Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Multienzyme Biocatalytic Cascade as a Route Towards Synthesis of α, ω-diamines from Corresponding Cycloalkanols

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Experimental enzymes

Table S1. List of enzymes used in this study.

Enzyme	Organism		Sequence	Plasmid	Ref.
ADH	*Lactobacillus brevis*	F	ATTA CATATG AGTAATCGTCTAGATG	pET24ma	1
		R	ATTA CTCGAG CTACTGCGCAGTATAG		
AcCHMO	*Acinetobacter calcoaceticus*	F	ATTA CATATGTCTCAGAAAATGGATTTC	pET24ma	1
		R	ATTA GGATCCCGCGTTAGCCGGCTGTTT		
Lactonase	*Rhodococcus* sp. HI-31	F	ATTA CATATGACCAATATTAGCGAAACC	pET24ma	1
		R	ATTA GGATCCTTACTCGAGTGCTTTCTG		
		F	ATTA CATATGACCAATATTAGCGAAACC	pETDuet1 and pCDFDuet1	
		R	ATTA GGTACCTTACTCGAGTGCTTTCTG		
AHR	*Synechocystis* species	F	ATTACATATGATGATTAAAGCCTACGCTG	pET24ma	1&2
		R	ATTACTCGAGATTTTTACTATGGCTGAG		
		F	ATTAAGGATCCATGATTAAAGCCTACGCT	pETDuet1 and pCDFDuet1	1&2
		R	ATTAGAGCTCATTTTTACTATGGCTGAG		
SPTA	*Silicibacter pomeroyi*	F	ATATCATATGATGGCTACTATCACCAACCACATG	pET24ma and pCDFduet1	[1]
		R	ATATCTCGAGTTAGGCGCTTTTCATCAGGC		
F_SPTA-AHR	-	SPTA_F	ATTACATATGATGGCTACTATCACCAACCACATGC	pET24ma	2
		SPTA_R	ACTACCTCCACCTCCGGCGCTTTTCATC		
		AHR_F	GCGCCGGAGGTGGAGGTAGTATGATTAAAGC		
		AHR_R	ATTACTCGAGTTAATTTTTACTATGGCTGA		
MaCAR	*Mycobacterium abscessus*		Gene synthesis	pETDuet1	
MaCAR_L342E	*Mycobacterium abscessus*	F	ATTACATATGGAGCAGGTGAAGCGACGC	pET24ma	4
		R	ATTACTCGAGCGCCAGTTCGGCGTACAG		
PPTA	*Phaeobacter porticola*	F	ATTAGGATCCAATGACCGCTATCACCAAC	pET24ma	1
		R	ATTAGAGCTCAGCAGAATGCAGCAGGTC		

SMTA	*Shimia marina*	F	ATTAGGATCCAATGAACGCAATCACCAAC	pET24ma	1
		R	ATTAGAGCTC TTTGTACAGGTCTTCAGC		
SPTA_R417A	*Silicibacter pomeroyi*	F	ATAATCTGATCATGGCCCACGTGGGCGATC	pET24ma	This study
		R	GATCGCCCACGTGGGCCATGATCAGATTAT		
SPTA_R417V	*Silicibacter pomeroyi*	F	ATAATCTGATCATGGTCCACGTGGGCGATC	pET24ma	This study
		R	GATCGCCCACGTGGACCATGATCAGATTAT		
SPTA_R417F	*Silicibacter pomeroyi*	F	ATAATCTGATCATGTTCCACGTGGGCGATC	pET24ma	This study
		R	GATCGCCCACGTGGAACATGATCAGATTAT		

Table S2. List of in-house TAs used in this study.

Entry	Organism	Abbreviation	Protein ID No.
1	*Chromobacterium violaceum*	CVTA	WP_011135573.1
2	*Vibrio fluvialis JS17*	VFTA	WP_040602310.1
3	*Agrobacterium tumefaciens*	ATTA	WP_010972924.1
4	*Sphaerobacter thermophilus*	STTA	WP_012871332.1

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, 6-hydroxy hexanoic acid, cyclohexanone, cyclohexylamine, ε-caprolactone, benzylamine, 1,6-Hexamethylenediamine (HMD), 6-amino-1-hexanol, 8-amino-1-octanol, 8-aminooctanoic acid, 1,8-diaminooctane, 1,8-octanediol, pyridine, NAD^+, $NADP^+$, isopropyl-thio-β-D-galactopyranoside (IPTG), N, O-Bis (trimethylsilyl)-trifluoroacetamide (BSTFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA), 6-aminohexanoic acid was purchased from alfa aesar, cyclooctanol, cycloheptanol, 1,6-hexandiol, 1,7-heptanediol, 1,7-diaminoheptane, Adenosine triphosphate (ATP) 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, Δ fadD cells were grown in a 100 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_{600} reached at 0.6. IPTG solution (20 µL, 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 µm 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_2PO4, 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 NaH2PO4, 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)[2]

Codon optimized DNA sequence is given below:

ATGTCTCAGAAAATGGATTTCGACGCTATCGTTATCGGTGGTGGTTTCGGTGGTCTGTACGCGGTTAAAA
AACTGCGTGATGAACTGGAACTGAAAGTTCAGGCATTCGATAAAGCGACCGACGTAGCGGGTACCTGG
TACTGGAACCGTTACCCAGGTGCTCTGACTGACACCGAAACCCACCTGTACTGTTACTCCTGGGATAAAG
AACTGCTGCAGTCTCTGGAAATCAAGAAAAAATACGTGCAGGGTCCGGATGTTCGTAAATACCTGCAGC
AGGTGGCTGAAAAACACGATCTGAAAAAATCCTACCAGTTCAACACCGCAGTTCAGTCTGCACACTACA
ACGAAGCTGACGCACTGTGGGAAGTTACCACTGAATACGGCGATAAATACACCGCGCGTTTCCTGATCA
CTGCGCTGGGTCTGCTGTCTGCGCCGAACCTGCCGAACATCAAAGGTATCAACCAGTTCAAAGGTGAAC
TGCACCACACCAGCCGTTGGCCGGATGACGTTTCTTTCGAAGGTAAACGTGTTGGTGTTATCGGCACCG
GTAGCACCGGTGTTCAGGTTATTACCGCTGTTGCTCCGCTGGCTAAACACCTGACCGTTTTCCAGCGTTC
TGCTCAGTACTCTGTTCCGATCGGTAACGATCCGCTGAGCGAAGAAGATGTAAAGAAAATTAAAGACAA
CTACGACAAAATCTGGGACGGTGTTTGGAACTCTGCTCTGGCGTTCGGTCTGAACGAATCTACCGTTCCG
GCTATGTCTGTTTCCGCTGAAGAACGTAAAGCAGTTTTCGAAAAAGCTTGGCAGACCGGTGGCGGTTTC
CGTTTCATGTTCGAAACCTTCGGCGATATCGCTACCAACATGGAAGCTAACATCGAAGCGCAGAACTTCA
TCAAAGGTAAAATCGCAGAAATCGTTAAAGATCCGGCAATCGCGCAGAAACTGATGCCGCAGGACCTGT
ACGCTAAACGTCCGCTGTGCGACTCCGGCTACTACAACACCTTCAACCGTGACAACGTTCGCCTGGAAG
ACGTGAAAGCTAACCCGATCGTTGAAATCACCGAAAACGGTGTTAAACTGGAAAACGGTGACTTCGTTG
AACTGGATATGCTGATCCTGGCGACCGGCTTCGACGCGGTTGACGGCAACTACGTTCGTATGGATATCC
AGGGTAAAAACGGTCTGGCTATCAAAGACTACTGGAAAGAAGGCCCGTCTTCTTACATGGGTGTTACCG
TTAACAACTACCCGAACATGTTCATGGTTCTGGGCCCGAACGGTCCGTTCACCAACCTGCCGCCGTCCAT
CGAATCCCAGGTTGAATGGATCTCTGACACTATCCAGTACACCGTTGAAAACAACGTTGAATCTATCGAA
GCAACCAAAGAAGCTGAAGAACAGTGGACCCAGACCTGCGCGAACATCGCGGAAATGACCCTGTTCCC
GAAAGCGCAGTCCTGGATCTTCGGTGCTAACATTCCGGGCAAAAAGAACACCGTTTACTTCTACCTGGG
TGGCCTGAAAGAATACCGTAGCGCTCTGGCTAACTGCAAAAACCACGCTTACGAAGGTTTCGACATCCA
GCTGCAGCGTTCTGACATTAAACAGCCGGCTAACGCG

Aldehyde reductase from *Synechocystis* species (slr1192) (AHR)[3]

Codon optimized DNA sequence is given below:

ATGATTAAAGCCTACGCTGCCCTGGAAGCCAACGGAAAACTCCAACCCTTTGAATACGACCCCGGTGCC
CTGGGTGCTAATGAGGTGGAGATTGAGGTGCAGTATTGTGGGGTGTGCCACAGTGATTTGTCCATGATT
AATAACGAATGGGGCATTTCCAATTACCCCCTAGTGCCGGGTCATGAGGTGGTGGGTACTGTGGCCGCC
ATGGGCGAAGGGGTGAACCATGTTGAGGTGGGGGATTTAGTGGGGCTGGGTTGGCATTCGGGCTACT
GCATGACCTGCCATAGTTGTTTATCTGGCTACCACAACCTTTGTGCCACGGCGGAATCGACCATTGTGGG
CCACTACGGTGGCTTTGGCGATCGGGTTCGGGCCAAGGGAGTCAGCGTGGTGAAATTACCTAAAGGCA

TTGACCTAGCCAGTGCCGGGCCCCTTTTCTGTGGAGGAATTACCGTTTTCAGTCCTATGGTGGAACTGAG
TTTAAAGCCCACTGCAAAAGTGGCAGTGATCGGCATTGGGGGCTTGGGCCATTTAGCGGTGCAATTTCT
CCGGGCCTGGGGCTGTGAAGTGACTGCCTTTACCTCCAGTGCCAGGAAGCAAACGGAAGTGTTGGAAT
TGGGCGCTCACCACATACTAGATTCCACCAATCCAGAGGCGATCGCCAGTGCGGAAGGCAAATTTGACT
ATATTATCTCCACTGTGAACCTGAAGCTTGACTGGAACTTATACATCAGCACCCTGGCGCCCCAGGGACA
TTTCCACTTTGTTGGGGTGGTGTTGGAGCCTTTGGATCTAAATCTTTTTCCCCTTTTGATGGGACAACGCT
CCGTTTCTGCCTCCCCAGTGGGTAGTCCCGCCACCATTGCCACCATGTTGGACTTTGCTGTGCGCCATGA
CATTAAACCCGTGGTGGAACAATTTAGCTTTGATCAGATCAACGAGGCGATCGCCCATCTAGAAAGCGG
CAAAGCCCATTATCGGGTAGTGCTCAGCCATAGTAAAAAT

Lactonase from *Rhodococcus sp. HI-31*

Codon optimized DNA sequence is given below:

catATGACCAATATTAGCGAAACCCTGAGCACCGCACCGGGTGGTGCAGCAGGTCCGGATGTTCTGCGT
GATCTGTATGCAGATTGGAGCGAAATTATGGCAGCAACACCGGATCTGACCATTCGTCTGCTGCGTAGC
CTGTTTGATGAATGGCATCAGCCGACCGTTGAACCGGAAGGTGTTACCTATCGTGAAGAAACCGTTGGT
GGTGTTCCGGGTATTTGGTGTCTGCCGCAGGGTGCAGATGGTAGCAAAGTTCTGCTGTATACCCATGGT
GGTGGTTTTGCAGTTGGTAGCGCAGCAAGCCATCGTAAACTGGCAGGTCATGTTGCAAAACACTGGGTG
CAGTTGGTTTTGTTCTGGATTATCGTCGTGCACCGGAATTTCAGCATCCGGCACAGATTGAAGATGGTGT
TGCAGCATTTGATGCACTGGTTGCAAATGGTATTGCACCGCAGGATATTACCACCATTGGTGATAGTGCC
GGTGGTAATCTGGCAGTTGCAATTGCACTGAGCCTGCGTGAACAGGGTAAACAGGGTCCGGGTAGCGT
TATTGCATTTAGCCCGTGGCTGGATATGGAAAATAAAGGTGAAACCCTGGCCACCAATAATGATACCGA
TGCACTGATTACACCGGAACTGCTGGAAGGCATGATTGCCGGTGTTCTGGGTGATACCATTGATCCGAA
AACACCGCTGGCAAATCCGCTGTATGCCGATTTTACCGGTTTTCCGCGTCTGTACATCACCGCAGGTAGC
GTTGAAAGCCTGCTGGATAATGCAACCCGTCTGGAAAAACTGGCAGCAAGTGCCGGTGTTGATGTTACC
CTGAGCATTGGTGAAGGTCAGCAGCATGTTTATCCGTTTCTGGCAGGTCGTAGCGCACTGGTTGATGAT
GAATTTGCCAAACTGGCAGCCTGGTATCAAAAGCACTCGAGcaccaccaccaccaccactga

Transaminase from *Silicibacter pomeroyi* (SPTA)[1]

Codon optimized DNA sequence is given below:

ATGGCTACTATCACCAACCACATGCCTACCGCGGAACTGCAGGCTCTGGATGCCGCCCATCATCTGCACC
CATTCTCTGCAAACAACGCTCTGGGTGAAGAGGGTACTCGTGTAATCACGCGTGCACGTGGTGTATGGC
TGAACGATTCTGAGGGTGAGGAGATCCTGGACGCAATGGCAGGTCTGTGGTGTGTAAACATCGGTTAC
GGTCGTGACGAGCTGGCAGAAGTAGCAGCACGTCAAATGCGTGAGCTGCCTTACTACAACACGTTCTTC
AAGACCACGCACGTCCCAGCAATCGCACTGGCTCAGAAGCTGGCAGAGCTGGCTCCAGGTGATCTGAA
CCATGTATTCTTCGCTGGCGGTGGTTCTGAGGCAAACGACACTAACATCCGCATGGTCCGTACTTACTGG
CAGAACAAGGGCCAGCCTGAAAAAACCGTCATCATCTCCCGTAAAAACGCCTACCACGGCTCCACCGTC
GCTTCCTCCGCTCTGGGTGGTATGGCTGGTATGCATGCTCAGTCTGGTCTGATCCCGGATGTTCATCACA
TCAACCAGCCGAACTGGTGGGCTGAAGGTGGTGATATGGACCCGGAAGAATTTGGCCTGGCTCGTGCT
CGTGAACTGGAAGAAGCTATCCTGGAACTGGGTGAAAACCGCGTTGCTGCTTTCATCGCGGAACCGGTT
CAGGGTGCGGGTGGTGTTATTGTTGCCCCGGATTCTTACTGGCCGGAAATCCAACGTATCTGCGACAAA

TACGACATCCTGCTGATCGCGGACGAAGTGATCTGCGGTTTCGGCCGTACTGGTAACTGGTTCGGCACT
CAGACCATGGGCATCCGTCCGCACATTATGACCATCGCGAAAGGTCTGTCTTCTGGCTACGCGCCGATTG
GCGGCAGCATTGTTTGTGATGAAGTGGCCCACGTTATCGGCAAAGACGAATTTAACCACGGCTATACCT
ATAGCGGCCACCCGGTGGCCGCAGCAGTTGCGCTGGAAAATCTGCGTATTCTGGAAGAGGAGAACATC
CTGGACCACGTTCGTAACGTGGCGGCGCCGTATCTGAAAGAAAAATGGGAAGCGCTGACCGACCACCC
GCTGGTTGGCGAAGCGAAAATTGTTGGCATGATGGCGAGCATCGCGCTGACCCCGAATAAAGCCAGCC
GTGCGAAATTTGCGTCCGAACCGGGCACCATTGGCTATATTTGCCGCGAACGCTGCTTTGCGAATAATCT
GATCATGCGCCACGTGGGCGATCGCATGATTATCTCCCCGCCGCTGGTGATTACCCCGGCGGAAATCGA
TGAAATGTTCGTGCGCATTCGCAAAAGCCTGGACGAAGCCCAGGCGGAAATTGAAAAACAGGGCCTGA
TGAAAAGCGCCT

Transaminase from *Phaeobacter porticola* (PPTA)[4]

Codon optimized DNA sequence is given below:

ATGACCGCTATCACCAACCACCTGCCGACCGCTGAACTGCAGGCGCTGGACGCGGCGCACCACATGCAC
CCGTTCACCGCGAACGGTGAACTGGCTGAAAAAGGTGTGCGTGTTATCACCCGCGCGTCTGGTGTTACT
CTGACCGATTCTGAAGGTCACGAAATCCTGGACGCGATGGCGGGTCTGTGGTGCGTTAACATCGGCTAC
GGCCGTGATGAACTGGCGGACGTTGCGGCACGTCAGATGCGTGAACTGCCGTACTACAACACCTTCTTC
CAGACCACCCATGCTCCGGCTATCGCGCTGGCGGCGAAAATCGCGGAACTGGCGCCGGAAGGTCTGAA
CCACGTTTTCTTCGCTGGTTCTGGTTCTGAAGCTAACGACACTAACATCCGTATGGTTCGTCACTACTGGG
CGATGAAAGGTAAACCGACTAAATCTGTGATCATTTCTCGTAAAAACGGTTACCACGGTTCCTCTGTTGG
TTCCGGTAGCCTGGGTGGTATGACCGCTATGCACGAACAGGGCGGTCTGCCGATCCCAGATATTCACCA
CATCAACCAGCCGAACTGGTGGGCGGAAGGCGGTGACACCAACCCGGAAGATTTCGGTCTGGCGCGTG
CCCAGGAACTGGAAAAAGCGATTCTGGAACTGGGTGAAGACCGCGTTGCGGCGTTCATCGCGGAACCG
GTTCAGGGCGCAGGTGGTGTAATCGTGCCGCCGGCGACCTATTGGCCGGAAATCCAGCGTATCTGCGA
CAAATACGAAATCCTGCTGATCGCTGACGAAGTGATCTGCGGTTTCGGTCGTACCGGTAACTGGTTCGG
TAGCCAGACCATGGGCATCCGTCCGGATATTATGACCATCGCGAAAGGTCTGTCTTCCGGCTACGCTCCG
ATCGGTGGTAGCATCGTATCCGATGAAATCGCGTCCGTTATCGGCAGCGGCGAATTCAACCACGGTTAC
ACCTACTCTGGCCACCCGGTTGCGTCTGCTGTGGCTCTGGAAAACCTGCGTATCCTGGAGGAAGAAAAC
ATCATCGGTCACGTTCAGGACGTTGCGGCACCGTACCTGAAAGAACAGTGGGAAGCTTTGGCGGACCA
CCCGCTGGTCGGTGAAGCTAAAATCGTGGGCATGATGGGCTCTATCGCACTGACCCCGAACAAAGAAAC
CCGCGCTACCTTTGCGGCAGCGGGCGGCACCGTTGGTTACATCTGCCGTGAACGTTGCTTCGCAAACAA
CCTGGTGATGCGTCACGTTGGCGATCGTATGATCATCTCTCCGCCGCTGGTTATCACCACCGATGAAATC
GATATGCTGATCTCTCGTGCTCGTCGTTCCCTGGATGAATGCTACGCTGCGCTGAAAGAACAGGACCTGC
TGCATTCTGCT

Transaminase from *Shimia marina* (SMTA)

Codon optimized DNA sequence is given below:

ATGAACGCAATCACCAACCACCTGCCGACCGCTGAACTGCAGGCTCTGGACGTTGCTCACCACATGCAC
CCGTTCTCTACCCAGAACGACTTCAACGACACCGGTGCGCGTGTTATCACCCAGGCGAAAGGTGTTACCC
TGACTGACTCTGAAGGTGCTCAGATCCTGGATGCGATGGCTGGTCTGTGGTGCGTTAACATCGGTTACG

GTCGTGAAGAACTGGCAGACGTGGCGGCTCGTCAGATGCGTGAACTGCCGTACTACAACACCTTCTTCA
AAACCACTCACGTTCCGGTTATCGCACTGTCCGCGAAACTGGCTGAACTGGCTCCGGGTGATCTGAACC
ACGTGTTCTACGCGGGTTCTGGCTCCGAAGCGAACGACACTAACATGCGTCTGGTTCGTCACTATTGGTC
CGCGAAAGGTAAACCGTCTAAAACTATCTTCATTTCCCGTAAAAACGCGTACCACGGTTCTACCATGGCG
GGTGCATCTCTGGGCGGTATGGTTCCTATGCACCAGCAGGGTTCTCTGCCGATCCCGGACGTTCACCACA
TTAACCAACCGAACTGGTGGGCTGAAGGTGGTGACATGAGCCCGGAAGAATTCGGTCTGCAGCGTGCG
CAGGAACTGGAAGAAGCTATCCTGGAACTGGGTGAAGATCGTGTAGCGGCTTTCATCGCTGAACCGATT
CAGGGCGCTGGCGGTGTTATCGTTCCGCCGGAAACTTACTGGCCGGAAATCCAGCGTATCTGCGACAAA
TACGAAATCCTGCTGATCGCGGACGAAGTGATCTGCGGTTTCGGTCGCACCGGTAACTGGTTCGGCTCC
CAGACTGTTGGTATCAAACCGCACATCATGTCTATCGCGAAAGGTCTGTCCTCCGGTTACGCGCCGATCG
GCGGTTCTATCGTTTGCGACGAAGTGGCTGAAGTTGTTGCAGCAACTGAATTCAACCACGGTTACACCTA
CAGCGGCCACCCGGTTGCTTGCGCGGTTGCACTGGAAAACCTGCGTATCATCGAAGAAGAAAACATCAT
CGGTCACGTACGTGACGACGTAGCGCCGTACCTGAAAGAAAAATGGGAAGCTCTGGCAGACCACCCGC
TGGTTGGTGAAGCTAAAATCGTTGGTATGATGGGCTCTATCGCTCTGACCCCGAACAAAGAAACCCGTG
CGCCGTTCGCAGCTGACACCGGCACCGTTGGTTACAAATGTCGTGAACACTGCTTCGGTAACAACCTGG
TAATGCGTCACGTTGGTGATCGTATGATCATCTCTCCGCCGCTGGTTATGACCCGTGACGAAGTTGACAC
CCTGATCGAACGTGCGACCCGTGCGCTGGACCTGACCTTCGAACAGATCAAAGCTGAAGACCTGTACAA
A

CAR from *Mycobacterium abscessus* (MaCAR)[1]

DNA sequence is given below:

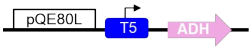
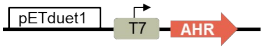
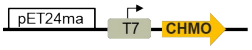
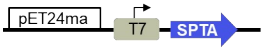
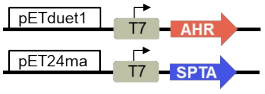
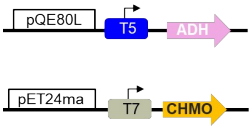
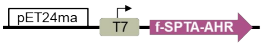
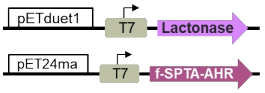
ATGACTGAAACGATCTCCACAGCGGCTGTCCCCACTACGGATCTCGAAGAGCAGGTGAAGCGACGCATC
GAGCAGGTCGTGTCCAACGATCCGCAGCTGGCGGCGCTTCTCCCGGAAGATTCGGTCACCGAGGCGGT
CAACGAGCCCGATCTACCGCTGGTCGAGGTGATCAGGCGACTGCTGGAGGGCTACGGTGACCGCCCGG
CACTCGGCCAGCGCGCCTTCGAGTTCGTCACCGGGGACGACGGTGCGACCGTGATCGCGCTGAAGCCC
GAATACACCACCGTCTCCTACCGCGAGTTGTGGGAACGTGCCGAGGCTATCGCTGCCGCGTGGCACGAG
CAGGGCATCCGTGACGGCGACTTCGTCGCTCAGTTGGGTTTCACCAGCACGGACTTCGCGTCGCTCGAC
GTCGCGGGATTGCGTCTGGGCACCGTCTCGGTGCCCCTGCAGACGGGCGCGTCGCTGCAGCAGCGCAA
CGCGATTCTCGAAGAGACCCGGCCCGCAGTCTTTGCCGCGAGTATCGAATACCTTGATGCCGCCGTCGA
TTCGGTGCTTGCGACCCCCTCGGTGCGACTCCTCTCGGTTTTCGACTATCACGCGGAGGTCGACAGCCAG
CGCGAAGCGCTGGAGGCTGTGCGGGCCCGGCTTGAGAGTGCCGGCCGGACGATCGTCGTCGAGGCCCT
GGCGGAGGCTCTCGCGCGGGGGCGGGACCTGCCCGCCGCGCCGCTGCCCAGTGCAGATCCCGATGCCT
TGCGTCTGCTCATCTACACCTCCGGCAGCACCGGTACCCCCAAGGGCGCCATGTATCCGCAATGGCTGGT
CGCCAACTTGTGGCAGAAGAAGTGGCTCACCGACGATGTGATTCCGTCCATAGGCGTGAACTTCATGCC
CATGAGCCACCTGGCGGGTCGCCTCACTCTCATGGGCACCCTTTCCGGTGGCGGAACCGCCTACTACATC
GCTTCGAGCGATCTTTCGACTTTCTTCGAGGACATCGCGCTCATCCGCCCCTCCGAAGTGCTCTTCGTGCC
GCGTGTGGTGGAGATGGTGTTCCAGCGTTTTCAGGCAGAATTGGACCGGTCCCTTGCCCCGGGTGAGA
GCAACTCCGAGATCGCGGAGCGAATCAAGGTCCGCATCCGGGAACAGGACTTCGGCGGGCGTGTGCTC
AGTGCTGGCTCCGGGTCGGCCCCGTTGTCTCCTGAGATGACGGAGTTCATGGAGTCGCTGCTGCAGGTG
CCGTTGCGCGACGGGTATGGGTCCACCGAGGCCGGTGGTGTGTGGCGTGACGGAGTCCTGCAGCGTCC

GCCCGTCACCGACTACAAGCTGGTTGACGTTCCGGAACTCGGATACTTCACCACAGATTCGCCGCATCCC
CGTGGCGAGCTGCGGTTGAAGTCGGAGACGATGTTCCCCGGCTACTACAAGCGCCCGGAGACCACTGC
CGATGTCTTCGATGACGAGGGGTACTACAAGACCGGTGACGTGGTCGCCGAGCTCGGGCCGGATCACC
TCAAGTACCTCGACCGCGTCAAGAACGTCCTCAAGCTCGCGCAGGGAGAGTTTGTCGCGGTGTCAAAGC
TGGAGGCCGCTTACACCGGCAGCCCGCTGGTCCGGCAGATCTTTGTGTACGGGAACAGTGAACGCTCGT
TCCTGCTGGCTGTCGTGGTCCCGACACCCGAAGTCCTTGAGCGGTACGCAGATTCGCCAGATGCGCTCA
AGCCCTTGATCCAGGATTCGCTGCAGCAGGTCGCCAAGGACGCGGAGCTGCAATCCTATGAGATACCGC
GCGACTTCATCGTTGAGACGGTGCCGTTCACCGTCGAGTCCGGATTGCTATCGGACGCGCGAAAGCTGC
TGCGCCCCAAGCTGAAGGATCACTACGGAGAGAGGCTGGAGGCGCTGTACGCCGAACTGGCGGAAAG
CCAGAATGAGCGGCTGCGCCAGTTGGCCAGGGAGGCAGCCACGCGCCCGGTCCTGGAGACGGTGACC
GATGCGGCCGCCGCGCTGCTGGGCGCATCGTCCTCGGATCTGGCTCCTGATGTGCGATTCATCGACCTC
GGTGGCGACTCACTGTCGGCGCTGTCGTACTCCGAGCTGCTGCGCGACATCTTTGAGGTGGACGTTCCG
GTGGGCGTCATCAACAGCGTCGCCAACGACCTTGCCGCGATCGCCCGGCACATCGAGGCGCAGCGGAC
CGGCGCCGCTACGCAGCCGACCTTTGCGTCGGTCCACGGCAAGGACGCGACGGTCATCACCGCCGGTG
AACTCACCCTCGACAAGTTCTTGGACGAGTCACTGTTGAAAGCGGCCAAGGACGTTCAGCCGGCAACGG
CCGATGTCAAGACCGTTCTAGTGACCGGCGGCAACGGCTGGTTGGGTCGTTGGCTGGTGCTCGATTGGC
TGGAGCGGTTGGCACCCAATGGTGGCAAGGTCTACGCCCTCATTCGTGGCGCCGATGCCGAAGCAGCC
CGGGCACGGTTGGACGCCGTGTACGAATCGGGTGATCCCAAGCTGTCCGCGCATTATCGTCAGCTGGCG
CAACAGAGTCTGGAAGTTATCGCCGGCGATTTCGGCGACCAGGATCTCGGTCTATCCCAGGAAGTTTGG
CAGAAGCTGGCCAAGGACGTGGACCTGATCGTGCACTCCGGTGCCTTGGTGAACCACGTGCTGCCGTAC
AGCCAGTTGTTCGGTCCGAATGTGGCGGGTACCGCCGAGATCATCAAGCTGGCAATTTCGGAGCGGCTC
AAGCCGGTCACCTACCTGTCGACGGTGGGCATCGCCGACCAGATTCCGGTGACGGAGTTCGAGGAAGA
CTCCGATGTTCGTGTGATGTCGGCCGAGCGCCAGATCAATGACGGCTACGCGAACGGATACGGCAACTC
AAAATGGGCCGGCGAGGTGCTGTTGCGGGAGGCTCATGACCTAGCGGGGCTGCCGGTGCGTGTGTTCC
GCTCCGACATGATCCTGGCGCACAGTGACTACCACGGACAGCTCAACGTCACCGACGTGTTCACCCGGA
GCATCCAGAGTCTGCTGCTCACCGGTGTTGCACCGGCCAGCTTCTATGAATTGGATGCCGACGGCAATC
GGCAGCGCGCTCACTATGACGGTGTGCCCGGCGATTTCACCGCCGCATCGATCACCGCCATCGGCGGTG
TGAACGTGGTAGACGGTTACCGCAGCTTCGACGTGTTCAACCCGCACCATGACGGTGTCTCGATGGATA
CCTTCGTCGACTGGCTGATCGACGCAGGCTACAAGATCGCGCGGATCGACGATTACGACCAGTGGCTCG
CCCGGTTCGAGCTGGCCCTCAAGGGATTGCCCGAGCAGCAGCGGCAACAGTCGGTGTTGCCACTTCTCA
AGATGTACGAGAAGCCGCAACCGGCGATCGACGGAAGTGCACTTCCGACCGCAGAATTCAGTCGCGCC
GTGCACGAGGCGAAGGTCGGAGACAGCGGTGAGATACCGCACGTCACCAAGGAGCTGATCCTCAAGTA
CGCCAGCGATATTCAGCTGTTGGGCCTGGTGTAG

Secondary alcohol dehydrogenase from *lactobacillus brevis* (r-ADH)

Codon optimized DNA sequence is given below:

ATGAGTAATCGTCTAGATGGTAAAGTAGCAATCATTACCGGGGGAACTCTGGGAATAGGTTTAGCAATC
GCCACAAAGTTCGTTGAGGAGGGGGCAAAAGTGATGATTACCGGTCGTCACAGCGATGTGGGTGAAAA
GGCGGCTAAATCTGTCGGCACTCCTGATCAGATCCAGTTTTTTCAACATGATTCATCCGATGAAGATGGA
TGGACGAAACTTTTCGATGCGACGGAAAAGGCCTTTGGACCAGTTAGTACACTCGTGAATAACGCAGGG
ATCGCGGTTAACAAGTCAGTCGAGGAAACCACAACTGCAGAATGGCGTAAACTGTTAGCCGTCAACCTG
GATGGTGTCTTTTTTGGTACCAGATTGGGTATTCAGCGCATGAAAAATAAGGGCTTAGGCGCCTCGATA
ATCAATATGTCTTCGATTGAAGGGTTTGTGGGCGACCCTAGCTTAGGCGCTTATAATGCAAGCAAAGGG
GCCGTACGCATTATGTCCAAATCAGCTGCCTTAGACTGCGCCCTGAAAGATTACGATGTTCGCGTAAATA
CCGTACATCCGGGATACATTAAAACACCGCTTGTTGATGACCTGCCGGGCGCAGAGGAGGCGATGTCAC
AACGGACCAAAACGCCCATGGGCCATATAGGAGAACCAAACGACATTGCTTATATTTGTGTTTATTTGGC
TTCTAACGAAAGTAAATTTGCAACGGGTTCTGAATTCGTGGTGGACGGTGGCTATACTGCGCAGTAG

Module 1 (oxidation module)				Module 2 (amination module)			
Entry	**Recombinant *E. coli* strain**		**Plasmid**	**Entry**	**Recombinant *E. coli* strain**		**Plasmid**
1	*E. Coli* (M1A)	M1A		**5**	*E. Coli* (M2A)	M2A	
2	*E. Coli* (M1B)	M1B		**6**	*E. Coli* (M2B)	M2B	
3	*E. Coli* (M1C)	M1C		**7**	*E. Coli* (M2A_M2B)	M2A M2B	
4	*E. Coli* (M1A_M1B)	M1A M1B		**8**	*E. Coli* (M2C)	M2C	
				9	*E. Coli* (M1C_M2C)	M1C M2C	

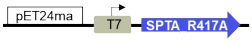
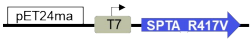
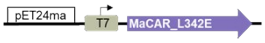
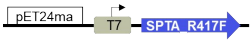
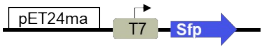
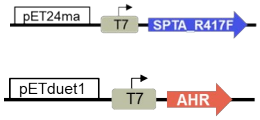
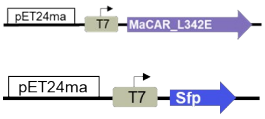
Module 2 (amination module)				Module 3 (reduction module)			
Entry	**Recombinant *E. coli* strain**		**Plasmid**	**Entry**	**Recombinant *E. coli* strain**		**Plasmid**
10	*E. Coli* (M2D)	M2D		**14**	*E. Coli* (M3A)	M3A	
11	*E. Coli* (M2E)	M3E		**15**	*E. Coli* (M3B)	M3B	
12	*E. Coli* (M2F)	M2F		**16**	*E. Coli* (M3C)	M3C	
13	*E. Coli* (M2F_M2A)	M2F M2A		**17**	*E. Coli* (M3B_M3C)	M3B M3C	

Figure S1. Design and construction of *E. coli* cells expressing or co-expressing desired enzymes involved in O_m and A_m

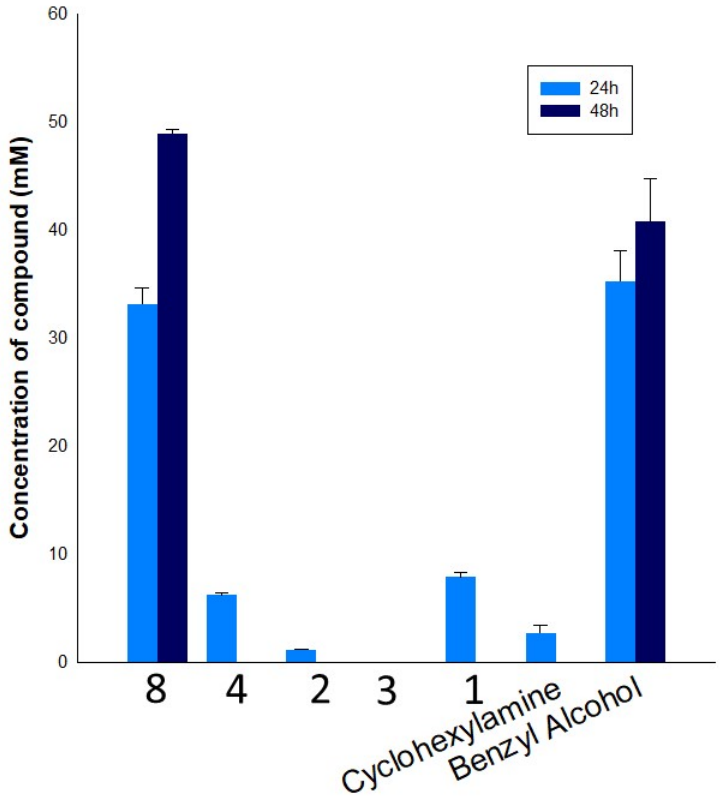


Figure S2. One-pot combined O_m_A_m reaction in potassium phosphate buffer to produce 6-AmHA (**8**) from Cyclohexanol. Reaction conditions (Total volume: 1 mL): 50 mM **1a**, 100 mM benzylamine, 0.2 mM PLP, *E. coli* M1A_M1B (27 mg_{CDW}/mL), *E. coli* M1C_M2C (36 mg_{CDW}/mL), 100 mM potassium phosphate buffer (pH 7.5) at 30 °C.

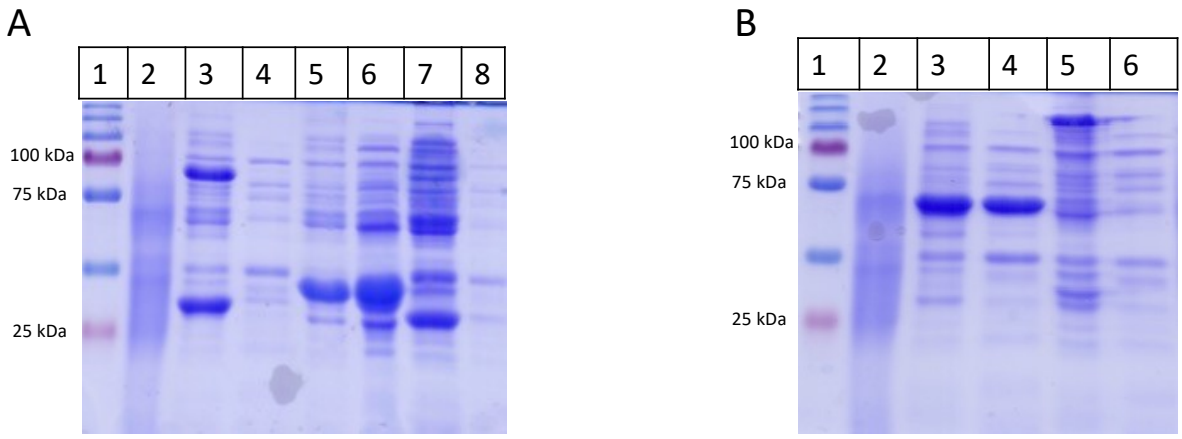


Figure S3. SDS-PAGE analysis of whole-cell expressions of desired proteins from O_m and A_m. A): Lane 1 Marker (M); Lane 2 E. coli whole-cell; Lane 3. E. coli (ADH, AcCHMO); Lane 4. E. coli (ADH, AcCHMO) soluble; Lane 5. *E. coli* (Lactonase); Lane 6. *E. coli* (Lactonase) soluble; Lane 7. *E. coli* (ADH, AcCHMO, Lactonase); Lane 8. *E. coli* (ADH, AcCHMO, Lactonase) soluble. B) Lane 1 Marker (M); Lane 2 *E. coli* whole-cell; Lane 3. *E. coli* (SPTA_R417F, AHR); Lane 4. *E. coli* (SPTA_R417F, AHR) soluble; Lane 5. *E. coli* (CARL342E, Sfp); Lane 6. *E. coli* (CARL342E, Sfp) soluble.

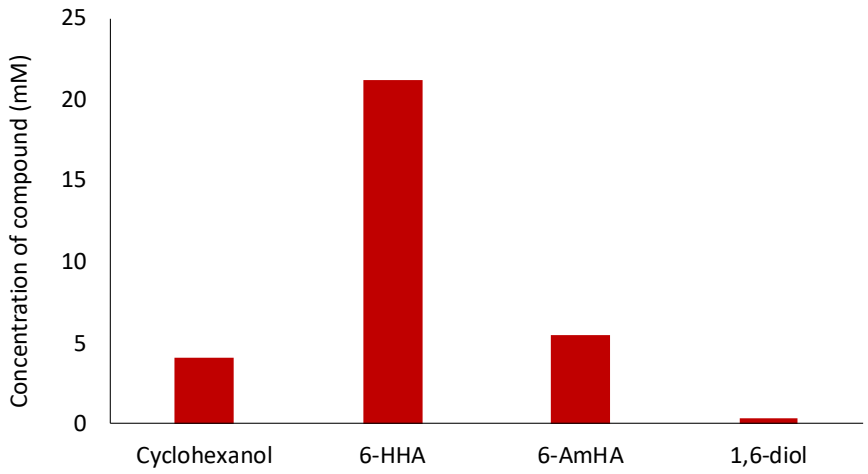


Figure S4. Initial one-pot one-step reaction using wild type SPTA and MaCAR to produce HMD from cyclohexanol. Reaction conditions (Total volume: 25 mL): 50 mM **1a**, 200 mM benzylamine, 0.2 mM PLP, *E. coli* M1A_M1B (18 mg_{CDW}/mL), *E. coli* M1C (6 mg_{CDW}/mL), *E. coli* M3A_M3C (27 mg_{CDW}/mL), *E. coli* M2A_M2B (18 mg_{CDW}/mL), 100 mM potassium phosphate buffer (pH 7.5) at 30°C.

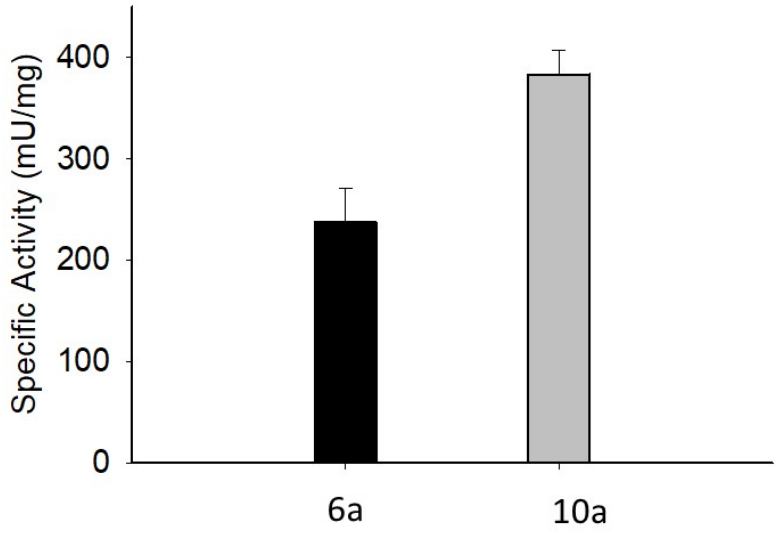


Figure S5. Specific activities of AHR towards 1,6-diol (**6a**) and 6-aminol (**10a**). Reaction conditions (Total volume: 0.5 mL): 5 mM substrate, 0.2 mM NADP+, 0.1 mg/mL AHR, 100 mM Tri-HCl buffer (8.0) 30°C. Absorbance was measured at 340 nm for 1 min.

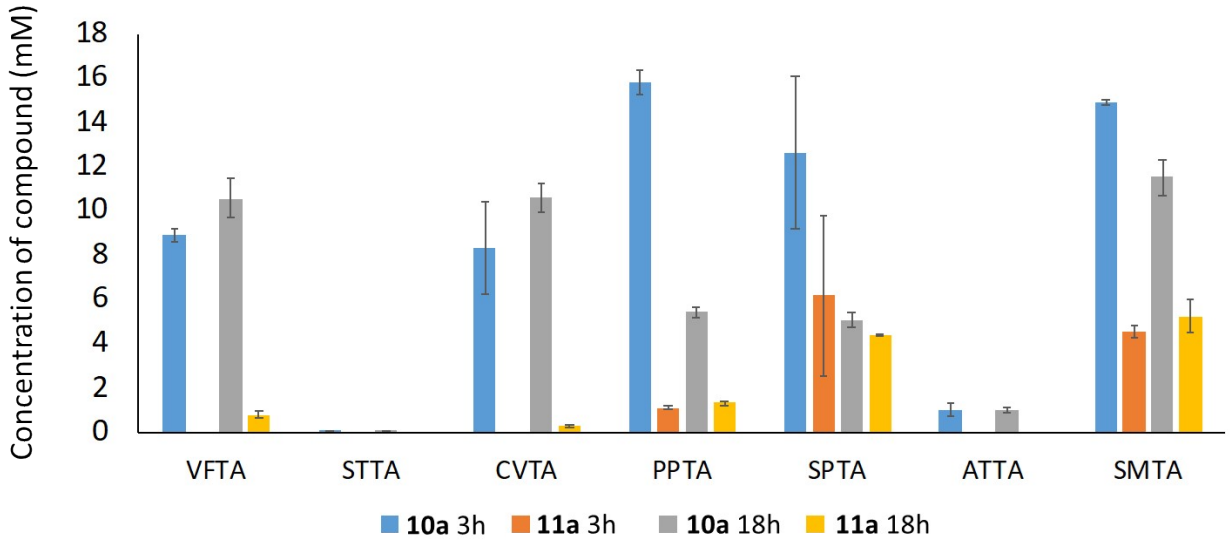


Figure S6. Screening of various TAs towards 1,6-diol (**6a**) Reaction condition (Total volume: 1 mL): 20 mM 1,6-diol, 40 mM benzylamine, 9 mg_{CDW}/mL E. coli M2A_M2B, 100 mM Tri-HCl buffer (8.0), 30 °C.

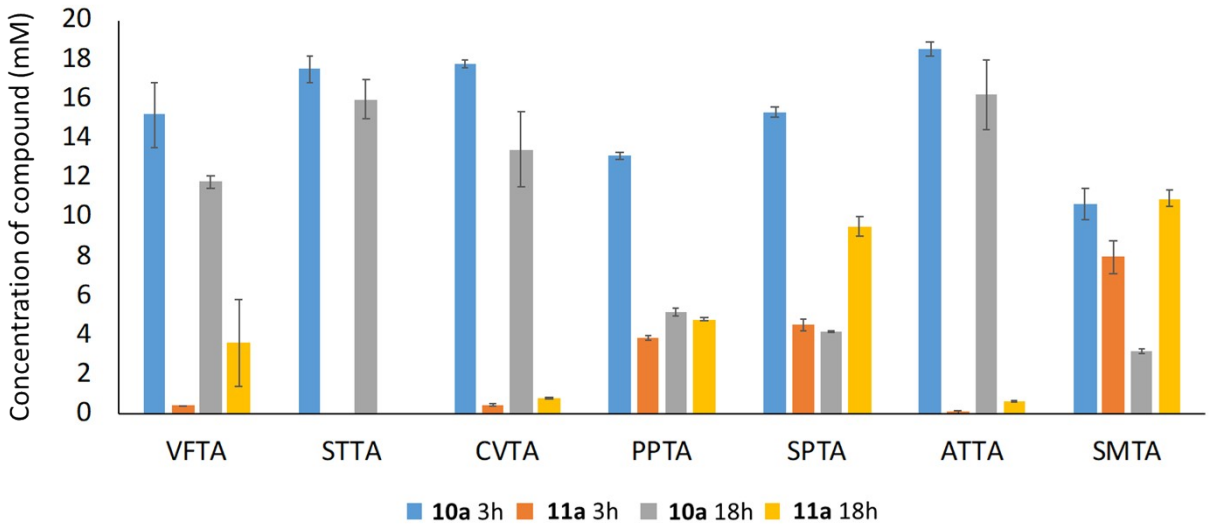


Figure S7. Screening of various TAs towards 6-aminol (**10a**) Reaction condition (Total volume: 1 mL): 20 mM 6-aminol, 40 mM benzylamine, 9 mg_{CDW}/mL E. coli M2A_M2B, 100 mM Tri-HCl buffer (8.0), 30 °C.

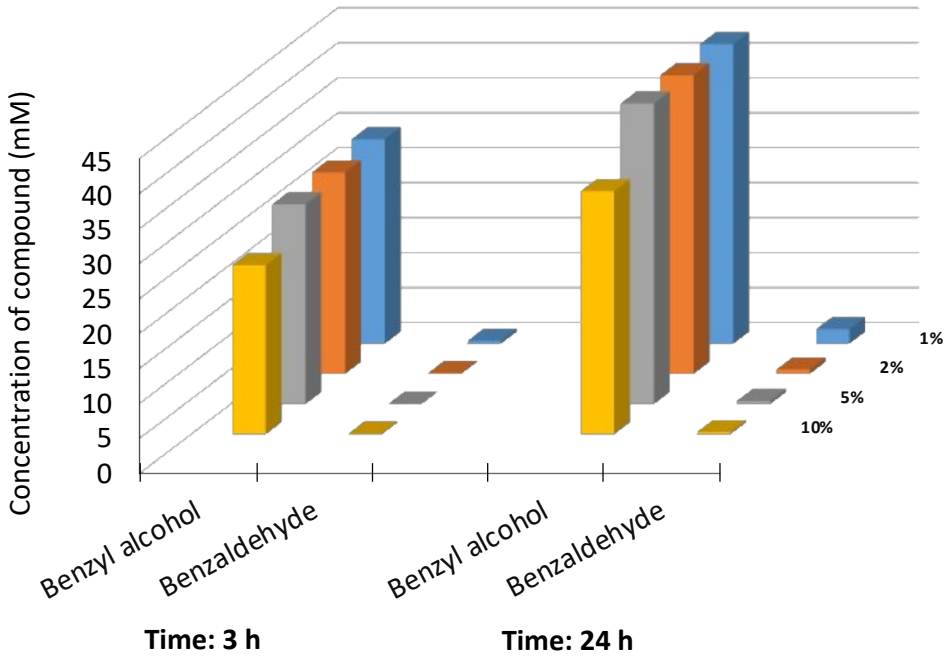


Figure S8. Effect of glucose concentration for cofactor recycling and formation of intracellular pyruvate in the cascade. Reaction conditions (Total volume: 25 mL): 200 mM benzylamine, *E. coli* M1C_M2C (27 mg_{CDW}/mL), *E. coli* M3B_M3C (27 mg_{CDW}/mL), 10 mM, $MgCl_2$, 1-10% (w/v) Glucose, 100 mM potassium phosphate buffer (7.5), 30 °C.

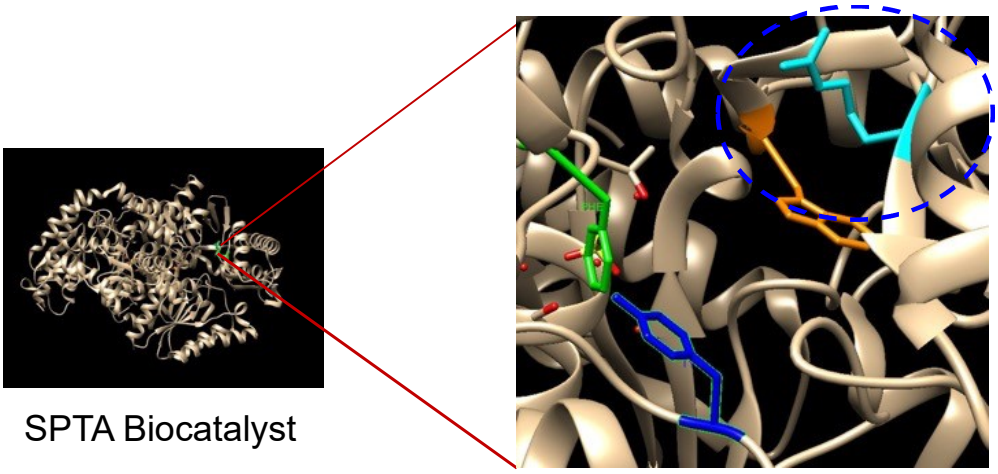


Figure S9. Active site of SPTA biocatalyst interacting with pyruvate substrate displaying arginine 417 as key interacting residue.[5]

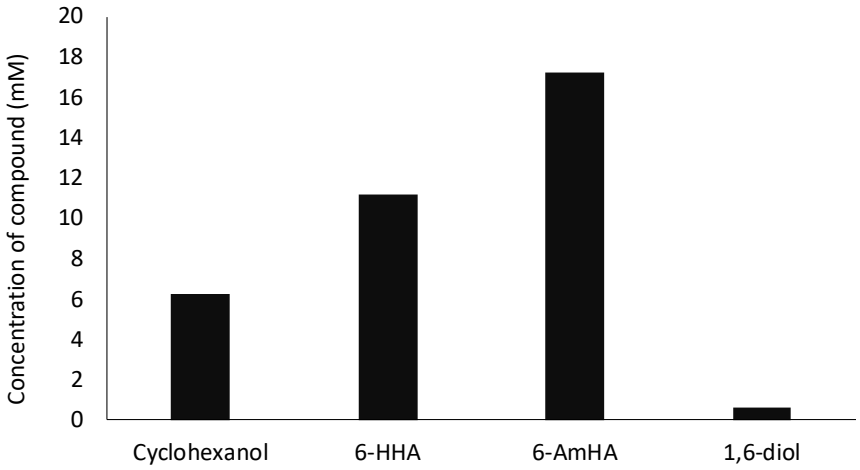


Figure S10. One-pot one-step reaction using SPTA_R417F and wild type MaCAR to produce HMD from cyclohexanol (Total volume: 10 mL). Reaction conditions: 50 mM **1a**, 200 mM benzylamine, 0.2 mM PLP, 1% glucose, *E. coli* M1A_M1B (18 mg_{CDW}/mL), E. coli M1C (6 mg_{CDW}/mL), *E. coli* M3A_M3C (27 mg_{CDW}/mL), *E. coli* M2F_M2A (18 mg_{CDW}/mL), 100 mM potassium phosphate buffer (pH 7.5) at 30°C.

Experimental procedure for sequential one-pot two step reaction for the synthesis of HMD (11a) (Figure 3A in main text):

Reaction conditions: 20 mM cyclohexanol (**1a)**, 80 mM benzylamine, 0.2 mM PLP, 1% (w/v) glucose, *E. coli* M1A_M1B (18 mg_{CDW}/mL), *E. coli* M1C (6 mg_{CDW}/mL), *E. coli* M3B_M3C (27 mg_{CDW}/mL), *E. coli* M2A_M2B (18 mg_{CDW}/mL), 100 mM potassium phosphate buffer (pH 7.5) at 30°C.

The reaction was performed at 25 mL of initial volume (step 1) which contains 20 mM cyclohexanol (**1a)**, 1% (w/v) glucose, whole cell biocatalyst from oxidation and reduction modules named as *E. coli* M1A_M1B (18 mg_{CDW}/mL), *E. coli* M1C (6 mg_{CDW}/mL), and *E. coli* M3B_M3C (27 mg_{CDW}/mL). The step 1 gave 7 mM of 1,6-diol as end product. After completion of step 1 the reaction was stopped by heating at 60 degrees for 10 min. Next, the 10 mL of step 1 reaction mixture was subjected for step 2 and reaction components from amination module were added which make up the total volume of reaction to 15 mL. The concentrations of final product and the reaction intermediates were calculated and multiplied by the dilution factors. This step 2 produced 3.8 mM of HMD (**11a**).

Site-directed mutagenesis:

Starting from pET24ma plasmid encoding desired cyclase and primers harbouring 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 Tm 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 harbouring the correct mutations identified by sequencing. For protein expression, pET24ma plasmid encoding desired CAR variant was transformed into *E. coli* BW25113 (DE3) Δ*fad*D strain and a single colony was used to inoculate an overnight culture for protein expression and purification[6].

Figure S11. Multiple sequence alignments confirming the MaCAR_L342E variant

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) trifluoro-acetamide) was added for derivatization followed by incubation at 55 °C for 45 min.

Sample preparation:

Preparation for analysis of 4,6, 10 and 11:

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.

Method 1: (For α, ω- diamine and ω- amino alcohols):

Injector 200°C; flow 1.5 mL/min; Temperature program 100°C/hold 0 min; 15°C per min to 180 °C/ hold 0 min and 5°C per min to 280°C / hold 5 min.

Table S3. The retention times of all observed reaction intermediates after derivatization.

Entry	Compound		Retention time	Method	Company
1	6-amino hexanoic acid	**8**	8.87	1	Alfa- aesar
2	ε-caprolactone	**3**	9.17	2	Sigma-Aldrich
3	6-hydroxyhexanoic acid	**4**	6.34	1	Alfa-aesar
4	Cyclohexylamine	**-**	4.78	2	Sigma-Aldrich
5	Cyclohexanol	**1**	5.18	2	Sigma-Aldrich
6	Cyclohexanone	**2**	5.25	2	Sigma-Aldrich
7	Benzylamine	**-**	7.18	2	Sigma-Aldrich
8	Benzyl alcohol	**-**	7.44	2	Sigma-Aldrich
9	6-aminol	**10**	7.5	3	Sigma-Aldrich
10	1,6-diol	**6**	12	2	Tokyo chemical industry
11	HMD	**11a**	10.5	3	Sigma-Aldrich
12	1,7-heptanediol	**6b**	13.2	2	Tokyo chemical industry
13	1,7-heptanediamine	**11b**	11.6	3	Tokyo chemical industry
14	8-aminol	**10c**	9.2	3	Tokyo chemical industry
15	1,8-octanediamine	**11c**	13.0	3	Sigma-Aldrich

Spectroscopic analysis:

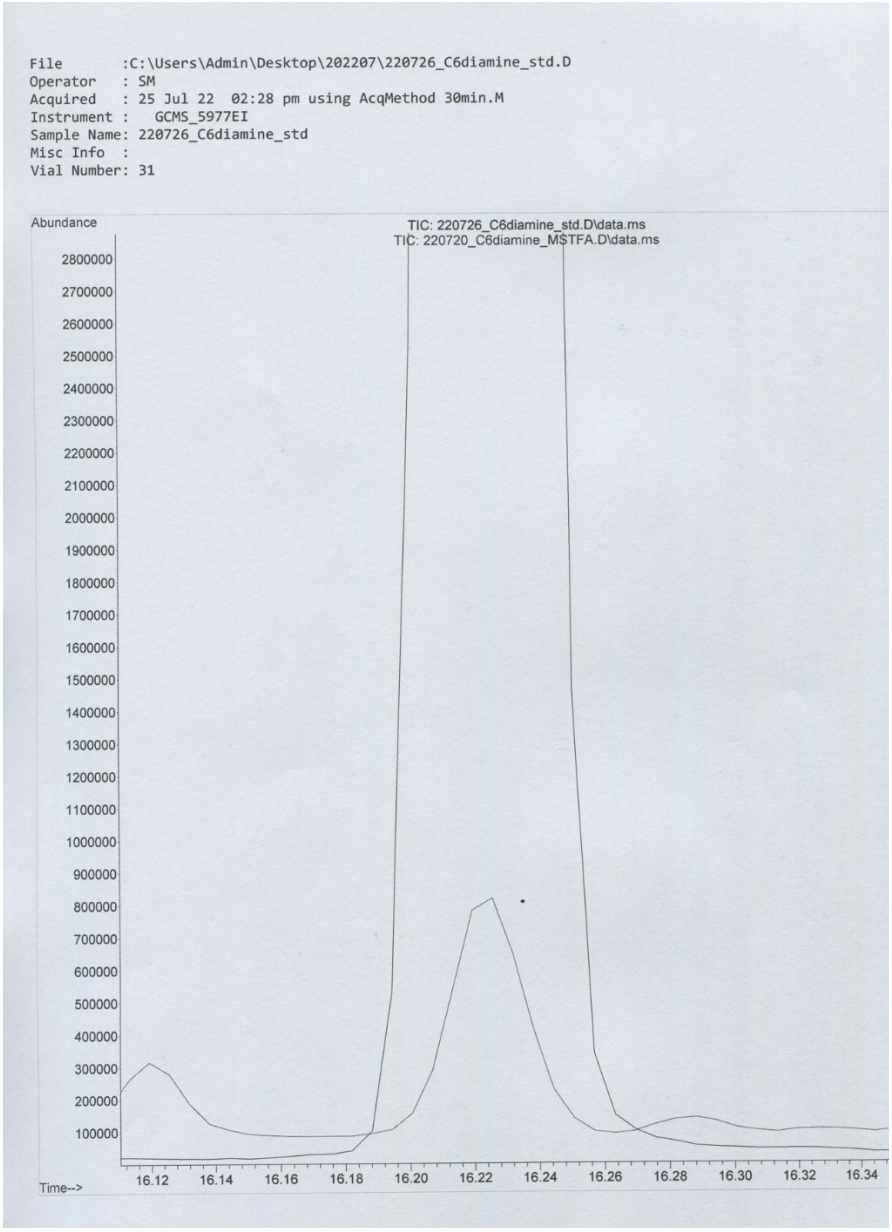


Figure S12. GC-MS analysis for **11a** overlayed with standard.

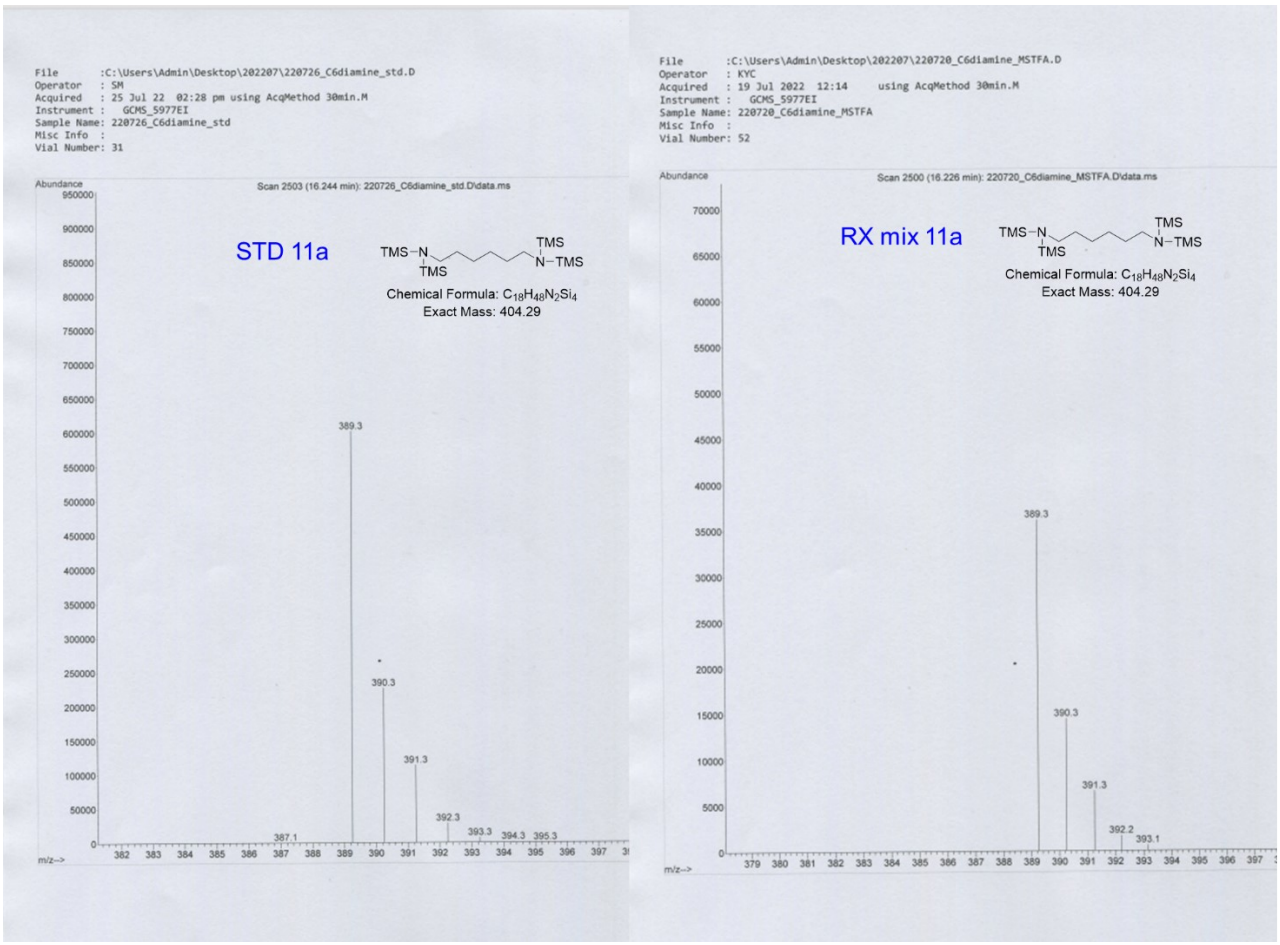


Figure S13. GC-MS analysis for **11a** overlayed with standard.

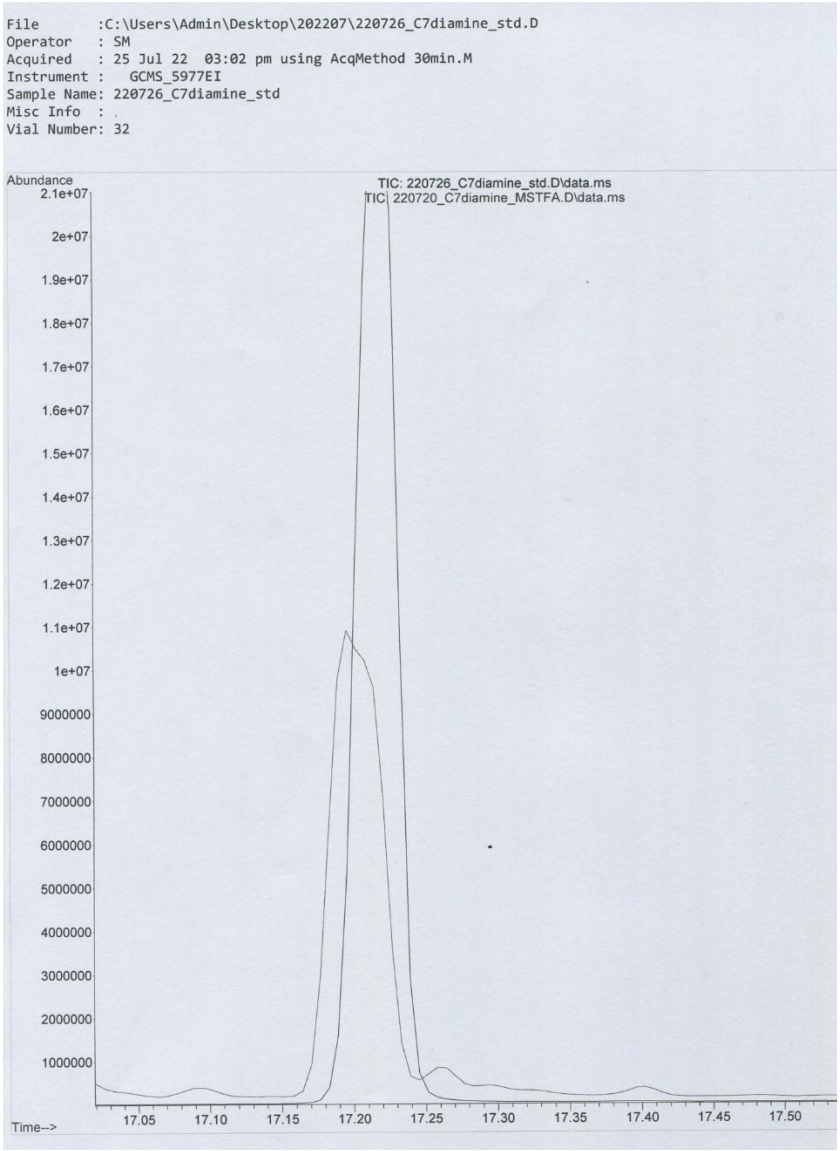


Figure S14. GC-MS analysis for **11b** overlayed with standard.

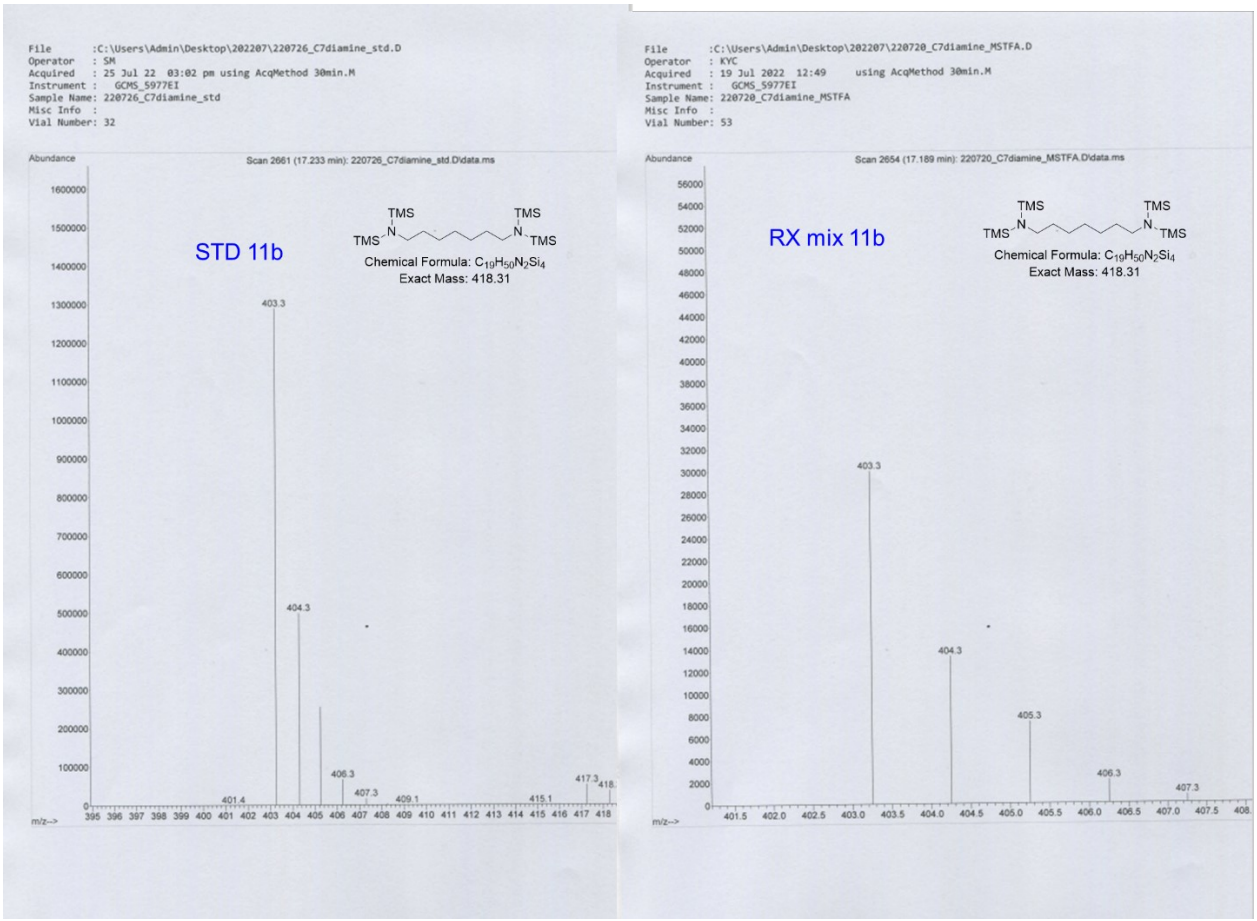


Figure S15. GC-MS analysis for **11b** overlayed with standard.

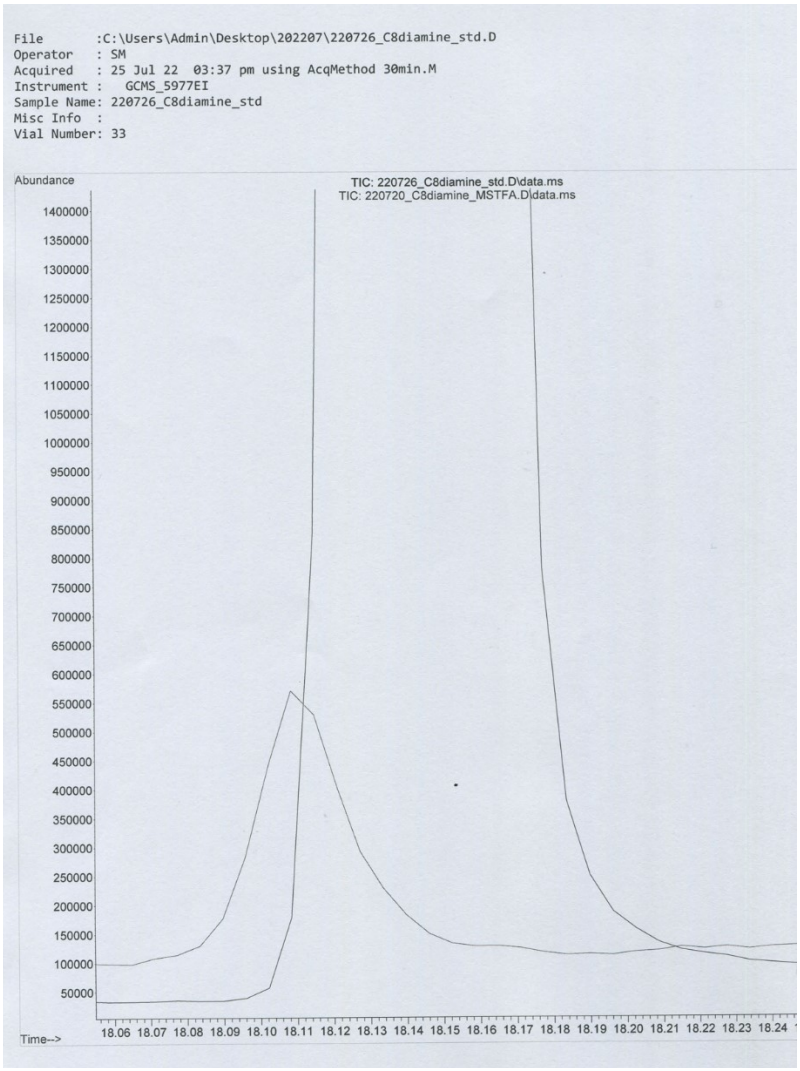


Figure S16. GC-MS analysis for **11c** overlayed with standard.

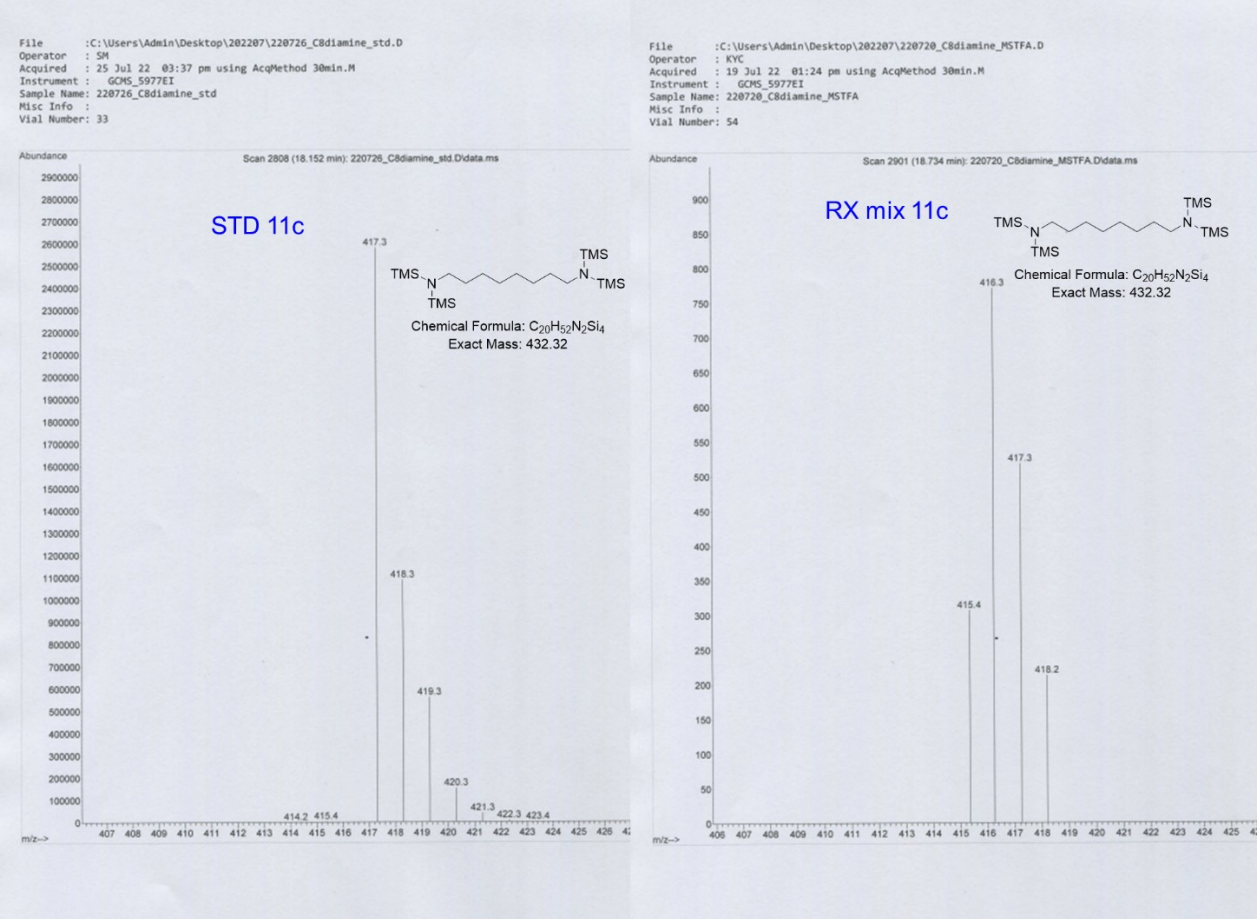


Figure S17. GC-MS analysis for **11c** overlayed with standard.

Figure S18. Gas Chromatogram showing peaks of substrate, desired product, and intermediates .

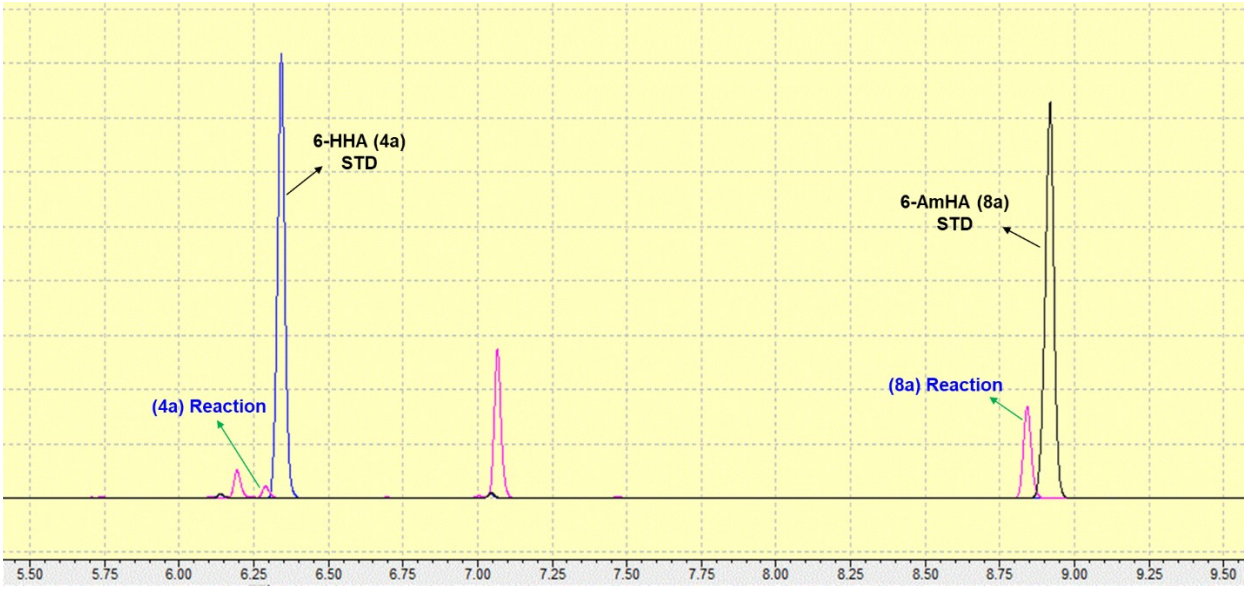


Figure S19. Gas Chromatogram showing desired products and intermediates (**4a** and **8a**).

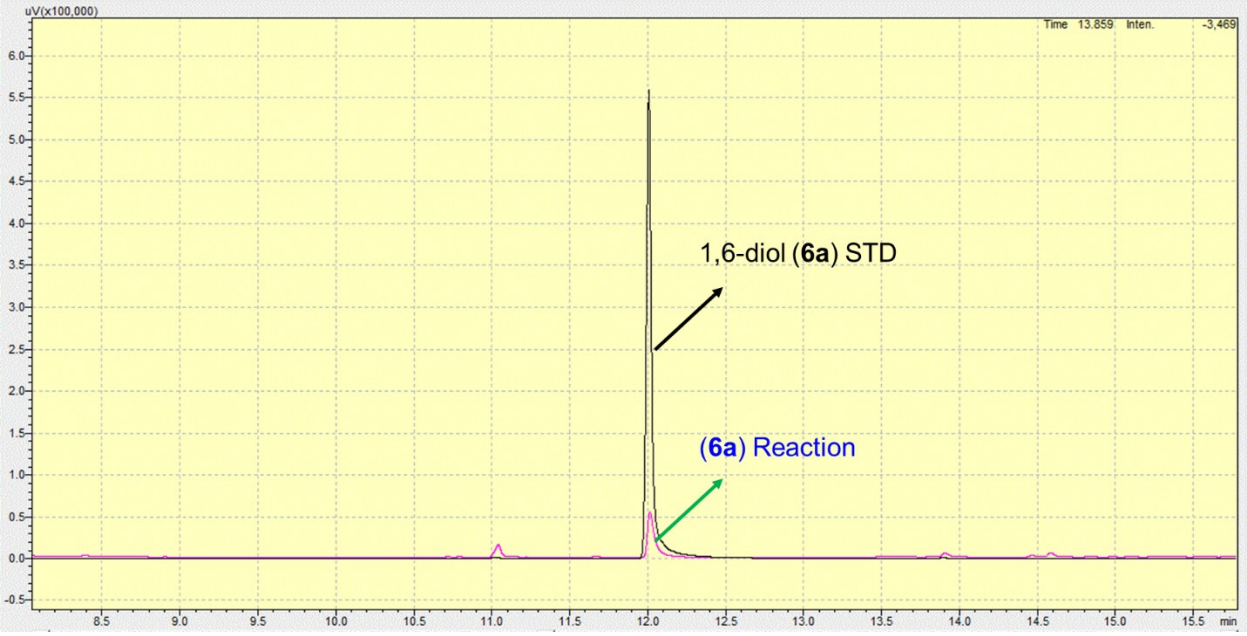


Figure S20. Gas Chromatogram showing desired products (**6a**).

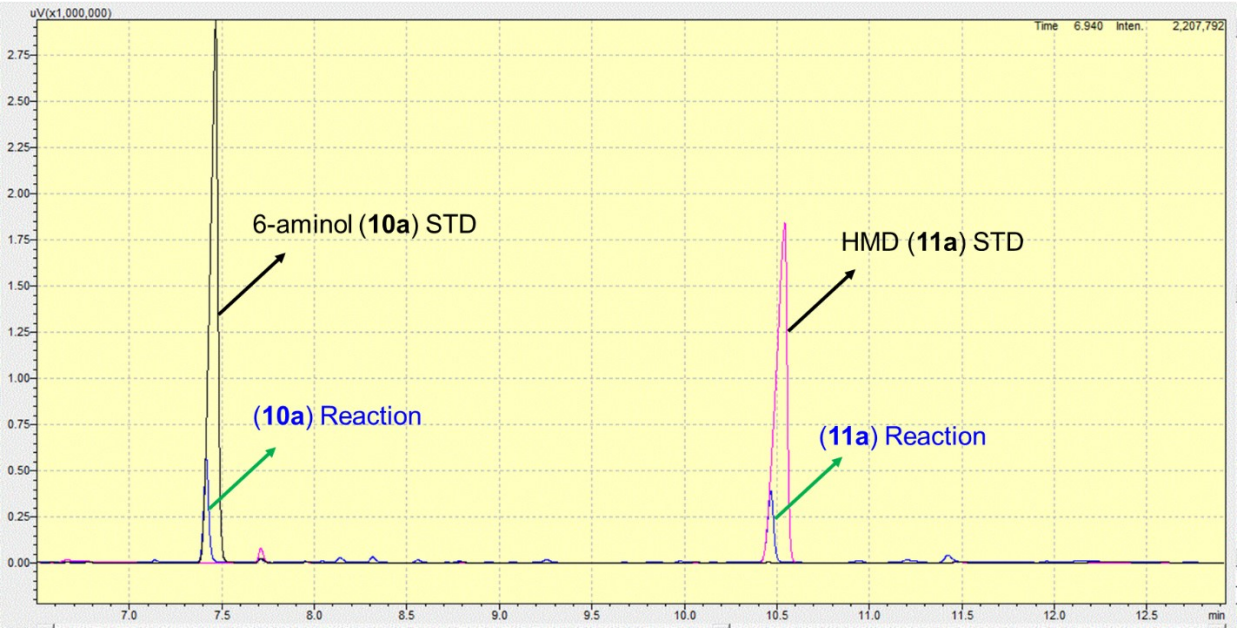


Figure S21. Gas Chromatogram showing desired products and intermediates (**10a** and **11a**).

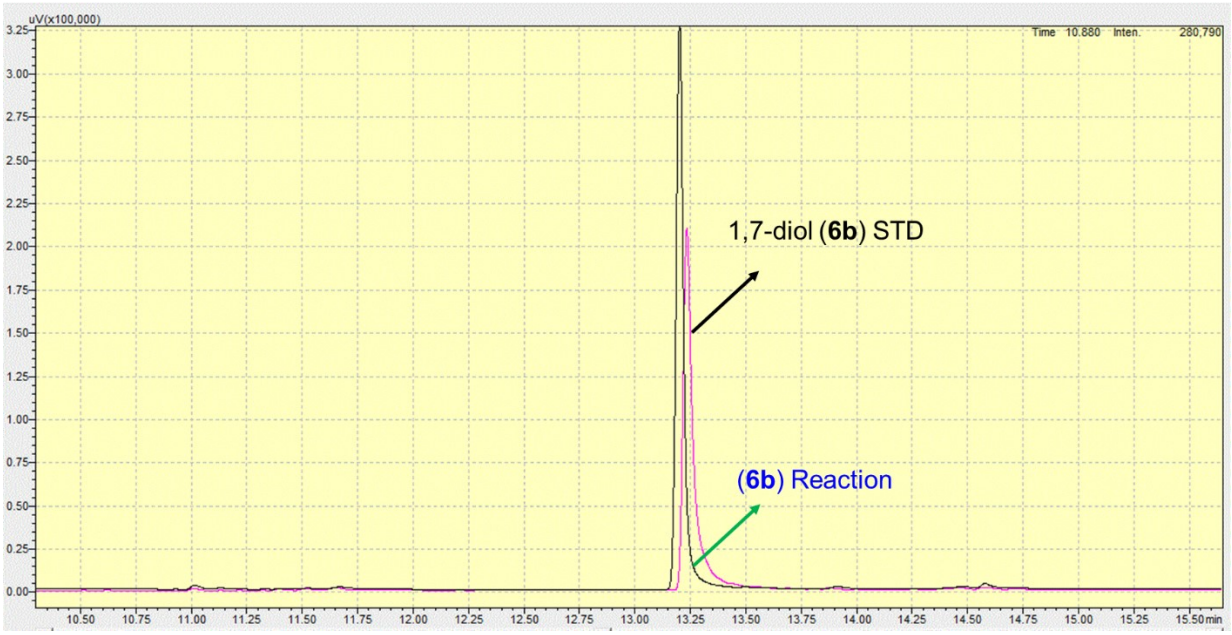


Figure S22. Gas Chromatogram showing desired products (**6b**).

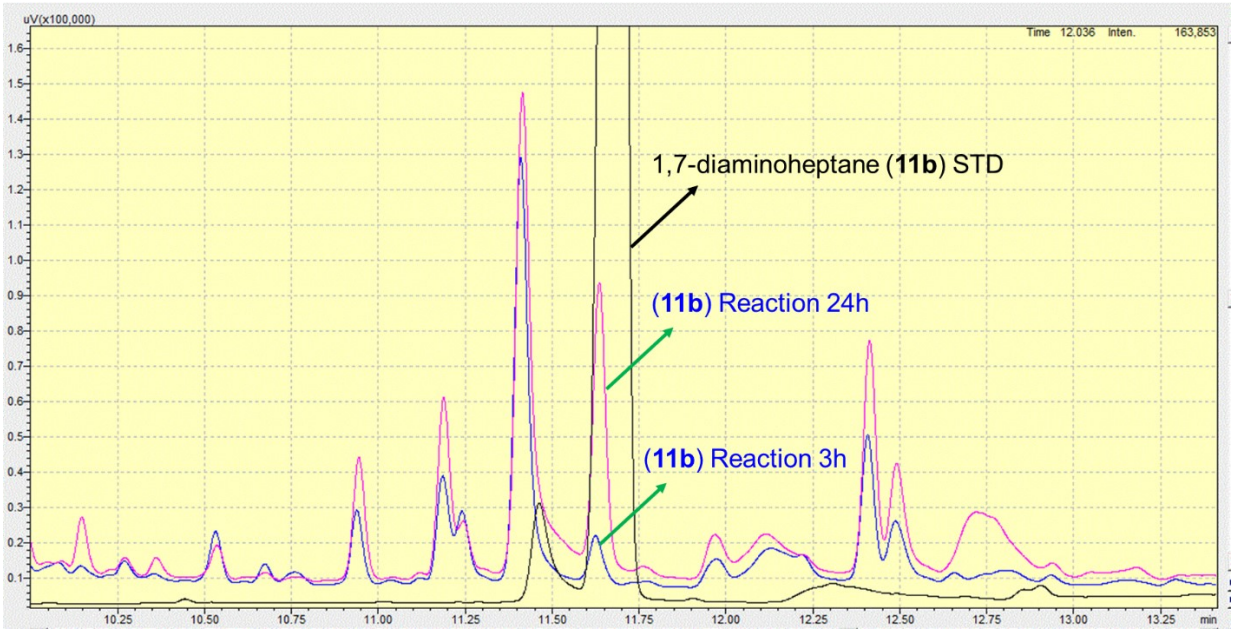


Figure S23. Gas Chromatogram showing desired products (**11b**).

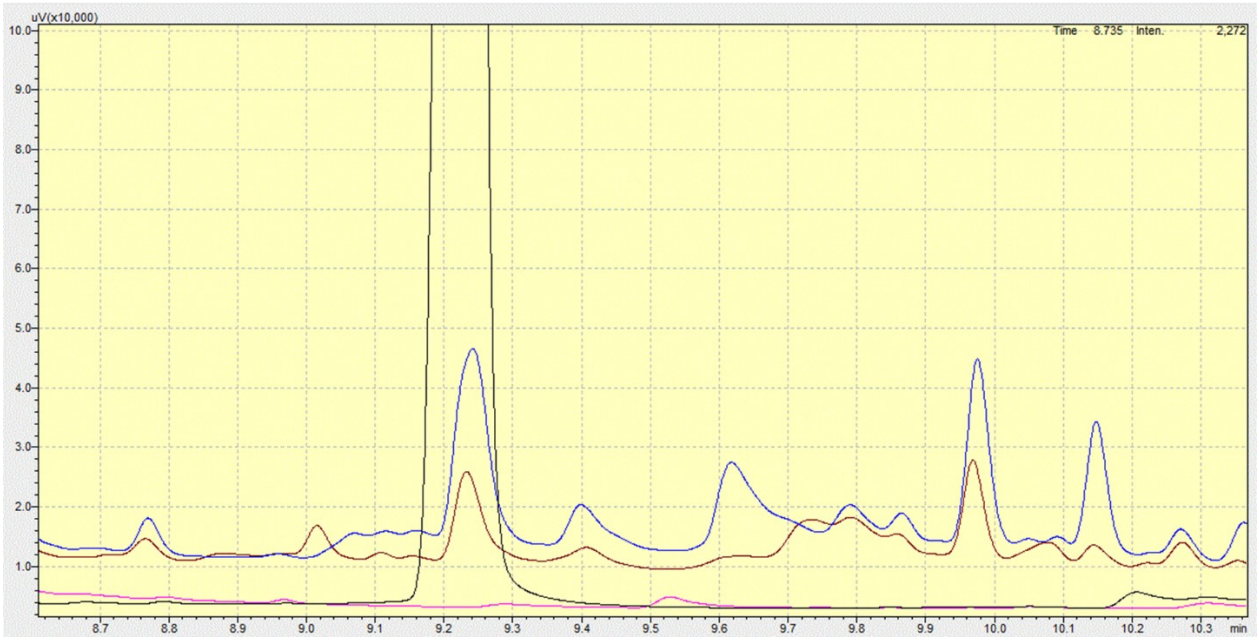


Figure S24. Gas Chromatogram showing desired products (**10c**).

References

[1] S. Sung, H. Jeon, S. Sarak, M. -M. Ahsan, M. D. Patil, W. Kroutil, B. G. Kim and H. Yun, *Green Chem.* 2018, **20**, 4591 – 4595.

[2] J. H. Sattler, M. Fuchs, F. G. Mutti, B. Grischek, P. Engel, J. Pfeffer, J. M. Woodley and W. Kroutil, *Angew. Chem. Int. Ed.* 2014, **53**, 14153 – 14157.

[3] M. K. Akhtar, N. J. Turner and P. R. Jones, *Proc. Natl. Acad. Sci. USA* 2013, **110**, 87 - 92.

[4] S. Sarak, S. Sung, H. Jeon, M. D. Patil, T. P. Khobragade, A. D. Pagar, P. E. Dawson and H. Yun, *Angew. Chem. Int. Ed.* 2021, **60**, 3481 – 3486.

[5] A. Łyskowski, C. Gruber, G. Steinkellner, M. Schürmann, H. Schwab, K. Gruber and K. Steiner, *PLoS One* 2014, **9**, e87350.

[6] T. P. Fedorchuk, A. N. Khusnutdinova, E. Evdokimova, R. Flick, R. Di-Leo, P. Stogios, A. Savchenko and A. F. Yakunin, *J. Am. Chem. Soc*. 2020, **142**, 1038–1048