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Asymmetric Synthesis of (*R*)-3-Hydroxy-2-methylpropanoate ('Roche Ester') and Derivatives *via* Biocatalytic C=C-Bond Reduction

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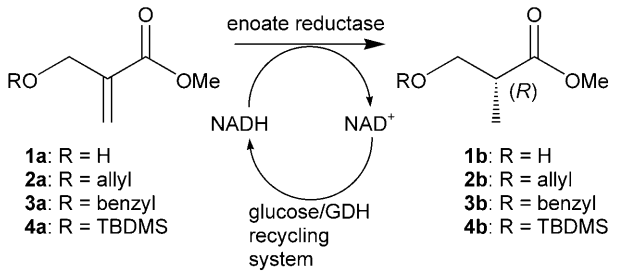
Abstract: Enoate reductases from the 'old yellow enzyme' family were employed for the asymmetric bioreduction of methyl 2-hydroxymethylacrylate and its *O*-allyl, *O*-benzyl and *O*-TBDMS derivatives to furnish (*R*)-configurated methyl 3-hydroxy-2-methylpropionate products in up to >99% *ee* Variation of the *O*-protective group had little influence on the stereoselectivity, but a major impact on the reaction rate.

Keywords: biocatalysis; C=C-bioreduction; enoate reductase; old yellow enzyme; substrate engineering

The asymmetric reduction of C=C bonds creates (up to) two chiral carbon centres and is thus one of the most widely employed strategies for the production of chiral materials. The biocatalytic variant, which is applicable to activated alkenes bearing an electron-withdrawing substituent is catalysed by enoate reductases [EC 1.3.1.X],[1,2] which are members of the 'old yellow enzyme' (OYE) family.[3] Over the past few years, increasing attention has been devoted to these flavoproteins[4] in view of their substrate scope,[5] encompassing α,β-unsaturated carbonyl compounds (such as enals and enones), as well as carboxylic acids and derivatives thereof (such as esters, cyclic imides, nitriles, lactones) and nitroalkenes. As a rule of thumb, the degree of activation of the C=C-bond exerted by the electron-withdrawing effect of the activating substituent goes hand in hand with the substrate acceptance, which ensures generally fast reaction rates for enals, enones and nitroalkenes, whereas (di)carboxylic acids and esters are transformed more slowly.

To illustrate the importance of this enzyme class for asymmetric synthesis, we aimed at their applicability for an industrially relevant product, that is, (*R*)-3-hydroxy-2-methylpropanoate, which is commonly denoted as the 'Roche ester'. The latter is a popular chiral building block for the synthesis of vitamins (e.g., α-tocopherol[6]), fragrance components (e.g., muscone[7]), and antibiotics (e.g., calcimycin,[8] palinurin,[9] rapamycin,[10] 13-deoxytedanolide,[11] dictyostatin[12]) and natural products (e.g., spiculoic acid A[13]). Classical methods for its preparation include the diastereoselective addition of non-racemic alcohols as chiral auxiliaries,[14] the transformation of a chiral homoallylic acetate[15] or involve aldol condensation[16] and – most prominent – the transition metal-catalysed asymmetric hydrogenation of acrylate esters using Rh[17] (*ee* up to 99%) or Ru[18] (*ee* up to 94%). For the biocatalytic synthesis of the 'Roche ester' only few examples are reported: the stereoselective oxidation of 2-methyl-1,3-propanediol by *Gluconobacter* and *Acetobacter* spp.[19] (*ee* up to 97%), the asymmetric reduction of ethyl 4,4-dimethoxy-3-methylcrotonate using baker's yeast[20] and the stereoseletive (formal) β-hydroxylation of isobutyric acid using *Pseudomonas putida* (ATCC 21244).[21] All of these biotransformations were performed using whole (fermenting) microbial cells with several enzymes being involved. Only recently, was it shown that a non-flavin NADH-dependent $\Delta^{4,5}$-steroid 5β-reductase from *Arabidopsis thaliana* was able to reduce ethyl 2-hydroxymethylacrylate, however, the stereochemistry of the product was not examined in regard to its absolute configuration and enantiomeric composition.[22]



Scheme 1. Asymmetric bioreduction of methyl 2-hydroxymethylacrylate derivatives **1a**–**4a**.

Herein we report on the synthesis of the 'Roche ester' *via* biocatalytic reduction of methyl 2-hydroxymethylacrylate using eleven OYE's. In order to test the influence of the overall substrate structure regarding its size and polarity on the relative rate and/or stereoselectivity, various protective groups on the hydroxy moiety were investigated.

Old yellow enzymes OYE1-3 from *Saccharomyces* sp.,[23] 12-oxophytodienoic acid reductase isoenzymes OPR1 and OPR3 from *Lycopersicon esculentum*,[24] YqjM from *Bacillus subtilis*,[25] nicotinamide-dependent cyclohexenone reductase (NCR) from *Zymomonas mobilis*,[26] xenobiotic reductases A and B (XenA, XenB) from *Pseudomonas putida* and *P. fluorescens*, respectively,[27] glycerol trinitrate reductase (NerA) from *Agrobacterium radiobacter*[28] and estrogen-binding protein (EBP1) from *Candida albicans*[29] were tested for their ability to reduce four methyl 2-hydroxymethacrylate derivatives (Scheme 1, Table 1).

The unprotected Roche ester precursor methyl 2-hydroxymethylacrylate (**1a**) was reduced to (*R*)-methyl 3-hydroxy-2-methylpropionate (**1b**) by four of the eleven enoate reductases tested (entries 1–8) with perfect stereoselectivity (*ee* up to >99%), NCR, NerA and OPT1 were inactive and XenA showed limited stereoselectivites (ee_{max} 63%, entry 15). Despite these encouraging selectivities, insufficient reaction rates led to incomplete conversion of this substrate (c_{max} 37%, entry 5). Since the *primary* allylic alcohol group of **1a** is considerably hydrated in aqueous solution, the corresponding allyl, benzyl and TBDMS ethers were tested as more lipophilic substrate analogues.

Overall, the masking of the hydroxy group of the initial substrate by easily removable allyl and benzyl ether groups enhanced the substrate acceptance drastically. The *O*-allyl analogue **2a** and *O*-benzyl ether **3a** were converted by OYEs1-3 and YqjM with approximately three-fold enhanced rates (c_{max} 90%, entry 5). In the case of NCR, NerA and OPR1 this substrate modification even turned the inactive substrate **1a** into a suitable target molecule for these enzymes (substrate **3a**, c_{max} 89%, entry 13), and the conversion of **2a** using XenA was almost quantitative (entry 16). In addition to improved reaction rates, the incomplete stereoselectivities of YqjM and XenA were corrected from *ee*s 94% and 60% to >99%, respectively (entries 8 and 16). However, the stereopreference remained constant for all modified substrates by invariably yielding (*R*)-**2b** and (*R*)-**3b**.

Since the stereopreference of enoate reductases sometimes may be controlled by the substrate shape, such as (*E/Z*)-configuration of the C=C-bond,[4a,5c,e] we attempted to obtain the (*S*)-'Roche ester' by increasing the steric bulkiness of the hydroxy protective

Table 1. Conversion and enantiomeric excess of bioreduction products **1b–4b**.

Entry	Enzyme	Cofactor[a]	**1b**		**2b**		**3b**		**4b**	
			c [%]	*ee* [%]	c [%]	*ee* [%]	c [%]	*ee* [%]	c [%]	*ee* [%]
1	OYE1	NADH	20	>99 (*R*)	14	>99 (*R*)	18	>99 (*R*)	<1	n.d.
2		NAD^+[b]	7	>99 (*R*)	5	>99 (*R*)	3	>99 (*R*)	n.d.	n.d.
3	OYE2	NADH	7	>99 (*R*)	38	>99 (*R*)	14	>99 (*R*)	<1	n.d.
4		NAD^+[b]	12	>99 (*R*)	10	>99 (*R*)	7	>99 (*R*)	n.d.	n.d.
5	OYE3	NADH	**37**	**>99 (*R*)**	90	>99 (*R*)	66	>99 (*R*)	<1	n.d.
6		NAD^+[b]	5	>99 (*R*)	32	>99 (*R*)	17	>99 (*R*)	n.d.	n.d.
7	YqjM	NADH	14	98 (*R*)	26	>99 (*R*)	67	>99 (*R*)	6	>99 (*R*)
8		NAD^+[b]	18	94 (*R*)	69	>99 (*R*)	55	>99 (*R*)	20	>99 (*R*)
9	NCR	NADH	<1	n.d.	34	>99 (*R*)	55	>99 (*R*)	14	>99 (*R*)
10		NAD^+[b]	n.d.	n.d.	22	>99 (*R*)	20	>99 (*R*)	24	>99 (*R*)
11	NerA	NADH	<1	n.d.	16	>99 (*R*)	39	>99 (*R*)	<1	n.d.
12		NAD^+[b]	n.d.	n.d.	30	>99 (*R*)	35	>99 (*R*)	n.d.	n.d.
13	OPR1	NADH	<1	n.d.	**77**	**>99 (*R*)**	**89**	**>99 (*R*)**	5	>99 (*R*)
14		NAD^+[b]	n.d.	n.d.	**76**	**>99 (*R*)**	**84**	**>99 (*R*)**	26	>99 (*R*)
15	XenA	NADH	17	63 (*R*)	**84**	**>99 (*R*)**	**76**	**>99 (*R*)**	**65**	**>99 (*R*)**
16		NAD^+[b]	28	60 (*R*)	**97**	**>99 (*R*)**	**90**	**>99 (*R*)**	**>99**	**>99 (*R*)**

[a] *Standard conditions:* substrate **1a**–**4a** (10 mM), NADH (15 mM), Tris-HCl-buffer 50 mM, pH 7.5, 30 °C, 24 h.
[b] NAD^+ (100 μM)/glucose dehydrogenase (10 U)/glucose (20 mM); c = conversion; n.d. = not determined.

group even further in order to enforce a flipped substrate orientation within the active site of the enzyme. Unfortunately, this attempt for selectivity control failed, as the bulky *tert*-butyldimethylsilyloxy derivative **4a** invariably furnished (*R*)-**4b** in >99% *ee*, going hand in hand with a negative effect on the reaction rates: The activities dropped significantly (for YqjM, NCR and OPR1) or were completely erased (for OYE1-3 and NerA), only XenA was able to reduce **4a** quantitatively with absolute selectivity for the (*R*)-enantiomer (entry 16). None of the substrates could be reduced with sufficient rates using OPR3, XenB and EBP1 (c <2%).

In conclusion, an efficient method for the preparation of (*R*)-methyl 3-hydroxy-2-methylpropionate ['(*R*)-Roche ester'] and related *O*-protected derivatives was developed *via* bioreduction of the corresponding acrylate precursors was developed using enoate reductases from the 'old yellow enzyme' family. In order to ensure full conversion, the allylic hydroxy moiety had to be protected with an allyl or benzyl ether group to render a more lipophilic substrate analogue.

Experimental Section

Source of Enzymes

OPR1 and OPR3 from *Lycopersicon esculentum* and YqjM from *Bacillus subtilis* were overexpressed and purified as reported recently.[5a,24,25] The cloning, purification and characterisation of OYEs from yeast (OYE1 from *Saccharomyces carlsbergensis*, OYE2 and OYE3 from *Saccharomyces cerevisiae*) and nicotinamide-dependent cyclohexenone reductase (NCR) from *Zymomonas mobilis* was performed according to the literature.[26,30] Xenobiotic reductases A (XenA) and B (XenB) from *Pseudomonas putida* and *P. fluorescens*, respectively,[27] glycerol trinitrate reductase NerA from *Agrobacterium radiobacter* and estrogen-binding protein (EBP1) from *Candida albicans* were obtained as recently published.[31]

General Procedure for the Bioreduction of 1a–4a

An aliquot of enzyme (OYE1–3, OPR1, OPR3, YqjM, NCR, XenA, XenB, NerA, and EBP1, protein concentration 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). 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. For cofactor recycling, the oxidized form of the cofactor (NAD^+, 100 μM), the cosubstrate (glucose 20 mM) and the recycling enzyme (glucose dehydrogenase, 10 U) were used.

Determination of Absolute Configurations

The absolute configurations of **1b**, **2b** and **4b** were determined by co-injection with reference material of known absolute configuration. The absolute configuration of **3b** was determined by comparison with a published chiral HPLC analysis (Chiralcel OD-H 0.46×25 cm, 98:2 heptane:2-propanol mobile phase at 1.5 mL min^{-1} flow): the (*R*)-enantiomer eluted at 3.88 min, the (*S*)-enantiomer eluted at 4.17 min.[32]

Supporting Information

General information on commercially obtained compounds and materials, the synthesis of substrates **1a**–**4a**, the synthesis of reference material for *rac*-**1b**, *rac*-**3b** and *rac*-**4b**, and analytical methods for the determination of conversion and enantiomeric excess are described in the Supporting Information.

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