This article is published as part of the *Dalton Transactions* themed issue entitled:

Bridging the gap in catalysis *via* multidisciplinary approaches

Guest Editors: Christophe Coperet and Rutger van Santen
Université de Lyon, France and Eindhoven University of Technology, The Netherlands

Published in issue 36, 2010 of *Dalton Transactions*

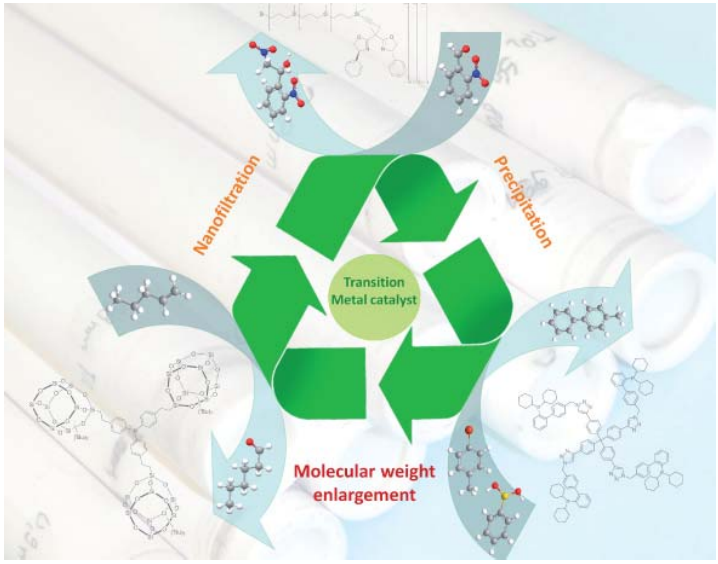


Image reproduced with the permission of Dieter Vogt

Articles in the issue include:

Visit the *Dalton Transactions* website for more cutting-edge inorganic and organometallic research
www.rsc.org/dalton

Bioreduction of α-methylcinnamaldehyde derivatives: chemo-enzymatic asymmetric synthesis of Lilial™ and Helional™†

Clemens Stueckler,[b] Nicole J. Mueller,[b] Christoph K. Winkler,[b] Silvia M. Glueck,[a,b] Karl Gruber,[c] Georg Steinkellner[a,c] and Kurt Faber*[b]

Received 11th February 2010, Accepted 14th April 2010
DOI: 10.1039/c002971h

Nonracemic aryl-substituted α-methyldihydrocinnamaldehyde derivatives employed as olfactory principles in perfumes (Lilial™, Helional™) were obtained *via* enzymatic reduction of the corresponding cinnamaldehyde precursors using cloned and overexpressed ene-reductases. (*R*)-Enantiomers were obtained using the old-yellow-enzyme (OYE) homolog YqjM from *Bacillus subtilis* and 12-oxophytodienoic acid reductase isoenzyme OPR1 from tomato (e.e.$_{max}$ 53%), and (*S*)-aldehydes were furnished in up to 97% e.e. using isoenzyme OPR3, nicotinamide 2-cyclohexene-1-one reductase NCR from *Zymomonas mobilis* and yeast OYE isoenzymes 1–3 under optimised reaction conditions in the presence of *t*-butyl methyl ether as the co-solvent. The stereochemical outcome of the reduction of α-methylcinnamaldehyde using NCR and OYEs 1–3 [previously reported to be (*R*)] was unambiguously corrected to be (*S*).

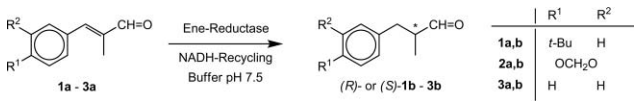
Introduction

Due to their volatility and their olfactory properties, aldehydes constitute important active ingredients in fragrance and flavour applications.[1] Since the enantiomers of α- and β-substituted aldehydes often considerably differ in odour,[2] their application in nonracemic form is required. Whereas β-substituted aldehydes are chirally stable, α-substituted analogues are prone to racemisation, which requires sophisticated methods for their preparation. Among them, the desymmetrisation of conjugated enals *via* asymmetric hydrogenation is the method of choice.[3] Whereas numerous protocols using chirally modified homogeneous (transition-metal) containing catalysts have been reported,[4] metal-independent organocatalysts for the reduction of enals at the expense of a nicotinamide-mimic ('Hantzsch-ester') as hydride source were developed more recently.[5] To date, chirally surface-modified heterogeneous catalysts are not competitive.[6] As an alternative to the variety of chemo-catalytic methods, bioreduction has been envisaged by using various types of redox enzymes.[7] In order to circumvent tedious protein purification and external cofactor-recycling, whole microbial cells — most prominently baker's yeast — were employed for the reduction of enals. Due to the presence of competing ene- and carbonyl-reductases, the chemo- and stereoselective bioreduction of enals was impossible, because undesired carbonyl reduction always overruled the desired C=C-bond reduction, thereby causing substrate- and product-depletion *via* formation of the corresponding allylic and/or saturated alcohols.[8]

It was only recently, that oxygen-stable ene-reductases from the Old Yellow Enzyme family became available in sufficient amounts, which allowed the chemo- and stereoselective bioreduction of activated C=C-bonds in enones and enals by leaving C=O-moieties untouched.[9,10] Encouraged by our recent results,[11] we investigated the application of these enzymes in the preparation of nonracemic α-methyl dihydrocinnamaldehyde derivatives used in perfumery applications.[10]

Results and discussion

The reduction product of *p-tert*-butylcinnamaldehyde (**1b**, Scheme 1) is the olfactory principle of the lily-of-the-valley[2c,12] and is marketed under the trade name Lilial™ or Lysmeral™, whereas the *m*,*p*-methylenedioxy aldehyde (**2b**) is the active ingredient of various perfumes and is marketed as Helional™ or Tropional™.[1,13]



Scheme 1 Asymmetric bioreduction of α-methylcinnamaldehyde derivatives **1a–3a**.

The bioreduction of **1a** under standard conditions in neat aqueous buffer pH 7.5 proved to be disappointingly slow using a variety of ene-reductases (data not shown). However, when the solubility of the lipophilic substrate was enhanced by addition of a small amount of di-isopropyl ether (5%, v:v), reaction rates picked up markedly (Table 1, entries 1–7). Among all enzymes, YqjM and isoenzyme OPR1 gave (*R*)-**1b**, albeit in low enantiomeric excess (e.e.$_{max}$ 21%). In contrast, OPR3, NCR and OYEs 1–3 furnished (*S*)-**1b** with slightly enhanced stereoselectivities, but

[a]*Austrian Centre of Industrial Biotechnology (ACIB), c/o Department of Chemistry, University of Graz, A-8010, Graz, Austria*
[b]*Department of Chemistry, Organic & Bioorganic Chemistry, Heinrichstrasse 28, A-8010, Graz, Austria. E-mail: Kurt.Faber@Uni-Graz.at; Fax: +43-316-380-9840; Tel: +43-316-380-5332*
[c]*Institute of Molecular Biosciences, University of Graz, Humboldtstrasse 50/III, A-8010, Graz, Austria*

† Electronic supplementary information (ESI) available: general experimental conditions, source of substrates, synthesis of reference compounds, spectroscopic data of substrates and products, and analytical procedures for the determination of conversion and enantiomeric excess of products. See DOI: 10.1039/c002971h

Table 1 Conversion and enantiomeric excess of bioreduction products **1b–3b**

				Product	
Entry	Substrate	Enzyme[a]	Conditions[b]	Conv. [%]	E.e. [%]
1	**1a**	YqjM	buffer/*i*-Pr_2O (95 : 5)	3	13 (*R*)
2	**1a**	OPR1	buffer/*i*-Pr_2O (95 : 5)	37	21 (*R*)
3	**1a**	OPR3	buffer/*i*-Pr_2O (95 : 5)	4	22 (*S*)
4	**1a**	NCR	buffer/*i*-Pr_2O (95 : 5)	66	59 (*S*)
5	**1a**	OYE1	buffer/*i*-Pr_2O (95 : 5)	48	52 (*S*)
6	**1a**	OYE2	buffer/*i*-Pr_2O (95 : 5)	75	64 (*S*)
7	**1a**	OYE3	buffer/*i*-Pr_2O (95 : 5)	67	64 (*S*)
8	**1a**	OPR1	buffer/EtOH (80 : 20)	62	21 (*R*)
9	**1a**	NCR	buffer/EtOH (80 : 20)	68	40 (*S*)
10	**1a**	OYE1	buffer/EtOH (80 : 20)	61	33 (*S*)
11	**1a**	OYE2	buffer/EtOH (80 : 20)	80	51 (*S*)
12	**1a**	OYE3	buffer/EtOH (80 : 20)	75	50 (*S*)
13	**1a**	YqjM	buffer/*t*-BuOMe (80 : 20)	0	n.d.
14	**1a**	OPR1	buffer/*t*-BuOMe (80 : 20)	3	17 (*R*)
15	**1a**	OPR3	buffer/*t*-BuOMe (80 : 20)	0	n.d.
16	**1a**	NCR	buffer/*t*-BuOMe (80 : 20)	26	83 (*S*)
17	**1a**	OYE1	buffer/*t*-BuOMe (80 : 20)	26	>**95** (*S*)
18	**1a**	OYE2	buffer/*t*-BuOMe (80 : 20)	26	>**95** (*S*)
19	**1a**	OYE3	buffer/*t*-BuOMe (80 : 20)	32	>**95** (*S*)
20	**2a**	YqjM	buffer/*t*-BuOMe (80 : 20)	10	13 (*R*)
21	**2a**	OPR1	buffer/*t*-BuOMe (80 : 20)	78	6 (*R*)
22	**2a**	OPR3	buffer/*t*-BuOMe (80 : 20)	8	34 (*S*)
23	**2a**	NCR	buffer/*t*-BuOMe (80 : 20)	86	88 (*S*)
24	**2a**	OYE1	buffer/*t*-BuOMe (80 : 20)	59	**95** (*S*)
25	**2a**	OYE2	buffer/*t*-BuOMe (80 : 20)	>99	**97** (*S*)
26	**2a**	OYE3	buffer/*t*-BuOMe (80 : 20)	72	**96** (*S*)
27	**3a**	YqjM	buffer/*t*-BuOMe (80 : 20)	26	33 (*R*)
28	**3a**	OPR1	buffer/*t*-BuOMe (80 : 20)	>99	53 (*R*)
29	**3a**	OPR3	buffer/*t*-BuOMe (80 : 20)	22	28 (*S*)
30	**3a**	NCR	buffer/*t*-BuOMe (80 : 20)	>99	76 (*S*)
31	**3a**	OYE1	buffer/*t*-BuOMe (80 : 20)	>99	**94** (*S*)
32	**3a**	OYE2	buffer/*t*-BuOMe (80 : 20)	>99	**96** (*S*)
33	**3a**	OYE3	buffer/*t*-BuOMe (80 : 20)	84	90 (*S*)

[a] YqjM = Old Yellow Enzyme homolog from *Bacillus subtilis*;[18] OPR1 and OPR3 = 12-oxophytodienoic acid reductase isoenzymes from *Lycopersicon esculentum* (tomato);[17] NCR = nicotinamide 2-cyclohexen-1-one reductase from *Zymomonas mobilis*;[10] OYE = Old Yellow Enzymes from *Saccharomyces carlsbergensis* (OYE1) and from *S. cerevisiae* (OYE2, OYE3).[19] [b] Tris-HCl buffer 50 mM, pH 7.5.

they still were insufficient for synthetic purposes (e.e.$_{max}$ 64%). Since the co-solvent seemed to have a strong influence on the reaction rate, we anticipated that it might also have an impact on the stereoselectivity of the ene-reductases. An increased amount of di-isopropyl ether (20%, v:v) caused a drop in reaction rates, without altering the stereoselectivities singnificantly, similar effects (reduced rates and slightly diminished stereoselectivities) were observed when *i*-Pr_2O was replaced with ethyl acetate or n-hexane (20%, v:v, data not shown). A switch to the water-miscible co-solvent ethanol (20%, v:v) enhanced the rates (c_{max} 80%) for OPR1, NCR and OYEs 1–3 going in hand with a decrease of stereoselectivities (e.e.$_{max}$ 51%, entries 8–12). YqjM and OPR3 were only marginally active (data not shown). Finally, a switch to *t*-butyl methyl ether proved to be an ideal solution: excellent stereoselectivities were obtained with OYEs 1–3 going in hand with a significant drop in reaction rates (e.e.$_{max}$ >95%, entries 13–19).

In order to tune the system, **1a** was reduced using OYE3 at increasing proportions of *t*-butyl methyl ether. As may be deduced from Fig. 1, a clear inverse correlation between reaction rate and stereoselectivity (plotted as conversion and e.e. *versus* TBME concentration) was observed at increasing amounts of co-solvent. Overall, a fraction of 20% (v:v) of *t*-BuOMe seemed to be a good compromise between a decrease of activity and an increase of stereoselectivity. Consequently, all further studies were performed at this co-solvent ratio.

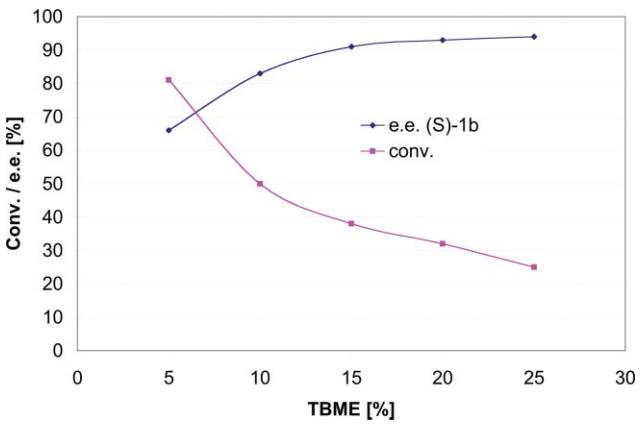


Fig. 1 Dependence of reaction rate and stereoselectivity on the proportion of organic co-solvent (*t*-BuOMe, v:v) in the reduction of **1a** using OYE3.

Under optimised conditions, **2a** was accepted by all ene-reductases (entries 20–26). In line with previous observations,

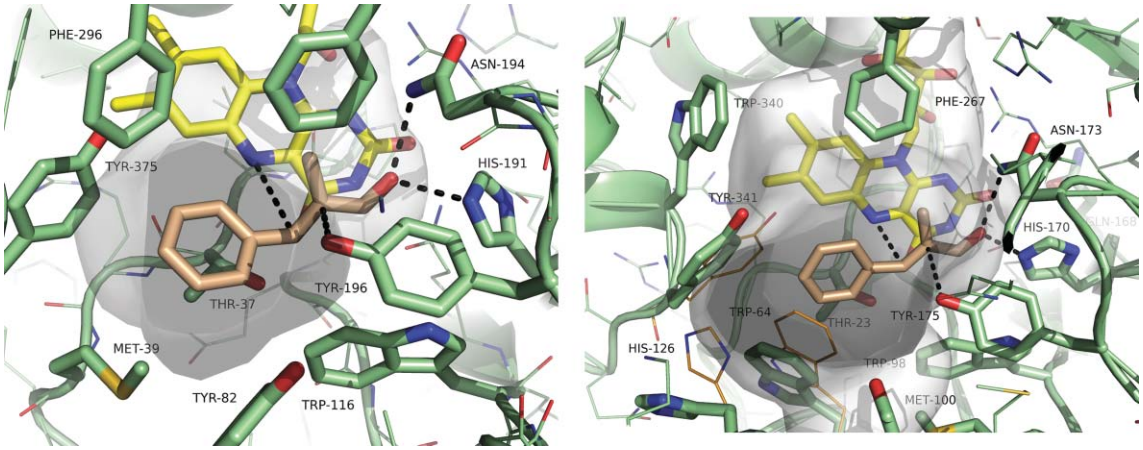


Fig. 2 Modelled binding modes of **3a** in the active sites of OYE1 from *Saccharomyces cerevisiae* (left) and NCR from *Zymomonas mobilis* (right). The active site pockets are shown in a semi-transparent surface representation. Amino acids are shown in green, the FMN cofactor in yellow and the bound substrate in pink. Hydrogen bonding interactions of **3a** with Asn-194 and His-191 in OYE1 and with Asn-173 and His-170 in NCR respectively are indicated as dashed lines. Close contacts (3.4–3.8 Å) between Cα of **3a** and Tyr-196 (in OYE1) or Tyr-175 (in NCR) as well as between Cβ of **3a** and N5 of the corresponding FMN-cofactor are also shown as dashed lines. The conformations of Trp-64, His-126 and Tyr-341 as observed in the NCR structure before molecular mechanics optimization are shown as orange thin lines. The figures were prepared using the programme PyMOL (http://www.pymol.org/).

YqjM and OPR1 showed a weak preference to furnish (*R*)-**2b**. Excellent stereoselectivities (e.e.$_{max}$ 97%) and rates (up to full conversion) were obtained with NCR and OYEs 1–3 for (*S*)-**2b**. Interestingly, the structurally and mechanistically closely related OYE-homologs *N*-ethylmaleimide-(NEM)-reductase, morphinone reductase and pentaerythritol tetranitrate-(PETN)-reductase showed insufficient stereoselectivities (e.e.s 0–14%) with good activities (conversion up to 79%) with **1a–3a**, or *vice versa*. NEM-reductase furnished (*S*)-**1b** and (*S*)-**2b** with e.e.$_{max}$ 57% (conversion 18%) and e.e.$_{max}$ 18% (conversion 64%), respectively.[14]

The absolute configuration of products **1b** and **2b** was deduced by comparison of optical rotation values of **1b** and **2b** obtained using OYE2 with literature data (for details see experimental part), which proved to be (*S*) for both substrates. However, the pronounced stereochemical preference of NCR and OYEs 1–3 to yield (*S*)-**1b** and (*S*)-**2b** is in conflict with the (*R*)-preference of these enzymes on the close homolog α-methyldihydrocinnamaldehyde (**3a**), as reported by B. Rosche *et al.*[10] According to this report, the bioreduction of **3a** using NCR and OYEs 1–3 furnished (*R*)-**3b** in 50% e.e. and *ca.* 75% e.e. respectively, using slightly different reaction conditions (recombinant whole cells of *E. coli* expressing NCR, NADPH, MES-buffer pH 6.8, 50 mM, 1-PrOH 10% v:v). In order to clarify this discrepancy, we re-investigated substrate **3a** using all ene-reductases (entries 27–33). Again, the absolute configuration of **3b** obtained by using OYE2 was deduced by comparison of optical rotation values with literature data and proved to be (*S*). This result was double-checked by chemical reduction of the aldehyde (*S*)-**3b** obtained *via* bioreduction using $NaBH_4$ to yield 2-methyl-3-phenyl-1-propanol (**3c**), which was proven to be (*S*)-configurated on the basis of its optical rotation. Overall, the stereochemical outcome of the reduction of **3a** nicely matched our previous results, as may be expected since substrates **1a–3a** represent a structurally homologous series: while YqjM and OPR1 furnished (*R*)-**3b** with modest stereoselectivities (e.e.$_{max}$ 53% using OPR1), OPR3, NCR and OYE1–3 gave predominantly (*S*)-**3b** in up to 96% e.e. In view of these results, the stereochemical assignment of **3b** — reported to be (*R*)[10] — has to be corrected to be (*S*).

In order to test whether the stereochemical preference of OYE1 and NCR to yield (*S*)-**3b** from **3a** could be predicted *via* molecular modelling, substrate complexes were modelled using docking simulations and molecular mechanics optimization (see Experimental Section). In the case of OYE1, a single binding mode of **3a** was obtained (Fig. 2, left) which clearly indicates that the (*S*)-configured product will be formed by *trans*-hydrogenation (hydride transfer from the flavin onto Cβ of **3a** and protonation of Cα by Tyr-196). Initial attempts of docking **3a** into the active site of NCR (assuming a rigid enzyme structure) failed to produce a productive binding mode, because the active site region was partially blocked by side chains of neighbouring residues. Thus, the modelled structure of the OYE1 complex (Fig. 2, left) was used to build a model of the corresponding complex with NCR. Only small movements of the interfering residues (Trp-64, His-126 and Tyr-341) were enough to yield exactly the same optimized binding mode as in the case of OYE1, again predicting the formation of (*S*)-**3b** (Fig. 2 right). Especially in the case of His-126, the crystallographic B-factors indicate significant flexibility of this part of the structure.

Conclusions

A convenient chemo-enzymatic synthesis for the fragrance aldehydes Lilial™ (**1b**) and Helional™ (**2b**) was developed *via* asymmetric bioreduction of α-methyldihydrocinnamaldehyde derivatives **1a** and **2a** catalysed by cloned and overexpressed ene-reductases. Whereas (*R*)-**1b** and (*R*)-**2b** were formed in modest e.e.s using YqjM and OPR1; NCR and OYEs 1–3 yielded (*S*)-antipodes in up to 97% e.e., when the reactions were run in an aqueous–organic biphasic system containing *t*-butyl methyl ether (20%, v:v). The stereochemical outcome of the reduction of α-methylcinnamaldehyde **3a** using NCR and

OYE1–3 — previously reported to be (*R*)[10] — was unambiguously corrected to be (*S*). Our biocatalytic method compares favourably with asymmetric hydrogenation protocols based on iridium-phosphanodihydrooxazole catalysts (e.e.s up to 94%),[15] asymmetric alkylation using SAMP/RAMP-hydrazone (e.e.s up to 90%)[13] and counteranion-directed organocatalytic transfer hydrogenation (e.e.s up to 98%).[16]

Experimental

Source of enzymes

The open reading frame of *Lycopersicon esculentum* OPR1 was cloned into pET-21a and overexpressed as a C-terminal hexahistidine tagged protein in *E. coli* BL21 cells. The overexpressed recombinant protein was purified on a Ni-NTA affinity column (Invitrogen) according to the manufacturer's protocol. *Lycopersicon esculentum* OPR3 and YqjM from *Bacillus subtilis* were overexpressed and purified as reported recently.[17,18] The cloning, purification and characterisation of old yellow iso-enzymes from yeast (OYE1 from *Saccharomyces carlsbergensis*, OYE2 and OYE3 from *Saccharomyces cerevisiae*) and *Zymomonas mobilis* reductase (NCR) were performed according to literature.[10,19] NEM-reductase (from *E. coli*), PETN-reductase (from *Enterobacter cloacae*) and morphinone-reductase (from *Pseudomonas putida* M10) were provided by N. C. Bruce (Department of Biology, University of York, York, UK).[20]

General procedure for the enzymatic bioreduction under standard conditions

An aliquot of enzyme (OPR1, OPR3, YqjM, OYE1–3, NCR, NEM-reductase, morphinone reductase, PETN-reductase, protein concentration in biotransformations: 75–125 μg mL^{-1}) was added to a Tris-HCl buffer solution (0.8 mL, 50 mM, pH 7.5) containing the substrate (10 mM) and the cofactor NADH (15 mM). The mixture was shaken at 30 °C and 120 rpm. After 24 h the products were extracted with EtOAc (2 × 0.5 mL). The combined organic phases were dried over Na_2SO_4 and analysed on achiral GC to determine the conversion and on chiral GC or HPLC, respectively, to determine the enantiomeric excess.

General procedure for cofactor recycling

An aliquot of enzyme (see above) was added to a Tris-HCl buffer solution (0.8 mL, 50 mM, pH 7.5) containing the substrate (10 mM) the oxidized form of the cofactor (NAD$^+$, 100 μM), the cosubstrate (glucose, 20 mM) and the recycling enzyme (glucose dehydrogenase, 10 U). The mixture was shaken at 30 °C and 120 rpm for 24 h and worked up as described above.

General procedure for the enzymatic bioreduction using organic cosolvents

An organic co-solvent (EtOH, *i*-Pr_2O, *tert*-BuOMe, ethyl acetate and n-hexane) was employed in a ratio of 20% (v:v). The substrate (10 mM) was dissolved in the organic solvent (200 μL, for the cosolvent concentration study in 50–250 μL of *t*-BuOMe) and added to a Tris-HCl buffer solution (0.75–0.95 mL, 50 mM, pH 7.5) containing either the cofactor NADH (10 mM) or the cofactor recycling system (see above) followed by the addition of an aliquot of enzyme (see above). The mixture was shaken at 30 °C and 120 rpm for 24 h and worked up as described above.

Table 2 Optical rotation values of products

Compound	$[\alpha]_D^{20}$	Conditions[a]	E.e. [%]	Config.	Reference
1b[b]	+4.5	$c = 1.8$, $CHCl_3$	83	(*S*)	this study
1b	−5.2	$c = 1$, $CHCl_3$	95	(*R*)	ref.21
2b[b]	−2.9	$c = 2.2$, $CHCl_3$	95	(*S*)	this study
2b	−2.8	$c = 1.07$, $CHCl_3$	≥90	(*S*)	ref.13
3b[b]	−4.1	$c = 0.5$, MeOH	96	(*S*)	this study
3b	+7.0	$c = 0.7$, MeOH	76	(*R*)	ref.22
3b	−4.42	$c = 4$, MeOH	94	(*S*)	ref.23
3c[c]	−11.3	$c = 1.0$, $CHCl_3$	96	(*S*)	this study
3c	−14.0	$c = 0.25$, $CHCl_3$	87	(*S*)	ref.24

[a] Concentration [g/100 mL]; [b] obtained by using OYE2; [c] 2-methyl-3-phenyl 1-propanol.

Determination of the absolute configuration

The absolute configuration of the products **1b–3b** was determined by comparison of its optical rotation value ($[\alpha]_D^{20}$) with literature data (Table 2). Enantioenriched material of **1b–3b** was obtained by repeating the bioreduction of **1a–3a** 40 times in parallel experiments in the presence of cofactor recycling. The combined aqueous phases were extracted, dried (Na_2SO_4) and evaporated. Compound **1b** was purified by flash chromatography (petroleum ether/ethyl acetate 20 : 1), **2b** and **3b** were directly used for optical rotation measurements.

The absolute configuration of **3b** was independently double-checked by comparison of the optical rotation value of **3c** (2-methyl-3-phenylpropan-1-ol), which was obtained by chemical reduction of **3b** (derived *via* bioreduction of **3a** using OYE2) using $NaBH_4$.

Docking and minimization

A molecular model of **3a** was docked into the structures of OYE1 from *Saccharomyces cerevisiae* and of NCR from *Zymomonas mobilis* using AutoDock 4.0[25] as implemented in YASARA Structure.[26,27] Protein coordinates for OYE1[28] were taken from the PDB (entry code: 1OYB), while the structure of NCR was kindly provided by Wolfgang Hoeffken (BASF SE, unpublished results). The flavin cofactor was modelled as reduced $FMNH^-$ in both structures, protonation and tautomerisation states of His residues were chosen according to hydrogen bonding networks. Asp, Glu, Arg, and Lys residues were treated as charged. A model of the substrate was built and optimized within YASARA, and AM1-BCC partial charges[29] were applied. The position and orientation of the ligand as well as one torsion angle were allowed to vary while the protein was kept rigid. The search was restricted to a 15 Å^3 box around the N5 atom of the cofactor. Twenty independent simulations were performed employing a genetic algorithm (population size 150, number of generations 20000). The lowest energy structures of each independent run were clustered with an r.m.s-tolerance of 2.0 Å. The docking mode with the overall lowest energy was subjected to an additional molecular mechanics optimization in YASARA. Rigid docking failed to yield meaningful results in the case of NCR. Thus, the final structure

of the complex of **3a** and OYE1 was used to build a model of the corresponding complex with NCR.

Acknowledgements

The authors would like to express their cordial thanks for financial support by BASF SE and to R. Stürmer, B. Hauer and K. Ditrich for stimulating discussions, and to W. Hoeffken (Ludwigshafen) for providing the coordinates of NCR. N. C. Bruce and H. Housden (York) are thanked for the generous donation of PETN-, NEM- and morphinone reductase.

References

1 (*a*) C. Chapuis and D. Jacoby, *Appl. Catal., A*, 2001, **221**, 93–117; (*b*) D. Pybus and C. Sell, *The chemistry of fragrances*, RSC Paperbacks, Royal Society of Chemistry, Cambridge, 1999.
2 (*a*) A. Abate, E. Brenna, C. Fuganti, F. G. Gatti and S. Serra, *Chem. Biodiversity*, 2004, **1**, 1888–1898; (*b*) L. Doszczak, P. Kraft, H. P. Weber, R. Bertermann, A. Triller, H. Hatt and R. Tacke, *Angew. Chem., Int. Ed.*, 2007, **46**, 3367–3371; (*c*) E. Brenna, C. Fuganti and S. Serra, *Tetrahedron: Asymmetry*, 2003, **14**, 1–42.
3 L. A. Saudan, *Acc. Chem. Res.*, 2007, **40**, 1309–1319.
4 For example see: (*a*) S. Akutagawa, *Appl. Catal., A*, 1995, **128**, 171–207; (*b*) S. Bovo, A. Scrivanti, M. Bertoldini, V. Beghetto and O. Matteoli, *Synthesis*, 2008, 2547–2550; (*c*) W. S. Knowles and R. Noyori, *Acc. Chem. Res.*, 2007, **40**, 1238–1239; (*d*) A. J. Minnaard, B. L. Feringa, L. Lefort and J. G. de Vries, *Acc. Chem. Res.*, 2007, **40**, 1267–1277.
5 (*a*) J. W. Yang, M. T. H. Fonseca, N. Vignola and B. List, *Angew. Chem., Int. Ed.*, 2005, **44**, 108–110; (*b*) H. Adolfsson, *Angew. Chem., Int. Ed.*, 2005, **44**, 3340–3342; (*c*) S. G. Ouellet, A. M. Walji and D. W. C. MacMillan, *Acc. Chem. Res.*, 2007, **40**, 1327–1339; (*d*) K. Akagawa, H. Akabane, S. Sakamoto and K. Kudo, *Tetrahedron: Asymmetry*, 2009, **20**, 461–466.
6 (*a*) D. J. Watson, R. J. B. R. J. Jesudason, S. K. Beaumont, G. Kyriakou, J. W. Burton and R. M. Lambert, *J. Am. Chem. Soc.*, 2009, **131**, 14584–14589; (*b*) A. Tungler, E. Sipos and V. Hada, *Curr. Org. Chem.*, 2006, **10**, 1569–1583.
7 S. Serra, C. Fuganti and E. Brenna, *Trends Biotechnol.*, 2005, **23**, 193–198.
8 (*a*) M. Majeric, A. Avdagic, Z. Hamersak and V. Sunjic, *Biotechnol. Lett.*, 1995, **17**, 1189–1194; (*b*) C. Fuganti and S. Serra, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3758–3764; (*c*) P. D'Arrigo, C. Fuganti, G. Pedrocchi-Fantoni and S. Servi, *Tetrahedron*, 1998, **54**, 15017–15026; (*d*) G. Fronza, C. Fuganti, P. Grasselli, L. Majori, G. Pedrocchi-Fantoni and F. Spreafico, *J. Org. Chem.*, 1982, **47**, 3289–3296; (*e*) G. Fronza, C. Fuganti, M. Pinciroli and S. Serra, *Tetrahedron: Asymmetry*, 2004, **15**, 3073–3077; (*f*) V. Sunjic, M. Majeric and Z. Hamersak, *Croat. Chem. Acta*, 1996, **69**, 643–660.
9 For a review see: (*a*) R. Stuermer, B. Hauer, M. Hall and K. Faber, *Curr. Opin. Chem. Biol.*, 2007, **11**, 203–213; (*b*) S. K. Padhi, D. J. Bougioukou and J. D. Stewart, *J. Am. Chem. Soc.*, 2009, **131**, 3271–3280; (*c*) J. F. Chaparro-Riggers, T. A. Rogers, E. Vazquez-Figueroa, K. M. Polizzi and A. S. Bommarius, *Adv. Synth. Catal.*, 2007, **349**, 1521–1531; (*d*) M. Kataoka, A. Kotaka, R. Thiwthong, M. Wada, S. Nakamori and S. Shimizu, *J. Biotechnol.*, 2004, **114**, 1–9.
10 For the stereoselective bioreduction of α-methylcinnamaldehyde (**3a**) see: A. Müller, B. Hauer and B. Rosche, *Biotechnol. Bioeng.*, 2007, **98**, 22–29.
11 (*a*) M. Hall, C. Stueckler, W. Kroutil, P. Macheroux and K. Faber, *Angew. Chem., Int. Ed.*, 2007, **46**, 3934–3937; (*b*) M. Hall, C. Stueckler, H. Ehammer, E. Pointner, G. Oberdorfer, K. Gruber, B. Hauer, R. Stuermer, W. Kroutil, P. Macheroux and K. Faber, *Adv. Synth. Catal.*, 2008, **350**, 411–418; (*c*) M. Hall, C. Stueckler, B. Hauer, R. Stuermer, T. Friedrich, M. Breuer, W. Kroutil and K. Faber, *Eur. J. Org. Chem.*, 2008, 1511–1516.
12 A. Scrivanti, M. Bertoldini, V. Beghetto and U. Matteoli, *Tetrahedron*, 2008, **64**, 543–548.
13 D. Enders and M. Backes, *Tetrahedron: Asymmetry*, 2004, **15**, 1813–1817.
14 PETN-reductase gave (*R*)-**3b** with $e.e._{max}$ 14%.
15 A. Lightfoot, P. Schnider and A. Pfaltz, *Angew. Chem., Int. Ed.*, 1998, **37**, 2897–2899.
16 B. List and M. Stadler, *Synlett*, 2008, 597–599.
17 C. Breithaupt, R. Kurzbauer, H. Lilie, A. Schaller, J. Strassner, R. Huber, P. Macheroux and T. Clausen, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 14337–14342.
18 K. Kitzing, T. B. Fitzpatrick, C. Wilken, J. Sawa, G. P. Bourenkov, P. Macheroux and T. Clausen, *J. Biol. Chem.*, 2005, **280**, 27904–27913.
19 (*a*) K. Saito, D. J. Thiele, M. Davio, O. Lockridge and V. Massey, *J. Biol. Chem.*, 1991, **266**, 20720–20724; (*b*) K. Stott, K. Saito, D. J. Thiele and V. Massey, *J. Biol. Chem.*, 1993, **268**, 6097–6106; (*c*) Y. S. Niino, S. Chakraborty, B. J. Brown and V. Massey, *J. Biol. Chem.*, 1995, **270**, 1983–1991.
20 (*a*) C. E. French, S. Nicklin and N. C. Bruce, *J. Bacteriol.*, 1996, **178**, 6623–6627; (*b*) K. Miura, Y. Tomioka, H. Suzuki, M. Yonezawa, T. Hishinuma and M. Mizugaki, *Biol. Pharm. Bull.*, 1997, **20**, 110–112; (*c*) C. E. French and N. C. Bruce, *Biochem. J.*, 1994, **301**, 97–103.
21 D. Enders and H. Dyker, *Liebigs Ann. Chem.*, 1990, **1990**, 1107–1110.
22 A. Baeza, C. Najera and J. M. Sansano, *Eur. J. Org. Chem.*, 2007, (7), 1101–1112.
23 M. V. Rangaishenvi, B. Singaram and H. C. Brown, *J. Org. Chem.*, 1991, **56**, 3286–3294.
24 S. D. Bull, S. G. Davies, R. L. Nicholson, H. J. Sanganee and A. D. Smith, *Org. Biomol. Chem.*, 2003, **1**, 2886–2899.
25 G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *J. Comput. Chem.*, 1998, **19**, 1639–1662.
26 E. Krieger, T. Darden, S. B. Nabuurs, A. Finkelstein and G. Vriend, *Proteins: Struct., Funct., Bioinf.*, 2004, **57**, 678–683.
27 E. Krieger, G. Koraimann and G. Vriend, *Proteins: Struct., Funct., Genet.*, 2002, **47**, 393–402.
28 K. M. Fox and P. A. Karplus, *Structure*, 1994, **2**, 1089–1105.
29 A. Jakalian, D. B. Jack and C. I. Bayly, *J. Comput. Chem.*, 2002, **23**, 1623–1641.

