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Asymmetric Synthesis of *O*-Protected Acyloins Using Enoate Reductases: Stereochemical Control through Protecting Group Modification

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O-Protected cyclic acyloins were obtained in nonracemic form through asymmetric bioreduction of α,β-unsaturated alkoxy ketones by using 11 different enoate reductases from the "Old Yellow Enzyme" family. The stereochemical outcome of the biotransformation could be switched by variation of the *O*-protecting group or by the ring size of the substrate, which allows access to both stereoisomers in up to >97 % *ee* Whereas α-alkoxy enones were readily accepted as substrates, β-analogs were not converted. Overall, α-alkoxy enones represent a novel type of substrate for flavin-dependent ene-reductases.

Introduction

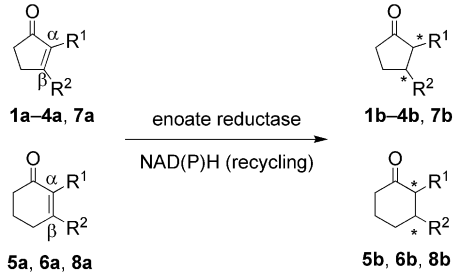
Due to their bifunctionality containing a nucleophilic and an electrophilic group, nonracemic acyloins are important building blocks in organic synthesis. They can be converted into alcohols, diols, epoxides, amines, hydroxylamines, and haloketones, usually with a high degree of chirality transfer. Consequently, they are frequently employed as building blocks for the asymmetric synthesis of bioactive compounds.[1–3] For the synthesis of nonracemic acyloins, classic approaches include the acyloin and the benzoin condensation, both of which have been performed in an asymmetric fashion.[4,5] The most common strategy to obtain nonracemic acyloins is based on the oxidation of enolates by chiral *N*-sulfonyloxaziridines derived from camphor.[6–8]

For the production of nonracemic acyloins, a broad range of enzymes belonging to different classes, including oxidoreductases (peroxidases, carbonyl reductases and oxidases), hydrolases (lipases), and lyases (hydroxynitrile lyases and transketolases)[3] were successfully used, including most prominently the thiamine-dependent C-C lyases.[9]

During our investigation of the exploration of the substrate spectrum of flavin-dependent enoate reductases from the old yellow enzyme family [EC 1.3.1.X],[10,11] we envisaged that *O*-functionalized α,β-unsaturated ketones might be suitable substrates for these enzymes, which would furnish *O*-protected acyloins as the reduction product. Although the asymmetric hydrogenation of α,β-unsaturated α-alkoxycarboxylic acids and esters using rhodium catalysts bearing chiral phosphane ligands[12] was reported, to the best of our knowledge, α-alkoxy-functionalized enones were not investigated as substrates for asymmetric (bio)reductions.[13]

Results and Discussion

In contrast to electronically activated enol ethers derived from open-chain β-dicarbonyl compounds, which proved to undergo spontaneous hydrolysis in aqueous buffer under standard conditions, cyclic analogs were sufficiently stable under the reaction conditions. Thus, alkoxy-functionalized cyclohexen-2-ones **1a**–**4a** and cyclopenten-2-ones **5a** and **6a**



Compound	R^1	R^2
1a,**b**	OMe	H
2a,**b**	O-allyl	H
3a,**b**	O-*n*Pr	H
4a,**b**	OBn	H
5a,**b**	OMe	H
6a,**b**	OBn	H
7a,**b**	H	OMe
8a,**b**	H	OMe

Scheme 1. Asymmetric bioreduction of α- and β-alkoxy substituted enones **1a**–**8a**.

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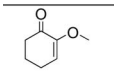
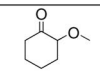
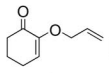
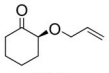
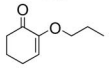
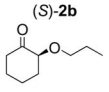
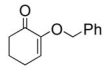
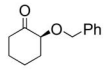
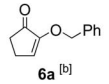
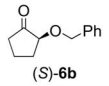
Table 1. Conversion, absolute configuration, and enantiomeric excess of products **1b**–**6b**.

Substrate	Product	Cofactor[a]	OYE1		OYE2		OYE3		OPR1		OPR3		YqjM		NCR		XenA		XenB		NerA		EBP1	
			c [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]	*c* [%]	*ee* [%]
 **1a**	 (*R*)- or (*S*)-**1b**	NADH	37	92 (*R*)	26	90 (*R*)	25	84 (*R*)	22	<5	9	59 (*R*)	31	24 (*R*)	46	77 (*R*)	77	47 (*S*)	21	67 (*R*)	nc	nd	24	85 (*R*)
		GDH	59	97 (*R*)	66	90 (*R*)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
 **2a**	 (*S*)-**2b**	NADH	<3	nd	<3	nd	<3	nd	91	77 (*S*)	11	64 (*S*)	26	64 (*S*)	94	97 (*S*)	80	91 (*S*)	74	98 (*S*)	<3	nd	nc	nd
 **3a**	 (*S*)-**3b**	NADH	<3	nd	<3	nd	<3	nd	99	83 (*S*)	15	38 (*S*)	31	62 (*S*)	82	96 (*S*)	99	92 (*S*)	86	98 (*S*)	<3	nd	nc	nd
 **4a**	 (*S*)-**4b**	NADH	13	87 (*S*)	12	87 (*S*)	6	67 (*S*)	98	70 (*S*)	56	64 (*S*)	91	71 (*S*)	99	93 (*S*)	93	85 (*S*)	78	96 (*S*)	12	83 (*S*)	12	18 (*S*)
		GDH	–	–	–	–	–	–	96	74 (*S*)	52	66 (*S*)	–	–	99	92 (*S*)	92	81 (*S*)	44	93 (*S*)	–	–	–	–
 **5a**	 (*S*)-**5b**	NADH	10	58 (*S*)	<3	nd	<3	nd	25	66 (*S*)	nc	nd	<3	nd	55	84 (*S*)	10	88 (*S*)	76	90 (*S*)	<3	nd	nc	nd
		FDH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9	88 (*S*)	30	98 (*S*)	–	–	–	–
 **6a** [b]	 (*S*)-**6b**	NADH	<3	nd	<3	nd	<3	nd	98	95 (*S*)	5	>99 (*S*)	3	92 (*S*)	74	>99 (*S*)	9	97 (*S*)	13	98 (*S*)	<3	nd	nc	nd
		FDH	–	–	–	–	–	–	>99	97 (*S*)	–	–	–	–	10	>99 (*S*)	–	–	–	–	–	–	–	–

[a] Standard condition: substrate (10 mM), enzyme (75–125 μg/mL), Tris-HCl-buffer (0.8 mL, 50 mM, pH 7.5), NADH (15 mM); GDH = NAD^+ (100 μM)/glucose dehydrogenase or formate dehydrogenase (10U)/glucose or formate, respectively, (20 mM). [b] TBME was added as a cosolvent (v:v 20%) to solubilize the substrate; abbreviation of enzymes: old yellow isoenzyme OYE1 from *Saccharomyces carlsbergensis*, OYE2 and OYE3 from *Saccharomyces cerevisiae*, 12-oxophytodienoic acid reductase isoenzymes OPR1 and OPR3 from *Lycopersicon esculentum*, old yellow enzyme homolog YqjM from *Bacillus subtilis*; nicotinamide-dependent cyclohexenone reductase NCR from *Zymomonas mobilis*, xenobiotic reductase XenA and XenB from *Pseudomonas putida* and *P. fluorescens*, respectively, glycerol trinitrate reductase NerA from *Agrobacterium radiobacter*, estrogen-binding protein EBP1 from *Candida albicans*; *c* = conversion; *ee* = enantiomeric excess; nc = no conversion; nd = not determined.

together with the corresponding β-alkoxy analogs **7a** and **8a** were synthesized from the corresponding 1,2- and 1,3-diones. In order to explore the influence of the size of the α/β-substituent on the stereoselectivity, the *O*-protecting group was varied from small (methyl) via medium (*n*-propyl/allyl) to large (benzyl). In addition, the allyl[14] and benzyl moieties[15] were chosen for their easy removal without endangering the newly generated stereocenter (Scheme 1). The results from the enzymatic reduction are summarized in Table 1.

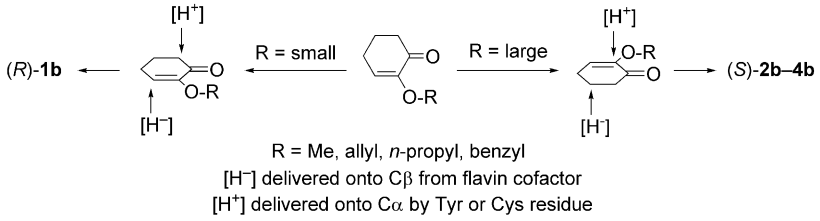
The identity of the products was confirmed by comparison with racemic reference material obtained through catalytic hydrogenation and the absolute configuration of the products was elucidated by co-injection with authentic non-racemic samples synthesized from (1*S*,2*S*)-cyclohexane-1,2-diol and (1*S*,2*S*)- or (1*R*,2*R*)-cyclopentane-1,2-diol through monoalkylation of the diol, followed by Jones oxidation of the unprotected hydroxy moiety to the corresponding *O*-protected acyloin as described in the Experimental Section.

Overall, the influence of the substrate size on the stereochemical outcome of the bioreduction followed a very consistent pattern (Table 1): The cyclohexenone bearing the shortest protecting group (i.e., **1a**) was accepted by all enoate reductases, except for NerA. However, the reactivities ranged from low (OPR3) to good (XenA). Interestingly, both enantiomeric products were obtained depending on the enzyme: Whereas most enzymes furnished (*R*)-**1b** in up to 92%*ee* (OYE1), XenA gave the mirror-image product (*S*)-**1b** in 47%*ee* The best data sets obtained by using OYE1 and OYE2 were repeated in the presence of cofactor-recycling [glucose dehydrogenase (GDH)/glucose/NAD^+], which yielded satisfactory results.

Substrates bearing a medium-sized allyl (**2a**) or *n*-propyl protecting group (**3a**) gave almost identical results: Whereas EBP1 was unreactive, the majority of the enzymes showed low to modest reactivities, only OPR1, NCR, XenA, and XenB were sufficiently active. No stereochemical switch was observed with XenA and all enzymes furnished (*S*)-**2b** and (*S*)-**3b**; excellent selectivities were obtained by using XenB (98%*ee*).

The cyclohexenone derivative bearing the largest benzyl group (**4a**) could be reduced by all enzymes with varying efficiencies; the best conversions were obtained with OPR1, YqjM, NCR, and XenA. Again, only (*S*)-**4b** was formed in up to 96%*ee* by using XenB. The preparative applicability of this method was exemplified by repeating the best data sets in the presence of the GDH/glucose-cofactor recycling system (Scheme 2).

Cyclopentenone derivatives bearing a short *O*-methyl protecting group (i.e., **5a**) could not be reduced with sufficient rates by most enzymes, only NCR and XenB were reasonably active, showing conversions of 55 and 76%, respectively, with *ee* values of 84 and 90%. In contrast to cyclohexenone analog **1a**, no stereoselectivity switch de-



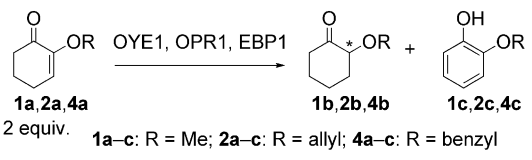
Scheme 2. Substrate-based stereocontrol for α-alkoxycyclohex-2-enone derivatives due to "flipped" orientation of the substrate within the active site, forced by a gradual increase in the steric demand of the *O*-protecting group R.

pending on the enzyme was observed with **5a** and all enzymes produced (*S*)-**5b** in up to 98%*ee* (XenB). This trend is in line with the results obtained by using structurally closely related α-methyl-substituted cyclohexenone and cyclopentenone derivatives by using OYE1–3, NCR,[10c] OPR1, OPR3, YqjM,[10a,10b] and PETN-reductase,[10e,16] which predominantly formed (*R*)-2-methylcyclohexanone but (*S*)-2-methylcyclopent-2-anone. This stereochemical switch was recently explained by a "flipped" orientation of the substrate in the active site.[16,17]

When the steric demand of the α-alkoxy substituent was increased from methyl (i.e., **5a**) to benzyl (i.e., **6a**), the stereoselectivity of all active enzymes increased, and the best overall results were obtained with OPR1 and NCR. Again, cofactor recycling proved the preparative feasibility of the method.

With β-alkoxy-functionalized cyclohexenone **7a** and cyclopentenone **8a** derivatives, no conversion could be achieved. This might be explained by a +M effect[18] exerted by the O atom, which enhances the electron density at Cβ, thereby quenching its δ^+ charge, which is required to allow nucleophilic attack of the hydride delivered from N5 of the flavin cofactor. Again, an analogous effect was observed for β-methyl-substituted cyclic alkenones, which were reduced at considerably lower rates than the corresponding α-substituted substrate analogs.[10a–10c,10e,16]

Careful monitoring of the bioreductions occasionally revealed the formation of phenolic byproducts **1c**, **2c**, and **4c**. In most cases they were only observed in trace amounts, with the exception of OYE1, OPR1, and EBP1, which formed **1b**, **2b**, and **4b** together with **1c**, **2c**, and **4c** from **1a**, **2a**, and **4a**, respectively, in significant amounts (OYE1: 29% **1c**; OPR1: 22% **1c**; OYE1: **2c** 20%; EBP1: **4c** 30%). The latter represents a disproportionation of enones, which was reported as a side activity of OYEs,[19] and which was recently exploited as a nicotinamide-independent method for the asymmetric bioreduction of activated alkenes by using β-methylcyclohexenone or cyclohexane-1,4-dione as a hydrogen donor (Scheme 3).[20]



Scheme 3. Disproportionation of 2-methoxycyclohex-2-enone derivatives catalyzed by OYE1, OPR1, and EBP1.

Conclusions

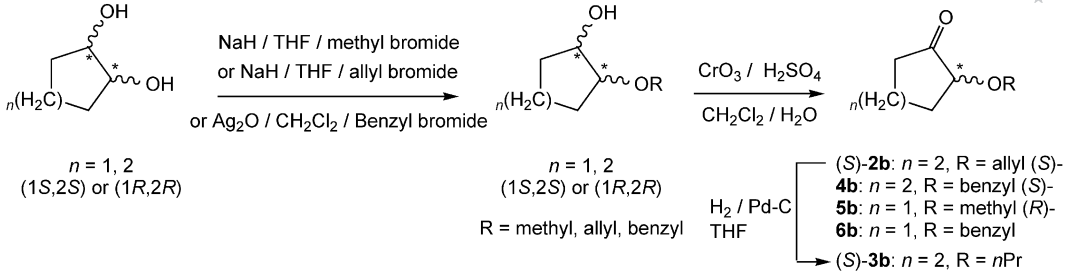
We could demonstrate that conjugated α-alkoxycycloenones are a novel type of substrate for flavin-dependent enoate reductases, which upon bioreduction yield *O*-protected acyloins in up to >99%*ee* The stereochemistry could be efficiently controlled by variation of the size of the *O*-protecting group, which – for cyclohexenone derivatives – furnished (*R*)-configured acyloins with short-chain (methyl) groups and mirror-image (*S*)-products with long-chain (*n*-propyl, allyl or benzyl) analogs in very high *ee* values (97–98%). No switch was detected with cyclopentenone derivatives, which invariably produced (*S*)-acyloins in up to >99%*ee*.

Experimental Section

General: TLC plates were run on silica gel Merck 60 (F_{254}) and compounds were visualized by spraying with Mo-reagent [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (100 g/L), $Ce(SO_4)_2{\cdot}4H_2O$ (4 g/L) in H_2SO_4 (10%)] or by UV light (254 nm). Silica gel 60 from Merck was used for flash chromatography. GC–MS analyses were performed with an Agilent 7890A GC system equipped with an Agilent 5975C mass-selective detector (electron impact, 70 eV) by using a (5%-phenyl)–methylpolysiloxane phase column (Agilent HP-5 ms, 30 m × 0.25 mm, 0.25 μm film). Helium was used as the carrier gas (column flow: 2 mL/min). GC–FID analyses were carried out with a Varian 3800 by using H_2 as a carrier gas (14.5 psi). NMR spectra were recorded with a Bruker Avance III 300 MHz NMR spectrometer. Chemical shifts are reported relative to TMS (δ = 0.00 ppm). Cyclohexane-1,2-dione, hexamethyl disilazane (HMDS), 2-chlorocyclopentane-1-ol, cyclohexane-1,3-dione, cyclopentane-1,3-dione, pyrocatechol, 2-(benzyloxy)phenol, 2-methoxyphenol, (1*S*,2*S*)-cyclohexane-1,2-diol, (1*S*,2*S*)-cyclopentane-1,2-diol, and (1*R*,2*R*)-cyclopentane-1,2-diol were purchased from Aldrich.

Source of Enzymes: 12-Oxophytodienoic acid reductase isoenzymes OPR1 and OPR3 from *Lycopersicon esculentum* and the OYE-homolog YqjM from *Bacillus subtilis* were overexpressed and purified as reported.[10a,21] The cloning, purification, and characterization of old yellow isoenzymes from yeast (OYE1 from *Saccharomyces carlsbergensis*, OYE2 and OYE3 from *Saccharomyces cerevisiae*) and nicotinamide-dependent cyclohexenone reductase (NCR) *Zymomonas mobilis* reductase were performed according to the literature.[22] Xenobiotic reductases XenA and XenB from *Pseudomonas putida* and *P. putida*, respectively, glycerol trinitrate reductase NerA from *Agrobacterium radiobacter* and estrogen binding protein EBP1 from *Candida albicans* were obtained as recently published.[10e,23]

General Procedure for the Enzymatic Bioreduction of 1a–8a: An aliquot of the enzyme (OYE1–3, OPR1, OPR3, YqjM, NCR, XenA,



Scheme 4. Asymmetric synthesis of reference material for (*S*)-**2b**, (*S*)-**4b**, (*S*)-**5b**, and (*R*)-**6b**.

XenB, NerA, and EBP1, 60–100 μg, protein concentration in biotransformations 75–125 μg/mL) 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). In the case of **6a**, the substrate was solubilized by addition of *t*BuOMe (v:v 20%). 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 with Na_2SO_4 and analyzed on achiral GC to determine the conversion and on chiral GC to determine the enantiomeric excess. For experiments in the presence of cofactor recycling, NADH was replaced by the oxidized form of the cofactor (NAD^+, 100 μM), the cosubstrate (glucose or formate, 20 mM), and the recycling enzyme (glucose dehydrogenase or formate dehydrogenase, 10 U), respectively.

Determination of Absolute Configuration of Products 1b–6b

(1*S*,2*S*)-2-(Allyloxy)cyclohexanol and (1*S*,2*S*)-2-Methoxycyclopentanol: The corresponding (1*S*,2*S*)-diol (0.86 mmol) was dissolved in THF (5 mL) and NaH (40 mg, 1 mmol, 60% in mineral oil) was added. After 10 min of stirring, allyl or methyl bromide (0.5 mmol) was added, and the mixture was stirred for 1 h at room temperature. Then, more NaH was added (40 mg; 1 mmol; 60% in mineral oil), and the mixture was stirred at room temperature for 16 h. Then, HCl (5 mL, 1 M) was added, and the mixture was extracted with ethyl acetate (3 × 10 mL). The organic phase was dried with Na_2SO_4, and the solvent was removed in vacuo to yield a mixture containing 15% of the desired product monoalkylated diol, which was used without further purification for the next step.

(1*S*,2*S*)-2-(Benzyloxy)cyclohexanol and (1*R*,2*R*)-2-(Benzyloxy)-cyclopentanol: The corresponding (1*S*,2*S*)- and (1*R*,2*R*)-diol (0.86 mmol) and benzyl bromide (152.9 mg, 0.9 mmol) were dissolved in CH_2Cl_2 (5 mL) and silver(I)oxide (1.35 mmol, 310 mg) was added. The mixture was stirred at room temperature for 24 h and then HCl (2 mL, 1 M) was added, and the mixture was extracted with ethyl acetate (2 × 10 mL). The organic phase was dried with Na_2SO_4, and the solvent was removed in vacuo to yield a mixture containing 80% of the of monobenzylated diol, which was used without further purification for the next step.[24]

The absolute configuration of **1b** was determined as (*R*) by comparison of the optical rotation value of **1b** {$[a]_D^{20}$ = +29.5 (CH_2Cl_2)} obtained by bioreduction of **1a** using EBP1 with literature data.[25]

General Procedure for the Oxidation of Monoethers of Cyclopentane- and Cyclohexane-1,2-diols to the Corresponding *O*-Protected Acyloins (*S*)-2b, (*S*)-4b, (*S*)-5b, and (*R*)-6b: (*S*)-2-(Allyloxy)cyclohexanone [(*S*)-**2b**], (*S*)-2-(benzyloxy)cyclohexanone [(*S*)-**4b**], (*S*)-2-methoxycyclopentanone [(*S*)-**5b**], and (*R*)-2-benzyloxycyclopentanone [(*R*)-**6b**] were obtained by Jones oxidation of the corresponding monoether derivatives of 1,2-diols as prepared above: Jones reagent CrO_3 (7 g, 70 mmol) was dissolved in water (50 mL) and H_2SO_4 (6.1 mL, conc.) was added dropwise under cooling. The monoether derivative was dissolved in CH_2Cl_2 (2 mL) and Jones reagent (2 mL) was added dropwise over 30 min with stirring. Excess oxidant was quenched by the addition of 2-butanol (2 mL). Water (10 mL) was added, and the products were extracted with CH_2Cl_2 (2 × 10 mL). The organic phase was filtered through Celite and dried with Na_2SO_4, and the solvent was removed in vacuo to afford (*S*)-2-(allyloxy)cyclohexanone [(*S*)-**2b**], (*S*)-2-(benzyloxy)-cyclohexanone [(*S*)-**4b**], (*S*)-2-methoxycyclopentanone [(*S*)-**5b**], and (*R*)-2-benzyloxycyclopentanone [(*R*)-**6b**].

(*S*)-2-Propoxycyclohexanone [(*S*)-3b]: (*S*)-2-(Allyloxy)cyclohexanone [(*S*)-**2b**] was hydrogenated according to method B (see Supporting Information), yielding (*S*)-2-propoxycyclohexanone [(*S*)-**3b**]. The absolute configuration of **1b** was determined by co-injection of enantioenriched reference material obtained by reduction of **1a** with EBP1 [20 mg, 78%*ee* (*R*)]. $[a]_D^{20}$ (CH_2Cl_2) = +29.5, for (*R*)-**1a** (Scheme 4).[25]

Supporting Information (see footnote on the first page of this article): Synthesis of substrates **1a**–**8a**; synthesis of reference material for *rac*-**1b**–**6b**, **2c**, and **3c**; analytical data for the GC-determination of conversion of substrates **1a**–**8a** and the enantiomeric composition of products **1b**–**6b**.

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[1] R. N. Patel, *Curr. Org. Chem.* **2006**, *10*, 1289–1321.
[2] A. S. Demir, P. Ayhan, S. B. Sopaci, *Clean* **2007**, *35*, 406–412.
[3] W. Adam, M. Lazarus, C. R. Saha-Möller, P. Schreier, *Acc. Chem. Res.* **1999**, *32*, 837–845.
[4] D. Enders, O. Niemeier, T. Balensiefer, *Angew. Chem. Int. Ed.* **2006**, *45*, 1463–1467.
[5] D. Enders, U. Kallfass, *Angew. Chem. Int. Ed.* **2002**, *41*, 1743–1745.
[6] F. A. Davis, M. S. Haque, T. G. Ulatowski, J. C. Towson, *J. Org. Chem.* **1986**, *51*, 2402–2404.
[7] F. A. Davis, B.-C. Chen, *Chem. Rev.* **1992**, *92*, 919–934.
[8] C. C. Hughes, A. K. Miller, D. Trauner, *Org. Lett.* **2005**, *7*, 3425–3428.
[9] J. Sukumaran, U. Hanefeld, *Chem. Soc. Rev.* **2005**, *34*, 530–542.
[10] For recent studies, see: a) M. Hall, C. Stueckler, W. Kroutil, P. Macheroux, 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, K. Faber, *Adv. Synth. Catal.* **2008**, *350*, 411–418; c) M. Hall, C. Stueckler, B. Hauer, R. Stuermer, T. Friedrich, M. Breuer, W. Kroutil, K. Faber, *Eur. J. Org. Chem.* **2008**,

1511–1516; d) C. Stueckler, M. Hall, H. Ehammer, E. Pointner, W. Kroutil, P. Macheroux, K. Faber, *Org. Lett.* **2007**, *9*, 5409–5411; e) N. J. Mueller, C. Stueckler, B. Hauer, N. Baudendistel, H. Housden, N. C. Bruce, K. Faber, *Adv. Synth. Catal.* **2010**, *352*, 387–394; f) C. Stueckler, N. J. Mueller, C. K. Winkler, S. M. Glueck, K. Gruber, G. Steinkellner K. Faber, *Dalton Trans.* **2010**, *39*, 8472–8476.

[11] For a historical study and reviews, see: a) O. Warburg, W. Christian, *Biochem. Z.* **1933**, *266*, 377–411; b) R. E. Williams, N. C. Bruce, *Microbiology* **2002**, *148*, 1607–1614; c) R. Stuermer, B. Hauer, M. Hall, K. Faber, *Curr. Opin. Chem. Biol.* **2007**, *11*, 203–213.

[12] a) M. J. Burk, C. S. Kalberg, A. Pizzano, *J. Am. Chem. Soc.* **1998**, *120*, 4345–4353; b) W. Chen, P. J. McCormack, K. Mohammed, W. Mbafor, S. M. Roberts, J. Whittall, *Angew. Chem. Int. Ed.* **2007**, *46*, 4141–4144.

[13] The bioreduction of diethyl oxaloacetate (which is in equilibrium with its enol form) by using baker's yeast proceeded through the carbonyl reductases acting on the keto form of the substrate: E. Santaniello, P. Ferraboschi, P. Grisenti, F. Aragozzini, E. Maconi, *J. Chem. Soc. Perkin Trans. 1* **1991**, 601–605.

[14] A. Dahlen, A. Sundgren, M. Lahmann, S. Oscarson, G. Hilmersson, *Org. Lett.* **2003**, *5*, 4085–4088; H. Tsukamoto, T. Suzuki, Y. Kondo, *Synlett* **2007**, 3131–3132.

[15] P. K. Mandal, J. S. McMurray, *J. Org. Chem.* **2007**, *72*, 6599–6601.

[16] A. Fryszkowska, H. Toogood, M. Sakuma, J. M. Gardiner, G. M. Stephens, N. S. Scrutton, *Adv. Synth. Catal.* **2009**, *351*, 2976–2990.

[17] S. K. Padhi, D. J. Bougioukou, J. D. Stewart, *J. Am. Chem. Soc.* **2009**, *131*, 3271–3280.

[18] R. C. Kerber, *J. Chem. Educ.* **2006**, *83*, 223–227.

[19] a) A. D. N. Vaz, S. Chakraborty, V. Massey, *Biochemistry* **1995**, *34*, 4246–4256; b) J. Buckman, S. M. Miller, *Biochemistry* **1998**, *37*, 14326–14336; c) P. A. Karplus, K. M. Fox, V. Massey, *FASEB J.* **1995**, *9*, 1518–1526.

[20] C. Stueckler, T. C. Reiter, N. Baudendistel, K. Faber, *Tetrahedron* **2010**, *66*, 663–667.

[21] a) C. Breithaupt, R. Kurzbauer, H. Lilie, A. Schaller, J. Strassner, R. Huber, P. Macheroux, T. Clausen, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 14337–14342; b) K. Kitzing, T. B. Fitzpatrick, C. Wilken, J. Sawa, G. P. Bourenkov, P. Macheroux, T. Clausen, *J. Biol. Chem.* **2005**, *280*, 27904–27913.

[22] a) K. Saito, D. J. Thiele, M. Davio, O. Lockridge, V. Massey, *J. Biol. Chem.* **1991**, *266*, 20720–20724; b) K. Stott, K. Saito, D. J. Thiele, V. Massey, *J. Biol. Chem.* **1993**, *268*, 6097–6106; c) Y. S. Niino, S. Chakraborty, B. J. Brown, V. Massey, *J. Biol. Chem.* **1995**, *270*, 1983–1991; d) A. Müller, B. Hauer, B. Rosche, *Biotechnol. Bioeng.* **2007**, *98*, 22–29.

[23] K. Durchschein, B. Ferreira-da Silva, S. Wallner, P. Macheroux, W. Kroutil, S. M. Glueck, K. Faber, *Green Chem.* **2010**, *12*, 616–619.

[24] A. Bouzide, G. Sauvé, *Tetrahedron Lett.* **1997**, *38*, 5945–5948.

[25] T. Rossi, C. Marchioro, A. Paio, R. J. Thomas, P. Zarantonello, *J. Org. Chem.* **1997**, *62*, 1653–1661.

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