Supporting Tables

Table S1. Ene reductases (ERs) used in this study.

Enzyme	Protein identifier	*Organism*	Ref.
*Ac*ER	CEL06147.1	*Aspergillus calidoustus*	This work
*Pb*ER	CEO58823.1	*Penicillium brasilianum*	This work
*Ar*ER	*XP*.040639288.1	*Aspergillus ruber*	This work
*Ap*ER	*XP*.031917154.1	*Aspergillus pseudotamarii*	This work
*Aa*2ER	KAE8394831.1	*Aspergillus alliaceus*	This work
*Af*ER	*XP*.041146091.1	*Aspergillus flavus*	This work
*Ap*2ER	*XP*.041551140.1	*Aspergillus puulaauensis*	This work
*Cf*ER	*XP*.018704979.1	*Cordyceps fumosorosea*	This work
*Ap*3ER	*XP*.031919233.1	*Aspergillus pseudotamarii*	This work
*Tc*ER	*XP*.024753128.1	*Trichoderma citrinoviride*	This work
*Lc*ER	WP_035455337.1	*Lactobacillus composti*	[1]
*Lp*ER	WP_057822429.1	*Lactobacillus perolens*	[1]
*Pd*ER	WP_100078324.1	*Pediococcus damnosus*	[1]
*Ka*ER	WP_035893563.1	*Kluyvera ascorbata*	[1]
*Bp*ER	WP_033496060.1	*Bifidobacterium psychraerophilum*	[1]
*Ga*ER	WP_065565632.1	*Gilliamella apicola*	[1]
*Ci*ER	WP_085558767.1	*Carnobacterium iners*	[1]
*Re*ER	XP_013324131.1	*Rasamsonia emersonii CBS 393.64*	[1]
*Aw*ER	RDH29236.1	*Aspergillus welwitschiae*	[1]
*Pf*ER	KUM55621.1	*Penicillium freii*	[1]
*Tv*ER	KUL85056.1	*Talaromyces verruculosus*	[1]
OYE1	CAA37666.1	*Saccharomyces pastorianus*	[2]
Ncr	AAV90509.1	*Zymomonas mobilis subsp. mobilis ZM4 ATCC 31821*	[3]
*Nt*DBR	Q9SLN8.1	*Nicotiana tabacum*	[4]
NEM A2	AHC69715.1	*Pseudomonas putida*	[1]
X8-2	WP_099593618.1	*Pseudomonas putida*	[1]
Xen A C25G	WP_016711963.1	*Pseudomonas putida strain NCIMB1007*	[5]
OYE2.6	XP_001384055.1	*Scheffersomyces stipitis CBS 6054*	[6]
*Se*ER	XP_018218866.1	*Saccharomyces eubayanus]*	[1]
*Nd*ER	XP_003668369.1	*Naumovozyma*	[1]
*Zb*ER	CDH15933.1	*dairenensis CBS 421*	[1]
*Ka*2ER	XP_003957301.1	*Zygosaccharomyces bailii ISA1307*	[1]
*Yl*ER	NP_194153.1	*Yarrowia lipolytica CLIB122*	[1]
*At*ER	NP_194153.1	*Arabidopsis thaliana*	[1]
OPR1	NP_001234781.1	*Solanum lycopersicum*	[7]
*Ss*2ER	KAG6388890.1	*Salvia splendens*	This work
*Ga*2ER	EPS65369.1	*Genlisea aurea*	This work
*Zj*ER	XP_015876252.1	*Ziziphus jujuba*	This work
*Hs*ER	XP_038993294.1	*Hibiscus syriacus*	This work
*Pp*ER	XP_020418184.1	*Prunus persica*	This work
*Pa*ER	XP_028801622.1	*Prosopis alba*	This work
*Pv*ER	XP_031270232.1	*Pistacia vera*	This work

*Plp*ER	WP_074296224.1	*Paraburkholderia phenazinium*	This work
*Lr*ER	WP_046277761.1	*Limnoraphis robusta*	This work
*Ps*ER	TAL55334.1	*Pandoraea sp.*	This work
*Rb*ER	WP_112325249.1	*Rhodothermaceae bacterium*	This work
*Cd*ER	WP_127081639.1	*Calothrix desertica*	This work
*Ms*ER	WP_044279457.1	*Myxococcus stipitatus*	This work
*Aa*ER	WP_129148644.1	*Achromobacter aloeverae*	This work
*Pse*ER	WP_093282826.1	*Pseudoxanthomonas sp. CF125*	This work
*Nk*ER	WP_109016915.1	*Novimethylophilus kurashikiensis*	This work
*Rb*1ER	HCH56748	*Rhodospirillaceae bacterium*	This work
*Nc*ER1	WP_010996029.1	*Nostoc sp. PCC7120*	[1]
*Ss*ER	WP_073925284.1	*Streptomyces sp. CB03911*	[1]
*Ao*ER	EIT78852.1	*Aspergillus oryzae 3.042*	[1]
*Aw*2ER	OJJ32706.1	*Aspergillus wentii DTO 134E9*	[1]
*Au*ER	GAO84519.1	*Aspergillus udagawae*	[1]
*Pf*2ER	KUM55621.1	*Penicillium freii*	[1]
*As*ER	RJE27435.1	*Aspergillus sclerotialis*	[1]
*Df*ER	TFY56562.1	*Dentipellis fragilis*	[1]
Yl-1ER	AOW07615.1	*Yarrowia lipolytica*	[1]
Yl-2ER	XP_502892.1	*Yarrowia lipolytica CLIB122*	[1]
Yl-3ER	RDW28493.1	*Yarrowia lipolytica*	[1]
Yl-4ER	XP_499654.1	*Yarrowia lipolytica CLIB122*	[1]
*Ptn*ER	ODV94697.1	*Pachysolen tannophilus NRRL Y-2460*	[1]
*Bz*ER	WP_078061662.1	*Bacillus zhangzhouensis*	[1]
*Ba*ER	WP_098797170.1	*Bacillus sp. AFS040349*	[1]
*Bf*ER	WP_123917522.1	*Bacillus sp. FJAT-42376*	[1]
*Pk*ER	WP_119883250.1	*Paenisporosarcina sp. K2R23-3*	[1]
*Pt*ER	WP_116554568.1	*Pueribacillus theae*	[1]
*Pg*ER	WP_090786729.1	*Paenibacillus sp. GP183*	[1]
Bac-OYE1	WP_003153250.1	*Bacillus sp.*	[8]
Bac-OYE2	WP_032863662.1	*Bacillus sp.*	[8]
*Gra*ER	NC_015064.1	*Granulicella tundricola MP5ACTX9*	[1]
NEM	P77258.1	*Escherichia coli*	[9]
OYE3	NC_001148.4	*Saccharomyces cerevisiae S288c*	[3]
YqjM	BAA12619.1	*Bacillus subtilis*	[10]
*Asp*ER	XP_001727650	*Aspergillus oryzae RIB40*	[1]
morB	AAC43569.1	*Pseudomonas putida*	[11]
*Lac*ER	YP_003789431.1	*Lactobacillus casei str Zhang*	[12]
*Bs*ER	AOR98769.1	*Bacillus subtilis*	[13]

>Bac-OYE1

MARKLFTPWTVKDVTIKNRIVMAPMCMYSSHEKDGKLQPFHMAHYISRAIGQVGLIIVE-
ATAVNPQGRISDQDLGIWSDDHIEGFAKLTEQVKAQGSKIGIQLAHAGRKAELEGDIYAPSAIPFDEQSKTPAEMTTE
QIKETIQEFKQAAARAKEAGFDIIELHAAHGYLMHEFLSPLSNHRTDEYGGSHENRYR-
FLGETIEAVKEVWDGPLFVRISASDYTDKGLDIADHIGFAKWMKEQGVDLIDCSSGALVQADINVFPGYQVSFAEKIR
EQAEIATGAVGLITTGTMAEEILQNNRADLIFVARELLRDPHFARSAAKQLNTEIPSPVQYDRAW

>YqjM
MARKLFTPITIKDMTLKNRIVMSPMCMYSSHEKDGKLTPFHMAHYIS-
RAIGQVGLIIVEASAVNPQGRITDQDLGIWSDEHIEGFAKLTEQVKEQGSKIGIQLAHAGRKAELEGDIFAPSAIAFDE
QSATPVEMSAEKVKETVQEFKQAAARAKEAGFDVIEIHAAHGYLIHEFLSPLSNHRTDEYGG-
SPENRYRFLREIIDEVKQVWDGPLFVRVSASDYTDKGLDIADHIGFAKWMKEQGVDLIDCSSGALVHADINVFPGYQ
VSFAEKIREQADMATGAVGMITDGSMAEEILQNGRADLIFIGRELLRDPFFARTAAKQLNTEIPAPVQYERGW

>*Bz*ER
ETKLFSPWTLKGVTLKNRIVMSPMCMYSSYDRDGKLQPFHFAHYIS-
RAQGQAGLIMIEASAVSPEGRISDHDLGIWSDEHIEGFASLNEQIKAYGTKTAIQLAHAGRKAELDGDILAPSSIPFDE
RSKTPVEMSVHQIKDTVQAFQDAAVRAKKAGFDIIEIHGAHGYLINEFLSPLANQRTDEYGG-
SPENRYRFLREVVDAVNDVWDGPLFVRVSASDYTTKGLDIADYIGIATWLKEQGVDLIDVSSGAVVQAKINIFPGYQ
VTFAEKIKEGAGIQTGAVGLITSGVQAEEILQNHRADLIFIGREFLRDPYFPKTAAKELRTSIEAPHQYDRAW

>*Af*ER
MGSISSTSPSVVKADSTPYFTPANNAGAAVNPDDPNTPTLFKPLRIRDVTLK-
NRIIVSPMCMYSAESDPTSPFVGALTDYHIAHLGQFALKGAGLVFVEAQAVQPNGRISPHDVGLWQDGADSEQFKG
LQRIVQFSHSQGAKVAVQLAHA-
GRKASVLPPWVAAQAGKHSLRADESVFGWPKDVVGPSGGEENIWDPAEGTYWAPRELSTAEIKEVVQAFAKSAEL
AVKAGVDVIEIHAAHGYLLNQFLSPATNKRTDEYGGSFENRVRIVREVATAVRAV-
IPKGMPLFLRISATDWLEGQPVAAESGSWDLESSLRLVEILPEVGIDLVDVSSGGVHKDQKIKLGPGYQVDLAGELRK
AIRKAGASTLVGGVGLITEAEQAQSIVQGADEAHQAEAIVTAKADVVLLARQFLREPEWVIT-
TAKKLGVKVTHPHQFWRAL

>*Se*ER
MPFVKDFEPVSLENTNLFKPIKVGNTQLAHRVVMPPLTRMRASH-
PGNVPNKQWAAEYYRQRAQRPGTMIITEGTFISPQAGGYDNAPGIWSDEQVAQWKDILLAIHDKKSFAWVQLWA
LGRASFPDAMARDGLRYDSASDAVYMEDTANAAKVPAHTLTKDDIKQYVKEYVHAAK-
NAVFLAGADGVEIHSANGYLLNQFLDPRSNTRNDEYGGSIENRARFTLEVVXAVSDAIGHDRVGLRLSPYGTFNSMS
GGAEPHIVAQYAYVLGELERRAKAGKRLAFVHLVEPRVTDLTLDEGEGDYDEGSNDFAY-
SIWKGPIVKAGNFALHPDAVKEEVKRDRTLIAYGRFFIANPDLVDRLEKGLPLNKYDRATFYTMSAEGYIDYPTYGE
AVELGWDKN

Table S2. GC conditions for ERs-catalyzed reduction.

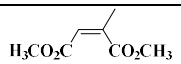
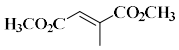
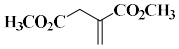
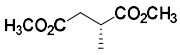
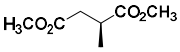
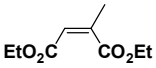
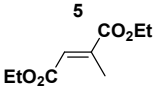
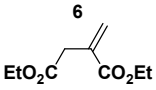
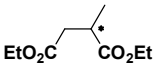
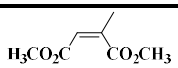
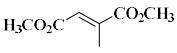
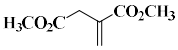
Entry	Substrate	Procedure	Peak retention time
1	 2	90°C for 4 min, 2°C /min,115°C for 2 min, 30°C /min, 160°C for 2 min.	12.372 min
2	 3		11.505 min
3	 4		12.210 min
4	 *R*-1		10.130 min
5	 *S*-1		10.292 min
6	 5		18.805 min
7	 6		19.065 min
8	 7		18.367 min
9	 8		16.079 min

Table S3. Screening results of substrates **2**[a],**3**[a] and **4**[b] for ERs library.

Enzyme	 2		 3		 4	
	Conv. [c]	*ee*[c]	Conv. [c]	*ee*[c]	Conv. [c]	*ee*[c]
Bac-OYE1	>99	99^{R}	>99	99^{S}	>99	99^{R}
Bac-OYE2	>99	99^{R}	33.7	98^{S}	24.5	99^{R}
GraER	1.5	99^{R}	n.r[d]	n.r[d]	2.9	99^{R}
NEM	>99	99^{R}	99.6	70^{R}	90.6	99^{R}
OYE3	>99	99^{R}	71.0	99^{S}	48.4	99^{R}
YqjM	>99	99^{R}	>99	98^{S}	>99	99^{R}
AspER	>99	99^{R}	>99	99^{S}	>99	99^{R}
morB	20.2	35^{R}	n.r[d]	n.r[d]	10.9	71^{R}
LacER	34.0	99^{R}	n.r[d]	n.r[d]	>99	99^{R}
BsER	>99	99^{R}	50.4	99^{S}	13.3	85^{R}
AcER	49.7	99^{R}	99.4	99^{S}	99.7	99^{R}
PbER	95.2	99^{R}	>99	99^{S}	>99	99^{R}
ArER	2.8	99^{R}	n.r[d]	n.r[d]	1.2	99^{R}
ApER	2.9	99^{R}	0.8	99^{S}	1.0	99^{R}
Aa2ER	18.0	99^{R}	9.9	81^{S}	19.6	99^{R}
AfER	>99	99^{R}	>99	99^{S}	>99	99^{R}

Ap2ER	19.7	99^{R}	19.5	99^{S}	20.1	99^{R}
CfER	>99	99^{R}	19.4	99^{S}	89.0	99^{R}
Ap3ER	2.2	99^{R}	0.7	99^{S}	0.7	99^{R}
TcER	>99	99^{R}	80.5	99^{S}	>99	99^{R}
LcER	2.2	39^{R}	n.r[d]	n.r[d]	n.r[d]	n.r[d]
LpER	2.9	99^{R}	0.5	99^{R}	2.4	99^{R}
PdER	3.0	99^{R}	0.6	99^{S}	n.r[d]	n.r[d]
KaER	61.1	99^{R}	0.9	99^{S}	>99	99^{R}
BpER	>99	99^{R}	39.9	98^{S}	19.1	99^{R}
CiER	3.8	99^{R}	0.6	99^{S}	1.3	99^{R}
ReER	95.3	99^{R}	>99	99^{S}	>99	99^{R}
AwER	67.7	99^{R}	92.7	99^{S}	>99	99^{R}
PfER	15.5	99^{R}	7.5	84^{S}	12.5	99^{R}
TvER	>99	99^{R}	>99	99^{S}	>99	99^{R}
OYE1	>99	97^{R}	>99	98^{S}	86.8	99^{R}
Ncr	>99	99^{R}	98.4	45^{R}	87.4	99^{R}
NtDBR	3.3	99^{R}	n.r[d]	n.r[d]	2.8	46^{R}
NEM A2	9.5	99^{R}	2.3	55^{R}	1.1	99^{R}
X8-2	>99	99^{R}	99.7	96^{R}	77.2	99^{R}
Xen A C25G	2.5	99^{R}	n.r[d]	n.r[d]	2.8	73^{R}
OYE2.6	n.r[d]	n.r[d]	n.r[d]	n.r[d]	n.r[d]	n.r[d]
SeER	57.8	54^{R}	>99	99^{S}	27.0	99^{R}
NdER	46.1	60^{R}	99.2	97^{S}	29.6	99^{R}
ZbER	>99	99^{R}	97.9	77^{S}	17.8	98^{R}
Ka2ER	44.0	59^{R}	>99	97^{S}	27.7	99^{R}
YlER	>99	99^{R}	>99	81^{S}	3.9	99^{R}
AtER	3.0	99^{R}	0.8	99^{S}	1.1	99^{R}
SsER	89.7	99^{R}	39.9	80^{S}	61.3	99^{R}
AoER	7.4	99^{R}	30.4	83^{S}	73.6	99^{R}
Aw2ER	4.6	99^{R}	2.4	28^{S}	11.3	99^{R}
AuER	>99	99^{R}	98.7	99^{S}	>99	99^{R}
Pf2ER	8.7	99^{R}	3.0	40^{S}	6.4	99^{R}
AsER	98.7	99^{R}	>99	99^{S}	>99	99^{R}
DfER	31.6	91^{R}	>99	99^{S}	42.2	99^{R}
Yl-1ER	>99	99^{R}	>99	82^{R}	8.3	93^{R}
Yl-2ER	6.7	99^{R}	4.5	51^{S}	16.1	99^{R}
Yl-3ER	3.5	99^{R}	0.6	99^{S}	1.8	99^{R}
Yl-4ER	>99	99^{R}	>99	75^{R}	46.5	98^{R}
PtnER	3.0	99^{R}	n.r[d]	n.r[d]	1.2	99^{R}
BzER	>99	99^{R}	>99	98^{S}	>99	99^{R}
BaER	>99	99^{R}	99.3	98^{S}	>99	99^{R}
BfER	n.r[d]	n.r[d]	97.0	98^{S}	>99	99^{R}
PkER	>99	99^{R}	>99	98^{S}	>99	99^{R}
PtER	>99	99^{R}	96.8	98^{S}	60.1	99^{R}

PgER	30.4	99^{R}	12.7	88^{S}	46.6	99^{R}
OPR1	27.1	97^{R}	92.2	71^{R}	68.3	99^{R}
Ss2ER	8.3	99^{R}	3.9	33^{S}	2.9	99^{R}
Ga2ER	3.5	99^{R}	1.4	27^{R}	3.5	99^{R}
ZjER	7.8	99^{R}	15.1	30^{R}	14.0	99^{R}
HsER	2.3	99^{R}	n.r[d]	n.r[d]	1.3	99^{R}
PpER	3.1	99^{R}	n.r[d]	n.r[d]	1.3	99^{R}
PaER	30.9	27^{R}	14.7	24^{S}	85.4	95^{R}
PvER	>99	97^{R}	>99	8^{R}	92.0	99^{R}
PlpER	66.0	99^{R}	17.8	90^{R}	1.5	99^{R}
LrER	>99	99^{R}	54.6	88^{R}	0.6	99^{R}
PsER	99.1	99^{R}	71.3	46^{R}	96.0	99^{R}
RbER	8.4	99^{R}	15.1	76^{R}	6.1	99^{R}
CdER	2.0	99^{R}	n.r[d]	n.r[d]	0.6	99^{R}
MsER	83.8	99^{R}	96.4	92^{R}	18.3	99^{R}
AaER	82.7	99^{R}	96.5	92^{R}	25.3	99^{R}
PseER	3.3	99^{R}	1.4	23^{R}	2.2	99^{R}
NkER	14.0	99^{R}	29.8	56^{R}	4.7	99^{R}
Rb1ER	1.9	99^{R}	n.r[d]	n.r[d]	1.0	99^{R}
NcER1	39.1	99^{R}	>99	90^{R}	6.3	99^{R}

[a] Reaction conditions: 50 mM **2**, **3** or **4**, 50 mg/mL wet cells, 2 U/mL LbFDH, 0.5 mM $NAD(P)^{+}$, 65 mM sodium formate, potassium phosphate buffer (100 mM, pH 7.0), 1 mL reaction volume, 30°C, 200 rpm, 24 h. [b] 10 mM **4**, 13 mM sodium formate，5% (v/v) DMSO, other conditions unchanged. [c] The conversion and *ee* were determined by chiral GC. [d] No Reaction.

Table S4. Analytical scale reaction results of ERs towards **2** with 500 mM substrate concentration.[a]

Enzyme	Conv. [b]	*ee* [b]	by-product ratio[b]
Bac-OYE1	>99	99*R*	-
Bac-OYE2	23.6	99*R*	-
NEM	99.4	99*R*	8.60%
OYE3	38.2	99*R*	0.60%
YqjM	75.4	99*R*	1.30%
AspER	30.8	99*R*	-
BsER	24.5	99*R*	0.40%
BzER	>99	99*R*	-
BaER	96.0	99*R*	-
PkER	40.2	99*R*	0.60%
PtER	61.2	99*R*	0.20%
BpER	16.1	99*R*	-
AuER	68.3	99*R*	-
TvER	46.6	99*R*	-
OYE1	20.7	99*R*	2.20%
Ncr	66.6	99*R*	1.20%
X8-2	41.7	99*R*	3.50%
ZbER	25.9	99*R*	2.20%
Y1ER	96.2	99*R*	1.80%
Y1-1ER	>99	99*R*	1.10%
Y1-4ER	25.6	99*R*	2.73%
PvER	31.1	99*R*	9.30%
LrER	18.3	99*R*	9.10%
AfER	62.0	99*R*	-
CfER	88.0	99*R*	-
TcER	69.4	99*R*	-

[a] Reaction conditions: potassium phosphate buffer (1 mL, 100 mM, pH 7.0) contained 500 mM substrate **2**, 650 mM sodium formate, 2 U/mL LbFDH, 0.5 mM $NADP^+$, and 50 mg /mL wet cells at 30°C for 24 h. [b] The conversion and *ee* values were determined by GC analysis.

Table S5. Reaction results of Bac-OYE1 and *Bz*ER with increasing substrate concentration.

Enzyme	600 mM[a] Conv. [d]	700 mM[b] Conv. [d]	800 mM[c] Conv. [d]
Bac-OYE1	>99	99.7	81.7
BzER	97.0	74.2	-

[a] Reaction conditions: potassium phosphate buffer (1 mL, 100 mM, pH 7.0) contained 600 mM substrate **2**, 780 mM sodium formate, 2 U/mL LbFDH, 0.5 mM $NADP^+$, and 50 mg /mL wet cells at 30°C for 24 h. [b] 700 mM **2**, 910 mM sodium formate, other conditions unchanged. [c] 800 mM **2**, 1.04 M sodium formate, other conditions unchanged. [d] The conversion was determined by GC analysis.

Table S6. Conversion and *ee* for screening with increasing substrate concentration of **3**.

Enzyme	100 mM[a]		300 mM[b]		400 mM[c]		500 mM[d]	
	Conv. [e]	*ee*[e]	Conv. [e]	*ee*[e]	Conv. [e]	*ee*[e]	Conv. [e]	*ee*[e]
Bac-OYE1	>99	98^{S}	99.6	99^{S}	80.4	98^{S}	-[f]	-[f]
NEM	>99	69^{R}	70.4	72^{R}	-[f]	-[f]	-[f]	-[f]
YqjM	>99	98^{S}	99.7	99^{S}	41.1	97S	-[f]	-[f]
BzER	31.2	97^{S}	4.8	96^{S}	-[f]	-[f]	-[f]	-[f]
BaER	98.2	98^{S}	29.5	97^{S}	-[f]	-[f]	-[f]	-[f]
PkER	99.4	98^{S}	31.1	97^{S}	-[f]	-[f]	-[f]	-[f]
AspR	97.2	98^{S}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
AcER	76.0	99^{S}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
PbER	80.3	98^{S}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
AfER	>99	99^{S}	99.6	99^{S}	52.7	98^{S}	-[f]	-[f]
ReER	>99	99^{S}	45.3	98^{S}	-[f]	-[f]	-[f]	-[f]
AsER	99.7	99^{S}	16.9	98^{S}	-[f]	-[f]	-[f]	-[f]
TvER	>99	98^{S}	83.5	98^{S}	-[f]	-[f]	-[f]	-[f]
OYE1	>99	96^{S}	61.5	96^{S}	-[f]	-[f]	-[f]	-[f]
X8-2	53.2	75^{R}	4.3	94^{R}	-[f]	-[f]	-[f]	-[f]
SeER	>99	99^{S}	>99	99^{S}	99.8	98^{S}	>99	98^{S}
NdER	95.6	97^{S}	40.9	97^{S}	-[f]	-[f]	-[f]	-[f]
Ka2ER	36.0	97^{S}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
Y1ER	75.9	44^{R}	33.6	52^{R}	-[f]	-[f]	-[f]	-[f]
DfER	>99	99^{S}	15.9	99^{S}	-[f]	-[f]	-[f]	-[f]
Y1-1ER	>99	79^{R}	98.7	78^{R}	69.9	71^{R}	-[f]	-[f]
Y1-4ER	>99	72^{R}	99.9	73^{R}	63.9	75^{R}	-[f]	-[f]
PvER	88.2	13^{R}	21.4	22^{R}	-[f]	-[f]	-[f]	-[f]
NcER1	25.8	89^{R}	5.2	89^{R}	-[f]	-[f]	-[f]	-[f]

[a] Reaction conditions: potassium phosphate buffer (1 mL, 100 mM, pH 7.0) contained 100 mM substrate **3**, 130 mM sodium formate, 2 U/mL LbFDH, 0.5 mM $NADP^{+}$, and 50 mg /mL wet cells at 30°C for 24 h. [b] 300 mM **3**, 390 mM sodium formate, other conditions unchanged. [c] 400 mM **3**, 520 mM sodium formate, other conditions unchanged. [d] 500 mM **3**, 650 mM sodium formate, other conditions unchanged. [e] The conversion and *ee* values were determined by GC analysis. [f] The reaction did not take place.

Table S7. Conversion and *ee* for screening with increasing substrate concentration of **4**.

Enzyme	50 mM[a]		200 mM[b]		300 mM[c]		400 mM[d]		500 mM[e]	
	Conv.	*ee*	Conv.	*ee*	Conv.	*ee*	Conv.	*ee*	Conv.	*ee*
Bac-OYE1	96.9	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
YqjM	>99	99^{R}	>99	99^{R}	>99	99^{R}	98.5	99^{R}	-[f]	-[f]
LacER	>99	99^{R}	99.9	99^{R}	97.7	99^{R}	93.1	99^{R}	-[f]	-[f]
AspER	78.1	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
AcER	>99	99^{R}	97.2	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
PbER	>99	99^{R}	94.5	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
AfER	>99	99^{R}	>99	99^{R}	>99	99^{R}	>99	99^{R}	88.3	99^{R}
TcER	>99	99^{R}	99.7	99^{R}	97.9	99^{R}	77.2	99^{R}	-[f]	-[f]
BzER	66.5	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
BaER	>99	99^{R}	53.1	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
BfER	86.8	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
PkER	>99	99^{R}	54.2	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
KaER	63.5	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
ReER	>99	99^{R}	99.9	99^{R}	96.4	99^{R}	43.8	99^{R}	-[f]	-[f]
AuER	95.7	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
AsER	>99	99^{R}	26.5	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
AwER	64.4	99^{R}	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]	-[f]
TvER	>99	99^{R}	99.9	99^{R}	99.9	99^{R}	88.3	99^{R}	-[f]	-[f]

[a] Reaction conditions: potassium phosphate buffer (1 mL, 100 mM, pH 7.0) contained 50 mM substrate **4**, 5% (v/v) DMSO, 65 mM sodium formate, 2 U/mL LbFDH, 0.5 mM $NADP^{+}$, and 50 mg /mL wet cells at 30°C for 24 h. [b] 200 mM **4**, 260 mM sodium formate, other conditions unchanged. [c] 300 mM **4**, 390 mM sodium formate, other conditions unchanged. [d] 400 mM **4**, 520 mM sodium formate, other conditions unchanged. [e] 500 mM **4**, 650 mM sodium formate, other conditions unchanged. [f] The conversion and *ee* values were determined by GC analysis. [g] The reaction did not take place.

Table S8. Specific activity of ERs towards **2**.

Enzyme	Enzyme activity (U/mL)	Protein concentration (mg/mL)	specific activity (U/mg)
Bac-OYE1	6.95	3.20	2.17
YqjM	4.43	4.20	1.06
BzER	2.82	5.12	0.55
AfER	0.99	3.98	0.25
SeER	0.27	7.85	0.03

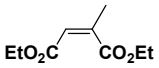
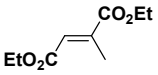
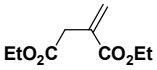
Table S9. Specific activity of ERs towards **3**.

Enzyme	Enzyme activity (U/mL)	Protein concentration (mg/mL)	specific activity (U/mg)
Bac-OYE1	1.14	3.20	0.36
YqjM	0.23	4.20	0.05
BzER	0.52	5.12	0.10
AfER	0.54	3.98	0.14
SeER	2.96	7.85	0.38

Table S10. Specific activity of ERs towards **4**.

Enzyme	Enzyme activity (U/mL)	Protein concentration (mg/mL)	specific activity (U/mg)
Bac-OYE1	3.06	3.20	0.95
YqjM	2.57	4.20	0.61
BzER	2.74	5.12	0.53
AfER	5.69	3.98	1.43
SeER	0.18	7.85	0.02

Table S11. Asymmetric reduction of **5**, **6** and **7** catalyzed by 3 ERs[a].

ERs	 **5**		 **6**		 **7**	
	Conv. (%)[b]	***ee* (%)**[c]	**Conv. (%)**[b]	***ee* (%)**[c]	**Conv. (%)**[b]	***ee* (%)**[c]
Bac-OYE1	>99	99^{R}	5	41^{S}	14	90^{R}
*Af*ER	>99	97^{R}	35	*98*^{S}	74	51^{R}
*Se*ER	>99	97^{R}	12	71^{R}	-	-

[a] Reaction conditions: 50 mM substrate (**5**, **6** or **7**) at varied concentration in potassium phosphate buffer (1 mL, 100 mM, pH 7.0), 1.3 eq sodium formate, 2 U/mL LbFDH, 0.5 mM $NADP^{+}$ (0.39 g/L), and 50 mg wet cells at 30°C for 24 h. [b] The conversion values were determined by GC analysis. [c] The *ee* values were determined by GC analysis after converted into corresponding dimethyl ester.

Table S12. Analytical scale reaction results of Bac-OYE1, *Af*ER and *Se*ER towards **5** with different concentration.

Enzyme	100 mM[a] Conv. [e]	300 mM[b] Conv. [e]	500 mM[c] Conv. [e]	700 mM[d] Conv. [e]
Bac-OYE1	>99	>99	>99	48%
*Af*ER	69	53	48	-
*Se*ER	>99	95	81	-

[a] Reaction conditions: potassium phosphate buffer (1 mL, 100 mM, pH 7.0) contained 100 mM substrate **2**, 130 mM sodium formate, 2 U/mL LbFDH, 0.5 mM $NADP^+$, and 50 mg /mL wet cells at 30°C for 24 h. [b] 300 mM **2**, 390 mM sodium formate, other conditions unchanged. [c] 500 mM **2**, 650 mM sodium formate, other conditions unchanged. [d] 700 mM **2**, 910 mM sodium formate, other conditions unchanged. [e] The conversion was determined by GC analysis.

Supporting Figures

SDS-PAGE analysis of the selected ERs and mutants

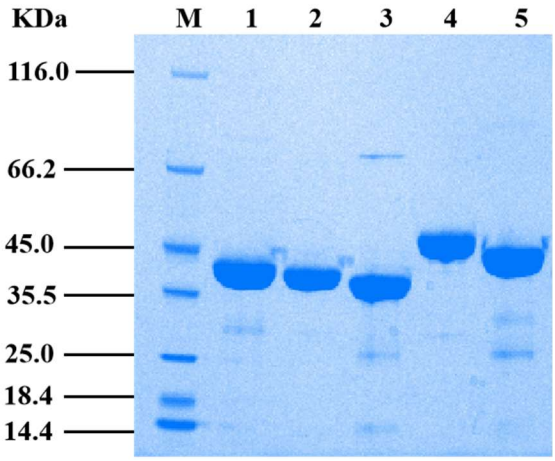


Figure S1. SDS-PAGE analysis of the selected ERs recombinantly expressed in *E. coli* BL21 (DE3). All samples were made up of pure enzymes. Lane M: Molecular Weight Marker (Page Ruler Plus™ Prestained Protein Ladder), 1: Bac-OYE1, 2: YqjM, 3: *Bz*ER; 4: *Af*ER, 5: *Se*ER.

Effects of pH and temperature on the activity and stability of purified ERs

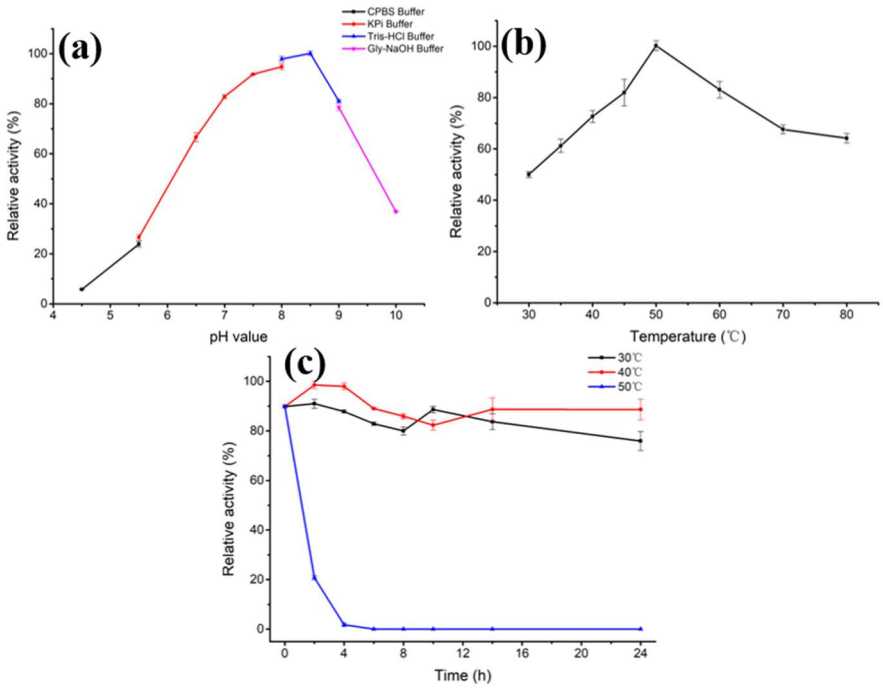


Figure S2. Effects of pH and temperature on the activity and stability of purified *Af*ER. (a) The optimum pH for the reduction of **4**. (b) The optimum temperature for the reduction of **4**. (c) The thermostability of *Af*ER at different temperatures.

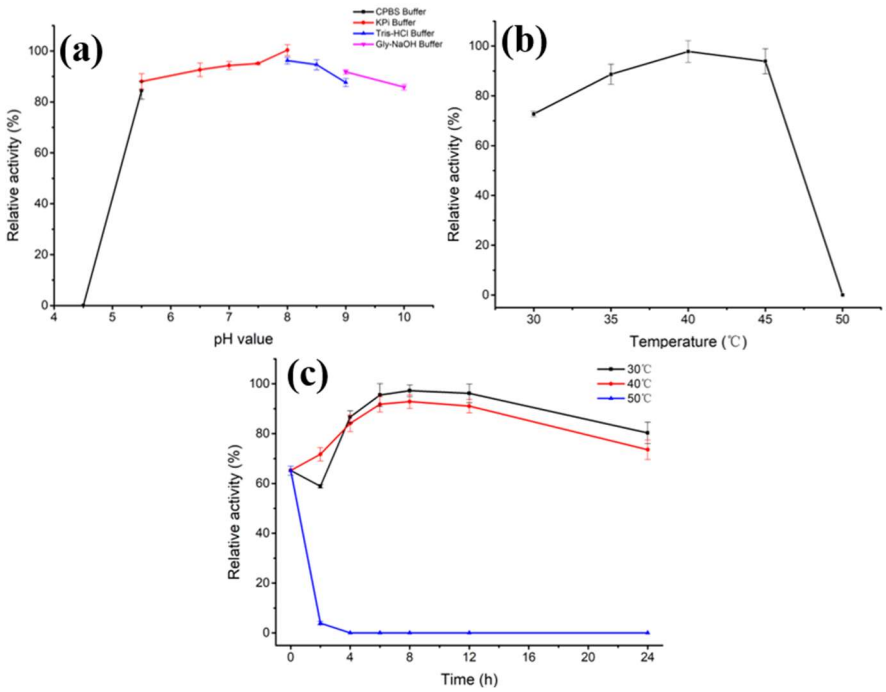
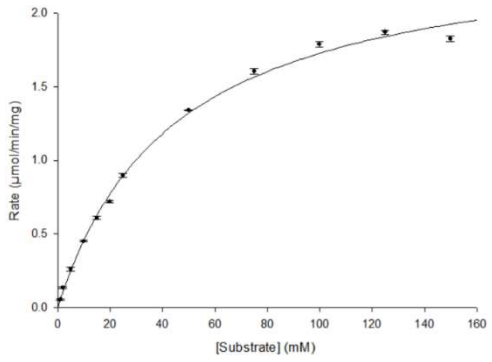


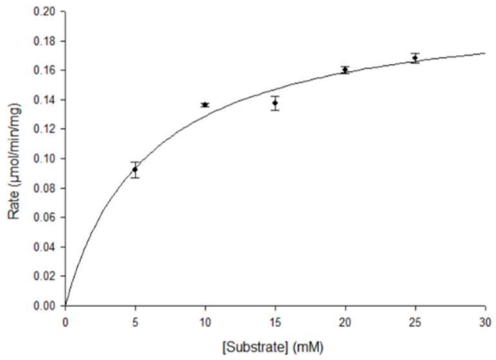
Figure S3. Effects of pH and temperature on the activity and stability of purified *Se*ER. (a) The optimum pH for the reduction of **3**. (b) The optimum temperature for the reduction of **3**. (c) The thermostability of *Se*ER at different temperatures.

K_m and V_{max} values were obtained from non-linear regression of Michaelis–Menten plots using SigmaPlot 12.0 of purified ERs

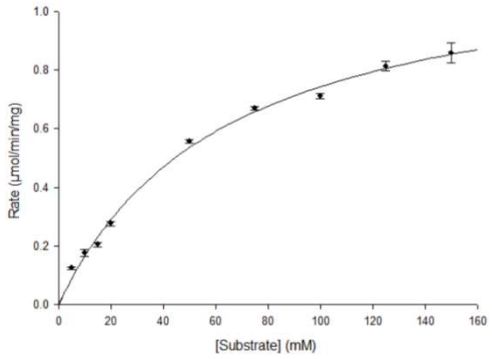
(A) Bac-OYE1 with substrate **2**



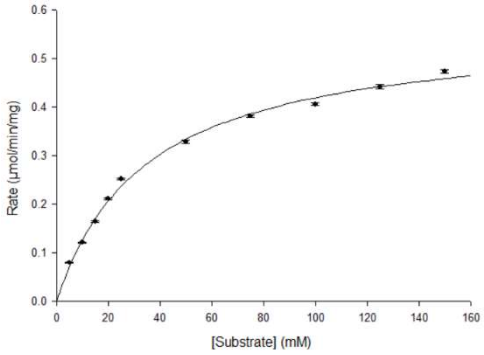
(B) Bac-OYE1 with substrate **3**



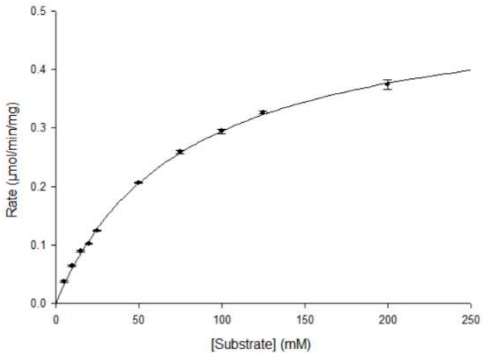
(C) Bac-OYE1 with substrate **4**



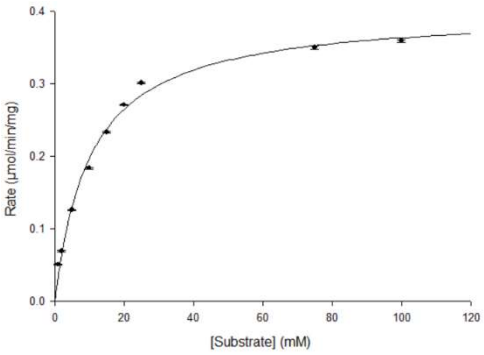
(D) YqjM with substrate **2**



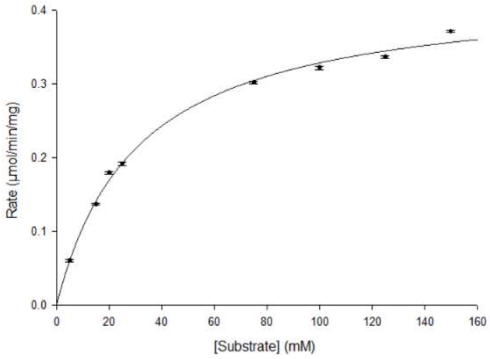
(E) YqjM with substrate **4**



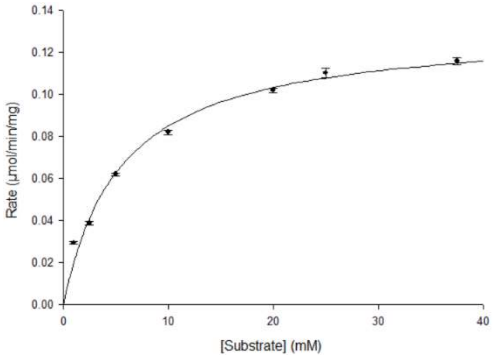
(F) *Se*ER with substrate **3**



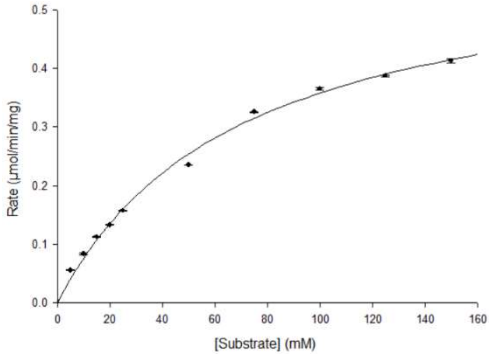
(G) *Bz*ER with substrate **2**



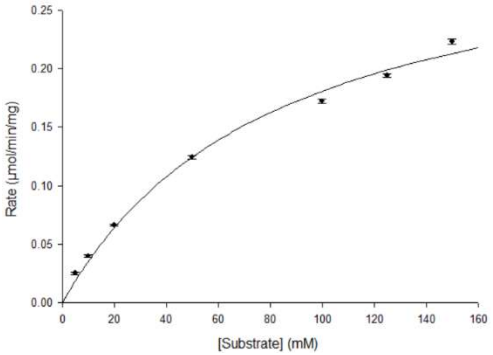
(H) *Bz*ER with substrate **3**



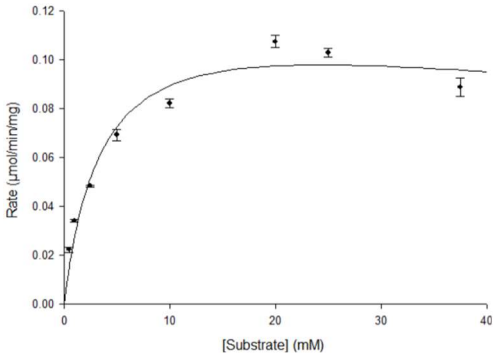
(I) *Bz*ER with substrate **4**



(J) *Af*ER with substrate **2**



(K) *Af*ER with substrate **3**



(L) *Af*ER with substrate **4**

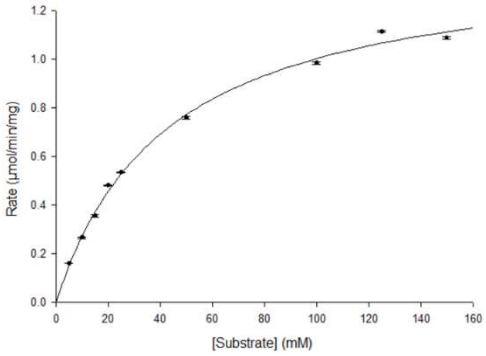


Figure S4. Non-linear regression analysis of ERs towards **2**, **3** and **4**.

^{1}H-NMR and ^{13}C-NMR spectra of substrate 3

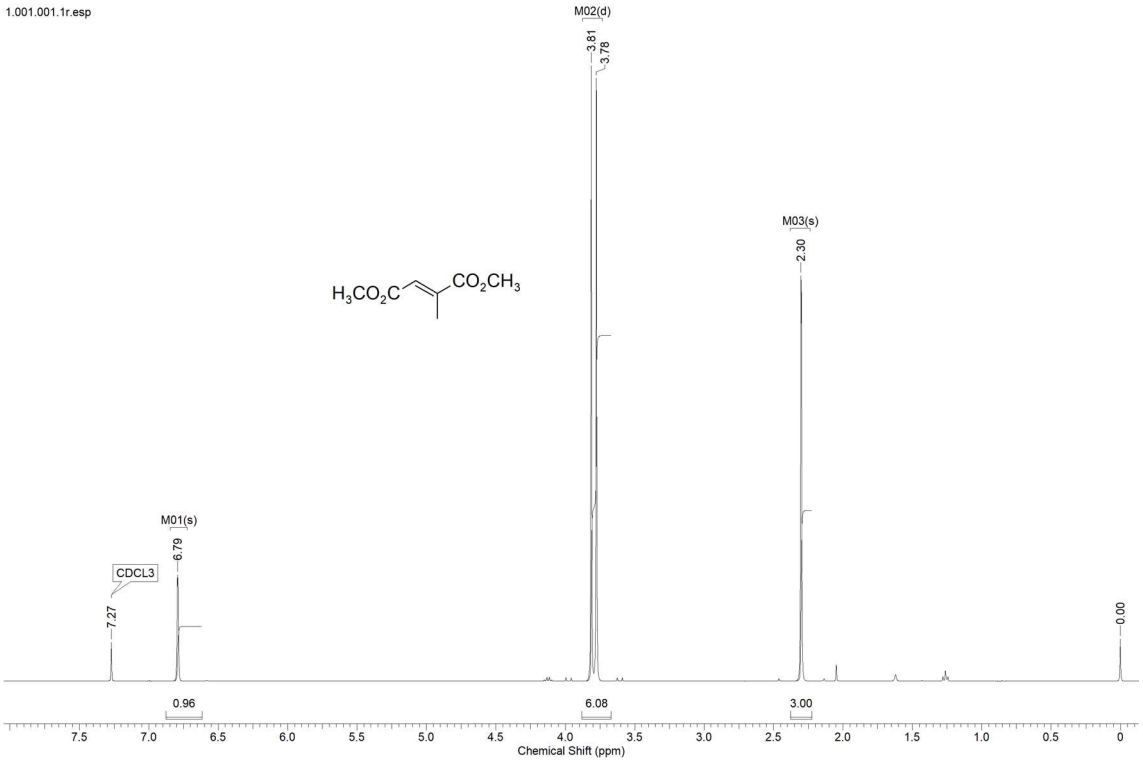


Figure S5. ^{1}H-NMR (400MHz) spectra of substrate dimethyl mesaconate (**3**) in $CDCl_3$.

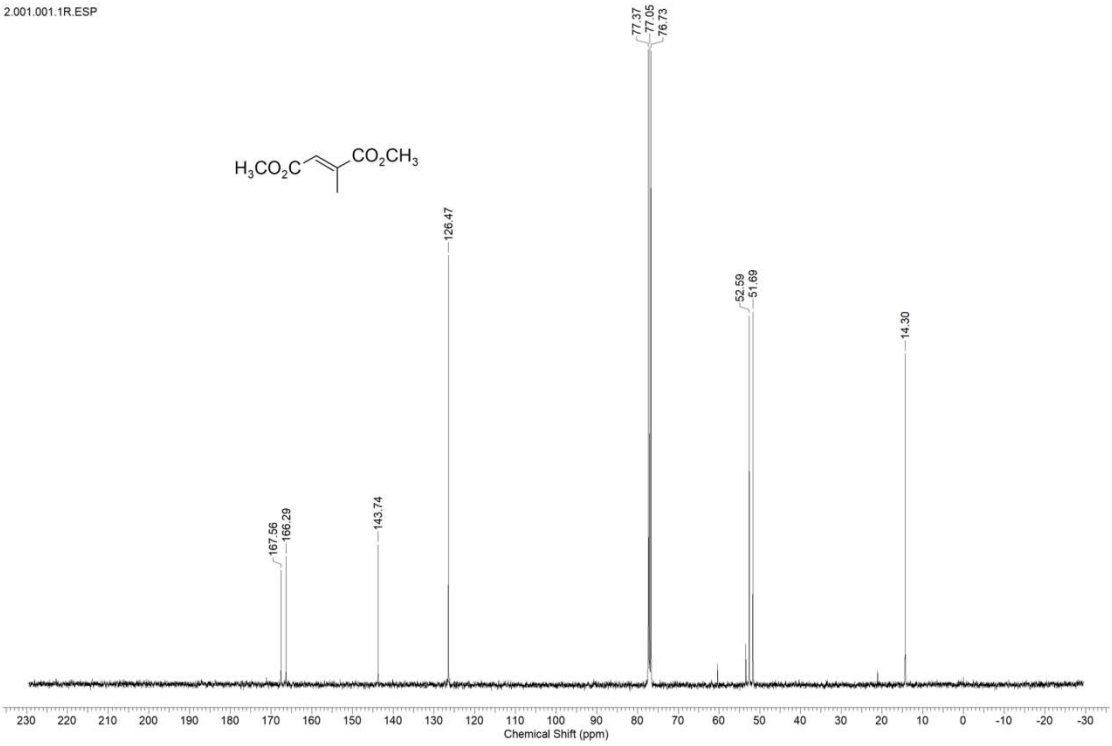


Figure S6. ^{13}C-NMR (100MHz) spectra of substrate methyl dimethyl mesaconate (**3**) in $CDCl_3$.

GC chromatograms of substrates 2, 3 and 4

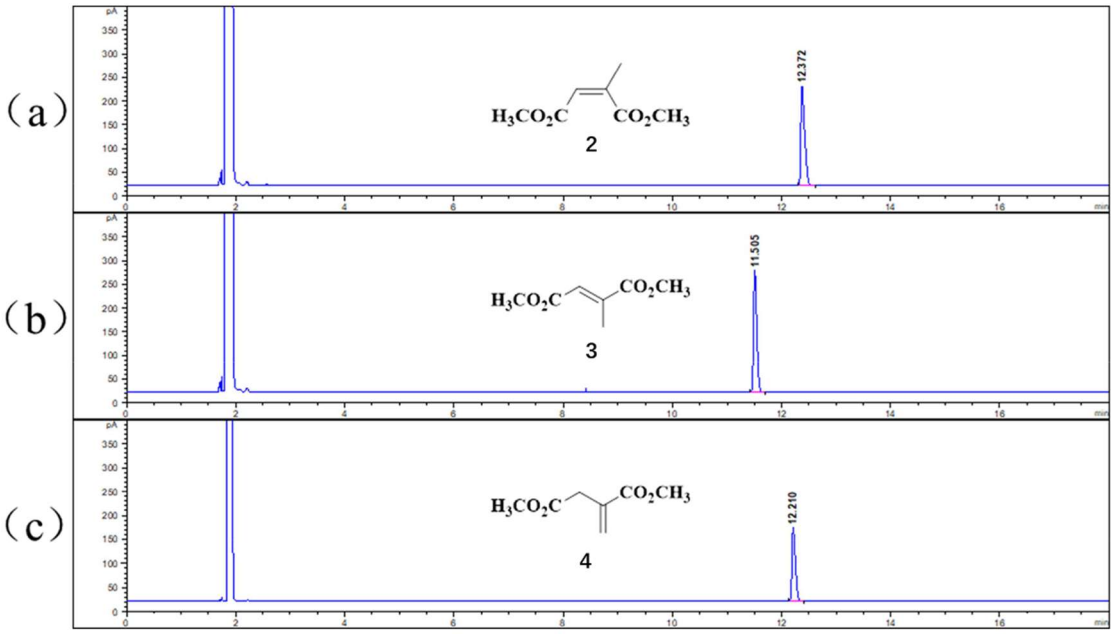


Figure S7. GC chromatograms of substrate dimethyl citraconate (a), dimethyl mesaconate (b) and dimethyl itaconate (c).

GC chromatograms, ^{1}H-NMR and ^{13}C-NMR spectra of biocatalytic products catalysed by ERs

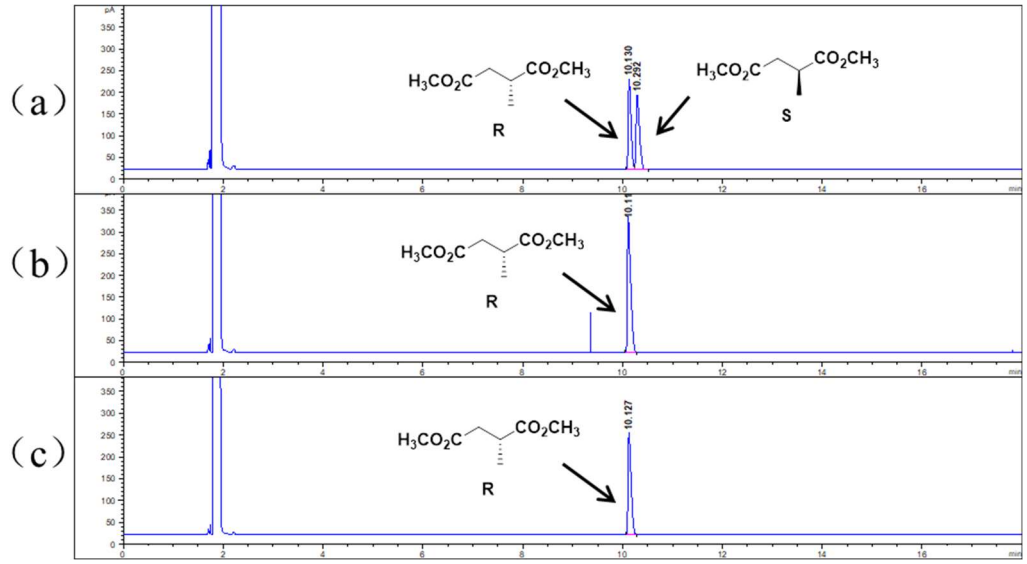


Figure S8. GC chromatograms of racemic dimethyl 2-methylsuccinate (a), (*R*)-dimethyl 2-methylsuccinate (b) and products of Bac-OYE1 (c).

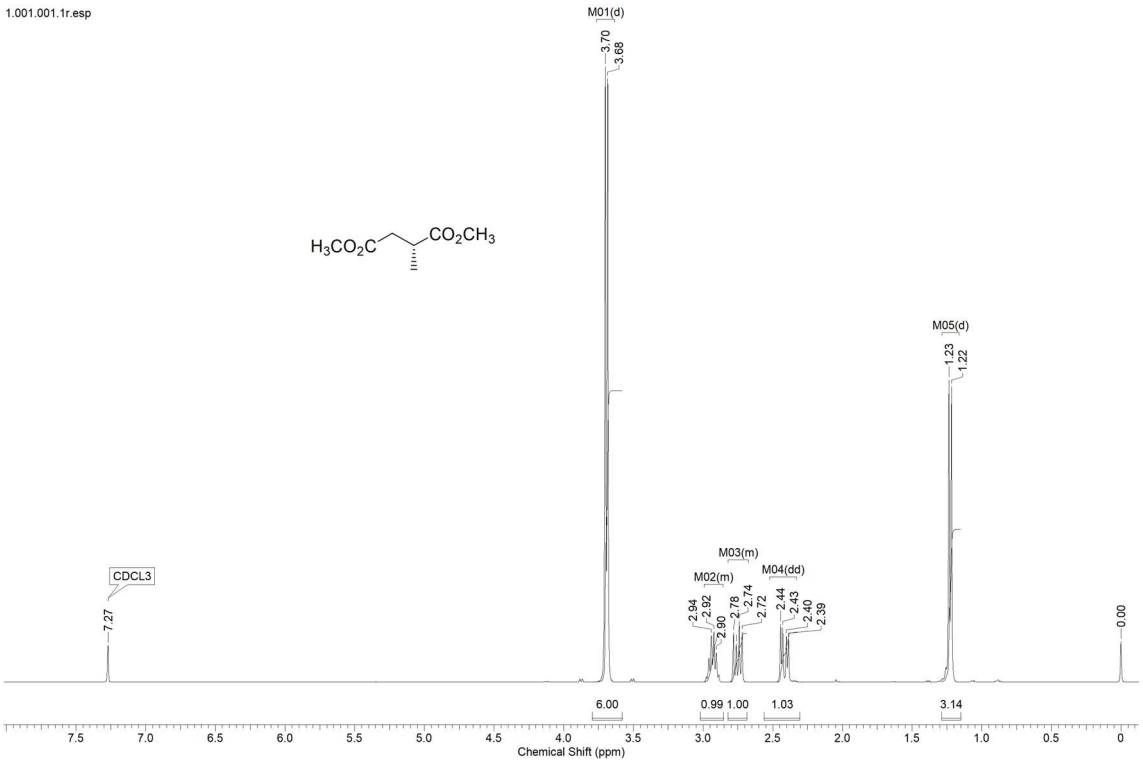


Figure S9. ^{1}H-NMR (400MHz) spectra of biocatalytic products catalysed by Bac-OYE1 in $CDCl_3$.

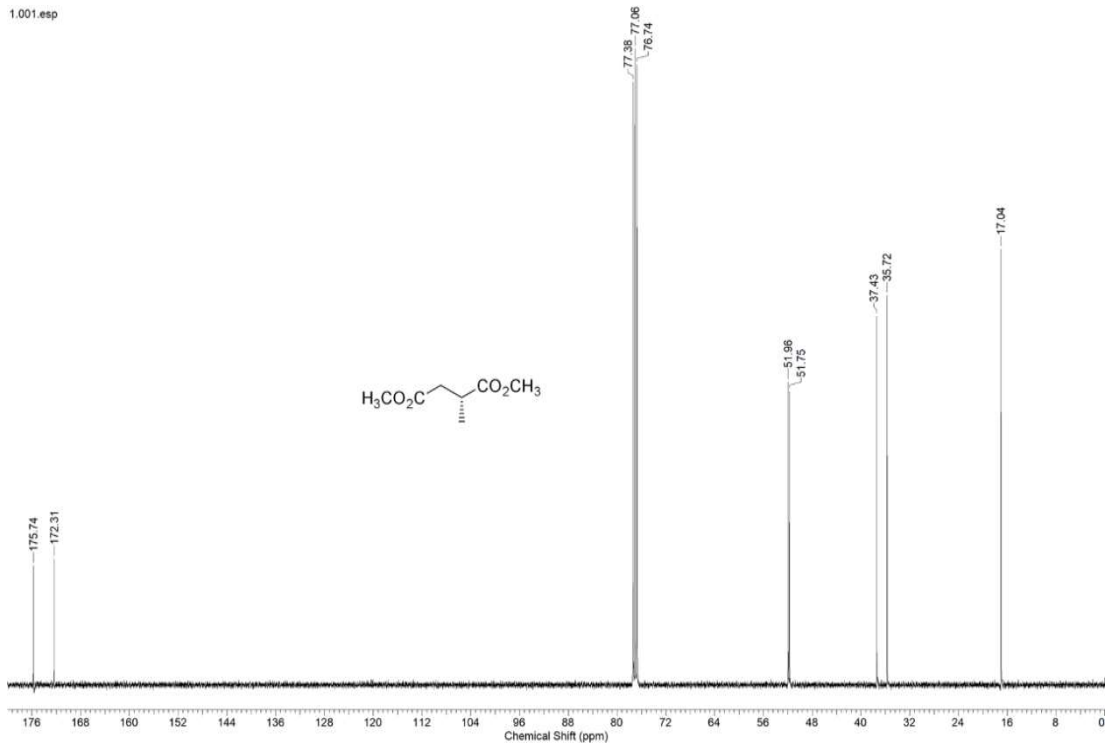


Figure S10. ^{13}C-NMR (100MHz) spectra of biocatalytic products catalysed by Bac-OYE1 in $CDCl_3$.

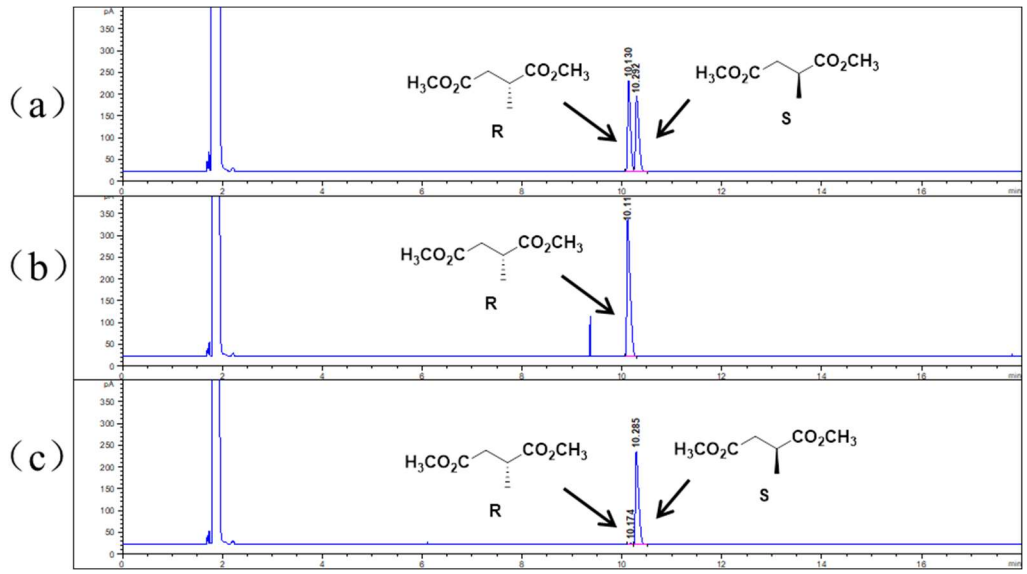


Figure S11. GC chromatograms of racemic dimethyl 2-methylsuccinate (a), (*R*)-dimethyl 2-methylsuccinate (b) and products of *Se*ER (c).

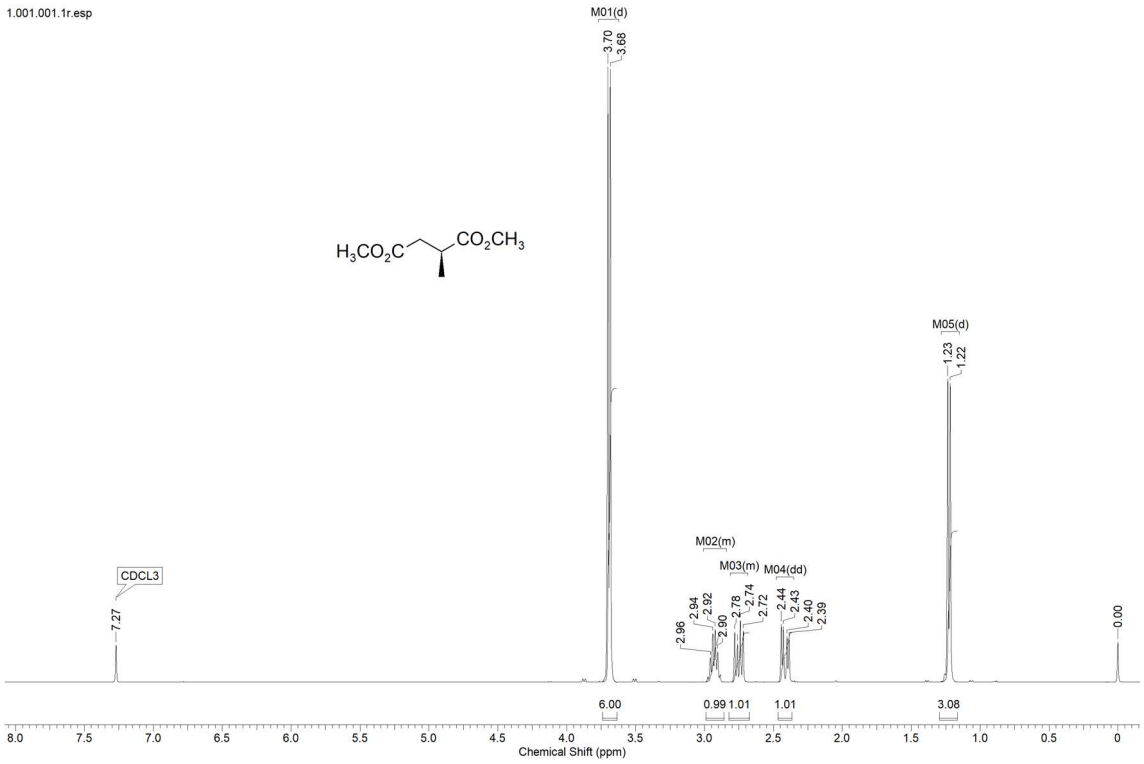


Figure S12. ^{1}H-NMR (400MHz) spectra of biocatalytic products catalysed by *Se*ER in $CDCl_3$.

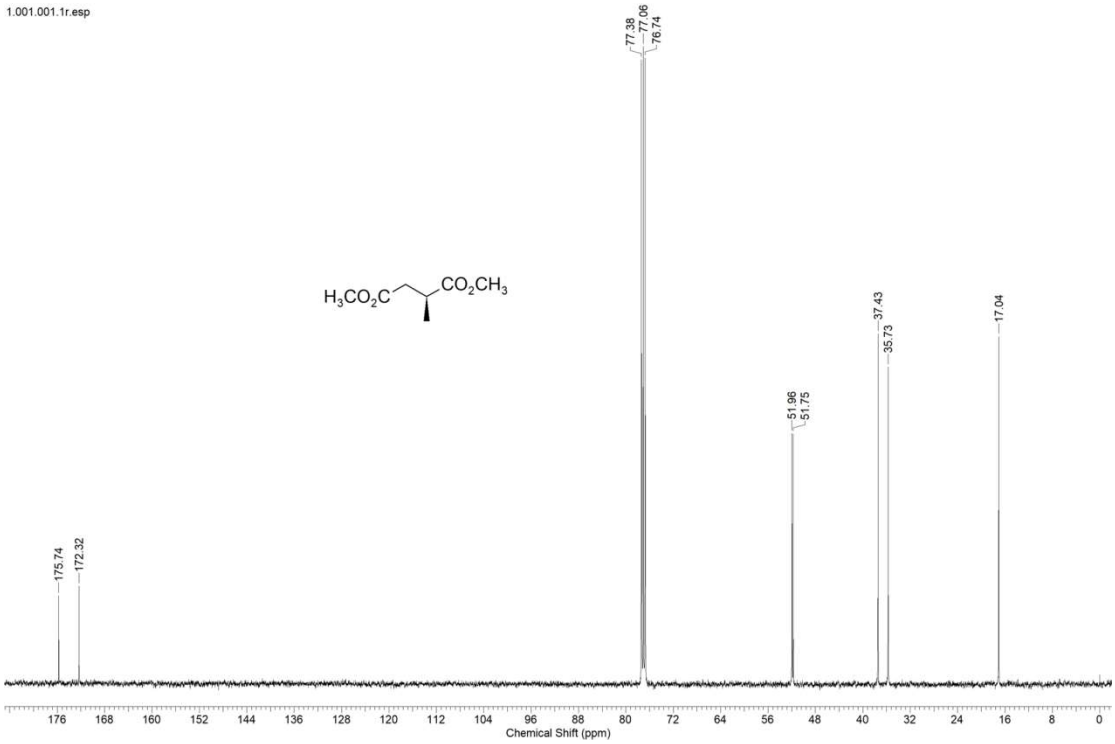


Figure S13. ^{13}C-NMR (100MHz) spectra of biocatalytic products catalysed by *Se*ER in $CDCl_3$.

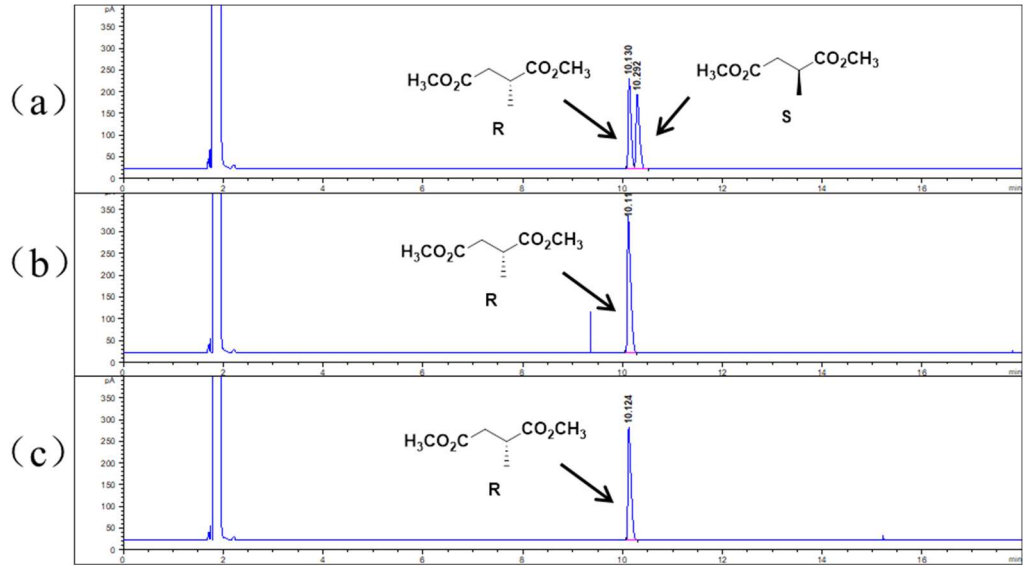


Figure S14. GC chromatograms of racemic dimethyl 2-methylsuccinate (a), (*R*)-dimethyl 2-methylsuccinate (b) and products of *Af*ER (c).

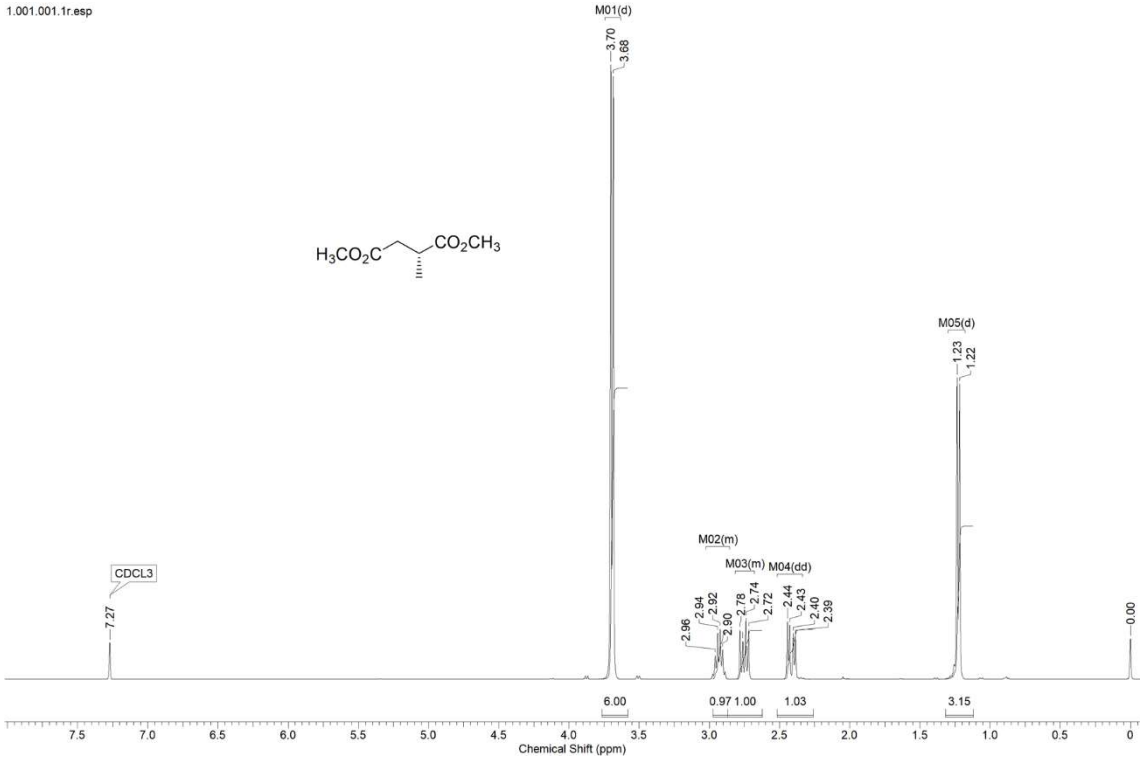


Figure S15 ^{1}H-NMR (400MHz) spectra of biocatalytic products catalysed by *Af*ER in $CDCl_3$.

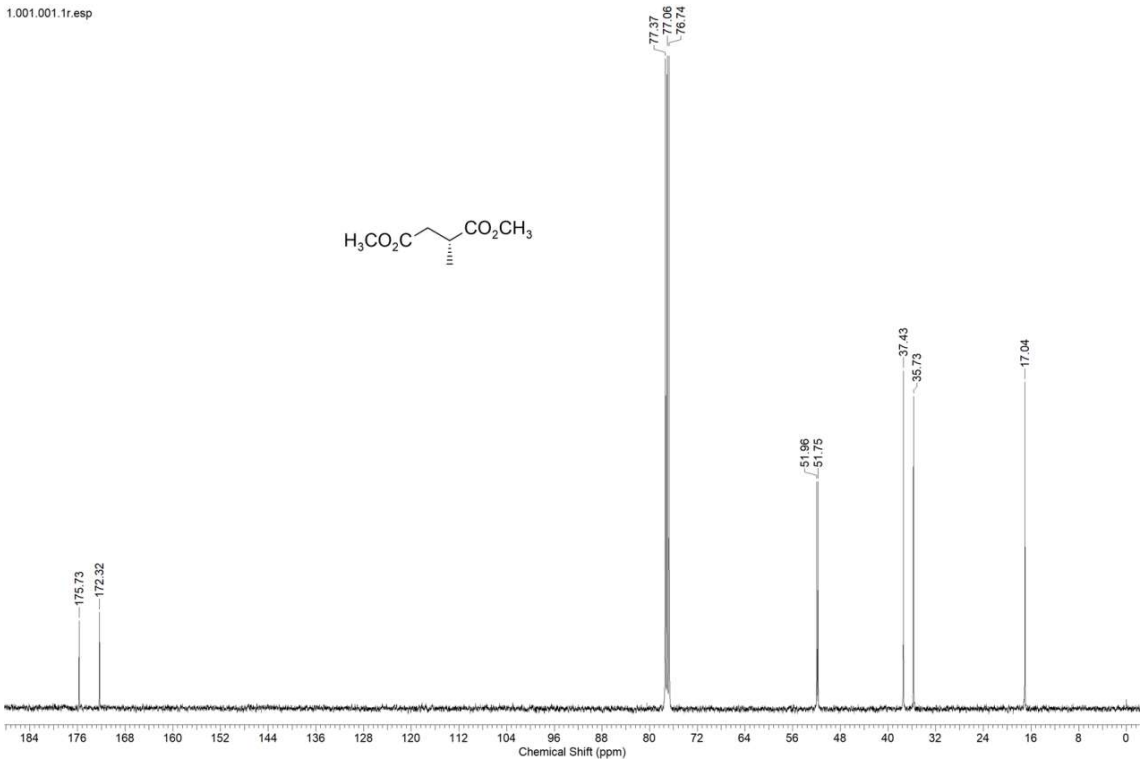


Figure S16. ^{13}C-NMR (100MHz) spectra of biocatalytic products catalysed by *Af*ER in $CDCl_3$.

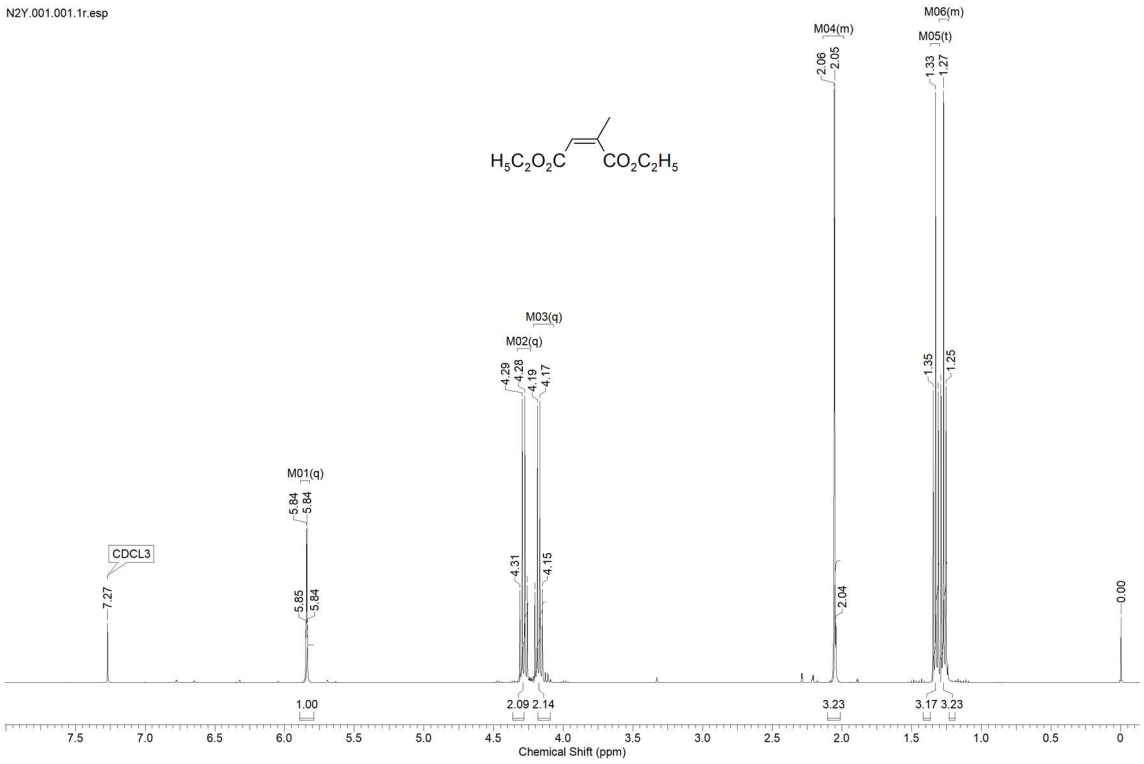


Figure S17. ^{1}H-NMR (400MHz) spectra of substrate diethyl citraconate in $CDCl_3$.

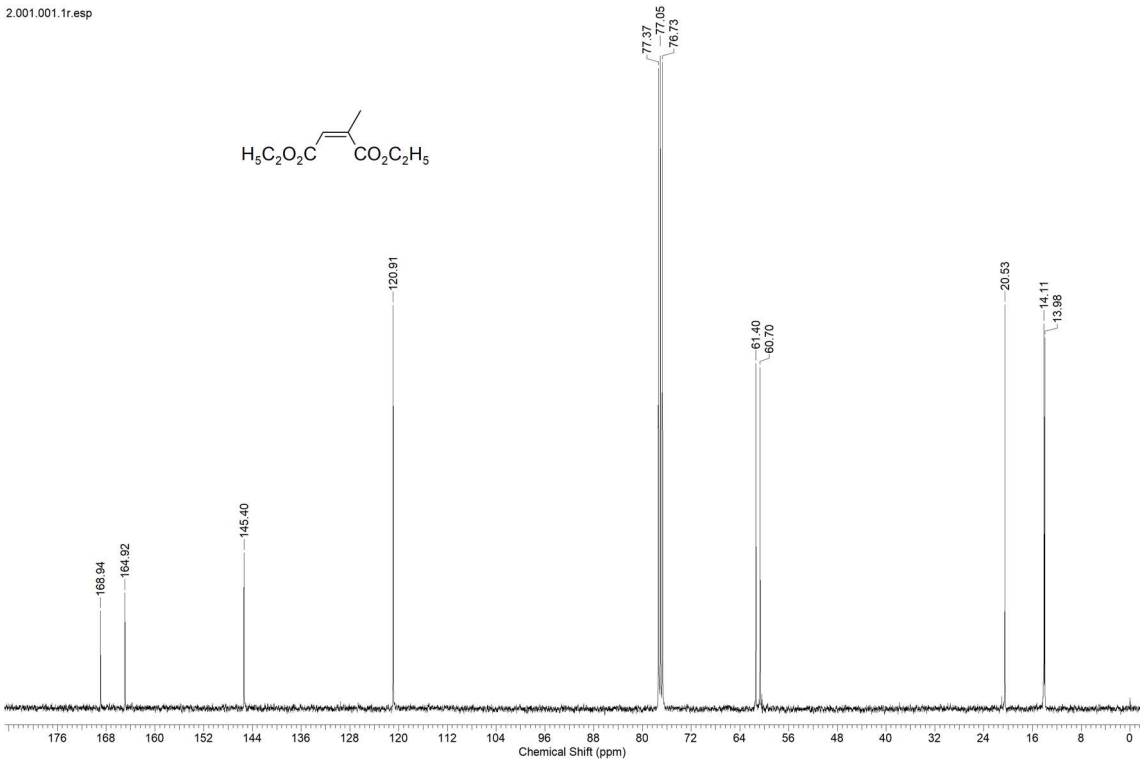


Figure S18. ^{13}C-NMR (100MHz) spectra of substrate diethyl citraconate in $CDCl_3$.

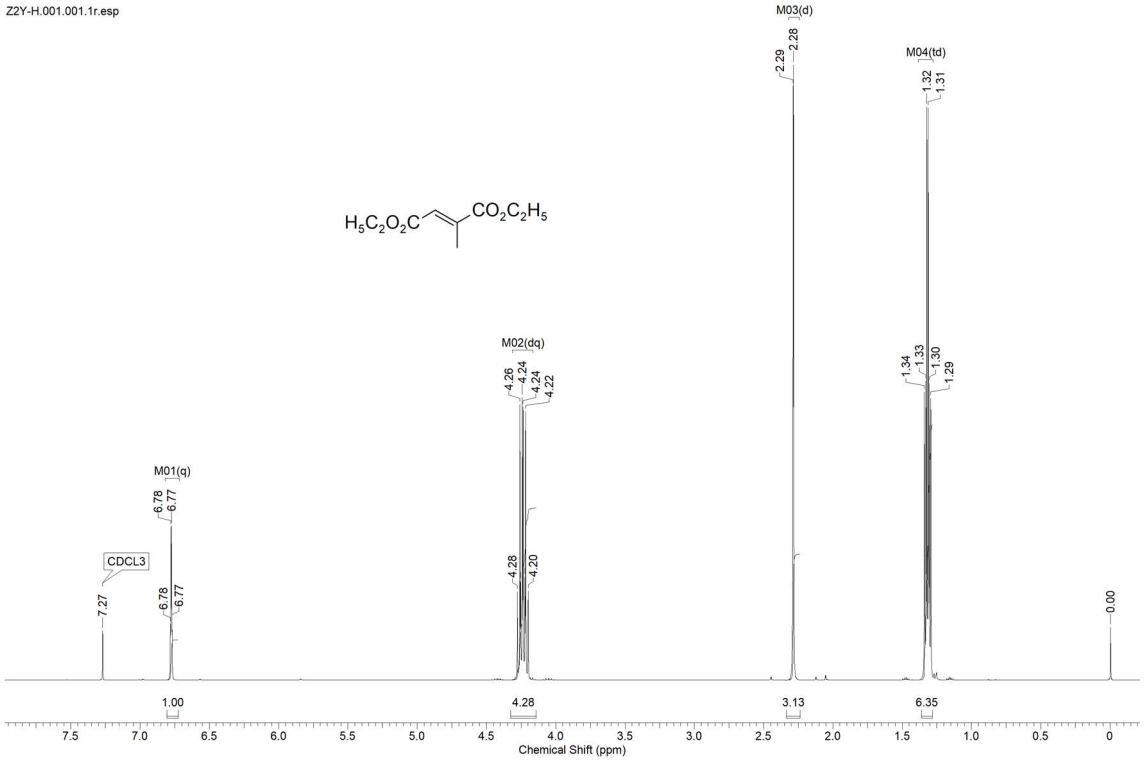


Figure S19. ^{1}H-NMR (400MHz) spectra of substrate diethyl mesaconate in $CDCl_3$.

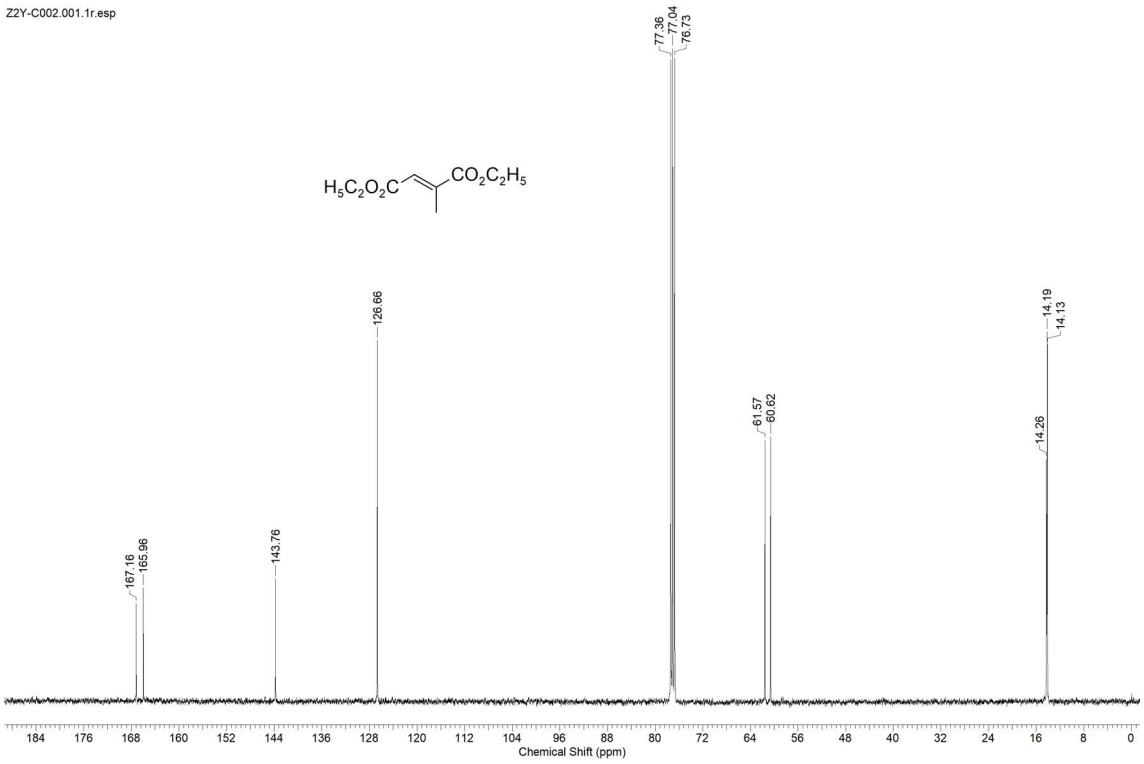


Figure S20. ^{13}C-NMR (100MHz) spectra of substrate diethyl mesaconate in $CDCl_3$.

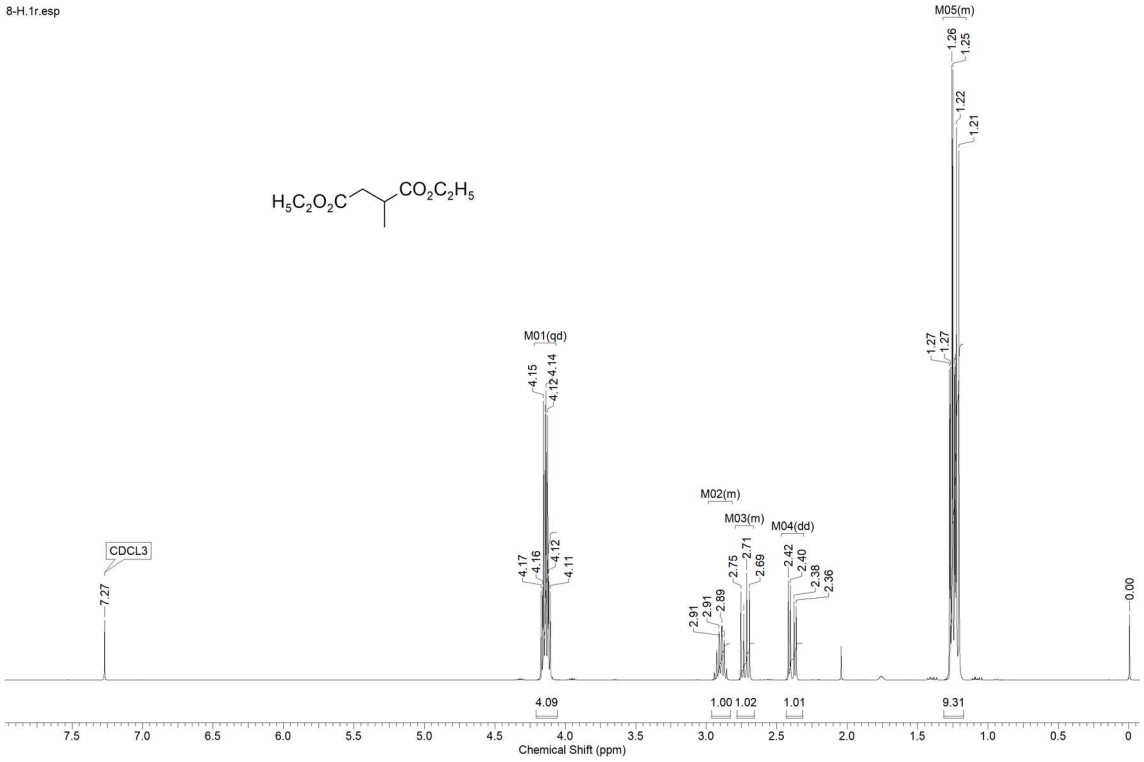


Figure S21. ^{1}H-NMR (400MHz) spectra of products diethyl methylsuccinate in $CDCl_3$.

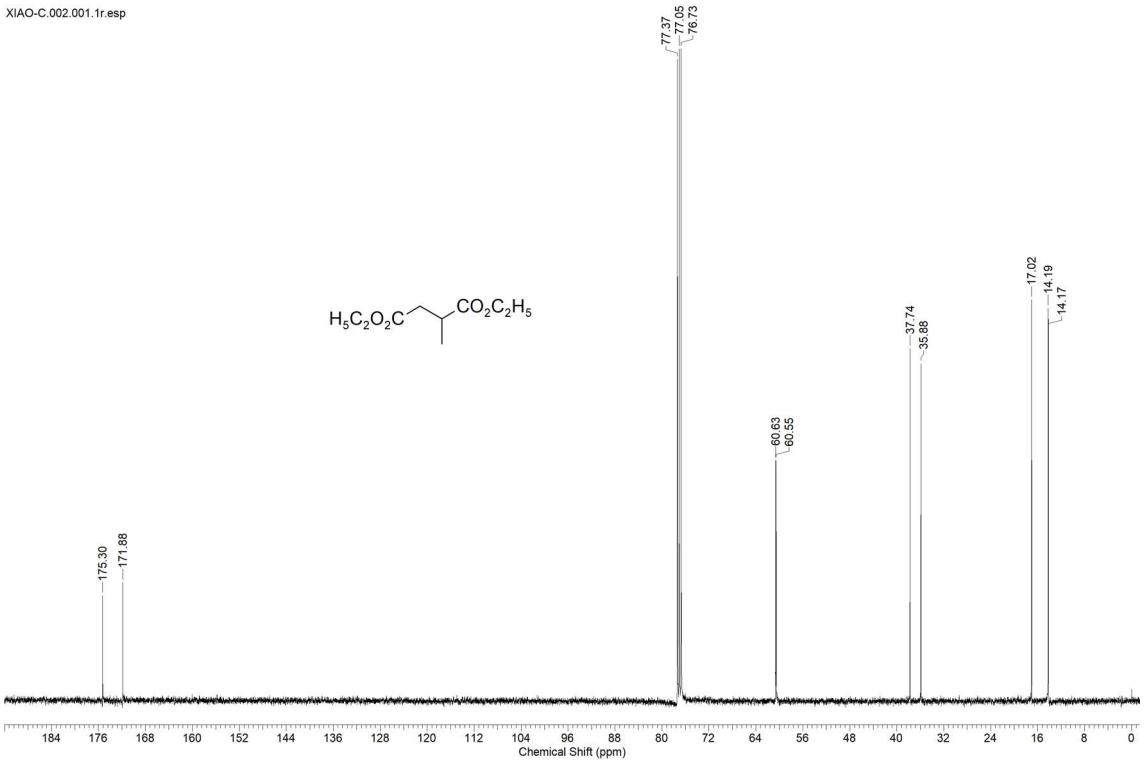


Figure S22. ^{13}C-NMR (100MHz) spectra of products diethyl methylsuccinate in $CDCl_3$.

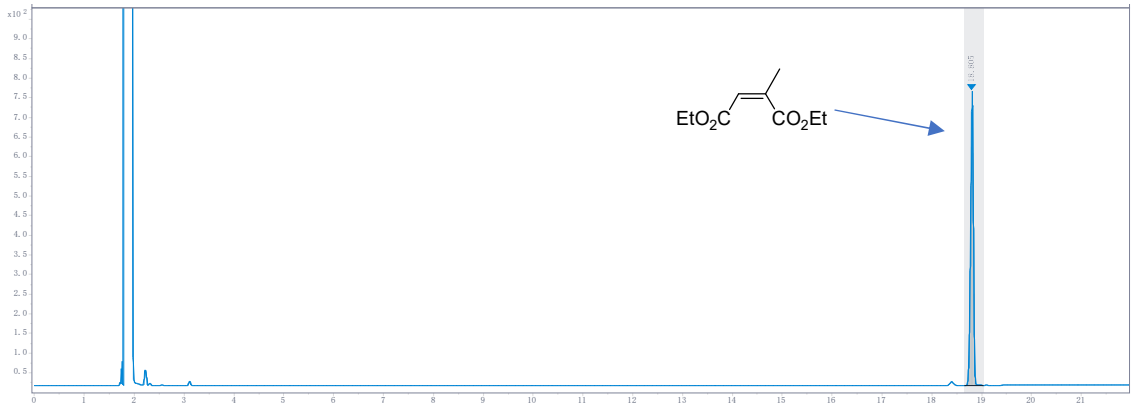


Figure S23. GC chromatogram of substrate diethyl citraconate.

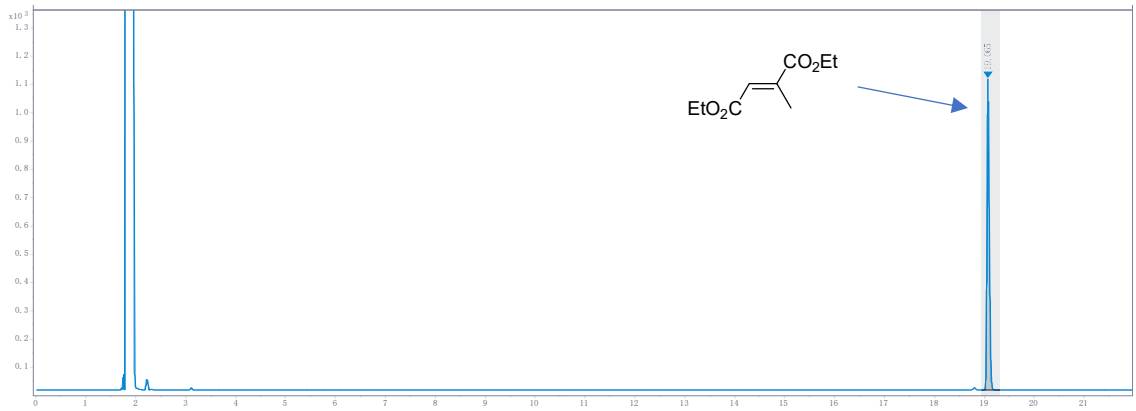


Figure S24. GC chromatogram of substrate diethyl mesaconate.

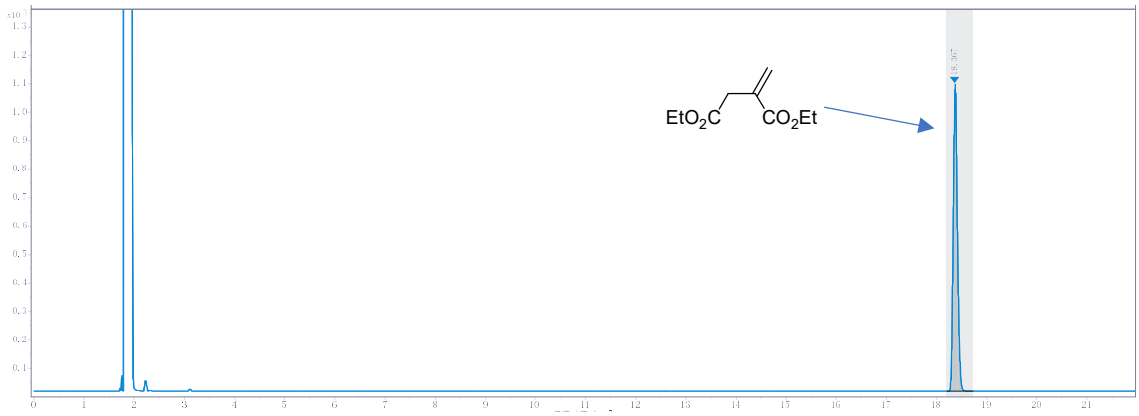


Figure S25. GC chromatogram of substrate diethyl itaconate.

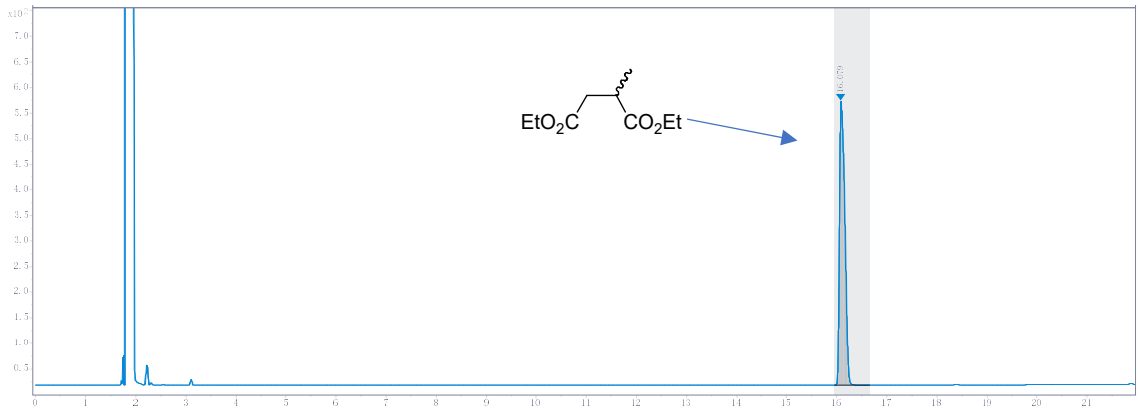


Figure S26. GC chromatogram of racemic diethyl 2-methylsuccinate.

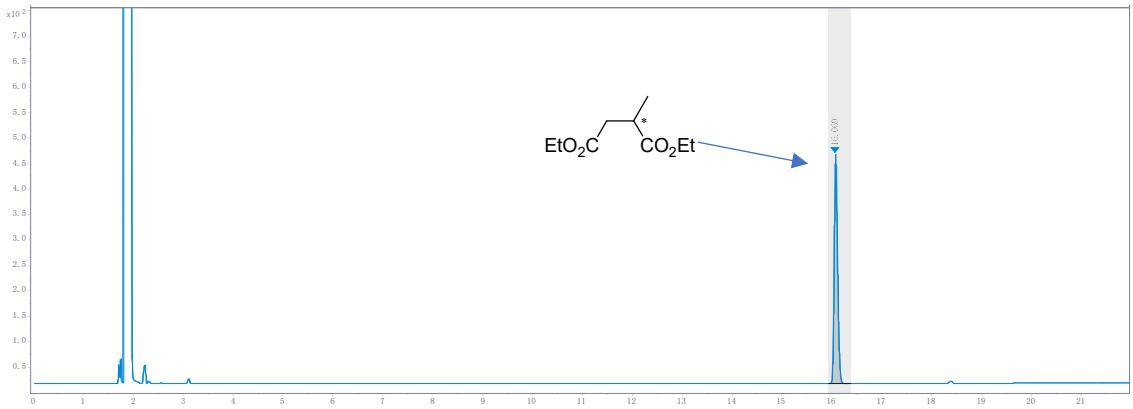


Figure S27. GC chromatogram of the analytical reaction product of Bac-OYE1 towards diethyl citraconate.

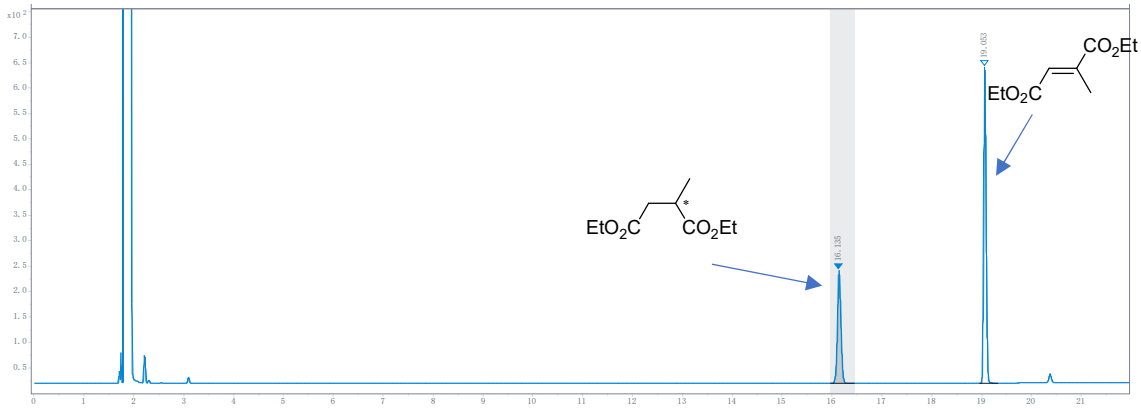


Figure S28. GC chromatogram of the analytical reaction product of *Af*ER towards diethyl mesaconate.

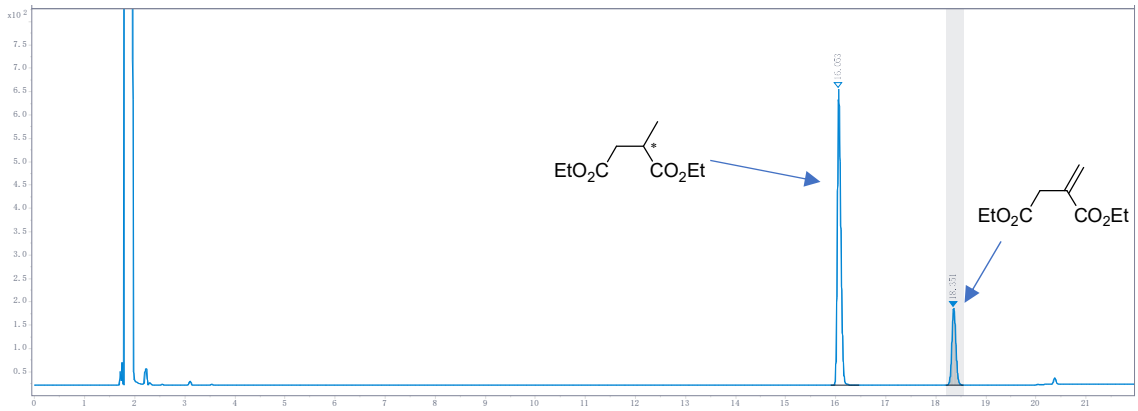


Figure S29. GC chromatogram of the analytical reaction product of *Af*ER towards diethyl itaconate.

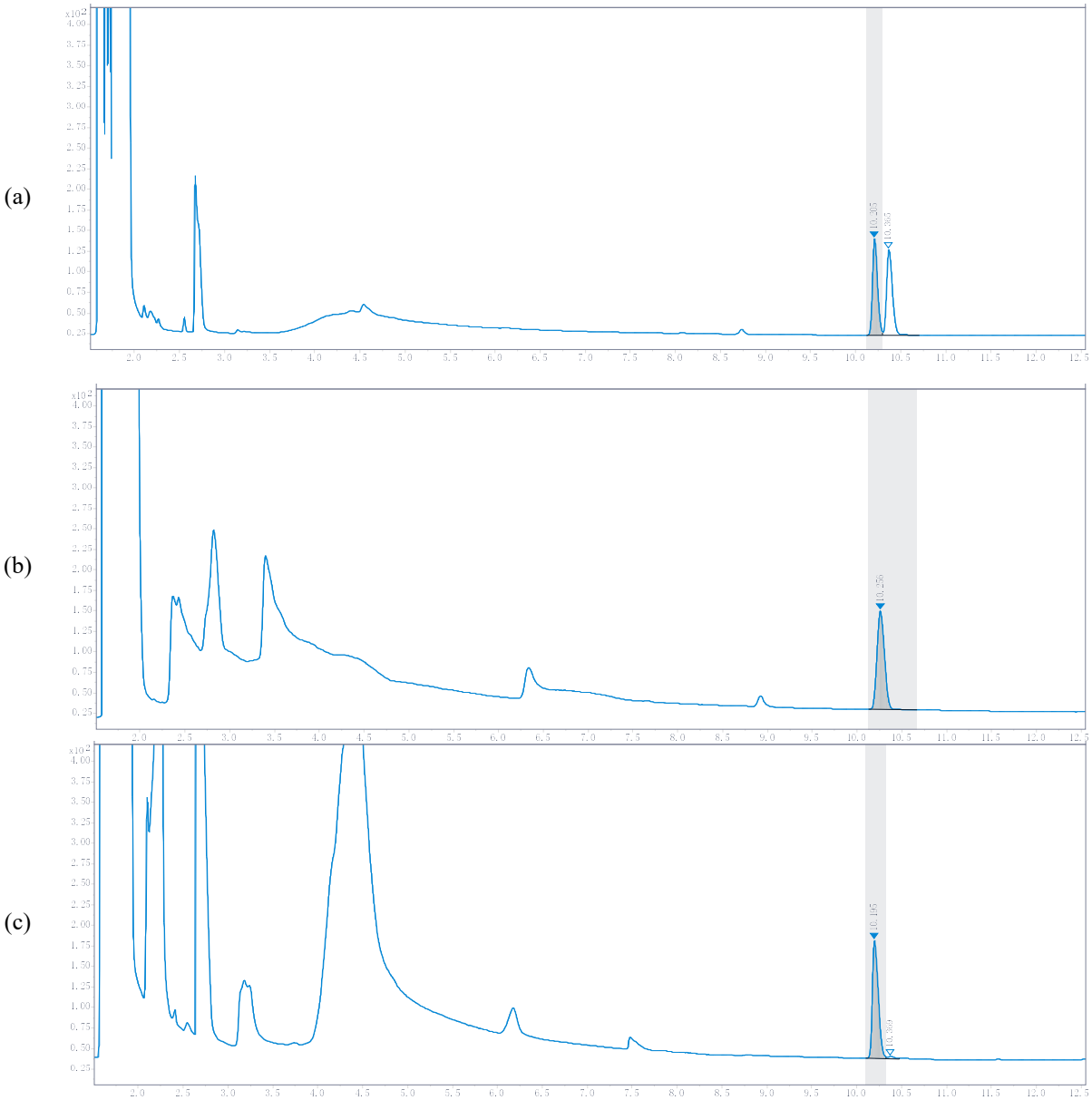


Figure S30. GC chromatograms of racemic dimethyl 2-methylsuccinate (a), analytical reaction products of Bac-OYE1 (b) and *Af*ER (c) towards diethyl citraconate after hydrolyzation and esterification to corresponding dimethyl esters.

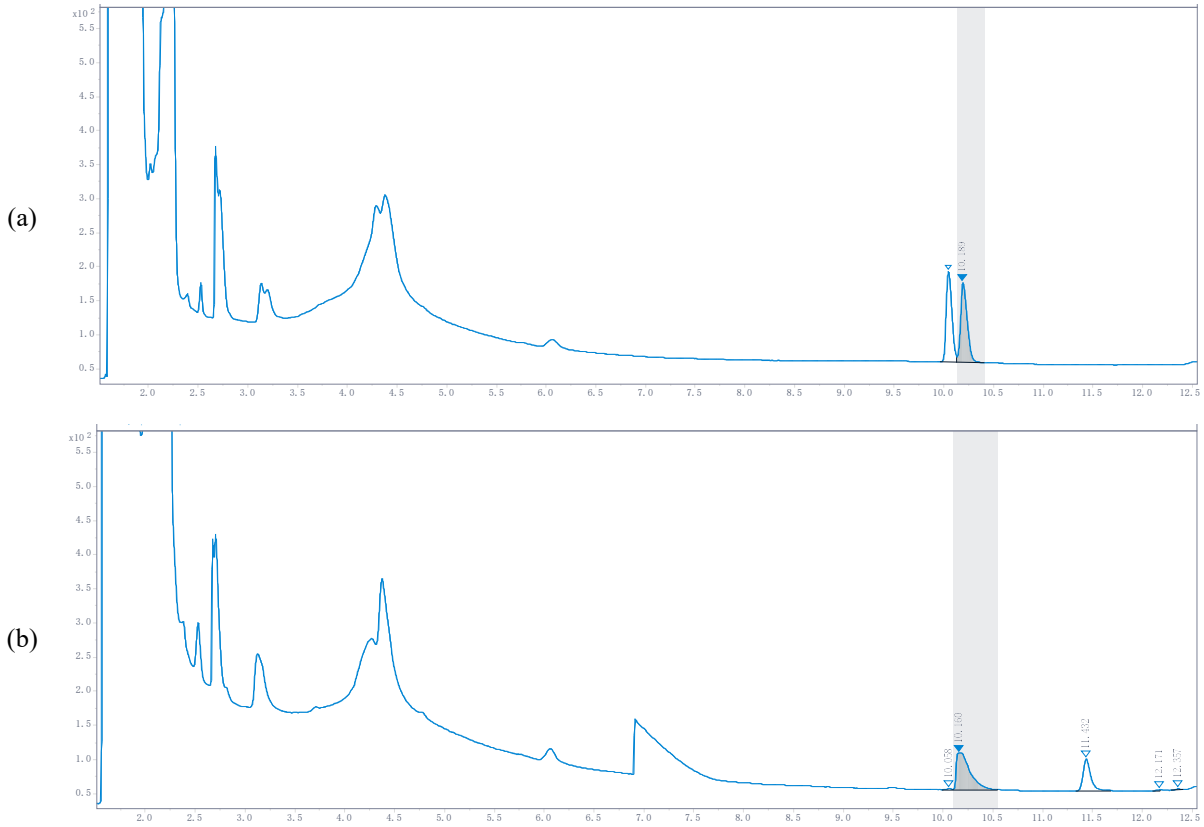


Figure S31. GC chromatograms of racemic dimethyl 2-methylsuccinate (a), analytical reaction products of *Af*ER towards diethyl mesaconate after hydrolyzation and esterification to corresponding dimethyl esters (b).

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