RESEARCH ARTICLE

To Investigate Methodologies that Integrate Biocatalysis with Flow Chemistry, Aiming to Leverage the Advantages of Both Techniques

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ABSTRACT
In this research paper will examine the "TO INVESTIGATE METHODOLOGIES THAT INTEGRATE BIOCATALYSIS WITH FLOW CHEMISTRY, AIMING TO LEVERAGE THE ADVANTAGES OF BOTH TECHNIQUES". In order to build the molecular structures, we want by connecting various organic species, new techniques have emerged as an indispensable tool, complementing more conventional ways. When it comes to pharmaceutical components in particular, air-and moisture-sensitive organometallic species might be problematic due to their inherent contamination, functional group compatibility issues, and stringent reaction conditions.
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INTRODUCTION

Quantitative measurement of improvements in the sustainability of biocatalytic industrial processes is necessary. In fact, benchmarking and improvement goal definition become impractical in the absence of a means to assess a process's sustainability. The same holds true for industrial implementation: quantifiable metrics are needed to evaluate process changes in order to justify the necessary expenditure. Two of the first green measures were the E factor (1992) and atom economy (1991). The atomic economy (AE) is determined by dividing the product's molecular weight by the total molecular weights of all chemicals generated in the reaction, as shown in the stoichiometric equation. Assuming a chemical yield of 100% and the utilization of precise stoichiometric amounts of the initial ingredients, the theoretical value of AE is derived. We ignore solvents and auxiliary chemicals that aren't part of the product recovery process since they aren't in the stoichiometric calculation. Still, it's a great statistic to use when you're just starting to assess potential resource use and waste creation in different paths to your product of interest, before you dive into experimentation.

On the other hand, the quantity of trash actually created is known as the E factor. Factors such as product output and waste from all auxiliary activities, such as solvent losses and chemicals utilized in workup, are taken into consideration. "Everything but the desired product," excluding water, was originally described as waste in the publication. The reasoning for leaving water out was that it may distort the E variables, which would make it hard to compare procedures meaningfully. That instance, a method that doesn't produce any waste but uses a lot of water might be seen as less environmentally friendly than one that does, even if it uses a lot less water. But adding water to the E factor is really rather trendy right now in the pharmaceutical sector.

More waste and a bad effect on the environment are the results of an E factor that is high. A zero E factor is optimal. One product, one manufacturing location, or even an entire business may have this figure computed. It is worth noting that lower E factors are positively correlated with lower API production costs. This is likely due to a combination of reasons, including lower process materials input, greater capacity utilization, decreased energy consumption, and lower costs of hazardous and toxic waste disposal.

There have been efforts to standardize the many green metrics, and there have been a number of alternative measures put out for gauging processes' impacts on the environment. For instance, researchers at GlaxoSmithKline (GSK) including Constable advocated for the application of reaction mass efficiency—a more nuanced version of atom economy that accounts for yield and the use of excess reagents—and mass intensity (MI), which is the ratio of the total mass (including water) used in a process to the mass of the product (MI = E factor + 1). With the goal of comparing the ecological footprints of API processes and using this information to propel the pharmaceutical sector toward greater sustainability, the Green Chemistry Institute Pharmaceutical Round Table rebranded this measure as Process Mass Intensity (PMI). When it comes to defining a process's wastefulness, nevertheless, none of these other measures really shines compared to the E factor. It is possible that the ideal E Factor value of zero better represents the end objective of zero waste than the PMI value of one. The E factor has the added benefit of being able to evaluate multi-step processes with additive E

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factors, unlike PMIs which do not include step products in their mass balance calculations. There are two sides to the coin that is PMI and the E factor. In contrast to the E factor's focus on waste, the Pharmaceutical Round Table's preferred PMI measures resource efficiency. It is more accurately represented in the E factor because the chemical industry aimed for "zero-waste production plants" to produce chemicals in the early 1990s, when waste removal was clearly the focus.

Literature Review

Rajeswari, Mookan et.al. (2025). The use of photo-redox catalysts has recently been recognized as a potential approach to green chemical synthesis. In this chapter of the book, the revolutionary power of photo-redox catalysts in more environmentally friendly synthetic processes is discussed. This chapter provides an overview of photo-redox catalysis, its principles and applications, and the core mechanism that drives these reactions. It explains how difficult chemical transformations may be driven with great efficiency and selectivity by using light.

Sharma, Dr et.al. (2025). More environmentally friendly and long-term sustainable approaches to organic synthesis are in high demand these days. By reducing the production of harmful byproducts and increasing the use of renewable or recyclable materials, green synthesis promotes the ecologically responsible creation of vital organic frameworks.

Micol Santi et.al (2021) Both academics and businesses are beginning to see biocatalysts as a more sustainable, environmentally friendly, and selective replacement for metal catalysts. A growing need for greener, more environmentally friendly industrial processes has piqued researchers' interest in biocatalytic transformations during the last 20 years.

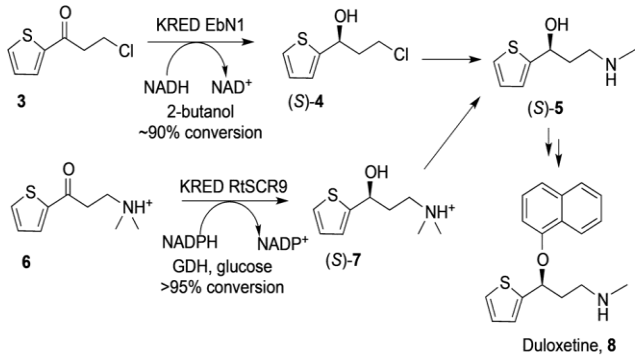
Laila Rubab et.al (2022) For chemical engineers, medicinal chemists, pharmacists, and others working in green (sustainable) chemistry, the field offers a framework within which to develop synthetic techniques, protocols, and procedures that contribute to various aspects of global sustainability. Catalysis and other environmentally friendly synthesis conditions provide the backbone of green chemistry.

Krishna Chandra Panda et.al (2024) The area of green chemistry has been a ray of hope in the fight for environmental sustainability, providing new approaches to reducing the negative effects of chemicals on the environment. This abstract delves into the interdependent nature of sustainable practices and green chemistry concepts, illuminating how these two work hand in hand to promote a more balanced connection between humans and the environment.

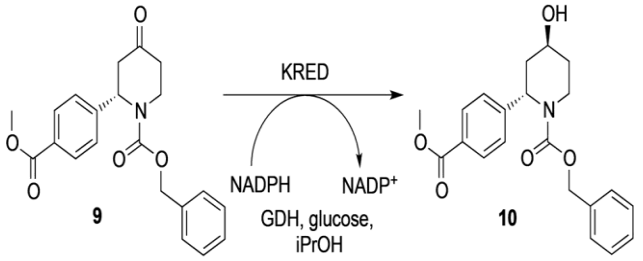
RESEARCH METHODOLOGY

As structural and functional motifs, chiral alcohols are widely used in medicines, fine chemicals, and agro-chemicals. In the pharmaceutical sector, chiral hydroxyl moieties are essential building blocks for various active pharmaceutical ingredients (APIs) of potential new drugs. The use of biocatalysis to get chiral alcohols is gaining popularity for good reason: it allows for stereo-controlled synthesis under moderate circumstances, does not need catalysts based on metals, and has a less impact on the environment.

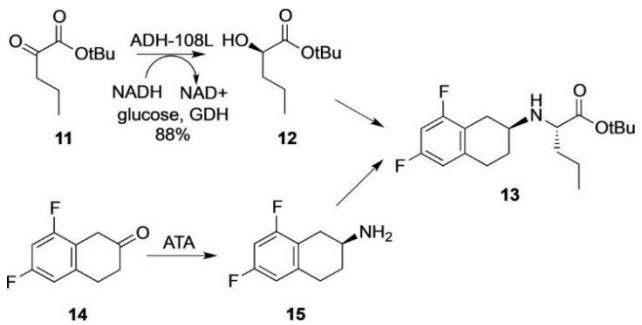
Case studies will show that the most common enzymatic



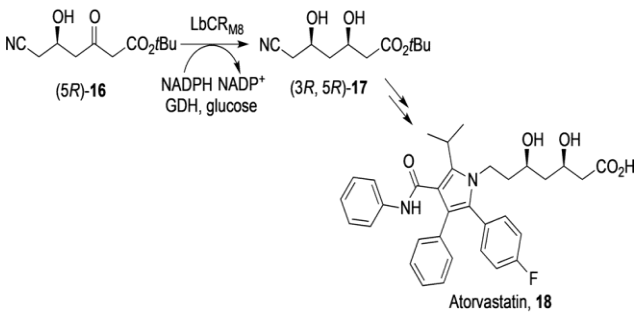
Scheme 1: Routes to key precursor (S)-5 for the anti-depressant Duloxetine using KREDs



Scheme 2: Synthesis of the alcohol intermediate 10 of LNP023 by a KRED



Scheme 3: A route to the chiral intermediate 13 of a gamma-secretase inhibitor using a KRED and a transaminase



Scheme 4: An improved KRED process for the synthesis of t-butyl 6- cyano-(3R,5R)-dihydroxy hexanoate 17 for the production of Atorvasta- tin 18

strategies for making chiral alcohols are kinetic resolution by lipases and asymmetric synthesis by KREDs. The results of a recent study on patent activity (2014–2019) show that these enzymatic processes are becoming more common. The stoichiometric need of the costly cofactors NADH or NADPH is one point to consider when employing KREDs. It is now possible to recycle NADH efficiently on an industrial scale using isopropanol as a hydride donor, eliminating the need for a second enzyme. In contrast, NADPH recycling typically involves a glucose dehydrogenase (GDH) co-substrate. As a substitute, hydroxylating enzymes will be covered.

Biocatalytic Reactions for Potential Industrialization

There have been many new enzymes found or rediscovery in the last ten years that might be useful in organic synthesis in the road. Although many of these compounds have been known for a long time and have been biochemically studied, their use in biocatalysis has just lately gained attention. For a variety of reasons, including but not limited to: their potential being underappreciated, the recent discovery of new types of reactions catalyzed by them, the development of intelligent solutions to overcome practical limitations, and most importantly, the development of methodology to find and produce high-quality enzymes. This includes things like improved expression systems, new tools for enzyme discovery in sequence databases, and enzyme engineering to enhance performance, selectivity, and substrate scope. A recent example of an efficient enzymatic process for recycling PET (polyethylene terephthalate) is the isolation of the monomer terephthalic acid (TA) following 90% depolymerization at an average productivity of 17 gL-1 h -1. This was made possible through extensive enzyme engineering, bioprocess development, and pretreatment of PET from waste bottles. Despite the extra expense of the enzyme and base required for depolymerization, the new PET produced from this monomer is of same quality as virgin PET, and the process as a whole lays the groundwork for a greater degree of circularity for the ubiquitous polyester PET. [200] In addition, biocatalysts now include a variety of innovative, frequently referred to as "new-to-nature" chemical reactions, made possible by breakthroughs in enzyme engineering, computational enzyme design, and research across catalytic disciplines. So, this section will provide a quick example of a situation where biocatalysts are not being employed on a big scale (industrial size), but they have the potential to be.

Biocatalytic Synthesis of the Ibuprofen Esters 3a/3b & 6

Making Ibuprofen Acid from Ibuprofen Salt

Submerged in 50 mL of water with agitation (100 rpm) at 50 ◦C, 5 g of sodium ibuprofen salt was dissolved in a flask. A further addition of 37% HCl brought the pH of the solution down to 1. Fifty millilitres of toluene was added to the contents of the flask after they were transferred to a separatory funnel. The organic component containing ibuprofen was isolated by solvent extraction and agitation. To conduct a second extraction, 50 mL of toluene was added to the water phase. After combining the organic phases, anhydrous Na2SO4 was used to dry them. A rotary evaporator was used to evaporate the solvent after filtering the sodium sulphate. The result was 3 grammes of ibuprofen;

Reaction Conditions

Twenty millilitre screw-top vials were used for the reactions; these were set in an oil bath on a magnetic stirrer, and the bath's temperature was controlled. Following the evaluation of enzymatic stability, the impact of the esterification parameter was examined; however, efforts to create a synthetic combination of ibuprofen and erythritol with a melting point lower than 100 ◦C were unsuccessful. In a 5-milliliter (2M2B) solution of 147 mM IBU 1, erythritol 2 (1, 3.5, or 6 equivalents) was added. The concentration of the biocatalyst in relation to the ibuprofen ranged from 20% to 60% w/w. Various temperatures (50, 70, or 90 ◦C) were used to warm the reaction. For the first 24 hours (and then for the next 144 hours), the reaction was maintained at the measured temperature and 400 RPM. In order to conduct further molar ratio experiments, 147 mM erythritol in 2M2B (5 mL) was mixed with three 1 equivalents. Here, 20% w/w biocatalyst was used in relation to 1. In order to synthesize 6a/6b, a 66 mM solution of 1 in 3 mL of 5 was mixed with 40% w/w biocatalyst relative to the mass of 1. In order to find the conversion, the esterified 1's CH3 proton signal integrals (δ = 1.36, 1.34, and 1.35 ppm for 3, 4, and 6 ppm, respectively) were compared to the unreacted 1's signal integral (δ = 1.32 ppm) using 1HNMR at 400 MHz after identification. The data is presented as the average plus or minus the standard deviation of three separate experiments' worth of measurements.

RESULTS AND DISCUSSION

Chiral Alcohols Produced by KREDs

An important building block for the antidepressant duloxetine is dulox alcohol (S)-5. BASF's method makes use of the selectivity for the reduction to the (S)-alcohol (S)-4 shown by the robust KREDs EbN1 from Aromatoleum aromaticum and LbADH from Lactobacillus brevis, which both accept the labile chloro-ketone 3.

In mixed solvent conditions, an evolved enzyme demonstrated rapid and robust activity. It takes rac-2-butanol or isopropanol for NADH recycling and shows decreased product inhibition. Aminating (S)-4 is a simple way to get (S)-5, as shown in Scheme 1. With the KRED RtSCR9 from Rhodosporidium toruloides, an alternate pathway based on the more stable dimethylammonium-ketone 6 may be accomplished at a concentration of 1 m with near-perfect enantioselectivity (Scheme 1). Afterwards, N-demethylation of (S)-7 is the main drawback of this pathway. In contrast to isopropanol, which still has trouble reaching quantitative conversion even after extensive distillation of acetone, glucose pushes the equilibrium of the carbonyl reduction to completion, despite its poor atom efficiency, making it an attractive terminal reductant choice. The co-enzyme GDH is very effective, and its consumption is often little as contrasted with the lead KRED. Enzyme production and co-factor recycling methods were the subject of recently published research.

Novartis researchers have looked at a number of potential

Table 1: Key performance indicators (KPIs) of different processes yielding chiral alcohols

Enzyme	Product	Product conc. [g L^{-1}]	STY [g L^{-1} h^{-1}]	TTN (estim.)[a]	Catalyst load [g kg^{-1} product][b]
KREDs:					
EbN1 from Aromatoleum aromaticum	(S)-4	62	8	40.000	13 (CDW)
RtSCR9 from Rhodosporidium toruloides	(S)-7	186	47	> 20.000	54 (CDW)
P450-monooxygenases:					
Beauveria bassiana	HPOPS	103	0.5	n. a.	1 (CDW)
recomb. Candida tropicalis	Dodecane diacid	150	1.4	n. a.	100 (CDW)
P450-BM3 var. in recomb. E. coli	4-HO-isophorone	6	1	18000	10^4 (CWW)
P450-BM3 var. in recomb. E. coli	5-HO-diclofenac	3	0.6	2750	10^4 (CWW)
Lipase:					
Lipase QL	22	140	21.5	n.a.	49
Oleate hydratase (OA):					
OA from Elizabethkingia meningoseptica in recomb. E. coli	(R)-10-hydroxy-stearate	100	4	n.a.	10^3 (CFE)

[a] Total turnover number (TTN); in the absence of more appropriate statistics: projected to represent one-third of the CDW for E. coli fermentation, the recombinant enzyme

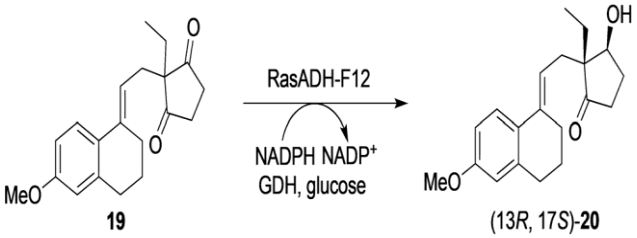
[b] Cell free extract (CFE); cell wet weight (CWW); and cell dry weight (CDW).

alternate approaches in their pursuit of effective and stereoselective.

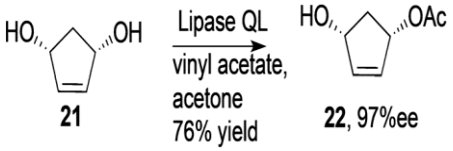
Obtaining access to LNP023, a medication used in the therapy of inflammatory kidney illness (other indications are now being evaluated in clinical studies). Poor enantio- and Dia stereoselectivity of the stages, resulting to undesired stereoisomers, and the employment of dangerous substances (such sodium hydride or dimethylacetamide) were two problems with the earlier synthesis technique.

To establish one of the two stereocenters, the technique of enzymatic ketone reduction was used (Scheme 2). By including this KRED stage, the procedure becomes more efficient, achieving complete selectivity, convergency, and simple execution compared to the earlier approaches.

The literature provides descriptions of industrial procedures that showcase the potential of enzymatic processes, such as using a mixture of biocatalysts to establish numerous chiral centres. Pfizer scientists developed a gamma secretase inhibitor, for instance, by providing very stereopure



Scheme 5: KRED-catalyzed desymmetrization of ethyl secodione 19 for the synthesis of intermediates for steroidal drugs



Scheme 6: Selective mono-acylation of diol 21 by lipase QL

fragments of a chiral amine building block 13 synthesized by a transaminase and an a-ketoester reduced by a KRED (Scheme 3). The screening enzymes used by the Pfizer team were already on the market, so they could be certain that the most promising candidate would be ready for rapid application in enough quantities to be used on a multi-kilogram scale. we go into further detail on the synthetic readiness and other uses of transaminases.

Imbruvica (25 g L-1 h-1), (R)-a-lipoic acid (24 g L-1 h-1), and Atorvastatin (17) (44 g L-1 h-1, Scheme 4) are among the significant chiral alcohols with high STY that the Xu and Zheng group recently reported synthesising using directed evolution of KREDs. The manufacturing process for 17 was based on the blueprints provided by Codexis. A KRED (LbCR) derived from Lactobacillus brevis was engineered to be more active and thermostable via directed evolution. Thanks to a synergistic effect of the useful mutants, LbCRM8 was created, which has a 3.2-fold increase in kcat/KM and a half-life 1900-fold higher at 40 8C. (5R)-16 (300 g L-1) was completely reduced to (3R, 5R)-17 in 6 hours with a STY of 44 g L-1 h-1 using E. coli cells co-expressing this mutant and GDH (1 g CDW L-1). When it comes to producing the chiral alcohol moiety for Atorvastatin in an industrial setting, the KRED technique is clearly very efficient.

A critical chiral intermediary in the manufacture of numerous steroidal medicines, ethyl secodione 19, may be desymmetrized to (13R,17S)-ethyl secol 20 (Scheme 5), as shown by Chen et al. using KREDs in desymmetrization. Since only one keto function needs to be lowered in order for four diastereomers to be generated, the reduction is also quite demanding in terms of regio- and stereoselectivity. A RasADH-F12 mutant with 183-fold activity compared to the wild-type and exceptional selectivity towards (13R,17S)-20 was obtained by performing many rounds of directed evolution on an alcohol dehydrogenase (RasADH) from Ralstonia sp. On a 1 L scale, it took 6 hours to transform 19 (20 g L-1) E. coli cells co-expressing

RasADH- F12 and GDH to (20) (13R,17S). There is potential for the enzyme and technique to be further refined for use in industrial settings.

Chiral Alcohols Produced by Lipases

There is a wealth of literature on the topic of lipase-catalyzed chiral alcohol synthesis via kinetic resolution of racemates, including reviews, chapters, and an entire book. Due to the widespread availability of ketoreductases (KREDs) and their use in enantioselective ketone reduction, lipase-mediated resolution is no longer considered a significant synthetic tool, unless there is a chance for dynamic kinetic resolution, desymmetrization of prochiral material, or the production of valuable products from both enantiomers in the synthetic route. In order to prove that lipases are still useful in organic synthesis and biocatalyze crucial reactions, such as the regioselective synthesis of alcohols and desymmetrization, we chose a handful of instances to illustrate these points.

A very new lipase approach involves selectively acylating the cyclopentene diol 21 to reach a critical prostaglandin intermediate (Scheme 6). Optimal conversion and selectivity were attained with lipase QL from Alcaligenes sp. after optimisation using commercial enzymes as catalysts. At a starting material size of 200 kg, the enzymatic process was carried out. Table 1 summarizes the key performance indicator factors for comparing this process to others that produce chiral alcohols by means of enzymes.

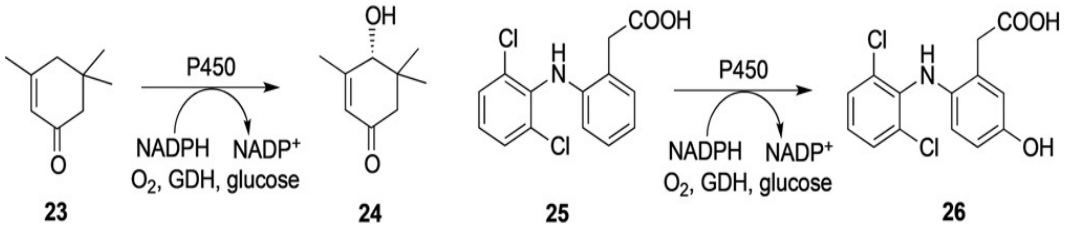
Chiral Alcohols Produced by Enzymatic Hydroxylations

When it comes to synthesising enantiomerically pure secondary, and particularly tertiary, alcohols on an industrial scale, there is a lack of powerful, regio- and stereoselective hydroxylations. Among the many types of enzymes involved in these monohydroxylations, cytochrome P450 monooxygenases stand out. When compared to more traditional chemical approaches, the selectivities shown by P450 and related flavin-dependent monooxygenases for particular hydroxylation products are much superior. There has been very little advancement in the use of recombinantly produced P450 enzymes for kilogramme scale synthesis and beyond, despite decades of reports of applications for whole-cell biotransformations of steroids for pharmaceutical synthesis without identifying the responsible enzymes. Schema 7 shows two instances of DSM/Innosyn processes: one for the 100 L scale oxidation of aisophorone 23 to the (R)-4-hydroxy isophorone 24 and another for diclofenac 25 to its 5-hydroxy-metabolite 26. These processes are based on P450-BM3 mutants. Exclusion of this oxidation approach from fine chemical applications was due to the significant biocatalyst consumption (10 times more E. coli biomass than product), even after meticulous process optimisation. So far, there have been relatively few instances of the effective transfer of monooxygenase-catalyzed hydroxylation of unactivated hydrocarbons: by the end of the '90s.

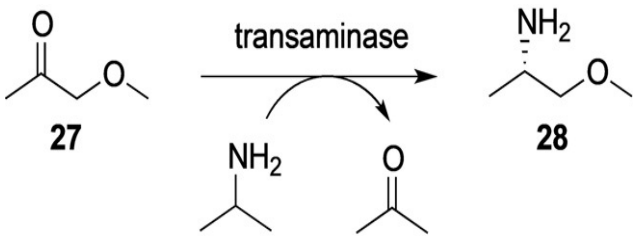
Cathay effectively implemented the oxidation of alkanes and fatty acids to diacids, beginning mechanistically with a P450-mediated terminal hydroxylation whereas BASF pioneered the p-hydroxylation of (R)-2-phenoxypropionic acid (POPS to HPOPS). Both methods outperform all instances using P450 enzymes recombinantly generated in E. coli (Table 1), thanks to their reliance on the metabolic network of naturally potent eukaryotic strains.

The slow turnover and typically average TTN of cytochrome c 450s are due to a combination of factors, including the enzyme's complicated mechanism (the catalytic cycle requires a reducing step requiring a coupled reductase and NAD(P)H prior to oxygen uptake) and the enzyme's often-observed low stability.

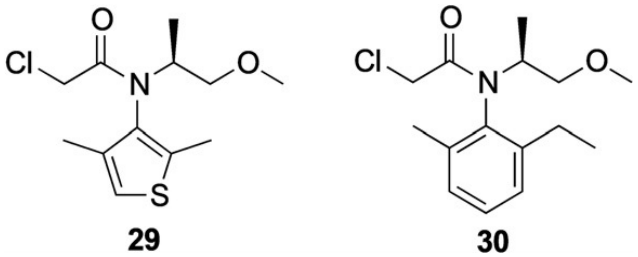
Instead of attempting to circumvent P450s' catalytic restrictions, it might be more attractive to focus on developing other kinds of enzymes, such as peroxygenases and a-ketoglutarate dependent oxygenases. Like P450 monooxygenases, peroxygenases are able to selectively hydroxylate and carry out additional oxidations thanks to their catalytic heme moiety. Since hydrogen peroxide (H2O2) is already reduced to oxygen, peroxygenases may use it as a substrate without any co-reductant. A factor of



Scheme 7: Hydroxylation of a-isophorone 23 or diclofenac 25 by a P450-monooxygenase from Bacillus megaterium expressed in E. coli



Scheme 8: Transaminase-catalyzed reductive amination exemplified for (S)-Moipa 28



Scheme 9: (S)-Dimethenamide 29 (BASF) and (S)-Metolachlor 30 (Syngenta)

Table 2: Key performance indicators (KPIs) of different processes yielding chiral amines[a]

Technology	Product	Product conc. [g L^{-1}]	STY [g L^{-1} h^{-1}]	TTN (estim.)	Catalyst load [g kg^{-1} product]
Crystallization of diasteromeric salts	(R)- or (S)-1-PEA 31	50 (0.4 m)	low	–	n.d. (90–95 % recovery of mandelic acid reported)
Lipase	1-PEA 31	(neat)	> 1000	10^7	< 0.5 (immob Enzyme)
Transaminase	(S)-1-PEA (94 % conv.)	6	1	10^3	800 (dry CFE)
Transaminase	(R)-1-PEA (80 % conv.)	40	2	–	125 (dry CFE)
Transaminase	(R)- or (S)-1-PEA (> 90 % conv.)	50	3	10^4	100 (dry CFE)
Transaminase	l-Alanine	90 (1 m)	5	–	50 (wet cells)
Transaminase	(S)-Moipa 28	170 (2 m)	25	10^5	20 CDW
Transaminase	36	156	7.8	–	20 (lyophilized CFE)
Lipase	(S)-Moipa 28	(neat)	300	10^7	< 1.0 (immob Enzyme)
Transaminase	Sitagliptin 34	190	8	25000	32 (dry CFE)
Rh-cat enamine hydroge-nation	Sitagliptin 34	110	7	670	3 (Rh-cat with chiral ligand)
RedAm	42	35	9	–	8 CDW
Aspartase (lyase)	Aspartate	166	140	–	< 0.5 (immob E. coli)

[a] 1-PEA: 1-phenylethylamine; RedAm: reductive aminase; STY: space-time-yield; TTN: total turnover number; CDW: cell dry weight; CFE: cell free extract.

ten or more quicker turnover rates is possible than oxidations catalyzed by P450. Although H2O2 is more expensive than O2 for fine chemical applications, it is simpler and quicker to dose as a liquid in many industrial setups than to use aeration. Therefore, the future of peroxygenases as catalysts for industrial applications is far brighter than that of P450s. Regrettably, the majority of studies have focused on a single version of Agrocybe aegerita's so-called "unspecific peroxygenase" (UPO), which exhibits strong overall resilience but has a poor tolerance for higher concentrations of H2O2. To get around this stability problem and make peroxygenase more practical, in situ H2O2 production technologies are being created.

The increased TTN compared to P450s is an immediate result of these efforts, and this family of enzymes has the potential to become the gold standard for hydroxylation biocatalysis with further research into enzymes and protein engineering. The a-ketoglutarate-dependent oxygenases are another kind of enzymes that can activate C-H reactions. With the help of a high-energy Fe(IV)-oxo intermediate, which is produced during catalytic oxidative breakdown of a-ketoglutarate, a hydroxylated product may be obtained by homolyzing unactivated C-H bonds and then undergoing radical recombination. The hydroxylation of several amino acids has shown that these enzymes have economic use. For instance, it has been shown that many hydroxylases can convert L-proline to each of its four monohydroxy isomers. A few of the biocatalysts have been modified to work better with 4-hydroxy prolines when produced on a big scale. A substrate concentration of 20–40 g L-1 was used for the examples. Compared to a normal P450-catalyzed reaction, these titers are already many orders of magnitude greater.

Hydratases are an intriguing family of enzymes that add water to double bonds, forming hydroxyl groups. The flavin-dependent oleate hydra-tases (OA) are noteworthy examples; they selectively convert oleic acid to (R)-10-hydroxystearic acid, with product yields of up to 100 g L-1 recorded (Table 1). Long chain aliphatic amines and functionalised fatty acid derivatives were also afforded by cascade reactions made possible by the high activity of OAs. The mechanism of oleate hydratases was greatly illuminated by the recent solution of the first structure of an oleate hydratase for the OA from Elizabethkingia meningoseptica. In addition, this laid the groundwork for protein engineering, which allows for the asymmetric hydration of different terminal and internal alkenes, in contrast to the wildtype enzyme, which is only active in fatty acids when carbohydrates are present. To get around this, we activated the E. meningoseptica enzyme using a carboxylic acid decoy molecule. This allowed for the preparative-scale asymmetric hydration of unactivated alkenes to achieve 93% conversion with good selectivity (> 99.99 percent ee, > 95.0 percent regioselectivity).

Optically Active Amines

The transaminase process is the most flexible biocatalytic method for primary amines; it involves converting carbonyl substrates to the desired amine via a reductive amination reaction. It also needs a supply of sacrificial amines, as shown in Scheme 8. We have paid homage to its potential impact on large-scale chemistry and thoroughly examined its whole extent.

Celgene (subsequently "Celgro") was an industry trailblazer in pioneering the use of pyridoxal-5'-phosphate (PLP)-dependent transaminases for the preparative synthesis of enantiomerically pure chemicals. In the beginning, Celgene used the reversible transaminase process in a de-amination mode to polish or deracemize chiral amines, such as 1-arylethyl-amines and 1-phenyl-3-aminobutane. Although the resolution was effective up to 160 L scale, its commercial applicability was limited due to the need of costly amine acceptors such as pyruvate or oxaloacetic acid. Once CelgeneRs identified the unique benefits of isopropylamine as an amine donor, it was a game-changer for synthetic applications.

Utilising the transaminase technology, Celgene

demonstrated the preparative utility of enantiopure L-alanine (derived from pyruvate), (S)-Moipa ((S)-1-methoxy-isopropylamine, 28; Scheme 8), and (S)-2-amino-3-methylbutane (derived from the corresponding ketones). To make herbizides, the last two amines are used as building blocks. As a building block for (S)-Dimethenamide 29 and maybe for (S)-Metolachlor 30, (S)-Moipa is manufactured in the thousands of tonnes every year (Scheme 9). Overcoming product inhibition by enzyme engineering was a particularly noteworthy accomplishment of Celgene's research. Celgene has the potential to produce (S)-Moipa to a concentration of over 2 m in the future (Table 2). Even so, an extensively optimised Ir-catalyzed imine hydrogenation approach to (S)-Metolachlor and lipase technology for (S)-Moipa were more effective than transaminase technology.

CONCLUSION

Many biocatalytic processes now attain STY equivalent to traditional chemical transformations, which may delight process engineers. Because most enzymes are so efficient, a little amount of enzyme may do a great deal in the chemical realm. As a result, producing enough catalyst for continuous production requires just modest pilot-plant equipment (and quite common expression technology). There is an increasing variety of biocatalysts accessible on a kilogramme scale. The ability to process downstream is made easier by the high selectivities and clean reactions. Because fermentation requires massive fermenter volumes and complex metabolic engineering to generate the producing organism—resulting in a STY that is one to two orders of magnitude lower—biocatalysis is more amenable to use in chemical manufacturing.

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