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Supporting Information

Production of the Carboxylate Reductase from *Nocardia otitidiscaviarum* in a Soluble, Active Form for *in vitro* Applications

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1. Enzymes

The *No*CAR and *Ec*PPTase enzymes used in this work are listed in Table S1. The production and purification procedures are described in the experimental section of the main manuscript.

Table S1: Enzymes used in this study.

Entry	Enzyme	Organism of origin	Type	Accession Nr.	Size (kDa)	pET system	Ref.
1	*No*CAR	*Nocardia otitiscaviarum*	Bacterial	WP_029928026.1[1]	130.3	pET151	[2,3]
2	*Ec*PPTase	*Escherichia coli*	Bacterial	CAQ31055.1[4]	23.6	pET151	[5–7]

1.1. SDS gel analysis of the purified enzymes

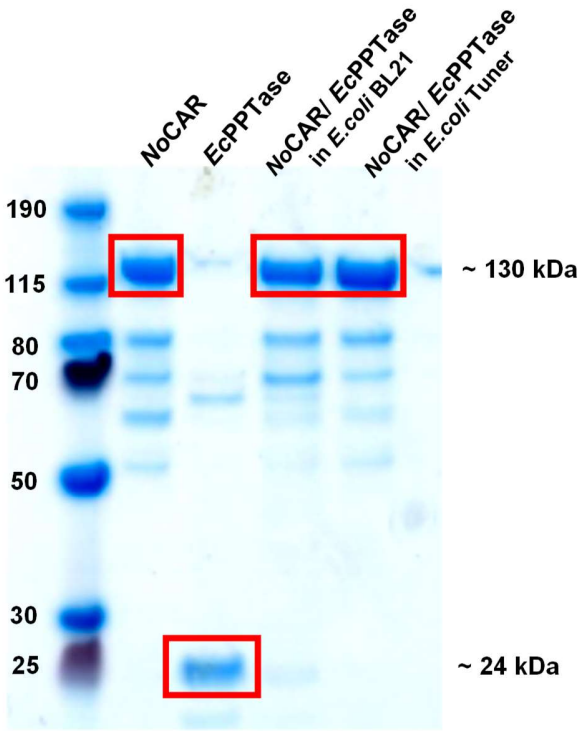

Figure S1. SDS gel analysis of the purified enzymes: *No*CAR expressed in *E.coli* Tuner, *Ec*PPTase expressed in *E.coli* Tuner, and the co-expressed *No*CAR/*Ec*PPTase expressed in *E.coli* BL21 or *E.coli* Tuner. It is important to mention that *Ec*PPTase in the petDuet does not have a His-tag.

2. Molecular biology

2.1. Vector maps

2.1.1. *No*CAR

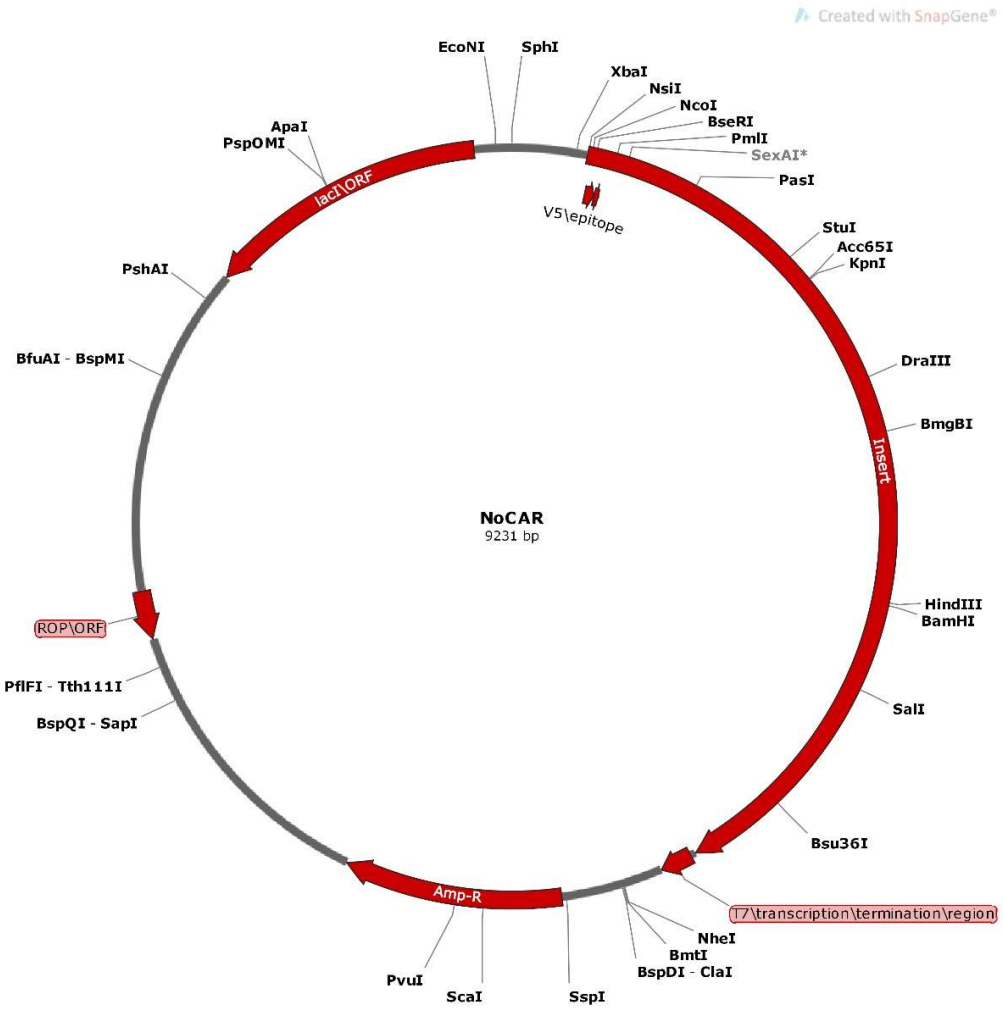

Figure S2. Illustration of the expression vector pET151 harboring the gene of interest coding for *No*CAR.

2.1.2. *Ec*PPTase

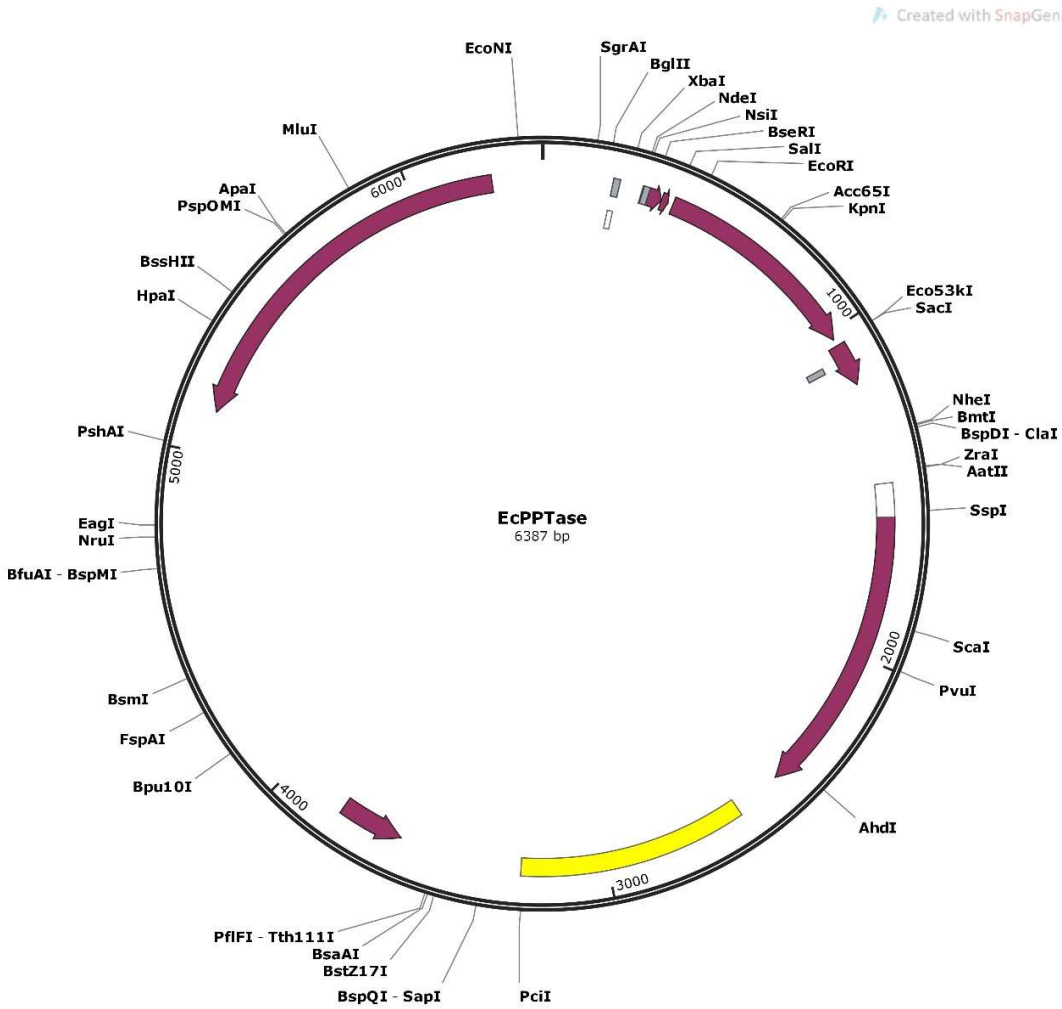

Figure S3. Illustration of the expression vector pET151 harboring the gene of interest coding for *Ec*PPTase.

2.1.3. *No*CAR/*Ec*PPTase

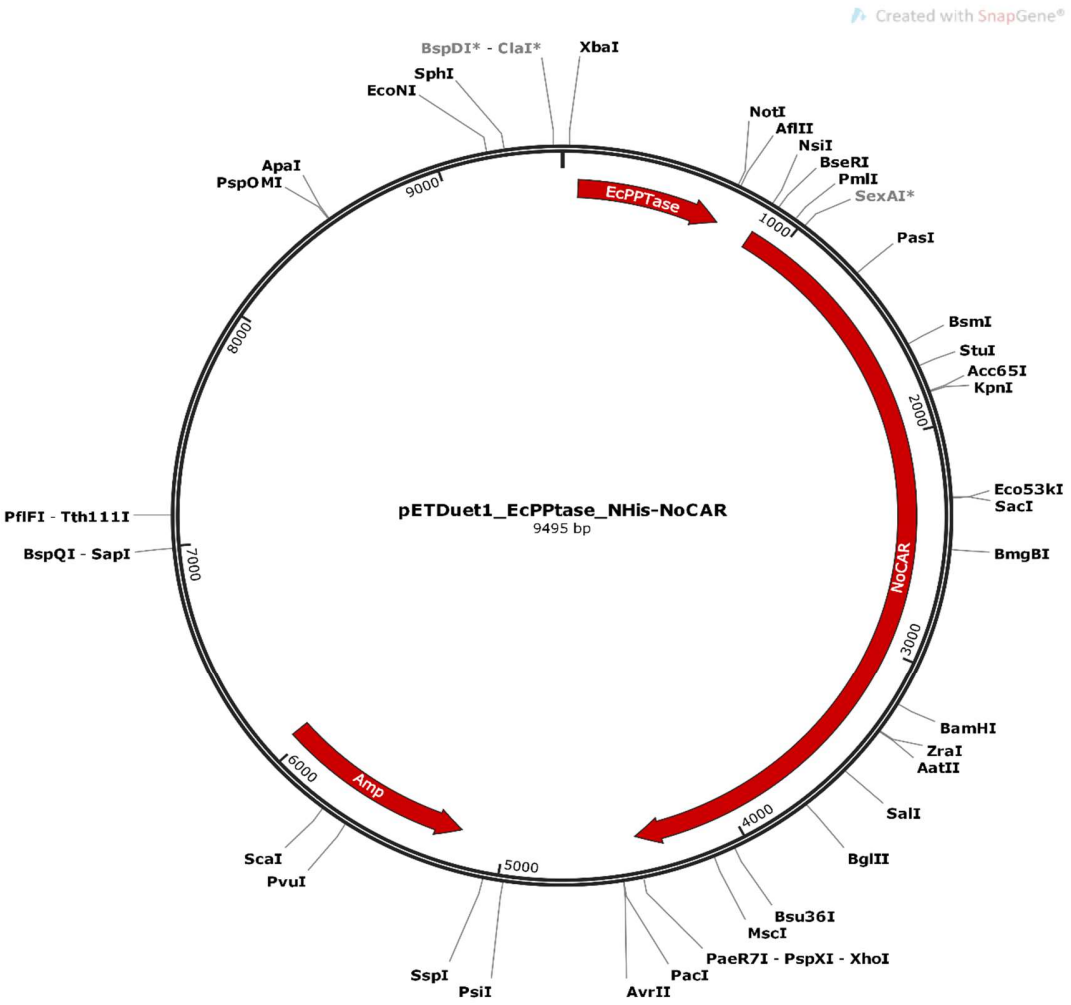

Figure S4. Illustration of the expression vector pETDuet harboring the genes of interest coding for *No*CAR and *Ec*PPTase.

2.2. Gene sequences

Table S2: Gene sequences used in this study. Inserts are highlighted in red and/or green.

Gene	Sequences
pET151_*No*CAR	CAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAA CAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATA GGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGG ATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACA ATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCATCATCACCATC ACCATGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACGGAAAACCTGTATTTTCA GGGAATTGATCCCTTCACCATGCTGGACGATGCACGTGCCGAACGTCGGGAACGTCGCATTG CGGACGCCTTAGCGGATGACCAGGTCGTGAAGCAGCCGCAGATGCCGCCGTTTCGGAAAGC GTCCGCCGTGTCGAAGTTCGCCTCGCTCGTATTGTTGATGCGGTGATGAGTGGCTACGGTGA CCGTGCCGCGTTAGCTTGGCGTCGTTCCGAACTGGTAGACGGCGCAGTTCGCCTCCTGCCGG AATACTCGACCATGACTTATCGTGAGCTGTGGCGTCAAGCGGGTGCTGTGGCGGCTGAATGG GGAGCGGATGCCGAAAGCCCTGTTCGCGCAGAAGATTTTGTGTGTACCCTGGGTTTTACGAGT CCGGACTACACCGTAGTGGACCTGGCCTTAATGCGTCTGGCCGCAGTTGCTGTTCCCCTTCAG GCGTCTGCCTCAGTTGCTCAGTGGCGCAGCATCATGGCCGAAACTGAACCGCGTATGCTTGC GGCGTCGGCGGAAACCCTGCCTGCCGCCGTGGAGGCTGTTCTGGGCGGTTTTGCGCCGCGT CGCGTGCTGGTCTTTGATTATCGCCCTGAACTTGAGGCACATCGTAGCGCCGTAGATAGCGCT CGTGAACGCCTGGCAGAAGTGGGTTGCACAGTAGCCACAGTTGCAGATGCCGTGGATCGCGG CGCTAATCTGCCAGCACCGCTTCGCATTCCCAGTGATCGCGAACGCTTGGCGTTATTAATTTA CACGAGCGGATCGACGGGAGCGCCGAAAGGCGCAATGTATACGGATCGTCTGGTCGCAGGC CTGTGGTTAAGCGCCAATGAGATCCGCGTGCCGGCACTGACCATGAACTATATGCCGTTAAGT CATATTGCGGGTCGCATGTCTCTGTATGGCACCCTGATGCGCGGTGGTACCGCGTACTTTGCT GCGGCGTCTGACATGTCTACCCTGCTGGACGACTTCGGCTTGGCACGGCCTACTGAGCTGTT TCTGGTCCCACGCGTGTGTGAACTGCTGCATCAGCGCTACCAGTCAGAGCTGGATCGCCGGG TCGTCGCCGGTGAAGATGCGGAGACAGCTGCCACTAACGTTAAAGCGGAACTGCGTGAACGC GTTCTGGGCGGTCGTTATCTGACAGCGTTGTCTGGGTCAGCCCCTCTGGCCGCGGAGATGAA AACGTTCATGGAATCTCTGCTGGACGATGAACTTCATGACGGCTACGGGTCAACGGAAGCGG GCGGCTCCGTACTGCTCGATAATCGCATCAAACGGCCACCGGTGCTTGATTATCGCCTGGTTG ATGTTCCGGAACTGGGGTATTTCCGCACAGACAAACCACATCCACGGGGTGAGCTCCTGTTGA AAACCGAGAGCATGTTCCCCGGTTACTACAAACGTCCGGAGATCACTGCAGAGATGTTTGACG CGGATGGCTTCTATCGTACTGGGGATGTCGTGGCGGAACTGGGTCCTGAACAGCTGGTTTAC GTGGACCGCCGTAACAACGTTTTAAAGCTGTCACAAGGCGAATTTGTGACTGTGGCGGCTTTA GAAGCAGTGTATGCCACGTCACCTCTGATTCGGCAGATTTTCGTATATGGGTCCAGCGAACGC GCGTATCTGCTTGCCGTGGTCGTGCCGACCGATGCGGTTCTGGCACTGCCGGCCGCTCGCG CGCGCGCGGAAGTGTCTGAAAGCCTGCAGCGTATCGCCAAAGAATCGGGGCTGCGTCCCTAC GAGATTCCGCGTGATCTGATCATCGAAAGCGAGCCCTTTACCATTGATAACGGCCTGTTGAGT GGCATTGGAAAGTTACTGCGCCCGAAACTGAAAGAGCACTATGGAGAACGTCTGGAGCAGTT GTACGCTGAACTCGCCGAACAGCGGGAGGATGAGTTGACTGCGTTGCGCCGTGGCGCCCAT GACCGCCCGATTCTGGATACGGTTACGCGTGCCGCGGGTGCAGTGTTAGACCTCACGGCAGG CGAGGTATCGCCGGATGCGCACTTCGCGGATTTGGGTGGGGACTCCTTATCTGCCCTGTCCT TCTCCACGCTGCTGCGCGATATCTTTGGCGTAGAAGTGCCAGTCGGTTTCATCGTGGGGCCG GCAACTGATCTGGCGCGCATTGCTGAATATCTGGTGTCCGAACGCGACTCGGGGTCGCGCCC AACCGCTGCAACGGTACATGGCGATGATGGTCTGCTGCGCGCTGACGACCTTGCGCTGGAAG CTTTCCTGGATCCGGCAACCTTAGACGCAGCGGCGCACTTACCCAGCGCTCTGGAACCGCCT CGCACGGTTTTGCTGACGGGTGCGAATGGGTATCTGGGCCGCTTTCTGGCCTTGGAGTGGCT GCAACGGCTTGACGTCAGTGGTGGCACACTCATTTGCTTAATCCGCGGTAGCGATGCGGATA GTGCGCGCCGTCGCCTGGATGCTGTTTTCGCCACCGGTGATCCGGAACTCGAAGCACACTAT CGCGAACTGGCAGAACGTCGTCTGCGCGTACTGCCGGGCGATATCGGCGAACCTAATTTGGG CCTTCGCGAGCAAGATTGGCGCGATCTCGCAGAAACGGTGACCTTATCGTGCACCCGGCGGC CTTGGTTAACCATGTCTTGCCATATGCGCAGTTGTTTGGTCCGAACGTGGTGGGTACAGCGGA AGTGATTCGTCTGGCGCTGACCTCCCGTTTAAAACCGGTGACCTACCTGAGCACCGTGGCAGT CAGCGCGGGGATTGATCCGGAAACCTTTACCGAAGATGGCGACATTCGTGAGATCTCGCCAG TCCGCCGCTTGGACGACGGCTACGCTAACGGATATGGTAACAGCAAATGGGCCGGAGAAGTG CTGCTCCGTAATGCGCATGATCGCTTTGGCCTGCCGGTCGCCGTGTTCCGCAGTGATATGATT CTGGCCCATAGCCGTTACGCGGGGCAGCTGAATGTCCCCGACATGTTTACGCGCCTTCTTCTC TCAGTGCTCGCAACAGGTCTTGCGCCGGGCTCTTTTCACGATGCGCATGGCGAACGCCATCG TGCACACTATGATGGCCTTCCGGCAGACTTTACCGCCGCTGCGGTCACTACCCTTGGCAGTCG CGTAACCTCAGGCTACGAAACCTATGATGTATTGAATCCGCATGACGATGGGATCAGCCTCGA CACCTTTGTGGATTGGCTCATTGAAGCTGGCCACCCGATTGATCGCATTGATGACTATGCCGA GTGGTTCGCACGCTTCGATACCGCCCTGCGTGCCTTGCCGGAGCACCAACGTCAACACTCCC TCCTGCCCCTGTTGCACGCGTATCGGCGGCCAACCCCTCCATTACATGGAGTCGCACTGCCA GCTAAGCACTTTCGTGCGGCCGTACAACAGGCGAAACTGGGACCGGATGGTGATATCCCGCA TGTTACCCGCGAACTCATCGAGAAGTACGCCTCGGATTTACGCCTGTTAGGTCTGATTCAGGG

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pET151_*Ec*PPTase	CAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCT CATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGC ACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAA TTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTT TAAGAAGGAGATATACATATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTCTCCTCGGTCT CGATTCTACGGAAAACCTGTATTTTCAGGGAATTGATCCCTTCACCATGGTCGACATGAAAACCACCCATA CCTCTCTTCCGTTTGCTGGGCATACGCTGCACTTCGTGGAATTCGATCCGGCCAATTTCTGCGAACAGGAT CTGTTGTGGTTACCGCACTATGCACAGCTGCAGCATGCGGGTCGCAAACGCAAAACGGAACATCTGGCT GGTCGTATTGCCGCAGTGTATGCGCTGCGCGAGTATGGCTACAAATGCGTACCAGCCATTGGCGAACTG CGTCAACCCGTTTGGCCAGCGGAAGTGTACGGGAGCATTTCCCATTGTGGTACCACAGCACTTGCGGTAG TTAGTCGTCAGCCGATTGGCATCGACATTGAGGAAATCTTCAGCGTTCAAACAGCTCGGGAATTGACCGA TAACATCATTACCCCTGCGGAACACGAGCGCTTAGCCGATTGTGGACTGGCCTTTTCACTGGCACTGACTC TCGCGTTTAGTGCGAAAGAAAGCGCCTTTAAAGCGTCGGAAATTCAGACGGATGCTGGCTTTCTCGACTA CCAGATTATCTCGTGGAACAAACAGCAAGTCATCATTCACCGCGAGAATGAGATGTTTGCAGTCCATTGG CAGATCAAGGAAAAGATTGTGATCACTCTGTGCCAACACGATTAAAAGGGCGAGCTCAGATCCGGCTGC TAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGG GGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCCGCAAGAGG CCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGGTGCCGAGGATGACGATG AGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCGTTAGCAATTTAACTGTGATAAACTACCGCATTA AAGCTAGCTTATCGATGATAAGCTGTCAAACATGAGAATTAATTCTTGAAGACGAAAGGGCCTCGTGATA CGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAA TGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACC CTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTC CCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTC GCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTT GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAG TCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTG ATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAA CATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGA GCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACT CTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCG GCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTG CAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTA TGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACC AAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCC TTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAA AAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACC GCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGC AGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAG CACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTT ACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTG CACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAG CGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAG CGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGAC

	TTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCT TTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTC AGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACAC CGCAATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGC TACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCT GCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCG TCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGATG TCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCG GGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATG GGGGTAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCCG GTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCACTC AGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGA TGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAAAC CGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCG TATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACGACAGGAGC ACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCCGAGATGCGCCGCGTGCGGCTGCTGGAGATG GCGGACGCGATGGATATGTTCTGCCAAGGGTTGGTTTGCGCATTCACAGTTCTCCGCAAGAATTGATTGG CTCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTCAGGTCGAGGTGGCCCG GCTCCATGCACCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCCTACAATCCATGCCA ACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCCAATGATCGAAGTTA GGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTCATCTACCTGCCTGGACAGCAT GGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGAATCATAATGGGGAAGGCCATCCAGC CTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCATGCCGGCGATAATGGCCTGCT TCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAAT ACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGACCCAGAGC GCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATG CCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCC TAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTG CCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTT TTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAA GCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACA TGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGCCCGGACTCGGTA ATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCA TTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTG AATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGAACTTAATGG GCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCT TCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATTA GTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATCAGCCCACTGACGC GTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCAC CACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGC CAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGG AATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGT TCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTACTGGTTT CACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATT CGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGT TGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATG
pETDuet_*No*CAR/ *Ec*PPTase	GGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA TATACCATGGTCGATATGAAAACTACGCATACCTCCCTCCCCTTTGCCGGACATACGCTGCATT TTGTTGAGTTCGATCCGGCGAATTTTTGTGAGCAGGATTTACTCTGGCTGCCGCACTACGCAC AACTGCAACACGCTGGACGTAAACGTAAAACAGAGCATTTAGCCGGACGGATCGCTGCTGTTT ATGCTTTGCGGGAATATGGCTATAAATGTGTGCCCGCAATCGGCGAGCTACGCCAACCTGTCT GGCCTGCGGAGGTATACGGCAGTATTAGCCACTGTGGGACTACGGCATTAGCCGTGGTATCT CGTCAACCGATTGGCATTGATATAGAAGAAATTTTTTCTGTACAAACCGCAAGAGAATTGACAG ACAACATTATTACACCAGCGGAACACGAGCGACTCGCAGACTGCGGTTTAGCCTTTTCTCTGG CGCTGACACTGGCATTTTCCGCCAAAGAGAGCGCATTTAAGGCAAGTGAGATCCAAACTGATG CAGGTTTTCTGGACTATCAGATAATTAGCTGGAATAAACAGCAGGTCATCATTCATCGTGAGAA TGAGATGTTTGCTGTGCACTGGCAGATAAAAGAAAAGATAGTCATAACGCTGTGCCAACACGA TTAAGCTTGCGGCCGCATAATGCTTAAGTCGAACAGAAAGTAATCGTATTGTACACGGCCGCA TAATCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCATCTTAGT

	ATATTAGTTAAGTATAAGAAGGAGATATACATATGCATCATCACCATCACCATGGTAAGCCTATC CCTAACCCTCTCCTCGGTCTCGATTCTACGGAAAACCTGTATTTTCAGGGAATTGATCCCTTCA CCATGCTGGACGATGCACGTGCCGAACGTCGGGAACGTCGCATTGCGGACGCCTTAGCGGAT GACCAGGTTCGTGAAGCAGCCGCAGATGCCGCCGTTTCGGAAAGCGTCCGCCGTGTCGAAGT TCGCCTCGCTCGTATTGTTGATGCGGTGATGAGTGGCTACGGTGACCGTGCCGCGTTAGCTT GGCGTCGTTCCGAACTGGTAGACGGCGCAGTTCGCCTCCTGCCGGAATACTCGACCATGACT TATCGTGAGCTGTGGCGTCAAGCGGGTGCTGTGGCGGCTGAATGGGGAGCGGATGCCGAAA GCCCTGTTCGCGCAGAAGATTTTGTGTGTACCCTGGGTTTTACGAGTCCGGACTACACCGTAG TGGACCTGGCCTTAATGCGTCTGGCCGCAGTTGCTGTTCCCCTTCAGGCGTCTGCCTCAGTTG CTCAGTGGCGCAGCATCATGGCCGAAACTGAACCGCGTACTTTGCTGCGGCGTCGGCGGAAACC CTGCCTGCCGCCGTGGAGGCTGTTCTGGGCGGTTTTGCGCCGCGTCGCGTGCTGGTCTTTGA TTATCGCCCTGAACTTGAGGCACATCGTAGCGCCGTAGATAGCGCTCGTGAACGCCTGGCAG AAGTGGGTTGCACAGTAGCCACAGTTGCAGATGCCGTGGATCGCGGCGCTAATCTGCCAGCA CCGCTTCGCATTCCCAGTGATCGCGAACGCTTGGCGTTATTAATTTACACGAGCGGATCGACG GGAGCGCCGAAAGGCGCAATGTATACGGATCGTCTGGTCGCAGGCCTGTGGTTAAGCGCCAA TGAGATCCGCGTGCCGGCACTGACCATGAACTATATGCCGTTAAGTCATATTGCGGGTCGCAT GTCTCTGTATGGCACCCTGATGCGCGGTGGTACCGCGTACTTTGCTGCGGCGTCTGACATGT CTACCCTGCTGGACGACTTCGGCTTGGCACGGCCTACTGAGCTGTTTCTGGTCCCACGCGTG TGTGAACTGCTGCATCAGCGCTACCAGTCAGAGCTGGATCGCCGGGTCGTCGCCGGTGAAGA TGCGGAGACAGCTGCCACTAACGTTAAAGCGGAACTGCGTGAACGCGTTCTGGGCGGTCGTT ATCTGACAGCGTTGTCTGGGTCAGCCCCTCTGGCCGCGGAGATGAAAACGTTCATGGAATCTC TGCTGGACGATGAACTTCATGACGGCTACGGGTCAACGGAAGCGGGCGGCTCCGTACTGCTC GATAATCGCATCAAACGGCCACCGGTGCTTGATTATCGCCTGGTTGATGTTCCGGAACTGGGG TATTTCCGCACAGACAAACCACATCCACGGGGTGAGCTCCTGTTGAAAACCGAGAGCATGTTC CCCGGTTACTACAAACGTCCGGAGATCACTGCAGAGATGTTTGACGCGGATGGCTTCTATCGT ACTGGGGATGTCGTGGCGGAACTGGGTCCTGAACAGCTGGTTTACGTGGACCGCCGTAACAA CGTTTTAAAGCTGTCACAAGGCGAATTTGTGACTGTGGCGGCTTTAGAAGCAGTGTATGCCAC GTCACCTCTGATTCGGCAGATTTTCGTATATGGGTCCAGCGAACGCGCGTATCTGCTTGCCGT GGTCGTGCCGACCGATGCGGTTCTGGCACTGCCGGCCGCTCGCGCGCGCGCGGAAGTGTCT GAAAGCCTGCAGCGTATCGCCAAAGAATCGGGGCTGCGTCCCTACGAGATTCCGCGTGATCT GATCATCGAAAGCGAGCCCTTTACCATTGATAACGGCCTGTTGAGTGGCATTGGAAAGTTACT GCGCCCGAAACTGAAAGAGCACTATGGAGAACGTCTGGAGCAGTTGTACGCTGAACTCGCCG AACAGCGGGAGGATGAGTTGACTGCGTTGCGCCGTGGCGCCCATGACCGCCCGATTCTGGAT ACGGTTACGCGTGCCGCGGGTGCAGTGTTAGACCTCACGGCAGGCGAGGTATCGCCGGATG CGCACTTCGCGGATTTGGGTGGGGACTCCTTATCTGCCCTGTCCTTCTCCACGCTGCTGCGC GATATCTTTGGCGTAGAAGTGCCAGTCGGTTTCATCGTGGGGCCGGCAACTGATCTGGCGCG CATTGCTGAATATCTGGTGTCCGAACGCGACTCGGGGTCGCGCCCAACCGCTGCAACGGTAC ATGGCGATGATGGTCTGCTGCGCGCTGACGACCTTGCGCTGGAAGCTTTCCTGGATCCGGCA ACCTTAGACGCAGCGGCGCACTTACCCAGCGCTCTGGAACCGCCTCGCACGGTTTTGCTGAC GGGTGCGAATGGGTATCTGGGCCGCTTTCTGGCCTTGGAGTGGCTGCAACGGCTTGACGTCA GTGGTGGCACACTCATTTGCTTAATCCGCGGTAGCGATGCGGATAGTGCGCGCCGTCGCCTG GATGCTGTTTTCGCCACCGGTGATCCGGAACTCGAAGCACACTATCGCGAACTGGCAGAACGT CGTCTGCGCGTACTGCCGGGCGATATCGGCGAACCTAATTTGGGCCTTCGCGAGCAAGATTG GCGCGATCTCGCAGAAACGGTCGACCTTATCGTGCACCCGGCGGCCTTGGTTAACCATGTCTT GCCATATGCGCAGTTGTTTGGTCCGAACGTGGTGGGTACAGCGGAAGTGATTCGTCTGGCGC TGACCTCCCGTTTAAAACCGGTGACCTACCTGAGCACCGTGGCAGTCAGCGCGGGGATTGAT CCGGAAACCTTTACCGAAGATGGCGACATTCGTGAGATCTCGCCAGTCCGCCGCTTGGACGA CGGCTACGCTAACGGATATGGTAACAGCAAATGGGCCGGAGAAGTGCTGCTCCGTAATGCGC ATGATCGCTTTGGCCTGCCGGTCGCCGTGTTCCGCAGTGATATGATTCTGGCCCATAGCCGTT ACGCGGGGCAGCTGAATGTCCCCGACATGTTTACGCGCCTTCTTCTCTCAGTGCTCGCAACAG GTCTTGCGCCGGGCTCTTTTCACGATGCGCATGGCGAACGCCATCGTGCACACTATGATGGC CTTCCGGCAGACTTTACCGCCGCTGCGGTCACTACCCTTGGCAGTCGCGTAACCTCAGGCTA CGAAACCTATGATGTATTGAATCCGCATGACGATGGGATCAGCCTCGACACCTTTGTGGATTG GCTCATTGAAGCTGGCCACCCGATTGATCGCATTGATGACTATGCCGAGTGGTTCGCACGCTT CGATACCGCCCTGCGTGCCTTGCCGGAGCACCAACGTCAACACTCCCTCCTGCCCCTGTTGC ACGCGTATCGGCGGCCAACCCCTCCATTACATGGAGTCGCACTGCCAGCTAAGCACTTTCGTG CGGCCGTACAACAGGCGAAACTGGGACCGGATGGTGATATCCCGCATGTTACCCGCGAACTC ATCGAGAAGTACGCCTCGGATTTACGCCTGTTAGGTCTGATTCAGGGTTAACTCGAGTCTGGT AAAGAAACCGCTGCTGCGAAATTTGAACGCCAGCACATGGACTCGTCTACTAGCGCAGCTTAA TTAACCTAGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGG GTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCT GTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGC CAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTT TCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCT CGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGT TTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACA ACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTG GTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAAT TTCTGGCGGCACGATGGCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAA AATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTA

	ATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCG TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGC GAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAG CGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT AGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTG GTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTT ACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGA AGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCA TGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTG TATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCA GAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACC GCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACT TTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGG GCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATCATGATTGAAGCATTTATCAGG GTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGTCATGAC CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGG ATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTAC CAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCA GCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGA ACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTG GCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGG TCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACT GAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACA GGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAA CGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCT GGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAAC CGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGA GTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCG GTATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGC CAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACA CCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGAC CGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGC TGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGT CCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAA GGGCGGTTTTTTCCTGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGG TAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCC GGTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAA ATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAG CAGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAG ACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCA GCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACC CCGCCAGCCTAGCCGGGTCCTCAACGACAGGAGCACGATCATGCTAGTCATGCCCCGCGCCC ACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAA TGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTG TCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCG CCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGC CCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTG ATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAG ATGTCCGCACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTG ATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTG AAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGT GAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTA ACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCT TCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGA ACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATC AGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCT TCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGC GACAATTTGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACT GTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTT CCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCT GATAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCT GAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGT GTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGT TGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAG TCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGT GGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTG GCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCGATCTCGATCCCG CGAAATTAATACGACTCACTATA

2.3. Cloning

Applying the Gibson Assembly, *No*CAR was cloned into a previous prepared pETDuet containing *Ec*PPTase in one of the cloning sites.[8,9] The original pETDuet plasmid harbored the gene for another CAR enzyme (carboxylate reductase from *Nocardia iowensis*, *Ni*CAR). *Ni*CAR gene was cut with the appropriate restriction enzymes to prepare the vector backbone. All primers and DNA templates were diluted in water. The plasmid pETDuet_*No*CAR/*Ec*PPTase is shown in Figure S4. The PCR program used to amplify was as follows:

1) Backbone (using carboxylate reductase from *Nocardia iowensis*, *Ni*CAR):
   pETDuet1_PPTase (5922 base pairs)
   - Primers:
   - Forward: CTCGAGTCTGGTAAAGAAAC
   - Reverse: ATGTATACTCCTTCTTATACTTAAC
   - PCR program:
   - 98°C for 30 s
   - 98°C for 10 s
   - 57.2°C for 20 s annealing
   - 72°C for 2 min extension
   - 72°C for 2 min final extension

   25x
2) Second fragment (*No*CAR):
   3613 base pairs
   - Primers:
   - Forward: TATAAGAAGGAGATATACATATGCATCATCACCATCAC
   - Reverse: GTTTCTTTACCAGACTCGAGTTAACCCTGAATCAGACC
   - PCR program:
   - 98°C for 30 s
   - 98°C for 10 s
   - 58.8°C for 20 s annealing
   - 72°C for 2 min extension
   - 72°C for 2 min final extension

   25x

3. Optimization of the *No*CAR production in *E. coli*

3.1. Medium optimization in *E. coli* BL21

Table S3: Protein content (mg mL^{-1}) of soluble and insoluble fractions of *No*CAR produced in different medium and induction conditions in *E. coli* BL21

Medium	Soluble fractions		Insoluble fractions	
	Samples	**(mg/mL)**	**Samples**	**(mg/mL)**
LB	3h	1.76	3h	1.7
	6h	1.4	6h	2.25
	24h	1.74	24h	7.3
	48h	1.94	48h	9.56
	72h	1.11	72h	9.55
AI	3h	1.55	3h	1.79
	6h	5.64	6h	1.01
	24h	7.92	24h	12.5
	48h	5	48h	12.9
	72h	7.1	72h	9.21
TB	3h	2	3h	2.27
	6h	1.93	6h	3.91
	24h	5.1	24h	9.34
	48h	7.71	48h	17.6
	72h	5.7	72h	19.1

3.2. Optimization in *E. coli* Tuner

As already mentioned in the main manuscript, producing soluble *No*CAR in sufficient amounts turned out to be challenging, hindering purification and characterization attempts. Thus, it was essential to find cultivation conditions that could increase the amount of soluble recombinant protein expression as much as possible. Those were the cultivation temperature, OD_{600} at induction, and IPTG concentration. All of them can be varied smoothly and without much effort, compared to co-expression of molecular chaperons or codon optimization, which would be much more time consuming.[10] After deciding on the parameters, the experiments were designed. This step was done according to a central composite design (CCD). The table below shows the cultivation conditions for a CCD in 15 experiments (great part of them were performed in duplicates). Therefore, the operational window was determined empirically or by suggestions from literature.[10,11]

Table S4: CCD for the optimization of the expression of *No*CAR in *E. coli* Tuner

Run Nr.	T (°C)	IPTG (mM)	OD_{600}
1	15	0.1	0.6
2	17	0.05	0.4
3	17	0.15	0.4
4	17	0.05	0.8
5	17	0.15	0.8
6	20	0.03	0.6
7	20	0.1	0.2
8	20	0.1	1.0
9	20	0.1	0.6
10	20	0.2	0.6
11	23	0.05	0.4
12	23	0.15	0.4
13	23	0.05	0.8
14	23	0.15	0.8
15	25	0.1	0.6

All the data was gathered from the small-scale cultivation (100 mL) of *No*CAR. Densitometric analysis with the aid of the *ImageJ* software, in combination with protein concentrations determined by Bradford assay (described in the experimental section in the main manuscript), was used to estimate the total soluble *No*CAR protein concentration (in mg/mL). For modeling and optimization, only the soluble fractions collected after 48 h of cultivation were considered. The quadratic surface obtained is shown in Figure 2A in the main manuscript. To obtain the optimal process conditions for the cultivation of soluble *No*CAR, the quadratic had to be maximized using a genetic algorithm. Genetic algorithms are a group of models that are inspired by the process of natural selection and are capable of generating high-quality solutions for both, constrained and unconstrained optimization and search problems[12]. The output of this was the cultivation conditions depicted in the main manuscript (0.9-1.0 OD_{600} at induction, 0.18 mM IPTG concentration, and a cultivation temperature of 15°C). It is essential to evaluate the performance of a model to develop an understanding of how well it generalizes to new data. Thus, a very common and popular metric for the evaluation of the performance of a regression model is the mean squared error (mse). It describes the average of the squares of errors and is an accurate measure of a model's predictive power. In other words, mse is the average squared difference between the estimated values and the actual value. In our work, the quadratic model showed an mse score of 0.19. The equation[13] used to fit the data is shown below:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum \sum_{i<j} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \epsilon.$$

in which *y* corresponds to the response of interest, x_i and x_j are numbers of associated control (or input) variables, β is a vector of *p* unknown constant coefficients referred to as parameters, and ϵ is a random experimental error assumed to have a zero mean. All this additional information were added to the SI material.

Thus, a mean squared error of 0.19 mg/mL and a standard deviation of 0.18 were determined by a 10-fold cross-validation for the quadratic system.

4. Optimization of the post-translational modification of *No*CAR *via* incubation assay

The incubation of purified *No*CAR expressed in *E.coli* Tuner with purified *Ec*PPTase and acetyl-CoA was performed under different conditions. Measuring parameters were the amount of *Ec*PPTase, concentration of acetyl-CoA, and incubation time. In each case, the specific activity using the photometric-based assay described in the experimental section of the main manuscript. Variables, conditions tested, and the best conditions are shown in Table S5. The initial parameters that were evaluated to determine the best *in vitro* conditions for the activation of *No*CAR were decided based on previous works.[7] Besides, these initial parameters were chosen because we believed they were the ones which could have the highest influence on the *in vitro* activation of *No*CAR. The PPTase from *E. coli* was produced and purified to be used in this study. Here, we were limited by the protein content of the protein preparation containing the PPTase. In this case, the protein concentration of this preparation was about 0.272 mg mL^{-1} (determined by the Bradford assay). Therefore, the operational window for this specific parameter was decided based on this limiting protein content.

Table S5: Post-translational modification of *No*CAR[a]: optimization of the incubation assay

Entry	Construct	Experimental	Variable	Specific activity (U/mg)	Best condition
1	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (**1 mM**) + 25 µL PPTase (0.143 mg/mL) → incubation (1 h, 28°C, 600 rpm)	acetyl-CoA concentration	0.010	2 mM acetyl-CoA
2	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (**2 mM**) + 25 µL PPTase (0.143 mg/mL) → incubation (1 h, 28°C, 600 rpm)		0.012	
3	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (**5 mM**) + 25 µL PPTase (0.143 mg/mL) → incubation (1 h, 28°C, 600 rpm)		0.011	
4	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (2 mM) + 25 µL PPTase **(0.075 mg/mL)** → incubation (1 h, 28°C, 600 rpm)	*Ec*PPTase concentration	0.0092	0.143 mg/mL
5	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (2 mM) + 25 µL PPTase **(0.143 mg/mL)** → incubation (1 h, 28°C, 600 rpm)		0.0108	
6	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (2 mM) + 25 µL PPTase **(0.272 mg/mL)** → incubation (1 h, 28°C, 600 rpm)		0.0107	
7	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (2 mM) + 25 µL PPTase (0.143 mg/mL) → incubation (**0.5 h**, 28°C, 600 rpm)	Time of incubation	0.010	2 h
8	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (2 mM) + 25 µL PPTase (0.143 mg/mL) → incubation (**1 h**, 28°C, 600 rpm)		0.011	
9	*No*CAR + acetyl-CoA + PPTase	50 µL + 25 µL acetyl-CoA (2 mM) + 25 µL PPTase (0.143 mg/mL) → incubation (**2 h**, 28°C, 600 rpm)		0.022	

a] Protein concentration of *No*CAR stock solution was always determined via Bradford assay prior to each incubation assay. The protein concentration ranged from 18 to 25 mg/mL.

5. Biocatalytic reduction reactions with *in vitro* recycling of cofactors

5.1. Auxiliary enzymes for the regeneration of both cofactors

The full recycling of both cofactors was achieved using the following auxiliary enzymes: the simultaneous action of polyphosphate kinases from *Meiothermus ruber* (*Mr*PPK) and *Sinorhizobium meliloti* (*Sm*PPK) for the regeneration of ATP and a glucose dehydrogenase (GDH) for the regeneration of NADPH. Finally, the inhibitory effects of pyrophosphate (PP), which is generated *in situ* during the catalytic cycle of CARs, is surpassed by using the pyrophosphatase from *Escherichia coli* (*Ec*PPase). GDH from *Pseudomonas* sp. was commercially available and obtained from Sigma-Aldrich (Steinheim, Germany).

His-tagged *Mr*PPK was used similarly as described previously by Parnell *et al*.[14]. *Sm*PPK was expressed from the pET28+ vector constructed previously by Mordhorst *et*

al.[14,15] *E. coli* BL21 (DE3) Star served as the production host. Cells were cultivated in LB medium and PPKs expression was induced by addition of 0.5 mM IPTG at 28°C. Cell disruption and purification was performed as described for *No*CAR. Liquid stocks of purified PPKs were stored at -20°C. Protein concentrations were determined with the Bradford assay following the procedure described in the experimental section of the main manuscript.

Strep-tagged *Ec*PPase was expressed from the pETSTREP3 vector as constructed previously by Pfeiffer et al.[16] Similarly, *E.coli* BL21(DE3) Star served as the expression host and was cultivated in LB medium at 37°C. *Ec*PPase expression was induced by addition of 0.5 mM IPTG. Purification was performed under standard conditions using a gravity flow strep tactin sepharose column as the stationary phase. Protein concentrations were determined with the Bradford assay following the procedure described in the experimental section of the main manuscript.

Liquid stocks of purified *Ec*PPase was stored at -20°C. Full characterization of both PPKs and *Ec*PPase in terms of enzyme activity was determined and described by Strohmeier *et al*.[8]

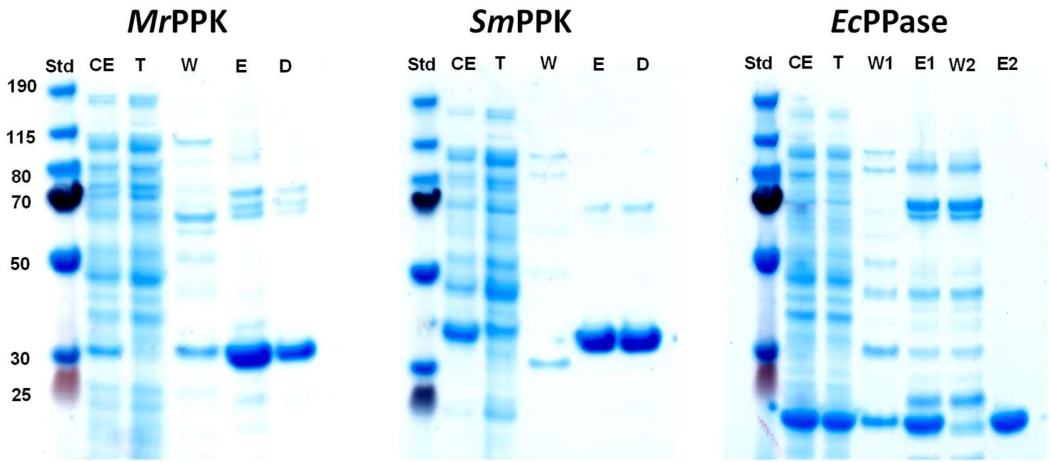

Figure S5. SDS gel analysis for the purification steps of the ATP-recycling enzymes used in this study. Legend: CE: crude cell extract; T: throughput fraction; W: washed fraction; E: eluted fraction; D: desalted fraction. *Ec*PPase had in total two washing and elution steps.

5.2. Reaction components and setup

The components and stock solutions prepared for the biotransformations with *in vitro* recycling of ATP and NADPH are shown in Tables S5 and S6.

Table S5: Components and stock concentrations for the *No*CAR-catalyzed biotransformations

Reaction mixture preparation		
Key components	**Concentration in reaction (mM)**	**Concentration in stock (mM)**
4xMOPS buffer pH 7.5	100	400
Sodium benzoate	5	250
NADPH	0.5	50
ATP	1	50
$MgCl_2$	6.25	50
Sodium polyphosphate	4 mg/mL	50 mg/mL
β-D-(+)-glucose	50	200
Auxiliary enzymes	**Concentration in reaction (µg/mL)**	**Concentration in the stock (µg/mL)**
*Mr*PPK	100	2220
*Sm*PPK	40	1100
*Ec*PPase	25	754
GDH	0.2	50

Table S6: Reaction mixture for the *No*CAR-catalyzed biotransformations

Mastermix for all reactions	
Components	**Volume/reaction (µL)**
H_2O Milli-Q	36.4
4xMOPS buffer containing $MgCl_2$ and β-D-(+)-glucose	62.5
Coenzyme stock (GDH and ATP-recycling enzymes)	98.6
NADPH	2.5
ATP	5
Sodium polyphosphate	20
Bromothymol blue (indicator)	5
Reaction vial/eppi	**Volume (µL)**
Mastermix	230
Substrate (250 mM stock)	5
*No*CAR/*Ec*PPTase (2.5 mg/mL stock)	10
Addition of KOH (1 M)	5
Total	**250**

The components $MgCl_2$ and β-D-(+)-glucose were added to the 4x buffer. The total volume of reaction was 250 µL. 5 µL of 1 M KOH was added after starting the reaction for pH adjustment.

Figure S6. Reaction mixture (250 µL total volume) performed in a 1.5 mL Eppendorf tube. The blue colour results from the dissociation of Bromothymol blue in solutions that are more basic.

5.3. Investigation of optimal reaction conditions

5.3.1. Type of buffer

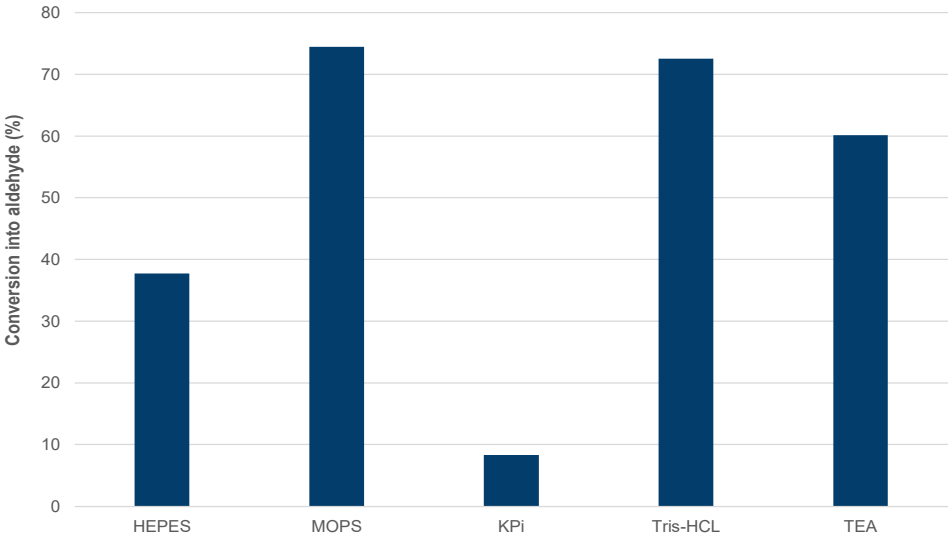

Figure S7. Evaluation of different types of buffer. Composition in 250 µL reaction volume: 100 mM buffer (pH 7.5), 6.25 mM $MgCl_2$, 50 mM glucose, 5 mM sodium benzoate, 4 mg mL^{-1} sodium polyphosphate, 1 mM ATP, 0.5 mM NADPH, 100 µg mL^{-1} *No*CAR/*Ec*PPTase, 100 µg mL^{-1} *Mr*PPK, 40 µg mL^{-1} *Sm*PPK, 25 µg mL^{-1} *Ec*PPase, 50 µg mL^{-1} GDH, 30°C, 750 rpm. Buffers: KPi: potassium phosphate, TEA: triethanolamine. Sampling after 1 h of reaction with subsequently HPLC analysis. Values shown are mean values of technical triplicates ($n_{biological}$ = 1). Error bars for the technical replicates were too small to indicate.[17][18]

5.3.2. Temperature of reaction

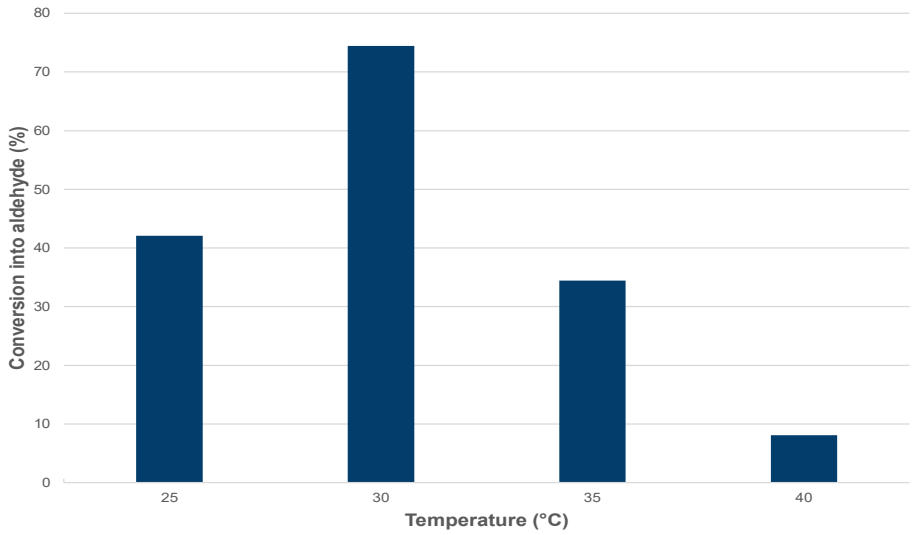

Figure S8. Evaluation of different reaction temperatures. Composition in 250 µL reaction volume: 100 mM MOPS buffer (pH 7.5), 6.25 mM $MgCl_2$, 50 mM glucose, 5 mM sodium benzoate, 4 mg mL^{-1} sodium polyphosphate, 1 mM ATP, 0.5 mM NADPH, 100 µg mL^{-1} *No*CAR/*Ec*PPTase, 100 µg mL^{-1} *Mr*PPK, 40 µg mL^{-1} *Sm*PPK, 25 µg mL^{-1} *Ec*PPase, 50 µg mL^{-1} GDH, 750 rpm. Sampling after 1 h of reaction with subsequently HPLC analysis. Values shown are mean values of technical triplicates ($n_{biological}$ = 1). Error barsfor the technical replicates were too small to indicate.[17][18]

5.3.3. Concentration of *No*CAR/*Ec*PPTase

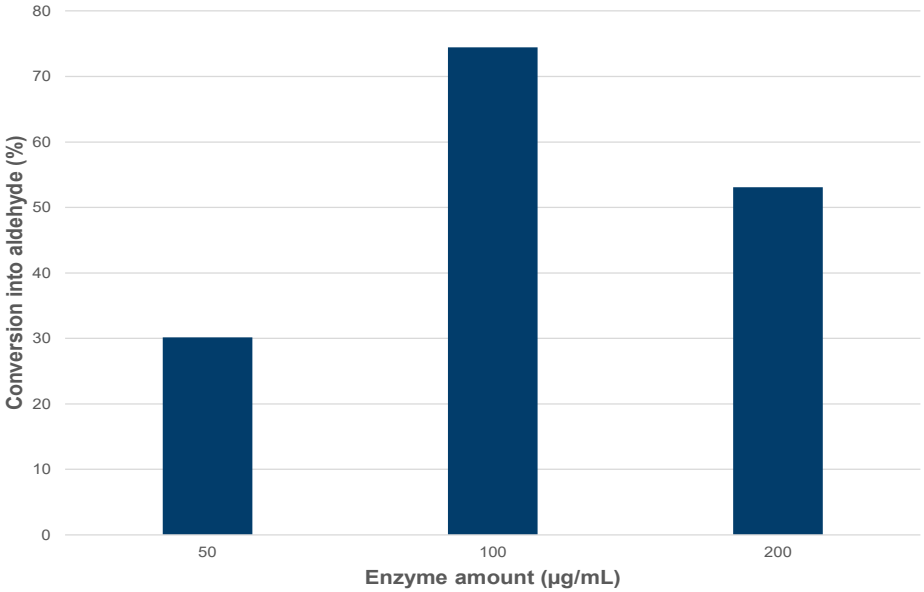

Figure S9. Evaluation of varying concentrations of *No*CAR/*Ec*PPTase. Composition in 250 µL reaction volume: 100 mM MOPS buffer (pH 7.5), 6.25 mM $MgCl_2$, 50 mM glucose, 5 mM sodium benzoate, 4 mg mL^{-1} sodium polyphosphate, 1 mM ATP, 0.5 mM NADPH, *No*CAR/*Ec*PPTase, 100 µg mL^{-1} *Mr*PPK, 40 µg mL^{-1} *Sm*PPK, 25 µg mL^{-1} *Ec*PPase, 50 µg mL^{-1} GDH, 30°C, 750 rpm. Sampling after 1 h of reaction with subsequently HPLC analysis. Values shown are mean values of technical triplicates ($n_{biological}$ = 1). Error bars for the technical replicates were too small to indicate.[17][18]

6. Analytics

6.1. Calibration curves

The calibration curves constructed to determine the conversions of sodium benzoate (NaBZ), benzaldehyde (BA) and benzyl alcohol (BZ alcohol) are shown below. The internal standard used was ethyl *para*-hydroxybenzoate.

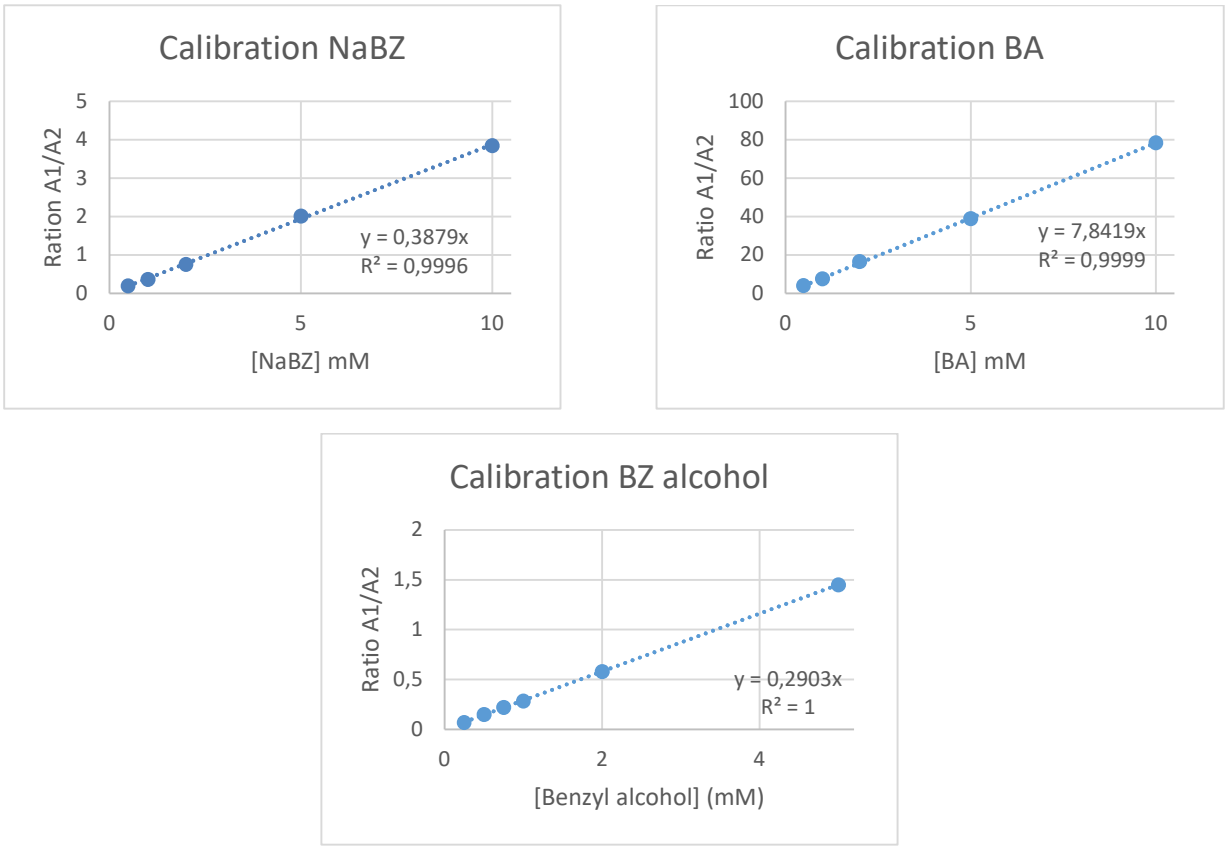

Figure S10. Calibration curves for sodium benzoate (NaBZ), benzaldehyde (BA), and benzyl alcohol (BZ alcohol). The *y*-axis corresponds to the ration of the area of one of these compounds (A1) and the area of the internal standard (A2).

6.2. Chromatogram

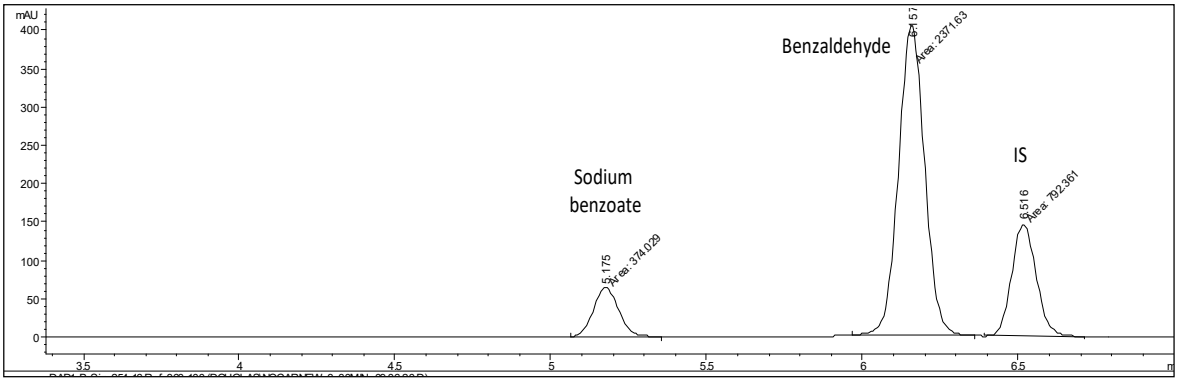

Figure S11. Exemplary chromatogram for an *in vitro* reaction.

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