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PAPER

Investigation of asymmetric alcohol dehydrogenase (ADH) reduction of acetophenone derivatives: effect of charge density†

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In an effort to study the effect of substituent groups of the substrate on the alcohol dehydrogenase (ADH) reductions of aryl–alkyl ketones, several derivatives of acetophenone have been evaluated against ADHs from *Lactobacillus brevis* (LB) and *Thermoanaerobacter sp*. (T). Interestingly, ketones with non-demanding (neutral) *para*-substituents were reduced to secondary alcohols by these enzymes in enantiomerically pure form whereas those with demanding (ionizable) substituents could not be reduced. The effect of substrate size, their solubility in the reaction medium, electron donating and withdrawing properties of the ligand and also the electronic charge density distribution on the substrate molecules have been studied and discussed in detail. From the results, it is observed that the electronic charge distribution in the substrate molecules is influencing the orientation of the substrate in the active site of the enzyme and hence the ability to reduce the substrate.

Introduction

Enantiopure alcohols are valuable intermediates and have found important applications in the synthesis of pharmaceuticals and other fine chemicals.[1–10] The asymmetric reduction of prochiral ketones is one of the straightforward approaches used to access enantiomerically pure alcohols. In this regard, a variety of chiral metal complexes have been developed as catalysts for asymmetric ketone reductions.[11–16] These methods predominantly use toxic metals and expensive metal hydrides, which require special reaction conditions. In the search for alternatives, several biocatalytic methods for stereoselective reduction of ketones have been developed,[17–19] which hold great potential with respect to environmental compatibility and catalytic efficiency.

Dehydrogenases are non-heme redox enzymes which catalyze hydrogen-transfer reactions in the presence of a coenzyme as hydrogen donor or acceptor. A number of different dehydrogenases (isolated from different sources) have been utilized for the asymmetric reduction of carbonyl functionalities. Stereoselective reduction of ketones using alcohol dehydrogenases (ADH) has become an important method for industrial preparation of optically pure alcohols.[20–24] We have started to investigate ADH reductions for the synthesis of chiral monomers in order to access polymers with defined chiral compositions.[25–27] Unfortunately, most of the biocatalytically applicable ADHs show a rather narrow substrate pattern: preferentially ketones which bear small, non-ionizable, non polar, non-demanding substituent functional groups have been reduced successfully.[28] The influence of different functional substituent groups on the enzymatic reduction of ketones has not yet been studied systematically. There is thus an increasing interest in understanding the effect of substituent groups on the biocatalytic reduction of ketones, which will eventually help in understanding the reduction mechanism and the development of suitable enzymatic reduction strategies for substituted ketones.

We therefore synthesized a series of acetophenone derivatives with different demanding substituent functional groups. These ketones were reduced by ADH enzymes from *Lactobacillus brevis* (*R* selective) and *Thermoanaerobacter sp*. (*S* selective) and the effect of different parameters on the reduction investigated in detail. To the best of our knowledge, the available literature on the ADH reductions does not satisfactorily disclose the effect of different functional groups on the biocatalytic reduction. Moreover, not much has been explored on the substrate specificity of ADHs from *Lactobacillus brevis* (ADH-LB) and *Thermoanaerobacter sp* (ADH-T). Correlating substrate profiles of the ADHs with their sequential and structural information would provide valuable insight into the understanding of how these enzymes control activity and enantioselectivity. With this perspective in mind, we have studied substrate specificity and enantioselectivity for the carbonyl reductases from *Lactobacillus brevis* and *Thermoanaerobacter sp* with various ketones of diverse structures.

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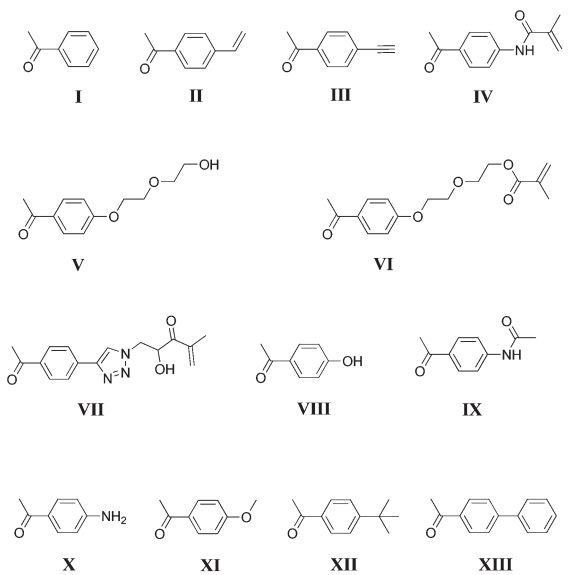
Results and discussion

The *R*-alcohol dehydrogenase (RADH) from *Lactobacillus brevis* (ADH-LB) is a NADP-dependent member of the extended superfamily of short-chain dehydrogenases/reductases (SDRs)[29–31] (enzyme class EC 1.1.1.2, CAS 9031-72-5[32]). It is a homotetrameric enzyme[32,33] with 251 amino acid residues and a molecular mass of 26 627 Da[33,34] or 22.5 kDa per subunit depending on the source. ADH-LB was discovered during a search for biotechnologically interesting alcohol dehydrogenases.[35] The active site of the enzyme contains a four member catalytic tetrad (Asn113, Ser142, Tyr155 and Lys159).[36] The complimentary *S*-alcohol dehydrogenase from *Thermoanaerobacter sp.* (ADH-T) is a NADP dependent alcohol dehydrogenase from the family of thermophillic enzymes with 352 amino acid series.

The binding site for the substrate for ADH-LB is reported to be formed by the nicotinamide moiety of NAD(P) and a hydrophobic patch on the enzyme surface, more precisely at the interface of helix αG and the loop between strand βE and helix αF.[34]

Scheme 1 shows the library of the functional ketones employed in the systematic study of ADH reductions. Initially the ketones were all reduced in a PBS buffer (pH 7.4)/IPA solution using ADH-LB and ADH-T in presence of NADPH as cofactor at 37 °C.[25,27] The progress of the reaction was monitored by ^{1}H NMR spectroscopy and gas chromatography (GC). The stereoselectivity of the enzymes was further analyzed by chiral GC yielding the enantiomeric excess.

In the first attempt to reduce ketones from **I** to **IX**, only ketones **I**, **II** and **III** could be reduced successfully by ADH-LB and ADH-T whereas ketones from **IV** to **X** could not be reduced at all. Changes in temperature, pH and ionic strength of the reaction medium did not improve the results. Although all substrates are from the same family of acetophenone derivatives with only difference in *p*-substituent functional group, the results on enzymatic reductions were completely contradictory. This prompted us to study the effect of different parameters on the enzymatic reduction of acetophenone derivatives in detail. The major factors influencing the reduction according to the literature[37] are pH of the medium, temperature of the reaction, the size of the substrate and their solubility in the reaction medium. The effect of pH and reaction temperature on the enzymatic reduction of ADH-LB and ADH-T has already been studied extensively.[38,39] In the literature it is reported that although the activity of these enzymes increases with increase in temperature the half-life of the enzyme decreases steadily. Hence, an optimum temperature of 37 °C was chosen for the reaction. An increase in reaction temperature to 42 °C for ketones **IV** to **X** did not improve the reaction (results not shown). All reactions were carried out at pH 7.0 due to the stability and reactivity of the ketones and enzymes at lower and higher pH (note: ADH-LB has two activity maxima at pH 5.5 and 9.0). The other obvious parameters, which influence the reaction, are the size of the substrate and their solubility in the reaction medium. Complete insolubility of the substrate in the reaction medium or substrates bigger than the cavity of enzyme will prevent reduction. Whether these parameters can explain the results obtained for the investigated substituted ketones will be discussed in the following.



Scheme 1 Various *para*-substituted acetophenone derivatives employed in the reduction by ADH-LB and ADH-T.

Effect substrate size

It is well known that all enzymatic reactions are performed through interaction of the active site of the enzyme with the ligands or substrate molecules. These active sites are normally buried inside the enzyme and are only accessible through a small channel called cavity. The size and shape of these cavities play a crucial role in the binding specificity.[40] For the ketone to be reduced it must first reach the active site through the cavity and then the carbonyl group of the ketone should bind to the active site of the ADH through a ternary-complex with the co-substrate NADPH. Hence, the size of the ketone is a very important factor in the enzymatic reduction.[41] However, proteins are not rigid but are in dynamic flexible state and ligand binding causes variation in protein binding cavity volume (PCV).[42] Binding of the ligand to a protein may cause conformational changes that align the residues involved in binding in their correct orientation and hence changes in cavity volume.[43–45] The size of the substrate does influence the binding specificity and hence the enantioselectivity or stereospecificity[46] but does not reject the substrate completely from binding to the active site. From the results of reduction of ketones of different sizes against ADH-LB and ADH-T presented in Table 1, it seems that though we cannot completely rule out the effect of size, the enzymatic reduction did not show any dependence on size of the ketone substrate at least in this study.

The ADH-LB has a large hydrophobic cavity, next to the nicotinamide ring of the co-factor, leading to the active site. The volume of the cavity has been determined to be ~310 Å^3 using Q-SiteFinder[47] and pocket-finder.[48] The phenyl ring of substrate interacts with hydrophobic side-chains (Ala93, Leu152, Val195, Leu198 and Met205) and with the aromatic ring of Tyr189, while its carbonyl group forms a hydrogen bond (distance 3.3 Å) with the terminal hydroxyl group of Tyr155, which is the most conserved residue of the whole SDR superfamily and the

Table 1 Enzymatic reduction of acetophenone derivatives of different molecular size by ADT-LB and ADH-T enzymes

Ref. no.	Ketone	Molecular size[a] (Å^3)	Conversion (%) by NMR
1	**I**	138.14	99
2	**VIII**	144.07	0
3	**X**	147.07	0
4	**II**	168.15	97
5	**III**	165.84	99
6	**IX**	187.49	0
7	**XII**	210.23	26
8	**IV**	219.59	0
9	**XIII**	219.78	15
10	**VI**	234.78	0
11	**VII**	306.78	0
12	**V**	309.64	0

[a] The sizes of the ketones were calculated by SPARTAN program after minimization of energy of the structure.

established catalytic base of the oxidative reaction direction.[36] The molecular size of all the ketones, in the present study, is less than ~310.0 Å^3 as calculated by SPARTAN program[49] after applying Hartee–Fock model of density functional theory and minimization of energy. The active cavity therefore has sufficient space to accommodate all ketone substrates investigated in this study. However, ketones **I** to **III** with molecular size ranging between ~140.0 Å^3–170.0 Å^3 were successfully reduced by ADH-LB as well as by ADH-T, whereas ketones **VIII** and **X** although smaller than 150.0 Å^3 molecular sizes were not reduced. Moreover, ketone **XII** and **XIII** with molecular sizes higher than 200 Å^3 were reduced to some extent. This shows that not only the size determines the reactivity but that there might be an influence of the nature of the substrate on the reaction.

Effect substrate solubility

The other important criterion that has significant influence on the reaction is the solubility of the substrate ketones. Practically the enzymatic reactions are generally carried out in a buffer solution to maintain the optimal conditions considering enzyme stability. However, the majority of ketones of interest are highly hydrophobic, and thus possesses low solubility in aqueous media leading to low substrate concentrations ranging from <5 to 10 mM. One of the traditional methods used to overcome this drawback is the usage of two phase system in which a co-solvent is be added to raise the substrate solubility in the reaction mixture.[50–56] This co-solvent can either be water-miscible[57–60] resulting in a one-phase system or, it can be water-immiscible[50,51,61] leading to an aqueous–organic two-phase system. Table 2 summarizes the effect of solubility of different ketones used in the present study on the enzymatic reduction. From the results it is observed that there is no specific trend on the reduction with solubility. Ketones **I**, **II** and **III**, though sparingly soluble in the buffer medium, are quantitatively reduced whereas **V** though soluble (and **VI** sparingly soluble) in the medium remain unreduced. However, **XIII**, which is a solid and is insoluble in the medium is reduced to an appreciable amount using the two-phase system with ethylacetate. In case of other ketones, we

Table 2 Solubility of different ketones in the buffer medium and different methods used for the ADH-LB and ADH-T reductions

Ketone	Solubility in buffer medium	Method used[a]	Solvent used in Method B[a]	Conversion (%) by NMR
V	Soluble	A & B	Ethylacetate	0
I	Sparingly soluble	A & B	Ethylacetate	99[b]
II	Sparingly soluble	A & B	Ethylacetate	97[b]
III	Sparingly soluble	A & B	Ethylacetate	99[b]
VI	Sparingly soluble	A & B	Ethylacetate, diisopropylether	0
XII	Sparingly soluble	A & B	Ethylacetate	26[c]
IV	Insoluble	B	Ethylacetate, diisopropylether	0
XIII	Insoluble	B	Ethylacetate	15[c]

[a] Method A: Consists of 20% solution of 2-propanol in 50 mM PBS buffer (pH 7.0); Method B: Consists of 50/50 mixture of an organic solvent (ethylacetate or dichloromethane or diisopropylether) and 20% solution of 2-propanol in 50 mM PBS buffer (pH 7.0). [b] The conversion data obtained from method A. [c] The product was not isolated (see Fig. 4 and 5 in ESI†).

did not observe any change in results by changing the reaction condition to a two-phase system with an organic solvent. This indicates that solubility is also not the criterion which is influencing the reaction as the use of a suitable two-phase solvent system will aid the reaction in case the substrate is insoluble in the reaction medium. For **XII** and **XIII** the reaction stopped at conversions of 26 and 15%, respectively and further addition of NADPH or enzyme did not increase conversion. We speculate that due to the reversibility of the reaction an equilibrium position under these conditions has been reached.

Effect of electron withdrawing or electron donating property of substituent

In a recent study on effects of *para*-substituted acetophenones on the catalytic activity of 3α-hydroxysteroid dehydrogenase (3α-HSD) from rat liver (*Rattus norvegicus*) Uwai *et al*. showed that the electron donating/withdrawing property of *para*-substituent group influence the rate of reduction.[62] The introduction of an electron withdrawing group increases the rate of reduction whereas an electron donating reduces. This is further supported by a study on the reduction of substituted acetophenones against a carbonyl reductase from *Candida magnolia* by Zhu *et al*.[63,64] This is because the electron donating substituent group at the *para*-position suppresses the delocalization of the partially polarized aryl-conjugated carbonyl (C═O) > C–O bond either by resonance or by induction whereas an electron withdrawing substituent promotes the delocalization and hence the reduction. However, in the present study we did not observe any relation between the property of the *p*-substituent and the reaction. Of the ketones **II**, **III**, **IV**, **VII**, **IX** and **XIII** with electron withdrawing functional groups at the *para*-position to the carbonyl group in conjugation with aromatic ring only ketones **II**, **III**, **XIII** were successfully reduced. Again ketones **XI** and **XII** although

Table 3 Enzymatic reduction of acetophenone derivatives of different *p*-substituted functional group by ADT-LB and ADH-T enzymes

Ketone	Property of *p*-substituent	Conversion (%) by NMR
I	None	99
II	Electron withdrawing	97
III	Electron withdrawing	99
IV	Electron withdrawing	0
VII	Electron withdrawing	0
IX	Electron withdrawing	0
XIII	Electron withdrawing	15[a]
V	Mildly electron donating	0
VI	Mildly electron donating	0
XII	Mildly electron donating	26[a]
VIII	Strongly electron donating	0
X	Strongly electron donating	0
XI	Strongly electron donating	89

[a] The product is not isolated (see Fig. 4 and 5 in ESI†).

contain strongly electron donating functional groups were successfully reduced whereas reduction of ketones **V**, **VI**, **VIII** and **X**, also containing electron donating groups, were unsuccessful (Table 3). These observations are also supported by earlier literature.[65–67]

Electronic charge distribution

If we carefully analyze the results and compare them with the nature of the *p*-substituent groups of the substrate ketones, the ketones with a neutral functional group at the *para*-position were reduced successfully while reduction of ketones with ionically demanding functional groups were unsuccessful. These observations suggest that there is indeed a strong electronic effect of the nature of the *para*-substituent on the catalytic activity of alcohol dehydrogenases from *Lactobacillus brevis* and *Thermoanaerobacter sp* but not through the electron withdrawing or donating properties.

Tidor *et al.*[68,69] observed that the electrostatic interactions between ligand and biomolecules play an important role in determining binding affinities and specificities. The electrostatic interaction between the receptor protein and a ligand was described as a screened Coulombic interaction between two charges separated by an effective distance.[70] Therefore, the electrostatic interaction between the ligand and protein hugely depends on the charge density which is simply electron charge distribution on the ligand. Careful observation of the structure of the acetophenone derivatives (**I** to **XIII**) and their results suggests that a higher electronic charge density far away from the carbonyl group of acetophenone may be playing a critical role in the reduction of the substrates. Therefore the direct information about the electronic charge distribution of the ligand molecule should provide complete insight into its interaction with the enzyme. The electrostatic potential map, an overlaying of a quantity called the electrostatic potential (energy of interaction of a point positive charge with the nuclei and electrons of a molecule, depends on the location of the point positive charge) onto the electron density, is valuable for describing overall molecular charge distribution.[49] Another important parameter which provides valuable information on the molecular charge

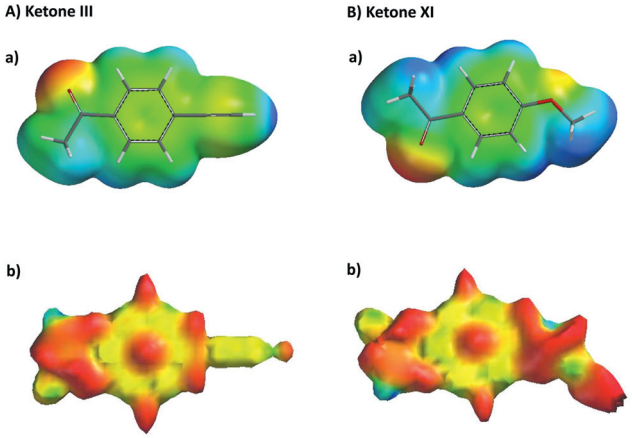


Fig. 1 Calculated electrostatic density potential (a) (in kJ mol^{-1} at the default isovalue 0.002 electron per Bohr^3) and local ionization density (b) (in kJ mol^{-1} at the default isovalue 20.0 electron per Bohr^3) surfaces showing the electron charge distribution on the molecule for ketone **III** (A), ketone **XI** (B).

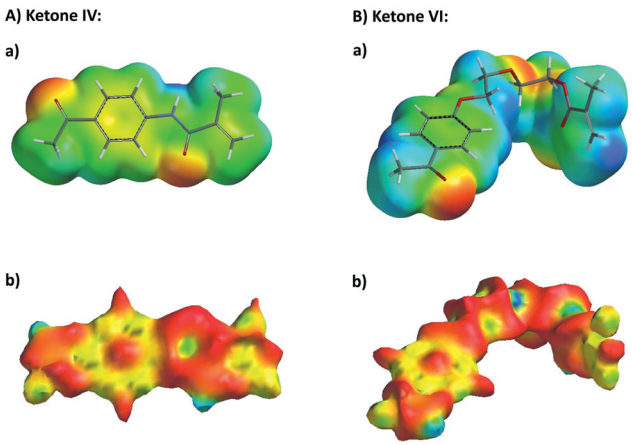


Fig. 2 Calculated electrostatic density potential (a) (in kJ mol^{-1} at the default isovalue 0.002 electron per Bohr^3) and local ionization density (b) (in kJ mol^{-1} at the default isovalue 20.0 electron per Bohr^3) surfaces showing the electron charge distribution on the molecule for ketone **IV** (A), ketone **VI** (B).

distribution is the local ionization potential map which is an overlay of the energy of electron removal ("ionization") onto the electron density.

The electrostatic potential and local ionization potential surfaces for the acetophenone derivatives in the present study have been determined using SPARTAN program[49] and are presented in Fig. 1 and 2. The figures show the electrostatic density potential map which is a direct representation of distribution of electron charge density throughout the molecule and local ionization density surface which is again a measure of charge distribution at any location around the molecule. The color red depicts regions of most negative electrostatic potential, while the color blue depicts the regions of most positive electrostatic potential. Intermediate colors represent intermediate values: red < orange < yellow < green < blue.

The Fig. 1A and 1B show the electrostatic density potential and local ionization density maps for ketones **III** and **XI** which were reduced easily by the ADH enzymes under study. From the figures it is observed that there is a high electron density (low ionization potential energy = red color) around the carbonyl group of the ketones. In contrast, Fig. 2A and 2B show the electrostatic density potential and local ionization density maps of ketones **IV** and **VI**, which remain unreduced by the enzymes in this study. In this case it is observed that the electron density is situated at the *para*-position, far away from the carbonyl group of the ketone. Similar observations were noticed for rest of the ketones (see Fig. 1–3 in ESI†).

In case of ketone **IV** the local ionization density map (Fig. 2A (b)) gives valuable information on the electron charge distribution though the density potential map (Fig. 2A(a)) does not show a clear information. The same is true for ketone **XI** where the density potential map (Fig. 1B(a)) provides complete information about the charge distribution over the molecule though the local ionization density map (Fig. 1B(b)) does not show a clear difference. Therefore electrostatic density potential and local ionization density maps together provide the information on the electronic charge distribution on a molecule.

These observations strongly suggest that the electron charge density might be playing an important role on the enzymatic reduction of ketones. With this information we hypothesize that the molecule may be entering the cavity of the enzyme with the part of the molecule having higher electron charge density facing the active site of the enzyme. Therefore in case of ketones **I**, **II**, **III** and **XI**, it is the carbonyl group with higher electron density which is facing the active site while the molecule is entering the cavity. This allows the carbonyl group of ketone to bind to the active site and get reduced. However, in case of ketones **IV**, **V** and **VI** it is the *para*-substituent group with higher electron density which is facing the active site while the molecule is entering the cavity of the enzyme. This leaves the carbonyl group of these ketones far away from binding to the active site and hence it cannot be reduced.

Conclusions

From the analysis of the various factors like solubility, electronic effects and molecular size that could explain substrate acceptance by the alcohol dehydrogenase enzymes from *Lactobacillus brevis* and *Thermoanaerobacter sp*. it is more evident that non-polar *para* substituents are favoured over polar or ionisable *para* substituents. With these results we conclude that there is an effect of the nature of the substituent group on the reduction of aromatic ketones using alcohol dehydrogenases from *Lactobacillus brevis* and *Thermoanaerobacter sp*, which were enantio-complimentary ketoreductases giving (*R*-) and (*S*-) enantiomers of the same ketone, making them useful in the synthesis of enantiopure alcohol intermediates of pharmaceutical and agricultural interest. The binding of substrates to the specificity pocket of an enzyme involves a combination of chemical forces including hydrogen bonds and electrostatic, hydrophobic, and steric interactions. Shape and charge complementarity between enzyme and substrate have been proposed as keys to enzyme function. The difference in any of these complementarities influences the binding. In the present study, we observed that there is a charge complementarity difference between enzyme and reactive group of some of the substrates induced by their electronic charge distribution. Thus, the electronic charge distribution in the molecules might be influencing the orientation of the substrate in the active site of the enzyme and hence the ability to reduce the substrate. Further investigations on influence of electron charge density and also the actual mechanism by which the nature of the substituent influences the enzyme activity are being studied and will be reported in our upcoming manuscript. These studies will help in the development of a suitable enzymatic reduction strategy for substituted acetophenones.

Experimental

Materials

Commercial reagents were used as received. Solvents for reactions (distilled THF, Et_2O; commercial benzene, toluene and CH_2Cl_2) were filtered over columns of dried alumina under a positive pressure of argon. Solvents for extractions and flash column chromatography were of technical grade and were distilled prior to use. The 4-vinylacetophenone (**II**) was synthesized as described previously.[25] Acetophenone (**I**), 4-ethynylacetophenone (**III**), *N*-(4-acetylphenyl)acetamide (**IX**) and 4-hydroxy acetophenone (**VIII**) were purchased from Acros. 4-Aminoacetophenone (**X**), 1-(4-methoxyphenyl)ethanone (**XI**), 1-(4-*tert*-butylphenyl)ethanone (**XII**), 1-(biphenyl-4-yl)ethanone (**XIII**), 2-chloroethyl benzene, sodium azide (NaN_3), glycidylmethacrylate (GMA), pMDETA, aluminum oxide (Al_2O_3), copper(I) bromide (Cu(I)Br), cesium carbonate ($CsCO_3$), triethylamine (TEA), hydrochloric acid (HCl) and $NaHCO_3$ were purchased from Aldrich whilst ammonium chloride (NH_4Cl), methacryloyl chloride and monochlorodiethyleneglycol were obtained from Fluka and all used as received. *N*-(4-Acetylphenyl)methacrylamide (**IV**), 1-(4-(2-(2-hydroethoxy)ethoxy)phenyl)ethanone (**V**), 2-(2-(4-acetylphnoxy)ethoxy)ethyl methacrylate (**VI**) and 5-(4-(4-acetylphenyl)-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-2-methylpent-1-en-3-one (**VII**), were synthesized in our lab. NADPH and alcohol dehydrogenase from *Lactobacillus brevis* (4100 U mL^{-1}) (ADH-LB) and *Thermoanaerobacter sp*. (331 U mL^{-1}) (ADH-T) were purchased from Julich Chiral Solutions GmbH, a Codexis company, Germany. All the solvents are obtained from Biosolve.

Computational details

The SPARTAN 06 program[49] running on Workstation Intel® Core™2 Quad CPU Q9550 2.66 GHz processor with 4GB RAM under Windows XP operating system was used to carry out HF and DFT-B3LYP calculations. The geometries were fully optimized at the DFT B3LYP level of theory with a 6-311G* basis set and using an *ab-initio* HF method with 6-311G* basis set. Electrostatic density potential and local ionization density surfaces were calculated at both HF/6-31G* and DFT/B3LYP/6-31G* levels. The electrostatic density potential surfaces are represented in kJ mol^{-1} at default isovalue 0.002 electron per Bohr.

Enantioselective reductions of ketones using alcohol dehydrogenase

General procedure A: (in a homogeneous medium)

Using alcohol dehydrogenase from LB (ADH-LB). 1 g of the substrate (ketone) was dissolved/suspended in a reaction mixture of 2-propanol (40 mL) and PBS buffer solution (pH 7.4, 160 mL) containing 20 mM NADPH and 0.5 mM $MgCl_2$ and maintained at 37 °C with uniform mixing. The enzyme ADH-02 (50 μL, 4100 U mL^{-1}) was then added to the reaction mixture and the mixture was allowed to stir overnight. The progress of the reaction was monitored by TLC and Chiral GC (Varian 430-GC) measurements and the mixture was treated with excess of water and extracted in methyl *t*-butyl ether. The organic layer was washed with brine, dried over magnesium sulfate and concentrated under reduced pressure.

Using alcohol dehydrogenase from T (ADH-T). Substrates were reduced by ADH-05 using the procedure similar to the one used for ADH-02 enzyme but without $MgCl_2$. In brief, 1 g of the substrate (ketone) was dissolved/suspended in a reaction mixture of 2-propanol (40 mL) and PBS buffer solution (pH 7.4, 160 mL) containing 20 mM NADPH and maintained at 37 °C with uniform mixing, followed by the addition of ADH-05 enzyme (285 μL, 331 U mL^{-1}). The reaction mixture was stirred overnight and the progress of the reaction was monitored by TLC and Chiral GC (Varian 430-GC) measurements. The mixture was treated with excess water and extracted in methyl *t*-butyl ether. The organic layer was washed with brine, dried over magnesium sulfate and concentrated under reduced pressure.

General procedure B: (in a heterogeneous medium). In this method, the substrates were reduced by the respective enzymes in a heterogeneous organic–aqueous biphasic medium to improve the solubility of the poorly water-soluble or water insoluble ketones. The reaction conditions were similar to those in procedure A for both the enzymes, except that the medium contained a varying aqueous (PBS buffer pH 7.4/2-propanol) to an organic solvent (dichloromethane, diisopropylether, ethylacetate or ionic liquid (AMMOENG™ 100)) ratio by volume.

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