DOI: 10.1002/adsc.201200139

Access to Enantiopure α-Alkyl-β-hydroxy Esters through Dynamic Kinetic Resolutions Employing Purified/Overexpressed Alcohol Dehydrogenases

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Received: February 17, 2012; Published online: May 29, 2012

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201200139.

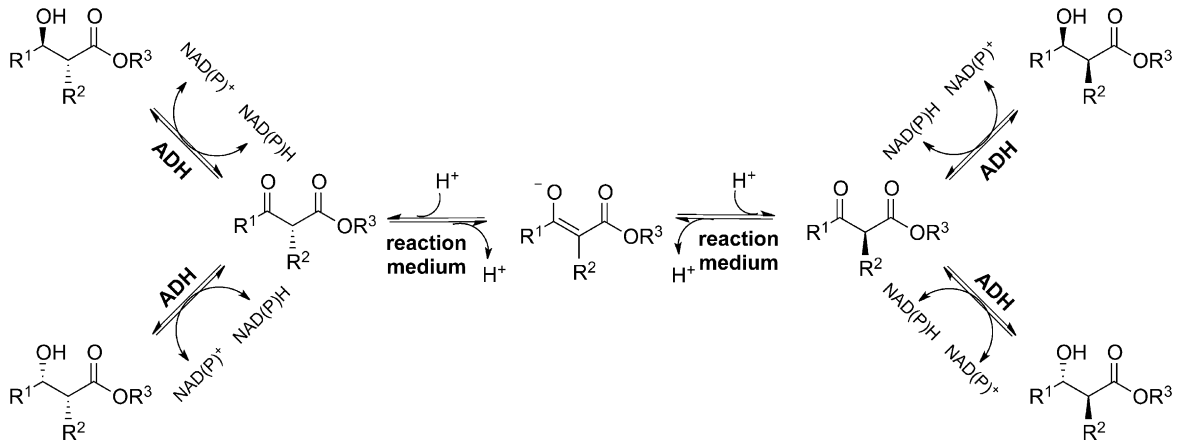
Abstract: α-Alkyl-β-hydroxy esters were obtained *via* dynamic kinetic resolution (DKR) employing purified or crude *E. coli* overexpressed alcohol dehydrogenases (ADHs). ADH-A from *R. ruber*, CPADH from *C. parapsilosis* and TesADH from *T. ethanolicus* afforded *syn*-(2*R*,3*S*) derivatives with very high selectivities for sterically not impeded ketones ('small-bulky' substrates), while ADHs from *S. yanoikuyae* (SyADH) and *Ralstonia* sp. (RasADH) could also accept bulkier keto esters ('bulky-bulky' substrates). SyADH also provided preferentially *syn*-(2*R*,3*S*) isomers and RasADH showed in some cases good selectivity towards the formation of *anti*-(2*S*,3*S*) derivatives. With *anti*-Prelog ADHs such as LBADH from *L. brevis* or LKADH from *L. kefir*, *syn*-(2*S*,3*R*) alcohols were obtained with high conversions and diastereomeric excess in some cases, especially with LBADH. Furthermore, due to the thermodynamically favoured reduction of these substrates, it was possible to employ just a minimal excess of 2-propanol to obtain the final products with quantitative conversions.

Keywords: alcohol dehydrogenases; biocatalysis; dynamic kinetic resolution; hydrogen transfer; β-hydroxy esters

Introduction

During the last years, the development of dynamic protocols in order to obtain enantio- or diastereomerically pure compounds starting from easily available racemic substrates has been intensified. An increasing number of processes involving enzymatic and homogeneous catalysis in dynamic conditions has been designed to achieve successful transformations for synthesising optically pure derivatives in quantitative yields. It is accepted that when these reactions involve the deracemisation of a racemic mixture, a *dynamic kinetic resolution* (DKR) is taking place,[1] while the de-epimerisation of a mixture of diastereomers is classified as a *dynamic kinetic asymmetric transformation* (DYKAT).[2] Thus, employing DKRs, the deracemisation of *sec*-alcohols[3] and *rac*-primary amines[4] among others have been performed, while DYKATs have often been applied for the de-epimerisation of polyalcohols.[5]

One example of a dynamic process is the reduction of an α-substituted β-keto ester (Scheme 1) to obtain the corresponding alcohols with high diastereomeric excess (*de*).[1g,6] This reaction has been successfully achieved using metal and enzyme catalysis, due to the high acidity of the α-hydrogen that ensures a fast substrate racemisation even at neutral pH. Historically this transformation has been categorised as a DKR-type process.[7] In fact, the first time where this term was implemented by Noyori,[8] corresponds to an Ru-catalysed hydrogenation of α-substituted β-keto esters. Initial examples of the biocatalysed reduction of these compounds were shown by Deol et al. in 1976 employing baker's yeast.[9] In the 1980s and 1990s, whole cells were mainly employed as biocatalysts, especially baker's yeast,[6b,10] and despite the excellent conversions and enantiomeric excess (*ee*) achieved in some cases, the presence of several active enzymes with different selectivities depleted the global stereoselectivity. More recently, the development of dynamic protocols using isolated or overex-



Scheme 1. ADH-catalysed reduction of α-substituted β-keto esters to afford a mixture of two diastereomeric pairs of alcohols through base-catalysed racemisation.

pressed alcohol dehydrogenases (ADHs),[11] has overcome this drawback along with the development of efficient techniques[12] to recycle the expensive cofactor required in these processes.

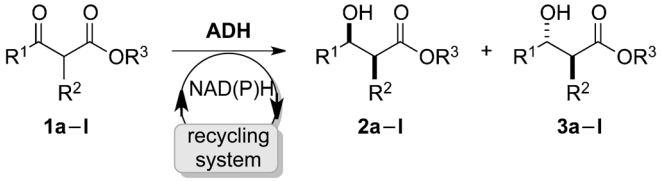
Herein, a set of α-substituted β-keto esters has been successfully reduced with several purified or crude preparations containing overexpressed ADHs in *E. coli* affording the corresponding β-hydroxy esters in many cases with excellent enantio- and diastereoselectivities. Taking into account that enantioenriched α-alkyl-β-hydroxy esters are building blocks of many natural and bioactive structures such statins,[13] pheromones,[11h] polyketides, and other pharmaceuticals,[13] we selected a series of β-keto esters for their preparation. On the other hand, the ADHs studied here have not been previously assayed toward this type of derivative.[14]

Results and Discussion

Initially, several α-alkylated β-keto esters were synthesised following a methodology previously described,[15] consisting of the treatment of the β-keto ester with the corresponding alkyl halide in basic medium (**1a–l**, Scheme 2). Then, the biotransformation conditions were optimised in order to ensure a successful DKR process. As previously shown with isolated enzymes,[11] neutral pH is enough to achieve a fast substrate racemisation (Scheme 1). Thus, the enantiomers of the α-substituted β-keto ester are quickly interconverted through an achiral intermediate, giving access to each of the four diastereomeric products after reduction. Since, at first, ADH-catalysed redox transformations are reversible and are mediated by the action of either the reduced or the oxidised form of the nicotinamide cofactor,[12a] it is necessary the use of a huge excess of 2-propanol in a 'coupled-substrate' approach or to employ, e.g., glucose dehydrogenase (GDH)/glucose in a 'coupled-enzyme' methodology to both recycle the nicotinamide cofactor and force the thermodynamic equilibrium to the product side. Therefore, Tris·HCl buffer 50 mM pH 7.5 was chosen as a suitable medium to carry out these bioreductions. In all cases, a catalytic amount of NAD(P)H (1 mM) was added.

DKRs with 'Small-Bulky' Prelog ADHs

Rhodococcus ruber ADH (ADH-A),[16] *Candida parapsilosis* ADH (CPADH),[17] and *Thermoanaerobacter*



1a, R^1 = Me, R^2 = Me, R^3 = Me; **1b**, R^1 = Me, R^2 = Me, R^3 = Et; **1c**, R^1 = Me, R^2= Me, R^3 = *i*-Pr;
1d, R^1 = Me, R^2 = Me, R^3 = Bn; **1e**, R^1 = Me, R^2 = Et, R^3 = Me; **1f**, R^1 = Me, R^2 = Et, R^3 = Et;
1g, R^1 = Me, R^2 = Et, R^3 = *i*-Pr; **1h**, R^1 = Me, R^2 = allyl, R^3 = *i*-Pr; **1i**, R^1 = Me, R^2 = Bn, R^3 = *i*-Pr;
1j, R^1 = Et, R^2 = Me, R^3 = Me; **1k**, R^1 = Ph, R^2 = Me, R^3 = Et; **1l**, R^1 = Me, R^2– R^3 = -[$(CH_2)_2$]-

Scheme 2. DKRs of β-keto esters **1a–l** using ADHs purified or overexpressed on *E. coli*.

Table 1. Selected bioreductions of α-substituted β-keto esters **1a–l** employing 'small-bulky' Prelog ADHs through DKR processes ($t = 24$ h).[a]

Substrate	ADH	Conversion [%][b]		*ee* [%][c,d]	*de* [%][c,d]
		2a–l	**3a–l**		
1a	CPADH	77	3	>99 (2*R*,3*S*)	93 (2*R*,3*S*)
1a	TesADH	90	3	>99 (2*R*,3*S*)	93 (2*R*,3*S*)
1b	CPADH	99	1	>99 (2*R*,3*S*)	98 (2*R*,3*S*)
1c	CPADH	97	<1	>99 (2*R*,3*S*)	99 (2*R*,3*S*)
1d	CPADH	68	<1	>99 (2*R*,3*S*)	99 (2*R*,3*S*)
1e	TesADH	97	<1	>99 (2*R*,3*S*)	98 (2*R*,3*S*)
1f	TesADH	97	<1	>99 (2*R*,3*S*)	98 (2*R*,3*S*)
1g	TesADH	97	<1	>99 (2*R*,3*S*)	98 (2*R*,3*S*)
1h	TesADH	99	<1	>99 (2*R*,3*S*)	99 (2*R*,3*S*)
1i	TesADH	65	<1	>99 (2*R*,3*S*)	99 (2*R*,3*S*)
1j	ADH-A	19	1	>99 (2*R*,3*S*)	90 (2*R*,3*S*)
1l	ADH-A	95	2	>99 (2*R*,3*S*)	96 (2*R*,3*S*)

[a] For experimental details and other bioreduction results, see Experimental Section and Supporting Information.
[b] Measured by GC.
[c] Measured by GC or HPLC on a chiral phase.
[d] The major diastereomer appears in brackets.

ethanolicus ADH (TesADH),[18] were firstly studied as biocatalysts to perform these reactions. While the first two ADHs were used in a purified form, the last one was employed as a lyophilised *E. coli* preparation containing the overexpressed enzyme.[19] These enzymes present in general an excellent Prelog selectivity,[20] and while the first two are highly active toward methyl alkyl or aryl ketones,[16,17,21] the last one can also accept ethynyl ketones and ethynyl keto esters.[18,22] Furthermore, all of them have demonstrated tolerance against organic solvents, and therefore they can be used in a 'coupled-substrate' approach employing an excess of 2-propanol (5% $v v^{-1}$) to recycle the cofactor.[16–18,19a,21,22] Selected results of the bioreduction of substrates **1a–l** with these three biocatalysts are shown in Table 1.

These ADHs displayed excellent stereoselectivities leading to the Prelog products, exclusively affording an (*S*)-configured centre at position 3 with very high conversions in many cases. For α-methylated β-keto esters **1a–d**, CPADH showed excellent enantio- and diastereoselectivities towards the formation of *syn*-(2*R*,3*S*)-**2a–d** alcohols, while TesADH and ADH-A achieved excellent *ee* although lower *de*, especially in the case of the last one (entries 1–5 in Table 1 and Table S1 in the Supporting Information). However, with α-ethylated ketones **1e–g** TesADH allowed the selective formation of alcohols (2*R*,3*S*)-**2e–g** (entries 6–8) while CPADH and ADH-A showed lower diastereoselectivities (see the Supporting Information, Table S1). There is a remarkable trend observed for CPADH toward this family of compounds (Figure 1).

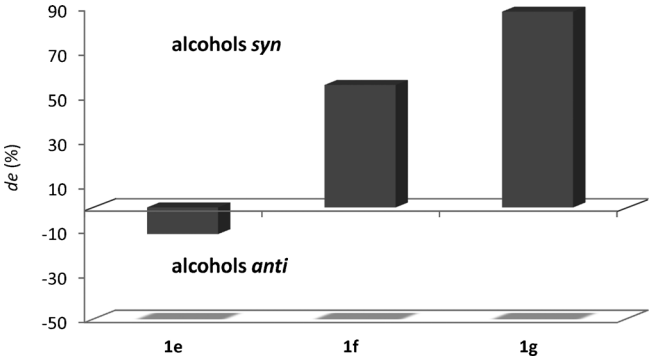


Figure 1. Influence of alkyl ester chain in the bioreductions of ketones **1e–g** with CPADH.

Depending on the ester alkyl moiety, a pronounced change in *de* was observed. The bulkier the alkyl chain was, the more favoured was the formation of *syn* isomer **2e–g**. In fact, for methyl ester derivative **1e**, a slight preference for *anti* isomer **3e** was found, while the isopropyl derivative **1g** afforded a 15:1 mixture of **2g** and **3g**. Interestingly, bulkier ketones **1h** and **1i** could be reduced by these ADHs, although TesADH afforded the best stereoselectivities to achieve (2*R*,3*S*)-**2h** and **2i** (entries 9 and 10, Table 1). Ethyl ketone **1j** could only be reduced by ADH-A with very high *ee* and *de* (entry 11, Table 1), although at low conversion. Phenyl keto ester **1k** was not a suitable substrate for any of these ADHs, as expected due to high sterical hindrance (see the Supporting Information, Table S1). Finally, cyclic derivative **1l** was stereoselectively reduced by ADH-A at very high conversions to form *syn* alcohol **2l** (entry 12, Table 1), while CPADH and especially TesADH gave lower conversions and *de* (see the Supporting Information, Table S1).

DKRs with 'Bulky-Bulky' Prelog ADHs

The study was continued employing *Sphingobium yanoikuyae* ADH (SyADH),[23] and *Ralstonia* sp. ADH (RasADH).[24] These enzymes are known to accept 'bulky-bulky' ketones. Therefore, we expected in some cases a complementary behaviour to the previous ADHs (Table 2 and the Supporting Information, Table S2). The glucose/GDH system was employed to recycle the nicotinamide cofactor in the case of RasADH,[24] while for SyADH-catalysed bioreductions, a 'coupled-substrate' approach using 2-propanol was chosen.[23]

SyADH showed a very high preference for the formation of *syn*-(2*R*,3*S*) isomers of the α-methylated, ethylated, allylated, and benzylated derivatives **2a–i** with excellent conversions, while RasADH showed lower diastereoselectivities with the exception of sub-

Table 2. Selected bioreductions of α-substituted β-keto esters **1a–k** employing 'bulky-bulky' ADHs overexpressed on *E. coli* through DKR processes ($t = 24$ h).[a]

Substrate	ADH	Conversion [%][b]		*ee* [%][c,d]	*de* [%][c,d]
		2a–k	**3a–k**		
1a	SyADH	90	2	>99 (2*R*,3*S*)	96 (2*R*,3*S*)
1b	SyADH	98	2	>99 (2*R*,3*S*)	96 (2*R*,3*S*)
1c	SyADH	76	4	>99 (2*R*,3*S*)	90 (2*R*,3*S*)
1d	SyADH	99	<1	>99 (2*R*,3*S*)	99 (2*R*,3*S*)
1f	SyADH	96	4	>99 (2*R*,3*S*)	92 (2*R*,3*S*)
1h	RasADH	38	62	>99 (2*R*,3*S*)	
				>99 (2*S*,3*S*)	24 (2*S*,3*S*)
1i	RasADH	5	95	>99 (2*S*,3*S*)	90 (2*S*,3*S*)
1j	RasADH	37	29	78 (2*R*,3*S*)	
				>99 (2*S*,3*S*)	12 (2*R*,3*S*)
1k[e]	RasADH	4	72	>99 (2*S*,3*R*)	89 (2*S*,3*R*)

[a] For experimental details and other bioreduction results, see Experimental Section and Supporting Information.
[b] Measured by GC.
[c] Measured by GC or HPLC on a chiral phase.
[d] The major diastereomer appears in brackets.
[e] Switch in Cahn–Ingold–Prelog (CIP) priority at position 3.

strate **1i**, leading to *anti*-(2*S*,3*S*) β-hydroxy ester **3i** with 90% *de* (entry 7, Table 2). β-Keto esters **1j** and **1k**, which could not be transformed by the 'small-bulky' ADHs, were reduced by RasADH at some extent, and although for **1j** the diastereoselectivity was low, bulky ketone **1k** was accepted showing good conversion and very high stereoselectivity for the formation of *anti*-(2*S*,3*R*) isomer **3k** (entry 9, Table 2). Finally, **1l** was not a suitable substrate for these biocatalysts (see the Supporting Information, Table S2). Both ADHs displayed excellent stereoselectivities leading to the Prelog products, exclusively affording an (*S*)-configured centre at position 3 for products **a–j** and an (*R*)-configured centre for **k**, due to a switch in the CIP priority (Scheme 2).

DKRs with *anti*-Prelog ADHs

Until this stage, all ADHs employed showed Prelog selectivity, therefore affording only 2 out of 4 possible diastereomers presenting an (*S*)-configured carbon at position 3. So, we tested two ADHs with *anti*-Prelog selectivity, namely *Lactobacillus brevis* ADH (LBADH)[25] and *Lactobacillus kefir* ADH (LKADH)[26] as purified enzymes. For the first one 2-propanol was used to recycle the cofactor while for the second one a system with GDH/glucose was employed. Results with LBADH and LKADH are summarised in Table 3 and the Supporting Information, Table S3.

As expected, in most cases the (3*R*)-isomers were obtained with high preference, but with some substrates there were noticeable changes in the stereoselectivity, especially with LKADH (see the Supporting Information, Table S3). The nature of the alkyl ester moiety has a significant influence, leading to diminished enantio- and diastereoselectivities the bigger the alkyl ester moiety was. In fact, a clear trend can be observed with ketones **1a–d** and **1e–g**. Especially for methyl, although also with ethyl ester derivatives, the stereoselectivity of the enzymes usually favoured the formation of (2*S*,3*R*)-*syn* isomers, but for bulkier compounds such as **1c**, **1d** and **1g**, the Prelog β-hydroxy esters were formed in high amounts. These biocatalysts did not accept bulky substrates showing in

Table 3. Selected bioreductions of α-substituted β-keto esters **1a–l** employing LBADH through DKR processes ($t = 24$ h).[a]

Substrate	ADH	Conversion [%][b]		*ee* [%][c,d]	*de* [%][c,d]
		2a–l	**3a–l**		
1a	LBADH	68	7	94 (2*S*,3*R*)	81 (2*S*,3*R*)
1b	LBADH	34	15	>99 (2*S*,3*R*)	
				20 (2*R*,3*R*)	38 (2*S*,3*R*)
1c	LBADH	6	8	n.d.	n.d.
1d	LBADH	21	35	24 (2*R*,3*S*)	
				49 (2*R*,3*R*)	25 (2*R*,3*R*)
1e	LBADH	38	3	>99 (2*S*,3*R*)	85 (2*S*,3*R*)
1f	LBADH	11	4	45 (2*S*,3*R*)	47 (2*S*,3*R*)
1g	LBADH	12	3	83 (2*S*,3*R*)	60 (2*S*,3*R*)
1j	LBADH	27	2	>99 (2*S*,3*R*)	86 (2*S*,3*R*)
1l	LBADH	95	5	>99 (2*S*,3*R*)	90 (2*S*,3*R*)

[a] For experimental details and other bioreduction results, see Experimental Section and Supporting Information.
[b] Measured by GC.
[c] Measured by GC or HPLC on a chiral phase.
[d] The major diastereomer appears in brackets.

addition low selectivity. Apart from methyl β-keto esters **1a** and **1e**, cyclic substrate **1l** (entry 9, Table 3) was nicely reduced by LBADH affording (2*S*,3*R*)-**2l** with very high stereoselectivity.

At this point it has to be mentioned that in order to improve the diastereoselectivity in some of these dynamic processes, a lower temperature (20 °C) was tried, but in all cases just lower conversions were achieved with no improvement in the selectivities (data not shown). When using lyophilised *E. coli* cells containing overexpressed ADHs, where some lipases or esterases can be present in the host microorganism, a study of the substrate stability was performed to discard hydrolysis or transesterifications of methyl or ethyl esters with 2-propanol employed in the 'coupled-substrate' approach. Due to the easy handling of lyophilised *E. coli* cells, these reactions could be readily performed at a 50-mg scale isolating the enantioenriched α-substituted β-hydroxy esters with high *de* and isolated yields (60–80%).

DKRs under 'Quasi-Irreversible' Conditions

In previous contributions we have shown that biocatalysed hydrogen transfer processes using ADHs in a 'coupled-substrate' fashion can be carried out employing a small excess of the co-substrate at determined conditions. Since these transformations mainly rely on the thermodynamic equilibrium between the redox pairs of substrate and co-substrate,[27] depending on the chemical structure of compounds reduced/oxidised, the reaction equilibrium will be easily (or not) displaced into the final products. Thus, we have observed that carbonyl groups presenting an electron-withdrawing moiety at the α-position can be quasi-irreversibly reduced by ADHs.[23,27,28]

This effect has also been observed in the case of bioreductions of 1,2- and 1,3-diketones, probably due to the fact that an intramolecular H-bond is formed between the formed alcohol moiety and the remaining carbonyl group, hampering their oxidation reaction.[29] With this in mind, we envisaged that bioreductions of these β-keto ester derivatives could be highly favoured due to the formation of an intramolecular H-bond affording a stabilised 6-membered ring (Figure 2), and therefore a small excess of 2-propanol could be used to achieve quantitative conversions of the corresponding β-hydroxy esters.

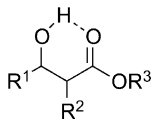


Figure 2. Intramolecular H-bond interaction in β-hydroxy esters **2** or **3**.

Consequently, substrate **1b** was reduced with several ADHs that accept 2-propanol as co-substrate (ADH-A, CPADH, and LBADH), using only 2 equivalents of the hydrogen donor (corresponds to 0.45% vv^{-1}). In all cases similar conversions were obtained as when employing an excess of donor (5% vv^{-1}, 23 equivalents). For instance, ADH-A and CPADH gave conversions around 95% after 24 h. This shows that a large excess of 2-propanol is not required for these substrates, thus being possible to improve both atom[30a] and redox[30b] economies. To the best of our knowledge, this is the first example of a DKR process *via* hydrogen transfer under 'quasi-irreversible' conditions.

Conclusions

Enantioenriched α-alkyl-β-hydroxy esters are building blocks of many natural and pharmaceutical compounds, so studies about catalysts that allow their preparation with high selectivity under mild conditions are of high relevance. Herein we have shown the DKR process *via* reduction of various α-alkyl-β-keto esters employing purified or lyophilised *E. coli* cells containing overexpressed ADHs, affording the corresponding β-hydroxy esters generally with excellent conversions and stereoselectivities. The high acidity of the α-proton ensured a fast substrate racemisation yielding the enantioenriched products at conversions close to 100% even at almost neutral pH.

Depending on the biocatalyst, the substrate scope and selectivity were different. While 'small-bulky' ADHs such as ADH-A, CPADH and TesADH rendered *syn*-(2*R*,3*S*) derivatives with very high diastereoselectivities for substrates that are not very sterically demanding, 'bulky-bulky' ADHs from *S. yanoikuyae* and *Ralstonia* sp. could also accept bulkier keto esters. While the former also afforded preferentially *syn*-(2*R*,3*S*) isomers, the latter showed in some cases a good selectivity towards the formation of *anti*-(2*S*,3*S*) derivatives. Finally, with *anti*-Prelog ADHs such as LBADH or LKADH, *syn*-(2*S*,3*R*) alcohols were obtained in some cases with high conversions and *ee*. Furthermore, with some of these enzymes a trend could be observed, which can be very useful for further applications. While for CPADH isopropyl esters gave better diastereoselectivities than methyl ones, for LBADH methyl esters were more appropriate than bulkier alkyl chains.

On the other hand, processes employing lower amounts of reagents are advisable for economical and environmental reasons. Since 1,2- or 1,3-diketones could be reduced with ADHs under quasi-irreversible conditions,[29] i.e., by employing a low excess of the required co-substrate (2-propanol), we took advantage of this principle to show that this methodology

can also work for performing DKRs over these α-substituted β-keto esters.

Experimental Section

General Remarks

Ketones **1b**, **1e**, **1f** and **1l** were purchased from commercial sources. All other reagents and solvents were of the highest quality available. Glucose dehydrogenase (GDH 002, 30 U mg^{-1}), ADH-A from *Rhodococcus ruber* (20 U mg^{-1}), CPADH from *Candida parapsilosis* (0.4 U μL^{-1}), and LBADH from *Lactobacillus brevis* (3.7 U μL^{-1}) were obtained from Jülich-Codexis. LKADH from *Lactobacillus kefir* (0.42 U mg^{-1}) was obtained from Fluka. Overexpressed ADHs have been obtained following the methodology previously described.[23,24] One unit (U) of ADH reduces 1.0 μM of 2-octanone to 2-octanol (for TesADH) or propiophenone to 1-phenylpropanol (for RasADH or SyADH) per minute at pH 7.5 and 30 °C in the presence of NADPH.

General Procedure for the DKR over the Racemic α-Alkyl-β-keto Esters 1a–l Employing Purified ADHs

The corresponding racemic α-alkyl-β-keto ester (50 mg) was dissolved in Tris-HCl buffer (50 mM, pH 7.5, 12 mL). Then, NAD(P)H (1 mM), the ADH (30 U), and 2-propanol (650 μL) for ADH-A, CPADH or LBADH [glucose dehydrogenase (50 μL) and glucose (50 mM) for LKADH] were added. The mixture was shaken at 250 rpm at 30 °C from 24 to 72 h. The reaction was stopped by extraction with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure and conversions were measured by GC. The final products were isolated after flash chromatography on silica gel, using hexane/EtOAc or diethylether/CH_2Cl_2 mixtures as eluent with yields ranging from 60 to 80%.

General Procedure for the DKR over the Racemic α-Alkyl-β-keto Esters 1a–l Employing Overexpressed ADHs

The corresponding racemic α-alkyl-β-keto ester (50 mg) was dissolved in Tris-HCl buffer (50 mM, pH 7.5, 12 mL). Then, NADPH (1 mM), the lyophilised cells of *E. coli* overexpressing the ADH (150 mg), and 2-propanol (650 μL) for TesADH or SyADH [glucose dehydrogenase (50 μL) and glucose (50 mM) for RasADH] were added. The mixture was shaken at 250 rpm at 30 °C from 24 to 72 h. The reaction was stopped by centrifugation and extraction with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure and conversions were measured by GC. The final products were isolated after flash chromatography on silica gel, using hexane/EtOAc or diethylether/CH_2Cl_2 mixtures as eluent with yields ranging from 60 to 80%.

Acknowledgements

A.C. thanks the Principado de Asturias for his predoctoral fellowship. A.R.-M. (FPU program) and I.L. (Ramón y Cajal Program) thank the Spanish MICINN for personal funding. Financial support from MICINN (Project CTQ2007-61126) is gratefully acknowledged. This project is supported from BIOTRAINS Marie Curie ITN, financed by the European Union through the Seventh Framework People Programme (Grant Agreement no. 238531).

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