Supporting Information

Enzymatic Assembly of Diverse Lactone Structures: An Intramolecular C–H Functionalization Strategy

Daniel J. Wackelin[1†], Runze Mao[1†], Kathleen M. Sicinski[1], Yutao Zhao[1,2], Anuvab Das[1], Kai Chen[1,3*] and Frances H. Arnold[1*]

[1]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, United States.

[2]Present address: Department of Chemistry, The University of Chicago, Chicago, IL 60637, United States.

[3]Present address: Innovative Genomics Institute, University of California, Berkeley, CA 94720, United States.

[†]These authors contributed equally.

*Corresponding author. E-mails: chenkaikc1990@gmail.com and frances@cheme.caltech.edu

Table of Contents

1. General Procedures

Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, VWR, TCI America, Fischer Scientific, Alfa Aesar, Acros, and Combi Blocks) and used without further purification. Silica gel chromatography was carried out using AMD Silica Gel 60, 230–400 mesh. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Prodigy 400 MHz instrument (400 MHz for ^{1}H and 101 MHz for ^{13}C NMR) or a Varian 300 MHz Spectrometer (300 MHz for ^{1}H NMR). Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane, using the solvent resonance as the internal standard (^{1}H NMR: δ = 7.26, ^{13}C NMR: δ = 77.16 for $CDCl_3$). Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets), coupling constant (Hz), integration. Sonication was performed using a Qsonica Q500 sonicator. High-resolution mass spectra were obtained at the California Institute of Technology Mass Spectral Facility. Samples were analyzed by field ionization (FI) using a JEOL AccuTOF GC-Alpha (JMS-T2000GC) mass spectrometer interfaced with an Agilent 8890 GC system. Ions detected by FI are radical cations.

Escherichia coli cells were grown using Luria-Bertani medium or HyperBroth (AthenaES) with 100 μg/mL ampicillin (LB_{amp} or HB_{amp}). Primer sequences are available upon request. T5 exonuclease, Phusion polymerase, and *Taq* ligase were purchased from New England Biolabs (NEB, Ipswich, MA). M9-N minimal media (abbreviated as M9-N buffer, pH = 7.4) were used as buffering systems for whole cells, unless otherwise specified. M9-N buffer was used without a carbon source, it contains 47.7 mM Na_2HPO_4, 22.0 mM KH_2PO_4, 8.6 mM NaCl, 2.0 mM $MgSO_4$, and 0.1 mM $CaCl_2$.

1.1 Chromatography

Chemical reactions were monitored using thin layer chromatography (Merck 60 silica gel plates) and a UV lamp for visualization. Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out using an Agilent 1200 series instrument and a Kromasil 100 C18 column (4.6 mm × 50 mm, 3 μm) or an Agilent C18 column (InfinityLab Poroshell 120 EC-C18, 4.6 x 50 mm, 2.7 μm; Part Number: 699975-902T) with water and acetonitrile, both

containing 0.1% acetic acid, as the mobile phase. Analytical chiral normal-phase HPLC was conducted using either an Agilent 1200 series instrument with *n*-hexane and isopropanol as the mobile phase or JASCO SF-2000 integrated analytical supercritical fluid chromatography (SFC) system with supercritical CO_2 and isopropanol as the mobile phase. Enantiomers were separated using one of the following chiral columns: Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak AD-H, and Daicel CHIRALCEL OJ-H (all 4.6 mm × 250 mm, 5 µm).

1.2 Cloning and Site-Saturation Mutagenesis

The genes encoding all enzymes described in this study were cloned using Gibson assembly[1] into vector pET22b(+) (Novagen) between restriction sites *Nde*I and *Xho*I in frame with a *C*-terminal 6×His-tag. Site-saturation mutagenesis was performed using the "22c-trick"[2] or "NNK" as degenerative codons. The PCR products were digested with *Dpn*I, gel purified, and ligated using Gibson Mix™.[1] Without further purification after the Gibson step, 1 µL of the Gibson product was used to transform 50 µL of electrocompetent *E. coli* BL21 E. cloni® (Lucigen) cells.

1.3 Expression of P411 Variants in 96-Well Plates

Single colonies from LB_{amp} agar plates were picked using sterile toothpicks and cultured in deep-well 96-well plates containing LB_{amp} (300 µL/well) at 37 °C, 80% humidity, and 220 rpm shaking overnight. Subsequently, HB_{amp} (1000 µL/well) in a deep-well plate was inoculated with an aliquot (50 µL/well) of these overnight cultures and allowed to shake for 2.5 hours at 37 °C, 80% humidity, and 220 rpm. The plates were then cooled on ice for 30 minutes, and the cultures were induced with 0.5 mM isopropyl *β*-D-1-thiogalactopyranoside (IPTG) (final concentration). The expression was supplemented with 1.0 mM 5-aminolevulinic acid (ALA) (final concentration). Expression was then conducted at 20 °C, 220 rpm for 20–22 hours.

1.4 Plate Reaction Screening in Whole-Cell Format

E. coli cells harboring P411 variants in deep-well 96-well plates were pelleted (3,500 g, 5 min, 4 ºC) and resuspended in M9-N buffer (360 µL, pH 7.4) by gentle vortexing. The 96-well plates were then transferred to an anaerobic chamber. To deep-well plates of cell suspensions was added the substrate (20 µL/well, 200 mM in acetonitrile). The plates were sealed with aluminum sealing foil immediately after the addition and shaken in the anaerobic chamber at room temperature and 600 rpm. After 24 hours, the seals were removed and acetonitrile (600 µL/well) was added. The

plates were tightly sealed with silicone mats, vigorously vortexed, and centrifuged (4,500 g, 5 min) to precipitate proteins and cell debris. The supernatant (200 µL/well) was filtered through an AcroPrep 96-well filter plate (0.2 µm) into a 96-well assay plate for reverse-phase HPLC analysis to determine the yield.

1.5 Flask Expression of P411 Variants

E. coli (E. cloni BL21(DE3)) cells carrying plasmid encoding the appropriate P411 variant were grown overnight in 5 mL Luria-Bertani medium supplemented with 0.1 mg/mL ampicillin (LB_{amp}). The preculture (1 mL) was used to inoculate 50 mL of Hyperbroth medium supplemented with 0.1 mg/mL ampicillin (HB_{amp}) in a 125-mL Erlenmeyer flask. This culture was incubated at 37 °C and 230 rpm for 2.5 hours. The culture was then cooled on ice for 45 min and induced with 0.5 mM IPTG and 1.0 mM ALA (final concentrations). Expression was conducted at 24 °C, 140 rpm for 16–18 hours. Subsequently, the *E. coli* cells were pelleted by centrifugation (5,000 g, 5 min, and 4 °C). Media were removed, and the pellets were resuspended to an optical density at 600 nm (OD_{600}) of 30 in M9-N minimal medium. Aliquots of the cell suspension (3–4 mL) were used to determine protein concentration after lysis by sonication.

1.6 Hemochrome Assay for the Determination of Hemoprotein Concentration

Protein concentration in the cell was determined using the hemochrome assay on cell lysate.[3] Lysate was obtained by sonication using using a Qsonica Q500 sonicator (6 minutes, 1 second on, 2 seconds off, 35% amplitude, on wet ice). The cell debris was removed by centrifugation (14,000 g, 10 minutes, 4 °C). To a cuvette, 500 µL of the lysate and 500 µL of solution I [0.2 M NaOH, 40% (v/v) pyridine, 0.5 mM $K_3Fe(CN)_6$] were added. The UV-Vis spectrum (380–620 nm) of the oxidized state Fe(III) was recorded immediately. Sodium dithionite (10 µL of 0.5 M solution in water) was added, and the UV-Vis spectrum of the reduced state Fe(II) was recorded immediately. The protein concentration was calculated using the extinction coefficient and dilution factor (2× dilution in volume): $\varepsilon\_[557_{reduced} - 540_{oxidized}] = 23.98\ mM^{-1}cm^{-1}$. The hemochrome assay detects total heme level, which is a good approximation of over-expressed heme enzyme.

1.7 Biotransformation Using Whole *E. coli* Cells

Suspension of *E. coli* (*E. cloni* BL21(DE3)) cells expressing the appropriate hemoprotein variant in M9-N buffer (typically OD_{600} = 2.5) were transferred to a reaction vial. Enzymatic

reactions were then set up in an anaerobic chamber. To an Agilent's 2-mL screw top vial were added degassed suspension of *E. coli* expressing P411 variant (typically OD_{600} = 2.5, 395 μL) and substrate (typically 5 μL of 200 mM stock solution in acetonitrile). The final volume of the biotransformation was set to be 400 μL, with 1.25% (v/v) acetonitrile. The reaction vials were then capped and shaken in the anaerobic chamber at room temperature and 600 rpm for 24–48 hours. After the completion of the reaction, 600 μL acetonitrile containing 0.67 mM 1,3,5-trimethoxybenzene internal standard were added to the vial. The resulting mixture was transferred to a 1.7-mL microcentrifuge tube, vigorously vortexed, and centrifuged (14,000 g, 5 min, 4 °C). A sample of the supernatant (0.6 mL) was transferred to a vial for HPLC analysis.

To determine the enantiomeric excess (e.e.), three parallel analytical reactions were conducted. Each reaction mixture was extracted with an equal volume of a hexane/ethyl acetate (3:1) solution. The organic extracts were combined and transferred to a 2-dram vial, and the solvent was removed by blowing air. Residue was resuspended in 100 μL isopropanol and subjected to normal-phase HPLC to determine the e.e.

1.8 Enzymatic Preparative Synthesis

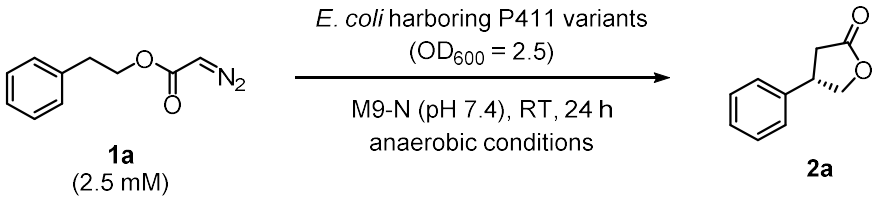
The *E. coli* cell suspension harboring a P411 variant was prepared as described in **Sections 1.5** and **1.6**. The cell suspension (*E. coli* whole cells harboring P411 variants, OD_{600} = 2.5, suspended in M9-N aqueous buffer) was placed in a flask covered loosely with aluminum foil and then bubbled with argon for at least 15 minutes. The reaction flask was then transferred to an anaerobic chamber. To the reaction flask with cell suspension (OD_{600} = 2.5), substrate (200 mM acetonitrile stock solution, final concentration = 2.5 mM, 1.0 equiv.) was added to make 1.25% v/v acetonitrile (co-solvent). The reaction vial was immediately capped and sealed with parafilm and stirred in the anaerobic chamber at room temperature for 48 hours. The reaction solution was filtered through celite then extracted with an equal volume of ethyl acetate (× 3 times). The combined organic layer was then washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*, and the product was purified by flash chromatography.

Note: Many of the lactone standard products involved in this study are challenging to prepare using existing chemical methods, particularly those with 6-membered and 7-membered rings, and complex structures. Therefore, those challenging products were synthesized using enzymatic

catalysis developed in this work. However, for most of these preparative-scale biosyntheses, we did not use the final enzyme variants, but rather used variants earlier in the evolution trajectory. This is because: 1. We need to obtain standard products early on to draw HPLC standard curves and determine the activity of enzyme variants during directed evolution. 2. The final enzymes usually afford nearly enantiopure products, so we would have been unable to obtain two enantiomers and separate them using chiral HPLC. In order to obtain imperfect e.e. values, we used earlier enzyme variants to biosynthesize lactone standards in preparative scales. Therefore, most of the NMR spectra were obtained from products catalyzed by non-final (less active) enzyme variants.

2. Discovery of Initial Activity and Directed Evolution

Table S1. Initial activity screening with engineered P411s for γ-lactone synthesis.[a]



A panel of cytochrome P450 and P411 (P450 with an axial serine ligand) variants previously engineered for carbene nitrene transformations were screened in whole *E. coli* cells against **1a** under anaerobic conditions.[4-6] Among these, P411-LAS-5239 (**C10**), known for its promiscuous activities in different carbene-transfer reactions such as internal alkyne cyclopropenation[5] and lactone-carbene C–H insertion,[4] was effective in producing the γ-lactone product **2a** (8% yield).

Entry	Variant / Catalyst	Yield of 3a[a]	Standard deviation of yield	TTN	Standard deviation of TTN
1	P411-LAS-5239	7.5%	0.5%	530	35
2	[b] hemin (20 μM)	N.D.	-	N.D.	-
3	[c] hemin (20 μM) + $Na_2S_2O_4$	N.D.	-	N.D.	-
4	[c] hemin (20 μM) + BSA (20 μM)	N.D.	-	N.D.	-
5	[c] hemin (20 μM) + $Na_2S_2O_4$ + BSA (20 μM)	N.D.	-	N.D.	-
6	[d] cellular background	N.D.	-	N.D.	-

[a] Experiments were performed using whole *E. coli* cells according to the protocol described in **Sections 1.3** and **1.4**. The yields of **2a** were calculated based on comparing the signal integration ratio of the products and an added internal standard compound (see **section 6** for more details). All reactions were performed in triplicate, with the reported yields representing the average of three

experiments. The signal calibration curves correlating the products and the internal standard compound can be found in **Section 6**. To enhance systematic management of different enzyme variants within the Arnold lab, we recently implemented a new nomenclature system. Variants are named as follows: family name-chemistry abbreviation-entry code. All enzyme variants in this study follow this nomenclature.

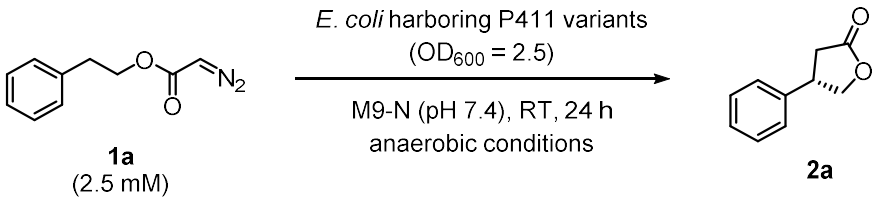
[b] Negative control experiments (with free hemin) were performed without the addition of $Na_2S_2O_4$. Under this condition, the resting oxidation state of hemin in aqueous buffer should be Fe(III).

[c] Negative control experiments using free hemin under reduced conditions (Fe(II)) were performed using an excess amount of $Na_2S_2O_4$ (20 mM).

[d] Cellular background control experiments were performed in whole-cell format, using *E. coli* (*E. cloni* BL21(DE3)) cells harboring an engineered tryptophan synthase β-subunit (Tm9D8*). The gene of this enzyme was also cloned into the pET22b(+) vector (Novagen) between restriction sites *Nde*I and *Xho*I. The protein expression protocol for this experiment followed the standard P411 expression conditions as described in **Sections 1.5**, **1.6**, and **1.7**.

N.D. – no product was detected.

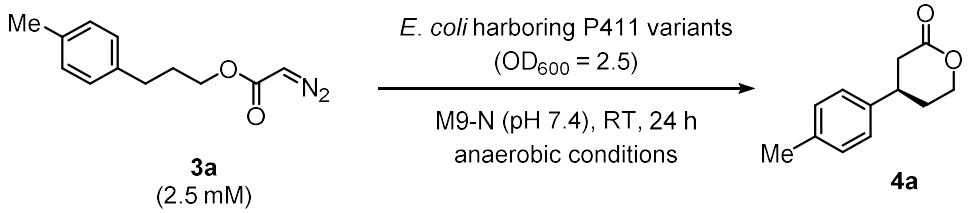
Table S2. Directed evolution of P411-LAS-5247 for γ-lactone synthesis.[a]



Entry	Round #	Variant	Yield of 2a[a]	Standard deviation of yield	TTN	Standard deviation of TTN
1	0	P411-LAS-5239	7.5%	0.5%	530	35
2	1	P411-LAS-5240	14%	1.7%	2200	260
3	2	P411-LAS-5241	20%	0.1%	1100	8
4	3	P411-LAS-5242	35%	0.4%	2700	28
5	4	P411-LAS-5243	41%	1.5%	2900	200
6	5	P411-LAS-5244	52%	1.5%	3900	120
7	6	P411-LAS-5245	64%	2.9%	4100	190
8	7	P411-LAS-5246	65%	2.0%	4900	150
9	8	P411-LAS-5247	66%	0.8%	3800	49

[a] Experiments were performed using a suspension of *E. coli* cells harboring enzyme variants prepared according to the protocol described in **Sections 1.5**, **1.6**, and **1.7**. Yields were calculated from HPLC calibration curves and the average of triplicate experiments (n = 3).

Table S3. Directed evolution of P411-LAS-5246 for *δ*-lactone **4a** synthesis.[a]



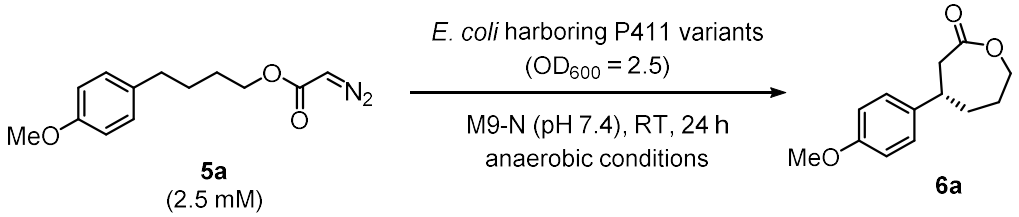
Entry	Round #	Variant	Yield of 4a[a]	Standard deviation of yield	TTN	Standard deviation of TTN
1	0	P411-LAS-5244 [b]	52%	12.4%	3900	930
2	1	P411-LAS-5248	50%	6.2%	2900	360
3	2	P411-LAS-5249 [c]	50%	1.1%	2800	60

[a] Experiments were performed using a suspension of *E. coli* cells harboring enzyme variants prepared according to the protocol described in **Sections 1.5**, **1.6**, and **1.7**. Yields were calculated from HPLC calibration curves and the average of triplicate experiments (n = 3).

[b] 46% e.e. for product **4a**.

[c] 83% e.e. for product **4a**.

Table S4. Directed evolution of P411-LAS-5265 for ε-lactone **6a** synthesis.[a]



Entry	Round #	Variant	Yield of 6a[a]	Standard deviation of yield	TTN	Standard deviation of TTN
1	0	P411-LAS-5250	16%	1.9%	940	110
2	1	P411-LAS-5251	26%	1.2%	1100	52
3	2	P411-LAS-5252	21%	0.5%	1100	28
4	3	P411-LAS-5253	31%	1.9%	1500	94
5	4	P411-LAS-5254	31%	4.0%	1300	170
6	5	P411-LAS-5255	36%	3.8%	1500	160
7	6	P411-LAS-5256	44%	2.3%	1700	90
8	7	P411-LAS-5257	52%	2.7%	2200	110
9	8	P411-LAS-5258	45%	2.2%	2300	120
10	9	P411-LAS-5259[b]	58%	0.3%	2400	14
11	10	P411-LAS-5260[c]	12%	0.3%	700	19

12	11	P411-LAS-5261	18%	0.8%	1200	51
13	12	P411-LAS-5262	33%	1.1%	2700	93
14	13	P411-LAS-5263	39%	2.2%	2900	160
15	14	P411-LAS-5264 [d]	54%	0.5%	3100	27

[a] Experiments were performed using a suspension of *E. coli* cells harboring enzyme variants prepared according to the protocol described in **Sections 1.5**, **1.6**, and **1.7**. Yields were calculated from HPLC calibration curves and the average of triplicate experiments (n = 3).

[b] 51% e.e. for product **6a**.

[c] 96% e.e. for product **6a**.

[d] 95% e.e. for product **6a**.

3. Nucleotide and Amino Acid Sequences

The genes encoding the heme proteins shown below were cloned using Gibson assembly[1] into vector pET-22b(+) (Novagen) between restriction sites *Nde*I and *Xho*I in frame with a *C*-terminal 6×His-tag.

Table S5. Mutations of the P411 variants in this study.

Name	**Mutations relative to the wild-type P450$_{BM3}$**	**Mutations relative to P411-C10**
P411-LAS-5239	N70E, A74G, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, I401L, T436L, L437Q, E442K, ΔFAD	-
P411-LAS-5240	N70E, A74G, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, I401L, T436L, **L437I**, E442K, ΔFAD	**Q437I**
P411-LAS-5241	N70E, A74G, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, **A328I**, A330Y, L353V, I366V, C400S, I401L, T436L, **L437I**, E442K, ΔFAD	**V328I, Q437I**
P411-LAS-5242	N70E, A74G, **V78M**, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G,	**L78M, V328I, Q437I**

	I263Y, H266V, T268G, A290V, **A328I**, A330Y, L353V, I366V, C400S, I401L, T436L, **L437I**, E442K, ΔFAD	
P411-LAS-5243	N70E, A74G, **V78M**, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, **A328I**, A330Y, L353V, I366V, C400S, I401L, **T436R**, **L437I**, E442K, ΔFAD	**L78M, V328I, L436R, Q437I**
P411-LAS-5244	N70E, A74G, **V78M**, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, **A328I**, A330Y, L353V, I366V, C400S, **I401V**, **T436R**, **L437I**, E442K, ΔFAD	**L78M, V328I, L401V, L436R, Q437I**
P411-LAS-5245	N70E, A74G, **V78M**, A82L, F87A, M118S, P142S, **F162I**, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, **A328I**, A330Y, L353V, I366V, C400S, **I401V**, **T436R**, **L437I**, E442K, ΔFAD	**L78M, L162I, V328I, L401V, L436R, Q437I**
P411-LAS-5246	N70E, A74G, **V78M**, A82L, F87A, M118S, P142S, **F162I**, T175I, M177L, A184V, **R190L**, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, **A328I**, A330Y, L353V, I366V, C400S, **I401V**, **T436R**, **L437I**, E442K, ΔFAD	**L78M, L162I, R190L, V328I, L401V, L436R, Q437I**
P411-LAS-5247	**N70S,** A74G, **V78M**, A82L, F87A, M118S, P142S, **F162I**, T175I, M177L, A184V, **R190L**, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, **A328I**, A330Y, L353V, I366V, C400S, **I401V**, **T436R**, **L437I**, E442K, ΔFAD	**E70S, L78M, L162I, R190L, V328I, L401V, L436R, Q437I**
P411-LAS-5248	N70E, A74G, **V78M**, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G,	**L78M, L401V, L436R, Q437I**

	I263Y, H266V, T268G, A290V, **A328V**, A330Y, L353V, I366V, C400S, **I401V**, **T436R**, **L437I**, E442K, ΔFAD	
P411-LAS-5249	N70E, A74G, **V78M**, A82L, F87A, **T88S,** M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, **A328V**, A330Y, L353V, I366V, C400S, **I401V**, **T436R**, **L437I**, E442K, ΔFAD	**L78M, T88S, L401V, L436R, Q437I**
P411-LAS-5250	N70E, S72F, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263W, H266V, T268G, A290V, A328V, A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72F, Y263W, S332G, L436R, Q437I**
P411-LAS-5251	N70E, S72F, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72F, Y263W, T327P, S332G, L436R, Q437I**
P411-LAS-5252	N70E, **S72V**, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, Y263W, T327P, S332G, L436R, Q437I**
P411-LAS-5253	N70E, **S72V**, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263W, H266V, T268G, A290V, **T327P**, A328V, **P329P**	**S72V, Y263W, T327P, S332G, L436R, Q437I**

	(silent mutation), A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	
P411-LAS-5254	N70E, **S72V**, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, H236Q, E252G, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, R226S, Y263W, T327P, S332G, L436R, Q437I**
P411-LAS-5255	N70E, **S72V**, V78L, A82L, F87A, **H92F**, M118S, P142S, F162L, T175I, M177L, A184V, H236Q, E252G, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, H92F, R226S, Y263W, T327P, S332G, L436R, Q437I**
P411-LAS-5256	N70E, **S72V**, V78L, A82L, F87A, **H92F**, M118S, P142S, T175I, M177L, A184V, H236Q, E252G, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, H92F, L162F, R226S, Y263W, T327P, S332G, L436R, Q437I**
P411-LAS-5257	N70E, **S72V**, V78L, A82L, F87A, **H92F**, M118S, P142S, T175I, M177L, A184V, H236Q, **E252R**, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, I366V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, H92F, L162F, R226S, G252R, Y263W, T327P, S332G, L436R, Q437I**
P411-LAS-5258	N70E, **S72V**, V78L, A82L, F87A, **H92F**, M118S, P142S, T175I, M177L, A184V, H236Q, **E252R**, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, H92F, L162F, R226S, G252R, Y263W, T327P, S332G,**

		V366I, L436R, Q437I
P411-LAS-5259	N70E, **S72V**, V78L, A82L, F87A, **H92F**, M118S, **P142G**, T175I, M177L, A184V, H236Q, **E252R**, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, H92F, S142G, L162F, R226S, G252R, Y263W, T327P, S332G, V366I, L436R, Q437I**
P411-LAS-5260	N70E, **S72V**, V78L, A82L, **F87V**, **H92F**, M118S, **P142G**, T175I, M177L, A184V, H236Q, **E252R**, I263W, H266V, T268G, A290V, **T327P**, A328V, A330Y, S332G, L353V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, A87V, H92F, S142G, L162F, R226S, G252R, Y263W, T327P, S332G, V366I, L436R, Q437I**
P411-LAS-5261	N70E, **S72V**, V78L, A82L, **F87V**, **H92F**, M118S, **P142G**, T175I, M177L, A184V, H236Q, **E252R**, I263W, H266V, T268G, A290V, **T327P**, **A328I**, A330Y, S332G, L353V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, A87V, H92F, S142G, L162F, R226S, G252R, Y263W, T327P, V328I, S332G, V366I, L436R, Q437I**
P411-LAS-5262	N70E, **S72V**, V78L, **D80E**, A82L, **F87V**, **H92F**, M118S, **P142G**, T175I, M177L, A184V, **I219I (silent mutation)**, H236Q, **G240R**, **E252R**, I263W, H266V, T268G, A290V, **T327P**, **A328I**, A330Y, S332G, L353V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**S72V, D80E, A87V, H92F, S142G, L162F, R226S, G240R, G252R, Y263W, T327P, V328I,**

		S332G, V366I, L436R, Q437I
P411-LAS-5263	**I2T**, N70E, **S72V**, V78L, **D80E**, A82L, **F87V**, **H92F**, M118S, **P142G**, T175I, M177L, A184V, H236Q, **G240R**, **E252R**, I263W, H266V, T268G, **F279L**, A290V, **T327P**, **A328I**, A330Y, S332G, L353V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**I2T, S72V, D80E, A87V, H92F, S142G, L162F, R226S, G240R, G252R, Y263W, F279L, T327P, V328I, S332G, V366I, L436R, Q437I**
P411-LAS-5264	**I2T**, N70E, **S72V**, V78L, **D80E**, A82L, **F87V**, **H92F**, M118S, **P142G**, T175I, M177L, A184V, **A191A (silent mutation)**, H236Q, **G240R**, **E252R**, I263W, H266V, T268G, **F279L**, A290V, **T327P**, **A328I**, A330Y, S332G, L353V, C400S, I401L, T436R, L437I, E442K, ΔFAD	**I2T, S72V, D80E, A87V, H92F, S142G, L162F, R226S, G240R, G252R, Y263W, F279L, T327P, V328I, S332G, V366I, L436R, Q437I**
P411-LAS-5265	N70E, A74G, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, **I263W**, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, I401L, T436L, **L437I**, E442K, ΔFAD	**Y263W, Q437I**

P411-LAS-5266	N70E, A74G, V78L, A82L, **F87P**, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, **A264S**, H266V, **E267D**, T268G, A290V, **T327P**, A328V, A330Y, **S332A**, L353V, I366V, C400S, I401L, T436L, E442K ΔFAD	**A87P, A264S, E267D, T327P, S332A, Q437L**

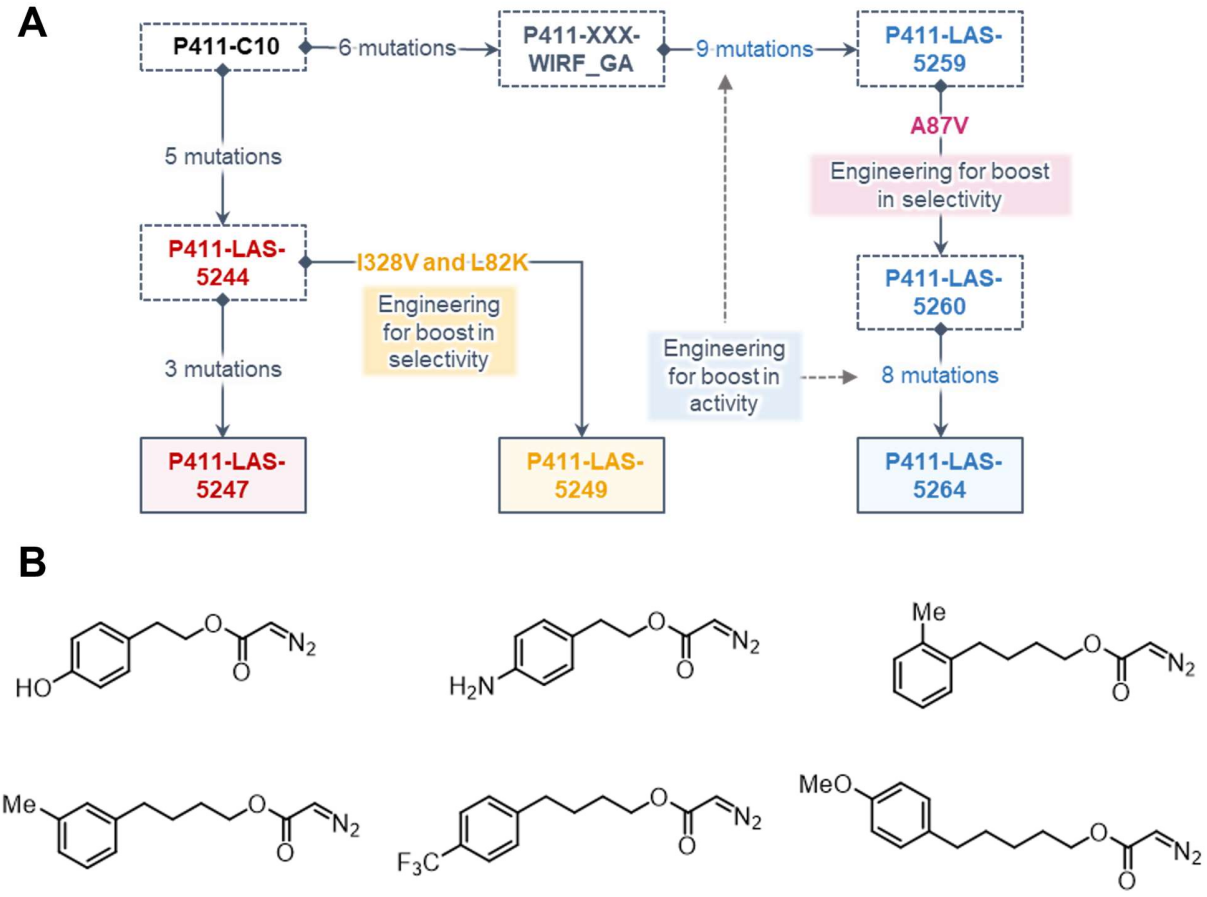


Figure S1. (A) Evolutionary trajectories of lactone synthases P411-LAS-5247, P411-LAS-5249, and P411-LAS-5264 from P411-C10. (B) Less reactive and unreactive substrates.

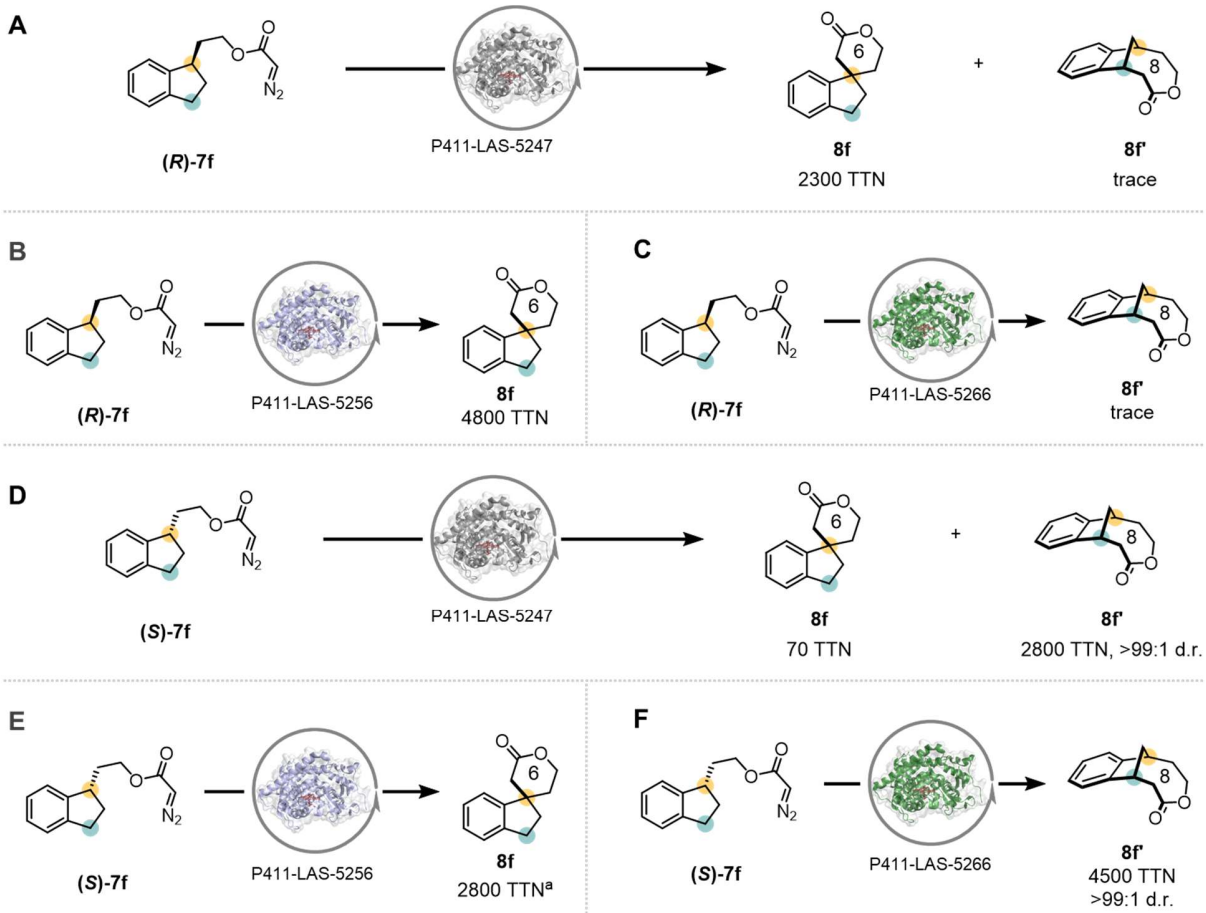


Figure S2. Reaction of stereopure 7f with P411-LAS-5247, P411-LAS-5256, and P411-LAS-5266.

[a]550 TTN for product **8f'**

DNA and amino acid sequences of P411-LAS-5239 (P411-C10)[5]:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGA
AATTTCTGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGCATGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCAT
CCATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGA
GCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC
AAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGA
ACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTG
AGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATATGCGGGAGT
TGAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACAT
GTATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGC
TACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGC
TTATGGCCAACGGTTCCTTATTTTTCCCTATATGCAAAAGAAGATACGGTGCTTGGAG
GAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACC
GTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAA
ATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGT
CTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAA
ACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACTGCAGAC
GTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGG
TATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGA
ACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCA
ACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACG
GCGTCTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACC

AAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATA
AAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGC
TAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTG
AAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTA
ACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGA
CAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCA
CCACCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELSQGLKFLRDFLGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLYAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPTVPYFSLYAKEDTVLGGEYPLEKGDEVMV
LIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLVLG
MMLKHFDFEDHTNYELDIKELQTLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAE
NAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTA
SYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAA
KGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSA
ADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5244:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGA
AATTTATGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGCATGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCAT
CCATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGA
GCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC
AAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGA
ACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTG
AGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATATGCGGGAGT
TGAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACAT
GTATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGC
TACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGC
TTATGGCCAACGATTCCTTATTTTTCCCTATATGCAAAAGAAGATACGGTGCTTGGAG
GAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACC
GTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAA
ATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGT
CTGTGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAA
ACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTAC
GTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGG
TATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGA
ACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCA
ACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACG
GCGTCTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACC

AAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATA
AAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGC
TAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTG
AAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTA
ACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGA
CAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCA
CCACCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELSQGLKFMRDFLGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLYAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPTIPYFSLYAKEDTVLGGEYPLEKGDEVMV
LIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASVGQQFALHEATLVLG
MMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAE
NAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTA
SYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAA
KGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSA
ADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5247:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAAGTTTAAGTCAAGGTCTGA
AATTTATGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGCATGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCATTAACAGCTTTTACCGAGATCAGCCTCAT
CCATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCTTG
CAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCA
AGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGAA
CAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTGA
GCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATATGCGGGAGTT
GAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATG
TATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCT
ACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCT
TATGGCCAACGATTCCTTATTTTTCCCTATATGCAAAAGAAGATACGGTGCTTGGAGG
AGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACCGT
GATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAAT
CCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCT
GTTGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAAC
ACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTACGTT
AAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTAT
TCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGC
TCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAAC
GGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAAC
GCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGC
GTCTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAA

GCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAA
AACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTA
AAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTGAA
GGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAAC
CTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACA
GCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACC
ACCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKSLSQGLKFMRDFLGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRINSFYRDQPHPFIISLVR
ALDEVMNKLQLANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLYAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPTIPYFSLYAKEDTVLGGEYPLEKGDEVMV
LIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASVGQQFALHEATLVLG
MMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAE
NAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTA
SYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAA
KGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSA
ADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5249:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGA
AATTTATGCGTGATTTTCTTGGAGACGGGTTAGCCAGTAGCTGGACGCATGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCAT
CCATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGA
GCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC
AAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGA
ACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTG
AGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATATGCGGGAGT
TGAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACAT
GTATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGC
TACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGC
TTATGGCCAACGGTTCCTTATTTTTCCCTATATGCAAAAGAAGATACGGTGCTTGGAG
GAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACC
GTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAA
ATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGT
CTGTGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAA
ACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTAC
GTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGG
TATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGA
ACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCA
ACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACG
GCGTCTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACC

AAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATA
AAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGC
TAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTG
AAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTA
ACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGA
CAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCA
CCACCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELSQGLKFMRDFLGDGLASSWTHEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLYAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPTVPYFSLYAKEDTVLGGEYPLEKGDEVMV
LIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASVGQQFALHEATLVLG
MMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAE
NAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTA
SYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAA
KGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSA
ADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5250 (P411-C10-WIRF_GA)[5]:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTATTTCAAGCGCTGA
AATTTCTGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGCATGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCAT
CCATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGA
GCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC
AAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGA
ACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTG
AGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATGGGCGGGAGT
TGAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACAT
GTATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGC
TACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGC
TTATGGCCAACGGTTCCTTATTTTGGTCTATATGCAAAAGAAGATACGGTGCTTGGAG
GAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACC
GTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAA
ATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGT
CTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAA
ACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTAC
GTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGG
TATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGA
ACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCA
ACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACG
GCGTCTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACC

AAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATA
AAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGC
TAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTG
AAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTA
ACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGA
CAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCA
CCACCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELFQALKFLRDFLGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLWAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPTVPYFGLYAKEDTVLGGEYPLEKGDEVM
VLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLVL
GMMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKA
ENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVT
ASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLA
AKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5256:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAGTGCAAGCGCTGA
AATTTCTGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGTTTGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATC
CATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAG
CAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCA
AGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGTGGTGAAC
AAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTGAG
CCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATGGGCGGGAGTTG
AAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGT
ATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTA
CAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTT
ATGGCCACCGGTTCCGTATTTTGGTCTATATGCAAAAGAAGATACGGTGCTTGGAGG
AGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACCGT
GATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAAT
CCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTC
TGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACA
CTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTACGTTA
AAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATT
CCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGCT
CATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACG
GCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACG
CTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGT
CTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAG

CGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAA
ACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAA
AGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTGAAG
GCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAACC
TCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAG
CGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCA
CCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELVQALKFLRDFLGDGLATSWTFEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKASGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLWAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPPVPYFGLYAKEDTVLGGEYPLEKGDEVM
VLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLVL
GMMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKA
ENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVT
ASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLA
AKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5266:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAGTGCAAGCGCTGA
AATTTCTGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGTTTGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATC
CATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAG
CAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCA
AGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGTGGTGAAC
AAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTGAG
CCGCTTGATGACCGTAACATTCGCTATCAAATTATTACATTCTTATGGGCGGGAGTTG
AAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGT
ATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTA
CAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTT
ATGGCCACCGGTTCCGTATTTTGGTCTATATGCAAAAGAAGATACGGTGCTTGGAGG
AGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACCGT
GATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAAT
CCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTC
TGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACA
CTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTACGTTA
AAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATT
CCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGCT
CATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACG
GCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACG
CTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGT
CTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAG

CGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAA
ACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAA
AGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTGAAG
GCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAACC
TCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAG
CGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCA
CCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELVQALKFLRDFLGDGLATSWTFEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKASGEQSDDLLTQM
LNGKDPETGEPLDDRNIRYQIITFLWAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPPVPYFGLYAKEDTVLGGEYPLEKGDEVM
VLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLVL
GMMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKA
ENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVT
ASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLA
AKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5259:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAGTGCAAGCGCTGA
AATTTCTGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGTTTGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTAGGTGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATC
CATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAG
CAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCA
AGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGTGGTGAAC
AAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTGAG
CCGCTTGATGACCGTAACATTCGCTATCAAATTATTACATTCTTATGGGCGGGAGTTG
AAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGT
ATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTA
CAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTT
ATGGCCACCGGTTCCGTATTTTGGTCTATATGCAAAAGAAGATACGGTGCTTGGAGG
AGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACCGT
GATAAAACAATTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAAT
CCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTC
TGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACA
CTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTACGTTA
AAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATT
CCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGCT
CATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACG
GCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACG
CTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGT
CTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAG

CGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAA
ACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAA
AGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTGAAG
GCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAACC
TCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAG
CGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCA
CCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELVQALKFLRDFLGDGLATSWTFEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVGEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISLV
RALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKASGEQSDDLLTQ
MLNGKDPETGEPLDDRNIRYQIITFLWAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAAR
VLVDPVPSYKQVKQLKYVGMVLNEALRLWPPVPYFGLYAKEDTVLGGEYPLEKGDEV
MVLIPQLHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLV
LGMMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKK
AENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIV
TASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLA
AKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5264:

ATGACAACTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAGTGCAAGCGCTGA
AATTTCTGCGTGAATTTCTTGGAGACGGGTTAGTGACAAGCTGGACGTTTGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTAGGTGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATC
CATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAG
CGAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCA
AGGTGATGAACGACCTAGTAGATAAAATCATTGCAGATCGCAAAGCAAGTGGTGAAC
AAAGCGATGATTTATTAACGCAGATGCTAAACAGAAAAGATCCAGAAACGGGTGAG
CCGCTTGATGACCGTAACATTCGCTATCAAATTATTACATTCTTATGGGCGGGAGTTG
AAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATCTCTTAGTGAAAAATCCACATGT
ATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGCTA
CAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTT
ATGGCCACCGATTCCGTATTTTGGTCTATATGCAAAAGAAGATACGGTGCTTGGAGG
AGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACCGT
GATAAAACAATTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAAT
CCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTC
TGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAACA
CTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACGTATTACGTTA
AAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATT
CCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGCT
CATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACG
GCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACG
CTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGT
CTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAG

CGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATAAAA
ACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAA
AGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTGAAG
GCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAACC
TCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAG
CGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCA
CCACCACCACCACTGA

MTTKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELVQALKFLREFLGDGLVTSWTFEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVGEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISLV
RALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKASGEQSDDLLTQ
MLNRKDPETGEPLDDRNIRYQIITFLWAGVEGTSGLLSFALYLLVKNPHVLQKVAEEAAR
VLVDPVPSYKQVKQLKYVGMVLNEALRLWPPIPYFGLYAKEDTVLGGEYPLEKGDEVM
VLIPQLHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLVLG
MMLKHFDFEDHTNYELDIKERITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAE
NAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTA
SYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAA
KGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSA
ADMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5265 (P411-C10-WI)[5]:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGA
AATTTCTGCGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGCATGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCAT
CCATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGA
GCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC
AAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGA
ACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTG
AGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATGGGCGGGAGT
TGAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACAT
GTATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGC
TACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGC
TTATGGCCAACGGTTCCTTATTTTTCCCTATATGCAAAAGAAGATACGGTGCTTGGAG
GAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACC
GTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAA
ATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGT
CTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAA
ACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACTGATTAC
GTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGG
TATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGA
ACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCA
ACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACG
GCGTCTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACC

AAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATA
AAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGC
TAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTG
AAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTA
ACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGA
CAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCA
CCACCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELSQGLKFLRDFLGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLWAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARV
LVDPVPSYKQVKQLKYVGMVLNEALRLWPTVPYFSLYAKEDTVLGGEYPLEKGDEVMV
LIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLVLG
MMLKHFDFEDHTNYELDIKELITLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAEN
AHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTAS
YNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAAK
GAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSAA
DMPLAKMHGAFSTLEHHHHHH*

DNA and amino acid sequences of P411-LAS-5266:

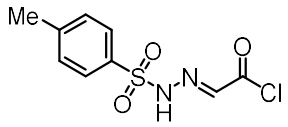
ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCG
TTATTAAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGA
GAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTC
TAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGA
AATTTCTGCGTGATTTTCTTGGAGACGGGTTACCGACAAGCTGGACGCATGAAAAAA
ATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAG
GCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAGTGGGAGCGTCT
AAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGATAC
AATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCAT
CCATTTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGA
GCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC
AAGGTGATGAACGACCTAGTAGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGA
ACAAAGCGATGATTTATTAACGCAGATGCTAAACGGAAAAGATCCAGAAACGGGTG
AGCCGCTTGATGACGGGAACATTCGCTATCAAATTATTACATTCTTATATAGTGGAGT
TGATGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACAT
GTATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTCCAAGC
TACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGC
TTATGGCCACCGGTTCCTTATTTTGCGCTATATGCAAAAGAAGATACGGTGCTTGGAG
GAGAATATCCTTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACC
GTGATAAAACAGTTTGGGGAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAA
ATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGT
CTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAA
ACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAACTGCTTACG
TTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGT
ATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAAC
GCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGA
ACGGCGCGTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCA
ACGCTTGATTCACACGCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACG
GCGTCTTATAACGGTCATCCGCCTGATAACGCAAAGCAATTTGTCGACTGGTTAGACC

AAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTATTTGGATGCGGCGATA
AAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGC
TAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGACTTTG
AAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTA
ACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGA
CAGCGCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCA
CCACCACCACCACCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKE
ACDESRFDKELSQGLKFLRDFLGDGLPTSWTHEKNWKKAHNILLPSFSQQAMKGYHAS
MVDIAVQLVQKWERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVR
ALDEVMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQM
LNGKDPETGEPLDDGNIRYQIITFLYSGVDGTSGLLSFALYFLVKNPHVLQKVAEEAARVL
VDPVPSYKQVKQLKYVGMVLNEALRLWPPVPYFALYAKEDTVLGGEYPLEKGDEVMV
LIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRASLGQQFALHEATLVLG
MMLKHFDFEDHTNYELDIKELLTLKPKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAE
NAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTA
SYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAA
KGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSA
ADMPLAKMHGAFSTLEHHHHHH*

4. Synthesis of Substrates

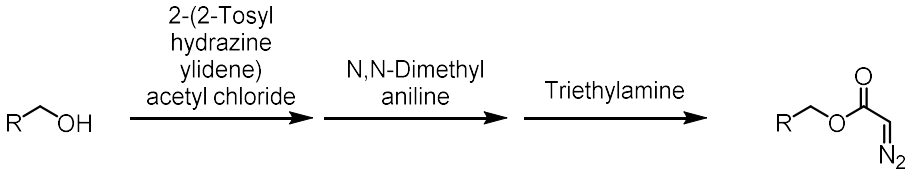
2-(2-Tosylhydrazineylidene)acetyl chloride



4-Methylbenzenesulfonohydrazide (55.9 g, 0.3 mol, 1.0 equiv.) was dissolved in aqueous hydrochloric acid (2 M, 180 mL) and warmed to 50 °C (solution 1). 2-Oxoacetic acid (44.4 g of 50% in water, 0.3 mol, 1.0 equiv.) was dissolved in water (300 mL) and heated to 50 °C (solution 2). Pre-warmed solution 1 was slowly transferred to solution 2. The reaction mixture was then stirred at 60 °C for 4 h until all the hydrozone product had crashed out. The mixture was cooled to 0 °C and kept for 2 h. The product 2-(2-tosylhydrazineylidene) acetic acid (~70 g, 97% yield) was collected by filtration, washed with hexane: ether (10:1, 20 mL × 3) and dried under vaccum.

2-(2-Tosylhydrazineylidene)acetic acid (70 g, 0.29 mmol, 1.0 equiv.) was dissolved in dry dichloromethane (300 mL). Thionyl chloride (50 mL) and N,N-dimethyl formaldehyde (4 drops, cat.) were added to the solution. The reaction mixture was stirred at room temperature for 1 h and then heated to reflux (~ 50 °C) for 5 h until the starting material was completely dissolved and the reaction turned clear and light yellow. After the reaction was cooled to room temperature, organic solvent and the excess thionyl chloride were removed under reduced pressure. The resulting mixture was treated with ether (20 mL) and sonicated for 5 min. Hexane (150 mL) was then slowly added to the mixture to completely crash out the acyl chloride product. The mixture was cooled to 0 °C and kept for 2 h. The product 2-(2-tosylhydrazineylidene)acetyl chloride (~74 g, 98% yield, pale yellow) was collected by filtration, washed with hexane (20 mL × 2) and dried under vacuum.

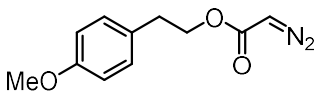
Diazo Synthesis



An alcohol substrate (1.0 equiv.) was dissolved in dry dichloromethane (conc. ~ 0.2–0.5 M) and kept at 0 °C. 2-(2-Tosylhydrazineylidene)acetyl chloride (1.05 equiv.) was then added to the solution. The mixture was stirred at 0 °C for 10 min before the addition of N,N-dimethyl aniline (1.3 equiv.). The resulting mixture was then stirred for another 10 min. Triethylamine (2.0 equiv.) was added to the reaction, which was then allowed to slowly warm up to room temperature over 20 min. The reaction was concentrated under reduced pressure and quenched by citric acid (saturated aqueous solution). The resulting mixture was transferred to a separatory funnel. Dichloromethane and water were used in minimum amount to wash the reaction container and transfer everything to the separatory funnel. Hexane/ethyl acetate (13:1) was used for extraction for three times. The combined organic layer was then washed with saturated citric acid solution and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude product was purified through a silica column using pentane/ether (1:0 to 10:1) as eluents. The yellow-colored fractions were concentrated to afford the diazo product as a yellow liquid (80–96% yield).

Compounds **1a**,[7] **1b**,[8] **1e**,[9] **1f,**[10] and **3b**[11] were known and spectral data were in accordance with literature values.

4-methoxyphenethyl 2-diazoacetate (1c)

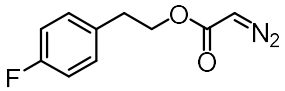


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 4.72 (s, 1H), 4.33 (t, *J* = 7.0 Hz, 2H), 3.79 (s, 3H), 2.89 (t, *J* = 7.0 Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.46 , 130.02 , 129.78 , 114.06 , 65.67 , 55.40 , 46.33, 34.56 .

HRMS (FI): calcd for $C_{11}H_{12}N_2O_3$ $[M]^{+\bullet}$ 220.08479; found 220.08468.

4-fluorophenethyl 2-diazoacetate (1d)

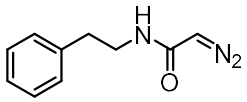


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.21 – 7.12 (m, 2H), 7.04 – 6.94 (m, 2H), 4.72 (s, 1H), 4.34 (t, *J* = 6.9 Hz, 2H), 2.92 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.86 (d, *J* = 244.6 Hz), 133.45 (d, *J* = 3.3 Hz), 130.47 (d, *J* = 8.0 Hz), 115.46 (d, *J* = 21.3 Hz), 65.30 , 46.35, 34.64 .

HRMS (FD): calcd for $C_{10}H_9N_2O_2F$ $[M]^{+\bullet}$ 208.06481; found 208.06525.

2-diazo-*N*-phenethylacetamide (1g)

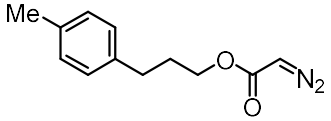


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.21 (m, 5H), 5.11 (s, 1H), 4.68 (s, 1H), 3.65 – 3.53 (m, 2H), 2.85 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.54 , 138.85 , 128.91 , 126.70 , 47.27 , 41.22 , 47.27, 36.15 .

HRMS (FI): calcd for $C_7H_{13}N_2O_4$ $[M]^{+\bullet}$ 189.08753; found 189.09037.

3-(*p*-tolyl)propyl 2-diazoacetate (3a)

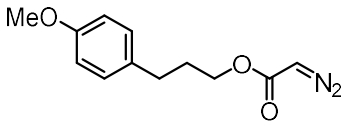


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.14 – 7.03 (m, 4H), 4.74 (s, 1H), 4.18 (t, *J* = 6.5 Hz, 2H), 2.65 (dd, *J* = 8.6, 6.7 Hz, 2H), 2.32 (s, 3H), 2.03 – 1.89 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 138.13 , 135.62 , 129.26 , 128.40 , 64.35 , 46.30, 31.73 , 30.63 , 21.13 .

HRMS (FI): calcd for $C_{12}H_{14}N_2O_2$ $[M]^{+\bullet}$ 218.10553; found 218.10529.

3-(4-methoxyphenyl)propyl 2-diazoacetate (3c)

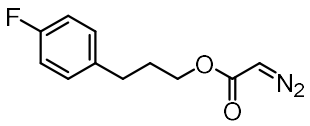


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 – 6.98 (m, 2H), 6.88 – 6.79 (m, 2H), 4.74 (s, 1H), 4.17 (t, *J* = 6.5 Hz, 2H), 3.79 (s, 3H), 2.63 (dd, *J* = 8.5, 6.8 Hz, 2H), 2.00 – 1.88 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.05 , 133.27 , 129.43 , 114.00 , 64.32 , 55.41 , 46.31, 31.27 , 30.74 .

HRMS (FI): calcd for $C_{12}H_{14}N_2O_3$ $[M]^{+\bullet}$ 234.10044; found 234.10034.

3-(4-fluorophenyl)propyl 2-diazoacetate (3d)

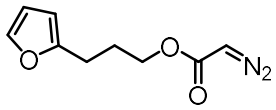


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 – 7.09 (m, 2H), 7.02 – 6.92 (m, 2H), 4.74 (s, 1H), 4.17 (t, *J* = 6.5 Hz, 2H), 2.66 (dd, *J* = 8.5, 6.8 Hz, 2H), 2.01 – 1.89 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.49 (d, *J* = 243.6 Hz), 136.81 (d, *J* = 3.2 Hz), 129.85 (d, *J* = 7.8 Hz), 115.33 (d, *J* = 21.2 Hz), 64.13 , 46.31, 31.42 , 30.63 .

HRMS (FD): calcd for $C_{11}H_{11}N_2O_2F$ $[M]^{+\bullet}$ 222.08046; found 222.07990.

3-(furan-2-yl)propyl 2-diazoacetate (3e)



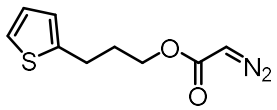
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.29 (m, 1H), 6.28 (dd, *J* = 3.2, 1.9 Hz, 1H), 6.01 (dt, *J* = 2.8, 0.9 Hz, 1H), 4.74 (s, 1H), 4.20 (t, *J* = 6.4 Hz, 2H), 2.76 – 2.69 (m, 2H), 2.00 (dq, *J* = 7.4, 6.4

Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 154.89 , 141.21 , 110.29 , 105.40 , 64.15 , 46.31, 27.42 , 24.57 .

HRMS (ESI): calcd for $C_9H_{10}N_2O_3Na$ $[M+Na]^{+\bullet}$ 217.0613; found 217.0596.

3-(thiophen-2-yl)propyl 2-diazoacetate (3f)

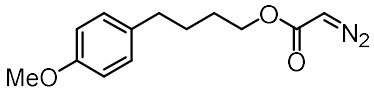


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.92 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.80 (dq, *J* = 3.3, 1.1 Hz, 1H), 4.75 (s, 1H), 4.22 (t, *J* = 6.4 Hz, 2H), 2.96 – 2.87 (m, 2H), 2.03 (dq, *J* = 7.5, 6.4 Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 143.86 , 126.96 , 124.65 , 123.42 , 63.93 , 46.31, 30.85 , 26.34 .

HRMS (FI): calcd for $C_9H_{10}N_2O_2S$ $[M]^{+\bullet}$ 210.04630; found 210.04534.

4-(4-methoxyphenyl)butyl 2-diazoacetate (5a)

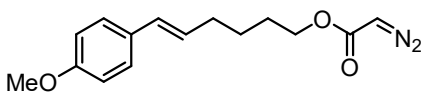


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.12 – 7.05 (m, 2H), 6.87 – 6.79 (m, 2H), 4.72 (s, 1H), 4.21 – 4.13 (m, 2H), 3.79 (s, 3H), 2.63 – 2.54 (m, 2H), 1.66 (d, *J* = 6.9 Hz, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 157.91 , 134.19 , 129.39 , 113.89 , 64.90 , 55.39 , 46.29, 34.62 , 28.45 , 27.95 .

HRMS (FI): calcd for $C_{13}H_{16}N_2O_3$ $[M]^{+\bullet}$ 248.11609; found 248.11580.

(*E*)-6-(4-methoxyphenyl)hex-5-en-1-yl 2-diazoacetate (5b)

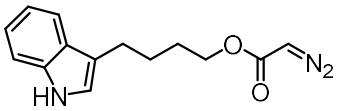


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.27 (d, *J* = 9.0 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.33 (d, *J* = 15.8 Hz, 1H), 6.11 – 5.96 (m, 1H), 4.73 (s, 1H), 4.18 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 3H), 2.22 (q, *J* = 6.9 Hz, 2H), 1.70 (dt, *J* = 15.3, 6.8 Hz, 2H), 1.58 – 1.49 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.85 , 130.64 , 129.82 , 128.13 , 127.15 , 114.05 , 64.94 , 55.43 , 46.30, 32.64 , 28.45 , 25.83 .

HRMS (FI): calcd for $C_{15}H_{18}N_2O_3$ $[M]^{+\bullet}$ 274.13174; found 274.13224.

4-(1*H*-indol-3-yl)butyl 2-diazoacetate (5c)

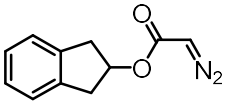


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 (s, 1H), 7.52 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.28 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.21 – 7.08 (m, 1H), 7.04 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 6.93 – 6.87 (m, 1H), 4.64 (s, 1H), 4.13 (t, *J* = 6.2 Hz, 2H), 2.77 – 2.68 (m, 2H), 1.76 – 1.62 (m, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 136.48 , 127.58 , 122.04 , 121.34 , 119.27 , 119.00 , 116.37 , 111.21 , 65.01 , 46.31, 28.75 , 26.45 , 24.84 .

HRMS (FI): calcd for $C_{14}H_{15}N_3O_2$ $[M]^{+\bullet}$ 257.11643; found 257.11652.

2,3-dihydro-1*H*-inden-2-yl 2-diazoacetate (7a)

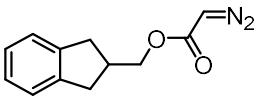


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.31 – 7.22 (m, 4H), 5.65 (dt, *J* = 6.5, 3.3 Hz, 1H), 4.75 (s, 1H), 3.36 (dd, *J* = 17.0, 6.5 Hz, 2H), 3.07 (dd, *J* = 17.0, 3.1 Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 140.31 , 126.80 , 124.65 , 75.91 , 46.51, 39.70 .

HRMS (FI): calcd for $C_{11}H_{10}N_2O_3$ $[M]^{+\bullet}$ 202.07423; found 202.07387.

(2,3-dihydro-1*H*-inden-2-yl)methyl 2-diazoacetate (7b)

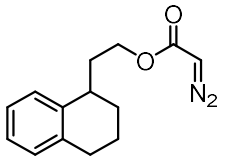


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.31 – 7.21 (m, 2H), 7.17 (dt, *J* = 5.2, 3.6 Hz, 2H), 4.78 (s, 1H), 4.23 (d, *J* = 6.8 Hz, 2H), 3.16 – 3.05 (m, 2H), 2.92 – 2.72 (m, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 142.25 , 126.41 , 124.61 , 67.94 , 46.22, 38.40 , 35.89 .

HRMS (FI): calcd for $C_{12}H_{12}N_2O_2$ $[M]^{+\bullet}$ 216.08988; found 216.08981.

2-(1,2,3,4-tetrahydronaphthalen-1-yl)ethyl 2-diazoacetate (7c)

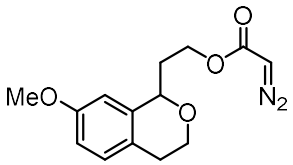


^{1}H NMR δ 7.17 – 7.04 (m, 4H), 4.76 (s, 1H), 4.29 (ddd, *J* = 7.2, 6.3, 3.7 Hz, 2H), 2.92 (dd, *J* = 9.4, 4.9 Hz, 1H), 2.81 – 2.72 (m, 2H), 2.13 – 2.02 (m, 1H), 1.95 – 1.81 (m, 3H), 1.73 (dtd, *J* = 10.0, 5.9, 3.8 Hz, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 140.16 , 137.18 , 129.36 , 128.64 , 125.90 , 125.78 , 63.38 , 46.36, 35.78 , 34.50 , 29.63 , 27.68 , 19.72 .

HRMS (FI): calcd for $C_{14}H_{16}N_2O_2$ $[M]^{+\bullet}$ 244.12118; found 244.12117.

2-(7-methoxyisochroman-1-yl)ethyl 2-diazoacetate (7d)

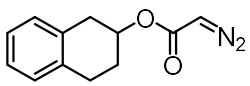


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.03 (d, *J* = 8.4 Hz, 1H), 6.74 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.59 (d, *J* = 2.6 Hz, 1H), 4.81 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.72 (s, 1H), 4.36 (ddd, *J* = 7.4, 5.9, 4.0 Hz, 2H), 3.82 – 3.67 (m, 4H), 2.96 – 2.83 (m, 1H), 2.63 (dt, *J* = 15.9, 3.8 Hz, 1H), 2.26 (ddd, *J* = 15.0, 7.3, 3.2 Hz, 1H), 2.13 (ddd, *J* = 9.0, 6.6, 5.3 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.07 , 138.55 , 130.02 , 126.08 , 112.59 , 109.91 , 72.84 , 63.51 , 61.87 , 55.46 , 46.32, 35.07 , 28.31 .

HRMS (FI): calcd for $C_{14}H_{16}N_2O_4$ $[M]^{+\bullet}$ 276.1101; found 276.11068.

1,2,3,4-tetrahydronaphthalen-2-yl 2-diazoacetate (7e)

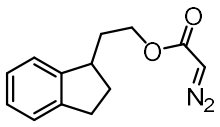


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 – 7.03 (m, 4H), 5.30 (dddd, *J* = 8.4, 7.0, 5.1, 3.2 Hz, 1H), 4.74 (s, 1H), 3.14 (dd, *J* = 16.7, 5.2 Hz, 1H), 2.99 – 2.80 (m, 3H), 2.12 – 1.92 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 135.92 , 133.97 , 129.77 , 129.04 , 126.51 , 126.40 , 70.83 , 46.93, 35.15 , 28.44 , 26.81 .

HRMS (FI): calcd for $C_{12}H_{12}N_2O_2$ $[M]^{+\bullet}$ 216.08988; found 216.08980.

2-(2,3-dihydro-1H-inden-1-yl)ethyl 2-diazoacetate (7f)



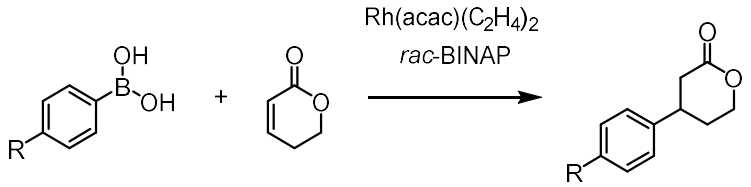
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.20 – 7.05 (m, 4H), 4.67 (s, 1H), 4.23 (t, *J* = 6.8 Hz, 2H), 3.17 – 3.08 (m, 1H), 2.91 – 2.71 (m, 2H), 2.31 – 2.07 (m, 2H), 1.75 – 1.53 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 146.48 , 143.96 , 126.66 , 126.28 , 124.65 , 123.59 , 63.71 , 46.32, 41.73 , 33.98 , 32.27 , 31.51 .

HRMS (FI): calcd for $C_{13}H_{14}N_2O_2$ $[M]^{+\bullet}$ 230.10553; found 230.10561.

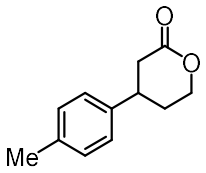
5. Synthesis of Reference Products

Compounds **2a**–**2g** and **4b** were purchased from commercial suppliers including Sigma-Aldrich, VWR, TCI America, Fischer Scientific, Alfa Aesar, Acros, and Combi Blocks and were used without additional purification. Other reference products were obtained using the method described below or through enzymatic preparative synthesis.



A typical procedure is given for the preparation of 6-membered lactones: 1,4-Dioxane (2.0 mL) was added to a flask charged with $Rh(acac)(C_2H_4)_2$ (12 μmol), rac-BINAP (14 μmol), and phenylboronic acid (2.00 mmol) and flushed with nitrogen, which was followed by addition of water (0.2 mL) and 5,6-dihydro-2H-pyran-2-one (0.40 mmol). The resulting mixture was then stirred at 100 °C for 3 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate. The solution was washed with saturated aqueous sodium bicarbonate and dried over anhydrous Na_2SO_4. Chromatography on silica gel (hexane:EtOAc 2:1 to 1:1) gave products as a colorless oil.

4-(*p*-Tolyl)tetrahydro-2*H*-pyran-2-one (4a)

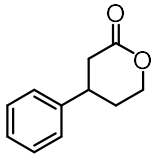


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.35 (m, 2H), 7.33 – 7.28 (m, 1H), 7.25 – 7.21 (m, 2H), 4.67 (dd, *J* = 9.1, 7.8 Hz, 1H), 4.28 (dd, *J* = 9.1, 7.9 Hz, 1H), 3.79 (p, *J* = 8.4 Hz, 1H), 2.93 (dd, *J* = 17.5, 8.7 Hz, 1H), 2.68 (dd, *J* = 17.5, 9.1 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 176.51 , 139.54 , 129.30 , 127.88 , 126.84 , 41.27 , 35.86 .

Spectral data were in accordance with literature values.[12]

4-Phenyltetrahydro-2*H*-pyran-2-one (4b)

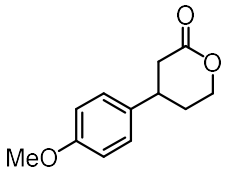


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.32 (m, 2H), 7.31 – 7.25 (m, 2H), 7.24 – 7.18 (m, 2H), 4.51 (ddd, *J* = 11.4, 4.9, 3.9 Hz, 1H), 4.40 (ddd, *J* = 11.4, 10.4, 3.8 Hz, 1H), 3.24 (tdd, *J* = 10.5, 5.9, 4.6 Hz, 1H), 2.93 (ddd, *J* = 17.7, 6.0, 1.7 Hz, 1H), 2.64 (dd, *J* = 17.6, 10.6 Hz, 1H), 2.19 (dqd, *J* = 14.0, 3.9, 1.6 Hz, 1H), 2.11 – 1.99 (m, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.78 , 142.89 , 129.13 , 127.38 , 126.59 , 68.78 , 37.65 , 37.60 , 30.46 .

Spectral data were in accordance with literature values.[13]

4-(4-Methoxyphenyl)tetrahydro-2*H*-pyran-2-one (4c)

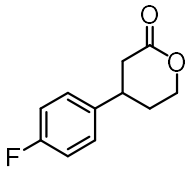


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 – 7.09 (m, 2H), 6.94 – 6.85 (m, 2H), 4.50 (ddd, *J* = 11.4, 4.9, 3.9 Hz, 1H), 4.38 (ddd, *J* = 11.4, 10.4, 3.8 Hz, 1H), 3.80 (s, 3H), 3.19 (tdd, *J* = 10.5, 5.9, 4.5 Hz, 1H), 2.90 (ddd, *J* = 17.6, 5.9, 1.7 Hz, 1H), 2.59 (dd, *J* = 17.7, 10.6 Hz, 1H), 2.15 (ddtd, *J* = 13.9, 4.5, 3.9, 1.7 Hz, 1H), 2.00 (dtd, *J* = 14.1, 10.5, 4.9 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.90 , 158.79 , 134.99 , 127.56 , 114.45 , 68.80 , 55.46 , 37.91 , 36.81 , 30.67 .

Spectral data were in accordance with literature values.[14]

4-(4-Fluorophenyl)tetrahydro-2*H*-pyran-2-one (4d)



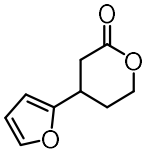
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 – 7.13 (m, 2H), 7.10 – 7.00 (m, 2H), 4.51 (ddd, *J* = 11.5, 4.9, 3.8 Hz, 1H), 4.39 (ddd, *J* = 11.5, 10.5, 3.7 Hz, 1H), 3.23 (tdd, *J* = 10.6, 5.9, 4.5 Hz, 1H), 2.91 (ddd, *J* = 17.6, 6.0, 1.7 Hz, 1H), 2.59 (dd, *J* = 17.6, 10.6 Hz, 1H), 2.21 – 2.13 (m, 1H), 2.01 (dtd, *J* = 14.1, 10.6, 4.9 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.51 , 163.21 , 160.76 , 138.60 , 128.13 , 128.05 , 116.09 , 115.88 , 68.68 , 37.82 , 36.98 , 30.56 .

^{19}F NMR (376 MHz, Chloroform-*d*) δ -115.32 .

Spectral data were in accordance with literature values. [14]

4-(Furan-2-yl)tetrahydro-2*H*-pyran-2-one (4e)

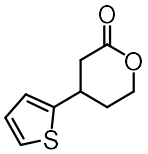


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (dd, *J* = 2.0, 0.8 Hz, 1H), 6.32 (dd, *J* = 3.2, 1.8 Hz, 1H), 6.09 (dt, *J* = 3.2, 0.9 Hz, 1H), 4.47 – 4.33 (m, 2H), 3.37 (dddd, *J* = 11.6, 6.1, 3.6, 1.0 Hz, 1H), 2.93 (ddd, *J* = 17.5, 6.2, 1.2 Hz, 1H), 2.72 (dd, *J* = 17.5, 9.2 Hz, 1H), 2.28 – 2.20 (m, 1H), 2.04 (dtd, *J* = 13.8, 8.8, 4.7 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.13 , 155.53 , 142.13 , 110.38 , 105.20 , 68.08 , 34.60 , 31.14 , 27.99 .

Spectral data were in accordance with literature values. [15]

4-(Thiophen-2-yl)tetrahydro-2*H*-pyran-2-one (4f)



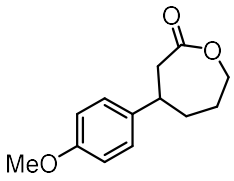
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.98 (dd, *J* = 5.1, 3.5 Hz, 1H), 6.90 – 6.84 (m, 1H), 4.50 (dt, *J* = 11.6, 4.6 Hz, 1H), 4.39 (ddd, *J* = 11.7, 10.1, 3.8 Hz, 1H), 3.58 – 3.49 (m, 1H), 3.03 (ddd, *J* = 17.7, 5.9, 1.6 Hz, 1H), 2.70 (dd, *J* = 17.6, 10.3 Hz, 1H), 2.29 (ddq, *J* = 11.0, 4.2, 2.6, 2.1 Hz, 1H), 2.06 (dtd, *J* = 14.5, 10.1, 4.8 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.89 , 146.43 , 127.18 , 124.10 , 123.48 , 68.39 , 38.24 ,

33.19 , 31.46 .

Spectral data were in accordance with literature values.[16]

4-(4-Methoxyphenyl)oxepan-2-one (6a)

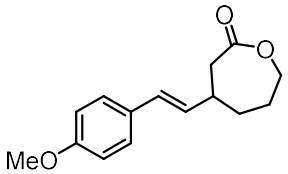


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 – 7.07 (m, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 4.42 – 4.20 (m, 2H), 3.79 (s, 3H), 3.05 – 2.71 (m, 3H), 2.21 – 2.01 (m, 2H), 1.99 – 1.70 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 174.76 , 158.47 , 137.84 , 128.01 , 127.35 , 114.39 , 114.24 , 69.23 , 55.43 , 41.96 , 40.00 , 38.09 , 29.17 .

Spectral data were in accordance with literature values.[17]

(*E*)-4-(4-Methoxystyryl)oxepan-2-one (6b)

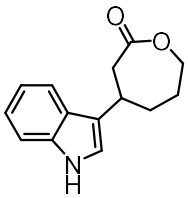


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.27 (d, *J* = 8.5 Hz, 3H), 6.88 – 6.82 (m, 2H), 6.40 (dd, *J* = 15.7, 1.1 Hz, 1H), 5.99 (dd, *J* = 15.9, 7.4 Hz, 1H), 4.38 – 4.27 (m, 1H), 4.23 (ddd, *J* = 12.7, 9.3, 1.1 Hz, 1H), 3.80 (s, 3H), 2.84 – 2.69 (m, 2H), 2.67 – 2.55 (m, 1H), 2.13 – 1.99 (m, 2H), 1.95 – 1.80 (m, 1H), 1.73 – 1.60 (m, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 174.69 , 159.29, 130.35 , 129.76 , 129.34 , 127.48 , 114.14 , 69.24 , 55.45 , 40.59 , 37.52 , 35.81 , 28.16 .

HRMS (FI): calcd for $C_{15}H_{18}O_3$ $[M]^{+\bullet}$ 246.12559; found 246.12724.

4-(1*H*-Indol-3-yl)oxepan-2-one (6c)

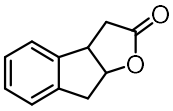


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.03 (s, 1H), 7.65 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.38 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.22 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.14 (ddd, *J* = 8.0, 7.1, 1.1 Hz, 1H), 7.05 – 7.01 (m, 1H), 4.44 – 4.29 (m, 2H), 3.33 (ddd, *J* = 11.6, 7.8, 4.5 Hz, 1H), 3.09 – 2.99 (m, 2H), 2.40 – 2.27 (m, 1H), 2.19 – 2.06 (m, 1H), 2.05 – 1.95 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 175.16 , 136.48 , 125.98 , 122.56 , 120.25 , 119.97 , 119.79 , 119.04 , 111.47 , 69.41 , 41.51 , 36.13 , 32.24 , 28.93 .

HRMS (FI): calcd for $C_{14}H_{15}NO_2$ $[M]^{+\bullet}$ 229.11028; found 229.11145.

3,3a,8,8a-Tetrahydro-2*H*-indeno[2,1-*b*]furan-2-one (8a)

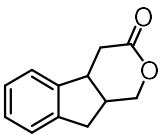


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.20 (m, 5H), 5.30 (ddd, *J* = 5.8, 3.8, 2.7 Hz, 1H), 4.02 (ddt, *J* = 9.3, 5.7, 1.3 Hz, 1H), 3.35 – 3.29 (m, 2H), 3.05 (dd, *J* = 17.8, 9.3 Hz, 1H), 2.75 (dd, *J* = 17.8, 1.5 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 142.15 , 128.46 , 127.85 , 125.43 , 124.79 , 84.43 , 45.58 , 39.07 , 35.48 .

HRMS (FI): calcd for $C_{11}H_{10}O_2$ $[M]^{+\bullet}$ 174.06808; found 174.06856.

4,4a,9,9a-Tetrahydroindeno[2,1-*c*]pyran-3(1*H*)-one (8b)

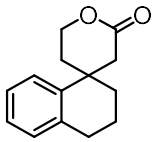


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.26 – 7.16 (m, 4H), 4.39 (dd, *J* = 11.4, 5.0 Hz, 1H), 4.09 (dd, *J* = 11.4, 8.8 Hz, 1H), 3.81 (dt, *J* = 9.8, 7.8 Hz, 1H), 3.30 (dd, *J* = 16.8, 9.7 Hz, 1H), 3.10 – 2.97 (m, 2H), 2.81 (dd, *J* = 16.8, 3.9 Hz, 1H), 2.58 (dd, *J* = 15.2, 7.8 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.79 , 143.96 , 141.31 , 127.91 , 127.56 , 125.06 , 124.27 , 70.26 , 41.04 , 35.81 , 34.93 , 34.90 .

HRMS (FI): calcd for $C_{12}H_{12}O_2$ $[M]^{+\bullet}$ 188.08373; found 188.08404.

3,4,5',6'-Tetrahydro-2*H*-spiro[naphthalene-1,4'-pyran]-2'(3'*H*)-one (8c)

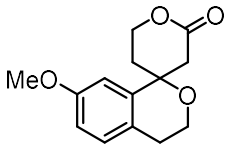


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 – 7.06 (m, 4H), 4.50 – 4.39 (m, 2H), 2.87 – 2.76 (m, 3H), 2.65 (dd, *J* = 16.7, 1.1 Hz, 1H), 2.26 (ddd, *J* = 14.2, 8.1, 5.8 Hz, 1H), 1.95 (dddd, *J* = 14.5, 5.3, 4.4, 1.1 Hz, 1H), 1.91 – 1.77 (m, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.01 , 142.44 , 136.63 , 126.81 , 126.68 , 77.48 , 66.40 , 37.20 , 36.23 , 18.98 .

HRMS (FI): calcd for $C_{12}H_{16}O$ $[M]^{+\bullet}$ 176.12012; found 176.12080.

7-methoxy-5',6'-dihydrospiro[isochromane-1,4'-pyran]-2'(3'H)-one (8d)

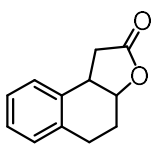


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.16 – 6.99 (m, 1H), 6.77 (ddd, *J* = 8.3, 2.7, 0.7 Hz, 1H), 6.58 (d, *J* = 2.7 Hz, 1H), 5.20 – 5.03 (m, 2H), 4.15 (dd, *J* = 11.2, 1.4 Hz, 1H), 3.92 – 3.85 (m, 2H), 3.82 (d, *J* = 1.5 Hz, 3H), 3.06 (d, *J* = 4.1 Hz, 1H), 2.68 (dd, *J* = 13.1, 5.3 Hz, 1H), 2.55 (ddd, *J* = 13.1, 2.6, 1.5 Hz, 1H), 2.51 – 2.41 (m, 1H), 2.41 – 2.27 (m, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 175.49 , 158.82 , 141.79 , 129.26 , 128.51 , 111.83 , 110.31 , 72.66 , 67.06 , 61.47 , 55.43 , 40.17 , 36.74 , 34.99 .

HRMS (FI): calcd for $C_{14}H_{16}O_4$ $[M]^{+\bullet}$ 248.10486; found 248.10591.

3a,4,5,9b-Tetrahydronaphtho[2,1-*b*]furan-2(1*H*)-one (8e)

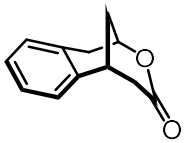


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 – 7.09 (m, 4H), 4.98 (td, *J* = 6.8, 3.4 Hz, 1H), 3.81 (dt, *J* = 9.6, 6.3 Hz, 1H), 3.09 (dd, *J* = 17.6, 9.5 Hz, 1H), 2.91 (ddd, *J* = 16.4, 9.5, 4.4 Hz, 1H), 2.75 – 2.65 (m, 1H), 2.58 (dd, *J* = 17.6, 5.6 Hz, 1H), 2.19 (dtd, *J* = 13.2, 6.4, 4.4 Hz, 1H), 1.99 (dddd, *J* = 14.0, 9.7, 4.6, 3.4 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 176.51 , 136.03 , 135.68 , 129.17 , 128.98 , 127.21 , 126.98 , 78.87, 37.86 , 37.74 , 26.54 , 24.67 .

HRMS (FI): calcd for $C_{12}H_{12}O_2$ $[M]^{+\bullet}$ 188.08373; found 188.08441.

1,2,5,6-tetrahydro-4*H*-2,6-methanobenzo[*d*]oxocin-4-one (8e')

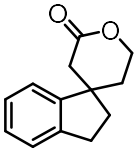


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 – 7.16 (m, 2H), 7.15 – 7.07 (m, 2H), 5.13 (dtd, *J* = 5.2, 3.6, 1.8 Hz, 1H), 3.35 – 3.21 (m, 2H), 3.10 (dd, *J* = 17.9, 3.8 Hz, 1H), 2.83 (dd, *J* = 17.9, 5.7 Hz, 1H), 2.65 (dt, *J* = 17.9, 2.2 Hz, 1H), 2.33 (ddt, *J* = 13.6, 4.7, 2.1 Hz, 1H), 2.23 – 2.10 (m, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.97 , 138.37 , 130.92 , 130.92 , 130.02 , 130.02 , 128.73 , 127.75 , 127.23 , 74.81 , 40.19 , 36.32 , 31.25 , 27.95 .

HRMS (FI): calcd for $C_{12}H_{12}O_2$ $[M]^{+\bullet}$ 188.08373; found 188.08414.

2,3,5',6'-Tetrahydrospiro[indene-1,4'-pyran]-2'(3'*H*)-one (8f)

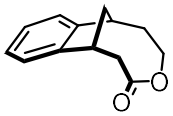


^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.19 (m, 4H), 7.19 – 7.11 (m, 1H), 4.57 – 4.42 (m, 2H), 2.96 (tt, *J* = 6.8, 0.7 Hz, 2H), 2.73 – 2.59 (m, 2H), 2.26 – 2.13 (m, 1H), 2.08 (t, *J* = 7.1 Hz, 2H), 1.91 (dddd, *J* = 14.1, 5.8, 4.6, 1.1 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.86 , 147.65 , 142.73 , 127.63 , 127.04 , 125.03 , 122.29 , 77.23 , 67.00 , 45.58 , 41.67 , 38.82 , 33.76 , 29.62 .

HRMS (FI): calcd for $C_{13}H_{14}O_2$ $[M]^{+\bullet}$ 202.09938; found 202.09993.

(7)-1,5,6,7-Tetrahydro-1,7-methanobenzo[*e*]oxonin-3(2*H*)-one (8f')



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.13 (m, 5H), 4.14 – 3.97 (m, 2H), 3.68 (dt, *J* = 9.4, 4.9 Hz, 1H), 3.53 – 3.44 (m, 1H), 2.90 (dd, *J* = 12.5, 4.9 Hz, 1H), 2.66 – 2.55 (m, 2H), 2.39 (dddd, *J* = 14.8, 11.4, 5.0, 3.5 Hz, 1H), 2.22 (dt, *J* = 13.7, 1.0 Hz, 1H), 2.00 (ddt, *J* = 14.8, 4.5, 3.1 Hz, 1H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 175.54 , 145.68 , 144.08 , 127.80 , 127.57 , 124.89 , 124.59 , 66.44 , 42.68 , 42.55 , 41.15 , 36.87 , 36.32 .
HRMS (FI): calcd for $C_{13}H_{14}O_2$ $[M]^{+\bullet}$ 202.09938; found 202.10012.

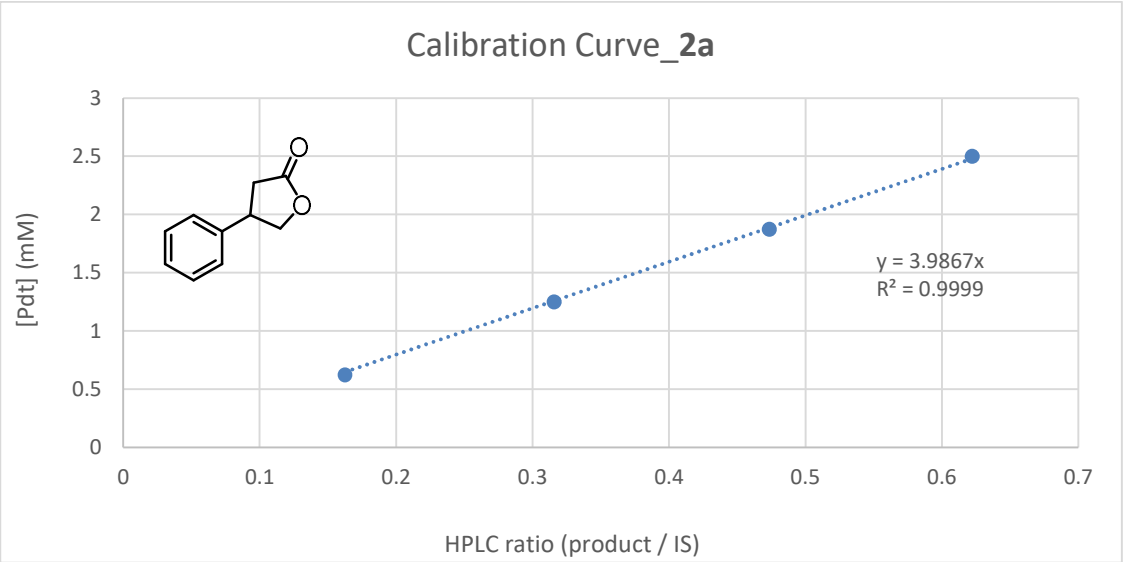
6. HPLC Calibration Curves

Calibration curves of synthesized reference compounds were created for the determination of yield and TTN.

For compounds **2a**–**6c**: For each substrate, four different concentrations of product (0.625, 1.25, 1.875, and 2.5 mM) with 0.67 mM internal standard in 600 μL acetonitrile solutions were mixed each with 400 μL water. The mixtures were vortexed and then analyzed by HPLC based on UV absorbance at 210 nm. All data points represent the average of duplicate runs. The calibration curves depict the ratio of product area to internal standard area (x-axis) against product concentration in mM (y-axis). Notes: Pdt = product area, IS = internal standard area, [Pdt] = product concentration in reaction, [PC] = protein concentration in reaction, Avg. TTN = average total turnover number, SD TTN = standard deviation of TTN, Avg. Yield = average yield, SD Yield = standard deviation of yield.

For compounds **8a**–**8f'**: For each substrate, four different concentrations of product (0.625, 1.25, 1.875, and 2.5 mM) with 0.67 mM internal standard in 600 μL hexane solutions were mixed each with 400 μL water. The mixtures were vortexed and centrifuged. The supernatant was transferred to a new vial and analyzed by HPLC based on UV absorbance at 210 nm. All data points represent the average of duplicate runs. The calibration curves depict the ratio of product area to internal standard area (x-axis) against product concentration in mM (y-axis). Notes: Pdt = product area, IS = internal standard area, [Pdt] = product concentration in reaction, [PC] = protein concentration in reaction, Avg. TTN = average total turnover number, SD TTN = standard deviation of TTN, Avg. Yield = average yield, SD Yield = standard deviation of yield.

4-Phenyldihydrofuran-2(3*H*)-one (2a)



Data analysis for P411-LAS-5239-catalyzed lactonization of **1a**; Table S1, entry 1 and Table S2, entry 1:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	264.9	6113.2	0.043	0.173	0.35	7.5%	0.5%	530	35
2	298.1	6053.8	0.049	0.196	0.35				
3	288.3	6053.9	0.048	0.190	0.35				

Data analysis for P411-LAS-5240-catalyzed lactonization of **1a**; Table S2, entry 2:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	475.1	6152.5	0.077	0.308	0.16	14%	1.7%	2200	260
2	594.4	6115.2	0.097	0.388	0.16				
3	565.8	6111.0	0.093	0.369	0.16				

Data analysis for P411-LAS-5241-catalyzed lactonization of **1a**; Table S2, entry 3:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	766.1	6119.3	0.125	0.499	0.45	20%	0.1%	1100	8
2	759.5	6120.2	0.124	0.495	0.45				

3	748.6	6070.1	0.123	0.492	0.45				

Data analysis for P411-LAS-5242-catalyzed lactonization of **1a**; Table S2, entry 4:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1370.9	6197.7	0.221	0.882	0.33	35%	0.4%	2700	28
2	1346.0	6084.3	0.221	0.882	0.33				
3	1391.7	6177.4	0.225	0.898	0.33				

Data analysis for P411-LAS-5243-catalyzed lactonization of **1a**; Table S2, entry 5:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1578.8	6156.9	0.256	1.022	0.37	41%	1.5%	2900	200
2	1525.3	6143.5	0.248	0.990	0.37				
3	1610.5	6044.4	0.266	1.062	0.37				

Data analysis for P411-LAS-5244-catalyzed lactonization of **1a**; Table S2, entry 6:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1982.7	6142.6	0.323	1.287	0.33	52%	1.5%	3900	120
2	2099.3	6182.1	0.340	1.354	0.33				
3	1989.2	6160.0	0.323	1.287	0.33				

Data analysis for P411-LAS-5245-catalyzed lactonization of **1a**; Table S2, entry 7:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	2383.0	6059.9	0.393	1.568	0.39	64%	2.9%	4100	190
2	2380.6	6097.7	0.390	1.556	0.39				
3	2610.4	6164.0	0.423	1.688	0.39				

Data analysis for P411-LAS-5246-catalyzed lactonization of **1a**; Table S2, entry 8:

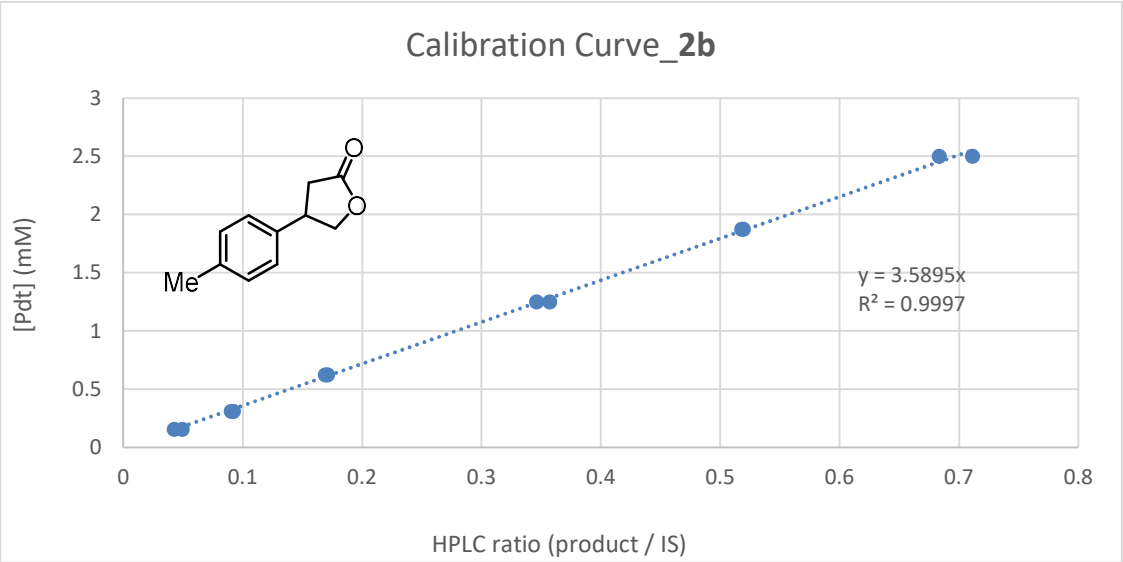
Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	2412.5	6093.9	0.396	1.578	0.33	65%	2.0%	4900	150
2	2522.5	5995.2	0.421	1.677	0.33				

3	2523.0	6134.8	0.411	1.640	0.33				

Data analysis for P411-LAS-5247-catalyzed lactonization of **1a**; Table S2, entry 9:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	2548.7	6054.8	0.421	1.678	0.43	66%	0.8%	3800	49
2	2512.5	6114.5	0.411	1.638	0.43				
3	2513.1	6086.4	0.413	1.646	0.43				

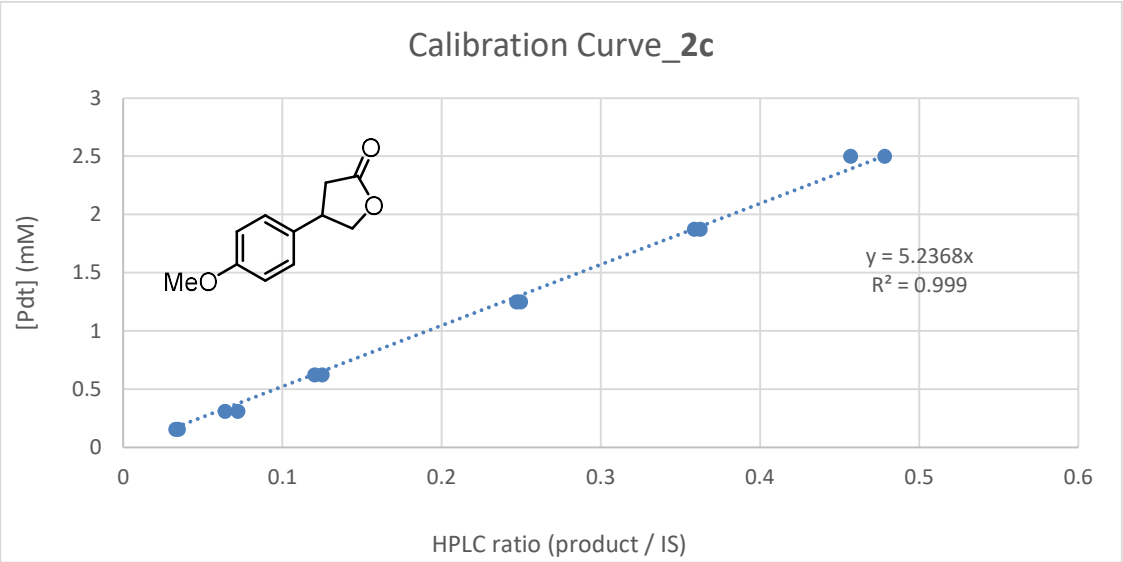
4-(*p*-Tolyl)dihydrofuran-2(3*H*)-one (2b)



Data analysis for P411-LAS-5247-catalyzed lactonization of **1b**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1780.8	5986.7	0.297	1.068	0.36	41%	1.4%	2900	97
2	1780.8	6166.7	0.289	1.037	0.36				
3	1797.3	6465.0	0.278	0.998	0.36				

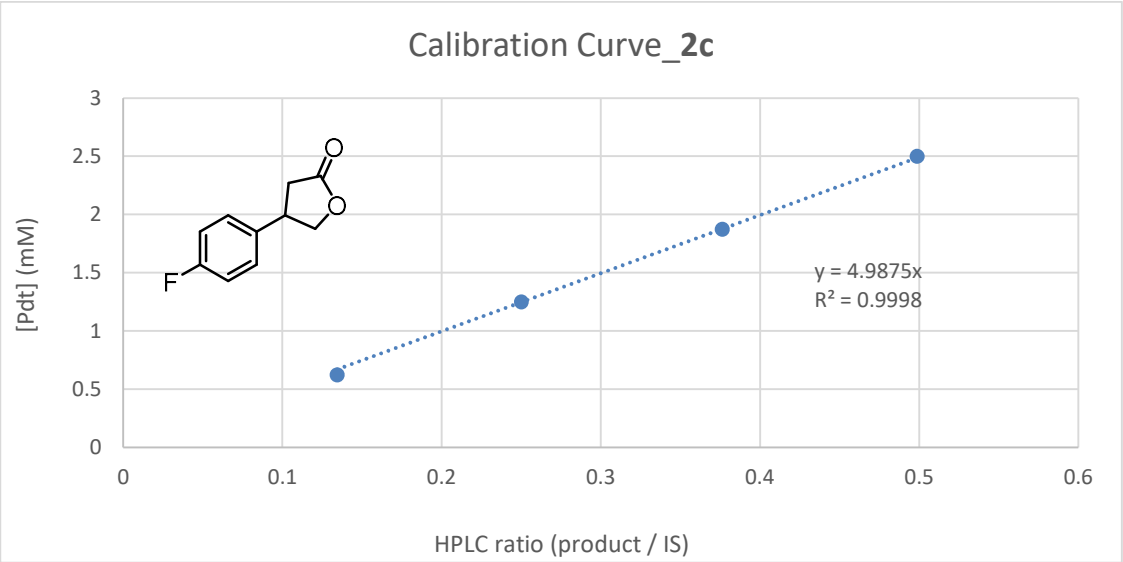
4-(4-Methoxyphenyl)dihydrofuran-2(3*H*)-one (2c)



Data analysis for P411-LAS-5247-catalyzed lactonization of **1c**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1699.8	6305.0	0.270	1.412	0.36	57%	0.3%	3900	17
2	1771.9	6513.9	0.272	1.425	0.36				
3	1746.2	6448.6	0.271	1.418	0.36				

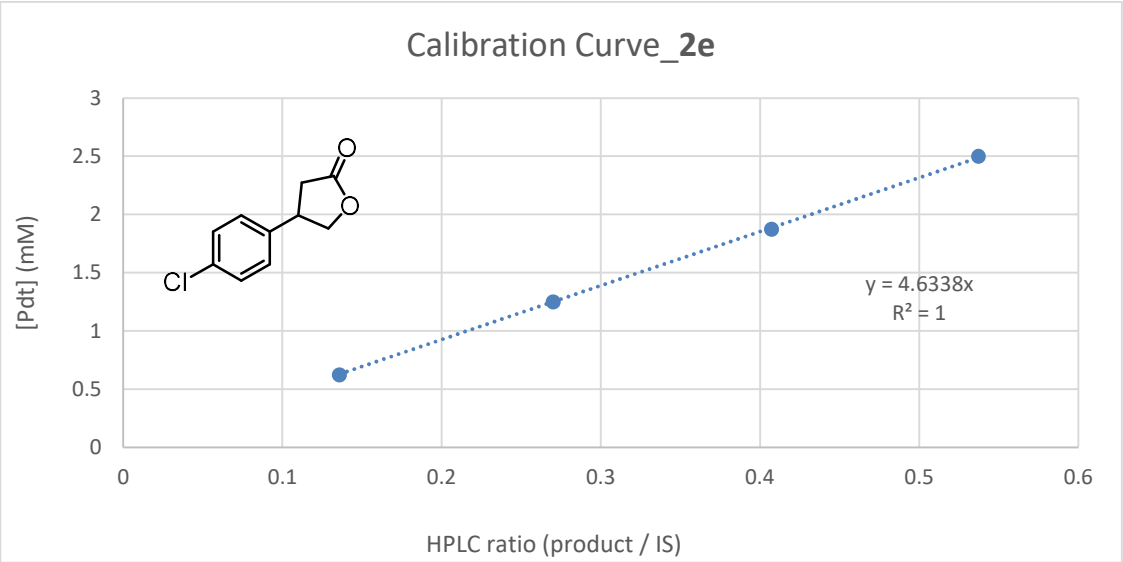
4-(4-Fluorophenyl)dihydrofuran-2(3*H*)-one (2d)



Data analysis for P411-LAS-5247-catalyzed lactonization of **1d**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1853.3	6071.5	0.305	1.522	0.36	59%	2.6%	4100	180
2	1764.3	6291.9	0.280	1.399	0.36				
3	1863.3	6193.4	0.301	1.501	0.36				

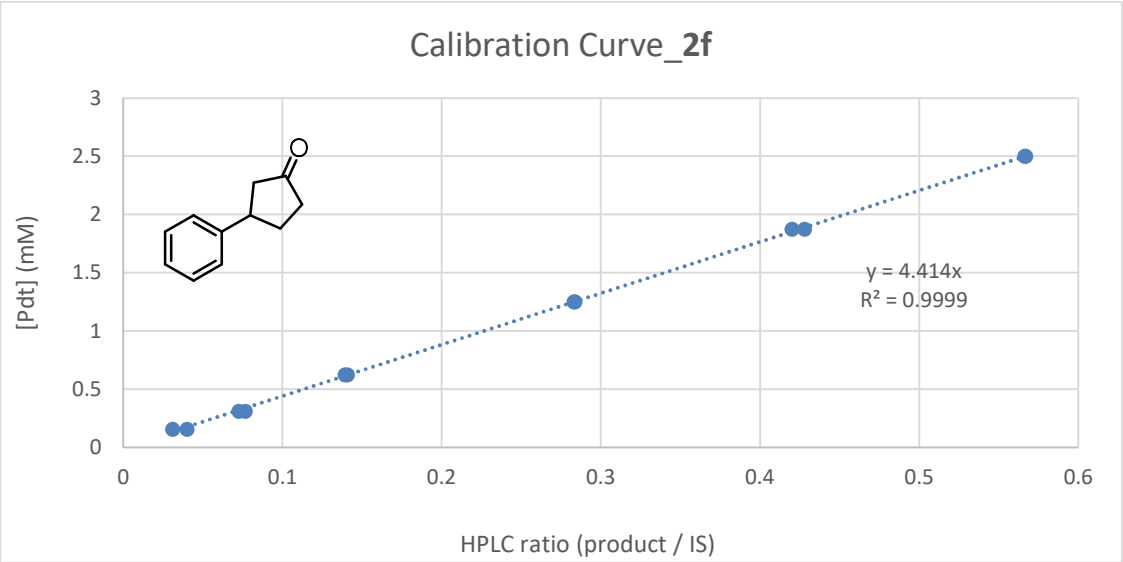
4-(4-Chlorophenyl)dihydrofuran-2(3*H*)-one (2e)



Data analysis for P411-LAS-5247-catalyzed lactonization of **1e**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	522.5	6060.0	0.086	0.400	0.36	16%	0.2%	1100	11
2	539.9	6222.4	0.087	0.402	0.36				
3	540.7	6154.0	0.088	0.407	0.36				

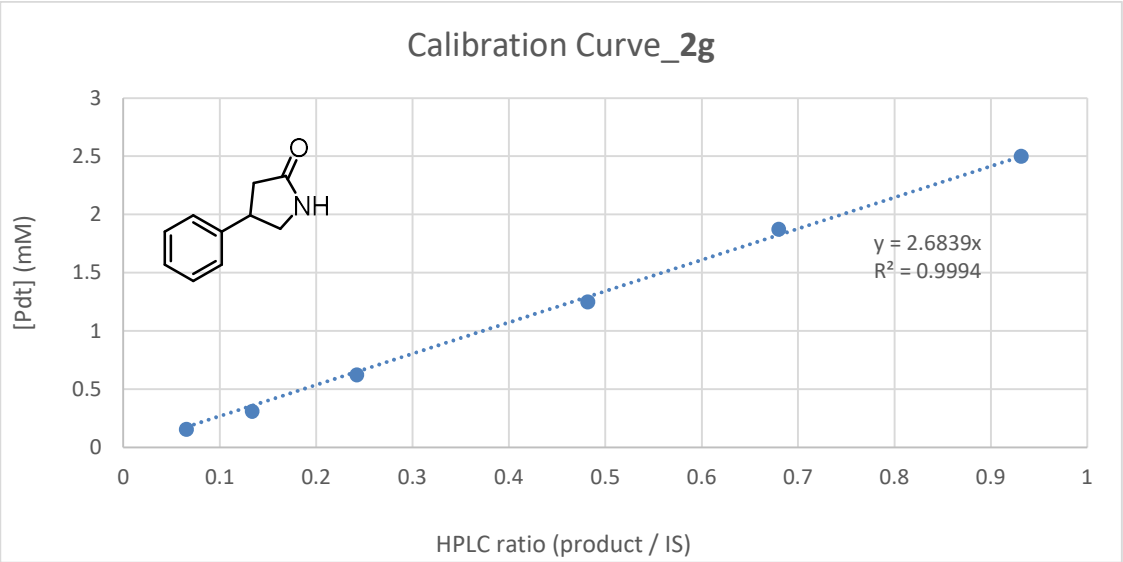
3-Phenylcyclopentan-1-one (2f)



Data analysis for P411-LAS-5247-catalyzed lactonization of **1f**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	3160.6	7132.0	0.443	1.956	0.36	80%	1.9%	5500	130
2	3265.6	7035.3	0.464	2.049	0.36				
3	3256.1	7160.5	0.455	2.007	0.36				

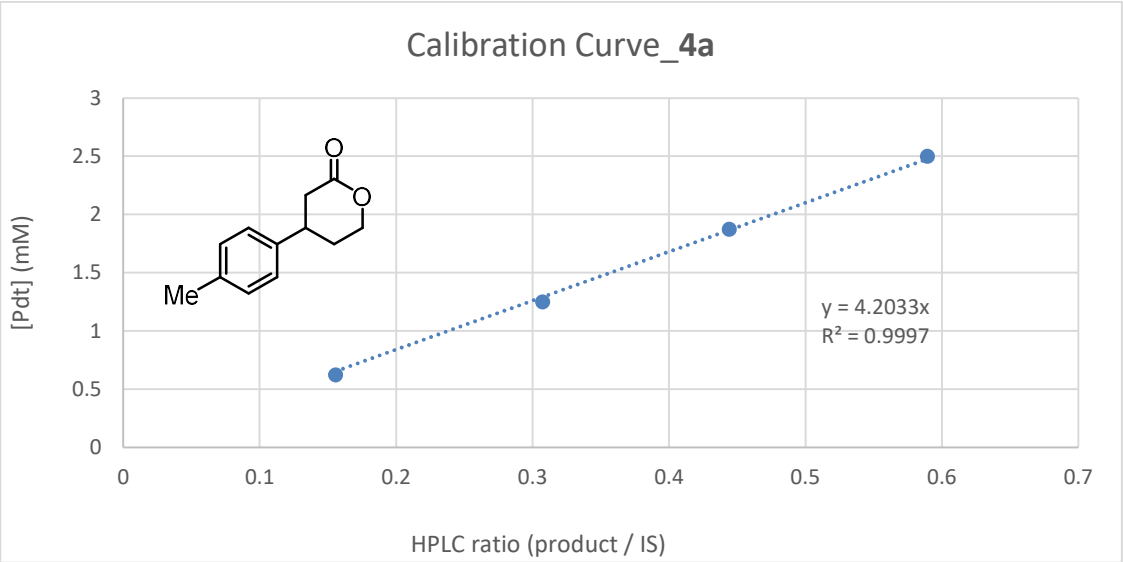
4-Phenylpyrrolidin-2-one (2g)



Data analysis for P411-LAS-5247-catalyzed lactonization of **1g**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1094.2	4986.8	0.219	0.589	0.36	22%	1.7%	1500	120
2	1071.8	5423.0	0.198	0.530	0.36				
3	922.1	4884.8	0.189	0.507	0.36				

4-(*p*-Tolyl)tetrahydro-2*H*-pyran-2-one (4a)



Data analysis for P411-LAS-5244-catalyzed lactonization of **3a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1481.7	6087.0	0.243	1.023	0.33	52%	12%	3900	930
2	2345.7	6020.0	0.390	1.638	0.33				
3	1801.6	6036.7	0.298	1.254	0.33				

Data analysis for P411-LAS-5248-catalyzed lactonization of **3a**:

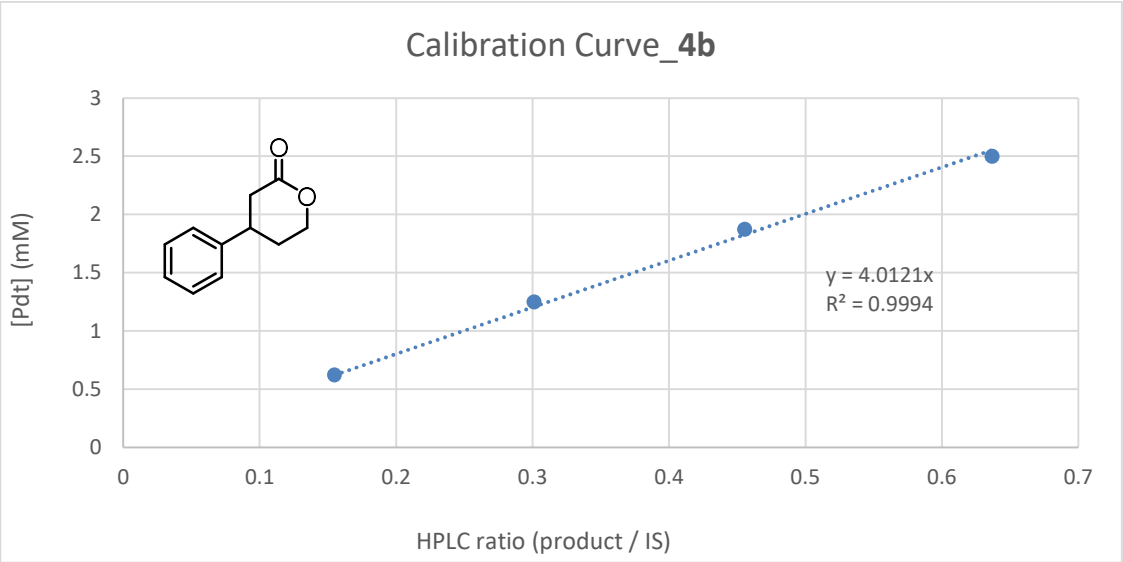
Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	2084.1	6464.7	0.322	1.355	0.43	50%	6.2%	2900	360
2	1584.3	6192.3	0.256	1.075	0.43				
3	1950.1	6176.5	0.316	1.327	0.43				

Data analysis for P411-LAS-5249-catalyzed lactonization of **3a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1801.0	6187.4	0.291	1.223	0.44	50%	1.1%	2800	60
2	1862.7	6140.9	0.303	1.275	0.44				

3	1799.9	6114.0	0.294	1.237	0.44				

4-Phenyltetrahydro-2*H*-pyran-2-one (4b)



Data analysis for P411-LAS-5244-catalyzed lactonization of **3b**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1620.3	5949.3	0.272	1.093	0.33	48%	5.0%	3600	370
2	1978.5	5925.5	0.334	1.340	0.33				
3	1781.6	5974.4	0.298	1.196	0.33				

Data analysis for P411-LAS-5248-catalyzed lactonization of **3b**:

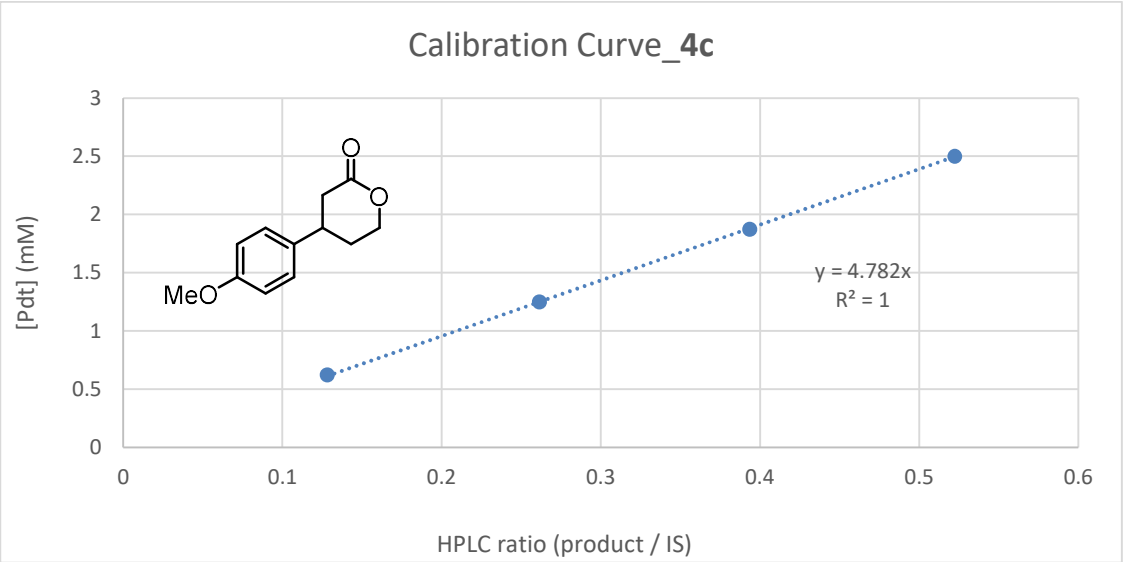
Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1677.7	6099.6	0.275	1.104	0.43	44%	0.7%	2600	39
2	1646.9	6092.6	0.270	1.085	0.43				
3	1691.7	6074.0	0.279	1.117	0.43				

Data analysis for P411-LAS-5249-catalyzed lactonization of **3b**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	997.9	6122.6	0.163	0.654	0.44	25%	1.2%	1400	69
2	937.9	6169.0	0.152	0.610	0.44				

3	908.2	6127.3	0.148	0.595	0.44				

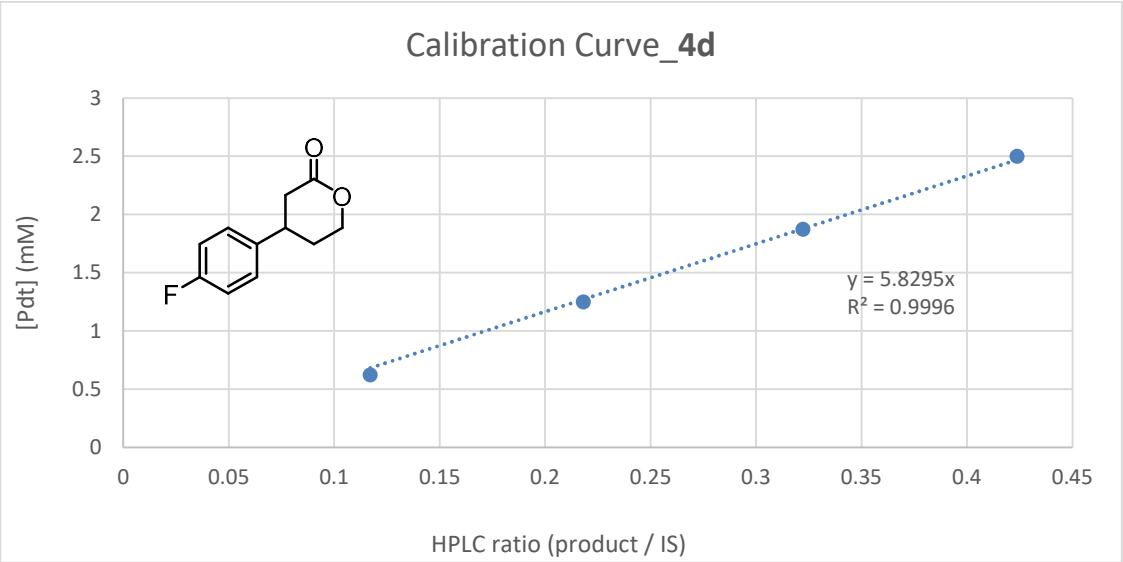
4-(4-Methoxyphenyl)tetrahydro-2*H*-pyran-2-one (4c)



Data analysis for P411-LAS-5249-catalyzed lactonization of **3c**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1600.1	5835.8	0.274	1.311	0.44	53%	3.1%	3000	170
2	1744.1	5891.3	0.296	1.416	0.44				
3	1584.1	5987.1	0.265	1.265	0.44				

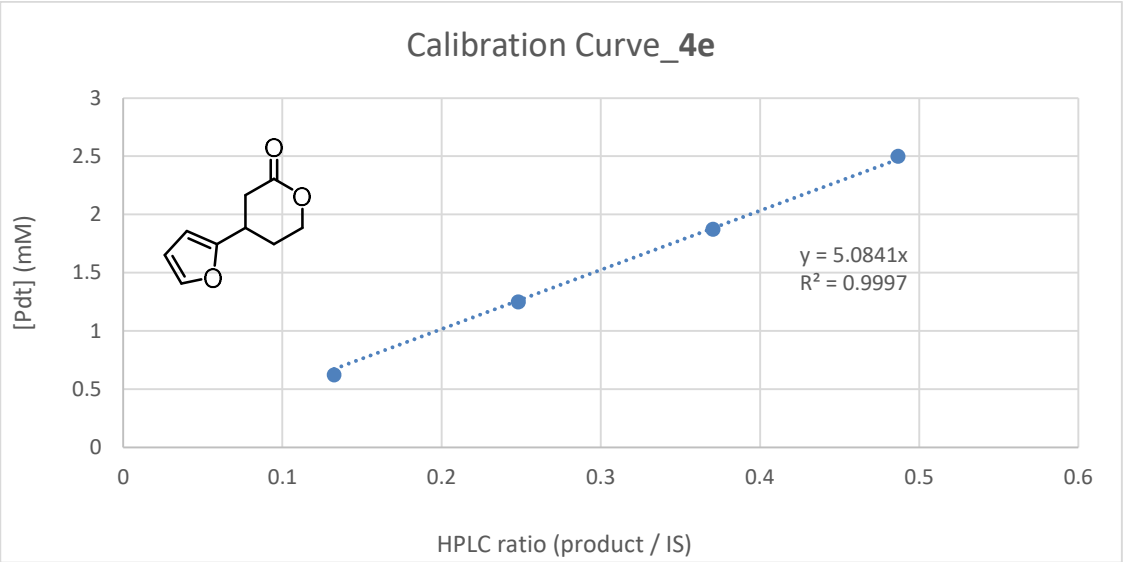
4-(4-Fluorophenyl)tetrahydro-2*H*-pyran-2-one (4d)



Data analysis for P411-LAS-5249-catalyzed lactonization of **3d**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	491.3	5720.4	0.086	0.501	0.44	21%	1.0%	1200	55
2	503.8	5389.0	0.093	0.545	0.44				
3	474.2	5481.1	0.087	0.504	0.44				

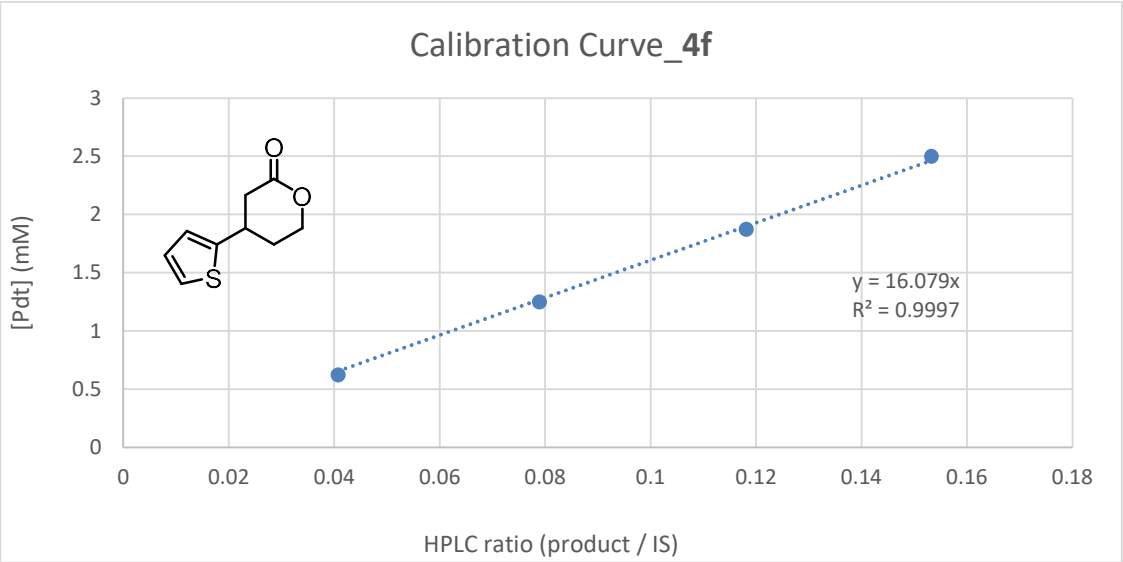
4-(Furan-2-yl)tetrahydro-2*H*-pyran-2-one (4e)



Data analysis for P411-LAS-5249-catalyzed lactonization of **3e**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1286.9	7507.0	0.171	0.872	0.44	41%	5.1%	2300	290
2	1172.1	5582.4	0.210	1.067	0.44				
3	1187.0	5436.8	0.218	1.110	0.44				

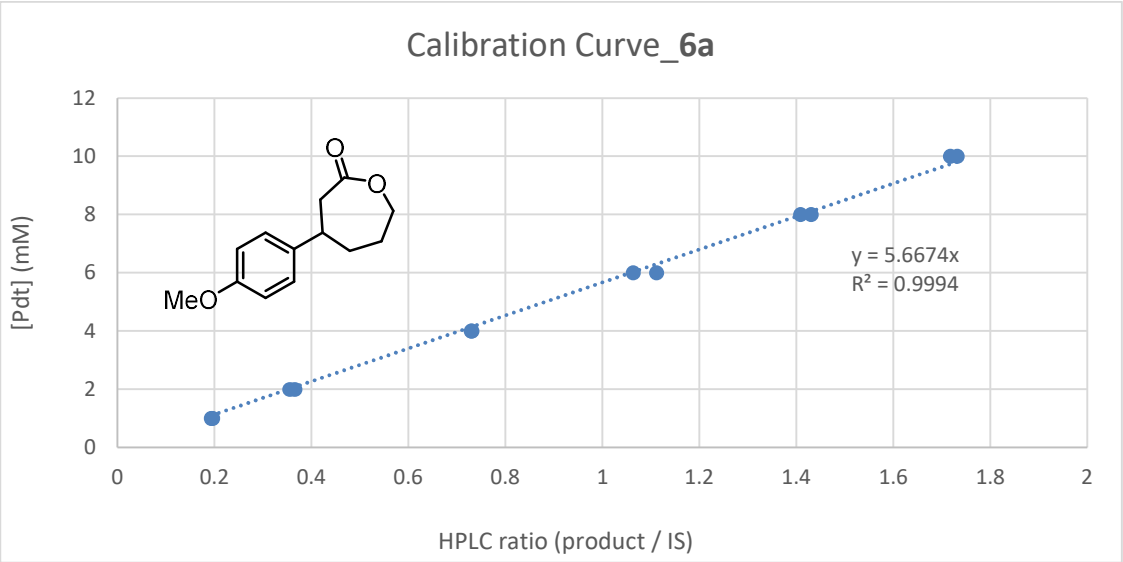
4-(Thiophen-2-yl)tetrahydro-2*H*-pyran-2-one (4f)



Data analysis for P411-LAS-5249-catalyzed lactonization of **3f**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	562.4	5383.0	0.104	1.680	0.44	71%	8.1%	4000	460
2	636.3	5093.9	0.125	2.009	0.44				
3	539.4	5284.6	0.102	1.641	0.44				

4-(4-Methoxyphenyl)oxepan-2-one (6a)



Data analysis for P411-LAS-5250-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	359.9	5927.4	0.061	0.344	0.42	16%	1.9%	940	111
2	452.7	6102.1	0.074	0.420	0.42				
3	484.2	6388.5	0.076	0.430	0.42				

Data analysis for P411-LAS-5251-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	732.6	6417.9	0.114	0.647	0.59	26%	1.2%	1100	52
2	718.9	6558.8	0.110	0.621	0.59				
3	788.2	6552.1	0.120	0.682	0.59				

Data analysis for P411-LAS-5252-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	603.8	6563.4	0.092	0.521	0.46	21%	0.5%	1100	28
2	600.4	6736.5	0.089	0.505	0.46				

3	625.1	6680.7	0.094	0.530	0.46				

Data analysis for P411-LAS-5253-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	902.5	6670.6	0.135	0.767	0.51				
2	827.5	6557.1	0.126	0.715	0.51	31%	1.9%	1500	94
3	936.6	6551.0	0.143	0.810	0.51				

Data analysis for P411-LAS-5254-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	782.1	5819.3	0.134	0.762	0.59				
2	976.6	6180.9	0.158	0.895	0.59	31%	4.0%	1300	169
3	777.4	6307.1	0.123	0.699	0.59				

Data analysis for P411-LAS-5255-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	981.8	6519.0	0.151	0.854	0.59				
2	1161.9	6458.8	0.180	1.020	0.59	36%	3.8%	1500	158
3	1007.9	6633.7	0.152	0.861	0.59				

Data analysis for P411-LAS-5256-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1234.1	6580.0	0.188	1.063	0.65				
2	1268.3	6630.9	0.191	1.084	0.65	44%	2.3%	1700	90
3	1341.1	6481.3	0.207	1.172	0.65				

Data analysis for P411-LAS-5257-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN

1	1536.3	6894.4	0.223	1.263	0.60	52%	2.7%	2200	110
2	1557.1	6936.0	0.225	1.272	0.60				
3	1585.7	6500.6	0.244	1.382	0.60				

Data analysis for P411-LAS-5258-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1427.9	6825.7	0.209	1.186	0.48	45%	2.2%	2300	115
2	1299.7	6783.4	0.192	1.086	0.48				
3	1320.3	6846.9	0.193	1.093	0.48				

Data analysis for P411-LAS-5259-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1701.9	6649.2	0.256	1.451	0.60	58%	0.3%	2400	14
2	1708.4	6745.3	0.253	1.435	0.60				
3	1686.3	6646.8	0.254	1.438	0.60				

Data analysis for P411-LAS-5260-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	459.3	8604.6	0.053	0.302	0.44	12%	0.3%	700	19
2	449.4	8536.2	0.053	0.298	0.44				
3	473.2	8540.4	0.055	0.314	0.44				

Data analysis for P411-LAS-5261-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	973.7	11628.9	0.084	0.475	0.38	18%	0.8%	1200	51
2	862.5	11202.6	0.077	0.436	0.38				
3	913.0	11158.4	0.082	0.464	0.38				

Data analysis for P411-LAS-5262-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1004.5	6735.3	0.149	0.845	0.30	33%	1.1%	2700	93
2	957.7	6833.3	0.140	0.794	0.30				
3	1002.6	6763.7	0.148	0.840	0.30				

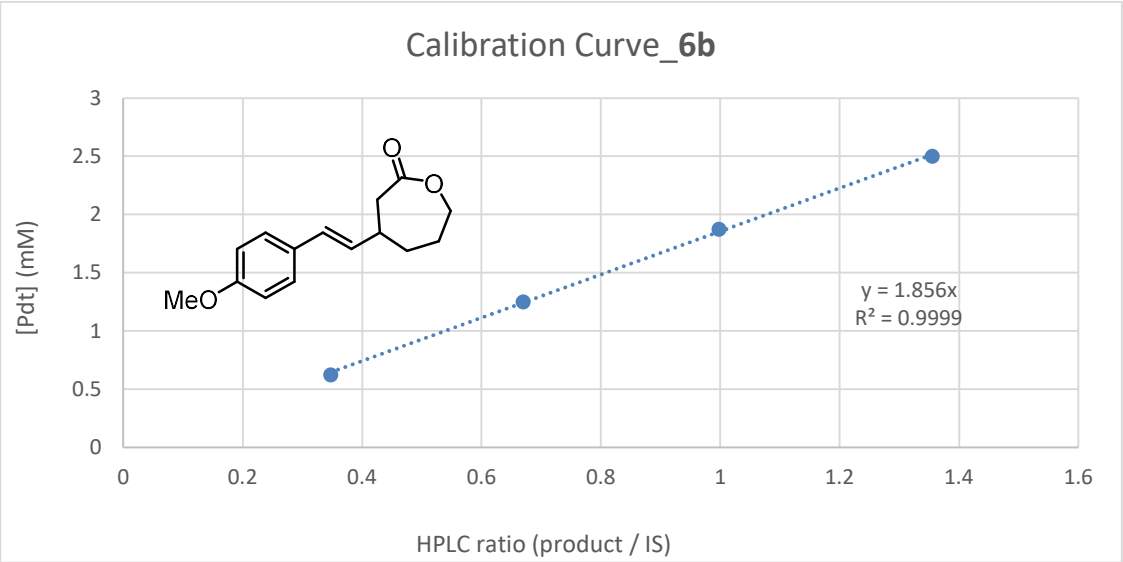
Data analysis for P411-LAS-5263-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1237.1	6865.1	0.180	1.021	0.34	39%	2.2%	2900	160
2	1138.2	7037.4	0.162	0.917	0.34				
3	1242.0	7022.0	0.177	1.002	0.34				

Data analysis for P411-LAS-5264-catalyzed lactonization of **5a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1554.7	6540.6	0.238	1.347	0.43	54%	0.5%	3100	27
2	1589.5	6571.2	0.242	1.371	0.43				
3	1574.8	6557.0	0.240	1.361	0.43				

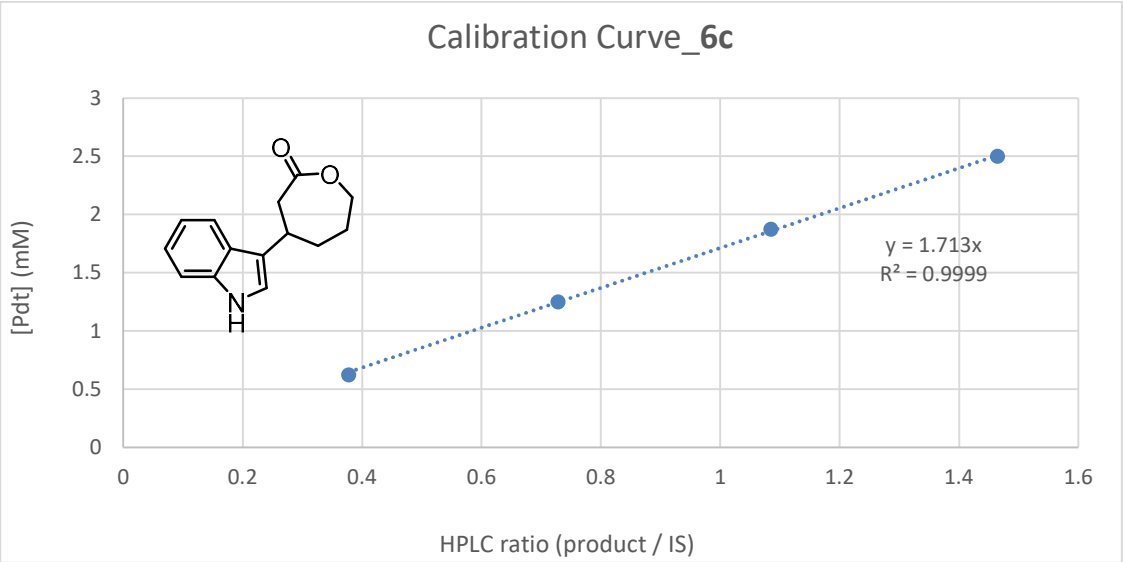
(*E*)-4-(4-Methoxystyryl)oxepan-2-one (6b)



Data analysis for P411-LAS-5264-catalyzed lactonization of **5b**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	4650.4	7286.5	0.638	1.185	0.38	51%	3.3%	3300	210
2	5572.1	7877.2	0.707	1.313	0.38				
3	5097.0	7072.8	0.721	1.338	0.38				

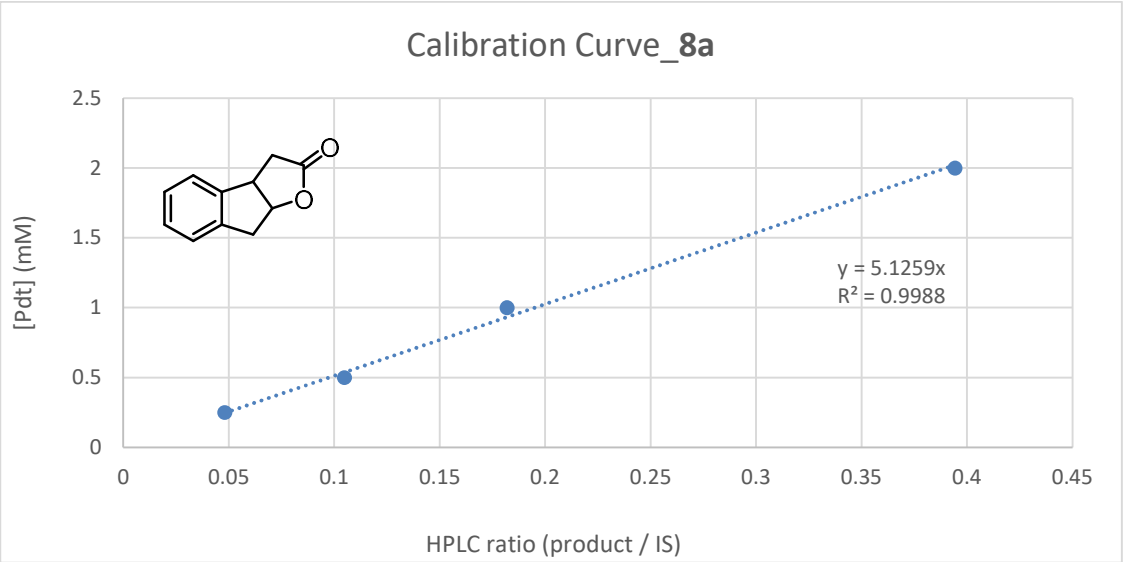
4-(1*H*-Indol-3-yl)oxepan-2-one (6c)



Data analysis for P411-LAS-5264-catalyzed lactonization of **5c**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	4710.8	7474.0	0.630	1.080	0.38	43%	3.3%	2800	220
2	5239.7	7653.6	0.685	1.173	0.38				
3	4474.1	7602.0	0.589	1.008	0.38				

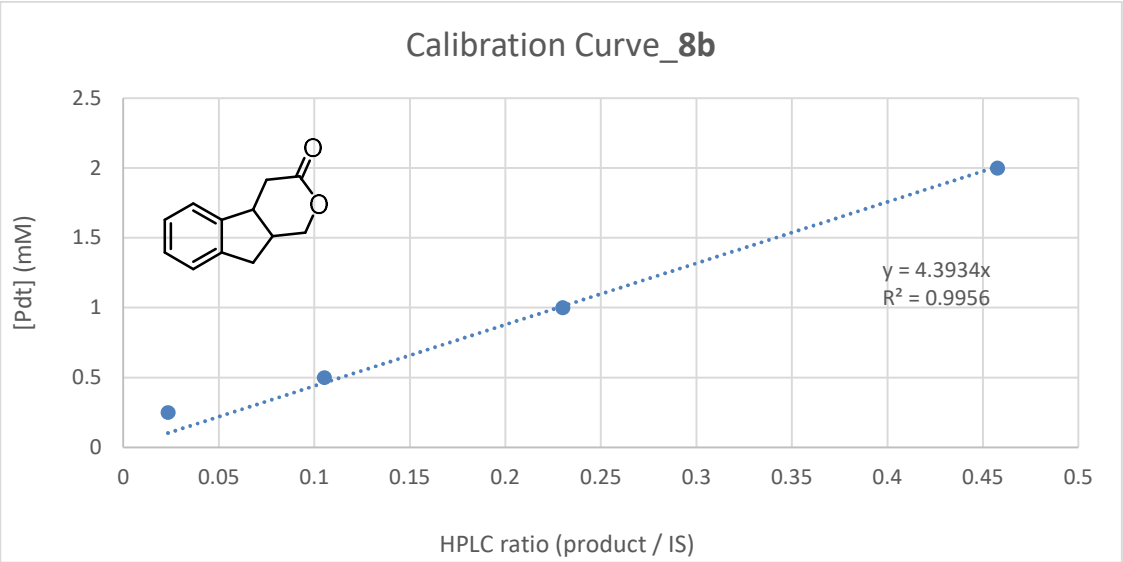
3,3a,8,8a-Tetrahydro-2*H*-indeno[2,1-*b*]furan-2-one (8a)



Data analysis for P411-LAS-5265-catalyzed lactonization of **7a**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1751.0	3491.1	0.502	2.571	0.37	98%	4.2%	6500	280
2	1603.7	3452.6	0.464	2.381	0.37				
3	1624.4	3476.7	0.467	2.395	0.37				

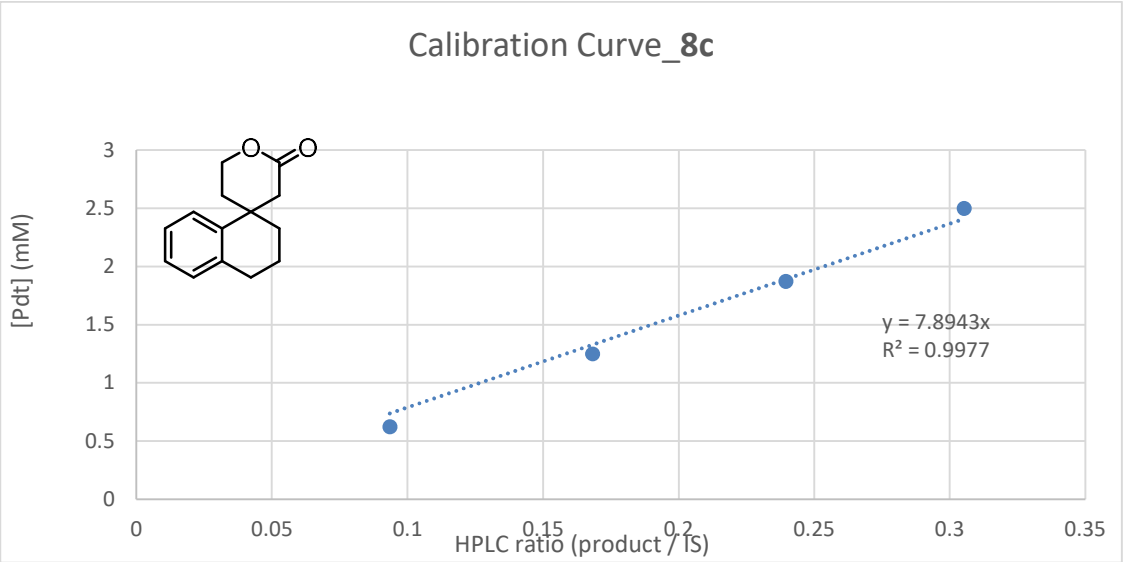
4,4a,9,9a-Tetrahydroindeno[2,1-*c*]pyran-3(1*H*)-one (8b)



Data analysis for P411-LAS-5265-catalyzed lactonization of **7b**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1442.1	3458.6	0.417	1.832	0.37	73%	2.9%	4800	200
2	1360.2	3451.3	0.394	1.732	0.37				
3	1473.1	3452.3	0.427	1.875	0.37				

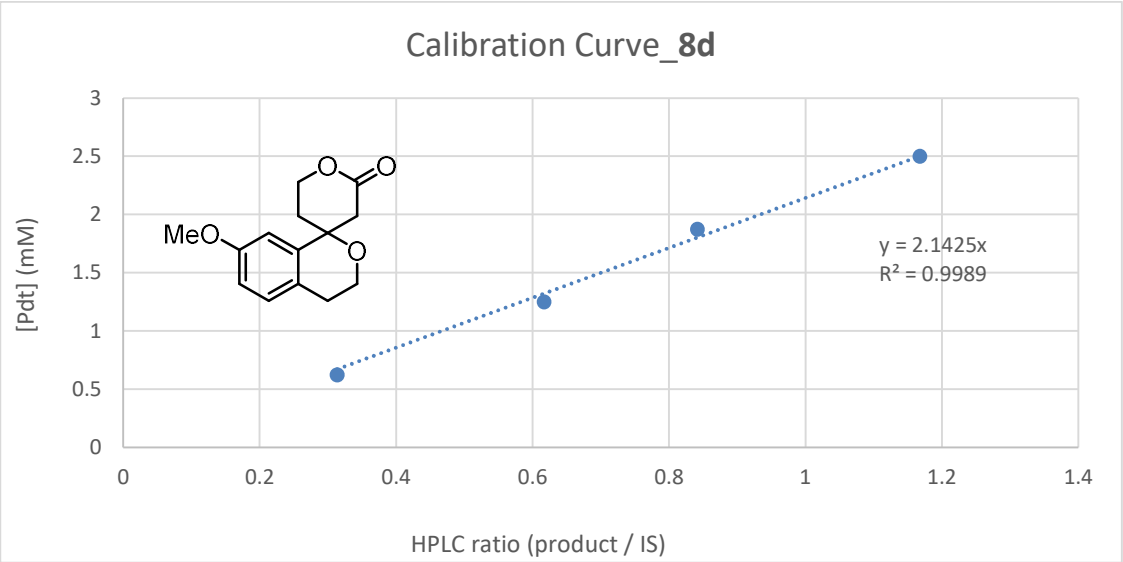
3,4,5',6'-Tetrahydro-2*H*-spiro[naphthalene-1,4'-pyran]-2'(3'*H*)-one (8c)



Data analysis for P411-LAS-5259-catalyzed lactonization of **7c**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	123.1	3824.5	0.032	0.254	0.46	11%	0.6%	591	31
2	134.5	3813.9	0.035	0.278	0.46				
3	136.4	3872.6	0.035	0.278	0.46				

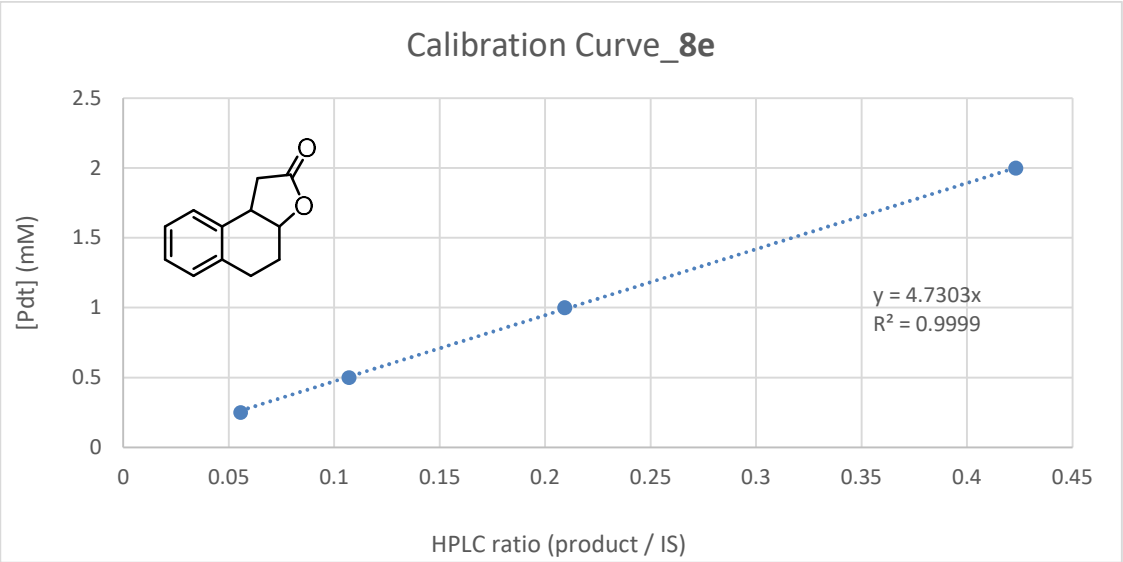
7-methoxy-5',6'-dihydrospiro[isochromane-1,4'-pyran]-2'(3'H)-one (8d)



Data analysis for P411-LAS-5257-catalyzed lactonization of **7d**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1554.7	3507.3	0.443	0.950	0.40	39%	1.8%	2500	110
2	1589.3	3286.4	0.484	1.036	0.40				
3	1599.7	3530.1	0.453	0.971	0.40				

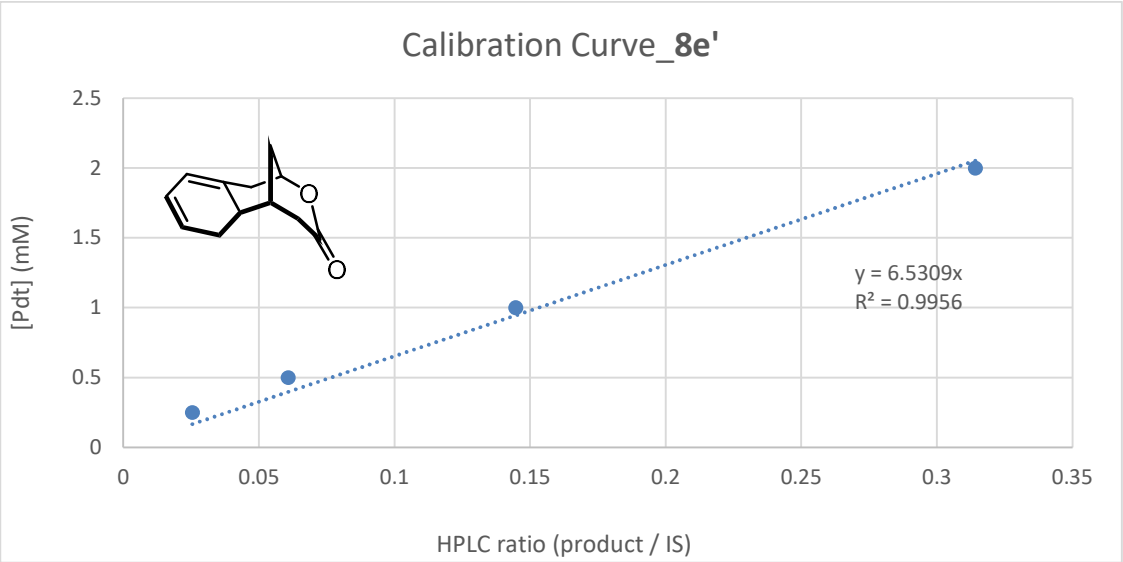
3a,4,5,9b-Tetrahydronaphtho[2,1-*b*]furan-2(1*H*)-one (8e)



Data analysis for P411-LAS-5259-catalyzed lactonization of **7e** toward the synthesis of **8e**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	717.4	3203.8	0.224	1.059	0.38	43%	1.1%	2900	76
2	740.4	3138.2	0.236	1.116	0.38				
3	720.8	3151.0	0.229	1.082	0.38				

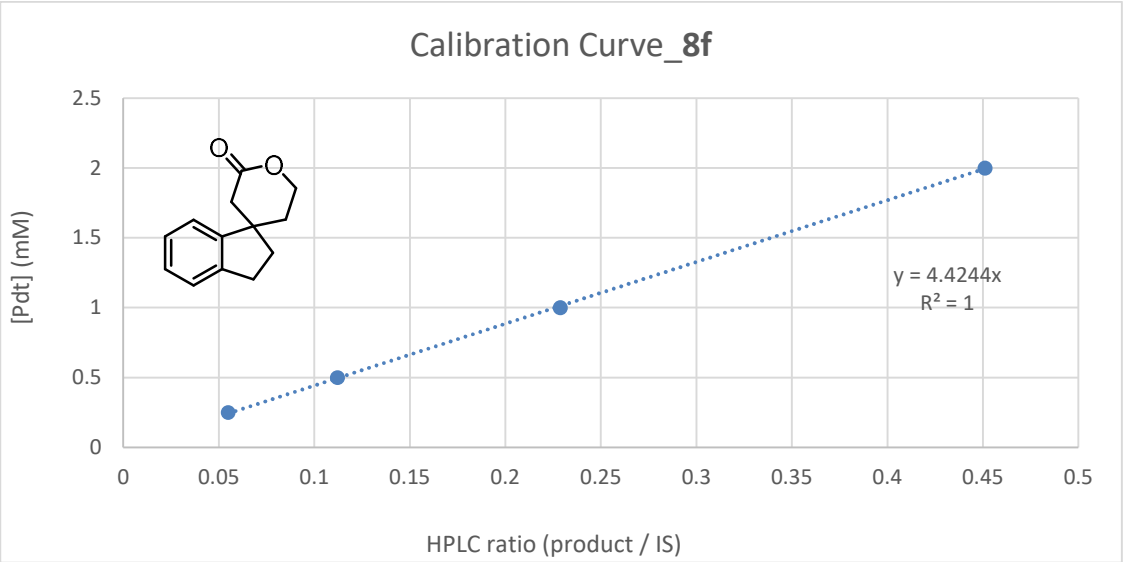
1,2,5,6-tetrahydro-4*H*-2,6-methanobenzo[*d*]oxocin-4-one (8e')



Data analysis for P411-LAS-5259-catalyzed lactonization of **7e** toward the synthesis of **8e'**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	619.5	3203.8	0.193	1.263	0.38	48%	2.3%	3200	150
2	571.5	3138.2	0.182	1.189	0.38				
3	554.6	3151.0	0.176	1.149	0.38				

2,3,5',6'-Tetrahydrospiro[indene-1,4'-pyran]-2'(3'*H*)-one (8f)



Data analysis for P411-LAS-5247-catalyzed lactonization of **7f** toward the synthesis of **8f**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	581.6	4155.9	0.140	0.619132	0.36	25%	2.3%	1700	160
2	532.8	4224.1	0.126	0.558086	0.36				
3	640.2	4219.2	0.152	0.671355	0.36				

Data analysis for P411-LAS-5256-catalyzed lactonization of **7f** toward the synthesis of **8f**:

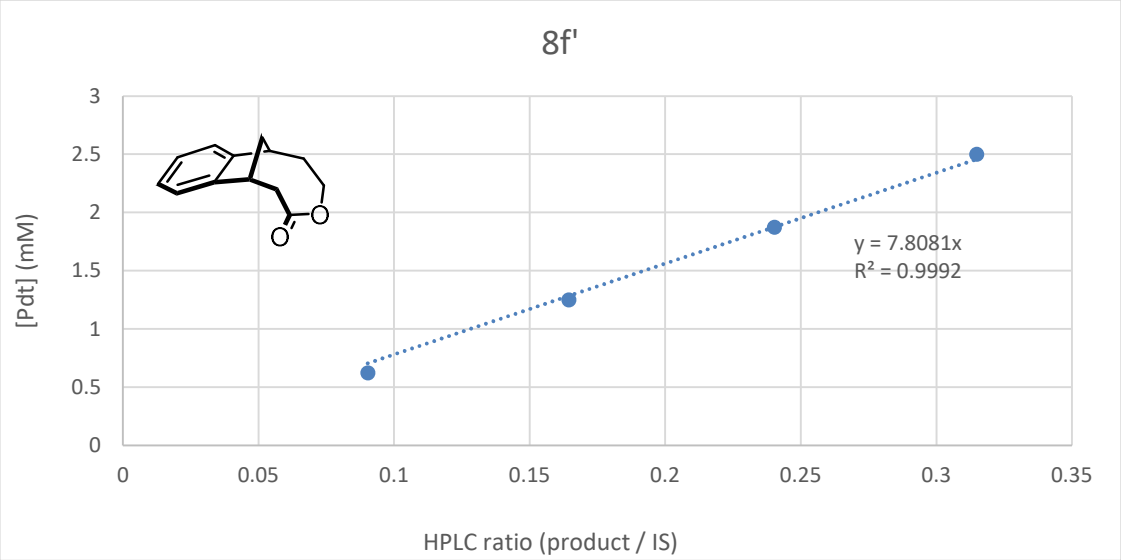
Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	1280.4	4008.6	0.319	1.413	0.36	62%	4.9%	4300	340
2	1473.9	3961.7	0.372	1.646	0.36				
3	1448.6	4034.8	0.359	1.588	0.36				

Data analysis for P411-LAS-5266-catalyzed lactonization of **7f** toward the synthesis of **8f**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	0	3090.1	-	-	0.17	-	-	-	-
2	0	3288.8	-	-	0.17				

3	0	3269.2	-	-	0.17				

(7)-1,5,6,7-Tetrahydro-1,7-methanobenzo[*e*]oxonin-3(2*H*)-one (8f')



Data analysis for P411-LAS-5247-catalyzed lactonization of **7f** toward the synthesis of **8f'**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	581.6	4155.9	0.121	0.945	0.36	34%	3.2%	2400	220
2	532.8	4224.1	0.109	0.848	0.36				
3	640.2	4219.2	0.101	0.787	0.36				

Data analysis for P411-LAS-5256-catalyzed lactonization of **7f** toward the synthesis of **8f'**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	0	4008.6	-	-	0.36	-	-	-	-
2	0	3961.7	-	-	0.36				
3	0	4034.8	-	-	0.36				

Data analysis for P411-LAS-5266-catalyzed lactonization of **7f** toward the synthesis of **8f'**:

Entry	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. Yield [%]	SD Yield [%]	Avg. TTN	SD TTN
1	205.0	3090.1	0.066	0.518	0.17	21%	0.3%	3100	39
2	221.8	3288.8	0.067	0.526	0.17				

3	222.3	3269.2	0.068	0.531	0.17				

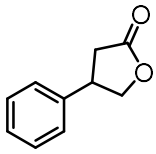
7. Enantioselectivity Determination

The absolute configuration of enzymatic product **2c** was assigned to be *S* by comparing the elution order of two enantiomers with a literature report under same elution conditions using the same column (Chiralpak IB).[18] The other *γ*-lactone products **2a**–**2b**, **2d**–**2m** were assigned by analogy.

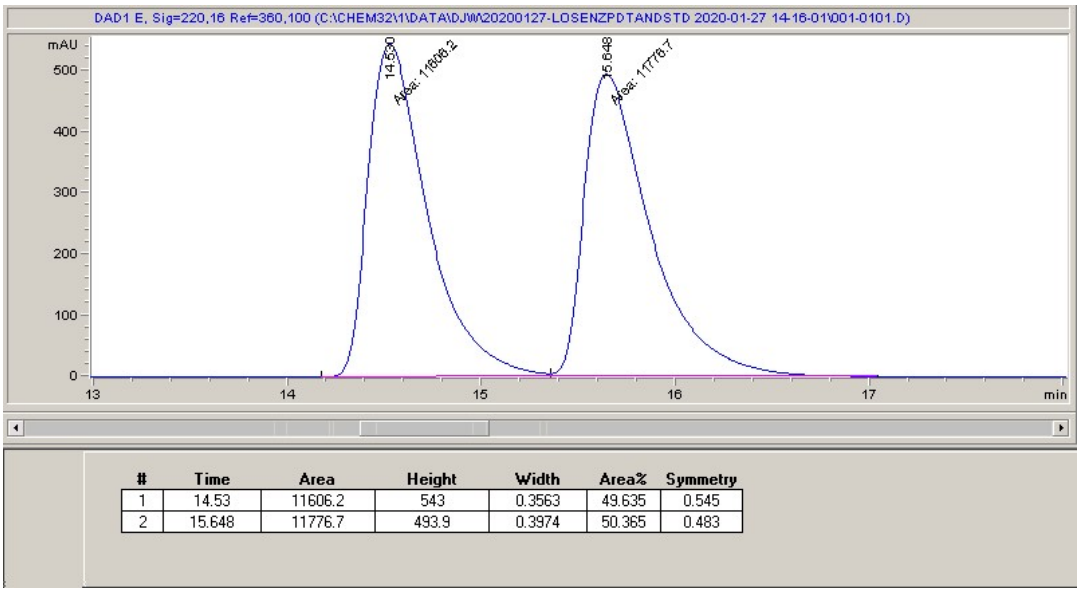
The absolute configuration of enzymatic product **4b** was assigned to be *R* by comparing the elution order of two enantiomers with a literature report under same elution conditions using the same column (Chiralpak AS-H).[18] The other *δ*-lactone products **4a**, **4c**–**4f** were assigned by analogy.

The absolute stereochemistry for enzymatic product **6a** was assigned as *S* through X-ray crystallography (see section 8). The other *ε*-lactone products **6b** and **6c** were assigned by analogy.

4-Phenyldihydrofuran-2(3*H*)-one (2a)

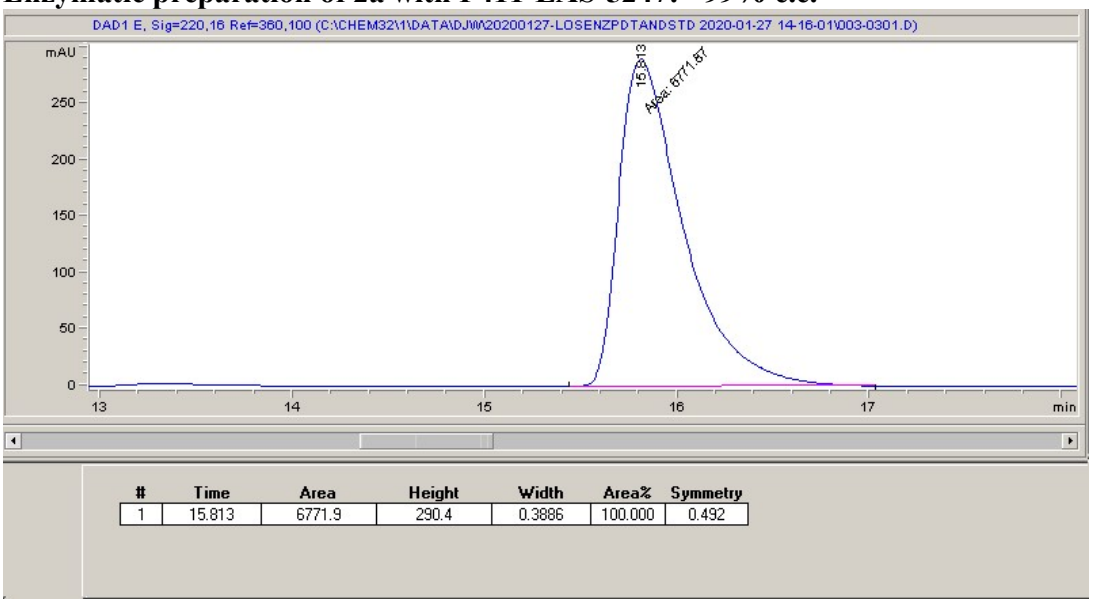


HPLC conditions: Chiralpak IA, 2.5% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm



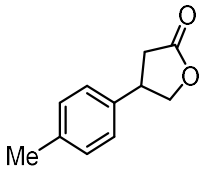
#	Time	Area	Height	Width	Area%	Symmetry
1	14.53	11606.2	543	0.3563	49.635	0.545
2	15.648	11776.7	493.9	0.3974	50.365	0.483

Enzymatic preparation of 2a with P411-LAS-5247: >99% e.e.

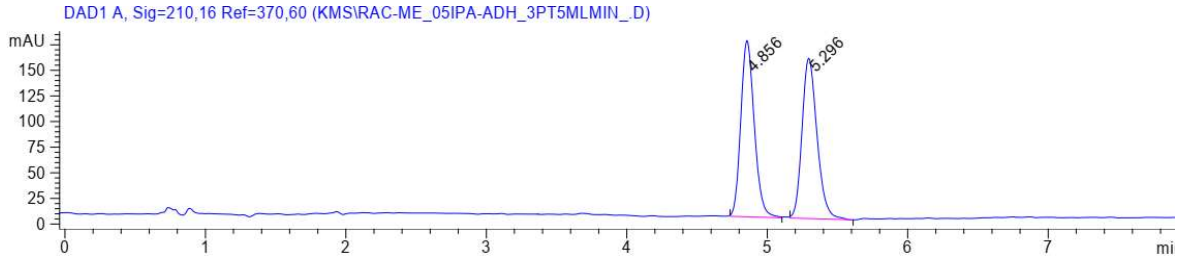


#	Time	Area	Height	Width	Area%	Symmetry
1	15.813	6771.9	290.4	0.3886	100.000	0.492

4-(*p*-Tolyl)dihydrofuran-2(3*H*)-one (2b)

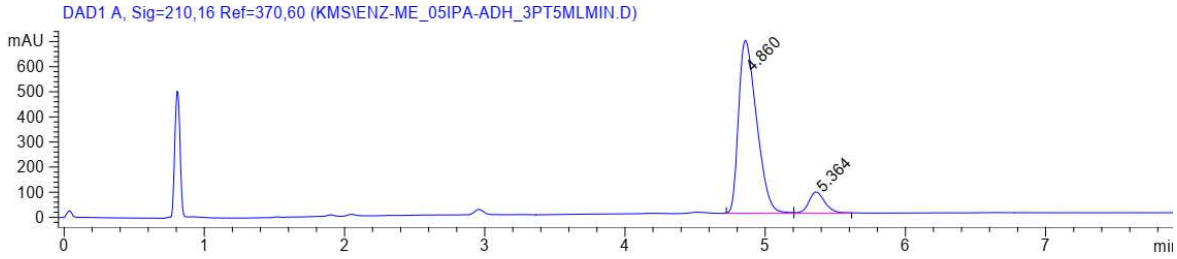


SFC conditions: Chiralpak AD-H, 5% *i*-PrOH in supercritical CO_2, 2.5 mL/min, 210 nm



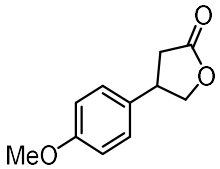
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.856	BB	0.1019	1146.25952	171.62752	49.6720
2	5.296	BB	0.1147	1161.39697	156.22182	50.3280

Enzymatic preparation of 2b with P411-LAS-5247: 80% e.e.

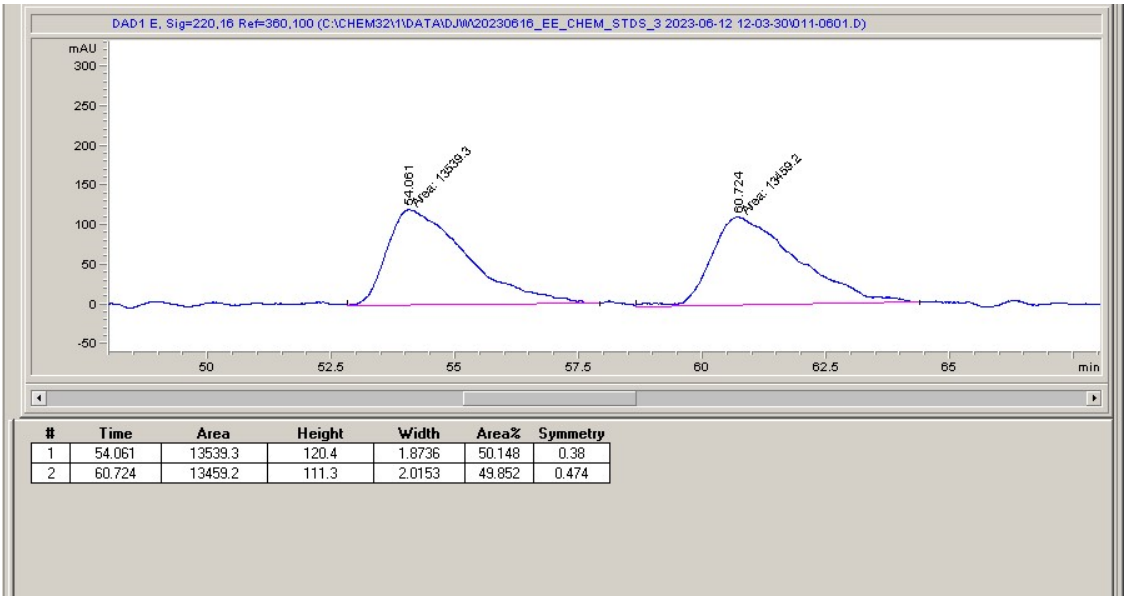


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.860	BV	0.1360	6139.50293	688.93280	90.1516
2	5.364	VB	0.1209	670.69690	84.23247	9.8484

4-(4-Methoxyphenyl)dihydrofuran-2(3*H*)-one (2c)

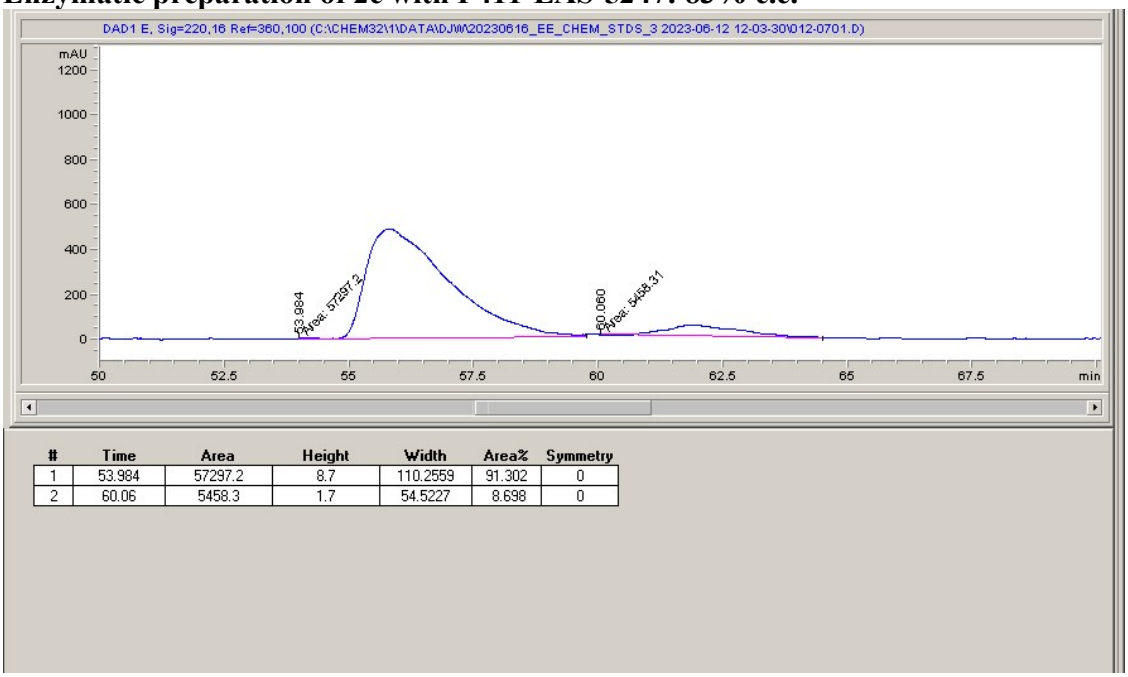


HPLC conditions: Chiralpak ODH, 2.5% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm



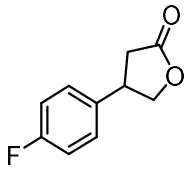
#	Time	Area	Height	Width	Area%	Symmetry
1	54.061	13539.3	120.4	1.8736	50.148	0.38
2	60.724	13459.2	111.3	2.0153	49.852	0.474

Enzymatic preparation of 2c with P411-LAS-5247: 83% e.e.

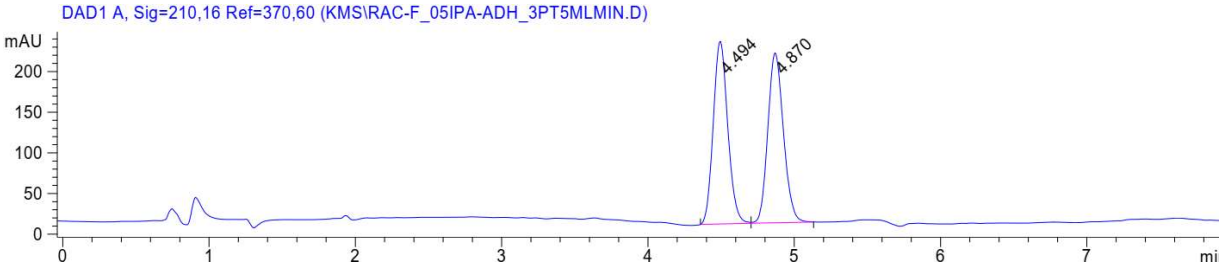


#	Time	Area	Height	Width	Area%	Symmetry
1	53.984	57297.2	8.7	110.2559	91.302	0
2	60.06	5458.3	1.7	54.5227	8.698	0

4-(4-Fluorophenyl)dihydrofuran-2(3*H*)-one (2d)

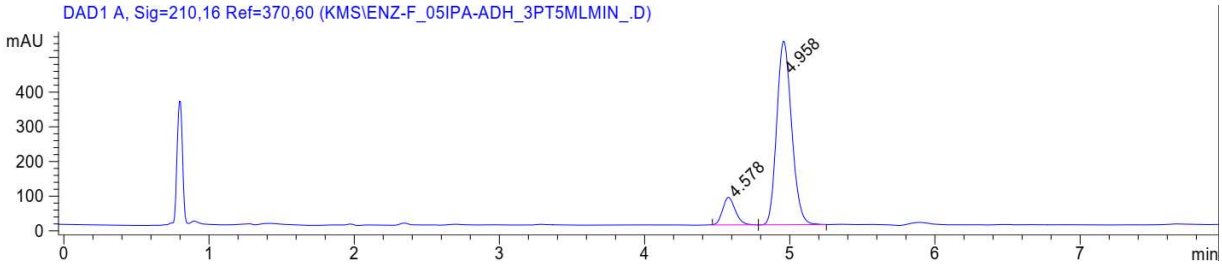


SFC conditions: Chiralpak AD-H, 5% *i*-PrOH in supercritical CO_2, 2.5 mL/min, 210 nm



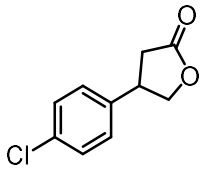
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.494	BV	0.1086	1548.69226	224.07057	50.0417
2	4.870	VB	0.1145	1546.11169	208.46843	49.9583

Enzymatic preparation of 2d with P411-LAS-5247: 77% e.e.

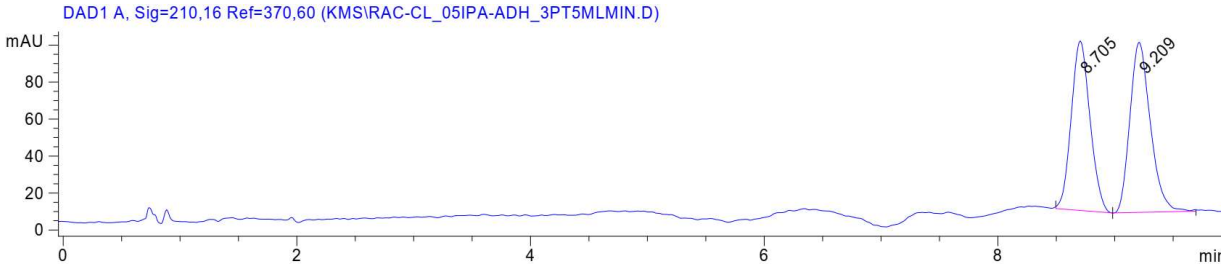


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.578	BV	0.0951	482.87766	79.23338	11.3347
2	4.958	VB	0.1113	3777.29224	528.91382	88.6653

4-(4-Chlorophenyl)dihydrofuran-2(3*H*)-one (2e)

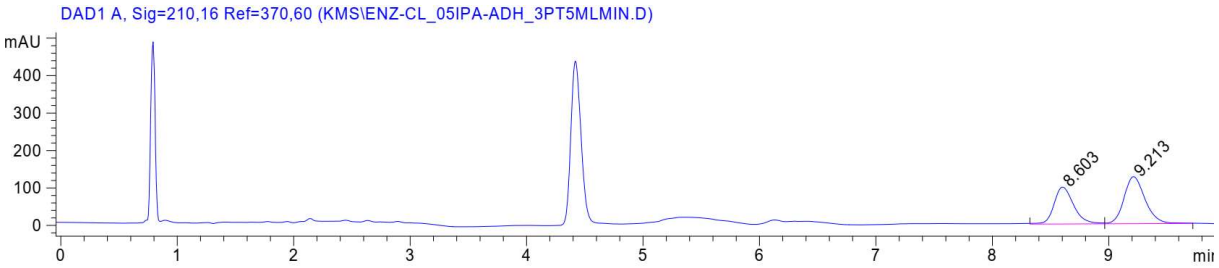


SFC conditions: Chiralpak AD-H, 5% *i*-PrOH in supercritical CO_2, 2.5 mL/min, 210 nm



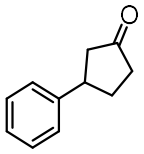
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.705	BV	0.1705	1023.10614	91.49740	47.7289
2	9.209	VB	0.1863	1120.47363	91.84954	52.2711

Enzymatic preparation of 2e with P411-LAS-5247: 16% e.e.

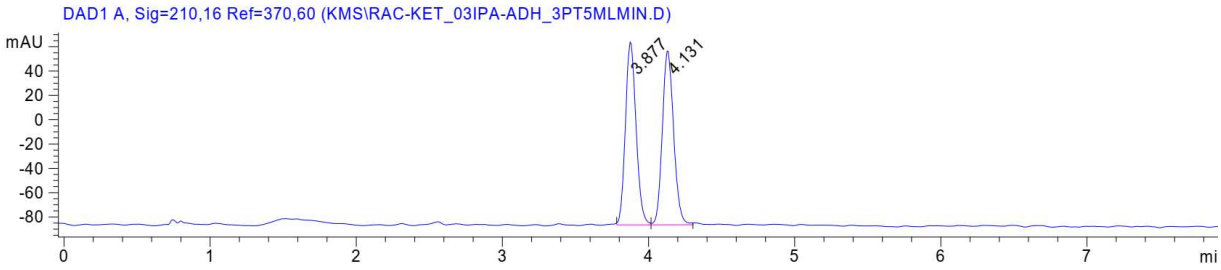


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.603	BV	0.1806	1152.80786	98.45173	42.0722
2	9.213	VB	0.1914	1587.26416	125.60918	57.9278

4-Phenyldihydrofuran-2(3*H*)-one (2f)

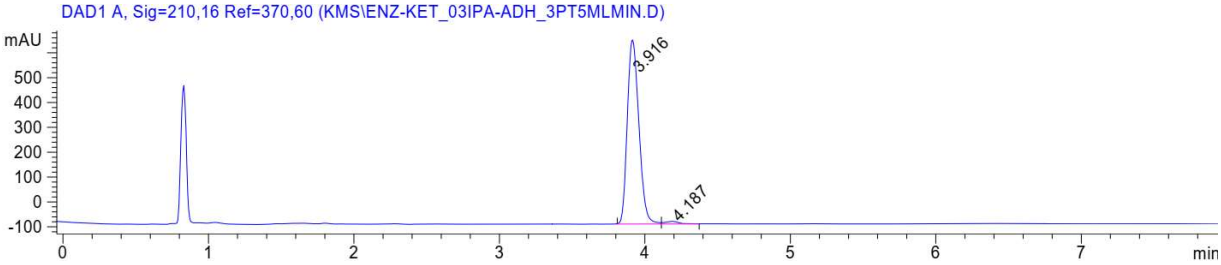


SFC conditions: Chiralpak AD-H, 5% *i*-PrOH in supercritical CO_2, 2.5 mL/min, 210 nm



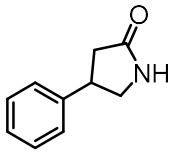
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.877	BV	0.0794	761.17230	149.62044	49.7216
2	4.131	VB	0.0829	769.69629	142.76801	50.2784

Enzymatic preparation of 2f with P411-LAS-5247: 97% e.e.

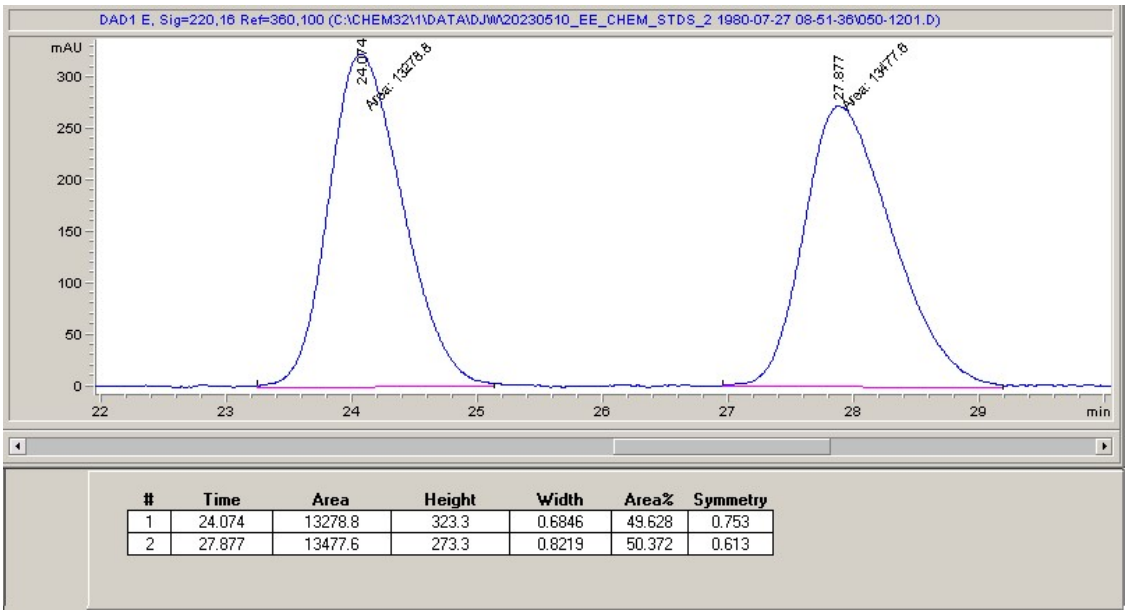


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.916	BV	0.0890	4094.99829	735.15277	98.3037
2	4.187	VB	0.0982	70.66048	10.54307	1.6963

4-Phenylpyrrolidin-2-one (2g)

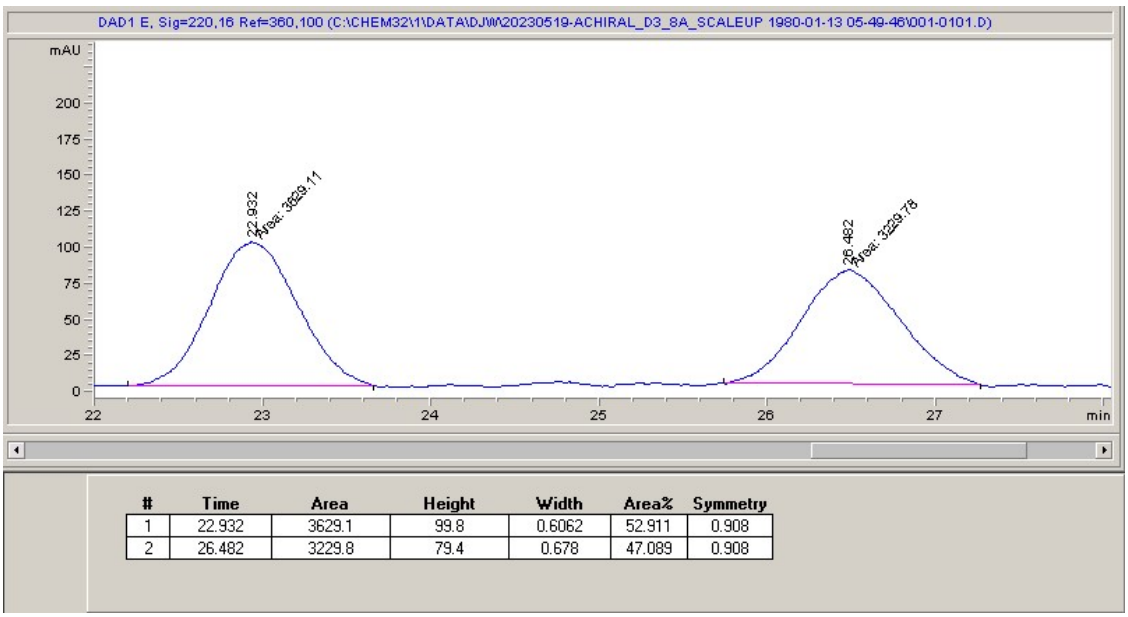


HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm



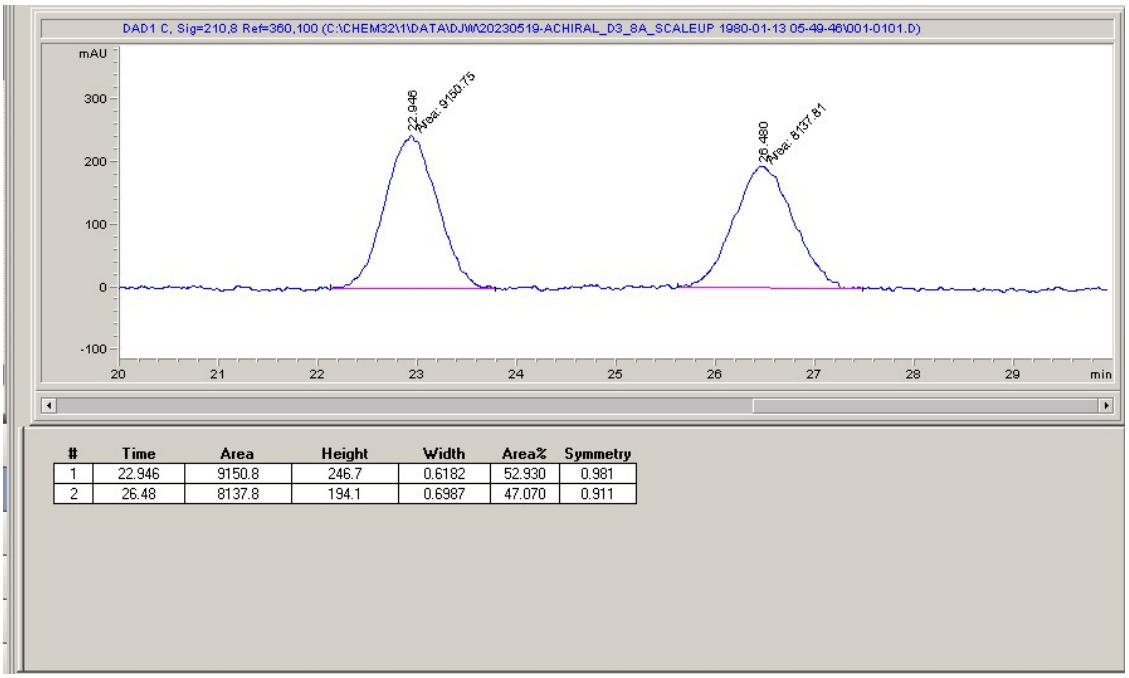
#	Time	Area	Height	Width	Area%	Symmetry
1	24.074	13278.8	323.3	0.6846	49.628	0.753
2	27.877	13477.6	273.3	0.8219	50.372	0.613

Enzymatic preparation of 2g with P411-LAS-5247, racemic
HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm



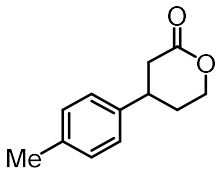
#	Time	Area	Height	Width	Area%	Symmetry
1	22.932	3629.1	99.8	0.6062	52.911	0.908
2	26.482	3229.8	79.4	0.678	47.089	0.908

Enzymatic preparation of 2g with P411-LAS-5247, racemic
HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 210 nm

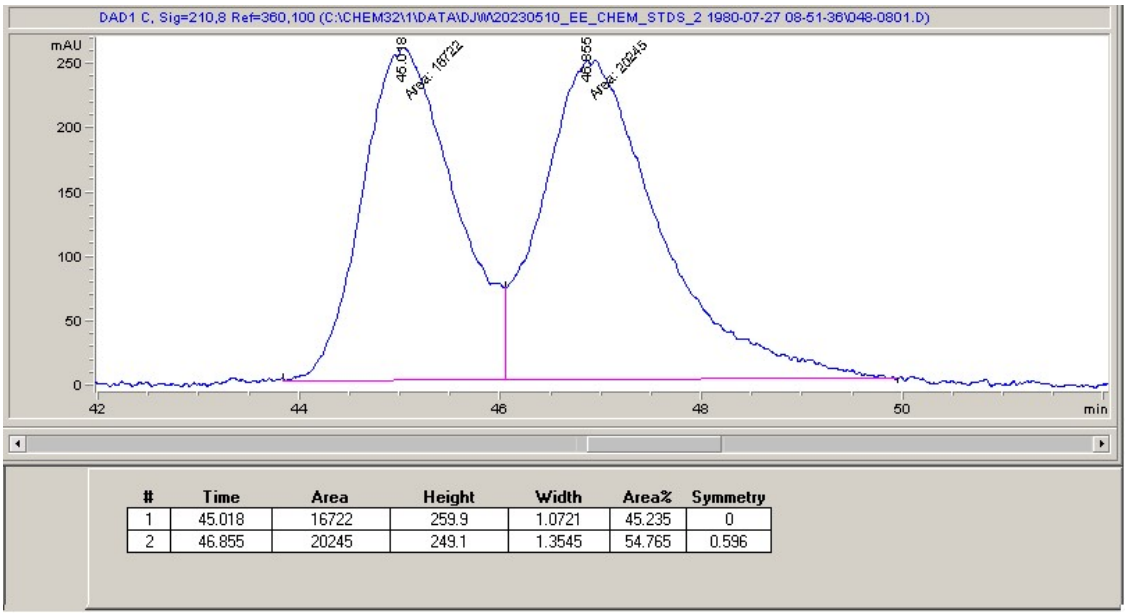


#	Time	Area	Height	Width	Area%	Symmetry
1	22.946	9150.8	246.7	0.6182	52.930	0.981
2	26.48	8137.8	194.1	0.6987	47.070	0.911

4-(*p*-Tolyl)tetrahydro-2*H*-pyran-2-one (4a)

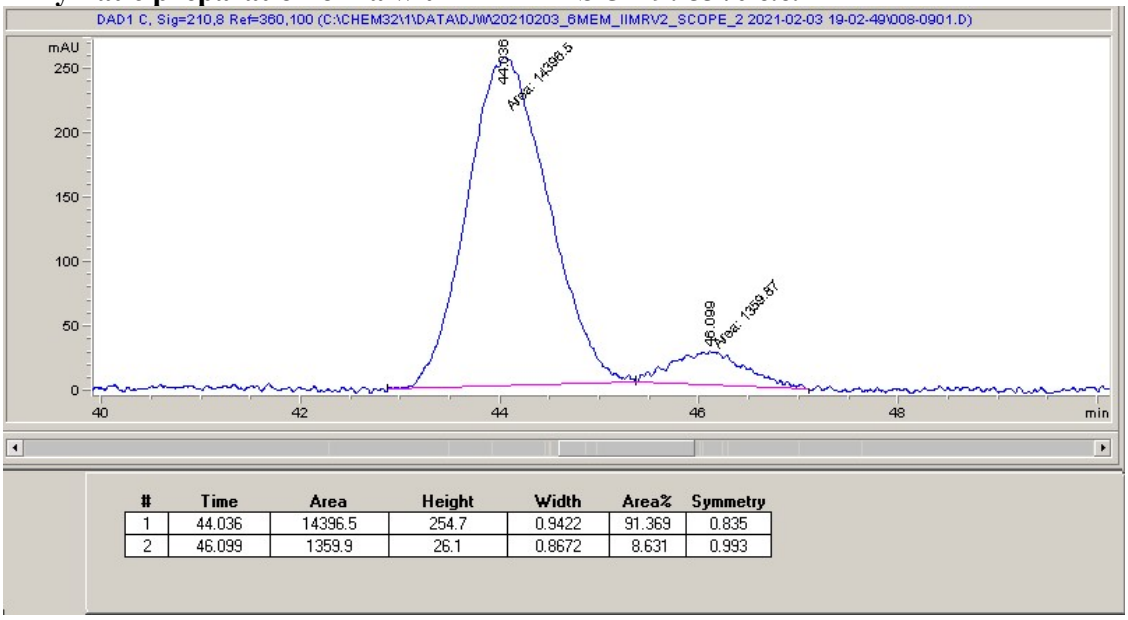


HPLC conditions: Chiralpak IC, 15% *i*-PrOH in hexane, 1.2 mL/min, 28 ºC, 220 nm



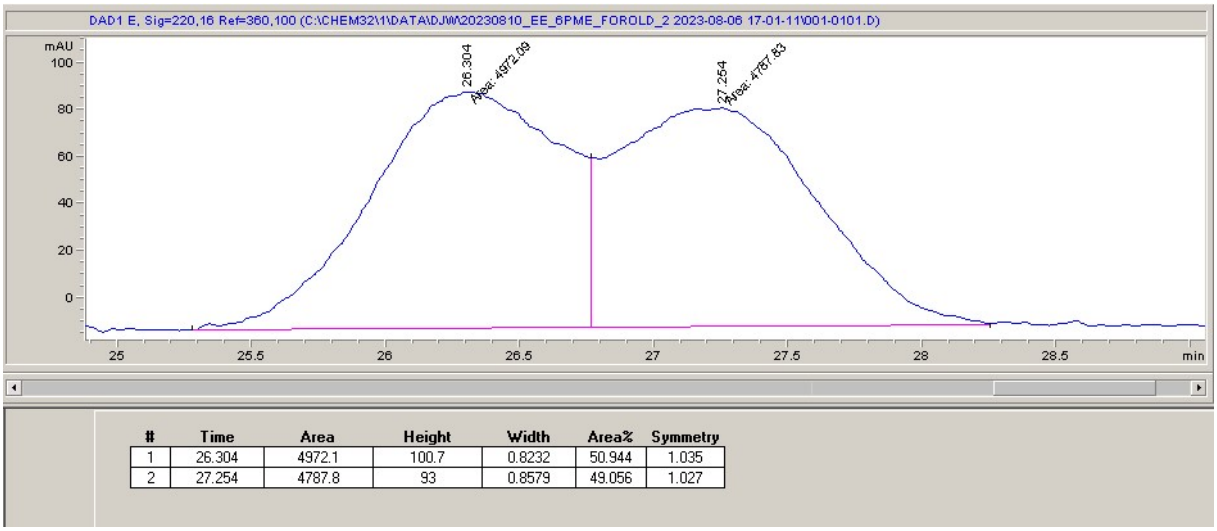
#	Time	Area	Height	Width	Area%	Symmetry
1	45.018	16722	259.9	1.0721	45.235	0
2	46.855	20245	249.1	1.3545	54.765	0.596

Enzymatic preparation of 4a with P411-LAS-5249: 83% e.e.



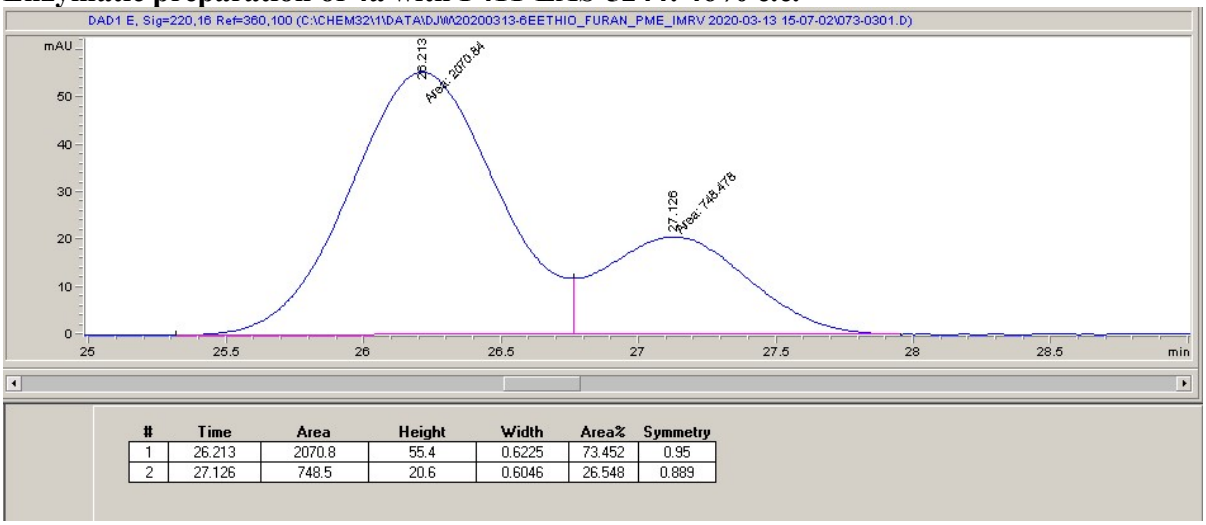
#	Time	Area	Height	Width	Area%	Symmetry
1	44.036	14396.5	254.7	0.9422	91.369	0.835
2	46.099	1359.9	26.1	0.8672	8.631	0.993

HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 28 ºC, 220 nm



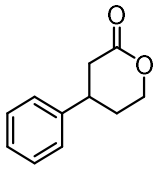
#	Time	Area	Height	Width	Area%	Symmetry
1	26.304	4972.1	100.7	0.8232	50.944	1.035
2	27.254	4787.8	93	0.8579	49.056	1.027

Enzymatic preparation of 4a with P411-LAS-5244: 46% e.e.

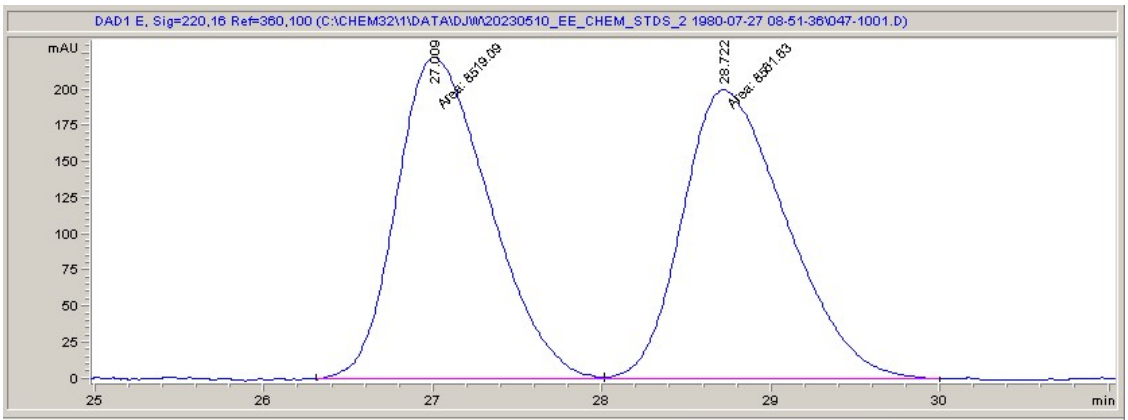


#	Time	Area	Height	Width	Area%	Symmetry
1	26.213	2070.8	55.4	0.6225	73.452	0.95
2	27.126	748.5	20.6	0.6046	26.548	0.889

4-Phenyltetrahydro-2*H*-pyran-2-one (4b)

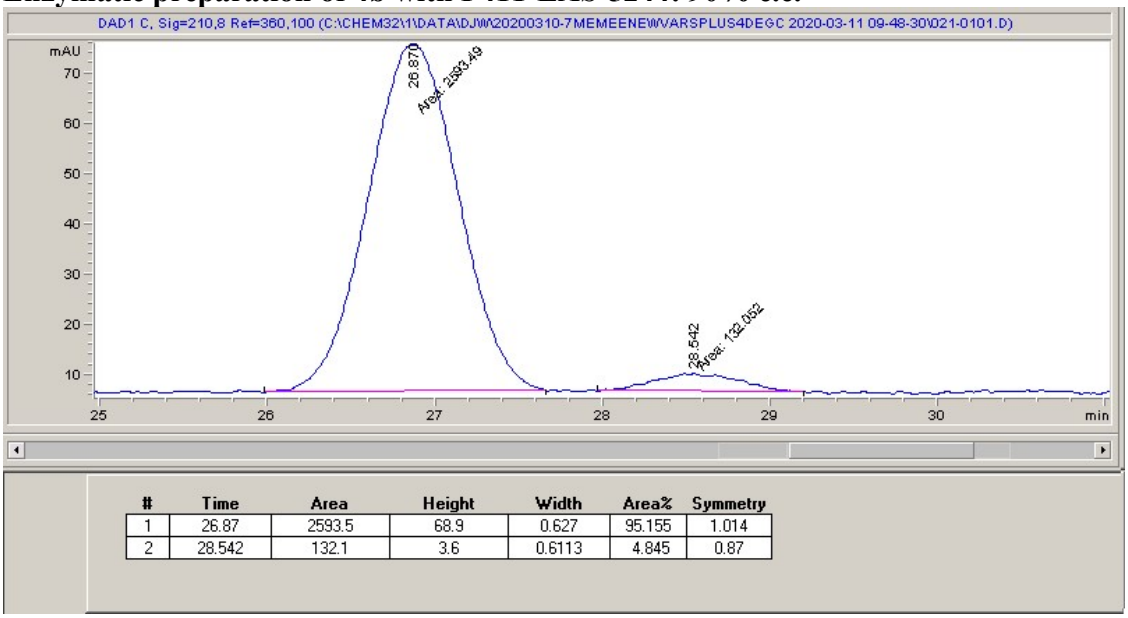


HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 28 ºC, 220 nm



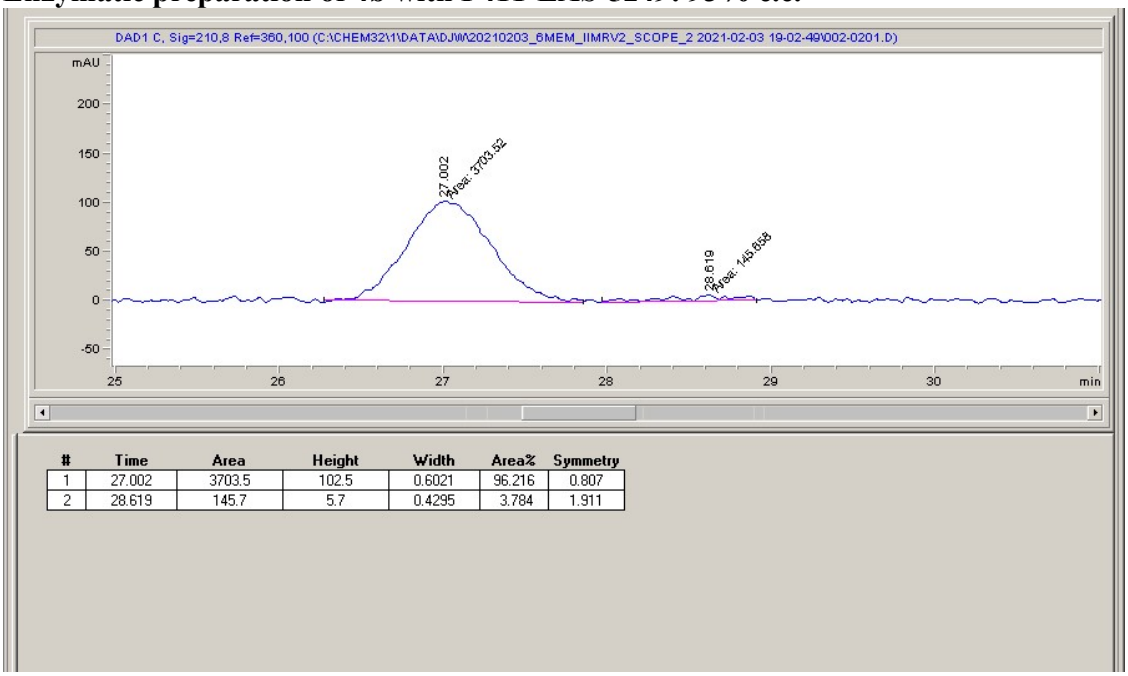
#	Time	Area	Height	Width	Area%	Symmetry
1	27.009	8519.1	222.4	0.6383	49.875	0.668
2	28.722	8561.6	200.4	0.7121	50.125	0.641

Enzymatic preparation of 4b with P411-LAS-5244: 90% e.e.



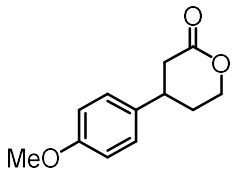
#	Time	Area	Height	Width	Area%	Symmetry
1	26.87	2593.5	68.9	0.627	95.155	1.014
2	28.542	132.1	3.6	0.6113	4.845	0.87

Enzymatic preparation of 4b with P411-LAS-5249: 93% e.e.

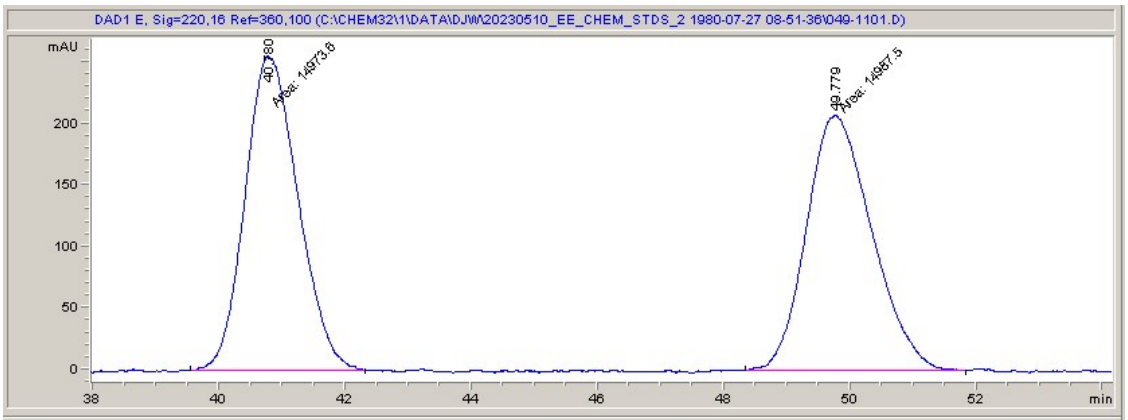


#	Time	Area	Height	Width	Area%	Symmetry
1	27.002	3703.5	102.5	0.6021	96.216	0.807
2	28.619	145.7	5.7	0.4295	3.784	1.911

4-(4-Methoxyphenyl)tetrahydro-2*H*-pyran-2-one (4c)

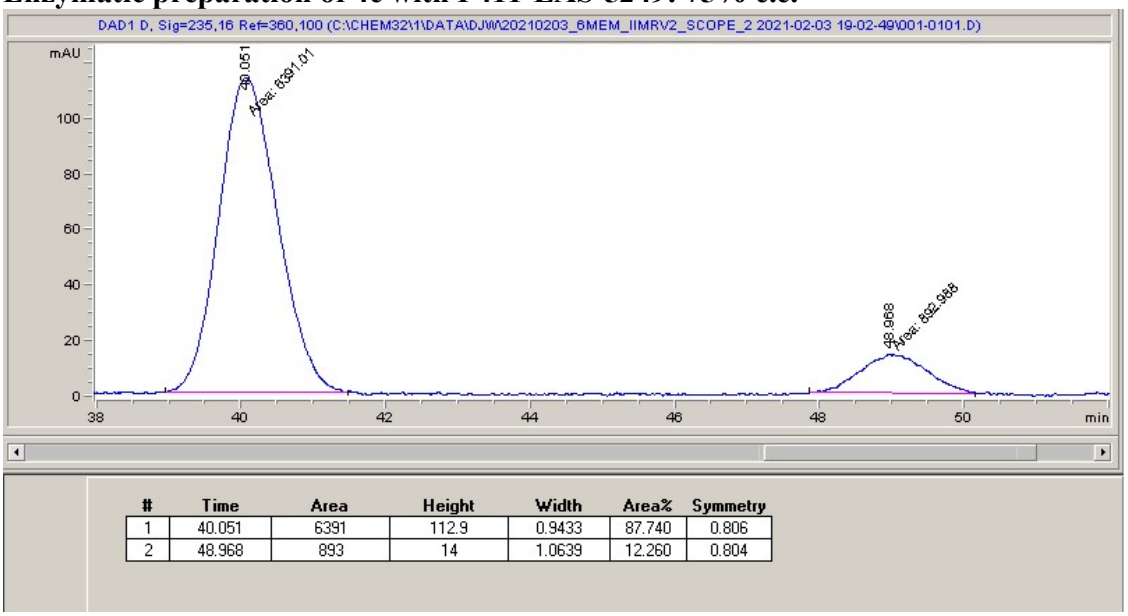


HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm



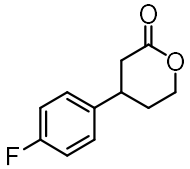
#	Time	Area	Height	Width	Area%	Symmetry
1	40.78	14973.6	255.9	0.9752	49.977	0.733
2	49.779	14987.5	207.7	1.2027	50.023	0.773

Enzymatic preparation of 4c with P411-LAS-5249: 75% e.e.



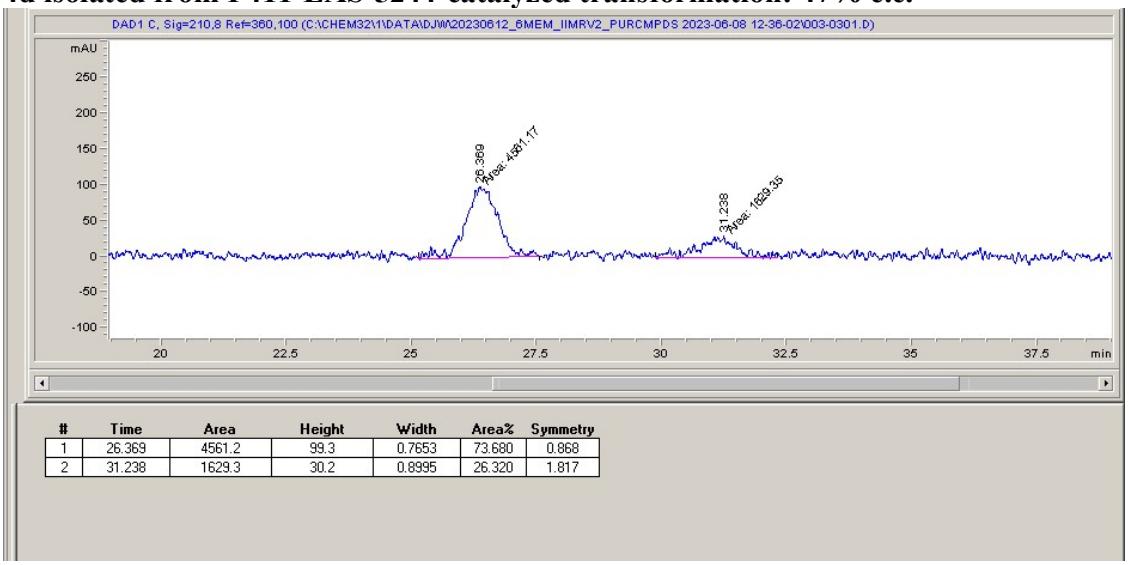
#	Time	Area	Height	Width	Area%	Symmetry
1	40.051	6391	112.9	0.9433	87.740	0.806
2	48.968	893	14	1.0639	12.260	0.804

4-(4-Fluorophenyl)tetrahydro-2*H*-pyran-2-one (4d)



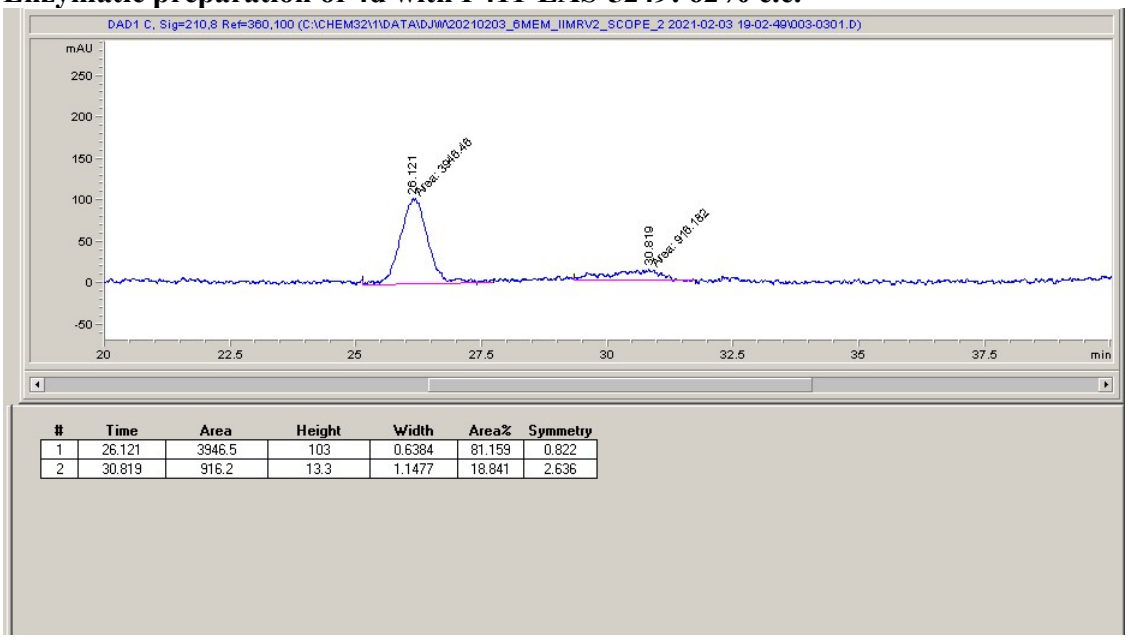
HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm

4d isolated from P411-LAS-5244-catalyzed transformation: 47% e.e.



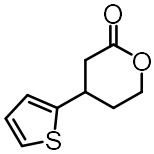
#	Time	Area	Height	Width	Area%	Symmetry
1	26.369	4561.2	99.3	0.7653	73.680	0.868
2	31.238	1629.3	30.2	0.8995	26.320	1.817

Enzymatic preparation of 4d with P411-LAS-5249: 62% e.e.



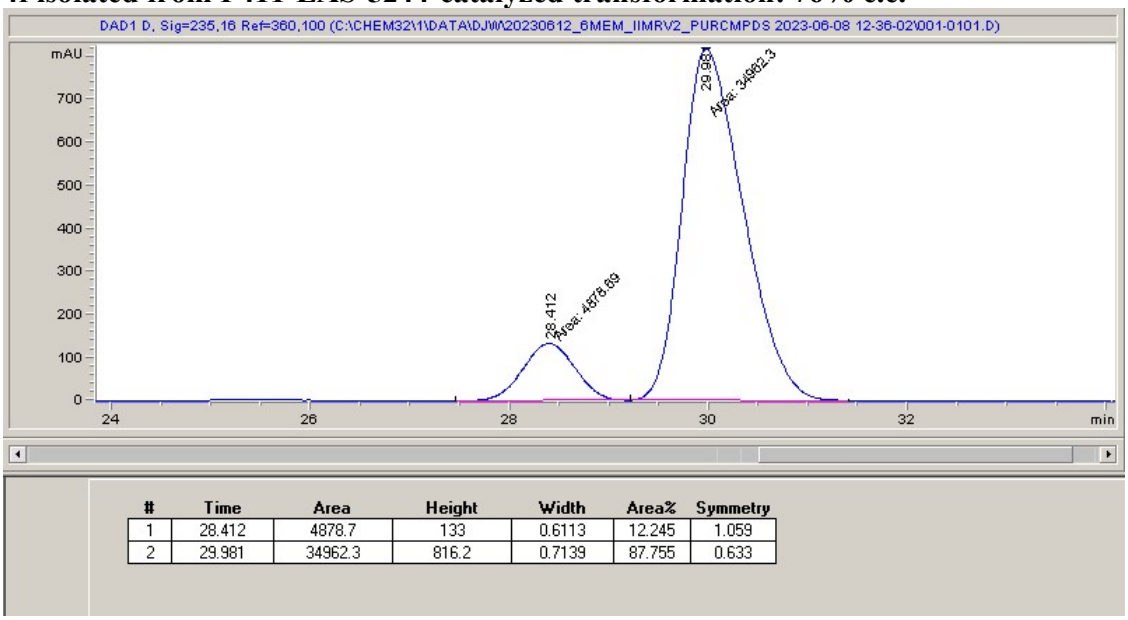
#	Time	Area	Height	Width	Area%	Symmetry
1	26.121	3946.5	103	0.6384	81.159	0.822
2	30.819	916.2	13.3	1.1477	18.841	2.636

4-(Thiophen-2-yl)tetrahydro-2H-pyran-2-one (4f)



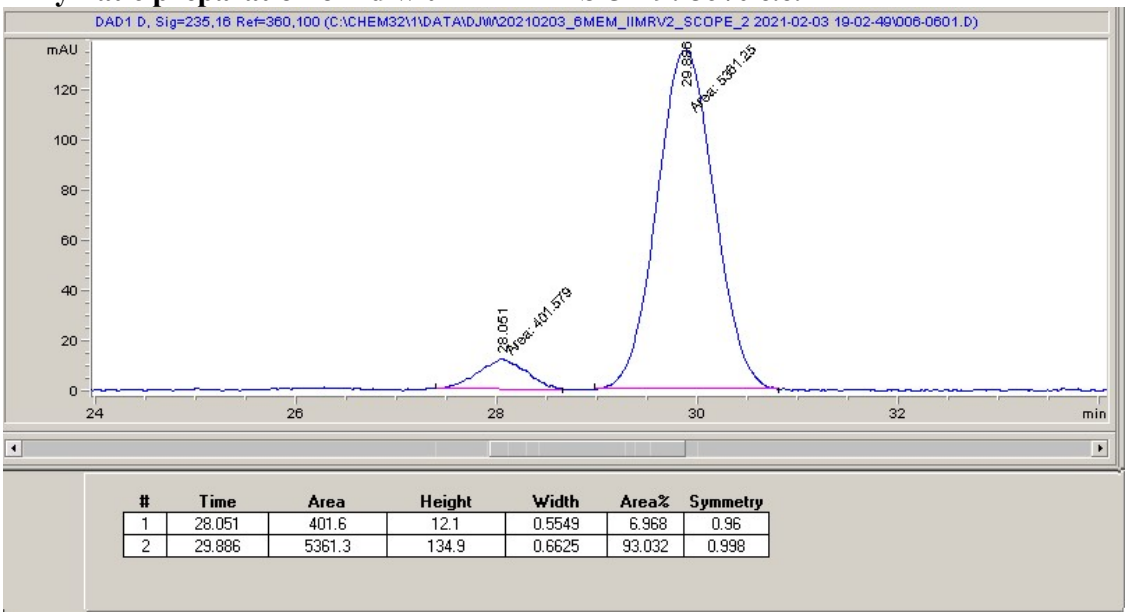
HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm

4f isolated from P411-LAS-5244-catalyzed transformation: 76% e.e.



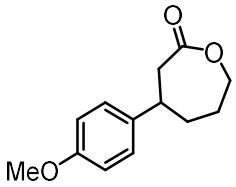
#	Time	Area	Height	Width	Area%	Symmetry
1	28.412	4878.7	133	0.6113	12.245	1.059
2	29.981	34962.3	816.2	0.7139	87.755	0.633

Enzymatic preparation of 4d with P411-LAS-5249: 86% e.e.



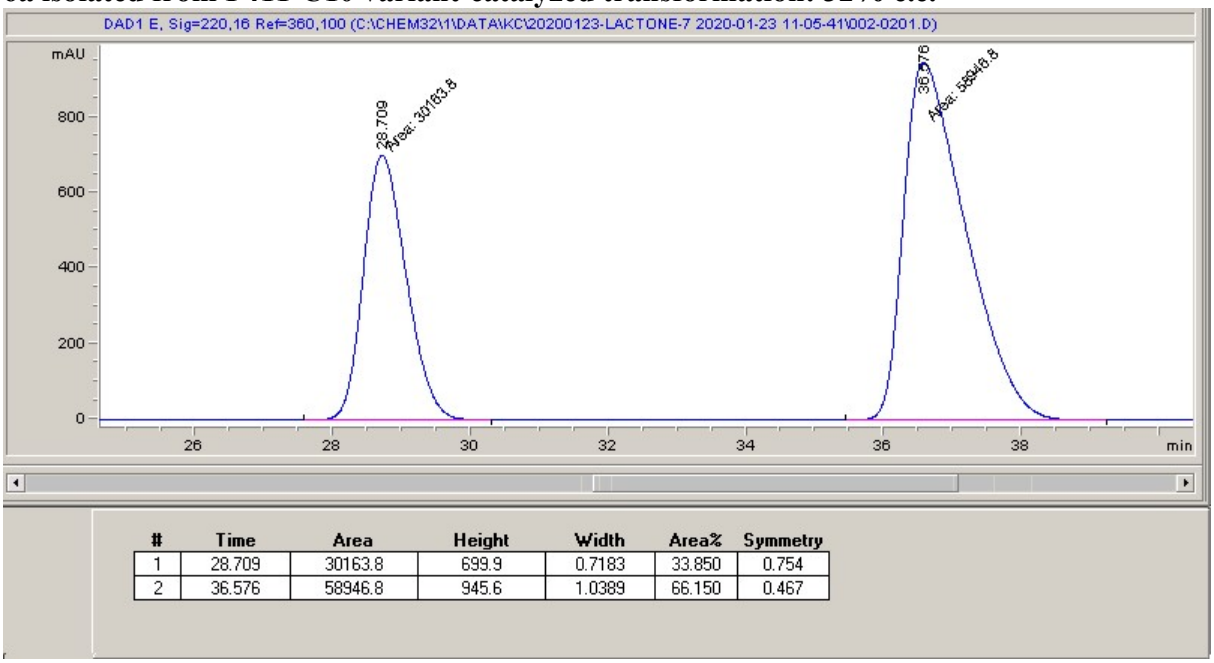
#	Time	Area	Height	Width	Area%	Symmetry
1	28.051	401.6	12.1	0.5549	6.968	0.96
2	29.886	5361.3	134.9	0.6625	93.032	0.998

4-(4-Methoxyphenyl)oxepan-2-one (6a)



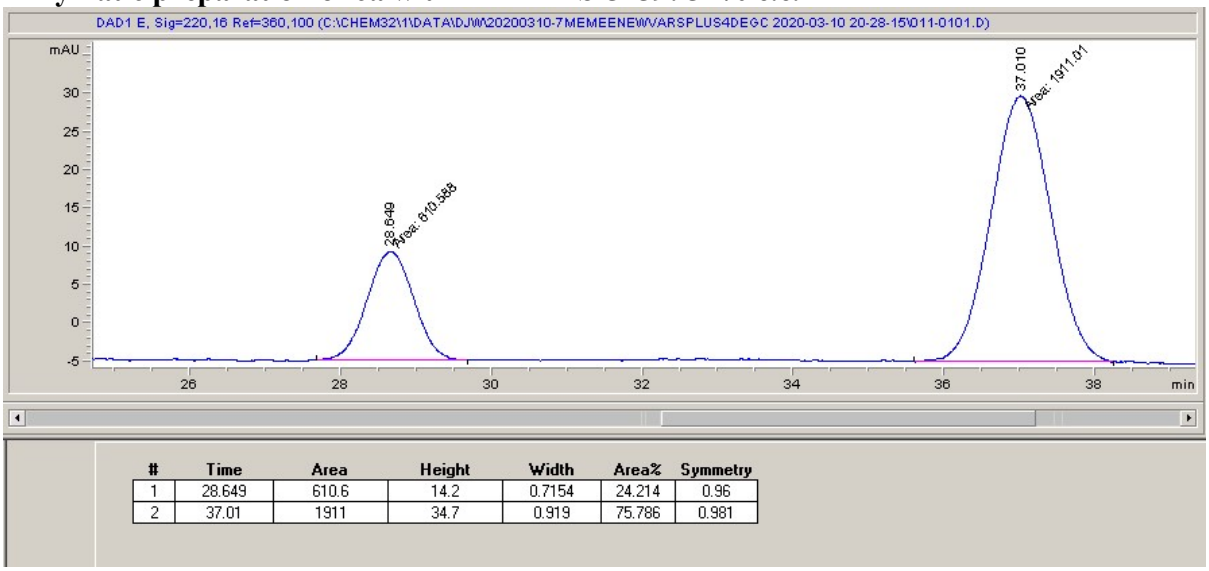
HPLC conditions: Chiralpak IC, 25% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm

6a isolated from P411-C10 variant-catalyzed transformation: 32% e.e.



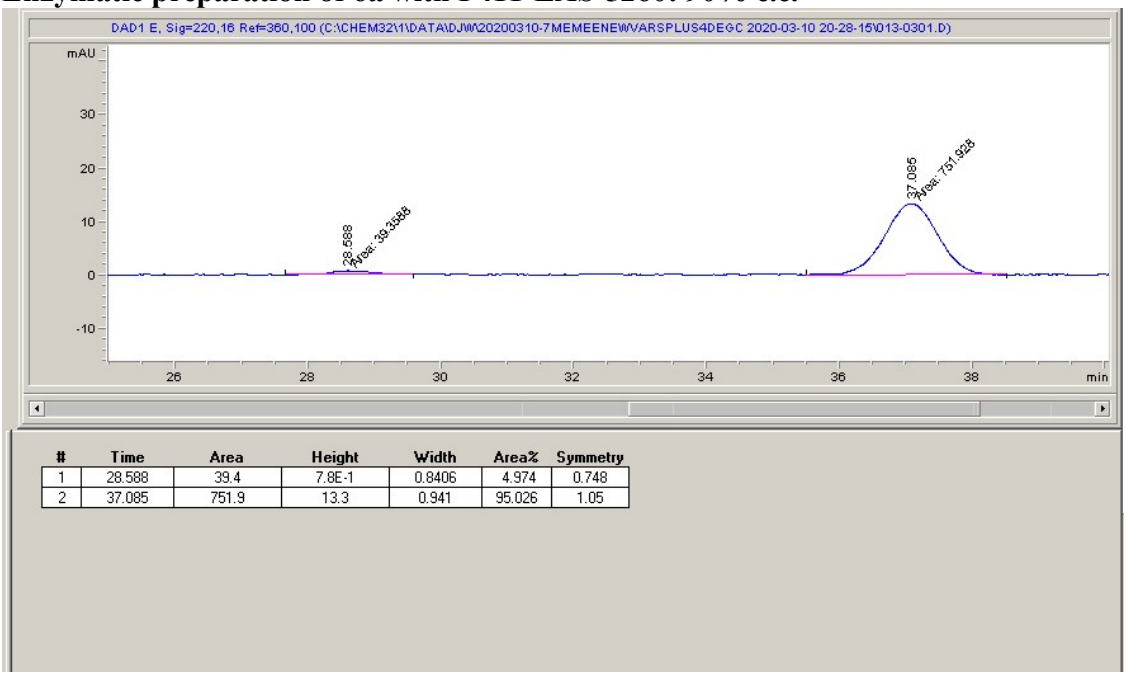
#	Time	Area	Height	Width	Area%	Symmetry
1	28.709	30163.8	699.9	0.7183	33.850	0.754
2	36.576	58946.8	945.6	1.0389	66.150	0.467

Enzymatic preparation of 6a with P411-LAS-5259: 51% e.e.



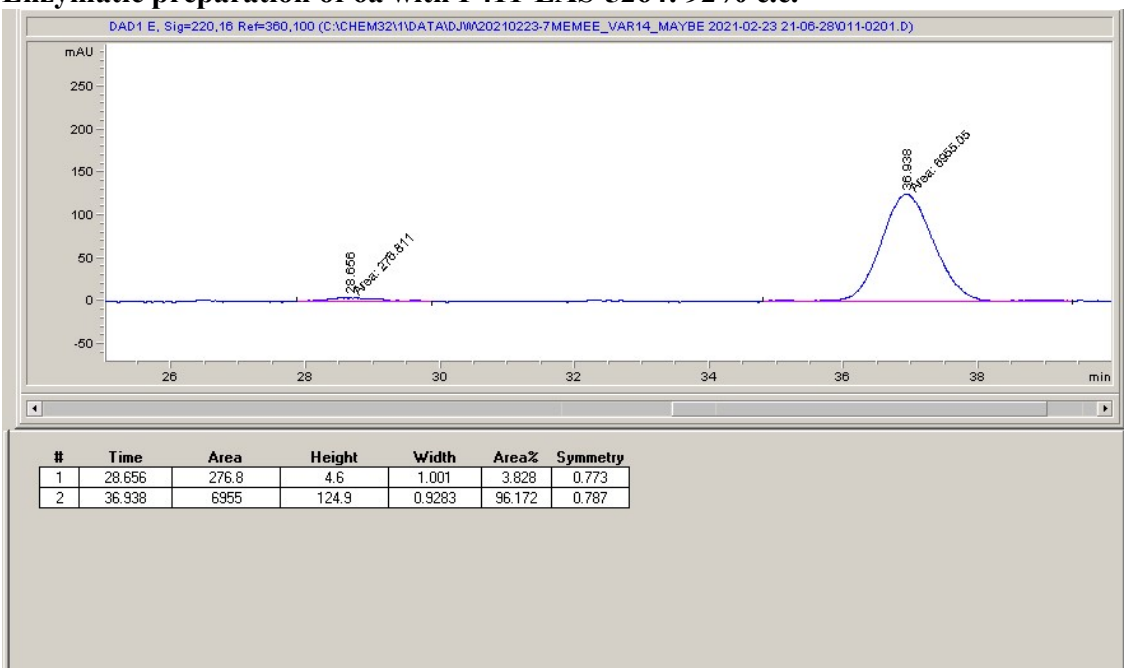
#	Time	Area	Height	Width	Area%	Symmetry
1	28.649	610.6	14.2	0.7154	24.214	0.96
2	37.01	1911	34.7	0.919	75.786	0.981

Enzymatic preparation of 6a with P411-LAS-5260: 90% e.e.



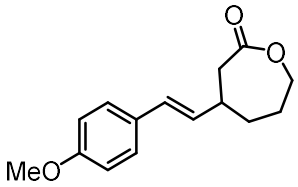
#	Time	Area	Height	Width	Area%	Symmetry
1	28.588	39.4	7.8E-1	0.8406	4.974	0.748
2	37.085	751.9	13.3	0.941	95.026	1.05

Enzymatic preparation of 6a with P411-LAS-5264: 92% e.e.



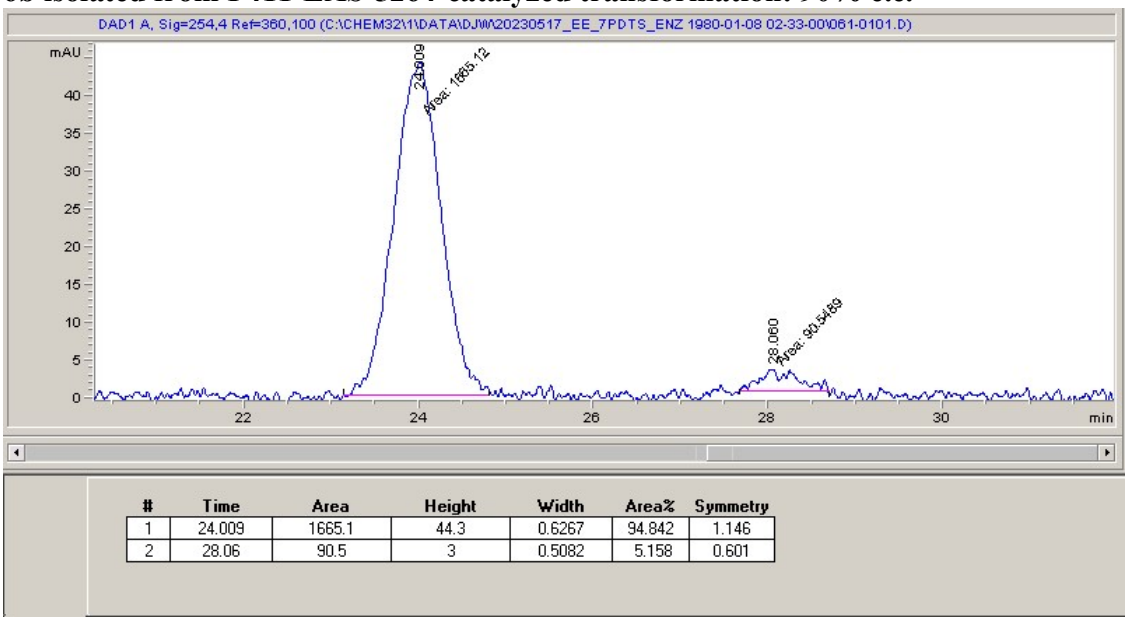
#	Time	Area	Height	Width	Area%	Symmetry
1	28.656	276.8	4.6	1.001	3.828	0.773
2	36.938	6955	124.9	0.9283	96.172	0.787

(*E*)-4-(4-Methoxystyryl)oxepan-2-one (6b)



HPLC conditions: Chiralpak IC, 35% *i*-PrOH in hexane, 1.2 mL/min, 32 ºC, 220 nm

6b isolated from P411-LAS-5264-catalyzed transformation: 90% e.e.



#	Time	Area	Height	Width	Area%	Symmetry
1	24.009	1665.1	44.3	0.6267	94.842	1.146
2	28.06	90.5	3	0.5082	5.158	0.601

8. X-ray Crystallography

Low-temperature diffraction data (ϕ-and ω-scans) were collected on a Bruker AXS D8 VENTURE KAPPA or Bruker APEX-II diffractometer coupled to a PHOTON II CPAD detector with Cu K_α radiation (λ = 1.54178 Å) from an IμS micro-source for the structure of compounds. The structure was solved by direct methods using SHELXS[20] and refined against F^2 on all data by full-matrix least squares with SHELXL-2019[21] using established refinement techniques.[22] All non-hydrogen atoms were refined anisotropically. Unless otherwise noted, all hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U value of the atoms they are linked to (1.5 times for methyl groups).

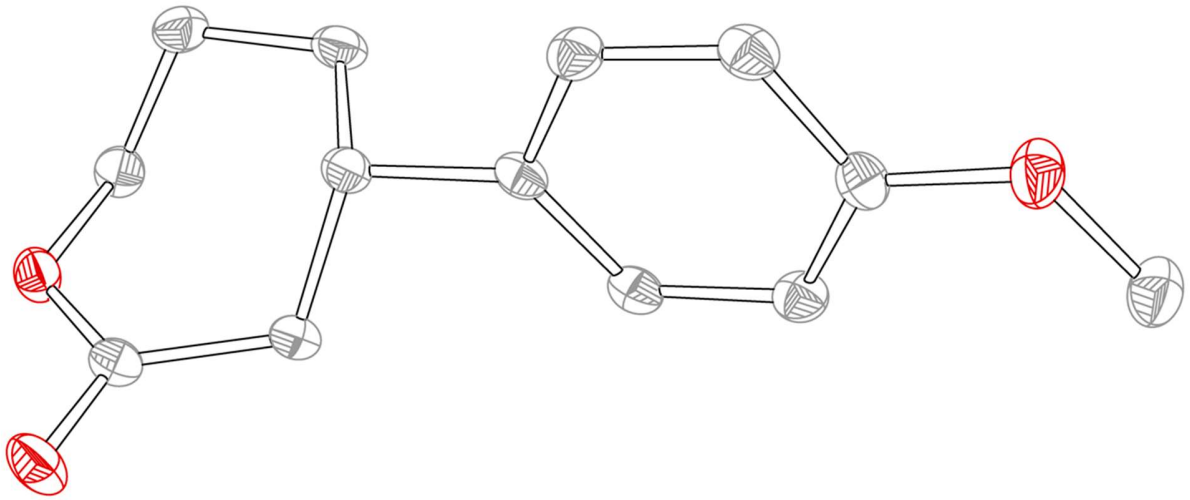


Figure S3. Displacement ellipsoid plot for **6a** plotted at 50% probability. Single crystals were obtained from slow evaporation of **6a** dissolved in $CHCl_3$. Compound **6a** crystallizes in the orthorhombic space group $P2_12_12_1$ with one molecule in the asymmetric unit.

Table S6. X-ray experimental details of **6a** (CCDC 2288858).

Crystal data	
Chemical formula	$C_{13}H_{16}O_3$
M_r	220.26
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	100
a, b, c (Å)	6.4683 (7), 9.5514 (11), 18.433 (2)
V (Å^3)	1138.8 (2)
Z	4
Radiation type	Cu $K\alpha$
μ (mm^{-1})	0.74
Crystal size (mm)	0.25 × 0.2 × 0.1
Data collection	
Diffractometer	Bruker *APEX*-II CCD
Absorption correction	
T_{min}, T_{max}	0.662, 0.754
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	14831, 2298, 2272
R_{int}	0.036
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.625
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.026, 0.066, 1.05
No. of reflections	2298
No. of parameters	146
H-atom treatment	H-atom parameters constrained
Γ_{max}, Γ_{min} (e Å^{-3})	0.14, -0.17
Absolute structure	Flack x determined using 932 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	-0.09 (6)

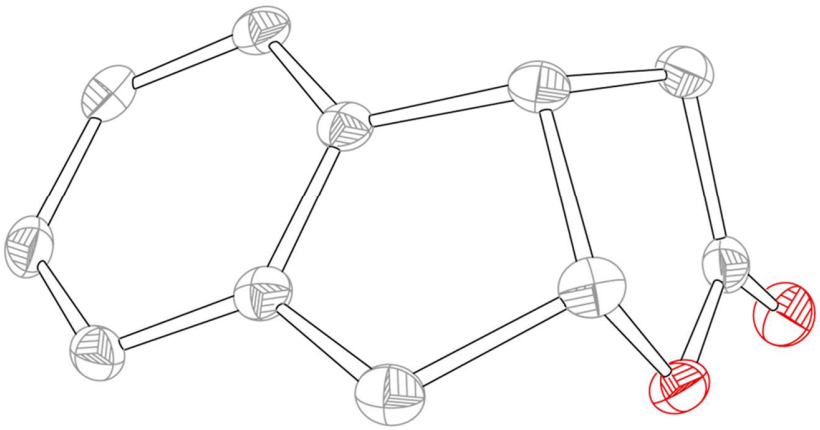


Figure S4. Displacement ellipsoid plot for **8a** plotted at 50% probability. Single crystals were obtained from evaporation of hexane into **8a** dissolved in ethyl acetate. Compound **8a** crystallizes in the orthorhombic space group $P2_12_12_1$ with one molecule in the asymmetric unit.

Table S7. X-ray experimental details of **8a** (CCDC 2287785).

Crystal data	
Chemical formula	$C_{11}H_{10}O_2$
M_r	179.19
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	100
a, b, c (Å)	4.7240 (4), 12.2962 (14), 14.3829 (12)
V (Å^3)	835.46 (14)
Z	4
Radiation type	Cu $K\alpha$
μ (mm^{-1})	0.768
Crystal size (mm)	0.15 × 0.1 × 0.1
Data collection	
Diffractometer	Bruker D8 VENTURE Kappa Duo PHOTON II CPAD
Absorption correction	Multi-scan SADABS2016/2 (Sheldrick, 2014)
T_{min}, T_{max}	0.6762, 0.7538
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	15040, 1711, 1670
R_{int}	0.0367
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.625
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.0261, 0.0668, 1.079
No. of reflections	1711
No. of parameters	118
H-atom treatment	H-atom parameters constrained
Γ_{max}, Γ_{min} (e Å^{-3})	0.15, -0.15
Absolute structure	Flack x determined using 667 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	0.05 (7)

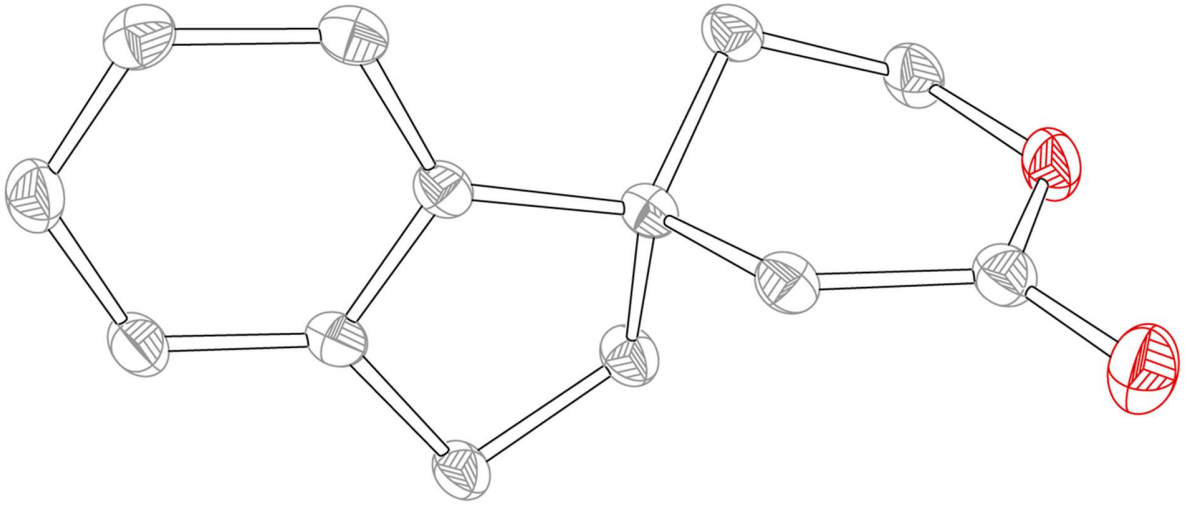


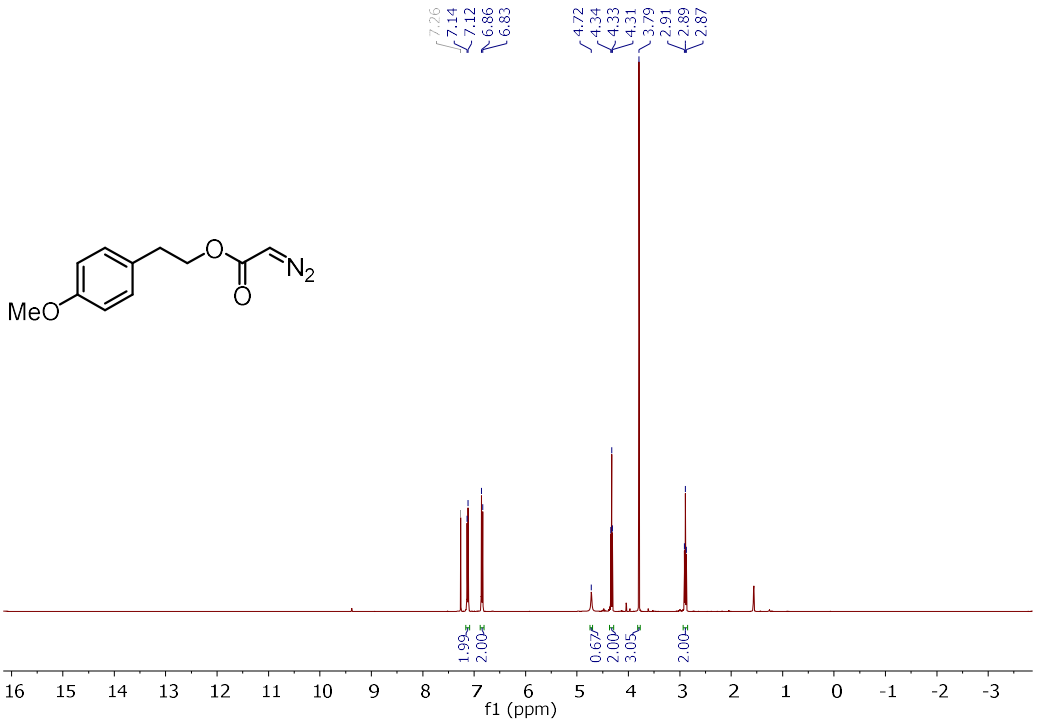
Figure S5. Displacement ellipsoid plot for **8f** plotted at 50% probability. Single crystals were obtained from evaporation of hexane into **8f** dissolved in ethyl acetate. Compound **8f** crystallizes in the monoclinic space group $P2_1/c$ with one molecule in the asymmetric unit.

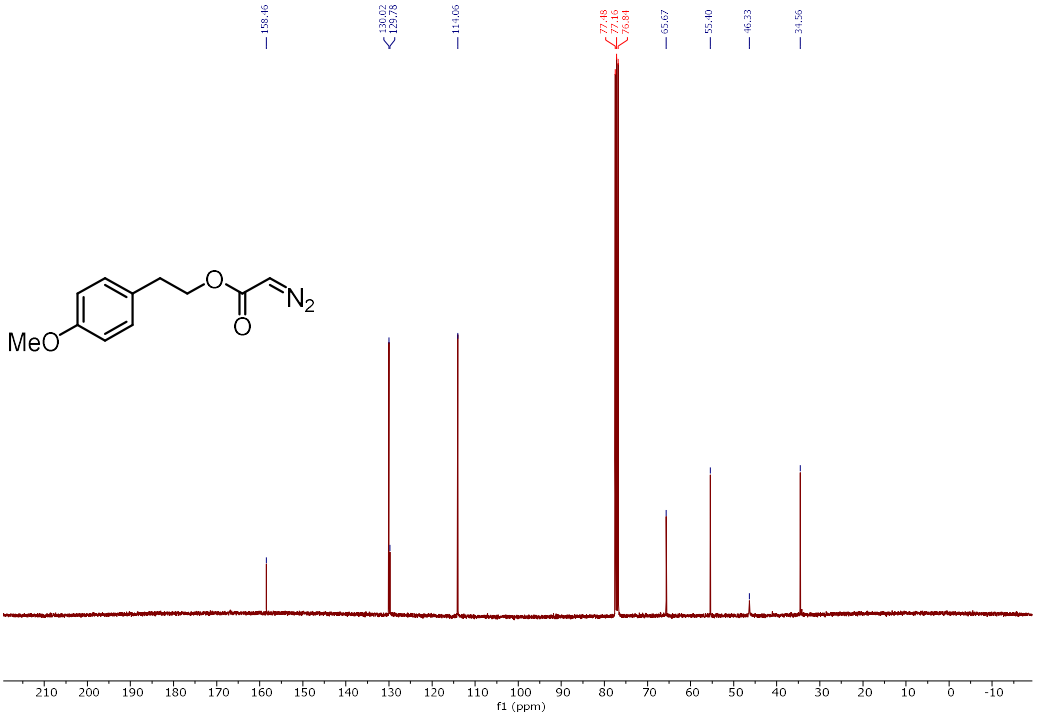
Table S8. X-ray experimental details of **8f** (CCDC 2287784).

Crystal data	
Chemical formula	$C_{13}H_{14}O_2$
M_r	202.24
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	100
a, b, c (Å)	13.0013 (8), 6.6891 (6), 12.8888 (10)
α, β, γ (°)	90, 113.787 (3), 90
V (Å^3)	1025.68 (14)
Z	4
Radiation type	Cu $K\alpha$
μ (mm^{-1})	0.696
Crystal size (mm)	0.2 × 0.15 × 0.05
Data collection	
Diffractometer	Bruker D8 VENTURE Kappa Duo PHOTON II CPAD
Absorption correction	Multi-scan SADABS2016/2 (Sheldrick, 2014)
T_{min}, T_{max}	0.5155, 0.7538
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	13396, 2098, 1884
R_{int}	0.0632
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.625
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.0449, 0.1248, 1.07
No. of reflections	2098
No. of parameters	136
H-atom treatment	H-atom parameters constrained
Γ_{max}, Γ_{min} (e Å^{-3})	0.41, -0.23

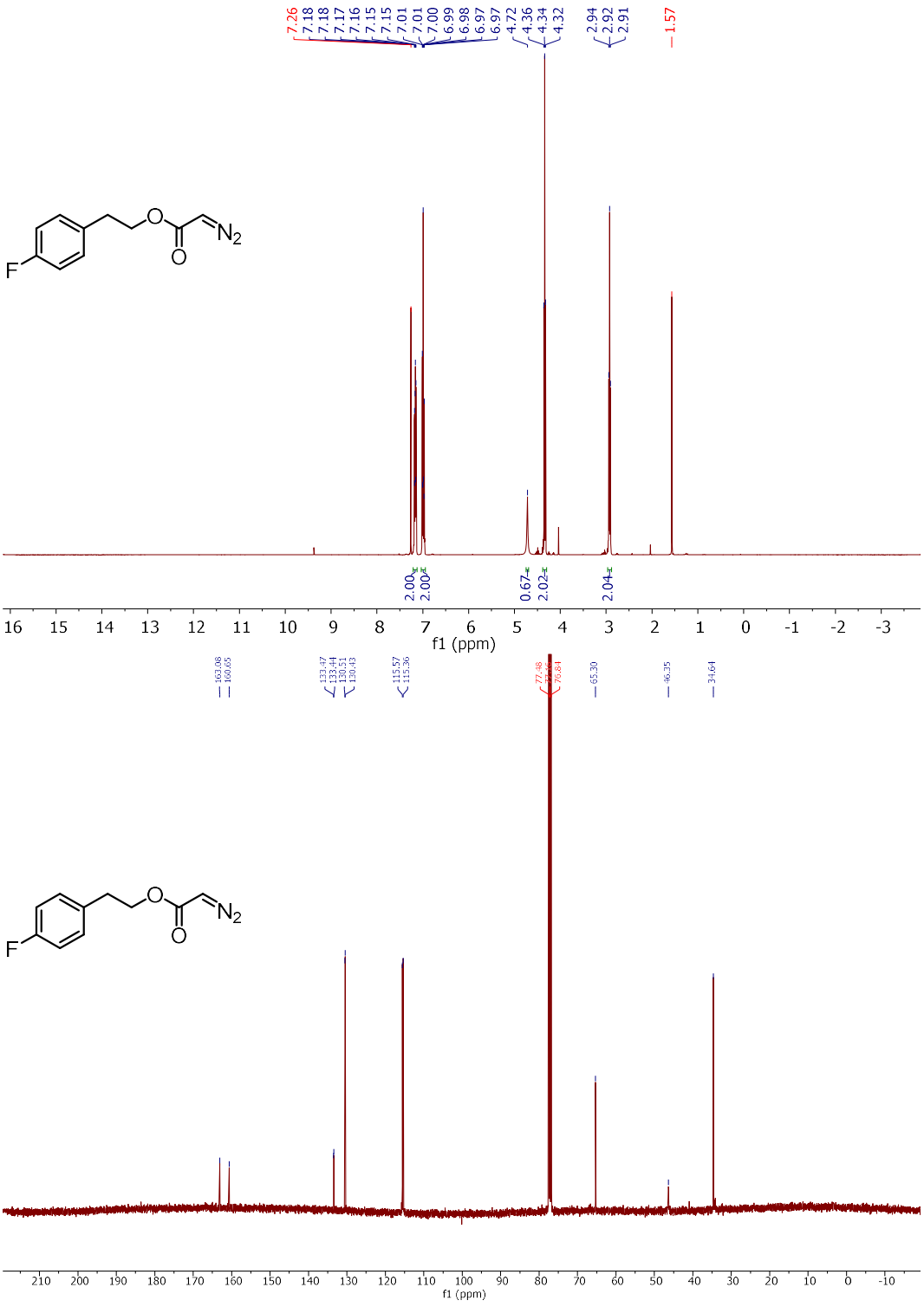
9. NMR Spectra

4-methoxyphenethyl 2-diazoacetate (1c)

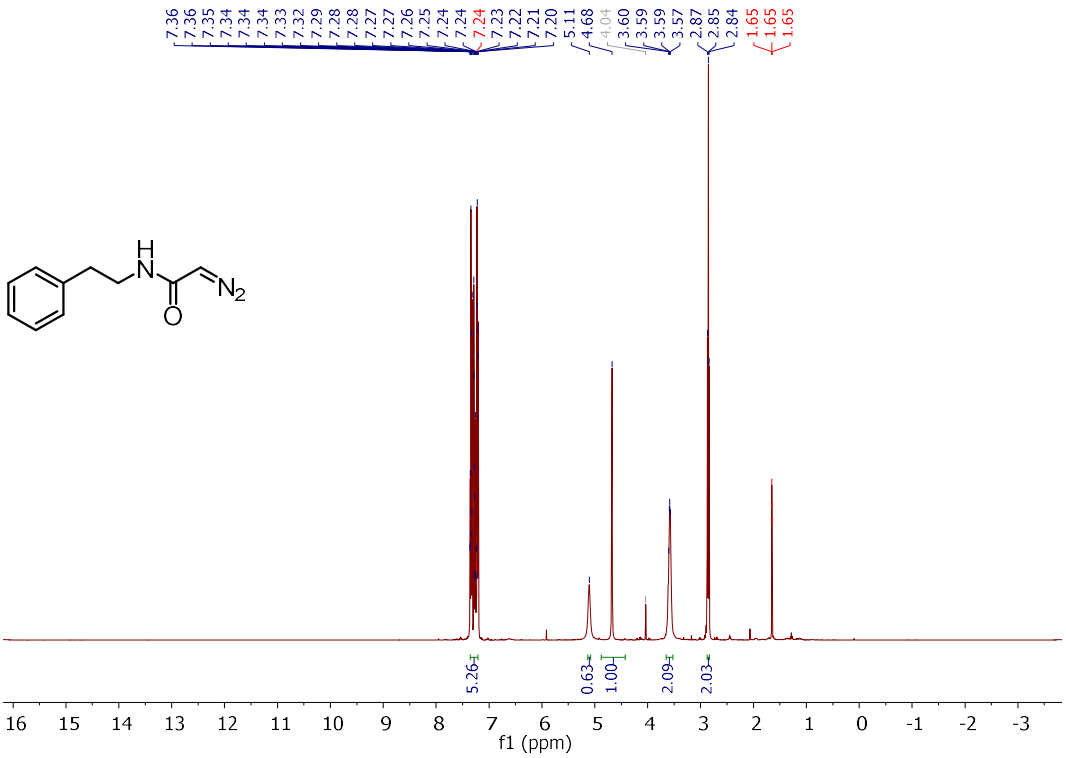


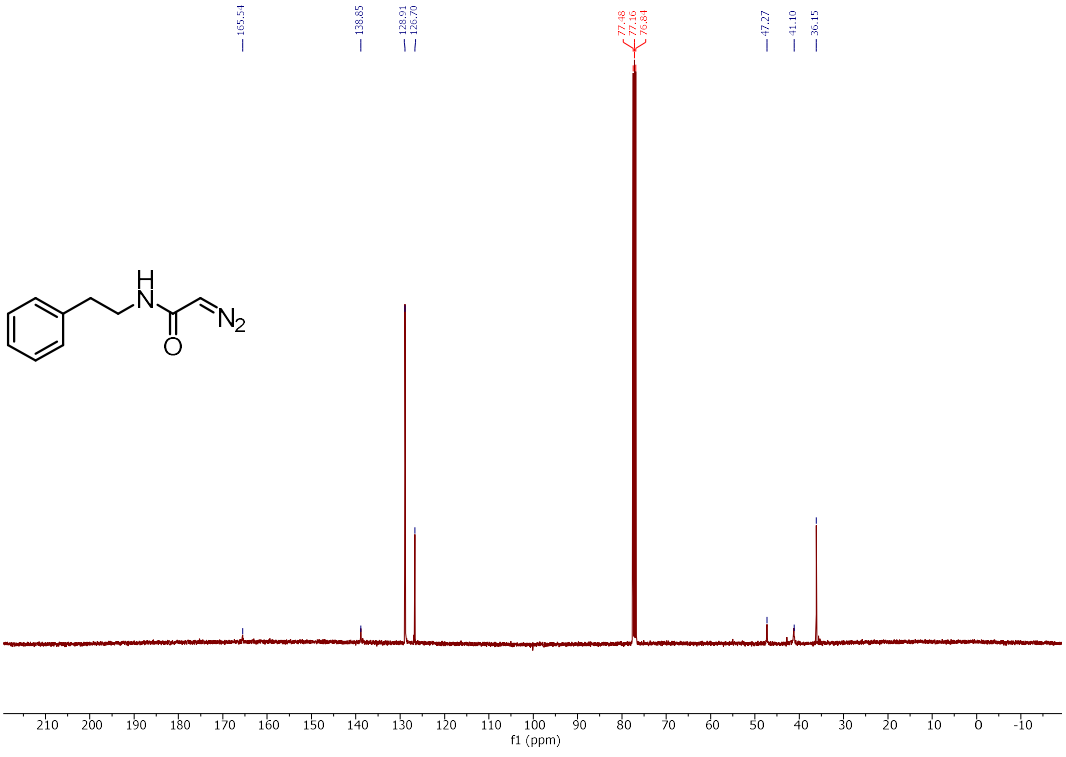


4-fluorophenethyl 2-diazoacetate (1d)

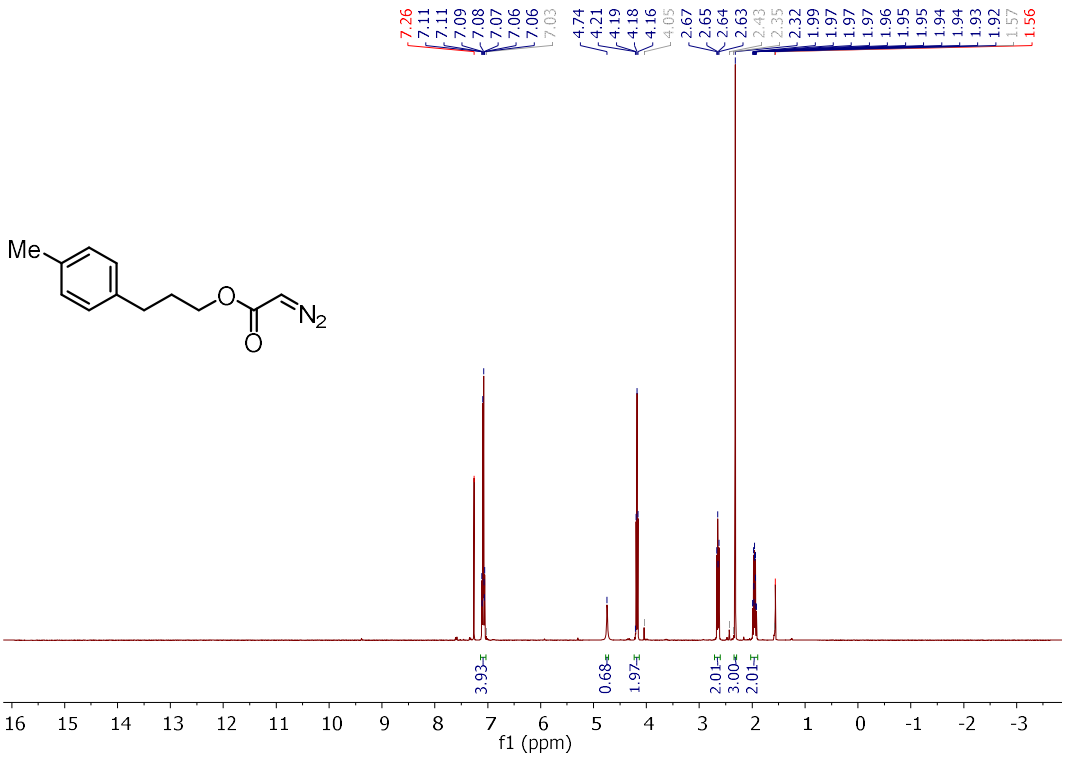


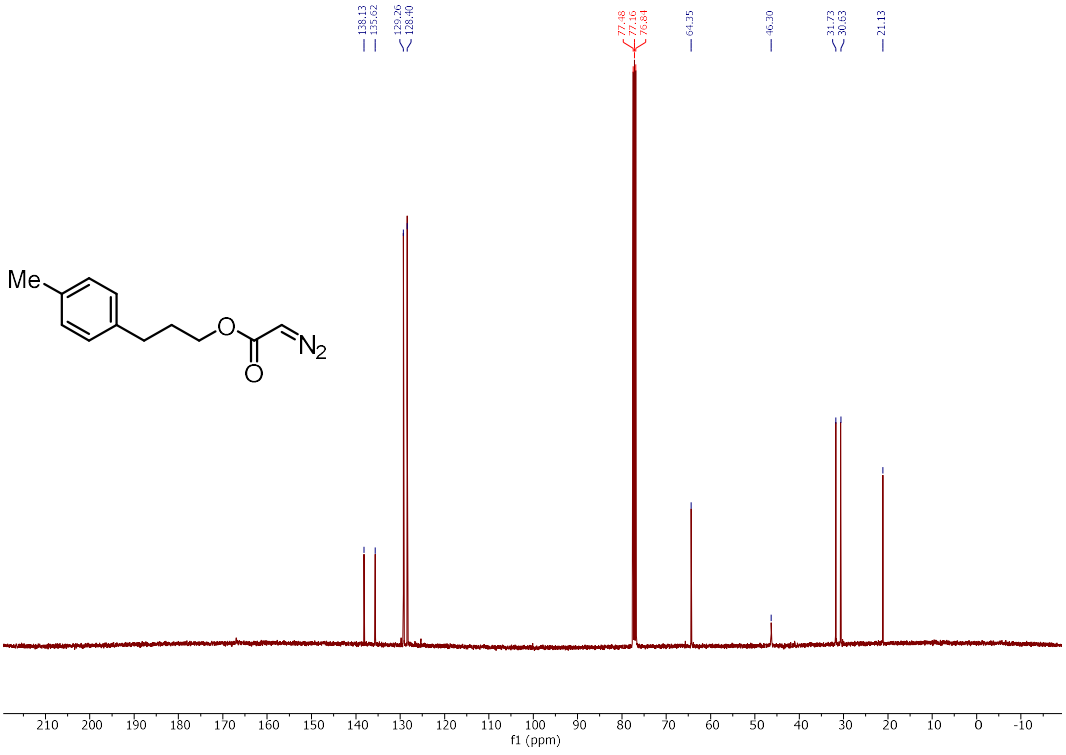
2-diazo-N-phenethylacetamide (1g)



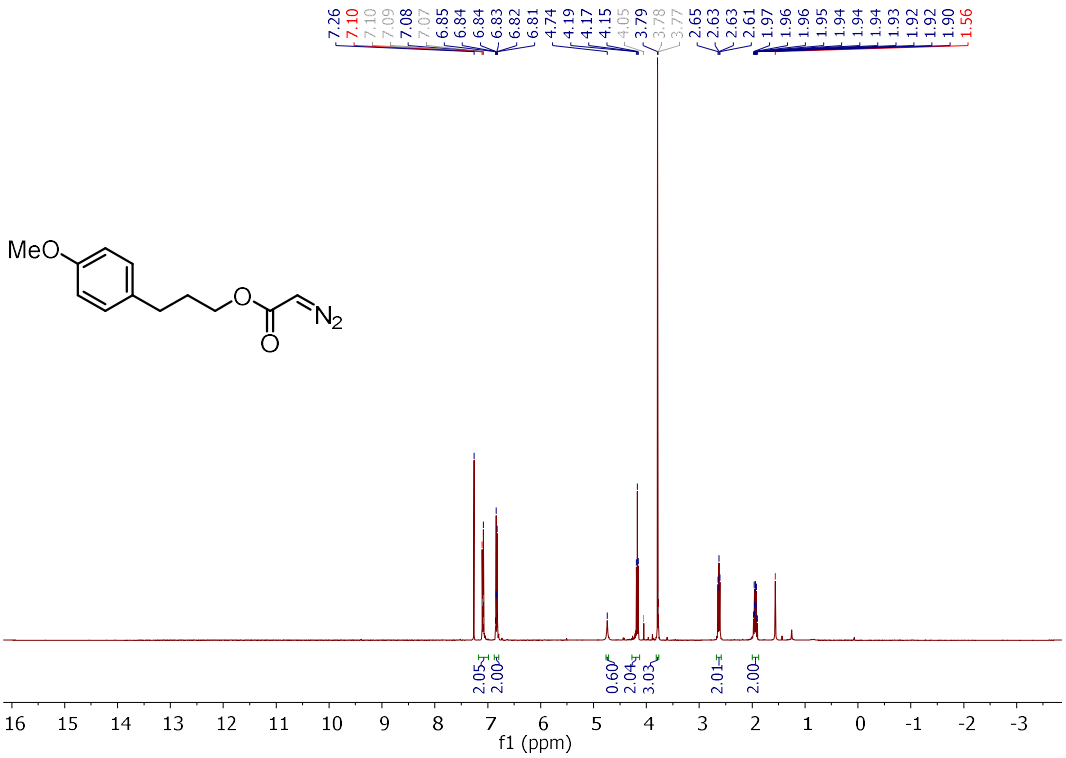


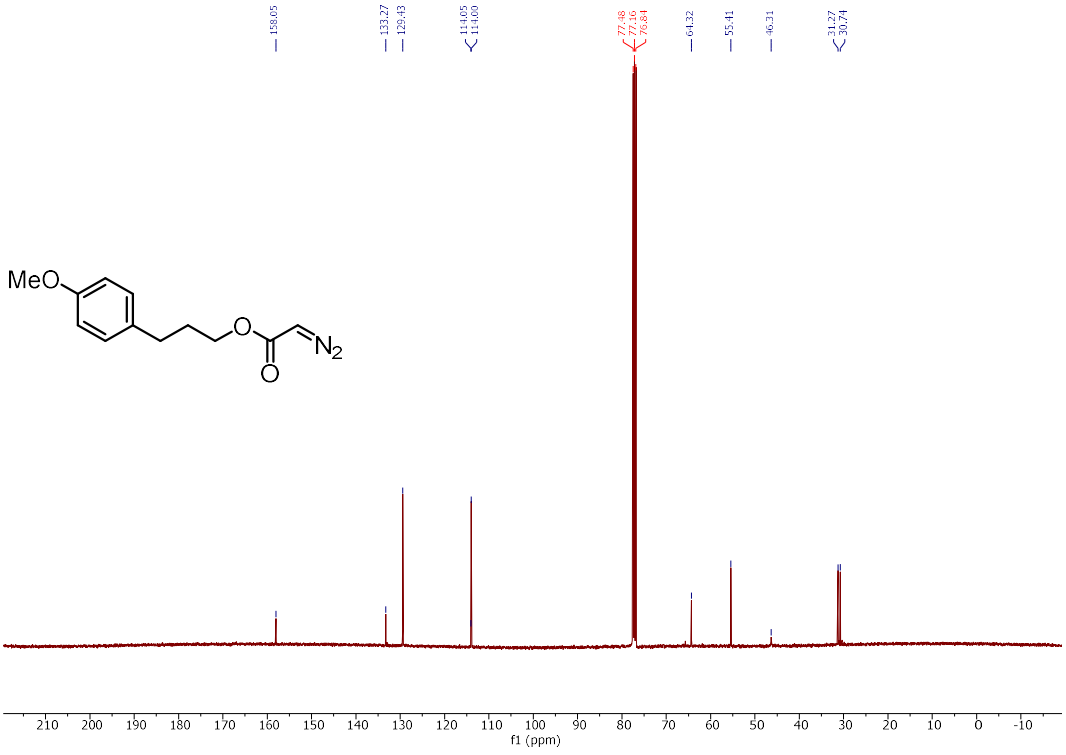
3-(*p*-tolyl)propyl 2-diazoacetate (3a)



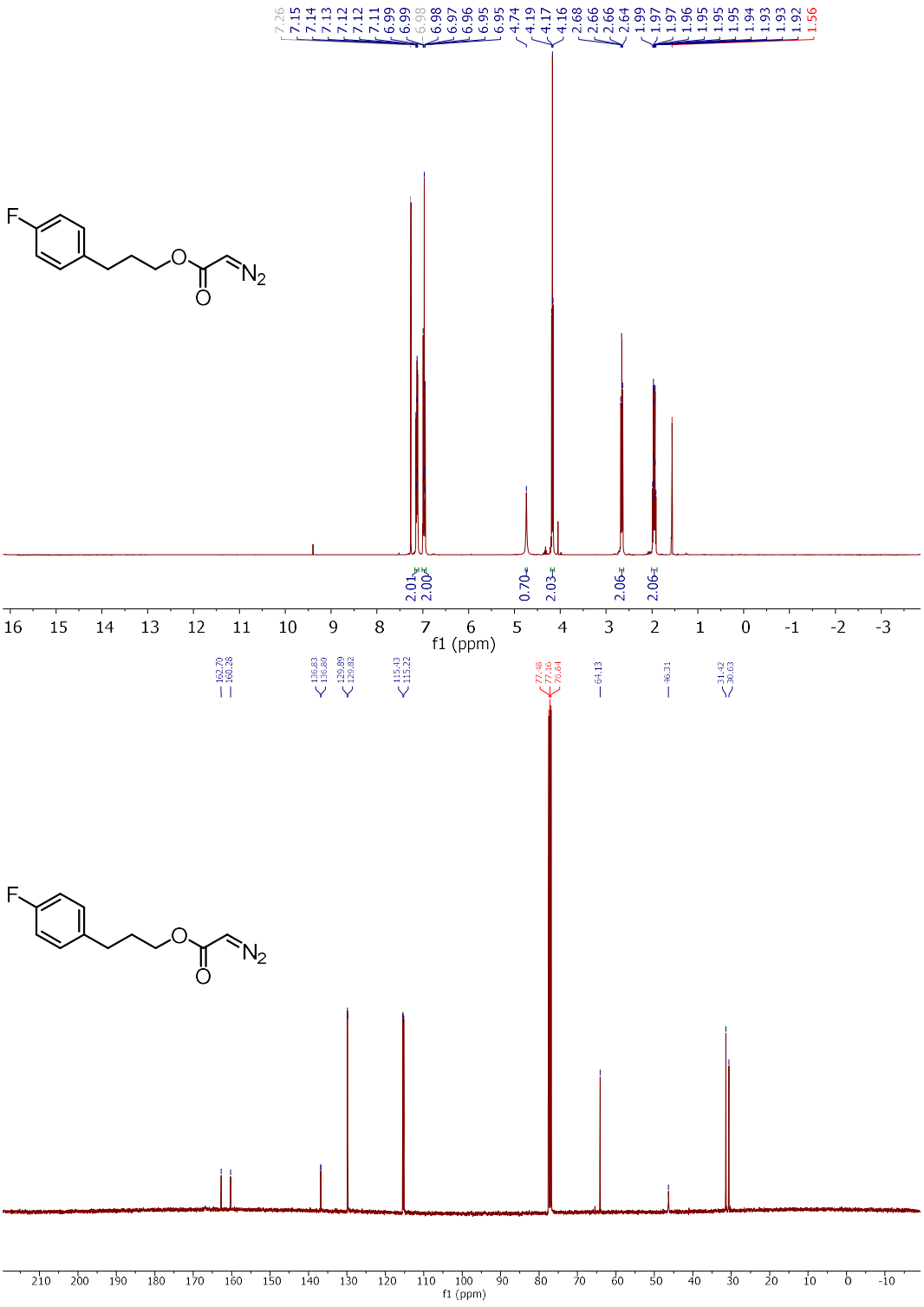


3-(4-methoxyphenyl)propyl 2-diazoacetate (3c)

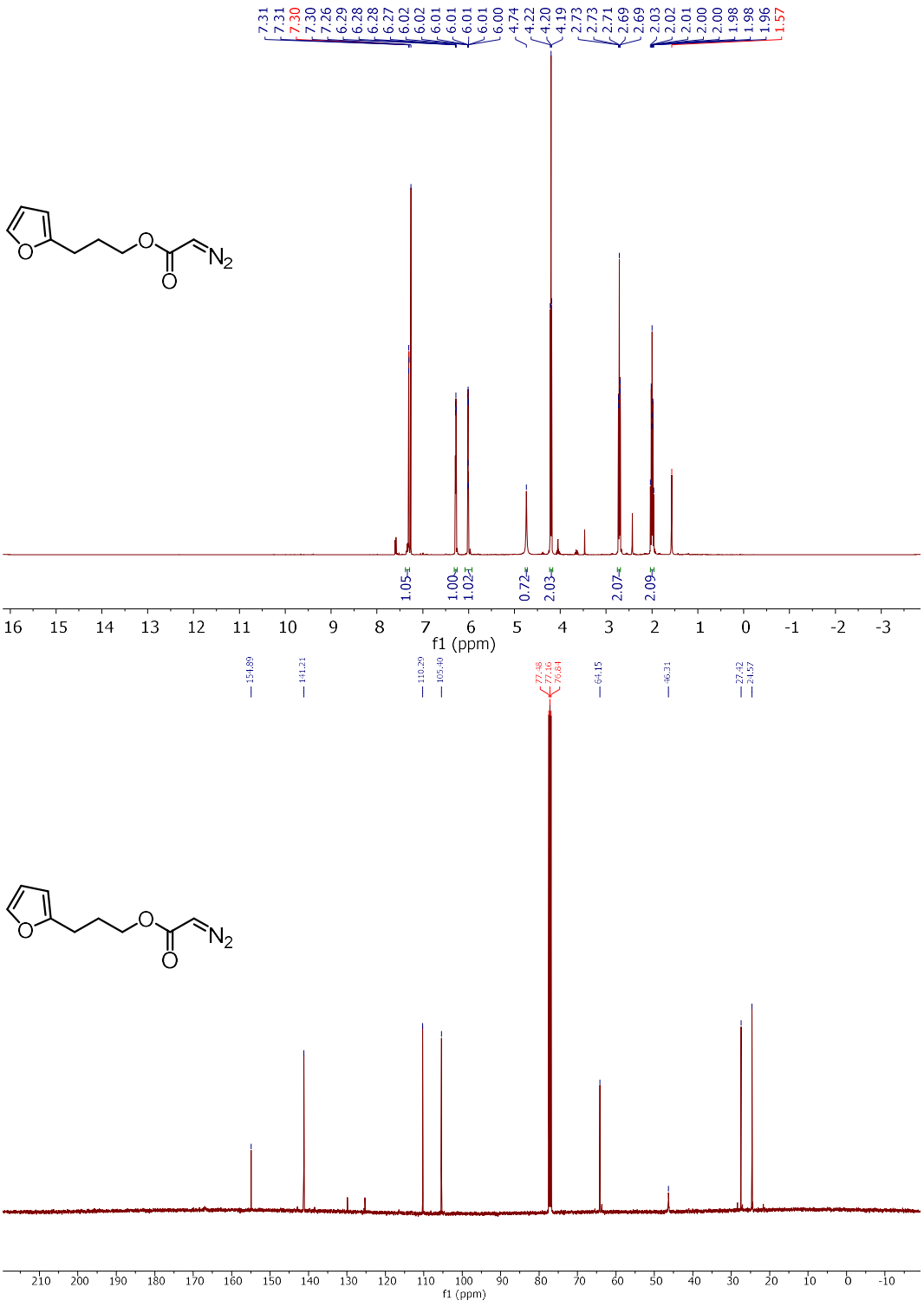




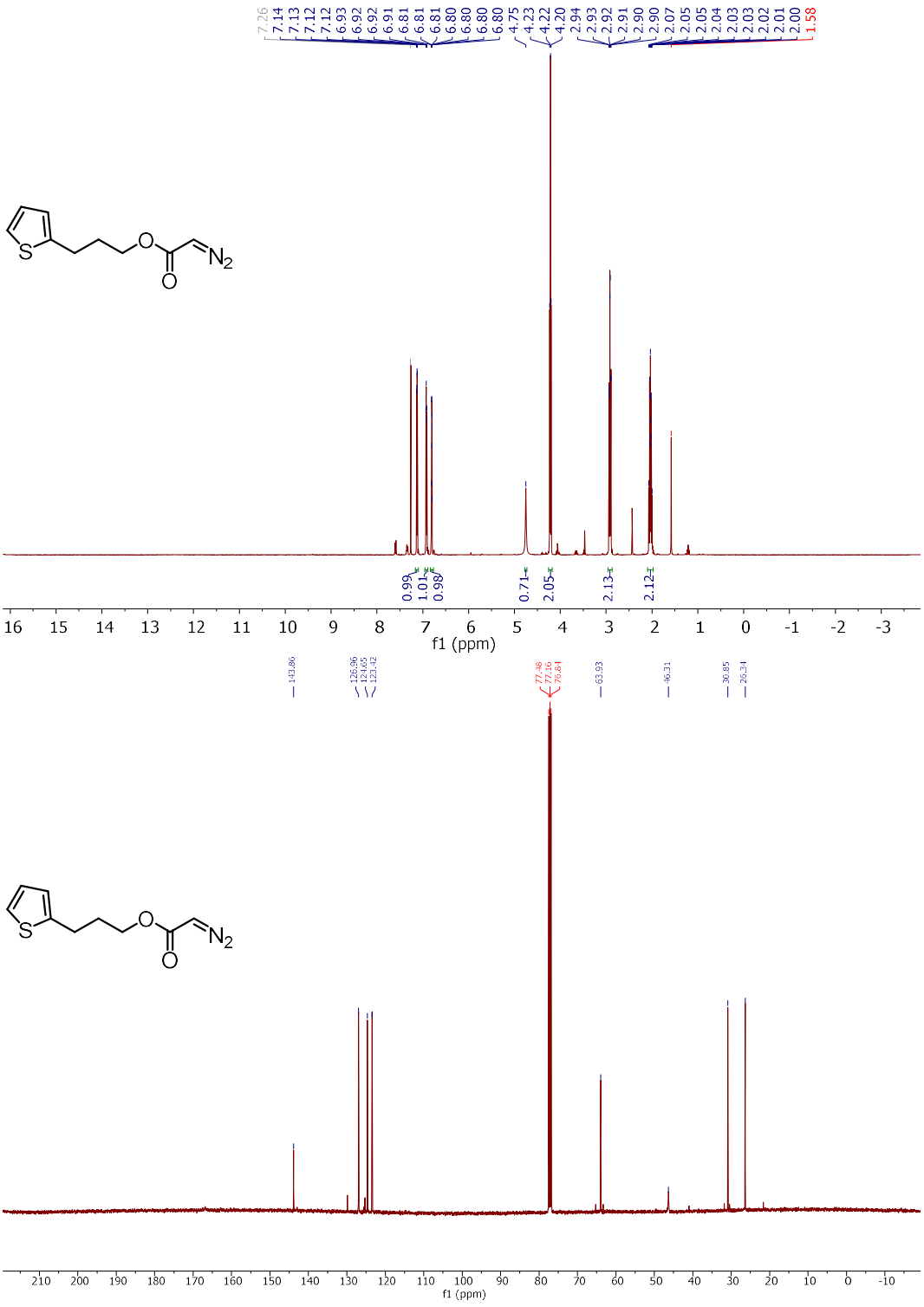
3-(4-fluorophenyl)propyl 2-diazoacetate (3d)



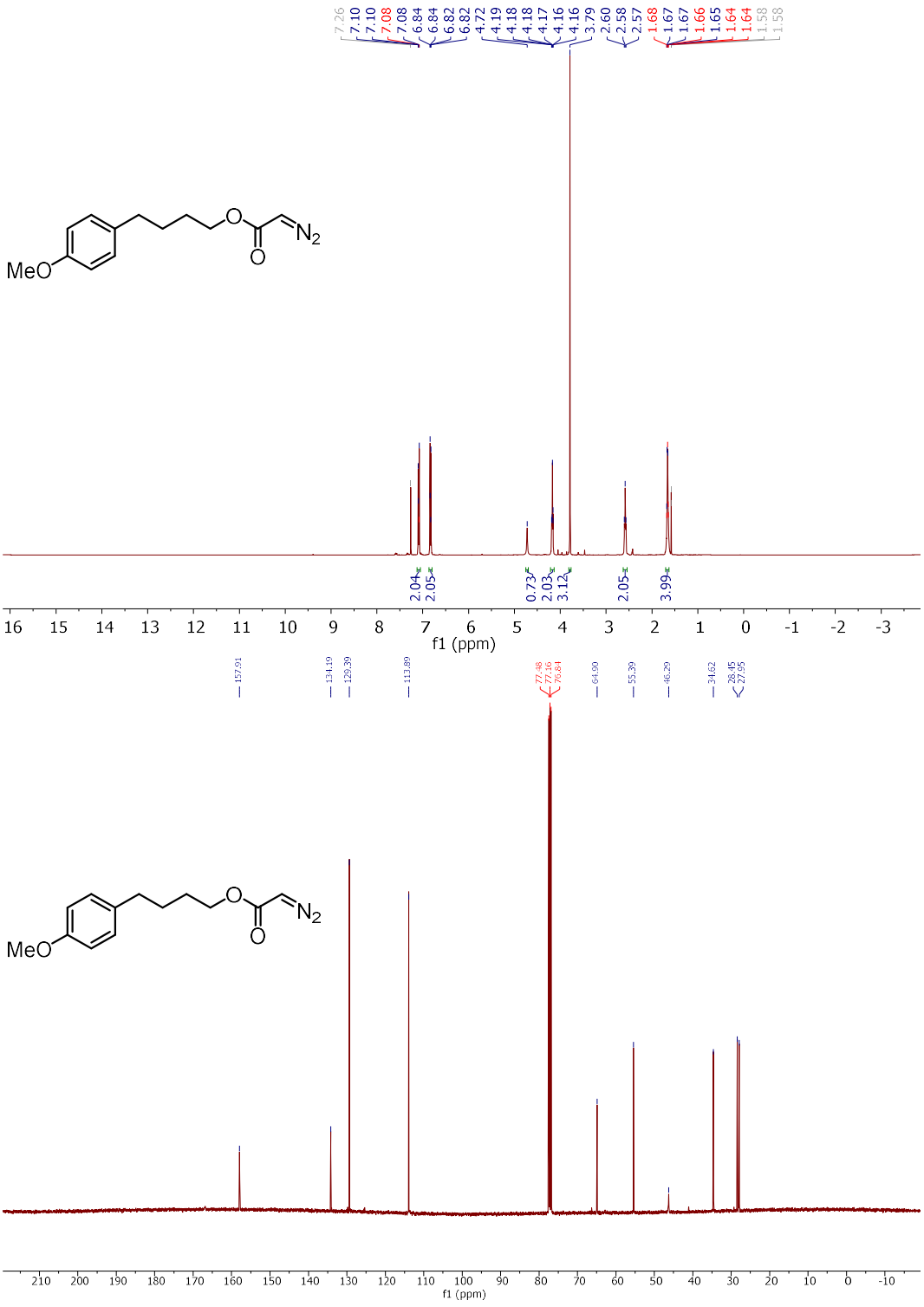
3-(furan-2-yl)propyl 2-diazoacetate (3e)



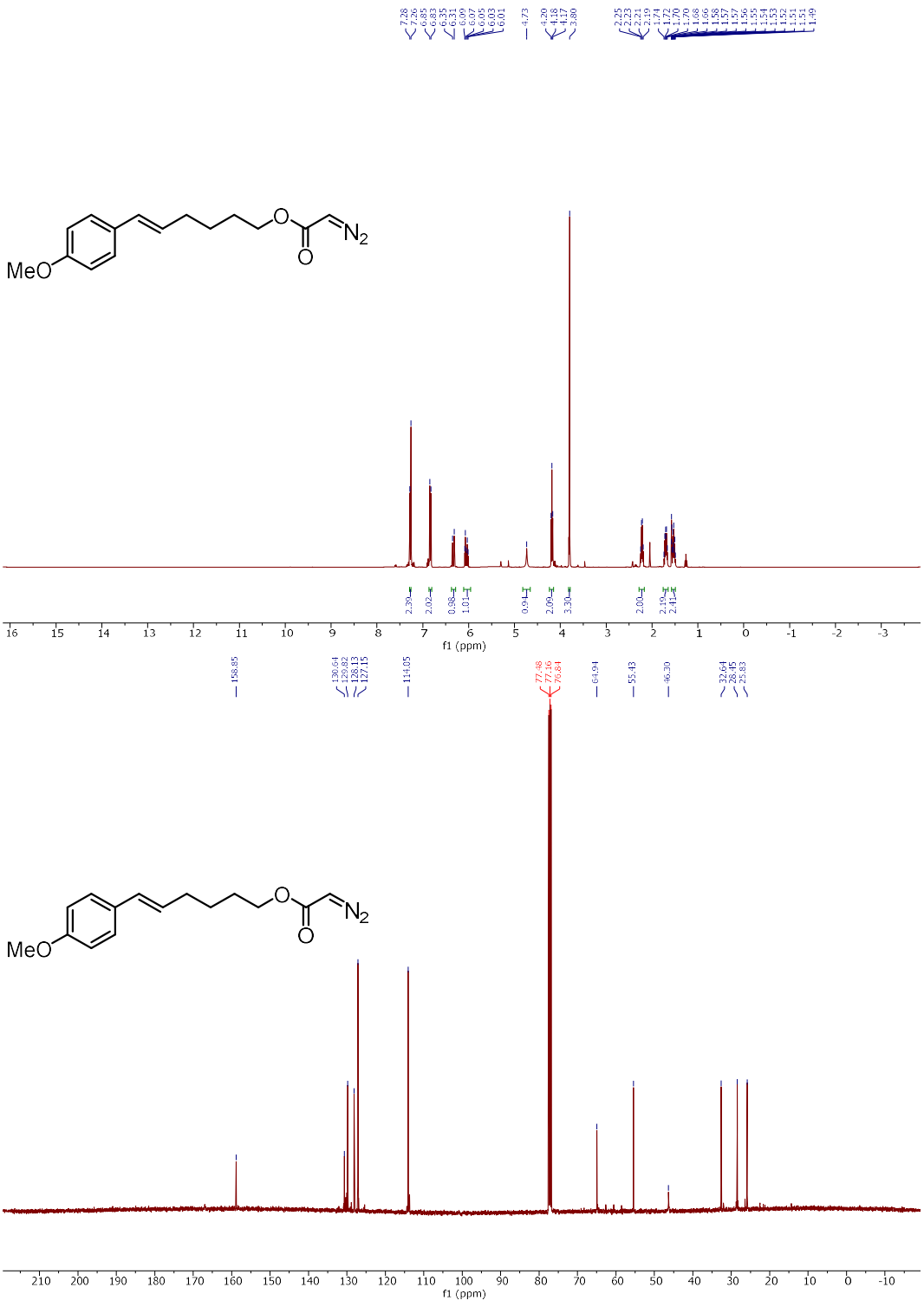
3-(thiophen-2-yl)propyl 2-diazoacetate (3f)



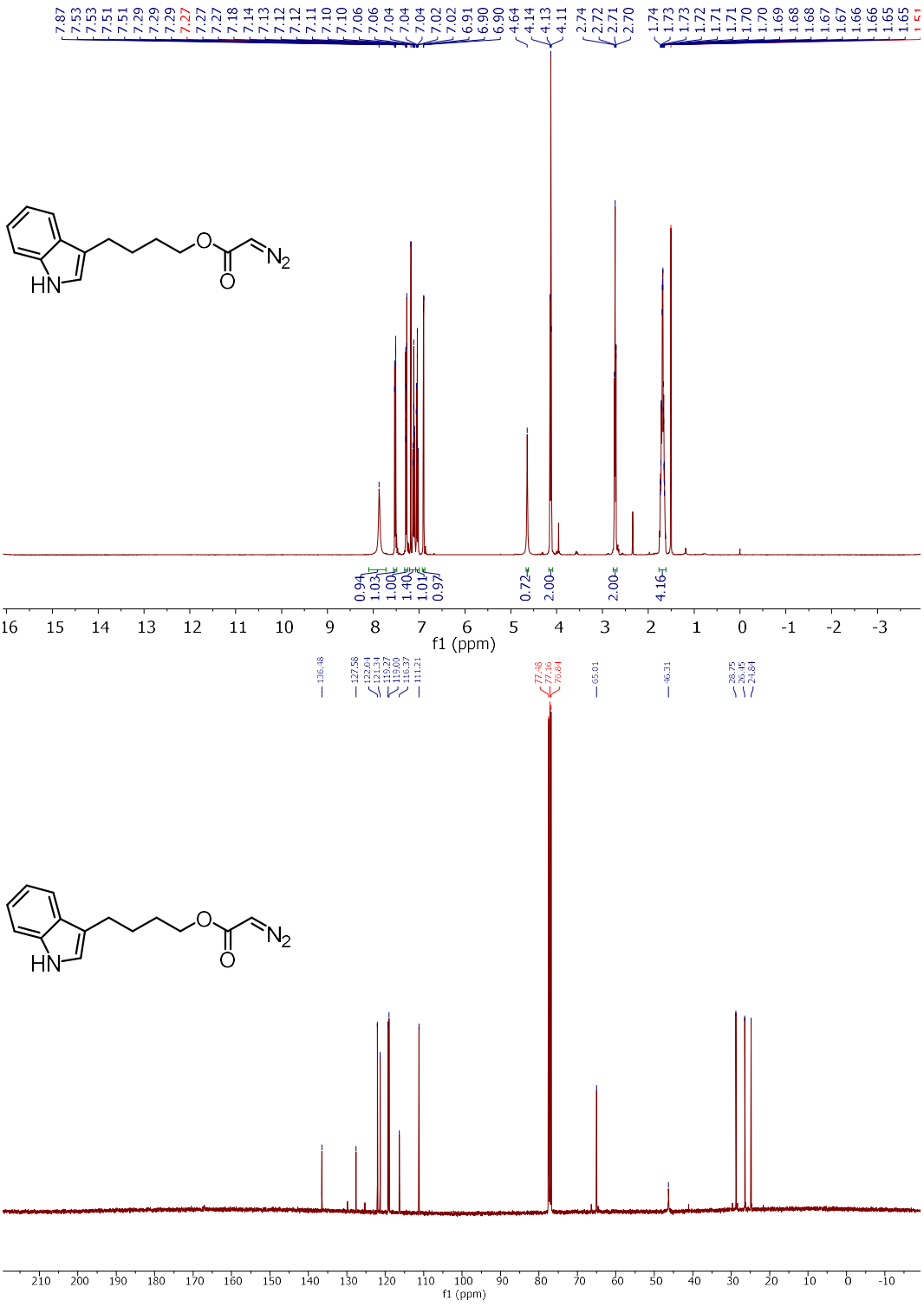
4-(4-methoxyphenyl)butyl 2-diazoacetate (5a)



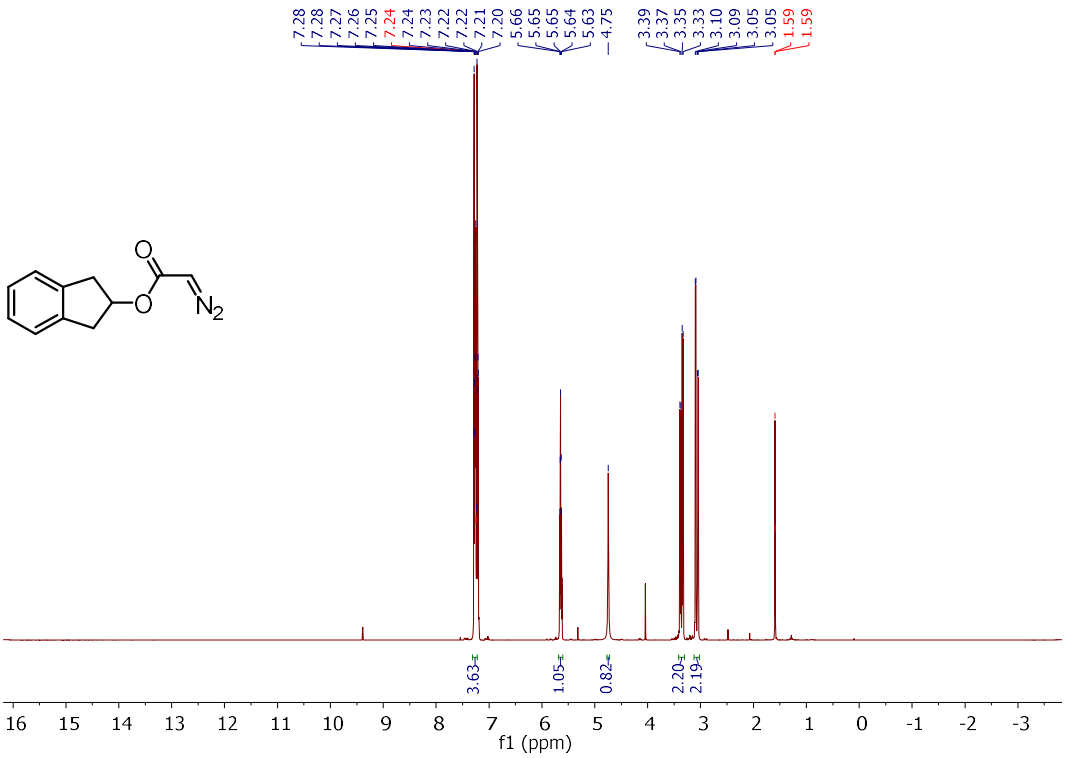
(*E*)-6-(4-methoxyphenyl)hex-5-en-1-yl 2-diazoacetate (5b)

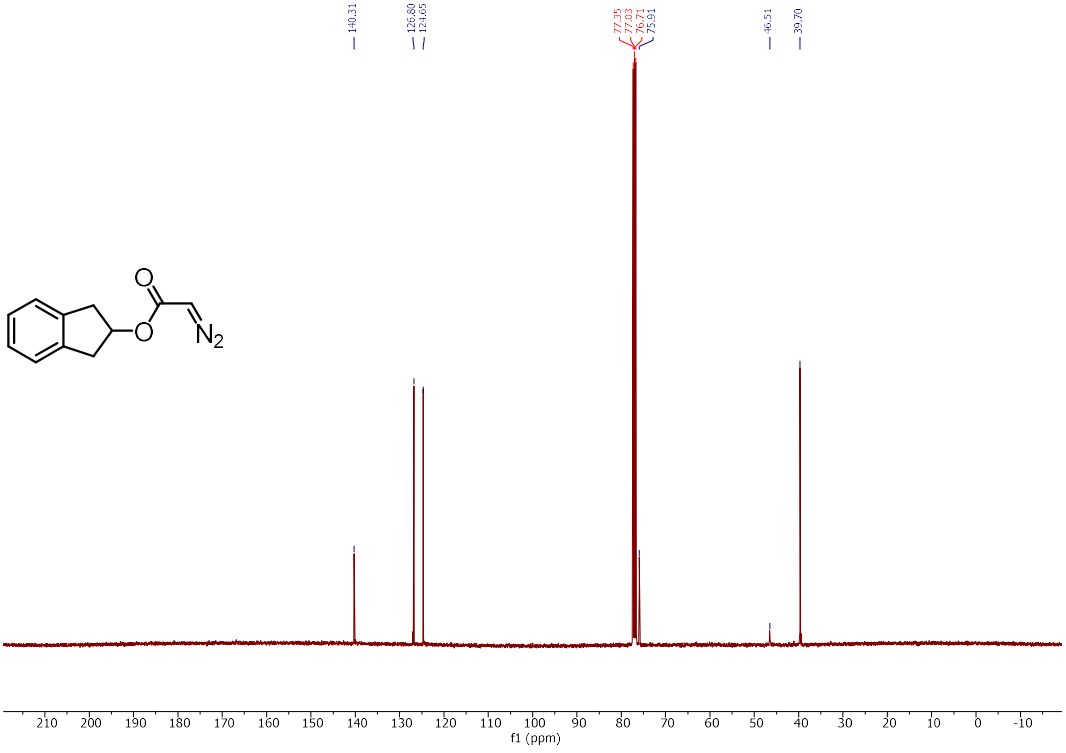


4-(1*H*-indol-3-yl)butyl 2-diazoacetate (5c)

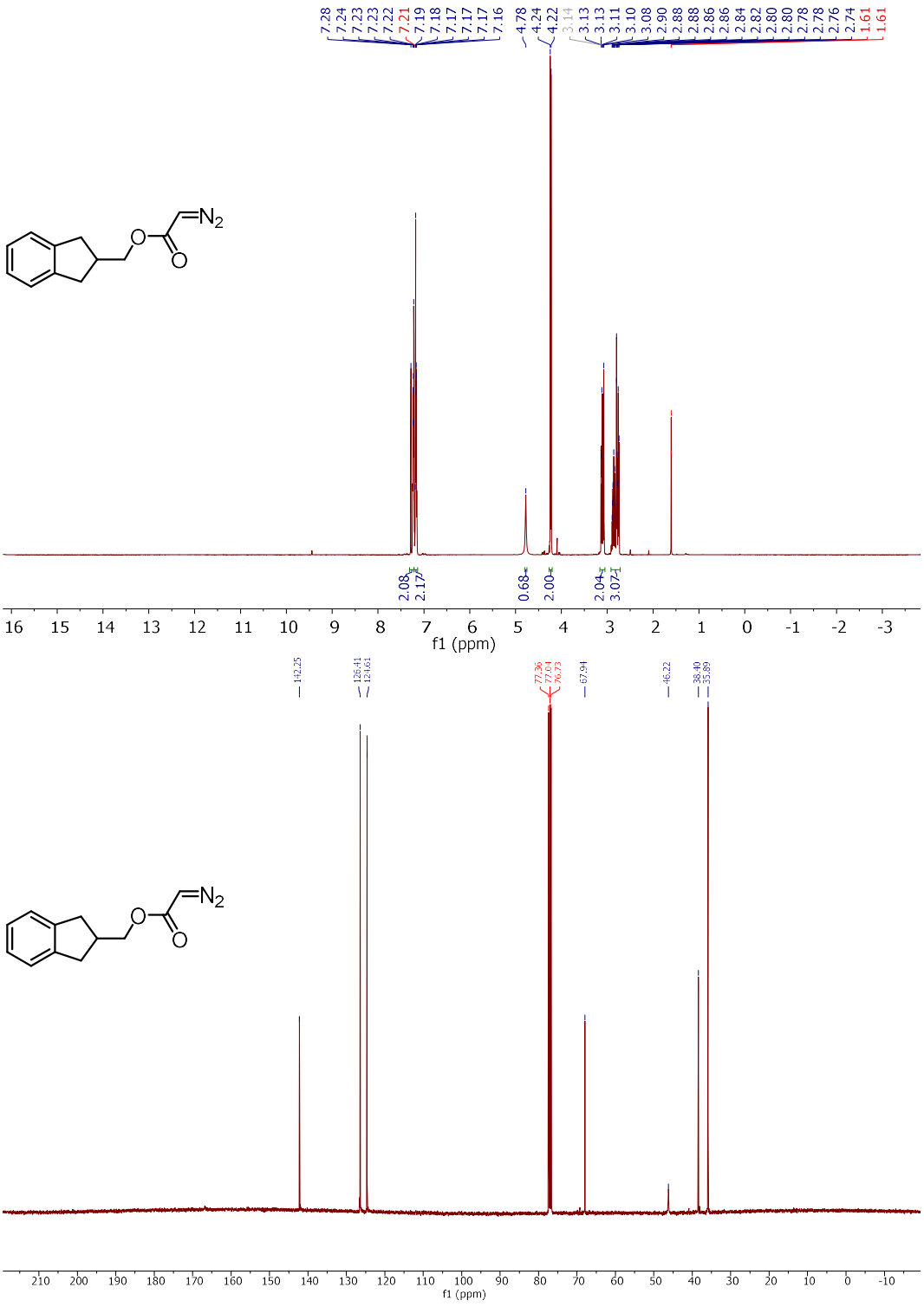


2,3-dihydro-1*H*-inden-2-yl 2-diazoacetate (7a)

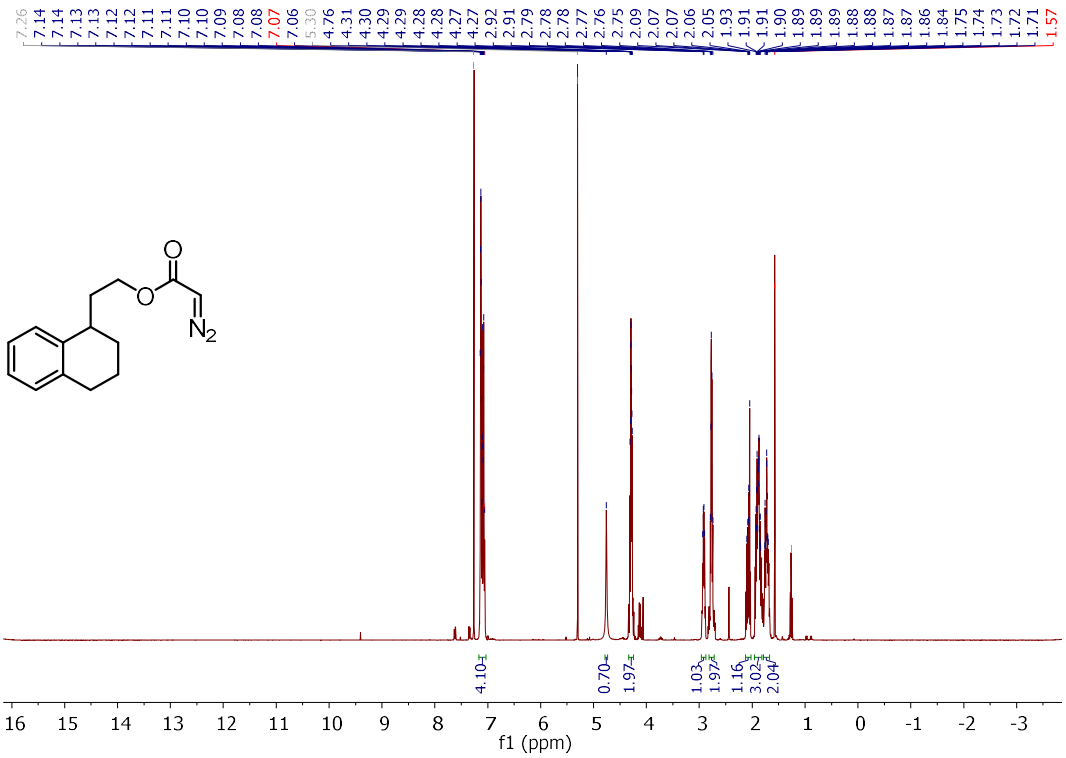


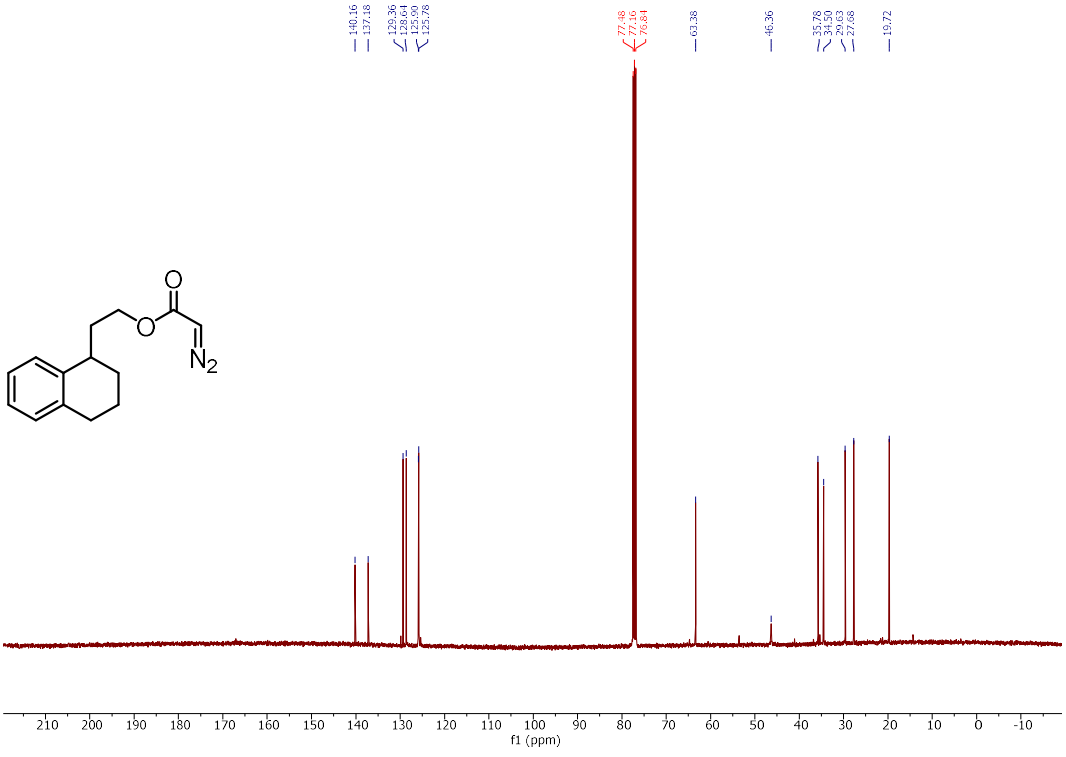


(2,3-dihydro-1*H*-inden-2-yl)methyl 2-diazoacetate (7b)

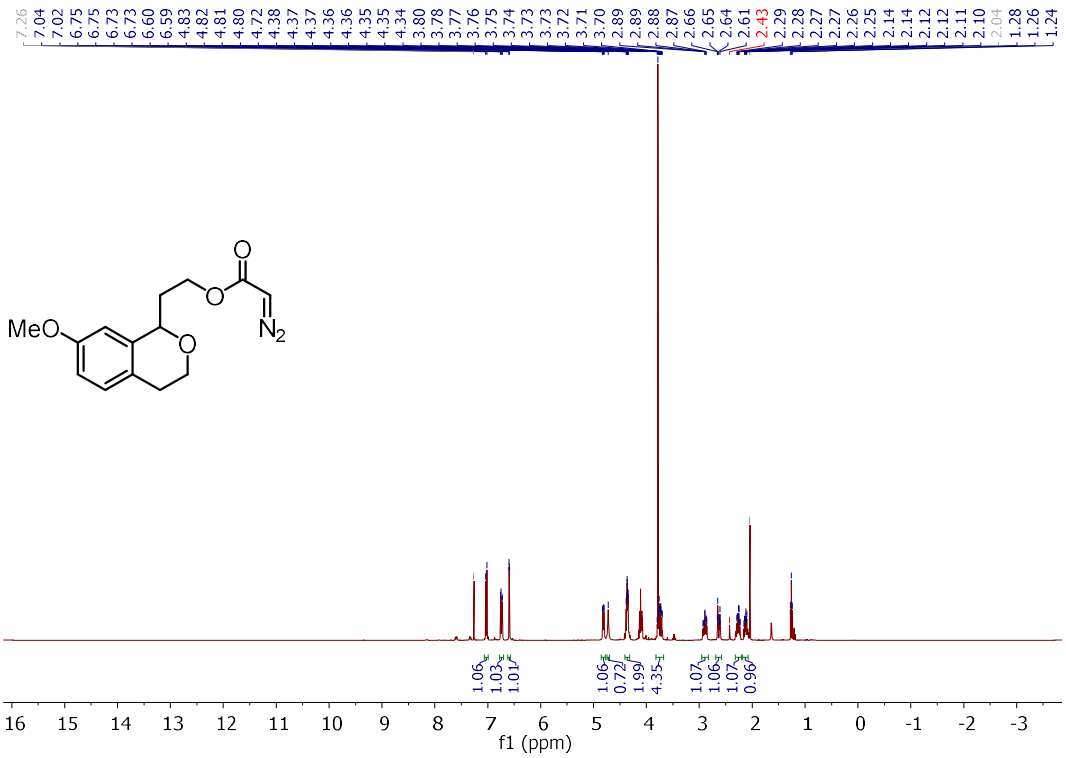


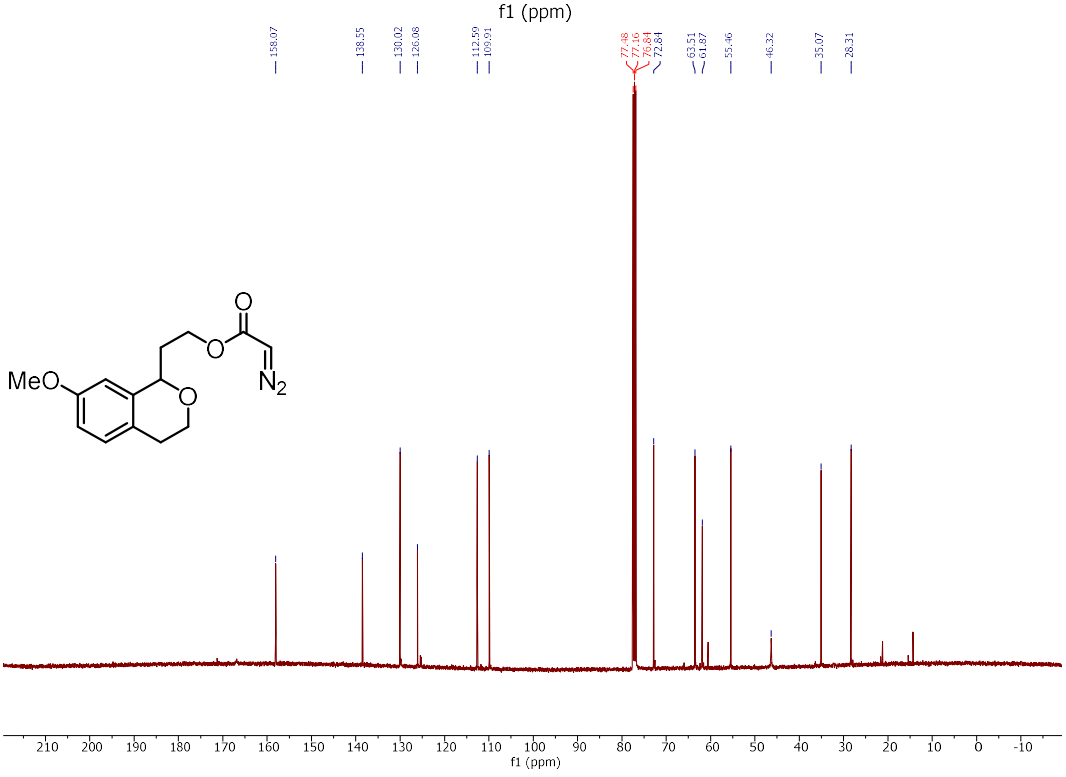
2-(1,2,3,4-tetrahydronaphthalen-1-yl)ethyl 2-diazoacetate (7c)



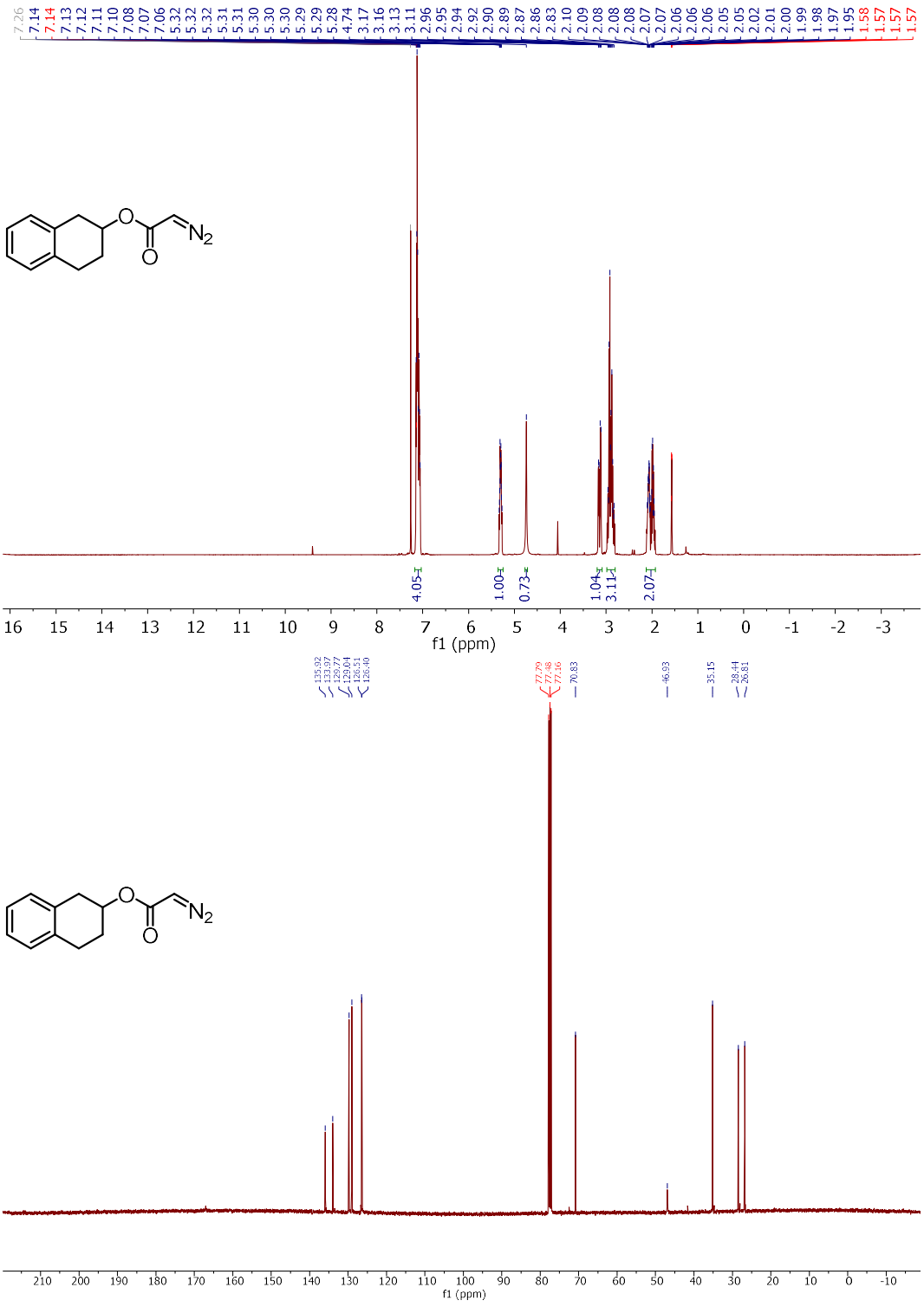


2-(7-methoxyisochroman-1-yl)ethyl 2-diazoacetate (7d)

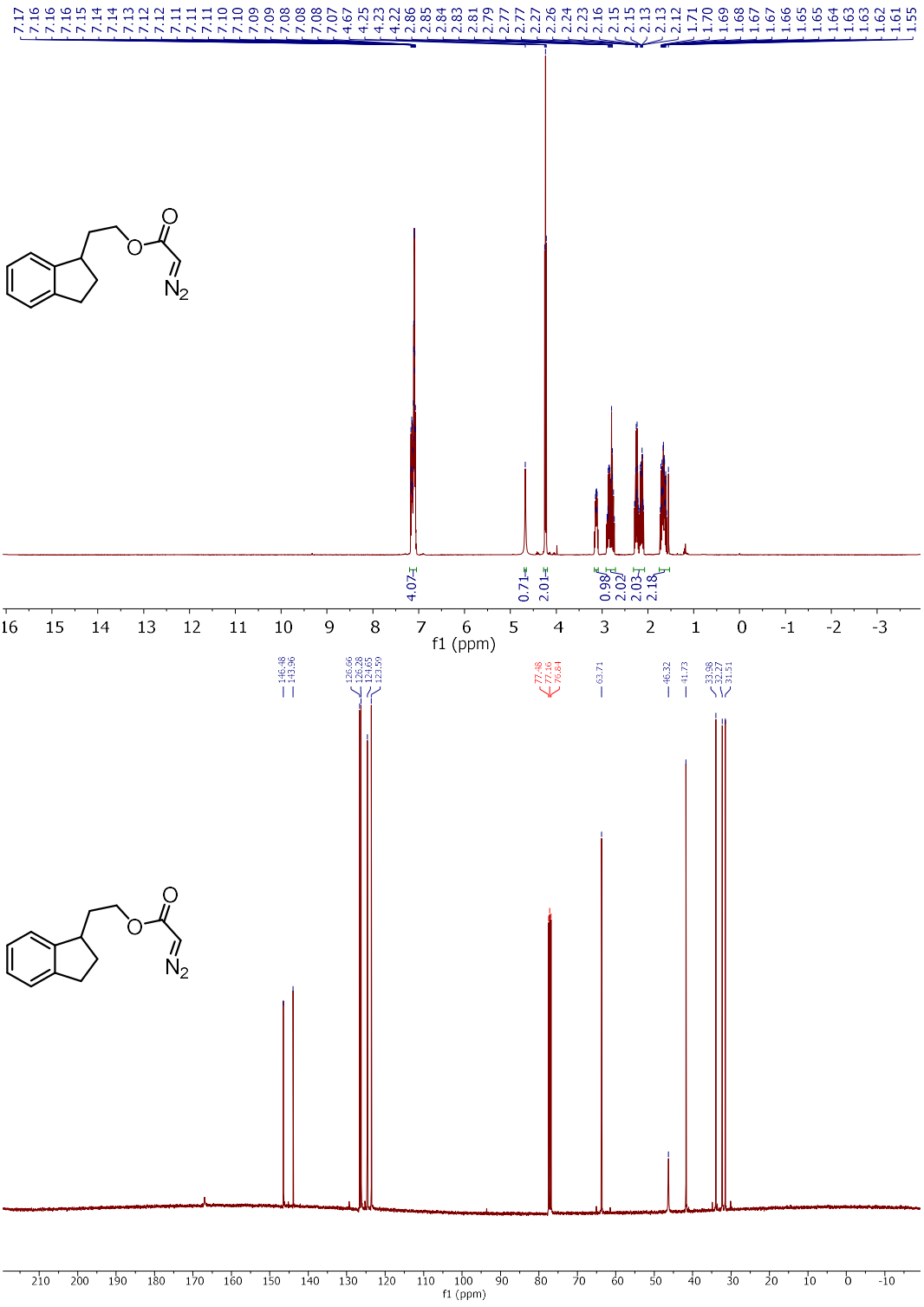




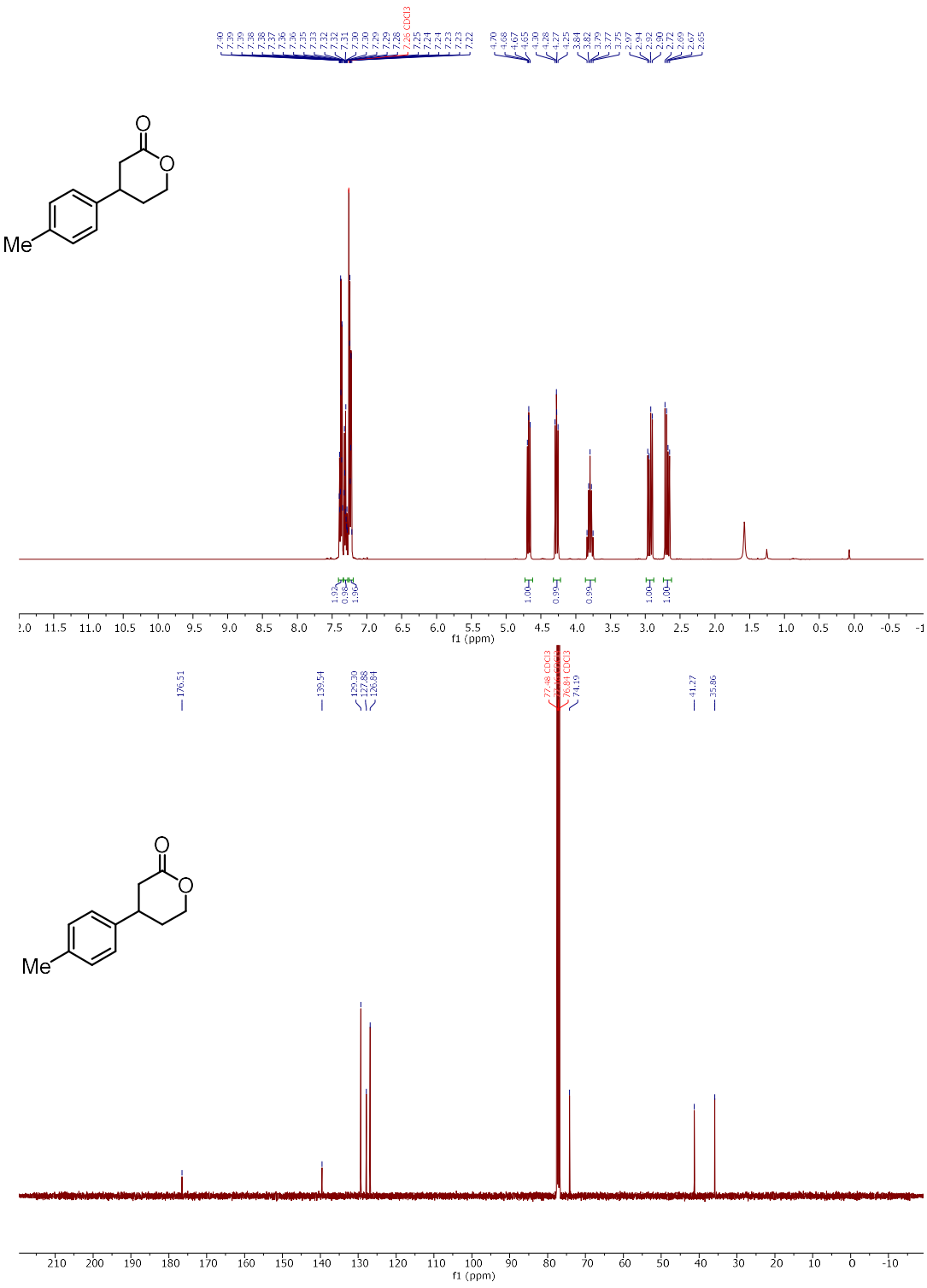
1,2,3,4-tetrahydronaphthalen-2-yl 2-diazoacetate (7e)



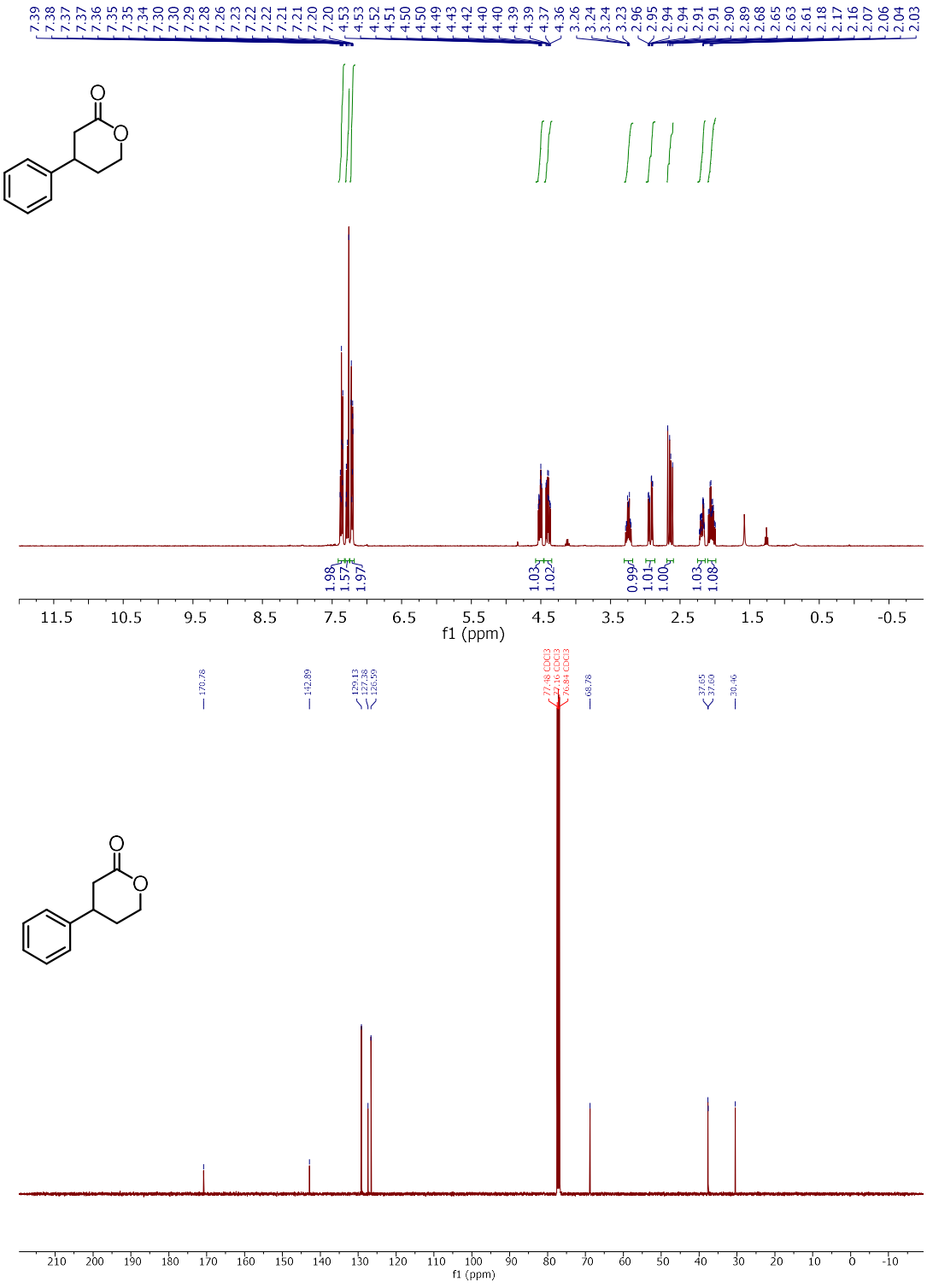
2-(2,3-dihydro-1H-inden-1-yl)ethyl 2-diazoacetate (7f)



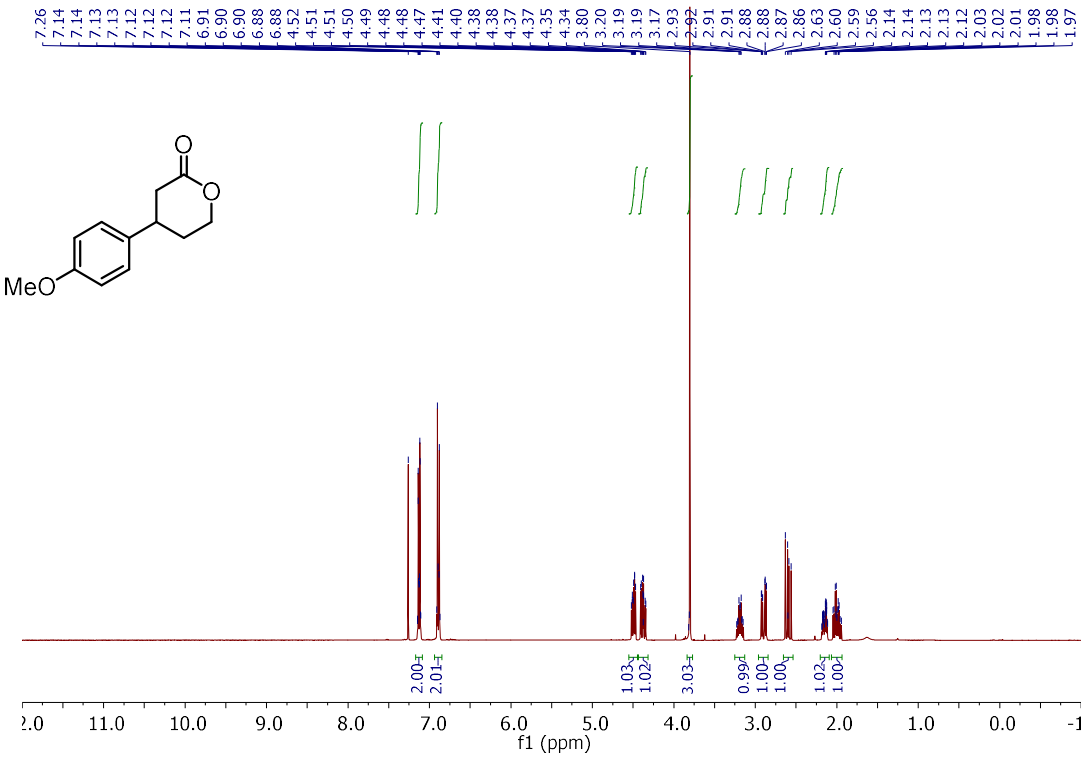
4-(*p*-tolyl)tetrahydro-2*H*-pyran-2-one (4a)

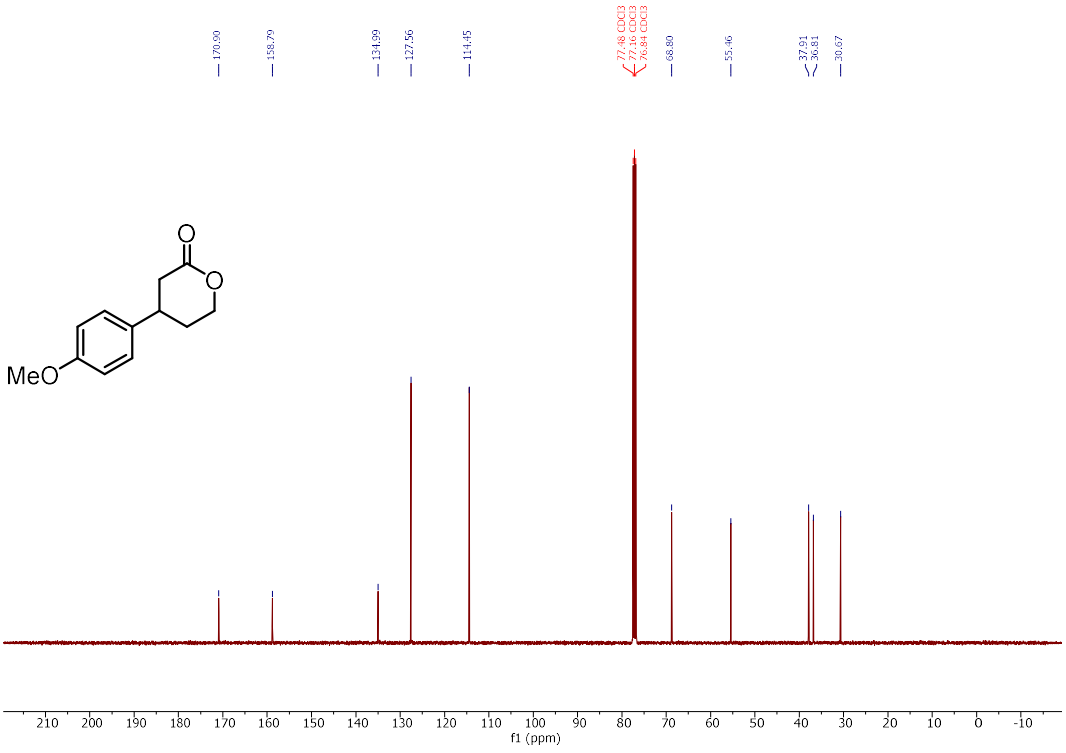


4-phenyltetrahydro-2H-pyran-2-one (4b)

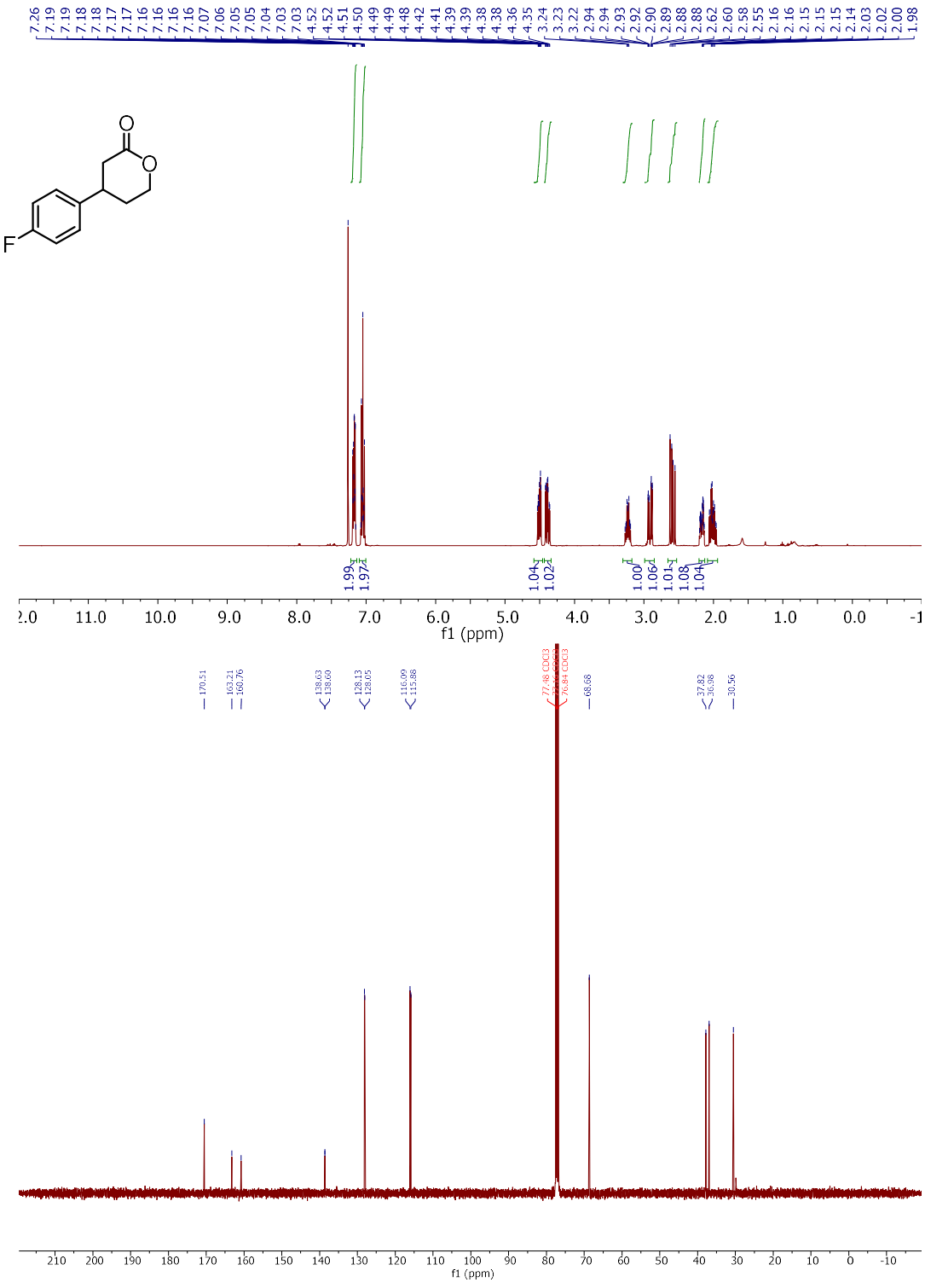


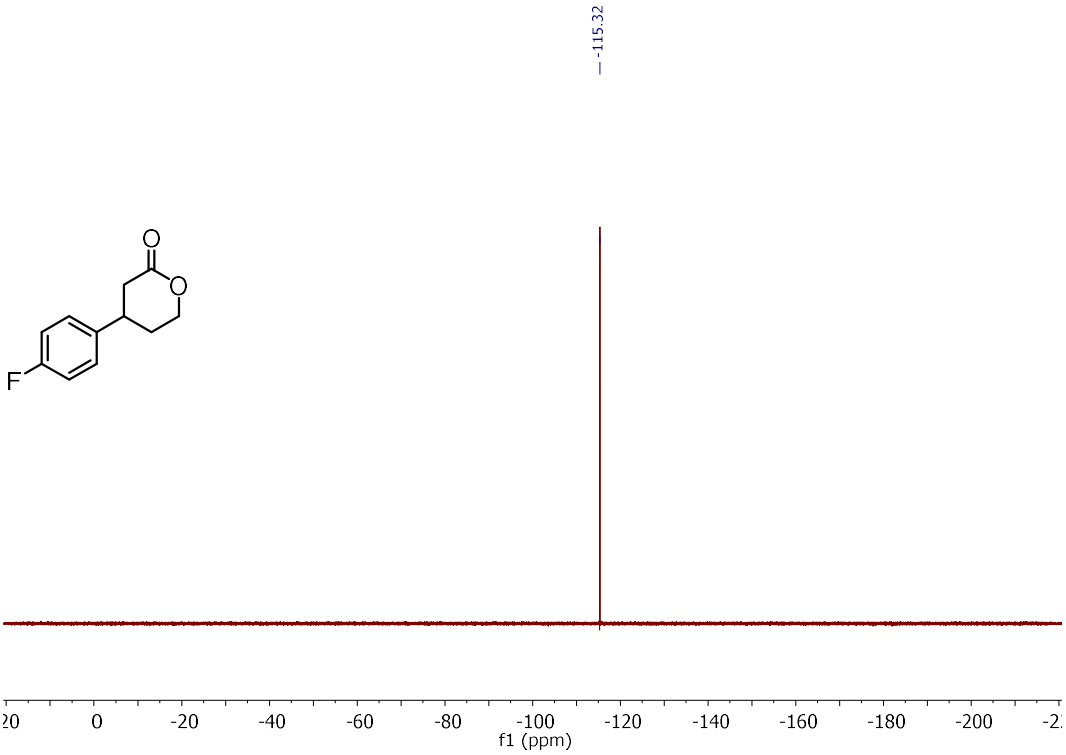
4-(4-methoxyphenyl)tetrahydro-2H-pyran-2-one (4c)



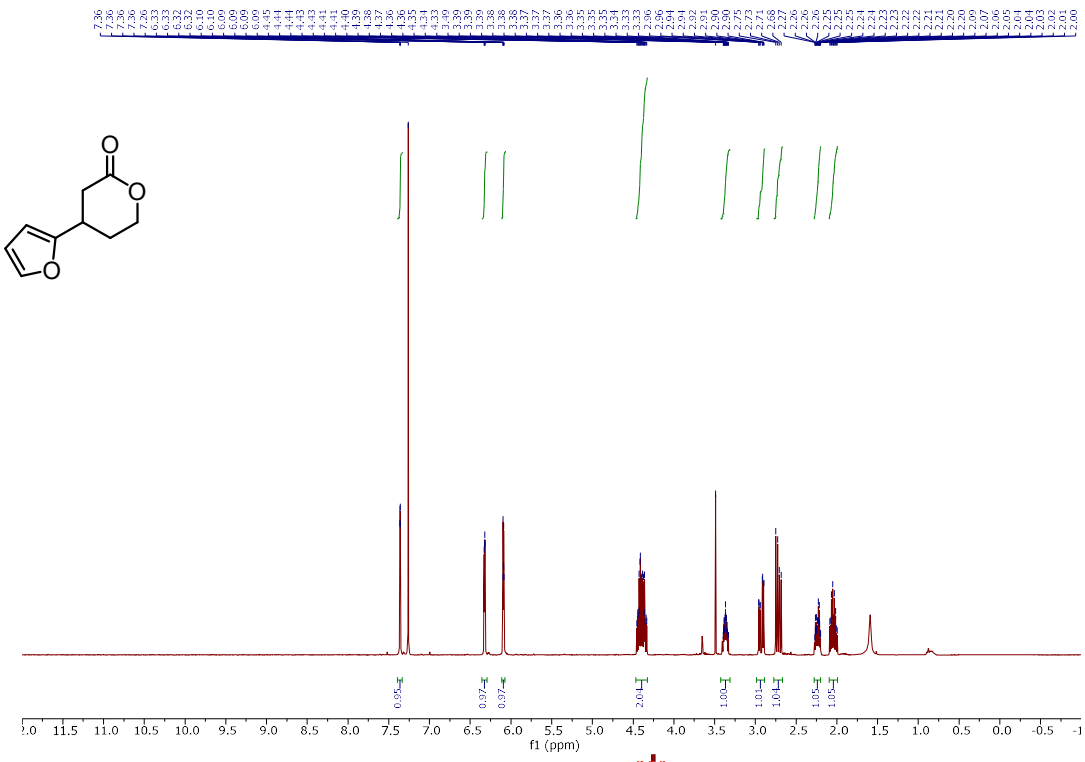


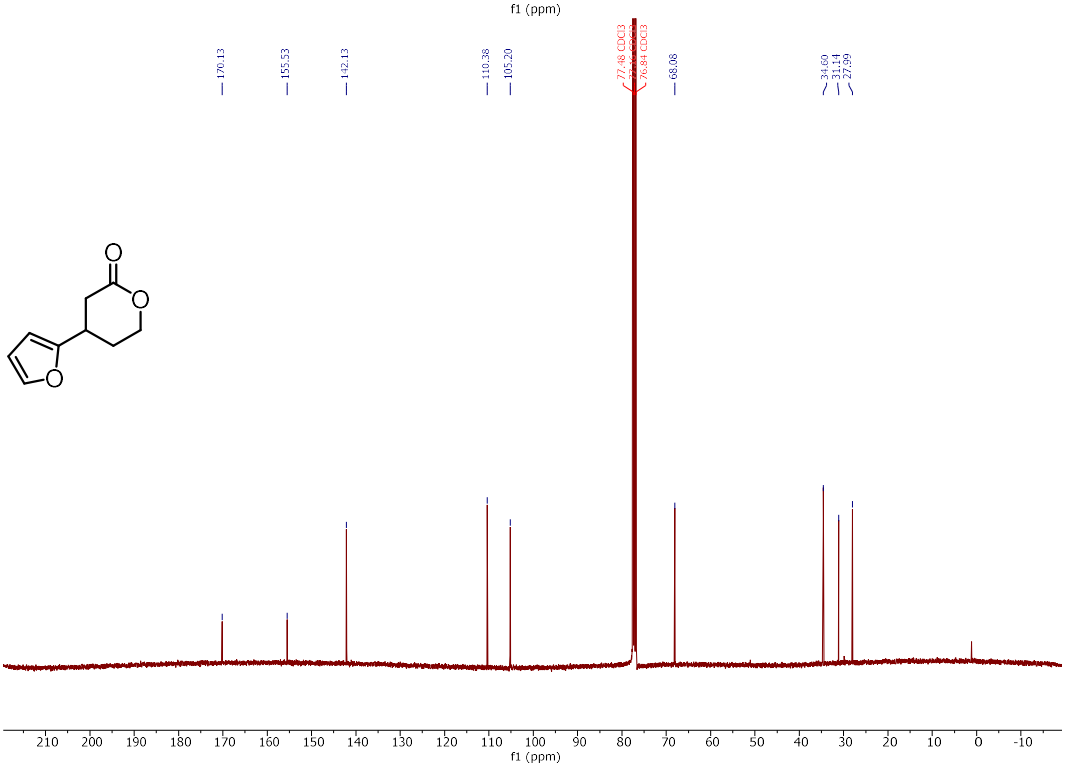
4-(4-fluorophenyl)tetrahydro-2H-pyran-2-one (4d)



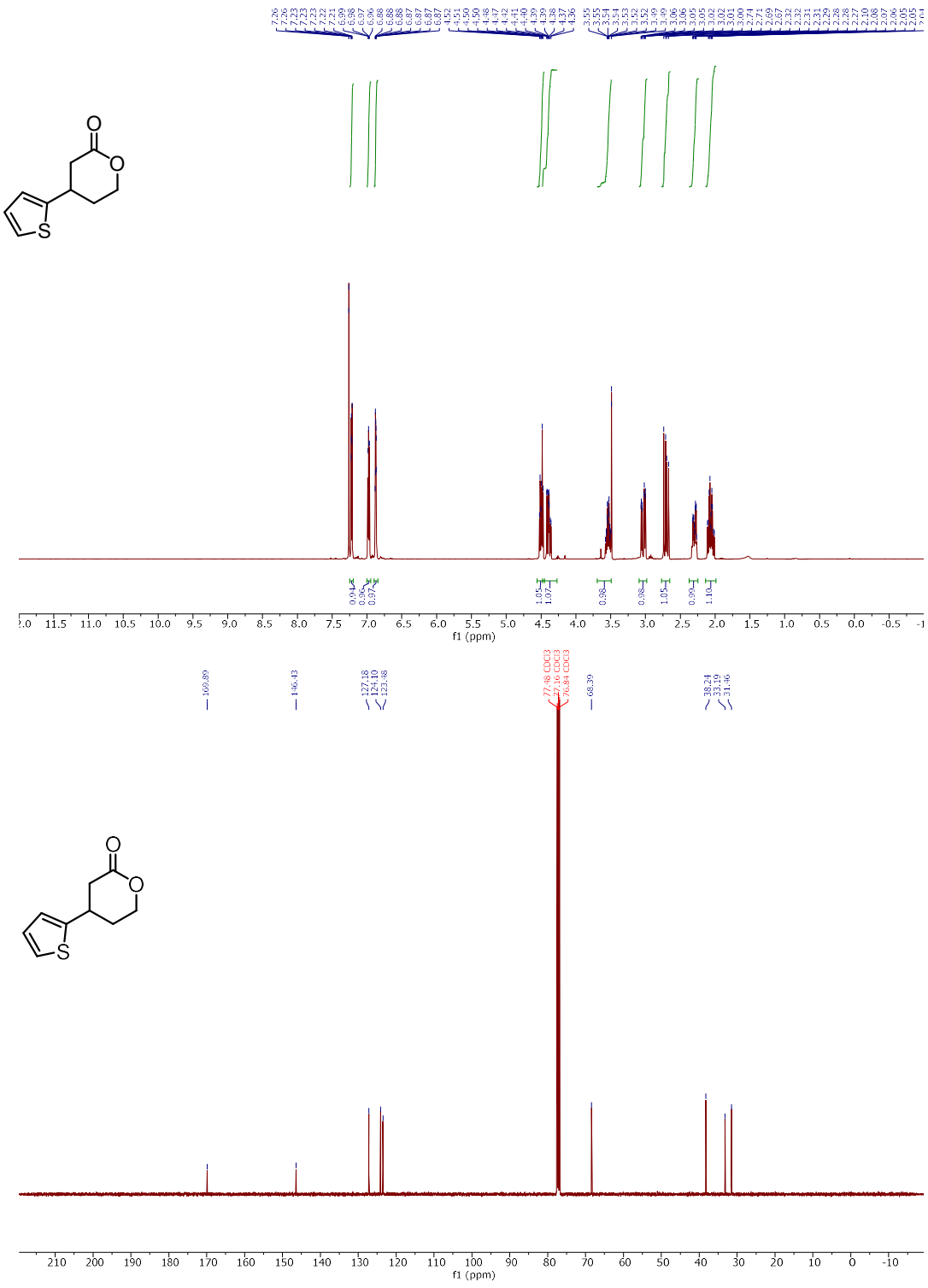

-115.32
O
O
F
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
f1 (ppm)

4-(furan-2-yl)tetrahydro-2*H*-pyran-2-one (4e)

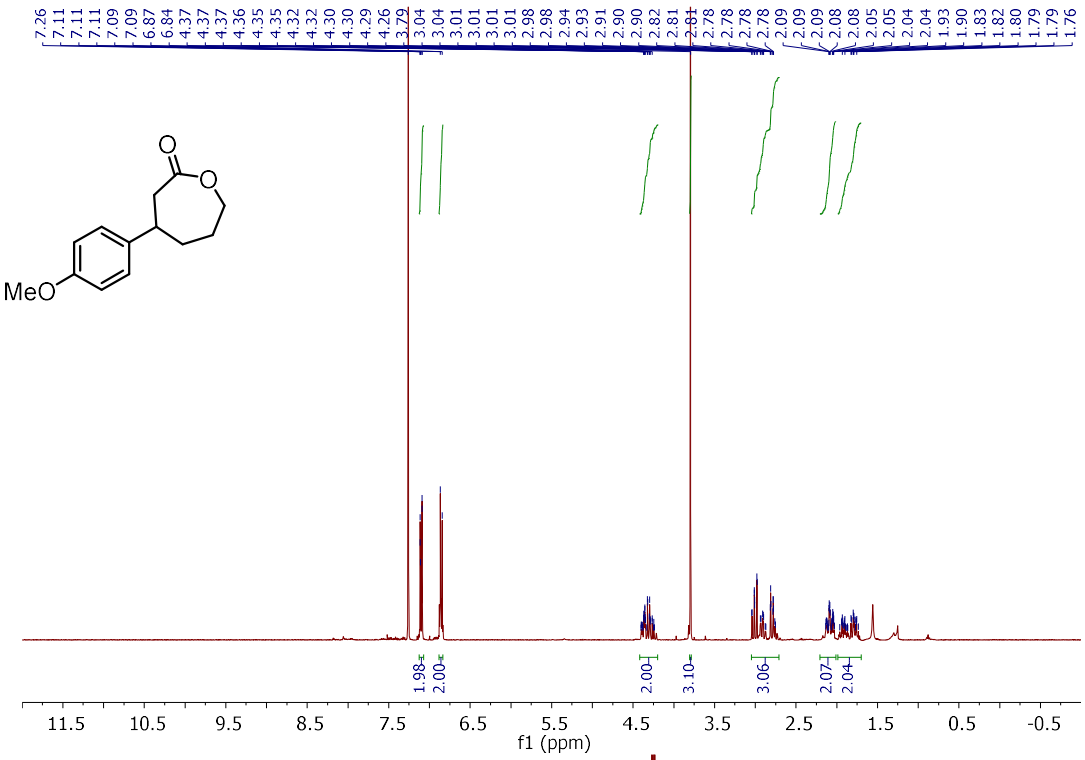


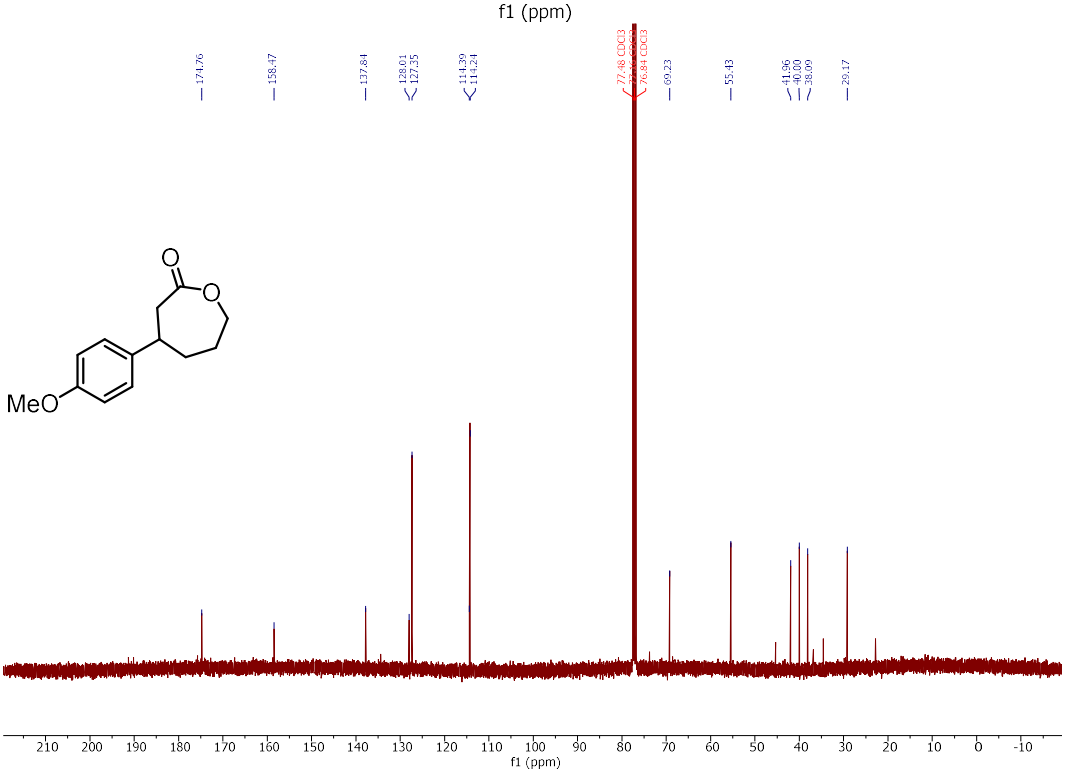


4-(thiophen-2-yl)tetrahydro-2*H*-pyran-2-one (4f)

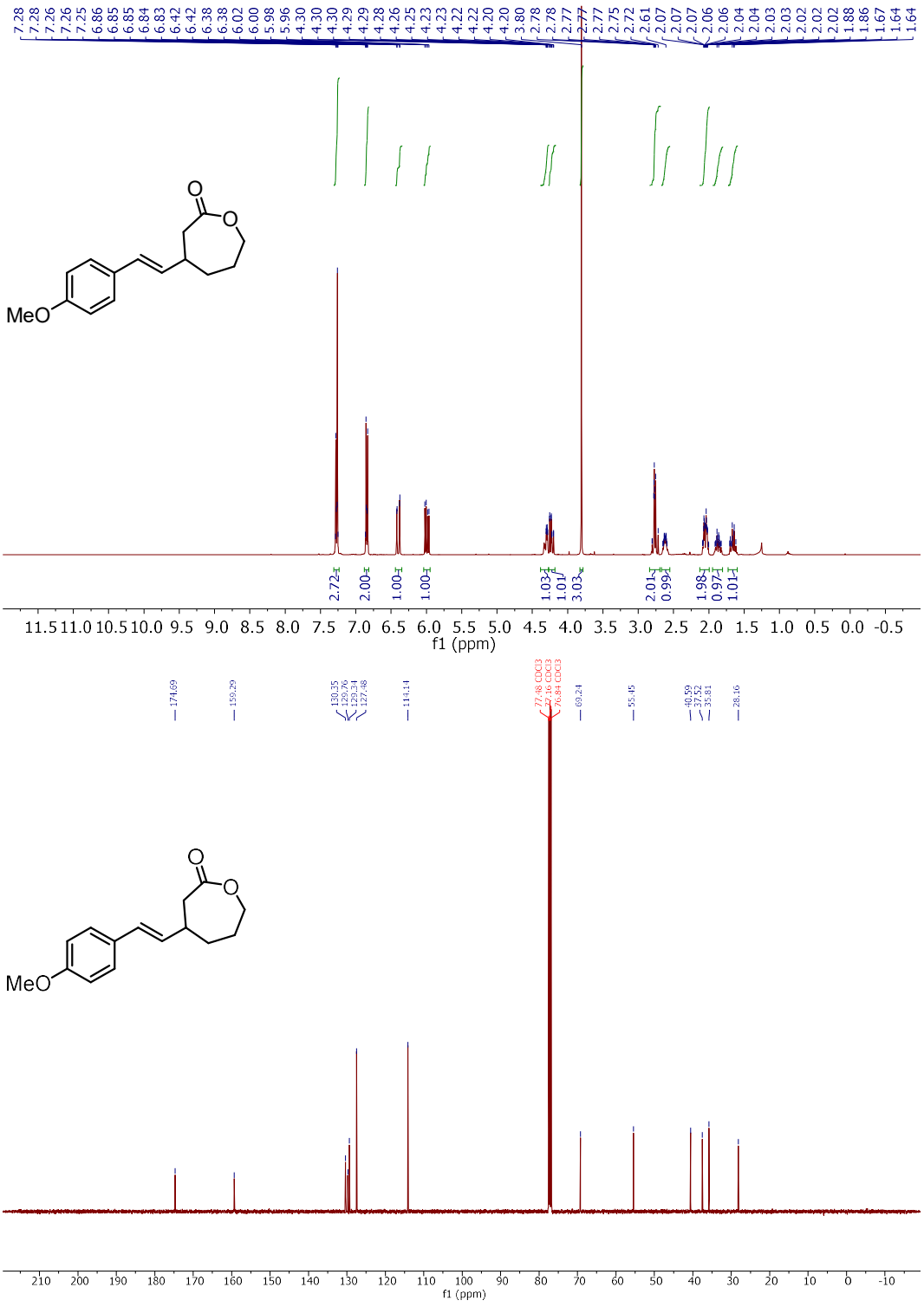


4-(4-methoxyphenyl)oxepan-2-one (6a)

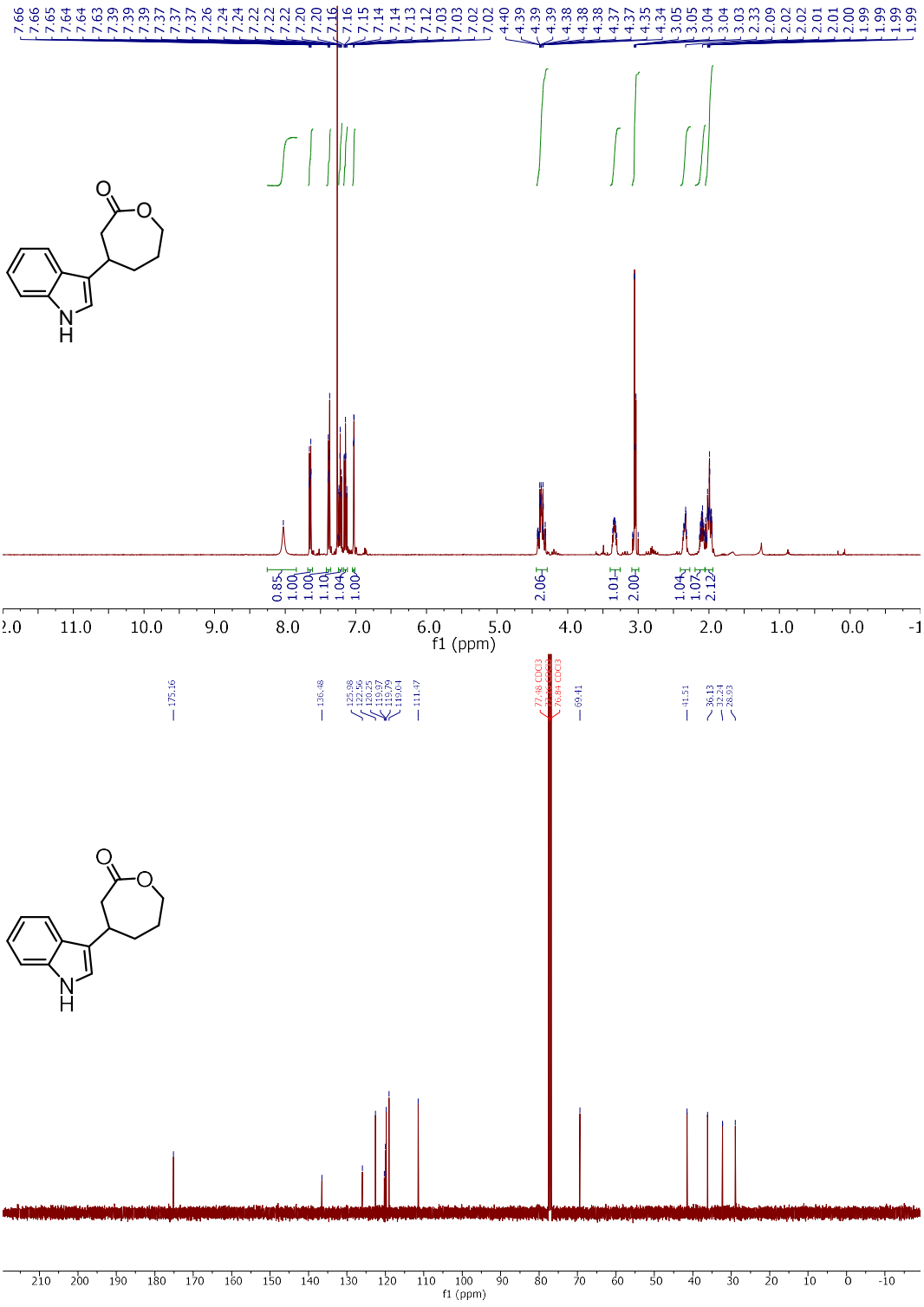




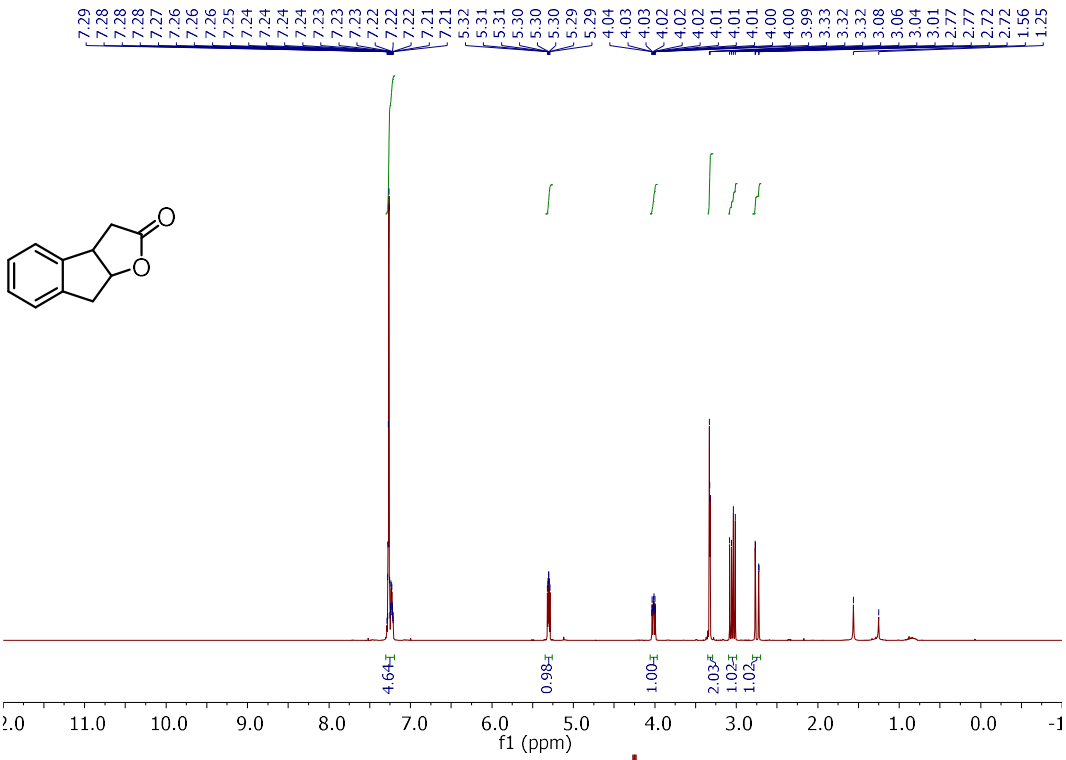
(*E*)-4-(4-methoxystyryl)oxepan-2-one (6b)

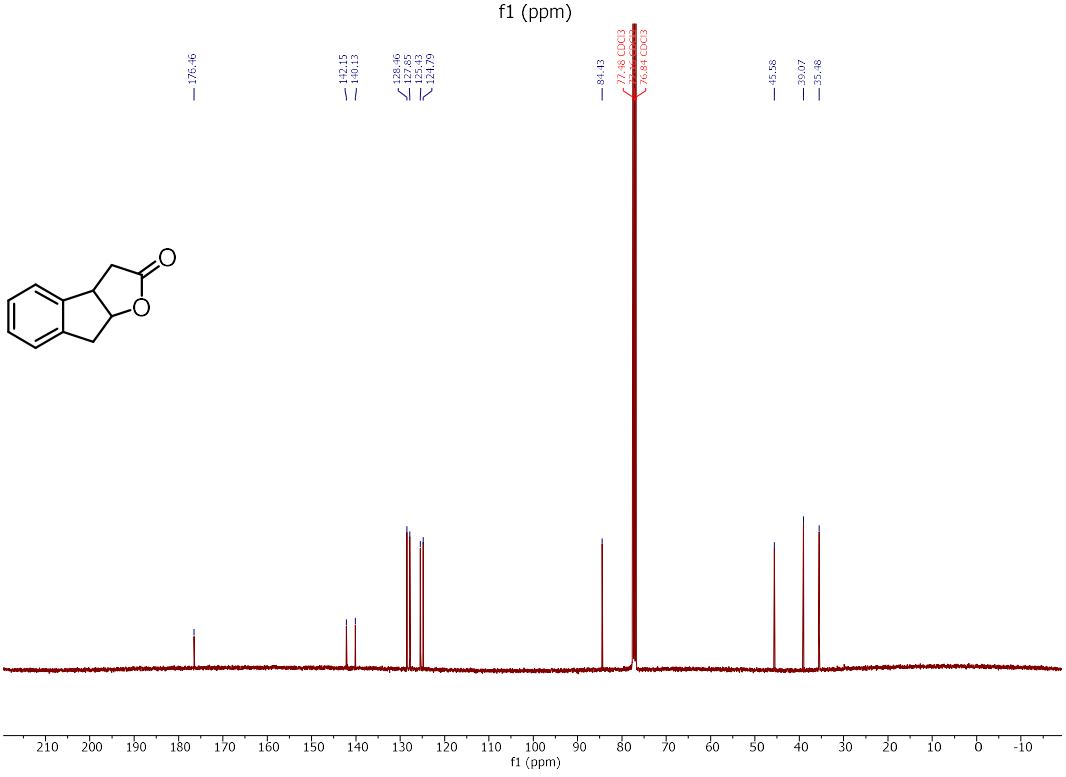


4-(1*H*-indol-3-yl)oxepan-2-one (6c)

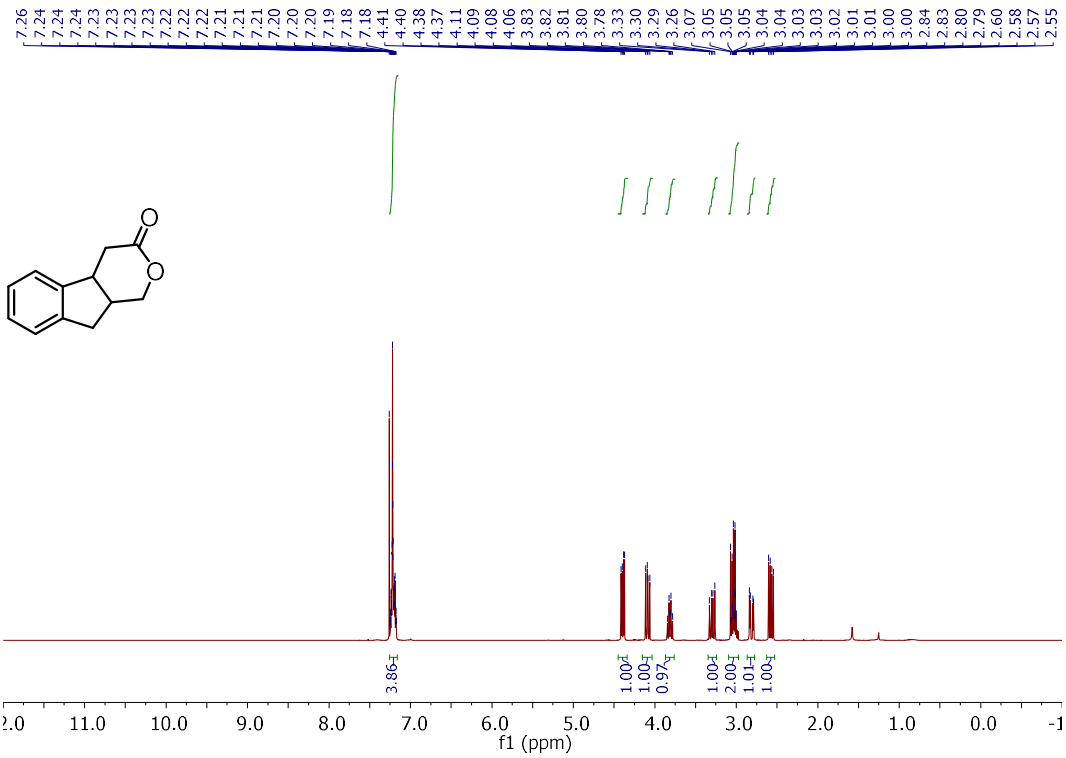


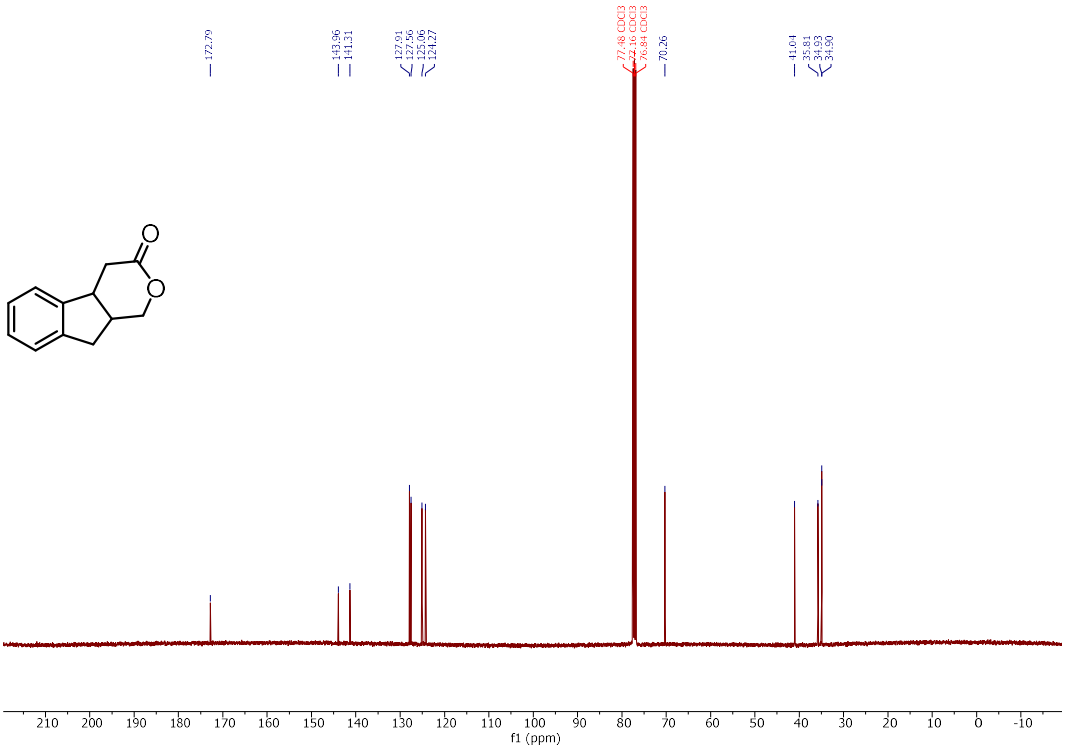
3,3a,8,8a-Tetrahydro-2*H*-indeno[2,1-*b*]furan-2-one (8a)



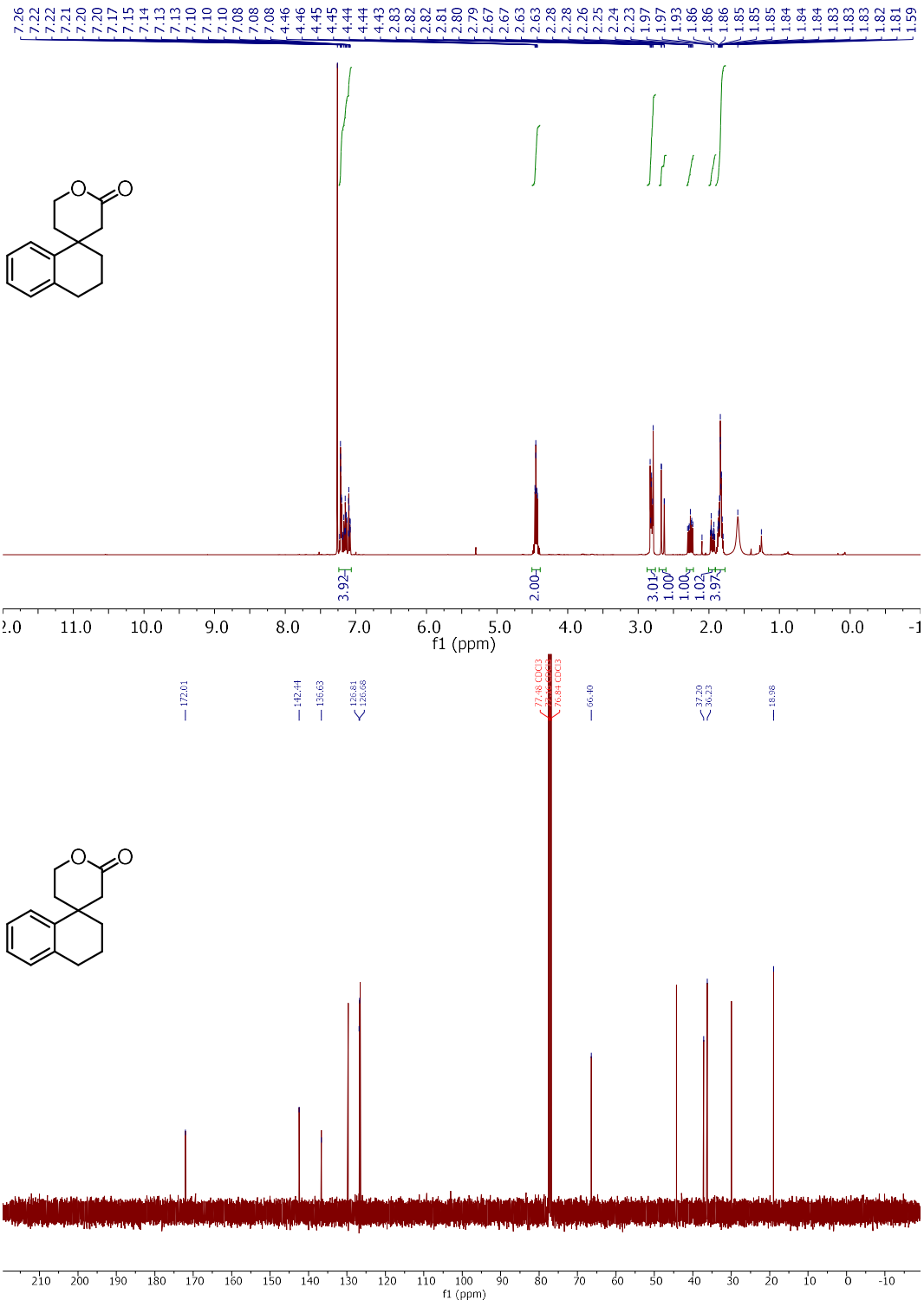


4,4a,9,9a-Tetrahydroindeno[2,1-*c*]pyran-3(1*H*)-one (8b)

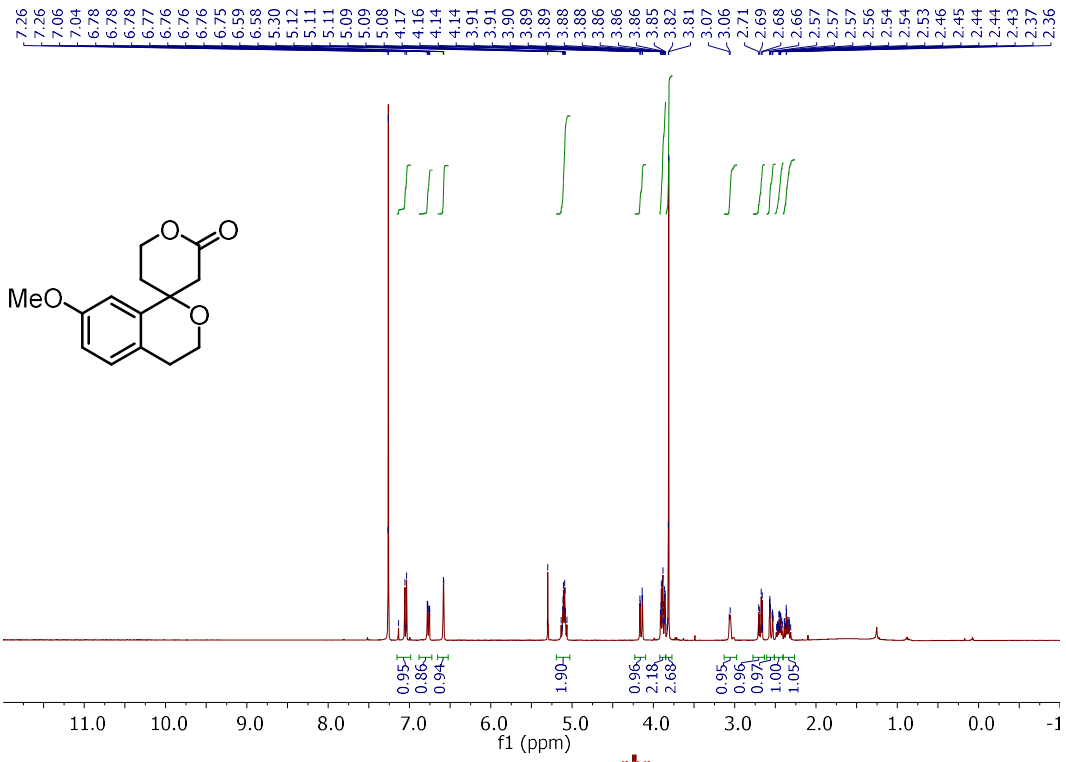


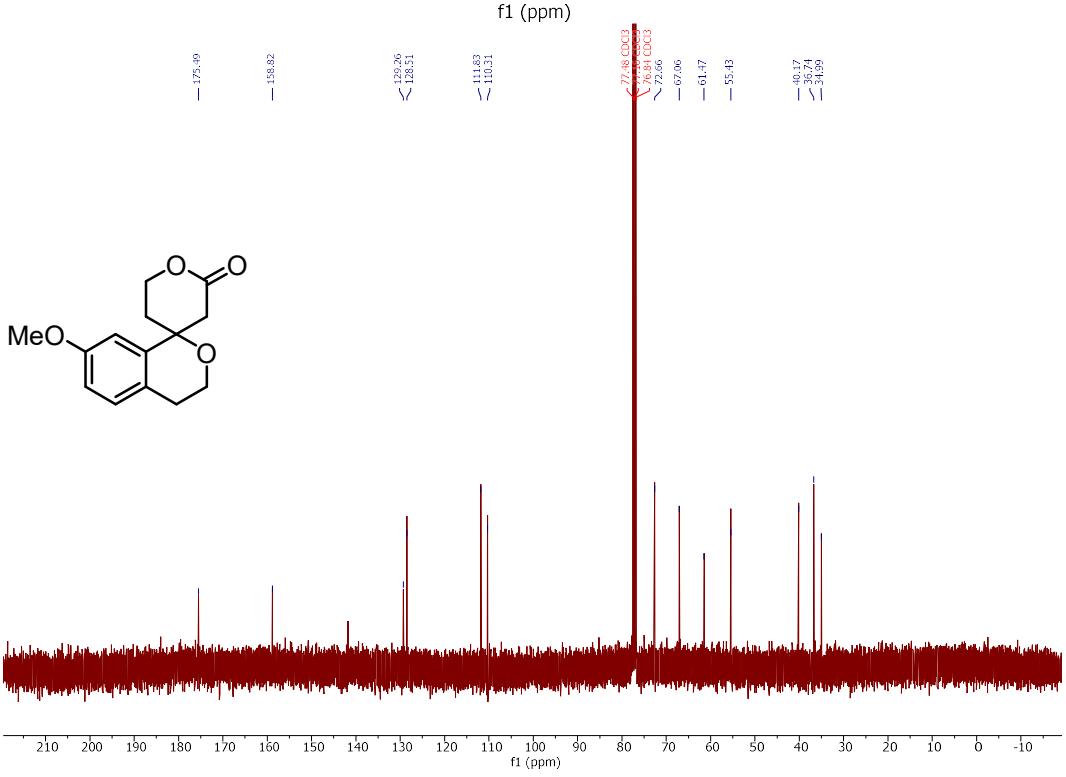


3,4,5',6'-tetrahydro-2H-spiro[naphthalene-1,4'-pyran]-2'(3'*H*)-one (8c)

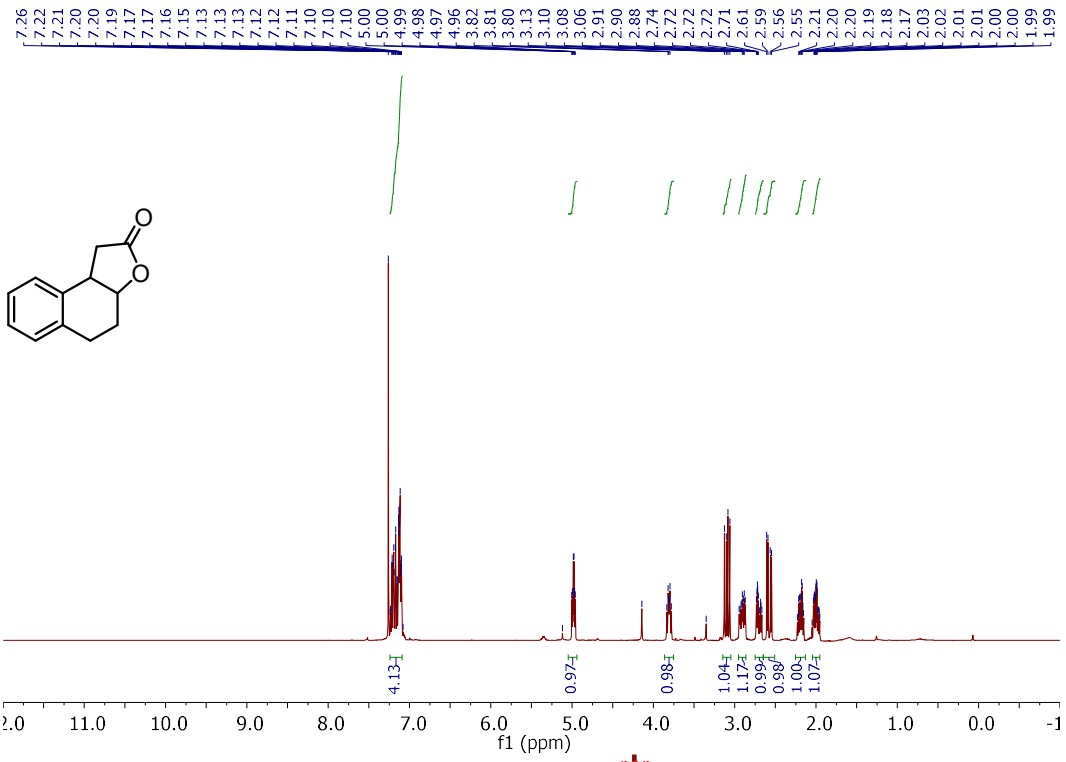


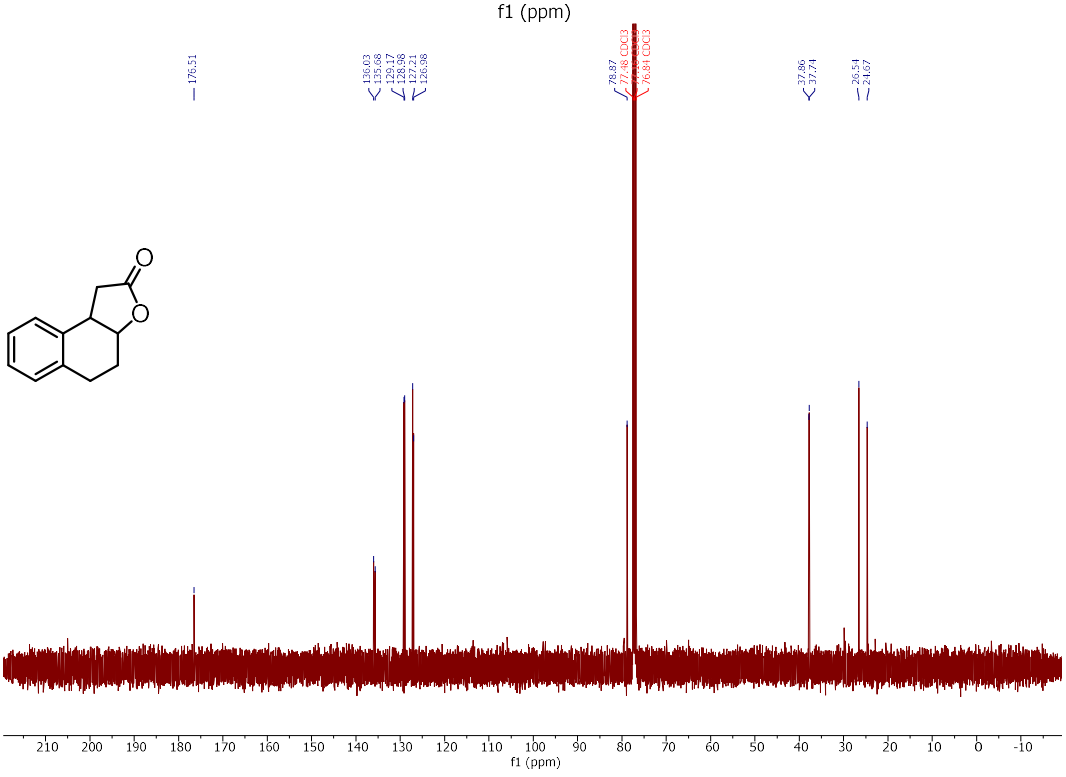
7-methoxy-5',6'-dihydrospiro[isochromane-1,4'-pyran]-2'(3'H)-one (8d)



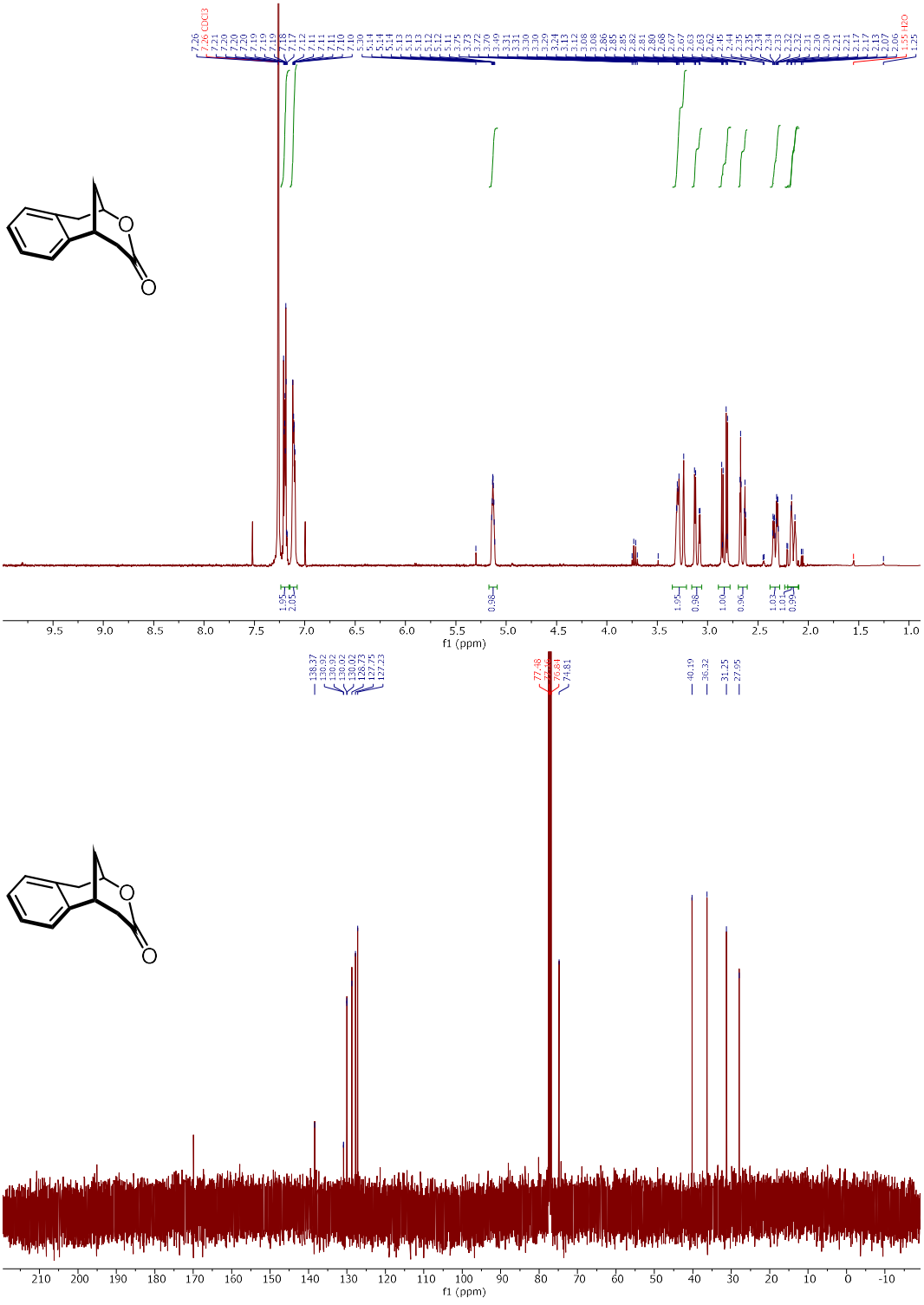


3a,4,5,9b-tetrahydronaphtho[2,1-*b*]furan-2(1*H*)-one (8e)

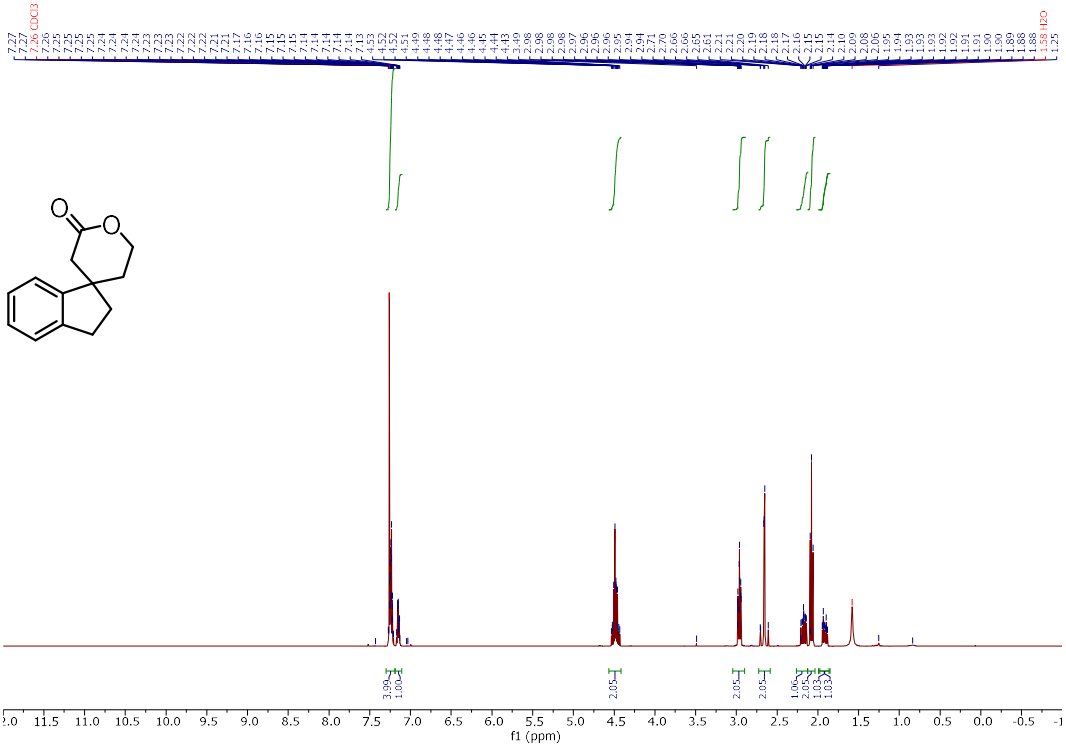


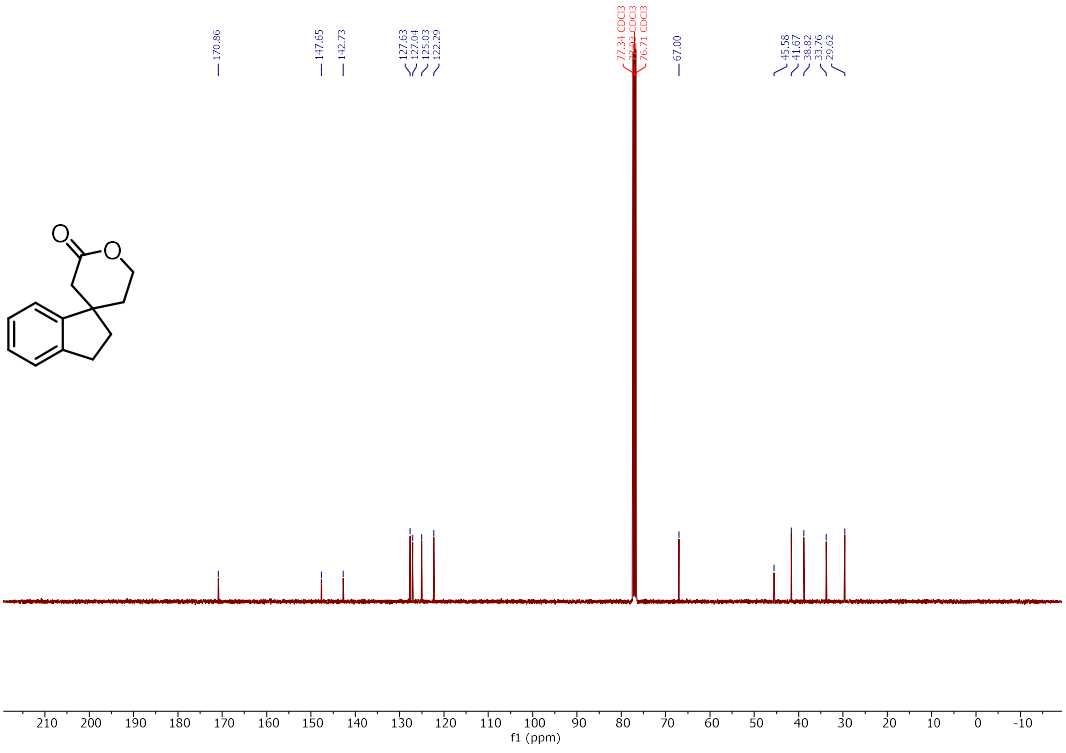


1,2,5,6-tetrahydro-4*H*-2,6-methanobenzo[*d*]oxocin-4-one (8e')

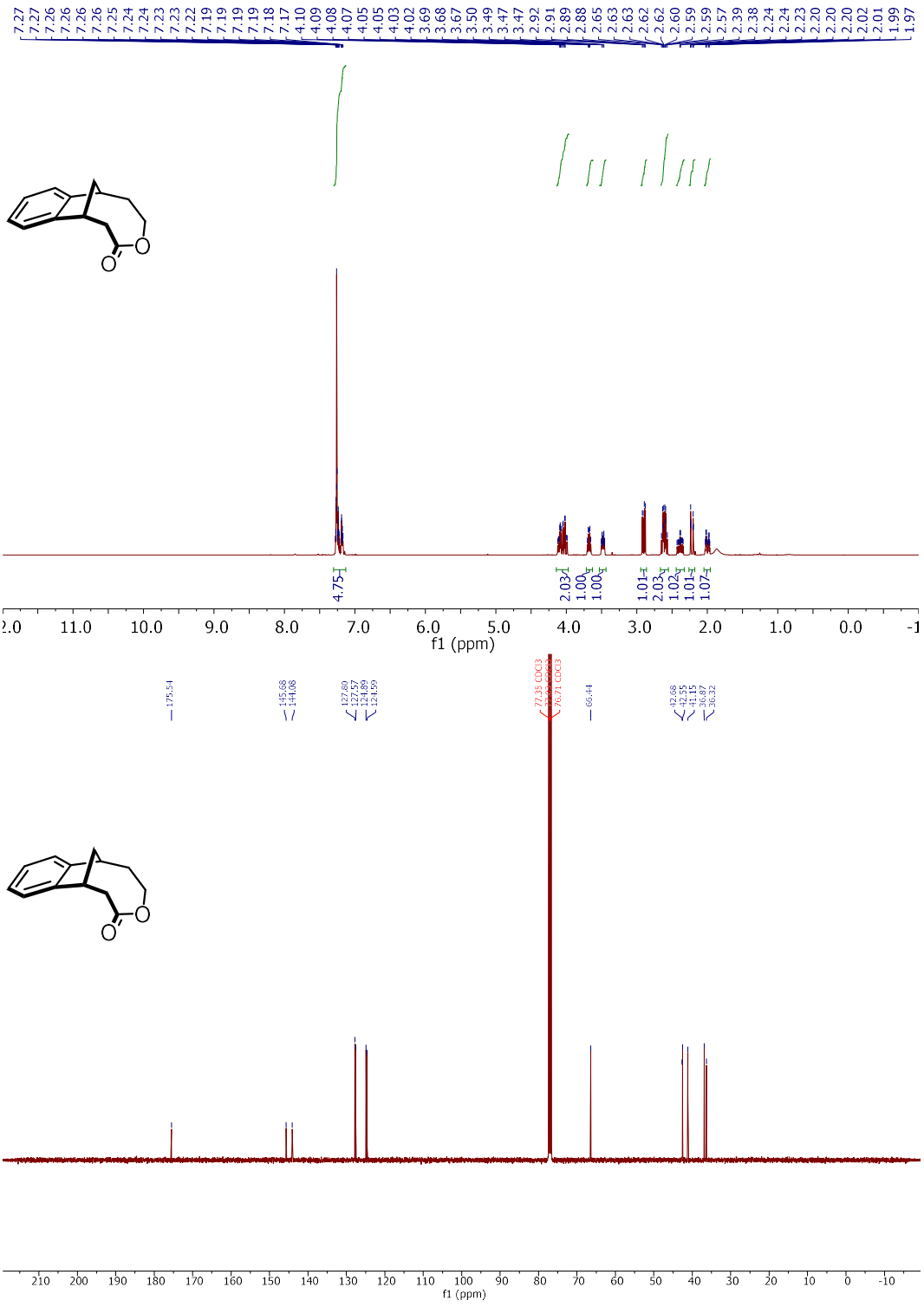


2,3,5',6'-tetrahydrospiro[indene-1,4'-pyran]-2'(3'*H*)-one (8f)





1,5,6,7-tetrahydro-1,7-methanobenzo[*e*]oxonin-3(2*H*)-one (8f')



10. References

1 Gibson, D. G. *et al.* Enzymatic Assembly of DNA Molecules up to Several Hundred Kilobases. *Nat. Methods* **6**, 343-345 (2009).

2 Kille, S. *et al.* Reducing Codon Redundancy and Screening Effort of Combinatorial Protein Libraries Created by Saturation Mutagenesis. *ACS Synth. Biol.* **2**, 83–92 (2013).

3 Barr, I. & Guo, F. Pyridine Hemochromagen Assay for Determining the Concentration of Heme in Purified Protein Solutions. *Bio. Protoc.* **5**, e1594 (2015).

4 Zhou, A. Z., Chen, K. & Arnold, F. H. Enzymatic Lactone-Carbene C–H Insertion to Build Contiguous Chiral Centers. *ACS Catal.* **10**, 5393-5398 (2020).

5 Chen, K. & Arnold, F. H. Engineering Cytochrome P450s for Enantioselective Cyclopropenation of Internal Alkynes. *J. Am. Chem. Soc.* **142**, 6891-6895 (2020).

6 Jia, Z. J., Gao, S. & Arnold, F. H. Enzymatic Primary Amination of Benzylic and Allylic C(s*p3*)–H Bonds. *J. Am. Chem. Soc.* **142**, 10279–10283 (2020).

7 Yoshinaga, Y., Yamamoto, T. & Suginome, M. Chirality-Switchable 2,2'-Bipyridine Ligands Attached to Helical Poly(quinoxaline-2,3-diyl)s for Copper-Catalyzed Asymmetric Cyclopropanation of Alkenes. *ACS Macro Lett* **6**, 705-710 (2017).

8 Wang, X. D. *et al.* Copper-Catalyzed Cascade Cyclization Reactions of Diazo Compounds with tert-Butyl Nitrite and Alkynes: One-Pot Synthesis of Isoxazoles. *J. Org. Chem.* **84**, 16214-16221 (2019).

9 Doyle, M. P. & Hu, W. Enantioselective Carbon–Hydrogen Insertion is an Effective and Efficient Methodology for the Synthesis of (R)-(-)-Baclofen. *Chirality* **14**, 169-172 (2002).

10 Nani, R. R. & Reisman, S. E. alpha-Diazo-beta-ketonitriles: uniquely reactive substrates for arene and alkene cyclopropanation. *J. Am. Chem. Soc.* **135**, 7304-7311 (2013).

11 Zhu, K. *et al.* Visible light-induced carbene reactivity of acceptor diazoalkanes: deconstructive difunctionalizations of cyclic ethers with nucleophiles. *Chem. Commun.* **59**, 631-634 (2023).

12 Feng, C. G., Wang, Z. Q., Tian, P., Xu, M. H. & Lin, G. Q. Easily accessible C2-symmetric chiral bicyclo[3.3.0] dienes as ligands for rhodium-catalyzed asymmetric 1,4-addition. *Asian J. Chem.* **3**, 1511-1516 (2008).

13 Khiar, N. *et al.* Asymmetric Rhodium-Catalyzed 1,4- and 1,2-Additions of Arylboronic Acids to Activated Ketones in Water at Room Temperature Using a Mixed Sulfur-Olefin Ligand. *Adv. Synth. Catal.* **355**, 1303-1307 (2013).

14 Díaz-Rodríguez, A. *et al.* From Diols to Lactones under Aerobic Conditions using a Laccase/TEMPO Catalytic System in Aqueous Medium. *Adv. Synth. Catal.* **354**, 3405-3408 (2012).

15 Smith, A. J., Abbott, L. K. & Martin, S. F. Enantioselective Conjugate Addition Employing 2-Heteroaryl Titanates and Zinc Reagents. *Org. Lett.* **11**, 4200-4203 (2009).

16 Le Notre, J., Allen, J. C. & Frost, C. G. Enantioselective rhodium-catalysed 1,4-additions of 2-heteroarylzinc donors using Me-DUPHOS. *Chem. Commun.*, 3795-3797 (2008).

17 Wu, W. *et al.* Asymmetric Baeyer-Villiger oxidation: classical and parallel kinetic resolution of 3-substituted cyclohexanones and desymmetrization of meso-disubstituted cycloketones. *Chem. Sci.* **10**, 7003-7008 (2019).

18 Zhou, Y. *et al.* Facile access to chiral gamma-butyrolactones via rhodium-catalysed asymmetric hydrogenation of gamma-butenolides and gamma-hydroxybutenolides. *Chem. Sci.* **14**, 4888-4892 (2023).
19 Wang, Y., Hu, X. & Du, H. Vicinal-Diamine-Based Chiral Chain Dienes as Ligands for Rhodium(I)-Catalyzed Highly Enantioselective Conjugated Additions. *Org. Lett.* **12**, 5482-5485 (2010).
20 Sheldrick, G. M. Phase Annealing in SHELX-90: Direct Methods for Larger Structures. *Acta Cryst.* **A46**, 467-473 (1990).
21 Sheldrick, G. M. Crystal Structure Refinement With SHELXL. *Acta Cryst.* **C71**, 3-8 (2015).
22 Müller, P. Practical Suggestions for Better Crystal Structures. *Crystallogr. Rev.* **15**, 57-83 (2009).