Supplementary Material:

Structure-guided evolution of carbonyl reductase for efficient biosynthesis of (*R*)-ethyl 2-hydroxy-4-phenylbutyrate

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Experiments:

Asymmetric reduction of ketone substrates

The reaction condition was described as following: 100 mM of substrate (2'-Fluoroacetophenone, 2'-Bromoacetophenone, 4'-Chloroacetophenone, 3',5'-Bis(trifluoromethyl)acetophenone) or 10 mM of substrate (Ethyl benzoylformate, 2-oxo-4-phenylbutyric,(4S)-3-[5-(4-fluorophenyl)-1,5-dioxophentyl]-4-phenyl-1,3-oxazolidin-2-one), NAD^+(0.2 mM), D-glucose (1.5 equiv.), lyophilized *E. coli* cells co-expressing $GDH_{E170K/Q252L}$ and mut-W193L/C93I/I187L (27.5 g/L). The reactions were conducted for 24 h at 30^{o}C. The conversion and stereoselectivity were determined by HPLC spectra according to previous studies [1-5].

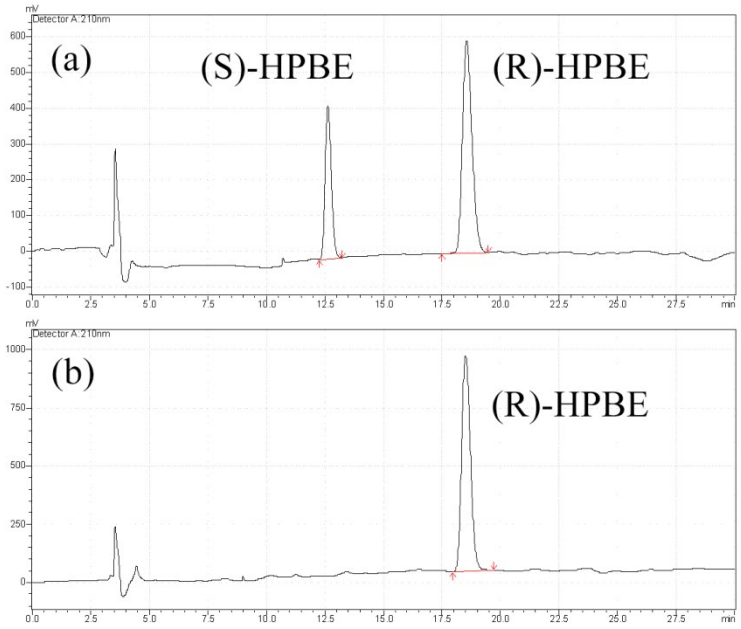


Fig. S1. Enantioselectivity determination of (a) *Go*CR and (b) mut-W193L/C93I/I187L toward OPBE.

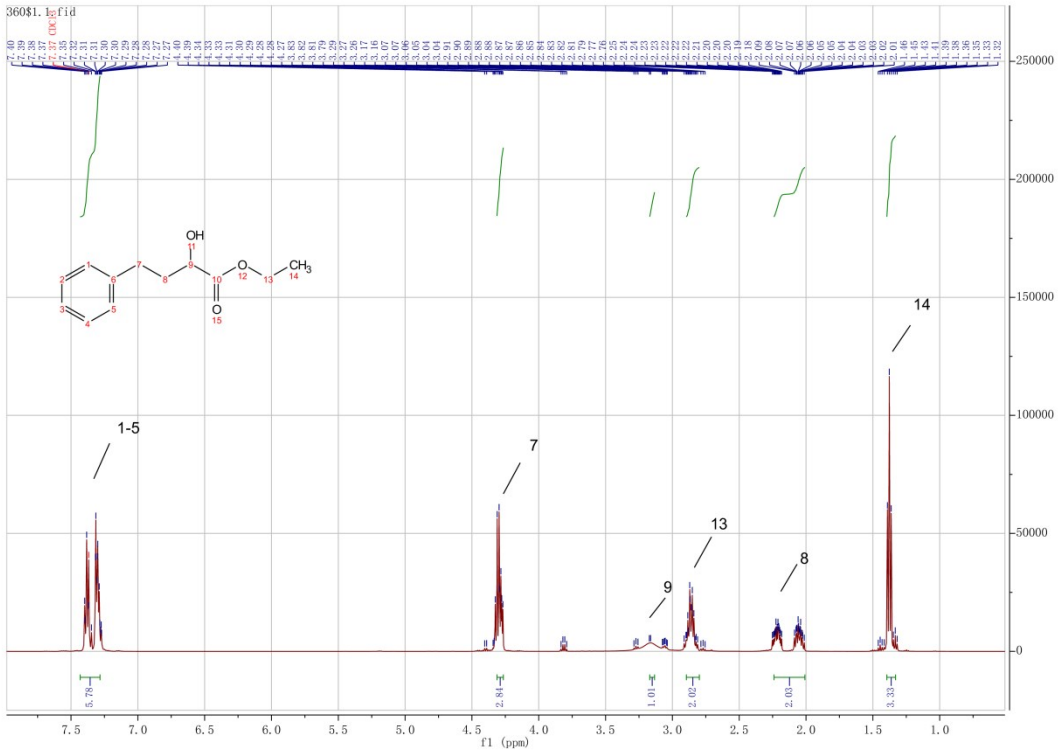


Fig. S2. ^{1}H NMR analysis of (*R*)-HPBE produced by mut-W193L/C93I/I187L .

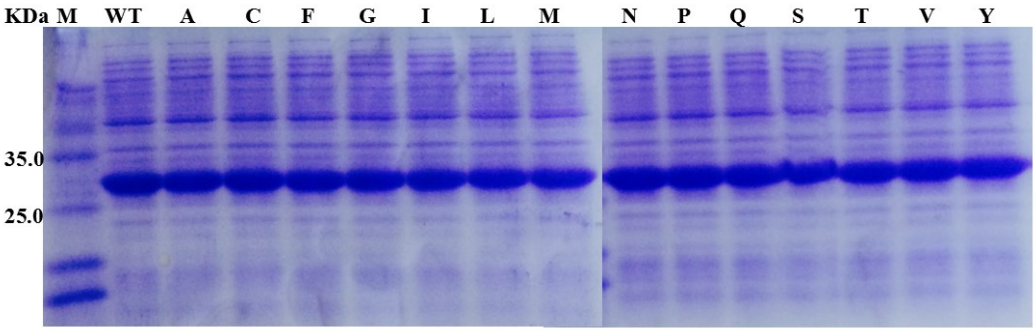


Fig. S3. SDS-PAGE analysis of supernatant of the 193 site mutants. The expected molecular weight (MW) of mutants were around 28 kDa.

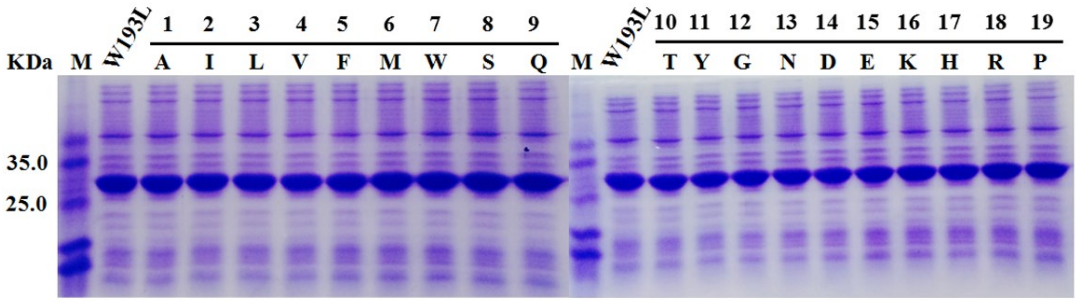


Fig. S4. SDS-PAGE analysis of supernatant of the 93 site mutants. The expected molecular weight (MW) of mutants were around 28 kDa.

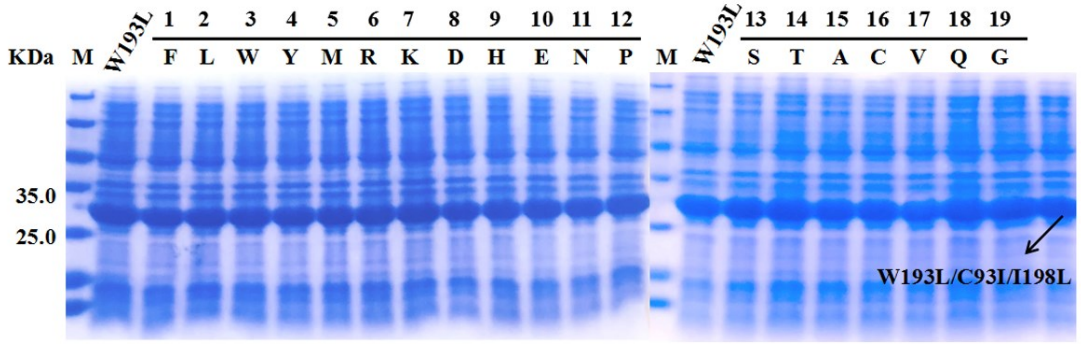


Fig. S5. SDS-PAGE analysis of supernatant of the 187 site mutants and mut-W193L/C93I/I187L. The expected molecular weight (MW) of mutants were around 28 kDa.

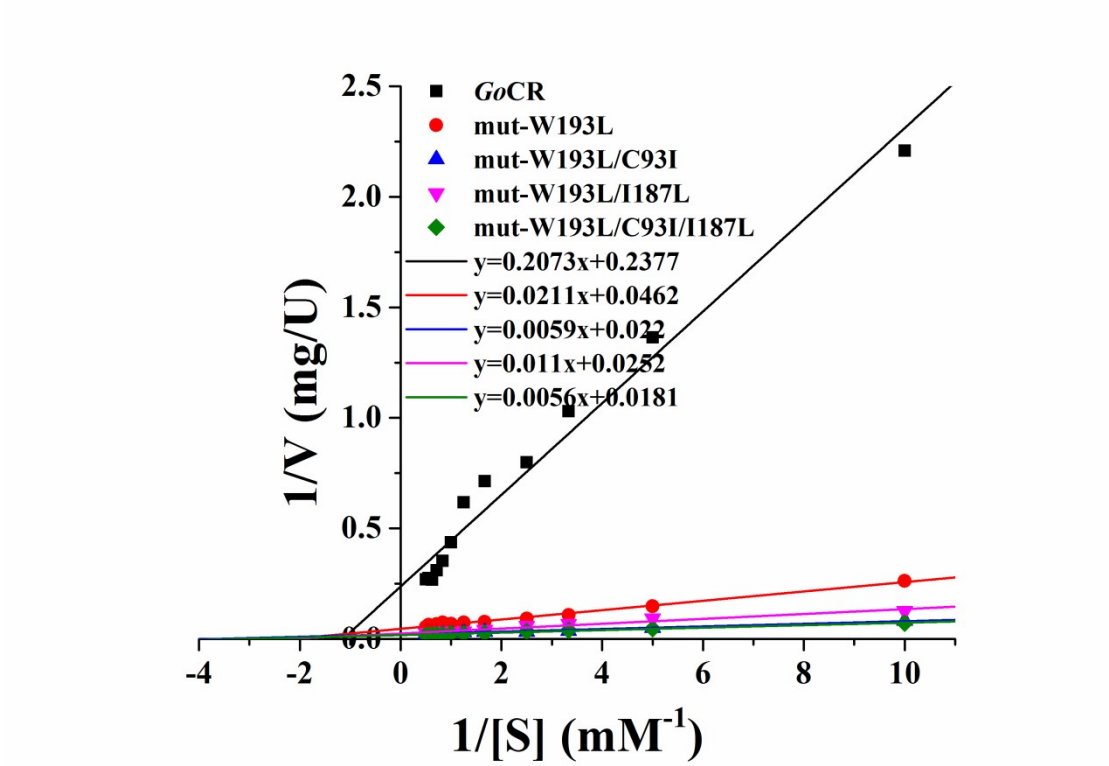


Fig. S6. Lineweaver-Burk plots of *Go*CR and its variants.

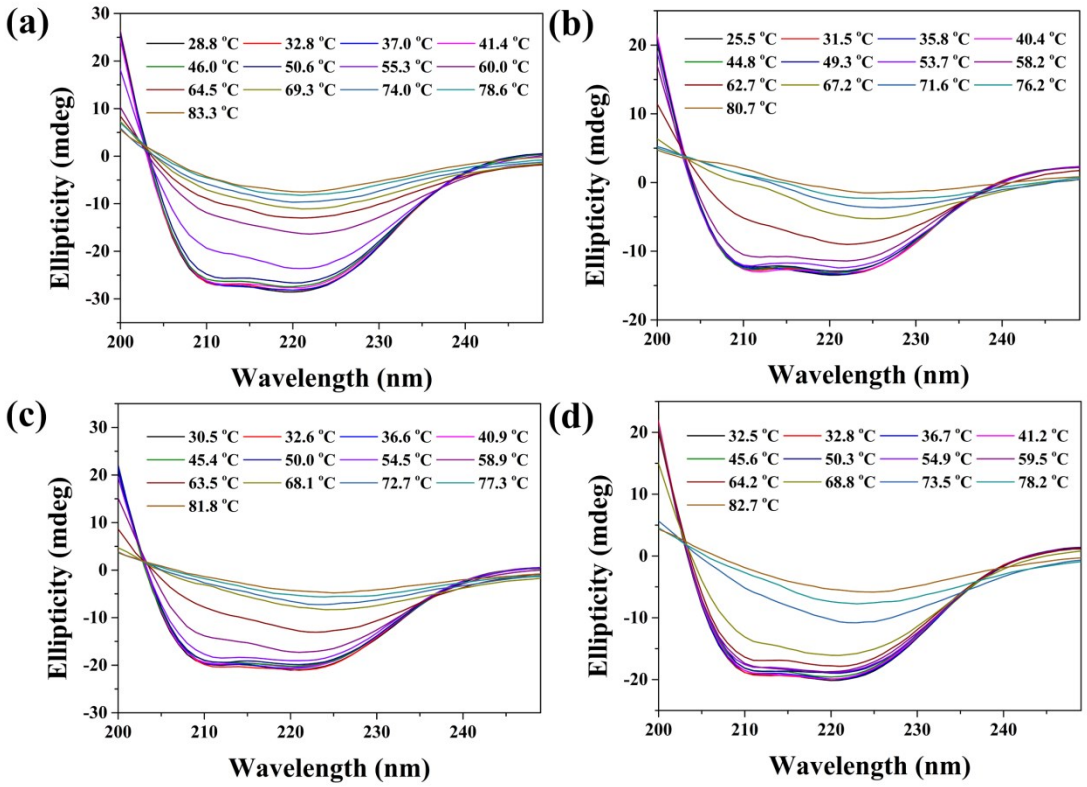


Fig. S7. CD analysis of *Go*CR variants at different temperatures. (a) mut-W193L, (b) mut-W193L/C93I, (c) mut-W193L/I187L and (d) mut-W193L/C93I/I1187L.

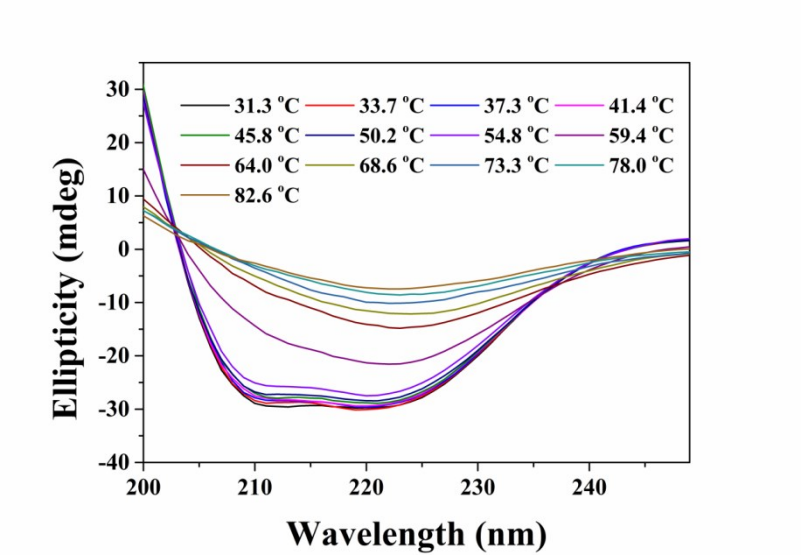


Fig. S8. CD analysis of wild-type *Go*CR at different temperatures.

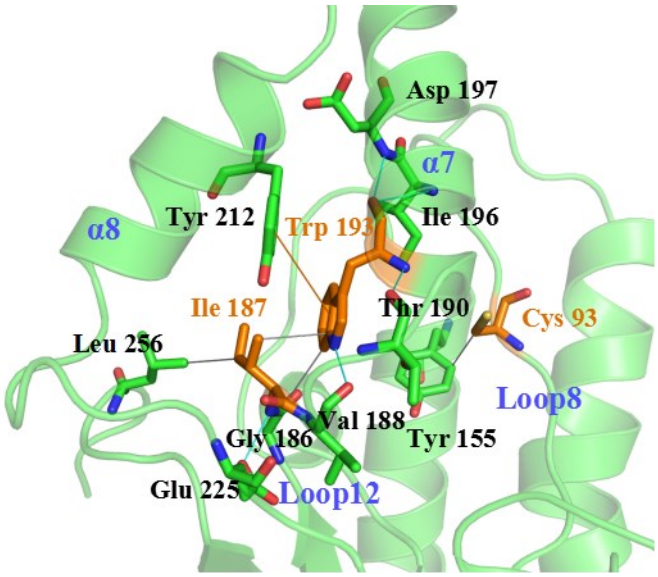


Fig. S9. Local residue interactions of wild-type *Go*CR predicted by the RING 2.0 web server. The hydrogen bonds, Van der Waals's interactions and pi-pi stack are indicated by blue, gray and orange lines, respectively.

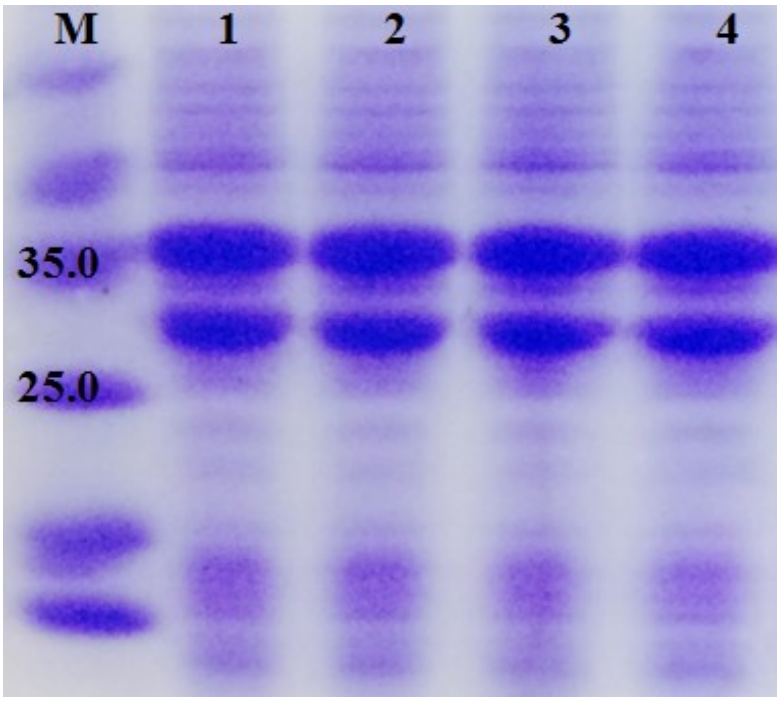


Fig. S10. The SDS-PAGE analysis of co-expression system in *E. coli* cell harboring $GDH_{E170K/Q252L}$ and *Go*CR variants. Lane 1: mut-W193L, lane 2: mut-W193L/C93I, lane 3: mut-W193L/I187L and lane 4: mut-W193L/C93I/I187L.

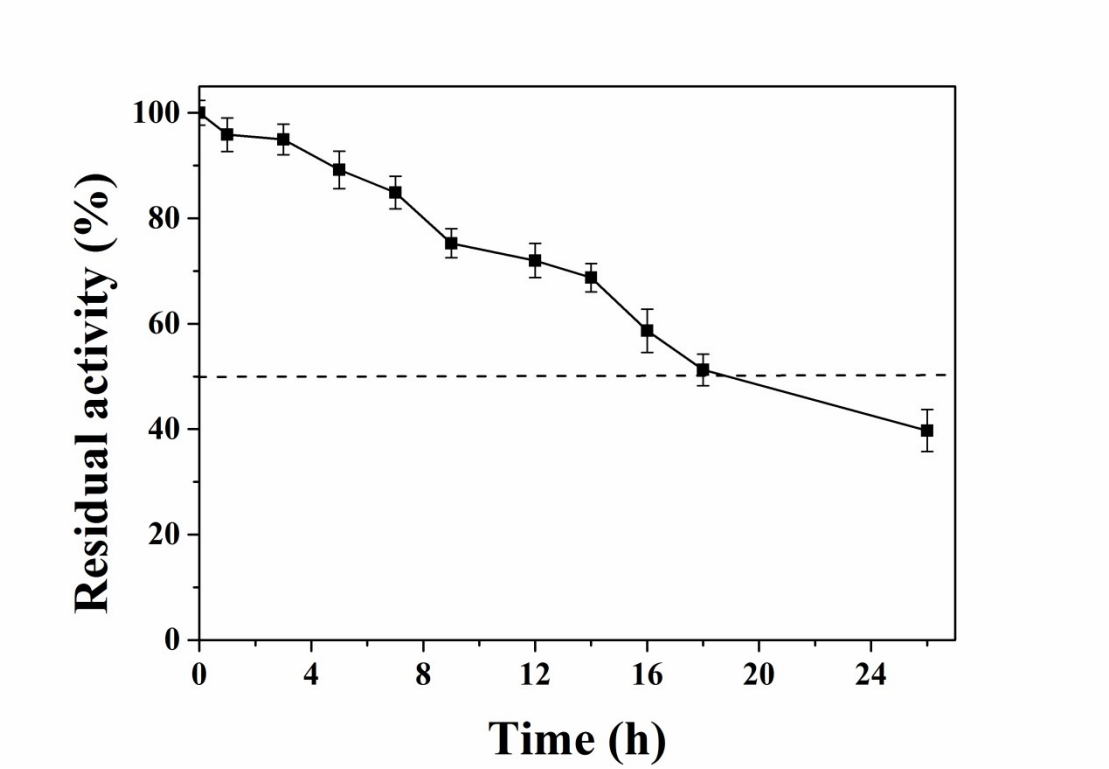


Fig. S11. Thermostability of purified mut-W193L/C92I/I187L at 40°C.

Table S1. The primers for mutagenesis at 193 site, 93 site and 187 site.

Primer name	Primer sequence[a]
193-F	5’-NNKGTCACGATCGACAAGCGCATGGCCGAA-3’
193-R	5’-CATGTCGGTTCCGACAATGCCGGGGCAGTA-3’
93-F	5’-ATCNNKCAGGTCAAGCCGATCCTGG-3’
93-R	5’-CCCCGCATTGTTGACCATGATGTCC-3’
187-F	5’-GGCNNKGTCGGAACCGACATGCTTG-3’
187-R	5’-GGGGCAGTAGGAATTGACGGTAATG-3’

[a] NNK represented degenerate bases.

Table S2. The primers used in the construction of recombinant plasmids.

Entry	Primer name	Primer sequence[a]
1	**GDH-F**	5’-GGAATTCATGTATCCGGATTTAAAAGGAAAAG-3’
2	**GDH-R**	5’-CGAGCTCTTAACCGCGGCCTGCC-3’
3	**mutant-F**	5’-CGGAATTCATGTCCCTTTCTGGAAAAATCG-3’
4	**mutant-R**	5’-CCCAAGCTTTCAGCGGAAAACGAGACC-3’
5	**T7-GDH-F**	5’-CCCAAGCTTTAATACGACTCACTATAGGGGAAT-3’
6	**T7-GDH-R**	5’-CCTCGAGTTAACCGCGGCCTGCCTG-3’

[a] The underlined bases are restriction sites.

Table S3. The distances between key residues of *Go*CR or its variants and OPBE.

Entry	Mutants	Distance 1[a] (Å)	Distance 2[b] (Å)	Distance 3[c] (Å)
1	***Go*CR**	4.4	3.8	5.5
2	**W193L**	3.8	3.2	4.0
3	**W193L/C93I**	3.6	2.5	3.3
4	**W193L/I187L**	3.5	2.9	2.7
5	**W193L/C93I/I187L**	3.3	2.5	3.2

[a] the distance between the stereogenic carbon atom of OPBE and a hydrogen from the C4 atom of the nicotinamide ring of NADH.

[b] the distance between the carbonyl oxygen atom of OPBE and the hydroxyl group of Tyr155.

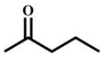
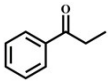
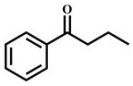
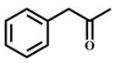
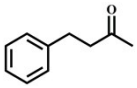
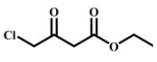
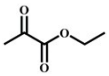
[c] the distance between carbonyl oxygen atom of OPBE and hydroxyl groups of Ser142.

Table S4. Comparison on asymmetric reduction of OPBE for the synthesis of (*R*)-HPBE

OPBE (M)	**Time (h)**	**Conv. (%)**	**Yield (%)**	**ee (%)**	**Biocatalyst**	**References**
1.8	14	100	84.2	99.2	*E. coli*/mut-W193L/C93I/I187L	This study
1.6[a]	12	>99	--	99.5	*E. coli*/IolS	[6]
1.0	24.5	97.1	88.0	>99	*Pichia pastoris*/CgKR2	[7]
1.0	6	>99	84	>99	*E. coli*/pCgKR2	[8]
0.4	48	>99	--	87.5	*Saccharomyces cerebisiae*	[9]
0.10	16	--	82	97.4	*Candida krusei* SW2026	[10]
0.02	12	--	92	99	*Candida boidinii* CIOC21	[11]
0.02	24	<100	--	>99	*E. coli*/PpADH	[12]

[a] feeding batch

Table S5. Substrate spectrum of *Go*CR and mut-W193L/C93I/I187L toward various ketones.

Entry	Substrate		Specific activity (U/mg)	
			***Go*CR**[13]	**mut-W193L/C93I/I187L**
1	Acetone		0.78 ± 0.06	0.94 ± 0.23
2	2-Butanone		0.96 ± 0.12	1.93 ± 0.44
3	2-Pentanone		7.48 ± 0.25	7.14 ± 0.36
4	Acetophenone		1.29 ± 0.15	5.21 ± 0.32
5	Propiophenone		1.76 ± 0.18	6.29 ± 0.45
6	Butyrophenone		2.44 ± 0.14	7.41 ± 1.27
7	1-Phenyl-2-propanone		3.53 ± 0.23	6.72 ± 0.72
8	4-Phenyl-2-butanone		8.89 ± 0.41	14.78 ± 1.16
9	Ethyl 4-chloro-3-oxobutanoate		2.82 ± 0.22	4.63 ± 0.42
10	Acetoin		4.34 ± 0.14	5.71 ± 0.56
11	Ethyl pyruvate		6.7 ± 0.25	7.0 ± 1.45
12	2, 3-Pentanedione		39.8 ± 0.33	47.3 ± 2.76
13	2, 3-Butanedione		121.2 ± 0.36	28.9 ± 1.93

14	Acetaldehyde		0 ± 0.01	N.A.[a]

[a] No measurable activity.

References

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[11] Y. Chen, H. Lin, X. Xu, S. Xia, L. Wang, Preparation the key intermediate of angiotensin-converting enzyme (ACE) inhibitors: high enantioselective production of ethyl (*R*)-2-hydroxy-4-phenylbutyrate with *Candida boidinii* CIOC21, Adv. Synth. Catal. 350 (2008) 426-430.

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[13] X. Liu, R. Chen, Z.W. Yang, J.L. Wang, J.P. Lin, D.Z. Wei, Characterization of a putative stereoselective oxidoreductase from gluconobacter oxydans and its application in producing ethyl (*R*)-4-chloro-3-hydroxybutanoate ester, Mol. Biotechnol. 56 (2014) 285-295.