Electronic Supplementary Material (ESI) for Green Chemistry.


## Supplementary Information

# One-Pot Biosynthesis of 1,6-Hexanediol from Cyclohexane by de novo Designed Cascade Biocatalysis

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## Materials

**Commercial materials.** Isopropyl *β*-D-1-thiogalactopyranoside (IPTG, >99%) and kanamycin disulfate salt (>99%) were purchased from Sangon (Shanghai, China). 5-aminolevulinic acid (ALA, >98%) and Imidazole (>99%) were purchased from Solarbio (Beijing, China). Tryptone and yeast extract were purchased from OXOID (Shanghai, China). Primer STAR Max DNA Polymerase was purchased from Takara (Shanghai, China). T5 exonuclease and restriction enzymes were obtained from New England Biolabs (Beverley, MA, USA). Plasmid Miniprep Purification kit and DNA Clean/Extraction kit were purchased from Genemark (USA). Gene synthesis was performed by Wuhan GeneCreate Biological Engineering Co., Ltd. (Wuhan, China). Synthesis of oligonucleotides and DNA sequencing were performed by TSINGKE Biological Technology (Wuhan, China). All other chemicals were of chemical purity and commercially available.

**Strains and plasmids.** *E. coli* DH5α and *E. coli* BL21 (DE3) were used as hosts for gene cloning and protein expression, respectively. The genes encoding alcohol dehydrogenases (ADH) from *Acinetobacter* sp. NCIMB9871, lactonase from *Rhodococcus* sp. HI-31, glucose dehydrogenase (GDH)[1] from *Bacillus megaterium*, double mutant (C376L/M400I) of Baeyer-Villiger monooxygenase (BVMO) from *Acinetobacter* sp. NCIMB9871[2, 3], Carboxylic acid reductase (CAR)[4], Phosphopantetheinyl transferase (PPT)[4] and Aldo-keto reductase (AKR)[4] were chemically synthesized and ligated to plasmid pRSFDuet-1 or pETDuet-1. The P450BM3 variants A82F and A82F/A328F were stored in our laboratory[5] and P450BM3 variants 19A12[6] was provided by professor Huilei Yu in East China University of Science and technology. Details of strains and plasmids used in this study are summarized in Table S1.

**Data availability**. The data that supports the plots within this paper as well as other findings of this study are available from the corresponding author upon reasonable request.

## Table S1. Kinetic parameters of purified enzymes

| Enzyme | Cofactor | Substrate | $K_m$ (mM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($mM^{-1}$ $min^{-1}$) |
|---|---|---|---|---|---|
| $P450_{BM3}$19A12 | NADPH | Cyclohexane | 1.75 ± 0.24 | 271.4 ± 8 | 155 |
| ADH | $NADP^+$ | Cyclohexanol | 7.20 ± 0.89 | 84.8 ± 2.0 | 11.8 |
| BVMO | NADPH | Cyclohexanone | 0.035 ± 0.011 | 1073 ± 147 | 30659 |
| Lac | - | ε-caprolactone | 0.68 ± 0.43 | 5236 ± 322 | 7758 |
| CAR | NADPH | 6-hydroxyhexanoic | 10.31 ± 1.07 | 337 ± 10 | 32.6 |
| GDH | $NADP^+$ | Glucose | 17.22 ± 1.224 | 37.8 ± 1.2 | 2.2 |
| AKR [a] | NADPH | Adipaldehyde | 0.29 ± 0.02 | 361.8 ± 15 | 1247 |

[a] Data was adopted from reference **[4]**.

**Table S2. Strains and plasmids**

| Strain | Description | Source |
|---|---|---|
| *E. coli* BL21 (DE3) | *F⁻ompT gal dcm lon hsdS$_B$ (r$_B^-$ m$_B^-$) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])* | Invitrogen |

| Plasmid | Description | Source |
|---|---|---|
| M1A | pRSFDuet-1 carrying $P450_{BM3}$A82F | This study |
| M1B | pRSFDuet-1 carrying $P450_{BM3}$A82F/A328F | This study |
| M1C | pRSFDuet-1 carrying $P450_{BM3}$19A12 | This study |
| M2A | pETDuet-1 carrying ADH and BVMO | This study |
| M2B | pRSFDuet-1 carrying Lactonase | This study |
| M2C | pRSFDuet-1 carrying ADH and BVMO | This study |
| M2D | pETDuet-1 carrying Lactonase | This study |
| M2E | pRSFDuet-1 carrying ADH, BVMO and Lactonase | This study |
| M2F | pETDuet-1 carrying ADH, BVMO and Lactonase | This study |
| M3A | pRSFDuet-1 carrying CAR, PPT and AKR | This study |
| M3B | pRSFDuet-1 carrying CAR, PPT, AKR and GDH | This study |
| M3C | pETDuet-1 carrying CAR, PPT and AKR | This study |
| M3D | pETDuet-1 carrying CAR, PPT | This study |
| M3E | pRSFDuet-1 carrying AKR | This study |
| M3F | pRSFDuet-1 carrying CAR, PPT | This study |
| M3G | pETDuet-1 carrying AKR | This study |
| M3H | pETDuet-1 carrying AKR and PPT | This study |
| M3I | pRSFDuet-1 carrying CAR | This study |
| pETDuet-1 | Double T7 promoters, pBR322 ori, $Amp^R$ | Novagen |
| pRSFDuet-1 | Double T7 promoters, RSF ori, $Kan^R$ | Novagen |

## Table S3. Oligonucleotide sequences

| Name | Sequence (5' →3') |
|---|---|
| pETDuet-Lac-RA-F | ATGACCAATATTAGCGAAACCCTGAG |
| pETDuet-Lac-RA-R | GCTAATATTGGTCATGTGGTGATGATGGTGATGGCTG |
| pETDuet-RA-F | GCCAGGATCCGAATTCGAGCTC |
| pETDuet-RA-R | GTGGTGATGATGGTGATGGCTGCTG |
| pRSFDuet-RA-F | GCCAGGATCCGAATTCGAGCTC |
| pRSFDuet-RA-R | GTGGTGATGATGGTGATGGCTGCTG |
| ADH to pRSFDuet/pETDuet-OL-F | CACCATCATCACCACATGAGCAATCGTCTGGATGGTAAAGTTG |
| ADH to pRSFDuet/pETDuet-OL-R | GATATATCTCCTTAGGTACCTTACTGTGCGGTATAACCACCATCCAC |
| BVMO to pRSFDuet/pETDuet-OL-F | GGTACCTAAGGAGATATATCATGTCACAAAAAATGGATTTTGATGCTATCGTG |
| BVMO to pRSFDuet/pETDuet-OL-R | GAATTCGGATCCTGGCTTAGGCATTGGCAGGTTGCTTGATATC |
| Lac to pRSFDuet-ADH-BVMO-OL-F | GGTACCTAAGGAGATATATCATGACCAATATTAGCGAAACCCTGAGC |
| Lac to pRSFDuet-ADH-BVMO-OL-R | GCTCGAATTCGGATCCTGGCTTATTCCAGGGCTTTCTGATACCATGCTG |
| Lac to pRSFDuet-ADH-BVMO-RA-F | GCCAGGATCCGAATTCGAGC |
| Lac to pRSFDuet-ADH-BVMO-RA-R | GATATATCTCCTTAGGTACCTTAGGCATTGGCAGGTTGCTTG |
| GDH to pRSF-CSA-RA -F | AAGCTTGCGGCCGCATAATG |
| GDH to pRSF-CSA-RA -R | GATATATCTCCTTAGGTACCTCATTTAAAACCGGTAACTGCATGCGGAAC |
| GDH to pRSF-CSA-OL-F | GGTACCTAAGGAGATATATCATGTATACAGATTTAAAAGATAAAGTAGTAGTAAT TAC |
| GDH to pRSF-CSA-OL-R | CATTATGCGGCCGCAAGCTTTTATCCGCGTCCTGCTTGGAATG |
| pETDuet-CAR-PPT-RA-R | TTACAGCAGTTCTTCATAGCTAACCATGGTAATATCTTCC |
| pETDuet-CAR-PPT-RA-F | GAAGAACTGCTGTAAAAGCTTGCGGCCGCATAATG |
| CSA to pETDuet-OL-F | CAGGATCCGAATTCGATGACCGAAACCATTAGCACCG |
| CSA to pETDuet-OL-R | TGCGGCCGCAAGCTTTCATTTAAAACCGGTAACTGCATGCG |
| CSA to pETDuet-RA-F | AAGCTTGCGGCCGCATAATG |
| CSA to pETDuet-RA-R | CGAATTCGGATCCTGGCTGTGG |
| AKR to pRSF-CAR-SFP-OL-F | GGTACCTAAGGAGATATATCATGCAGTATGTTAAACTGGGTAGCACC |
| AKR to pRSF-CAR-SFP -OL-R | CATTATGCGGCCGCAAGCTTTCATTTAAAACCGGTAACTGCATGCG |
| AKR to pRSF-CAR-SFP -RA-F | AAGCTTGCGGCCGCATAATG |
| AKR to pRSF-CAR-SFP -RA-R | GATATATCTCCTTAGGTACCTTACAGCAGTTCTTCATAGCTAACCATGGTAATATCT TC |
| pRSFDuet-AKR-RA-F | ATGCAGTATGTTAAACTGGGTAGCACC |
| PRSFDuet-AKR-RA-R | TTTAACATACTGCATCGAATTCGGATCCTGGCTGTG |
| PPT to pETDuet-AKR-OL-F | AGAAGGAGATATACAGGATCCGATGAAAATCTATGGCATCTATATG |
| PPT to pETDuet-AKR-OL-R | CTTTACCAGACTCGAGAATTCTTACAGCAGTTCTTCATAGCTAACC |
| PPT to pETDuet-AKR-RA-F | TCGAGTCTGGTAAAGAAACCGCTG |
| PPT to pETDuet-AKR-RA-R | TGTATATCTCCTTCTTATACTTAACTAATATACTAAGATGGGGA |
| PPT to pRSFDuet-CAR-OL-F | GGTACCTAAGGAGATATATCATGAAAATCTATGGCATCTATATGGATCGTCC |
| PPT to pRSFDuet-CAR-OL-R | CATTATGCGGCCGCAAGCTTTTACAGCAGTTCTTCATAGCTAACCATGG |
| PPT to pRSFDuet-CAR-RA-F | AAGCTTGCGGCCGCATAATGCTTAAG |
| PPT to pRSFDuet-CAR-RA-R | GATATATCTCCTTAGGTACCTTAAACTAAACCCAGCAGCTGAATATCGCTGGCATA TT |

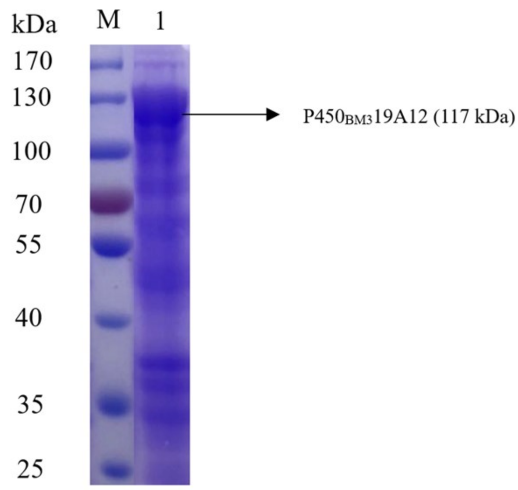


**Figure S1. SDS-PAGE analysis of whole-cell proteins of Module 1 expressed in *E. coli*.** Lane M: protein marker (kDa); Lane 1: *E. coli* (M1C).

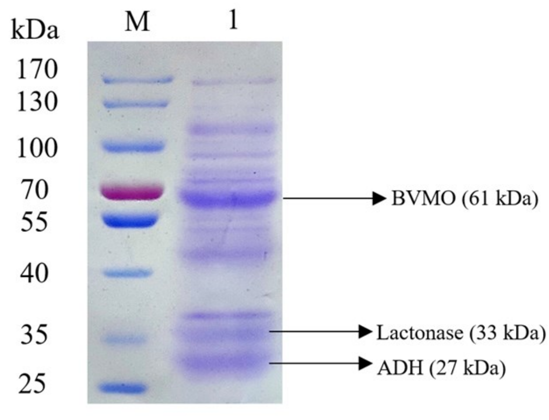


**Figure S2. SDS-PAGE analysis of whole-cell proteins of Module 2 expressed in *E. coli*.** Lane M: protein marker (kDa); Lane 1: *E. coli* (M2E).

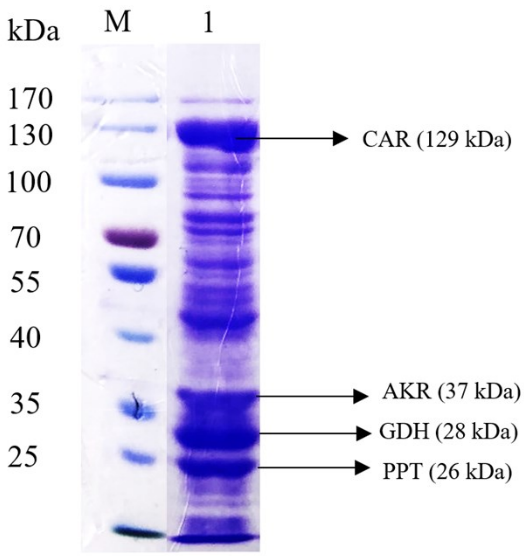


**Figure S3. SDS-PAGE analysis of whole-cell proteins of Module 3 expressed in *E. coli*.** Lane M: protein marker (kDa); Lane 1: *E. coli* (M3B).

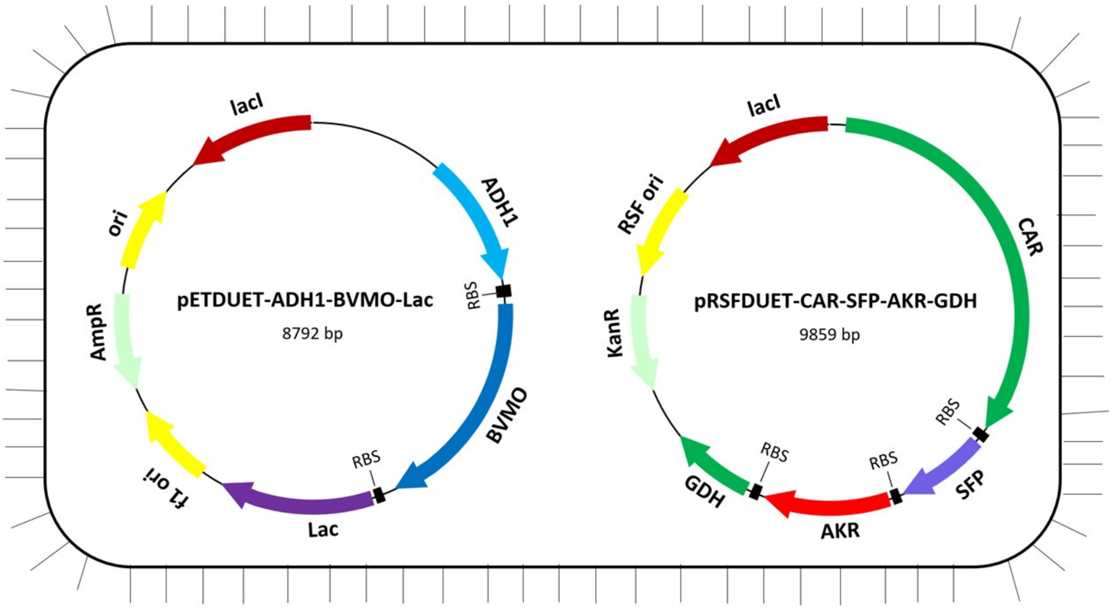


**Figure S4. Plasmid configuration of the *E. coli* cell expressing the enzymes of modules 2 and 3**

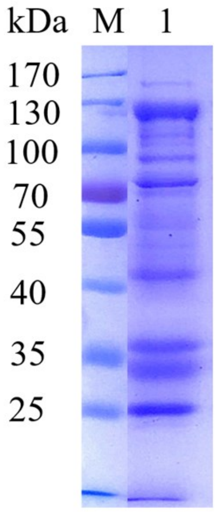


**Figure S5. SDS-PAGE analysis of whole-cell proteins of *E. coli* which expressing the enzymes of modules 2 and 3.** Lane M: protein marker (kDa); Lane 1: *E. coli* (M2E_M3B). Some protein bands are missed in the SDS-PAGE due to the imbalanced protein expression.

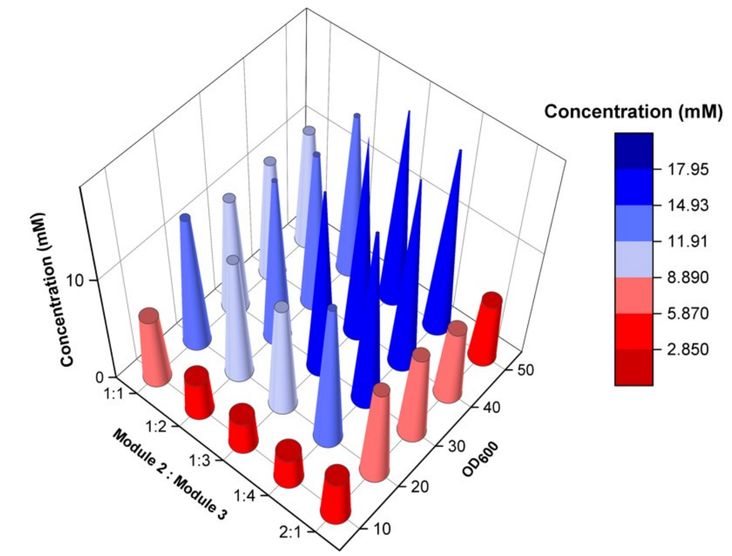


**Figure S6. Optimization of conditions for *E. coli* consortium 2_3 (EC2_3) catalyzed the conversion of 30 mM CHOL to HDO for 5 h.** The reaction conditions including the ratios of different modular cells and total cell density were likewise investigated. (Optimal function: modules 2:3 = 1:3, cell density of $OD_{600}$ = 40)

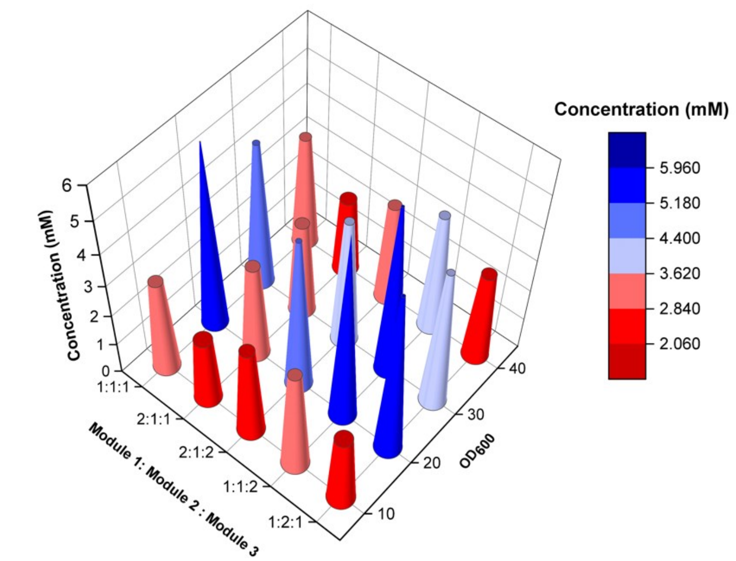


**Figure S7. Optimization of conditions for *E. coli* consortium 1_2_3 (EC1_2_3) catalyzed the conversion of 30 mM CH to HDO for 5 h.** The reaction conditions including the ratios of different modular cells and total cell density were likewise investigated. (Optimal function: modules 1:2:3 = 1:1:2, cell density of $OD_{600}$ = 20)

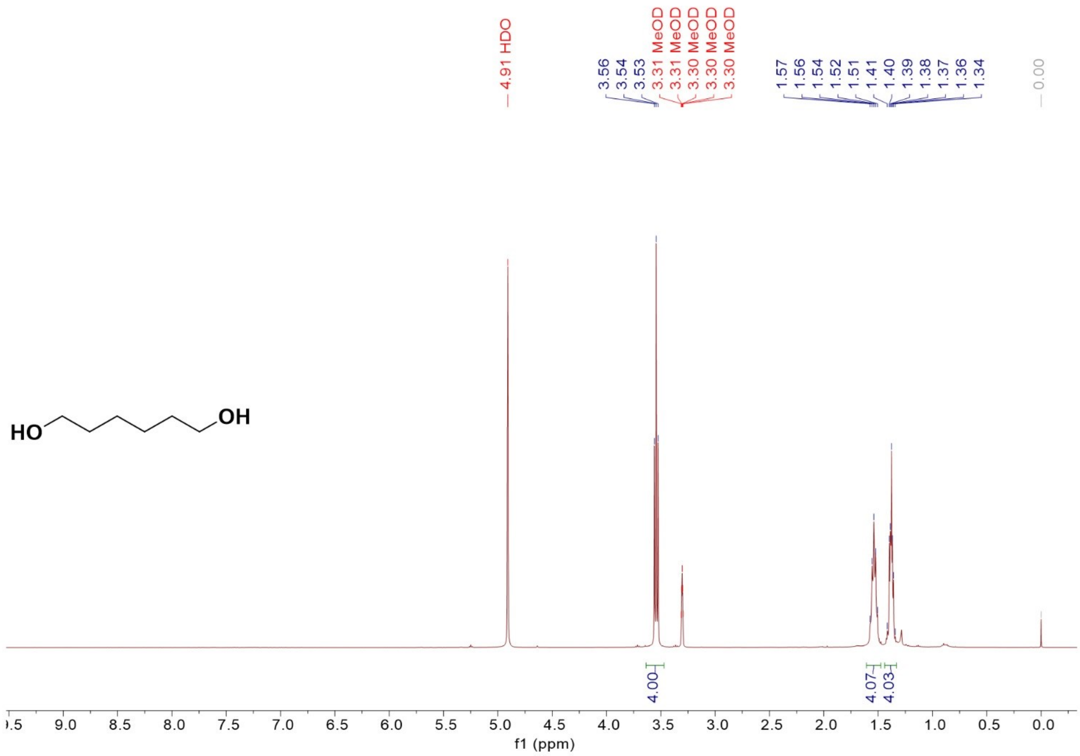


**Figure S8. NMR spectra of HDO.** 1,6-Hexanediol: $^{1}$H NMR (400 MHz, Methanol-$d_4$) δ 3.54 (t, $J$ = 6.6 Hz, 4H), 1.54 (m, 4H), 1.44 -1.33 (m, 4H).

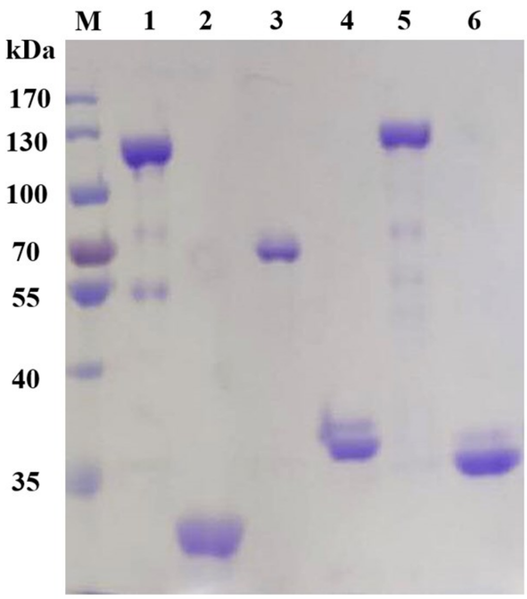


**Figure S9. SDS-PAGE analysis of purified proteins.** Lane M: protein marker (kDa); Lane 1: 19A12; Lane 2: ADH; Lane 3: BVMO; Lane 4: Lac; Lane 5: CAR; Lane 6: GDH.

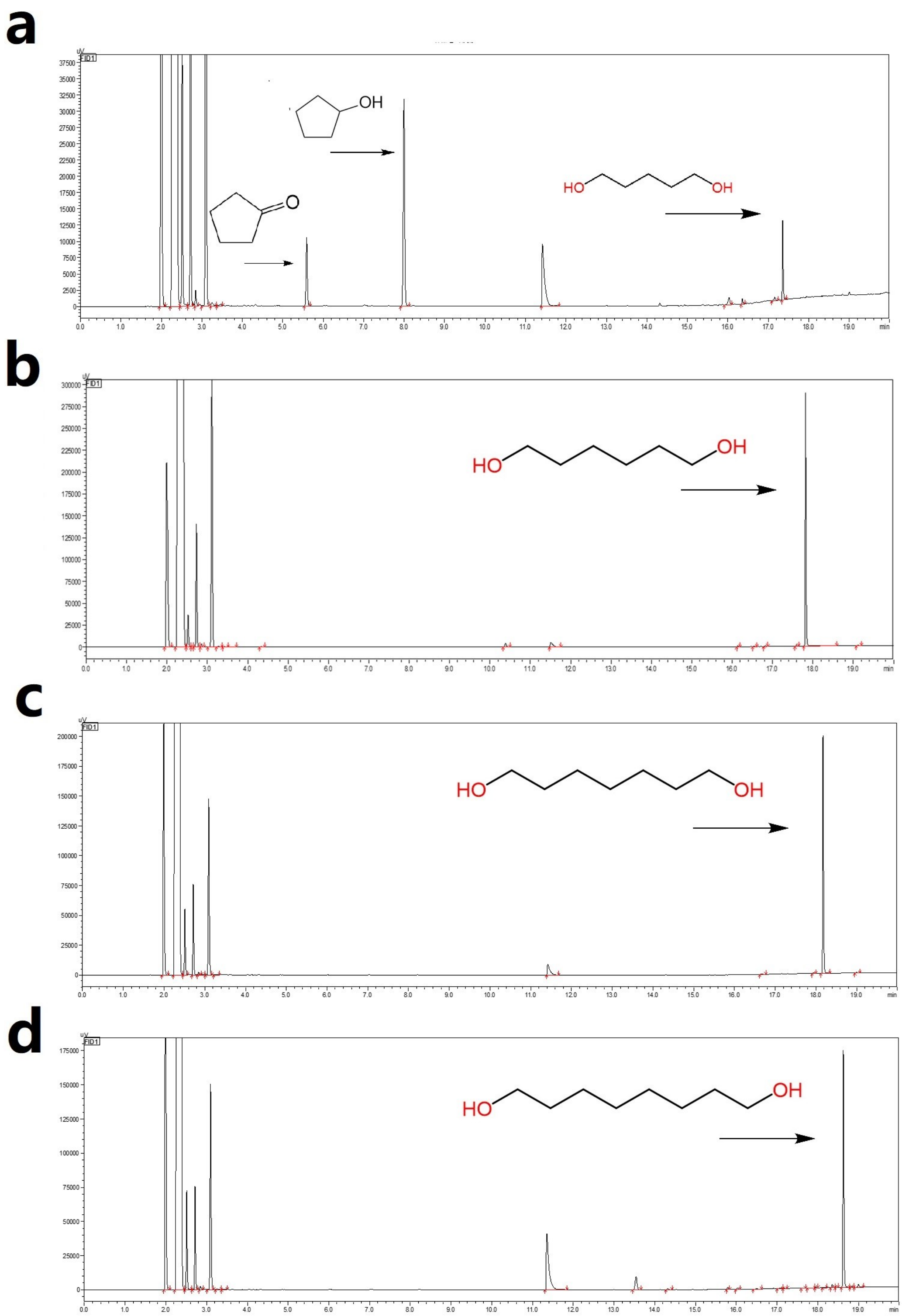


**Figure S10. GC analysis of reaction mixtures from *E. coli* consortium 2_3 catalyzed the conversion of (2a-2d) to (7a-7d) with SH-Rtx-WAX column.**

a: GC chromatograms of *E. coli* consortium 2_3 catalyzed cascade reactions of **2a** to **7a**.

b: GC chromatograms of *E. coli* consortium 2_3 catalyzed cascade reactions of **2b** to **7b**.

c: GC chromatograms of *E. coli* consortium 2_3 catalyzed cascade reactions of **2c** to **7c**.

d: GC chromatograms of *E. coli* consortium 2_3 catalyzed cascade reactions of **2d** to **7d**.

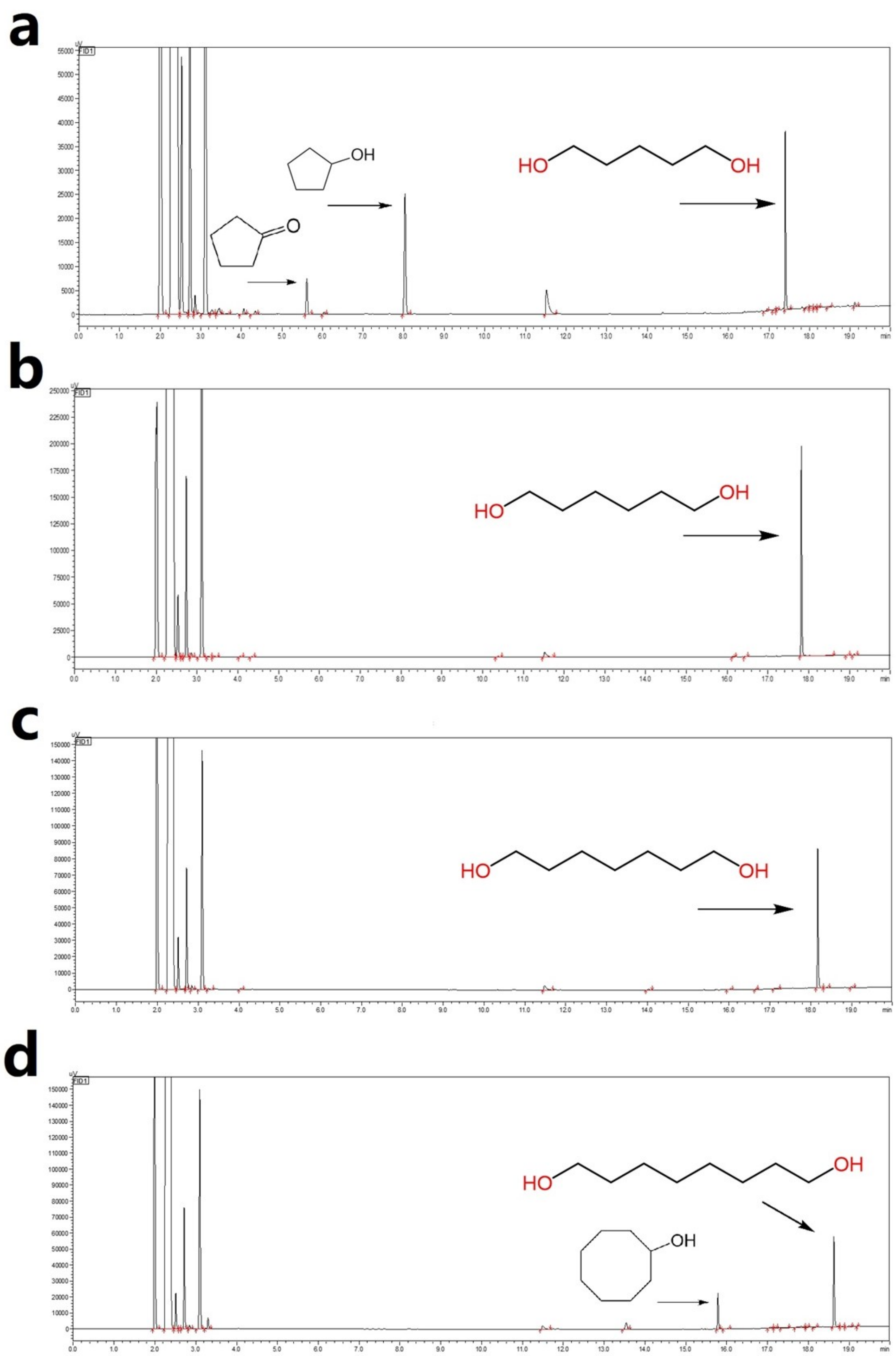


**Figure S11. GC analysis of reaction mixtures from *E. coli* consortium 1_2_3 catalyzed the conversion of (1a-1d) to (7a-7d) with SH-Rtx-WAX column.**

a: GC chromatograms of *E. coli* consortium 1_2_3 catalyzed cascade reactions of **1a** to **7a**.

b: GC chromatograms of *E. coli* consortium 1_2_3 catalyzed cascade reactions of **1b** to **7b**.

c: GC chromatograms of *E. coli* consortium 1_2_3 catalyzed cascade reactions of **1c** to **7c**.

d: GC chromatograms of *E. coli* consortium 1_2_3 catalyzed cascade reactions of **1d** to **7d**.

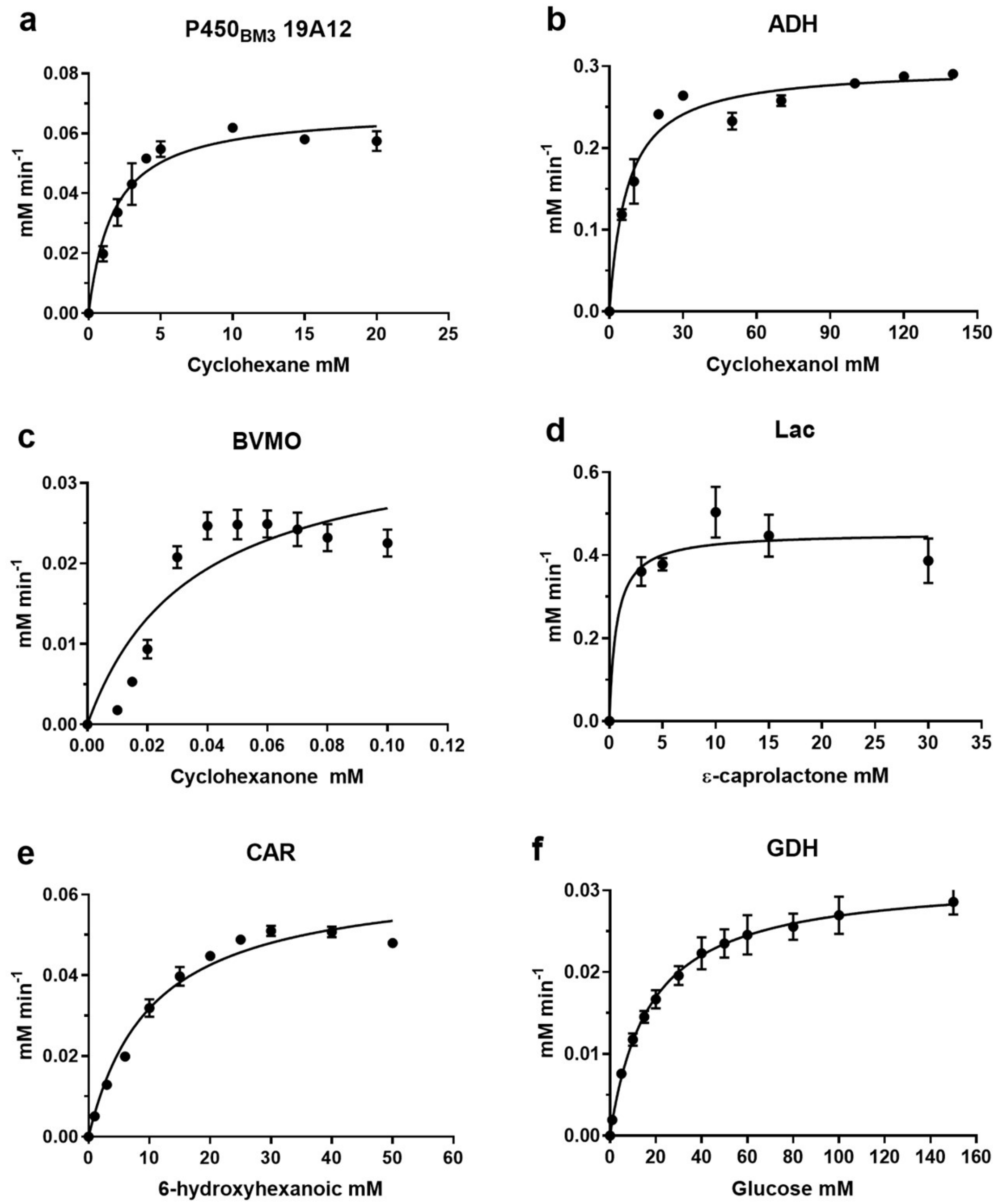


**Figure S12. Kinetic behavior of purified enzymes.**

## Synthetic gene sequences

### Glucose dehydrogenase (GDH) from *Bacillus megaterium*

ATGTATACAGATTTAAAAGATAAAGTAGTAGTAATTACAGGTGGATCAACAGGTTTA
GGACGCGCAATGGCTGTTCGTTTCGGTCAAGAAGAAGCAAAAGTTGTTATTAACTATT
ACAACAATGAAGAAGAAGCTTTAGATGCGAAAAAAGAAGTAGAAGAAGCAGGCGGA
CAAGCAATCATCGTTCAAGGCGACGTAACAAAAGAAGAAGATGTTGTAAACCTTGTT
CAAACAGCTATTAAAGAATTCGGTACATTAGACGTTATGATTAATAACGCTGGTGTTG
AAAACCCAGTTCCTTCTCATGAGTTATCTTTAGACAACTGGAATAAAGTTATTGATAC
AAACTTAACAGGTGCATTCTTAGGAAGCCGTGAAGCAATCAAATATTTTGTTGAAAA
CGACATTAAAGGAAACGTTATTAACATGTCTAGTGTTCATGAAATGATTCCTTGGCCA
TTATTTGTTCATTACGCAGCAAGTAAAGGCGGTATGAAACTAATGACGGAAACATTG
GCTCTTGAATATGCGCCAAAAGGTATCCGCGTAAATAACATTGGACCAGGTGCGATG
AACACACCAATTAACGCAGAGAAATTTGCAGATCCTGTACAACGTGCAGACGTAGAA
AGCATGATTCCAATGGGTTACATCGGTAAACCAGAAGAAGTAGCAGCAGTTGCAGCA
TTCTTAGCATCATCACAAGCAAGCTATGTAACAGGTATTACATTATTTGCTGATGGTG
GTATGACGAAATACCCATCATTCCAAGCAGGACGCGGATAA

### Alcohol dehydrogenase from *Acinetobacter* sp. NCIMB9871

ATGAGCAATCGTCTGGATGGTAAAGTTGCAATTATTACCGGTGGCACCTTAGGTATTG
GTCTGGCAATTGCAACCAAATTTGTTGAAGAGGGTGCCAAAGTTATGATTACCGGTC
GTCATAGTGATGTTGGTGAAAAAGCAGCAAAAAGCGTTGGTACACCGGATCAGATTC
AGTTTTTTCAGCATGATAGCAGTGATGAAGATGGTTGGACCAAACTGTTTGATGCAAC
CGAAAAAGCATTTGGTCCGGTTAGCACCCTGGTTAATAATGCAGGTATTGCAGTGAA
TAAGAGCGTTGAAGAAACCACCACCGCAGAATGGCGTAAACTGCTGGCAGTTAATCT
GGATGGCGTTTTTTTTGGTACACGTCTGGGTATTCAGCGCATGAAAAACAAAGGTCTG
GGTGCAAGCATTATCAACATGAGCAGCATTGAAGGTTTTGTTGGTGATCCGAGCCTG
GGTGCATATAATGCAAGCAAAGGTGCAGTTCGTATTATGAGCAAAAGCGCAGCACTG
GATTGTGCACTGAAAGATTATGATGTTCGTGTGAATACCGTTCATCCGGGTTATATCA
AAACACCGCTGGTTGATGATCTGCCTGGTGCCGAAGAAGCAATGAGCCAGCGTACAA
AAACCCCGATGGGTCATATTGGTGAACCGAATGATATTGCCTATATCTGTGTTTATCT
GGCCAGCAACGAAAGTAAATTTGCAACCGGTAGCGAATTTGTTGTGGATGGTGGTTA
TACCGCACAGTAA

### Baeyer-Villiger monooxygenase (BVMO) from *Acinetobacter* sp. NCIMB9871

ATGTCACAAAAAATGGATTTTGATGCTATCGTGATTGGTGGTGGTTTTGGCGGACTTT

ATGCAGTCAAAAAATTAAGAGACGAGCTCGAACTTAAGGTTCAGGCTTTTGATAAAG
CCACGGATGTCGCAGGTACTTGGTACTGGAACCGTTACCCAGGTGCATTGACGGATA
CAGAAACCCACCTCTACTGCTATTCTTGGGATAAAGAATTACTACAATCGCTAGAAAT
CAAGAAAAAATATGTGCAAGGCCCTGATGTACGCAAGTATTTACAGCAAGTGGCTGA
AAAGCATGATTTAAAGAAGAGCTATCAATTCAATACCGCGGTTCAATCGGCTCATTA
CAACGAAGCAGATGCCTTGTGGGAAGTCACCACTGAATATGGTGATAAGTACACGGC
GCGTTTCCTCATCACTGCTTTAGGCTTATTGTCTGCGCCTAACTTGCCAAACATCAAA
GGCATTAATCAGTTTAAAGGTGAGCTGCATCATACCAGCCGCTGGCCAGATGACGTA
AGTTTTGAAGGTAAACGTGTCGGCGTGATTGGTACGGGTTCCACCGGTGTTCAGGTTA
TTACGGCTGTGGCACCTCTGGCTAAACACCTCACTGTCTTCCAGCGTTCTGCACAATA
CAGCGTTCCAATTGGCAATGATCCACTGTCTGAAGAAGATGTTAAAAAGATCAAAGA
CAATTATGACAAAATTTGGGATGGTGTATGGAATTCAGCCCTTGCCTTTGGCCTGAAT
GAAAGCACAGTGCCAGCAATGAGCGTATCAGCTGAAGAACGCAAGGCAGTTTTTGAA
AAGGCATGGCAAACAGGTGGCGGTTTCCGTTTCATGTTTGAAACTTTCGGTGATATTG
CCACCAATATGGAAGCCAATATCGAAGCGCAAAATTTCATTAAGGGTAAAATTGCTG
AAATCGTCAAAGATCCAGCCATTGCACAGAAGCTTATGCCACAGGATTTGTATGCAA
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AGAAGATGTGAAAGCCAATCCGATTGTTGAAATTACCGAAAACGGTGTGAAACTCGA
AAATGGCGATTTCGTTGAATTAGACATGCTGATACTGGCCACAGGTTTTGATGCCGTC
GATGGCAACTATGTGCGCATGGACATTCAAGGTAAAAACGGCTTGGCCATTAAAGAC
TACTGGAAAGAAGGTCCGTCGAGCTATATGGGTGTCACCGTAAATAACTATCCAAAC
ATGTTCATGGTGCTTGGACCGAATGGCCCGTTTACCAACCTGCCGCCATCAATTGAAT
CACAGGTGGAATGGATCAGTGATACCATTCAATACACGGTTGAAAACAATGTTGAAT
CCATTGAAGCGACAAAAGAAGCGGAAGAACAATGGACTCAAACTTGCGCCAATATTG
CGGAAATGACCTTATTCCCTAAAGCGCAATCCTGGATTTTTGGTGCGAATATCCCGGG
CAAGAAAAACACGGTTTACTTCTATCTCGGTGGTTTAAAAGAATATCGCAGTGCGCTA
GCCAACTGCAAAAACCATGCCTATGAAGGTTTTGATATTCAATTACAACGTTCAGATA
TCAAGCAACCTGCCAATGCCTAA

**Lactonase from *Rhodococcus* sp. HI-31**

ATGACCAATATTAGCGAAACCCTGAGCACCGCACCTGGTGGTGCAGCAGGTCCGGAT
GTTCTGCGTGATCTGTATGCAGATTGGAGCGAAATTATGGCAGCAACACCGGATCTG
ACCATTCGTCTGCTGCGTAGCCTGTTTGATGAATGGCATCAGCCGACCGTTGAACCGG
AAGGTGTTACCTATCGTGAAGAAACCGTTGGTGGTGTTCCTGGTATTTGGTGTCTGCC
GCAGGGTGCAGATGGTAGCAAAGTTCTGCTGTATACCCATGGTGGTGGTTTTGCAGTT

GGTAGCGCAGCAAGCCATCGTAAACTGGCAGGTCATGTTGCAAAAGCACTGGGTGCC
GTTGGTTTTGTTCTGGATTATCGTCGTGCACCGGAATTTCAGCATCCGGCACAGATTG
AAGATGGTGTTGCAGCATTTGATGCACTGGTTGCAAATGGTATTGCACCGCAGGATAT
TACCACCATTGGTGATAGTGCCGGTGGTAATCTGGCAGTTGCAATTGCCCTGAGCCTG
CGTGAACAGGGTAAACAAGGTCCGGGTAGCGTTATTGCATTTAGCCCGTGGCTGGAT
ATGGAAAATAAAGGTGAAACCCTGGCCACCAATAATGATACCGATGCACTGATTACA
CCGGAACTGCTGGAAGGCATGATTGCCGGTGTGCTGGGTGATACCATTGATCCGAAA
ACACCGCTGGCAAATCCGCTGTATGCCGATTTTACCGGTTTTCCGCGTCTGTATATCA
CCGCAGGTAGCGTTGAAAGCCTGCTGGATAATGCAACCCGTCTGGAAAAATTAGCAG
CATCTGCCGGTGTTGATGTTACCCTGAGTATTGGTGAAGGTCAGCAGCATGTTTATCC
GTTTCTGGCAGGCCGTAGCGCACTGGTGGATGATGAATTTGCAAAGCTGGCAGCATG
GTATCAGAAAGCCCTGGAATAA

**Carboxylic acid reductase from *Mycobacterium abscessus* ATCC 19977**

ATGACCGAAACCATTAGCACCGCAGCAGTTCCGACCACCGATCTGGAAGAACAGGTT
AAACGTCGTATTGAACAGGTTGTTAGCAATGATCCGCAGCTGGCAGCCCTGCTGCCG
GAAGATAGCGTTACCGAAGCAGTTAATGAACCGGATCTGCCGCTGGTTGAAGTTATT
CGTCGTCTGCTGGAAGGTTATGGTGATCGTCCGGCACTGGGTCAGCGTGCATTTGAAT
TTGTTACCGGTGATGATGGTGCAACCGTTATTGCACTGAAACCGGAATACACCACCGT
TAGCTATCGTGAACTGTGGGAACGTGCCGAAGCAATTGCAGCAGCATGGCATGAACA
GGGTATTCGTGATGGTGATTTTGTTGCACAGCTGGGTTTTACCAGCACCGATTTTGCA
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CCAGCCTGCAGCAGCGTAATGCAATCCTGGAAGAAACCCGTCCGGCAGTTTTTGCAG
CAAGCATTGAATATCTGGATGCAGCAGTTGATAGCGTTCTGGCAACCCCGAGCGTGC
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GAAGCGCTGGCACGTGGTCGTGATCTGCCAGCAGCACCGCTGCCGAGTGCCGATCCG
GATGCACTGCGCCTGCTGATTTATACCAGCGGTAGCACCGGTACACCGAAAGGTGCC
ATGTATCCGCAGTGGCTGGTTGCAAATCTGTGGCAGAAAAAATGGCTGACCGATGAT
GTTATTCCGAGCATTGGTGTGAATTTTATGCCGATGAGCCATCTGGCAGGTCGTCTGA
CCCTGATGGGCACCCTGAGCGGTGGTGGCACCGCCTATTATATCGCAAGCAGCGATC
TGAGCACCTTTTTTGAAGATATTGCCCTGATTCGTCCGAGCGAAGTTCTGTTTGTTCCG
CGTGTTGTGGAAATGGTTTTTCAGCGTTTTCAGGCAGAACTGGATCGTAGCCTGGCTC
CGGGTGAAAGCAATAGCGAAATTGCAGAACGTATTAAAGTGCGTATTCGCGAACAGG
ATTTTGGTGGTCGTGTGCTGAGCGCAGGTAGCGGTAGTGCACCGCTGAGTCCGGAAA

TGACCGAATTTATGGAAAGCCTGCTGCAAGTGCCGCTGCGTGATGGTTATGGTAGTAC
CGAAGCCGGTGGTGTTTGGCGTGATGGCGTTCTGCAGCGTCCGCCTGTTACCGATTAT
AAACTGGTTGATGTGCCGGAACTGGGTTATTTACCACCGATAGTCCGCATCCGCGTG
GTGAACTGCGTCTGAAAAGCGAAACCATGTTTCCGGGTTATTACAAACGTCCGGAAA
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GTTCGTCAGATTTTTGTTTATGGTAATAGCGAACGTAGCTTTCTGCTGGCCGTTGTTGT
TCCGACACCGGAAGTTCTGGAACGTTATGCAGATAGTCCGGATGCGCTGAAACCGCT
GATTCAGGATAGTCTGCAGCAGGTTGCAAAAGATGCAGAACTGCAGAGCTATGAAAT
TCCGCGTGATTTTATTGTTGAAACCGTTCCGTTTACCGTTGAAAGCGGACTGCTGAGT
GATGCACGTAAACTGTTACGTCCGAAACTGAAAGATCATTATGGTGAACGCCTGGAA
GCCCTGTATGCCGAACTGGCAGAAAGCCAGAATGAACGTCTGCGTCAGCTGGCACGC
GAAGCAGCAACACGTCCGGTTCTGGAAACCGTTACCGATGCCGCAGCCGCACTGCTG
GGTGCAAGCAGCTCCGATCTGGCACCAGATGTTCGTTTTATTGATTTAGGTGGTGATA
GCCTGAGCGCACTGAGCTATAGCGAGCTGCTGCGCGATATTTTTGAAGTTGATGTTCC
GGTTGGTGTGATTAATAGCGTTGCAAATGATCTGGCAGCAATTGCCCGTCATATTGAA
GCACAGCGTACAGGTGCAGCAACCCAGCCGACCTTTGCAAGCGTTCATGGTAAAGAT
GCCACCGTTATTACCGCAGGCGAACTGACCCTGGATAAATTTCTGGATGAAAGTCTGC
TGAAAGCAGCCAAAGATGTTCAGCCTGCGACAGCAGATGTTAAAACCGTGCTGGTGA
CCGGTGGTAATGGCTGGTTAGGTCGTTGGCTGGTTCTGGATTGGCTGGAACGTCTGGC
ACCGAATGGTGGTAAAGTTTATGCACTGATTCGTGGTGCAGATGCGGAAGCAGCACG
CGCACGCCTGGATGCCGTTTATGAAAGCGGTGATCCTAAACTGAGTGCACATTATCGT
CAACTGGCCCAGCAGAGCCTGGAAGTTATTGCAGGCGATTTTGGCGATCAGGATCTG
GGTCTGAGCCAAGAAGTTTGGCAGAAACTGGCGAAAGATGTTGATCTGATTGTTCAT
AGCGGTGCCCTGGTTAATCATGTTCTGCCGTATAGCCAGCTGTTTGGTCCGAATGTTG
CCGGTACAGCAGAAATTATCAAACTGGCAATTAGCGAACGCCTGAAACCTGTTACCT
ATCTGAGTACCGTTGGTATTGCAGATCAGATTCCGGTTACCGAATTTGAAGAGGATAG
TGATGTTCGCGTTATGAGCGCAGAACGTCAGATTAATGATGGCTATGCAAATGGCTAT
GGCAATAGCAAATGGGCTGGTGAAGTTCTGCTGCGTGAAGCCCATGATTTAGCCGGT
CTGCCGGTTCGTGTTTTTCGTAGCGATATGATTCTGGCACATAGCGATTATCACGGTC
AGCTGAATGTGACCGATGTTTTTACCCGTAGCATTCAGAGTCTGCTGCTGACAGGTGT
TGCACCGGCAAGTTTTTATGAACTGGATGCGGATGGTAATCGCCAGCGTGCCCATTAT
GATGGTGTTCCAGGTGATTTTACCGCAGCCAGCATTACCGCAATTGGTGGTGTTAATG
TTGTGGATGGTTATCGCAGCTTTGATGTGTTTAATCCGCATCACGATGGTGTTAGCAT

GGATACCTTTGTTGATTGGCTGATTGATGCCGGTTACAAAATTGCACGCATCGATGAT
TATGATCAGTGGTTAGCACGTTTTGAACTGGCCCTGAAAGGCCTGCCTGAACAGCAG
CGTCAGCAGAGCGTTCTGCCACTGCTGAAAATGTATGAAAAACCGCAGCCTGCAATT
GATGGTAGCGCACTGCCGACCGCAGAATTTAGCCGTGCAGTTCATGAAGCAAAAGTG
GGTGATAGTGGTGAAATCCCGCATGTTACCAAAGAACTGATTCTGAAATATGCCAGC
GATATTCAGCTGCTGGGTTTAGTTTAA

**Phosphopantetheinyl transferase from *Bacillus subtilis***

ATGAAAATCTATGGCATCTATATGGATCGTCCGCTGAGCCAAGAAGAAAACGAACGT
TTTATGAGCTTTATCAGTCCGGAAAAACGTGAAAAATGCCGTCGCTTTTATCACAAAG
AAGATGCACATCGTACCCTGCTGGGTGATGTTCTGGTTCGTAGCGTTATTAGCCGTCA
GTATCAGCTGGATAAATCCGATATTCGTTTTAGCACCCAAGAATATGGTAAACCGTGT
ATTCCGGATCTGCCGGATGCACATTTTAACATTAGCCATAGCGGTCGTTGGGTTATTT
GTGCATTTGATAGCCAGCCGATTGGCATTGATATCGAAAAAACCAAACCGATCAGCC
TGGAAATTGCCAAACGTTTTTTTAGCAAAACCGAGTATAGCGATCTGCTGGCCAAAG
ATAAAGATGAACAGACCGATTATTTCTATCATCTGTGGTCCATGAAAGAGAGCTTCAT
TAAACAAGAAGGTAAAGGTCTGAGCCTGCCGCTGGATAGCTTTAGCGTTCGTCTGCA
TCAGGATGGTCAGGTTAGCATTGAACTGCCGGATAGCCATAGTCCGTGTTATATCAAA
ACCTATGAAGTTGATCCGGGTTACAAAATGGCAGTTTGTGCAGCACATCCGGATTTTC
CGGAAGATATTACCATGGTTAGCTATGAAGAACTGCTGTAA

**Aldo-keto reductase from *Pseudomonas putida***

ATGCAGTATGTTAAACTGGGTAGCACCGGTCTGGATATTAGCCGTCTGTGTCTGGGTT
GTATGACCTTTGGTGAACCGGATGCAGGCACCCATCCGTGGACACTGGGTGAAGATG
CAAGCCGTCCGATTATTCGTCATGCAGTTGAACAGGGCATCAACTTTTTTGATACCGC
AAATAGCTATAGCGCAGGCACCAGCGAAATCATTCTGGGTAAACTGCTGCGTGAATT
TACCCGTCGTGAAGAAACCGTTATTGCCACCAAAGTTTTTTTCCGGCAAATATGTGG
GAAGGTGCATCACGTCCGAATGAACAGGGTCTGAGCCGTAAAGCAATTATGGCAAAT
ATTGATGCCAGCCTGAGCCGTCTGGGCACCGATTATGTTGATCTGTATCAGATTCATC
GTTGGGATTATCATACCCCGATTGAAGAAACAATGGAAGCACTGCATGATGTTGTTA
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ATCTGAACCTGATCTATCGTGAAGAGGAACGTGAAATGATTCCTCTGTGCCTGGATCA
AGGTGTTGGTCTGATGCCGTGGTCACCGATGGCACGTGGTCGTCTGACCCGTCCGCAT
GGTCAGCAGACCCAGCGTACCCGTACCGATGTTAGCGGTCAGAGCTTTTATGAAAAA

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GGTGTTCCGATGGCGCAGGTTGCCCTGGCATGGGTTCTGGGTCGTGCCGGTGTTAGCG
CACCGATTGTTGGTGCCAGCAAACCGGCACATCTGGATGATGCACTGGGTGCACTGA
GCCTGCAGCTGAGCGAAGATGAAGTTGCACGTCTGCAAGCACCGTATGTTCCGCATG
CAGTTACCGGTTTTAAATGA

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