Electronic Supplementary Information

Reductive Dehalogenation of β-Haloacrylic Ester Derivatives Mediated by Ene-Reductases

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General remarks

Silica gel 60 from Merck was used for column chromatography. GC-MS analyses were performed on an HP 6890 Series GC system equipped with a 5973 mass selective detector and a 7683 Series injector using a (5% phenyl)-methylpolysiloxane capillary column (HP-5MS, 30 m x 0.25 mm, 0.25 μm film). GC-FID analyses were carried out on a Varian 3800 using He as carrier gas. NMR spectra were measured on a Bruker Avance III 300 MHz NMR spectrometer. Chemical shifts are reported relative to TMS (δ = 0.00 ppm); *J* values are given in Hz.

Methyl *rac*-2,3-dichloropropionate (rac-**3b**), methyl *rac*-2,3-dibromopropionate (*rac*-**5b**), methyl *rac*-2-chloropropionate (*rac*-**7b**), methyl *rac*-2-bromopropionate (*rac*-**9b**), (*S*)-2-bromopropionic acid, dimethyl fumarate [(*E*)-**8a**], dimethyl maleate [(*Z*)-**8a**], tetrabutylammonium iodide and methyl 3-bromopropionate (**13**) were from Sigma, methyl (*S*)-lactate [(S)-**11**] was from Alfa Aesar, methyl (*E*)-2,3-dibromofumarate [(*E*)-**2a**] was purchased from TCI Europe. Methyl 2,3,3-trichloroacrylate (**1a**) was kindly provided by BASF. Methyl (*Z*)-2,3-dibromopropionate [(*Z*)-**5a**)],[1] methyl (*E*)-2,3-diiodopropionate [(*E*)-**6a**)],[2] methyl *rac*-2-iodopropionate (*rac*-**10b**)[3] and methyl (*S*)-2-chloropropionate [(*S*)-**7b**][4] were synthesized as previously reported. All reagents and solvents were of analytical grade.

The absolute configurations of **7b**, **9b** and **10b** were determined by co-injection with reference materials of known absolute configuration. (*S*)-2-Bromopropionic acid was derivatized with $TMSCHN_2$ prior to analysis to yield ester (*S*)-**9b**.

Source of Enzymes

12-Oxophytodienoate reductase isoenzymes OPR1 and OPR3 from *Lycopersicon esculentum* and the OYE homologue YqjM from *Bacillus subtilis* were overexpressed and purified as reported.[5-7] The cloning, purification and characterisation of OYE isoenzymes from yeast (OYE1 from *Saccharomyces pastorianus*, OYE2 and OYE3 from *Saccharomyces cerevisiae*) and nicotinamide-dependent cyclohexenone reductase (NCR) from *Zymomonas mobilis* were performed according to literature methods.[8, 9]

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Synthesis of chiral reference compound (*R*)-10b

(*S*)-Methyl lactate [(*S*)-**11**] (1.96 g, 14.4 mmol, *ee* >97%), DMAP (176 mg, 1.44 mmol) and triethylamine (1.75 g, 17.3 mmol) were added to a solution of tosyl chloride (3.03 g, 15.9 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C. The reaction was stirred for 18 h at room temperature, then washed with sat. NH_4Cl (2 × 15 mL) and 5% $NaHCO_3$ (1 × 15 mL). The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, and purified by column chromatography (toluene/EtOAc 4:1) to give tosylate (*S*)-**12** (42%, 1.76 g, colourless oil).[10]
δ_H (300 MHz, $CDCl_3$) δ 7.83 (2H, d, *J* = 8.3 Hz, Ar), 7.36 (2H, d, *J* = 8.0 Hz, Ar), 4.97 (1H, q, *J* = 6.9 Hz, CH), 3.70 (3H, d, *J* = 9.9 Hz, $COOCH_3$), 2.45 (3H, s, Ar-CH_3), 1.52 (3H, d, *J* = 6.9 Hz, CH_3CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 145.1, 133.3, 129.8, 128.0, 74.0, 52.6, 21.7, 18.4.

To a solution of (*S*)-**12** (100 mg, 0.34 mmol) in MeCN (10 mL), tetrabutylammonium iodide (127 mg, 0.34 mmol) was added in several portions at 0 °C. The reaction was stirred for 2 h at 0 °C, then EtOAc (10 mL) and dil. NH_4Cl (5 mL) were added and the mixture was extracted. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated to give (*R*)-**10b** (quantitative yield, *ee* 59%).
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (1H, q, *J* = 7.0 Hz, CH), 3.76 (3H, s, $COOCH_3$), 1.98 (3H, d, *J* = 7.0 Hz, CH_3CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 52.9, 23.4, 12.4.

Determination of absolute configuration

The absolute configurations of **7b**, **9b** and **10b** were determined by co-injection with reference materials of known absolute configuration.

Analytical methods

Table S1 Achiral GC-analysis for determination of conversion[a]

Compound	Condition[b]	retention time [min]	
		Substrate (**1a-10a**)	Product (**1b-10b**)
1	a	6.64	n.d.
2	b	19.07 (*E*)-isomer	n.d.
3	a	5.84[c]	5.72
4	b	15.99 and 17.00[c,d]	15.95[c]
5	a	7.30 (*Z*)-isomer	7.08
6	a	8.83 (*E*)-isomer	n.d.
7	a	4.13	4.09
8	b	12.86 (*E*) and 12.92 (*Z*)[c]	13.09[c]
9	a	4.96[c]	4.84
10	a	5.95[c]	5.75
13	a	5.54	

[a] J&W HP-5, 5% phenylmethylpolysiloxane capillary column, 30 m x 0.32 mm, 0.25 µm film. Detector temperature 300 °C, injector temperature 300 °C.
[b] a = split ratio 20:1, 40 °C, hold for 2 min, then heat rate 20 °C min^{-1} to 180 °C, hold for 2 min (total: 11 min)
b = split ratio 20:1, 40 °C, hold for 7 min, then heat rate 10 °C min^{-1} to 180 °C, hold for 1 min (total: 22 min).
[c] Identified by GC-MS.
[d] *E*/*Z*-configuration was not determined.

Table S2 Chiral chromatographic analysis for determination of enantiomeric excess[a]

Compound	retention time [min]	
	Product [(*R*)-**7b**, **9b**, **10b**]	Product [(*S*)-**7b**, **9b**, **10b**]
7b	5.51	5.27
9b	6.88	6.44
10b	9.24	8.37

[a] Chrompack Chirasil-DEX CB, β-cyclodextrin capillary column, 25 m x 0.32 mm, 0.25 µm film. Detector temperature 250 °C, injector temperature 250 °C. Condition: split ratio 50:1, 40 °C, then heat rate 10 °C min^{-1} to 100 °C, hold for 4 min (total: 10 min)

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Table S3 Reduction of methyl 2,3,3-trichloroacrylate (**1a**) with ene-reductases

Entry	Enzyme	Cofactor						*ee* (%)
1	OYE1[b]	NADH	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	OYE2[b]		n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	OYE3		-	-	-	-	-	>99	89 (*S*)
4	YqjM		>99	-	-	-	-	-	n.d.
5	NCR		81	-	4	-	-	15	>99 (*R*)
6	OPR1		>99	-	-	-	-	-	n.d.
7	OPR3		>99	-	-	-	-	-	n.d.
8	OYE1	NAD^+/GDH	52	-	6	-	-	42	52 (*S*)
9	OYE2		31	-	5	-	-	64	39 (*S*)
10	OYE3		28	-	-	-	1	71	89 (*S*)
11	YqjM		97	-	-	-	-	3	n.d.
12	NCR		54	-	4	-	-	42	>99 (*R*)
13	OPR1		95	-	3	-	-	2	n.d.
14	OPR3		95	-	2	-	-	3	n.d.
15	OYE1	$NADP^+$/GDH	12	-	2	-	-	86	45 (*S*)
16	OYE2		14	-	3	-	-	83	38 (*S*)
17	OYE3		4	-	-	-	-	96	89 (*S*)
18	YqjM		92	-	-	-	-	8	91 (*R*)
19	NCR		47	-	3	-	-	50	>99 (*R*)
20	OPR1		97	-	3	-	-	-	n.d.
21	OPR3		97	-	2	-	-	1	n.d.
blank1	-	NADH	>99	-	-	-	-	-	-
blank2	-	NAD^+/GDH	>99	-	-	-	-	-	-
blank3	-	-	>99	-	-	-	-	-	-
blank4	-	$NADP^+$/GDH	>99	-	-	-	-	-	-

Reaction conditions: 10 mM substrate, 35 mM NADH or cofactor recycling system in Tris-HCl-buffer (50 mM, pH 7.5), 30°C, 24 h. $NAD(P)^+$/GDH = glucose/glucose dehydrogenase was used for cofactor recycling; n/a not applicable

[a] *E*/*Z*-configuration was not determined. [b] Significant polymerization.

blank1: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing 3.5 eq. NADH

blank2: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system (NAD^+, 35 mM glucose)

blank3: no enzyme, 50 mM Tris-HCl buffer (pH 7.5)

blank4: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system ($NADP^+$, 35 mM glucose)

Table S4 Reduction of methyl 2,3-dichloropropionate (*rac*-**3a**) with ene-reductases

Entry	Enzyme	Cofactor				*ee* (%)
1	OYE1	NADH	-	-	>99	43 (*S*)
2	OYE2		-	22	78	37 (*S*)
3	OYE3		-	-	>99	86 (*S*)
4	YqjM		-	-	>99	89 (*R*)
5	NCR		-	-	>99	>99 (*R*)
6	OPR1		-	-	>99	93 (*R*)
7	OPR3		-	57	43	>99 (*R*)
8	OYE1	NAD^+/GDH	-	24	76	41 (*S*)
9	OYE2		7	11	82	29 (*S*)
10	OYE3		-	26	74	85 (*S*)
11	YqjM		8	1	91	89 (*R*)
12	NCR		8	1	91	>99 (*R*)
13	OPR1		1	22	77	92 (*R*)
14	OPR3		5	49	46	>99 (*R*)
15	OYE1	$NADP^+$/GDH	25	3	72	45 (*S*)
16	OYE2		22	2	76	39 (*S*)
17	OYE3		15	2	83	90 (*S*)
18	YqjM		22	3	75	89 (*R*)
19	NCR		20	2	78	>99 (*R*)
20	OPR1		12	9	79	90 (*R*)
21	OPR3		6	27	67	>99 (*R*)
blank1	-	NADH	-	>99	-	-
blank2	-	NAD^+/GDH	-	>99	-	-
blank3	-	-	-	>99	-	-
blank4	-	$NADP^+$/GDH	-	>99	-	-

Reaction conditions: 10 mM substrate, 15 mM NADH or cofactor recycling system in Tris-HCl-buffer (50 mM, pH 7.5), 30°C, 24 h. $NAD(P)^+$/GDH = glucose/glucose dehydrogenase was used for cofactor recycling

blank1: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing 1.5 eq. NADH

blank2: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system (NAD^+, 35 mM glucose)

blank3: no enzyme, 50 mM Tris-HCl buffer (pH 7.5)

blank4: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system ($NADP^+$, 35 mM glucose)

Table S5 Reduction of methyl (*Z*)-2,3-dibromoacrylate [(*Z*)-**5a**] with ene-reductases

Entry	Enzyme	Cosolvent	Cofactor						*ee* (%)
1	OYE1	2% TBME	NADH	-	-	-	97	3	89 (*S*)
2	OYE2			-	-	-	>99	-	90 (*S*)
3	OYE3			-	-	-	89	11	92 (*S*)
4	YqjM			88	-	2	10	-	56 (*R*)
5	NCR			-	-	-	83	17	92 (*R*)
6	OPR1			95	-	4	1	-	n.d.
7	OPR3			99	-	1	-	-	n.d.
8	OYE1	-	NAD^+/GDH	16	-	9	75	-	93 (*S*)
9	OYE2			<1	-	<1	>99	-	92 (*S*)
10	OYE3			-	-	2	98	-	93 (*S*)
11	YqjM			60	-	5	35	-	53 (*R*)
12	NCR			<1	-	-	97	3	96 (*R*)
13	OPR1			71	-	15	14	-	75 (*R*)
14	OPR3			95	-	5	<1	-	n.d.
15	OYE1	-	$NADP^+$/GDH	<1	-	<1	>99	<1	93 (*S*)
16	OYE2			<1	-	-	>99	-	93 (*S*)
17	OYE3			<1	-	-	98	2	95 (*S*)
18	YqjM			28	-	3	69	-	57 (*R*)
19	NCR			-	-	1	98	1	96 (*R*)
20	OPR1			70	-	15	14	<1	75 (*R*)
21	OPR3			96	-	3	1	-	n.d.
blank1	-	2% TBME	NADH	98	-	2	-	-	-
blank2	-	-	NAD^+/GDH	>99	-	-	-	-	-
blank3	-	2% TBME	-	>99	-	-	-	-	-
blank4	-	-	$NADP^+$/GDH	>99	-	-	-	-	-

Reaction conditions: 10 mM substrate, 25 mM NADH or cofactor recycling system in Tris-HCl-buffer (50 mM, pH 7.5), 30°C, 24 h. $NAD(P)^+$/GDH = glucose/glucose dehydrogenase was used for cofactor recycling

[a] Compound was identified on GC-MS and confirmed by co-injection with authentic sample.

blank1: no enzyme, 50 mM Tris-HCl buffer (pH 7.5)containing 2.5 eq. NADH

blank2: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system NAD^+ (35 mM glucose)

blank3: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system $NADP^+$ (35 mM glucose)

blank4: no enzyme, 50 mM Tris-HCl buffer (pH 7.5)

Table S6 Reduction of methyl 2,3-dibromopropionate (*rac*-**5b**) with ene-reductases

Entry	Enzyme	Cofactor				*ee* (%)
1	OYE1	NADH	-	-	>99	92 (*S*)
2	OYE2		-	-	>99	93 (*S*)
3	OYE3		-	-	>99	95 (*S*)
4	YqjM		-	-	>99	61 (*R*)
5	NCR		-	-	>99	96 (*R*)
6	OPR1		-	6	94	91 (*R*)
7	OPR3		-	56	44	90 (*R*)
8	OYE1	NAD^+/GDH	-	-	>99	93 (*S*)
9	OYE2		-	1	99	92 (*S*)
10	OYE3		-	-	>99	94 (*S*)
11	YqjM		-	-	>99	59 (*R*)
12	NCR		-	-	>99	96 (*R*)
13	OPR1		-	6	94	87 (*R*)
14	OPR3		-	69	31	90 (*R*)
15	OYE1	$NADP^+$/GDH	3	1	96	94 (*S*)
16	OYE2		2	1	97	94 (*S*)
17	OYE3		1	1	98	95 (*S*)
18	YqjM		2	1	97	59 (*R*)
19	NCR		-	-	>99	96 (*R*)
20	OPR1		-	26	74	87 (*R*)
21	OPR3		-	73	27	91 (*R*)
blank1	-	NADH	-	>99	-	-
blank2	-	NAD^+/GDH	-	>99	-	-
blank3	-	-	-	>99	-	-
blank4	-	$NADP^+$/GDH	-	>99	-	-

Reaction conditions: 10 mM substrate, 15 mM NADH or cofactor recycling system in Tris-HCl-buffer (50 mM, pH 7.5), 30°C, 24 h. $NAD(P)^+$/GDH = glucose/glucose dehydrogenase was used for cofactor recycling

blank1: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing 1.5 eq. NADH

blank2: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system (NAD^+, 35 mM glucose)

blank3: no enzyme, 50 mM Tris-HCl buffer (pH 7.5)

blank4: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system ($NADP^+$, 35 mM glucose)

Table S7 Reduction of dimethyl (*E*)-dibromofumarate [(*E*)-**2a**] with ene-reductases

Entry	Enzyme	Cosolvent	Cofactor						
1	OYE1	10% TBME	NADH	22	-	-	6	8	64
2	OYE2			62	-	-	10	8	20
3	OYE3			-	-	-	9	3	88
4	YqjM			88	-	8	-	4	-
5	NCR			78	-	1	6	12	3
6	OPR1			93	-	3	1	3	-
7	OPR3			87	-	8	1	4	-
8	OYE1	1% DMF	NAD^+/GDH	>99	-	-	-	-	-
9	OYE2			92	-	-	3	5	-
10	OYE3			71	-	-	7	17	5
11	YqjM			93	-	-	3	-	4
12	NCR			61	-	1	23	13	2
13	OPR1			91	-	-	1	6	2
14	OPR3			95	-	-	-	5	-
15	OYE1	1% DMF	$NADP^+$/GDH	58	-	-	24	13	5
16	OYE2			74	-	-	11	12	2
17	OYE3			10	-	1	71	15	3
18	YqjM			92	-	1	1	3	3
19	NCR			56	-	-	28	14	2
20	OPR1			86	-	-	2	10	2
21	OPR3			92	-	-	1	7	-
blank1	-	10% TBME	NADH	15	-	60	-	25	-
blank2	-	1% DMF	NAD^+/GDH	>99	-	-	-	-	-
blank3	-	10% TBME	-	>99	-	-	-	-	-
blank4	-	1% DMF	$NADP^+$/GDH	>99	-	-	-	-	-

Reaction conditions: 10 mM substrate, 35 mM NADH or cofactor recycling system in Tris-HCl-buffer (50 mM, pH 7.5), 30°C, 24 h. $NAD(P)^+$/GDH = glucose/glucose dehydrogenase was used for cofactor recycling

[a] *E*/*Z*-configuration was not determined. [b] Absolute configuration was not determined.

blank1: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing 3.5 eq. NADH

blank2: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system (NAD^+, 35 mM glucose)

blank3: no enzyme, 50 mM Tris-HCl buffer (pH 7.5)

blank4: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system ($NADP^+$, 35 mM glucose)

Table S8 Reduction of methyl (*E*)-diiodopropenoate [(*E*)-**6a**] with ene-reductases

Entry	Enzyme	Cosolvent	Cofactor				*ee* (%)
1	OYE1	10% TBME	NADH	45	-	1	54	90 (*S*)
2	OYE2			82	-	1	17	90 (*S*)
3	OYE3			2	-	-	98	86 (*S*)
4	YqjM			98	-	1	1	n.d.
5	NCR			35	-	35	30	*rac*
6	OPR1			78	-	18	4	n.d.
7	OPR3			96	-	3	1	n.d.
8	OYE1	1% DMF	NAD^+/GDH	>99	-	-	-	n.d.
9	OYE2			98	-	-	2	n.d.
10	OYE3			99	-	-	1	n.d.
11	YqjM			99	-	-	1	n.d.
12	NCR			98	-	1	1	n.d.
13	OPR1			97	-	2	1	n.d.
14	OPR3			>99	-	-	-	n.d.
15	OYE1	1% DMF	$NADP^+$/GDH	82	-	-	18	86 (*S*)
16	OYE2			87	-	-	13	88 (*S*)
17	OYE3			3	-	-	97	70 (*S*)
18	YqjM			93	-	1	6	60 (*S*)
19	NCR			69	-	27	4	n.d.
20	OPR1			82	-	13	5	40 (*R*)
21	OPR3			94	-	4	2	n.d.
blank1	-	10% TBME	NADH	99	-	1	-	-
blank2	-	1% DMF	NAD^+/GDH	>99/>99	-	-	-	
blank3	-	10% TBME	-	>99/>99	-	-	-	
blank4	-	1% DMF	$NADP^+$/GDH	>99/>99	-	-	-	

Reaction conditions: 10 mM substrate, 25 mM NADH or cofactor recycling system in Tris-HCl-buffer (50 mM, pH 7.5), 30°C, 24 h. $NAD(P)^+$/GDH = glucose/glucose dehydrogenase was used for cofactor recycling

blank1: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing 2.5 eq. NADH

blank2: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system (NAD^+, 35 mM glucose)

blank3: no enzyme, 50 mM Tris-HCl buffer (pH 7.5)

blank4: no enzyme, 50 mM Tris-HCl buffer (pH 7.5) containing cofactor recycling system ($NADP^+$, 35 mM glucose)