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Asymmetric Bioreduction of Activated C=C Bonds Using *Zymomonas mobilis* NCR Enoate Reductase and Old Yellow Enzymes OYE 1–3 from Yeasts

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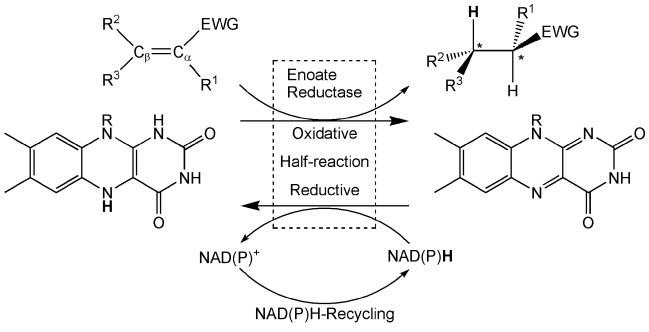
The asymmetric bioreduction of C=C-bonds bearing an electron-withdrawing group, such as an aldehyde, ketone, imide, nitro, carboxylic acid, or ester moiety by a novel enoate reductase from *Zymomonas mobilis* and Old Yellow Enzymes OYE 1–3 from yeasts furnished the corresponding saturated products in up to >99 % *ee*. Depending on the substrate type, stereocontrol was achieved by variation of the substrate structure, by switching the (*E*/*Z*) geometry of the alkene or by choice of the appropriate enzyme. This substrate- or enzyme-based stereocontrol allowed access to the opposite enantiomeric products.

Introduction

The asymmetric reduction of functionalized alkenes goes in hand with the creation of up to two stereogenic centers and thus constitutes a method of choice for the synthesis of chiral carbon centers. This transformation has been achieved by homogeneous catalysis by using (transition) metal-catalyzed *cis* hydrogenation,[1] and more recently by organocatalysis, which has shown promising results in the *trans* specific conjugate reduction of enals, enones, and nitroalkenes at the expense of a sacrificial dihydropyridine hydride donor.[2] Biocatalytic protocols for the bioreduction of activated alkenes bearing an electron-withdrawing substituent have gained growing interest with the recent "rediscovery" of Old Yellow Enzyme (OYE), a flavoprotein first described in 1932,[3,4] and OYE homologs from yeasts, bacteria, and plants.[5–15] Mechanistically, the stereoselective reduction of activated alkenes by these so-called enoate reductases [EC 1.3.1.31] has been investigated in great detail and was shown to proceed by a ping–pong bi–bi mechanism (Scheme 1).[15,16] First, the flavin cofactor is reduced at the expense of a nicotinamide cofactor NAD(P)H, which is followed by hydride transfer onto C-β of the substrate, whereas a Tyr residue (conserved along the enoate reductase enzyme family) adds a proton to C-α from the opposite side. Both steps are termed the "reductive" and the "oxidative" half-reaction, respectively. Overall, this mechanism results in *trans* addition of [2H] with absolute stereospecificity.



Scheme 1. Asymmetric bioreduction of activated alkenes bearing an electron-withdrawing group (EWG) by enoate reductases (the hydride being transferred is shown in bold).

Although the remarkable synthetic potential of enoate reductases has been recognized long ago,[17] preparative-scale applications were severely impeded by two major problems: Simple to use whole-cell systems (most prominent baker's yeast, but also fungi and yeasts, such as *Geotrichum candidum*, *Rhodotorula rubra*, *Beauveria bassiana*, *Aspergillus niger*, etc.) are plagued by undesired side reactions, such as carbonyl reduction (catalyzed by competing alcohol dehydrogenases/carbonyl reductases)[7] or ester hydrolysis (mediated by carboxyl ester hydrolases).[18] On the other hand, the first generation of isolated (cloned) enoate reductases were obtained from (strict or facultative) an-

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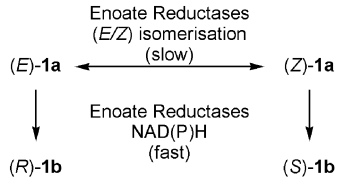
aerobes, which were inapplicable to preparative-scale transformations owing to their sensitivity towards traces of molecular oxygen. It was only recently that this bottleneck was resolved by providing oxygen-stable OYEs from yeasts.[8–12]

We recently demonstrated the potential of these enzymes in the reduction of various activated olefins by using 12-oxophytodienoate reductase isoenzymes OPR1 and OPR3 from *Lycopersicon esculentum* (tomato)[11] and the OYE-homolog YqjM from *Bacillus subtilis*.[12] Here we report on the substrate spectrum of a newly discovered enoate reductase from *Zymomonas mobilis* in comparison to Old Yellow Enzymes OYE-1 (*Saccharomyces carlsbergensis*), OYE-2, and OYE-3 (*Saccharomyces cerevisiae*).[19] Although OYEs 1–3 are biochemically well characterized, they are rather underexploited in preparative biotransformations.

Recently, Rosche et al. discovered enoate reductase activity in the bacterium *Zymomonas mobilis*.[8] The responsible OYE-homolog (termed NCR reductase)[20] reduced enals or enones and even a conjugated ynone[13] with high stereoselectivities. In order to broaden the applicability of this new biocatalyst we investigated its substrate spectrum in more detail by (i) using a wide range of activating groups, such as aldehyde, ketone, imide, nitro, carboxylic acid, or ester moieties; (ii) varying the main substrate (cyclic vs. acyclic); (iii) switching the (*E*/*Z*) configuration of the alkene moiety; and (iv) modifying the substitution pattern (mono- vs. di-, α- vs. β-) relative to the activating group.

Results and Discussion

In the reduction of the α,β-unsaturated aldehyde citral (**1a**), the (*E*/*Z*) configuration of the substrate played a crucial role in the stereoselectivity of OYEs 1–3.[8] When we tested the (*Z*)-configured substrate neral [(*Z*)-**1a**], *Zymomonas mobilis* NCR-reductase reduced **1a** to (*S*)-citronellal (**1b**) with high conversion (93 to >99%) and excellent stereoselectivity (>95%) (Table 1, Entries 1–4). The three OYEs, however, displayed different reactivities depending on the cofactor and the recycling system employed. Both OYE-1 and OYE-2 produced enantiomeric (*S*)-**1b** and (*R*)-**1b** with molar equivalents of NAD(P)H, albeit with low stereoselectivity (max. 16%*ee*), whereas OYE-3 displayed modest stereoselectivity leading to (*S*)-**1b** (max. 51%*ee*). All OYEs produced (*R*)-**1b** in moderate-to-fair stereoselectivities (20–70%*ee*) with the NAD^+/GDH system, whereas the $NADP^+$/G6PDH system gave the opposite enantiomer (*S*)-**1b** up to 49%*ee* with OYE-3. These variations in stereoselectivities can be explained by in situ (nonspecific) enzymatic (*E*/*Z*) isomerization of citral (**1a**).[21] As OYEs 1–3 are known to produce (*R*)- and (*S*)-**1b** from (*E*) and (*Z*) isomers of citral (**1a**), respectively,[8] the relative rate of competing (fast) C=C-bond reduction versus (slow) (*E*/*Z*) isomerization determines the overall outcome of the process (Scheme 2). Short reaction times furnish high stereoselectivities (but low conversion), whereas extended exposure times result in reduced *ee* values (but enhanced yields) of **1b**.



Scheme 2. Competing (*E*/*Z*) isomerization of citral during C=C-bond reduction.

Whereas OYE-1 and OYE-2 displayed low stereoselectivities in the reduction of 2-methylcyclopentenone (**2a**; Table 1, Entries 5–8), OYE-3 and *Zymomonas mobilis* NCR-reductase delivered the (*S*) enantiomer in low-to-moderate enantiopurity (34 and 48%*ee*, respectively). A sharp switch of stereopreference was observed when the ring size was increased from five (**2a**) to six carbon atoms (**3a**): whereas **2a** led to (*S*)-**2b**, **3a** was converted into (*R*)-**3b** in a highly stereoselective fashion (conversion 78–97%, up to 98%*ee*) by all four enzymes (Table 1, Entries 9–12), which is remarkable bearing in mind that the difference in the total molar volume of cyclopentene versus Cyclohexene substrates **2a** and **3a**, respectively, is only 18%.[22] Although such a stereodivergent behavior caused by small modification of the substrate framework is rare for enzymes, it was previously noted with OPR1, OPR3, YqjM,[12] and OYE-1.[9]

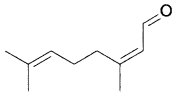
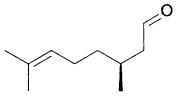
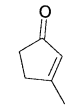
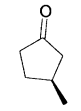
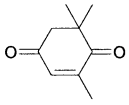
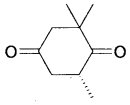
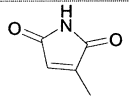
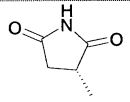
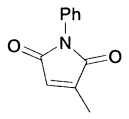
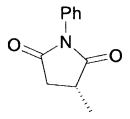
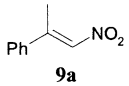
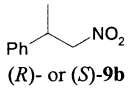
In contrast to α-substituted cyclic enones, β-substituted analogs (**4a**, **5a**) were converted at reduced reaction rates, but greatly enhanced stereoselectivities (99 to >99%*ee*) and furnished exclusively (*S*) products **4b** and **5b** (Table 1, Entries 13–16), which is in line with observations of Swiderska and Stewart.[9] Among all enzymes, OYE-1 was most active (conversion up to 96%; Table 1, Entries 17–20).

The reduction of ketoisophorone (**6a**) led to the formation of levodione (**6b**), the (*R*) enantiomer of which is an important building block in the synthesis of carotenoids used as feed additive for salmon and trout. Previously, (*R*)-**6b** was produced by using baker's yeast.[23] More recently, cloned enoate reductases from *Candida macedoniensis* were investigated in this context.[14,24] All four enzymes formed (*R*)-**6b** in up to 98%*ee* with excellent conversion (Table 1, Entries 21–24).

2-Methylmaleimide derivatives **7a** and **8a** were smoothly transformed into (*R*)-**7b** and (*R*)-**8b**, respectively, by all enzymes, but *Zymomonas mobilis* NCR-reductase was clearly superior in terms of activities and stereoselectivities (Table 1, Entries 25–32). As previously observed,[12] an attempt to alter the stereoselectivity by substrate engineering through variation of the size of the N substituent was unsuccessful. Overall, the influence of the N-substituent regarding its size (N-H vs. N-Ph) was only marginal.

With the substrates investigated so far, *Zymomonas* NCR-reductase and OYEs 1–3 displayed various, but not greatly different behaviors in terms of activities and stereoselectivities. For nitroalkene **9a**, however, a switch of stereopreference was observed, which was first detected with isoenzymes OPR1 and OPR3,[11] whereas *Zymomonas mobilis*

Table 1. Asymmetric bioreduction of activated alkenes **1a**–**9a** by using *Zymomonas mobilis* NCR-reductase and Old Yellow Enzymes OYE 1–3 from yeasts.

Entry	Substrate	Product	Cofactor	*Zymomonas* NCR-reductase		OYE-1		OYE-2		OYE-3	
				c %	*ee* %	*c* %	*ee* %	*c* %	*ee* %	*c* %	*ee* %
1			NADH	93	(*S*) >95	92	(*S*) 15	90	(*R*) 7	87	(*S*) 40
2			NADPH	94	(*S*) >95	98	(*S*) 16	95	(*R*) 16	96	(*S*) 51
3			NAD^+/GDH[a]	98	(*S*) >95	49	(*R*) 77	97	(*R*) 20	97	(*R*) 42
4	**1a**	(*S*)-**1b**	$NADP^+$/G6PDH[b]	>99	(*S*) >95	89	(*S*) 20	90	(*R*) 8	76	(*S*) 49
5			NADH	80	(*S*) 15	40	*rac*	47	*rac*	46	(*S*) 5
6			NADPH	92	(*S*) 31	69	*rac*	99	*rac*	70	(*S*) 16
7			NAD^+/GDH[a]	99	(*S*) 35	49	*rac*	99	(*R*) 9	51	(*S*) 30
8	**2a**	(*S*)-**2b**	$NADP^+$/G6PDH[b]	99	(*S*) 48	97	*rac*	99	(*R*) 16	50	(*S*) 34
9			NADH	89	(*R*) 88	78	(*R*) 89	81	(*R*) 91	78	(*R*) 98
10			NADPH	91	(*R*) 89	81	(*R*) 89	95	(*R*) 91	85	(*R*) 90
11			NAD^+/FDH[c]	97	(*R*) 85	94	(*R*) 87	92	(*R*) 87	97	(*R*) 85
12	**3a**	(*R*)-**3b**	$NADP^+$/G6PDH[b]	97	(*R*) 93	90	(*R*) 93	95	(*R*) 94	94	(*R*) 92
13			NADH	45	(*S*) 97	41	(*S*) 98	22	(*S*) 97	22	(*S*) 97
14			NADPH	39	(*S*) 98	64	(*S*) >99	30	(*S*) 99	22	(*S*) >99
15			NAD^+/FDH[c]	43	(*S*) >99	16	(*S*) >99	36	(*S*) >99	25	(*S*) >99
16	**4a**	(*S*)-**4b**	$NADP^+$/G6PDH[b]	16	(*S*) >99	54	(*S*) >99	27	(*S*) >99	18	(*S*) >99
17			NADH	58	(*S*) >99	74	(*S*) >99	60	(*S*) >99	37	(*S*) >99
18			NADPH	40	(*S*) >99	88	(*S*) >99	46	(*S*) >99	25	(*S*) >99
19			NAD^+/FDH[c]	61	(*S*) >99	72	(*S*) >99	91	(*S*) >99	43	(*S*) >99
20	**5a**	(*S*)-**5b**	$NADP^+$/G6PDH[b]	20	(*S*) >99	96	(*S*) >99	71	(*S*) >99	27	(*S*) >99
21			NADH	95	(*R*) 31	88	(*R*) 40	96	(*R*) 29	59	(*R*) 39
22			NADPH	97	(*R*) 33	93	(*R*) 39	93	(*R*) 43	81	(*R*) 46
23			NAD^+/FDH[c]	>99	(*R*) 38	99	(*R*) 41	98	(*R*) 33	>99	(*R*) 43
24	**6a**	(*R*)-**6b**	$NADP^+$/G6PDH[b]	>99	(*R*) 95	98	(*R*) 98	>99	(*R*) 97	56	(*R*) 98
25			NADH	>99	(*R*) 99	>99	(*R*) 73	>99	(*R*) 90	>99	(*R*) 92
26			NADPH	>99	(*R*) 99	>99	(*R*) 72	>99	(*R*) 92	>99	(*R*) 93
27			NAD^+/FDH[c]	>99	(*R*) 98	>99	(*R*) 75	>99	(*R*) 84	>99	(*R*) 90
28	**7a**	(*R*)-**7b**	$NADP^+$/G6PDH[b]	>99	(*R*) 99	>99	(*R*) 73	>99	(*R*) 92	>99	(*R*) 89
29			NADH	94	(*R*) >98	98	(*R*) >98	>99	(*R*) >98	96	(*R*) >98
30			NADPH	95	(*R*) >98	>95	(*R*) >98	90	(*R*) >98	97	(*R*) >98
31			NAD^+/FDH[c]	99	(*R*) >98	74	(*R*) >98	>99	(*R*) >98	96	(*R*) >98
32	**8a**	(*R*)-**8b**	$NADP^+$/G6PDH[b]	>99	(*R*) >98	>99	(*R*) >98	>99	(*R*) >98	>99	(*R*) >98
33			NADH	>99	(*S*) >99	>99	(*R*) 77	>99	(*R*) 74	>99	(*R*) 68
34			NADPH	>99	(*S*) >99	>99	(*R*) 72	>99	(*R*) 83	>99	(*R*) 68
35			NAD^+/FDH[c]	>99	(*S*) >99	>99	(*R*) 90	>99	(*R*) 81	>99	(*R*) 80
36	**9a**	(*R*)- or (*S*)-**9b**	$NADP^+$/G6PDH[b]	98	(*S*) 98	97	(*R*) 83	98	(*R*) 83	97	(*R*) 85

[a] NADH was recycled by glucose/glucose dehydrogenase. [b] NADPH was recycled by glucose-6-phosphate/glucose-6-phosphate dehydrogenase. [c] NADH was recycled by formate/formate dehydrogenase.

NCR-reductase quantitatively formed (*S*)-**9b** in >99%*ee*. OYEs 1–3 produced (*R*)-**9b** with moderate-to-good stereoselectivities (68–90%*ee*, Table 1, Entries 33–36). Thus, combination of *Zymomonas mobilis* NCR-reductase with OPR1 provides a stereocomplementary biocatalytic system for the reduction of 1-nitro-2-phenylpropene (**9a**).

We recently showed that α,β-unsaturated dicarboxylic acids were readily accepted by enoate reductases depending

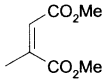
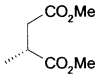
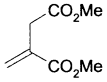
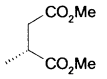
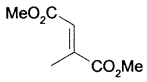
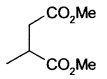
on the biocatalyst and substrate configuration.[6] Structurally related diacids – 2-methylmaleic acid (**10a**, "citraconic acid"), 2-methylenesuccinic acid (**11a**, "itaconic acid"), and 2-methylfumaric acid (**12a**, "mesaconic acid") – were investigated to extend the substrate spectrum of the four enzymes. Unfortunately, the expected product 2-methylsuccinic acid (**10b**) could not be detected, as all enzymes were inactive. Despite close structural similarities among the OYE family,[4] even small changes in the architecture of the active site seem to be sufficient to cause dramatic effects in substrate recognition.[12]

In contrast, the dimethyl ester counterparts **13a**–**15a** were all smoothly reduced to dimethyl 2-methylsuccinate (**13b**) by the four enzymes. The reaction rates, absolute configurations of the product, and the stereoselectivities were very much dependent on the enzyme and the (*E*/*Z*) configuration of the substrate. Whereas *Zymomonas mobilis* NCR-reductase quantitatively converted (*Z*)-**13a** into (*R*)-**13b** with absolute stereoselectivity (>99%*ee*), OYEs 1–3 displayed somewhat reduced reaction rates (conversion 25–87%), albeit the stereoselectivities remained perfect for the (*R*) enantiomer (>99%*ee*; Table 2, Entries 1–4). The *exo* methylene analogue **14a** was reduced to enantiopure (*R*)-**13b**, although it appeared to be a slow substrate (c_{max} 16, 21, and 43% with OYE-1, OYE-2 and *Zymomonas* NCR-reductase, respectively). OYE-3 was clearly the best enzyme (*c* 82%, Table 2, Entries 5–8). As first noted during whole-cell reduction of 2-chloroacrylic esters using baker's yeast,[18] the (*E*/*Z*) configuration of the substrate had a tremendous effect on the stereochemical outcome of the reaction.

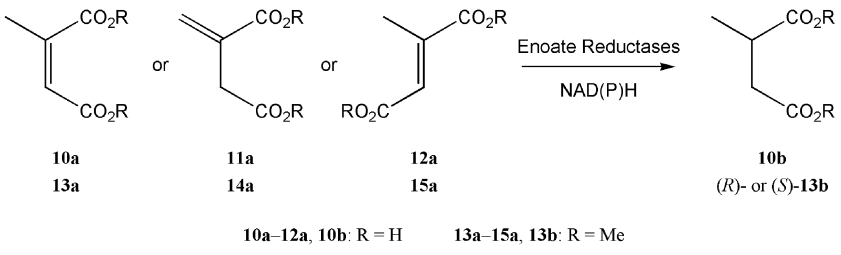
In contrast to (*Z*)-**13a**, which gave (*R*)-**13b**, (*E*)-**15a** was converted into furnished (*S*)-**13b** by OYEs 1–3 with high reaction rates and perfect stereoselectivities as observed with YqjM from *Bacillus subtilis*[6] (>99%*ee*; Table 1, Entries 9–12). Quite remarkably, this switch of stereopreference was absent for *Zymomonas mobilis* NCR-reductase, which gave (*R*)-**13b** in low *ee* or in racemic form (Scheme 3).

Overall, all four enoate reductases seemed to accept either NADH or NADPH about equally well. In contrast to carbonyl reductases, which show a rather pronounced preference for either NADH or NADPH,[25] enoate reductases are less specific in this respect: Their preference for NADH over NADPH may vary within a significant range;[26] some enzymes are very specific,[27] others are able to accept both cofactors equally well.[14] Nicotinamide co-

Table 2. Asymmetric bioreduction of α,β-unsaturated dicarboxylic esters **13a**–**15a** by using *Zymomonas mobilis* NCR-reductase and Old Yellow Enzymes OYE 1–3 from yeasts.

Entry	Substrate	Product	Cofactor	*Zymomonas* NCR-reductase		OYE1		OYE2		OYE3	
				c %	*ee* %	*c* %	*ee* %	*c* %	*ee* %	*c* %	*ee* %
1			NADH	99	(*R*) >99	68	(*R*) >99	74	(*R*) >99	98	(*R*) 96
2			NADPH	99	(*R*) >99	72	(*R*) >99	32	(*R*) >99	99	(*R*) 94
3			NAD^+/FDH[a]	99	(*R*) >99	25	(*R*) >99	87	(*R*) >99	91	(*R*) 97
4	**13a**	(*R*)-**13b**	$NADP^+$/G6PDH[b]	99	(*R*) >99	58	(*R*) >99	64	(*R*) >99	30	(*R*) >99
5			NADH	29	(*R*) >99	16	(*R*) >99	19	(*R*) >99	60	(*R*) >99
6			NADPH	28	(*R*) >99	10	(*R*) >99	5	(*R*) >99	82	(*R*) >99
7			NAD^+/FDH[a]	43	(*R*) >99	7	(*R*) >99	21	(*R*) >99	39	(*R*) >99
8	**14a**	(*R*)-**13b**	$NADP^+$/G6PDH[b]	9	(*R*) >99	8	(*R*) >99	5	(*R*) >99	7	(*R*) >99
9			NADH	54	*rac*	99	(*S*) >99	99	(*S*) >99	99	(*S*) >99
10			NADPH	57	*rac*	99	(*S*) >99	68	(*S*) >99	99	(*S*) >99
11			NAD^+/FDH[a]	80	(*R*) 11	62	(*S*) >99	99	(*S*) >99	99	(*S*) >99
12	**15a**	(*R*)- or (*S*)-**13b**	$NADP^+$/G6PDH[b]	14	(*R*) 18	99	(*S*) >99	80	(*S*) >99	99	(*S*) >99

[a] NADH was recycled by formate/formate dehydrogenase. [b] NADPH was recycled by glucose-6-phosphate/glucose-6-phosphate dehydrogenase.



Scheme 3. Asymmetric bioreduction of α,β-unsaturated dicarboxylic acids **10a**–**12a** and corresponding dimethyl esters **13a**–**15a** by enoate reductases.

factor recycling was successfully carried out by using glucose/GDH or formate/FDH for NADH and glucose-6-phosphate/G6PDH for NADPH. So far, the variations in stereoselectivities observed with the different cofactor systems remain unexplained but are consistent with previous works with related enzymes.[9,10,14,24,28]

Conclusions

A novel enoate reductase from *Zymomonas mobilis* and three Old Yellow Enzymes (OYE1-OYE3) from *Saccharomyces* spp. were shown to accept a large variety of α,β-unsaturated compounds, such as enals, enones, cyclic imides, nitroalkenes, and α,β-unsaturated dicarboxylic diesters for the conjugate reduction of the C=C bond. Depending on the substrate type, remarkable differences in reaction rates and stereoselectivities were observed. In several cases, the stereochemical outcome of the reaction could be controlled by a "substrate-based stereocontrol", that is, the ring-size of cycloalkenones, position of substituents on the C=C bond, and its (*E*/*Z*) configuration. Alternatively, "enzyme-based stereocontrol" was achieved with a nitroalkene, which led to the formation of opposite enantiomeric products by *Zymomonas mobilis* NCR-reductase and OYEs 1–3.

Experimental Section

General: Column chromatography was performed by using silica gel 60 (0.040–0.063 mm), TLC plates were run on silica gel 60 F_{254} (aluminium sheets) from Merck. Compounds were visualized either by spraying with Mo reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (100 g L^{-1}), $Ce(SO_4)_2\cdot 4H_2O$ (4 g L^{-1}) in H_2SO_4 (10%)], or by UV light.

GC–MS analyses were performed with a HP 6890 Series GC system equipped with a 5973 mass selective detector and a 7683 Series injector by using a (5%-phenyl)methylpolysiloxane capillary column (HP-5Msi, 30 m, 0.25 mm ID, 0.25 μm film). GC–FID analyses were carried out with a Varian 3800 by using H_2 as a carrier gas (14.5 psi). HPLC analyses were performed by using a Shimadzu system equipped with a Chiralcel OD-H column (25 cm, 0.46 cm). Circular dichroism spectra were measured with a JASCO spectropolarimeter J-715. NMR spectra were measured with a Bruker AMX spectrometer at 360 MHz. Chemical shifts are reported relative to TMS (δ = 0.00 ppm) and coupling constants are given in Hz. Petroleum ether (b.p. 60–90 °C) and EtOAc used for chromatography were distilled prior to use.

Citral **1a**, 2-methylcyclopentanone (**2b**), 2-methylcyclohexenone (**3a**), 2-methylcyclohexanone (**3b**), and levodione (**6b**) were provided by BASF (Ludwigshafen). (*R*)- and (*S*)-Citronellal (**1b**), (*R*)-3-methylcyclopentanone (**4b**), 3-methyl-2-cyclohexenone (**5a**), (*R*)-3-methylcyclohexanone (**5b**), *N*-phenyl-2-methylmaleimide (**8a**), (*S*)-2-methylsuccinic acid (**10b**), mesaconic acid (**12a**), BF_3–methanol solution, NAD^+, and ammonium formate were obtained from Aldrich. 4-Ketoisophorone (**6a**) was purchased from ABCR; 2-methyl-2-cyclopentenone (**2a**) was obtained from Acros; citraconic anhydride was purchased from Alfa Aesar; itaconic acid (**11a**), 3-methyl-2-cyclopentenone (**4a**), *rac*-3-methylcyclohexanone (**5b**), ammonium acetate, and glucose were purchased from Fluka; citraconic acid (**10a**) was purchased from Alfa Aesar. NADH, NADPH, and $NADP^+$ were purchased from Biocatalytics (Order-No 004642, 041939 and 004669, respectively); glucose-6-phosphate and glucose-6-phosphate dehydrogenase (Order-No 49272) were obtained from Biochemica; formate dehydrogenase (Order-No 09.11 and 24.11) and glucose dehydrogenase (Order-No 29.10 and 22.10) were obtained from Jülich Chiral Solutions.

Biocatalysts: NCR-Enoate reductase from *Zymomonas mobilis* was obtained as reported before.[8]

Preparation of Old Yellow Enzymes OYE 1–3 from Baker's Yeast: An enzyme exhibiting activity in reducing citral to citronellal was purified from commercial baker's yeast by activity guided purification procedure. The *N*-terminal amino acid sequence of a single active protein band was shown to be SFVKDFKPQALGDTNLFKPI by Edman sequencing. A database search identified this sequence as Old Yellow Enzyme 1 (Genbank ID Q02899). The whole amino acid sequence of OYE1 as well as of its close relatives OYE2 and OYE3 (Genbank ID Q03558 and P41816, respectively) was translated into DNA by using the backtranslation program of the Vector NTI package (Invitrogen Corp.). The three genes were obtained as custom-synthesized material from Geneart (Regensburg, OYE1 and OYE2) and Entelechon (Regensburg, OYE3), respectively.

General Procedure for the Enzymatic Bioreduction of Substrates 1a–15a: An enzyme aliquot (*Zymomonas mobilis* NCR-reductase, OYE1, OYE2 or OYE3, protein purity >90%, protein content 75–125 μg mL^{-1}) was added to a Tris-HCl buffer solution (0.8 mL, 50 mM, pH 7.5) containing the substrate (5 mM) and the cofactor NADH or NADPH (15 mM). *N*-Phenyl-2-methylmaleimide (**8a**) was added as a 0.5 M DMF solution (1% final DMF conc.) to overcome its poor solubility in water. The mixture was shaken at 30 °C and 140 rpm. After 48 h, products were extracted with EtOAc (2 × 0.5 mL) containing 0.05% (v/v) of 1-octanol (for **1a**/**1b**) or (*R*)-limonene (for **2a**/**2b**–**9a**/**9b**) as internal GC standard. The combined organic phases were dried (Na_2SO_4), and the resulting samples were analyzed by achiral GC. Products were identified by comparison with authentic reference materials (which were either commercially available or were independently synthesized as described below) by co-injection on GC–MS and achiral GC.

General Procedure for Cofactor Recycling: An aliquot of enzyme (*Zymomonas mobilis* NCR-reductase, OYE1, OYE2, or OYE3, protein purity >90%, protein content 75–125 μg mL^{-1}) was added to a Tris-HCl buffer solution (0.8 mL, 50 mM, pH 7.5) containing the substrate (5 mM), the oxidized form of the cofactor (NAD^+ or $NADP^+$, 100 μM), the cosubstrate (ammonium formate, glucose, or glucose-6-phosphate; 20 mM), and the corresponding recycling enzyme (formate dehydrogenase, glucose dehydrogenase, or glucose-6-phosphate dehydrogenase; 10 U). The mixture was shaken at 30 °C and 140 rpm for 24 h and worked up as described above.

Supporting Information (see footnote on the first page of this article): A detailed description of the purification of Old Yellow Enzymes 1–3 from baker's yeast; their gene sequences and cloning; preparation of compounds **6b**, **7a**,**b**, **8b**, **9a**,**b**, **10b**, **13b**, **13a**–**15a**; analytical data for compounds **1a**–**9a** and **13a**–**15a** and determination of the absolute configuration of products.

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