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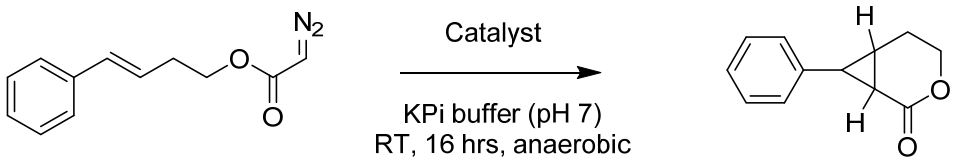
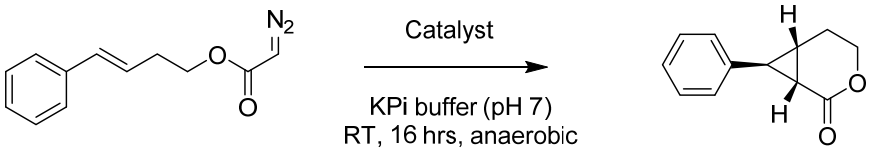
Table S1. Activity of hemin and hemoproteins in the intramolecular cyclopropanation of (*E*)-4-phenylbut-3-en-1-yl 2-diazoacetate (**1a**). Reaction conditions: 20 μM catalyst, 2.5 mM **1a**, 10 mM $Na_2S_2O_4$, in KPi buffer (50 mM, pH 7), room temperature, 16 hours, in anaerobic chamber. The reaction with Fe(TPP)Cl (= meso-Tetraphenylporphyrin iron(III) chloride, CAS Number 16456-81-8) was performed in dichloromethane.



1a **2a / 3a**

Entry	Catalyst	Yield (GC)	TON	% *ee*
1	Hemin	0%	0	n.d.
2	Fe(TPP)Cl in DCM	0%	0	n.d.
3	Mb	0%	0	n.d.
4	Catalase	0%	0	n.d.
5	Cytochrome *c* (equine heart)	0%	0	n.d.
6	Cytochrome *c* (*Hydrogenobacter thermophilus*)	0%	0	n.d.
7	$P450_{BM3}$	0%	0	n.d.
8	P450 XplA	0%	0	n.d.
9	P450 BezE	0%	0	n.d.

Table S2. Activity and selectivity of a library of engineered Mb variants in the intramolecular cyclopropanation of (*E*)-4-phenylbut-3-en-1-yl 2-diazoacetate (**1a**). Reaction conditions: 20 μM protein, 2.5 mM **1a**, 10 mM $Na_2S_2O_4$, in KPi buffer (50 mM, pH 7), room temperature, 16 hours, in anaerobic chamber. Negative % *ee* values refer to formation of (1*R*,6*R*,7*R*)-isomer **3a** as the major enantiomer.



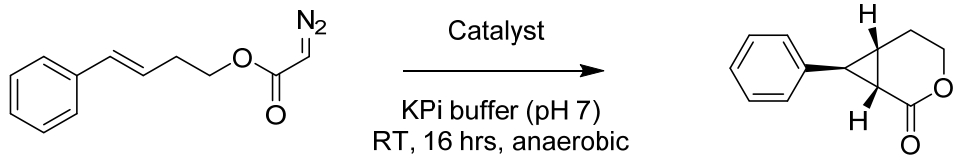
1a **2a**

Entry	Mutations	Yield	TON	*% ee* (1*S*,6*S*,7*S*)
1	none (WT)	0.0%	0	n.d.
2	H64V/V68A	0.1%	0	n.d.
3	H64V	0.1%	0	n.d.
4	H64G	0.2%	0	n.d.
5	V68A	0.0%	0	n.d.
6	L29A	0.0%	0	n.d.
7	L29A/H64V/V68A	0.0%	0	n.d.
8	H64A/V68A	0.2%	0	n.d.
9	H64V/V68G	1.0%	1	4
10	L29T/H64V/V68L	0.3%	0	n.d.
11	L29T/H64V/V68F	0.4%	0	4
12	L29T/H64V/V68F/I107L	0.0%	0	n.d.
13	L29S/H64V/V68F	0.0%	0	n.d.
14	L29T/F43W/H64V/V68F	0.3%	0	n.d.
15	L29F/F43S/H64V	0.2%	0	n.d.
16	H64G/V68A	0.4%	0	53
17	L29F/H64V/V68A	0.0%	0	n.d.
18	F43V/H64A/V68A	0.3%	0	n.d.
19	H64V/I107S	0.0%	0	n.d.
20	L29T/H64V	0.0%	0	n.d.

21	F43Y/H64V/V68A	0.0%	0	n.d.
22	V68F	0.4%	0	-26
23	F43A/V68F	0.0%	0	n.d.
24	F43A/H64W/V68F	0.9%	1	-65
25	H64V/I107Y	0.0%	0	n.d.
26	L29S/H64V	0.9%	1	57
27	F43V/V68F	0.0%	0	n.d.
28	L29C/H64V	0.7%	1	15
29	L29A/H64V/I107S	0.0%	0	n.d.
30	L29W/F43V/V68F	0.5%	1	-7
31	F43A/H64W/T67S/V68F	0.4%	1	-49
32	V68G	1.1%	1	16
33	H64A	0.8%	1	-27
34	F43A	0.0%	0	n.d.
35	F43V/H64W	0.7%	1	16
36	L29F/H64V/V68F	0.0%	0	n.d.
37	F43G	0.8%	1	9
38	F43V/H64V/V68F	0.5%	1	-1
39	F43V/H64F	0.9%	1	1
40	H64V/V68F	0.5%	2	0
41	F43Y/H64V/V68A/I107V	0.0%	0	n.d.
42	F43I/H64V	0.9%	1	33
43	F43Y/H64V/V68F	0.8%	1	-18
44	F43V/V68L	0.7%	1	25
45	F43Y/V68F	0.9%	1	-23
46	H64V/F43W	0.1%	0	n.d.
47	H64V/F43Q	0.0%	0	n.d.
48	H64V/F43C	0.2%	0	n.d.
49	H64V/F43G	0.0%	0	n.d.
50	H64V/F43M	0.4%	1	n.d.
51	H64V/F43T	0.0%	0	n.d.

52	H64V/F43Y	0.2%	0	n.d.
53	H64V/F43D	0.0%	0	n.d.
54	H64V/F43A	0.0%	0	n.d.
55	H64V/F43S	0.0%	0	n.d.
56	H64V/I107N	0.0%	0	n.d.
57	H64V/I107K	0.0%	0	n.d.
58	H64V/I107E	0.0%	0	n.d.
59	H64V/I107D	0.0%	0	n.d.
60	H64V/I107V	0.4%	1	n.d.
61	H64V/I107H	0.0%	0	n.d.
62	H64V/I107W	0.0%	0	n.d.
63	H64V/I107T	0.0%	0	n.d.
64	H64V/I107P	0.0%	0	n.d.
65	H64V/I107L	0.0%	0	n.d.
66	H64V/V68D	0.0%	0	n.d.
67	H64V/V68T	0.2%	0	n.d.
68	H64V/V68S	0.3%	0	n.d.
69	H64V/V68W	0.4%	1	-23
70	H64V/V68E	0.0%	0	n.d.
71	H64V/V68N	0.0%	0	n.d.
72	H64V/V68R	0.0%	0	n.d.
73	H64V/V68C	0.5%	1	11
74	H64V/L29P	0.0%	0	n.d.
75	H64V/L29M	0.0%	0	n.d.
76	H64V/L29A	0.3%	0	n.d.
77	H64V/L29Y	0.0%	0	n.d.
78	H64V/L29F	0.5%	1	−7
79	H64V/L29N	0.0%	0	n.d.
80	H64V/L29H	0.1%	0	n.d.
81	H64V/L29G	0.4%	1	n.d.
82	H64V/L29Q	0.0%	0	n.d.

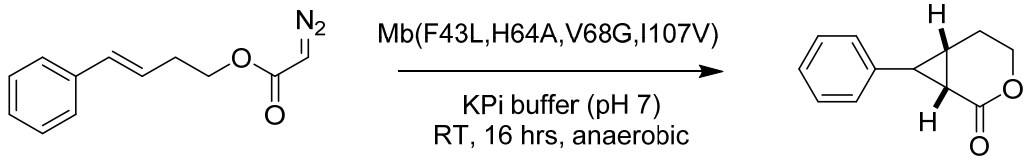
Table S3 Activity and stereoselectivity of selected Mb variants identified during the catalyst optimization process (**Figures 2 and 3** in main text) toward the intramolecular cyclopropanation of **1a**. Reaction conditions: 2.5 mM **1a**, 20 μM purified protein in 0.5 mL KPi buffer (50 mM, pH 7), room temperature, 16 hours in anaerobic chamber. Positive and negative % *ee* values refer to formation of (1*S*,6*S*,7*S*)-isomer **2a** and (1*R*,6*R*,7*R*)-isomer **3a**, respectively, as the major enantiomer.



1a **2a**

Mutations	Yield (GC)	TON	*% ee* (1*S*,6*S*,7*S*)
- (WT)	0%	0	n.d.
V68G	1.1%	1	16
H64A/V68G	1.9%	2	33
H64A/V68G/I107V	17%	21	61
F43L/H64A/V68G/I107V	63%	79	99
H64L/V68G	1.5%	2	-31
H64L/V68G/I107F	24%	30	-89
F43H/H64L/V68G/I107F	43%	54	-99
H64A/V68G/I107F	37%	46	-74
F43Y/H64A/V68G/I107F	69%	86	-98

Table S4. Optimization studies for Mb(F43L,H64A,V68G,I107V) -catalyzed intramolecular cyclopropanation of **1a** in reactions with purified protein or Mb-expressing *E. coli* cells (C41(DE3)). Reaction conditions: 1-10 mM **1a**, 20 μM purified protein or Mb-expressing *E. coli* cells (C41(DE3)) at the indicated cell density (OD_{600}) in 0.5 mL KPi buffer (50 mM, pH 7), room temperature, 16 hours in anaerobic chamber.

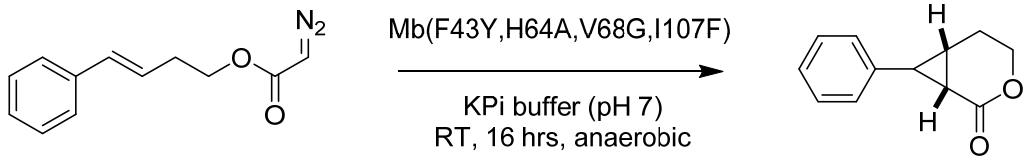


1a **2a**

Catalyst	Protein/cell conc.	[1a] (mM)	Yield (GC)	TON	*e.e.* (1S, 6S, 7S)
Purified protein	20 μM	1	99%	49	99%
Purified protein	20 μM	2.5	63%	79	99%
Purified protein	20 μM	5	38%	96	99%
Purified protein	20 μM	10	22%	112	99%
Whole cells	OD = 5	1	>99%	506	99%
Whole cells	OD = 5	2.5	92%	1164	99%
Whole cells	OD = 5	5	50%	1253	99%
Whole cells	OD = 5	10	14%	709	98%
Whole cells	OD = 10	1	>99%	253	99%
Whole cells	OD = 10	2.5	94%	595	99%
Whole cells	OD = 10	5	71%	898	99%
Whole cells	OD = 10	10	28%	697	99%
Whole cells	OD = 20	1	>99%	127	99%

Whole cells	OD = 20	2.5	>99%	316	99%
Whole cells	OD = 20	5	66%	417	99%
Whole cells	OD = 20	10	42%	535	99%
Whole cells	OD = 40	1	>99%	63	99%
Whole cells	OD = 40	2.5	87%	137	99%
Whole cells	OD = 40	5	80%	253	99%
Whole cells	OD = 40	10	46%	292	99%

Table S5. Optimization studies for Mb(F43Y, H64A,V68G, I107F) -catalyzed intramolecular cyclopropanation of **1a** in reactions with purified protein or Mb-expressing *E. coli* cells (C41(DE3)). Reaction conditions: 1-10 mM **1a**, 20 μM purified protein or Mb-expressing *E. coli* cells (C41(DE3)) at the indicated cell density (OD_{600}) in 0.5 mL KPi buffer (50 mM, pH 7), room temperature, 16 hours in anaerobic chamber.



1a **2a**

Catalyst	Protein/cell conc.	[1a] (mM)	Yield (GC)	TON	*e.e.* (1R, 6R, 7R)
Purified protein	5 μM	1	70%	140	98%
Purified protein	5 μM	2.5	21%	105	98%
Purified protein	5 μM	5	9.2%	92	98%
Purified protein	10 μM	1	82%	82	98%
Purified protein	10 μM	2.5	36%	90	98%
Purified protein	10 μM	5	16%	78	98%
Purified protein	20 μM	1	>99%	50	98%
Purified protein	20 μM	2.5	69%	86	98%
Purified protein	20 μM	5	37%	93	98%
Whole cells	OD = 5	1	70%	436	98%
Whole cells	OD = 5	2.5	21%	328	97%
Whole cells	OD = 5	5	9.2%	289	97%
Whole cells	OD = 5	10	5.2%	326	96%

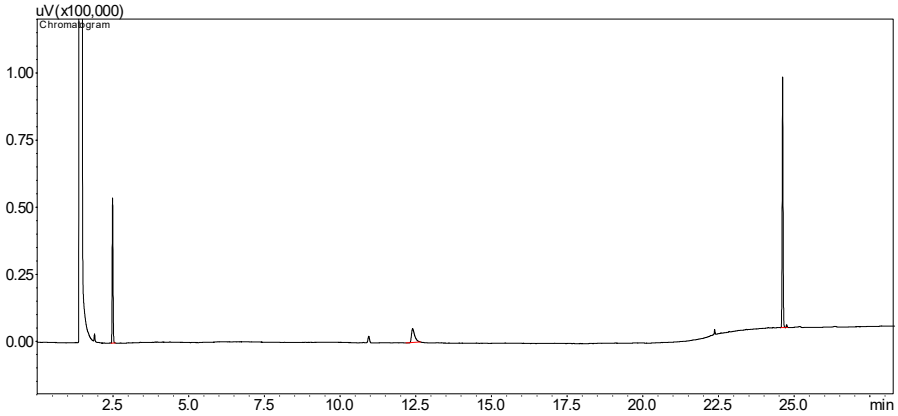
Whole cells	OD = 10	1	82%	257	98%
Whole cells	OD = 10	2.5	36%	280	98%
Whole cells	OD = 10	5	16%	243	97%
Whole cells	OD = 10	10	6%	186	97%
Whole cells	OD = 20	1	93%	145	98%
Whole cells	OD = 20	2.5	42%	163	97%
Whole cells	OD = 20	5	20%	156	96%
Whole cells	OD = 20	10	8.2%	128	96%
Whole cells	OD = 40	1	97%	77	98%
Whole cells	OD = 40	2.5	66%	129	98%
Whole cells	OD = 40	5	45%	174	98%
Whole cells	OD = 40	10	15%	118	98%

Table S6. Oligonucleotide used for site saturation mutagenesis

Oligonucleotide	**sequence (5' – 3')**
XhoI Rev	GGCTTTGTTAGCAGCCGGAT
L29NNK Fwd	GTCACGGTCAGGACATCNNKATCCGTCTGTTC
F43NNK Fwd	CAC CCG GAAACCCTGGAAAAANNKGACCGTTTC
H64NNK Fwd	GAAGGCTTCTGAAGACCTGAAAAAANNKGGTGTTACCG
V68NNK Fwd	CCTGAAAAAACACGGTGTTACCNNKCTGACCGCT
I107NNK Fwd	CCCGATCAAATACCTGGAGTTCNNKTCTGAAGCTATC

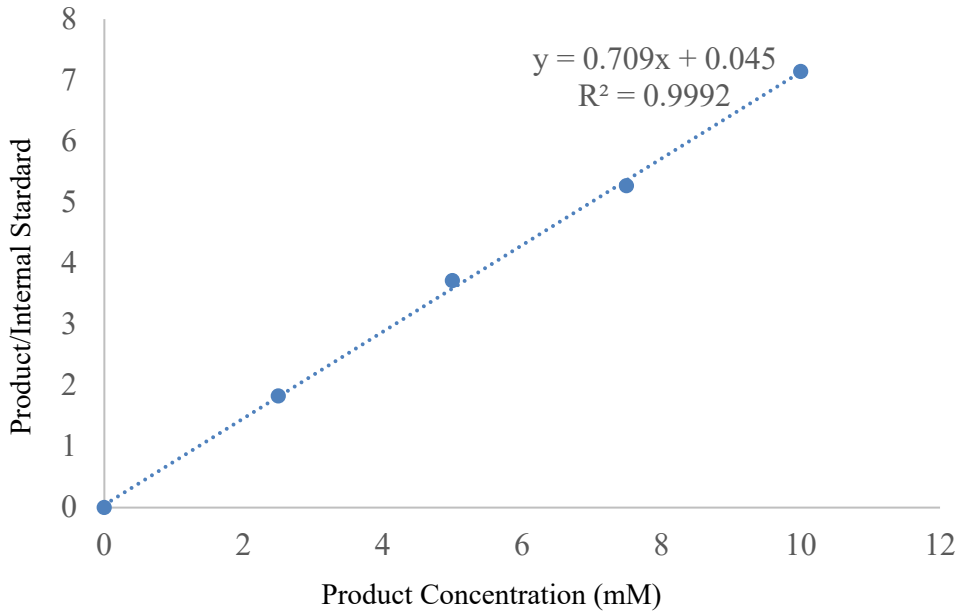
Figure S1. Full chiral GC chromatograms of Mb(F43L, H64A, V68G, I107V)-catalyzed reactions with the homoallylic diazoacetates. Calibration curves were prepared using isolated product and conversions were calculated from the ratio of the peak areas corresponding to the products and internal standard.

- Enzymatic reaction of **1a** (2.5 mM)

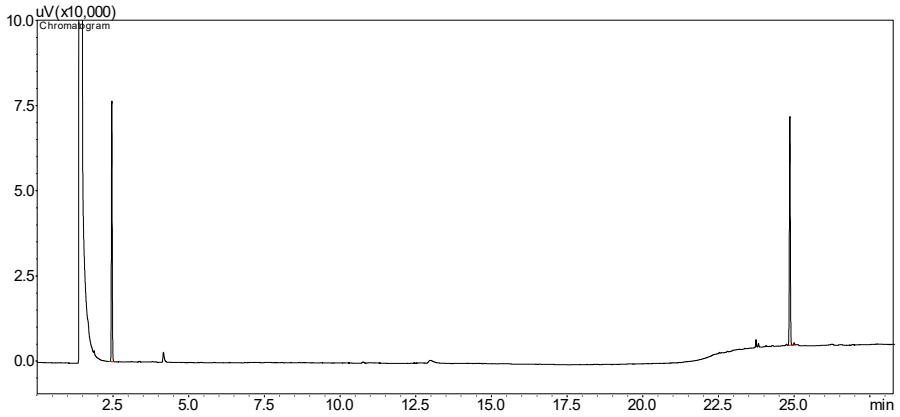


I.S.	107012.2
Products	189708.7
Conversion	>99%

Calibration curve:

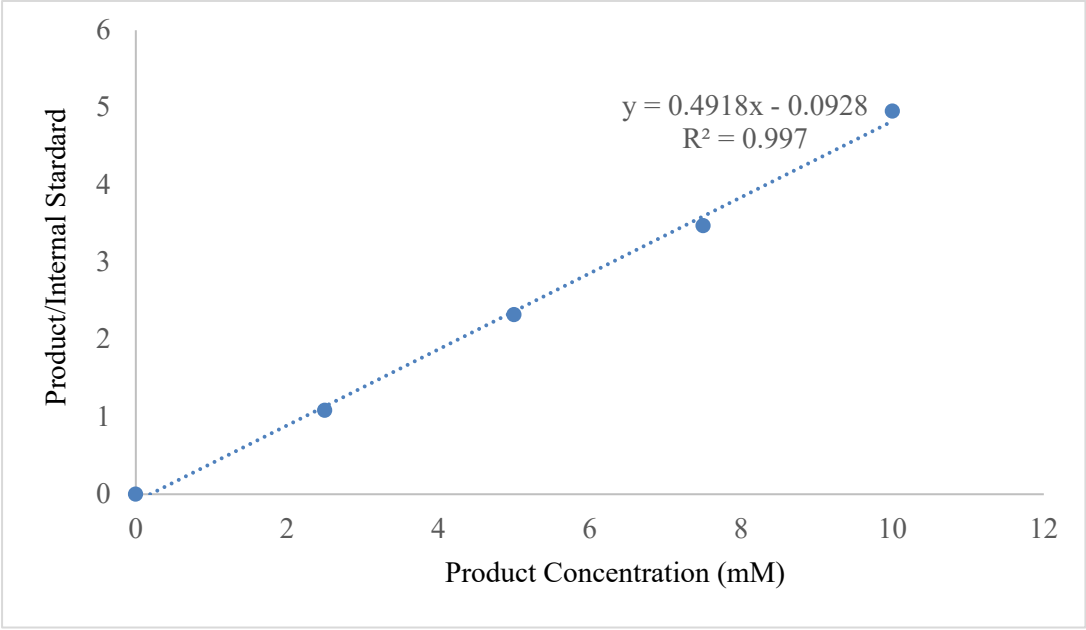


- Enzymatic reaction of **1b** (2.5 mM)

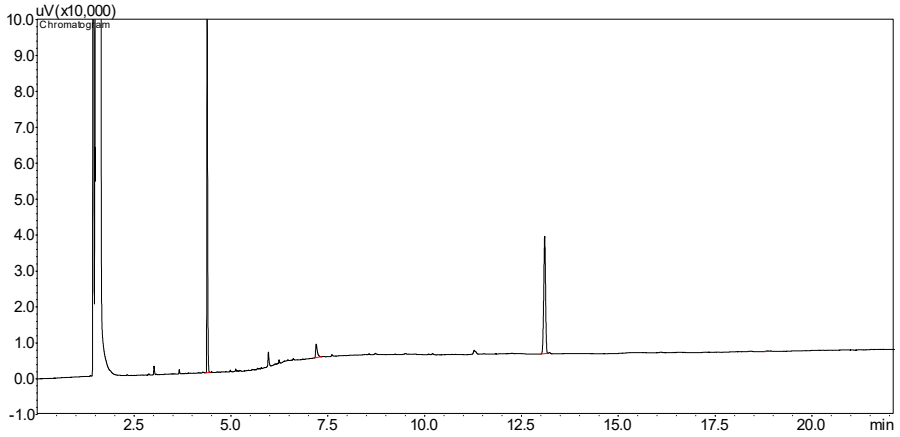


I.S.	146773.2
Products	141761.4
Conversion	79%

Calibration curve:

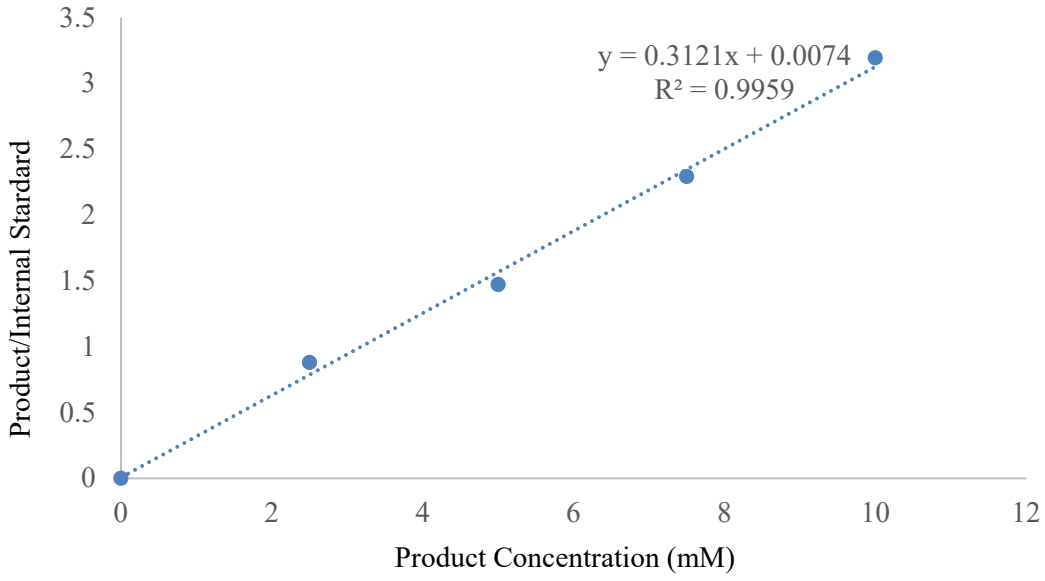


- Enzymatic reaction of **1c** (2.5 mM)

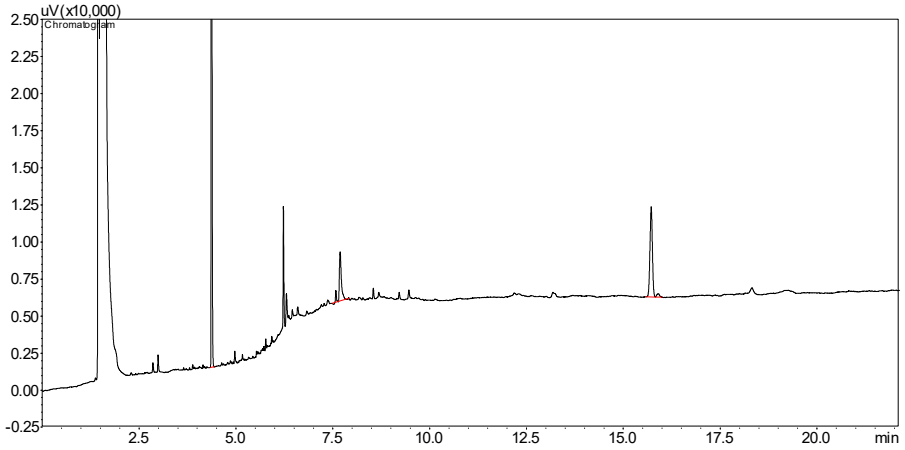


I.S.	154606.4
Products	106145.0
Conversion	88%

Calibration curve:

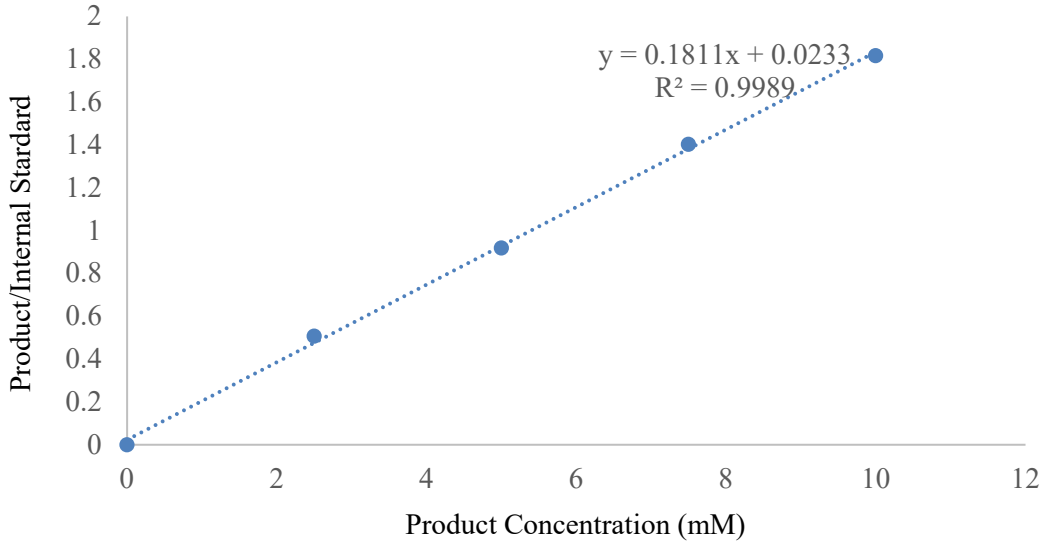


- Enzymatic reaction of **1d** (2.5 mM)

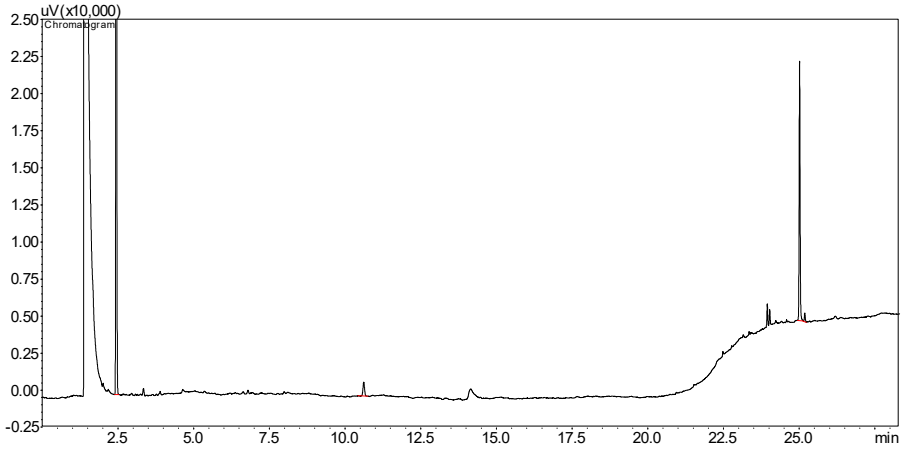


I.S.	109028.7
Products	26324.8
Conversion	54%

Calibration curve:

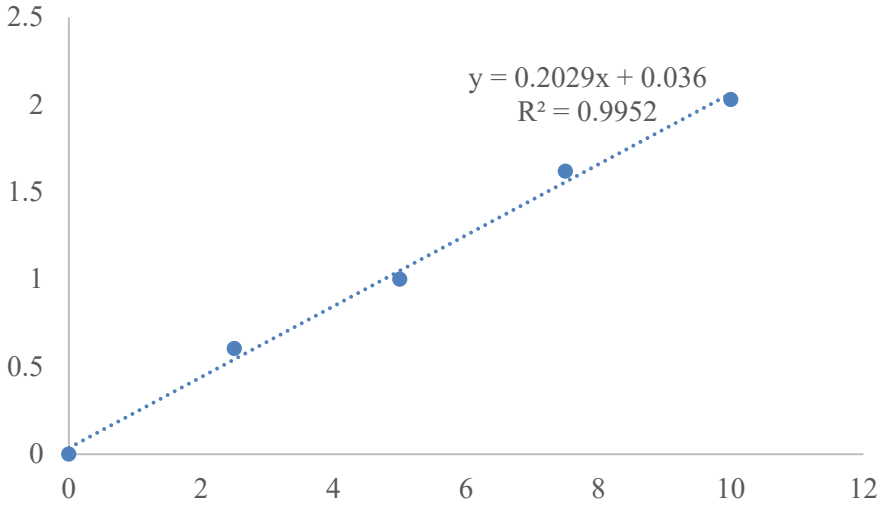


- Enzymatic reaction of **1e** (2.5 mM)

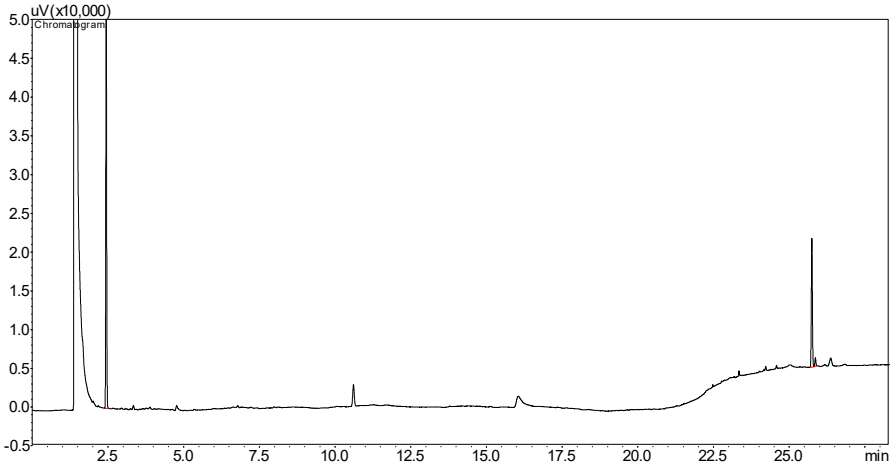


I.S.	136336.7
Products	38088.0
Conversion	55%

Calibration curve:

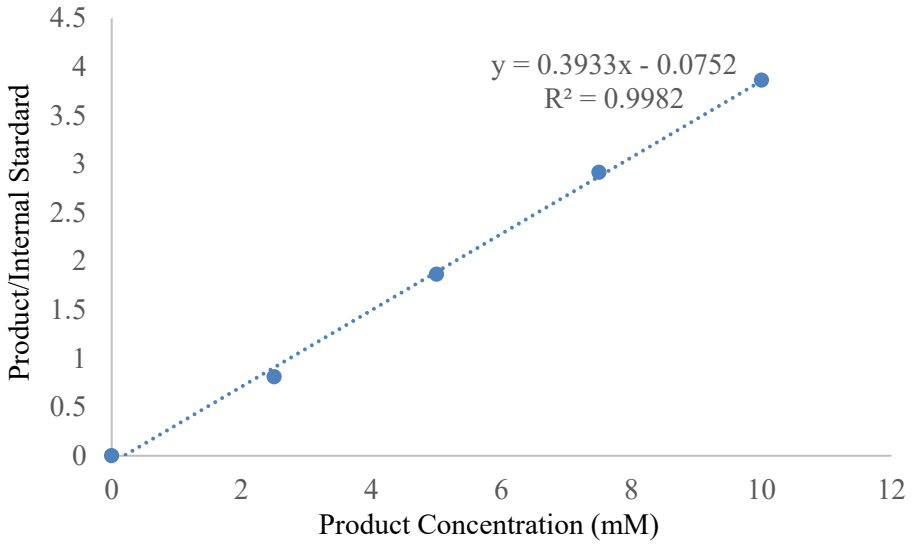


- Enzymatic reaction of **1f** (2.5 mM)

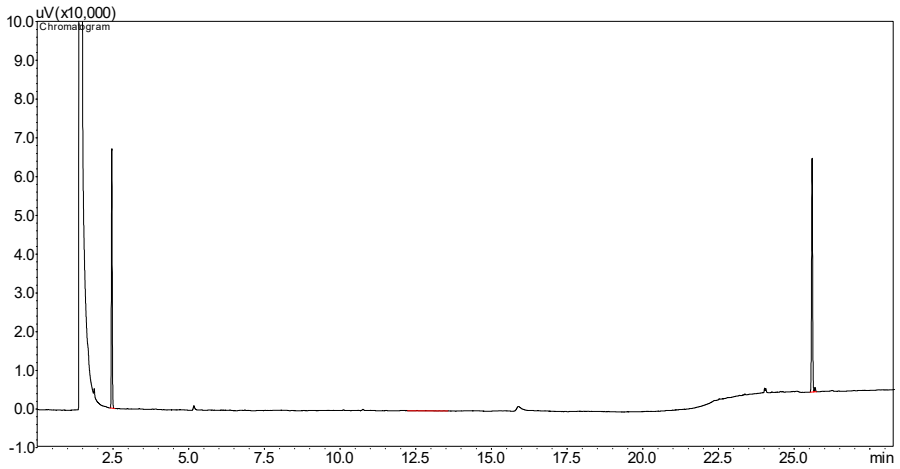


I.S.	111830.7
Products	39503.0
Conversion	36%

Calibration curve:

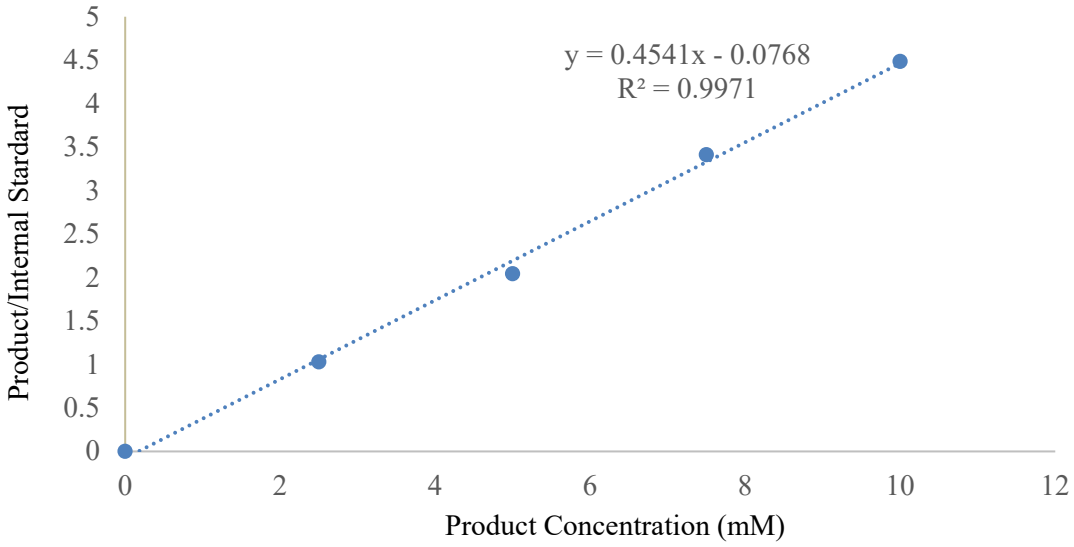


- Enzymatic reaction of **1g** (2.5 mM)

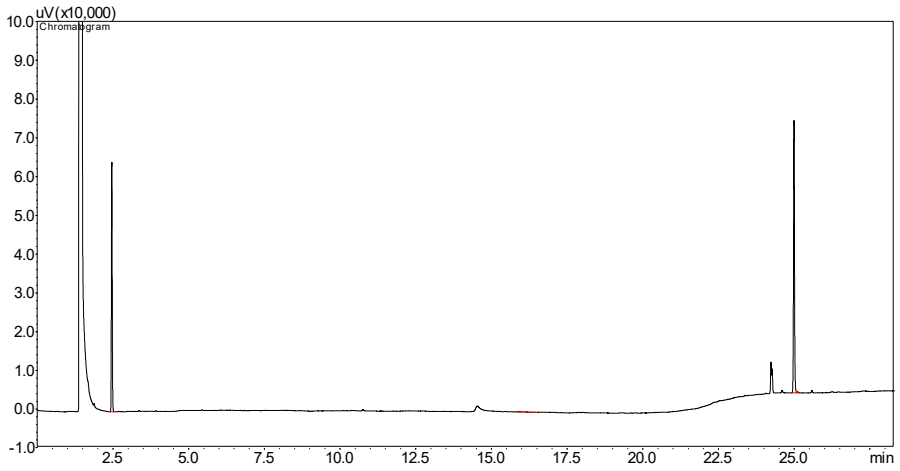


I.S.	128891.2
Products	139934.9
Conversion	95%

Calibration curve:

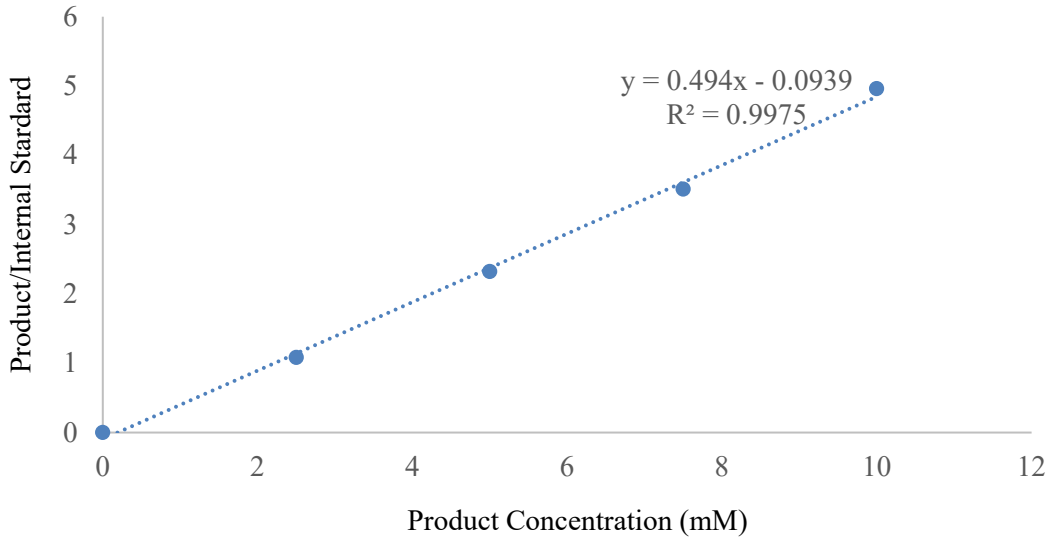


- Enzymatic reaction of **1h** (2.5 mM)

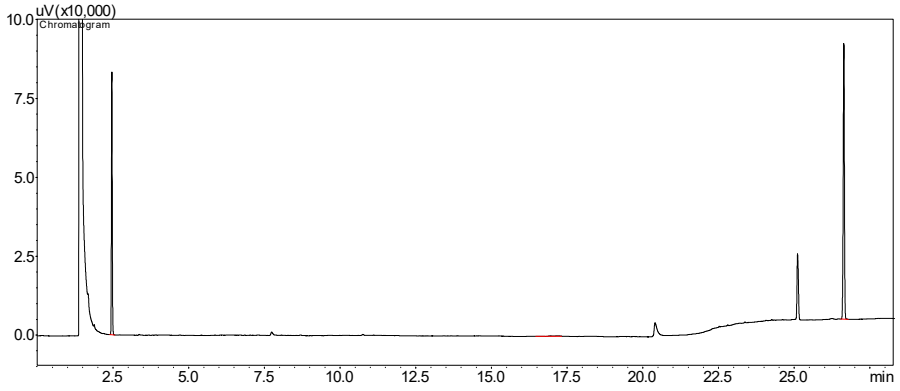


I.S.	123900.1
Products	156088.1
Conversion	99%

Calibration curve:

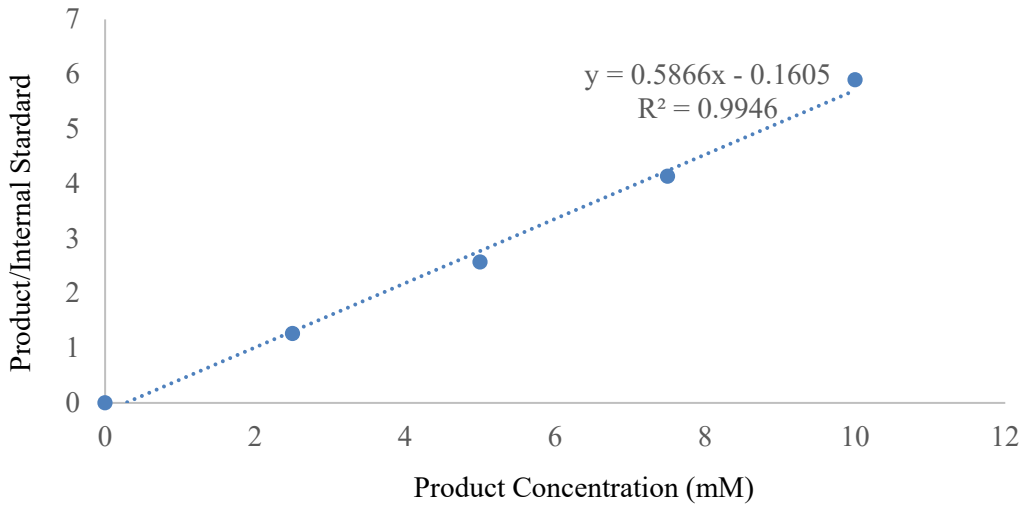


- Enzymatic reaction of **1i** (2.5 mM)

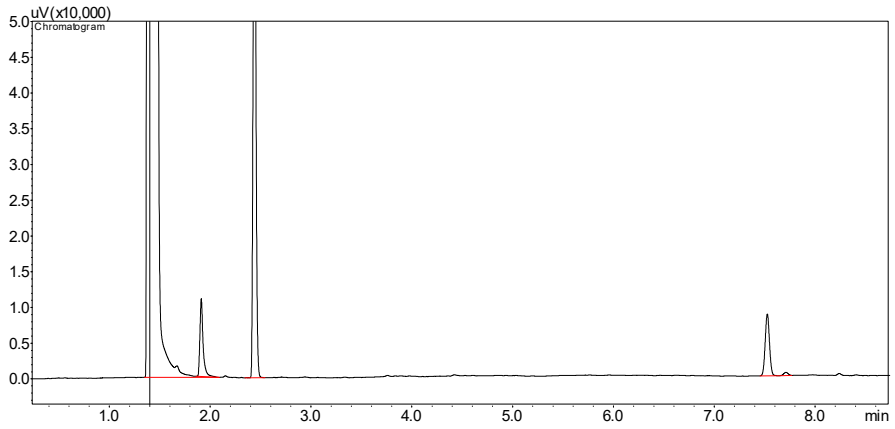


I.S.	162053.8
Products	239348.2
Conversion	99%

Calibration curve:



- Enzymatic reaction of **1j** (1 mM)



I.S.	172074.5
Products	25495.7
Conversion	41%

Calibration curve:

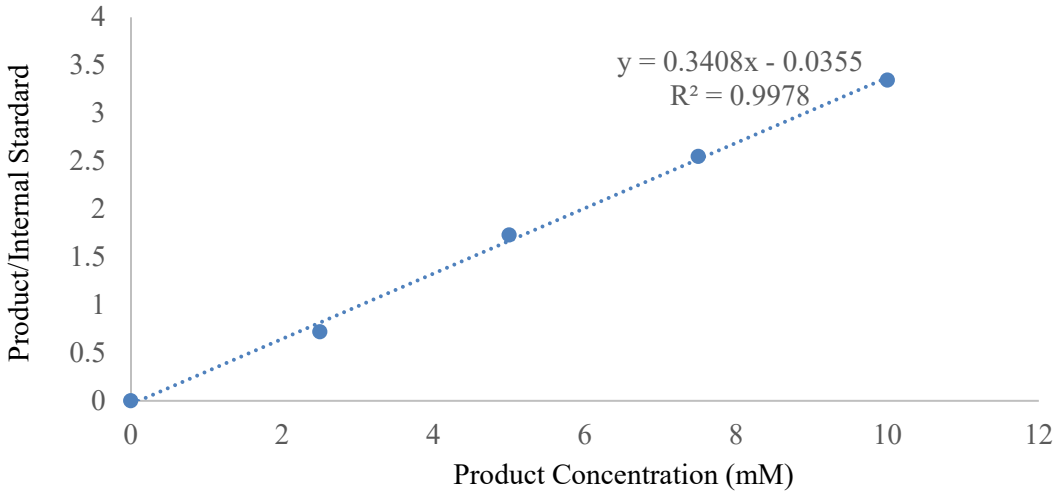
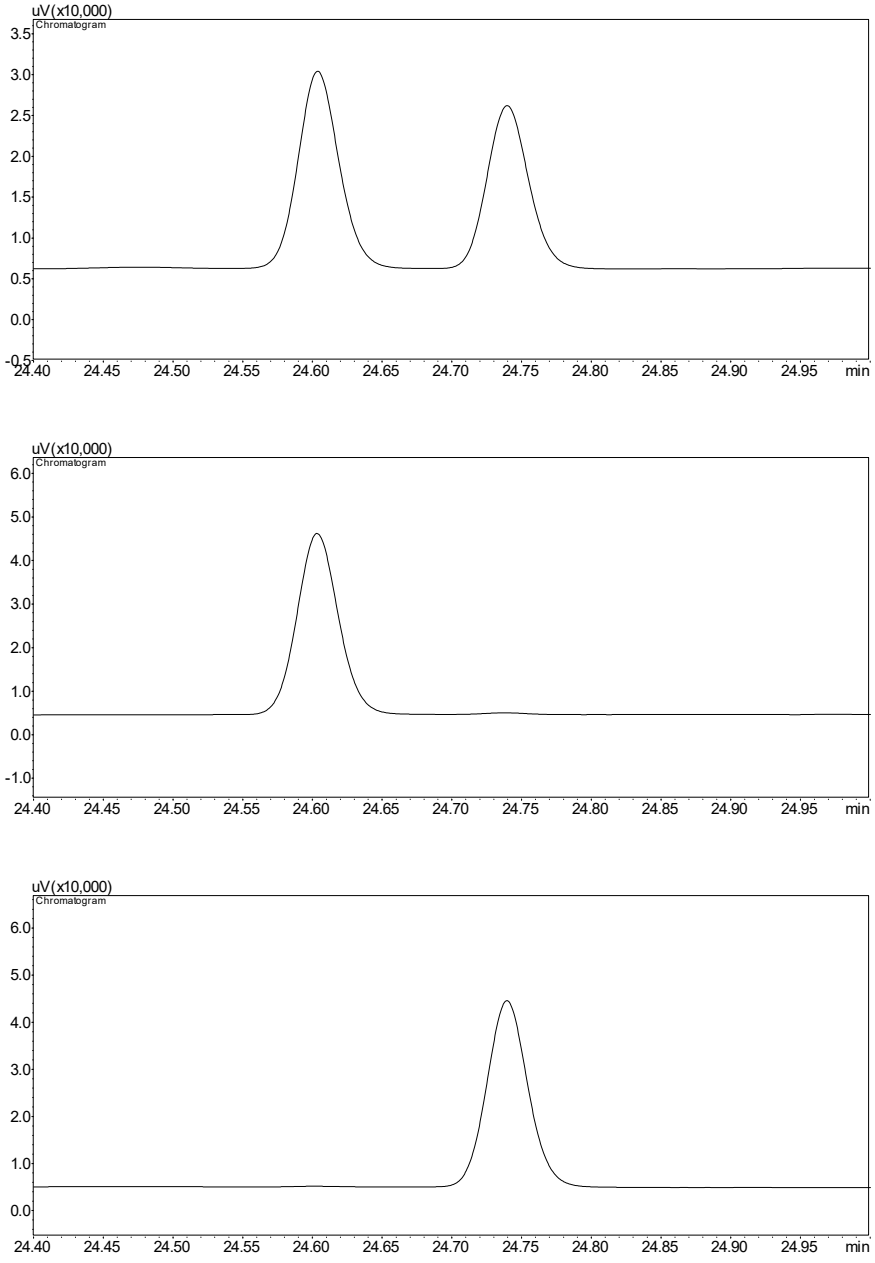
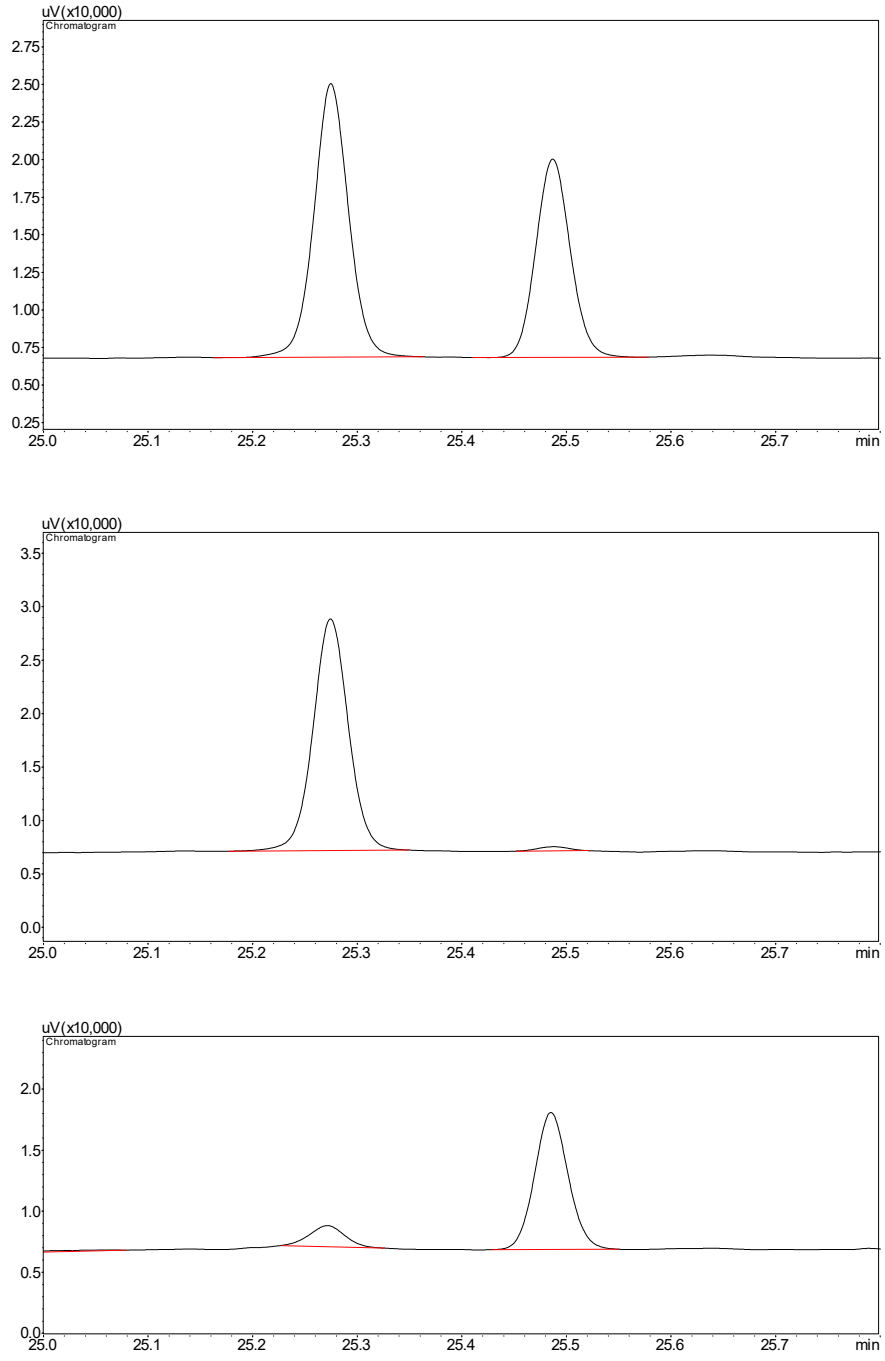


Figure S2. Chiral GC analysis for the determination of an enantiomeric excess in the Mb-catalyzed intramolecular cyclopropanation reactions. The reference racemic samples were prepared with engineered myoglobin variant as described in the following figures.

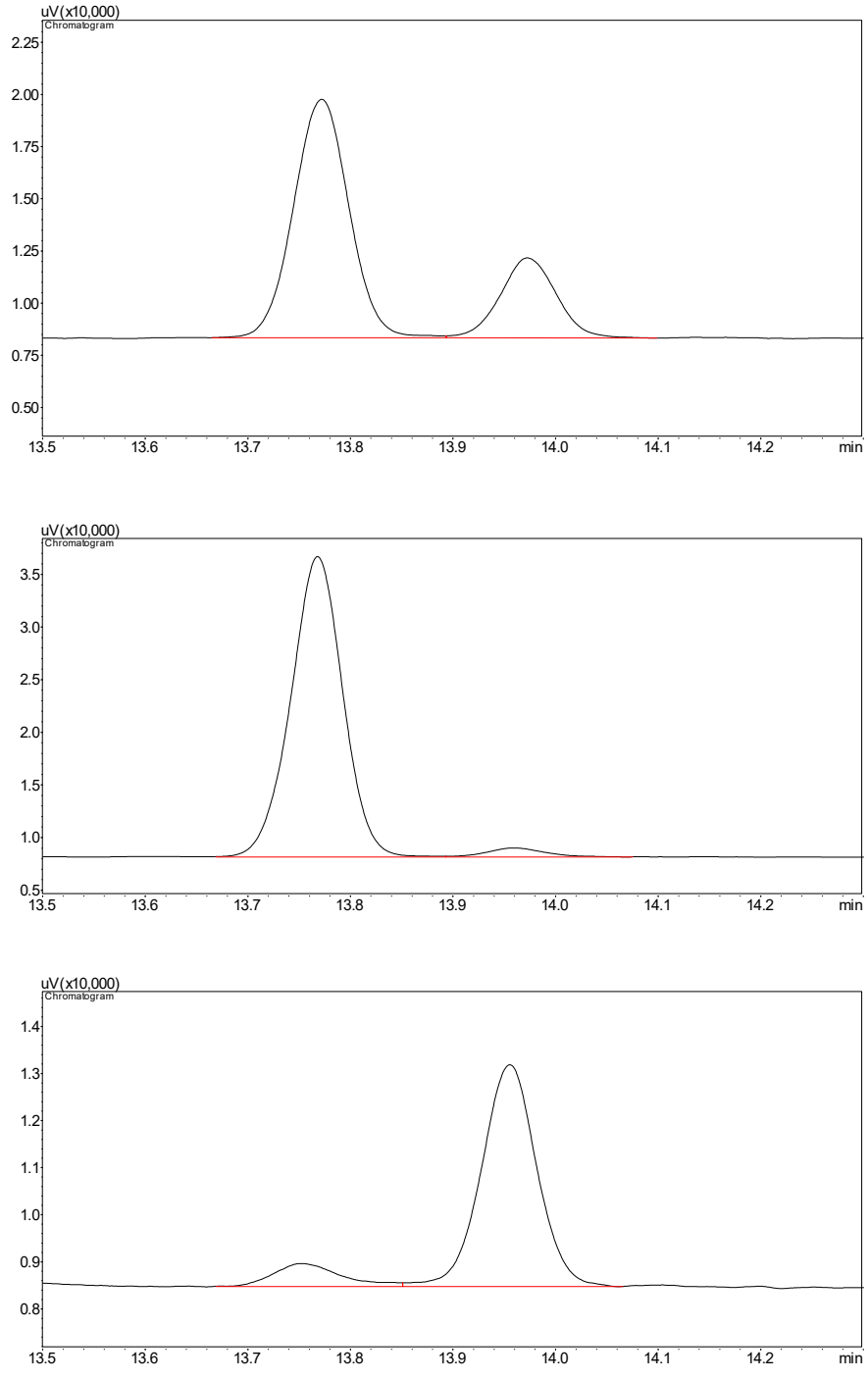
- Chiral GC analysis of racemic **2a** + **3a** (using Mb(H64A,V68G,I107L; *top*) and enantioenriched **2a** (using Mb(F43L, H64A, V68G, I107V); *middle*) and enantioenriched **3a** product (using Mb(F43H,H64L,V68G,I107F); *bottom*):



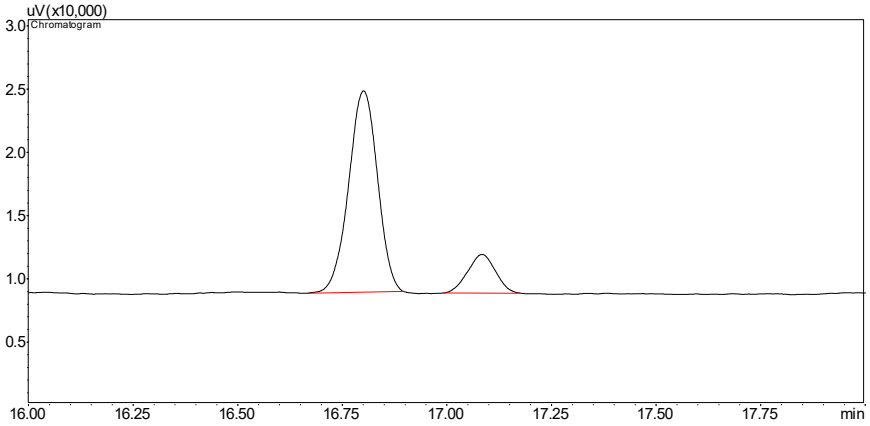
- Chiral GC analysis of racemic (Mb-H64A/V68G/I107L catalyzed protein reaction) **2b**/**3b** (*top*) and enzymatically produced **2b** (using Mb(F43L, H64A, V68G, I107V); *middle*) and **3b** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):

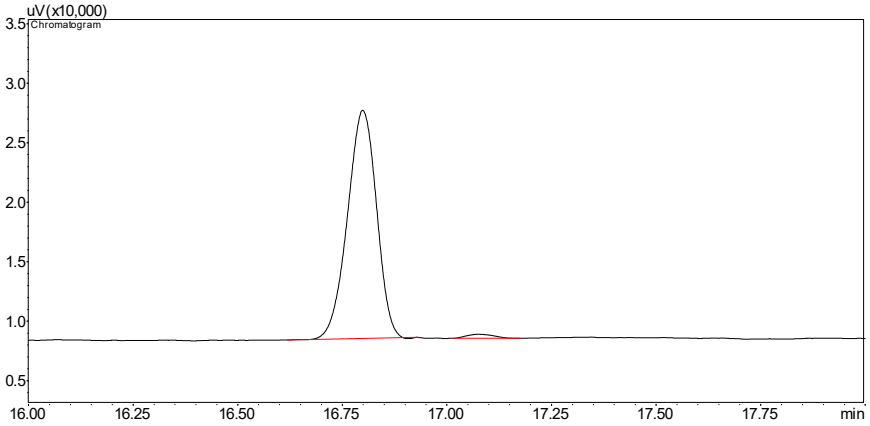


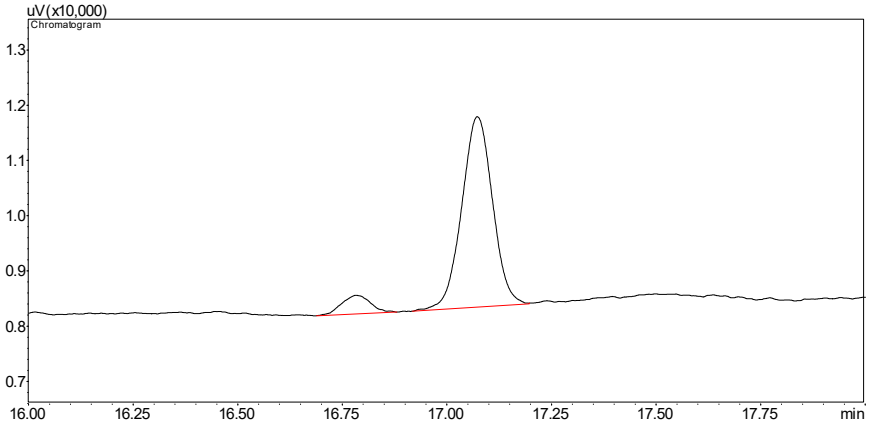
- Chiral GC analysis of racemic (Mb-H64A/V68G/I107L catalyzed protein reaction) **2c/3c** (*top*) and enzymatically produced **2c** (using Mb(F43L, H64A, V68G, I107V); *middle*) and **3c** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):



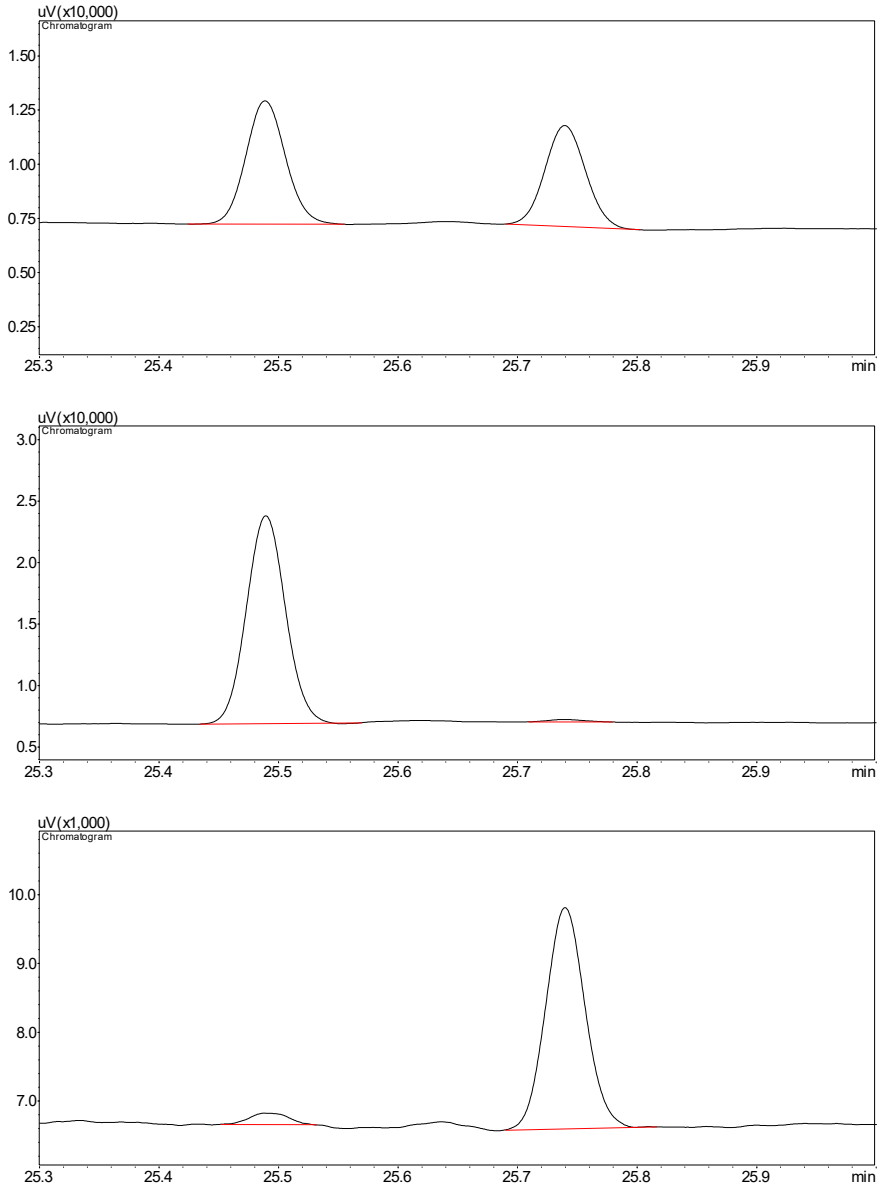
- Chiral GC analysis of racemic (Mb-H64A/V68G/I107L catalyzed protein reaction) **2d**/**3d** (*top*) and enzymatically produced **2d** (using Mb(F43L, H64A, V68G, I107L); *middle*) and **3d** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):



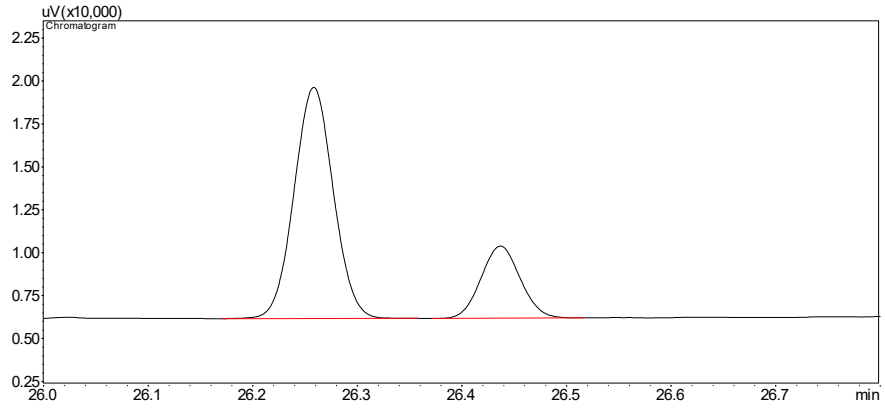


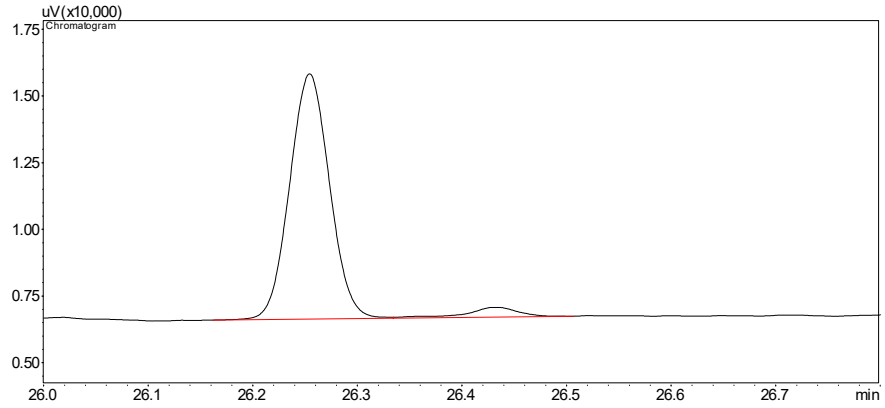


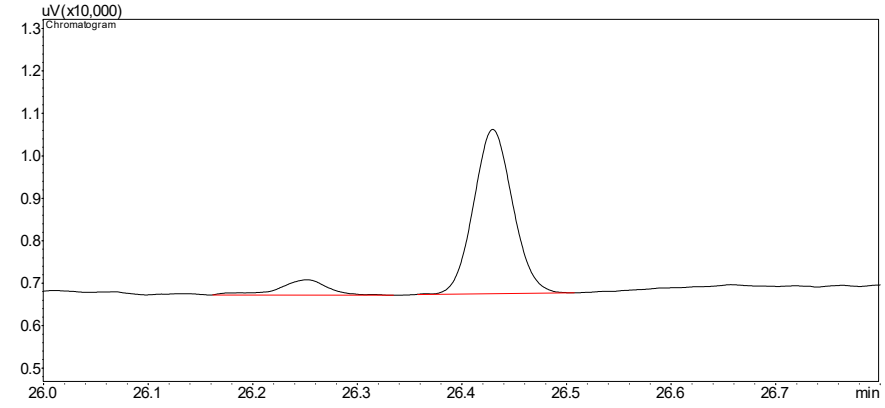
- Chiral GC analysis of racemic (Mb-H64A/V68G/I107F catalyzed protein reaction) **2e**/**3e** (*top*) and enzymatically produced **2e** (using Mb(F43L, H64A, V68G, I107L); *middle*) and **3e** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):



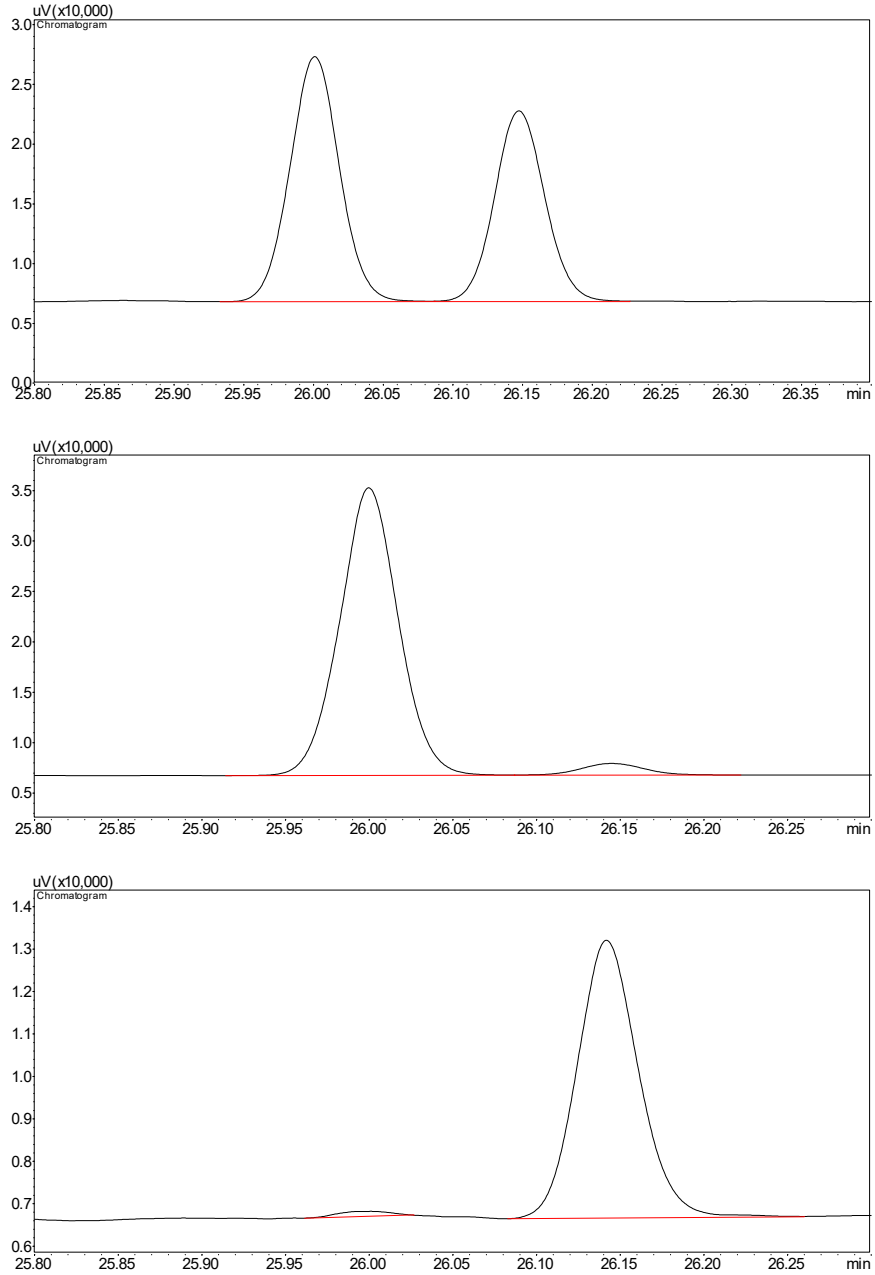
- Chiral GC analysis of racemic (Mb-H64A/V68G/I107L catalyzed protein reaction) **2f**/**3f** (*top*) and enzymatically produced **2f** (using Mb(F43L, H64A, V68G, I107L); *middle*) and **3f** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):



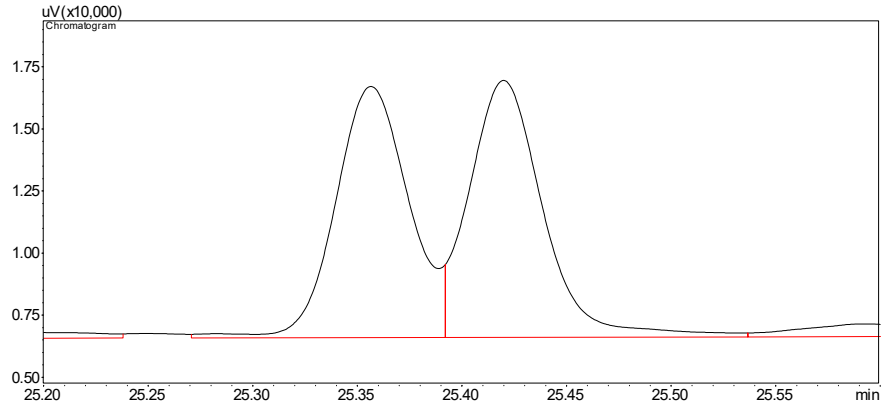


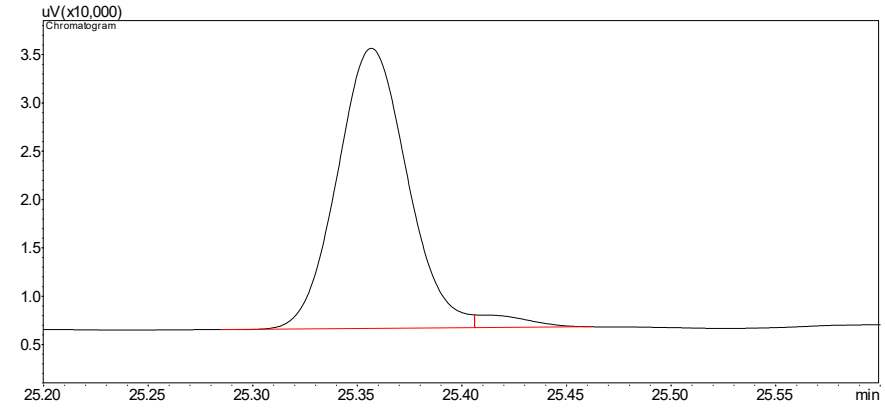


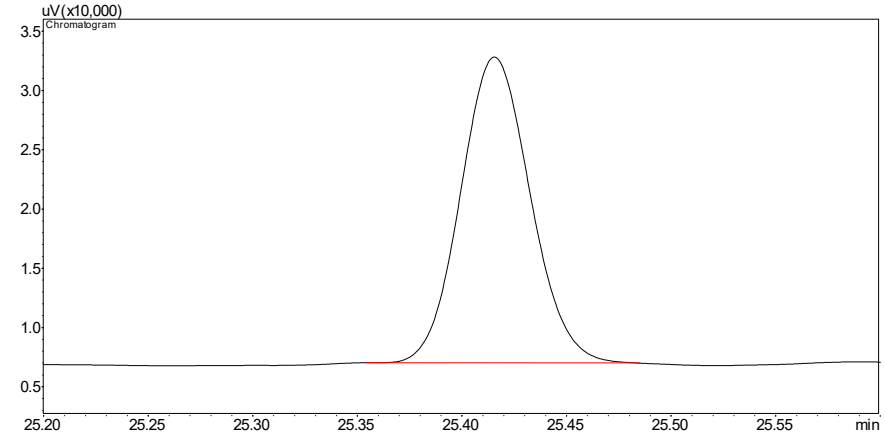
- Chiral GC analysis of racemic (Mb-H64A/V68G/I107V catalyzed protein reaction) **2g**/**3g** (*top*) and enzymatically produced **2g** (using Mb(F43L, H64A, V68G, I107L); *middle*) and **3g** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):



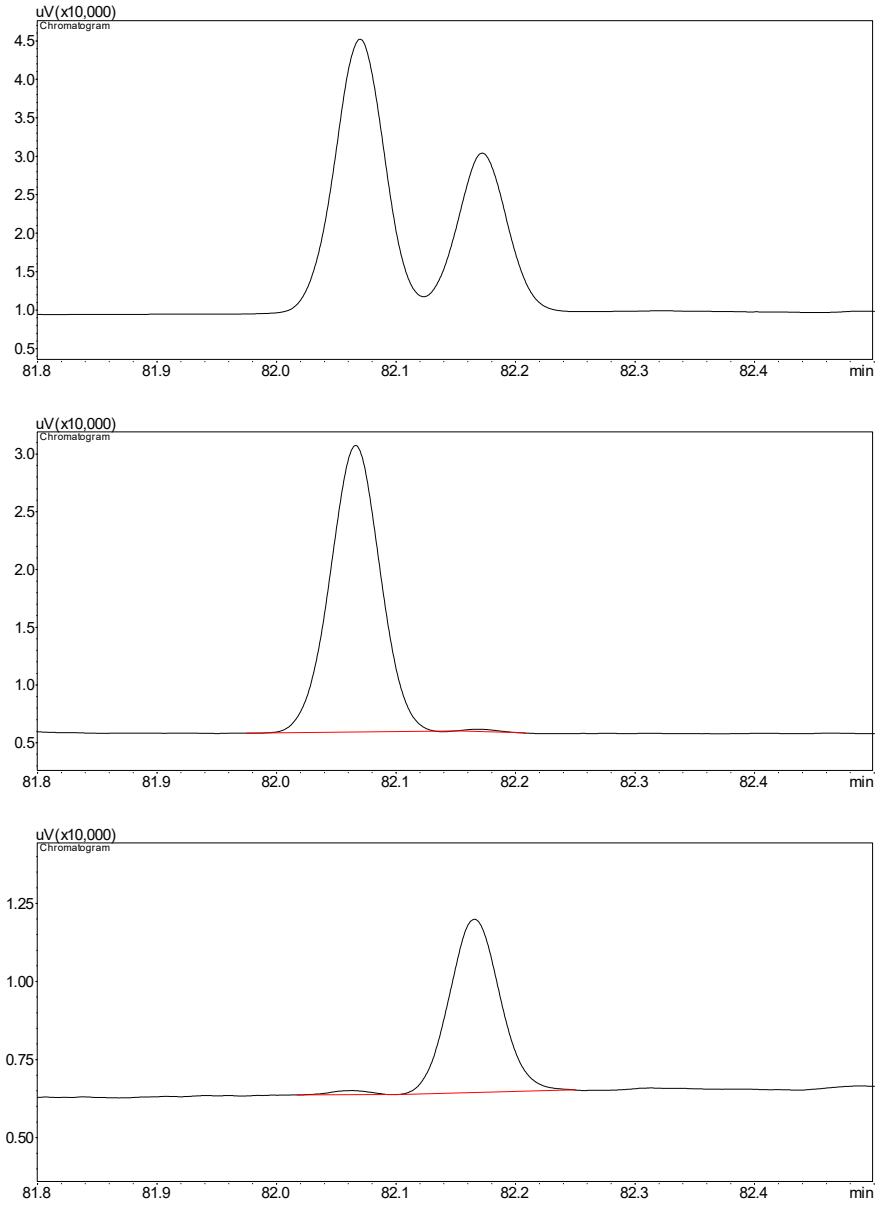
- Chiral GC analysis of racemic (Mb-H64A/V68G/I107L catalyzed protein reaction) **2h**/**3h** (*top*) and enzymatically produced **2h** (using Mb(F43L, H64A, V68G, I107L); *middle*) and **3h** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):



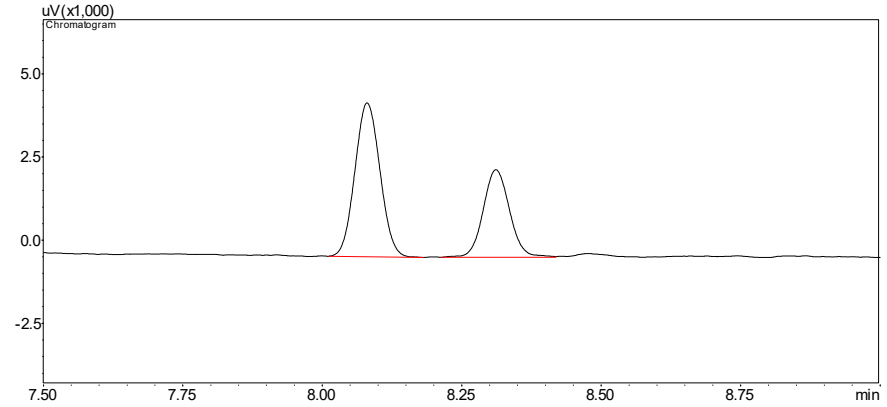


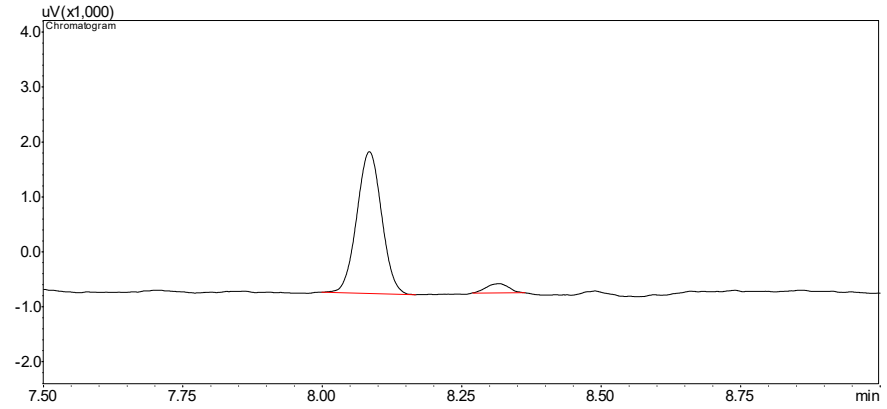


- Chiral GC analysis of racemic (Mb-H64A/V68G/I107V catalyzed protein reaction) **2i**/**3i** (*top*) and enzymatically produced **2i** (using Mb(F43L, H64A, V68G, I107L); *middle*) and **3i** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):



- Chiral GC analysis of racemic (Mb-H64W/V68G/I107F catalyzed protein reaction) **2j**/**3j** (*top*) and enzymatically produced **2j** (using Mb(F43L, H64A, V68G, I107L); *middle*) and **3j** product (using Mb(F43Y,H64A,V68G,I107F); *bottom*):





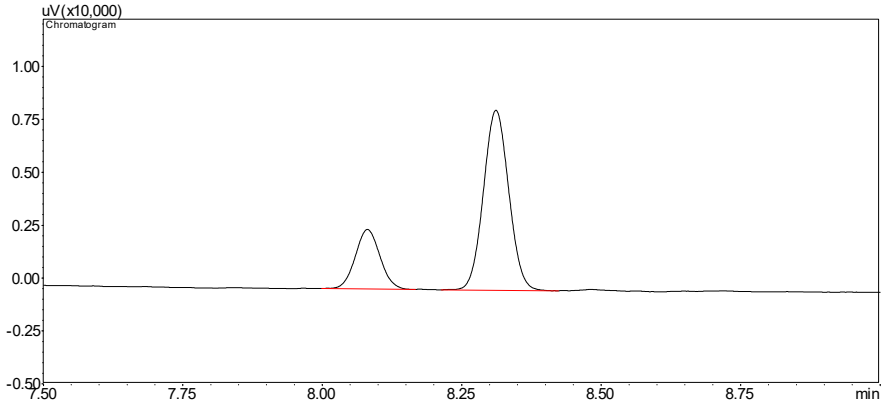


Figure S3. ORTEP of (1S,6S,7S)-7-(4-(trifluoromethyl)phenyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2e)** with ellipsoids drawn at the 50% probability level. Hydrogen atoms were located in the difference Fourier map and refined freely. They are represented here as spheres of arbitrary radius for clarity.

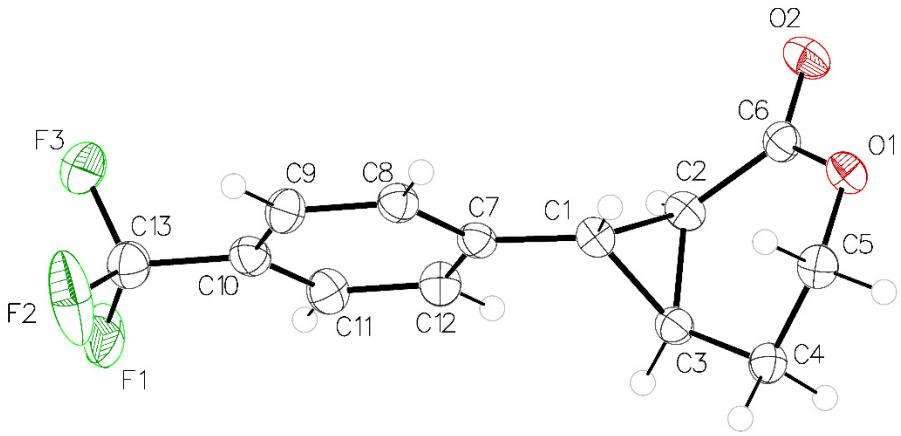
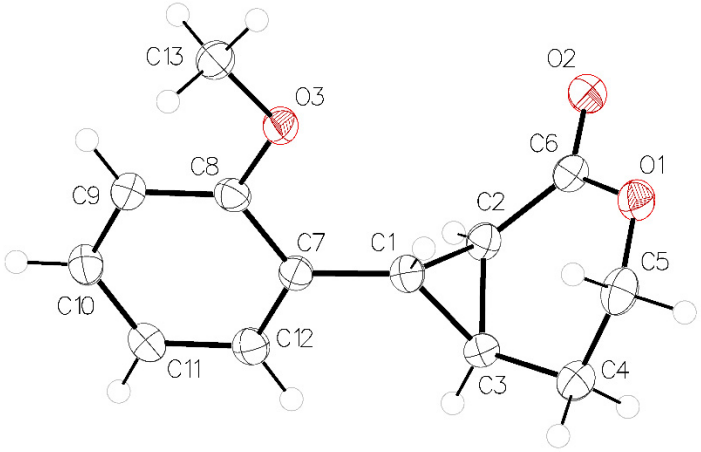


Figure S4. ORTEP of (1S,6S,7S)-7-(2-methoxyphenyl)-3-oxabicyclo[4.1.0]heptan-2-one (**2i**) with ellipsoids drawn at the 50% probability level. Hydrogen atoms were located in the difference Fourier map and refined freely. They are represented here as spheres of arbitrary radius for clarity.



Experimental Procedures

General Information

All chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, AK Scientific, Alfa Aesar, TCI, Acros) and used without any further purification, unless otherwise stated. ^{1}H, and ^{13}C NMR spectra were measured on a Bruker DPX-500 instrument (operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C). Tetramethylsilane (TMS) served as the internal standard (0 ppm) for ^{1}H NMR and $CDCl_3$ was used as the internal standard (77.0 ppm) for ^{13}C NMR. Flash column chromatography purification was carried out using AMD Silica Gel 60 Å 230-400 mesh or Alumina, (Fisher adsorption) 80-200 mesh. Thin Layer Chromatography (TLC) was carried out using Merck Millipore TLC silica gel 60 F254 glass plates.

Protein Expression

Cloning procedures of the Mb variants investigated in this work were described previously.[1, 2] The oligonucleotides used for site saturation mutagenesis are shown in **Table S6**. The Mb variants were expressed in *E. coli* BL21(DE3) or C41(DE3) cells as follows. After transformation, cells were grown in TB medium (ampicillin, 100 mg L^{-1}) at 37 °C (200 rpm) until OD_{600} reached 0.6. Cells were then induced with 0.25 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) and 0.3 mM δ-aminolevulinic acid (ALA). After induction, cultures were shaken at 180 rpm and 27 °C and harvested after 20 h by centrifugation at 4,000 rpm at 4 °C. Myoglobin concentration was determined after cell lysis by sonication, followed by CO-binding assay using an extinction coefficient ε_{424} = 187 $mM^{-1}cm^{-1}$.

Protein engineeering

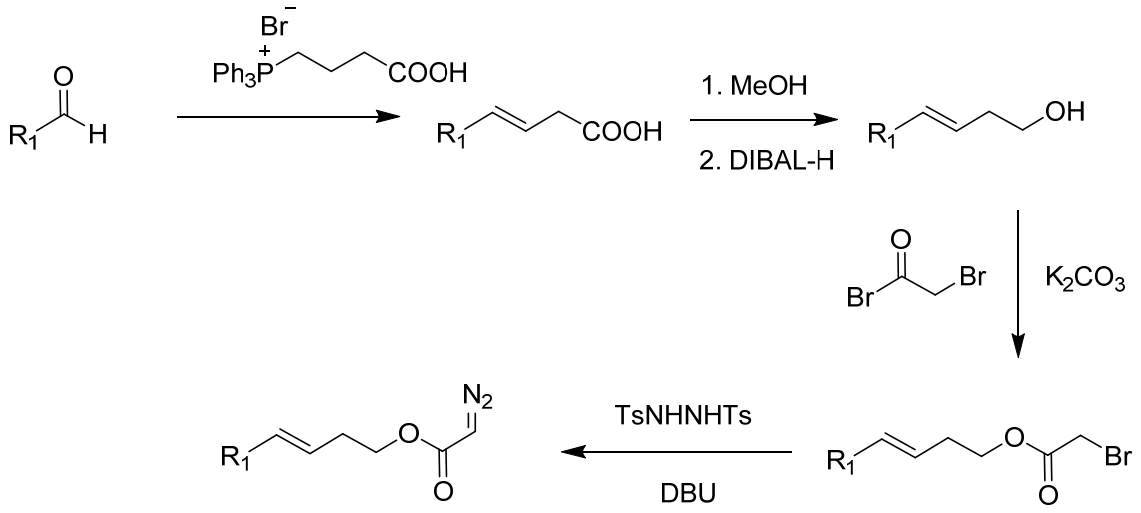
The protein evolution was conducted through iterative rounds of site saturation mutagenesis. In each round, the site-saturation library of Mb variants was constructed and transformed into *E.coli* DH5α cells. The colonies were collected in LB medium (ampicillin, 100 mg L^{-1}) and plasmids were extracted by QIAprep Spin Miniprep Kit. The library coverage was assessed by DNA sequencing. The library of Mb variants was then transformed into *E.coli* DH5α cells and expressed in 96-well plates under the conditions described above. After expression, the cells were pelleted by centrifuge and resuspended in potassium phosphate buffer (50 mM, pH 7).

The reactions were carried out by adding the substrate to each well of the plate and by shaking the plates for 5 hours at room temperature in an anaerobic chamber. The reactions mixtures were extracted by DCM and analyzed by chiral GC-FID. The Mb variant that showed improved activity and enantioselectivity was sequenced and used as template for next round of mutagenesis and screening.

Synthetic Procedures:

Synthesis of *trans*-homoallylic diazoacetates:

All diazo-compounds were synthesized by the following procedures. Firstly, benzaldehyde substrates were reacted with 2-carboxyethyl triphenylphosphonium bromide to form *trans*-styrylacetic acids as major products. The acids then underwent esterification and reduction to give the corresponding alcohols, which were converted to *trans*- homoallylic diazoacetates by the reported procedures.



General Procedure A: Synthesis of *trans*-styrylacetic acid from benzaldehyde (Wittig r eaction):

To a solution of (2-carboxyethyl)triphenylphosphonium bromide (2.7g, 6.6 mmol, 1.1 eq.) in tetrahydrofuran (20 ml), a solution of sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran,

13.2 ml, 13.2 mmol, 2.2 eq.) was added slowly under argon in ice bath. The solution was stirred for 30 min, then cooled to -78 °C. Benzaldehyde (6 mmol, 1 eq.) was then added slowly and stirred overnight. The reaction mixture was warmed up to room temperature prior to the addition of water and diethyl ether. The aqueous layer was washed with ethyl acetate three times and subsequently was acidified with 1 M hydrochloric acid to pH =1, which was then extracted three times with ethyl acetate. The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was used in the next step without further purification.

General Procedure B: Synthesis of *trans*-homoallylic alcohol from carboxylic acid

To a solution of *trans*-homoallylic carboxylic acid (1.0 equiv) in methanol, conc. H_2SO_4 solution was added slowly. The reaction was heated under reflux for 5 h, then was cooled to room temperature and concentrated in *vacuo*. The residue was neutralized with saturated $NaHCO_3$ aqueous solution, extracted with ethyl acetate three times. The combined organic layer was then washed with brine, dried over $MgSO_4$, filtered, and concentrated in *vacuo* to obtain the homoallylic ester which was used in next step without any further purification.

To a solution of the homoallylic ester (1.0 equiv) in dry DCM at −78 °C under argon, DIBAL-H (1.0 M in hexane, 2.3 equiv) was added in dropwise. The reaction was stirred at −78 °C until the reaction was completed (2-6 h). The reaction mixture was diluted with 1 M HCl and then warmed to room temperature and stirred overnight. The aqueous layer was extracted with DCM three times. The combined organic phase was washed with 1 M HCl, sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated in *vacuo* to afford *trans*-homoallylic alcohol.

General Procedure C: Synthesis of trans-homoallylic diazoacetate from *trans*-homoallylic alcohol:

To a solution of *trans*-homoallylic alcohol (3 mmol) and K_2CO_3 (2.08 g, 15 mmol) in DCM (15.0 mL), bromoacetyl bromide (780 µl, 9 mmol) was added slowly at 0 °C and stirred for 30 min. The reaction mixture was quenched with H_2O and extracted with DCM three times. The organic phase was washed with brine and dried over $MgSO_4$. The solvent was removed in *vacuo* and the obtained bromoacetate residue was used in the next step without any future purification. To the solution of the resulting bromoacetate and N',N-ditosylhydrazine (2.04 g, 6.0 mmol) in THF (15.0 mL), DBU (2.28 mL, 15 mmol) was added dropwise at 0 °C and stirred for 30 min. Reaction was quenched

by aqueous saturated solution of $NaHCO_3$. Reaction mixture was extracted with Et_2O three times. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to give crude diazoacetate. The crude product was purified by column chromatography on silica gel with ethyl acetate/hexanes to afford the desired *trans*-homoallylic diazoacetate product.

General Procedure D: Biocatalytic intramolecular cyclopropanation reactions using whole cells expressing myoglobin on preparative scale:

These reactions were carried out on a 80 mL-scale using Mb(F43L,H64A,V68G,I107V) (otherwise mentioned) expressing *E. coli* cells, 2.5 mM of diazoacetate. In a typical procedure, the homoallylic diazoacetate (0.2 mmol in 1 mL of ethanol) was added slowly to a 125 mL Erlenmeyer flask containing a suspension of Mb-expressing cells (OD_{600} = 20 in KPi, pH 7) under argon pressure, equipped with a magnetic stir bar and sealed with a rubber septum. Reaction mixture stirred at room temperature for 3-5 hours. The reaction mixtures were extracted with ethyl acetate (80 mL x 3) and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The TON for the whole-cell reactions were calculated based on Mb concentration in the reaction mixture as measured via UV-vis spectroscopy using the CO-binding assay (ε_{424} = 187 $mM^{-1}cm^{-1}$) after cell lysis. The crude product was purified by flash column chromatography using silica gel and ethyl acetate/hexanes as the eluent to isolate the intramolecular cyclopropanation product. The purified product was characterized by NMR, GC-MS, and chiral SFC or GC for stereoselectivity determination and they were used as authentic standards for the construction of the calibration curves (TON and % conversion determination).

General Procedure E: Synthesis of racemic standards by Engineered Mb variants.

Under standard reaction conditions, 500 µL scale reactions were carried out using 20 µM engineered Mb variant, 2.5 mM diazoacetate, and 10 mM sodium dithionite. In a typical procedure, a solution containing Mb in potassium phosphate buffer (50 mM, pH 7.0) with sodium dithionite was prepared in an anaerobic chamber. Reactions were initiated by addition of 10 µL of diazoacetate from 0.5 M stock solutions, and the reaction mixtures were stirred in the chamber for 12 h at room temperature. Reaction mixtures were extracted by using DCM and used for analysis.

Reaction Analysis

The reactions were analyzed by adding 25 μL of internal standard (benzodioxole, 50 mM in methanol) to a 500 μL aliquot of the reaction mixture, followed by extraction with 500 μL dichloromethane (DCM) and centrifugation at 14,000 rpm. The organic layer was collected and analyzed by SFC or GC-FID. The TON for the whole-cell reactions were calculated based on Mb concentration in the reaction mixture as measured via UV-vis using the CO-binding assay (ε_{424} = 187 $mM^{-1}cm^{-1}$) after cell lysis. Calibration curves of the different intramolecular cyclopropane products were constructed using authentic standards from the whole cell reactions (procedure F). Enantioselectivity was determined by using SFC or GC-FID using a chiral column as described below.

Analytical Methods

Gas chromatography (GC) analysis were carried out using a Shimadzu GC-2010 gas chromatograph equipped with a FID detector, and a Cyclosil-B column (30 m x 0.25 mm x 0.25 μm film). The following GC methods were used for TON analysis and stereoisomer separation (% *ee* analysis)**,** 1 μL injection, injector temp.: 200 ºC, detector temp: 300 ºC.

Gradient for method A: column temperature set at 140 ºC for 3 min, then to 160 ºC at 1.8 ºC/min, then to 165 ºC at 1.0 ºC/min, then to 245 at 25 ºC/min, then 245 ºC for 6 min. Total run time was 28 min.

Gradient for method B: column temperature set at 160ºC for 2 min, then to 245 °C at 7 ºC/min, 245 ºC hold for 8 min. Total run time was 22 min.

Gradient for method C: column temperature set at 100ºC for 3 min, then to 140 ºC at 0.4 ºC/min then to 245 ºC at 25 ºC/min, 245 ºC hold for 2 min. Total run time was 109 min.

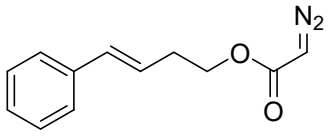
Table S7. Enantiomer resolution via chiral GC analysis.

Product	**Method**	**t_R for 1st isomer (min)**	**t_R for 2nd isomer (min)**
2a/3a	A	24.61	24.74
2b/3b	A	25.28	25.49
2c/3c	B	13.78	13.99

2d/3d	B	16.79	17.10
2e/3e	A	25.49	25.74
2f/3f	A	26.25	26.43
2g/3g	A	26.01	26.15
2h/3h	A	25.36	25.42
2i/3i	C	82.07	82.18
2j/3j	A	8.05	8.31

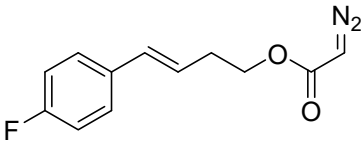
Compound Characterization Data

(E)-4-phenylbut-3-en-1-yl 2-diazoacetate (1a):



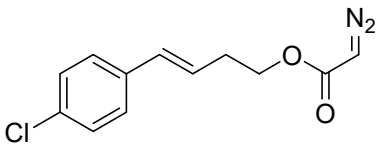
(E)-4-phenylbut-3-en-1-yl 2-diazoacetate (**1a**) was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 – 7.11 (m, 5H), 6.48 (d, *J* = 15.9 Hz, 1H), 6.17 (dt, *J* = 15.9, 6.8 Hz, 1H), 4.75 (s, 1H), 4.29 (t, *J* = 6.7 Hz, 2H), 2.57 (dt, *J* = 6.8, 6.3 Hz, 2H). ^{13}C NMR (126 MHz, CDCl3) δ 137.4, 132.7, 128.7, 127.5, 126.2, 125.5, 64.2, 46.3, 32.7. Carbonyl carbon is not observed.

(E)-4-(4-fluorophenyl)but-3-en-1-yl 2-diazoacetate (1b):



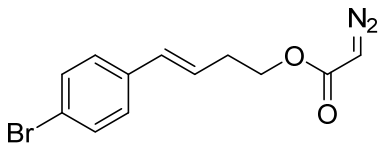
(E)-4-(4-fluorophenyl)but-3-en-1-yl 2-diazoacetat (**1b**) was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 – 7.23 (m, 2H), 7.11 – 6.92 (m, 2H), 6.43 (d, *J* = 15.7 Hz, 1H), 6.07 (dt, *J* = 15.7, 6.8 Hz, 1H), 4.75 (s, 1H), 4.27 (t, *J* = 6.7 Hz, 2H), 2.55 (dt, *J* = 6.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) 133.5, 131.5, 127.7 (d, *J* = 4.57 Hz), 125.3, 115.6 (d, *J* = 20.1 Hz), 64.1, 46.4, 32.7. Carbonyl carbon and F-C* carbon are not observed. ^{19}F NMR (376 MHz, $CDCl_3$) δ -113.2.

(E)-4-(4-chlorophenyl)but-3-en-1-yl 2-diazoacetate (1c):



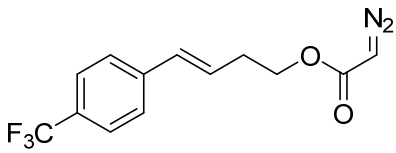
(E)-4-(4-chlorophenyl)but-3-en-1-yl 2-diazoacetate (**1c**) was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (s, 4H), 6.43 (d, *J* = 15.9 Hz, 1H), 6.12 (dt, *J* = 15.9, 7.2 Hz, 1H), 4.74 (s, 1H), 4.27 (t, *J* = 6.7 Hz, 2H), 2.54 (dt, *J* = 7.2, 6.7 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.1, 131.5, 128.8, 127.5, 126.3, 64.0, 46.4, 32.7. Carbonyl carbon is not observed

(E)-4-(4-bromophenyl)but-3-en-1-yl 2-diazoacetate (1d):



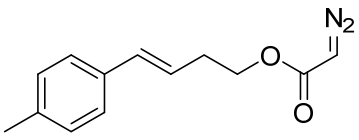
(E)-4-(4-bromophenyl)but-3-en-1-yl 2-diazoacetate (**1d**) was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 6.40 (d, *J* = 15.9 Hz, 1H), 6.15 (dt, *J* = 15.9, 6.7 Hz, 1H), 4.75 (s, 1H), 4.28 (t, *J* = 6.6 Hz, 2H), 2.55 (dt, *J* = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 131.8, 131.5, 127.8, 126.4, 121.1, 63.9, 46.3, 32.7.

(E)-4-(4-(trifluoromethyl)phenyl)but-3-en-1-yl 2-diazoacetate (1e):



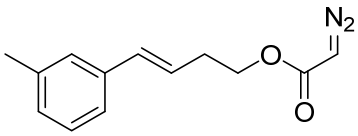
(E)-4-(4-(trifluoromethyl)phenyl)but-3-en-1-yl 2-diazoacetate **(1e)** was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 6.50 (d, *J* = 15.9 Hz, 1H), 6.27 (dt, *J* = 15.9, 6.9 Hz, 1H), 4.75 (s, 1H), 4.30 (t, *J* = 6.6 Hz, 2H), 2.59 (dt, *J* = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.8, 133.5, 131.5, 129.2 (q, *J* = 34.64 Hz), 128.5, 126.4, 125.6 (q, *J* = 3.36 Hz), 124.4 (q, *J* = 270.64 Hz), 63.8, 46.4, 32.7. Carbonyl carbon is not observed. ^{19}F NMR (376 MHz, $CDCl_3$) δ -63.3.

(E)-4-(p-tolyl)but-3-en-1-yl 2-diazoacetate (1f):



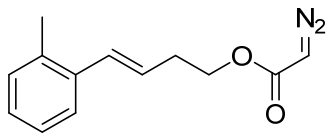
(E)-4-(*p*-tolyl)but-3-en-1-yl 2-diazoacetate **(1f)** was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 6.44 (d, *J* = 15.8 Hz, 1H), 6.09 (dt, *J* = 15.8, 6.7 Hz, 1H), 4.75 (s, 1H), 4.27 (t, *J* = 6.7 Hz, 2H), 2.55 (dt, *J* = 6.7 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.2, 134.6, 132.6, 129.4, 126.1, 124.4, 64.3, 46.4, 32.7, 21.3. Carbonyl carbon is not observed

(E)-4-(m-tolyl)but-3-en-1-yl 2-diazoacetate (1g):



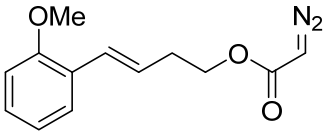
(E)-4-(*m*-tolyl)but-3-en-1-yl 2-diazoacetate **(1g)** was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.01 (m, 4H), 6.46 (d, *J* = 15.8 Hz, 1H), 6.16 (dt, *J* = 15.8, 6.9 Hz, 1H), 4.76 (s, 1H), 4.29 (t, *J* = 6.6 Hz, 2H), 2.57 (dt, *J* = 6.6 Hz, 2H), 2.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 138.2, 137.3, 132.7, 128.5, 128.2, 126.9, 125.2, 123.3, 64.2, 46.3, 32.7, 21.5.

(E)-4-(o-tolyl)but-3-en-1-yl 2-diazoacetate (1h):



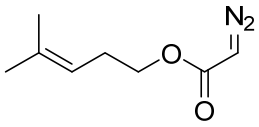
(E)-4-(o-tolyl)but-3-en-1-yl 2-diazoacetate (**1h**) was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.35 (m, 1H), 7.21 – 7.08 (m, 3H), 6.67 (d, *J* = 15.6 Hz, 1H), 6.03 (dt, *J* = 15.7, 7.0 Hz, 1H), 4.74 (s, 1H), 4.30 (t, *J* = 6.7 Hz, 2H), 2.58 (dt, *J* = 6.7 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.6, 135.2, 130.7, 130.3, 127.4, 126.9, 126.2, 125.8, 64.2, 46.3, 33.0, 19.9. Carbonyl carbon is not observed

(E)-4-(2-methoxyphenyl)but-3-en-1-yl 2-diazoacetate (1i):



(E)-4-(2-methoxyphenyl)but-3-en-1-yl 2-diazoacetate **(1i)** was prepared according to the general procedure for the synthesis of *trans*-homoallylic diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 7.4 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 6.92 (t, *J* = 7.2 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 15.9 Hz, 1H), 6.16 (dt, *J* = 15.9, 7.0 Hz, 1H), 4.74 (s, 1H), 4.29 (t, *J* = 6.6 Hz, 2H), 3.84 (s, 3H), 2.58 (dt, *J* = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 156.6, 139.2, 128.5, 127.5, 126.8, 126.1, 120.8, 110.9, 64.4, 55.6, 33.1, 31.7.

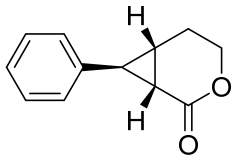
4-methylpent-3-en-1-yl 2-diazoacetate (1*J*):



4-methylpent-3-en-1-yl 2-diazoacetate **(1J)** was prepared from hydroxylation of 5-bromo-2-methyl-2-pentene, then the general procedure C for the synthesis of diazoacetate. Reaction mixture was purified by silica gel column chromatography with 10% EtOAc/hexanes as an eluent to give the desired product as pale-yellow. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.09 (t, *J* = 7.2 Hz, 1H), 4.73 (s, 1H), 4.12 (t, *J* = 7.0 Hz, 2H), 2.33 (dt, *J* = 7.0 Hz, 2H), 1.71 (s, 3H), 1.63 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.9, 119.2, 64.7, 46.3, 27.9, 25.9, 17.9. Carbonyl carbon is not observed.

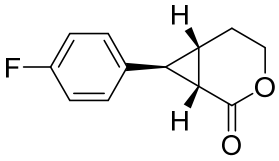
Data for the intramolecular cyclopropanation product synthesizes by using Mb-catalyst on preparative scale.

(1S,6S,7S)-7-phenyl-3-oxabicyclo[4.1.0]heptan-2-one (2a):



(1S,6S,7S)-7-phenyl-3-oxabicyclo[4.1.0]heptan-2-one **(2a)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) in 1200 mL to afford the product as a colorless oil, 85% yield (480 mg). GC-MS m/z (% relative intensity): 188(13.2), 144(16.8), 143(19.6), 130(12.0), 129(100), 128(37.0), 127(10.5), 115(21.3), 91(7.7), 77(8.5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.05 (m, 5H), 4.36 (dd, *J* = 12.0, 6.3 Hz, 1H), 4.27 (td, *J* = 12.5, 3.5 Hz, 1H), 2.93 (t, *J* = 4.1 Hz, 1H), 2.41 – 2.21 (m, 1H), 2.16 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 138.2, 128.9, 127.2, 126.4, 64.7, 26.7, 25.1, 23.8, 20.5.

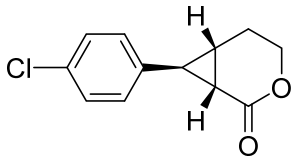
(1S,6S,7S)-7-(4-fluorophenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2b):



(1S,6S,7S)-7-(4-fluorophenyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2b)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a white solid, 65% yield (26.8 mg). GC-MS m/z (% relative intensity): 206(13.1), 162(21.4), 161(28.2), 148(12.1), 147(100), 146(36.5), 133(31.4), 127(12.9), 109(15.7); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 – 6.95 (m, 4H), 4.36 (dd, *J* = 12.2, 6.2 Hz, 1H), 4.25 (td, *J* = 12.6, 3.7 Hz, 1H), 2.91 (t, *J* = 4.5 Hz, 1H), 2.38 – 2.20 (m, 1H), 2.20 – 2.06 (m, 3H); ^{13}C NMR (101 MHz,

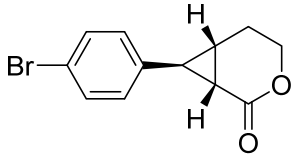
$CDCl_3$) δ 169.5, 133.9, 127.9, 115.8 (d, J = 22.3 Hz), 64.7, 26.6, 24.4, 23.6, 20.5. F-C* carbon is not observed; ^{19}F NMR (376 MHz, C_6D_6) δ -115.4.

(1S,6S,7S)-7-(4-chlorophenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2c):



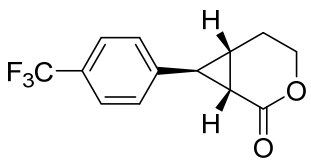
(1S,6S,7S)-7-(4-chlorophenyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2c)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a white solid, 71% yield (31.6 mg). GC-MS m/z (% relative intensity): 222(13.1), 163(14.9), 144(12.4), 143(100), 142(13.9), 141(13.1), 129(19.8), 128(78.0), 127(29.9), 115(27.6), 101(6.5), 89(8.4), 75(8.2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 4.35 (dd, J = 12.2, 6.2 Hz, 1H), 4.25 (td, J = 12.5, 3.5 Hz, 1H), 2.89 (t, J = 4.5 Hz, 1H), 2.37 – 2.21 (m, 1H), 2.18 – 2.08 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.3, 136.7, 132.9, 128.9, 127.7, 64.7, 26.7, 24.5, 23.8, 20.4.

(1S,6S,7S)-7-(4-bromophenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2d):



(1S,6S,7S)-7-(4-bromophenyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2d)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a white solid, 42% yield (22.4 mg). GC-MS m/z (% relative intensity): 268(13.1), 266(13.2), 207(7.1), 144(10.1), 143(81.8), 142(14.9), 141(12.5), 129(20.4), 128(100), 127(16.7), 115(27.0), 102(8.5), 89(7.7), 77(6.2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 4.35 (dd, J = 12.2, 6.2 Hz, 1H), 4.25 (td, J = 12.5, 3.6 Hz, 1H), 2.88 (t, J = 4.5 Hz, 1H), 2.27 (tdd, J = 13.8, 6.2, 3.1 Hz, 1H), 2.18 – 2.06 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 135.3, 129.9, 126.1, 118.9, 62.7, 24.7, 22.6, 21.8, 18.4.

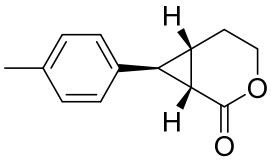
(1S,6S,7S)-7-(4-(trifluoromethyl)phenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2e):



(1S,6S,7S)-7-(4-(trifluoromethyl)phenyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2e)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a white solid, 40% yield (20.5 mg). GC-MS m/z (% relative

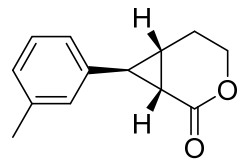
intensity): 256(4.3), 237(10.0), 212(16.3), 211(8.3), 197(61.5), 191(12.7), 183(9.8), 177(62.1), 159(9.3), 151(13.1), 144(13.0), 143(100), 142(9.8), 129(129.0), 128(66.3), 127(13.6), 115(24.7); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.2 Hz, 1H), 7.21 (d, *J* = 8.1 Hz, 1H), 4.38 (dd, *J* = 12.2, 6.2 Hz, 1H), 4.28 (td, *J* = 12.6, 3.5 Hz, 1H), 2.96 (t, *J* = 4.2 Hz, 1H), 2.35 – 2.25 (m, 1H), 2.25 – 2.11 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 142.4, 126.7, 125.8 (q, *J* = 4.6 Hz), 124.1 (q, *J* = 273.6 Hz), 64.7, 26.9, 24.7, 24.1, 20.4. CF3-C* is not observed; ^{19}F NMR (376 MHz, C_6D_6) δ -63.4.

(1S,6S,7S)-7-(p-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one (2f):



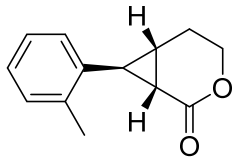
(1S,6S,7S)-7-(p-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2f)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a white solid, 25% yield (10.1 mg). GC-MS m/z (% relative intensity): 202(30.5), 158(8.0), 157(15.5), 144(12.8), 143(100), 142(16.4), 141(12.0), 129(26.7), 128(57.4), 127(12.8), 115(25.4), 91(10.4), 77(13.0); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 7.6 Hz, 2H), 7.00 (d, *J* = 7.8 Hz, 2H), 4.34 (dd, *J* = 12.5, 6.0 Hz, 1H), 4.26 (td, *J* = 12.5, 3.3 Hz, 1H), 2.90 (t, *J* = 4.6 Hz, 1H), 2.32 (s, 3H), 2.28 – 2.20 (m, 1H), 2.16 – 2.08 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 134.7, 131.7, 129.5, 126.3, 64.7, 26.6, 24.9, 23.7, 22.7, 20.6.

(1S,6S,7S)-7-(m-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one (2g):



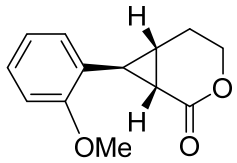
(1S,6S,7S)-7-(m-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2g)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a white solid, 77% yield (31.1 mg). GC-MS m/z (% relative intensity): 202(28.1), 158(7.4), 157(13.0), 144(12.8), 143(100), 142(15.2), 141(11.6), 129(27.1), 128(59.9), 127(13.2), 115(25.2), 91(10.0), 77(13.1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (t, *J* = 7.6 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 6.93 (s, 1H), 6.90 (d, *J* = 7.8 Hz, 1H), 4.35 (dd, *J* = 12.1, 6.3 Hz, 1H), 4.26 (td, *J* = 12.5, 3.5 Hz, 1H), 2.89 (t, *J* = 4.4 Hz, 1H), 2.33 (s, 3H), 2.31 – 2.21 (m, 1H), 2.19 – 2.09 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 136.6, 136.1, 126.8, 125.9, 125.2, 121.4, 62.7, 24.6, 23.1, 21.7, 19.5, 18.5.

(1S,6S,7S)-7-(o-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one (2h):



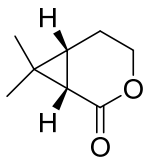
(1S,6S,7S)-7-(o-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2h)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a green solid, 82% yield (33.2 mg). GC-MS m/z (% relative intensity): 202(47.4), 158(6.3), 157(27.0), 144(12.8), 143(100), 142(17.0), 141(14.0), 130(11.4), 129(51.2), 128(80.2), 127(18.6), 116(12.8), 115(38.5), 91(14.5), 77(17.6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 – 7.12 (m, 3H), 7.07 (d, *J* = 6.6 Hz, 1H), 4.38 (dd, *J* = 12.1, 6.1 Hz, 1H), 4.27 (td, *J* = 12.6, 3.7 Hz, 1H), 2.93 (t, *J* = 4.7 Hz, 1H), 2.40 (s, 3H), 2.38 – 2.26 (m, 1H), 2.22 – 2.05 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 138.1, 136.1, 130.3, 127.5, 126.5, 126.3, 64.9, 24.8, 23.6, 22.1, 20.7, 19.8.

(1S,6S,7S)-7-(2-methoxyphenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2i):



(1S,6S,7S)-7-(2-methoxyphenyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2i)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) to afford the product as a white liquid, 84% yield (36.7 mg). GC-MS m/z (% relative intensity): 218(89.8), 187(17.3), 174(19.2), 173(74.4), 159(100), 158(24.3), 145(24.7), 144(64.9), 143(51.8), 141(27.5), 132(16.3), 131(56.9), 129(27.5), 128(44.3), 127(23.0), 116(21.3), 115(64.2), 91(63.9), 89(16.9), 77(32.6), 65(15.5), 51(16.1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (ddd, *J* = 9.0, 6.6, 2.6 Hz, 1H), 6.94 – 6.83 (m, 3H), 4.36 – 4.29 (m, 2H), 3.85 (s, 3H), 3.21 (t, *J* = 4.7 Hz, 1H), 2.31 – 2.20 (m, 1H), 2.17 – 2.08 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.1, 128.2, 126.8, 126.5, 125.9, 120.7, 110.5, 64.8, 55.6, 25.5, 22.7, 20.7, 19.7.

(1S,6R)-7,7-dimethyl-3-oxabicyclo[4.1.0]heptan-2-one (2j):



(1S,6R)-7,7-dimethyl-3-oxabicyclo[4.1.0]heptan-2-one **(2j)** was prepared according to the general **Procedure D** with *E. coli* cells expressing Mb(F43L,H64A,V68G,I107V) in 200 mL with 1 mM of **1j** to afford the product as a white solid, 32% yield (9.0 mg). GC-MS m/z (% relative intensity): 140(3.5), 125(8.5), 97(7.2), 96(28.9), 95(23.8), 82(16.8), 81(100), 79(22.7), 67(35.2), 57(20.3), 56(21.3), 55(15.6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.33 – 4.08 (m, 2H), 2.35 (t, *J* = 7.4 Hz, 1H), 1.89 – 1.72 (m, 1H), 1.59 –

1.44 (m, 2H), 1.20 (s, 3H), 1.13 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 69.5, 27.6, 25.0, 23.9, 22.8, 19.3, 16.5.

2-((1S,2S,3S)-2-(hydroxymethyl)-3-phenylcyclopropyl)ethan-1-ol (4):

2-((1S,2S,3S)-2-(hydroxymethyl)-3-phenylcyclopropyl)ethan-1-ol **(4)** was prepared according to a reported procedure.[3] (1S,6S,7S)-7-phenyl-3-oxabicyclo[4.1.0]heptan-2-one **(2a)** (40 mg, 0.21 mmol, 1 equiv) in dry THF was added dropwise to a suspension of $LiAlH_4$ (1 equiv) in dry THF at 0 °C. The resulting mixture was stirred for 2 h at room temperature and then quenched with aqueous diethyl ether and stirred for 1 h at room temperature. After filtration through a pad of Celite, the filtrate was dried over $MgSO_4$ and concentrat ed to give a residue, which was further purified by silica-gel chromatography using 80% EtOAc/hexanes as eluent to afford the product as a white solid, 36 mg, 89% yield. GC-MS m/z (% relative intensity): 192(0.6), 162(11.8), 161(50.8), 143(35.2), 129(54.2), 128(58.4), 117(100), 115(61.6), 91(75.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, J = 6.3 Hz, 2H), 7.19 – 7.09 (m, 1H), 7.04 (d, J = 6.5 Hz, 1H), 4.16 – 4.00 (m, 1H), 3.88 (s, 1H), 3.80 – 3.60 (m, 1H), 3.42 (t, J = 10.5 Hz, 1H), 2.09 (d, J = 13.6 Hz, 1H), 1.69 (d, J = 3.8 Hz, 1H), 1.65 – 1.53 (m, 1H), 1.47 (d, J = 3.6 Hz, 1H), 1.21 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.1, 129.1, 126.4, 126.3, 63.7, 61.9, 30.9, 30.5, 26.7, 26.5.

(1S,2S,3S)-2-(2-hydroxyethyl)-3-phenylcyclopropane-1-carboxylic acid (5):

(1S,2S,3S)-2-(2-hydroxyethyl)-3-phenylcyclopropane-1-carboxylic acid **(5)** was prepared according to a modified version of a reported procedure.[3] To a solution of LiOH (5 equiv) in water:THF (1:5) at RT was slowly added the (1S,6S,7S)-7-phenyl-3-oxabicyclo[4.1.0]heptan-2-one **(2a)** (40 mg, 0.21 mmol, 1 equiv). The mixture was refluxed for 10 h. Solvent was removed under reduced pressure. The residue was dissolved in water and acidified with 2M HCl to pH 1 followed by extraction with DCM. The crude product was purified by column chromatography on silica gel with Ethyl acetate to afford the product as a white solid, 41 mg, 94% yield. ^{1}H NMR

(500 MHz, MeOD) δ 7.23 (d, *J* = 7.6 Hz, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 7.10 (d, *J* = 7.4 Hz, 2H), 3.62 (t, *J* = 6.5 Hz, 2H), 2.43 – 2.35 (m, 1H), 2.06 – 1.93 (m, 2H), 1.92 – 1.83 (m, 1H), 1.81 – 1.70 (m, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.7, 141.1, 128.8, 126.7, 126.6, 61.8, 31.8, 30.5, 28.5, 28.3.

X-ray crystallographic analyses

X-ray crystal diffraction data were collected using a XtaLab Synergy-S Dualflex diffractometer equipped with a HyPix-6000HE HPC area detector for data collection at 100.00(10) K. A preliminary set of cell constants and an orientation matrix were calculated from reflections harvested from a sampling of reciprocal space (*CrysAlisPro*, version 171.40.71a; Rigaku Corporation: Oxford, UK, 2020.). The full data collection was carried out using a PhotonJet (Cu) X-ray Source with frame times of 0.07 and 0.26 seconds and a detector distance of 31.2 mm. Series of frames were collected in 0.50º steps in ω at different 2θ, κ, and ϕ settings. The intensity data were scaled and corrected for absorption, and final cell constants were calculated from the xyz centroids of strong reflections from the actual data collections after integration. Space groups were determined based on systematic absences and intensity statistics.

Structures were solved using SHELXT(Sheldrick, G. M. *SHELXT*, version 2018/2; *Acta. Crystallogr*. 2015, A71, 3-8.)and refined using SHELXL (against F^2) (Sheldrick, G. M. *SHELXL*, version 2018/3; *Acta. Crystallogr.* 2015, C71, 3-8.).All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters. See **Figure S3-S4** and **Table S8-S9** for additional crystal data and structure refinement information for **2e** and **2i**.

Table S8. Crystal data and structure refinement for (1S,6S,7S)-7-(4-(trifluoromethyl)phenyl)-3-oxabicyclo[4.1.0]heptan-2-one **(2e)**. Cambridge Crystallographic Data Centre (CCDC) entry: 1998584.

Identification code	**2e**	
Empirical formula	C13 H11 F3 O2	
Formula weight	256.22	
Temperature	100.00(10) K	
Wavelength	1.54184 Å	
Crystal system	orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 5.19720(10) Å	α = 90°
	b = 10.3107(2) Å	β = 90°
	c = 21.0437(4) Å	γ = 90°
Volume	1127.66(4) Å^3	
Z	4	
Density (calculated)	1.509 Mg/m^3	
Absorption coefficient	1.163 mm^{-1}	
F(000)	528	
Crystal color, morphology	colourless, plate	
Crystal size	0.202 x 0.154 x 0.038 mm^3	
Theta range for data collection	4.202 to 77.587°	
Index ranges	$-5 \le h \le 6$, $-12 \le k \le 13$, $-26 \le l \le 25$	
Reflections collected	11981	
Independent reflections	2383 [R(int) = 0.0502]	
Observed reflections	2258	
Completeness to theta = 74.504°	100.0%	
Absorption correction	Multi-scan	
Max. and min. transmission	1.00000 and 0.84871	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2383 / 0 / 163	
Goodness-of-fit on F^2	1.068	
Final R indices [I>2sigma(I)]	$R1$ = 0.0416, $wR2$ = 0.1114	
R indices (all data)	$R1$ = 0.0437, $wR2$ = 0.1135	
Absolute structure parameter	0.08(7)	
Largest diff. peak and hole	0.326 and -0.438 e.Å^{-3}	

Table S9. Crystal data and structure refinement for (1S,6S,7S)-7-(2-methoxyphenyl)-3-oxabicyclo[4.1.0]heptan-2-one (**2i**). Cambridge Crystallographic Data Centre (CCDC) entry: 1998585.

Identification code	**2i**	
Empirical formula	C13 H14 O3	
Formula weight	218.24	
Temperature	100.00(10) K	
Wavelength	1.54184 Å	
Crystal system	orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 6.97510(10) Å	α = 90°
	b = 9.07040(10) Å	β = 90°
	c = 17.0695(2) Å	γ = 90°
Volume	1079.94(2) Å^3	
Z	4	
Density (calculated)	1.342 Mg/m^3	
Absorption coefficient	0.774 mm^{-1}	
F(000)	464	
Crystal color, morphology	colourless, plate	
Crystal size	0.458 x 0.238 x 0.036 mm^3	
Theta range for data collection	5.182 to 77.833°	
Index ranges	$-7 \le h \le 8$, $-11 \le k \le 11$, $-21 \le l \le 21$	
Reflections collected	35394	
Independent reflections	2297 [R(int) = 0.0730]	
Observed reflections	2219	
Completeness to theta = 74.504°	100.0%	
Absorption correction	Multi-scan	
Max. and min. transmission	1.00000 and 0.45048	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2297 / 0 / 147	
Goodness-of-fit on F^2	1.054	
Final R indices [I>2sigma(I)]	R1 = 0.0315, wR2 = 0.0827	
R indices (all data)	R1 = 0.0325, wR2 = 0.0837	
Absolute structure parameter	-0.12(9)	
Extinction coefficient	0.0077(11)	
Largest diff. peak and hole	0.194 and -0.166 e.Å^{-3}	

References:

(1) Bordeaux, M., Tyagi, V., and Fasan, R. Highly Diastereoselective and Enantioselective Olefin Cyclopropanation Using Engineered Myoglobin-Based Catalysts, *Angew. Chem. Int. Ed.* **2015**, *54*, 1744–1748.

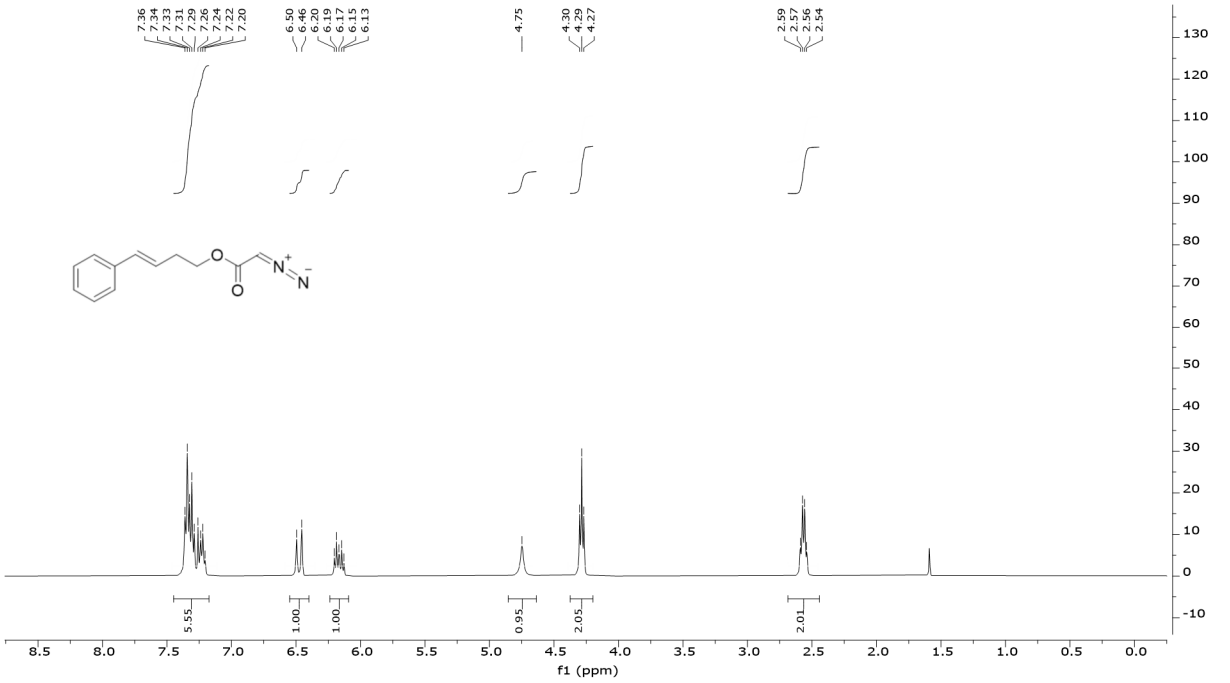
(2) Bajaj, P., Sreenilayam, G., Tyagi, V., and Fasan, R. Gram-Scale Synthesis of Chiral Cyclopropane Containing Drugs and Drug Precursors with Engineered Myoglobin Catalysts Featuring Complementary Stereoselectivity, *Angew. Chem. Int. Ed.* **2016**, *55*, 16110–16114.

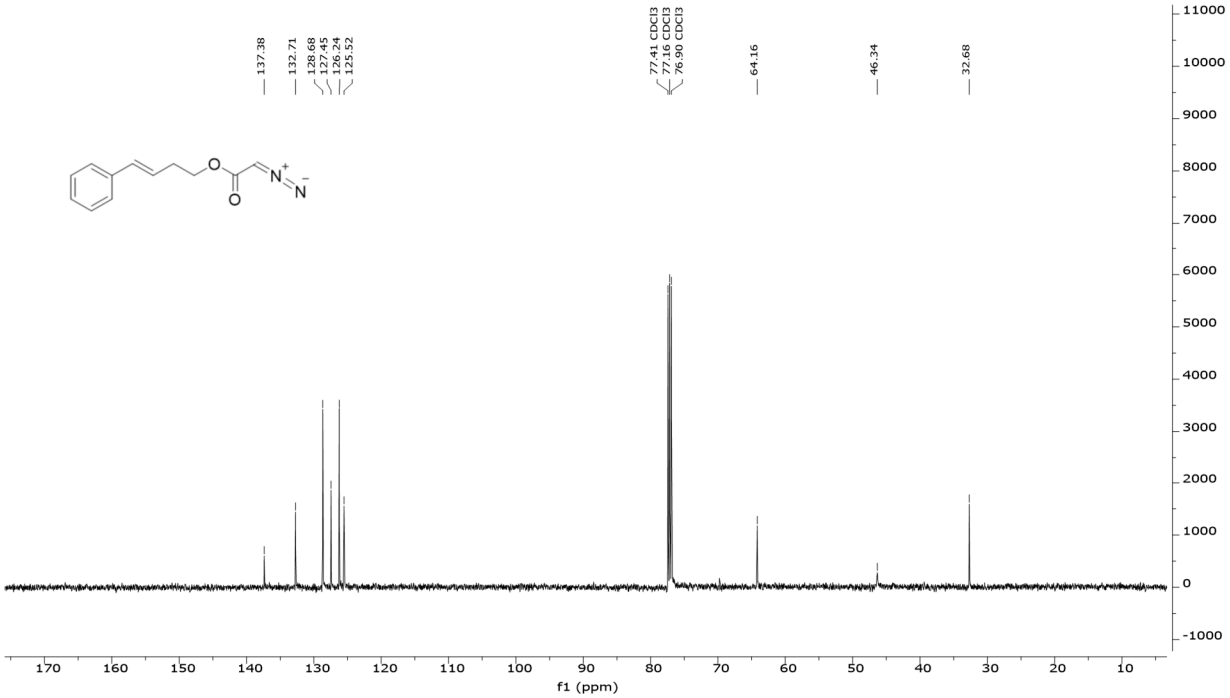
(3) Chandgude, A., Ren, X., and Fasan, R. Stereodivergent Intramolecular Cyclopropanation Enabled by Engineered Carbene Transferases *J. Am. Chem. Soc.* **2019**, 141, 9145-9150.

(4) Mandour, H. S. A., Chanthamath, S., Shibatomi, K., Iwasa, S., Inter- and Intramolecular Cyclopropanations of Diazo Weinreb Amides Catalyzed by Ruthenium(II)-Amm-Pheox, *Adv. Synth. Catal.* **2017**, 359, 1742–1746.

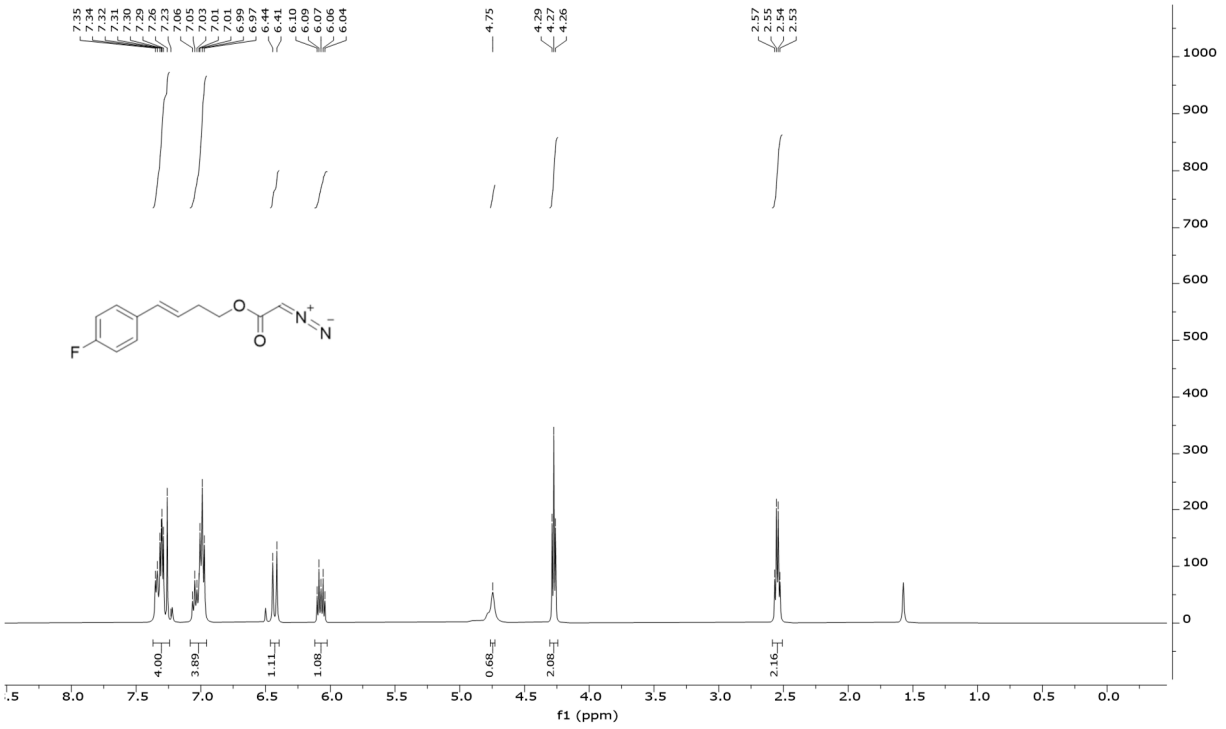
NMR Spectra

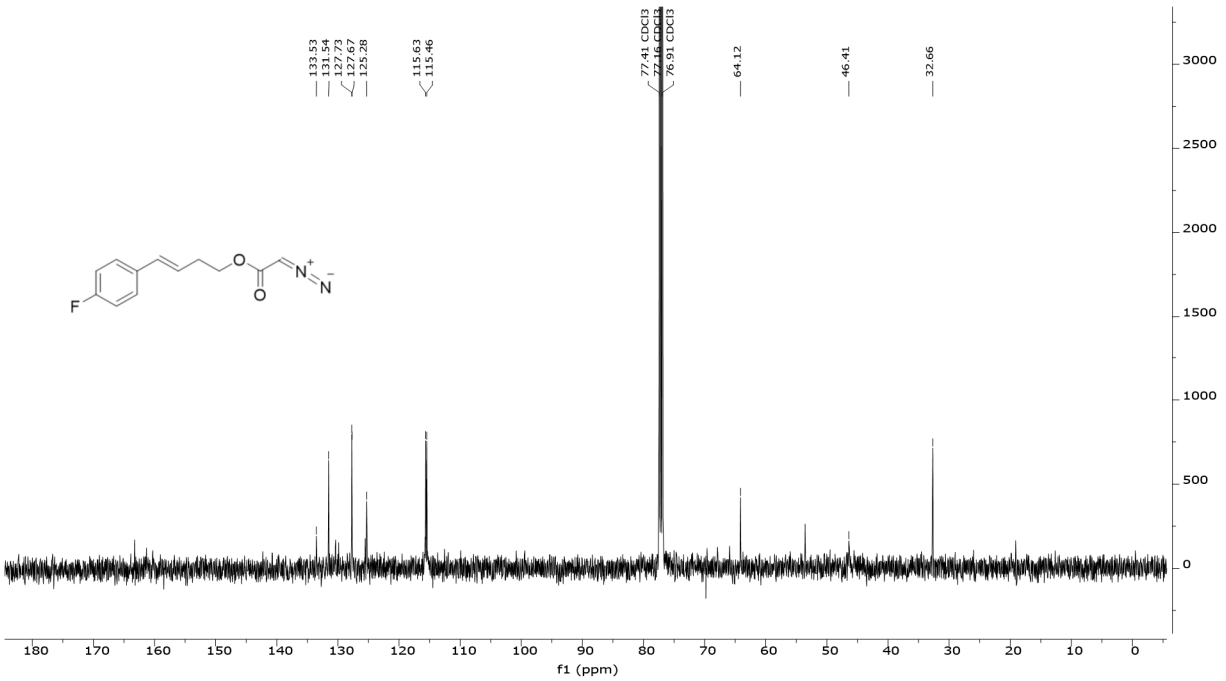
(E)-4-phenylbut-3-en-1-yl 2-diazoacetate (1a): 500 MHz ^{1}H spectrum and 126 MHz ^{13}C spectrum in $CDCl_3$ solvent

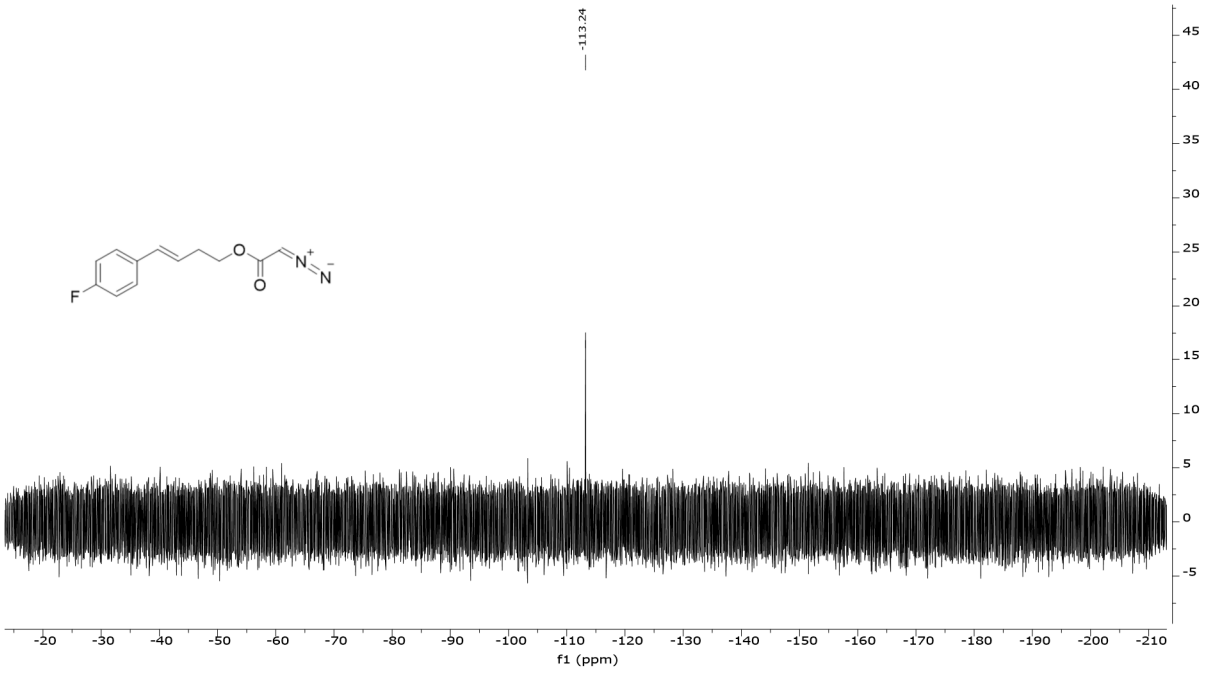




(E)-4-(4-fluorophenyl)but-3-en-1-yl 2-diazoacetate (1b): 500 MHz ^{1}H spectrum, 126 MHz ^{13}C spectrum and 376 MHz ^{19}F spectrum in $CDCl_3$ solvent






-113.24
F
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O
N+
N-
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40
35
30
25
20
15
10
5
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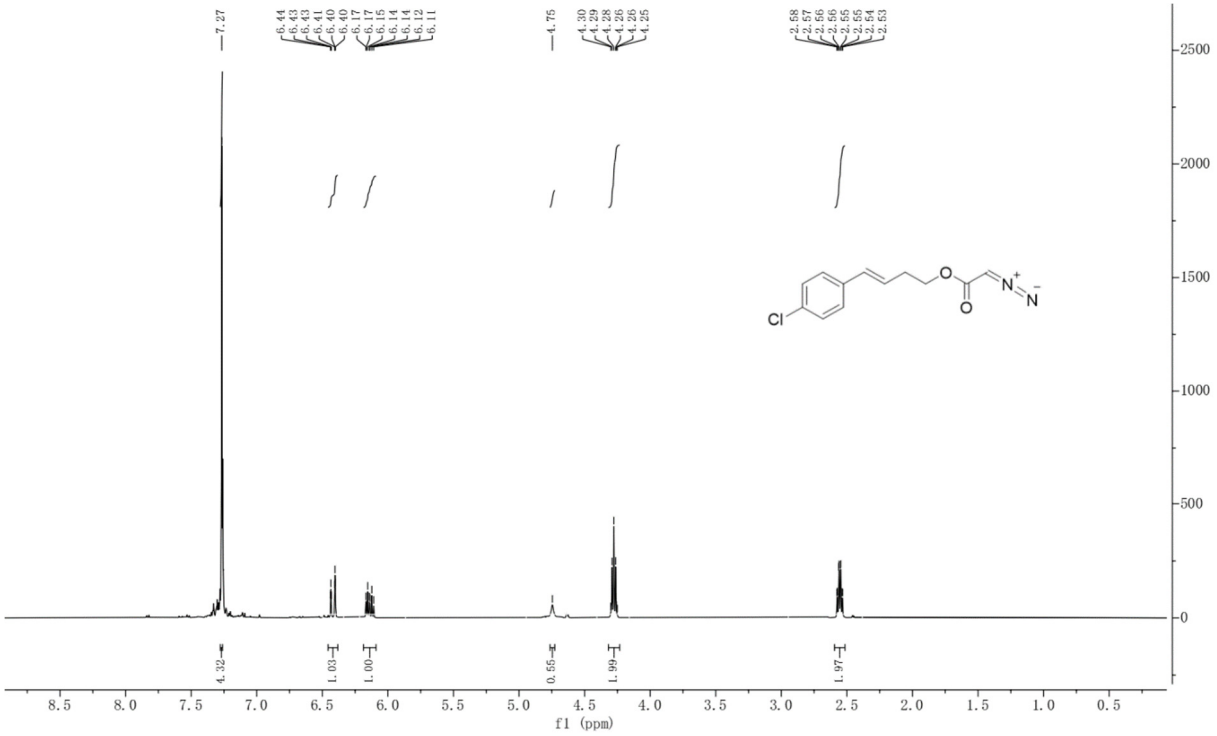
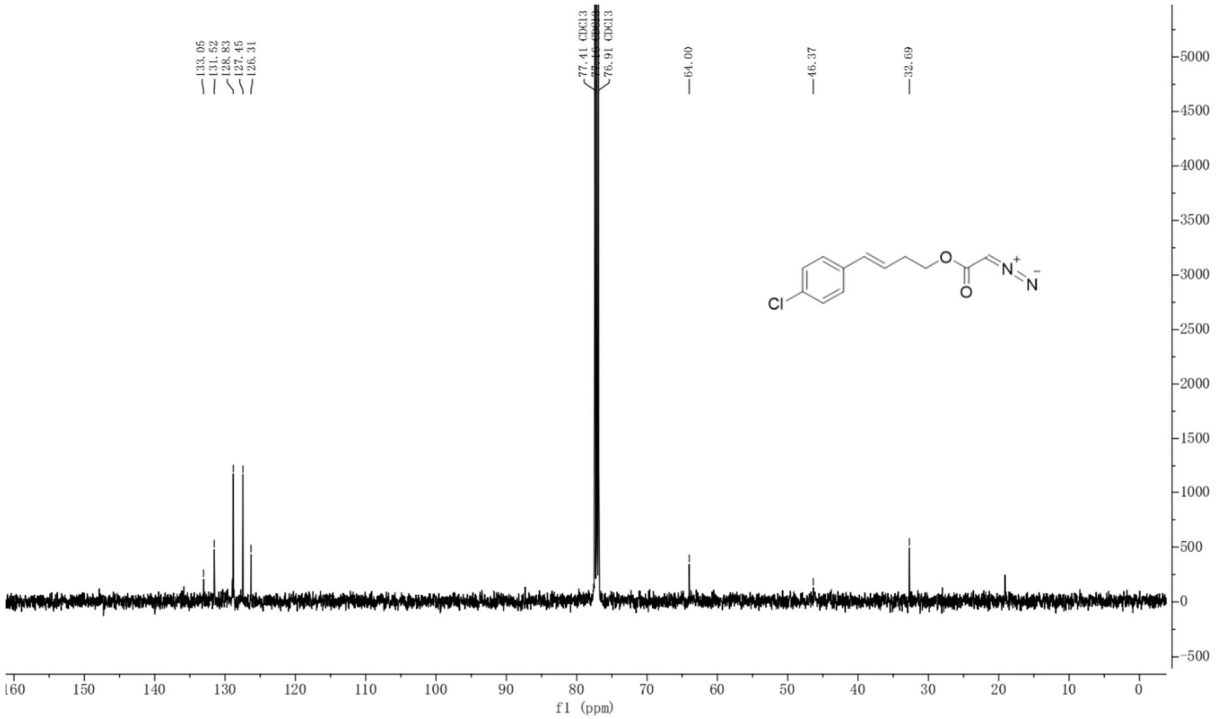
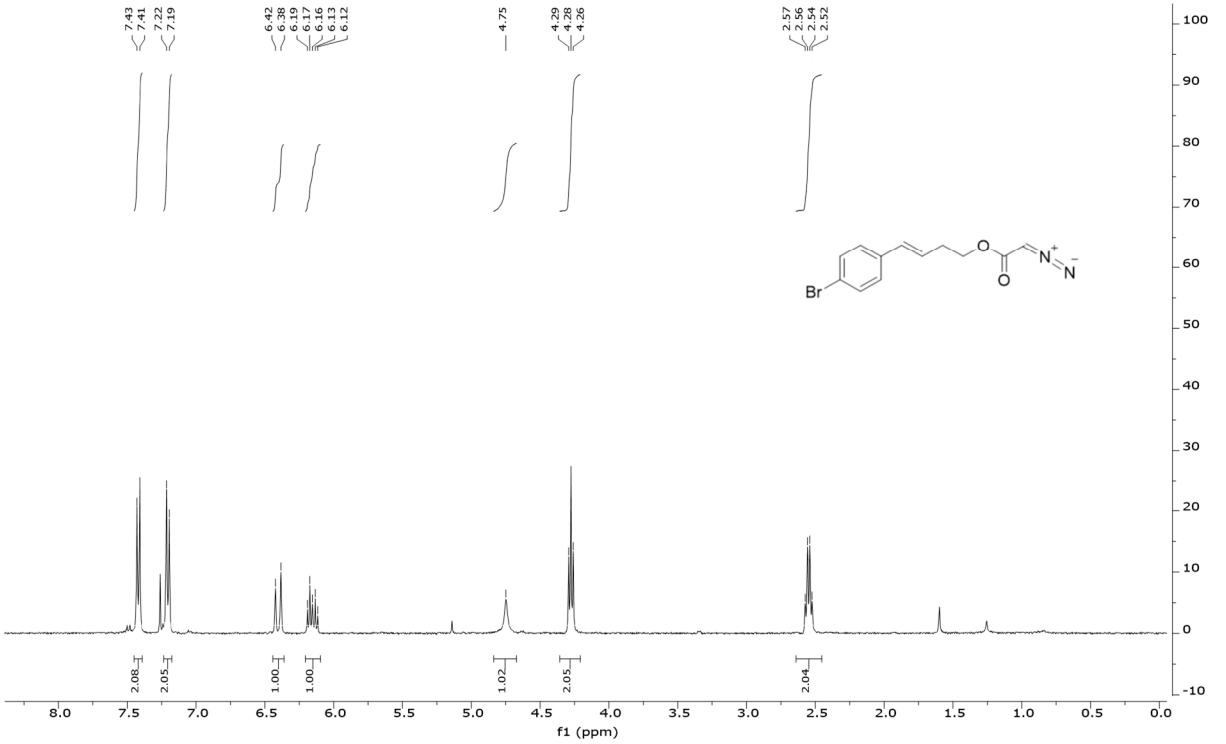
-5
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
f1 (ppm)

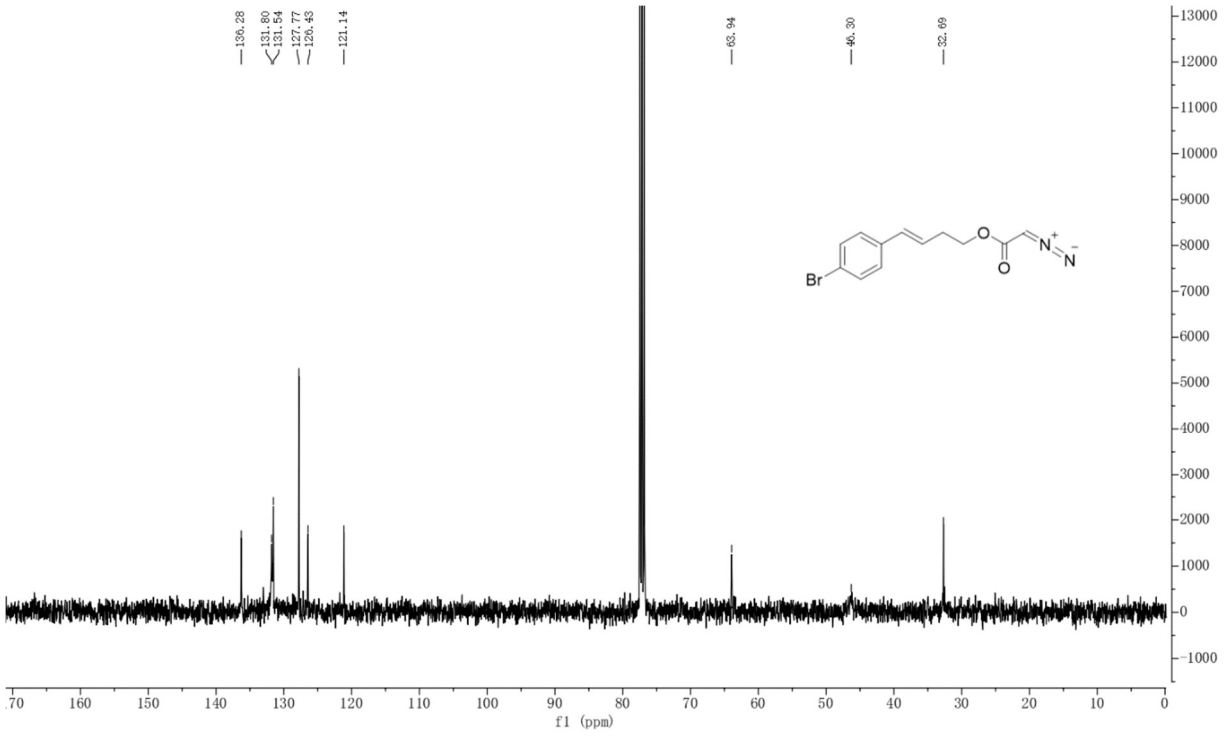
(E)-4-(4-chlorophenyl)but-3-en-1-yl 2-diazoacetate (1c): 500 MHz ^{1}H spectrum and 126 MHz ^{13}C spectrum in $CDCl_3$ solvent



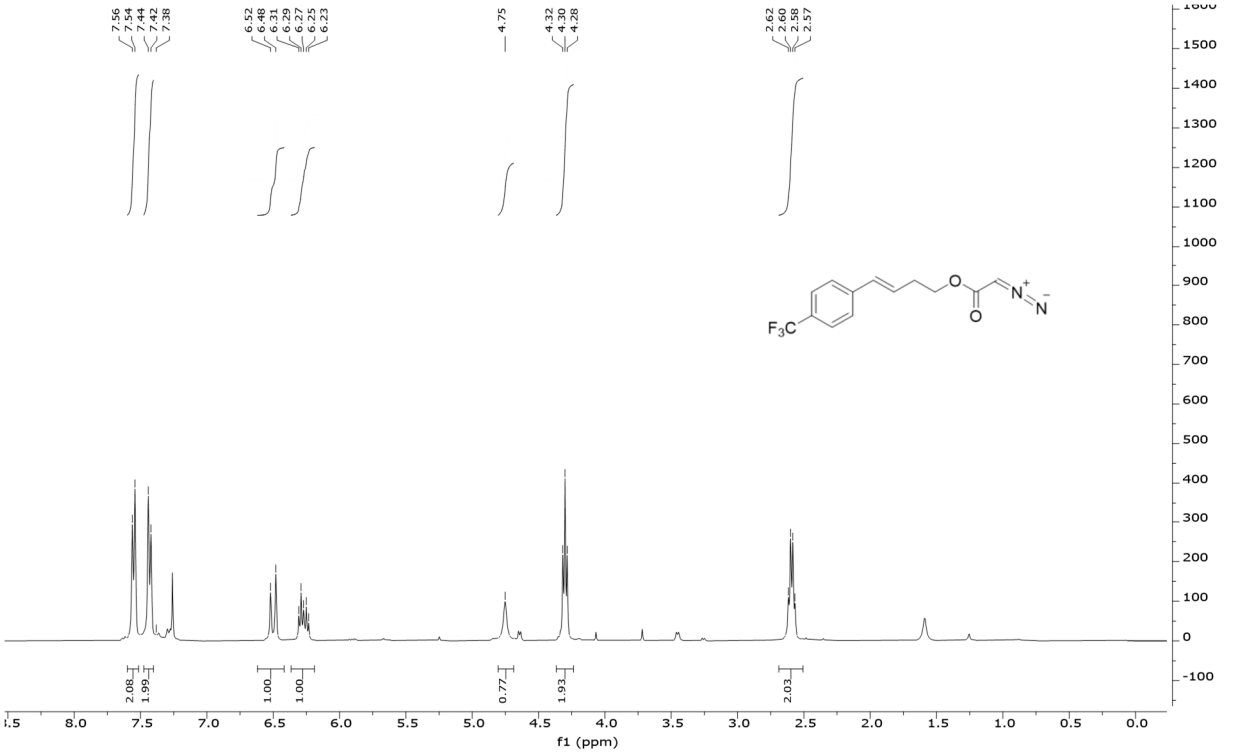


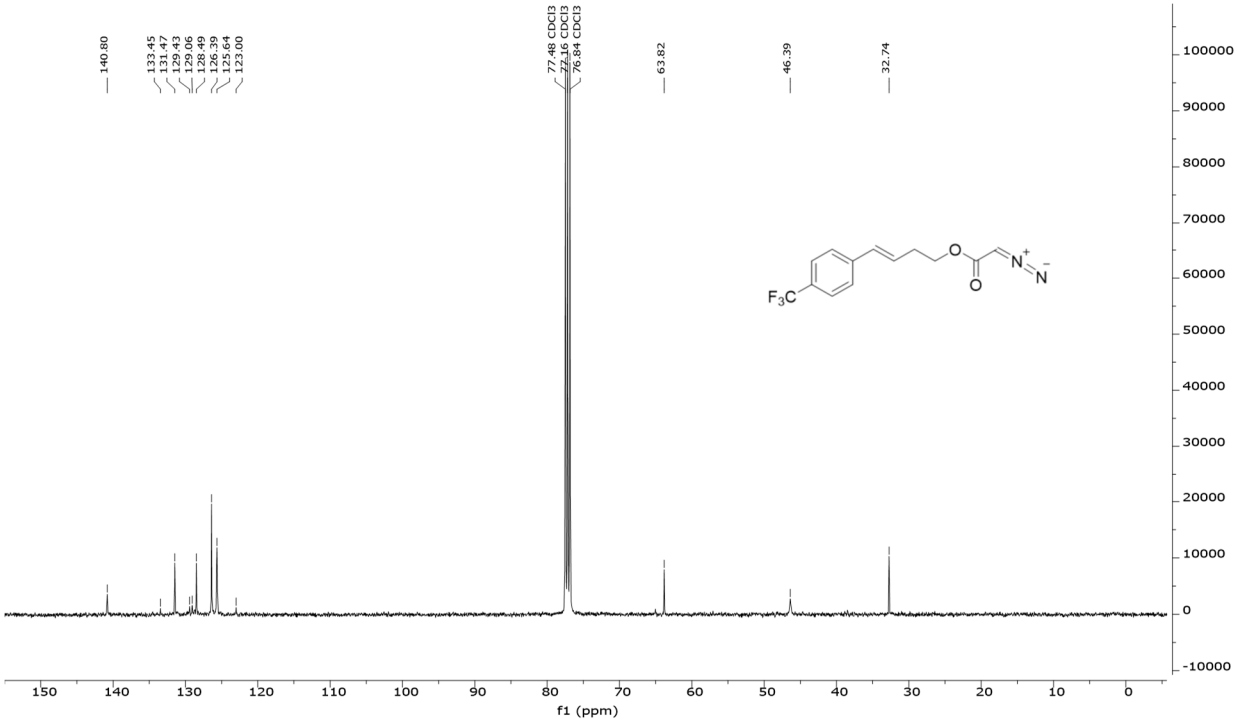
(E)-4-(4-bromophenyl)but-3-en-1-yl 2-diazoacetate (1d): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

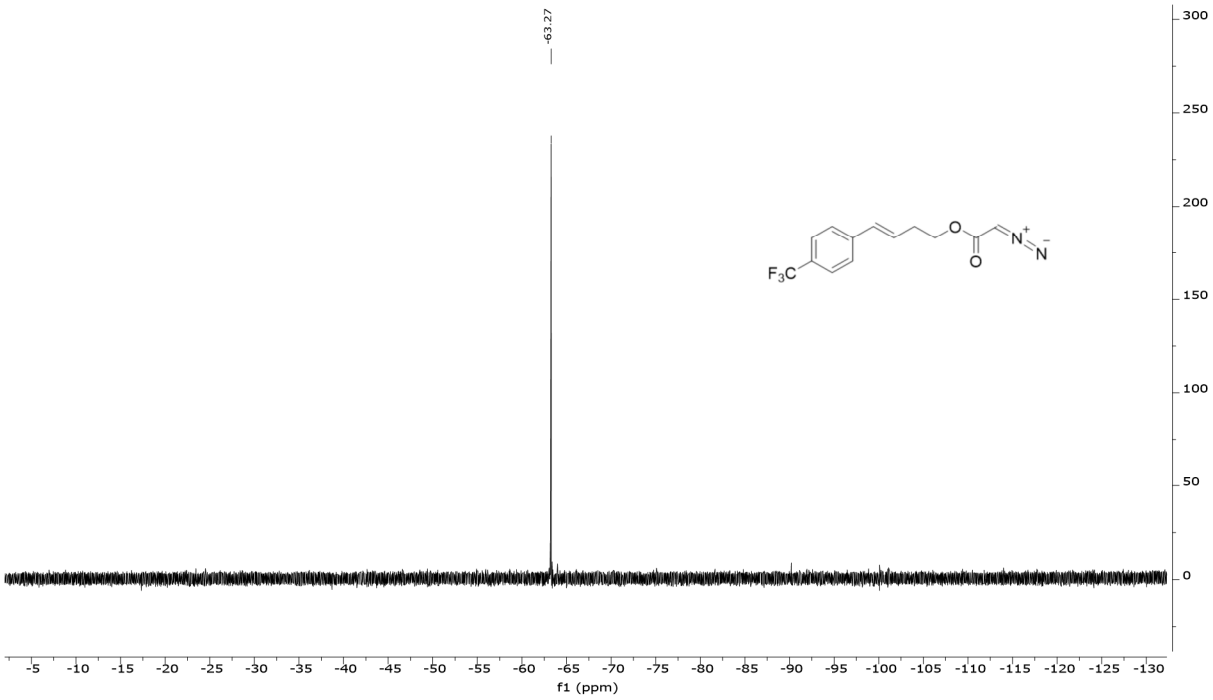




(E)-4-(4-(trifluoromethyl)phenyl)but-3-en-1-yl 2-diazoacetate (1e): 400 MHz ^{1}H spectrum, 101 MHz ^{13}C spectrum and 376 MHz ^{19}F spectrum in $CDCl_3$ solvent



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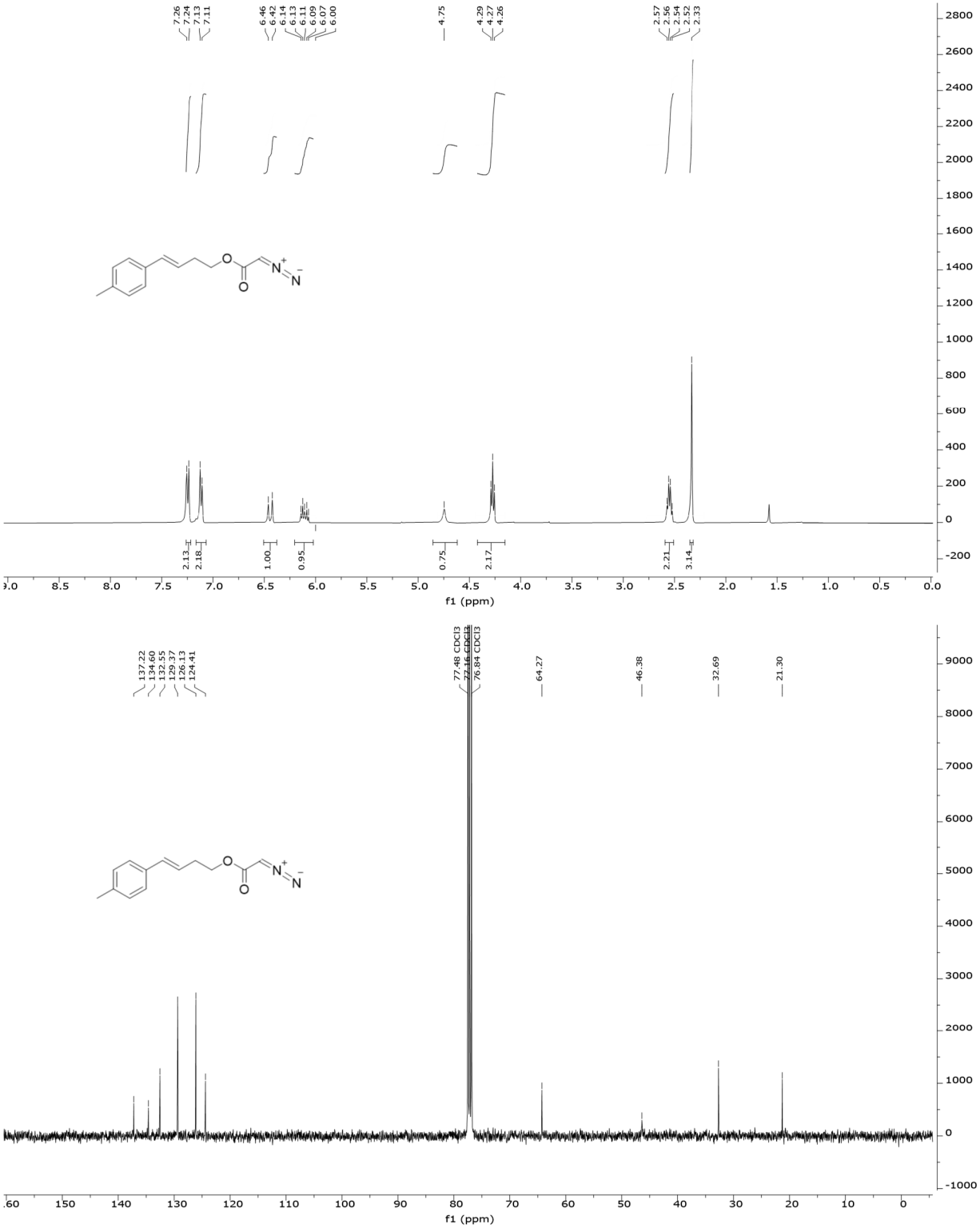
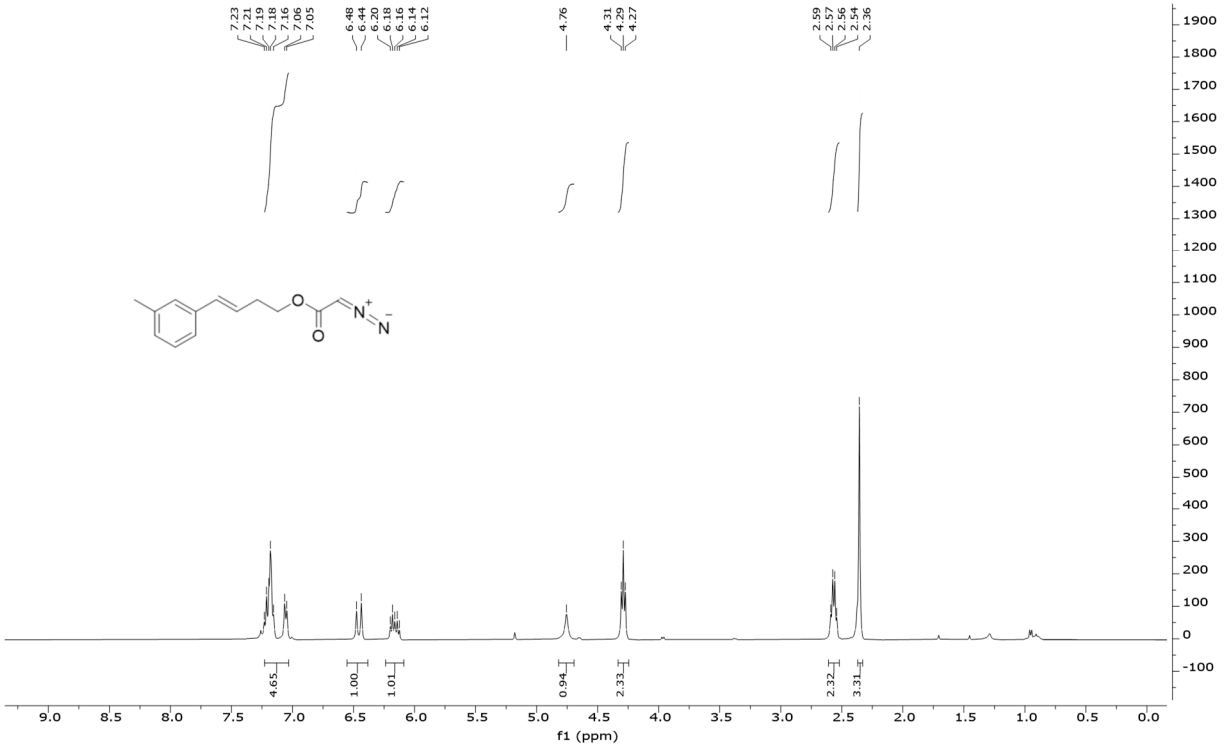
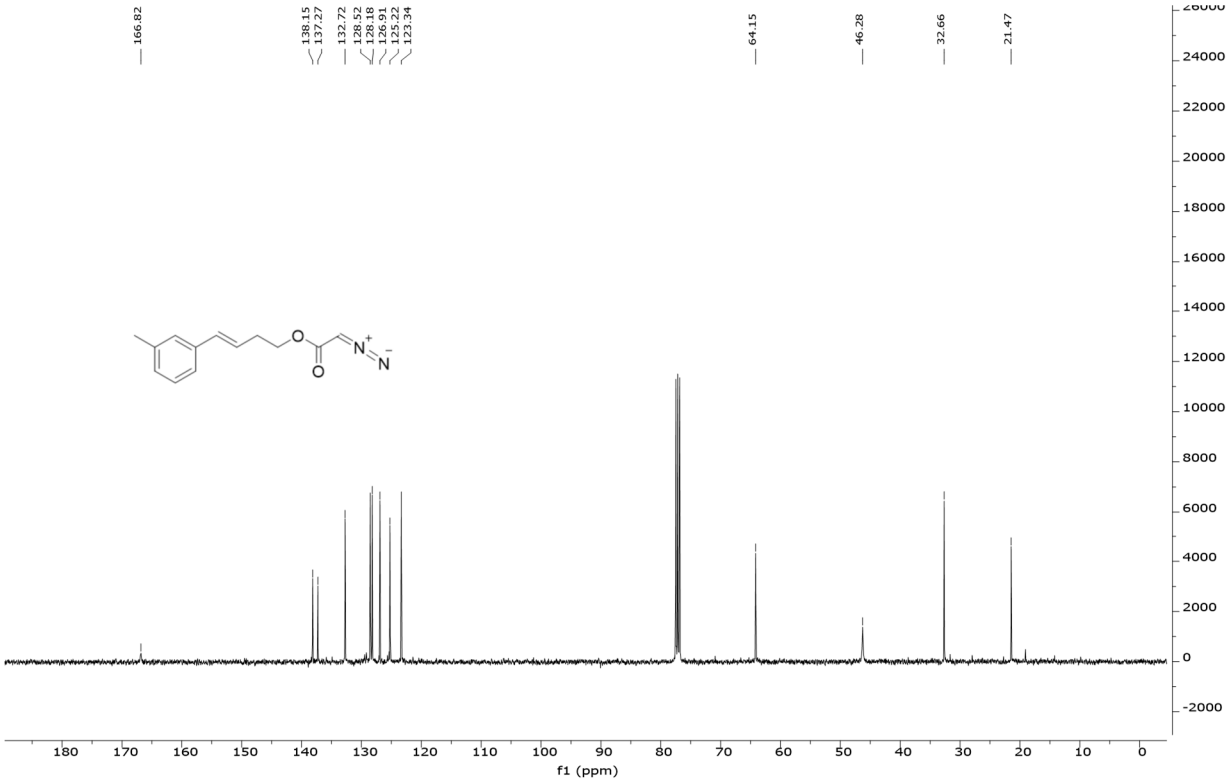
200
150
100
50
0
-5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130
f1 (ppm)
F3C
O
O
N+
N-

(E)-4-(p-tolyl)but-3-en-1-yl 2-diazoacetate (1f): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

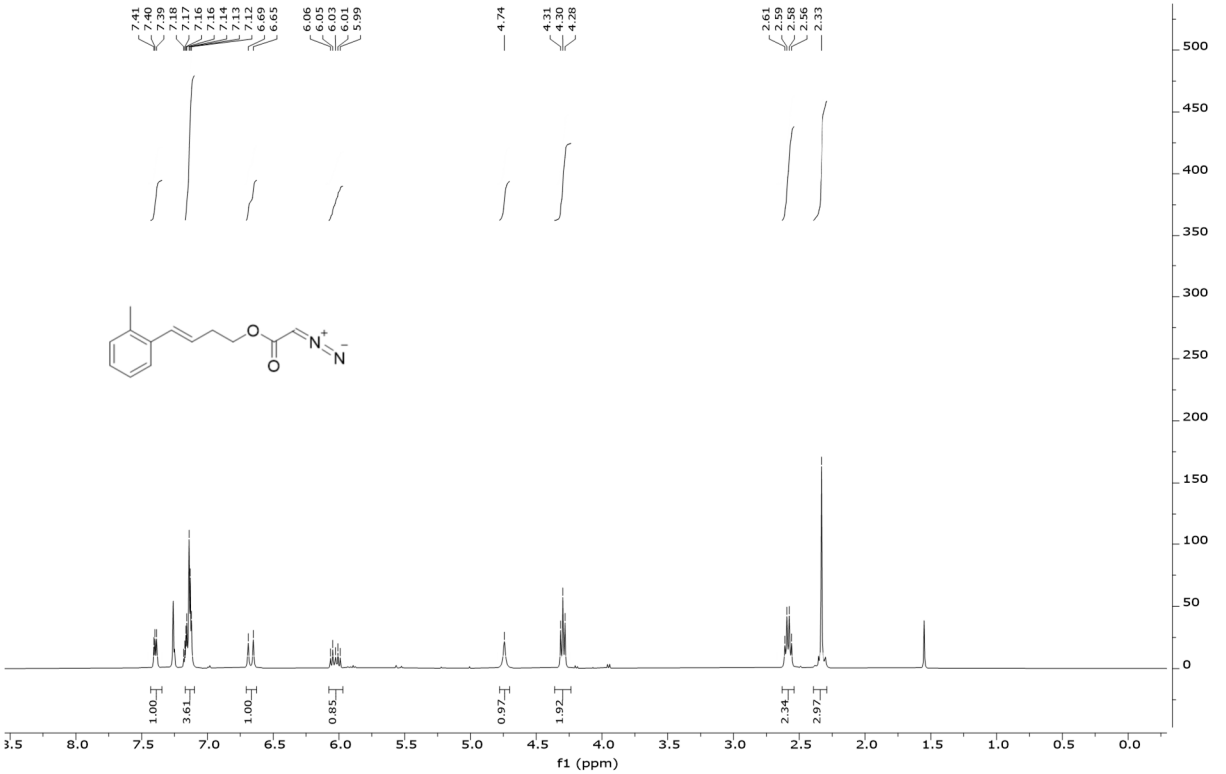


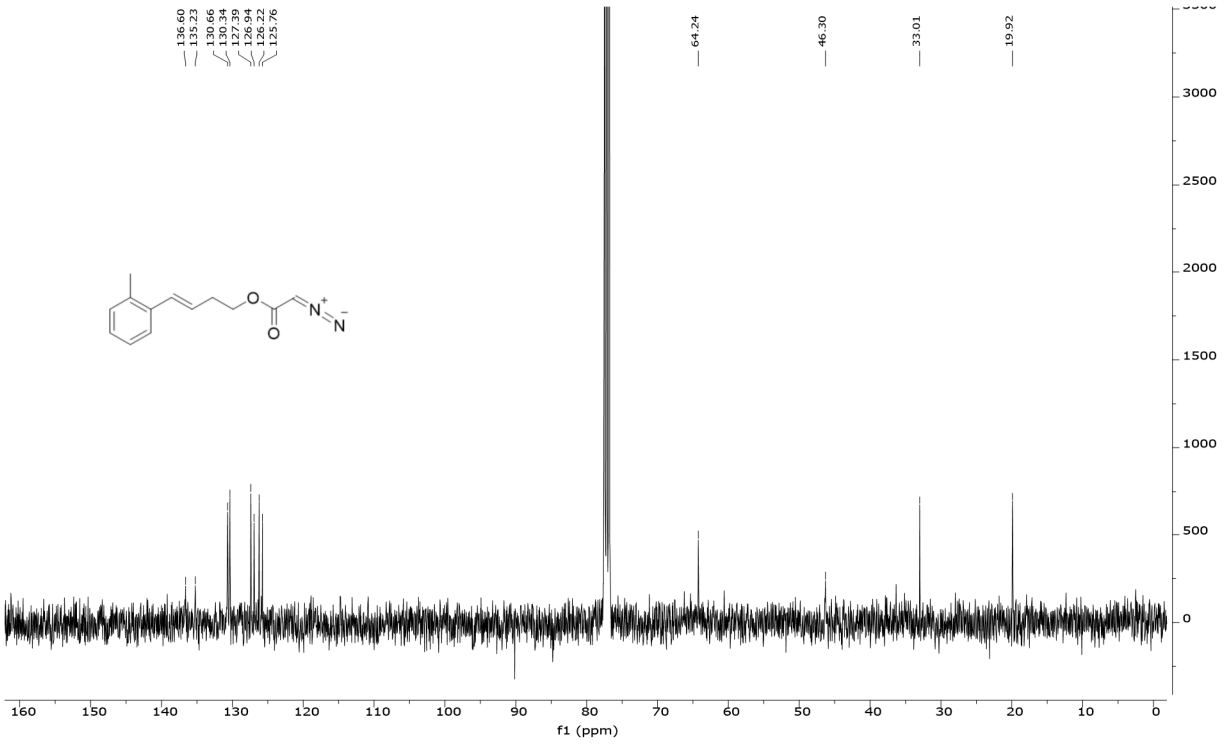
(E)-4-(m-tolyl)but-3-en-1-yl 2-diazoacetate (1g): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent



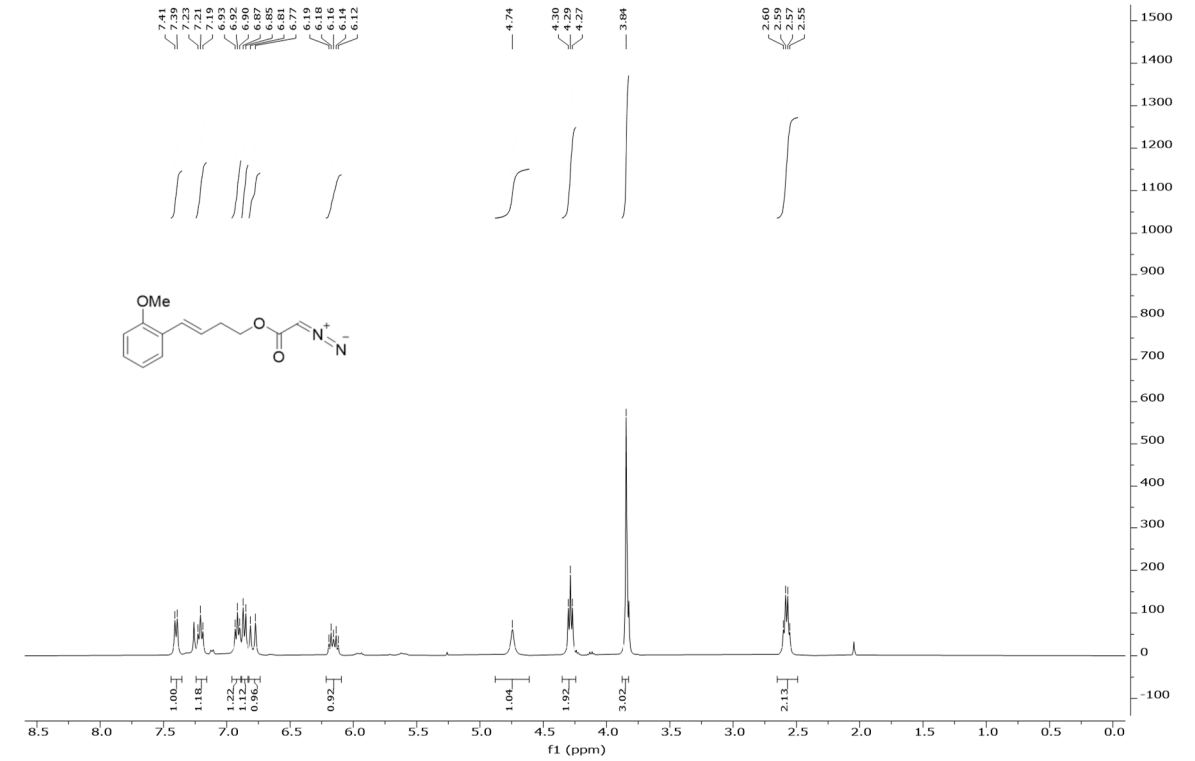


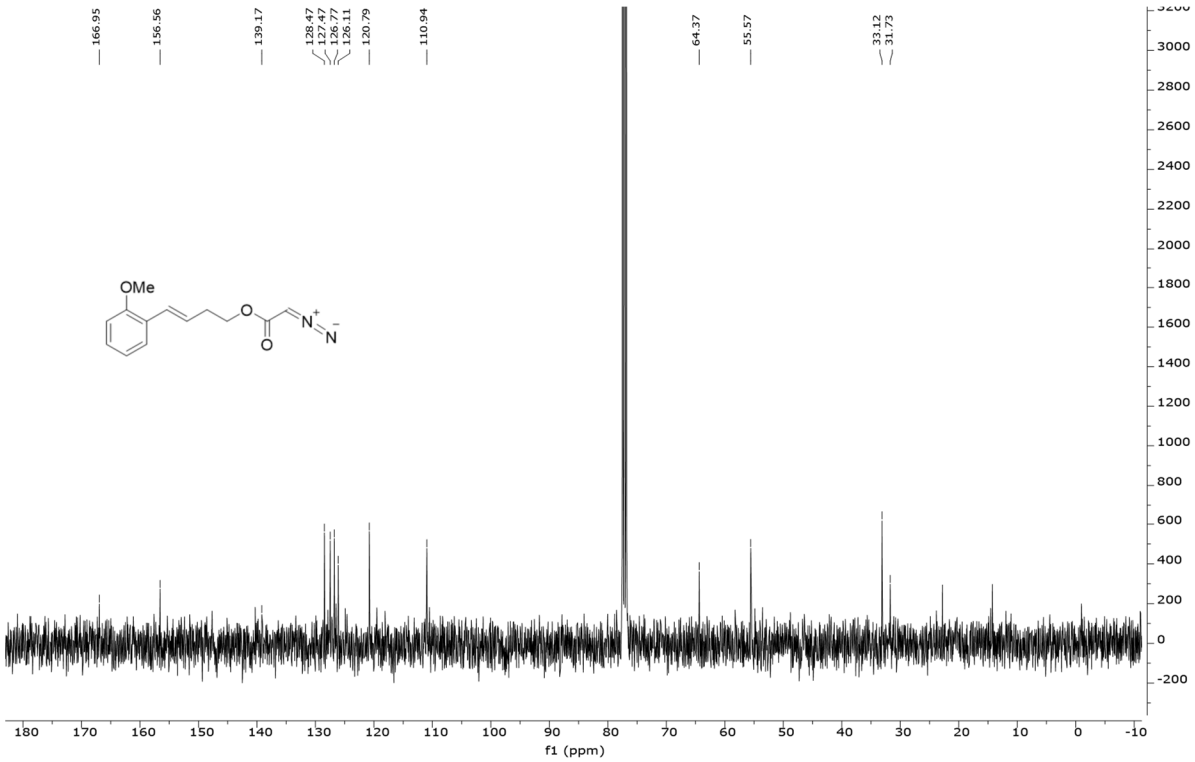
(E)-4-(o-tolyl)but-3-en-1-yl 2-diazoacetate (1h): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent



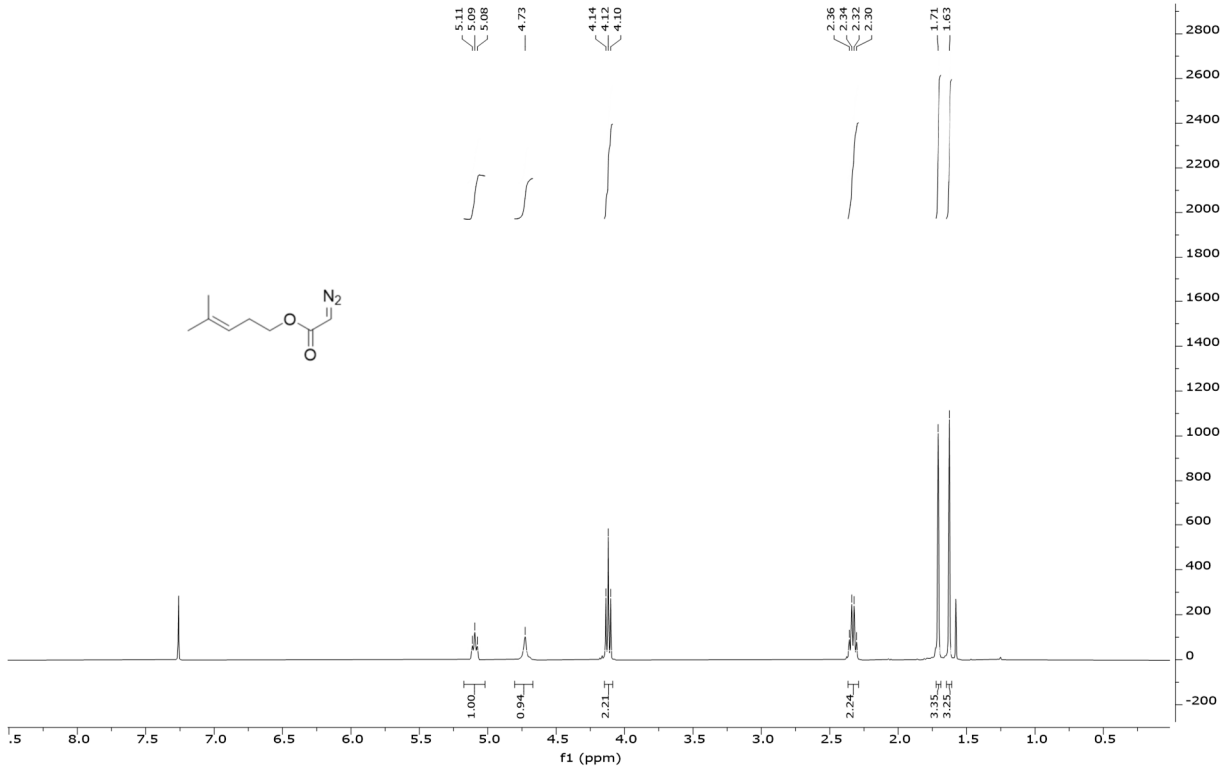


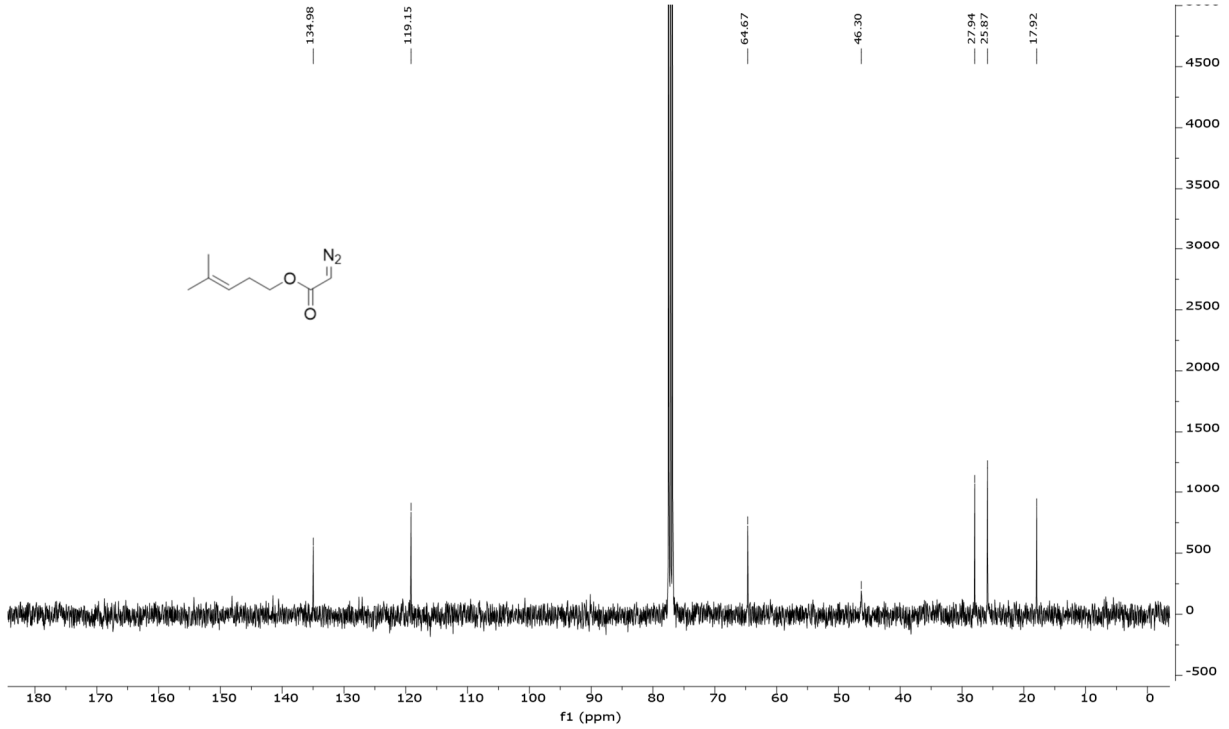
(E)-4-(2-methoxyphenyl)but-3-en-1-yl 2-diazoacetate (1i): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent



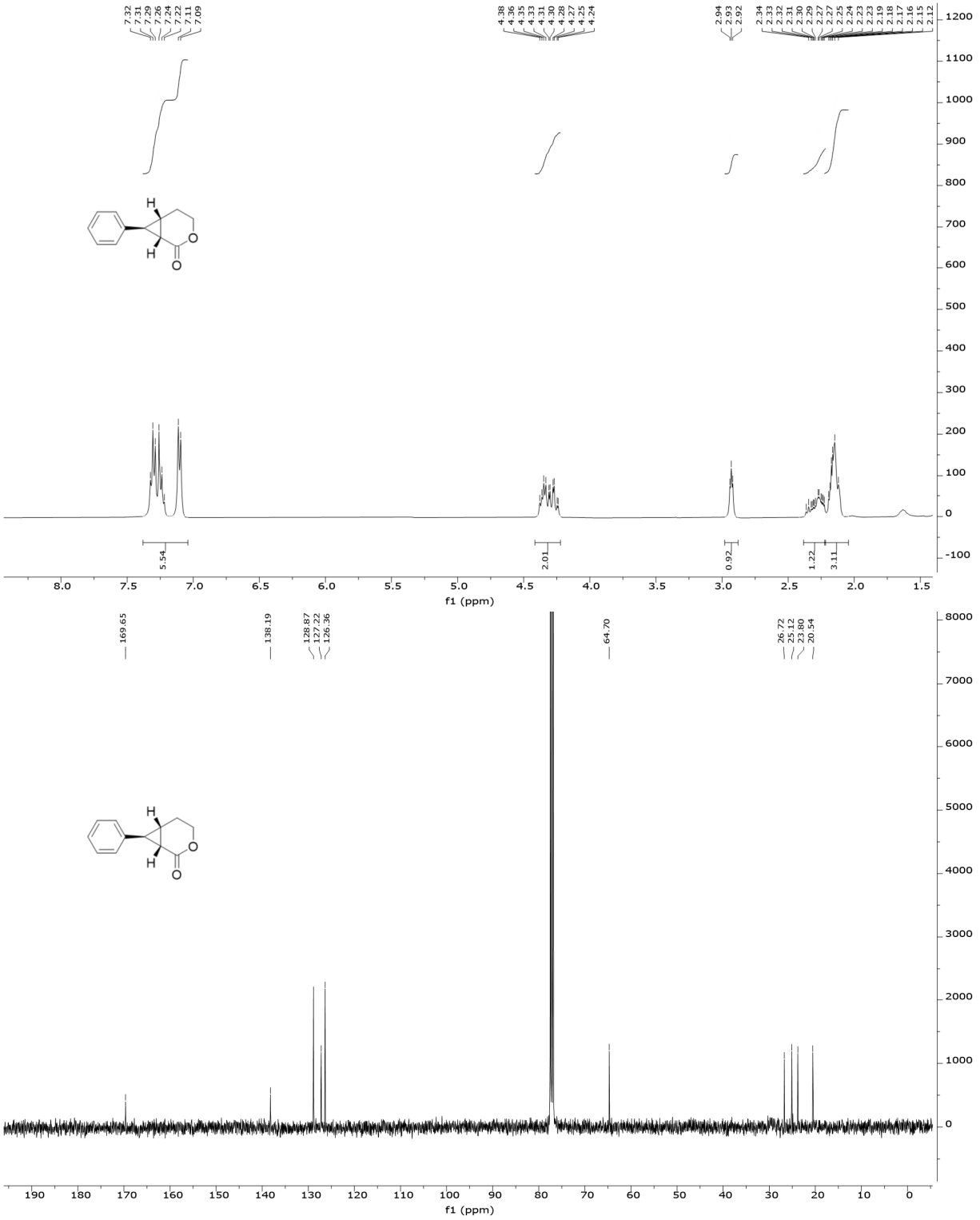


4-methylpent-3-en-1-yl 2-diazoacetate (1j): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

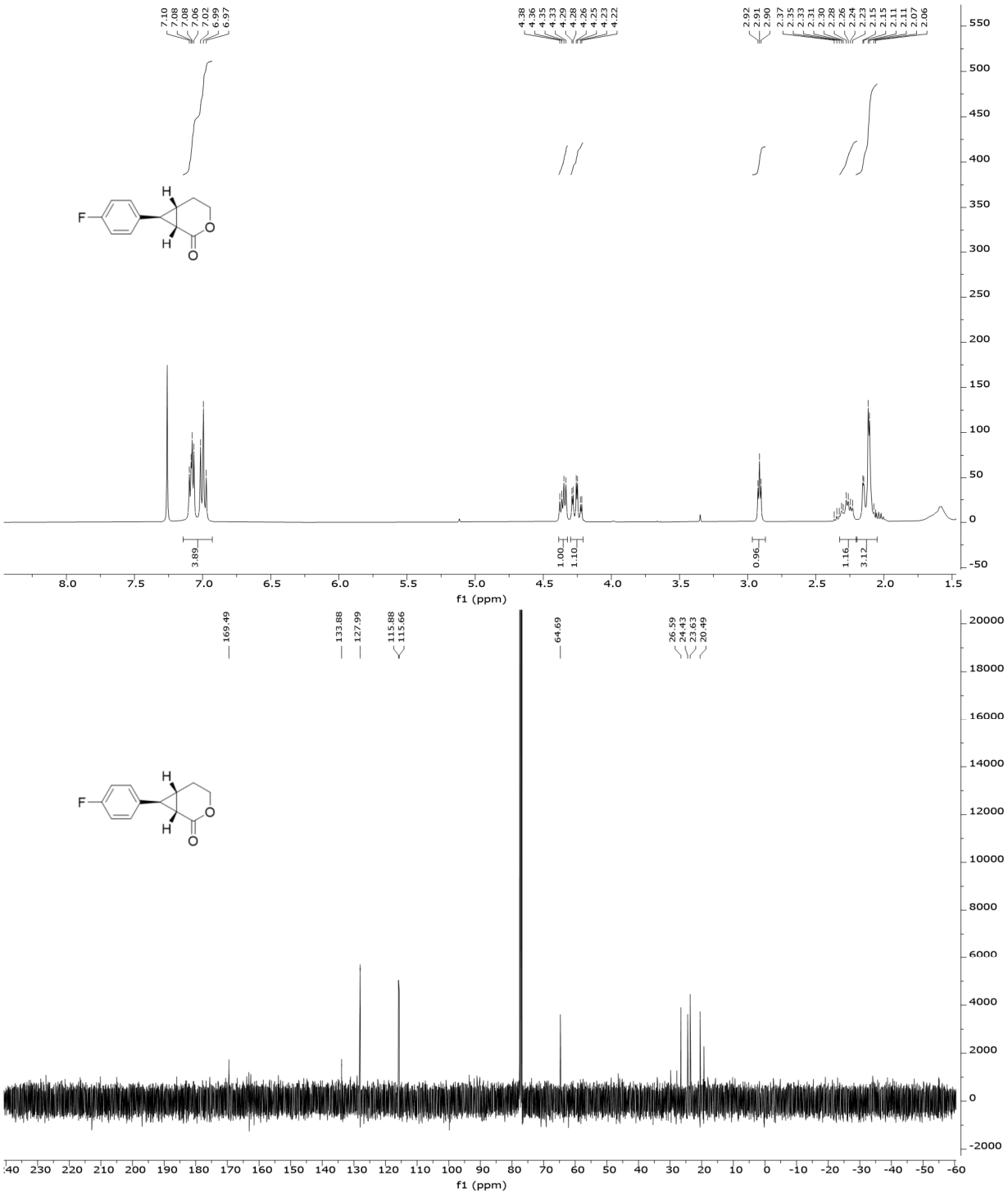


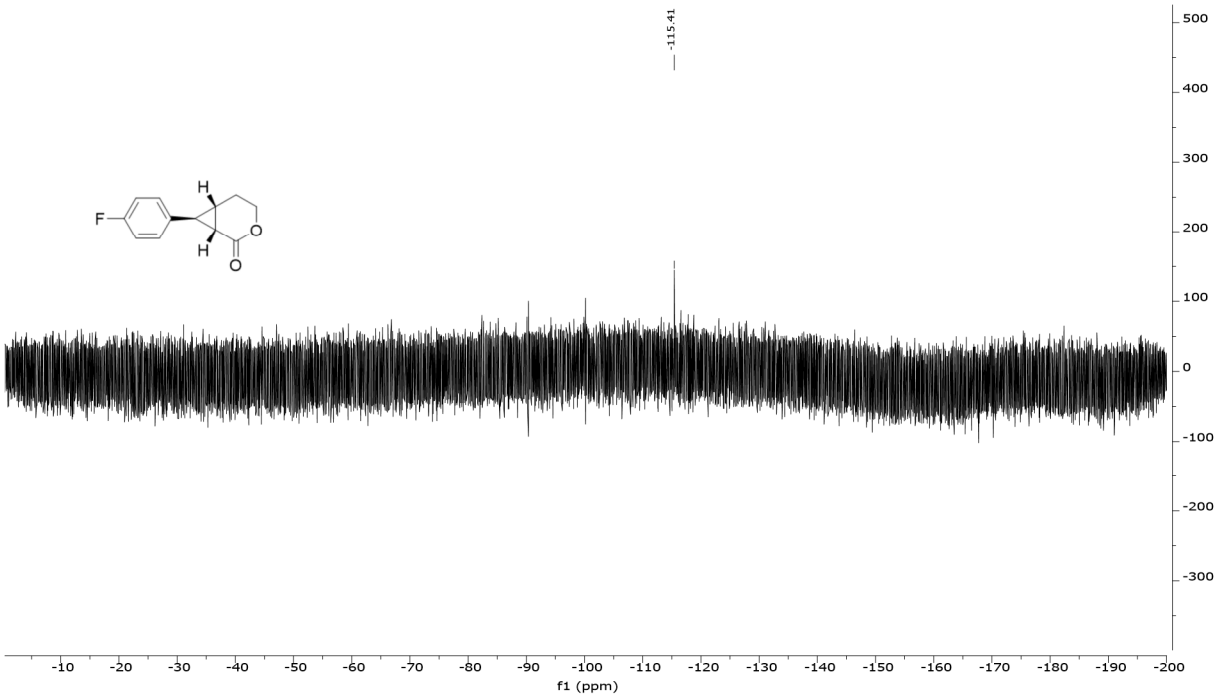


(1S,6S,7S)-7-phenyl-3-oxabicyclo[4.1.0]heptan-2-one (2a): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

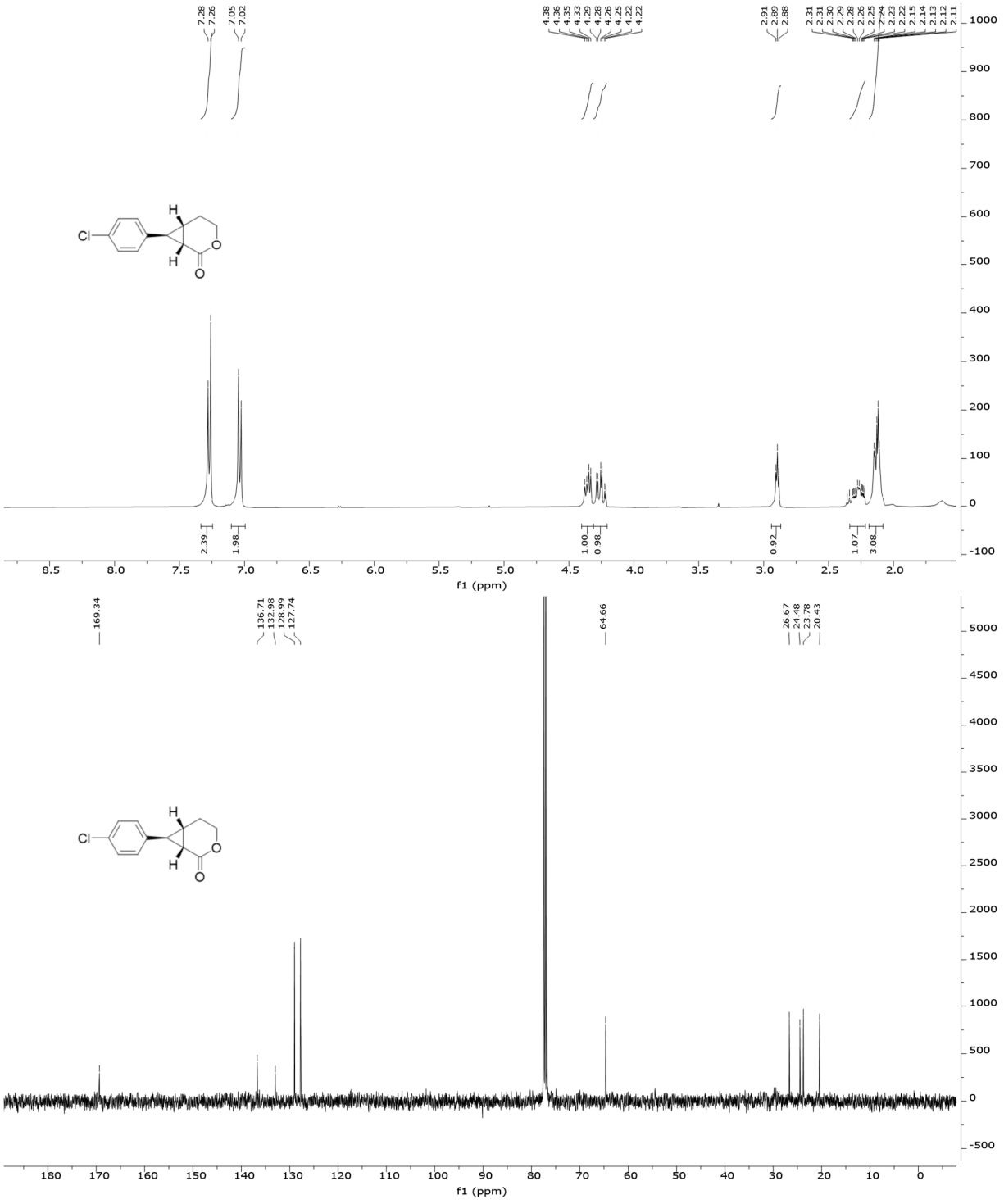


(1S,6S,7S)-7-(4-fluorophenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2b): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent, 376 MHz ^{19}F spectrum in C_6D_6

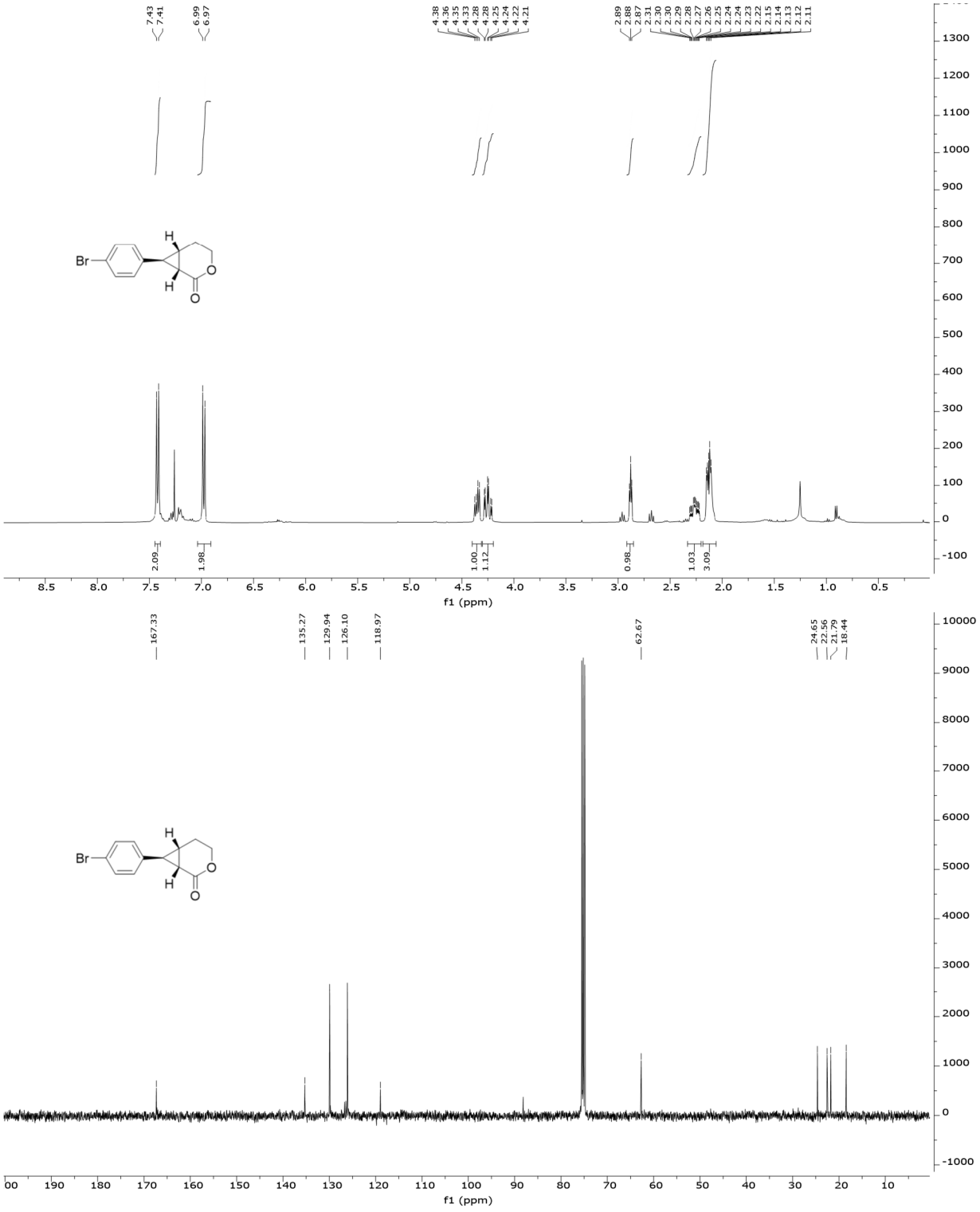



-115.41
F
H
H
O
O
500
400
300
200
100
0
-100
-200
-300
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

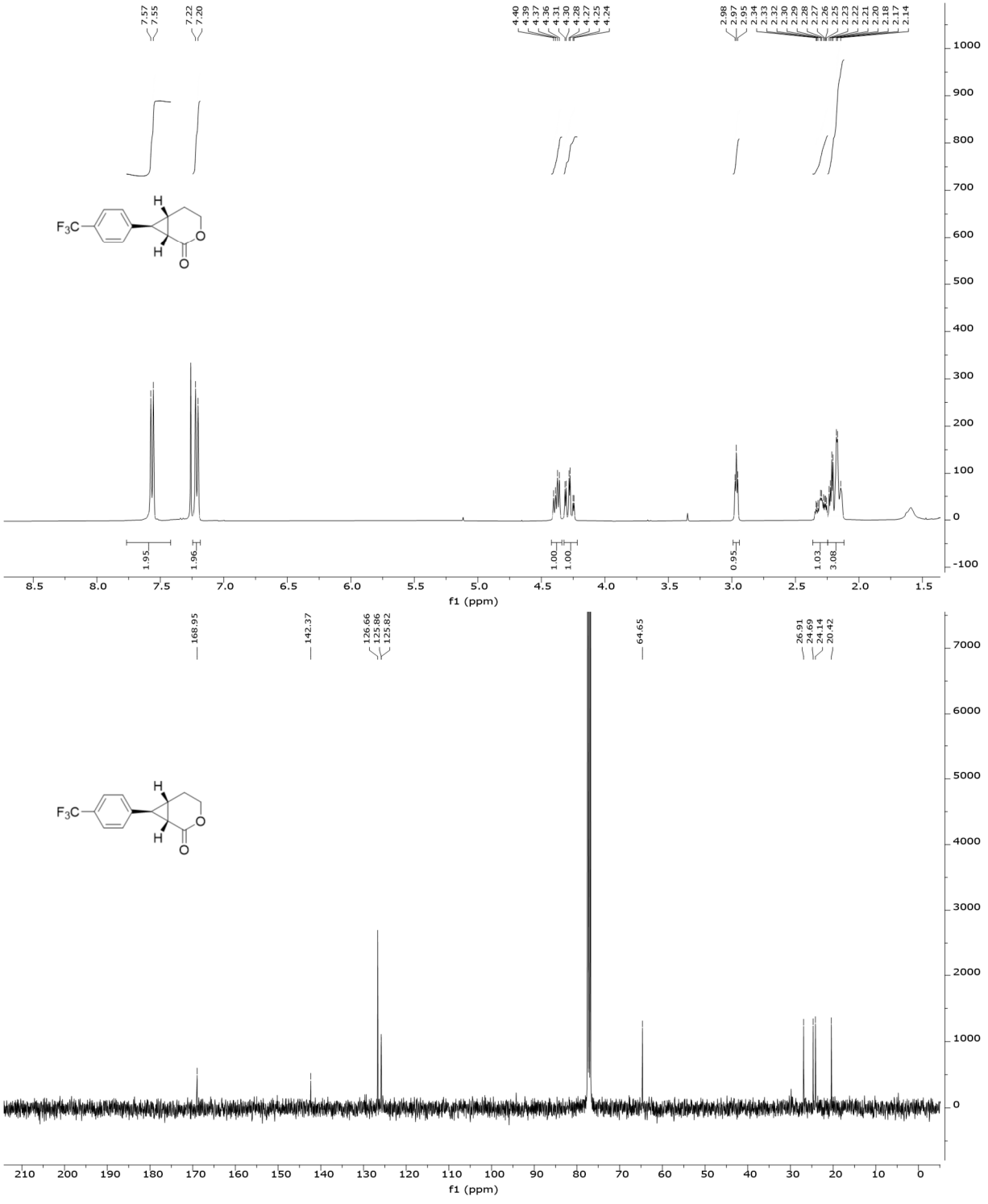
(1S,6S,7S)-7-(4-chlorophenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2c): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

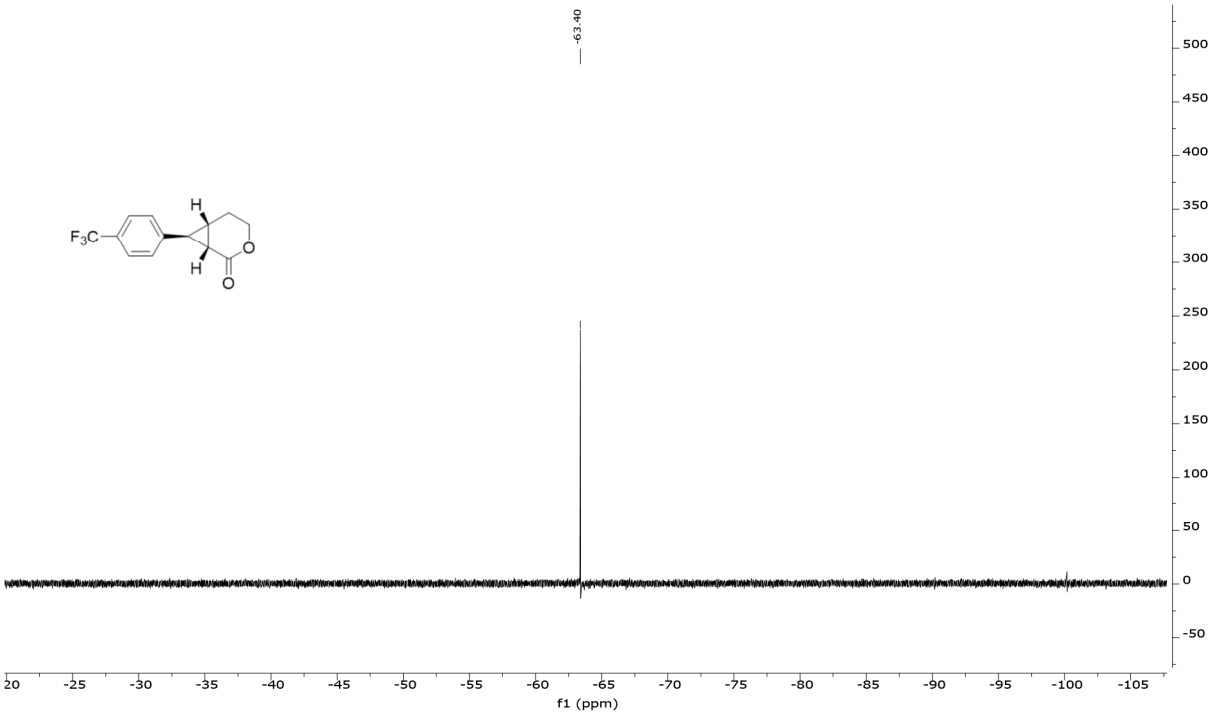


(1S,6S,7S)-7-(4-bromophenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2d): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

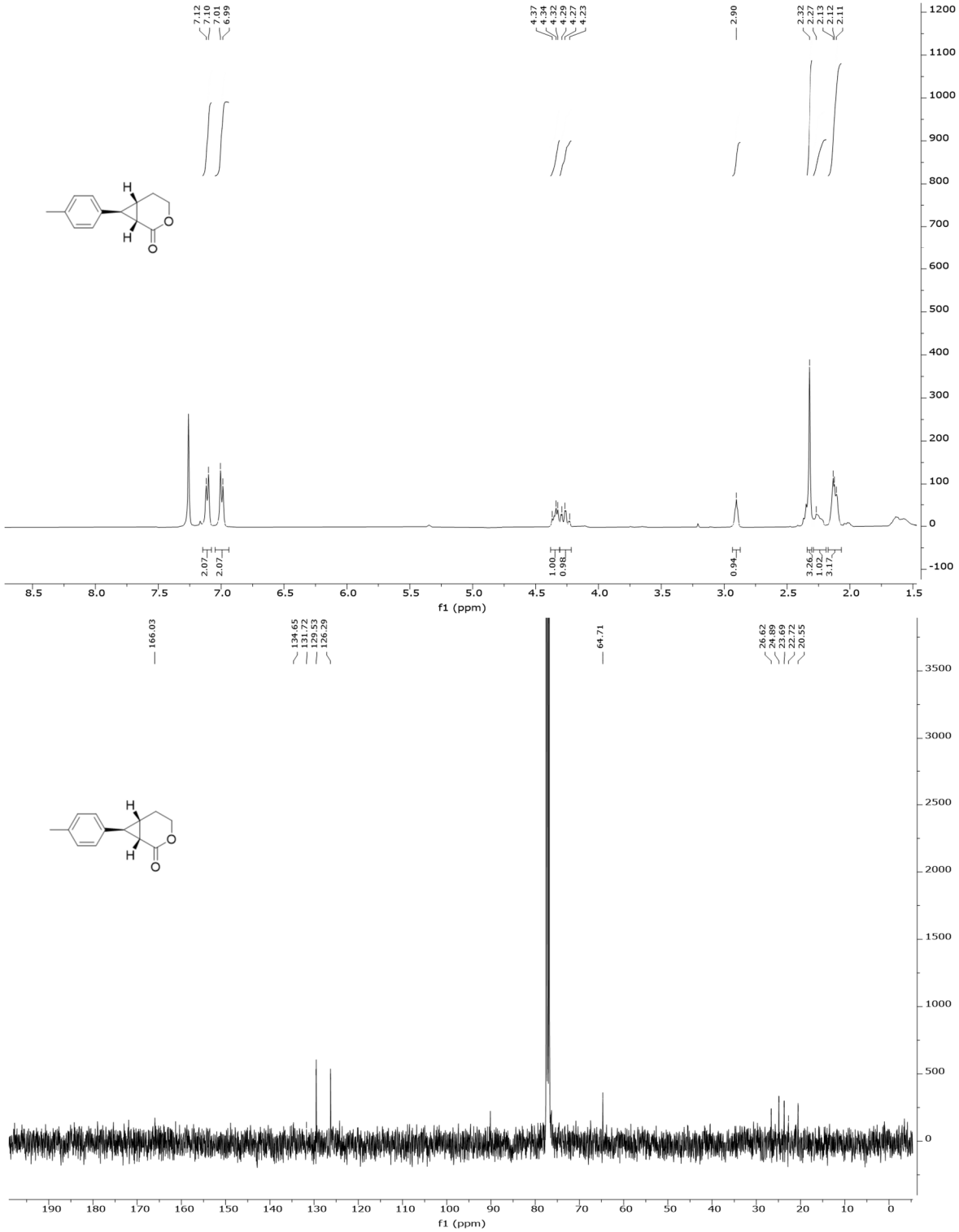


(1S,6S,7S)-7-(4-(trifluoromethyl)phenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2e): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent**,** 376 MHz ^{19}F spectrum in C_6D_6

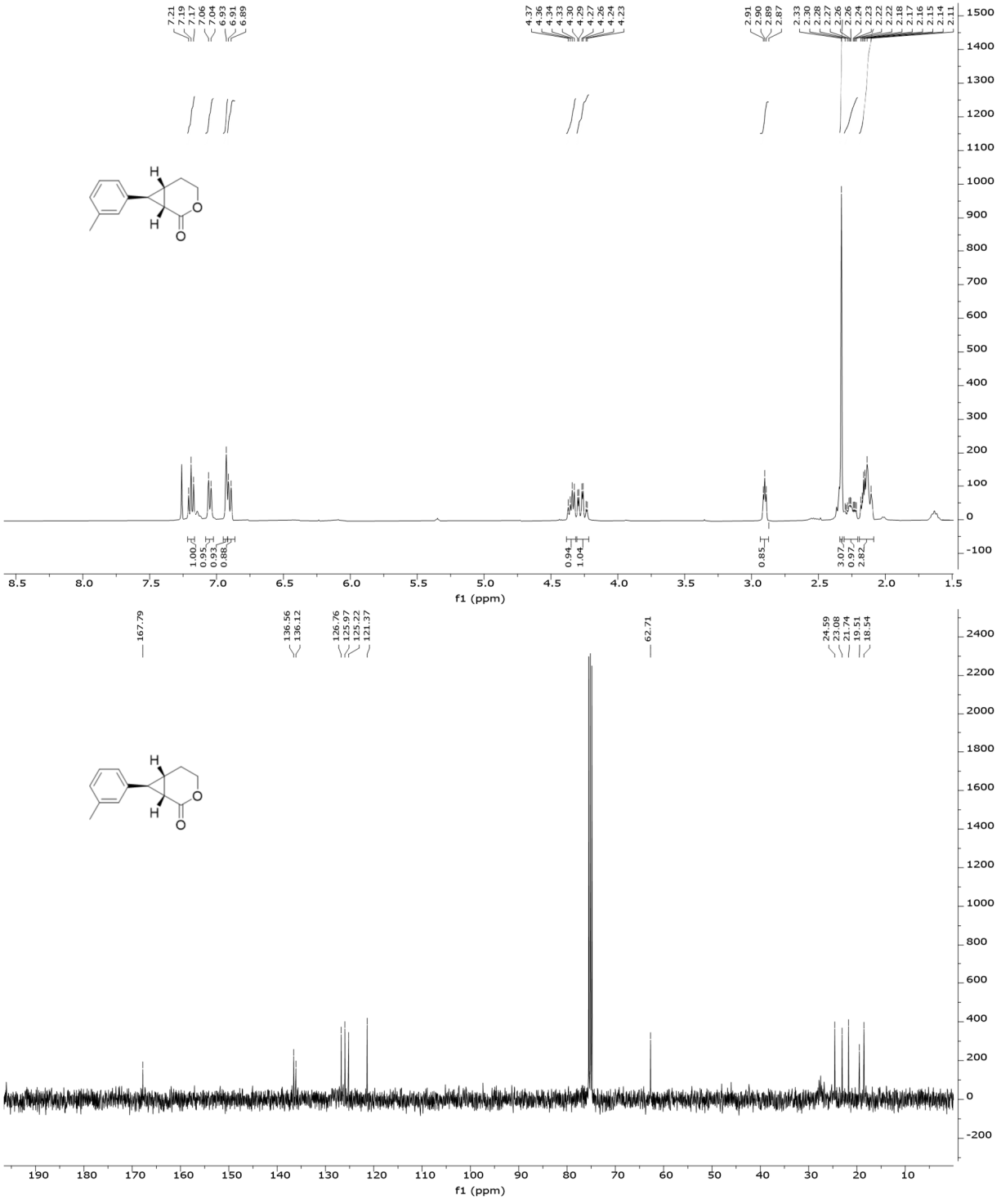



-63.40
F3C
H
H
O
O
500
450
400
350
300
250
200
150
100
50
0
-50
20
-25
-30
-35
-40
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
f1 (ppm)

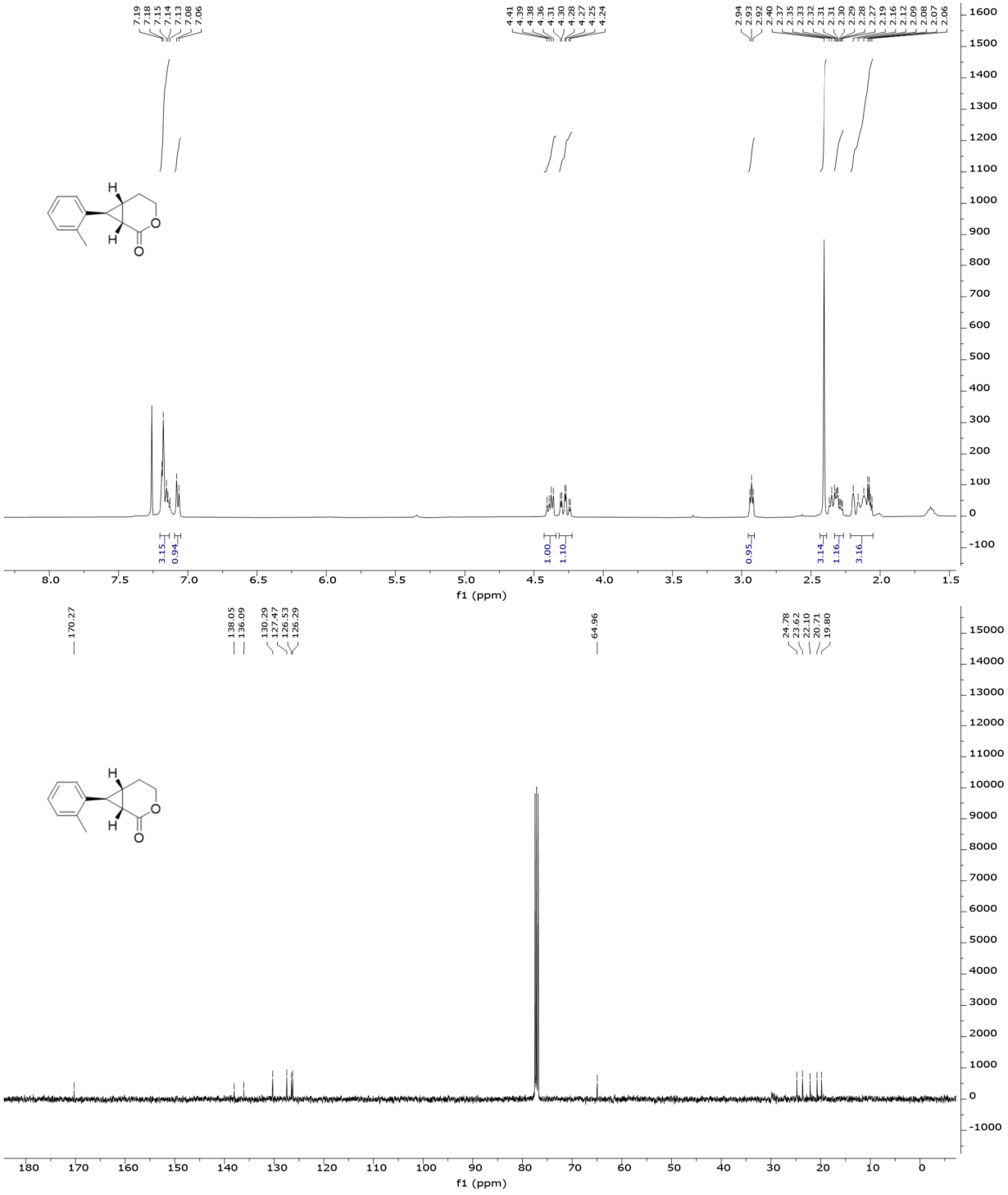
(1S,6S,7S)-7-(p-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one (2f): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent



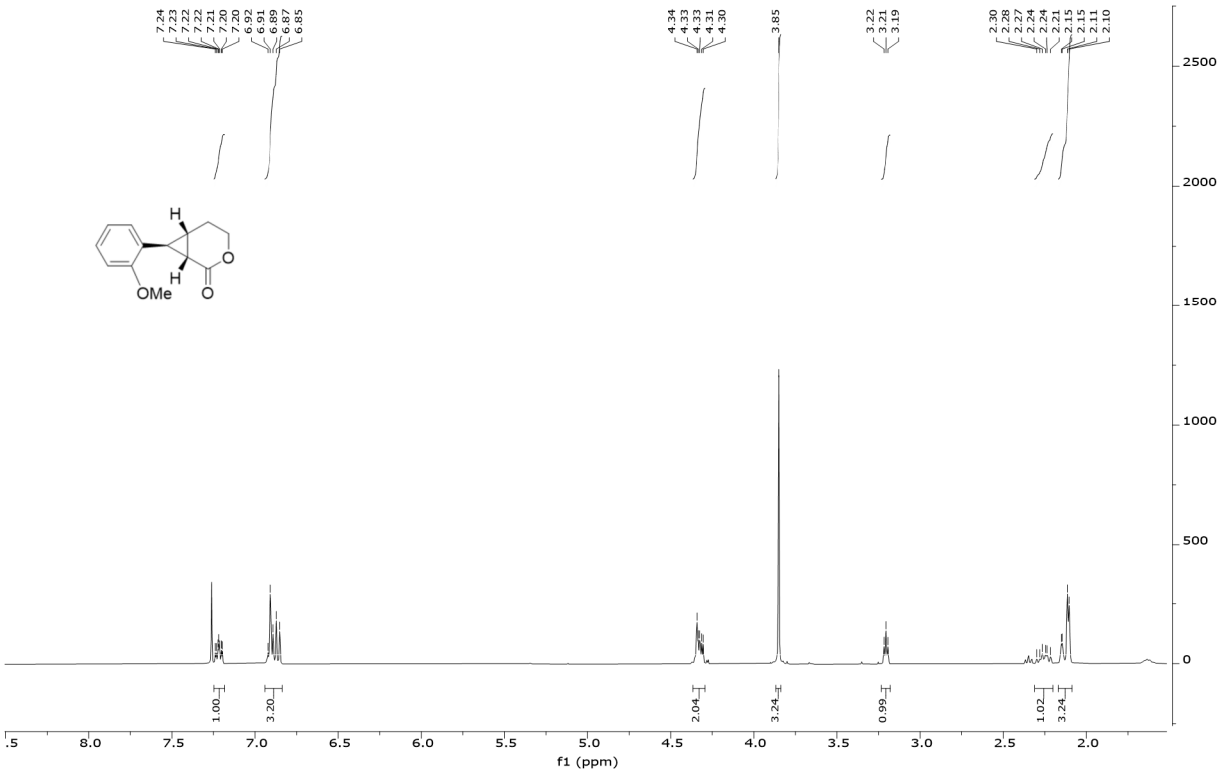
(1S,6S,7S)-7-(m-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one (2g): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

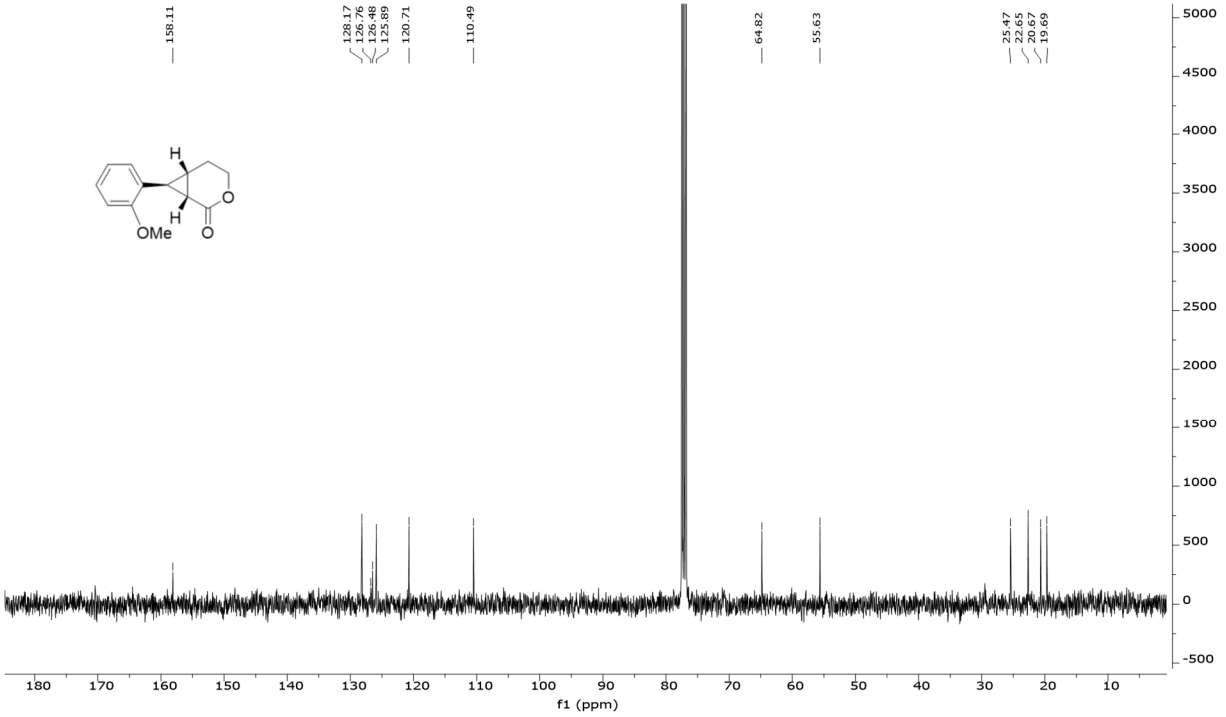


(1S,6S,7S)-7-(o-tolyl)-3-oxabicyclo[4.1.0]heptan-2-one (2h): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

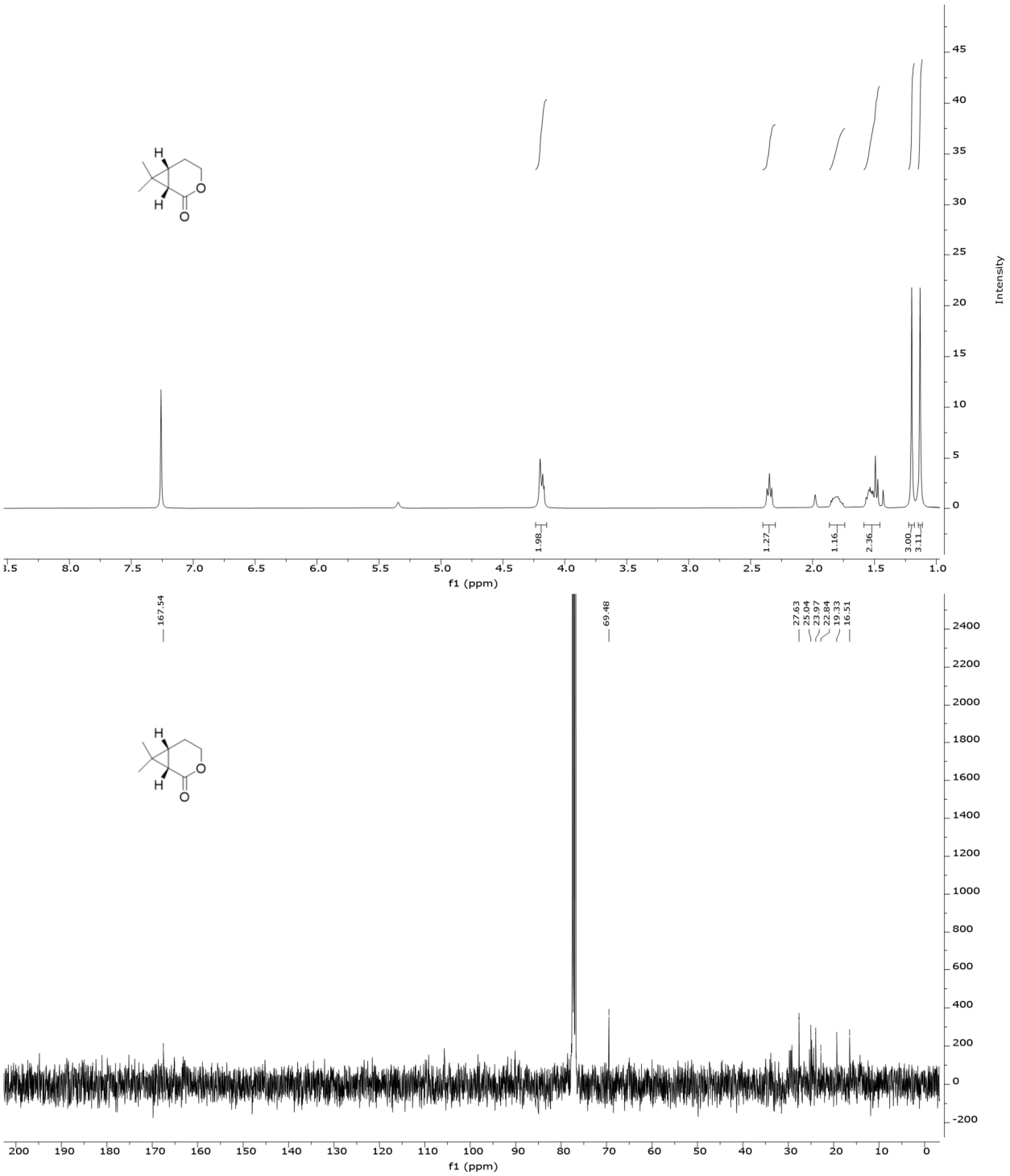


(1S,6S,7S)-7-(2-methoxyphenyl)-3-oxabicyclo[4.1.0]heptan-2-one (2i): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent

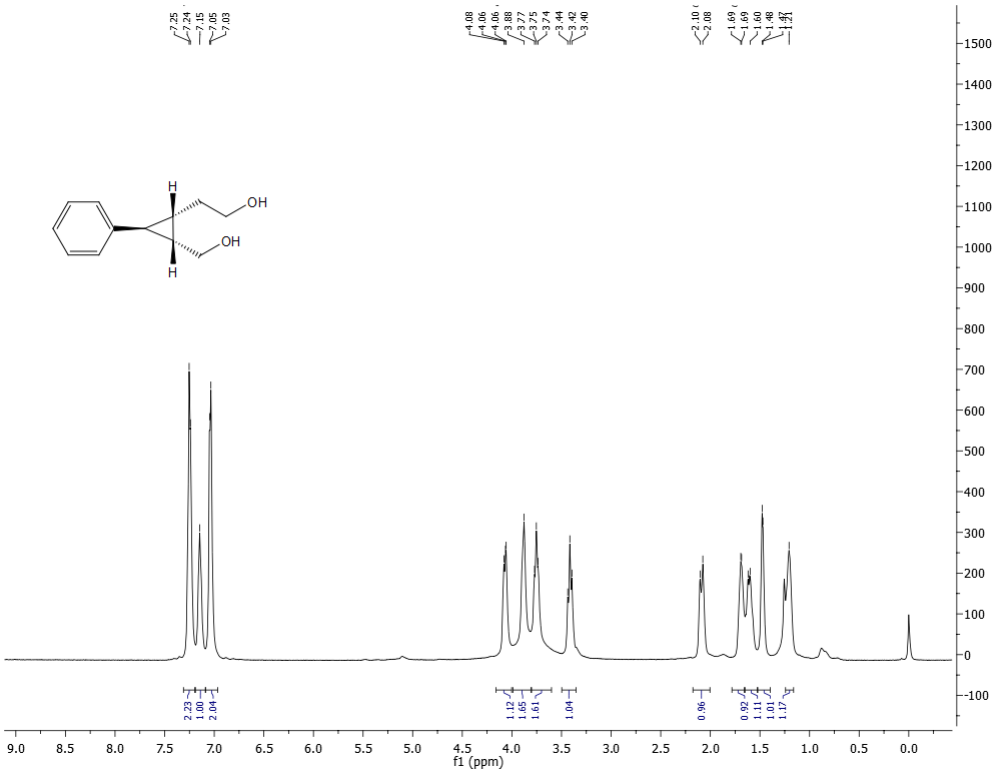


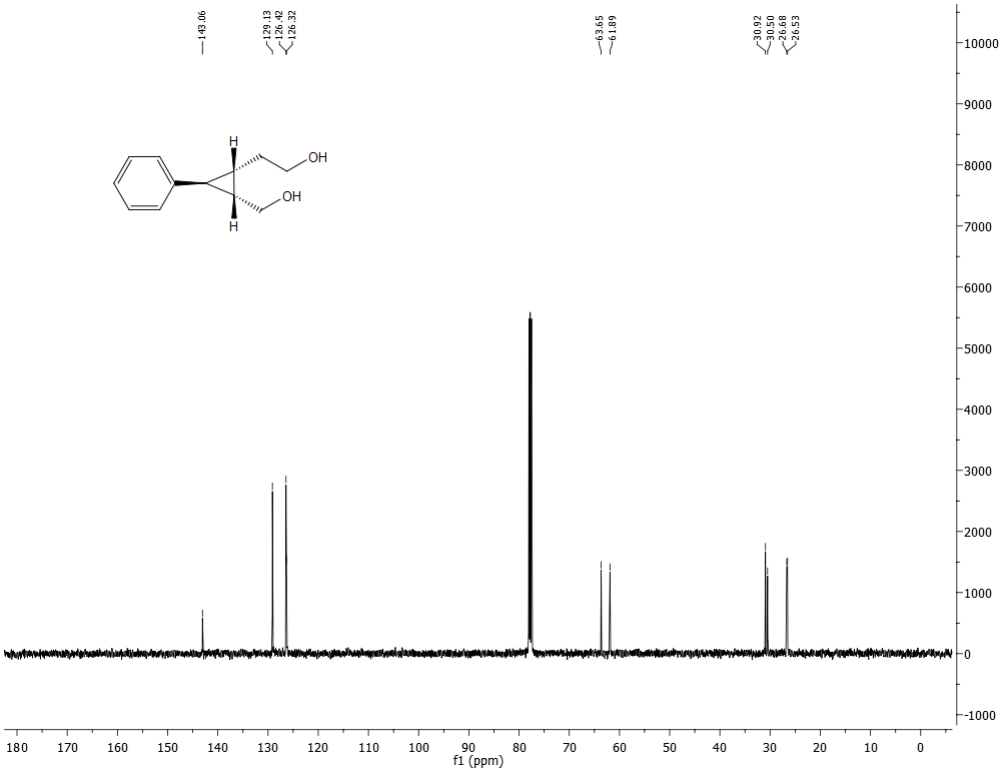


(1S,6R)-7,7-dimethyl-3-oxabicyclo[4.1.0]heptan-2-one (2j): 400 MHz ^{1}H spectrum and 101 MHz ^{13}C spectrum in $CDCl_3$ solvent



2-((1S,2S,3S)-2-(hydroxymethyl)-3-phenylcyclopropyl)ethan-1-ol (4): 500 MHz ^{1}H spectrum and 126 MHz ^{13}C spectrum in $CDCl_3$ solvent





(1S,2S,3S)-2-(2-hydroxyethyl)-3-phenylcyclopropane-1-carboxylic acid (5): 500 MHz ^{1}H spectrum and 126 MHz ^{13}C spectrum in MeOD solvent