Supporting Information

Characterization of a Carbonyl Reductase from *Rhodococcus erythropolis* WZ010 and Its Variant Y54F for Asymmetric Synthesis of (*S*)-*N*-Boc-3-Hydroxypiperidine

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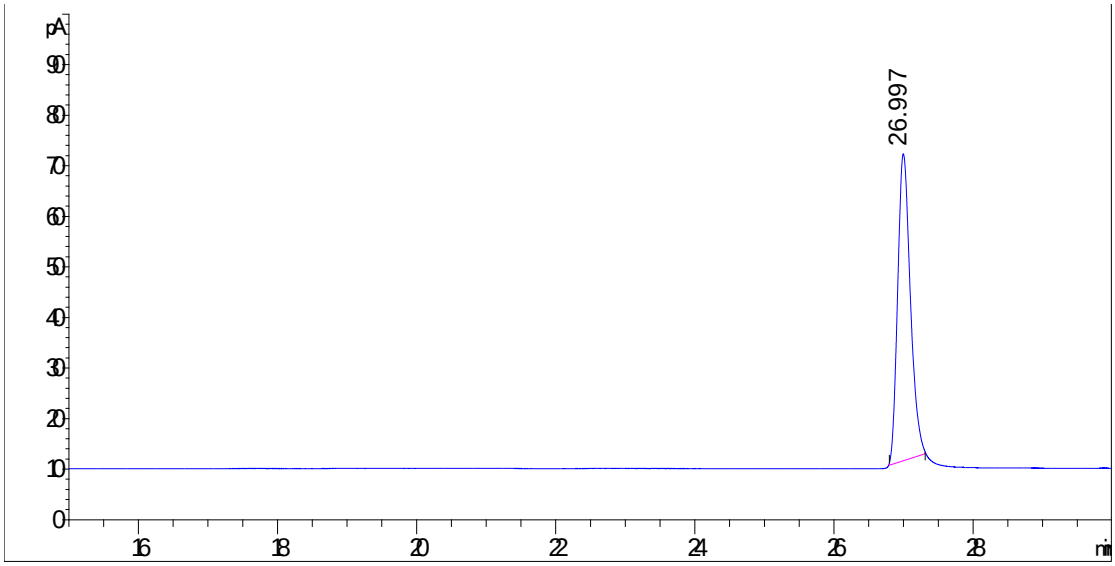
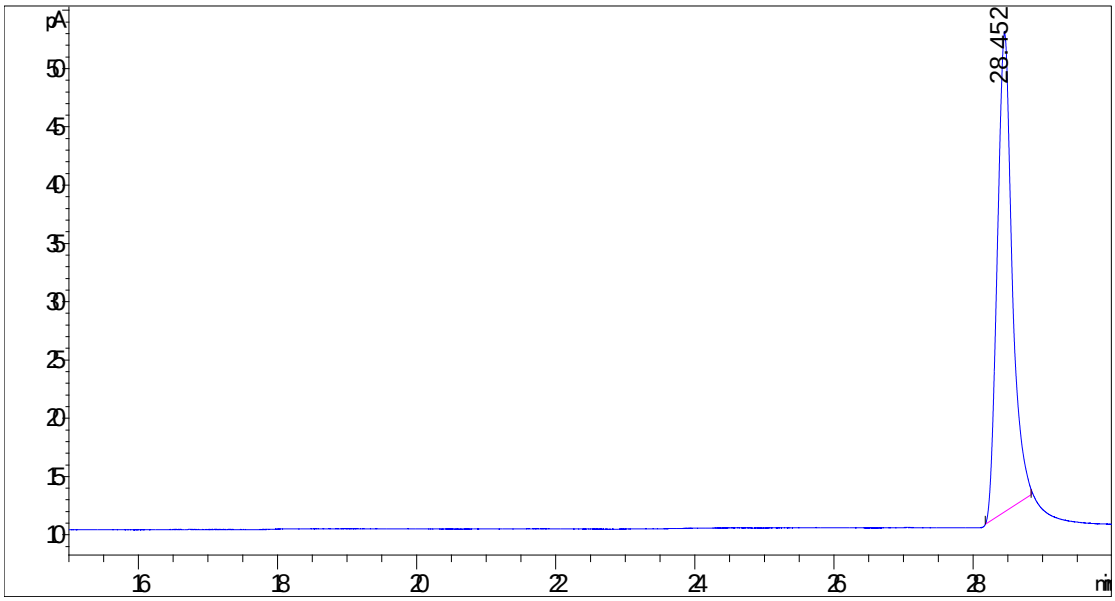


A



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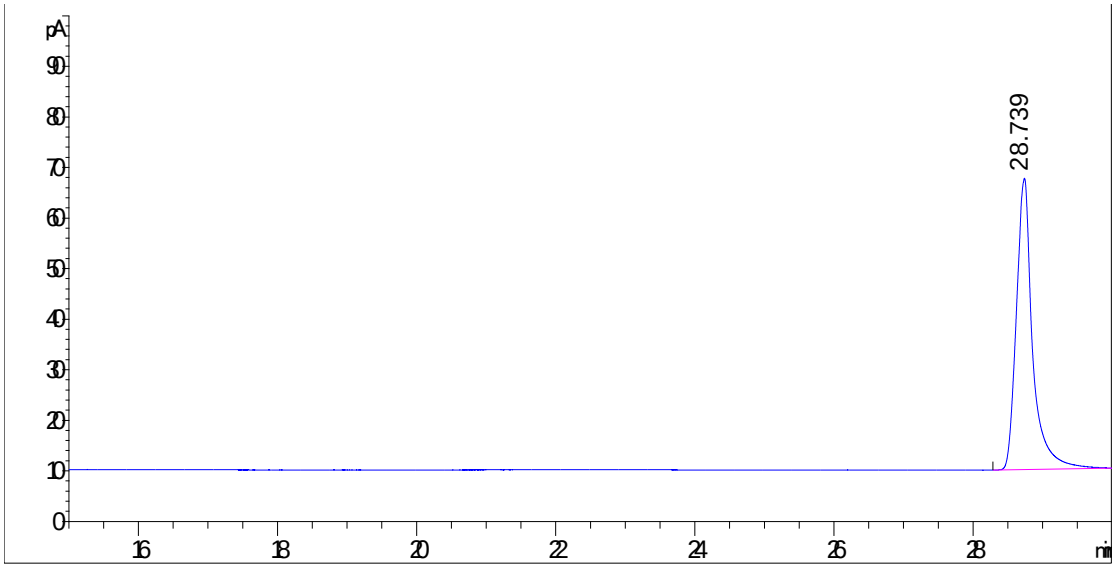

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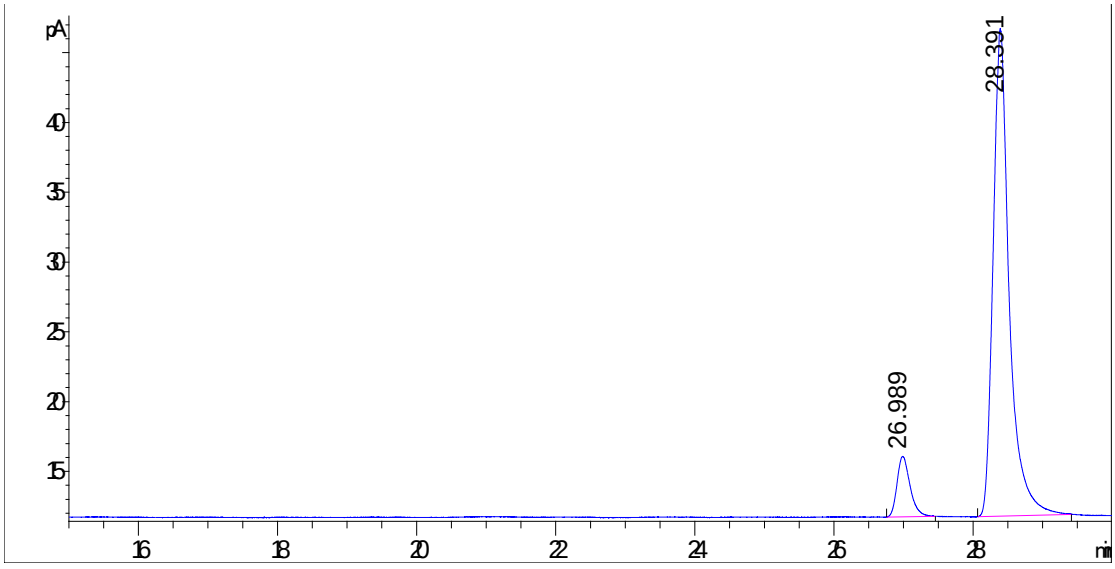


D

Figure S1. Gas **c**hromatograph analysis for standards *N*-Boc-3-piperidone (A), (*S*)-*N*-Boc-3-hydroxypiperidine and (*R*)-*N*-Boc-3-hydroxypiperidine. Figure 1D represents a typical chromatograph of the formation of (S)-*N*-Boc-3-hydroxypiperidine (retention time 28.391 min) from the reduction of *N*-Boc-3-piperidone (26.989).

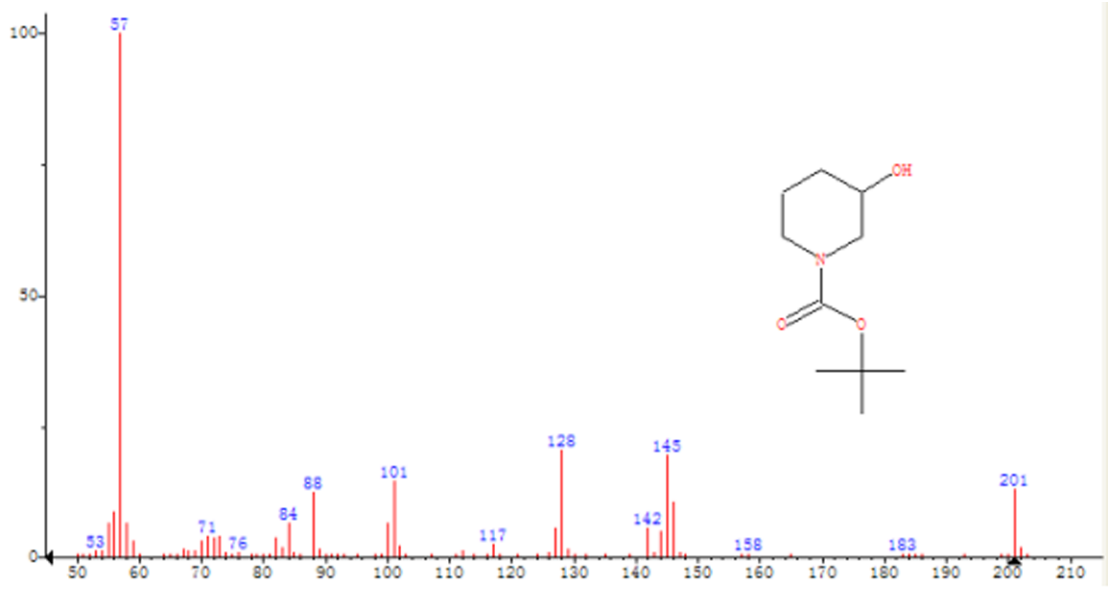
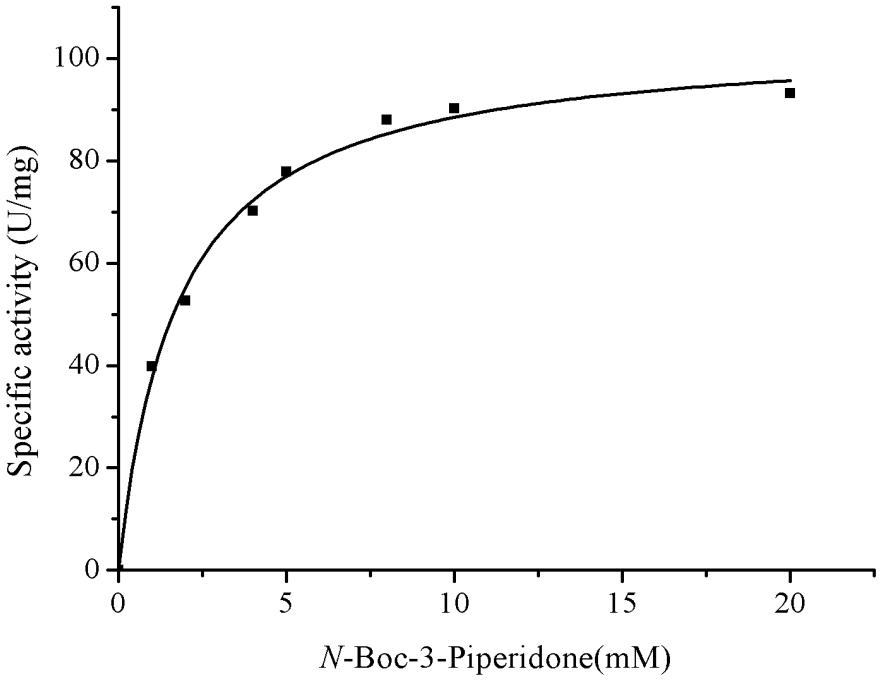
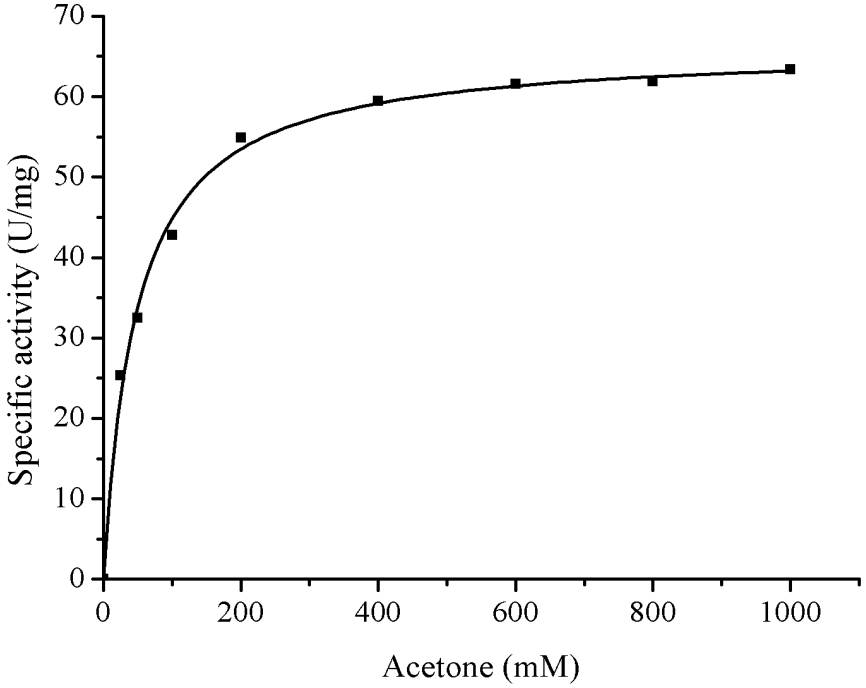


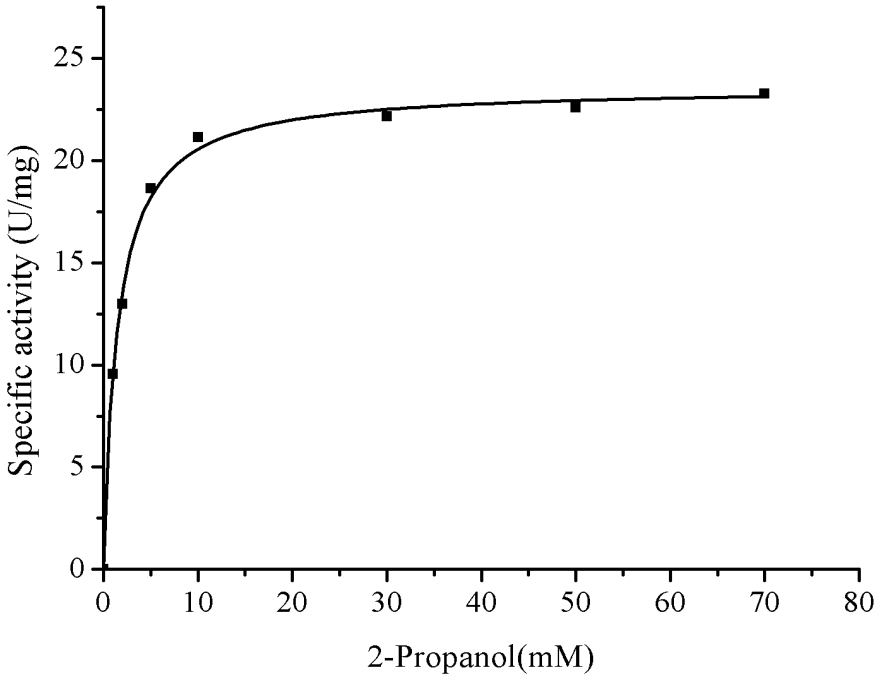
Figure S2. Gas chromatograph-mass spectrometry analysis of (*S*)-*N*-Boc-3-hydroxypiperidine in asymmetric reduction of *N*-Boc-3-piperidone. We analyzed the reaction product by using gas chromatography mass spectrometry (GC–MS; Agilent7890A/5975C, Agilent Technologies, USA). The analysis method of (S)-*N*-Boc-3-hydroxypiperidine comprised the following steps: GC column, BGB174; injection port temperature, 250°C; injection volume, 1 μL; split ratio, 50:1; column flow rate, 1 mL/min; column oven temperature, 5 ℃/min from 100 ℃ to 125 ℃, hold 3 min; 2 ℃/min to 140 ℃, hold 8 min; 1 ℃/min to 150 ℃; auxiliary heating zone temperature, 250 ℃; MS quadrupole temperature,150 ℃; ion source temperature, 230 ℃; scan quality range, 30–500 amu; emission current, 200 μA; and electron energy, 70 eV.


Specific activity (U/mg)
100
80
60
40
20
0
0
5
10
15
20
N-Boc-3-Piperidone(mM)

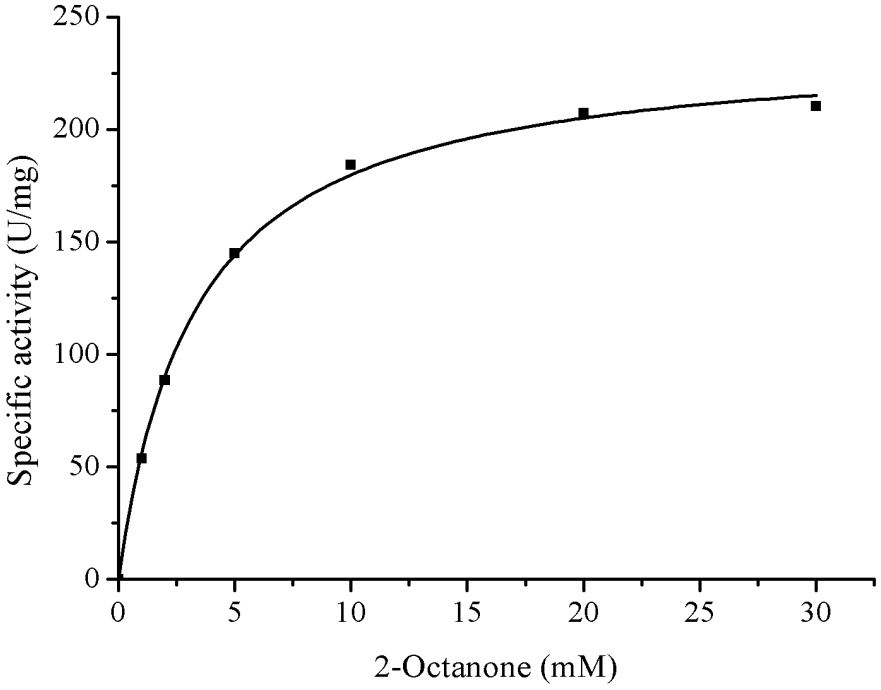
A


Specific activity (U/mg)
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
Acetone (mM)

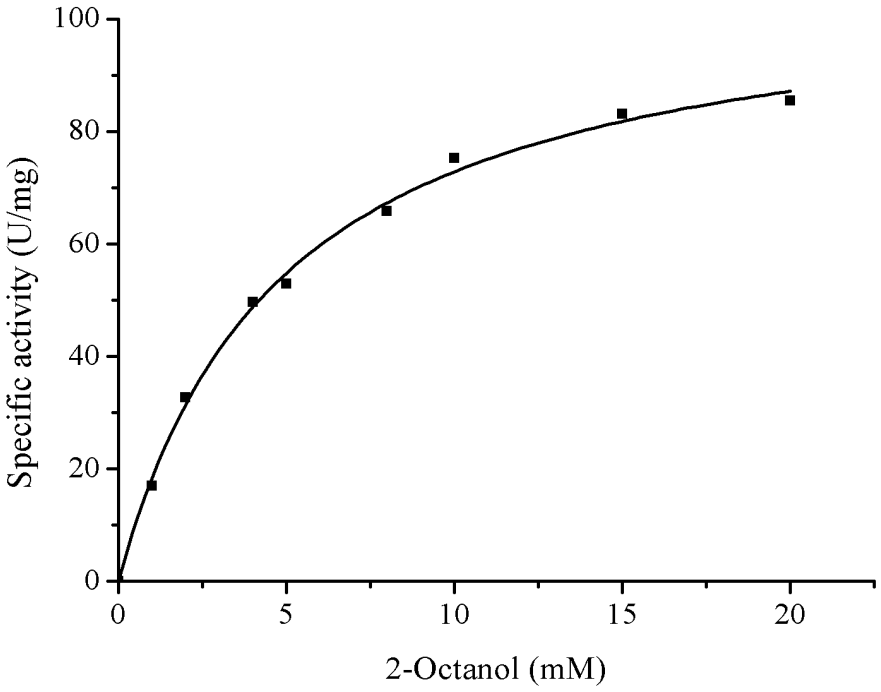
B


Specific activity (U/mg)
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
2-Propanol(mM)

C

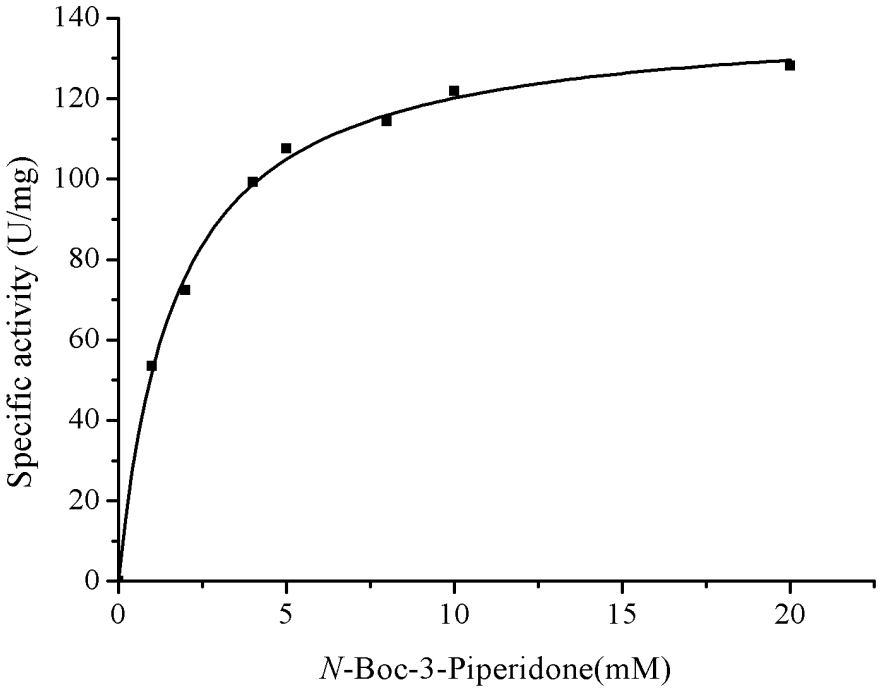

Specific activity (U/mg)
250
200
150
100
50
0
0
5
10
15
20
25
30
2-Octanone (mM)

D

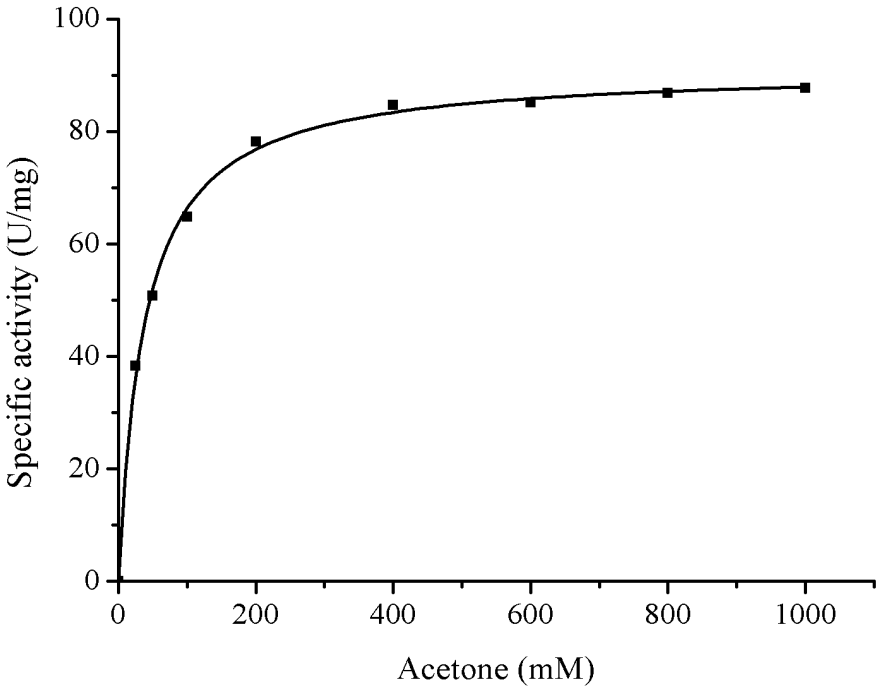


E

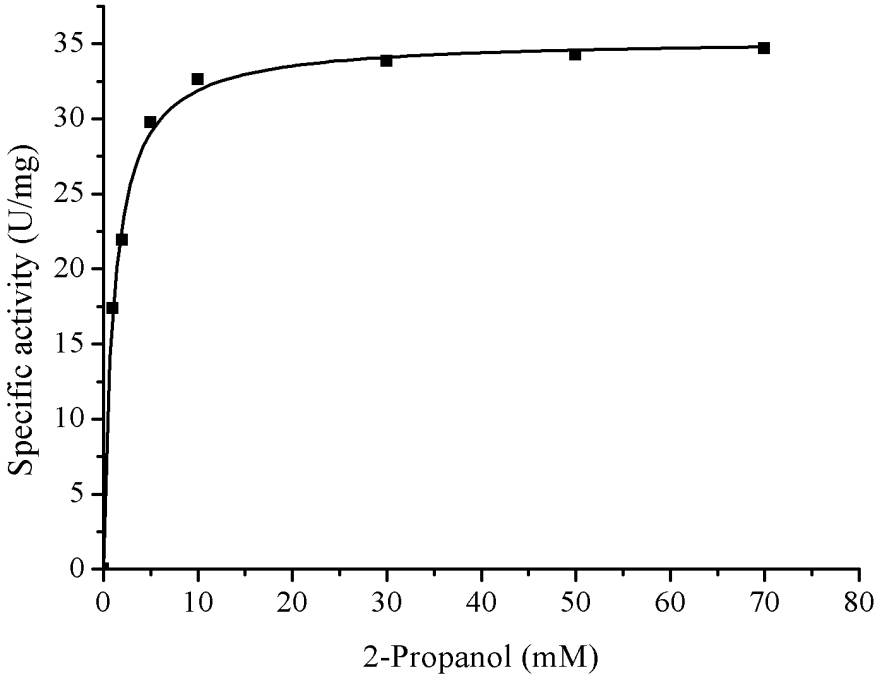
Figure S3. The Michaelis-Menten kinetics of ReCR. The tested substrates: A, *N*-Boc-3-piperidone; B, acetone; C, 2-propanol; D, 2-octanone; E, 2-octanol.


Specific activity (U/mg)
140
120
100
80
60
40
20
0
0
5
10
15
20
N-Boc-3-Piperidone(mM)

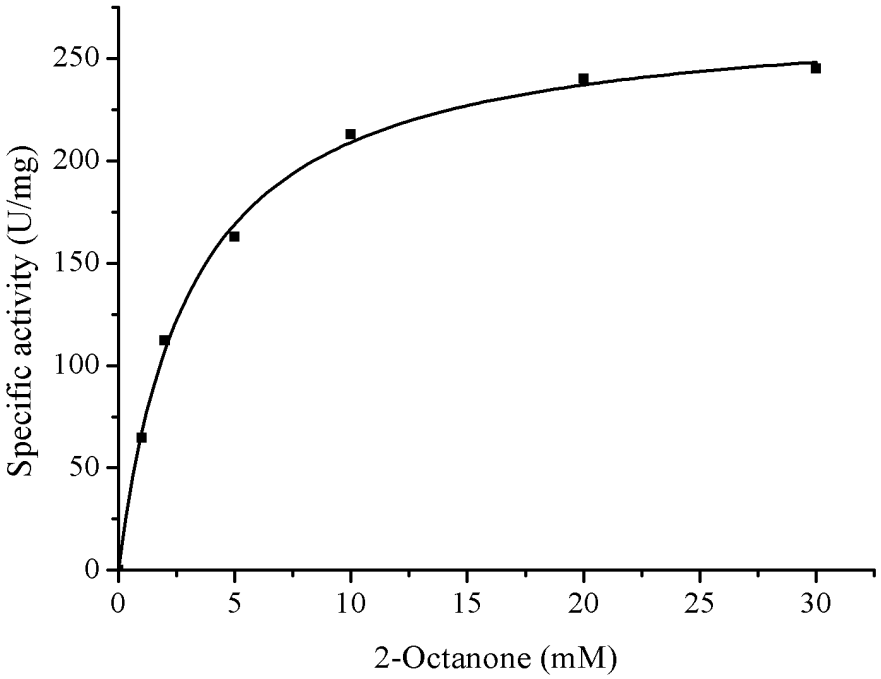
A


Specific activity (U/mg)
0
20
40
60
80
100
0
200
400
600
800
1000
Acetone (mM)

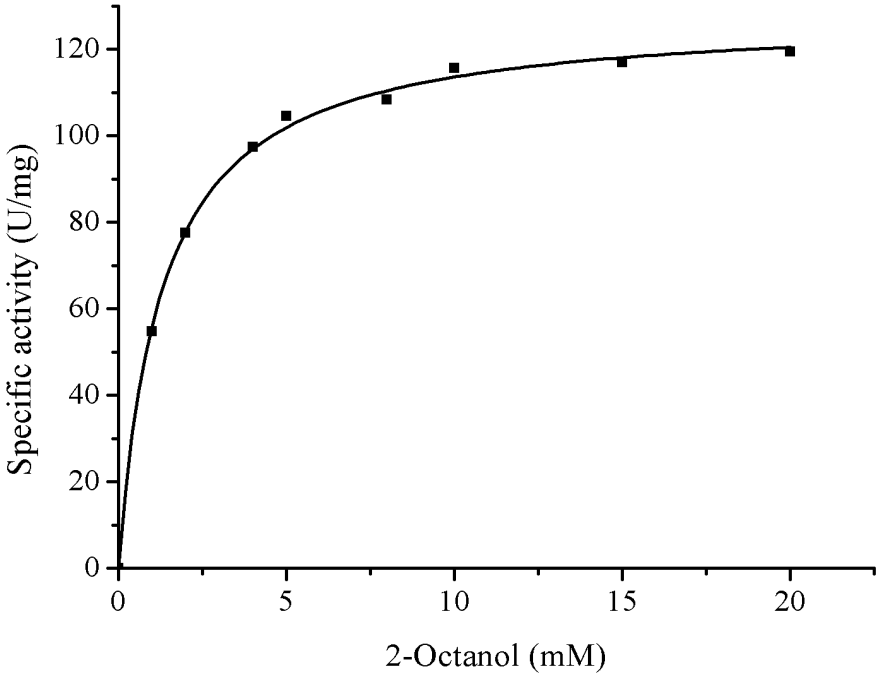
B


Specific activity (U/mg)
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
80
2-Propanol (mM)

C


Specific activity (U/mg)
250
200
150
100
50
0
0
5
10
15
20
25
30
2-Octanone (mM)

D



E

Figure S4. The Michaelis-Menten kinetics of ReCR variant Y54F. The tested substrates: A, *N*-Boc-3-piperidone; B, acetone; C, 2-propanol; D, 2-octanone; E, 2-octanol.

Supplementary table

Table S1. Effect of metal ions, EDTA, dithiothreitol and sodium iodoacetate on the activity of recombinant ReCR

Chemicals (mM)	Relative activity (%)
None	100.0[a] ± 2.6
EDTA (1)	144.4 ± 3.7
$CaCl_2$ (1)	116.1 ± 4.2
Dithiothreitol (1)	113.0 ± 1.1
$MnCl_2$ (1)	110.1 ± 0.9
KCl (1)	107.0 ± 1.8
$CoCl_2$ (1)	61.9 ± 2.1
$AlCl_3$ (1)	30.0 ± 0.5
$CuSO_4$ (1)	20.0 ± 0.7
$FeSO_4$ (1)	19.5 ± 0.2
$ZnCl_2$ (1)	13.2 ± 0.5
Sodium iodoacetate (1)	9.7 ± 0.4

[a] The enzyme activity assay was performed at 60℃ in duplicate using the assay mixture (2.5 ml) containing 10 mM *N*-Boc-3-piperidone, 0.4 mM NADH, and 50 mM PIPES buffer (pH 6.0). The relative activity of 100% represents 85.8 U/mg for *N*-Boc-3-piperidone reduction at 60℃ and pH 6.0. Data present mean values ± s.d. from two independent experiments.