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The Swiss Industrial Biocatalysis Consortium (SIBC) turns 20!

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Abstract: In 2024, the Swiss Industrial Biocatalysis Consortium (SIBC), celebrated its 20 years of bringing together experts from the pharma, flavor and fragrance, fine chemicals, and agrochemicals industries to discuss enzyme technology developments. In this perspective, we share recent examples of how our member organizations utilize biocatalysis in their respective industries. While the motivations for employing enzymatic synthesis and the end goals of various production processes may vary, we aim to emphasize the shared aspects that we are coming across. Over the past 20 years, those synergies have provided us with a fruitful basis for pre-competitive knowledge sharing around biocatalysis as a technology. We look forward to many more years of the SIBC and the surprises that await us through the potential of our enzymes.

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1. Introduction

The Swiss Industrial Biocatalysis Consortium was founded in 2004 to promote biocatalysis as a technology across various industries within Switzerland (Box 1).[1] Over these 20 years, one of our key goals has been to provide biocatalysis teams, as well as process and research chemists within organizations, a peer group for sharing pre-competitive knowledge and exchanging the challenges and opportunities of the technology.

We have come to realize that irrespective of the type of industry – be it fine or agro chemicals, flavor and fragrance, or pharmaceuticals – there are plenty of common denominators when discussing biocatalysis:

1. How to access robust, highly productive, and selective enzymes for a given reaction in a fast and cost-efficient manner at scale.

2. How to educate chemists at all levels in the use of biocatalysis to create realistic opportunities in processes early on.

3. How to tackle possible regulatory challenges when applying GMOs as catalysts.

4. How to expand the scope of scalable chemical reactions catalyzed by enzymes.

In the past 20 years of SIBC's existence, the field has experienced rapid changes in methods and working models, particularly influenced by (Nobel prize recognized!) advances in enzyme engineering,[2] bioinformatics, predictive structural biology and protein design.[3] Given the expanding opportunities to address biological challenges in the field, the synthetic and process chemistry experience of biocatalysis experts becomes increasingly important in finding and executing opportunities to add value to company portfolios.

In this perspective, several companies in diverse sectors of the industry, applying biocatalysis in Switzerland, share recent examples of how they have applied enzymes and biocatalysis to solve (stereo)chemical challenges at both lab and production scale. Authors are from Givaudan, Johnson & Johnson, Lonza, Merck KGaA, Novartis, Roche, and Syngenta.

Box 1: Swiss Industrial Biocatalysis Consortium (SIBC)

Founded: 2004

Founding companies: Lonza, Ciba, Novartis, Givaudan, Sigma-Aldrich, Roche, Syngenta

Mission: Promote biocatalysis inside and outside of Switzerland by bringing together scientists from the Pharma, Fine Chemicals, Agrochemicals and Flavor and Fragrance industries

Goal: Provide mutual benefits in the form of pre-competitive knowledge sharing

Contributing companies in 2025: Givaudan, Johnson & Johnson, Lonza, Merck KGaA, Novartis, Roche, Syngenta, dsm-firmenich

2. Where and When to use Biocatalysis?

The *agrochemical industry* recognizes the value of biocatalysis for its potential to simplify routes to increasingly complex and chiral active ingredients while improving sustainability metrics. However, high volumes and low costs requirements are often perceived as barriers to implementation in production. The main drivers for considering biocatalysts are their high regio- and stereoselectivities and the volatility in the prices of certain metals such as Pd or Ru, often required for metal catalysis. In the past decades, the application rates of agrochemicals have been steadily decreasing, allowing for the consideration of more complex modalities and ingredients. In parallel, the need to reduce residues in the environment has increased the use of single enantiomers over racemates and led to higher sp^3 content in active ingredients.[4]

In early research, commercial and internal enzyme screens are readily tested using automated platforms to provide proof-of-concept for specific transformations. As the demand for active ingredients rapidly escalates from milligrams (for early phenotypic and *in vitro* screening) to hundreds of grams (for field applications), prior to the involvement of process or development teams, synthetic routes relying on metal catalysis are implemented. At this stage, only simple enzyme classes, typically lipases or nitrilases, continue to be utilized. The requirement to evolve enzymes is not justified, as the final ingredient is not yet identified.

Once a product reaches the development phase, aiming for its first ton-scale production, the decision to choose a biocatalytic process over a chemocatalytic one can be reconsidered. However, the barrier to opt for a technology still perceived as novel, and therefore riskier and requiring higher upfront investment, remains high. The main challenges, recently reviewed,[5] include: lack of biocatalysis training for chemists during their academic studies and dedicated teams in industry, potential need to find process partners for enzyme process and engineering, development of new supply chains, especially to access enzymes at scale without relying on a single supplier, downstream processing, which may be better achieved with continuous and immobilization technologies, and waste management, critical at multi-ton scales.

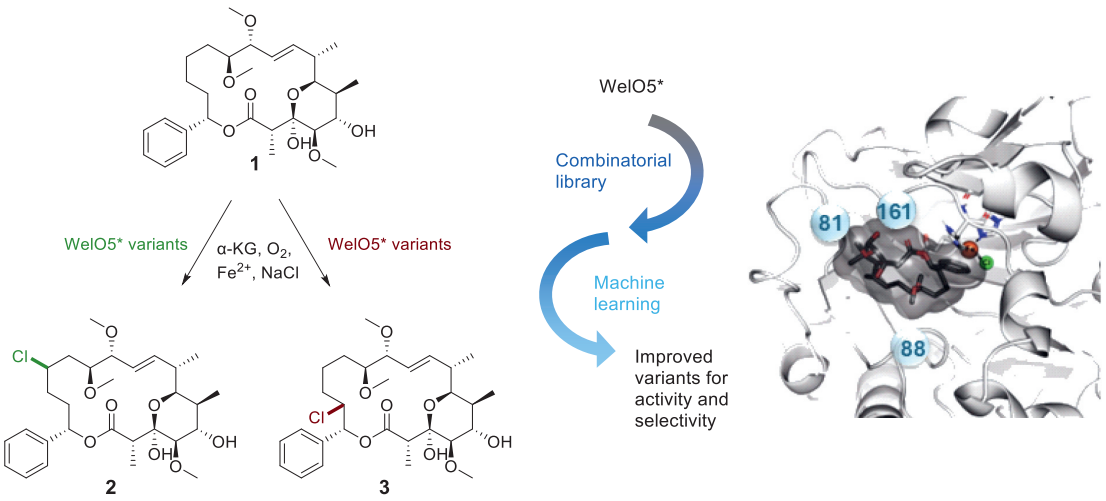
Fortunately, the production under non-GMP conditions allows for continuous process improvement and step changes even after market introduction. Introducing biocatalytic steps as second or third-generation processes may offer more time to overcome these hurdles, assuming these changes provide better sustainability and/or profitability.

With this challenge in mind, Syngenta has acknowledged the necessity to increase the internal expertise and develop a strategic framework to bring biocatalysis proofs-of-concept all the way to production. Crossbreeding between different pipeline stages – research, metabolites (mid- to late-stage pipeline), and process development – served as a driver. Syngenta has currently set out to explore biocatalysis in early research as it provides a flexible environment where riskier long-term options can be investigated.

To complement and build biocatalysis culture within Syngenta but also through partnerships, Syngenta teamed up with Prof. Buller's group (CCBio, ZHAW) and Novartis colleagues to evolve halogenases in a late-stage functionalization strategy.[6] The collaboration partners selected a complex natural product, soraphen A (**1**, Scheme 1), with a structure significantly different from the enzyme natural substrate. Soraphen A is a very potent fungicide, acting through inhibition of acetyl-coenzyme A carboxylase, however its use as a commercial fungicide is limited by off-target selectivity concerns and sensitization in mammals. WelO5* variants were obtained through a 3-site combinatorial library, yielding two distinct chlorinated products (**2**, **3**).[7] A machine learning approach was then employed to improve activity and selectivity towards one of the regioisomers. The predicted variants with either best activity or selectivity were expressed, leading to the identification of a variant with a 300-fold higher total turnover number (TTN) than the initial variant (Scheme 1).

These initial results encouraged further investigation of the WelO5* halogenase class and applying it on renewable building blocks. Such collaborative efforts facilitate the exploration of forward-looking approaches and enable the advantages and limitations of specific enzyme classes to be assessed prior to committing substantial internal resources to a full research program.

In the *flavor and fragrance* (*F&F*) industry white biotechnology processes are attractive for the innovative and sustainable approach they offer for the discovery and production of perfumery ingredients. Developing new odorant molecules and novel processes for their production allows perfumers to create unique olfactive signatures, instantly recognizable in many products including famous fine fragrance perfumes. For that, Givaudan takes advantage of recent developments in the fields of biocatalysis, synthetic biology, and metabolic engineering



Scheme 1. Halogenase WelO5* catalyzed chlorination reactions of soraphen A (**1**). Different level of selectivity towards formation of one regioisomer or the other were obtained depending on the variants employed.[6]

to design efficient processes for the production, at reduced or low environmental impact, of high value, everyday everywhere synthetic odorant molecules irreplaceable in personal care and household products.

Taking into account the urgency and the complexity of the environmental impact challenge, Givaudan has recently launched the FiveCarbon Path™, a new vision that drives the development of fragrance molecules while delivering on the environmental commitment of using today's carbon resources wisely.[8–11] The FiveCarbon Path™ measures the ambition in developing efficient processes according to green chemistry principles[12] focusing on five criteria: (1) increased use of renewable carbon, (2) increased carbon efficiency in synthesis, (3) maximized biodegradable carbon, (4) increased 'odor per carbon ratio' with high-impact materials, and (5) use of upcycled carbon from side streams.[7]

Challenges remain and although not *per se* sustainable,[13,14] Givaudan recognizes the potential of fermentation and biocatalysis to influence positively the metrics used to measure the efficiency of the processes to reduce waste generation, energy consumption, or increase the use of green solvents.[13] Fermentative processes provide an access to terpene mixtures similar to essential oils, or single compounds, which can be transformed further into odorant molecules.[9,11]

In the *pharmaceutical industry*, there are several instances during the drug discovery and development process where biocatalysis proves valuable. Often, companies place the biocatalysis team within the development sector, and these teams may support earlier drug discovery programs as needed. Within SIBC, Roche and Johnson & Johnson follow such a model, whereas Novartis[16] has biocatalysis teams in both the research and development departments. Lonza hosts biocatalysis mainly in their Small Molecules Manufacturing Science and Technology (MSAT) group. MSAT specializes in three key areas. Firstly, it manages the efficient transfer of customer processes to Lonza, including activities such as scale-up, plant fit, and process optimization. Secondly, the group maintains scientific ownership of projects within the production setting, thus ensuring active monitoring, continuous streamlining, troubleshooting, and close collaboration with internal stakeholders (operations, quality, regulatory and commercial) while, at the same time, serving as the primary scientific contact for the customer. Finally, the MSAT group drives innovation by modifying synthetic routes (including the development of second-generation processes) and integrating new technologies to enhance overall efficiency.

As a compound progresses through the pharmaceutical development pipeline, the required amounts of active pharmaceutical ingredient (API) increase, while the costs of synthesis per kg of material must decrease to meet commercial production targets. During the discovery phase, chemists design target compound families to ensure desired properties such as biological activity, pharmacokinetics, solubility, metabolic stability, and low toxicity. At this stage, the development of the route is of lesser priority, while ensuring the rapid, safe, and preparatively effective delivery of the material is deemed sufficient. In addition, synthetic routes often focus on efficient synthesis of key building blocks to support structure-activity relationship (SAR) studies, while allowing for the easy generation of diversity in the final synthetic steps. Biocatalysis for SAR exploration is arguably one of the most underexplored areas, yet it holds significant potential, particularly for applications such as late-stage modifications of advanced substructures, especially within oligonucleotides, peptides or bioconjugates. As the compound moves into early development, structural modifications become less likely, shifting the focus on timelines. Delivering material for first tox studies quickly is often more important than cost-efficiency and scalability. When entering late-stage development, cost reduction and scalability become even more critical, prompting the development of highly efficient synthetic routes. In addition, sustainability, and the 'greenness' of the process – particularly the selection of solvents, reagents, and catalysts – gain increasing importance. As the synthetic requirements evolve during API development, the opportunities for incorporating biocatalytic steps also wchanges.

In the discovery phase, transformations that are difficult to achieve with conventional organic chemistry but possible through enzymatic transformations are highly valued. Examples include the selective reduction of substituted acids to aldehydes by carboxylic acid reductases (CARs), which can be used in subsequent transformations, as well as the application of late-stage C-H hydroxylations or halogenations for further derivatization of lead compounds or for identifying positions critical for biological activity or metabolic stability. However, biocatalysts generally show substrate scope limitations, which restrict their broader application beyond specific cases, making them more suited for later development phases. Additionally, while stereo- and enantioselectivities are some of the key strengths of biocatalysis, they are less relevant at this stage of development. In practice, both enantiomers are often tested after being synthesized

as a racemic mixture and separated using chiral preparative chromatography.

During early development, bulk API availability is on the critical path of the preclinical and clinical development. Therefore, API development is focused on speed. The urgent project timelines often make the implementation of new chemistries – especially biocatalysis – particularly challenging. A key challenge is achieving the desired stereo- and enantioselectivities. High-throughput screening is frequently used to identify suitable catalysts, ligands, or reaction conditions, but in many cases, only moderate selectivities are observed and further improvements can be difficult to attain. If salt resolutions cannot be developed to enrich the desired stereoisomer, chiral chromatographic separation becomes necessary, representing a significant burden for timelines and costs. In this context, biocatalysis can outperform traditional chemical methods by offering higher process efficiency in terms of selectivity and yield, and for certain structural motifs, it could be the preferred choice over chemistry that relies on auxiliaries or costly chiral ligands. Due to time constraints, enzyme engineering is often not feasible at this stage, so off-the-shelf enzymes with the required selectivity and sufficient activity for scale-up are typically used to meet immediate synthetic needs. Mature biocatalysts, such as hydrolases, nitrilases, keto-reductases, and transaminases, are particularly advantageous due to the availability of large panels of robust, highly active variants and extensive scale-up experience, making them highly effective in this context. Emerging enzyme classes, such as imine or enoate reductases, may show promise in screenings, but their catalytic performance is often insufficient for successful scale-up to synthetically relevant levels and would require additional engineering. Although sub-optimal processes with high catalyst loadings and diluted conditions are typically acceptable at this stage – given that the targeted batch sizes are relatively small, usually ranging from hundreds of grams or low-kilogram range – these enzymes still face limitations for widespread applications. In addition to sub-optimal processes, enzymatic kinetic resolutions are a common approach to improve enantiomeric excess (*ee*) values and avoid the need for chiral chromatographic separations.

Once a compound enters the later phases of early development, the main objective of route scouting is to identify a commercial process. At this point, no further changes to the compound's structure are expected, and previous synthetic routes are carefully analyzed and reassessed. This provides an opportunity to explore entirely new synthetic strategies that maximize efficiency. Arguably, biocatalysis can have the most profound impact at this stage, offering the potential to significantly streamline synthetic processes. Even biocatalytic steps with a low probability of success can be considered and tested if they have the potential to significantly improve the synthetic route thanks to the extended time allocated to commercial manufacturing route development. With slightly longer timelines in this development stage, enzyme engineering becomes a viable option to enhance catalytic performance, and measurable activity, even very low activity, is often enough to justify further investment in biocatalytic steps. High process intensities and low catalyst loadings are the primary goals of engineering campaigns, but downstream considerations must also be factored into the process. However, long development timelines and uncertain engineering outcomes – particularly for enzyme classes with limited or no successful scale-up demonstrations – remain significant obstacles to the implementation of biocatalytic steps.

3. Recent Examples from Industrial Applications of Biocatalysis Within the Swiss Industrial Biocatalysis Consortium

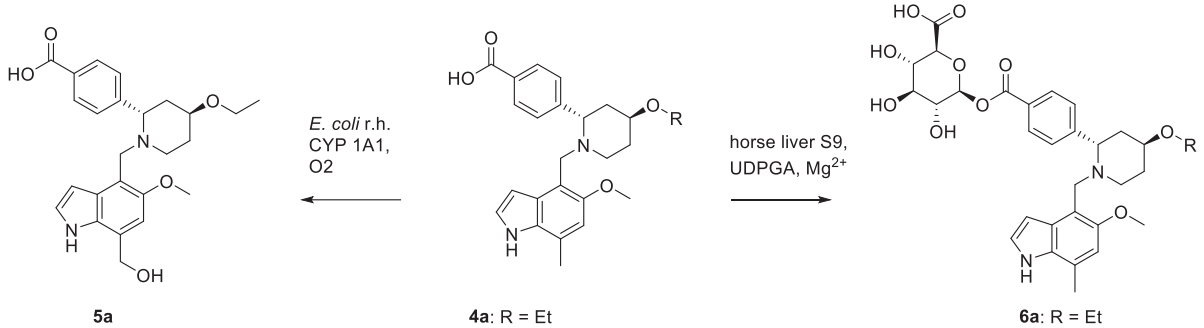
3.1 *Metabolites*

One key area of biocatalytic application in pharma as well as in the agrochemical industry, at which Roche, Novartis, Syngenta and Merck KGaA, have a long-standing experience, is the use of biocatalytic systems to access drug metabolites. As the pharmaceuticals are metabolized in the human body by oxidative and glucuronidating enzymes, it is intuitive to use similar enzymatic systems for synthetic purposes. Agrochemicals undergo metabolism under different environmental conditions, including soil ecosystems and a wide range of organisms such as beneficial insects, fish, birds, and mammals. The increasing regulatory pressure on maximum residue levels, exemplified by the European Union's standard maximum residue level of 0.01 mg/kg and precautionary groundwater quality standard of 0.1 µg/L, has made early identification of metabolites crucial. In pharma, these synthetic metabolites can be used for structure elucidation purposes or as analytical references when analyzing toxicological or clinical study samples. Biocatalysis is widely considered as an effective and technically mature approach to efficiently acquire such drug metabolite standards.

In a recent example from Novartis, the structural characterization of metabolites from Fabhalta® (**4a**, Scheme 2)[17] was supported by the biocatalytic production of reference standards. **4a** absorption, distribution, metabolism, and excretion (ADME) studies were successfully conducted using synthetic reference standards generated *via* biocatalytic routes utilizing animal liver S9 fractions, recombinantly expressed human CYP450 enzymes and co-fermentations of the compound with select Actinomycetes species.

To obtain the oxidized metabolite **5a**, (Scheme 2) a panel of recombinant human CYP450s was screened revealing that the isoform CYP1A1 can successfully produce the desired metabolite. The biocatalyst production and subsequent biocatalytic steps were carried out as described in ref.[18] to obtain the metabolite **5a** in mg scale.

Glucuronidated metabolites (**6a** and **6b**) were obtained by incubation of Iptacopan **4a** and **4b** with horse liver S9 as



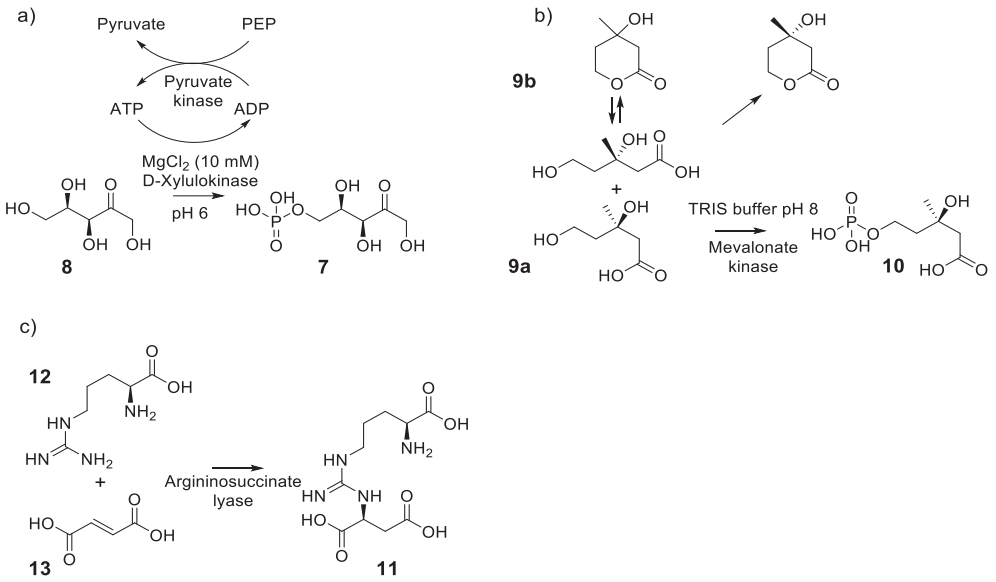
Scheme 2. Biocatalytic methods for the production of metabolite references of Fabhalta® (**4a**).

previously described.[19] Liver S9 is a supernatant fraction obtained by centrifuging a liver homogenate in a suitable medium, such as sodium chloride solution, and can be applied in metabolite synthesis. However, after screening a panel of microorganisms, a Hypha proprietary bacterial strain of the genus *Streptomyces*, was identified that could also make the glucuronide **6a**. The fermentation and down-stream process methods as described by Papp *et al*.[20] enabled the production of glucuronide metabolites, replacing the use of UDPGA and animal liver fractions on a larger scale.

Merck KGaA has recently applied kinases and a lyase in the synthesis of four important metabolites (Scheme 3). The first example showcases the efficient synthesis of D- and L-xylulose-5-phosphate (**7**) catalyzed by xylulokinases. Both enantiomers of xylulose-5-phosphate play an important role as substrates to investigate biochemical pathways as well as standards for dried blood spot analysis, *e.g*. to detect errors in the pentose phosphate pathway, such as transaldolase deficiency.[21] For both, the continuous availability of highly pure compounds is essential. Merck developed a one-step phosphorylation of D- and L-xylulose (**8**) applying recombinant xylulokinases with ATP as a phosphate donor showing high turnover rates.[22] Monitoring of the conversion using ^{1}H and ^{31}P NMR spectroscopy showed complete reaction after 2–4 h. Work-up was performed by adding calcium acetate for precipitation of the final product. Because this step is not selective for the desired xylulose phosphates, it would lead to unwanted coprecipitation of ADP and ATP. To avoid that, a cofactor recycling system (phosphoenolpyruvate (PEP)/pyruvate kinase (PK)) was applied (Scheme 3). This allowed the use of a minimum of ATP for the reaction that would remain as ADP in the precipitate. By limiting PEP to 0.9 eq. the complete conversion to pyruvate was guaranteed. Traces of inorganic phosphate were removed by an additional step, where the calcium salt was converted to the respective lithium salt. Phosphoric acid monoesters are generally prone to hydrolysis under acidic conditions. Lithium conditioned Dowex resin was ideal for the conversion because it minimizes contact of the product to acidic conditions. With this procedure a highly selective conversion of L-xylulose and D-xylulose to the respective 5-phosphates was reached in high yields of 91% and excellent purities of approximately 100%, thus providing valuable intermediates of metabolism for various applications in life science.

A second example involves the use of a kinase for the resolution of mevalonic acid (**9a**) to make (*R*)-5-phosphomevalonate (**10**), a critical intermediate in the biosynthesis of terpenes and sterols.[23] For this reaction Merck applied recombinant mevalonate kinase from the hyperthermophilic archaeon *Thermococcus kodakarensis*. It showed excellent turnover rates and almost complete conversion of (*R*)-mevalonate **9a** from racemic mevalolactone **9b** to the corresponding phosphate **10**. The remaining (*S*)-**9b** could also be isolated in high purity, providing access to this enantiopure species. For the reaction, mevalolactone, ATP, magnesium chloride, and a suitable buffer, such as Tris buffer at pH 8.0, were mixed and the reaction initiated by the addition of mevalonate kinase. During the conversion, the pH was controlled and adjusted to 8 by slowly adding LiOH solution. The slow reaction at room temperature can be accelerated by raising the temperature to 55 °C to obtain complete conversion after 25 h. Reaction monitoring can be done applying ^{31}P-NMR, TLC or by measurement of LiOH consumption during the conversion. (*R*)-**10** can be isolated by fractional crystallization and is further purified by silica gel chromatography to obtain purities greater than 95%. This biocatalytic approach simplifies traditional multi-step synthetic routes, avoiding the need for protecting group strategies and extensive purification. Furthermore, from the remaining mother liquor, enantiomerically pure (*S*)-**9b** can be obtained with yields higher than 80% from the enantiomeric starting material (Scheme 3b). This metabolite has been studied in clinical settings, particularly for its role in lipid metabolism and its potential implications in cardiovascular diseases.[24] Elevated levels of mevalonic acid have been associated with certain metabolic disorders, making it a target for clinical testing and therapeutic intervention. This process to produce (*R*)-5–**10** and (*S*)-**9b** from racemic mevalolactone **9b** can also use the aforementioned PEP/ PK system for enzymatic ATP regeneration, facilitating the upscale to large scale production.

As a last example an enzymatic version of the Michael addition is illustrated. The Michael addition is a fundamental reaction in organic chemistry and involves the nucleophilic addition of a compound to an electrophilic partner to form new carbon-carbon bonds. The enzymatic Michael addition is particularly appealing due to its high selectivity and mild reaction conditions. In our study, we aimed to synthesize L-argininosuccinic acid (**11**), a key metabolite in the urea cycle, through the biocatalytic asymmetric Michael addition of L-arginine (**12**) to fumarate (**13**) (Scheme 3c).[25] L-Argininosuccinate (**11**) plays a crucial role in nitrogen metabolism and is a precursor for various amino acids and other nitrogen-containing compounds. It is also of clinical interest, elevated levels of **11** are associated with argininosuccinic aciduria, a metabolic disorder resulting from a deficiency in argininosuccinate lyase (ASL).[26] Merck utilized recombinant ASL from *Saccharomyces cerevisiae* as the biocatalyst for this reaction, which was successfully developed at a 1.1 gram scale. A



Scheme 3. Reaction schemes to show the biocatalytic synthesis of four different metabolites. a) xylulokinase/PEP/PK system for the synthesis of D-xylulose-5-phosphate (**7**); b) Mevalonate kinase catalyzed reaction to synthesize (*R*)-5-phosophomevalonate ((*R*)-**10**). The byproduct is enantiomerically pure (*S*)-mevalonic acid ((*S*)-**9a/b**); c) enzymatic Michael type reaction to argininosuccinic acid catalyzed by Argininosuccinate lyase.

reaction mixture consisting of L-arginine (**12**) and fumarate (**13**) in a suitable buffer was prepared, and the pH was adjusted to 7.5 with LiOH. The ASL enzyme solution was then added to this mixture, and the reaction was stirred at room temperature. The progress of the reaction was monitored using ^{1}H NMR spectroscopy, allowing the tracking of the formation of L-argininosuccinate in real time. After six days of reaction time, the conversion plateaued, and the crude product could be obtained through precipitation. The final product was isolated by flash chromatography with a yield of 0.77 g (70%) of **11** and a purity higher than 95%.

Overall, the use of recombinantly expressed human CYP450s or the liver S9 fractions in metabolite synthesis not only enabled challenging chemistry but also reduced the need for hazardous reagents and extensive purification steps. These approaches align with the sustainable practices in chemical synthesis, making biocatalysis a valuable strategy for producing high purity metabolites essential for various applications in life sciences and clinical settings. However, while recombinant enzymes are valuable tools for generating putative metabolites, they do not encompass the diversity of metabolism found in the environment. Moreover, scaling-up CYP450s catalyzed oxidations remains challenging, as grams or even kilograms of metabolites may be required during (re)-registration of crop protection ingredients, particularly for dietary toxicological studies. Hence alternative classes of oxidative enzymes that offer improved scalability, such as unspecific peroxygenases or monooxygenases, are particularly of interest for the agro- but also for the pharmaceutical industry.

3.2 *Kinetic Resolutions*

Kinetic resolutions are some of the earliest biocatalytic applications reported in the organic chemistry literature and remain highly relevant in the SIBC members' methodology, as described below by Lonza, Givaudan, Johnson & Johnson and Roche.

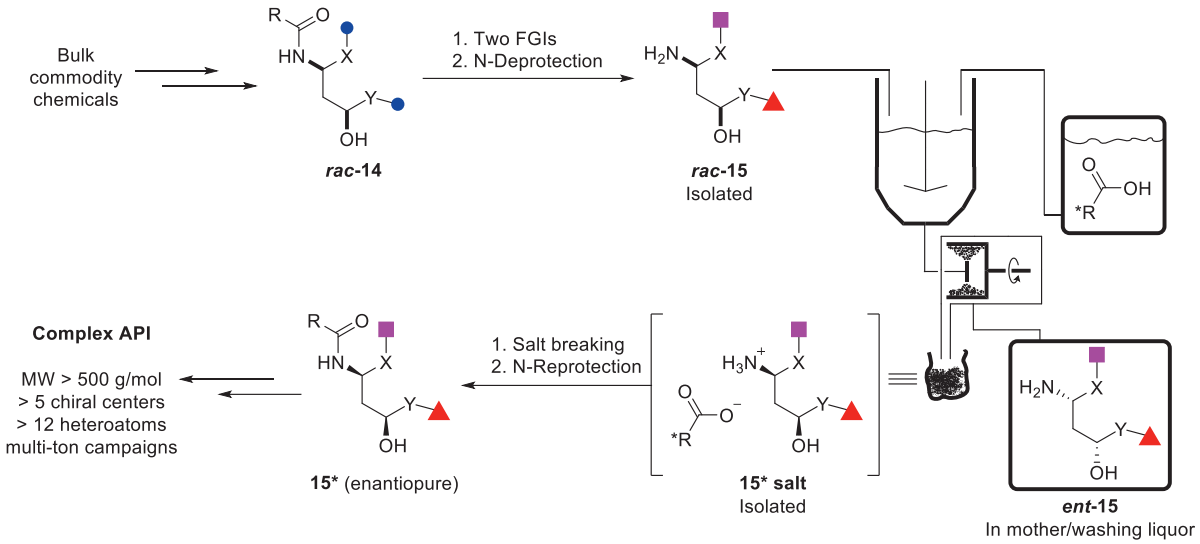
A decade ago, Lonza's MSAT group received a manufacturing request for a complex, enantiopure Active Pharmaceutical Ingredient (API). While the core chemistry has remained largely intact, MSAT has refined various elements of the process, including adjusting reactant charges, optimizing the sequence of operations, concentrations, and crystallization parameters. These enhancements have not only improved yield as well as quality and significantly reduced waste, but have also streamlined the process, ensuring scalability and efficient transfer between production trains within Lonza.

A key intermediate in the synthesis of this API is the racemic aminoalcohol *rac*-**14**, manufactured at 10 m³ scale from bulk commodity chemicals (Scheme 4). The process continued with two functional group interconversions followed by *N*-deprotection to enable a classical resolution of *rac*-**15** using an enantiomerically pure acid. Salt breaking and re-protection of the *N*-functionality finally delivers enantiopure **15***, which is processed further to the API.

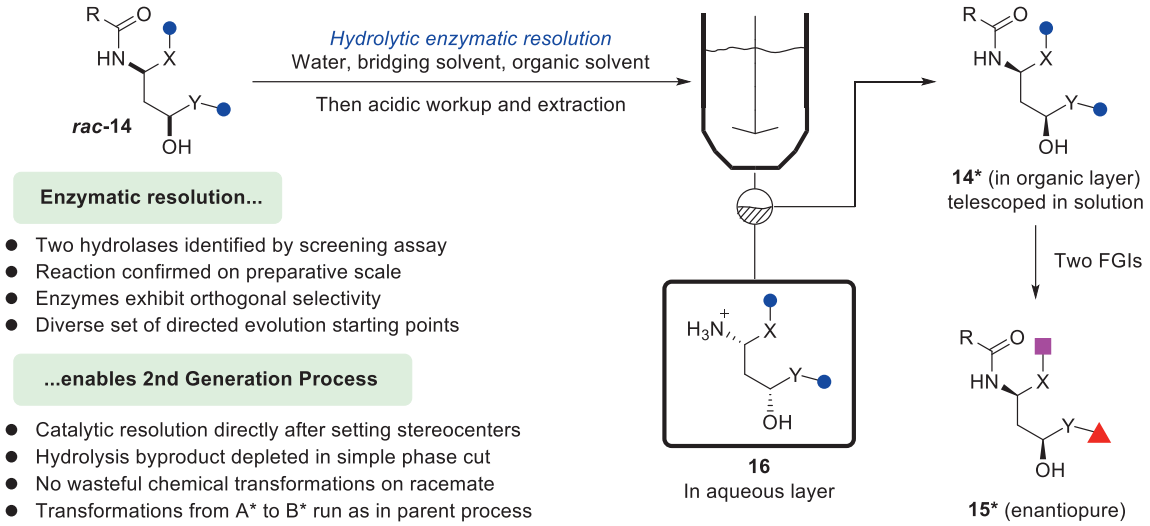
While effective for delivering the API at commercial scale, this approach was resource-intensive, as 50% of the material and reactor space upstream of the resolution are consumed by the undesired enantiomer, and stoichiometric amounts of chiral acid were required. While a recycling process was developed and successfully implemented on production scale to recover the chiral acid after salt breaking, a streamlined process was desired to minimize resource consumption and to optimize manufacturing capabilities.

To tackle the inefficiency, a redesign of the synthetic route to **15*** was proposed, aiming to combine the best of two worlds by incorporating enzymes while preserving as much of the established process as possible. This approach (Scheme 5) involves kinetic resolution of *rac*-**14** using a hydrolytic enzyme, thus bringing the resolution step forward to immediately follow the formation of *rac*-**14**, and, at the same time, eliminating functional group interconversions of racemic material, the classical resolution, and the deprotection-reprotection sequence. This strategy is also intended to leverage the significant difference in lipophilicity between *rac*-**14** and its hydrolysis product **16**, enabling an efficient separation of **14*** and **16** through phase separation. This enzymatic approach not only reduces waste but also streamlines the process, improving both sustainability and cost-efficiency.

Building on this concept, the next step involved screening for potential enzymes capable of facilitating the hydrolytic resolution shown in Scheme 5. Among the candidates screened, two enzymes demonstrated effective kinetic resolution of substrate *rac*-**14**, preferentially consuming one of the enantiomers and providing proof of concept for this approach. Interestingly, the enzymes exhibited orthogonal selectivities: one hydrolyzed the undesired enantiomer (the desired pathway), while the other preferentially hydrolyzed the desired enantiomer. Both enzymes were advanced as starting points for efforts to enhance their



Scheme 4. The synthesis of an API manufactured at Lonza involves functional group interconversions on a racemic intermediate prior to a classical resolution with several isolated intermediates. FGI: Functional group interconversion. The colored pictograms represent non-disclosable functional groups.



Scheme 5. The direct enzymatic hydrolysis of *rac*-**14** addresses many of the limitations of the original process while fully leveraging the robustness gained through years of development and production. The colored pictograms represent non-disclosable functional groups. This project successfully established proof of concept for a second-generation synthesis of a complex API intermediate, which was designed to address the increasing demands on efficiency, sustainability, and cost reduction to which a manufacturing process is exposed throughout its life cycle. This endeavor exemplifies how the MSAT team of Lonza not only adapts to challenges but also paves the way for future advances, ultimately contributing to Lonza's mission of enabling a healthier world through science.

suitability for industrial applications, focusing on key factors such as concentration, turnover, and solvent resistance.

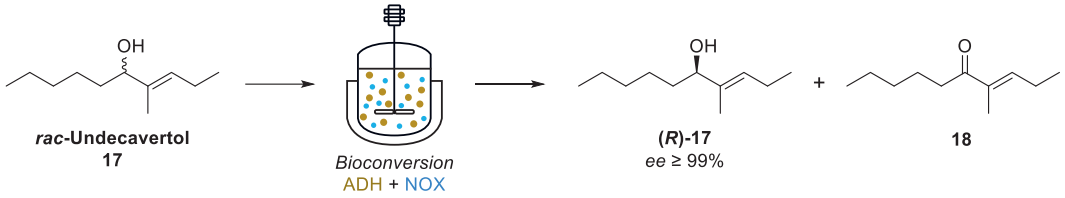
Alcohols, aldehydes, and ketones are found in many olfactive families and are among the most important odorants.[27] While the chemical reduction of aldehydes or ketones to alcohols can be very selective, the chemical oxidation of alcohols is much less selective. It requires stoichiometric amounts of oxidizing agent, thus producing large amounts of waste.

Alcohols can be enzymatically oxidized using alcohol dehydrogenases (ADH), enzymes which catalyze both the reduction of ketones and aldehydes using NAD(P)H, and the oxidation of alcohols using $NAD(P)^+$ as a cofactor.[28] The stoichiometric requirement for the cofactor is not economically viable, therefore a cofactor regeneration system is required for cofactor recycling *via* the oxidation/reduction of a cost-effective co-substrate.

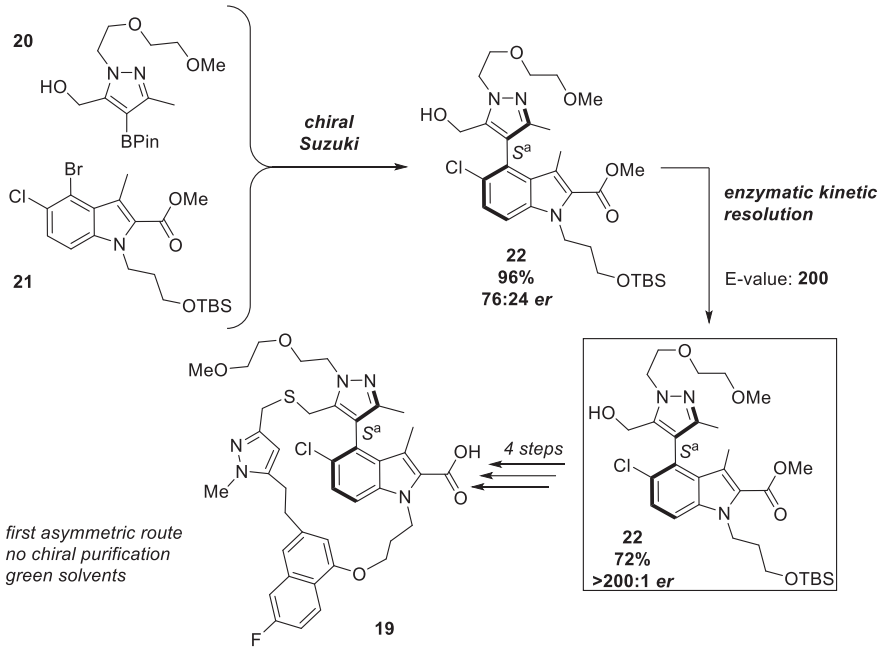
The different stereoisomers of a fragrance ingredient can differ significantly in their olfactive properties. ADHs are hence useful enzymes for the synthesis of odorants,[9,29,30] in particular when addressing differences in olfactive character between isomers rather than in the threshold between the isomers of an odorant, keeping in mind that 50% of the material gets lost during racemic resolution.

An enzymatic racemic resolution of Undecavertol ((*E*)-4-methyldec-3-en-5-ol, **17**) using an (*S*)-selective ADH in the oxidative direction was developed at Givaudan[31] in which an engineered oxygen-dependent NAD(P)H oxidase is used for cofactor regeneration producing only water as a by-product and driving the reaction in the desired direction. In this process (*S*)-**17** is fully oxidized by the ADH enzyme to Undecavertone (**18**), (*R*)-**17** is left untouched (Scheme 6). Fractionated distillation allows the separation of the two reaction products. The ADH used is highly stable in the presence of high concentrations of substrate and products. The developed process proved highly productive as, for example, on a laboratory scale, up to 680 g/l of the substrate was fully oxidized within 16 h with only 5.2 g/l of the ADH enzyme (Scheme 6).

Axially chiral biaryl fragments are common scaffolds in drug design. These key synthons are typically constructed through metal-catalyzed Csp^2–Csp^2 bond formation, followed by the separation of atropisomers, a process that often leads to low yields and generates excess waste. Process chemists at Johnson & Johnson reported an unprecedented chiral Suzuki–Miyaura coupling/enzymatic kinetic resolution sequence for the synthesis of the highly potent MCL-1 inhibitor JNJ-4355 (**19**) and other atropisomeric biaryls, achieving high enantiopurity of the targets without the need for chiral separation (Scheme 7).[32] The initial efforts focused on screening chiral ligands in combination with different solvents and bases using high-throughput experimentation to establish the chiral axis. The best result yielded the desired target with a 76:24 *er* (*S*/*R*) and 96% yield. Although highly beneficial compared to the previous racemic approach, the method did not, on its own, eliminate the need for a chiral enrichment step. The team subsequently turned to biocatalysis, aiming for an enzymatic kinetic acylation of the hydroxyl group on the undesired atropisomer, with the goal of bypassing the need for chiral resolution or purification. Comparable efforts focusing on the stereoselective hydrolysis of the methyl ester had previously failed, due to the low enzymatic activities and selectivities observed during the screening stage. Fortunately, several commercially available immobilized enzyme formulations exhibited the desired selectivity, with LIPX-T2-150XL (lipase Lipex 100L) from ChiralVision outperforming other candidates when catalyzing the envisaged enantioselective



Scheme 6. Enzymatic, racemic resolution of Undecavertol. The two products are produced in a 1:1 ratio.



Scheme 7. Synthesis of atropisomeric biaryls using the combination of a chiral Suzuki-Miyaura coupling with an enzyme-catalysed kinetic resolution enabled an efficient delivery of **19**.

acylation in organic media. This chemistry was demonstrated on a 2 g scale using 1 wt% immobilized catalyst and two equivalents of vinyl propionate as the acyl donor in heptane, achieving 72% assay yield with excellent selectivity as quantified by E-values >200. The E-value is a constant describing the enantioselectivity of a kinetic resolution. The approach was then extended to eight structurally different atropisomeric biaryls of pharmaceutical interest. The implementation of the enantioenriched coupling, combined with lipase catalysis, significantly reduced the overall process mass intensity (PMI) and aligned with the principles of modern green chemistry during the early development of **19** (Scheme 7) a highly potent MCL-1 inhibitor.

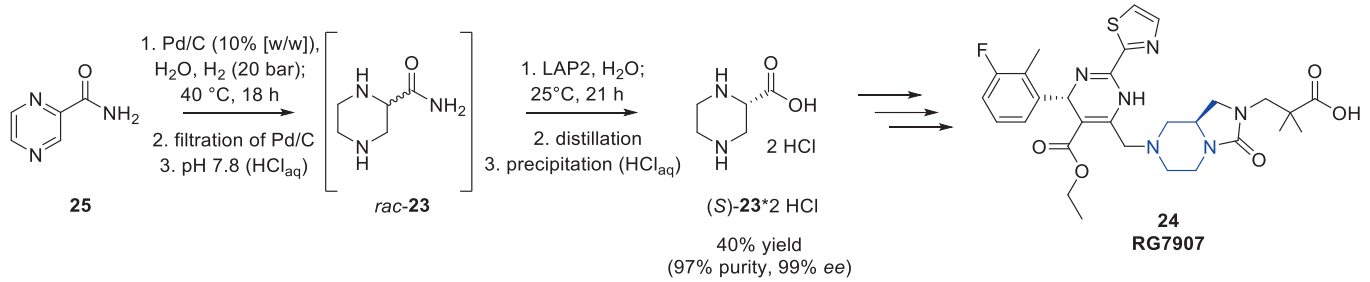
(*S*)-piperazine-2-carboxylic acid ((*S*)-**23**) is a precursor in the synthesis of Linvencorvir (**24**, RG7907)[33] an active pharmaceutical ingredient (API) under investigation as a hepatitis B virus (HBV) core protein allosteric modulator for the treatment of chronic HBV infection. Roche developed an all-catalytic, cost-efficient approach to (*S*)-**23** consisting of quantitative hydrogenation of the readily available pyrazine-2-carboxamide (**25**) with 10% Pd/C delivering *rac*-**23** (Scheme 8, step 1), followed by its enzymatic resolution at 20%[*w/w*] substrate loading using the leucine peptidase 2 of *Aspergillus oryzae* (LAP2) (Scheme 8, Step 2).[34,35]

The enzymatic process was inspired by early resolution work using whole cells of *Klebsiella* and *Burkholderia* strains.[36] At Roche, two formulations of leucine amino peptidase 2 from *Aspergillus oryzae*, the commercial Flavourzyme® 1000L (Novozyme) and a partially purified enzyme formulation of the heterologously expressed and secreted LAP2 in *Pichia pastoris*, were successfully applied in this resolution process on 100 g scale (see Table 1), delivering (*S*)-**23**·2HCl in 97–98% purity, >99% *ee* and 40% yield.

Table 1. Kinetic resolutions on 100 g scale using different leucine aminopeptidase (LAP) preparations.

Leucine aminopeptidase (LAP)		***(S)*-23·2HCl**			
Liquid formulation	Loading	Mass Yield	HPLC Purity	Corrected Yield	(*S*)-**23** *ee*
Flavourzyme® 1000L	100 mL	69 g	97%	40%	>99%
Recombinant LAP2	25 mL	69 g	98%	41%	>99%

The process was applied for a supply campaign in various batches on the ton scale using Flavourzyme® 1000L at a substrate loading of 20% [*w/w*] and the different batches delivered the intermediate (*S*)-**23**·2HCl in 97–98% purity, 97–98% *ee* and 37–41% yield. At scale the temperature control during the di-HCl-salt formation was not ideal (addition of ~1.8 eq. concentrated HCl (35%) was needed to form the dihydrochloride salt in the presence of the substrate and the salt crystals were washed with 3 N HCl) and the chiral purity decreased by minor hydrolysis of (*R*)-**23**·2HCl under these very acidic conditions (pH <1).



Scheme 8. Optimized all-catalytic process to (*S*)-**23** a precursor of RG7907 (**24**). LAP2 = Leucine aminopeptidase 2.

Table 2. Productivity / sustainability metrics for the different experimental setups.

Catalyst form	Process type	Productivity (g product g enzyme^{-1} h^{-1})	Ideal STY (g product /L/h)	Real Production Rate (g product/h)	E-factor
LAP2	Batch	0.48	2.9	1.6	14.5
Immobilized LAP2	Batch	1.37	3.4	0.003	12.8
	SpinChem®	2.78	16.7	1.7	4.0
	Flow (8 mL PBR)	3.67	50.4	0.3	5.3
	Flow (20 mL PBR)			1.1	3.6

Due to the high demand of (*S*)-**23**·2HCl, Roche aimed for further potential process intensification by immobilizing semi-purified LAP2 (95% purity as assessed by SDS-PAGE gel electrophoresis) and applying the heterogeneous biocatalyst (HFA/S-amino aldehyde-LAP2) to the chiral resolution of *rac*-**23**, delivering enantiopure (*S*)-**23**. Satisfyingly, the immobilized biocatalyst retained its activity even after multiple uses. Ultimately, the biotransformation was integrated both in continuous flow mode and a stirred tank reactor (SpinChem®), which allowed for an increased real production rate (1.1 and 1.7 g product/L, respectively). Importantly, its employment not only allowed to increase productivity (Table 2), but reduced waste formation by a factor of 4.

However, the real productivity rate was hampered by product inhibition of (*S*)-**23** and ammonia (by-product), hence suggesting that even higher productivity could be obtained by reducing the inhibition effects and increasing the activity by enzyme engineering.

All these examples highlight the benefits of enzymatic stereoselectivity, across different enzyme classes. Furthermore, as demonstrated by Roche and Johnson & Johnson, the combination of metal- and biocatalysis can be powerful, particularly when applied in a telescoped processes to yield efficiently key chiral building blocks for API synthesis. Moreover, they outline the advantages that immobilization can have on the catalyst reusability and stability, and as a key enabling technology to perform such transformations in continuous mode.

3.3 *Asymmetric Synthesis and Dynamic Kinetic Resolution*

Even if the above examples show great benefits of resolution processes, asymmetric synthesis and dynamic kinetic resolution are the preferred methods of choice across SIBC, because the theoretical yield is not limited to 50% but 100% conversion can be reached, often saving materials, and improving cost and environmental impact of the synthesis.

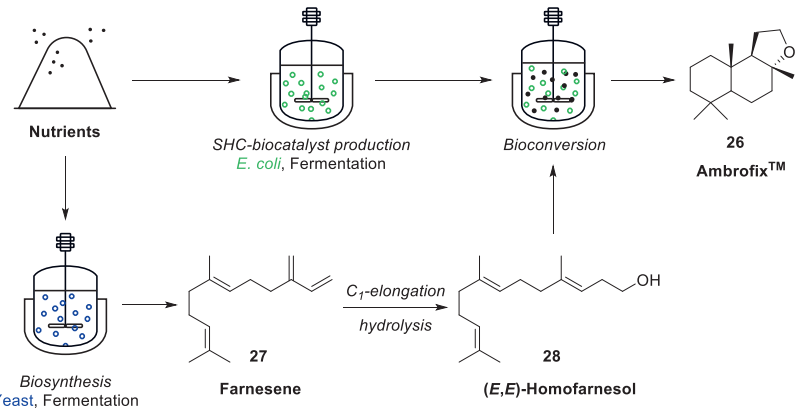
The much-appreciated perfumery ingredient (–)-ambrox has first kept generations of chemists on their toes before biochemists started to toe the line.[37] This compound, first discovered as a result of degradation studies of sclareol, was identified later as the key odorant of mature ambergris tincture, a coprolith produced by the sperm whale.[37] In a recent study Givaudan demonstrated the usefulness of Squalene Hopene Cyclase (SHC) enzymes for the chemoenzymatic synthesis and olfactive characterization of ambrox isomers, and that the commercial product (–)-ambrox, produced by Givaudan as Ambrofix™ (**26**), is by far the strongest isomer in terms of odor strength.[38]

The synthesis of this most widely used biodegradable ambery fragrance ingredient has depended so far on a well-established process developed in the 1950s, starting from sclareol extracted from clary sage. The required access to large quantities of sclareol and the availability of the new and renewable feedstock (*E*)-*β*-farnesene (**27**), produced by fermentation, triggered efforts at Givaudan towards the identification of alternative and sclareol-independent routes to produce Ambrofix™ (**26**).[37]

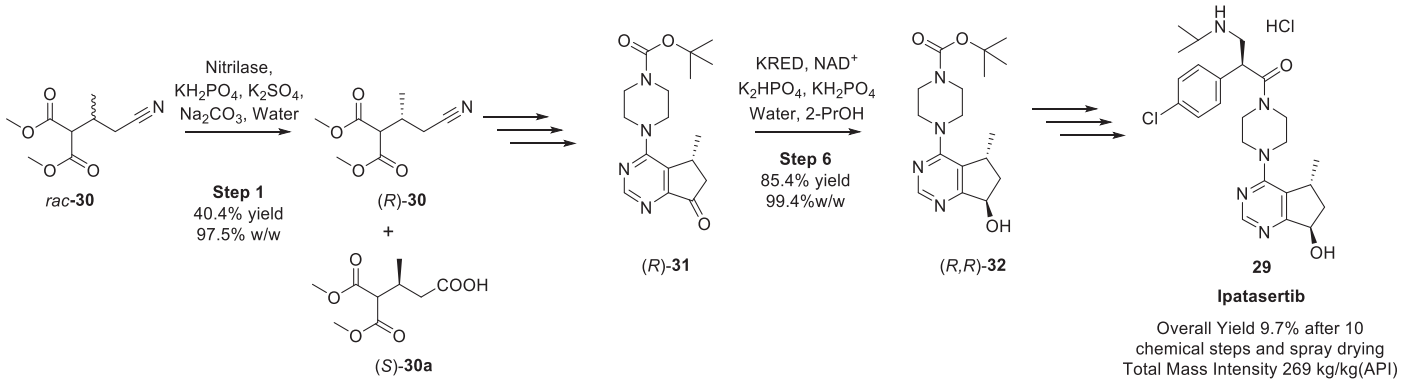
Since the first ever observation that an SHC enzyme can cyclize in a single step the linear C16 (*E*,*E*)-homofarnesol (**28**) to the tricyclic **26**, the development of an innovative and cost-effective chemoenzymatic synthesis process at Givaudan for Ambrofix™ production from **27** *via* **28** brought a long-standing desire of the fragrance industry to reality[37,39] (Scheme 9).

Iterative cycles of SHC enzyme evolution and reaction engineering created biocatalysts suited to operate under process-relevant conditions and at industrial scale in biotransformation systems with whole cells of *E. coli* producing an SHC enzyme variant. Up to 450 g/l **28** are fully converted in 72 h at a cells to substrate ratio of as low as 0.4:1 depending on the SHC variant used.[39] Ambrofix™ directly crystallizes out of the reaction mixture in this new production process: this allows for an efficient and environmentally-friendly Ambrofix™ recovery by means of filtration, as no solvent is required for extraction.

The optimized Ambrofix™ production process developed at Givaudan resulted in atom and step economy, reduced waste production, solvent, and energy consumption, making a significant step forward in terms of sustainability compared to the traditional chemical method of synthesis of (-)-ambrox from sclareol. It illustrated the complementarity of biocatalysis and chemical synthesis, and how their combination enables one to develop and implement efficient, sustainable, and environmentally friendly production processes at a multi-metric ton scale. Metrics, productivity and selectivity of the enzymatic cyclization of **28** to Ambrofix™ with an SHC enzyme[39] can be estimated for the time being as by far more efficient than recent advances



Scheme 9. Givaudan process for Ambrofix™ (**26**) production from (*E*,*E*)-homofarnesol (**28**) with Squalene Hopene Cyclase.



Scheme 10. Biocatalysis steps in the GMP manufacturing of Ipatasertib (**29**).

reported on (-)-ambrox production from **28** *via* chemical polyene cyclization.[40]

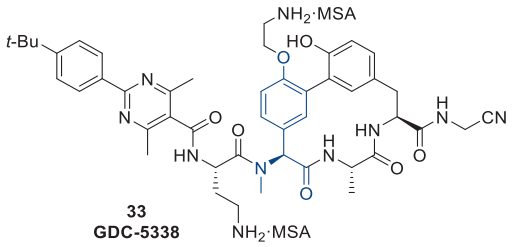
Roche's Ipatasertib (**29**),[41,42] a potent small molecule Akt kinase inhibitor, contains three stereocenters and two are established by enzymatic catalysis. The kinetic resolution of *rac*-**30** using an evolved nitrilase starts the validated GMP process[43,44] (see Scheme 10).

The nitrilase process successfully manufactured (*R*)-**30** on a commercial scale, producing ~11 tons of material with 40.4% yield, 97.5% *w/w* assay, 99.5 area% purity and >98.0% *ee*. The team at Roche applied a liquid formulation of the nitrilase to significantly reduce the safety precaution of enzyme handling. Therefore, the enzyme engineering targeted a reduced inhibition of glycerol (principal component in the liquid formulation) besides a high volumetric activity, selectivity, and stability. In a collaborative effort with the company cLecta and Roche, all the engineering targets were successfully achieved.

The second enzymatic process, a diastereoselective ketoreduction of (*R*)-**31** using an evolved Codexis ketoreductase (CDX-040), produced ~5 tons of white (*R*,*R*)-**32** after crystallization with 85.4% yield, 99.4% *w/w* assay, 99.8 area% purity, and >99.9% *de*. During the target-specific ketoreductase CDX-040 engineering the NADP cofactor specificity vanished and allowed use of the significantly cheaper NAD. Moreover, a further alternative Roche proprietary engineered KRED[45] could be identified for the cost-competitive ketoreduction process and it highlights a Swiss university-industry collaboration:[46] Excelzyme, which is a collaboration with Prof. Bullers' group, located at the Zurich University of Applied Sciences.

In another example, the pipeline of Roche includes antibiotics with peptide core structures such as Zosurabalpin, a small molecule, novel chemical class antibiotic for the treatment of *Acinetobacter baumannii* infections and LepB inhibitors, such as GDC-5338 (**33**), an arylomycin antibiotic (see Scheme 11).

The molecule of interest contains *N*-alkyl and unnatural amino acids and new synthetic routes for these building blocks are highly sought after. One of Roche's contributions in this field is a lipase-catalyzed dynamic kinetic resolution (DKR) of PEGylated *N*-alkyl amino esters (**34**).[47] This method was developed for substituted



Scheme 11. GDC-5338 (**33**) an arylomycin antibiotic.

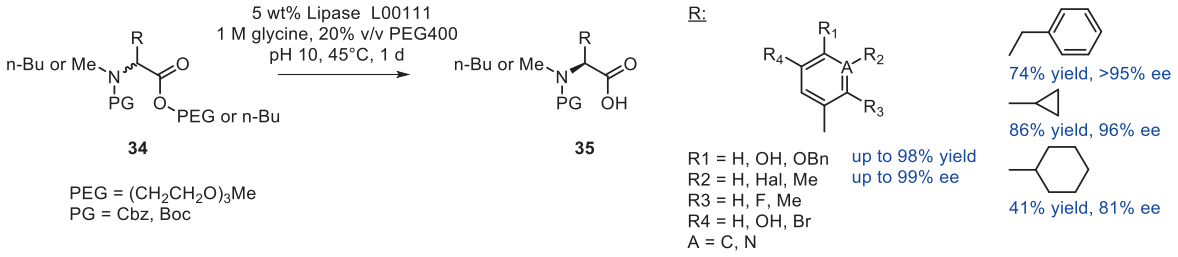
N-methyl hydroxyphenylglycines (**35**), enabling the macrocyclic ring closure,[48] and proved to be applicable for the preparation of a broad range of aromatic and aliphatic enantiomerically enriched *N*-alkyl unnatural amino acids, in up to 98% yield and 99% *ee* (see Scheme 12).

The commercial lipase L00111 [evo1.3.147.S, Evoxx] displays a remarkably high enantioselectivity and stability at elevated temperatures (up to 55 °C) and basic conditions (up to pH 11) but lacks an acceptable solvent tolerance. The *in situ* racemization rate has to outperform the enzymatic hydrolysis rate for an efficient DKR, while avoiding chemical hydrolysis (ester stability). This might require a substrate-dependent fine-tuning of pH and temperature as key racemization drivers. The PEGylated esters have a significant solubility advantage and an improved reactivity. The 'classical' *N*-butyl esters offer the advantage of a higher hydrolysis stability resulting in decreased solubility and enzymatic activity. In summary, PEGylated esters offer a practical and scalable DKR procedure for *N*-alkylated non-proteinogenic amino acids.

3.4 *Emerging Modalities: Biocatalysis for Oligonucleotides*

The portfolio of pharmaceutical companies has traditionally been dominated by low-molecular-weight compounds (LMWs) and the biocatalytic applications focus largely on asymmetric synthesis and kinetic resolutions as exemplified above. However, the increasing significance of technology platforms like biotherapeutics, peptides, oligonucleotides, radioligands, and cell & gene therapy has started impacting pharmaceutical industries' focus on biocatalytic research and development (R&D). Enzymatic bioconjugation, for example, is particularly intriguing for biotherapeutic antibody drug conjugates (ADCs) or radioligand therapies.[16]

Oligonucleotides are becoming increasingly important as therapeutic agents.[49] Examples include antisense oligonucleotides (ASOs) and double stranded RNA (dsRNA), which effectively silence gene expression, targeting mRNA by diverse mechanisms.[50] The global market is growing rapidly, estimated at $5.2 billion in 2020 and is expected to reach $26.1 billion in 2030.[51] As demand for therapeutic oligonucleotides will likely reach metric ton amounts, there is an acute need for robust and sustainable synthetic methods.[52] Solid phase phosphoramidite chemistry (SPOS), has been the method of choice for the synthesis of oligonucleotides for several decades,[53] but there are inherent problems in applying this process for large scale manufacture. Errors in coupling efficiency are typically minimal (<1% per coupling cycle), but these accumulate, leading to complex impurity profiles and reduction in yield as the length of the oligonucleotide increases. Furthermore, sustainable and economically viable manufacturing remains a challenge due to the excessive need of acetonitrile (>1000 Kg per



Scheme 12. Dynamic kinetic resolution of non-proteinogenic *N*-alkyl amino acid esters.

Kg product) and the difficulties in obtaining high product purity.[54]

Biocatalytic approaches have been proposed as an alternative to solid phase synthesis, and examples have included the use of template-independent polymerases, particularly terminal deoxynucleotidyl transferase (TdT) and poly(U) polymerase (PUP) for DNA and RNA oligos respectively.[55–57] In addition, self-templating approaches using bacterial DNA polymerases have also shown promise.[58] Many therapeutic oligonucleotides contain modifications of the phosphodiester backbone (phosphorothioates), ribose (2'-substitutions including fluoro, O-methyl, methoxyethyl), and these often present problems for polymerases.[59]

An alternative chemo-enzymatic approach employs template-dependent DNA or RNA ligases for assembly of fragments synthesized by SPOS, which has the advantage that the 'shortmer' fragments can be prepared in high purity leading to improved impurity profiles in the final product. In addition, ligases are generally more tolerant of ribose modifications than polymerases.[58] Examples include assembly of ASO gapmers on a DNA template,[59] dsRNA products[60,61] and long single guide (sgRNAs) used in *ex vivo* Crispr-CAS mediated gene therapy.

Roche collaborated with the group of Dr. Marcel Hollenstein at the Institut Pasteur in Paris. After some investigations into employing polymerases for synthesis of ASOs,[55,62] the focus was switched to assembly of heavily modified ASOs using short 5mer fragments on DNA templates and commercially available T3 DNA ligase as the biocatalyst.[63] The method developed has a high tolerance for chemical modifications on the sugar, base and backbone of the 'shortmer' fragments. The addition of 'crowding agents' such as DMSO and PEG8000 proved to be critical in improving conversions.

A proof of concept was demonstrated by synthesizing Fomiversen, which is a fully phosphorothioated 21mer ASO. The DNA template was biotinylated at the 5'-end, allowing a simple magnetic separation from the product (Fig. 1). In addition, variants of Fomiversen containing 3'- and 5'- conjugates were also synthesized in moderate to good yields, typically 60–70% after magnetic separation. Purities were in the range 60–90%. The method could also be used to produce very long oligos, up to 159 nucleotides in length. Further improvements in the method would involve enzyme engineering to improve tolerance to less well accepted modifications, such as 2'-MOE, and on recycling of the DNA template.

Novartis recently described a related, T4 RNA ligase method where a highly chemically modified siRNA was synthesized starting from short oligonucleotide fragments (≤9 nt).[60] RNA ligase was able to ligate short fragments with minimal overlap between the fragments and containing extensive chemical modifications. Two different ligation methods were evaluated to assemble the ligation reaction to avoid potential mismatch between the fragments. In a first approach complementary pairs of oligonucleotide fragments are first annealed together. The resulting overlapping, partially double-stranded fragments are then subsequently combined and ligated together in a single reaction. In the second approach the

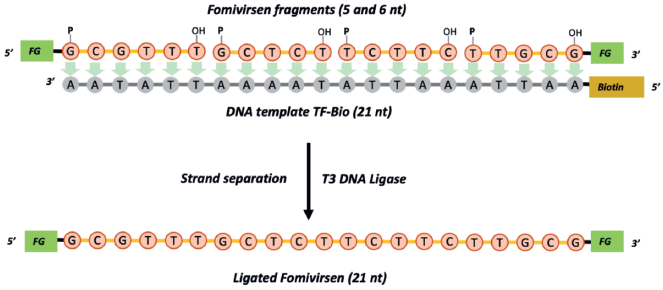


Fig. 1. Adapted from ref. [63]: Synthesis of Fomivirsen and 5' or 3'-conjugates. Isolated Yields: Fomiversen; 74% yield, 76% purity. 5'-FG = FAM; 72% yield, 86% purity. 5'-FG = NH_2 or N_3; 68-71% yield, 74-86% purity. 5'-FG = C16 fatty acid; 61% yield, 56% purity. 3'-FG = cholesterol; 27% yield, 63% purity.

ligation is performed in a stepwise manner. In the first step two independent ligation reactions with three fragments each are set up. Two of the fragments are ligated together, while the third complementary fragment functions as template. In a second step, the two resulting partially double-stranded products are combined and ligated together to make the full-length product. Both ligation methods were optimized and this resulted in conversion to the desired product in the range of 40–80% (Fig. 2).[60]

4. Conclusions and Outlook

As the SIBC celebrates 20 years of fostering collaboration among industries such as pharma, flavor and fragrance, fine chemicals, and agrochemicals, with a focus on biocatalysis applications, we have shared recent examples of using biocatalysis to solve (stereo)chemical challenges, highlighting the innovative approaches and successful implementations in various industries. Asymmetric synthesis and (dynamic) kinetic resolution are preferred methods in biocatalysis, offering higher yields and improved cost and environmental benefits compared to traditional resolution processes. With the growing significance of new technology platforms like biotherapeutics and oligonucleotides, biocatalysis research and development are expanding to include enzymatic bioconjugation and sustainable synthetic methods. At the same time, biocatalysis is widely used in the pharma and agrochemical industries to synthesize drug metabolites for structure elucidation and as analytical references, showcasing its technical maturity and effectiveness.

We also note that the Swiss research ecosystem offers favorable conditions for fostering collaborations between academia and industry. The SIBC offers a privileged place to discuss potential new collaborations between industries around specific topics that have been identified during our discussions. Even if the advances in enzyme engineering, bioinformatics, and predictive structural biology have significantly influenced biocatalysis methods and models and enhanced opportunities for addressing biological questions, challenges in implementing biocatalysis remain, including the need for robust enzymes, regulatory issues with

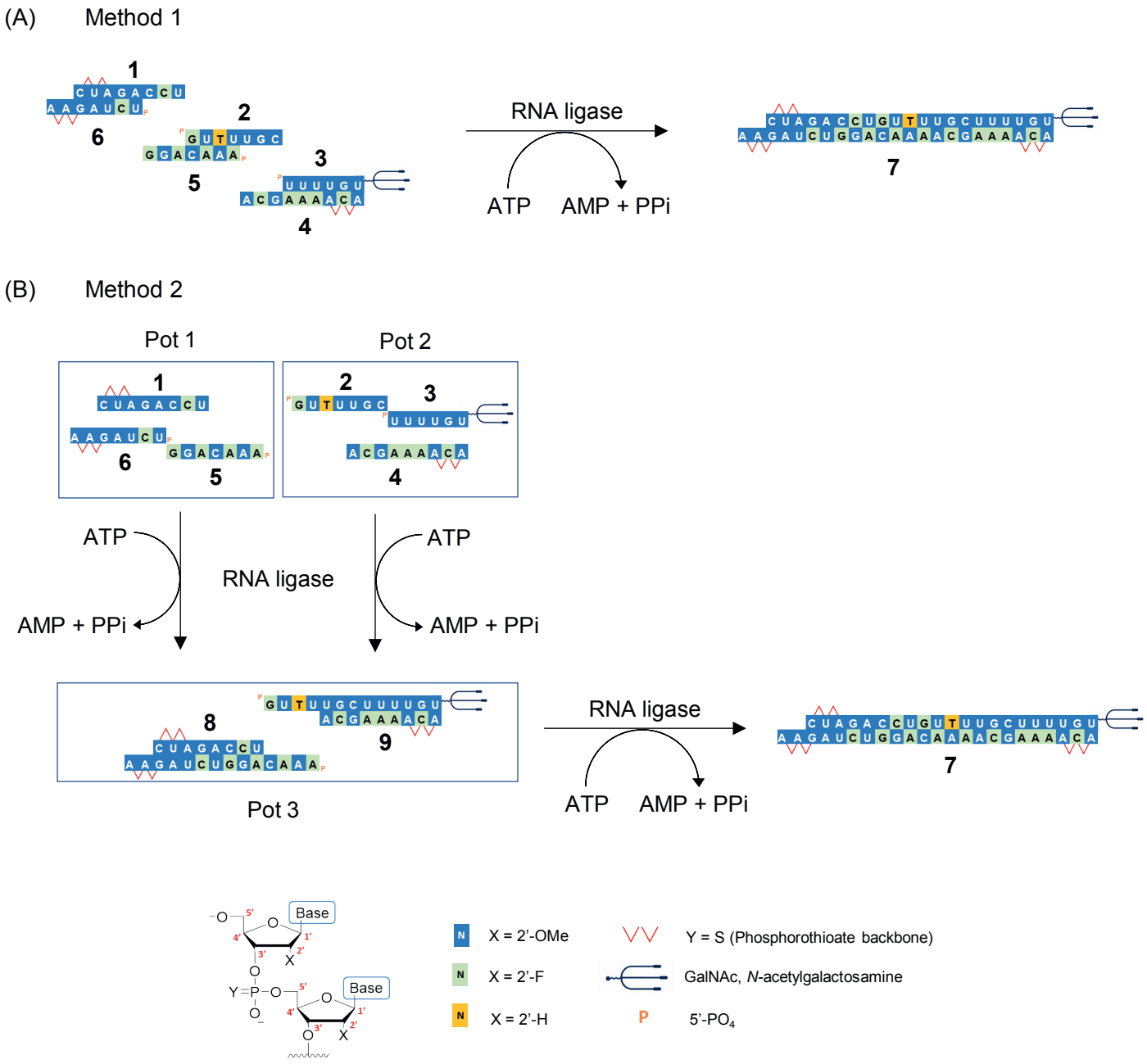


Fig. 2. Assembly of short oligonucleotide fragments by RNA ligase to yield highly chemically modified RNA 7.[60] (A) Method 1: Complementary pairs of oligonucleotide fragments are first annealed together, subsequently combined and ligated together in a single reaction. (B) Method 2: Stepwise ligation to first form double-stranded intermediate fragments 8 and 9, which are subsequently combined and ligated to form 7.

GMOs, and expanding the scope of scalable enzyme-catalyzed reactions. We hope that SIBC provides a platform to further share and discuss the challenges, as well as the benefits and new developments in the technology, between industrial partners and academic collaborators.

Acknowledgement

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