Supporting Information

Synthesis of a Sacubitril Precursor by Construction of Chemoenzymatic Cascades

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1.General information

All chemicals were purchased from Bide Pharmatech Ltd., Shanghai Macklin Biochemical Co., or Anhui Zesheng Technology Co., Ltd. and used without further purification unless otherwise stated. All solvents were dried and degassed by standard methods and stored under nitrogen.

HPLC analysis was performed on a Shimadzu system equipped with an LC-40B XR binary pump, SIL-40C autosampler unit, SPD-40V diode array detector, and CTO-40C temperature-controlled column oven.

Flash chromatography was performed using EM Science silica gel 60 (100-300 mesh). NMR spectra were recorded on a Bruker AVANCE III 400 MHz NMR spectrometer (Bruker Biospin, Germany, 7.47 μs 90 pulses, 1.00 s relaxation delay and 16 scans) in $CDCl_3$ and DMSO-d_6.

2. Screening of Enzyme Library for ene-reductases

Colonies were picked and incubated in 700 μL LB medium containing kanamycin (50 μg mL^{-1}), shaken in a 96-well plate at 180 rpm and cultured at 37°C for 8 hours. Then, 300 μL of TB medium was added to a 96-well plate, and IPTG and kanamycin were added to make the final concentrations of 0.2 mM and 50 μg mL^{-1} (TB medium, IPTG and antibiotics were pre-mixed and sub-packed), and the protein was cultured at 30°C at 800 rpm for 16 h. The cell precipitate was collected and washed. The cell pellets were harvested and washed with 500 μL of 50 mM, pH 7.5 PBS buffer and centrifuged for 10 min with 4000 rpm at 4°C. Then, 6 units mL^{-1} DNase I and 1 mg mL^{-1} lysozyme, 10 mM **5**, 20 mM glucose, 2.5 g L^{-1} GDH, 1 mM $NADP^+$ and 20% DMSO were added to the 96-well plate and reacted at 30°C at 800 rpm for 24 hours. The remaining substrates and products were extracted with MTBE and analyzed by HPLC (OJ-H, eluents: heptane/i-PrOH = 70 : 30, 254 nm, 30 °C, flow rate: 1.0 mL min^{-1}).

3. Preparation of the biocatalysts

3.1 Sequence information

DNA sequence of *Go*ER (WP_011252080.1):

ATGCCGACACTGTTTGATCCGATTGATTTTGGTCCGATTCATGCCAAAAATCGCAT
TGTTATGAGTCCGCTGACACGTGGTCGTGCAGATAAAGAAGCAGTTCCGACACCG
ATTATGGCAGAATATTATGCACAGCGTGCAAGCGCAGGTCTGATTATTACCGAAG
CAACCGGTATTAGCCGTGAAGGTTTAGGTTGGCCGTTTGCACCTGGTATTTGGAG
TGATGCACAGGTTGAAGCATGGAAACCGATTGTTGCCGGTGTTCATGCAAAAGGT
GGTAAAATTGTTTGTCAGCTGTGGCACATGGGTCGTATGGTTCATAGCAGCGTTA
CCGGTACACAGCCGGTTAGCAGCAGCGCAACCACCGCACCGGGTGAAGTTCATA
CCTATGAAGGTAAAAAACCGTTTGAACAGGCACGTGCAATTGATGCAGCAGATA
TTAGTCGTATCCTGAACGATTATGAAAATGCAGCACGTAATGCAATTCGTGCAGG
TTTTGATGGTGTTCAGATTCATGCAGCAAATGGCTATCTGATTGATGAATTTCTGC
GTAATGGCACCAATCATCGTACCGATGAATATGGTGGTGTTCCGGAAAATCGTAT
TCGCTTTCTGAAAGAAGTTACCGAACGCGTTATTGCAGCAATTGGTGCAGATCGT
ACCGGTGTTCGTCTGAGCCCGAATGGTGATACCCAGGGTTGTATTGATAGCGCAC
CGGAAACCGTTTTTGTTCCGGCAGCAAAACTGCTGCAGGATCTGGGTGTTGCATG
GCTGGAACTGCGTGAACCGGGTCCGAATGGCACCTTTGGTAAAACCGATCAGCC
GAAACTGAGTCCGCAGATTCGTAAAGTTTTTCTGCGTCCGCTGGTTCTGAATCAG
GATTATACCTTTGAAGCAGCACAGACCGCACTGGCCGAAGGTAAAGCAGATGCA
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TGGTATTGCACTGCAGCCGGATGATATGAAAACCTGGTATAGCCAGGGTCCTGAA
GGTTATACCGATTATCCGAGCGCGACCAGCGGTCCGAATCTCGAGCACCACCACC
ACCACCACTGA

Protein sequence of *Go*ER:

MPTLFDPIDFGPIHAKNRIVMSPLTRGRADKEAVPTPIMAEYYAQRASAGLIITEATGI
SREGLGWPFAPGIWSDAQVEAWKPIVAGVHAKGGKIVCQLWHMGRMVHSSVTGTQ
PVSSSATTAPGEVHTYEGKKPFEQARAIDAADISRILNDYENAARNAIRAGFDGVQIH
AANGYLIDEFLRNGTNHRTDEYGGVPENRIRFLKEVTERVIAAIGADRTGVRLSPNGD
TQGCIDSAPETVFVPAAKLLQDLGVAWLELREPGPNGTFGKTDQPKLSPQIRKVFLRP
LVLNQDYTFEAAQTALAEGKADAIAFGRKFISNPDLPERFARGIALQPDDMKTWYSQ
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DNA sequence of ω-ATA CDX-043:

ATGAACAAACCGCAGAGCTGGGAAACGCGTGCAGAAACCTATAGCCTGATGGGT
CATACCGATATGCCGAGCCTGCATCAGCGTGGCACCATGGTGGTGACCCATGGCG
AAGGCCCGTATATTTTTGATGTGCATGGCCGTCGTTATCTGGATGCGTGCAGCGG
CGCTGCGAACATGGTGGCGGGCTTTGATCATCCAGGCCTGATTGATGCGGCGAAA
ACGCAGTATGAACGTTTTCCGGGCTATCACGCGGTTAATGGCCGTATGAGCGATC
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AACGTCCGAACATTGGCGAATATCGTGGCATTGGCTTTATGTGGGCGCTGGAAGT
CGTGAAAGATAAAGCGAGCAAAGCCCCGTTTCCCGGCAACCTGAGCGTGAGCGA
ACGTATTGCGAACACCTGCACCGATCTGGGCCTGATTGTGCGTGCGCTGGGCCAG
AGCGTGGCGCTGGCGCCGCCGTTTATTCTGACCGAAGCGCAGATGGATGAAATGT
TCGATAAACTGGAAAAAGCGCTGGATAAAGTGTTCGCGGAAGTGGCGAAGCTTG
CGGCCGCACTCGAGCACCACCACCACCACCACTGA

Protein sequence of ω-ATA CDX-043[1]:

MNKPQSWETRAETYSLMGHTDMPSLHQRGTMVVTHGEGPYIFDVHGRRYLDACSG
AANMVAGFDHPGLIDAAKTQYERFPGYHAVNGRMSDQTVMLSEKLVEVSPFDSGR
VFYTNSGSEANDTMVKMLWFLHAAEGKPQKRKILTHWNAYHGATAVSASMTGFP

WNSVFGLPLPGFLHLTCPHYWRYGEEGETEEQFVARLAHELEETIQKEGADTIAGFF
AEPVMGAGGVIPPAKGYFQAILPILRKYDIPVISDEVITGFGRTGNTWGCVTYDFTPD
AIISAKGLTAGYFAVGAVILGPELSKRLETAIELREWFPHAFTTGGHPVGCAIALKAID
VVMNEGLAENVRRLAPRFEERLKHIAERPNIGEYRGIGFMWALEVVKDKASKAPFPG
NLSVSERIANTCTDLGLIVRALGQSVALAPPFILTEAQMDEMFDKLEKALDKVFAEV
AKLAAALEHHHHHH*

Protein sequence of *Nt*ER:

MAEEVSNKQVILKNYVTGYPKESDMEIKNVTIKLKVPEGSNDVVVKNLYLSCDPYM
RSRMRKIEGSYVESFAPGSPITGYGVAKVLESGDPKFQKGDLVWGMTGWEEYSIITPT
QTLFKIHDKDVPLSYYTGILGMPGMTAYAGFHEVCSPKKGETVFVSAASGAVGQLV
GQFAKMLGCYVVGSAGSKEKVDLLKSKFGFDEAFNYKEEQDLSAALKRYFPDGIDI
YFENVGGKMLDAVL

Protein sequence of *Cp*ER:

MSSPLSDTLVFEPIKVGDVTLSNRIVLCPTTRFRAAKSGNDVSNHLPSDLILDHYAQR
AQYPGTLLVTEATYVSPQAGGYDGVPGIYTVEQTKAWKKVVDGVHAKKSFISLQG
WFLGRVGDPRVLKKEGQKYVSVSPIYPDDRSKKLSEKAGLRLTELSIEDLKQIIHQDY
ANAAKNAMEAGFDFFELHGAHGYLLDTFLHENTNHRTDQYGGSIEKRASFILELIDH
LSTVIPSSKLAIRLSPWAEIQGVVEETSPIPQFSYLLAQLQKRADSGKPLGYISVVEPRV
QGVVTVSLDSIKGTNDFVESVWKGVTLRAGNYTYDAPEFNQIKKDVSNGRTLVGFS
RFFTSNPDLVYKLKNDPSKLVKYDRKSFYQPYNWGYNTFDGQSYDEEAEKKRYAQP
IKRSEANL

Protein sequence of *Cl*ER

MVAVKPLKDTEIFKPTKVGNHELSNKIVYAPTTRMRAIADHTPSDLAYKYYDDRTK
YPGSLVITEATLMSPKTGLYDRVPGIYTDEHVAGWKKITDKIHANGSKVSMQLWPLG
RVADPVATKKAGYPLVAPSLIYPSEEAKKAAEEAGNPIHVLTTEEVEDLVNDFVHAA
KKAVAAGVDYVEVHGAHGYLVDTFFQVSTNKRTDKYGGSIENRARFALEILDRLIEE
IGAERVAIRISPWAKFQGILAEEGEVNPVAQFGYFLSELENRARAGKRIAYVSIVEPRV
SGVIDVAGEDIQGDNSFVRSVWKGIVIKAGNYTYDAPEFKTLLQDVSDGKTLVAFAR
YFTSNPDLVQRLHDGAD
LTPYKRELFYAPSNWGYNTFTNAGETKTFSEEEESKRLPAPIDTKA

Protein sequence of *CiER*

MVAVRALKDTQVFTPTTVGAHTFSNKIVYAPTTRRRALENNVPSDLAAQYYDDRSK
FPGSLVITEATIASPRFGMYERVPGIYNDEQVQAWKKITDKVHANGSFASIQLWNLGR
VADPKYTKATGYPLVAPSAVYHSDEAKRAAEEAGNPLRELTTQEVEEFVQDFIKAG
KNSVAAGFDYVEVHGAHGYLVDQFFNPSTNQRTDKYGGSIENRARFALEVIDGLIAA
IGADKVAIRLSPWAKFQNVKAENEDVSPVAQLGYFLGELQKRAKEGKELAYVSIVEP
RVSGIVNVDVSEQFGDNSFVRSIWKGVVIKAGNYTYDAPEFKTALEDISDGKTLVAFS
RFFTSNPD
LVQRLHDGVDLVAYNRDTFYNSDNWGYNTFNASGSNIVFDEAVERQRIPLPIH

Protein sequence of *SsER*

MSESLKLLTPVQVGRYELRNRIVMAPLTRNRATGPDNIPNDLNVLYYQQRASAGLIIT
EASQISPQGQGYPLTPGIHSPEQVEGWKPIVQAVHDRGGCIFLQLWHVGRISHPSLQP
DGALPVAPSAIQPAGMAATFQGEQPFVTPRALETEEIAGIVEDYRRAAENALAAGFD
GVEVHGANGYLIDQFLQDGTNQRSDRYGGSFENRSRFLREVLDAVISVWGSDRVGL
RLSPWGQFNDMRDSDPVGLFSYVAQMLNPYNLAYLHWIEPRWDKAEESPEFNQMA
TPVFRSLYNGPVIAAGGYSRSTAEAAIASGAADLVAFGRLYISNPDLVERFALDAPLN
PYDRNTFYGGDEHGYTDY PSLEAASV

Protein sequence of *ClER-2*

MVAVKALKNTDVFKPIKVGKHELSNRIVYAPTTRKRALPDHTPSDLQYKYYDDRTK
FPGSLVITEATILSPKTGLYENVPGIYTESHVAAWKKITDKIHANKSRVSMQLWPLGR
VADPVATKEAGYPLIAPTASYESEESRKAAEAAGNPVHELTTSEVEDLVDDFVNTAK
NAVAAGVDYVEVHGAHGYLVDTFFQVSANSRTDKYGGSIENRSRLALEIIDRLIEEIG
ADRVAIRISPWVKFQGILAEEGEVSPVAQFGHFLGELENRARAGKRIAYVSIVEPRFG
GGGDISPENIYGDNSFAKSVWKGILIKAGNYTYDAPEFNTLVEDVSDGKTLVAFARY
FTSNPD
LVQRLHDGTDLTPYNRALFYNNSNWGYNTFNSAGEATVFDEEKEQKRLPAPIDVTK
AQL

Protein sequence of *ChER*

MAPPPTPVVARPLEDTVIFKPLQVGKNELSNRIVFAPSTRFRALEDHSPSDLQLEYYDE
RSKYPGTLLTVEGTLPSKKTGSYAFVPGIYTDKHVREWKKVTDKIHENKSFVTVQLW

GLGRVADPAQNKKEGQKLKGPSALYDHPRSEKAAKKAENEIEAYTTEEIDDLVNEYS
KAAKNSVAAGFDYVEFHCAHGYLFNQFFAPSANKRTDKYGGSIENRARFILSVIDRL
SDEIGSERLAVRISPWATVYGIQAQKDEVHPITIYSHFLNELQKRADAGRPIAYVSVVE
PRVSGVFDVAEKDIAGDNDFVKAVWKGVVMKAGNYTYDAPKFKSLLDDTEDGRTL
VGFSRYFISNPDLVYRLRDGRELTPYDRKTFYKTTNWGYNTYKRFEDERQFDEEAEK
KRRAAPIAVAATHAKL

Protein sequence of *YbER*

MKTAKLFSPLKVGALTLPNRVFMAPLTRLRSIEPGDIPTPLMAEYYRQRASAGLIITEA
TQISFQAKGYAGAPGLHTQEQLNAWKKITQAVHEEGGHIAVQLWHVGRISHSSLQPG
QQAPVAPSAIAADTRTTVRDENGAWVRVPCSTPRALETEEIPGIINDFRQATANAREA
GFDYIELHAAHGYLLHQFMSPASNQRTDQYGGSIENRTRLTLEVVDATAAQWSAERI
GIRISPLGPFNGLDNGEDQEEAALYLIDELNKRHIAYLHISEPDWAGGKPYSEAFRDA
VRARFKGVIIGAGAYTAEKAEELIEKGFIDAVAFGRSYISNPDLVARLQQHAPLNEPD
GETFYGGGAKGYT DYPTL

Protein sequence of *CsER*

MKDKYKVLYDPIKIGKLEIKNRYVLAPMGPGGMCNADGSFNKRGIEFYVERAKGGT
GLIMTGVTMVENNIEKCALPSMPCPTINPLNFITTGNEMTERVHAYGSKIFLQLSAGF
GRVSIPSIVGKVAVAPSKIPHRFLPGVTCRELTTEEVKEYVKAFGESAEIAKKAGFDG
VEIHAVHEGYLLDQFAISFFNHRTDEYGGSLENRLRFACEVVQEIKKRCGQDFPVSLR
YSIKSFIKDWCKGGLPDEEFEEKGRDIPEGIEAAKILVAAGYDALNGDVGSYDSWYW
SHPPMYQKKGLYLPYNEILKKVVDVPIITAGRMEDPELSSDAILSGKTDMIALGRPLL
ADAEIPNKIFEDKYDKVRPCLSCQEGCMGRLQNFATVSCAVNPACGREKEYGLKKA
EQIKKVLVVGGGVAGMEAARVTAVRGHKVTLIEKNGYLGGNIVPGGIPDFKDDDRA
LVKWYEGILKDLGVEIKLNVGASKENIKEFGADEVLLATGSSPRTLTIEGADKVYSAE
DVLMERKTVGEKVIVIGGGLVGCETALWLKQQGKEITIVEMQNDILQVGGPLCHAN
HDMLVDLIKFNKIDVKTSSYISKKTDEGFVLNTNGEESIINADSAVVAIGYLSEKDLYS
EVRFDIPNARLIGDANKVQNIMYAIWSAYEVAKNI

Protein sequence of *RoER*

MSVLFEPITFRGVTVPNRVWMAPMCQYSADVTGRDVGVPGDWHRTHLVTRAIGGA
GLILTEATAVSPEGRISPADLGIWNDTQTEAFAEINAQLEYFGAVPGIQLGHAGRKGS

AHVPWRGGGSLDGDDRLSWQTVAPSAIGFGDHTPPAAATTADIRKVVADFAAAAER
ASRAGFKVVEIHAAHGYLLHQFLSPVSNHRTDEYGGSFAGRIRLLLEVVDAVRGVWP
AELPVFVRVSATDWLSEEPGLDADSWTPDQTVSLVQALADLGVDLVDVSSGGVASA
RIPIGPGYQVPFARRIQNETTVPAAAVGLITEPEQAERIVESGEAVAVFLGRELLRDPY
WPRKAALVLNAQVTPQIPAQYARAY

3.2 Heterologous expression of ene-reductase (*Go*ER)

The plasmid containing ene-reductase from *Gluconobacter oxydans* on *E. coli* pET 24a. was expressed in *E. coli* BL21 (DE3) on agar plates spiked with kanamycin (50 μg mL^{-1}), individual colonies were selected and inoculated into pre-cultures (50 mL LB medium), and then the pre-cultures were inoculated into 1 L of TB medium containing kanamycin (50 μg mL^{-1}). Cultures were grown at 37 °C, 220 rpm until OD600 = 0.6-0.8, then induced with IPTG (0.1 mM) and continued to incubate for 16 h at 20 °C, 220 rpm. Subsequently, cells were collected and washed with 50 mM, pH 7.5 PBS buffer and centrifuged for 20 min with 5000 rpm at 4 °C. cell pellets were packaged and stored at -80°C for further use.

3.3 Heterologous expression of amine transaminase (ω-ATA CDX-043)

The plasmid containing amine transaminase (ω-ATA CDX-043) on *E. coli* pET 28a. was expressed in *E. coli* BL21 (DE3) on agar plates spiked with kanamycin (50 μg mL^{-1}), individual colonies were selected and inoculated into pre-cultures (50 mL LB medium), and then the pre-cultures were inoculated into 1 L of TB medium containing kanamycin (50 μg mL^{-1}). Cultures were grown at 30°C, 220 rpm until OD600 reach 0.6-0.8, then induced with IPTG (1 mM) and continued to incubate for 16 h at 20°C, 220 rpm. Subsequently, cells were collected and washed with 50 mM, pH 7.5 PBS buffer and centrifuged for 20 min with 5000 rpm at 4°C. cell pellets were packaged and stored at -80°C for further use.

3.4 Construction of *E. coli* strains co-expressing ene-reductase (*Go*ER) and amine transaminase (ω-ATA CDX-043)

The fragments with corresponding homologous arms were amplified by PCR from the previously constructed genomes of ene-reductase (*Go*ER) and amine transaminase (ω-ATA CDX-043). All primers used in the studies are listed in Table S3. The PCR products were digested with restriction enzyme (DpnI) and recovered by gel-cutting. The vector (pET 24a,

pET 28a, pET Duet 1 and pACYC Duet 1) was also subjected to the above treatment, thereby obtaining a fragment with a homologous arm. For the vector (pET 24a and pET 28a), the resulting three fragments were then ligated using ClonExpress II One Step Cloning Kit C113 to obtain a recombinant plasmid containing two genes (enoate reductase and amine transaminase). For the vector (pET Duet 1 and pACYC Duet 1), two gene fragments were connected to the vector fragment separately using ClonExpress II One Step Cloning Kit C112 to obtain a recombinant plasmid containing two genes (enoate reductase and amine transaminase).

3.5 Purification of ene-reductase (*Go*ER) and amine transaminase (ω-ATA CDX-043)

Protein purification was accomplished with AKTA pure equipped with a 5 mL His Trap ™ FF column pre-equilibrated with washing buffer. After loading with crude extract, the column was washed with 5 column volumes (CV) of washing buffer followed by protein elution with 3 CV of elute buffer. Subsequently, the collected protein samples were dialyzed into desalted solution at 4 ℃ for 12 hours, and then transferred to a flexible dialysis tube (30 kDa cut-off value). After SDS-PAGE analysis, protein samples were divided into equal parts and frozen in liquid nitrogen before storage at -80℃.

Table S1. Buffer compositions used for protein purification.

Buffer	Composition
Washing buffer	25 mM PBS, 30 mM iminazole, pH 7.4
Elute buffer	25 mM PBS, 500 mM iminazole, pH 7.4
Desalination solution	25 mM PBS, 5% glycerol , pH 7.4

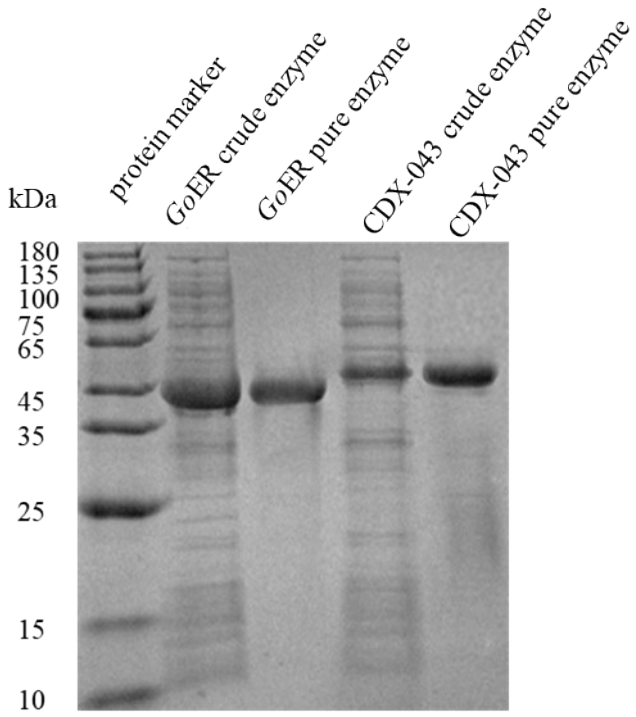
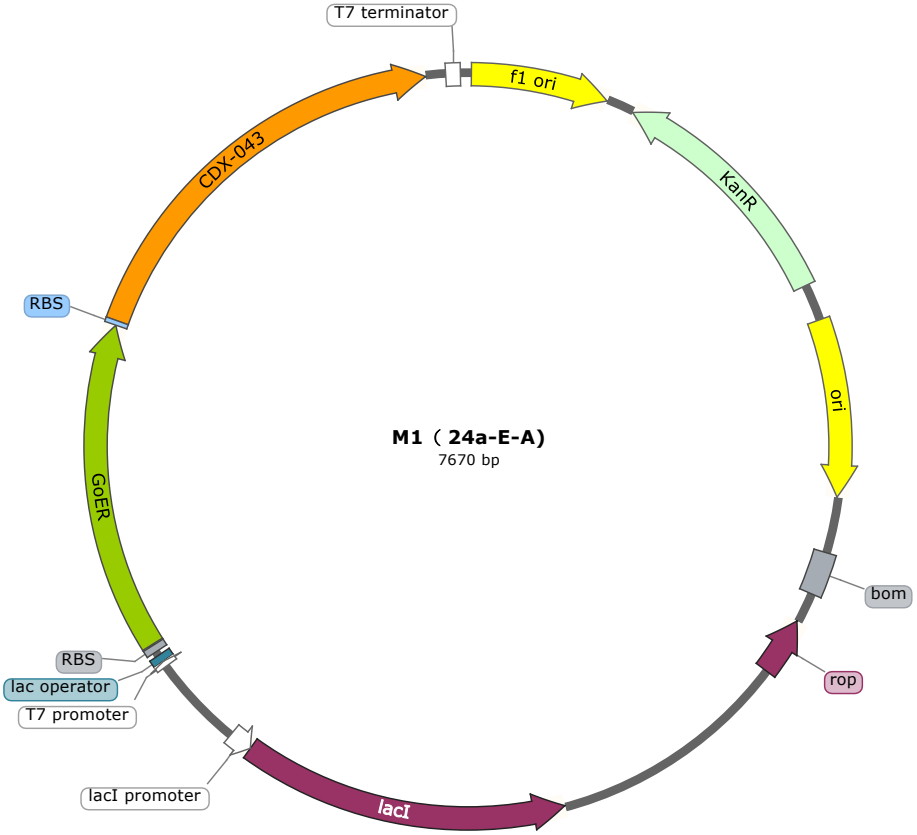


Figure S1. SDS-Page of crude and purified ene-reductase (*Go*ER) and amine transaminase (ω-ATA CDX-043).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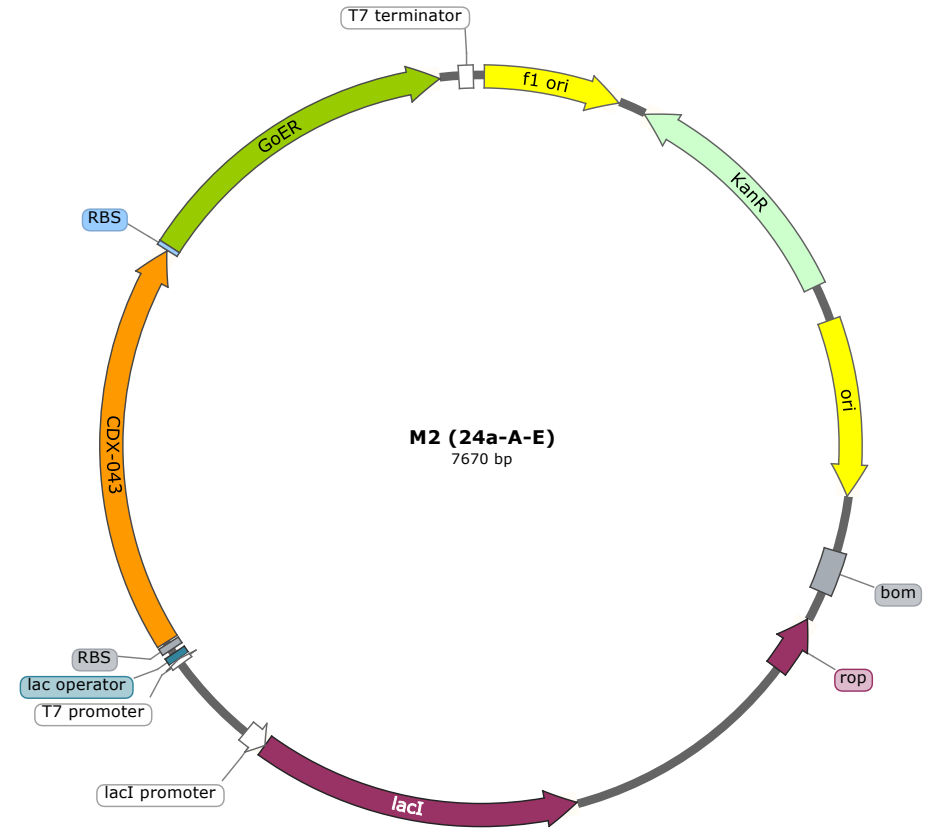
f1 ori
KanR
ori
bom
rop
lacI
lacI promoter
T7 promoter
lac operator
RBS
GoER
RBS
CDX-043
M1（24a-E-A)
7670 bp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f1 ori
KanR
ori
bom
rop
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RBS
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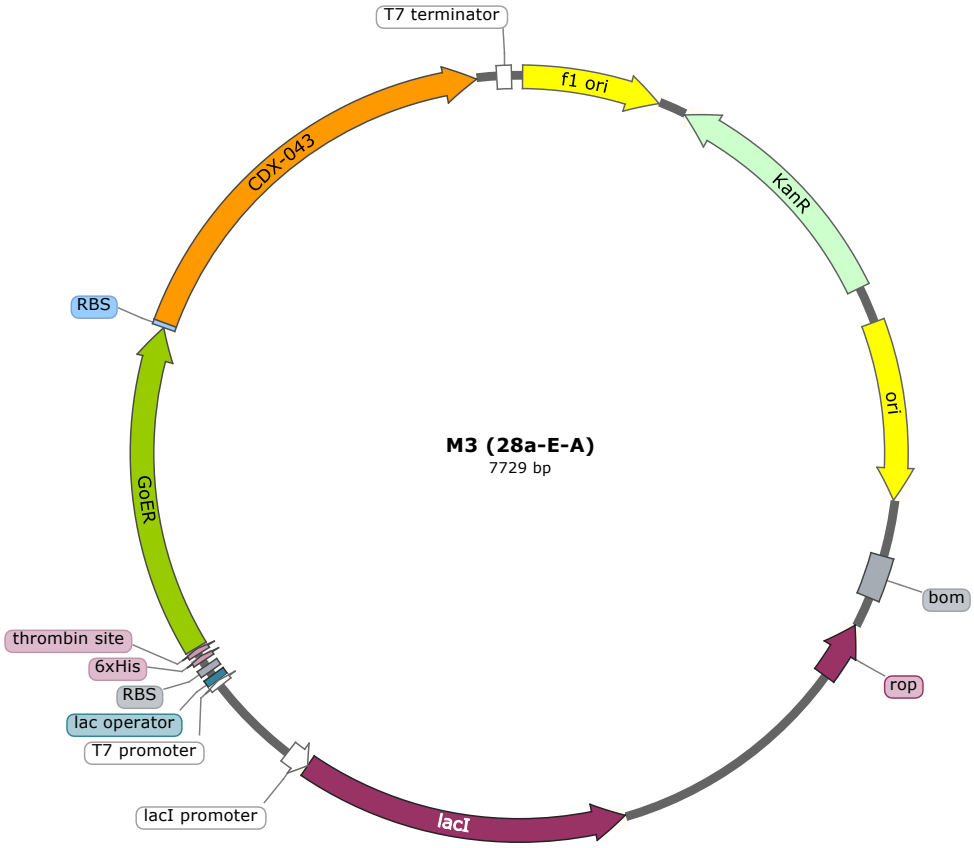
T7 terminator
f1 ori
KanR
ori
bom
rop
lacI
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T7 promoter
lac operator
RBS
6xHis
thrombin site
RBS
GoER
CDX-043
M3 (28a-E-A)
7729 bp


T7 terminator
f1 ori
KanR
ori
bom
rop
lacI
lacI promoter
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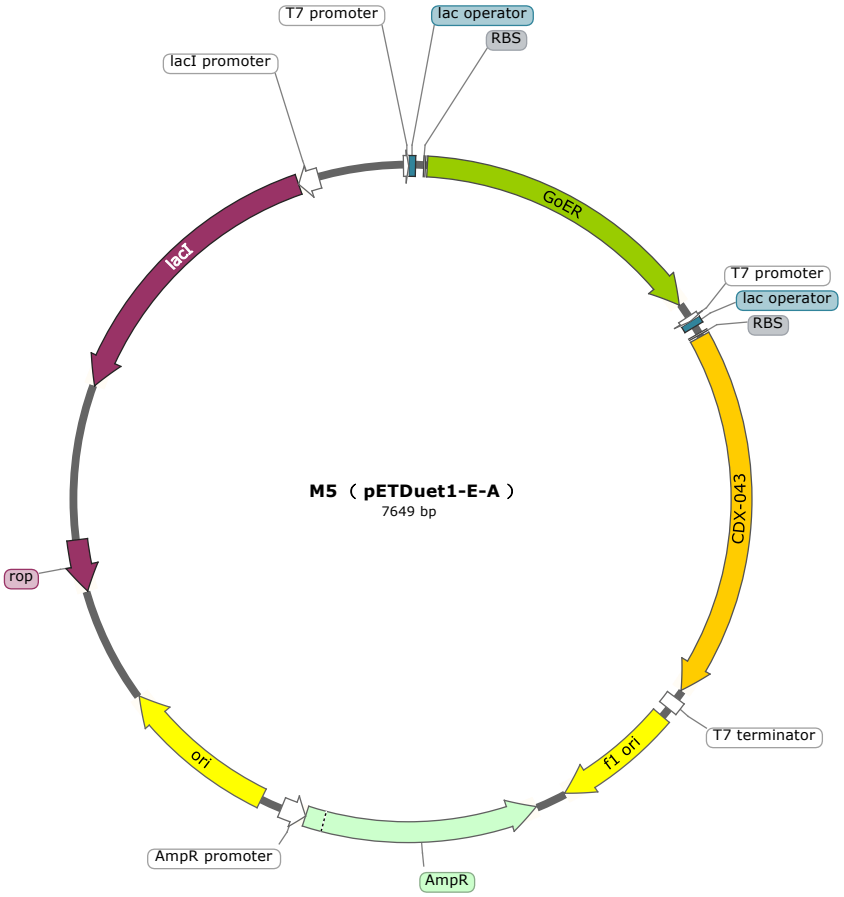
CDX-043
GoER
M4 (28a-A-E)
7729 bp


T7 promoter
lac operator
RBS
lacI promoter
GoER
lacI
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lac operator
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CDX-043
rop
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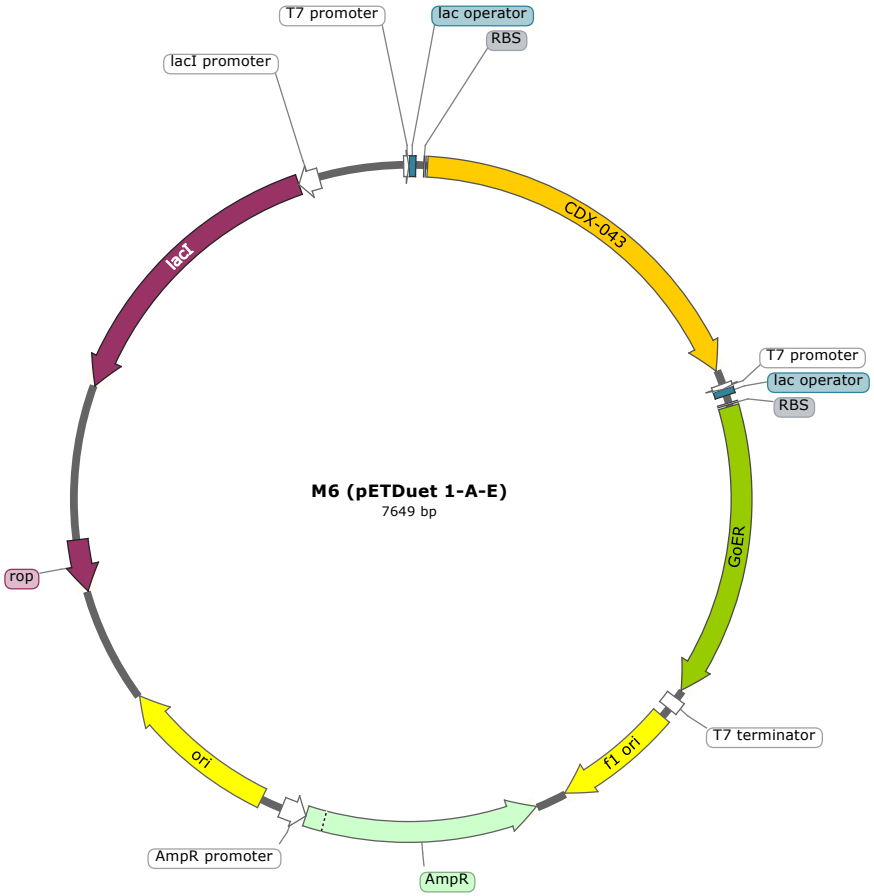
ori
AmpR promoter
AmpR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lac operator
RBS
lacI promoter
CDX-043
lacI
T7 promoter
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rop
T7 terminator
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ori
AmpR promoter
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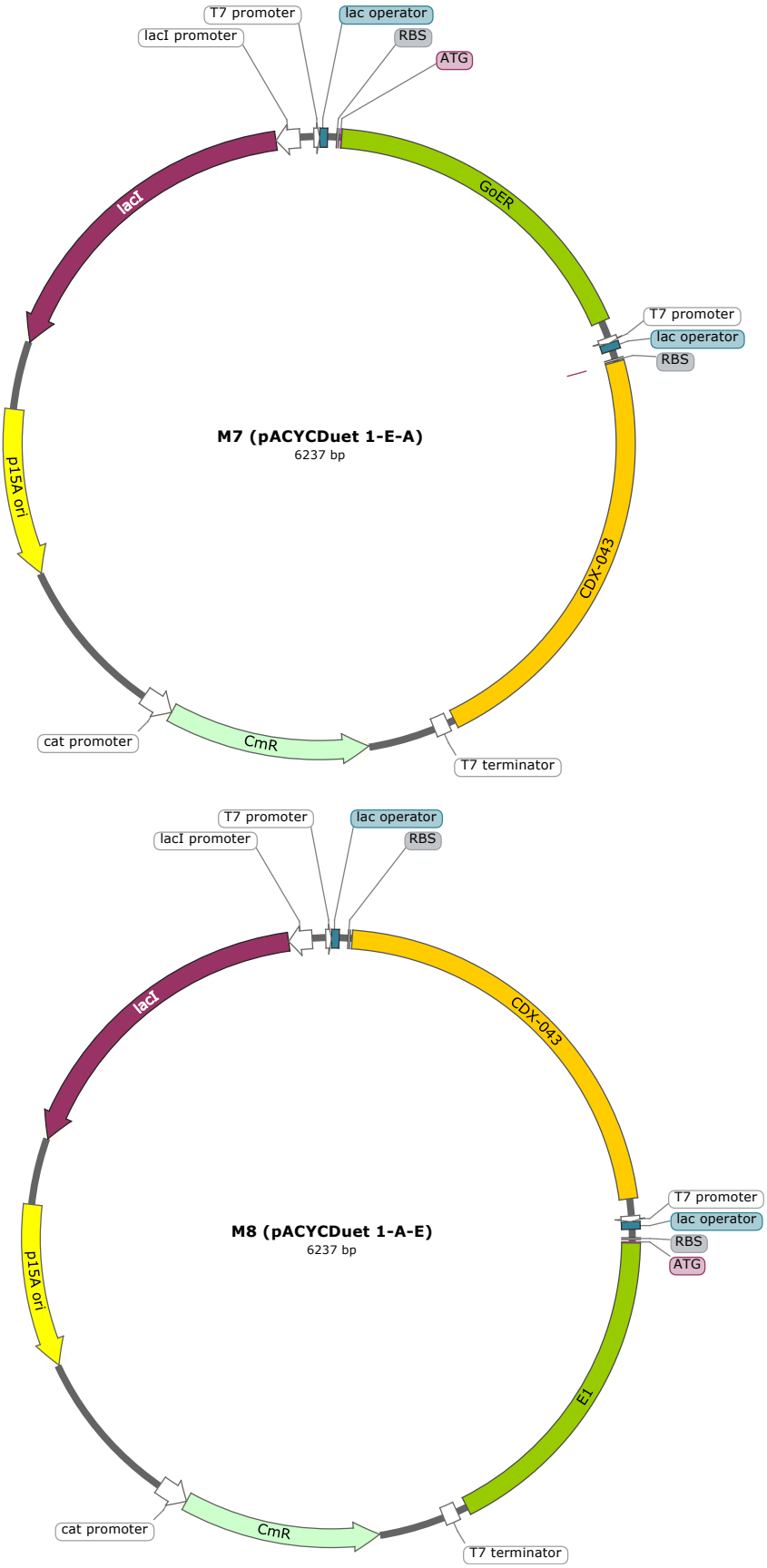


Figure S2. Plasmid maps of Module M1-8

Table S2. Key primers used in this study

plasmid	Name of primers	Sequences (5'-3')
M1	M1-A-F	gaattaaggaggtgacaatatgatgaacaaaccgcagagctgggaaacgcg
	M1-V-R	catcatattgtcacctccttaattcggaccgctggtcgcgctcggataatcgg
	M1-V-F	cggaagtggcgtgagatccggctgctaacaaagcccgaaaggaagctgagttg
	M1-A-R	cggatctcacgccacttccgcgaacactttatccagcgctttttcc
M2	M2-V-R	gcggtttgttcatatgtatatctccttcttaaagttaaacaaaattatttctagagggg
	M2-A-F	gatatacatatgaacaaaccgcagagctgggaaacgcgtg
	M2-A-R	gcatcatattgtcacctcctcacgccacttccgcgaacactttatccagc
	M2-E-F	gcgtgaggaggtgacaatatgatgccgacactgtttgatccgattgattttg
	M2-E-R	cagccggatcttaattcggaccgctggtcgcgctcggataatcggtata
	M2-V-F	gtccgaattaagatccggctgctaacaaagcccgaaaggaagct
M3	M3-V-R	cagtgtcggcatatggctgccgcgcggcaccaggccgctgctgtgatgatgatgatg
	M3-E-F	gcagccatatgccgacactgtttgatccgattgattttgg
	M3-E-R	catcatattgtcacctccttaattcggaccgctggtcgcgctcggataatcgg
	M3-A-F	gaattaaggaggtgacaatatgatgaacaaaccgcagagctgggaaacgcg
	M3-A-R	cggatctcacgccacttccgcgaacactttatccagcgctttttcc
	M3-V-F	cggaagtggcgtgagatccggctgctaacaaagcccgaaaggaagctgagttg
M4	M4-V-R	gcatcatattgtcacctcctcacgccacttccgcgaacactttatccagc
	M4-E-F	gcgtgaggaggtgacaatatgatgccgacactgtttgatccgattgattttg
	M4-E-R	cagccggatcttaattcggaccgctggtcgcgctcggataatcggtata
	M4-V-F	gtccgaattaagatccggctgctaacaaagcccgaaaggaagct
M5	M5-V-R	cagtgtcggcatggtatatctccttcttaaagttaaac
	M5-E-F	gatataccatgccgacactgtttgatccgattgattttggtccgattc
	M5-E-R	cttaagcattaattcggaccgctggtcgcgctcggataatcg
	M5-V-F	ggtccgaattaatgcttaagtcgaacagaaagtaatcgtattgtacacggc
	M5-V-R2	gcggtttgttcatatgtatatctccttcttatacttaactaatatac
	M5-A-F	gatatacatatgaacaaaccgcagagctgggaaacgcgtgcagaaac

	M5-A-R	ggttaattacgccacttccgcgaacactttatccagcgcttttc
	M5-V-F2	cgcggaagtggcgtaattaacctaggctgctgccaccgctgagc
	M6-V-R	gcggtttgttcatggtatatctccttcttaaag
	M6-A-F	gatataccatgaacaaaccgcagagctgggaaacgcgtgcagaaac
	M6-A-R	cttaagcattacgccacttccgcgaacactttatccagcg
M6	M6-V-F	gaagtggcgtaatgcttaagtcgaacagaaagtaatcg
	M6-V-R2	cagtgtcggcatatgtatatctccttcttatacttaactaatatac
	M6-E-F	gatatacatatgccgacactgtttgatccgattgattttggtccg
	M6-E-R	ctaggttaattaattcggaccgctggtcgcgctcggataatcggtataac
	M6-V-F2	ggtccgaattaattaacctaggctgctgccaccgctgagc
	M7-V-R	cagtgtcggcatggtatatctccttattaaagttaaac
	M7-E-F	gatataccatgccgacactgtttgatccgattgattttggtccg
	M7-E-R	cttaagcattaattcggaccgctggtcgcgctcggataatc
M7	M7-V-F	ggtccgaattaatgcttaagtcgaacagaaagtaatcgtattgtacacgg
	M7-V-R2	gcggtttgttcatatgtatatctccttcttatacttaactaatatactaagatgggg
	M7-A-F	atatacatatgaacaaaccgcagagctgggaaacgcgtgcagaaacctatagcc
	M7-A-R	ctaggttaattacgccacttccgcgaacactttatccagcgcttttc
	M7-V-F2	aagtggcgtaattaacctaggctgctgccaccgctgagcaataactag
	M8-V-R	ggtttgttcatggtatatctccttattaaag
	M8-A-F	agatataccatgaacaaaccgcagagctgggaaacgcgtgcagaaacctatag
	M8-A-R	cttaagcattacgccacttccgcgaacactttatccagcgcttttc
M8	M8-V-F	gaagtggcgtaatgcttaagtcgaacagaaagtaatcgtattg
	M8-V-R2	agtgtcggcatatgtatatctccttcttatacttaactaatatac
	M8-E-F	gatatacatatgccgacactgtttgatccgattgattttggtcc
	M8-E-R	ctaggttaattaattcggaccgctggtcgcgctcggataatcgg
	M8-V-F2	gtccgaattaattaacctaggctgctgccaccgctgagcaataa

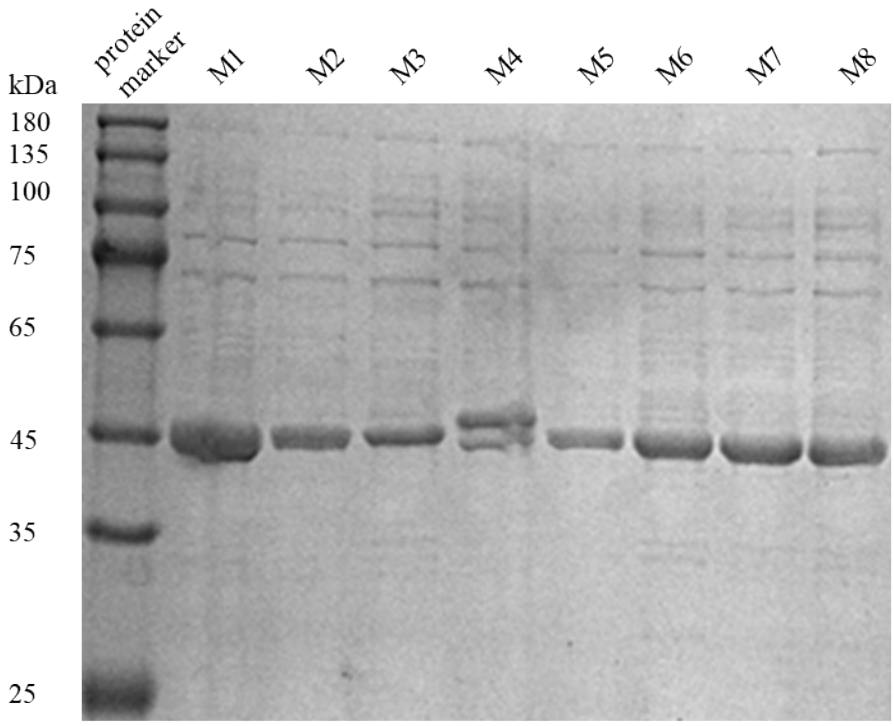
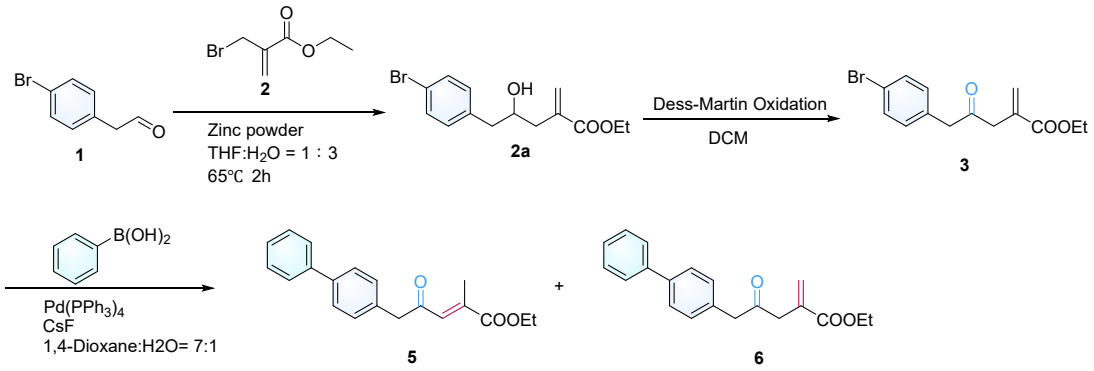


Figure S3. SDS-Page analysis of cell-free extract of *E. coli* co-expressing *Go*ER and ω-ATA CDX-043 (M1-M8).

4. General procedures for the synthesis of substrate 5

We designed two routes. Due to the more steps and lower conversions in route 2, we chose route 1 to synthesize substrate 5.

4.1 General procedures for Route 1



4.1.1 General procedures for the synthesis of 2a

2-(4-bromophenyl)acetaldehyde **1** (2 g, 10 mmol) and ethyl 2-(bromomethyl)acrylate

(2.88 g, 15 mmol) in 20 mL of THF and 90 mL saturated ammonium chloride solution were added to a suspension of Zn (0.98 g, 15 mmol) in anhydrous THF (10 mL) under reflux. The THF solution containing Zn was added dropwise to the reaction mixture and stirred under reflux for 2 h. Finally, the reaction mixture was cooled to room temperature, and quenched with 50 mL 10% AcOH. The reaction mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine and dried with anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:9) to yield **2a** (2.835 g, 90%) as a pale green oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 2H), 7.12 (d, *J* = 6.4 Hz, 2H), 6.27 (s, 1H), 5.67 (s, 1H), 4.22 (q, *J* = 6.4 Hz, 2H), 3.99 (dq, *J* = 8.7, 4.3 Hz, 1H), 2.82 – 2.68 (m, 2H), 2.61 (dd, *J* = 14.6, 4.2 Hz, 1H), 2.39 (dd, *J* = 14.0, 8.5 Hz, 1H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.66, 137.52, 137.45, 131.51, 131.23, 127.93, 120.31, 71.23, 61.11, 43.04, 39.84, 14.20.

4.1.2 General procedures to the synthesis of 3

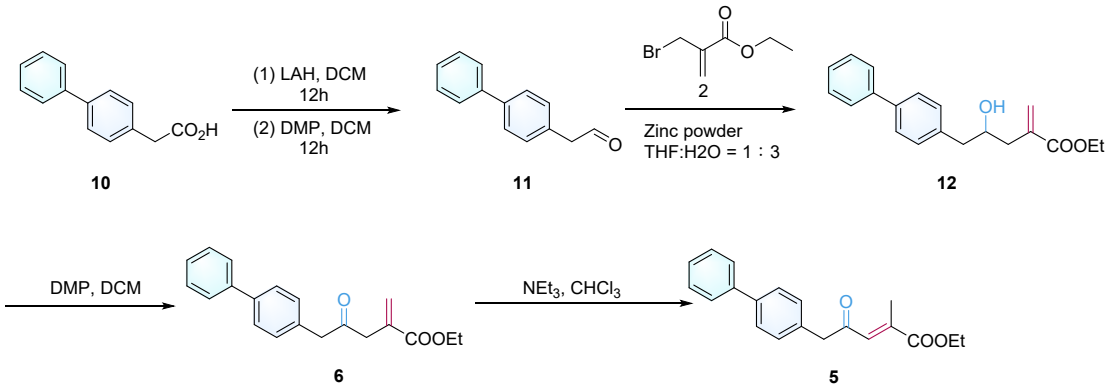
To a solution of ethyl 5-(4-bromophenyl)-4-hydroxy-2-methylenepentanoate **2a** (2 g, 6.4 mmol) in dichloromethane (50 mL) was added the Dess-Martin periodinane (3.26 g, 7.68 mmol), stirred for 2 h, filtered and washed with saturated sodium bicarbonate aqueous solution (50 mL) and brine (50 mL), then dried over anhydrous Na_2SO_4. The organic layer was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:9) to yield **3** (1.85 g, 93%) as a pale green oil. ^{1}H NMR (400 MHz, CDCl3) δ 7.48 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H), 6.36 (s, 1H), 5.64 (s, 1H), 4.20 (q, J = 7.1 Hz, 2H), 3.77 (s, 2H), 3.45 (s, 2H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 204.14, 166.26, 134.28, 132.89, 131.81, 131.33, 128.84, 121.18, 61.15, 48.98, 45.25, 14.16.

4.1.3 General procedures to the synthesis of 5

To a 100 mL round-bottom flask was charged with ethyl 5-(4-bromophenyl)-2-methylene-4-oxopentanoate (1 g, 3.22 mmol), phenylboronic acid (589 mg, 4.83mmol), CsF (978 mg, 6.44 mmol) and $Pd(PPh_3)_4$ (111 mg, 0.00966 mmol) and was evacuated and charged with argon three times. Then 1,4- dioxane (28 mL) and water (4 mL) were added and the reaction was heated at

105°C for 16h under argon atmosphere. The mixture was poured into water and extracted with EtOAc three times. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:19 to 1: 9) to yield **5** (594 mg, 60%) as a pale green oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.60 (m, 4H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 1.6 Hz, 1H), 4.29 (q, *J* = 7.2 Hz, 2H), 3.93 (s, 2H), 2.28 (d, *J* = 1.6 Hz, 3H), 1.36 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.71, 167.53, 142.44, 140.77, 140.23, 132.54, 131.34, 130.02, 128.84, 127.61, 127.38, 127.12, 61.70, 51.29, 14.57, 14.19.

4.2 General procedures for Route 2



4.2.1 General procedures to the synthesis of 11

To a cooled (0°C) solution of **10** (1.0 g, 4.7 mmol) in 30 mL of anhydrous DCM, LAH (0.53 g, 14 mmol) was added portion wise while stirring, under argon. The resulting reaction mixture was stirred at r.t. for 12 h and then cooled to 0 °C. The mixture was poured into water, washed with 1.0 M HCl and extracted with EtOAc three times. The organic layer was washed with brine and dried over anhydrous Na_2SO_4, evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1: 4) to yield 2-([1,1'-biphenyl]-4-yl)ethanol **11a** (835 mg, 90 %) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, *J* = 11.9, 7.5 Hz, 4H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.37 (dd, *J* = 14.8, 7.7 Hz, 3H), 3.95 (t, *J* = 6.5 Hz, 2H), 2.96 (t, *J* = 6.5 Hz, 2H).

To the solution of ([1,1'-biphenyl]-4-yl)ethanol (835 mg, 4.21 mmol) in dichloromethane (30 mL) was added the Dess-Martin periodinane (2.33 g, 5.5 mmol), stirred for 3 h, filtered and

washed with saturated sodium bicarbonate aqueous solution (50 mL) and brine (50 mL), then dried over anhydrous Na_2SO_4. The organic layer was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:5) to yield **11** (500 mg, 60 %) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.67 – 7.60 (m, 4H), 7.48 (s, 2H), 7.34 (d, *J* = 8.0 Hz, 3H), 3.78 (d, *J* = 1.8 Hz, 2H).

4.2.2 General procedures to the synthesis of 12

11 (500 mg, 2.55 mmol) and ethyl 2-(bromomethyl)acrylate (2.88 g, 3.825 mmol) in 10 mL of THF and 60 mL of H_2O were added to a suspension of Zn (0.98 g, 15 mmol) in anhydrous THF (10 mL) at reflux. The THF solution containing Zn was added dropwise to the reaction mixture and stirred under reflux for 2 h. Finally, the reaction mixture was cooled to room temperature, and quenched with 50 mL 10% AcOH. The reaction mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine and dried with anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:5) to yield **12** (515 mg, 65 %) as a pale green oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.55 (m, 4H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.36 (dd, *J* = 12.7, 7.7 Hz, 3H), 6.32 (d, *J* = 1.4 Hz, 1H), 5.72 (s, 1H), 4.29 – 4.23 (m, 2H), 4.10 (s, 1H), 2.88 (qd, *J* = 13.7, 6.5 Hz, 2H), 2.71 (dd, *J* = 14.0, 2.7 Hz, 1H), 2.48 (dd, *J* = 13.7, 8.5 Hz, 1H), 2.29 (s, 1H), 1.35 (d, *J* = 7.2 Hz, 3H).

4.2.3 General procedures to the synthesis of 6

To the solution of **12** (515 mg, 1.66 mmol) in dichloromethane (20 mL) was added Dess-Martin periodinane (844 mg, 1.99 mmol), stirred at room temperature for 3 hours, filtered and washed with saturated sodium bicarbonate aqueous solution (50 mL) and brine (50 mL), then dried over anhydrous Na_2SO_4. The organic layers was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:9) to yield **6** (440 mg, 86 %) as a pale green oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.55 (m, 4H), 7.48 (s, 2H), 7.34 (d, *J* = 8.2 Hz, 3H), 6.43 – 6.31 (m, 1H), 5.78 – 5.56 (m, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.87 (s, 2H), 3.51 (s, 2H), 1.32 (d, *J* = 7.1 Hz, 3H).

4.2.4 General procedures to the synthesis of 5

To the solution of **6** (440 mg, 1.43 mmol) in chloroform (20 mL) was added triethylamine(0.994 mL, 7.15 mmol), stirred at room temperature for 12 hours. the solvent was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:9) to yield **5** (132 mg, 30 %) as a pale green oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.60 (m, 4H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 1.6 Hz, 1H), 4.29 (q, *J* = 7.2 Hz, 2H), 3.93 (s, 2H), 2.28 (d, *J* = 1.6 Hz, 3H), 1.36 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.71, 167.53, 142.44, 140.77, 140.23, 132.54, 131.34, 130.02, 128.84, 127.61, 127.38, 127.12, 61.70, 51.29, 14.57, 14.19.

5. Comparison of oxidants used for synthesis of 3

For the oxidation reaction of 3, different oxidants (DMP, Pyridinium Chlorochromate (PCC), Pyridinium Dichromate (PDC), Jones reagent) were compared. We found that the conversions by DMP and PCC were comparable, and Jones reagent afforded slightly lower conversion. We finally chose to use DMP for the oxidation reaction.

Table S3. Screening of oxidants

Reagents	Solvents	equiv	temperature (°C)	Reaction time (h)	Yields (%)
DMP	DCM	1.2	rt	2	93
PCC	DCM	1.2	rt	2	92
Jones reagent	DCM	1.2	0-20	2	83
PDC	DCM	1.2	rt	2	25

5.1 General procedure for synthesis of 3 with DMP

Detailed reaction procedure was described in Section 4.1.2

5.2 General procedure for synthesis of 3 with Jones reagent

In a 100 mL reaction flask, **2a** (3 mmol) was dissolved in DCM (20 mL) and the solution was cooled to 0°C. The Jones reagent (1.2 eq) was then added dropwise. The reaction was monitored by TLC analysis. After completion of the reaction, i-PrOH (1 mL) was added to quench the excess Jones reagent. The mixture was poured into water and extracted with EtOAc three times. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:19 to 1: 9) to yield **3** (775 mg, 83%).

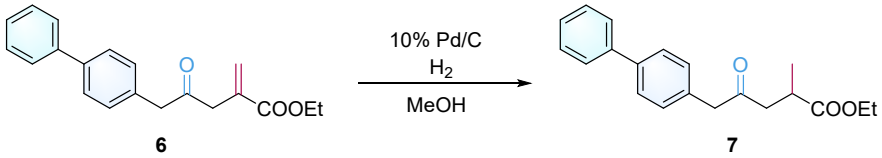
5.3 General procedure for synthesis of 3 with PCC

To a solution of **2a** (3 mmol) in dichloromethane (50 mL) was added the PCC (1.5 eq), stirred for 2 h, filtered and extracted with EtOAc three times, then dried over anhydrous Na_2SO_4. The organic layer was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:19 to 1: 9) to yield **3** (858 mg, 92%).

5.4 General procedure for synthesis of 3 with PDC

To a solution of **2a** (3 mmol) in dichloromethane (50 mL) was added the PDC (1.5 eq), stirred for 2 h, filtered and extracted with EtOAc three times, then dried over anhydrous Na_2SO_4. The organic layer was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc : petroleum ether = 1:19 to 1: 9) to yield **3** (234 mg, 25%).

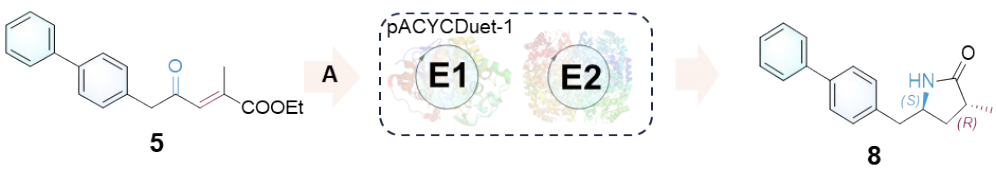
6. General procedures for the chemical synthesis of product 7



6 **7**

A mixture of ethyl 5-([1,1'-biphenyl]-4-yl)-2-methylene-4-oxopentanoate **6** (1 g, 3.24 mmol) and 10% Pd/C (50 mg) was subjected to standard hydrogenation condition at room temperature and atmospheric pressure (balloon) for 12 h. On completion, the reaction mixture was filtered and evaporated to give a precipitate, which was purified by column chromatography to yield **7** as a colorless oil (0.96 g, 95 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.58 (m, 4H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 3.80 (s, 2H), 3.05 – 2.93 (m, 2H), 2.61 – 2.50 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.19 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.37, 175.78, 140.79, 140.08, 133.06, 129.95, 128.83, 127.52, 127.35, 127.11, 77.30, 60.68, 49.86, 45.18, 34.91, 17.14, 17.10, 14.21.

7. General procedures for Route A



5 **8**

The reaction components were added by stock solution, in which the substrate **5** was dissolved in 20% v/v DMSO, while GDH, $NADP^+$, glucose, PLP, isopropylamine and lysozyme were all suspended in PBS buffer. In a 2-mL Eppendorf tube, the following components were combined: The substrates **5** (10-50 mM), GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), DMSO (20% v/v), glucose (100 mM), isopropylamine solution (1 M, pH 9.0), PLP (0.5 mg mL^{-1}), resting *E.coli* cells containing M7 (0.2 g mL^{-1}), lysozyme (1 mg mL^{-1}) and DNase I (6 units mL^{-1}) were added to a 2-mL-Eppendorf tube, supplemented to 1 mL with PBS buffer (pH 7.5, 50 mM), and cultured at 30°C and 1000 rpm for 24 h. Then the temperature was elevated to 58°C, and reacted for additional 24 h. For the one-pot two-step reaction sequence, isopropylamine solution and PLP were added after 24 h of reaction commencement with elevation of temperature to 58°C. Samples were taken and extracted with MTBE (extraction ratio = 1:4), then dried with anhydrous sodium sulfate, and analyzed by normal phase HPLC. For the one-pot two-step reaction sequence, sSTY is 198 g $L^{-1}d^{-1}$ g_{cat}^{-1}, which is calculated by overall conversion (40 mM, 83%), the reaction times (48 h) and the total amount of catalysts used (Figure S2, approx. 18.504 mg pure enzyme) for the substrate **5** (40 mM) in 1.2 mL; simplified E factors (sEF) is 42, which is calculated by using substrate in DMSO (50 mM, 100 mg), co-expressed whole cell (200 mg), ^{i}PrNH2 (59 mg), GDH (2.5 mg), Glu (19.8 mg), $NADP^+$ (0.8 mg) and PLP (0.5 mg); Finally, 8.798 mg of product was obtained.

$$sSTY = \frac{m_{product}}{V_t \cdot t \cdot m_{cat}} \times 24$$

$m_{product}$: the quantity of products;

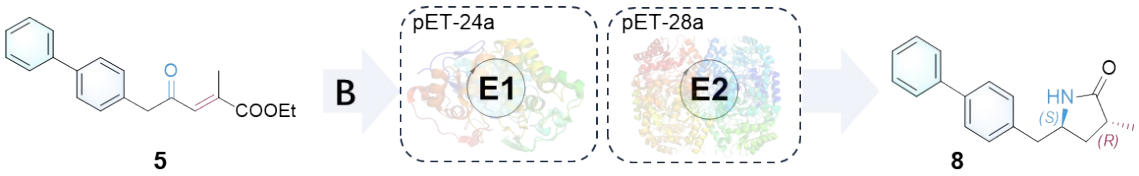
V_t: the total reaction volume;

t: overall reaction times;

m_{cat}: the quantity of catalyst used.

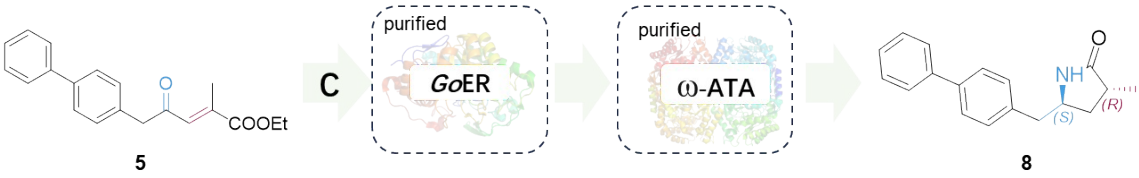
$$sEF = \frac{\sum m(raw\ materials) + \sum m(reagents) - m(product)}{m(product)}$$

8. General procedures for Route B



In a 2-mL Eppendorf tube, the following components were combined: The substrates **5** (10-80 mM), GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), DMSO (20% v/v), glucose (100 mM), isopropylamine solution (1M, pH 9.0), PLP (0.5 mg mL^{-1}), *Go*ER whole cell (0.2 g mL^{-1}) and ω-ATA CDX-043 whole cell (0.1 g mL^{-1}) were added to a 2-mL-Eppendorf tube, supplemented to 1 mL with PBS buffer (pH 7.5, 50 mM), and cultured at 30°C and 1000 rpm for 24 h. Then the temperature was elevated to 58°C, and reacted for additional 24 h. For the one-pot two-step reaction sequence, isopropylamine solution, PLP, ω-ATA CDX-043 whole cell, lysozyme (1 mg mL^{-1}) and DNase I (6 units mL^{-1}) were added after 24 h of reaction, and then the temperature was adjusted to 58°C. Samples were taken and extracted with MTBE (extraction ratio = 1:4), then dried with anhydrous sodium sulfate, and analyzed by normal phase HPLC. For the one-pot two-step reaction sequence, sSTY is 217 g $L^{-1}d^{-1}$ g_{cat}^{-1}; simplified E factors (sEF) is 42.

9. General procedures for Route C



In a 2-mL Eppendorf tube, the following components were combined: The substrates **5** (10-80 mM), GDH (2.5 g L^{-1}), $NADP^+$ (1 m), DMSO (20% v/v), glucose (100 mM), isopropylamine solution (1M, pH 9.0), PLP (0.5 mg mL^{-1}), *Go*ER pure enzyme (10 mg mL^{-1}), ω-ATA CDX-043 pure enzyme (6 mg mL^{-1}) and lysozyme (1 mg mL^{-1}) were added to a 2-mL-Eppendorf tube, supplemented to 1 mL with PBS buffer (pH 7.5, 50 mM), and cultured at 30°C and 1000 rpm for 24 h. Then the temperature was elevated to 58°C, and reacted for additional 24 h. For the one-pot two-step reaction sequence, isopropylamine solution, PLP

and ω-ATA CDX-043ere added after 24 h of reaction, and then the temperature was adjusted to 58°C. Samples were taken and extracted with MTBE (extraction ratio = 1:4), then dried with anhydrous sodium sulfate, and analyzed by normal phase HPLC. For the one-pot two-step reaction sequence, sSTY is 272 g $L^{-1}d^{-1}$ g_{cat}^{-1}; simplified E factors (sEF) is 16.

10. General procedures for Semi-preparative reaction (chemoenzymatic cascades one-pot two step reaction sequence)

To a 500 mL reaction flask, the substrates **5** (50 mM) dissolved in DMSO(20% v/v), then GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), DMSO (20% v/v), glucose (100 mM) were accurately weighed and added into the reaction mixture. *Go*ER whole cell (0.2 g mL^{-1}) were added, and finally PBS buffer (pH 7.5, 50 mM) was added to reach a final volume of 100 mL. The mixture was stirred for 24h at 30°C, 220 rpm, After 24 hours of reaction, added ω-ATA CDX-043 whole cell (0.1 g mL^{-1}), isopropylamine solution (1M, pH 9) and PLP (0.5 mg mL^{-1}), lysozyme (1 mg mL^{-1}) and DNase I (6 units mL^{-1}) changed the temperature to 58 ℃, and then reacted for another 24 h.. The solution was mixed with a mixture of acetic acid and concentrated hydrochloric acid (200 mL ratio 1 :1) and stirred under reflux for about 20 hours. Then the mixture was extracted three times with ethyl acetate[2-3], and the organic layers were combined, and subjected to column chromatography (MeOH : DCM =1: 8) for purification to yield **9** (979mg, 68.5%) as a white soild. ^{1}H NMR (400 MHz, DMSO) δ 8.23 (s, 2H), 7.68 (td, *J* = 8.4, 1.7 Hz, 4H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.42 – 7.33 (m, 3H), 3.44 (m, 1H), 3.07 (dd, *J* = 13.9, 5.7 Hz, 1H), 2.87 (dd, *J* = 13.9, 7.6 Hz, 1H), 2.68 (dq, *J* = 13.2, 7.0 Hz, 1H), 1.88 (ddd, *J* = 14.2, 8.8, 5.4 Hz, 1H), 1.60 (ddd, *J* = 13.9, 7.9, 5.6 Hz, 1H), 1.09 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 177.04, 140.27, 139.11, 136.08, 130.54, 129.47, 127.90, 127.30, 127.03, 50.83, 38.61, 36.25, 35.40, 18.01. We separated **8** separately in a step-by-step reaction,^{1}H NMR (400 MHz, CDCl3) δ 7.59 (dd, J = 10.7, 7.6 Hz, 4H), 7.48 (t, J = 7.5 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 7.28 (d, J = 8.9 Hz, 2H), 3.94 – 3.85 (m, 1H), 2.90 (dd, J = 13.4, 5.5 Hz, 1H), 2.78 (dd, J = 13.4, 8.3 Hz, 1H), 2.53 (h, J = 7.5 Hz, 1H), 2.19 (ddd, J = 12.6, 8.7, 3.6 Hz, 1H), 1.97 (dt, J = 12.9, 7.8 Hz, 1H), 1.24 (d, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 180.38, 140.72, 139.86, 136.73, 129.58, 128.86, 127.53, 127.36, 127.06, 53.48, 42.46, 35.17, 34.97, 16.28.

11. Thermostability and kinetic parameters assay

The melting point (Tm) of the *Go*ER was measured by circular dichroism analysis (Figure S8). The target protein was subjected to nickel column affinity chromatography. After desalination, the pure enzyme solution was diluted to 0.2-0.5 mg mL^{-1} with desalination solution (50 mM PBS buffer, pH 7.5), and the pure enzyme solution with high purity and good homogeneity was obtained by high-speed centrifugation (12000 rpm, 10min, 4℃). Calculate and fit Tm according to the curve recorded by circular dichroism. The kinetic parameters were obtained by measuring the initial velocities of **7** generation (detected by HPLC) in the enzymatic reaction and fitting the curve according to the Michaelis-Menten equation (Figure S9). The activity assay was performed in a mixture containing 50 mM NADPH, 1.25~20 mM **5** and the purified enzyme (1 mg mL^{-1}). The reaction was initiated by the addition of the enzyme and was monitored for 10 min at 30°C. The activity was determined by measuring **7** generation (detected by HPLC at 254 nm). 1 unit (1 U) of activity is defined as the amount of enzyme required to consume 1 μM **5** in one minute.

12. Supplementary data

Compounds **5** and **6** were screened using the ene-reductases library and the results were shown in Tables S4 and S5. *Go*ER had the highest activity against substrate **5**, with 65% conversion to 10 mM substrate **5** in a 96-well plate reaction. *Ec*ER had the highest activity against substrate **6**, with 57% conversion to 10 mM substrate **6** in a 96-well plate reaction. To further compare the activity of the two enzymes, a resieve was performed and the activity of the two enzymes was tested in a 1 mL reaction containing 10 mM substrate concentration. In the end, it was found that *Ec*ER had 70% conversion for 10 mM substrate **6** in a 1 mL reaction, and *Go*ER had a higher activity and 99% conversion for 10 mM substrate **5**.

Table S4. The conversions of ERs screen for **5**.

Entry	ERED	GenBank	Origins	Typical selectivity	Conversion [%]	ee [%]	Ref
1	*Go*ER	WP_011252080.1	*Gluconobacter oxydans*	R	65	>99	[4]
2	*Nt*ER	XP_016514079.1	*Nicotiana tabacum*	R	42	98.76	[5]
3	*Cp*ER	KAF6043514.1	*Candida*	R	22	>99	[6]

			parapsilosis				
4	*Cl*ER	XM_002615435.1	*Clavispora lusitaniae* ATCC 42720	R	14	>99	[7]
5	*Ci*ER	SGZ52755.1	*Candida intermedia*	R	14	>99	
6	*Ss*ER	WP_011377589.1	*Synechococcus* sp. PCC 7942	R	12	98.55	[8]
7	*Cl*ER2	QFZ29493.1	*Clavispora lusitaniae*	R	9	>99	[7]
8	*Ch*ER	XP_025340861.1	*Candida haemuloni*	R	7	98.43	
9	*Yb*ER	WP_032896199.1	*Yersinia bercovieri*	R	5	96.93	[9]
10	*Cs*ER	WP_033059898.1	*Clostridium sporogenes*	R	3	98.75	[10]
11	*Ro*ER	WP_064080187.1	*Rhodococcus opacus*	R	2	>99	[11]
12	*Sp*ER	CAA37666.1	*Saccharomyces pastorianus*		0	0	
13	*Av*ER	ABA25236.1	*Anabaena variabilis*		0	0	
14	*Am*ER	WP_012165090.1	*Acaryochloris marina MBIC11017*		0	0	
15	*Kl*ER	XP_451397.1	*Kluyveromyces lactis*		0	0	
16	*Pp*ER	WP_016711963.1	*Pseudomonas putida*		0	0	
17	*Ec*ER	AAB38683.1	*Enterobacter cloacae*		0	0	
18	*Km*ER	BAD24850.1	*Kluyveromyces marxianus/Candida macedoniensis*		0	0	
19	*Tp*ER	WP_012268805	*Thermoanaerobacter pseudethanolicus*		0	0	
20	*St*ER	BAM99302.1	*Streptomyces sp. GF3587*		0	0	
21	*Ad*ER	XP_045278909.1	*Ajellomyces dermatitidis*		0	0	
22	*Ss*ER2	XP_001385078.1	*Scheffersomyces stipitis CBS 6054*		0	0	
23	*Tt*ER	WP_011173882.1	*Thermus thermophilus*		0	0	
24	*Ph*ER	WP_010884948.1	*Pyrococcus horikoshii*		0	0	
25	*Mg*ER	XP_001482000.1	*Meyerozyma guilliermondii ATCC 6260*		0	0	
26	*Fe*ER	WP_056929840.1	*Ferrovum sp. JA12*		0	0	
27	*Pf*ER	AAF02539.1	*Pseudomonas*		0	0	

28	*Cm*ER	WP_011519282.1	*fluorescens* *Cupriavidus metallidurans*	0	0

Reaction conditions: 5 (10 mM), DMSO (20 %), $NADP^+$ (1 mM), glucose (100 mM) and GDH (2.5 g L^{-1}), ERs (0.1 g mL^{-1} whole cells), DNase I (6 units mL^{-1}) and lysozyme (1 mg mL^{-1}), PBS buffer (50 mM, pH 7.5), 30°C, 24 h, 800 rpm, final volume: 0.5 mL.

Table S5 The conversions of ERs screen for **6**.

Entry	ERED	GenBank	Origins	Typical selectivity	Conversion [%]	ee [%]
1	*Go*ER	WP_011252080.1	*Gluconobacter oxydans*	R	18	>99
2	*Nt*ER	XP_016514079.1	*Nicotiana tabacum*		0	0
3	*Cp*ER	KAF6043514.1	*Candida parapsilosis*		0	0
4	*Cl*ER	XM_002615435.1	*Clavispora lusitaniae* ATCC 42720		0	0
5	*Ci*ER	SGZ52755.1	*Candida intermedia*		0	0
6	*Ss*ER	WP_011377589.1	*Synechococcus* sp. PCC 7942		0	0
7	*Cl*ER2	QFZ29493.1	*Clavispora lusitaniae*		0	0
8	*Ch*ER	XP_025340861.1	*Candida haemuloni*		0	0
9	*Yb*ER	WP_032896199.1	*Yersinia bercovieri*		0	0
10	*Cs*ER	WP_033059898.1	*Clostridium sporogenes*	R	36	>99
11	*Ro*ER	WP_064080187.1	*Rhodococcus opacus*		0	0
12	*Sp*ER	CAA37666.1	*Saccharomyces pastorianus*		0	0
13	*Av*ER	ABA25236.1	*Anabaena variabilis*		0	0
14	*Am*ER	WP_012165090.1	*Acaryochloris marina MBIC11017*		0	0
15	*Kl*ER	XP_451397.1	*Kluyveromyces lactis*		0	0
16	*Pp*ER	WP_016711963.1	*Pseudomonas putida*	R	44	>99
17	*Ec*ER	AAB38683.1	*Enterobacter cloacae*	R	57	>99
18	*Km*ER	BAD24850.1	*Kluyveromyces marxianus/Candida macedoniensis*		0	0
19	*Tp*ER	WP_012268805	*Thermoanaerobacter pseudethanolicus*		0	0

20	*St*ER	BAM99302.1	*Streptomyces sp. GF3587*	0	0
21	*Ad*ER	XP_045278909.1	*Ajellomyces dermatitidis*	0	0
22	*Ss*ER2	XP_001385078.1	*Scheffersomyces stipitis CBS 6054*	0	0
23	*Tt*ER	WP_011173882.1	*Thermus thermophilus*	0	0
24	*Ph*ER	WP_010884948.1	*Pyrococcus horikoshii*	0	0
25	*Mg*ER	XP_001482000.1	*Meyerozyma guilliermondii ATCC 6260*	0	0
26	*Fe*ER	WP_056929840.1	*Ferrovum sp. JA12*	0	0
27	*Pf*ER	AAF02539.1	*Pseudomonas fluorescens*	0	0
28	*Cm*ER	WP_011519282.1	*Cupriavidus metallidurans*	0	0

Reaction conditions: **6** (10 mM), DMSO (20 %), $NADP^+$ (1 mM), glucose (100 mM) and GDH (2.5 g L^{-1}), ERs (0.1 g mL^{-1} whole cells), DNase I (6 units mL^{-1}) and lysozyme (1 mg mL^{-1}), PBS buffer (50 mM, pH 7.5), 30°C, 24 h, 800 rpm, final volume: 0.5 mL.

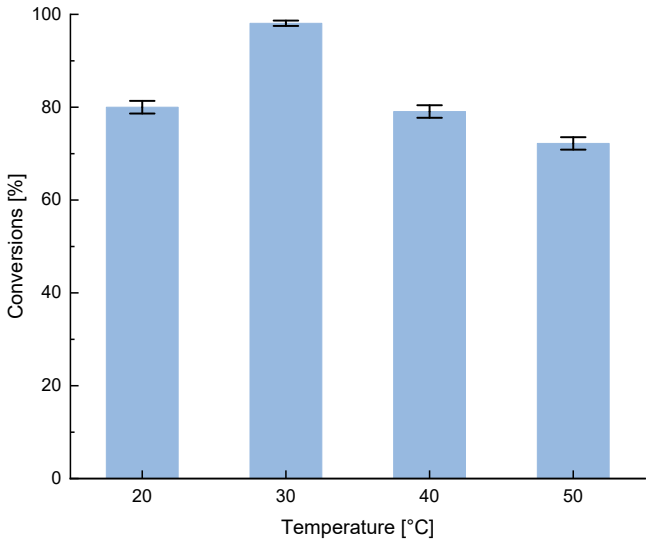


Figure S4. Optimization of temperatur of the *Go*ER catalyzed reduction of **5**. Reaction conditions: **5** (20 mM), *Go*ER whole cell (0.1 g mL^{-1}), DNase I (6 units mL^{-1}) and lysozyme (1 mg mL^{-1}), $NADP^+$ (1 mM), glucose (100 mM) and PBS buffer (50 mM, pH 7.5), 30-50°C, 24 h.

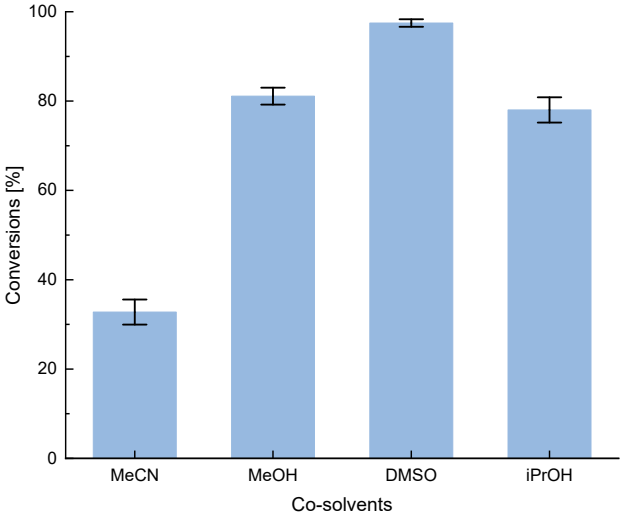


Figure S5. Optimization of co-solvents of the *Go*ER catalyzed reduction of **5**. Reaction conditions: **5** (20 mM in 20% solvents), *Go*ER whole cell (0.1 g mL^{-1}), DNase I (6 units mL^{-1}) and lysozyme (1 mg mL^{-1}), GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), glucose (100 mM) and PBS buffer (50 mM, pH 7.5), 30°C, 24 h.

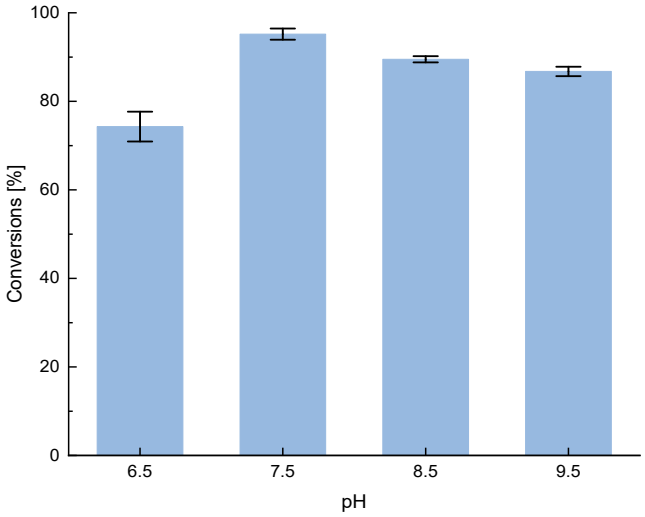


Figure S6. Optimization of pH of the *Go*ER catalyzed reduction of **5**. Reaction conditions: **5** (20 mM), *Go*ER whole cell (0.1 g mL^{-1}), DNase I (6 units mL^{-1}) and lysozyme (1 mg mL^{-1}), GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), glucose (100 mM) and PBS buffer(50 mM, pH 6.5-9.5), 30℃, 24 h.

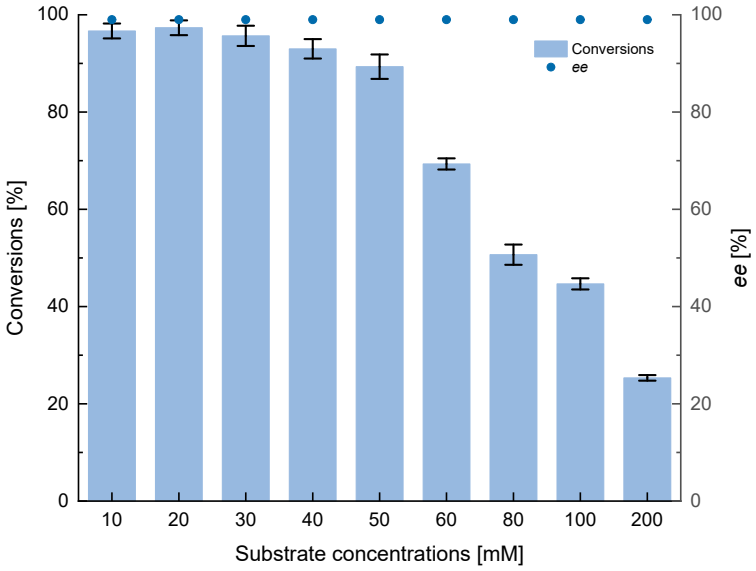


Figure S7. Optimization of substrate concentrations of the *Go*ER catalyzed reduction of **5**. Reaction conditions: **5** (10-200 mM), *Go*ER whole cell (0.2 g mL^{-1}), DNase I (6 units mL^{-1}) and lysozyme (1 mg mL^{-1}), GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), glucose (100 mM) and PBS buffer (50 mM, pH 7.5), 30℃, 24 h.

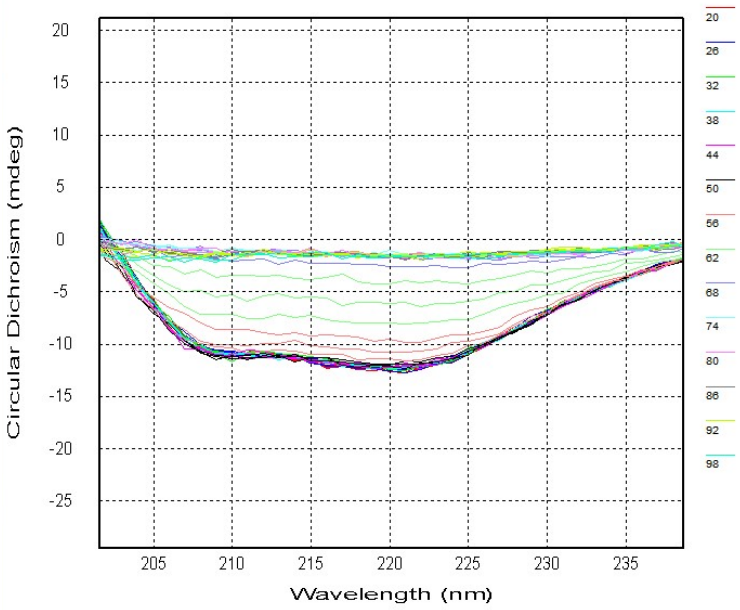


Figure S8 Circular dichroism spectrum of *Go*ER.

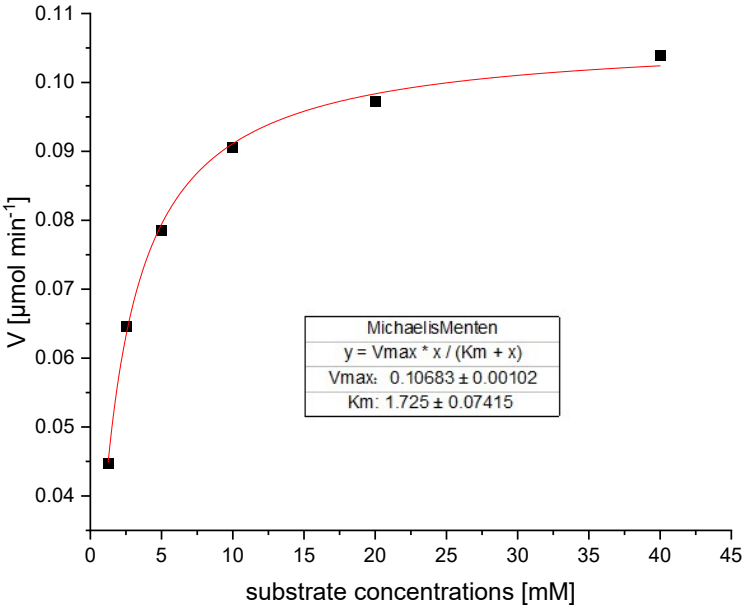


Figure S9. Determination of kinetic parameters of *Go*ER.

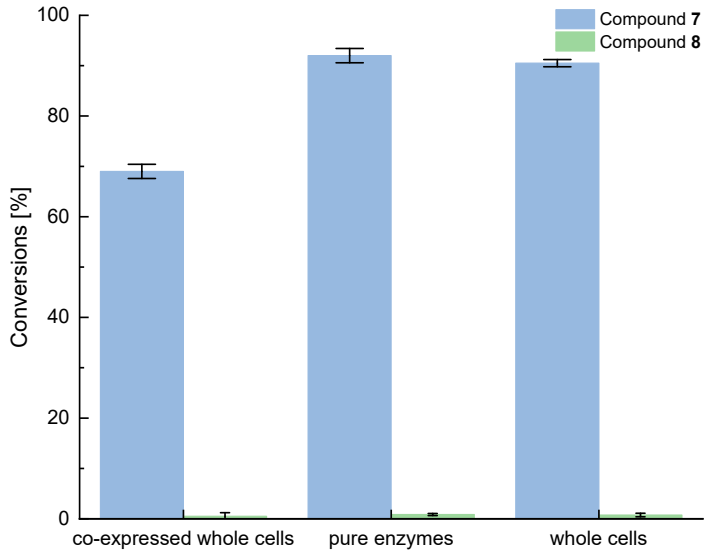


Figure S10. The one-pot two step biotransformation of **5** catalyzed by co-expressed whole cells, pure enzymes and whole cells, the whole reaction is carried out at 30°C without adjusting the temperature to 58°C at 24 h.

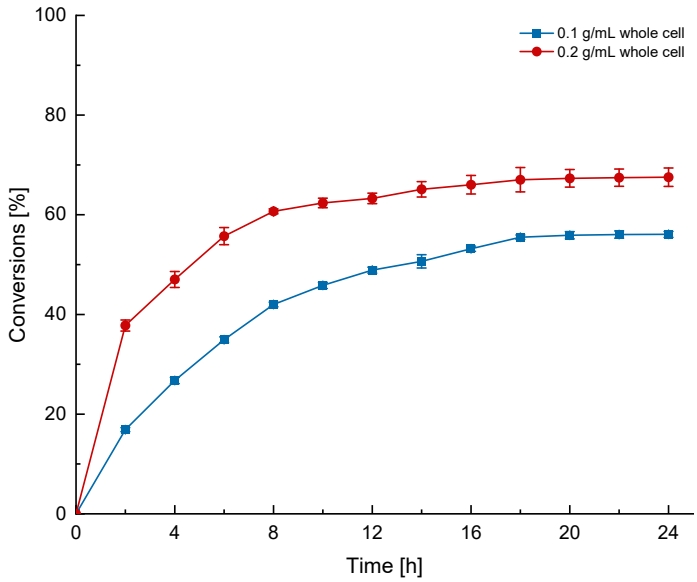


Figure S11. Optimization of co-expression whole-cell concentrations. Reaction conditions: **5** (50 mM), co-expressed whole cell (0.1-0.2 g mL^{-1}), DNase I (6 units mL^{-1}) and lysozyme (1 mg mL^{-1}), GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), glucose (100 mM) and PBS buffer (50 mM, pH 7.5), 30°C 24 h.

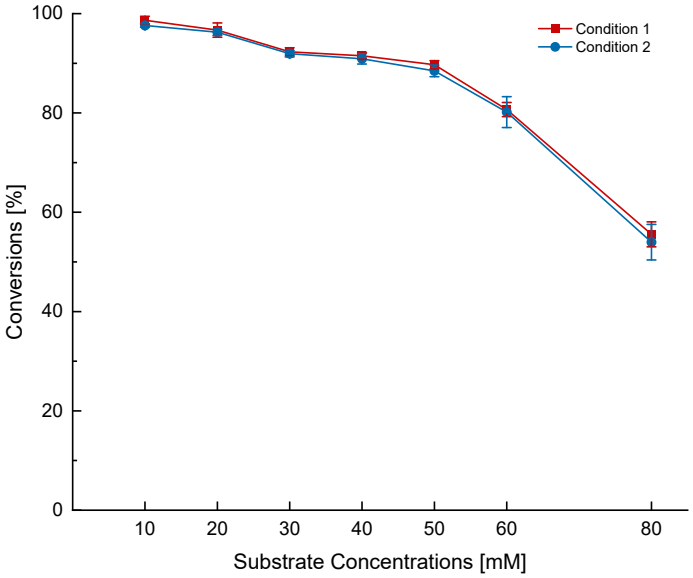


Figure S12. Effect of the system of the reduction reaction by *Go*ER on the transamination reaction by ω-ATA CDX-043. Condition 1: transamination reaction by ω-ATA CDX-043; Condition 2: transamination reaction with addition of GDH (2.5 g L^{-1}), $NADP^+$ (1 mM), glucose (100 mM).

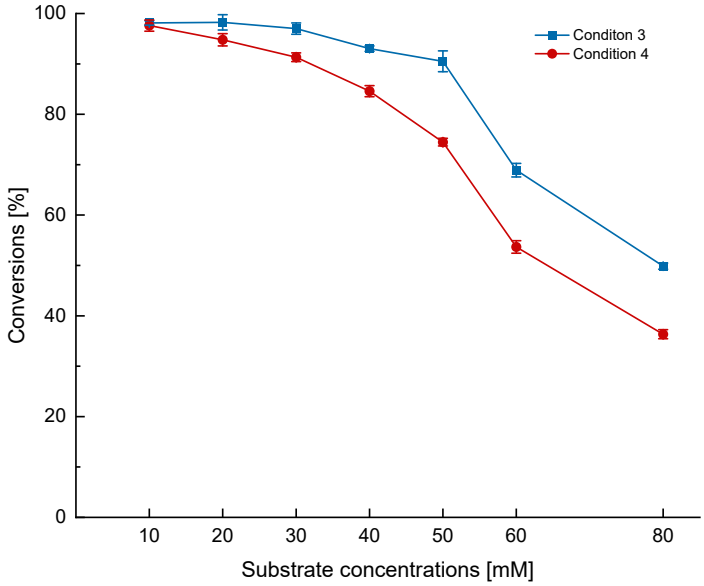


Figure S13. Effect of the system of the transamination reaction by ω-ATA CDX-043 on the reduction reaction by *Go*ER. Condition 3: Reduction reaction by *Go*ER; Condition 4: Reduction reaction with addition of PLP (0.5 g L^{-1}) and $^{i}PrNH_2$ (1 M, pH 9.0).

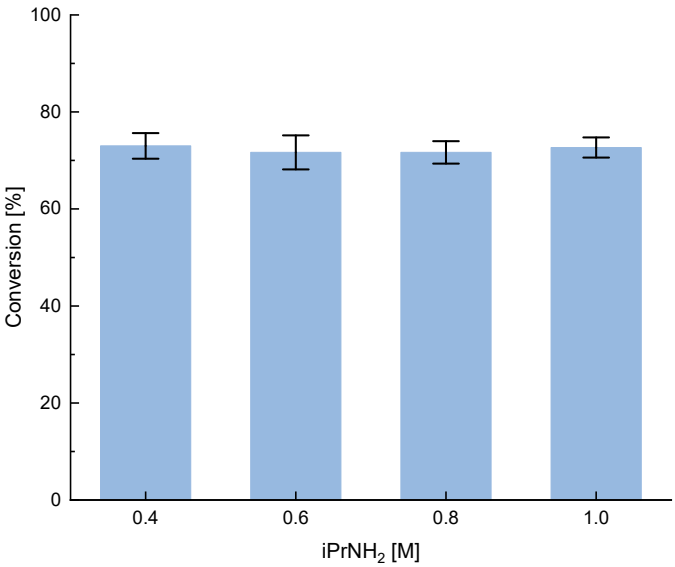


Figure S14. The effect on enoate reductase activity at different concentrations of isopropylamine (0.4-1.0 M). Reaction conditions: **5** (50 mM), DMSO (20 %), $^{i}PrNH_2$ (0.4 M-1.0 M), *Go*ER (0.2 g mL^{-1} whole cell with 6 units mL^{-1} DNase I and 1 mg mL^{-1} lysozyme), $NADP^+$ (1 mM), glucose (100 mM) and GDH (2.5 g L^{-1}) in PBS buffer (50 mM, pH 7.5), 30°C, 24 h, 1000 rpm, final volume: 1.0 mL.

13. HPLC chromatograms

Samples with various concentrations of *rac*-**7** and **8** with PBS buffer containing 20% DMSO were run by HPLC. The yields of all target products (**7** and **8**) were calculated according to the standard curve of the corresponding products.

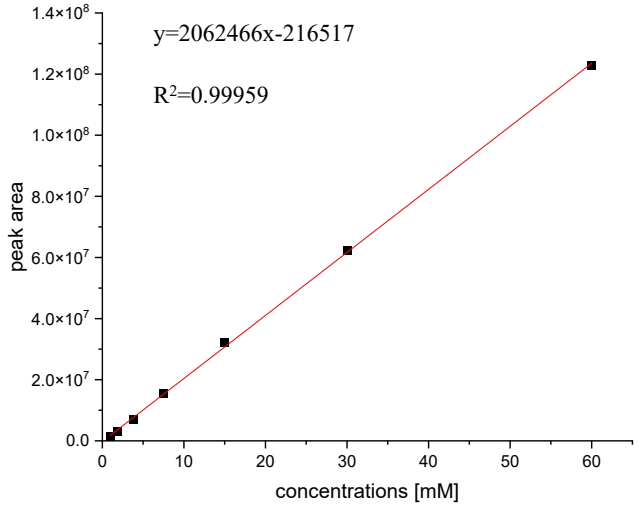


Figure S15. Standard curve for the compound *rac*-**7**

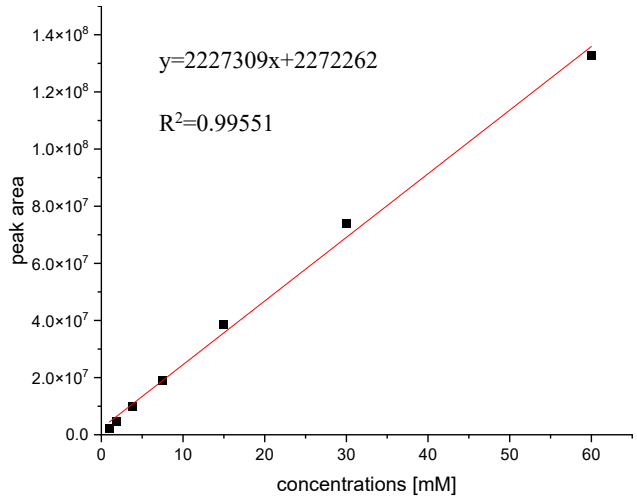


Figure S16. Standard curve for the compound **8**

Rac-7 was prepared by Pd/C catalyzed hydrogenation and was analyzed by HPLC (Figure S21), and transamination of rac-7 was performed to yield (5*S*)-5-([1,1'-biphenyl]-4-ylmethyl)-3-methylpyrrolidin-2-one (5*S*)-**8** (Figure S19). The final product (3*R*,5*S*)-5-([1,1'-biphenyl]-4-ylmethyl)-3-methylpyrrolidin-2-one (3*R*,5*S*)-**8** from enzymatic biotransformations was determined by HPLC (Figure S23), where the retention time is comparable with one enantiomer of (5*S*)-**8**. The absolute configuration of (3*R*,5*S*)-**8** can be determined by X-ray chromatography (Table S6).

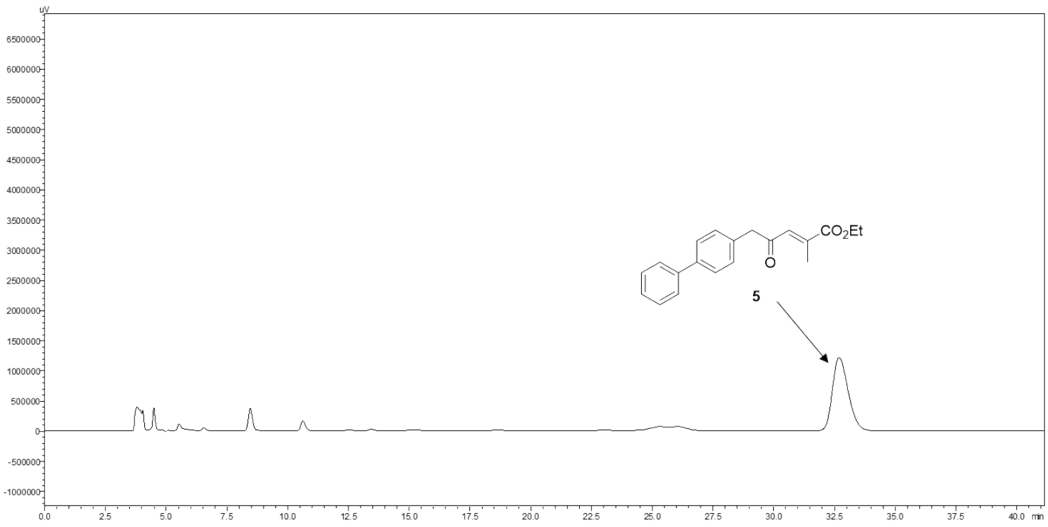


Figure S17. HPLC standards for **5** (Chiralpak® OJ-H, eluents: n-hexane/i-PrOH = 70/30, 254 nm, 30°C, flow rate: 1.0 mL/min，retention time [min] for **5**: 32.984).

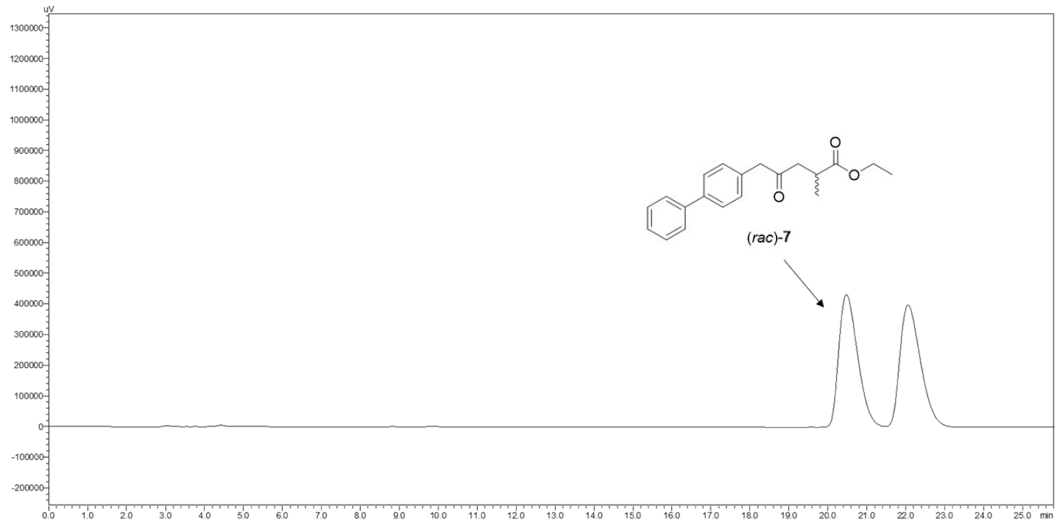


Figure S18. HPLC standards for *rac*-**6** (Chiralpak® OJ-H, eluents: n-hexane/i-PrOH = 70/30, 254 nm, 30°C, flow rate: 1.0 mL/min，retention time [min] for *rac*-**7**: 20.443/22.186).

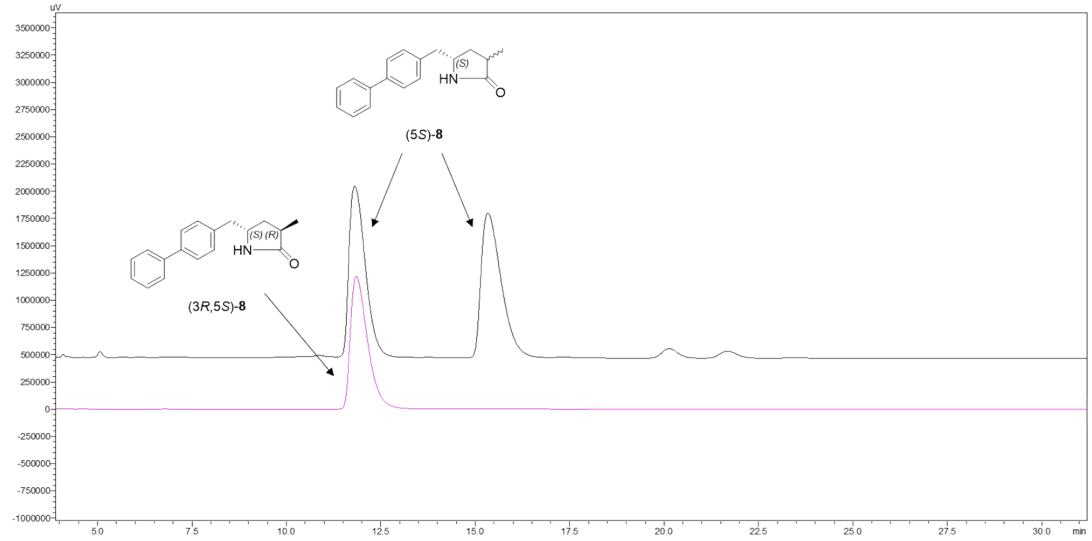


Figure S19. HPLC standards for (5*S*)-**8** and (3*R*,5*S*)-**8** (Chiralpak® OJ-H, eluents: n-hexane/i-PrOH = 70/30, 254 nm, 30°C, flow rate: 1.0 mL/min，retention time [min] for (3*R*,5*S*)-**8**: 11.961 and (5*S*)-**8**: 11.961/15.323).

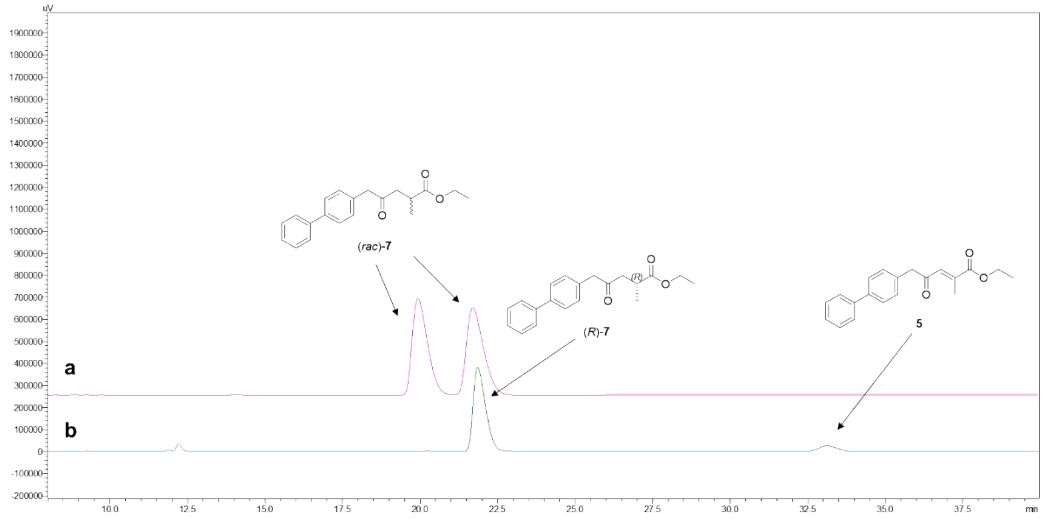


Figure S20. Representative chiral HPLC chromatograms for biotransformation of **5** produced by *Go*ER-catalyzed (**a**: compound (*rac*)**-7**; **b**: the reaction produced by whole cell of *Go*ER).

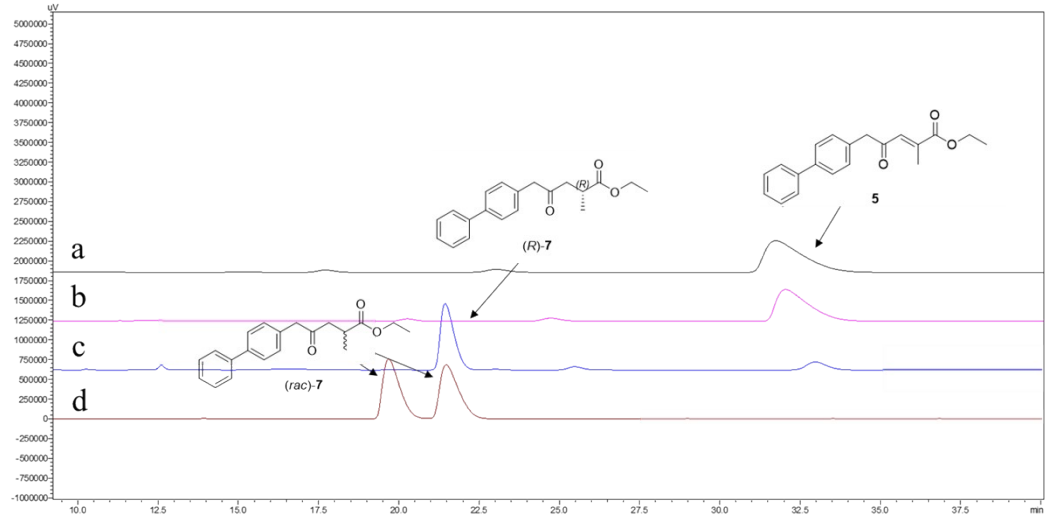


Figure S21. Representative chiral HPLC chromatograms for biotransformation of *Go*ER (**a**: compound **5**; **b**: the reaction produced by empty plasmid pET 24a; **c**: the reaction produced by whole cell of *Go*ER; **d**: compound (*rac*)-**7**).

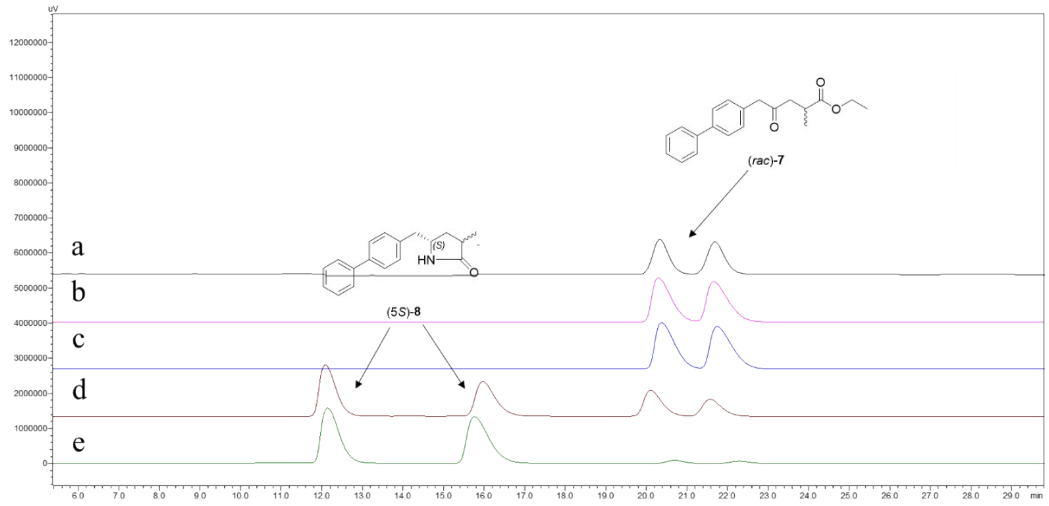


Figure S22. Representative chiral HPLC chromatograms for biotransformation of ω-ATA CDX-043 (**a**: compound (*rac*)**-7**; **b**: the reaction produced by empty plasmid pET 28a; **c**: the reaction produced by ω-ATA CDX-043 without PLP and $^{i}PrNH_2$; **d**: the reaction mixture produced by ω-ATA CDX-043 without PLP; **e**: the reaction mixture at 48 h produced by ω-ATA CDX-043 with PLP and $^{i}PrNH_2$;).

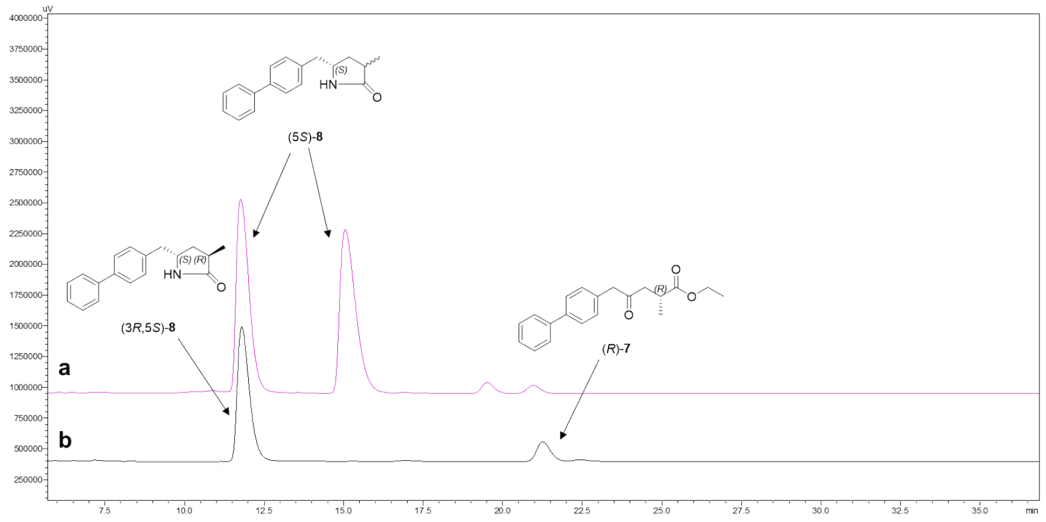


Figure S23. Representative chiral HPLC chromatograms for biotransformation of (*R*)**-7** produced by ω-ATA CDX-043 catalyzed (**a**: compound (5*S*)**-8**; **b**: the reaction produced by whole cell of ω-ATA CDX-043).

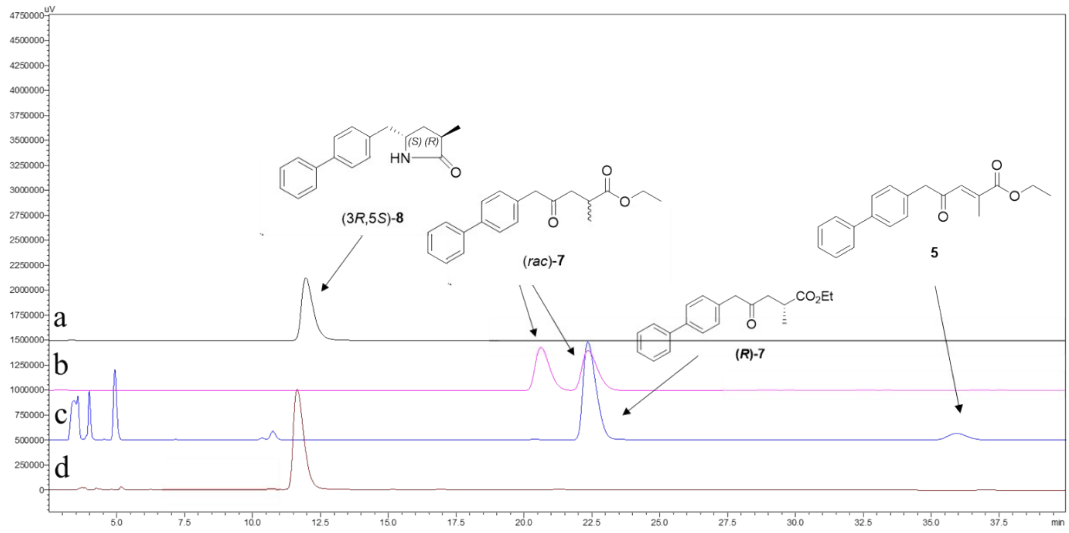


Figure S24. Representative chiral HPLC chromatograms of (2*R*,4*S*)-**8** produced by Semi-preparative reaction (the whole cells cascades). (**a**: compound (3*R*,5*S*)-**8**; **b**: compound ***(rac)*-7**; **c**: 24h; **d**: 48h).

14. ^{1}H and ^{13}C NMR spectra

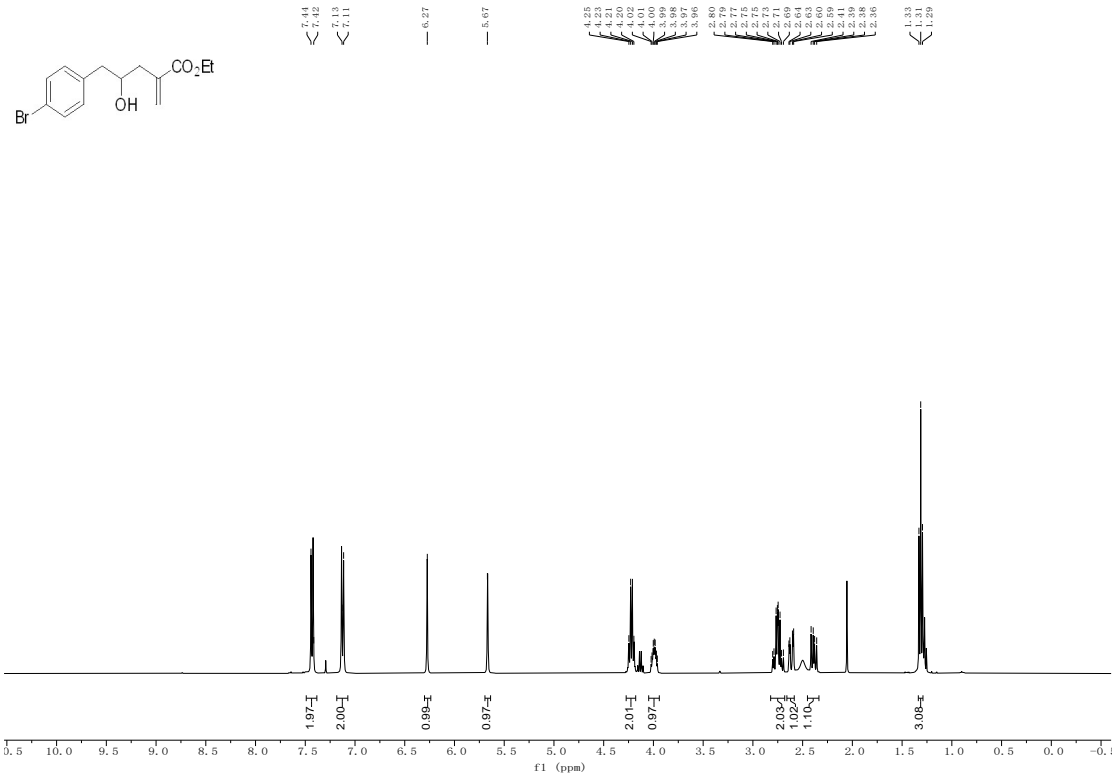


Figure S25. ^{1}H NMR spectra of **2a** in $CDCl_3$.

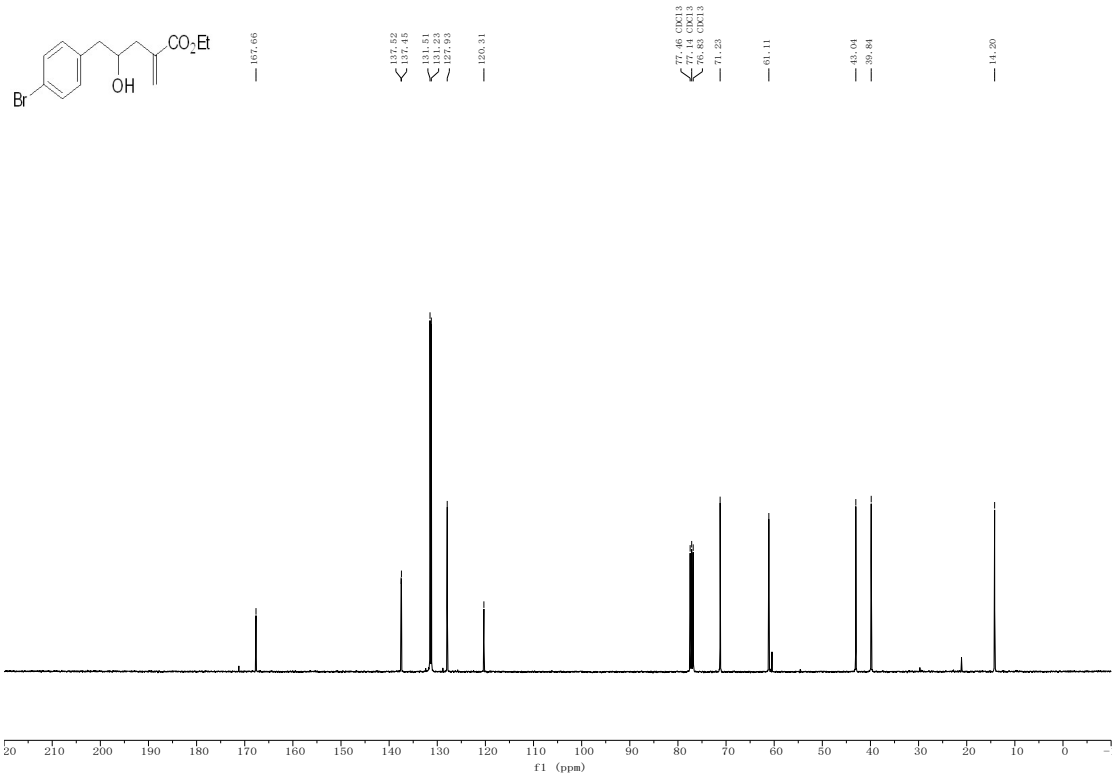


Figure S26. ^{13}C NMR spectra of **2a** in $CDCl_3$.

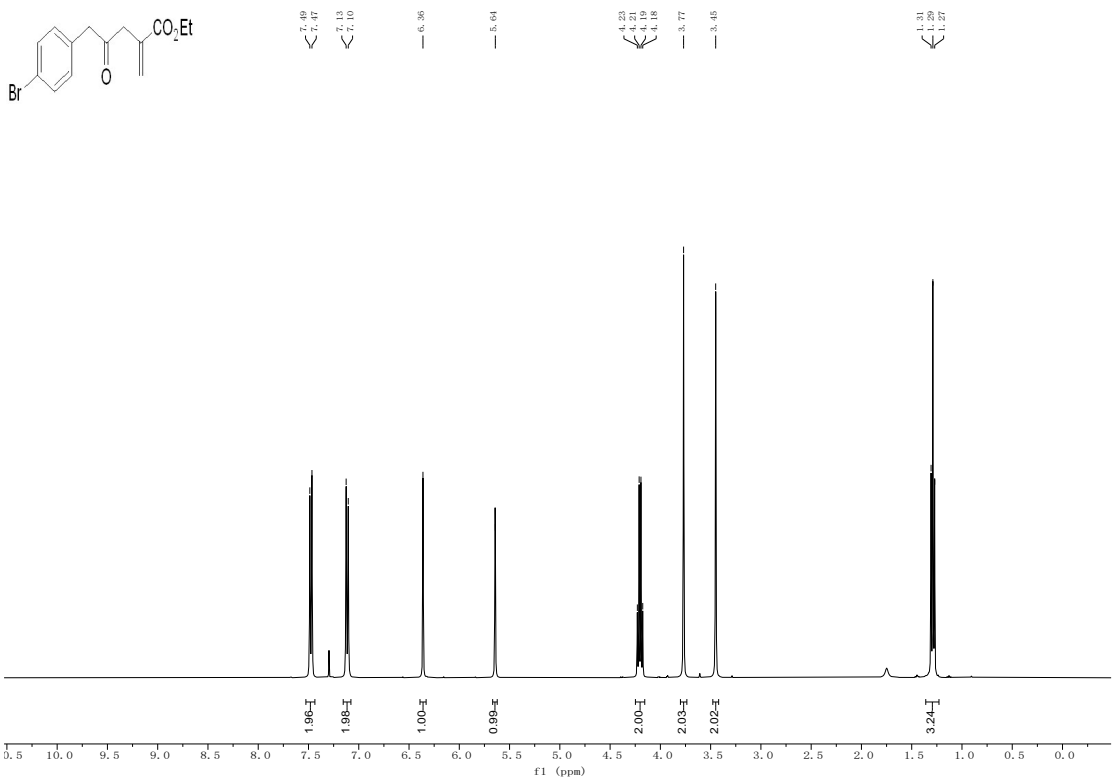


Figure S27. ^{1}H NMR spectra of **3** in $CDCl_3$.

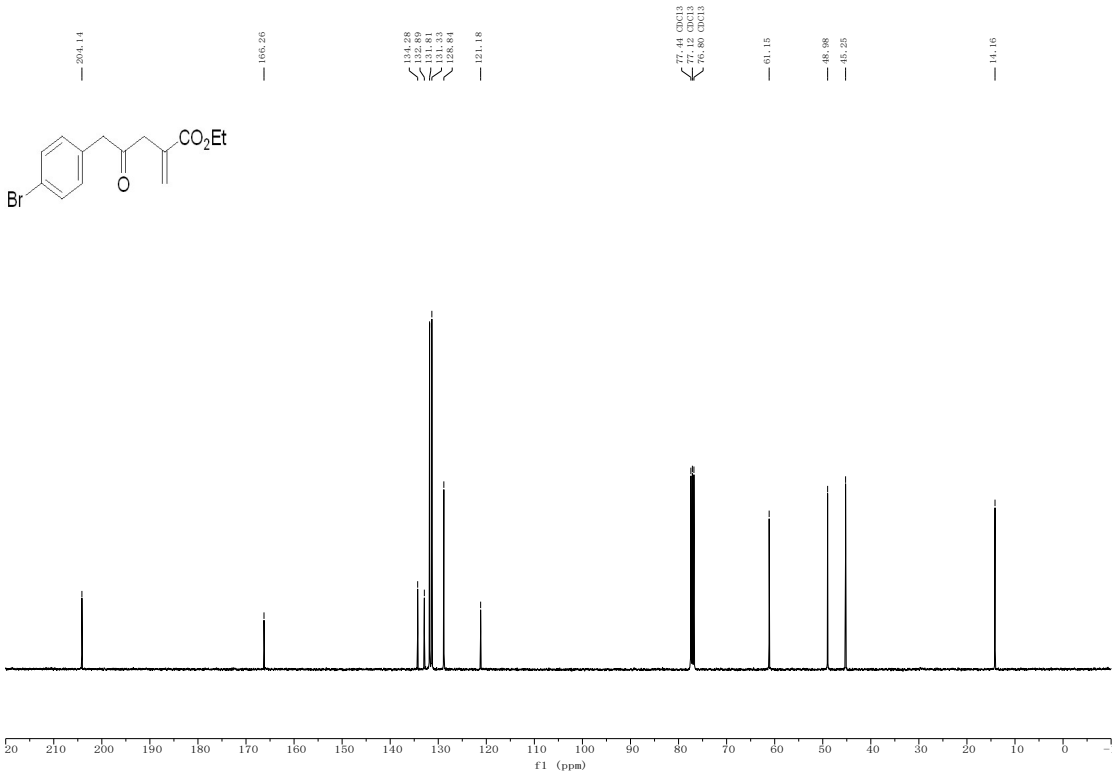


Figure S28. ^{13}C NMR spectra of **3** in $CDCl_3$.

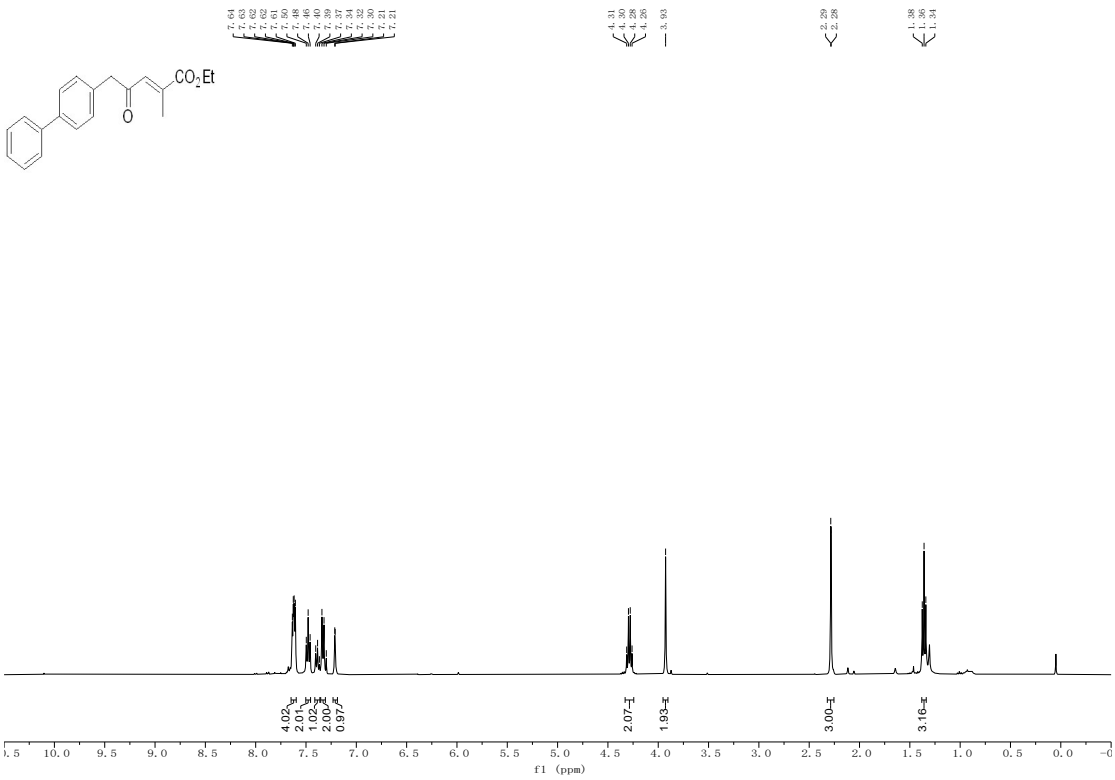


Figure S29. ^{1}H NMR spectra of **5** in $CDCl_3$.

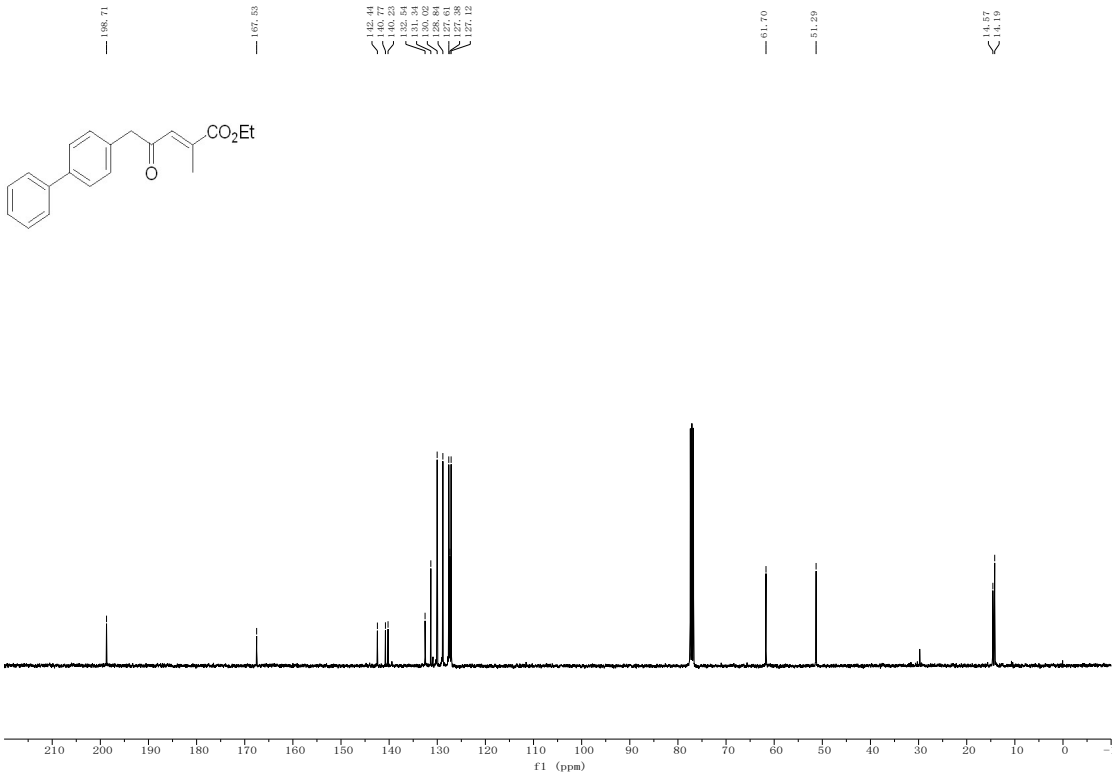


Figure S30. ^{1}H NMR spectra of **5** in $CDCl_3$.

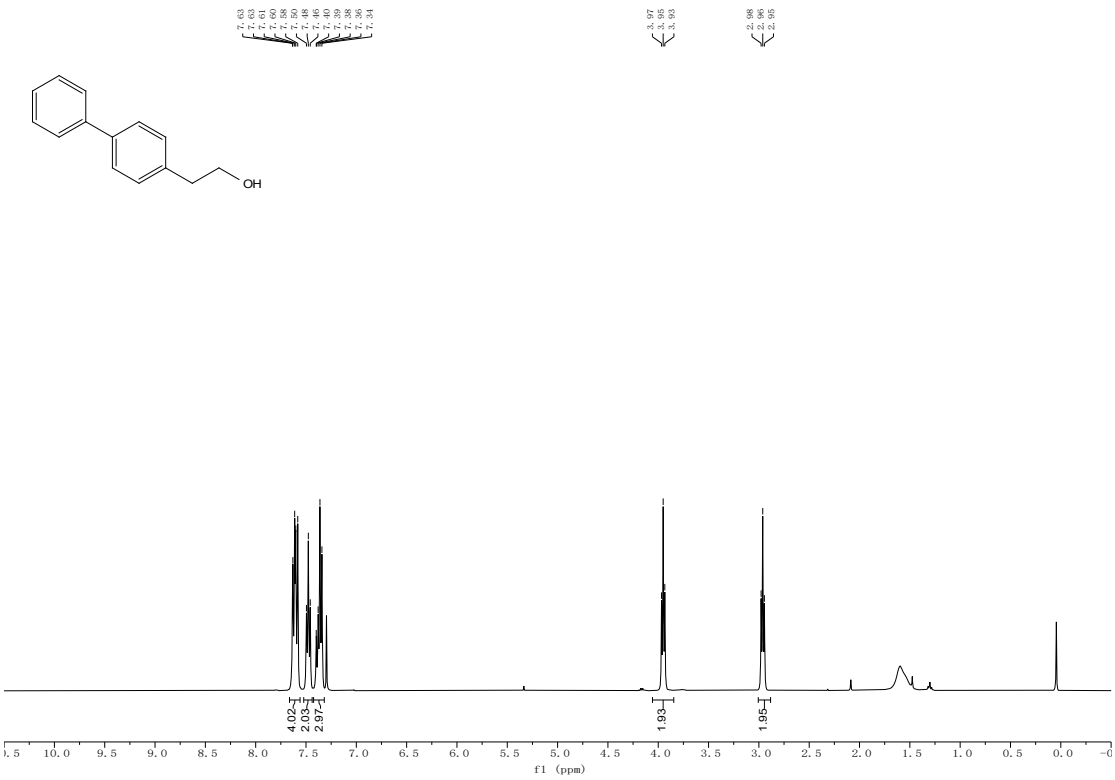


Figure S31. ^{1}H NMR spectra of **11a** in $CDCl_3$.

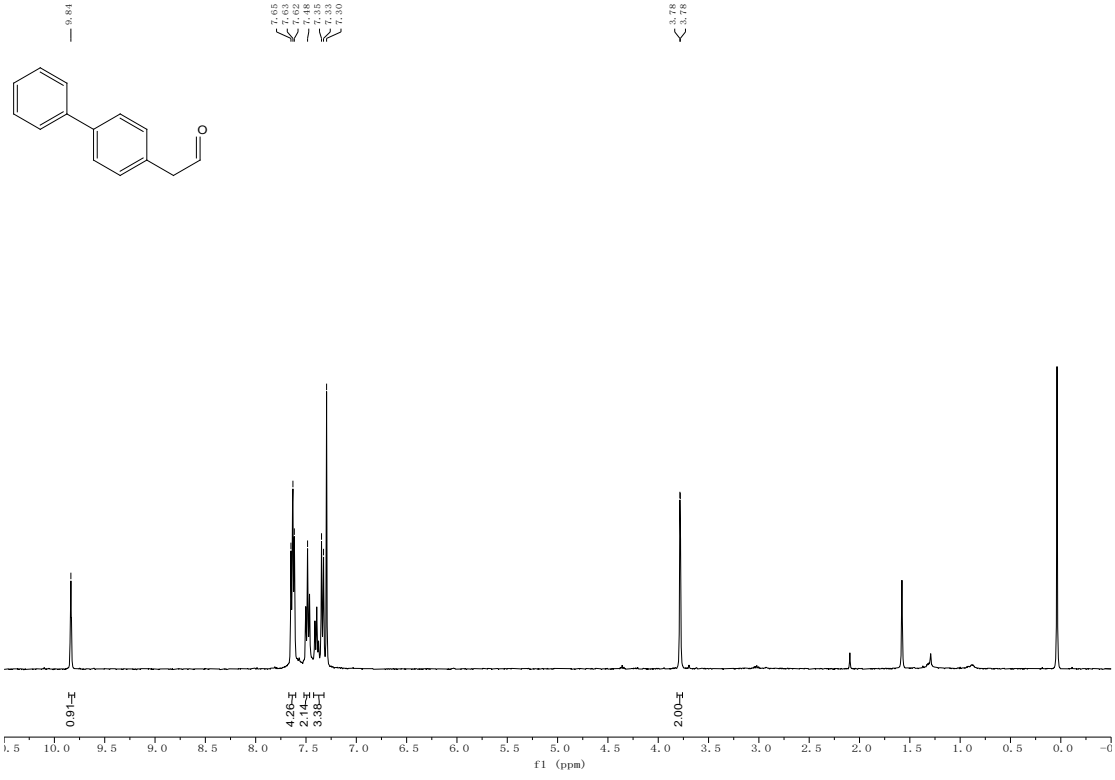


Figure S32. ^{1}H NMR spectra of **11** in $CDCl_3$.

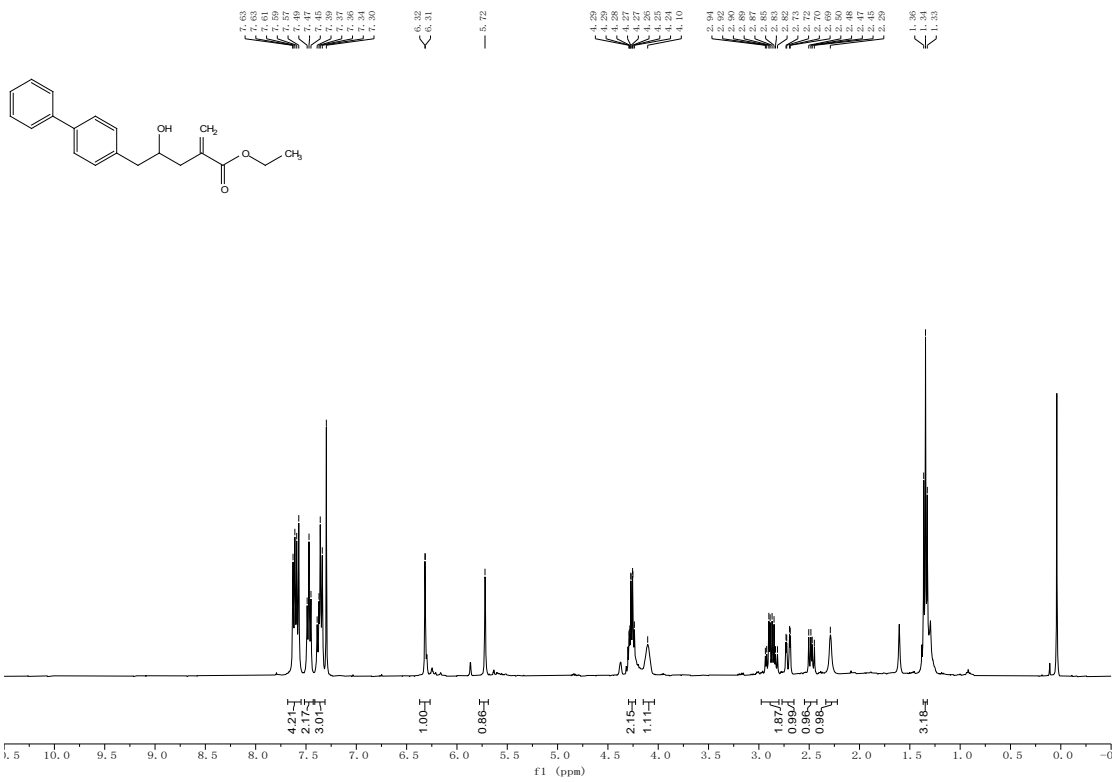


Figure S33. ^{1}H NMR spectra of **12** in $CDCl_3$.

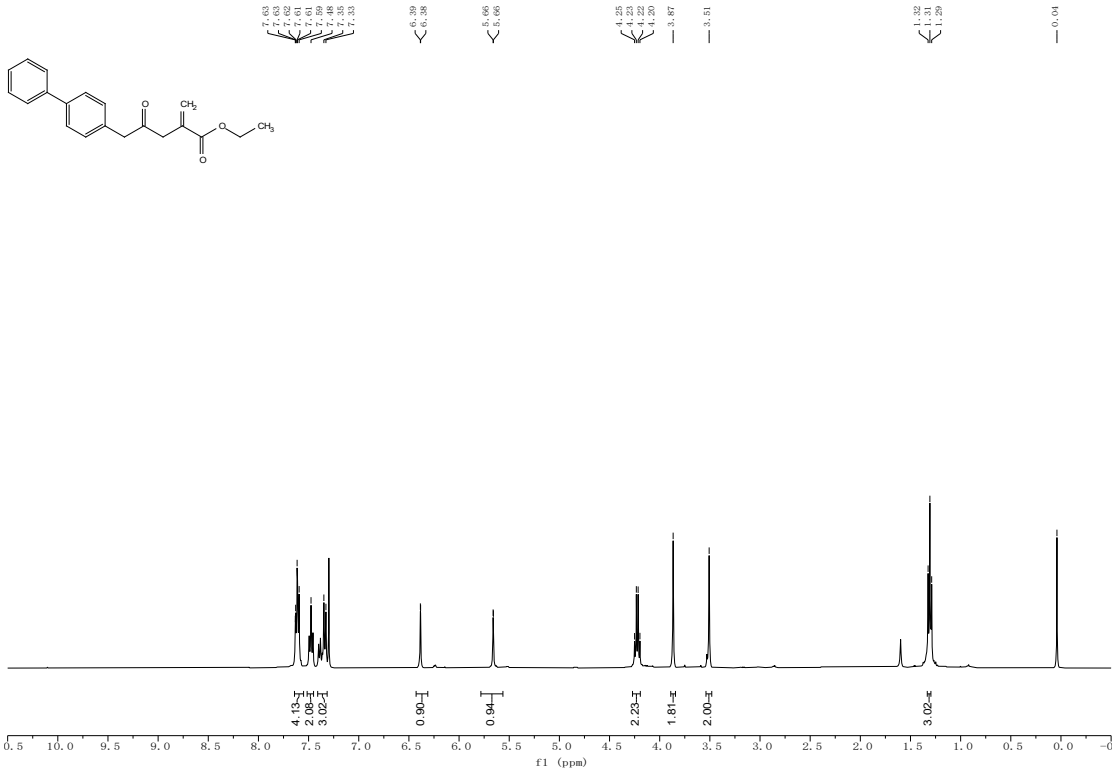


Figure S34. ^{1}H NMR spectra of **6** in $CDCl_3$.

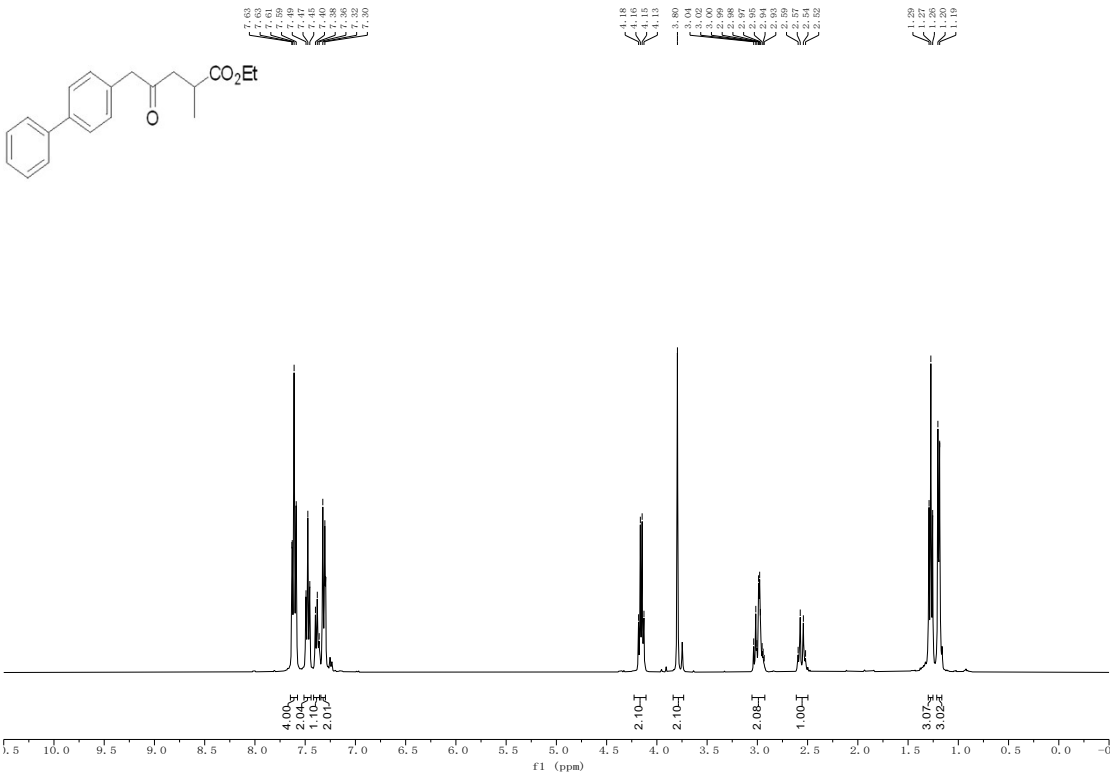


Figure S35. ^{1}H NMR spectra of **7** in $CDCl_3$.

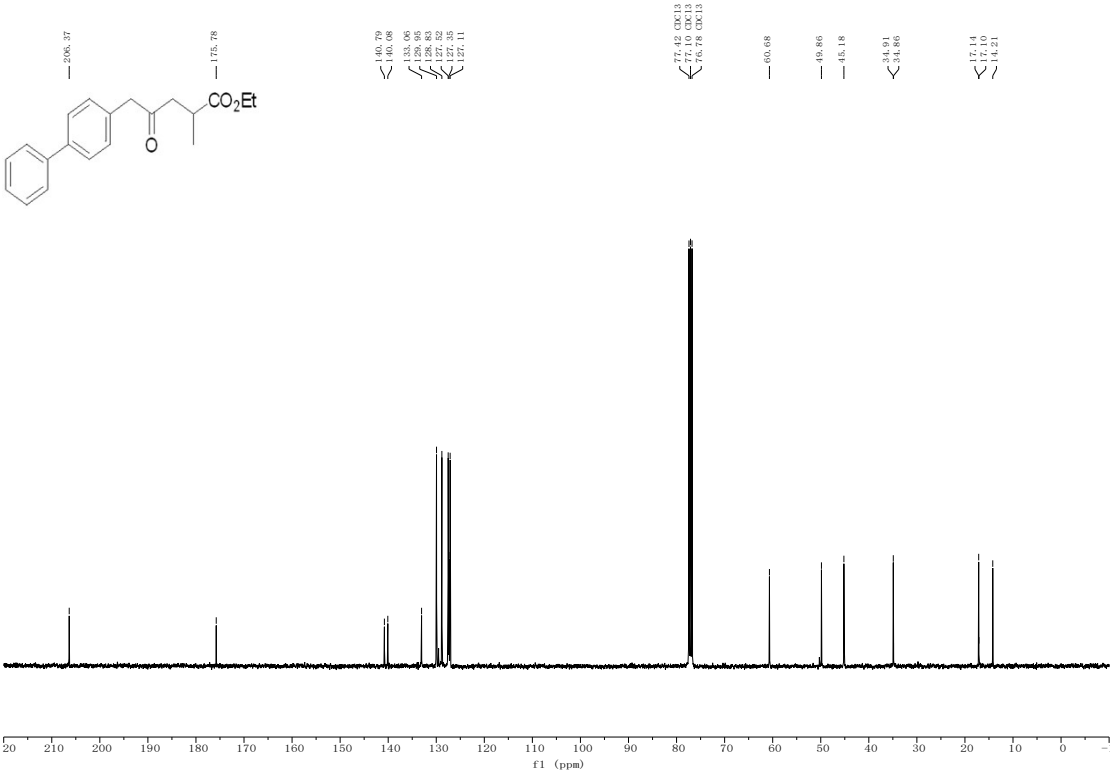


Figure S36. ^{13}C NMR spectra of **7** in $CDCl_3$.

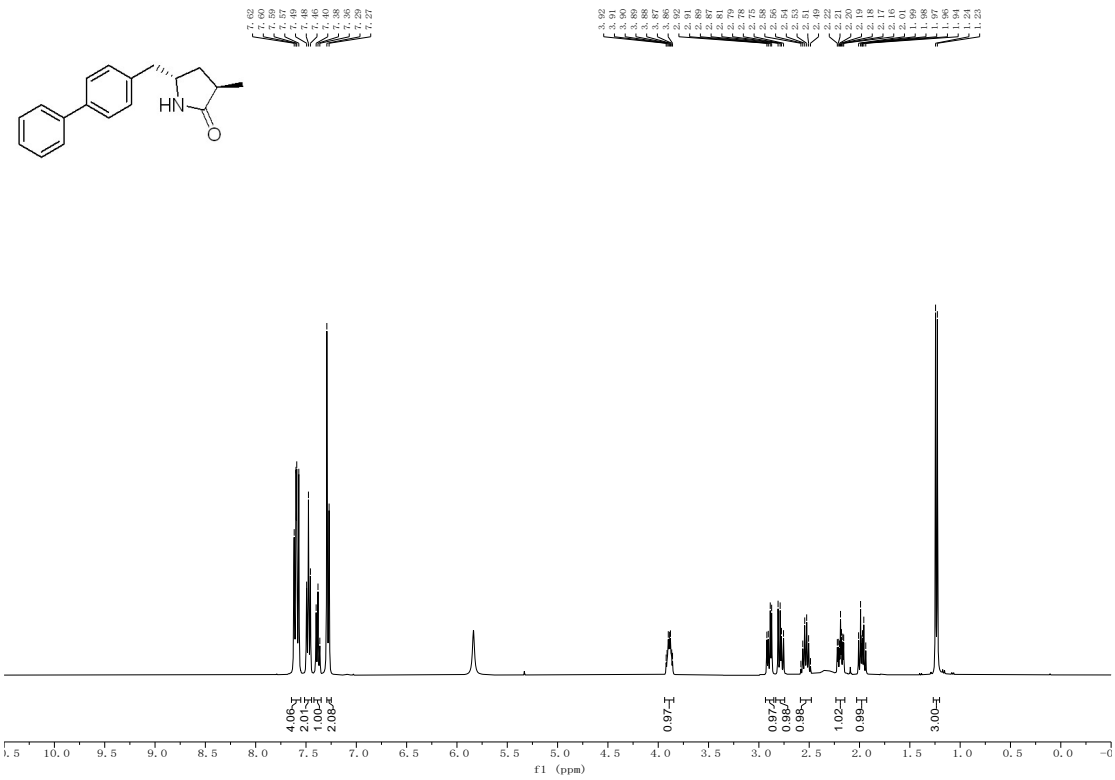


Figure S37. ^{1}H NMR spectra of **8** in $CDCl_3$.

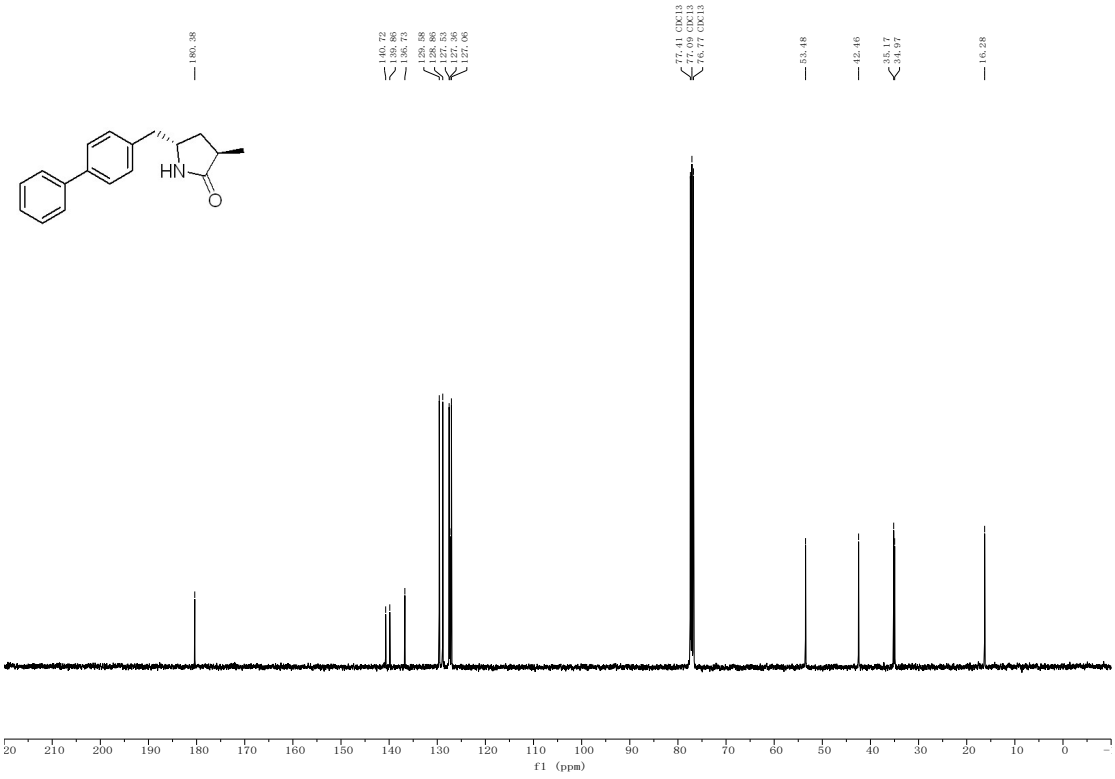


Figure S38. ^{13}C NMR spectra of **8** in $CDCl_3$.

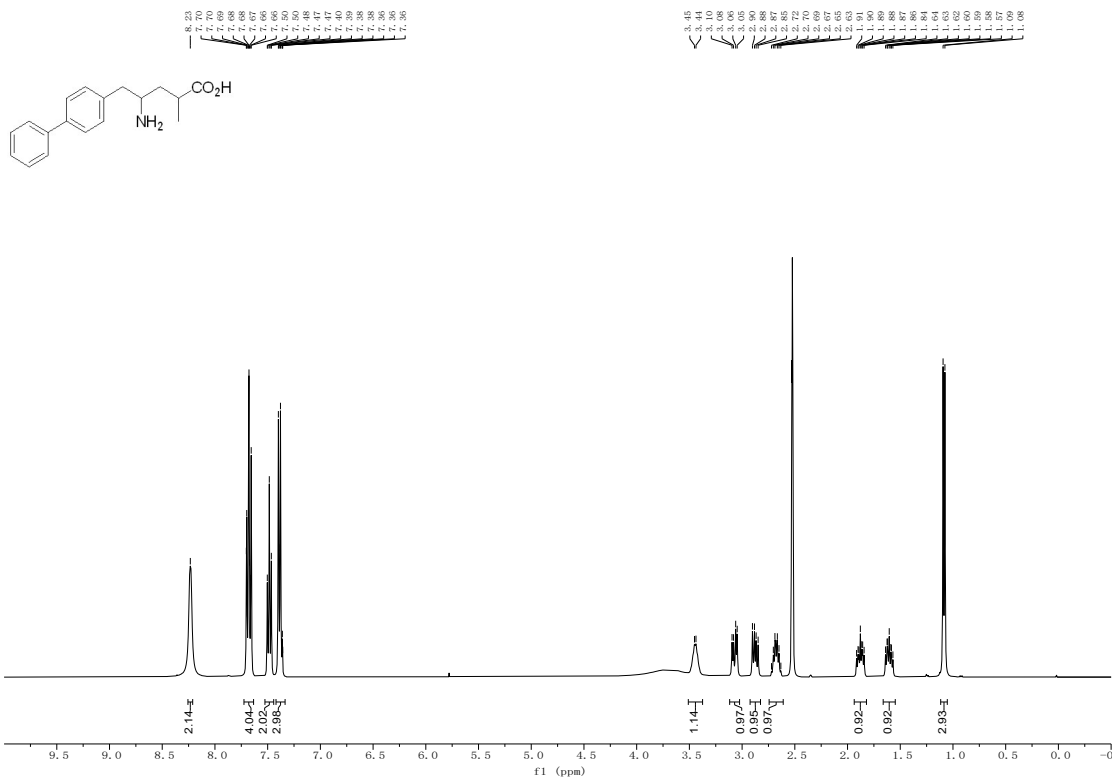


Figure S39. ^{1}H NMR spectra of **9** in DMSO-d_6.

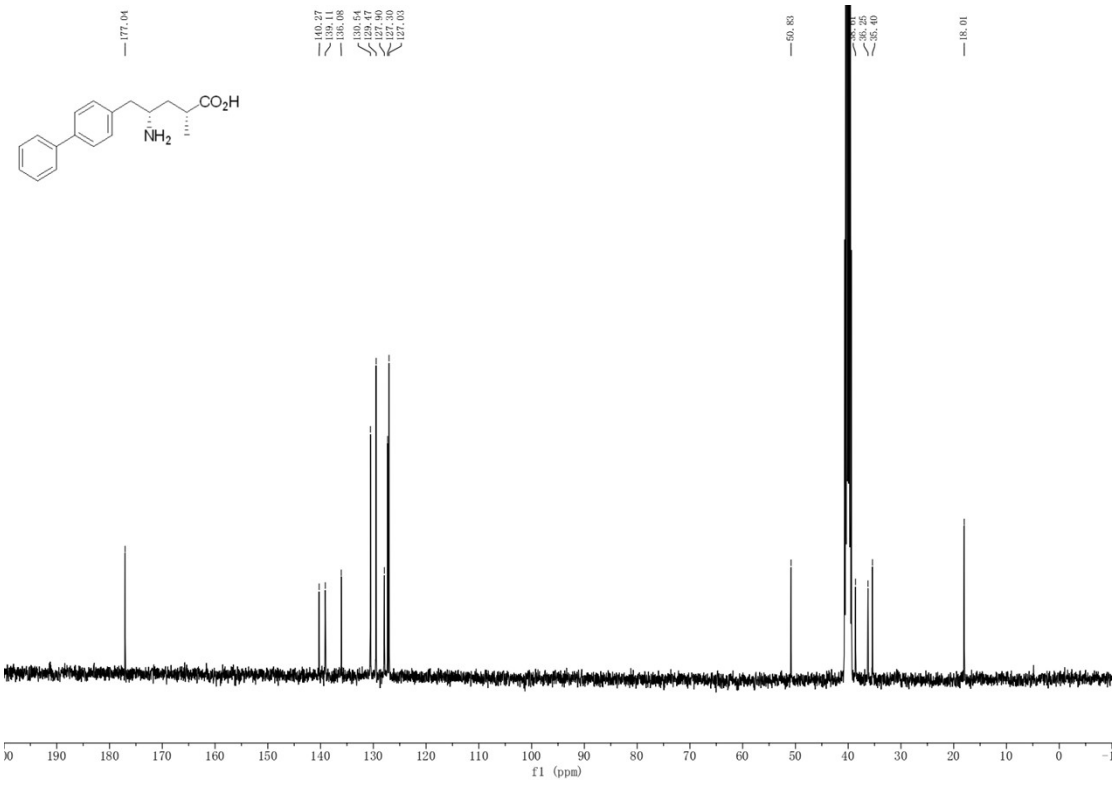
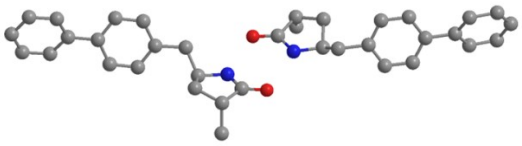


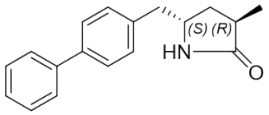
Figure S40. ^{13}C NMR spectra of **9** in DMSO-d_6.

15. Crystal structures of product 8

The products of the biocatalytic reaction were separated and purified to present a white solid. Subsequently, we used the liquid-phase diffusion method to cultivate the single crystals, and the good solvent was EtOAc and the poor solvent was n-hexane. Three sets of experiments were carried out at the same time, and fortunately, one of them successfully grew a single crystal, and then we performed crystal diffraction and successfully obtained the crystal structure.

Suitable crystal was selected and on a Bruker APEX-II CCD diffractometer equipped with CuKα radiance source. The crystal was kept at 296 K during data collection. Using Olex2[12], the structure was solved with the XS[13] structure solution program using Direct Methods and refined with the XLrefinement package using Least Squares minimization





(3*R*,5*S*)-5-([1,1'-biphenyl]-4-ylmethyl)-3-methylpyrrolidin-2-one

Table S6. Crystal chromatography information for compound **8**

Empirical formula	$C_{18}H_{19}NO$
Formula weight	265.34
Temperature/K	100.01(10)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	10.6039(3)
b/Å	8.8338(2)
c/Å	15.3191(3)
α/°	90
β/°	92.902(2)
γ/°	90
Volume/Å^3	1433.14(6)
Z	4
ρ_{calc}mg/mm^3	1.23
μ/mm^{-1}	0.588
F(000)	568
Crystal size/mm^3	0.1 × 0.1 × 0.1

2Θ range for data collection	5.776 to 148.376°
Index ranges	$-12 \leq h \leq 12, -10 \leq k \leq 10, -18 \leq l \leq 18$
Reflections collected	27270
Independent reflections	5318[R(int) = 0.0798]
Data/restraints/parameters	5318/1/364
Goodness-of-fit on F^2	1.14
Final R indexes [I>=2σ (I)]	$R_1 = 0.0355$, $wR_2 = 0.1035$
Final R indexes [all data]	$R_1 = 0.0405$, $wR_2 = 0.1061$
Largest diff. peak/hole / e $Å^{-3}$	0.22/-0.17
Flack parameter	0.16(14)

16. References

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