Supporting information

An Engineered Self-sufficient Biocatalyst Enables Scalable Production of Linear Alpha Olefins from Carboxylic Acids

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1 Materials and methods

1.1 Enzymes and chemicals

Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, EMD Millipore, and Tokyo Chemical Industry). Restriction enzymes for plasmid construction were purchased from New England Biolabs. Catalase from bovine liver was obtained from Sigma-Aldrich.

1.2 Molecular cloning

$OleT_{JE}$, P450BM3 and PTDH genes were ordered from Genewiz. The PCR fragments containing $OleT_{JE}$, BM3R, and PTDH (carried 18 mutations that led to enhanced activity towards $NADP^+$) genes [1-4] were double digested by *NdeI* and *XhoI* and subsequently ligated into *NdeI*/*XhoI* pre-treated pET28a to afford pET28a-$OleT_{JE}$, pET28a-BM3R and pET28a-PTDH, respectively. For molecular cloning of OleT-BM3R, the PCR fragment of $OleT_{JE}$ (residues 1−418) and P450BM3 reductase domain (residues 451−1048) were fused by In-Fusion technique, resulting in expression construct pET28a-OleT-BM3R. Successful plasmid constructions were verified by DNA sequencing. The plasmids were transformed into *E. coli* BL21 (Rosetta) and *E. coli* BL21 (DE3) for expressions of OleT-BM3R and PTDH, respectively.

1.3 Protein Expression and purification

After pre-culturing, the cells carrying pET28a-$OleT_{JE}$, pET28a-BM3R, and pET28a-OleT-BM3R were transferred into Terrific Broth (TB) medium that contained 50 μg mL^{-1} kanamycin and 34 μg mL^{-1} chloramphenicol. When OD_{600} reaching 0.6, the cultures were supplemented with 0.5 mM δ-aminolevulinic acid (only for $OleT_{JE}$- and OleT-BM3R-expressing cultures) and 0.1 mM IPTG. The cells were harvested by centrifugation (5000 rpm, 10 min) after 20 h incubation at 22 ℃ and 220 rpm shaking. The pellets were then resuspended in buffer A (0.1 M KPi buffer, pH 7.0, 20% glycerol, 0.3 M KCl) and disrupted by sonication. After centrifugation at 10000 rpm for 45 min at 4 ℃, the cell-free lysates were loaded onto a Ni-NTA column. The columns were then washed with buffer A containing 35mM imidazole. Finally the proteins were eluted by buffer B (0.1 M KPi buffer, pH 7.0, 20% glycerol, 0.3 M KCl, 250 mM imidazole). In order to remove imidazole, dialysis was performed against buffer A. The purified proteins were concentrated and stored at -80 ℃. Protein

concentrations for $OleT_{JE}$ and OleT-BM3R were determined by the P450-CO spectra method developed by Omura and Sato.[5] Protein concentration of BM3R was determined by flavin absorbance (ε=21 mM^{-1} cm^{-1}).[6]

After pre-culturing, the cells carrying pET28a-PTDH were transferred into LB medium that contained 50 μg mL^{-1} kanamycin. When OD_{600} reached 0.6, the culture was induced with 0.3 mM IPTG. The cells were harvested by centrifugation (5000 rpm, 10 min) after 10 h incubation at 25 ℃ and 220 rpm shaking. The pellet was then resuspended in buffer A and disrupted by sonication. After centrifugation at 10000 rpm for 45 min at 4 ℃, the cell-free lysate was loaded onto a Ni-NTA column. The column was then washed with buffer A containing 80mM imidazole. The PTDH protein was eluted by buffer B and dialyzed against buffer A supplemented with 1mM DTT. The protein was concentrated and stored at -80 ℃. Enzyme concentration was determined by measuring A_{280} (ε=26.5 mM^{-1} cm^{-1}).[1]

1.4 General procedure for the oxidative decarboxylation of carboxylic acids with OleT-BM3R

The carboxylic acid decarboxylation reaction contained buffer A, OleT-BM3R (3 μM), carboxylic acid (1 mM), bovine liver catalase (100 U mL^{-1}), PTDH (2 μM), NADPH (200 μM), sodium phosphite (10 mM), 5% EtOH (v/v) as co-solvent (except for the case of FA C6:0 where DMF was used as co-solvent). Although a 1.5 min^{-1} of the H_2O_2 formation rate via the peroxide shunt pathway in the monooxygenase catalytic cycle was observed during the reaction, the turnover number of OleT-BM3R for stearic acid in the absence of catalase was similar to that measured in the presence of catalase, indicating that there was no significant additive effect from NADPH and H_2O_2 on the activity of OleT-BM3R. However, addition of catalase could prevent generation of H_2O_2, which would demonstrate that the efficient transformation was achieved via the H_2O_2 independent reaction path (the NADPH/O_2 path) and help interpretation of the results. To increase the solubility of the substrate in the reaction solution, 1.5% Triton X-100 (v/v) was supplemented for FAs (fatty acids) C12:0-C20:0. The reactions were performed in septum-sealed glass vials at room temperature at 120 rpm for 12 h in a final volume of 1 mL.

For quantitative analyses of α-olefins C11-C19, reaction mixtures were extracted with ethyl acetate. The organic phases were dried over anhydrous $MgSO_4$ and then analyzed by gas chromatography (GC). α-olefins C5-C10 and terminally functionalized olefins were quantified directly by headspace-GC. For quantitative GC

analyses of the products of decarboxylation of FAs C4:0 and C5:0, propene and 1-butene produced in the reactions were transformed into less volatile 1,2-dibromopropane and 1,2-dibromobutane by reacting with bromine, respectively.

To examine the conversions of the carboxylic acids and the hydroxylation side reactions, reactions were quenched by addition of 100 μL 6 N HCl, and then ethyl acetate was added to extract the substrates and products. Extraction was performed at least three times for each sample, achieving >98% recovery of the products and the unreacted substrate. The organic phases were dried over anhydrous $MgSO_4$. Derivatization of hydroxy fatty acids and unreacted substrates to the corresponding methyl esters was achieved by mixing the dried organic phase with MeOH followed by supplementation of $TMSCHN_2$ as described elsewhere.[7] After incubated at 25℃ for 1 h, the composition of the mixture was first examined by GC-MS. Then the corresponding alkene product and fatty acid methyl ester (derivatized substrate) were quantified by GC with authentic standards. To minimize evaporation, liquid was transferred to septum-sealed vials with gastight Hamilton syringes during the whole process. Overall, the recoveries of alkene products and unreacted substrates were found to be greater than 95%.

All the yields were determined as the average of three experiments with a maximum Δyield = ± 5%.

1.5 Determination of total turnover numbers (TON), turnover frequency (TOF) and NADPH coupling efficiency

Determination of TON: The reaction mixture contained buffer A, OleT-BM3R (1 μM), FA C18:0 (10 mM), bovine liver catalase (100 U mL^{-1}), NADPH (20 mM) as well as 5% EtOH (v/v) and 1.5% Triton X-100 (v/v) as co-solvents. For the OleT-BM3R/PTDH reaction system, 20 mM NADPH was substituted by NADPH (200 μM), PTDH (2 μM), and sodium phosphite (50 mM). The reactions were performed at room temperature at 120 rpm for 12 h in a final volume of 1 mL. TON = molar concentration of α-olefin formed / molar concentration of OleT-BM3R.

Determination of TOF: The reaction mixture contained buffer A, OleT-BM3R or $OleT_{JE}$/BM3R (1 μM), FA C18:0 (10 mM), bovine liver catalase (100 U mL^{-1}), NADPH (2.5 mM), 5% EtOH (v/v) and 1.5% Triton X-100 (v/v). The reaction was performed at room temperature at 120 rpm in a final volume of 1 mL. After 1 h, the reaction was quenched as mentioned above and analyzed by GC. TOF= TON /

reaction time.

Determination of NADPH coupling efficiency: The reaction mixture contained buffer A, OleT-BM3R or $OleT_{JE}$/BM3R (3 μM), FA C18:0 (1 mM), bovine liver catalase (100 U mL^{-1}), NADPH (0.8 mM) as well as 5% EtOH (v/v) and 1.5% Triton X-100 (v/v) as co-solvents. The reaction was performed at room temperature at 120 rpm for 12 h in a final volume of 1 mL. NADPH coupling efficiency = molar concentration of α-olefin formed / molar concentration of NADPH supplied in reaction system.[7]

1.6 Enzymatic activity assay

Ferricyanide and cytochrome c reduction assays were performed as described by Klein and Fulco.[8] The concentration of H_2O_2 formed in the reaction was determined by the horseradish peroxidase/phenol/4-aminoantipyrine assay as described by Xu et al.[9]

1.7 ^{1}H-NMR analyses

^{1}H NMR spectra were recorded on a Bruker Avance 600 MHz instrument.

1.8 GC-FID and headspace GC analyses

For quantitative analyses of decarboxylation of FAs C12-C20, GC analyses were performed on an Agilent 7890A GC system with FID detector using a DB-5MS column (30 m × 0.25 mm, 0.25 μm film) with N_2 as carrier gas. Injection temperature: 250 ℃. Splitless mode. Detector temperature: 300 ℃. Oven temperature: 60 ℃ hold 1 min, 20 ℃ min^{-1} to 320 ℃, 14 min total.

Headspace gas chromatography was carried out on an Agilent 7890A GC equipped with 7697A Headspace Sampler and FID detector using a HP-5 column (30 m × 0.32 mm, 0.25 μm film) with N_2 as carrier gas. Injection temperature: 150 ℃. Split mode with a split ratio of 20. Detector temperature: 300 ℃. The oven temperature programs are as follows: i) for quantitative analyses of LAOs C5-C11, 40 ℃ hold 5 min, 20 ℃ min^{-1} to 170 ℃, 11.5 min total. ii) for quantitative analyses of terminal functionalized olefins, 40 ℃ hold 5 min, 20 ℃ min^{-1} to 170 ℃, 30 ℃ min^{-1} to 300 ℃ hold 3 min, 18.8 min total.[7]

Propene and 1-butene, produced by decarboxylation of FAs C4-C5, were converted into less volatile 1,2-dibromopropane and 1,2-dibromobutane and then quantified on an Agilent 7890A GC system with FID detector using DB-5MS column (30 m × 0.25 mm, 0.25 μm film) with N_2 as carrier gas. Injection temperature: 200 ℃. Split mode with a split ratio of 10. Detector temperature: 300 ℃. Oven temperature: 60 ℃ hold 1 min, 20 ℃ min^{-1} to 64 ℃ hold 5 min, 20 ℃ min^{-1} to 320 ℃, 19 min total.

For product distribution analyses, GC were performed on an agilent 7890A GC system with FID detector using DB-WAX column (30 m × 0.25 mm, 0.25 μm film) with N_2 as carrier gas. Injection temperature: 250 ℃. Splitless mode. Detector temperature: 300 ℃. Oven temperature: 40 ℃ hold 1 min, 10 ℃ min^{-1} to 250 ℃ hold 15 min, 40 min total.[10]

Gas chromatography-mass spectrometry (GC-MS) analyses were carried out on Thermo Scientific TRACE 1310-ISQ: LT system (70 eV) equipped with a TG-5MS column (30 m × 0.25 mm, 0.25 μm film). Injection temperature: 250 ℃. Splitless mode. Oven temperature: 40 ℃ hold 2 min, 10 ℃ min^{-1} to 320 ℃ hold 1 min, 31 min total.

Authentic standards of olefins and fatty acid methyl esters were treated in the same way and used as reference for compound identification and preparation of calibration curves. All authentic standards of products and samples were injected by the Agilent 7693A autosampler or 7697A Headspace Sampler during quantitative analyses.

1.9 Bioconversion using cell-free lysate

Cell culture that overexpressed the OleT-BM3R fusion protein was prepared as described in "**Expression and purification of OleT-BM3R and PTDH**". 400 mL cell culture was centrifuged and the pellet was resuspended in 40 mL buffer A. The cell-free lysate was then achieved by sonication and centrifugation. FA C18:0 (10 mM), bovine liver catalase (100 U mL^{-1}), sodium phosphite (50 mM), PTDH (2 μM), 5% EtOH (v/v), and 1.5% Triton X-100 (v/v) were added to the cell-free lysate to set up the reaction. The reaction and quantitative analysis of the product were performed as described in "**General procedure for the oxidative decarboxylation of carboxylic acids with OleT-BM3R**".

1.10 Stability of OleT-BM3R in reaction buffer

Purified OleT-BM3R was incubated at 37℃ in buffer A. At 0 h, 1 h, 3 h, 5 h, 12 h , and 24 h, 1 mL enzyme solution was taken out to test the remaining decarboxylation activity. The reaction mixture contained buffer A, OleT-BM3R (3 μM), FA C18:0 (10 mM), bovine liver catalase (100 U mL^{-1}), PTDH (2 μM), NADPH (200 μM), sodium phosphite (50 mM), as well as 5% EtOH (v/v) and 1.5% Triton X-100 (v/v) as co-solvents. The activities were deterimined as mentioned in "**General procedure for the oxidative decarboxylation of carboxylic acids with OleT-BM3R**".

1.11 Preparation of lyophilized OleT-BM3R

OleT-BM3R was purified as described in '**Expression and purification of OleT-BM3R and PTDH**', except that the glycerol was not included in all solutions. After dialysis and concentration, the protein sample was transferred to a Freeze Dryer for lyophilization. Protein powder obtained was stored at -20℃. .

1.12 General procedure for preparative scale reaction with stearic acid (FA C18:0)

Conversion of 1 g (3.52 mmol) stearic acid (FA C18:0) was performed under the same reaction condition as described in "**Stability of OleT-BM3R in reaction buffer**" on 400 ml reaction scale. After 20 h reaction time at room temperature, the product was extracted with ethyl acetate. The organic phase was dried over anhydrous $MgSO_4$ and then concentrated by vacuum. The product was purified by flash chromatography on silica gel using hexane as eluent. The product was isolated as a colorless oil. Yield: 60% (503.6 mg). The NMR spectrum is showed in **Figure S25**.

1-Heptadecene (2b)[11]**:** ^{1}H NMR (600 MHz, Chloroform-d) δ 5.81 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H), 4.99 (d, J = 17.1 Hz, 1H), 4.92 (d, J = 10.2 Hz, 1H), 2.04 (q, J = 7.1 Hz, 2H), 1.37 (q, J = 6.8 Hz, 2H), 1.31–1.22 (m, 24H), 0.88 (t, J = 6.9 Hz, 3H).

1.13 15-Oxopentadecanoic acid synthesis and quantification for its decarboxylative product

15-oxopentadecanoic acid was prepared with Dess-Martin reagent.[12,13] 0.01 M 15-Hydroxypentadecanoic acid and 0.02 M Dess-Martin periodinane were added in 150 ml CH_2Cl_2 and the mixture was stirred at room temperature for 4 h. The reaction

mixture was washed by 10% $Na_2S_2O_3$ twice and the saturated NaCl solution once. The product was purified by column chromatography (silica gel, 2:98 CH_3OH:CH_2Cl_2). The title compound was obtained as a white solid. Yield: 98% (147 mg). The NMR spectrum is showed in **Figure S26**.

15-Oxopentadecanoic acid (3i)[14]**:** ^{1}H NMR (600 MHz, Chloroform-d) δ 9.79 (s, 1H), 2.44 (td, J = 7.4, 1.9 Hz, 2H), 2.38 (t, J = 7.5 Hz, 2H), 1.65 (h, J = 7.2 Hz, 4H), 1.36–1.28 (m, 18H).

The conversion of 76.9 mg (0.3 mmol) 15-oxopentadecanoic acid was performed under the same reaction condition as described in "**Stability of OleT-BM3R in reaction buffer**" on 30 ml reaction scale. After 20 h reaction time at room temperature at 120 rpm, the product was extracted by CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$. After concentrated by vacuum, the organic phase was purified by column chromatography (silica gel, 10:90 ethyl acetate:hexane) based on method of *Hon et al*.[15] Yield: 11% (6.9 mg). The NMR spectrum was showed in **Figure S27**.

13-Tetradecenal (4i)[15]**:** ^{1}H NMR (600 MHz, Chloroform-d) δ 9.77 (t, J = 1.9 Hz, 1H), 5.82 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 4.99 (dq, J = 17.1, 1.7 Hz, 1H), 4.93 (ddt, J = 10.2, 2.3, 1.2 Hz, 1H), 2.42 (td, J = 7.4, 1.9 Hz, 2H), 2.04 (q, J = 6.9 Hz, 2H), 1.63 (p, J = 7.4 Hz, 2H), 1.41 – 1.27 (m, 16H).

2 Analytic data

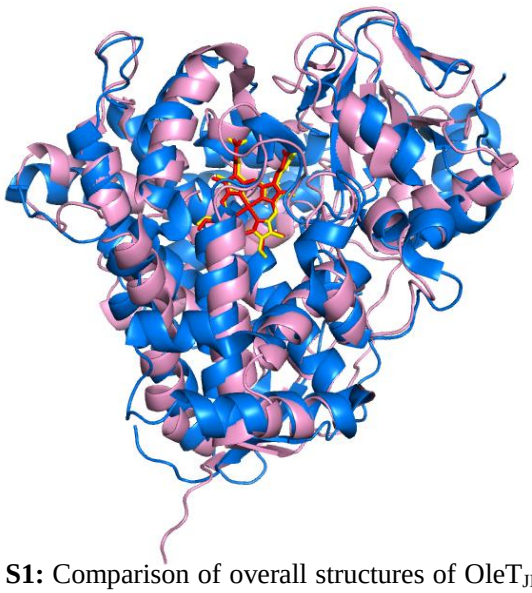


Figure S1: Comparison of overall structures of $OleT_{JE}$ (in pink; PDB code 4L54)[16] and the heme domain of P450BM3 (in blue; PDB code 2IJ2)[17]. The heme groups are represented in sticks, colored in red for $OleT_{JE}$ and in yellow for P450BM3. The RMSD between these two structures is 3.6 Å.

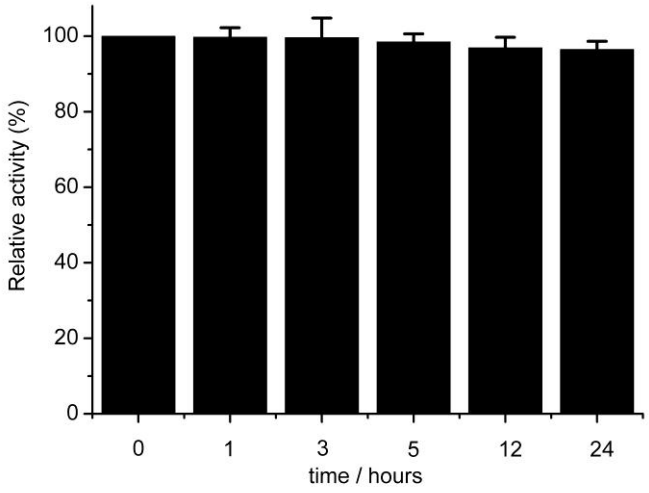


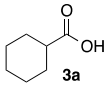
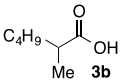
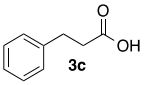
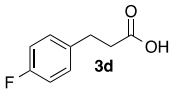
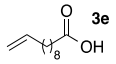
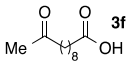
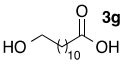
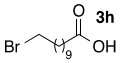
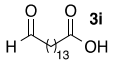
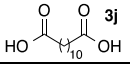
Figure S2： Stability of the fusion protein OleT-BM3R. The decarboxylation activity of the enzyme was tested after incubation at 37 ℃ for indicated time. Reaction condition: buffer A, OleT-BM3R (3 μM), FA C18:0 (10 mM), catalase (100 U ml^{-1}), PTDH (2 μM), NADPH (200 μM), sodium phosphite (50 mM), as well as 5% EtOH (v/v) and 1.5% Triton X-100 (v/v) as co-solvents, 12 h, room temperature. Reactions were done three times and the product 1-heptadecene was detected and quantified by GC-FID.

Table S1. Comparison of redox systems for the decarboxylation of FAs with OleT.

Entry	Catalyst [μM]	FAs [mM]	Oxidant/ Electron source	Auxiliary proteins	TON	TOF (h^{-1})	Cofactor coupling [%]	Reaction time	Ref.
1	$OleT_{JE}$ (0.32)	C18:0 (1)	H_2O_2 batch	Not required	72	4.5	2.3	16 h	[7]
2	$OleT_{JE}$ (0.32)	C18:0 (1)	H_2O_2 fed-batch	Not required	99	n.d.	4.6	27×15 min	[7]
3	$OleT_{JE}$ (200)	C18:0 (0.5)	EDTA/FMN/ *hv*	Not required	2.5	1.25	n.d.	2 h	[18]
4	$OleT_{JE}$ (1)	C18:0 (1)	NADPH	CamAB	118	47	11	2.5 h	[7]
5	$OleT_{JE}$-RhFRED (0.2)	C18:0 (0.2)	NADPH	Not required	~100	~50	~4	2 h	[10]
6[a]	OleT-BM3R (1)	C18:0 (1)	NADPH	Not required	617	370	62	100 min	this study
7	$OleT_{JE}$ (1)	C16:0 (1)	NADPH	CamAB	33	13	3	2.5 h	[7]
8	$OleT_{JE}$-RhFRED (0.2)	C16:0 (0.2)	NADPH	Not required	~180	~90	~7.2	2 h	[10]
9[a]	OleT-BM3R (1)	C16:0 (1)	NADPH	Not required	326	196	33	100 min	this study
10	$OleT_{JE}$ (1)	C14:0 (0.2)	NADPH	*E. coli* Flavodoxin and flavodoxin reductase	124	62	24.8	2 h	[10]
11	$OleT_{JE}$-HRV3C-AldO (5)	C14:0 (0.5)	H_2O_2 recycling system	Not required	37	110	36.6	20 min	[19]
12	$OleT_{JE}$-RhFRED (0.2)	C14:0 (0.2)	NADPH	Not required	~400	~200	~16	2 h	[10]
13	$OleT_{JE}$ (6)	C4:0 (10)	FDH-based NADH recycling system	CamAB	82	3.4	n.d.	24 h	[7]
14[b]	OleT-BM3R (5)	C4:0 (10)	PTDH-based NADPH recycling system	Not required	362	18	n.d.	20 h	this study

[a] Entries 6 and 9: bovine liver catalase (100 U ml^{-1}), 1 mM NADPH, 5% EtOH (v/v), 1.5% Triton X-100 (v/v), buffer A, RT. [b] Entry 14: bovine liver catalase (100 U ml^{-1}), PTDH (2 μM), NADPH (200 μM), sodium phosphite (50 mM), buffer A, RT.

Table S2. Product distribution of carboxylic acid transformation by OleT-BM3R.[a]

Entry	Substrate	1-Alkene[%]	α-OH+β-OH side products [%][b]
1	$CH_3(CH_2)_{18}CO_2H$ (**1a**)	91	9
2	$CH_3(CH_2)_{16}CO_2H$ (**1b**)	85	15
3	$CH_3(CH_2)_{14}CO_2H$ (**1c**)	78	22
4	$CH_3(CH_2)_{12}CO_2H$ (**1d**)	73	27
5	$CH_3(CH_2)_{10}CO_2H$ (**1e**)	54	46
6	$CH_3(CH_2)_9CO_2H$ (**1f**)	64	36
7	$CH_3(CH_2)_8CO_2H$ (**1g**)	>99[c]	n.d.
8	$CH_3(CH_2)_7CO_2H$ (**1h**)	>99[c]	n.d.
9	$CH_3(CH_2)_6CO_2H$ (**1i**)	>99[c]	n.d.
10	$CH_3(CH_2)_5CO_2H$ (**1j**)	>99[c]	n.d.
11	$CH_3(CH_2)_4CO_2H$ (**1k**)	>99[c]	n.d.
12	$CH_3(CH_2)_3CO_2H$ (**1l**)	>99[c]	n.d.
13	$CH_3(CH_2)_2CO_2H$ (**1m**)	>99[c]	n.d.
14	 **3a**	>99[c]	n.d.
15	 **3b**	>99[c]	n.d.
16	 **3c**	95	5
17	 **3d**	92	8
18	 **3e**	82	18
19	 **3f**	75	25
20	 **3g**	75	25
21	 **3h**	>99	0
22	 **3i**	>99[d]	n.d.
23	 **3j**	43[e]	57[f]

[a] Reaction conditions: purified OleT-BM3R (3 μM), substrate (1mM), catalase (100 U mL^{-1}), NADPH (200 μM), PTDH (2 μM), sodium phosphite (0.01 M), 1 mL scale, rt, 12 h. [b] Determined from subtraction of 1-alkene from the total substrate converted. [c] Volatile fraction analyzed. [d] Isolated as the only identifiable product by column chromatography. [e] Only terminal diene was formed; no ω-alkenoic acid intermediate was found. [f] α-/β-hydroxy diacids and α-/β-hydroxy ω-enoic acids were found. n.d.: not determined.

3 GC / Headspace-GC results and calibration curves

3.1 Conversion of eicosanoic acid (FA C20:0) 1a

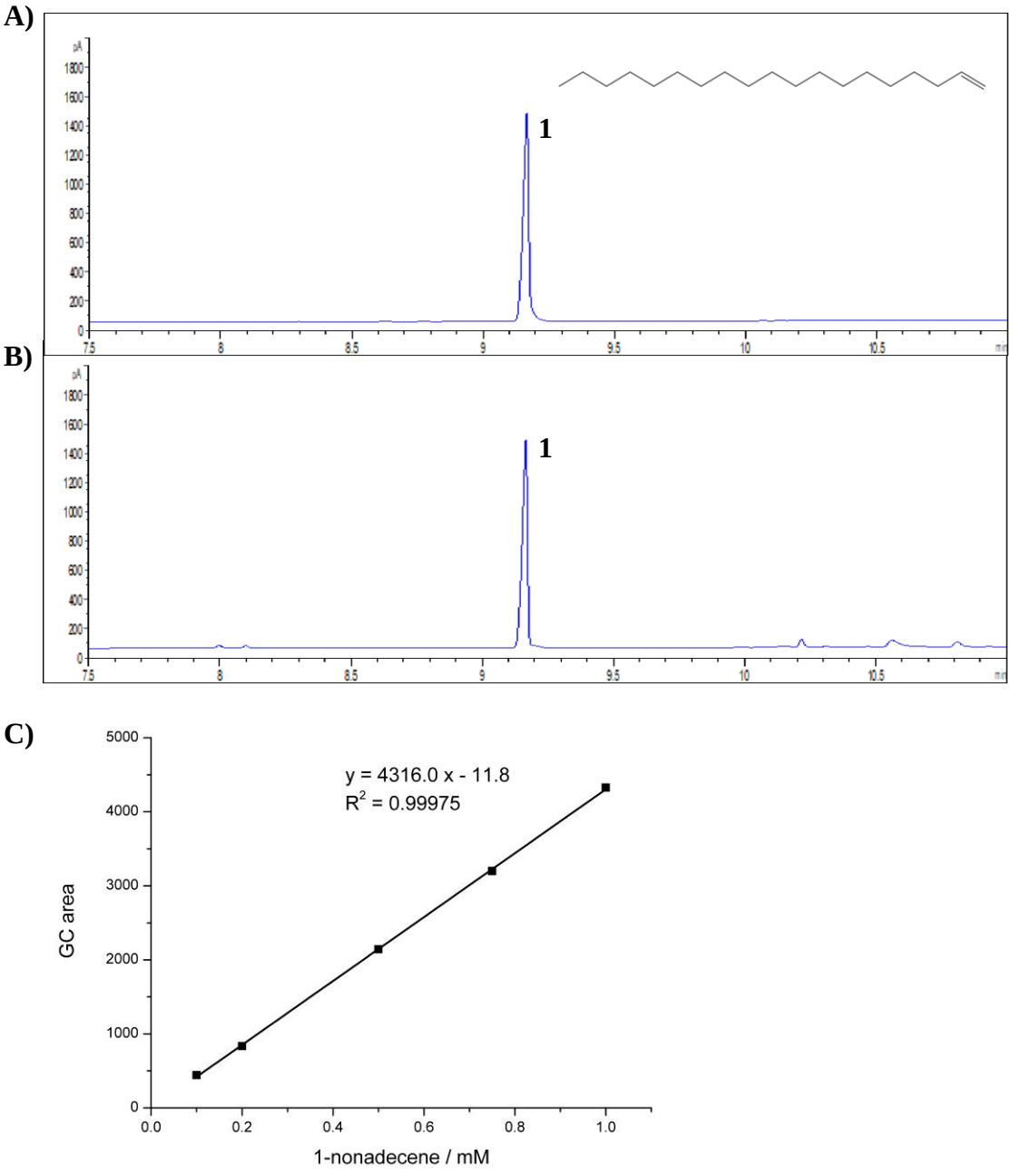


Figure S3: GC results of decarboxylation of FA C20:0 by OleT-BM3R with NADPH recycling. A) 1-nonadecene standard; B) Conversion of eicosanoic acid (FA C20:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-nonadecene via GC-FID analysis. Compounds were injected via automatic injection into a GC-FID chromatograph. **1**: 1-nonadecene.

3.2 Conversion of stearic acid (FA C18:0) 1b

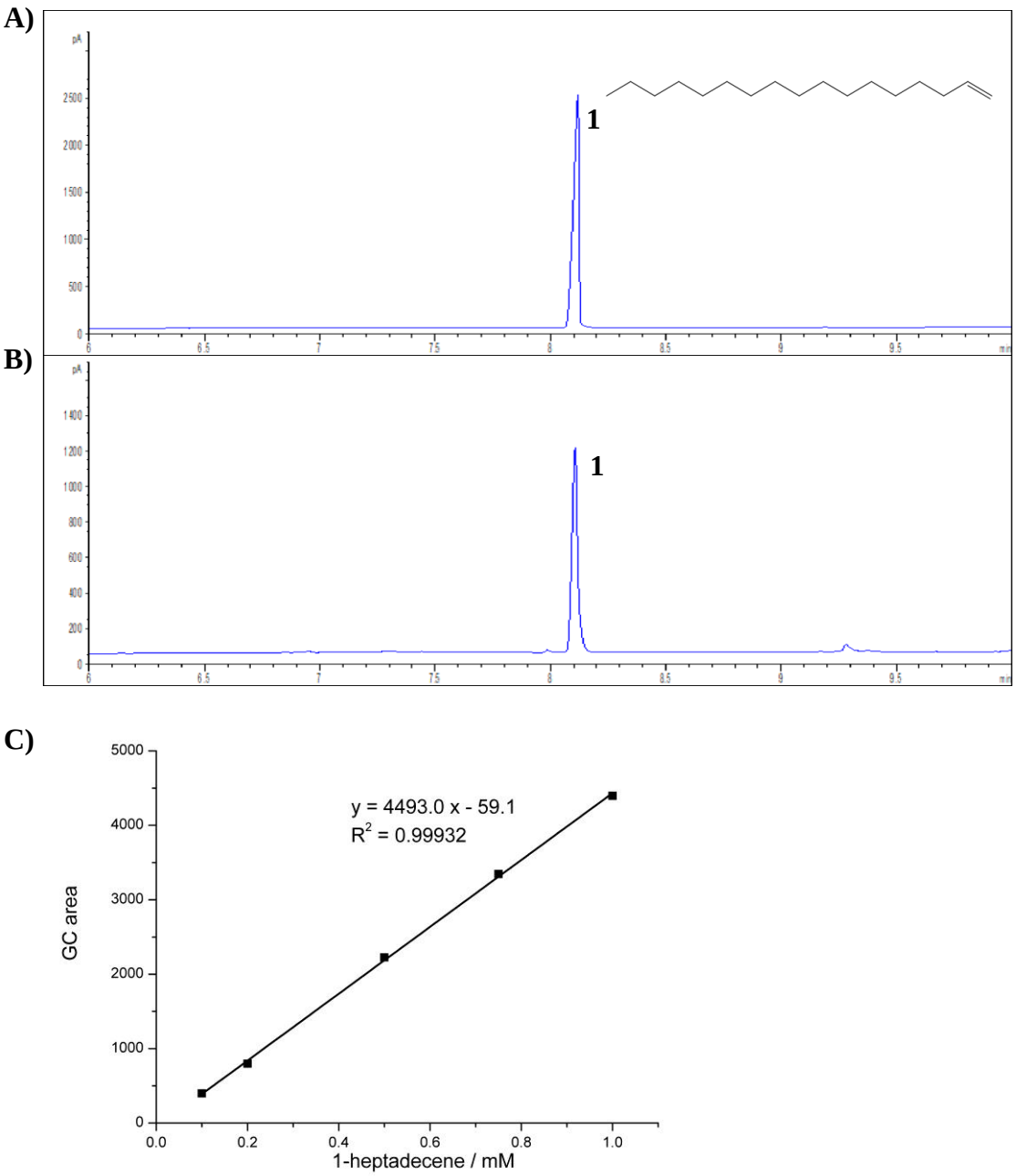


Figure S4: GC results of decarboxylation of FA C18:0 by OleT-BM3R with NADPH recycling. A) 1-heptadecene standard; B) Conversion of stearic acid (FA C18:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-heptadecene via GC-FID analysis. Compounds were injected via automatic injection into a GC-FID chromatograph. **1**: 1-heptadecene.

3.3 Conversion of hexadecanoic acid (FA C16:0) 1c

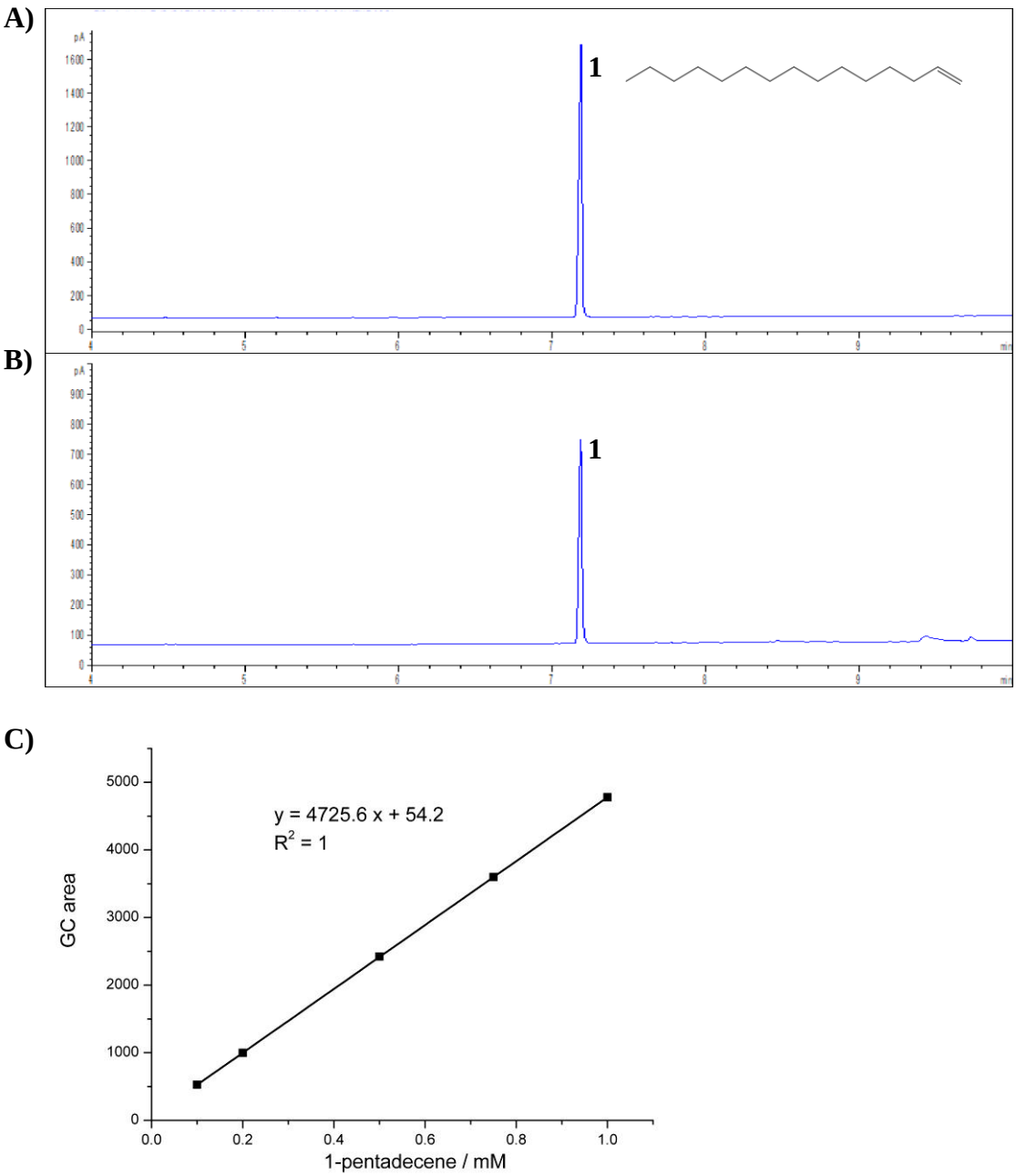


Figure S5: GC results of decarboxylation of FA C16:0 by OleT-BM3R with NADPH recycling. A) 1-pentadecene standard; B) Conversion of hexadecanoic acid (FA C16:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-pentadecene via GC-FID analysis. Compounds were injected via automatic injection into a GC-FID chromatograph. **1**: 1-pentadecene.

3.4 Conversion of tetradecanoic acid (FA C14:0) 1d

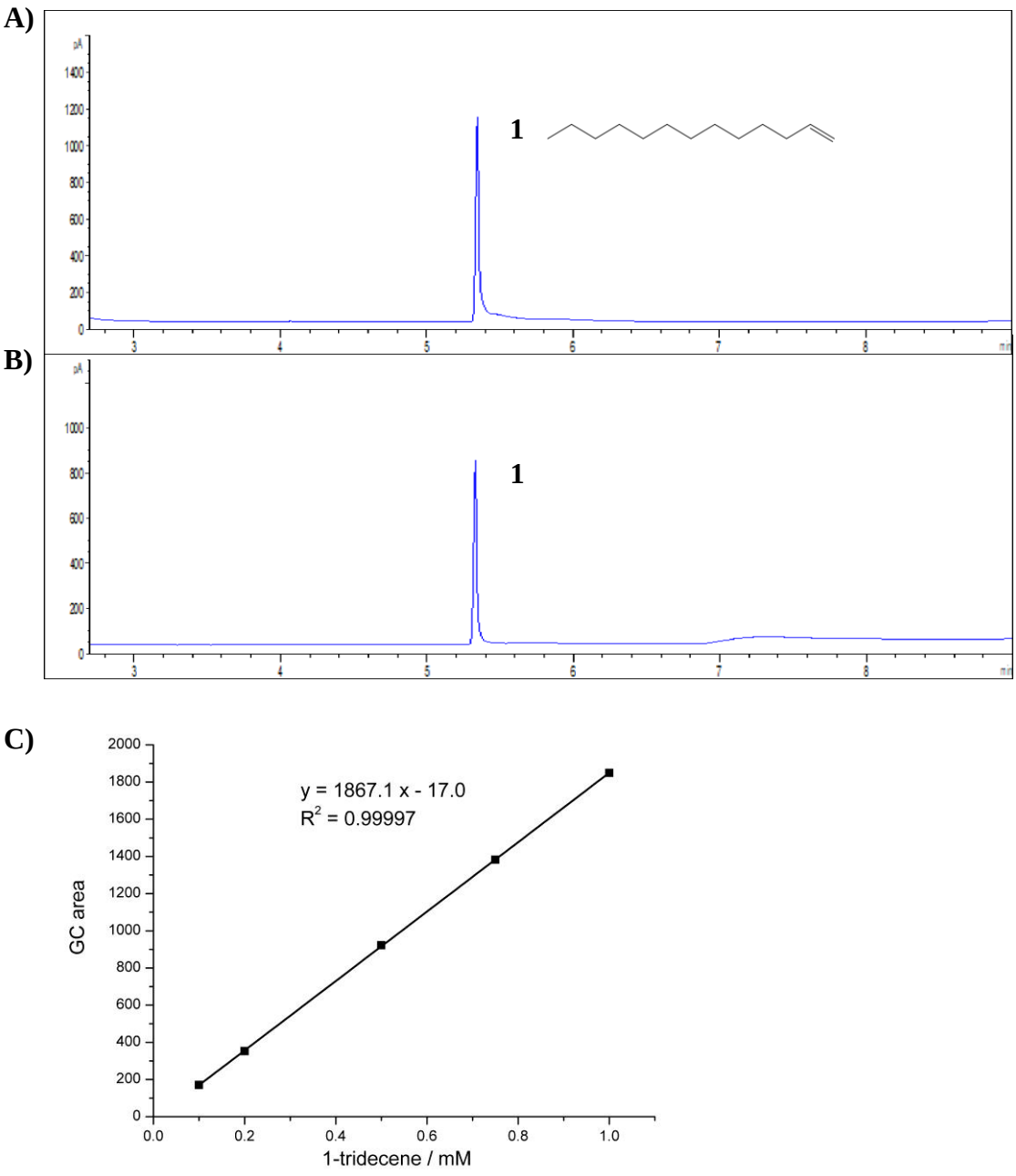


Figure S6: GC results of decarboxylation of FA C14:0 by OleT-BM3R with NADPH recycling. A) 1-tridecene standard; B) Conversion of tetradecanoic acid (FA C14:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-tridecene via GC-FID analysis. Compounds were injected via automatic injection into a GC-FID chromatograph. **1**: 1-tridecene.

3.5 Conversion of dodecanoic acid (FA C12:0) 1e

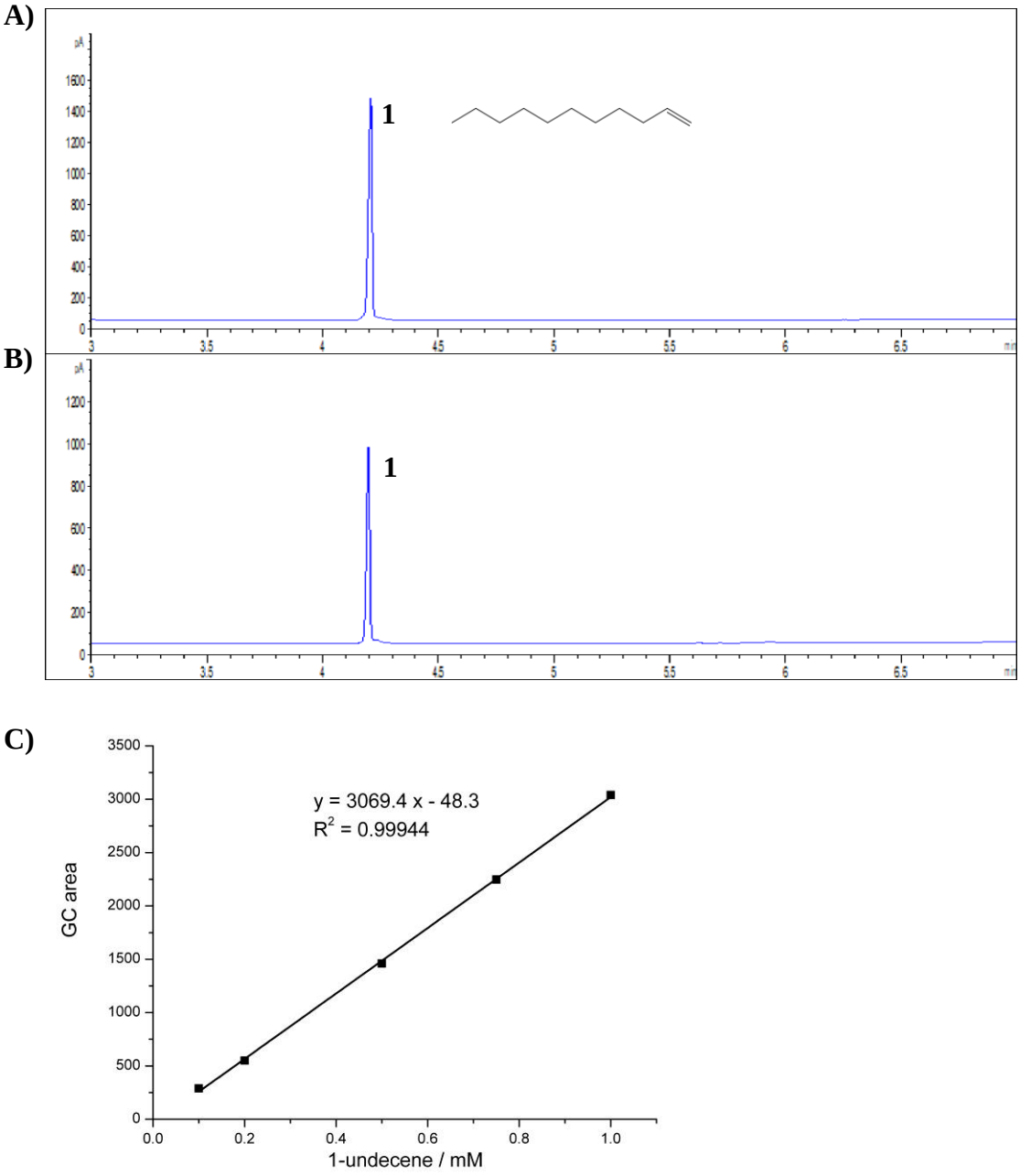


Figure S7: GC results of decarboxylation of FA C12:0 by OleT-BM3R with NADPH recycling. A) 1-undecene standard; B) Conversion of dodecanoic acid (FA C12:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-undecene via GC-FID analysis. Compounds were injected via automatic injection into a GC-FID chromatograph. **1**: 1-undecene.

3.6 Conversion of undecanoic acid (FA C11:0) 1f

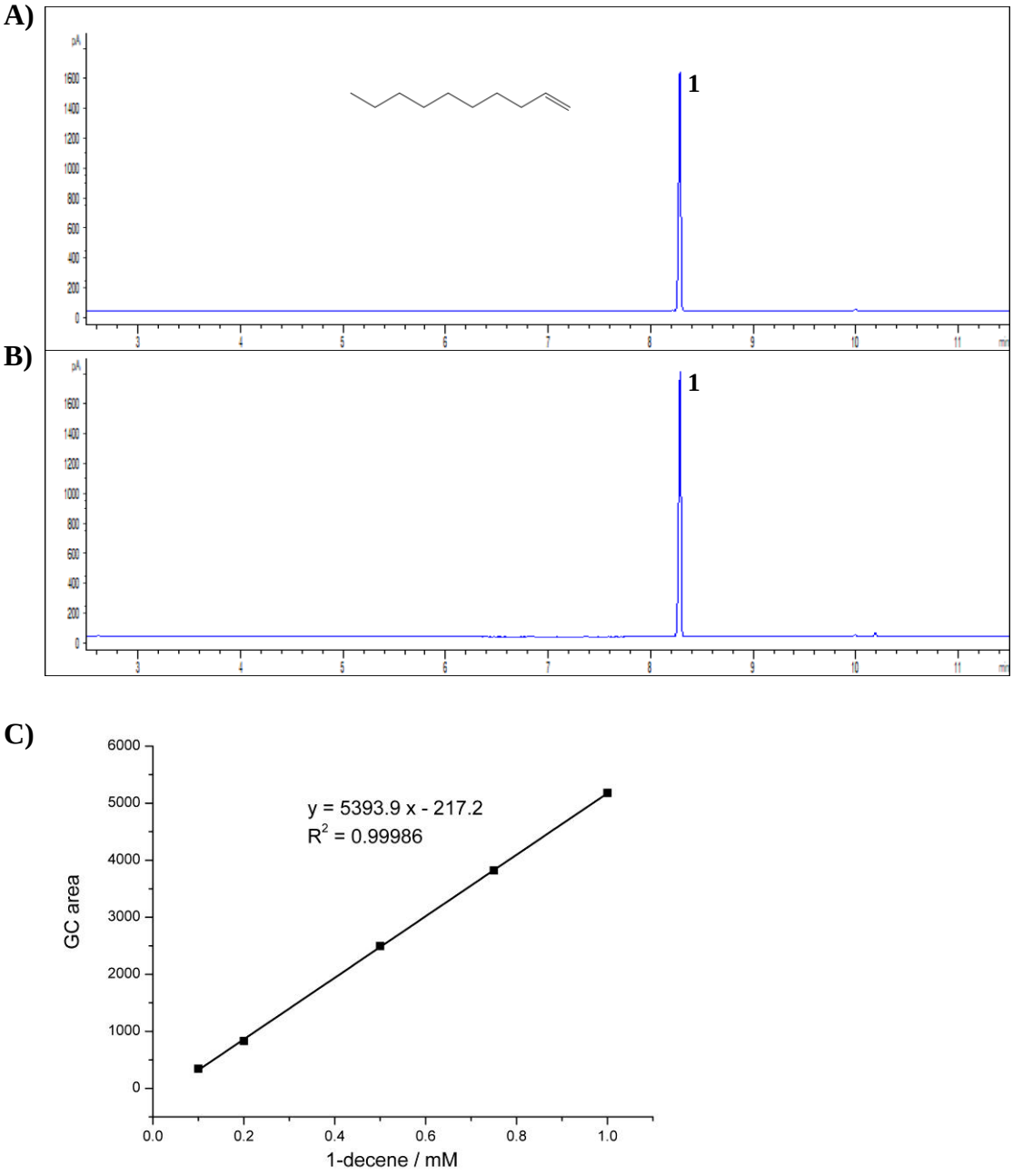


Figure S8: Headspace GC results of decarboxylation of FA C11:0 by OleT-BM3R with NADPH recycling. A) 1-decene standard; B) Conversion of undecanoic acid (FA C11:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-decene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 1-decene.

3.7 Conversion of decanoic acid (FA C10:0) 1g

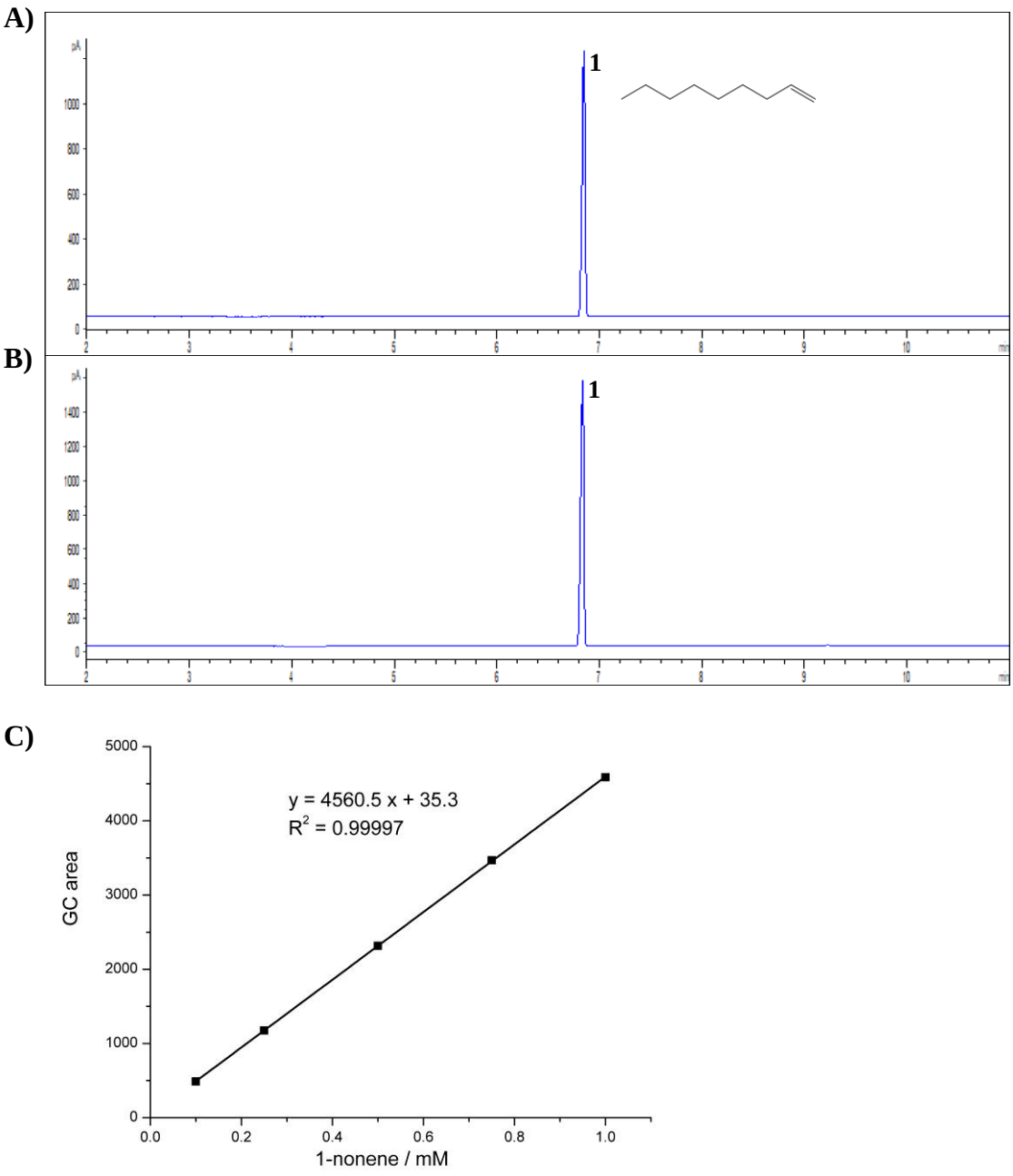


Figure S9: Headspace GC results of decarboxylation of FA C10:0 by OleT-BM3R with NADPH recycling. A) 1-nonene standard; B) Conversion of decanoic acid (FA C10:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-nonene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 1-nonene.

3.8 Conversion of nonanoic acid (FA C9:0) 1h

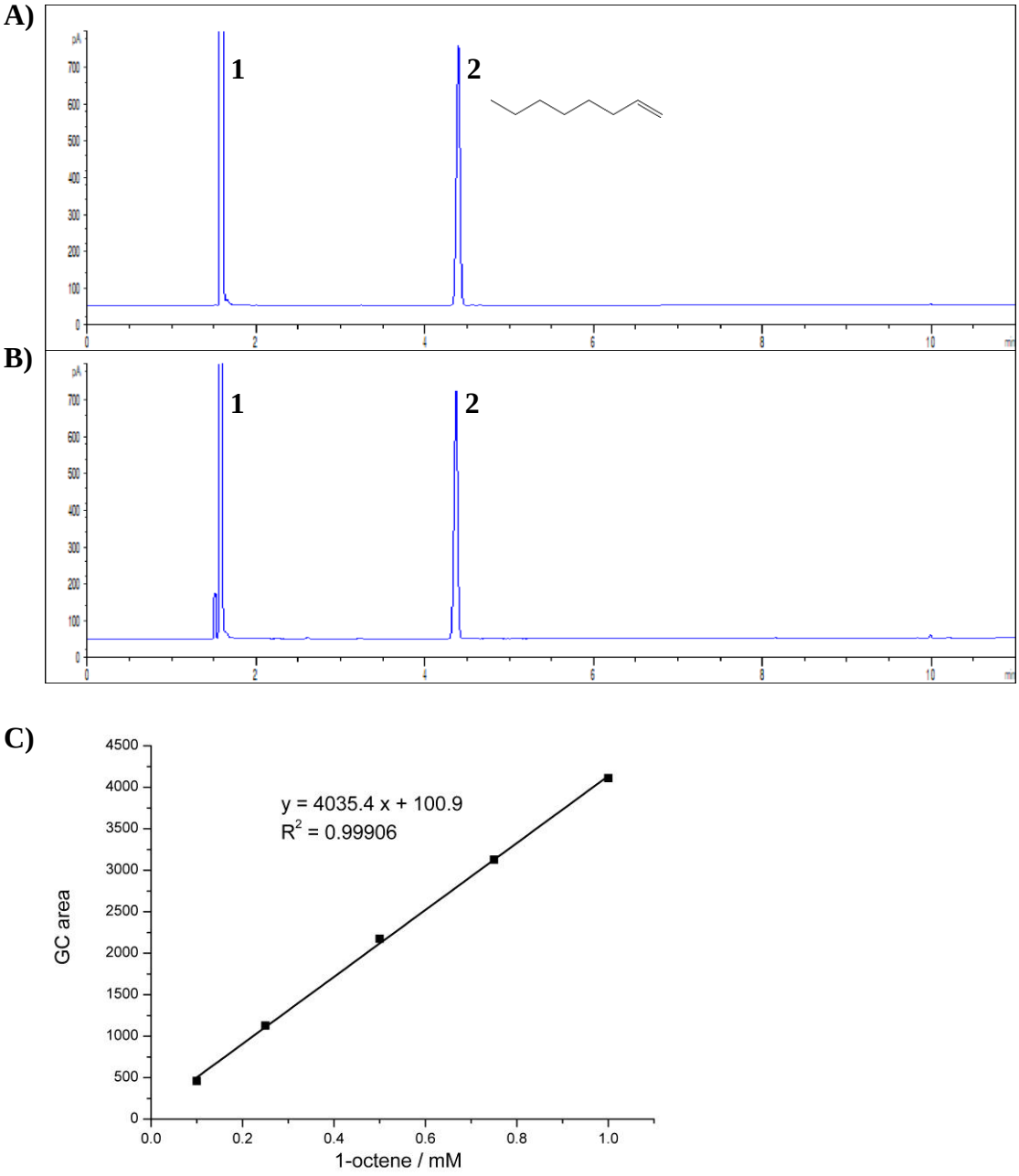


Figure S10: Headspace GC results of decarboxylation of FA C9:0 by OleT-BM3R with NADPH recycling. A) 1-octene standard; B) Conversion of nonanoic acid (FA C9:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-octene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: solvent peak (5 %EtOH, v/v). **2**: 1-octene.

3.9 Conversion of octanoic acid (FA C8:0) 1i

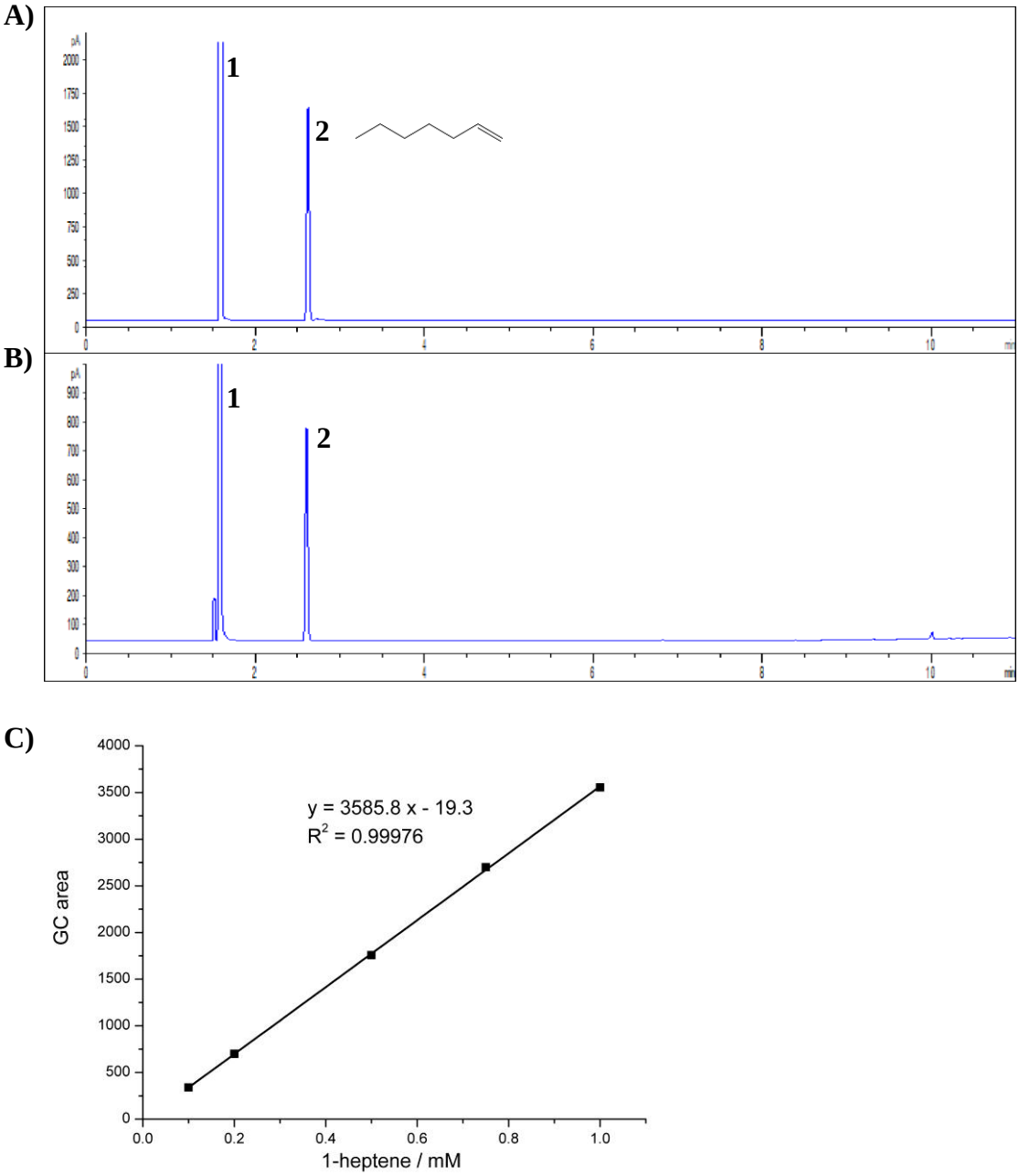


Figure S11: Headspace GC results of decarboxylation of FA C8:0 by OleT-BM3R with NADPH recycling. A) 1-heptene standard; B) Conversion of octanoic acid (FA C8:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-heptene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: solvent peak (5 %EtOH, v/v). **2**: 1-heptene.

3.10 Conversion of heptanoic acid (FA C7:0) 1j

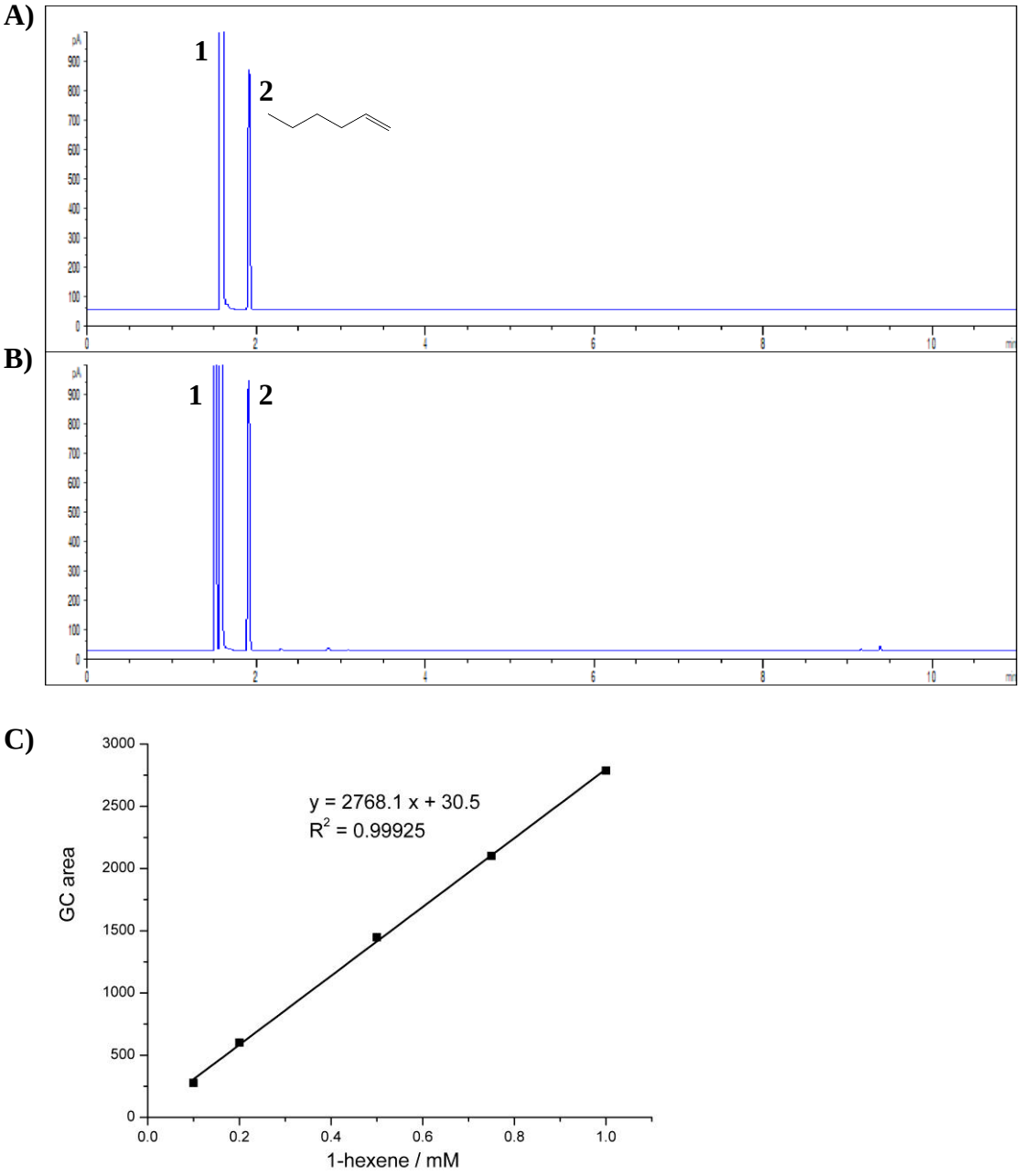


Figure S12: Headspace GC results of decarboxylation of FA C7:0 by OleT-BM3R with NADPH recycling. A) 1-hexene standard; B) Conversion of heptanoic acid (FA C7:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-hexene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: solvent peak (5% EtOH, v/v). **2**: 1-hexene.

3.11 Conversion of hexanoic acid (FA C6:0) 1k

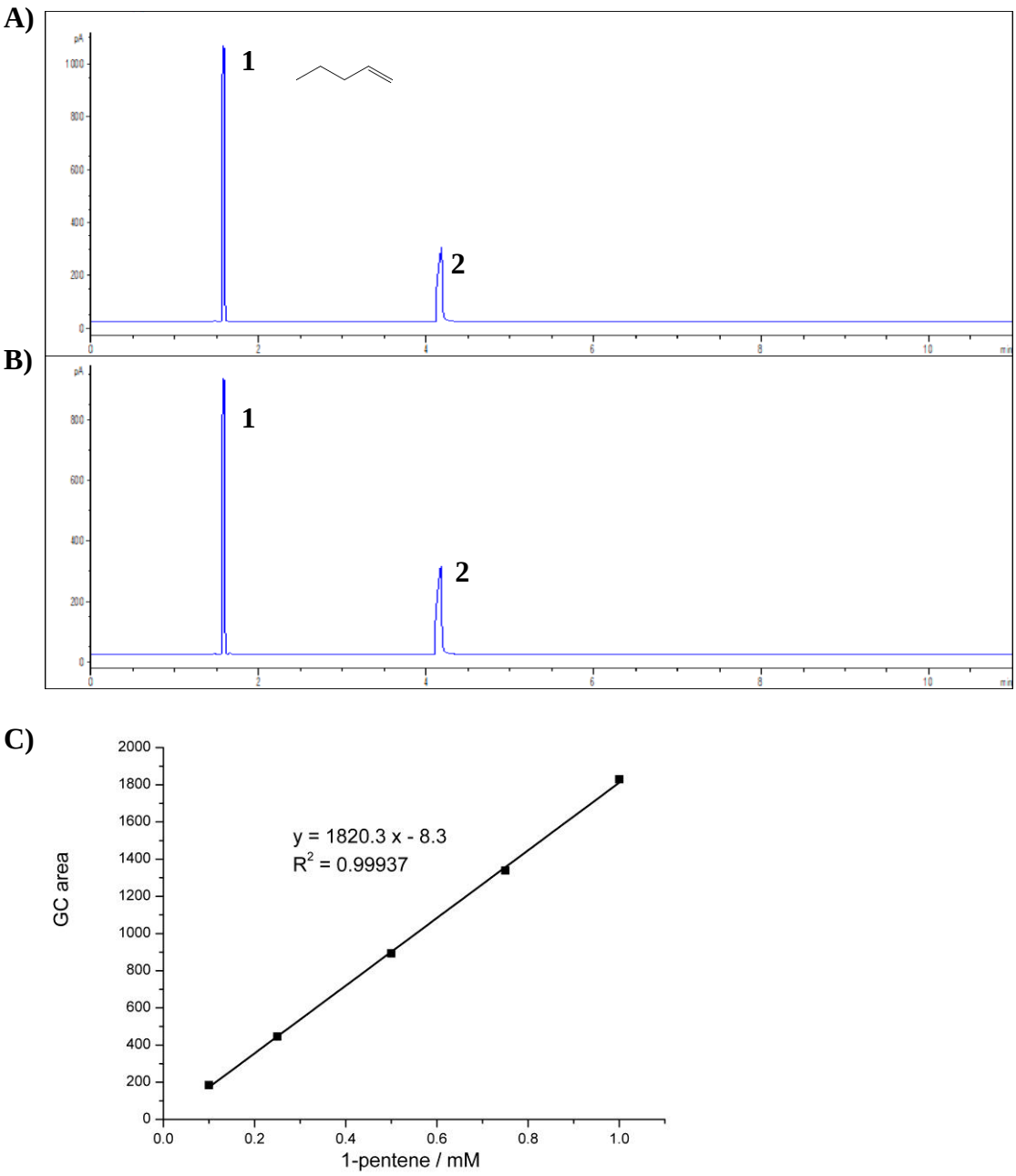


Figure S13: Headspace GC results of decarboxylation of FA C6:0 by OleT-BM3R with NADPH recycling. A) 1-pentene standard; B) Conversion of hexanoic acid (FA C6:0) by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1-pentene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 1-pentene. **2**: solvent peak (5% DMF, v/v).

3.12 Conversion of pentanoic acid (FA C5:0) 1l

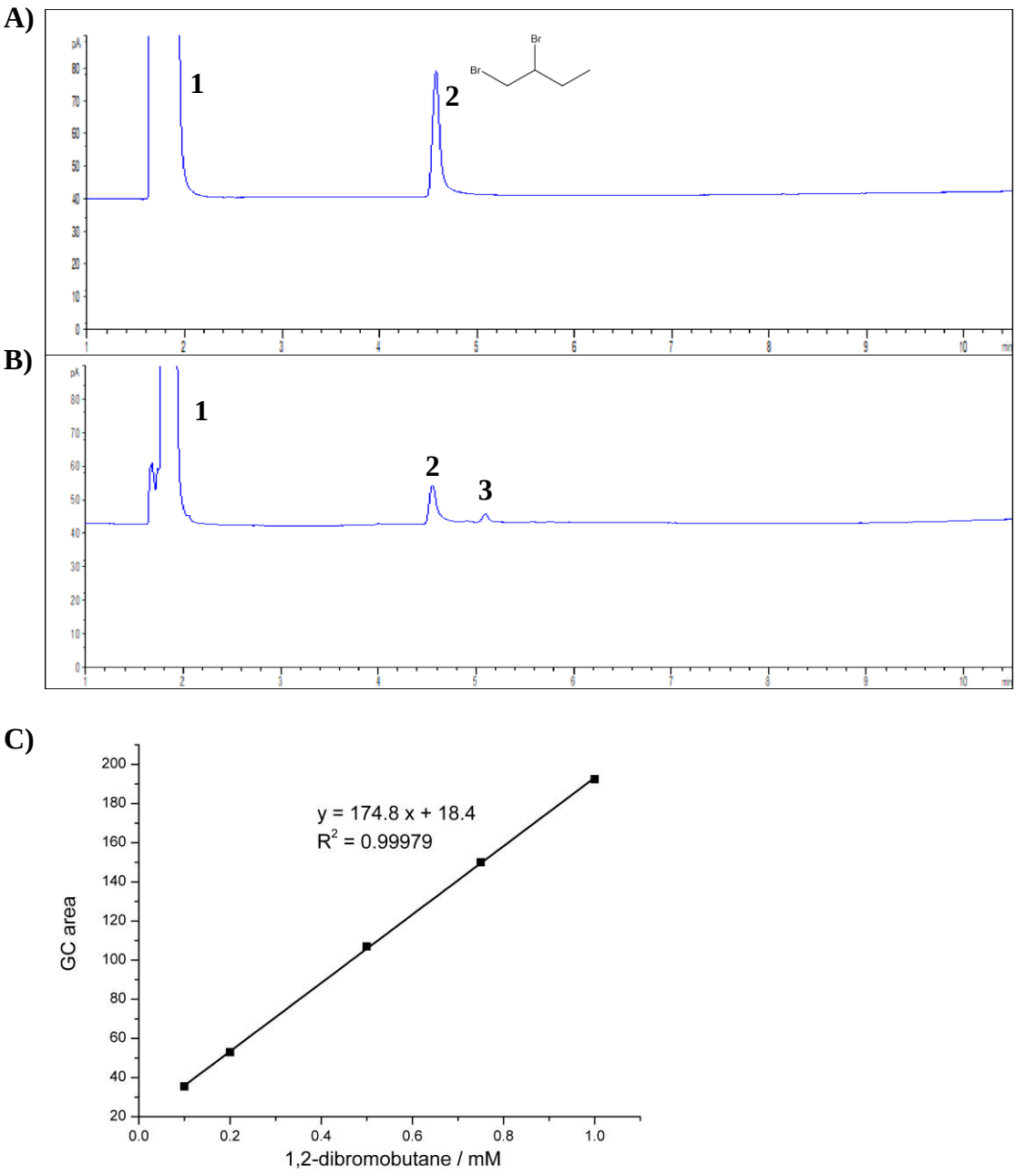


Figure S14: GC results of decarboxylation of FA C5:0 by OleT-BM3R with NADPH recycling. A) 1, 2-dibromobutane standard in CH_2Cl_2; B) 1,2-dibromobutane after derivatization of 1-butene derived from conversion of pentanoic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1,2-dibromobutane via GC-FID analysis. Compounds were injected via automatic injection into a GC-FID chromatograph. **1**: solvent peak (CH_2Cl_2). **2**: 1, 2-dibromobutane. **3**: impurity from Br_2.

3.13 Conversion of butanoic acid (FA C4:0) 1m

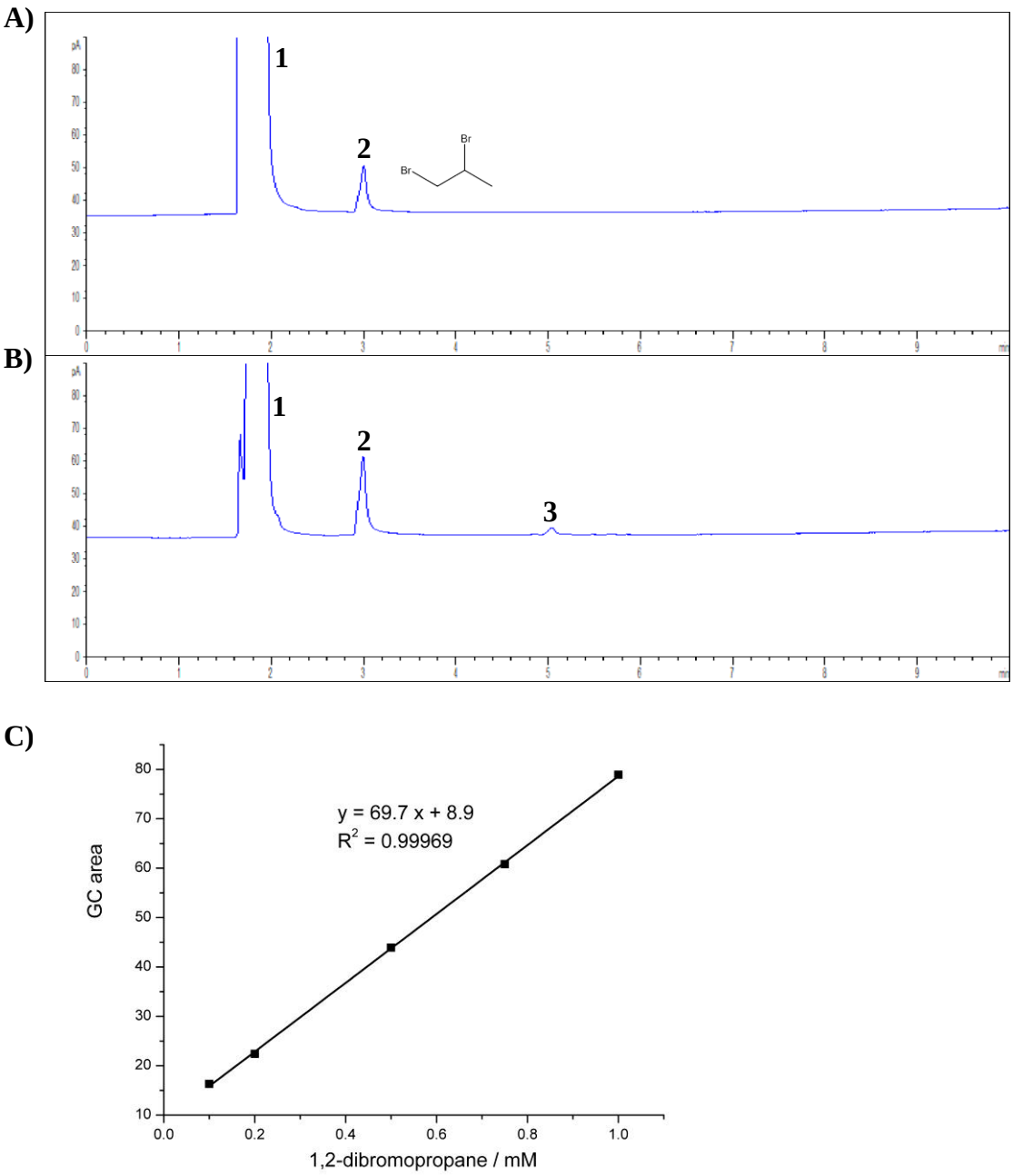


Figure S15: GC results of decarboxylation of FA C4:0 by OleT-BM3R with NADPH recycling. A) 1,2-dibromopropane standard in CH_2Cl_2; B) 1,2-dibromopropane after derivatization of 1-propane derived from conversion of butanoic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1,2-dibromopropane via GC-FID analysis. Compounds were injected via automatic injection into a GC-FID chromatograph. **1**: solvent peak (CH_2Cl_2). **2**: 1, 2-dibromopropane. **3**: impurity from Br_2.

3.14 Conversion of cyclohexanecarboxylic acid 3a

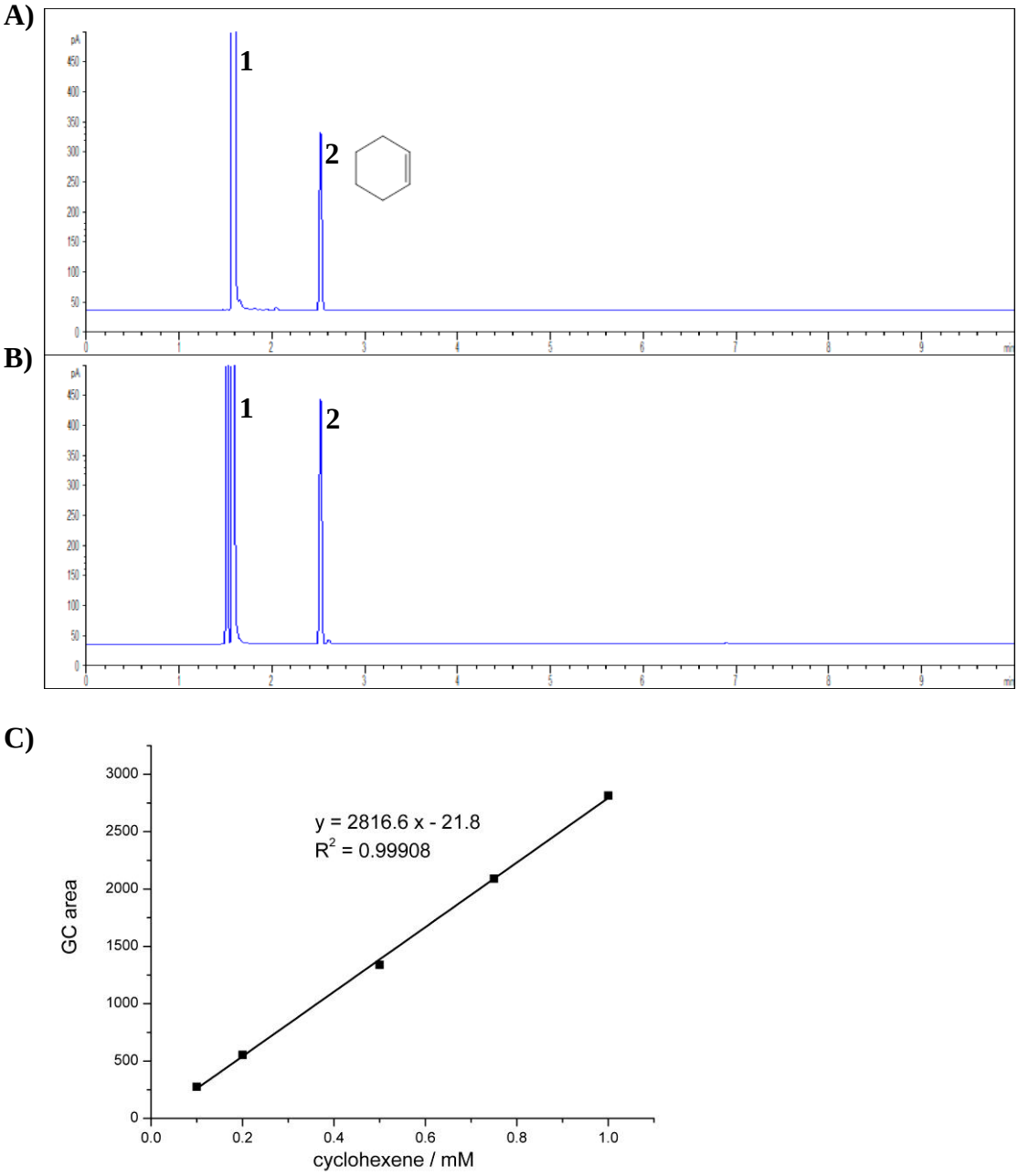


Figure S16: Headspace GC results of decarboxylation of cyclohexanecarboxylic acid by OleT-BM3R with NADPH recycling. A) cyclohexene standard; B) Conversion of cyclohexanecarboxylic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of cyclohexene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: solvent peak (EtOH, 5 % v/v). **2**: cyclohexene.

3.15 Conversion of 2-methlyhexanoic acid 3b

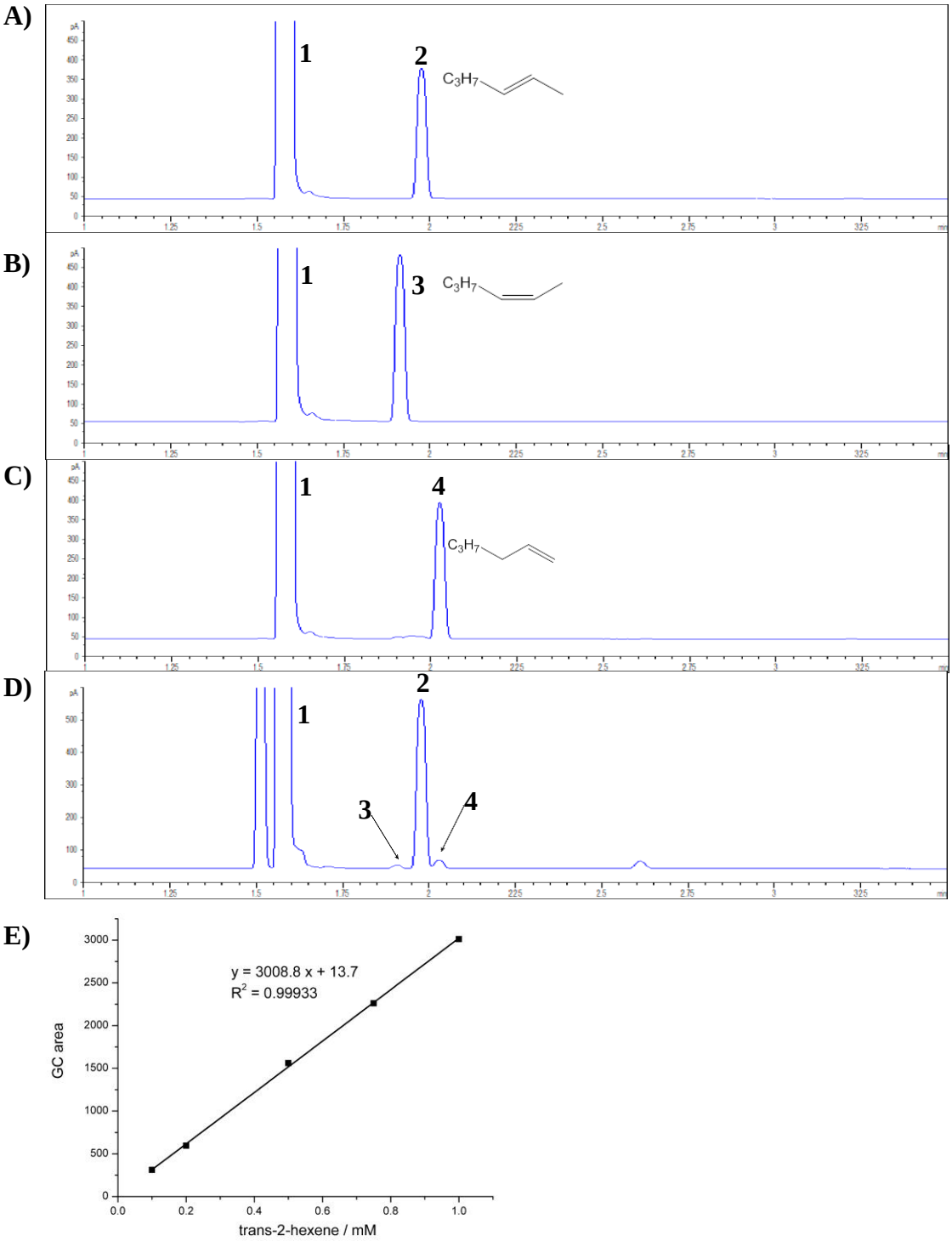


Figure S17: Headspace GC results of decarboxylation of 2-Methlyhexanoic acid by OleT-BM3R with NADPH recycling. A) *trans*-2-hexene standard; B) *cis*-2-hexene standard C) 1-hexene standard D) Conversion of 2-Methlyhexanoic acid by OleT-BM3R with NADPH recycling; E) Calibration curve for quantification of *trans*-2-hexene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: solvent peak (5% EtOH, v/v). **2**: *trans*-2-hexene. **3**: *cis*-2-hexene. **4**: 1-hexene.

3.16 Conversion of 3-phenylpropionic acid 3c

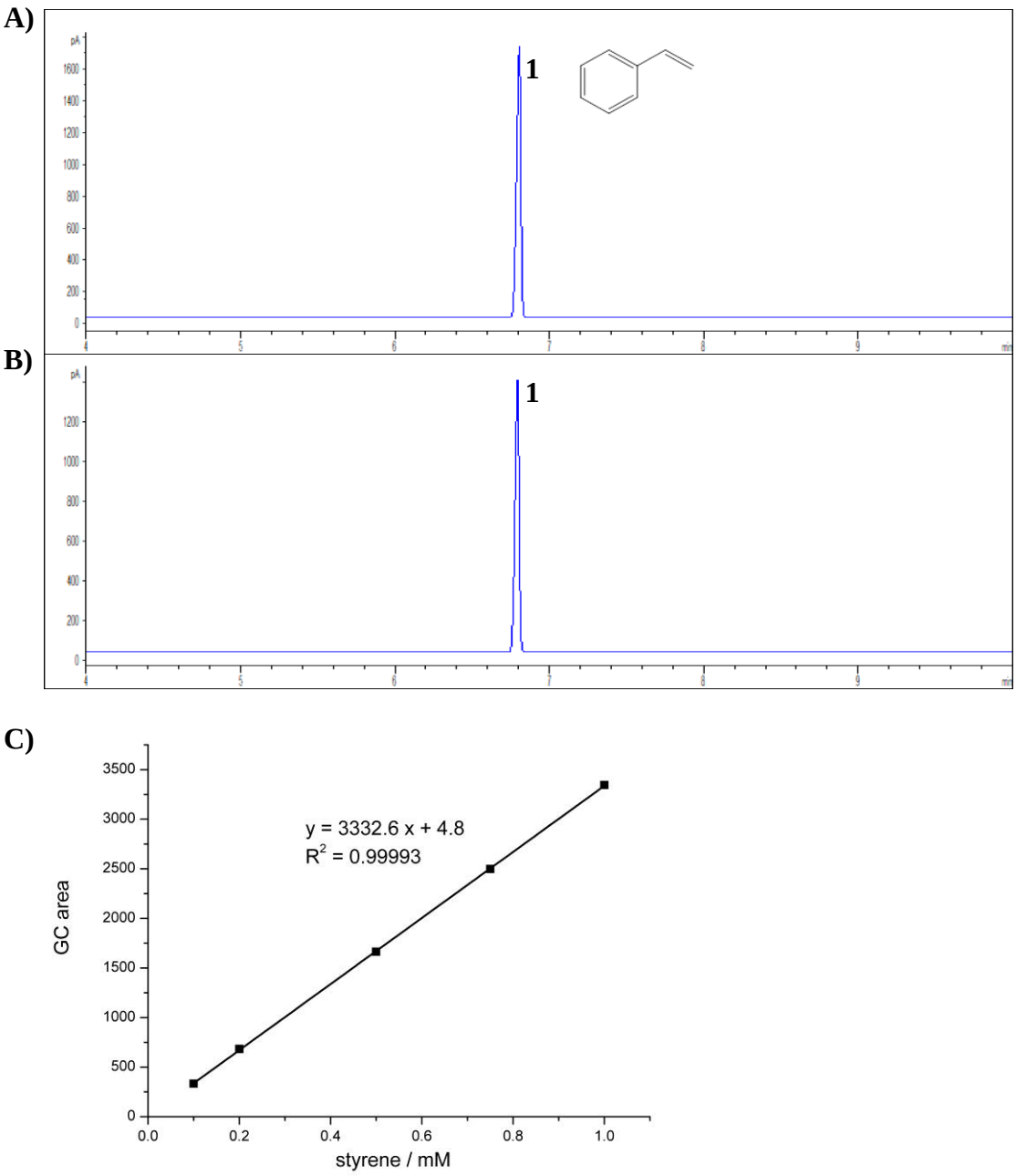


Figure S18: Headspace GC results of decarboxylation of 3-phenylpropionic acid by OleT-BM3R with NADPH recycling. A) styrene standard; B) Conversion of 3-phenylpropionic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of styrene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: styrene.

3.17 Conversion of 3-(4-Fluorophenyl) propionic acid 3d

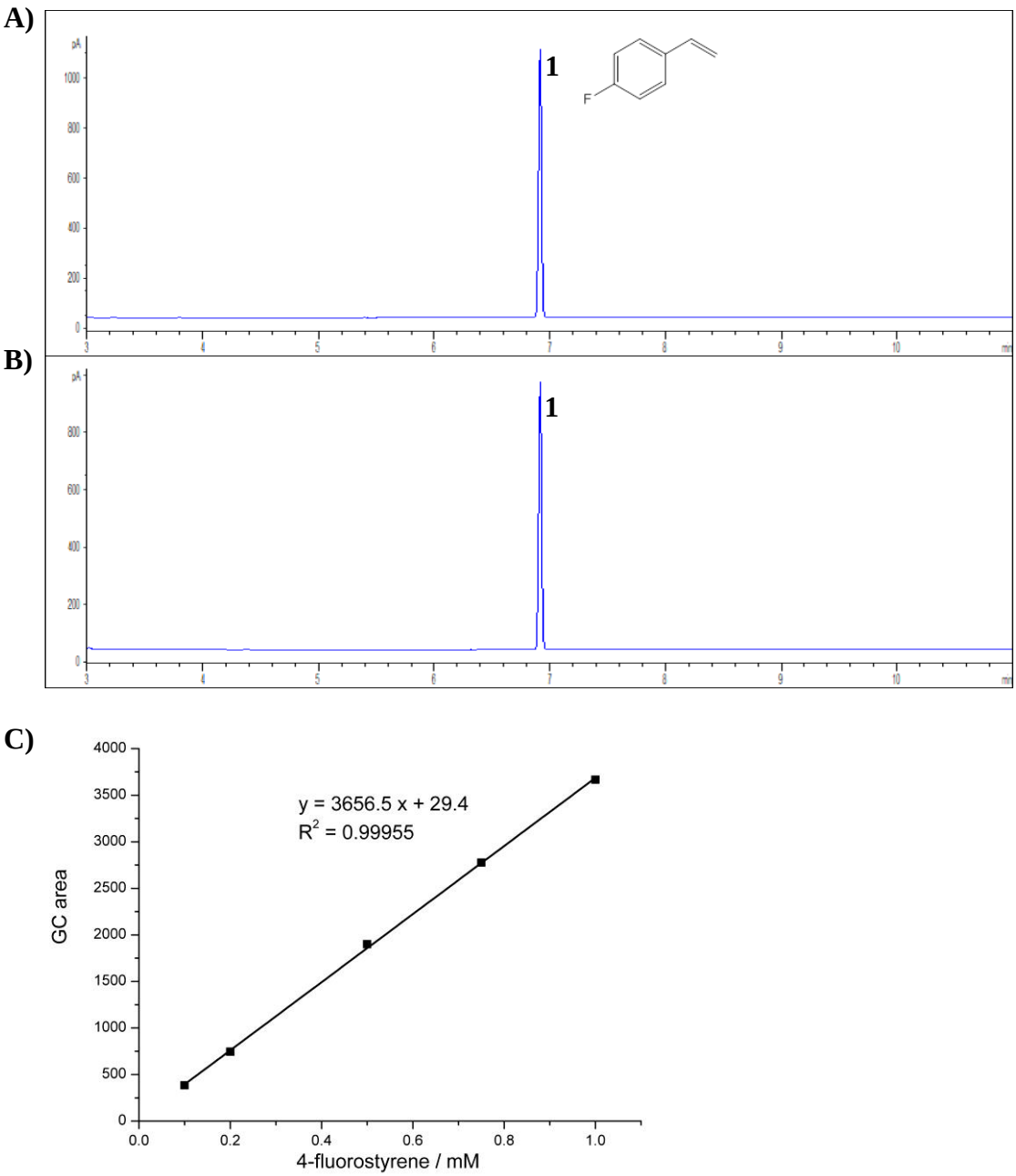


Figure S19: Headspace GC results of decarboxylation of 3-(4-Fluorophenyl) propionic acid by OleT-BM3R with NADPH recycling. A) 4-fluorostyrene standard; B) Conversion of 3-(4-fluorophenyl) propionic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 4-fluorostyrene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 4-fluorostyrene.

3.18 Conversion of 10-undecenoic acid 3e

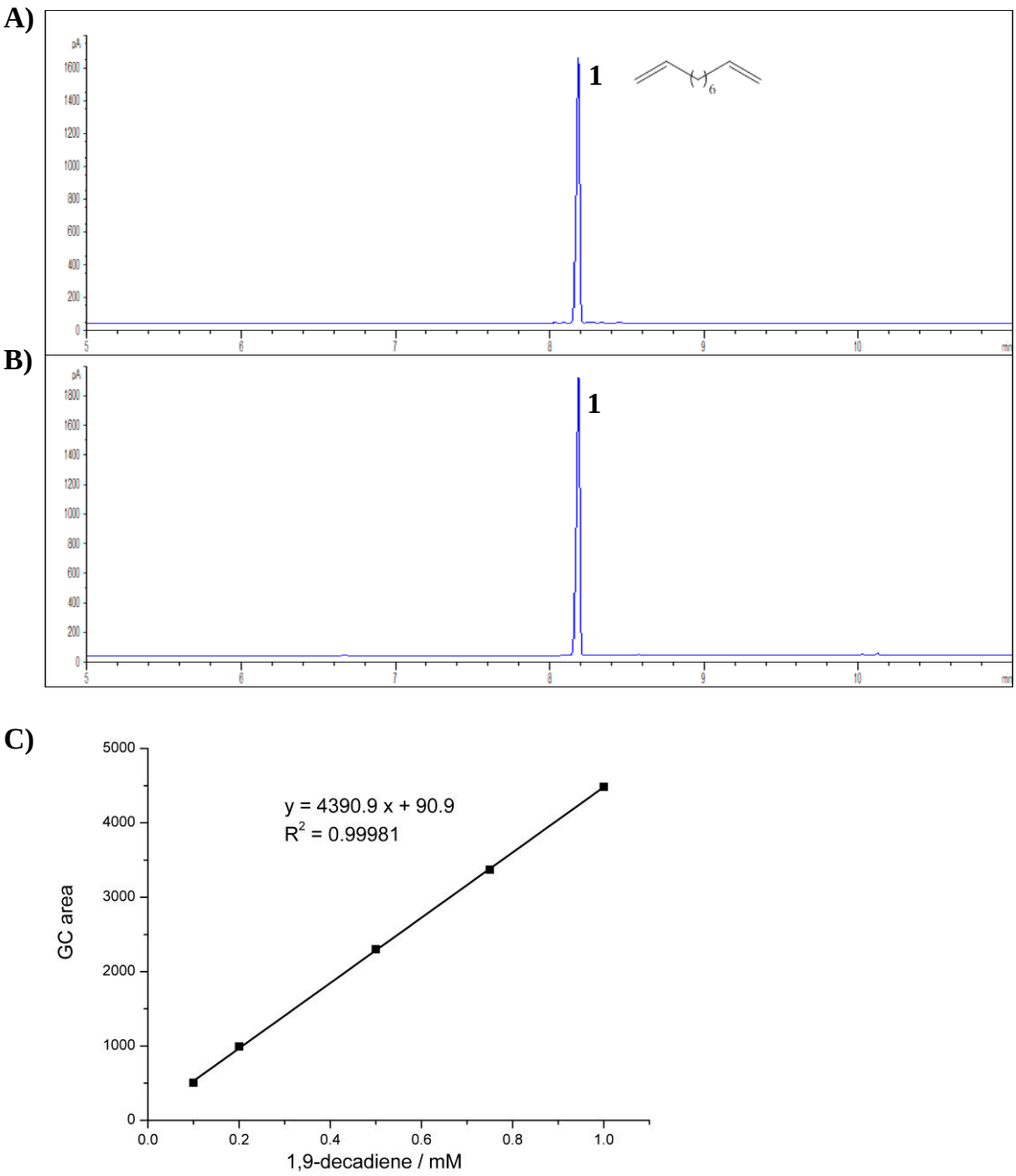


Figure S20: Headspace GC results of decarboxylation of 10-undecenoic acid by OleT-BM3R with NADPH recycling. A) 1,9-decadiene standard; B) Conversion of 10-undecenoic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1,9-decadiene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 1, 9-decadiene.

3.19 Conversion of 10-oxoundecanoic acid 3f

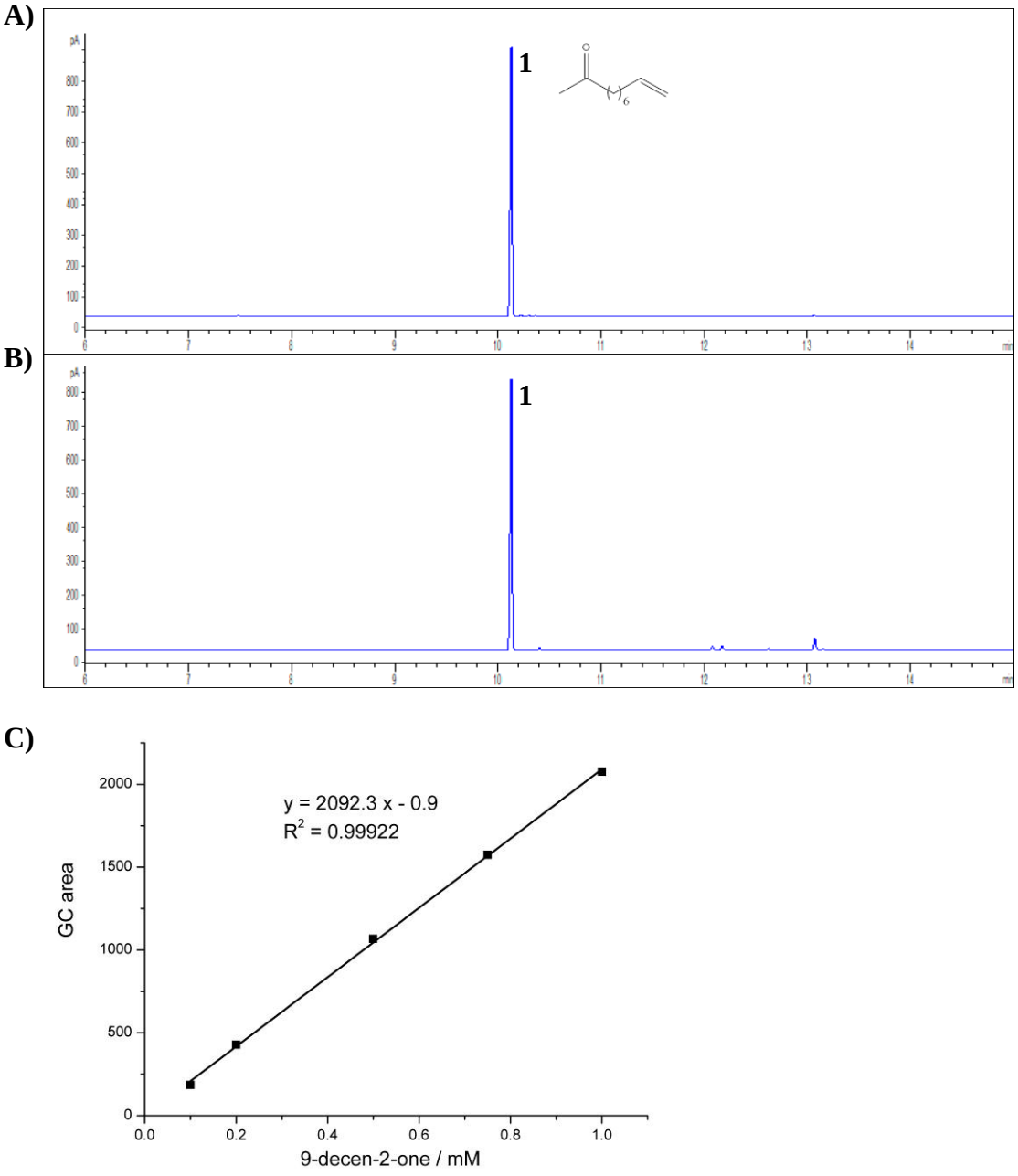


Figure S21: Headspace GC results of decarboxylation of 10-oxoundecanoic acid by OleT-BM3R with NADPH recycling. A) 9-decen-2-one standard; B) Conversion of 10-oxoundecanoic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 9-decen-2-one via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 9-decen-2-one.

3.20 Conversion of 12-hydroxydodecanoic acid 3g

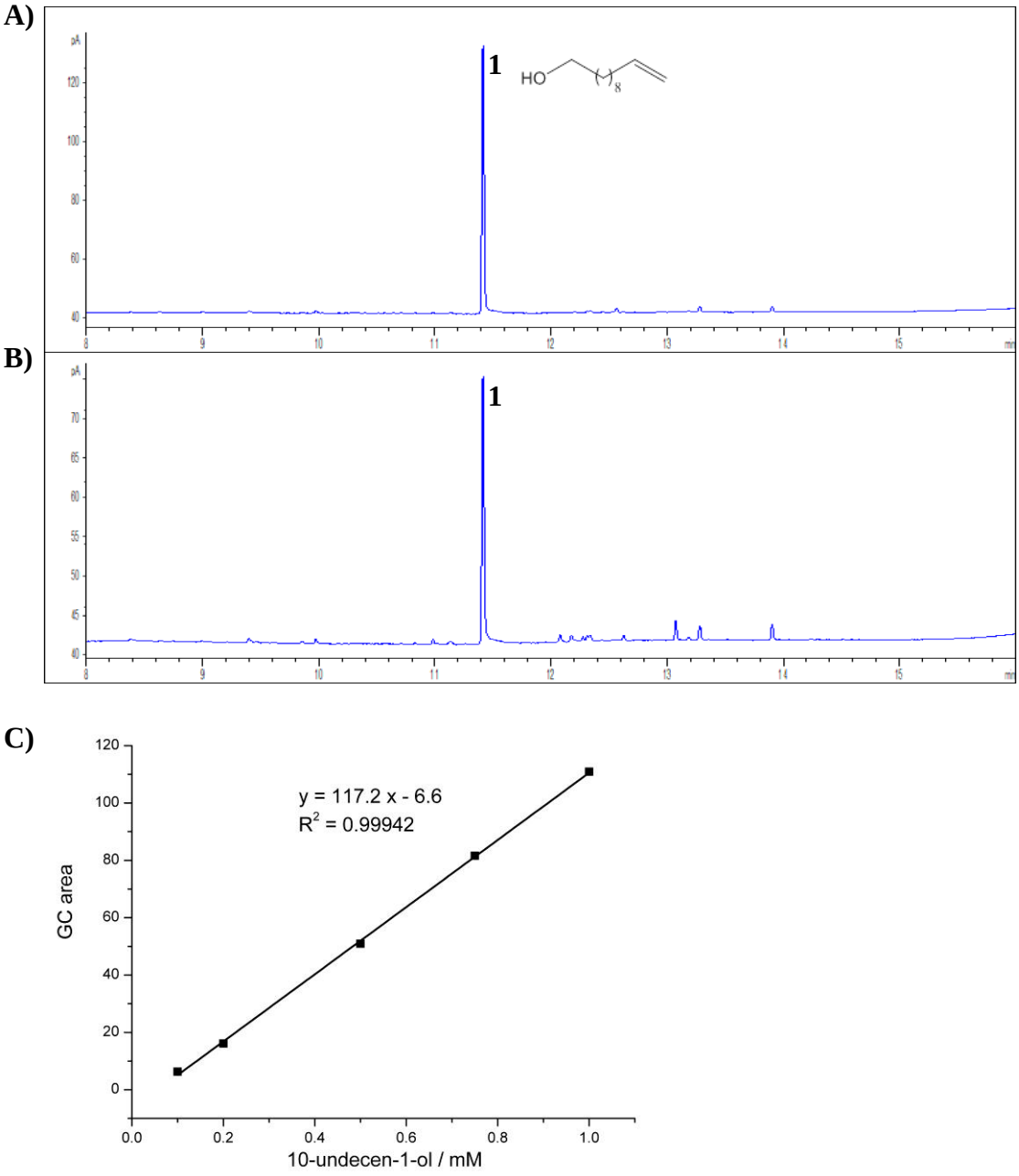


Figure S22: Headspace GC results of decarboxylation of 12-hydroxydodecanoic acid by OleT-BM3R with NADPH recycling. A) 10-undecen-1-ol standard; B) Conversion of 12-hydroxydodecanoic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 10-undecen-1-ol via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 10-undecen-1-ol.

3.21 Conversion of 11-bromoundecanoic acid 3h

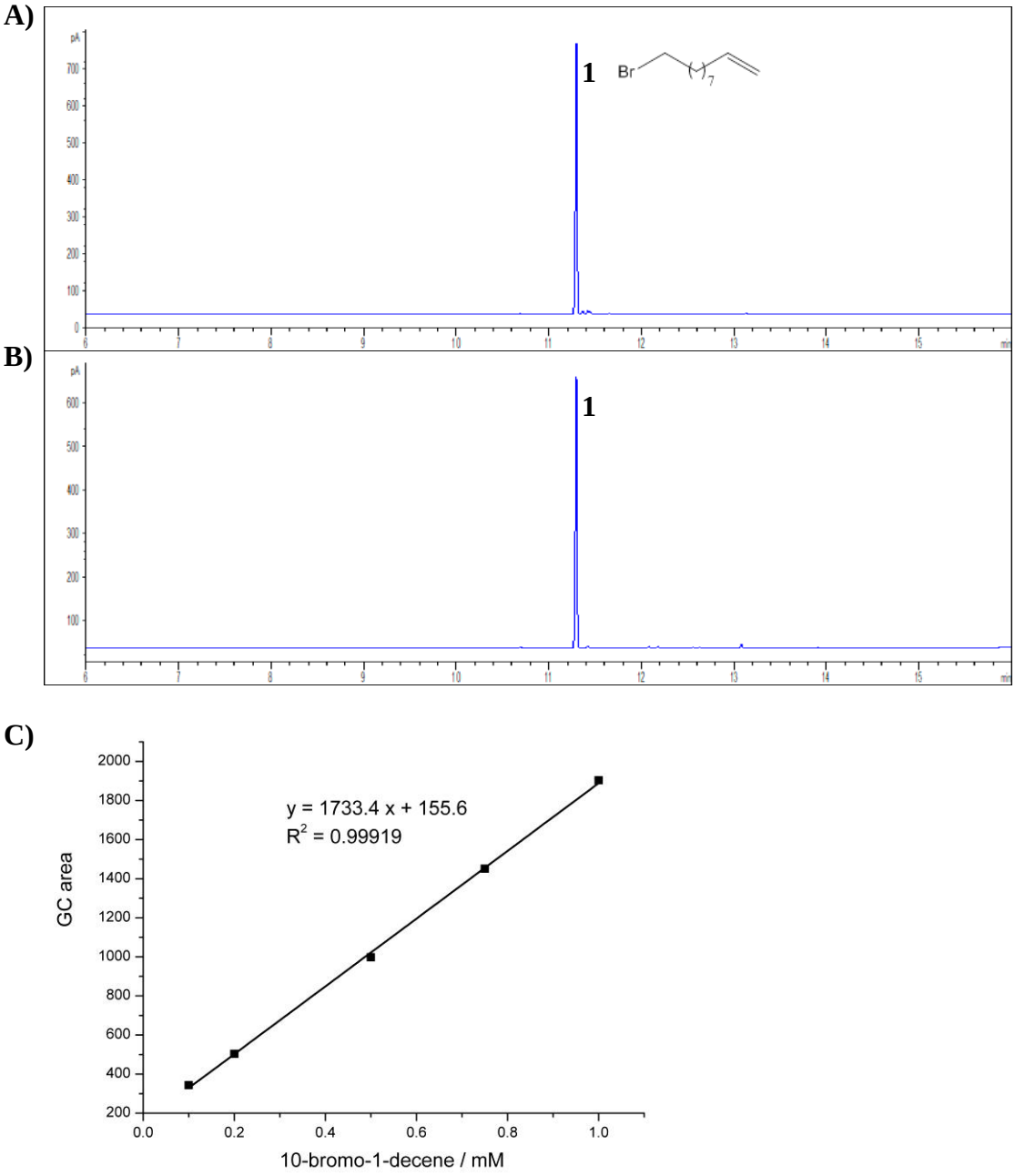


Figure S23: Headspace GC results of decarboxylation of 11-bromoundecanoic acid by OleT-BM3R with NADPH recycling. A) 10-bromo-1-decene standard; B) Conversion of 11-bromoundecanoic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 10-bromo-1-decene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 10-bromo-1-decene.

3.22 Conversion of dodecanedioic acid 3j

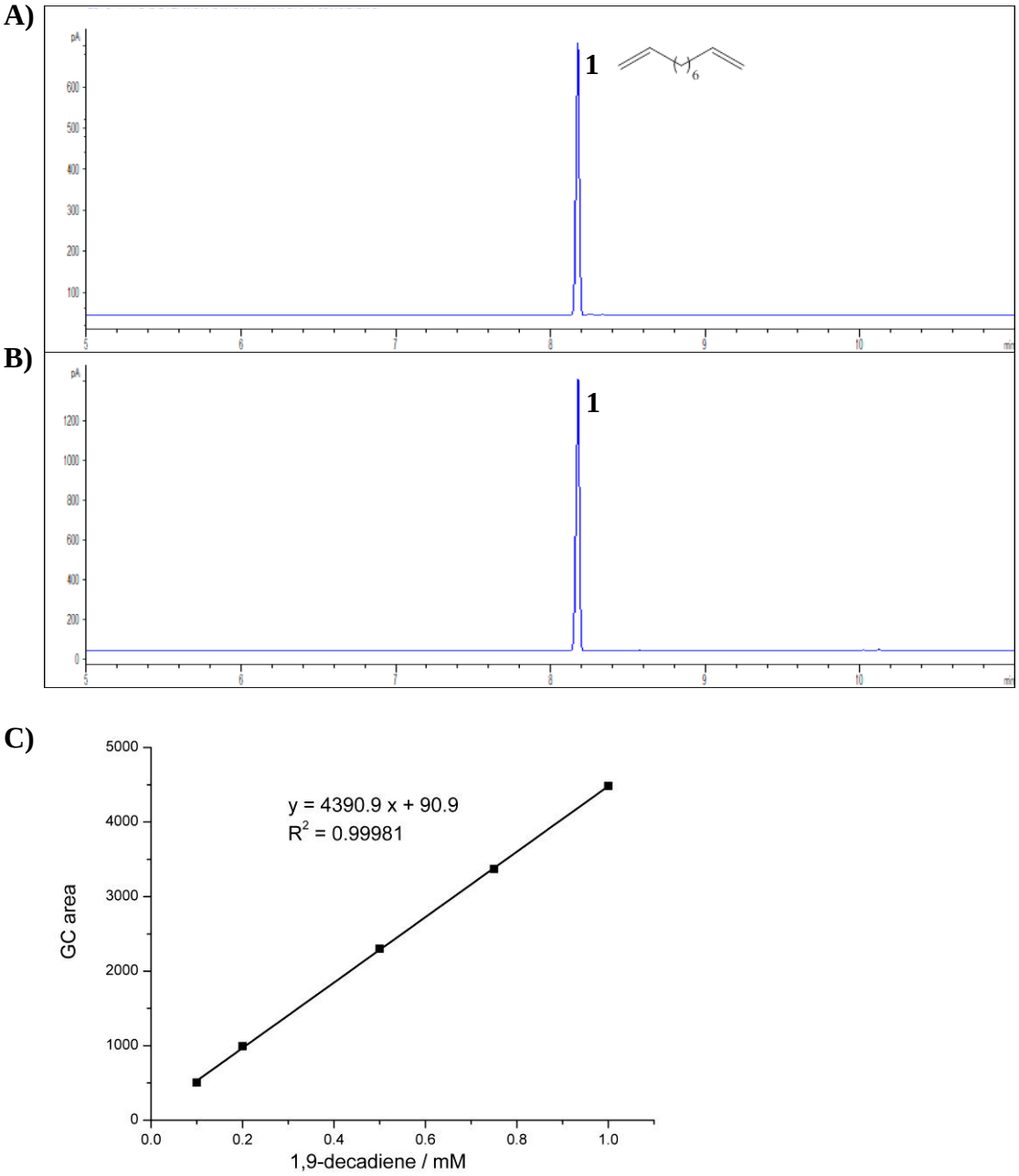


Figure S24: Headspace GC results of decarboxylation of dodecanedioic acid by OleT-BM3R with NADPH recycling. A) 1, 9-decadiene standard; B) Conversion of dodecanedioic acid by OleT-BM3R with NADPH recycling; C) Calibration curve for quantification of 1, 9-decadiene via headspace GC-FID analysis. Compounds were injected via Headspace Sampler into a GC-FID chromatograph. **1**: 1, 9-decadiene.

4 NMR Spectra

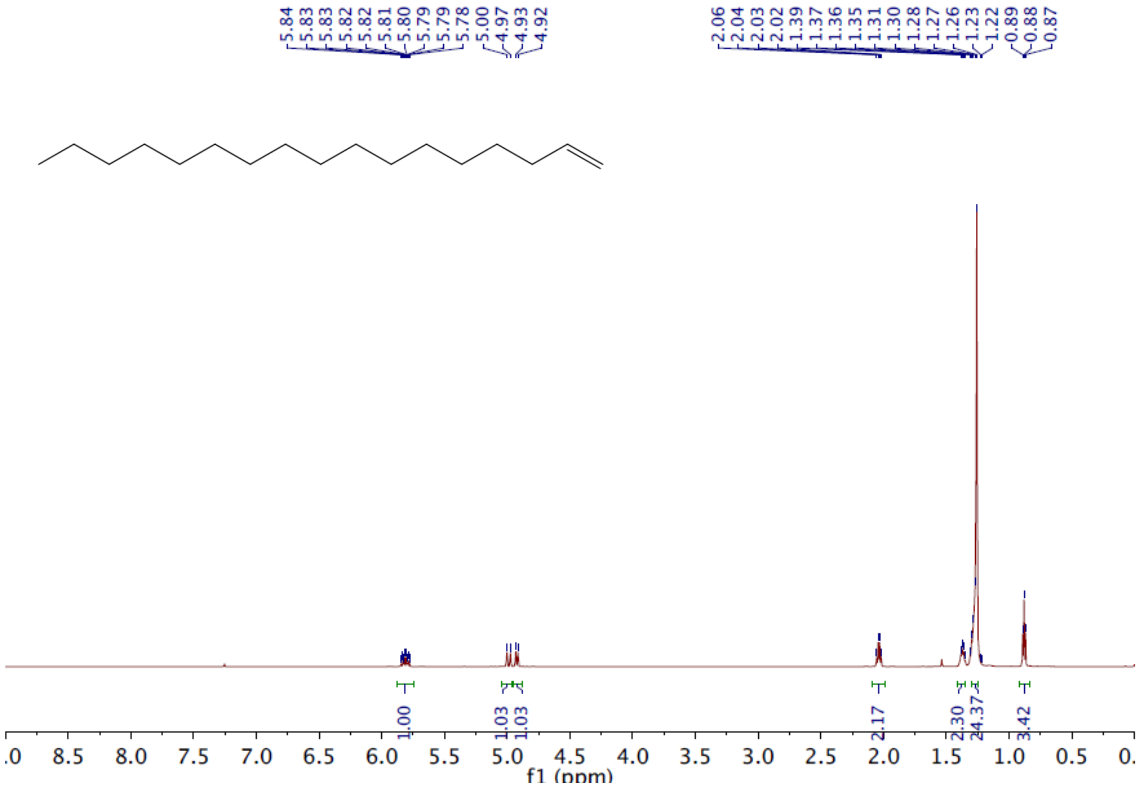


Figure S25: NMR spectrum of purified 1-heptadecene from conversion of octadecanoic acid.

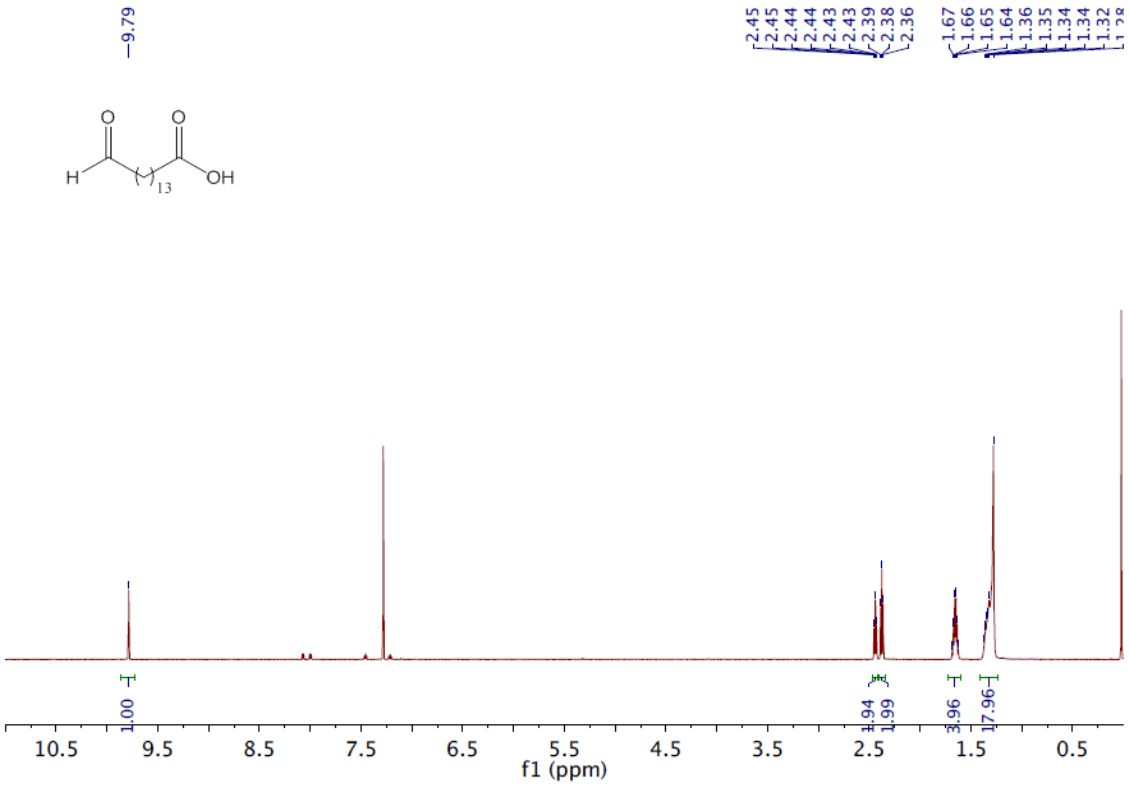


Figure S26: NMR spectrum of purified 15-oxopentadecanoic acid produced by using reagent Dess–Martin periodinane for oxidation of the corresponding primary alcohol.

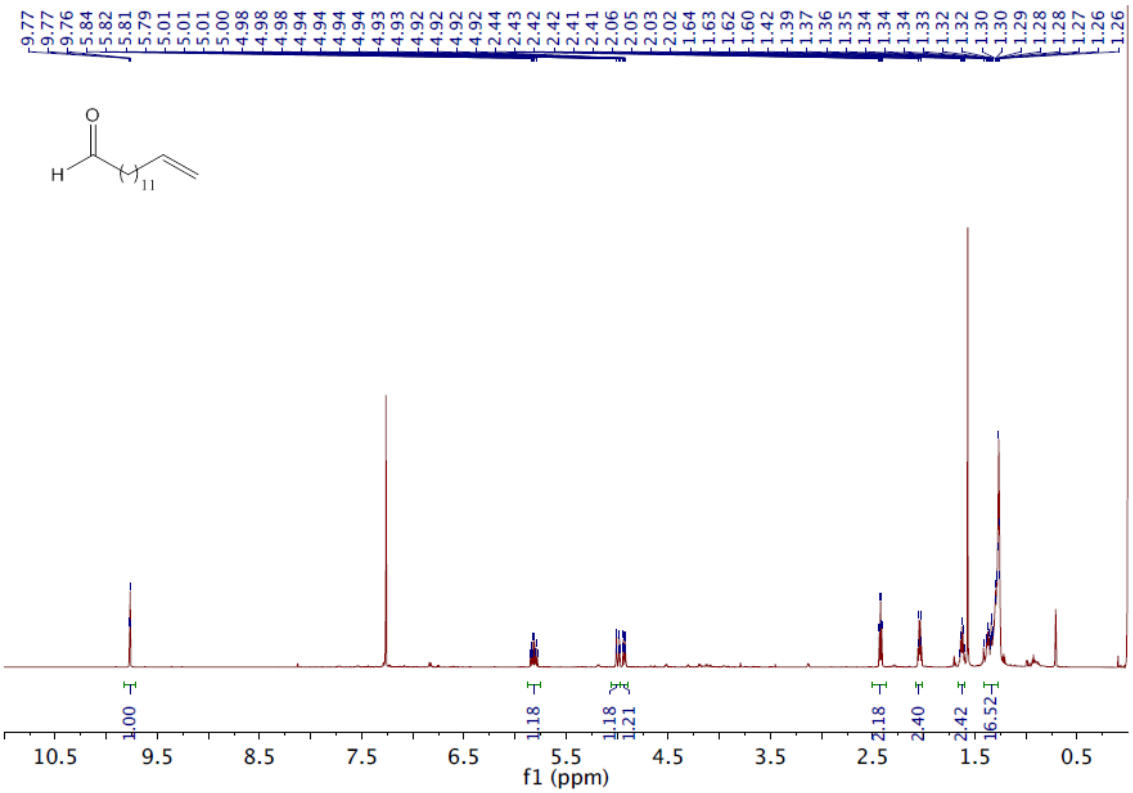


Figure S27: NMR spectrum of purified 13-tetradecenal from conversion of 15-oxopentadecanoic acid

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