catalysts

MDPI

Review

Evolving New Chemistry: Biocatalysis for the Synthesis of Amine-Containing Pharmaceuticals

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Abstract: Biocatalysis has become an attractive tool in modern synthetic chemistry both in academic and industrial settings, offering access to enantiopure molecules. In industry, biocatalysis found use in small molecule pharmaceutical development. For several amine-containing drugs, biotransformations were applied in the process routes, improving the original syntheses employing classical chemical methods. This review illustrates how and why biocatalysis has been applied to create safer, more efficient and less costly processes for the manufacture of chiral amine-containing pharmaceuticals and alkaloids. Several enzyme classes have been applied to syntheses of natural products, pharmaceutical products and their intermediates, including transaminases, imine reductases, monoamine oxidases and Pictet-Spenglerases. The routes with and without application of biocatalysis are compared, and the potential of these enzyme classes in redesigned synthetic routes to natural products, alkaloids and high-value chemicals is evaluated, using syntheses of sitagliptin, suvorexant, PF-04449913, MK-7246, vernakalant, GSK-2879552, boceprevir and (−)-strictosidine as examples. Application of biocatalysis in the synthesis of amine-containing pharmaceuticals constitutes a greener alternative to transition metal-catalysed routes, facilitates installation of chiral amine functionalities at a late stage of the synthesis and provides exquisite stereocontrol. Opportunities and challenges of biocatalysis for the synthesis of chiral amines are reviewed with respect to use in drug discovery and development.

Keywords: biocatalysis; chiral amine; transaminase; imine reductase; monoamine oxidase; Pictet-Spenglerase; enzyme; pharmaceutical; drug development; API

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1. Introduction

Innovations in synthetic chemistry at different stages of drug discovery and development play an important role in the identification and production of new therapeutic agents [1–7]. New methodologies have the power to change practice in drug discovery, expanding the chemical space available to scientists and facilitating quick access to it. Many of these advances are now driven by partnerships between pharmaceutical companies and leading academic groups [1]. Biocatalysis is one area in which such collaborations have been particularly effective, accelerating the pipeline from enzyme discovery to large-scale practical application in synthesis [8]. The use of molecular biology, protein engineering and bioinformatics tools helps to tailor the properties of a biocatalyst, such as activity and selectivity, with the influence of the directed evolution of enzymes being highlighted by the 2018 Nobel Prize in Chemistry awarded to Frances Arnold [8,9]. One of landmark achievements of using biotransformations in industrial research was the application of a recombinant engineered transaminase in the manufacture of sitagliptin (Januvia) by Merck and Codexis, and this success drove further interest in the industrial use of biocatalysts [10].

Amine moieties, in particular, are common in drugs and biologically active compounds, and can also be valuable synthetic intermediates [11–13]. Thus enantiomerically enriched chiral amines are of particular interest to the pharmaceutical and agrochemical industries [14,15]. Their classic chemical methods of asymmetric synthesis include asymmetric

reduction, nucleophilic addition or diastereomeric crystallization [11,16–19]. However, the last decade witnessed a significant growth of portfolio of biocatalytic processes for the preparation of chiral amines. These enzymes include transaminases, imine reductases, amine dehydrogenases, monoamine oxidases, Picter-Spenglerases and lipases [20–25]. This review examines applications of these enzyme classes in the preparation of active pharmaceutical ingredients (APIs) and alkaloids, and investigates how the synthetic routes evolved. The examples of sitagliptin, suvorexant, PF-04449913, MK-7246, and vernakalant are used to demonstrate applications of transaminases, whereas GSK-2879552, boceprevir and (−)-strictosidine show synthetic use of imine reductases, monoamine oxidases and Pictet-Spenglerases, respectively (Table 1). The purpose of the review is to summarise the extent to which amine biocatalysis is being applied in the pharmaceutical industry and scrutinize why enzymatic methods superseded existing chemical processes.

Table 1. List of compounds discussed in this review and enzymes used during their synthesis.

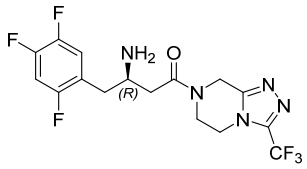
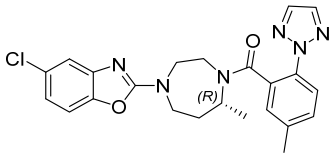
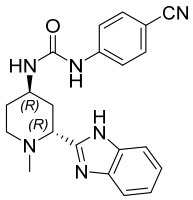
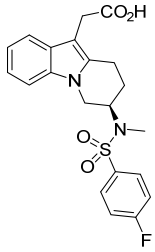
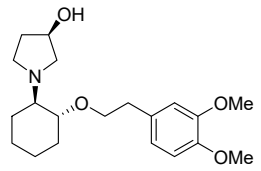
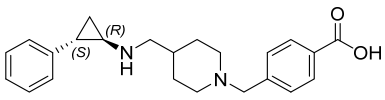
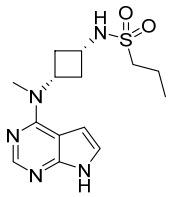
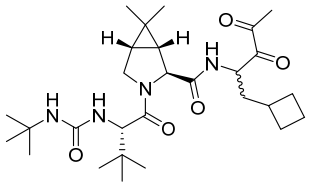
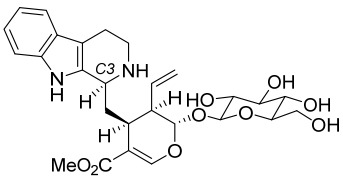
Compound	Enzyme Class	Enzyme Used
 sitagliptin	Transaminase	ω-TAm ArRMut11
 suvorexant	Transaminase	ω-TAm (CDX-017)
	Imine reductase	IR1 *Leishmania major* from Y194F/D232H
 PF-0444913	Transaminase	ATA-036
 MK-7246	Transaminase	ω-TAm (CDX-017)

Table 1. *Cont.*

Compound	Enzyme Class	Enzyme Used
 vernakalant	Transaminase	ATA-303
 GSK2879552	Imine reductase	IRED-M3
 abrocitinib	Imine reductase	*Sp*RedAm-V6
 boceprevir	Monoamine oxidase	MAON401
 (−)-strictosidine	Picter-Spenglerase	Strictosidine synthase cell-free lysate

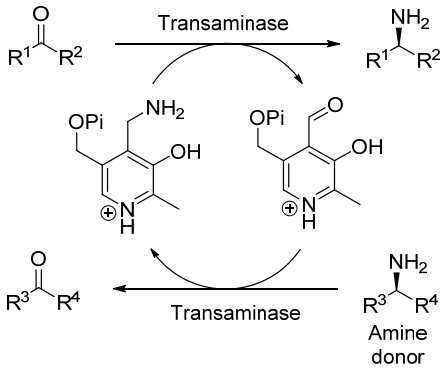
2. Transaminases

2.1. Overview

Transaminases offer access to primary amines by converting carbonyl substrates to the target amine. They require a sacrificial amine donor and vitamin B_6 derivative pyridoxal-5′ phosphate (PLP) as a cofactor (Scheme 1) [24]. This class of enzymes has found a number of applications in the preparation of complex pharmaceutical intermediates [15], and commercially available panels of transaminases can be used without specialist equipment to solve challenging synthetic problems [25].

However, transaminases catalyse the reversible transfer of the amino group, so it might be necessary to shift the equilibrium towards the product by using excess amounts of the amine donor. If isopropylamine is used as the donor, giving acetone as the side-product, reaction mixture sparging can be performed to shift the equilibrium. An alternative approach uses diamines as 'smart' amine donors which cyclise on deamination [26]. A final way of overcoming the unfavourable thermodynamics is to set up a cascade which uses the reaction product directly [27]. Various enzyme classes can be used in cascades with transaminases,

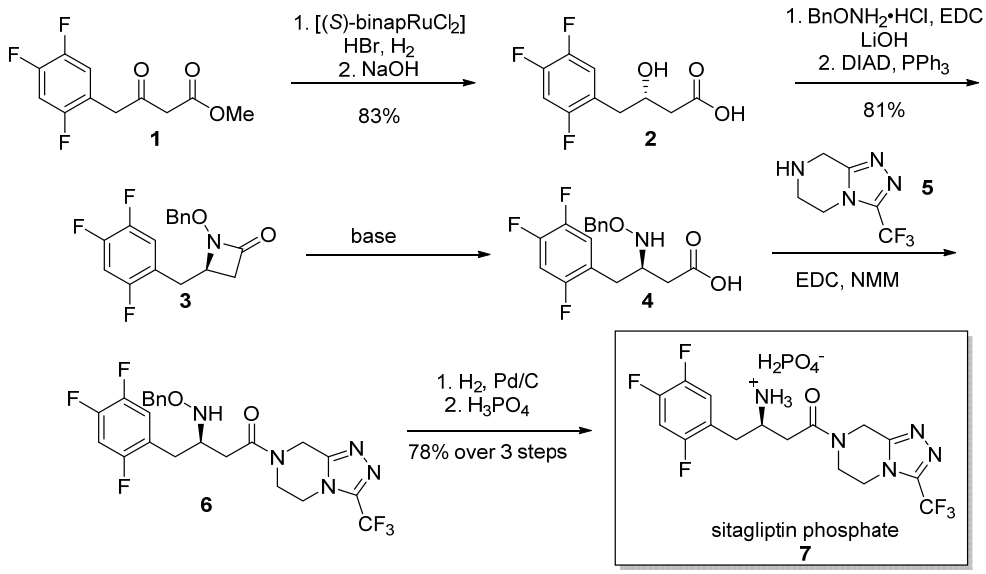
e.g., alanine dehydrogenase, lactate dehydrogenase, pyruvate dehydrogenase, alcohol dehydrogenase [28], carboxylic acid reductase [29], monoamine oxidase [30,31] or imine reductase [32]. From the synthetic point of view, the key limitation of transaminases is that only primary amines can be generated from the ketone congeners [18]. As a result, although transaminases have found widespread use in industry, they are beginning to face competition from emerging enzyme classes, such as amine dehydrogenases and imine reductases, which do not have this limitation [25].



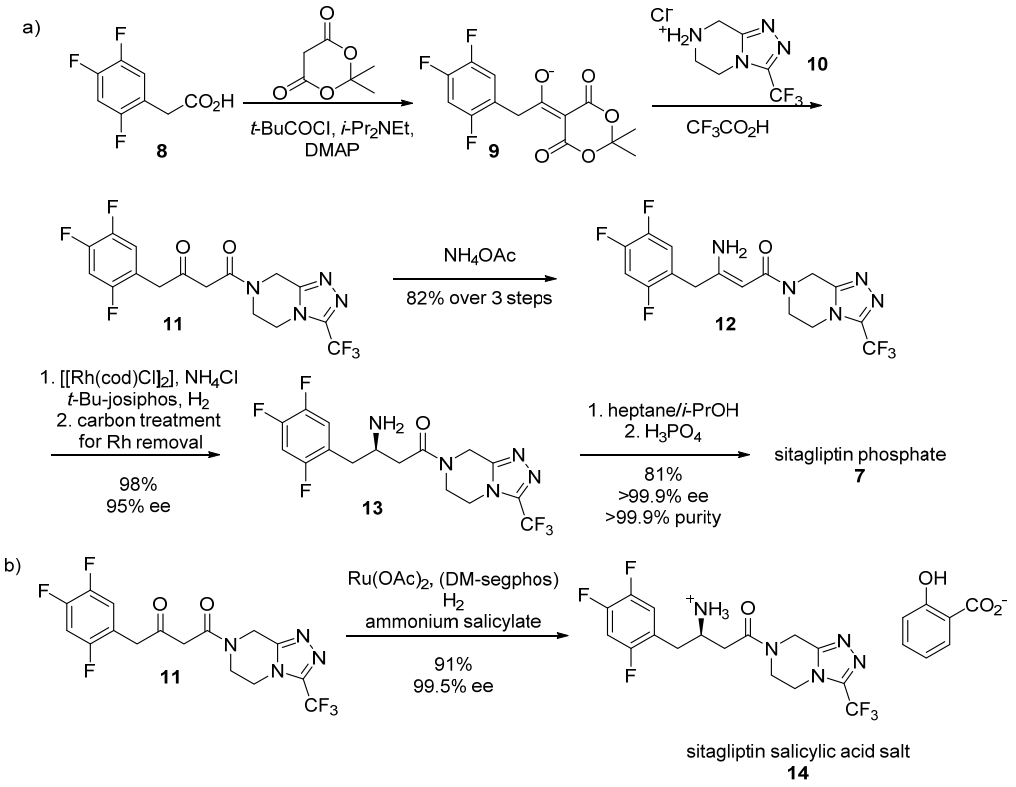
Scheme 1. Mechanism of transaminase-catalysed reaction with PLP as a cofactor.

2.2. Sitagliptin

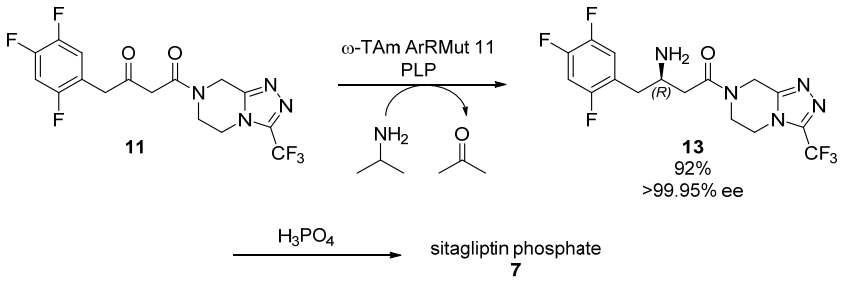
Sitagliptin, the active ingredient in Merck's Januvia, is used for treatment of type II diabetes [33–35]. The manufacturing process of the phosphate salt of sitagliptin was developed by Merck in collaboration with Selvias and Codexis [35]. In synthetic terms, the evolution through three generations of process research and development shows moving from setting the stereochemistry early in the synthesis to installation of the amine functionality late in the synthesis by asymmetric catalytic hydrogenation and finally the application of a transaminase (Schemes 2–4).



Scheme 2. First-generation sitagliptin process synthesis.



Scheme 3. Second-generation sitagliptin process syntheses with (**a**) stereoselective hydrogenation using a rhodium catalyst and (**b**) stereoselective amination using a ruthenium-based catalyst.



Scheme 4. Third-generation sitagliptin process synthesis.

The first-generation process route introduced the asymmetric bias by means of a ruthenium-catalysed asymmetric hydrogenation of β-keto ester **1** (Scheme 2) [36]. The amine functionality was then introduced by the formation of a benzyl-protected hydroxyamide followed by the stereospecific displacement of the alcohol by the NH of the hydroxyamide. The subsequent hydrolysis of the resulting protected cyclic hydroxyamide **3**, amide coupling, deprotection and salt formation gave sitagliptin as the phosphate salt **7**. The total 52% yield over eight steps provided 100 kg of sitagliptin phosphate for early clinical studies. However, the first-generation process had some significant drawbacks, particularly in the sequence employing the EDC coupling and the Mitsunobu reaction, which suffers from poor atom economy and with the by-product being difficult to remove. The chiral amine was also introduced in a circuitous way.

Improvements with respect to the first-generation process led to the second-generation sitagliptin synthesis, the key feature of which was three-step, one-pot transformation starting from triflurophenyl acetic acid **8** and leading to dehydrositagliptin **12** (Scheme 3a) [37]. The overall yield of the process was 82%. Dehydrositagliptin **12** was then subjected to rhodium-catalysed asymmetric hydrogenation, followed by crystallization of sitagliptin phosphate **7**. The yield over three steps from **8** to **12** was 82%. Then an asymmetric,

rhodium-catalysed hydrogenation step installed the chiral amine, using 0.15 mol% of the catalyst to give sitagliptin **13** in 98% yield and 95% ee. Crystallisation of the final product sitagliptin phosphate **7** enhanced ee to 99.9% ee. The rhodium catalyst was subsequently removed using activated carbon. The protecting-group-free, second-generation process had a total yield of 65% for the three-step process. With respect to the first-generation route, a significant reduction of waste was achieved. The total waste produced per kilogram of the final product was reduced from 250 kg to 50 kg.

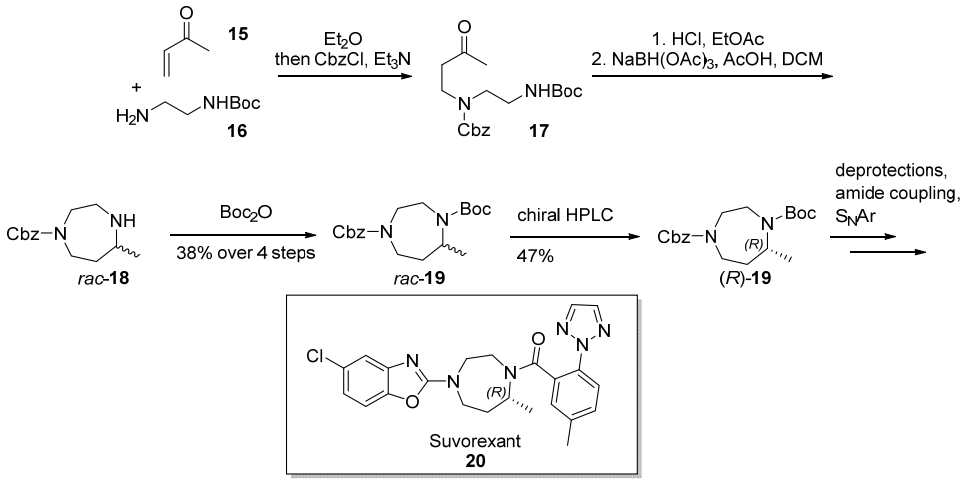
Further improvements to the second-generation route were then made [37]. β-Ketoamide **11**, which was previously an intermediate en route to dehydrositagliptin **12**, was isolated in order to be subjected to a ruthenium-catalysed asymmetric reductive amination reaction, thus directly converting the β-keto amide to β-amino amide **14** (Scheme 3b).

Despite improvement with respect to the first-generation process, the disadvantages of the second-generation route were inherent to the use of the transition metal catalysts, including the necessity of removal of ruthenium or rhodium from the product stream using specialized, high-pressure equipment. The rhodium-catalysed step (95% ee) also required an extra crystallization step to enhance the optical purity of the final product.

The aforementioned challenges were solved by the application of a highly evolved transaminase to transform **11** to sitagliptin **13** in the third-generation process, which was developed by means of collaboration between Merck and Codexis [10]. The research started with *R*-selective transaminase that was evolved utilizing computation modelling and iterative directed evolution. The final enzyme applied in the route contained 27 mutations. The optimized process employed 6 g/L of the evolved transaminase and 200 g/L concentration of β-keto amide **11** in 50% DMSO at 40 °C to afford sitagliptin **13** in 92% yield and >99.95% ee (Scheme 4). The third-generation process increased the yield and reduced the total amount of waste produced.

2.3. Suvorexant

Suvorexant **20** (Scheme 5) is an orexin antagonist used to treat insomnia, formerly known as MK-4305 [38]. It was approved for sale by the FDA in 2014. Key structural features include a 1,4-diazepine core with a stereogenic centre at the 7-position bearing a methyl group alpha to one of the nitrogens. While attaching the benzoxazole and aromatic amide moieties to the core is relatively straightforward, the requirement for an enantiopure chiral diazepine core with (*R*)-configuration constituted a significant challenge on scale-up from the medicinal chemistry preparation to the process route.

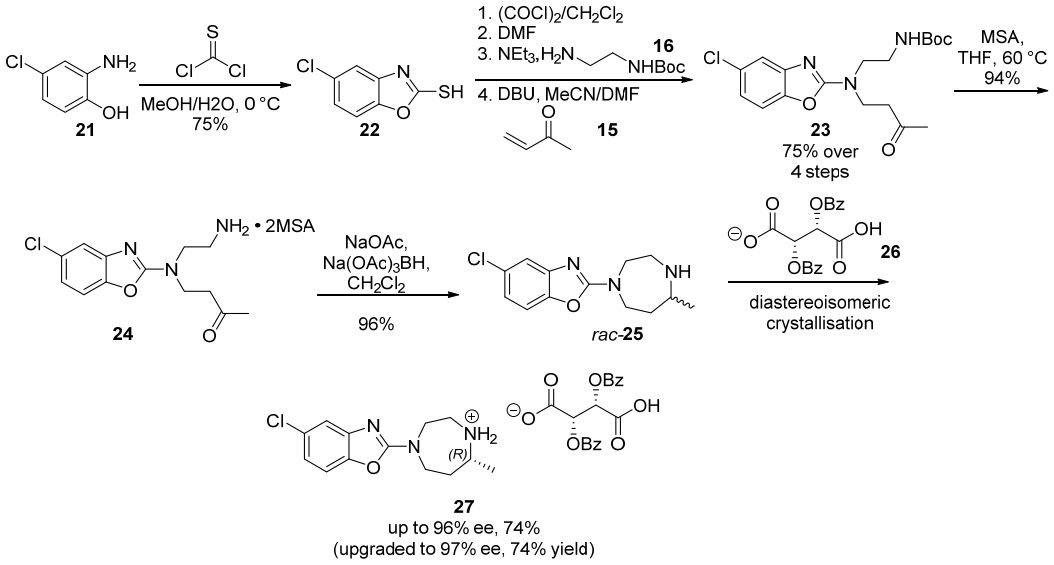


Scheme 5. Medicinal chemistry route to suvorexant.

Originally, the (*R*) enantiomer of intermediate **19** was prepared by preparative chiral HPLC separation of the Cbz- and Boc-protected *rac*-**19** following the low-yielding aza-Michael reaction and was successful for preparation of multigram quantities of **20** during

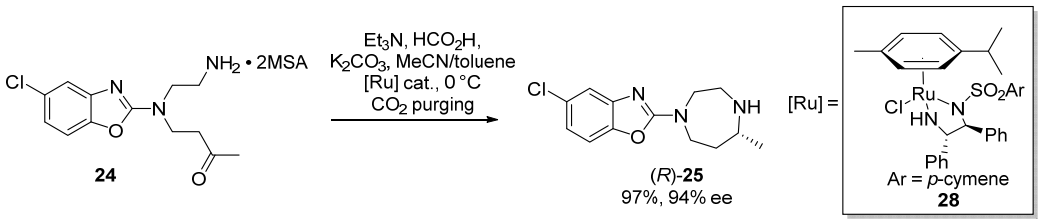
early development phase and quick exploration of structure activity relationships thanks to the orthogonal protection of nitrogen atoms of the diazepane core (Scheme 5) [38,39].

The first kilogram-scale synthesis of **20** replaced the Cbz group with the benzoxazole functionality as a surrogate protecting groups during the aza-Michael reaction (Scheme 6). The diazepane ring system was made by Boc deprotection of **23**, followed by reductive amination to afford the racemic precursor of suvorexant *rac*-**25**. The racemic mixture was subsequently subjected to classical resolution using dibenzoyl-D-tartartic acid to obtain **27** with a maximum enantiomeric excess of 74% and in 39% yield on scale. The enantiomeric excess value was further increased to 96% with 74% yield after reslurring using methanol and isopropyl acetate (27% yield from aminoketone **24**) [38].



Scheme 6. First kilogram-scale synthesis of diazepane core of MK-4305.

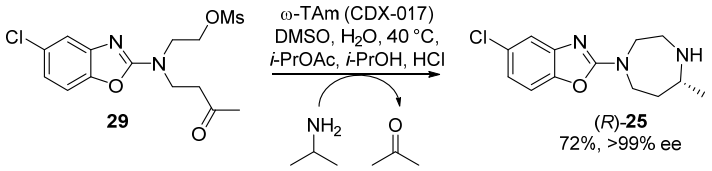
Scientists at Merck sought further improvements in the synthesis of the chiral diazepane core [40,41]. The intramolecular asymmetric reductive amination of aminoketone **24** mediated by a ruthenium-based transfer hydrogenation catalysts **28** afforded (*R*)-**25** in 97% yield and 94.5% enantiomeric excess. The catalyst employed was a new variant of Noyori's (*S*,*S*)-RuCl(*p*-cymene)ArSO_2DPEN) catalyst and the reaction was run on >100 kg scale (Scheme 7) [40].



Scheme 7. Ru-catalysed asymmetric reductive amination of suvorexant precursor.

Merck also reported the first enantioselective synthesis of suvorexant that employed a tandem transamination and seven-membered ring annulation in the key step (Scheme 8) [41]. The crucial sequence of reactions posed several challenges. Firstly, a suitable (*R*)-selective transaminase had to be found. Installation of a primary amine with transamination could trigger ring opening of the benzoxazole, which was previously found to be labile to nucleophilic attacks. Amine **25** was previously found to be prone to isomerisation, and the mesylate leaving group could undergo hydrolysis. Ultimately, (*R*)-selective transaminase evolved during the manufacturing process of sitagliptin (CDX-017) [10] gave good conver-

sion and excellent enantioselectivity (>99% ee). Employing moderate pH and slow addition of a DMSO solution of the substrate to the reaction mixture gave 71% yield reported on a multigram scale (Scheme 8) [41].

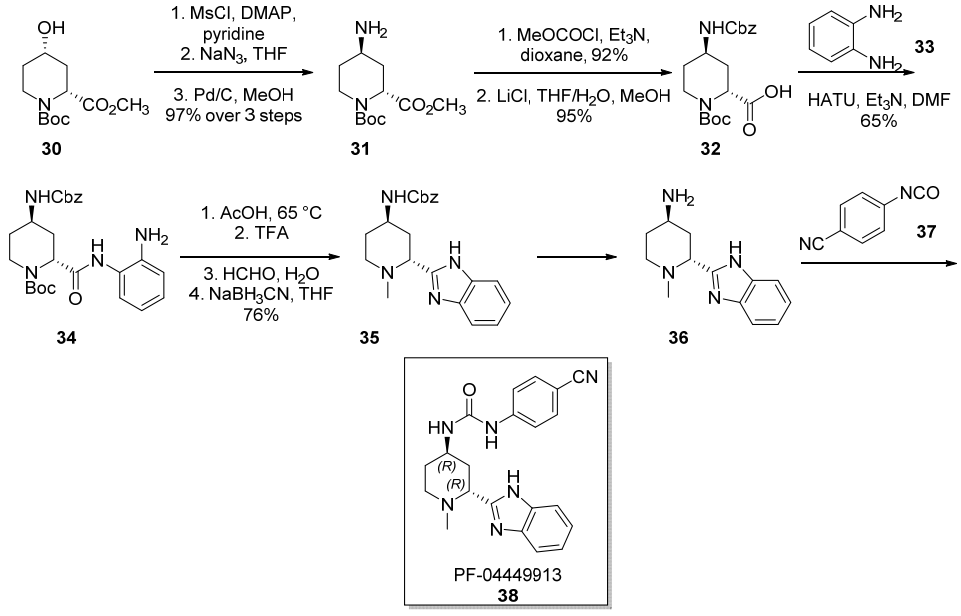


Scheme 8. Tandem transamination/annulation synthesis of the diazepane suvorexant core.

Imine reductases constitute another class of enzymes that were used to synthesise suvorexant (Section 3.4) [42]. The synthetic routes employing the ruthenium catalyst, transaminase and imine reductase all achieved excellent enantiopurity of the chiral diazepane intermediate and went beyond the 50% yield that is the limit for the classic resolution approach initially employed by Merck (Scheme 6). Ruthenium-catalysed intramolecular reductive amination requires transition metal and organic solvents, and biocatalytic routes help to decrease the environmental impact of preparation of the key intermediate. Even though the reported scales of transaminase and imine reductase-mediated biotranformations were much lower than that of the ruthenium-catalysed transfer hydrogenation, the example of suvorexant shows that enzymes are viable synthetic solutions when moving from drug discovery to development.

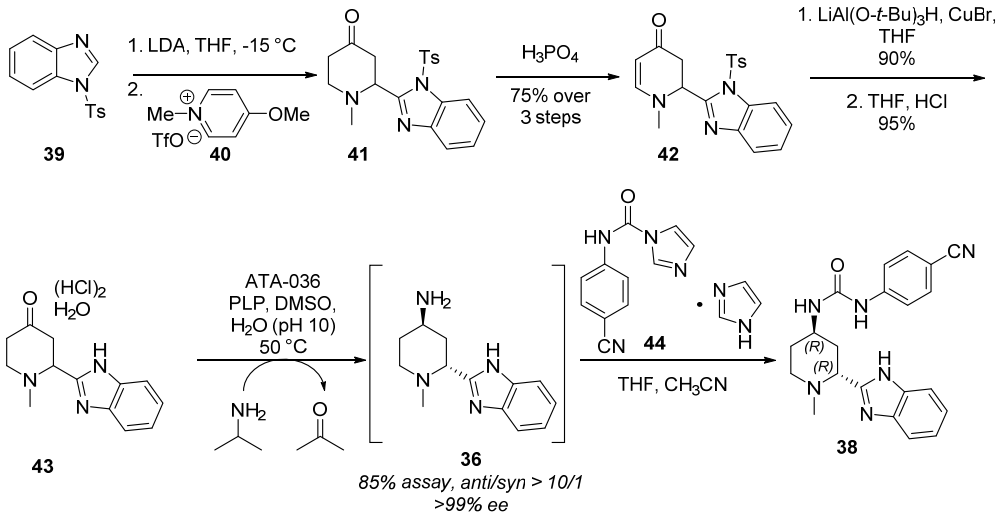
2.4. PF-0444913

PF-0444913 **38** (also known as glasdegib, Scheme 9) is an inhibitor of the Hedgehog signaling pathway developed by Pfizer that is used for the treatment of acute myeloid leukemia. The original medicinal chemistry route started with commercially available (2*R*,4*S*)-1-*tert*-butyl 2-methyl 4-hydroxypiperidine-1,2-dicarboxylate **30** with two stereogenic centres allowing synthetic manipulations to obtain PF-0444913 with desired configuration. The *syn* relationship between chiral centres in **30** was changed to *anti* by means of alcohol activation and S_N2 chemistry, with facile transformations following to install the benzoimidazole alpha to the nitrogen and form the final urea (Scheme 9) [43].



Scheme 9. Medicinal chemistry route to PF-04449913.

In the drug development phase, it was necessary to provide a practical and scalable synthesis of PF-04449913 **38**. The key challenge was establishing that the *anti* relationship between the stereogenic centres in **38** and **30** employed in the original route was not readily available on scale. Installing the primary amine functionality on the piperidine core required three steps and the use of sodium azide posed safety concerns. In order to generate the primary amine and also establish the *anti* relationship between the chiral centres by means of dynamic kinetic resolution, transaminases were employed (Scheme 10) [44].



Scheme 10. Synthesis of PF-04449913 by transamination and DKR.

The 4-pyridinone substrate for transaminase application **43** was made by addition of benzimidazole **39** to pyridinium salt **40**, followed by acidic hydrolysis to yield enone **42**. The latter species **42** was converted to **43** by conjugate reduction and acidic deprotection of the tosylate group. Commercially available transaminase ATA-036 was used for the amine formation and dynamic kinetic resolution step, giving amine **36** in 85% assay yield, with the *anti*/*syn* ratio exceeding 10:1 and >99% ee. Due to low stability of 4-pyridinone **43** at elevated temperatures (>60 °C), the reaction was performed at 50 °C for 50 to 60 h. Product **36** displayed very high aqueous solubility, making organic extraction difficult, so the DMSO/water mixture was subjected directly to the last step. Here, a suitable *N*-carbamoylimidazole reagent was employed instead of the previously used isocyanate to address the safety issues. The entire synthesis required five linear steps, affording **38** in 40% overall yield without a requirement for chromatography and on a reported multigram scale [44].

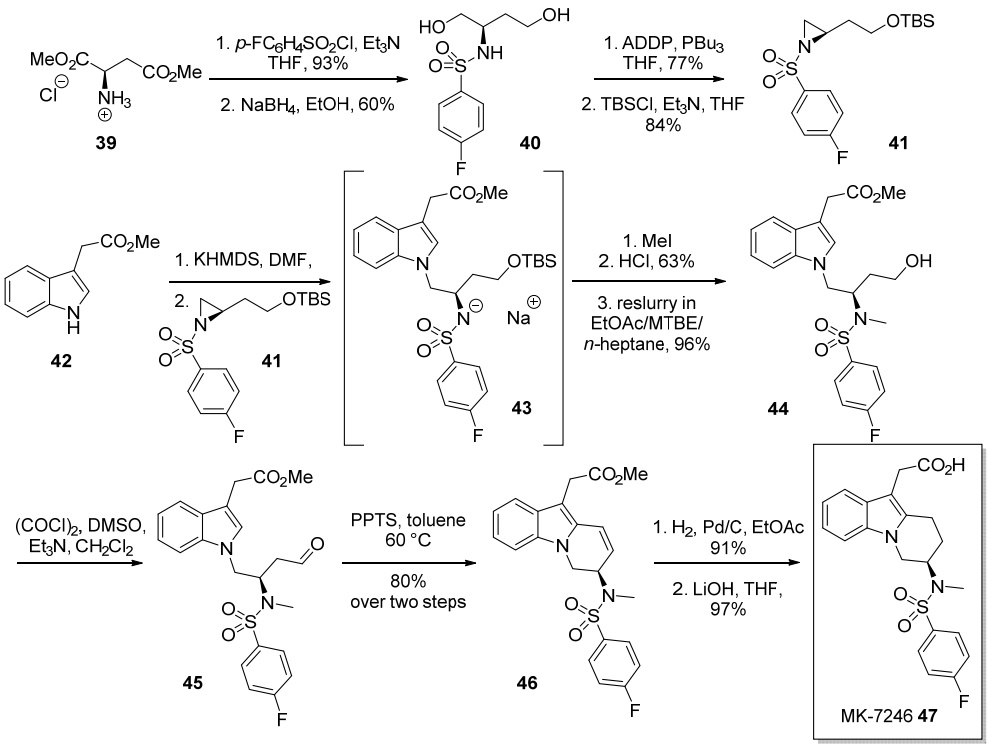
2.5. MK-7246

MK-7246 **47** is a CRTH2 antagonist developed for treatment of respiratory diseases by Merck [45,46]. This example demonstrates how enzymes were employed to evolve the synthesis during drug development to obtain quantities of exceeding 10 kg for late-stage development into phase II of clinical trials and beyond.

The original medicinal chemistry route of MK-7246 **47** involved HPLC separation at the last step. The synthesis scalable to 1-10 kg and developed to supply the animal toxicity studies and phase I clinical trials involved the ring-opening of enantiopure aziridine **41** as the key strategic step, followed by Friedel-Crafts cyclisation onto the C2 position of indole **45** (Scheme 11) [46].

The aziridine **41** was prepared from D-aspartic acid **39** available from the chiral pool in a synthesis involving sulfonamide formation, ester reduction to form a diol that was subjected to the intramolecular Mitsunobu reaction to form the target aziridine **41**. Addition of the aziridine 42 to the *N*-anion prepared from indole acetic ester **42** resulted in regioselective ring opening to give sulfonamide anion **43** that was methylated. After the

removal of the TBS protecting group and oxidation of the alcohol **44** to the corresponding aldehyde **46**, the latter species was subjected to mild acidic conditions to facilitate cyclisation and subsequent dehydration to afford allylic sulfonamide **46**. This was reduced and deprotected to give MK-7246 **47** (Scheme 11).



Scheme 11. Preparation of MK-7246 by aziridine ring-opening.

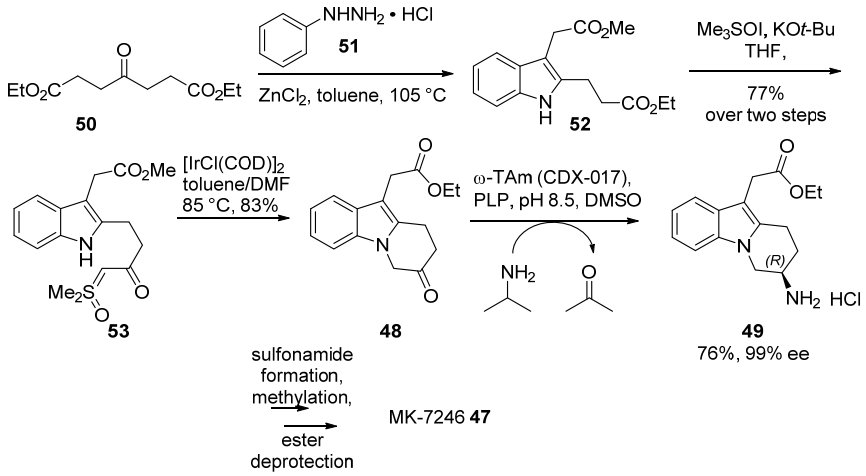
The route had a few limitations, including processing difficulties for a few steps and instability of intermediates in the route, which made it impractical for preparation of >10 kg quantities of **47**. At the same time, the medicinal chemistry program was still identifying potential backups for MK-7246, and the route installed the sulfonamide moiety early in the synthesis, which complicated SAR exploration.

For the second-generation approach, the chiral amine was installed from prochiral ketone **48** with the aid of a transaminase (Scheme 12). Prochiral ketone **48** was screened against an array of *R*-selective transaminases, with CDX-017 giving 98–99% ee and 81% HPLC yield. Control of pH was required to prevent ester hydrolysis and the biotransformation was performed with nitrogen purging to minimize alpha-hydroxylation of ketone **48** under the basic conditions while shifting the equilibrium of the biotransformation towards amine **49**. Biocatalytic preparation of amine **49** facilitated SAR exploration around the amine functionality at a late stage of the synthesis, and gave access to laboratory-scale quantities of **49**. However, the bottleneck of the synthesis was preparation of ketone **48**, which was complicated by dearth of crystalline intermediates and necessity of purification by chromatography after each step. In the manufacture route to MK-7246 **47,** the synthesis of ketone **48** was altered while keeping the transaminase step unchanged. The route employed Fischer indolization to yield diester **52**. Subsequently, sulfoxonium ylide **53** was prepared, and it was subjected to Ir-catalysed N-H insertion giving ketone **48**. The biotransformation employing transaminase was reproducible on scale-up, and isolation was facilitated by hydrochloride salt formation, giving amine **49** in 76% yield and >99% ee (Scheme 12).

2.6. Vernakalant

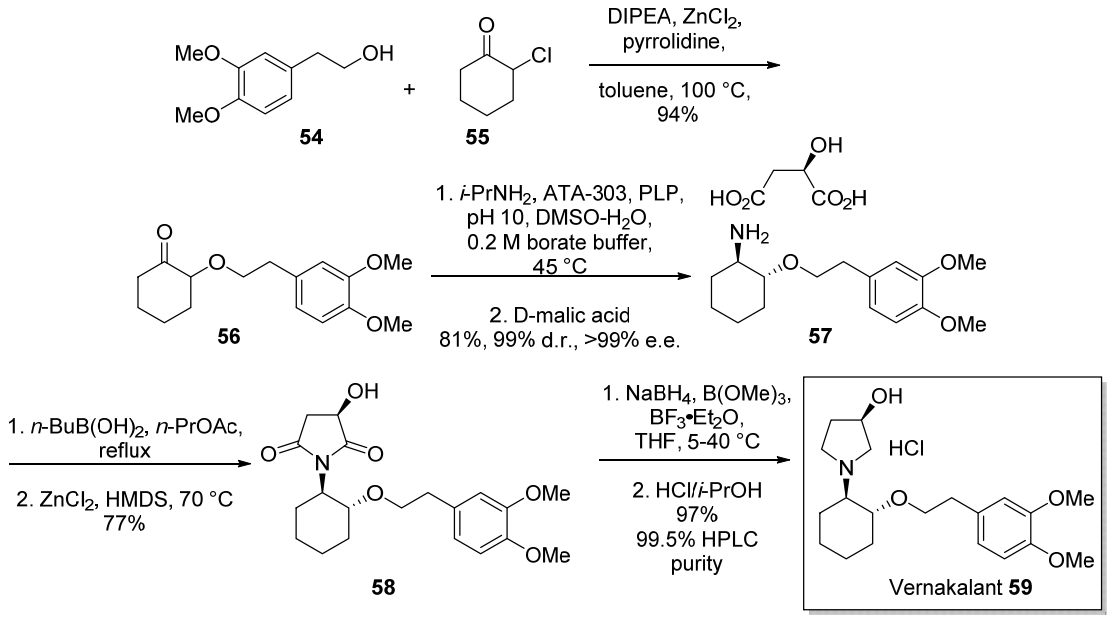
Vernakalant **59** is an antiarrhytmic agent to treat atrial fibrillation. It was originally developed by Cardiome Pharma Corp [47]. Various approaches to the aminoether core of

the molecule were reported and usually utilized classic resolution or separation of racemic intermediate or included an expensive chiral starting material [48–51].



Scheme 12. Manufacturing route to MK-7246 including the transaminase step.

Merck reported a novel approach to the drug with a key step including dynamic kinetic resolution mediated by a transaminase (Scheme 13). The synthesis was commenced by the alpha-etherification of alpha-chloroketone **55** with alcohol nucleophile **54**. The ketone **56** was screened against various transaminases. Interestingly, initially it was the amine with *syn* relationship between the stereogenic centres that was the major product. In silico design and directed evolution of transaminase ATA-013 gave a new variant that displayed reverse diastereoselectivity furnishing the anti product. Concurrent enhancement of reactivity and selectivity as well as high pH tolerance were observed. The optimized conditions included isolation of the amine as the D-malate salt **57** to give **58** in 81% yield with 99.6:0.4 dr and >99% ee. Apart from the purity upgrade, the malate salt facilitates direct formation of the hydroxypyrrolidine ring. The malate salt was converted to succinimide intermediate in a step catalysed by alkyl boronic acid catalyst and subsequently reduced to obtain vernakalant (Scheme 13).

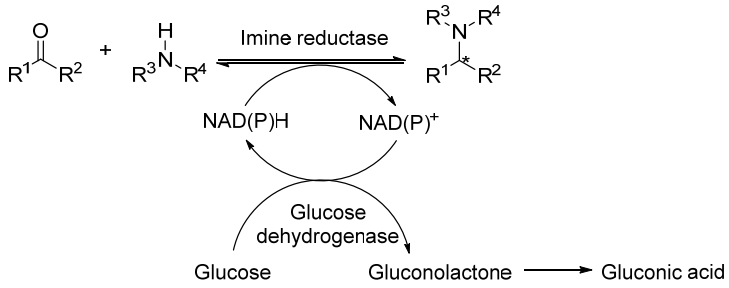


Scheme 13. Synthesis of vernakalant utilising dynamic kinetic resolution mediated by a transaminase.

3. Imine Reductases

3.1. Overview

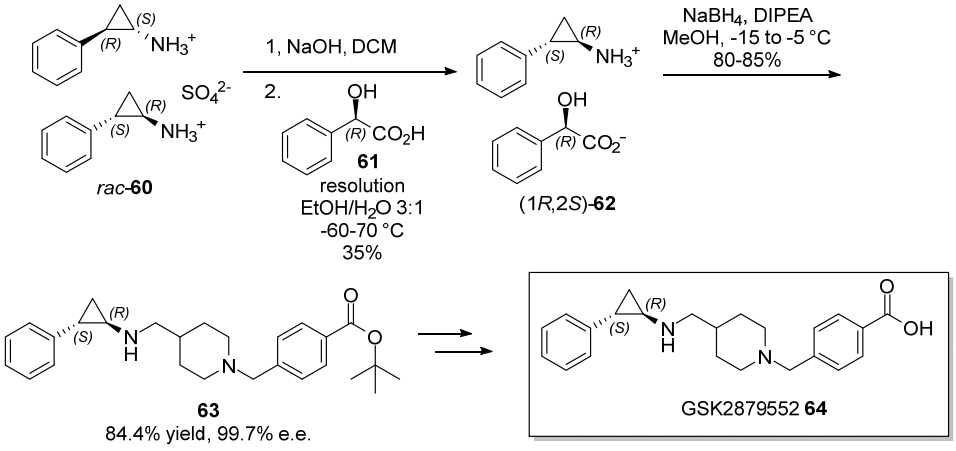
As pointed out previously, the main limitation of transaminases is their ability to generate only primary amines. This difficulty can be overcome by employing imine reductases (IREDs), being NAD(P)H-dependent oxidoreductases that reduce imines to the corresponding substituted amines. This class of enzymes can perform both imine reduction and reductive amination (Scheme 14). The later process is particularly impressive considering the unfavourable equilibrium of imine formation in the aqueous medium [52]. For this reaction to be efficient, imine reductases must be chemoselective to avoid reduction of the carbonyl to the corresponding alcohol. Since 2010, when Mitsukura and co-workers published the application of an imine reductase isolated from the *Streptomyces* species to the reduction of 2-methyl-1-pyrrolidine [53,54], several publications and patents have been issued each year [20]. However, until recently few industry-relevant applications have been reported. The section below describes the use of imine reductases in the manufacture of GSK2879552 [55], and a team from Pfizer Worldwide Research and Development have also applied this family of enzymes to the synthesis of JAK1 inhibitor abrocitinib [56].



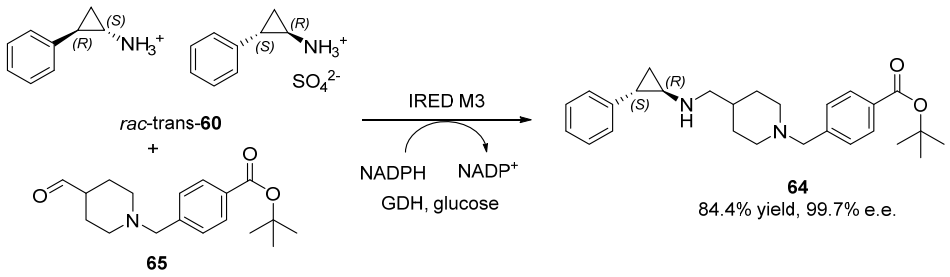
Scheme 14. Reductive amination using imine reductases.

3.2. GSK2879552

Imine reductases facilitate production of chiral amines by catalysing asymmetric reductive amination of ketones or aldehydes with amines. Despite a lot of momentum in their research since the disclosure of the first imine reductase by Mitsukura in 2010 [53,54], few industrial applications were exemplified until recently. The application of an imine reductase by the GSK in the manufacture of lysine-specific demethylase-1 LSD1 inhibitor GSK2879552 **64** is a spectacular demonstration of the potential of this class of enzymes (Schemes 15 and 16) [55].



Scheme 15. Original chemical route to GSK2879552.



Scheme 16. Application of imine reductases to the manufacture of GSK2879652.

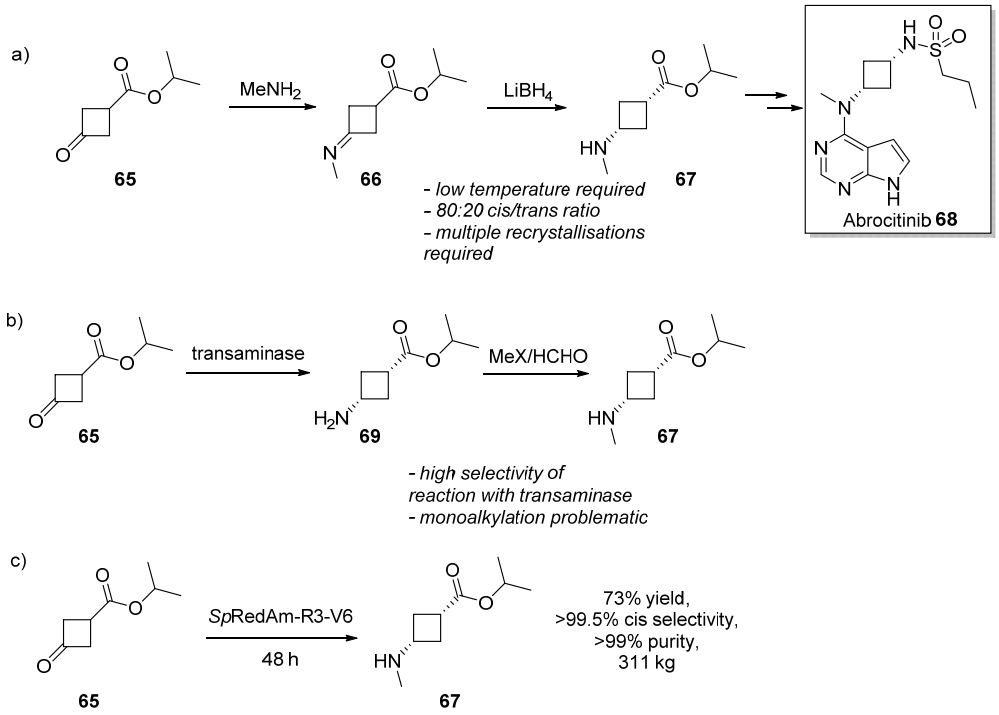
The imine reductase used was a wild-type enzyme that was then engineered to a more stable and active variant in three rounds of evolution, giving access to kilogram quantities of GSK2879552 **64**. The key intermediate in the synthesis was afforded in 84% yield, 99.9% purity and >99.7% ee. The reductive amination step also performed amine resolution of the chiral substrate amine. At the time of publication, GSK2879552 was in Phase II clinical trials to treat small cell lung cancer and acute leukaemia [57].

The advantage of using biocatalysis in the preparation of the compound is clear when the enzymatic route is compared to the original chemical process requiring resolution of racemate of amine tranylcypromine sulfate (*rac-trans*-**60**) to the (1*R*, 1*S*)-**62** enantiomer. Following the resolution of **62**, chemical reductive amination at low temperature including several workups and generating boron-containing waste was performed to give **63** (Scheme 15).

After screening a panel of IREDs, IR-**46** gave the best results on a 1 g scale with cell-free lysate, affording (1*R*,2*S*)-**64** with 73% conversion and >99% e.e. after 2 h. However, in order to give a commercially viable process, it was concluded that enzyme evolution had to be performed. The desired improvements included a reduced catalyst loading of biocatalyst, increased substrate concentration and higher isolated yield while maintaining the high enantioselectivity of the reaction. A lower, moderately acidic pH was desired to enhance product stability as well as solubility of both the substrate and product. After three rounds of directed evolution the best variant M3 met the target process criteria and it was used in 20 L scale manufacture of **64**. With the biotransformation run in three batches, 1.4 kg of **64** was afforded in 84.4% isolated yield, >99.9% purity and 99.7% enantiomeric excess with excellent batch-to-batch reproducibility (Scheme 16). To avoid the risk of emulsion formation and maximise product isolation, a product precipitation work-up was implemented. The desired susbtrate loading in the process was lower compared to other industrial processes because of the low solubility of aldehyde **65** and product **65**. However, the developed process was still highly advantageous with respect to the original chemical one.

3.3. Abrocitinib

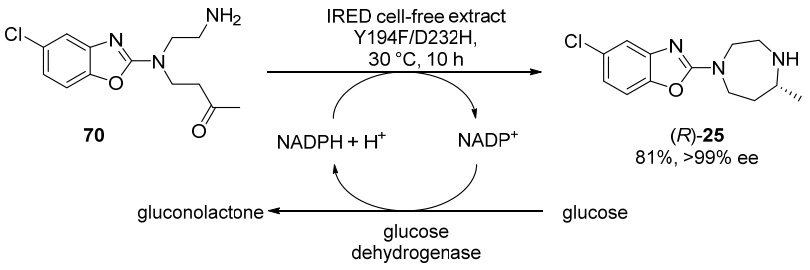
In 2021, Weaver and coworkers published a synthesis of late-stage drug candidate abrocintinib, a Janus kinase 1 (JAK1) inhibitor, using an engineered IRED, *Sp*RedAm-R3-V6 (Scheme 17c) [56]. Some IRED enzymes have been referred to as reductive aminases, or RedAms, to highlight their activity in intermolecular reductive amination where the ratio of amine to ketone or aldehyde is close to 1:1. The Pfizer team describes a >200-fold improvement in performance for their evolved enzyme over the wild-type IRED in the key reductive amination of isopropyl 3-oxocyclobutane-1-carboxylate **65** with methylamine. The process was carried out on metric tonne scale and replaced a chemical reduction with $LiBH_4$ which was not sufficiently diastereoselective (Scheme 17a) and required a low reaction temperature. A transaminase route was also explored (Scheme 17b); however since the product is a secondary amine, it would have required further alkylation after the transaminase step. Kumar et al. sought to avoid this due to the risk of over-alkylation and the genotoxicity of typical alkylating agents.



Scheme 17. Chemical and enzymatic routes to abrocitinib precursor: (**a**) chemical amination and reduction with subsequent recrystallisations to afford a single diastereomer; (**b**) highly selective transaminase-catalysed amination with subsequent alkylation; and (**c**) imine reductase-catalysed reductive amination using methylamine to afford **67** in a single step with excellent diastereoselectivity.

3.4. Suvorexant

Imine reductases were also used to synthesise suvorexant, which was described in Section 2.3. Zhu and coworkers reported syntheses of both (*R*)-**25** and (*S*)-**25** by biocatalytic asymmetric intramolecular reductive amination using enantiocomplementary imine reductases (Scheme 18) [42]. An (*R*)-Selective IRED from *Leishmania major* (IR1) was evolved, and its double mutant Y194F/D232H was identified by saturation mutagenesis and iterative combinatorial mutagenesis. A preparative biotransformation using the Y194F/D232H mutant was performed on 100 mL scale with 100 mM substrate concentration to give (*R*)-25 in 81% isolated yield with excellent > 99% ee.



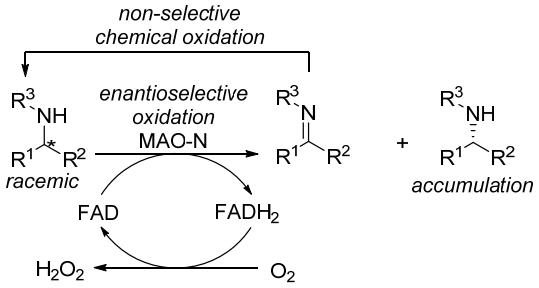
Scheme 18. Synthesis of the suvorexant diazepane core using imine reductases.

4. Monoamine Oxidase

4.1. Overview

Monoamine oxidases are flavin-dependent enzymes that catalyse oxidation of amines to imines using molecular oxygen, which is associated with concomitant FAD cofactor reduction and hydrogen peroxide generation (Scheme 19) [21,23]. MAO-N isolated from *Aspergillus niger* was amenable to protein engineering and gave several synthetically useful

biocatalysts, which were applied in various transformations, including kinetic resolutions [30], deracemisations [58] or oxidative desymmetrisations [59].



Scheme 19. Oxidation of amines to imines by monoamine oxidases and application in deracemisation of chiral amines. Chiral center is indicater by *.

The deracemization of chiral amines involves enzyme-mediated cycles of repeated enantioselective oxidation followed by non-selective chemical reduction using reagents, such as ammonia borane (Scheme 19) [60], and this process can be applied to primary, secondary and tertiary amines. Preparative scale deracemisations of precursors to levocetirizine, solifenacin [59] and telaprevir [61] were demonstrated. The section below describes the application of monoamine oxidases to the preparation of a precursor to boceprevir by Merck.

4.2. Boceprevir

Boceprevir **71** (trade name Victrelis) is a peptidomimetic protease inhibitor used for the treatment of chronic hepatitis C [62]. Its structure consists of three units and a Cap (Figure 1). Unit P1 is a racemic β-aminoamide, P2 is a chiral dimethylcyclopropylproline analogue, and P3 is (*S*)-*tert*-leucine, with the latter being capped with a *tert*-butylcarbamoyl group. The P2 moiety was particularly challenging synthetically, and biocatalysis was successfully employed in the synthesis of P2 to improve the sustainability and performance of the manufacturing process of boceprevir.

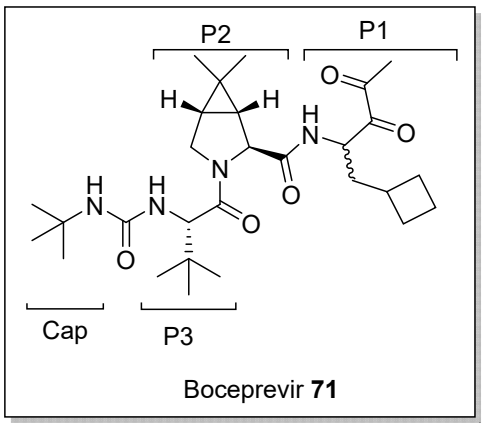
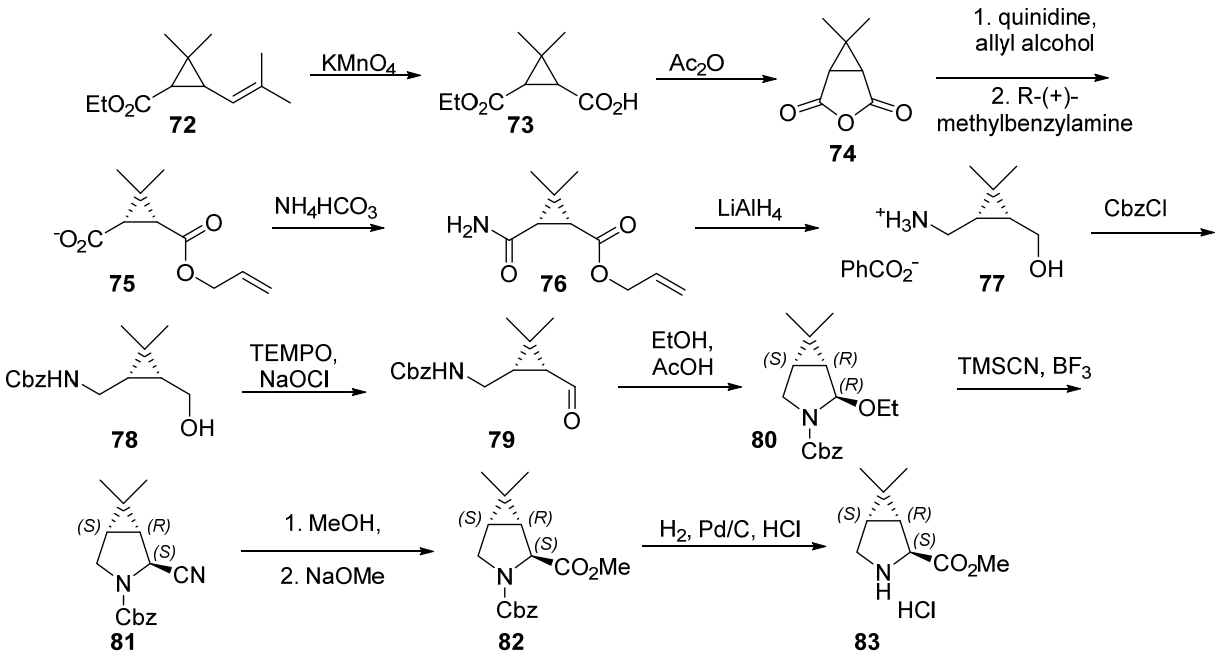


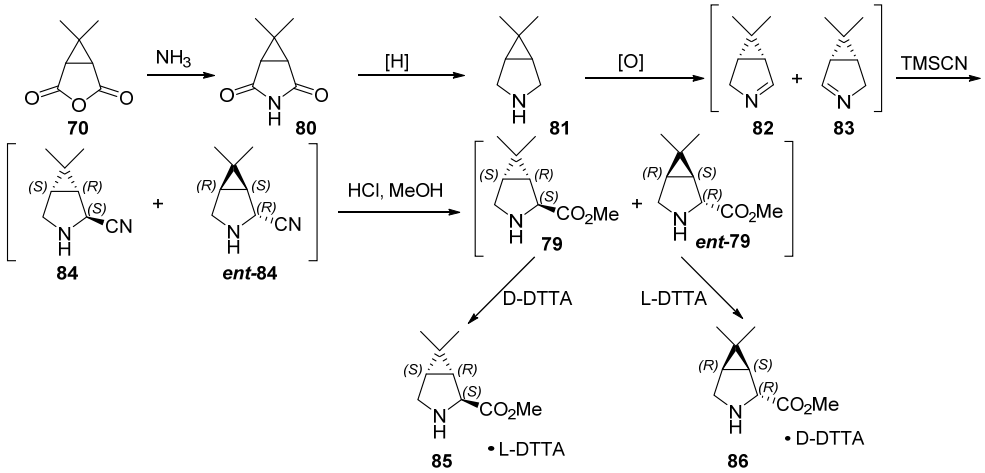
Figure 1. Structure of Boceprevir.

The hydrochloride salt of the dimethylcyclopropylproline methyl ester, being the precursor for the P2 unit, was originally prepared on a large scale using caronic acid **74** as the starting material (Scheme 20) [63]. The latter species can be accessed in two steps. Caronic acid **74** was desymmetrised using (*R*)-(+)-methylbenzylamine to obtain enantiopure species **75** with two stereogenic centres. After four more steps, species **79** containing Cbz-protected amine and aldehyde functionalities was converted to **80**, which was subjected to diastereoselective cyanation to obtain **81**. Nitrile hydrolysis and Cbz-deprotection gave **83**.



Scheme 20. First-generation process route to intermediate P1 in the synthesis of Boceprevir.

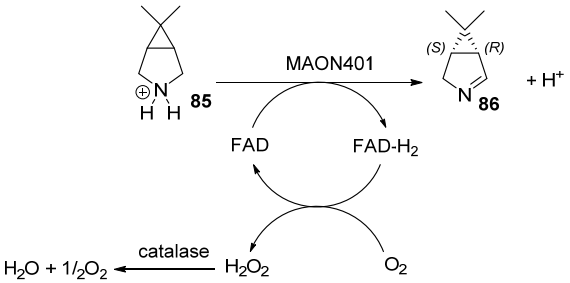
The second-generation route performed the classical resolution of a racemic mixture of aminoester **83** using tartrates at the end of the route (Scheme 21) [64,65]. The classical resolution of the target enantiomer in the last step of the second-generation synthesis meant the automatic loss of 50% of material, and the precursor of dimethylcyclopropylpyrrolidine **85** from the ethyl chrysanthemate **72** precursor (Scheme 20) used excess amounts of oxidants and reductants, thus further increasing the amounts of the material lost in the preparation of **83**. Hence, more practical approaches involving desymmetrisation of dimethylcyclopropylpyrrolidine **85** were explored. The application of asymmetric phase-transfer catalysis for the dehydrohalogenation of the chloroamine congener gave low enantiomeric excess of 20%.



Scheme 21. Second-generation synthesis of boceprevir. D-DTTA-di-*p*-toluoyl-D-tartaric acid; L-DTTA-di-*p*-toluoyl-L-tartaric acid.

Biocatalytic asymmetric oxidation was investigated as another approach [62,66]. FAD-dependent monoamine oxidase from *Aspergillus niger* (MAO-N) uses molecular oxygen to oxidise the amine to the corresponding imine, giving hydrogen peroxide as a side-product (Scheme 22). The mechanism of the biotransformation created challenges from the process

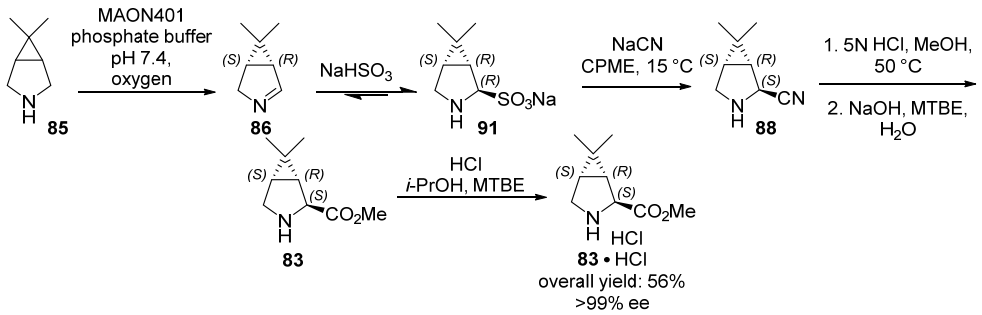
development point of view, the key points being application of molecular oxygen and releasing a proton per turnover. The former aspect precluded using most organic solvents, whereas the latter one necessitated monitoring the pH of the process.



Scheme 22. Desymmetrisation of dimethylcyclopyrrolidine by oxidation with monoamine oxidase MAO-N.

The enzyme optimization, initiated with the *Aspergillus niger* MAO-N enzyme, led to variant MAON401 after four rounds of evolution. The variant met the stability and activity targets and gave an eight-fold increase in activity with respect to the parent enzyme [62].

Due to the low solubility of oxygen in the aqueous medium of biotransformation (approx. 250 µM in pure water), the supply of oxygen was facilitated by passing air through a gas dispersion tube, which maximized mass transfer. Catalase was used to decompose the byproduct hydrogen peroxide, and the pH 7.4 was maintained by sodium hydroxide titration. The biotransformation had limited conversion since the imine product **86** irreversibly inhibited MAO-N. Imine 86 had also low aqueous solubility and was volatile, constituting a potential hazard upon exposure to oxygen. To solve this problem, imine **86** was converted to a water-soluble amino sulfonate **91** upon the addition of sodium bisulfite to the reaction mixture. The formation of amino sulfonate **91** helped to drive the conversion to 100% by keeping the concentration of MAO-N inhibitor imine **86** low throughout the process. The chiral amino sulfonate was telescopically subjected to cyanation, and the resulting *trans*-nitrile 88 was obtained in approx. 90% yield from dimethylcyclopropylpyrrolidine 85. This was followed by conversion of the nitrile to the methyl ester and formation of the hydrochloride salt, giving **83 HCl** a 56% overall yield and >99% ee (Scheme 23).



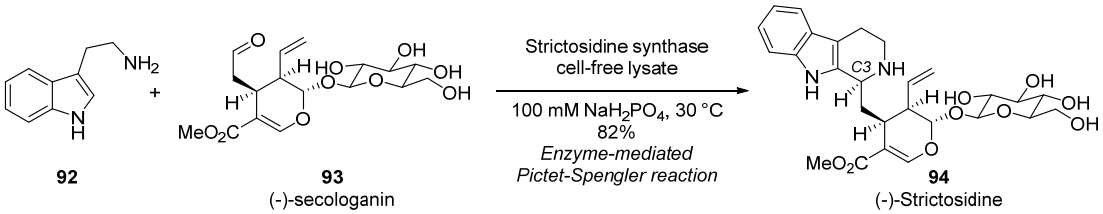
Scheme 23. Synthesis of P2 intermediate in the synthesis of Boceprevir.

5. Pictet-Spenglerases

The Pictet-Spengler reaction plays an important role in the biosynthesis of alkaloids. These reactions are catalysed by Pictet-Spenglerases. The biotransformations employing them offer stereo-and regiocontrol with respect to the classical organic synthetic approaches. Products of reactions mediated by Pictet-Spenglerases are tetrahydroisoquinoline and β-carboline scaffolds, which are of interest to the pharmaceutical industry. Several enzymes of this class have been identified in the literature but only two of them (norcoclaurine synthase and strictosidine synthase) have been used to prepare novel alkaloids. Application of Pictet-

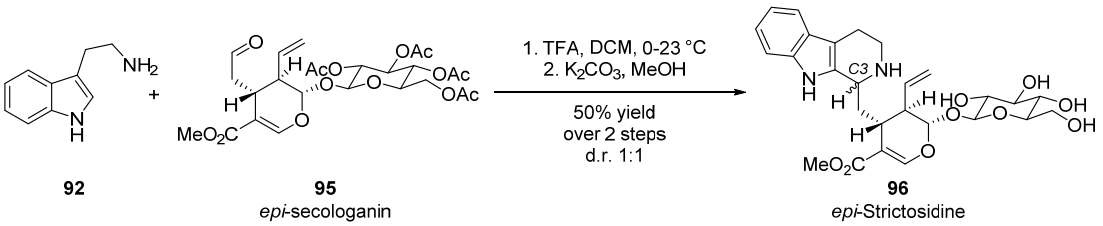
Spenglerases as biocatalysts is limited due to a narrow substrates scope, problems with isolations and enzyme stability [22].

Nonetheless, their use was recently demonstrated in a total synthesis of (−)-strictosidine **94** by Tang, Houk, Garg and co-workers [67], in which the last step of the 10-step synthesis includes an enzymatic Pictet-Spengler reaction. The biocatalyst applied was strictosidine synthase used as a cell free lysate with the intermediate (−)-secologanin **93** and tryptamine **92** on a milligram scale to afford the natural product **94** in 82% yield (Scheme 24). Strictosidine is a natural substrate for this transformation that was previously employed in de novo production of the alkaloid in yeast [68].



Scheme 24. Enzymatic Pictet-Spengler reaction in the total synthesis of (-)-strictosidine.

It is worth emphasizing that the biotransformation furnishes a single diastereomer due to the fully stereoselective formation of the chiral centre at position C3 (Scheme 25). However, the same procedure was unsuccessful when applied to the synthesis of the epimer of strictosidine **96**, which shows the substrate specificity of the enzyme. In this case, the tetrahydro-*beta*-carboline scaffold was prepared using TFA on the acetate-protected *epi*-secologanin **95** and **92** to yield **96** as a 1:1 diastereomeric ratio with respect to position C3.



Scheme 25. TFA-mediated Pictet-Spengler reaction in the total synthesis of epi-strictosidine.

6. Conclusions

The examples shown in this review demonstrate that biocatalysis found use in small molecule pharmaceutical development CMC (Chemistry, Manufacturing and Controls). In several cases biotransformations were not applied in the initial process route, which can be rationalized by high substrate specificity of enzymes and having to evolve them in order to meet the target requirements. With progress in protein engineering, these timelines are getting shorter. It can be envisaged that the impact of biocatalysis on the pharmaceutical industry will increase, particularly taking into consideration the potential to provide greener, more sustainable and less expensive processes. Biocatalysis may be a useful tool in drug discovery, but high enzyme specificity can hinder the SAR exploration, and the potential necessity to evolve enzymes does not match the timelines of many projects [9,25]. However, medicinal chemists should think about biocatalysis particularly to bridge the gap between discovery chemistry and process chemistry, when the breadth of chemical space becomes less important and more emphasis is put on robustness and productiveness [3].

Transaminases are currently the most commonly used enzymes for the synthesis of amine-containing drugs. Their application in the manufacture of sitagliptin helped to develop a greener alternative to transition metal-catalysed hydrogenation when installing the chiral primary amine, and introduced asymmetric bias at a late stage of the synthesis.

Similarly, employing transaminases in the preparation of suvorexant gave an alternative to transition metal-catalysed reductive amination and overcame the limitations of classical enantiomer resolution originally employed in the synthesis. Both in the case of PF-0444913 and vernakalant, transaminases helped to establish the *anti* relationship between the chiral centres by means of dynamic kinetic resolution. For MK-7246, application of transaminases removed the necessity of installing chiral amine moiety at the beginning of the synthesis, which supported the medicinal chemistry program and facilitated SAR exploration around the sulfonamide functionality at a late stage of the synthesis. Despite their common use in industry, application of transaminases faces challenges resulting from unfavourable equilibria and being limited to preparation of primary amines. This means that they are facing competition from emerging enzyme classes, most notably imine reductases. It was demonstrated very well in the case of abrocitinib, in synthesis of which both transaminases and imine reductases were investigated. It was the latter enzyme class that was applied in the multi-kilogram-scale preparation of the drug intermediate by giving directly a secondary amine and removing necessity of an extra alkylation of primary amine step with respect to the transaminase route. The number of applications of imine reductases for large-scale preparation of chiral amines is likely to grow in the next few years. Monoamine oxidases have also found industrial applications, as shown for boceprevir, but their mechanism of action employing oxygen and generating hydrogen dioxide as well as a proton makes their use on a large scale challenging.

It is important to note that many of the final processes described here relied on enzyme evolution to achieve the desired yield and turnover. Enantioselectivity is not typically the limiting factor, but rather the stability of the enzyme preparation under intensive process conditions and its activity towards a particular pharmaceutical intermediate. This protein engineering can be outsourced but is increasingly carried out in-house, typically in a semi-rational way which cuts down the number of mutants to be screened while still relying on automation to meet drug development timelines. Methods such as the Combinatorial Active-Site Saturation Test, Iterative Saturation Mutagenesis, and Focused Rational Iterative Site-specific Mutagenesis combine the Nobel Prize-recognised directed evolution approach with modern computational tools [69]. Fortunately, biotransformations generally lend themselves to high-throughput screening due to the aqueous reaction media and non-toxic nature of the catalysts themselves.

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