

Promiscuous Substrate Binding Explains the Enzymatic Stereo- and Regiocontrolled Synthesis of Enantiopure Hydroxy Ketones and Diols

Marcela Kurina-Sanz,^{a,*} Fabricio R. Bisogno,^b Iván Lavandera,^b
Alejandro A. Orden,^a and Vicente Gotor^{b,*}

^a INTEQUI-CONICET, Área de Química Orgánica, Facultad de Química, Bioquímica y Farmacia, UNSL, Chacabuco y Pedernera, 5700 San Luis, Argentina

Fax: (+54)-2652-426-711; e-mail: mkurina@unsl.edu.ar

^b Departamento de Química Orgánica e Inorgánica, Instituto Universitario de Biotecnología de Asturias, University of Oviedo, 33006 Oviedo, Spain

Fax: (+34)-985-103-448; e-mail: vgs@fq.uniovi.es

Received: April 2, 2009; Revised: May 29, 2009; Published online: July 27, 2009



Supporting information for this article is available on the WWW under
<http://dx.doi.org/10.1002/adsc.200900218>.

Abstract: Regio- and stereoselective reductions of several diketones to afford enantiopure hydroxy ketones or diols were accomplished using isolated alcohol dehydrogenases (ADHs). Results could be rationalised taking into account different (promiscuous) substrate-binding modes in the active site of the enzyme. Furthermore, interesting natural cyclic diketones were also reduced with high regio- and stereoselectivity. Some of the 1,2- and 1,3-diketones used

in this study were reduced by employing a low excess of the hydrogen donor (2-propanol) due to the quasi-irreversibility of these ADH-catalysed processes. Thus, using lower quantities of co-substrate, scale-up could be easily achieved.

Keywords: alcohol dehydrogenases; diols; hydrogen transfer; hydroxy ketones; quasi-irreversible reduction

Introduction

For decades, synthetic organic chemists have devoted their efforts in developing new and versatile protecting-deprotecting strategies in order to selectively modify similar reacting moieties in the same molecule. Hence, considering the loss of the yields and the laborious separation techniques in multistep protocols, a different approach is always desired. Biocatalysis has become a powerful tool for highly stereo- and regioselective transformations due to the intrinsic chirality of enzymes.^[1] In particular, biocatalysed regioselective transformations using hydrolases have been widely described in the literature,^[1,2] while the use of oxidoreductases has less frequently been performed.

It is well established that enantioenriched *anti* diols can be readily available through epoxide ring-opening starting from the suitable oxirane.^[3] On the other hand, *syn* diols can be obtained *via* osmium-catalysed oxidation of olefins, among other methods.^[4] Enantioselective epoxide hydrolase-catalysed oxirane opening

leads to the preparation of *anti* diols,^[5] but the intrinsic handicap of the maximum 50% yield still remains. Aldolases have also been employed to afford *syn*- or *anti*-derivatives, although the reversibility of these processes appears as the main drawback.^[6]

However, the use of alcohol dehydrogenases (ADHs, also called ketoreductases or carbonyl reductases) applied to the synthesis of such important compounds has been scarcely developed. In the last few years, the use of purified or overexpressed ADHs have gained increasing relevance.^[7–14] The requirement of expensive nicotinamide cofactors [NAD(P)H] in those processes has slowed down their industrial applicability, although the use of several methodologies to recycle the cofactor have greatly improved the efficacy of these biotransformations.^[9–18] In the particular case of the synthesis of enantiopure hydroxy ketones and/or diols starting from the corresponding diketones employing isolated ADHs, there are few examples available in the literature.^[19–26] In other cases plants or micro-organisms are employed,^[27–32] frequently affording a mixture of stereo-

and regioisomers due to the action of several enzymes with different and/or opposite selectivities. These derivatives are important building blocks of many natural compounds,^[33,34] such as pheromones^[35,36] or antitumour agents like discodermolide.^[37] As a result, they are used as chiral precursors for fine chemicals in the flavour and fragrance, agrochemical, and pharmaceutical industries.^[38] α -Hydroxy ketones (also called acyloins), constitute well-known derivatives for the synthesis of 1,2-amino alcohols through diastereoselective reductive amination.^[39] Furthermore, short-chain diols can be employed as starting materials for chiral polymers^[40] or as backbone for chiral ligands applied to asymmetric transition metal catalysis.^[41]

Herein we present a stereo- and regioselective ADH-catalysed reduction of several diketones of interest (Figure 1) with excellent *ees* and *des*, using internal recycling of cofactor (*substrate-coupled* approach). We will focus on the different substrate recognition modes in order to explain the experimental results obtained, and we will show for the first time the quasi-irreversible reduction concept^[42] applied to the ADH-catalysed reduction of 1,2- and 1,3-diketones.

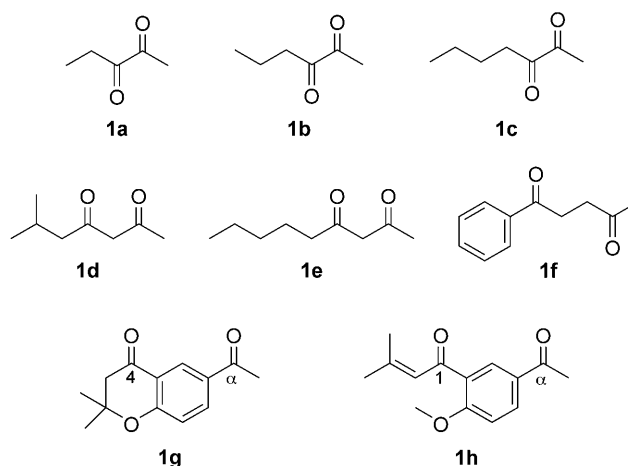
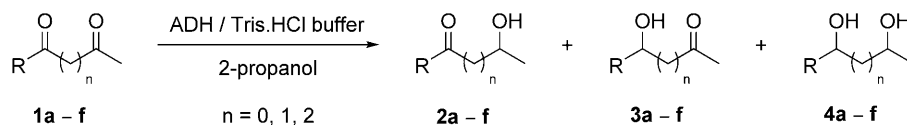


Figure 1. Diketones employed in this study.

Results and Discussion

As a first step, several diketones (Figure 1) were chosen as suitable substrates to be reduced by commercial ADHs.^[43] Thus, 1,2- (**1a–c**), 1,3- (**1d** and **e**), and 1,4-diketones (**1f**) were firstly used in order to study the acceptance of the tested enzymes (Scheme 1 and Table 1). *Lactobacillus brevis* ADH (LBADH),^[44]



Scheme 1. Regio- and stereoselective reduction of 1,2-, 1,3-, and 1,4-diketones using ADHs and 2-propanol.

Table 1. ADH-catalysed reduction of diketones **1a–f** with 2-propanol.^[a]

Entry	Substrate	ADH	2 [%] ^[b]	<i>ee</i> [%] ^[c]	3 [%] ^[b]	<i>ee</i> [%] ^[c]	4 [%] ^[b]	<i>de</i> [%] ^[c]	<i>ee</i> [%] ^[c]
1	1a	LBADH	26	> 99 (<i>R</i>)	3	99 (<i>R</i>)	69	91 (2 <i>R</i> ,3 <i>R</i>)	> 99
2	1a	ADH-‘A’	68	> 99 (<i>S</i>)	–	–	32	62 (2 <i>S</i> ,3 <i>R</i>)	> 99
3	1a	ADH-T	3	99 (<i>S</i>)	6	66 (<i>R</i>)	89	74 (2 <i>S</i> ,3 <i>R</i>)	> 99
4	1b	LBADH	92	> 99 (<i>R</i>)	–	–	6	99 (2 <i>R</i> ,3 <i>S</i>)	> 99
5	1b	ADH-‘A’	49	> 99 (<i>S</i>)	2	99 (<i>R</i>)	46	95 (2 <i>S</i> ,3 <i>R</i>)	> 99
6	1b	ADH-T	3	99 (<i>S</i>)	3	99 (<i>R</i>)	91	96 (2 <i>S</i> ,3 <i>R</i>)	> 99
7	1c	LBADH	42	> 99 (<i>R</i>)	–	–	10	99 (2 <i>R</i> ,3 <i>S</i>)	> 99
8	1c	ADH-‘A’	4	99 (<i>S</i>)	–	–	49	99 (2 <i>S</i> ,3 <i>R</i>)	> 99
9	1c	ADH-T	–	–	–	–	62	99 (2 <i>S</i> ,3 <i>R</i>)	> 99
10	1d	LBADH	> 97	> 99 (<i>R</i>)	–	–	–	–	–
11	1d	ADH-‘A’	> 97	> 99 (<i>S</i>)	–	–	–	–	–
12	1e	LBADH	> 97	> 99 (<i>R</i>)	–	–	–	–	–
13	1e	ADH-‘A’	> 97	> 99 (<i>S</i>)	–	–	–	–	–
14	1f ^[d]	LBADH	25	> 99 (<i>R</i>)	–	–	–	–	–
15	1f ^[d]	ADH-‘A’	60	> 99 (<i>S</i>)	–	–	–	–	–
16	1f ^[d]	ADH-T	62	> 99 (<i>S</i>)	–	–	–	–	–

^[a] *Reaction conditions*: the corresponding diketone (**1a–f**, 100 mM) was added to the 50 mM Tris-HCl buffer pH 7.5 with the corresponding cofactor (1 mM) and 2-propanol (5% v/v).

^[b] Measured by GC.

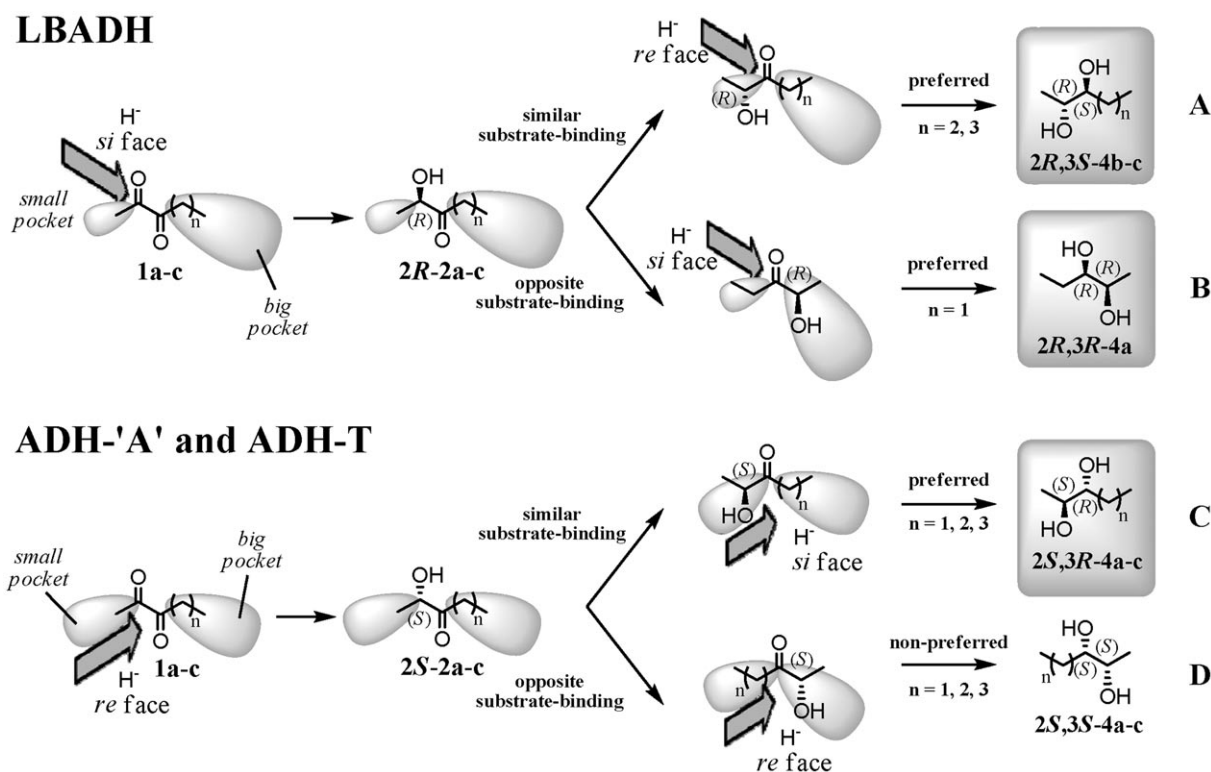
^[c] Calculated by chiral GC.

^[d] Less than 5% of a by-product was formed.

alcohol dehydrogenase from *Rhodococcus ruber* (ADH-‘A’),^[45] and ADH from *Thermoanaerobacter* sp. (ADH-T)^[46] were selected as biocatalysts to perform the corresponding reductions using an excess of 2-propanol as hydrogen donor, through a ‘substrate-coupled’ or ‘biocatalytic hydrogen transfer’ approach.^[14] The first enzyme shows a perfect anti-Prelog selectivity, that is, it catalyses the transfer of the hydride to the carbonyl moiety through its *si* face, while the second and third ADHs display an excellent Prelog profile.

As can be noted, these ADHs accepted all the tested substrates although showing different behaviour. Thus, with diketone **1a**, LBADH mainly afforded enantiopure *syn* derivative **2R,3R-4a** (entry 1), while ADH-‘A’ mainly provided enantiopure hydroxy ketone **S-2a** (entry 2) and ADH-T *anti* diol **2S,3R-4a** (entry 3). For the other 1,2-diketones **1b** and **1c**, LBADH showed a high preference for the regio- and stereoselective reduction of the carbonyl moiety at position 2, providing **R-2b** and **R-2c** and a small quantity of *anti* diols **2R,3S-4b** and **2R,3S-4c** (entries 4 and 7). Meanwhile, Prelog biocatalysts allowed us to obtain enantiopure *anti* diols **2S,3R-4b** and **2S,3R-4c** (entries 6, 8, and 9). On the other hand, the reduction of diketone **1b** with ADH-‘A’ afforded 49% of the enantiopure hydroxy ketone **S-2b** (entry 5).

Looking at these results, it was obvious that the substrate structure greatly influenced the ADH-catalysed reductions. For instance, LBADH mainly provided the unexpected *syn* diol **4a** starting from diketone **1a**, but when the substrate presented an additional methylene group in the alkyl chain (**1b**), hydroxy ketone **2b** resulted in the main product and enantiopure *anti* diol **4b** as minor product. Especially the formation of *syn-4a* was very remarkable since this enzyme has always been described as a perfect anti-Prelog enzyme.^[44] These data can be explained on the basis of promiscuous substrate binding of 1,2-diketones at the active site of the enzyme (Scheme 2). Thus, these substrates possess two reactive positions, and therefore different binding modes can be involved. For 1,2-diketones such as **1a–c**, the carbonyl moiety at position 2 will be reduced following the standard fashion, that is, with the methyl group located in the small pocket and the ethylcarbonyl moiety in the big one. The conflict arises when the ketone at position 3 is also reduced. Since location of the nicotinamide cofactor is well defined in the active site of ADHs,^[47] the hydride attack will take place through the same side, but two possible substrate orientations can exist: *i*) a similar one as that previously described, locating the hydroxyethyl group into the small binding site and the ethyl moiety into the bigger site, thus affording the corresponding *anti* diols **4a–c**



Scheme 2. Promiscuous binding modes of diketones **1a–c** and hydroxy ketones **2a–c** in the active site of alcohol dehydrogenases: LBADH (binding modes A and B); and ADH-‘A’ and ADH-T (binding modes C and D).

(Scheme 2, binding-modes A and C), or *ii*) the opposite substrate binding providing the *syn* derivatives (Scheme 2, binding modes B and D). Depending on the pocket sizes of the biocatalyst, one mode will be favoured at the expense of the second one, and therefore different regio- and stereoisomer ratios will be obtained.

From the obtained results, LBADH favoured the opposite disposition of hydroxy ketone **2a** with regards to diketone **1a** (Scheme 2, mode B), while for alcohols **2b** and **2c** a similar binding was preferred (Scheme 2, mode A). For **2b** and **2c**, since in the reverse binding mode a propyl or a butyl moiety, respectively, should be located in the small pocket, this biocatalyst was not able to afford the corresponding *syn* diols. The hydroxyethyl group already seems to crowd the small pocket of this ADH due to the low conversions obtained (less than 10%) for **4b** and **4c** (Table 1, entries 4 and 7). On the other hand, ADH-‘A’ and ADH-T always preferred a similar disposition for **1a–c** and **2a–c** placing the larger acyl chain into the bigger binding site (Scheme 2, mode C), thus mainly affording the corresponding *anti* diols **4a–c**. The larger the difference between the acyl chain and the hydroxyethyl moiety was, the better were the *des* favouring the *anti* derivatives (Table 1, compare entries 2–3 with 5–6 and 8–9).

A clearer scenario appeared when 1,3- or 1,4-diketones (**1d–f**) were subjected to the hydrogen transfer conditions (Table 1, entries 10–16). ADHs showed perfect regio- and stereoselectivity achieving the reduction of the carbonyl moiety at position 2 to afford enantiopure *R*-**2d–f** (in the case of LBADH) or *S*-**2d–f** (in the case of ADH-‘A’ and ADH-T) alcohols. Diketone **1f** was less well accepted, obtaining moderate (with LBADH) or good conversions (with both Prelog enzymes). In this case another by-product (less than 5%) was formed.

The selective modification of natural compounds in order to obtain novel derivatives with enhanced biological activities is a difficult task which organic chemists must often face.^[48,49] In our particular case, the natural product 6-acetyl-2,2-dimethyl-2,3-dihydro-4*H*-chromen-4-one (**1g**) and the derivative 1-(5-acetyl-2-methoxyphenyl)-3-methylbut-2-en-1-one (**1h**) were also chosen as substrates to study the regio- and stereoselectivity in the bioreduction processes catalysed by isolated ADHs (Table 2). The former was isolated from *Ophryosporus axilliflorus* (Griseb.) Hieron (Asteraceae). These and other related compounds showed anti-inflammatory activity.^[50] Previous reductions of diketone **1g** using plants were reported.^[27,51] It remained clear that LBADH was not a suitable enzyme to perform these bioreductions due to low conversions (entries 1 and 4). This is in concordance with the small active site of this biocatalyst. On the contrary, ADH-‘A’ and ADH-T were able to re-

Table 2. ADH-catalysed reduction of natural compounds **1g** and **1h** with 2-propanol.^[a]

Entry	Substrate	ADH	2 [%] ^[b]	<i>ee</i> [%] ^[c]
1	1g	LBADH	9	> 99 (<i>R</i>)
2	1g	ADH-‘A’	90	94 (<i>S</i>)
3	1g	ADH-T	86	90 (<i>S</i>)
4	1h	LBADH	3	n. d.
5	1h	ADH-‘A’	95	> 99 (<i>S</i>)
6	1h	ADH-T	45	> 99 (<i>S</i>)

^[a] Reaction conditions: the corresponding diketone (**1g** and **1h**, 50 mM) was added to the 50 mM Tris-HCl buffer pH 7.5 with the corresponding cofactor (1 mM) and 2-propanol (5% v/v).

^[b] Measured by GC.

^[c] Calculated by chiral HPLC. n. d. not determined.

gioselectively reduce both substrates on the acetyl moiety (C_α) affording highly pure (*S*)-**2g** and (*S*)-**2h** alcohols (entries 2, 3, 5 and 6).

Until this point, we were pleased to find out that different kinds of diketones could regio- and stereoselectively be reduced by the tested ADHs. Having in mind the scale-up of these processes, we tried to perform the bioreductions at lower co-substrate concentrations, using less harmful conditions for the biocatalyst and maximising the *atom efficiency*^[52] of the process. We envisaged the possible irreversibility in the reduction of 1,2- and 1,3-diketones since it has previously been described that 1-chloro-2-propanol^[42] cannot be oxidised by ADHs due to an intramolecular H-bond interaction between the hydroxy group and the halogen atom.^[53] Thus, 1,2- or 1,3-diketones could quasi-irreversibly be reduced since it is well known that the corresponding hydroxy ketones show a strong intramolecular interaction *via* H-bonding network (Figure 2).^[54]

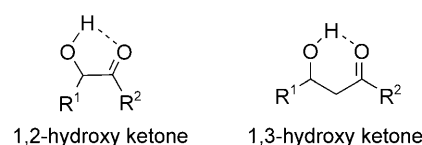


Figure 2. Intramolecular H-bond interactions in 1,2- and 1,3-hydroxy ketones.

In order to demonstrate this fact,^[55] we repeated the LBADH-catalysed reduction of diketones **1b**, **1d**, and **1e** employing different concentrations of 2-propanol (from 2 equiv. to 10 equiv.), and results were compared with that obtained in the case of a non-activated ketone such as 2-octanone (**1i**). As can be noticed (Table 3), diketones were reduced into the corresponding hydroxy ketones (>90%) even at low 2-propanol concentrations (entries 1, 4 and 7). On the contrary, for ketone **1i** (entry 10) conversion was 67%

Table 3. LBADH-catalysed reduction of 1,2- (**1b**), 1,3-diketones (**1d** and **1e**) and 2-octanone (**1i**), using different concentrations of 2-propanol.^[a]

Entry	Substrate	[2-propanol] [M]	2 [%] ^[b]
1	1b	0.2	94
2	1b	0.5	95
3	1b	1	97
4	1d	0.2	92
5	1d	0.5	91
6	1d	1	94
7	1e	0.2	95
8	1e	0.5	94
9	1e	1	92
10	1i	0.2	67

^[a] *Reaction conditions:* the corresponding diketone (100 mM) was added to the 50 mM Tris-HCl buffer pH 7.5 with the corresponding cofactor (1 mM) and 2-propanol (200 mM–1 M).

^[b] Measured by GC.

due to the similar equilibrium constants between the 2-octanone/2-octanol and acetone/2-propanol pairs.^[56]

Since a small amount of 2-propanol (2 equiv.) can be employed to quantitatively reduce this type of substrates, enantiopure (*R*)-alcohols **2b**, **2d**, and **2e** were prepared on a higher scale starting from 25 mg of the corresponding diketones with isolated yields higher than 80%.

Conclusions

The bioreduction of diketones has not been extensively studied,^[19–26] and in several cases micro-organisms or plants^[27–32] were used for this purpose. Thus, the obtained results in terms of regio- and stereoselectivity usually remained unclear. Herein we have shown the bioreduction of several diketones of interest catalysed by isolated alcohol dehydrogenases. With the advantage of using a single enzyme, we have proposed that in reductions of 1,2-diketones, these substrates can be suited within the active site of the enzyme in two different orientations depending on the size of the enzyme pockets. Hence, just by choosing the proper catalyst, *syn* or *anti* products could be obtained. For instance, due to its small binding site, LBADH presents a promiscuous binding for the diketone **1a** and the hydroxy ketone intermediate to afford the corresponding *syn*-diol. On the other hand, ADH-‘A’ and ADH-T favour a similar recognition mode for both species, thus mainly providing the *anti*-derivatives. This is an elegant example of enzyme-based stereocontrol.^[57] In the case of the other diketones studied, perfect regio- and stereoselectivity was achieved obtaining the corresponding enantiopure hydroxy ketones.

In addition, it has been demonstrated that 1,2- or 1,3-diketones can be reduced *via* ADH-catalysed hydrogen transfer using quasi-irreversible conditions,^[42] that is, employing a low excess of the required co-substrate (2-propanol). This is due to the difficulty of the enzyme to oxidise backwards the formed hydroxy ketone due to an intramolecular H-bond interaction between the hydroxy group and the remaining carbonyl moiety. Since a lower amount of the hydrogen donor (usually used in a huge excess) is needed, the scale-up of the ADH-catalysed reductions of such compounds is feasible.

Experimental Section

General Remarks

Alcohol dehydrogenases and ketones **1a–1f**, **1i** were purchased from commercial sources. Ketone **1g** was isolated from aerial parts of *Ophryosporus axilliflorus* (Griseb.) Hieron according to Favier et al.^[50] The same procedure also afforded the diketone 1-(5-acetyl-2-hydroxyphenyl)-3-methylbut-2-en-1-one which was methylated by classical methods (Me₂SO₄, K₂CO₃, under acetone reflux, 2 h) to obtain **1h**. Racemic alcohols **2a–h**, **3a–c** and **4a–c** were purchased or synthesised by conventional reduction from the corresponding ketones (NaBH₄, MeOH, 0°C or room temperature). All other reagents and solvents were of the highest quality available. 1 unit (U) of ADH reduces 1.0 μM of acetophenone to 1-phenylethanol per minute at pH 7.5 and 30°C in the presence of NAD(P)H. Flash chromatography was performed using silica gel 60 (230–400 mesh).

General Protocol for the Biocatalytic Reduction of Diketones Employing ADHs and 2-Propanol

In a 1.5-mL Eppendorf vial, 3 U of ADH (*Lactobacillus brevis* ADH, *Rhodococcus ruber* ADH-‘A’, or *Thermoanaerobacter* sp. ADH) in Tris-HCl buffer [50 mM, pH 7.5, 1 mM NAD(P)H, 1 mM MgCl₂ for LB-ADH] were mixed with 2-propanol (32 μL, 5% v/v) and the corresponding diketone (**1a–1f**, and **1i**, 100 mM; **1g**, **1h**, 50 mM). Reactions were shaken at 30°C and 120 rpm for 24 h and stopped by extraction with ethyl acetate (2×0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried (Na₂SO₄). Conversions and enantiomeric excesses of the corresponding alcohols were determined by GC or HPLC analysis on an achiral or a chiral stationary phase, respectively (see Supporting Information).

General Protocol for the Biocatalytic Reduction of Diketones Employing LBADH with Different Concentrations of 2-Propanol

In a 1.5-mL Eppendorf vial, 3 U of LBADH in Tris-HCl buffer (50 mM, pH 7.5, 1 mM NADPH, 1 mM MgCl₂) were mixed with 2-propanol (200 mM–1 M) and the corresponding diketone (**1b**, **1d**, **1e** or **1i**, 100 mM). Reactions were shaken at 30°C and 120 rpm for 24 h and stopped by extraction with ethyl acetate (2×0.5 mL). The organic layer was

separated by centrifugation (2 min, 13000 rpm) and dried (Na_2SO_4). Conversions were determined by GC analysis on an achiral stationary phase (see Supporting Information).

Representative Example for Biocatalytic Reduction of a Diketone: Preparation of (R)-2d

In a 5-mL screw-capped tube 5 U of LBADH in Tris-HCl buffer (1.8 mL, 50 mM, pH 7.5, 1 mM NADPH, 1 mM MgCl_2) were mixed with 2-propanol (27.5 μL , 0.36 mmol) and **1d** (28 μL , 0.18 mmol). Reactions were shaken at 30 °C and 120 rpm for 24 h and stopped by extraction with ethyl acetate (3 \times 1 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried (Na_2SO_4). Alcohol **2d** was purified by flash chromatography (petroleum ether: EtOAc 4:1) obtaining thus enantiopure (R)-**2d** as a colourless oil; yield: 21.5 mg (83%); $[\alpha]_{\text{D}}^{20}$: -13.7 (c 1.5, CHCl_3); IR (neat): ν =3420, 2960, 2874, 1707, 1467, 1370, 1125 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz): δ =0.92 (d, 6H, H-7, $^3J_{\text{H,H}}$ =6.6 Hz), 1.18 (d, 3H, H-1, $^3J_{\text{H,H}}$ =6.4 Hz), 2.07–2.20 (m, 1H, H-6), 2.30 (d, 2H, H-5, $^3J_{\text{H,H}}$ =6.8 Hz), 2.48 (dd, 1H, H-3, $^2J_{\text{H,H}}$ =17.8, $^3J_{\text{H,H}}$ =8.8 Hz), 2.59 (dd, 1H, H-3, $^2J_{\text{H,H}}$ =17.8, $^3J_{\text{H,H}}$ =3.1 Hz), 4.22 (m, 1H, H-2); ^{13}C NMR (CDCl_3 , 75.5 MHz): δ =22.2 (C-1), 22.4 (2C, C-7), 24.5 (C-6), 50.8 (C-3 or C-5), 52.4 (C-3 or C-5), 63.7 (C-2), 212.2 (C-4); MS (APCI⁺): m/z (%)=144 (3) $[\text{M}]^+$, 129 (6) $[\text{M}-\text{CH}_3]^+$, 126 (8) $[\text{M}-\text{H}_2\text{O}]^+$, 111 (22), 87 (42), 69 (61), 57 (80), and 43 (100).

Acknowledgements

M.K.-S. is member of the Research Career of CONICET. A.A.O. is a postdoctoral CONICET fellow. F.R.B. is supported by the Programme Alþan, the European Union Program of High Level Scholarships for Latin America (scholarship No. E07D402519AR). I.L. thanks Principado de Asturias for personal funding (Clarín Program). Financial support from the Spanish Ministerio de Ciencia e Innovación (MICINN, Project CTQ2007-61126) and the Spanish Ministerio de Asuntos Exteriores y de Cooperación (Programa de Cooperación Interuniversitaria, PCI Iberoamérica MAEC-AECID, Project A/8856/07) is gratefully acknowledged.

References

- [1] *Asymmetric Organic Synthesis with Enzymes*, (Eds.: V. Gotor, I. Alfonso, E. García-Urdiales), Wiley-VCH, Weinheim, **2008**.
- [2] U. T. Bornscheuer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis*, 2nd edn., Wiley-VCH, Weinheim, **2005**.
- [3] M. Tokunaga, J. F. Larrow, F. Kakiuchi, E. N. Jacobsen, *Science* **1997**, 277, 936–938.
- [4] C. Bolm, J. P. Hildebrand, K. Muniz, in: *Catalytic Asymmetric Synthesis*, (Ed.: I. Ojima), 2nd edn., Wiley-VCH, New York, **2000**, pp 399–428.
- [5] R. Chênevert, P. Morin, N. Pelchat, in: *Asymmetric Organic Synthesis with Enzymes*, (Eds.: V. Gotor, I. Alfonso, E. García-Urdiales), Wiley-VCH, Weinheim, **2008**, pp 133–169.
- [6] W.-D. Fessner, in: *Asymmetric Organic Synthesis with Enzymes*, (Eds.: V. Gotor, I. Alfonso, E. García-Urdiales), Wiley-VCH, Weinheim, **2008**, pp 275–318.
- [7] H. Li, D. Zhu, L. Hua, E. R. Biehl, *Adv. Synth. Catal.* **2009**, 351, 583–588.
- [8] M. J. Sorgedraeger, F. van Rantwijk, G. W. Huisman, R. A. Sheldon, *Adv. Synth. Catal.* **2008**, 350, 2322–2328.
- [9] S. Buchholz, H. Gröger, in: *Biocatalysis in the Pharmaceutical and Biotechnology Industry*, (Ed.: R. N. Patel), CRC Press, Boca Raton, **2007**, pp 757–790.
- [10] S. M. A. de Wildeman, T. Sonke, H. E. Schoemaker, O. May, *Acc. Chem. Res.* **2007**, 40, 1260–1266.
- [11] J. C. Moore, D. J. Pollard, B. Kosjek, P. N. Devine, *Acc. Chem. Res.* **2007**, 40, 1412–1419.
- [12] K. Goldberg, K. Schroer, S. Lütz, A. Liese, *Appl. Microbiol. Biotechnol.* **2007**, 76, 237–248.
- [13] W. Kroutil, H. Mang, K. Edegger, K. Faber, *Adv. Synth. Catal.* **2004**, 346, 125–142.
- [14] W. Kroutil, H. Mang, K. Edegger, K. Faber, *Curr. Opin. Chem. Biol.* **2004**, 8, 120–126.
- [15] I. Lavandera, A. Kern, M. Schaffenberger, J. Gross, A. Glieder, S. de Wildeman, W. Kroutil, *ChemSusChem* **2008**, 1, 431–436.
- [16] H. Gröger, C. Rollmann, F. Chamouleau, I. Sebastien, O. May, W. Wienand, K. Drauz, *Adv. Synth. Catal.* **2007**, 349, 709–712.
- [17] M. M. Musa, K. I. Ziegelmann-Fjeld, C. Vieille, J. G. Zeikus, R. S. Phillips, *Angew. Chem.* **2007**, 119, 3151–3154; *Angew. Chem. Int. Ed.* **2007**, 46, 3091–3094.
- [18] W. A. van der Donk, H. Zhao, *Curr. Opin. Biotechnol.* **2003**, 14, 421–426.
- [19] S. Lüdeke, M. Richter, M. Müller, *Adv. Synth. Catal.* **2009**, 351, 253–259.
- [20] M. Katzberg, K. Wechler, M. Müller, P. Dünkelfmann, J. Stohrer, W. Hummel, M. Bertau, *Org. Biomol. Chem.* **2009**, 7, 304–314.
- [21] B. T. Grau, P. N. Devine, L. N. DiMichele, B. Kosjek, *Org. Lett.* **2007**, 9, 4951–4954.
- [22] D. Kalaitzakis, J. D. Rozzell, I. Smonou, S. Kambourakis, *Adv. Synth. Catal.* **2006**, 348, 2309–2313.
- [23] J. Haberland, A. Kriegesmann, E. Wolfram, W. Hummel, A. Liese, *Appl. Microbiol. Biotechnol.* **2002**, 58, 595–599.
- [24] A. Matsuyama, H. Yamamoto, N. Kawada, Y. Kobayashi, *J. Mol. Catal. B: Enzym.* **2001**, 11, 513–521.
- [25] Y. Kawai, K. Hida, M. Tsujimoto, S. Kondo, K. Kitano, K. Nakamura, A. Ohno, *Bull. Chem. Soc. Jpn.* **1999**, 72, 99–102.
- [26] O. Bortolini, G. Fantin, M. Fogagnolo, P. P. Giovannini, A. Guerrini, A. Medici, *J. Org. Chem.* **1997**, 62, 1854–1856.
- [27] A. A. Orden, F. R. Bisogno, O. S. Giordano, M. Kurina-Sanz, *J. Mol. Catal. B: Enzym.* **2008**, 51, 49–55.
- [28] K. Edegger, W. Stampfer, B. Seisser, K. Faber, S. F. Mayer, R. Oerhrlein, A. Hafner, W. Kroutil, *Eur. J. Org. Chem.* **2006**, 8, 1904–1909.
- [29] P. Boutoute, G. Mousset, H. Veschambre, *New J. Chem.* **1998**, 22, 247–251.
- [30] H. Ikeda, E. Sato, T. Sugai, H. Ohta, *Tetrahedron* **1996**, 52, 8113–8122.

- [31] P. Besse, J. Bolte, A. Fauve, H. Veschambre, *Bioorg. Chem.* **1993**, *21*, 342–353.
- [32] J.-X. Gu, Z.-Y. Li, G.-Q. Lin, *Tetrahedron* **1993**, *49*, 5805–5816.
- [33] M. Kalesse, M. Christmann, *Synthesis* **2002**, 981–1003.
- [34] S. D. Rychnovsky, *Chem. Rev.* **1995**, *95*, 2021–2040.
- [35] D. A. Higgins, M. E. Pomianek, C. M. Kraml, R. K. Taylor, M. F. Semmelhack, B. L. Bassler, *Nature* **2007**, *450*, 883–886.
- [36] W. S. Leal, X. Shi, K. Nakamuta, M. Ono, J. Meinwald, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 1038–1042.
- [37] M. Kalesse, *ChemBioChem* **2000**, *1*, 171–175.
- [38] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Kessler, R. Stürmer, T. Zelinski, *Angew. Chem.* **2004**, *116*, 806–843; *Angew. Chem. Int. Ed.* **2004**, *43*, 788–824.
- [39] A. J. Smallridge, M. A. Trewalla, A. Maurice, A. K. Wilkinson, WO Patent 2003018531 A1 20030306; *Chem. Abstr.* **2003**, *138*, 205242.
- [40] G. N. Bennet, K. Y. San, *Appl. Microbiol. Biotechnol.* **2001**, *55*, 1–9.
- [41] M. J. Burk, M. F. Gross, J. P. Martinez, *J. Am. Chem. Soc.* **1995**, *117*, 9375–9376.
- [42] I. Lavandera, A. Kern, V. Resch, B. Ferreira-Silva, A. Glieder, W. M. F. Fabian, S. de Wildeman, W. Kroutil, *Org. Lett.* **2008**, *10*, 2155–2158.
- [43] ADHs have been purchased from Codexis or Jülich, a Codexis company.
- [44] M. Wolberg, W. Hummel, C. Wandrey, M. Müller, *Angew. Chem.* **2000**, *112*, 4476–4478; *Angew. Chem. Int. Ed.* **2000**, *39*, 4306–4308.
- [45] W. Stampfer, B. Kosjek, C. Moitzi, W. Kroutil, K. Faber, *Angew. Chem.* **2002**, *114*, 1056–1059; *Angew. Chem. Int. Ed.* **2002**, *41*, 1014–1017.
- [46] Z. Findrik, D. Vasić-Racki, S. Lütz, T. Dausmann, C. Wandrey, *Biotechnol. Lett.* **2005**, *27*, 1087–1095.
- [47] See, for instance: N. H. Schlieben, K. Niefind, J. Müller, B. Riebel, W. Hummel, D. Schomburg, *J. Mol. Biol.* **2005**, *349*, 801–813.
- [48] S. Riva, *J. Mol. Catal. B: Enzym.* **2002**, *19–20*, 43–54.
- [49] M. Ferrero, V. Gotor, in: *Enzymes in Action. Green Solutions for Chemical Problems*, (Eds.: B. Zwanenburg, M. Mikolajczyk, P. Kielbasinski), Kluwer Academic Publishers, Dordrecht, The Netherlands, **2000**, pp 347–363.
- [50] L. Favier, C. Tonn, E. Guerreiro, A. Rotelli, L. Pelzer, *Planta Med.* **1998**, *64*, 657–659.
- [51] A. A. Orden, F. R. Bisogno, D. Cifuentes, O. S. Giordano, M. Kurina-Sanz, *J. Mol. Catal. B: Enzym.* **2006**, *42*, 71–77.
- [52] B. M. Trost, *Science* **1991**, *254*, 1471–1477.
- [53] T. Goldstein, M. S. Snow, B. J. Howard, *J. Mol. Spectrosc.* **2006**, *236*, 1–10.
- [54] In fact, in a very recently published patent β -keto esters were used as oxidants: C. Peschko, J. Stohrer, (Wacker Chemie AG), German Patent DE102006009743, A1 20070906; *Chem. Abstr.* **2007**, *147*, 321414.
- [55] Further experiments were carried out in order to prove that ADHs clearly favoured the reduction of diketones over the oxidation of hydroxy ketones. Thus, the corresponding reverse ADH-catalysed oxidations with hydroxy ketones **2b**, **2d**, and **2e** were made using an excess of acetone, obtaining that **2b** was not oxidised backwards while **2d** and **2e** were slightly oxidised (around 15% conversion).
- [56] M. F. Eckstein, M. Peters, J. Lembrecht, A. C. Spiess, L. Greiner, *Adv. Synth. Catal.* **2006**, *348*, 1591–1596.
- [57] M. Hall, C. Stueckler, H. Ehammer, E. Pointner, G. Oberdorfer, K. Gruber, B. Hauer, R. Stuermer, W. Kroutil, P. Macheroux, K. Faber, *Adv. Synth. Catal.* **2008**, *350*, 411–418.