Biocatalytic synthesis of chiral cyclic γ-oxoesters by sequential C-H hydroxylation, alcohol oxidation and alkene reduction

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Contents **Page**

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General methods

GC-MS analyses were performed using a HP-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent). The following temperature program was employed: 60°C (1 min) / 6°C min^{-1} / 150°C (1 min) / 12°C min^{-1} / 280°C (5 min). ^{1}H and ^{13}C NMR spectra were recorded on a 400 or 500 MHz spectrometer and the chemical shift scale was based on internal tetramethylsilane. TLC analyses were performed on Merck Kieselgel 60 F254 plates. All the chromatographic separations were carried out on silica gel columns. Chiral GC analyses of compounds **7a,b**-**8a,b** as acetyl derivatives (obtained by treatment with acetic anhydride in pyridine) were performed on a Chirasil DEX CB (25m × 0.25mm × 0.25 μm, Chrompack) column, installed on HP 6890 gas chromatographs, with the following temperature program: 80°C / 1.0°C min^{-1} / 118°C (1 min) / 90°C min^{-1} / 220°C (5 min): compound **7a** t_R minor enantiomer = 20.7 min, t_R major enantiomer = 22.2 min; compound **7b** t_R minor enantiomer = 25.6 min, t_R major enantiomer = 27.3 min; compound **8a** t_R minor enantiomer = 29.8 min, t_R major enantiomer = 30.2 min; compound **8b** t_R minor enantiomer = 35.3 min, t_R major enantiomer = 35.9 min.

Chemical synthesis of 1a,b-8a,b

Methyl cyclopentenecarboxylate (**3a**) and methyl cyclohexenecarboxylate (**4a**) were commercial products. The corresponding ethyl esters (**3b** and **4b**) were prepared by transesterification with ethanol and catalytic sulfuric acid. Methyl and ethyl esters of compounds **4**-**7**, to be used as reference standards, were prepared according to literature procedures:

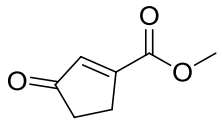
(i) oxidation of **3a,b**-**4a,b** with chromium(VI) oxide, with acetic anhydride and acetic acid in CH_2Cl_2 at 0-5°C;[1,2]

(ii) reduction of **5a,b**-**6a,b** with $NaBH_4$ and cerium(III) chloride heptahydrate in MeOH at 0°C;[3] racemic compounds **1a,b**-**2a,b** were prepared by hydrogenation of derivatives **5a,b**-**6a,b** in the presence of Pd/C in EtOAc solution.

Conversion of allylic alcohols 7a,b-8a,b into derivatives 5a,b-6a,b

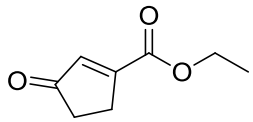
A solution of crude allylic alcohols **7a,b**-**8a,b**, obtained by *R. oryzae* mediated hydroxylation (procedure C, main text), in DMSO (0.42 mL, 500 mM) was added to an acetate buffer (6 mL, 50 mM, pH 5), containing $TEMPO^+BF_4^-$ (42 μmol, 0.2 eq.) and laccase Amano M120 (30 mg). After stirring at 30 °C for 24 h under O_2 atmosphere, the reaction mixture was extracted with EtOAc, and the combined organic solutions were dried over anhydrous Na_2SO_4, to give derivatives **5a,b**-**6a,b**, which were submitted to purification by column chromatography (silica gel), eluting with *n*-hexane and increasing amounts of EtOAc, to afford pure compounds for analytical characterisation.

Methyl 3-oxocyclopent-1-enecarboxylate (5a)



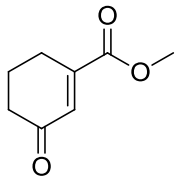
From compound **8a** (0.030 g, 0.21 mmol) derivative **5a** was obtained (0.026 g, 89%): ^{1}H NMR ($CDCl_3$, 400 MHz):[4] δ = 6.76 (t, J = 2.1 Hz, 1H, C*H*=), 3.87 (s, 3H, COO*CH*$_3$), 2.88 (m, 2H, CC*H*$_2$), 2.56 (m, 2H, COC*H*$_2$); ^{13}C NMR ($CDCl_3$, 100.6 MHz):[4] δ = 209.1, 164.9, 163.9, 138.4, 52.7, 35.7, 27.6; GC/MS (EI) t_R = 11.20 min: *m*/*z* (%) = 140 (M^+, 69), 112 (75), 109 (38), 53 (100).

Ethyl 3-oxocyclopent-1-enecarboxylate (5b)



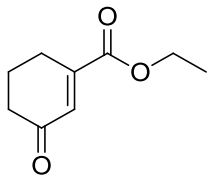
From compound **7b** (0.033 g, 0.21 mmol) derivative **5b** was obtained (0.030 g, 92%): ^{1}H NMR ($CDCl_3$, 400 MHz): [4] δ = 6.75 (t, J = 2.1 Hz, 1H, C*H*=), 4.32 (q, J = 7.1 Hz, 2H, COOC*H*$_2$CH$_3$), 2.85 (m, 2H, CC*H*$_2$), 2.53 (m, 2H, COC*H*$_2$), 1.35 (t, J = 7.1 Hz, 3H, COOCH$_2$*CH*$_3$); ^{13}C NMR ($CDCl_3$, 100.6 MHz): [4] δ = 209.1, 164.4, 138.1, 61.7, 35.7, 27.6, 14.2; GC/MS (EI) t_R = 12.92 min: *m*/*z* (%) = 154 (M^+, 44), 126 (50), 109 (44), 98 (100).

Methyl 3-oxocyclohex-1-enecarboxylate (6a)



From compound **8a** (0.033 g, 0.21 mmol) derivative **6a** was obtained (0.028 g, 87%):^{1}H NMR ($CDCl_3$, 400 MHz): [4] δ = 6.73 (t, *J* = 1.9 Hz, 1H, C*H*=), 3.83 (s, 3H, COO*CH*$_3$), 2.58 (m, 2H, COC*H*$_2$), 2.44 (m, 2H, CC*H*$_2$), 2.06 (m, 2H, $CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$, 100.6 MHz): [4] δ = 200.0, 167.0, 148.8, 133.0, 52.6, 37.7, 24.9, 22.2; GC/MS (EI) t_R = 13.78 min: *m*/*z* (%) = 154 (M^+, 60), 126 (100), 98 (80).

Ethyl 3-oxocyclopent-1-enecarboxylate (6b)



From compound **8b** (0.036 g, 021 mmol) derivative **6b** was obtained (0.032 g, 91%): ^{1}H NMR ($CDCl_3$, 400 MHz): [4] δ = 6.74 (t, *J* = 1.9 Hz, 1H, C*H*=), 4.28 (q, *J* = 7.1 Hz, COOC*H*$_2$CH$_3$), 2.58 (m, C*H*$_2$CO), 2.45 (m, 2H, C*H*$_2$C), 2.06 (m, $CH_2CH_2CH_2$), 1.33 (t, *J* = 7.1 Hz, $COOCH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100.6 MHz):[4] δ = 200.2, 166.2, 149.3, 133.0, 61.8, 37.8, 25.0, 22.3, 14.2; GC/MS (EI) t_R = 15.51 min: *m*/*z* (%) = 168 (M^+, 26), 140 (26), 133 (11), 123 (16), 112 (100).

General procedures for *R. oryzae* mediated hydroxylations of substrates 3a,b-4a,b

Procedure A

Rhizopus oryzae CBS 112.07 was cultured in 500 mL Erlenmeyer flasks containing 100 mL of MEA (malt extract agar) medium (malt extract 20 g L^{-1}, glucose 20 g L^{-1}, peptone 2 g L^{-1}, agar 20 g L^{-1}), and incubated at 25°C on a rotary shaker at a rotational speed of 160 rpm. Suspensions of spores (1.6×10^4 / mL) were used as inoculum. After 2 days of pre-growth, a solution of the substrate in DMSO (1 mL, 500 mM) was added. For each substrate, three biological replicates were run. The reaction was monitored by GC/MS: 1 mL samples, taken at specified intervals (usually every 24 h), were extracted with EtOAc (500 μL), the organic phase was dried over anhydrous Na_2SO_4 and analysed by means of GC/MS after acetylation with acetic anhydride and pyridine.

Procedure B

R. oryzae beads, prepared according to the procedure described in the Experimental Section (see Entrapment of *R. oryzae*), were pre-cultured in a flask (ca. 10 g of beads per 40 mL of MEA medium in a 100 mL flask) at 25°C on a rotary shaker at a rotational speed of 160 rpm. After 2 days of pre-growth, a solution of the substrate in DMSO (400 μL, 500 mM) was added. For each substrate, three biological replicates were run. The reaction was monitored by GC/MS: 1 mL samples, taken at specified intervals (usually every 24 h), were extracted with EtOAc (500 μL), the organic phase was dried over anhydrous Na_2SO_4 and analysed by means of GC/MS after acetylation with acetic anhydride and pyridine.

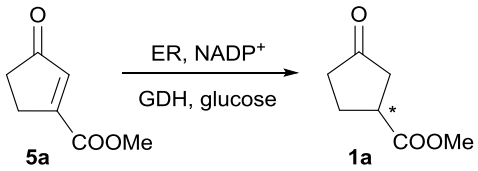
Procedure C

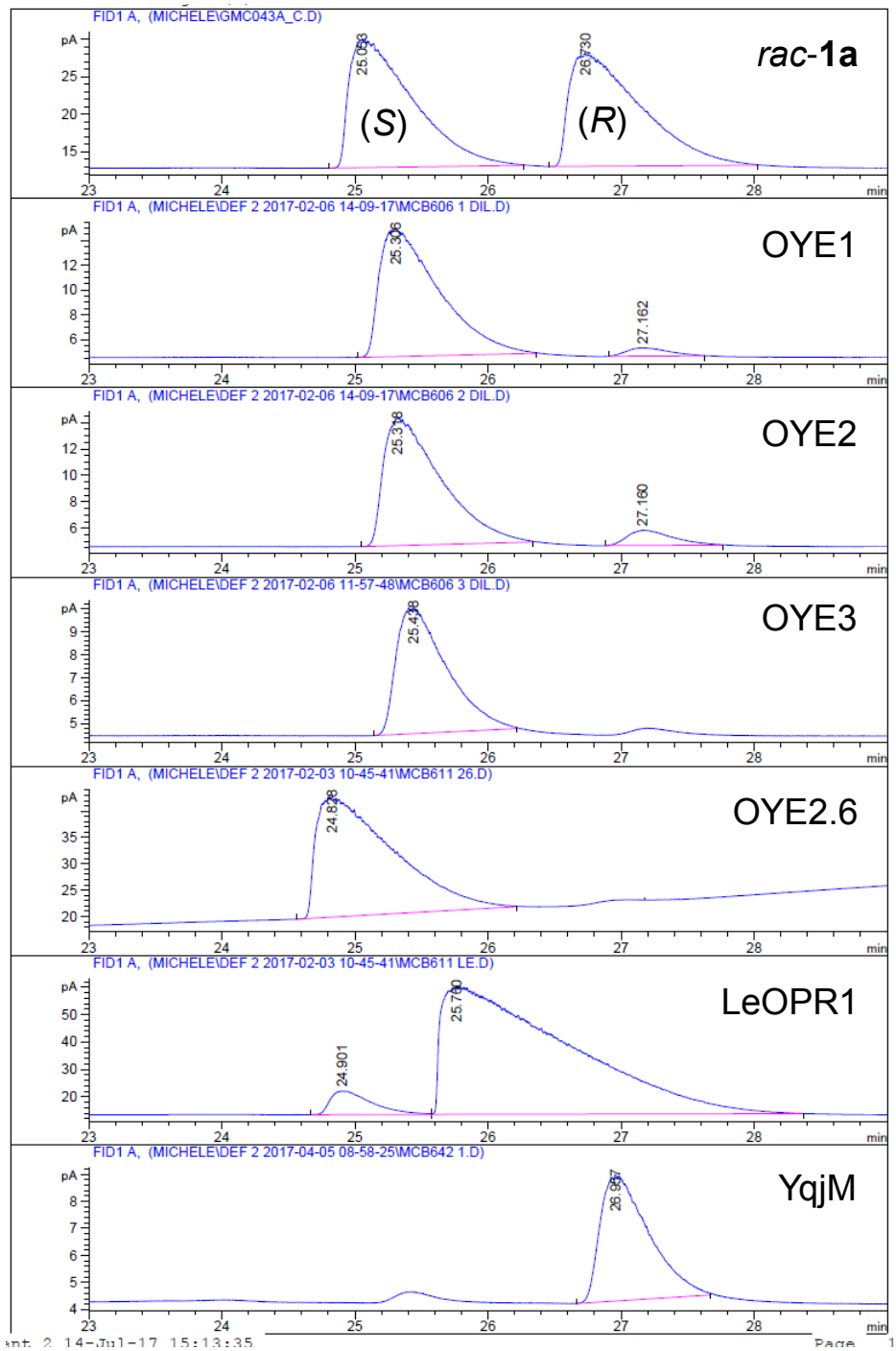
Pre-cultivated *R. oryzae* beads (corresponding to ca. 10 g of immobilised spore beads) were suspended in of acetate buffer (40 mL, 20 mM, pH 6.0) in a 100 mL flask, and a solution of the substrate (400 μL, 500 mM) in MeOH (for compound **3a**) or DMSO (for compounds **3b-4a,b**) was added. The flask was shaken at 160 rpm at 25°C. For each substrate, three biological replicates were run. The reaction was monitored by GC/MS: 1 mL samples, taken at specified intervals (usually every 24 h), were extracted with EtOAc (500 μL), the organic phase was dried over anhydrous Na_2SO_4 and analysed by means of GC/MS after acetylation with acetic anhydride and pyridine.

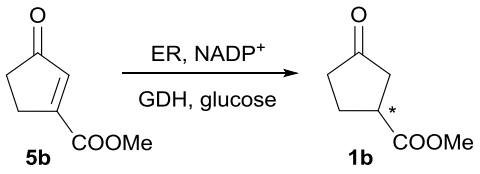
General procedure for ER mediated hydrogenations of substrates 5a,b-6a,b (screening)

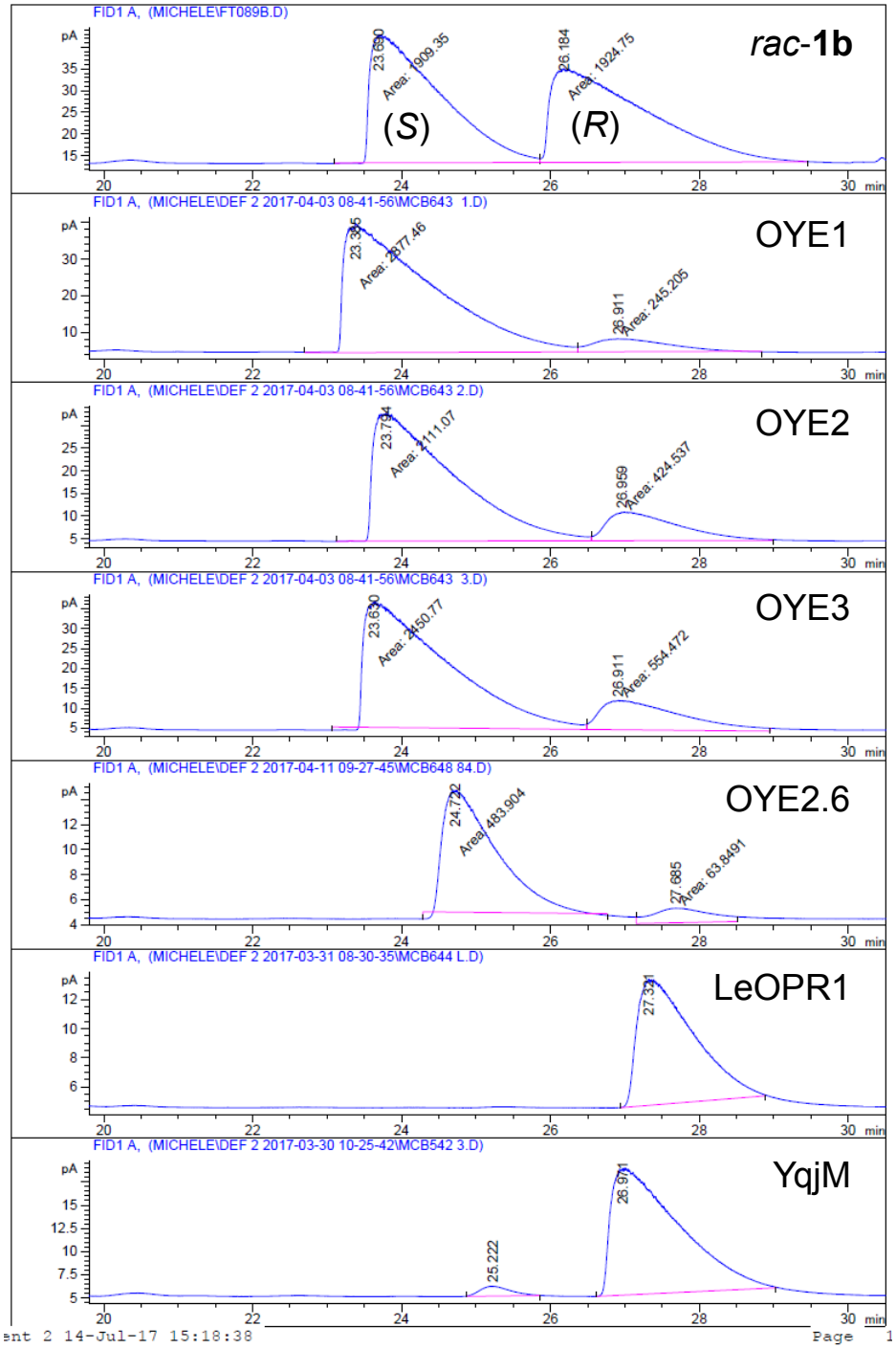
A solution of the substrate in DMSO (10 μL, 500 mM) was added to a potassium phosphate buffer solution (1.0 mL, 50 mM, pH 7.0) containing glucose (20 μmol), $NADP^+$ (0.1 μmol), GDH (4 U) and the required purified or cell-free extract OYE (80-120 μg). The mixture was incubated for 24 h in an orbital shaker (160 rpm, 30°C). The solution was extracted with EtOAc (2 × 250 μL), centrifuging after each extraction (15000 *g*, 1.5 min), and the combined organic solutions were dried over anhydrous Na_2SO_4. Two replicates were performed for each biotransformation: no significant differences (less than 5%) were observed for conversion and enantiomeric excess values.

Representative GC chromatograms






O
ER, NADP+
GDH, glucose
O
COOMe
5b
*
COOMe
1b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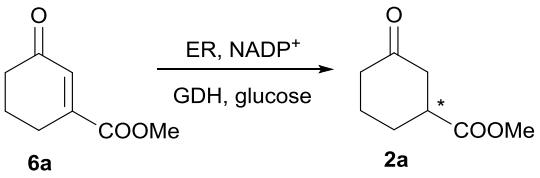
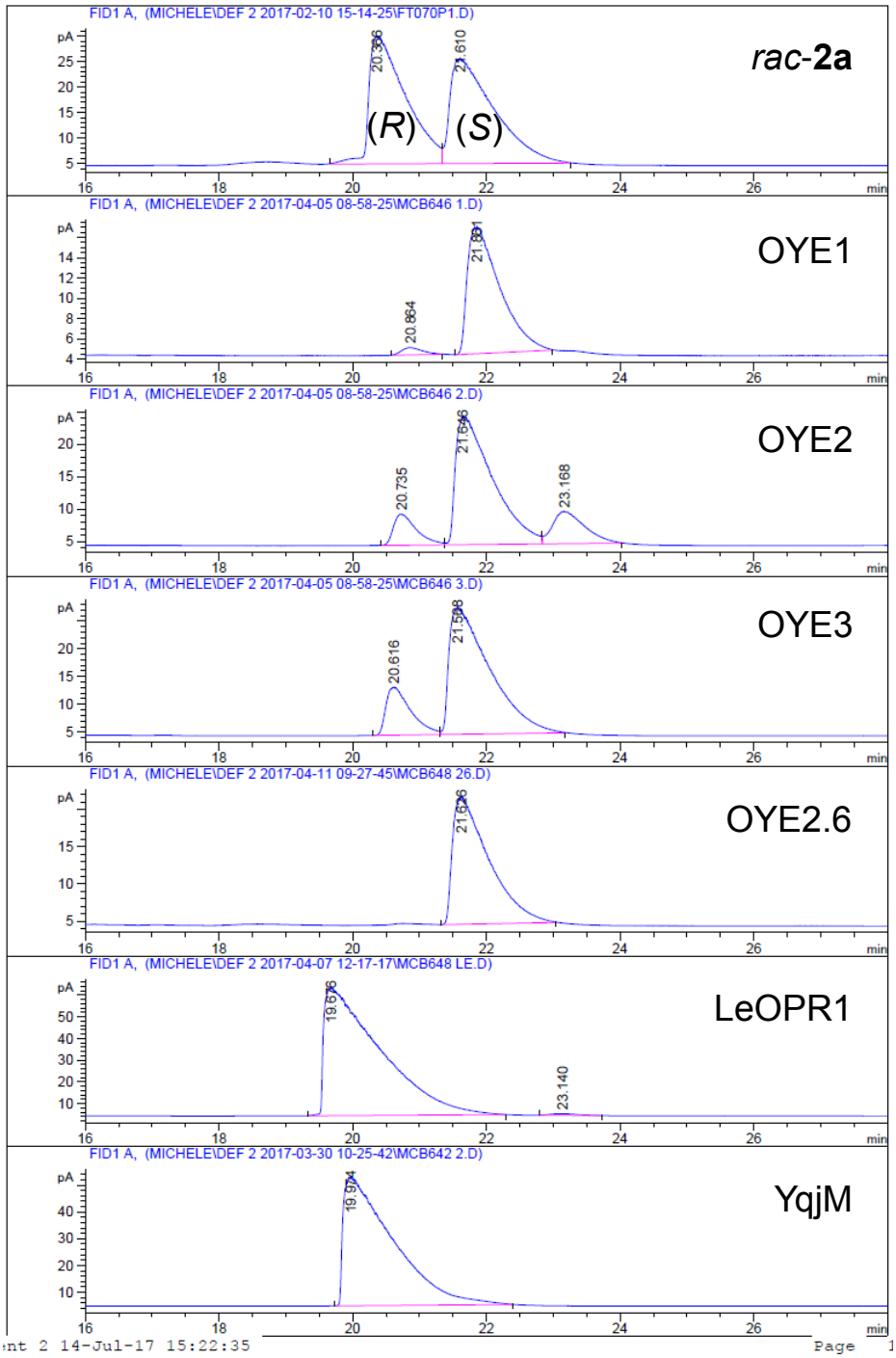
rac-1b
(S)
(R)
FID1 A, (MICHELE\DEF 2 2017-04-03 08-41-56\MCB643 1.D)
OYE1
FID1 A, (MICHELE\DEF 2 2017-04-03 08-41-56\MCB643 2.D)
OYE2
FID1 A, (MICHELE\DEF 2 2017-04-03 08-41-56\MCB643 3.D)
OYE3
FID1 A, (MICHELE\DEF 2 2017-04-11 09-27-45\MCB648 84.D)
OYE2.6
FID1 A, (MICHELE\DEF 2 2017-03-31 08-30-35\MCB644 L.D)
LeOPR1
FID1 A, (MICHELE\DEF 2 2017-03-30 10-25-42\MCB542 3.D)
YqjM
ent 2 14-Jul-17 15:18:38
Page 1


O
ER, NADP+
GDH, glucose
COOMe
6a
O
*
COOMe
2a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(R)
(S)
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OYE2
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21.646
23.168
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OYE3
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FID1 A, (MICHELE\DEF 2 2017-04-11 09-27-45\MCB648 26.D)
OYE2.6
21.626
FID1 A, (MICHELE\DEF 2 2017-04-07 12-17-17\MCB648 LE.D)
LeOPR1
19.676
23.140
FID1 A, (MICHELE\DEF 2 2017-03-30 10-25-42\MCB642 2.D)
YqjM
19.974
pA
min
nt 2 14-Jul-17 15:22:35
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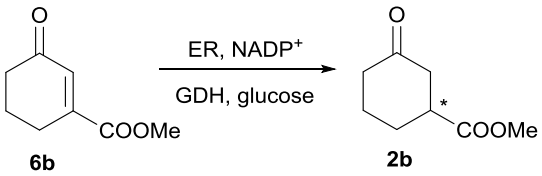

O
ER, NADP+
GDH, glucose
COOMe
6b
O
*
COOMe
2b

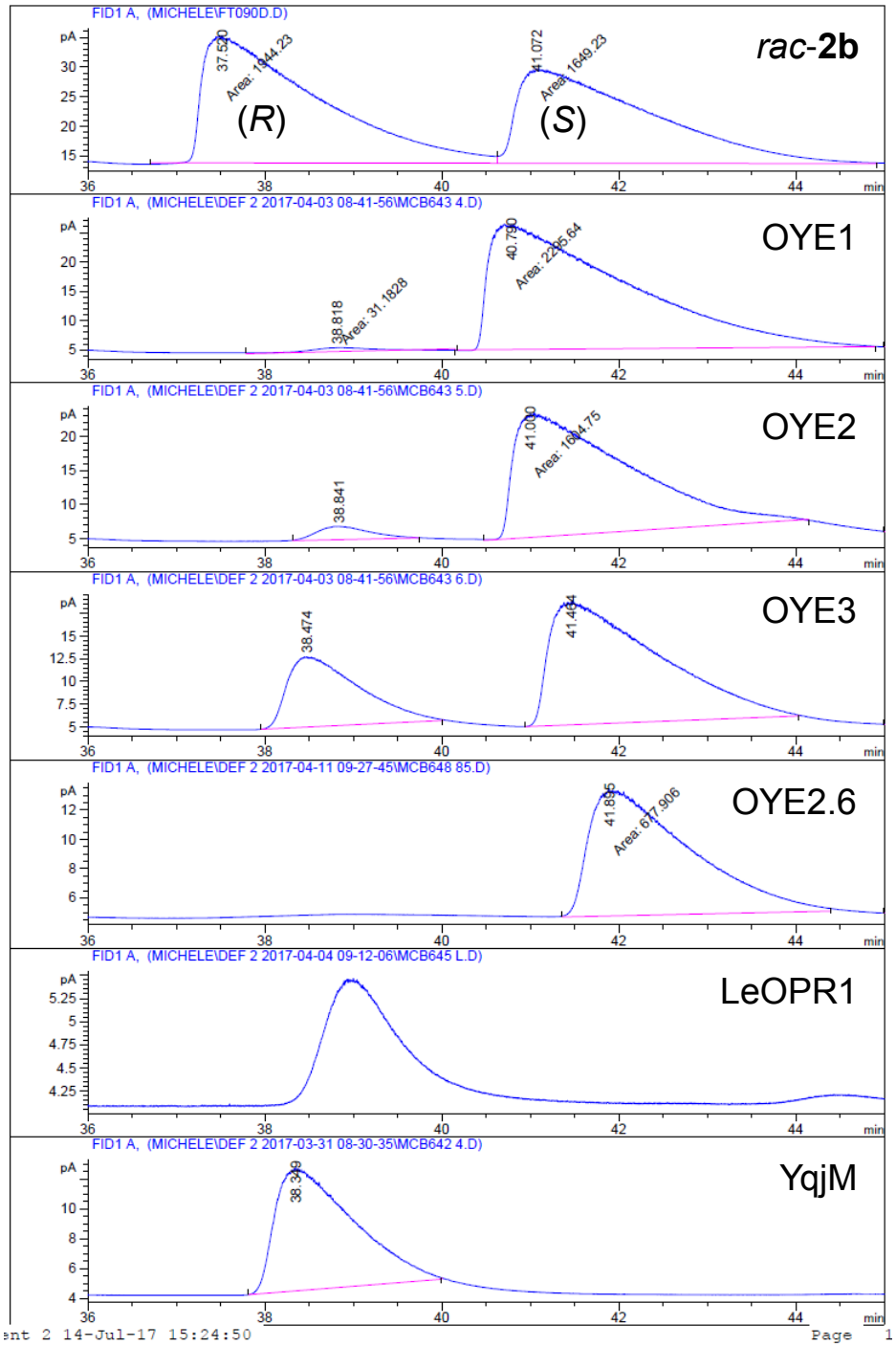
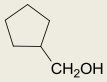
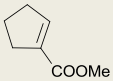
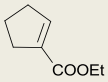
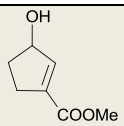
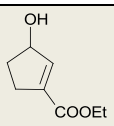
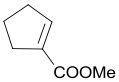
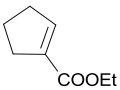
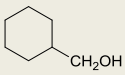
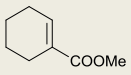
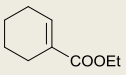
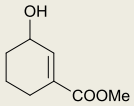
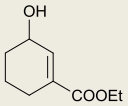
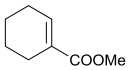
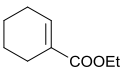

FID1 A, (MICHELE\FT090D.D)
rac-2b
(R)
(S)
FID1 A, (MICHELE\DEF 2 2017-04-03 08-41-56\MCB643 4.D)
OYE1
FID1 A, (MICHELE\DEF 2 2017-04-03 08-41-56\MCB643 5.D)
OYE2
FID1 A, (MICHELE\DEF 2 2017-04-03 08-41-56\MCB643 6.D)
OYE3
FID1 A, (MICHELE\DEF 2 2017-04-11 09-27-45\MCB648 85.D)
OYE2.6
FID1 A, (MICHELE\DEF 2 2017-04-04 09-12-06\MCB645 L.D)
LeOPR1
FID1 A, (MICHELE\DEF 2 2017-03-31 08-30-35\MCB642 4.D)
YqjM
pA
min

Table S1. Results of *R. oryzae* mediated biotransformations of compounds **3a,b**-**4a,b**.

Substrate	Procedure (reaction time)	 **9**	 **3a**	 **3b**	 **7a**	 **7b**	Other products
 **3a**	A (5 days)	14	-	-	41 (60% ee)[c]	30 (46% ee)[c]	15[d]
	B (4 days)	3	-	-	16 (48% ee)	71 (46% ee)	10
	C (5 days)	-	-	-	95 (75% ee)	-	5
 **3b**	A (5 days)	10	-	-	-	35 (45% ee)	55[e]
	B (2 days)	-	-	2	-	77 (44% ee)	21[f]
	C (2 days)	-	-	8	-	78 (35% ee)	14[g]
Substrate	**Procedure (reaction time)**	 **10**	 **4a**	 **4b**	 **8a**	 **8b**	**Other products**
 **4a**	A (5 days)	5	-	-	95 (72% ee)[c]	-	-
	B (4 days)	4	1	1	82 (74% ee)	11 (83% ee)	1
	C (3 days)	-	3	-	97 (70% ee)	-	-
 **4b**	A (5 days)	-	-	55	-	45 (86 % ee)[c]	-
	B (4 days)	1	-	2	-	97 (60% ee)	-
	C (3 days)	-	-	4	-	96 (60% ee)	

[a] Procedure A: 5 mM substrate in reaction medium (DMSO as cosolvent), *R. oryzae* whole cells, 25°C; Procedure B: 5 mM substrate in reaction medium (DMSO as cosolvent), *R. oryzae* beads in culture medium, 25°C; Procedure C: 5 mM substrate in reaction medium (MeOH or DMSO as cosolvent), *R. oryzae* beads in acetate buffer pH 6.0, 25°C.

[b] Conversion calculated on the basis of GC analysis of the crude mixture, acetylated with acetic anhydride and pyridine.

[c] Enantiomeric excess calculated on the basis of GC analysis (see Experimental Section) of the corresponding acetyl derivatives on a chiral stationary phase.

[d] Unknown products.

[e] 10% of unknown compounds + 45% of ethyl 3-hydroxycyclopentane-1-carboxylate.

[f] 11% of unknown compounds + 10% of ethyl 3-hydroxycyclopentane-1-carboxylate.

[g] 10% of unknown compounds + 4% of ethyl 3-hydroxycyclopentane-1-carboxylate.

References

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