Supporting Information

A chemo-enzymatic strategy for efficient synthesis of amphenicol antibiotic chloramphenicol mediated by an engineered L-threonine transaldolase with high activity and stereoselectivity

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Running title: Chemo-enzymatic strategy for synthesis of chloramphenicol

Table S1. Primers used for site-directed mutagenesis of PsLTTA.

Primer	Sequence (5'-3')
N35A F [a]	GACCGCAgcaGAAAATTATCCGAGCGC
N35A R	AATTTTCtgcTGCGGTCAGGCTCAGGC
N35S F	GACCGCAagcGAAAATTATCCGAGCGC
N35S R	AATTTTCgctTGCGGTCAGGCTCAGGC
N35C F	GACCGCAtgtGAAAATTATCCGAGCGC
N35C R	AATTTTCacaTGCGGTCAGGCTCAGGC
N35V F	GACCGCAgttGAAAATTATCCGAGCGC
N35V R	AATTTTCaacTGCGGTCAGGCTCAGGC
N35I F	GACCGCAattGAAAATTATCCGAGCGC
N35I R	AATTTTCaatTGCGGTCAGGCTCAGGC
N35G F	GACCGCAggcGAAAATTATCCGAGCGC
N35G R	AATTTTCgccTGCGGTCAGGCTCAGGC
Y55N F	GCATTTaatCATTGTAGCTTTCCGTTT
Y55N R	ACAATGattAAATGCGCCTGCGGTGCT
Y55L F	GCATTTctgCATTGTAGCTTTCCGTTT
Y55L R	ACAATGcagAAATGCGCCTGCGGTGCT
Y55T F	GCATTTaccCATTGTAGCTTTCCGTTT
Y55T R	ACAATGggtAAATGCGCCTGCGGTGCT
Y55S F	GCATTTagcCATTGTAGCTTTCCGTTT
Y55S R	ACAATGgctAAATGCGCCTGCGGTGCT
Y55A F	GCATTTgcaCATTGTAGCTTTCCGTTT
Y55A R	ACAATGtgcAAATGCGCCTGCGGTGCT
C57I F	TTATCATattAGCTTTCCGTTTGAAGTTCC
C57I R	GAAAGCTaatATGATAAAATGCGCCTGC
C57M F	TTATCATatgAGCTTTCCGTTTGAAGTTCC
C57M R	GAAAGCTcatATGATAAAATGCGCCTGC
C57V F	TTATCATgttAGCTTTCCGTTTGAAGTTCC
C57V R	GAAAGCTaacATGATAAAATGCGCCTGC
C57A F	TTATCATgcaAGCTTTCCGTTTGAAGTTCC
C57A R	GAAAGCTtgcATGATAAAATGCGCCTGC
F59A F	TGTAGCgcaCCGTTTGAAGTTCCGGCA
F59A R	AAACGGtgcGCTACAATGATAAAATGC
F59I F	TGTAGCattCCGTTTGAAGTTCCGGCA
F59I R	AAACGGaatGCTACAATGATAAAATGC
F59V F	TGTAGCgttCCGTTTGAAGTTCCGGCA
F59V R	AAACGGaacGCTACAATGATAAAATGC
F59S F	TGTAGCagcCCGTTTGAAGTTCCGGCA
F59S R	AAACGGgctGCTACAATGATAAAATGC
F59L F	TGTAGCctgCCGTTTGAAGTTCCGGCA
F59L R	AAACGGcagGCTACAATGATAAAATGC
C57I-F59A F	TATCATattAGCgcaCCGTTTGAAGTTCCGGCA
C57I-F59A R	AAACGGtgcGCTaatATGATAAAATGCGCCTGC
F61A F	TTTCCGgcaGAAGTTCCGGCAGGCGAA
F61A R	AACTTCtgcCGGAAAGCTACAATGATA
F61I F	TTTCCGattGAAGTTCCGGCAGGCGAA
F61I R	AACTTCaatCGGAAAGCTACAATGATA
F61V F	TTTCCGgttGAAGTTCCGGCAGGCGAA
F61V R	AACTTCaacCGGAAAGCTACAATGATA
F61S F	TTTCCGagcGAAGTTCCGGCAGGCGAA
F61S R	AACTTCgctCGGAAAGCTACAATGATA
F61L F	TTTCCGctgGAAGTTCCGGCAGGCGAA
F61L R	AACTTCcagCGGAAAGCTACAATGATA
P64A F	GAAGTTagcGCAGGCGAATGGCATTTT
P64A R	GCCTGCgctAACTTCAAACGGAAAGCT

P64A F	GAAGTTagcGCAGGCGAATGGCATTTT
P64A R	GCCTGCgctAACTTCAAACGGAAAGCT
P64G F	GAAGTTggtGCAGGCGAATGGCATTTT
P64G R	GCCTGCaccAACTTCAAACGGAAAGCT
P64V F	GAAGTTgttGCAGGCGAATGGCATTTT
P64V R	GCCTGCaacAACTTCAAACGGAAAGCT
W68A F	GGCGAAgcaCATTTTCCGGAACCGGGT
W68A R	AAAATGtgcTTCGCCTGCCGGAACTTCAAA
W68F F	GGCGAAtttCATTTTCCGGAACCGGGT
W68F R	AAAATGaaaTTCGCCTGCCGGAACTTCAAA
W68L F	GGCGAActgCATTTTCCGGAACCGGGT
W68L R	AAAATGcagTTCGCCTGCCGGAACTTCAAA
W68Q F	GGCGAAcagCATTTTCCGGAACCGGGT
W68Q R	AAAATGctgTTCGCCTGCCGGAACTTCAAA
W68S F	GGCGAAagcCATTTTCCGGAACCGGGT
W68S R	AAAATGgctTTCGCCTGCCGGAACTTCAAA
H69F F	GAATGGtttTTTCCGGAACCGGGTCAT
H69F R	CGGAAAaaaCCATTCGCCTGCCGGAAC
H69Y F	GAATGGtatTTTCCGGAACCGGGTCAT
H69Y R	CGGAAAataCCATTCGCCTGCCGGAAC
H69V F	GAATGGgttTTTCCGGAACCGGGTCAT
H69V R	CGGAAAaacCCATTCGCCTGCCGGAAC
H69L F	GAATGGctgTTTCCGGAACCGGGTCAT
H69L R	CGGAAAcagCCATTCGCCTGCCGGAAC
H69A F	GAATGGgcaTTTCCGGAACCGGGTCAT
H69A R	CGGAAAtgcCCATTCGCCTGCCGGAAC
H69I F	GAATGGattTTTCCGGAACCGGGTCAT
H69I R	CGGAAAaatCCATTCGCCTGCCGGAAC
H126A F	TTTGCAgcaCGTGATGGTGGTCATTTTGCC
H126A R	ATCACGtgcTGCAAAATGAACAAAACCTTC
H126T F	TTTGCAaccCGTGATGGTGGTCATTTTGCC
H126T R	ATCACGggtTGCAAAATGAACAAAACCTTC
H126S F	TTTGCAagcCGTGATGGTGGTCATTTTGCC
H126S R	ATCACGgctTGCAAAATGAACAAAACCTTC
H126L F	TTTGCActgCGTGATGGTGGTCATTTTGCC
H126L R	ATCACGcagTGCAAAATGAACAAAACCTTC
H132A F	GGTCATgcaGCCCTGGAAAGCCTGGCA
H132A R	CAGGGCtgcATGACCACCATCACGATG
H132V F	GGTCATgttGCCCTGGAAAGCCTGGCA
H132V R	CAGGGCaacATGACCACCATCACGATG
H132L F	GGTCATctgGCCCTGGAAAGCCTGGCA
H132L R	CAGGGCcagATGACCACCATCACGATG
H132N F	GGTCATaatGCCCTGGAAAGCCTGGCA
H132N R	CAGGGCattATGACCACCATCACGATG
S180V F	GACCAGgttTTTAAACTGCGTTGGCAG
S180V R	TTTAAAaacCTGGTCCAGAATAACAATACGAAT
S180I F	GACCAGattTTTAAACTGCGTTGGCAG
S180I R	TTTAAAaatCTGGTCCAGAATAACAATACGAAT
S180A F	GACCAGgcaTTTAAACTGCGTTGGCAG
S180A R	TTTAAAtgcCTGGTCCAGAATAACAATACGAAT
S180G F	GACCAGggcTTTAAACTGCGTTGGCAG
S180G R	TTTAAAgccCTGGTCCAGAATAACAATACGAAT
S180V F	GACCAGgttTTTAAACTGCGTTGGCAG
S180V R	TTTAAAaacCTGGTCCAGAATAACAATACGAAT
C262A F	TGGGTTgcaCCGCATCTGCAGAGCAAT
C262A R	ATGCGGtgcAACCCACAGGCTGGTATC
C262I F	TGGGTTattCCGCATCTGCAGAGCAAT

C262I R	ATGCGGaatAACCCACAGGCTGGTATC
C262V F	TGGGTTgttCCGCATCTGCAGAGCAAT
C262V R	ATGCGGaacAACCCACAGGCTGGTATC
C262L F	TGGGTTctgCCGCATCTGCAGAGCAAT
C262L R	ATGCGGcagAACCCACAGGCTGGTATC
P263A F	GTTTGTgcaCATCTGCAGAGCAATTGT
P263A R	CAGATGtgcACAAACCCACAGGCTGGT
P263G F	GTTTGTggcCATCTGCAGAGCAATTGT
P263G R	CAGATGgccACAAACCCACAGGCTGGT
P263V F	GTTTGTgttCATCTGCAGAGCAATTGT
P263V R	CAGATGaacACAAACCCACAGGCTGGT
S267A F	TGCAGgcaAATTGTCATGCCGAACAGCTGC
S267A R	CAATTtgcGATGCGGACAAACCCACAGG
S267V F	TGCAGgttAATTGTCATGCCGAACAGCTGC
S267V R	CAATTaacGATGCGGACAAACCCACAGG
S267G F	TGCAGggcAATTGTCATGCCGAACAGCTGC
S267G R	CAATTgccGATGCGGACAAACCCACAGG
S267I F	TGCAGattAATTGTCATGCCGAACAGCTGC
S267I R	CAATTaatGATGCGGACAAACCCACAGG

[a]: the replacement nucleotide sequences were shown in lower case text.

Figure S1.

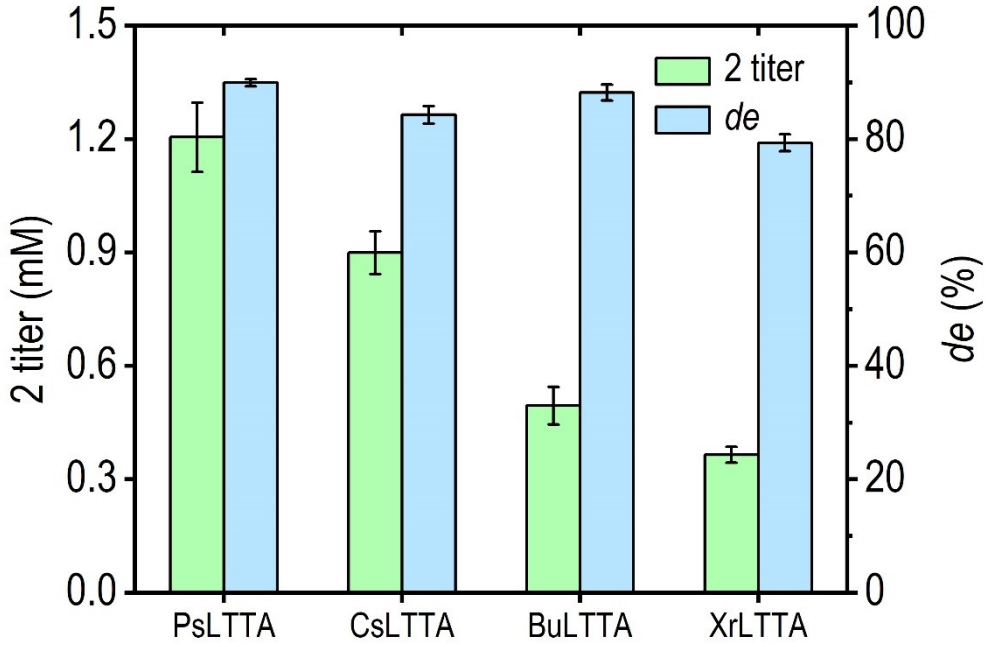

Figure S1. Four L-threonine transaldolase (PsLTTA, CsLTTA, BuLTTA and XrLTTA) were screened for synthesizing of **2**. Transaldol reaction was performed in a 1 ml volume comprising 6 mM of L-threonine, 5 mM of **1**, 0.1 mM of PLP and 25 mg of whole-cell catalyst in Tris-HCl buffer (50 mM, pH 7.5) and incubated at 30℃ with shaking for 2 h. After reaction, the pellets were removed by centrifugation and the supernatant was submitted for evaluating the conversion and stereoselectivity of **2** by chromatography analysis.

Figure S2.

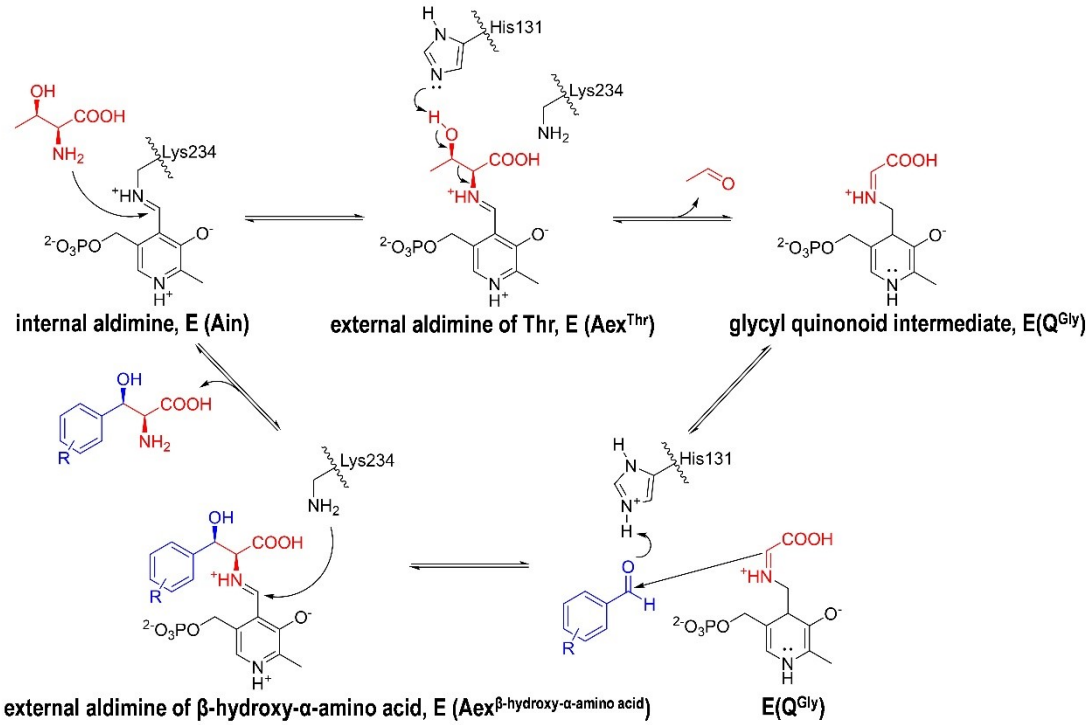

Figure S2. Based on the crystal structure of ObiH (PDB code: 7K34), the catalytic mode of L-threonine transaldolase was a multi-stage reaction following a Ping-Pong mechanism. The reaction is initiated by the binding of L-threonine to phosphopyridoxal (PLP) to form an external aldimine of L-threonine (Aex^{Thr}) and a re-aldol cleavage is followed to produce a highly basic glycyl quinonoid intermediate Q^{Gly} and acetaldehyde. Then Q^{Gly} attacks on substrate aldehyde to form an external aldimine of β-hydroxy-α-amino acid ($Aex^{β\text{-hydroxy-α-amino acid}}$) and the reintegration of catalytic residue K234 with PLP is occurred to form an internal aldimine (Ain), leading to the release of β-hydroxy-α-amino acids.

Figure S3.

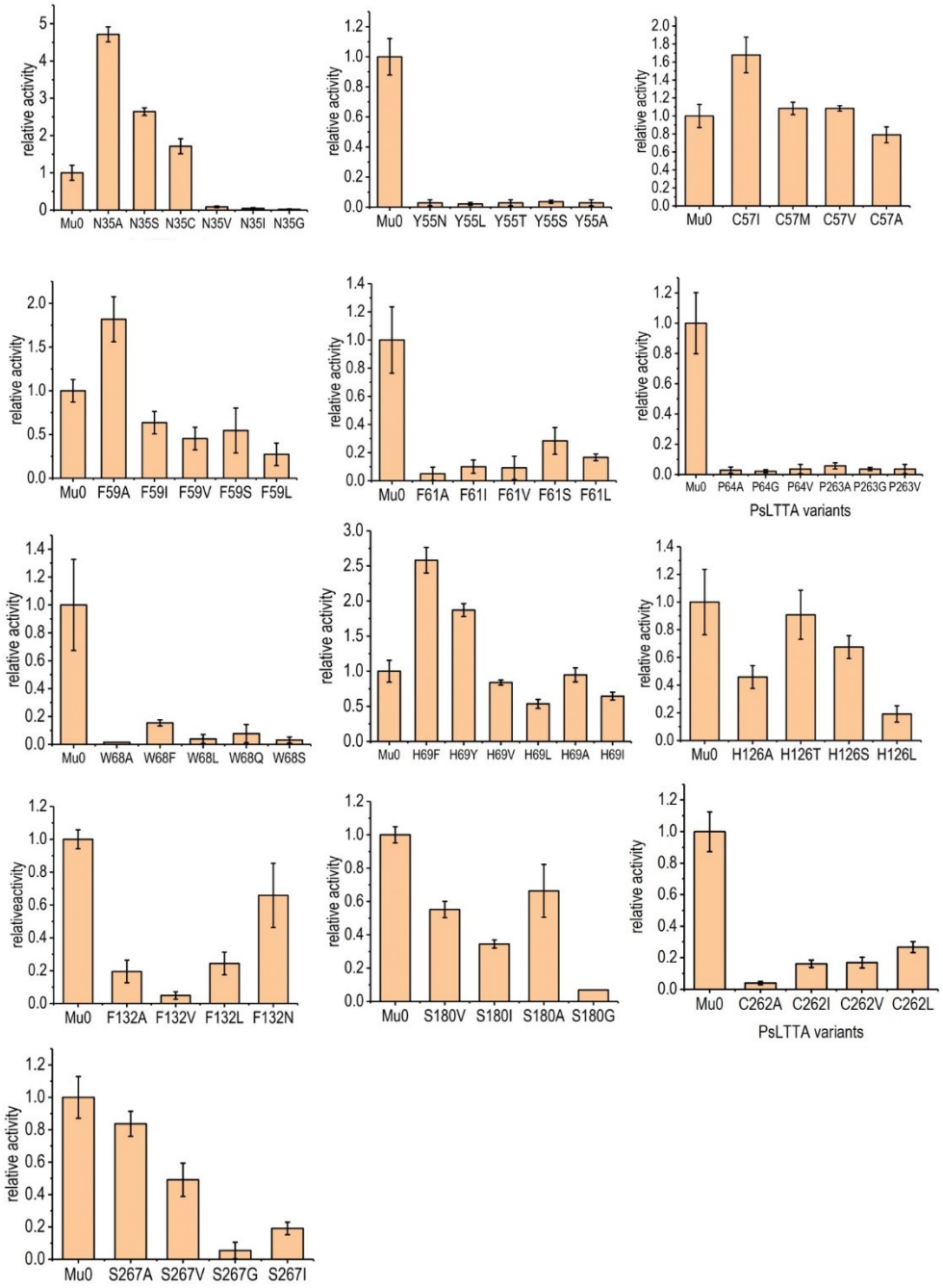

Figure S3. The activities of PsLTTA variants were screened by high-throughput screening method. The reaction catalyzed by wild-type PsLTTA (Mu0) was used as a control. All experiments were conducted in triplicate and the results were represented as the mean ± standard deviation.

Figure S4.

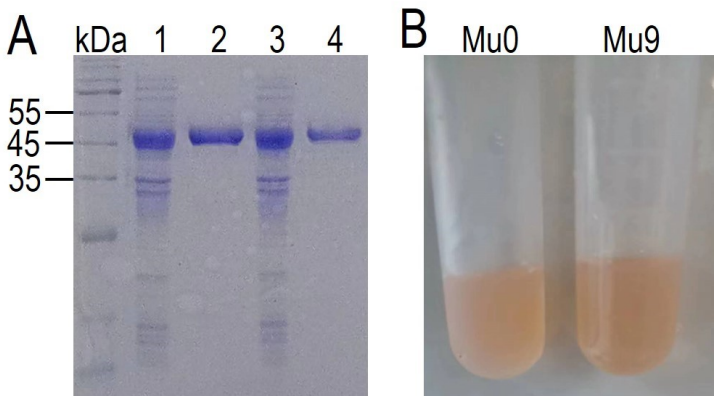

Figure S4. (A) SDS-PAGE analysis of PsLTTA-Mu0 and PsLTTA-Mu9. Lane 1, whole cell extract of PsLTTA-Mu0; Lane 2, purified PsLTTA-Mu0; Lane 3, whole cell extract of PsLTTA-Mu9; Lane 4, purified PsLTTA-Mu9. (B) The purified PsLTTA-Mu0 and PsLTTA-Mu9 were pink in solution.

Figure S5.

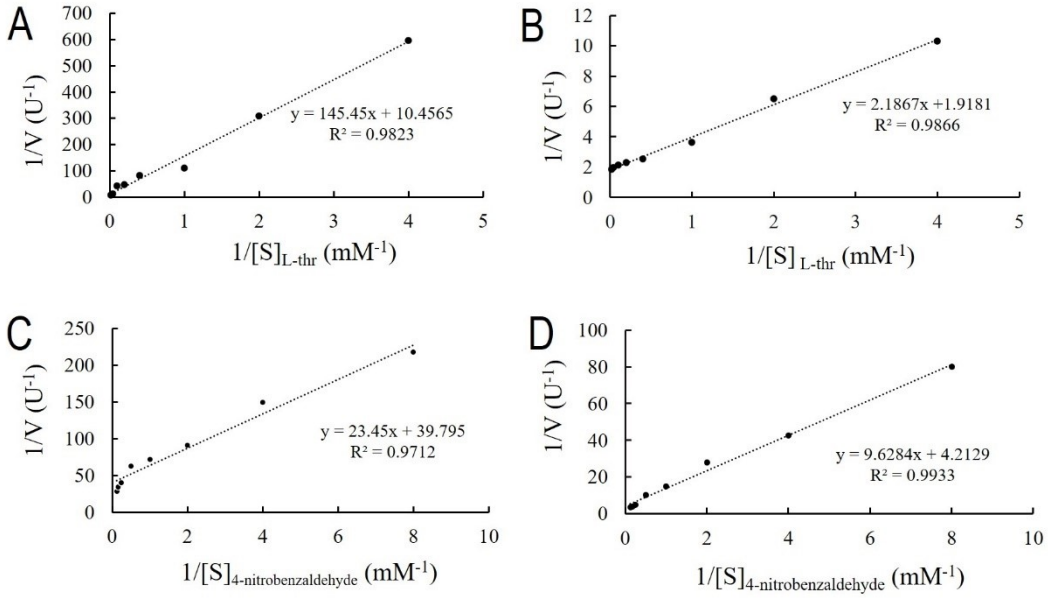

Figure S5. The kinetic plots of PsLTTA-Mu0 and PsLTTA-Mu9 toward substrates **1** and L-threonine. V: reaction rate; [S]: substrate concentration.

Figure S6.

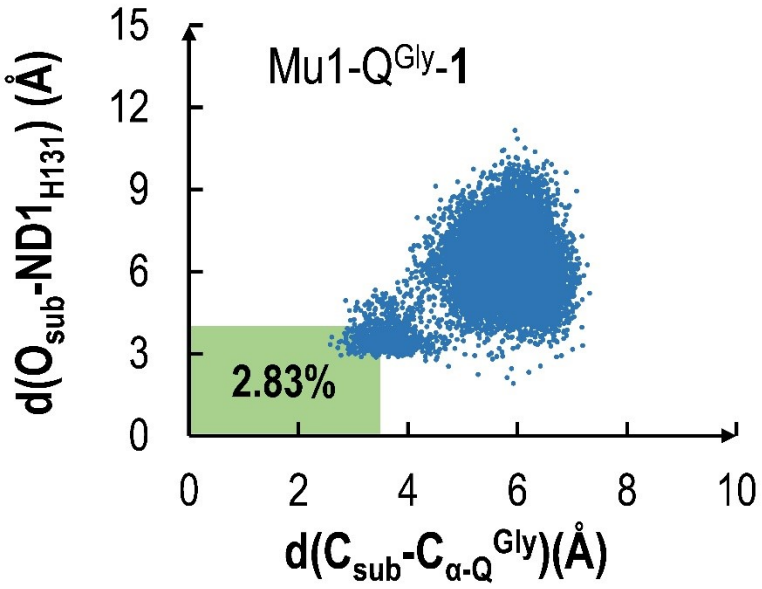

Figure S6. Conformation maps of Mu1-Q^{Gly}-**1**. The limit of "catalytic distance" d(O_{sub}-$ND1_{H131}$) <4.0 Å and d(C_{sub}-$C_{α-Q^{Gly}}$) <3.5 Å are colored by green.

Figure S7.

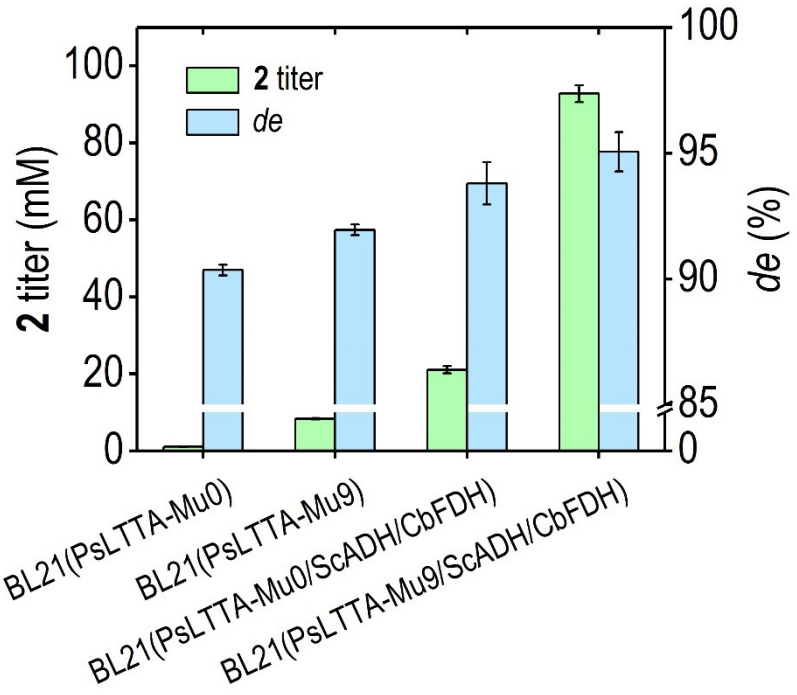

Figure S7. The yield and *de* of **2** synthesized by whole-cell catalyst BL21(PsLTTA-Mu0), BL21(PsLTTA-Mu9), BL21(PsLTTA-Mu0/ScADH/CbFDH) and BL21(PsLTTA-Mu9/ScADH/CbFDH).

Figure S8.

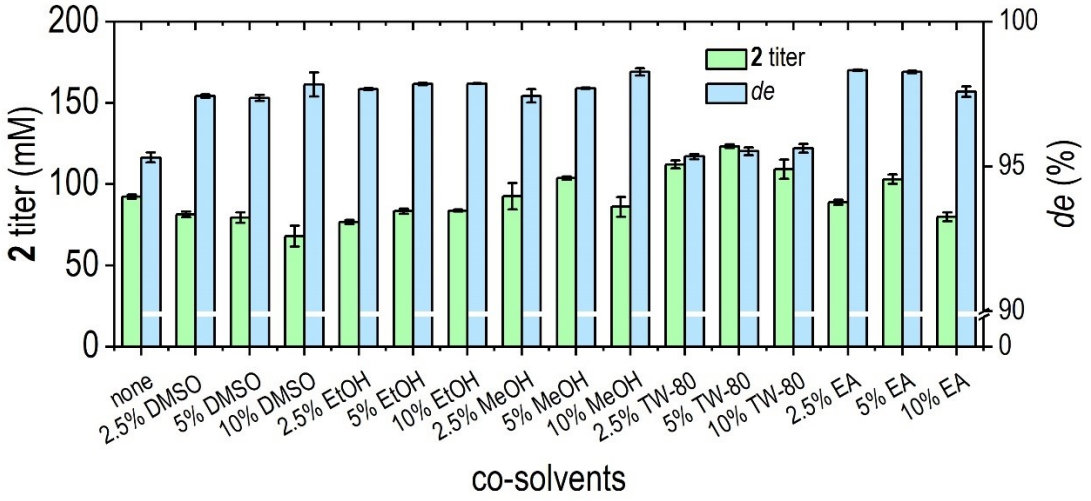

Figure S8. The optimization of co-solvents addition on the synthesis of **2**. Transaldol reaction was performed at 30 °C and 200 rpm for 2 h, in 1 mL of Tris-HCl buffer (50 mM, pH 7.5) containing 50 mg/mL of whole-cell catalyst, 200 mM **1**, 220 mM L-threonine, 250 mM sodium formate, 0.1 mM PLP and 0.3 mM NAD^+. The conversion and stereospecificity of **2** were detected by chromatography analysis.

Figure S9.

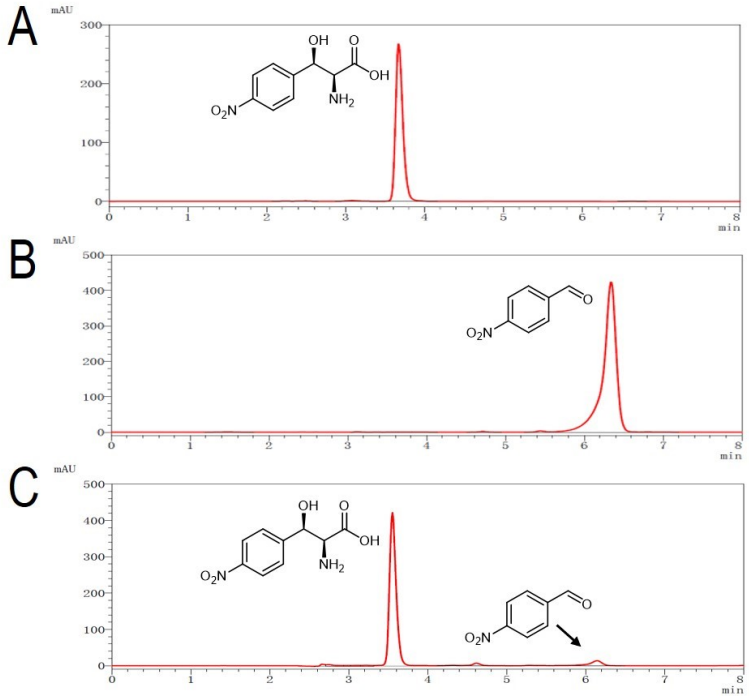

Figure S9. HPLC chromatogram of sample from bio-catalysis of **1** to **2** with whole cell catalyst BL21(PsLTTA-Mu9/ScADH/CbFDH). (A) HPLC chromatogram of **2** standard (t=3.6 min). (B) HPLC chromatogram of **1** standard (t=6.1 min). (C) HPLC chromatogram of sample catalyzed by whole cell catalyst BL21(PsLTTA-Mu9/ScADH/CbFDH).

Figure S10.

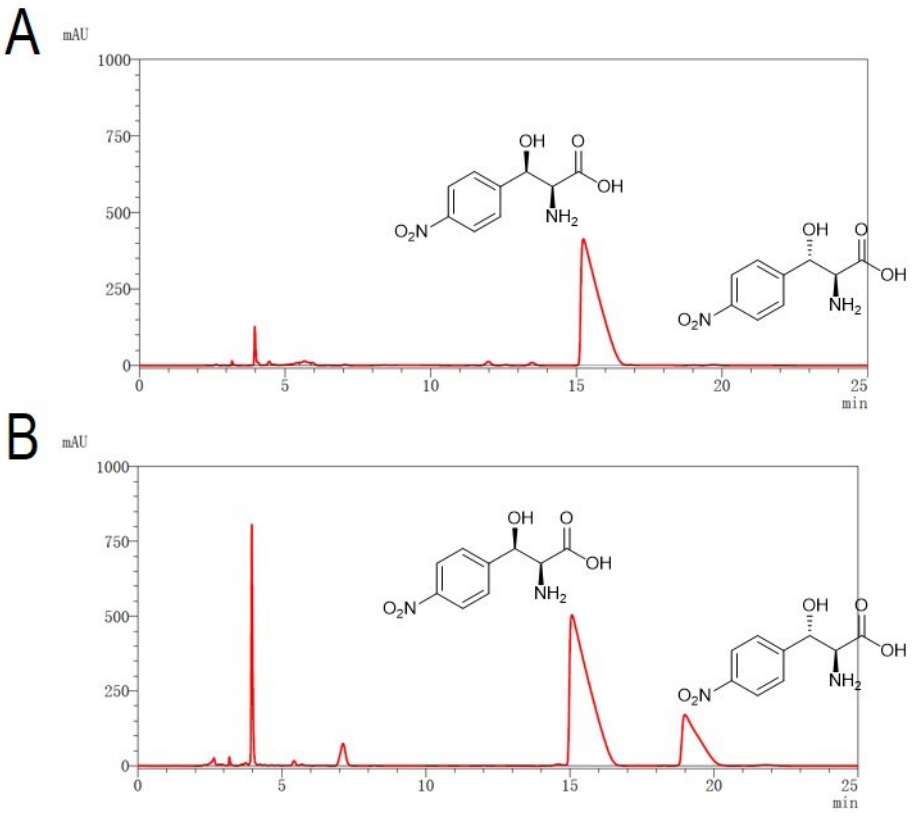

Figure S10. Chiral HPLC chromatograms of 2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid. (**A**) Transaldol reaction catalyzed by whole cell catalyst BL21(PsLTTA-Mu9/ScADH/CbFDH) with 98.9% *de*. 2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid ($t_{(2S,\ 3R)}$=15.3 min, $t_{(2S,\ 3S)}$=19.2 min). (**B**) Transaldol reaction catalyzed L-Threonine aldolase with 62% *de* was selected as a control. 2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid ($t_{(2S,\ 3R)}$=15.2 min, $t_{(2S,\ 3S)}$=19.1 min).

Figure S11.

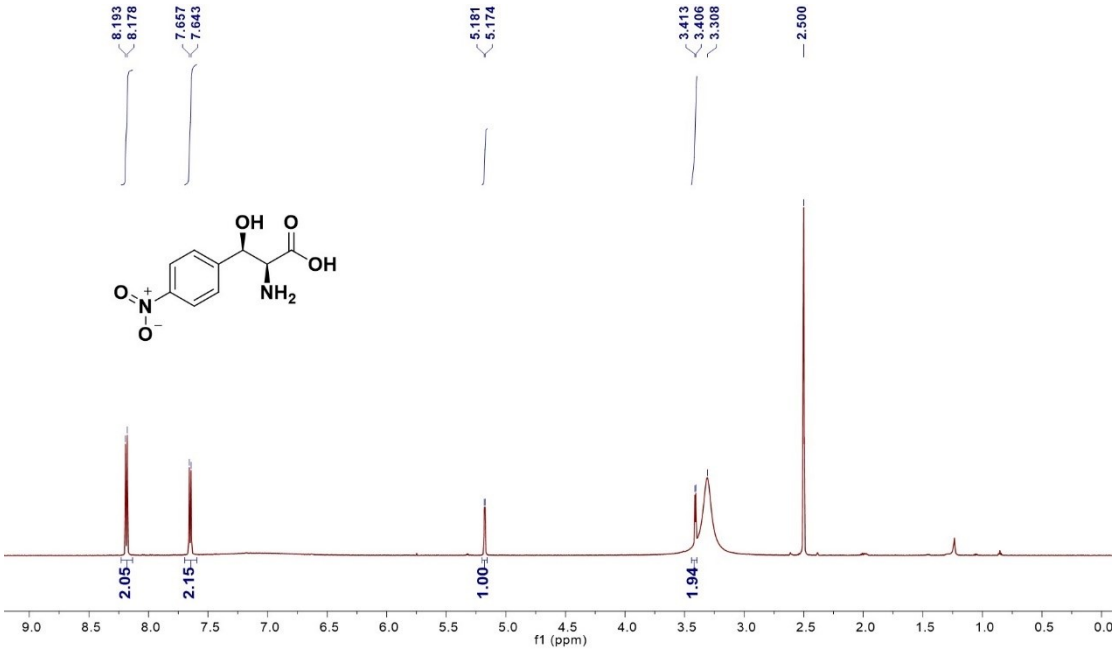

Figure S11. ^{1}H- NMR spectra of (2*S*,3*R*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid (**2**). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) = 8.18 (d, *J* = 9.0 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 1H), 5.18 (d, *J* = 4.2 Hz, 1H), 3.41 (d, *J* = 4.2 Hz, 1H).

Figure S12.

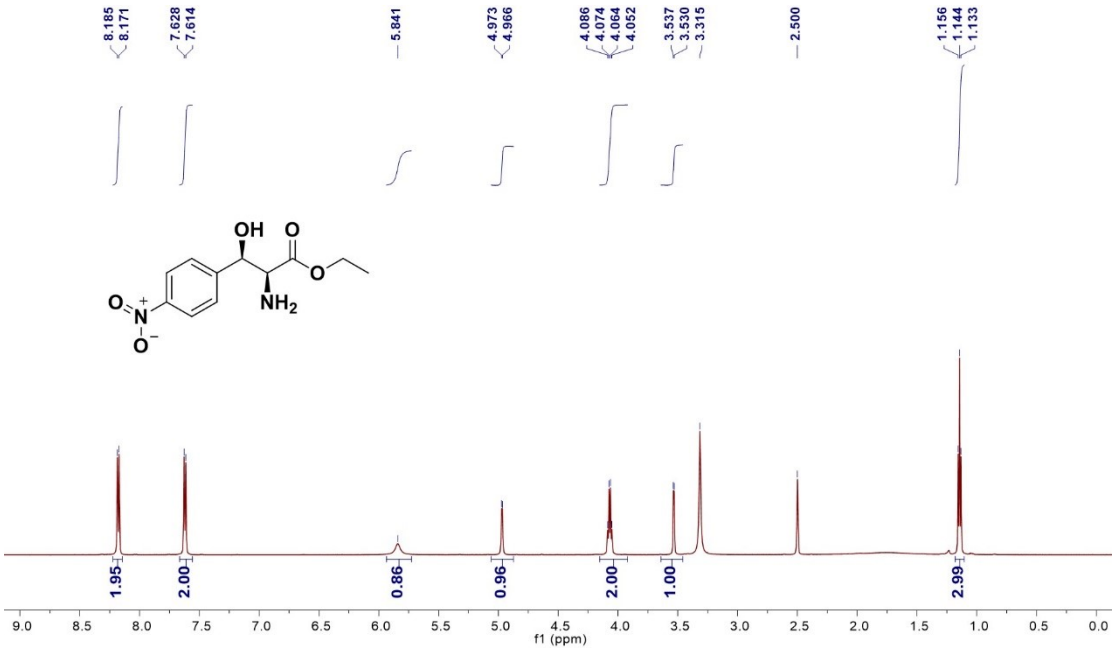

Figure S12. ^{1}H- NMR spectra of ethyl (2*S*,3*R*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propanoate (**3**). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) = 8.18 (d, J = 8.6 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 5.84 (s, 1H), 4.97 (d, J = 4.2 Hz, 1H), 4.07 (q, J = 7.2 Hz, 2H), 3.53 (d, J = 4.2 Hz, 1H), 1.14 (t, J = 7.2 Hz, 3H).

Figure S13.

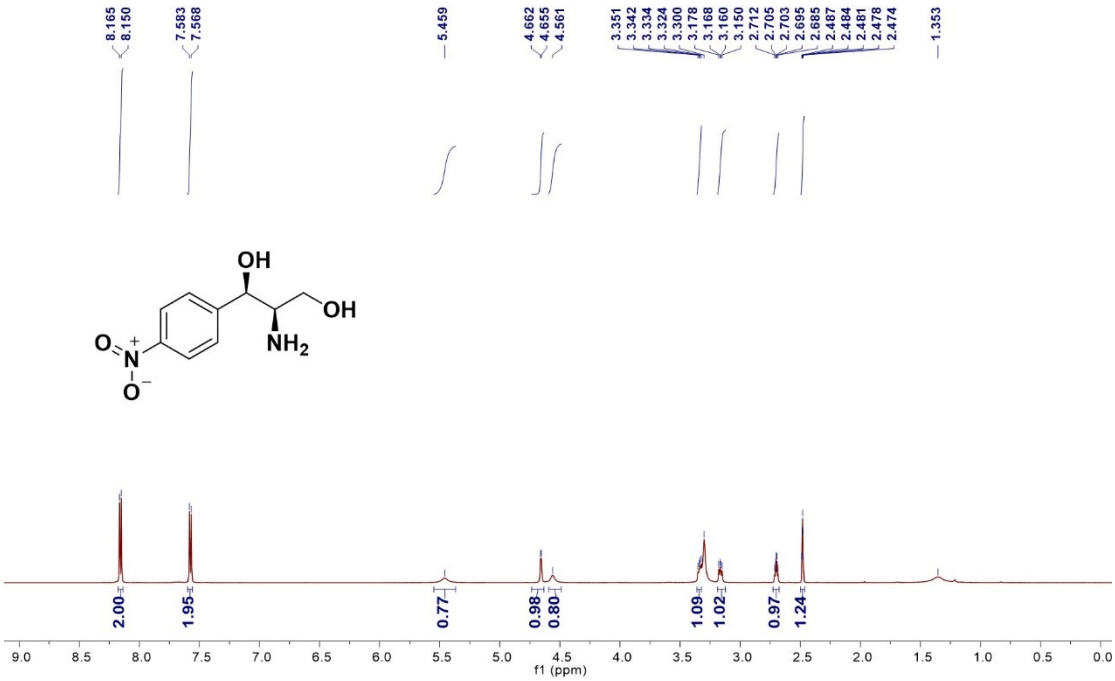

Figure S13. ^{1}H- NMR spectra of (1*R*,2*R*)-2-amino-1-(4-nitrophenyl)propane-1,3-diol (**4**). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) = 8.16 (d, *J* = 9.0 Hz, 2H), 7.58 (d, *J* = 9.0 Hz, 2H), 5.46 (s, 1H), 4.66 (d, *J* = 4.2 Hz, 1H), 4.56 (s, 1H), 3.34 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.16 (dd, *J* = 10.2, 6.0 Hz, 1H), 2.70 (q, *J* = 5.4 Hz, 1H), 2.48-2.47 (m, 2H).

Figure S14.

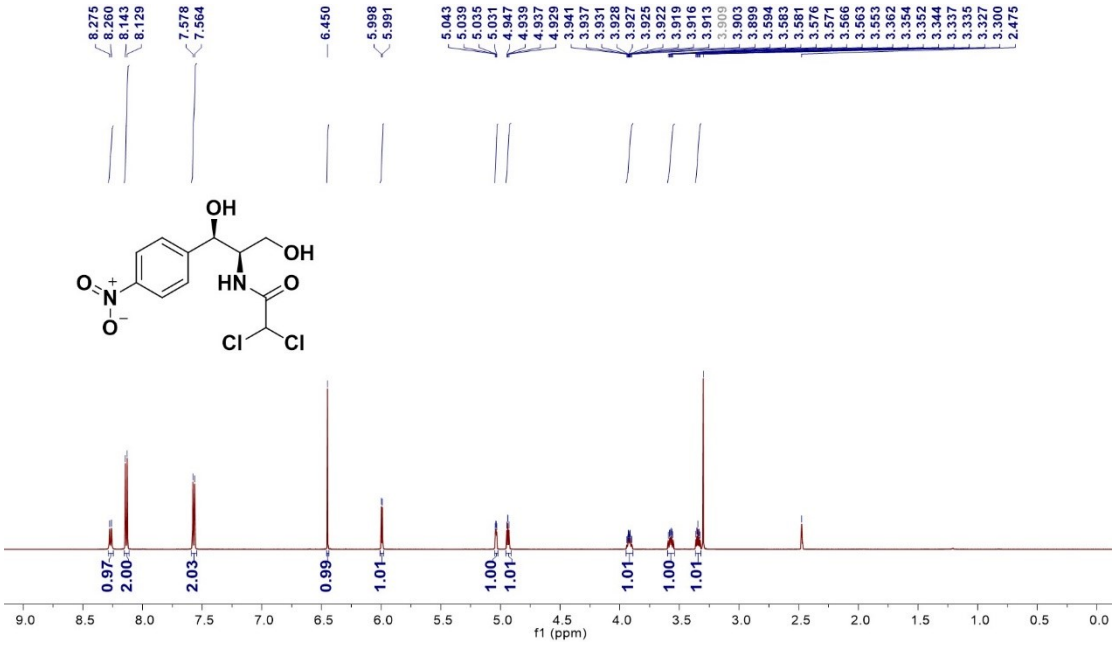

Figure S14. ^{1}H- NMR spectra of 2,2-dichloro-N-((1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)acetamide (**5**). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) = 8.27 (d, J = 9.0 Hz, 1H), 8.14 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 6.45 (s, 1H), 5.99 (d, J = 4.2 Hz, 1H), 5.04 (dd, J = 4.8, 2.4 Hz, 1H), 4.94 (dd, J = 6.0, 4.8 Hz, 1H), 4.11-3.88 (m, 1H), 3.59-3.55 (m, 1H), 3.36-3.33 (m, 1H).