Site-Selective Functionalization of (sp^3)C–H Bonds Catalyzed by Artificial Metalloenzymes Containing an Iridium-Porphyrin Cofactor

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Supporting Information

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1.1: Protein Expression, Purification

General Methods. Unless otherwise noted, the chemicals, salts, and solvents used were reagent grade and used as received from commercial suppliers without further purification. All expression media and buffers were prepared using ddH_2O (MilliQ A10 Advantage purification system, Millipore). Expression media was sterilized either by autoclave (45 min, 121^oC) or a sterile syringe filter (0.22 um). To maintain sterile conditions, sterile materials and E. coli cells were manipulated near a lit Bunsen burner.

Genes and Cloning. The WT CYP119 gene was cloned into the vector 2BT (6xHis-TEV-ORF; AddGene #29666), which was purchased from GenScript with codon optimization for *E. coli*.

Media Preparation. Preparation of the optimized minimal expression media: Salts (15 g Na_2HPO_4, 7.5 g K_2HPO_4, 0.3 g NaH_2PO_4, 0.3 g KH_2PO_4, 1.5 g NaCl, 5.0 g NH_4Cl) were dissolved in 2 L ddH_2O and autoclaved to give a media with pH ~8.0 - 8.2. Solutions of glucose (20%), casamino acids (BD Company, low Fe, 20%), and $MgSO_4$ (1 M), were autoclaved separately. Solutions of ampicillin (100 mg/ mL) and $CaCl_2$ (1 M) were sterilized by syringe filter. The following amounts of the listed solutions were added per 2 L of sterile salt solution: 40 mL glucose, 20 mL casamino acids, 4 mL $MgSO_4$, 100 uL $CaCl_2$, 2 mL ampicillin. Stock solutions of antibiotics were stored for several weeks; prepared media was stored for less than 1 day. Minimal media plates or Lysogeny broth were prepared as previously described.[1]

CYP119 Protein Expression. Optimized expression of Apo CYP119: BL21 Star competent *E. coli* cells (50 uL, QB3 Macrolab, UC Berkeley) were thawed on ice, transferred to 14 mL Falcon tubes, and transformed with the desired plasmid solution (2 uL, 50-250 ng/uL). The cells were incubated on ice (30 min), heat shocked (30 sec, 42°C), re-cooled on ice (2 min), and recovered with supper optimal broth (SOB) media (37 °C, 1 h, 250 rpm). Aliquots of the cultures (100 uL) were plated on minimal media plates (expression media supplemented with 17 g agar/L) and incubated (20 h, 37 °C) to produce approximately 10-100 colonies per plate. Single colonies were used to inoculate starter cultures (4 mL, expression media), which were grown (6-8 h, 37°C, 275 rpm) and used to inoculate 100 mL cultures for overnight growth (minimal media, 37° C, 275 rpm). Each culture grown overnight was used to inoculate 750 mL of minimal media, which was then grown further (9 h, 37 °C, 275 rpm). Expression was induced with IPTG (800 uL, 1M), and the cultures were grown further (15 h, 30 °C, 275 rpm). Cells were harvested by centrifugation (5000 rpm, 15

min, 4° C), and the pellets were resuspended in 25 mL Ni-NTA lysis buffer (50 mM NaPi, 250 mM NaCl, 10 mM Imidazole, pH = 8.0) and stored at -80 °C until purification.

Protein Purification. Cell suspensions were thawed in a room-temperature water bath, decanted into 50 mL glass beakers, and lysed on ice by sonication (3x30 sec on, 2x2 min off, 65% power). Cell debris was removed by centrifugation (10,000 rpm, 30 min, 4 °C), and Ni-NTA (5 mL, 50% suspension per 850 mL cell culture) was added. The lysates were briefly incubated with Ni-NTA (30 min, rt, 20 rpm) and poured into glass frits (coarse, 50 mL). The resin was washed with Ni-NTA lysis buffer (3 x 35 mL), and the wash fractions were monitored using a Bradford assay dye. The desired protein was eluted with 18 mL Ni-NTA elution buffer (50 mM NaPi, 250 mM NaCl, 250 mM Imidazole, pH = 8.0), dialyzed twice against Tris buffer (10 mM, pH = 8.0, 1 h, rt). Gel electrophoreses was then preformed to monitor protein purify (Figure S1). Purified samples were then concentrated using a spin concentrator, and metallated within several hours. Apo protein was not stored for more than 8 hours.

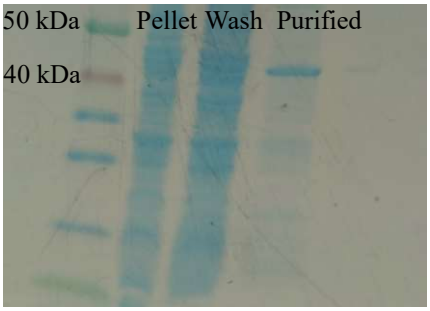


Figure S1. Representative SDS-Page gel ran at 300 V.

After following the procedure above, apo-protein samples for spectroscopic characterization were purified further on an ÄKTA FPLC system using a 5 mL GE HisTrap HP column. Proteins were loaded using a Tris Buffer (100 mM, 50 mM NaCl, 30 mM Imidazole, pH = 7.0) and eluted with a steady gradient increase of an imidazole rich Tris buffer (100 mM, 50 mM NaCl, 500 mM Imidazole, pH = 7.0) over 30 column volumes. Fractions containing the desired protein were then pooled together and spin concentrated.

Metallation of the Apo-CYP119 (General Method). A stock solution of Ir(Me)-MPIX cofactor dissolved in DMF (5.7 mM) was added to solutions of apo-CYP119 in Tris buffer (10 mM, pH = 8.0) at room temperature. The ratio of CYP119 to cofactor was always ≥2:1 and the final DMF concentration ≤ 2%. The Ir(Me)-CYP119 mixture was briefly incubated at room temperature (5 minutes), and

DMF was removed by using a NAP column equilibrated with the NaPi buffer (100 mM NaPi, 100 mM NaCl, pH = 6.0).

Protein Storage. Glycerol was added to a solution of the protein (3:1 v:v protein solution: 50% glycerol), the solution was divided into 1.5 mL Eppendorf tubes (0.5 mL per aliquot), and the tubes were flash frozen in liquid nitrogen and stored at -80 °C until further use. Frozen aliquots of the protein were thawed in a room-temperature water bath

2.1 CYP119 Mutant Library Construction

Library construction by Site-directed mutagenesis

Site-directed mutagenesis was performed using the QuickChange Lightning mutagenesis kit (Agilent); requisite double-stranded DNA primers were designed according to the Agilent Primer Design Program and purchased from Integrated DNA Technology. PCR reactions were performed according to the manufacturer's directions. PCR reactions contained 5 uL reaction buffer, 34 uL ddH_2O, 1.5 uL QuickSolution, 1 uL plamids (50 ng/uL), 1.25 uL forward primer (100 ng/uL), 1.25 uL reverse primer (100 ng/ uL), 5 uL dNTPs (2 mM/base), and 1 uL polymerase.

PCR Program: Phase 1 (1 cycle): 95 °C, 1.5 min; Phase 2 (18 cycles): 95 °C, 20 sec, 60 °C, 10 sec, 68 °C, 4.5 min; Phase 3 (1 cycle): 68 °C, 3 min; Phase 4 (storage): 4 °C.

DNA Isolation and Storage: Following the completion of the above set of PCR procedures, 1.5 uL DPN I was added to each reaction, and the reactions were further incubated (3 h, 37 °C). The crude PCR mixture was used to transform XL-1 Blue Ultracompetent cells (45 uL cells, 2 uL PCR reactions, QB3 Macrolab, UC Berkeley). The mixture was incubated on ice (30 min), heat shocked (30 s, 42 °C), recovered with SOB media (1 h, 37 °C, 275 rpm), and plated on LB plates. Plates were grown (18 h, 37 °C), and individual colonies were used to inoculate 1 mL LB cultures, which were grown in 96-well plates (13 h, 37 °C, 300 rpm). DNA was isolated from the 96-well cultures using magnetic bead technology at the UC Berkeley DNA Sequencing Facility. Alternatively, individual colonies were used to inoculate 4 mL LB and grown overnight (13 h, 37 °C, 300 rpm), and the plasmids were purified using a Qiagen DNA Miniprep kit (Cat No./ID: 27106) according to the manufacturer's instructions.

Primers sequence: all the primers used in the site-directed mutagenesis were designed from the QuikChange® Primer Design Program provided by Agilent. The primers used in this paper as the following ones:

Table S1. The sequence of the primers used in this paper

mutant	Forward primer	Reverse primer
A152L	CGGCTAGCTCCTGTTCCTTTTAAGGATCTTACCTCGCTAGACC	GCCGATCGAGGACAAGGAAAATTCCTAGAATGGAGCGATCTGG
A152V	AGCTCCTGTTCCTTTTAAGCACCTTACCTCGCTAGACCAA	TCGAGGACAAGGAAAATTCGTGGAATGGAGCGATCTGGTT
A152G	AGCTCCTGTTCCTTTTAAGCCCCTTACCTCGCTAGACCAA	TCGAGGACAAGGAAAATTCGGGGAATGGAGCGATCTGGTT
A152Y	GCTAGCTCCTGTTCCTTTTAAGATACTTACCTCGCTAGAC	CGATCGAGGACAAGGAAAATTCTATGAATGGAGCGATCTG
A152W	AGCTCCTGTTCCTTTTAAGACCCTTACCTCGCTAGACCAA	TCGAGGACAAGGAAAATTCTGGGAATGGAGCGATCTGGTT
A152F	CGGCTAGCTCCTGTTCCTTTTAAGAAGCTTACCTCGCTAGACC	GCCGATCGAGGACAAGGAAAATTCTTCGAATGGAGCGATCTGG
A152T	GCTAGCTCCTGTTCCTTTTAAGTGCCTTACCTCGCT	CGATCGAGGACAAGGAAAATTCACGGAATGGAGCGA
A209V	ATTCTGCTGCTGATTGTGGGCAACGAAGGCAC	GTGCCTTCGTTGCCCACAATCAGCAGCAGAAT
A209L	GTGCCTTCGTTGCCTAGAATCAGCAGCAGAATGTAACCCAG	CTGGGTTACATTCTGCTGCTGATTCTAGGCAACGAAGGCAC
A209F	GTGCCTTCGTTGCCGAAAATCAGCAGCAGAATGATGTAACCCAG	CTGGGTTACATCATTCTGCTGCTGATTTTCGGCAACGAAGGCAC
A209W	CTGGGTTACATCATTCTGCTGCTGATTTATGGCAACGAAGGCAC	GTGCCTTCGTTGCCATAAATCAGCAGCAGAATGATGTAACCCAG
A209Y	GTGCCTTCGTTGCCATAAATCAGCACCAGAATGATGTAACCCAG	CTGGGTTACATCATTCTGGTGCTGATTTATGGCAACGAAGGCAC
A209G	GCCTTCGTTGCCCCCAATCAGCAGCAGA	TCTGCTGCTGATTGGGGGCAACGAAGGC
A209T	CTTCGTTGCCCGTAATCAGCAGCAGAATGATGTAAC	GTTACATCATTCTGCTGCTGATTACGGGCAACGAAG
L155A	AGGAAAATTCAAGGAATGGGCCGATCTGGTTGCGTTTCGT	TCCTTTTAAGTTCCTTACCCGGCTAGACCAACGCAAAGCA
L155V	AAGGAAAATTCAAGGAATGGGTCGATCTGGTTGCGTTTCGTC	TTCCTTTTAAGTTCCTTACCCAGCTAGACCAACGCAAAGCAG
L155G	GGAAAATTCAAGGAATGGGGCGATCTGGTTGCGTTTC	AAGGAAAATTCAAGGAATGGGTCGATCTGGTTGCGTTTCGTC
L155Y	ACAAGGAAAATTCAAGGAATGGTATGATCTGGTTGCGTTTCGTCTGG	TGTTCCTTTTAAGTTCCTTACCATACTAGACCAACGCAAAGCAGACC
L155W	AGGAAAATTCAAGGAATGGTGGGATCTGGTTGCGTTTCGTC	TCCTTTTAAGTTCCTTACCACCCTAGACCAACGCAAAGCAG
L155T	AGGAAAATTCAAGGAATGGACCGATCTGGTTGCGTTT	TCCTTTTAAGTTCCTTACCTGGCTAGACCAACGCAAA
L155F	AAGGAAAATTCAAGGAATGGTTCGATCTGGTTGCGTTTCGTC	TTCCTTTTAAGTTCCTTACCAAGCTAGACCAACGCAAAGCAG
L318A	CACCTGAGCTTCGCTAGCGGCATCCAC	GTGGACTCGAAGCGATCGCCGTAGGTG
L318V	GCACCTGAGCTTCGTTAGCGGCATCCACC	CGTGGACTCGAAGCAATCGCCGTAGGTGG
L318G	ACCTGAGCTTCGGGAGCGGCATCCACC	TGGACTCGAAGCCCTCGCCGTAGGTGG
L318Y	CCGCACCTGAGCTTCTATAGCGGCATCCACCT	GGCGTGGACTCGAAGATATCGCCGTAGGTGGA
L318W	CGCACCTGAGCTTCTGGAGCGGCATCCACCT	GCGTGGACTCGAAGACCTCGCCGTAGGTGGA
L318T	CGCACCTGAGCTTCACTAGCGGCATCCACC	GCGTGGACTCGAAGTGATCGCCGTAGGTGG
L318F	CCGCACCTGAGCTTCTTTAGCGGCATCCACCT	GGCGTGGACTCGAAGAAATCGCCGTAGGTGGA
F310A	CGAGAAATTTATCCCGGATCGTGCCCCGAACCCGCA	GCTCTTTAAATAGGGCCTAGCACGGGGCTTGGGCGT
F310V	CGAGAAATTTATCCCGGATCGTGTCCCGAACCCGCA	GCTCTTTAAATAGGGCCTAGCACAGGGCTTGGGCGT
F310G	CGAGAAATTTATCCCGGATCGTGGCCCGAACCCGCA	GCTCTTTAAATAGGGCCTAGCACCGGGCTTGGGCGT
F310Y	GAAATTTATCCCGGATCGTTATCCGAACCCGCACCTG	CTTTAAATAGGGCCTAGCAATAGGCTTGGGCGTGGAC
F310W	GAGAAATTTATCCCGGATCGTTGGCCGAACCCGCACCTGA	CTCTTTAAATAGGGCCTAGCAACCGGCTTGGGCGTGGACT
F310T	AGAAATTTATCCCGGATCGTACCCCGAACCCGCA	TCTTTAAATAGGGCCTAGCATGGGGCTTGGGCGT
F310L	GCGAGAAATTTATCCCGGATCGTCTACCGAACCCGCACCTGAG	CGCTCTTTAAATAGGGCCTAGCAGATGGCTTGGGCGTGGACTC
L69A	CCGACCCGTTATACCGCGCTGACCAGCGACCC	GGCTGGGCAATATGGCGCGACTGGTCGCTGGG
L69F	CGACCCGTTATACCTTCCTGACCAGCGACCC	GCTGGGCAATATGGAAGGACTGGTCGCTGGG
L69G	CCGACCCGTTATACCGGGCTGACCAGCGACCC	GGCTGGGCAATATGGCCCGACTGGTCGCTGGG
L69T	TCCGACCCGTTATACCACGCTGACCAGCG	AGGCTGGGCAATATGGTGCGACTGGTCGC
L69W	CCGACCCGTTATACCTGGCTGACCAGCGACCC	GGCTGGGCAATATGGACCGACTGGTCGCTGGG
L69V	CGACCCGTTATACCGTGCTGACCAGCGAC	GCTGGGCAATATGGCACGACTGGTCGCTG
L69Y	TCCGACCCGTTATACCTATCTGACCAGCGACCCGC	AGGCTGGGCAATATGGATAGACTGGTCGCTGGGCG
V254A	AGCGATTGAGGAAGCACTGCGTTATAGCCCG	TCGCTAACTCCTTCGTGACGCAATATCGGGC
V254F	CTGTACCTGAAAGCGATTGAGGAATTCCTGCGTTATAGCCCGC	GACATGGACTTTCGCTAACTCCTTAAGGACGCAATATCGGGCG
V254G	AAGCGATTGAGGAAGGGCTGCGTTATAGCCC	TTCGCTAACTCCTTCCCGACGCAATATCGGG
V254T	TGTACCTGAAAGCGATTGAGGAAACGCTGCGTTATA	ACATGGACTTTCGCTAACTCCTTTGCGACGCAATAT
V254W	TGTACCTGAAAGCGATTGAGGAATGGCTGCGTTATAGCC	ACATGGACTTTCGCTAACTCCTTACCGACGCAATATCGG
V254L	CTGTACCTGAAAGCGATTGAGGAACTACTGCGTTATAGCCCGC	GACATGGACTTTCGCTAACTCCTTGATGACGCAATATCGGGCG
V254Y	CTGTACCTGAAAGCGATTGAGGAATATCTGCGTTATAGCCCGC	GACATGGACTTTCGCTAACTCCTTATAGACGCAATATCGGGCG
I208A	CGATATCGAGAAGCTGGCTTACATCATTCTGCTGC	GCTATAGCTCTTCGACCGAATGTAGTAAGACGACG
I208V	CGATATCGAGAAGCTGGTTTACATCATTCTGCTGC	GCTATAGCTCTTCGACCAAATGTAGTAAGACGACG
I208F	GAGCGATATCGAGAAGCTGTTTTACATCATTCTGCTGCTG	CTCGCTATAGCTCTTCGACAAAATGTAGTAAGACGACGAC
I208G	GATATCGAGAAGCTGGGGTACATCATTCTGCTGCT	CTATAGCTCTTCGACCCCATGTAGTAAGACGACGA
I208T	GAGCGATATCGAGAAGCTGACTTACATCATTCTGCTGCTG	CTCGCTATAGCTCTTCGACTGAATGTAGTAAGACGACGAC
I208W	GAGCGATATCGAGAAGCTGTGGTACATCATTCTGCTGCTGA	CTCGCTATAGCTCTTCGACACCATGTAGTAAGACGACGACT
I208L	CTGAGCGATATCGAGAAGCTGCTATACATCATTCTGCTGCTGATT	GACTCGCTATAGCTCTTCGACGATATGTAGTAAGACGACGACTAA
I208Y	GAGCGATATCGAGAAGCTGTATTACATCATTCTGCTGCTG	CTCGCTATAGCTCTTCGACATAATGTAGTAAGACGACGAC
L205A	CAACCTGAGCGATATCGCGAAGCTGGGTTACATCA	GTTGGACTCGCTATAGCGCTTCGACCCAATGTAGT
L205V	CAACCTGAGCGATATCGTGAAGCTGGGTTACATCA	GTTGGACTCGCTATAGCACTTCGACCCAATGTAGT
L205F	AACAGCAACCTGAGCGATATCTTCAAGCTGGGTTACATCATTCTG	TTGTCGTTGGACTCGCTATAGAAGTTCGACCCAATGTAGTAAGAC
L205G	CAACCTGAGCGATATCGGGAAGCTGGGTTACATCA	GTTGGACTCGCTATAGCCCTTCGACCCAATGTAGT
L205T	CAGCAACCTGAGCGATATCACGAAGCTGGGTTACATCATT	GTCGTTGGACTCGCTATAGTGCTTCGACCCAATGTAGTAA
L205W	CAGCAACCTGAGCGATATCTGGAAGCTGGGTTACATCATT	GTCGTTGGACTCGCTATAGACCTTCGACCCAATGTAGTAA
L205Y	CAGCAACCTGAGCGATATCTATAAGCTGGGTTACATCATTC	GTCGTTGGACTCGCTATAGATATTCGACCCAATGTAGTAAG

Library expression, lysis and metalation from site-directed mutagenesis with 24-well plates

BL21 Star competent *E. coli* cells (50 uL, QB3 Macrolab, UC Berkeley) were thawed on ice, transferred to 14 mL Falcon tubes, and transformed with the desired plasmid solution (2 uL, 50-250 ng/uL). The cells were incubated on ice (30 min), heat shocked (30 sec, 42°C), re-cooled on ice (2 min), and recovered with SOC media (37 °C, 1 h, 250 rpm). Aliquots of the cultures (100 uL) were plated on M9 media plates and incubated (20 h, 37 °C) to produce approximately 10-100 colonies per plate. Single colonies were used to inoculate starter cultures (LB media). For each 24 well plate, 4 wells were used as a positive control containing a previously evolved CYP119 mutant that catalyzes the reaction with high selectivity. The 0.1 mL overnight seed culture was used to inoculate pre-warmed, MagicMedia TM (4 ml containing 100 ug/mL ampicillin). The resulting cell culture was shaken at 37 °C for 8 h at 300 rpm and then 25 °C for 24 h at 300 rpm. The cultures were harvested by centrifugation (4000 rpm, 5min, 4 ºC) and frozen at -80 ºC before further manipulation.

Cells were pelleted by centrifugation (4000 RPM, 10 min, 4 °C) and resuspended in 1 mL of lysis buffer. The lysis buffer contained 10 mM tris buffer (pH = 8), 1 mg/mL lysozyme, 500 µg/mL polymixin B sulfate (PMBS), and 25 U/mL Benzonase® Nuclease. The cell mixtures were shaken at rt for 1 hour before centrifuging (4 ºC, 4000 rpm) for 15 min. The supernatant was transferred to glass vials arrayed in a 96-well plate, and 4 uL of a solution of the Ir(Me)-PIX cofactor (4.4 mg/mL in DMF) was added to each vial. After binding the cofactor at 4 ºC for 1 hour, the stock solution was used directly for the reactions.

Library construction by error-prone PCR

Mutant libraries were constructed by conducting error-prone PCR using the GeneMorph II EzClone Domain Mutagenesis Kit (Agilent catalog #200552).

Mutant Megaprimer Synthesis: Megaprimer synthesis reactions contained 5 µL of 10× Mutazyme II reaction buffer, 5µL template, 1 µL of sample primers (125 ng/µL of each primer), 1 µL of 40 mM dNTP mix (200 µM each final), 35 µL of ddH_2O, and 1 µL of Mutazyme II DNA polymerase (2.5 U/µl).

PCR Program: Phase 1 (1 cycle): 95 °C, 2 min; Phase 2 (30 cycles): 95 °C, 30 sec, 60 °C, 30 sec, 72 °C, 5 min; Phase 3 (1 cycle): 72 °C, 10 min.

EZClone Reaction: EZClone reactions contained 25 µL of the 2×EZClone enzyme

mix, 50 ng of template plasmid, 500 ng of megaprimer from megaprimer synthesis, 3 μL of EZClone solution, and the appropriate volume of ddH_2O to give a final volume of 50 μL.

PCR Program: Phase 1 (1 cycle): 95 °C, 2 min; Phase 2 (25 cycles): 95 °C, 50 sec, 60 °C, 50 sec, 68 °C, 10 min.

Primers sequence:

Forward primer: GACTGGTCCAGCGAGATGCGTAAGAAG

Reverse primer: CACCACCAGACGCTTGTAACCATTCAGAAC

Generation of Ir(Me)-CYP119 constructs from error-prone PCR.

1 μL of the EZClone reaction mixture were added to 25 μL of E. cloni® EXPRESS BL21(DE3) competent cells (Lucigen catalog # 60300-1) on ice. 25 μL of the cell/DNA mixture was carefully pipetted into a chilled electroporation cuvette without introducing bubbles. Within 10 seconds of the pulse, 975 μL of Expression Recovery Medium was added to the cuvette, and the cells were re-suspended by pipetting up and down three times. The cells and recovery medium were transferred to a culture tube. The culture tube was placed in a shaking incubator at 250 rpm for 1 hour at 37 °C. 100 uL the mixture of cells and recovery medium was plated on LB agar plates containing 100 ug/mL ampicillin. The plates were incubated overnight at 37 °C. Individual colonies were used to inoculate 0.5 mL Magicmedia™ medium (Invitrogen catalog # K6815) containing 100 ug/mL ampicillin. The cell cultures were grown in 96-well plates (13 h, 37 °C, 300 rpm). In each 96-well plate, 4 wells were inoculated with colonies containing the parent plasmids as positive control. The 0.1 mL overnight seed culture was used to inoculate pre-warmed, MagicMedia™ (4 ml containing 100 ug/mL ampicillin). The resulting cell culture was shaken at 37 °C for 8 hours at 300 rpm and then 25 °C for 24 h at 300 rpm. The cultures were harvested by centrifugation (4000 rpm, 5min, 4 ºC) and frozen at -80 ºC before further manipulation. Cells were lysed by resuspending in 250 μL lysis buffer, which contained 10 mM tris buffer, pH = 8.0, 1 mg/mL lysozyme, 500 μg/mL polymixin B sulfate (PMBS), and 25 U/mL Benzonase® Nuclease. The cell mixtures were shaken at rt for 1 hour before centrifuging the mixture for 15 min (4 ºC, 4000 rpm). The supernatant was transferred to glass vials arrayed in a 96-well plate, and 1 uL of a solution of the Ir(Me)-PIX cofactor (4.4 mg/mL in DMF) was added to each vial. After binding the cofactor at 4 ºC for 1 hour, the stock solution was used directly for the reactions.

If any of the mutants catalyze the reaction of **1a** with EDA with higher selectivity than any of the prior mutants, then the plasmid was amplified from the separate cell culture

and purified using a Qiagen DNA Miniprep kit (Cat No./ID: 27106) according to the manufacturer's instructions. After sequencing the plasmid, the improved mutant was used for the next round of evolution.

3.1: Catalytic Experiments

General Methods. Unless otherwise noted, catalytic reactions were performed in 4 mL individually capped vials or in 1.2 mL vials as part of a sealed 96-well array. Reactions were either (1) assembled under a nitrogen atmosphere wet box or (2) assembled on the bench. In the latter case, the head space of the vial was purged with nitrogen through a septum cap. Solutions of Ir(Me)-PIX-CYP119 were gently degassed on a Schlenk line (3 cycles vacuum/refill) before being pumped into a glove box in sealed vials. Organic reagents were added as stock solutions in DMF, such that the final amount of DMF in the reaction was approximately 2% by volume (unless noted otherwise). Protein catalysts were diluted to reaction concentrations in sodium phosphate buffer (100 mM, pH = 6.0) before being added to reaction vials. Unless otherwise noted, all reactions were performed with catalysts generated from a 1:2 ratio of Ir(Me)-cofactor:apo CYP119 protein, with 0.05 mol % catalyst loading, based on the ratio of limiting reagent to metal cofactor. Unless otherwise noted, all reactions were conducted in a shaking incubator (20 °C, 16 h, 275 rpm).

Procedure for typical catalytic experiments: The Ir(Me)-CYP119 catalyst was prepared by addition of a stock solution of Ir(Me)-PIX (3.1 mM in DMF) to a solution of the apo CYP119 protein (0.13 mM in 10 mM Tris buffer, pH = 8.0), such that the resulting solution had a 1 : 2 ratio of Ir(Me)PIX : CYP119. This ratio was used to ensure no free Ir(Me)-PIX in solution. The mixture was incubated for 5 min and desalted with a NAP-10 desalting column equilibrated with reaction buffer (100 mM NaPi, 100 mM NaCl, pH = 6.0). The protein mixture was diluted to the required reaction concentration with the same reaction buffer.

Screening the Mutants in 96-well plates or 24-well plates: 5 uL of phthalan derivatives (0.5 M in DMF solution) and 5 uL of EDA (60% vol in DMF) were added to the 250 uL of the catalyst stock solution in 96-well plates. The 96-well array was fitted with a cover that is attached by screws, incubated in a shaker (20 °C, 275 rpm) for 16 h. After the conclusion of the reaction, the reaction mixture was extracted with ethyl acetate and analyzed as described. The reaction with the most selective mutants were repeated with purified proteins according the following procedure.

Table S2. Representative data on site selectivities from reactions run in a 24-well plate.

ratio	1	2	3	4	5	6
A	1.3	1	1	0.9	0.9	1.1
B	1	0.9	1	0.9	1	1.9
C	2.7	2.3	2.2	0.9	0.9	1
D	0.9	0.9	1.7	2	1.2	2.1

Numbers: the ratio of **2a** to **3a**; red one: parent mutant; green: hit mutants.

Table S3. Directed-evolution pathway

run number	Parent plamides	mutanted position	the mutangenies methods	screening methods	numbers of mutant (colonies)	better mutants
1	T213G, C317G	69, 209, 254, 208, 205	Site-directed mutagenesis	cell lysis	36	V254A,T213G,C317G for meta V254L, T213G, C317G for para
2	V254A,T213G,C317G; V254L, T213G, C317G	209, 152, 155, 310, 318	Site-directed mutagenesis	cell lysis	36	A152L, V254A,T213G,C317G for meta A152F,V254L, T213G, C318G for para
3	A152L, V254A,T213G,C317G; A152F,V254L, T213G, C318G	208, 310, 318, 155, 209, 205, 69	Site-directed mutagenesis	cell lysis	48	no better one found
4	A152L, V254A,T213G,C317G; A152F,V254L, T213G, C318G	random	error-prone PCR	cell lysis	900 colonies	10 mutants for meta selectivity; 6 mutants for para seletivity
5	The mutants found in run 4	random	error-prone PCR	cell lysis	1350 colonies	no better one found for meta selectivity; 5 mutants found for para selectivity
6	The mutants found in run 4 and run 5	no new mutants		purified proteins with slow addition of EDA	21 mutants	A247S,A152L, V254A,T213G,C317G for meta; D117H, H340L, A152F,V254L, T213G, C318G for para

Slow addition of EDA to the reaction mixture with purified proteins: 1 mL of the catalyst stock solution (0.005 mM to 0.0005 mM Ir(Me)CYP119 in 100 mM NaPi, 100 mM NaCl, pH = 6.0) was added to a 4 mL vial containing a micro stir bar. The vial was sealed with a cap containing a septum, 10 uL phthalan derivatives (0.5 M in DMF solution) were added to the vial. The reaction vial was covered with septa caps and removed from the glove box. A syringe pump outfitted with a series of gas tight syringes was used to add the solution of EDA (10 uL of 60% vol DMF solution) to each reaction vial over 1 hour. After this time, the reaction mixture was extracted with ethyl acetate and analyzed as described.

Scale up of the reactions with 1a and 1b:

60 mL of the catalyst stock solution (0.0025 mM Ir(Me)CYP119 in 100 mM NaPi, 100 mM NaCl, pH = 6.0) was added to a 250 mL flask containing a stir bar. 0.60 mL phthalan derivatives (0.5 M in DMF solution) were added to the reaction mixture, and the reaction mixture was covered with septa caps. A syringe pump outfitted with a series of gas tight syringes was used to add the solution of EDA (0.60 mL of 60% vol DMF solution) to the reaction mixture over 4 hour. After this time, the reaction mixture was extracted with ethyl acetate (50 ml×3). The organic phase was dried with Na_2SO_4 and concentrated under vacuum, and purified by flash chromatography (Hexane: EtOAc = 4:1) to provide compounds as the light yellow oil mixture (65 mg, 76% yield with 3.1 :1 ratio of **2a** to **3a**; 53 mg, 72% yield with 3.4:1 ratio of **2b** to **3b**, respectively).

Analysis of yield and site-selectivity: Yields were determined by achiral GC using dodecane as an internal standard. Site-selectivity was determined by achiral GC or chiral HPLC. A 500 ul solution of EtOAc (0.1% v/v dodecane) was added to each reaction vial, and the contents of the vial were mixed and transferred to a 96-well plate or to a 1.5 ml micro-centrifugal tube. After centrifuging, the mixture was then frozen at -80 ºC. A portion of the organic layer (250 uL) was removed from the organic solvent layer and then transferred to a new vial for GC analysis or filtered through a silica gel pad with a 25% EtOAC in hexane mixture for chiral HPLC analysis. In the case of experiments performed in a 96-well array, all manipulations were performed using a multichannel pipet.

4.1: Organic Synthesis and Characterization

General methods and materials. Unless stated otherwise, all reactions and manipulations were conducted on the laboratory bench in air with reagent grade solvents. Reactions under inert gas atmosphere were conducted with oven dried glassware in a nitrogen-filled glove box or by standard Schlenk techniques under nitrogen. Unless noted otherwise, all reagents and solvents were purchased from commercial suppliers and used without further purification. If required, dichloromethane (DCM) and tetrahydrofuran (THF) were degassed by purging with argon for 15 minutes and dried with a solvent purification system containing a one-meter column of activated alumina; dried and degassed acetonitrile, 1,2-xylene, toluene, N,N-dimethylformamide (DMF), ethanol and methanol were purchased form commercial suppliers and used as received.

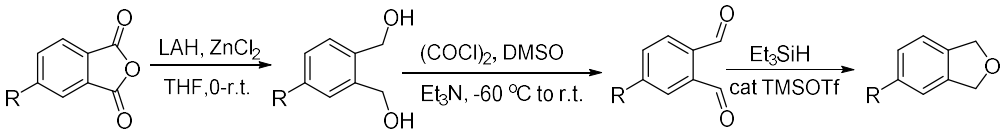
NMR spectra were acquired on 400 MHz, 600 MHz Bruker instruments at the University of California, Berkeley. NMR spectra were processed with MestReNova 9.0 (Mestrelab Research SL). Chemical shifts are reported in ppm and referenced to residual solvent peaks. Coupling constants are reported in hertz. Chiral HPLC analysis was conducted on an Agilent 1260 Infinity II Prime LC. GC analyses was obtained on an Agilent 6890 GC equipped with an HP-5 column (25 m x 0.20 mm ID x 0.33 um film) and an FID detector. GC yields and ratios were calculated using dodecane as the internal standard. The GC method started at 100 ℃ , was held for 3 min, and then ramped to 300 ℃ with a 40 ℃ /min rate and finally held at 300 for 4 min. High-resolution mass spectra were obtained via the Micro-Mass/Analytical Facility operated by the College of Chemistry, University of California, Berkeley.

The synthetic procedures and characterization of Ir(Me)-PIX were reported previously.[2]

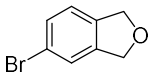
4.2 Substrate Synthesis

Unless noted otherwise, substituted phthalans were prepared following the reported process comprising three steps starting from the corresponding commercially available phthalic anhydrides: first reduction with LAH in the presence of $ZnCl_2$ to give the corresponding diol, followed by Swern oxidation and final cyclization of the resulting dialdehyde by treatment with Et_3SiH in the presence of Me_3SiOTf (Scheme S1).[3]

Scheme S1. General synthetic scheme for substituted phthalans

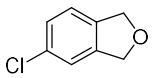


5-Bromo-1,3-dihydroisobenzofuran (**1a**)[3]



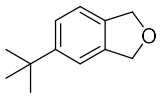
Following the general procedure **1a** (2.1 g, 43% yield for 3 steps) was synthesized from 5.5 g of 5-bromoisobenzofuran-1,3-dione.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 8.0 Hz, 1H), 7.38 (s, 1H),7.11 (d, J = 8.0 Hz, 1H), 5.08 (s, 2H), 5.06 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 141.71, 138.27, 130.44, 124.40, 122.59, 121.15, 73.36, 73.20.

5-Chloro-1,3-dihydroisobenzofuran (**1b**)



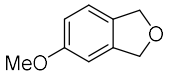
Following the general procedure **1b** (0.50 g, 35% yield for 3 steps) was synthesized from 1.7 g 5-chloroisobenzofuran-1,3-dione.
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23 (d, J = 8.0 Hz, 1H), 7.22 (s, 1H), 7.16 (d, J = 8.0 Hz, 1H), 5.07 (s, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 141.30, 137.70, 133.26, 127.61, 122.19, 121.47, 73.32, 73.31.
HR MS (EI): calcd. for C_8H_7ClO [M]+: 154.0185, found: 154.0186.

5-(*tert-butyl*)-1,3-Dihydroisobenzofuran (**1c**)



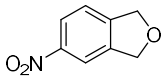
Following the general procedure **1c** (0.74 g, 43% yield for 3 steps) was synthesized from 2.0 g 5-(*tert-butyl*)isobenzofuran-1,3-dione.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 8.0 Hz, 1H), 7.28 (s, 1H), 7.19 (d, J = 8.0 Hz, 1H), 5.11 (s, 2H), 5.10 (s, 2H), 1.34 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 150.79, 139.26, 136.34, 124.61, 120.62, 117.85, 73.81, 73.58, 34.86, 31.69.
HR MS (EI): calcd. for $C_{12}H_{16}O$ [M]+: 176.1201, found: 176.1201.

5-Methoxy-1,3-dihydroisobenzofuran (**4a**)



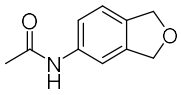
Following the general procedure **4a** (0.62 g, 36% yield for 3 steps) was synthesized from 2.0 g 5-methoxyisobenzofuran-1,3-dione.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, J = 8.0 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.77 (s, 1H), 5.08 (s, 2H), 5.06 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.59, 140.88, 131.15, 121.73, 113.58, 106.38, 73.70, 73.36, 55.66.
HR MS (APCI): calcd. for $C_9H_{11}O_2$ [M+H]+: 151.0759, found: 151.0767.

5-Nitro-1,3-dihydroisobenzofuran (**1d**)



Following a reported procedure **1d** (2.5 g, 56% yield) was synthesized from 2.63 g phthalan **7b**.[4]
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 8.0 Hz, 1H), 8.11 (s, 1H), 7.39 (d, J = 8.0 Hz, 1H), 5.11 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.17, 146.52, 141.21, 123.43, 121.79, 116.75, 73.31, 73.13.

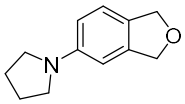
N-(1,3-dihydroisobenzofuran-5-yl)acetamide (**4b**)



The **4b** was prepared from the reaction of 5-aminophthalan[4] with acetyl chloride: Acetyl chloride (1.20 g, 10.00 mmol) was added drop wise to a mixture of 5-aminophthalan (675 mg, 5.00 mmol) and Et_3N (0.80 g) in 30 mL DCM. The reaction mixture was stirred at room temperature for 2.5 hour and quenched with 20 mL water. The solution was extracted with DCM (50 mL x3) and the organic phase was dried with Na_2SO_4. A light yellow solid (800 mg, 90% yield) was obtained after removal of solvent under reduced pressure.

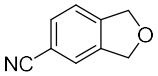
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (br, 1H), 7.55 (s, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 5.05 (s, 4H), 2.16 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$), ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.79, 140.20, 137.48, 134.92, 121.30, 119.32, 113.05, 73.60, 73.39, 24.58.
HR MS (APCI): calcd. for $C_{10}H_{12}NO_2$ [M+H]+: 178.0868, found: 178.0862.

1-(1,3-Dihydroisobenzofuran-5-yl)pyrrolidine (**4c**)



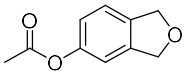
Following a previously reported procedure **4c** (0.24 g, 32% yield) was synthesized from 1.05 g of 5-aminophthalan.[5]
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, J = 8.0 Hz, 1H), 6.49 (d, J = 8.0 Hz, 1H), 6.42 (s, 1H), 5.07 (s, 2H), 5.05 (s, 2H), 3.28 (br, 4H), 2.01 (br, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 147.98, 140.57, 125.84, 121.47, 111.28, 103.77, 73.90, 73.51, 48.10, 25.57.

1,3-Dihydroisobenzofuran-5-carbonitrile (**1e**)



To a dissolved solution of compound **1e** (100 mg, 0.54 mmol) in 3 mL of DMF was added copper (I) cyanide (137 mg, 1.50 mmol). The mixture was refluxed at 160 ^{0}C for 20 hours. After being cooled to room temperature, 10 mL water, 0.50 g iron (III) chloride and 0.25 mL concentrated HCl were added to the reaction mixture, and the resulting reaction was stirred for another 45 minutes. The reaction mixture was then basified to pH > 10 using commercial ammonium hydroxide solution. The basic solution was then extracted with ethyl acetate (4 x 400 mL). The combined organic extracts were washed with water, dried over magnesium sulfate, filtered and concentrated in vacuo. The residue obtained was purified using flash chromatography (Hexane: EtOAc = 2:1) to provide compound **1e** as the white solid (52.0 mg, 66% yield)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.7 Hz, 1H), 7.54 (s, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 5.14 (br, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.69, 140.62, 131.74, 125.00, 122.10, 118.98, 111.49, 73.55, 73.18.
HR MS (EI): calcd. for C_9H_7NO [M]+: 145.0528, found: 145.5027.

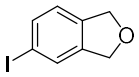
1,3-Dihydroisobenzofuran-5-yl acetate (**4c**)



To a solution of 1,3-dihydroisobenzofuran-5-ol[6] (68 mg, 0.50 mmol) in dry pyridine (0.15 mL) was added 0.24 mL of acetic anhydride (2.5 mmol). The mixture was stirred overnight at room temperature. The reaction mixture was gently heated under

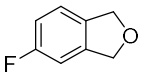
vacuum to evaporate excess acetic anhydride and pyridine thus obtaining **4c** (78 mg, 88% yield) without further purification.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 5.10 (s, 4H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.86, 150.28, 140.89, 136.73, 121.84, 120.81, 114.69, 73.50, 73.38, 21.24.
HR MS (APCI): calcd. for $C_{10}H_{11}O_3$ [M+H]+: 179.0708, found: 179.0719.

5-Iodo-1,3-dihydroisobenzofuran (**1f**)



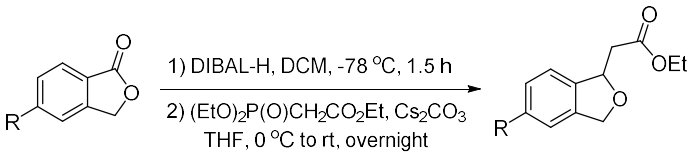
A Schlenk tube was charged with Cu_2O (7.20 mg, 0.05 mmol), L-proline (11.5 mg, 0.10 mmol), **1a** (98.0 mg, 0.50 mmol), potassium iodide (249 mg, 0.75 mmol), and EtOH (1.5 mL) under a nitrogen atmosphere. The Schlenk tube was sealed with a teflon cap, and the reaction mixture was stirred at 110℃ for 24 h. The solvent was removed under reduced pressure. The residue obtained was purified via silica gel chromatography (Hexane: EtOAc = 5:1) to afford **1f** as pink solid (58.0 mg, 47% yield).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.3 Hz, 2H), 7.01 (d, *J* = 8.3 Hz, 1H), 5.07 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.99, 139.05, 136.32, 130.31, 122.96, 92.37, 73.45, 73.01.
HR MS (EI): calcd. for C_8H_7IO [M]+: 245.9542, found: 245.9543.

5-Fluoro-1,3-dihydroisobenzofuran (**7a**)[3]



Following the general procedure **7a** (0.32 g, 35% yield for 3 steps) was synthesized from 1.1 g 5-methoxyisobenzofuran-1,3-dione.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (dd, *J* = 8.6, 5.0 Hz, 1H), 6.95 (dd, *J* = 17.2, 8.6 Hz, 2H), 5.08 (s, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.72 (d, *J* = 244.4 Hz), 141.49 (d, *J* = 8.7 Hz), 134.58 (d, *J* = 2.1 Hz), 122.18 (d, *J* = 8.9 Hz), 114.51 (d, *J* = 23.1 Hz), 108.41 (d, *J* = 23.7 Hz), 73.48 (d, *J* = 2.4 Hz), 73.24.

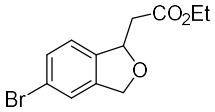
4.3 General procedure for the synthesis of phthalan derivative standards.



Following a previously reported procedure,[6] the *para*- or *meta*-C-H insertion products were synthesized independently in two steps starting from the corresponding 5-or 6-substituted phthalide. To a stirred solution of phthalide derivative in dry dichloromethane (10 mL) was added 1.2 equiv of DIBAL-H (25 wt % solution in toluene) at –78 °C. The reaction mixture was kept at –78 °C for 1.5 h. The reaction

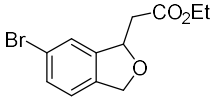
was quenched by the addition of 10 mL of saturated aqueous solution of sodium sulfate and allowed to warm to room temperature. To the resulting mixture was added anhydrous sodium sulfate. The mixture was stirred for 1 h, then filtered and concentrated under reduce pressure. The residue was dissolved in THF (4 mL) and cooled to 0° C. Triethyl phosphonoacetate (3.1 equiv) and cesium carbonate (3.1 equiv) were added. After 1 hour the cold bath was removed, and the reaction mixture was allowed to stir at room temperature overnight. The reaction was quenched with a saturated solution of ammonium chloride and extracted with ethyl acetate. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated under reduce pressure. The products were purified by column chromatography, eluting with ethyl acetate/hexane (1:4)

Ethyl 2-(5-bromo-1,3-dihydroisobenzofuran-1-yl)acetate (**2a**)



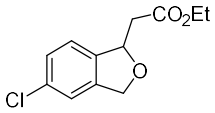
Following the general procedure **2a** (85 mg, 54% yield for 2 steps) was synthesized from 117 mg of 5-bromoisobenzofuran-1(*3H*)-one.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 8.0 Hz, 1H), 7.31 (s, 1H), 7.04 (d, J = 8.0 Hz, 1H), 5.57~5.54 (m, 1H), 5.07 (d, J = 12 Hz, 1H), 4.98 (d, J = 12 Hz, 1H), 4.15 (q, J = 8.0 Hz, 2H), 2.76~2.65 (m, 2H), 1.22 (t, J = 8.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.46, 141.68, 139.87, 130.51, 124.36, 122.76, 121.69, 79.99, 72.08, 60.71, 41.28, 14.17.
HR MS (ESI): calcd. for $C_{12}H_{13}BrNaO_3$ [M+Na]+: 306.9946, found: 306.9931

Ethyl 2-(6-bromo-1,3-dihydroisobenzofuran-1-yl)acetate (**3a**)



Following the general procedure **3a** (78 mg, 49% yield for 2 steps) was synthesized from 119 mg 6-bromoisobenzofuran-1(*3H*)-one.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.0 Hz, 1H), 7.34 (s, 1H), 7.09 (d, J = 8.0 Hz, 1H), 5.66~5.59 (m, 1H), 5.08 (d, J = 12 Hz, 1H), 4.99 (d, J = 12 Hz, 1H), 4.19 (q, J = 8.0 Hz, 2H), 2.80~2.69 (m, 2H), 1.27 (t, J = 8.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.59, 143.35, 138.42, 131.08, 124.75, 122.74, 121.30, 80.05, 72.51, 60.93, 41.45, 14.31.
HR MS (ESI): calcd. for $C_{12}H_{13}BrNaO_3$ [M+Na]+: 306.9946, found: 306.9944.

Ethyl 2-(5-chloro-1,3-dihydroisobenzofuran-1-yl)acetate (**2b**)

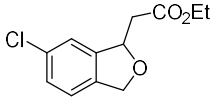


Following the general procedure **2b** (67 mg, 44% yield for 2 steps) was synthesized from 106 mg of 5-chloroisobenzofuran-1(*3H*)-one as following the general procedure.[7]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 8.0 Hz, 1H), 7.21 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 5.63~5.59 (m, 1H), 5.11 (d, J = 12 Hz, 1H), 5.03 (d, J = 12 Hz, 1H), 4.19 (q, J = 8.0 Hz, 2H), 2.80~2.69 (m, 2H), 1.27 (t, J = 8.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.53, 141.28, 139.29, 133.79, 127.70, 122.35, 121.42, 79.96, 72.19, 60.74, 41.38, 14.14.

HR MS (ESI): calcd. for $C_{12}H_{13}ClNaO_3$ [M+Na]+: 263.0451, found: 263.0457

Ethyl 2-(6-chloro-1,3-dihydroisobenzofuran-1-yl)acetate (**3b**)

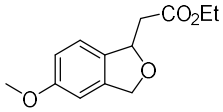


Following the general procedure **3b** (85 mg, 56% yield for 2 steps) was synthesized from 106 mg of 6-chloroisobenzofuran-1(*3H*)-one.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 8.0 Hz, 1H), 7.16 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 5.62~5.57 (m, 1H), 5.09(d, J = 12 Hz, 1H), 5.01(d, J = 12 Hz, 1H), 4.18(q, J = 8.0 Hz, 2H), 2.80~2.67(m, 2H), 1.25(t, J = 8.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.59, 142.92, 137.81, 133.38, 128.19, 122.31, 121.76, 80.07, 72.41, 60.88, 41.37, 14.24.

HR MS (ESI): calcd. for $C_{12}H_{13}ClNaO_3$ [M+Na]+: 263.0451, found: 263.0462

Ethyl 2-(5-methoxy-1,3-dihydroisobenzofuran-1-yl)acetate (**5d**)

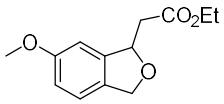


Following the general procedure **5d** (98 mg, 52% yield for 2 steps) was synthesized from 128 mg 5-methoxyisobenzofuran-1(*3H*)-one.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.75 (s, 1H), 5.63~5.57 (m, 1H), 5.11 (d, J = 12 Hz, 1H), 5.03 (d, J = 12 Hz, 1H), 4.17 (q, J = 8.0 Hz, 2H), 3.81 (s, 3H)， 2.78~2.67 (m, 2H), 1.27 (t, J = 8.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.90, 159.88, 140.92, 132.83, 121.93, 113.62, 106.25, 80.04, 72.61, 60.65, 55.53, 41.89, 14.21.

HR MS (APCI): calcd. for $C_{13}H_{17}O_4$ [M+H]+: 237.1127, found: 237.1124.

Ethyl 2-(6-methoxy-1,3-dihydroisobenzofuran-1-yl)acetate (**6d**)

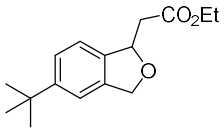


Following the general procedure **6d** (53 mg, 28% yield for 2 steps) was synthesized from 128 mg of 6-methoxyisobenzofuran-1(*3H*)-one.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.12 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.72 (s, 1H), 5.65~5.60 (m, 1H), 5.09 (d, J = 12 Hz, 1H), 5.01 (d, J = 12 Hz, 1H), 4.20 (q, J = 8.0 Hz, 2H), 3.80 (s, 3H)， 2.79~2.71 (m, 2H), 1.27 (t, J = 8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.98, 159.72, 142.57, 131.26, 121.90, 114.16, 106.73, 80.43, 72.53, 60.84, 55.69, 41.76, 14.35.

HR MS (APCI): calcd. for $C_{13}H_{17}O_4$ [M+H]+: 237.1127, found: 237.1135.

Ethyl 2-(5-(tert-butyl)-1,3-dihydroisobenzofuran-1-yl)acetate (**2c**)

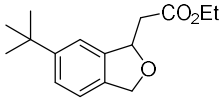


Following the general procedure **2c** (17 mg, 34% yield for 2 steps) was synthesized from 36.2 mg of 5-(tert-butyl)isobenzofuran-1(*3H*)-one.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (d, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 5.67~5.60 (m, 1H), 5.14 (d, J = 12 Hz, 1H), 5.06 (d, J = 12 Hz, 1H), 4.20 (q, J = 8.0 Hz, 2H), 2.81~2.69 (m, 2H), 1.32 (s, 9H), 1.27 (t, J = 8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.08, 151.39, 139.34, 137.98, 124.79, 120.81, 117.94, 80.32, 72.94, 60.78, 41.86, 34.87, 31.62, 14.32

HR MS (ESI): calcd. for $C_{16}H_{22}NaO_3$ [M+Na]+: 285.1467, found: 285.1467

Ethyl 2-(6-(tert-butyl)-1,3-dihydroisobenzofuran-1-yl)acetate (**3c**)

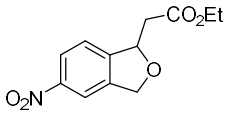


Following the general procedure **3c** (10 mg, 29% yield for 2 steps) was synthesized from 25 mg of 6-(tert-butyl)isobenzofuran-1(*3H*)-one.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (d, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 5.67~5.61 (m, 1H), 5.14 (d, J = 12 Hz, 1H), 5.06 (d, J = 12 Hz, 1H), 4.20 (q, J = 8.0 Hz, 2H), 2.81~2.69 (m, 2H), 1.32 (s, 9H), 1.27 (t, J = 8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.13, 151.45, 139.39, 138.02, 124.83, 120.85, 117.97, 80.36, 72.98, 60.82, 41.90, 34.90, 31.65, 14.35

HR MS (ESI): calcd. for $C_{16}H_{23}O_3$ [M+H]+: 263.1647, found: 263.1643.

Ethyl 2-(5-nitro-1,3-dihydroisobenzofuran-1-yl)acetate (**2d**)

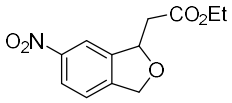


Following the general procedure **2d** (102 mg, 56% yield for 2 steps) was synthesized from 130 mg of 5-nitroisobenzofuran-1(*3H*)-one.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (d, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 5.67~5.61 (m, 1H), 5.14 (d, J = 12 Hz, 1H), 5.06 (d, J = 12 Hz, 1H), 4.20 (q, J = 8.0 Hz, 2H), 2.87~2.77 (m, 2H), 1.27 (t, J = 8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.18, 148.34, 147.86, 141.32, 123.50, 122.16, 116.77, 80.01, 72.14, 60.98, 40.85, 14.20.

HR MS (ESI): calcd. for $C_{12}H_{13}NNaO_5$ [M+Na]+: 274.0691, found: 274.0698.

Ethyl 2-(6-nitro-1,3-dihydroisobenzofuran-1-yl)acetate (**3d**)

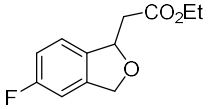


Following the general procedure **3d** (152 mg, 42% yield for 2 steps) was synthesized from 260 mg of 6-nitroisobenzofuran-1(*3H*)-one as following the general procedure.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.18 (d, J = 8.0 Hz, 1H), 8.10 (s, 1H), 7.37 (d, J = 8.0 Hz, 1H), 5.73~5.68 (m, 1H), 5.22(d, J = 12 Hz, 1H), 5.13(d, J = 12 Hz, 1H), 4.20 (q, J = 8.0 Hz, 2H), 2.81~2.69 (m, 2H), 1.27 (t, J = 8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.06, 148.10, 146.53, 142.75, 123.78, 121.83, 116.98, 79.83, 72.32, 60.91, 40.92, 14.11.

HR MS (ESI): calcd. for $C_{12}H_{13}NNaO_5$ [M+Na]+: 274.0691, found: 274.0684.

Ethyl 2-(5-fluoro-1,3-dihydroisobenzofuran-1-yl)acetate (**8a**)

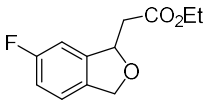


Following the general procedure **8a** (52 mg, 39% yield for 2 steps) was synthesized from 90 mg of 5-fluoroisobenzofuran-1(*3H*)-one.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.16 (dd, J = 8.0 , 4.8 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 5.65~5.60 (m, 1H), 5.10 (d, J = 12 Hz, 1H), 5.03 (d, J = 12 Hz, 1H), 4.20 (q, J = 8 Hz, 2H), 2.79~2.73 (m, 2H), 1.27 (t, J = 8.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.78, 163.04 (d, *J* = 245.3 Hz), 141.69 (d, *J* = 8.5 Hz), 136.41, 122.61 (d, *J* = 9.2 Hz), 114.76 (d, *J* = 23.1 Hz), 108.51 (d, *J* = 23.7 Hz), 80.06, 72.48 (d, *J* = 2.7 Hz), 60.87, 41.77, 14.32.

HR MS (ESI): calcd. for $C_{12}H_{13}FNaO_3$ [M+Na]+: 247.0746, found: 247.0751.

Ethyl 2-(6-fluoro-1,3-dihydroisobenzofuran-1-yl)acetate (**9a**)



Following the general procedure **8a** (25 mg, 38% yield for 2 steps) was synthesized from 45 mg of 6-fluoroisobenzofuran-1(*3H*)-one.

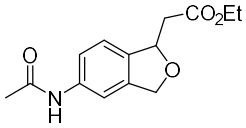
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.13 (dd, J = 8.0 Hz; 4.8 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 5.65~5.60 (m, 1H), 5.12 (d, J = 12 Hz, 1H), 5.03 (d, J = 12 Hz, 1H), 4.19 (q, J = 8 Hz, 2H), 2.80~2.69(m, 2H), 1.27(t, J = 8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.68, δ 162.74 (d, *J* = 244.8 Hz), 143.15 (d, *J* = 8.4 Hz), 134.69 (d, *J* = 2.2 Hz), 122.37 (d, *J* = 9.0 Hz), 115.20 (d, *J* = 23.1 Hz), 108.78 (d, *J* = 23.8 Hz), 80.23 (d, *J* = 2.7 Hz), 72.43, 60.94, 41.48, 14.33.

HR MS (ESI): calcd. for $C_{12}H_{13}FNaO_3$ [M+Na]+: 247.0746, found: 247.0735.

General Procedure for amide and pyrrolidine derivatives 4a,b and 5a,b:

A 25 ml vial containing acetamide (28 mg, 0.474 mmol) or pyrrolidine (33.7 mg, 0.474 mmol), palladium acetate (13.0 mg, 0.058 mmol), XantPhos (50 mg, 0.087 mmol) and cesium carbonate (170 mg, 0.521 mmol) was degassed and purged with nitrogen twice. In 5mL of dry dioxane **2a** or **2b** (105 mg, 0.368 mmol) was added by syringe. The reaction was heated to 105 °C overnight and then allowed to cool to room temperature. Dichloromethane was added, and the mixture was stirred vigorously for 1 h. The mixture was filtered, and the filtrate was concentrated to dryness. Silica-gel chromatography eluting with 25% ethyl acetate in hexane yielded the desired products.

Ethyl 2-(5-acetamido-1,3-dihydroisobenzofuran-1-yl)acetate (**5a**)

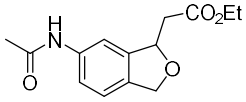


Following the general procedure **5a** (61 mg, 63% yield) was synthesized from **2a** .

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.42 (s, 1H), 7.23 (d, J = 8.0 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 5.64~5.58 (m, 1H), 5.11(d, J = 12 Hz, 1H), 5.03(d, J = 12 Hz, 1H), 4.19(q, J = 8 Hz, 2H), 2.78~2.67(m, 2H), 2.17 (s, 3H), 1.26(t, J = 8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.84, 168.45, 140.41, 137.82, 136.62, 121.57, 119.23, 112.88, 80.11, 72.63, 60.75, 41.69, 24.61, 14.22.

HR MS (ESI): calcd. for $C_{14}H_{17}NNaO_4$ [M+Na]+: 286.1055, found: 286.1057.

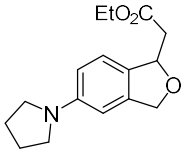
Ethyl 2-(6-acetamido-1,3-dihydroisobenzofuran-1-yl)acetate (**6a**)



Following the general procedure **6a** (50 mg, 52% yield) was synthesized from of **3a** .

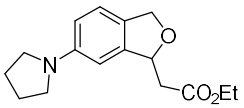
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.39 (s, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 5.66~5.60 (m, 1H), 5.11(d, J = 12 Hz, 1H), 5.03(d, J = 12 Hz, 1H), 4.19(q, J = 8 Hz, 2H), 2.82~2.68(m, 2H), 2.17 (s, 3H), 1.26 (t, J = 8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.95, 168.49, 141.93, 137.62, 135.10, 121.55, 119.81, 113.10, 80.43, 72.61, 60.90, 41.63, 24.73, 14.32.
HR MS (ESI): calcd. for $C_{14}H_{18}NO_4$ [M+H]+: 264.1236, found: 264.1227.

Ethyl 2-(5-(pyrrolidin-1-yl)-1,3-dihydroisobenzofuran-1-yl)acetate (**5b**)



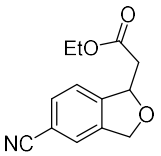
Following the general procedure 5**b** (52 mg, 51% yield) was synthesized from **2a**
^{1}H NMR (600 MHz, CDCl3) δ 7.02 (d, J = 8.0 Hz, 1H), 6.48 (d, J = 8.0 Hz, 1H), 6.38 (s, 1H), 5.62~5.56 (m, 1H), 5.09(d, J = 12 Hz, 1H), 5.01(d, J = 12 Hz, 1H), 4.20(q, J = 8 Hz, 2H), 3.27(br, 4H), 2.75~2.65(m, 2H), 2.00 (br, 4H), 1.28(t, J = 8 Hz, 3H); δ^{13}C NMR (151 MHz, $CDCl_3$) δ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.23, 150.75, 142.19, 127.18, 121.56, 112.91, 105.09, 80.60, 72.60, 60.77, 41.97, 41.15, 14.37.
HR MS (ESI): calcd. for $C_{16}H_{22}NO_3$ [M+H]+: 276.1600, found: 276.1604.

Ethyl 2-(6-(pyrrolidin-1-yl)-1,3-dihydroisobenzofuran-1-yl)acetate (**6b**)



Following the general procedure 6**b** (57 mg, 56% yield) was synthesized from **2a**
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.02 (d, J = 8.0 Hz, 1H), 6.48 (d, J = 8.0 Hz, 1H), 6.39 (s, 1H), 5.65~5.58 (m, 1H), 5.10(d, J = 12 Hz, 1H), 5.02(d, J = 12 Hz, 1H), 4.19(q, J = 8 Hz, 2H), 3.27(br, 4H), 2.75~2.65(m, 2H), 2.00 (br, 4H), 1.27(t, J = 8 Hz, 3H); δ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.31, 148.26, 140.71, 127.55, 121.73, 111.28, 103.57, 80.31, 72.96, 60.68, 48.02, 42.33, 25.58, 14.36.
HR MS (ESI): calcd. for $C_{16}H_{21}NNaO_3$ [M+Na]+: 298.1419, found: 298.1427.

Ethyl 2-(5-cyano-1,3-dihydroisobenzofuran-1-yl)acetate (**2e**)



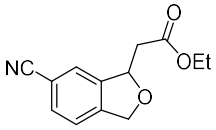
Compound **2a** (100 mg, 0.54 mmmol) was dissolved in DMF (3 mL), and to this solution was added copper (I) cyanide (137mg, 1.5 mmol). The mixture was heated to 160 °C and allowed to stir at this temperature for 20 h. After being cooled to room temperature, with water (10 mL), iron (III) chloride (0.5 g) and concentrated hydrochloric acid (0.25 ml) were added to the reaction mixture, and the resulting

reaction was stirred for 45 min. The reaction mixture was then basified to pH > 10 using commercial ammonium hydroxide. The basic solution was then extracted with ethyl acetate (4 x 40 mL). The combined organic extracts were washed with water, dried over magnesium sulfate, filtered, and concentrated under reduce pressure. The residue obtained was purified by flash chromatography eluting with 30% EtOAc in Hexane to provide **2e** as a white solid (75 mg, 60% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 5.68 (t, *J* = 5.9 Hz, 1H), 5.13 (dd, *J* = 33.4, 12.7 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 2.86 – 2.73 (m, 2H), 1.26 (t, *J* = 6.6 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.34, 146.18, 140.81, 131.96, 125.13, 122.47, 118.81, 112.13, 80.36, 72.33, 61.09, 41.03, 14.31.

HR MS (EI): calcd. for $C_{13}H_{13}NO_3$ [M]+: 231.0899, found: 231.0895.

Ethyl 2-(6-cyano-1,3-dihydroisobenzofuran-1-yl)acetate (**3e**)

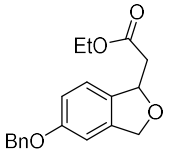


Following the same procedure to prepare **2e**, **3e** (32 mg, 34% yield) was syntheszied from 75 mg **3a**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.8 Hz, 1H), 7.53 (s, 1H), 7.35 (d, *J* = 7.8 Hz, 1H), 5.67 (t, *J* = 6.1 Hz, 1H), 5.15 (dd, *J* = 33.7, 13.7 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.80 (m, 2H), 1.30 – 1.23 (t, *J* = 7.1 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.33, 144.86, 142.38, 132.29, 125.50, 122.25, 118.86, 111.76, 80.07, 72.73, 61.10, 41.17, 14.32.

HR MS (EI): calcd. for $C_{13}H_{13}NO_3$ [M]+: 231.0896, found: 231.0895.

Ethyl 2-(5-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

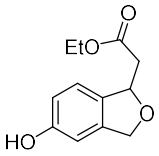


Following the general procedure ethyl 2-(5-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate (180 mg, 45% yield for 2 steps) was synthesized from 320 mg 5-(benzyloxy)isobenzofuran-1(*3H*)-one.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.30 (m, 5H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.82 (s, 1H), 5.64 – 5.57 (m, 1H), 5.13 – 4.99 (m, 4H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.80 – 2.66 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.02, 159.22, 159.22, 141.09, 141.09, 136.99, 133.29, 128.76, 128.17, 127.55, 122.12, 114.66, 107.45, 80.18, 72.74, 70.47, 60.78, 42.00, 14.34.

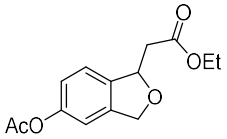
HR MS (ESI): calcd. for $C_{19}H_{20}O_4$ [M+H]+: 313.1440, found: 313.1453.

Ethyl 2-(5-hydroxy-1,3-dihydroisobenzofuran-1-yl)acetate



Ethyl 2-(6-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate (180 mg, 0.60 mmol) was stirred under an atmosphere of hydrogen in the presence of 10% Pd/C (36 mg, 0.036 mmol) for 16 h. The catalyst was removed by vacuum filtration, and the filtrate concentrated at reduced pressure to give 118 mg (91% yield) of the title compound as a colourless viscous oil without further purification.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (d, *J* = 8.1 Hz, 1H), 6.73 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.69 (s, 1H), 5.60 (s, 1H), 5.32 (s, 1H), 5.09 (dd, *J* = 12.4, 2.5 Hz, 1H), 5.01 (d, *J* = 12.4 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 2.79 – 2.67 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.16, 156.00, 141.27, 132.96, 122.25, 114.89, 108.10, 80.19, 72.61, 60.88, 42.03, 14.33.
HR MS (ESI): calcd. for $C_{12}H_{25}O_4$ [M+H]+: 223.0970, found: 223.0957.

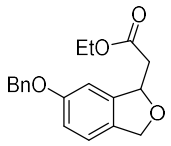
Ethyl 2-(5-acetoxy-1,3-dihydroisobenzofuran-1-yl)acetate **(5c)**



To a solution of ethyl 2-(5-hydroxy-1,3-dihydroisobenzofuran-1-yl)acetate (68 mg, 0.50 mmol) in dry pyridine (0.15 mL) was added acetic anhydride (0.24 mL, 2.5 mmol). The resulting solution was then stirred at r.t. overnight. The reaction mixture was gently heated under vacuum to evaporate the excess acetic anhydride and pyridine. The resulting 1,3-dihydroisobenzofuran-5-yl acetate (78 mg, 88% yield) was used without further purification

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.2 Hz, 1H), 6.98 (d, *J* = 8.2 Hz, 1H), 6.95 (s, 1H), 5.64 (t, *J* = 6.2 Hz, 1H), 5.10 (dd, *J* = 31.3, 11.2 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.84 – 2.67 (m, 2H), 2.31 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.74, 169.64, 150.60, 140.94, 138.29, 122.10, 120.90, 114.65, 80.05, 72.42, 60.78, 41.58, 29.73, 21.13, 14.21.
HR MS (ESI): calcd. for $C_{14}H_{16}NaO_5$ [M+Na]+: 287.0895, found: 287.0897.

Ethyl 2-(6-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

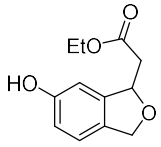


Following the general procedure, 2-(6-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate (20 mg, 43% yield for 2 steps) was synthesized from 40 mg of 5-(benzyloxy)isobenzofuran-1(*3H*)-one.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.39 (m, 4H), 7.36 – 7.32 (m, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.81 (s, 0H), 5.65 – 5.61 (m, 1H), 5.14 – 4.99 (m, 4H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.82 – 2.68 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.91, 158.82, 142.52, 136.90, 131.50, 128.70, 128.13, 127.55, 121.89, 114.98, 107.77, 80.35, 72.48, 70.45, 60.80, 41.67, 14.31.

HR MS (ESI): calcd. for $C_{19}H_{20}O_4$ [M+H]+: 313.1440, found: 313.1426.

Ethyl 2-(6-hydroxy-1,3-dihydroisobenzofuran-1-yl)acetate

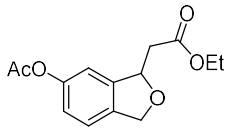


Ethyl 2-(5-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate (16.0 mg, 0.050 mmol) was stirred under an atmosphere of hydrogen in the presence of 10 % Pd/C (3.0 mg, 0.003 mmol) for 16 h. The catalyst was removed by vacuum filtration, and the filtrate concentrated at reduced pressure to give 12 mg (93% yield) of the title compound as a colourless viscous oil.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.05 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.67 (s, 1H), 6.11 (br, 1H), 5.61 (br, 1H), 5.08 (d, *J* = 11.4 Hz, 1H), 5.00 (d, *J* = 11.4 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 2.78 – 2.70 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.37, 155.91, 142.45, 130.77, 122.04, 115.47, 108.32, 80.34, 72.54, 61.07, 41.68, 14.26.

HR MS (ESI): calcd. for $C_{12}H_{25}O_4$ [M+H]+: 223.0970, found: 223.0961.

Ethyl 2-(6-acetoxy-1,3-dihydroisobenzofuran-1-yl)acetate (**6c**)



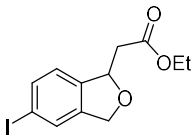
Following a similar procedure to the synthesis of **5c**, **6c** was prepared from 14 mg of ethyl 2-(6-hydroxy-1,3-dihydroisobenzofuran-1-yl)acetate in a 98% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.1 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.94 (s, 1H), 5.64 (t, *J* = 6.1 Hz, 1H), 5.08 (dd, *J* = 31.4, 12.2 Hz, 2H), 4.19 (q, *J* = 7.1 Hz, 2H), 2.81 – 2.70 (m, 2H), 2.30 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 4H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ 170.77, 169.68, 150.37, 142.50, 136.79, 121.94, 121.49, 114.99, 80.25, 72.52, 60.88, 41.49, 29.82, 21.22, 14.29.

HR MS (ESI): calcd. for $C_{14}H_{17}O_5$ [M+H]+: 265.1076, found: 265.1062.

Ethyl 2-(5-iodo-1,3-dihydroisobenzofuran-1-yl)acetate (**2f**)

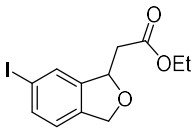


A Schlenk tube was charged with Cu_2O (7.2 mg, 0.05 mmol), L-proline (11.5 mg, 0.1 mmol), Compound **2a** (49mg, 0.25 mmol), potassium iodide (249 mg, 0.75 mmol), and EtOH (1.5 mL) under a nitrogen atmosphere. The Schlenk tube was sealed with a Teflon valve, and the reaction mixture was stirred at 110 ℃ for 24 h. The solvent was removed under reduced pressure. The residue obtained was purified via silica gel chromatography to afford 5-iodo-1,3-dihydroisobenzofuran as pink solid (37 mg, 41% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.9 Hz, 1H), 7.54 (s, 1H), 6.99 (d, *J* = 7.9 Hz, 1H), 5.61 (t, *J* = 6.1 Hz, 1H), 5.05 (dd, *J* = 33.8, 12.6 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.80 – 2.70 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.51, 143.48, 139.10, 136.84, 130.51, 122.98, 92.37, 79.78, 72.49, 60.84, 41.38, 14.22.

HR MS (ESI): calcd. for $C_{12}H_{13}NaIO_3$ [M+Na]+: 354.9807, found: 354.9803.

Ethyl 2-(6-iodo-1,3-dihydroisobenzofuran-1-yl)acetate (**3f**)



The compound was synthesized by following the same process as 2e, but using Compound 3a as starting material.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 10.4 Hz, 1H), 7.60 (s, 1H), 6.96 (d, *J* = 7.9 Hz, 1H), 5.60 (m, 1H), 5.06 (m, 2H), 4.19 (q, *J* = 7.1 Hz, 1H), 2.78 – 2.70 (m, 1H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.56, 141.94, 140.64, 136.46, 130.34, 123.10, 93.10, 80.15, 71.99, 60.81, 41.34, 14.21.

HR MS (ESI): calcd. for $C_{12}H_{13}NaIO_3$ [M+Na]+: 354.9807, found: 354.9794.

4.4 Calibration curve

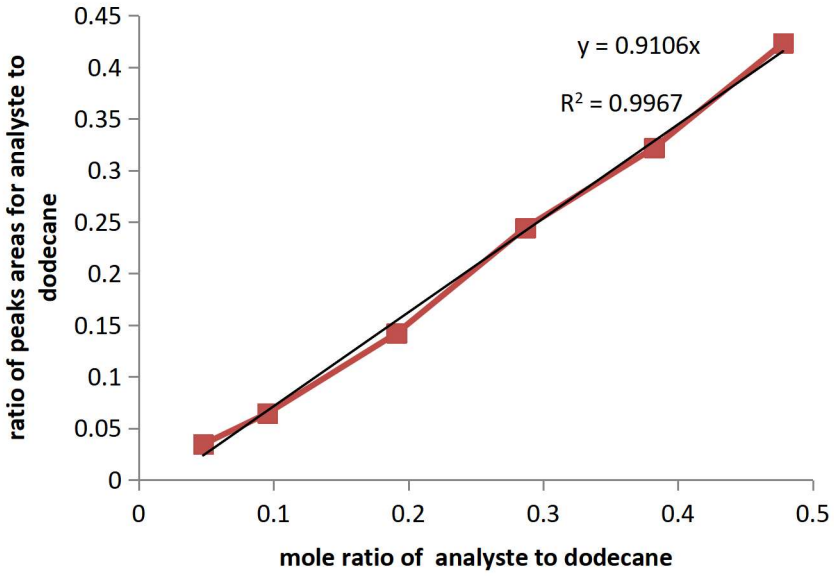


Figure S2. Calibration curve for products from the reaction of **1a** with EDA.

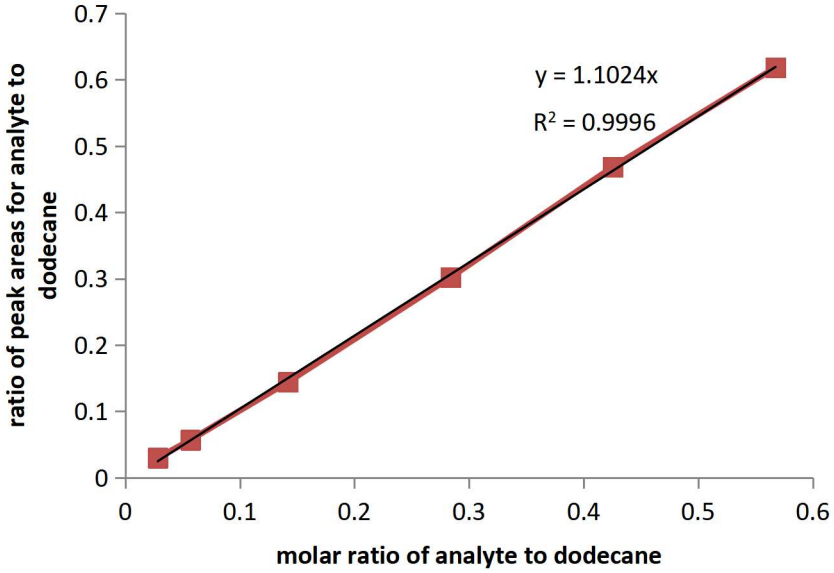


Figure S3. Calibration curve for products from the reaction of **1b** with EDA.

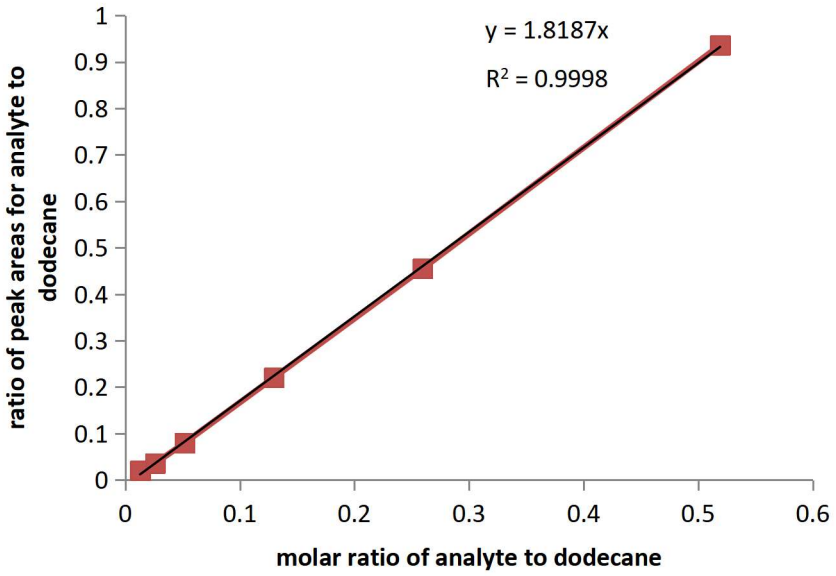


Figure S4. Calibration curve for products from the reaction of **1c** with EDA.

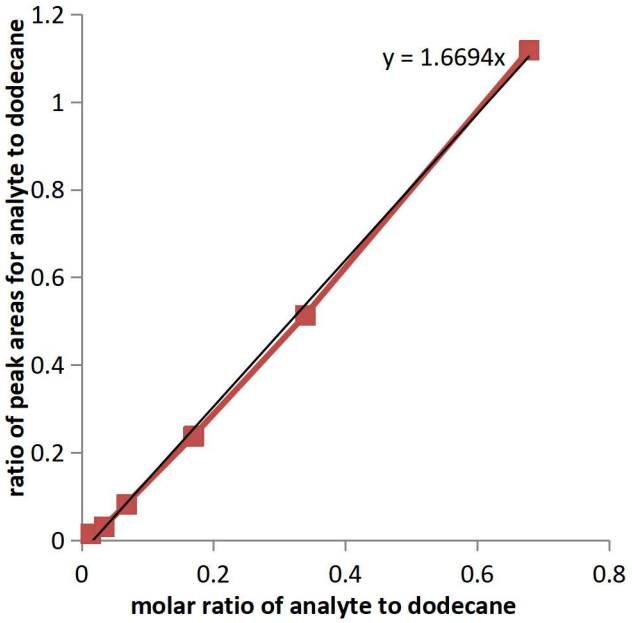


Figure S5. Calibration curve for products from the reaction of **1d** with EDA.

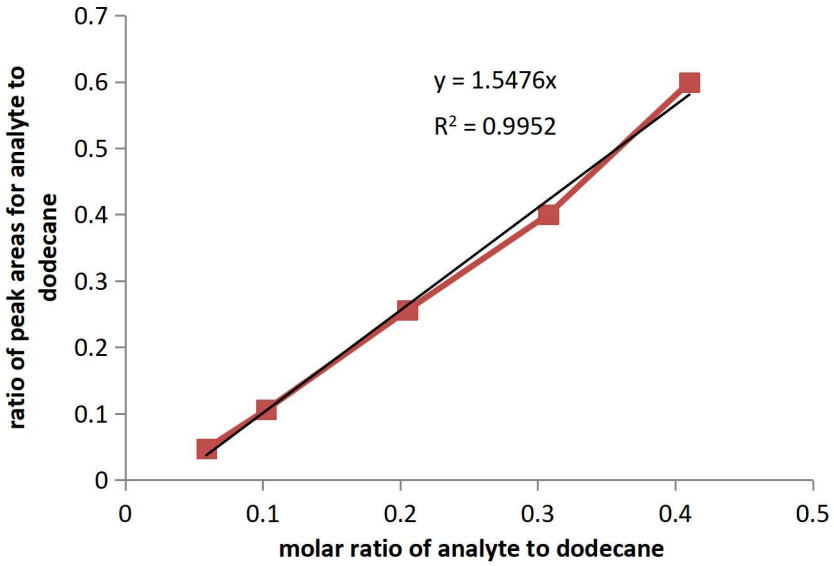


Figure S6. Calibration curve for products from the reaction of **1e** with EDA.

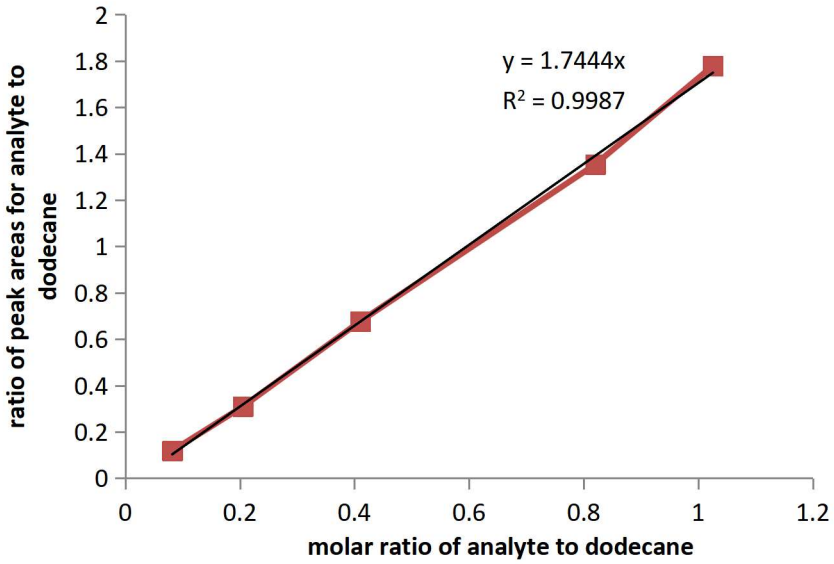


Figure S7. Calibration curve for products from the reaction of **1f** with EDA.

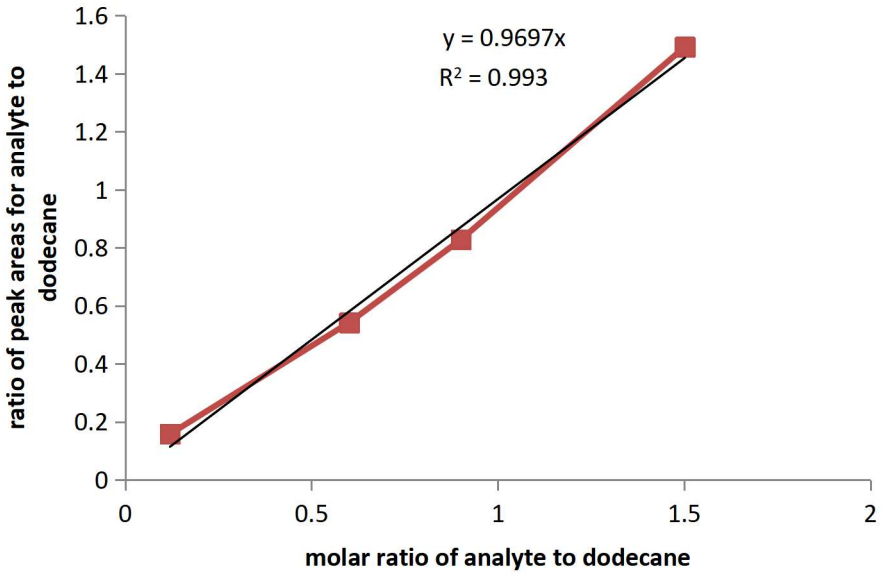


Figure S8. Calibration curve for products from the reaction of **4a** with EDA.

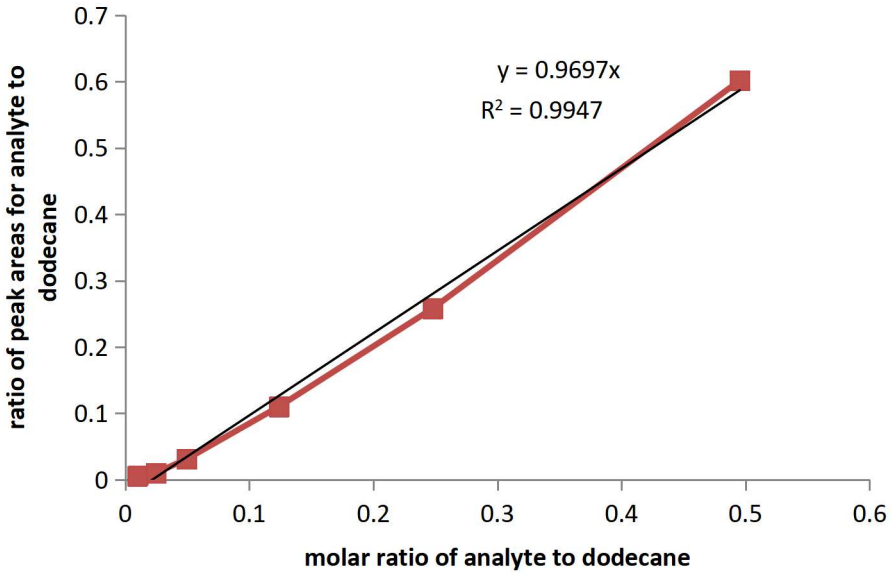


Figure S9. Calibration curve for products from the reaction of **4b** with EDA.

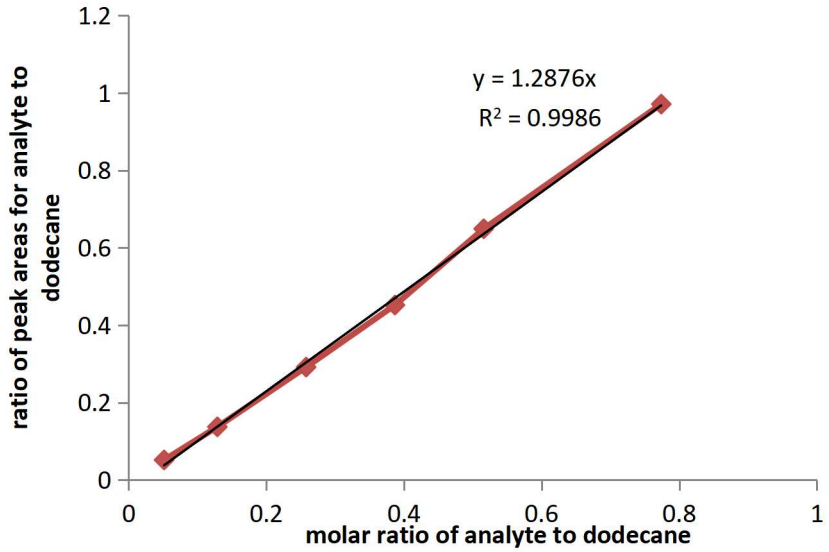


Figure S10. Calibration curve for products from the reaction of **4c** with EDA

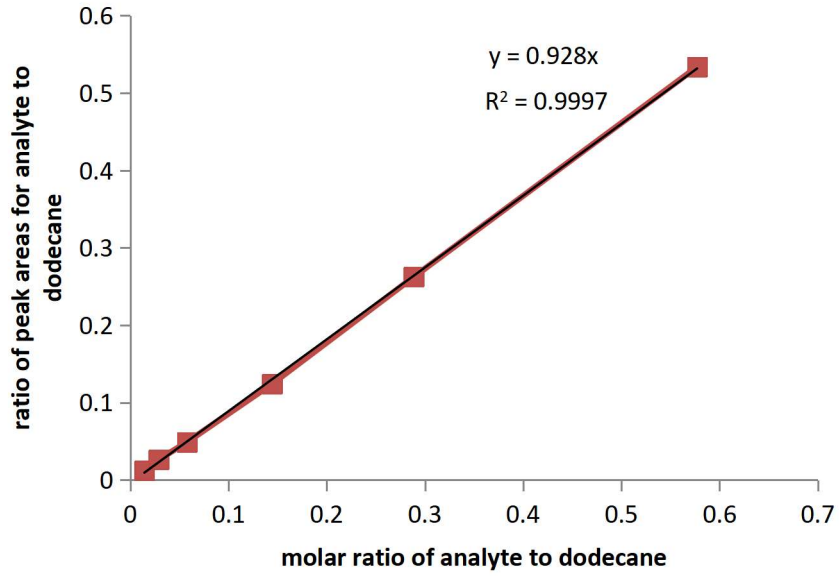


Figure S11. Calibration curve for products from the reaction of **4d** with EDA.

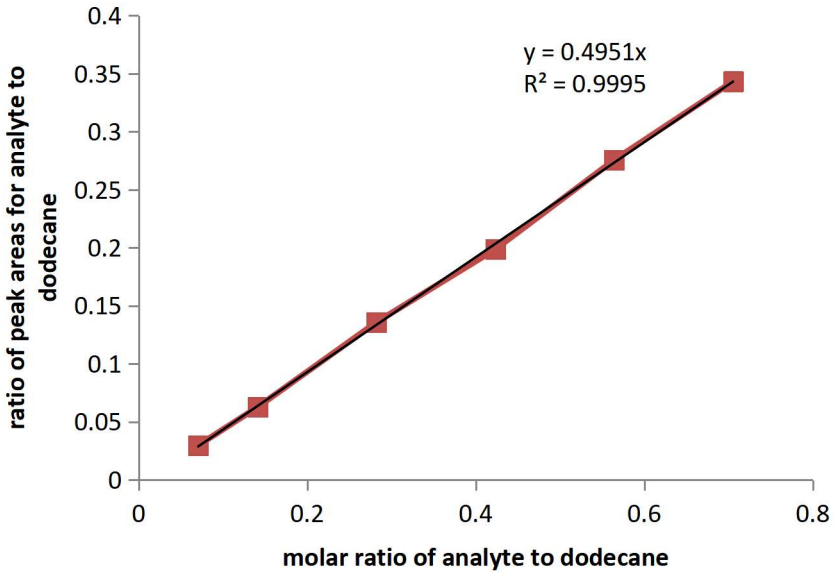


Figure S12. Calibration curve for products from the reaction of **7a** with EDA.

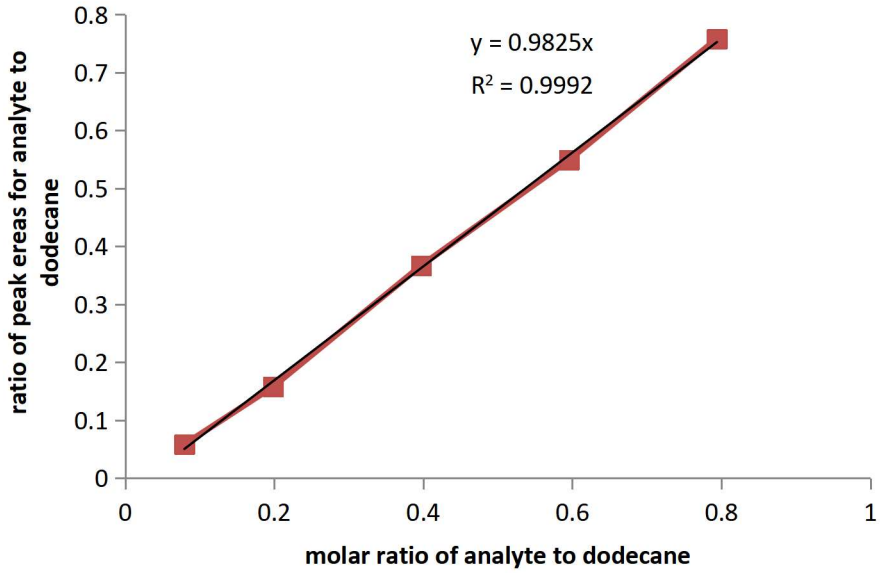


Figure S13. Calibration curve for products from the reaction of **7b** with EDA.

4.5 Selectivity Traces

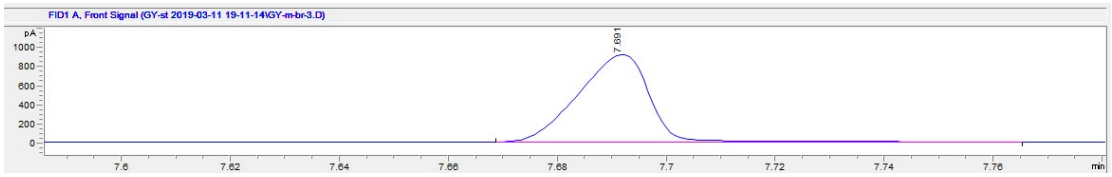


Figure S14. GC trace of synthesized **2a**.

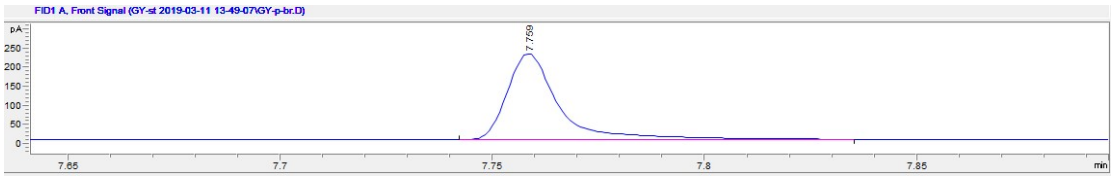


Figure S15. GC trace of synthesized **3a**.

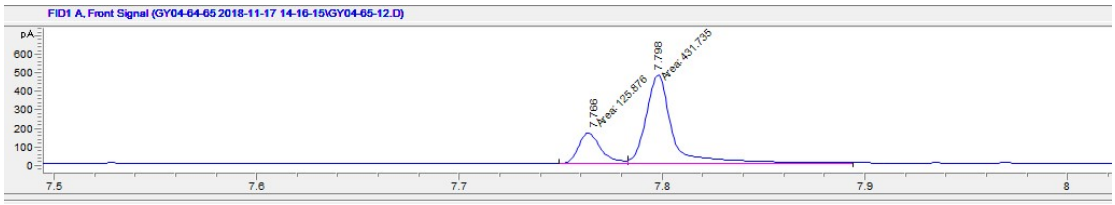


Figure S16. GC trace of the reaction **1a** with EDA catalyzed by P_{G2}.

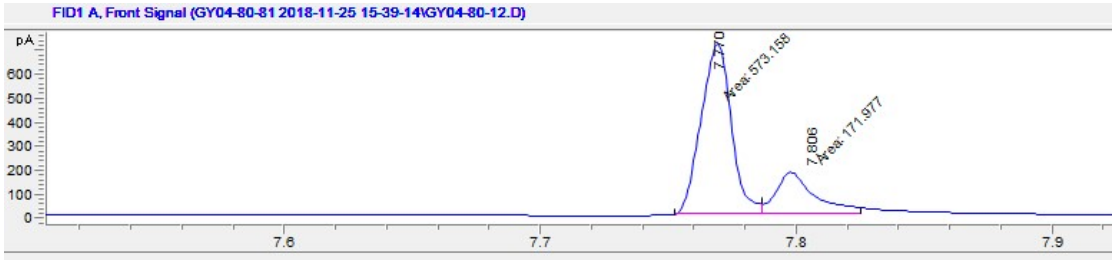


Figure S17. GC trace of the reaction **1a** with EDA catalyzed by M_{G2}.

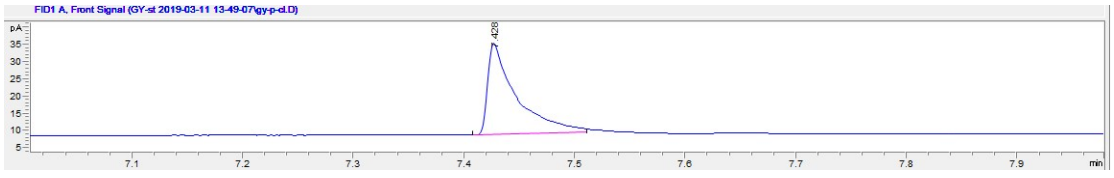


Figure S18. GC trace of synthesized **2b**.

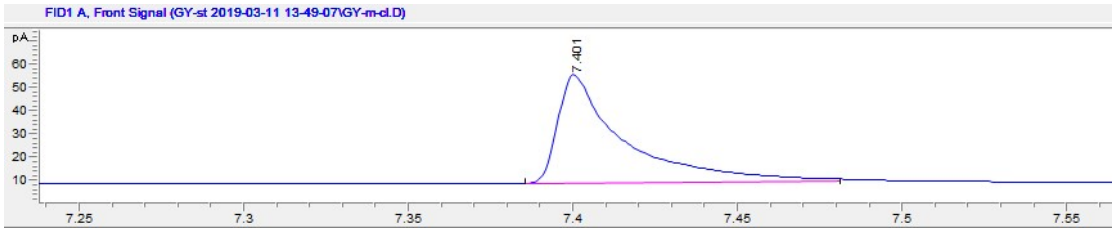


Figure S19 GC trace of synthesized **3b**.

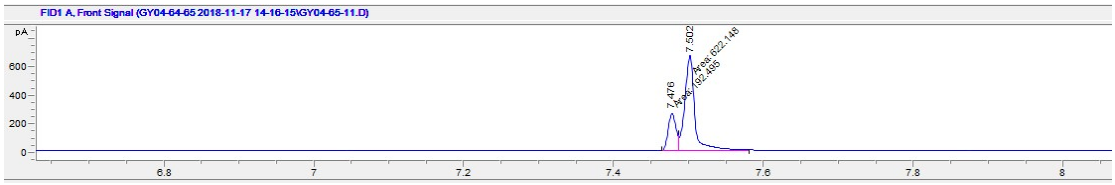


Figure S20 GC trace of the reaction **1b** with EDA catalyzed by P_{G2}.

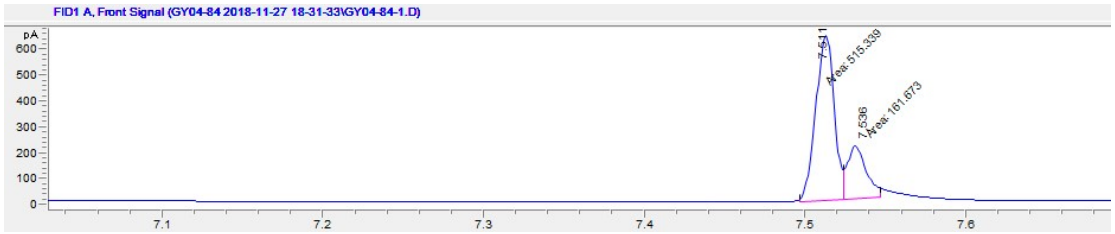


Figure S21. GC trace of the reaction **1b** with EDA catalyzed by M_{G2}.

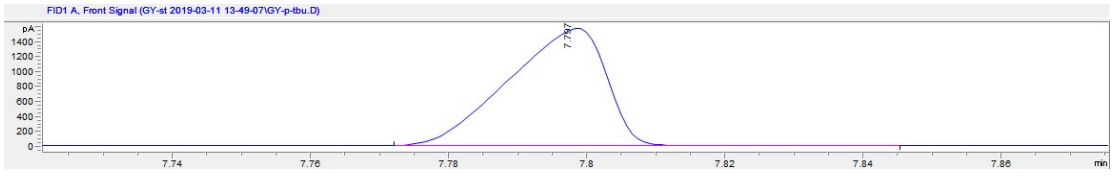


Figure S22. GC trace of synthesized **2c**.

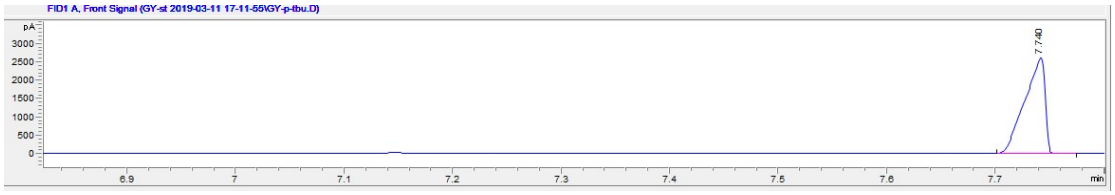


Figure S23. GC trace of synthesized **3c**.

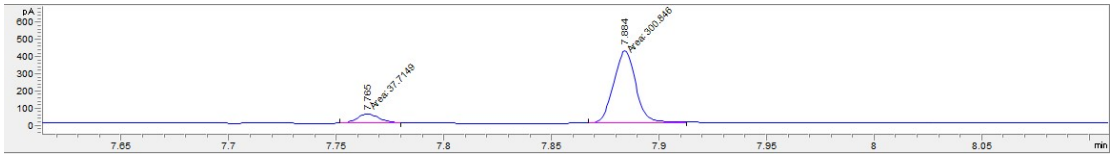


Figure S24. GC trace of the reaction **1c** with EDA catalyzed by P_{G2}.

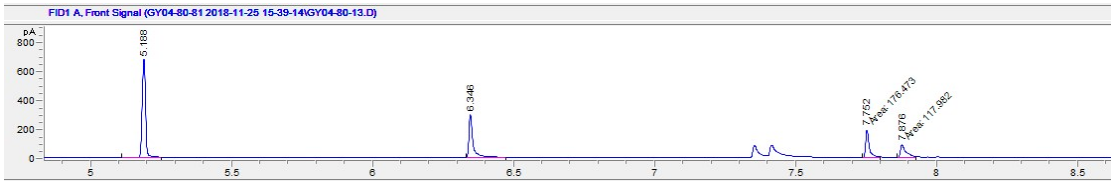


Figure S25. GC trace of the reaction **1c** with EDA catalyzed by M_{G2}.

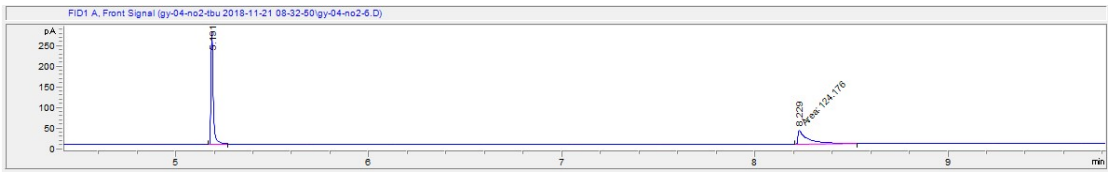


Figure S26. GC trace of synthesized **2d**.

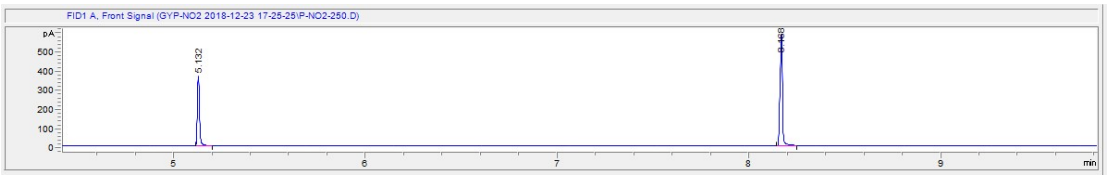


Figure S27. GC trace of synthesized 3**d**.

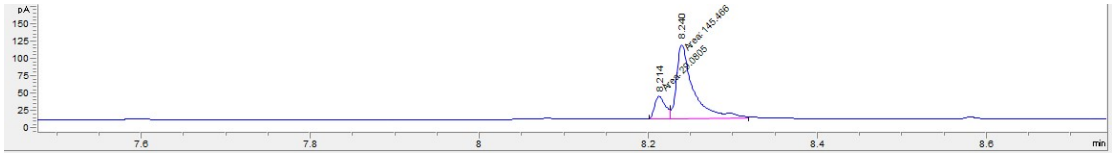


Figure S28. GC trace of the reaction **1d** with EDA catalyzed by P_{G2}.

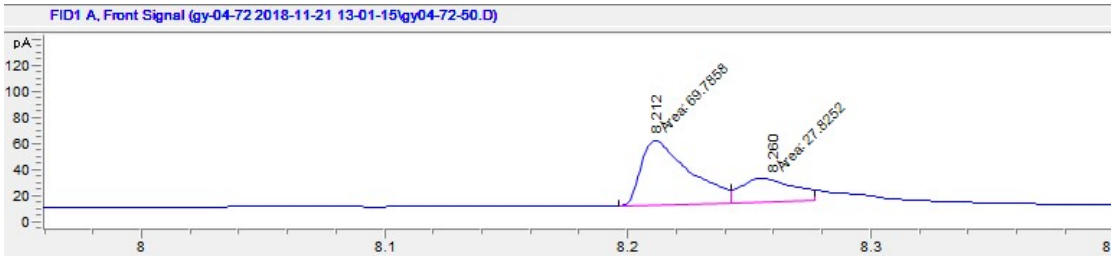


Figure S29. GC trace of the reaction **1d** with EDA catalyzed by M_{G2}.

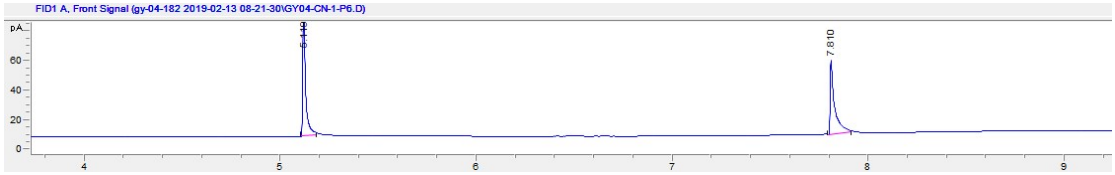


Figure S30. GC trace of synthesized 2**e**.

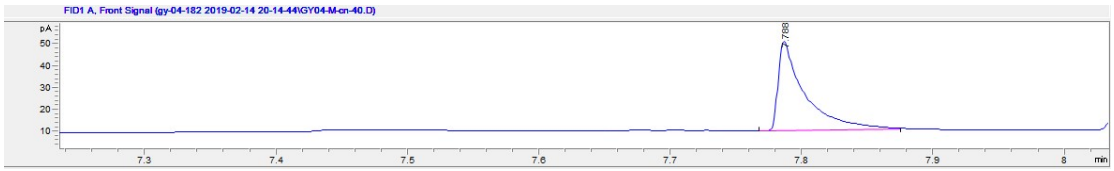


Figure S31. GC trace of synthesized **3e**.

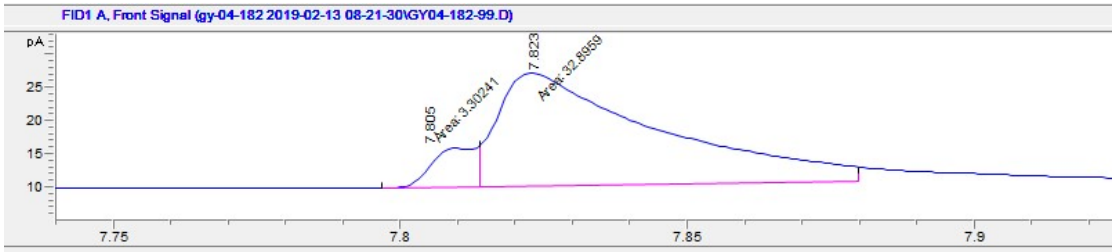


Figure S32. GC trace of the reaction **1e** with EDA catalyzed by $\mathbf{P_{G2}}$.

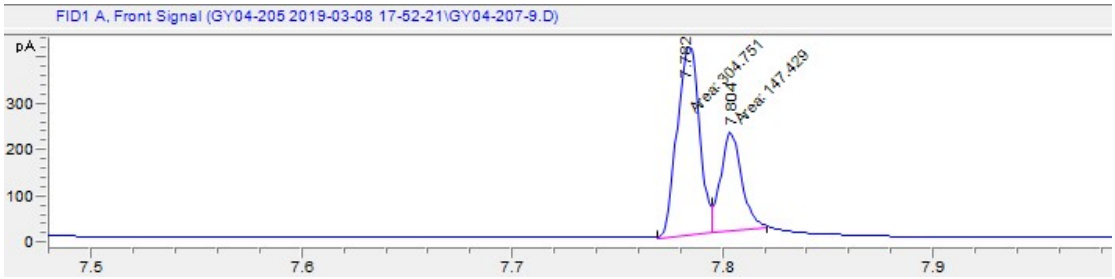


Figure S33. GC trace of the reaction **1e** with EDA catalyzed by $\mathbf{M_{G2}}$.

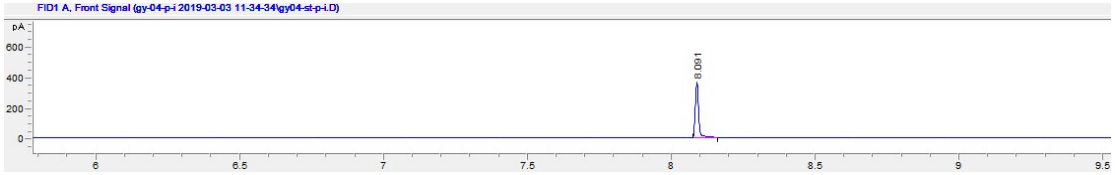


Figure S34. GC trace of synthesized **2f**.

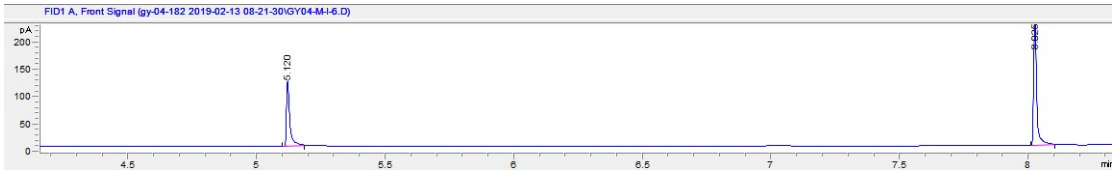


Figure S35. GC trace of synthesized **3f**.

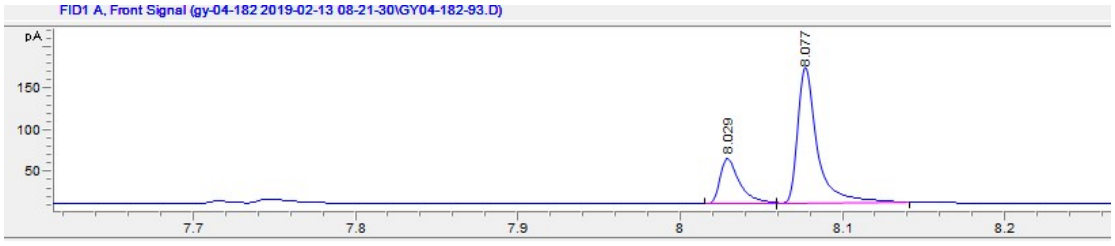


Figure S36. GC trace of the reaction **1f** with EDA catalyzed by P_{G2}.

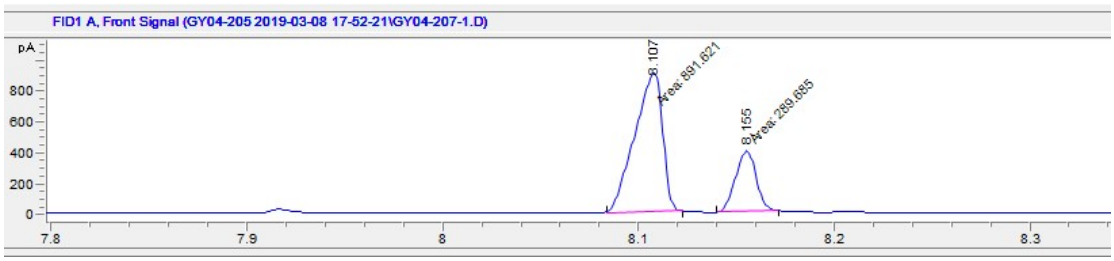


Figure S37. GC trace of the reaction **1f** with EDA catalyzed by M_{G2}.

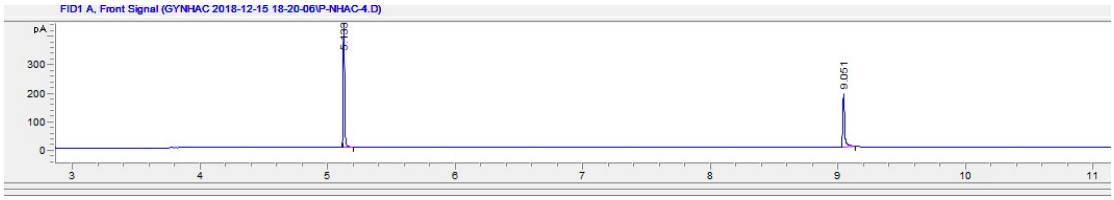


Figure S38. GC trace of synthesized **5a**.

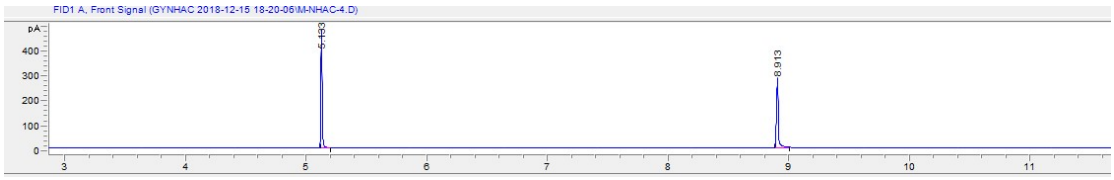


Figure S39. GC trace of synthesized **6a**.

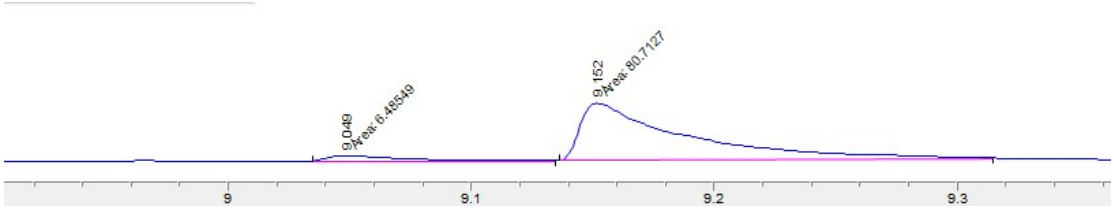


Figure S40. GC trace of the reaction **4a** with EDA catalyzed by P_{G2}.

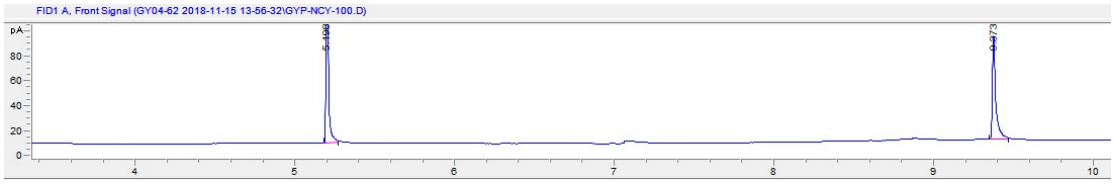


Figure S41. GC trace of synthesized **5b**.

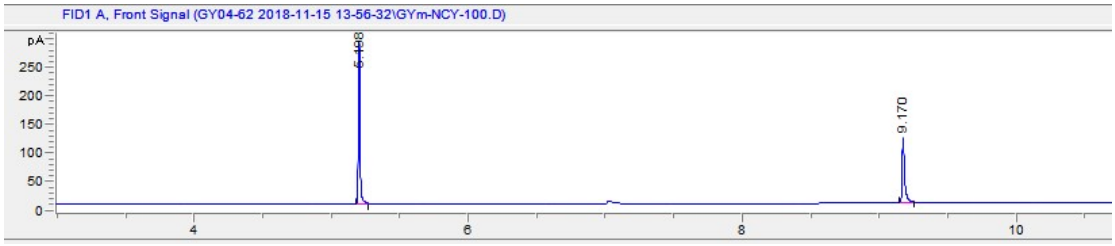


Figure S42. GC trace of synthesized **6b**.

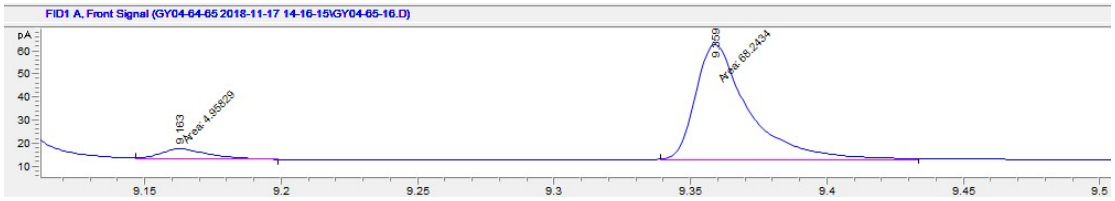


Figure S43. GC trace of the reaction **4b** with EDA catalyzed by P_{G2}.

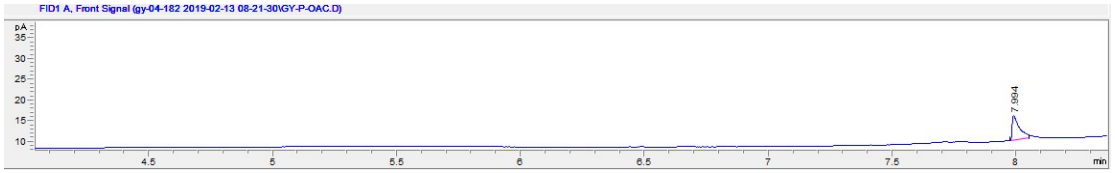


Figure S44. GC trace of synthesized **5c**.

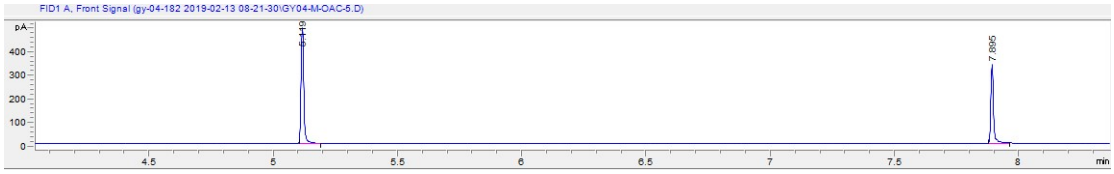


Figure S45. GC trace of synthesized **6c**.

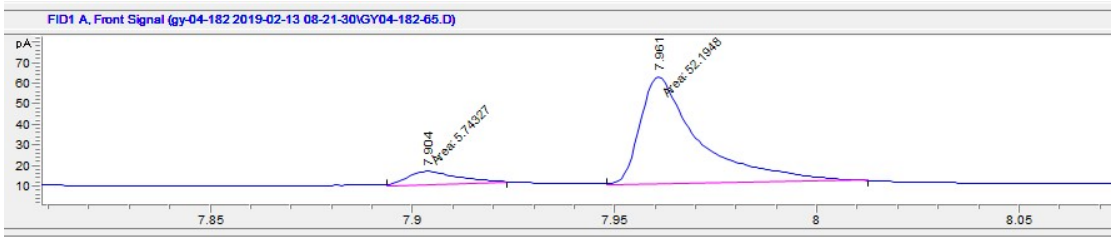


Figure S46. GC trace of the reaction **4c** with EDA catalyzed by P_{G2}.

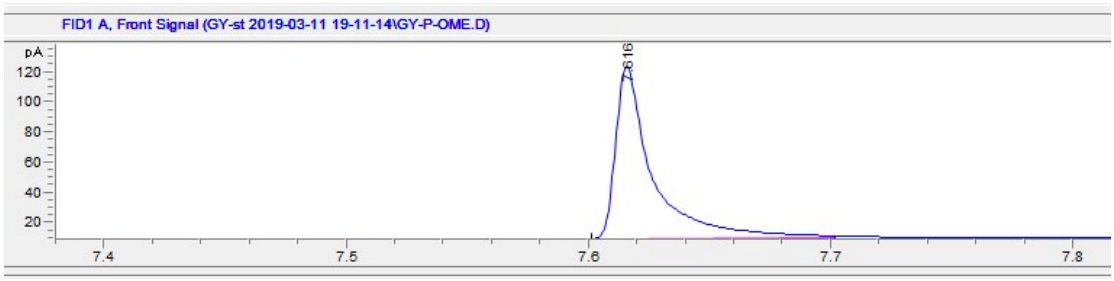


Figure S47. GC trace of synthesized **5d**.

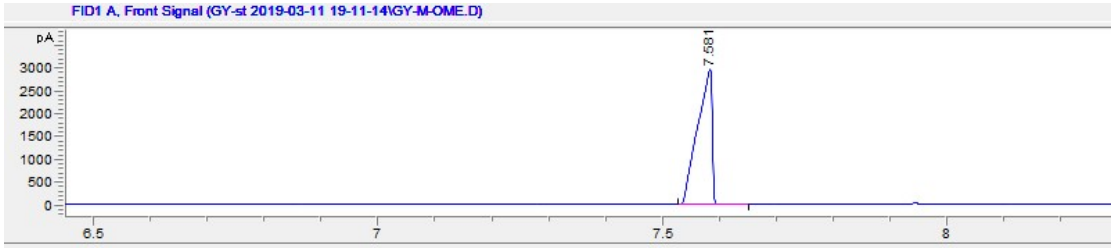


Figure S48. GC trace of synthesized **6d**.

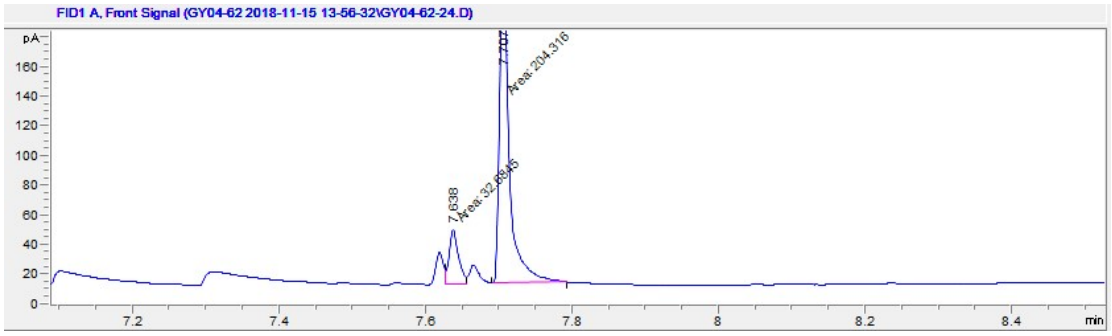


Figure S49. GC trace of the reaction **4b** with EDA catalyzed by P_{G2}.

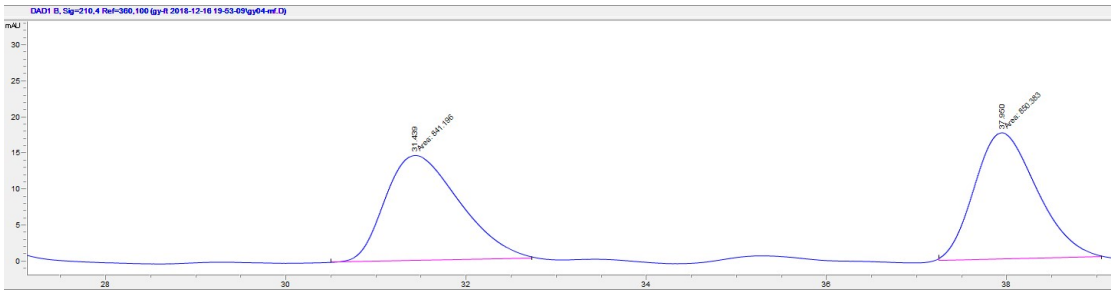


Figure S50. HPLC trace of racemic **8a** (Chiracel OD-H, 0.5% *i*-PrOH in hexane with 0.75 ml/min flow).

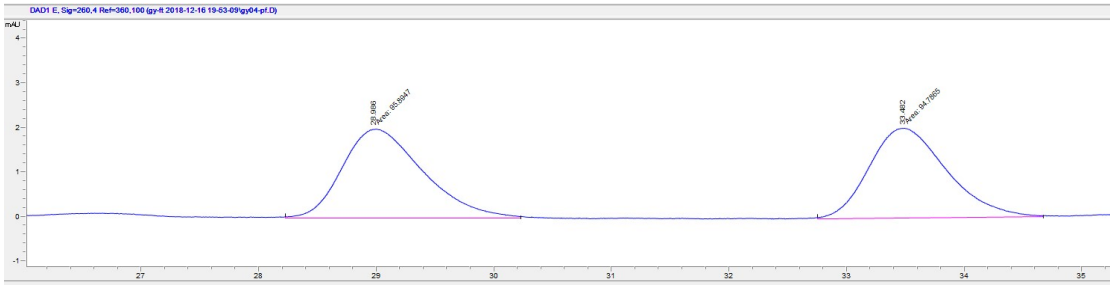


Figure S51. HPLC trace of racemic **9a** (Chiracel OD-H, 0.5% *i*-PrOH in hexane with 0.75 ml/min flow).

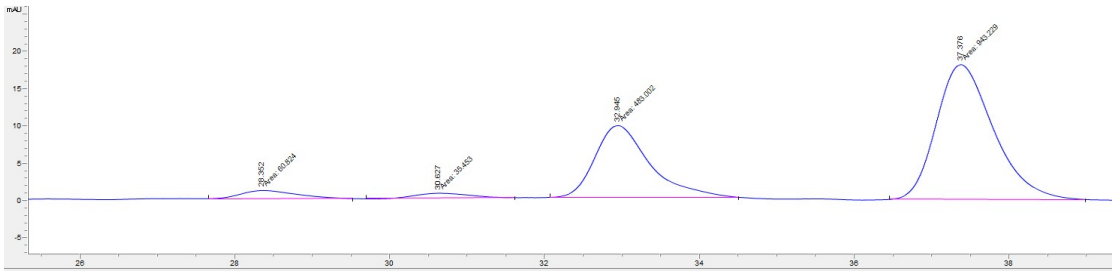


Figure S52. HPLC trace of enatioselective C-H insertion of **7a** with EDA catalyzed by P252S+ M_{G1} (Chiracel OD-H, 0.5% *i*-PrOH in hexane with 0.75 ml/min flow).

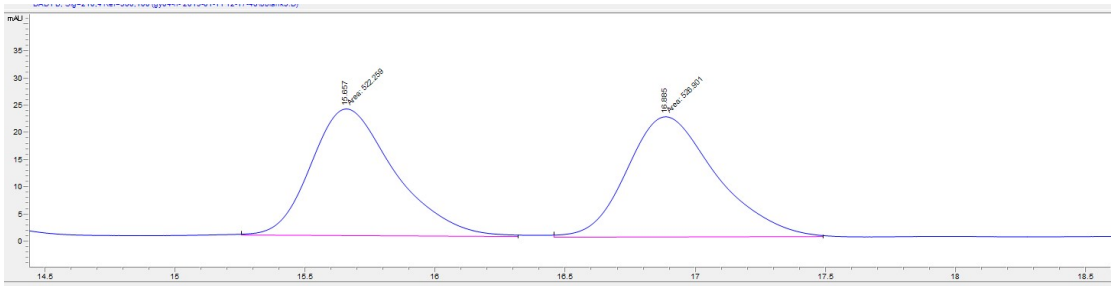


Figure S53. HPLC trace of racemic **8b** (Chiracel OD-H, 1.0% *i*-PrOH in hexane with 0.75 ml/min flow).

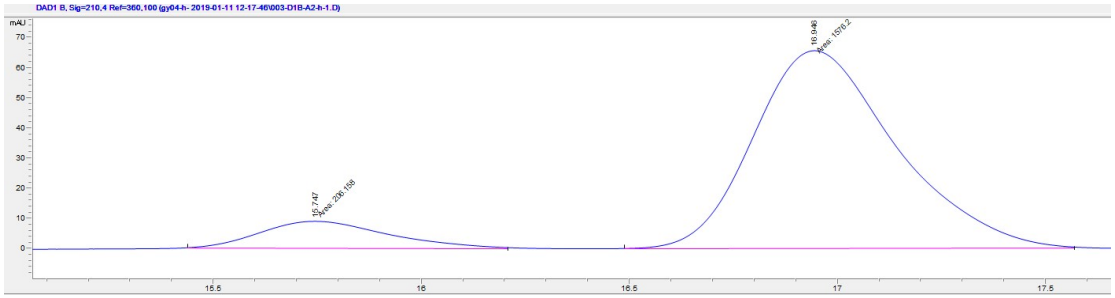
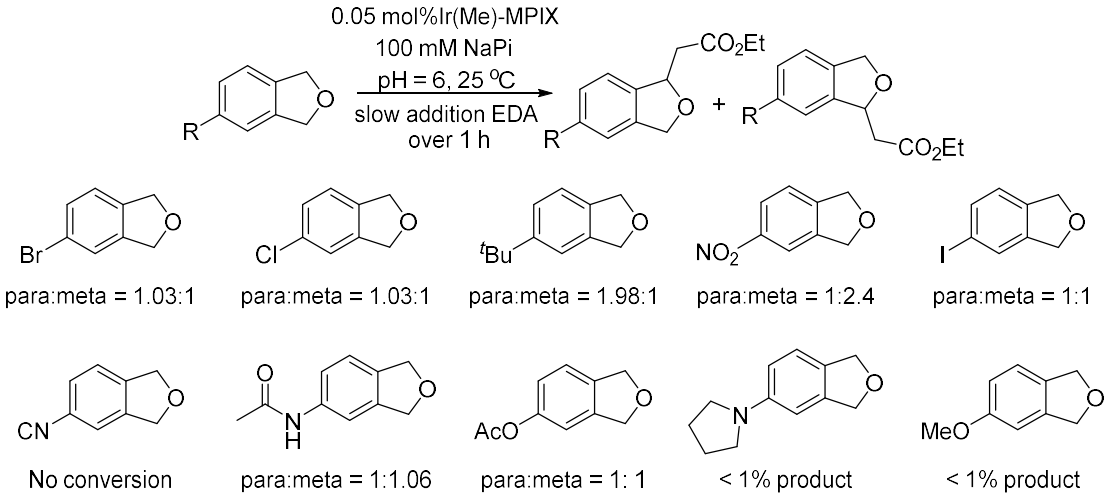
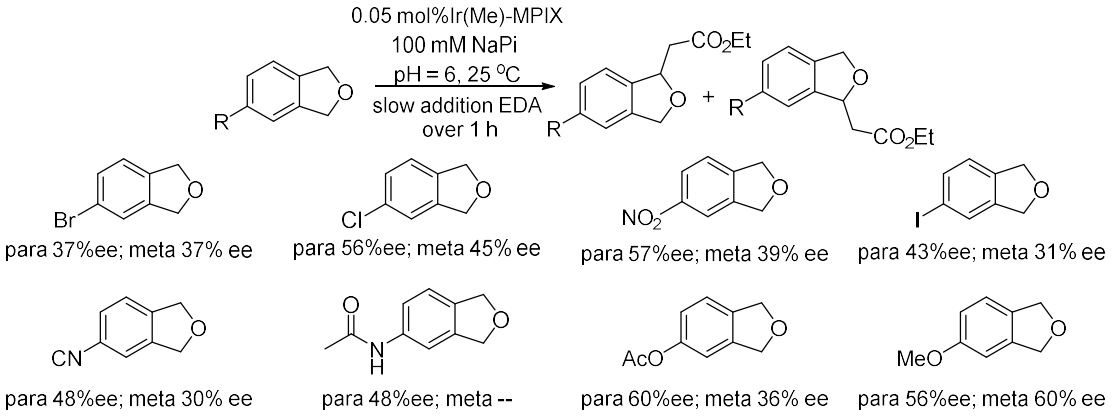


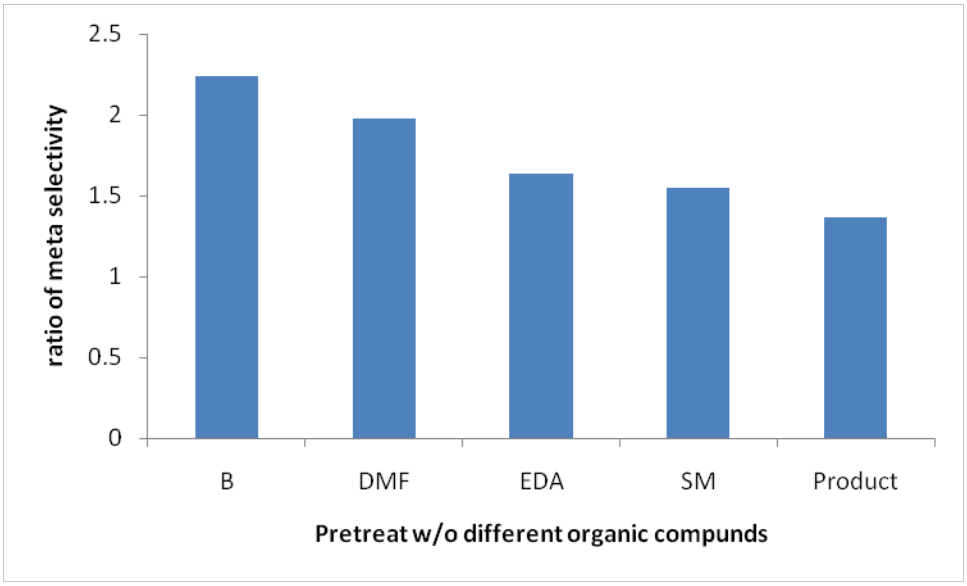
Figure S54. HPLC trace of enatioselective C-H insertion with **7b** with EDA catalyzed by P252S+ M_{G1} (Chiracel OD-H, 1.0% *i*-PrOH in hexane with 0.75 ml/min flow).



Scheme S2. The site-selectivity of the reactions from phathalan derivatives with EDA catalyzed by the free MPIX-Ir-Me cofactor.

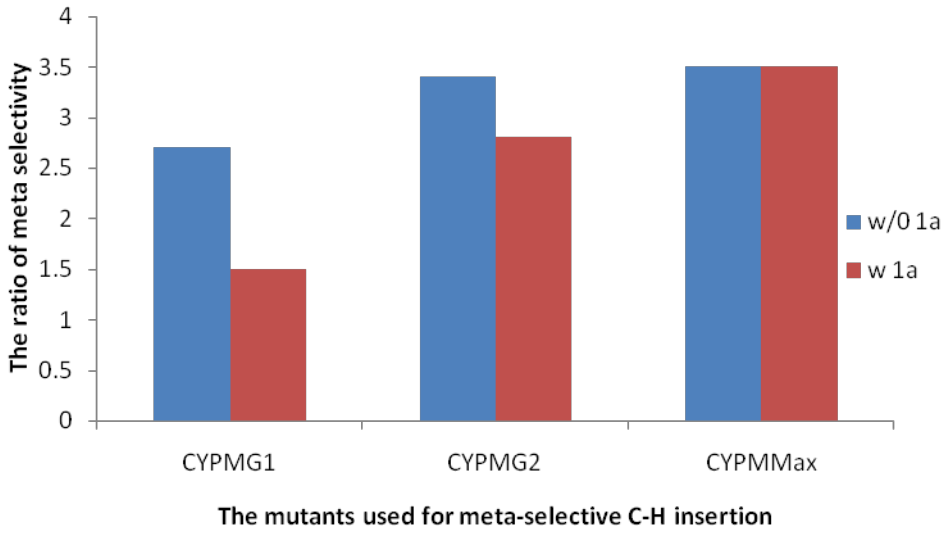


Scheme S3. Enantioselectivity of the reactions from selected phathalan derivatives with EDA catalyzed by P_{G2}.



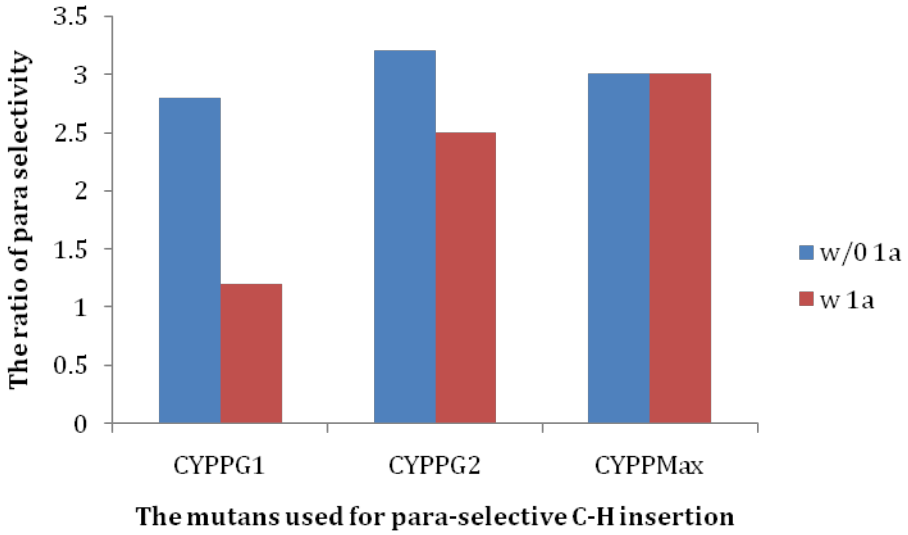
Conditions: 1 mL stock solutions of $\mathbf{M_{G1}}$ were pretreated with 10 uL of DMF, 50% EDA in DMF, 0.5 M **1a** or **2b** and stirring at rt for 1 hour before setting the reactions as following the general progress.

Figure S55. Ratio of meta selectivity to the reactions **1a** with EDA catalyzed by $\mathbf{M_{G1}}$ after exposure to the individual reaction components for 1 hour.



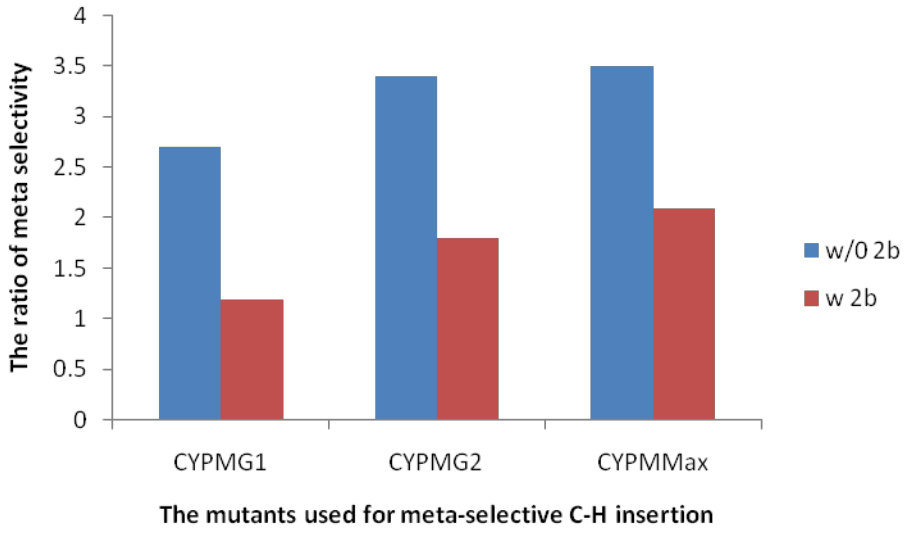
Conditions: 1 mL stock solutions of different ArMs were pretreated with or without 10 uL of 0.5 M **1a** DMF solutions and stirring at rt for 1 hour before setting the reactions as following the general progress.

Figure S56. Ratio of meta selectivity to the reactions **1a** with EDA catalyzed by different mutants that pretreated with or without 10 uL **1a** (0.5 M in DMF) for 1 hour.



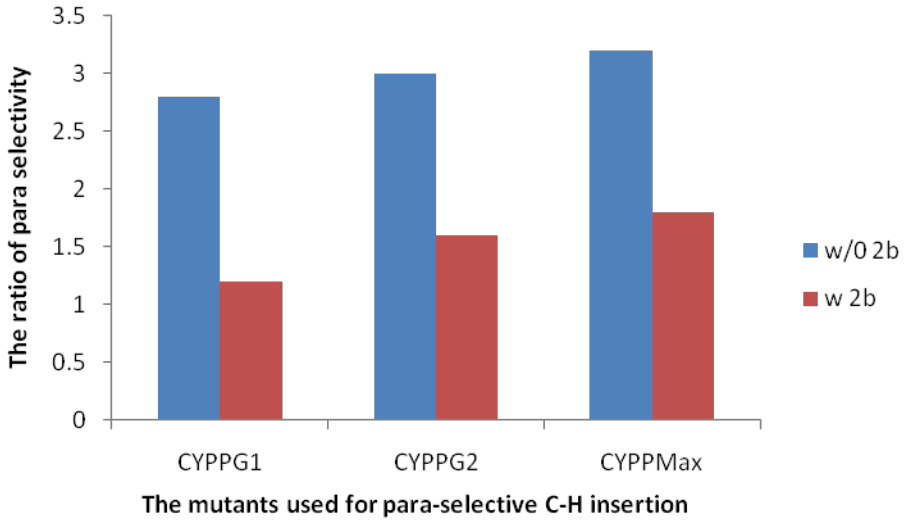
Conditions: 1 mL stock solutions of different ArMs were pretreated with or without 10 uL of 0.5 M **1a** DMF solutions and stirring at rt for 1 hour before setting the reactions as following the general progress.

Figure S57. Ratio of para-selectivity to the reactions **1a** with EDA catalyzed by different mutants that pretreated with 10 uL **1a** (0.5 M in DMF) for 1 hour.



Conditions: 1 mL stock solutions of different ArMs were pretreated with or without 10 uL of 0.5 M **2b** DMF solutions and stirring at rt for 1 hour before setting the reactions as following the general progress.

Figure S58. Ratio of meta selectivity to the reactions **1a** with EDA catalyzed by different mutants that pretreated with or without 10 uL **2b** (0.5 M in DMF) for 1 hour.



Conditions: 1 mL stock solutions of different ArMs were pretreated with or without 10 uL of 0.5 M **2b** DMF solutions and stirring at rt for 1 hour before setting the reactions as following the general progress.

Figure S59. Ratio of para selectivity to the reactions **1a** with EDA catalyzed by different mutants that pretreated with or without 10 uL **2b** (0.5 M in DMF) for 1 hour.

5.1: DNA sequence and Amino Acid sequence

Protein	Organism	Construction	Vector	Code Name	Sequence
mOCR-Myo	*Physeter macrocephalus*	6xHis-TEV-mOCR-Myo	2BT	**Myo**	EGDIHMKSSHHHHHHENLYFQSNMSNMTYNNVFDHAYEMLKENIRYDDIRDTDDLHDAIHMAADNAVPHYYADIRSVMASEGIDLEFEDSGLMPDTKDDIRILQARIYEQLTIDLWEDAEDLLNEYLEEVEEYEEDEEGTGSETPGTSESGVLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPETLEKFDRFKHLKTEAEMKASEDLKKHGVTVLTALGAILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISEAIIHVLHSRHPGDFGADAQGAMNKALELFRKDIAAKYKELGYQG

CYP 119	*Sulfolobus solfataricus*	6xHis-TEV-CYP119	2BT	**WT**	EGDIHMKSSHHHHHHENLYFQSN AMYDWFSEMRKKDPVYYDGNI WQVFSYRYTKEVLNNFSKFSSDL TGYHERLEDLRNGKIRFDIPTRYT MLTSDPPLHDELRSMSADIFSPQK LQTLETFIRETTRSLLDSIDPREDDI VKKLAVPLPIIVISKILGLPIEDKEK FKEWSDLVAFRLGKPGEIFELGK KYLELIGYVKDHLNSGTEVVSRV VNSNLSDIEKLGYIILLLIAGNETT TNLISNSVIDFTRFNLWQRIREENL YLKAIEEALRYSPPVMRTVRKTK ERVKLGDQTIEEGEYVRVWIASA NRDEEVFHDGEKFIPDRNPNPHLS FGSGIHLCLGAPLARLEARIAIEEF SKRFRHIEILDTEKVPNEVLNGYK RLVVRLKSNE
CYP 119	*Sulfolobus solfataricus*	6xHis-TEV-CYP119	2BT	**P_{G1}**	E G D I H M K S S H H H H H H E N L Y F Q S N Y D W F S E M R K K D P V Y Y D G N I W Q V F S Y R Y T K E V L N N F S K F S S D L T G Y H E R L E D L R N G K I R F D I P T R Y T M L T S D P P L H D E L R S M S A D I F S P Q K L Q T L E T F I R E T T R S L L D S I D P R E D D I V K K L A V P L P I I V I S K I L G L P I E D K E K F K E W S D L V F F R L G K P G E I F E L G K K Y L E L I G Y V K D H L N S G T E V V S R V V N S N L S D I E K L G Y I I L L L I A G N E G T T N L I S N S V I D F T R F N L W Q R I R E E N L Y L K A I E E A L R Y S P P L M R T V R K T K E R V K L G D Q T I E E G E Y V R V W I A S A N R D E E V F H D G E K F I P D R N P N P H L S F G S G I H L G L G A P L A R L E A R I A I E E F S K R F R H I E I L D T E K V P N E V L N G Y K R L V V R L K S N E
CYP 119	*Sulfolobus solfataricus*	6xHis-TEV-CYP119	2BT	**P_{G2}**	E G D I H M K S S H H H H H H E N L Y F Q S N Y D W F S E M R K K D P V Y Y D G N I W Q V F S Y R Y T K E V L N N F S K F S S D L T G Y

					H E R L E D L R N G K I R F D I P T R Y T M L T S D P P L H D E L R S M S A D I F S P Q K L Q T L E T F I R E T T R S L L D S I D P R E D D I V K K L A V P L P I I V I S K I L G L P I E D K E K F K E W S D L V F F R L G K P G E I F E L G K K Y L E L I G Y V K N H L N S G T E V V S R V V N S N L S D I E K L G Y I I L L L I A G N E G T T N L I S N S V I D F T R F N L W Q R I R E E N L Y L K A I E E A L R Y S P P L M R T V R K T K E R V K L G D Q T I E E G E Y V R V W I A S A N R D E E V F H D G E K F I P D R N P N P H L S F G S G I H L G L G A P L A R L E A R I A I E E F S K R F R L I E I L D T E K V P N E V L N G Y K R L V V R L K S N E
CYP 119	*Sulfolobus solfataricus*	6xHis-TEV-CYP119	2BT	**P_{max}**	E G D I H M K S S H H H H H H E N L Y F Q S N Y D W F S E M R K K D P V Y Y D G N I W Q V F S Y R Y T K E V L N N F S K F S S D L T G Y H E R L E D L R N G K I R F D I P T R Y T M L T S D P P L H D E L R S M S A D I F S P Q K L Q T L E T F I R E T T R S L L D S I D P R E D D I V K K L A V P L P I I V I S K I L G L P I E D K E K F K E W S D L V F F R L G K P G E I F E L G K K Y L E L I G Y V K N H L N S G T E V V S R V V N S N L S D I E K L G Y I I L L L I A G N E G T T N L I S N S V I D F T R F N L W Q R I R E E N L Y L K A I E E S L R Y S P P L M R T V R K T K E R V K L G D Q T I E E G E Y V R V W I A S A N R D E E V F H D G E K F I P D R N P N P H L S F G S G I H L G L G A P L A R L E A R I A I E E F S K R F R L I E I L D T E K V P N E V L N G Y K R L V V R L K S N E

CYP 119	*Sulfolobus solfataricus*	6xHis-TEV-CYP119	2BT	**M_{G1}**	E G D I H M K S S H H H H H H E N L Y F Q S N Y D W F S E M R K K D P V Y Y D G N I W Q V F S Y R Y T K E V L N N F S K F S S D L T G Y H E R L E D L R N G K I R F D I P T R Y T M L T S D P P L H D E L R S M S A D I F S P Q K L Q T L E T F I R E T T R S L L D S I D P R E D D I V K K L A V P L P I I V I S K I L G L P I E D K E K F K E W S D L V L F R L G K P G E I F E L G K K Y L E L I G Y V K D H L N S G T E V V S R V V N S N L S D I E K L G Y I I L L L I A G N E G T T N L I S N S V I D F T R F N L W Q R I R E E N L Y L K A I E E A L R Y S P P A M R T V R K T K E R V K L G D Q T I E E G E Y V R V W I A S A N R D E E V F H D G E K F I P D R N P N P H L S F G S G I H L G L G A P L A R L E A R I A I E E F S K R F R H I E I L D T E K V P N E V L N G Y K R L V V R L K S N E
CYP 119	*Sulfolobus solfataricus*	6xHis-TEV-CYP119	2BT	**M_{G2}**	E G D I H M K S S H H H H H H E N L Y F Q S N Y D W F S E M R K K D P V Y Y D G N I W Q V F S Y R Y T K E V L N N F S K F S S D L T G Y H E R L E D L R N G K I R F D I P T R Y T M L T S D P P L H D E L R S M S A D I F S P Q K L Q T L E T F I R E T T R S L L D S I D P R E D D I V K K L A V P L P I I V I S K I L G L P I E D K E K F K E W S D L V L F R L G K P G E I F E L G K K Y L E L I G Y V K D H L N S G T E V V S R V V N S N L S D I E K L G Y I I L L L I A G N E G T T N L I S N S V I D F T R F N L W Q R I R E E N L Y L K A I E E S L R Y S P P A M R T V R K T K E R V K L G D Q T I E E G E Y V R V W I A S A N R D E E V F H D G E K F I P D R N P N P H L S F G S G I H L G L G A P L A R L E A R I A I E E F S K R F R H I E I L D T E

					K V P N E V L N G Y K R L V V R L K S N E
CYP 119	*Sulfolobus solfataricus*	6xHis-TEV-CYP119	2BT	**M_{max}**	E G D I H M K S S H H H H H H E N L Y F Q S N Y D W F S E M R K K D P V Y Y D G N I W Q V F S Y R Y T K E V L N N F S K F S S D L T G Y H E R L E D L R N G K I R F D I P T R Y T M L T S D P P L H D E L R S M S A D I F S P Q K L Q T L E T F I R E T T R S L L D S I D P R E D D I V K K L A V P L P I I V I S K I L G L P I E D K E K F K E W S D L V L F R L G K P G E I F E L G K K Y L E L I G Y V K N H L N S G T E V V S R V V N S N L S D I E K L G Y I I L L L I A G N E G T T N L I S N S V I D F T R F N L W Q R I R E E N L Y L K A I E E S L R Y S P P A M R T V R K T K E R V K L G D Q T I E E G E Y V R V W I A S A N R D E E V F H D G E K F I P D R N P N P H L S F G S G I H L G L G A P L A R L E A R I A I E E F S K R F R L I E I L D T E K V P N E V L N G Y K R L V V R L K S N E

Representative DNA sequence (CYP M_{G2}):

GGCTCCGGAGAGCTCTTTAATTAAGCGGCCGCCCTGCAGGACTCGAGTTC
TAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGAAATCTTC
TCACCATCACCATCACCATGAAAACCTGTACTTCCAATCCAATTATGACTG
GTTCAGCGAGATGCGTAAGAAGGACCCGGTTTATTATGACGGCAACATTT
GGCAAGTTTTCAGCTATCGTTATACCAAAGAGGTGCTGAACAACTTCAGC
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TAACGGCAAAATCCGTTTTGATATTCCGACCCGTTATACCATGCTGACCAG
CGACCCGCCGCTGCACGATGAACTGCGTAGCATGAGCGCGGATATCTTTA

GCCCGCAGAAGCTGCAAACCCTGGAGACCTTCATTCGTGAAACCACCCGT
AGCCTGCTGGACAGCATCGATCCGCGTGAGGACGATATTGTGAAGAAACT
GGCGGTTCCGCTGCCGATCATTGTGATCAGCAAAATTCTGGGTCTGCCGAT
CGAGGACAAGGAAAAATTCAAGGAATGGAGCGATCTGGTTCTATTTCGTC
TGGGTAAACCGGGCGAGATCTTCGAACTGGGTAAGAAATACCTGGAGCTG
ATTGGCTATGTGAAGGACCACCTGAACAGCGGCACCGAAGTGGTTAGCCG
TGTGGTTAACAGCAACCTGAGCGATATCGAGAAGCTGGGTTACATCATTC
TGCTGCTGATTGCGGGCAACGAAGGCACCACCAACCTGATCAGCAACAGC
GTTATTGACTTCACCCGTTTTAACCTGTGGCAGCGTATCCGTGAGGAAAAC
CTGTACCTGAAAGCGATTGAGGAATCGCTGCGTTATAGCCCGCCGGCGAT
GCGTACCGTTCGTAAAACCAAGGAGCGTGTGAAGCTGGGTGACCAAACCA
TCGAGGAAGGCGAATATGTGCGTGTTTGGATTGCGAGCGCGAACCGTGAC
GAGGAAGTTTTCCACGATGGCGAGAAATTTATCCCGGATCGTAACCCGAA
CCCGCACCTGAGCTTCGGTAGCGGCATCCACCTGGGCCTGGGTGCGCCGC
TGGCGCGTCTGGAAGCGCGTATCGCGATTGAGGAATTCAGCAAACGTTTT
CGTCACATCGAGATTCTGGATACCGAAAAGGTTCCGAATGAAGTTCTGAA
TGGTTACAAGCGTCTGGTGGTGCGTCTGAAGAGCAATGAGTAAGGATCCG
CGATCGCGGCGCGCCACCTGGTGGCCGGCCGGTACCACGCGTGCGCGCTG
ATCCGGCTGCTAACAAAGCCCGAAA

6.1: Reconstitution Circular Dichroism (CD) Spectrum

General Procedure: CD spectrum were recorded on a Jasco J-815 spectrometer using a 0.1 mm quartz cuvette. Ir(Me)-CYP119s were prepared at a concentration of 5 μM using the procedure described in section 1.1, except in a 10 mM phosphate buffer (10 mM NaPi, pH = 6.0). CD measurements were recorded from 260–190 nm with a scan rate of 100 nm/min, a bandwidth of 1nm, and averaged over 8 accumulations.

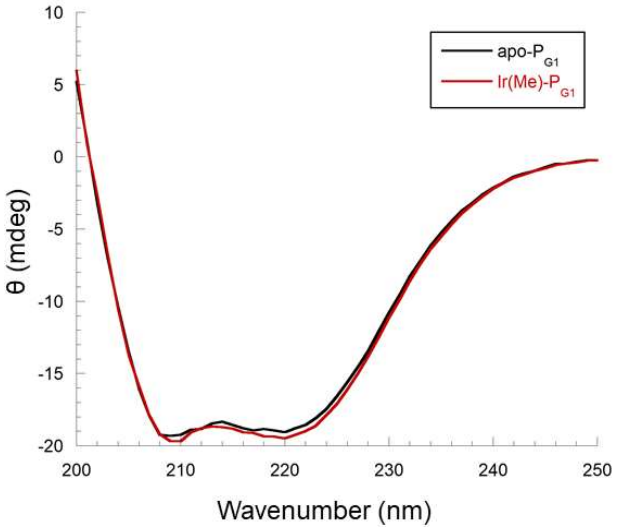


Figure S60. Circular dichroism spectrum of apo-$\mathbf{P_{G1}}$ and Ir(Me)-$\mathbf{P_{G1}}$ recorded in 10 mM NaPi (pH = 6.0).

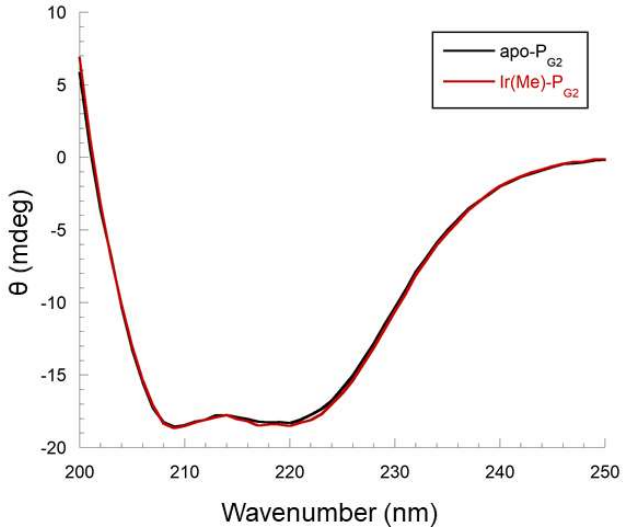


Figure S61. Circular dichroism spectrum of apo-$\mathbf{P_{G2}}$ and Ir(Me)-$\mathbf{P_{G2}}$ recorded in 10 mM NaPi (pH = 6.0).

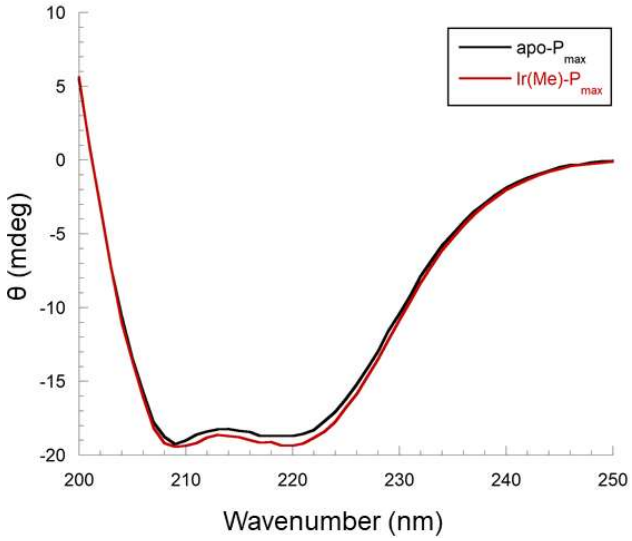


Figure S62. Circular dichroism spectrum of apo-$\mathbf{P_{max}}$ and Ir(Me)-$\mathbf{P_{max}}$ recorded in 10 mM NaPi (pH = 6.0).

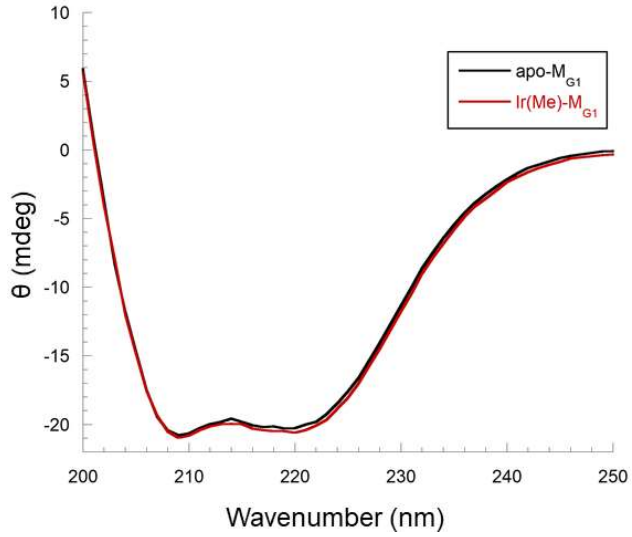


Figure S63. Circular dichroism spectrum of apo-$\mathbf{M_{G1}}$ and Ir(Me)-$\mathbf{M_{G1}}$ recorded in 10 mM NaPi (pH = 6.0).

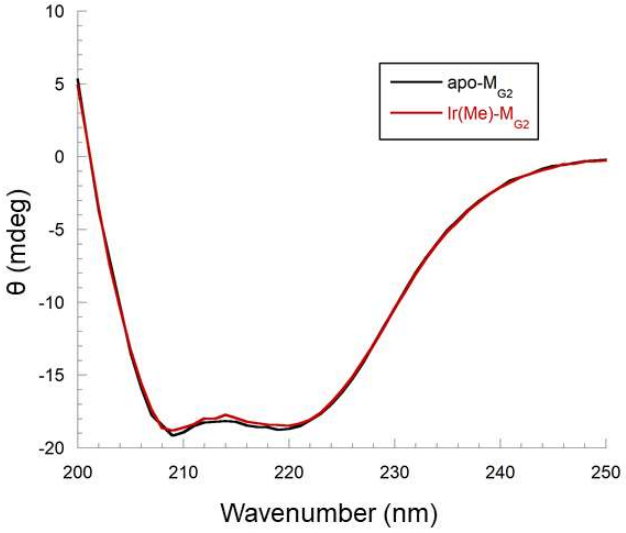


Figure S64. Circular dichroism spectrum of apo-$\mathbf{M_{G2}}$ and Ir(Me)-$\mathbf{M_{G2}}$ recorded in 10 mM NaPi (pH = 6.0).

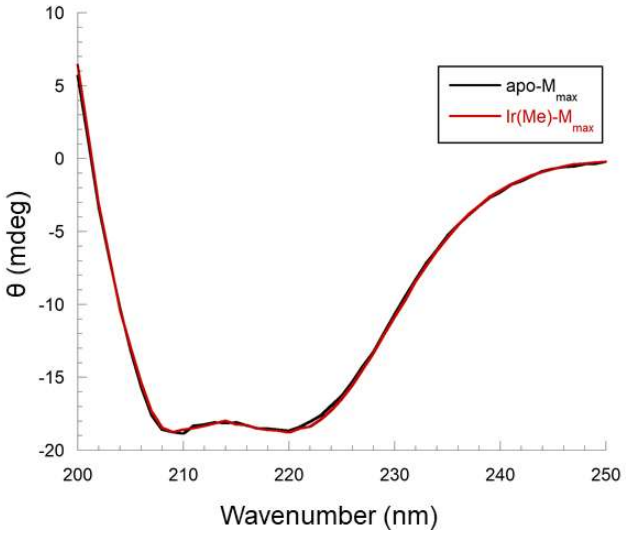


Figure S65. Circular dichroism spectrum of apo-$\mathbf{M_{max}}$ and Ir(Me)-$\mathbf{M_{max}}$ recorded in 10 mM NaPi (pH = 6.0).

6.2: EDA Protein Stability Studies: Circular Dichroism (CD) and Mass Spectrum

General Procedure: CD spectrum were recorded on a Jasco J-815 spectrometer using a 0.1 mm quartz cuvette. CD measurements were recorded from 260 – 190 nm at a scan rate of 100 nm/min, a bandwidth of 1 nm, and averaged over 8 accumulations. Acetonitrile (Optima grade, 99.9%, Fisher, Waltham, MA), formic acid (1 mL ampules, 99+%, Pierce, Rockford, IL), and water purified to a resistivity of 18.2 MΩ·cm (at 25 °C) using a Milli-Q Gradient ultrapure water purification system (Millipore, Billerica, MA) were used to prepare mobile phase solvents for LC-MS. Electrospray ionization mass spectrometry (ESI-MS) of protein samples was performed using an Agilent 1260 series liquid chromatograph outfitted with an Agilent 6224 time-of-flight (TOF) LC-MS system (Santa Clara, CA). The LC was equipped with a Proswift RP-4H (monolithic phenyl, 1.0 mm × 50 mm, Dionex) analytical column. Solvent A was 99.9% water/0.1% formic acid and solvent B was 99.9% acetonitrile/0.1% formic acid (v/v). For each sample, approximately 15 to 30 picomoles of analyte were injected onto the column. Following sample injection, a 5-100% B elution gradient was run at a flow rate of 0.30 mL/min over 12 min. Data was collected and analyzed by deconvolution of the entire elution profile in order to provide reconstructed mass spectra that are representative of the entire sample using Agilent MassHunter Qualitative Analysis B.05.00. Protein samples were prepared as described in section 1.1, except in a 0.2 mM NaPi buffer to decrease the amount of salt added to the column during analysis. An excess of EDA (1000 equiv, 2.5 M MeCN stock solution) was added to the protien samples (1 mL, 5 μM) and allowed to shake at room temperature and 300 rpm for 1h and then directly analyzed by CD and mass spectrometry. No protein masses associated with the insertion of EDA into the protein framework were ever observed over numerous trials (>10). Reactions with **1a** (0.5 M, MeCN) and EDA (1 M, MeCN) were conducted in a similar manner as described in section 3.1 and allowed to go for 1h before being directly analyzed by both CD and mass spectrometry. Yields for EDA dimerization were determined from reaction of either free cofactor (Ir(Me)-MPIX) or Ir(Me)-CYP119 in the presence of 1000 equiv of EDA (1M in DMF) in NaPi buffer (100mM NaPi, 100 mM NaCl, pH = 6.0). Reactions were quenched with 250 μl of a 50% HBr solution followed by neutralization using 250 μl of a saturated $NaHCO_3$ solution and a 500 μl stock solution of EtOAc containing dodecane as an internal standard (1 μl dodecane to 500 μL of EtOAc). Results were analyzed as described in section 3.1.

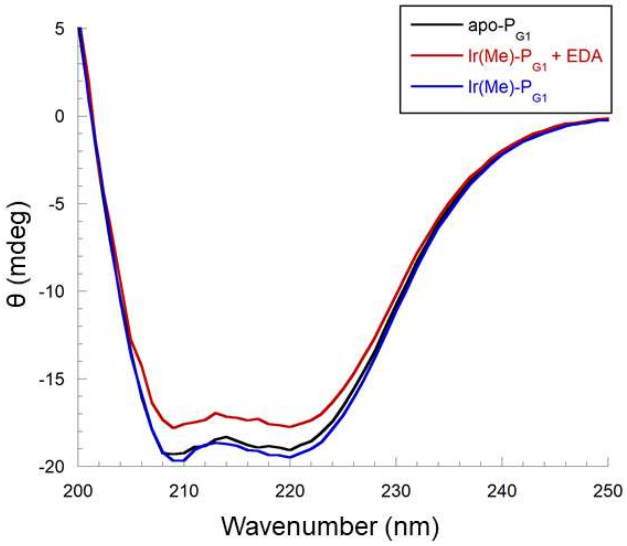


Figure S66. Circular dichroism spectrum of apo-$\mathbf{P_{G1}}$ (black), Ir(Me)-$\mathbf{P_{G1}}$ (red), and Ir(Me)-$\mathbf{P_{G1}}$ + 1000 equiv of EDA (blue) recorded in 10 mM NaPi (pH = 6.0).

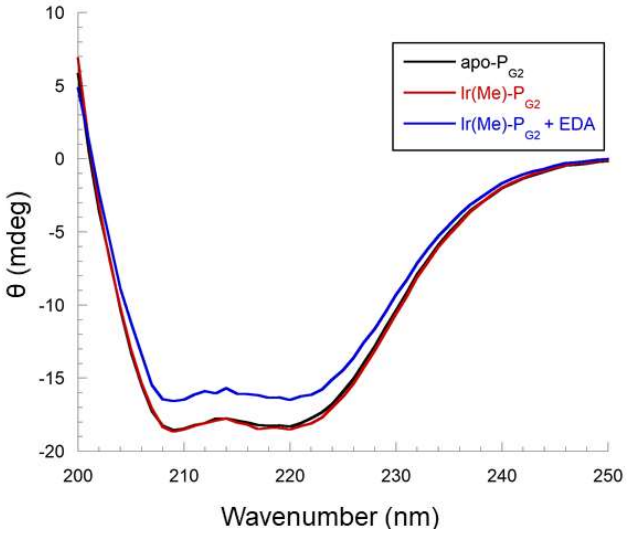


Figure S67. Circular dichroism spectrum of apo-$\mathbf{P_{G2}}$ (black), Ir(Me)-$\mathbf{P_{G2}}$ (red), and Ir(Me)-$\mathbf{P_{G2}}$ + 1000 equiv of EDA (blue) recorded in 10 mM NaPi (pH = 6.0).

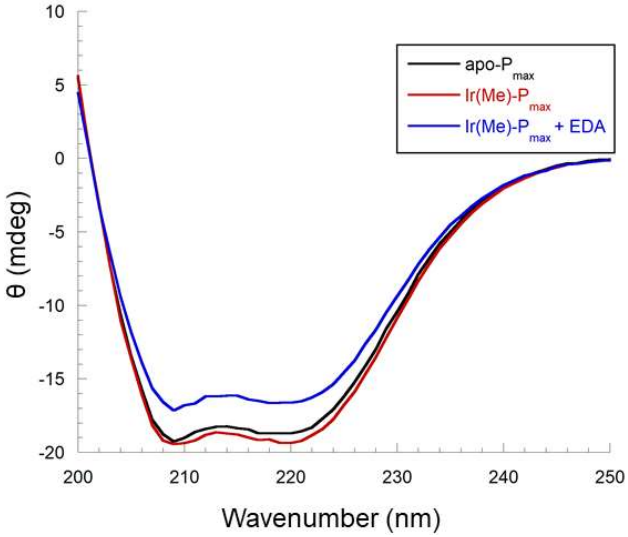


Figure S68. Circular dichroism spectrum of apo-$\mathbf{P_{max}}$ (black), Ir(Me)-$\mathbf{P_{max}}$ (red), and Ir(Me)-$\mathbf{P_{max}}$ + 1000 equiv of EDA (blue) recorded in 10 mM NaPi (pH = 6.0).

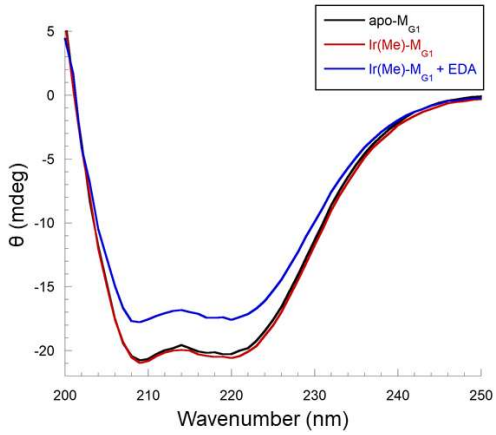


Figure S69. Circular dichroism spectrum of apo-$\mathbf{M_{G1}}$ (black), Ir(Me)-$\mathbf{M_{G1}}$ (red), and Ir(Me)-$\mathbf{M_{G1}}$ + 1000 equiv of EDA (blue) recorded in 10 mM NaPi (pH = 6.0).

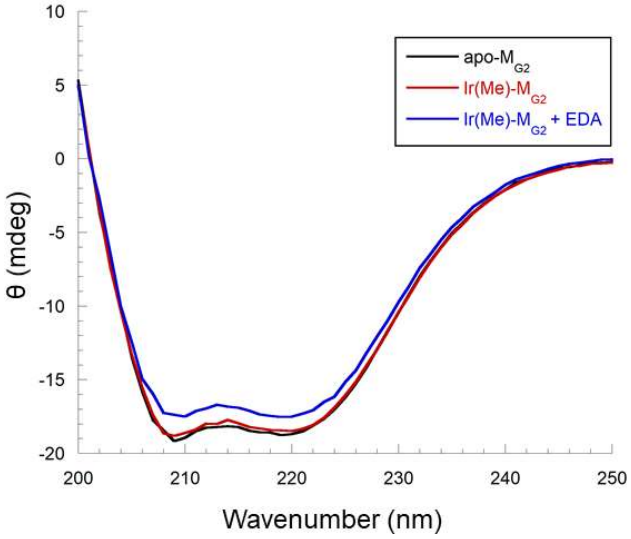


Figure S70. Circular dichroism spectrum of apo-$\mathbf{M_{G2}}$ (black), Ir(Me)-$\mathbf{M_{G2}}$ (red), and Ir(Me)-$\mathbf{M_{G2}}$ + 1000 equiv of EDA (blue) recorded in 10 mM NaPi (pH = 6.0).

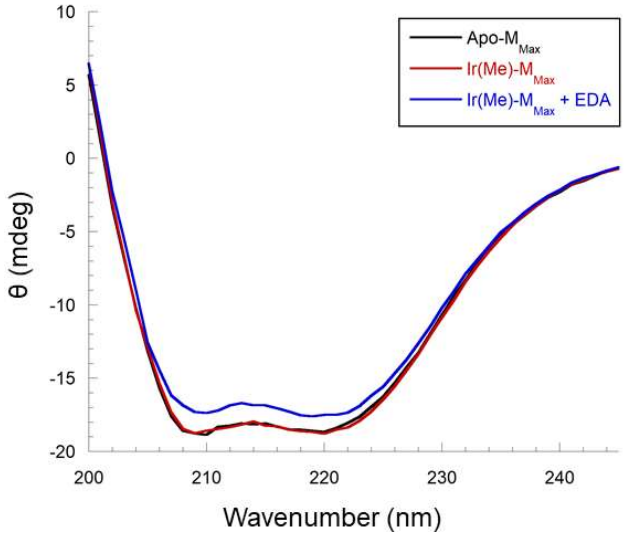


Figure S71. Circular dichroism spectrum of apo-$\mathbf{M_{max}}$ (black), Ir(Me)-$\mathbf{M_{max}}$ (red), and Ir(Me)-$\mathbf{M_{max}}$ + 1000 equiv of EDA (blue) recorded in 10 mM NaPi (pH = 6.0).

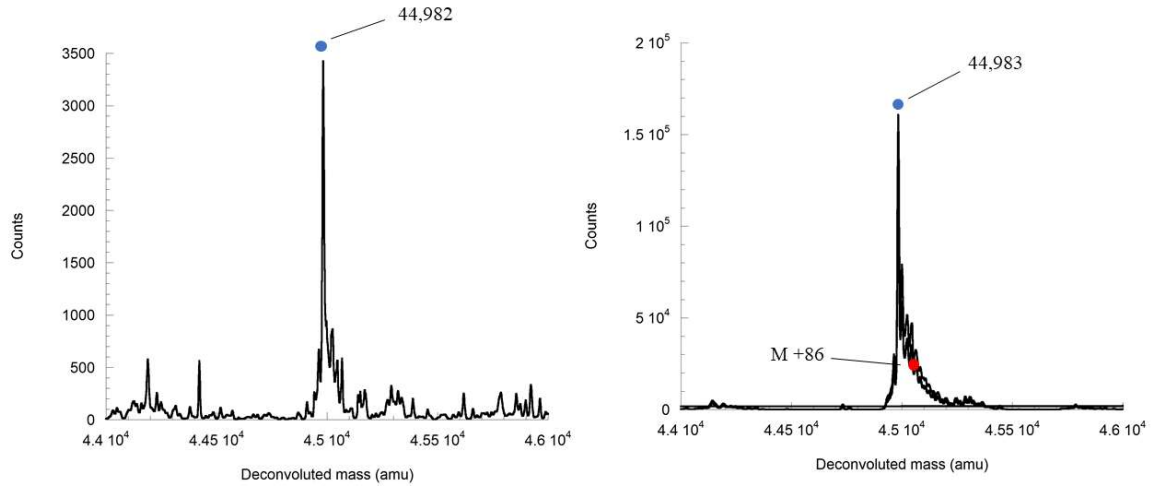


Figure S72. Deconvoluted mass spectrum of $\mathbf{P_{G1}}$ before (left) and after 1h rxn with EDA and **1a** (right) (calcd mass for apo-$\mathbf{P_{G1}}$ = 44984).

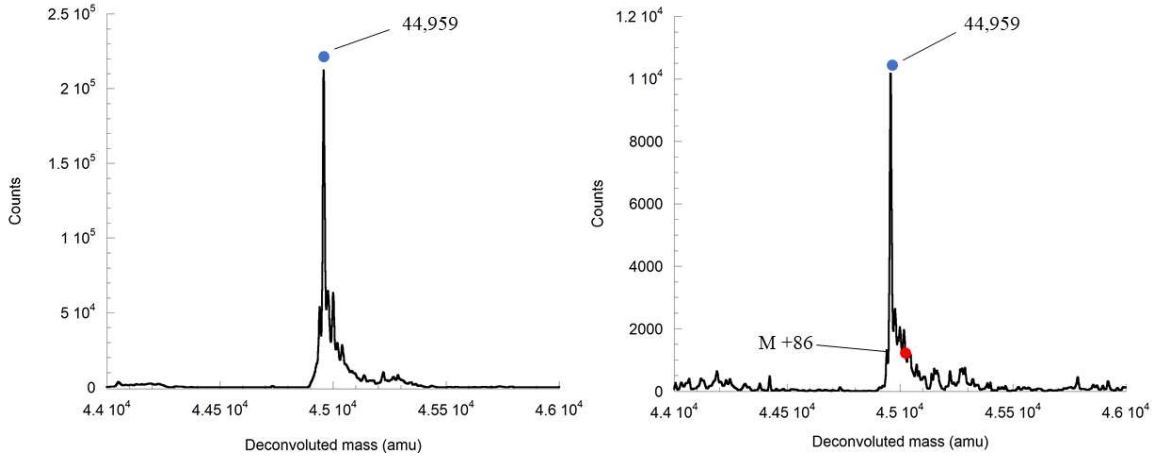


Figure S73. Deconvoluted mass spectrum of $\mathbf{P_{G2}}$ before (left) and after 1h rxn with EDA and **1a** (right) (calcd mass for apo-$\mathbf{P_{G2}}$ = 44959).

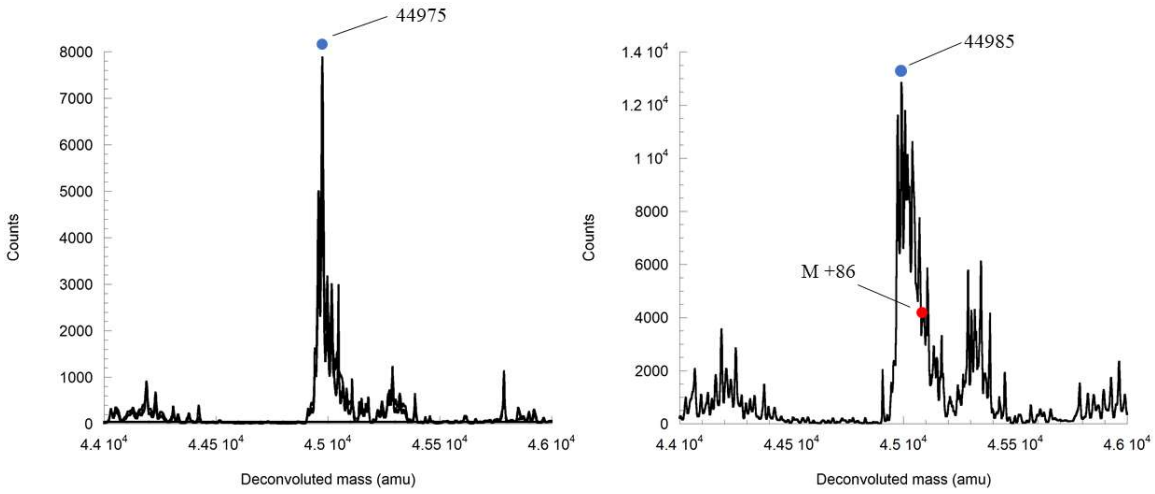


Figure S74. Deconvoluted mass spectrum of $\mathbf{P_{max}}$ before (left) and after 1h rxn with EDA and **1a** (right) (calcd mass for apo-$\mathbf{P_{max}}$ = 44975).

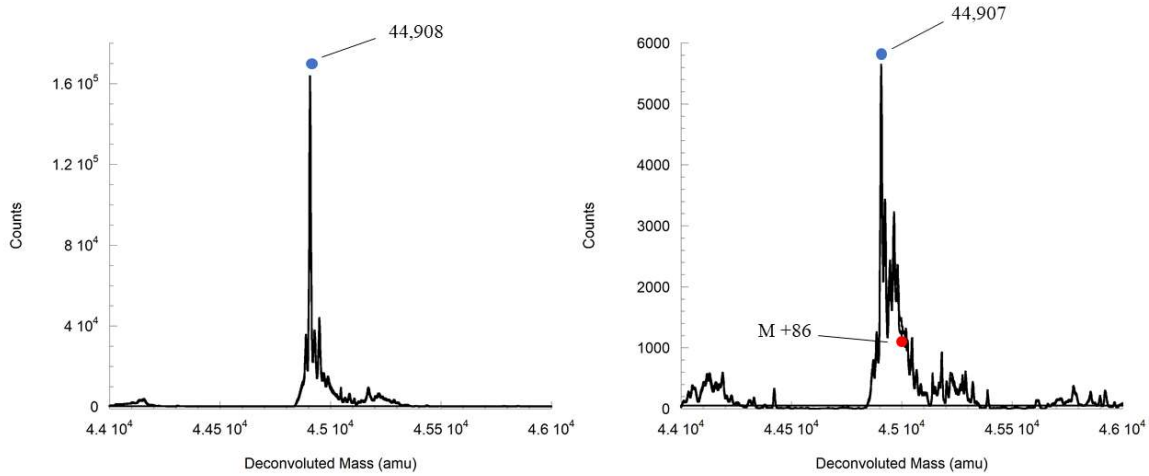


Figure S75. Deconvoluted mass spectrum of $\mathbf{M_{G1}}$ before (left) and after 1h rxn with EDA and **1a** (right) (calcd mass for apo-$\mathbf{M_{G1}}$ = 44908).

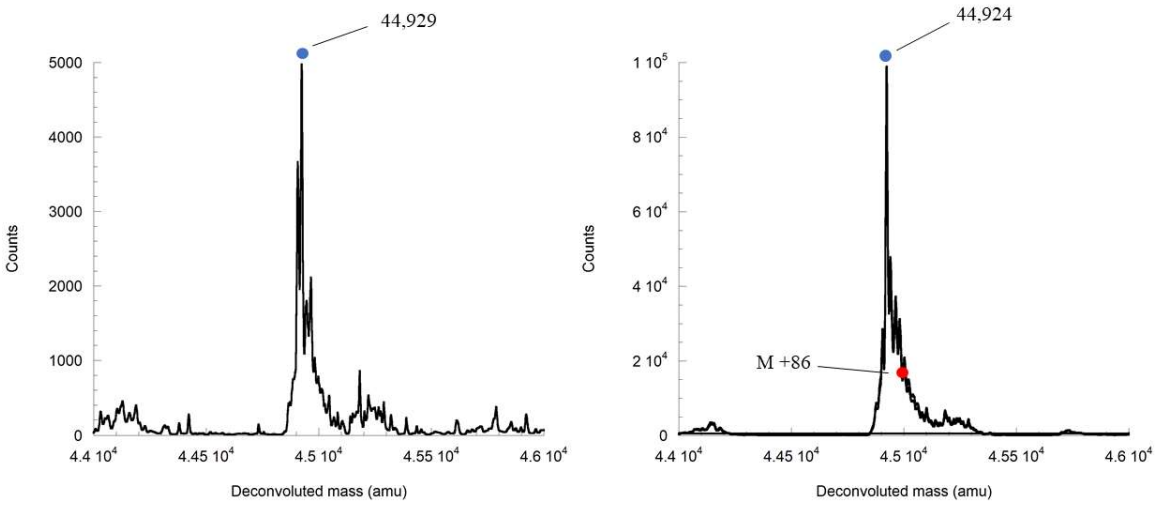


Figure S76. Deconvoluted mass spectrum of $\mathbf{M_{G2}}$ before (left) and after 1h rxn with EDA and **1a** (right) (calcd mass for apo-$\mathbf{M_{G2}}$ = 44924).

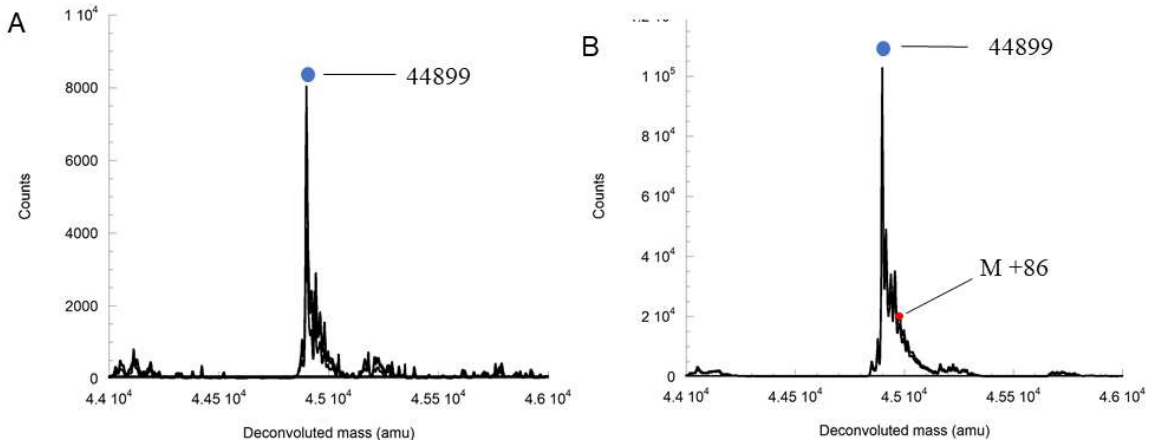


Figure S77. Deconvoluted mass spectrum of $\mathbf{M_{max}}$ before (A) and after 1h rxn with EDA and **1a** (B) (calcd mass for apo-$\mathbf{M_{max}}$ = 44899).

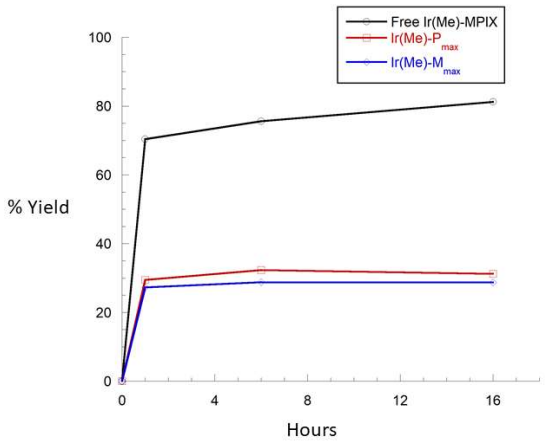


Figure S78. Dimerization of 1000 equiv of EDA by free cofactor (black), Ir(Me)-$\mathbf{P_{max}}$ (red), and Ir(Me)-$\mathbf{M_{max}}$ (blue).

6.3: Ir(Me)-CYP119 Stability Towards Product

General Procedure: CD spectrum were recorded on a Jasco J-815 spectrometer using a 0.1 mm quartz cuvette. CD measurements were recorded from 260 – 190 nm at a scan rate of 100 nm/min, a bandwidth of 1 nm, and averaged over 8 accumulations. Reactions were carried out using a 1 mL solution of Ir(Me)-CYP119 (5 μM, 10mM NaPi, pH = 6.0) to which was added 10 equiv of **4c** (10 mM, MeCN). The mixture was then stirred at 250 rpm with a 3 mm octagon magnetic stir bar at room temperature for the allotted time. After the appropriate time interval, 800 ul of sample was centrifuged (1min, 5000rpm) through a 0.45 μm cellulose acetate spin-x filter. The cuvette was washed with 250 ul of solution sample and then loaded with 400 ul of sample.

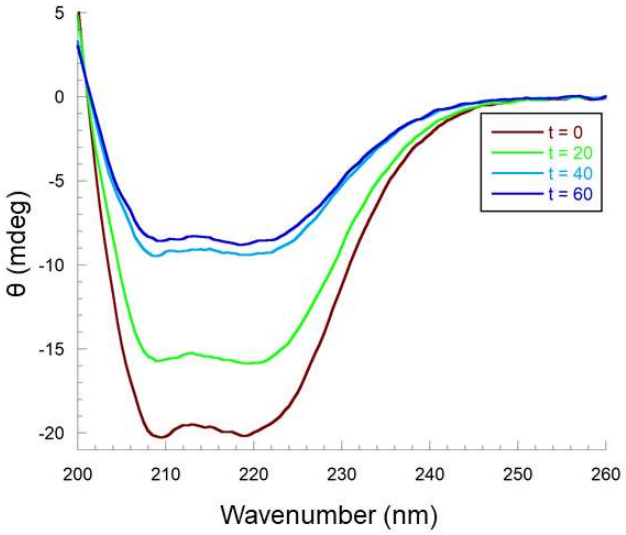


Figure S79. Circular dichroism spectrum of Ir(Me)-$\mathbf{P_{G1}}$ with 10 equiv of **4c** (10 mM, MeCN) measured over 1h recorded in 10 mM NaPi (pH = 6.0). A decrease in ellipticity of 56% (± 1) is observed from t = 0 to t = 60 min at 222 nm.

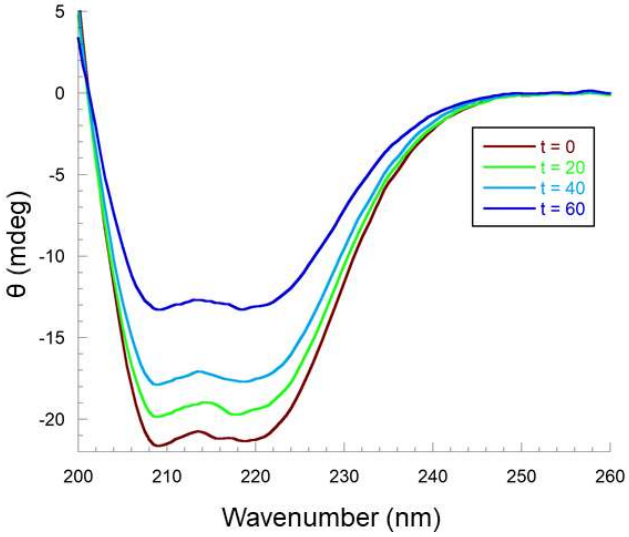


Figure S80. Circular dichroism spectrum of Ir(Me)-$\mathbf{P_{max}}$ with 10 equiv of **4c** (10 mM, MeCN) measured over 1h recorded in 10 mM NaPi (pH = 6.0). A decrease in ellipticity of 38% (± 1) is observed from t = 0 to t = 60 min at 222 nm.

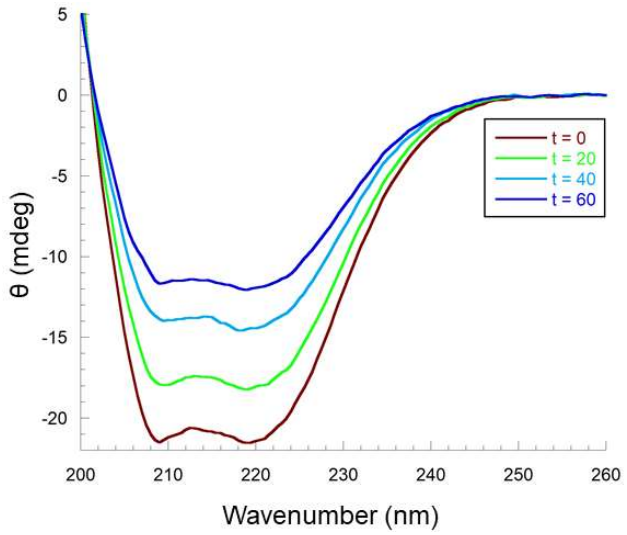
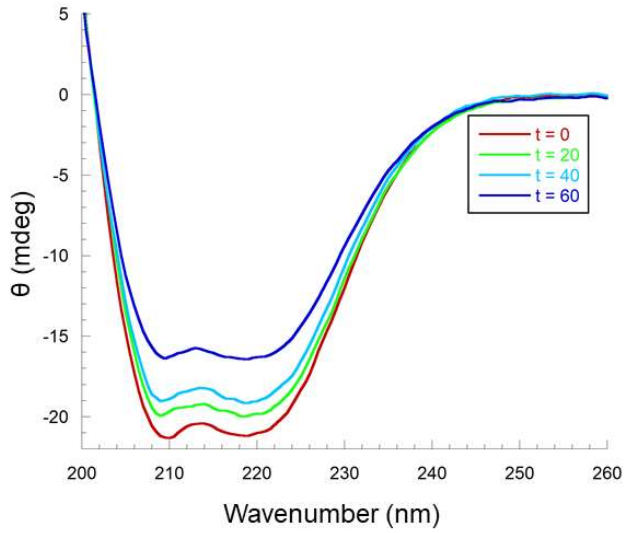


Figure S81. Circular dichroism spectrum of Ir(Me)-$\mathbf{M_{G1}}$ with 10 equiv of **4c** (10 mM, MeCN) measured over 1h recorded in 10 mM NaPi (pH = 6.0). A decrease in ellipticity of 44% (± 1) is observed from t = 0 to t = 60 min at 222 nm.



Figures S82. Circular dichroism spectrum of Ir(Me)-$\mathbf{M_{max}}$ with 10 equiv of **4c** (10 mM, MeCN) measured over 1h recorded in 10 mM NaPi (pH = 6.0). A decrease in ellipticity of 21% (± 2) is observed from t = 0 to t = 60 min at 222 nm.

6.4: Differential Scanning Coulometry (DSC) For Tm values

General Procedure: Protein samples were prepared as described in section 1.1. Ir(Me)-CYP119 was exchanged into 100 mM Kpi, pH 7.0, by passage over a Sephadex G-25 gel-filtration column. The Nano-DSC unit from TA Instruments (New Castle, DE) was employed for all experiments. DSC scans were taken with a scan rate of 70°C/h from 25 to 100°C for all Ir(Me)-CYP119 samples. The data were fit using Nano Analyzer software (Ta Instruments) to gaussian model after baseline subtraction. The WT-CYP119 has a Tm = 91.9°C under similar conditions.[8]

Table S4. Experimental and Gaussian Fit thermal melting (Tm) points for $\mathbf{P_{G1}}$, $\mathbf{P_{G2}}$, $\mathbf{P_{max}}$, $\mathbf{M_{G1}}$, $\mathbf{M_{G2}}$, and $\mathbf{M_{max}}$ under the conditions described above.

Compound	T_m exp	T_m model	Compound	T_m exp	T_m model
$\mathbf{P_{G1}}$	67.4 °C	67.6 °C	$\mathbf{M_{G1}}$	67.3 °C	67.1 °C
$\mathbf{P_{G2}}$	67.8 °C	67.9 °C	$\mathbf{M_{G2}}$	67.4 °C	67.5 °C
$\mathbf{P_{max}}$	67.3 °C	67.3 °C	$\mathbf{M_{max}}$	67.9 °C	67.9 °C

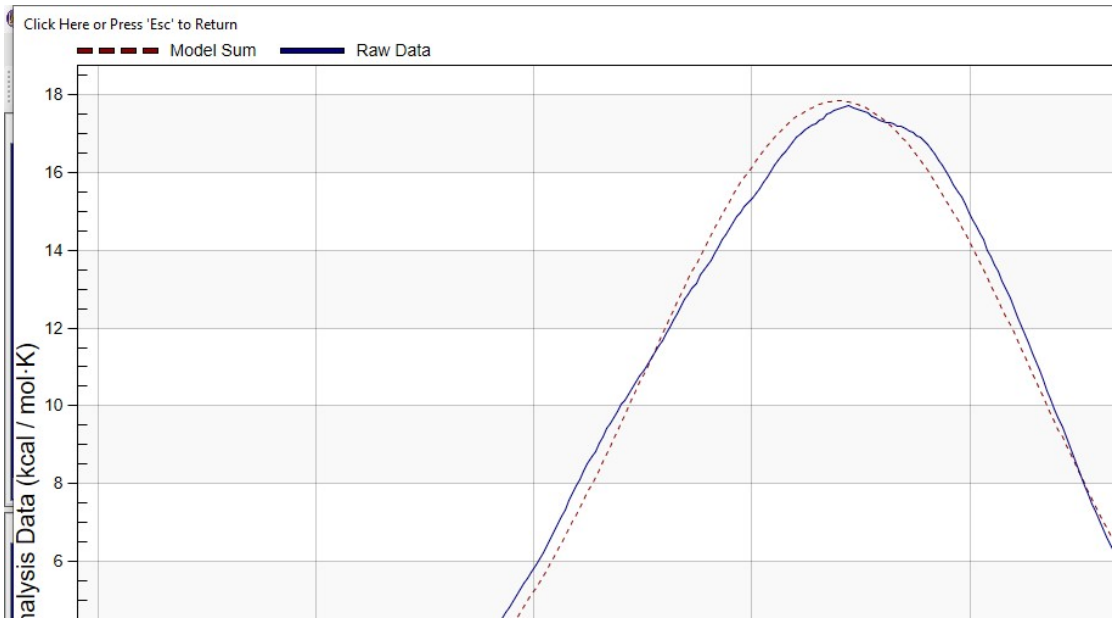


Figure S83. DSC measurement for $\mathbf{P_{G1}}$ under conditions described above. Experimental data (blue trace) and calculated fit (Red Trace).

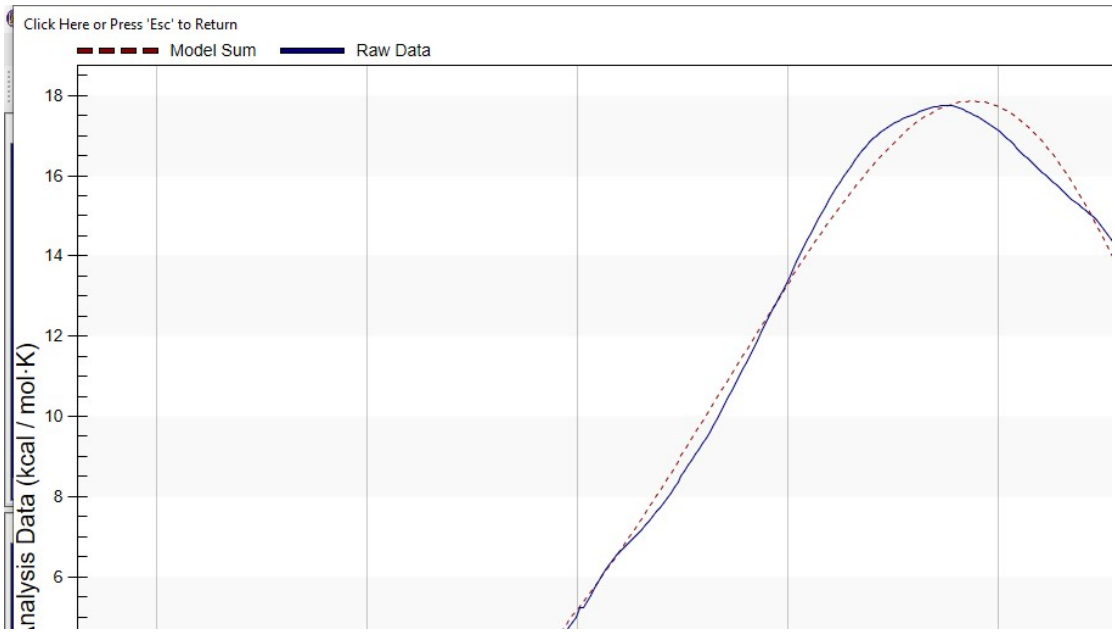


Figure S84 DSC measurement for $\mathbf{P_{G2}}$ under conditions described above. Experimental data (blue trace) and calculated fit (Red Trace).

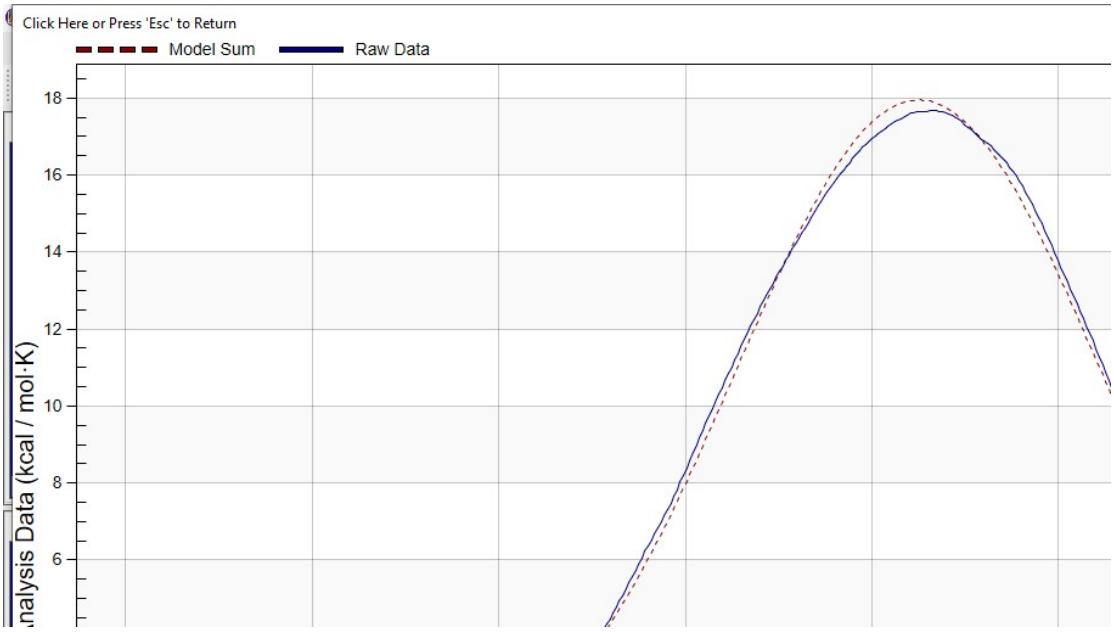


Figure S85. DSC measurement for $\mathbf{P_{max}}$ under conditions described above. Experimental data (blue trace) and calculated fit (Red Trace).

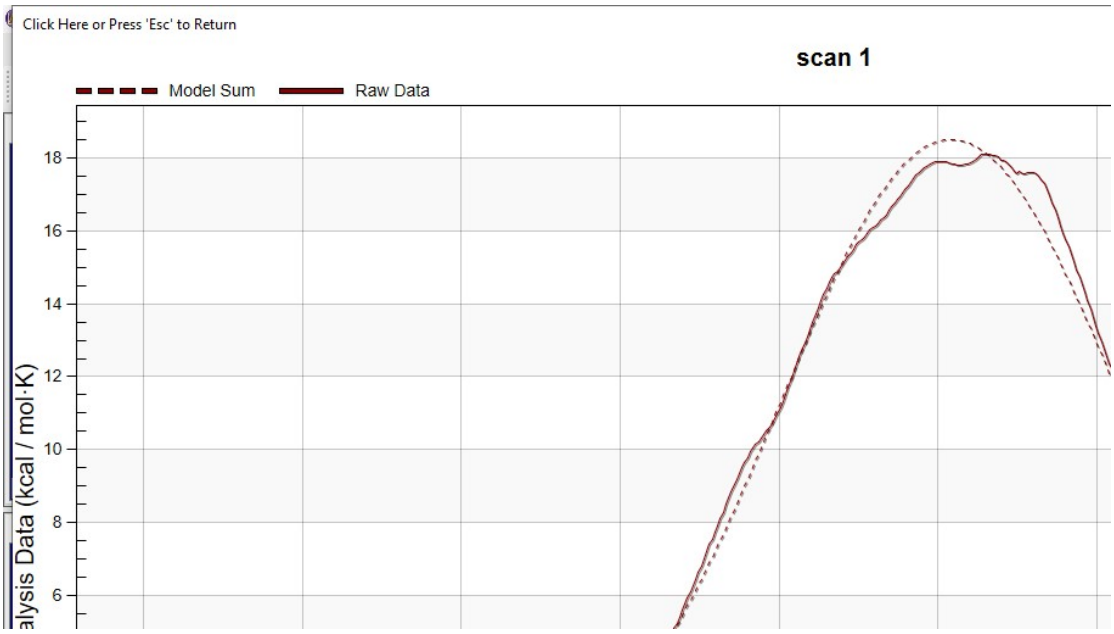


Figure S86. DSC measurement for $\mathbf{M_{G1}}$ under conditions described above. Experimental data (blue trace) and calculated fit (Red Trace).

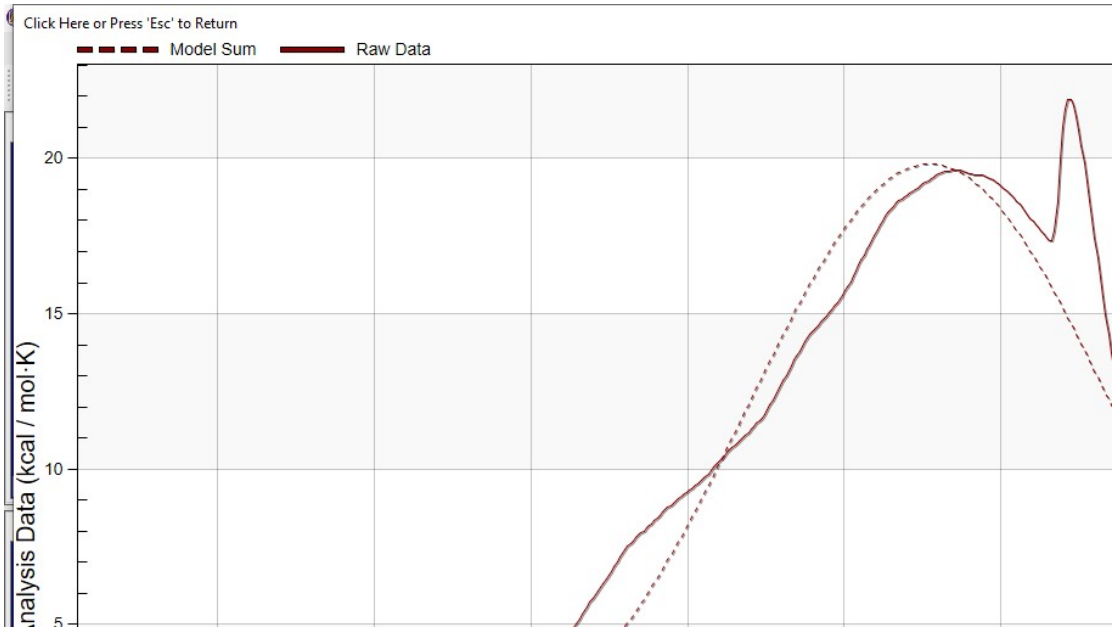


Figure S87. DSC measurement for $\mathbf{M_{G2}}$ under conditions described above. Experimental data (blue trace) and calculated fit (Red Trace).

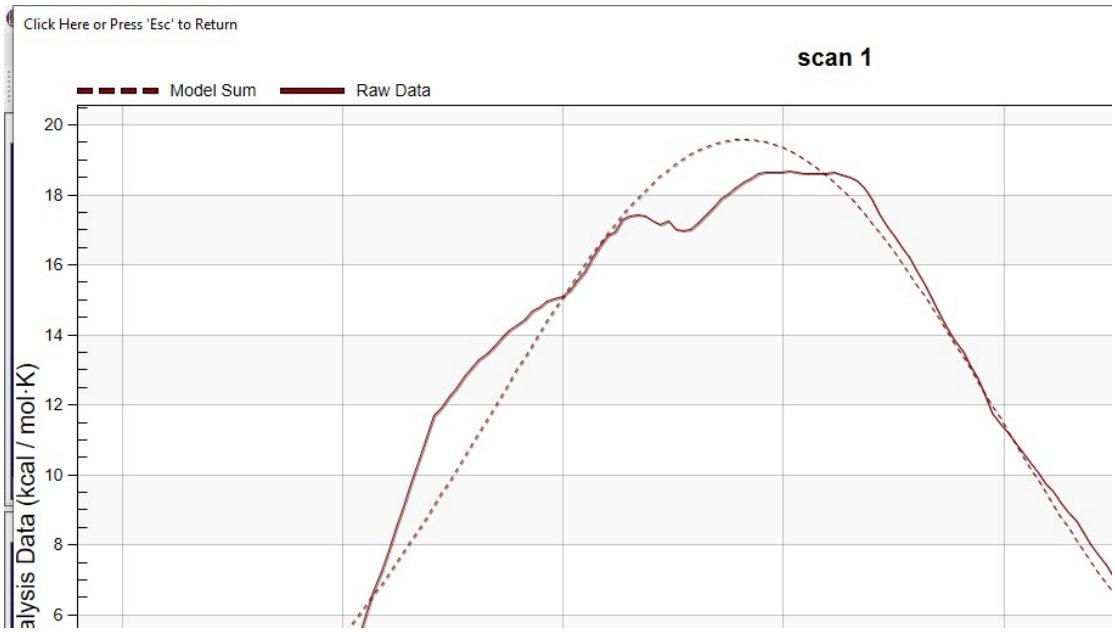


Figure S88. DSC measurement for $\mathbf{M_{max}}$ under conditions described above. Experimental data (blue trace) and calculated fit (Red Trace).

7.1:NMR spectra

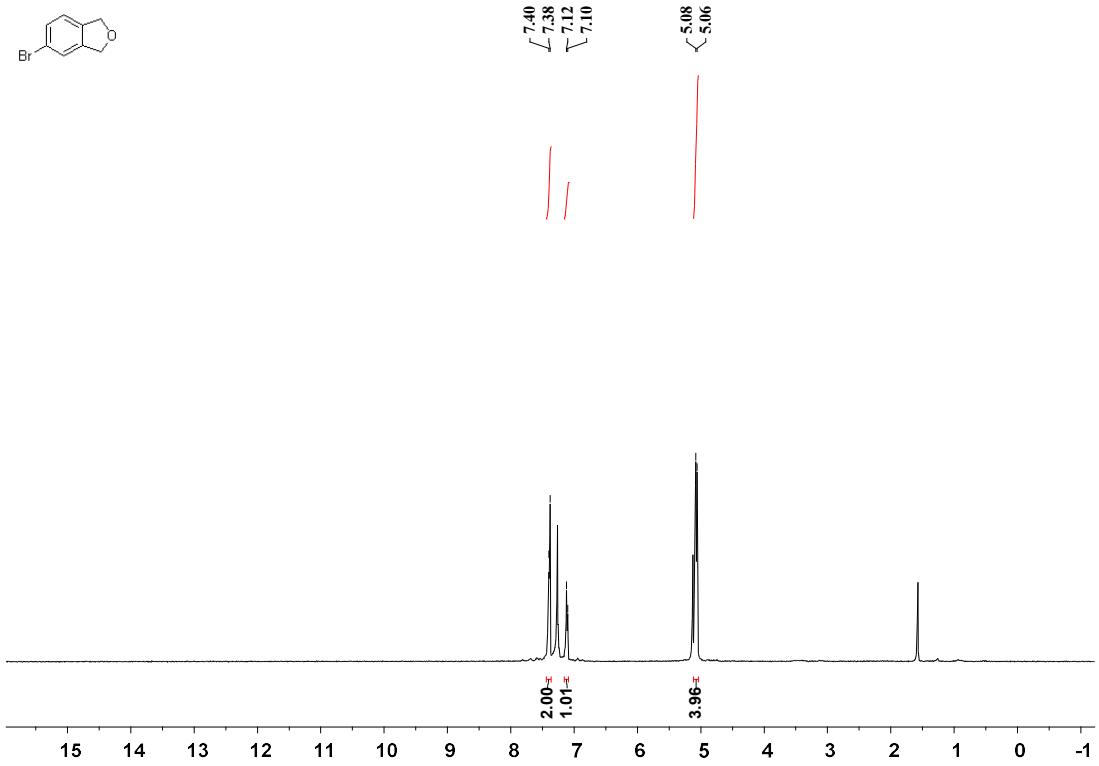


Figure S89. ^{1}H NMR (400 MHz, $CDCl_3$) of **1a**

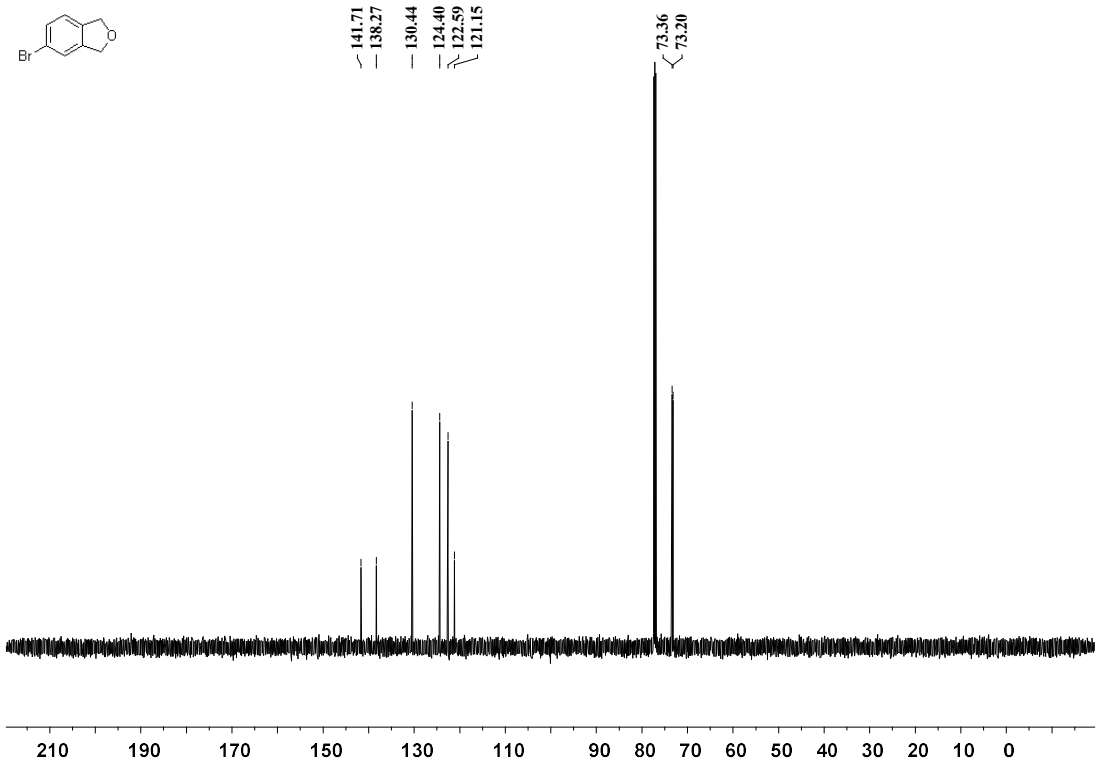


Figure S90. ^{13}C NMR (151 MHz, $CDCl_3$) of **1a**

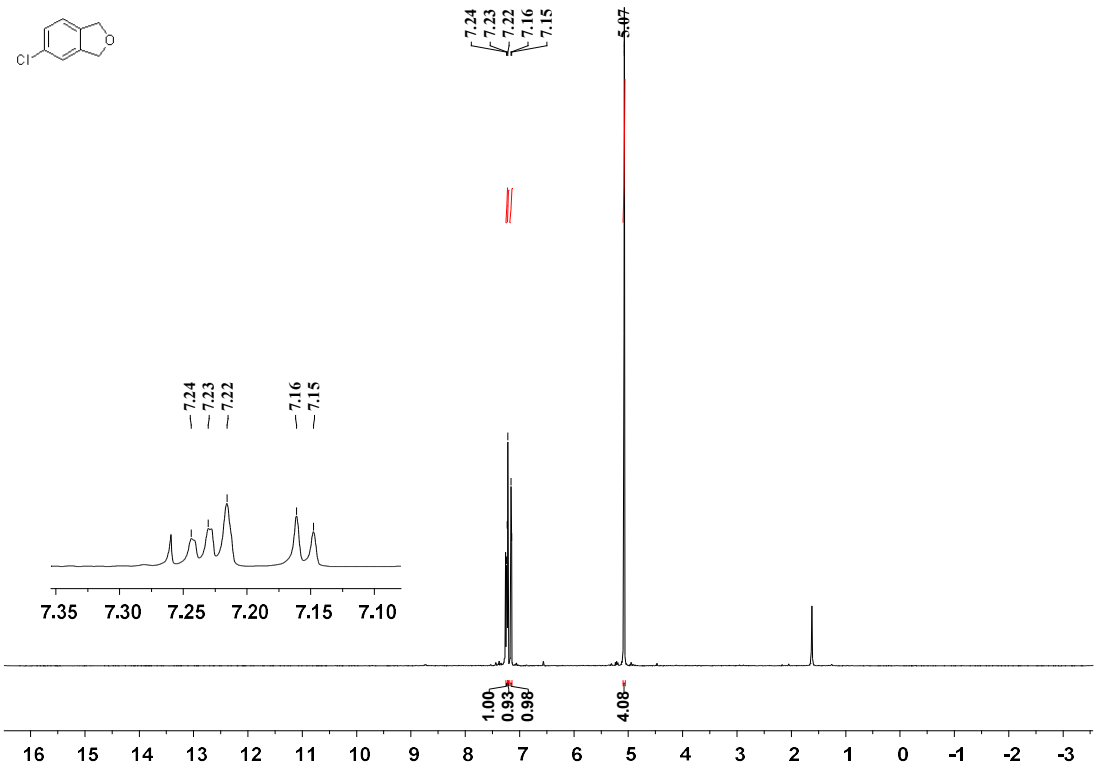


Figure S91. ^{1}H NMR (600 MHz, $CDCl_3$) of **2a**

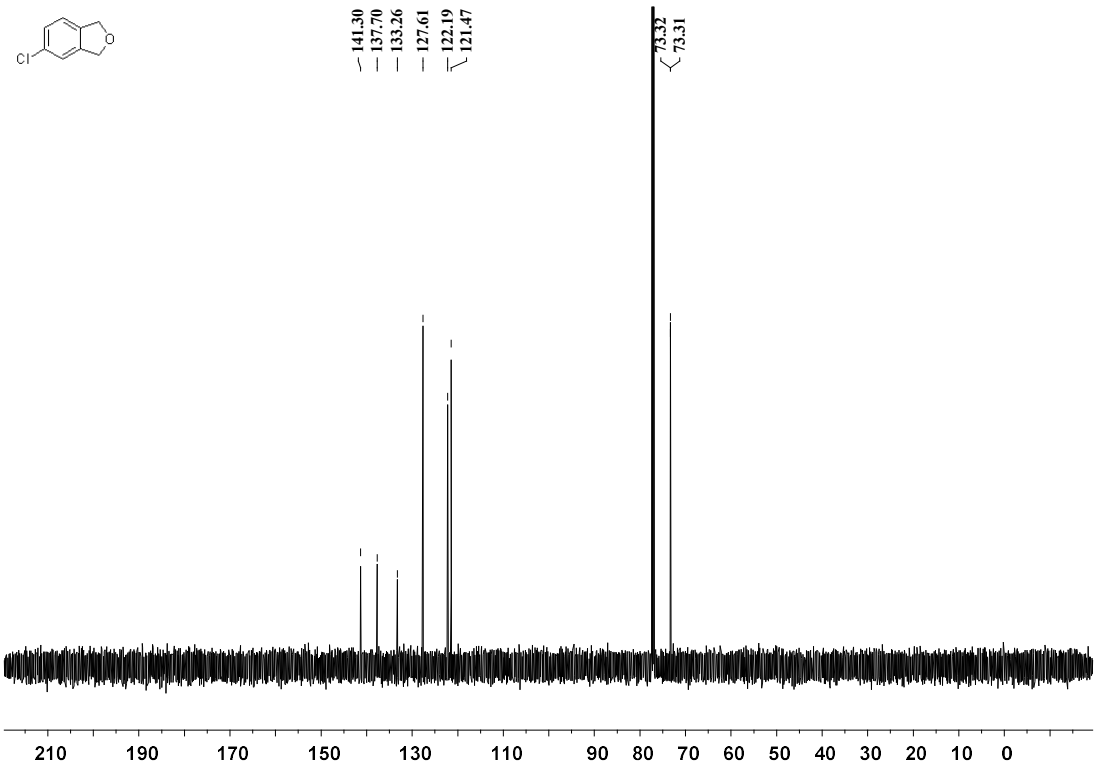


Figure S92. ^{13}C NMR (151 MHz, $CDCl_3$) of **2a**

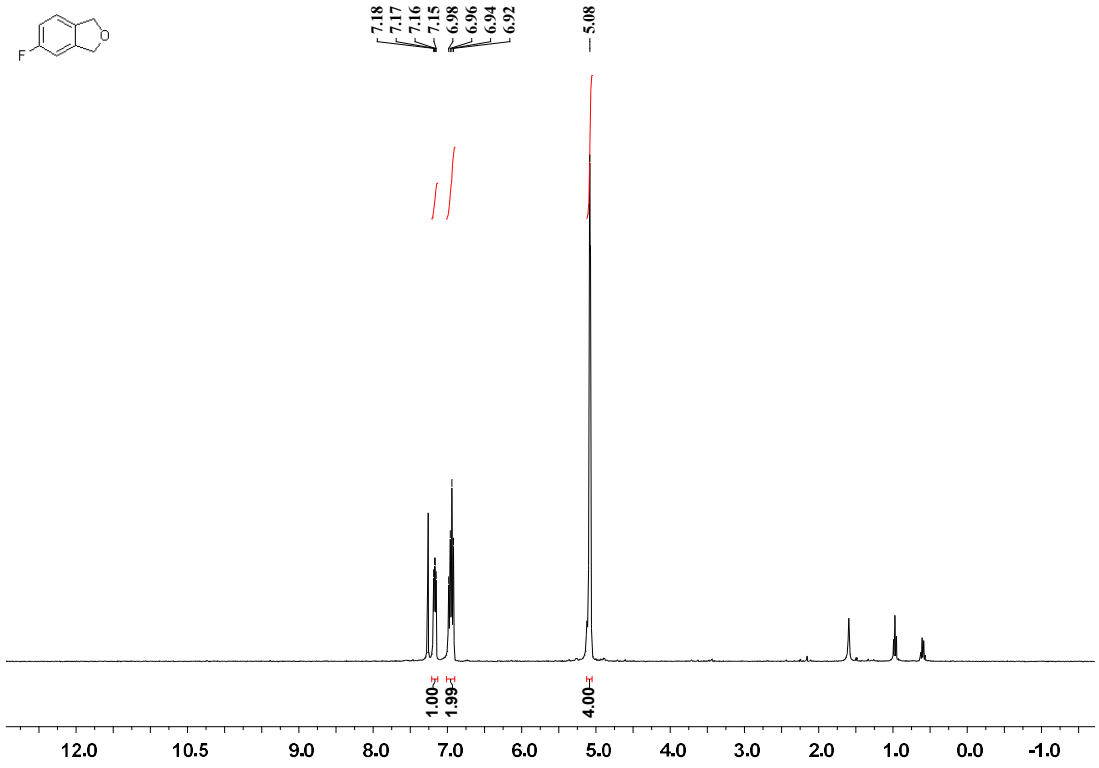


Figure S93. ^{1}H NMR (400 MHz, $CDCl_3$) of 7**a**

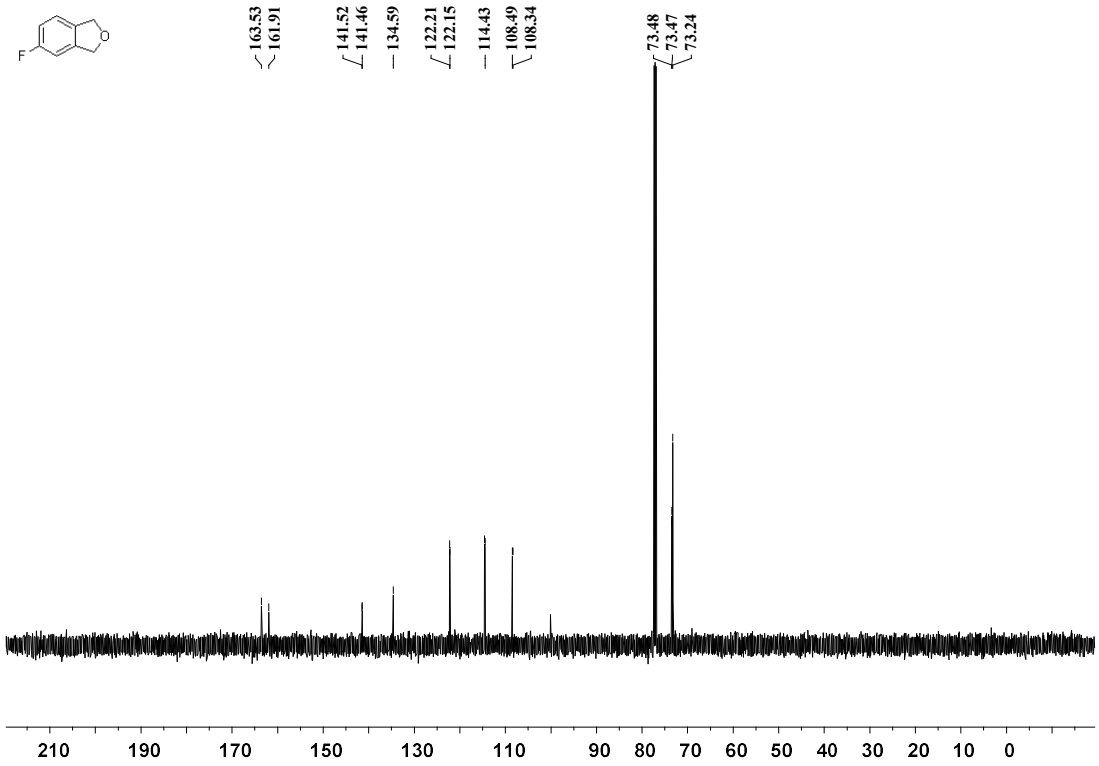


Figure S94. ^{13}C NMR (151 MHz, $CDCl_3$) of 7**a**

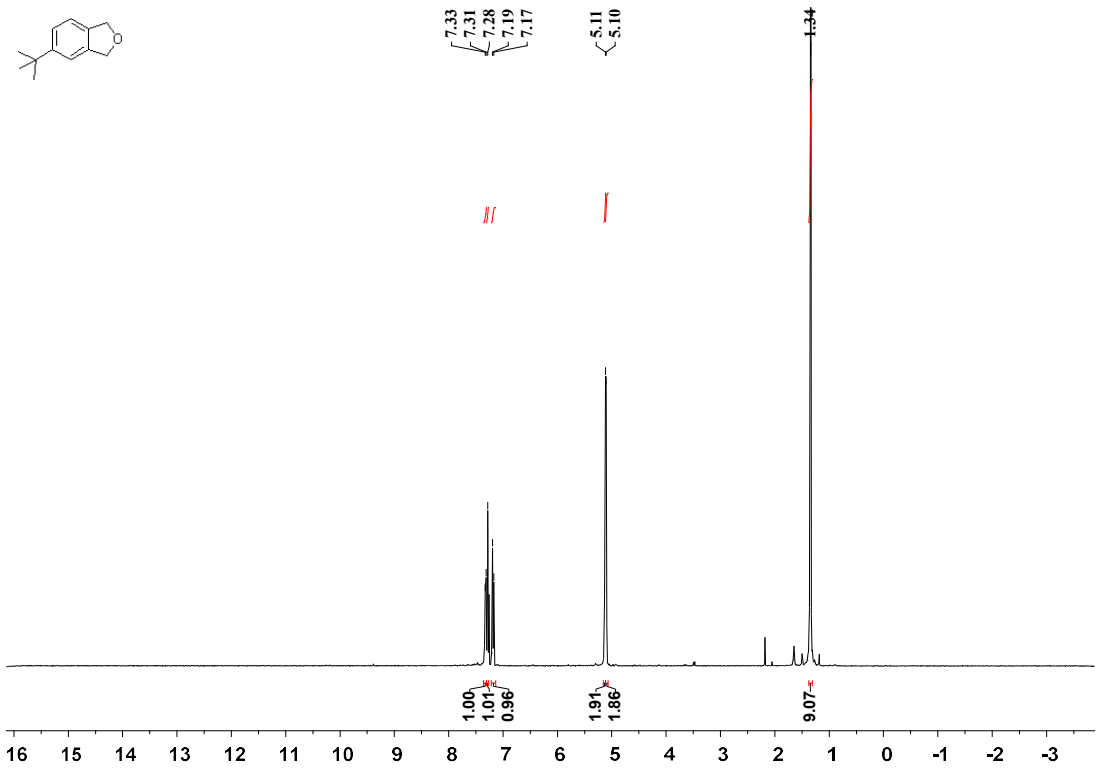


Figure S95. ^{1}H NMR (400 MHz, $CDCl_3$) of **1c**

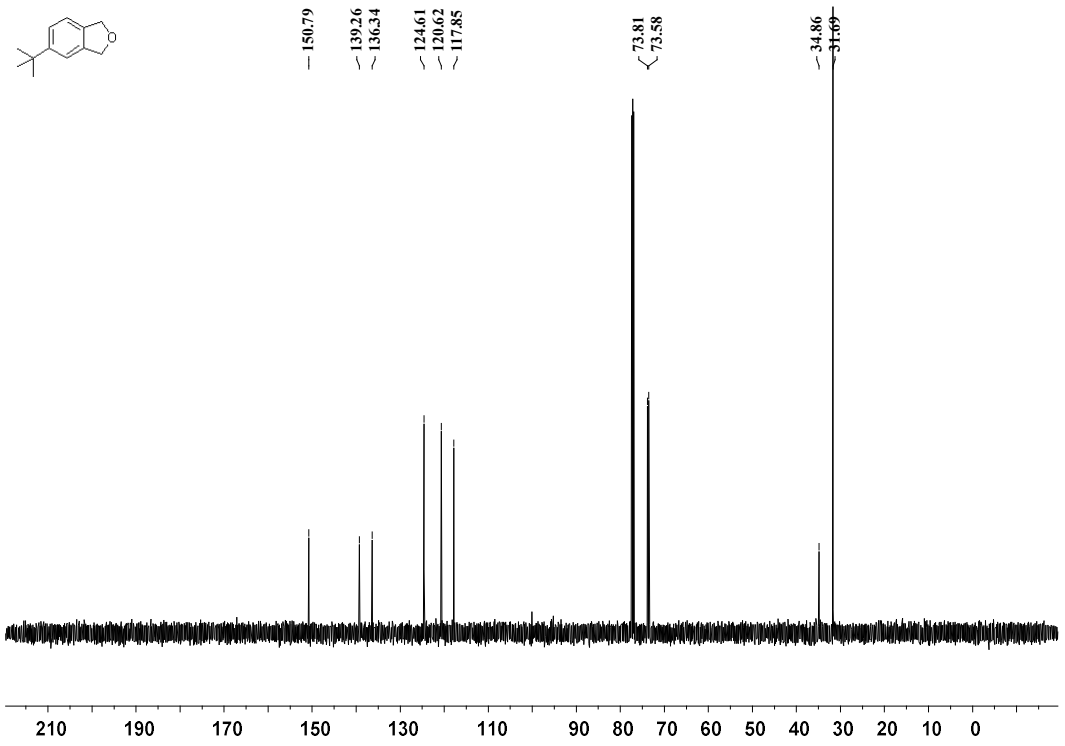


Figure S96. ^{13}C NMR (151 MHz, $CDCl_3$) of **1c**

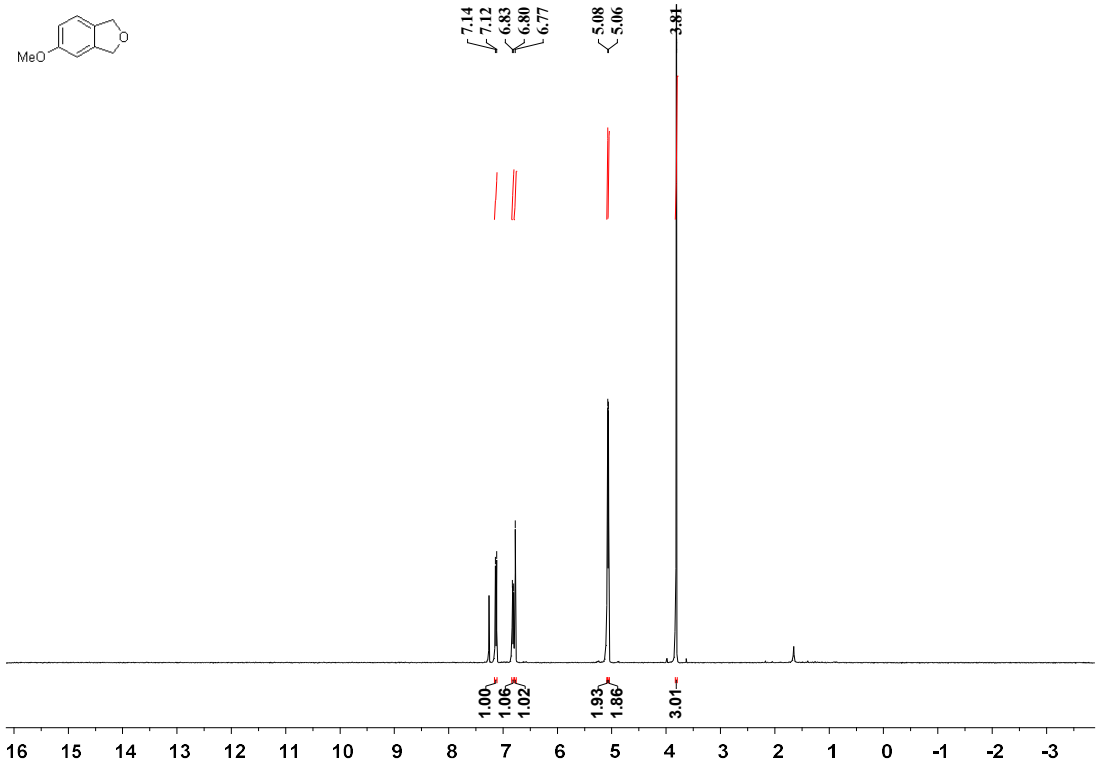


Figure S97. ^{1}H NMR (400 MHz, $CDCl_3$) of **4a**

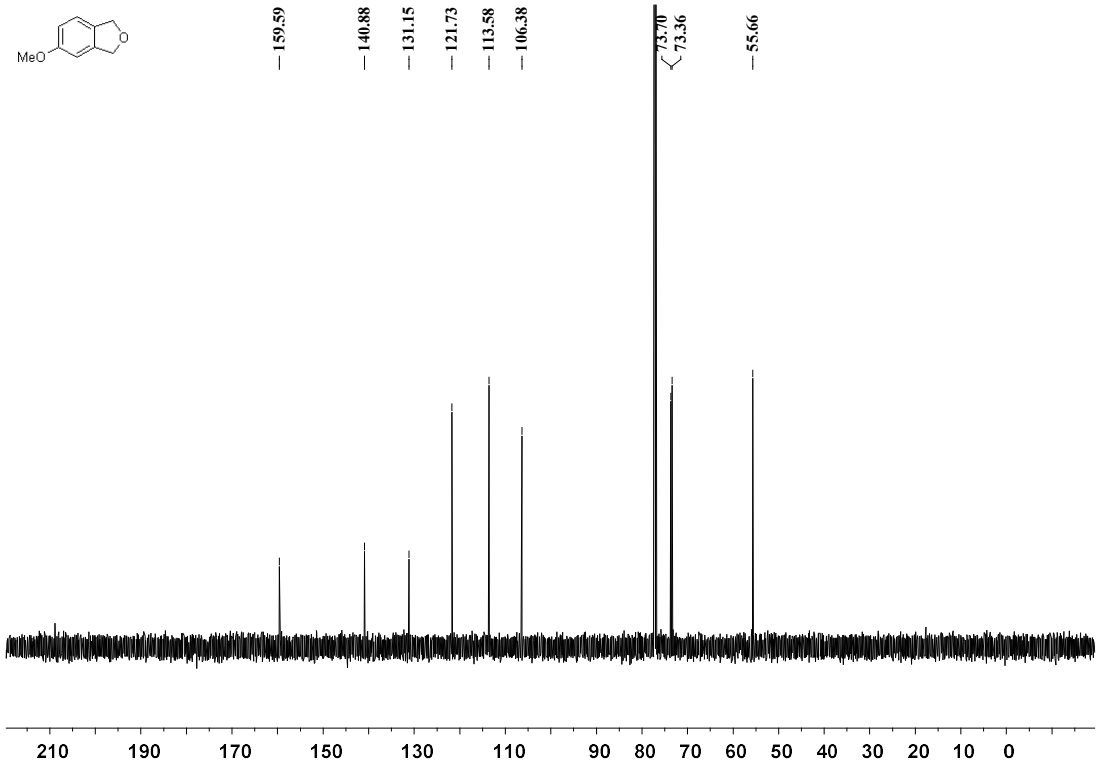


Figure S98. ^{13}C NMR (151 MHz, $CDCl_3$) of **4a**

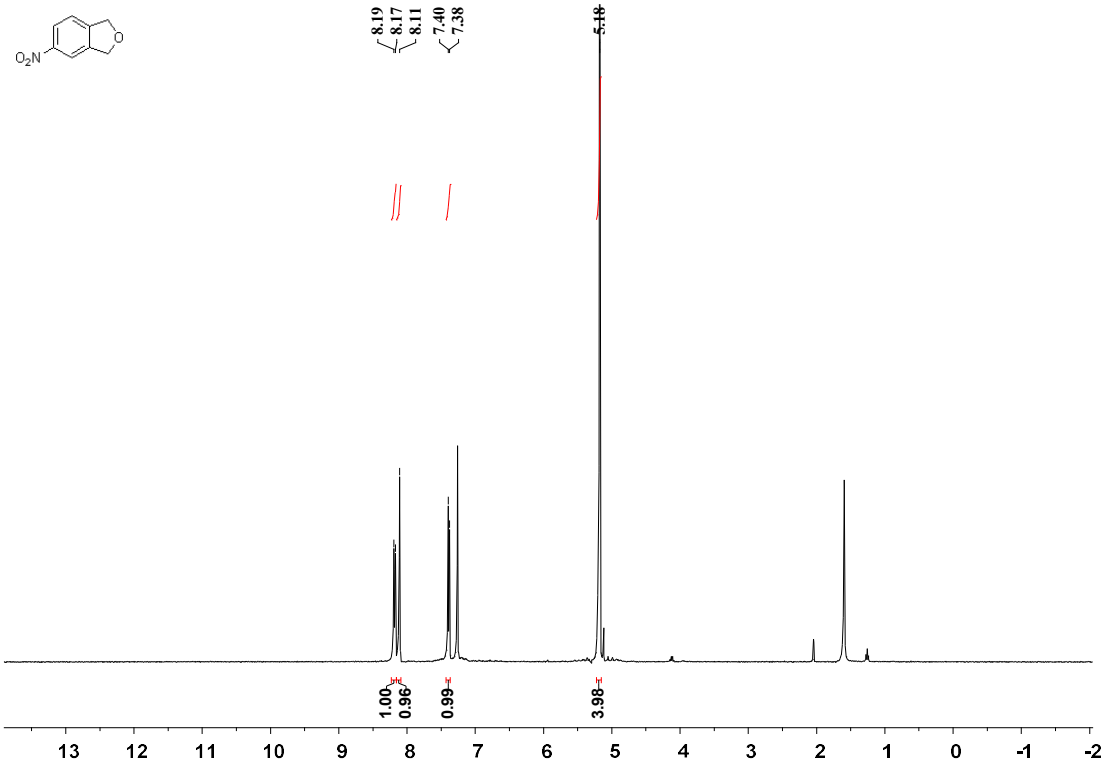


Figure S99.[1]H NMR (400 MHz, $CDCl_3$) of **1d**

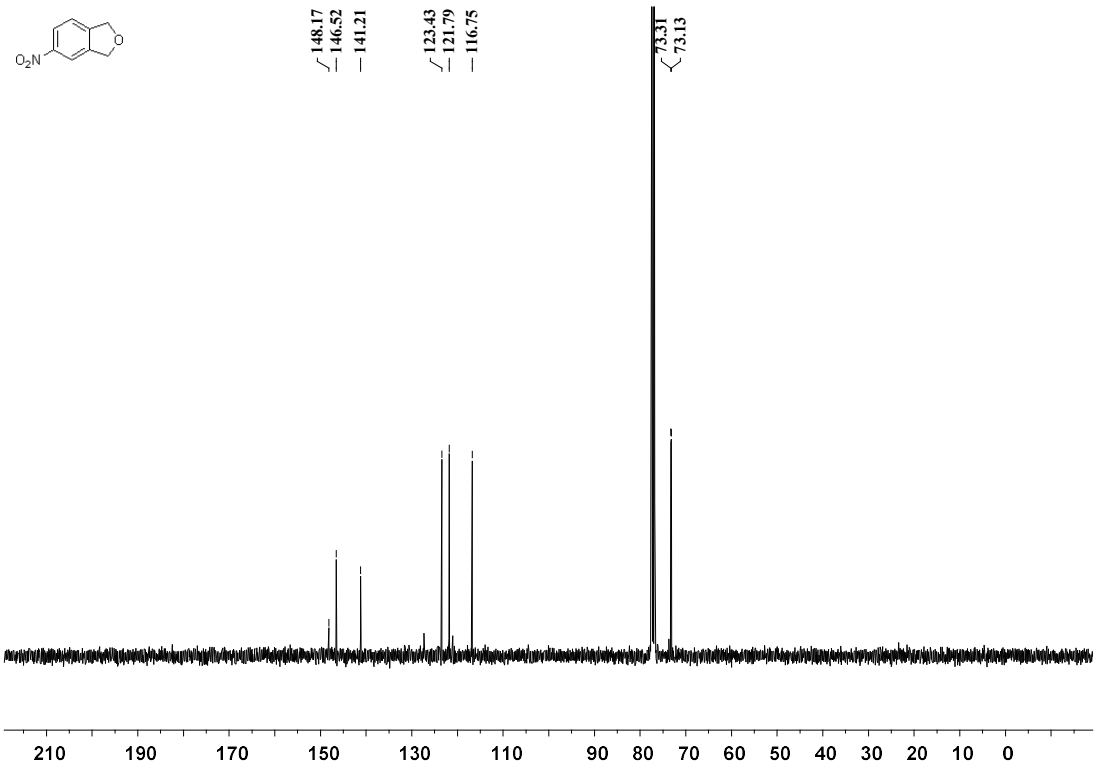


Figure S100. [13]C NMR (101 MHz, $CDCl_3$) of **1d**

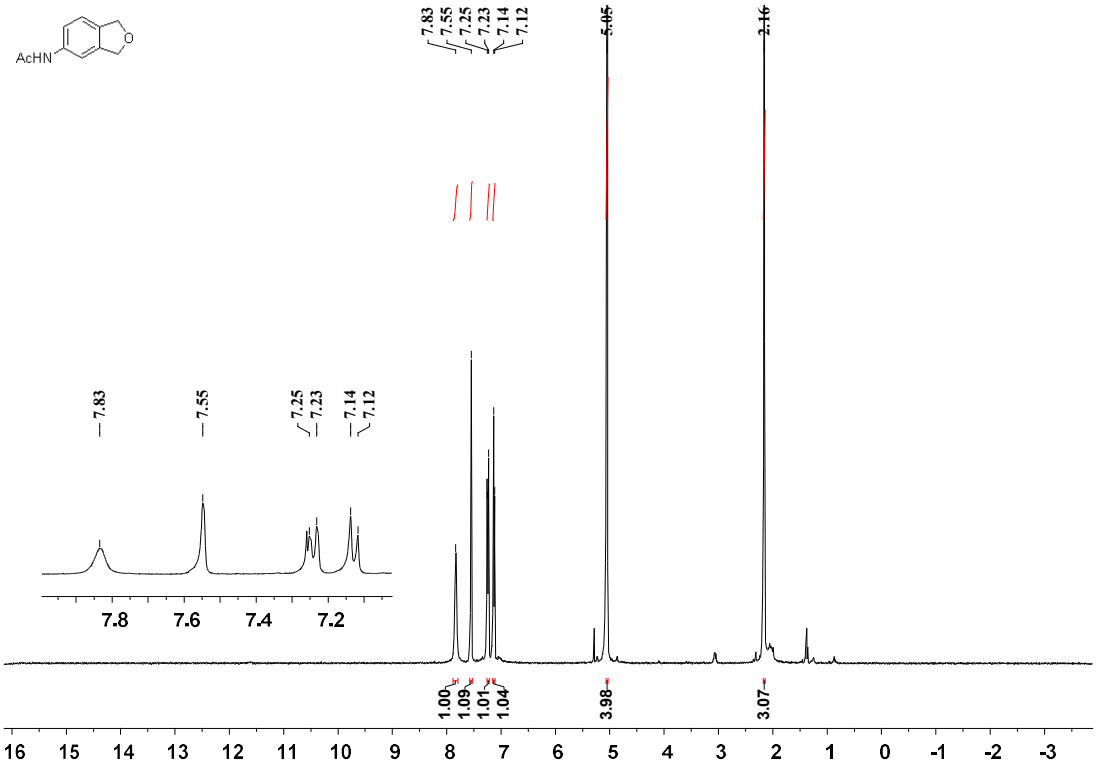


Figure S101.[1]H NMR (400 MHz, $CDCl_3$) of **4b**

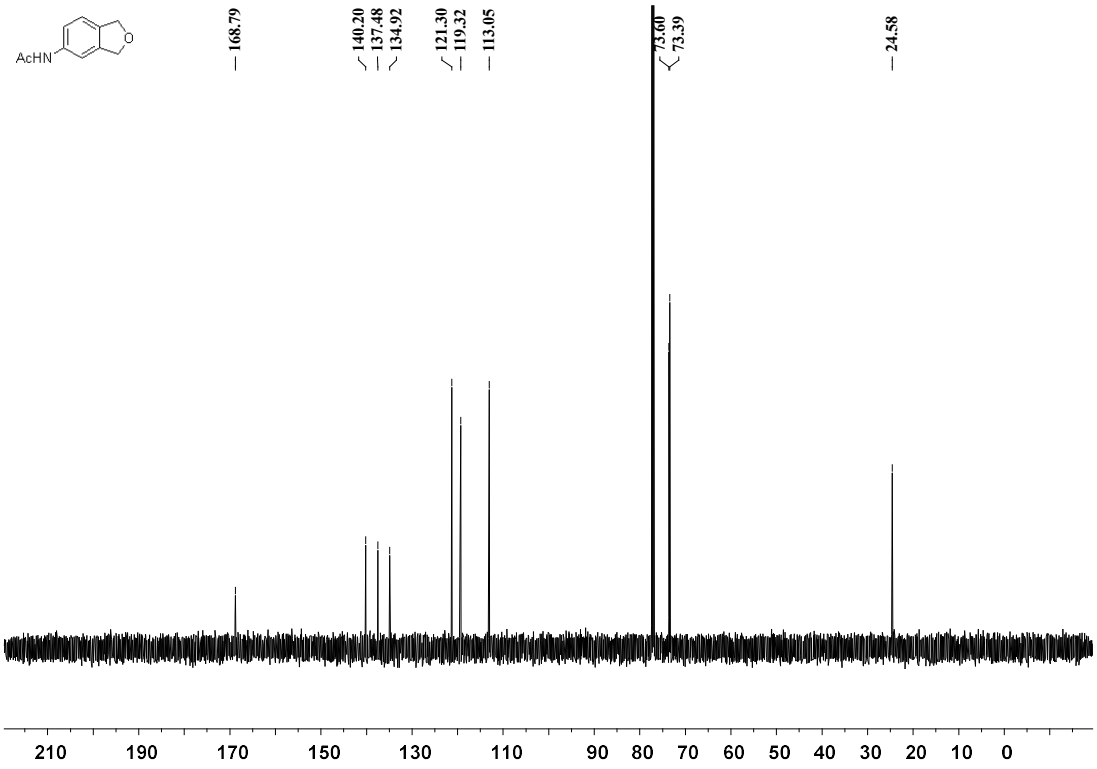


Figure S102. [13]C NMR (101 MHz, $CDCl_3$) of **4b**

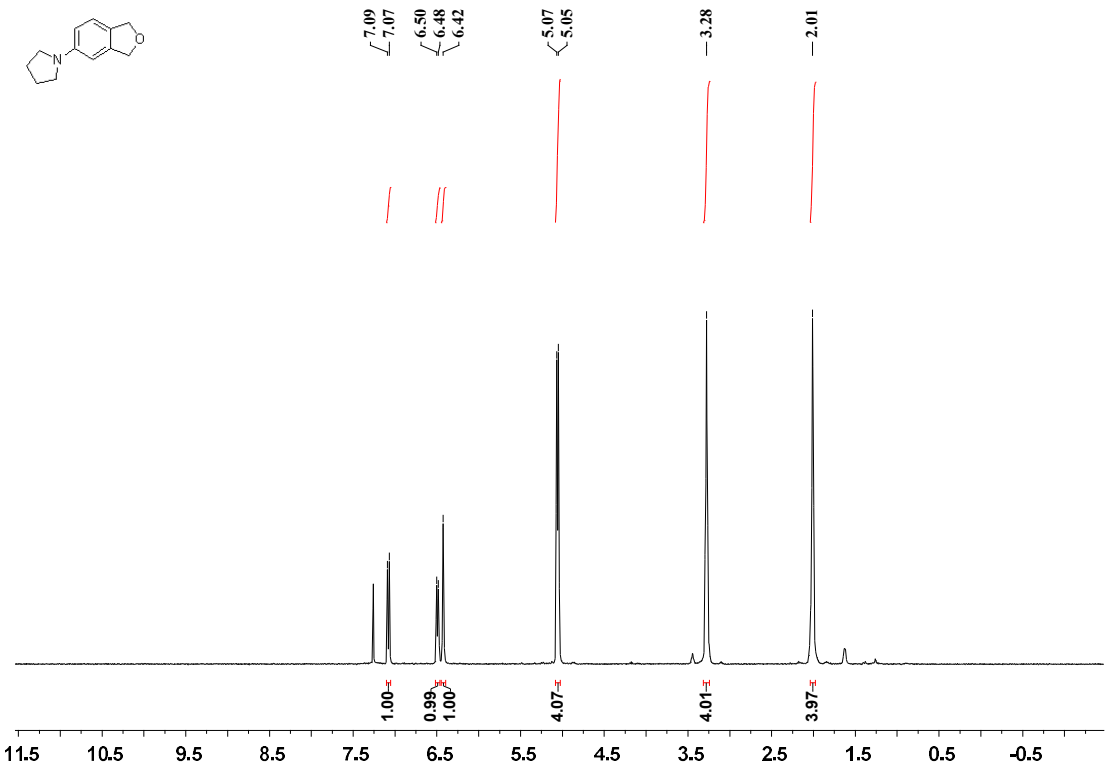


Figure S103.[1]H NMR (400 MHz, $CDCl_3$) of **4c**

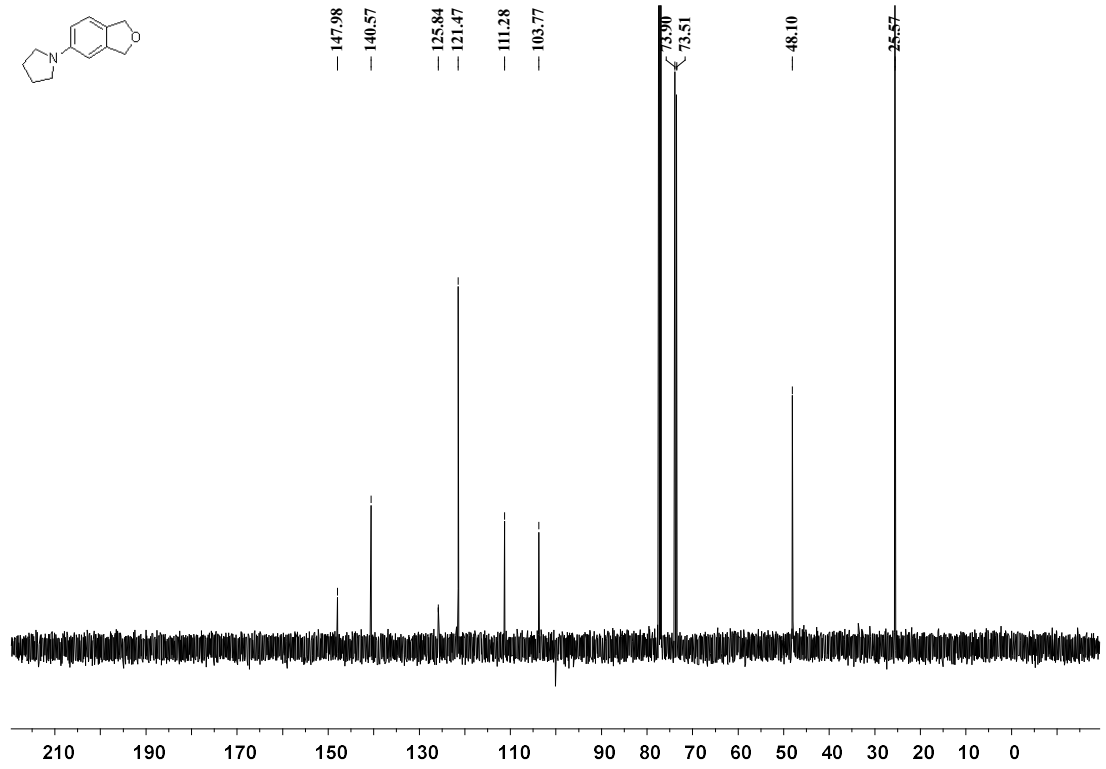


Figure S104.[13]C NMR (151 MHz, $CDCl_3$) of **4c**

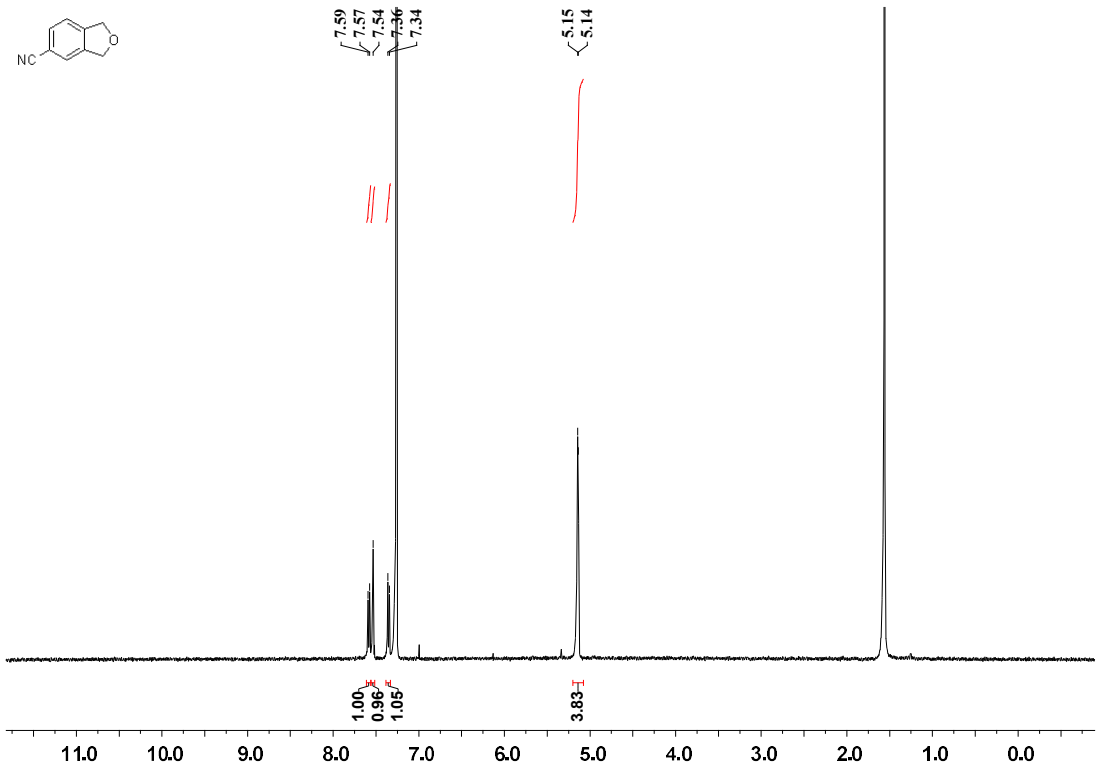


Figure S105.[1]H NMR (400 MHz, $CDCl_3$) of **1e**

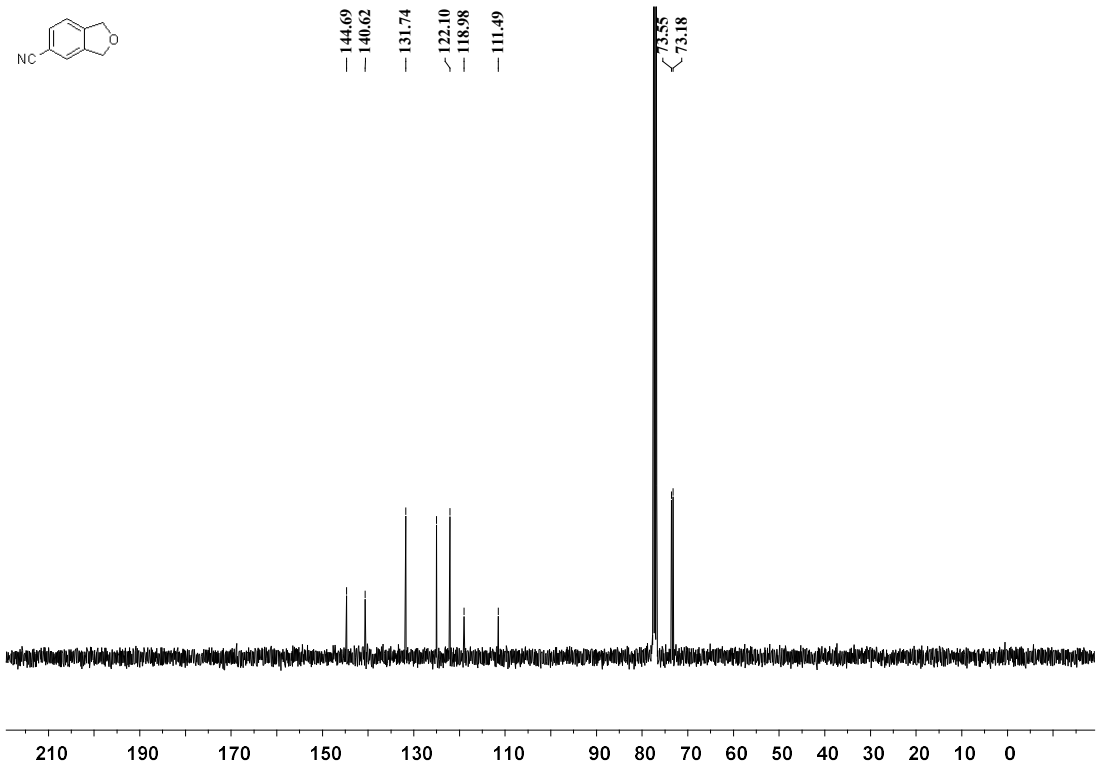


Figure S106.[13]C NMR (101 MHz, $CDCl_3$) of **1e**

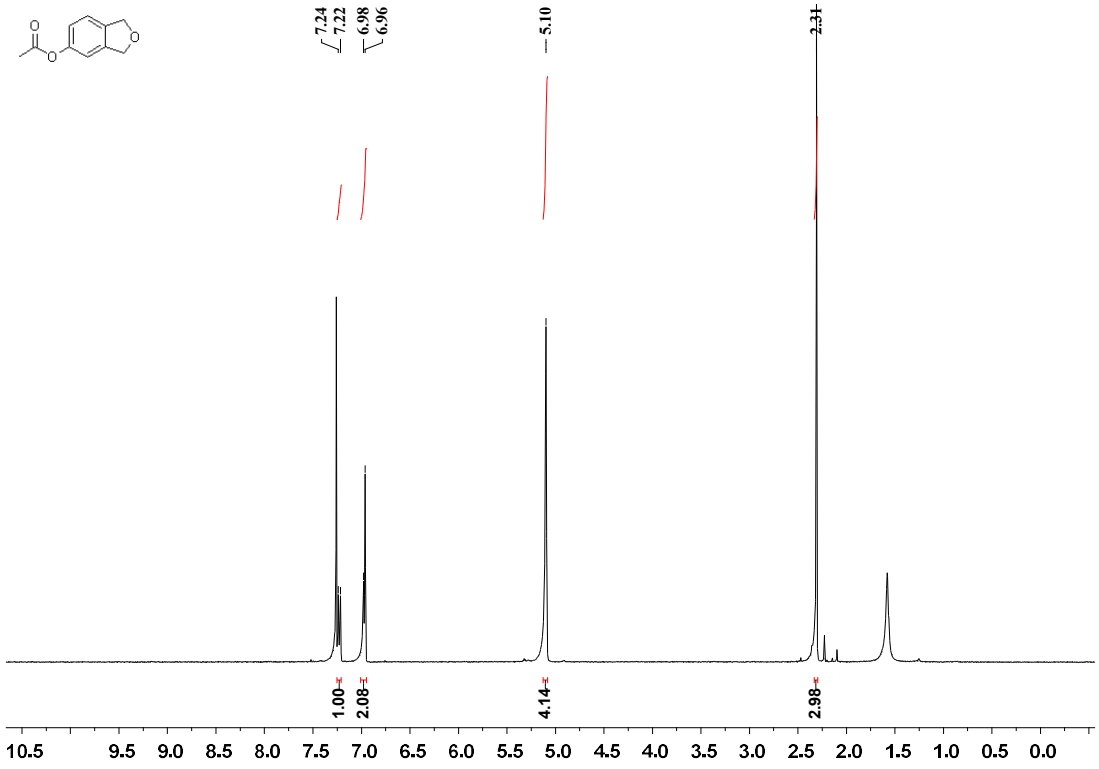


Figure S107.[1]H NMR (400 MHz, $CDCl_3$) of **4c**

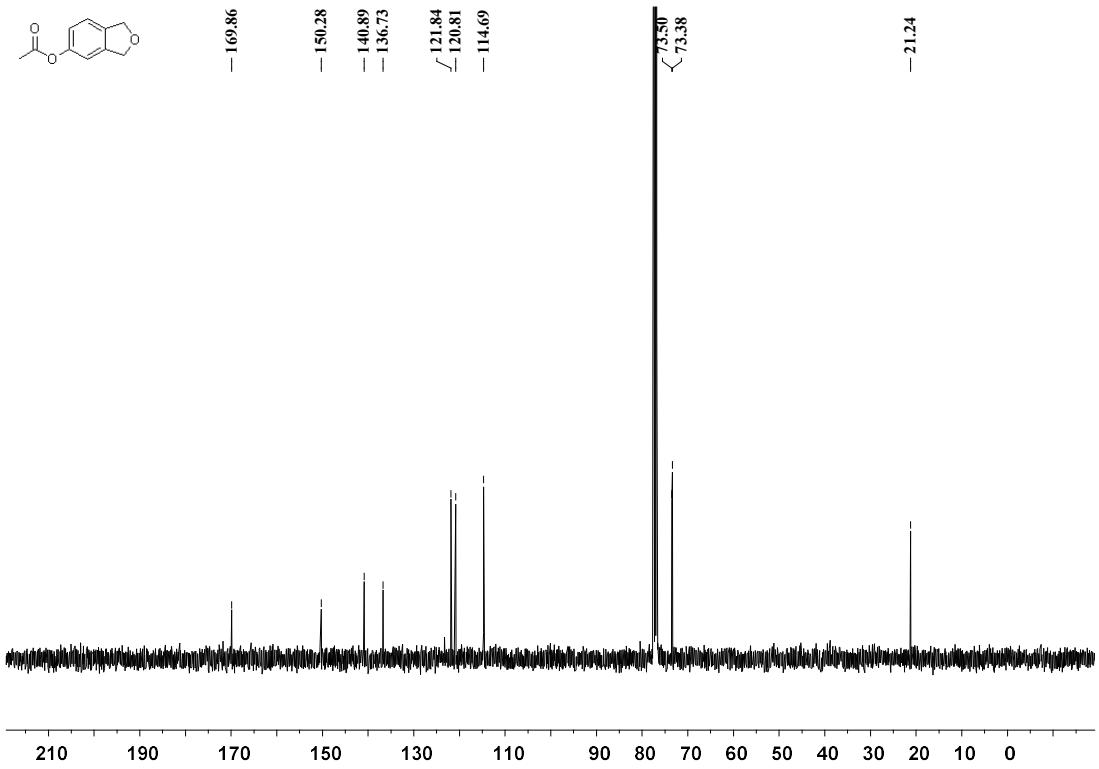


Figure S108. [13]C NMR (101 MHz, $CDCl_3$) of **4c**

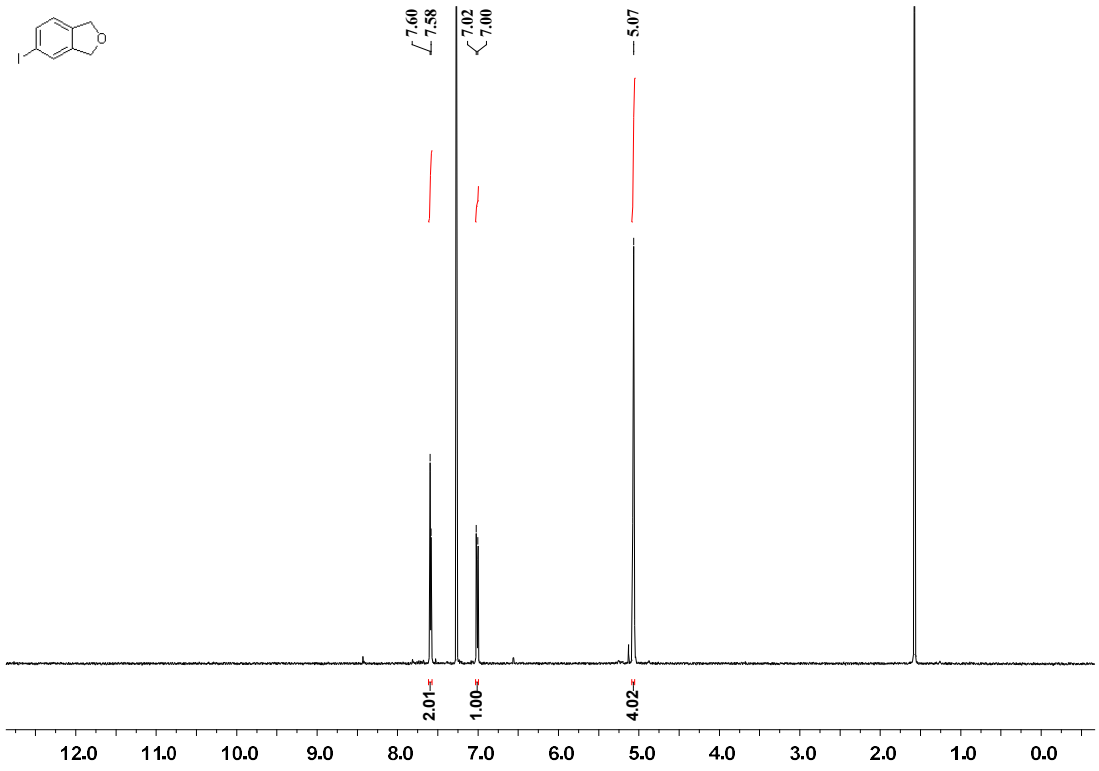


Figure S109.[1]H NMR (400 MHz, $CDCl_3$) of **1f**

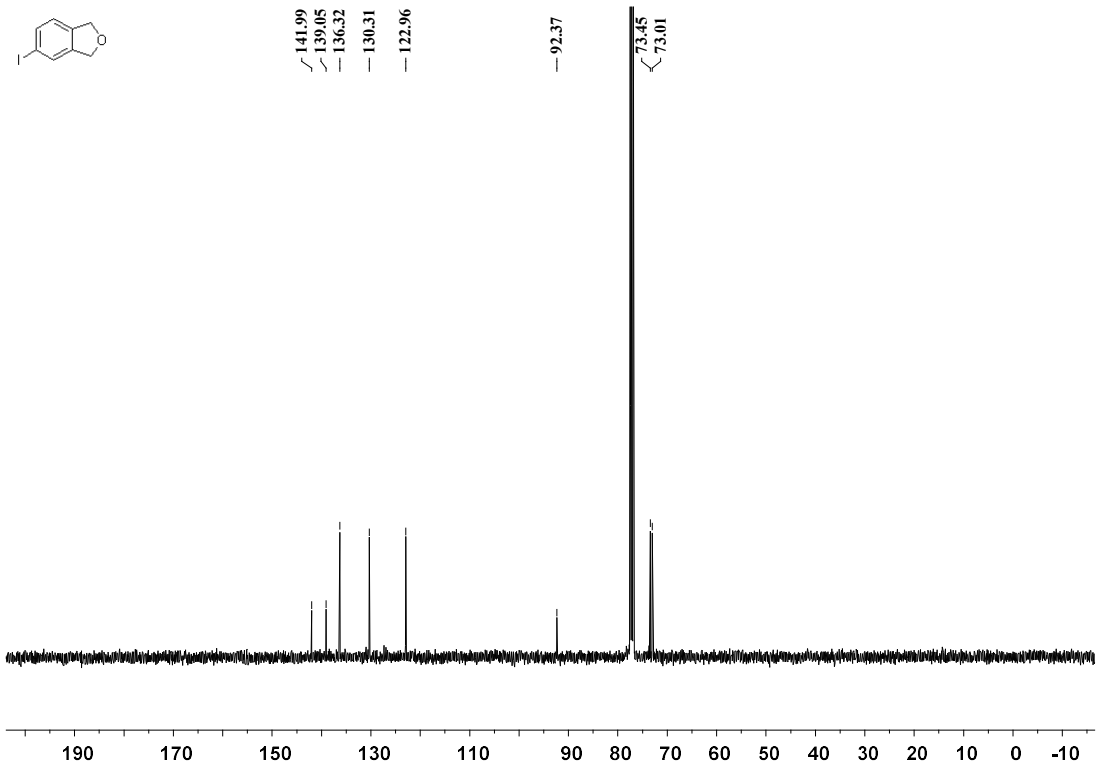


Figure S110. [13]C NMR (101 MHz, $CDCl_3$) of **1f**

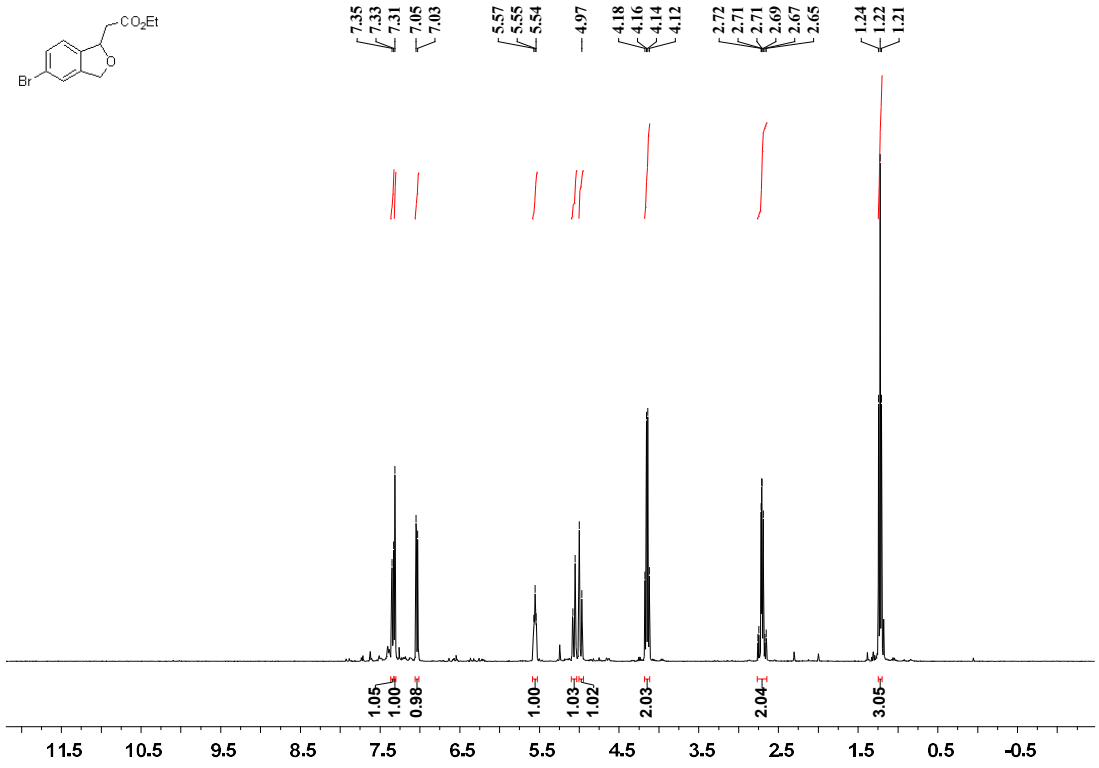


Figure S111.[1]H NMR (400 MHz, $CDCl_3$) of **2a**

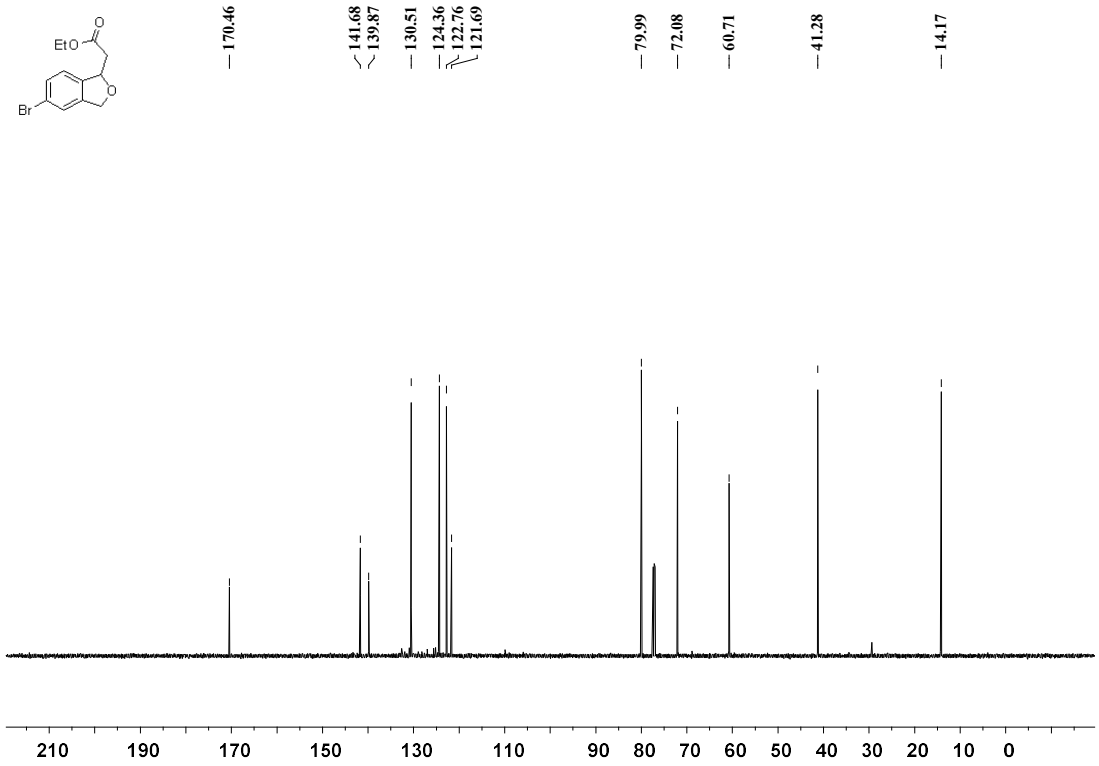


Figure S112.[13]C NMR (151 MHz, $CDCl_3$) of **2a**

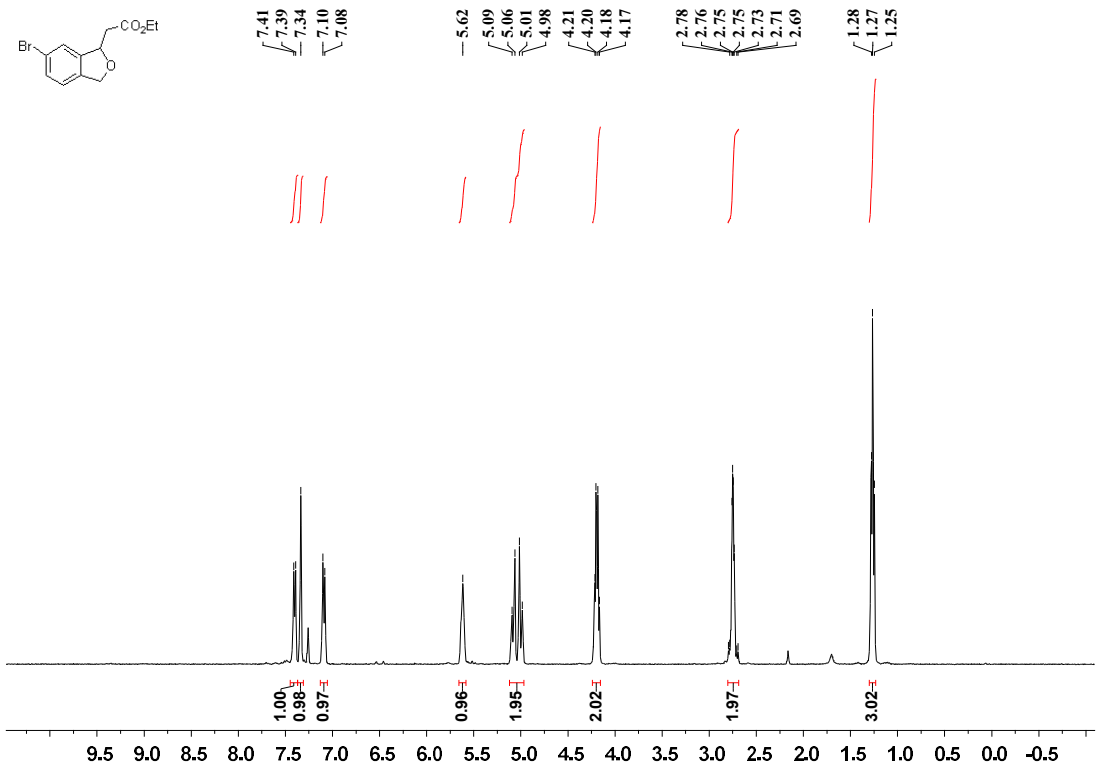


Figure S113.[1]H NMR (400 MHz, $CDCl_3$) of **3a**

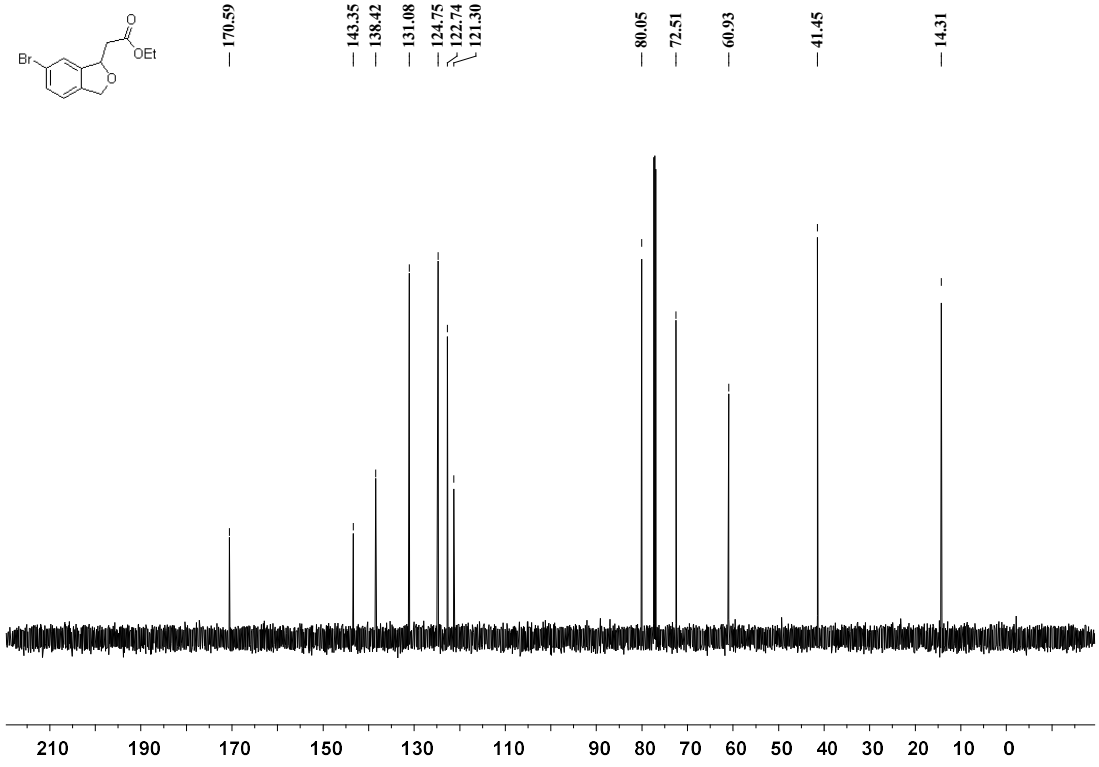


Figure S114.[13]C NMR (151 MHz, $CDCl_3$) of **3a**

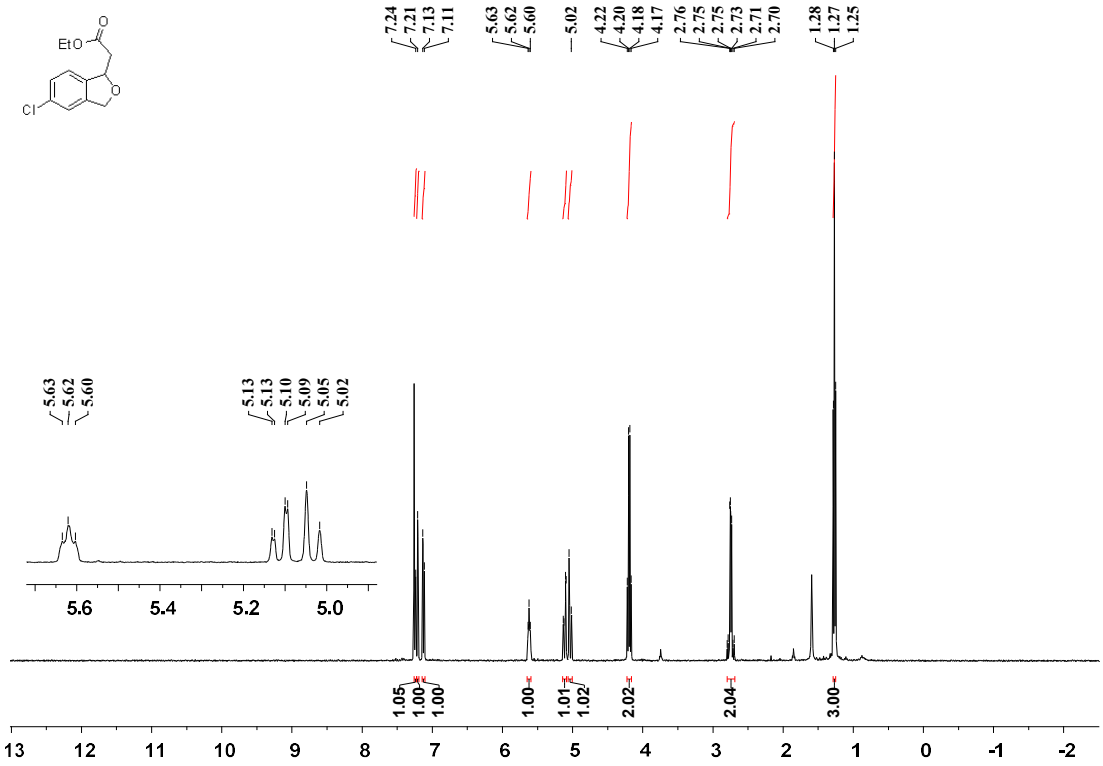


Figure S115.[1]H NMR (400 MHz, $CDCl_3$) of **2b**

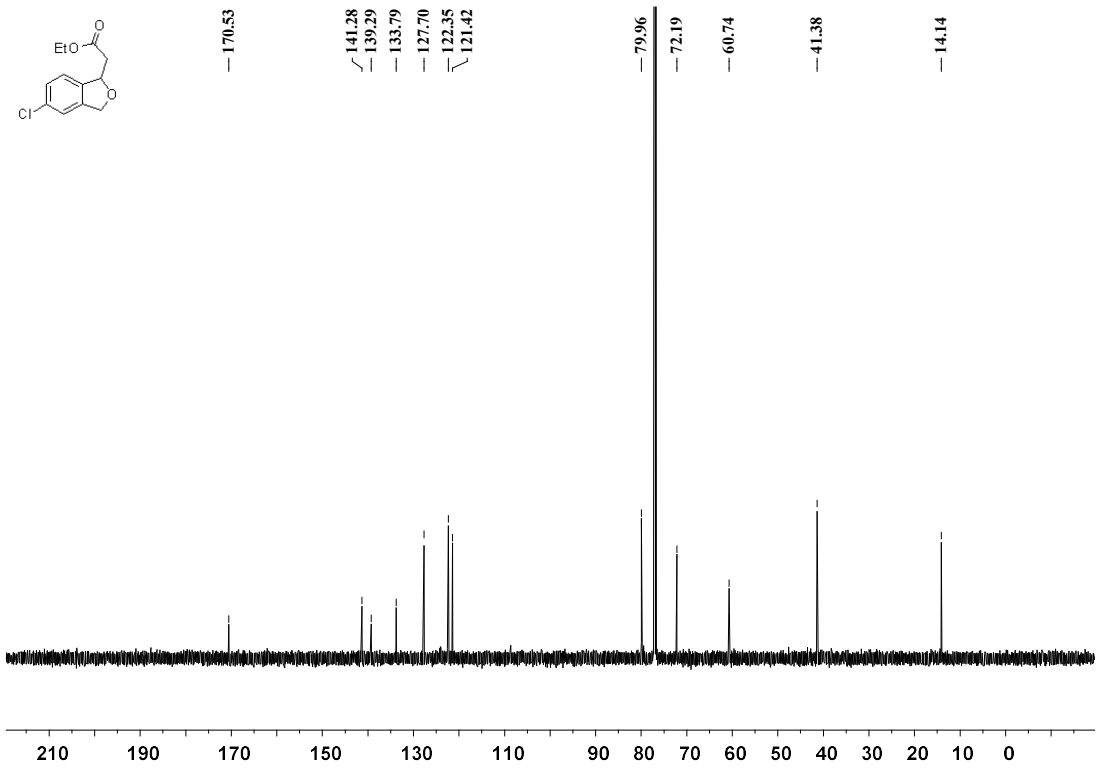


Figure S116.[13]C NMR (151 MHz, $CDCl_3$) of **2b**

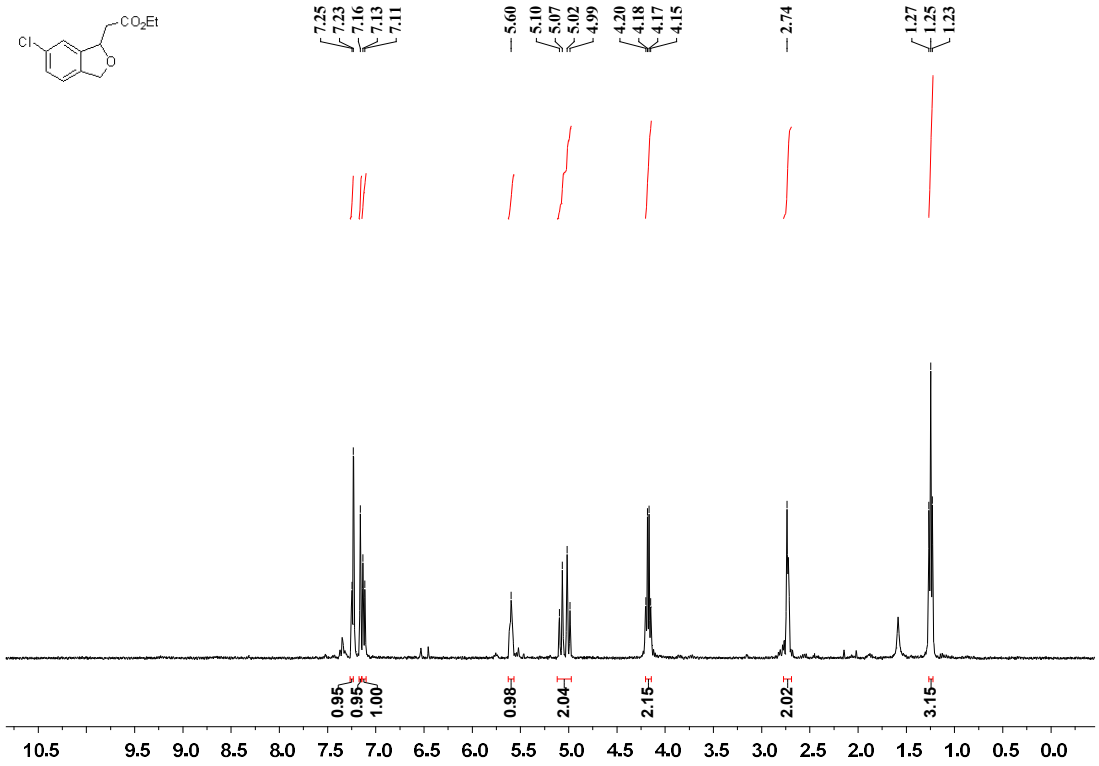


Figure S117.[1]H NMR (400 MHz, $CDCl_3$) of **3b**

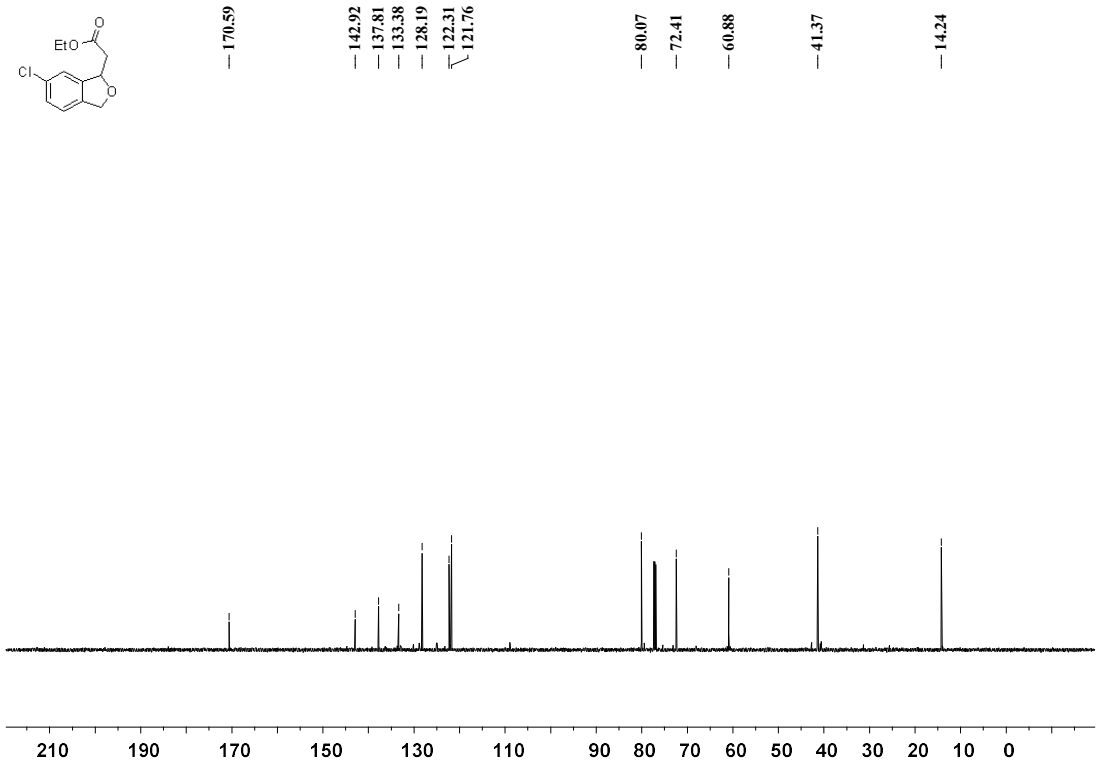


Figure S118.[13]C NMR (151 MHz, $CDCl_3$) of **3b**

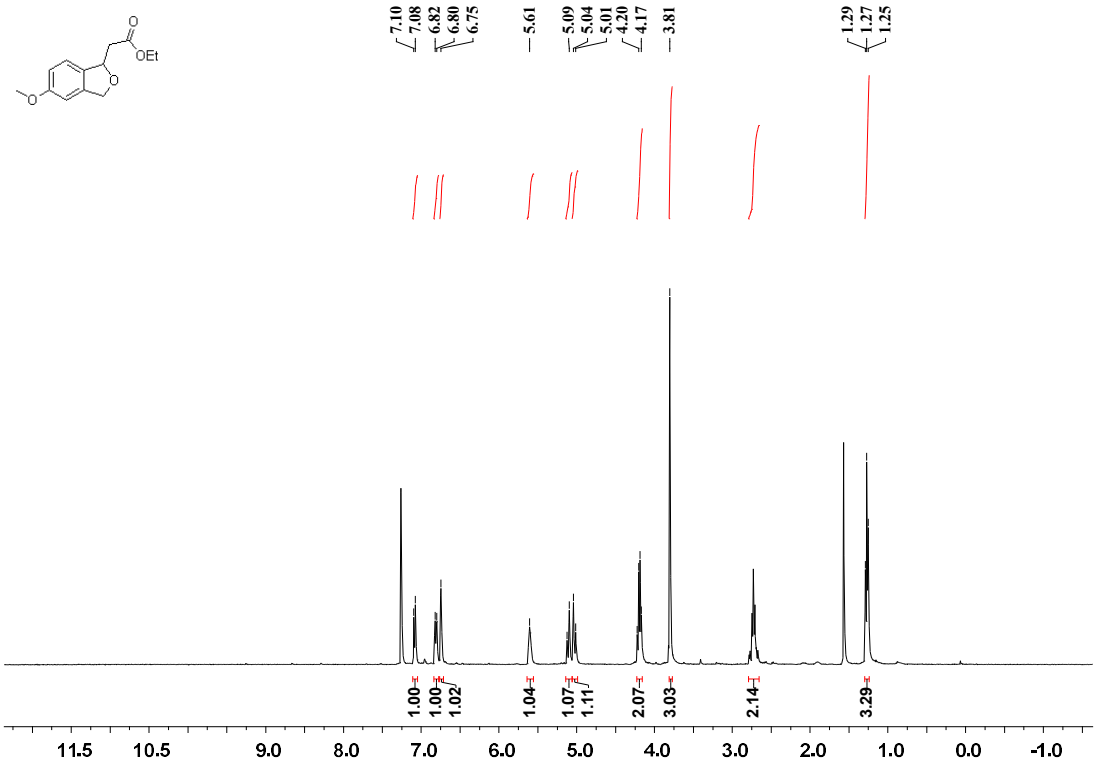


Figure S119.[1]H NMR (400 MHz, $CDCl_3$) of **5d**

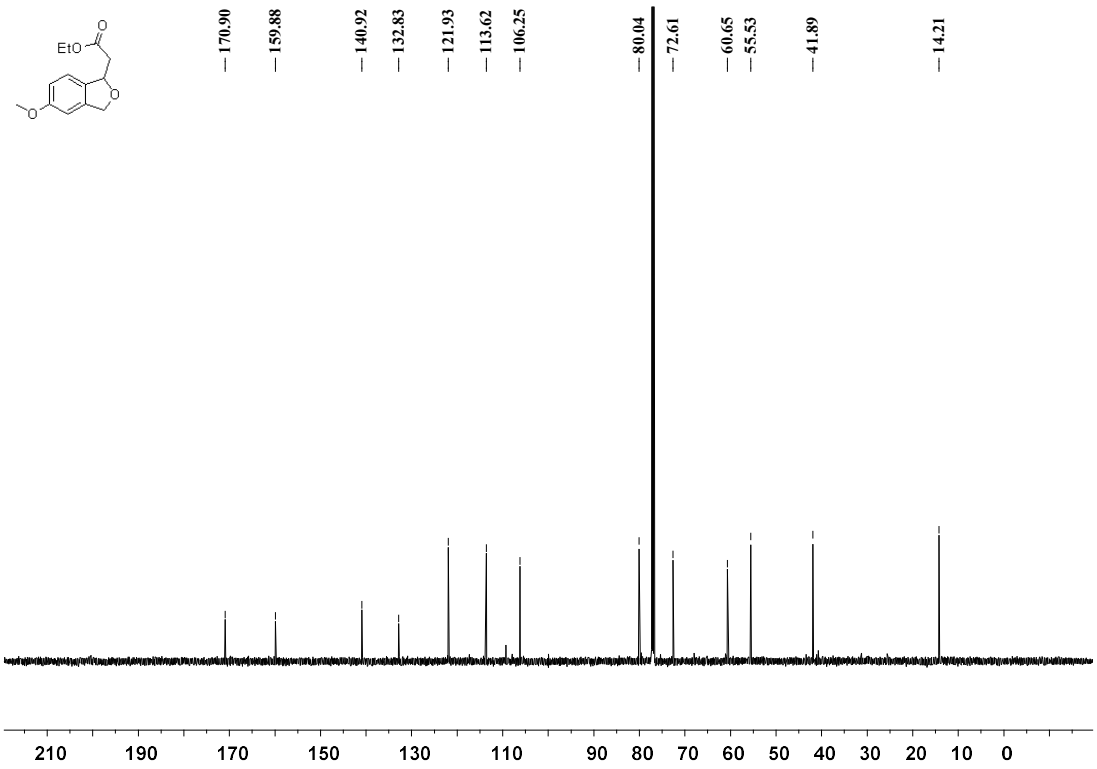


Figure S120.[13]C NMR (151 MHz, $CDCl_3$) of **5d**

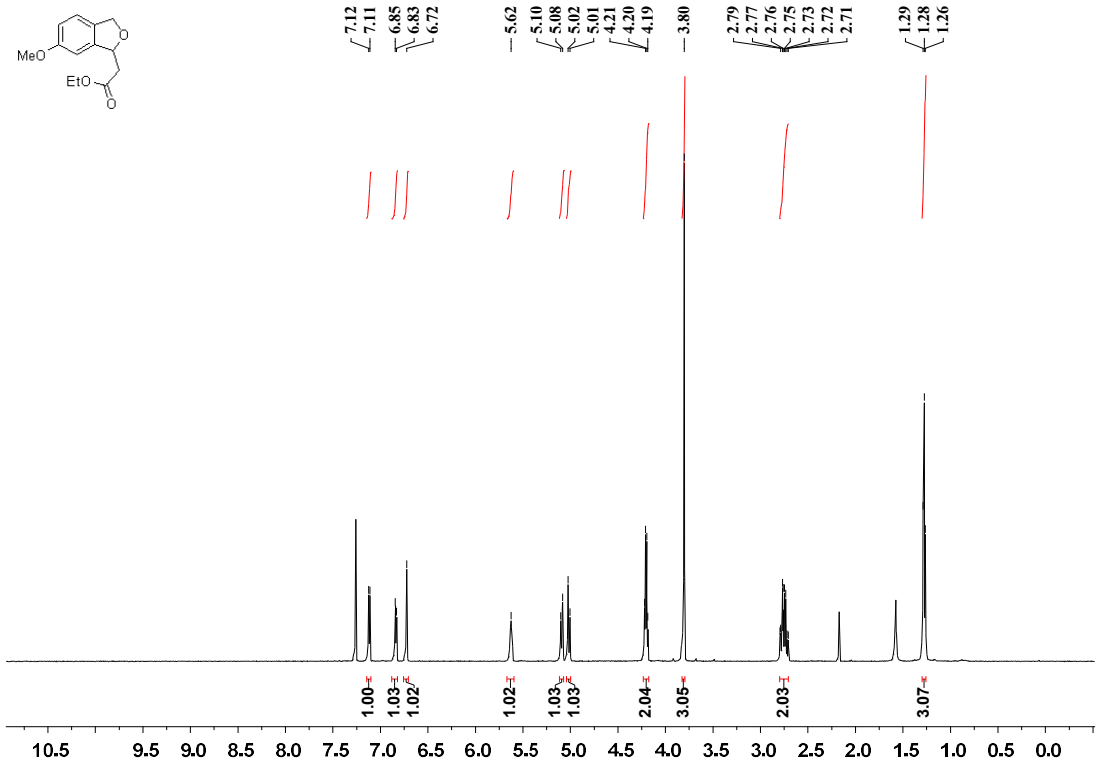


Figure S121.[1]H NMR (600 MHz, $CDCl_3$) of **6d**

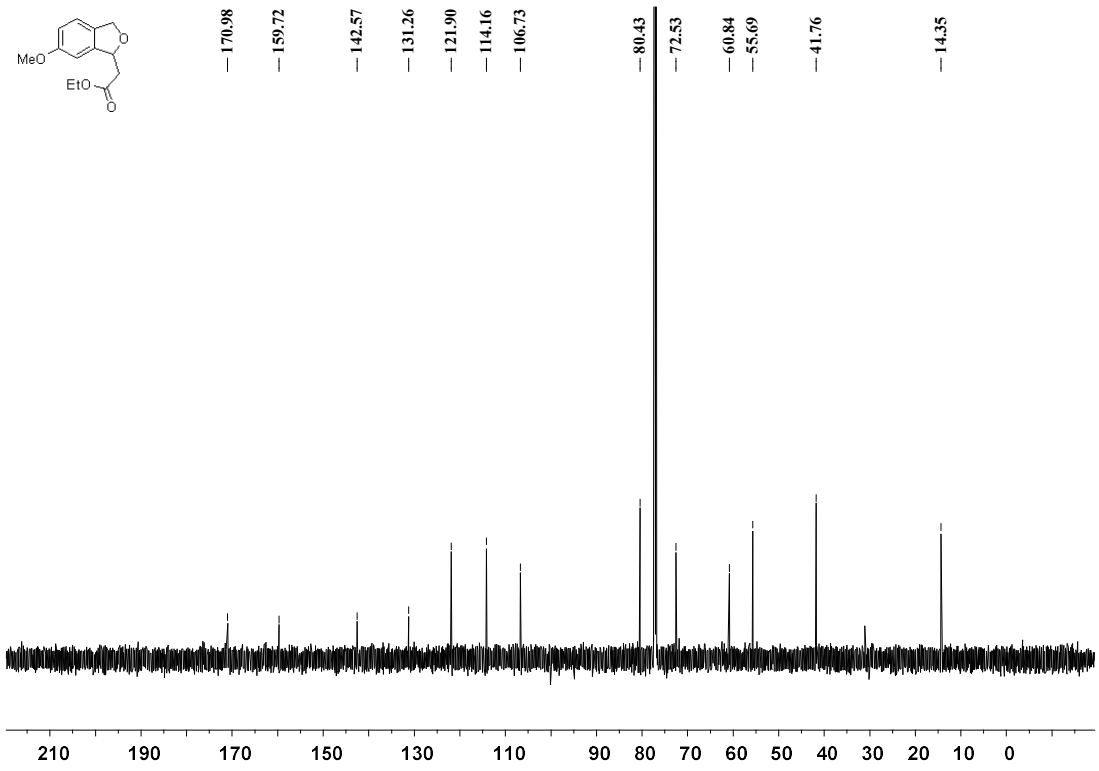


Figure S122.[13]C NMR (151 MHz, $CDCl_3$) of **6d**

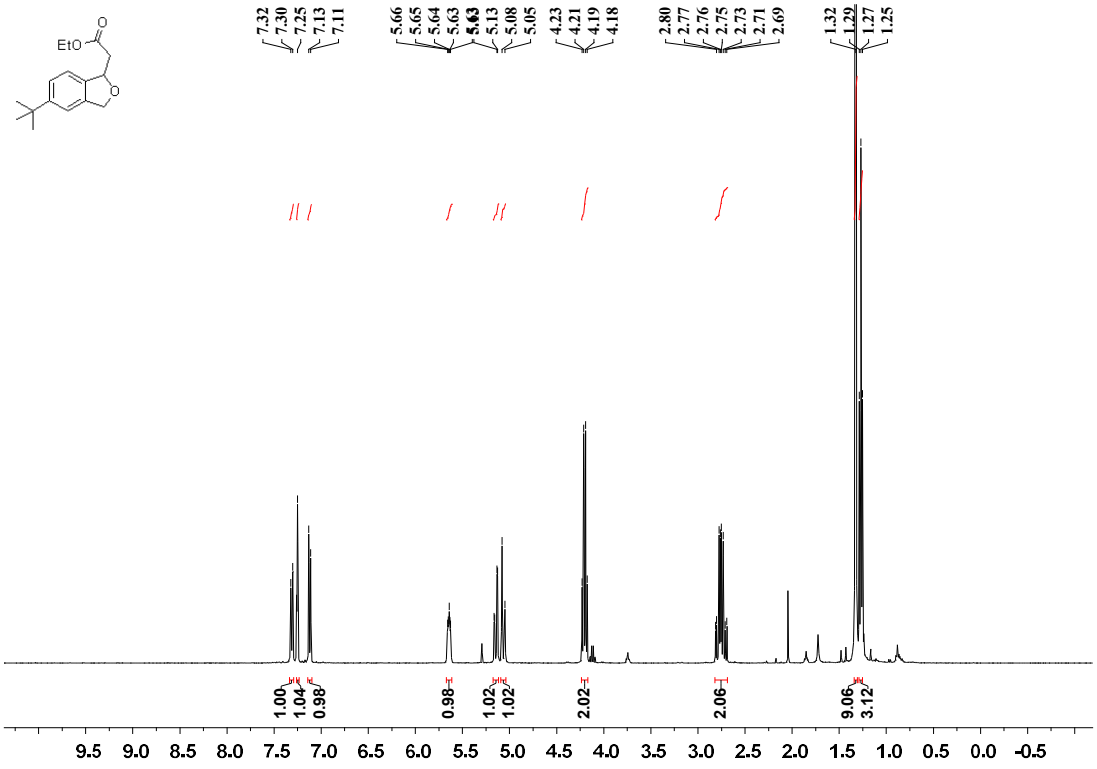


Figure S123.[1]H NMR (600 MHz, $CDCl_3$) of **2c**

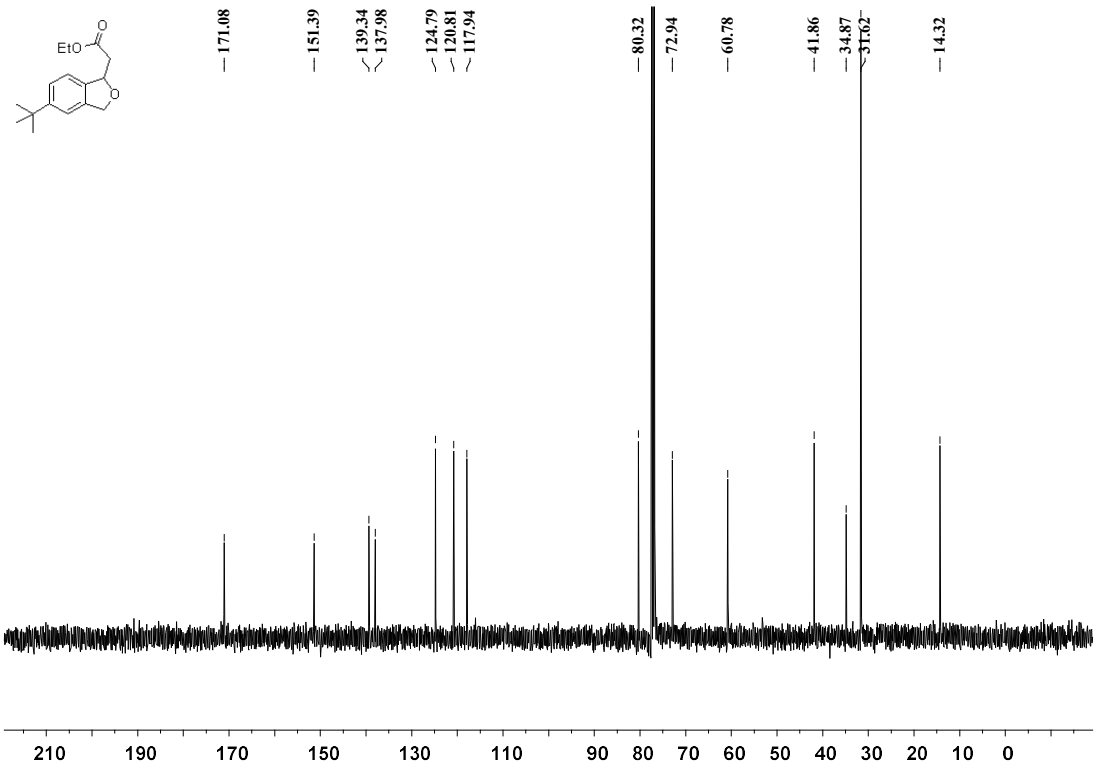


Figure S124.[13]C NMR (101 MHz, $CDCl_3$) of **2c**

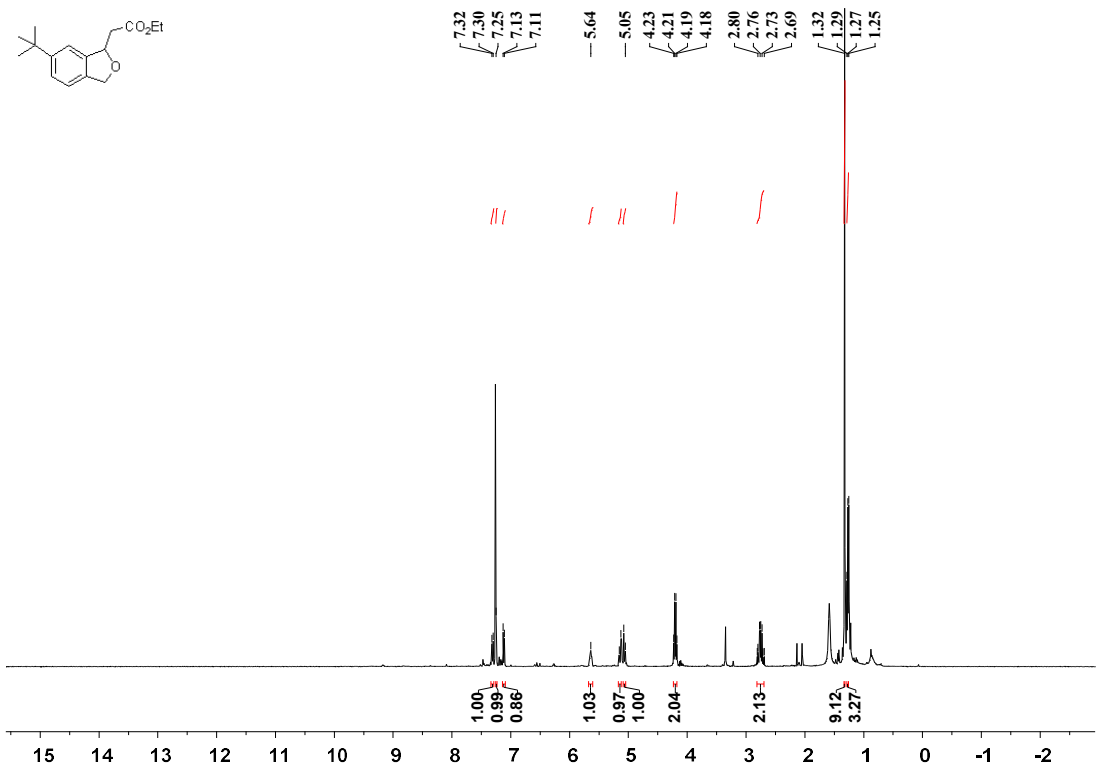


Figure S125.[1]H NMR (600 MHz, $CDCl_3$) of **3c**

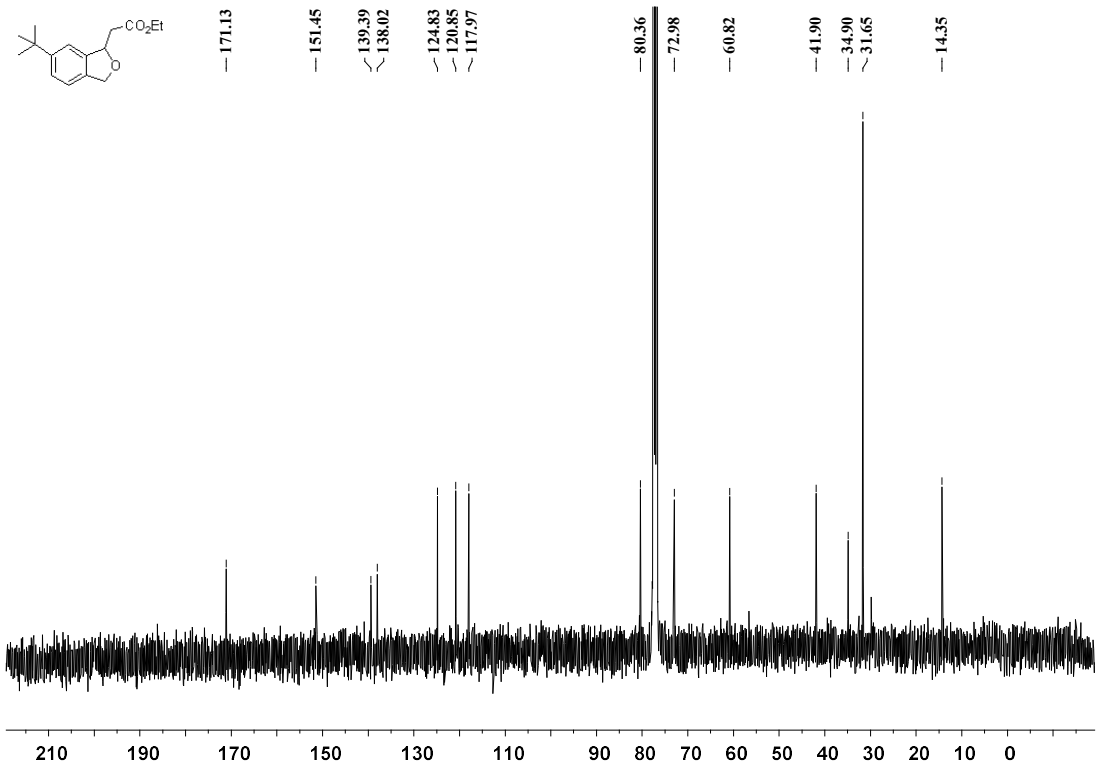


Figure S126.[13]C NMR (101 MHz, $CDCl_3$) of **3c**

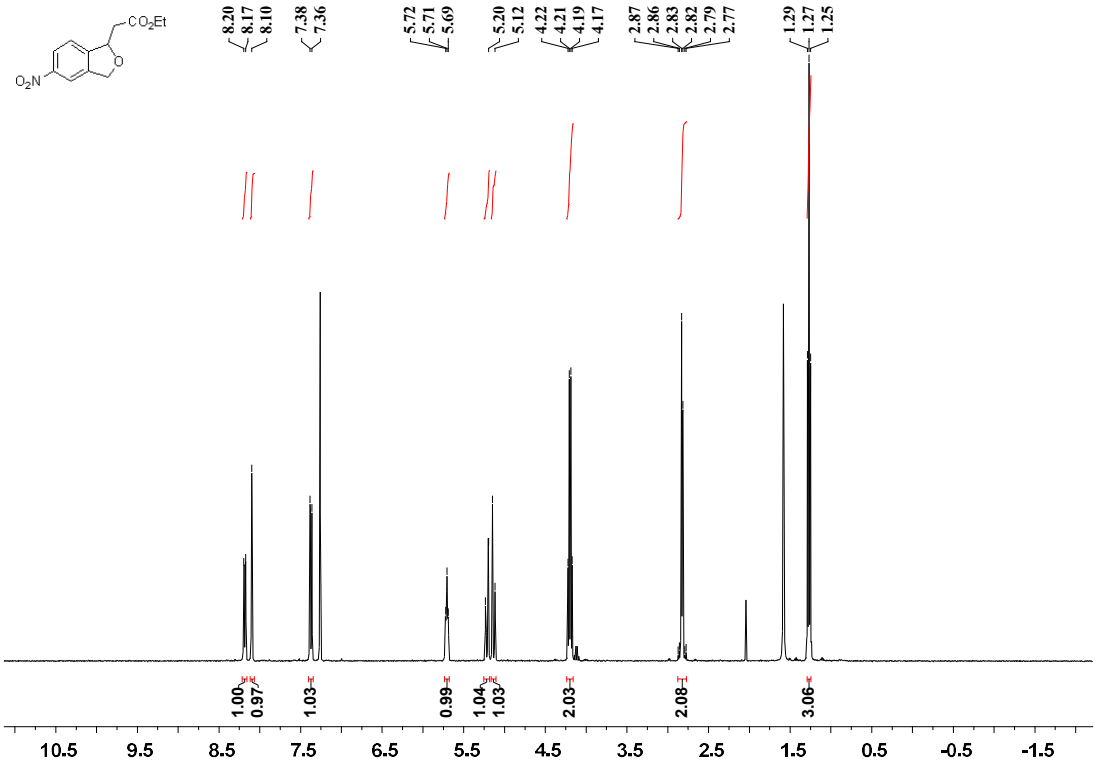


Figure S127.[1]H NMR (600 MHz, $CDCl_3$) of **2d**

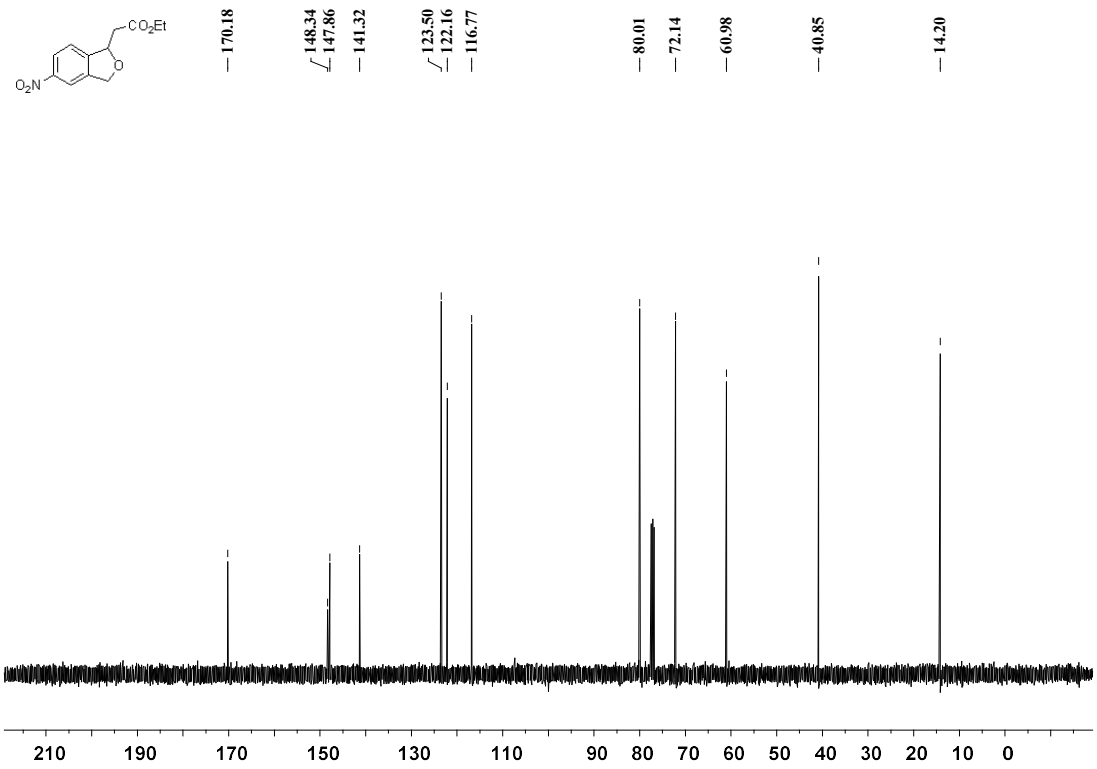


Figure S128.[13]C NMR (101 MHz, $CDCl_3$) of **2d**

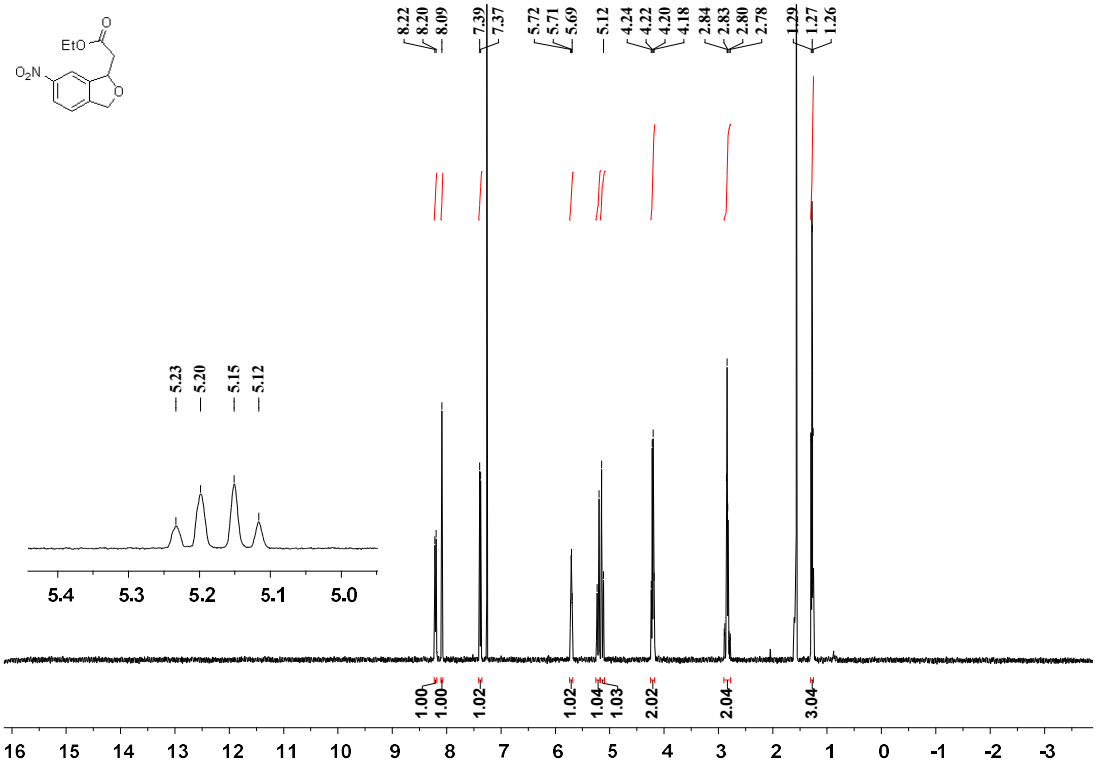


Figure S129.[1]H NMR (600 MHz, $CDCl_3$) of **3d**

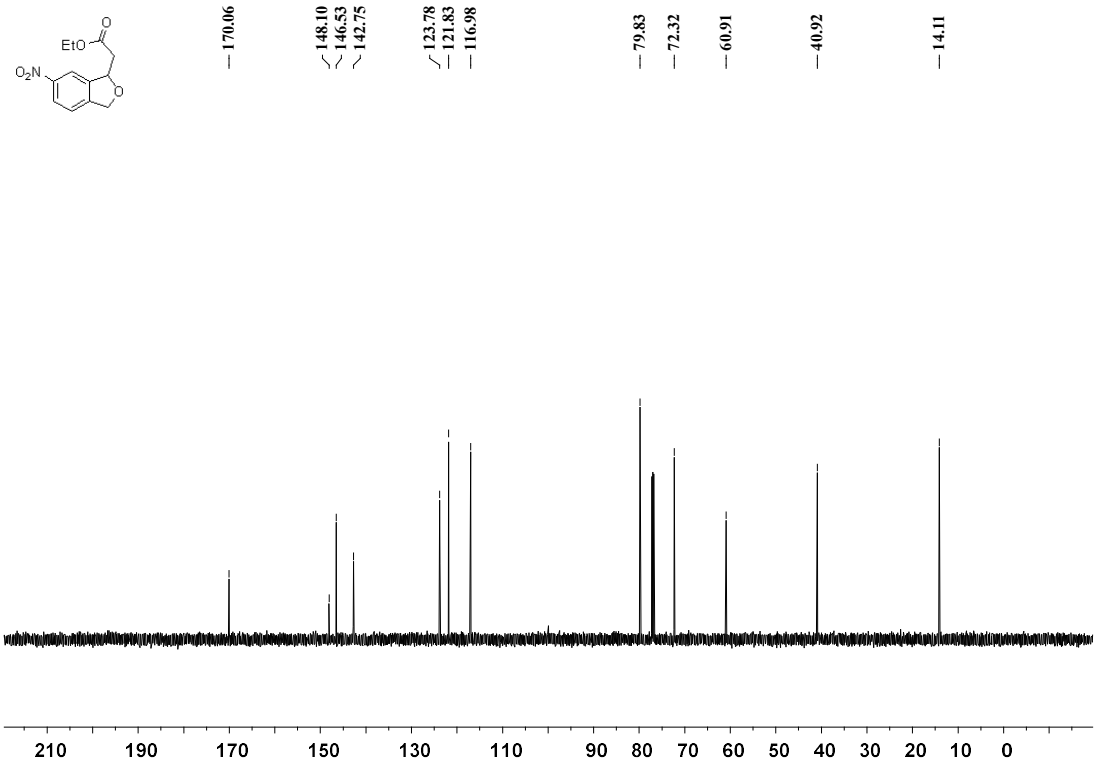


Figure S130.[13]C NMR (101 MHz, $CDCl_3$) of **3d**

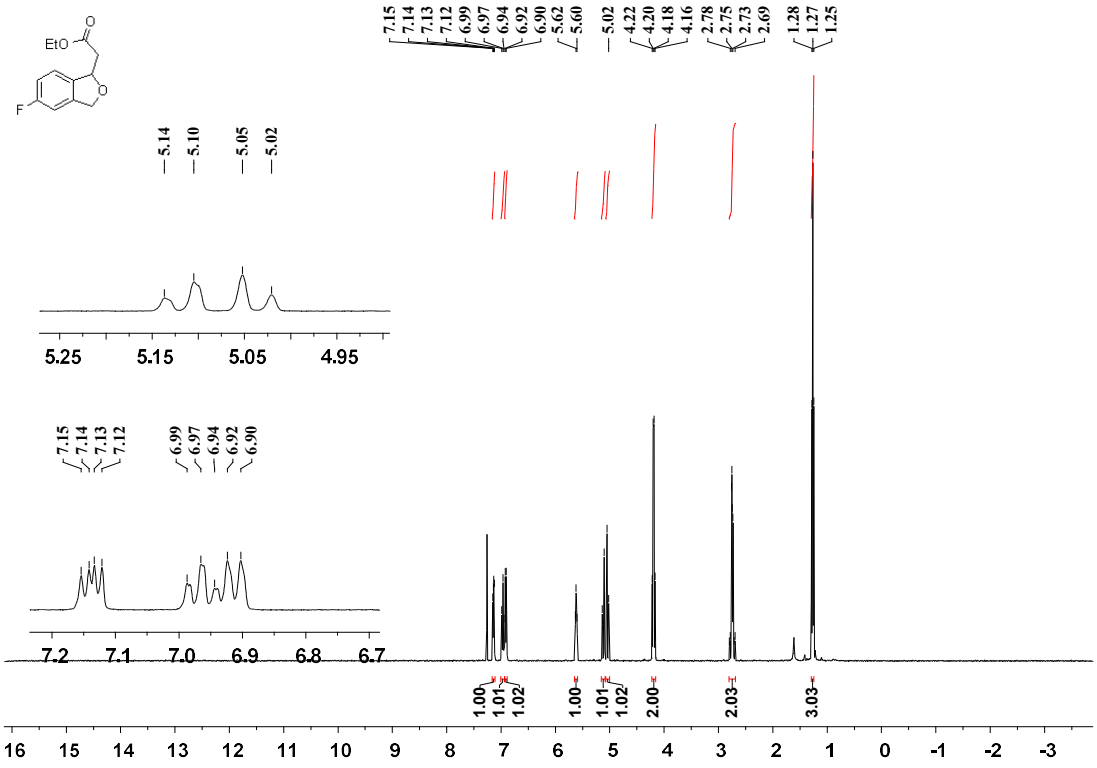


Figure S131.[1]H NMR (600 MHz, $CDCl_3$) of **8a**

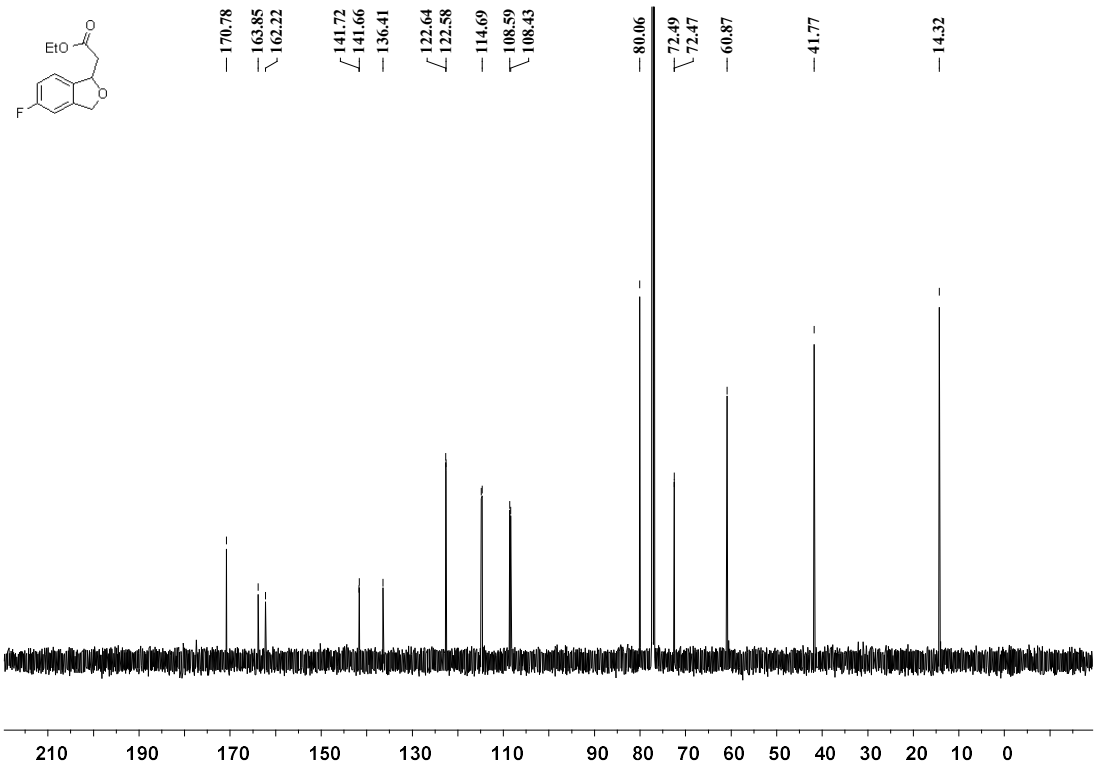


Figure S132.[13]C NMR (151 MHz, $CDCl_3$) of **8a**

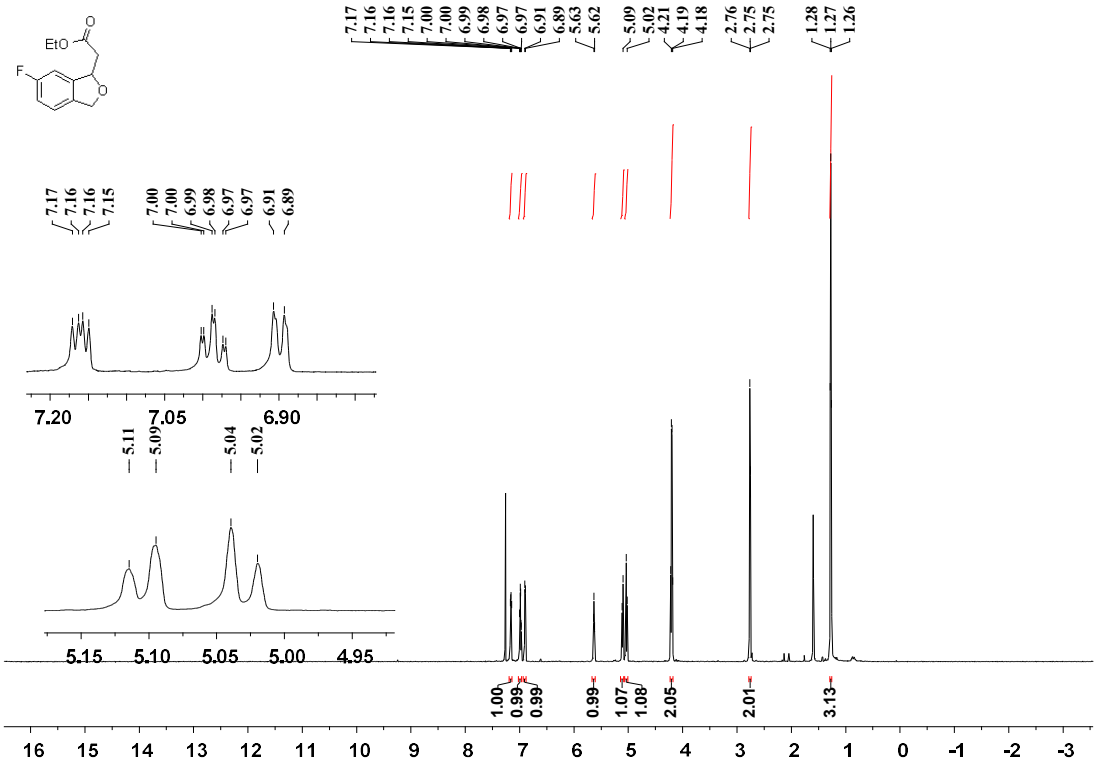


Figure S133.[1]H NMR (600 MHz, $CDCl_3$) of **9a**

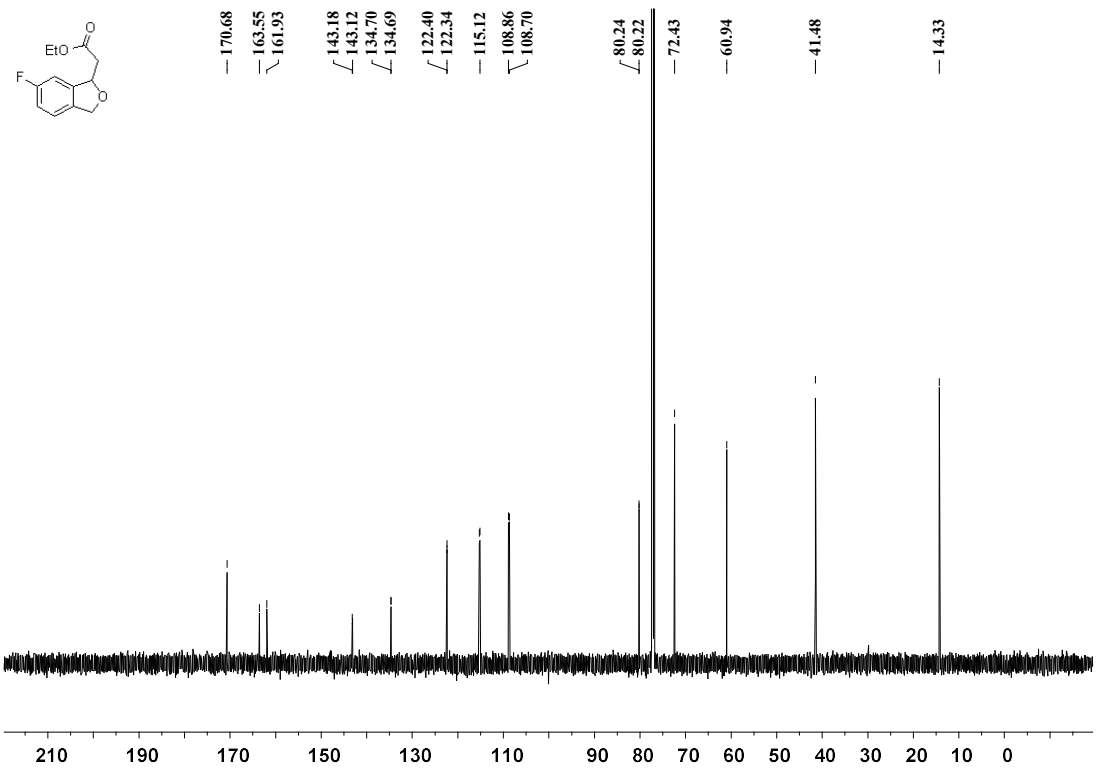


Figure S134.[13]C NMR (151 MHz, $CDCl_3$) of **9a**

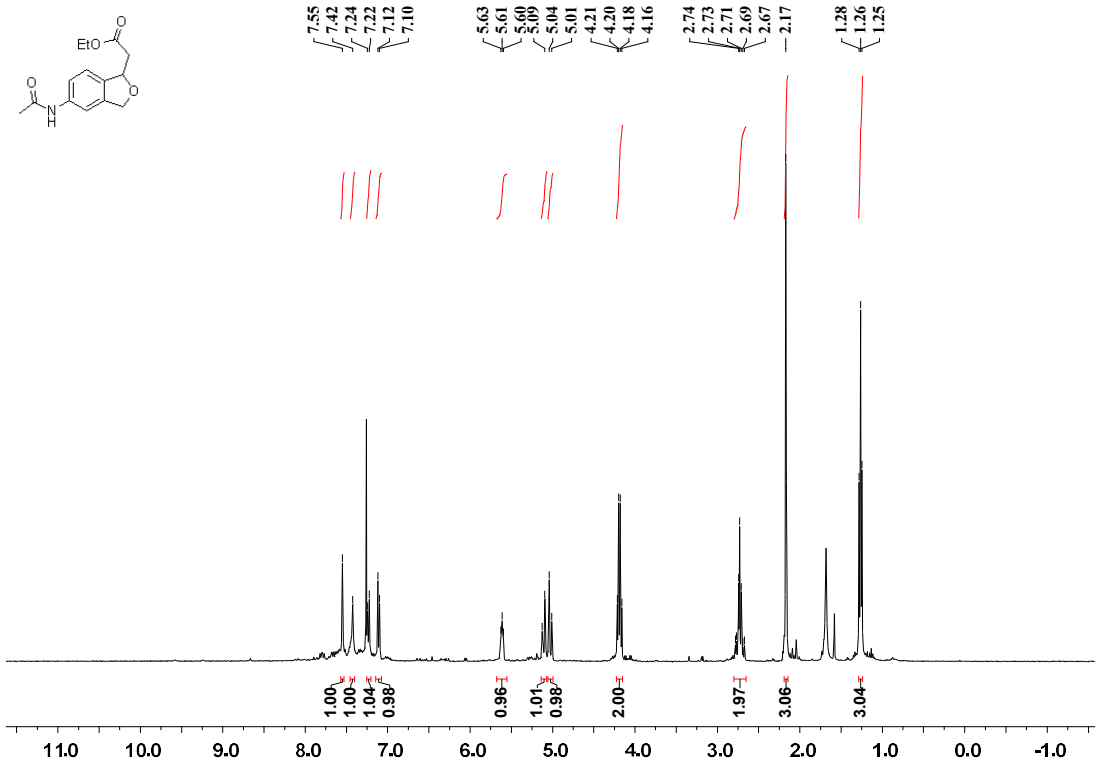


Figure S135.[1]H NMR (600 MHz, $CDCl_3$) of **5a**

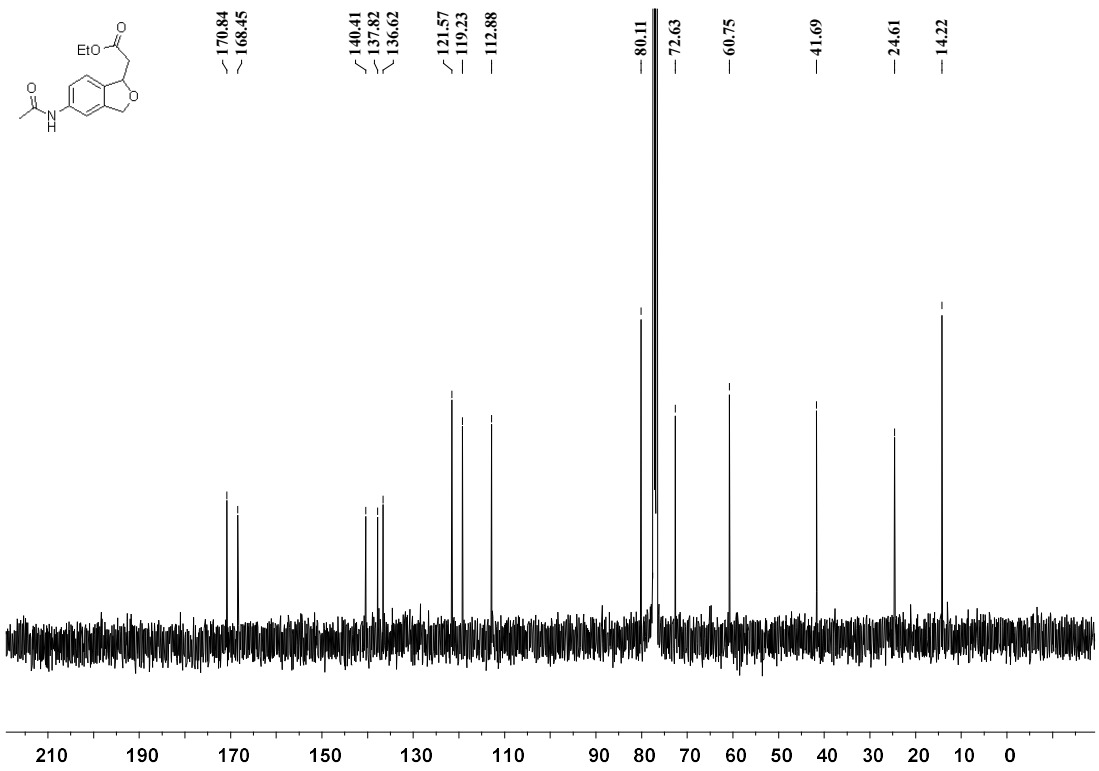


Figure S136.[13]C NMR (101 MHz, $CDCl_3$) of **5a**

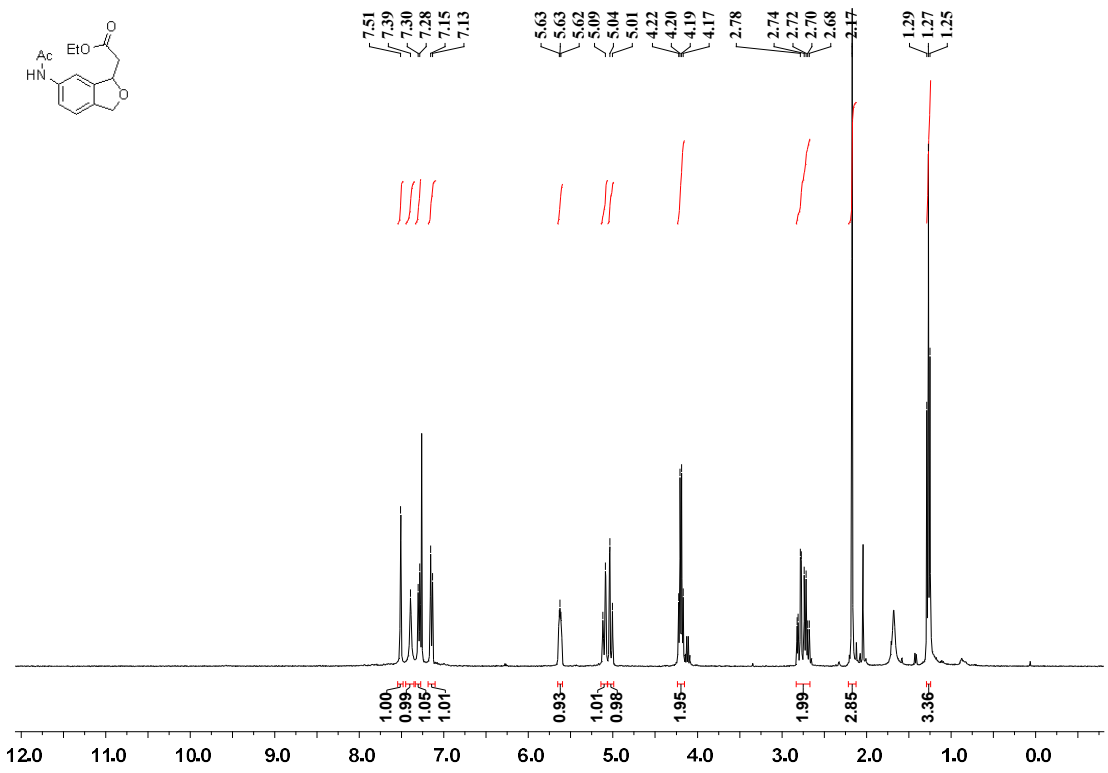


Figure S137.[1]H NMR (600 MHz, $CDCl_3$) of **6a**

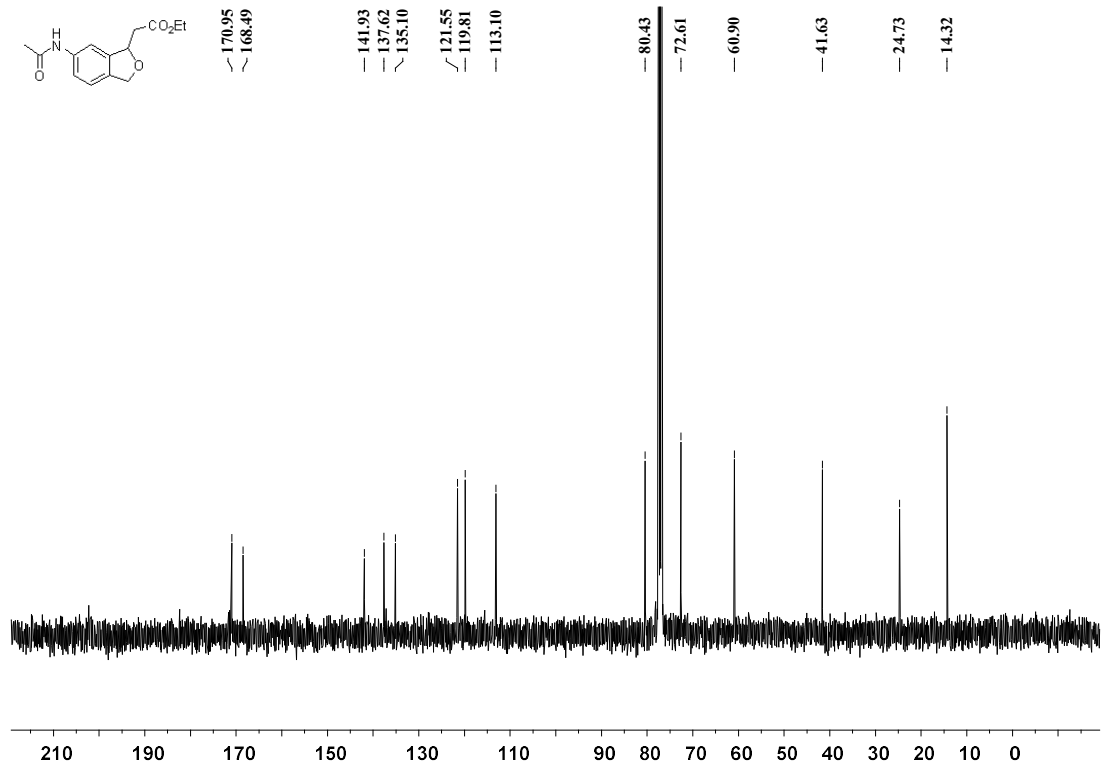


Figure S138.[13]C NMR (101 MHz, $CDCl_3$) of **6a**

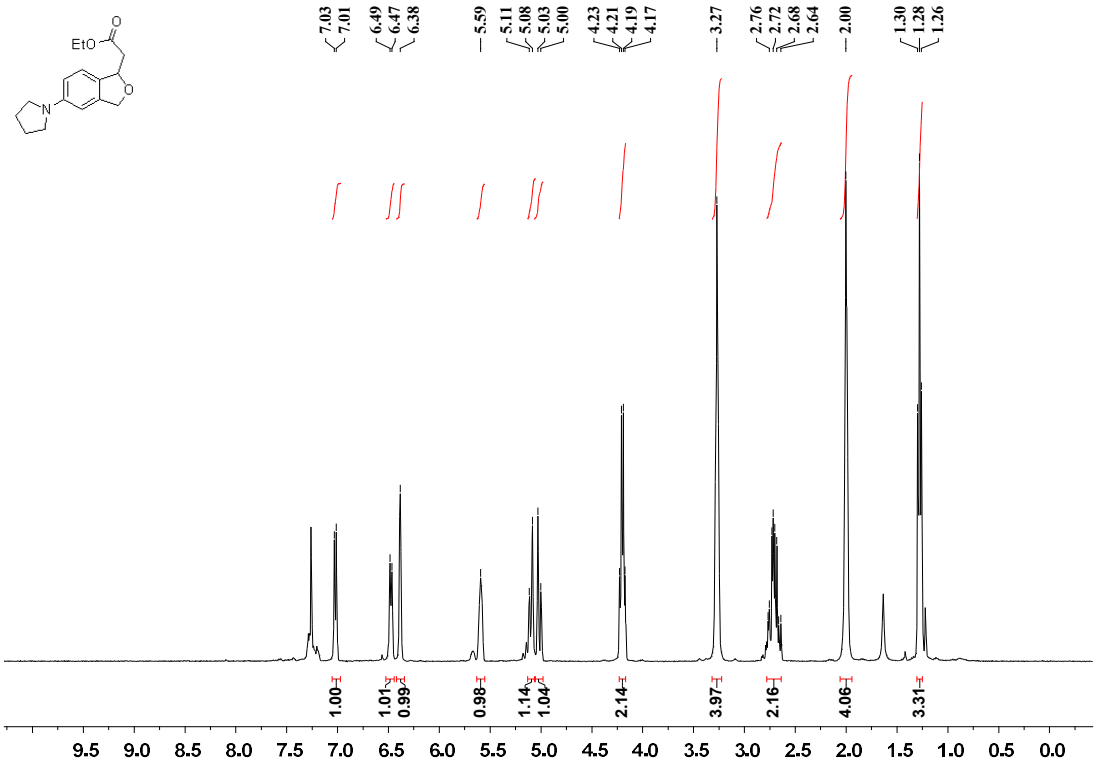


Figure S139.[1]H NMR (600 MHz, $CDCl_3$) of **6a**

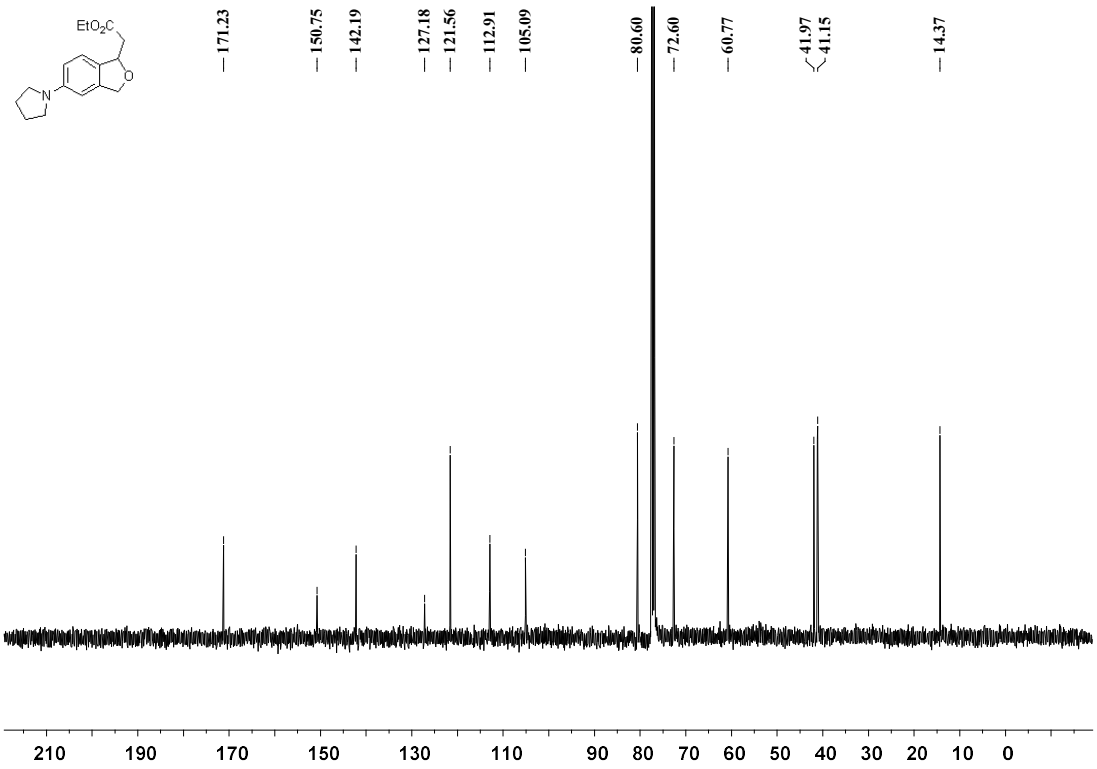


Figure S140. [13]C NMR (151 MHz, $CDCl_3$) of **6a**

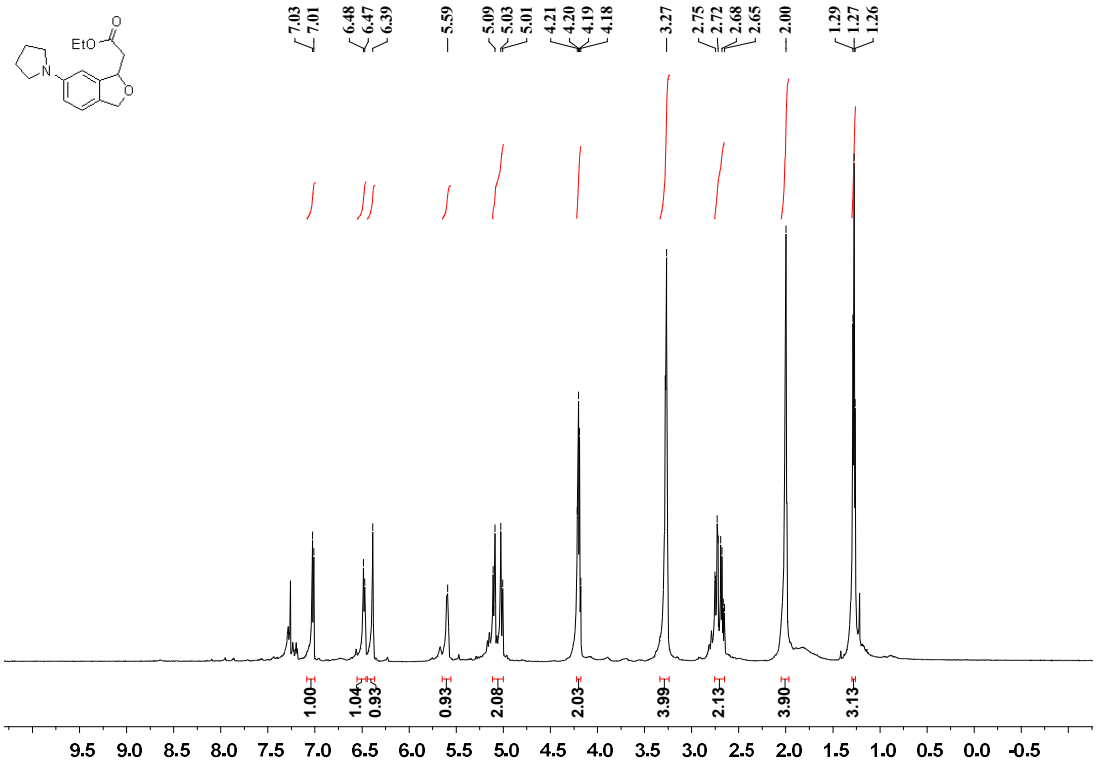


Figure S141.[1]H NMR (600 MHz, $CDCl_3$) of **6b**

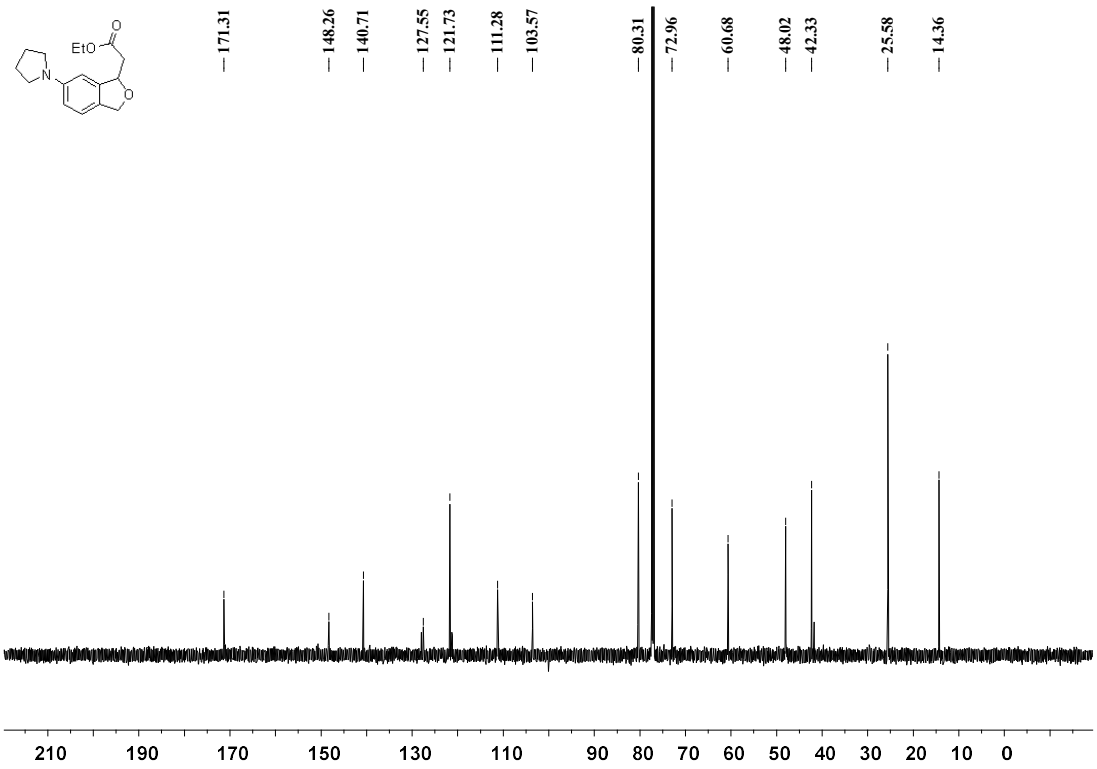


Figure S142. [13]C NMR (151 MHz, $CDCl_3$) of **6b**

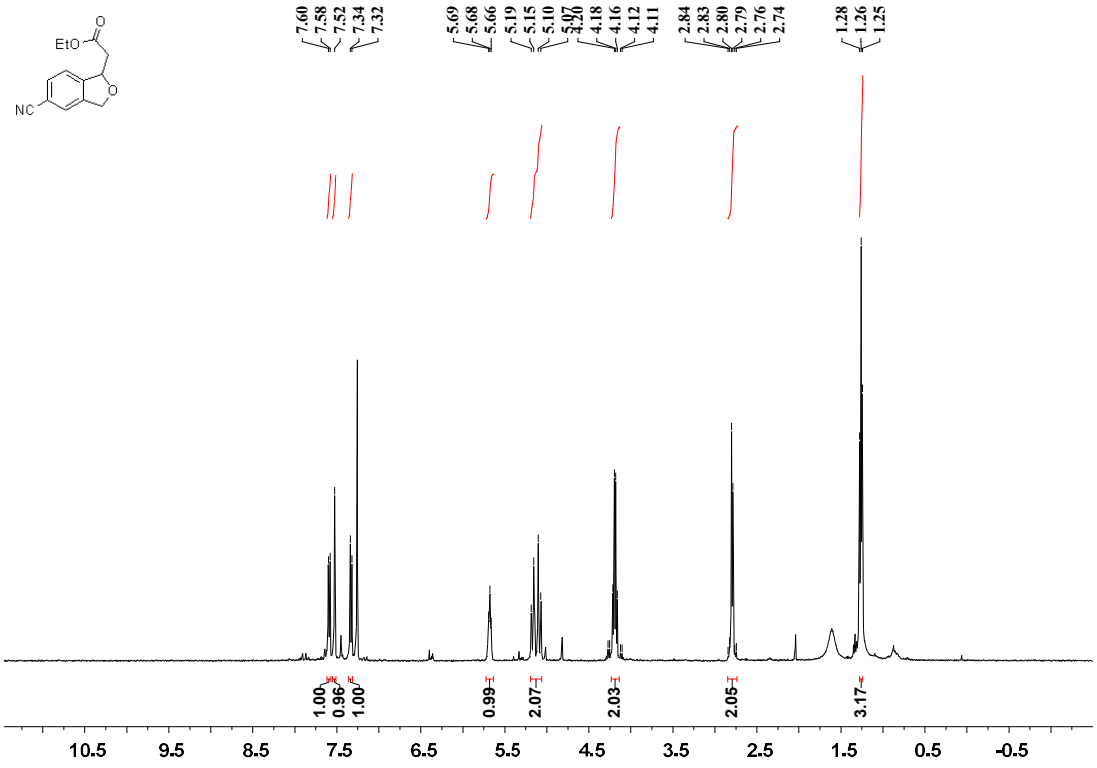


Figure S143.[1]H NMR (400 MHz, $CDCl_3$) of **2e**

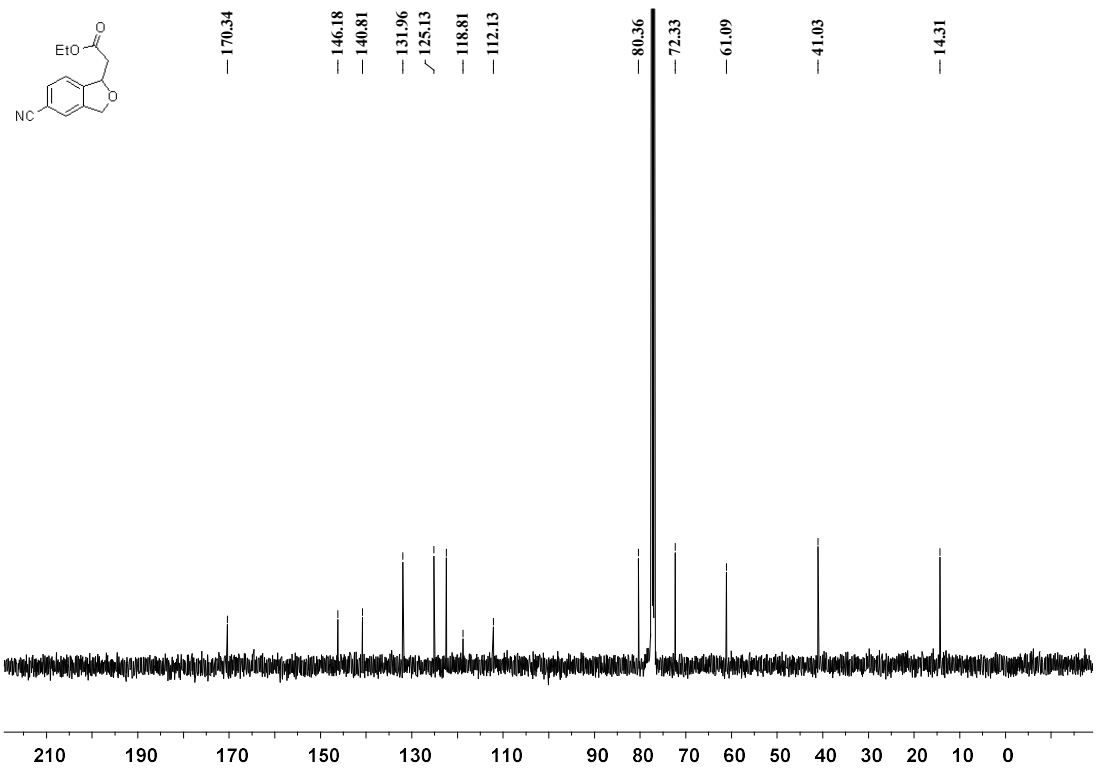


Figure S144.[13]C NMR (101 MHz, $CDCl_3$) of **2e**

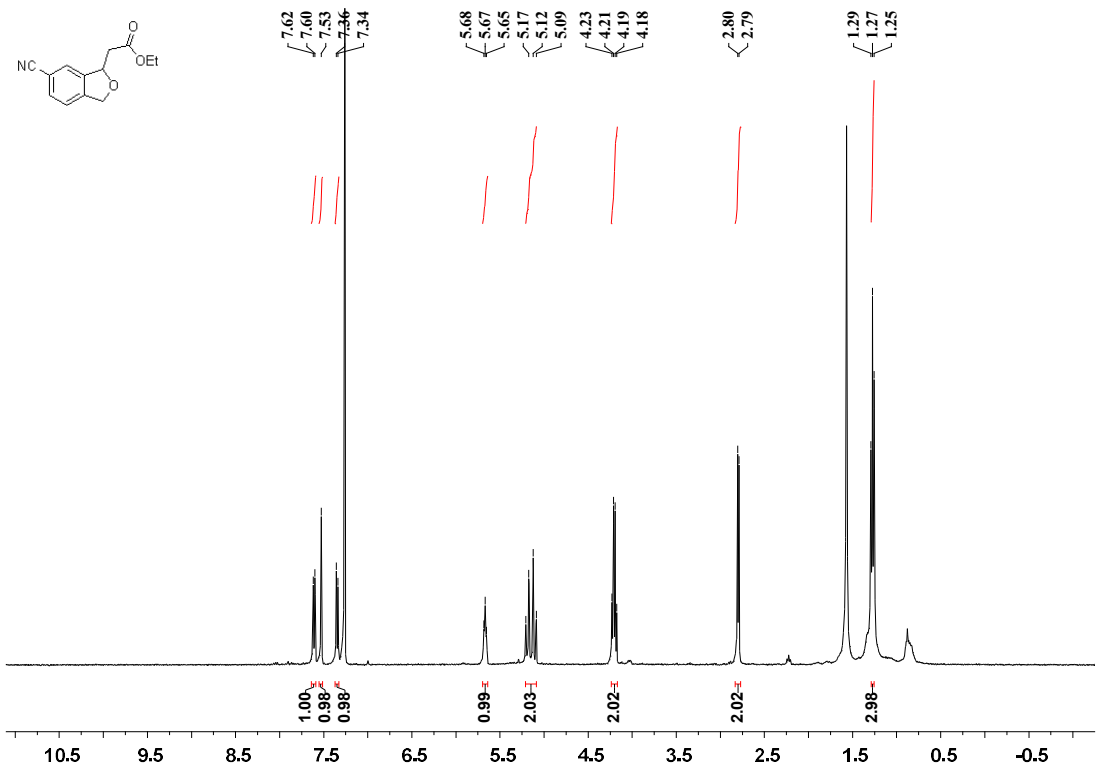


Figure S145 ^{1}H NMR (400 MHz, $CDCl_3$) of **3e**

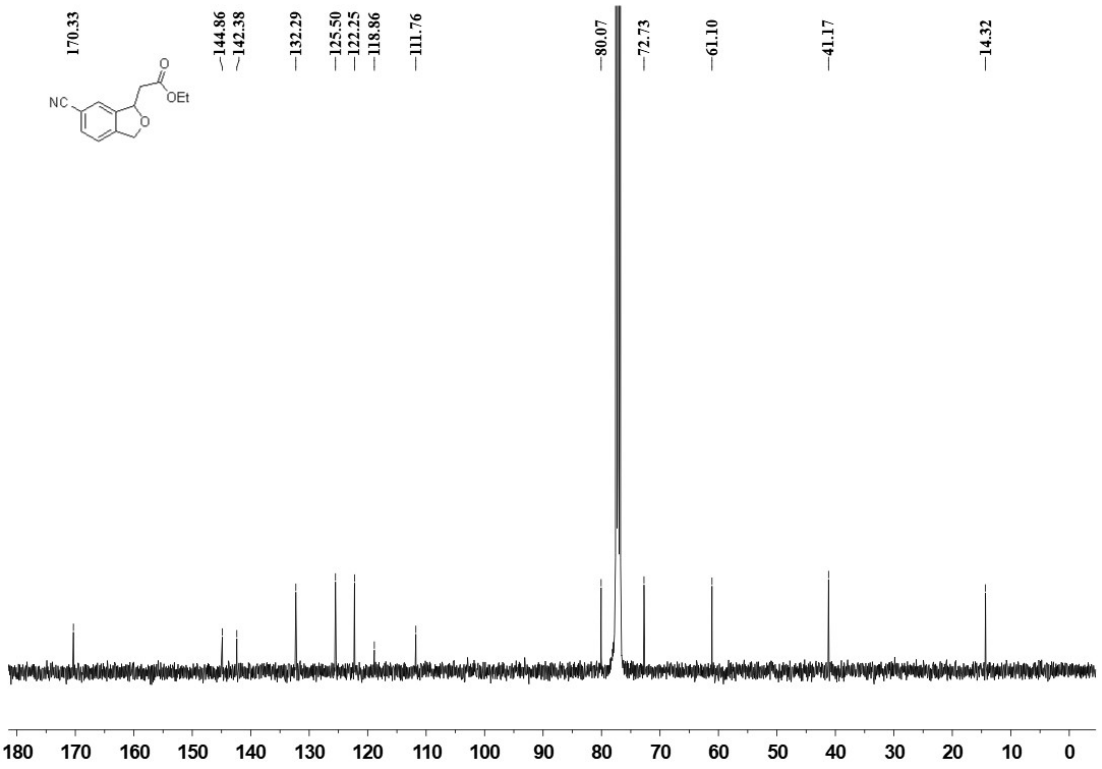


Figure S146 ^{13}C NMR (101 MHz, $CDCl_3$) of **3e**

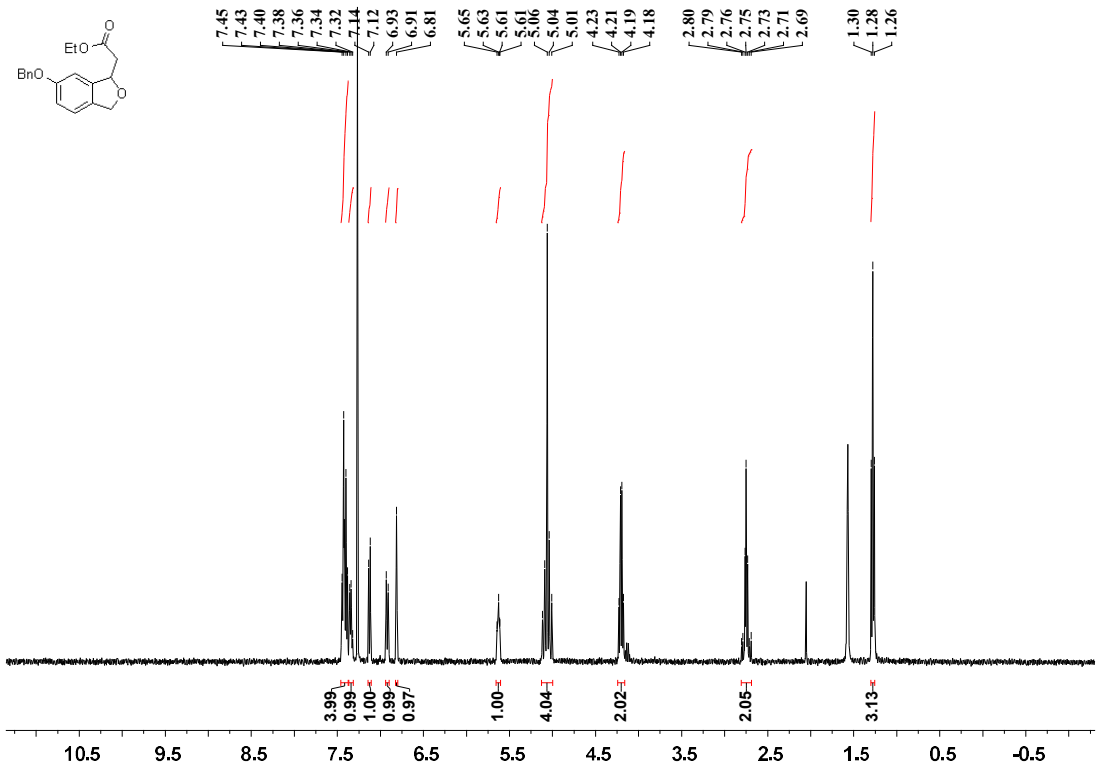


Figure S147. ^{1}H NMR (400 MHz, $CDCl_3$) of Ethyl 2-(6-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

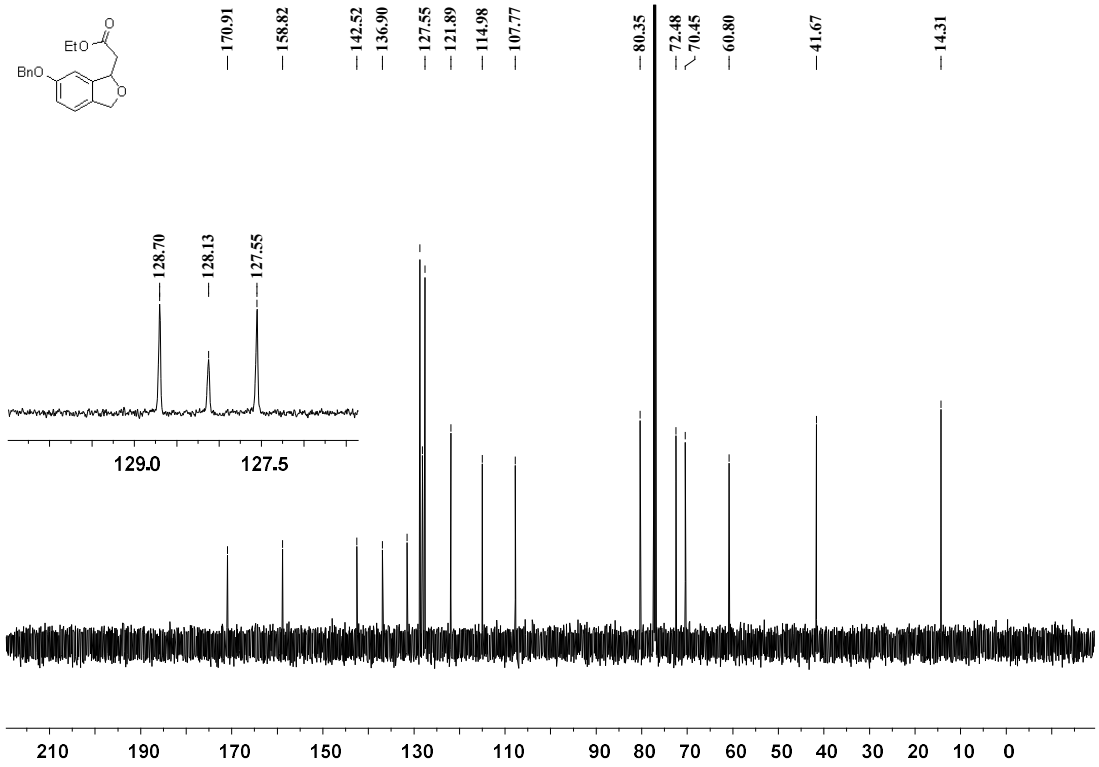


Figure S148 ^{13}C NMR (151 MHz, $CDCl_3$) of Ethyl 2-(6-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

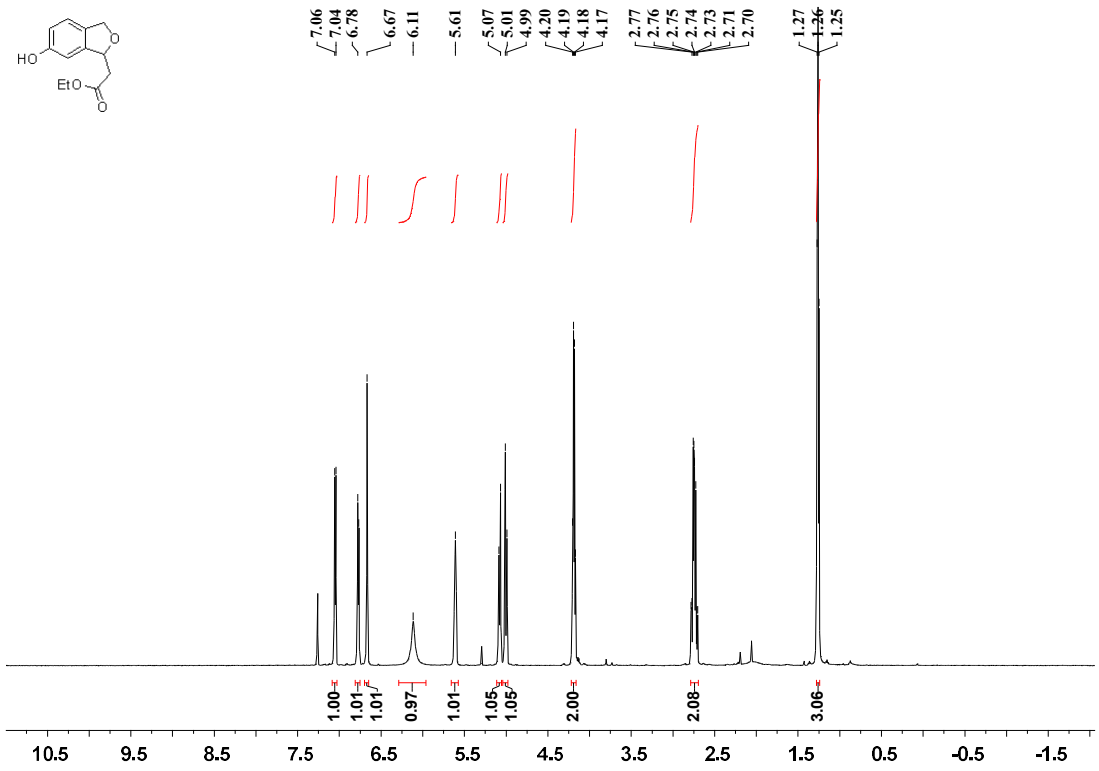


Figure S149. ^{1}H NMR (600 MHz, $CDCl_3$) of Ethyl 2-(6-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

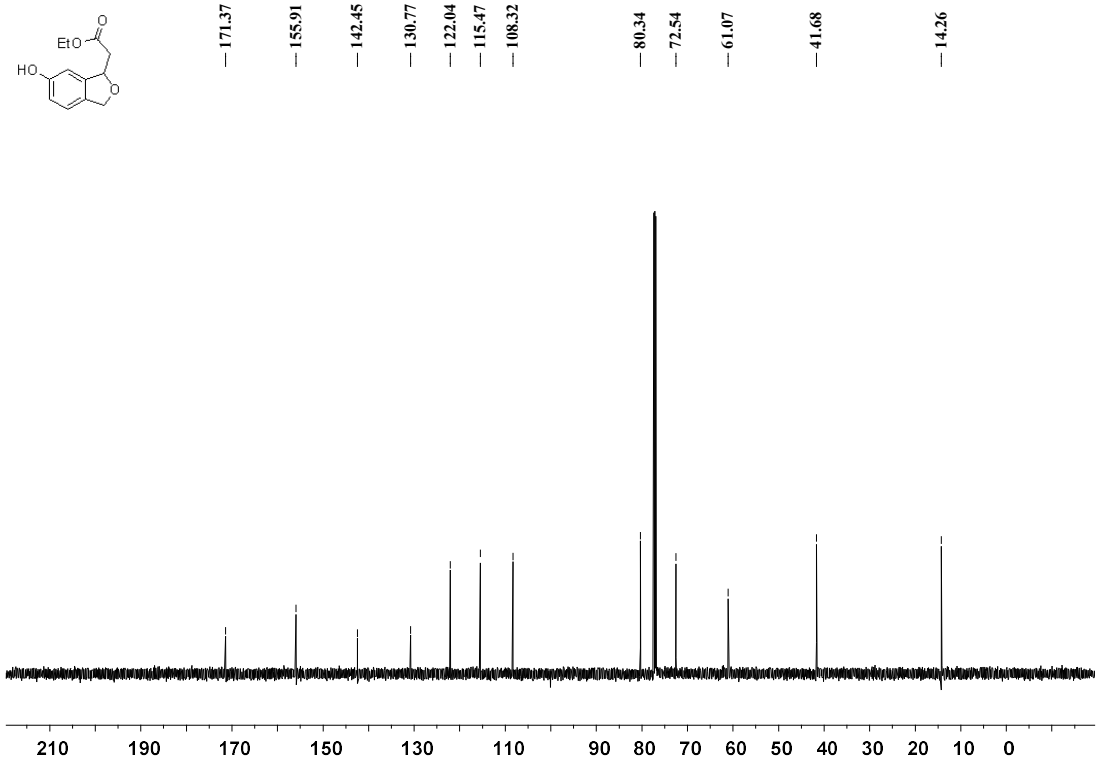


Figure S150. ^{13}C NMR (151 MHz, $CDCl_3$) of Ethyl 2-(6-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

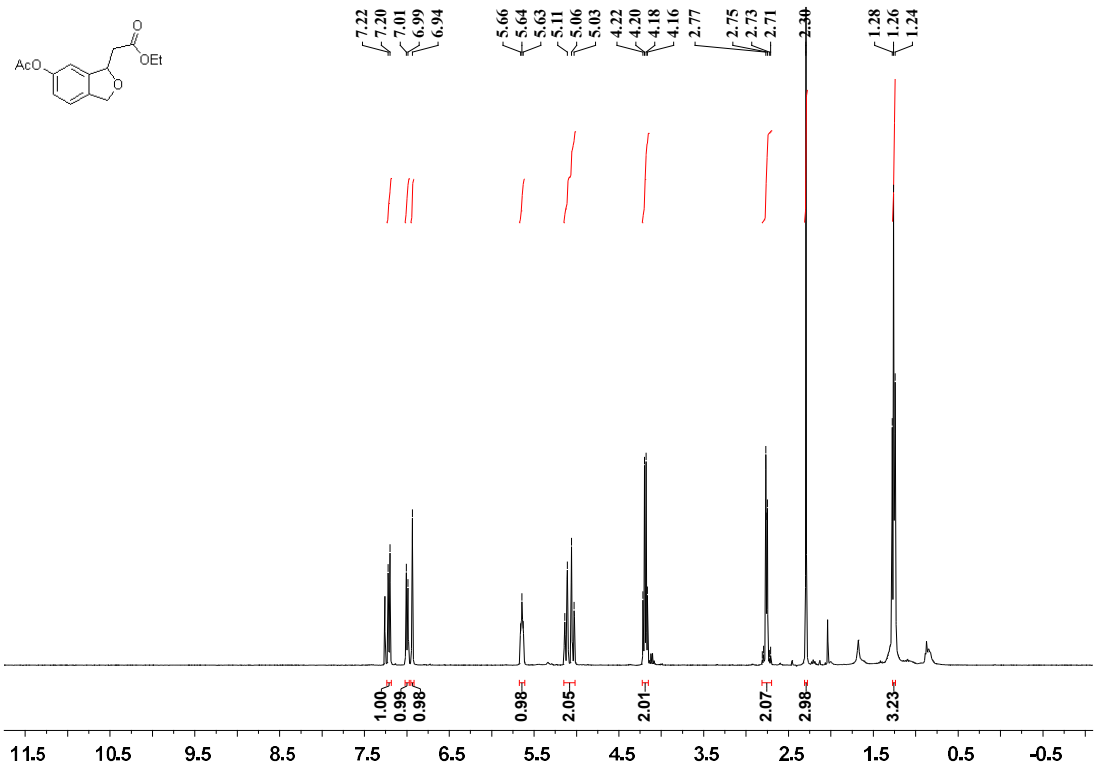


Figure S151 ^{1}H NMR (400 MHz, $CDCl_3$) of **6c**

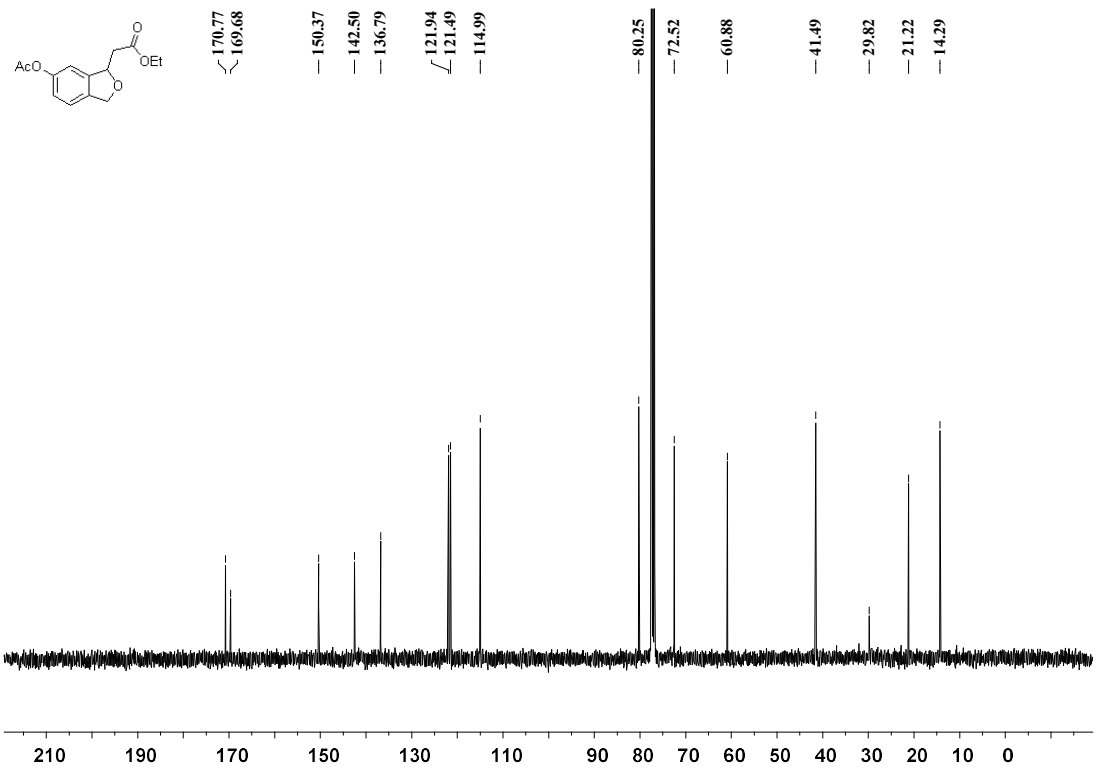


Figure S152 ^{13}C NMR (101 MHz, $CDCl_3$) of **6c**

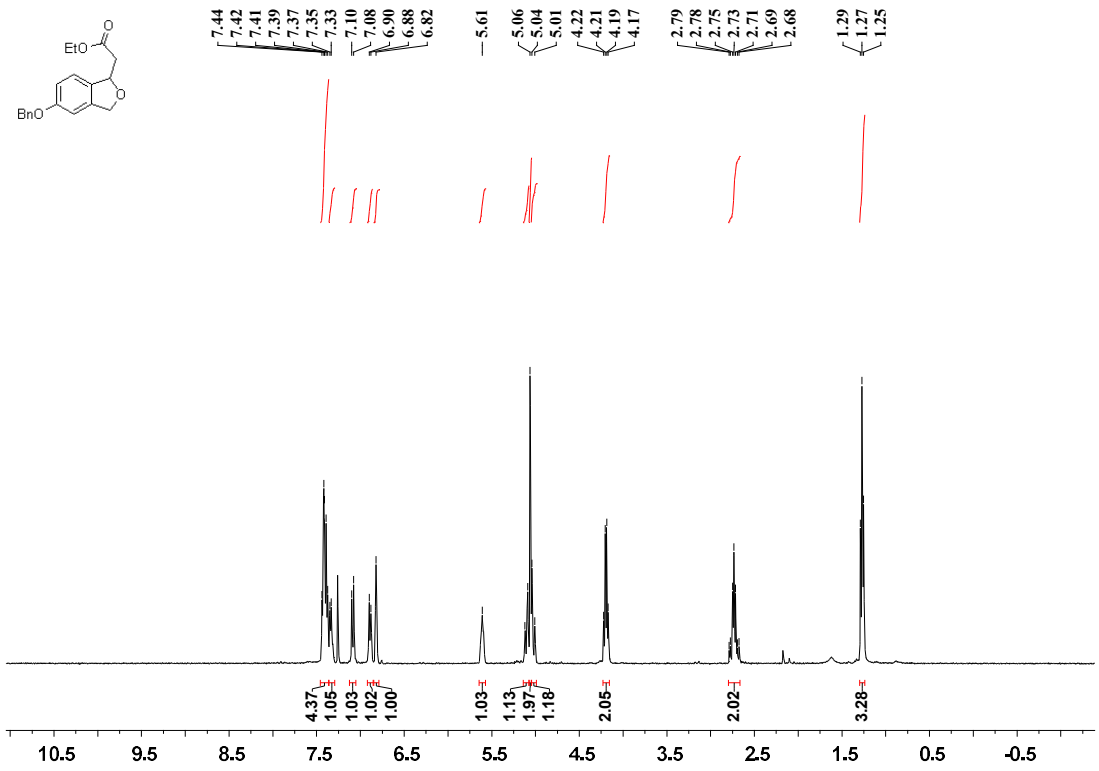


Figure S153. ^{1}H NMR (400 MHz, $CDCl_3$) of Ethyl 2-(5-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

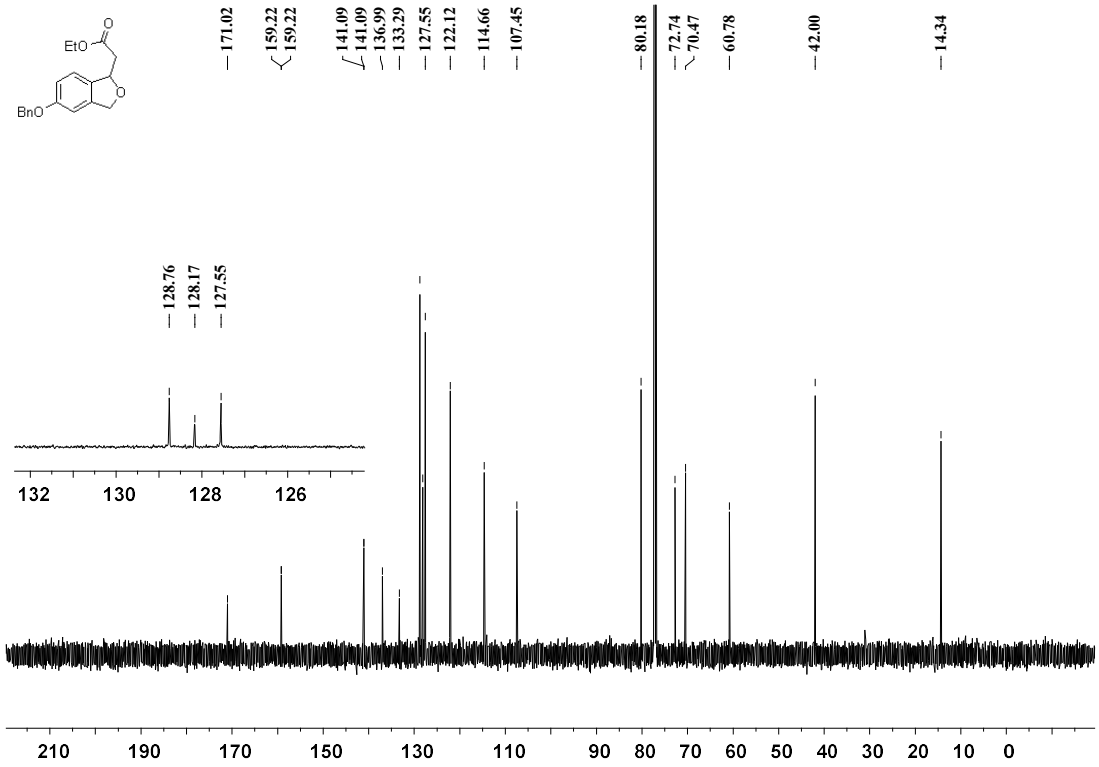


Figure S154. ^{13}C NMR (151 MHz, $CDCl_3$) of Ethyl 2-(5-(benzyloxy)-1,3-dihydroisobenzofuran-1-yl)acetate

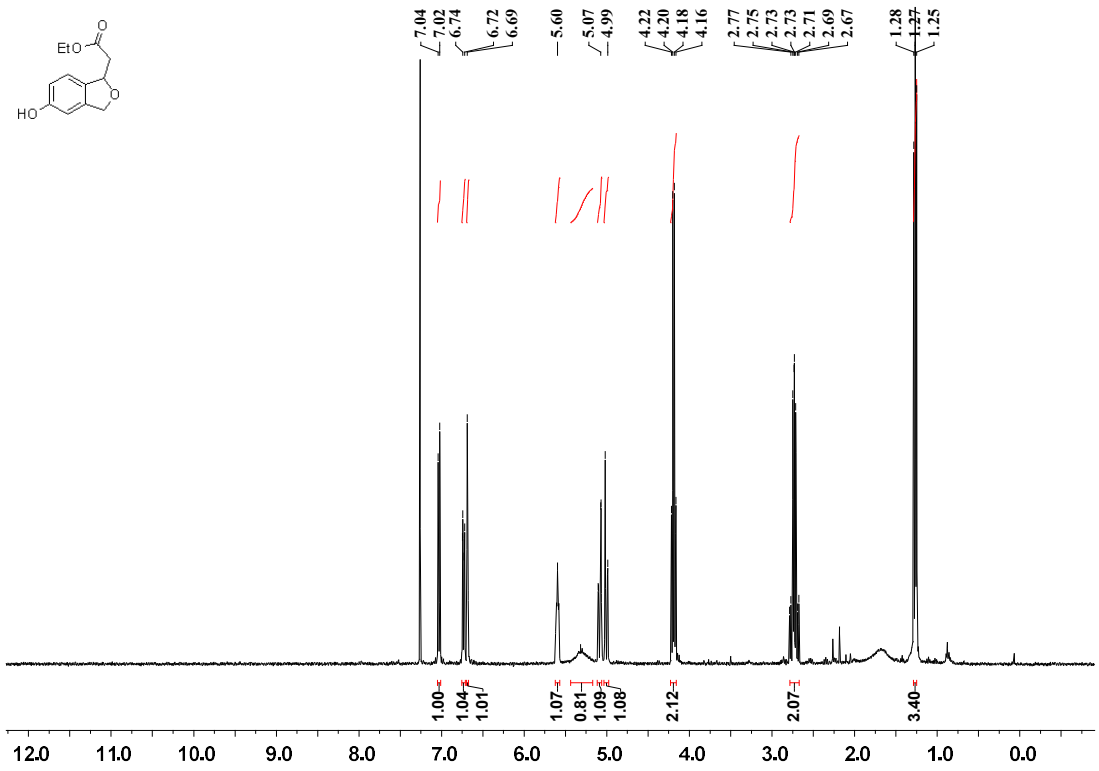


Figure S155. ^{1}H NMR (400 MHz, $CDCl_3$) of Ethyl 2-(5-hydroxy-1,3-dihydroisobenzofuran-1-yl)acetate

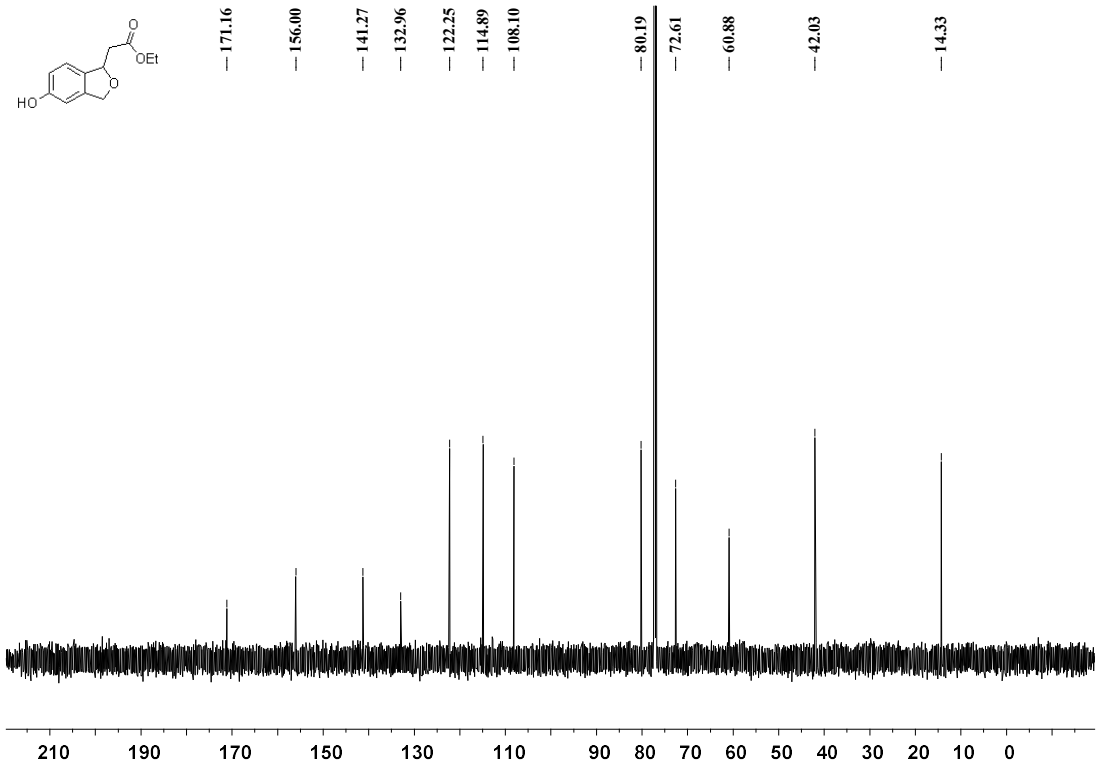


Figure S156. ^{13}C NMR (151 MHz, $CDCl_3$) of Ethyl 2-(5-hydroxy-1,3-dihydroisobenzofuran-1-yl)acetate

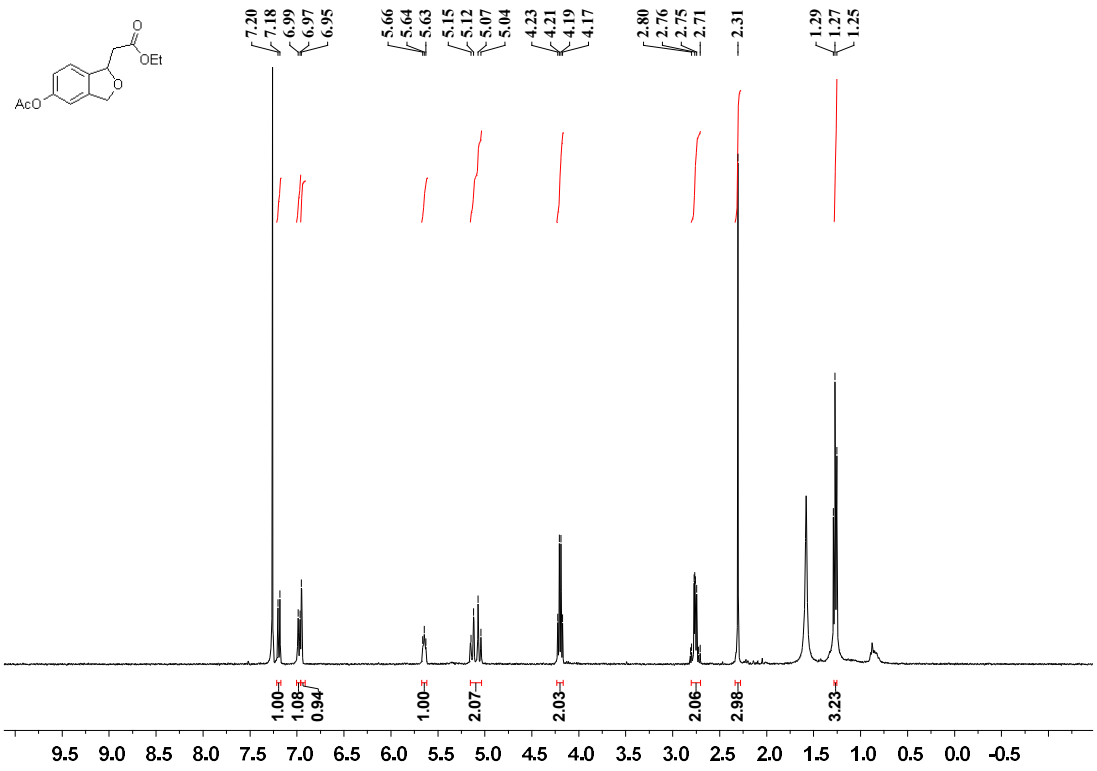


Figure S157 ^{1}H NMR (400 MHz, $CDCl_3$) of **5c**

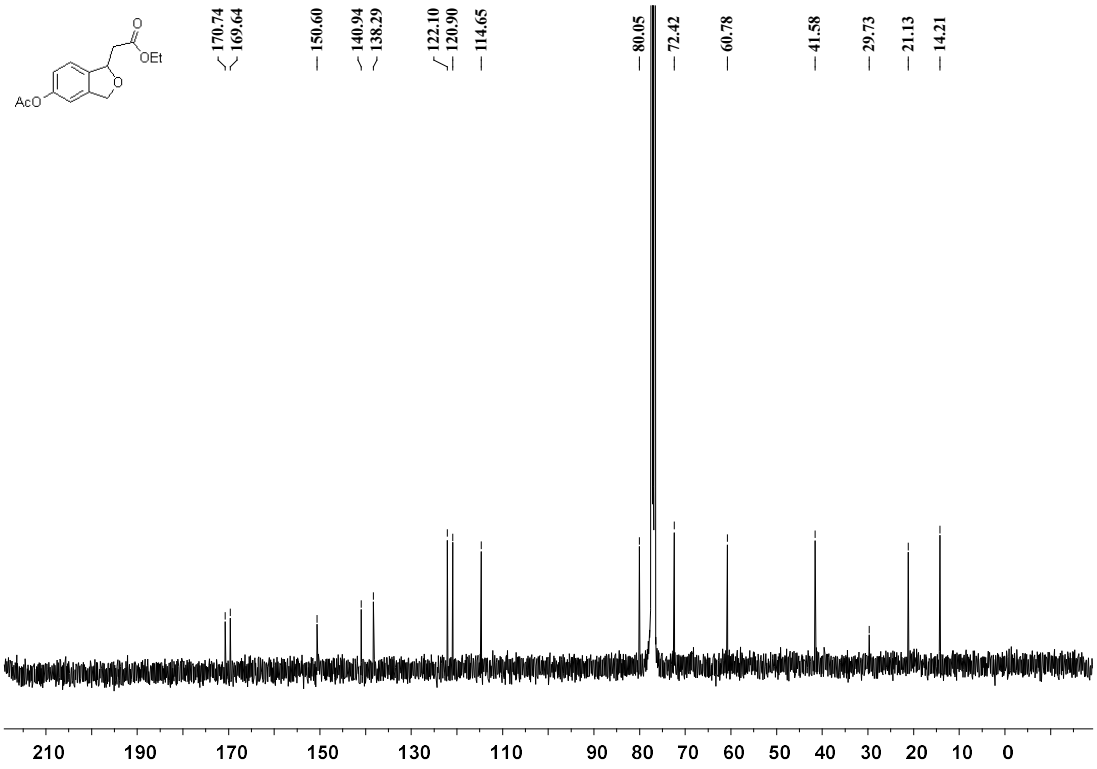


Figure S158 ^{13}C NMR (101 MHz, $CDCl_3$) of **5c**

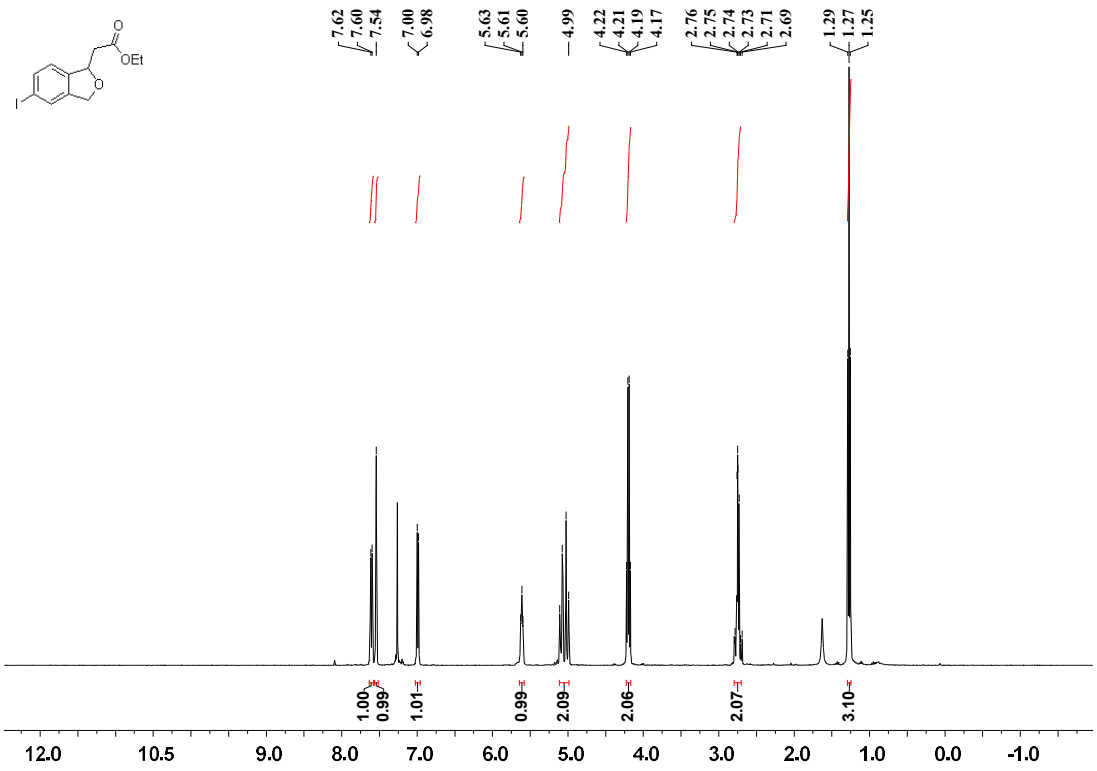


Figure S159 ^{1}H NMR (400 MHz, $CDCl_3$) of **2f**

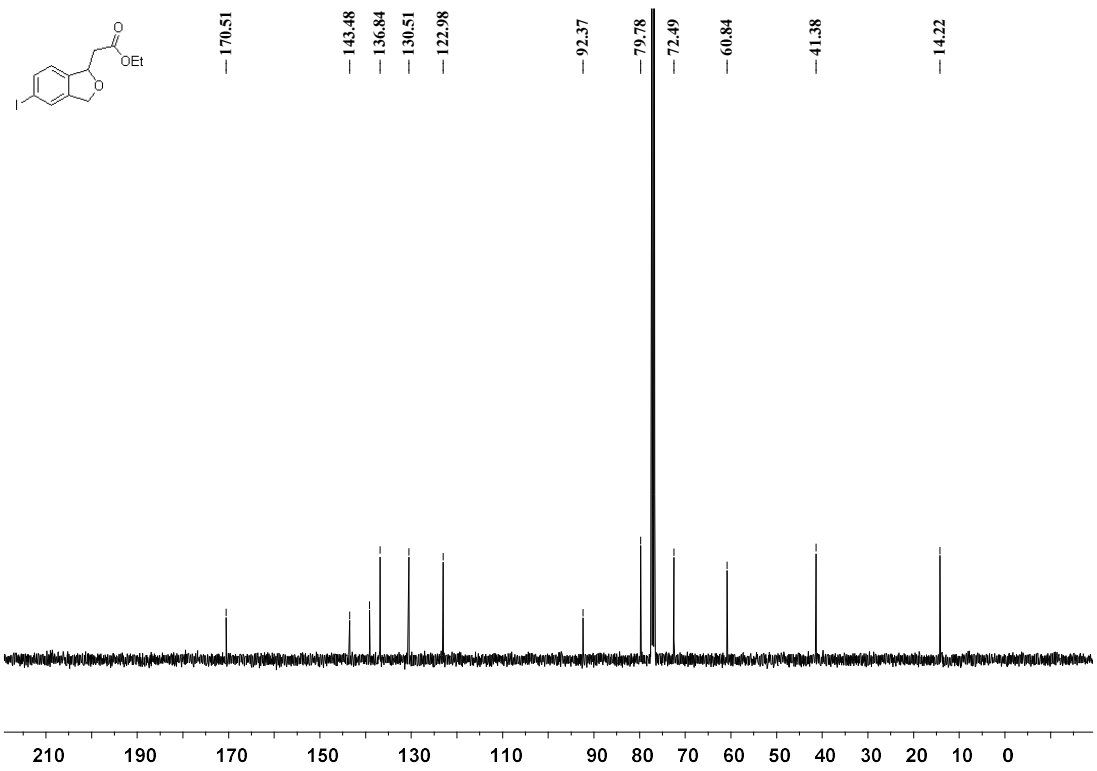


Figure S160 ^{13}C NMR (101 MHz, $CDCl_3$) of **2f**

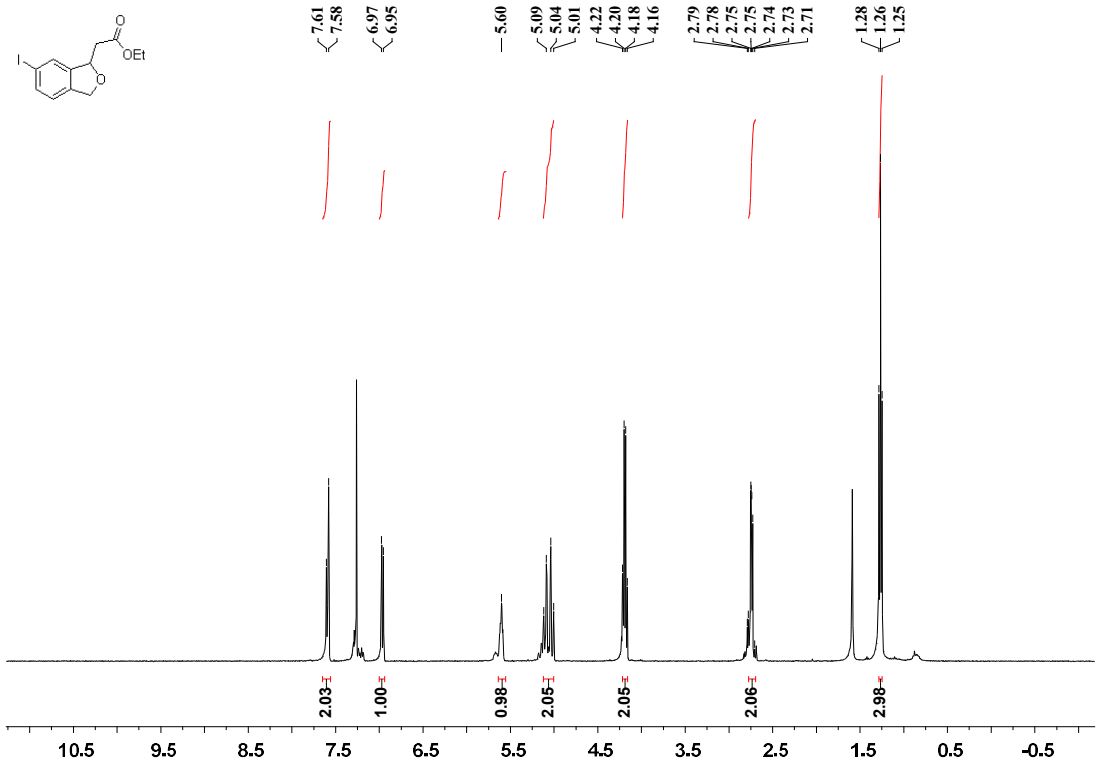


Figure S161 ^{1}H NMR (400 MHz, $CDCl_3$) of 3**f**

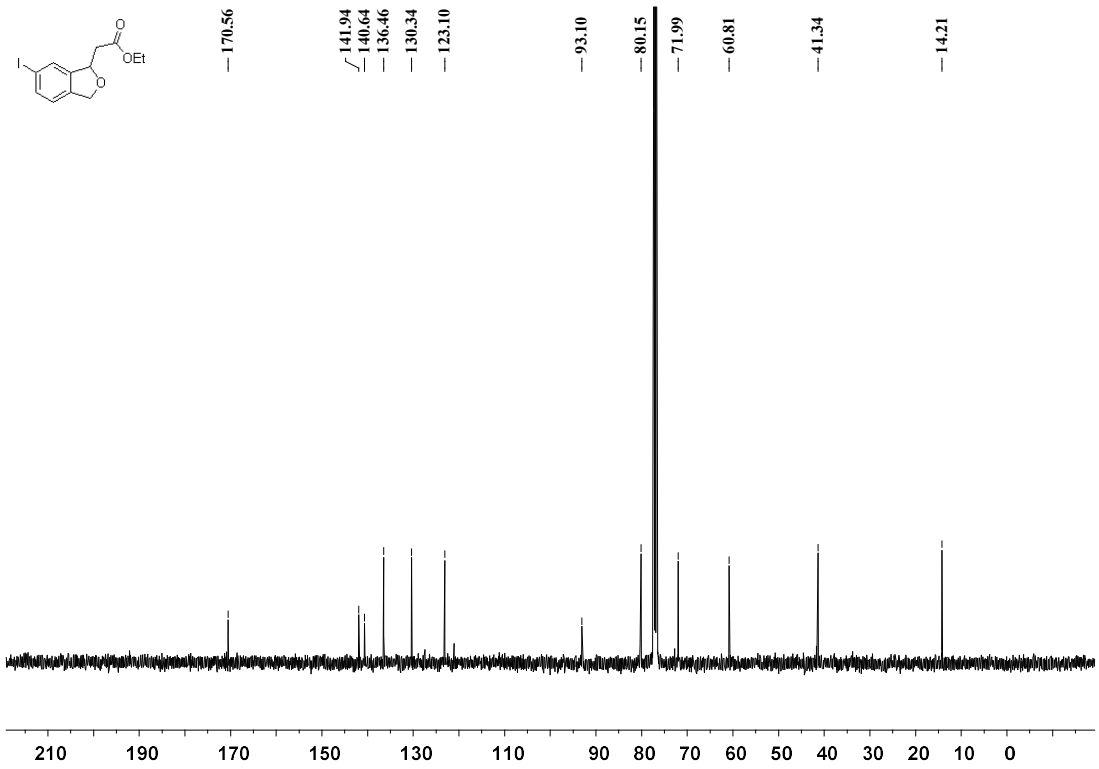


Figure S162 ^{13}C NMR (101 MHz, $CDCl_3$) of 3**f**

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