Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 276–281

A biocatalytic one-pot oxidation/reduction sequence for the deracemisation of a *sec*-alcohol

Constance V. Voss, Christian C. Gruber and Wolfgang Kroutil*

Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria

Received 18 December 2006; accepted 8 January 2007
Available online 7 February 2007

Abstract—Biocatalytic deracemisation via inversion of *rac*-2-decanol was accomplished by a combined oxidation/reduction sequence using the same 'single' catalyst for both steps. Overall, the (*R*)-alcohol was inverted to the corresponding (*S*)-alcohol. Lyophilised cells of various *Rhodococci* spp. were tested for the unselective oxidation of the racemic *sec*-alcohol using acetone as the hydrogen acceptor in the first step. For the second step, the stereoselective asymmetric reduction of the corresponding ketone, 2-propanol was employed as the hydrogen donor. Employing lyophilised cells of *Rhodococcus* sp. CBS 717.73 racemic 2-decanol was transformed to (*S*)-2-decanol with excellent enantiomeric excess (92% ee) and yield (82% isolated yield) in the combined one-pot oxidation/reduction sequence.
© 2007 Elsevier Ltd. All rights reserved.

1. Introduction

Enantiopure chiral secondary alcohols are commonly used in the pharmaceutical and food industry as well as for flavour and fragrances and liquid crystals. They can be synthesised by the asymmetric reduction of their corresponding prochiral ketone using chemical[1] as well as biocatalytic methods.[2] Besides the biocatalytic reduction of ketones, the kinetic resolution of the racemic alcohols (i) via acyltransfer employing, for example, lipases[3] or (ii) via oxidation[4] is also widely applied; however, the yield of a kinetic resolution is limited to 50% of each enantiomer.

To circumvent this severe limitation when starting from the alcohol, various deracemisation techniques have been developed,[5] for instance, by applying two different fermenting micro-organisms in a one-pot one-step process[6] or two different enzymes or micro-organisms in two separated steps.[7] A chemical two-step deracemisation protocol was described by Williams,[8] employing a 'single' metal-catalyst for the non-enantiospecific oxidation; thus both enantiomers are oxidised to a ketone intermediate, followed by a stereoselective reduction step leading ideally to one product enantiomer in a 100% yield. The catalyst, a complex of Ru/BINAP/DPEN, was able to oxidise various *sec*-alcohols to their corresponding ketones by non-selective hydrogen transfer employing cyclohexanone as a hydrogen acceptor. After the oxidation step, pressurisation with hydrogen led to the stereoselective reduction of the ketone.

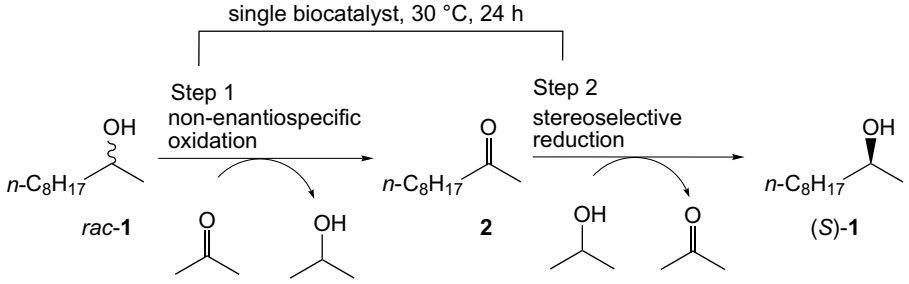
A drawback of the above protocol is the involvement of high quantities of metal and the limitations for metals in active pharmaceutical ingredients.[9] We recently reported an enzymatic oxidation/reduction system employing a *sec*-alcohol dehydrogenase showing exceptionally high tolerance towards elevated concentrations of co-substrates used as hydrogen donors or acceptors, such as 2-propanol and acetone, respectively.[10] Employing lyophilised cells of *Rhodococcus ruber* DSM 44541 as a catalyst and acetone as the hydrogen acceptor, a variety of *rac*-alcohols were resolved by kinetic resolution to furnish non-reacted (*R*)-alcohols and ketone. For the reduction of ketones the over-expressed ADH-'A' of *R. ruber* DSM 44541 showed excellent stereoselectivity using 2-propanol as the hydrogen donor.[11]

2. Results and discussion

We envisaged a deracemisation via an oxidation/reduction sequence to be carried out in a one-pot two-step process by employing a 'single biocatalyst' (Scheme 1). As a model compound *rac*-2-decanol was chosen. To achieve efficient and quick transformations, the biocatalyst has to display on the one hand a low, or in the ideal case, no

* Corresponding author. Tel.: +43 316 380 5332; fax: +43 316 380 9840; e-mail: wolfgang.kroutil@uni-graz.at

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.01.013



Scheme 1. Oxidation/reduction deracemisation in a one-pot two-step fashion.

enantioselectivity for the oxidation while on the other hand perfect stereoselectivity for the reduction step.

2.1. Testing of lyophilised cells

To identify a suitable non-selective biocatalyst for oxidation, various *Rhodococci* strains were tested for their ability to deracemise *rac*-2-decanol (~10 g/l) via a one-pot two-step oxidation/reduction sequence. Acetone was employed as the hydrogen acceptor for the possible non-selective oxidation via hydrogen transfer, whereby the obvious aim was to oxidise both enantiomers, since any racemic alcohol remaining, would decrease the ee of the alcohol recovered after the oxidation/reduction sequence. The reduction reaction was performed by an excess of 2-propanol, which acted as a hydrogen donor and shifted the equilibrium back to the alcohol.

Various concentrations of acetone (10, 25 and 55 μl; 0.3, 0.7 and 1.5 M) were tested employing lyophilised *Rhodococci* cells for the oxidation at 30 °C. After 16 h of oxidation, 2-propanol (120 μl, 3.2 M) was added for reduction. The results of this oxidation/reduction sequence with various *Rhodococci* are listed in Table 1.

Almost all the *Rhodococci* species favoured the oxidation of the (*S*)-enantiomer, leading to an excess of the (*R*)-enantiomer of the remaining alcohol. An exception was *Rhodococcus* sp. NCIMB 11216, which preferentially oxidised the (*R*)-enantiomer, while *Rhodococcus* sp. 312 CBS 71773

Table 1. Testing of *Rhodococci* strains for deracemisation of *rac*-2-decanol by non-selective oxidation and 2-propanol mediated reduction

Micro-organism	Acetone (M)	After oxidation[a]		*E*[b]	After reduction	
		Ketone (%)	ee (%)		Alcohol (%)	ee (%)
Rhodococcus ruber DSM 44541	0.3	80	15 (*R*)	1.2	87	82 (*S*)
	0.7	90	28 (*R*)	1.3	70	93 (*S*)
	1.5	92	40 (*R*)	1.4	55	88 (*S*)
Rhodococcus erythropolis FCC 173	0.3	82	7 (*R*)	1.1	87	81 (*S*)
	0.7	89	31 (*R*)	1.3	76	82 (*S*)
	1.5	71	88 (*R*)	5.5	76	82 (*S*)
Rhodococcus ruber DSM 44540	0.3	67	23 (*R*)	1.5	89	77 (*S*)
	0.7	86	50 (*R*)	1.7	74	78 (*S*)
	1.5	72	84 (*R*)	4.7	60	68 (*S*)
Rhodococcus ruber DSM 44539	0.3	48	36 (*R*)	3.2	93	57 (*S*)
	0.7	68	68 (*R*)	3.7	80	52 (*S*)
	1.5	53	90 (*R*)	27	71	30 (*S*)
Rhodococcus sp. NCIMB 11216	0.3	43	63 (*S*)	21	93	3 (*S*)
	0.7	47	84 (*S*)	98	83	25 (*S*)
	1.5	40	64 (*S*)	95	83	50 (*S*)
Rhodococcus sp. 312 CBS 71773	0.3	87	4 (*S*)	1.0	89	88 (*S*)
	0.7	98	5 (*R*)	1.0	73	88 (*S*)
	1.5	100	na	1.0	55	87 (*S*)
Rhodococcus erythropolis DSM 312	0.3	81	10 (*R*)	1.1	90	78 (*S*)
	0.7	96	17 (*R*)	1.1	74	90 (*S*)
	1.5	99	34 (*R*)	1.2	53	90 (*S*)
Rhodococcus equi IFO 3730	0.3	67	5 (*R*)	1.1	90	54 (*S*)
	0.7	89	15 (*R*)	1.1	78	56 (*S*)
	1.5	94	35 (*R*)	1.3	62	46 (*S*)
Rhodococcus ruber DSM 43338	0.3	84	26 (*R*)	1.3	87	74 (*S*)
	0.7	96	25 (*R*)	1.2	74	83 (*S*)
	1.5	98	9 (*R*)	1.0	54	80 (*S*)

na: not applicable.
[a] Reaction time: 16 h.
[b] Enantioselectivity calculated from conversion and ee of the substrate according to the literature.[12]

oxidised both enantiomers non-specifically, allowing a fast transformation of both enantiomers to the ketone in up to 100% (55 μl acetone was added).

Between the added amount of acetone (0.3, 0.7 or 1.5 M) and the generated amount of ketone, no clear correlation was found in the oxidation step. Sometimes the addition of 0.7 M acetone led to the highest conversion, sometimes the addition of 1.5 M.

After the reduction, all strains gave the (*S*)-alcohol in excess. In this first test, the enantiomeric excess achieved, reached up to 94%, while the highest amount of alcohol achieved was 93%. Although in the oxidation reaction, no clear correlation between the added amount of acetone and the amount of generated ketone could be recognised, there was a clear trend for the reduction: the amount of ketone was lower, the smaller amount of acetone was added during the oxidation step; this is easily explainable since less acetone in the first step, allows in the second step to shift the equilibrium more easily to the product (alcohol) side.

It can be summarised that the oxidation process with acetone as the hydrogen acceptor was very efficient with most of the *Rhodococci* species, especially *Rhodococcus* sp. 312 CBS 71773 showed an exceptional low enantioselectivity, making it an interesting candidate. Nevertheless, the stereoselectivity of the reduction of this strain had to be determined separately.

2.2. Reduction

Therefore, *Rhodococcus* sp. 312 CBS 71773 as well as *R. ruber* DSM 44541, and *Rhodococcus* sp. NCIMB 11216 were used for the evaluation of the stereoselectivity of the reduction process with 2-propanol. The lyophilised cells were incubated with 2-decanone and 2-propanol at 30 °C (Table 2). In all cases conversions of >94% were achieved. As expected from previous experiments *R. ruber* DSM 44541 showed perfect stereoselectivity. Surprisingly *Rhodococcus* sp. NCIMB 11216 displayed low (*R*)-selectivity, which seems at first to contradict the results obtained after the reduction in Table 1, since there, the (*S*)-enantiomer was in excess. Nevertheless, a closer inspection of the results of this strain in Table 1 revealed, that the conversion of the oxidation in the first step was low (<47%), so that after reduction, the amount of the (*R*)-enantiomer formed could not get higher than the amount of the (*S*)-enantiomer, also due to the low stereoselectivity of the reduction. The third strain, *Rhodococcus* sp. 312 CBS 71773, showed very good stereoselectivity (ee 96%). Due to its low enantioselectivity *E* in the oxidation combined with the very good stereoselectivity in reduction, this strain is a perfect candidate for the oxidation/reduction sequence in order to achieve deracemisation.

Table 2. Biocatalytic reduction of 2-decanone via hydrogen transfer employing 2-propanol

Micro-organism	Conv. (%)	ee (%)
Rhodococcus ruber DSM 44541	94.9	>99 (*S*)
Rhodococcus sp. NCIMB 11216	95.3	44 (*R*)
Rhodococcus sp. 312 CBS 717.73	94.2	96 (*S*)

2.3. Improvement of the deracemisation sequence

Different strategies were used to improve the oxidation/reduction sequence concerning the yield and ee of the reaction. As already stated above, these two are closely related, since if in the ideal case both enantiomers are oxidised in the first step, then the second step determines the final ee; otherwise the non-oxidised enantiomer of the first step diminishes the final ee. Furthermore, as discussed in Section 2.1 a smaller amount of alcohol was obtained after the total sequence when higher concentrations of acetone were applied, since the remaining amount of acetone of the oxidation step shifts the equilibrium of the second step in the wrong direction.

For improvements, we considered several strategies: (i) The improvement of the availability of oxygen; (ii) the implementation of an evaporation step of the remaining acetone after the first step; and (iii) different reaction conditions such as temperature and pH.

2.3.1. Reaction vessel. Taking into account that molecular oxygen could be a part of the non-enantiospecific oxidation, we investigated different reaction vessels (1.5 ml Eppendorf tubes, 1.5 ml GC-vials or 8 ml glass vials), varying in size and, therefore, the amount of available oxygen. As shown in Table 3, there was a clear difference in the results depending upon the reaction vessel with respect to the amount of ketone formed in the oxidation step and the ee at the end of the sequence. When the oxidation/reduction sequence was performed in Eppendorf tubes or GC-vials, an ee of 73% was achieved, due to lower conversion in the oxidation step. The best results were obtained with 8 ml glass vials (97% conversion in the oxidation step), which resulted in a 94% ee of the (*S*)-enantiomer after the reduction step. These observations supported the presumption that oxygen benefits the enzymatic alcohol oxidation.

Table 3. Oxidation/reduction sequence with *Rhodococcus ruber* DSM 44541, accomplished in different reaction vessels

Sample	After oxidation ketone **2** (%)	After reduction ee (%)
Eppendorf tube (1.5 ml)	75	73 (*S*)
Glass vial (8 ml)	97	94 (*S*)
GC-vial (1.5 ml)	87	72 (*S*)

2-Decanol was used as a substrate, acetone (0.3 M) as a hydrogen acceptor and 2-propanol (3.2 M) as hydrogen donor.

2.3.2. Implementation of an evaporation step after the oxidation reaction. Since the final amount of the obtained alcohol depends on the amount of added acetone as the hydrogen acceptor in the first step, removal of the acetone after the first step was envisaged. Therefore, this oxidation/reduction sequence consisted of three steps, namely, oxida-

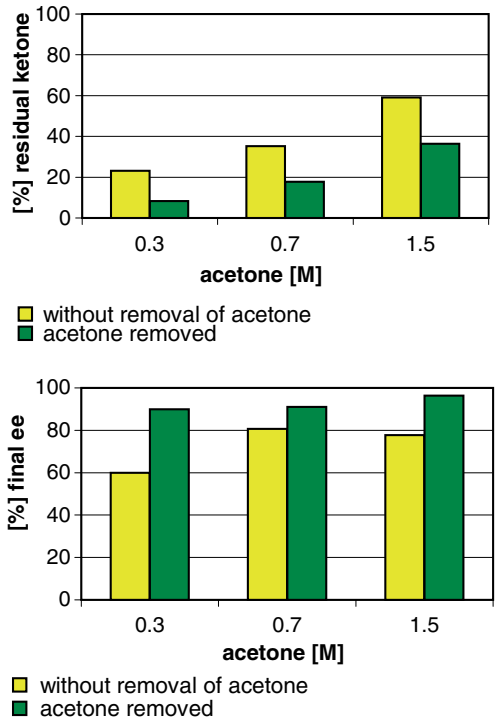


Figure 1. Comparison of the amounts of ketone and ee obtained after an oxidation/reduction sequence, accomplished with (■) or without (□) removal of acetone after the first step. The reactions were performed employing *Rhodococcus ruber* DSM 44541. Acetone (0.3, 0.7 or 1.5 M) was used as the hydrogen acceptor in the oxidation step and 2-propanol (3.2 M) as the hydrogen donor in the reduction step.

tion, evaporation and reduction. The three-step reaction was compared with an oxidation/reduction sequence that proceeded without an evaporation step. As depicted in Figure 1 the amount of ketone was up to 50% lower when acetone was removed by evaporation, compared to the sample without acetone removal. The removal of acetone resulted in a higher ee after the reduction step, due to a higher conversion in the second step. The best results for the ee (97%) were obtained when 1.5 M acetone was used to assist the oxidation reaction, followed by an evaporation step and the addition of 2-propanol. Although the amount of ketone was still quite high (36%) at the end of the reaction, it was almost 25% lower than in the non-evaporated sample.

2.3.3. Comparison of different reaction conditions (*T*, pH). Since parameters such as pH and temperature can influence the course of the biocatalytic transformation, an oxidation/reduction sequence was performed at different pH values and temperatures to find the optimum conditions for the biocatalytic reaction (Table 4). The best results concerning the ee and recovered products were obtained at 40 °C and pH 7.5, but in general the pH had no influence on the stereoselectivity. Elevated temperatures were found to have a positive influence on the oxidation/reduction reaction.

2.4. Scale-up of the deracemisation sequence

In order to prove the applicability of the method on a preparative scale, the deracemisation of *rac*-2-decanol via oxidation/reduction sequence was performed with 414 mg (500 µl, 51 mM) substrate in the presence of 1% (v/v) acetone (step 1) and 24% (v/v) 2-propanol (step 2) employing the best strain, namely *Rhodococcus* sp. 312 CBS 71773. A centrifuge peaker (440 ml volume) was employed as the reaction vessel. During oxidation, 86% of the alcohol was converted to the ketone. At this stage, the alcohol showed an ee of 7%. After the reduction, 338 mg of highly enantioenriched (*S*)-2-decanol (82% isolated yield, 92% ee) was obtained.

Table 4. Deracemisation sequence of *rac*-2-decanol via oxidation/reduction with *Rhodococcus ruber* DSM 44541 at different pH values and temperatures

Temperature (°C)	pH	Alcohol (%)	ee (%)
30	2	74	72 (*S*)
30	6	73	72 (*S*)
30	7.5	73	72 (*S*)
30	8.5	75	74 (*S*)
30	10	73	71 (*S*)
30	12	76	70 (*S*)
25	7.5	39	67 (*S*)
30	7.5	27	72 (*S*)
40	7.5	34	89 (*S*)

3. Conclusion

This study has shown that an oxidation/reduction sequence with a single biocatalyst and acetone and 2-propanol as co-substrates represents a highly efficient method for the deracemisation of a racemic secondary alcohol in a one-pot two-step process. Various *Rhodococci* species were found to have the desired activity and tolerance towards acetone as the hydrogen acceptor and 2-propanol as the hydrogen donor. The deracemisation of racemic 2-decanol occurred in high yield and enantiomeric excess. Some attempts for improvement were accomplished, whereby the implementation of an evaporation step after the oxidation reaction led to the best results. The applicability of deracemisation via an oxidation/reduction sequence was proven on a 400 mg scale. Since the reaction is performed in an environmentally friendly aqueous–organic solvent mixture at ambient temperatures, the reaction provides a practical applicable method for the synthesis of enantiopure secondary alcohols in 100% yield starting from the racemic alcohol. Investigation of the enzymes involved in the oxidation and reduction may lead to a one-pot *one*-step process for the efficient deracemisation of *sec*-alcohols.

4. Experimental

4.1. General

The following chemicals were purchased and used as received: *rac*-2-decanol (Lancaster), 2-octanone (Aldrich).

4.1.1. Bacterial strains. Strains were obtained from the Deutsche Sammlung für Mikroorganismen and Zellkultu-

ren (Braunschweig, Germany, http://www.dsmz.de), the National Collections of Industrial Food and Marine Bacteria (Aberdeen, Scotland, http://www.ncimb.co.uk), the Institute for Fermentation, Osaka (Osaka, Japan, http://www.ifo.or.jp), the Centraalbureau voor Schimmelcultures (CBS) (Utrecht, The Netherlands, http://www.cbs.knaw.nl/) and FCC, which stands for our in-house culture collection.

4.1.2. Cultivation media. Group I: Yeast extract (10 g/l, Oxoid L21), bacteriological peptone (10 g/l, Oxoid L37); group II: glucose (10 g/l, Fluka 49150); group III: sodium chloride (2 g/l, Roth 9265.1), $MgSO_4 \cdot 7H_2O$ (0.15 g/l, Fluka 63140); group IV: NaH_2PO_4 (1.3 g/l, Fluka 71496), KH_2PO_4 (4.4 g/l, Merck 5101).

4.1.3. Growth of micro-organisms. Strains were grown in 250 ml medium in baffled flasks (1 l) with shaking at 30 °C and 120 rpm for 3 days. The cells were centrifuged (6000 rpm, 4 °C, 20 min), washed with Tris–buffer (pH 7.5. 50 mM) and lyophilised.

4.2. General procedure for the biocatalytic deracemisation via an oxidation/reduction sequence

4.2.1. Oxidation step. The lyophilised cells (40 mg) were rehydrated in Tris–HCl–buffer (0.5 ml, pH 7.5. 50 mM) for 1 h (25 °C, 170 rpm). Afterwards substrate (5 µl, *rac*-2-decanol, 51 mM) and acetone (10 µl, 0.3 M; 25 µl, 0.7 M, 55 µl, or 1.5 M respectively) were added and the mixture shaken at 30 °C and 150 rpm for 16 h.

4.2.2. Reduction step. To the reaction mixture, 2-propanol (120 µl, 3.2 M) was added and the solution was shaken at the respective temperatures for 3 days. The biotransformation was stopped by the addition of ethyl acetate (0.8 ml, 16 M) and centrifugation (13,000 rpm, 5 min).

4.2.3. Derivatisation. For separation of the two enantiomers, the alcohols were derivatised by the addition of acetic anhydride (250 µl, 5 M) and a catalytic amount of DMAP (4-dimethylaminopyridine, 2 mg, 33 mM) to the organic phase. The reaction mixture was shaken for 1 h at 170 rpm and 25 °C. Afterwards, water (300 µl) and an internal standard (1 mg, 20 µl of a standard solution with 50 mg/ml 2-octanone in ethyl acetate) were added. The solution was centrifuged (2 min) and the organic phase dried over Na_2SO_4 and analysed on GC.

4.3. Variations of the procedure for improvement of deracemisation

4.3.1. Comparison of different reaction vessels. The experiment was accomplished as described above (with 0.3 M acetone and 3.2 M 2-propanol), but different reaction vessels were used: 1.5 ml Eppendorf tubes, 1.5 ml GC-vials or 8 ml Glass vials.

4.3.2. Comparison of different reaction conditions (*T*, pH). The experiments were performed as described above, but at different temperatures (25, 30 or 40 °C) or pH-values (Tris–buffer, 0.5 ml, 50 mM, pH 2, 6, 7.5, 8.5, 10 or 12).

4.3.3. Implementation of an evaporation step. After the oxidation step, the acetone was removed under reduced pressure. To achieve that, the reaction mixture was transferred into an adequate glass flask and acetone was removed for 2–4 min under vacuum on a rotary evaporator. Afterwards 2-propanol (120 µl, 3.2 M) was added to the samples and the reaction mixture was shaken at 30 °C and 150 rpm for 3 days. Further procedures were performed as described before.

4.4. Reduction of 2-decanone

Lyophilised cells (40 mg) were rehydrated in Tris–HCl–buffer (0.5 ml, pH 7.5, 50 mM) for 1 h (25 °C, 170 rpm). Subsequently, 2-decanone (5 µl, 51 mM) and 2-propanol (120 µl) were added and the mixture was shaken at 30 °C and 150 rpm for 3 days. The biotransformation was stopped by the addition of ethyl acetate (0.8 ml) and centrifugation (5 min, 13,000 rpm). Derivatisation and analysis were carried out as described before.

4.5. Standard procedure for preparative deracemisation

4.5.1. Oxidation step. Lyophilised cells (3 g) were rehydrated in a Tris–HCl–buffer (50 ml, pH 7.5, 50 mM) for 1 h (25 °C, 170 rpm) in a closed centrifuge peaker (440 ml volume). Afterwards substrate (500 µl, 414 mg *rac*-2-decanol, 51 mM) and acetone (0.5 ml, 0.14 M) were added and the mixture was shaken at 30 °C and 150 rpm for 1 day (the peaker was placed in horizontal position on the shaker). For analysis of the oxidation process a sample (1 ml) of the reaction mixture was taken, derivatised and analysed by GC.

4.5.2. Reduction step. To the reaction mixture (49 ml) mentioned above, 2-propanol (12 ml, 3.2 M) was added and the solution was shaken at 30 °C and 150 rpm for 3 days. The biotransformation was stopped by the addition of ethyl acetate (50 ml) and centrifugation (13,000 rpm for 5 min). A sample (1 ml) of this solution was used for derivatisation and GC analysis.

4.5.3. Determination of yield. After evaporation and column chromatography (silica gel) 338 mg of (*S*)-2-decanol (82%, 92% ee) was obtained. $[\alpha]_D^{20} = +9.8$ (*c* 1.00, EtOH) 92% ee, lit.[13] +10.3 (*c* 0.80, EtOH) 99% ee.

4.5.4. GC analysis conditions. GC analytic was performed using an internal standard.

Chiral column: Chirasil-Dex CB-cyclodextrin; temperature program: 100/1/2.5/120/10/150/1 (start temperature °C/holding time [min]/heating rate [°C/min]/plateau temperature [°C]/holding time [min]/heating rate [°C/min]/final temperature [°C]/holding time [min]). 2-Decanol was measured as its acetate derivative. Retention time: (*R*)-isomer: 7.2 min; (*S*)-isomer: 7.7 min; 2-decanon: 5.4 min; 2-octanon: 2.4 min.

Acknowledgement

This study was financed by the Austrian Science Fund (FWF Project P18537-B03).

References

1. (a) Gladiali, S.; Alberico, E. *Chem. Soc. Rev.* **2006**, *35*, 226–236; (b) Samec, J. S. M.; Bäckvall, J.-E.; Andersson, P. G.; Brandt, P. *Chem. Soc. Rev.* **2006**, *35*, 237–248; (c) Noyori, R.; Ohkuma, T. *Angew. Chem., Int. Ed.* **2001**, *40*, 40–73; (d) Ma, Y.; Liu, H.; Chen, L.; Cui, X.; Zhu, J.; Deng, J. *Org. Lett.* **2003**, *5*, 2103–2106.
2. (a) Nakamura, K.; Matsuda, T. Reduction of Ketones. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 3, pp 991–1047; (b) Patel, R. N. *Curr. Opin. Biotechnol* **2001**, *12*, 587–604; (c) Kula, M.-R.; Kragl, U. Dehydrogenases in the Synthesis of Chiral Compounds. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, 2000; pp 839–866; (d) Kroutil, W.; Mang, H.; Edegger, K.; Faber, K. *Curr. Opin. Chem. Biol.* **2004**, *8*, 120–126.
3. (a) Bornscheuer, U. T.; Bessler, C.; Srinivas, R.; Krishna, S. H. *Trends Biotechnol* **2002**, *20*, 433–437;; (b) Faber, K. *Biotransformations in Organic Chemistry*, 5th ed.; Springer: Berlin, Heidelberg, New York, 2004.
4. (a) Faller, J. W.; Lavoie, A. R. *Org. Lett.* **2001**, *3*, 3703–3706; (b) Schmid, A.; Hollmann, F.; Bühler, B. Oxidation of Alcohols. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 3, pp 1108–1170; (c) Nishibayashi, Y.; Yamauchi, A.; Onodera, G.; Uemura, S. *J. Org. Chem.* **2003**, *68*, 5875–5880; (d) Kroutil, W.; Mang, H.; Edegger, K.; Faber, K. *Adv. Synth. Catal.* **2004**, *346*, 125–142; (e) Mandal, S. K.; Sigman, M. S. *J. Org. Chem.* **2003**, *68*, 7535–7537; (f) Arterburn, J. B. *Tetrahedron* **2001**, *57*, 9765–9788.
5. (a) Faber, K. *Chem. Eur. J.* **2001**, *7*, 5004–5010; (b) Pamies, O.; Bäckvall, J.-E. *Trends. Biotechnol.* **2004**, *22*, 130–135; (c) Gruber, C. C.; Lavandera, I.; Faber, K.; Kroutil, W. *Adv. Synth. Catal.* **2006**, *348*, 1789–1805.
6. (a) Takahashi, E.; Nakamichi, K.; Furui, M. *J. Ferm. Bioeng.* **1995**, *80*, 247–250; (b) Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Medici, A.; Pedrini, P. *Tetrahedron: Asymmetry* **1995**, *6*, 3047–3053.
7. (a) Hummel, W.; Riebel, B. *Ann. N. Y. Acad. Sci.* **1996**, *799*, 713–716; (b) Adam, W.; Lazarus, M.; Boss, B.; Saha-Möller, C. R.; Humpf, H. U.; Schreier, P. *J. Org. Chem.* **1997**, *62*, 7841–7843; (c) Adam, W.; Lazarus, M.; Saha-Möller, C. R.; Schreier, P. *Tetrahedron: Asymmetry* **1998**, *9*, 351–355; (d) Tsuchiya, S.; Miyamoto, K.; Ohta, H. *Biotechnol. Lett.* **1992**, *14*, 1137–1142; (e) Shimizu, S.; Hatori, S.; Hata, H.; Yamada, H. *Enzyme Microb. Technol.* **1987**, *9*, 411–416.
8. Adair, G. R. A.; Williams, J. M. J. *Chem. Commun.* **2005**, 5578–5579.
9. Garrett, C. E.; Prasad, K. *Adv. Synth. Catal.* **2004**, *346*, 889–900.
10. Stampfer, W.; Kosjek, B.; Faber, K.; Kroutil, W. *J. Org. Chem.* **2003**, *68*, 402–406.
11. Edegger, K.; Gruber, C. C.; Poessl, T. M.; Wallner, S. R.; Lavandera, I.; Faber, K.; Niehaus, F.; Eck, J.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Chem. Commun.* **2006**, 2402–2404.
12. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
13. Nakamura, K.; Matsuda, T. *J. Org. Chem.* **1998**, *63*, 8957–8964.