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Evolution of Biocatalysis at Novartis over the last 40 Years

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Abstract: The fortieth anniversary of biocatalysis started at Ciba-Geigy and later at Novartis is a great time to pause and reflect on the development of science and technology in this field. Enzyme-based synthesis became a highly valued enabling tool for pharmaceutical research and development over the last decades. In this perspective we aim to discuss how the scientific approaches and trends evolved over the time and present future challenges and opportunities.

Keywords: Biocatalysis · Bioinformatics · Enzyme evolution · Late-stage functionalization · Metabolite synthesis

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1. How it all started

There was no Big Bang event in 1982, but rather the visionary mindset of Oreste Ghisalba who started his industrial employment at Ciba-Geigy, and believed in the power of enzymes and founded the Bioreactions group at the Zentrale Forschung Lab of Ciba-Geigy.[1] Oreste's scientific interest went initially beyond enzyme applications in chemical synthesis for pharmaceutical purposes and became mostly oriented on environmental technology and sustainability.[2–4]

The expertise built during the 1980s in the isolation and identification of microbial strains capable of degradation of chemical compounds (*e.g.* DMF, dimethyl sulfate) likely helped the group to switch its attention from biodegradation to the use of enzymes for synthetic purposes.

Microbial strains that were originally isolated for the biodegradation of chemical waste turned out to be a rich source for novel enzymes such as methylsulfatase, N,N-dimethylformamidase (DMFase), esterases, methanol dehydrogenase, *etc*.[5]

Purification and characterization of novel enzymes for the identification of substrate scope was another important step which paved the way towards the enzymatic synthesis of building blocks and chiral intermediates.[6]

With the discovery of novel enzymes internally and also through external companies and collaboration partners, the toolbox of available biocatalysts started to grow over time (Fig. 1). The expanding enzymatic toolbox, growing skillset in the group and the evolution occurring in life science in general, enabled the team to prepare the first references of drug metabolites such as glucuronides[7] in the early 1990s, which would be otherwise difficult to synthesize by conventional methods.

With further progress in molecular biology, DNA-reading and writing and the development of bioinformatics tools in the early part of this century, the team was allowed to further expand into the area of genome-mining and preparation of novel recombinant enzymes for synthesis of lead molecules, drug candidates and APIs. To prepare an efficient catalyst for large-scale synthesis using enzymes found in nature, it was also necessary to embark on enzyme engineering activities, resulting in internal efforts starting in 2019.

Nowadays, enzymes are not simply limited to the manufacture of chiral molecules. They are also used to enable a novel class of reaction (enabling technology), improve productivity and selectivity (higher yield, shortened synthesis route) and offer a great cost saving potential, with simplified processing and purifications, 'greening' our processes (replace costly chiral auxiliaries, reduce waste streams, offer mild reaction conditions). Over the years, biocatalysis has become a mature technology, with commercial kits allowing for fast screening available from many established vendors, as well as large numbers of 'off-the-shelf' enzymes ready for upscaling. It was a successful journey over the last four decades for biocatalysis at Ciba-Geigy and Novartis and we can proudly say that we are in a unique position in the pharmaceutical industry and have teams of experts along the entirety of the drug life cycle- from drug discovery, through early- and late-phase development.

In this article, we would like to demonstrate the power of enzymatic technology, and show how the science has evolved over the last four decades and to celebrate 40 years of biocatalysis. We don't aim to cover the whole evolution of entire science happening externally in the last four decades, but rather focus on key scientific achievements within the company that had a crucial impact on the implementation of enzymes throughout the drug development cycle. For more details, the original research articles and patents are cited.

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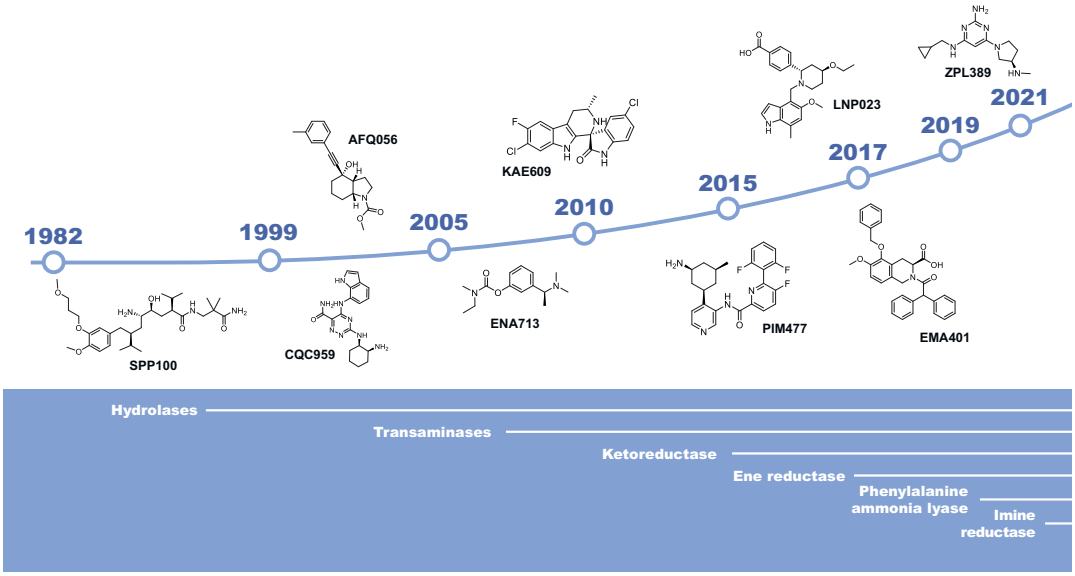
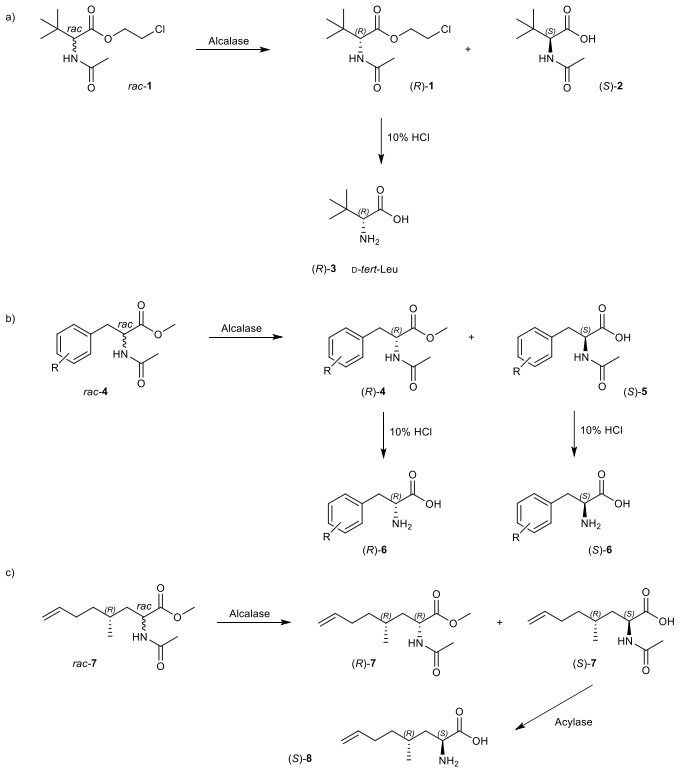


Fig. 1. Evolution of the enzymatic toolbox over the past four decades at Novartis. The first tailored enzyme by protein engineering was used for the synthesis of intermediate of KAE609 in 2010.

2. Evolution of Biocatalytic Approaches

2.1 *Early Approaches to Chiral Building Blocks*

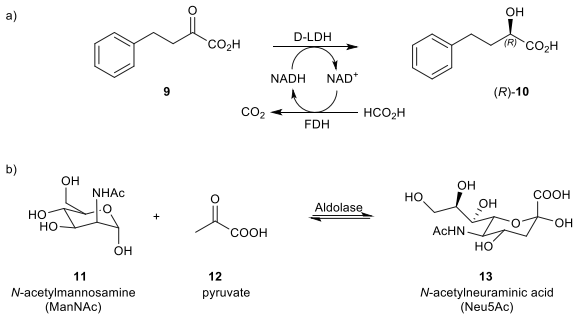
Early work on chiral building blocks relied largely on hydrolytic resolutions. Chiral, non-natural amino acids, such as D-*tert*-leucine ((*R*)-**3**),[8] phenylalanine derivatives (**6**)[9] or a β-secretase 1 (BACE1) inhibitor building block ((*S*)-**8**)[10] were accessed *via* selective hydrolysis of *N*-acetyl or ester bonds using commercial alcalase and acylase (Scheme 1). Chiral phenylethylamine derivatives were obtained with three novel amidohydrolases isolated directly from microbes, such as *Rhodococcus*[11] or *Arthrobacter*.[12] On a preparative scale (50–100 g of several chiral amines), amidohydrolases were applied as wet cells.[13] During drug discovery, enzyme-mediated hydrolytic resolutions yielding chiral acids or esters are still widely applied at Novartis and several commercial hydrolases



Scheme 1. Kinetic resolutions of chiral amino acid synthesis at Novartis in the early 2000s.

are in monthly use. In addition to lipases, proteases and esterases, amidases and nitrilases are often encountered in our chiral building block syntheses *via* resolutions. However, direct asymmetric alternatives, such as amino acid dehydrogenases, transaminases or ammonia lyases are nowadays preferred, if the corresponding carbonyl- or carboxylic acid starting material can be accessed easily.

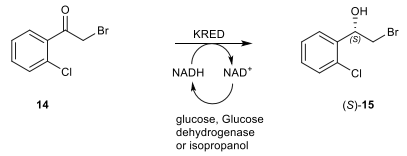
In addition to resolutions, early work in the group focused on asymmetric reductions and intensification of the processes. For example, in 1991, (*R*)-2-hydroxy-4-phenylbutyric acid ((*R*)-**10**), an intermediate of several angiotensin converting enzyme (ACE) inhibitors, was synthesized by selectively reducing the corresponding keto acid **9** with a D-lactate dehydrogenase (D-LDH, Scheme 2).[14] The cofactor, NADH, was recycled by formate dehydrogenase (FDH). A continuous process in an enzyme membrane reactor (EMR) allowed a mean space-time-yield of 165 g L^{-1} d^{-1}. Four years later an EMR approach and thorough kinetic analysis of aldolase catalyzed *N*-acetylneuraminic acid (Neu5Ac, **13**) synthesis (Scheme 2) allowed for a multi-kg process at a space-time yield of 650 g L^{-1} d^{-1}.[15] At the time Neu5Ac was an important building block of sialidase inhibitors or antiallergic agents.



Scheme 2. Enzyme membrane reactor (EMR) processes developed at Ciba-Geigy: a) D-Lactate dehydrogenase (D-LDH) catalyzed asymmetric reduction to (*R*)-2-hydroxy-4-phenylbutyric acid (*R*)-**10** and b) Aldolase catalysed N-acetylneuraminic acid (**13**) synthesis at multi-kg scale.

One of the major milestones, increasing the interest in biocatalysis, not only at Novartis but in many chemistry groups, was the implementation of enzymes optimized *via* directed evolution and their subsequent commercial availability. In the Novartis Bioreactions group this initially started with a small set of engineered ketoreductases (KREDs) from Jülich Fine Chemicals

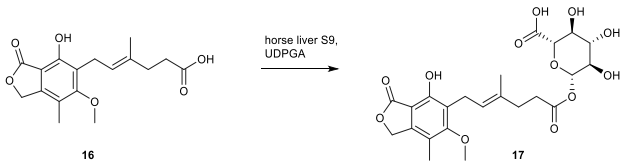
which were evaluated for the enantioselective reduction of building blocks (Scheme 3).[16]



Scheme 3. Simple access to an enantiopure chiral alcohol (*S*)-**15** using KRED variants.

2.2 *Metabolite Synthesis*

At several time points in a drug development project, the investigation of drug metabolism and pharmacokinetics is of vital importance. In an early lead-finding phase, the identification of a metabolite from the lead compound can support the medicinal chemistry teams and influence the design of the next lead compound with improved properties. In a later phase, metabolite identification is needed to study drug-clearance and to fulfill regulatory requirements. Even later, in clinical studies, drug-metabolite references as analytical standards are required for precise quantification of metabolites in often complex biological matrices. The chemical synthesis of metabolites, in particular glucuronides,[17] can be very challenging, often including completely new multi-step synthesis routes, likely using complex protection group strategies. The use of enzymes to initially produce and characterize small amounts of Phase I as well as Phase II drug metabolites was therefore implemented quite early in the Novartis Bioreactions portfolio. Classically, for the preparation of glucuronides, which are the most common Phase II metabolites in human drug metabolism, liver S9 preparation of different animal species were (and still are) applied. An early example of such drug metabolite projects was the glucuronidation of mycophenolic acid (**16**) using UDP glucuronosyltransferases (UGT) in horse liver S9 (Scheme 4).[18]



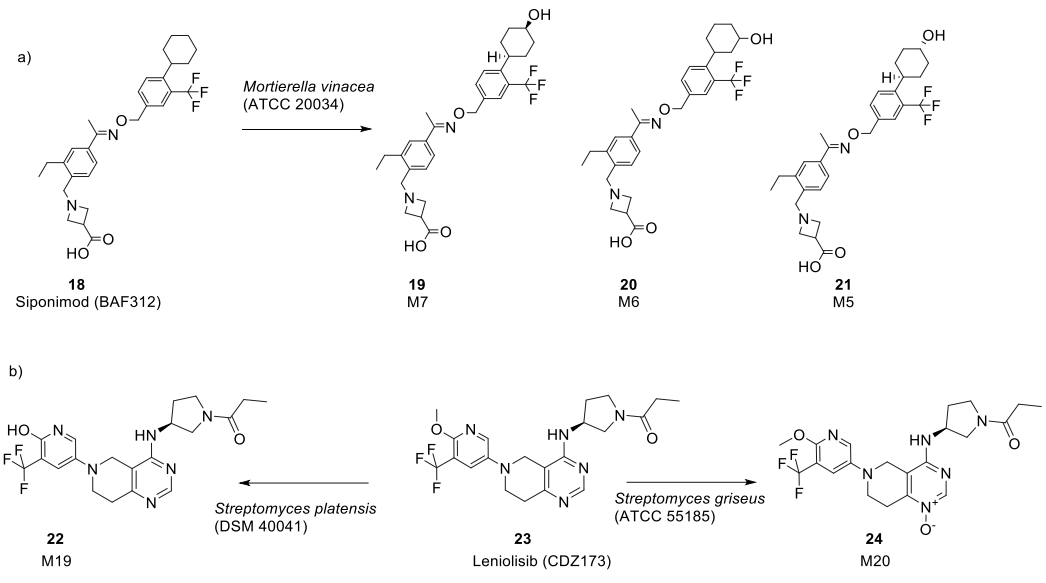
Scheme 4. Production of mycophenolic acid acyl-glucuronide **17** catalyzed by UGTs in horse liver S9.

The preparative synthesis of oxidative Phase I metabolites was first embarked upon by the natural capability of microorganisms to oxidize xenobiotics, such as drug molecules or natural products. Many prokaryotic microorganisms, such as *Streptomyces* sp., but also yeast and fungi possess monooxygenase systems similar to those found in mammalian hepatic microsomes. Therefore, wild-type microorganisms can be applied for the synthesis of oxidative metabolites.[19] In contrast to other Phase I drug metabolizing enzyme systems, oxidative wild-type organisms are characterized by typically higher turnover numbers and better operational stability. Wild-type microorganisms were applied for example in metabolism studies of Siponimod (BAF312) (**18**)[20] and Leniolisib (CDZ173) (**22**)[21] (Scheme 5).

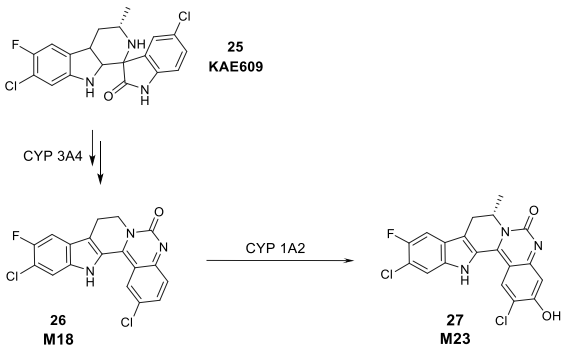
An important milestone in the enzymatic production of drug metabolites, was the implementation of the recombinant human CYP-P450 enzymes expressed in *E. coli*.[22] The constructs of human CYP-P450 enzymes that are applied in the Bioreactions group of Novartis for more than two decades now, were developed by the Research Centre of the University of Dundee, (UK) in the course of a collaboration of this institute with 14 pharmaceutical companies. Compared to biotransformations using wild-type microorganisms when a mixture of regioisomers is often produced, the use of recombinant human enzymes enabled the production of a specific drug metabolite. Metabolites produced in reactions catalyzed by recombinant human CYP-P450, can also be compared directly with *e.g. in vivo* samples helping to identify new metabolites, as demonstrated in the formation of metabolite M23 (**27**) of Cipargamin (KAE609) (**25**) (Scheme 6).[23] The broader and more versatile use of this technology has been described by our group previously.[24]

Expanding the toolbox of metabolizing enzymes for drug development,[25] further non-CYP P450 oxidative enzymes were accessed through a collaboration with Roche and ACIB. Hereby the human recombinant flavine monooxygenases (FMOs),[26] aldehyde- and xanthine oxidases (AO,[27] XO[28]) and monoamine oxidases (MAOs, unpublished work) were implemented (Scheme 7).

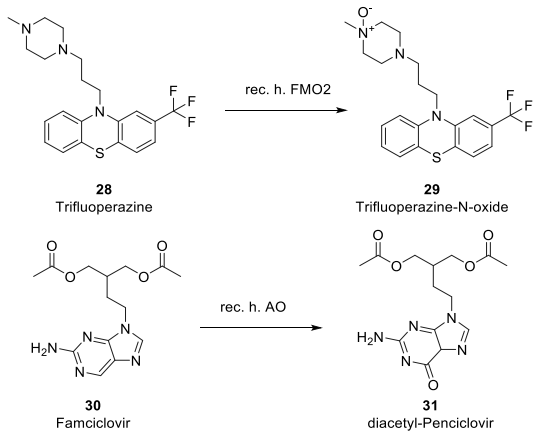
Besides the synthesis of human drug metabolites, oxidative biocatalysts gained importance for late-stage functionalization (LSF) of drug molecules and natural products. This approach generally leads to fast and easy access to derivatives of a drug candidate with potentially improved potency, physicochemical properties or altered absorption-distribution-metabolism-excretion (ADME) properties.[29] Therefore, it can significantly accelerate the drug discovery process when applied in the last step of synthesis.



Scheme 5. Drug metabolites of a) Siponimod (BAF312) (**18**) and b) Leniolisib (CDZ173) (**22**) synthesized by biotransformation using wild-type microorganisms.



Scheme 6. Production and identification of metabolite M23 (**27**) of Cipargamin (KAE609) (**25**) using recombinant human CYP-P450 1A2.



Scheme 7. Non CYP-P450 mediated oxidative drug metabolism, demonstrated with a) recombinant human flavin-containing monooxygenase 2 (FMO2) and b) recombinant human aldehyde oxidase (AO).

Recently, late-stage functionalization of cladosporin, a natural product functioning as a potent and selective inhibitor of lysyl-tRNA synthetase, by biotransformation was reported.[30] The new compounds covered a wide chemical space, showed activity in a cell-based synthetase assay and thus highlighted positions in the molecular structure suitable for modification while maintaining the biological activity and selectivity towards the drug target. The fast and easy access to cladosporin derivatives gave new insights into the structure–activity relationship and thus provided guidance towards further chemical optimization of this promising antimalarial lead compound.

In parallel to the expansion of the Bioreactions group's toolbox for biocatalytic metabolite synthesis, persistent efforts have been made to implement powerful biocatalysts for late-stage functionalization of drug molecules. With the exploration of versatile and promiscuous bacterial P450s, the oxidative biocatalyst collection was further expanded.[31] Most recently a collaboration with the ZHAW focused on evolving halogenases for LSF, demonstrated by the synthesis of chlorinated derivatives of a martinelline-derived fragment.[32]

2.3 *New Era of Enzyme Discovery and Engineering*

The impact of genome sequencing coupled with recent advances in DNA synthesis technologies has had a revolutionary impact on several disciplines of biological science. This impact is now being experienced in the biocatalysis field as well. At the time of writing, the National Center for Biotechnology Information (NCBI) lists over 600,000 completed and annotated genomes in its repository.[33] Taking this number into account we can roughly estimate that many hundreds of millions genes encoding enzymes are available in this database that could potentially be applied to biocatalytic endeavors. At the same time, the past decade has seen DNA synthesis costs drop significantly to levels that facilitate the construction and expression of enzymes in numbers that permit the development of screening libraries for classes of biotechnology relevant enzymes. The challenge therefore is deciding how to apply bioinformatic approaches to choose the best enzymes from this massive global inventory when developing an enzyme screening library.

Early efforts in utilizing genomic sequence data to support biocatalysis efforts were relatively small in scope and focused primarily on opportunistic applications such as onboarding enzymes from literature. One example of this was the identification of a threonine aldolase from *Vanrija humicola* capable of catalyzing the formation of β-hydroxy α-amino acid chiral building blocks. This enzyme had been reported, purified and crystallized almost 50 years ago directly from whole cells, however, no gene had subsequently been reported to facilitate expression in a heterologous host, thereby limiting its application. We identified the gene encoding this enzyme in the genome sequence data of this organism and successfully expressed it in *E. coli*.[34]

In 2017 the decision was taken to investigate the use of imine reductases (IREDs) for reductive amination in the Bioreactions group of Novartis. At the time, there were only a small number of published enzymes from this emerging class available. It was therefore decided to utilize a genome mining strategy to identify novel IREDs from the NCBI database.[35] As such, a Hidden Markov model was developed and applied to populate a database of approximately 15,000 potential IREDs (Fig. 2). These were then binned into gene clusters based on the amino acid sequence space that defined the substrate binding sites for these examples. From these clusters individual examples were chosen for DNA synthesis and subsequent expression. This IRED library has served as the primary screening starting point for several projects at Novartis in the early drug discovery and development projects.

In the past several years, approaches similar to that used to build the IRED screening library have been applied to build Novartis enzyme screening libraries for KREDs, EREDs, nitrilases and proline hydroxylases.[36] The full impact of this strategy, while currently significant, will likely increase as facilitating technologies continue to improve and automation schemes are applied to enzyme screening and associated analytical methods. However, 'wild type' enzyme screening hits pose significant challenges when considered for deployment at scale. Enzyme characteristics such as activity, stability and stereoselectivity are often not sufficient to be efficiently applied to a pharmaceutical production process. As such, engineering enzymes to improve these industrially relevant properties is generally required.

In recognition of the fact that enzymes are being applied with increasing frequency in pharmaceutical production, development and research efforts at Novartis, and also in the context of our long-term environmental sustainability commitment, the Chemical and Analytical Development department in-licensed the Codexis enzyme engineering platform into Novartis in 2019. This platform supports the engineering of enzymes in the drug development pipeline.

Around the same time, the Bioreactions group began developing a complementary capability to support enzyme engineering for early-stage drug discovery efforts. This enzyme engineering platform set out to compare machine-directed evolution approaches to deep mutational scanning and error-prone PCR based techniques.[37] It was evident from this effort that artificial intelligence, as applied with a large enough training dataset, provides a significant efficiency advantage over more traditional mutagenesis strategies. This platform is now able to be utilized for a variety of efforts to support early-stage drug discovery.

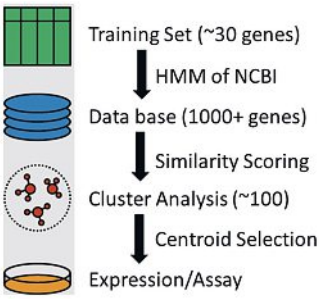
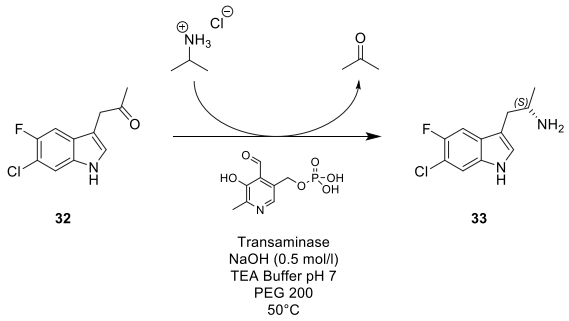


Fig. 2. Generalized approach to genome mining for novel enzyme libraries.



Scheme 8. Enzymatic transamination of key ketone intermediate **32** in the synthesis of Cipargamin (**25**).

Both enzyme engineering technologies allow us to increase our capabilities in this key biocatalysis expertise and to develop improved enzymes to enable novel types of reactions and shorten the synthesis timelines of drug candidates and APIs. Already several projects have been completed and highly engineered enzymes have been used on scale successfully.[38]

2.4 *Enzymes in the Manufacture of APIs*

Harnessing the exceptional catalytic power of natural enzymes is one of the most exciting opportunities in pharmaceutical manufacturing.

The beauty of biocatalysis is that we can produce higher yields of a medicine in a more timely and cost-efficient way than the chemical route, whilst being more environmentally friendly at the same time. It is a great example of using the natural world to complement pharmaceutical ingenuity.

Additionally, regulatory landscape and strategies for Health Authority approvals for enzymatic transformation are now well established for commercial manufacturing. This was a fantastic opportunity for low-molecular-weight compound development and manufacturing at Novartis as 50% of the small-molecule-portfolio have molecules containing chiral centers and it is estimated that 30% of those molecules can potentially benefit from biocatalysis technology to produce them in an enantiomerically pure form.

Since March 2016, the newly created Biocatalysis Technology Platform in Process R&D, was given the objective to fully demonstrate applicability of biocatalysis in the development and manufacturing of APIs. Developing 'know-how' and application awareness as well as further strengthening our working model with strong internal and external partnering platforms are in the focus.

One of the very first projects using enzyme engineering technology towards manufacturing, was the new route towards Cipargamin (KAE609) (**25**), a very effective anti-malaria drug bearing an aspiroindolone structural core (Scheme 6).[39] One key intermediate of the synthesis was a chiral tryptamine (**33**, Scheme 8) which was the focus of intensive alternative route scouting.[39] Although (*S*)-**33** could be obtained *via* an L-amino acylase or lipase catalyzed kinetic resolution, an asymmetric reductive amination starting from the corresponding ketone (**32**) was desired. Through a collaboration with Codexis, an active and selective transaminase enabling the preparation of **33** was found and developed. This solution was retained as the best method for the final synthesis.[39] Adequate process development was needed to ensure high productivity and performance of this step on scale. The utilization of PEG200 was found to be crucial for the success of the scale-up, due to the low solubility of the ketone substrate in the reaction buffer.

Another prominent example highlighting the benefit and potential of biocatalytic transformations were the two enantioselective enzymatic transaminations in the alternative synthesis of two key building blocks of Sacubitril (**34**), a key component of LCZ696, the active ingredient in Entresto (Scheme 9).[40]

Two distinct routes and associated follow-up development programs have proceeded in parallel, namely Process 1 and Process 2 (Scheme 10).

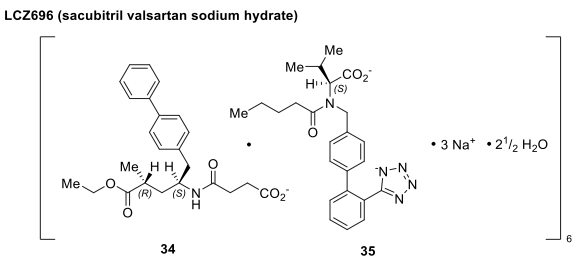
Both processes were successfully launched at the multi-ton level within exactly 18 months after identification of the initial enzyme hit during screening. Thanks to a smooth transition into Manufacturing Operations between Novartis R&D and Manufacturing operations, it was possible to achieve implementation of this engineered enzyme at commercial scale.

Several tons of material were produced in very high quality with perfect control of enantioselectivity.

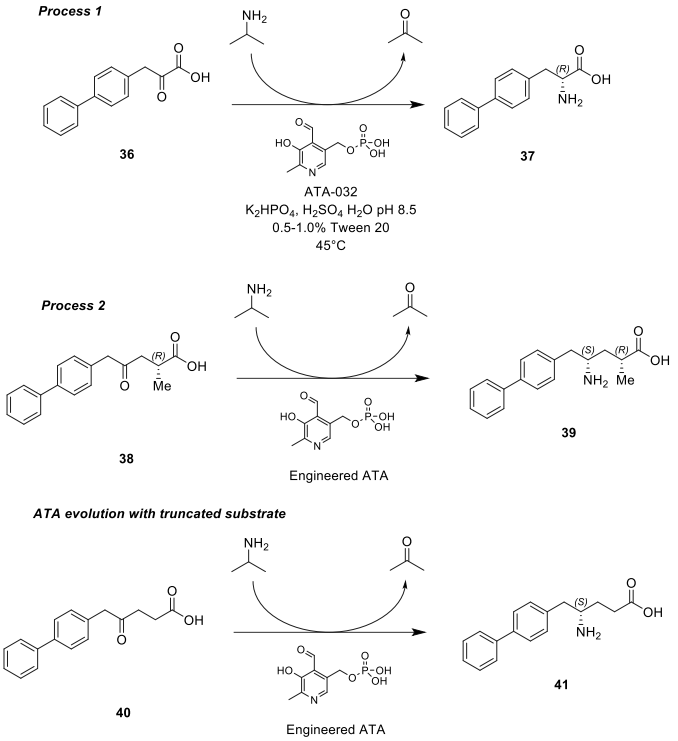
Process 1: In the case of keto acid **36**, a transaminase with adequate activity and enantioselectivity could be identified by commercial enzyme library screening. Transaminase ATA-032 was found to provide optimum performance towards **37**. Process development was conducted to enable sufficient productivity (60g/L substrate) with minimum enzyme loading (1% w/w) in less than 20 h. A multi-ton process has used the same substrate concentration and substrate/enzyme ratio.

Process 2: The structure analogy between **37** and **39** made it tempting to evaluate transamination, as well. Unfortunately, no enzyme from all tested screening kits gave any conversion. Therefore, the team started to perform enzyme evolution with the external partner, Codexis, using a truncated substrate approach. When screening the least truncated substrate **40** (missing the methyl group and thus the stereogenic center), low activity was observed.

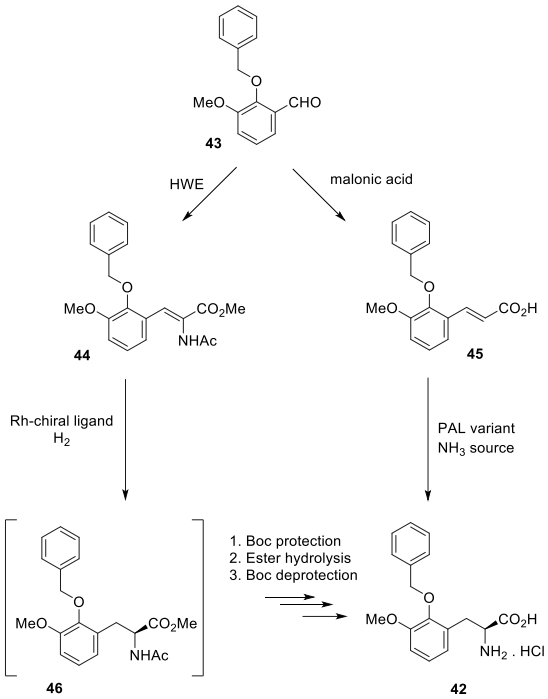
Codexis then screened the complete target substrate **38** again (as the least truncated substrate provided a hit for an (*S*)-selective ATA, *i.e.* the desired selectivity), using modified screening conditions and an improved sample preparation. Under these modified conditions, activity was observed, but the (*S*)-selective ATA provided the undesired (*R*) product (with 100% diastereoselectivity). Interestingly, a hit with lower activity from the (*R*)-selective ATA panels provided the desired (*S*)-product. With such a result in hand, an enzyme evolution campaign was initiated.[41] The enantioselectivity could be reversed in the first round of evolution. Overall, 11 rounds of evolution were needed to result in an enzyme with ~500,000-fold improved activity. Aspirational target performance specifications were met enabling process development.



Scheme 9. LCZ696 (Sacubitril (**34**), Valsartan (**35**) sodium hydrate, LCZ696) and two enzymatic transamination processes.



Scheme 10. Two enzymatic transamination processes and the truncated substrate **40** which was used as a starting point for enzyme evolution.



Scheme 11. Engineered PAL mediated synthesis afforded chiral amino acid **42** in two steps. In comparison the initial rhodium catalyzed hydrogenation required several protection and deprotection steps.

Process development was conducted to enable sufficient productivity (75g/L substrate) with an enzyme loading of 1.5% w/w in about 20 h. The same substrate concentration and substrate/enzyme ratio was kept as such on large scale.

Overall, using process 2, unlocking the transamination allowed for reducing the number of synthetic steps towards **39** by 50%.

The third example in which enzyme engineering was recently used in process development at Novartis was a chemo-enzymatic synthesis of EMA401.[42]

In the project EMA401, the key intermediate was a chiral amino acid **42** which was initially produced through five steps using an asymmetric hydrogenation catalyzed by a chiral rhodium complex (Scheme 11).

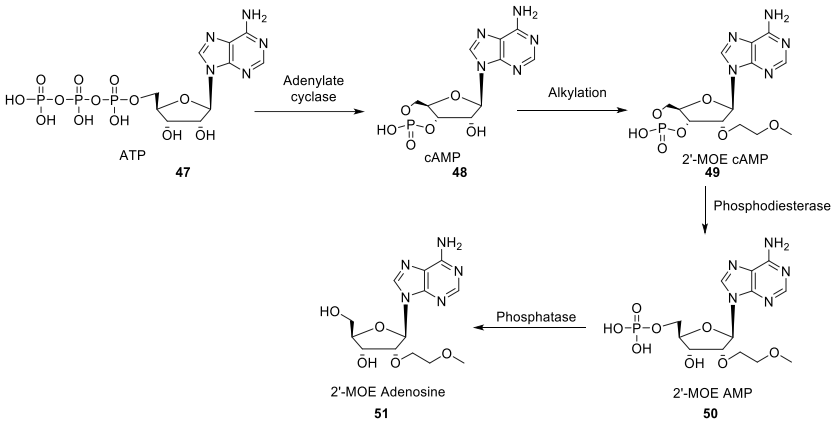
To remove the requirement to employ a costly precious metal catalyst and to develop a more sustainable process, a biocatalytic route was initiated to significantly shorten the synthesis using a phenylalanine ammonia lyase (PAL) (Scheme 11). PAL is capable of adding ammonia to cinnamic acid derivatives enantioselectively in one step. Starting from no activity at all, through a collaboration with Codexis and after 13 rounds of evolution, it was possible to produce a very active enzyme leading to a highly pure amino-acid intermediate **42**.

A large excess of ammonia is crucial for completion of the reaction, however, this excess needs to be efficiently removed without a tedious and low productivity neutralization process. It was possible after a thorough process development to find high yielding conditions while minimizing the aqueous waste *via* a smart ammonia recovery process. The process has been confirmed on the multi-kilogram scale.

2.5 *Developing Enzymatic Solutions for Increased Molecular Complexity*

Low-molecular-weight compounds (LMWs) still hold the largest share in the Novartis portfolio and are also accounting for half of the new drug approvals in the US in 2022. Nevertheless, increasing importance of technology platforms such as biotherapeutic, peptides, oligonucleotides, radioligands and cell & gene therapy, are consequently also influencing the focus of Novartis' efforts in the field of biocatalytic R&D. Enzymatic bioconjugation[43] is especially interesting for biotherapeutic antibody drug conjugates (ADCs) or radioligand therapies,[44] whereas in the area of oligonucleotide therapeutics not only the linkage, but also the enzymatic synthesis of the monomers can be envisaged.[45] It's fascinating that the Ghisalba's group had already anticipated the increasing importance of these fields and, for example, published and patented a chemoenzymatic synthesis of their building blocks, modified ribonucleosides, already 23 years ago![46] Scheme 12 shows the described synthesis approach starting with the enzymatic cyclization of adenosine triphosphate (ATP, **47**) to cyclic adenosine monophosphate (cAMP, **48**). Subsequently **48** was chemically O-methoxylated to yield 2'-O-cAMP (**49**) and then decyclized to 2'-O-methoxyethyl adenosine 5'-monophosphate (2'-O-MOE AMP, **50**) with a phosphodiesterase from *Serratia marcescens*. In a last step, the phosphate moiety can be cleaved off using a phosphatase to form the modified nucleoside (2'-O-methoxyethyl adenosine, **51**).

The phosphorylated nucleosides can then function as the building blocks for oligotherapeutics like small interfering RNAs (siRNAs) or antisense oligonucleotides (ASOs). Despite the fact that chemical synthesis methodologies for modified oligonucleotides are well-established, large-scale manufacturing in a sustainable and economically feasible manner remains challenging.[47] Errors in the coupling efficiency, despite being low (typically <1% per coupling cycle), accumulate as the length of the oligonucleotide increases. Several companies have developed the technology to enzymatically assemble chemically modified oligonucleotides.[43,48,49] The approach utilizes the well-known catalytic activity of RNA ligases to form an ATP-dependent covalent bond between the 3'-OH and 5'-PO_4 termini of two oligoribonucleotides to form one larger, continuous strand.[50] Mann *et al*. describe a method where such a RNA ligase was utilized to synthesize a chemically modified RNA starting from short (≤9 nt) oligonucleotide fragments.[49] The authors report a conversion of 40–80% to the desired product in aqueous solution (Scheme 13).



Scheme 12. Ghisalba's chemo-enzymatic synthesis of modified ribonucleosides, here shown on the example of methoxyethyl adenosine 5'-monophosphate (2'-MOE AMP).

Furthermore, unmodified or post-translationally modified peptides can be accessed *via* biosynthetic routes. Novartis has a long history with these naturally derived peptides. The most prominent example might be the cyclosporine identified in 1971 and derivatives thereof such as the cyclophilin inhibitor Nim811.[51] The aforementioned new biotechnological tools are also having a dramatic impact on this field. The selective Gq/11 protein inhibitor FR900359 is of great pharmacological interest and a potential lead molecule for targeted therapies of cancers. So far, neither the isolation process from plant leaves, nor heterologous expression in *E. coli,* has shown scalability in an economic viable manner. Recently Novartis scientists addressed this challenge with a high-yielding, scalable and sustainable biotechnological process in *Chromobacterium vaccinii* DSM 25150.[52] Strain identification *via* genome mining and a promoter-driven overexpression of the biosynthetic gene cluster, encoding the nonribosomal peptide assembly line for the cyclic depsipeptide FR900359 improved product titers 8-fold compared to the wild-type.[52,53]

3. Future Outlook and Opportunities

The biocatalysis field and underlying scientific disciplines have evolved dramatically over the last 40 years. Thanks to the fast-paced development of novel life science tools, enzymes are the enabling power for synthesis of lead compounds, drug candidates and APIs. Enzymatic approaches have become the preferred options in drug discovery and development being implemented as the 1st route of choice and no longer are they considered applicable only for life-cycle management projects. We have experienced how powerful enzyme engineering can be when starting from near zero conversion, all the way to full implementation of the perfect biocatalyst into manufacturing scale production![40]

Not only has the science evolved, but so did the Biocatalysis teams at Novartis over the years. To reflect on the changes, new skillsets such as bioinformatics, molecular biology and protein engineering have become necessary to acquire and will continue to expand into the field of other computational methods, such as artificial intelligence, machine learning, *in silico* predictions and data management.

We are on a journey to target the ever more difficult to drug biological space with low-molecular-weight compounds and as an outcome our molecules across the portfolio are growing in complexity – having *e.g.* innovative 3D scaffolds or molecules that require increasing functionality. The same can be claimed in the space of bioconjugates where payloads and other chemical modifiers need to be attached to biopolymers, RNA molecules need to be decorated with targeting groups, small peptide binders need to be charged with radioactive payloads and even surfaces of large protein complexes need to be modified.

We intend to use biocatalysis as the base for solving the presented synthetic challenges, but to do so, we need to push the boundaries of what has been achieved to date. We need to apply creativity and teamwork, to bring about this step change in how we build our molecules.

This challenge is best tackled if forces are joined and coordinated hand-in-hand with academic and industrial partners towards the same goal.

We are in a fortunate position to take advantage of the close proximity of other Pharma, Fine Chemicals, Agrochemicals and Flavor and Fragrance Industries which have their own internal biocatalysis groups (Swiss Industrial Biocatalysis Consortium).[54] Furthermore, we have many excellent academic groups that have a focus on biocatalysis and related areas in a relatively small footprint of Switzerland.[55]

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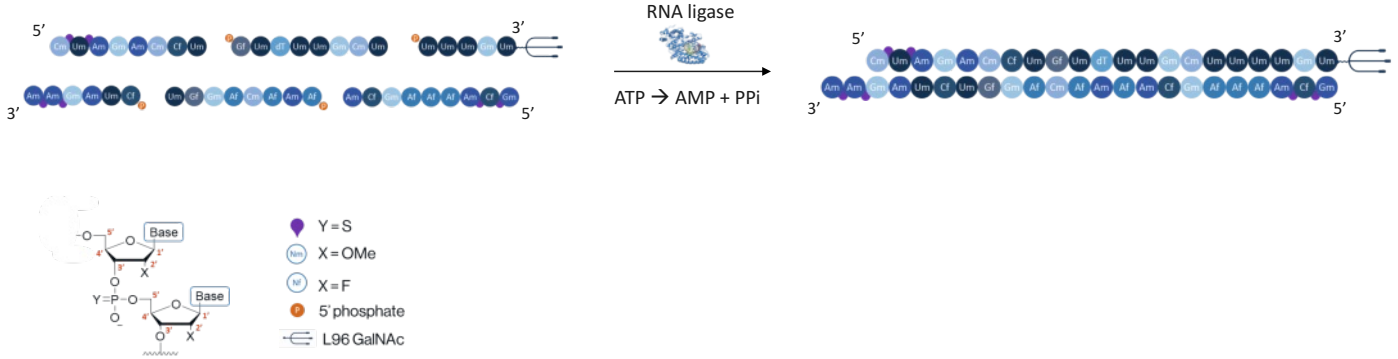

Scheme 13. T4 RNA ligase 2 (Rnl2) catalyzed assembly of short oligonucleotide fragments to yield highly chemically modified RNA.[49]

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