FULL PAPER

A Substrate-Driven Approach to Determine Reactivities of α , β -Unsaturated **Carboxylic Esters Towards Asymmetric Bioreduction**

Gábor Tasnádi,^[a] Christoph K. Winkler,^[b] Dorina Clay,^[b] Nargis Sultana,^[b] Walter M. F. Fabian,^[b] Mélanie Hall,^[b] Klaus Ditrich,^[c] and Kurt Faber^{*[b]}

Abstract: The degree of C=C bond activation in the asymmetric bioreduction of α,β -unsaturated carboxylic esters by ene-reductases was studied, and general recommendations to render these "borderline-substrates" more reactive towards enzymatic reduction are proposed. The concept of "supported substrate activation" was developed. In general, an additional *a*-halogenated substituent proved to be beneficial for enzymatic activity, whereas β-alkyl or β-aryl substituents were detrimental for

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the reactivity of nonhalogenated substrates, and α -cyano groups showed little effect. The alcohol moiety of the ester functionality was found to have a strong influence on the reaction rate. Overall, activities were determined by both steric and electronic effects.

Introduction

Modern synthetic strategies rely on stereoselective transformations to increase molecular complexity through generation of optical activity. In this context, asymmetric reduction involves the transformation of prochiral sp²-centers into chiral sp³-moieties, which creates complexity and added value in the formed product. Hence, reduction is widely applied in the chemical and pharmaceutical industry, in which single enantiomers are highly desired.^[1-3] The asymmetric catalytic reduction of C=C bonds is particularly powerful, because it (theoretically) leads to the generation of up to two stereogenic centers.^[1] On industrial scale, transitionmetal-catalyzed processes are most sophisticated, [4-6] whereas organocatalytic asymmetric transfer hydrogenations are still at the stage of development.^[7-11] The biocatalytic variant, that is, the asymmetric bioreduction, is gaining in-

[a] Dr. G. Tasnádi ACIB GmbH c/o Biocatalytic Synthesis, University of Graz Heinrichstrasse 28, 8010-Graz (Austria) [b] C. K. Winkler, D. Clay, N. Sultana, Prof. Dr. W. M. F. Fabian, Dr. M. Hall, Dr. K. Faber

Department of Chemistry, Organic & Bioorganic Chemistry University of Graz, Heinrichstrasse 28, 8010-Graz (Austria) Fax: (+43) 316-380-9840 E-mail: Kurt.Faber@Uni-Graz.at

[c] Dr. K. Ditrich Fine Chemicals & Biocatalysis Research BASF SE

Carl-Bosch-Strasse 38, 67056 Ludwigshafen (Germany)

Supporting information for this article contains description of the source of enzymes, chemicals and materials, chiral and nonchiral analytics, synthesis of substrates, and reference compounds, determination of absolute configurations, additional data on organic cosolvents, and DFT calculations and is available on the WWW under http:// dx.doi.org/10.1002/chem.201200990.

creased attention due to its exquisite chemo-, regio-, and stereoselectivity, further strengthened by mild operational conditions and independence from heavy metals.^[12] The enzymes catalyzing this reaction are ene-reductases [EC 1.3.1.X], members of the "Old Yellow Enzyme" (OYE) family.^[13] Over the past few years, these flavoproteins have been proven to be powerful catalysts for the synthesis of valuable chiral building blocks, amino acid derivatives, terpenoids, and fragrances.^[14] Ene-reductases catalyze the reduction of C=C bonds that are electronically activated by a conjugated electron-withdrawing group (EWG), which also serves as anchor for substrate binding in the enzyme active site; nonactivated (isolated) alkenes are unreactive (Scheme 1). Well-accepted substrates include enals, enones,



Scheme 1. Asymmetric bioreduction of α,β -unsaturated esters by using ene-reductases.

 α , β -unsaturated carboxylic acids and esters, nitriles, and nitroalkenes. Although enzyme- and substrate-based stereocontrol techniques are now well understood, no rules regarding substrate activation have been defined, which would allow the prediction of reactivities and thus assist to use these enzymes more systematically.^[15] Based on their high degree of polarization, enals, enones, and nitroalkenes constitute excellent Michael acceptors and are thus well accepted by OYEs. On the other hand, carboxylic acids and derivatives thereof, such as esters and nitriles, are less activated and often behave as "borderline substrates". The data avail-

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able to date indicate that alkenes with a single carboxylic acid or ester group are generally poorly converted by OYEs.^[16-18] The insufficient degree of substrate activation in α,β -unsaturated carboxylic acids may be overcome by using so-called "enoate-reductases", which possess an $[Fe_4S_4]$ cubane cluster in addition to the flavin cofactor. Unfortunately, this motif renders these enzymes extremely sensitive towards traces of O₂, which makes them inapplicable to preparative-scale bioreductions.^[19,20]

Although a single carboxylic acid/ester/nitrile moiety is barely sufficient for alkene activation, addition of a second electron-withdrawing moiety may help to enhance the degree of polarization of the C=C bond to facilitate the bioreduction of these "borderline-substrates". Recently, OYEs 1-3 were shown to catalyze the stereoselective bioreduction of methyl α-halo-2-alkenoates and 3-arylacrylates.^[21-23] Comparable methods for the preparation of chiral α -halo esters include organocatalytic^[24-26] and metal-catalyzed^[27] α -halogenation, stereoselective substitution^[28-31] as well as resolution of racemic esters by enzymatic hydrolysis.[32,33] Asymmetric hydrogenation of alkenes is restricted to fluoro derivatives^[34,35] due to reductive dehalogenation in the presence of reducing metal agents. Additionally, stereoselective bromination leads to enantiopure α,β -dihalogenated compounds.^[36,37] Chiral α-halo esters have found application in the synthesis of biologically active compounds^[38,39] and herbicides^[40] as well as in natural-product synthesis,^[41] whereas stereocontrolled nucleophilic substitution on enantiopure α halo esters gives access to optically active N-, O-, and S-containing compounds.^[42-47]

Herein, we propose the concept of "supported substrate activation" as a tool to probe the reactivity of α,β -unsaturated carboxylic esters with ene-reductases and to test the influence of additional substituents on the C=C bond activation. The electronic properties and the size of the $\alpha\text{-}$ and $\beta\text{-}$ substituents of the unsaturated acid moiety together with

Table 1. Bioreduction of acrylic-ester derivatives with ene-reductases.

variations in the alcohol part of the ester functionality were investigated, and the degree of C=C bond activation was related to enzymatic activity. Additionally, both reaction rate and stereoselectivity could be tuned by a switch of substrate E/Z-configuration and type of organic co-solvent to produce enantiopure α -halo esters.

Results

Seven isolated ene-reductases (OYEs 1-3, YqjM, NCR, OPR1, and OPR3) known to display broad substrate acceptance in the reduction of α,β -unsaturated compounds were investigated by using a series of acrylic ester derivatives 1a-8a (Scheme 1 and Table 1). The overall poor reactivity of acrylonitrile (1a; only NCR, OYE1, and OYE2 showed modest activities, Table 1, entry 1)^[48] indicated that the nitrile moiety was a poor activating group and prompted us to test various alkyl acrylates (2a-7a). The change from nitrile to carboxylic ester as activating group had a huge influence on the reaction rates, because alkyl esters 2a-4a were almost quantitatively converted by the ene-reductases tested, except with YqjM and OPR1 (Table 1, entries 2-4). Surprisingly, the chain length of the alcohol moiety had a strong positive effect on YqjM and OPR1 activities, in which conversions improved from 27 to 91% and <1 to 55% going from methyl to *n*-butyl. An analogous effect was observed with Roche' ester precursors and a-alkoxy-functionalized enones,^[49,50] which may be caused by alternative substrate-binding modes induced by steric effects. The sterically cumbersome *tert*-butyl ester (5a) was only accepted by NCR (87% conversion), whereas the other enzymes were poorly active ($c_{\text{max}} = 7\%$; Table 1, entry 5). The presence of an α -methyl group in methyl methacrylate (6a) diminished the reactivity of OYE2, OYE3, NCR, and OPR3 (2a: 89–99% conversion; 6a: 14–91% conversion; Table 1,

Entry	Substrate	Product	Cofactor	OYE1 Conv. <i>ee</i> [%]	OYE2 Conv. <i>ee</i> [%]	OYE3 Conv. ee [%]	YqjM Conv. ee [%]	NCR Conv. ee [%]	OPR1 Conv. ee [%]	OPR3 Conv. <i>ee</i> [%]
2	COOMe 2a	COOMe 2b	NADH	>99	>99	97	27	>99	<1	89
3	COOEt 3a	COOEt 3b	NADH	>99	>99	80	42	>99	5	98
4	COOnBu 4a	COO <i>n</i> Bu 4b	NADH	>99	98	98	91	>99	55	>99
5	COO <i>t</i> Bu 5a	COO <i>t</i> Bu 5b	NADH	7	6	n.c.	1	87	<1	7
6	COOMe 6a	COOMe 6b	NADH	>99	91	58	46	89	26	14
	COOMe			98	>99	83	>99	>99	>99	33
7	 Cl 7a	⊂l (<i>R</i>)- or (<i>S</i>)- 7b	NADH	40 (<i>S</i>)	45 (<i>S</i>)	83 (<i>S</i>)	86 (R)	>99 (<i>R</i>)	93 (<i>R</i>)	>99 (<i>R</i>)
8		COOMe	NADH	93	90	>99	9	34	21	n.c.
				>99(S)	>99(S)	>99(S)	>99(S)	57 (R)	53 (R)	
9	ĊI (<i>Z</i>)-8a		NAD+/GDH	83	97	88	37	74	19	4
				99 (S)	98 (S)	>99(S)	98 (S)	72 (R)	54 (R)	45 (S)
10		Cl (<i>B</i>)- or (<i>S</i>)- 8b	NADH	97	92	>99	33	>99	87	35
10	COOMe			39 (R)	43 (R)	57 (S)	93 (R)	97 (R)	90 (R)	69 (R)
11			NAD±/CDU	14	79	9	54	77	19	56
11	UI (<i>E)-</i> 08		NAD 7/GDH	75 (R)	14 (R)	59 (S)	69 (R)	94 (R)	84 (R)	94 (R)

Reaction conditions: substrate (10 mm), NADH (15 mm) in Tris-HCl buffer (50 mm, pH 7.5), 30 °C, 24 h; n.c. = no conversion; NAD+/GDH = glucose/glucose dehydrogenase was used for cofactor recycling.

entries 2 and 6). Changing the electron-donating α -methyl moiety to an electron-withdrawing α -chloro substituent translated into greatly improved reactivity: all enzymes showed enhanced conversion with methyl 2-chloroacrylate (7a) compared with 6a, in particular OYE3, YqjM, OPR3, and OPR1, which displayed a 1.4 to 3.8-fold improvement in conversions (Table 1, entry 7). It is tempting to relate this effect of electronic activation to the mechanism of OYEs, because the reaction proceeds through a Michael-type addition through nucleophilic attack of a hydride onto the β carbon followed by proton addition onto the α -position.^[51] Thus, the additional electron-withdrawing α -chloro group (in contrast to the methyl moiety) enhances the degree of C=C polarization, thereby favoring the overall addition of [2H]. However, comparing 7a with unsubstituted methyl acrylate 2a, it appears that also steric factors play a role. Although (electronically expected) higher reaction rates were achieved with YqjM and OPR1, OYE3 and OPR3 showed reduced conversion with nonactivated 7a. Moderate-to-high S-stereoselectivity was obtained with OYEs 1-3 (40-83% enantiomeric excess (ee)), whereas YqjM, NCR, and OPRs showed excellent opposite stereopreference (86 up to >99% *ee* for (*R*)-**7b**).

Next, we investigated the effects of β -substituents. Interestingly, the addition of an alkyl substituent, regardless of its size, totally deactivated the system (compounds 9a-12a, <1% conversion, Figure 1A). Herein, the result can also be rationalized by considering the reaction mechanism. Indeed, the electron-donating group diminished the δ^+ at the $\beta\text{-}$ carbon; in addition, the β -position may be particularly sensitive towards steric hindrance, because the hydride has to be delivered at this point from N5 of the flavin cofactor. In need of additional activation of unreactive β -alkyl- or β aryl-substituted acrylic esters, we looked for potential α-activating groups. Because methyl (Z)-2-ethoxy-3-(4-methoxyphenyl)propenoate with a moderately electron donating α alkoxy group was not converted by OYE3,^[52] a-cyano-substituted acrylates, such as methyl 2-cyanoacrylate (15a), methyl 2-cyano-3,3-dimethylacrylate (16a), and methyl 2cyano-3-phenylpropenoate [(E)-17a], were tested, expecting



Figure 1. A) Nonsubstrates for ene-reductases (<1% conversion, data not shown). B) Substrates undergoing polymerization or decomposition.

that the α -cyano group would enhance the C=C bond polarization (Figure 1B). Unfortunately, substrates **15a–17a** underwent decomposition and/or polymerization under screening conditions (data not shown) and were not further investigated.

As an alternative, we tested β -substituted α -haloacrylic ester derivatives. To our delight, methyl 2-chloropentenoate [(Z)-8a] was nicely accepted and yielded (S)-8b with excellent stereoselectivity (conversion up to >99%, >99% ee) in the presence of OYEs 1-3 and YqjM (Table 1, entry 8). This supports the hypothesis that C=C bond activation can be tuned by the presence of α -halo substituents, because the corresponding α -unsubstituted derivative [(E)-12a] was not converted at all. By using the glucose/glucose dehydrogenase (NAD⁺/GDH) cofactor recycling system, 97% conversion and 98% ee were obtained by using OYE2 (Table 1, entry 9). Interestingly, NCR and OPR1 showed moderately expressed opposite stereopreference [ee up to 72% for (R)-**8b**]. The *E*-isomer was similarly well accepted, and the change of isomer geometry had a strong influence on the stereochemical outcome for some of the enzymes: OYE1, OYE2, YqjM, and OPR3 showed a strong switch, that is, (Z)-8a gave (S)-8b and (E)-8a led to (R)-8b. In contrast, OYE3 always formed (S)-8b, and NCR and OPR1 were always R-selective, regardless of the E/Z-configuration of substrate 8a (Table 1, entry 10). This behavior has been previously observed with OYEs.^[53] The cofactor recycling system yielded noticeable variations in conversion and ee values (Table 1, entry 11). Such an influence of the nicotinamide regeneration system on the bioreduction reaction has been observed in numerous cases.^[54-57] Although not fully understood, it may result from differences in the (bi-bi Ping-Pong) kinetics between ene-reductase and recycling enzyme, leading to variations in the ratio oxidized/reduced cofactor.

Prompted by these results, a series of α -halogenated cinnamic esters (18a-20a) were tested to overcome the insufficient degree of activation in (unreactive) methyl cinnamate ((E)-13a, <1% conversion, Figure 1A). Again, the introduction of an α -halo substituent proved to be successful, because all substrates were converted with OYEs 1-3 (Table 2, entries 1-8). Among them, OYE3 showed highest activities (conversion 36-97%) and S-stereoselectivity (79% up to >99% ee) with methyl α -chloro-, bromo-, and iodocinnamates. The ability of OYE3 to accept larger substrates is likely due to an enlarged binding pocket caused by an exchange of phenylalanine F296 (in OYE1 and OYE2) by serine S297 in OYE3.^[58,59] The type of halogen had a strong effect on the activity of OYE3: although α -fluoro-substitution led to an unreactive substrate ((Z)-14a, <1% conversion, Figure 1A), the Cl-, Br-, and I-analogues (Z)-18a, (Z)-19a, and (Z)-20a were successfully converted at different rates, and the α -bromo derivative (Z)-19a was the most reactive of the series (Table 2, entry 3). The S-stereopreference of OYEs 1-3 was conserved throughout the series 18a-20 a, reduced stereoselectivity was observed with the sterically most demanding iodo analogue (Z)-20a (ee 79-80%). The E/Z-configuration of the brominated substrate had no

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Entry	Substrates	Product	Cofactor	OYE1 Conv. <i>ee</i> [%]	OYE2 Conv. ee [%]	OYE3 Conv. <i>ee</i> [%]	YqjM Conv. <i>ee</i> [%]	NCR Conv. ee [%]	OPR1 Conv. <i>ee</i> [%]	OPR3 Conv. <i>ee</i> [%]
1	COOMe	COOMe	NADH	9 92 (S)	7 >99 (S)	36 98 (S)	n.c.	5 >99 (S)	5 94 (<i>R</i>)	n.c.
2	(Z)-18a	(<i>R</i>)- or (<i>S</i>)-18b	NAD+/GDH	n.c.	1 n.d.	14 >99 (<i>S</i>)	n.c.	<1 n.d.	<1 n.d.	n.c.
3	COOMe		NADH	n.c.	11 95 (S)	79 99 (S)	<1 n.d.	6 48 (S)	15 90 (<i>R</i>)	<1 n.d.
4	(<i>Z</i>)-19a	COOMe	NAD+/GDH	4 98 (S)	18 97 (S)	18 99 (S)	<1 n.d.	10 94 (S)	8 95 (R)	n.c.
5	Br	Br (<i>R</i>)- or (<i>S</i>)- 19b	NADH	15 90 (S)	9 92 (S)	97 98 (S)	<1 n.d.	13 >99 (<i>R</i>)	45 66 (<i>S</i>)	4 99 (R)
6	(<i>E</i>)-19a		NAD+/GDH	15 89 (S)	33 90 (S)	39 95 (S)	8 > 99 (R)	42 >99 (<i>R</i>)	29 74 (S)	28 > 99 (R)
7	COOMe	COOMe	NADH	24 85 (S)	7 85 (S)	43 79 (<i>S</i>)	n.c.	<1 n.d.	2 n.d.	n.c.
8	(<i>Z</i>)-20a	(<i>R</i>)- or (<i>S</i>)- 20b	NAD+/GDH	12 85 (S)	8 84 (S)	12 80 (S)	n.c.	<1 n.d.	8 n.d.	n.c.
9	CI COOMe	CI	NADH	14 96 (S)	3 93 (S)	58 98 (S)	n.c.	n.c.	<1 n.d.	n.c.
10	Cl NO ₂ (Z)-21a	Cl NO ₂ (<i>R</i>)- or (<i>S</i>)- 21b	NAD ⁺ /GDH	16 96 (S)	21 98 (S)	9 98 (S)	n.c.	n.c.	n.c.	n.c.
11	NO ₂ COOMe		NADH	<1 n.d.	<1 n.d.	<1 n.d.	n.c.	n.c.	n.c.	n.c.
12	Br (<i>Z</i>)-22a		NAD+/GDH	1 n.d.	2 n.d.	3 n.d.	n.c.	n.c.	n.c.	n.c.

Reaction conditions: substrate (10 mM), NADH (15 mM) or cofactor-recycling system in Tris-HCl buffer (50 mM, pH 7.5), 30 °C, 24 h; n.c. = no conversion; n.d. = not determined; NAD⁺/GDH = glucose/glucose dehydrogenase was used for cofactor recycling.

effect on the stereorecognition by OYEs 1–3 (Table 2, entries 3–6), which is in line with similar observations on methyl α -bromo-butenoate, hexenoate, and heptenoate.^[23] In contrast, NCR and OPR1 converted the geometric E/Zisomers of **19a** in a stereocomplementary fashion (Table 2, entries 3–6). YqjM and OPR3 were not active on (Z)-**18a**– **20a**, whereas (E)-**19a** was reduced slowly (8 and 28% conversion) with excellent *ee* to (R)-**19b**. The use of a cofactor recycling system improved the performance of OYE2, YqjM, NCR, and OPR3 in the bioreduction of (E)-**19a** giving access to (R)-**19b** in up to 42% conversion and >99% *ee*.

Additionally, two α -halo-cinnamic ester derivatives [(Z)-**21a** and (Z)-**22a**] with electron-withdrawing substituents on the aromatic ring were tested (Table 2, entries 9-12). Interestingly, the presence of two substituents on the phenyl moiety enhanced the reactivity of (Z)-21a compared with (Z)-18a, but did not influence the enzyme stereoselectivity. OYE1 and OYE3 showed exactly the same trend with NADH as cofactor: 55% enhanced conversion through ring activation. This effect was more pronounced with OYE2 in presence of a recycling system (Table 2, entries 9-10). All enzymes tested were inactive on (Z)-22 a. It is an interesting aspect that the o-nitro group supports an (aci-nitro-like) mesomeric form leading to the delocalization of positive charge on the 1'-phenyl carbon and α -C thereby reducing the electrophilicity on β -C (which is impossible with the *m*nitro group, cf. substrate 21a). Although beneficial on smaller unsubstituted alkyl acrylates (2a-4a), the variation of the alcohol functionality in cinnamic ester derivatives was detrimental; only methyl esters reacted, ethyl esters of **19a**, **21a**, and **22a** were not accepted by the enzymes (data not shown).

To summarize the observed trends, the properties of the α -halo substituent in methyl cinnamates, covalent radius and electronegativity, were plotted against reactivity (i.e., conversion) and stereoselectivity (*ee*; Figure 2). Although the electronegativity of the α -halogen substituent correlates with the reactivity from bromo to iodo, it fails to predict the reactivity of the fluoro and chloro derivatives. Likewise, the size of the substituent⁶⁰ explains the drop in reactivity



Figure 2. Effect of α -halogen substituent on the bioreduction of methyl (*Z*)- α -halo cinnamates by OYE3 (14a: X=F; 18a: X=Cl; 19a: X=Br, 20a: X=I).

(from bromo to iodo) going in hand with a drop in stereoselectivity, pointing at binding issues in the active site and imperfect substrate recognition.^[49,59] Thus, electronic effects influencing C=C bond polarization alone cannot account for observed trends in substrate reactivity—steric effects obviously play an important role.

We envisaged to rationalize the "supported-substrate activation" concept by using conceptual DFT^[61] to estimate the reactivity of the investigated compounds towards nucleophilic addition at β -C in α , β -unsaturated carboxylic esters. The global electrophilicity index (ω) was used to provide a reactivity scale for a series of different molecules. Overall, no strong relationship with enzyme activity was found, though clusters of enzyme–substrate combinations could be identified, in which electrophilicity reflected substrate reactivity (Figures S1 and S2 in the Supporting Information).

Because all substrates tested possess low solubility in aqueous media, we tested various organic cosolvents (10% v/v) to enhance their solubility thereby overcoming masstransfer limitations. In addition, organic cosolvents are known to modulate the catalytic properties of enzymes.^[62] We selected OYE3 for the cosolvent study with substrates showing particularly low-to-moderate conversions [(Z)-18a-22a] (Figure 3). Although stereoselectivity of OYE3 was unchanged, significant increase in reactivity was observed, especially in biphasic systems (Figure 3 and Table S3 in the Supporting Information), which supports the beneficial effect of water-immiscible solvents on the performance of OYEs as well as enzyme robustness.^[63] In general, *tert*-butyl methyl ether (TBME), di-isopropyl ether (DIPE), and nhexane afforded superior conversion: notable improvements were observed in the case of (Z)-18a and (Z)-20a with nhexane (from 36 to 84%, from 43 to 67%, respectively), only a slight enhancement was obtained with (Z)-19a (from 79 to 84%). Compound (Z)-21a also showed enhanced reaction rate with most cosolvents including EtOAc, although other substrates were poorly converted in the presence of this cosolvent. An even more pronounced increase was achieved with (Z)-22 a, unreactivity of which in neat aqueous buffer was completely overcome by addition of TBME or DIPE (\geq 96% conversion, >99% *ee*). Interestingly, THF, EtOAc, acetone, TBME, DIPE, toluene, and dichloromethane provided usually higher conversions for substrates with substituents on the aromatic ring [(Z)-21 a and (Z)-22 a], in contrast to unsubstituted derivatives [(Z)-18 a-20 a].^[22] Overall, DMF was a poor cosolvent.

Conclusion

Investigation of the de/activating effects of α - and β -substituents on the asymmetric bioreduction of acrylic and cinnamic esters by using ene-reductases was investigated and condensed in the following trends: 1) the tuning of the reactivity is possible to some extent by influencing the polarization of the C=C bond through electronic substituent effects; 2) the presence of an α -halogen atom strongly improved reaction rates, whereas 3) a-cyano groups were largely ineffective; 4) an electron-donating group on β -C tended to deactivate nonhalogenated derivatives. However, electronic effects could not be disentangled from steric effects. Though structurally highly related, ene-reductases yield specific binding modes with each substrate, thus preventing a general statement on specific interactions involved with a given compound. Overall, *a*-halo-substituted acrylic and cinnamic esters were reduced with high-to-excellent conversion and stereoselectivity, occasionally even in a stereocomplementary fashion through enzyme- or substrate-based stereocontrol. Additionally, organic cosolvents had a strong enhancing effect on enzyme activity.



Experimental Section

Source of enzymes, chemicals and materials, chiral and nonchiral analytics, synthesis of substrates, and reference compounds, determination of absolute configurations, additional data on organic co-solvents, and DFT calculations are available in the Supporting Information.

General procedure for the bioreduction: An aliquot of enzyme (OYE 1–3, YqjM, NCR, OPR1, OPR3; 6.6–31 μ L of the stock solution, final protein concentration 75–125 μ gmL⁻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 Na₂SO₄ and analyzed on achiral GC to deter-

Figure 3. Effect of cosolvents (10% v/v) on the bioreduction of α , β -unsaturated carboxylic esters with OYE3; water-miscible solvents (blue); water-immiscible solvents (red).

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mine the conversion and on chiral GC or HPLC, respectively, to determine the *ee.* For cofactor recycling, the oxidized form of the cofactor (NAD⁺, 100 μ M), the cosubstrate (glucose 20 mM), and the recycling enzyme (glucose dehydrogenase, 10 U) were used.

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- [1] C. A. Busacca, D. R. Fandrick, J. J. Song, C. H. Senanayake, Adv. Synth. Catal. 2011, 353, 1825–1864.
- [2] H. Shimizu, I. Nagasaki, K. Matsumura, N. Sayo, T. Saito, Acc. Chem. Res. 2007, 40, 1385–1393.
- [3] N.B. Johnson, I.C. Lennon, P.H. Moran, J.A. Ramsden, Acc. Chem. Res. 2007, 40, 1291–1299.
- [4] W. S. Knowles, R. Noyori, Acc. Chem. Res. 2007, 40, 1238-1239.
- [5] R. Noyori, Angew. Chem. 2002, 114, 2108–2123; Angew. Chem. Int. Ed. 2002, 41, 2008–2022.
- [6] W. S. Knowles, Angew. Chem. 2002, 114, 2096–2107; Angew. Chem. Int. Ed. 2002, 41, 1998–2007.
- [7] Q.-A. Chen, M.-W. Chen, C.-B. Yu, L. Shi, D.-S. Wang, Y. Yang, Y.-G. Zhou, J. Am. Chem. Soc. 2011, 133, 16432–16435.
- [8] N. J. A. Martin, L. Ozores, B. List, J. Am. Chem. Soc. 2007, 129, 8976–8977.
- [9] J. B. Tuttle, S. G. Ouellet, D. W. C. MacMillan, J. Am. Chem. Soc. 2006, 128, 12662–12663.
- [10] J. W. Yang, M. T. Hechavarria Fonseca, N. Vignola, B. List, Angew. Chem. 2005, 117, 110–112; Angew. Chem. Int. Ed. 2005, 44, 108– 110.
- [11] S. G. Ouellet, J. B. Tuttle, D. W. C. MacMillan, J. Am. Chem. Soc. 2005, 127, 32–33.
- [12] M. Hall, A. S. Bommarius, Chem. Rev. 2011, 111, 4088-4110.
- [13] R. Stuermer, B. Hauer, M. Hall, K. Faber, Curr. Opin. Chem. Biol. 2007, 11, 203–213.
- [14] C. K. Winkler, G. Tasnádi, D. Clay, M. Hall, K. Faber, J. Biotechnol. 2012, DOI: 10.1016/j.jbiotec.2012.03.023.
- [15] M. Hall, Y. Yanto, A. S. Bommarius in *The Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology* (Ed.: M. C. Flickinger), Wiley, New York, **2010**, p. 2234.
- [16] P. Ferraboschi, P. Grisenti, R. Casati, A. Fiecchi, E. Santaniello, J. Chem. Soc. Perkin Trans. 1 1987, 1743–1748.
- [17] S. Koul, D. H. G. Crout, W. Errington, J. Tax, J. Chem. Soc. Perkin Trans. 1 1995, 2969–2988.
- [18] M. Utaka, S. Konishi, A. Mizuoka, T. Ohkubo, T. Sakai, S. Tsuboi, A. Takeda, J. Org. Chem. 1989, 54, 4989–4992.
- [19] H. Simon, J. Bader, H. Günther, S. Neumann, J. Thanos, Angew. Chem. 1985, 97, 541–555; Angew. Chem. Int. Ed. Engl. 1985, 24, 539–553.
- [20] H. Simon, Pure Appl. Chem. 1992, 64, 1181-1186.
- [21] E. Brenna, G. Fronza, C. Fuganti, D. Monti, F. Parmeggiani, J. Mol. Catal. B: Enzym. 2011, 73, 17–21.
- [22] E. Brenna, F. G. Gatti, A. Manfredi, D. Monti, F. Parmeggiani, *Eur. J. Org. Chem.* 2011, 2011, 4015–4022.
- [23] E. Brenna, F. G. Gatti, A. Manfredi, D. Monti, F. Parmeggiani, Org. Process Res. Dev. 2012, 16, 262–268.
- [24] N. Halland, A. Braunton, S. Bachmann, M. Marigo, K. A. Jørgensen, J. Am. Chem. Soc. 2004, 126, 4790–4791.
- [25] N. T. Reynolds, T. Rovis, J. Am. Chem. Soc. 2005, 127, 16406–16407.
 [26] T. Kano, M. Ueda, K. Maruoka, J. Am. Chem. Soc. 2008, 130, 3728–
- 3729.
- [27] J. Erb, D. H. Paull, T. Dudding, L. Belding, T. Lectka, J. Am. Chem. Soc. 2011, 133, 7536–7546.
- [28] W. Gaffield, W. G. Galetto, Tetrahedron 1971, 27, 915-934.

K. Faber et al.

- [29] A. Focella, F. Bizzarro, C. Exon, Synth. Commun. 1991, 21, 2165– 2170.
- [30] N. Yoshikawa, Y. M. A. Yamada, J. Das, H. Sasai, M. Shibasaki, J. Am. Chem. Soc. 1999, 121, 4168–4178.
- [31] M. Tanasova, Q. Yang, C. C. Olmsted, C. Vasileiou, X. Li, M. Anyika, B. Borhan, *Eur. J. Org. Chem.* 2009, 4242–4253.
- [32] L. Haughton, J. M. J. Williams, Synthesis 2001, 0943-0946.
- [33] H. M. R. Gardimalla, D. Mandal, P. D. Stevens, M. Yen, Y. Gao, *Chem. Commun.* 2005, 4432–4434.
- [34] M. Engman, J. S. Diesen, A. Paptchikhine, P. G. Andersson, J. Am. Chem. Soc. 2007, 129, 4536–4537.
- [35] P. Kaukoranta, M. Engman, C. Hedberg, J. Bergquist, P. G. Andersson, Adv. Synth. Catal. 2008, 350, 1168–1176.
- [36] J. P. Shaw, E. W. Tan, J. Org. Chem. 1996, 61, 5635-5637.
- [37] L. See Wong, B. Chan, E. Wui Tan, *Tetrahedron Lett.* 2000, 41, 2671–2674.
- [38] W. Liu, K. Liu, H. B. Wood, M. E. McCann, T. W. Doebber, C. H. Chang, T. E. Akiyama, M. Einstein, J. P. Berger, P. T. Meinke, *J. Med. Chem.* **2009**, *52*, 4443–4453.
- [39] J. Surtees, D. Bouvy, A. Thomas, Y. Combret, M. Frank, G. Schmidt, EP 1806 339 A1, 2007.
- [40] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, Angew. Chem. 2004, 116, 806–843; Angew. Chem. Int. Ed. 2004, 43, 788–824.
- [41] S. Mukherjee, R. Sivappa, M. Yousufuddin, C. J. Lovely, Org. Lett. 2010, 12, 4940–4943.
- [42] M. Seki, T. Yamanaka, K. Kondo, J. Org. Chem. 2000, 65, 517-522.
- [43] D. Yang, B. Li, F.-F. Ng, Y.-L. Yan, J. Qu, Y.-D. Wu, J. Org. Chem. 2001, 66, 7303–7312.
- [44] Y.-M. Cui, J.-Y. Li, L.-L. Chen, J. Li, Q.-Z. Ye, F.-J. Nan, Bioorg. Med. Chem. 2004, 12, 2853–2861.
- [45] G. Righi, S. Ciambrone, C. D'Achille, A. Leonelli, C. Bonini, *Tetrahedron* 2006, 62, 11821–11826.
- [46] N. Narendra, H. S. Lalithamba, V. V. Sureshbabu, *Tetrahedron Lett.* 2010, 51, 6169–6173.
- [47] H. Kunz, H.-G. Lerchen, Tetrahedron Lett. 1987, 28, 1873-1876.
- [48] B. Kosjek, F. J. Fleitz, P. G. Dormer, J. T. Kuethe, P. N. Devine, *Tetra-hedron: Asymmetry* 2008, 19, 1403–1406.
- [49] C. K. Winkler, C. Stueckler, N. J. Mueller, D. Pressnitz, K. Faber, *Eur. J. Org. Chem.* 2010, 6354–6358.
- [50] C. Stueckler, C. K. Winkler, M. Bonnekessel, K. Faber, Adv. Synth. Catal. 2010, 352, 2663–2666.
- [51] A. D. N. Vaz, S. Chakraborty, V. Massey, Biochemistry 1995, 34, 4246–4256.
- [52] M. Bechtold, E. Brenna, C. Femmer, F. G. Gatti, S. Panke, F. Parmeggiani, A. Sacchetti, Org. Process Res. Dev. 2012, 16, 269–276.
- [53] C. Stueckler, M. Hall, H. Ehammer, E. Pointner, W. Kroutil, P. Macheroux, K. Faber, Org. Lett. 2007, 9, 5409–5411.
- [54] M. Hall, C. Stueckler, B. Hauer, R. Stuermer, T. Friedrich, M. Breuer, W. Kroutil, K. Faber, *Eur. J. Org. Chem.* 2008, 1511–1516.
- [55] J. Bernard, E. van Heerden, I. W. C. E. Arends, D. J. Opperman, F. Hollmann, *Chemcatchem* 2012, 4, 196–199.
- [56] G. Tasnádi, C. K. Winkler, D. Clay, M. Hall, K. Faber, *Catal. Sci. Technol.*, DOI: 10.1039/C1032CY20079 A.
- [57] 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.
- [58] K. M. Fox, P. A. Karplus, Structure 1994, 2, 1089-1105.
- [59] C. Stueckler, C. K. Winkler, M. Hall, B. Hauer, M. Bonnekessel, K. Zangger, K. Faber, Adv. Synth. Catal. 2011, 353, 1169–1173.
- [60] P. Pyykkö, M. Atsumi, Chem. Eur. J. 2009, 15, 186-197.
- [61] P. Geerlings, F. De Proft, W. Langenaeker, Chem. Rev. 2003, 103, 1793–1874.
- [62] A. M. Klibanov, Nature 2001, 409, 241–246.
- [63] Y. Yanto, C. K. Winkler, S. Lohr, M. Hall, K. Faber, A. S. Bommarius, Org. Lett. 2011, 13, 2540–2543.

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FULL PAPER



Biotransformation: The asymmetric bioreduction of α , β -unsaturated carboxylic esters by ene-reductases could be tuned by varying the degree of C=C bond activation (see scheme). An additional α -halogenated substituent proved to be beneficial for enzymatic activity, whereas β -alkyl or β -aryl substituents were detrimental for the reactivity of nonhalogenated substrates.

Biocatalysis -

G. Tasnádi, C. K. Winkler, D. Clay, N. Sultana, W. M. F. Fabian, M. Hall, *K. Ditrich, K. Faber**........

A Substrate-Driven Approach to Determine Reactivities of α,β-Unsaturated Carboxylic Esters Towards **Asymmetric Bioreduction**

