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Is literature data useful for identifying enzyme catalysts for new substrates?-A case study on reduction of 1-aryl-2-alkanoates

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Abstract: The use of literature data to identify catalysts for a novel transformation is a commonly used approach. Herein, we have evaluated if this is a viable strategy in enzyme catalysis, using asymmetric reduction of 1-aryl-2-alkanoates as a model system. The study, which includes data from 24 ketone substrates and 108 enzymes, clearly identifies pitfalls with this approach, but anyhow shows that literature data is highly useful for identification of enantioselective catalysts. By combining data for selectivity and rate useful catalyst for converting different substrates to their corresponding (*R*)- and (*S*)-enantiomers are highlighted.

1. Introduction

In the search for an efficient enzyme catalyst for a specific transformation, screening of commercially or in-house libraries is one option [1, 2]. Alternatively, new enzymes can be evolved by genome mining or guided by bioinformatics, which might increase enantioselectivity and activity [3, 4]. Modelling of asymmetric catalytic processes can also be useful [5], however given the low energy differences between the transition states of such processes, this is inherently difficult. Therefore, molecular docking have mostly been used as guidelines in mutagenesis experiments [6, 7] and for explaining the observed experimental results [8-10]. For a chemist without advanced bioengineering capabilities, however, commercial or easily available catalysts is the only viable option.

Enzyme catalysed asymmetric reduction of ketones is among the preferred strategies for production of enantiomerically enriched secondary alcohols [11-13]. These enzymes belong to the group of oxidoreductases, and are named as alcohol dehydrogenases (ADH), but also referred to as carbonyl reductases (CR) or ketoreductases (KRED) and are dependent on nicotinamides (NADH or NADPH) as cofactors [14]. Although some reductase enzymes are highly specific, others display activity and enantioselectivity towards a range of different substrates [15-17]. Herein, we have evaluated the usefulness of literature data in search for catalyst enzyme catalysis using asymmetric transformation of 1-aryl-2-alkanoates as a model.

2. Materials and methods

2.1 Data for enzymatic reductions

All data are shown in the Supplementary Material File. The enzymes used were carbonyl reductase from *Candida magnolia* [17]; carbonyl reductases from Codexis (KRED) [18, 19]; alcohol dehydrogenase from *Pyrococcus furiosus* (PFADH) [20]; wild type and mutants carbonyl reductases from *Sporobolomyces salmonicolor* [16, 21, 22]; carbonyl reductase from *Kluyveromyces termotolerans* [23]*;* alcohol dehydrogenase from *Saccharomyces cerevisiae* (YMRC226c) [15]; phenylacetaldehyde oxidoreductase from *Corynebacterium strain*, ST-10 [24]; alcohol dehydrogenase from *Leifsonia sp.* [25]; carbonyl reductase from *Streptomyces coelicolor* [26]; carbonyl reductase from *Pichia guilliermondii* NRRL Y-324 (PgCR) [27]; carbonyl reductases from *Candida glabrata* [28, 29]; wild type and mutant carbonyl reductases from *Candida parapsilosis* [30]; additional carbonyl reductases from *Candida parapsilosis* (CPAR1-CPAR8) [31]; (*S*)-1-phenylethanol dehydrogenase from *Aromatoleum aromaticum* [32]; medium chain alcohol dehydrogenase from *Kuraishia capsulate* (CBS1993) [33]; carbonyl reductase from Pichia pastoris, GS115 [34]; short chain alcohol dehydrogenases from *Burholderia gladioli* [35]; ketoreductase from *Scheffersomyces stipitis* CBS

6045 [36]; short chain alcohol dehydrogenases from *Chryseobacterium sp* CA49 (ChKRED20) [37]; alcohol dehydrogenase from *Lactobacillus brevis* [38, 39]; carbonyl reductase from *Bacillus sp* ECU0013 [40]; alcohol dehydrogenases from *Thermoanaerobacter brockii* (WT and mutants) [3]; (*R*)-specific alcohol dehydrogenase from *Lactobacillus kefir* [41], alcohol dehydrogenases from ADH *Pseudomonas sp*. [42]; alcohol dehydrogenases from *Thermus thermophiles*, isolate ADH1 [43]; alcohol dehydrogenases from *Candida maris* [44]; diketoreductase from *Acinetobacter baylyi* ATC 3305 alcohol dehydrogenases from *Thermus thermophiles*, isolate ADH1 [45]; carbonyl reductase from *Lactobacillus brevis* [46]; recombinant alcohol dehydrogenase, ADH evo-1.1.200, from evocatal GmbH [47]; □-ketoacetyl-APC reductase from *Bacillus sp* ECU0013 (FabG) [48]; carbonyl reductase from *Yarrowia lipolytica* ACA-DC 50109, β-Ketoacetyl-APC reductase from *Bacillus sp* ECU0013 (FabG) [49]; carbonyl reductase from *Yarrowia lipolytica* ACA-DC 50109 [49]; β-ketoacetyl-APC reductase from *Bacillus sp* ECU0013 (FabG) [50]; Xylose reductase from *Candida tennis* (CtXR AKR2B5) [51]; mutant *I86A of alcohol dehydrogenase from Thermoanaerobacter ethanolicus* [52] and (*R*)-specific alcohol dehydrogenase from *Rhodococcus erytropolis* [53, 54].

2.2 Data treatment

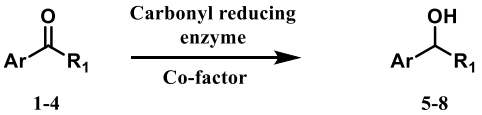
Pearson correlation and regression analysis were performed using Minitab 17 from Minitab Inc. at a confidence level of 95%. Plotting was done using Minitab 17 from Minitab Inc. and Microsoft Excel 2016.

2.3 Supplementary material.

This file contains all raw data on enantiomeric excess and rate/relative rate alongside additional statistical results and plots from this study.

3. Result and Discussion

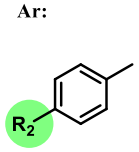
Asymmetric reduction of 1-aryl-2-alkanoates was selected as model system for our investigation due to the simplicity of the substrate structures, having few rotatable bonds, the abundance of available literature and the importance of this compound class as building block in synthesis. Data for 108 different carbonyl reductase enzymes were identified using SciFinder [1, 3, 15-38, 40, 41]. The primary selection criterion was that enantiomeric excess (ee) or conversion data should be provided for several of the pre-selected substrates shown in Scheme 1.



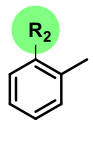
1-4 **5-8**

R_1: CH_3 (**1**, **5**), CH_2Cl (**2**, **6**), CH_2CH_3 (**3**, **7**), CF_3 (**4**, **8**)

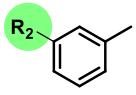
Ar:



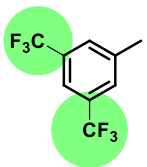
R_2 = H (**a**), F (**b**), Cl (**c**), Br (**d**), Me (**e**), OMe (**f**), CF_3 (**g**), *t*-Bu (**h**)



R_2 = Cl (**i**), Me (**j**), MeO (**k**)



R_2 = Cl (**l**), Me (**m**),



n

Scheme 1. Main compounds included in the study

The data contains information both on wild-type (WT) and mutated purified or semi-purified enzymes, while whole cell reduction protocols were excluded. Reactions are in water, but in a number of cases 2-propanol has been used as a co-substrate.

In the early days of biocatalysis, most catalysts identified displayed what is called Prelog selectivity [55]. In the experimental data included herein acetophenone had Prelog selectivity in 58 (64 %) of the cases, anti-Prelog selectivity was noted for 26 enzyme-substrate pair (29 %), while low reactivity or a racemic mixture was seen for 13 enzymes (14 %). The distribution of data (Figure 1), shows that finding suitable catalysts with opposite enantiopreference is no major concern.

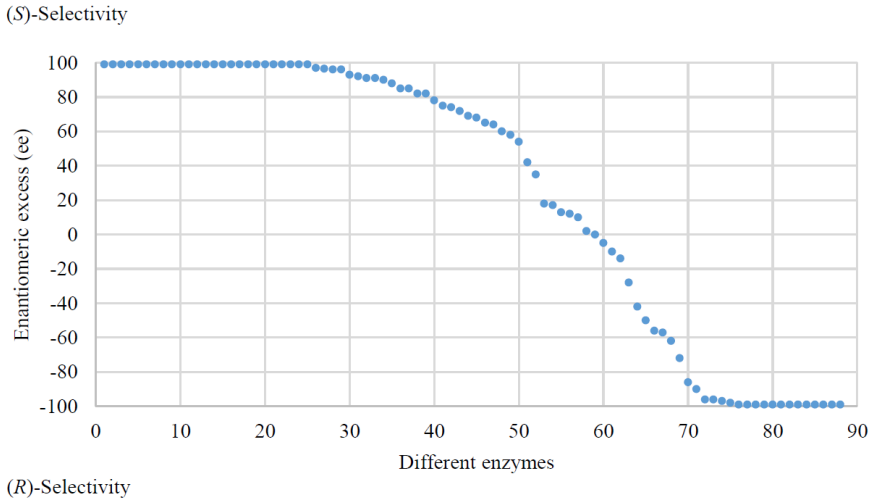


Figure 1. Distribution of enantiomeric excess (ee) in reduction of acetophenone (**1a**) using 97 enzymes. Anti-Prelog selectivity (*R*-stereochemistry) is shown as negative values.

Figure 2 shows how the enantiomeric excess (ee) of reduction processes varies within this series of closely related analogues using different enzyme. Obviously, any analogue cannot be used to identify useful enzymes for enantioselective reduction.

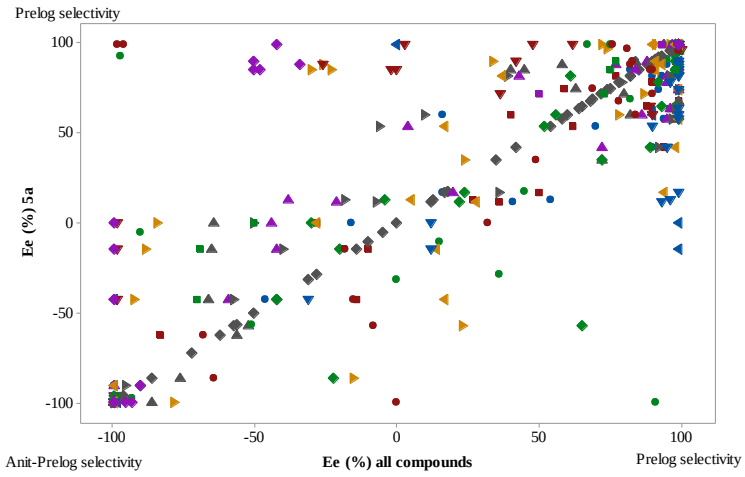


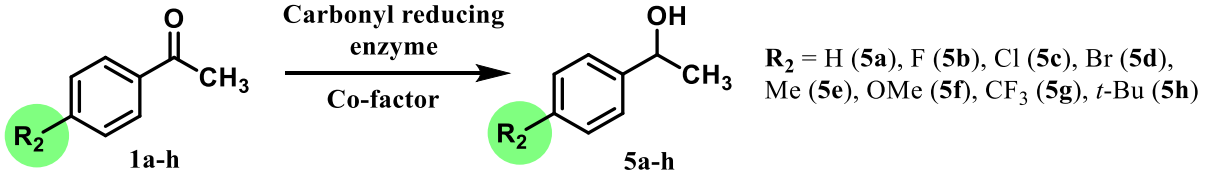
Figure 2. Ee (%) of 1-phenylethanol (**5a**) plotted as a function of the ee of the other products. Anti-Prelog selectivity is shown as negative values.

In an attempt to identify if certain analogues are more useful for predicting enantioselectivity than others, Pearson correlation analysis was performed using ee data for pairs of “model” and “target” compounds. Relative rate of reaction was also compared in cases where this data was provided.

3.1 *para*-Substituted derivatives

The collected data includes information on reduction of eight *para*-substituted acetophenones, **1a-h**, with different enzymes. To identify model compounds for predicting enantioselectivity, Pearson coefficients for various "model-target" pair were calculated, see Table 1.

Table 1. Pearson correlation coefficient for ee data of compounds bearing 8 different substituents. The number of enzymes included in the correlation varies from 46-21, see Supplementary Material.



Comp.	**5a** (H)	**5b** (F)	**5c** (Cl)	**5d** (Br)	**5e** (Me)	**5f** (OMe)	**5g** (CF_3)
5b (F)	0.973						
5c (Cl)	0.979	0.978					
5d (Br)	0.937	0.965	0.992				
5e (Me)	0.964	0.931	0.953	0.978			
5f (OMe)	0.953	0.952	0.966	0.975	0.980		
5g (CF_3)	0.946	0.909	0.951	0.935	0.955	0.946	
5h (*t*-Bu)	0.909	0.926	0.929	0.897	0.829	0.831	0.875

The Pearson coefficients, which should have a value > 0.95 to be useful in this setting, indicate that the chloro-substituted **5c** is most efficient in predicting the selectivity for the series **5a-h**. This suggests compound **5c** as a model for identifying new catalysts with a broad substrate scope. A Scatterplot showing the correlation between the ee of **5c** and the ee of the *para*-bromo derivative **5d** is given in Figure 3.

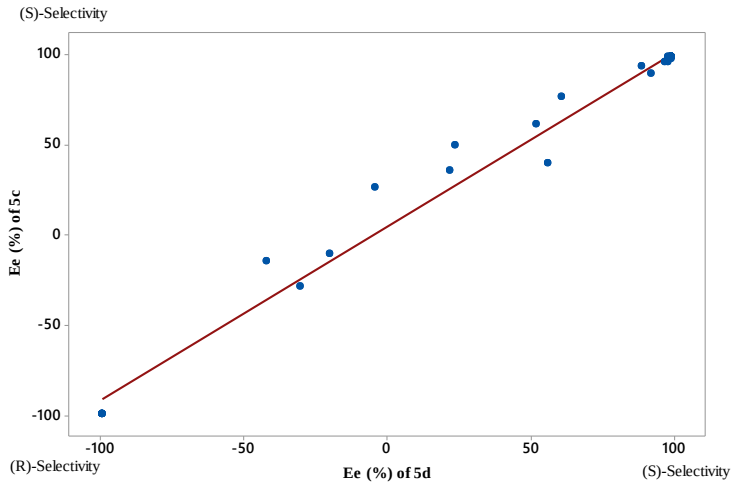


Figure 3. Scatterplot for ee data for compounds **5c** (*p*-Cl) and **5d** (*p*-Br) based on 40 examples. Regression line: Ee **5c** = 4.88+ 0.96×ee-**5d**, R^2 = 98.5, n = 40.

The methyl and methoxy-substituted compounds **5e** and **5f** displayed very similar selectivity profile, which also could be well predicted by the data for the bromo analogue **5d**. Overall, the ee data for compounds **5a-f** are all suited to identify enantioselective enzymes for other derivatives in this series. On the other hand, despite the fact that the trifluoromethyl and the *tert*-butyl group are rather bulky [56, 57], **5g** and **5h** fails to predict the ee-value of the other. Obviously, electronic effects comes into play.

Of equal importance to the selectivity is the rate of reaction. Substituents could affect rate in carbonyl reductions by a) providing additional binding forces or repulsive interaction with respect to the preferred orientations of the substrate and its transition state in conversion to the product; b) affecting the electrophilicity of the carbonyl carbon; or c) modifying the ability for coordination of the carbonyl oxygen to the oxyanion hole, which either contains zinc and serine [58, 59], or tyrosine and serine [60, 61]. Inhibition phenomenon might also be encountered [62, 63]. Given the high level of complexity of protein structures and the catalytic process, it is obvious that universal guidelines concerning substituent effects on rate is not likely to be found. Plapp *et al.* [64] studied reduction of substituted benzaldehydes using an ADH from *S. carlsbergensis* and observed an increased rate when substituents were electron withdrawing. This was also observed for reduction using 3α-hydroxysteroid dehydrogenase [65]. Hua *et al.* [18] investigating 24 KRED enzymes showed that the rate of some enzymes were positively affected by electron withdrawing substituents, while the rate of others were largely unaffected by the nature of the *para*-substituent. Further, the rate of reduction of 27 acetophenones using *Candida tenuis* xylose reductase was found to correlate with the σ-Hammett coefficients and the position of the substituent [51].

To evaluate substituent effects on rate we analysed data where the activity of a specific enzymes was reported for at least six of the derivatives **5a-h** (32 enzymes). The rates were normalised as relative to that of reduction to **5a**. On average, the relative rates followed the trend CF_3 > Cl, Br > F, H, Me > OMe, *t*-Bu, see selected examples in Figure 4. This indicate that increasing electrophilicity of the carbonyl carbon by electron withdrawing substituents is very important for rate. Moreover, it provides further evidence that the *tert*-butyl group is a poor model for the trifluoromethyl group and vice versa. It also indicates that development of efficient processes for electron rich ketones is more challenging than for electron deficient analogues. Besides one outlier in the *p*-chloro series, the relative activity in reduction of **1c**, **1d** and **1g** could be reasonably well predicted by rate data of each other, see Supplementary Material.

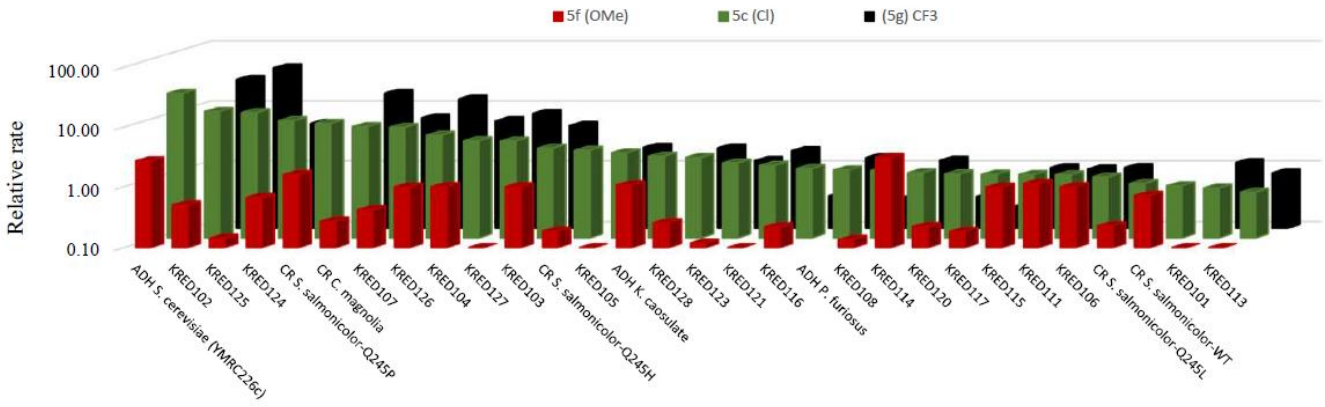
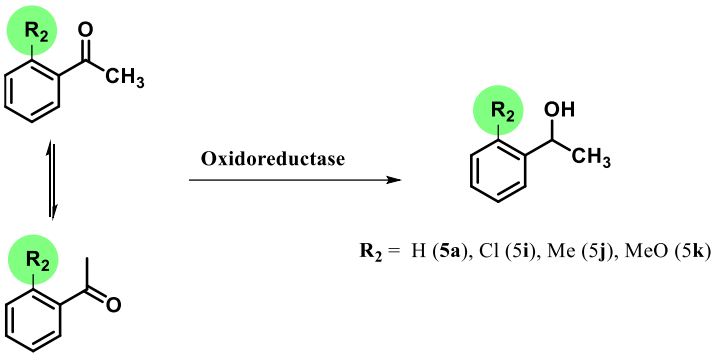


Figure 4. Relative activity of enzymes in reduction to **5c** (*p*-Cl), **5f** (*p*-OMe) and **5g** (*p*-CF_3) plotted in log scale. Activity for reduction to compound **5a** is used as reference (relative rate = 1). The raw data can be found in the Supplementary material. In case of **5g** (*p*-CF_3) there are no data for six of the enzymes.

In conclusion, for the *para* substituted derivatives, high predictability is noted for enantioselectiviy, except when using data for the *tert*-butyl containing analogue. The *tert*-butyl group in this setting is a poor bioisostere for the trifluoromethyl group and vice versa. In rate of reduction both the steric bulk and electronic properties of the substituents is of importance. Generally, electron withdrawing groups increase rate of reaction. Thus, when identifying a literature model reaction, mechanistical aspects also must be taken into consideration.

3.1 *ortho*-Substituted derivatives

In case of *ortho*-substituted derivatives, less experimental data exist, thus the analysis was focused on **5i-5k**, see Scheme 2. These structures might prefer a *cis* or *trans*-conformation [66, 67].



R$_2$ = H (**5a**), Cl (5**i**), Me (5**j**), MeO (5**k**)

Scheme 2. Structure of compounds **5i-5k**.

Pearson correlation analysis of the ee data for the *ortho* derivatives showed a decent fit between **5a** and the *ortho*-methyl derivative **5j** using a quadratic model, Figure 5.

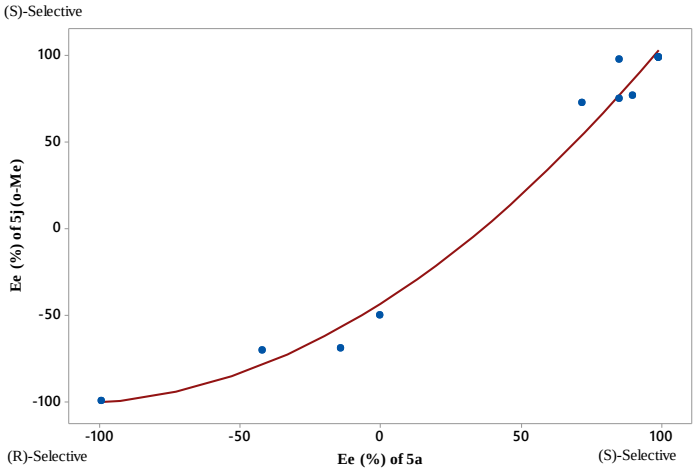


Figure 5. Scatterplot for ee data for compounds **5j** (*o*-Me) and **5a** based on 12 examples. Regression line, quadratic model: Ee **5j** = -43.24+1.03×ee-**5a**+ (0.005×(ee-**5a**)^2), R^2 = 98.3, n = 12.

Compound **5i** (*o*-Cl) mostly displays selectivity similar to that of **5a**, but with a few outliers. When removing the two most extreme cases (reported for two ketoreductases from *Candida glabrata* [28]) a Pearson coefficient of 0.957 (n = 32) was obtained (Supplementary Material). Interestingly, the presence of an *ortho*-methoxy substituent was found to favour anti-Prelog selectivity in a number of cases. It has been deduced that 2-methoxyacetophenone (**1k**) prefers a *trans*-orientation [66], whereas the *cis*-conformation is more favoured in 2-chloroacetophenone (**1i**) [67], which might explain this effect. This clearly show that if literature data should be useful, the conformation of the system has to be evaluated.

Brecker *et al.* [51] using *C. tenuis* xylose reductase observed higher rate in reduction of *ortho* than in *meta* and *para* substituted acetophenones, which were explained by mesomeric and inductive effects causing a polarisation of the carbonyl group. In contrast, in study of three CR from *C. papapsilosis* the effect of *ortho*-substituents on rates varied from enzyme to enzyme. Additionally, for KRED enzymes elevated rates were seen for some *ortho*-methoxy derivatives, which were postulated to be due to hydrogen-bonding ability [18].

The rate of reduction to **5a** and the *ortho*-substituted **5j-k** was compared using data for 41, 30 and 32, enzymes, respectively. The reactivity followed the order **5i** (*o*-Cl) > **5k** (*o*-OMe) > **5a** > **5j** (*o*-Me). Figure 6 compares the reactivity using the KRED enzymes.

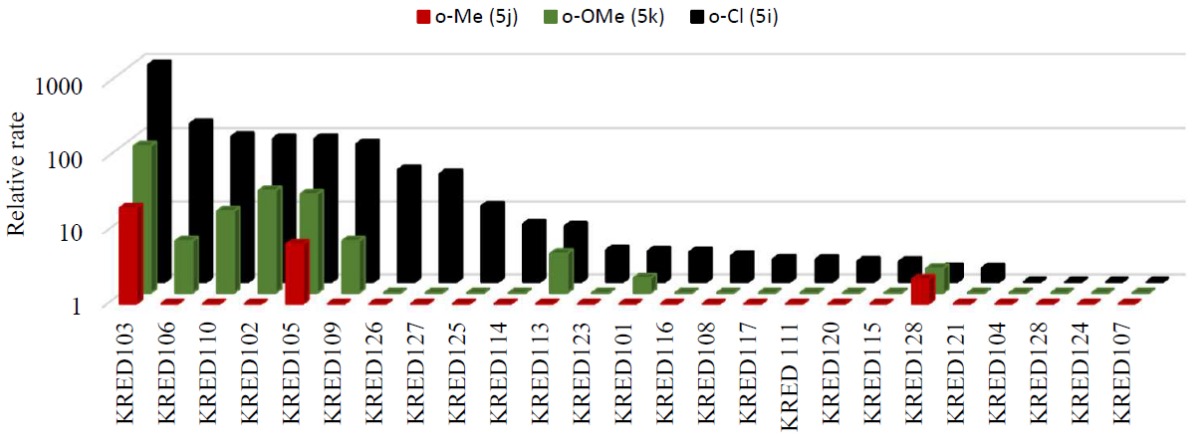


Figure 6. Relative rate of 22 KRED enzymes in reduction to **5i** (*o*-Cl), **5j** (*o*-Me) and **5k** (*o*-OMe). Activity for reduction to compound **5a** is used as reference (relative rate = 1). The raw data can be found in the Supplementary material.

3.3 Other aromatic substitution patterns

In case of other substitution patterns, only derivatives with a *meta*-chloro, *meta*-methyl, and 3,5-ditrifluoromethyl substitution have been studied in a sufficient number of reaction with several enzymes, see Figure 7.

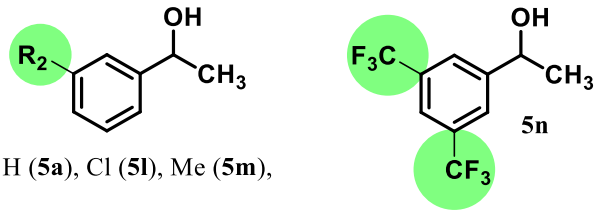


Figure 7. Structure of the *meta* substituted derivatives **5l** and **5m,** and the 3,5-trifluorometyl substituted **5n**.

The selectivity obtained in reduction to the *meta*-chloro derivative **5l** was reasonable predicted by the ee of **5a**, **5c** (*p*-Cl) and **5e** (*p*-Me) (Pearson coefficients 0.953-0.975, see Supplementary Material). Surprisingly, the ee for the corresponding *meta*-methyl derivative **5m** could not be predicted accurately with data for any compounds (Supplementary Material). Figure 8 shows how the ee of **5j** differs from that of the reference compound **5a**. A possible reason for these differences could be that the substrate ketones prefers different orientations in analogy with that discussed for the *ortho*-derivatives [68].

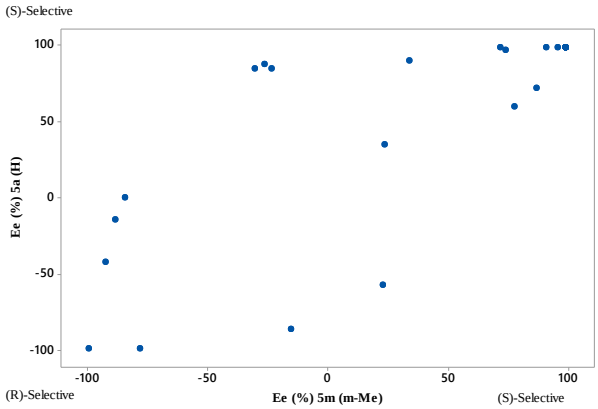


Figure 8. Comparison of ee data of **5a** and **5m** after reduction with 28 enzymes.

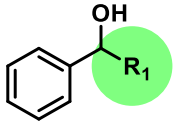
The 3,5-bistrifluoromethyl derivative **5n** (Figure 9) is a highly valuable building block for Aprepitant. Thus, its production by various methods have been thoroughly investigated [12, 69, 70]. We identified data in which the enzymes selectivity had been experimentally determined in reduction to **5a, 5g** (*p*-CF3) and **5n** (25 examples, excluding those inactive towards these three derivatives). Notably, in eight of the cases the reactivity was too low to measure ee of **5n**. The corresponding numbers of "inactive" enzymes for **5a** and **5g** were 3 and 4, respectively. Most of the reported reductions to **5n** proceeds in excellent ee and it appears that the steric bulk induced by the substituents, either increase selectivity or prevents reaction. The enantiomeric excess could not be predicted by the data for other derivatives.
The rates of reduction to **5l-n** was compared to the rate seen for compound **5a**. The key findings are highlighted in Table 2. On average, the *meta*-chloro derivative **5l** was formed 11 times faster than **5a** (42 pairs compared). Only in five of the examples the rate was lower than for **5a**. Reduction to compounds **5m** also often proceed with a high rate, but with a larger fraction of processes proceeding with a lower rate than **5a**. However, the relative rates could well be predicted by the relative activity of **5l** (see Supplementary material). Reduction to the 3,5-ditrifluoromethyl substituted compound **5n** in most cases proceeds with a rate comparable to that of **5a**, but in a few instances clearly with elevated rates.

Table 2. Statistic of conversion rates of **5l-n** relative to that of **5a**.

Parameter	**5l (*m*-Cl)**	**5m (*m*-Me)**	**5n (3,5-diCF$_3$)**
Examples compared	42	35	29
Average rel. rates as compared to **5a**	11.0	4.7	1.8
Numbers with lower rate than **5a**	5	12	12
% with lower rate than **5a**	12	34	41

3.4 Effect of the alkyl chain

Next, we evaluated processes in which oxidoreductases had been used in reduction of various 1-phenyl-alkanoates, see Figure 9.



R$_1$:
CH_3 (**5a**)
CH_2Cl (**6a**)
CH_2CH_3 (**7a**)
CF_3 (**8a**)

Figure 9. Structure of the compounds evaluated

The enantioselectivity of processes towards 2-chloro-1-phenylethanol (**6a**), and 1-phenylpropan-1-ol (**7a**) had Pearson coefficient < 0.90 with the selectivity seen for compound **5a** ($R_1 = CH_3$). Figure 10 illustrates the variance in selectivity observed for **5a**, **6a** and **7a**. The correlation between data for 2-chloro-1-phenylethanol (**6a**) and 1-phenyl-2-propanol (**7a**) had Pearson coefficient of 0.98. However, more examples are needed to verify this effect as the number of enzymes compare is low and most of these display excellent ee.

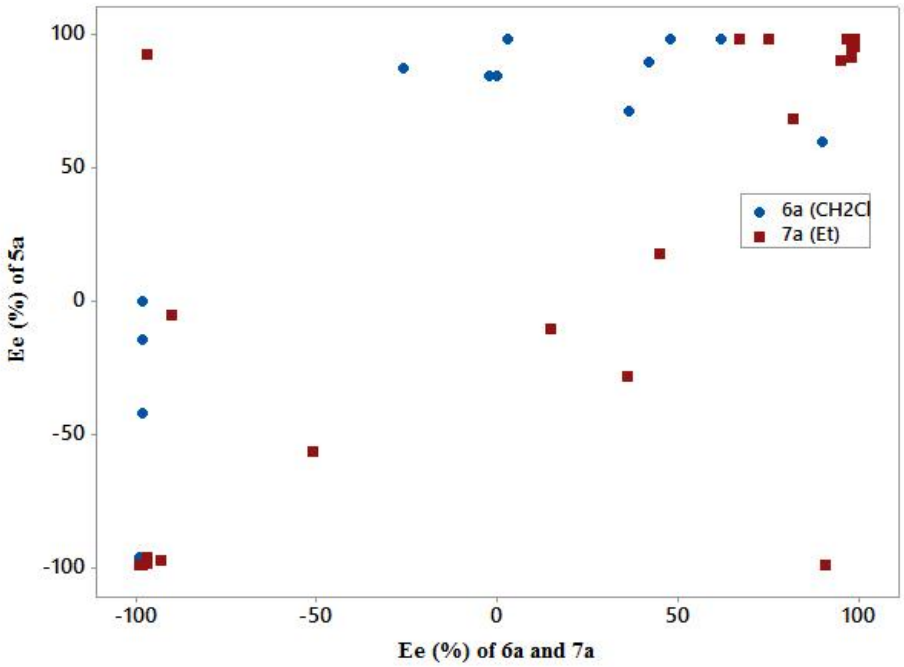


Figure 10. Scatterplot for ee data for compounds 1-phenylethanol (**5a**) vs. **6a** ($R_1 = CH_2Cl$) and **7a** (R_1 = Et).

Many of the studies cited provide activity or conversion data for the asymmetric reductions. These measurements were normalised to relative rate/conversion using the data for **5a** as a baseline with a value of 1. α-Chloroacetophenone (**2a**) reacted on average 9.7 times faster than the reference (16 examples compared), while propiophenone (**3a**) was reduced with almost the same rate (2.1 times faster on average, 34 examples). α,α,α-Trifluoroacetophenone (**4a**) however was on average reduced >100 times faster than acetophenone in the nine comparable examples. Obviously, electron withdrawing group increase rate of reaction in most of these examples. There is also a risk that equilibration have affected some of the rate measurements [38, 71], as the equilibrium position depend on the electronic properties of the ketone Anyhow, these numbers are highly indicative of the ease of performing such reductions. This shows that even though reductions are slow with acetophenones, the enzymes might be highly valuable for reduction of more activated substrates as α-chloroacetophenone (**2a**) and α,α,α-trifluoroacetophenone (**4a**). Figure 11 compares the relative rates of **5a**-**8a** where data exist for at least 3 of these four products.

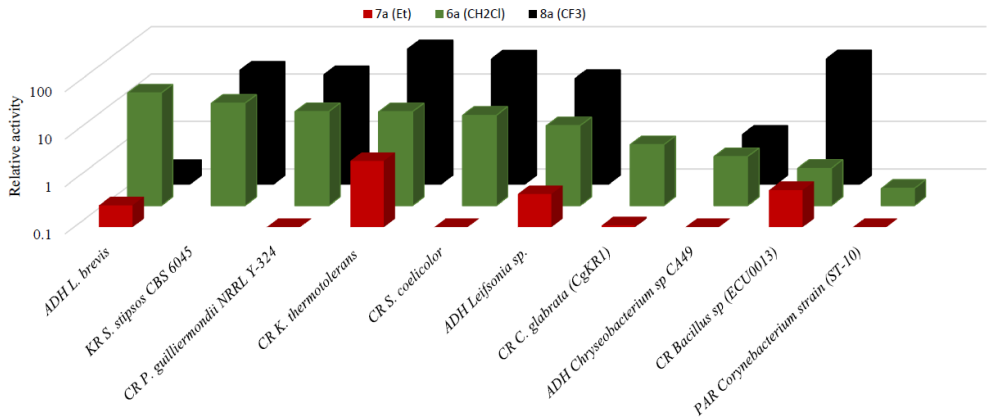


Figure 11. Relative rate in reduction to **6a** ($R_1 = CH_2Cl$), **7a** ($R_1 = CH_2CH_3$) and **8a** ($R_1 = CF_3$) with different enzymes. Activity for reduction to compound **5a** ($R_1 = CH_3$) is used as reference (relative rate = 1). The raw data can be found in the Supplementary Material.

The effect of mutation on enantioselectivity in reduction to the derivatives **5a** ($R_1 = CH_3$) and **7a** ($R_1 = CH_2CH_3$) was studied by Reetz *et al*. [3]. Wild-type ADH from *T. brockii* displayed ee values of 18 and 45% for **5a** and **7a**, respectively. With the correct single mutation, high Prelog or anti-Prelog selectivity was achieved. In most cases **5a** and **7a** had similar ee values, but using four mutants large differences were seen. The Pearson coefficient for the ee values of **5a** and **7a** was as low as 0.69 (n = 19). Thus, this series of data had a higher variance than that seen for the whole collection of ee values for **7a** (n = 30). Further, it clearly shows that a small methyl group can have profund effect on the chiral recognition processes. Interestingly, those mutations, which led to higher selectivity, also increased rate of reaction. The ee and conversion for **5a** are shown as a bar plot in Figure 12. Conversion data for compound **7a** had a similar profile.

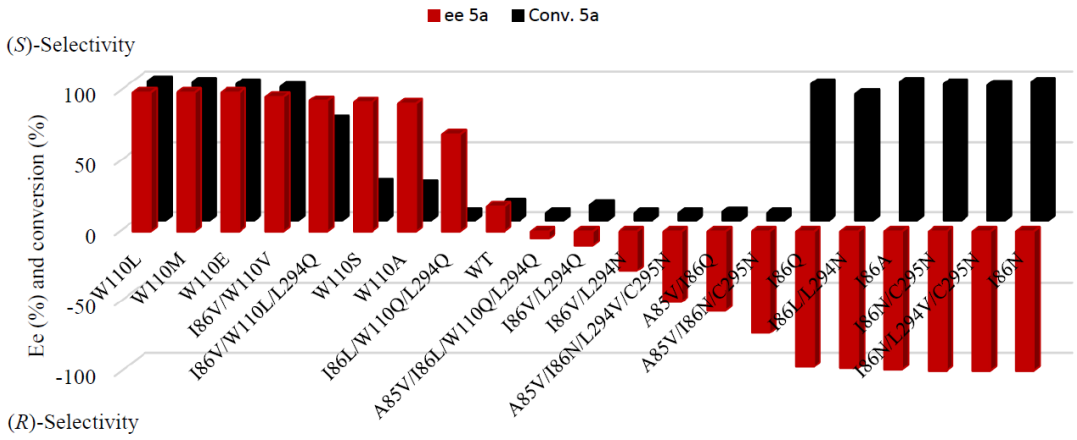


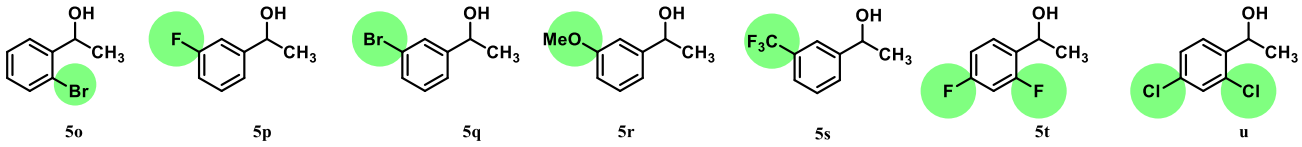
Figure 12. Effect of mutation on ee and conversion in reduction of **5a** using WT and mutants of alcohol dehydrogenase *T. brockii*. Data is taken from Reetz *et al.* [3].

Thus, also for variation of the alkyl part of the ketone the selectivity could not be precisely predicted based data for closely related analogues. However, again due to mechanistically aspects an increase in rate of reaction was seen for substrates having electron withdrawing substituents.

3.5 Estimation of ee based on models

To evaluate the usefulness of literature information and models in predicting enantioselectivity, we used ee for **5a-f**, **5i** and **5l** as input to compute enantioselectivity for the other derivatives reported in the same studies using linear or quadratic models (46 examples). These data was not part of the initial study. The Pearson correlation coefficient for the computed and experimental ee values was 0.99. However, the same high correlation was seen when only using the ee value of the model compound as input. (See supplementary information for the equations used and residual plots following regression analysis.) It was also analysed if the ee of compounds **5o**-**5t** (Table 3), could be predicted by models of adapted from analogues. Table 3 shows the input data, the model used and the calculated and experimentally determined ee values. The ee of the *ortho*-bromo derivative **5o** was estimated using a linear model for **5i** (*o*-Cl). The calculated ee values were in good correspondence with the experimentally determined data (entries 1-2). The *meta* substituted derivatives **5p**-**5s** (entries 3-14) were modelled using the equation for the *meta*-chloro derivative **5l**. The largest deviation between the computed and experimental ee was seen for the *meta*-bromo derivative **5q** (entry 5). The ee of the 2,4-difluoro substituted derivative **5t** was modelled using data for the *para*-fluoro derivative **5b**. The equation slightly underestimated the ee value (entry 15). Lastly, the ee of the 2,4-dichloro derivative **5u** was computed with a model for **5i** (*o*-Cl) (entries 16-19), showing larger deviation for the (*R*)-enantiomer than the (*S*)-enantiomer.

Table 3. Estimation of ee-values of processes towards **5o-t** using data for **5a** as input.



5o 5p 5q 5r 5s 5t u

Entry	Target comp.	Model	Model: ee (%)	Equation for ee	Calcd.ee (%)[a)]	Exp. ee (%)[b)]	Enzyme/reference
1	(*S*)-**5o**	**5a**	99	15.01+0.84×ee-**5a**[c)]	98	99	*C. tenuis* xylose reductase/[51]
2	(*S*)-**5o**	**5a**	96	15.01+0.84×ee-**5a**[c)]	95	99	PAR *Coryneb. strain* (ST-10)/ [24]
3	(*S*)-**5p**	**5a**	99	3.91+ 0.99×ee-**5a**[d)]	102	99	CR *C. glabrata* (CgKR1)/ [28]
4	(*S*)-**5p**	**5a**	99	3.91+ 0.99×ee-**5a**[d)]	102	98	CR *C. glabrata* (CgKR2)/ [28]
5	(*S*)-**5q**	**5a**	96	3.91+ 0.99×ee-**5a**[d)]	99	87	PAR *Coryneb. strain* (ST-10)/ [24]
6	(*S*)-**5q**	**5a**	99	3.91+ 0.99×ee-**5a**[d)]	102	97	CR *C. glabrata* (CgKR1)/ [28]
7	(*S*)-**5q**	**5a**	99	3.91+ 0.99×ee-**5a**[d)]	102	94	CR *C. glabrata* (CgKR2)/ [28]
8	(*R*)-**5q**	**5a**	-99	3.91+ 0.99×ee-**5a**[d)]	-94	-99	ADH *Leifsonia sp./* [72]
9	(*S*)-**5r**	**5a**	99	3.91+ 0.99×ee-**5a**[d)]	102	99	CR *C. glabrata* (CgKR1)/ [28]
10	(*S*)-**5r**	**5a**	99	3.91+ 0.99×ee-**5a**[d)]	102	99	CR *C. glabrata* (CgKR2)/ [28]
11	(*S*)-**5r**	**5a**	96	3.91+ 0.99×ee-**5a**[d)]	99	99	PAR *Coryneb. strain* (ST-10)/[73]
12	(*R*)-**5r**	**5a**	-99	3.91+ 0.99×ee-**5a**[d)]	-94	-99	ADH *Leifsonia sp./* [72]
13	(*R*)-**5s**	**5a**	-98	3.91+ 0.99×ee-**5a**[d]	-93	-99	*ADH L. brevis/[39]*
14	(*S*)-**5s**	**5a**	99	3.91+ 0.99×ee-**5a**[d)]	102	99	*CR Chryseob. sp*. CA49/[37]
15	(*R*)-**5t**	**5a**	-98	4.36+1.02×ee-**5a**[e)]	-95	-99	*ADH T. ethanolicus/* [52]
16	(*R*)-**5u**	**5a**	-99	15.01+0.84×ee-**5a**[c)]	-68	-79	ADH *Leifsonia sp./* [72]
17	(*S*)-**5u**	**5a**	99	15.01+0.84×ee-**5a**[c)]	98	99	CR *C. glabrata* (CgKR1)/ [28]
18	(*S*)-**5u**	**5a**	99	15.01+0.84×ee-**5a**[c)]	98	93	CR *C. glabrata* (CgKR2)/ [28]
19	(*S*)-**5u**	**5a**	96	15.01+0.84×ee-**5a**[c)]	95	99	PAR *Coryneb. strain* (ST-10)/[73]

[a)]Calculated ee based on the described model.
[b)]Experimentally determined ee-value
[c)]Model for computing ee of **5i** (*o*-Cl) based on ee of **5a**, R^2=91.6.
[d)]Model for computing ee of **5l** (*m*-Cl) based on ee of 5a, R^2 = 92.8.
[e)]Model for computing ee of **5b** (*p*-F) based on ee of **5a**, R^2=94.6.

Also in the case for **5o-u,** the ee could well be predicted by the models (Pearson coefficient: 0.999). However the ee could equally well be predicted by the ee of the starting material (Pearson coefficient: 0.999). Thus, generalised models to predict ee precisely seems to be of little value. On the other hand, this section shows that reference data on ee is highly useful when searching for enantioselectivity catalysts. If the reference model compound is transformed with a high ee, there is a high likelihood that also the target molecule will be converted with excellent selectivity.

To further verify this, the performance of 52 catalysts, showing ≥96% ee in reduction of acetophenone (**1a**), was compared with the ee for other derivatives reduced with the same enzyme (251 examples). Overall, 92% of the reactions had ee ≥ 96%. All but one of the reductions to the *para* substituted compounds **5b-h** (115 examples) had ee > 97% (Figure 13). The corresponding figures for the *ortho* substituted, **5i-5k**, **5l-5n** and the α-substituted **6a-8a** were 84% (38 examples), 86% (44 examples) and 85% (54 examples), respectively (Figure 14).

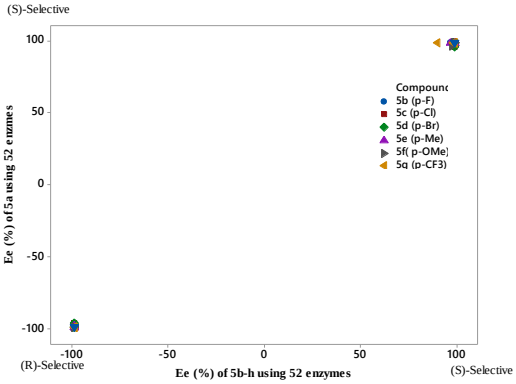


Figure 13. Scatterplot of ee of 52 processes with **5a** as substrate correlated with the ee of processes towards **5b-5h** (115 examples). One reaction proceeded with <96 % ee.

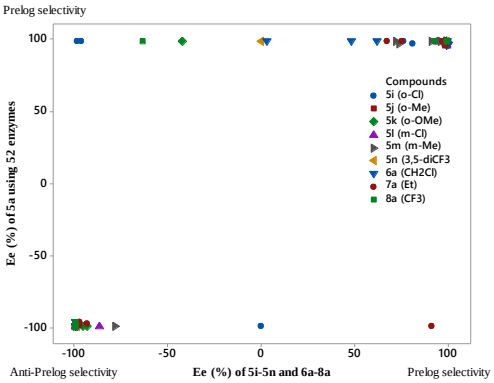


Figure 14. Scatterplot of ee of 52 processes with **5a** as substrate correlated with the ee of processes towards **5i-5n** and **6a-8a** (136 examples). Twenty reactions proceeded with < 96% ee.

3.6 Most useful catalysts

By combining data for activity and enantioselectivity, useful candidate enzymes for further optimisation can be identified as shown for **5a** in Figure 15. Here the reported specific activity is plotted versus ee (%). ADH *T. ethanolicus*-I86A, *(S)*-ADH *R. erytropolis* and CR LbCR (from *L. brevis)* are indicated as useful enzymes for preparation of (*S*)-**5a**, while ADH-R from *L. kefir*, ADH *C. maris* and two ADH's from *B. gladioli* were found efficient for preparation of (*R*)-**5a**. ADH *C. maris* also displayed high ee in preparation of *(S)*-**8a** and heterocyclic 1-arylethanols [44]. Similar data was gathered for the other products. The study of KRED enzymes by Hua *et al*. [18] only reported relative activity. However, since this is the largest single study of substituted acetophenones in terms of number of enzymes and substrates, the best KRED enzymes were also identified. Figure 16 highlights those enzymes having both ≥ 99 % ee and good rates.

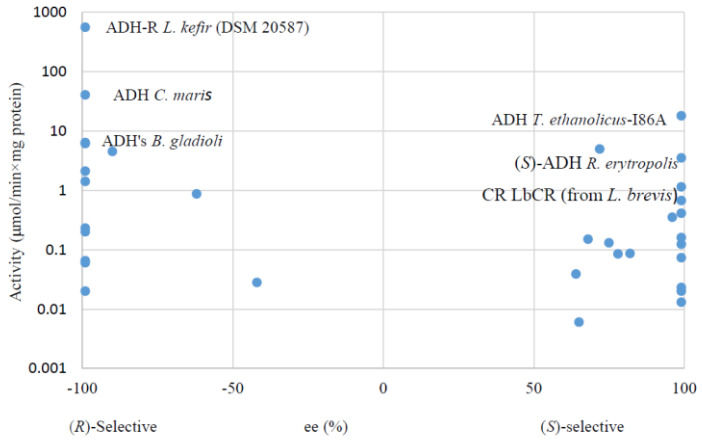


Figure 15. Activity (µmol/min×mg protein) plotted as a function of ee (%) for **5a**. The most promising enzymes are highlighted.

(*S*)-ADH *R. erytropolis* has Prelog selectivity. Although good rates and excellent selectivity was seen in most cases, the enzyme seems especially useful for preparation of (*S*)-**5l** (*m*-Cl) and (*S*)-**5d** (*p*-Br) [53]. CR *C. parapsilosis* (CPAR4) [31] have only been evaluated towards five acetophenones and showed good activity and excellent selectivity in every case. It will be interesting to see if more bulky substituents as R_1 and R_2 can be accepted. ADH *K. capsulate* [33] displayed excellent selectivity towards a range of substrates, but had a low rate for preparation of (*S*)-**5a**, (*S*)-**5f**, (*S*)-**5i**, **(***S*)-**5j** and (*R*)-**6a**. However, reaction engineering might improve processes to a range of products. PAR *Corynebacterium st.* (ST-10) [24] displayed highest activity towards *para* and *meta* halogenated derivatives. Lower rate was seen in production of **5a** ($R_1 = CH_3$), **6a** ($R_1 = CH_2Cl$) and **7a** ($R_1 = CH_2CH_3$). The other Prelog selective enzymes shown in Figure 15 have a narrower activity/selectivity profile. Generally, few efficient enzymes exist for *ortho*-substituted derivatives. Promising catalysts appear to be KRED105 [18] and *C. tenuis* xylose reductase [51, 74]. None of the enantioselective enzymes have notable rate of reaction in production of the *ortho* methyl derivative (*S*)-**5j** and (*S*)-1-phenyl-2-propanol (**7a**). In the case of the latter, ADH *T. brockii*-W110L might be an enzyme worth further studies.

ADH *B. gladioli* (BgADH2) and the isoenzyme BgADH1 display high anti-Prelog selectivity with a broad range of substrates accepted. A slight lowering of ee (95%) was see for the *ortho*-methoxy derivative **5k**. ADH *Chryseobacterium sp.* CA49[37] appeared efficient for making (*R*)-**5a**, but also for compounds having more bulky groups in the aromatic and the aliphatic part. A lower rate of reaction was however seen for the process towards (*R*)-**7a** (R_1= Et). KRED107 also demonstrated high anti-Prelog selectivity and good rates towards *para*-substituted acetophenones. R-specific ADH from *L. kefir* [41] proved efficient for making (*R*)-**5a** and (*R*)-**7a**. No other ee data was reported in this paper.

ADH from *Leifsonias sp.* [72] displayed the highest activity towards *para* and *meta* halogenated derivatives. However, (*R*)-**8a** was also formed with a decent rate. KRED101 [18] showed low selectivity for *para*-substituted derivatives, however it displayed excellent enantioselectivity and rate for production of the *ortho*-methoxy analogue (*R*)-**5k**. CR *K. thermotolerans* [23] was tested towards 14 acetophenone analogues showing excellent ee in all cases, but besides those compounds shown in Figure 15, a low rate was seen. The enzyme was especially efficient for making the (*R*)-2,2,2-trifluoro-1-phenylethanol (**8a**). The Supplementary file of this article [23] also summarises activity/enantioselectivity data for many enzymes in reduction of

acetophenone (**1a**). CR *P. guilliermondii* NRRL Y-324 [27] displays high selectivity and activity towards similar substrates, although not all analogues were included in both studies.

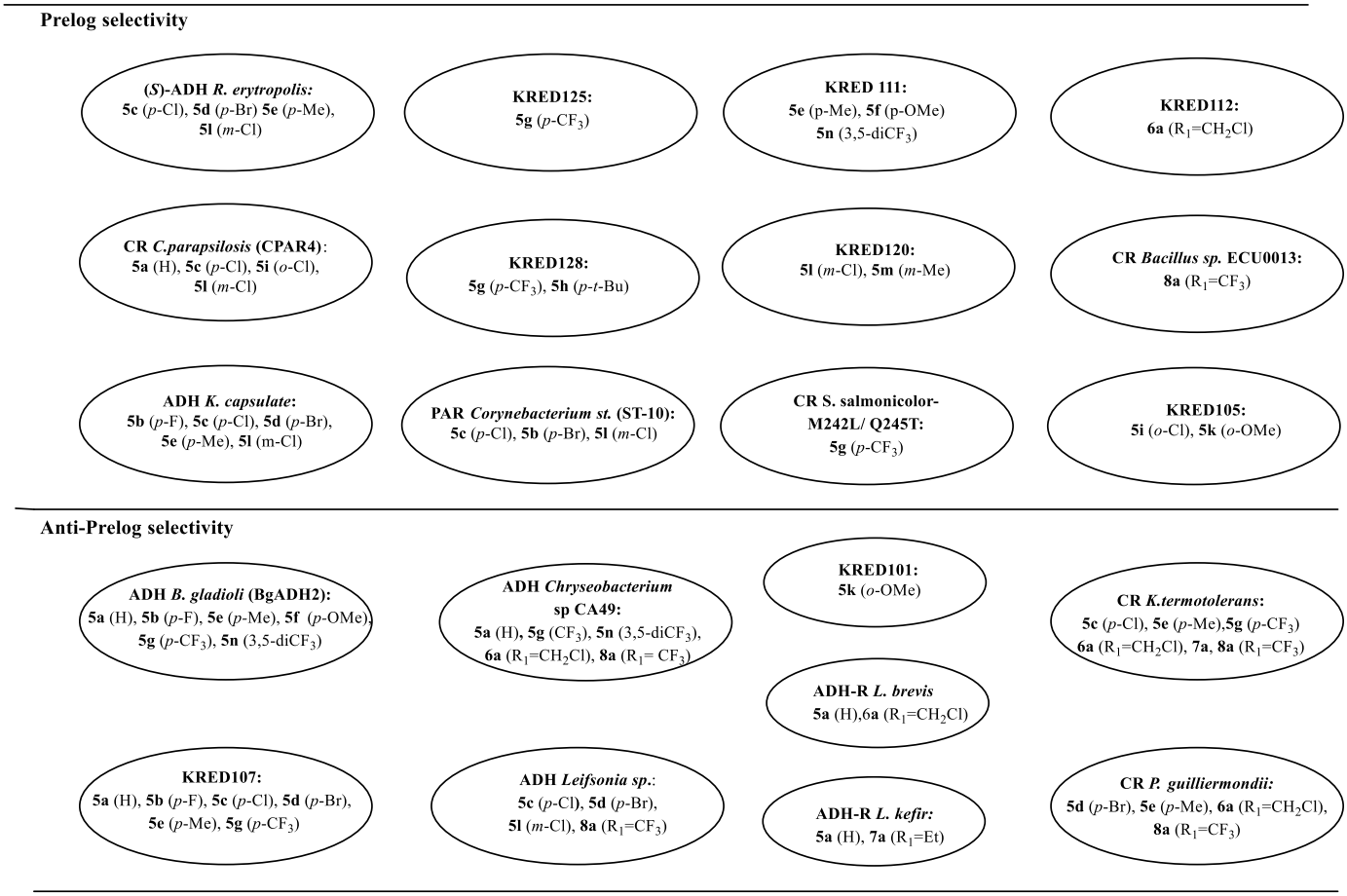


Figure 16. Suitable enzymes for preparation of different acetophenones.

There are no efficient enzymes for preparation of the *tert*-butyl substituted (*R*)-**5h,** *ortho*-chloro derivative (*R*)-**5i** and *ortho*-methyl derivative (*R*)-**5j**. Evidently, more efficient enzymes needs to be developed for these substrates. Other classes of acetophenones in which there is a lack of protocols includes compounds having two or more electron donating substituents and derivatives with the 2,6-substitution pattern.

Conclusion

Based on a data set of 108 enzymes used in reduction of 24 different aromatic ketones we have evaluated to what degree selectivity and reactivity can be deduced from data of closely related analogues. It can be assumed that the available data is somewhat biased by the fact that acetophenone (**1a**) is a commonly used model compound, and the challenge and reluctance to publish information on mediocre selectivity. The enantiomeric excess in reduction of *para*-substituted acetophenones could be well predicted by the ee for the structurally related ketones. The most pronounced difference was seen in catalysis of substrates containing a *tert*-butyl or a CF_3 group. These derivatives are poor model compound for each other both in terms of selectivity and rate. Of the eight *para*-substituted derivatives investigated, 1-(4-trifluoromethyphenyl)ethanol (**5g**) on average displayed the highest rate, while the 4-*tert*-butyl derivative displayed the lowest rate. The enantiomeric excess in reduction of *ortho* and *meta* substituted acetophenones were more difficult to predict. This is likely due to different preference for *cis*- and *trans*- conformations between the substituents and the carbonyl oxygen. The selectivity seen for the *ortho*-chloro, *ortho*-methyl and *meta*-chloro- acetophenones could be predicted by data for other derivatives, while this was not the case for the *meta*-methyl and *ortho*-methoxy acetophenones. It was observed that 1-(2-methoxyophenyl)ethanone more often induced anti-Prelog

selectivity in reduction than the other analogues evaluated. Variation of the alkyl chain also induce changes in enantioselectivity, and the ee could not be precisely predicted based on reference data. Clearly, subtle changes in conformation, size and electronic properties can have pronounced effects on the catalytic process. Anyhow, despite the fact that precise modelling of ee is not possible, literature data is highly useful for identification of enantioselective catalysts. Using enantioselective enzymes for reduction of acetophenone as models, a high ee was seen for 99% of *para*-substituted derivatives prepared by the same enzymes. In the case of *ortho*-, *meta*- and α-substitution, the predictability of a high ee was in the range of 84-86%. The selection of enzyme catalysts should also be based on rates. Generally, the rate of conversion was highest when having electron withdrawing groups as aromatic substituents or in the α-position. Thus, enzymes unsuited to reduce electron rich acetophenones might be well suited to reduce more activated derivatives. Finally, by combining data for selectivity and rate, suitable enzymes for conversion of different substrates have been identified.

References

[1] D. Pollard, M. Truppo, J. Pollard, C.y. Chen, J. Moore, Effective synthesis of (*S*)-3,5-bistrifluoromethylphenyl ethanol by asymmetric enzymatic reduction, Tetrahedron: Asymmetry, 17 (2006) 554-559.

[2] M.J. Homann, R.B. Vail, E. Previte, M. Tamarez, B. Morgan, D.R. Dodds, A. Zaks, Rapid identification of enantioselective ketone reductions using targeted microbial libraries, Tetrahedron, 60 (2004) 789-797.

[3] Z. Sun, G. Li, A. Ilie, M.T. Reetz, Exploring the substrate scope of mutants derived from the robust alcohol dehydrogenase TbSADH, Tetrahedron Lett., 57 (2016) 3648-3651.

[4] A. Li, L. Ye, X. Yang, B. Wang, C. Yang, J. Gu, H. Yu, Reconstruction of the Catalytic Pocket and Enzyme-Substrate Interactions To Enhance the Catalytic Efficiency of a Short-Chain Dehydrogenase/Reductase, ChemCatChem, 8 (2016) 3229-3233.

[5] G.V. Dhoke, C. Loderer, M.D. Davari, M. Ansorge-Schumacher, U. Schwaneberg, M. Bocola, Activity prediction of substrates in NADH-dependent carbonyl reductase by docking requires catalytic constraints and charge parameterization of catalytic zinc environment, J. Comput.-Aided Mol. Des., 29 (2015) 1057-1069.

[6] J. Deng, Z. Yao, K. Chen, Y.A. Yuan, J. Lin, D. Wei, Towards the computational design and engineering of enzyme enantioselectivity: A case study by a carbonyl reductase from Gluconobacter oxydans, J. Biotechnol., 217 (2016) 31-40.

[7] D. Zhang, X. Chen, J. Chi, J. Feng, Q. Wu, D. Zhu, Semi-rational engineering a carbonyl reductase for the enantioselective reduction of β-amino ketones, ACS Catal., 5 (2015) 2452-2457.

[8] F. Qin, B. Qin, T. Mori, Y. Wang, L. Meng, X. Zhang, X. Jia, I. Abe, S. You, Engineering of Candida glabrata Ketoreductase 1 for Asymmetric Reduction of α-Halo Ketones, ACS Catal., 6 (2016) 6135-6140.

[9] Z. Sun, R. Lonsdale, A. Ilie, G. Li, J. Zhou, M.T. Reetz, Catalytic Asymmetric Reduction of Difficult-to-Reduce Ketones: Triple-Code Saturation Mutagenesis of an Alcohol Dehydrogenase, ACS Catal., 6 (2016) 1598-1605.

[10] N.-Q. Wang, J. Sun, J. Huang, P. Wang, Cloning, expression, and directed evolution of carbonyl reductase from Leifsonia xyli HS0904 with enhanced catalytic efficiency, Appl. Microbiol. Biotechnol., 98 (2014) 8591-8601.

[11] S. de Wildeman, N. Sereinig, Enzymatic reduction of carbonyl groups, Sci. Synth. , Stereosel. Synth, 2 (2011) 133-208.

[12] B.H. Hoff, E. Sundby, Preparation of pharmaceutical important fluorinated 1-arylethanols using isolated enzymes, Bioorg. Chem., 51 (2013) 31-47.

[13] Y.-G. Zheng, H.-H. Yin, D.-F. Yu, X. Chen, X.-L. Tang, X.-J. Zhang, Y.-P. Xue, Y.-J. Wang, Z.-Q. Liu, Recent advances in biotechnological applications of alcohol dehydrogenases, Appl. Microbiol. Biotechnol., 101 (2017) 987-1001.

[14] A. Berenguer-Murcia, R. Fernandez-Lafuente, New trends in the recycling of NAD(P)H for the design of sustainable asymmetric reductions catalyzed by dehydrogenases, Curr. Org. Chem, 14 (2010) 1000-1021.

[15] Y. Yang, D. Zhu, T.J. Piegat, L. Hua, Enzymatic ketone reduction: mapping the substrate profile of a short-chain alcohol dehydrogenase (YMR226c) from *Saccharomyces cerevisiae*, Tetrahedron: Asymmetry, 18 (2007) 1799-1803.
[16] D. Zhu, Y. Yang, J.D. Buynak, L. Hua, Stereoselective ketone reduction by a carbonyl reductase from *Sporobolomyces salmonicolor*. Substrate specificity, enantioselectivity and enzyme-substrate docking studies, Org. Biomol. Chem, 4 (2006) 2690-2695.
[17] D. Zhu, Y. Yang, L. Hua, Stereoselective enzymatic synthesis of chiral alcohols with the use of a carbonyl reductase from *Candida magnoliae* with anti-Prelog enantioselectivity, J. Org. Chem, 71 (2006) 4202-4205.
[18] D. Zhu, B.E. Rios, J.D. Rozzell, L. Hua, Evaluation of substituent effects on activity and enantioselectivity in the enzymatic reduction of aryl ketones, Tetrahedron: Asymmetry, 16 (2005) 1541-1546.
[19] D. Zhu, C. Mukherjee, L. Hua, 'Green' synthesis of important pharmaceutical building blocks: enzymatic access to enantiomerically pure a-chloro alcohols, Tetrahedron: Asymmetry, 16 (2005) 3275-3278.
[20] D. Zhu, H.T. Malik, L. Hua, Asymmetric ketone reduction by a hyperthermophilic alcohol dehydrogenase. The substrate specificity, enantioselectivity and tolerance of organic solvents, Tetrahedron: Asymmetry, 17 (2006) 3010-3014.
[21] D. Zhu, Y. Yang, S. Majkowicz, T.H.-Y. Pan, K. Kantardjieff, L. Hua, Inverting the enantioselectivity of a carbonyl reductase via substrate-enzyme docking-guided point mutation, Org. Lett, 10 (2008) 525-528.
[22] H. Li, Y. Yang, D. Zhu, L. Hua, K. Kantardjieff, Highly enantioselective mutant carbonyl reductases created via structure-based site-saturation mutagenesis, J. Org. Chem, 75 (2010) 7559-7564.
[23] G.C. Xu, H.L. Yu, X.Y. Zhang, J.H. Xu, Access to Optically Active Aryl Halohydrins Using a Substrate-Tolerant Carbonyl Reductase Discovered from Kluyveromyces thermotolerans, ACS Catal, 2 (2012) 2566-2571.
[24] N. Itoh, N. Mizuguchi, M. Mabuchi, Production of chiral alcohols by enantioselective reduction with NADH-dependent phenylacetaldehyde reductase from Corynebacterium strain, ST-10, J. Mol. Catal. B: Enzym, 6 (1999) 41-50.
[25] K. Inoue, Y. Makino, T. Dairi, N. Itoh, Gene cloning and expression of *Leifsonia* alcohol dehydrogenase (LSADH) involved in asymmetric hydrogen-transfer bioreduction to produce (*R*)-form chiral alcohols, Biosci. Biotechnol. Biochem, 70 (2006) 418-426.
[26] L.J. Wang, C.X. Li, Y. Ni, J. Zhang, X. Liu, J.H. Xu, Highly efficient synthesis of chiral alcohols with a novel NADH-dependent reductase from *Streptomyces coelicolor*, Bioresour. Technol, 102 (2011) 7023-7028.
[27] G. Xu, H. Yu, J. Xu, Facile Access to Chiral Alcohols with Pharmaceutical Relevance Using a Ketoreductase Newly Mined from Pichia guilliermondii, Chin. J. Chem., 31 (2013) 349-354.
[28] P. Liang, B. Qin, M. Mu, X. Zhang, X. Jia, S. You, Prelog and anti-Prelog stereoselectivity of two ketoreductases from Candida glabrata, Biotechnol. Lett., 35 (2013) 1469-1473.
[29] H. Ma, L. Yang, Y. Ni, J. Zhang, C.-X. Li, G.-W. Zheng, H. Yang, J.-H. Xu, Stereospecific Reduction of Methyl O-Chlorobenzoylformate at 300 g/L-1 without Additional Cofactor using a Carbonyl Reductase Mined from Candida glabrata, Adv. Synth. Catal., 354 (2012) 1765-1772.
[30] S. Wang, Y. Nie, Y. Xu, R. Zhang, T.-P. Ko, C.-H. Huang, H.-C. Chan, R.-T. Guo, R. Xiao, Unconserved substrate-binding sites direct the stereoselectivity of medium-chain alcohol dehydrogenase, Chem. Commun. (Cambridge, U. K.), 50 (2014) 7770-7772.
[31] R. Guo, Y. Nie, X.Q. Mu, Y. Xu, R. Xiao, Genomic mining-based identification of novel stereospecific aldo-keto reductases toolbox from Candida parapsilosis for highly enantioselective reduction of carbonyl compounds, J. Mol. Catal. B: Enzym., 105 (2014) 66-73.
[32] A. Dudzik, W. Snoch, P. Borowiecki, J. Opalinska-Piskorz, M. Witko, J. Heider, M. Szaleniec, Asymmetric reduction of ketones and β-keto esters by (S)-1-phenylethanol dehydrogenase from denitrifying bacterium Aromatoleum aromaticum, Appl. Microbiol. Biotechnol., 99 (2015) 5055-5069.
[33] K. Wu, L. Chen, H. Fan, Z. Zhao, H. Wang, D. Wei, Synthesis of enantiopure epoxide by 'one pot' chemoenzymatic approach using a highly enantioselective dehydrogenase, Tetrahedron Lett., 57 (2016) 899-904.

[34] Z. Li, W. Liu, X. Chen, S. Jia, Q. Wu, D. Zhu, Y. Ma, Highly enantioselective double reduction of phenylglyoxal to (R)-1-phenyl-1,2-ethanediol by one NADPH-dependent yeast carbonyl reductase with a broad substrate profile, Tetrahedron, 69 (2013) 3561-3564.
[35] X. Chen, Z.-Q. Liu, J.-F. Huang, C.-P. Lin, Y.-G. Zheng, Asymmetric synthesis of optically active methyl-2-benzamido-methyl-3-hydroxy-butyrate by robust short-chain alcohol dehydrogenases from Burkholderia gladioli, Chem. Commun. (Cambridge, U. K.), 51 (2015) 12328-12331.
[36] Y.-P. Shang, Q. Chen, X.-D. Kong, Y.-J. Zhang, J.-H. Xu, H.-L. Yu, Efficient Synthesis of (R)-2-Chloro-1-(2,4-dichlorophenyl)ethanol with a Ketoreductase from Scheffersomyces stipitis CBS 6045, Adv. Synth. Catal., 359 (2017) 426-431.
[37] T.-X. Tang, Y. Liu, Z.-L. Wu, Characterization of a robust anti-Prelog short-chain dehydrogenase/reductase ChKRED20 from Chryseobacterium sp. CA49, J. Mol. Catal. B: Enzym., 105 (2014) 82-88.
[38] C. Rodriguez, W. Borzecka, J.H. Sattler, W. Kroutil, I. Lavandera, V. Gotor, Steric vs. electronic effects in the Lactobacillus brevis ADH-catalyzed bioreduction of ketones, Org. Biomol. Chem., 12 (2014) 673-681.
[39] S. Cacchi, R. Cirilli, G. Fabrizi, S. Sgalla, A. Macone, A. Bonamore, A. Boffi, (R)-1-Arylethanols from aryl iodides through a two-step one-pot enantioselective chemoenzymatic process, J. Mol. Catal. B: Enzym, 61 (2009) 184-187.
[40] Y. Ni, C.X. Li, L.J. Wang, J. Zhang, J.H. Xu, Highly stereoselective reduction of prochiral ketones by a bacterial reductase coupled with cofactor regeneration, Org. Biomol. Chem, 9 (2011) 5463-5468.
[41] A. Weckbecker, W. Hummel, Cloning, expression, and characterization of an (R)-specific alcohol dehydrogenase from Lactobacillus kefir, Biocatal. Biotransform, 24 (2006) 380-389.
[42] C.W. Bradshaw, C.H. Wong, G.J. Shen, Pseudomonas sp. ATCC No. 49794 alcohol dehydrogenase and use in producing an alcohol, in, The Scripps Research Institute, US, 1992, pp. 17.
[43] A. Pennacchio, B. Pucci, F. Secundo, F. La Cara, M. Rossi, C.A. Raia, Purification and characterization of a novel recombinant highly enantioselective short-chain NAD(H)-dependent alcohol dehydrogenase from Thermus thermophilus, Appl. Environ. Microbiol, 74 (2008) 3949-3958.
[44] S. Kawano, M. Yano, J. Hasegawa, Y. Yasohara, Purification and characterization of an NADH-dependent alcohol dehydrogenase from Candida maris for the synthesis of optically active 1-(pyridyl)ethanol derivatives, Biosci., Biotechnol., Biochem., 75 (2011) 1055-1060.
[45] X. Wu, Y. Wang, J. Ju, C. Chen, N. Liu, Y. Chen, Enantioselective synthesis of ethyl (S)-2-hydroxy-4-phenylbutyrate by recombinant diketoreductase, Tetrahedron: Asymmetry, 20 (2009) 2504-2509.
[46] X.-M. Gong, G.-W. Zheng, Y.-Y. Liu, J.-H. Xu, Identification of a Robust Carbonyl Reductase for Diastereoselectively Building syn-3,5-Dihydroxy Hexanoate: a Bulky Side Chain of Atorvastatin, Org. Process Res. Dev., (2017) Ahead of Print.
[47] S. Kara, D. Spickermann, A. Weckbecker, C. Leggewie, I.W.C.E. Arends, F. Hollmann, Bioreductions Catalyzed by an Alcohol Dehydrogenase in Non-aqueous Media, ChemCatChem, 6 (2014) 973-976.
[48] Y. Ni, C.X. Li, J. Zhang, N.D. Shen, U.T. Bornscheuer, J.H. Xu, Efficient Reduction of Ethyl 2-Oxo-4-phenylbutyrate at 620 g.L-1 by a Bacterial Reductase with Broad Substrate Spectrum, Adv. Synth. Catal, 353 (2011) 1213-1217.
[49] Q. Xu, X. Xu, H. Huang, S. Li, Efficient synthesis of (R)-2-chloro-1-phenylethol using a yeast carbonyl reductase with broad substrate spectrum and 2-propanol as cosubstrate, Biochem. Eng. J., 103 (2015) 277-285.
[50] Y. Ni, C.X. Li, H.M. Ma, J. Zhang, J.H. Xu, Biocatalytic properties of a recombinant aldo-keto reductase with broad substrate spectrum and excellent stereoselectivity, Appl. Microbiol. Biotechnol, 89 (2011) 1111-1118.
[51] M. Vogl, R. Kratzer, B. Nidetzky, L. Brecker, Candida tenuis xylose reductase catalyzed reduction of acetophenones: the effect of ring-substituents on catalytic efficiency, Org. Biomol. Chem., 9 (2011) 5863-5870.
[52] M.M. Musa, N. Lott, M. Laivenieks, L. Watanabe, C. Vieille, R.S. Phillips, A single point mutation reverses the enantiopreference of *Thermoanaerobacter ethanolicus* secondary alcohol dehydrogenase, ChemCatChem, 1 (2009) 89-93.
[53] W. Hummel, New alcohol dehydrogenases for the synthesis of chiral compounds, Adv. Biochem. Eng./Biotechnol., 58 (1997) 145-184.

[54] H. Gröger, W. Hummel, C. Rollmann, F. Chamouleau, H. Husken, H. Werner, C. Wunderlich, K. Abokitse, K. Drauz, S. Buchholz, Preparative asymmetric reduction of ketones in a biphasic medium with an (*S*)-alcohol dehydrogenase under in situ-cofactor-recycling with a formate dehydrogenase, Tetrahedron, 60 (2004) 633-640.
[55] V. Prelog, Specification of the stereospecificity of some oxidoreductases by diamond lattice sections, Pure Appl. Chem., 9 (1964) 119-130.
[56] F. Leroux, P. Jeschke, M. Schlosser, a-Fluorinated Ethers, Thioethers, and Amines: Anomerically Biased Species, Chemical Reviews (Washington, DC, United States), 105 (2005) 827-856.
[57] C. Hansch, A. Leo, D. Hoekman, Exploring QSAR.Hydrophobic, Electronic and Steric Constants, 1 ed., ACS Professional Reference Book, American Chemical Society, Washington, DC, 1995.
[58] V. Leskovac, S. Trivic, D. Pericin, The three zinc-containing alcohol dehydrogenases from bakers' yeast, *Saccharomyces cerevisiae*, FEMS Yeast Res, 2 (2002) 481-494.
[59] L.A. LeBrun, B.V. Plapp, Control of coenzyme binding to horse liver alcohol dehydrogenase, Biochemistry, 38 (1999) 12387-12393.
[60] C. Filling, K.D. Berndt, J. Benach, S. Knapp, T. Prozorovski, E. Nordling, R. Ladenstein, H. Jornvall, U. Oppermann, Critical residues for structure and catalysis in short-chain dehydrogenases/reductases, J. Biol. Chem, 277 (2002) 25677-25684.
[61] N.H. Schlieben, K. Niefind, J. Mueller, B. Riebel, W. Hummel, D. Schomburg, Atomic resolution structures of *R*-specific alcohol dehydrogenase from *Lactobacillus brevis* provide the structural bases of its substrate and cosubstrate specificity, J. Mol. Biol, 349 (2005) 801-813.
[62] H. Weiner, S. Freytag, J.M. Fox, J.H.J. Hu, Reversible inhibitors of aldehyde dehydrogenase, Prog. Clin. Biol. Res., 114 (1982) 91-102.
[63] R.W. MacKintosh, C.A. Fewson, Benzyl alcohol dehydrogenase and benzaldehyde dehydrogenase II from Acinetobacter calcoaceticus. Substrate specificities and inhibition studies, Biochem. J., 255 (1988) 653-661.
[64] S. Pal, D.-H. Park, B.V. Plapp, Activity of yeast alcohol dehydrogenases on benzyl alcohols and benzaldehydes: characterization of ADH1 from Saccharomyces carlsbergensis and transition state analysis, Chem Biol Interact, 178 (2009) 16-23.
[65] K. Uwai, N. Konno, Y. Yasuta, M. Takeshita, Electronic effects of para-substitution on acetophenones in the reaction of rat liver 3α-hydroxysteroid dehydrogenase, Bioorg. Med. Chem., 16 (2008) 1084-1089.
[66] T. Schaefer, J. Peeling, T.A. Wildman, Conformational preferences of 2-methoxyacetophenone, Org. Magn. Reson., 22 (1984) 477-479.
[67] P.J. Krueger, Substituent effects on the acetyl group conformation and the carbonyl frequency in acetophenones, Can. J. Chem., 51 (1973) 1363-1367.
[68] M. Grimaud, G. Pfister-Guillouzo, Influence of substitution on the structure of benzophenone. X. Conformational study of substituted benzaldehydes, acetophenones, and benzophenones by complexation with lanthanides, Org. Magn. Reson., 7 (1975) 386-391.
[69] P.J. Vankawala, N. Kolla, C.R. Elati, M. Sreenivasulu, K.A. Kumar, Y. Anjaneyulu, S. Venkatraman, A. Bhattacharya, V.T. Mathad, Efficient Synthesis of (1R)-[3,5-Bis(trifluoromethyl)phenyl]ethanol, a Key Intermediate for Aprepitant, an NK-1 Receptor Antagonist, Synth. Commun, 37 (2007) 3439-3446.
[70] M.M. Zhao, J.M. McNamara, G. Ho, K.M. Emerson, Z.J. Song, D.M. Tschaen, M.J. Brands Karel, U. Dolling, J.J. Grabowski Edward, P.J. Reider, I.F. Cottrell, M.S. Ashwood, B.C. Bishop, Practical asymmetric synthesis of aprepitant, a potent human NK-1 receptor antagonist, via a stereoselective Lewis acid-catalyzed trans acetalization reaction, J Org Chem, 67 (2002) 6743-6747.
[71] F.R. Bisogno, E. Garcia-Urdiales, H. Valdes, I. Lavandera, W. Kroutil, D. Suirez, V. Gotor, Ketone-alcohol hydrogen-transfer equilibria: Is the biooxidation of halohydrins blocked?, Chem. Eur. J, 16 (2010) 11012-11019, S11012/11011.
[72] K. Inoue, Y. Makino, N. Itoh, Production of (*R*)-chiral alcohols by a hydrogen-transfer bioreduction with NADH-dependent *Leifsonia* alcohol dehydrogenase (LSADH), Tetrahedron: Asymmetry, 16 (2005) 2539-2549.
[73] N. Itoh, M. Matsuda, M. Mabuchi, T. Dairi, J. Wang, Chiral alcohol production by NADH-dependent phenylacetaldehyde reductase coupled with in situ regeneration of NADH, Eur. J. Biochem., 269 (2002) 2394-2402.

[74] M. Vogl, R. Kratzer, B. Nidetzky, L. Brecker, Candida tenuis Xylose Reductase Catalyzed Reduction of Aryl Ketones for Enantioselective Synthesis of Active Oxetine Derivatives, Chirality, 24 (2012) 847-853.