Biocatalytic deuterium- and hydrogen-transfer using over-expressed ADH-'A': enhanced stereoselectivity and ^{2}H-labeled chiral alcohols†

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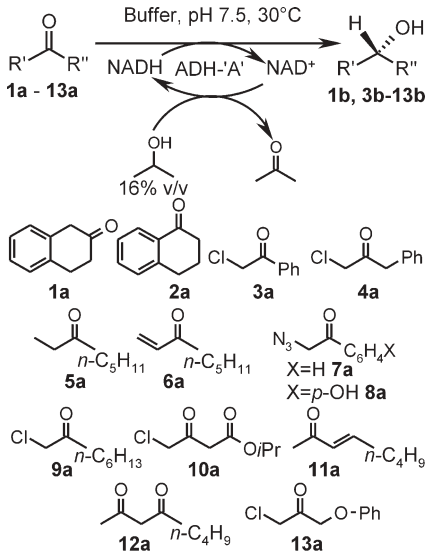
Employing the over-expressed highly organic solvent tolerant alcohol dehydrogenase ADH-'A' from *Rhodococcus ruber* DSM 44541, versatile building blocks, which were not accessible by the wild type catalyst, were obtained in > 99% e.e.; furthermore, employing d_8-2-propanol as deuterium source, stereoselective biocatalytic deuterium transfer was made feasible to furnish enantiopure deuterium labeled *sec*-alcohols on a preparative scale employing a single enzyme.

Stereoselective reduction of ketones is one of the most widely employed methods for the synthesis of non-racemic chiral alcohols in organic synthesis.[1] In this context, biocatalytic approaches for ketone reduction employing alcohol dehydrogenases (ADHs) have very recently gained increasing importance.[2] However, most of the identified alcohol dehydrogenases need a further ADH or a sophisticated set-up for co-factor recycling.[2d,3] Only very few ADHs allow the most simple approach, namely the simultaneous recycling of the co-factor and the reduction of the desired ketone by a single enzyme in the coupled substrate approach (Scheme 1).[4] Recently, we have successfully employed whole cells of *Rhodococcus ruber* DSM[5] 44541 in this approach[6] and have demonstrated that a single alcohol dehydrogenase named ADH-'A' is catalyzing both reactions in a hydrogen transfer like fashion. Partially purified ADH-'A' showed impressive operational stability in the presence of high concentrations of organic compounds, for instance it is active in 2-propanol up to 80% v/v, thereby allowing a shift in the equilibrium to the desired product and simultaneously solubilizing the substrate in the aqueous phase.[7]

Employing lyophilized cells of *E. coli* containing the over-expressed ADH-'A', selected substrates, which were initially transformed by the wild type-catalyst (WT) with low stereoselectivity, were reinvestigated. For instance, employing the WT-catalyst reduction of β-tetralone **1a** furnished (*S*)-β-tetralol **1b** in only 83% e.e. To our delight, when employing *E. coli*/ADH-'A' enantiopure (*S*)-β-tetralol (*S*)-**1b** with e.e. > 99% was found[8] (Table 1, Scheme 1), which indicates that further ADHs competed for this substrate in the WT. Interestingly, the regioisomer α-tetralone **2a** was neither accepted by the WT nor by *E. coli*/ADH-'A'. Encouraged by the selectivity enhancement for substrate **1a**, other previously moderate substrates like **3a–6a** were reduced, and again the corresponding alcohols (*R*)-**3b**, (*R*)-**4b**,



Scheme 1 Biocatalytic hydrogen transfer using *E. coli*/ADH-'A'.

Table 1 Comparison of selectivity and conversion for over-expressed ADH-'A' in *E. coli* with the wild type catalyst, *i.e.* whole cells of *Rhodococcus ruber* DSM 44541

	R. ruber DSM 44541 WT			*E. coli*/ADH-'A'		
Substr.	*t*/h	Conv. (%)	e.e. (%)	*t*/h	Conv.[e] (%)	e.e. (%)
1a	22	20[a]	83 (*S*)	19	89	> 99 (*S*)
2a	22	<1[a]	n.d.	19	< 1	n.d.
3a	24	21[b]	43 (*R*)	2	> 99	> 99 (*R*)[f]
4a	24	82[b]	73 (*R*)	2	> 99	> 99 (*R*)[f]
5a	22	78[a]	97 (*S*)	1	76	> 99 (*S*)
6a	24	8	85 (*S*)	2	81	> 99 (*S*)
7a	48	7	89 (*R*)	48	> 99	> 99 (*R*)[f]
8a	48	2	n.d.	48	> 99	> 99 (*R*)[f]
9a	24	99[b]	99 (*R*)	2	> 99	> 99 (*R*)[f]
10a	24	>99[b]	> 99 (*R*)	2	> 99	> 99 (*R*)[f]
11a	96	67[c]	> 99 (*S*)	1	66	> 99 (*S*)
12a	48	97[d]	> 99 (*S*)	2	98	> 99 (*S*)
13a	24	<1[b]	n.d.	2	> 99	> 99 (*R*)[f]

[a] Ref. 13*a*. [b] Ref. 13*c*. [c] Ref. 6. [d] Ref. 13*d*. [e] Substrate concentration ~ 15 g l^{-1}, 16% v v^{-1} 2-propanol. [f] Switch in CIP priority. n.d.: not determined, due to low conversion.

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(*S*)-**5b** and (*S*)-**6b** were obtained with absolute stereoselectivity (> 99% e.e.) using *E. coli*/ADH-'A' (Table 1). The most significant enhancement (43% e.e. for the WT to > 99% e.e. with *E. coli*/ADH-'A') was observed for 2-chloro-1-phenylethanol **3b**. Derivatives of **3b** are quite frequently employed as intermediates in the synthesis of pharmaceuticals, *e.g.* for the treatment of obesity and depression,[9] or for the synthesis of (*R*)-Salmeterol, employed to treat asthma and chronic bronchitis.[10]

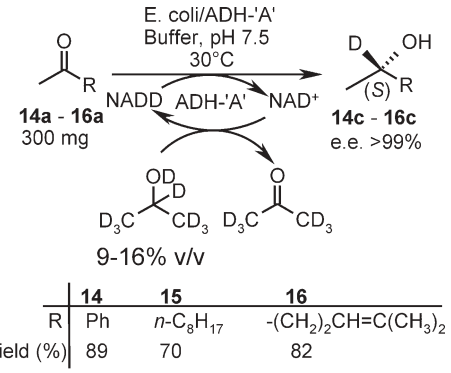
Encouraged by the higher activity and higher enantioselectivity for more sterically demanding ketones, we tested the reduction of sterically demanding α-azido ketones, *e.g.* 2-azido-1-phenylethanone **7a** and its *para*-hydroxy derivative **8a**. The corresponding azido alcohol (*R*)-**7b** is a building block for selective β_2-adrenoceptor agonists like KUR-1246[11] or denopamine, a β_1-receptor agonist effective in the treatment of congestive heart failure.[12] Indeed both ketones **7a–8a** could be stereoselectively reduced to the corresponding enantiopure (*R*)-alcohols with > 99% e.e. at complete conversion.

All substrates recently investigated[13] which were converted by the wild type catalyst *Rhodococcus ruber* DSM 44541 (WT) were transformed by lyophilized cells of *E. coli*/ADH-'A' with excellent e.e. > 99%, too, however now at significantly reduced reaction times. For instance, reaction times of one to two hours were required instead of 24 hours or more for substrates **9a–12a** (Table 1). Furthermore, dione **12a** was reduced regioselectively at the (ω−1)-position to the corresponding hydroxy ketone, (*S*)-2-hydroxy-4-octanone (*S*)-**12b**, while the oxo-moiety at carbon C-4 remained untouched.

The (*R*)-alcohol (*R*)-**13b** is an important precursor for adrenergic β-blockers.[14] Unfortunately, the corresponding ketone **13a** was not reduced by the WT-catalyst. Surprisingly, *E. coli*/ADH-'A' did display excellent activity and selectivity forming (*R*)-**13b** in > 99% e.e. The reason for this discrepancy requires further investigation. Overall, employing *E. coli*/ADH-'A' a broad variety of pharmaceuticals and related intermediates became accessible in optically pure form, which could not be obtained with the WT-catalyst.

To analyze drug metabolism or to elucidate reaction mechanisms, isotopic deuterium labeling of (bioactive) compounds is a common strategy. Biocatalytic recycling of the labeled cofactor NAD(P)D has already been successfully demonstrated with d_2-formate DCOOD and 1,1-d_2-ethanol for the preparation of primary alcohols by two enzymes.[15] To the best of our knowledge, a biocatalytic deuterium transfer approach has never been employed before for the synthesis of secondary alcohols deuterium labeled at the chiral center[16] by a single enzyme. We envisaged that d_8-2-propanol might serve as deuterium donor in an analogous fashion to 2-propanol (Scheme 2). Indeed, enantiopure (e.e. > 99%) deuterium labeled (*S*)-alcohols **14c–16c** were prepared starting from 300 mg of the corresponding ketones **14a–16a** on a preparative scale in 70 to 89% isolated yield. When comparing the reaction rate of the reduction of acetophenone **14a** with labeled and with unlabeled 2-propanol, an apparent kinetic isotope effect[17] of 3.3 was measured, as expected for a primary isotope effect.

For the identification of a suitable *E. coli*/ADH-'A' catalyst, which was employed in the experiments described above as a lyophilized powder, various *E. coli* hosts were tested employing a pET-vector (pET22b+) and the cultivation conditions were thoroughly optimized to reach the highest apparent activity per



	14	15	16
R	Ph	n-C_8H_{17}	-$(CH_2)_2CH{=}C(CH_3)_2$
yield (%)	89	70	82

Scheme 2 Preparation of deuterium labeled enantiopure alcohols *via* biocatalytic deuterium transfer.

mg lyophilized cells. The host *E. coli* Tuner™ (DE3) gave the best results, reaching under optimized reaction conditions, 2.0 U mg^{-1} cell dry weight for the coupled reduction of acetophenone and recycling of the cofactor. For comparison, employing wild type cells of *R. ruber* just 0.01–0.03 U mg^{-1} were obtained, which corresponds to an improvement up to 200-fold. It has to be emphasized that the increase of activity, as for instance observed for the shorter reaction times, resulted from the higher expression level in *E. coli* and was not due to an improved enzyme.

In summary, we could access for the first time enantiopure secondary alcohols deuterium labeled on the chiral center by biocatalytic deuterium transfer from d_8-2-propanol using a single enzyme. Lyophilized *E. coli* cells containing the over-expressed ADH-'A' were shown to be an excellent catalyst for biocatalytic deuterium and hydrogen transfer, allowing the synthesis of versatile building blocks which were not accessible by the wild type catalyst.

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