Supporting Information

Enzyme Cascades in Whole Cells for the Synthesis of Cyclic Chiral Amines.

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1. Materials and equipment.

1.1. Chemicals and equipment.

Commercial reagents and solvents were purchased from Sigma Aldrich, Alfa Aesar or Fluorochem and used without further purification. Specifically substrates **1a** and **1f** and amine standards **4f** and **4g** were purchased from Sigma Aldrich, **1c** was purchased from Fluorochem, and amine standard **4a** was purchased from TCI Chemicals. Substrates **1b**-**e** and amine standards **4b**-**e** were prepared as previously reported.[1–3] NMR spectra were recorded using a Bruker Avance 400 spectrometer with chemical shifts reported in ppm relative to residual protic solvent signals ($CHCl_3$ in $CDCl_3$, ^1H = 7.27; $CDCl_3$, ^{13}C = 77.0; CHD_2OD in CD_3OD, ^1H = 3.31; CD_3OD, ^{13}C = 49.0; CHD_2SOCD_3 in $(CD_3)_2SO$, ^1H = 2.50; $(CD_3)_2SO$, ^{13}C = 39.52).[4] The coupling constants (*J*) are quoted in Hz to the nearest 0.1 Hz. Signal multiplicities are assigned as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), sextet (sxt), multiplet (m), broad (br) or a combination of the above. Low resolution mass spectrometry (MS) was performed on a HP-6890 GC connected to a HP5973 MS detector.

1.2. HPLC, GC, and GCMS analysis.

Normal phase HPLC was performed on an Agilent system equipped with a G1379A degasser, G1312A binary pump, a G1367A well plate autosampler unit, a G1316A temperature controlled column compartment and a G1315C diode array detector. CHIRALPAK®IC analytical column was purchased from Daicel (Osaka, Japan). The column possesses dimensions of 250 mm length, 4.6 mm diameter, 5 µm particle size. An injection volume of 10 µL was used and chromatograms were monitored at 265 nm.

Gas chromatography was performed on an Agilent 6850 GC system using an Agilent Technologies 6850 Series Auto Sampler for injections and equipped with a flame ionization detector (FID) for detection, using a 25 m CP-Chirasil-DEX CB column with 0.25 mm inner diameter and 0.25 µm film thickness (Agilent, Santa Clara, CA, USA). Helium was used as the carrier gas (1.2 mL min^{-1}). Derivatization of samples for chiral GC-FID analysis was achieved using acetic anhydride and an excess of triethylamine at room temperature, where stated.

GCMS analysis was performed on a HP-6890 Series GC coupled to a HP5973 MS detector, EI positive mode with helium as the carrier gas.

Analysis and determination of ee for amines **4a**-**e** based on previously reported HPLC or GC-FID analysis on a chiral stationary phase.[1–3]

2. Molecular biology, whole cell biocatalyst preparation, and protein expression.

2.1. Nucleotide sequences for plasmid pPB01, BioBrick prefix and suffix, operons and genes for MCAR, NCAR, *Bs*Sfp, ATA-117, SitATA, (*R*)-IRED and (*S*)-IRED.

pPB01 DNA sequence:

GGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCC
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ACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAA
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BioBrick prefix DNA sequence:

GAATTCGAGCAGTGTCTCTAGAAGAGACGTAC

EcoRI *XbaI*

BioBrick suffix DNA sequence:

ACTGGGCCTTTCGTTTTATCTGACTAGTTAGCATCGTTCACTGCAG

SpeI *PstI*

MCAR operon sequence:

GAATTCGAGCAGTGTCTCTAGAAGAGACGTACGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGA
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MCAR open reading frame (ORF) sequence [gene with N-terminal His_6 tag]:

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NCAR operon sequence:

GAATTCGAGCAGTGTCTCTAGAAGAGACGTACGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGA
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GTGACCTGGCCAACTATATTGAAGCGGAACGTAACAGCGGCGCGAAACGTCCGACCTTTACCAGCGTGCATG
GCGGCGGTAGCGAAATTCGTGCGGCCGATCTGACCCTGGATAAATTTATTGATGCGCGTACCCTGGCCGCAG
CGGATAGCATTCCGCATGCACCGGTTCCGGCACAGACCGTCCTGCTGACGGGCGCAAATGGCTATCTGGGCC
GTTTTCTGTGCCTGGAATGGCTGGAACGTCTGGATAAAACCGGTGGCACCCTGATTTGCGTGGTGCGTGGCA
GCGATGCGGCGGCAGCCCGTAAACGCCTGGATAGCGCGTTTGATAGCGGCGATCCGGGCCTGCTGGAACATT
ATCAGCAGCTGGCCGCACGCACCCTGGAAGTTCTGGCCGGTGATATTGGCGATCCGAACCTGGGCCTGGATG
ATGCCACCTGGCAGCGTCTGGCCGAAACCGTGGATCTGATTGTGCACCCGGCTGCTCTGGTGAATCATGTGCT
GCCGTATACCCAGCTGTTTGGCCCGAACGTTGTGGGCACCGCGGAAATCGTTCGTCTGGCTATTACCGCGCGT
CGTAAACCGGTGACCTATCTGAGCACCGTGGGCGTGGCGGATCAGGTTGATCCGGCGGAATATCAGGAAGAT
AGCGACGTCCGCGAAATGAGCGCAGTCCGCGTCGTTCGCGAAAGTTATGCAAACGGTTATGGTAACAGCAAA
TGGGCGGGTGAAGTGCTGCTGCGTGAAGCGCATGATCTGTGCGGTCTGCCGGTGGCGGTGTTTCGTAGCGAT
ATGATTCTGGCCCATAGCCGTTATGCGGGCCAGCTGAACGTGCAGGATGTGTTTACCCGTCTGATTCTGAGCC
TGGTGGCGACCGGCATTGCGCCGTATAGCTTTTATCGCACCGATGCGGATGGCAACCGTCAGCGTGCGCATTA
TGATGGCCTGCCGGCGGATTTTACCGCAGCAGCTATCACCGCACTGGGCATTCAGGCGACCGAAGGCTTTCGT
ACCTATGATGTGCTGAATCCGTATGATGATGGCATTAGCCTGGATGAATTTGTCGATTGGCTGGTCGAATCTG
GTCACCCGATTCAGCGCATTACCGATTATAGCGATTGGTTTCACCGCTTTGAAACCGCGATTCGTGCGCTGCCG
GAAAAACAGCGTCAGGCGAGCGTTCTGCCGCTGCTGGATGCGTATCGTAATCCGTGTCCGGCGGTCCGTGGT
GCAATTCTGCCGGCGAAAGAATTTCAGGCGGCGGTGCAGACCGCGAAAATTGGCCCGGAACAGGATATTCCG
CATCTGAGCGCACCGCTGATTGATAAATATGTGAGCGATCTGGAACTGCTGCAACTGCTGTAACCGGCTTATC
GGTCAGTTTCACCTGATTTACGTAAAAACCCGCTTCGGCGGGTTTTTGCTTTTGGAGGGGCAGAAAGATGAAT
GACTGTCCACGACGCTATACCCAAAAGAAAGCGGCTTATCGGTCAGTTTCACCTGGTTTACGTAAAAACCCGC
TTCGGCGGGTTTTTGCTTTTGGAGGGGCAGAAAGATGAATGACTGTCCACGACACTATACCCAAAAGAAAGC
GGCTTATCGGTCAGTTTCACCTGTTTTACGTAAAAACCCGCTTCGGCGGGTTTTTACTTTTGGAGGGGCAGAAA
GATGAATGACTGTCCACGACACTATACCCAAAAGAAAACTGGGCCTTTCGTTTTATCTGACTAGTTAGCATCGT
TCACTGCAG

NCAR open reading frame (ORF) sequence [gene with N-terminal His_6 tag]:

ATGCACCATCATCATCATCATTCTTCTGGTGCGGTGGATAGCCCGGATGAACGTCTGCAACGTCGTATTGCGCA
GCTGTTTGCGGAAGATGAACAGGTGAAAGCAGCACGCCCGCTGGAAGCGGTTAGCGCAGCGGTGAGCGCAC
CGGGTATGCGTCTGGCCCAGATTGCGGCGACCGTGATGGCGGGCTATGCGGATCGTCCGGCAGCGGGTCAG
CGTGCGTTTGAACTGAACACCGATGATGCGACCGGCCGTACCAGCCTGCGTCTGCTGCCGCGTTTTGAAACCA
TTACCTATCGTGAACTGTGGCAGCGTGTGGGTGAAGTTGCGGCAGCGTGGCATCACGATCCGGAAAATCCGC
TGCGTGCGGGCGATTTTGTGGCGCTGCTGGGCTTTACCAGCATTGATTATGCGACCCTGGATCTGGCCGATAT
TCATCTGGGCGCGGTGACCGTTCCGCTGCAAGCGAGCGCAGCAGTCAGCCAACTGATTGCGATTCTGACCGA
AACGAGTCCGCGCCTGCTGGCATCTACCCCGGAACATCTGGATGCGGCGGTGGAATGTCTGCTGGCAGGTAC
GACGCCGGAACGCCTGGTGGTGTTTGATTATCATCCGGAAGATGATGATCAGCGTGCGGCGTTTGAAAGCGC
GCGTCGTCGTCTGGCCGATGCGGGCAGCCTGGTGATTGTGGAAACCCTGGATGCGGTGCGTGCGCGTGGTCG
TGATCTGCCGGCTGCTCCGCTGTTTGTGCCGGATACCGATGATGATCCGCTGGCCCTGCTGATTTATACCTCTG
GTAGCACGGGTACGCCGAAAGGCGCCATGTATACCAACCGCCTGGCAGCAACGATGTGGCAAGGTAACAGC
ATGCTGCAAGGCAATAGCCAGCGTGTGGGCATTAACCTGAACTATATGCCGATGAGCCATATTGCGGGCCGT
ATTAGCCTGTTTGGCGTGCTGGCCCGTGGTGGCACCGCGTATTTTGCGGCGAAAAGCGATATGAGCACCCTGT
TTGAAGATATTGGCCTGGTGCGTCCGACCGAAATTTTTTTTGTGCCGCGTGTGTGCGATATGGTGTTTCAGCGT
TATCAGAGCGAACTGGATCGTCGTAGCGTGGCGGGTGCGGATCTGGATACCCTGGATCGTGAAGTGAAAGC
GGATCTGCGTCAGAACTATCTGGGCGGTCGTTTTCTGGTGGCGGTGGTGGGTAGCGCACCGCTGGCCGCGGA
AATGAAAACCTTTATGGAAAGCGTGCTGGATCTGCCGCTGCATGATGGCTATGGCAGCACCGAAGCGGGTGC
GAGCGTGCTGCTGGATAACCAGATTCAGCGTCCGCCGGTGCTGGATTATAAACTGGTGGACGTCCCGGAACT
GGGCTATTTTCGTACCGATCGTCCGCATCCGCGTGGCGAACTGCTGCTGAAAGCGGAAACCACCATTCCGGGC
TATTATAAACGTCCGGAAGTGACCGCGGAAATTTTTGATGAAGATGGCTTCTATAAAACCGGCGATATTGTGG
CGGAACTGGAACATGATCGTCTGGTGTATGTGGATCGTCGCAACAACGTGCTGAAACTGAGCCAGGGCGAAT
TTGTGACCGTGGCGCATCTGGAAGCGGTGTTTGCGAGCAGCCCGCTGATTCGTCAGATTTTTATCTACGGCTC
TAGTGAACGCTCTTATCTGCTGGCAGTGATTGTGCCGACCGATGATGCCCTGCGTGGCCGTGATACCGCGACC
CTGAAAAGCGCGCTGGCCGAAAGCATTCAGCGTATTGCGAAAGATGCGAACCTGCAACCGTATGAAATTCCG
CGTGATTTTCTGATTGAAACCGAACCGTTCACCATTGCGAACGGCCTGCTGTCTGGCATTGCGAAACTGCTGC
GTCCGAACCTGAAAGAACGTTATGGCGCGCAGCTGGAACAAATGTATACCGATCTGGCCACCGGCCAGGCGG
ATGAACTGCTGGCCCTGCGTCGTGAAGCGGCGGATCTGCCGGTTCTGGAAACCGTTAGCCGTGCGGCGAAAG
CCATGCTGGGTGTGGCGAGCGCGGATATGCGTCCGGATGCGCATTTTACCGATCTGGGCGGCGATAGCCTGA
GCGCCCTGAGCTTTAGCAACCTGCTGCATGAAATTTTTGGCGTGGAAGTGCCGGTGGGTGTGGTTGTGAGCC
CGGCAAACGAACTGCGTGACCTGGCCAACTATATTGAAGCGGAACGTAACAGCGGCGCGAAACGTCCGACCT
TTACCAGCGTGCATGGCGGCGGTAGCGAAATTCGTGCGGCCGATCTGACCCTGGATAAATTTATTGATGCGCG
TACCCTGGCCGCAGCGGATAGCATTCCGCATGCACCGGTTCCGGCACAGACCGTCCTGCTGACGGGCGCAAA
TGGCTATCTGGGCCGTTTTCTGTGCCTGGAATGGCTGGAACGTCTGGATAAAACCGGTGGCACCCTGATTTGC
GTGGTGCGTGGCAGCGATGCGGCGGCAGCCCGTAAACGCCTGGATAGCGCGTTTGATAGCGGCGATCCGGG
CCTGCTGGAACATTATCAGCAGCTGGCCGCACGCACCCTGGAAGTTCTGGCCGGTGATATTGGCGATCCGAAC
CTGGGCCTGGATGATGCCACCTGGCAGCGTCTGGCCGAAACCGTGGATCTGATTGTGCACCCGGCTGCTCTG
GTGAATCATGTGCTGCCGTATACCCAGCTGTTTGGCCCGAACGTTGTGGGCACCGCGGAAATCGTTCGTCTGG
CTATTACCGCGCGTCGTAAACCGGTGACCTATCTGAGCACCGTGGGCGTGGCGGATCAGGTTGATCCGGCGG
AATATCAGGAAGATAGCGACGTCCGCGAAATGAGCGCAGTCCGCGTCGTTCGCGAAAGTTATGCAAACGGTT
ATGGTAACAGCAAATGGGCGGGTGAAGTGCTGCTGCGTGAAGCGCATGATCTGTGCGGTCTGCCGGTGGCG
GTGTTTCGTAGCGATATGATTCTGGCCCATAGCCGTTATGCGGGCCAGCTGAACGTGCAGGATGTGTTTACCC
GTCTGATTCTGAGCCTGGTGGCGACCGGCATTGCGCCGTATAGCTTTTATCGCACCGATGCGGATGGCAACCG
TCAGCGTGCGCATTATGATGGCCTGCCGGCGGATTTTACCGCAGCAGCTATCACCGCACTGGGCATTCAGGCG
ACCGAAGGCTTTCGTACCTATGATGTGCTGAATCCGTATGATGATGGCATTAGCCTGGATGAATTTGTCGATT

GGCTGGTCGAATCTGGTCACCCGATTCAGCGCATTACCGATTATAGCGATTGGTTTCACCGCTTTGAAACCGC
GATTCGTGCGCTGCCGGAAAAACAGCGTCAGGCGAGCGTTCTGCCGCTGCTGGATGCGTATCGTAATCCGTG
TCCGGCGGTCCGTGGTGCAATTCTGCCGGCGAAAGAATTTCAGGCGGCGGTGCAGACCGCGAAAATTGGCCC
GGAACAGGATATTCCGCATCTGAGCGCACCGCTGATTGATAAATATGTGAGCGATCTGGAACTGCTGCAACTG
CTGTAA

***Bs*Sfp operon sequence:**

GAATTCGAGCAGTGTCTCTAGAAGAGACGTACTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATT
GTGAGCGGATAACAATTTCACACAGGAAACAGCGCCGCTGAGAAAAAGCGAAGCGGCACTGCTCTTTAACAA
TTTATCAGACAATCTGTGTGGGCACTCGACCGGAATTATCGATTAACTTTATTATTAAAAATTAAAGAGGTATA
TATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGGTAAAATCTACGGGATCTATATGGATCGCCCCCT
CAGCCAGGAAGAGAATGAACGCTTTATGTCATTTATTAGTCCCGAGAAACGTGAAAAGTGCCGCCGCTTTTAC
CATAAAGAAGATGCACATCGTACCCTGTTAGGTGATGTGTTAGTCCGGTCGGTGATTAGTCGCCAGTACCAAT
TGGATAAAAGCGATATCCGTTTTTCCACCCAGGAATATGGCAAACCGTGCATTCCGGATCTCCCTGACGCTCAT
TTTAACATTTCTCATTCAGGTCGCTGGGTCATCTGCGCATTTGACTCTCAGCCAATTGGCATTGATATTGAAAAA
ACAAAACCCATTTCCCTGGAGATTGCAAAACGTTTCTTTGCTAAAACGGAGTATAGCGATCTGCTGGCGAAAG
ACAAAGACGAACAGACGGATTACTTCTATCATTTGTGGAGCATGAAGGAAAGTTTTATCAAGCAAGAGGGAA
AGGGCCTTTCTCTTCCTCTGGATTCTTTCAGCGTCCGTCTTCACCAGGACGGCCAAGTTAGTATCGAACTCCCA
GACTCACACAGTCCGTGTTATATCAAGACGTATGAGGTCGATCCGGGTTATAAAATGGCGGTATGTGCCGCAC
ATCCAGATTTTCCGGAGGATATTACAATGGTGTCGTATGAGGAGTTGCTGCGCGGTAGCGGCGATTATAAGG
ACGATGATGATAAATAATACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACAGAATTTGCCTGGCGG
CAGTAGCGCGGTGGTCCCACCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTAGCGCCGATGGTAGTGT
GGGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGG
GCCTTTCGTTTTATCTGACTAGTTAGCATCGTTCACTGCAG

***Bs*Sfp open reading frame (ORF) sequence [gene with C-terminal FLAG tag]:**

ATGGGTAAAATCTACGGGATCTATATGGATCGCCCCCTCAGCCAGGAAGAGAATGAACGCTTTATGTCATTTA
TTAGTCCCGAGAAACGTGAAAAGTGCCGCCGCTTTTACCATAAAGAAGATGCACATCGTACCCTGTTAGGTGA
TGTGTTAGTCCGGTCGGTGATTAGTCGCCAGTACCAATTGGATAAAAGCGATATCCGTTTTTCCACCCAGGAAT
ATGGCAAACCGTGCATTCCGGATCTCCCTGACGCTCATTTTAACATTTCTCATTCAGGTCGCTGGGTCATCTGC
GCATTTGACTCTCAGCCAATTGGCATTGATATTGAAAAAACAAAACCCATTTCCCTGGAGATTGCAAAACGTTT
CTTTGCTAAAACGGAGTATAGCGATCTGCTGGCGAAAGACAAAGACGAACAGACGGATTACTTCTATCATTTG
TGGAGCATGAAGGAAAGTTTTATCAAGCAAGAGGGAAAGGGCCTTTCTCTTCCTCTGGATTCTTTCAGCGTCC
GTCTTCACCAGGACGGCCAAGTTAGTATCGAACTCCCAGACTCACACAGTCCGTGTTATATCAAGACGTATGA
GGTCGATCCGGGTTATAAAATGGCGGTATGTGCCGCACATCCAGATTTTCCGGAGGATATTACAATGGTGTCG
TATGAGGAGTTGCTGCGCGGTAGCGGCGATTATAAGGACGATGATGATAAATAA

ATA-117 operon sequence:

GAATTCGAGCAGTGTCTCTAGAAGAGACGTACTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATT
GTGAGCGGATAACAATTTCACACAGGAAACAGCGCCGCTGAGAAAAAGCGAAGCGGCACTGCTCTTTAACAA
TTTATCAGACAATCTGTGTGGGCACTCGACCGGAATTATCGATTAACTTTATTATTAAAAATTAAAGAGGTATA
TATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGAACAAAAACTTATTTCTGAAGAAGATCTGACCAG
CGAAATTGTTTATACCCATGATACCGGTCTGGATTATATCACCTATAGCGATTATGAACTGGATCCGGCAAATC
CGCTGGCAGGCGGTGCAGCATGGATTGAAGGTGCATTTGTTCCGCCTAGCGAAGCACGTATTAGCATTTTTGA
TCAGGGCTATCTGCATAGTGATGTTACCTATACCGTGTTTCATGTGTGGAATGGTAATGCATTTCGCCTGGATG
ATCATATTGAACGTCTGTTTAGCAATGCAGAAAGCATGCGTATTATTCCGCCTCTGACCCAGGATGAAGTTAAA
GAAATTGCACTGGAACTGGTTGCAAAAACCGAACTGCGTGAAGCATTTGTTAGCGTTAGCATTACCCGTGGTT
ATAGCAGCACACCGGGTGAACGTGATATTACCAAACATCGTCCGCAGGTTTATATGTATGCAGTTCCGTATCA
GTGGATTGTTCCGTTTGATCGTATTCGTGATGGTGTTCATGCAATGGTTGCACAGAGCGTTCGTCGTACACCGC
GTAGCAGCATTGATCCGCAGGTTAAAAATTTTCAGTGGGGTGATCTGATTCGTGCAGTTCAAGAAACCCATGA
TCGTGGTTTTGAAGCACCGCTGCTGCTGGATGGTGATGGTCTGCTGGCCGAAGGTAGCGGTTTTAATGTTGTT
GTGATTAAAGATGGTGTGGTTCGTAGTCCGGGTCGTGCAGCACTGCCTGGTATTACCCGTAAAACCGTTCTGG
AAATTGCAGAAAGCCTGGGTCATGAAGCAATTCTGGCAGATATTACCCTGGCAGAACTGCTGGATGCAGATG
AAGTTCTGGGTTGTACCACCGCAGGCGGTGTTTGGCCGTTTGTTAGCGTGGATGGTAATCCGATTTCAGATGG
TGTTCCGGGTCCGATTACCCAGAGCATTATTCGTCGTTATTGGGAACTGAATGTTGAAAGCAGCAGCCTGCTG
ACACCGGTTCAGTATAACTGGTCTCACCCGCAGTTCGAAAAATAATACAGATTAAATCAGAACGCAGAAGCGG
TCTGATAAAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGACCCCATGCCGAACTCAGAAGT
GAAACGCCGTAGCGCCGATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAAATA
AAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGACTAGTTAGCATCGTTCACTGCAG

ATA-117 open reading frame (ORF) sequence [gene with c-Myc epitope and C-terminal Strep II tag]:

ATGGAACAAAAACTTATTTCTGAAGAAGATCTGACCAGCGAAATTGTTTATACCCATGATACCGGTCTGGATT
ATATCACCTATAGCGATTATGAACTGGATCCGGCAAATCCGCTGGCAGGCGGTGCAGCATGGATTGAAGGTG
CATTTGTTCCGCCTAGCGAAGCACGTATTAGCATTTTTGATCAGGGCTATCTGCATAGTGATGTTACCTATACC
GTGTTTCATGTGTGGAATGGTAATGCATTTCGCCTGGATGATCATATTGAACGTCTGTTTAGCAATGCAGAAA
GCATGCGTATTATTCCGCCTCTGACCCAGGATGAAGTTAAAGAAATTGCACTGGAACTGGTTGCAAAAACCGA
ACTGCGTGAAGCATTTGTTAGCGTTAGCATTACCCGTGGTTATAGCAGCACACCGGGTGAACGTGATATTACC
AAACATCGTCCGCAGGTTTATATGTATGCAGTTCCGTATCAGTGGATTGTTCCGTTTGATCGTATTCGTGATGG
TGTTCATGCAATGGTTGCACAGAGCGTTCGTCGTACACCGCGTAGCAGCATTGATCCGCAGGTTAAAAATTTT
CAGTGGGGTGATCTGATTCGTGCAGTTCAAGAAACCCATGATCGTGGTTTTGAAGCACCGCTGCTGCTGGATG
GTGATGGTCTGCTGGCCGAAGGTAGCGGTTTTAATGTTGTTGTGATTAAAGATGGTGTGGTTCGTAGTCCGG
GTCGTGCAGCACTGCCTGGTATTACCCGTAAAACCGTTCTGGAAATTGCAGAAAGCCTGGGTCATGAAGCAAT
TCTGGCAGATATTACCCTGGCAGAACTGCTGGATGCAGATGAAGTTCTGGGTTGTACCACCGCAGGCGGTGTT
TGGCCGTTTGTTAGCGTGGATGGTAATCCGATTTCAGATGGTGTTCCGGGTCCGATTACCCAGAGCATTATTC
GTCGTTATTGGGAACTGAATGTTGAAAGCAGCAGCCTGCTGACACCGGTTCAGTATAACTGGTCTCACCCGCA
GTTCGAAAAATAA

SitATA operon sequence:

GAATTCGAGCAGTGTCTCTAGAAGAGACGTACCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGC
GGATAACAATTCCCCTCTTGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGGGCCATCATCATCAT
CATCATCATCATCATCACAGCAGCGGCCATATCGAAGGTCGTATGGCATTTAGCGCAGATACACCGGAAATTG

TTTATACCCATGATACCGGTCTGGATTATATCACCTATAGCGATTATGAACTGGACCCTGCAAATCCGCTGGCA
GGCGGTGCAGCATGGATTGAAGGTGCATTTGTTCCGCCTAGCGAAGCACGTATTAGCATTTTTGATCAGGGCT
TTTATACCAGTGATGCAACCTATACCACCTTTCATGTGTGGAATGGTAATGCATTTCGTCTGGGTGATCATATT
GAACGTCTGTTTAGCAATGCCGAAAGCATTCGTCTGATTCCGCCTCTGACCCAGGATGAAGTTAAAGAAATTG
CACTGGAACTGGTTGCAAAAACCGAACTGCGTGAAGCAATGGTTACCGTTACCATTACCCGTGGTTATAGCAG
CACCCCGTTTGAACGTGATATTACCAAACATCGTCCGCAGGTTTATATGAGCGCATGTCCGTATCAGTGGATTG
TTCCGTTTGATCGTATTCGTGATGGTGTTCATCTGATGGTTGCACAGAGCGTTCGTCGTACACCGCGTAGCAGC
ATTGATCCGCAGGTTAAAAATTTTCAGTGGGGTGATCTGATTCGTGCAATTCAGGAAACCCACGATCGCGGTT
TTGAACTGCCGCTGCTGCTGGATTGTGATAATCTGCTGGCCGAAGGTCCGGGTTTTAATGTTGTTGTTATTAAA
GATGGCGTGGTTCGTAGTCCGGGTCGTGCAGCACTGCCTGGTATTACCCGTAAAACCGTTCTGGAAATTGCAG
AAAGCCTGGGTCATGAAGCAATTCTGGCAGATATTACACCGGCAGAACTGTATGATGCAGATGAAGTTCTGG
GTTGTAGCACCGGTGGTGGTGTTTGGCCGTTTGTTAGCGTTGATGGTAATAGCATTAGTGATGGCGTTCCGGG
TCCGGTTACCCAGAGCATTATTCGTCGTTATTGGGAACTGAATGTTGAACCGAGCAGCCTGCTGACACCGGTT
CAGTATTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTT
GGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGACTGGGCCTTTCGTTTTATCTGACTAGTTAGCATCGTTCAC
TGCAG

SitATA open reading frame (ORF) sequence [gene with N-terminal His_6 tag]:

ATGGGCCATCATCATCATCATCATCATCATCACAGCAGCGGCCATATCGAAGGTCGTATGGCATTTAGCGC
AGATACACCGGAAATTGTTTATACCCATGATACCGGTCTGGATTATATCACCTATAGCGATTATGAACTGGACC
CTGCAAATCCGCTGGCAGGCGGTGCAGCATGGATTGAAGGTGCATTTGTTCCGCCTAGCGAAGCACGTATTA
GCATTTTTGATCAGGGCTTTTATACCAGTGATGCAACCTATACCACCTTTCATGTGTGGAATGGTAATGCATTT
CGTCTGGGTGATCATATTGAACGTCTGTTTAGCAATGCCGAAAGCATTCGTCTGATTCCGCCTCTGACCCAGGA
TGAAGTTAAAGAAATTGCACTGGAACTGGTTGCAAAAACCGAACTGCGTGAAGCAATGGTTACCGTTACCATT
ACCCGTGGTTATAGCAGCACCCCGTTTGAACGTGATATTACCAAACATCGTCCGCAGGTTTATATGAGCGCAT
GTCCGTATCAGTGGATTGTTCCGTTTGATCGTATTCGTGATGGTGTTCATCTGATGGTTGCACAGAGCGTTCGT
CGTACACCGCGTAGCAGCATTGATCCGCAGGTTAAAAATTTTCAGTGGGGTGATCTGATTCGTGCAATTCAGG
AAACCCACGATCGCGGTTTTGAACTGCCGCTGCTGCTGGATTGTGATAATCTGCTGGCCGAAGGTCCGGGTTT
TAATGTTGTTGTTATTAAAGATGGCGTGGTTCGTAGTCCGGGTCGTGCAGCACTGCCTGGTATTACCCGTAAA
ACCGTTCTGGAAATTGCAGAAAGCCTGGGTCATGAAGCAATTCTGGCAGATATTACACCGGCAGAACTGTATG
ATGCAGATGAAGTTCTGGGTTGTAGCACCGGTGGTGGTGTTTGGCCGTTTGTTAGCGTTGATGGTAATAGCAT
TAGTGATGGCGTTCCGGGTCCGGTTACCCAGAGCATTATTCGTCGTTATTGGGAACTGAATGTTGAACCGAGC
AGCCTGCTGACACCGGTTCAGTATTAA

(*R*)-IRED operon sequence:

GAATTCGAGCAGTGTCTCTAGAAGAGACGTACGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGA
ATTGTGAGCGGATAACAATTTCACACAGGAAACAGAATTGTAGAGGAGATATACATATGGGCAGCAGCCATC
ATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGGCGACAATCGTACGCCGGTCACGG
TTATCGGTCTGGGTCTGATGGGTCAAGCACTGGCAGCAGCATTTCTGGAAGCAGGTCACACCACGACCGTGT
GGAACCGTAGCGCGGGTAAAGCCGAACAGCTGGTTTCTCAGGGTGCGGTTCAGGCCGCAACCCCGGCAGAT
GCTGTTGCAGCTTCAGAACTGGTGGTTGTCTGCCTGTCGACCTATGATAACATGCATGACGTCATTGGTAGTCT

GGGCGAATCCCTGCGTGGTAAAGTCATCGTGAATCTGACGAGCGGTAGCTCTGATCAGGGTCGTGAAACCGC
CGCATGGGCAGAAAAACAGGGTGTTGAATACCTGGACGGCGCAATTATGATCACGCCGCCGGGTATTGGCAC
GGAAACCGCAGTCCTGTTTTATGCTGGTACCCAGTCTGTGTTCGAAAAATACGAACCGGCTCTGAAACTGCTG
GGCGGTGGCACGACCTATCTGGGTACCGATCATGGCATGCCGGCCCTGTACGACGTGTCACTGCTGGGTCTG
ATGTGGGGCACGCTGAACTCGTTTCTGCATGGCGTGGCAGTGGTTGAAACCGCGGGTGTTGGCGCCCAGCAA
TTTCTGCCGTGGGCACACATGTGGCTGGAAGCTATTAAAATGTTCACCGCGGATTATGCAGCTCAAATCGATG
CGGGTGACGGCAAATTCCCGGCAAATGACGCTACGCTGGAAACCCACCTGGCGGCCCTGAAACATCTGGTTC
ACGAATCAGAAGCGCTGGGCATTGATGCCGAACTGCCGAAATACAGTGAAGCGCTGATGGAACGCGTGATCT
CCCAGGGTCACGCTAAAAACAGCTATGCGGCAGTCCTGAAAGCCTTCCGTAAACCGTCCGAATAACCGGCTTA
TCGGTCAGTTTCACCTGATTTACGTAAAAACCCGCTTCGGCGGGTTTTTGCTTTTGGAGGGGCAGAAAGATGA
ATGACTGTCCACGACGCTATACCCAAAAGAAAGCGGCTTATCGGTCAGTTTCACCTGGTTTACGTAAAAACCC
GCTTCGGCGGGTTTTTGCTTTTGGAGGGGCAGAAAGATGAATGACTGTCCACGACACTATACCCAAAAGAAA
GCGGCTTATCGGTCAGTTTCACCTGTTTTACGTAAAAACCCGCTTCGGCGGGTTTTTACTTTTGGAGGGGCAGA
AAGATGAATGACTGTCCACGACACTATACCCAAAAGAAAACTGGGCCTTTCGTTTTATCTGACTAGTTAGCATC
GTTCACTGCAG

***(R)*-IRED open reading frame (ORF) sequence [gene with N-terminal His_6 tag]:**

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGGCGACAAT
CGTACGCCGGTCACGGTTATCGGTCTGGGTCTGATGGGTCAAGCACTGGCAGCAGCATTTCTGGAAGCAGGT
CACACCACGACCGTGTGGAACCGTAGCGCGGGTAAAGCCGAACAGCTGGTTTCTCAGGGTGCGGTTCAGGCC
GCAACCCCGGCAGATGCTGTTGCAGCTTCAGAACTGGTGGTTGTCTGCCTGTCGACCTATGATAACATGCATG
ACGTCATTGGTAGTCTGGGCGAATCCCTGCGTGGTAAAGTCATCGTGAATCTGACGAGCGGTAGCTCTGATCA
GGGTCGTGAAACCGCCGCATGGGCAGAAAAACAGGGTGTTGAATACCTGGACGGCGCAATTATGATCACGCC
GCCGGGTATTGGCACGGAAACCGCAGTCCTGTTTTATGCTGGTACCCAGTCTGTGTTCGAAAAATACGAACCG
GCTCTGAAACTGCTGGGCGGTGGCACGACCTATCTGGGTACCGATCATGGCATGCCGGCCCTGTACGACGTG
TCACTGCTGGGTCTGATGTGGGGCACGCTGAACTCGTTTCTGCATGGCGTGGCAGTGGTTGAAACCGCGGGT
GTTGGCGCCCAGCAATTTCTGCCGTGGGCACACATGTGGCTGGAAGCTATTAAAATGTTCACCGCGGATTATG
CAGCTCAAATCGATGCGGGTGACGGCAAATTCCCGGCAAATGACGCTACGCTGGAAACCCACCTGGCGGCCC
TGAAACATCTGGTTCACGAATCAGAAGCGCTGGGCATTGATGCCGAACTGCCGAAATACAGTGAAGCGCTGA
TGGAACGCGTGATCTCCCAGGGTCACGCTAAAAACAGCTATGCGGCAGTCCTGAAAGCCTTCCGTAAACCGTC
CGAATAA

(*S*)-IRED operon sequence:

GAATTCGAGCAGTGTCTCTAGAAGAGACGTACGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGA
ATTGTGAGCGGATAACAATTTCACACAGGAAACAGAATTGTAGAGGAGATATACATATGGGCAGCAGCCATC
ATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGAGCAAACAGTCAGTTACGGTGATTG
GTCTGGGTCCGATGGGTCAAGCGATGGTCAATACCTTTCTGGATAATGGTCACGAAGTGACCGTGTGGAACC
GTACGGCGTCAAAAGCAGAAGCTCTGGTGGCGCGCGGCGCAGTTCTGGCACCGACCGTCGAAGATGCTCTGA
GCGCGAATGAACTGATTGTTCTGTCTCTGACCGATTATGACGCCGTGTACGCAATCCTGGAACCGGTTACGGG
CTCACTGTCGGGTAAAGTGATTGCAAACCTGAGCTCTGATACCCCGGACAAAGCGCGTGAAGCGGCCAAATG
GGCAGCTAAACATGGTGCGAAACATCTGACCGGCGGTGTGCAGGTTCCGCCGCCGCTGATCGGCAAACCGGA

AAGTTCCACCTATTACTCCGGTCCGAAAGATGTTTTTGACGCCCATGAAGATACCCTGAAAGTCCTGACGAAC
GCCGATTATCGTGGTGAAGATGCAGGTCTGGCCGCAATGTATTACCAGGCGCAAATGACCATTTTCTGGACCA
CGATGCTGAGCTATTACCAGACGCTGGCTCTGGGCCAAGCGAATGGTGTTAGTGCTAAAGAACTGCTGCCGT
ATGCCACCATGATGACGTCCATGATGCCGCATTTTCTGGAACTGTATGCTCAGCACGTCGATTCTGCGGACTAT
CCGGGTGATGTGGACCGTCTGGCGATGGGCGCAGCTTCAGTCGATCACGTGCTGCATACCCACCAAGATGCG
GGTGTTAGCACCGTCCTGCCGGCCGCAGTGGCCGAAATCTTCAAAGCCGGTATGGAAAAAGGCTTTGCTGAA
AATTCGTTCTCCTCTCTGATTGAAGTCCTGAAAAAACCGGCAGTGTAACCGGCTTATCGGTCAGTTTCACCTGA
TTTACGTAAAAACCCGCTTCGGCGGGTTTTTGCTTTTGGAGGGGCAGAAAGATGAATGACTGTCCACGACGCT
ATACCCAAAAGAAAGCGGCTTATCGGTCAGTTTCACCTGGTTTACGTAAAAACCCGCTTCGGCGGGTTTTTGCT
TTTGGAGGGGCAGAAAGATGAATGACTGTCCACGACACTATACCCAAAAGAAAGCGGCTTATCGGTCAGTTT
CACCTGTTTTACGTAAAAACCCGCTTCGGCGGGTTTTTACTTTTGGAGGGGCAGAAAGATGAATGACTGTCCA
CGACACTATACCCAAAAGAAAACTGGGCCTTTCGTTTTATCTGACTAGTTAGCATCGTTCACTGCAG

***(S)*-IRED open reading frame (ORF) sequence [gene with N-terminal His_6 tag]:**

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGAGCAAACAG
TCAGTTACGGTGATTGGTCTGGGTCCGATGGGTCAAGCGATGGTCAATACCTTTCTGGATAATGGTCACGAAG
TGACCGTGTGGAACCGTACGGCGTCAAAAGCAGAAGCTCTGGTGGCGCGCGGCGCAGTTCTGGCACCGACC
GTCGAAGATGCTCTGAGCGCGAATGAACTGATTGTTCTGTCTCTGACCGATTATGACGCCGTGTACGCAATCC
TGGAACCGGTTACGGGCTCACTGTCGGGTAAAGTGATTGCAAACCTGAGCTCTGATACCCCGGACAAAGCGC
GTGAAGCGGCCAAATGGGCAGCTAAACATGGTGCGAAACATCTGACCGGCGGTGTGCAGGTTCCGCCGCCG
CTGATCGGCAAACCGGAAAGTTCCACCTATTACTCCGGTCCGAAAGATGTTTTTGACGCCCATGAAGATACCCT
GAAAGTCCTGACGAACGCCGATTATCGTGGTGAAGATGCAGGTCTGGCCGCAATGTATTACCAGGCGCAAAT
GACCATTTTCTGGACCACGATGCTGAGCTATTACCAGACGCTGGCTCTGGGCCAAGCGAATGGTGTTAGTGCT
AAAGAACTGCTGCCGTATGCCACCATGATGACGTCCATGATGCCGCATTTTCTGGAACTGTATGCTCAGCACG
TCGATTCTGCGGACTATCCGGGTGATGTGGACCGTCTGGCGATGGGCGCAGCTTCAGTCGATCACGTGCTGC
ATACCCACCAAGATGCGGGTGTTAGCACCGTCCTGCCGGCCGCAGTGGCCGAAATCTTCAAAGCCGGTATGG
AAAAAGGCTTTGCTGAAAATTCGTTCTCCTCTCTGATTGAAGTCCTGAAAAAACCGGCAGTGTAA

2.2. Operon designs.

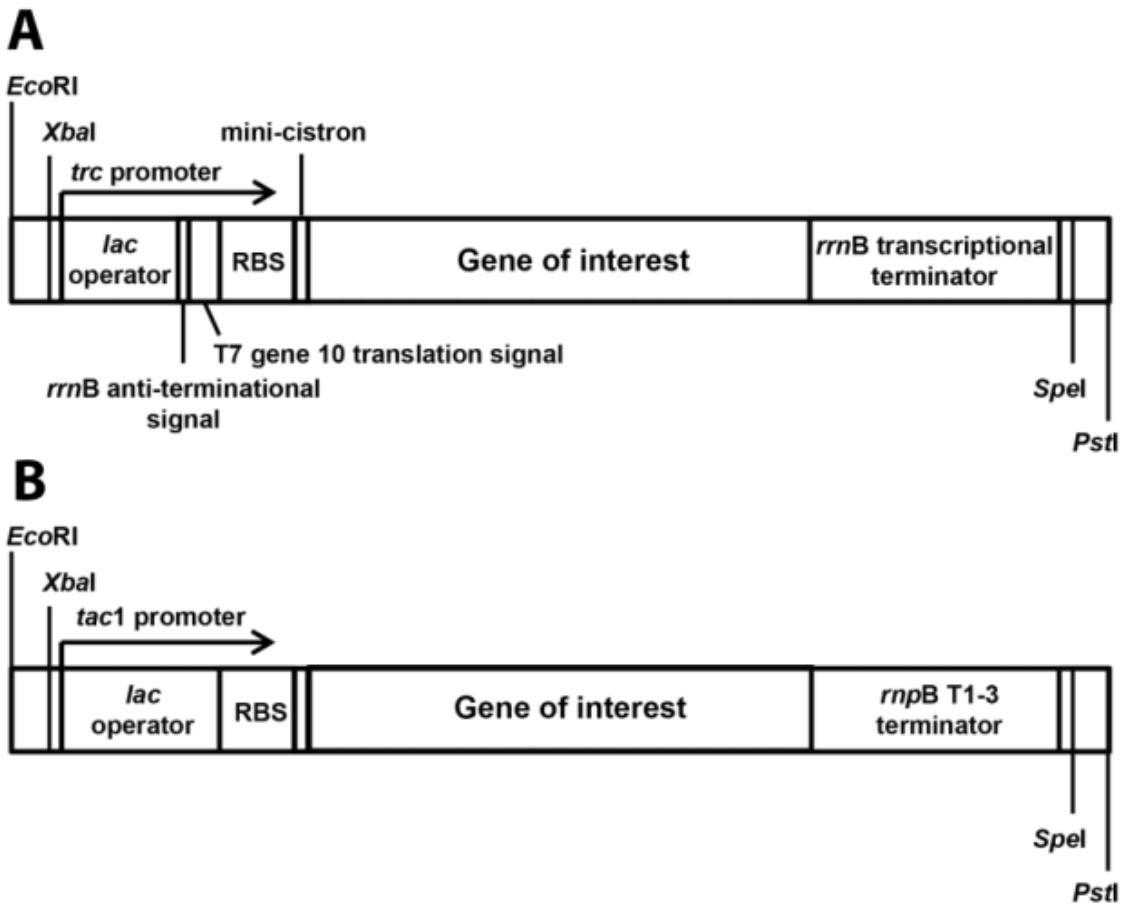


***Figure S1*.** Operon designs for the genes used in this study. A) Operon design used for ATA-117, *Bs*Sfp. B) Operon design used for MCAR, (*R*)-IRED.

2.3. Cloning of operons into pPB01/*Bs*Sfp using BioBrick strategy to give pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp.

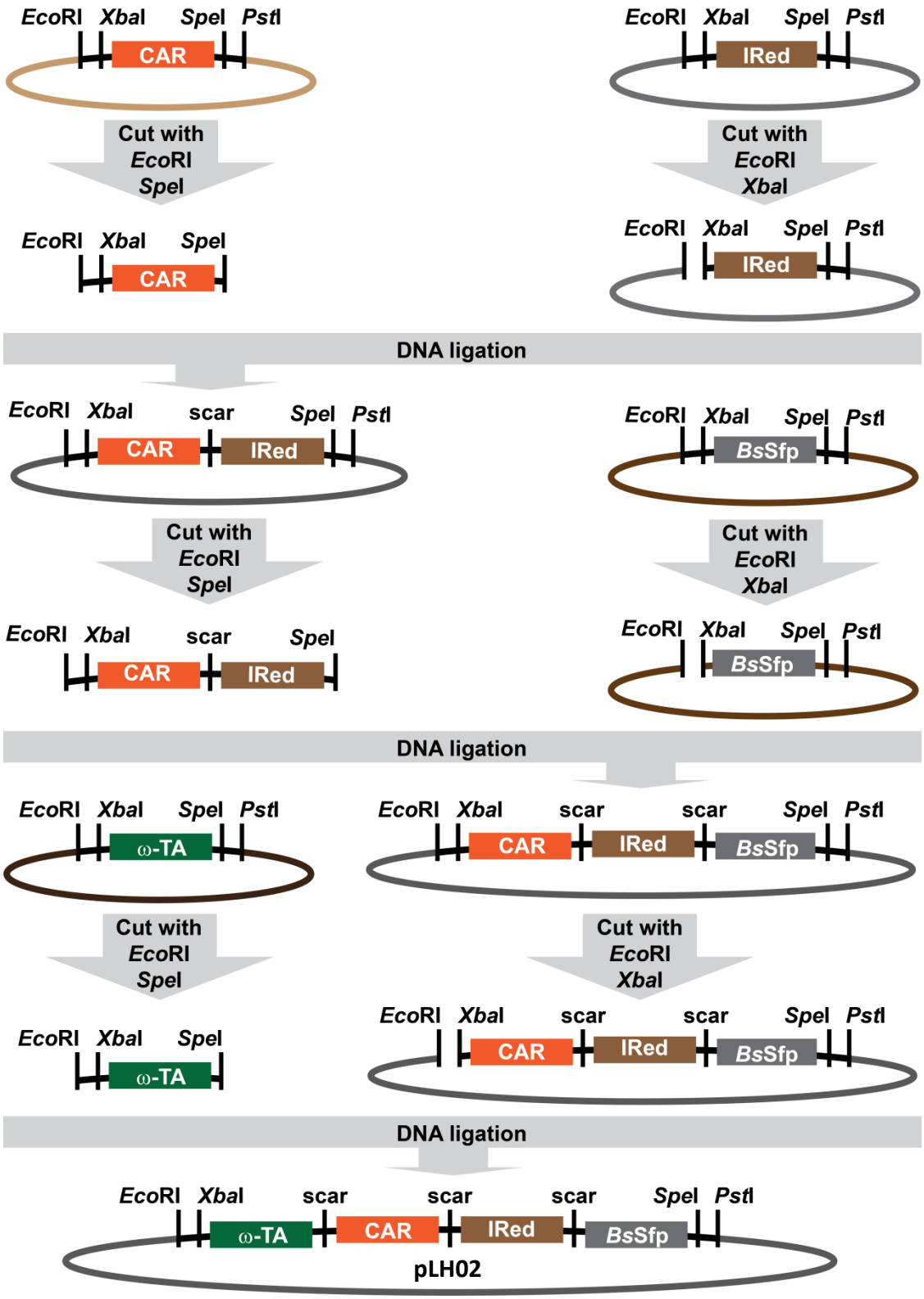


***Figure S2*.** Subcloning of operons into pPB01/(*R*)-IRED using the BioBrick cloning strategy to generate the final expression plasmid pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH02). *Eco*RI and *Xba*I (BioBrick prefix) and *Spe*I and *Pst*I (BioBrick suffix) are restriction endonucleases that recognize and cleave specific palindromic DNA sequences. **Scar** denotes the mixed ligation site of *Spe*I and *Xba*I

which is no longer recognized by any of these endonucleases. DNA ligation was performed by T4 DNA ligase.

2.4. Cloning of duplicate ATA-117 and (*R*)-IRED operons into pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp using BioBrick™ strategy.

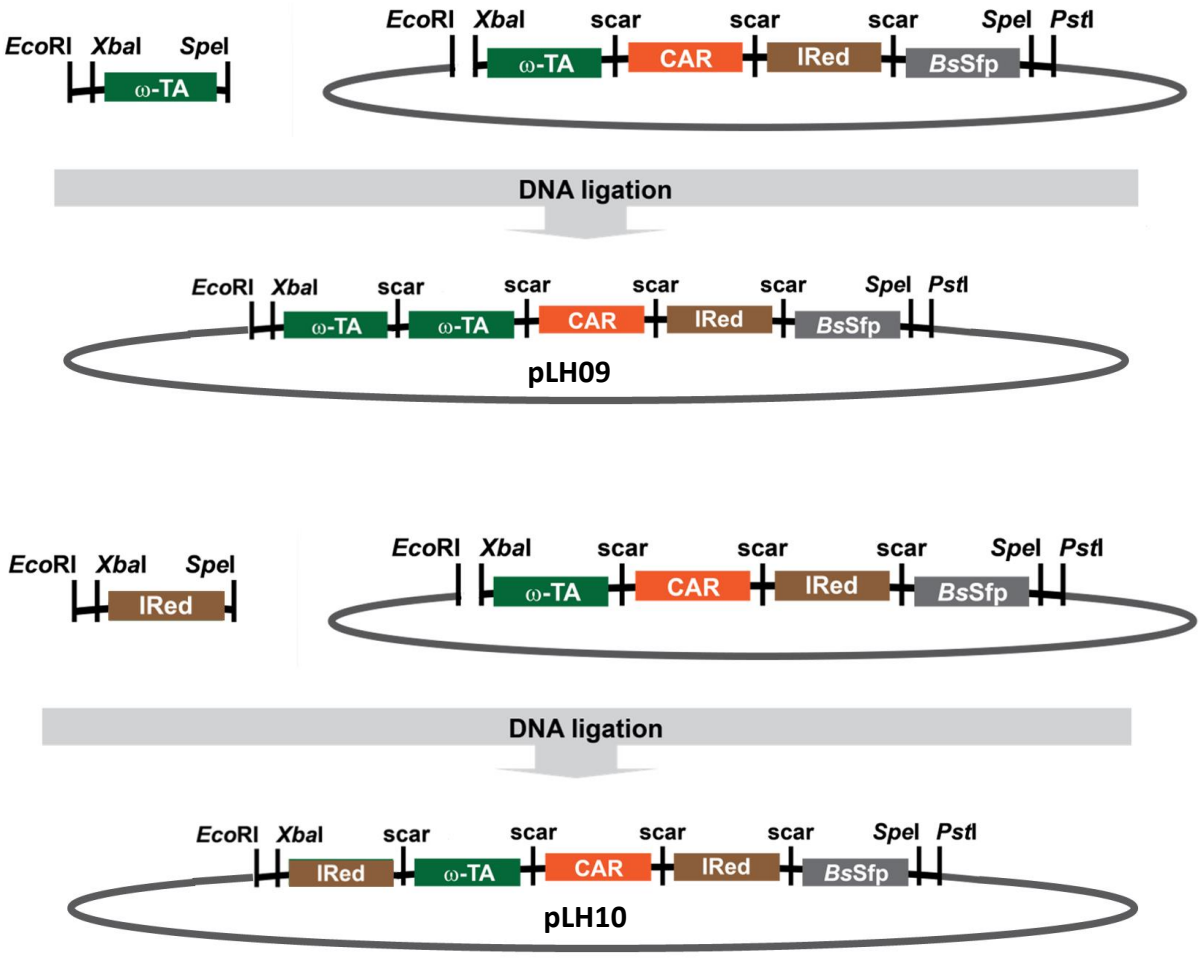


***Figure S3*.** Subcloning of operons into pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp using the BioBrick cloning strategy to generate the two new expression plasmids pPB01/ATA-117 + ATA117 + MCAR+ (*R*)-IRED + *Bs*Sfp (pLH09) and pPB01/(*R*)-IRED + ATA117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH10). *Eco*RI and *Xba*I (BioBrick prefix) and *Spe*I and *Pst*I (BioBrick suffix) are restriction endonucleases that recognize and cleave specific palindromic DNA sequences. **Scar** denotes the mixed ligation site of *Spe*I and *Xba*I which is no longer recognized by any of these endonucleases. DNA ligation was performed by T4 DNA ligase.

2.5. Preparation of whole cell biocatalyst.

Chemically competent *E. coli* BL21 (DE3) expression cells were transformed with plasmid pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH02), pPB01/ATA-117 + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH09) or pPB01/(*R*)-IRED + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH10) following the manufacturer's protocol (NEB). Pre-cultures were inoculated using either a single colony picked from a transformation plate or from a glycerol stock and grown in LB media (supplemented with 100 µg mL^{-1} ampicillin) for 16 h at 37 °C. 400 mL LB media (supplemented with 100 µg mL^{-1} ampicillin) was inoculated with pre-culture (1:100 dilution) and grown at 37 °C until an optical density (OD_{600}) of 0.6 was reached. Flasks were then cooled for 20 min at 20 °C before induction of protein expression using isopropyl-β-D-1-thiogalactopyranoside (IPTG, 0.4 mM). Protein expression at 20 °C and 250 rpm was stopped after 16 h by centrifugation (3200 rcf, 4 °C, 20 min). Wet cell mass was recorded and resuspended in 500 mM sodium phosphate buffer (pH 7) to a final concentration of 40 mg mL^{-1} cells, ready to be used in biotransformation reactions.

2.6. Protein expression profile of transformed *E. coli* BL21 (DE3) cells via western blot analysis.

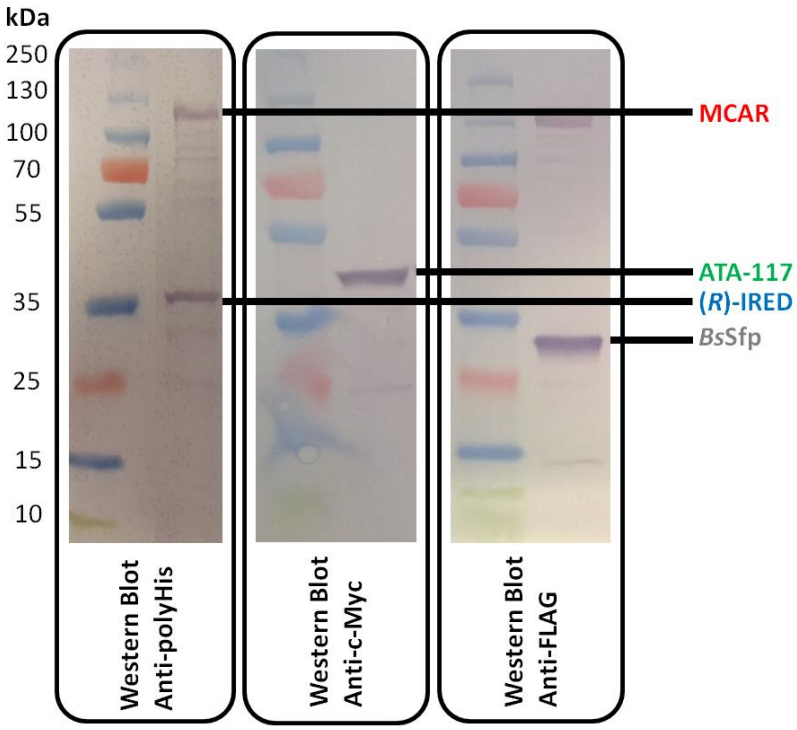


Figure S4 Western blot analysis of protein expression profile of *E. coli* BL21 (DE3) cells harboring plasmid pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH02) using Anti-polyHis, Anti-c-Myc IgG and Anti-FLAG antibodies. Analysis shown for the soluble fraction of cell lysate.

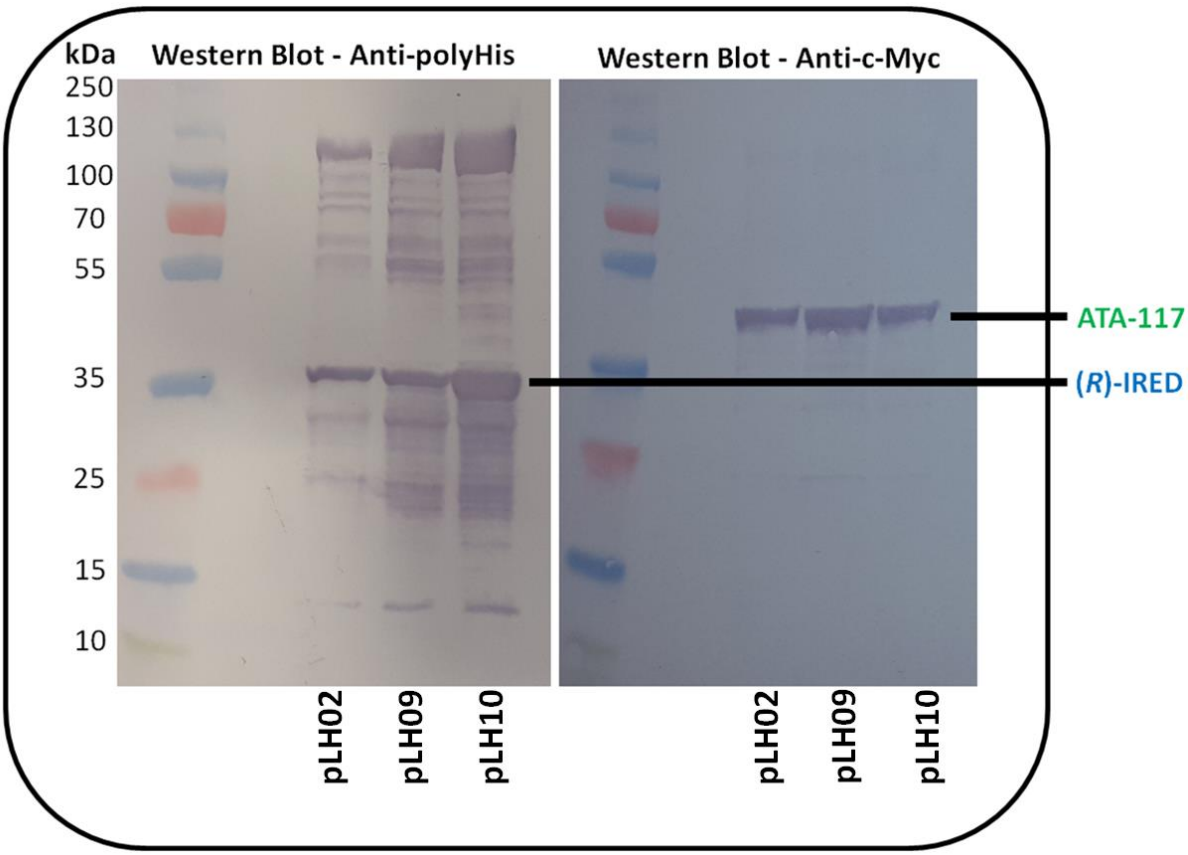


Figure S5 Western blot analysis of protein expression profile of *E. coli* BL21 (DE3) cells harboring plasmid pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH02), pPB01/ATA-117 + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH09), or pPB01/(*R*)-IRED + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (pLH10) using Anti-polyHis and Anti-c-Myc IgG antibodies. Increased expression of (*R*)-IRED is seen when pLH10 is used, and increased expression of ATA-117 is seen when pLH09 is used. Analysis shown for the soluble fraction of cell lysate.

3. Analytical methods and sample preparation

3.1. Gas chromatography methods for conversion and ee determination.

Chiral GC equipped with a 25 m CP-Chirasil-DEX CB column with 0.25 mm inner diameter and 0.25 µm film thickness (Agilent, Santa Clara, CA, USA) was used for the detection of all imine intermediates and piperidine products, and for the determination of **4a, 4f and 4g** production using a calibration curve (sections **3.3**, **3.4** and **3.5**). Determination of conversion and ee for **4a**: injector temperature 200°C, detector temperature 250 °C. Method: 90 °C - 200 °C, 4 °C min^{-1}, hold at 200 °C for 5 min. Determination of ee for **4d**-**e**: injector temperature 200°C, detector temperature 250 °C. Method: 50 °C - 200 °C, 5 °C min^{-1}, hold at 200 °C for 2 min.

GCMS equipped with a HP1-MS column (Agilent, 30.0 m x 320 µm x 0.25 µm) was used for the detection of all keto alcohol and imine intermediates, and piperidine products (method: 50 °C – 175 °C at a rate of 5 °C min^{-1}, followed by 175 °C – 250 °C at a rate of 10 °C min^{-1}, inlet temperature 270 °C).

Percentage conversion of keto acid to imine, keto alcohol and amine was determined based on integration of these corresponding peaks (after comparison with authentic commercial or chemically obtained standards), except for amine **4a**, **4f** and **4g**, where amine production was calculated by integration of its peak with decane as an external standard (sections **3.3**, **3.4** and **3.5**).

3.2. High performance liquid chromatography methods for consumption of keto acid substrates and ee determination.

HPLC equipped with a 250 mm x 4.6 mm, 5 µm CHIRALPAK®IC column was used for the analysis of keto acid substrate consumption. Solvent: n-hexane/isopropanol/trifluoroacetic acid (90/10/0.1), 1 mL min^{-1}, 265 nm. Retention times for keto acids **1a**-**e** using this method were reported previously.[1]

Determination of ee for **4b**-**c** was carried out by normal phase HPLC using a Daicel CHIRALPAK®IC column (250 mm × 4.6 mm, 5 µm), solvent: n-hexane/isopropanol/diethylamine = 98/2/0.1 (**4b**) or 90/10/0.1 (**4c**), 1 mL/min, 265 nm.

3.3. Calibration curve for the determination of conversion to product using compound 4a.

Reactions of compound **1a** were extracted in the presence of external standard decane (1 mg mL^{-1}) to enable the calculation of conversion to product **4a** by use of a calibration curve of decane standard vs. **4a** authentic standard.

The calibration curve was constructed using 1 mg mL^{-1} decane against varying concentrations of **4a** authentic standard in triplicate.

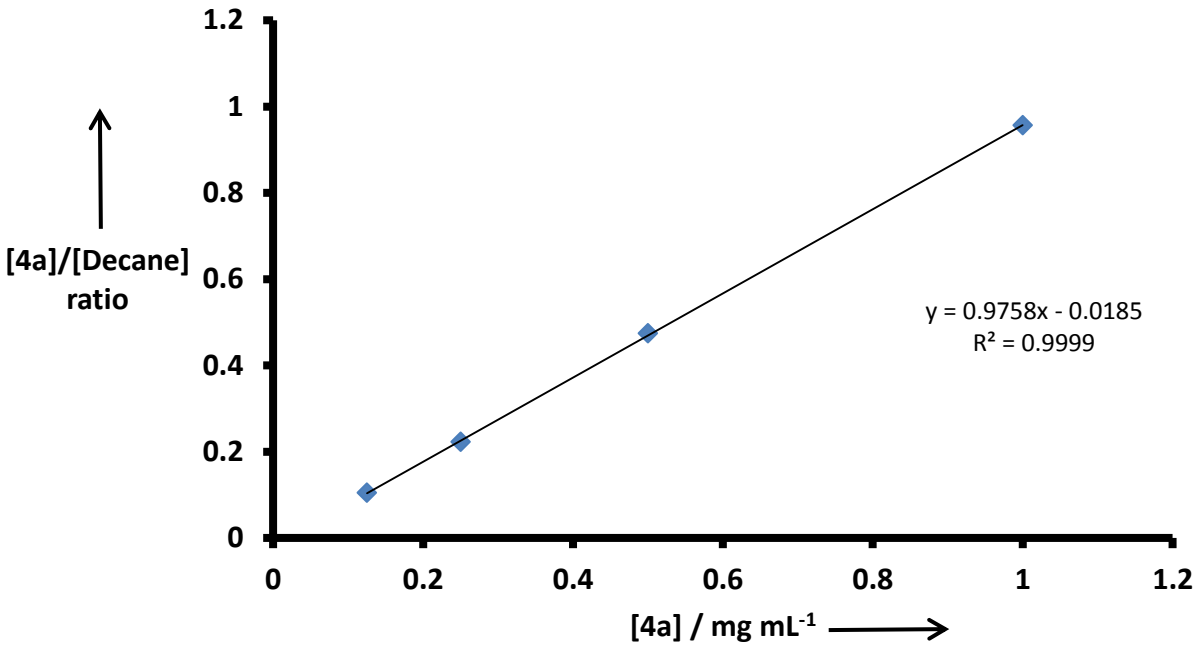


***Figure S6*.** Calibration curve used to determine the conversion to product amine **4a** for the reactions of substrate **1a**.

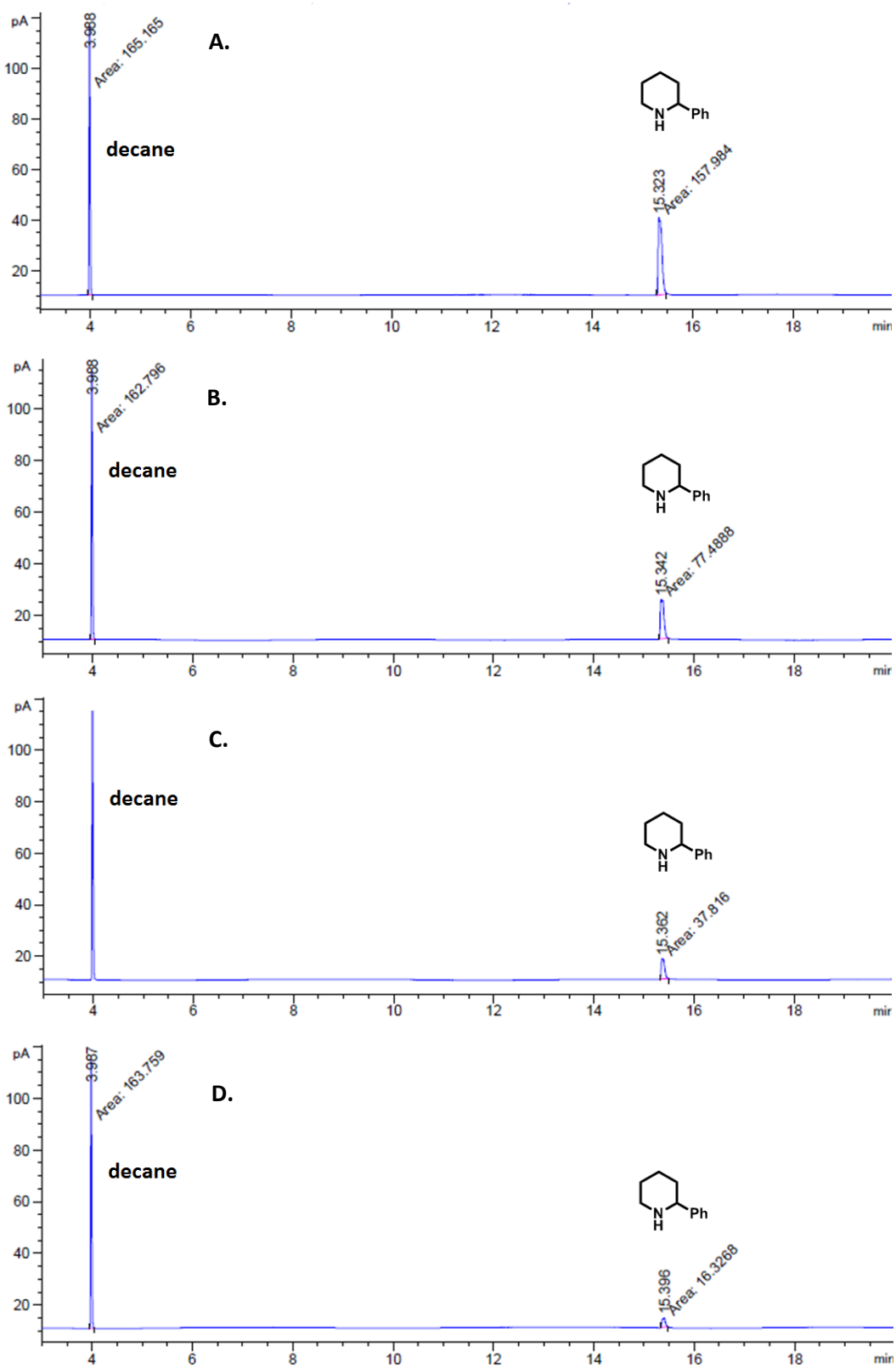


Figure S7. GC chromatograms used for the construction of the calibration curve used to determine the conversion to product amine **4a** for reactions using substrate **1a**. A) 1 mg mL^{-1} **4a**. B) 0.5 mg mL^{-1} **4a**. C) 0.25 mg mL^{-1} **4a**. D) 0.125 mg mL^{-1} **4a**. (CP-Chirasil-DEX CB (Agilent, 25.0 m x 0.25 mm x 0.25 µm), injector temperature 200°C, detector temperature 250 °C . Method: 90 °C - 200 °C, 4 °C min^{-1}, hold at 200 °C for 5 min; carrier gas helium.)

3.4. Calibration curve for the determination of conversion to product using compound 4f.

Reactions of compound **1f** were extracted in the presence of external standard decane (1 mg mL^{-1}) to enable the calculation of conversion to product **4f** by use of a calibration curve of decane standard vs. **4f** authentic standard.

The calibration curve was constructed using 1 mg mL^{-1} decane against varying concentrations of **4f** authentic standard in triplicate.

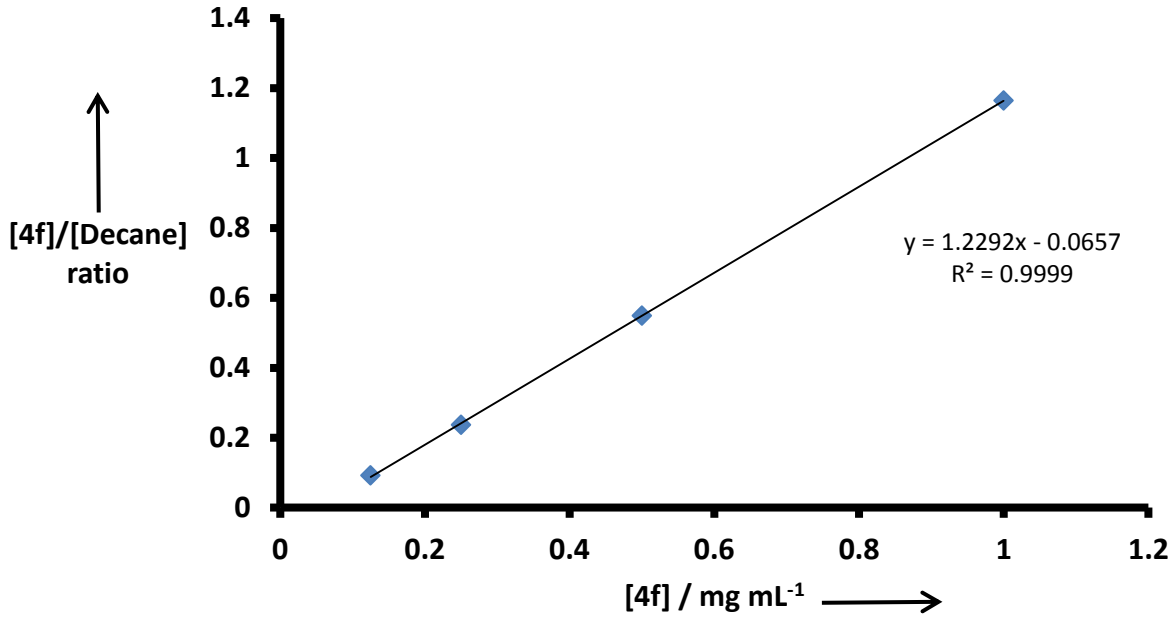


***Figure S8*.** Calibration curve used to determine the conversion to product amine **4f** for the reactions of substrate **1f**.

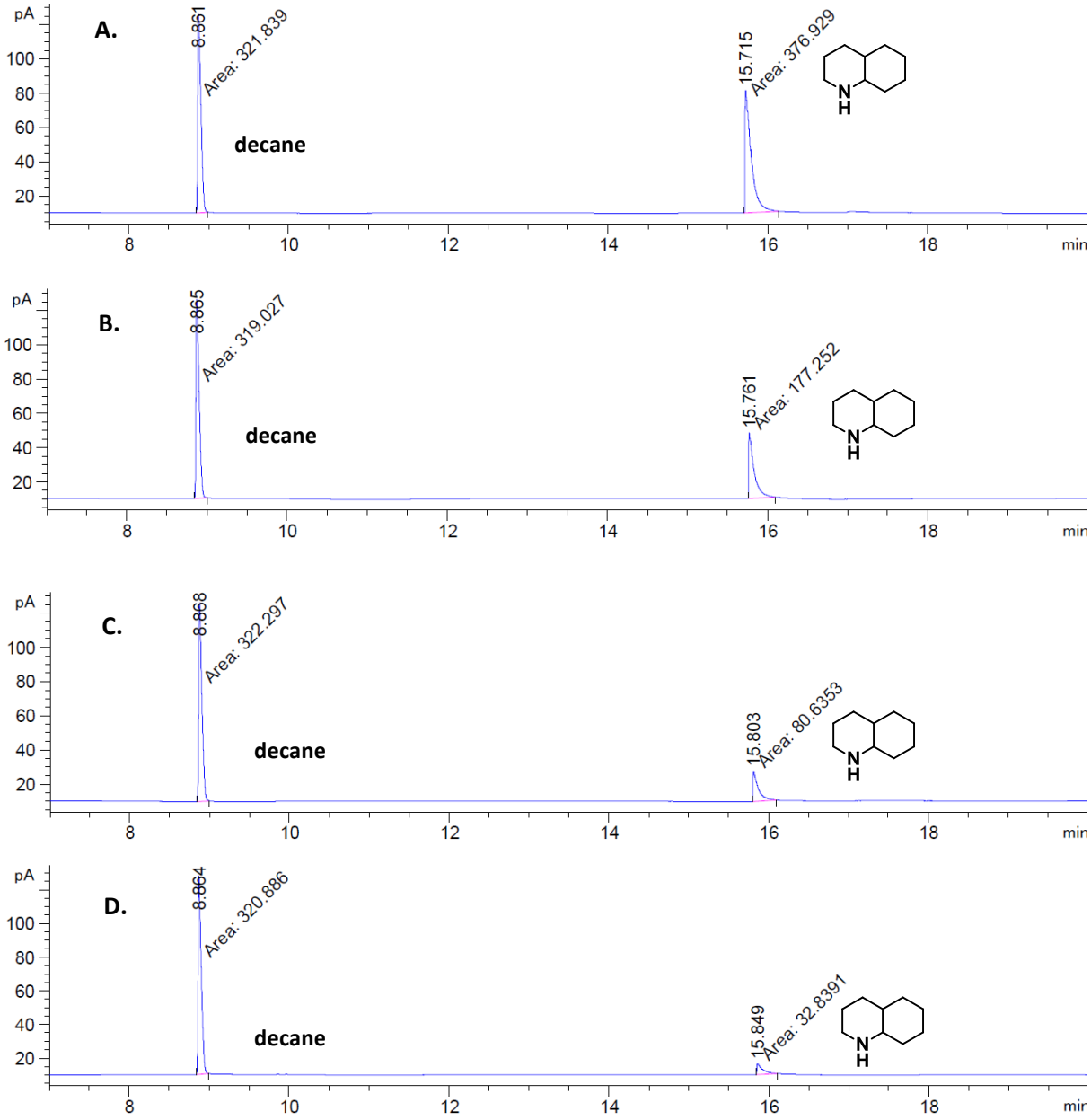


Figure S9. GC chromatograms used for the construction of the calibration curve used to determine the conversion to product amine **4f** for reactions using substrate **1f**. A) 1 mg mL^{-1} **4f**. B) 0.5 mg mL^{-1} **4f**. C) 0.25 mg mL^{-1} **4f**. D) 0.125 mg mL^{-1} **4f**. (CP-Chirasil-DEX CB (Agilent, 25.0 m x 0.25 mm x 0.25 µm), injector temperature 200°C, detector temperature 250 °C . Method: 90 °C - 200 °C, 4 °C min^{-1}, hold at 200 °C for 5 min; carrier gas helium.)

3.5. Calibration curve for the determination of conversion to product using compound 4g.

Reactions of compound **1g** were extracted in the presence of external standard decane (1 mg mL^{-1}) to enable the calculation of conversion to product **4g** by use of a calibration curve of decane standard vs. **4g** authentic standard.

The calibration curve was constructed using 1 mg mL^{-1} decane against varying concentrations of **4g** authentic standard in triplicate.

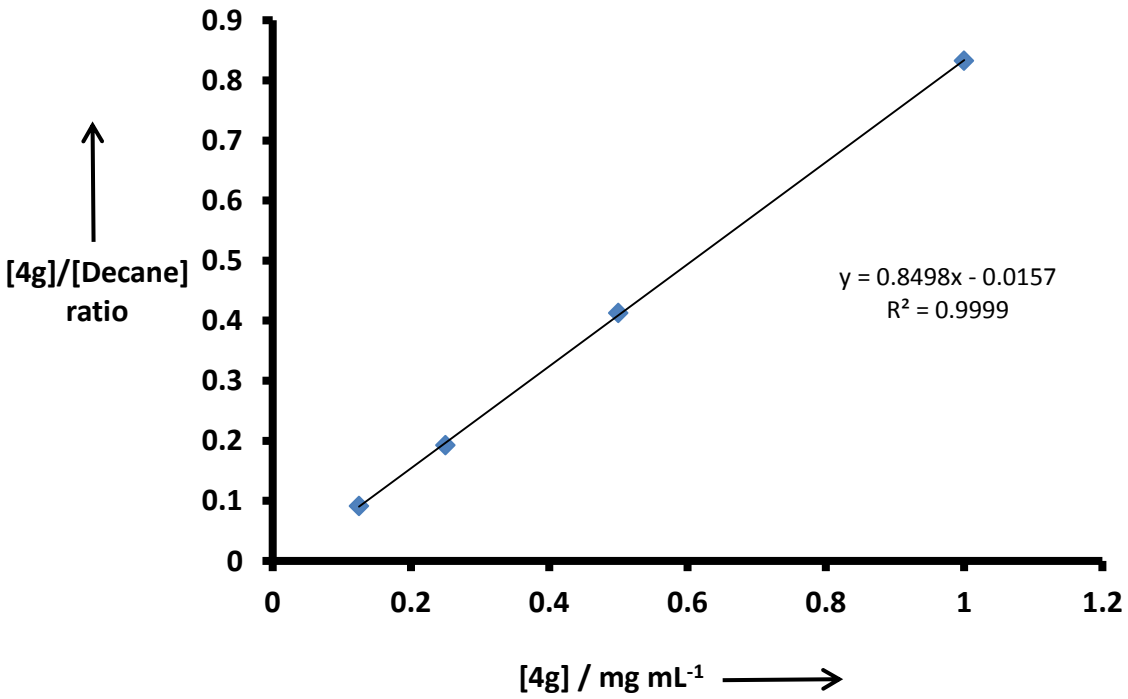


Figure S10. Calibration curve used to determine the conversion to product amine **4g** for the reactions of substrate **1g**.

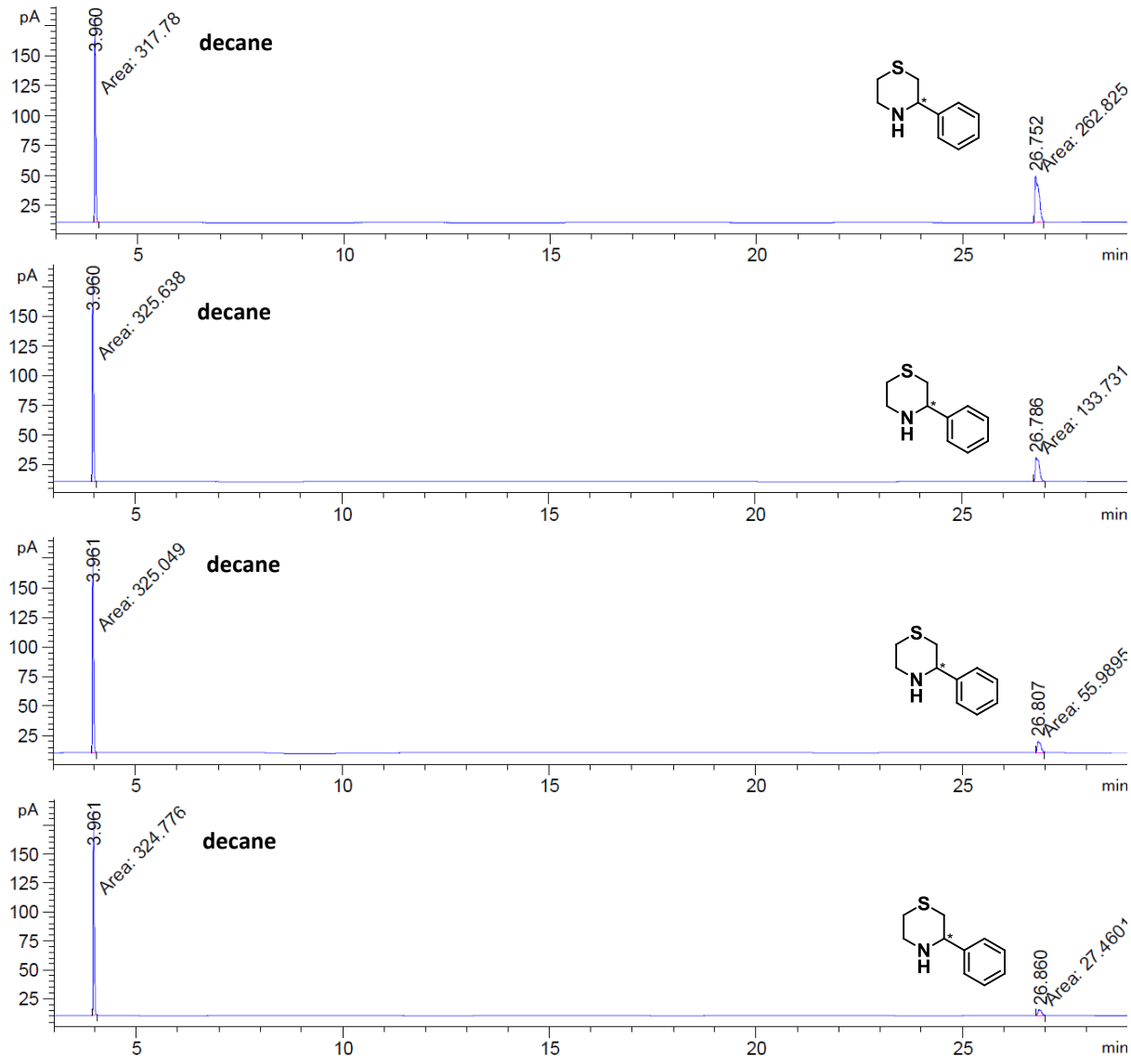


Figure S11. GC chromatograms used for the construction of the calibration curve used to determine the conversion to product amine **4g** for reactions using substrate **1g**. A) 1 mg mL^{-1} **4g**. B) 0.5 mg mL^{-1} **4g**. C) 0.25 mg mL^{-1} **4g**. D) 0.125 mg mL^{-1} **4g**. (CP-Chirasil-DEX CB (Agilent, 25.0 m x 0.25 mm x 0.25 µm), injector temperature 200°C, detector temperature 250 °C . Method: 90 °C - 300 °C, 3 °C min^{-1}, hold at 300 °C for 5 min; carrier gas helium.)

3.6. Extraction protocol and sample preparation for GC and HPLC analysis.

For extraction of the amine product (and keto aldehyde, imine and keto alcohol intermediates), reactions were quenched after 24 h with 500 µL ethyl acetate (EtOAc, containing 1 mg mL^{-1} decane external standard for compound **1a**) and basified to pH 12.0

using sodium hydroxide (NaOH, 10M). Samples were vortexed (2 m), separated using a benchtop centrifuge (16000 rcf, 5 m), and the organic layer dried over anhydrous magnesium sulfate ($MgSO_4$) before being transferred to a clean vial ready for analysis by GC-FID on a chiral column and GCMS.

For analysis of keto acid (starting material) consumption, samples were extracted as before but acidified to pH 4.0 using hydrochloric acid (HCl, 37%). Analysis of keto acid was performed using HPLC.

To determine the ee of some the chiral amine products by GC-FID on a chiral column, derivatisation of the sample was required. In these cases, the samples were extracted as described above and acetic anhydride (5 µL) and triethylamine (10 µL) were added. The mixture was vortexed (2 m) before the addition of H_2O (100 µL). The mixture was vortexed again and separated using a benchtop centrifuge (16000 rcf, 5 m), with the organic phase then dried over anhydrous $MgSO_4$ and transferred to a clean vial ready for analysis.

4. Whole cell biotransformation optimization and final optimized conditions.

5-Oxo-5-phenylvaleric acid (compound **1a**) was used as the model substrate for all optimization experiment screens due to the commercial availability of both substrate and expected amine product **4a**.

4.1. Preliminary screen of expression constructs pLH01-08 using keto acid 1a.

An initial screen of whole cell biocatalysts harboring expression constructs pLH01-08 against the selected keto acid substrate **1a** was used in order to select the construct displaying the highest conversion to product amine **4a**.

Expression Construct	Relative conversion to 2-phenylpiperidine, **4a** (%)
pPB01/ATA-117 + MCAR + (*S*)-IRED + *Bs*Sfp (**pLH01**)	n.d.
pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp (**pLH02**)	100
pPB01/ATA-117 + NCAR + (*S*)-IRED + *Bs*Sfp (**pLH03**)	13
pPB01/ATA-117 + NCAR + (*R*)-IRED + *Bs*Sfp (**pLH04**)	88
pPB01/SitATA + MCAR + (*S*)-IRED + *Bs*Sfp (**pLH05**)	n.d.

pPB01/SitATA + MCAR + (*R*)-IRED + *Bs*Sfp (**pLH06**)	n.d.
pPB01/SitATA + NCAR + (*S*)-IRED + *Bs*Sfp (**pLH07**)	n.d.
pPB01/SitATA + NCAR + (*R*)-IRED + *Bs*Sfp (**pLH08**)	n.d.

n.d., not detected.

The lack of conversion for the cascades utilizing SitATA is likely a result of the intermediate aldehyde produced from the CAR reaction not being a suitable substrate for this heavily mutated variant of ATA-117. The expression constructs utilizing (*S*)-IRED resulted in significantly lower conversions to **4a** than their (*R*)-IRED counterparts. However, the nucleotide sequence of (*S*)-IRED was verified and biotransformations using whole cells containing the (*S*)-IRED individually demonstrated the expected activity against model IRED substrate 1-methyl-3,4-dihydroisoquinoline. Therefore it can be concluded that the use of (*S*)-IRED in combination with ATA-117 and either MCAR or NCAR results in a much less efficient cascade than with (*R*)-IRED, and also metabolic burden may affect the expression levels of (*S*)-IRED when it is expressed in conjunction with the other cascade proteins. Further experimentation revealed that inserting an additional copy of the (*S*)-IRED gene into constructs pLH01 and pLH03 resulted in conversions to **4a** comparable to those seen for pLH02 and pLH04 (around 40 % conversion, calculated using the calibration curve described in section **3.3**), suggesting that an increased expression level of (*S*)-IRED is needed for these cascades to be successful.

This preliminary screen revealed that pLH02 gave the highest conversion to product **4a**, and so this construct was selected for all further optimization experiments.

4.2. Optimization of protein expression.

E. coli BL21 (DE3) cells harboring plasmid pLH02 (pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp) were used as the biocatalyst for all optimization experiment screens.

4.2.1. Effect of expression temperature on product formation.

An investigation into the effect of different expression temperatures for the cascade proteins, ranging from 20 – 37 ^{o}C, on final amine production revealed that lower expression temperatures (20 ^{o}C) are needed for effective conversion to amine product. Temperatures of 30 ^{o}C still yielded some active protein, yet accumulation of imine suggested that (*R*)-IRED is not expressed optimally at higher temperatures. Neither amine product nor imine intermediate were detected when cells harboring protein expressed at 37 ^{o}C were used in biotransformations.

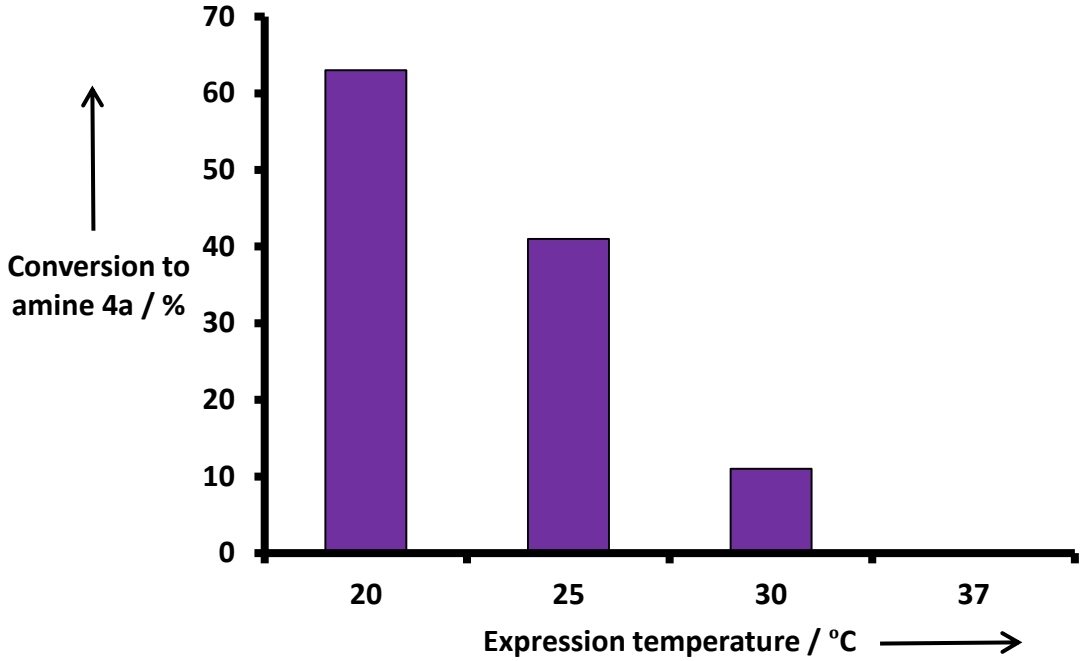


***Figure S12*.** Effect of protein expression temperature on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.2.2. Effect of optical density (OD_{600}) at protein expression induction on product formation.

Probing the effect of the optical density of the *E. coli* BL21 (DE3) cells prior to expression of the cascade proteins, with OD_{600} values ranging from 0.4 to 1.3, it was seen that an OD_{600} value of 0.6 was optimal for increased conversion to amine product. Optical densities higher than 0.6 still resulted in conversion to amine product, yet to a lesser extent, potentially due to the fact that fewer cells are in the exponential growth stage when higher cell densities are reached.

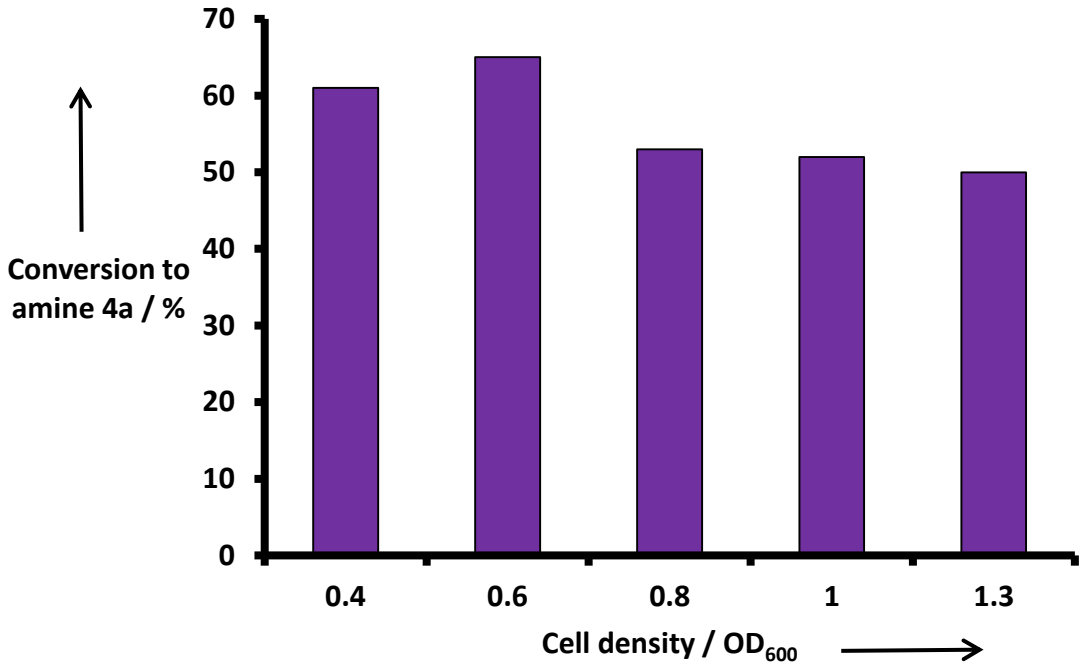


***Figure S13*.** Effect of cell culture density on final amine production. *Expression conditions*: 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.2.3. Effect of expression time length on product formation.

An investigation into the effect of increased protein expression time lengths was conducted, with cell cultures being allowed to express protein for 2 hours, 3.5 hours, or 16 hours. It was apparent that protein expression needed to be carried out for at least 3.5 hours to improve biocatalyst efficiency and increase conversion to product amine. Alternatively, leaving cells expressing protein overnight for 16 hours showed no detrimental effect when the cells were used for production of amine **4a**.

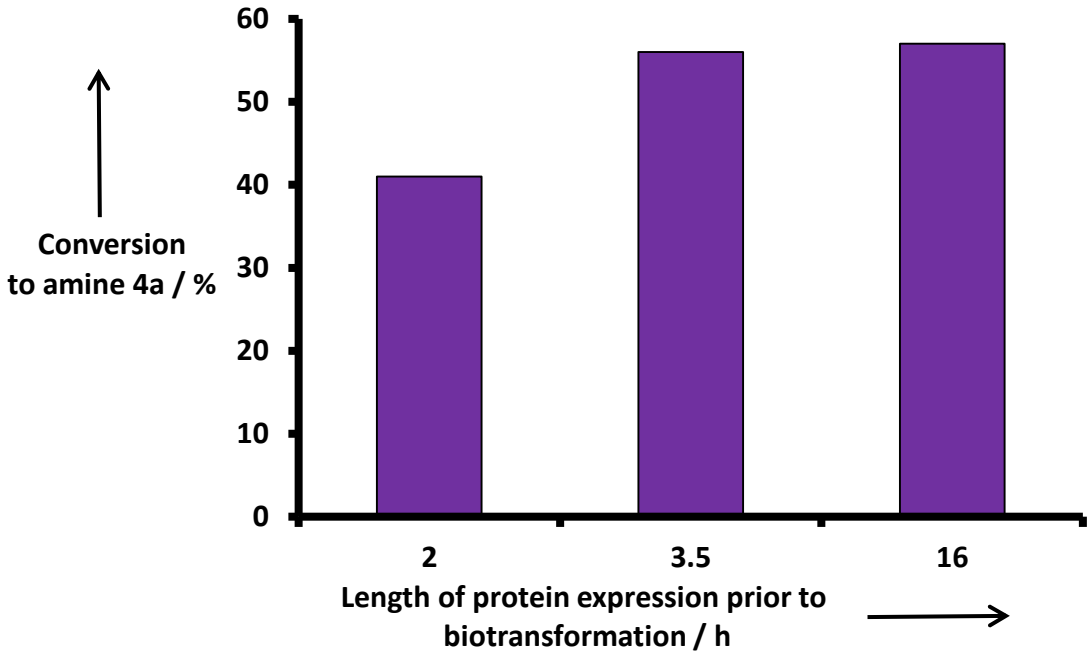


***Figure S14*.** Effect of protein expression time length on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.2.4. Effect of IPTG concentration on product formation.

Probing the effect of the concentration of IPTG used to induce protein expression in the whole cell biocatalyst, using 0.2 – 0.8 mM IPTG, demonstrated that a concentration of 0.8 mM resulted in higher conversion to amine product **4a**.

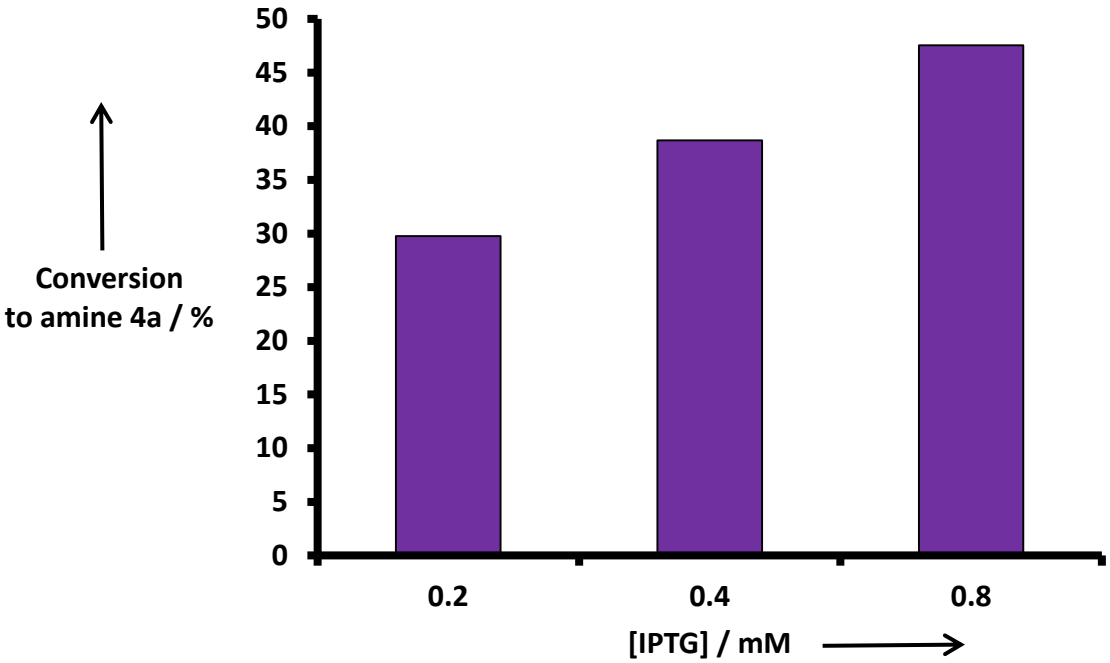


***Figure S15*.** Effect of IPTG concentration used to induce protein expression on final amine production. *Expression conditions*: OD_{600} 0.6, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3. Optimization of reaction conditions.

4.3.1. Analysis of cell pellet and reaction supernatant to determine product diffusion out of the cell.

Separate extractions (500 µL of ethyl acetate for 500 µL reaction volume) for both the cell pellet and the reaction buffer after 24 hour reaction indicated that very little amine product remained within the cell (around 4%), suggesting that the amine product freely diffuses out of the cell and into the reaction buffer.

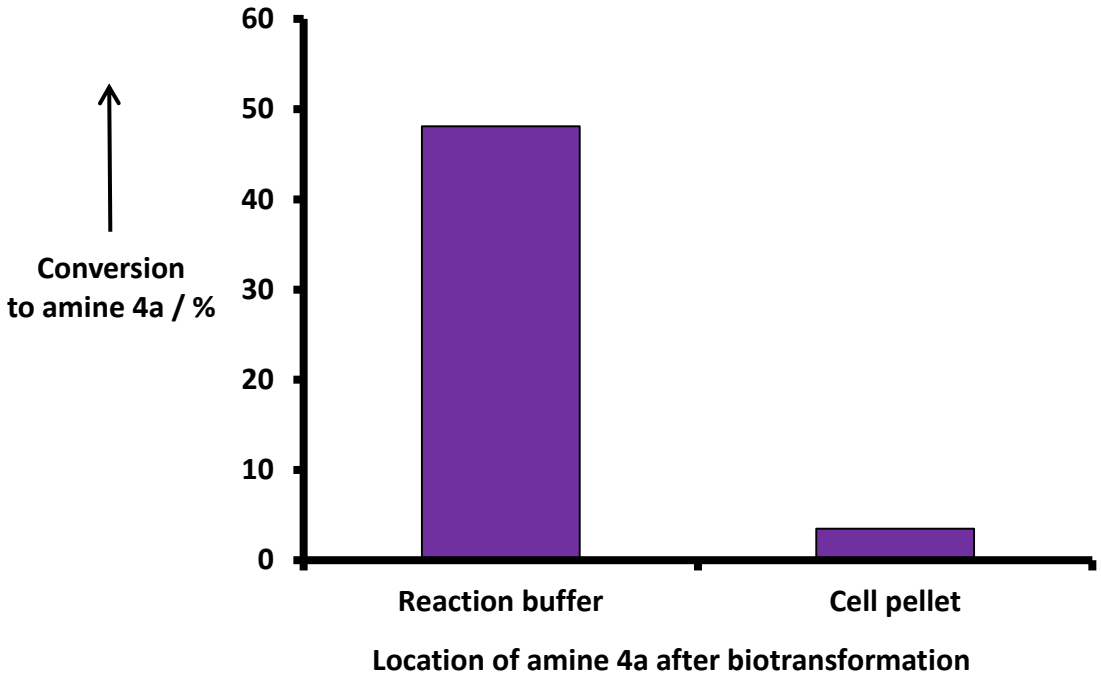


***Figure S16*.** Extraction into ethyl acetate of reaction buffer and of cell pellet after biotransformation. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.2. Comparison of sacrificial amine donors for the ATA-117 reaction.

Both D/L-alanine and isopropylamine (IPA) are known to be accepted as amine donors for ATA-117.[5,6] It was seen that using either of these amine donors gave a similar conversion to amine, so we opted for use of D/L-alanine for all further experiments.

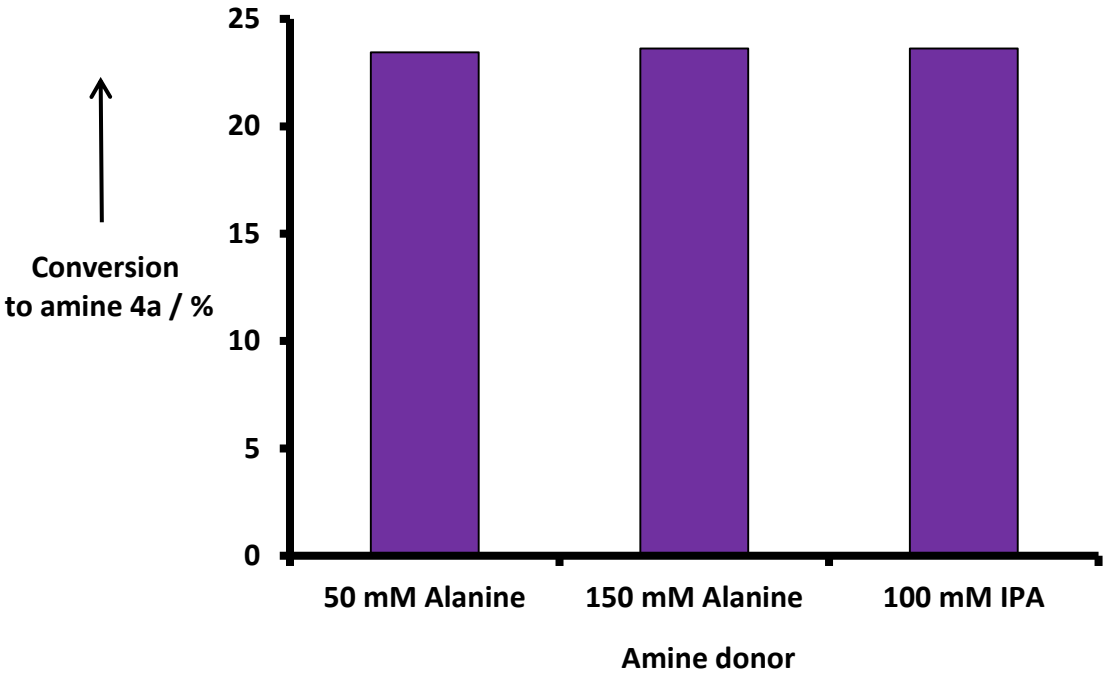


***Figure S17*.** Comparison of D/L-alanine and IPA as amine donor for ATA-117. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.3. Effect of PLP concentration on product formation.

PLP is an essential cosubstrate for *ω*-transaminases, and is also found endogenously within *E. coli* bacterial cells.[7] Assessing the effect of PLP concentration on product formation, ranging from 0 - 2 mM, revealed that the supplementation of PLP resulted in a decrease in conversion to amine when compared to biotransformations performed in the absence of additional PLP. This suggests that the presence of higher concentrations of PLP may inhibit one or more of the enzymes in this cascade.

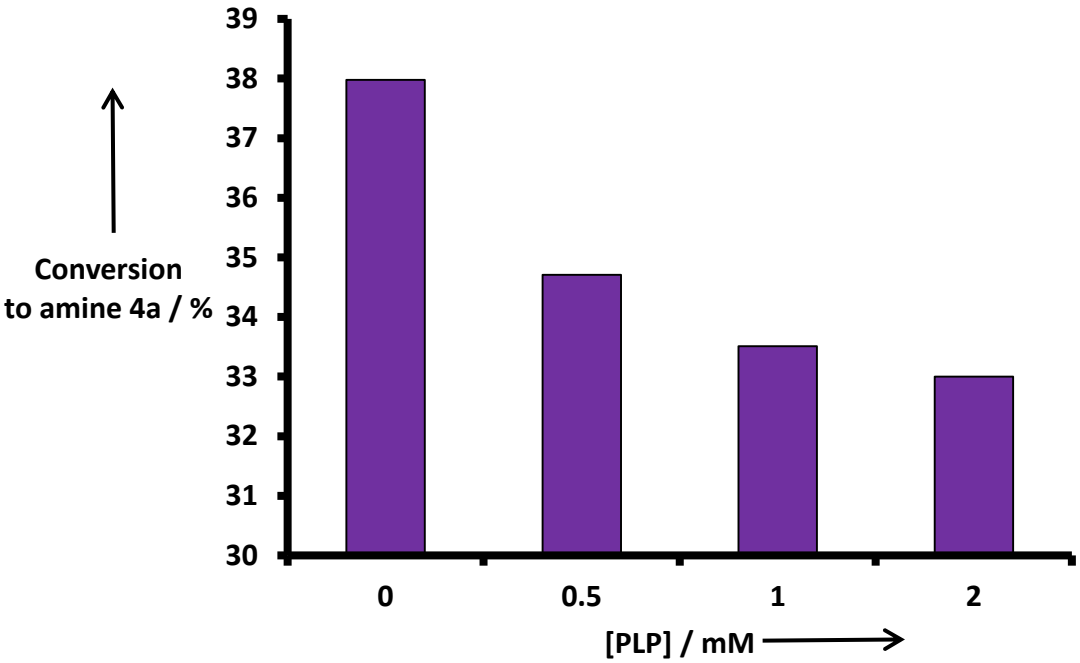


***Figure S18*.** Effect of different concentrations of PLP ranging from 0 - 2 mM on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.4. Effect of D/L-alanine concentration on product formation.

Probing the effect of D/L-alanine concentration on the conversion to amine, ranging from 0 - 350 mM, showed that an increase in the concentration of D/L-alanine lead to an increased conversion to amine, peaking at 250 mM. Neglecting to supplement the reactions with D/L-alanine still yielded some conversion to amine (around 7 %), but addition of extra D/L-alanine was needed to obtain the best results.

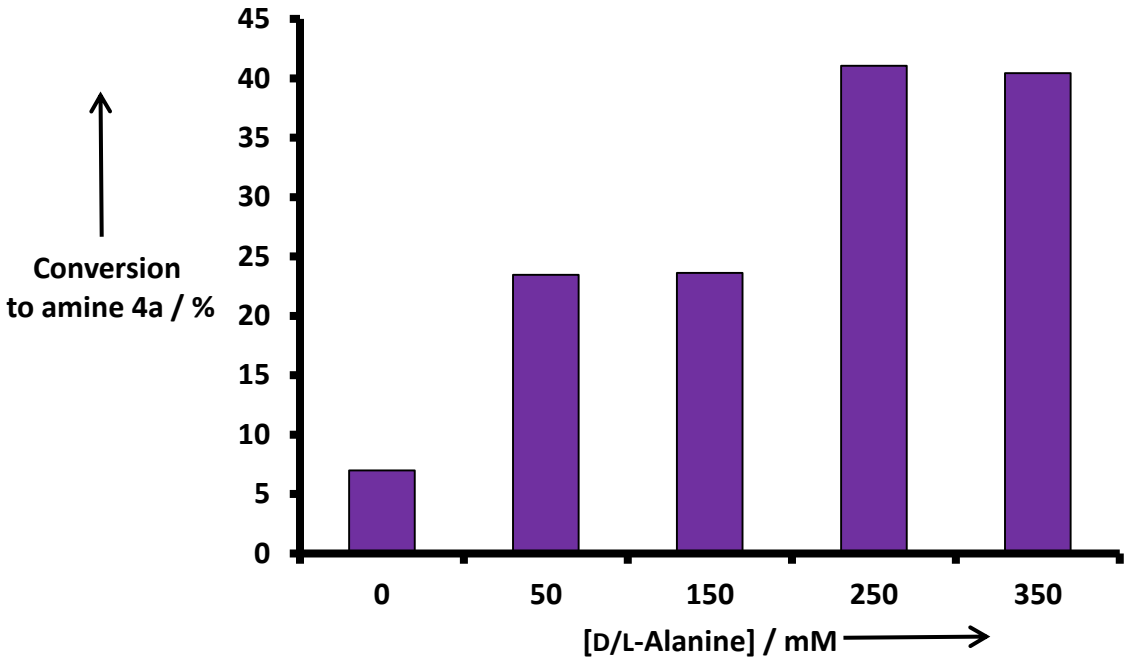


***Figure S19*.** Effect of D/L-alanine concentration ranging from 0 - 350 mM on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.5. Effect of glucose concentration on product formation.

Probing the effect of glucose concentrations ranging from 0 - 100 mM on the conversion to amine demonstrated that a higher level of glucose equates to a higher conversion to amine, but peaks at 50 mM. As seen with the D/L-alanine concentration experiment, the absence of exogenous glucose supplementation still leads to some production of amine (around 12 %) but a concentration of 50 mM glucose yields the best conversion.

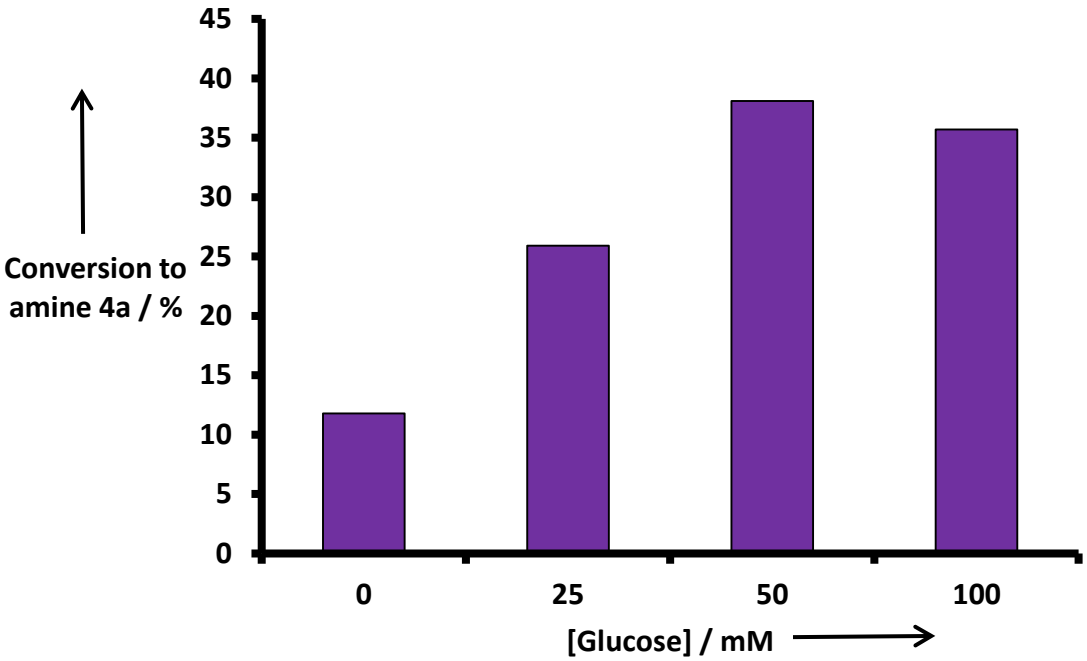


***Figure S20*.** Effect of glucose concentration ranging from 0 - 100 mM on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.6. Effect of keto acid substrate concentration on product formation.

Probing the effect of substrate concentration on conversion to amine product, various concentrations of keto acid were investigated ranging from 1 - 50 mM. 3 mM concentration resulted in the highest conversion to amine product, with conversion to amine product dropping as substrate concentration increases above 3 mM.

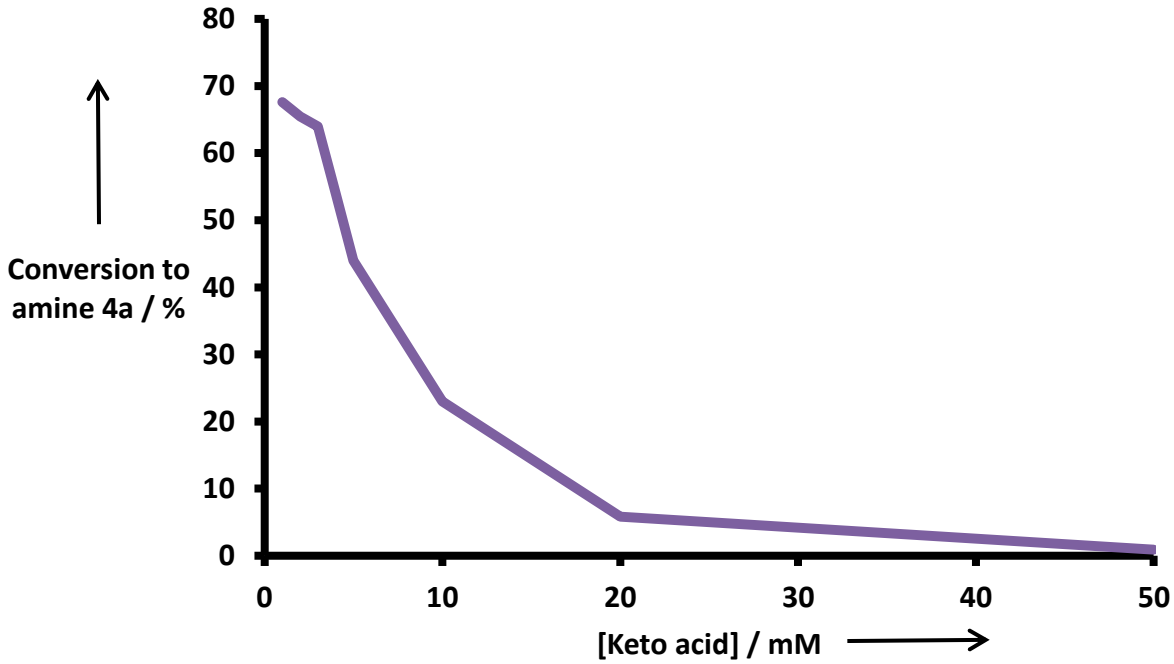


***Figure S21*.** Effect of keto acid **1a** concentration ranging from 0 - 50 mM on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.7. Effect of keto acid substrate concentration on extent of substrate consumption.

The same substrate concentration range as in **4.3.6.** was also assessed for keto acid consumption by CAR. It was seen that keto acid substrate is completely consumed after 24 hours for concentrations up to 5 mM, with concentrations higher than 5 mM leading to incomplete consumption of substrate for the same 24 hour reaction length. Increasing the reaction length to 48 hours gave only a negligible increase in consumption of keto acid, suggesting that the whole cell system loses some activity after the initial 24 hour period.

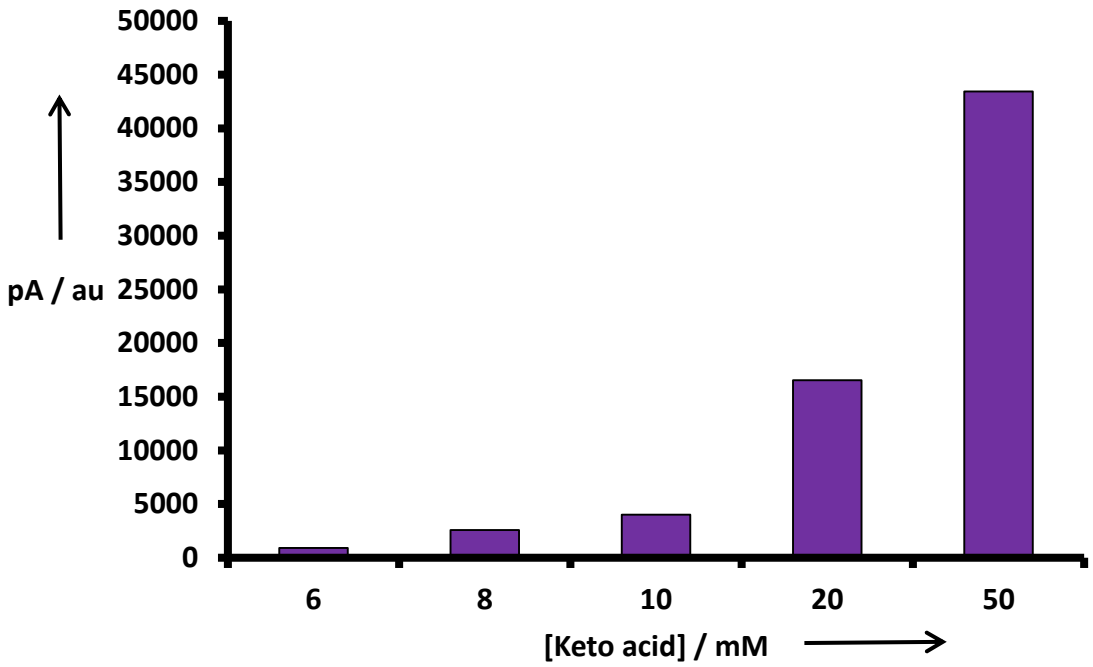


***Figure S22*.** Effect of keto acid **1a** concentration ranging from 0 - 50 mM on substrate consumption. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.8. Effect of wet cell mass on product formation.

Probing the effect of wet cell concentrations, ranging from 20 – 240 mg mL^{-1}, on product formation it was seen that 40 mg mL^{-1} was the optimum amount of cells needed to maximize product output. Higher concentrations of cells (120 mg mL^{-1}, 240 mg mL^{-1}) resulted in a significantly lower apparent conversion to amine, possibly due to the fact that it is increasingly more difficult to efficiently extract amine product from higher masses of cells.

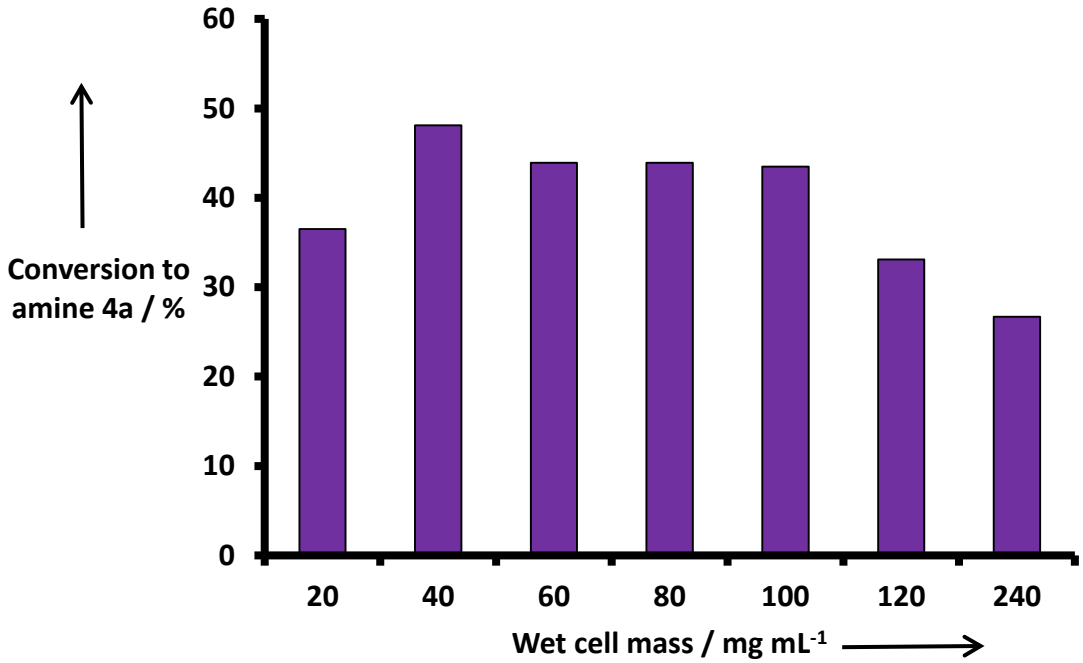


***Figure S23*.** Effect of wet cell mass, ranging from 20 – 240 mg mL^{-1}, on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 24 h, 30 °C, 250 rpm.

4.3.9. Effect of reaction time length on product formation.

Probing the effect of reaction time length, ranging from 0.5 - 24 h, on product formation indicated that the biotransformation proceeds steadily, reaching maximum conversion to amine **4a** at around 9 h and remaining this way until 24 h is reached. Increasing the time length to 48 h resulted in no further conversion to amine compared with 24 h (not shown).

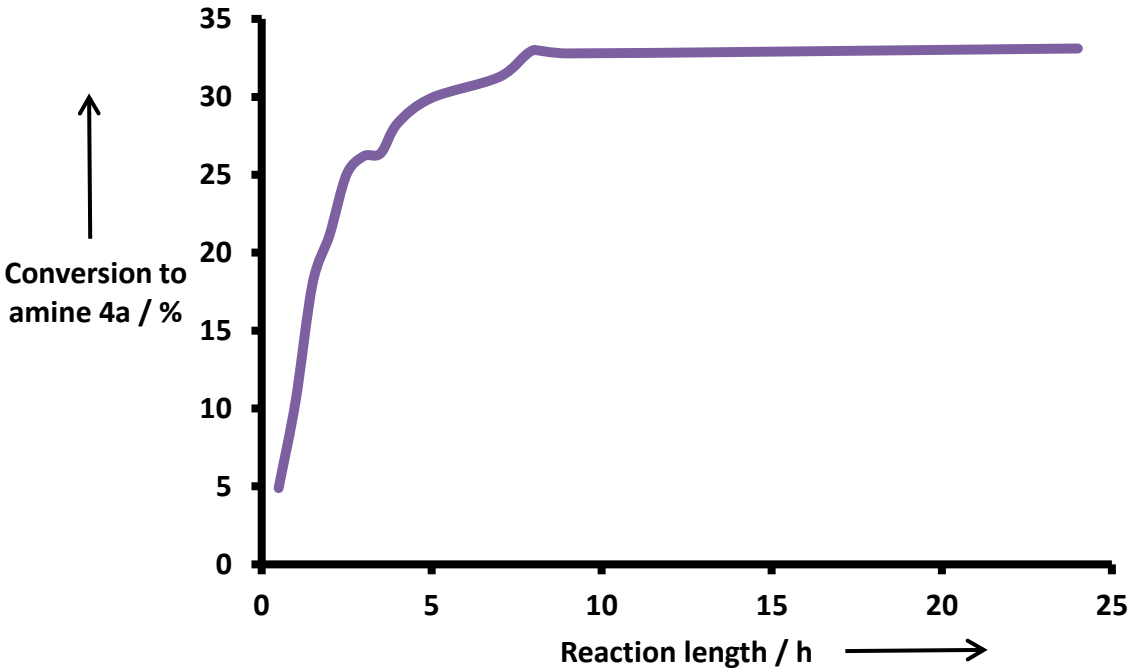


***Figure S24*.** Effect of reaction time length, ranging from 0.5 - 24 h, on final amine production. *Expression conditions*: OD_{600} 0.6, 0.4 mM IPTG, 16 h expression, 20 °C, 250 rpm. *Reaction conditions*: 5 mM **1a**, 50 mM glucose, 250 mM D/L-alanine, 500 mM NaP_i pH 7, 30 °C, 250 rpm.

4.4. Optimized expression and reaction conditions.

Transformed *E. coli* BL21 (DE3) cells harboring plasmid pLH02, pLH09 or pLH10 were grown in a shaking incubator (37 °C, 250 rpm) until an optical density (OD_{600}) of 0.6 was achieved. The cultures were then cooled to 20 °C before protein induction with 0.8 mM IPTG and left to express for 16 h.

For analytical-scale biotransformations, 3 mM keto acid substrate, 50 mM glucose, 250 mM D/L-alanine, 40 mg mL^{-1} *E. coli* BL21 (DE3) cells containing plasmid pLH02, pLH09 or pLH10 in 500 mM sodium phosphate (pH 7.0) reaction buffer (300 µL) was added to a 1.7 mL Eppendorf tube, with a final volume of 500 µL. Reactions were then incubated at 30 °C with shaking (250 rpm) for 24 h to allow full consumption of starting material.

5. Comparison of ATA-117 and ATA-113 in multi-component one pot hybrid reactions.

5.1. Protein expression and reaction conditions for multi-component hybrid one pot reactions.

Biotransformations containing 5 mM keto acid substrate, 75 mg mL^{-1} MCAR wet whole cells, 50 mg mL^{-1} (*R*)-IRED wet whole cells, 2.5 mg mL^{-1} ATA-113 or ATA-117, 1 mg mL^{-1} GDH (CDX-901), 0.5 mg mL^{-1} LDH (LDH-103), 250 mM racemic D/L-alanine, 100 mM glucose, 1.5 mM, NAD+ and 1 mM PLP in 500 mM pH 7.0 sodium phosphate buffer and 1% v/v DMSO (from addition of substrate as a solution in DMSO) were incubated at 30°C, 250 rpm for 24 h. Reactions were then extracted following the procedure in section **3.6** and analyzed using GC-FID.

5.2. Comparison of hybrid cascade reactions using ATA-117 or ATA-113 against keto acid 1a.

5 mM keto acid **1a** was transformed to **4a** following the method described in section **5.1**, and conversion was calculated using the calibration curve shown in section **3.3**. The % conversion to amine **4a** for each reaction is shown below.

Cascade System	Conv. To **4a**/%
Hybrid (ATA-117 + MCAR + (*R*)-IRED)	26
Hybrid (ATA-113 + MCAR + (*R*)-IRED)	58

6. Preparative-scale biotransformations.

6.1. Synthesis of (*S*)-2-phenylpiperidine, (*S*)-4a, using whole cell biocatalyst.

Following the optimized expression and reaction conditions described in section **4.4**, preparative-scale synthesis of (*S*)-2-phenylpiperidine, (*S*)-**4a**, was achieved through conversion of **1a** (144 mg, 0.75 mmol) using cells harboring pLH10 in a 500 mL baffled flask. After 24 h the biotransformation was basified to pH 12.0 using 10 M NaOH and extracted twice into EtOAc. The crude extract was subjected to further purification by dissolving the residue into EtOAc (20 mL) and extracting the amine product into 1 M HCl (3 x 20 mL). The aqueous layers were then combined, basified with 10 M NaOH to pH 12.0 and the product extracted into EtOAc (4 x 25 mL). The organic layers were then combined, dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to yield (*S*)-**4a** (70 mg, 0.43 mmol, 58%, ee 30%) as a yellow oil: **^{1}H-NMR** δH (400 MHz, $CDCl_3$) 7.42-7.30 (m, 4H), 7.30-7.23 (m, 1H), 3.70-3.58 (m, 1H), 3.24-3.15 (m, 1H), 2.80 (td, *J* = 11.6, 3.0 Hz, 1H), 1.95-1.87 (m, 2H), 1.86-1.77 (m, 1H), 1.72-1.65 (m, 1H), 1.65-1.45 (m, 3H); **^{13}C-NMR** δC (100 MHz $CDCl_3$) 145.3, 128.4, 127.1, 126.7, 62.3, 47.7, 34.8, 25.8, 25.4; **MS** *m/z* 161 [M^+]. Data consistent with literature values.[8]

6.2. Synthesis of (±)-*cis*-4-methyl-2-phenylpiperidine, (±)-*cis*-4e, using whole cell biocatalyst.

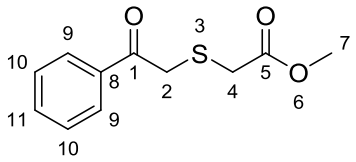
Following the optimized expression and reaction conditions described in section **4.4**, preparative-scale synthesis of (±)-*cis*-4-methyl-2-phenylpiperidine, (±)-*cis*-**4e**, was achieved through conversion of **1e** (100 mg, 0.49 mmol) using cells harboring pLH10 in a 500 mL baffled flask. After 24 h the biotransformation was basified to pH 12.0 using 10 M NaOH and extracted twice into EtOAc. The crude extract was subjected to further purification by dissolving the residue into EtOAc (20 mL) and extracting the amine product into 1 M HCl (3 x 20 mL). The aqueous layers were then combined, basified with 10 M NaOH to pH 12.0 and the product extracted into EtOAc (4 x 25 mL). The organic layers were then combined, dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to yield (±)-*cis*-**4e** (50 mg, 0.29 mmol, 59%, de >98%) as a yellow oil: **^{1}H-NMR** δH (400 MHz, $CDCl_3$) 7.41-7.30 (m, 4H), 7.29-7.23 (m, 1H), 3.62 (dd, *J* = 11.3, 2.5 Hz, 1H), 3.22 (ddd, *J* = 11.6, 4.1, 2.3 Hz, 1H), 2.82 (app td, *J* = 12.0, 2.5 Hz, 1H), 2.15 (br s, 1H), 1.85-1.77 (m, 1H), 1.74-1.58 (m, 2H), 1.30-1.15 (m, 2H), 0.97 (d, *J* = 6.4 Hz, 3H) ; **^{13}C-NMR δC** (100 MHz $CDCl_3$) 145.2, 128.4, 127.1, 126.7, 61.9, 47.3, 43.5, 34.4, 32.0, 22.5. **MS** *m/z* 175[M^+]. Data consistent with literature values.[1]

6.3. Synthesis of keto alcohol intermediate using whole cell biocatalyst and 1a as substrate.

An adaptation of the reaction conditions outlined in section **4.3** was used for the preparative-scale synthesis of keto alcohol intermediate 5-hydroxy-1-phenyl-1-pentanone, through conversion of **1a** (75 mg, 0.46 mmol) using glucose (50 mM) and cells harboring plasmid pPB01/MCAR + *Bs*Sfp in a 250 mL baffled flask. After 24 h the biotransformation was basified to pH 12.0 using 10 M NaOH and extracted twice into EtOAc. The organic layers were then dried over $MgSO_4$ and the solvent removed under reduced pressure to yield 5-hydroxy-1-phenyl-1-pentanone (63 mg, 0.35 mmol, 91 %) as a yellow oil: **^{1}H-NMR** δH (400 MHz, CDCl3) 8.00-7.94 (m, 2H), 7.60-7.55 (m, 1H), 7.51-7.44 (m, 2H), 3.69 (t, *J* = 6.4 Hz, 2H), 3.05 (t, *J* = 7.1 Hz, 2H) 2.63 (2H, s), 1.91-1.81 (m, 2H), 1.72-1.63 (m , 2H); **^{13}C-NMR δC** (100 MHz $CDCl_3$) 200.5, 136.9, 133.0, 128.6, 128.0, 62.4, 38.1, 32.3, 20.1; **MS** *m/z* 160 $[M - H_2O]^+$. Data consistent with literature values.[9]

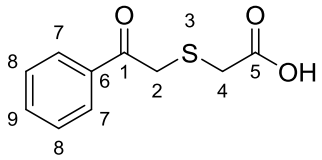
7. Synthesis of substrate 2-((2-oxo-2-phenylethyl)thio)acetic acid, 1g.

Preparation of methyl 2-((2-oxo-2-phenylethyl)thio)acetate



To a flask under N_2 were added 2-bromoacetophenone (3.34 g, 16.8 mmol), methyl thioglycolate (1.5 mL, 16.8 mL) and anhydrous THF (22 mL). Oven-dried K_2CO_3 (5 x, 11.6 g, 83.9 mmol) was added to the flask and the mixture was stirred for 24 h. The mixture was then filtered and the residue filter cake was washed with ethyl acetate (20 mL). The organic layers were pooled together and concentrated *in vacuo* to yield the crude product. The final product was obtained by Kugelrohr distillation (180°C under high vacuum, 3.39 g, 90% yield) as clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.99 – 7.94 (m, 2H, C9-CH), 7.62 – 7.56 (m, 1H, C11-CH), 7.51 – 7.45 (m, 2H, C10-CH), 4.03 (s, 2H, C2-CH_2), 3.73 (s, 3H, C7-CH_3) 3.53 (s, 2H, C4-CH_2); **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 194.2 (C1), 170.5 (C5), 135.5 (C8), 133.7 (C11), 128.9 (C9), 128.8 (C10), 52.6 (C2), 37.9 (C4), 33.3 (C7).

Preparation of 2-((2-oxo-2-phenylethyl)thio)acetic acid, **1g**



Methyl 2-((2-oxo-2-phenylethyl)thio)acetate (1.55g, 6.91 mmol) was dissolved in a flask containing 2 M NaOH (20 mL). The mixture was stirred for 4 h before the pH was adjusted to pH 2 by addition of 1 M HCl. The mixture was extracted with ethyl acetate (3 x 15 mL). The organic phases were pooled together, dried over $MgSO_4$ and concentrated *in vacuo* to yield the ketoacid **1g** (1.31 g, 90%) as a crystalline orange solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.95 – 7.90 (m, 2H, C7-CH), 7.63 – 7.57 (m, 1H, C9-CH), 7.51 – 7.46 (m, 2H, C8-CH), 4.06 (s, 2H, C4-CH_2), 3.38 (s, 2H, C2-CH_2); **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 194.4 (C5), 175.6 (C1), 135.3 (C8), 133.9 (C9), 129.0 (C7), 128.8 (C8), 37.9 (C4), 33.4 (C2).

8. GC and HPLC traces.

8.1. GC traces and mass spectra for preparative-scale syntheses.

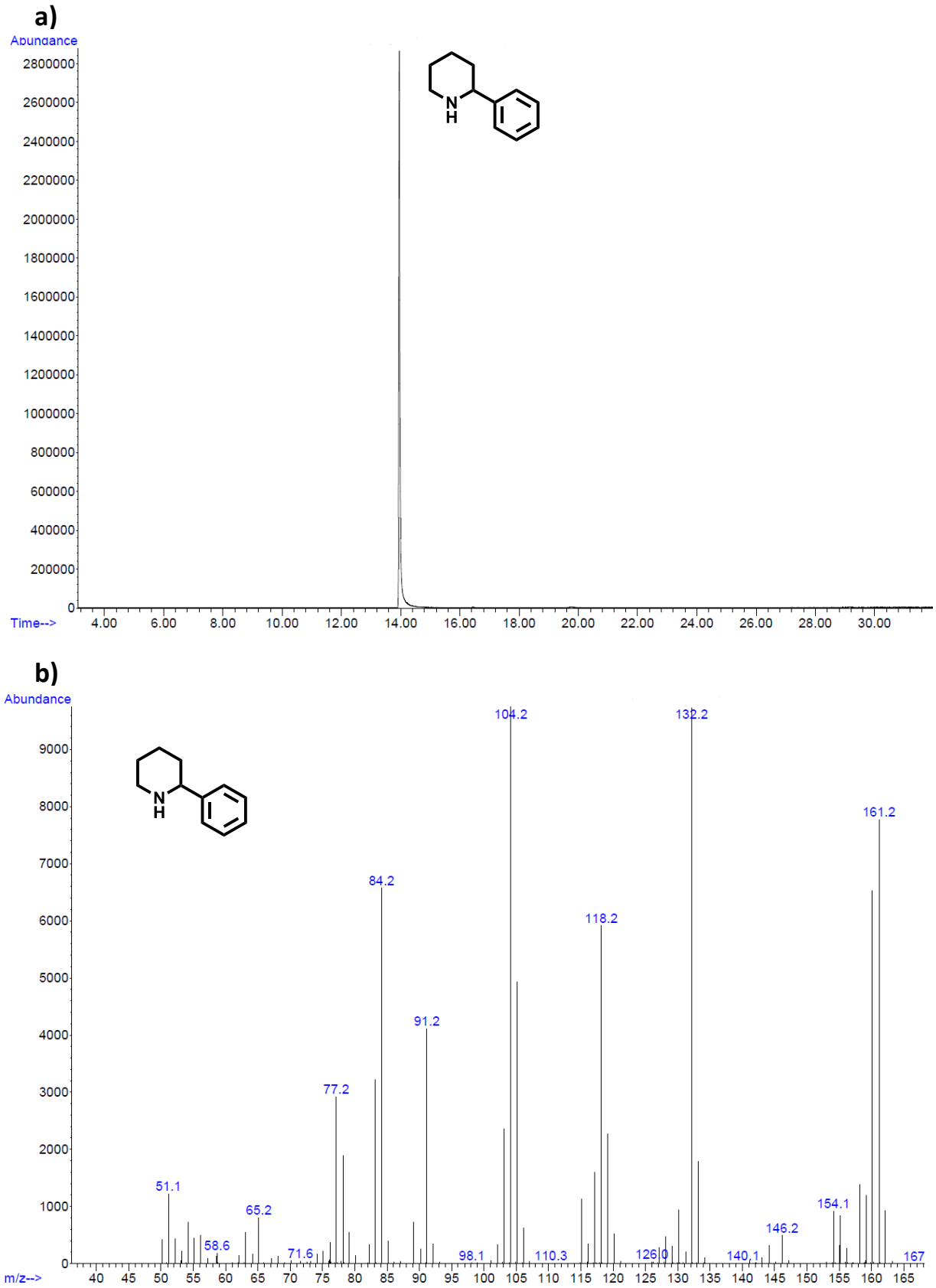


Figure S25 a) GC trace and b) MS trace from GCMS analysis for purified **4a** from preparative-scale conversion of **1a** using *E. coli* BL21 (DE3) cells harboring plasmid pLH10. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temperature 270°C. Method: 50 °C - 175 °C, 5 °C min^{-1}, 175 °C – 250 °C, 10 °C min^{-1}).

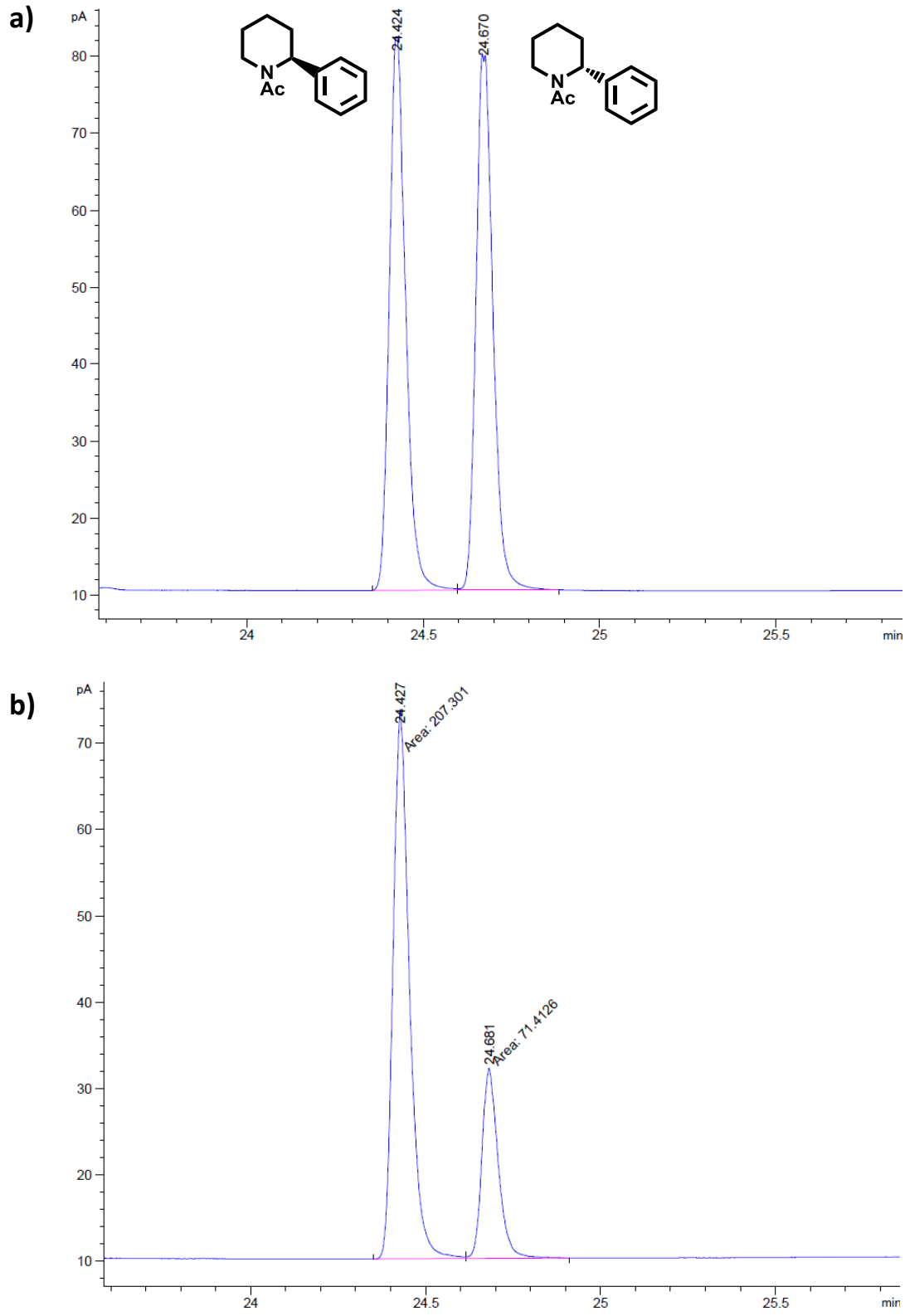


Figure S26 GC-FID analysis of cascade biotransformation of **1a** to determine ee. (CP-Chirasil-DEX CB (Agilent, 25.0 m x 0.25 mm x 0.25 µm), injector temperature 200°C, detector temperature 250 °C . Method: 90 °C - 200 °C, 4 °C min^{-1}, hold at 200 °C for 5 min). Samples were derivatized with acetic anhydride prior to analysis. a) racemic amine standard; b) biotransformation of **1a** using *E. coli* BL21 (DE3) cells harboring plasmid pLH10.

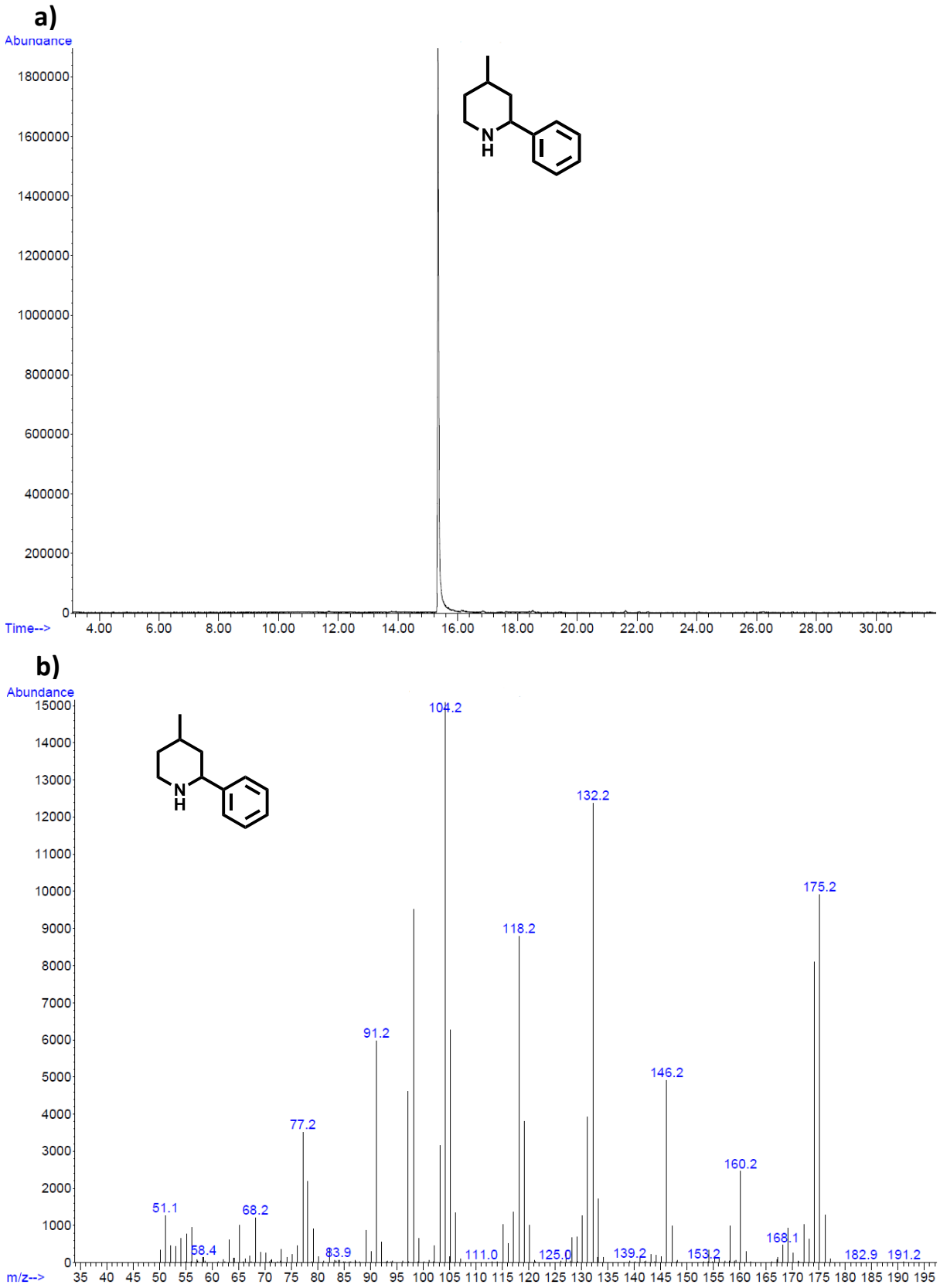


Figure S27 a) GC trace and b) MS trace from GCMS analysis for purified **4e** from preparative-scale conversion of **1e** using *E. coli* BL21 (DE3) cells harboring plasmid pLH10. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temperature 270°C. Method: 50 °C - 175 °C, 5 °C min^{-1}, 175 °C – 250 °C, 10 °C min^{-1}).

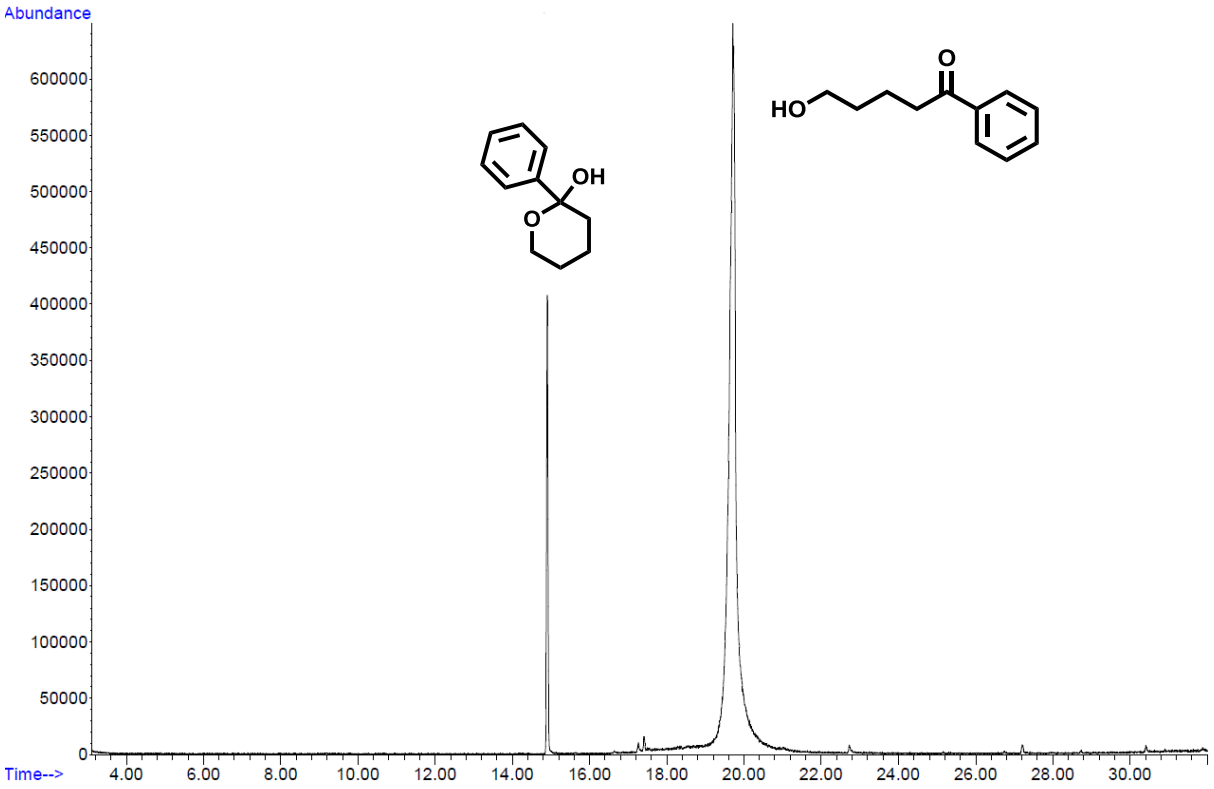
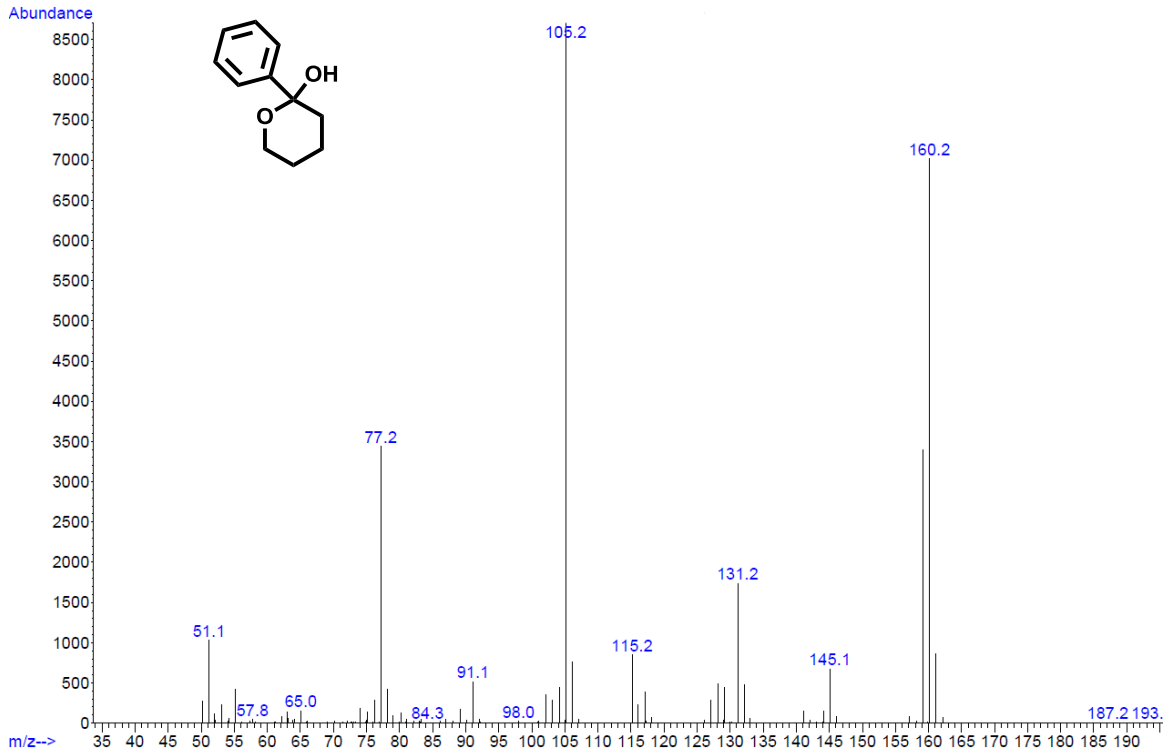


Figure S28 GC trace from GCMS analysis for purified keto alcohol over-reduction product from preparative-scale conversion of **1a** using *E. coli* BL21 (DE3) cells harboring pPB01/MCAR +*Bs*Sfp. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temperature 270°C. Method: 50 °C - 175 °C, 5 °C min^{-1}, 175 °C – 250 °C, 10 °C min^{-1}). This compound is known to form the cyclic hemiacetal over time.[10]

a)



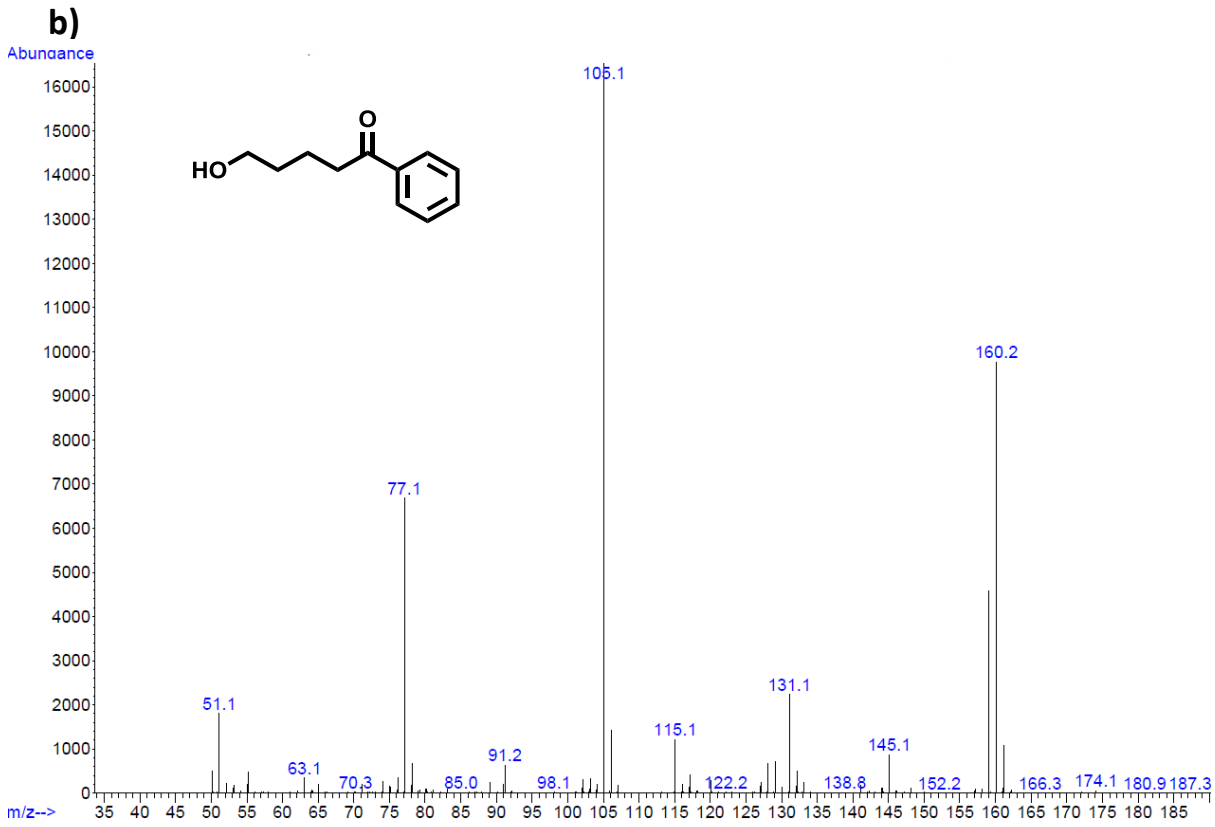
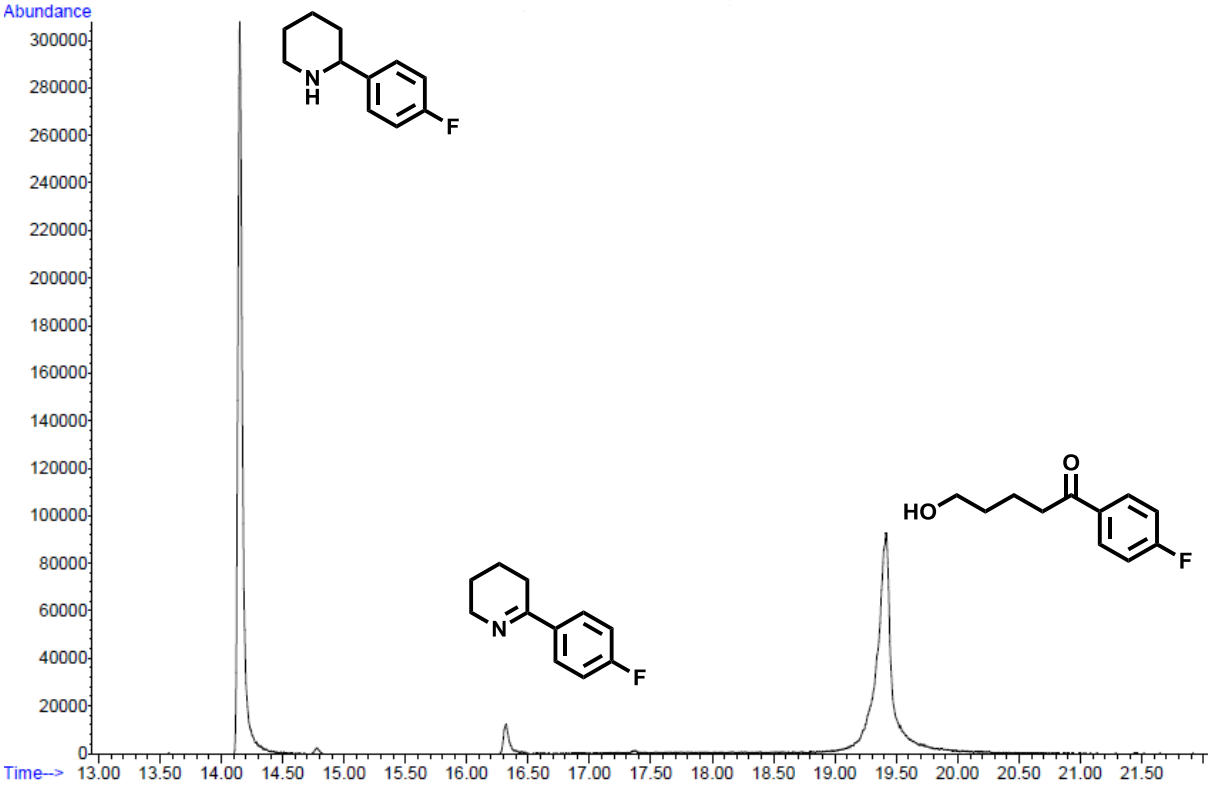


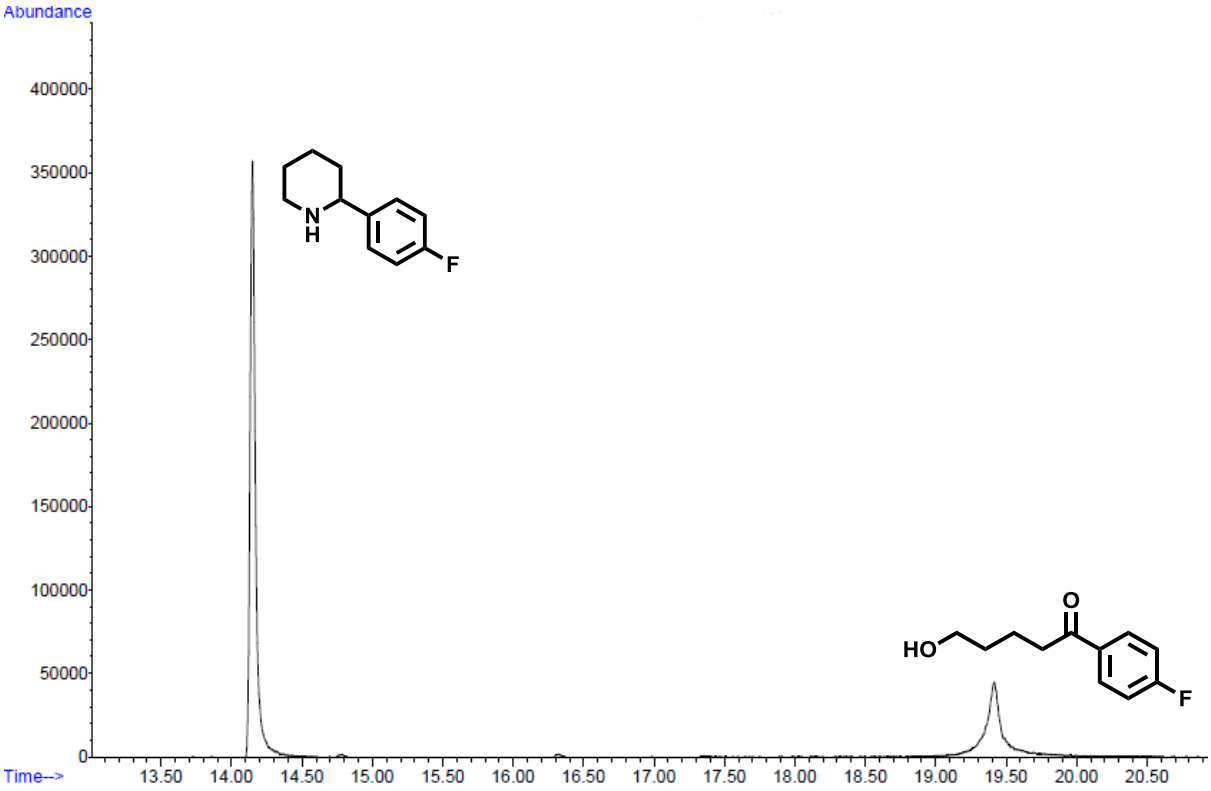
Figure S29 MS traces from GCMS analysis for purified keto alcohol over-reduction product from preparative-scale conversion of **1a** using *E. coli* BL21 (DE3) cells harboring pPB01/MCAR +*Bs*Sfp. a) cyclized keto alcohol product; b) linear keto alcohol product.

8.2. GC traces, HPLC traces and mass spectra for analytical-scale syntheses.

a)


Abundance
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Time-->
13.00 13.50 14.00 14.50 15.00 15.50 16.00 16.50 17.00 17.50 18.00 18.50 19.00 19.50 20.00 20.50 21.00 21.50
N
H
F
N
F
HO
O
F

b)


Abundance
400000
350000
300000
250000
200000
150000
100000
50000
0
Time-->
13.50 14.00 14.50 15.00 15.50 16.00 16.50 17.00 17.50 18.00 18.50 19.00 19.50 20.00 20.50
N
H
F
HO
O
F

c)

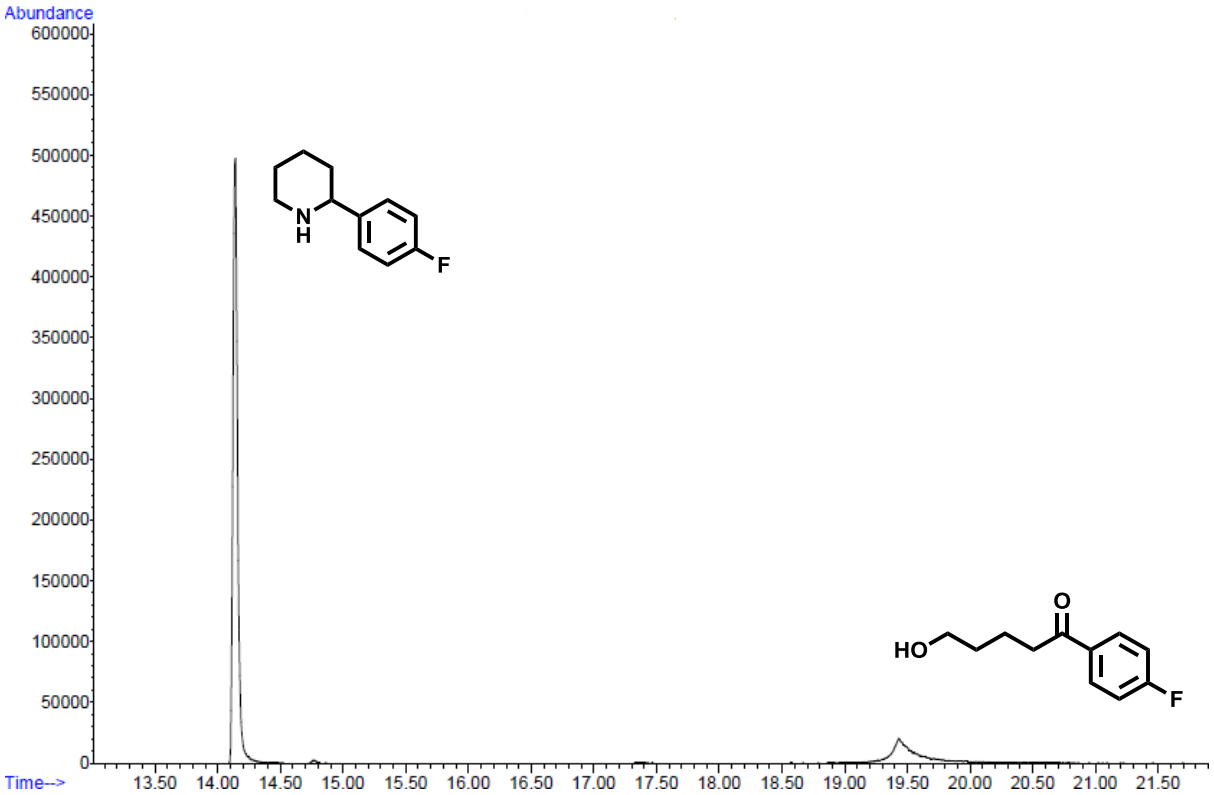
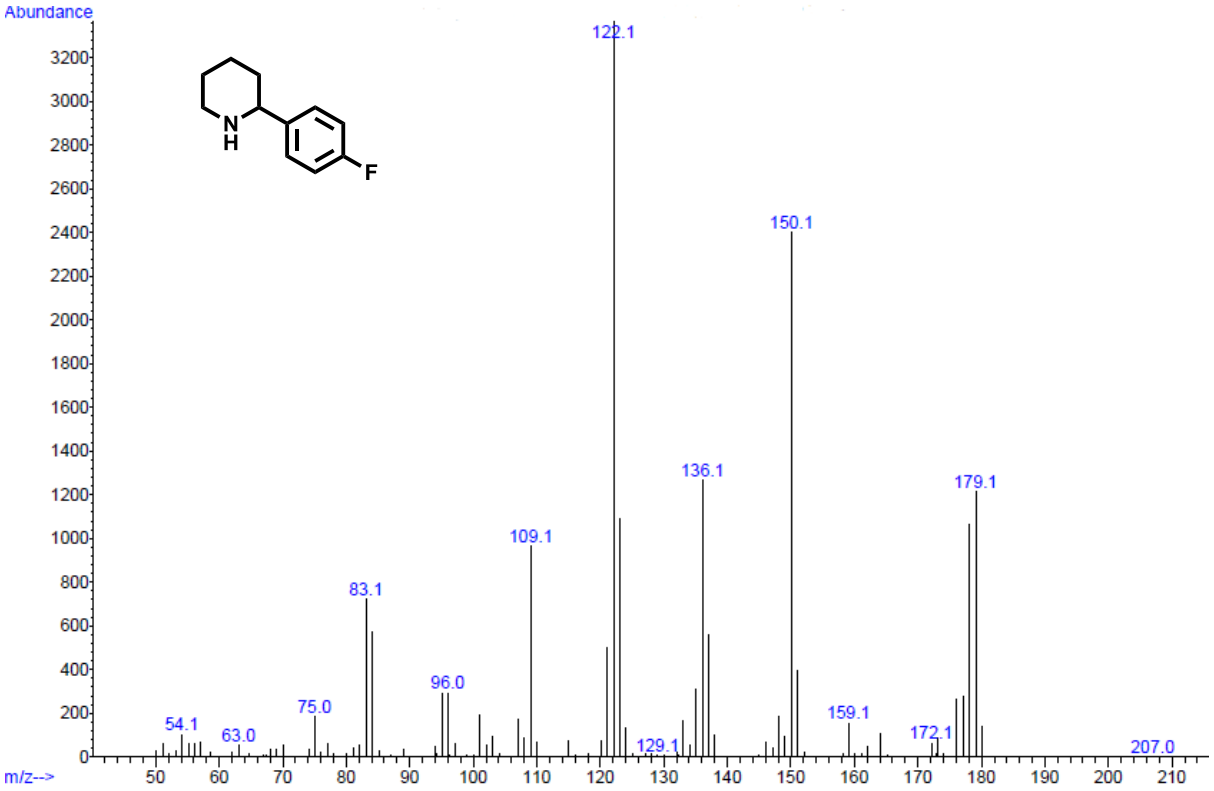


Figure S30 GC traces from GCMS analysis of cascade biotransformation of **1b** to determine conversion. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temperature 270°C. Method: 50 °C - 175 °C, 5 °C min^{-1}, 175 °C – 250 °C, 10 °C min^{-1}). a) cascade using cells harboring plasmid pLH02 (pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); b) cascade using cells harboring plasmid pLH09 (pPB01/ATA-117 + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); c) cascade using cells harboring plasmid pLH10 (pPB01/(*R*)-IRED + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp).

a)


Abundance
m/z-->
122.1
150.1
136.1
179.1
109.1
83.1
96.0
75.0
54.1
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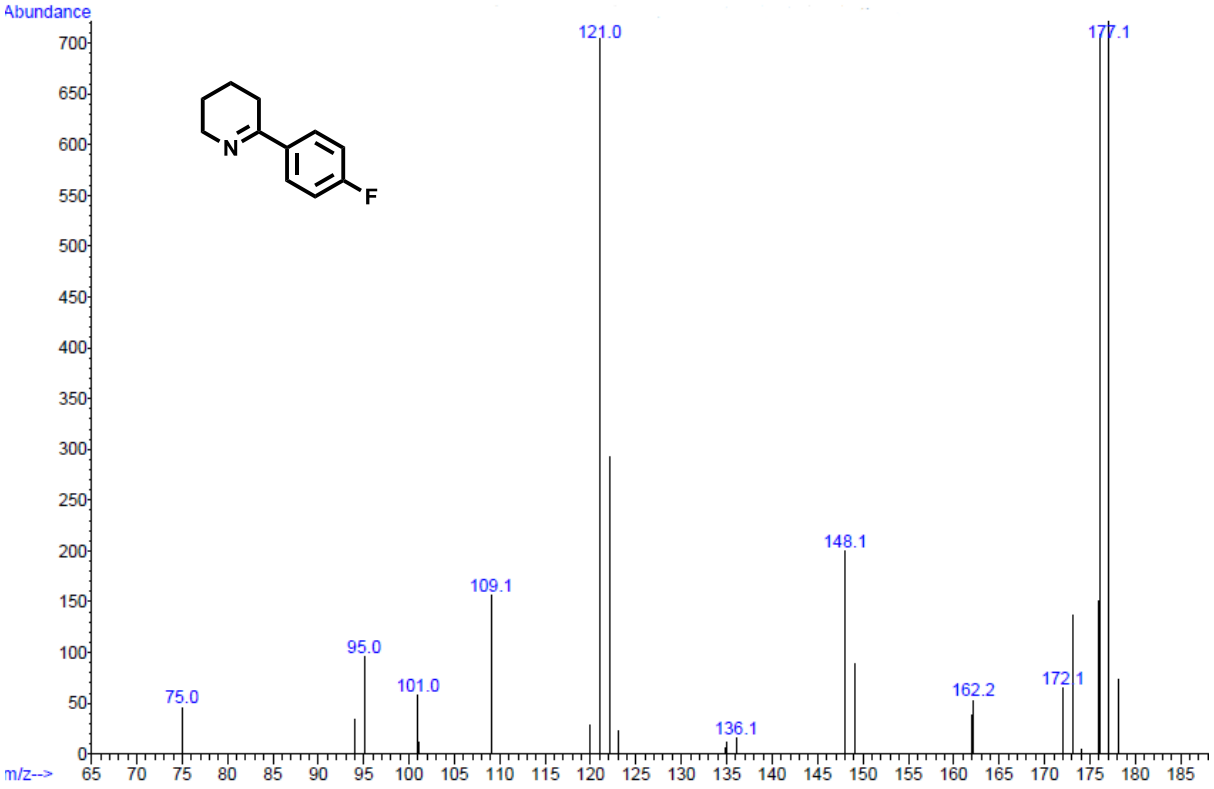
172.1
129.1
207.0
N
H
F

b)


Abundance
m/z-->
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177.1
148.1
109.1
95.0
101.0
75.0
162.2
172.1
136.1
N
F

c)

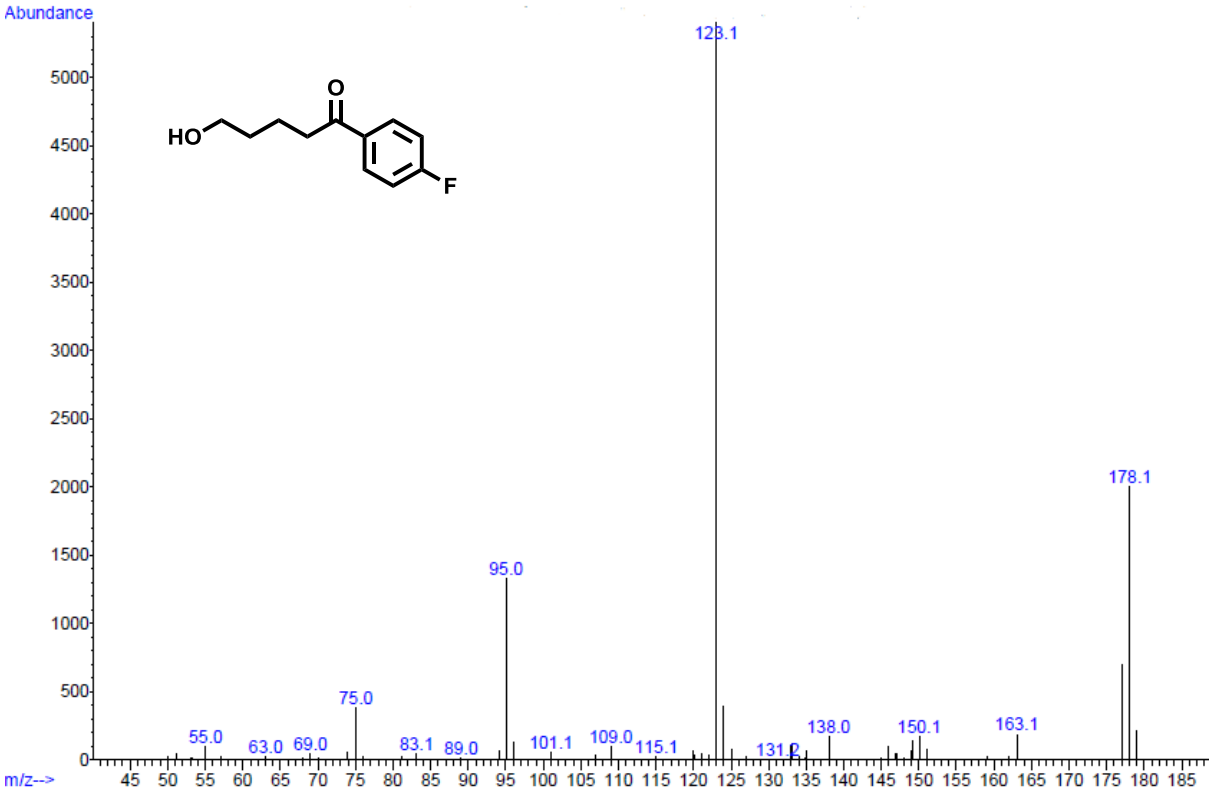


Figure S31 MS traces from GCMS analysis of cascade biotransformation of **1b**. a) MS data for amine; b) MS data for imine; c) MS data for linear keto alcohol.

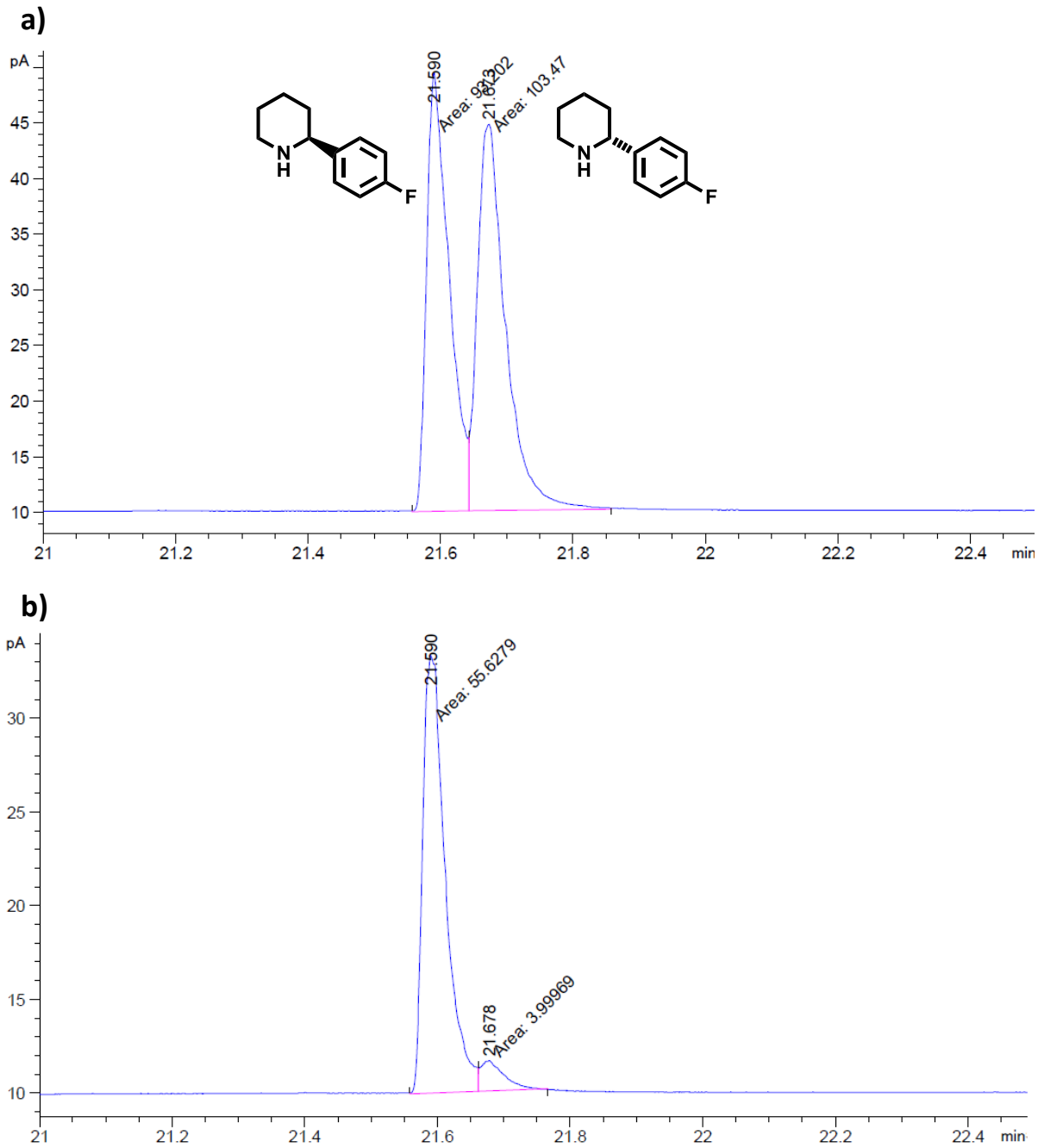
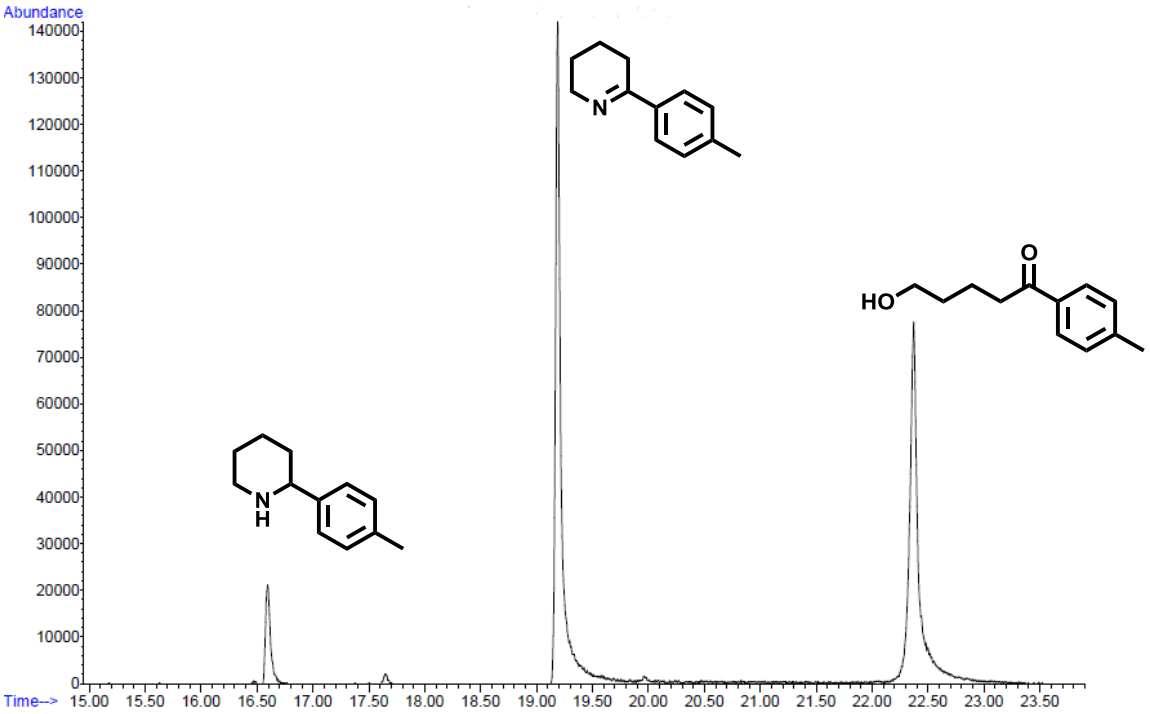
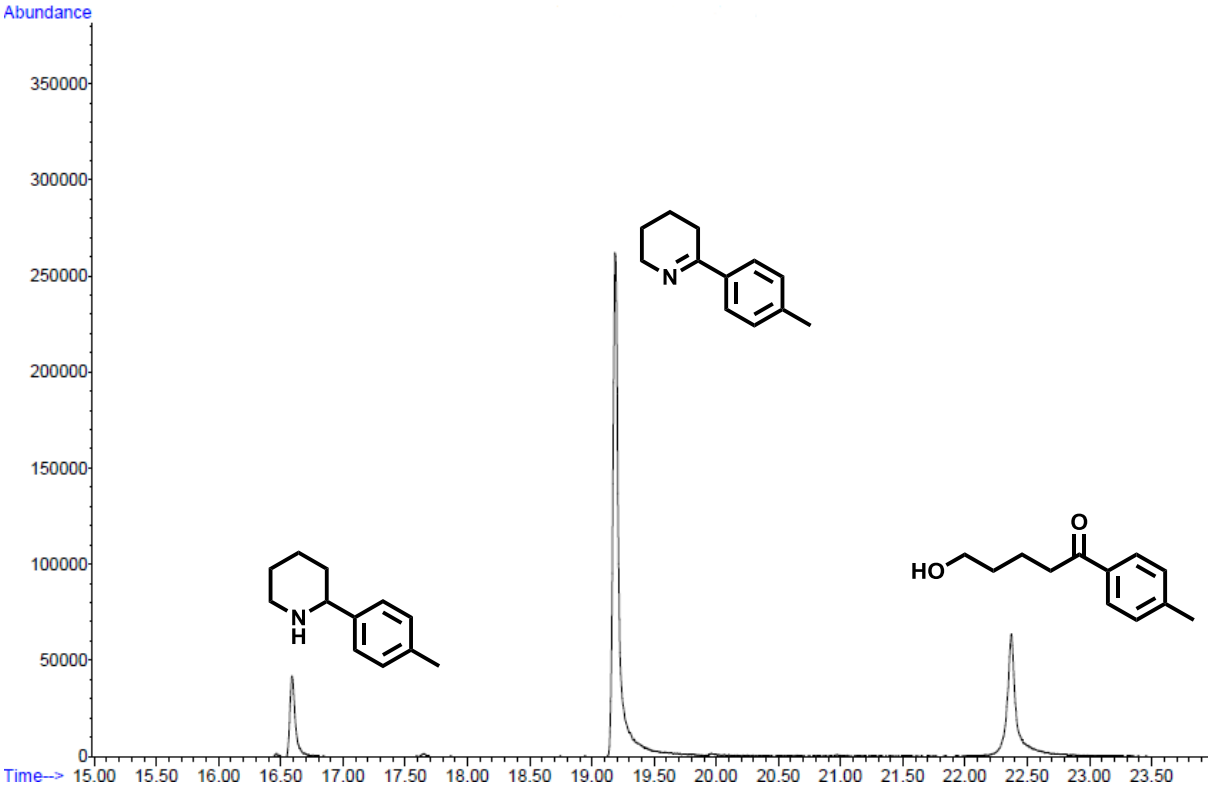


Figure S32 GC-FID analysis of cascade biotransformation of **1b** to determine ee. (CP-Chirasil-DEX CB (Agilent, 25.0 m x 0.25 mm x 0.25 µm), injector temperature 200°C, detector temperature 250 °C . Method: 50 °C - 200 °C, 5 °C min^{-1}, hold at 200 °C for 2 min). a) racemic amine standard; b) biotransformation of **1b** using *E. coli* BL21 (DE3) cells harboring plasmid pLH10. Absolute configuration based on known selectivity of (*R*)-IRED with **1b**.[2]

a)


Abundance
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Time-->
15.00 15.50 16.00 16.50 17.00 17.50 18.00 18.50 19.00 19.50 20.00 20.50 21.00 21.50 22.00 22.50 23.00 23.50
N
N
H
HO
O

b)


Abundance
350000
300000
250000
200000
150000
100000
50000
0
Time-->
15.00 15.50 16.00 16.50 17.00 17.50 18.00 18.50 19.00 19.50 20.00 20.50 21.00 21.50 22.00 22.50 23.00 23.50
N
N
H
HO
O

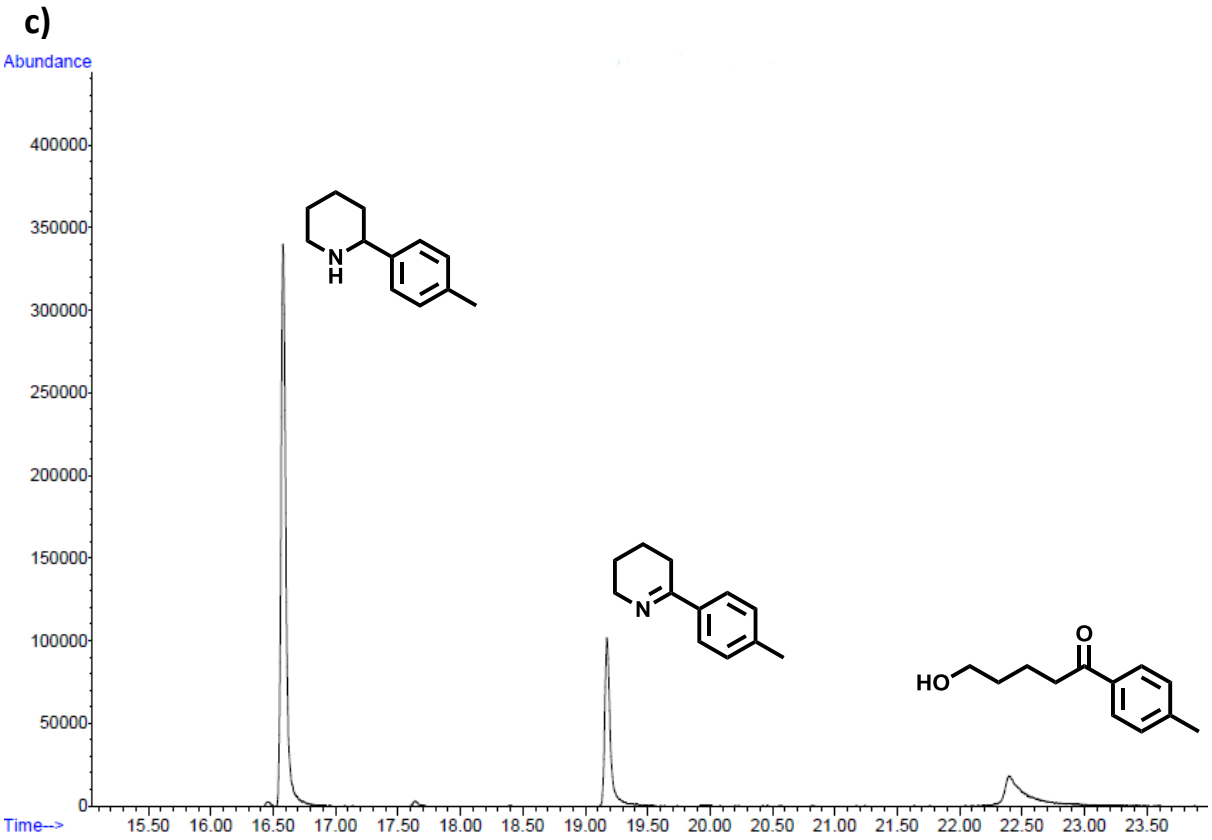
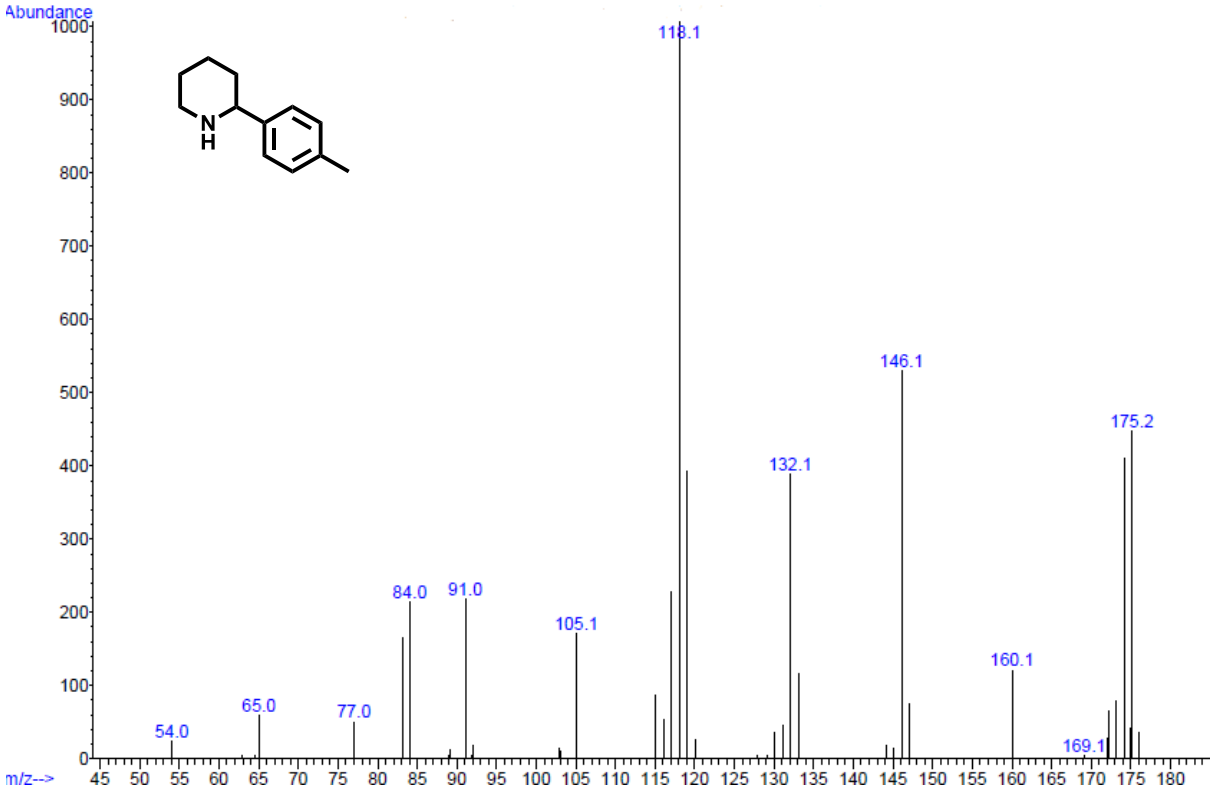
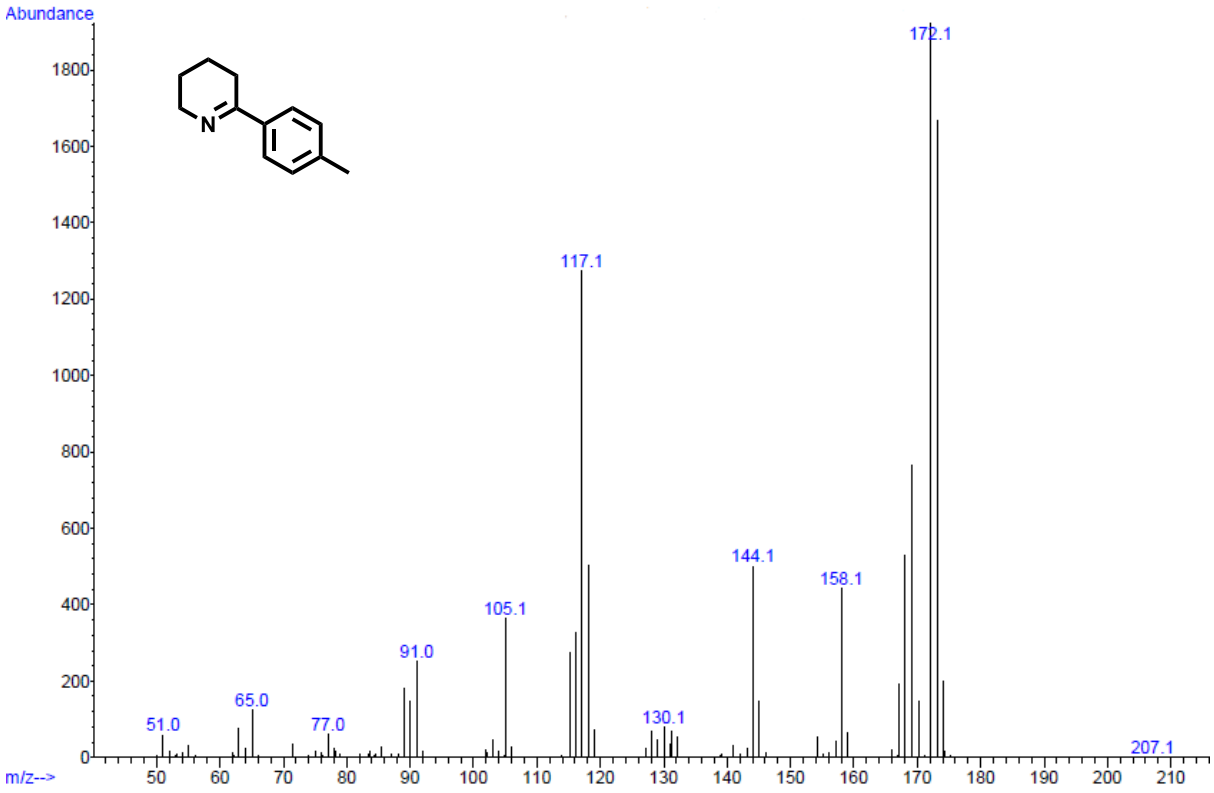


Figure S33 GC traces from GCMS analysis of cascade biotransformation of **1c** to determine conversion. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temperature 270°C. Method: 50 °C - 175 °C, 5 °C min^{-1}, 175 °C – 250 °C, 10 °C min^{-1}). a) cascade using cells harboring plasmid pLH02 (pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); b) cascade using cells harboring plasmid pLH09 (pPB01/ATA-117 + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); c) cascade using cells harboring plasmid pLH10 (pPB01/(*R*)-IRED + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp).

a)


Abundance
m/z-->
118.1
146.1
175.2
132.1
84.0
91.0
105.1
160.1
65.0
77.0
54.0
169.1

b)


Abundance
m/z-->
172.1
117.1
144.1
158.1
105.1
91.0
65.0
51.0
77.0
130.1
207.1

c)

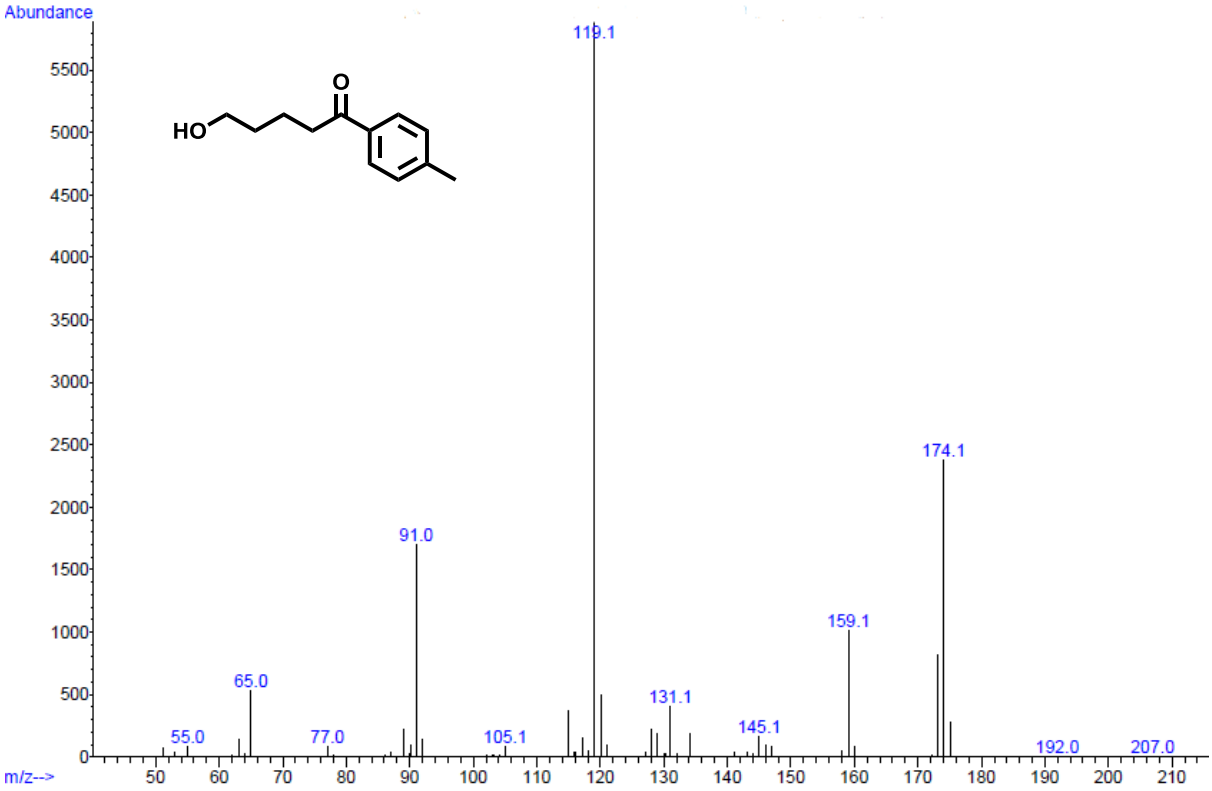


Figure S34 MS traces from GCMS analysis of cascade biotransformation of **1c**. a) MS data for amine; b) MS data for imine; c) MS data for linear keto alcohol.

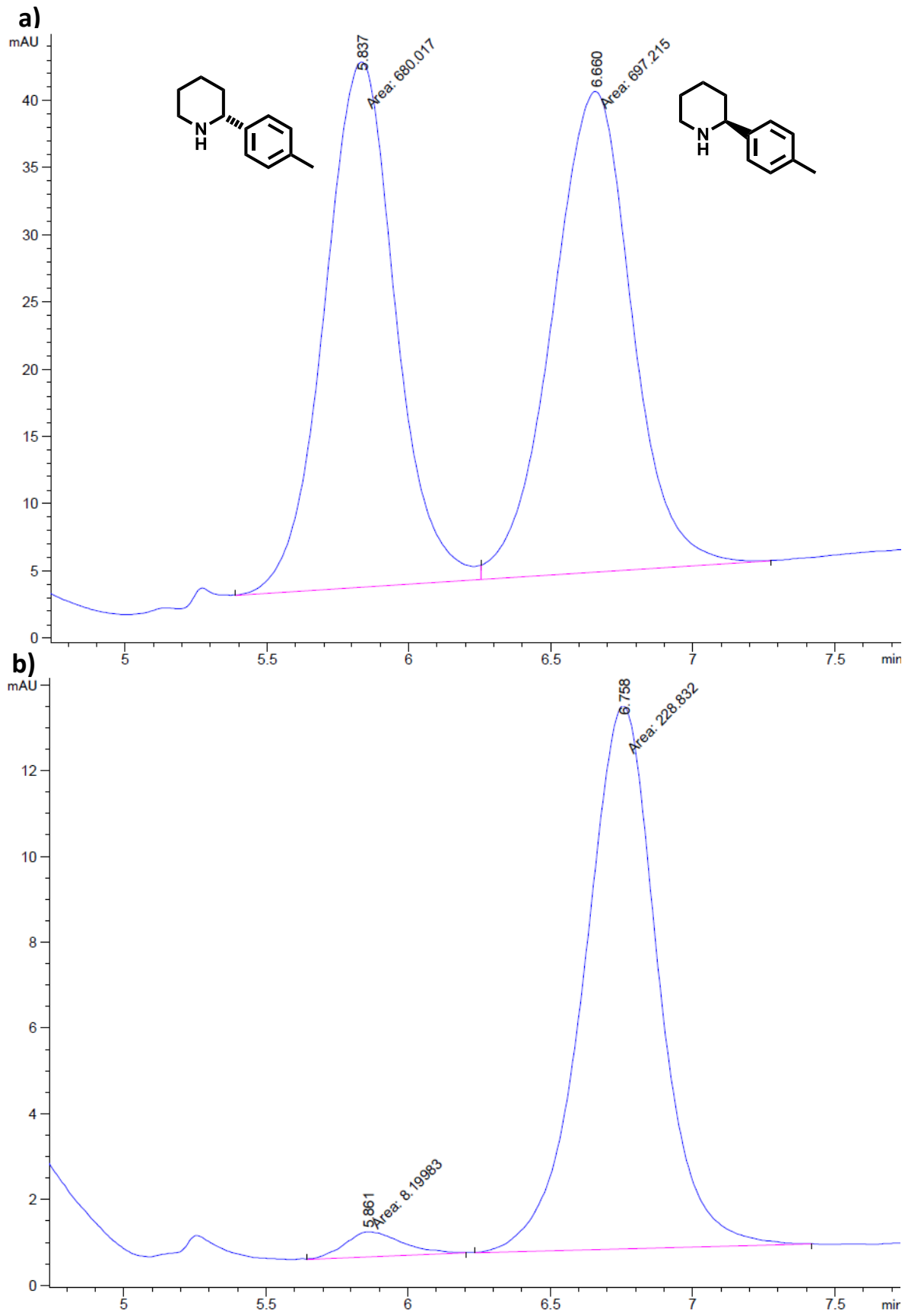
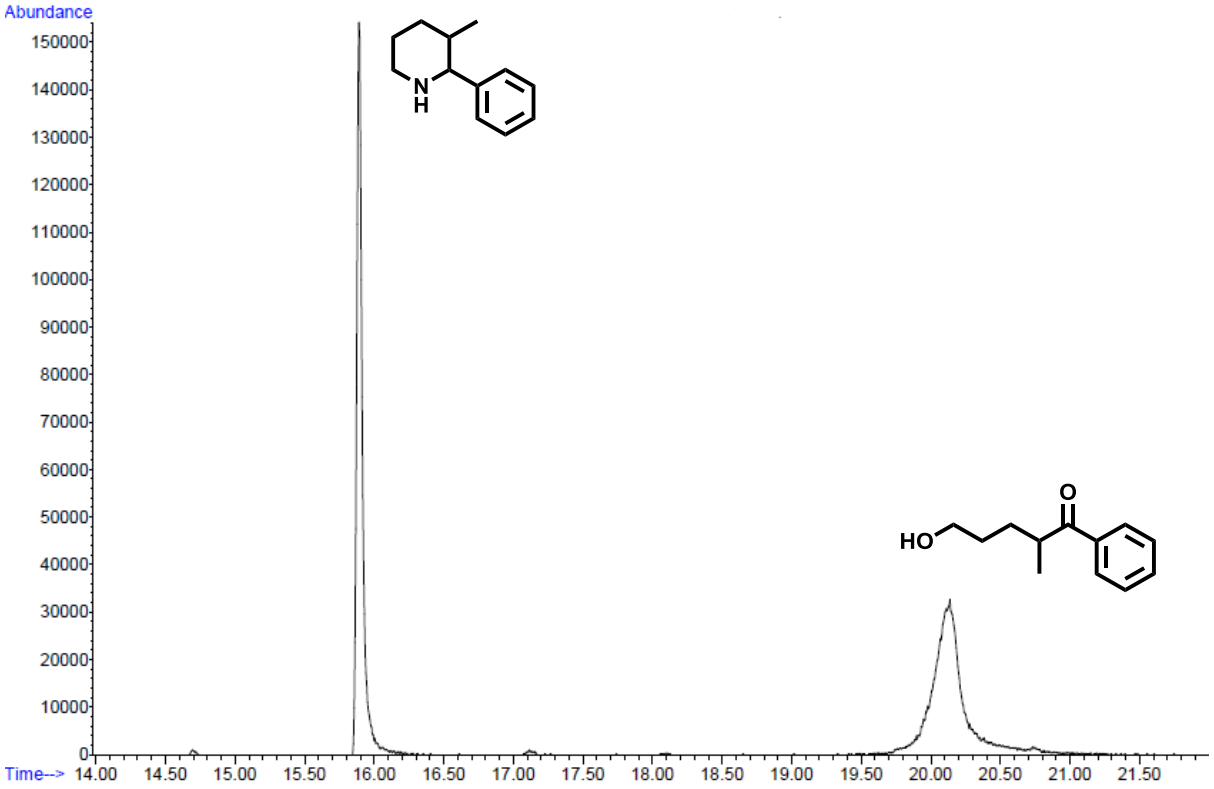
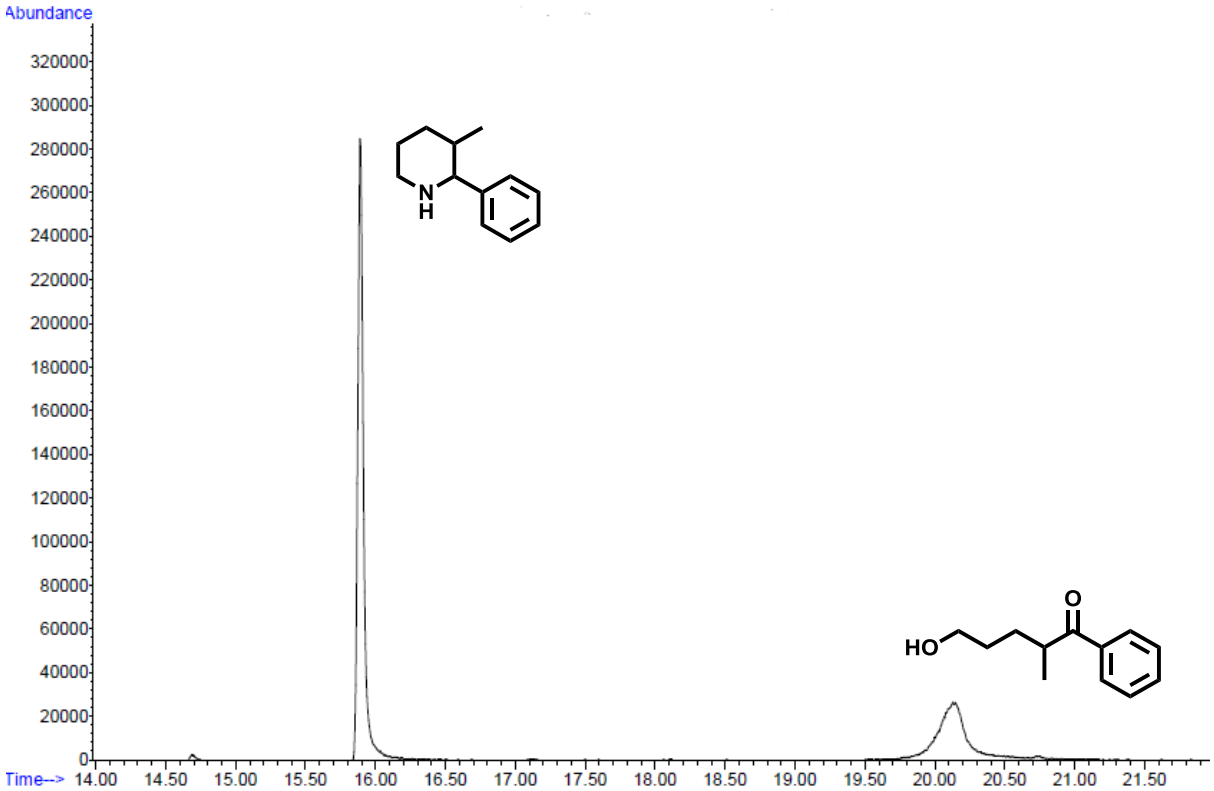


Figure S35 HPLC analysis of cascade biotransformation of **1c** to determine ee and de. (CHIRALPAK®IC column (250 mm × 4.6 mm, 5 µm), solvent: n-hexane/isopropanol/diethylamine = 90/10/0.1, 1 mL/min, 265 nm). a) racemic amine standard; b) biotransformation of **1c** using *E. coli* BL21 (DE3) cells harboring plasmid pLH10.

a)


Abundance
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Time-->
14.00
14.50
15.00
15.50
16.00
16.50
17.00
17.50
18.00
18.50
19.00
19.50
20.00
20.50
21.00
21.50
N
H
HO
O

b)


Abundance
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Time-->
14.00
14.50
15.00
15.50
16.00
16.50
17.00
17.50
18.00
18.50
19.00
19.50
20.00
20.50
21.00
21.50
N
H
HO
O

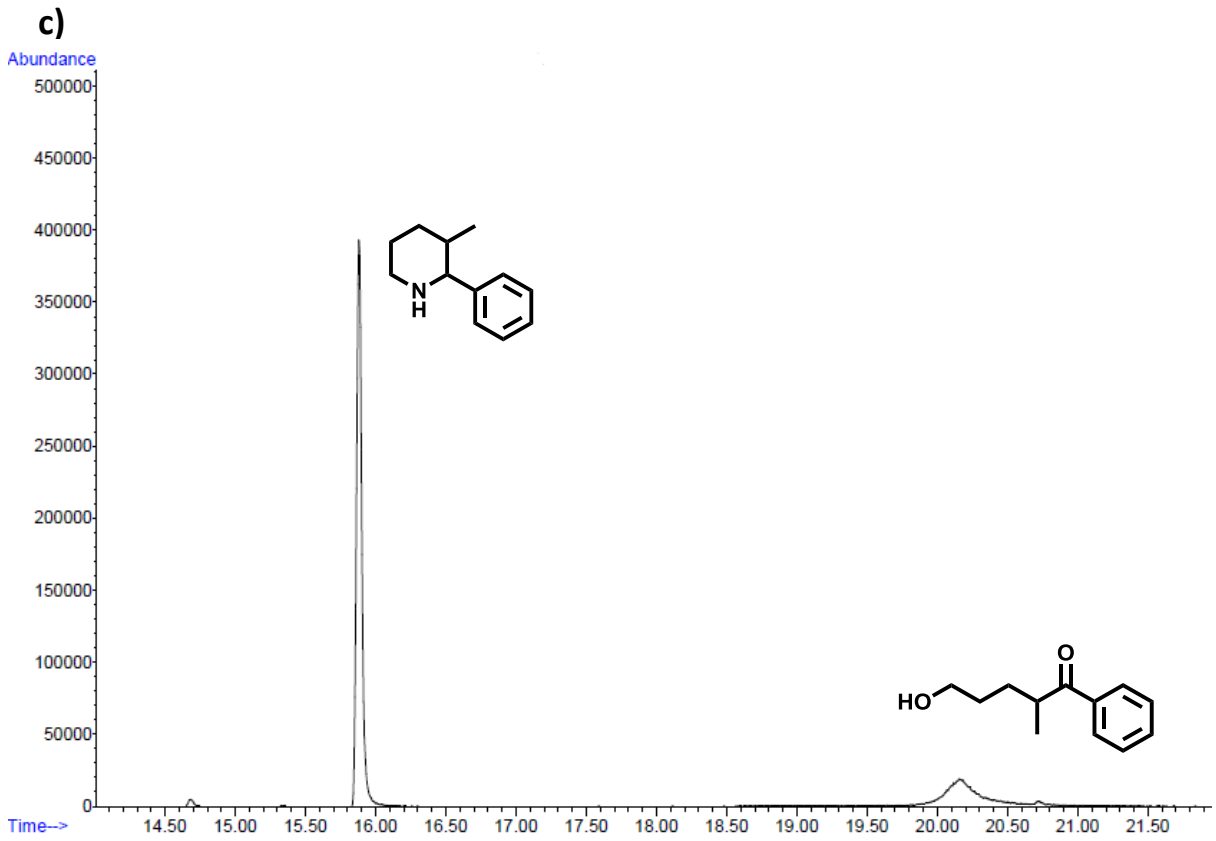


Figure S36 GC traces from GCMS analysis of cascade biotransformation of **1d** to determine conversion. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temperature 270°C. Method: 50 °C - 175 °C, 5 °C min^{-1}, 175 °C – 250 °C, 10 °C min^{-1}). a) cascade using cells harboring plasmid pLH02 (pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); b) cascade using cells harboring plasmid pLH09 (pPB01/ATA-117 + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); c) cascade using cells harboring plasmid pLH10 (pPB01/(*R*)-IRED + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp).

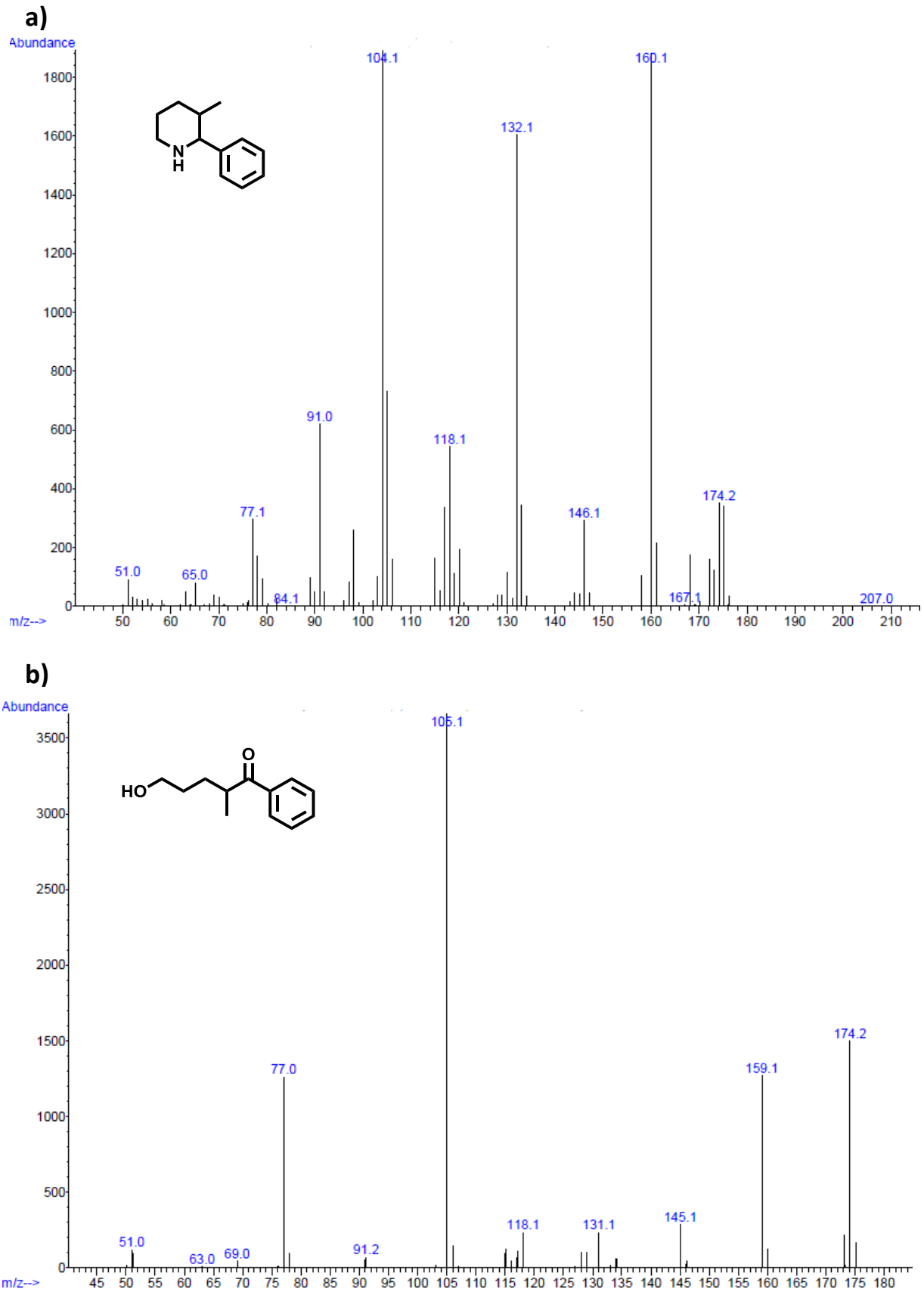
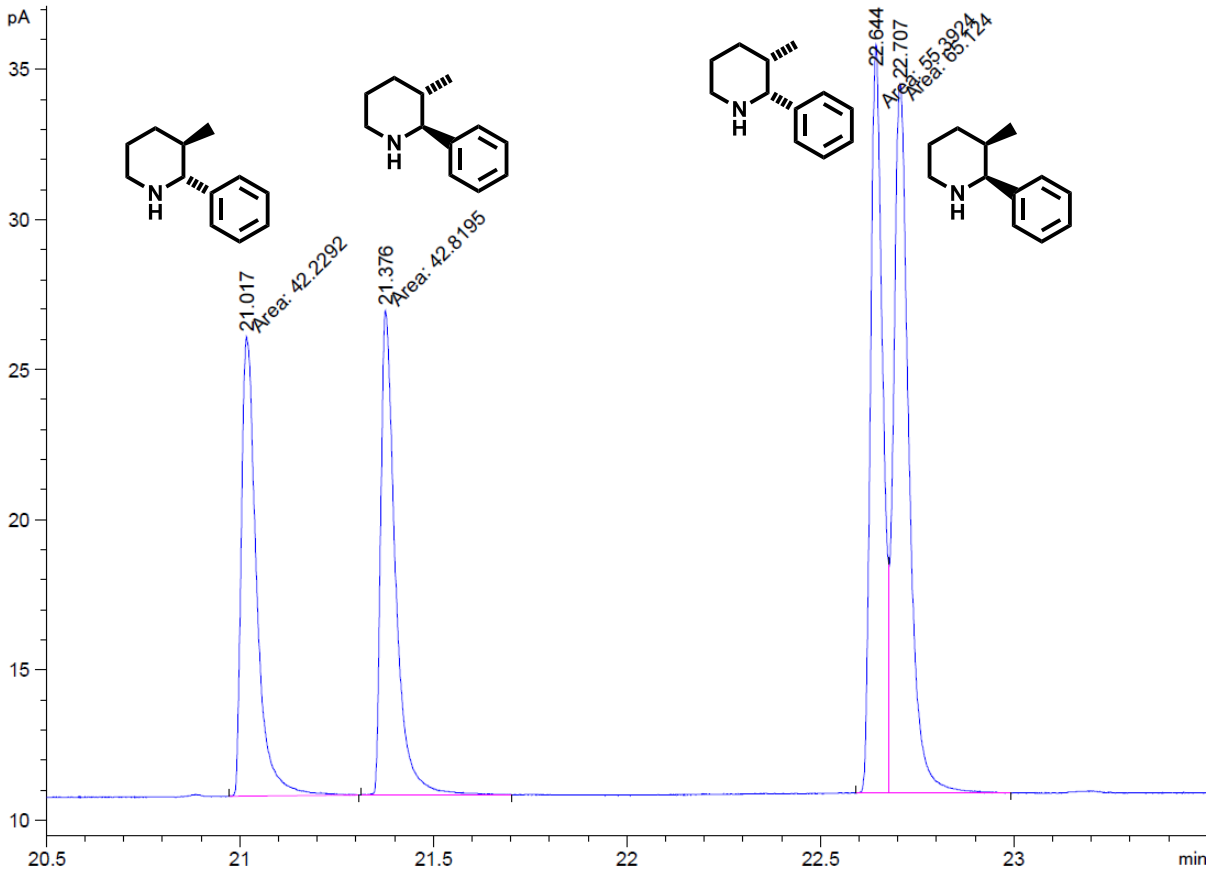


Figure S37 MS traces from GCMS analysis of cascade biotransformation of **1d** . a) MS data for amine; b) MS data for linear keto alcohol.

a)


pA
35
30
25
20
15
10
20.5
21
21.5
22
22.5
23
min
21.017
Area: 42.2292
21.376
Area: 42.8195
22.644
22.707
Area: 55.3924
Area: 65.124

b)

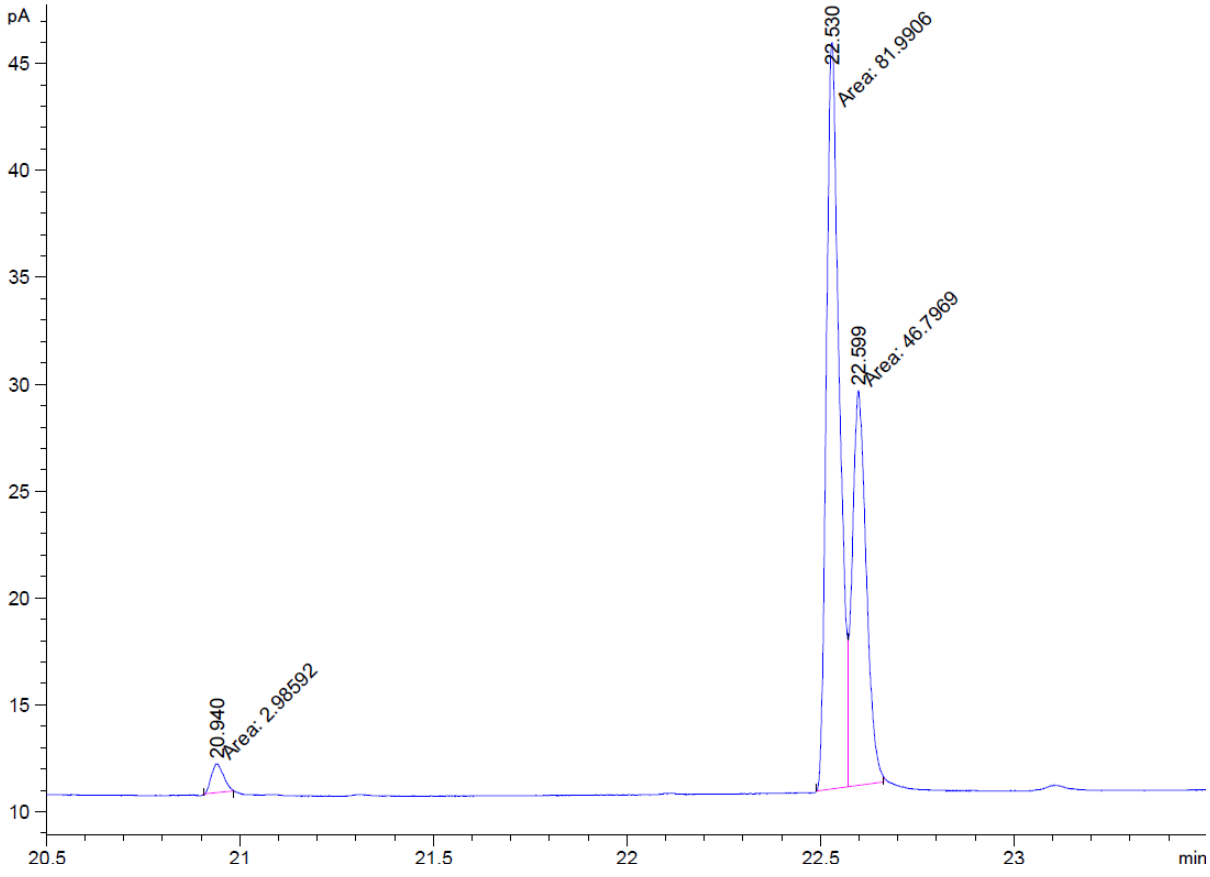
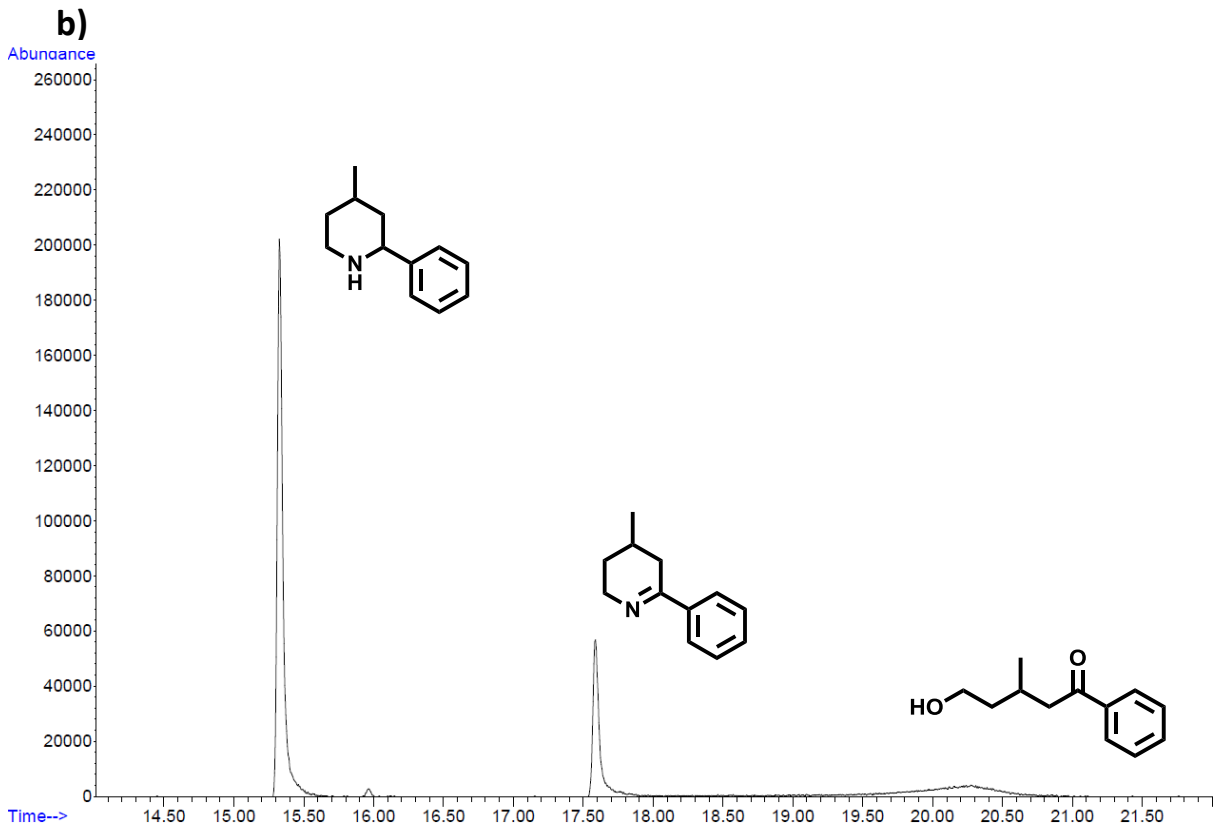

pA
45
40
35
30
25
20
15
10
20.5
21
21.5
22
22.5
23
min
20.940
Area: 2.98592
22.530
Area: 81.9906
22.599
Area: 46.7969

Figure S38 GC-FID analysis of cascade biotransformation of **1d** to determine ee and de. (CP-Chirasil-DEX CB (Agilent, 25.0 m x 0.25 mm x 0.25 µm), injector temperature 200°C, detector temperature 250 °C . Method: 50 °C - 200 °C, 5 °C min^{-1}, hold at 200 °C for 2 min). a) racemic amine standard; b) biotransformation of **1d** using *E. coli* BL21 (DE3) cells harboring plasmid pLH10. Absolute configuration based on known selectivity of (*R*)-IRED with **1d**.[1]


a)
Abundance
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Time-->
14.50
15.00
15.50
16.00
16.50
17.00
17.50
18.00
18.50
19.00
19.50
20.00
20.50
21.00
21.50
N
H
N
HO
O


b)
Abundance
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Time-->
14.50
15.00
15.50
16.00
16.50
17.00
17.50
18.00
18.50
19.00
19.50
20.00
20.50
21.00
21.50
N
H
N
HO
O

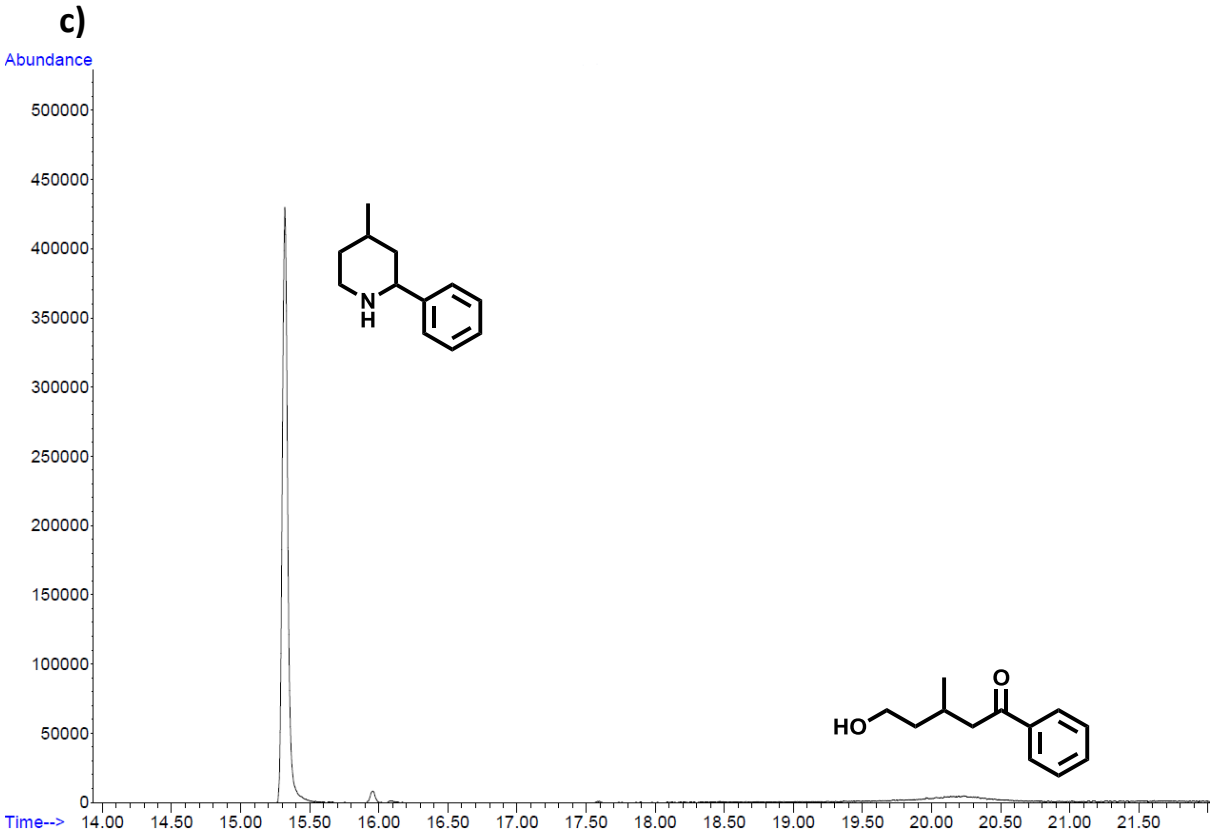
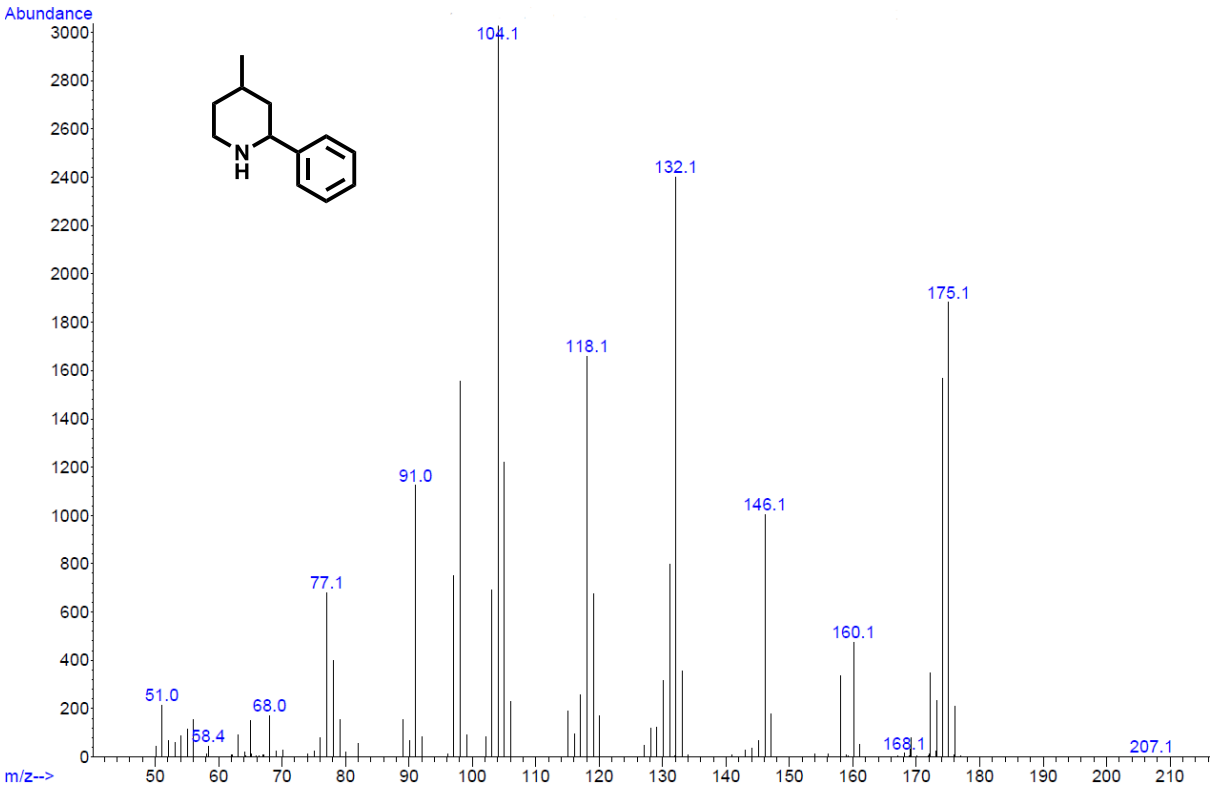
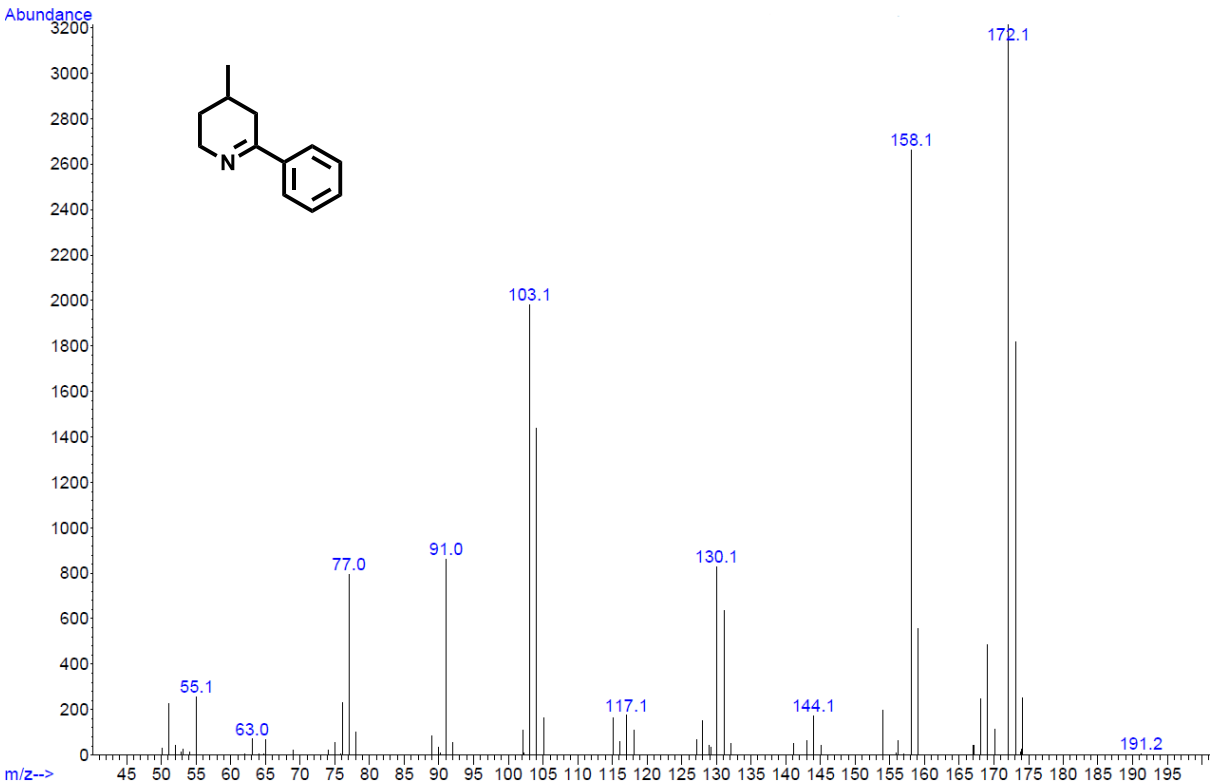


Figure S39 GC traces from GCMS analysis of cascade biotransformation of **1e** to determine conversion. (HP1-MS (Agilent, 30.0 m x 320 µm x 0.25 µm), Inlet temperature 270°C. Method: 50 °C - 175 °C, 5 °C min^{-1}, 175 °C – 250 °C, 10 °C min^{-1}). a) cascade using cells harboring plasmid pLH02 (pPB01/ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); b) cascade using cells harboring plasmid pLH09 (pPB01/ATA-117 + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp); c) cascade using cells harboring plasmid pLH10 (pPB01/(*R*)-IRED + ATA-117 + MCAR + (*R*)-IRED + *Bs*Sfp).

a)


Abundance
m/z-->
104.1
132.1
175.1
118.1
91.0
146.1
77.1
160.1
51.0
68.0
58.4
168.1
207.1

b)


Abundance
m/z-->
172.1
158.1
103.1
77.0
91.0
130.1
55.1
117.1
144.1
63.0
191.2

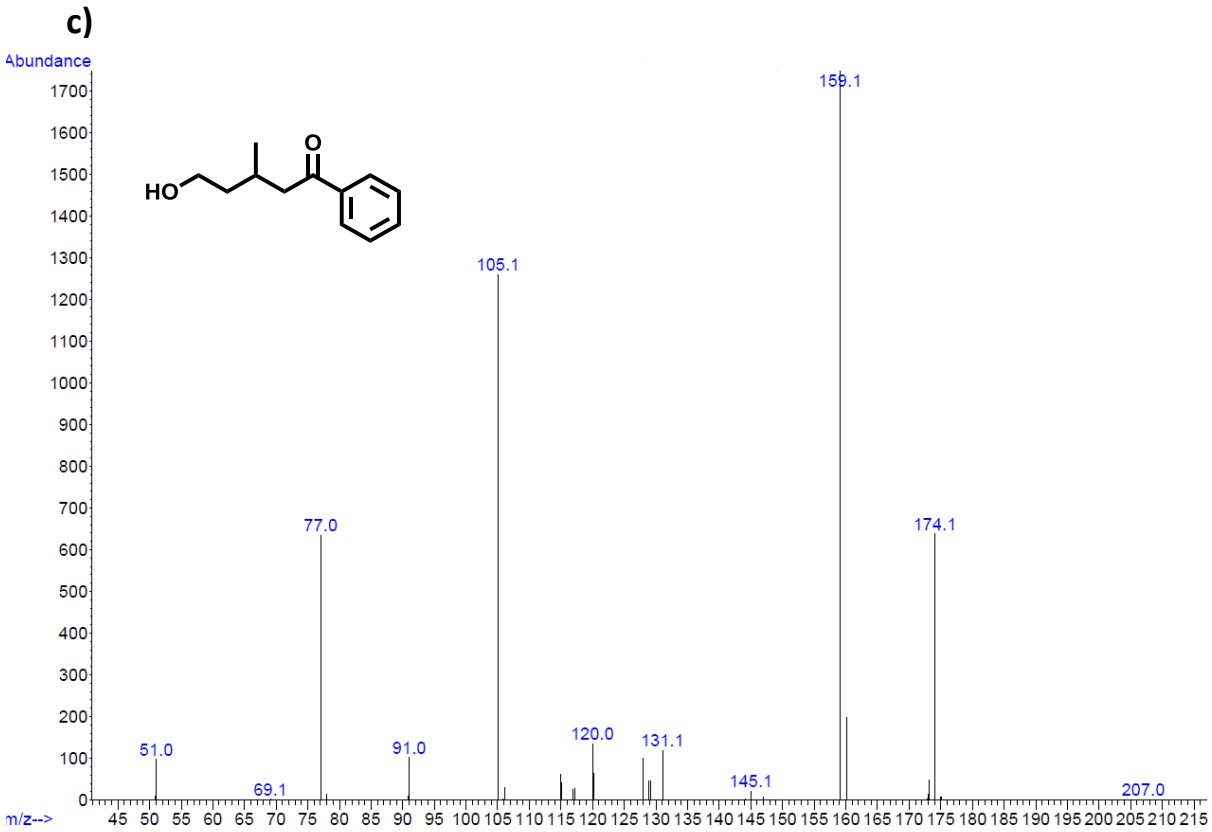


Figure S40 MS traces from GCMS analysis of cascade biotransformation of **1e**. a) MS data for amine; b) MS data for imine; c) MS data for linear keto alcohol.

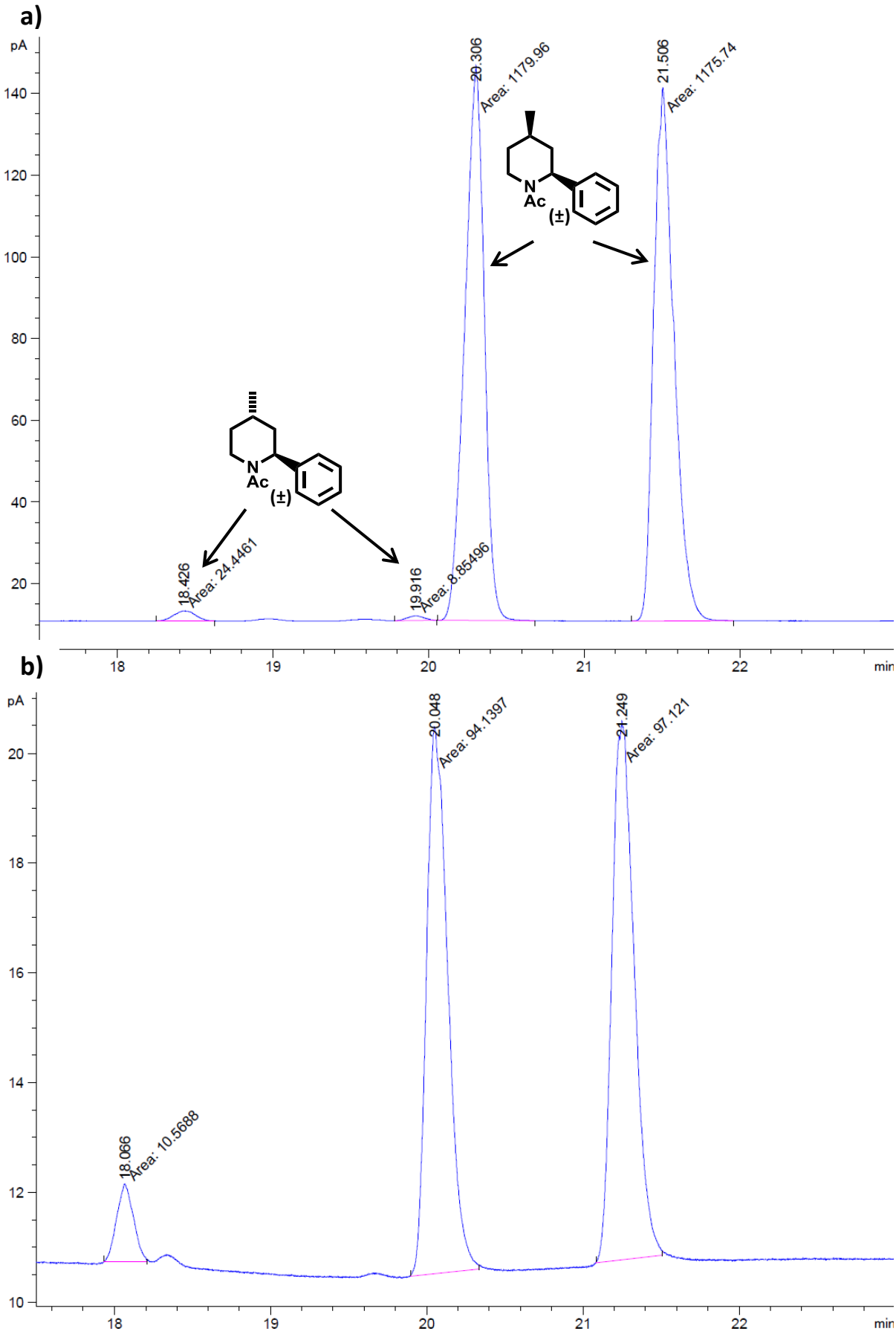
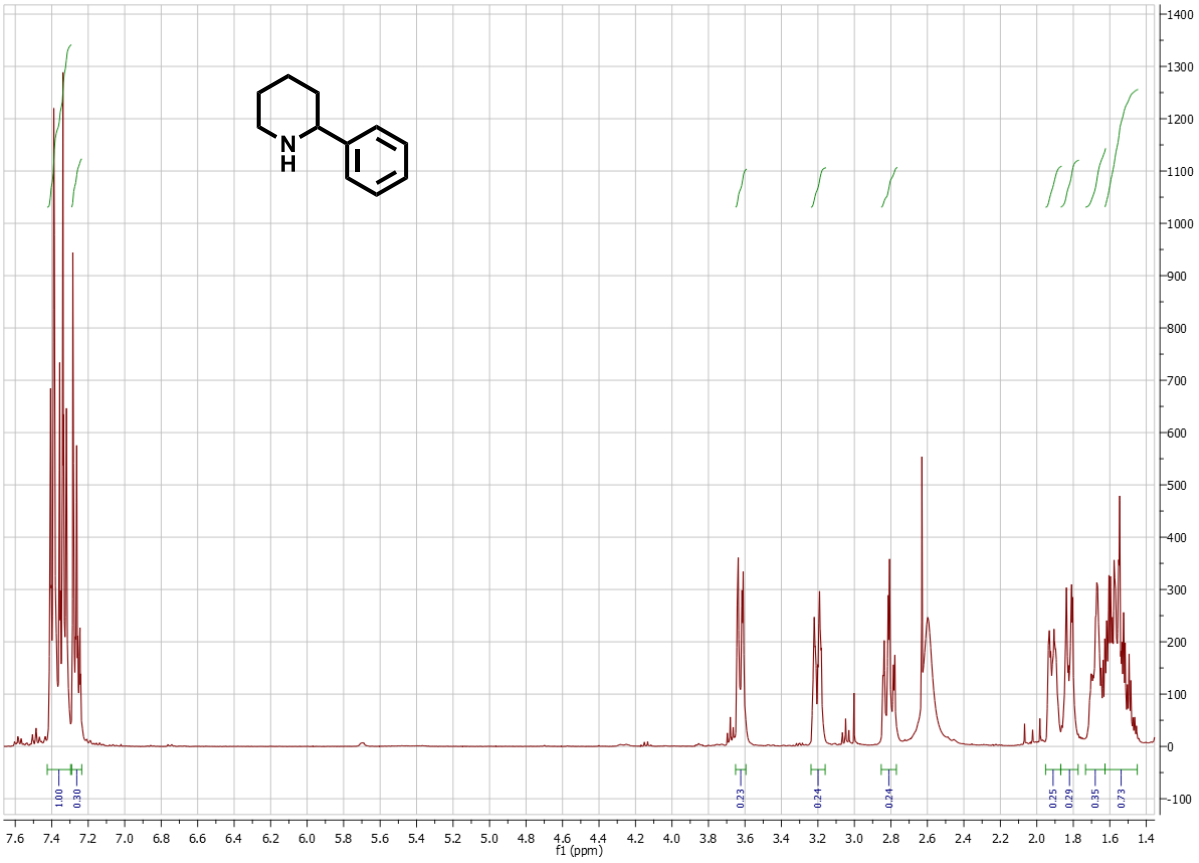
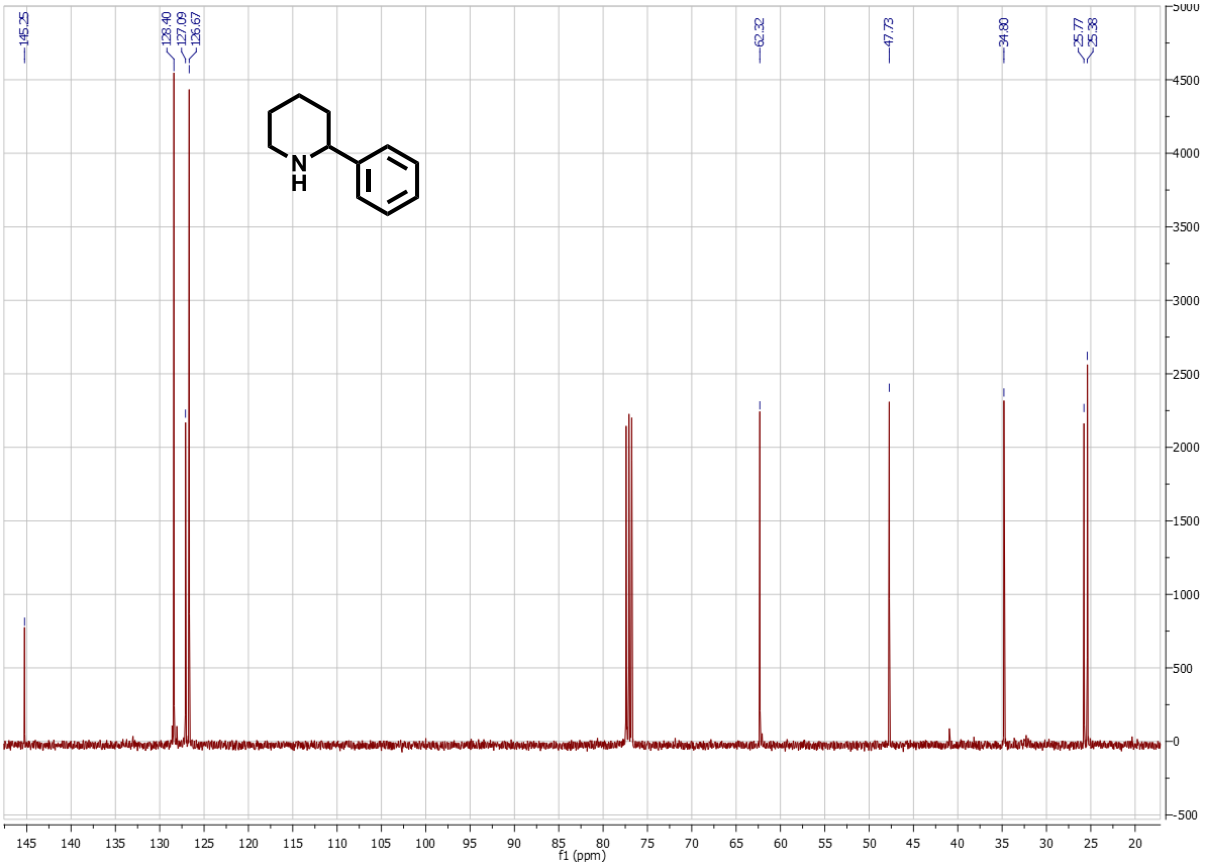
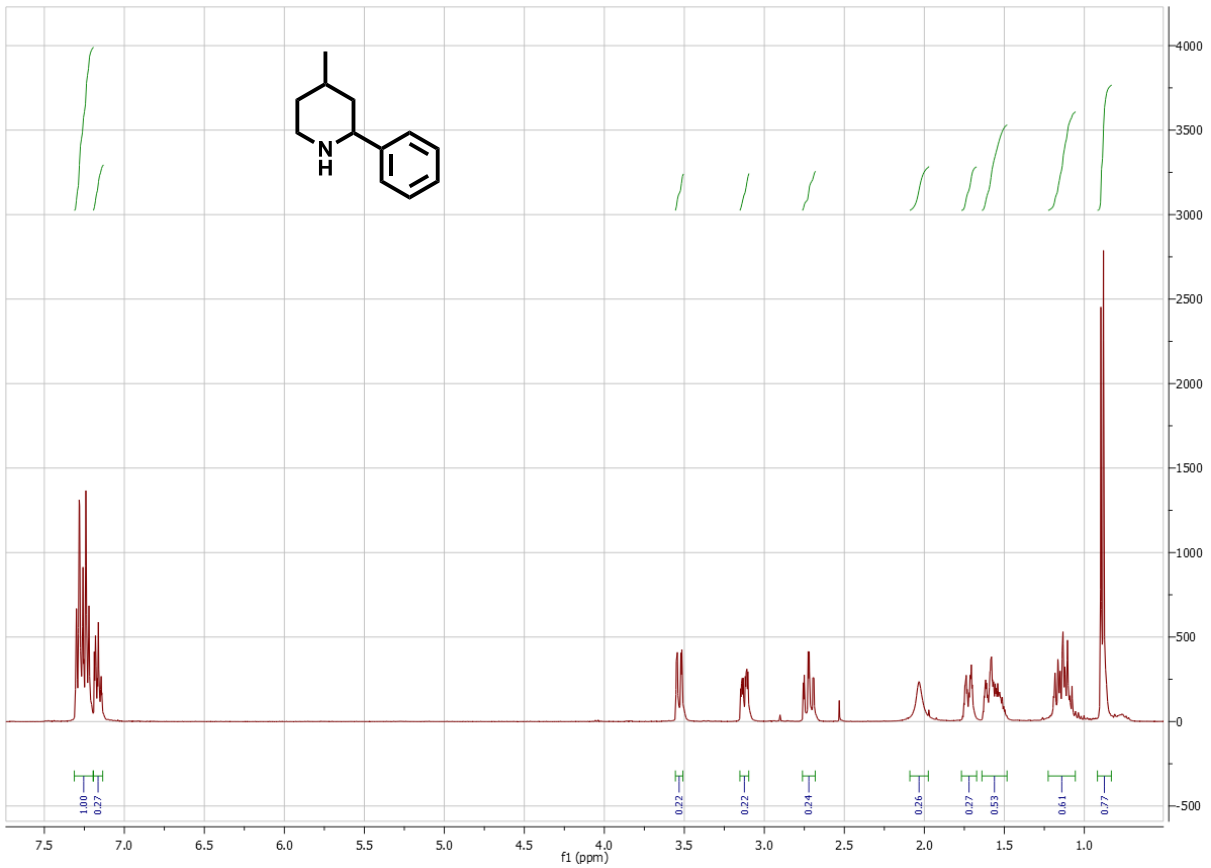

a)
pA
140
120
100
80
60
40
20
20.306
Area: 1179.96
21.506
Area: 1175.74
N
Ac
(±)
18.426
Area: 24.4461
19.916
Area: 8.85496
18
19
20
21
22
min
b)
pA
20
18
16
14
12
10
20.048
Area: 94.1397
21.249
Area: 97.121
18.066
Area: 10.5688
18
19
20
21
22
min

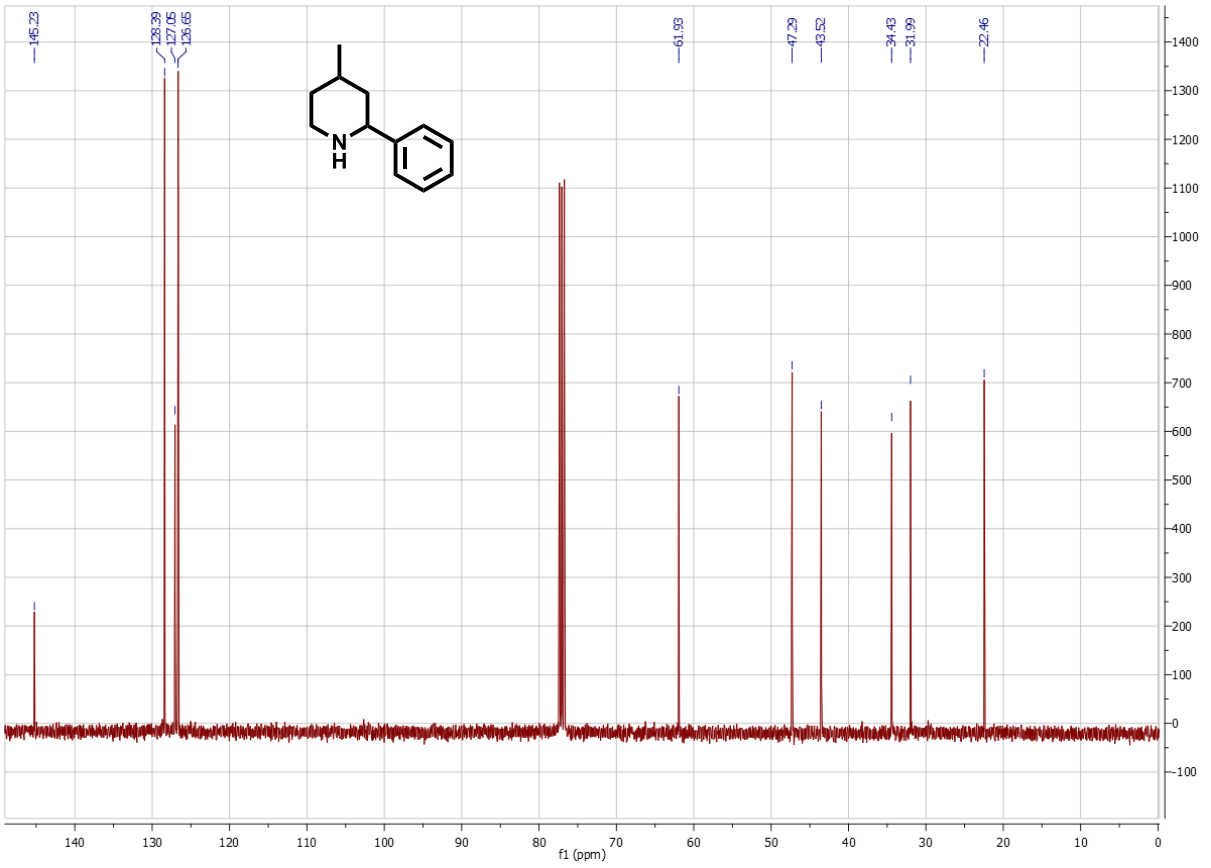
Figure S41 GC-FID analysis of cascade biotransformation of **1e** to determine ee and de. (CP-Chirasil-DEX CB (Agilent, 25.0 m x 0.25 mm x 0.25 µm), injector temperature 200°C, detector temperature 250 °C . Method: 50 °C - 200 °C, 5 °C min^{-1}, hold at 200 °C for 2 min). Samples were derivatized with acetic anhydride prior to analysis. a) racemic amine standard; b) biotransformation of **1e** using *E. coli* BL21 (DE3) cells harboring plasmid pLH02.

9. NMR of amine and keto alcohol products from preparative biotransformations.






1.00
0.27
0.22
0.22
0.24
0.26
0.27
0.53
0.61
0.77
f1 (ppm)


145.23
128.39
127.05
126.65
61.93
47.29
43.52
34.43
31.99
22.46
f1 (ppm)

10. References.

[1] S. P. France, S. Hussain, A. M. Hill, L. J. Hepworth, R. M. Howard, K. R. Mulholland, S. L. Flitsch, N. J. Turner, *ACS Catal.* **2016**, *6*, 3753–3759.

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[3] F. Leipold, S. Hussain, D. Ghislieri, N. J. Turner, *ChemCatChem* **2013**, *5*, 3505–3508.

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