Supporting Information for

Diverse engineered heme proteins enable stereodivergent cyclopropanation of unactivated alkenes

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Previous literature for stereocomplementary enzymatic cyclopropanation and enantioselective, intermolecular unactivated alkene cyclopropanation

Supplemental Table 1a. Literature precedent for stereocomplementary cyclopropanation of styrenyl alkenes via carbene transfer. Reference numbers are given for the main text reference numbers.

	Trans-cyclopropane		*Cis*-cyclopropane	
	(*R*,*R*)	(*S*,*S*)	(*R*,*S*)	(*S*,*R*)
Ref 11, 10.1126/science.1231434	**Not reported**	Up to 98% de, 96% *e.e.*	Up to 84% de, 97% *e.e.*	**Not reported**
Ref 13, doi/10.1002/cbic.201600528	Up to 86% de, **32% *e.e.***	Up to 98% de, 97% *e.e.*	Up to 78% de, 99% *e.e.*	Up to **42% de**, 95% *e.e.*
Ref 17, 10.1002/anie.201608680	Up to 99.9% de, 95% *e.e.*	Up to 99.9% de, 99.9% *e.e.*	**Not reported**	**Not reported**
Ref 34, 10.1021/jacs.7b00768	Up to 99.5% de, 92% *e.e.*	Up to 99.9% de, 99.9% *e.e.*	**Not reported**	**Not reported**

Supplemental Table 1b. Current state-of-the-art methods for the enantioselective, intermolecular cyclopropanation of unactivated alkenes. Because iron-catalyzed asymmetric examples are not known, achiral examples are listed for iron.

Entry	Metal Catalyst	Ref.	Note
1	Fe	1	Iron porphyrin-catalyzed, achiral. Two examples involved unactivated alkenes. Fe(PFP)Cl (0.02-0.05 mol%) catalyzed the cyclopropanation of 2-ethyl-1-butene (100 eq.) with EDA in 390 TON, d.r. not reported. PFP = *meso*-tetrakis(pentafluoropheny1)porphyrin
2	Fe	2	Iron porphyrin-catalyzed, achiral. One example involved unactivated alkenes. Fe(TPP)Cl (3 mol%) catalyzed the cyclopropanation of allyl benzene with *in situ* generated trifluoromethyl diazomethane (1.5 eq.) in water with 3.3 TON, d.r. not reported. TPP = 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine
3	Fe	3	Iron porphyrin-catalyzed, achiral. One example involved unactivated alkenes. This example is similar to entry 2, but a different *in situ* method was used to generate the trifluoromethyl diazomethane. No product was detected.
4	Fe	4	Iron porphyrin-catalyzed, achiral. Three examples involved unactivated alkenes.

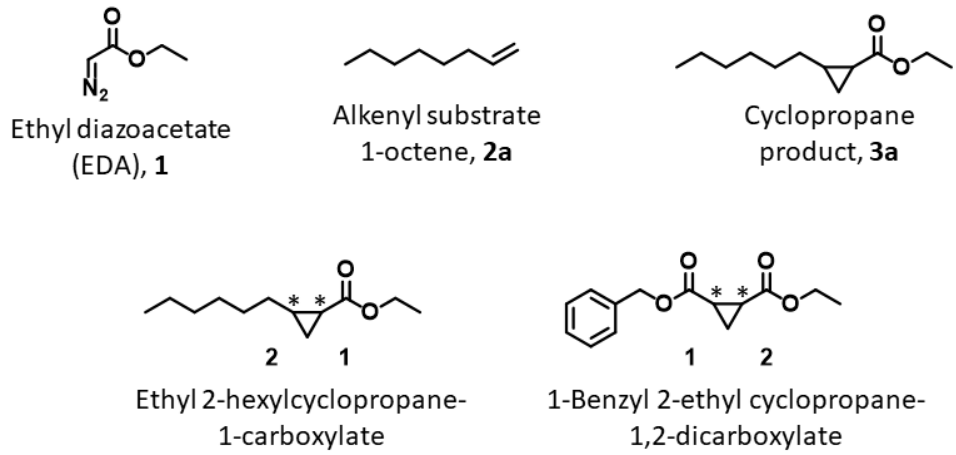
			Fe(TPP)Cl (10 mol%) catalyzed the cyclopropanation of aliphatic alkenes using *in situ* generated difluoromethylcarbene (2 eq.) in 6-8 TONs, 7:1 to 13: 1 d.r. (*trans*).
5	Rh	5-13	All Rh-carboxylate or Rh-carboxamidate-catalyzed (0.2-2 mol% Rh) examples of unactivated alkene cyclopropanation reported are ***trans*-selective**. The most efficient and selective examples are the cyclopropanation of 1-hexene (72 TTN, >20:1 d.r. (*trans*), 96% *e.e.*), and 1-octene (70 TTN, >20:1 d.r. (*trans*), 90 % *e.e.*).
7	Ir	14	Ir-salen (1 mol%) catalyzed the cyclopropanation of >10 unactivated alkenes with up to 93 TTN, 98:2 d.r. (*cis* major), 99% *e.e.*.
8	Ir	15	Ir(Me)PPIX in engineered myoglobin (0.5 mol%) catalyzed the cyclopropanation of 1-octene in 40 TON, 91:9 er, 40:1 d.r. (*trans* major). 6 equivalents of EDA were added *via* syringe pump over 12 h.
9	Ir	16	Ir(Me)PPIX in engineered CYP119 enzymes catalyzed the cyclopropanation of a variety of unactivated alkenes (terminal, internal and 1,1-disubstituted) with up to 1300 TON and 99% *e.e.*. 3 equivalents of EDA were added *via* syringe pump over 3 h.
10	Ru	17, 18	Ru-(iminophosphoranyl)ferrocene (2 mol%) catalyzed the cyclopropanation of 3 unactivated alkenes with up to 37 TON, 72:28 d.r. (*cis* major), 98% *e.e.*.
11	Co	19, 20	All Co-catalyzed (1-5 mol% Co) examples of unactivated alkene cyclopropanation reported are ***cis*-selective** and based on Co-porphyrins. The most efficient and selective example is the cyclopropanation of phenylbutene (90 TTN, >99:1 d.r. (*cis*), 96% *e.e.*).
12	Cu	21, 22	All Cu-catalyzed (1 mol% Cu) examples of unactivated alkene cyclopropanation reported are ***trans*-selective**. The most efficient and selective example is the cyclopropanation of 1-octene (80 TTN, 93:7 d.r. (*trans*), 90% *e.e.*).

Safety statement

No unexpected or unusually high safety hazards were encountered in these methods. While ethyl diazoester (EDA) has well-defined usage and risks, use of more volatile and reactive diazo compounds (e.g. diazoalkanes) should be performed with caution (for example procedures, see reference 23).

Nomenclature for compound labeling

Alkenyl substrates are named **2x** (where x is a-m). The corresponding cyclopropyl esters (from reaction with ethyl diazoacetate **1**) are named **3x**. Single diastereomer compounds are named *cis*-**3x** or *trans*-**3x**. The 1-octene cyclopropane products, whose absolute configuration is known (see *Compound chiral separation conditions and representative traces*), are labeled (***1***(***RlS***), ***2***(***RlS***))-**3a**. Alkenyl substrates with functional groups protected (e.g. 7-octen-1-ol, 7-octen-1-oic acid) are named **2xa**, and their corresponding cyclopropane products are named **3xa**. These are depicted in Supplemental Figure 1.



Supplemental Figure 1. Compound nomenclature used in this work.

Materials and Methods

Solvents and reagents were ordered from Sigma Aldrich, TCI, CombiBlocks, or Alfa Aesar and used without further purification. GC-FID data were collected on a Shimadzu GC-17A, Agilent 6850 GC system, and Agilent 7820A GC system. GC-MS data were collected on a Shimadzu GCMS-QP2010 SE. Screening HLPC-UV data were taken on an Agilent 1200 series HPLC. Normal-phase chiral HPLC data were taken on an Agilent 1100 series HPLC. NMR spectra were recorded on a Bruker Prodigy 400 MHz instrument or Varian 300 MHz instrument with $CDCl_3$ as solvent. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100

MHz. Chemical shifts were normalized to the chloroform solvent's protio impurity (^{1}H NMR 7.26 ppm, ^{13}C NMR 77.16 ppm). Optical rotation data were collected on a JASCO P-2000 Polarimeter.

Proteins tested in enzyme discovery

Genes encoding eleven heme-binding proteins were ordered as codon-optimized gBlocks (Integrated DNA Technologies, Coralville, Iowa) and assembled into pET22b(+) with the pelB leader sequence removed and a C-terminal 6xHis tag. As the putative distal ligand could interfere with the initially low substrate binding affinities, the proteins were ordered with the distal axial ligands mutated to smaller, nonpolar residues, found to be beneficial for vinylarene cyclopropanation in myoglobin[24]. Some gBlocks (Supplemental Table 2) were also ordered with a mutation in a putative entrance tunnel residue previously found to enhance styrene cyclopropanation activity in myoglobin.[24] These heme proteins were first tested for cyclopropanation activity using 1,7-octadiene and EDA as substrates (1,7-octadiene was chosen for its higher effective concentration of terminal olefin). The most active and selective proteins identified from these experiments, protoglobin from *Aeropyrum pernix* (ApePgb) and nitric oxide dioxygenase from *Rhodothermus marinus* (RmaNOD), were subsequently tested as wild-type proteins against 1-octene **2a**, a commonly used model substrate for unactivated alkene cyclopropanation studies.

Supplemental Table 2. Heme-binding proteins tested for unactivated alkene cyclopropanation activity using 1,7-octadiene and EDA as substrates.

UniProt ID	Organism	Annotation	Mutation(s) from WT	Cyclopropane product formation detected
Q3IDI7	*Pseudoalteromonas haloplanktis*	Putative hemoglobin-like oxygen-binding protein	Y42V F69A	No
Q7CX73	*Agrobacterium fabrum*	Uncharacterized protein	Y26V F53A	No
Q5L1S0	*Geobacillus kaustophilus*	Hypothetical conserved protein	Y29V Q50A	No
Q9NPG2	*Homo sapiens*	Neuroglobin	F28V F61I H64A	No
Q0PB48	*Campylobacter jejuni*	Truncated hemoglobin	none	No
B3DVC3	*Methylacidiphilum infernorum*	Hemoglobin IV	H71V L93A	Yes
D0MGT2	*Rhodothermus marinus*	Nitric oxide dioxygenase	Y32V Q52A	Yes
G7VHJ7	*Pyrobaculum ferrireducens*	Protoglobin	Y58V	Yes
Q9YFF4	*Aeropyrum pernix*	Protoglobin	Y60V	Yes
O66586	*Aquifex aeolicus*	Thermoglobin	Y29V Q50A	Yes

Screening previously engineered cytochromes P411

A composite plate of 36 P411-CIS proteins from lineages engineered for non-natural reactions such as aziridination, sulfimidation, amination, and non-styrenyl, activated alkene cyclopropanation[25] was screened for activity and selectivity in **3a** product formation. Most reactions showed trace activity and moderate diastereoselectivity, but the highest activities by a large margin were found in the lineage engineered for *N*-vinyl amide cyclopropanation (O. F. Brandenberg et al., unpublished results). Screening this lineage for stereoselectivity showed that P411-CIS L437F T438Q L75Y L181I, referred to in this study as P411-UA, had the highest activity and enantioselectivity. It was therefore used as a starting point in unactivated alkene cyclopropanation.

Homology models

ApePgb was modeled with the *Methanosarcina acetivorans* protoglobin (PDB ID: 3ZJL) and RmaNOD was modeled with *Alcaligenes eutrophus* flavohemoglobin (PDB ID: 1CQX) using SWISS-MODEL.[26] A homology model of P411-UA was generated through side-chain mutations to the P411-CIS crystal structure (PDB ID: 4H23). Figures generated from protein homology models and crystal structures were made with PyMOL (Schrödinger, Inc.).

Subcloning and transformation of genes

Genes for *Aeropyrum pernix* protoglobin (ApePgb) and *Rhodothermus marinus* nitric oxide dioxygenase (RmaNOD) were ordered as codon-optimized gBlocks (Integrated DNA Technologies, Coralville, Iowa). The gBlocks were amplified via polymerase chain reaction (PCR) and the PCR products were gel extracted and purified with Zymoclean Gel DNA Recovery Kit (Zymo Research Corp, Irvine, CA). The PCR product was subcloned into pET22b(+) via Gibson assembly.[27] Gibson assembly products were transformed into electrocompetent *E. cloni* EXPRESS BL21(DE3) cells (Lucigen, Middleton, WI) with a Gene Pulser Xcell (Bio-Rad, Hercules, CA). Aliquots of SOC medium (750 µL) were added and the cells were incubated at 37°C and 230 rpm for 45 minutes before being plated on LB-ampicillin (100 µg mL^{-1}) agar plates. Overnight cultures (5 mL LB-amp in culture tubes) were grown at 37°C and 230 rpm for 12-18 hours. Overnight cultures were used to inoculate flask cultures, prepare glycerol stocks, and isolate plasmids. Plasmids were isolated with Qiagen Miniprep kits and the genes were sequence verified (T7 promoter / terminator sequencing primers, Laragen, Inc.).

Protein expression

Cultures of Hyperbroth (HB, AthenaES) with 100 µg mL^{-1} ampicillin in unbaffled Erlenmeyer flasks were inoculated 1% (v/v) with stationary-phase overnight cultures and shaken in an Innova 42

shaker at 230 rpm, 37°C. At OD_{600} = 0.8, cultures were chilled on ice for 20 minutes. Protein expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and heme production was enhanced with supplementation of 1 mM 5-aminolevulinic acid (ALA). The cultures were shaken at 180 rpm and 22°C overnight (18-24 hours). Cells were pelleted via centrifugation at 4000 g for 10 minutes at 4°C. The supernatant was decanted and the cells were resuspended in M9-N buffer supplemented with 25 mM glucose.

Site-saturation library construction

Site-saturation mutagenesis was performed using the 22-codon method.[28] Briefly, oligonucleotides were ordered with NDT, VHG, and TGG codons in the coding strand at the amino acid position to be saturated. A reverse primer complementary to all three forward primers was also ordered. Two PCRs were performed for each library, the first containing a mixture of forward primers (12:9:1 NDT:VHG:TGG) and a pET22b(+) internal reverse primer and the second containing the complementary reverse primer and a pET22b(+) internal forward primer. The two PCR products were gel-purified with Zymoclean Gel DNA Recovery Kit (Zymo Research Corp, Irvine, CA) and ligated together via Gibson assembly. The Gibson assembly product was transformed into electrocompetent *E. cloni* EXPRESS BL21(DE3) cells (Lucigen, Middleton, WI). Aliquots of SOC medium (750 µL) were added and the cells were incubated at 37°C for 45 minutes before being plated on LB-ampicillin (100 µg mL^{-1}) agar plates.

Site-saturation library expression

Single colonies from the LB-ampicillin agar plates were picked using sterile toothpicks and grown in 300 µL LB-ampicillin in 2 mL 96 deep-well plates at 37°C, 250 rpm, 80% humidity overnight (12-18 hours). Multi-channel pipettes were used to transfer 30 µL of starter culture into deep-well plates containing 1 mL HB-amp per well. Glycerol stocks of these plates were prepared in parallel by adding starter culture (100 µL) and 50% (v/v) sterile glycerol (100 µL) to a 96-well microplate,

which was then stored at −80°C. The deep-well expression culture plate was incubated at 37°C, 250 rpm, 80% humidity for 2.5 hours. The plate was then chilled on ice for 30 minutes. The cultures were induced with 0.5 mM IPTG and supplemented with 1 mM ALA to increase cellular heme production. The plate was incubated at 22°C and 250 rpm overnight. The plate was centrifuged at 4000×g for 10 minutes at 4°C.

Site-saturation library reactions and screening

The pellets in the site-saturation library deep-well plates were resuspended in nitrogen-free M9 minimal medium (47.7 mM Na_2HPO_4, 22.0 mM KH_2PO_4, 8.6 mM NaCl, 2.0 mM $MgSO_4$, and 0.1 mM $CaCl_2$, abbreviated as M9-N, 400 µL). In an anaerobic chamber, 50 µL reactant mixture in ethanol (final concentrations in a 450 µL reaction: 20 mM EDA **1**, 20 mM 4-phenyl-1-butene **2b**) were added to the reaction plate. The reaction plate was covered with a pierceable foil cover (USA Scientific) and shaken at 500 rpm for 3 hours. To quench the reaction and extract the substrates, 400 µL of a mixture of acetonitrile (49 mL) and 3 M HCl (1 mL) was added to each well. The reaction plate was shaken for an additional 30 minutes, followed by centrifugation (4000×g, 10 minutes, 4°C). The supernatant was filtered through a 0.2 µm PTFE 96-well filter plate into a 96-well microplate (4000×g, 1 minute, RT). The microplate was sealed with a pierceable foil cover. The wells were screened for activity and diastereoselectivity of **3b** formation via HPLC using a Kromasil 100-5-C18 column, 4.6x50 mm with a 71% acetonitrile isocratic method (3 minutes). In later screening with higher enzymatic activity, the separation of *cis*- and *trans*-isomers of **3b** was improved with the use of an Eclipse XDB-C18 column, 5 µm particle size, 4.6x150 mm and a 6-minute 71% acetonitrile isocratic method. Wells with improved activity relative to the parent protein were streaked out from the glycerol stock onto LB-amp plates. A single colony was picked and grown in 5 mL LB-amp overnight (230 rpm, 37°C). These overnight cultures were used in flask protein expression and small-scale biocatalytic reactions to verify enhanced activity and/or selectivity relative to the parent sequence.

Sonicated lysate preparation and hemochrome assay

An aliquot of cells for protein concentration determination was sonicated (QSonica Q500 Sonicator, 1/8 in tip) for 2 minutes, 1 second on, 1 second off at 25% amplitude. Cells expressing ApePgb or RmaNOD variants had 0.1 eq. Bugbuster 10X protein extraction reagent (EMD Millipore) added prior to sonication. The sonicated lysate was clarified via centrifugation at 4500×g and 4°C for 10 minutes. The concentration of heme-loaded protein was determined with the pyridine hemochromagen (hemochrome) assay.[29] Briefly, sonicated and clarified lysate (500 µL) was sterile filtered and added to a cuvette. 500 µL of solution I (0.2 M NaOH, 40% (v/v) pyridine, 500 µM potassium ferricyanide) was added and the spectrum of this oxidized sample was taken from 350-600 nm. Sodium dithionite (10 µL of 0.5 M solution in 0.5 M NaOH) was added and the reduced spectrum was taken from 350-600 nm. The pyridine hemochromagen concentration was determined using its Q bands, with extinction coefficient 23.98 mM^{-1} cm^{-1} for (557 $nm_{reduced}$ – 540 $nm_{oxidized}$).[30]

Small-scale, whole-cell biocatalytic reaction preparation and work-up

Small-scale reactions were set up in 2 mL GC crimp vials. *E. coli* expressing the appropriate heme protein catalyst (380 µL, adjusted to the appropriate optical density or protein concentration) was added to the vials and they were brought into a Coy anaerobic chamber (~ 0-10 ppm O_2). To each vial was added alkene (final concentration 10 mM) followed by EDA **1** (final concentration 20 mM) with 5% ethanol as a cosolvent. Directly following addition of EDA, the reaction vial was crimped and shaken at 500 rpm at RT. Reactions were worked up by the addition of HCl (16 µL, 3 M stock) and internal standard (16 µL of 40 mM acetophenone in cyclohexane). Cyclohexane (700 µL) was added and the reaction was transferred into 1.7 mL Eppendorf tubes for extraction. The extraction was carried out with a Retsch MM 301 mixing mill (1 minute, 30 Hz / 1800 rpm). Samples were centrifuged at 20000×g for 5 minutes at RT and the organic layer was used for chromatographic analysis.

Preparative-scale, whole-cell biocatalytic reaction setup and product purification

Whole cells resuspended in M9-N supplemented with 25 mM glucose were brought into a Coy anaerobic chamber. Whole-cell catalyst was added to unbaffled Erlenmeyer flasks, followed by alkene (5 mM, 1.0 eq.) and EDA (10 mM, 2.0 eq.) diluted in ethanol (5% final ethanol cosolvent). The reactions were sealed and shaken at room temperature at 180 rpm for 16 hours. The product was extracted three times from the aqueous reaction mixture with 1 volume eq. of 2:1 pentane:diethyl ether. The organic layer was dried with sodium sulfate and concentrated via rotary evaporation. The concentrated reaction mixture was then purified via flash chromatography (Biotage, Inc.). Pentane:diethyl ether gradients were generally more effective at separating the cyclopropane products from the EDA dimer byproducts. Fractions containing cyclopropanation products were pooled and concentrated *in vacuo*.

Supplemental Table 3. Conditions for the preparative-scale reactions reported in the manuscript. **3k** and **3l** were run at higher reaction volume due to the lower molecular weight of the substrates (and corresponding products).

Product	Protein variant used	Reaction volume (mL)	Whole-cell OD_{600}
3i	ApePgb AGW	40	8
3j	ApePgb AGW	40	8
3k	RmaNOD Q52V	80	10
3l	ApePgb AGW	80	10
3m	ApePgb AGW	40	8

Large-scale protein expression and purification

HB-amp (1 L in 2.8 L unbaffled flask) was inoculated with 1% (v/v) overnight culture and shaken at 37°C, 160 rpm. At OD_{600} = 1.3 – 1.5 the flasks were chilled on ice for 20 minutes. Protein expression was then induced with 0.5 mM IPTG and 1 mM ALA and the cultures were grown for

24 hours at 22°C and 140 rpm. The cultures were pelleted (4000×g, 5 minutes, 4°C) and frozen at –20°C. The cells were resuspended in binding buffer (25 mM Tris HCl pH 7.5, 100 mM NaCl, 25 mM imidazole). Hemin (1 mg $g_{wet\ cells}^{-1}$) was added to increase heme loading of the protein and DNase I (0.1 mg mL^{-1}) was added to reduce lysate viscosity. The cells were sonicated, the lysate was clarified via centrifugation (20,000×g, 20 minutes, 4°C), and the clarified lysate was filtered (0.45 µm sterile filter). The protein was purified via HisTrap (1 mL column) on an ÄktaPurifier (GE Healthcare Life Sciences), using a 25 mM – 300 mM imidazole gradient over 10 column volumes. Fractions containing the protein of interest were pooled and buffer exchanged via centrifugal concentration to a 25 mM Tris HCl pH 7.5, 25 mM NaCl. The buffer-exchanged protein was flash-frozen and stored in 25 µL aliquots at –80°C. The purified protein concentration was determined using the bicinchoninic acid assay (BCA assay, Thermo Scientific) using the standard protocol provided.

Small-scale lysate and purified protein reactions

Small-scale reactions were set up in 2 mL GC crimp vials. Lysate or purified protein was diluted to the desired concentration with M9-N buffer (no glucose added), added to the vials, and brought into a Coy anaerobic chamber (~ 0-10 ppm O_2). To each vial was added sodium dithionite (final concentration 2 mM), alkene (final concentration 10 mM), and EDA **1** (final concentration 20 mM) with 5% ethanol as a cosolvent. Directly following addition of EDA, the reaction vial was crimped and shaken at 500 rpm at RT. Reactions were worked up by the addition of HCl (16 µL, 3 M stock) and internal standard (16 µL of 40 mM acetophenone in cyclohexane). Cyclohexane (700 µL) was added and the reaction was transferred into 1.7 mL Eppendorf tubes for extraction. The extraction was carried out with a Retsch MM 301 mixing mill (1 minute, 30 Hz / 1800 rpm). Samples were centrifuged at 20000×g for 5 minutes at RT and the organic layer was used for chromatographic analysis.

Determination of hemin-catalyzed cyclopropanation activity

Hemin-catalyzed cyclopropanation reactions were set up as small-scale reactions above; hemin (50 μM final concentration) in M9-N buffer, with or without 1 mg mL^{-1} bovine serum albumin (BSA), was brought into the Coy anaerobic chamber in 2 mL glass crimp vials. To each vial was added sodium dithionite (final concentration 2 mM), alkene (final concentration 10 mM), and EDA **1** (final concentration 20 mM) with 5% ethanol as a cosolvent. Directly following addition of EDA, the reaction vial was crimped and shaken at 500 rpm at room temperature for 16 hours.

Protein engineering strategies

***Rhodothermus marinus* nitric oxide dioxygenase (RmaNOD):**

3b and EDA **1** were used as substrates to engineer RmaNOD for improved activity and selectivity in the production of (***1S***, ***2S***)-**3b**. The wild-type gene was mutated at active-site positions Y32 (the putative distal axial ligand), Q52, and V97 using single-site site-saturation mutagenesis. Though modest improvements in activity in the Y32X site-saturation mutagenesis library was observed, the most significant increase in activity from this first round was the Q52V mutation. This mutation also enhanced the diastereo- and enantioselectivity to near-perfect.

***Aeropyrum pernix* protoglobin (ApePgb):**

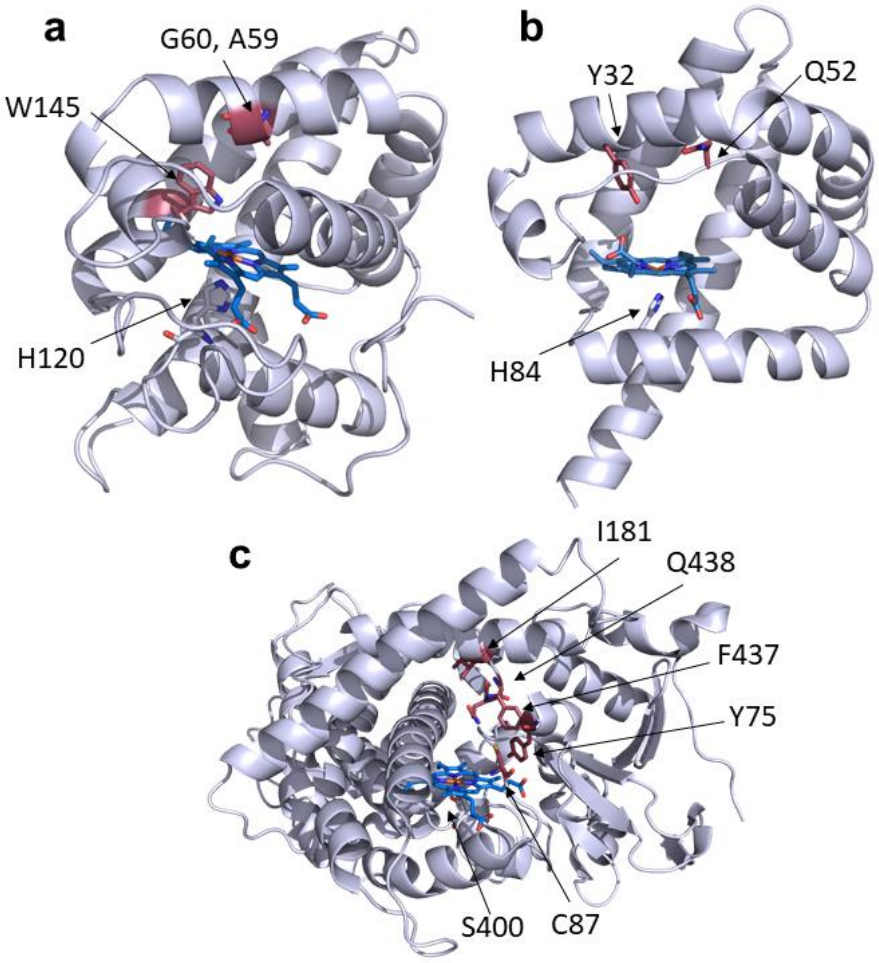
3b and EDA **1** were used as substrates to engineer ApePgb for improved activity and selectivity in the production of (***1R***, ***2S***)-**3b**. The wild-type gene underwent site-saturation mutagenesis at position Y60 (the putative distal axial ligand). This yielded the Y60G variant as the most active catalyst. ApePgb Y60G was then subjected to single-site site-saturation mutagenesis at W59, F73, F93, and F145 in parallel, all of which are positions known to modulate the gaseous ligand binding properties of the homologous *Methanosarcina acetivorans* protoglobin.[31] The variant that showed the greatest increase in activity while maintaining high selectivity was W59A Y60G. This

new variant was used to parent the next round of single-site site-saturation mutagenesis at positions F73, F93, and F145. After verification in small-scale biocatalytic reactions, ApePgb W59A Y60G F145W demonstrated a significantly increased in enantioselectivity for the production of (***1R, 2S***)-**3b**.

Previously engineered *Bacillus megaterium* variant P411-CIS L437F T438Q L75Y L181I (P411-UA):

3b and EDA **1** were used as substrates to screen P411-UA variants for improved selectivity in the production of (***1S***, ***2R***)-**3b**. The engineered P411-UA was first mutated at positions previously mutated in its lineage relative to P411-CIS. A simultaneous NDT double site-saturation mutagenesis library at F437/Q438 and single 22-codon site-saturation mutagenesis libraries[28] at Y75 and I181 were generated and screened for improved diastereoselectivity in the formation of **3b**. In each case P411-UA was among the most active and selective variants. Using P411-UA as parent protein, we next performed single-site site-mutagenesis at additional active site residues V87, I263, E267, and A328, screening for enhanced diastereoselectivity. The most significant changes in diastereoselectivity were in the V87X site-saturation library, in which an enhanced *cis* diastereoselectivity mutation (V87C) and an inversion in stereochemistry (V87F, producing the (***1R, 2R***)-isomer) were found.

Protein homology models



Supplemental Figure 2. Homology models of proteins engineered in the study. The protein and proximal ligand are shown in gray. Red indicates residues at which mutations were made for the engineered variants. The heme cofactor is shown in blue. a) ApePgb homology model with W59A, Y60G, and F145W mutations in red. b) RmaNOD homology model with Q52 and the putative distal ligand Y32 (where mutations also enhanced activity) in red. c) P411-UA homology model with V87C in red, as well as other mutated residues 75, 181, 437, and 438 colored.

Cyclopropanation activity and selectivity of whole-cell variants, purified proteins, and cell lysates

Supplemental Table 4. Activity and selectivity of hemin controls, wild-type protein, and engineering lineage intermediate proteins in **3a** product formation. Mean and standard deviation were determined from two biological replicates of technical duplicates. The reactions were performed on analytical scale (10 mM **2a**, direct addition of 20 mM **1**, 16-hour anaerobic reaction). n.d.: not determined. The diastereoselectivity ratio (d.r.) is given as *cis:trans* and the enantiomeric excess (*e.e.*) is given for the major diastereomer.

Catalyst	TTN	d.r.	*e.e.* (major)
Hemin	0.39 ± 0.04	25 : 75	n.d.
Hemin + BSA	0.41 ± 0.03	23 : 77	n.d.
RmaNOD WT	27 ± 9	3 : 97	89%
ApePgb WT	18 ± 2	81 : 19	69%
ApePgb Y60G	140 ± 40	82 : 18	96%
ApePgb W59A Y60G	360 ± 90	85 : 15	96%
P411-UA	500 ± 130	89 : 11	94%

Supplemental Table 5. Activity and selectivity of wild-type and engineering lineage intermediate proteins in **3b** product formation. Mean and standard deviation were determined from two biological replicates of technical duplicates. The reactions were performed on analytical scale (10 mM **2b**, direct addition of 20 mM **1**, 16-hour anaerobic reaction). The diastereoselectivity ratio (d.r.) is given as *cis:trans* and the enantiomeric excess (*e.e.*) is given for the major diastereomer.

Protein	TTN	d.r.	*e.e.* (major)
RmaNOD WT	34 ± 4	17 : 83	71%
ApePgb WT	85 ± 3	88 : 12	81%
ApePgb Y60G	125 ± 3	83 : 17	94%
ApePgb W59A Y60G	570 ± 50	80 : 20	94%
P411-UA	2500 ± 130	91 : 9	>99%

The four final protein variants were tested under whole-cell, aerobic conditions at analytical scale (400 µL) for their ability to form **3a**. The reactions were set up under the same conditions as the anaerobic analytical-scale reactions, with the exception that they were set up outside of the Coy anaerobic chamber. The aerobic conditions resulted in a nearly complete loss in cyclopropanation for the globins, with only traces of **3a** detected. The formation of EDA dimer was also severely attenuated, suggesting carbene formation was significantly slower under these conditions. The

P411-UA variants had a substantial loss in activity, but were still catalytically active (P411-UA V87C: 140 ± 5 TTN, 95:5 d.r.; P411-UA V87F: 25 ± 3 TTN, 6:94 d.r.). The loss of activity for the globins but not P411s could be due to the globins' high affinity for gaseous ligands like O_2, CO, and NO[32], whereas some engineered P411-BM3 variants have previously been shown to function, albeit with attenuated catalytic activity, in aerobic conditions[33]. In addition to being tested as anaerobic and aerobic whole-cell catalysts, the final protein variants were tested as sonicated cell lysates and as purified proteins. In cell lysate the proteins have decreased in activity relative to whole-cell catalysis. The enzymes also had decreased activity as purified proteins. In both lysate and purified protein, the catalysts maintained their stereoselectivity.

Supplemental Table 6. Activity and selectivity of final protein variants as whole-cell, sonicated cell lysate, and purified protein for **3a** product formation. The reactions were performed on analytical scale (10 mM **2a**, direct addition of 20 mM **1**, 16-hour anaerobic reaction). Whole-cell catalyst loading was OD_{600}= 5 (ApePgb AGW, RmaNOD Q52V) and OD_{600}=20 (P411-UA V87C, P411-UA V87F). Cell lysates were diluted to the apparent OD_{600}= 5 (ApePgb AGW, RmaNOD Q52V) and OD_{600}=20 (P411-UA V87C, P411-UA V87F), corresponding to protein concentrations of 0.9 – 2.1 µM. Catalyst loading in purified protein reactions was 5 µM. The diastereoselectivity ratio (d.r.) is given as *cis:trans*.

	Whole cell		Cell lysate		Purified protein	
Protein	TTN	d.r.	TTN	d.r.	TTN	d.r.
RmaNOD Q52V	100 ± 6	< 1 : 99	43 ± 2	< 1 : 99	53 ± 9	< 1 : 99
ApePgb AGW	490 ± 20	89 : 11	190 ± 9	89 : 11	80 ± 10	87 : 13
P411-UA V87C	270 ± 30	95 : 5	56 ± 4	95 : 5	6.9 ± 0.9	95 : 5
P411-UA V87F	310 ± 20	4 : 96	54 ± 5	5 : 95	13 ± 2	4 : 96

Decreased activity in lysate and purified protein relative to whole-cell reactions has been observed in many enzyme-catalyzed carbene transfer reactions[34,35] and is likely due to reduced carbene transfer-based enzyme inactivation in whole cells[36]. Whole cells could also be stabilizing the protein (through proper macromolecular crowding effects, chaperones, etc.). As our focus was on developing a straightforward system for biocatalysis, we opted for whole-cell catalysis that does not require additional catalyst purification steps.

Supplemental Table 7. Substrate scope and diastereoselectivity of the four final variants. Activities were confirmed via GC-MS. +: activity against the substrate. *: trace activity detected. –: no detectable activity. n.d.: not determined. Diastereoselectivity ratio is given as *cis*:*trans*.

Alkene, product	P411-UA V87C	ApePgb AGW	P411-UA V87F	RmaNOD Q52V
1-octene (**3a**)	+, 95:5 d.r.	+, 89:11 d.r.	+, 4:96 d.r.	+, <1:99 d.r.
4-phenyl-1-butene (**3b**)	+, 96:4 d.r.	+, 84:16 d.r.	+, <1:99 d.r.	+, 3:97 d.r.
benzyl acrylate (**3c**)	+, 91:9 d.r.	+, 71:29 d.r.	+, 2:98 d.r.	+, <1:99 d.r.
6-bromo-1-hexene (**3d**)	+, 92:8 d.r.	+, 92:8 d.r.	+, 4:96 d.r.	+, <1:99 d.r.
vinyl cyclohexane (**3e**)	*	+, 76:24 d.r.	*	*
methylenecyclohexane (**3f**)	*	+	+	*
1-penten-3-one (**3g**)	*	+, 89:11 d.r.	*	+, <1:99 d.r.
2-vinylpyridine (**3h**)	*	*	*	+, <1:99 d.r.
7-octen-1-ol (**3i**)	+	+, 91:9 d.r.	*	*
7-octen-1-oic acid (**3j**)	n.d.	+, 83:17 d.r.	n.d.	n.d.
(*E*)-penta-1,3-diene (**3k**)	+, 96:4 d.r.	+, 91:9 d.r.	+, 31:69 d.r.	+, 4:96 d.r.
(*Z*)-penta-1,3-diene (**3l**)	+, 55:45 d.r.	+, 93:7 d.r.	+, 35:65 d.r.	+, 35:65 d.r.
5-hexen-2-one (**3m**)	–	+, 94:6 d.r.	–	*

Supplemental Table 8. GC yields for analytical-scale reactions reported in Figure 2 in the manuscript. These yields are determined by comparing the GC yield to the calibration curves (*Calibration curves for analytical-scale TTN determination*). These reactions were run under the conditions given above (*Small-scale, whole-cell biocatalytic reaction preparation and work-up*). These reactions were run under conditions designed to demonstrate the catalysts' potential TTNs, rather than yields (TTN and stereoselectivity information in main text Figures 2 and 3).

Alkene, product	P411-UA V87C	ApePgb AGW	P411-UA V87F	RmaNOD Q52V
1-octene (**3a**)	4%	8%	7%	5%
4-phenyl-1-butene (**3b**)	26%	40%	44%	4%
benzyl acrylate (**3c**)	40%	91%	53%	71%
6-bromo-1-hexene (**3d**)		19%		
vinyl cyclohexane (**3e**)		18%		
methylenecyclohexane (**3f**)		10%		
1-penten-3-one (**3g**)		41%		
2-vinylpyridine (**3h**)				60%

Compound synthesis and characterization
General procedure A:

Rhodium acetate dimer (10 µmol, 4.4 mg) and a stir bar were added to a 5 mL dram vial and it was sealed with a septum. The sealed vial was purged with three cycles of vacuum and argon. Neat olefin (8 mmol) was added to the vial. EDA (2 mmol) was added in a 2-hour slow addition on ice and reacted overnight at room temperature. The crude reaction mixture was concentrated *in vacuo* and loaded on a SNAP Ultra silica flash cartridge. The reaction mix was separated on an Isolera flash purification system (Biotage, Charlotte, NC) with a hexane/ethyl acetate gradient. Fractions containing the desired product were pooled and concentrated *in vacuo*. Yields were approximately 5-30%, due in part to both significant EDA dimer formation and low conversion of the unactivated alkenes.

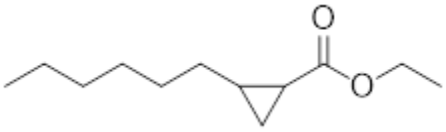
General procedure B:

Rhodium acetate dimer (40 µmol, 17 mg) and a stir bar was added to a scintillation vial and sealed with a septum. The sealed vial was purged with three cycles of vacuum and argon. The vial was charged with alkene (5 mmol) and dichloromethane (5 mL). EDA **1** (2.0 eq. diluted in 4 mL dichloromethane) was added at room temperature by slow addition over 2 hours and reacted overnight at room temperature. The crude reaction mixture was concentrated *in vacuo* and loaded on a SNAP Ultra silica flash cartridge. Using pentane/diethyl ether as eluents, the reaction mix was separated on an Isolera flash purification system (Biotage, Charlotte, NC). Fractions containing the desired product were pooled and concentrated *in vacuo*. Yields of pure fractions were approximately 5-30%, due in part to both significant EDA dimer formation and low conversion of the unactivated alkenes. This method is preferable for more volatile alkenes, and the pentane/diethyl ether gradients appeared to give better separation from the EDA dimer byproducts compared to the hexanes/ethyl acetate gradient.

Determination of absolute configurations of the cyclopropane products

Absolute configurations of **3a** were confirmed by comparison to literature chiral GC.[15] Cyclosil-B column, 90°C isothermal, absolute configuration of products elute in order: *cis* (***1S, 2R***)–**3a**, (***1R, 2S***)–**3a**, *trans*: (***1R, 2R***)–**3a**, (***1S, 2S***)–**3a**. The absolute configurations of other compounds in this study were not determined, but one could infer them by analogy, assuming the facial selectivity of the diazo reagents and olefins from which these products were made remains the same for each protein variant. The inferred absolute configurations provided here should be used with caution, understanding that substrate effects could have inverted the absolute stereochemistry. The chiral separation conditions for all cyclopropane products are detailed in the section below (*Compound chiral separation conditions and representative traces*), and can be utilized to compare the enzymatic products to an absolute configuration authentic standard in future studies. The optical rotations of some isolated products were collected for additional characterization of their chirality.

Ethyl 2-hexylcyclopropane-1-carboxylate (**3a**)

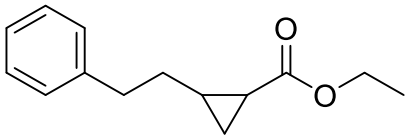


3a was synthesized with procedure B from 1-octene **2a** and **1** to give **3a** as a 58:42 mixture of *trans*/*cis*-isomers. Both *trans*- and *cis*-**3a** are known compounds.[15,16]

Cis-**3a**: ^{1}H NMR (300 MHz, Chloroform-*d*) δ 4.13 (q, *J* = 7.1 Hz, 2H), 1.66 (ddd, *J* = 8.9, 7.8, 5.5 Hz, 1H), 1.56 – 1.43 (m, 2H), 1.38 – 1.22 (m, 12H), 1.03 – 0.90 (m, 2H), 0.90 – 0.82 (m, 3H).

Trans-**3a**: ^{1}H NMR (300 MHz, Chloroform-*d*) δ 4.11 (q, *J* = 7.1 Hz, 2H), 1.36 – 1.22 (m, 15H), 1.14 (dt, *J* = 8.7, 4.5 Hz, 1H), 0.90 – 0.84 (m, 3H), 0.68 (ddd, *J* = 7.9, 6.1, 4.0 Hz, 1H). HR-MS (FAB+): fragment ion, loss of ethoxy group $[M - CH_3CH_2O]^+$ $C_{10}H_{17}O$ calculated 153.1279, found 153.127.

Ethyl 2-phenethylcyclopropane-1-carboxylate (**3b**)

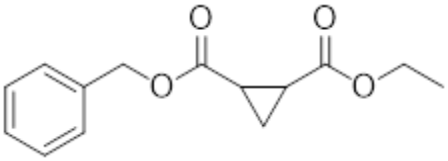


3b was synthesized with procedure B from 4-phenyl-1-butene **2b** and **1** to give **3b** as a 57:43 mixture of *trans*/*cis*-isomers. C*is*-**3b** is a known compound.[14]

Cis-**3b**: ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.37 – 7.22 (m, 5H), 4.20 (q, J = 7.1 Hz, 2H), 2.70 (t, J = 7.3 Hz, 2H), 1.93 (tq, J = 14.1, 6.7 Hz, 2H), 1.80 – 1.66 (m, 1H), 1.34 (td, J = 7.1, 0.7 Hz, 4H), 1.14 – 0.96 (m, 2H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 173.2, 142.1, 128.6, 128.4, 125.9, 60.5, 36.0, 29.0, 21.5, 18.4, 14.5, 13.6.

Trans-**3b** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.32 – 7.23 (m, 2H), 7.24 – 7.13 (m, 3H), 4.11 (q, *J* = 7.1 Hz, 2H), 2.72 (t, *J* = 7.7 Hz, 2H), 1.71 – 1.54 (m, 2H), 1.45 – 1.31 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.16 (ddd, *J* = 8.8, 4.8, 4.1 Hz, 1H), 0.69 (ddd, *J* = 8.2, 6.3, 4.1 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 174.5, 141.8, 128.5, 128.5, 126.0, 60.5, 35.6, 35.2, 22.6, 20.4, 15.6, 14.4. HR-MS (FAB+): $[M+H]^+$ $C_{14}H_{19}O_2$, calculated 219.1385, found 219.1382.

1-Benzyl 2-ethyl cyclopropane-1,2-dicarboxylate (**3c**)

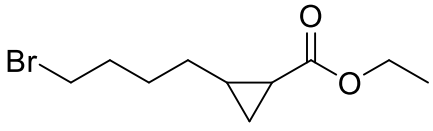


General procedure A only produced trace amounts of the cyclopropane product **3c**. As each final protein variant produced **3c** with high activity, preparative-scale reactions were carried out for each protein. The purified products were confirmed via NMR and used for chiral separation method development and analytical-scale calibration curves.

Cis-**3c** (prepared from P411-UA V87C) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 – 7.28 (m, 5H), 5.15 (d, *J* = 12.3 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 4.08 (qd, *J* = 7.1, 1.0 Hz, 2H), 2.16 – 1.99 (m, 2H), 1.71 (td, *J* = 6.7, 5.0 Hz, 1H), 1.28 – 1.23 (m, 1H), 1.20 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.9, 169.9, 135.9, 128.7, 128.5, 128.4, 67.0, 61.2, 21.9, 21.7, 14.3, 11.9. HR-MS: $[M+H]^+$ $C_{14}H_{17}O_4$,calculated 249.1127, found 249.1125. *Cis*-**3c** optical rotation from ApePgb AGW product: $[\alpha]_D^{22}$ = –17.0 ° (*c* 0.1, EtOAc). *Cis*-**3c** optical rotation from P411-UA V87C product: $[\alpha]_D^{22}$ = +9.8 ° (*c* 0.1, EtOAc).

Trans-**3c** (prepared from P411-UA V87F) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.29 (m, 5H), 5.13 (s, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 2.27 – 2.15 (m, 2H), 1.46 (ddd, *J* = 8.3, 6.2, 1.8 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.8, 171.8, 135.6, 128.8, 128.5, 128.5, 67.0, 61.3, 22.7, 22.5, 15.7, 14.3. HR-MS : $[M+H]^+$ $C_{14}H_{17}O_4$, calculated 249.1127, found 249.1123. *Trans*-**3c** optical rotation from P411-UA V87F product: $[\alpha]_D^{22}$ = –124.1 ° (*c* 0.1, EtOAc).

Ethyl 2-(4-bromobutyl)cyclopropane-1-carboxylate (**3d**)

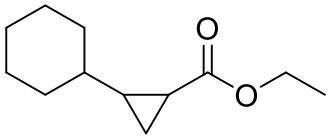


Both *cis*- and *trans*-**3d** are *k*nown compounds.[37] **3d** was synthesized with Procedure A from 6-bromo-1-hexene **2d** and EDA **1** to give **3d**. The crude *cis*/*trans* ratio of this reaction was not determined.

Cis-**3d** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.14 (q, *J* = 7.1 Hz, 2H), 3.40 (t, *J* = 6.8 Hz, 2H), 1.93 – 1.80 (m, 2H), 1.74 – 1.38 (m, 4H), 1.56 (s, 1H), 1.33 – 1.15 (m, 4H), 1.02 (td, *J* = 8.0, 4.5 Hz, 1H), 0.92 (ddd, *J* = 7.2, 5.4, 4.5 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.1, 60.5, 34.0, 32.6, 28.4, 26.3, 21.7, 18.3, 14.5, 13.5. HR-MS (FAB+): Fragment ion, loss of ethoxy group [M - CH_3CH_2O]$^+$ $C_8H_{12}{}^{79}BrO$,calculated 203.0072, found 203.0026.

3d fraction containing 71:29 *trans*/*cis* diastereomeric mixture, reporting shifts characterized as *trans*-**3d**. ^{1}H NMR (400 MHz, Chloroform-d) δ 4.21 – 4.04 (m, 2H), 3.40 (td, J = 6.8, 3.6 Hz, 2H), 1.94 – 1.79 (m, 2H), 1.72 – 1.46 (m, 3H), 1.39 – 1.31 (m, 2H), 1.31 – 1.19 (m, 4H), 1.22 – 1.12 (m, 1H), 0.74 – 0.64 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 174.5, 60.5, 33.8, 32.5, 32.3, 27.8, 22.6, 20.3, 15.6, 14.4.

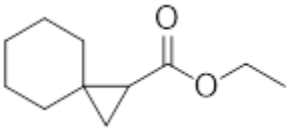
Ethyl 2-cyclohexylcyclopropane-1-carboxylate (**3e**)



Synthesized with general procedure A from vinylcyclohexane **2e** and EDA **1** to give **3e**. The crude *trans*/*cis* ratio of this reaction was not determined. *Cis*-**3e** is a known compound.[14]

Cis-**3e**: ^{1}H NMR (300 MHz, Chloroform-*d*) δ 4.18 – 4.04 (m, 1H), 1.85 – 1.51 (m, 6H), 1.29 – 0.92 (m, 2H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 173.2, 60.2, 35.8, 33.3, 33.2, 28.6, 26.4, 26.1, 25.9, 17.9, 14.4, 12.5. HR-MS (FAB+): [M]$^+$ $C_{12}H_{20}O_2$, calculated 196.1463, found 196.1464.

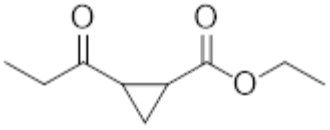
Ethyl spiro[2.5]octane-1-carboxylate (**3f**)



Synthesized with general procedure A from methylenecyclohexane **2f** and EDA **1**. **3f** is a known compound.[38]

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.11 (qd, *J* = 7.1, 1.0 Hz, 2H), 1.62 – 1.27 (m, 11H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.06 (dd, *J* = 5.4, 4.3 Hz, 1H), 0.79 (dd, *J* = 7.8, 4.3 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.1, 60.2, 37.4, 30.7, 28.8, 26.2 (2 overlapping carbons), 25.7, 25.7, 20.6, 14.4. HR-MS (FAB+): [M]$^+$ $C_{11}H_{18}O_2$, calculated 182.1307, found 182.1282

Ethyl 2-propionylcyclopropane-1-carboxylate (**3g**)



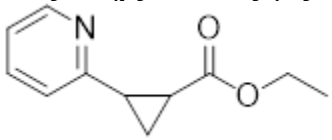
Running general procedure B with 1-penten-3-one **2h** produced no detectable cyclopropane product **3g**; the primary products were diethyl fumarate, diethyl maleate, and an apparent 1,3-dipolar [2+3] cycloaddition product (ethyl 3-propionyl-4,5-dihydro-1*H*-pyrazole-5-carboxylate). *Trans*-**3g** was synthesized on a 5 mmol scale with a racemic strategy adapted from the chiral synthesis previously reported.[39] Co(II) tetraphenylporphyrin (5 mol%, 168 mg) and 1,1,-dimethylaminopyridine (DMAP, 305 mg, 2.5 mmol) were added to a 40 mL scintillation vial with stir bar. The vial was sealed with a septum and placed under an Argon atmosphere. Dichloromethane (18 mL) was added, followed by 1-penten-3-one **2g** (510 µL, 5 mmol). EDA **1** (725 µL, 6 mmol) was added via syringe pump over 2 hours. The crude reaction mixture was concentrated *in vacuo* and separated via flash chromatography with a SNAP Ultra 25g column using a gradient of 0-20% diethyl ether in pentane. Fractions containing *trans*-**3g** were concentrated *in vacuo* to give the desired product.

Trans-**3g** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.14 (q, *J* = 7.2 Hz, 2H), 2.63 (q, *J* = 7.3 Hz, 2H), 2.49 – 2.39 (m, 1H), 2.21 – 2.11 (m, 1H), 1.45 – 1.35 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.08 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 208.2, 172.3, 61.2, 37.3, 28.9, 24.1, 17.2, 14.3, 7.8. HR-MS (ESI+): $[M+Na]^+$ $C_9H_{14}O_3Na$, calculated 193.0841 found 193.0819.

Cis-**3g** was synthesized based on a modified literature procedure of the methyl ketone analog.[40] 3-Oxabicyclo[3.1.0]hexane-2,4-dione (10 mmol) was added to anhydrous diethyl ether under Argon. Ethylmagnesium bromide (9 mL of 1 M in diethyl ether, diluted from 3 M solution in diethyl ether, 9 mmol) was added dropwise over 20 min at −78°C. The reaction mixture was allowed to warm to room temperature over 2 hours and stirred for 18 hours. The reaction was then quenched with aqueous ammonium chloride and extracted by diethyl ether (4 x 40 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude product was used for the esterification step without further purification. Half of the crude product was dissolved in ethanol (20 mL), thionyl chloride (1 mL) was added, and the reaction proceeded at room temperature for 12 hours. The reaction was then quenched with aqueous sodium bicarbonate and diluted with water, and extracted with diethyl ether. The crude mixture was purified via flash chromatography using a gradient of 0-50% diethyl ether in pentane. Fractions containing *cis*-**3g** were concentrated *in vacuo* to give the desired product.

Cis-**3g** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.20 – 4.04 (m, 2H), 2.69 – 2.47 (m, 2H), 2.22 (ddd, *J* = 9.3, 8.2, 6.7 Hz, 1H), 2.08 (ddd, *J* = 9.2, 8.4, 6.6 Hz, 1H), 1.70 (tdd, *J* = 6.7, 4.7, 0.6 Hz, 1H), 1.23 (t, *J* = 7.2 Hz, 3H), 1.22 – 1.15 (m, 1H), 1.07 (td, J = 7.3, 0.5 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 206.6, 170.1, 61.1, 36.9, 27.9, 23.4, 14.3, 12.2, 7.9.

Ethyl 2-(pyridin-2-yl)cyclopropane-1-carboxylate (3h)



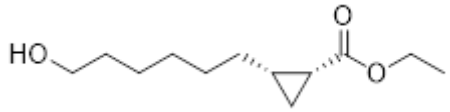
Both *trans*- and *cis*-**3h** are known compounds.[41] *Cis*- and *trans*-**3h** were produced by refluxing 2-vinylpyridine **2h** (10 mmol) with EDA **1** (10 mmol) in toluene (20 mL) overnight. The reaction yielded a 65:35 *trans/cis* mixture of diastereomers. The crude mixture was concentrated *in vacuo* and the product was purified using flash chromatography with a SNAP Ultra 10g Biotage column, 0-100% hexane:EtOAc gradient. Fractions containing pure *trans*-**3h** and *cis*-**3h** were separable

and concentrated *in vacuo* to give the desired products (391.7 mg *trans*-**3h**, 290.7 mg *cis*-**3h**, 36% yield).

Cis-**3h**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.49 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 1H), 7.58 (td, *J* = 7.7, 1.8 Hz, 1H), 7.32 – 7.22 (m, 1H), 7.10 (ddd, *J* = 7.4, 4.8, 1.1 Hz, 1H), 3.90 (qd, *J* = 7.2, 1.5 Hz, 2H), 2.72 (td, *J* = 9.0, 7.6 Hz, 1H), 2.22 – 2.11 (m, 1H), 1.87 – 1.77 (m, 1H), 1.47 – 1.36 (m, 1H), 1.02 (td, *J* = 7.1, 0.9 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.1, 156.7, 148.9, 135.9, 123.6, 121.6, 60.3, 27.2, 21.7, 14.0, 11.7.

Trans-**3h** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.43 (ddd, *J* = 4.9, 1.9, 1.0 Hz, 1H), 7.54 (td, *J* = 7.7, 1.8 Hz, 1H), 7.21 (dt, *J* = 7.8, 1.1 Hz, 1H), 7.07 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.56 (ddd, *J* = 8.9, 6.1, 3.9 Hz, 1H), 2.23 (ddd, *J* = 8.4, 5.5, 3.9 Hz, 1H), 1.59 (dddd, *J* = 13.8, 9.0, 5.8, 3.8 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.4, 158.9, 149.4, 136.0, 122.5, 121.3, 60.7, 27.2, 24.4, 17.3, 14.3. HR-MS (FAB+): $[M]^+$ $C_{11}H_{13}NO_2$, calculated 191.0946, found 191.092.

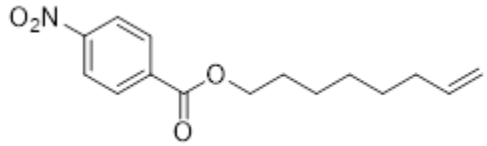
***Cis* Ethyl 2-(6-hydroxyhexyl)cyclopropane-1-carboxylate (*cis*-3i)**



Cyclopropanation of the free alcohol **2i** was performed via preparative-scale enzymatic reaction (ApePgb AGW) to give *cis*-**3i**.

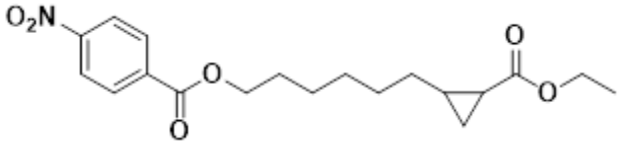
cis -**3i** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.12 (q, *J* = 7.1 Hz, 2H), 3.62 (t, *J* = 6.6 Hz, 2H), 1.78 – 1.61 (broad m, 1H), 1.66 (ddd, *J* = 8.9, 7.8, 5.5 Hz, 1H), 1.60 – 1.43 (m, 4H), 1.41 – 1.29 (m, 6H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.25 – 1.16 (m, 1H), 1.05 – 0.95 (m, 1H), 0.91 (ddd, *J* = 7.2, 5.5, 4.4 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.3, 63.1, 60.4, 32.8, 29.7, 29.2, 27.0, 25.8, 22.1, 18.3, 14.5, 13.5. HR-MS (FAB+): $[M+H]^+$ $C_{12}H_{23}O_3$, calculated 215.1647, found 215.1648.

Oct-7-en-1-yl 4-nitrobenzoate (2ia)



7-Octen-1-ol **2i** (577 mg, 4.5 mmol, 1.0 eq.) and triethylamine (0.82 mL, 1.3 eq.) were dissolved in anhydrous dichloromethane (45 mL). A solution of 4-nitrobenzoyl chloride (944 mg, 1.13 eq.) in anhydrous dichloromethane (5 mL) was added to the reaction. The resulting mixture was stirred at room temperature for 4 hours. The crude reaction mixture was extracted with dichloromethane and washed with brine and concentrated *in vacuo*. The product was purified by flash chromatography with a gradient of 0-8% ethyl acetate in hexane. Fractions containing **2ia** were concentrated *in vacuo* to give the desired product (1.24 g, 99% yield).

6-(2-(Ethoxycarbonyl)cyclopropyl)hexyl 4-nitrobenzoate (3ia)

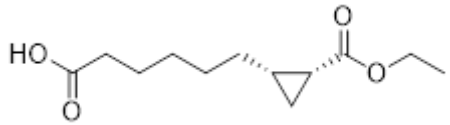


3ia standard (racemic mixture) was synthesized as a mixture of *trans*/*cis*-isomers using general procedure B from **2ia** and EDA **1** and purified via flash chromatography with a gradient of 0-13% ethyl acetate in hexane. Fractions containing *trans*-**3ia** and *cis*-**3ia** were separable and concentrated *in vacuo* to give the desired products (approximately 25% yield).

Cis-**3ia** derivatized enzymatic product was prepared by protecting the preparative-scale enzymatic product **3i**. **3i** (~16 mg, ~0.058 mmol, 1.0 eq.), 4-nitrobenzoyl chloride (43 mg, 4.0 eq.), and triethylamine (80 µL, ~10.0 eq.) were added to anhydrous dichloromethane and the reaction proceeded at room temperature for 3 hours. The crude reaction mixture was concentrated *in vacuo* and purified by flash chromatography with a gradient of 0-13% ethyl acetate in hexane. Fractions containing *cis*-**3ia** were concentrated *in vacuo* to give the desired product.

Cis-**3ia** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 – 8.25 (m, 2H), 8.25 – 8.17 (m, 2H), 4.36 (t, *J* = 6.7 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 1.82 – 1.74 (m, 2H), 1.77 – 1.62 (m, 1H), 1.49 – 1.32 (m, 9H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.00 (td, *J* = 8.2, 4.5 Hz, 1H), 0.94 – 0.87 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.2, 164.9, 150.6, 136.0, 130.8, 123.7, 66.2, 60.4, 29.6, 29.1, 28.7, 27.0, 26.1, 22.0, 18.3, 14.5, 13.6. HR-MS (FAB+): $[M+H]^+$ $C_{19}H_{26}NO_6$ calculated 364.1760, found 364.1775.

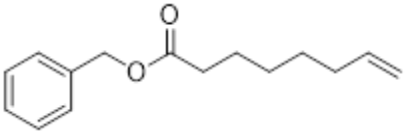
Cis 6-(2-(ethoxycarbonyl)cyclopropyl)hexanoic acid (***cis*-3j**)



Cyclopropanation of the free carboxylic acid **2j** was performed via preparative-scale enzymatic reaction (ApePgb AGW) to give *cis*-**3j**.

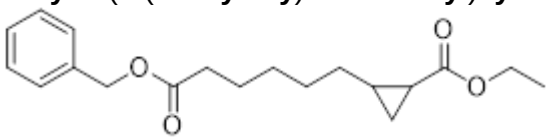
Cis-**3j** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.00 – 9.50 (broad, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 2.38 – 2.29 (m, 2H), 1.71 – 1.58 (m, 3H), 1.58 – 1.40 (m, 2H), 1.40 – 1.30 (m, 4H), 1.29 – 1.16 (m, 1H), 1.26 (t, 3H), 0.99 (td, *J* = 8.1, 4.4 Hz, 1H), 0.91 (ddd, *J* = 7.2, 5.4, 4.5 Hz, 1H). *Trans*-**3j** cyclopropane isomers (minor product in *cis*-**3j** ^{1}H NMR): 1.14 (tdd, *J* = 7.1, 4.8, 2.5 Hz), 0.68 (ddd, *J* = 7.8, 5.9, 4.0 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 179.4, 173.3, 60.4, 34.0, 29.4, 28.8, 26.9, 24.8, 21.9, 18.3, 14.5, 13.5. HR-MS (FAB+): $[M+H]^+$ $C_{12}H_{21}O_4$, calculated 229.1440, found 229.1448.

Benzyl oct-7-enoate (**2ja**)



2ja was produced by benzyl-protection of 7-octen-1-oic acid **2j**. **2j** (498 mg, 3.5 mmol, 1.0 eq.) and potassium carbonate (967 mg, 2.0 eq.) were added to anhydrous dimethylformamide (20 mL). A solution of benzyl bromide (720 mg, 1.2 eq.) in anhydrous dimethylformamide (4 mL) was added to the reaction slowly over 10 min. The resulting reaction mixture was stirred at room temperature for 20 hours. The crude reaction mixture was extracted with diethyl ether, washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The product was purified via flash chromatography with hexane:ethyl acetate (20:1) as eluent. Fractions containing **2ja** were concentrated *in vacuo* to give the desired product (805 mg, 99% yield).

Ethyl 2-(6-(benzyloxy)-6-oxohexyl)cyclopropane-1-carboxylate (3ja)



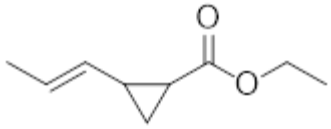
3ja racemic standard was synthesized with a modification of general procedure B. **2ja** (100 mg, ~0.43 mmol) and EDA **1** (~5.0 eq) were added to dichloromethane with $Rh_2(OAc)_4$ (2 mol%) and the reaction proceeded at room temperature for 20 hours. The crude reaction mixture was concentrated *in vacuo* and purified via flash chromatography with a gradient of 0-20% ethyl acetate in hexane. Fractions containing *trans*-**3ja** and *cis*-**3ja** were separable and concentrated *in vacuo* to give the desired products (<15% yield).

3ja derivatized enzymatic product was synthesized from the preparative-scale enzymatic reaction (ApePgb AGW) **3j**. **3j** (15 mg, ~0.065 mmol, 1.0 eq.) and potassium carbonate (27 mg, 3.0 eq.) were added to anhydrous dimethylformamide (2 mL). A solution of benzyl bromide (16 µL, 2.0 eq.) in anhydrous dimethylformamide (0.5 mL) was added to the reaction slowly. The resulting reaction mixture was stirred at room temperature for 20 hours. The crude reaction mixture was extracted with diethyl ether, washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The product was purified via flash chromatography with hexane:ethyl acetate (10:1) as eluent. Fractions containing **3ja** were concentrated *in vacuo* to give the desired product.

Cis-**3ja** from **3j** produced by ApePgb AGW. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35 (s, 3H), 7.41 – 7.27 (m, 2H), 5.11 (s, 2H), 4.17 – 4.06 (m, 2H), 2.35 (td, *J* = 7.5, 3.4 Hz, 2H), 1.69 – 1.59 (m, 3H), 1.55 – 1.28 (m, 6H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.20 (dtd, *J* = 8.6, 7.1, 1.5 Hz, 1H), 0.98 (td, *J* = 8.1, 4.4 Hz, 1H), 0.90 (ddd, *J* = 7.3, 5.5, 4.5 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 173.8, 173.2, 136.2, 128.7, 128.3, 128.3, 66.2, 60.4, 34.4, 29.4, 28.9, 26.9, 25.1, 21.9, 18.3, 14.5, 13.5. HR-MS (FAB+): $[M+H]^+$ $C_{19}H_{27}O_4$, calculated 319.1909, found 319.1903.

Trans-**3ja** from $Rh_2(OAc)_4$-catalyzed reaction. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.35 (s, 2H), 7.41 – 7.29 (m, 3H), 5.11 (s, 2H), 4.11 (qd, *J* = 7.2, 0.7 Hz, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.68 – 1.59 (m, 3H), 1.45 – 1.29 (m, 7H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.18 – 1.09 (m, 1H), 0.67 (ddd, *J* = 7.8, 6.0, 4.1 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 174.6, 173.6, 136.1, 128.6, 128.2, 66.1, 60.3, 34.2, 32.8, 28.8, 28.7, 24.9, 22.8, 20.2, 15.5, 14.3.

Ethyl (*E*)-2-(prop-1-en-1-yl)cyclopropane-1-carboxylate (3k)



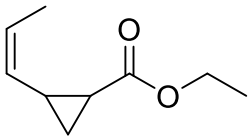
Both *trans*- and *cis*-**3k** are known compounds.[42] Terminal alkene cyclopropanation of (*E*)-penta-1,3-diene **2k** and EDA **1** accomplished via preparative-scale enzymatic reaction gave *cis*-**3k** (ApePgb AGW) and *trans*-**3k** (RmaNOD Q52V).

Cis-**3k** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.66 (dq, *J* = 15.4, 6.5 Hz, 1H), 5.41 (ddq, *J* = 15.3, 8.6, 1.7 Hz, 1H), 4.22 – 4.03 (m, 2H), 1.89 – 1.78 (m, 2H), 1.67 (dd, *J* = 6.5, 1.7 Hz, 3H), 1.26 (t, 3H), 1.23 – 1.10 (m, 2H).

Trans-**3k** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.60 (dqd, *J* = 15.1, 6.5, 0.8 Hz, 1H), 5.03 (ddq, *J* = 15.2, 8.3, 1.7 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 1.95 (dddd, *J* = 9.4, 8.5, 6.3, 4.0 Hz, 1H), 1.65 (dd, *J* = 6.5, 1.7 Hz, 3H), 1.57 – 1.53 (m, 1H), 1.31 (ddd, *J* = 9.2, 5.1, 4.3 Hz, 1H), 1.26 (t, J =

7.1 Hz, 3H), 0.91 (ddd, *J* = 8.3, 6.3, 4.3 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.9, 130.9, 126.1, 60.7, 25.0, 21.8, 17.9, 15.6, 14.4. HR-MS (EI+): $[M]^+$ $C_9H_{14}O_2$, calculated 154.0994, found 154.0974. *Trans*-**3k** optical rotation from RmaNOD Q52V product: $[\alpha]_D^{23}$ = +164 ° (*c* 0.1, EtOAc).

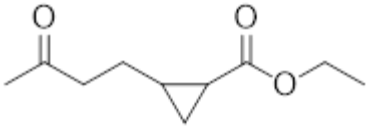
Ethyl (*Z*)-**2**-(**prop**-**1**-**en**-**1**-**yl**)**cyclopropane**-**1**-**carboxylate (3l**)



Both *trans*- and *cis*-**3l** are known compounds.[42] General procedure A was used to produce a mixture of 8 possible cyclopropanes (internal and terminal) for chiral GC standard from (*Z*)-penta-1,3-diene **2l** and EDA **1**. Stereoselective, terminal alkene cyclopropanation accomplished via preparative-scale enzymatic reaction (ApePgb AGW).

Cis-**3l** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.56 (dqd, *J* = 10.9, 6.9, 1.1 Hz, 1H), 5.34 (ddq, *J* = 11.0, 9.3, 1.8 Hz, 1H), 4.21 – 4.04 (m, 2H), 2.16 – 2.03 (m, 1H), 1.92 (ddd, *J* = 8.8, 7.8, 6.0 Hz, 1H), 1.72 (dd, *J* = 6.8, 1.8 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.23 – 1.17 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.3, 127.2, 126.3, 60.5, 21.0, 19.2, 14.5, 14.5, 13.3. *Cis*-**3l** optical rotation from ApePgb AGW product: $[\alpha]_D^{23}$ = −260 ° (*c* 0.1, EtOAc).

Ethyl 2-(**3**-**oxobutyl**)**cyclopropane**-**1**-**carboxylate** (**3m**)



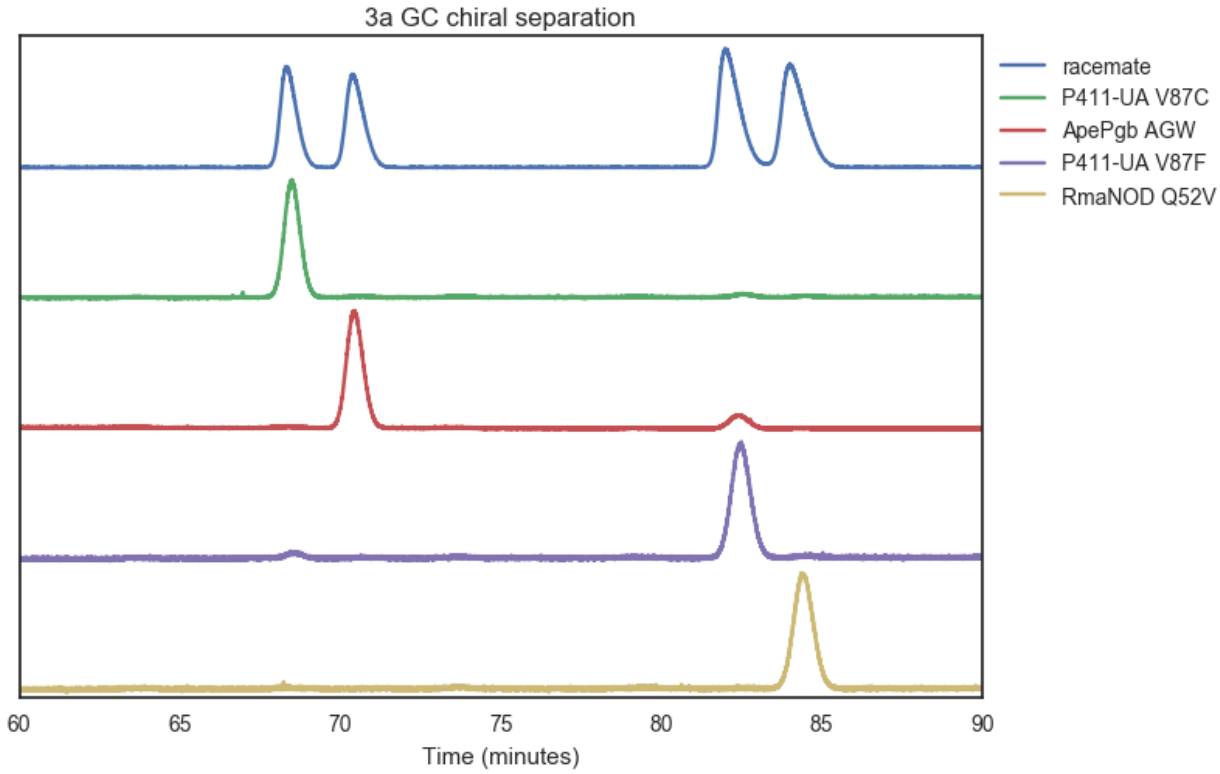
Cis-**3m** is a known compound.[16] General procedure B was used to produce a mixture of 55:45 *trans*/*cis* **3m**, and a preparative-scale enzymatic reaction (ApePgb AGW) produced *cis*-**3m**.

Cis-**3m** ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.13 (q, *J* = 7.1 Hz, 2H), 2.46 (td, *J* = 7.3, 2.3 Hz, 2H), 2.13 (s, 3H), 1.94 – 1.62 (m, 3H), 1.26 (t, *J* = 7.1 Hz, 4H), 1.06 – 0.86 (m, 2H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 208.9, 173.2, 60.7, 43.7, 30.2, 21.8, 21.3, 18.6, 14.7, 13.8. HR-MS (FAB+): $[M+H]^+$ $C_{10}H_{17}O_3$, calculated 185.1178, found 185.1171. *Cis*-**3m** optical rotation from ApePgb AGW product: $[\alpha]_D^{23}$ = −52 ° (*c* 0.1, EtOAc).

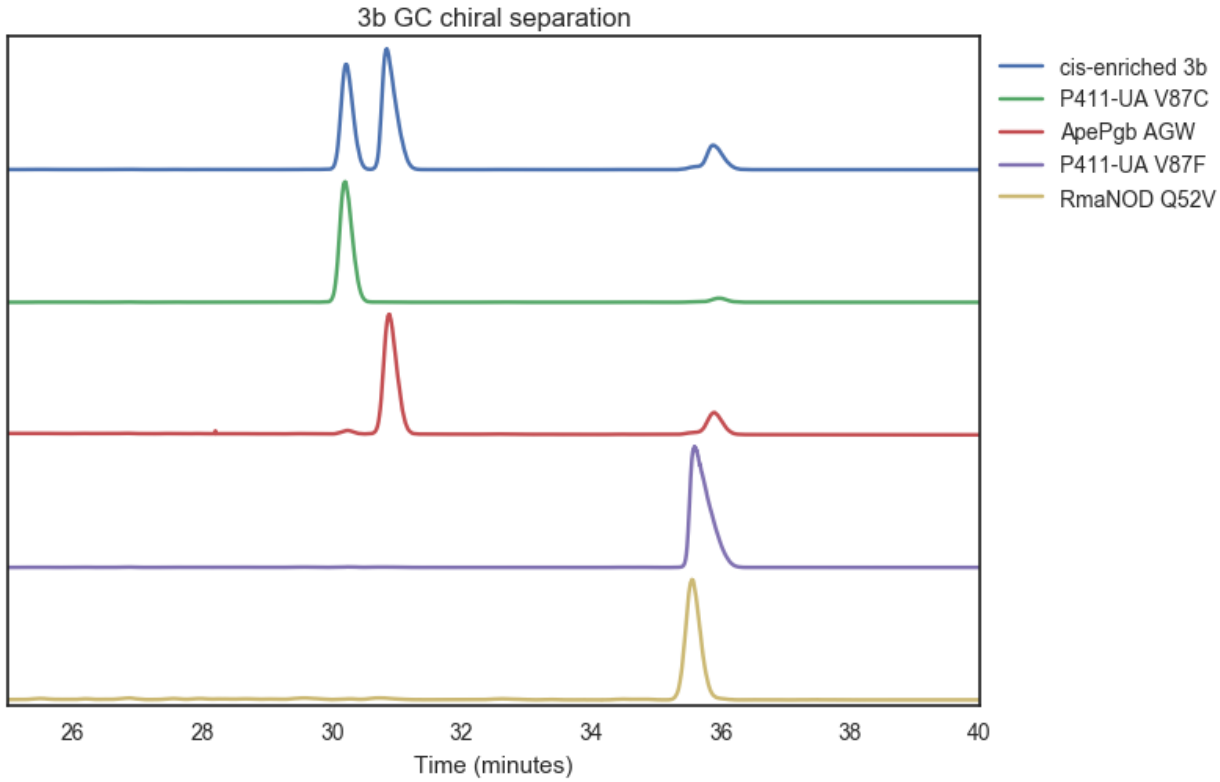
Compound chiral separation conditions and representative traces
Chiral trace figures here are produced via Jupyter Notebook. The Jupyter notebook and raw data traces are available as supplemental files.

Supplemental Table 9. Chromatographic separation conditions. Column dimensions: Cyclosil-B 30 m length x 0.32 mm ID x 0.25 µm film thickness; Chiraldex G-TA 30 m length x 0.25 mm ID x 0.12 µm film thickness; DB-WAXETR (15 / 30 m) length x 0.32 mm ID x 0.25 µm film thickness; Chiralpak IC 4.6 mm x 250 mm x 5 µm; Chiralpak IA 4.6 mm x 250 mm x 5 µm.

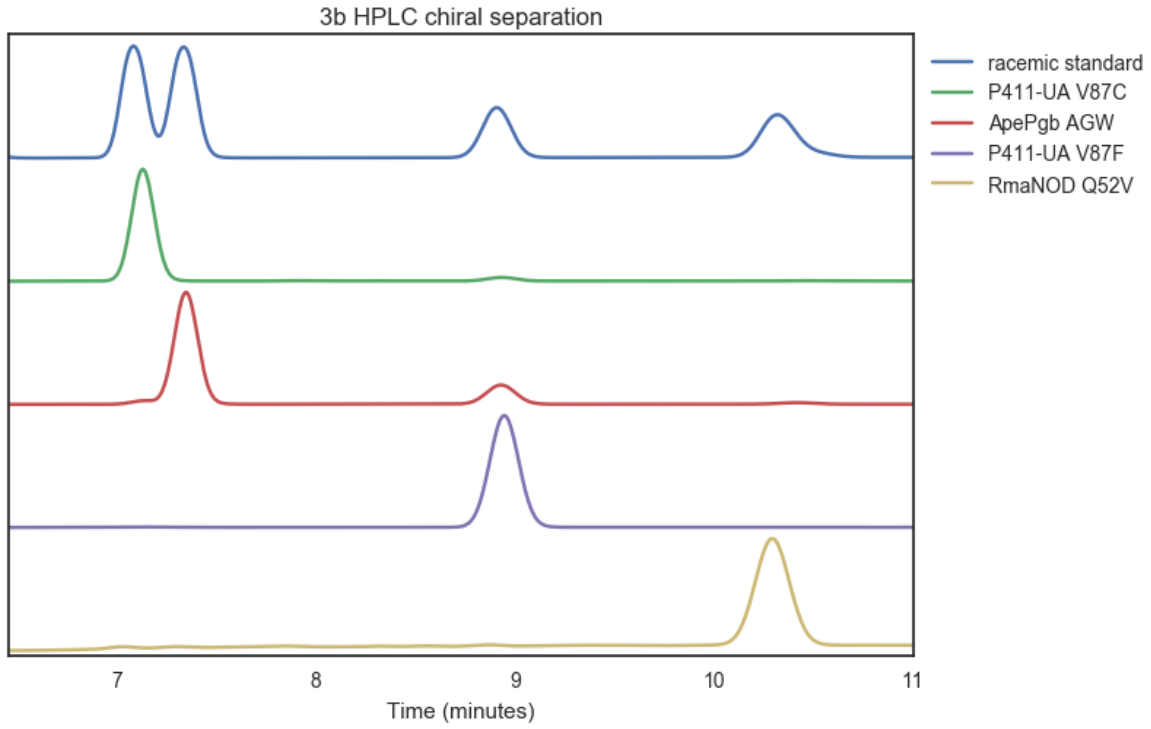
Compound	Instrument / column	Method	Retention times
3a	GC-FID, Cyclosil-B	90°C isothermal	*Cis* 67.9 min, 69.5 min; *trans* 81.7 min, 83.5 min
3b	GC-FID, Cyclosil-B	80°C hold 2 min, 30°C min^{-1} to 140°C hold 40 min	*cis* 30.2 min, 30.9 min; *trans* 35.7 min (unresolved)
	HPLC, Chiralpak IC	Hexane: 0.8% isopropanol isocratic	*cis* 7.1 min, 7.3 min (unresolved); *trans* 8.9 min, 10.3 min
3c	GC-FID, Cyclosil-B	80°C hold 2 min, 30°C min^{-1} to 170°C hold 30 min	*Trans* 24.5 min (unresolved); *cis* 26.4 min, 26.8 min
	HPLC, Chiralpak IC	Hexane:16% isopropanol isocratic	*Cis* 4.6 min (unresolved; *trans* 6.4 min, 7.2 min
3d	GC-FID, Chiraldex G-TA	100°C, 2°C min^{-1} to 170°C	*Cis* 25.1 min, 25.3 min; *trans* 27.1 min, 27.2 min
3e	GC-FID, Cyclosil-B	80°C hold 2 min, 30°C min^{-1} to 100°C hold 80 min	*Cis* 44.7 min, 46.8 min; *trans* 76.6 min, 78.2 min
3f	GC-FID, Chiraldex G-TA	80°C isothermal	50.05 min, 51.0 min
3g	HPLC, Chiralpak IC	Hexane: 9% isopropanol isocratic	*Trans* 8.7 min, 9.7 min (second peak coelution with EDA dimer)
	GC-FID, Cyclosil-B	80°C hold 2 min, 30°C min^{-1} to 125°C hold 12 min	*Cis* 12.5 min, 12.7 min
3h	GC-FID, Cyclosil-B	80°C hold 2 min, 20°C min^{-1} to 120°C, 6 °C min^{-1} to 180°C	*Trans* 13.7 min, 13.8 min
3i	GC-FID, DB-WAXETR (30 m)	80°C hold 2 min, 10°C min^{-1} to 170°C	**2i** 8.4 min; EDA dimer (byproducts) 8.9 min, 10.1 min; *cis*-**3i** 14.5 min, *trans*-**3i** 14.6 min
3ia	HPLC, Chiralpak IA	Hexane: 0.5% isopropanol isocratic	*Cis* 8.7 min, 9.0 min
3j	GC-FID, DB-WAXETR (15 m)	90°C hold 2 min, 10°C min^{-1} to 250°C	EDA dimer (byproducts) 5.0 min, 6.2 min, **2j** 9.7 min, *cis*-**3j** 16.6 min, *trans*-**3j** 16.7 min
3ja	HPLC, Chiralpak IC	Hexane: 1% isopropanol isocratic	*Cis* 24.9 min, 26.2 min
3k	GC-FID, Cyclosil-B	80°C hold 2 min, 5°C min^{-1} to 140°C	Terminal cyclopropane products: *cis* 9.5 min, 9.6 min; *trans* 9.9 min, 10.1 min
3l	GC-FID, Cyclosil-B	80°C hold 2 min, 10°C min^{-1} to 85°C hold 20 min	Terminal cyclopropane products: *cis* 15.8 min, 16.3 min; *trans* 17.6 min, 20.4 min
3m	GC-FID, Chiraldex G-TA	90°C isothermal	*Cis* 112.7 min, 114.6 min



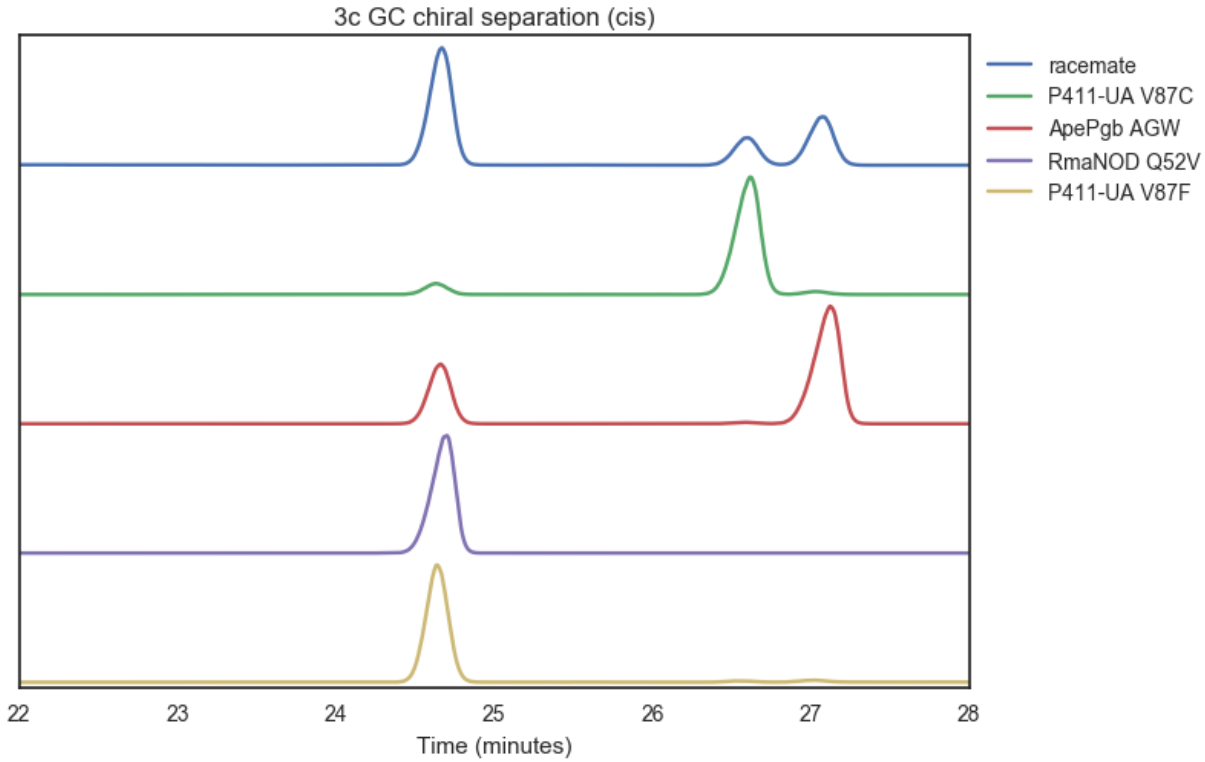
Supplemental Figure 3. Representative GC trace of **3a** (1-octene + EDA). Chiral GC trace of authentic standard ($Rh_2(OAc)_4$-catalyzed) and each of the final variants.



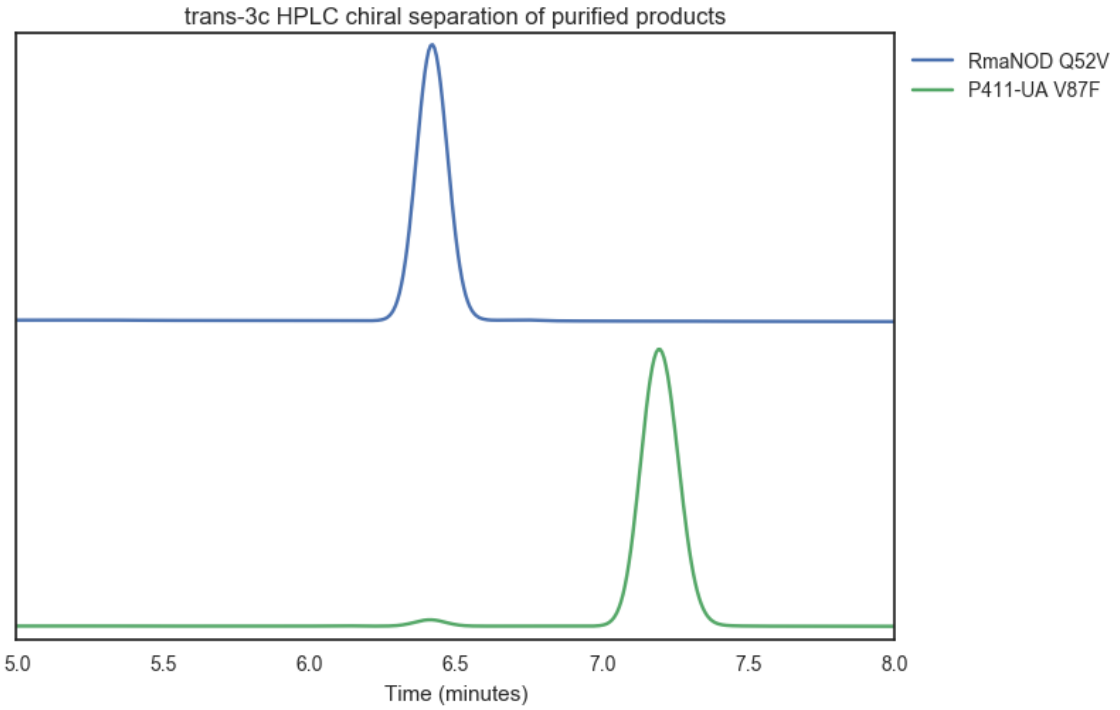
Supplemental Figure 4. Representative GC trace of **3b** (4-phenyl-1-butene + EDA). Chiral GC trace of **3b** product formation. *Trans*-**3b** peaks (35.7 min) are not baseline separated.



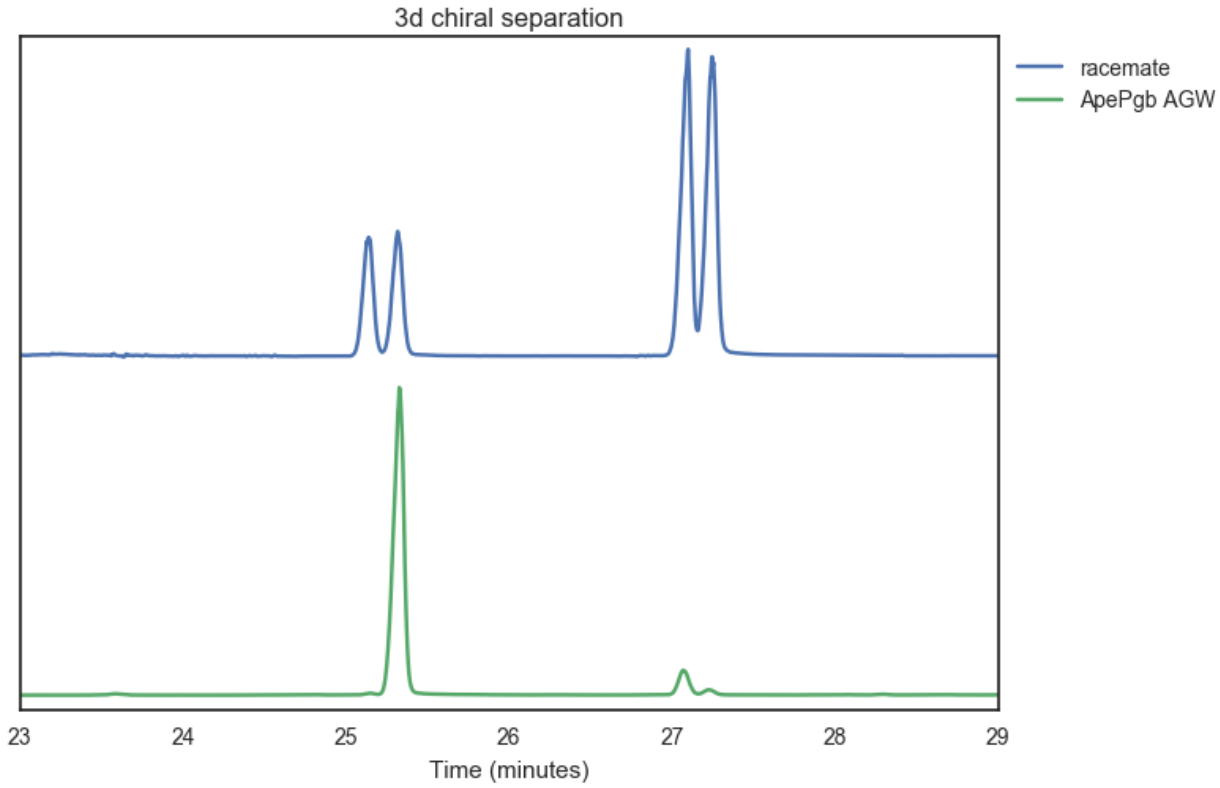
Supplemental Figure 5. Representative HPLC trace of **3b** (4-phenyl-1-butene + EDA). Chiral HPLC trace of **3b** product formation.



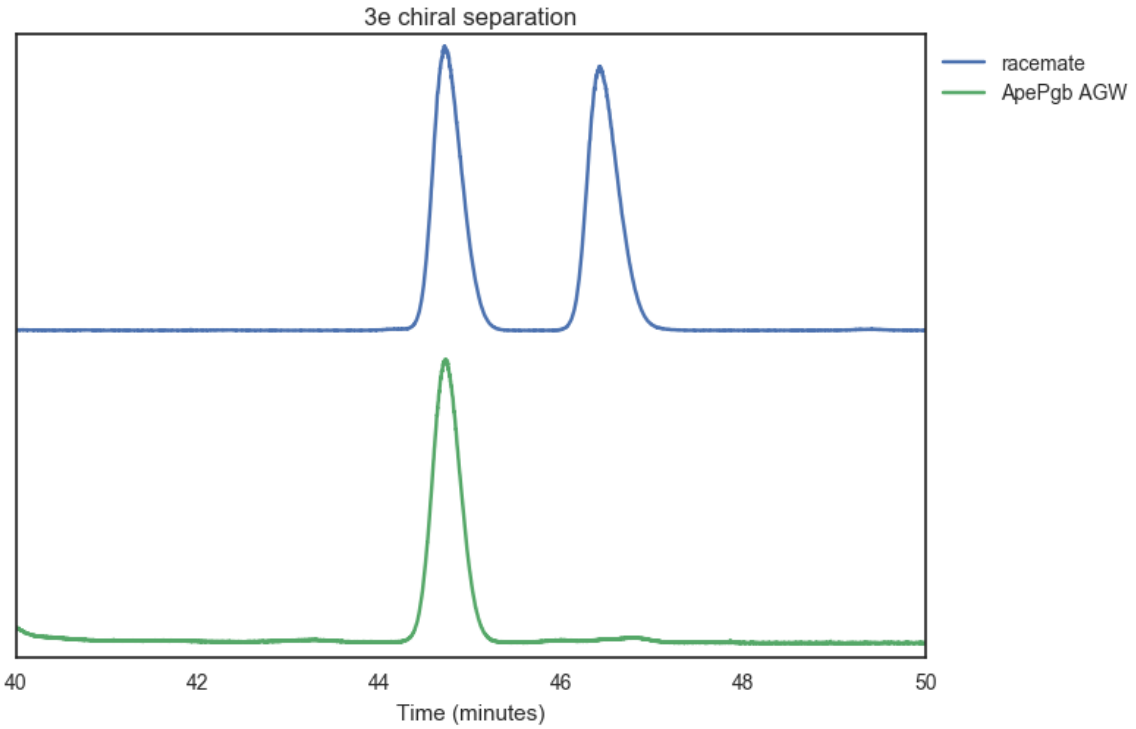
Supplemental Figure 6. Representative GC trace of **3c** (benzyl acrylate + EDA). *Trans*-**3c** (24.5 min) was not resolved on the Cyclosil-B column.



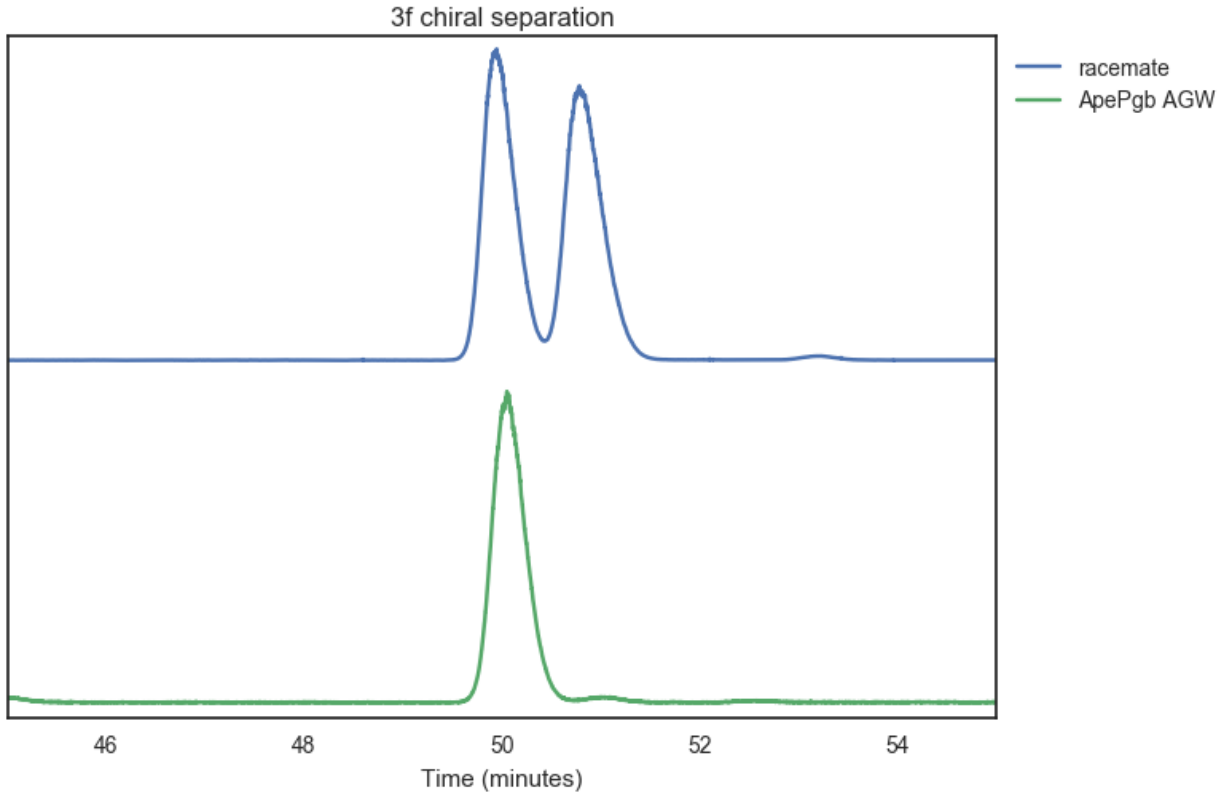
Supplemental Figure 7. Representative HPLC trace of *trans*-**3c** (benzyl acrylate + EDA). Enzymatic samples were from purified, preparative-scale enzymatic reactions.



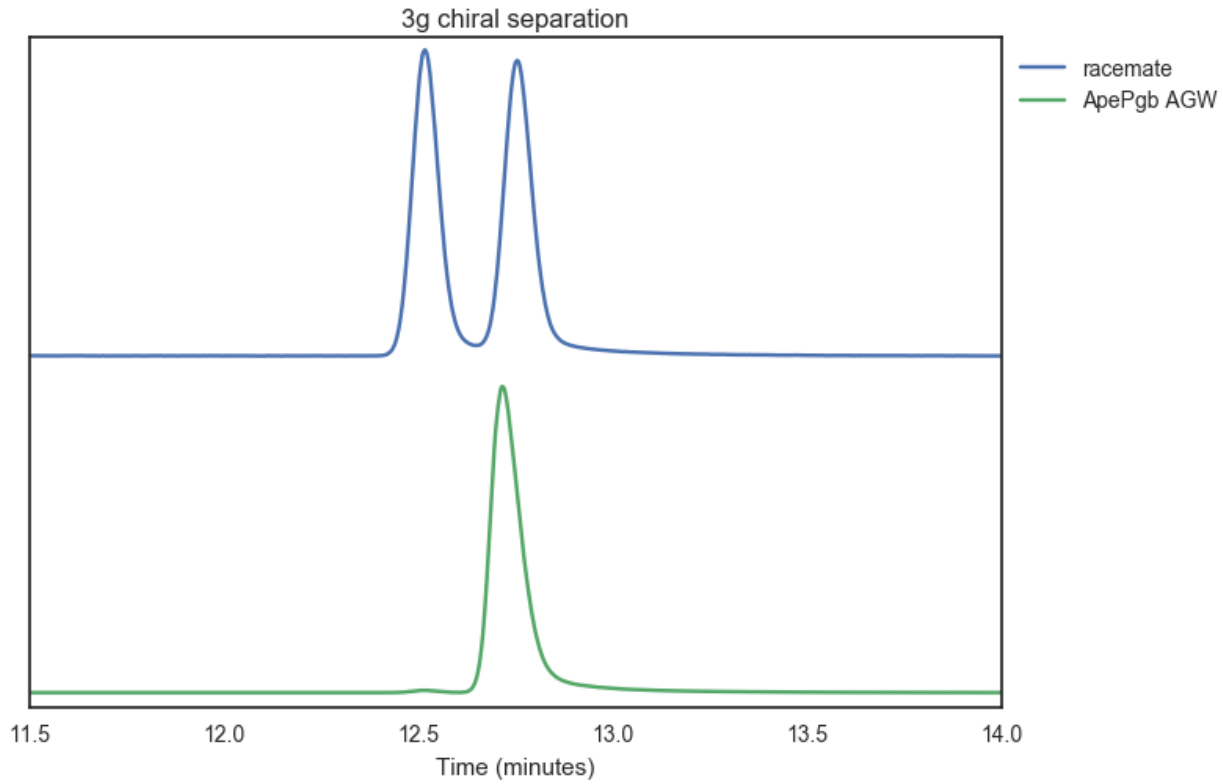
Supplemental Figure 8. Representative GC trace of **3d** (6-bromo-1-hexene + EDA).



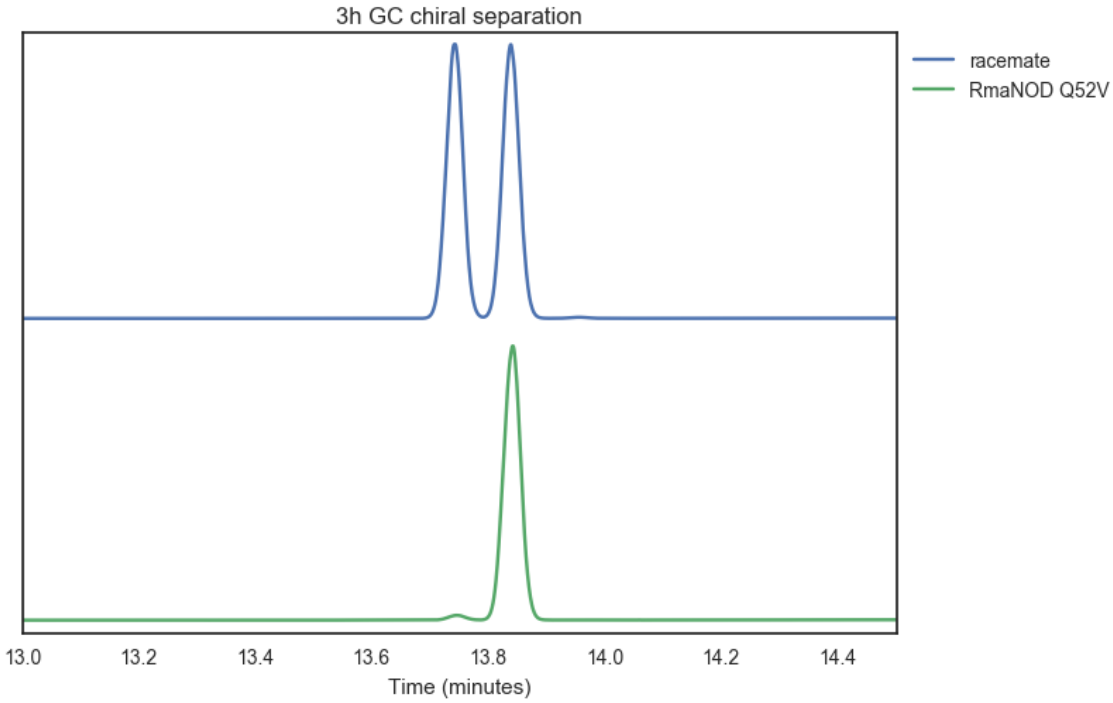
Supplemental Figure 9. Representative chiral GC trace of **3e** (vinylcyclohexane + EDA).



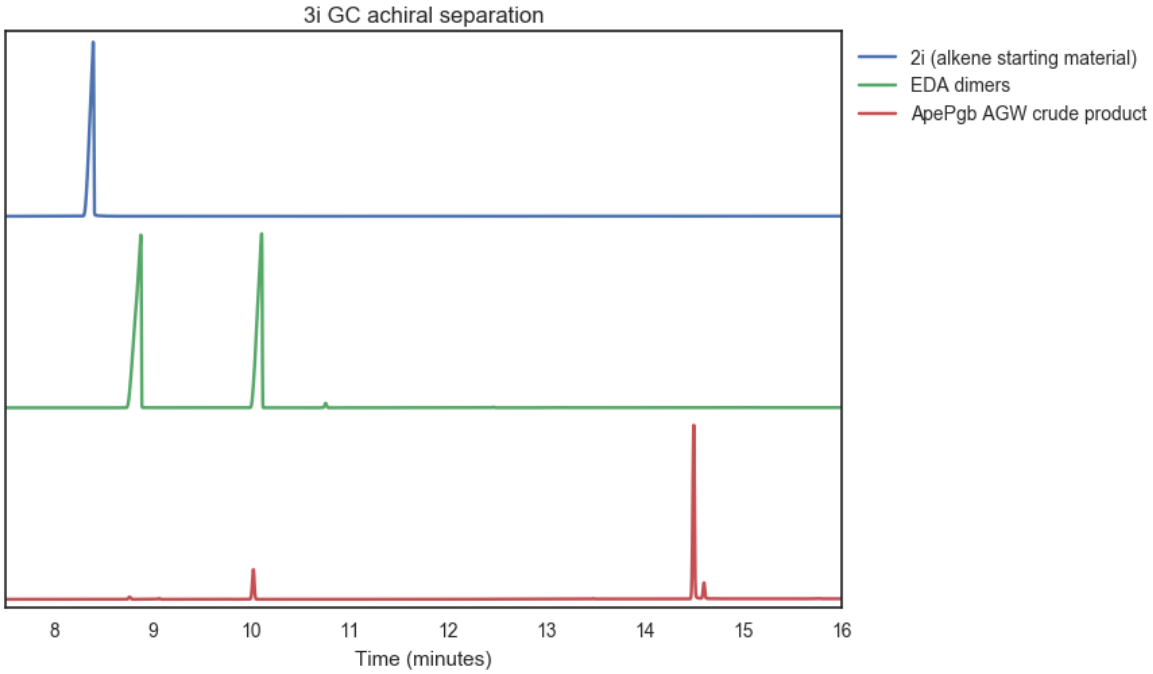
Supplemental Figure 10. Representative chiral GC trace of **3f** (methylenecyclohexane + EDA).



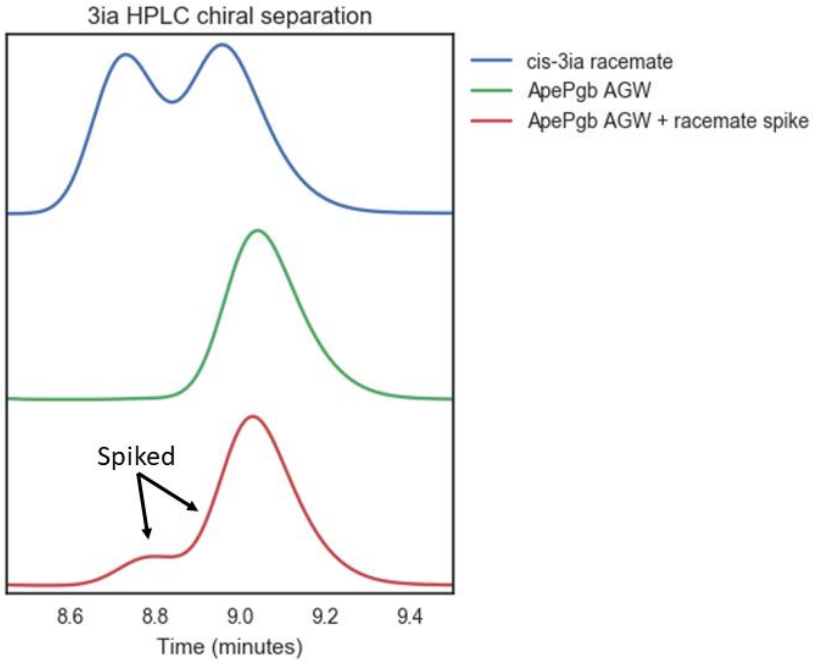
Supplemental Figure 11. Representative chiral GC trace of *cis*-**3g** (1-penten-3-one + EDA).



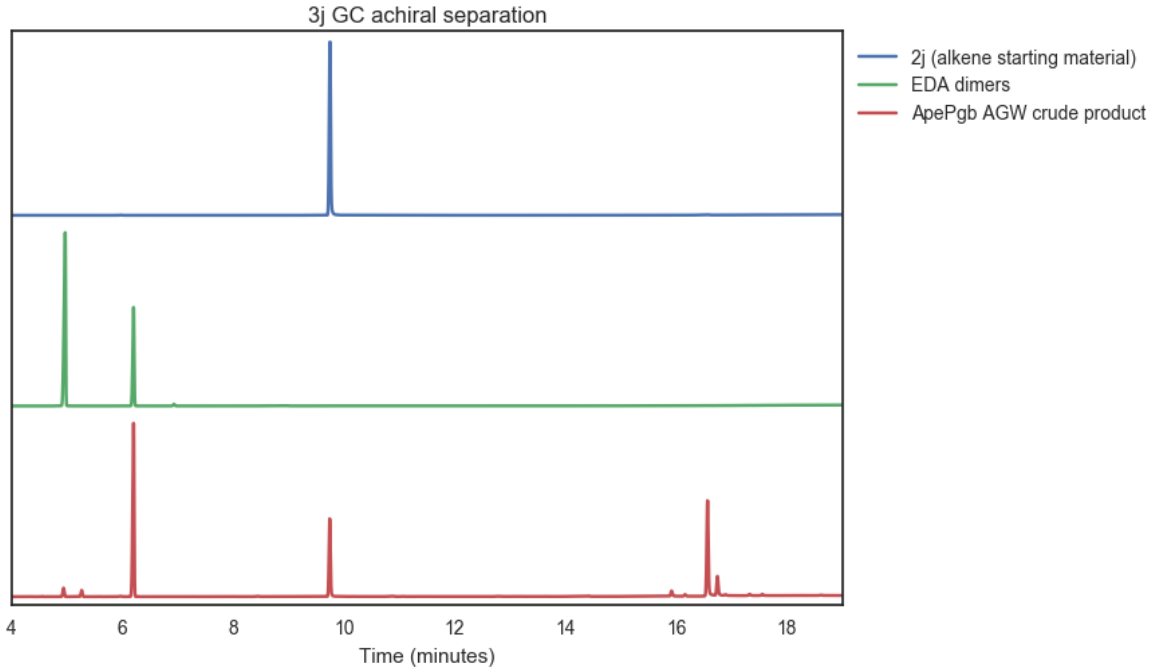
Supplemental Figure 12. Representative chiral GC trace of **3h** (2-vinylpyridine + EDA).



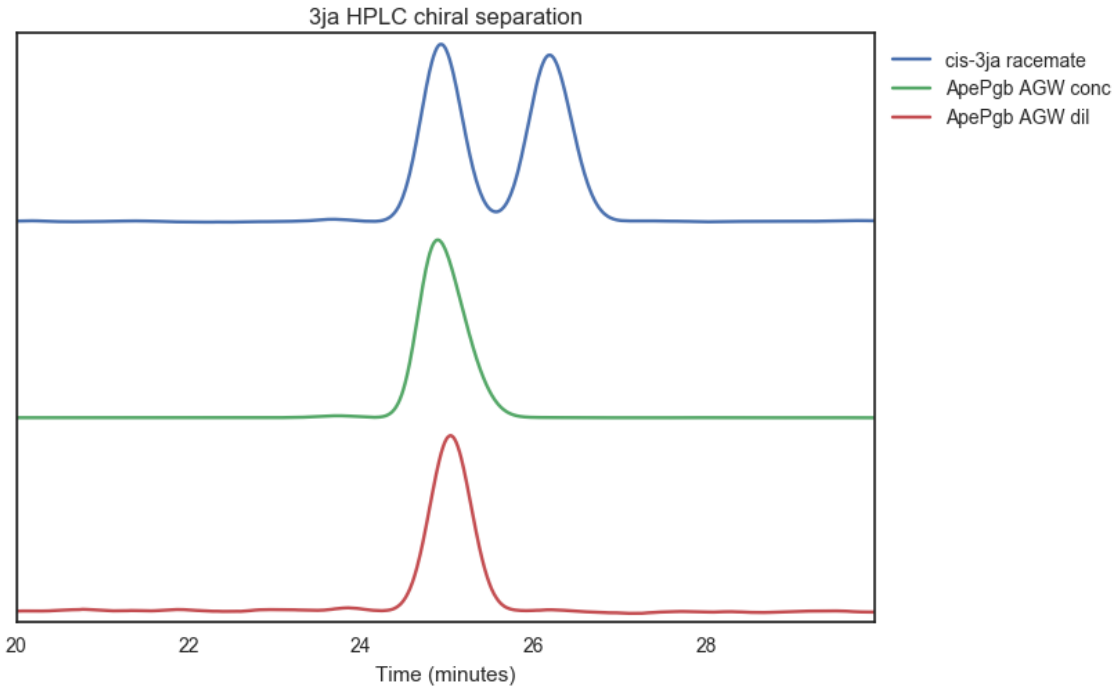
Supplemental Figure 13. Representative achiral GC trace of **3i** (7-octen-1-ol + EDA). Offset traces show 7-octen-1-ol starting material **2i**, EDA dimers (diethyl fumarate and diethyl maleate), and the crude reaction mixture from ApePgb AGW-catalyzed preparative-scale reaction.



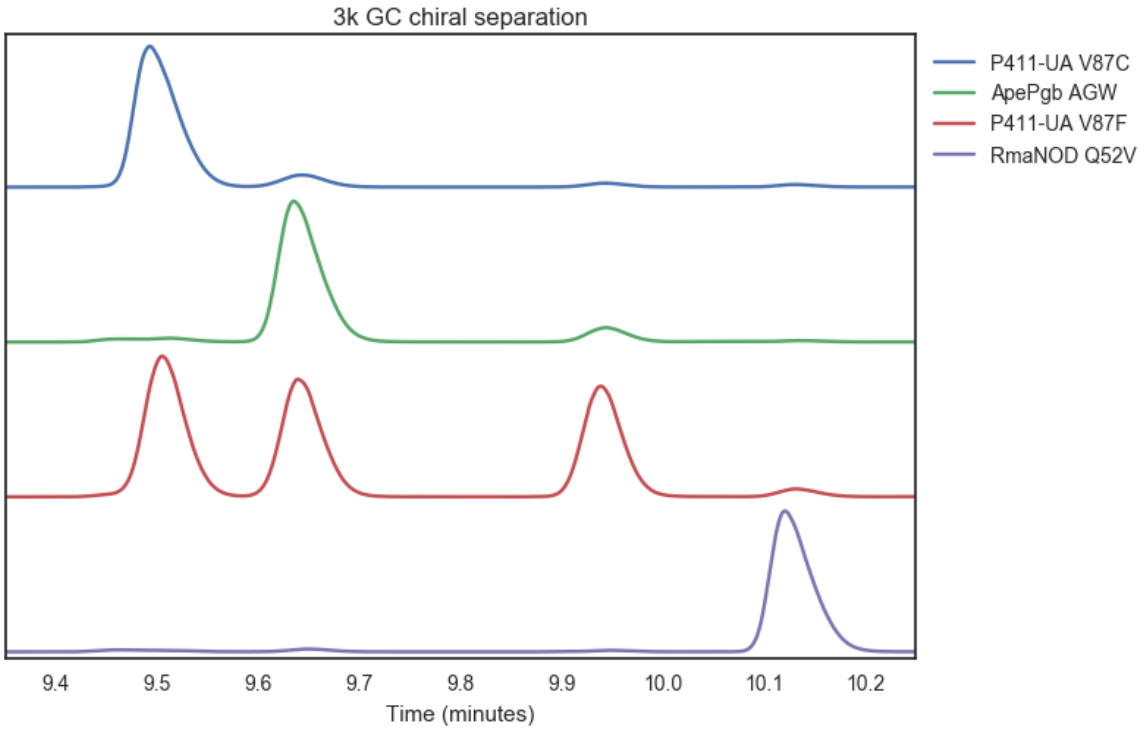
Supplemental Figure 14. Chiral HPLC trace of **3ia**. Conditions for the baseline separation of isomers were not found, but a spike-in of racemic standard into the ApePgb AGW-catalyzed preparative-scale reaction shows the first enantiomer peak was not previously observed and that the enzyme-catalyzed product has high enantiopurity.



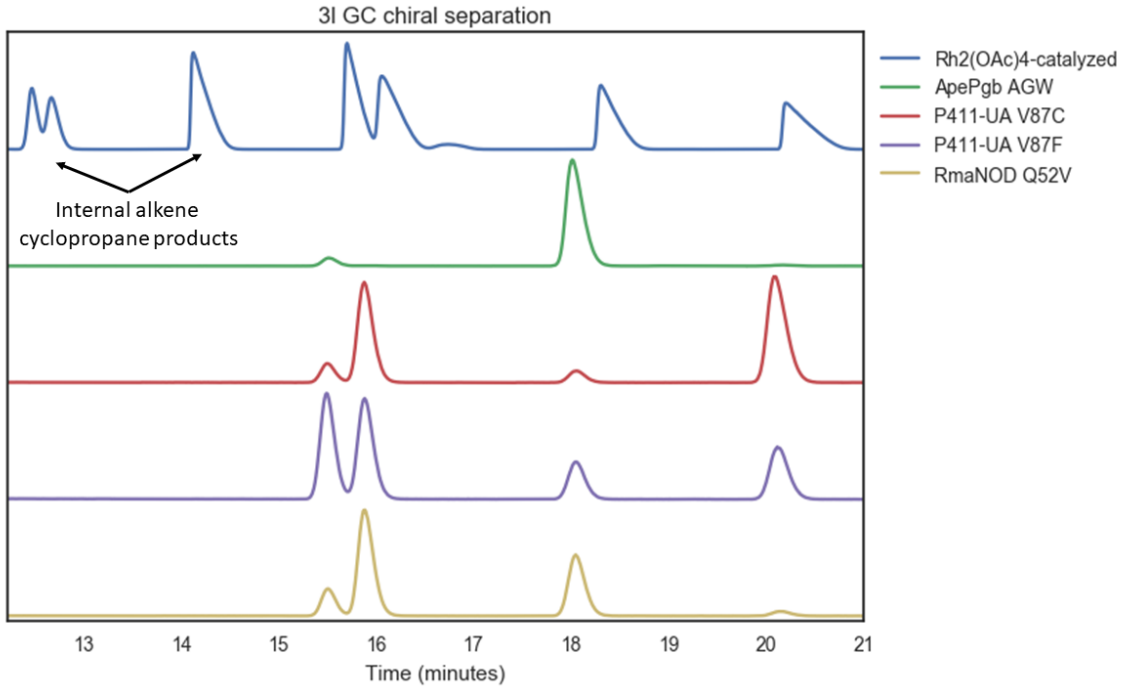
Supplemental Figure 15. Representative achiral GC trace of **3j** (7-octen-1-oic acid + EDA). Offset traces show 7-octen-1-oic acid starting material **2j**, EDA dimers (diethyl fumarate and diethyl maleate), and the crude reaction mixture from ApePgb AGW-catalyzed preparative-scale reaction.



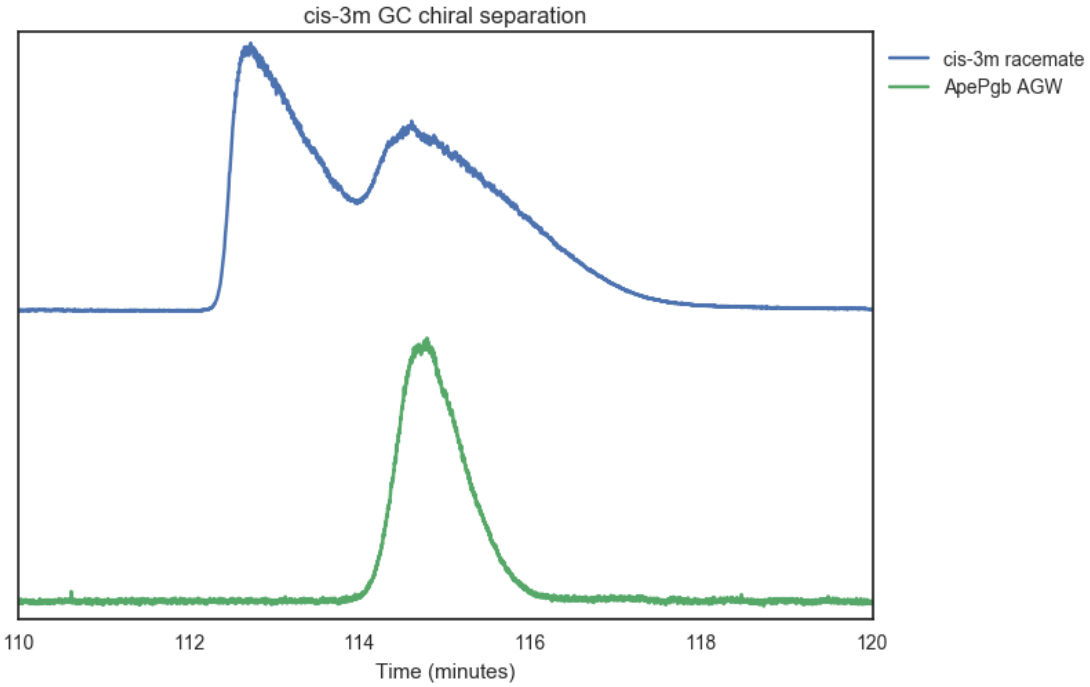
Supplemental Figure 16. Chiral HPLC trace of **3ja**. ApePgb AGW-catalyzed preparative-scale reaction was run at both concentrated (approximately 5 mg mL $^{-1}$, green) and dilute (approximately 0.5 mg mL^{-1}, red) conditions.



Supplemental Figure 17. Representative GC trace of **3k** ((*E*)-penta-1,3-diene + EDA). Offset chiral GC-FID trace of **3k** from the four final variants.



Supplemental Figure 18. Representative GC trace of **3l** ((*Z*)-penta-1,3-diene + EDA). Chiral GC-FID trace of **3l** authentic standard; 3 of 4 enantiomeric pairs had separation; the fourth was an internal cyclopropanation product not observed in the enzymatic reactions (14 minutes). Offset chiral GC-FID trace of **3l** from the four final variants.

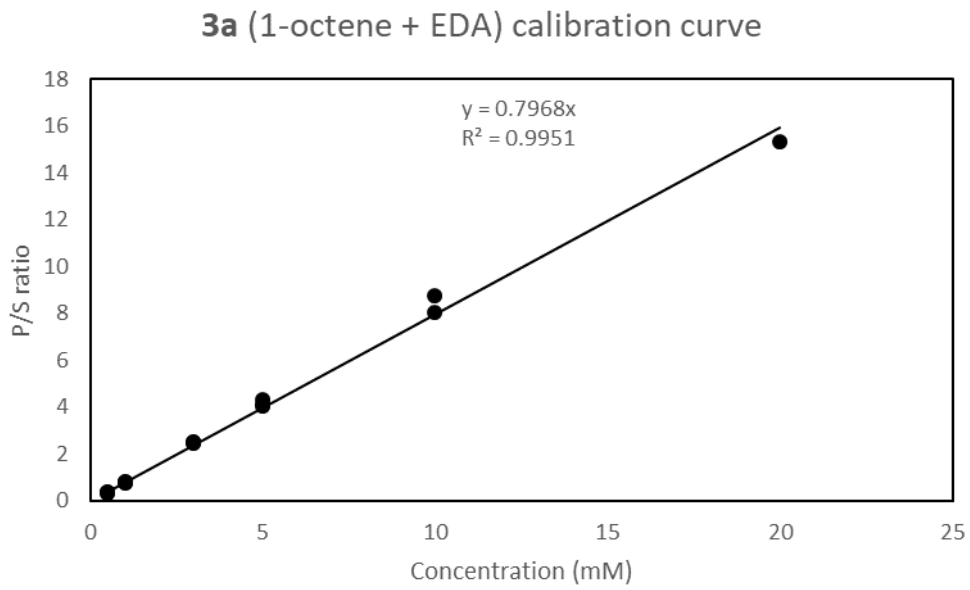


Supplemental Figure 19. Representative chiral GC trace of *cis*-**3m**.

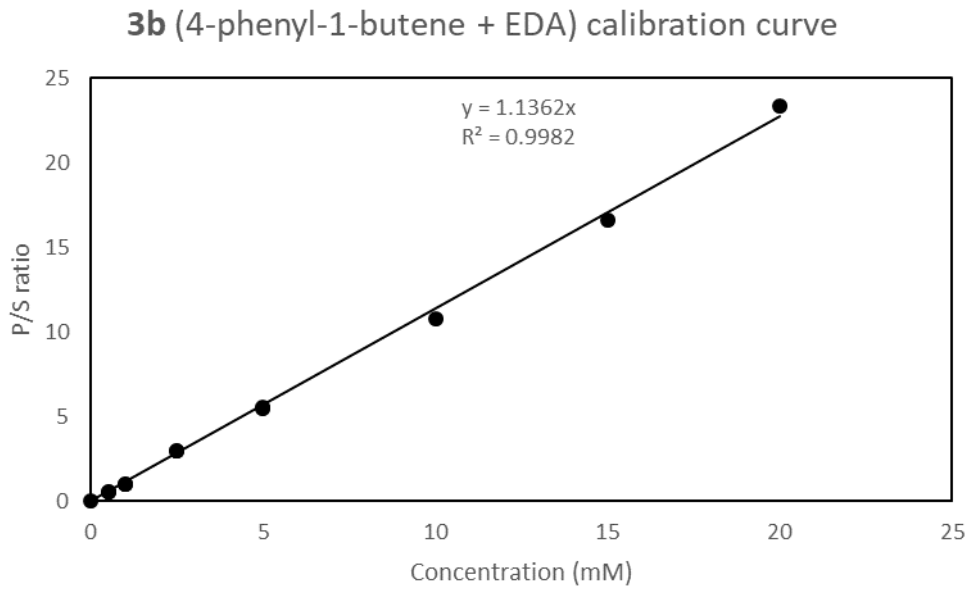
Calibration curves for analytical-scale TTN determination

Calibration curves were prepared with analogous conditions to the analytical scale reactions. Cyclopropane product standards were diluted in ethanol and added (20 µL) to M9-N buffer (380 µL), with final concentrations of product ranging from 0 – 20 mM (with two to three technical replicates). The samples were worked up the same as analytical-scale reactions, by the addition of HCl (16 µL, 3 M stock) and internal standard (16 µL of 40 mM acetophenone in cyclohexane). Cyclohexane (700 µL) was added and the samples were transferred into 1.7 mL Eppendorf tubes for extraction. The extraction was carried out with a Retsch MM 301 mixing mill (1 minute, 30 Hz / 1800 rpm). Samples were centrifuged at 20000×g for 5 minutes at RT and the organic layer was used for chromatographic analysis. Cyclopropane products for methylenecyclohexane **3g** and 2-vinylpyridine **3h** were set up in the same way, with the exception of running ten single replicates over the 0 to 20 mM product range.

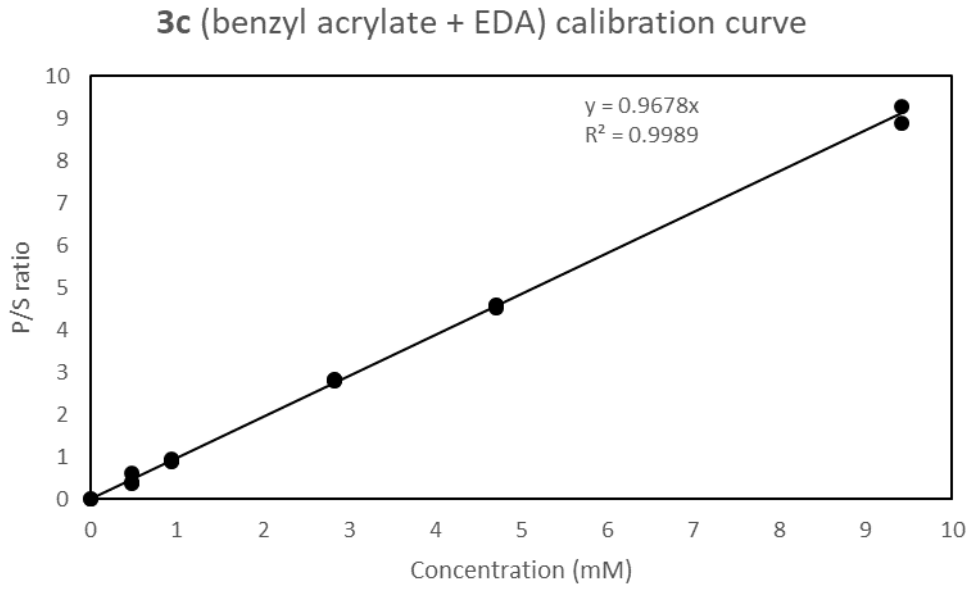
The ratio of the product area and internal standard (P/S ratio) was determined for each sample, and a linear regression was performed for the P/S ratio versus the known concentration in each reaction, with the y–intercept set to 0. The resulting slopes were used to determine the concentration of product in the analytical-scale reactions, which is in turn used to calculate the total turnover number (TTN) per enzyme.



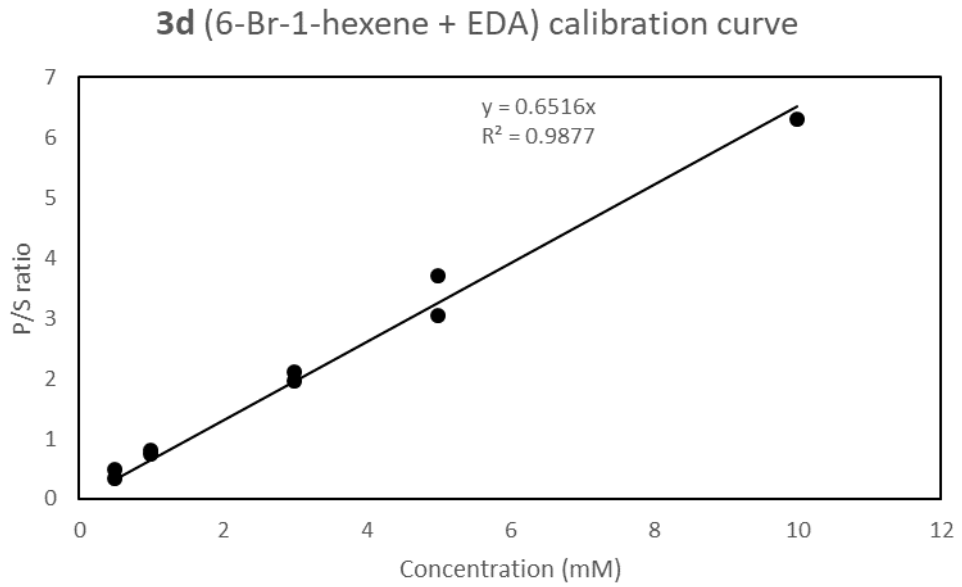
Supplemental Figure 20. Achiral GC calibration curve of **3a** (1-octene + EDA).



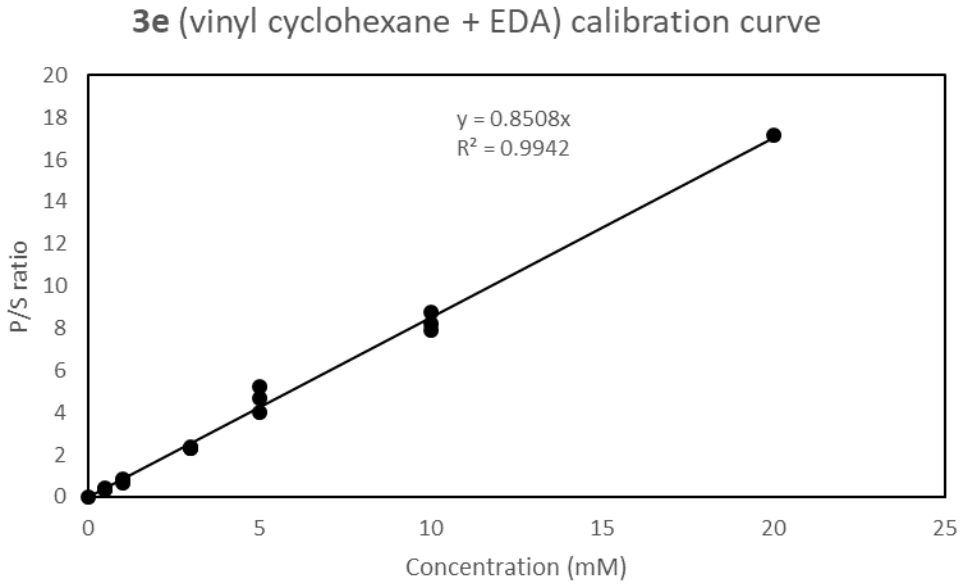
Supplemental Figure 21. Achiral GC calibration curve of **3b** (4-phenyl-1-butene + EDA).



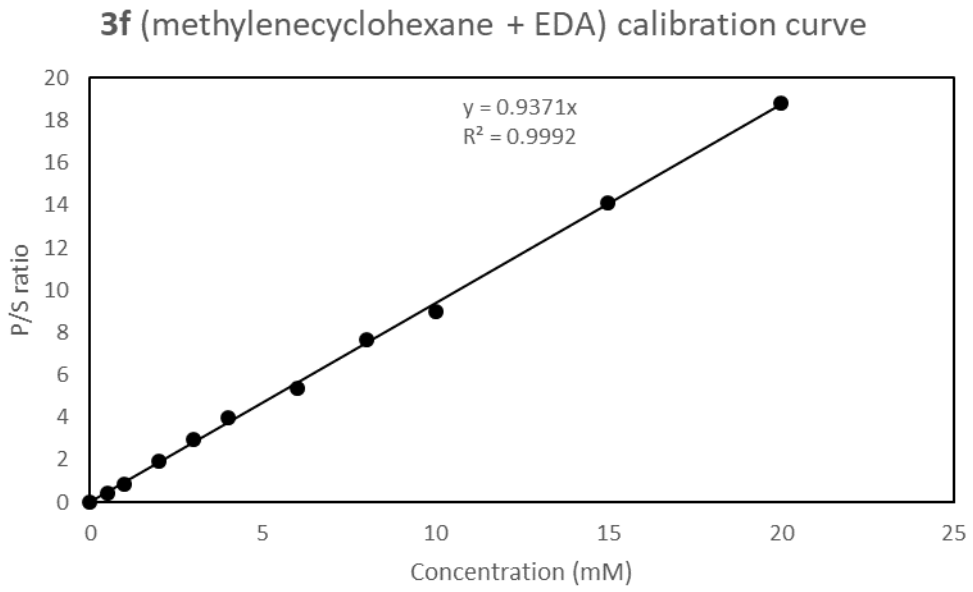
Supplemental Figure 22. Achiral GC calibration curve of **3c** (benzyl acrylate + EDA).



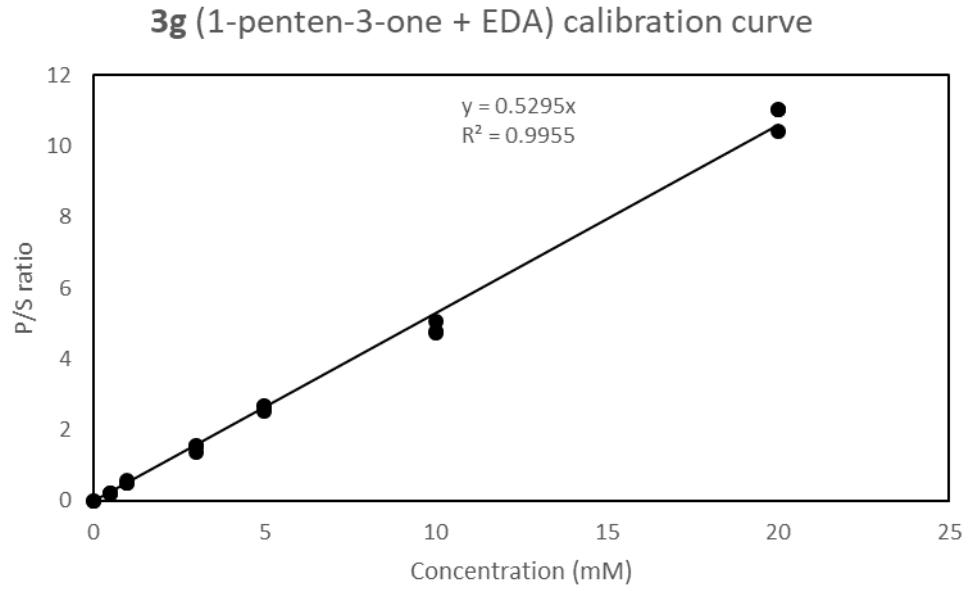
Supplemental Figure 23. Achiral GC calibration curve of **3d** (6-bromo-1-hexene + EDA).



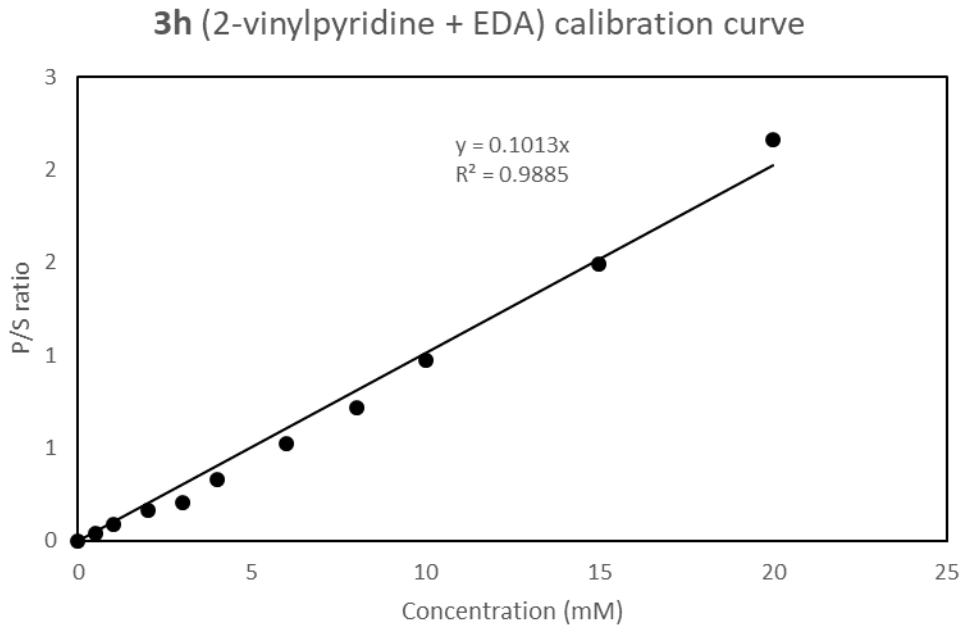
Supplemental Figure 24. Achiral GC calibration curve of **3e** (vinylcyclohexane + EDA).



Supplemental Figure 25. Achiral GC calibration curve of **3f** (methylenecyclohexane + EDA).



Supplemental Figure 26. Achiral GC calibration curve of **3g** (1-penten-3-one + EDA).



Supplemental Figure 27. Achiral GC calibration curve of **3h** (2-vinylpyridine + EDA).

Sequences of primers and variants

Supplemental Table 10. Primers used in the protein engineering described in the report. These primers are also available as a supplemental csv file.

Primer name	Primer sequence
ApePgb_Y60X_NDT_f	TTGACTTGTGGNDTGGTTGGGTAGCATCAAATGAGC
ApePgb_Y60X_VHG_f	TTGACTTGTGGVHGGGTTGGGTAGCATCAAATGAGC
ApePgb_Y60X_TGG_f	TTGACTTGTGGTGGGGTTGGGTAGCATCAAATGAGC
ApePgb_Y60X_univ_r	CCACAAGTCAAGGATCTCATCAACTTGATC
ApePgb_Y60G_W59X_NDT_f	TTGATGAGATCCTTGACTTGNDTGGTGGTTGGGTAGCATC
ApePgb_Y60G_W59X_VHG_f	TTGATGAGATCCTTGACTTGVHGGGTGGTTGGGTAGCATC
ApePgb_Y60G_W59X_TGG_f	N/A; WT codon is TGG
ApePgb_Y60G_W59X_univ_r	CAAGTCAAGGATCTCATCAACTTGATCTTTCAGAACGTC
ApePgb_F73X_NDT_f	ATGAGCATTTGATTTATTACNDTTCCAATCCGGATACAGGAG
ApePgb_F73X_VHG_f	ATGAGCATTTGATTTATTACVHGTCCAATCCGGATACAGGAG
ApePgb_F73X_TGG_f	ATGAGCATTTGATTTATTACTGGTCCAATCCGGATACAGGAG
ApePgb_F73X_univ_r	GTAATAAATCAAATGCTCATTTGATGCTACCCAACC
ApePgb_F93X_NDT_f	TGGAACGTGTACGCGCTCGCNDTGGAGCCTGGATTCTGGACAC
ApePgb_F93X_VHG_f	TGGAACGTGTACGCGCTCGCVHGGGAGCCTGGATTCTGGACAC
ApePgb_F93X_TGG_f	TGGAACGTGTACGCGCTCGCTGGGGAGCCTGGATTCTGGACAC
ApePgb_F93X_univ_r	GCGAGCGCGTACACGTTCCAGGTATTCCTTAATAGGCTCTC
ApePgb_F145X_NDT_f	CACTTCGTTATCTTATCGCANDTATCTATCCTATCACCGCCAC

ApePgb_F145X_VHG_f	CACTTCGTTATCTTATCGCAVHGATCTATCCTATCACCGCCAC
ApePgb_F145X_TGG_f	CACTTCGTTATCTTATCGCATGGATCTATCCTATCACCGCCAC
ApePgb_F145X_univ_r	TGCGATAAGATAACGAAGTGGGATATGGGGCACGGTGCGT
RmaNOD_Y32X_NDT_f	CGCCACGATGNDTCGGCTGCTTTTCGAACG
RmaNOD_Y32X_VHG_f	CGCCACGATGVHGCGGCTGCTTTTCGAACG
RmaNOD_Y32X_TGG_f	CGCCACGATGTGGCGGCTGCTTTTCGAACG
RmaNOD_Y32X_univ_r	CATCGTGGCGCTAATAGCGACTGAGTGTTTCTGC
RmaNOD_Q52X_NDT_f	CTTCCTGAGAGANDTATACACAAGCTTGCG
RmaNOD_Q52X_VHG_f	CTTCCTGAGAGAVHGATACACAAGCTTGCG
RmaNOD_Q52X_TGG_f	CTTCCTGAGAGATGGATACACAAGCTTGCG
RmaNOD_Q52X_univ_r	TCTCTCAGGAAGTTCAAACAAGCTCCGCG
RmaNOD_V97X_NDT_f	CCATTATCCGCTGNDTTGGGAATGTTTGAGAGACG
RmaNOD_V97X_VHG_f	CCATTATCCGCTGVHGTGGGAATGTTTGAGAGACG
RmaNOD_V97X_TGG_f	CCATTATCCGCTGTGGTGGGAATGTTTGAGAGACG
RmaNOD_V97X_univ_r	CAGCGGATAATGGACGGCCTGCACTCCTGC
P411_UA_437X_438X_NDT_NDT_f	GCTCGATATTAAAGAAACTNDTNDTTTAAAACCTAAAGGC
P411_UA_437X_438X_NDT_ACN_f	GCTCGATATTAAAGAAACTNDTACNTTAAAACCTAAAGGC
P411_UA_437X_438X_NDT_CAR_f	GCTCGATATTAAAGAAACTNDTCARTTAAAACCTAAAGGC
P411_UA_437X_438X_univ_r	AGTTTCTTTAATATCGAGCTCGTAGTTTGTATGATCTTC
P411_UA_A328X_NDT_f	GCTTATGGCCAACTNDTCCTGCGTTTTCC
P411_UA_A328X_VHG_f	GCTTATGGCCAACTVHGCCTGCGTTTTCC
P411_UA_A328X_TGG_f	GCTTATGGCCAACTTGGCCTGCGTTTTCC
P411_UA_A328X_univ_r	AGTTGGCCATAAGCGCAGCGCTTCG
P411_UA_I263X_NDT_f	CAAATTATTACATTCTTANDTGCGGGACACGAAGC
P411_UA_I263X_VHG_f	CAAATTATTACATTCTTAVHGGCGGGACACGAAGC
P411_UA_I263X_TGG_f	CAAATTATTACATTCTTATGGGCGGGACACGAAGC
P411_UA_I263X_univ_r	TAAGAATGTAATAATTTGATAGCGAATGTTCCCG
P411_UA_V87X_NDT_f	GCAGGAGACGGGTTANDTACAAGCTGGACGC
P411_UA_V87X_VHG_f	GCAGGAGACGGGTTAVHGACAAGCTGGACGC
P411_UA_V87X_TGG_f	GCAGGAGACGGGTTATGGACAAGCTGGACGC
P411_UA_V87X_univ_r	TAACCCGTCTCCTGCAAAATCACG
P411_UA_L181X_NDT_f	GTATGGTCCGTGCANDTGATGAAGTAATGAACAAG
P411_UA_L181X_VHG_f	GTATGGTCCGTGCAVHGGATGAAGTAATGAACAAG
P411_UA_L181X_TGG_f	GTATGGTCCGTGCATGGGATGAAGTAATGAACAAG
P411_UA_L181X_univ_r	TGCACGGACCATACTTATAATAAATGGATGAGG
P411_UA_L75X_NDT_f	GATAAAAACTTAAGTCAAGCGNDTAAATTTGCACGTGATTTTGCAGG
P411_UA_L75X_VHG_f	GATAAAAACTTAAGTCAAGCGVHGAAATTTGCACGTGATTTTGCAGG
P411_UA_L75X_TGG_f	GATAAAAACTTAAGTCAAGCGTGGAAATTTGCACGTGATTTTGCAGG
P411_UA_L75X_univ_r	CGCTTGACTTAAGTTTTTATCAAAGCGTGATTCATCGC
P411_UA_E267X_NDT_f	CTTAATCGCGGGACACNDTGCAACAAGTGGTCTTTTATC
P411_UA_E267X_VHG_f	CTTAATCGCGGGACACVHGGCAACAAGTGGTCTTTTATC

P411_UA_E267X_TGG_f	CTTAATCGCGGGACACTGGGCAACAAGTGGTCTTTTATC
P411_UA_E267X_univ_r	GTGTCCCGCGATTAAGAATGTAATAATTTGATAGC
pET_internal_primer_forward	GCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCC
pET_internal_primer_reverse	GGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGC
pET22_OPV_005	GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATG
pET22_OPV_006	GCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAG
pET22_OPV_007	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTC
pET22_OPV_008	CTCGAGCACCACCACCACCACCACTGAGATCCGGC

Supplemental Table 11. Information on engineered proteins in the report.

Protein	Original organism	Host strain	Vector	Mutation(s) with respect to WT
ApePgb WT	*Aeropyrum pernix*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	
ApePgb Y60G	*Aeropyrum pernix*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	Y60G
ApePgb W59A Y60G	*Aeropyrum pernix*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	W59A Y60G
ApePgb W59A Y60G F145W ("AGW")	*Aeropyrum pernix*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	W59A Y60G F145W
RmaNOD WT	*Rhodothermus marinus*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	
RmaNOD Q52V	*Rhodothermus marinus*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	Q52V
P411-CIS	*Bacillus megaterium*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	V78A, F87V, P142S, T175I, A184V, S226R, H236Q, E252G, T268A, A290V, L353V, I366V, C400S, E442K
P411-UA	*Bacillus megaterium*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	P411-CIS, L75Y, L181I, L437F, T438Q
P411-UA-V87C	*Bacillus megaterium*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	P411-CIS, L75Y, V87C, L181I, L437F, T438Q
P411-UA-V87F	*Bacillus megaterium*	E. cloni EXPRESS BL21(DE3)	pET22b(+)	P411-CIS, L75Y, V87F, L181I, L437F, T438Q

Supplemental Table 12. DNA sequences of reported biocatalysts. These sequences are also available as a supplemental csv file.

Protein	DNA sequence
ApePgb WT	ATGACTCCCTCGGACATCCCGGGATATGATTATGGGCGTGTCGAGAAGTCACCCATCACGGACCTTG AGTTTGACCTTCTGAAGAAGACTGTCATGTTAGGTGAAAAGGACGTAATGTACTTGAAAAAGGCGTG TGACGTTCTGAAAGATCAAGTTGATGAGATCCTTGACTTGTGGTATGGTTGGGTAGCATCAAATGAG CATTTGATTTATTACTTCTCCAATCCGGATACAGGAGAGCCTATTAAGGAATACCTGGAACGTGTAC GCGCTCGCTTTGGAGCCTGGATTCTGGACACTACCTGCCGCGACTATAACCGTGAATGGTTAGACTA CCAGTACGAAGTTGGGCTTCGTCATCACCGTTCAAAGAAAGGGGTCACAGACGGAGTACGCACCGTG CCCCATATCCCACTTCGTTATCTTATCGCATTTATCTATCCTATCACCGCCACTATCAAGCCATTTT TGGCTAAGAAAGGTGGCTCTCCGGAAGACATCGAAGGGATGTACAACGCTTGGTTCAAGTCTGTAGT TTTACAAGTTGCCATCTGGTCACACCCTTATACTAAGGAGAATGACTGGCTCGAGCACCACCACCAC CACCACTGA
ApePgb Y60G	ATGACTCCCTCGGACATCCCGGGATATGATTATGGGCGTGTCGAGAAGTCACCCATCACGGACCTTG AGTTTGACCTTCTGAAGAAGACTGTCATGTTAGGTGAAAAGGACGTAATGTACTTGAAAAAGGCGTG TGACGTTCTGAAAGATCAAGTTGATGAGATCCTTGACTTGTGGGGTGGTTGGGTAGCATCAAATGAG CATTTGATTTATTACTTCTCCAATCCGGATACAGGAGAGCCTATTAAGGAATACCTGGAACGTGTAC GCGCTCGCTTTGGAGCCTGGATTCTGGACACTACCTGCCGCGACTATAACCGTGAATGGTTAGACTA CCAGTACGAAGTTGGGCTTCGTCATCACCGTTCAAAGAAAGGGGTCACAGACGGAGTACGCACCGTG CCCCATATCCCACTTCGTTATCTTATCGCATTTATCTATCCTATCACCGCCACTATCAAGCCATTTT TGGCTAAGAAAGGTGGCTCTCCGGAAGACATCGAAGGGATGTACAACGCTTGGTTCAAGTCTGTAGT TTTACAAGTTGCCATCTGGTCACACCCTTATACTAAGGAGAATGACTGGCTCGAGCACCACCACCAC CACCACTGA
ApePgb W59A Y60G	ATGACTCCCTCGGACATCCCGGGATATGATTATGGGCGTGTCGAGAAGTCACCCATCACGGACCTTG AGTTTGACCTTCTGAAGAAGACTGTCATGTTAGGTGAAAAGGACGTAATGTACTTGAAAAAGGCGTG TGACGTTCTGAAAGATCAAGTTGATGAGATCCTTGACTTGGCGGGTGGTTGGGTAGCATCAAATGAG CATTTGATTTATTACTTCTCCAATCCGGATACAGGAGAGCCTATTAAGGAATACCTGGAACGTGTAC GCGCTCGCTTTGGAGCCTGGATTCTGGACACTACCTGCCGCGACTATAACCGTGAATGGTTAGACTA CCAGTACGAAGTTGGGCTTCGTCATCACCGTTCAAAGAAAGGGGTCACAGACGGAGTACGCACCGTG CCCCATATCCCACTTCGTTATCTTATCGCATTTATCTATCCTATCACCGCCACTATCAAGCCATTTT TGGCTAAGAAAGGTGGCTCTCCGGAAGACATCGAAGGGATGTACAACGCTTGGTTCAAGTCTGTAGT TTTACAAGTTGCCATCTGGTCACACCCTTATACTAAGGAGAATGACTGGCTCGAGCACCACCACCAC CACCACTGA
ApePgb W59A Y60G F145W ("AGW")	ATGACTCCCTCGGACATCCCGGGATATGATTATGGGCGTGTCGAGAAGTCACCCATCACGGACCTTG AGTTTGACCTTCTGAAGAAGACTGTCATGTTAGGTGAAAAGGACGTAATGTACTTGAAAAAGGCGTG TGACGTTCTGAAAGATCAAGTTGATGAGATCCTTGACTTGGCGGGTGGTTGGGTAGCATCAAATGAG CATTTGATTTATTACTTCTCCAATCCGGATACAGGAGAGCCTATTAAGGAATACCTGGAACGTGTAC GCGCTCGCTTTGGAGCCTGGATTCTGGACACTACCTGCCGCGACTATAACCGTGAATGGTTAGACTA CCAGTACGAAGTTGGGCTTCGTCATCACCGTTCAAAGAAAGGGGTCACAGACGGAGTACGCACCGTG CCCCATATCCCACTTCGTTATCTTATCGCATGGATCTATCCTATCACCGCCACTATCAAGCCATTTT TGGCTAAGAAAGGTGGCTCTCCGGAAGACATCGAAGGGATGTACAACGCTTGGTTCAAGTCTGTAGT TTTACAAGTTGCCATCTGGTCACACCCTTATACTAAGGAGAATGACTGGCTCGAGCACCACCACCAC CACCACTGA
RmaNOD WT	ATGGCGCCGACCCTGTCGGAACAGACCCGTCAGTTGGTACGTGCGTCTGTGCCTGCACTGCAGAAAC ACTCAGTCGCTATTAGCGCCACGATGTATCGGCTGCTTTTCGAACGGTATCCCGAAACGCGGAGCTT GTTTGAACTTCCTGAGAGACAGATACACAAGCTTGCGTCGGCCCTGTTGGCCTACGCCCGTAGTATC GACAACCCATCGGCGTTACAGGCGGCCATCCGCCGCATGGTGCTTTCCCACGCACGCGCAGGAGTGC AGGCCGTCCATTATCCGCTGGTTTGGGAATGTTTGAGAGACGCTATAAAAGAAGTCCTGGGCCCGGA TGCCACCGAGACCCTTCTGCAGGCGTGGAAGGAAGCCTATGATTTTTTAGCTCATTTACTGTCTACC AAGGAAGCGCAAGTCTACGCTGTGTTAGCTGAACTCGAGCATCACCATCACCATCACTGA
RmaNOD Q52V	ATGGCGCCGACCCTGTCGGAACAGACCCGTCAGTTGGTACGTGCGTCTGTGCCTGCACTGCAGAAAC ACTCAGTCGCTATTAGCGCCACGATGTATCGGCTGCTTTTCGAACGGTATCCCGAAACGCGGAGCTT GTTTGAACTTCCTGAGAGAGTTATACACAAGCTTGCGTCGGCCCTGTTGGCCTACGCCCGTAGTATC GACAACCCATCGGCGTTACAGGCGGCCATCCGCCGCATGGTGCTTTCCCACGCACGCGCAGGAGTGC AGGCCGTCCATTATCCGCTGGTTTGGGAATGTTTGAGAGACGCTATAAAAGAAGTCCTGGGCCCGGA

	TGCCACCGAGACCCTTCTGCAGGCGTGGAAGGAAGCCTATGATTTTTTAGCTCATTTACTGTCTACC AAGGAAGCGCAAGTCTACGCTGTGTTAGCTGAACTCGAGCACCACCACCACCACCACTGA
P411-CIS	ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAACA CAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCGAGGC GCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAATCACGC TTTGATAAAAACTTAAGTCAAGCGCTGAAATTTGCACGTGATTTTGCAGGAGACGGGTTAGTCACAA GCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGC AATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCTA AATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATACAATTGGTCTTT GCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATCCATTTATTATAAGTATGGT CCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGACGACCCAGCTTATGATGAA AACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATC GCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAAC GGGTGAGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGAA GCAACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAG TAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTACAAACAAGTCAAACAGCTTAA ATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATAT GCAAAAGAAGATACGGTGCTTGGAGGAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGA TTCCTCAGCTTCACCGTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTT TGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTATC GGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTG AAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTAACGTTAAAACCTAAAGGCTTTGTGGT AAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAA AAAGTACGCAAAAAGGCAGAAAACGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGG GTACCGCTGAAGGAACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGT CGCAACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCT TATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATG AAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAA AGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAA GCAGATGCAAGCGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAG CAGCCTACTTTAACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGT CGACAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGAACGTCGTAGCAAGC AAAGAACTTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAG CTTCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGTGT AACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAATTAGCT CATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTTCAAGATCCTG TTACGCGCACGCAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCCCGCCGCATAAAGTAGAGCTTGA AGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGTTTAACAATGCTTGAACTG CTTGAAAAATACCCGGCGTGTGAAATGAAATTCAGCGAATTTATCGCCCTTCTGCCAAGCATACGCC CGCGCTATTACTCGATTTCTTCATCACCTCGTGTCGATGAAAAACAAGCAAGCATCACGGTCAGCGT TGTCTCAGGAGAAGCGTGGAGCGGATATGGAGAATATAAAGGAATTGCGTCGAACTATCTTGCCGAG CTGCAAGAAGGAGATACGATTACGTGCTTTATTTCCACACCGCAGTCAGAATTTACGCTGCCAAAAG ACCCTGAAACGCCGCTTATCATGGTCGGACCGGGAACAGGCGTCGCGCCGTTTAGAGGCTTTGTGCA GGCGCGCAAACAGCTAAAAGAACAAGGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGT TCACCTCATGAAGACTATCTGTATCAAGAAGAGCTTGAAAACGCCCAAAGCGAAGGCATCATTACGC TTCATACCGCTTTTTCTCGCATGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGA CGGCAAGAAATTGATTGAACTTCTTGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAA ATGGCACCTGCCGTTGAAGCAACGCTTATGAAAAGCTATGCTGACGTTCACCAAGTGAGTGAAGCAG ACGCTCGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGCT CGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAA
P411-UA	ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAACA CAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCGAGGC GCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAATCACGC TTTGATAAAAACTTAAGTCAAGCGTATAAATTTGCACGTGATTTTGCAGGAGACGGGTTAGTGACAA GCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGC AATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCTA AATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATACAATTGGTCTTT

	GCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATCCATTTATTATAAGTATGGT CCGTGCAATTGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGACGACCCAGCTTATGATGAA AACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATC GCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAAC GGGTGAGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGAA GCAACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAG TAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTACAAACAAGTCAAACAGCTTAA ATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATAT GCAAAAGAAGATACGGTGCTTGGAGGAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGA TTCCTCAGCTTCACCGTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTT TGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTATC GGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTG AAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTTCAGTTAAAACCTAAAGGCTTTGTGGT AAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAA AAAGTACGCAAAAAGGCAGAAAACGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGG GTACCGCTGAAGGAACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGT CGCAACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCT TATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATG AAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAA AGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAA GCAGATGCAAGCGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAG CAGCCTACTTTAACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGT CGACAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGAACGTCGTAGCAAGC AAAGAACTTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAG CTTCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGTGT AACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAATTAGCT CATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTTCAAGATCCTG TTACGCGCACGCAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCCCGCCGCATAAAGTAGAGCTTGA AGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGTTTAACAATGCTTGAACTG CTTGAAAAATACCCGGCGTGTGAAATGAAATTCAGCGAATTTATCGCCCTTCTGCCAAGCATACGCC CGCGCTATTACTCGATTTCTTCATCACCTCGTGTCGATGAAAAACAAGCAAGCATCACGGTCAGCGT TGTCTCAGGAGAAGCGTGGAGCGGATATGGAGAATATAAAGGAATTGCGTCGAACTATCTTGCCGAG CTGCAAGAAGGAGATACGATTACGTGCTTTATTTCCACACCGCAGTCAGAATTTACGCTGCCAAAAG ACCCTGAAACGCCGCTTATCATGGTCGGACCGGGAACAGGCGTCGCGCCGTTTAGAGGCTTTGTGCA GGCGCGCAAACAGCTAAAAGAACAAGGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGT TCACCTCATGAAGACTATCTGTATCAAGAAGAGCTTGAAAACGCCCAAAGCGAAGGCATCATTACGC TTCATACCGCTTTTTCTCGCATGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGA CGGCAAGAAATTGATTGAACTTCTTGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAA ATGGCACCTGCCGTTGAAGCAACGCTTATGAAAAGCTATGCTGACGTTCACCAAGTGAGTGAAGCAG ACGCTCGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGCT CGAGCACCACCACCACCACCACTGA
P411-UA-V87C	ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAACA CAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCGAGGC GCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAATCACGC TTTGATAAAAACTTAAGTCAAGCGTATAAATTTGCACGTGATTTTGCAGGAGACGGGTTATGTACAA GCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGC AATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCTA AATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATACAATTGGTCTTT GCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATCCATTTATTATAAGTATGGT CCGTGCAATTGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGACGACCCAGCTTATGATGAA AACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATC GCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAAC GGGTGAGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGAA GCAACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAG TAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTACAAACAAGTCAAACAGCTTAA ATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATAT GCAAAAGAAGATACGGTGCTTGGAGGAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGA

	TTCCTCAGCTTCACCGTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTT TGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTATC GGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTG AAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTTCAGTTAAAACCTAAAGGCTTTGTGGT AAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAA AAAGTACGCAAAAAGGCAGAAAACGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGG GTACCGCTGAAGGAACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGT CGCAACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCT TATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATG AAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAA AGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAA GCAGATGCAAGCGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAG CAGCCTACTTTAACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGT CGACAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGAACGTCGTAGCAAGC AAAGAACTTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAG CTTCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGTGT AACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAATTAGCT CATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTTCAAGATCCTG TTACGCGCACGCAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCCCGCCGCATAAAGTAGAGCTTGA AGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGTTTAACAATGCTTGAACTG CTTGAAAAATACCCGGCGTGTGAAATGAAATTCAGCGAATTTATCGCCCTTCTGCCAAGCATACGCC CGCGCTATTACTCGATTTCTTCATCACCTCGTGTCGATGAAAAACAAGCAAGCATCACGGTCAGCGT TGTCTCAGGAGAAGCGTGGAGCGGATATGGAGAATATAAAGGAATTGCGTCGAACTATCTTGCCGAG CTGCAAGAAGGAGATACGATTACGTGCTTTATTTCCACACCGCAGTCAGAATTTACGCTGCCAAAAG ACCCTGAAACGCCGCTTATCATGGTCGGACCGGGAACAGGCGTCGCGCCGTTTAGAGGCTTTGTGCA GGCGCGCAAACAGCTAAAAGAACAAGGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGT TCACCTCATGAAGACTATCTGTATCAAGAAGAGCTTGAAAACGCCCAAAGCGAAGGCATCATTACGC TTCATACCGCTTTTTCTCGCATGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGA CGGCAAGAAATTGATTGAACTTCTTGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAA ATGGCACCTGCCGTTGAAGCAACGCTTATGAAAAGCTATGCTGACGTTCACCAAGTGAGTGAAGCAG ACGCTCGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGCT CGAGCACCACCACCACCACCACTGA
P411-UA-V87F	ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAACA CAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCGAGGC GCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAATCACGC TTTGATAAAAACTTAAGTCAAGCGTATAAATTTGCACGTGATTTTGCAGGAGACGGGTTATTTACAA GCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGC AATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCTA AATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATACAATTGGTCTTT GCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATCCATTTATTATAAGTATGGT CCGTGCAATTGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGACGACCCAGCTTATGATGAA AACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATC GCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAAC GGGTGAGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGAA GCAACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAG TAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTACAAACAAGTCAAACAGCTTAA ATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATAT GCAAAAGAAGATACGGTGCTTGGAGGAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGA TTCCTCAGCTTCACCGTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTT TGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTATC GGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTG AAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTTCAGTTAAAACCTAAAGGCTTTGTGGT AAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAA AAAGTACGCAAAAAGGCAGAAAACGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGG GTACCGCTGAAGGAACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGT CGCAACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCT TATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATG

	AAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAA AGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAA GCAGATGCAAGCGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAG CAGCCTACTTTAACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGT CGACAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGAACGTCGTAGCAAGC AAAGAACTTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAG CTTCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGTGT AACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAATTAGCT CATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTTCAAGATCCTG TTACGCGCACGCAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCCCGCCGCATAAAGTAGAGCTTGA AGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGTTTAACAATGCTTGAACTG CTTGAAAAATACCCGGCGTGTGAAATGAAATTCAGCGAATTTATCGCCCTTCTGCCAAGCATACGCC CGCGCTATTACTCGATTTCTTCATCACCTCGTGTCGATGAAAAACAAGCAAGCATCACGGTCAGCGT TGTCTCAGGAGAAGCGTGGAGCGGATATGGAGAATATAAAGGAATTGCGTCGAACTATCTTGCCGAG CTGCAAGAAGGAGATACGATTACGTGCTTTATTTCCACACCGCAGTCAGAATTTACGCTGCCAAAAG ACCCTGAAACGCCGCTTATCATGGTCGGACCGGGAACAGGCGTCGCGCCGTTTAGAGGCTTTGTGCA GGCGCGCAAACAGCTAAAAGAACAAGGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGT TCACCTCATGAAGACTATCTGTATCAAGAAGAGCTTGAAAACGCCCAAAGCGAAGGCATCATTACGC TTCATACCGCTTTTTCTCGCATGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGA CGGCAAGAAATTGATTGAACTTCTTGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAA ATGGCACCTGCCGTTGAAGCAACGCTTATGAAAAGCTATGCTGACGTTCACCAAGTGAGTGAAGCAG ACGCTCGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGCT CGAGCACCACCACCACCACCACTGA

Supplemental Table 13. Amino acid sequences of reported biocatalysts. These sequences are also available as a supplemental csv file.

Protein	Amino acid sequence
ApePgb WT	MTPSDIPGYDYGRVEKSPITDLEFDLLKKTVMLGEKDVMYLKKACDVLKDQVDEILDLWYGWVASNE HLIYYFSNPDTGEPIKEYLERVRARFGAWILDTTCRDYNREWLDYQYEVGLRHHRSKKGVTDGVRTV PHIPLRYLIAFIYPITATIKPFLAKKGGSPEDIEGMYNAWFKSVVLQVAIWSHPYTKENDWLEHHHH HH*
ApePgb Y60G	MTPSDIPGYDYGRVEKSPITDLEFDLLKKTVMLGEKDVMYLKKACDVLKDQVDEILDLWGGWVASNE HLIYYFSNPDTGEPIKEYLERVRARFGAWILDTTCRDYNREWLDYQYEVGLRHHRSKKGVTDGVRTV PHIPLRYLIAFIYPITATIKPFLAKKGGSPEDIEGMYNAWFKSVVLQVAIWSHPYTKENDWLEHHHH HH*
ApePgb W59A Y60G	MTPSDIPGYDYGRVEKSPITDLEFDLLKKTVMLGEKDVMYLKKACDVLKDQVDEILDLAGGWVASNE HLIYYFSNPDTGEPIKEYLERVRARFGAWILDTTCRDYNREWLDYQYEVGLRHHRSKKGVTDGVRTV PHIPLRYLIAFIYPITATIKPFLAKKGGSPEDIEGMYNAWFKSVVLQVAIWSHPYTKENDWLEHHHH HH*
ApePgb W59A Y60G F145W ("AGW")	MTPSDIPGYDYGRVEKSPITDLEFDLLKKTVMLGEKDVMYLKKACDVLKDQVDEILDLAGGWVASNE HLIYYFSNPDTGEPIKEYLERVRARFGAWILDTTCRDYNREWLDYQYEVGLRHHRSKKGVTDGVRTV PHIPLRYLIAWIYPITATIKPFLAKKGGSPEDIEGMYNAWFKSVVLQVAIWSHPYTKENDWLEHHHH HH*
RmaNOD WT	MAPTLSEQTRQLVRASVPALQKHSVAISATMYRLLFERYPETRSLFELPERQIHKLASALLAYARSI DNPSALQAAIRRMVLSHARAGVQAVHYPLVWECLRDAIKEVLGPDATETLLQAWKEAYDFLAHLLST KEAQVYAVLAELEHHHHHH*
RmaNOD Q52V	MAPTLSEQTRQLVRASVPALQKHSVAISATMYRLLFERYPETRSLFELPERVIHKLASALLAYARSI DNPSALQAAIRRMVLSHARAGVQAVHYPLVWECLRDAIKEVLGPDATETLLQAWKEAYDFLAHLLST KEAQVYAVLAELEHHHHHH*
P411-CIS	MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDESR FDKNLSQALKFARDFAGDGLVTSWTHEKNWKKAHNILLPSFSQQAMKGYHAMMVDIAVQLVQKWERL NADEHIEVSEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISMVRALDEVMNKLQRANPDDPAYDE NKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQIITFLIAGHE

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P411-UA	MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDESR FDKNLSQAYKFARDFAGDGLVTSWTHEKNWKKAHNILLPSFSQQAMKGYHAMMVDIAVQLVQKWERL NADEHIEVSEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISMVRAIDEVMNKLQRANPDDPAYDE NKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQIITFLIAGHE ATSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRLWPTAPAFSLY AKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASI GQQFALHEATLVLGMMLKHFDFEDHTNYELDIKETFQLKPKGFVVKAKSKKIPLGGIPSPSTEQSAK KVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTAS YNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAAKGAENIADRGE ADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSAADMPLAKMHGAFSTNVVAS KELQQPGSARSTRHLEIELPKEASYQEGDHLGVIPRNYEGIVNRVTARFGLDASQQIRLEAEEEKLA HLPLAKTVSVEELLQYVELQDPVTRTQLRAMAAKTVCPPHKVELEALLEKQAYKEQVLAKRLTMLEL LEKYPACEMKFSEFIALLPSIRPRYYSISSSPRVDEKQASITVSVVSGEAWSGYGEYKGIASNYLAE LQEGDTITCFISTPQSEFTLPKDPETPLIMVGPGTGVAPFRGFVQARKQLKEQGQSLGEAHLYFGCR SPHEDYLYQEELENAQSEGIITLHTAFSRMPNQPKTYVQHVMEQDGKKLIELLDQGAHFYICGDGSQ MAPAVEATLMKSYADVHQVSEADARLWLQQLEEKGRYAKDVWAGLEHHHHHH*
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P411-UA-V87F	MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDESR FDKNLSQAYKFARDFAGDGLFTSWTHEKNWKKAHNILLPSFSQQAMKGYHAMMVDIAVQLVQKWERL NADEHIEVSEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISMVRAIDEVMNKLQRANPDDPAYDE NKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQIITFLIAGHE ATSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRLWPTAPAFSLY AKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASI GQQFALHEATLVLGMMLKHFDFEDHTNYELDIKETFQLKPKGFVVKAKSKKIPLGGIPSPSTEQSAK KVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTAS YNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAAKGAENIADRGE ADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSAADMPLAKMHGAFSTNVVAS KELQQPGSARSTRHLEIELPKEASYQEGDHLGVIPRNYEGIVNRVTARFGLDASQQIRLEAEEEKLA HLPLAKTVSVEELLQYVELQDPVTRTQLRAMAAKTVCPPHKVELEALLEKQAYKEQVLAKRLTMLEL

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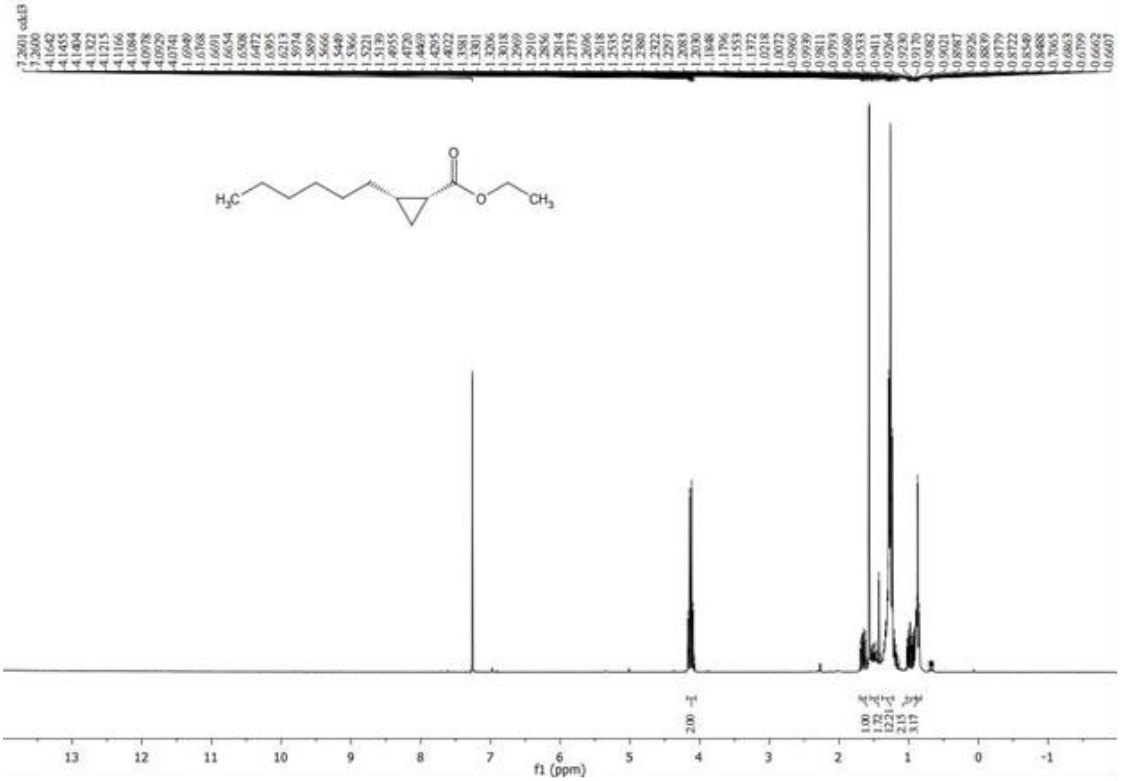
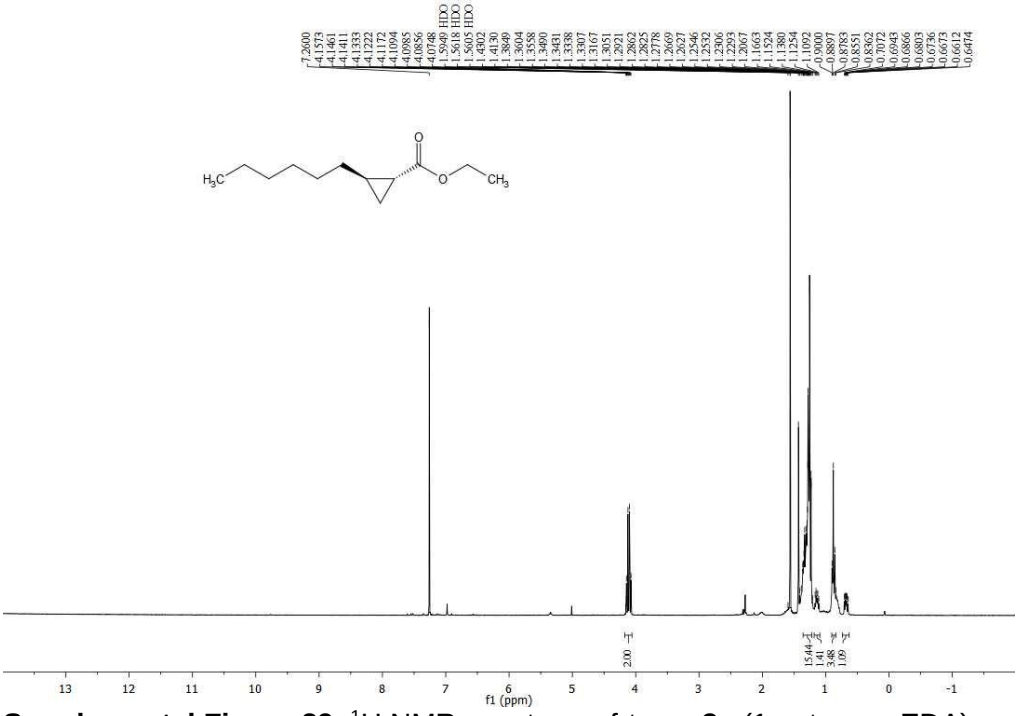
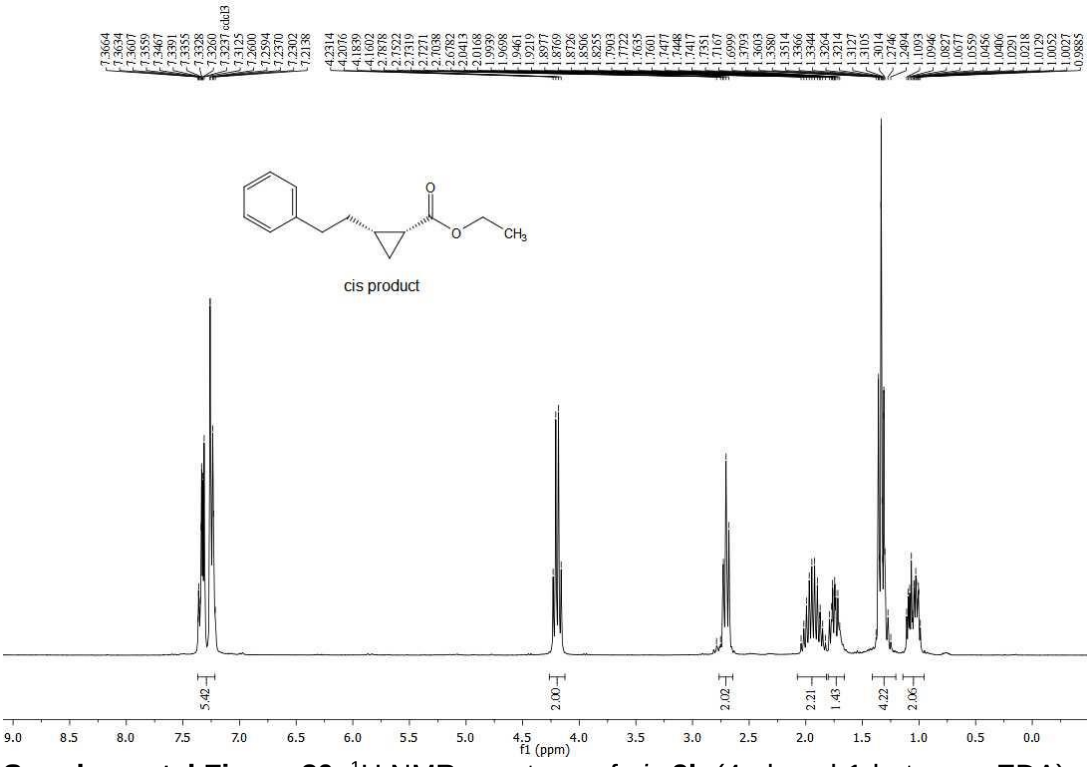
NMR characterization of cyclopropane products



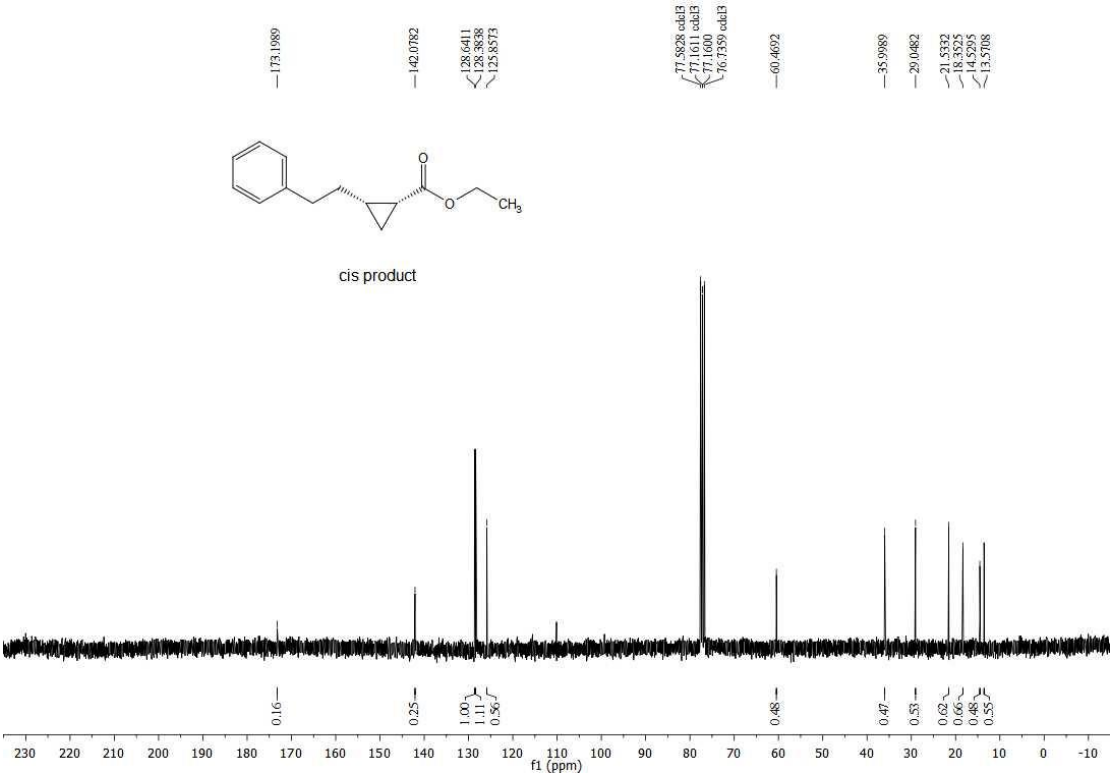
Supplemental Figure 28. ^{1}H NMR spectrum of *cis*-**3a** (1-octene + EDA).



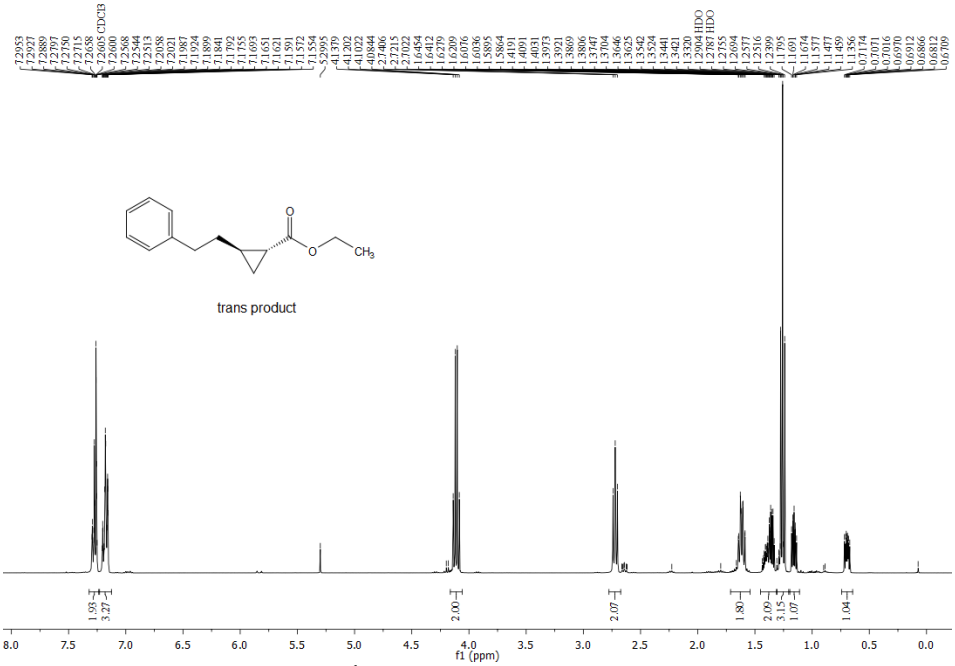
Supplemental Figure 29. ^{1}H NMR spectrum of *trans*-**3a** (1-octene + EDA).



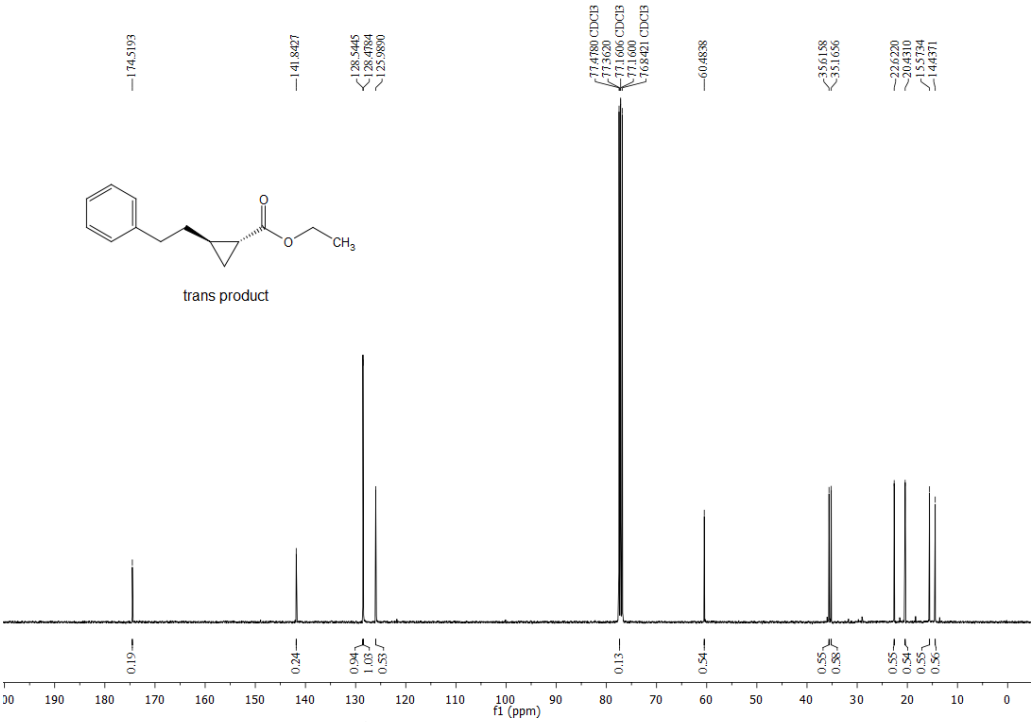
Supplemental Figure 30. ^{1}H NMR spectrum of *cis*-**3b** (4-phenyl-1-butene + EDA).



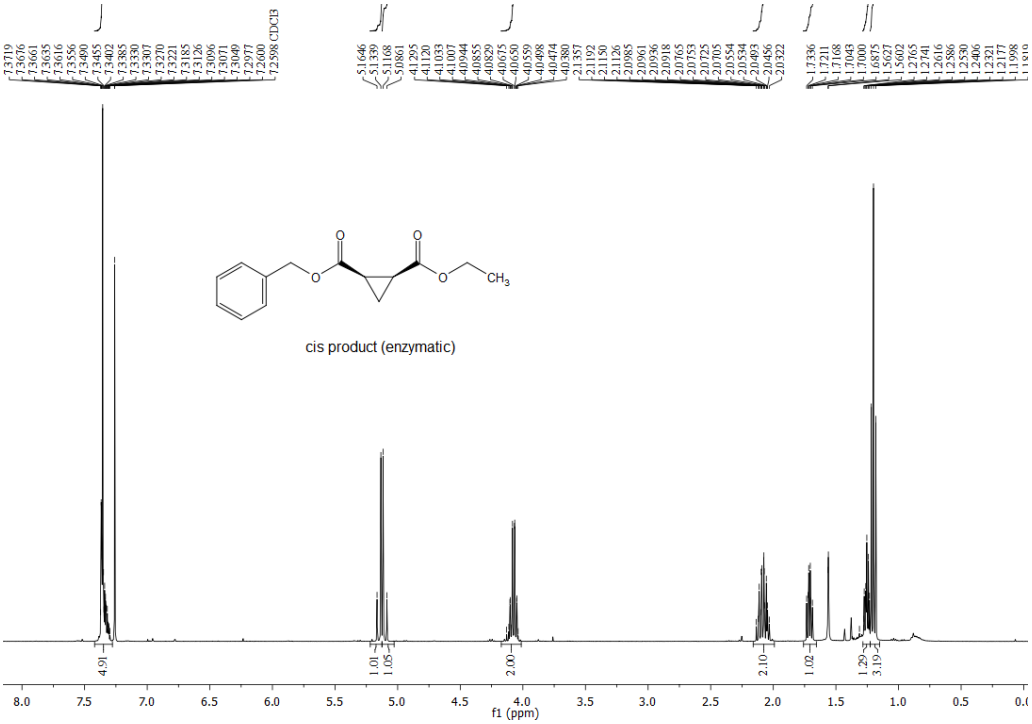
Supplemental Figure 31. ^{13}C NMR spectrum of *cis*-**3b** (4-phenyl-1-butene + EDA).



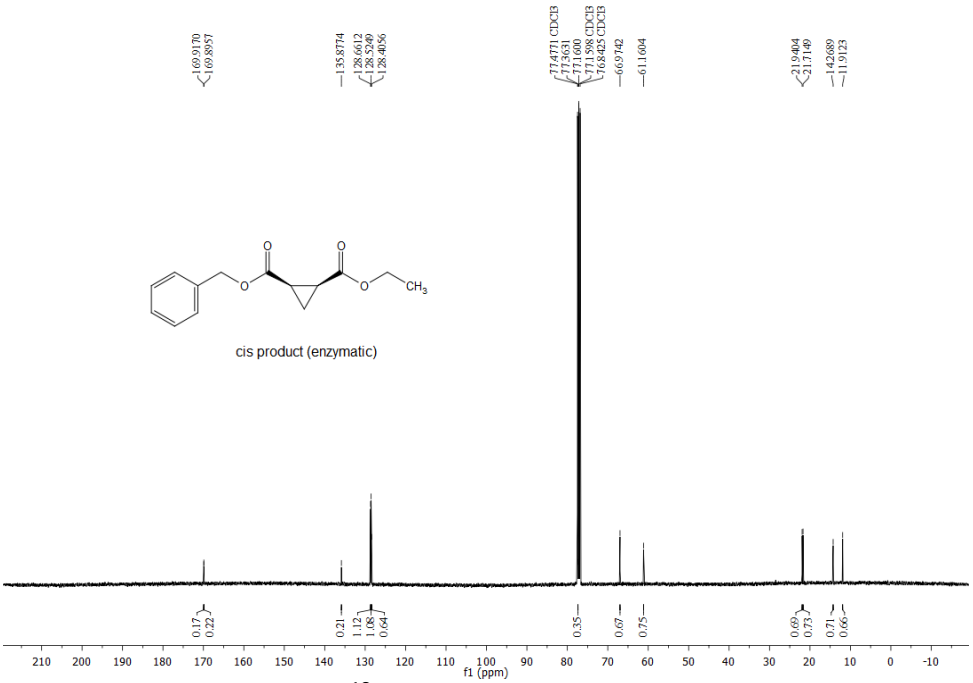
Supplemental Figure 32. ^{1}H NMR spectrum of *trans*-**3b** (4-phenyl-1-butene + EDA).



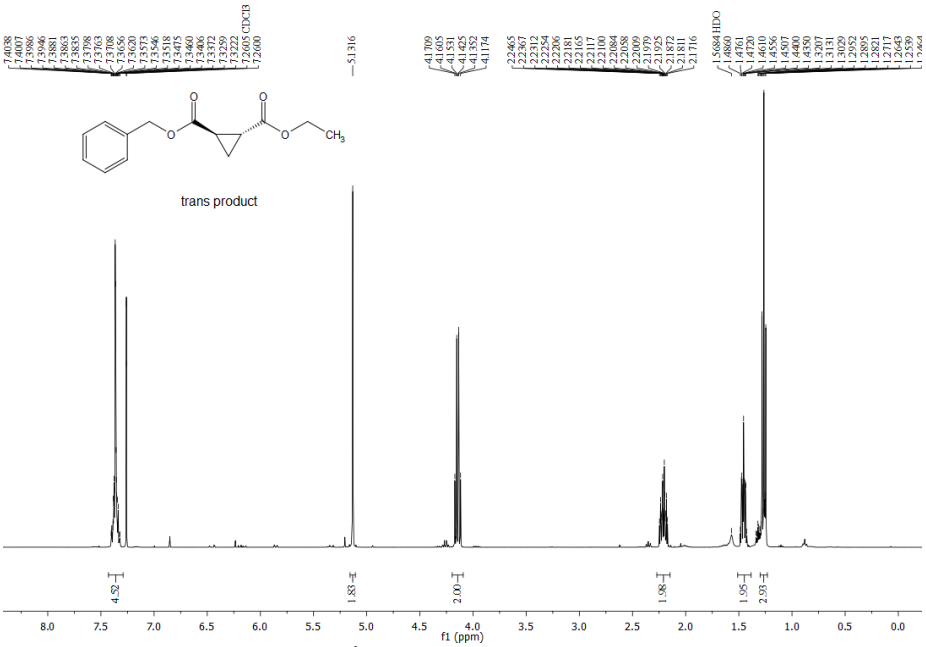
Supplemental Figure 33. ^{13}C NMR spectrum of *trans*-**3b** (4-phenyl-1-butene + EDA).



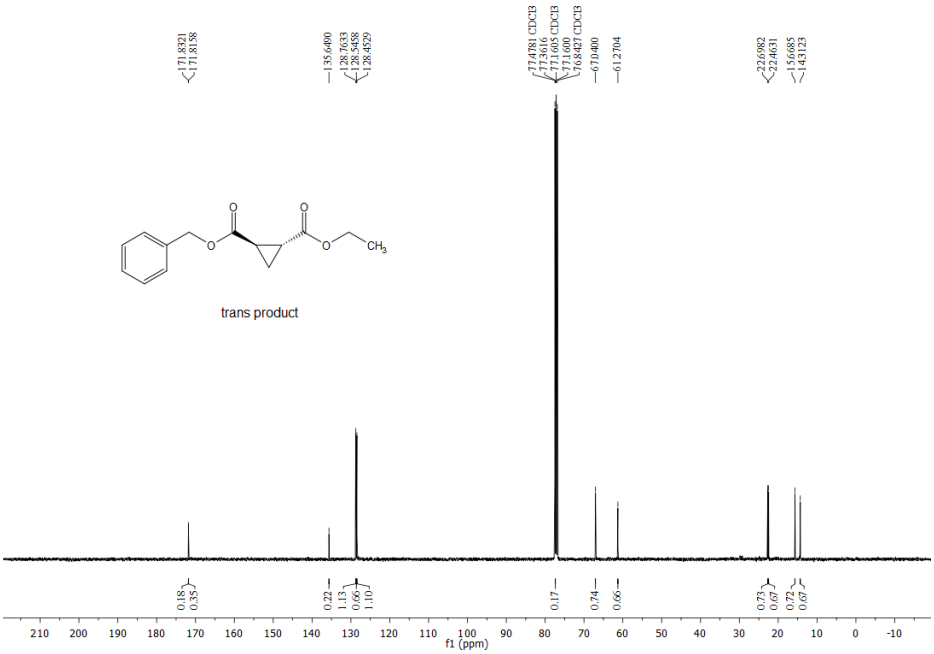
Supplemental Figure 34. ^{1}H NMR spectrum of *cis*-**3c** (benzyl acrylate + EDA, enzymatic from P411-UA V87C).



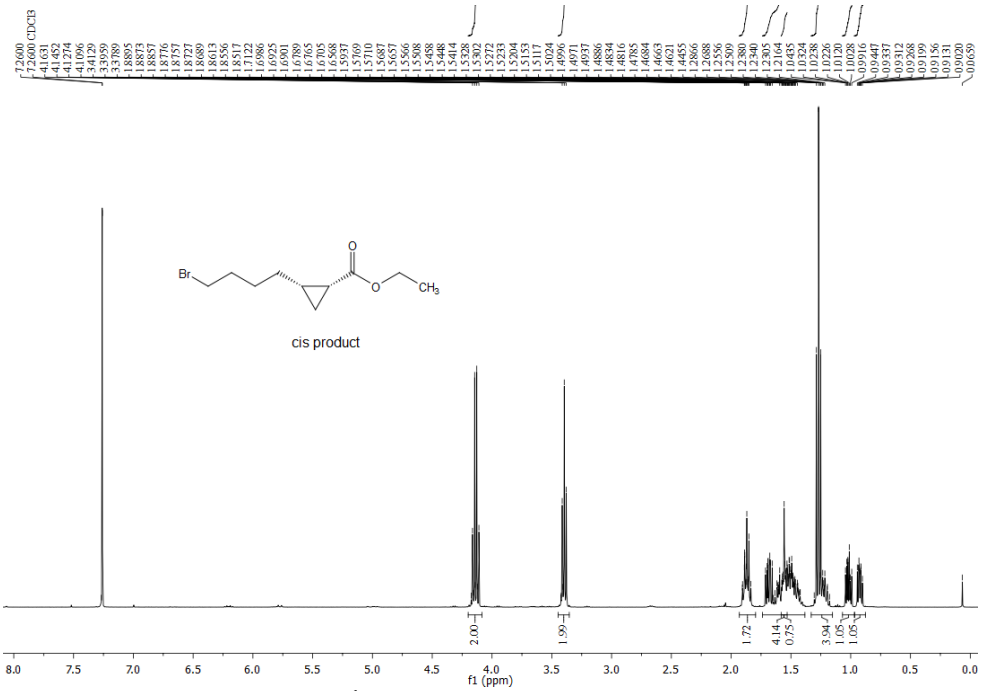
Supplemental Figure 35. ^{13}C NMR spectrum of *cis*-**3c** (benzyl acrylate + EDA, enzymatic from P411-UA V87C).



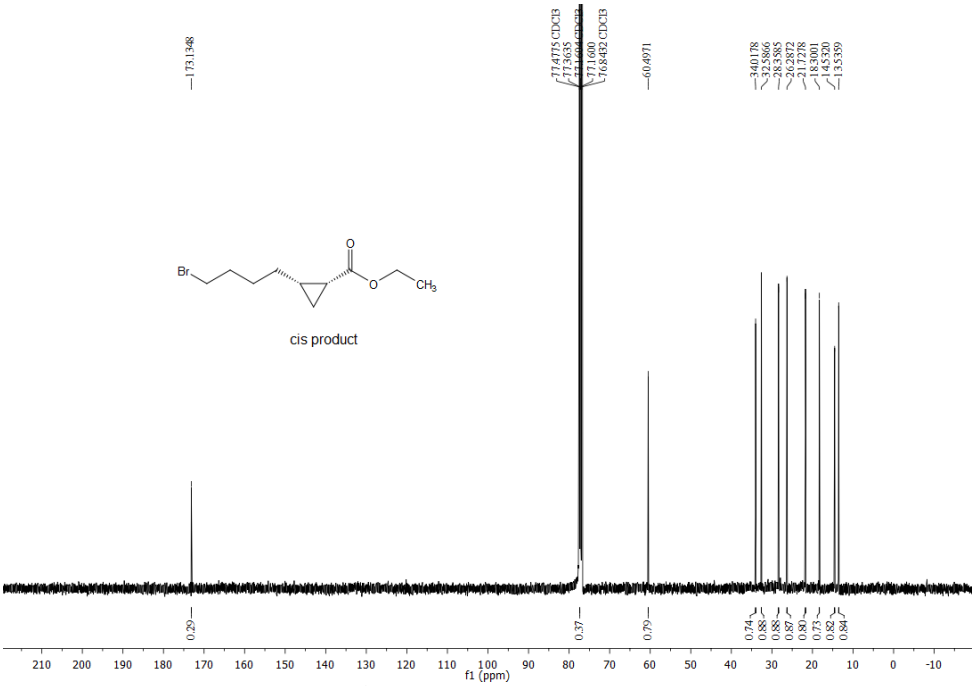
Supplemental Figure 36. ^{1}H NMR spectrum of *trans*-**3c** (benzyl acrylate + EDA, enzymatic from P411-UA V87F).



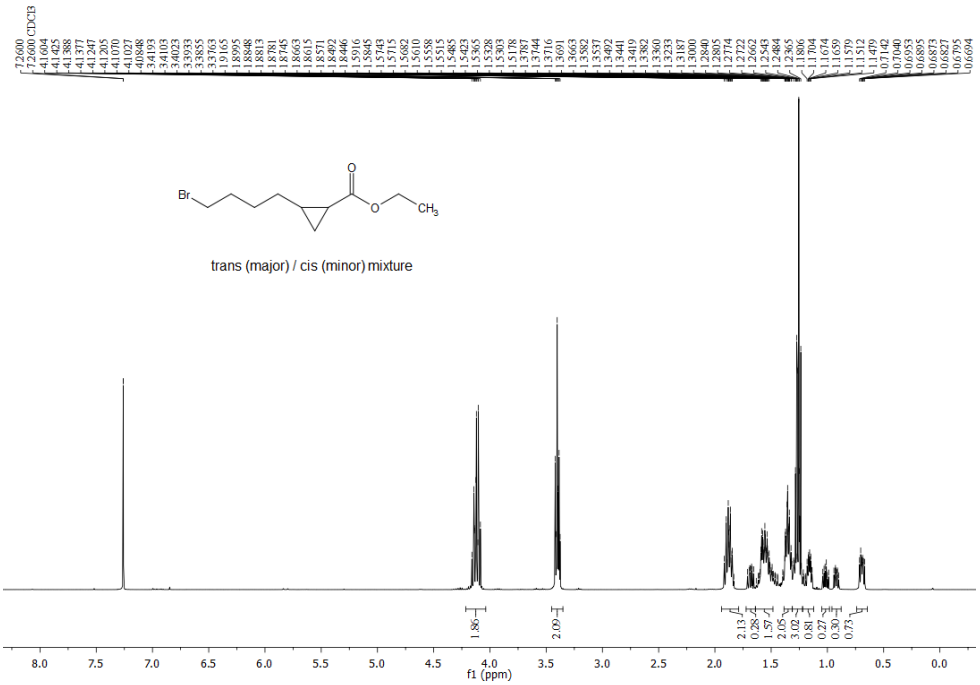
Supplemental Figure 37. ^{13}C NMR spectrum of *trans*-**3c** (benzyl acrylate + EDA, enzymatic from P411-UA V87F).



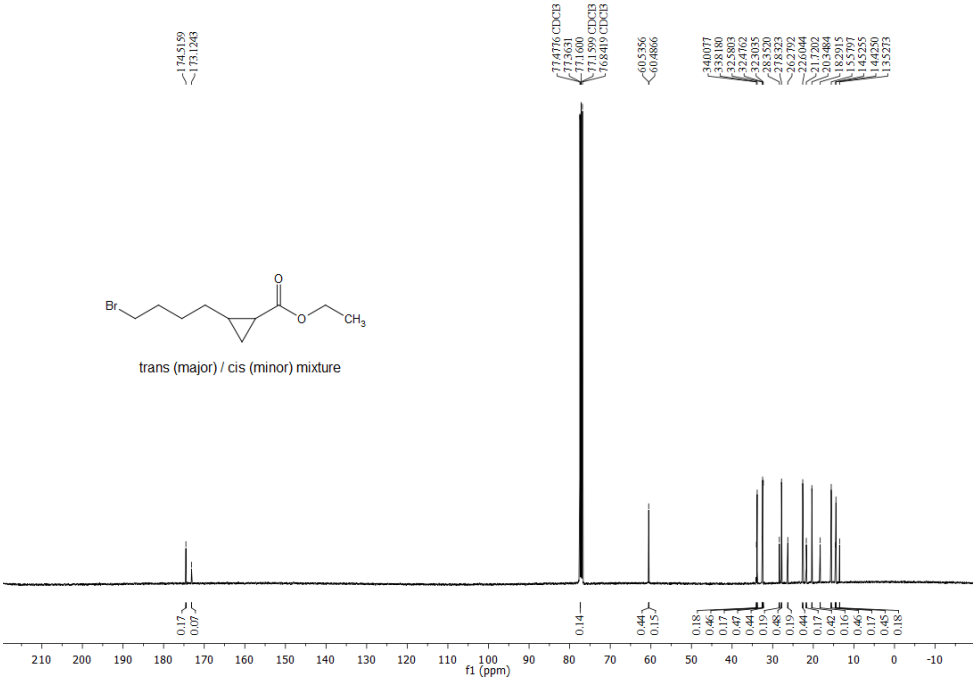
Supplemental Figure 38. ^{1}H NMR spectrum of *cis*-**3d** (6-bromo-1-hexene + EDA).



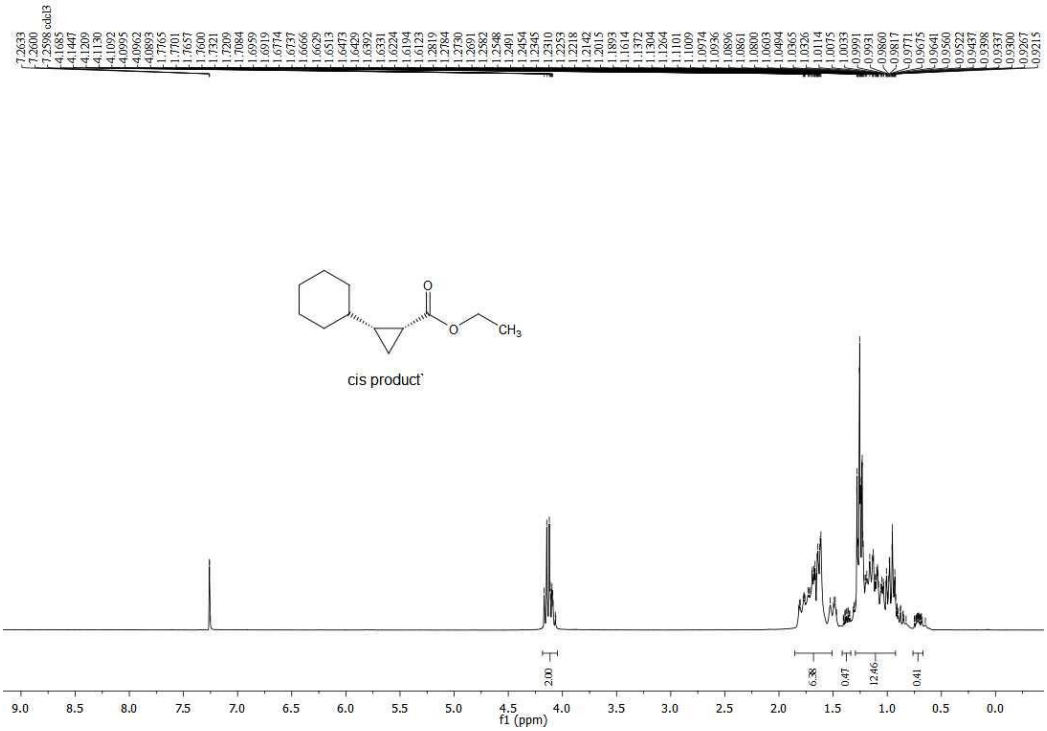
Supplemental Figure 39. ^{13}C NMR spectrum of *cis*-**3d** (6-bromo-1-hexene + EDA).



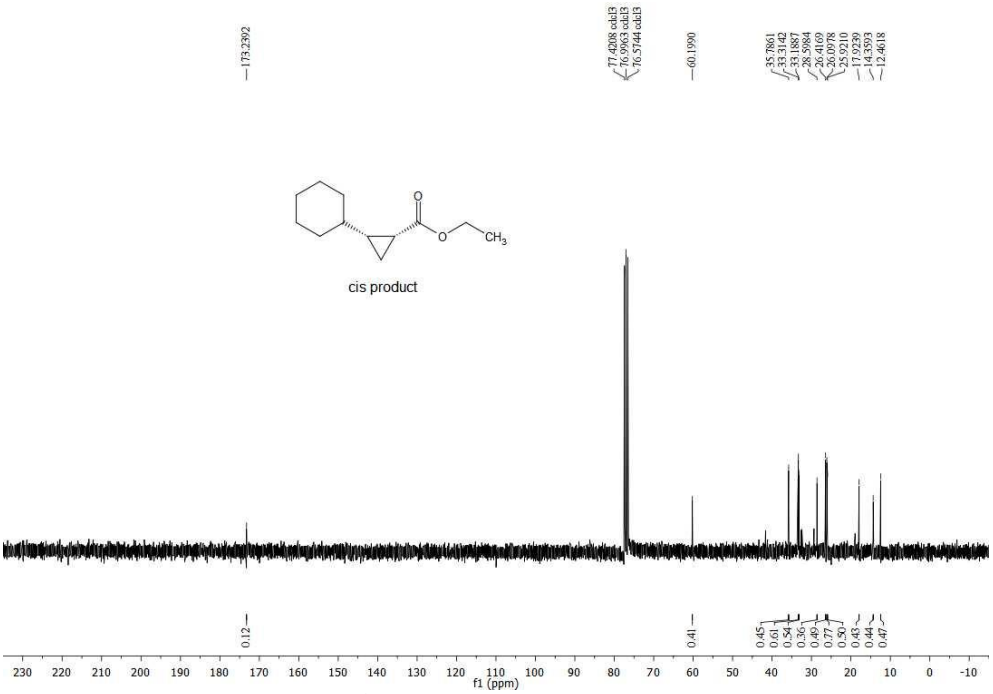
Supplemental Figure 40. ^{1}H NMR spectrum of **3d** (6-bromo-1-hexene + EDA) diastereomeric mixture (~71:29 *trans*:*cis*).



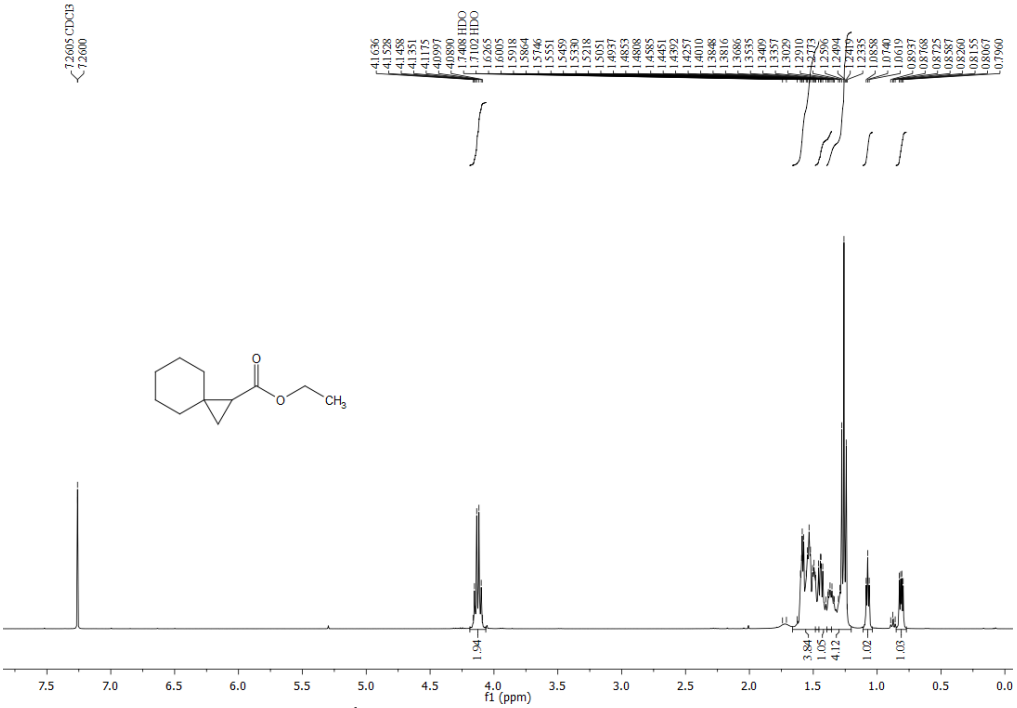
Supplemental Figure 41. ^{13}C NMR spectrum of **3d** (6-bromo-1-hexene + EDA) diastereomeric mixture (~71:29 *trans*:*cis*).



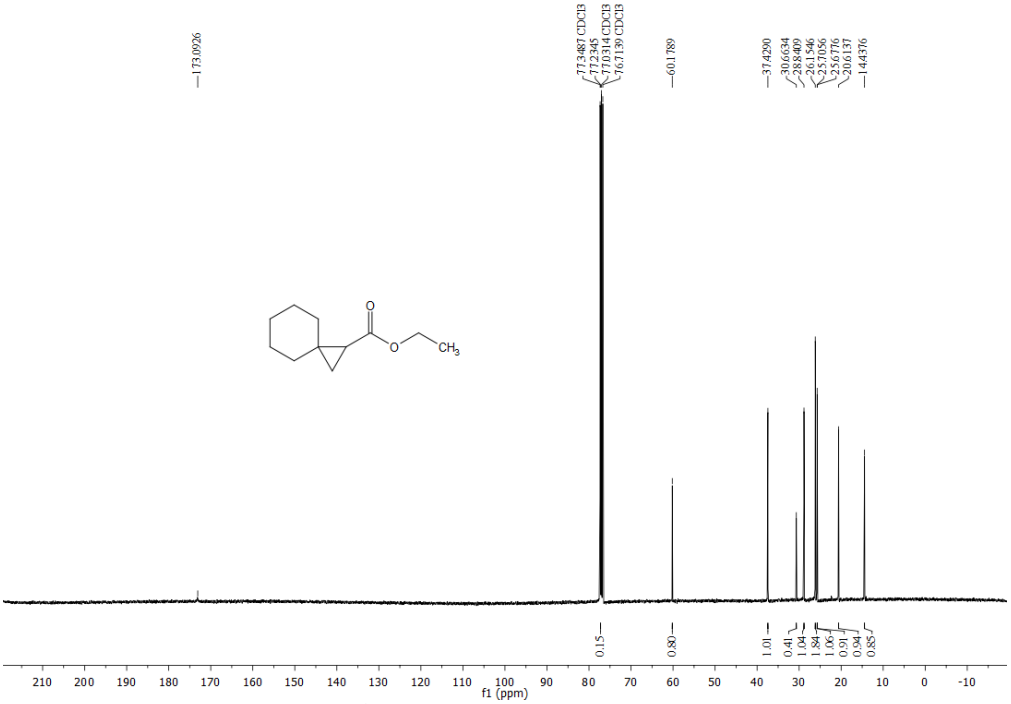
Supplemental Figure 42. ^{1}H NMR spectrum of *cis*-**3e** (vinylcyclohexane + EDA).



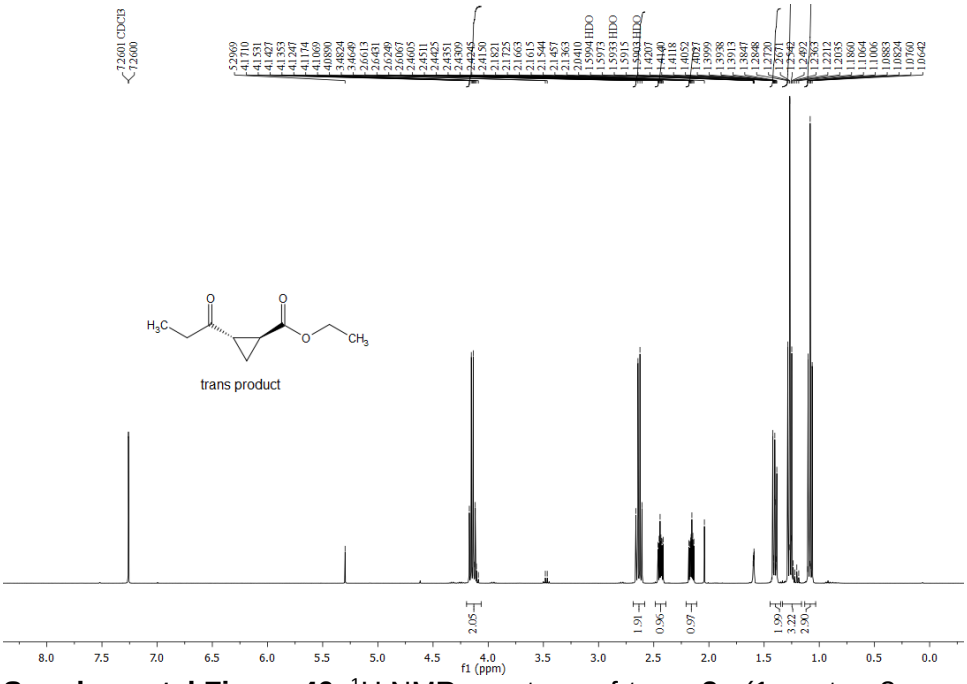
Supplemental Figure 43. ^{13}C NMR spectrum of *cis*-**3e** (vinylcyclohexane + EDA).



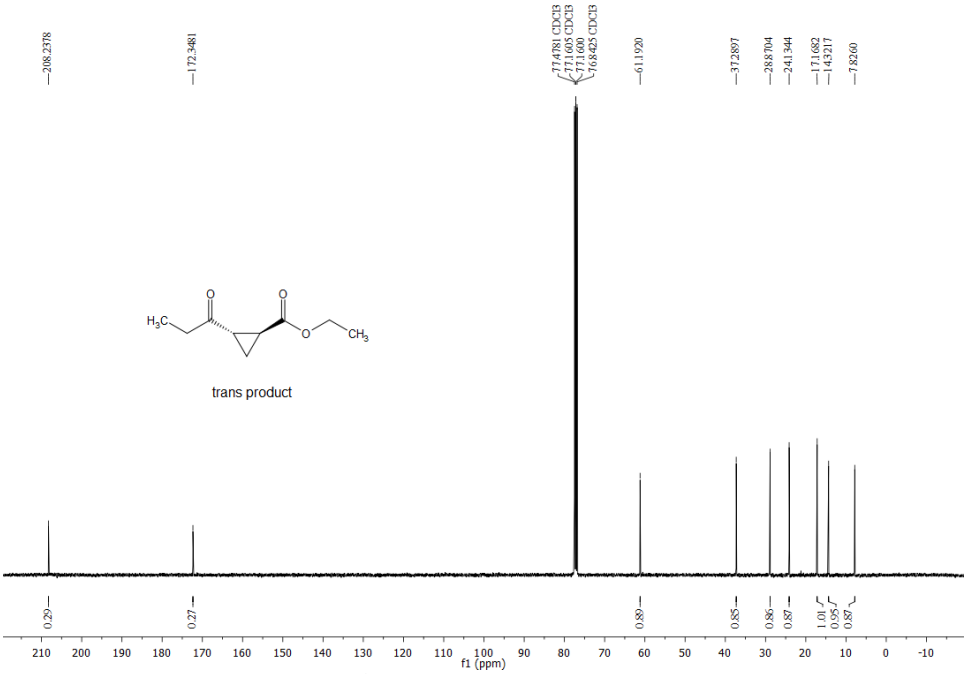
Supplemental Figure 44. ^{1}H NMR spectrum of **3f** (methylenecyclohexane + EDA, enzymatic from ApePgb AGW).



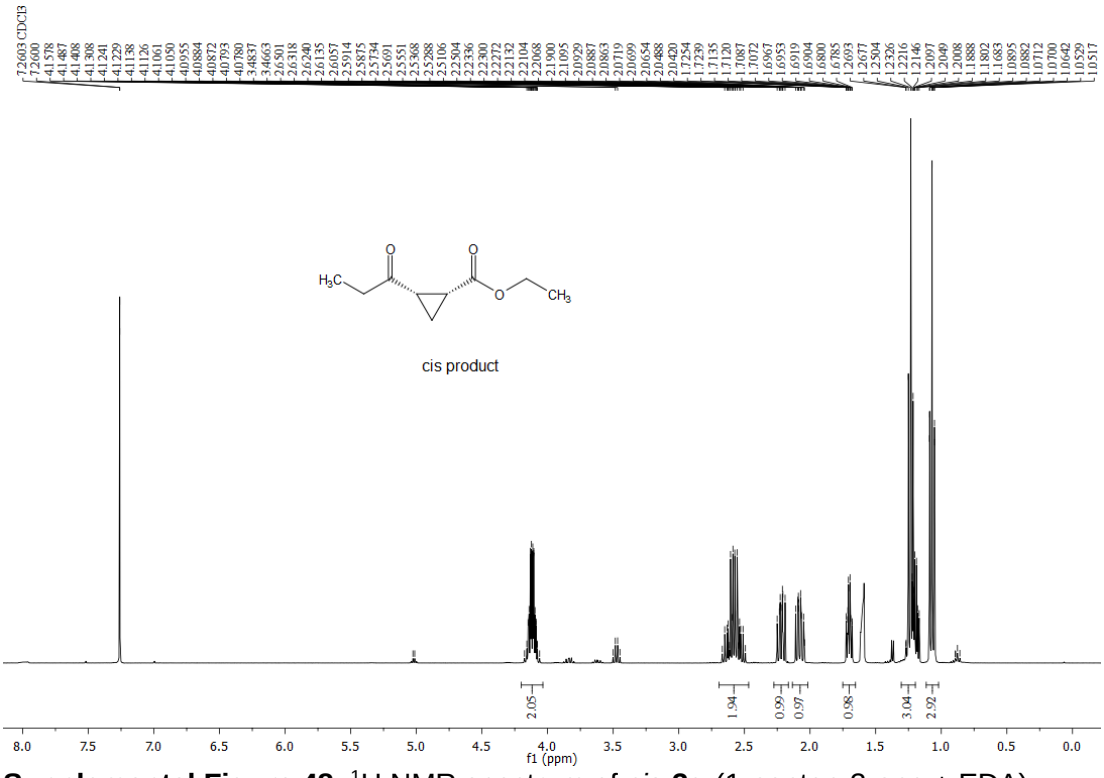
Supplemental Figure 45. ^{13}C NMR spectrum of **3f** (methylenecyclohexane + EDA, enzymatic from ApePgb AGW).



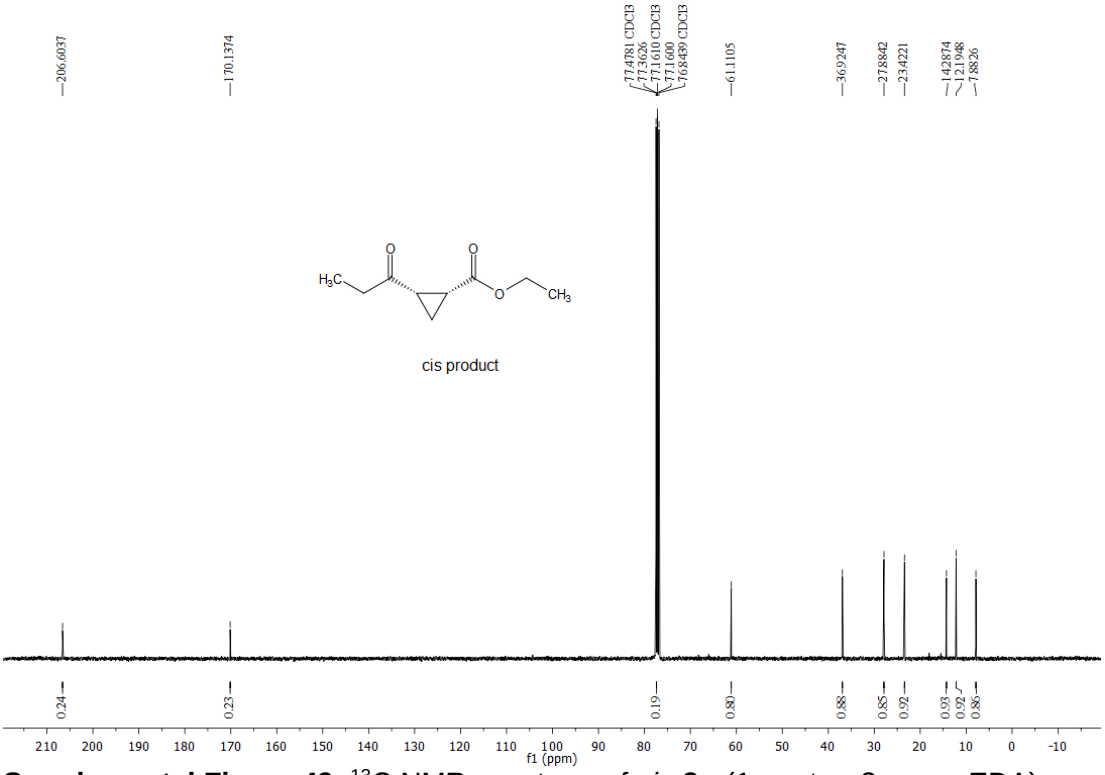
Supplemental Figure 46. ^{1}H NMR spectrum of *trans*-**3g** (1-penten-3-one + EDA).



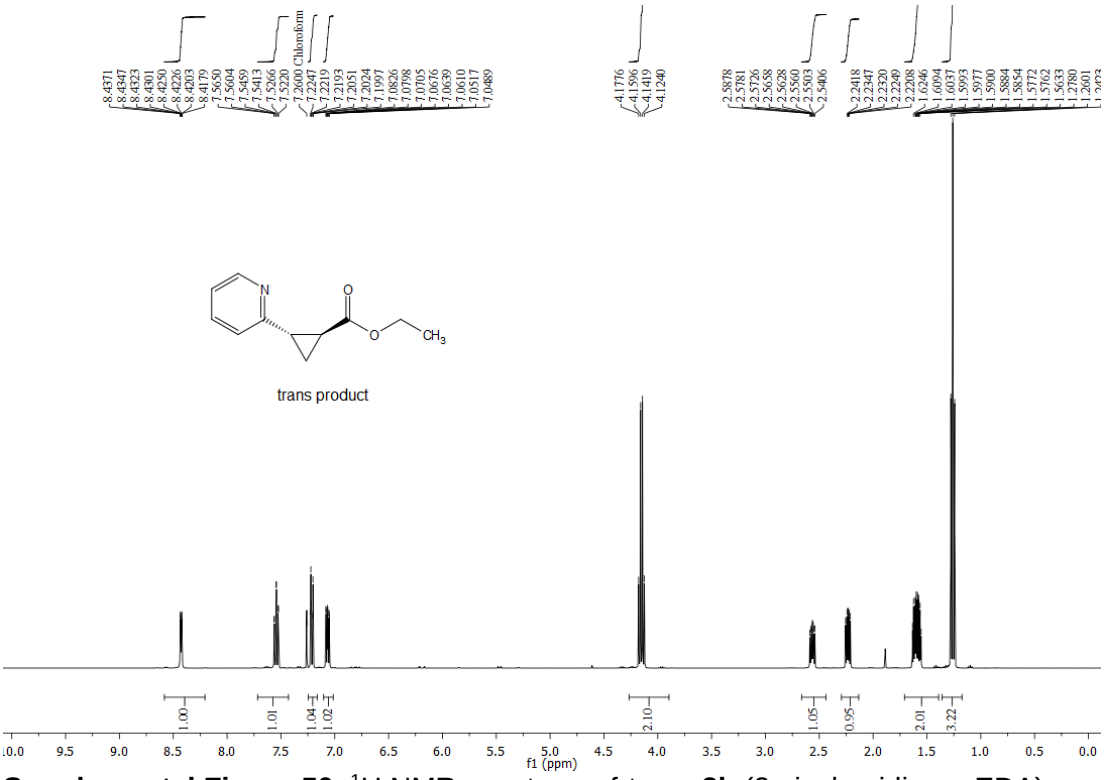
Supplemental Figure 47. ^{13}C NMR spectrum of *trans*-**3g** (1-penten-3-one + EDA).



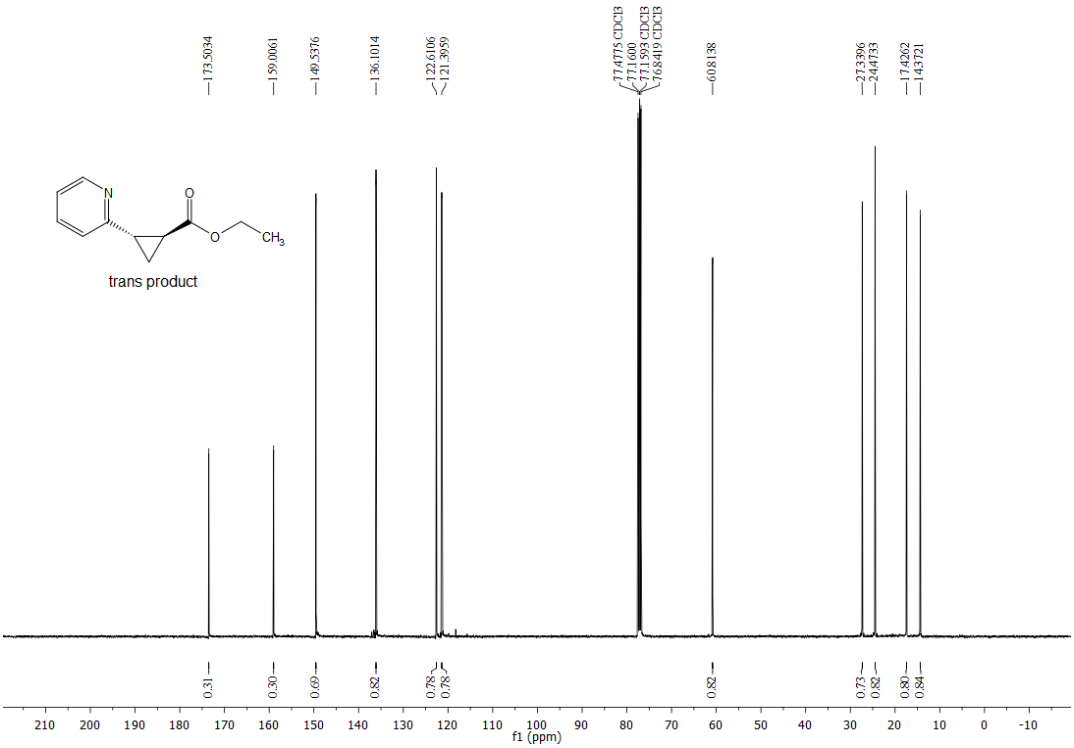
Supplemental Figure 48. ^{1}H NMR spectrum of *cis*-**3g** (1-penten-3-one + EDA).



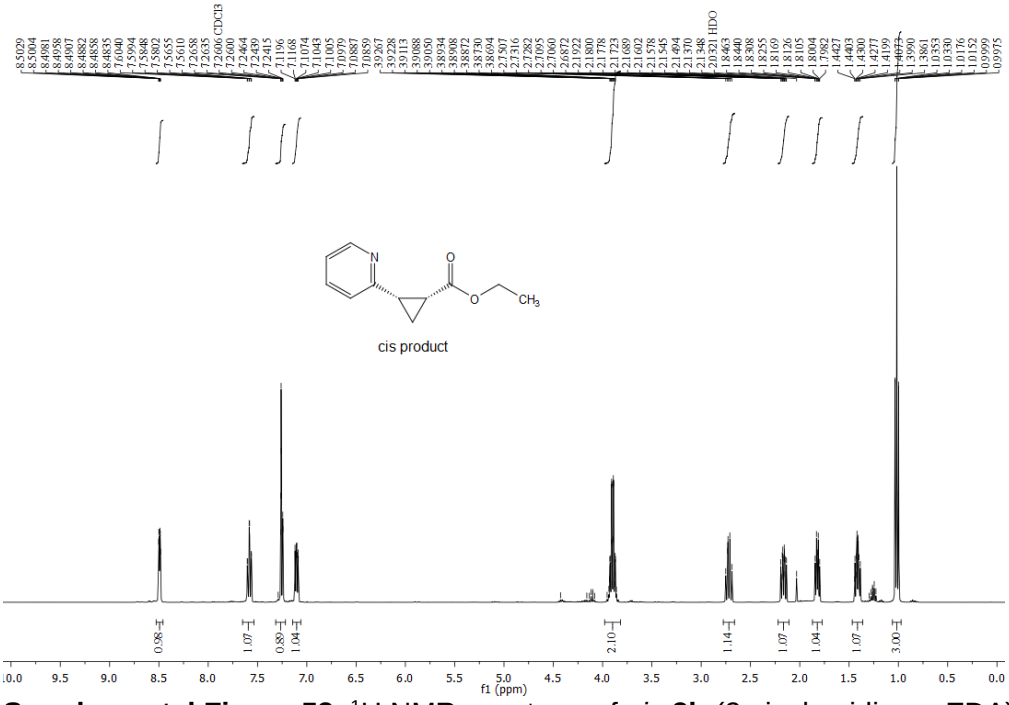
Supplemental Figure 49. ^{13}C NMR spectrum of *cis*-**3g** (1-penten-3-one + EDA).



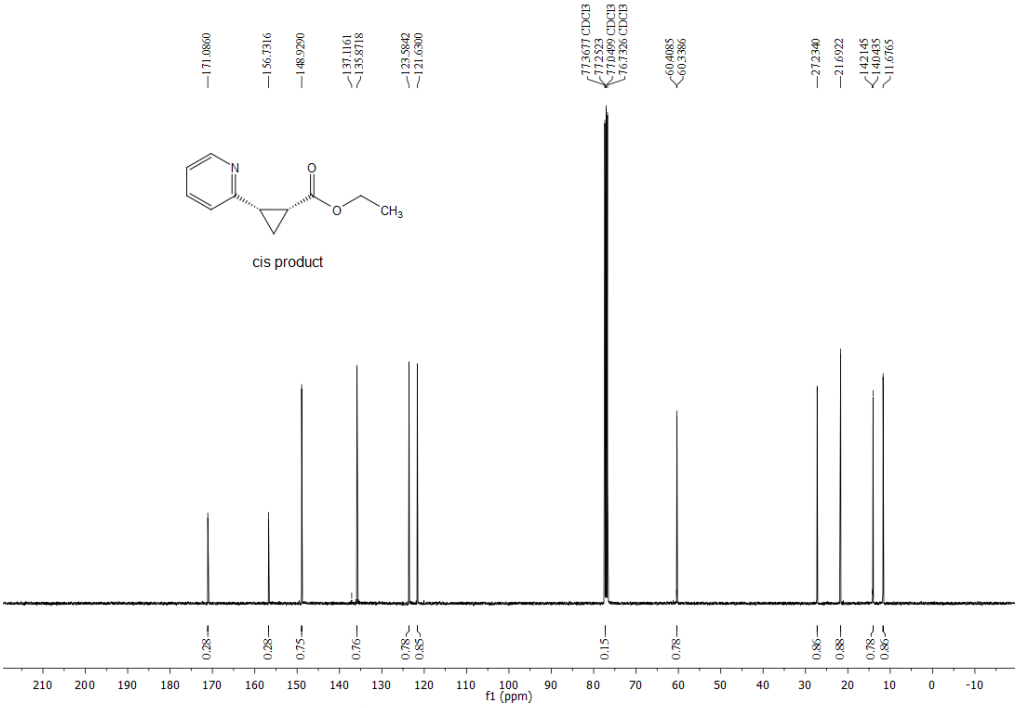
Supplemental Figure 50. ^{1}H NMR spectrum of *trans*-**3h** (2-vinylpyridine + EDA).



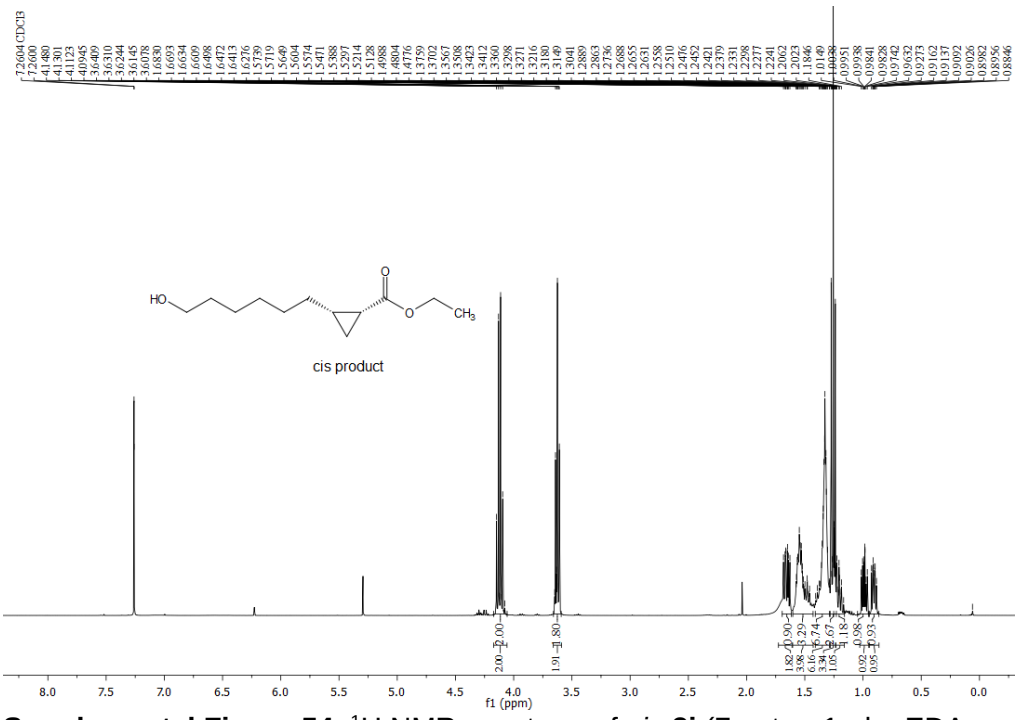
Supplemental Figure 51. ^{13}C NMR spectrum of *trans*-**3h** (2-vinylpyridine + EDA).



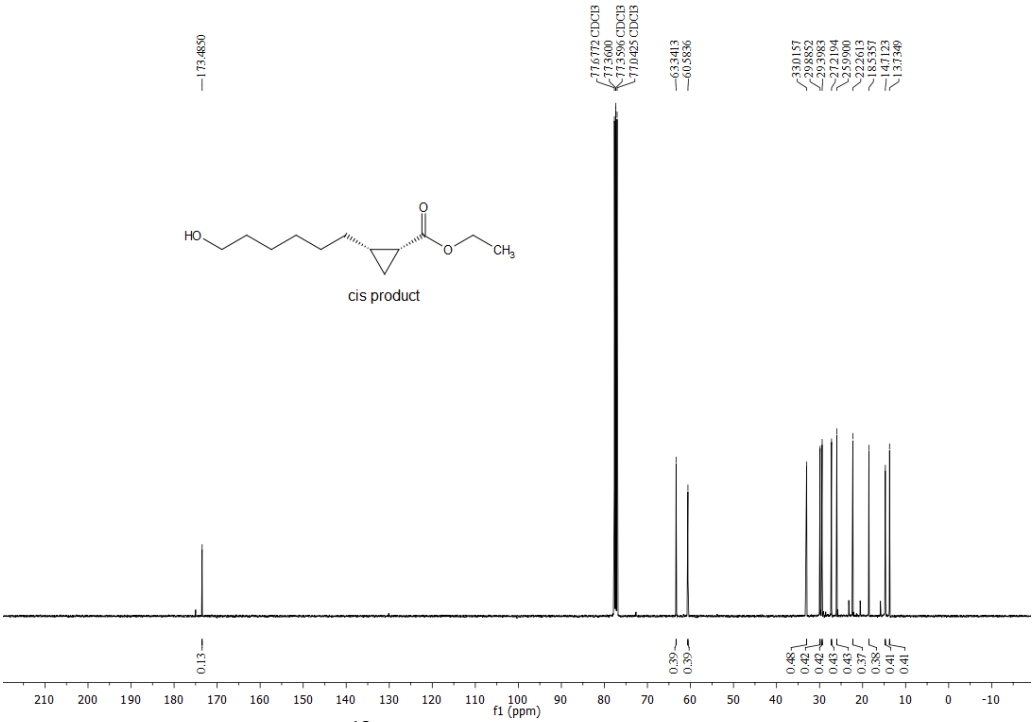
Supplemental Figure 52. ^{1}H NMR spectrum of *cis*-**3h** (2-vinylpyridine + EDA).



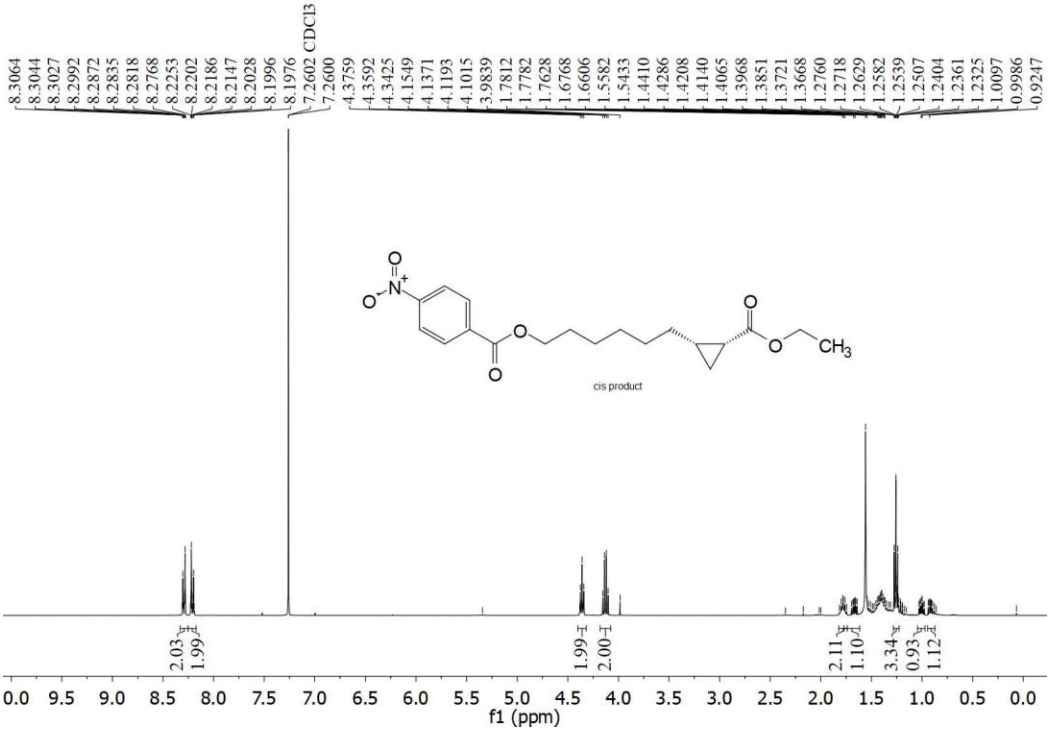
Supplemental Figure 53. ^{13}C NMR spectrum of *cis*-**3h** (2-vinylpyridine + EDA).



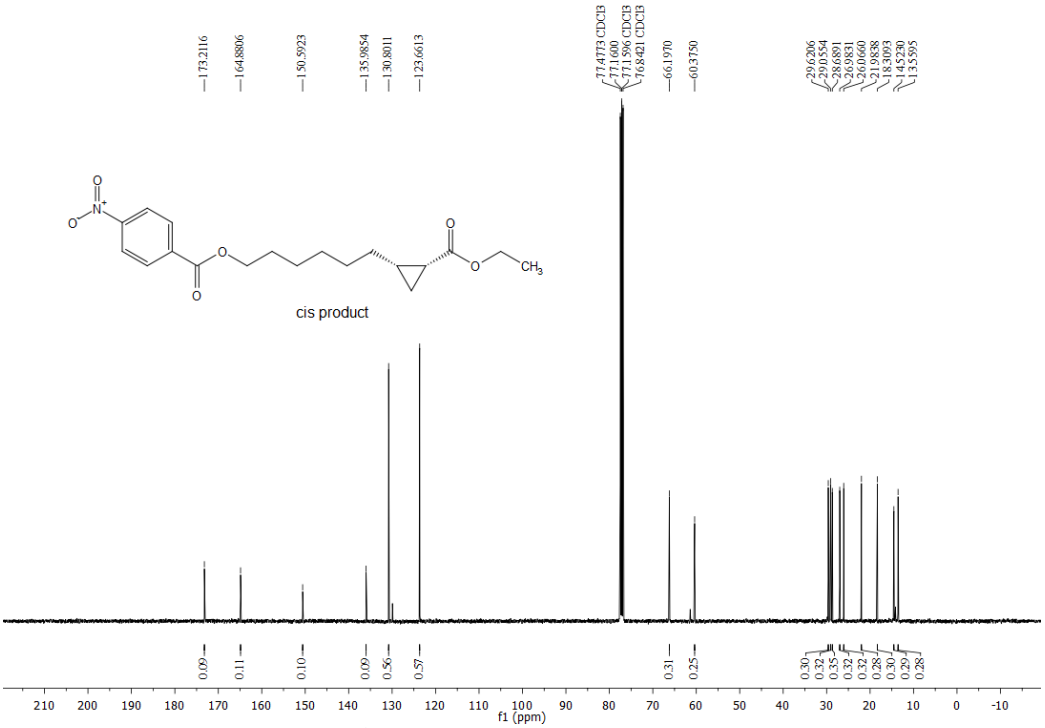
Supplemental Figure 54. ^{1}H NMR spectrum of *cis*-**3i** (7-octen-1-ol + EDA, enzymatic from ApePgb AGW).



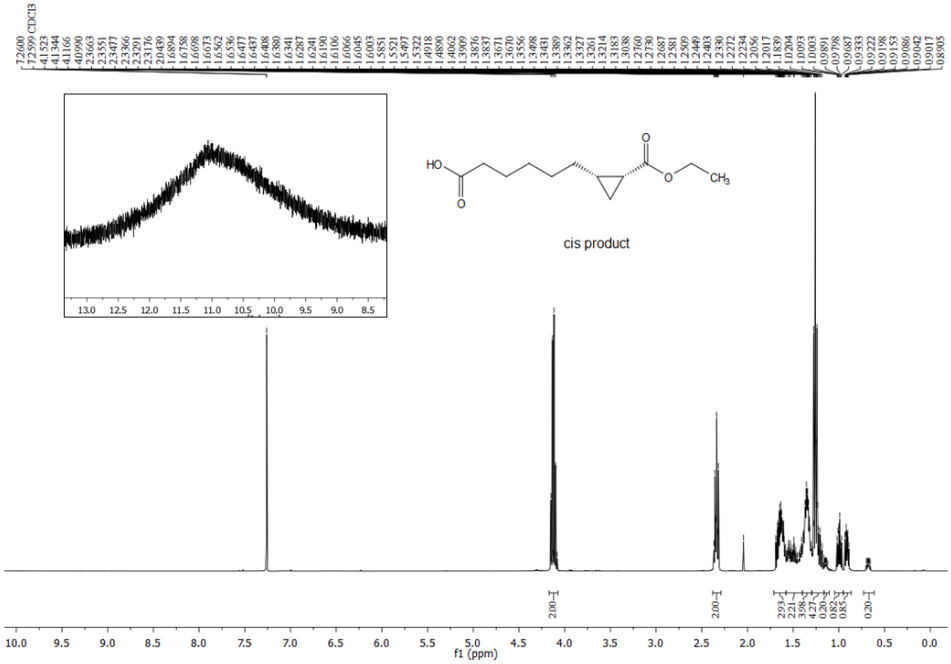
Supplemental Figure 55. ^{13}C NMR spectrum of *cis*-**3i** (7-octen-1-ol + EDA, enzymatic from ApePgb AGW).



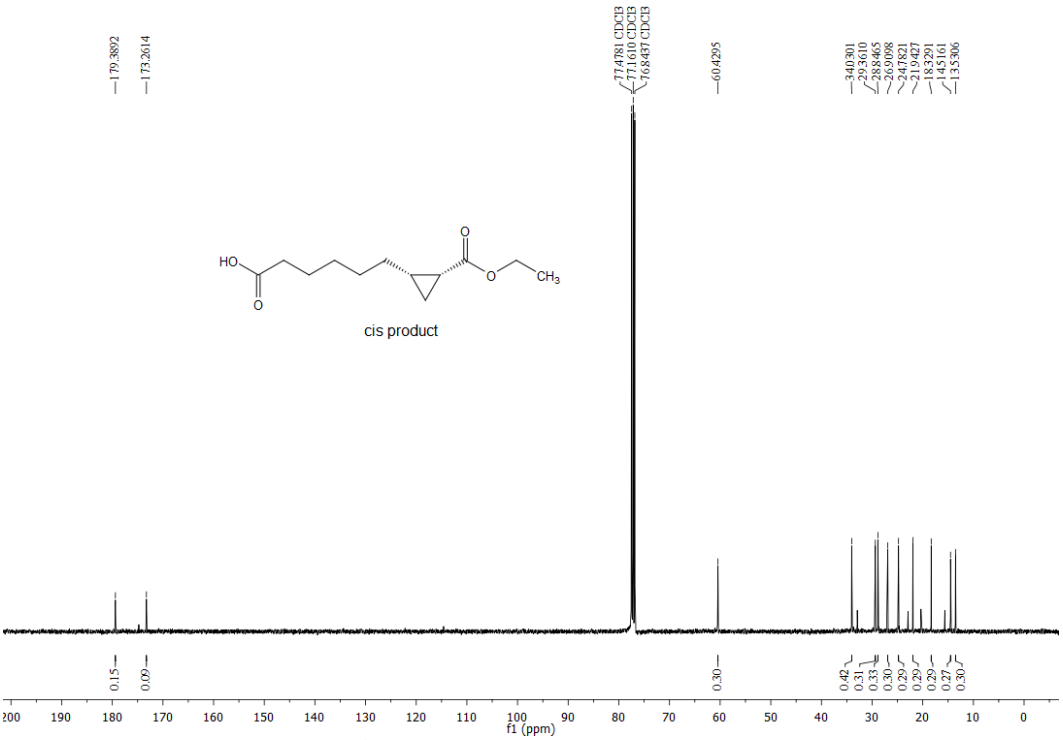
Supplemental Figure 56. ^{1}H NMR spectrum of *cis*-**3ia** (*para*-NO_2-benzoyl protected **3i**).



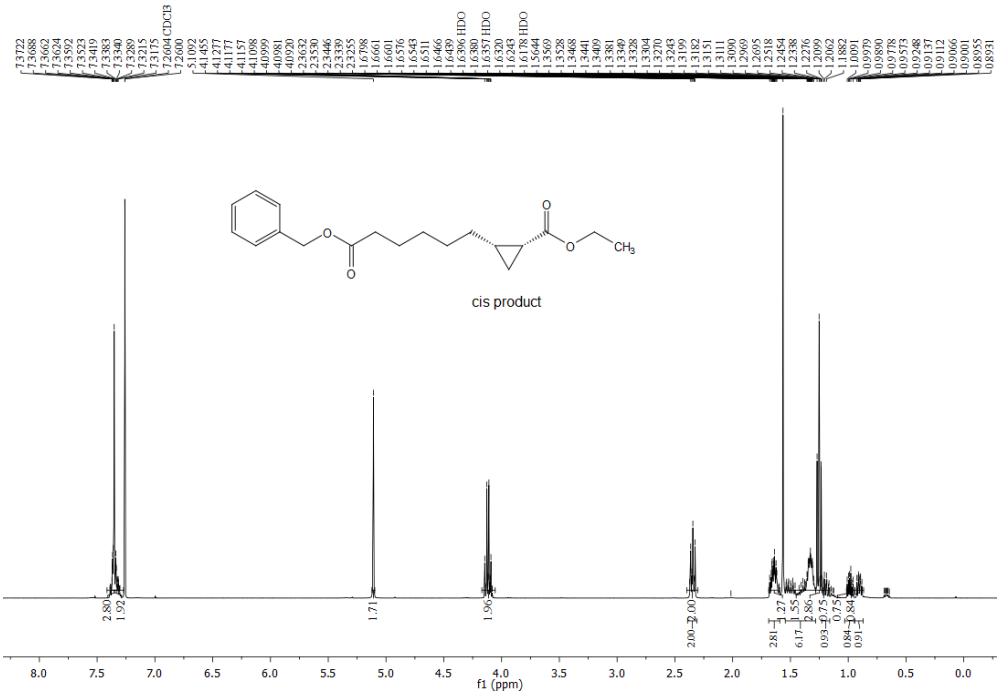
Supplemental Figure 57. ^{13}C NMR spectrum of *cis*-**3ia** (*para*-NO_2-benzoyl protected **3i**).



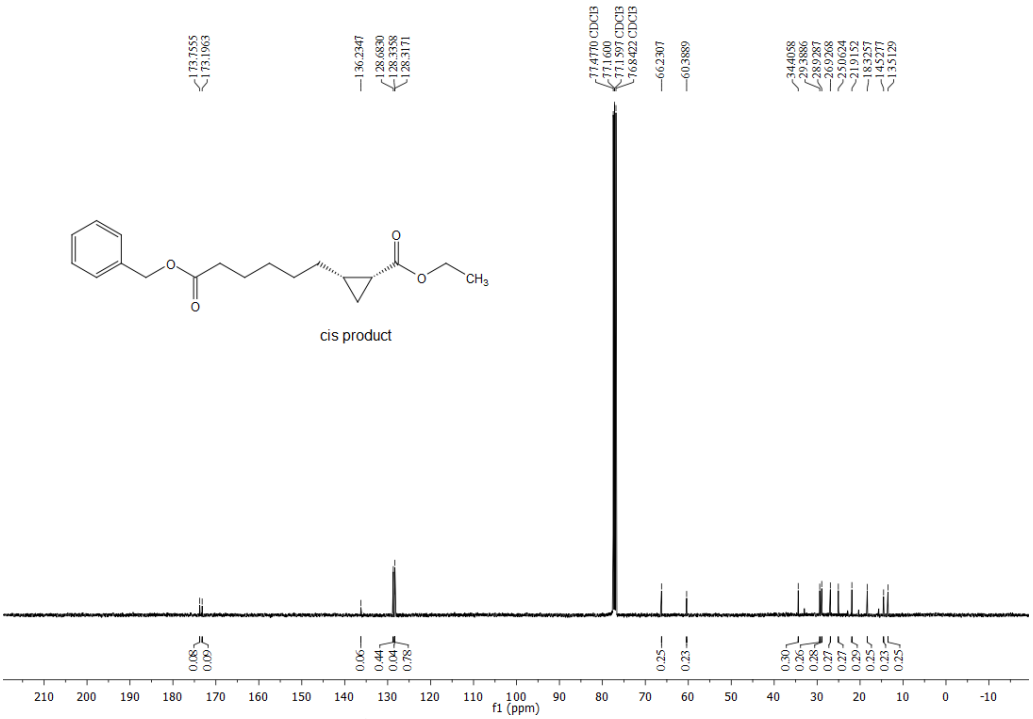
Supplemental Figure 58. ^{1}H NMR spectrum of **3j** (7-octen-1-oic acid + EDA, enzymatic from ApePgb AGW). The carboxylic acid proton at 11.0 ppm is broad and not visible unless the spectrum is magnified.



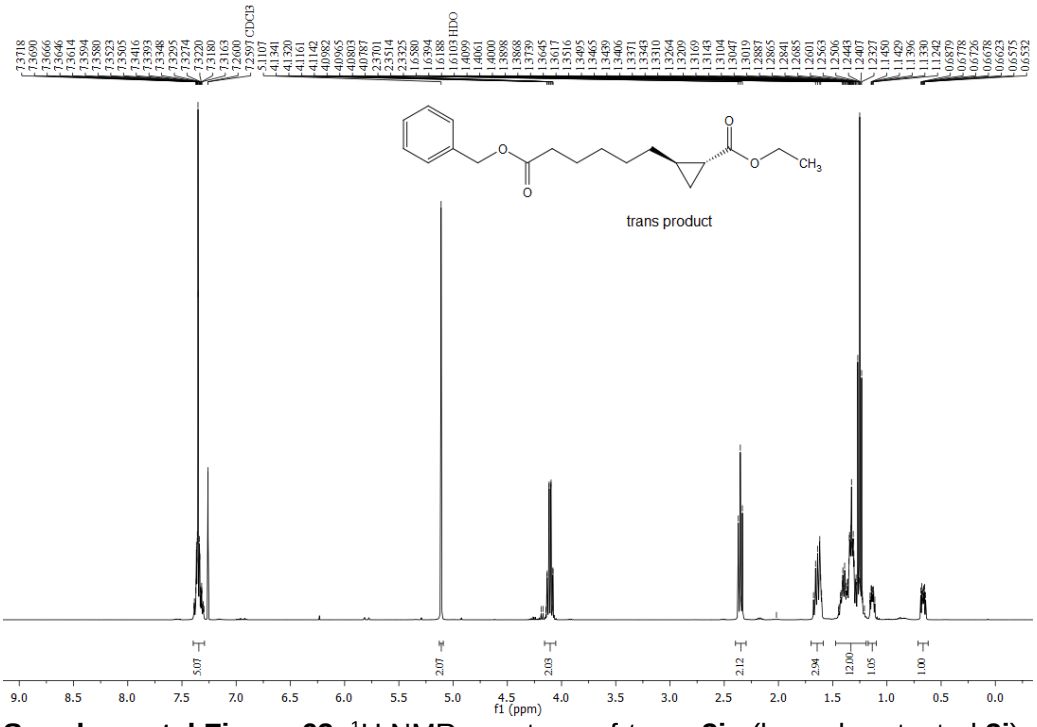
Supplemental Figure 59. ^{13}C NMR spectrum of **3j** (7-octen-1-oic acid + EDA, enzymatic from ApePgb AGW).



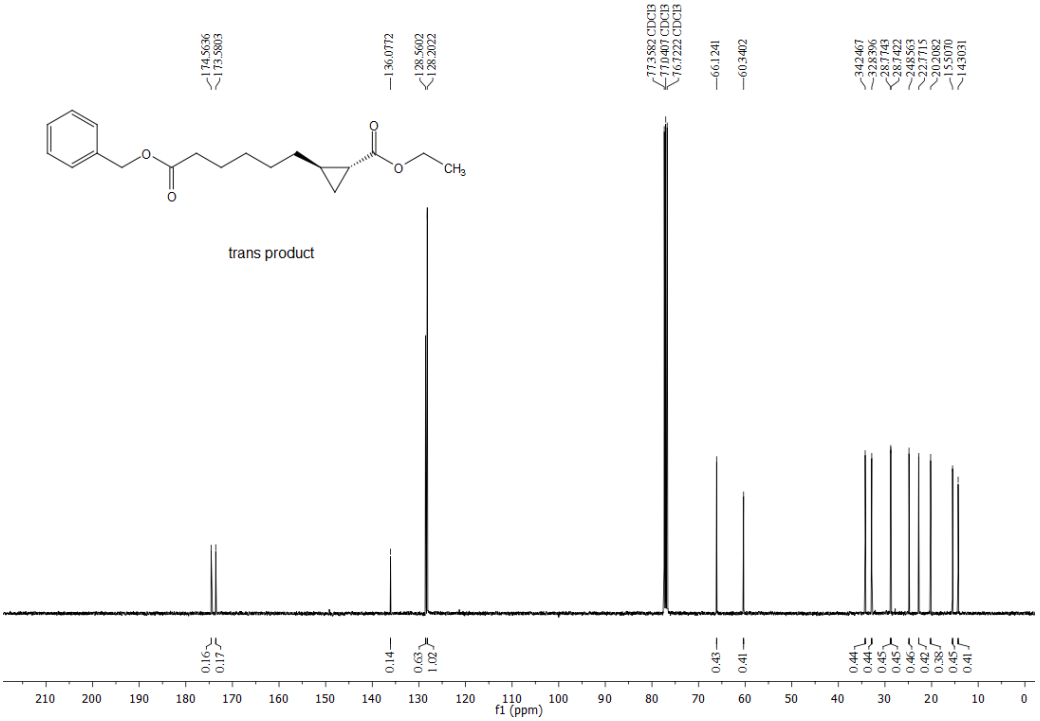
Supplemental Figure 60. ^{1}H NMR spectrum of *cis*-**3ja** (benzyl-protected **3j**).



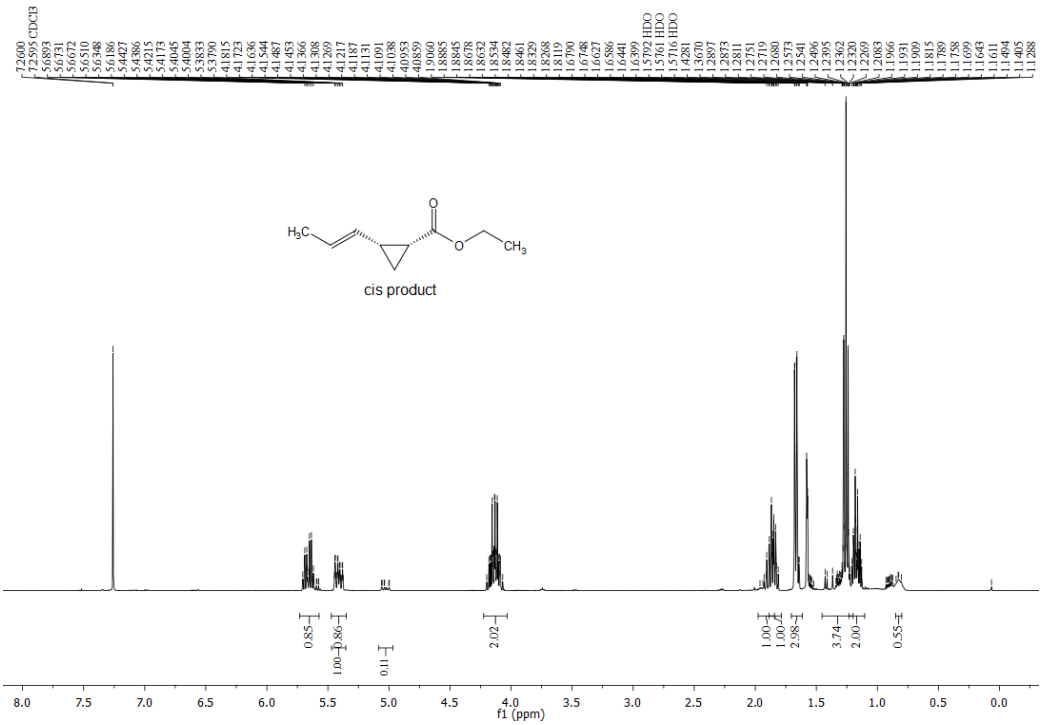
Supplemental Figure 61. ^{13}C NMR spectrum of *cis*-**3ja** (benzyl protected **3j**).



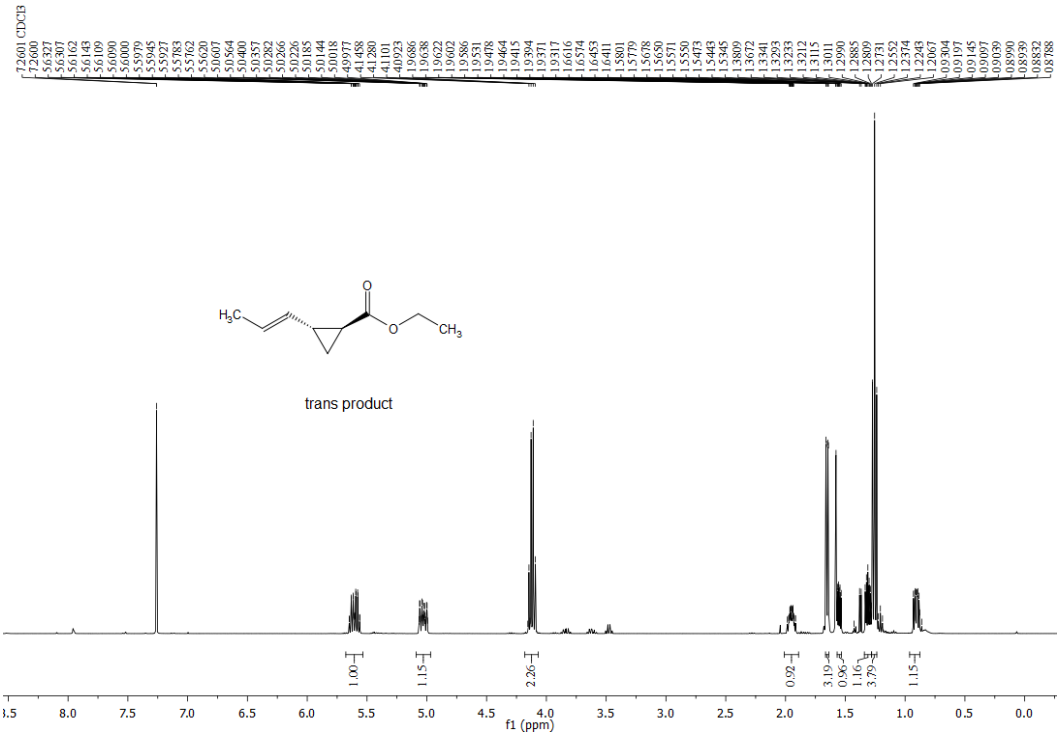
Supplemental Figure 62. ^{1}H NMR spectrum of *trans*-**3ja** (benzyl-protected **3j**).



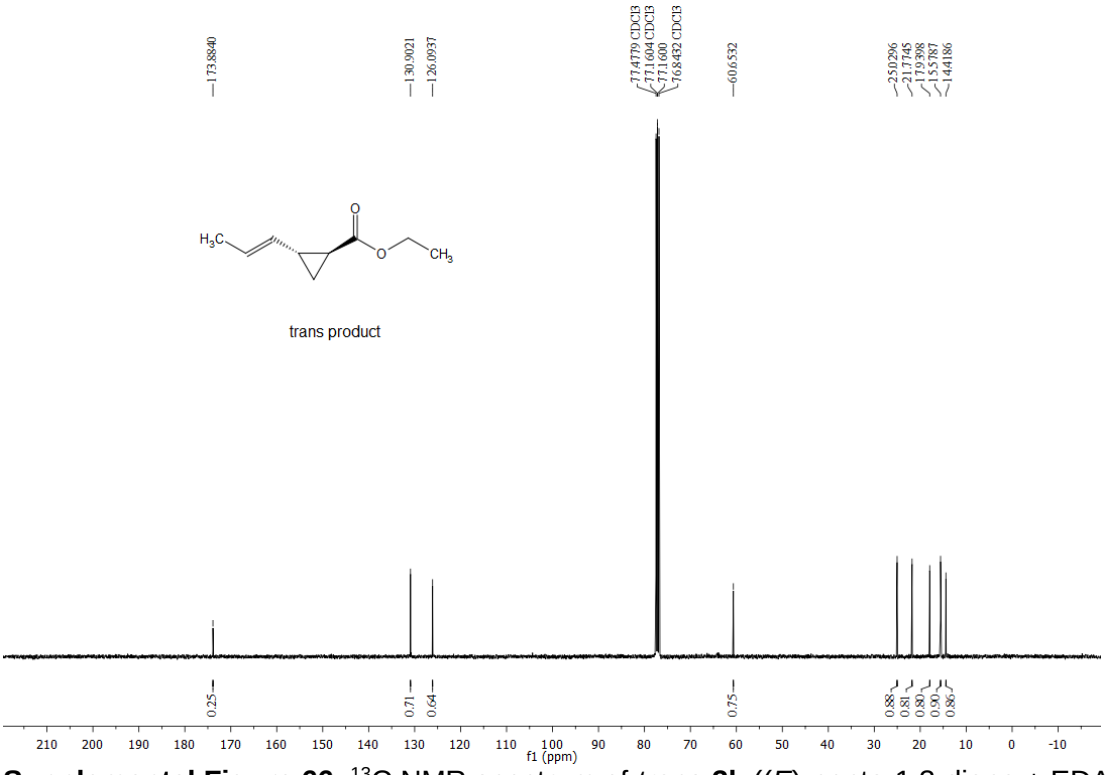
Supplemental Figure 63. ^{13}C NMR spectrum of *trans*-**3ja** (benzyl protected **3j**).



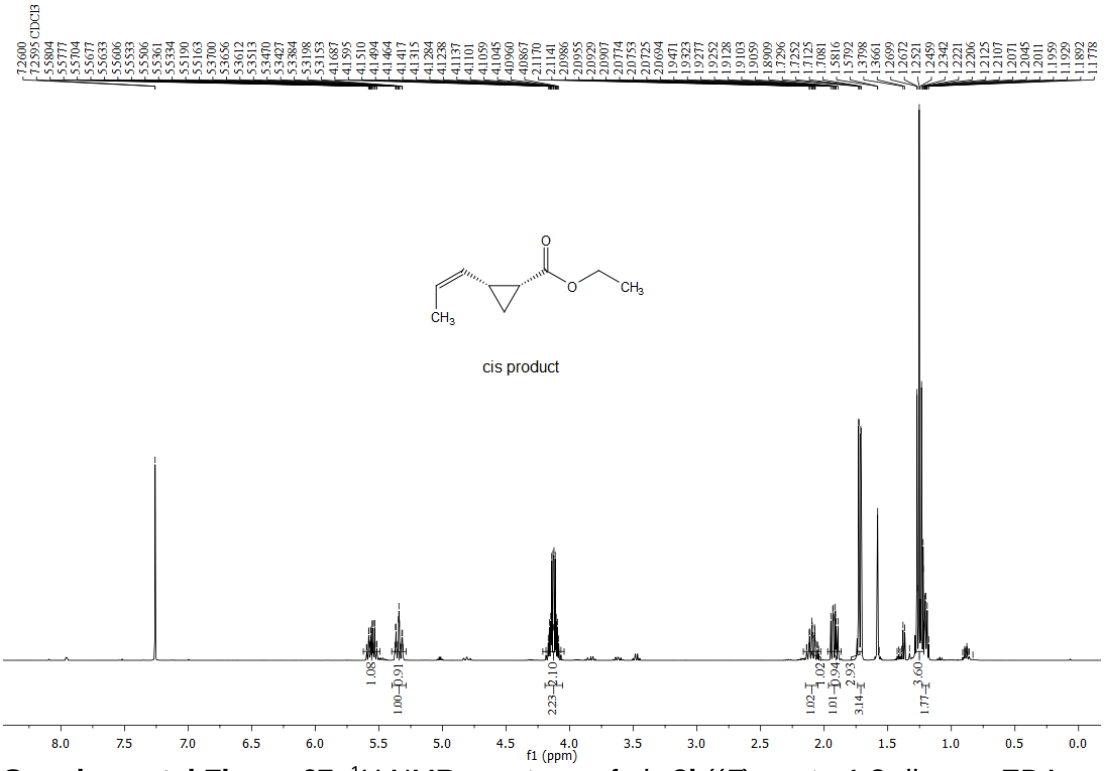
Supplemental Figure 64. ^{1}H NMR spectrum of *cis*-**3k** ((*E*)-penta-1,3-diene + EDA, enzymatic from ApePgb AGW).



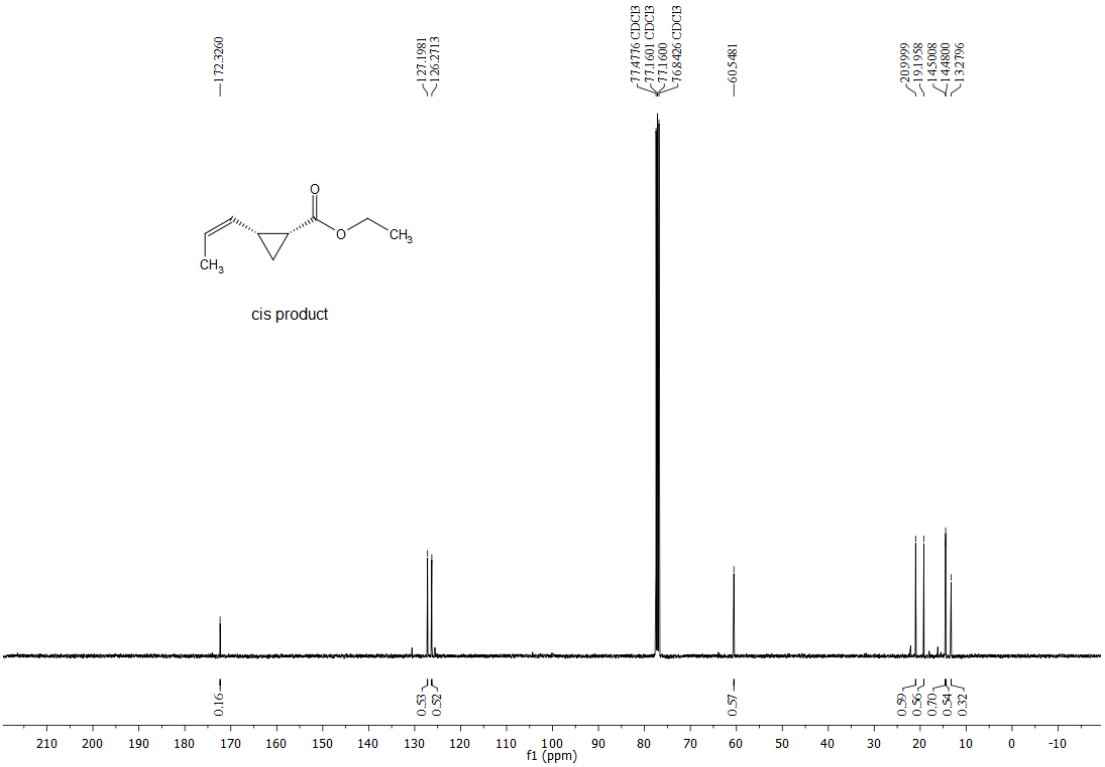
Supplemental Figure 65. ^{1}H NMR spectrum of *trans*-**3k** ((*E*)-penta-1,3-diene + EDA, enzymatic from RmaNOD Q52V).



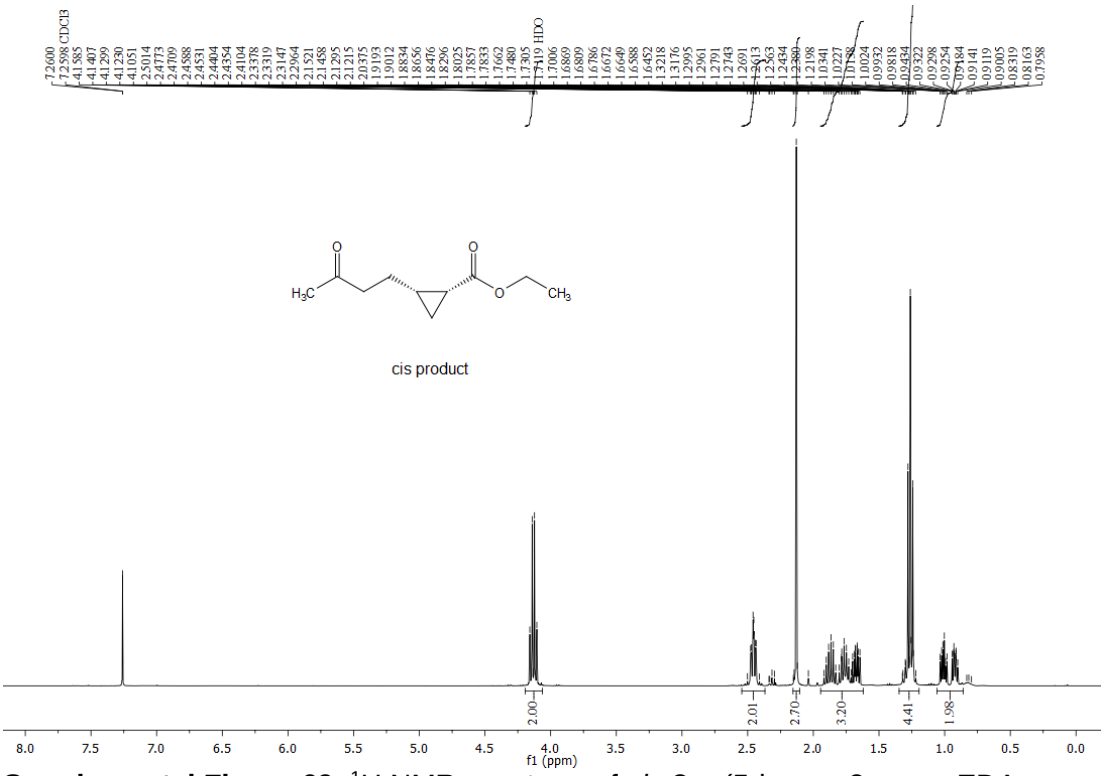
Supplemental Figure 66. ^{13}C NMR spectrum of *trans*-**3k** ((*E*)-penta-1,3-diene + EDA, enzymatic from RmaNOD Q52V).



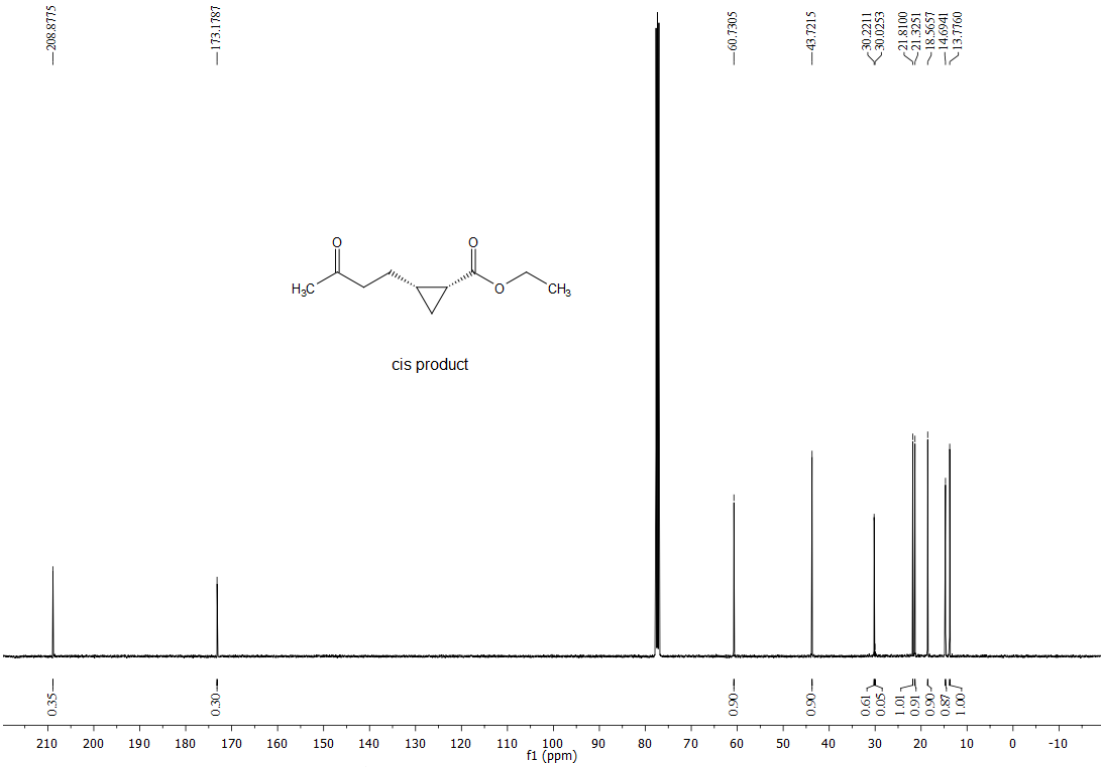
Supplemental Figure 67. ^{1}H NMR spectrum of *cis*-**3l** ((*Z*)-penta-1,3-diene + EDA, enzymatic from ApePgb AGW).



Supplemental Figure 68. ^{13}C NMR spectrum of *cis*-**3l** ((*Z*)-penta-1,3-diene + EDA, enzymatic from ApePgb AGW).



Supplemental Figure 69. ^{1}H NMR spectrum of *cis*-**3m** (5-hexen-2-one + EDA, enzymatic from ApePgb AGW).



Supplemental Figure 70. ^{13}C NMR spectrum of *cis*-**3m** (5-hexen-2-one + EDA, enzymatic from ApePgb AGW).

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