-fertilization between these distinct fields and complementary hybrid approaches to catalysts design have begun to address some of the inherent limitations of the traditional fields. These efforts have enabled combining some of the most attractive features of the disciplines, such as chemocatalysis.

In this article, enzymatic chemistry will be the main focus, highlighting the considerable progress that has been made over the past two decades leading to a mature technology in organic synthesis. Major advances in enzyme discovery and engineering have brought with them a surge in the development and implementation of biocatalytic reactions in organic syntheses executed in both academic and industrial settings. Moreover, they offer retrosynthetic disconnections that can be quite distinct from typical chemical small-molecule-mediated transformations.

2.1. *State-of-the-art*

The amazing efficiency with which living organisms build complex molecules from abundant starting materials has inspired chemists for centuries. To produce natural compounds, there are necessarily individual enzymes that have specific tasks for specific compounds. Enzymes excel at catalyzing chemical transformations in complex cellular media and, despite their selectivity, some of them are even evolving *in vivo* to specifically handle non-natural chemicals [7].

Innovation inspired by nature has led to recent significant achievements in the field of biocatalysis, enabling scientists to mimic this approach by using evolutionary strategies to create and tune enzymes for a broad and diversified applications [8–11]. One of the major advances has been the development of directed evolution of enzymes which has enabled the adaptation of enzymes and their optimization to the needs of catalysis. The work on directed evolution has been rewarded with the Nobel Prize in Chemistry in 2018 to Professor Frances Arnold[1].

[1] Nobel Price received with Gregory Winter and Georges P. Smith, for their work on directed evolution of peptides and antibodies via phages.

Enzymes have a promiscuous behavior which is useful for exploring other types of reactions that have not yet been discovered. Indeed, catalytic promiscuity [12] is defined as the ability of an enzyme to catalyze secondary reactions. Interestingly, promiscuous enzymes provide the opportunity for new reaction applications through molecular evolution in the laboratory, through protein engineering techniques and reaction engineering.

Another major breakthrough for the discovery of new enzymes has been the extraordinary advances in high-throughput DNA sequencing and also the development of computer-assisted tools for protein modeling combined with protein engineering techniques. All these technical advances have made it possible to enlarge the application of biocatalysis in multiple synthetic routes [13,14]. Today, the number of reactions accessible by a biocatalytic approach has considerably expanded [15]. However, for neophytes, its use seems restricted to certain experts, whereas it should be more widespread among synthetic chemists.

For the synthetic chemists, biocatalysis offers a number of advantages:

(1) enzymes as catalysts can typically deliver superior regio-, stereo-, and enantioselectivity,
(2) biocatalysis can significantly shorten multistep synthesis routes by allowing reactions and reaction sequences, which cannot be carried out with classical chemical means,
(3) biocatalysis is generally associated with mild reaction conditions, e.g., avoiding the use of toxic reagents and high temperature or pressure.

However, chemists have preconceived ideas on biocatalysis such as:

(1) enzymatic process is necessarily carried out in very dilute conditions and can only work in water,
(2) the enzymes are not stable,
(3) high-cost contribution of enzymes in the process,
(4) the reaction does not work for compounds that are not soluble in water,
(5) or native enzymes will not be active on substrates that are chemically different from the natural substrate.

These considerations are not anymore valid. Biocatalysis in organic synthesis has faced many challenges related to stability, substrate scope, and restricted reaction conditions. Enzymes can be easy to handle, and it should be acknowledged that enzymatic reactions can be carried out in combination of water with organic solvents (even fully excluding the presence of water) and, in addition the stability and productivity of biocatalysts for commercial applications are now widely demonstrated [16]. Cost contribution of the biocatalyst into the process is an important milestone. General rules for cost analysis have been established for biocatalyst production based on biocatalyst format (whole cells, soluble or immobilized enzyme), product cost, market size and value, and the requirements for the implementation of biocatalytic processes [17] have been carefully analyzed. Enzymes still are the exception rather than the rule in the synthesis of fine chemicals and pharmaceuticals; biocatalysis is often regarded as a second generation process. Although it holds great promise as a powerful tool for application in process chemistry, the number of applications at commercial scale is still low. By means of enzyme and/or reaction engineering, cost-effective process is achievable. For illustration, in one of the following case study referring to enantioselective aldolization to produce D-serine at manufacturing scale, the biocatalyst cost contribution is less than 5%.

2.2. *The experimental method for implementing biocatalysis*

When designing the retrosynthesis pathway to produce the targeted molecule, the integration of the enzymatic approach should be considered as it may enlarge the route alternatives and/or potential shortcuts. To select the enzymatic step, the disconnection approach through functional group consideration, will help to determine the appropriate enzyme family. To plan the required tests to be carried out, the following features have to be considered.

2.2.1. *Access to enzymes*

Enzyme providers, such as Protéus by Seqens, offer enzyme collections in single vials and/or microtiterplate format for rapid screening (Seqenzym® kits) and will ensure availability at larger scale.

Customized Seqenzym® kit.

Cofactor-independent enzymes are usually preferred for organic synthesis because they don't require cofactor recycling. Nevertheless, there are known methods to recycle cofactor-dependent enzymes:

- NADH can be recycled by adding *iso*propanol or using Formate DeHydrogenase (FDH) with formic acid.
- NADPH can be recycled using Glucose DeHydrogenase (GDH) and glucose or NADPH-dependent FDH variants.
- Pyridoxal-5′-phosphate (PLP)-dependent enzymes usually do not need cofactor recycling, but adding a small amount of PLP maybe recommended for stability.
- Thiamine diphosphate in lyases is sufficiently accessible and does not require recycling.
- Efficient ADP recycling systems for ATP-dependent enzymes are currently lacking, so whole cells with glucose supplementation are used.

2.2.2. *Screening conditions*

The enzyme screening approach needs to be combined with adequate reaction conditions to evaluate the technical feasibility and to determine the best enzyme candidates. Once the size of the enzyme library is addressed, the implementation of the most appropriate analytical method is required for activity measurement. Regarding the reaction conditions, the next features should be taken into account:

Most enzymes can tolerate the presence of organic solvents. Typically, the concentration of water-miscible organic solvents (like DMSO, DMF, lower molecular weight alcohols) should not exceed 10–20% (v/v). Enzymes may tolerate water-non-miscible solvents (toluene, ethers), leading to biphasic systems that can ease product isolation and potentially favor product formation. Certain enzymes, especially lipases, can be highly active in pure organic solvents. Noteworthy, maintaining appropriate pH and temperature is crucial for enzyme activity.

When working with enzymes, it is important to evaluate the risk of substrate and product inhibition.

According to the activity results, further confirmation and statement on enzymes performance will need to be carried out independently, in order to select the best enzyme candidate in respect of the targeted criteria, such as stability and robustness under the chosen reaction conditions. At this point, acceptable specifications can be reached within the selected enzyme. For industrial applications, the choice of criteria acceptance and relevance at the screening stage is very important prior to further development studies.

Stability, selectivity and activity, are the prominent criteria for all types of catalysis, homogeneous, heterogeneous and biocatalysis, but they may be evaluated in a very different way. Enzymes are proteins and they may be typically stable in the range of moderate temperatures and moderate pH values, unless using extremozymes [18]. Under these conditions they can be remarkably active. Several enzymes may be limited only by diffusion of the starting material to the active site, allowing however extremely high turnover frequencies once the medium composition is fulfilled. Air is often not a problem for enzymes, while many homogenous and heterogeneous catalysts will be oxidized and deactivated by air, even at room temperature.

Choosing the best route depends on factors like enzyme availability, starting material cost, solubility, product isolation, and productivity. Careful evaluation of the entire process is necessary before making a decision.

2.2.3. *Enzyme properties improvement*

To achieve both an excellent selectivity and a broad substrate scope, modern biocatalysis relies on libraries of enzymes, similar to the catalyst libraries used in chemoselective metal-based catalysis. Noteworthy, compared to other catalyst-based chemical transformations, when observing initial poor performance with an enzyme, such as very low conversion, it remains yet a good starting point for further remarkable improvement, as recognition (or affinity) between the enzyme and the substrate remains the key feature. In the case where the diversity of available libraries does not provide the desired

specifications, nor within studied reaction conditions, it may be required to engineer an existing biocatalyst for a specific substrate shape or even identify/evolve a new biocatalyst for a substrate for which no catalyst is available at all.

Moreover, enzymes have a chiral 3D structure that interacts at multiple contact points with the substrate, thus explaining activity and selectivity. As a result, through protein engineering, modifications of protein sequences and therefore of their 3D structures, will alter the properties of a biocatalyst indefinitely until reaching the targeted specifications. Conversely, the selectivity and reactivity of typical chemical catalysts are more limited to the available structures of the catalysts themselves or of their ligands.

2.3. *In the field of pharmaceuticals manufacturing*

The pharmaceutical industry has already adopted biocatalysis as an essential tool [19] for providing efficient and sustainable catalytic routes toward APIs. The synthesis of active pharmaceutical ingredients (APIs) can be particularly challenging as they become more complex molecules. Chemists recognize the value of enzymes related to exceptional selectivity, in terms of stereo-, regio-, and chemoselectivity in many biotransformations. This field has gained impressive developments offering robust, scalable processes along with a very diversified panel of enzymes that are required.

Today, the pharmaceutical industry is facing significant challenges associated with environmental regulations, including productivity and costs [20], which provides the opportunity to expand synthetic methods within green chemistry technologies. Consequently, the identification of innovative, cost-effective and environmentally responsible synthetic methods becomes a "Must-Have".

2.4. *How sustainable is biocatalysis?*

Enzyme catalysis is a clean, green, and sustainable alternative to traditional synthetic catalysts, as enzymes exhibit an exquisite selectivity and efficiency, and work under mild reaction conditions (significantly less energy consumption), while eliminating the use of hazardous solvents and reagents (less waste), making it a green alternative to classical strategies [21]. The conciseness, environmental friendliness, and atom economy of synthetic methods have become important aspects of synthetic chemistry (Figure 1).

In terms of process-based chemistry, biocatalysis is aligned with all the principles of green chemistry. Alignment with five of the principles is a consequence of enzymes generally exhibiting high chemo-, regio-, and stereoselectivities that are in general difficult to achieve with traditional catalytic methods. In particular, the (near) perfect enantioselectivities observed with highly engineered enzymes are practically inimitable.

The use of enzymes in organic synthesis has clear economic and environmental benefits. Enzymes are produced from readily available and inexpensive renewable resources and the cost of production is, therefore, essentially stable and predictable. This contrasts with the serious environmental costs of precious metals and the disruptive price fluctuations caused by competing demand from other industries [22]. Moreover, the significant costs associated with removing traces of noble metals from end products to the very low levels that are demanded by pharmaceutical regulatory authorities, are prohibitive.

Regarding the production of enzymes, you need to know how they are produced, but a synthetic chemist should focus on their use within the awareness of mechanistic insights in the chemical transformation.

2.5. *How are enzymes produced?*

An enzyme is a polypeptide chain that includes on average 200–600 amino-acids, hence it is necessarily produced by fermentation. At industrial scale, depending on the production host—for example: bacteria or yeast, the cultivation times are around 24 h–72 h.

In most enzyme-based catalysis, the term biocatalyst refers typically to a crude cell extract from the production strain (e.g., *E. coli*), which may contain many other enzymes that, in general, do not interfere with the intended reaction. The whole cells containing the non-natural expressed enzyme may also be used as catalyst preparation, thanks to the recombinant DNA technology. Enzyme purification by chromatography is prohibitively expensive on a large scale, explaining the prevalence of crude

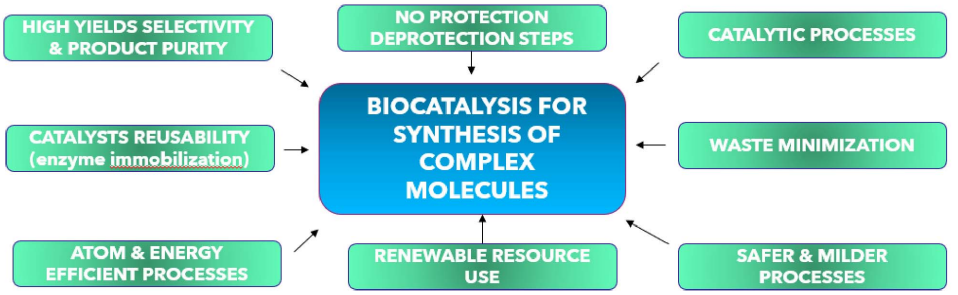


Figure 1. Biocatalysis is aligned with the green chemistry principles.

preparations for biocatalysis applications. Certain production strains (e.g., *Yarrowia lipolytica*, *Pichia pastoris*) allow the secretion of enzymes into the aqueous medium in which the production strain grows, making separation straightforward and leading directly to a rather pure enzyme preparation.

In the following case studies, the use of either a single enzyme (provided as crude preparation) or in combination of different enzymes in one-pot have been implemented. For illustration, below are cited some of our work, where biocatalysis was applied successfully with key considerations for limiting the number of chemical steps, such as protection and deprotection steps. But also, for replacing metal-based catalytic process by an enzyme in an asymmetric reduction of alkenes, desymmetrization of prochiral compounds, and in kinetic resolution steps to avoid separative chromatography.

3. Applications

3.1. *Biocatalytic synthesis of (−)-Ambrox*

The terpenoid molecule: (−)-8,12-epoxy-13,14,15, 16-tetranorlabdane, so-called (−)-Ambrox, is a valuable ingredient related to Ambergris, exhibiting amber and woody scent. It is widely used in the fragrance industry. For a longtime, it has been produced starting from the diterpenic alcohol, sclareol [23,24]. The latter is readily extracted in sufficient quantities from clary sage (*Salvia sclarea* L.). The historical and chemical synthesis consists of seven steps involving long reaction times and hazardous reagents such as peracetic acid, lithium aluminum hydride, and butyllithium, a stoichiometric oxidation with sodium periodate, and the generation of enormous amounts of waste in addition to the 76% global yield of the desired product (Scheme 1).

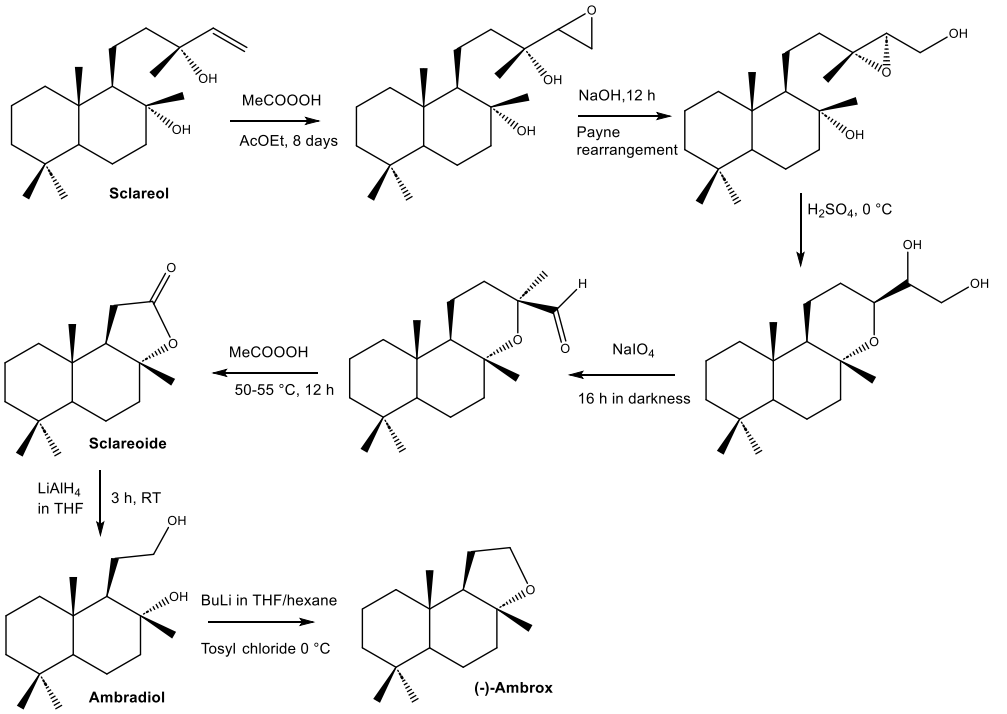
Later, a green two-step process has been reported [25], which involves the conversion of sclareol to ambradiol, catalyzed by whole cells of *Hyphozyma roseoniger*, followed by cyclization to (−)-Ambrox over a Ca–Y zeolite at ambient temperature, both steps proceeding in 98% yield (Scheme 2).

More recently, Eichhorn and Schroeder described a new industrial method for producing (−)-Ambrox starting from (*E*,*E*)-homofarnesol, which includes a biocatalytic step using a Squalene Hopene Cyclase (SHC) enzyme (Scheme 3) [26].

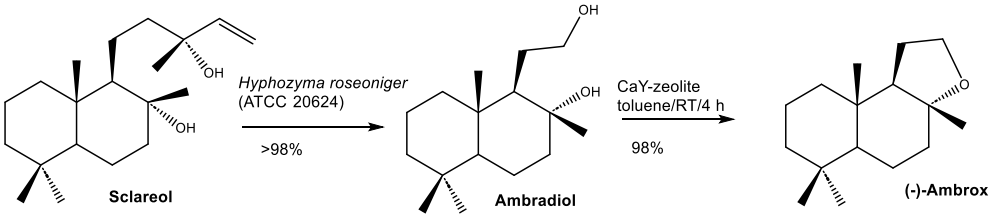
Squalene-Hopene Cyclase (SHC) enzymes are remarkable because they transform the C30 terpene squalene into the pentacyclic product hopene through a unique cationic polycyclization process involving multiple C–C bond formations. This transformation is initiated by the protonation of the C=C double bond of the terminal isoprene unit. The properties of the SHC enzyme were improved by evolving the wild type enzyme to produce a biocatalyst suited for application at industrial scale (Scheme 4) [27].

The engineering strategy generated a significant range of new variants that were then tested experimentally in the reaction [27]. As a result, the best variants resulted in the following:

- Improved (*E*,*E*)-homofarnesol conversion properties. With one round of random mutagenesis, 1.5- to 10-fold compared to the wild type enzyme.
- Conversion of 125 g/L (*E*,*E*)-homofarnesol. The maximal productivity observed was about 10 g/L/h, with approximately a 10-fold improvement with the best variant compared to the wild type enzyme.



Scheme 1. Chemical route to (−)-Ambrox starting from sclareol [1].



Scheme 2. Chemo-enzymatic route to (−)-Ambrox starting from sclareol.

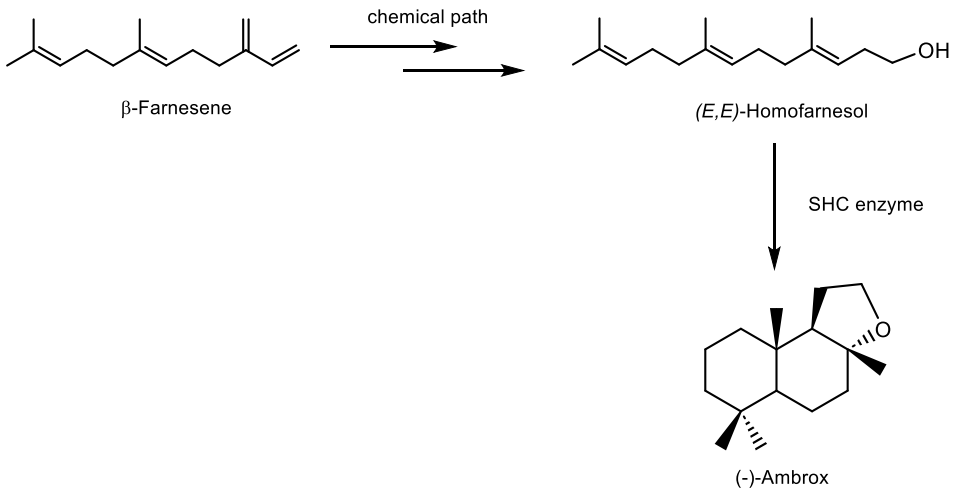
- Further optimization of the reaction conditions was carried out by DoE (Design of Experiments) with the best performing variant.

3.2. *Regioselective acylation*

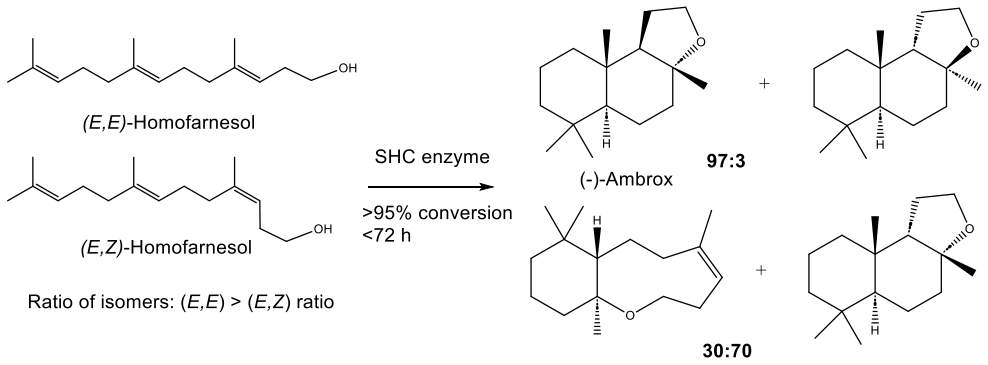
Simvastatin is a lipolipidemic compound that lowers the cholesterol and triglycerides circulating in the blood, this drug is marketed by Merck as Zocor. Historically, the manufacturing chemical route synthesis, involved six chemical steps starting from lovastatin, including protection and deprotection steps (Scheme 5).

Following the hydrolysis of the natural product lovastatin to give monacolin J, this latter was then converted to simvastatin by lactonization. Subsequent protection of the hydroxyl group followed by acylation to introduce the dimethylbutyryl side chain yields the protected form of simvastatin, which is then deprotected to yield simvastatin. The overall process needs six steps which are technically and economically demanding.

By integrating an enzymatic approach, the overall chemical steps could be reduced to only two steps (Scheme 6), thanks to a regioselective acylation using an acyl transferase with a cheap acyl donor, e.g.



Scheme 3. SHC-mediated (−)-Ambrox synthesis from β-farnesene via (*E*,*E*)-homofarnesol.



Scheme 4. Biocatalytic reaction with evolved SHC enzyme: work with Protéus by Seqens [4].

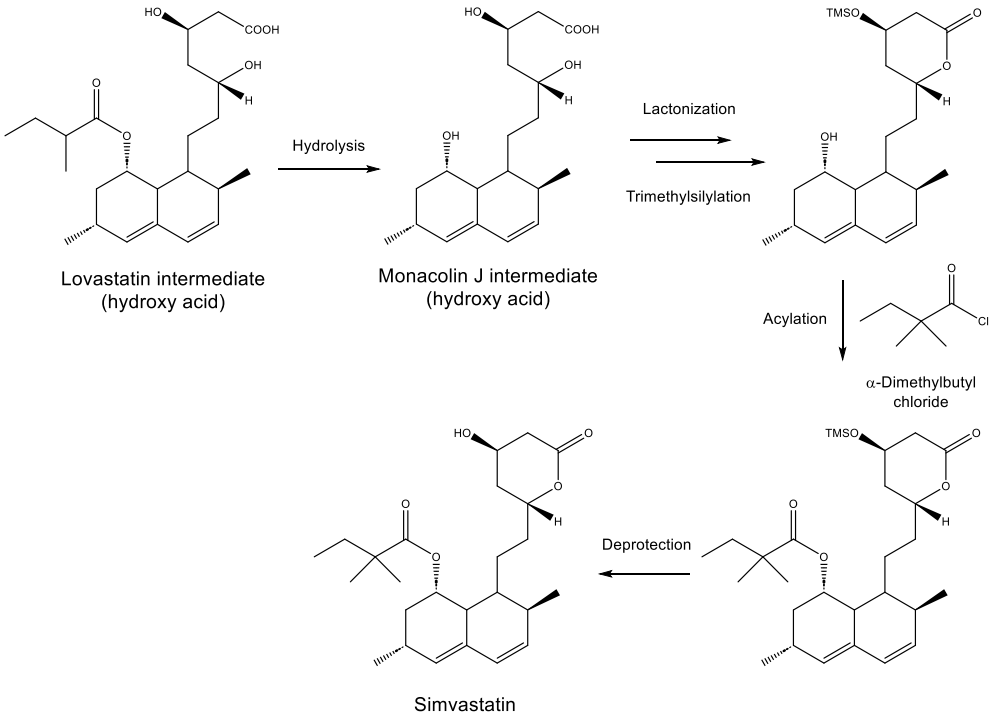
dimethylbutyryl phosphonate [28], and starting from the lactone-hydrolyzed form of lovastatin. The benefits of using acyl-phoshonates are:

- low cost acyl donors easy to produce,
- acyl transferases accommodate acyl-phosphonates donors,
- non reactive leaving groups.

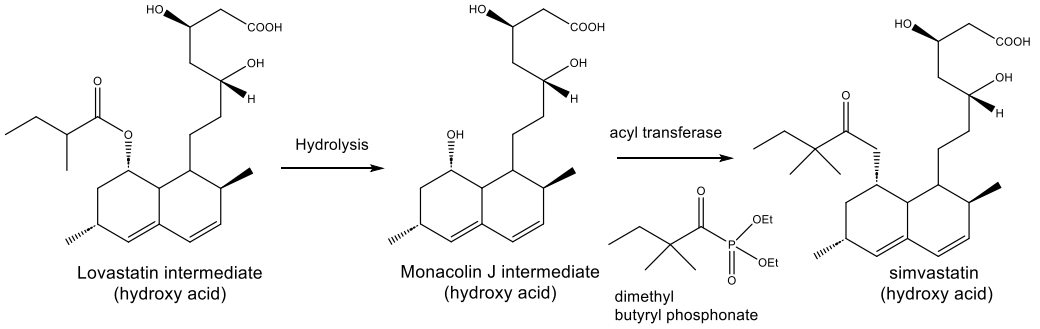
3.3. *Alkene asymmetric bioreduction*

Danoprevir is a protease inhibitor used for the treatment of hepatitis C virus (HCV). During the Covid-19 pandemic crisis, some antiviral drugs were evaluated to repositioning on Covid-19 treatment. This product was discovered by Array BioPharma (now Pfizer) and first licensed to Roche for development and commercialization. The modified-peptidic type macrocyclic structure of Danoprevir is built according to a 20 steps convergent synthesis [29]. To build Danoprevir, the retrosynthetic pathway considers three main disconnections, that lead to three fragments: an hydroxy proline **1**, a protected amino ester **2**, and a substituted cyclopropane **3**. The key steps will be two peptide couplings and a ring closing metathesis (Scheme 7).

Regarding the synthesis of **2**, the unnatural α-amino acid derivative possessing a terminal alkene, was produced by a rhodium-catalyzed asymmetric hydrogenation of the dehydroamino ester **4** [30] (Scheme 8).



Scheme 5. Chemical route to simvastatin starting from lovastatin.

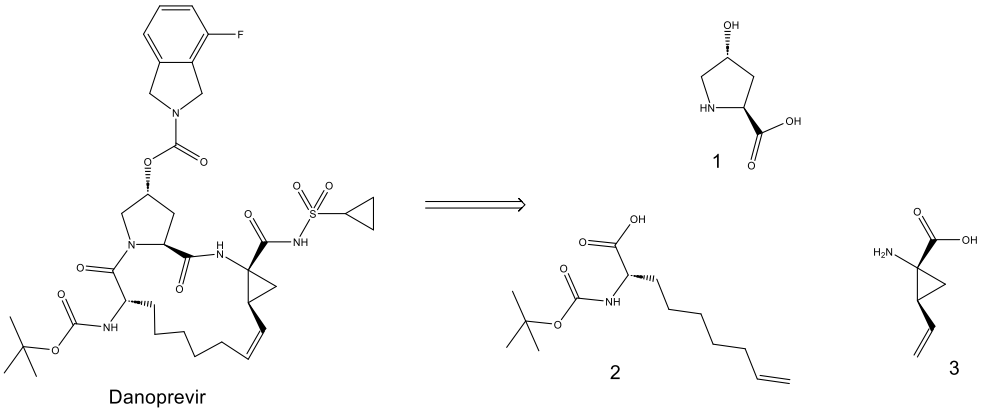


Scheme 6. Biocatalytic route to simvastatin starting from lovastatin.

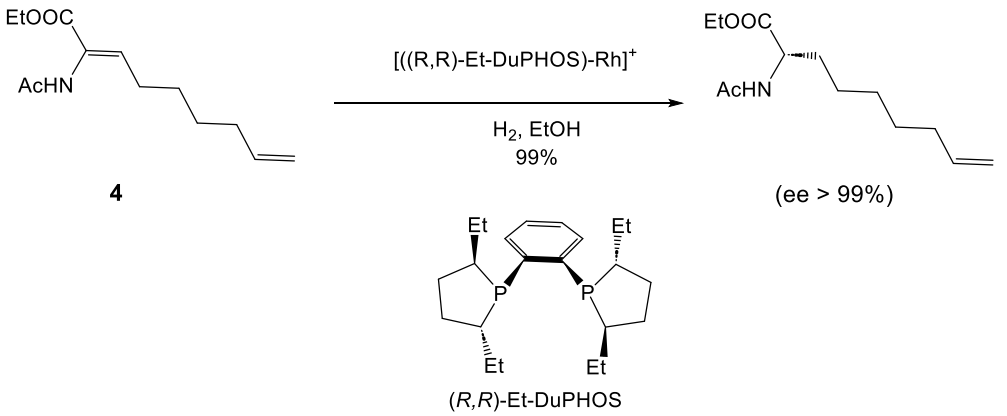
Despite a perfect enantio- and chemo-selectivity for the conjugated alkene, the use of this transition metal catalyst suffers from a tedious preparation of the chiral diphosphine ligand and from the actual price of rhodium, which has a commercial price that has increased by a factor of 8–13 between 2015–2020.

A cheaper alternative was envisaged by using a metal-free catalytic step, such as the reduction of the enoate analog **4** by an ene reductase (ERED) (Scheme 9) [31].

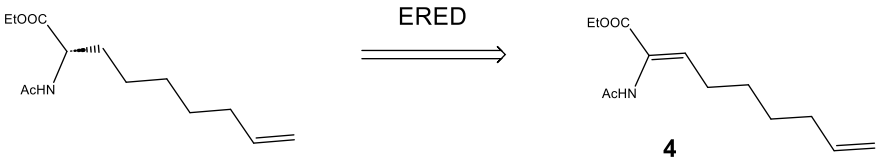
This NAD(P)-dependent ERED family of enzymes can reduce selectively substituted alkenes conjugated to an electron-withdrawing group meaning that chemo-selectivity between the two alkenes in **4** could be achieved. Generally, the ERED-catalyzed reactions proceed with high enantio-selectivity in mild conditions without hydrogen gas. An efficient system for the regeneration of the NAD(P)H cofactor could be easily implemented to afford a greener process.



Scheme 7. Retrosynthetic pathway of danoprevir compound.



Scheme 8. Asymmetric hydrogenation with a rhodium-based chiral catalyst.

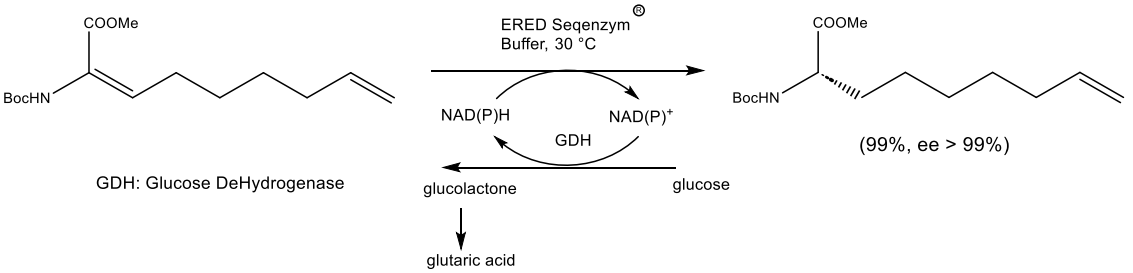


Scheme 9. Enzymatic reduction of a dehydro-amino intermediate.

At Protéus by Seqens, the asymmetric bioreduction of a dehydro-amino ester analog has been carried out (Scheme 10). The EREDs tested have allowed to selectively reduce only one of the alkene functions.

A panel of twenty ene reductases (EREDs) Seqenzym® have been tested. Within the exclusion of two enzyme candidates, all exhibited activity and the conversion rate during this first series of screening was moderate (around 30%) but still high degree of enantioselectivity were detected. Optimization of the reaction conditions is also on its way.

Depending on the scale of production, replacing a reaction catalyzed by a transition metal by an enzymatic reaction can result in a competitive process.



Scheme 10. Bioreduction of dehydro-amino acid derivative with ERED Seqenzym®.

This is explained by the volatility of the price of transition metals compared to the stable prices of raw materials used in the enzyme production (glucose, mineral salts, etc.). In addition, there is a strong scale effect with a cost reduction during the production of a biocatalyst making the latter competitive for the intermediate production on a large scale. Furthermore, when evaluating the manufacturing cost, the overall process must be considered, including potential purification steps to remove some impurities.

3.4. *Desymmetrization strategy*

Understanding that desymmetrization is the transformation of a substrate that results in the loss of a symmetry element, that precludes chirality (plane of symmetry, center of symmetry), this concept is one the most efficient strategy for obtaining enantioenriched molecules, within 100% attainable yield. The exquisite advantage of enzymes being highly specific, makes enzymatic desymmetrization of prochiral molecules an obvious and efficient method for obtaining enantiomerically pure compounds.

To desymmetrize 2-Me-propanedi-acetate and a cyclopentan-diester analog, a screening test of 80 enzymes, such as lipases, were realized and implemented at large scale. In both examples, we reached high enantioselectivity (ee > 98%) (Scheme 11).

Lipases are astonishing catalysts with a vast range of applications including the synthesis of esters/acids, and polymers. The broad specificity of the substrates, as well as their regio-, stereo-, and enantioselectivity, are the differentiating factors of these enzymes. They are also attractive biocatalysts in the kinetic resolution of racemic mixtures and they are highly robust in organic solvents.

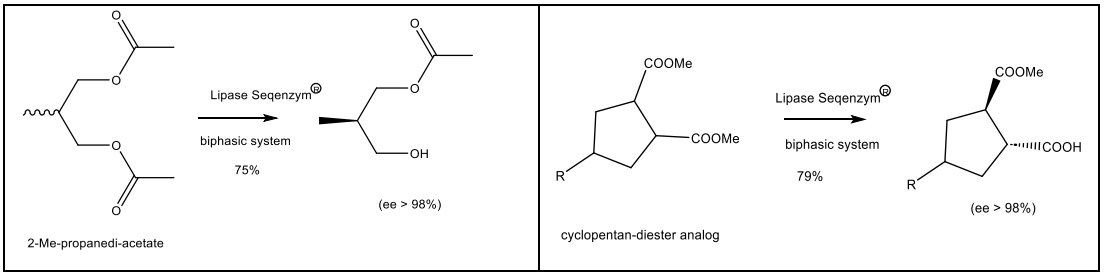
3.5. *Kinetic resolution*

For the substituted aminoester **5**, a lipase was identified in the collection of Seqenzym® for the kinetic resolution of racemic 4-amino-butanoate derivative, with high enantioselectivity (Scheme 12).

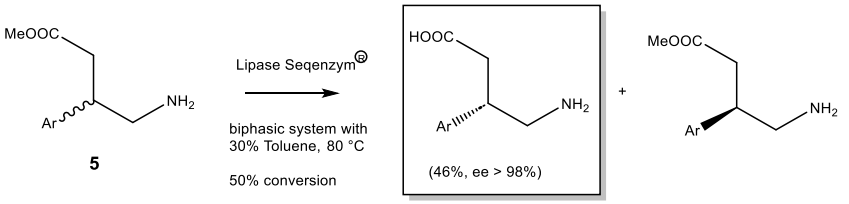
However, the lipase candidate suffered from thermal stability issue during the course of the reaction. Thus, it was decided to run an experimental statistical strategy with DoE approach (Design of Experiments), to immobilize the enzyme by absorption on the most efficient resin support. Ten different resin carriers were tested. This work resulted in implementing a new biocatalyst. As shown in Figure 2, the new immobilized enzyme, allowed running the resolution process without the presence of the undesired enantiomer, even at prolonged time. The resulting biocatalyst had exclusive stereopreference in favor of the (*R*)-configuration. Figure 2 exhibits the results obtained with the corresponding immobilized enzyme.

We were able to measure the enantiomeric ratio $E = (k^R_{cat}/K^R_m)/(k^S_{cat}/K^S_m)$, which is a measure of the intrinsic selectivity of the catalyst and therefore gives a concise representation of the enantioselective properties of an enzyme in reactions involving chiral compounds [32]. As such, we obtained a value of $E > 150$ with the new immobilized enzyme, to be compared with the initial value of $E = 60$. Consequently, we were able to improve the productivity by a factor of 44. By reaching such high enantiomeric ratio, this leads to the optimum process for ensuring full chirality control, without the presence of the undesired enantiomer at manufacturing scale and in multi-batch campaigns.

In the second example represented in Scheme 13, the kinetic resolution was carried out on a racemic



Scheme 11. Enzymatic desymmetrization with lipases starting from 2-Me-propanedi-acetate and cyclopentan-diester analog.



Scheme 12. Resolution of β-aminoester derivative.

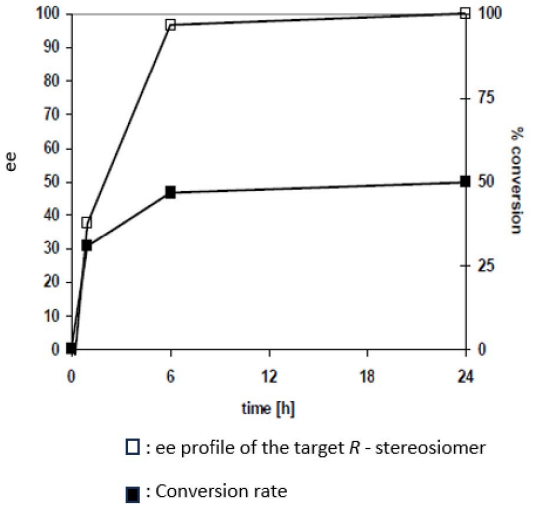


Figure 2. Conversion and enantioselectivity profiles during kinetic resolution with the corresponding immobilized enzyme.

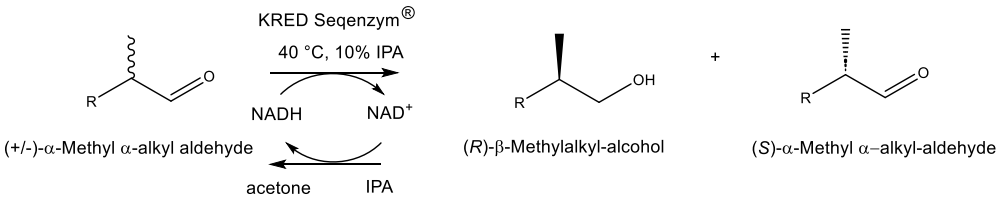
compound: α-methyl α-alkyl aldehyde derivative using a ketoreductase (KRED) from Seqenzym®, leading to the desired enantioenriched β-alcohol product (ee > 98%). By working on the process conditions, we were able to manufacture at large scale starting with 400 g/L of substrate, the cofactor system NAD^+/NADH was regenerated by using *iso*propanol (IPA) as co-substrate. Conversion was completed within 8 h, and the desired product was isolated within 47% yield, after trapping the remaining enantioenriched aldehyde intermediate with bisulfite salt reagent to make the isolation process easier.

The scope with other aldehyde substrates was investigated with KRED Seqenzym® family leading to preferential (*R*)- or (*S*)-enantiomers as presented below (Scheme 14).

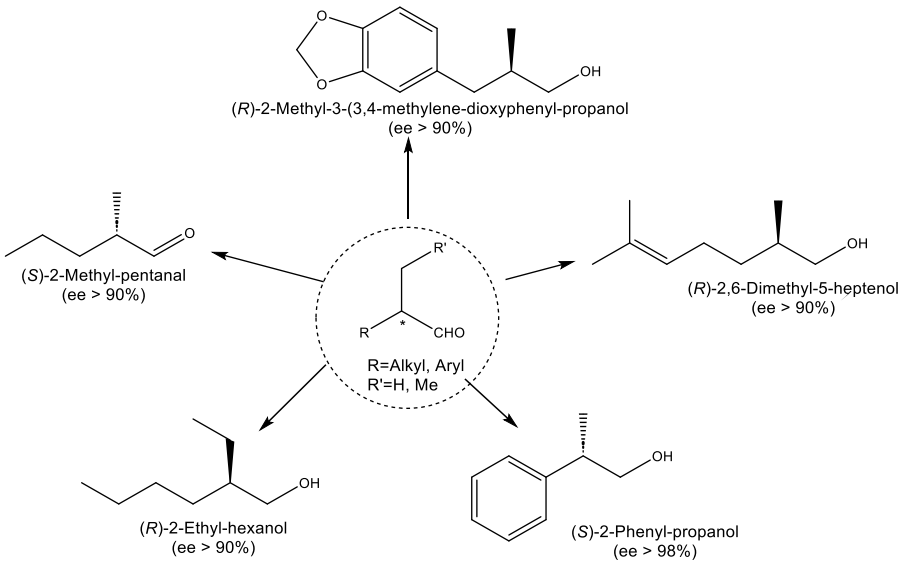
3.6. *Enantioselective aldolization*

In this work, an enzymatic process, for producing non-natural amino-acid e.g. D-serine, has been developed and implemented, which is a key building block for the synthesis of several APIs. The enzymatic aldolization step is carried out on glycine as starting material (Scheme 15). This approach is an alternative strategy to access D-serine and to avoid conventional chromatographic separation process.

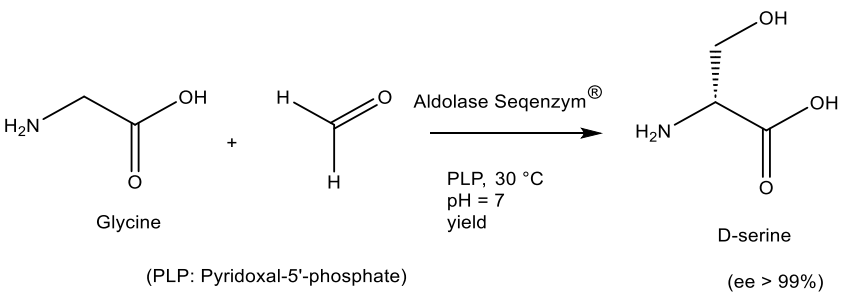
It is important to mention that this process leading to very high enantioselectivity is also carried out at high substrate concentration (>300 g/L), and that the cost impact of the biocatalyst added in the process contributes to less than 5% of the overall



Scheme 13. Kinetic resolution of (±)-α-methyl α-alkyl-aldehyde with KRED Seqenzym®.



Scheme 14. Scope of products formed with KRED Seqenzym® by kinetic resolution of racemic aldehydes.

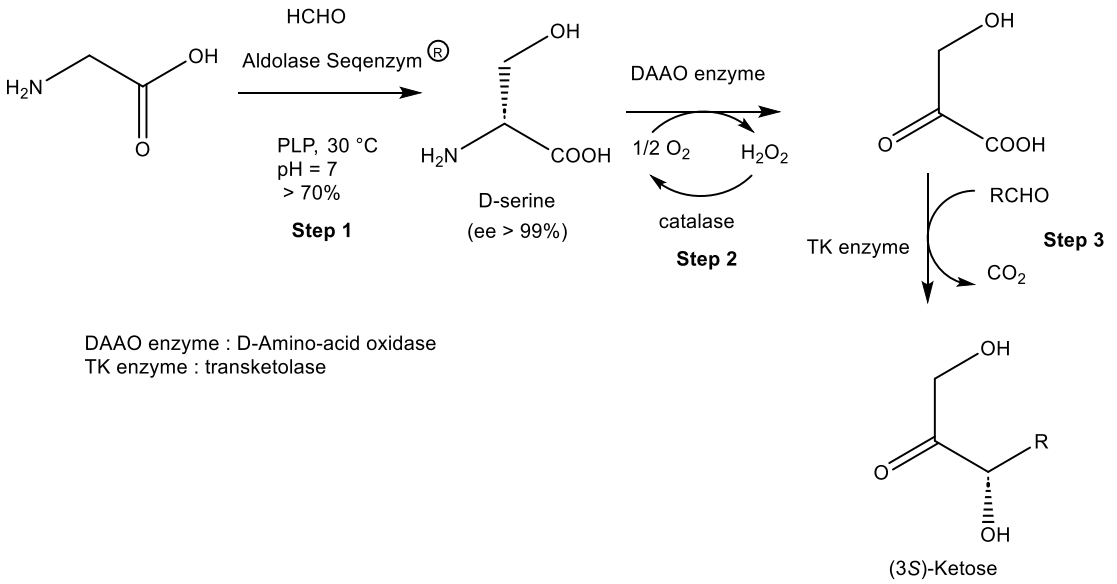


Scheme 15. Enzymatic aldolization of glycine substrate.

chemical production cost. Noteworthy, enzyme residues in the final product isolation are below detection limit. Besides, the enzymatic aldolization conditions were implemented into a cascade reaction for producing (*S*)-ketose [33] (Scheme 16).

4. The emergence of multi-catalysis systems

Driven by the demand for more efficient and sustainable chemical processes, and bringing solutions for more complex molecules, the field of catalysis



Scheme 16. Enzymatic cascade reaction from glycine to (3S)-ketose derivative. Step 2 and step 3 are simultaneous.

continues to evolve rapidly. With the aim of minimizing isolation and purification steps during multistep syntheses, concurrent catalytic cascades have attracted increasing attention and, recently, there has been a growing interest in developing multi-catalytic systems.

The biocatalytic route developed by Merck & Co. for the manufacture of the anti-HIV drug islatravir illustrates how efficient cascades reactions are, along with protein engineering for fine tuning enzyme performances (Scheme 17) [34].

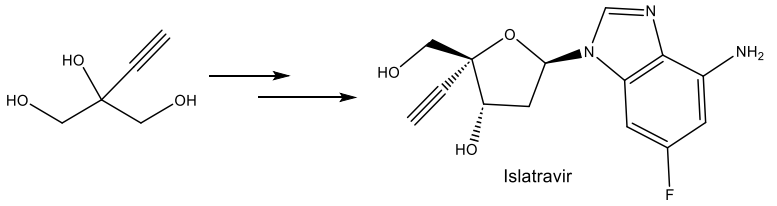
Multi-catalysis emerges not only to enlarge the avenue of new methods but also to offer potential new valuable short-cuts to the synthetic practitioner. Within multi-catalytic processes, either multiple catalysts execute single reactions, or precise sequences of multiple catalytic reactions occur in a one-pot fashion. Cascade reactions can achieve more than the separated individual steps. This is particularly the case when an equilibrium reaction is coupled with an irreversible catalytic step.

The combination of several catalysts from different fields, such as chemocatalysis and biocatalysis, has been developed. For example, Bornscheuer and coworkers developed a method for producing chiral biaryl amines by combining a biocatalytic method, with an amino transfer using an optimized transaminase enzyme, with a chemocatalysis Suzuki-Miyaura Coupling (SMC) reaction [35] (Scheme 18).

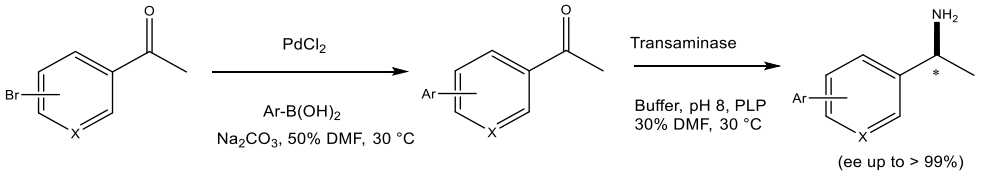
The ability of such species to work under "enzyme-compatible" conditions continues to trigger the development of linear cascades.

5. Perspectives

Remarkably, multi-catalytic sequences can undergo through otherwise unfeasible pathways thanks to, for instance, prospectively unstable intermediates being converted as soon as formed. Complex molecules need to be produced, especially in the pharmaceutical industry and the design of methods to access these compounds in a minimum of steps is highly desirable. For illustration, the synthesis of Eribulin (Figure 3), targeting anticancer treatment, is a highly complex molecule possessing 19 stereogenic centers. It was designed [36] thanks to synthetic chemist's skills. Still, among the first process generation, a 62-step sequence was required for the preparation of this drug which obviously leaves room for further improved productivity and route design, potentially by using some biocatalytic steps.



Scheme 17. Chemical structure of islatravir.



Scheme 18. Combination of the Suzuki–Miyaura cross-coupling reaction with engineered transaminases.

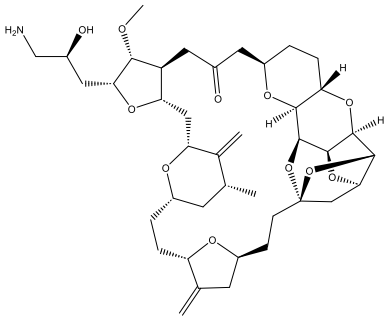


Figure 3. Eribulin structure.

6. The awareness of biocatalysis for synthetic chemists must be strengthen

Some synthetic chemists might be hesitant to adopt biocatalysis due to features like unfamiliarity with the techniques, limited access to biocatalysts, concerns about scalability, and perception that biocatalysis could be more complex than traditional chemical methods. However, as biocatalysis continues to progress, its benefits such as high selectivity (chemo- and enantioselectivity) and environmentally friendly conditions, begin to be recognized and more chemists are going to be motivated to explore its potential. However, most of synthetic chemists are not familiar with the principles, techniques, and applications of biocatalysis. Chemists who are more accustomed to traditional chemical methods might not have the necessary knowledge or experience to effectively use biocatalysis in their work. This lack of "familiarity" could lead to a reluctance to adopt biocatalysis as a viable option for their synthetic processes.

To address the unawareness of synthetic chemists with biocatalysis, several features should be considered:

- *Education and collaborations.* Some academic[2] and industrial organizations[3] can offer workshops, seminars, and training sessions focused on biocatalysis. This would help synthetic chemists understand the principles, advantages, and techniques involved, making them more comfortable with incorporating biocatalysis into their work.
- *Access to biocatalysts.* An increasing number of commercially available enzymes (see footnote 3)—ready to be used are available. Nevertheless, the need to establish repositories or networks that provide synthetic chemists with easy access to enzymes and biocatalyst-related tools.

[2] ESAB: European Society of Applied Biocatalysis.

[3] Such as SEQENS.

- *Practical experience.* Training opportunities where synthetic chemists can work directly with biocatalysts under the guidance of experts. Practical experience will demystify the process and boost confidence in using biocatalysis.

By addressing the unfamiliarity through education, cooperation, and practical experience, the barriers preventing synthetic chemists from using biocatalysis can be gradually overcome. Communicating on success stories with synthetic chemists can inspire others and demonstrate the feasibility and benefits of adopting biocatalytic approaches.

7. Concluding remarks

Current biotechnology offers a comprehensive arsenal of powerful approaches to discover, improve and apply enzymes to produce value-added compounds. Enzyme-catalyzed reactions have already expanded the landscape of attractive retrosynthetic disconnections to selectively build new bonds and design fundamentally new syntheses of complex molecular structures.

Despite the growing impact of biocatalysis in industrial chemistry, the full potential of this technology is yet to be unlocked, especially for reactions that are highly underexplored in enzyme catalysis or not yet known to the enzyme universe. We hope to bring this knowledge to the attention of chemists to bridge the educational gap between organic chemistry and biocatalysis [37–39].

Within industrial organizations, manufacturing active ingredients is paving the way by taking the best from different fields of expertise, including synthetic chemists, process engineers and technobiologists.

This leads to unique opportunity to collaborate across each competency by sharing their knowledge, know-how and perspectives for allowing the discovery of new strategic paths which will enable considerable synthetic economies. Interdisciplinarity is also highly associated with innovation.

Innovative organizations with integrated competencies and capacities, such as high throughput screening platform, can offer these services, such as Seqens.

Declaration of interests

The authors do not work for, advise, own shares in, or receive funds from any organization that could benefit from this article, and have declared no affiliations other than their research organizations.

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