Supporting Information

Multi-strategy engineering of transaminase enables a one-pot synergistic biocatalytic cascade to a key florfenicol chiral intermediate

Hong-Ru Zhao [a, c], Wen-Tao Shen [b, c], Bing-Mei Su [b, c], Lian Xu [b, c, *], Juan Lin [a, b, c, *]

[a] College of Chemical Engineering, Fuzhou University, Fuzhou 350108, China.

[b] College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China.

[c] Institute of Enzyme Catalysis and Synthetic Biotechnology, Fuzhou University, Fuzhou 350108, China.

*Corresponding author: Lian Xu

Mailing address: College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China.

*E-mail: xulian@fzu.edu.cn.

*Corresponding author: Juan Lin

Mailing address: College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China.

*E-mail: ljuan@fzu.edu.cn.

Table S1. Amino acid sequences of the desired enzymes used in this work.

AncLAAO, ancestral L-amino acid oxidase
Protein sequence: MTHYKFGNEISDKSIPKQVKVAIVGAGMSGLYSAWRLQNEANTQDLAIFERSDRTGGRLDSDLIEFKNQRSGPETPSTITVKEEQGGMRFLFEGMDDLMALFLKLDLQDQIVPFPMNSGGNNRLYFRGESFSVNDAQQDDYAIWSHLYNLDPSEQGVNPKDIINVVFNRILQANPQFDARPEVRGPEFWQSFRLECQWQGKTLNEWTLWDLFTDMGYSQECITMLYRVLGFNGTFLSKMNAGVAYQLLEDFPADVQFKTFKDGFSTLPNALVDKIGTDKIHLQTSIEEIDFDEASGKYVLHYTHTDEHGQVHKGQVKAEKVILGLPRLALEKLFVRSNAFNRLEKKRSEQLWNTLQSASNQPLLKINLYYDTAWWGRGITGRPAVEFGPNFADLPTGSVYPFYAVNDELAAALMYEERHTNPSQDTQHKLDGINSEKYERPAALTIYCDYLNINFWSALQNKGELYHHPHQDEYVESVPSDIYPASTAVVQQATKFFKDIFNTHYVPEPILTSARIWEGSVNFDVPASQQFGFGVHQWAVGANDKQVMEDLVEPLPNLFTCGEAFSDYQGWVEGALRSTDLVLEKGFGLAPLSEVYEQNTHISSSEAIKAVYEENSSKLINQYIDPNFSANTAPIEKLADVNSVIGVNLSYFDKP*
PvLAAD, L-amino acid deaminases from *Proteus vulgaris*
Protein sequence: MAISRRKFIIGGTVVAVAAGAGILTPMLTREGRFVPGTPRHGFVEGTEGALPKQADVVVVGAGILGIMTAINLVERGLSVVIVEKGNIAGEQSSRFYGQAISYKMPDETFLLHHLGKHRWREMNAKVGIDTTYRTQGRVEVPLDEEDLVNVRKWIDERSKNVGSDIPFKTRIIEGAELNQRLRGATTDWKIAGFEEDSGSFDPEVATFVMAEYAKKMGVRIYTQCAARGLETQAGVISDVVTEKGAIKTSQVVVAGGVWSRLFMQNLNVDVPTLPAYQSQQLISGSPTAPGGNVALPGGIFFREQADGTYATSPRVIVAPVVKESFTYGYKYLPLLALPDFPVHISLNEQLINSFMQSTHWNLDEVSPFEQFRNMTALPDLPELNASLEKLKAEFPAFKESKLIDQWSGAMAIAPDENPIISEVKEYPGLVINTATGWGMTESPVSAELTADLLLGKKPVLDPKPFSLYRF*
PmLAAD, L-amino acid deaminases from *Proteus mirabilis*
Protein sequence: MNISRRKLLLGVGAAGVLAGGAALVPMVRRDGKFVEAKSRASFVEGTQGALPKEADVVIIGAGIQGIMTAINLAERGMSVTILEKGQIAGEQSGRAYSQIISYQTSPEIFPLHHYGKILWRGMNEKIGADTSYRTQGRVEALADEKALDKAQAWIKTAKEAAGFDTPLNTRIIKGEELSNRLVGAQTPWTVAAFEEDSGSVDPETGTPALARYAKQIGVKIYTNCAVRGIETAGGKISDVVSEKGAIKTSQVVLAGGIWSRLFMGNMGIDIPTLNVYLSQQRVSGVPGAPRGNVHLPNGIHFREQADGTYAVAPRIFTSSIVKDSFLLGPKFMHLLGGGELPLEFSIGEDLFNSFKMPTSWNLDEKTPFEQFRVATATQNTQHLDAVFQRMKTEFPVFEKSEVVERWGAVVSPTFDELPIISEVKEYPGLVINTATVWGMTEGPAAGEVTADIVMGKKPVIDPTPFSLDRFKK*
ZmPDC, pyruvate decarboxylase from *Zymomonas mobilis*
Protein sequence: MSYTVGTYLAERLVQIGLKHHFAVAGDYNLVLLDNLLLNKNMEQVYCCNELNCGFSAEGYARAKGAAAAVVTYSVGALSAFDAIGGAYAENLPVILISGAPNNNDHAAGHVLHHALGKTDYHYQLEMAKNITAAAEAIYTPEEAPAKIDHVIKTALREKKPVYLEIACNIASMPCAAPGPASALFNDEASDEASLNAAVEETLKFIANRDKVAVLVGSKLRAAGAEEAAVKFADALGGAVATMAAAKSFFPEENPHYIGTSWGEVSYPGVEKTMKEADAVIALAPVFNDYSTTGWTDIPDPKKLVLAEPRSVVVNGVRFPSVHLKDYLTRLAQKVSKKTGALDFFKSLNAGELKKAAPADPSAPLVNAEIARQVEALLTPNTTVIAETGDSWFNAQRMKLPNGARVEYEMQWGHIGWSVPAAFGYAVGAPERRNILMVGDGSFQLTAQEVAQMVRLKLPVIIFLINNYGYTIQVMIHDGPYNNIKNWDYAGLMEVFNGNGGYDSGAGKGLKAKTGGELAEAIKVALANTDGPTLIECFIGREDCTEELVKWGKRVAAANSRKPVNKLL*
MhPDC, pyruvate decarboxylase from *Macrococcus hajekii*
Protein sequence: MKRRVGQYLIDQISVYGVDQIFGVPGDFNLAFLDDIVSHEQVEWIGNTNELNASYAADGYARIKGLGALVTTFGVGELSAVNGIAGSYAERVPVVAITGAPTTVVEQAKRYVHHSLGEGVFDDYQRMFEPITCGQIYLTADNAAIEIPRIIAKAIRFQRPVHIHLPIDVAMTEIEVKEATVQPMLSQDVSVFTDLIQERLKEAQQPVLIAGHEINSFKLHEELEQFVNQTHIPVAQLSLGKGAFNEENPHYIGIFDGKIAHKDVRDYVNSSDCIINLGAKLTDSATAGFSYEFKTEDVIFINDEDFRVDSTYHTDVSLRDMMRSLLTLNYENTANFTPFNREHMTDFILNDEALTQENYFKMINHFLQQDDVLLAEQGTSFFGAYDLVLYAGNQFIGQPLWGSIGYTLPALLGTQMADKKRRNVLLIGDGSFQLTAQELSTMIRHQLKPIIFLINNDGYTVERLIHGEKEIYNDIQMWDYTALPAVFGGKDHVQTHKASTSNELKTVMDTVDQQPDKMHFIEVTMGMLDAPEKLVNISKAFAAQNK *
SwPDC, pyruvate decarboxylase from *Staphylococcus warneri*
Protein sequence: MKQRVGQYLMDAVNAAGVDKIFGVPGDFNLAFLDDIISHDQVEWIGNTNELNASYAADGYARINGLGALVTTFGVGELSAVNGIAGSYAERVPVIAITGAPTRAVESAGKYVHHSLGEGTFDDYRKMFEPITTAQGYITPENATTEIPRLIQAAINERRPVHLHLPIDVAMTEIDVPKSFQPEARDDQDVSHYIQMIEDKLNSAKQPVIITGHEINSFGLHSELEQFVNQTHIPVAQLSLGKGAFNEENEHYIGIFDGSIAEENVKNYVNQSDAILNIGAKLTDSATAGFSFEFDIDDVVMINHNYFKMNETISEQVALPHLIKGLMSISYKNKSEFPMYQRPKEHDYQVDHEPLTQATYFKMMQDFLQLDDILIAEQGSSFFGAYDLALYKDNTFIGQPLWGSIGYTLPATLGTQIAAPHRRNVLLIGDGSLQLTVQSLSTMIRQQLKPIIFVVNNDGYTVERLIHGMKEPYNDIHMWDYKTLPAVFGGDNVVVHDVNTSHELKETFEKINAHSDCMHFVEVKMAIEDAPAKLSDIAKAFASQNK *
EcTK_YYH, a variant of transketolases from *E.coli*
Protein sequence:

MSSRKELANAIRALSMDAVQKAKSGYPGAPMGMADIAEVLWRDFLKHNPQNPSWADRDRFVLSNG HGSMLIYSLLHLTGYDLPMEELKNFRQLHSKTPGHPEVGYTAGVETTTGPLGQGIANAVGMAIAEKTL AAQFNRPGHDIVDHYTYAFMGDGCMMEGISHEVCSLAGTLKLGKLIAFYDDNGISIDGHVEGWFTDD TAMRFEAYGWHVIRDIDGHDAASIKRAVEEARAVTDKPSLLMCKTIIGFGSPNKAGTHDSHGAPLGDA EIALTREQLGWKYAPFEIPSEIYAQWDAKEAGQAKESAWNEKFAAYAKAYPQEAAEFTRRMKGEMPS DFDAKAKEFIAKLQANPAKIASRKASQNAIEAFGPLLPEFLGGSADLAPYNLTLWSGSKAINEDAAGN YIHYGVREFGMTAIANGISLHGGFLPYTSTYLMFVEYARNAVRMAALMKQRQVMVYTHDSIGHGET GPTHQPVEQVASLRVTPNMSTWRPCDQVESAVAWKYGVERQDGPTALILSQQNLAQQERTEEQLANI ARGGYVLKDCAGQPELIFIATGSEVELAVAAYEKLTAEGVKARVVSMPSTDAFDKQDAAYRESVLPK AVTARVAVEAGIADYWYKYVGLNGAIVGMTTFGESAPAELLFEEFGFTVDNVVAKAKELL*
ATA117, (*R*)-amine transaminase from Arthrobacter sp. KNK168
Protein sequence: MAFSADTSEIVYTHDTGLDYITYSDYELDPANPLAGGAAWIEGAFVPPSEARISIFDQGYLHSDVTYTV FHVWNGNAFRLDDHIERLFSNAESMRIIPPLTQDEVKEIALELVAKTELREACVSVSITRGYSSTPGERD ITKHRPQVYMYAVPYQWIVPFDRIRDGVHAMVAQSVRRTPRSSIDPQVKNFQWGDLIRAVQETHDRG FEAPLLLDGDGLLAEGSGFNVVVIKDGVVRSPGRAALPGITRKTVLEIAESLGHEAILADITLAELLDAD EVLGCTTAGGVWPFVSVDGNPISDGVPGPVTQSIIRRYWELNVESSSLLTPVQY*
ATA117_ACHH, a variant of (*R*)-amine transaminase from Arthrobacter sp. KNK168
Protein sequence: MAFSADTSEIVYTHDTGLDYITYSDYELDPANPLAGGAAWIEGACVPPSEARISIFDQGYLHSDVTYTA FHVWNGNAFRLDDHIERLFSNAESMRIIPPLTQDEVKEIALELVAKTELREACVSVSITRGYSSTPGERD ITKHRPQVYMYAVPYQWHVPFDRIRDGVHAMVAQSVRRTPRSSIDPQVKNFQWGDLIRAVQETHDR GFEAPLLLDGDGLLAEGSGHNVVVIKDGVVRSPGRAALPGITRKTVLEIAESLGHEAILADITLAELLD ADEVLGCTTAGGVWPFVSVDGNPISDGVPGPVTQSIIRRYWELNVESSSLLTPVQY*

Table S2. Primers used for site-directed mutagenesis of ATA117.

Primers	Sequence (5’-3’)
F122I_F	CGTGAAGCAATTGTTAGTGTTAGCATTACCCGC
F122I_R	AACACTAACAATTGCTTCACGCAGCTCGGTTTTTGC
F122L_F	CGTGAAGCACTGGTTAGTGTTAGCATTACCCGCGGT
F122L_R	AACACTAACCAGTGCTTCACGCAGCTCGGTTTTTGC
F122C_F	AACACTAACTGCTGCTTCACGCAGCTCGGTTTTTGC
F122C_R	AACACTAACGCATGCTTCACGCAGCTCGGTTTTTGC
F122M_F	AACACTAACATGTGCTTCACGCAGCTCGGTTTTTGC
F122M_R	AACACTAACCATTGCTTCACGCAGCTCGGTTTTTGC
F122A_F	AACACTAACGCGTGCTTCACGCAGCTCGGTTTTTGC
F122A_R	AACACTAACCGCTGCTTCACGCAGCTCGGTTTTTGC
F225Y_F	GGCAGCGGTTATAATGTTGTGGTTATTAAA
F225Y_R	CACAACATTATAACCGCTGCCTTCTGCCAG
F225H_F	GGCAGCGGTCATAATGTTGTGGTTATTAAA
F225H_R	CACAACATTATGACCGCTGCCTTCTGCCAG
Q155S_F	GTTCCGTATAGCTGGATTGTTCCGTTTGATCGTATT
Q155S_R	AACAATCCAGCTATACGGAACAGCATACATATAAAC
Q155T_F	GTTCCGTATACCTGGATTGTTCCGTTTGAT
Q155T_R	AACAATCCGGTGATACGGAACAGCATACAT
Q155N_F	GTTCCGTATAACTGGATTGTTCCGTTTGAT
Q155N_R	AACAATCCGTTGATACGGAACAGCATACAT
Q155C_F	GTTCCGTATTGCTGGATTGTTCCGTTTGAT
Q155C_R	AACAATCCGCAGATACGGAACAGCATACAT
Q155A_F	GTTCCGTATGCGTGGATTGTTCCGTTTGAT
Q155A_R	AACAATCCCGCGATACGGAACAGCATACAT
Y60F_F	GATCAGGGCTTTCTGCATAGCGATGTTACA
Y60F_R	GCTATGCAGAAAGCCCTGATCAAAGATGCT
Y60C_F	GATCAGGGCTGCCTGCATAGCGATGTTACA
Y60C_R	GCTATGCAGGCAGCCCTGATCAAAGATGCT
T178F_F	GTTCGTCGTTTTCCGCGCAGCTCTATTGAT
T178F_R	GCTGCGCGGAAAACGACGAACGCTCTGTGC
T178C_F	GTTCGTCGTTGCCCGCGCAGCTCTATTGAT
T178C_R	GCTGCGCGGGCAACGACGAACGCTCTGTGC

Table S3. Primers used for saturation mutagenesis of ATA117.

Primers	Sequence (5'-3')
Y23_F	TACATTACCNNKAGCGACTACGAGCTGGACCCTGCG
Y23_R	GTAGTCGCTMNNGGTAATGTAGTCCAGCCCCGTGTC
Y26_F	TACAGCGACNNKGAGCTGGACCCTGCGAACCCGCTG
Y26_R	GTCCAGCTCMNNGTCGCTGTAGGTAATGTAGTCCAG
L28_F	GACTACGAGNNKGACCCTGCGAACCCGCTGGCAGGT
L28_R	CGCAGGGTCMNNCTCGTAGTCGCTGTAGGTAATGTA
E42_F	GCATGGATTNNKGGCGCATTTGTTCCTCCGAGCGAA
E42_R	AAATGCGCCMNNAATCCATGCTGCCCCACCTGCCAG
R52_F	AGCGAAGCCNNKATTAGCATCTTTGATCAGGGCTAC
R52_R	GATGCTAATMNNGGCTTCGCTCGGAGGAACAAATGC
T101_F	CCGCCGCTGNNKCAGGATGAGGTTAAAGAAATTGCA
T101_R	CTCATCCTGMNNCAGCGGCGGGATAATACGCATTGA
P135_F	AGCAGCACCNNKGGCGAACGTGATATCACAAAACAT
P135_R	ACGTTCGCCMNNGGTGCTGCTATAACCGCGGGTAAT
V167_F	CGTGATGGTNNKCATGCAATGGTGGCACAGAGCGTT
V167_R	CATTGCATGMNNACCATCACGAATACGATCAAACGG
R180_F	CGTACCCCGNNKAGCTCTATTGATCCACAGGTTAAA
R180_R	AATAGAGCTMNNCGGGGTACGACGAACGCTCTGTGC
A209_F	GGTTTTGAANNKCCGCTGCTGCTGGATGGTGATGGC
A209_R	CAGCAGCGGMNNTTCAAAACCACGATCATGTGTTTC
L219_F	GATGGCCTGNNKGCAGAAGGCAGCGGTTATAATGTT
L219_R	GCCTTCTGCMNNCAGGCCATCACCATCCAGCAGCAG
G239_F	CGTAGTCCGNNKCGTGCAGCCCTGCCGGGTATTACC
G239_R	GGCTGCACGMNNCGGACTACGCACAACTCCATCTTT
L252_F	AAAACCGTTNNKGAAATTGCAGAAAGCCTGGGACAT
L252_R	TGCAATTTCMNNAACGGTTTTACGGGTAATACCCGG
F290_F	GTTTGGCCANNKGTTAGCGTTGATGGTAATCCTATT
F290_R	AACGCTAACMNNTGGCCAAACGCCGCCTGCTGTAGT
I310_F	ACCCAAAGCNNKATTCGTCGTTATTGGGAACTGAAT
I310_R	ACGACGAATMNNGCTTTGGGTAACCGGACCCGGAAC

Table S4. Engineered strains constructed in this study.

Engineered strains	Recombinant plasmids in the strain
BL21(01)	pRSFDuet-*ATA117-Mu9*, pETDuet-*Anclaao-Swpdc*
BL21(02)	pRSFDuet-*ATA117-Mu9*, pCDFDuet-*Anclaao-Swpdc*
BL21(03)	pRSFDuet-*ATA117-Mu9*, pACYCDuet-*Anclaao-Swpdc*
BL21(04)	pETDuet-*ATA117-Mu9*, pRSFDuet-*Anclaao-Swpdc*
BL21(05)	pETDuet-*ATA117-Mu9*, pCDFDuet-*Anclaao-Swpdc*
BL21(06)	pETDuet-*ATA117-Mu9*, pACYCDuet-*Anclaao-Swpdc*
BL21(07)	pCDFDuet-*ATA117-Mu9*, pRSFDuet-*Anclaao-Swpdc*
BL21(08)	pCDFDuet-*ATA117-Mu9*, pETDuet-*Anclaao-Swpdc*
BL21(09)	pCDFDuet-*ATA117-Mu9*, pACYCDuet-*Anclaao-Swpdc*
BL21(10)	pACYCDuet-*ATA117-Mu9*, pRSFDuet-*Anclaao-Swpdc*
BL21(11)	pACYCDuet-*ATA117-Mu9*, pETDuet-*Anclaao-Swpdc*
BL21(12)	pACYCDuet-*ATA117-Mu9*, pCDFDuet-*Anclaao-Swpdc*

Table S5. Comparison of E-factor for different synthetic routes to (1R, 2R)-3

Synthetic route	**Number of steps**	**E-factor (water-free)** [a]	**Main Solvents/Reagents**	**Reference**
This work	1	45	4-(Methylsulfonyl)benzaldehyde, D-Serine, L-Serine	This work
Chemical route	4	342	4-(Methylsulfonyl)benzaldehyde, Glycine, Sulfuric acid, Ethanol, Potassium borohydride, D-tartrate salt	[b]

[a] E-factor calculated excluding process water to enable consistent comparison across different methodologies.

[b] Xia, Yingqi, et al. "Continuous-Flow Synthesis of syn-2-Amino-1, 3-diol via Catalytic Hydrogenation: A Vital Intermediate of (+)-Thiamphenicol and (+)-Florfenicol." *Organic Process Research & Development* 26.9 (2022): 2656-2664.

Figure S1.

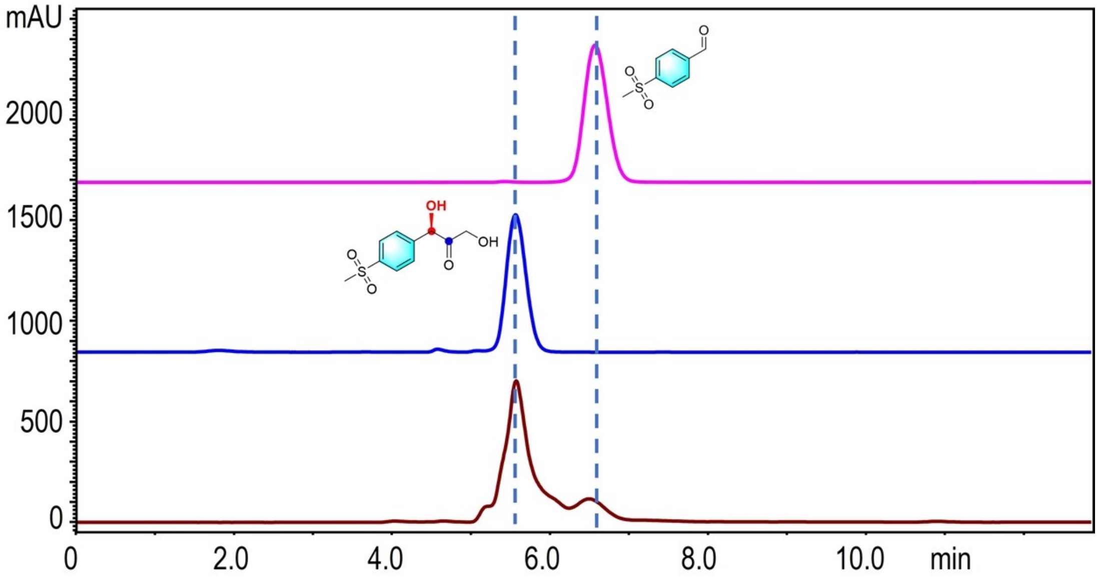


Figure S1. HPLC chromatogram for converting **1** and 4-Mb to (*R*)-**2** catalyzed by SwPDC. Pink line represents compound 4-Mb standard, blue line represents compound (*R*)-**2** standard, brown line represents experimental sample.

Figure S2.

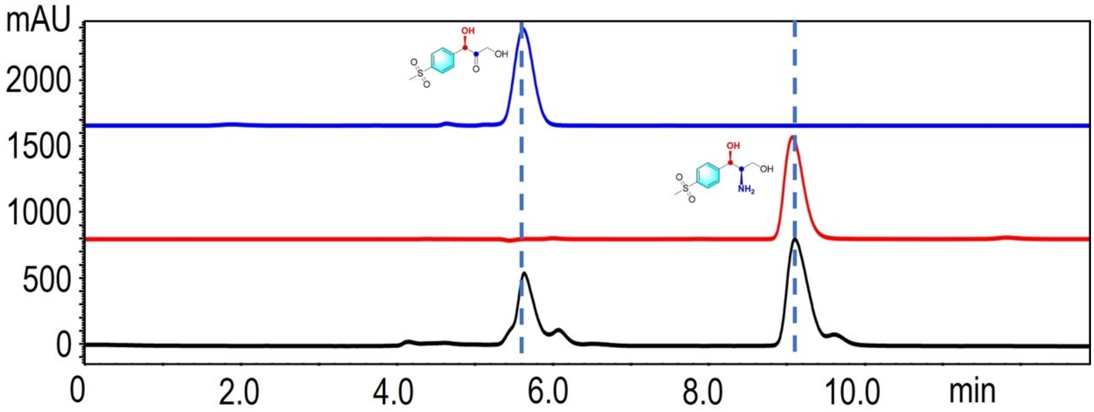


Figure S2. HPLC chromatogram for converting (*R*)-**2** to (1*R*,2*R*)-**3** catalyzed by ATA117. Blue line represents compound (*R*)-**2** standard, red line represents compound (1*R*,2*R*)-**3** standard, black line represents experimental sample.

Figure S3

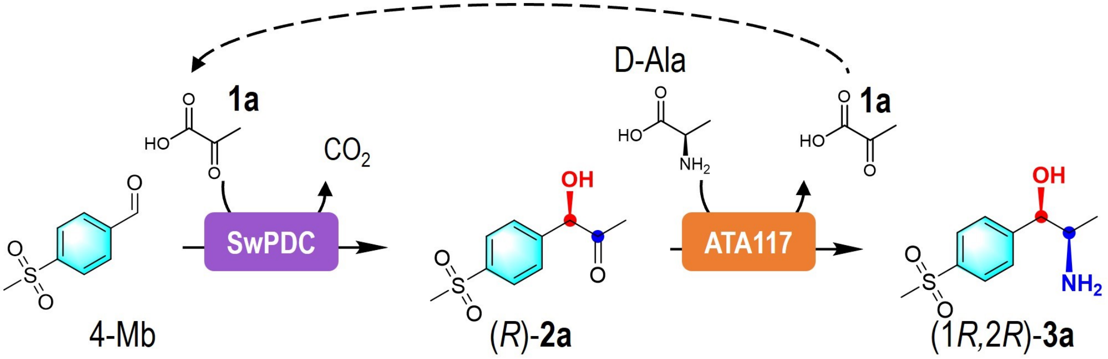


Figure S3. Scheme of the side reaction catalyzed by SwPDC and ATA117 when using D-Ala as the amine donor. (*R*)-**2a**: (*R*)-1-hydroxy-1-(4-(methylsulfonyl)phenyl)propan-2-one; (1*R*,2*R*)-**3a**: (1*R*,2*R*)-2-amino-1-(4-(methylsulfonyl)phenyl)propan-1-ol

Figure S4

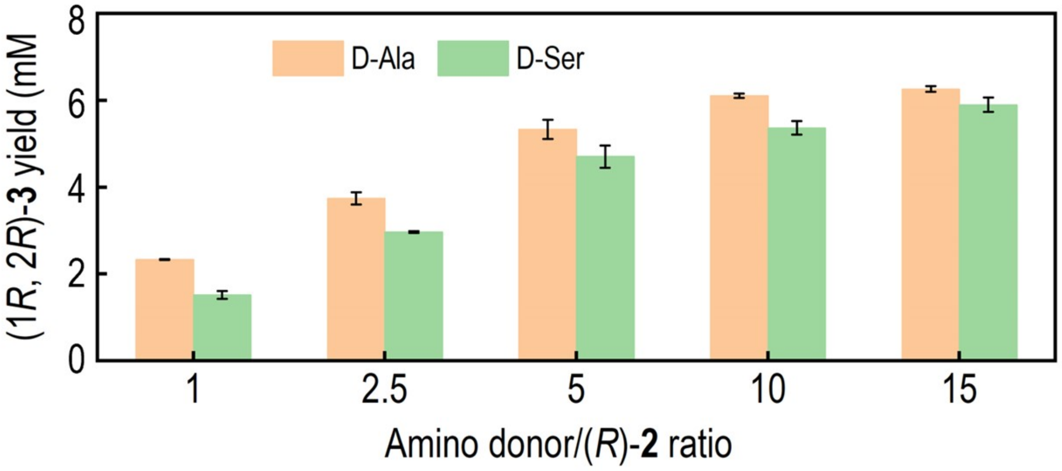


Figure S4. The production of (1*R*,2*R*)-**3** catalyzed by ATA117 from substrate (*R*)-**2** was investigated using D-Ala and D-Ser as ammonia donors. The initial concentration of (*R*)-**2** in the reaction system was set at 20 mM, and the addition amount of each ammonia donor was 1-15 molar equivalents relative to (*R*)-**2**. The reaction was conducted at 37°C for 2 hours, and the concentration of the resulting product (1*R*,2*R*)-**3** was quantified via HPLC.

Figure S5.

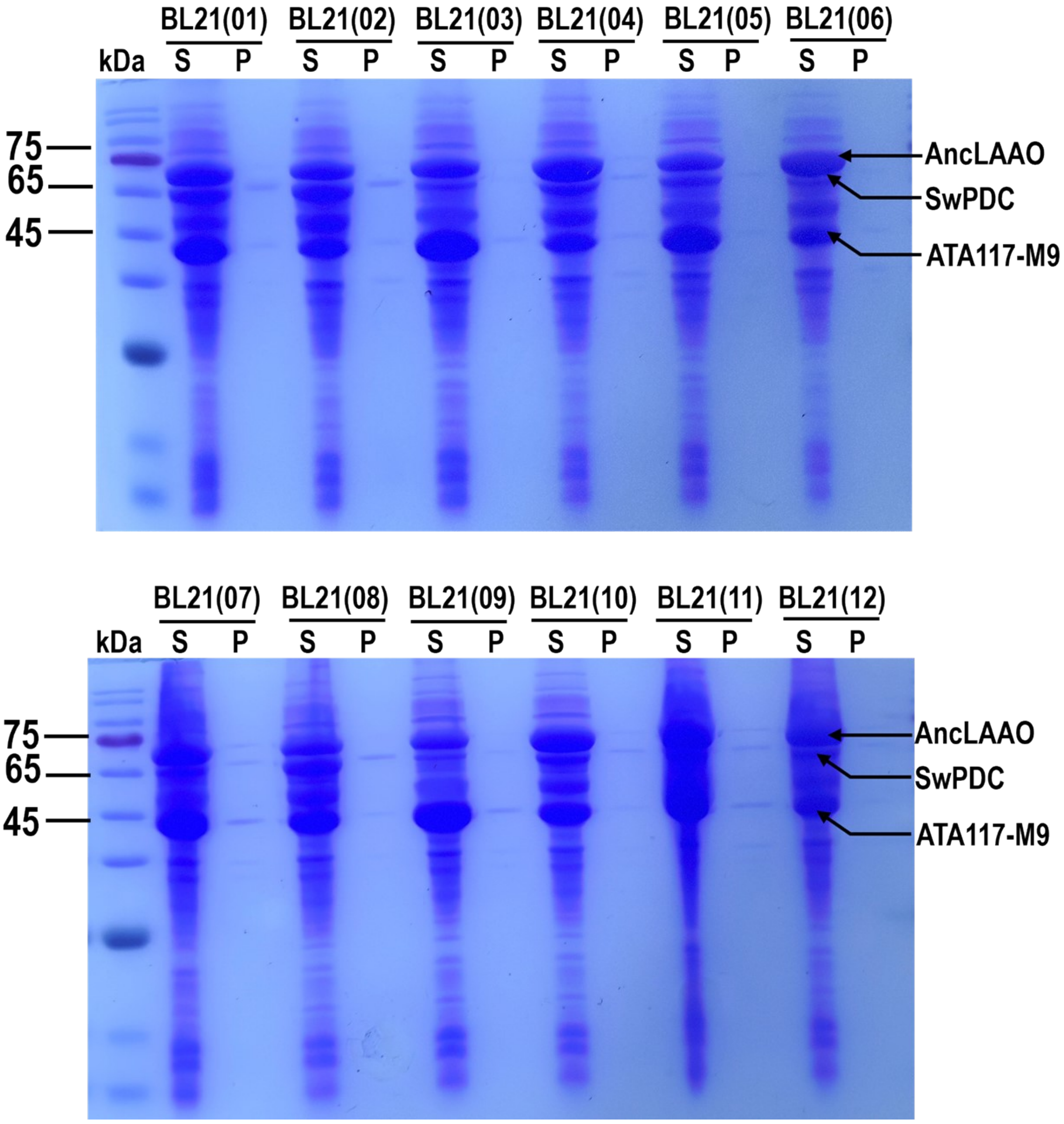


Figure S5. SDS-PAGE analysis of engineered strains BL21(01) to BL21(12) co-expressing AncLAAO (74 kDa), SwPDC (67 kDa) and ATA117-M9 (40 kDa). The theoretical molecular weights of AncLAAO, SwPDC and ATA117-M9 were calculated by SnapGene. S, supernatant of the lysate; P, precipitate of the lysate.

Figure S6

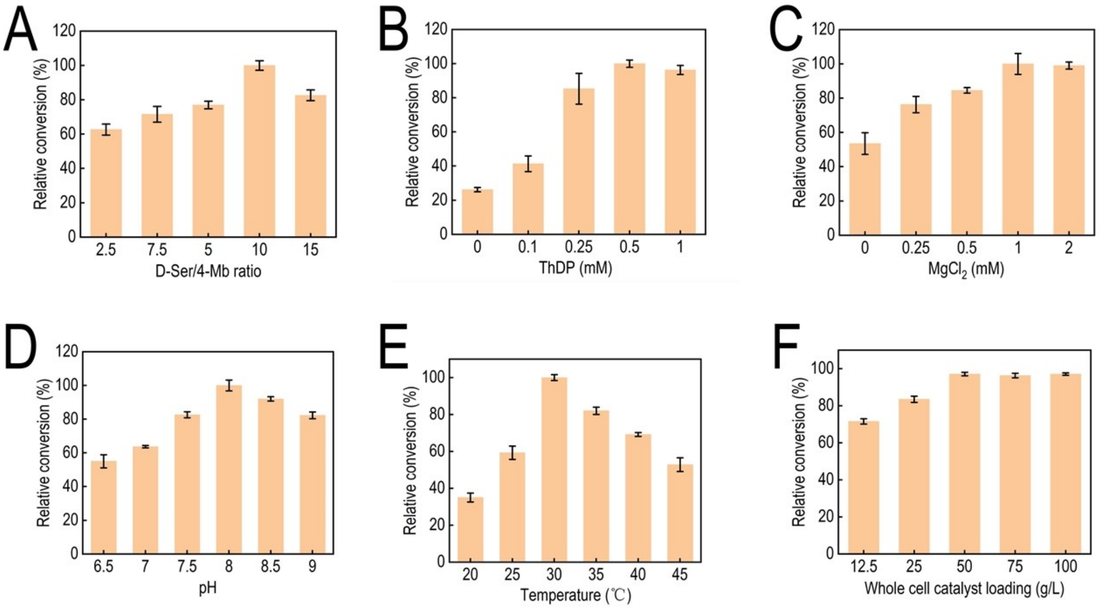


Figure S6. Effects of reaction parameters for the synthesis of (1*R*,2*R*)-**3** by engineering strain BL21(01). The effects of D-Ser/4-Mb ratio (A), ThDP addition (B), MgCl$_2$ addition (C), pH (D), Temperature (E), and whole cell catalyst (wet weight) loading (F) on the synthesis of (1*R*,2*R*)-**3** were analyzed. All experiments were performed in triplicate and the data were presented as the mean ± SEM.

Figure S7.

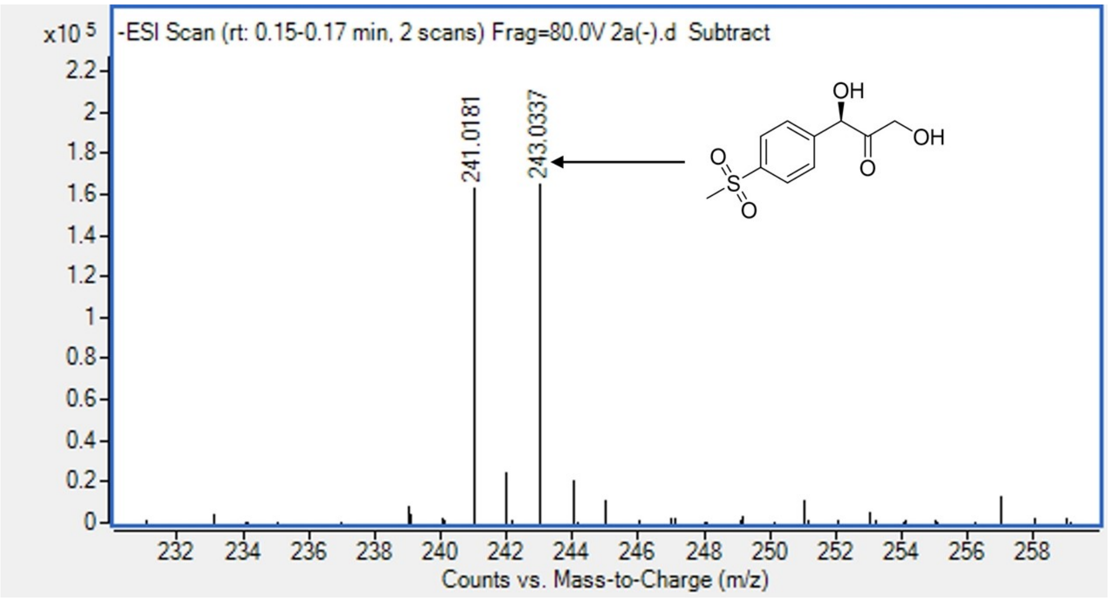
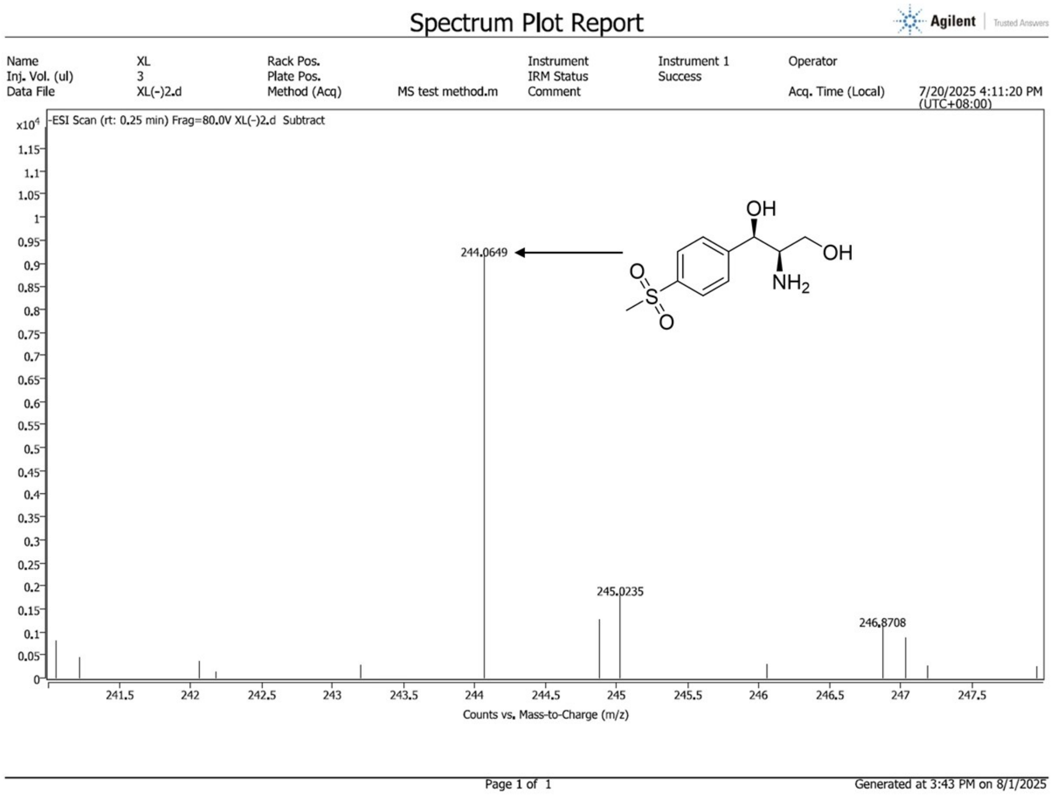


Figure S7. The experimental sample catalyzed by SwPDC using **1** and 4-Mb as substrates was evaluated using liquid chromatography-mass spectrometry (LC-MS). (*R*)-**2** (*m/z*) (M-H)$^-$: calcd. for $C_{10}H_{12}O_5S$, 243.0333, found 243.0337.

Figure S8.



Spectrum Plot Report

Name	XL	Rack Pos.		Instrument	Instrument 1	Operator	
Inj. Vol. (ul)	3	Plate Pos.		IRM Status	Success		
Data File	XL(-)2.d	Method (Acq)	MS test method.m	Comment		Acq. Time (Local)	7/20/2025 4:11:20 PM (UTC+08:00)

-ESI Scan (rt: 0.25 min) Frag=80.0V XL(-)2.d Subtract

Page 1 of 1

Generated at 3:43 PM on 8/1/2025

Figure S8. The experimental sample for one-pot synthesis of (1*R*,2*R*)-**3** was evaluated using liquid chromatography-mass spectrometry (LC-MS). (1*R*,2*R*)-**3** (*m/z*) $(M\text{-}H)^-$: calcd. for $C_{10}H_{15}NO_4S$, 244.0643, found 244.0649.

Figure S9.

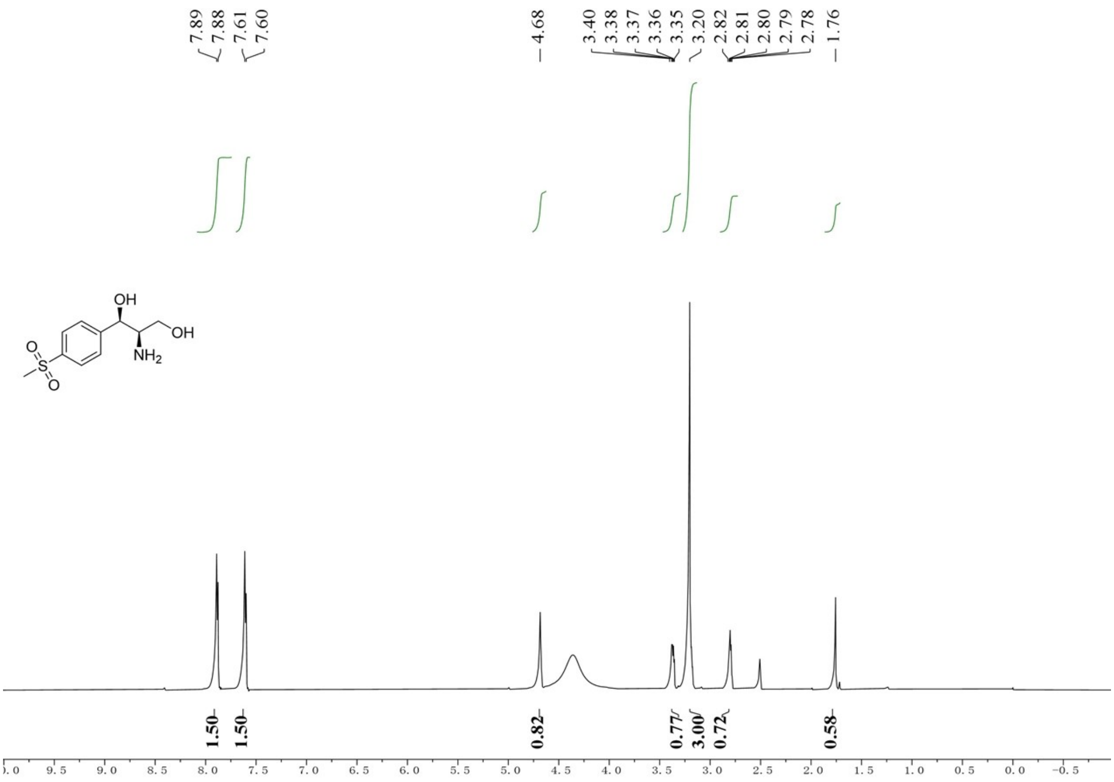


Figure S9. ^{1}H- NMR spectra of (1*R*,2*R*)-**3**. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 7.89 (d, *J* = 6.0 Hz, 2H), 7.61 (d, *J* = 6.0 Hz, 2H), 4.68 (d, *J* = 6.0 Hz, 2H), 3.40-3.35 (m, 1H), 3.20 (s, 3H), 2.82-2.78 (m, 1H), 1.76 (s, 1H).