

## Supporting Information

### **One-pot Biocatalytic Synthesis of Nylon Monomers from Cyclohexanol Using *Escherichia coli*-based Concurrent Cascade Consortia**

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## Table of Contents

Experimental enzymes.....	3
Bacterial Strains and Plasmid Construction .....	5
Chemicals and Media .....	5
Enzyme preparation .....	5
Site-directed mutagenesis:.....	22
Isolation and Purification of final products .....	28
Analytical Conditions: .....	29
Spectroscopic analysis: .....	33
References .....	36

## Experimental enzymes

**Table S1.** List of enzymes used in this study.

Enzyme	Organism	Recombinant <i>E. coli</i> Strain		Sequence	Plasmid	Ref.	
ADH	<i>Lactobacillus brevis</i>	<i>E. coli</i> (M1A)	F	ATTA CATATG AGTAATCGTCTAGATG	pET24ma	This study	
			R	ATTA CTCGAG CTA CTGCGCAGTATAG			
AcCHMO	<i>Acinetobacter calcoaceticus</i>	<i>E. coli</i> (M1B)	F	ATTA CATATGTCTCAGAAAATGGATTC	pET24ma	This study	
			R	ATTA GGATCCCGCGTTAGCCGGCTGTTT			
TmCHMO	<i>Thermocrisium municipale</i> DSM 44069	-	F	aaggagatatacatatgTCGACCACCCAGACCCCG	pET24ma	This study	
			R	gg <sup>t</sup> gg <sup>t</sup> gg <sup>t</sup> gctcagTGCAACTGCCTGCGCacg			
Lactonase	<i>Rhodococcus</i> sp. HI-31	<i>E. coli</i> (M1C)	F	ATTA CATATGACCAATATTAGCGAAACC	pET24ma	This study	
			R	ATTA GGATCCTTACTCGAGTGCTTTCTG			
			F	ATTA CATATGACCAATATTAGCGAAACC	pETDuet1 and pCDFDuet1		
			R	ATTA GGTACCTTACTCGAGTGCTTTCTG			
AHR	<i>Synechocystis</i> species	<i>E. coli</i> (M2A)	F	ATTACATATGATGATTAAGCCTACGCTG	pET24ma		
			R	ATTACTCGAGATTTTTACTATGGCTGAG			
			F	ATTAAGGATCCATGATTAAGCCTACGCT	pETDuet1 and pCDFDuet1		
			R	ATTAGAGCTCATTTTTACTATGGCTGAG			
SPTA	<i>Silicibacter pomeroyi</i>	<i>E. coli</i> (M2B)	F	ATATCATATGATGGCTACTATCACCAACCA CATG	pET24ma and pCDFduet1	[1]	
			R	ATATCTCGAGTTAGGCGCTTTTCATCAGGC			
F_SPTA-AHR	-	<i>E. coli</i> (M2C)	SPTA	F	ATTACATATGATGGCTACTATCACCAACCA CATGC	pET24ma	This study
				R	ACTACCTCCACCTCCGGCGCTTTTCATC		
			AHR	F	GCGCCGGAGGTGGAGGTAGTATGATTAA AGC		
				R	ATTACTCGAGTTAATTTTTACTATGGCTGA		

MaCAR	<i>Mycobacterium abscessus</i>	<i>E. coli</i> (M3A)	-	Gene synthesis	pETDuet1	This study
McCAR	<i>Mycobacterium chelonae</i>	<i>E. coli</i> (M3B)	-	Gene synthesis	pETDuet1	This study
MmCAR	<i>Mycobacterium marinum</i>	<i>E. coli</i> (M3C)	-	Gene synthesis	pETDuet1	This study
MaCAR_L 342E	<i>Mycobacterium abscessus</i>	<i>E. coli</i> (M3D)	F	ATTACATATGGAGCAGGTGAAGCGACGC	pET24ma	This study
			R	ATTACTCGAGCGCCAGTTCGGCGTACAG		
Cy- EK_Y152A	<i>Enterobacter kobei</i>	<i>E. coli</i> (M3E)	F	GAAAAAAGTGCAGCTGTGGCCGCA	pET24ma	This study
			R	TGCGGCCACAGCTGCACTTTTTTC		
Cy- EK_Y152A /S139C	<i>Enterobacter kobei</i>	<i>E. coli</i> (M1F)	F	ATTATTAATATTGCATGTGTTTCATGGC	pET24ma	This study
			R	GCCATGAACACATGCAATATTAATAAT		
Cy- CF_Y152A /S139C	<i>Citrobacter freundii</i>	<i>E. coli</i> (M1G)	F	ATTAATATTGCCTGTGTTTCATGGT	pET24ma	This study
			R	ACCATGAACACAGGCAATATTAAT		
Cy- Pst_Y152 A/S139C	<i>Pseudomonas stutzeri</i>	<i>E. coli</i> (M1H)	F	ATTAATGTTGCCTGTGTTTCATGGT	pET24ma	This study
			R	ACCATGAACACAGGCAACATTAAT		

## Bacterial Strains and Plasmid Construction

All bacterial strains and plasmid vectors used in this study are listed in Table 1. The concentration of DNA was quantified at 260 nm using a Nanodrop (ND-1000 spectrophotometer; Thermo Fisher Scientific, DE, USA). Each gene sequences were accessed from National Center for Biotechnology Information (NCBI). The genes encoding desired enzymes (listed in Table 1) were synthesized and codon optimized for *E. coli* from Bionics (Seoul, Korea). The genes for cloning were amplified from genomic DNA using oligonucleotides by PCR thermocycler. All primers used in this study were also purchased from Bionics (Seoul, Korea)

## Chemicals and Media

All chemicals, such as, cyclohexanol,  $\epsilon$ -caprolactam, 5 amino pentanoic acid, 6-hydroxy hexanoic acid (C6), cyclohexanone, cyclohexylamine,  $\epsilon$ -caprolactone, benzylamine, pyridine, NAD<sup>+</sup>, NADP<sup>+</sup>, isopropyl-thio- $\beta$ -D-galactopyranoside (IPTG), N, O-Bis (trimethylsilyl)-trifluoroacetamide (BSTFA) propylamine and all other aliphatic amine were purchased from Sigma-Aldrich (St. Louis, MO, USA), 6-aminohexanoic acid was purchased from alfa aesar, valerolactam was purchased from Tokyo chemical industry (TCI), Chloroform was obtained from Junsei (Tokyo, Japan). Bacteriological agar, Luria Bertani (LB) broth and terrific broth (TB) media were purchased from BD Difco (Franklin Lakes, NJ, USA). All chemicals used in this study were of analytical grade.

## Enzyme preparation

### Representative procedure for expression and purification of biocatalysts

For expression, transformed *E. coli* BW25113,  $\Delta$  fadD cells were grown in a 500 mL flask supplemented with LB/ medium (10 mL) at 37 °C, 200 rpm for 16 h. A sample (1 mL) of this culture was used to inoculate the experimental LB medium (400 mL) with desired antibiotic marker. The culture was shaken at 37 °C 200 rpm for 2 h and the induced when cell OD<sub>600</sub> reached at 0.6. IPTG solution (20  $\mu$ L, 500 mM stock) was added per 100 mL culture. The cultures were shaken overnight at 20 °C, 120 rpm. Cells were harvested by centrifugation (5000 rpm, 10 min, 4°C), washed with Tris-HCl buffer (100 mM, pH 8.0), centrifuged (5000 rpm, 10 min, 4 °C) and resuspended in Tris-HCl buffer (100 mM, pH 8.0).

### For Purified biocatalysts

The cells were grown as described above and subjected to sonication, the disrupted cells were centrifuged, and supernatant was transferred through 2  $\mu$ m fluted filter into new falcon tube (50 mL). after which the sample was loaded into charged nickel affinity column with flow rate of 1.5 mL/min. the column was washed with 100 mL of washing buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl and 20 mM imidazole) with flow rate of 2 mL/min. Next, the column was eluted by using elution buffer 1 (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl and 250 mM imidazole) with flow rate of 2 mL/min to obtain desired protein (first 5 mL sample was discarded and next 20 mL sample was collected), after this the collected sample was filtered through desired protein filter and kept overnight for

dialysis. after dialysis, the protein sample was concentrated, and protein concentration was measured by using standard BSA curve.

**Cyclohexanone Monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 (variant C376L M400I) (AcCHMO)<sup>[2]</sup>**

Codon optimized DNA sequence is given below:

```
ATGTCTCAGAAAATGGATTTGACGCTATCGTTATCGGTGGTGGTTTCGGTGGTCTGTACGCGGTTAAAA
AACTGCGTGATGAACTGGAAGTTCAGGCATTCGATAAAGCGACCGACGTAGCGGGTACCTGG
TACTGGAACCGTTACCCAGGTGCTCTGACTGACACCGAAACCCACCTGTACTGTTACTCCTGGGATAAAG
AACTGCTGCAGTCTCTGGAATCAAGAAAAAATACGTGCAGGGTCCGGATGTTTCGTAATACCTGCAGC
AGGTGGCTGAAAAACACGATCTGAAAAAATCCTACCAGTTCAACACCGCAGTTCAGTCTGCACACTACA
ACGAAGCTGACGCACTGTGGGAAGTTACCACTGAATACGGCGATAAATACACCGCGCGTTTCCTGATCA
CTGCGCTGGGTCTGCTGTCTGCGCCGAACCTGCCGAACATCAAAGGTATCAACCAGTTCAAAGGTGAAC
TGCACCACACCAGCCGTTGGCCGGATGACGTTTCTTTCGAAGGTAAACGTGTTGGTGTATCGGCACCG
GTAGCACCGGTGTTCAGGTTATTACCGCTGTTGCTCCGCTGGCTAAACACCTGACCGTTTTCCAGCGTTC
TGCTCAGTACTCTGTTCCGATCGGTAACGATCCGCTGAGCGAAGAAGATGTAAAGAAAATTAAGACAA
CTACGACAAAATCTGGGACGGTGTGGAACTCTGCTCTGGCGTTCGGTCTGAACGAATCTACCGTTCGG
GCTATGTCTGTTTCCGCTGAAGAACGTAAAGCAGTTTTTCGAAAAGCTTGGCAGACCGGTGGCGGTTTC
CGTTTCATGTTTCGAAACCTTCGGCGATATCGCTACCAACATGGAAGCTAACATCGAAGCGCAGAACTTCA
TCAAAGGTAAAATCGCAGAAATCGTTAAAGATCCGGCAATCGCGCAGAAACTGATGCCGCAGGACCTGT
ACGCTAAACGTCCGCTGTGCGACTCCGGCTACTACAACACCTTCAACCGTGACAACGTTTCGCTGGAAG
ACGTGAAAGCTAACCCGATCGTTGAAATCACCGAAAACGGTGTAAACTGGAAAACGGTGACTTCGTTG
AACTGGATATGCTGATCCTGGCGACCGGTTTCGACGCGGTTGACGGCAACTACGTTTCGTATGGATATCC
AGGGTAAAACGGTCTGGCTATCAAAGACTACTGGAAAGAAGGCCCGTCTTCTTACATGGGTGTTACCG
TTAACAACCTACCCGAACATGTTTCATGGTTCTGGGCCGAACGGTCCGTTACCAACCTGCCGCCGTCCAT
CGAATCCAGGTTGAATGGATCTCTGACACTATCCAGTACACCGTTGAAAACAACGTTGAATCTATCGAA
GCAACCAAAGAAGCTGAAGAACAGTGGACCCAGACCTGCGCGAACATCGCGGAAATGACCCTGTTCCC
GAAAGCGCAGTCTGGATCTTCGGTGCTAACATTCCGGGCAAAAAGAACACCGTTTACTTCTACCTGGG
TGGCCTGAAAGAATACCGTAGCGCTCTGGCTAACTGCAAAAACCACGCTTACGAAGGTTTCGACATCCA
GCTGCAGCGTTCTGACATTAACAGCCGGCTAACGCG
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**Cyclohexanone Monooxygenase from *Thermocrispum municipale* DSM 44069 (TmCHMO)<sup>[3]</sup>**

Codon optimized DNA sequence is given below:

```
ATGTCGACCACCCAGACCCCGGATCTGGATGCGATCGTAATTGGTGCGGGATTCGGCGGTATTTATATG
CTGCATAAACTGCGTAACGATCTGGGTCTGTCTGTTTCGTGTGTTTCGAAAAGGGTGGCGGTGTAGGTGGT
ACCTGGTACTGGAACAAATACCCAGGTGCTAAATCTGATACCGAAGGCTTCGTATACCGTTATAGCTTTG
ACAAAGAAGTCTGCGTGAATACGATTGGACACCCGTTACCTGGATCAGCCGGACGTTCTGGCGTACC
TGGAACACGTTGTTGAACGTTACGACCTGGCTCGTGATATCCAGCTGAACACCGAAGTTACTGATGCGA
TCTTCGATGAAGAAACCGAAGTGTGGCGTGTACCACCGCGGGTGGCGAAACGCTGACTGCGCGCTTCC
TGGTGACCGCGCTGGGCCTGCTGAGCCGCTCTAACATCCCGGATATTCCGGGTCTGATTCTTTCGACG
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GCCGTCTGGTGCACACCAACGCGTGGCCGGAAGATCTGGATATCACCGGTAAACGCGTTGGTGTGATC  
GGTACCGGTAGTACCGGCACCCAGTTCATCGTTGCGGCGGCAAAAATGGCGGAACAGCTGACCGTTTTTC  
CAGCGTACCCCGCAGTACTGCGTTCCGTCTGGCAATGGCCCGATGGATCCGGATGAAGTTGCGCGTATC  
AAACAGAACTTCGATAGCATTGGGATCAGTTTCGTTCTTCTACCGTGGCGTTCGGCTTCGAAGAAAGC  
ACCGTTGAAGCGATGTCTGTTTCTGAATCTGAACGTCAGCGTGTTCAGCAGGCGTGGGACAAAGGT  
AACGGCTTCCGTTTCATGTTCCGGTACCTTCTGCGATATCGCTACCAACCCGGAAGCTAACGCGGCAGCTG  
CGGCTTTCATTGCTCTAAAATCGCTGAAATCGTTAAAGACCCGGAACCGCCCGTAAACTGACCCCGAC  
CGATCTGTACGCGAAACGCCCGCTGTGCAACGAAGTTACTATGAAACCTACAACCGCGACAACGTTTC  
TCTGGTTAGCCTGAAAGAAACCCCGATCGAAGAAATCGTTCCGCAAGGTGTTGTTACCTCCGATGGCGT  
TGTTACGAACTGGATGTGTTAGTGTTCGCCACCGTTTTGATGCGGTTGATGGCAACTACCGTGCAT  
GAACCTGCGTGGCCGTGATGGCCGTCACATCAACGAACACTGGACCGAAGGTCCGACCAGCTACCTGG  
GCGTGACCAAAGCTGGCTTCCCGAACATGTTTCATGATCCTGGGCCCGAACGGCCCGTTACCAACCTGC  
CGCCGAGCATTGAAGCGCAGGTGCAATGGATCTCTGACCTGATTGATAAGGCAACCCGTGAAGGTCTG  
ACTACTGTTGAACCGACCGCAGATGCTGAACGTGAATGGACCGAAACCTGCGCGGAAATCGCGAACAT  
GACCCTGTTCCCGAAAGCGGATAGCTGGATCTTTGGCGCGAACATTCCGGGTAAACGTCATGCGGTTAT  
GTTCTATCTGGGTGGCCTGGGTAACCTACCGTCGCCAGCTGGCGGATGTTGCTGACGGTGGTTATCGAGG  
CTTCCAGCTGCGTGGTGAACGTGCGCAGGCAGTTGCA

**Aldehyde reductase from *Synechocystis* species (slr1192) (AHR)<sup>[4]</sup>**

Codon optimized DNA sequence is given below:

ATGATTAAGCCTACGCTGCCCTGGAAGCCAACGGAAAACCTCAACCCTTTGAATACGACCCCGGTGCC  
CTGGGTGCTAATGAGGTGGAGATTGAGGTGCAGTATTGTGGGGTGTGCCACAGTGATTTGTCCATGATT  
AATAACGAATGGGGCATTTCCAATTACCCCTAGTGCCGGGTCATGAGGTGGTGGGTTACTGTGGCCGCC  
ATGGGCGAAGGGGTGAACCATGTTGAGGTGGGGGATTTAGTGGGGCTGGGTTGGCATTCCGGGCTACT  
GCATGACCTGCCATAGTTGTTTATCTGGCTACCACAACCTTTGTGCCACGGCGGAATCGACCATTGTGGG  
CCACTACGGTGGCTTTGGCGATCGGGTTCGGGCCAAGGGAGTCAGCGTGGTGAATTACCTAAAGGCA  
TTGACCTAGCCAGTGCCGGGCCCTTTTCTGTGGAGGAATTACCGTTTTTCAGTCCATGTTGTTGAACTGAG  
TTTAAAGCCCACTGCAAAAGTGGCAGTGATCGGCATTGGGGGCTTGGGCCATTTAGCGGTGCAATTTCT  
CCGGCCTGGGGCTGTGAAGTGAAGTGCCTTTACCTCCAGTGCCAGGAAGCAAACCGGAAGTGTGGAAT  
TGGGCGCTCACCACATACTAGATTCCACCAATCCAGAGGCGATCGCCAGTGCAGGAAGGCAAATTTGACT  
ATATTATCTCCACTGTGAACCTGAAGCTTGAAGTACTGGAACCTATACATCAGCACCCCTGGCGCCCCAGGGACA  
TTTCCACTTTGTTGGGGTGGTGTGGAGCCTTTGGATCTAAATCTTTTTCCCTTTTGGTGGGACAACGCT  
CCGTTTCTGCCTCCCCAGTGGGTAGTCCCGCCACCATTGCCACCATGTTGGACTTTGCTGTGCGCCATGA  
CATTAAACCCGTGGTGGAAACAATTTAGCTTTGATCAGATCAACGAGGCGATCGCCCATCTAGAAAGCGG  
CAAAGCCCATTATCGGGTAGTGCTCAGCCATAGTAAAAAT

**Lactonase from *Rhodococcus* sp. HI-31<sup>[2]</sup>**

Codon optimized DNA sequence is given below:

catATGACCAATATTAGCGAAACCCTGAGCACCGCACCGGGTGGTGCAGCAGGTCCGGATGTTCTGCGT  
GATCTGTATGCAGATTGGAGCGAAATTATGGCAGCAACACCGGATCTGACCATTCGTCTGCTGCGTAGC  
CTGTTTGTATGAATGGCATCAGCCGACCGTTGAACCGGAAGGTGTTACCTATCGTGAAGAAACCGTTGGT  
GGTGTCCGGGTATTTGGTGTCTGCCGAGGGTGCAGATGGTAGCAAAGTTCTGCTGTATAACCATGGT  
GGTGGTTTTGCAGTTGGTAGCGCAGCAAGCCATCGTAAACTGGCAGGTCATGTTGCAAAACACTGGGTG  
CAGTTGGTTTTGTTCTGGATTATCGTCGTGCACCGGAATTTACGCATCCGGCACAGATTGAAGATGGTGT  
TGCAGCATTTGATGCACTGGTTGCAAATGGTATTGCACCGCAGGATATTACCACCATTGGTGATAGTGCC  
GGTGGTAATCTGGCAGTTGCAATTGCACTGAGCCTGCGTGAACAGGGTAAACAGGGTCCGGGTAGCGT  
TATTGCATTTAGCCCGTGGCTGGATATGGAAAATAAAGGTGAAACCCTGGCCACCAATAATGATACCGA  
TGCAGTATTACACCGGAACTGCTGGAAGGCATGATTGCCGGTGTCTGGGTGATACCATTGATCCGAA  
AACACCGCTGGCAAATCCGCTGTATGCCGATTTTACCGTTTTTCCGCGTCTGTACATCACCGCAGGTAGC  
GTTGAAAGCCTGCTGGATAATGCAACCCGCTGGAAAACTGGCAGCAAGTGCCGGTGTGATGTTACC  
CTGAGCATTGGTGAAGGTCAGCAGCATGTTTATCCGTTTTCTGGCAGGTCGTAGCGCACTGGTTGATGAT  
GAATTTGCCAAACTGGCAGCCTGGTATCAAAGCACTCGAGGcaccaccaccaccactga

**Transaminase from *Silicibacter pomeroyi* (SPTA)<sup>[1]</sup>**

Codon optimized DNA sequence is given below:

ATGGCTACTATCACCAACCACATGCCTACCGCGGAACTGCAGGCTCTGGATGCCGCCATCATCTGCACC  
CATTCTCTGCAAACAACGCTCTGGGTGAAGAGGGTACTCGTGTAAATCACGCGTGACGTGGTGTATGGC  
TGAACGATTCTGAGGGTGAGGAGATCCTGGACGCAATGGCAGGTCTGTGGTGTGTAAACATCGTTAC  
GGTCGTGACGAGCTGGCAGAAGTAGCAGCACGTCAAATGCGTGAGCTGCCTTACTACAACACGTTCTTC  
AAGACCACGCACGTCCCAGCAATCGCACTGGCTCAGAAGCTGGCAGAGCTGGCTCCAGGTGATCTGAA  
CCATGTATTCTTCGCTGGCGGTGGTTCTGAGGCAAACGACACTAACATCCGCATGGTCCGTACTIONACTGG  
CAGAACAAGGGCCAGCCTGAAAAACCGTCATCATCTCCCGTAAAAACGCCTACCACGGCTCCACCGTC  
GCTTCTCCGCTCTGGGTGGTATGGCTGGTATGCATGCTCAGTCTGGTCTGATCCCGGATGTTTCATCACA  
TCAACCAGCCGAACTGGTGGGCTGAAGGTGGTGTATGGACCCGGAAGAATTTGGCCTGGCTCGTGCT  
CGTGAAGTGAAGAAGCTATCCTGGAAGTGGGTGAAAACCGCGTTGCTGCTTTCATCGCGGAACCGGTT  
CAGGGTGCGGGTGGTGTATTATTGTTGCCCCGGATTCTTACTGGCCGAAATCCAACGTATCTGCGACAAA  
TACGACATCCTGCTGATCGCGGACGAAGTGTCTGCGGTTTTCGGCCGTACTIONACTGGTTCGGCACT  
CAGACCATGGGCATCCGTCCGCACATTATGACCATCGCGAAAGGTCTGTCTTCTGGCTACGCGCCGATTG  
GCGGCAGCATTGTTTGTGATGAAGTGGCCACGTTATCGGCAAAGACGAATTTAACACGGCTATACT  
ATAGCGGCCACCCGGTGGCCGAGCAGTTGCGCTGGAAAATCTGCGTATTCTGGAAGAGGAGAACATC  
CTGGACCACGTTTCGTAACGTGGCGGCGCCGTATCTGAAAGAAAAATGGGAAGCGCTGACCGACCC  
GCTGTTGGCGAAGCGAAAATTGTTGGCATGATGGCGAGCATCGCGCTGACCCGAATAAAGCCAGCC  
GTGCGAAATTTGCGTCCGAACCGGGCACCATTGGCTATATTTGCCGCGAACGCTGCTTTGCGAATAATCT  
GATCATGCGCCACGTGGGCGATCGCATGATTATCTCCCGCCGCTGGTATTACCCCGGCGGAAATCGA  
TGAAATGTTCTGCGCATTTCGAAAAGCCTGGACGAAGCCCAGGCGGAAATTGAAAAACAGGGCCTGA  
TGAAAAGCGCCT



**Cyclase from Transaminase from *Citrobacter freundii* (Cy-CF\_Y152A)<sup>[5]</sup>**

Codon optimized DNA sequence is given below:

ATGAATTTGACCGGTAAAACCGCACTAGTGACCGGTAGTACTAGTGGTATAGGCTTAGGTATTGCGCAG  
GTCTTAGCACAGGCAGGAGCCACGTTGATTCTTAACGGGTTTGGCGACGTCGATGCGGCGAAAGACGC  
CGTAGCGCAATATGGTAAAACCCCGGGCTATCATGGCGCTGATCTGAGTGATGAAGCACAGATAGCTG  
ACATGATGCGGTATGCAGAAAGTGAATTTGGTGGTGTGATATTCTGATTAATAATGCAGGTATTCAGC  
ATGTTAGCCCGATTGAAACCTTTCCGGTTGATAAATGGAATGCAATTATTGCAATTAATCTGAGCTCTGTT  
TTTCATACCACCCGGCTGGCACTGCCGGGTATGCGGGCACGGAATTGGGGTCGGATTATTAATATTGCC  
AGTGTTTCATGGTCTGGTTGCATCAAAGAAAAAAGCGCCGAGTTGCAGCCAAACATGGTGTGTTGGT  
CTGACCAAACCATTTGCACTGGAAACCGCACAGACCGAAATTACCTGTAATGCACTGTGTCCGGGTTGG  
GTGCTGACCCCGCTGGTTCAGCAGCAGATTGATAACGGATTGCAGAACGCGCAGAACCGGAAGCAGC  
ACGCGATGCACTGCTGGCTGAAAAACAGCCGAGCCGCGAATTTGTTACCCCGGAACAGCTGGGTAATCT  
GGCACTGTTTCTGTGTAGTGATGGTGCAGCACAGGTTCCGGGGTGTGCGTGGAATATGGATGGTGGCT  
GGTTGCACAG

**Cyclase from Transaminase from *Enterobacter kobei* (Cy-EK)**

Codon optimized DNA sequence is given below:

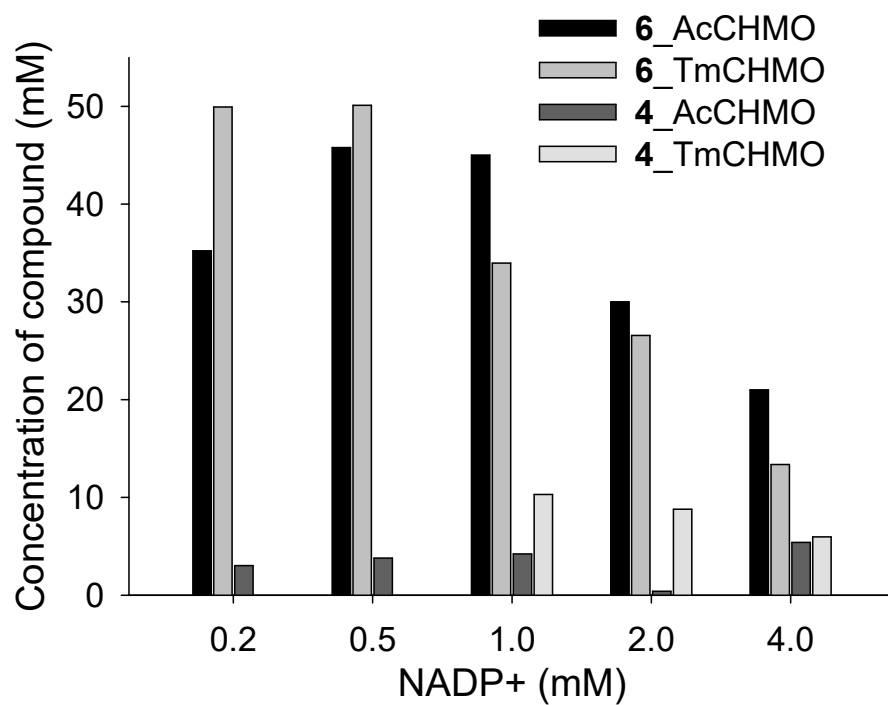
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AATTGCACAGCTGGGTAAAAACCGGTTATCATGATGCAGATCTGCGGGATGTTCTGCAGATTGAAGC  
GATGATGCGGTATGCAGAAAGTCAGTTTGGTGGTGTGATATTCTGGTTAATAATGCCGGTATTCAGCA  
TGTTGCACCTGTTGAAACCTTTCCGGTTGAAAAATGGAATGATATTATTGCGATTAATCTGTCAGCCGTTT  
TTCATACCAGCCGGCTGGCCCTGCCGGCAATGCGGGAACAGAATTGGGGTTCGGATTATTAATATTGCAT  
CTGTTTCATGGCCTGGTGGCAAGCAAAGAAAAAAGTGCATATGTGGCCGCAAACATGGTGTGGTTGGT  
TTTACCAAAGCCTGGCGCTGGAAACCGCCCGCACCGGTGTTACCGCAAATGCCCTGTGCCCGGGTTGG  
GTTCTGACCCCGCTGGTTCAGCAGCAGATTGATAACGGATTGCCGATGGTGTTCCTCCGGAACCTGGCC  
CAGGATCAGCTGCTGGGTGAAAAACAACCGAGTGGTGAATTTGTTACACCTGAACAGCTGGGTGAACT  
GGCACTGTTTCTGTGTAGCGATAGCGCAGTTAATGTTCCGGGTGCAGCATGGAATATGGATGGTGGTTG  
GGTTGCCAG

**Cyclase from Transaminase from *Pseudomonas stutzeri* (Cy-Pst)**

Codon optimized DNA sequence is given below:

ATGAACCTAACGGGGAAGACTGCTCTAGTAACTGGGTCGACTTCGGGAATAGGGCTAGGGATAGCATT  
AAAGCTCGCCGAAGCGGGGGCGGATTTAATCTTAAACGGATTCGGTGATGCATCTGCGGCACAGGCGG  
AAGTTGGCCGCATGGTTCGAAAGTTGGTTCATCATGGTGCAGATGTTAGTGATCCGGGTCAGATTGCC  
GAACTGTTTGCATATGCCGAACGGGAATTTGGTGGTGTGGATATTTTAGTAAATAATGCAGGTATTCAG  
CATGTTGCACCGGTGGAAGAATTTCCGCCGGAACGGTGGGATGCAATTATTGCCATTAATCTGAGTAGT  
GCTTTTCATACCATTAGGCTGGCCCTGCCGGGTATGCGGCAGCGGGGTTGGGGTTCGGATTATTAATGTT  
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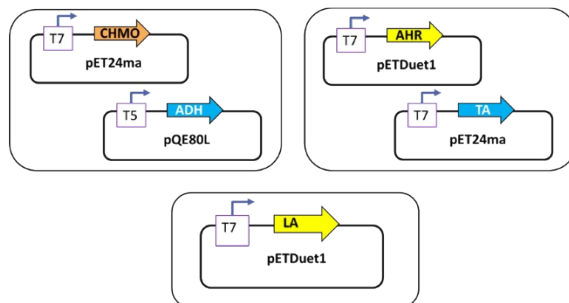
GTCTGACCAAAGTTGTTGCACTGGAAACCGCAACCACCCCTATTACTTGTAATGCAATTTGTCCGGGTTG  
GGTTTTAACCCCGTTAGTTCAGCAGCAGATTGATGAACGAGCCCGGCAGGATGGTGATGAACAGCGCG  
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TGGCACTGTTTCTGTGTTCAGAAGCAGGTGAT



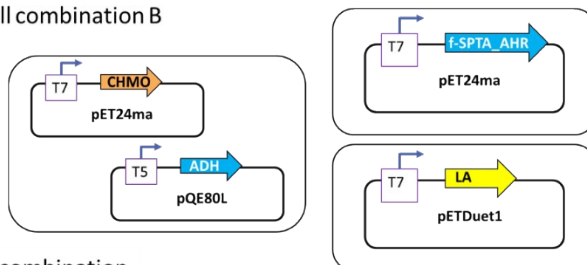
**Figure S1.** One-pot  $O_m$ - $A_m$  reaction for the synthesis of **6** from **1** using purified enzyme biocatalyst. Reaction condition (total volume 0.5 mL): 50 mM **1**, 100 mM benzylamine, 0.2-4.0 mM NADP+, 0.2 mM PLP, AcCHMO/TmCHMO (2 mg/mL), PMTA (2 mg/mL), AHR (1.5 mg/mL), LA (1.5 mg/mL), 1.0 mM NADP+, 100 mM Tris-HCl buffer (8.0) 25°C. See **Scheme 2** for numerically abbreviated compounds.

A) The *E. coli* strains for **Module 1** and **Module 2** (Oxidation module and Amination module; Om and A<sub>m</sub>)

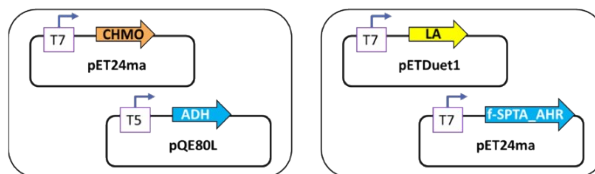
Three-cell combination A



Three-cell combination B

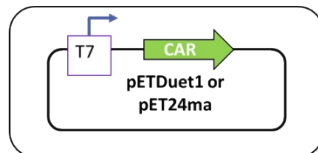


Two-cell combination

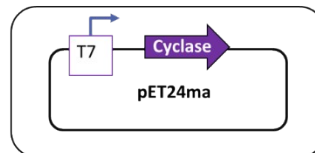


B) The *E. coli* strains for **Module 3** (Cyclization module; C<sub>m</sub>)

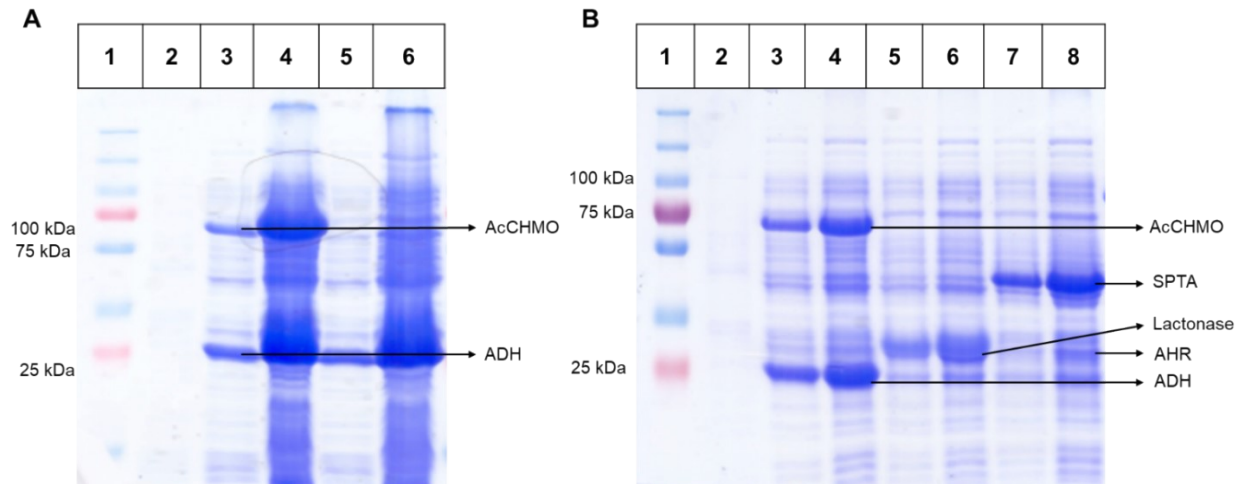
a) CAR biocatalysts



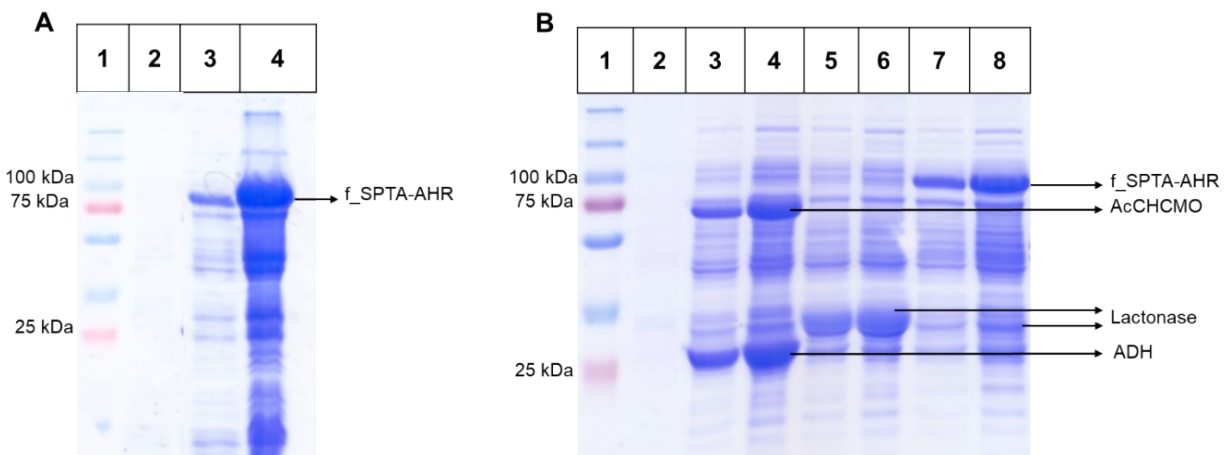
b) Cyclase biocatalysts



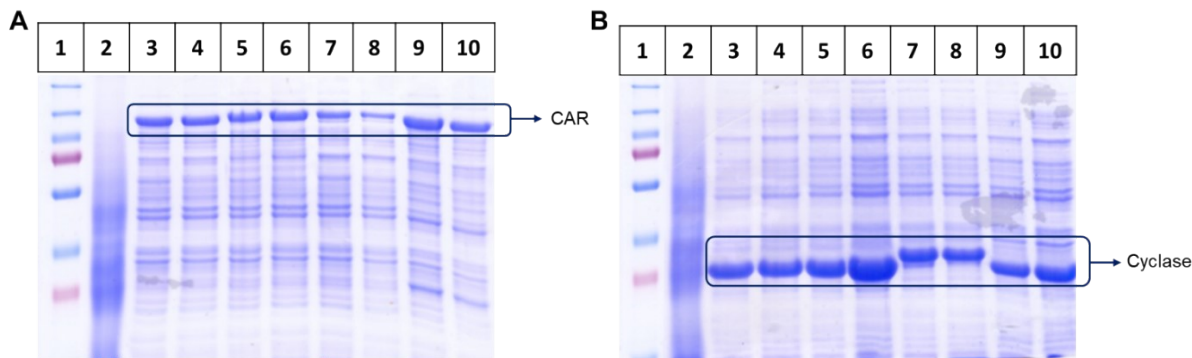
**Figure S2.** Vector designs to develop suitable *E. coli* expression system for desired enzymes A) The vector designs of *E. coli* strains for **Module 1** and **Module 2** (Oxidation module and Amination module; O<sub>m</sub> and A<sub>m</sub> to produce 6-AmHA (6) B) The vector designs of *E. coli* strains for **Module 3** (Cyclization module; C<sub>m</sub>) to produce lactams a) *E. coli* stains expressing CAR biocatalysts; b) *E. coli* stains expressing cyclase biocatalysts



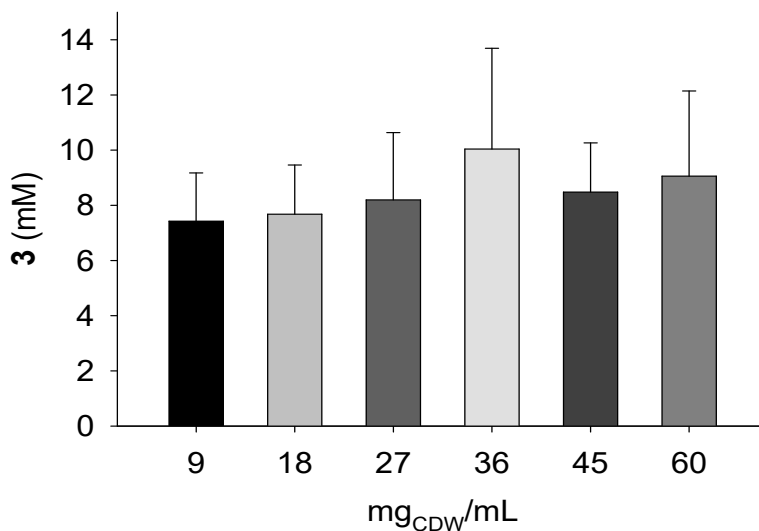
**Figure S3.** SDS-PAGE analysis of whole-cell expressions of desired proteins from  $O_m$  and  $A_m$ . A): Lane 1 Marker (M), Lane 2 control, Lane 3 *E. coli* (M1A\_M1B) whole-cell, Lane 4 *E. coli* (M1A\_M1B) soluble protein, Lane 5 co-expressed TmCHMO and ADH whole cell, Lane 6 Lane 5 co-expressed TmCHMO and ADH soluble protein; B) Lane 1 Marker (M), Lane 2 control, Lane 3 *E. coli* (M1A\_M1B) whole-cell, Lane 4 *E. coli* (M1A\_M1B) soluble protein, Lane 5 *E. coli* (M1C) whole-cell, Lane 6 *E. coli* (M1C) soluble protein, Lane 7 *E. coli* (M2A\_M2B) whole-cell, Lane 8 *E. coli* (M2A\_M2B) soluble protein.



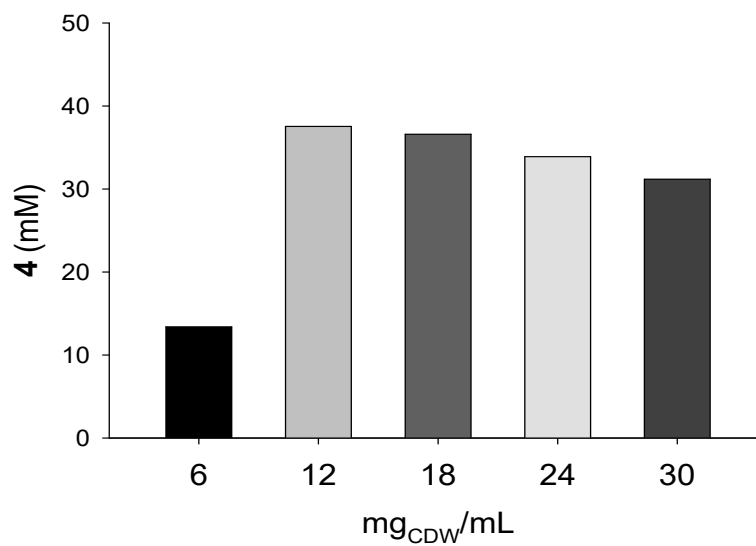
**Figure S4.** SDS-PAGE analysis of whole-cell expressions of desired proteins from  $O_m$  and  $A_m$ . A): Lane 1 Marker (M), Lane 2 control, Lane 3 *E. coli* (M2C) whole-cell, Lane 4 *E. coli* (M2C) soluble protein, B) Lane 1 Marker (M), Lane 2 control, Lane 3 *E. coli* (M1A\_M1B) whole-cell, Lane 4 *E. coli* (M1A\_M1B) soluble protein, Lane 5 *E. coli* (M1C) whole-cell, Lane 6 *E. coli* (M1C) soluble protein, Lane 7 *E. coli* (M1C\_M2C) whole-cell, Lane 8 *E. coli* (M1C\_M2C) soluble protein



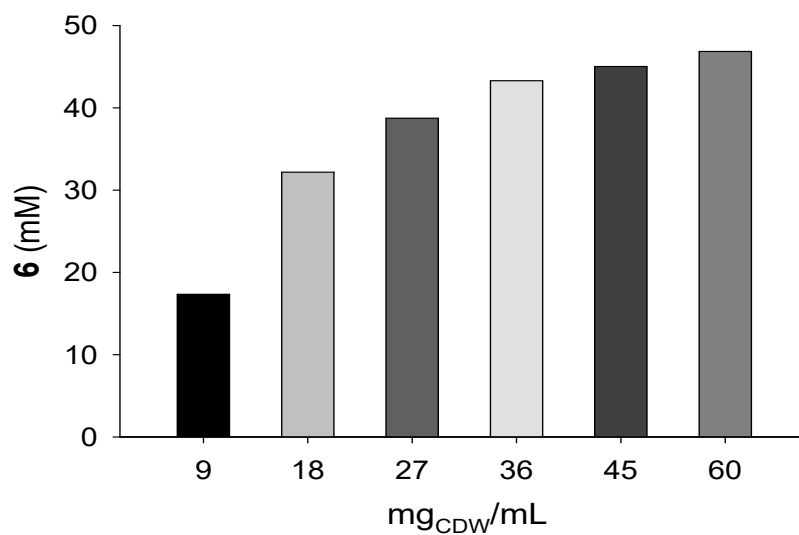
**Figure S5.** SDS-PAGE analysis of whole-cell expressions of desired proteins from  $C_m$ . A): Lane 1 Marker (M), Lane 2 control, Lane 3 *E. coli* (M3A) whole-cell, Lane 4 *E. coli* (M3A) soluble protein, Lane 5 *E. coli* (M3B) whole-cell, Lane 6 *E. coli* (M3B) soluble protein, Lane 7 *E. coli* (M3C) whole-cell, Lane 8 *E. coli* (M3C) soluble protein, Lane 9 *E. coli* (M3D) whole-cell, Lane 10 *E. coli* (M3D) soluble protein B) Lane 1 Marker (M), Lane 2 control, Lane 3 *E. coli* (M3E) whole-cell, Lane 4 *E. coli* (M3E) soluble protein, Lane 5 *E. coli* (M3F) whole-cell, Lane 6 *E. coli* (M3F) soluble protein, Lane 7 *E. coli* (M3G) whole-cell, Lane 8 *E. coli* (M3G) soluble protein, Lane 9 *E. coli* (M3H) whole-cell, Lane 10 *E. coli* (M3H) soluble protein



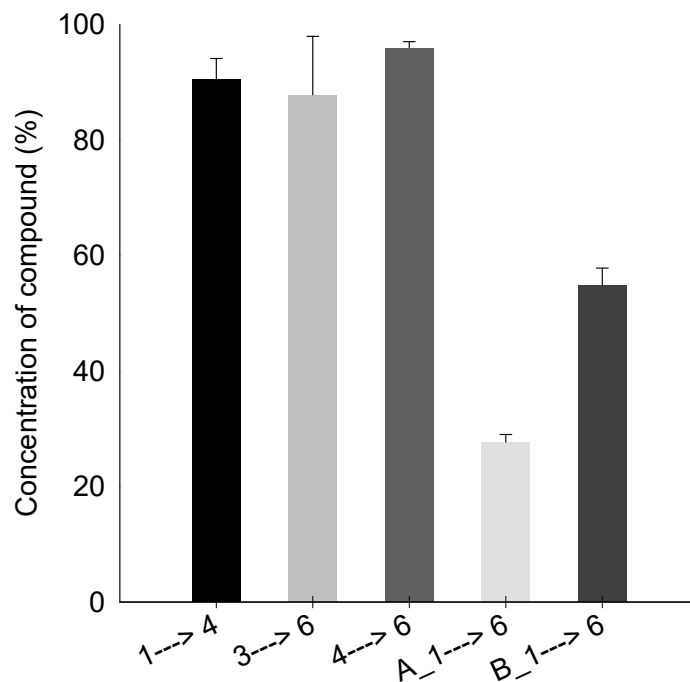
**Figure S6.** Optimization of desired amount of whole-cell biocatalyst for Module 1 ( $O_m$ ). Reaction condition: 50 mM **1**, 9-60 mg<sub>CDW</sub>/mL *E. coli* M1A\_M1B, 100 mM Tris-HCl buffer (8.0) 25°C



**Figure S7.** Optimization of desired amount of whole-cell biocatalyst for Module 1 ( $O_m$ ). Reaction condition: 50 mM **3**, 6-30 mg<sub>CDW</sub>/mL *E. coli* M1C, 100 mM Tris-HCl buffer (8.0) 25°C

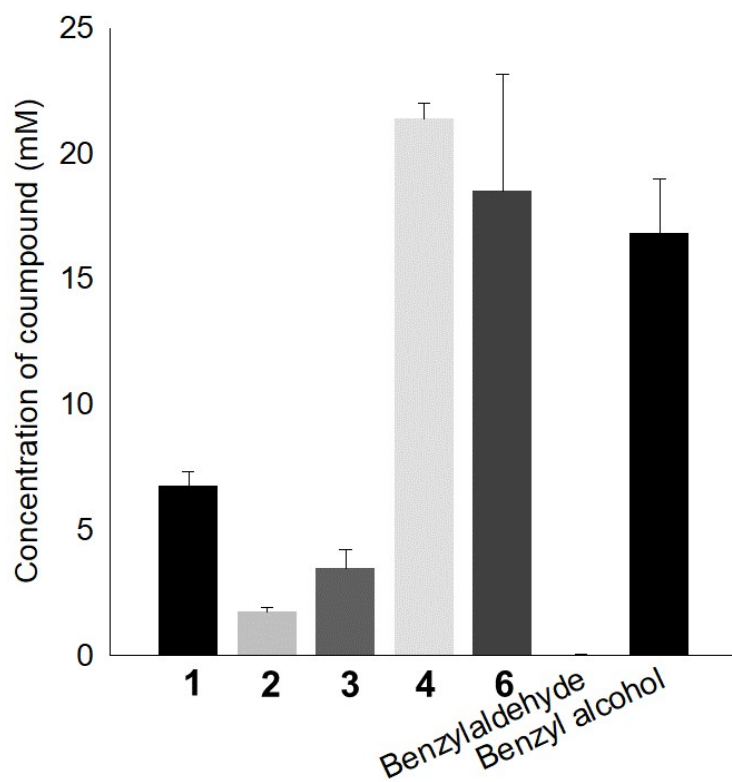


**Figure S8.** Optimization of desired amount of whole-cell biocatalyst for Module 2 ( $A_m$ ). Reaction condition: 50 mM **3**, 100 mM amino donor, 9-60 mg<sub>CDW</sub>/mL *E. coli* M1C\_M2C, 100 mM Tris-HCl buffer (8.0) 25°C

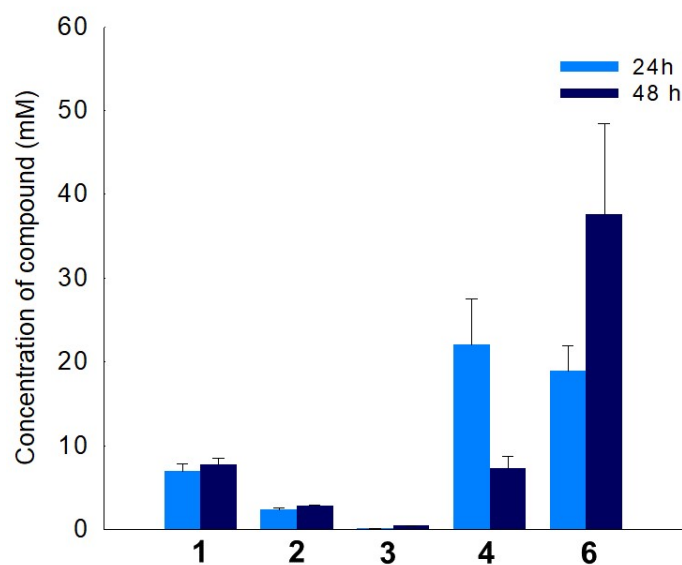


**Figure S9.** Combined three cell consortium reactions as well as model reactions catalysed by respective module. Reaction condition: **1— 4**: 50 mM **1**, *E. coli* M1A\_M1B (27 mg<sub>CDW</sub>/mL), *E. coli* M1C (9 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C; **3— 6**: 100 mM **3**, 200 mM benzylamine, *E. coli* M1C\_M2C (27 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C; **4— 6**: 20 mM **4**, 40 mM benzylamine, *E. coli* M2C (27 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C; **A<sub>1</sub>— 6**: 50 mM **1**, 100 mM benzylamine, *E. coli* M1A\_M1B (27 mg<sub>CDW</sub>/mL), *E. coli* M1C (9 mg<sub>CDW</sub>/mL), *E. coli* M2A\_M2B (27 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C; **B<sub>1</sub>— 6**: 50 mM **1**, 100 mM benzylamine, *E. coli* M1A\_M1B (27 mg<sub>CDW</sub>/mL), *E. coli* M1C (9 mg<sub>CDW</sub>/mL), *E. coli* M2C (27 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C; See **Scheme 2** for numerically abbreviated compounds.

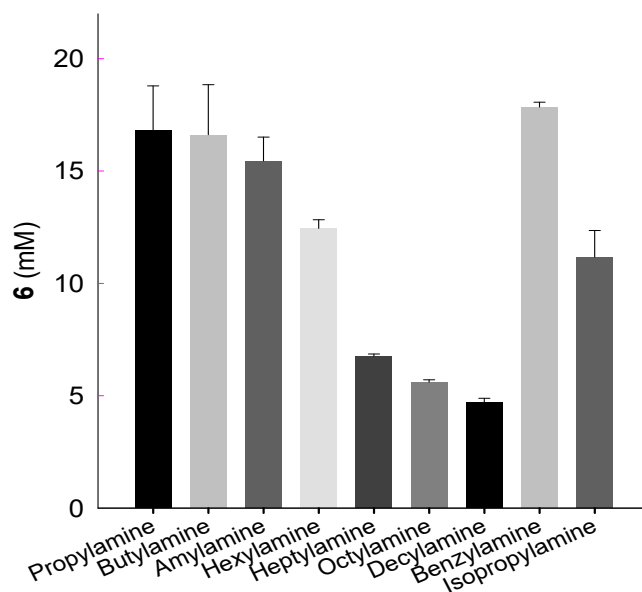




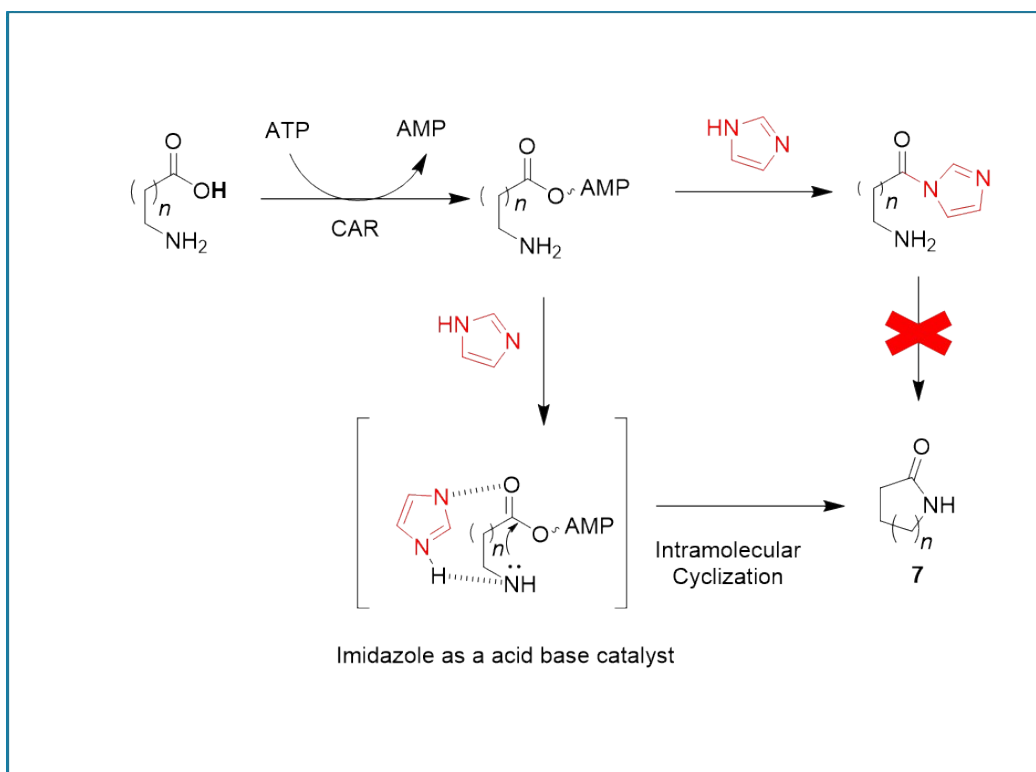
**Figure S10.** Initial one-pot two cell reaction for the synthesis of **6** from **1**. Reaction conditions: 50 mM **1**, 100 mM benzylamine, *E. coli* M1A\_M1B (27 mg<sub>CDW</sub>/mL), *E. coli* M1C\_M2C (27 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C; See **Scheme 2** for numerically abbreviated compounds.



**Figure S11.** One-pot two cell reaction for the synthesis of **6** from **1** at increased amount of  $A_m$  biocatalyst. Reaction conditions: 50 mM **1**, 100 mM benzylamine, *E. coli* M1A\_M1B (27 mg<sub>CDW</sub>/mL), *E. coli* M1C\_M2C (36 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C; See **Scheme 2** for numerically abbreviated compounds.



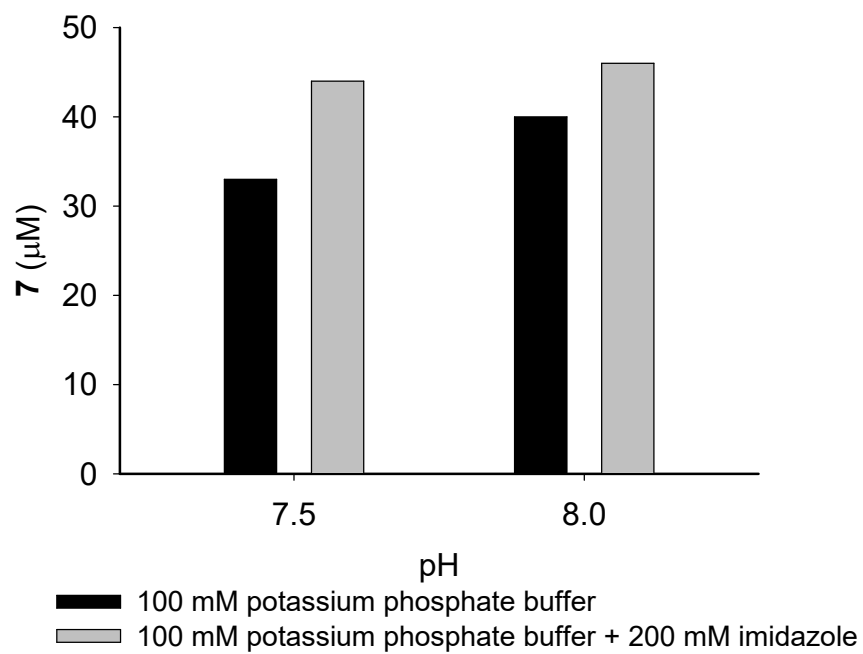
**Figure S12.** Screening of aliphatic amines as amine donor for Module 2 ( $A_m$ ). Reaction Condition: 20 mM **4**, 40 mM amino donor, *E. coli* M2A\_M2B (27 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (8.0) 25°C.



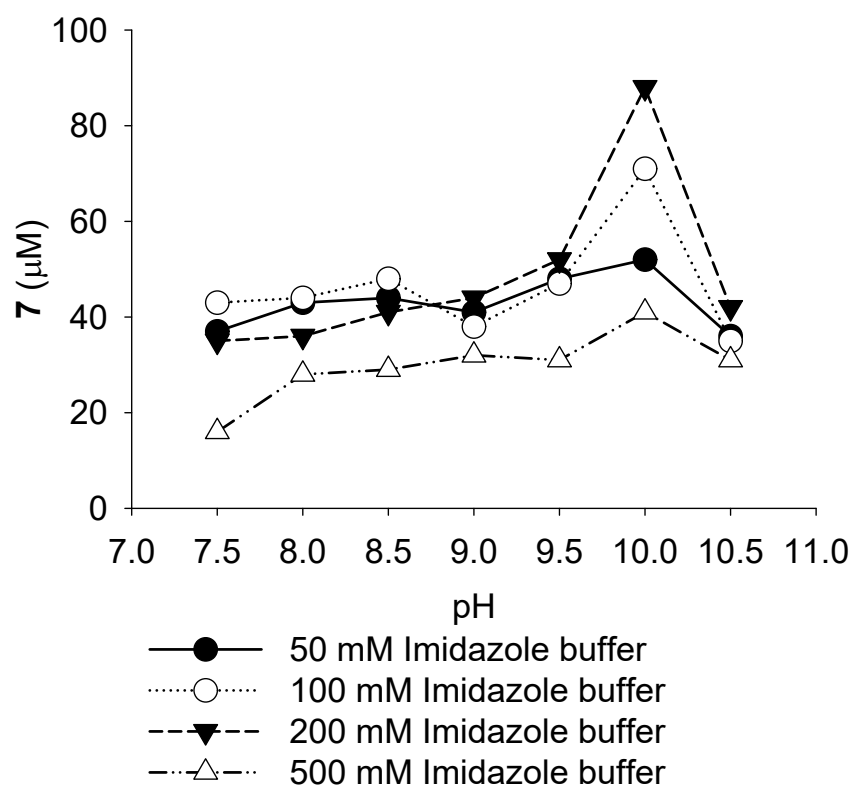
**Figure S13.** The plausible mechanism for imidazole catalysed intramolecular ring-cyclisation (lactamization) catalysed by CAR biocatalyst.

Entry	Recombinant <i>E. coli</i> strain		Plasmid	
1	<i>E. Coli</i> (M3A)	M3A	pETduet1	T7 → MaCAR_WT
2	<i>E. Coli</i> (M3B)	M3B	pETduet1	T7 → McCAR_WT
3	<i>E. Coli</i> (M3C)	M3C	pETduet1	T7 → MmCAR_WT
4	<i>E. Coli</i> (M3D)	M3D	pET24ma	T7 → MaCAR_L342E
5	<i>E. Coli</i> (M3E)	M3E	pET24ma	T7 → Cy-EK_Y152A
6	<i>E. Coli</i> (M3F)	M3F	pET24ma	T7 → Cy-EK_Y152A/S139C
7	<i>E. Coli</i> (M3G)	M3G	pET24ma	T7 → Cy-CF_Y152A/S139C
8	<i>E. Coli</i> (M3H)	M3H	pET24ma	T7 → Cy-Pst_Y152A/S139C

**Figure S14.** Design and construction of *E. coli* cells expressing or co-expressing desired enzymes involved in module 3 ( $C_m$ ).



**Figure S15.** The effect of imidazole on the lactamization using CAR biocatalyst. Reaction conditions: 5 mM **6**, 10 mM MgCl<sub>2</sub>, 1% Glucose, *E. coli* M3A (9 mg<sub>CDW</sub>/mL), 100 mM potassium phosphate buffer (7.5-8.0) 30°C



**Figure S16.** Optimization of suitable pH and optimal amount of imidazole buffer for lactamization using CAR biocatalyst. Reaction conditions: 5 mM **6**, 10 mM MgCl<sub>2</sub>, 1% Glucose, *E. coli* M3A (9 mg<sub>CDW</sub>/mL), 50-200 mM Imidazole buffer (7.5-10.0) 30°C

## Site-directed mutagenesis:

Starting from pET24ma plasmid encoding desired cyclase and primers harboring appropriate mutations were used to generate corresponding cyclase variants (a list of primers can be found in Table S1). The following PCR protocol was used: (1) initial denaturation at 95 °C for 2 min, (2) 18 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s (depending on the T<sub>m</sub> of the primers) and extension at 72 °C for 5 min; (3) a final extension at 72 °C for 5 min. The resulting PCR product was digested with DpnI for 2 hours at 37 °C and transformed into chemically competent *E. coli* DH5-alpha cells. A single colony was picked from LB plates containing Kanamycin (Km) (50 µg/mL) and used to inoculate 5 mL of LB medium containing the same concentration of Km. Bacteria were grown over night, plasmids isolated and variants harboring the correct mutations identified by sequencing. For protein expression, pET24ma plasmid encoding desired CAR variant was transformed into *E. coli* BW25113 (DE3)  $\Delta$ *fadD* strain and a single colony was used to inoculate an overnight culture for protein expression and purification<sup>[8]</sup>.

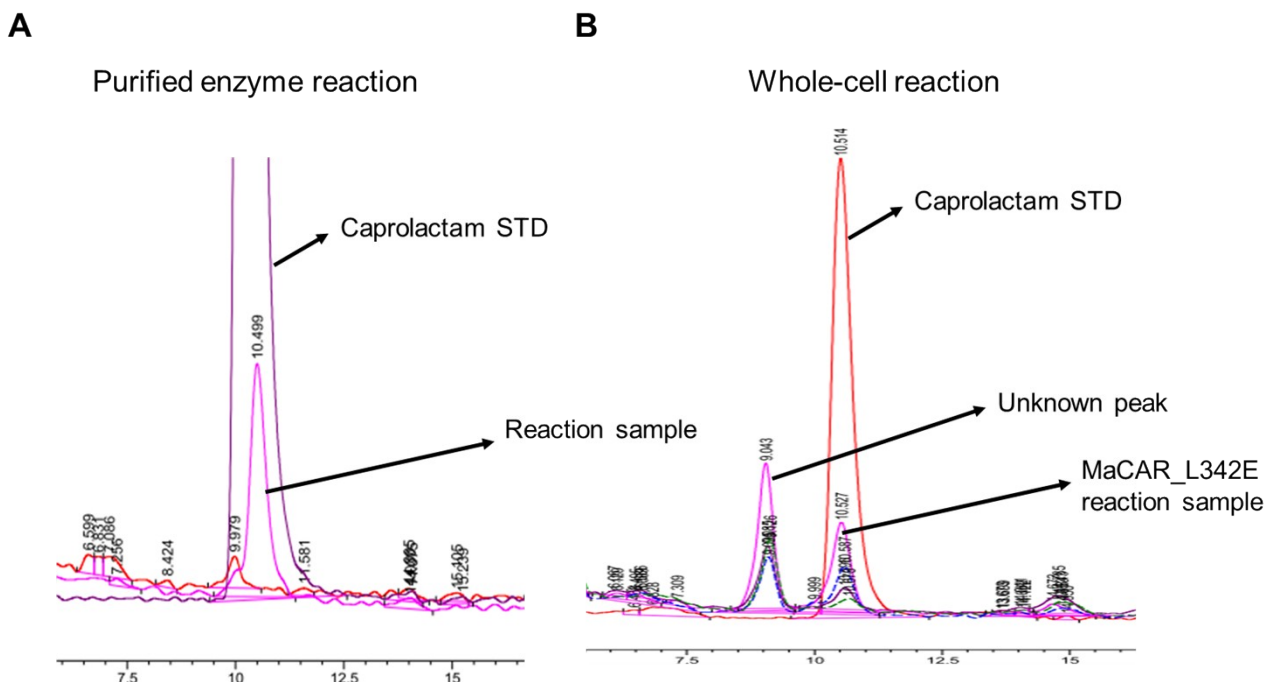
### Forward seq.

<b>MACAR F</b>	CGCTCATCCG	CCCCTCCGAA	GTGCTCTTCG	TGCCGCGTGT	GGTGGAGATG
<b>MaCAR L342</b>	CGCTCATCCG	CCCCTCCGAA	GTGGAAATTCG	TGCCGCGTGT	GGTGGAGATG
<b>Clustal Co</b>	*****	*****	***	*****	*****

### Reverse seq.

<b>MaCAR R</b>	CGGCACGAAG	AGCACTTCGG	AGGGGCGGAT	GAGCGCGATG	TCCTCGAAGA
<b>MaCAR L342</b>	CGGCACGAAT	TCCACTTCGG	AGGGGCGGAT	GAGCGCGATG	TCCTCGAAGA
<b>Clustal Co</b>	*****	*****	*****	*****	*****

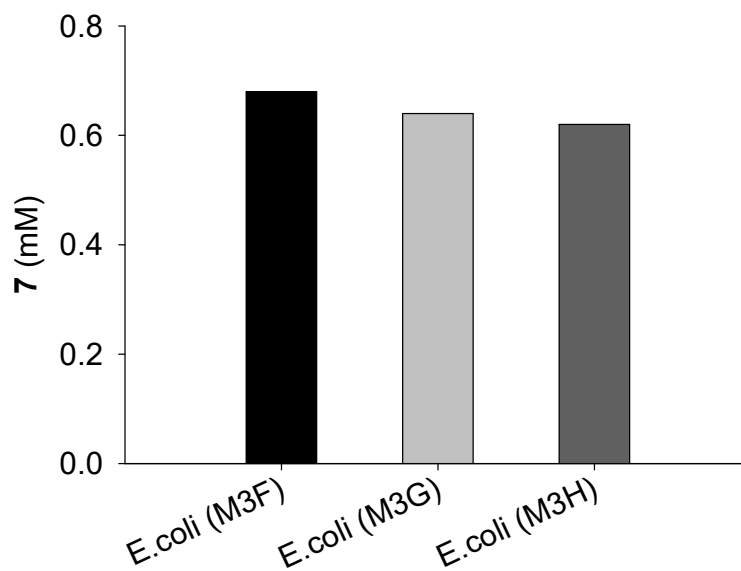
**Figure S17.** Multiple sequence alignments confirming the MaCAR\_L342E variant



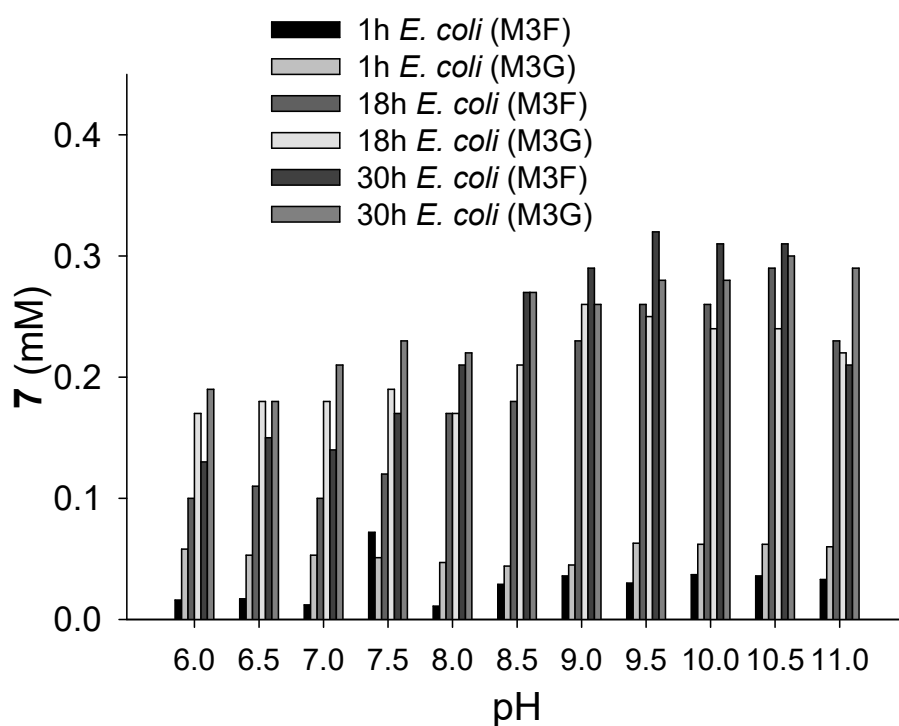
**Figure S18.** HPLC chromatograms for caprolactam showing the unknown peak A) purified enzyme reaction B) whole cell reaction. The control reactions for purified as well as whole cell reaction does not show any unknown peak confirming that the stand-alone unknown peak is not only from the host cell metabolism. Additionally, the purified enzyme reaction does not show any such peak but, in the whole cell reactions it can be seen as marked in figure B. therefore, we speculated that it might be due reaction with any of the nucleophile interacting with activated acyl-AMP intermediate.

<b>CF_STD_F</b>	TGGGGTCGGA	TTATTAATAT	TGCCAGT	GTT	CATGGTCTGG	TTGCATCAAA
<b>CF_2F</b>	TGGGGTCGGA	TTATTAATAT	TGCC	TGTGTT	CATGGTCTGG	TTGCATCAAA
<b>Clustal Co</b>	*****	*****	***	*****	*****	*****
<b>EK_STD_F</b>	GGTCGGATTA	TTAATATTGC	ATCT	GTTTCAT	GGCCTGGTGG	CAAGCAAAGA
<b>EK_2F</b>	GGTCGGATTA	TTAATATTGC	ATGT	GTTTCAT	GGCCTGGTGG	CAAGCAAAGA
<b>Clustal Co</b>	*****	*****	*	*****	*****	*****
<b>Pst_STD_F</b>	GTTGGGGTTCG	GATTATTAAT	GTTGCC	TCTG	TTCATGGTCT	GGTTGGTAGT
<b>Pst_2F</b>	GTTGGGGTTCG	GATTATTAAT	GTTGCC	TGTG	TTCATGGTCT	GGTTGGTAGT
<b>Clustal Co</b>	*****	*****	*****	***	*****	*****

**Figure S19.** Multiple sequence alignments confirming the Cyclase Y152A/S139C variants (See the protocol for site directed mutagenesis as described earlier)

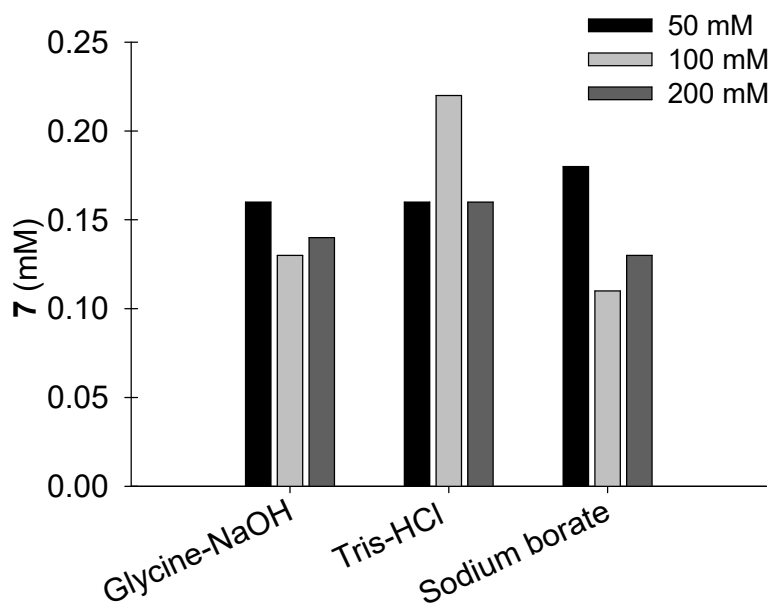


**Figure S20.** Comparison of activity of cyclase variants for the synthesis of caprolactam. Reaction Condition: 10 mM **6**, Cyclase (36 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (7.5) 30°C.

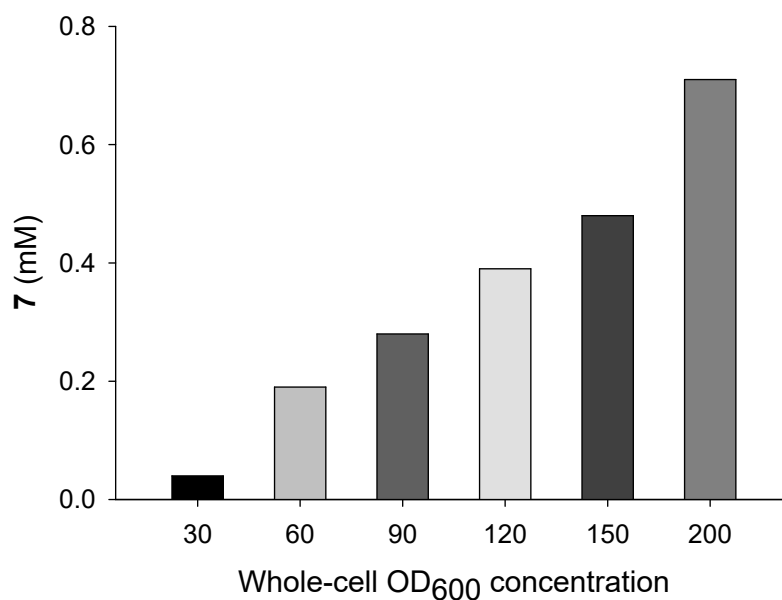


**Figure S21.** Optimization of suitable pH condition for cyclase catalysed lactamization. Reaction Condition: 10 mM **6**, *E. coli* (M3F) or *E. coli* (M3G) (18 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (6.0-11.0) 30°C

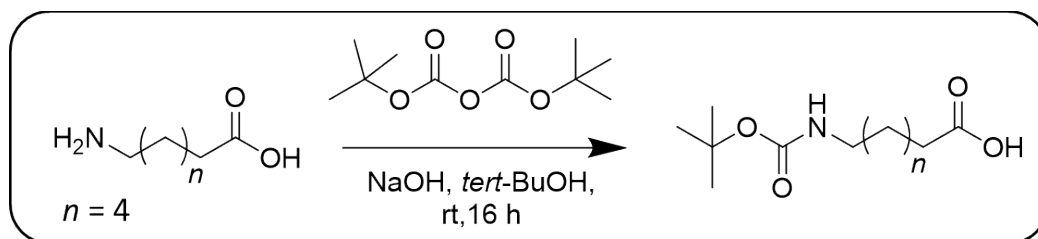




**Figure S22.** Optimization of suitable buffer species and concentration of buffer for cyclase catalysed lactamization. Reaction Condition: 10 mM **6**, *E. coli* (M3F) (18 mg<sub>CDW</sub>/mL), 50-100 mM Tris-HCl buffer or Glycine-NaOH buffer or Sodium borate buffer (10.0) 30°C



**Figure S23.** Optimization of desired amount of whole-cell biocatalyst for Module 3 ( $C_m$ ). using cyclase catalysed lactamization. Reaction Condition: 10 mM **6**, *E. coli* (M3F) (9-60 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (8.0) 30°C



**Scheme S1.** Reaction scheme for the synthesis of Boc-protected **6**

## Isolation and Purification of final products

### Representative procedure of upscaled reactions for isolation and purification of final products (25 mL total volume)

Cyclohexanol (**1**) (100 mM) was added to Tris-HCl buffer (100 mM pH 8.0) in reaction vessel (50 mL) followed by the addition of propylamine (amine donor; 200 mM), 0.2 mM PLP and respective whole-cell strains such as *E. coli* M1A\_M2B (27 mg<sub>CDW</sub>/mL) and *E. coli* M1C\_M2C (36 mg<sub>CDW</sub>/mL) were added. The reaction was incubated at 25°C on a pH titer and monitored for the pH drops at various time intervals. At the desired time intervals, the samples aliquots of the reaction mixture were withdrawn and derivatized as described in the analytical methods section and the conversions were determined by using GC.

After completion of the reaction the mixture was carefully acidified to pH 3.0 applying a 6 M HCl solution. Precipitated proteins were removed by filtration and the aqueous phase was extracted with ethyl acetate (3 x 15 mL). This separated organic phase contain the unreacted propylamine and its corresponding propanol generated co-product in the reaction along with non-consumed substrate and other organic impurities. Next the aqueous phase was neutralized by using 6 N NaOH and equal volume of *tert* butanol, NaOH (2.0 eq) and Di-*tert*-butyl dicarbonate (Boc-anhydride 2.0 eq) was added. Next the reaction mixture was stirred at room temperature (slightly heated at 60°C for 3-4 h) for 16 hours (See scheme S1), after which it was diluted with water (100 mL) and neutralized by using 6 M HCl, The resulting solution was extracted with ethyl acetate (1x100 mL + 2x100 mL), washed with brine, dried over magnesium sulfate (MgSO<sub>4</sub>) and concentrated in vacuum to obtain desired product<sup>[6]</sup> (673 mg of Boc-protected **6** isolated yields of 58.8%) The product was further characterized by using Mass and H<sup>1</sup> NMR spectroscopy.

### NMR-data of isolated Boc-protected products obtained from upscaled biotransformations

#### Boc-protected 6-aminohexanoic acid (Boc- protected 6a)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 0.97(m, 2H), 1.46 (s, 9H), 1.68 (m, 4H), 2.37 (t, *J* = 14.8 2H), 3.30 (m, 2H), 5.57 (s,1H), 8.10 (s, 1H)

## **Analytical Conditions:**

### **Derivatization**

For derivatization, reaction mixture was evaporated, 10 volumes of pyridine as a solvent was added. This mixture was subjected to sonication and (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) was added for derivatization followed by incubation at 55 °C for 45 min.

### **Sample preparation:**

#### **Preparation for analysis of 6 and 4:**

For the analysis of **6** acids and **4**, the acidified reaction samples were dried in a vacuum concentrator. After complete drying, the sample was dissolved again to the 5 volumes with pyridine. The sample was then mixed with an equal volume of MSTFA by vigorous vortexing for 1 min and converted to the TMS derivatives by incubation at 55 °C for 30 min. after this the samples were centrifuged and transformed to a new microcentrifuge tube for Gas chromatographic analysis

#### **Preparation for analysis of compound 1, 2 and 3:**

For the analysis of other compounds such as substrate **1** or intermediate **2** and **3**, the sample aliquots were basified by using 5 N NaOH at each sampling time. The products were extracted from time interval samples by centrifugation at 14,000 rpm for 30 min with a 10 volume of chloroform after vigorous vortexing for 1 min. After centrifugation, the extracted samples in chloroform (bottom layer) were pooled and transformed to a new microcentrifuge tube for Gas chromatographic analysis.

### **Determination of conversion**

Quantitative analysis was performed using a gas chromatography instrument with a flame ionization detector (GC/FID) fitted with an AOC-20i series auto sampler injector (GC 2010 plus Series, Shimadzu Scientific Instruments, Kyoto 604-8511, Japan). Two-microliter samples were injected by split mode (split ratio 20:1) and analyzed using a nonpolar capillary column (5% phenyl methyl siloxane capillary 30 m \* 320 µm i.d., 0.25-µm film thickness, HP-5)

### **GC program parameters:**

**Method 1:** (For ω- amino fatty acids and ω- hydroxy fatty acids ):

Injector 230°C; flow 1.5 mL/min; Temperature program 90°C/hold 0 min; 15°C per min to 200 °C/ hold 0 min and 5°C per min to 280°C / hold 5 min.

**Method 2:** (Ketones, and cycloalkylamines)

Injector 260°C; flow 1.5 mL/min; Temperature program 50°C/hold 1 min. and 10°C per min to 250 °C / hold 0 min and 30°C per min to 280 °C / hold 5 min.

**Table S2.** The retention times of all observed reaction intermediates after derivatization.

Entry	Compound		Retention time	Method	Company
1	6-amino hexanoic acid	<b>6</b>	8.87	1	Alfa- aesar
2	$\epsilon$ -caprolactone	<b>3</b>	9.17	2	Sigma-Aldrich
3	6-hydroxyhexanoic acid	<b>4</b>	6.34	1	Alfa-aesar
4	Cyclohexylamine	-	4.78	2	Sigma-Aldrich
5	Cyclohexanol	<b>1</b>	5.18	2	Sigma-Aldrich
6	Cyclohexanone	<b>2</b>	5.25	2	Sigma-Aldrich
7	Benzylamine	-	7.18	2	Sigma-Aldrich
8	Benzyl alcohol	-	7.44	2	Sigma-Aldrich

### Identification and quantitative analysis of lactams (7)

The analysis of lactams was performed by using HPLC with an Eclipse XDB-C18 column (5 $\mu$ , 4.6  $\times$  250 mm; Agilent Technologies, USA) and UV detector at 205 nm. The mobile phase composition of methanol: water (0.1% TFA) (90:10 v/v) at isocratic elution, with flow rate of 1 mL/min was used for compound **7**.

**Table S3.** The retention times observed for corresponding lactams.

Entry	Compound		Retention time	Method	Company
1	$\epsilon$ -caprolactam	<b>7</b>	10.5	-	Sigma-Aldrich
2	Valerolactam	-	5.4	-	Tokyo Chemical Industry

**Table S4:** E-factor calculations for biotransformation<sup>#</sup>

		This work (AmHA; <b>6</b> ) <sup>[a]</sup>	Kroutil and co-workers (AmHA; <b>6</b> ) <sup>[b,c]</sup>	This work (caprolactam; <b>7</b> ) <sup>[d]</sup>	Yun and co- workers (Caprolactam; <b>6</b> ) <sup>[e]</sup>	
Total number of reactants	Whole Cells (g <sub>CDW</sub> )	3.15	0.02532018	0.099	0.270	
	Oxygen (g)	0.16	0.0016	0.00032	0.00032	
	Substrate (g)	0.50	0.005	0.001	0.00297	
	Co-substrate (g)	0.59	0.01754	0.0012	0.00118	
	Sum (g)	4.40	0.04946018	0.10152	0.27447	
Amount of waste	Sum (g) (Amount of reactant – Amount of product)	With Co- product	3.44	0.04796018	0.1006	0.27197
	w/o co- product	3.74	-	0.1012	-	
Amount of product	Product (g)	0.66	0.0015	0.00032	0.0025	
	Co-product (g) <sup>[f]</sup>	0.30	-	0.00060	-	
	Sum (g)	0.96	0.0015	0.00092	0.0025	
E- factor_(Amount of waste/ Amount of product)	With Co-product	3.58	31.97	109.34	108.78	
	w/o co-product	5.66	-	316.25	-	

w/o: without water, E-factor: Amount of waste/ Amount of product

<sup>#</sup>Solvent (water) and buffer salts have been excluded from this calculation assuming that the same amount salt used in the beginning retain in the final stage.

<sup>[a]</sup>The O<sub>m</sub> and A<sub>m</sub>-catalyzed one-pot whole-cell biotransformation for synthesis of 6-AmHA (**6**) from cyclohexanol (**1**); Reaction conditions (Total volume 50 mL; 24h) : 100 mM **1**, 200 mM amine donor, *E. coli* M1A\_M1B (27 mg<sub>CDW</sub>/mL), *E. coli* M1C\_M2C (36 mg<sub>CDW</sub>/mL), 0.2 mM PLP, 100 mM Tris-HCl buffer (pH 8.0) at 25 °C.

<sup>[b]</sup>Modularized six-enzyme in vitro system for the synthesis of 6-AmHA (**6**) from Cyclohexanol (**1**); Reaction conditions (Total volume 1 mL): Reaction conditions: 50 mM **1**, Alanine (250 mM), 0.3 mg NADP<sup>+</sup>, 0.3 mg, NAD<sup>+</sup>, 250 mM NH<sub>4</sub>Cl, 0.2 mM PLP, 0.2 U LB-ADH and 0.2 U BVMO, 3.5 U Lactonase, 0.18 U<sub>6-hydroxyhexanoic acid</sub>

ADH-hT, 0.22 U<sub>alanine</sub> AlADH, 20 mg lyophilized cells of TA, 10% v/v MeOH, 2 mM MgCl<sub>2</sub>, Na<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub> buffer (pH 8.0), 21 °C, 170 rpm orbital shaker, 20 h, 1 bar O<sub>2</sub>.

<sup>[c]</sup>Isolated enzymes were used

<sup>[d]</sup>The whole-cell biotransformation using engineered cyclase for synthesis of caprolactam (**7**); Reaction conditions (Total volume 1 mL; 24h): 10 mM **1**, 20 mM propylamine, 0.2 mM PLP, *E. coli* M1A\_M1B (27 mg<sub>CDW</sub>/mL), *E. coli* M1C\_M2C (36 mg<sub>CDW</sub>/mL), *E. coli* M1E (36 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 8.0) at 25°C.

<sup>[e]</sup>An Integrated Cofactor/Co-Product Recycling Cascade for the for synthesis of caprolactam (**7**) from cycloalkylamine; (Whole cell biocatalysis; Total volume 3 mL) Reaction conditions: 10 mM Cyclohexylamine, 3.0 mM **4**, AcCHMO/AHR (30 mg<sub>CDW</sub>/mL); SPTA/LA (20 mg<sub>CDW</sub>/mL), Cy-EK\_Y152A (40 mg<sub>CDW</sub>/mL), 100 mM Tris-HCl buffer (pH 7.5) at 30 °C.

<sup>[f]</sup>Transamination reaction mainly requires addition of co-substrate (Amine donor) in the reaction, which leads to formation of corresponding deaminated co-product. In general, these co-products are considered as waste, but the use of AHR in this cascade resulted into corresponding alcohol as co-product which, could be then considered as desired co-product.

## Spectroscopic analysis:

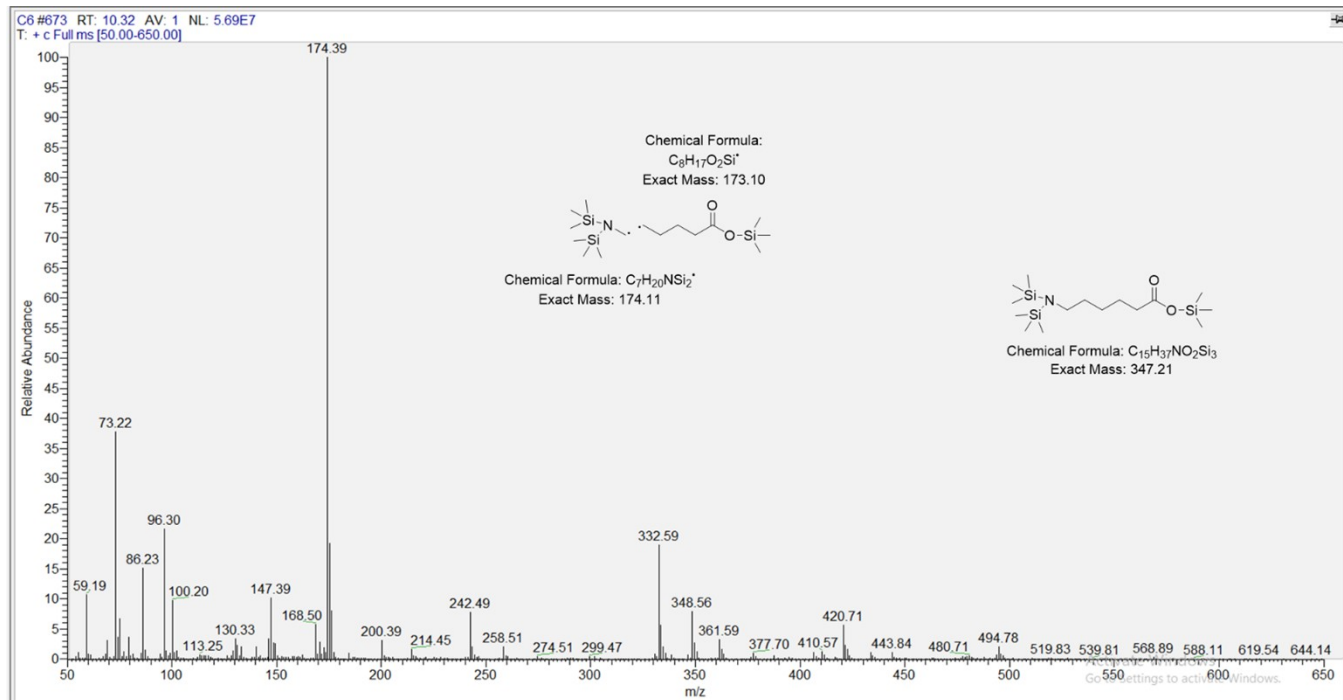


Figure S24. Mass spectrum of 6

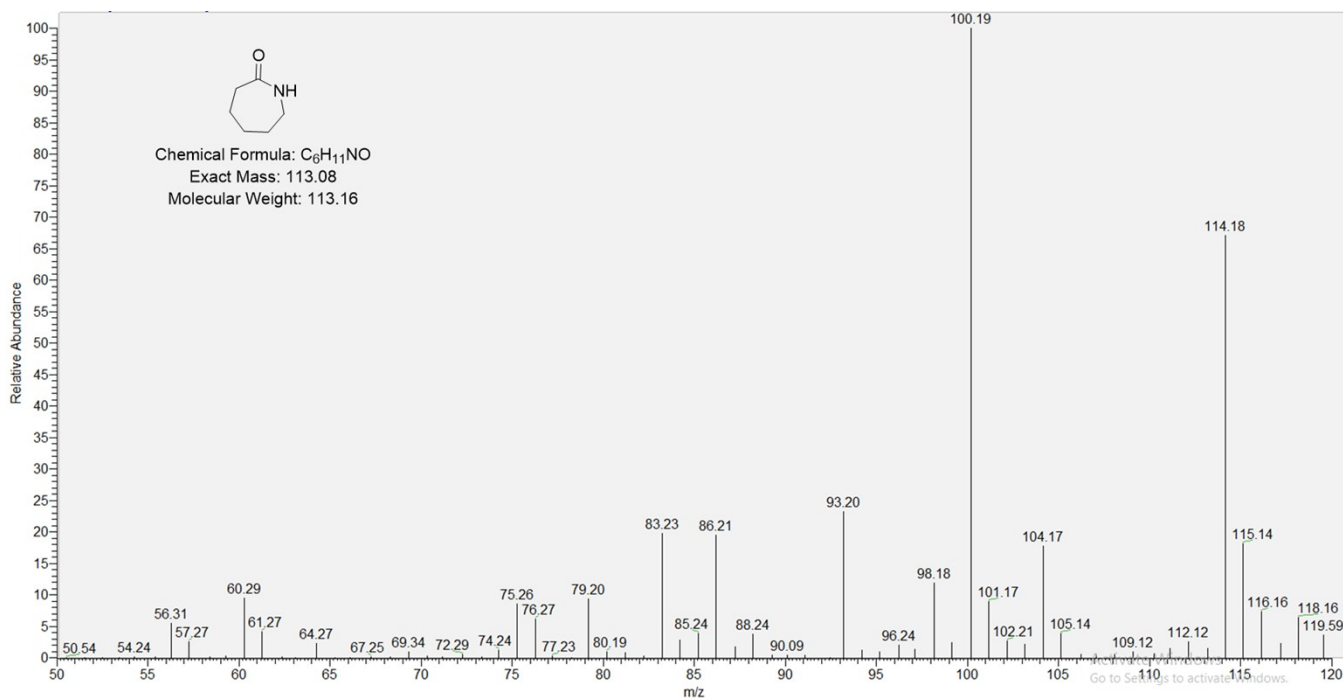
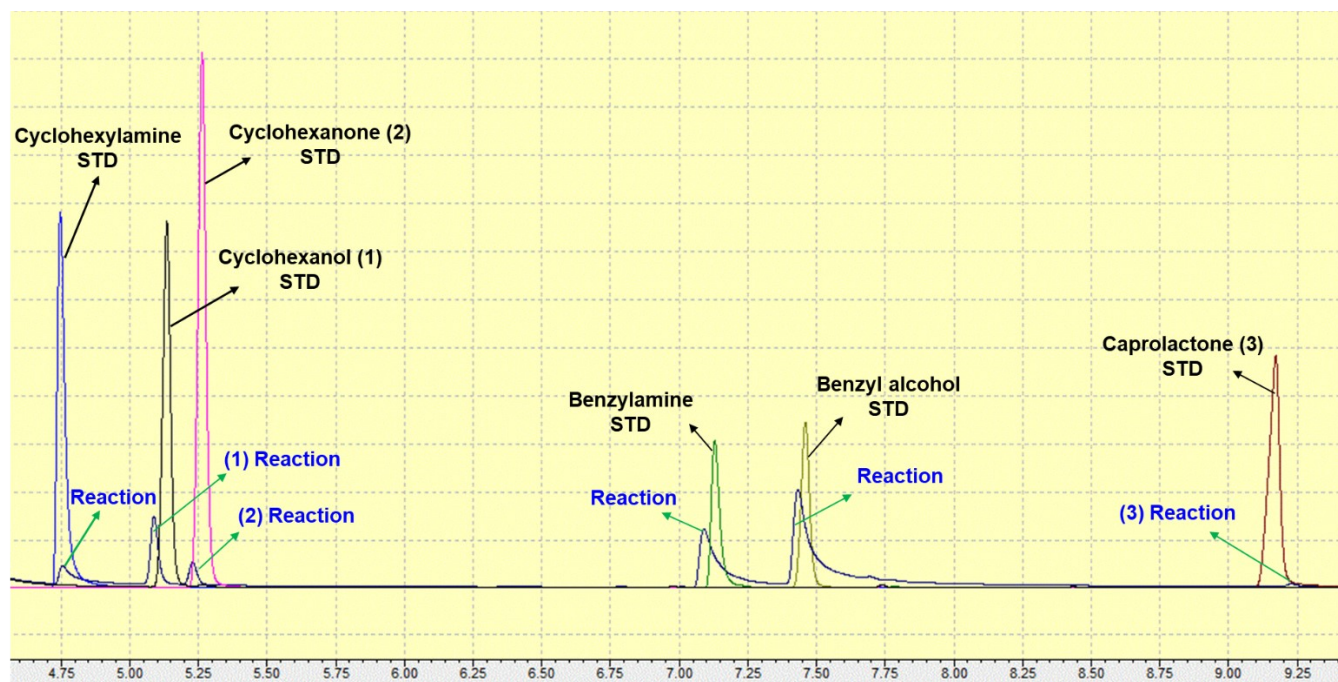
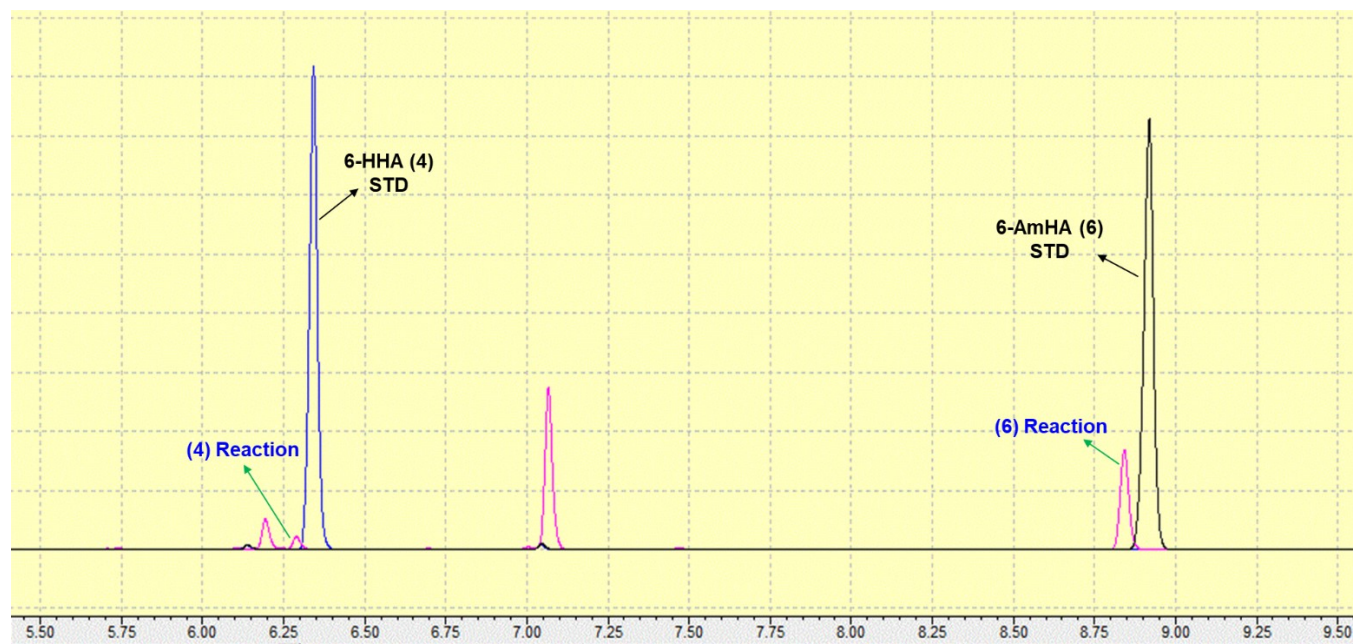


Figure S25. Mass spectrum of purified 7



**Figure S26.** Gas Chromatogram showing peaks of substrate, desired product, and intermediates .



**Figure S27.** Gas Chromatogram showing desired products and intermediates (4 and 6).



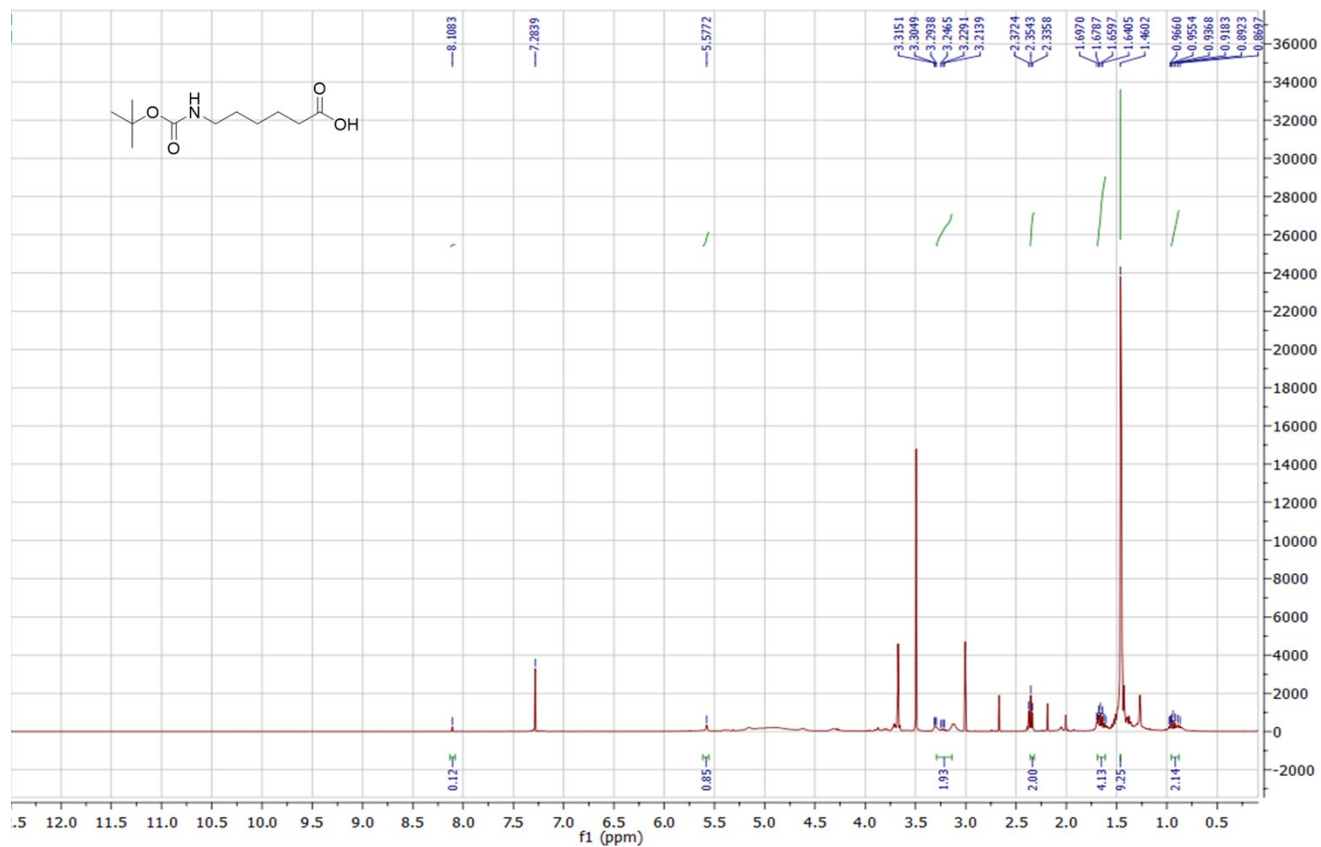


Figure S29. Overview of the  $^1\text{H}$  NMR of boc-protected 6.

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