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Reviews

Preparation of pharmaceutical important fluorinated 1-arylethanols using isolated enzymes

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ABSTRACT

Fluorinated 1-arylethanols are important building blocks in medicinal chemistry especially for preparing kinase inhibitors for cancer therapy, NK1 receptor antagonists and drugs used in treatment of osteoporosis. Asymmetric reduction of carbonyl groups using enzymes is one of the key technologies to obtain such molecules in enantiomerically pure form. By using isolated enzymes coupled with cofactor recycling, highly concentrated, robust and economical processes can be developed. The aim of this review is to give an overview of biocatalytic carbonyl reduction with special focus on processing of fluoro containing 1-arylethanones with enzymes in cell free systems. The methodologies applied to improve the reactions are highlighted, alongside potential application of the building blocks in bioactive compounds. Enzymatic ketone reduction is concluded to be most beneficial as compared to chemo catalytic methods in transformations of highly fluorinated and therefore electron deficient ketones. A high enantiomeric excess can be achieved, and by changing the catalyst, both enantiomers are accessible. High rates are often seen for such electron deficient ketones, and the reactions have a favourable equilibrium position.

Contents

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1. Introduction

A number of efficient catalytic tools are available for the preparation of chiral building blocks. In the case of 1-aryl-2-ethanols, chemo catalytic methods as catalytic addition of gaseous hydrogen [1], oxazaborolidine type catalysts [2–4], and asymmetric transfer hydrogenation [5,6], are very useful protocols. However, the need for establishing enantioselective, cost efficient and environmentally sound chemistry requires the adoption of the best available technology, and in a number of cases the use of carbonyl reducing enzymes is the most viable option. Although enzymes have not evolved to process artificial fluorinated molecules, enzymes catalyse these reactions provided that the substrate is sterically and electronically compatible with the active site structure of the enzyme. Herein, the literature on asymmetric reduction of fluorinated 1-arylethanones has been reviewed, with focus on the use of isolated enzymes.

Enzymes that reduce carbonyl groups belong to the group oxidoreductases, and are classified as alcohol dehydrogenases (ADH), but also referred to as carbonyl reductases (CR) or ketoreductases (KRED). They are dependent on nicotinamides (NADH or NADPH) as cofactors. Some of these enzymes do not catalyse oxidations, whereas others can catalyse both reduction and oxidation. Throughout this text, the enzymes are named as in the original articles or patents.

Biocatalytic reductions can be performed with whole cells in the presence of carbon and nitrogen sources (usually called fermenting conditions), with resting cells where the medium does not contain the necessary nutrition, or with isolated enzymes. Whole cells contain all necessary machinery for completing the catalytic task, and a high activity is dependent on the stability of the cell as well as the stability of the involved enzymes. Whole cells might contain several isoenzymes capable of performing the same transformation, but with different stereochemical outcome [7,8]. These isoenzymes might be expressed in varying levels depending on the growth conditions and growth phase, and they might also have different stability towards the reaction conditions. Consequently, the resulting enantiomeric excess (ee) of the product can vary as a function of reaction time and conversion. Moreover, whole cell catalysis is usually operated under dilute conditions (<2 g/L) and the work-up is often complicated by the separation of a large amount of biomass. Recombinant cell systems are also emerging, where the engineered cells contain both the reductase and the cofactor recycling enzyme [9]. Designed cells have fewer interfering enzymes, allowing for development of more efficient reduction processes.

Reduction using isolated enzymes represents a simpler system than whole cells and is therefore more easily optimised. Conventional type reactors can be used, and further processing is easier and cheaper due to less biomass and by-products. New enzymes have traditionally been discovered from screening of large collections of growing microorganisms. Nowadays, a larger diversity of genes and thereby enzymes can be accessed directly from environmental DNA by genome mining using activity- or homology based screens [10–12]. Following identification, the enzyme properties can then be improved by generating genomic libraries of variants using mutagenesis techniques, gene shuffling, de Novo gene synthesis or a combination of these [13,14]. High-throughput screening of the genetic libraries allows for identification of candidate catalysts, which are further characterised and evaluated by methods appropriate to the synthetic challenge in question. The production of the biocatalyst is then optimised by genetic engineering of a host cell and design of the fermentation process [15]. Besides technological progress in bioscience, improvements in enzymatic reduction of fluorinated 1-arylethanones have been triggered by the need of specific fluorinated building blocks, some of which their end uses are exemplified in Fig. 1 [16–18]. Also, large developmental programs focusing on intermediates for other top selling drugs have broadened the arsenal of available catalysts and methodology and thereby made an impact to this field [19–22].

In contrast to whole cell catalysis, the question of cofactor recycling must be actively addressed (see Section 1.3.2). However, as new more active enzymes is constantly emerging this is no longer a hurdle, as claimed in the past. A potentially cheaper alternative to using purified enzymes is the so call cell free extracts. These are semi purified protein mixtures that might prove efficient if the microbial source do not process isoenzymes with different enantiopreference [23,24].

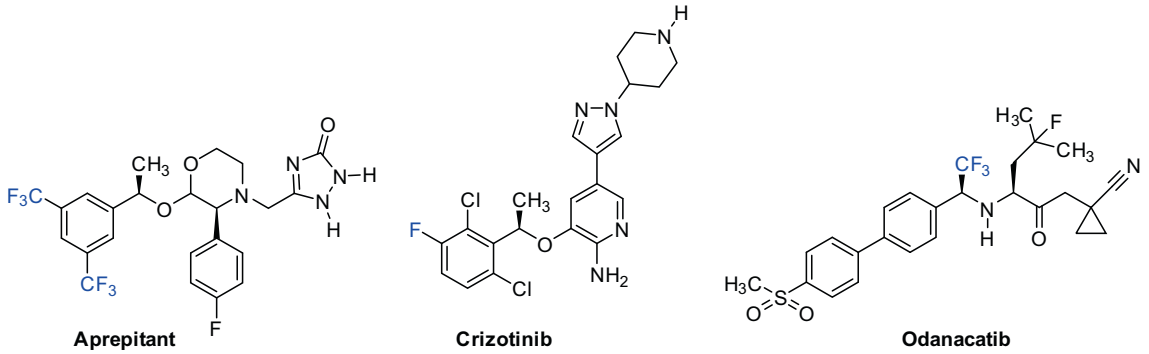


Fig. 1. Bioactive compounds derived from fluorinated 1-arylethanol building blocks. Aprepitant: NK1 antagonist/antiemetic/Merck [16]; Crizotinib: Mesenchymal epithelial transition factor/Anaplastic lymphoma kinase (c-Met/ALK)/cancer/Pfizer [17]; Odanacatib: Cathepsin K inhibitor/osteoporosis/bone metastasis/Merck [18].

The synthetic use of redox enzymes has recently been reviewed [25–29]. In addition to tabulated reduction data, one of these reviews contains representative reduction protocols, guidelines and recommendations for optimisation [25]. The use of these enzymes in an oxidative mode has also been covered [27,29,30]. However, to the best of our knowledge a thorough review covering enzymatic reduction of fluorinated analogues has not been published previously.

1.1. Effects of the fluoro and trifluoromethyl group

Fluoro or trifluoromethyl group insertion in a molecule affects the lipophilicity, electronic, steric and conformational properties of the molecule in question. Fluorinated derivatives can therefore be used as tools to investigate structure–activity relationships in catalysis or pharmaceutical science, or improve the properties of a bioactive compound. As a consequence of the modified properties, fluorinated molecules might require a different catalyst as compared to its non-fluorinated analogue. The effects exerted by a fluoro or a trifluoromethyl group in aromatic systems are conveniently discussed on the basis of free linear energy substituent constants as shown in Table 1.

1.1.1. Size

The interest in the fluoro group as a substitute for hydrogen is due to its high polarising effect, with very little change in steric bulk. Exemplified by Taft's Es [32,33], and the Charton volume [34,35], a fluoro substituent is intermediate between a proton and a chloro substituent. The effect of the trifluoromethyl group is more profound and measured by these substituent constants the size is intermediate between an *iso*-propyl and a *tert*-butyl group, while the van der Waals radius indicates a size similar to an ethyl group. As measured by the interference to rotation in *ortho*-substituted bi-aryls, the size of the CF_3 group is comparable to an *iso*-propyl group [36].

1.1.2. Electronic properties

The fluoro substituent is dualistic in nature since it is electron withdrawing by inductive effect, but also can engage in resonance stabilisation by its lone pairs. The electronic influence in aromatic systems depends on the position of substitution. In *para* position the net effect on a reaction centre is not much different to the hydrogen derivative, while in *meta* position the inductive effect is more evident. The trifluoromethyl group is strongly electron withdrawing by an inductive effect and a consequence is a change of pKa of neighbouring groups [37].

Table 1
Effect of fluoro and fluoro containing groups on lipophilicity, electronic properties and size. (Data taken from Ref. [31].)

R	Lipophilicity	Electronic properties		Size	
	π	σ-*meta*	σ-*para*	Taft's Es	Charton volume
H	0.00	0.00	0.00	0.00	0.00
F	0.14	0.34	0.06	−0.55	0.27
Cl	0.71	0.37	0.23	−0.97	0.55
CH_3	0.56	−0.09	−0.17	−1.24	0.52
CH_2F	ND	0.12	0.11	−1.32	0.62
CHF_2	ND	0.29	0.32	−1.91	0.68
CF_3	0.88	0.43	0.54	−2.4	0.91
i-Pr	1.53	−0.07	−0.15	−1.71	0.76
t-Bu	1.98	−0.10	−0.20	−2.78	1.24

1.1.3. Lipophilicity

Fluoro atom insertion modifies lipophilicity only slightly, but the effect of the trifluoromethyl group is again more profound. Inspection of 292 pairs of compounds with the hydrogen-fluoro exchange, revealed that the average lipophilicity (Log D) increased by 0.25 as reflected by the π-coefficient. However, compounds having a 1,2- or 1,3-fluoro-oxygen substitution pattern were found to have lowered lipophilicity [38].

1.1.4. Aliphatic α-fluoro substituents and catalysis

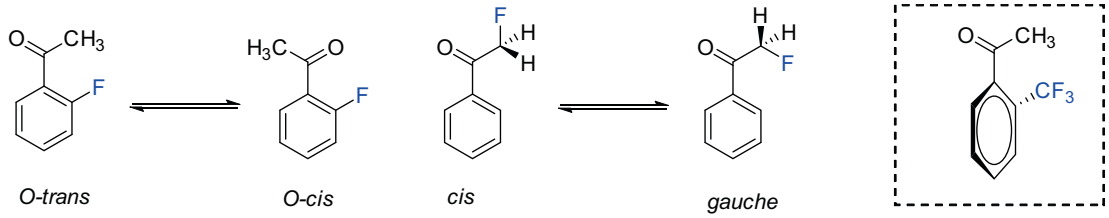
The introduction of aliphatic α-fluoro atoms in ketones could affect reactivity and selectivity by a size effect, and especially so in the case of the trifluoromethyl group. However, electronic effects are expected to be of equal importance. Density functional theory calculations of 1-phenylethanone and 2,2,2-trifluoro-1-phenylethanone orbitals have indicated that the energy of the HOMO and LUMO orbitals are lowered in the latter case [39,40]. The change in electron distribution has consequences for the reactivity. In non-catalysed reactions and in transformations having a late transition state, hydride delivery to electron deficient ketones such as 2,2,2-trifluoro-1-phenylethanone is expected to be more favoured. However, as the electron density at the carbonyl oxygen is lowered, the ability to coordinate to other atoms is reduced [39], which are likely to affect rates in cases where the transition state involves hydrogen bonding or interactions with metals.

1.1.5. Weak binding forces involving fluoro groups

The binding of a fluorinated molecule to the active site of the catalyst or to a bio-macromolecule as a receptor, might also involve a number of more weak forces. The fluoro substituent has been postulated to be involved in hydrogen bonding [41], dipolar interactions [41,42], and van der Waals interaction by filling apolar pockets [43]. Moreover, for aromatic compounds inserted fluoro atoms and especially the trifluoromethyl group could affect the strength of arene-arene interactions [44].

1.1.6. Conformation

Insertion of a fluoro or trifluoromethyl group also alter the conformation of the molecule in question [41], and thereby potentially affecting the rate and selectivity of the transformation, or the binding strength with a drug target. For instance, 1-(2-fluorophenyl)ethanone was found to mainly adopt an *O*-trans conformation [45,46], with an energy barrier which prohibit free rotation [45]. 1-(2-(Trifluoromethyl)-phenyl)ethanone is on the other hand postulated to adopt a conformation where the acetyl group is perpendicular to the plane of the benzene ring [45]. IR,



Scheme 1. Conformation of fluoro containing 1-arylethanones.

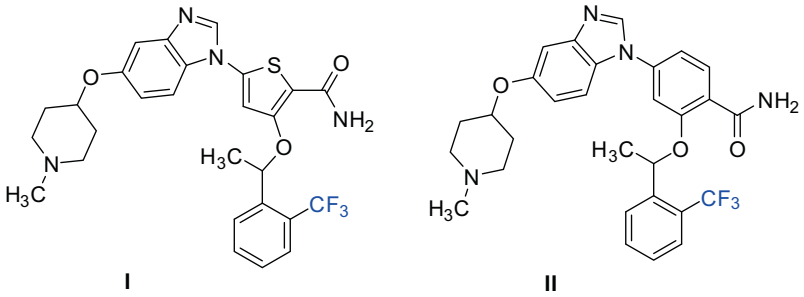


Fig. 2. NEK2 kinase inhibitor leads having a favourable conformation in solution.

NMR and theoretical calculations have shown that 2-fluoro-1-phenylethanone prefers a cis/syn conformation rather than a gauche conformation in polar solvent [47,48], see Scheme 1.

Fluoro induced conformational effects have among others been used in development of Nek2 kinase inhibitors [49]. Research identified compounds **I–II** as potential lead compounds, Fig. 2 [49]. To avoid A-strain between the trifluoromethyl and the methyl group, the molecule in solution adopts a conformation similar to that of the receptor-ligand complex. Thus, lower unfavourable conformation entropy on binding should be expected as compared to analogues without the methyl group.

1.1.7. Metabolism

Organic fluorinated molecules are usually less prone to oxidative and hydrolytic metabolism as compared to their hydrogen analogues [50]. This is normally beneficial in pharmaceutical active compounds and when performing biocatalysis under fermenting conditions. Using isolated ADH's, metabolism cannot take place.

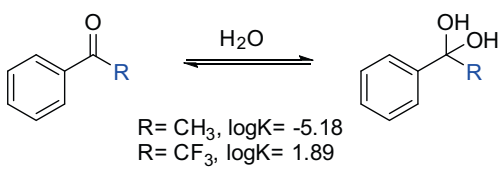
1.1.8. Hydrates

Ketones exist in equilibrium with their hydrate form, Scheme 2. In case of 1-arylethanones, the equilibrium constant increase in magnitude as the number of aliphatic fluoro atoms increase and more electron withdrawing groups are present in the aromatic ring [51–54].

1.1.9. Chemo catalysis

Enantioselective reductions of 1-arylethanones containing fluoro- and trifluoromethyl substituents in the aromatic ring have been extensively documented, some examples can be found in reference [1–6]. Less is published in the case of 1-aryl-2-fluoroethanones, however it seems that the enantioselectivity is comparable to that of the corresponding 1-arylethanones [55–58].

Chemo catalysis appears more challenging for electron deficient ketones, such as 2,2-difluoro- and 2,2,2-trifluoro-1-arylethanones, as mediocre ee of the products alcohols is usually observed [57–63]. A complicating factor is that electron deficient ketones are prone to non-catalysed reactions leading to racemic products [57,62], thus mild conditions are needed. Decent ee values have however been reported using oxazaborolidine [64] and Alpine-borane [65] catalysed reductions.



Scheme 2. Equilibration of ketones and their hydrate form and log*K* [51].

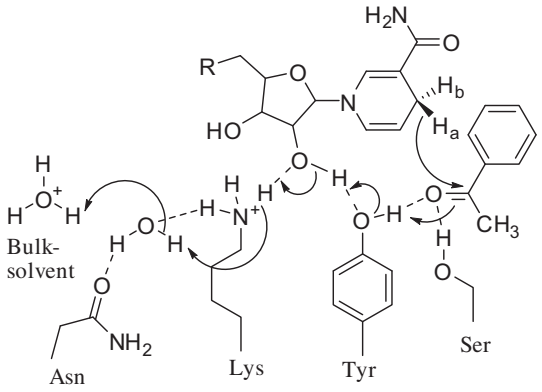


Fig. 3. Simplified substrate binding model of cofactor and substrate ketone to alcohol dehydrogenase. (Based on Refs. [71,72].)

1.2. Mechanistic aspects of carbonyl reducing enzymes

Various types of oxidoreductases are applicable to the reduction of carbonyl compounds [66]. Mainly used in synthetic preparative carbonyl reduction is the short chain zinc independent ADH and the medium and long chain zinc dependent ADH's [67,68].

The active sites in these enzymes are protected from the solvent by hydrophobic residues to enable hydrogen ion transfer. Substrate (ketone) binding occurs in a cavity where the cofactor forms one boundary. The cofactor binding site is rich in glycine to enable flexibility and close contact. The cofactor is bound by several hydrogen bonds to the ribose sugar, by electrostatic interactions with the pyrophosphate tail and the cofactor is further stabilised by the dipole moment of α-helices [69,70].

Metal independent ADH's have a catalytic triad/tetrad with highly conserved Tyr, Lys, Ser and often an Asn residue, Fig. 3 [68,71]. The substrate is coordinated to the enzyme by hydrogen bonding involving Tyr and Ser [71,72]. The pK_a of the Try-OH is modified by a proton relay system consisting of the Lys, a water molecule and other residues, see Fig. 3.

Hydride delivery can take place from the *re*- or *si*-side and either one of the hydrides (marked a and b, Fig. 3) can be transferred. Aided by the proton relay system, the Tyr residue protonates the alkoxide to give the product alcohol. Re-protonation of the Tyr leads to abstraction of one proton from the bulk solvent and an increase in pH. The size of the active site varies, but several of these enzymes have a small binding pocket suitable for positioning of a methyl group, whereas larger groups are positioned outwards the bulk solvent [72,73].

In contrast, zinc-containing ADH's have catalytic active zinc, and sometimes additional metals with structural roles. Horse liver alcohol dehydrogenase (HLADH) is one of the more studied

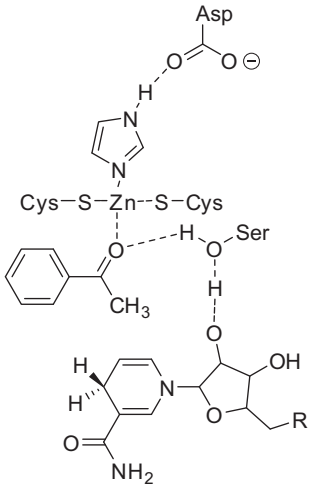


Fig. 4. Simplified substrate binding model for Zn-dependent ADH's. (Based on Ref. [74].)

enzymes of this class [69,70,74–76]. The catalytic zinc atom coordinates to a His, two Cys residues, and a water molecule. The ketone displaces water molecules upon binding, and the substrate is kept in place by the central zinc and a Ser residue, which is also involved in hydrogen bonding to the ribose part of the cofactor (Fig. 4). Also, the structure and function of three zinc-containing ADH's from *Saccharomyces cerevisiae* (Baker's yeast) have been studied in detail [8]. The ADH's from *S. cerevisiae* have similar catalytic mechanism to HLADH, but the structure is tetrameric in contrast to HLADH which is composed of two identical subunits.

1.3. How to perform an asymmetric reduction using isolated enzymes

The ideal catalyst should have a high selectivity, high activity under conditions where cofactor recycling is efficient, and tolerate high substrate concentration. To finally arrive at such a catalyst, it is important to evaluate a number of initial candidates.

1.3.1. Identifying catalyst

A suitable enzyme is first identified by screens for activity and selectivity. Currently keto-reducing enzymes can be sourced from Sigma–Aldrich [77], Syncozymes [78], Johnson Matthey Catalysis (X-Zyme) [79], Libragen [80], Codexis [81], Daicel [82], Evocatal GmbH [83], and Enzymlabs [84]. Possible starting point in terms of catalyst selection might be identified from Sections 2. For those with knowledge in the field, the enzymes can also be identified, modified and produced in-house [29]. The activity of commercial enzyme preparations is reported by the activity unit (U). 1 unit (U) is the amount of enzyme that catalyses the reaction of 1 μmol of substrate per minute under standard conditions. Care should be taken when comparing these units as both conditions and the substrate might be different. Among others, in the case of alcohol dehydrogenases both benzaldehyde and acetaldehyde are common substrates. Another measure of enzyme activity, katal (kat) [85], which is the amount of enzyme that catalyse the transformation of one mole of substance per second (1 kat = 60,000,000 U) is less commonly used. The specific activity of enzymes towards novel and artificial substrates are commonly given as converted substrate (mmol or nmol) per unit time (min) × enzyme amount (mg). For carbonyl reductase reactions conversion is often measured indirectly by spectrophotometric analysis of cofactor consumption.

Screenings are most conveniently performed using an excess of the cofactor, enabling the rapid spectrophotometric detection of activity [86]. Although the activity of the enzyme might depend on pH and the buffer type [87], screens are normally performed in phosphate buffer at pH from 5.5 to 8.5.

Varying (lowering) the reaction temperature is a common way of increasing selectivity in organic chemistry. Most ADH's have the highest activity around 30 °C. More flexibility in terms of temperature tuning is allowed for by using thermo stable enzymes [88]. Generally, the enantiomeric excess decrease up to a certain temperature (racemic temperature) due to a less favourable activation enthalpy, but increase above this temperature, now in favour of the opposite enantiomer due to more favourable activation entropy [89]. As a fine tuning tool the effect of temperature should always be considered. Moreover, an increase in temperature could increase productivity due to higher solubility leading to increase in mass transfer terms and might relive inhibition if this becomes a challenge.

In the early phases it is advised to evaluate the pH, temperature and water stability, and water solubility of the substrate and product. The pH stability points to the most suitable cofactor regenerating system. The solubility and stability of substrates can be increased by the use of organic cosolvents. If the substrate in question is likely to react with protein amino groups or cysteins, protection should be considered. Alkyl bromides and chlorides are often toxic to ADH-enzymes, and there is a rich literature on their effect in whole cell systems [90–94]. However, a new carbonyl reductase from *Kluyversomyces thermotolerans* discovered by genome data mining has been shown to tolerate the challenging substrate 2-chloro-1-phenylethanone in high concentrations [95]. Generally, kinetic studies and time studies of conversion is helpful in identifying enzyme stability issues and inhibition phenomenon's [25].

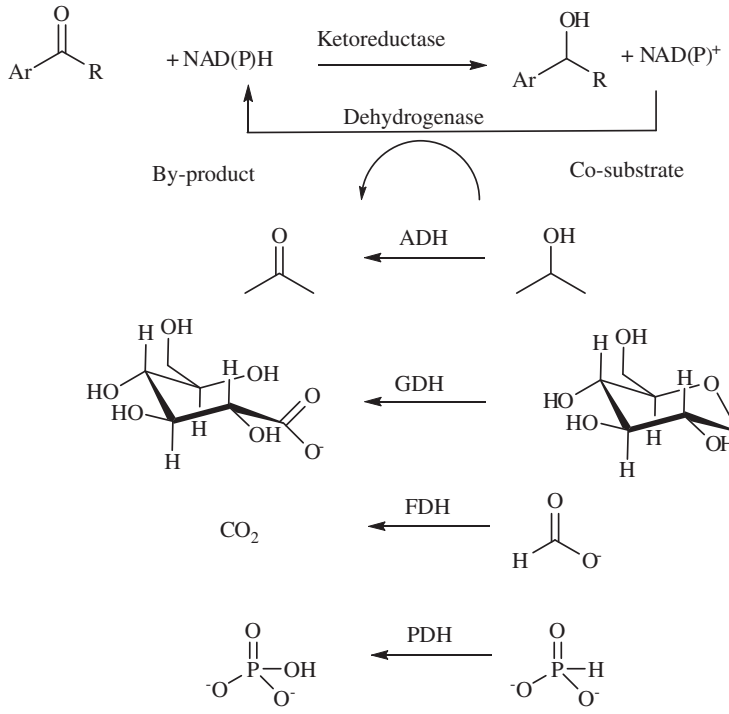
The purity of the enzyme preparation with respect to isoenzymes will affect the observed enantioselectivity, and the activity could also be lowered if the protein impurities compete for, or degrade the cofactor. The purity of the enzyme can be evaluated for instance by SDS–PAGE analysis. Also, the amount of trace impurities in the substrate could be a variable, and especially metal impurities from previous processing steps might interfere with the biocatalysts.

1.3.2. Selection of cofactor recycling system

Due to the price of the cofactors, and their limited stability [96], an economical enzymatic reduction process is highly dependent on their *in situ* regeneration. The enantioselectivity and rate might also depend on the type of cofactor used. Available technologies have recently been reviewed [97,98], and the most useful systems are shown in Scheme 3.

When the main enzyme is able to reduce the oxidised cofactor, regeneration can be solved by adding an alcohol, typically 2-propanol, which acts as a hydride donor. This is called the coupled substrate approach. The methodology is useful for those ADH's that tolerate high concentrations of the chosen alcohol and the corresponding carbonyl compound, and for reactions having a large equilibrium constant. In case when the catalyst is denaturated by 2-propanol, more lipophilic alcohols might improve the result. Primary alcohols could also be used as cosubstrates. However, the aldehyde coproducts are more prone to Schiff base formation with amino groups than ketones [99,100]. This reaction is accompanied by a loss of positive charge in the enzyme (Fig. 5), which might alter protein conformation and the activity of enzymes. Many ADH's have a catalytically important Lys-residue which might be involved in such reactions [68,71].

The equilibrium position for the reductions usually has to be displaced to the product side using an excess of the coupled-substrate. Fig. 6 shows how conversion is affected by the equilibrium constant and the molar excess of the co-substrate. In



Scheme 3. Commonly used cofactor regeneration systems.

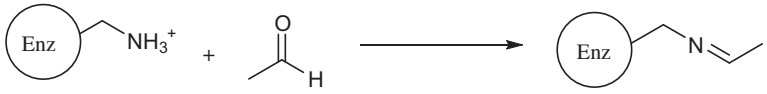


Fig. 5. Schiff base formation between acetaldehyde and lysine function in a catalyst.

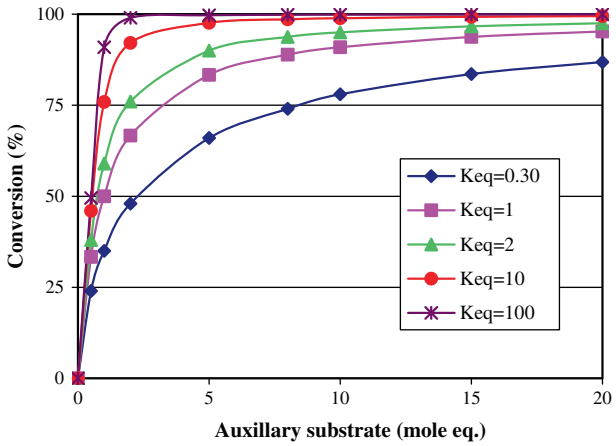


Fig. 6. Degree of conversion (%) as a function of excess auxiliary alcohol for different equilibrium constants.

reduction of 1-phenylethanone using 2-propanol as auxiliary substrate the equilibrium constant is close to 0.3 [101].

As seen from Fig. 6, when the equilibrium constant is low an excess of cosubstrate is needed. Thus, the enzyme must tolerate a high concentration of alcohol and the carbonyl by-product (aldehyde or ketone). As more electron withdrawing groups are included in the aromatic ring, a more favourable thermodynamic situation occurs. For the reduction of α-chlorinated and fluorinated ketones, Nature is conveniently helping the chemist. The thermodynamic stability of the halohydrins compared to the starting ketone makes the reaction quasi irreversible, allowing for the use of only a slight excess of cosubstrate [101]. The same effect can also be utilised in other systems with similar thermodynamic profiles [101–103].

If the enzyme in question remains active in the presence of substrates, but regeneration of cofactor is rate limiting, a better strategy is to use a second enzyme; the coupled enzyme approach. In this way the ketone substrate and the coupled-substrate do not compete for the same active site. As opposed to the coupled substrate approach, these processes are irreversible or have a high equilibrium constant [27]. Glucose dehydrogenases (GDH's) are frequently employed due to their general high stability and activity. GDH can regenerate both NADH and NADPH by employing glucose as cosubstrate. *D*-Gluconic acid is produced as a by-product. Neutralisation with base is usually performed, which is convenient for measuring conversion.

Formate dehydrogenases (FDH's) utilise formate for cofactor recycling and have the benefit that only CO_2 is formed, simplifying purification. FDH's only accept NADH, and generally show lower activity as compared to the GDH's. The pH is maintained by the addition of acid. Therefore, FDH's have mainly been used in catalysis in cases where the substrates or products are base sensitive.

Phosphite dehydrogenases (PDH's), a less studied class of cofactor regenerating enzymes, accept both NADH and NADPH and are generally more stable than the original FDH's. New variants are being developed [104–106], extending the opportunities in the field of biocatalytic reduction. Other coupled enzyme approaches include glucose 6-phosphate dehydrogenase and hydrogenases [97]. The enzyme class nicotinoprotein alcohol dehydrogenases contain non-dissociable cofactors in the active site [107], but their capabilities have been less explored.

1.3.3. Tuning the reaction in terms of space–time-yield

After identifying an enantioselective enzyme, optimisation of processes is focused on maximising throughput (space–time-yield), while maintaining a high ee of the product. This is nicely exemplified in work from Merck's Research Laboratories [86,108,109].

It is essential to acquire as much knowledge of the enzyme characteristics as possible from literature, or by determining properties such as temperature-activity profile, pH–activity profile, dependence of metal ions [110,111], and tolerance to organic substances. If the process depends on two enzymes the optimal conditions for productivity might be different to that of the individual enzymes.

On scaling, a common challenge is inactivation by inhibition caused by the substrate or product. Substrate or product inhibition are revealed by kinetics studies, and plotting for instance by the Lineweaver–Burk plot. Simple conversion vs. time curves can also identify substrate inhibition by a decrease in initial rate upon increasing substrate concentration, while inspection of the same type of curve at high conversion could reveal issues caused by the product.

Strategies which can be used to overcome competitive reversible substrate/product inhibition include slow feeding protocols, addition of hydrophobic polymers which absorb the components reversibly [112], or by applying a two-phase reaction [110]. By adding a non-polar solvent, the organic phase could acts as a reservoir for the substrate and product. Employing organic reaction medium only is however not possible, due to the polar nature of the cofactor. Water is simply needed for release of the oxidised cofactor from the enzyme. Product inhibition can also be relieved by removing the product by precipitation or evaporation. Gas/solid reactor system, typically fixed bed reactors, can also be a solution [113].

Low rate due to limited solubility of non-polar substrate is another common challenge. If 2-propanol or other alcohols can be used as cosubstrates this might increase rate of reaction due to increased solubility. Alternatively, other more hydrophobic solvents might be used. The activity of enzymes in the presence of organic solvents varies. Generally the highest activity is observed in non-polar solvents due to their lower ability to strip of the essential monolayer of water surrounding the catalyst. Usually, enzymes derived from thermophilic species also tolerate organic solvents, and some enzymes can even tolerate DMSO [88]. Generally, ionic liquids decrease the life time of enzyme preparations [114,115]. However, in specific examples ionic liquids have been shown to increase rate of reaction and increase the life time of the enzymes involved [108]. If the enzyme is denaturated by the reaction medium, alternative solvent systems should be sought or a slow feeding strategy of the biocatalyst might be applied.

The enzyme is a major cost driver in most processes and reuse of catalyst could be simplified by immobilisation to a solid support by physical absorption or covalent linkage [116,117]. As an example ADH from *Lactobacillus brevis* experienced a 60-fold increase in stability by immobilisation on an amino epoxy resign and cross-linking with glutardialdehyde [118]. An interesting concept is the co-immobilisation of both the main and the cofactor recycling enzyme on the same support, thereby reducing mass transfer limitations [119]. However, the cost-benefit of immobilisation should always be evaluated as yield in the immobilisation process and the remaining activity of the enzyme preparation is not always excellent.

Last, but not least the agitation type and speed must be carefully considered. Low agitation leads to low mass transfer, while to vigorous agitation might cause disruption and aggregation of the enzyme [120,121]. Alternative guidelines for how to optimise asymmetric reductions using isolated enzymes can also be found elsewhere [25].

2. Enzyme reduction of fluoro containing 1-arylethanones and potential end use of the chiral building blocks

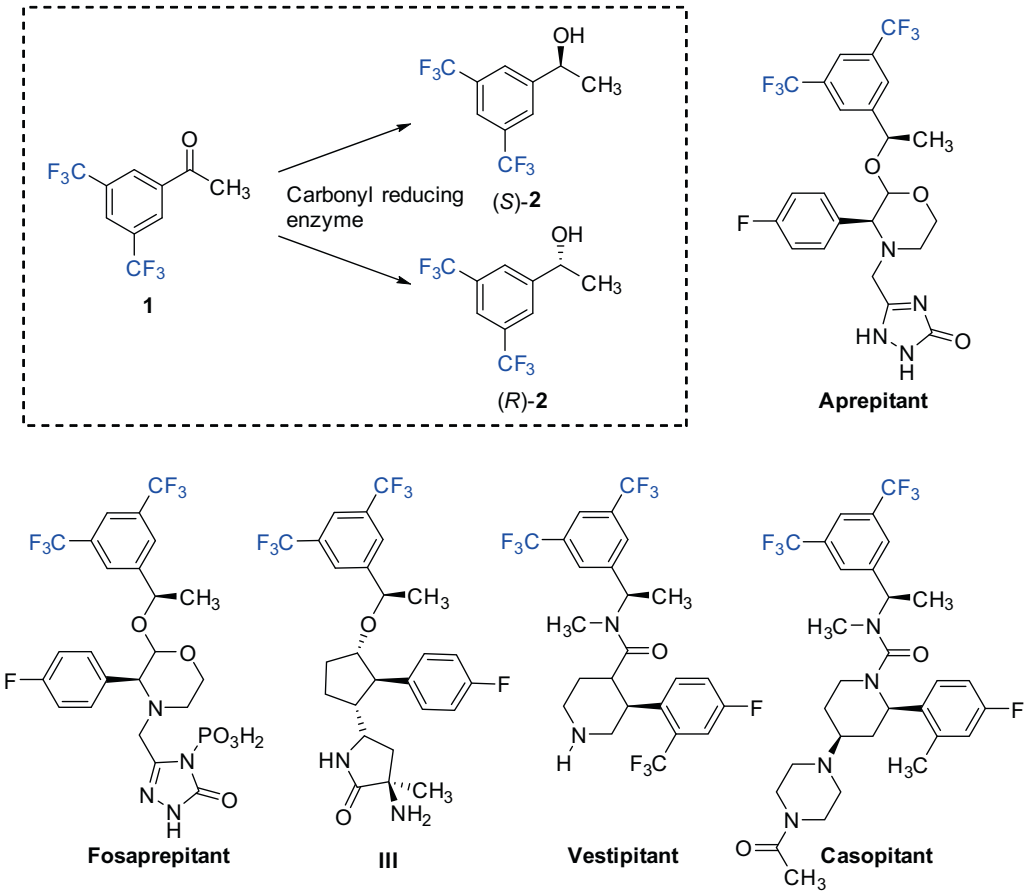
This chapter summarises enzymatic reductions performed on fluoro containing 1-arylethanones and presents a collection of possible end uses of the alcohol building blocks. Some characteristics of the enzymes are described, alongside tools applied to improve rate of reaction and enantioselectivity. First, in Section 2.1 reduction to 1-(3,5-bis(trifluoromethyl)phenyl)ethanol (**2**) is covered due to its importance in neurokinin 1 (NK1) receptor antagonists synthesis. Section 2.2 summarises data on reduction of 1-arylethanones substituted in *para* position with fluoro- and trifluoromethyl groups, Section 2.3 provides the limited data for 1-arylethanones substituted in *ortho or meta* position with fluoro- and trifluoromethyl groups, while Section 2.4 deals with reduction of α-fluorinated ketones.

2.1. Reduction of 1-(3,5-Bis(trifluoromethyl)phenyl)ethanone (1)

One of the more important and investigated chiral fluorinated building block is 1-(3,5-bis(trifluoromethyl)phenyl)ethanol (**2**), due to its use in NK1 receptor antagonists [122–126]. Development by Merck & Co. first led to Aprepitant, used for chemotherapy induced vomiting and nausea [16]. Compound **2** is also used in the pro-drug Fosaprepitant, the follow-up drug **III**, and might be used for similar compounds in the GSK pipeline including Vestipitant [127], and Casopitant [128], Scheme 4. *In vivo* the 3,5-ditrifluoromethylphenyl moiety ensures high lipophilicity and good brain penetration [124,129], while the (*R*)-stereochemistry at the benzylic carbon is required for high-affinity NK1 receptor binding. Both (*R*)- and (*S*)-**2** could be used depending on the route of synthesis. The preparation of enantioenriched **2** has been investigated among others using Corey's CBS method [130], catalytic hydrogenation [131], and asymmetric transfer hydrogenation [132], while the reductions employing isolated enzymes are compiled in Table 2.

At Merck & Co, screening a library of 40 commercial enzymes identified ADH from *Rhodococcus erythropolis* (entry 1) as the most suitable catalyst for obtaining (*S*)-**2** [109]. Employing formate dehydrogenase 101 (from former BioCatalytics, Inc.) for cofactor regeneration, the process was optimised to a space–time-yield of 100–110 g L^{-1} day^{-1}. Later, the availability of the more thermostable glucose dehydrogenase 103 (GDH-103), allowed for reaction at elevated temperatures [109]. After optimisation of reaction conditions with focus on enzyme activity and stability, a space–time-yield of 260 g L^{-1} day^{-1} could be achieved, and the process was further demonstrated to perform well in a 25 kg scale [86]. ADH from *R. erythropolis* is classified as a Zn-containing medium chain ADH. Other studies have shown that the activity was strongly substrate dependent, and especially aromatic OH and amine substituents were found to lower the conversion rate dramatically [133]. In terms of solvent tolerance, the enzyme appears active in aqueous media containing 20% hexane, which is conveniently used to relive inhibition phenomenons. Three other ADH's have been isolated from this species, however they are evidently less stable in the presence of organic solvents [134].

A recombinant CR from the microorganism DSMZ 20028 was efficient giving 99% ee at a high 1100 mM concentration of substrate, (entry 2). A rather unusual alcohol for cofactor recycling was used, possibly to increase solubility of **1** and **2**. The optimal pH



Scheme 4. Asymmetric reduction of 1-(3,5-bis(trifluoromethyl)phenyl)ethanone (**1**) and some NK1 receptor antagonists.

Table 2
Enatioselectivity in reduction of 1-(3,5-bis(trifluoromethyl)phenyl)ethanone (**1**) using isolated enzymes. In case of the KRED enzymes, only the highly enantioselective catalysts are shown.

Entry	Catalyst	Cofactor regeneration/pH	Temp. (°C)	Ee (%)	*R/S*	Ref.
1	ADH-*Rhodococcus erythropolis*	NAD^+/GDH-103/pH = 6.5	45	99	*S*	[86,109,133]
2	CR from DSMZ 20028	NAD^+/4-methyl-2-pentanol/pH = 7.0	25	99	*S*	[135]
3	ADH-*Pyrococcus furiosus*	NADH/ GDH/Glucose/pH = 6.5	37	99	*S*	[88]
4	ADH-*Candida parapsilosis*	NADH[b,c]	30	98[b]/96[c]	*S*	[21]/[109]
5	HLADH[a]	NADH[b,d]	30	99[d]/98[b]	*S*	[136]/[21]
6	ADH *Candida boidinii*	NADPH (1.2 equiv.)/pH = 7.0[c]	30	94[c]	*S*	[109]
7	KRED101	NADPH[c,e]	30/25	99[c]/99[e]	*R/S*	[109]/[137]
8	KRED110	NADPH[c]	30	98	*R*	[109]
9	KRED112	NADPH[c]	30	97	*R*	[109]
10	KRED113	NADPH[c,e]	25	96[c]/99[e]	*R/S*	[109]/[137]
11	KRED114	NADPH/GDH/glucose/pH = 6.75[e]	25	99	*S*[f]	[137]
12	KRED123	NADPH[e]	25	99	*S*[f]	[137]
13	ADH-*Pichia finlandica*	NAD^+/*i*-PrOH/pH = 8.5	25	99	*R*	[138]
14	ADH *Lactobacillus kefir*	NADPH[c,d]	30	99[d]/83[c]	*R*	[136]/[109]
15	ADH *Lactobacillus brevis*	NADPH[c]	30	87	*R*	[109]

[a] Horse live alcohol dehydrogenase.
[b] NADPH (1.1 equiv.), 30 °C, pH: 6.0 [21];
[c] NADPH (1.2 equiv.), pH: 7.0, 30 °C [109];
[d] NADH/*i*-PrOH, pH = 7.8, 30 °C [136];
[e] NADPH, GDH/glucose, pH: 6.75, 25 °C, [137];
[f] The absolute stereochemistry is uncertain.

for reduction was found to be 6.5–7.5, the enzyme was stable up to 60 °C and also tolerated non-polar solvents [135].

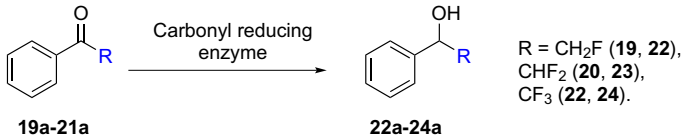
Various alcohol dehydrogenases have been identified from the hyperthermophilic archaeon *Pyrococcus furiosus* [139–141]. One particular isolate [139], was able to catalyse the reduction of **1** to (*S*)-**2** in 99% ee utilising GDH for recycling of NADH, (entry 3, Table 2) [88]. This enzyme was reported to be active from 50–75 °C and in the presence of a range of organic solvents [88]. (See also Table 3, entry 1 for other catalytic examples) ADH from *C. parapsilosis* employing NADH has been used in two studies reacting at pH 6 and 7, giving 96% and 98% ee respectively, (entry 4). Three different carbonyl reducing enzymes have now been isolated and

Table 3
Enantioselectivity in reduction of 1-(4-fluorophenyl)ethanone (**3**) and 1-(4-(trifluoromethyl)-phenyl)ethanone (**4**) using isolated enzymes. Cofactor regeneration was in every case performed using GDH's with glucose as the coupled-substrate. Only those KRED enzymes displaying more than 95% ee is shown.

Entry	Catalyst	Cofactor/pH	Temp (°C)	Ee 5 (4-F)	Ee[a] 6 (4-CF_3)	*R/S*	Ref.
1	ADH-*Pyrococcus furiosus*	NADH/ 6.5	37	99	99	*S*	[88]
2	ADH from *S. cerevisiae* (YMR22c)	$NADP^+$/6.5	25	–[b]	98	*S*	[150]
3	KRED123	NADPH/6.75	25	95	93	*S*	[137]
4	KRED128	NADPH/6.75	25	16	99	*S*	[137]
5	KRED126	NADPH/6.75	25	0	99	*S*	[137]
6	CR *S. salmonicolor* (M242L/Q245P)	NADPH/6.5	25	92	99	*S*	[151]
7	CR *S. salmonicolor* (Q245L)	NADPH/6.5	25	93	–[b]	*S*	[152]
8	KRED107	NADPH/6.75	25	99	99	*R*	[137]
9	CR *Candida magnoliae*	NADPH/6.5	25	99	99	*R*	[153]
10	CR *S. salmonicolor*-WT	NADPH/6.5	25	46	−17	*R*	[151]

[a] A negative sign of ee denotes a change of absolute configuration of the product as opposed to the given stereochemistry.
[b] Not performed.

Table 4
Reduction of the α-fluorinated 1-phenylethanones, **19a**, **20a** and **21a**, using isolated enzymes. The reaction temperature was from 25–30 °C.



19a-21a **22a-24a**

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Entry	Subst.	R	Catalyst	Conditions	Time (h)	Ee (%)	Conv. (%)	R/S	Ref.
1	**19a**	CH_2F	*G. candidum (purified)*	NADPH//G-6-P/[a] pH = 6.0	20	99	91	*S*	[7]
2	**20a**	CHF_2	*G. candidum (purified)*	NADPH//G-6-P/[a] pH = 6.0	20	99	95	*S*	[7]
3	**21a**	CF_3	*ADH-L. brevis*	NADPH/i-PrOH	24	99	87	*S*	[201]
4	**21a**	CF_3	*ADH-L.kefir (crude)*	NADPH/i-PrOH/pH = 7.1	12	99	–[b]	*S*	[200]
5	**21a**	CF_3	*ADH-Thermo-anaerobachter*	NADPH/i-PrOH	24	99	89	*S*	[201]
6	**21a**	CF_3	*ADH- Leifsonia sp. S749*	NAD+/i-PrOH/pH = 7.0	24	99	99	*S*	[202]
7	**21a**	CF_3	*CR-Streptomyces coelicolor*	NAD^+/i-PrOH/pH = 6.5	12	99	97	*S*	[110]
8	**21a**	CF_3	*ADH-Pseudomonas sp. (crude)*	NAD^+/i-PrOH/pH = 7.1	–[b]	92	–[b]	*S*	[203]
9	**21a**	CF_3	*CR-Bacillus sp. ECU0013*	$NADP^+$/GDH/glucose pH = 7.0	12	99	100	*R*	[87]/[204]
10	**21a**	CF_3	*ADH-Rhodococcus 2*	GDH/Glucose	24	98	12	*R*	[201]

[a] Glucose 6-phosphate.
[b] Not reported.

characterised from *C. parapsilosis*. They all belong to the class of short chain alcohol dehydrogenases [142]. HLADH (entry 5) has been tested in reduction of **1** giving high ee of the product. Using an excess of cofactor at pH 6.0 gave full conversion in 24 h [21], while at pH 7.8 the reaction appeared slower [136]. However, this might reflect enzyme loading/activity of the preparations rather than a pH effect. ADH from *Candida boidinii* in "screening mode" gave (*S*)-**2** in 94% ee (entry 6) [109].

Zhu et al. together with BioCatalysis Inc reported the selectivities of a number of recombinant reductase enzymes (KREDs), and two studies have dealt with the reduction of **1** using these enzymes [109,137]. However, different absolute stereochemistry of the product is reported. Presumably the assignment done by Merck & Co is more to be trusted as compound **2** is used for the synthesis of Aprepitant. Anyhow, both enantiomers of **2** can be obtained in a high ee by the proper selection of catalyst. These "KRED" enzymes and many more are now provided by Codexis Inc. The most interesting catalysts for reduction of **1** are shown in Table 2, entries 7–12.

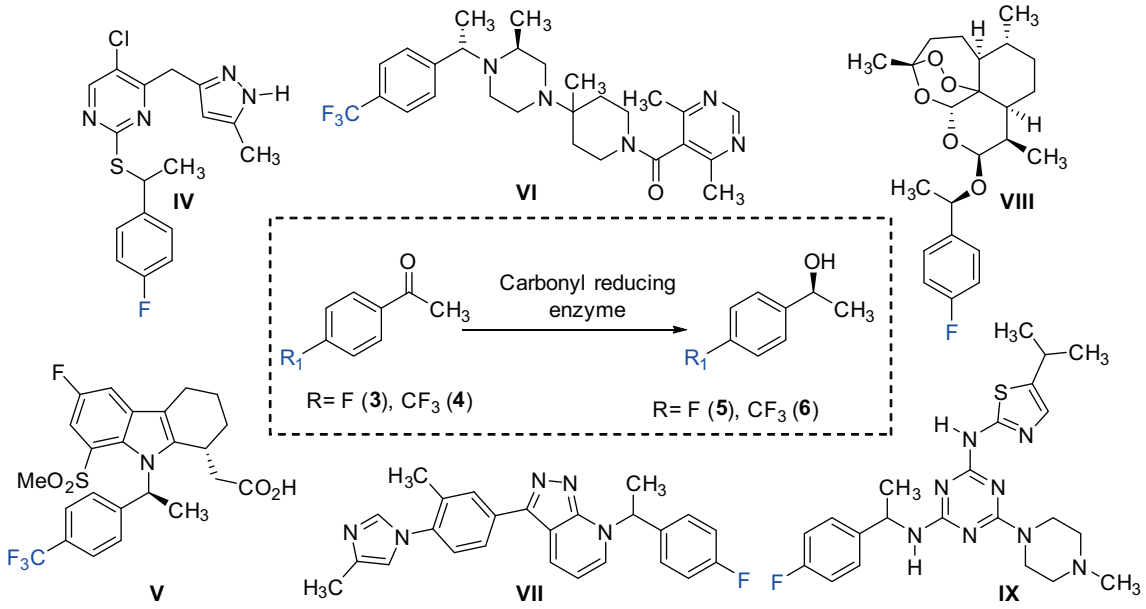
Highly enantioselective processes have also been identified for preparation of the (*R*)-enantiomer. Most impressively, ADH from *Pichia finlandica* (entry 13) at pH 8.5 catalysed the reduction of 1-(3,5-bis(trifluoromethyl)phenyl)ethanone (**1**) to (*R*)-**2** in 99% ee at a substrate concentration of 926 mM. NAD^+ was used as cofactor and recycling was achieved using an excess of 2-propanol [138]. Other catalysts converting the ketone **1** to (*R*)-**2** includes ADH from *L. kefir* and *L. brevis*. ADH-*L. kefir* has been used in two studies, giving different ee-values at slightly different conditions (entry 14). A further challenge was the low rate [136]. The use of ADH from *L. brevis* only resulted in mediocre ee (entry 15). Improvements in the catalytic properties of *L. brevis* have recently been achieved by point mutation [143].

2.2. Reduction of 1-(4-fluorophenyl)ethanone (3) and 1-(4-(trifluoromethyl)phenyl)ethanone (4)

1-(4-Fluorophenyl)ethanone (**3**) and 1-(4-(trifluoromethyl) phenyl)ethanone (**4**) have been incorporated in many chemo catalytic and enzymatic studies due to their difference in size and electronic properties, thereby shedding light on the mechanism of enantioselection in the investigated system. Some potential bioactive compounds where 1-(4-fluorophenyl)ethanol (**5**) and 1-(4-(trifluoromethyl)phenyl)ethanol (**6**) could be utilised as building blocks are shown in Scheme 5.

The most enantioselective enzymatic catalytic systems discovered are compiled in Table 3. All reactions tabulated used GDH/glucose in recycling of the cofactor.

Some enzymes have a broad substrate scope and ADH from *P. furiosus* has been used to prepare (*S*)-**5** and (*S*)-**6** in 99% ee by applying NADH as cofactor, (entry 1) [88]. *S. cerevisiae* is a rich



Scheme 5. Possible use of the alcohols **5** and **6** in bioactive molecules. **IV**: JAK kinase inhibitor/ Cancer/AstraZeneca [144]; **V**: Prostanoid receptors DP1 antagonist/allergic/ Merck [145]; **VI**: CCR5 antagonists/ human immunodeficiency virus/Schering [146]; **VII**: Gamma secretase modulators/Alzheimer's disease/Schering Corp. [147]; **VIII**: Anti-malaria/interfering with heme-function [148]; **IX**: Kinase inhibitor/undesired cellular proliferation/Abraxis Bioscience [149].

source of carbonyl reducing enzymes. A short chain ADH from *S. cerevisiae* (YMR22c, entry 2) reduced **4** in 98% ee [150]. The most interesting KRED catalysts (Codexis) for obtaining **5** and **6** are shown in entries 3–5 and 8 [137]. KRED126 and KRED128 were efficient for preparing (*S*)-**6**, while (*S*)-**5** was obtained in 95% ee using KRED123.

The stereospecificity of a short chain carbonyl reductase from *Sporobolomyces salmonicolor* has also been investigated [151,152]. Use of the wild type enzyme gave a low ee of the product (Entry 10). However, by point mutation guided by docking, the investigators were able to increase the enantioselectivity considerably and also invert the stereochemical outcome of the process. The most efficient of these designer enzymes in terms of ee of the product are shown in entries 6–7.

There are fewer examples of enzymes showing anti-Prelog selectivity. However, KRED107 (Entry 8) tested in reduction of **3** and **4** gave an impressive 99% ee for both alcohols. More examples on the performance of the KRED enzymes can be found in the original literature [137]. Catalysis with CR from *C. magnoliae* (Entry 9) also gave excellent enantioselectivity. Whereas the wild type enzyme only accepts NADPH as cofactor, an elegant study by Morikawa et al. showed that CR from *C. magnoliae* could be engineered for utilisation of the less expensive NADH cofactor [154].

2.3. Reduction of other fluoro and trifluoromethyl substituted 1-arylethanones

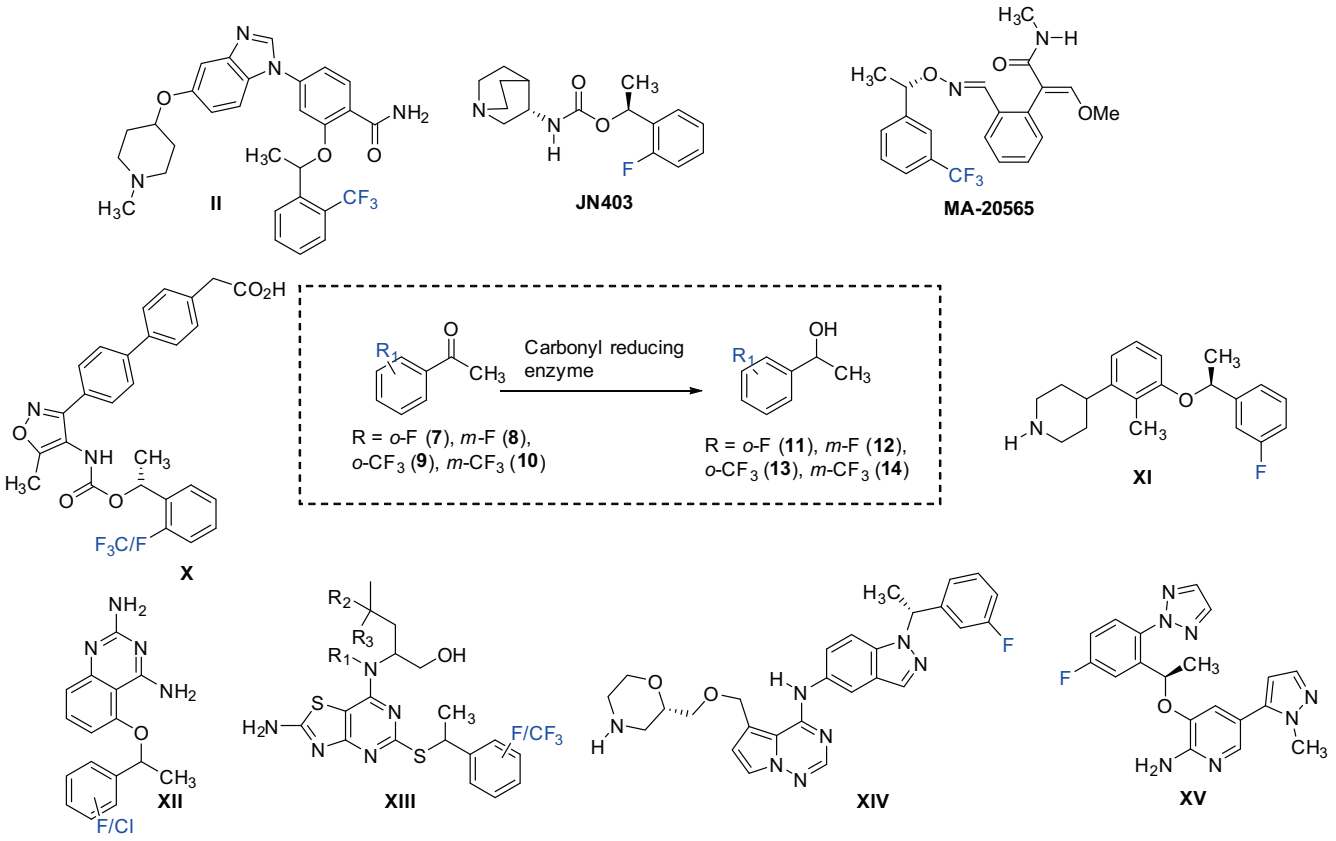
A number of whole cell asymmetric reductions of 1-(2-fluorophenyl)ethanone (**7**), and 1-(3-fluorophenyl)ethanone (**8**) have been reported [9,23,112,155–163]. However, little data is available for isolated enzyme catalysed reduction of **7**, **8** and other derivatives containing aromatic fluoro and trifluoromethyl substituents. *Ortho*-substituted derivatives due to their effect on conformation of the molecule might require alternative enzymes, and this is indicated by some of the whole cell studies [156,157,160,163]. Bioactive compounds requiring such chiral building blocks are shown in Scheme 6. The main use of the building blocks seems to be in the synthesis of kinase inhibitors.

Geotrichum candidum cells and enzyme preparations have been extensively explored by Nakamura and co-workers for the reduction of 1-arylethanones [7,23,112,173–176]. A crude enzyme isolate (acetone powder) containing several isoenzymes [7], catalysed reduction of many fluoro containing 1-arylethanones among others 1-(perfluorophenyl)ethanone, in 97–99% ee and 60–94% yield [23,173]. However, the reduction of the *ortho* trifluoromethyl derivative **9** was troubled by low conversion [23] Reduction of the *ortho*- and *meta*-fluoro ketones **7**–**8** has been performed using a crude ADH from *G. candidum* (NBRC 5767, APG5) in supercritical CO_2 [174]. Enantiopure alcohols (*S*)-**11**–**12** were obtained, again the challenge was getting a high conversion. The use of sodium carbonate to buffer the system was found to be of importance.

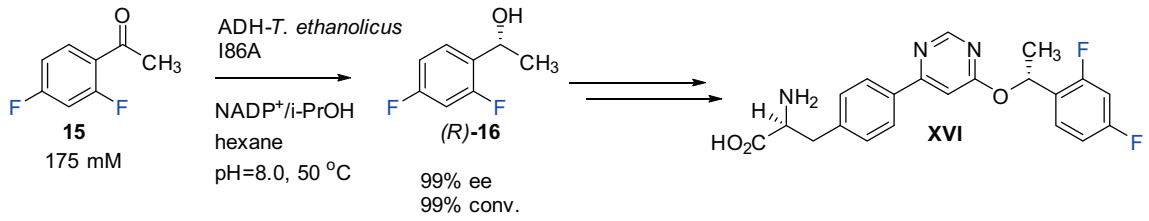
1-(3-(Trifluoromethyl)phenyl)ethanol (**14**) which can be used to construct the antifungal herbicide **MA-20565** (Scheme 6), has been obtained in (*S*)-form in 98% ee using enzymes from *C. parapsilios, Rhodoccocus* or KRED132 [21]. Catalysis with ADH *L. kefir* gave the antipode in 86% ee [21].

1-(2,4-Difluorophenyl)ethanol (**16**) is a building block for kinase inhibitors such as the substituted pyrimidine **XVI** (Scheme 7) [177]. The I86A mutant of ADH-from *Thermoanaerobacter ethanolicus* at 50 °C, pH 8 and a 175 mM concentration of **15** was used to obtain (*R*)-**16** in 99% ee [111]. The enzyme requires NADPH as cofactor, and was found to be zinc dependent. Both the activity and stability of the biocatalyst increased with $ZnCl_2$ and dithiothreitol as additives. To relive substrate inhibition in this transformation, hexane was used as a cosolvent. The high anti-Prelog selectivity is an interesting feature of this enzyme.

In preparation of mesenchymal epithelial transition factor/ anaplastic lymphoma kinase inhibitors (c-Met/ALK) as Crizotinib [17], and compound **XVII** [178], (*S*)-(2,6-dichloro-3-fluorophenyl) ethanol (**18**) is required. Chemo catalysis using Corey's Me-CBS gave a low ee, and enzyme catalysed kinetic resolution, though giving high ee, has the drawback of maximum 50% yield, and additional time consuming steps, see Scheme 8 [17,179]. A screen with 31 isolated enzymes and 97 yeast isolates identified HLADH as a possible catalyst, however the low substrate loading tolerated by the enzyme, did not encourage further process development [179]. Instead, new keto-reducing enzymes were constructed by



Scheme 6. The use of isolated enzymes in reduction of *ortho* and *meta* substituted 1-arylethanones **7**–**10**, and potential bioactive compounds and leads containing the building blocks **11**–**14**. **II**: NEK2 kinase/cancer/Solanki et al. [49]; **MA-20565**: Agricultural antifungal agent/Broussy et al. [21]; **X**: Antagonist of lysophosphatidic receptors/Amira Pharmaceuticals [164]; **JN403**: Nicotinic acetylcholine receptor agonist/Novartis [165,166]; **XI** Serotonin-6 (5-HT6) receptor/Pfizer [167]; **XII**: *SMN2* promoter activator/apinal muscular atrophy/Thurmond et al. [168]; **XIII**: Chemokine CX3CR1 receptor antagonists/AstraZeneca [169,170], **XIV**: EGFR and HER2 kinase inhibitor/Cancer/Bristol-Myers Squibb [171]; **XV**: Anaplastic lymphoma kinase (ALK) inhibitor/various diseases/Pfizer [172].

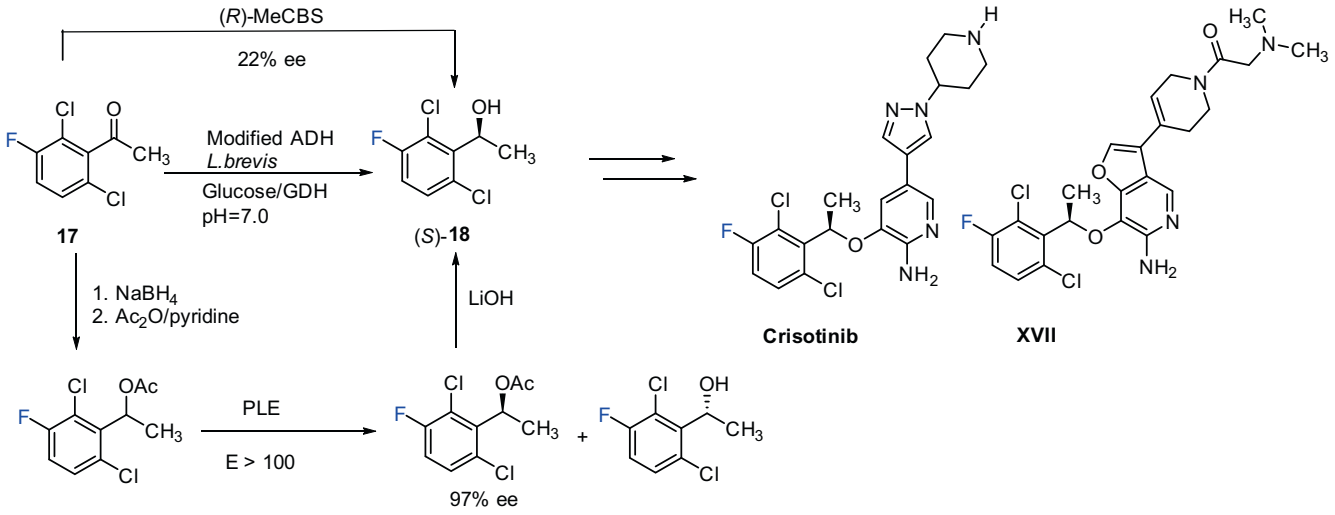


Scheme 7. Reduction of 1-(2,4-difluorophenyl)ethanone (**15**) and structure of the kinase inhibitor **XVI**.

protein engineering [143]. Mutation of the 170 tyrosine residue in ADH *L. brevis* to proline increased the activity and stability of the enzyme, enabling an efficient reduction of **17**. The invention was exemplified with a process using 50 g of ketone in 500 mL buffer employing 0.5 g each of the modified ADH and GDH catalysts [143].

2.4. Reduction of α-fluorinated 1-arylethanones

Bioactive compounds containing 1-arylethanols substituted with fluoro atoms in the α-position to the alcohol function have now emerged in the patent and open literature. Some of the compounds under development are shown in Fig. 7. Especially the trifluoromethyl alcohols have found their niche in synthesis of Cathepsin K inhibitors used in treatment of osteoporosis [18,19,65,180–182]. In these compounds the trifluoromethyl group is a bioisoster for an amide bond. The electron withdrawing properties of the trifluoromethyl group lower the basicity of the amino group. Thus, under physiological conditions the amine nitrogen is not protonated, but instead can act as a hydrogen bond acceptor, which in this case was found to be important for binding to the target protein. Moreover, a favourable conformation is induced by the bulky trifluoromethyl group, alongside the benefit of higher metabolic stability as compared to the amide bond [180]. Development first led to Odanacatib currently in phase III clinical studies. To modify the metabolic profile of Odanacatib, further investigation lead to MK-0674 as a back-up drug [19,65]. In contrast to Odanacatib, MK-0674 is partly excreted as its *O*-glucuronide conjugate [65]. Interestingly, the alcohol function adjacent to the difluoromethyl group underwent oxidation and reduction *in vivo*, thus the stereochemistry at this carbon had little effect on potency. Analogues compounds are now investigated as Cathepsin S inhibitors for treatment of neural diseases [183].



Scheme 8. Methods investigated for preparation of (*S*)-**18** and end use of the building block.

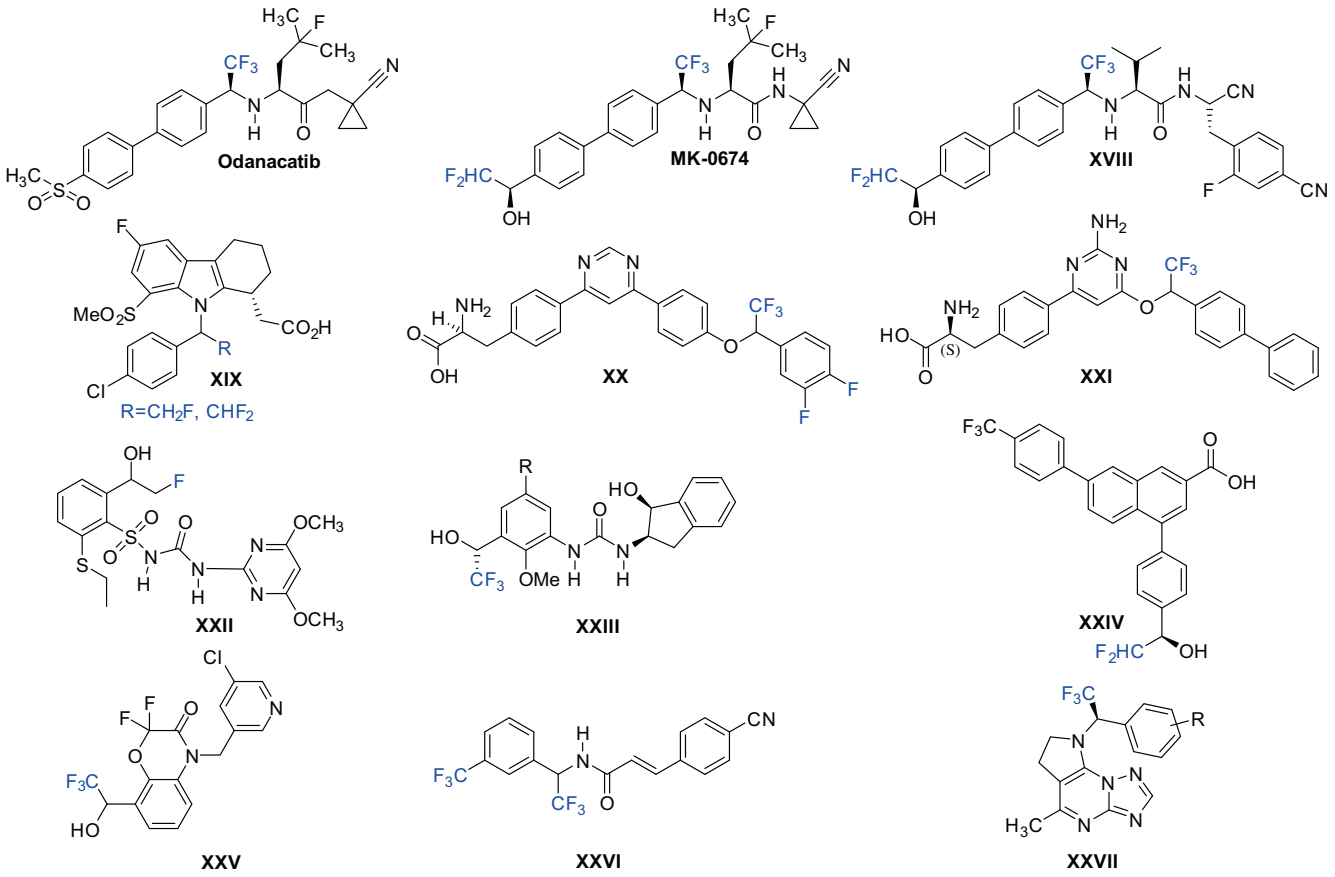


Fig. 7. Bioactive compounds containing the 1-aryl-2-fluoroalcohol fragment. **Odanacatib**: Cathepsin K inhibitor/osteoporosis/Merck [18]; **MK-0674**: Cathepsin K inhibitor/ Osteoporosis/Merck [65]; **XVIII:** Cruzipain inhibitors/Chagas disease/Merck [184]; **XIX**: Prostanoid receptors DP1 antagonists/allergic/Merck [145]; **XX**: Tryptophan hydroxylase inhibitor/cancer/Lexus Pharmaceuticals [185]; **XXI**: Tryptohane hydroxylase inhibitors/ Osteoporosis/Lexus Pharmaceuticals [181,182]; **XXII**: Acetohydroxyacid synthase inhibitor/ Mycobacterium tuberculosis [186]; **XXIII**: p38MAPK/allergic dermatitis/inflammatory bowel disease/Toray Industries Inc. [187,188]; **XXIV:** UDP-competitive antagonist [189]; **XXV:** Glycine transporter inhibitors/neurological disorders/ GlaxoSmithKline [190]; **XXVI**: Insecticide/Bayer Cropscience [191]; **XXVII**: Inhibitor of microglia activation/Alzheimer's/Senexis, BTG International [192].

Chiral building blocks for such bioactive compounds can be derived at by reduction of the corresponding ketones using reductase enzymes. The reported reductions of the α-fluorinated 1-phenylethanones **19a**, **20a** and **21a** with isolated enzymes are summarised in Table 4. As the priority of the groups at the stereocentre change by introduction of fluoro atoms in the

aliphatic side chain, the preferable formation of the (*R*)-enantiomer corresponds with a Prelog selectivity.

2-Fluorinated 1-phenylethanones have been reduced with Baker yeast [90,91,193–198], and other whole cell systems [159,176, 198,199]. Considerably less has been done with cell free biocatalysts.

A purified keto-reducing enzyme from *G. candidum* has been used and allowed for the reduction of 2-fluoro-1-phenylethanone (**19a**) and also 2,2-difluoro-1-phenylethanone (**20a**) in 99% ee, (entries 1–2, Table 4) [7]. The rather expensive glucose-6-phosphate was used as cosubstrate for recycling NADPH [7]. For the difluoro-alcohol **23a** this represents one of a few enantioselective methods both using enzyme and chemo catalysis.

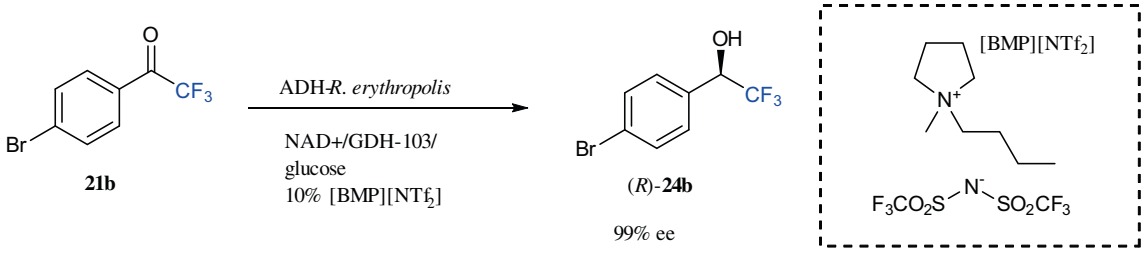
(*S*)-2,2,2-Trifluoro-1-phenylethanol ((*S*)-**24a**) has been obtained using several catalysts. ADH from *L. brevis* (entry 3) gave a high enantioselectivity, although the crystal structure indicated limited space for large substituents such as the trifluoromethyl group [72]. A crude ADH from *L. kefir* (entry 4) [200], ADH from *Thermoanaerobachter* (entry 5) [201], and ADH from *Leifsonia sp*. S749 (Entry 6), can also be used to prepare (*S*)-**24a** [202]. ADH from *Leifsonia sp*. S749 is also highly enantioselective in reduction of many other 1-arylalkanoanes [202], and might also be useful in reduction of **19**–**20**. Another useful enzyme for obtaining *(S)*-**24a** is *CR-Streptomyces coelicolor* (entry 7). The enzyme was identified by "genome mining" and purified by affinity chromatography using a His-tag, and shown to be Mg^{2+} dependent. Several 1-arylethanones could be reduced in high ee, however, a high activity was only observed in reduction of 2,2,2-trifluoro-1-phenylethanone (**21a**) [110]. A crude ADH from *Pseudomonas* gave only mediocre ee (entry 8), however this might be due to the purity of the enzyme preparation.

For preparing the (*R*)-enantiomer, a carbonyl reductase from *Bacillus sp.* ECU0013, could be used giving (*R*)-**24a** in 99 ee (Entry 9) [87,204]. The enzyme was found to have similar properties and 98% homology with *Bacillus cereus* benzil reductase. Only NADPH was accepted as a cofactor, and the highest activity was found at 45–50 °C [204]. Compound **21a** has also been reduced to (*R*)-**24a** in 99% ee using ADH from *Rhodococcus 2* [201], though at a low rate.

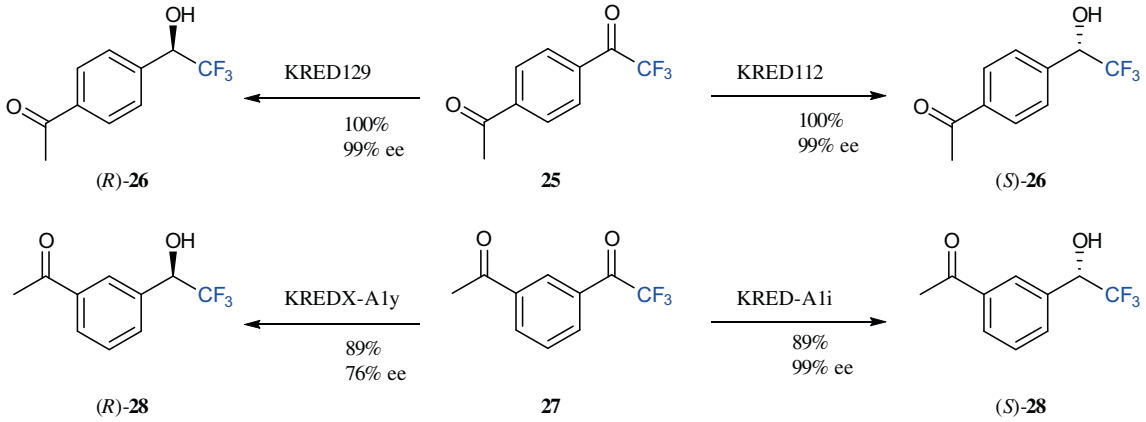
1-(4-Bromophenyl)-2.2.2-trifluoroethanol (**24b**) (Scheme 9) is an important building block for preparation of bioactive compounds such as the 2,2,2-trifluoroethanol biaryls shown in Fig. 4. The bromo substituent is used as the reactive handle in formation of the biaryls by metal catalysed coupling (Suzuki coupling). A screen (66 enzymes) identified ADH from *R. erythropolis* as the best biocatalyst for obtaining (*R*)-**24b** [108]. Faced with low productivity due to substrate inactivation of the catalyst, it was found that employing 10% of the ionic liquid, [BMP][NTf_2], allowed for full conversion of 50 g/L of the ketone **21b** to the corresponding alcohol in 85% isolated yield, see Scheme 9 [108]. A crucial point in the optimisation was to find the optimal trade-off for the stability and activity of the ADH and the GDH enzymes. It should be noted that the corresponding (*S*)-enantiomer can efficiently be produced using baker's yeast whole cell reduction [205].

Chemoselectivity can also be achieved at the same time as a chiral centre is introduced. The recombinant KRED enzymes (Codexis) were able to reduce 1-(4-acetylphenyl)-2,2,2-trifluoroethanone (**25**) with excellent ee, see Scheme 10. Most of the catalysts tested favoured reduction of the trifluoromethyl ketone moiety. The preferred enantiomer can be obtained by selecting the appropriate enzyme. Higher chemoselectivity, ee and yield were observed in reduction of **25** than for the 1,3-substituted derivatives **27** [206].

Table 5 compares the enantioselectivity and relative conversion rates for 1-phenylethanone (**29a**) and 2,2,2-trifluoro-1-phenylethanones (**21a**).



Scheme 9. The use of ionic liquids to relive inhibition in reduction of **21b** [108].



Scheme 10. Enantioselective and chemoselective reduction of 1,4 and 1,3 diacylated benzene derivatives. Condition: 3 eq. NAD(P)H, substrate 2.5 g/L, 5% DMF in phosphate buffer (pH = 7.0), 30 °C. Yield by GC [206].

Table 5
Comparison of ee-values and the relative rates in reduction of 1-phenylethanone (**29a**) and 2,2,2-trifluoro-1-phenylethanone (**21a**).

Entry	Catalyst	Conditions	Ee **21a** (CF_3)	Ee **29a** (CH_3)	Rel. rate **21a/29a**
1	*ADH-L. brevi*	NADPH/i-PrOH	99 (*S*)[a]	99 (*R*)[b]	[c]
2	*ADH-L. kefir (crude)*	NADPH/i-PrOH/ pH = 7.1	99 (*S*)[d]	99 (*R*)[b]	[c]
3	*ADH-Pseudomonas sp. (crude)*	NAD^+/i-PrOH/ pH = 7.1	92 (*S*)[e]	94 (*R*)[e]	1.2[e]
4	*ADH- Leifsonia sp. S749*	NAD^+/i-PrOH/ pH = 7.0	99 (*S*)[f]	99 (*R*)[f]	>16[f]
5	*CR-Streptomyces coelicolor*	NAD^+/i-PrOH/ pH = 6.5	99 (*S*)[g]	96 (*R*)[g]	43[g]
6	*CR-Bacillus sp. ECU0013*	$NADP^+$/GDH/ glucose/pH = 7.0	99 (*R*)[h]	99 (*S*)[h]	15[i]

[a] Ref. [201].
[b] Ref. [207].
[c] Data not given.
[d] Ref. [200].
[e] Ref. [203].
[f] Ref. [202].
[g] Ref. [110].
[h] Refs. [87,204].
[i] Ref. [204].

It is noteworthy that even though the enantioselectivity was similar in most cases, the trifluoroketone **21a** reacts at much higher rate than **29a**. If this is due to the low lying LUMO orbitals allowing for a fast hydride transfer in the case of **21a**, or also caused by the experimental set-up with the equilibrium position affecting the measure activity remains to be investigated. Therefore, in terms of enantioselectivity, enzymes used for reduction of 1-phenylethanone (**29a**) might provide efficient for reduction of 1-phenyl-2,2,2-trifluoroethanone (**21a**). Moreover, catalysts displaying a low activity in reduction of 1-arylethanone such as **29a**, are likely to perform better in the case of trifluoroketones such as **21a**.

3. Conclusion

An increasing number of bioactive compounds are based on fluorinated 1-aryl-alkanols, and the use of isolated keto-reducing enzyme is a viable and competitive strategy for their production in enantiomerically pure form. 1-Arylethanones containing fluoro or trifluoromethyl substituents in the aromatic part or in α-position to the ketone can be reduced in high ee, and by choosing the appropriate catalyst either the (*R*)- or the (*S*)-enantiomer might be obtained. In addition new enzymes allow for chemoselective asymmetric reduction of diketones. While the literature is extensive on the reduction of *para*-substituted derivatives, less information has been gathered for reduction of *ortho*- and *meta* substituted 1-arylethanones and α-fluorinated ketones. Reduction of α-fluorinated ketones proceeds with increased rate and a favourable thermodynamic profile as compared to their non-fluorinated counterparts. In contrast to chemo catalytic asymmetric reduction, these electron deficient ketones do not generally pose a challenge to isolated enzyme reduction, and high enantiomeric excess can be obtained. The major challenge on industrialisation is obtaining a high throughput, a field of research that is mainly addressed by the pharmaceutical companies. Increasing the substrate loadings in processes has been achieved by discovering and introducing new enzymes, altering the structure of established biocatalyst, by solvent engineering and slow addition protocols. Thermostable enzymes allow for higher reaction temperature and they often have higher tolerance towards organic solvents. Thus, higher substrate loadings can be used. Moreover, for these enzymes the investigators can fine tune enantioselectivity by playing on temperature effects. To broaden the use of enzyme catalysed asymmetric reduction even more, efficient cofactor recycling systems are needed. In this respect phosphite dehydrogenase development seems appealing since a buffer salt is produced as a by-product.

In the future, development of new reactor and enzyme immobilisation technology could ease reuse of catalyst and reduce enzyme consumption and cost. A challenge with membrane technology is the large difference in size of the cofactor and the enzyme, while immobilisation of the carbonyl reducing enzyme can lead to mass transfer limitations. A possible solution currently focused is co-immobilisation of both the enzyme and cofactor.

Given the capabilities in modern biotechnology artificial catalyst with improved selectivity, turnover numbers, stability and altered cofactor dependency, can be constructed. However, as enzymes often have to be tailor made for its specific commercial application, a decent scale of production must be anticipated before entering development. Thus, keto-reducing enzymes will in the short term be only one of several tools for preparing enantiopure building blocks in industry. However, once established enzymatic processes can be cost competitive and are especially attractive in the context of sustainable chemistry.

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