Highly efficient asymmetric reduction of arylpropionic aldehydes by Horse Liver Alcohol Dehydrogenase through dynamic kinetic resolution†‡

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The enantioselective synthesis of (2*S*)-2-phenylpropanol and (2*S*)-2-(4-*iso*-butylphenyl)propanol ((*S*)-Ibuprofenol) has been achieved by means of Horse Liver Alcohol Dehydrogenase (HLADH) in buffered aqueous solution or buffered organic solvent mixtures; under the reaction conditions, a dynamic kinetic resolution (DKR) process was realized with good reaction yields and enantiomeric ratios.

Dynamic kinetic resolution (DKR),[1] which combines resolution and *in situ* racemization of the unreacted enantiomer, is known to be a promising alternative process to overcome the limitations in kinetic resolution (KR) of a 50% yield. In DKR processes, a 100% yield of a single enantiomer can be reached whenever the racemization rate successfully competes with the resolution reaction. DKR processes are particularly attractive for the pharmaceuticals and fine chemicals industries in the production of enantiomerically pure drugs and intermediates because of increased yields (theoretically up to 100%), and the disappearance of the unwanted enantiomer (distomer) and related problems (waste or recycling).

In DKR processes, enzymes have been successfully employed as catalysts in the racemization or resolution steps.[2] Lipases have been used in esterification or hydrolysis reactions in highly stereoselective DKR, with good yields and under mild conditions.[3] Dehydrogenases have been employed less frequently in organic synthesis than lipases because of several drawbacks: (i) a limited number of commercially available purified enzymes, (ii) limited stability and activity in non-aqueous solvents, and (iii) the requirement for a cofactor and its associate recycling system that increases the complexity of the reaction.

However, recent applications of alcohol dehydrogenases and carbonyl reductases have shown that the productivity of these systems, in particular in the reduction mode, is constantly increasing,[4] and some examples of DKR by dehydrogenases have been successfully reported.[5]

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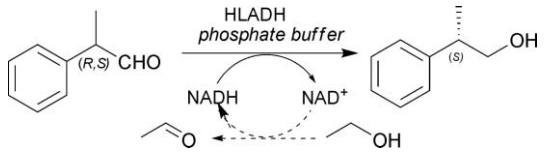
We present here our preliminary results in the asymmetric reduction of racemic 2-phenylpropanal and Ibuprofenal to their corresponding (2*S*)-2-phenyl-1-propanol and (*S*)-Ibuprofenol derivatives by means of Horse Liver Alcohol Dehydrogenase (HLADH) in an aqueous–organic medium, thus allowing an efficient biocatalysed DKR process. The value of this finding resides in the application of these two chiral intermediates to the synthesis of optically active pharmaceutical products of the Profen class (Ibuprofen) and fragrances.[6]

Two independent research groups have recently reported examples of the DKR of arylpropanols by means of a hydrogenation reaction catalysed by chiral ruthenium complexes. However, these methods employ very harsh conditions in comparison to a biocatalytic system.[7]

We recently reported a study on engineered Phenylalanine Dehydrogenase for the enantioselective reductive amination of phenylpyruvic acid in aqueous–organic solvents,[8] demonstrating a good tolerance for organic solvents in homogeneous or biphasic systems. Therefore, as a consequence of our efforts, we turned our attention to the use of alcohol dehydrogenases in organic solvents for studying solvent effects in enzymatic reactions.[9] Klibanov and co-workers reported the use of alcohol dehydrogenases in organic solvents for the asymmetric reduction of some aldehydes and ketones; but in di-*iso*-propylether (pre-saturated with aqueous buffer), the reaction proceeded with very low conversions (13–20%).[10] In a preliminary exploration, we selected commercially available Horse Liver Alcohol Dehydrogenase (HLADH Sigma) for the reduction of racemic 2-phenylpropanal (0.5 mM) in 0.1 M phosphate buffer solution (pH = 7.5) and 1 mM NADH. Reduction was monitored by HPLC analysis at the reaction times reported in Table 1, Table 2 and Table 3. HPLC analyses were performed on a C-8 column (eluent H_2O/CH_3CN), with alcohol yields being determined *via* a standard calibration curve. The (*S*)% and (*R*)% were obtained on a chiral column CHIRALCEL OF (eluent hexane/*iso*-propanol). Assignment of chromatographic peaks to the (*S*)- or (*R*)-alcohols was done by the HPLC analysis (CHIRALCEL OF) of an authentic sample of commercially available (2*S*)-2-phenyl-1-propanol (see ESI for further details‡).

Surprisingly, the yield after a 5 h reaction time exceeded 80%, with an enantiomeric ratio (*S*)/(*R*) of 82 : 18, which remained almost unvaried over 24 h with little increase in the yield (Table 1, entries 1 and 2). A reduced amount of the biocatalyst did not adversely affect the yield, and yet increased the enantiomeric ratio (Table 1, entries 3 and 4). The addition of ethanol to the reaction mixture allowed the use of a catalytic amount of cofactor, which was successfully regenerated *in situ* by the same HLADH. The

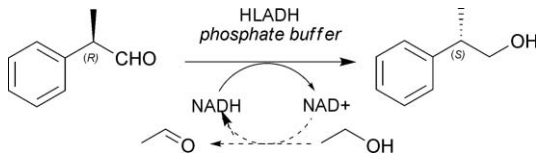
Table 1 DKR of racemic 2-phenylpropanal in phosphate buffer (pH = 7.5)



Entry	Alcohol yield (%)	(*S*)-Alcohol (%)	(*R*)-Alcohol (%)	HLADH/ mg ml^{-1}	Reaction time/h
1[a]	81.1	82	18	0.01	5
2[a]	88.5	81	19	0.01	24
3[a]	75.1	>99	not detected	0.002	5
4[a]	89.1	95	5	0.002	24
5[b]	88.7	84	16	0.01	5
6[b]	99.4	83	17	0.01	24

[a] 0.5 mM 2-phenylpropanal, 1 mM NADH. [b] 0.5 mM 2-phenylpropanal, 0.01 mM NADH, 0.5 M ethanol.

Table 2 DKR of (*R*)-2-phenylpropanal in phosphate buffer (pH = 7.5)



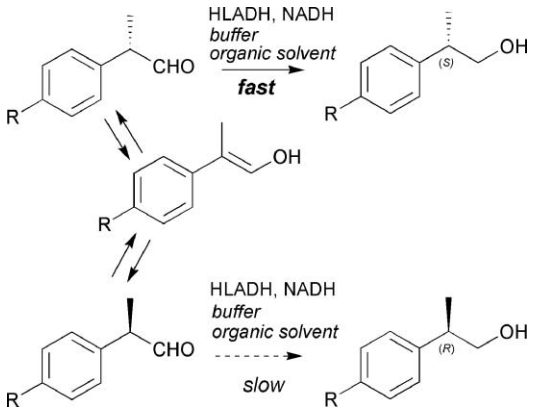
Entry	Alcohol yield (%)	(*S*)-Alcohol (%)	(*R*)-Alcohol (%)	HLADH/ mg ml^{-1}	Reaction time/h
1[a]	96.2	96	4	0.002	27
2[b]	94.7	95	5	0.002	27

[a] 0.5 mM (*R*)-2-phenylpropanal, 1 mM NADH. [b] 0.5 mM (*R*)-2-phenylpropanal, 0.01 mM NADH, 0.5 M ethanol.

yield in this case reached its maximum value in 24 h, with unvaried enantiomeric ratios (Table 1, entries 5 and 6).

Quantitative conversion of the racemic aldehyde to the preferred (*S*)-alcohol needed a DKR *via* a racemization reaction of the less reactive (*R*)-2-phenylpropanal (Scheme 1).

To confirm this mechanism, we prepared enantiomerically pure (*R*)-2-phenylpropanal, starting from (2*R*)-2-phenyl-1-propanol *via* a Dess–Martin periodinane oxidation,[11] and performed the enzymatic reduction. The reaction gave excellent results in terms of yields, enantiomeric ratios and cofactor recycling (Table 2), thus



Scheme 1

confirming the concurrent racemization reaction and reductive stereoinversion.[12]

The poor solubility of 2-phenylpropanal in buffer solutions is exacerbated in the scale-up of the process and in the application of the protocol to more lipophilic derivatives. We therefore tried to incorporate some organic co-solvents into the aqueous medium; CH_3CN, THF and *n*-hexane were chosen as representative examples of miscible, partially-miscible and immiscible solvents with water.

10% CH_3CN or THF (v/v) with respect to the buffer solution was well tolerated by HLADH (Table 3). Enantiomeric ratios were better with THF (10%) than with CH_3CN (10%), albeit with lower yields, whereas higher amounts of either of the co-solvents totally inhibited the reaction.

A good result was obtained in the attempt to scale-up the process to a millimolar preparative scale with CH_3CN (16%) and cofactor recycling (Table 3, entry 2); we obtained the desired alcohol in 90% isolated yield after flash chromatography with good enantioselectivity (94% of (2*S*)-2-phenyl-1-propanol).

The reaction did not work in *n*-hexane 10% (v/v with respect to the buffer). By increasing the percentage of hexane (90–99% v/v), and changing the protocol by adding an excess of lyophilised enzyme and solid NADH directly into the reaction mixture under vigorous stirring, the reaction proceeded well, and in some cases greatly exceeded 50% yield.

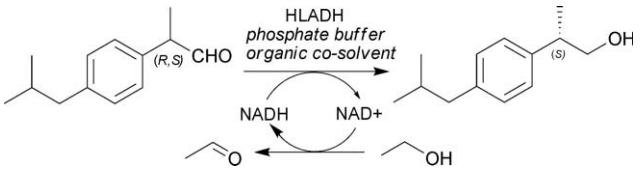
Direct application of our DKR enzymatic reduction of 2-phenylpropanal to interesting intermediates in the

Table 3 DKR of racemic 2-phenylpropanal in phosphate buffer/organic co-solvent (v/v)

Entry	Co-solvent (%)	Alcohol yield (%)	(*S*)-Alcohol (%)	(*R*)-Alcohol (%)	HLADH/mg ml^{-1}	Reaction time/h
1[a]	CH_3CN (10)	71.9	89	11	0.01	5
2[b]	CH_3CN (16)	90.3	94	6	0.09	96
3[a]	THF (10)	54.7	96	4	0.01	5
4[c]	THF (10)	54.1	95	5	0.01	5
5[a]	*n*-hexane (90)	69.9	74	26	0.2	5
6[a]	*n*-hexane (95)	80.3	64	36	0.2	5
7[c]	*n*-hexane (95)	68.0	72	28	0.2	5
8[c]	*n*-hexane (99)	54.6	90	10	0.2	5

[a] Reaction conditions: 0.5 mM 2-phenylpropanal, 1 mM NADH. [b] Reaction conditions: 2-phenylpropanal (1 mmol, 0.045 M), NADH (1 mmol), EtOH (5.1 mmol) in 24 ml of phosphate buffer–CH_3CN (16%). % yield after flash chromatography. [c] Reaction conditions: 0.5 mM 2-phenylpropanal, 0.01 mM NADH, 0.5 M ethanol.

Table 4 DKR of racemic Ibuprofenal in phosphate buffer (pH = 7.5) and phosphate buffer/organic solvents with EtOH-mediated NADH recycling. Reaction conditions: 0.5 mM 2-(4-*iso*-butylphenyl)propanal, 0.01 mg ml^{-1} HLADH, 0.01 mM NADH, 0.5 M EtOH



Entry	Co-solvent (%)	Alcohol yield (%)	(*S*)-Alcohol (%)	(*R*)-Alcohol (%)	Reaction time/h
1	—	48.8	—	—	5
2	—	69.3	97	3	24
3	THF (10)	36.6	—	—	5
4	THF (10)	45.6	>99	not detected	24
5	CH_3CN (10)	81.4	—	—	5
6	CH_3CN (10)	93.0	>99	not detected	24

pharmaceutical industry was preliminarily tested on racemic Ibuprofenal.[13]

The DKR enzymatic reaction worked well in buffered aqueous solution. In the presence of an organic co-solvent (CH_3CN or THF), the yields were excellent and enantiomeric ratios always in favour of (*S*)-Ibuprofenol (Table 4).[14] The oxidation of (*S*)-Ibuprofenol to (*S*)-Ibuprofen has already been reported in the literature,[15] and here we can claim to have established a chemoenzymatic process to obtain (*S*)-Ibuprofen *via* an efficient DKR of the parent aldehyde.

Work is in progress on the optimization of the reaction parameters (pH, solvent and temperature) for a more efficient scale-up and application to other relevant Profen derivatives.

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