Supporting Information

Enantioselective Conjugate Addition of Malonates to α,β-Unsaturated Aldehydes Catalyzed by 4-Oxalocrotonate Tautomerase

Ming-Zhu Yu,[1] Kai-Yue Chen,[1] Yi-Bin Zhang,[1] Chang-Xuan Zhang,[1] and Zheng Xiang[1,2,3]*

[1]State Key Laboratory of Chemical Oncogenomics, Guangdong Provincial Key Laboratory of Chemical Genomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China. [2]Institute of Chemical Biology, Shenzhen Bay Laboratory, Shenzhen 518132, China. [3]AI for Science (AI4S) Preferred Program, Peking University Shenzhen Graduate School, Shenzhen 518055, China.

*Email: zxiang@pku.edu.cn

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I. Supplementary figures

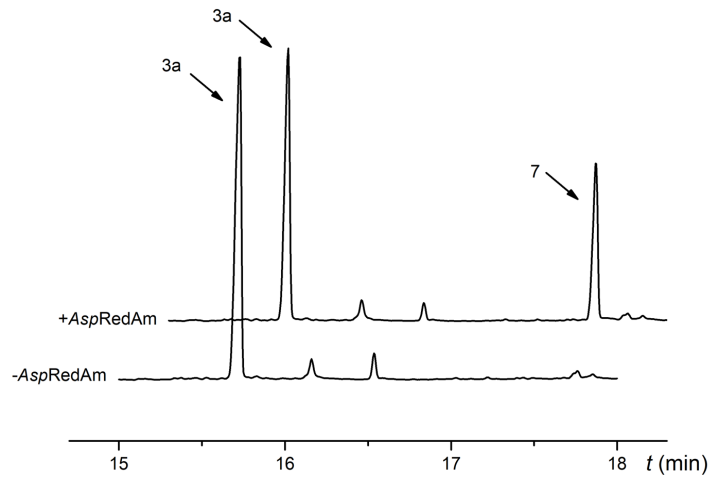


Figure S1. Enzymatic reductive amination of compound **3a** and methylamine catalyzed by *Asp*RedAm.

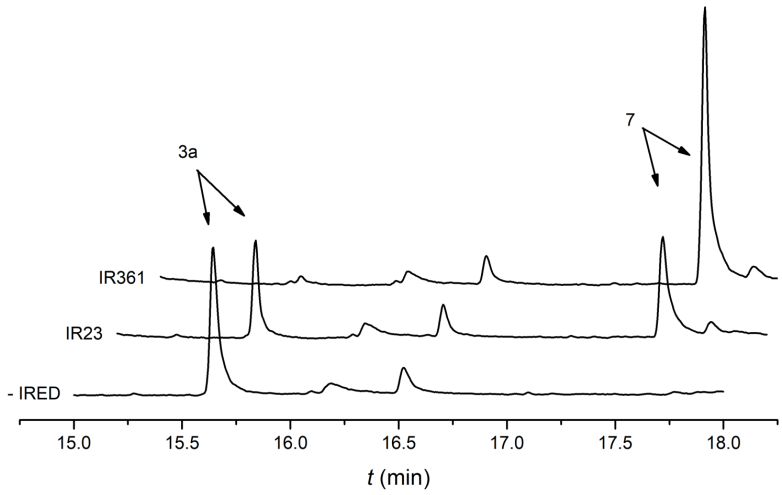


Figure S2. Enzymatic reductive amination of compound **3a** and methylamine catalyzed by IR-23 and IR-361.

II. Materials and methods

Bacterial strains, plasmids, and chemicals

Escherichia coli DH5α (Shenzhen KT Life technology) and BL21(DE3) (Shanghai Weidi Biotechnology Co. Ltd.) strains were used for cloning and protein expression, respectively. The codon-optimized genes of 4-OT, CHMI, DDT, and MIF were synthesized and subcloned into pET-29a(+) vector (Novagen) by *Nde*I and *BamH*I

restriction sites by Genscript (Nanjing, China). The 4-OT mutants were made from this plasmid using the QuikChange Site-Directed Mutagenesis and verified by DNA sequencing. The codon-optimized genes of *Asp*RedAm, IR23, and IR361 were synthesized and subcloned into pET-28a(+) vector (Novagen) by *Nde*I and *Xho*I restriction sites by Genscript (Nanjing, China). Protein and nucleotide sequences are listed in Table S1 and Table S2, respectively. PCR primers used in this work are listed in Table S3. DNA sequencing was conducted by Sangon Biotech. Chemicals used for protein expression, purification and enzymatic assays were purchased from Sangon Biotech, Sigma-Aldrich and Solarbio. Protein concentrations were determined using BCA Protein Assay Kit (Beyotime Biotechnology) according to the manufacturer's protocol.

Table S1. Protein sequences

Protein	Sequence
4-OT (63 aa)	MPIAQIHILEGRSDEQKETLIREVSEAISRSLDAPLTSVRVIITEMAKGHFGIGGELASKVRR*
CHMI (126 aa)	MPHFIVECSDNIREEADLPGLFAKVNPTLAATGIFPLAGIRSRVHWVDTWQMADGQHDYAFVHMTLKIGAGR SLESRQQAGEMLFELIKTHFAALMESRLLALSFEIEELHPTLNFKQNNVHALFK*
DDT (118 aa)	MPFLELDTNLPANRVPAGLEKRLCAAAASILGKPADRVNVTVRPGLAMALSGSTEPCAQLSISSIGVVGTAE DNRSHSAHFFEFLTKELALGQDRILIRFFPLESWQIGKIGTVMTFL*
MIF (115 aa)	MPMFIVNTNVPRASVPEGFLSELTQQLAQATGKPAQYIAVHVVPDQLMTFSGTNDPCALCSLHSIGKIGGAQ NRNYSKLLCGLLSDRLHISPDRVYINYYDMNAANVGWNGSTFA*
*Asp*RedAm (295 aa)	MSKHIGIFGLGAMGTALAAKYLEHGYKTSVWNRTTAKAIPLVEQGAKLASTISEGVNANDLIIICLLNNQVV EDALRDALQTLPSKTIVNLTNGTPNQARKLADFVTSHGARYIHGGIMAVPTMIGSPHAVLLYSGESLELFQS IESHLSLLGMSKYLGTDAGSASLHDLALLSGMYGLFSGFLHAVALIKSGQDTSTTATGLLPLLTPWLSAMTG YLSSIAKQIDDGDYATQGSNLGMQLAGVENIIRAGEEQRVSSQMILPIKALIEQAVGEGHGGEDLSALIEYF KVGKNVD*
IR-23 (291 aa)	MKPGISVLGTGRMGSALVGAFLKQGYNVAVWNRTKSKCAPLAALGARVATTVRDAVADAEVVVVNVNDYVTS EALLRQDDVTKGLRGKLIVQLTSGSPRQAREMAAWARQHELQYLDGAIMGTPNFIGEPGGTILYSGPGALFE KYKPVLLVLGGNSLHVGSDVGHASALDSALLSFLWGSMFGVLQAVSVCEAEGLPLGAYMEYVQATKPMVDGA VTDFVKRIQTGRFAGDEKTLATVEAHHGALRHLIELCEEHGIHHAVPAAFGQLFQAALQAGHAQDDFAVLNK FMK*
IR-361 (297 aa)	MSDPNADRPPVTVVGLGLMGQALAAAFLKGGHPTTVWNRSPEKAERLVADGAVLADTLESAVTASPLVIVCV SDYDAVHELIRPVESALAGRVLVNLTTATSTQARETAEWAAQRNIPYLDGAIMAIPPVIGTDGAVLLYSGHK SAFEAHESTLKAIAPAATTYLEEDHGLSSLYDMALLGIMWGILNGFLHGAALLGTAKVKAETFAPLANTMIS AITEYVTAYAPQVDEGRYEATDATMTVHQAAMEHLAEESEHLGIHSELPRFFKTLADRAVADGHAENSYAAM IELFRKPTA*

Table S2. Nucleotide sequences

Protein	Sequence

4-OT (192 bp)	ATGCCGATCGCGCAGATCCACATTCTGGAGGGTCGTAGCGACGAGCAAAAGGAAACCCTGATCCGTGAGGTG TCTGAAGCGATTAGCCGTAGCCTGGATGCGCCGCTGACCAGCGTGCGTGTTATCATTACCGAGATGGCGAAG GGTCACTTCGGCATTGGTGGCGAACTGGCGAGCAAAGTTCGTCGTTAA
CHMI (381 bp)	ATGCCCCACTTTATAGTAGAATGTTCAGATAACATTCGCGAAGAGGCAGACCTGCCGGGTCTGTTCGCCAAA GTGAATCCGACTTTGGCGGCTACCGGTATTTTTCCGCTGGCGGGTATTCGTTCTCGTGTTCATTGGGTTGAT ACCTGGCAGATGGCTGACGGCCAACACGATTACGCCTTCGTGCACATGACCCTGAAAATCGGCGCTGGCCGT AGCCTGGAATCCCGTCAGCAGGCAGGTGAAATGTTGTTCGAGCTTATCAAGACCCATTTTGCGGCGCTGATG GAATCGCGCTTGCTGGCGCTGAGCTTTGAGATCGAGGAGCTGCATCCAACGTTGAATTTTAAGCAAAACAAC GTCCACGCGCTCTTCAAGTAA
DDT (357 bp)	ATGCCCTTTTTGGAATTAGATACAAATCTACCGGCTAATCGTGTTCCGGCGGGTTTGGAGAAACGTCTGTGC GCTGCCGCGGCGAGCATCTTAGGTAAGCCGGCGGACCGCGTGAACGTGACCGTTCGCCCAGGTCTGGCTATG GCACTCTCCGGTTCCACCGAACCGTGTGCCCAACTGTCGATCAGCTCTATCGGTGTTGTGGGCACCGCGGAA GACAACAGAAGCCATAGCGCACACTTTTTCGAGTTCTTGACCAAAGAACTGGCGCTGGGCCAGGATCGTATT CTGATTCGTTTTTTCCCGCTGGAGAGCTGGCAGATTGGCAAGATCGGCACTGTCATGACGTTTTTGTAA
MIF (348 bp)	ATGCCCATGTTTATAGTAAACACAAATGTTCCACGTGCGAGCGTCCCGGAAGGTTTCTTGTCCGAGCTGACC CAACAGCTGGCACAGGCTACCGGTAAGCCGGCGCAATATATCGCTGTGCATGTTGTTCCGGACCAGCTGATG ACCTTCAGCGGCACTAATGATCCGTGTGCACTGTGCTCGCTGCACAGCATTGGCAAAATCGGCGGTGCGCAA AACCGTAATTACAGCAAACTGTTATGCGGTCTGCTCTCTGATCGCTTGCACATCTCCCCGGACCGTGTTTAT ATTAACTACTACGACATGAACGCCGCGAACGTGGGTTGGAATGGCAGCACGTTTGCGTAA
*Asp*RedAm (888 bp)	ATGTCCAAGCACATCGGTATCTTCGGTCTGGGTGCAATGGGTACCGCACTGGCTGCGAAATACCTGGAGCAT GGTTACAAAACCTCTGTTTGGAACCGTACTACCGCGAAAGCGATCCCGCTGGTTGAGCAGGGTGCTAAGCTG GCGTCTACCATCAGCGAAGGTGTTAACGCGAACGACCTGATCATTATCTGCCTGCTGAACAACCAGGTTGTT GAAGATGCGCTGCGTGACGCGCTGCAAACCCTGCCGTCTAAAACCATCGTTAACCTGACTAACGGTACTCCG AACCAGGCGCGTAAACTGGCAGACTTCGTTACCTCTCACGGTGCACGTTACATCCACGGTGGTATCATGGCG GTGCCGACCATGATTGGCTCTCCGCACGCAGTGCTGCTGTACTCTGGTGAATCCCTGGAACTGTTTCAGTCT ATTGAATCTCACCTGTCTCTGCTGGGTATGAGCAAGTATCTGGGCACTGACGCGGGCTCTGCGAGCCTGCAT GATCTGGCACTGCTGTCTGGCATGTACGGTCTGTTCTCTGGTTTCCTGCACGCGGTGGCTCTGATTAAATCT GGTCAGGACACCTCCACCACTGCAACTGGTCTGCTGCCGCTGCTGACTCCGTGGCTGAGCGCAATGACCGGT TACCTGAGCTCTATCGCGAAACAGATCGACGACGGTGATTACGCGACCCAGGGTTCTAACCTGGGCATGCAG CTGGCTGGTGTTGAAAACATCATCCGTGCGGGTGAAGAACAGCGTGTTTCTTCTCAGATGATCCTGCCGATC AAAGCACTGATTGAACAGGCGGTTGGTGAAGGTCATGGTGGCGAAGACCTGTCCGCGCTGATCGAATACTTC AAGGTGGGTAAAAACGTTGACTAA
IR-23 (876 bp)	ATGAAACCCGGGATATCAGTACTAGGTACAGGACGCATGGGCTCTGCGTTGGTCGGTGCGTTTCTGAAACAG GGTTATAACGTCGCAGTTTGGAATCGTACCAAGTCCAAGTGCGCTCCGCTGGCAGCGCTGGGCGCGCGTGTT GCGACCACCGTGCGTGATGCAGTAGCGGACGCAGAGGTGGTTGTTGTGAACGTGAACGATTACGTGACGAGC GAGGCTTTGTTGCGCCAAGATGACGTGACGAAGGGTCTGAGAGGCAAACTGATCGTGCAGCTGACCAGCGGC TCCCCGCGTCAAGCTCGTGAAATGGCAGCCTGGGCACGCCAGCATGAACTGCAATACCTGGACGGCGCAATT ATGGGTACACCGAATTTTATTGGTGAGCCGGGTGGTACGATCCTGTACAGCGGTCCGGGTGCCCTCTTCGAG AAGTATAAACCAGTGTTATTGGTTCTTGGCGGAAACAGCCTGCACGTGGGTTCGGATGTGGGCCACGCGTCC GCTCTTGACAGCGCTTTGCTGAGCTTCCTGTGGGGTTCTATGTTCGGCGTTCTGCAGGCGGTAAGCGTTTGT GAAGCGGAGGGCCTGCCGCTGGGCGCGTATATGGAATACGTTCAAGCGACTAAGCCGATGGTTGACGGCGCT GTCACCGATTTCGTGAAACGTATTCAGACCGGTCGTTTTGCGGGTGATGAAAAGACCCTGGCGACCGTTGAG GCGCATCACGGCGCCCTGCGCCATCTGATCGAACTTTGCGAGGAACATGGCATCCACCACGCCGTTCCGGCG GCATTCGGTCAGCTGTTTCAGGCTGCCCTGCAAGCCGGTCACGCGCAAGACGACTTCGCGGTCTTGAATAAA TTCATGAAATAA
IR-361 (894 bp)	ATGTCAGACCCTAATGCTGATAGGCCCCCAGTAACTGTCGTGGGTCTGGGCCTGATGGGCCAGGCACTCGCC GCGGCGTTCCTGAAGGGTGGTCATCCGACGACCGTTTGGAATCGTAGCCCGGAAAAAGCCGAACGTTTGGTG GCGGACGGTGCCGTGTTGGCGGACACCTTGGAGAGTGCTGTGACCGCATCCCCGCTAGTTATTGTTTGCGTT AGCGATTACGACGCTGTACACGAGCTGATTCGTCCGGTTGAATCCGCCTTGGCGGGTCGCGTTCTGGTTAAT CTGACCACCGCGACCAGCACCCAAGCTCGTGAAACGGCGGAGTGGGCGGCGCAGCGTAACATTCCGTATCTG GATGGCGCAATCATGGCCATCCCACCGGTGATCGGCACTGACGGCGCGGTGCTGCTGTACAGCGGTCACAAA AGCGCGTTCGAGGCACATGAGTCGACCCTGAAGGCCATCGCACCGGCAGCGACCACCTACCTGGAAGAGGAT CATGGCCTGTCCTCGCTGTATGATATGGCTCTTCTGGGCATCATGTGGGGTATTCTGAACGGTTTTCTGCAT GGCGCGGCCCTGTTGGGGACGGCTAAAGTCAAAGCGGAGACGTTTGCTCCGCTCGCGAACACCATGATCAGC GCGATTACCGAATACGTGACCGCGTATGCGCCTCAGGTTGATGAAGGTCGCTATGAAGCGACCGATGCCACC ATGACCGTGCACCAAGCTGCTATGGAACATCTGGCTGAGGAATCTGAGCACCTGGGCATCCACAGCGAATTA

	CCGCGTTTTTTCAAGACGTTGGCGGACAGAGCAGTCGCAGACGGTCACGCAGAGAACTCTTACGCAGCTATG ATTGAGCTGTTCCGCAAGCCGACTGCGTAA

Table S3. Primers used for site-directed mutagenesis.

Primer	Nucleotide sequence (5'-3')
F50A-F	ATGGCGAAGGGTCACGYGGGCATTGGTGG
F50A-R	CRCGTGACCCTTCGCCATCTCGGTAATG
F50V-F	ATGGCGAAGGGTCACGYGGGCATTGGTGG
F50V-R	CRCGTGACCCTTCGCCATCTCGGTAATG
F50I-F	ATGGCGAAGGGTCACATTGGCATTGGTGG
F50I-R	AATGTGACCCTTCGCCATCTCGGTAATG
F50L-F	ATGGCGAAGGGTCACCTGGGCATTGGTGG
F50L-R	CAGGTGACCCTTCGCCATCTCGGTAATG
P1A-F	GGAGATATACATATGGCGATCGCGCAGATC
P1A-R	CGCCATATGTATATCTCCTTCTTAAAGTTAAAC

Protein expression and purification

Expression and purification of the wild-type 4-OT and its mutants

The wild-type 4-OT and its mutants were expressed and purified according to a literature method.[1]

Expression and purification of MIF

MIF was expressed and purified according to modified literature protocols.[2] pET29a-MIF was freshly transformed into chemically competent *E.coli* BL21(DE3) cells for protein expression. An individual colony was used directly to prepare a 10 mL starter culture in LB medium supplemented with 50 μg/mL kanamycin. The starter culture was incubated overnight at 37 °C with 220 r.p.m. shaking. The overnight culture was diluted 1:100 into 1 L of LB medium containing kanamycin (50 mg/L). The culture was incubated at 37 °C with 220 r.p.m. shaking. When OD_{600} reached 0.6–0.8, protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. The culture was incubated at 37 °C for 12–15 hours with 230 r.p.m. shaking.

Cells from 1 L culture were harvested by centrifugation (6,000 × g, 20 min, 4 °C) and resuspended in 40 mL lysis buffer (20 mM Tris-HCl, pH 8.0, buffer A). Cells were lysed using a continuous-flow homogenizer (ATS Engineering Inc.) and the lysate was clarified by centrifugation (40,000 × g, 40 min, 4 °C). The supernatant was

applied to 8 mL of Q Beads 6 FF (Smart-Lifesciences) preequilibrated with lysis buffer. The column was then washed with lysis buffer (3 × 8 mL). Protein was eluted from column in elution buffer (lysis buffer containing 0.1 M–0.5 M NaCl) in multiple 2 mL fractions. Fractions containing the desired protein, as determined by Tricine-SDS-PAGE (18% resolving gel and 5% stacking gel), were combined and concentrated using an Amicon Ultra-15 Centrifugal Filter (Merck Millipore) with a 3 kDa molecular weight cut-off membrane. The buffer was exchanged against buffer B (20 mM Tris-HCl, pH 6.5) using a pre-packed PD-10 Sephadex G-25 gel filtration column (GE Healthcare). The protein was further purified using SP Beads 6 FF (Smart-Lifesciences) by gradient elution with buffer B containing 0.1 M–0.5 M NaCl. The fractions containing MIF, as determined by Tricine-SDS-PAGE (18% resolving gel and 5% stacking gel) were pooled and concentrated. The concentrated protein was further purified by size-exclusion chromatography on a Superdex 75 HR 10/300 gel filtration column (GE Healthcare) equilibrated in protein storage buffer (20 mM Tris-HCl, pH 7.4, 20 mM NaCl). The fractions containing pure MIF were pooled and concentrated to 3.7 mg/mL (1 mL). The protein was flash frozen with liquid nitrogen and stored at −80 °C.

Expression and purification of DDT

DDT was expressed and purified according to a modified literature protocol. [3] pET29a-DDT was freshly transformed into chemically competent *E.coli* BL21(DE3) cells for protein expression. An individual colony was used directly to prepare a 10 mL starter culture in LB medium supplemented with 50 μg/mL kanamycin. The starter culture was incubated overnight at 37 °C with 220 r.p.m. shaking. The overnight culture was diluted 1:100 into 1 L of LB medium containing kanamycin (50 mg/L). The culture was incubated at 37 °C with 220 r.p.m. shaking. When OD_{600} reached 0.6–0.8, protein expression was induced by adding IPTG to a final concentration of 0.5 mM. The culture was incubated at 37 °C for 12–15 hours with 230 r.p.m. shaking.

Cells from 1 L culture were harvested by centrifugation (6,000 × g, 20 min, 4 °C) and resuspended in 40 mL lysis buffer (20 mM Tris-HCl, pH 7.0, buffer A). Cells were lysed using the continuous-flow homogenizer and the lysate was clarified by centrifugation (40,000 × g, 40 min, 4 °C). The supernatant was applied to 8 mL of SP Beads 6FF (Smart-Lifesciences) preequilibrated with buffer A. The column was then washed with buffer A (3 × 8 mL). Protein was eluted from column in elution buffer (buffer A containing 0.1 M–0.5 M NaCl) in multiple 2 mL fractions. Fractions containing the desired protein, as determined by Tricine-SDS-PAGE (18% resolving gel and 5% stacking gel), were pooled and made 1.5 M in $(NH_4)_2SO_4$. After stirring for 2 hours at 4 °C, the precipitate was removed by centrifugation (20,000 × g, 20 min, 4 °C), and the supernatant was loaded onto 8 mL of phenyl Beads 6FF (High Sub) (Smart-Lifesciences) preequilibrated with buffer B (20 mM potassium phosphate, pH 7.0) containing 1.5 M $(NH_4)_2SO_4$. The column was first washed with buffer B containing 1.5 M $(NH_4)_2SO_4$ (3 × 8 mL) and then the protein was eluted by gravity flow by using buffer B containing 1.5 M–0.4 M $(NH_4)_2SO_4$ in multiple 2 mL fractions. Fractions containing the desired protein, as determined by Tricine-

SDS-PAGE were combined and concentrated using an Amicon Ultra-15 Centrifugal Filter (Merck Millipore) with a 3 kDa molecular weight cut-off membrane. The concentrated protein was further purified by size-exclusion chromatography on a Superdex 75 HR 10/300 gel filtration column (GE Healthcare) equilibrated in buffer A containing 50 mM NaCl. The fractions containing pure DDT were pooled and concentrated to 8.3 mg/mL (0.5 mL). The protein was flash frozen with liquid nitrogen and stored at −80 °C.

Expression and purification of CHMI

CHMI was expressed and purified according to modified literature protocols [4]. pET29a-CHMI was freshly transformed into chemically competent *E.coli* BL21(DE3) cells for protein expression. An individual colony was used directly to prepare a 10 mL starter culture in LB medium supplemented with 50 µg/mL kanamycin. The starter culture was incubated overnight at 37 °C with 220 r.p.m. shaking. The overnight culture was diluted 1:100 into 1 L of LB medium containing kanamycin (50 mg/L). The culture was incubated at 37 °C with 220 r.p.m. shaking. When OD_{600} reached 0.6–0.8, protein expression was induced by adding IPTG to a final concentration of 1 mM. The culture was incubated at 37 °C for 12–15 hours with 230 r.p.m. shaking.

Cells from 1 L culture were harvested by centrifugation (6,000 × g, 20 min, 4 °C) and resuspended in 40 mL lysis buffer (20 mM Tris-HCl, pH 7.5, buffer A). Cells were lysed using the continuous-flow homogenizer and the lysate was clarified by centrifugation (40,000 × g, 40 min, 4 °C). The supernatant was applied to 8 mL of Q Beads 6 FF (Smart-Lifesciences) preequilibrated with lysis buffer. The column was then washed with buffer A (3 × 8 mL). Protein was eluted from column in elution buffer (lysis buffer containing 0.1 M–0.5 M NaCl) in multiple 2 mL fractions. Fractions containing the desired protein, as determined by Tricine-SDS-PAGE (18% resolving gel and 5% stacking gel), were pooled and made 1.7 M in $(NH_4)_2SO_4$. After stirring for 2 hours at 4 °C, the precipitate was removed by centrifugation (20,000 × g, 20 min, 4 °C) and the supernatant was loaded onto 8 mL of phenyl Beads 6FF (High Sub) (Smart-Lifesciences) that had been previously equilibrated with buffer B (100 mM sodium phosphate, pH 7.5) containing 1.7 M $(NH_4)_2SO_4$. The column was first washed with buffer B containing 1.7 M $(NH_4)_2SO_4$ (3 × 8 mL) and then the protein was eluted by gravity flow by using buffer B containing 1.7 M–0.1 M $(NH_4)_2SO_4$ in multiple 2 mL fractions. Fractions containing the desired protein, as determined by Tricine-SDS-PAGE, were combined and concentrated using an Amicon Ultra-15 Centrifugal Filter (Merck Millipore) with a 3 kDa molecular weight cut-off membrane. The concentrated protein was further purified by size-exclusion chromatography on a Superdex 75 HR 10/300 gel filtration column (GE Healthcare) equilibrated in protein storage buffer (50 mM sodium phosphate, pH 7.5, 150 mM NaCl). The fractions containing pure CHMI were pooled and concentrated to 16.4 mg/mL (0.5 mL). The protein was flash frozen with liquid nitrogen and stored at −80 °C.

Expression and purification of *Asp*RedAm

*Asp*RedAm was expressed and purified based on a literature protocol.[5] pET28a-AspRedAm plasmid was transformed into chemically competent *E.coli* BL21(DE3) cells for protein expression. An individual colony was used directly to prepare a 5 mL starter culture in LB medium supplemented with 50 μg/mL kanamycin. The starter culture was incubated overnight at 37 °C with 220 r.p.m. shaking. The overnight culture was diluted 1:100 into 500 mL 2× YT broth medium with kanamycin (50 mg/L). The culture was incubated at 37 °C with 220 r.p.m. shaking. When OD_{600} reached 0.6–0.8, protein expression was induced by adding IPTG to a final concentration of 0.5 mM. The culture was incubated at 20 °C for 15 hours with 230 r.p.m. shaking.

Cells from 0.5 L culture were harvested by centrifugation (6,000 × g, 20 min, 4 °C) and resuspended in lysis buffer (100 mM Tris-HCl, pH 8.0, 300 mM NaCl, buffer A). Cells were lysed using the continuous-flow homogenizer and the lysate was clarified by centrifugation (40,000 × g, 40 min, 4 °C). The supernatant was applied to 4 mL of Ni-NTA beads (Smart-Lifesciences) preequilibrated with lysis buffer. The column was washed with lysis buffer (3 × 4 mL). The samples were gradient washed with elution buffer (buffer A containing 30 mM–300 mM imidazole). Fractions were collected and analyzed by SDS-PAGE (10 % polyacrylamide gel). Those containing purified *Asp*RedAm were combined and concentrated using an Amicon Ultra-15 Centrifugal Filter with a 10 kDa molecular weight cut-off membrane. The proteins were further purified by size-exclusion chromatography on Superdex 75 HR 10/300 gel filtration column with protein storage buffer (100 mM sodium phosphate, pH 7.5, 500 mM NaCl) as the eluent. The fractions containing pure *Asp*RedAm were pooled and concentrated to 32.4 mg/mL (2 mL). Then the proteins were flash frozen in liquid nitrogen and stored at −80 °C.

Expression and purification of IREDs (IR23, IR361)

IREDs were expressed and purified according to a literature protocol.[6] The IRED-containing pET-28a(+) plasmid was freshly transformed into chemically competent *E.coli* BL21(DE3) cells for protein expression. An individual colony was used directly to prepare a 10 mL starter culture in LB medium supplemented with 50 μg/mL kanamycin. The starter culture was incubated overnight at 37 °C with 220 r.p.m. shaking. The overnight culture was diluted 1:100 into 1 L of terrific broth medium containing kanamycin (50 mg/L). The culture was incubated at 37 °C with 220 r.p.m. shaking. When OD_{600} reached 0.6, protein expression was induced by adding IPTG to a final concentration of 0.5 mM. The culture was incubated at 23 °C for 15 hours with 230 r.p.m. shaking.

Cells from 1 L culture were harvested by centrifugation (6,000 × g, 20 min, 4 °C) and resuspended in lysis buffer (100 mM sodium phosphate, pH 7.0, 300 mM NaCl) containing 30 mM imidazole. Cells were lysed using the continuous-flow homogenizer and the lysate was clarified by centrifugation (40,000 × g, 40 min, 4 °C). The supernatant was applied to 10 mL of Ni NTA Beads (Smart-Lifesciences) preequilibrated with lysis buffer. The column was then washed with lysis buffer (3 × 10 mL). Protein was eluted from column in elution buffer (lysis buffer containing 30 mM–300 mM imidazole) in multiple 2 mL fractions. Fractions were analyzed by SDS-PAGE (10 % polyacrylamide gel). Those containing purified IRED proteins were combined and dialyzed at 4 °C

against 2 L of dialysis buffer (100 mM sodium phosphate, pH 7.0). Following dialysis purified proteins were concentrated to 75 mg/mL (5 mL) using an Amicon Ultra-15 Centrifugal Filter with a 10 kDa molecular weight cut-off membrane. The protein was flash frozen in liquid nitrogen and stored at −80 °C.

Reaction condition optimization of 4-OT mutants

Chemically competent *E. coli* BL21 (DE3) with 4-OT mutants-containing plasmids were grown in 1 L of terrific broth medium at 37 °C. The pelleted wet cells were weighed and suspended in 3 mL of lysis buffer (20 mM sodium phosphate, pH 7.3) per 1 g of wet cell mass. Cells were lysed using the continuous-flow homogenizer and the lysate was clarified by centrifugation (40,000 × g, 40 min, 4 °C). All extracts contained about 11.5 mg/mL of total *E. coli* proteins. All of prepared cell extract was flash frozen in liquid nitrogen and stored at −80 °C until use.

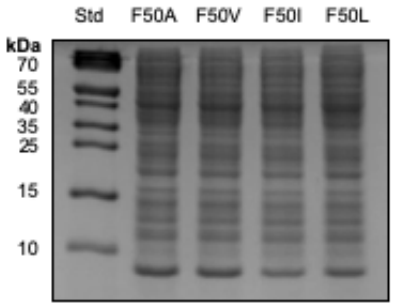


Figure S3. SDS-PAGE of 4-OT mutants

The single incubation mixtures consisting of a total volume of 50 mL contained CFE, 4 mM cinnamaldehyde (**1a**), 50 mM nucleophile, and 5% (v/v) co-solvent. The reaction volume was made up to 50 mL with buffer (20 mM sodium phosphate, pH 7.3). The mixture was incubated at 25 °C and 150 r.p.m for 12 hours, after which the reaction mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated under *vacuo*. The residue was purified by column chromatography (*n*-pentane/ethyl acetate = 15:1, silica gel) to afford the product.

Analytical scale reactions

Activity comparison of wild-type 4-OT and variants 4-OT F50A, 4-OT F50V, 4-OT F50I, 4-OT F50L, and 4-OT P1A with other tautomerases (CHMI, DDT, MIF) for addition of **2a** to **1a** was carried out on analytical scale reactions (0.2 mL reaction volume). Reaction mixtures consisted of 2 mM **1a**, 40 mM **2a**, 14.25% mM purified enzyme in 20 mM sodium phosphate buffer at pH 7.3 (except that 50 mM sodium phosphate buffer containing 150 mM NaCl, pH 7.5 for CHMI; 20 mM Tris-HCl buffer containing 50 mM NaCl, pH 7.0 for DDT; 20 mM Tris-HCl buffer containing 20 mM NaCl, pH 7.4 for MIF), and 5% DMSO (v/v). The mixtures were incubated at room temperature with 500 r.p.m. for 6 hours. The reaction was terminated by extraction with ethyl acetate (3

× 0.2 mL). The combined organic layers were concentrated by nitrogen Termovap Sample Concentrator and the samples were resolved in ethyl acetate (0.4 mL). Then the samples were centrifuged (17,000 × g, 10 min, 25 °C) and subjected to GC–MS analysis. The conversion was determined by GC–MS analysis based on consumption of **1a**. Measurements were performed in triplicates from the same protein batch.

For the reductive amination reaction catalyzed by *Asp*RedAm, a typical 200 µL reaction mixture contained 25 mM D-glucose, 3.5 U GDH (Beijing Aibixin Biotechnology Co., Ltd.), 1 mM $NADP^+$, 0.8 mg/mL *Asp*RedAm, 4 mM **3a**, 40 mM methylamine (in buffer adjusted to pH 9.0) and 5% (v/v) dimethyl sulfoxide. The reaction volume was made up to 200 µL with Tris-HCl buffer (100 mM, pH 9.0). Reactions were incubated at 29 °C with shaking at 450 r.p.m. for 18 hours, after which they were extracted three times with 200 µL ethyl acetate. The organic fractions were combined, centrifuged (17,000 × g, 10 min, 25 °C), and analyzed by GC–MS.

For the reductive amination reaction catalyzed by IRED, a typical 500 µL reaction mixture contained 50 mM D-glucose, 2.5 U GDH (Beijing aibixin Biotechnology Co., Ltd.), 0.5 mM $NADP^+$, 0.8 mg/mL IRED, 10 mM **3a**, 100 mM methylamine (in buffer adjusted to pH 8.0) and 5% (v/v) dimethyl sulfoxide. The reaction volume was made up to 500 µL with buffer (100 mM sodium phosphate, pH 8.0). Reactions were incubated at 30 °C with shaking at 120 r.p.m. for 18 hours, after which they were extracted three times with 500 µL ethyl acetate. The organic fractions were combined, centrifuged (17,000 × g, 10 min, 25 °C), and analyzed by GC–MS.

Biocatalytic synthesis of compounds 3a-p (preparative scale)

To test the synthetic applicability of 4-OT F50V, a series of preparative-scale reactions were performed. Preparative-scale reactions (50 mL) were run using **2a** (42 mM, except for **1d** and **1i** for which 28 mM **2a** was used), **1a-p** (6 mM, except for **1d** and **1i** which was used at 4 mM) and 4-OT F50V enzyme (57 mg, except for **1o**, **1d** and **1i** for which 89 mg enzyme was used and **1c** for which 64 mg enzyme was used) in buffer (20 mM sodium phosphate, pH 6.6) containing 5% (v/v) DMSO (except for **1d** and **1i** for which 10% (v/v) ethanol was used). Reactions were incubated at 25 °C with 150 r.p.m. shaking and monitored by GC–MS. The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated under *vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate from 20:1 to 5:1, silica gel) to afford **3a-p**.

GC–MS analysis

GC–MS was performed on a Shimadzu Nexis GC-2030 series GC system equipped with an SH-Rxi-5Sil column (30 m × 0.25 mm × 0.25 mm; Shimadzu) and coupled with a QP2020 NX mass spectrometer. The carrier gas

was helium at a constant linear velocity of 44.5 cm/sec. Injection was in split-less mode with the injector temperature set at 250 °C and the oven temperature was programmed from 80 °C to 300 °C at 10 °C/min and keep 2 min at 80 °C and 5 min at 300 °C. The identity of the product was confirmed based on the concordance of mass spectrum with standard samples from chemical synthesis. The quantification of products was confirmed by the external standard method.

(*S*)-Diethyl 2-(2-formyl-1-phenylethyl)malonate (3a) Yellow oil (87% isolated yield). **$[\alpha]_D^{25.0}$** = +22.10 (*c* 1.0, CH_2Cl_2). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.55 (d, *J* = 0.7 Hz, 1H), 7.28–7.12 (m, 5H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.02 – 3.84 (m, 3H), 3.67 (d, *J* = 10.0 Hz, 1H), 2.95 – 2.76 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H), 0.95 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 200.2, 168.1, 167.5, 139.9, 128.8, 128.2, 127.6, 61.9, 61.5, 57.6, 47.5, 39.6, 14.1, 13.8. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{20}O_5$+Na^+: 315.1208 [*M*+Na]$^+$; found: 315.1201.

(*S*)-Diethyl 2-(1-(4-fluorophenyl)-2-formylethyl)malonate (3b) Colorless oil (69% isolated yield). **$[\alpha]_D^{21.7}$** = +16.60 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.59 (dd, *J* = 2.1, 1.2 Hz, 1H), 7.22 (dd, *J* = 8.6, 5.4 Hz, 2H), 6.97 (t, *J* = 8.7 Hz, 2H), 4.23–4.15 (m, 2H), 4.05–3.91 (m, 3H), 3.66 (d, *J* = 10.0 Hz, 1H), 2.98 – 2.79 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.02 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 199.8, 168.0, 167.5, 163.3, 160.8, 135.8, 135.7, 130.0, 129.9, 115.8, 115.6, 62.0, 61.6, 57.6, 47.7, 38.8, 14.1, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{19}FO_5$+Na^+: 333.1114 [*M*+Na]$^+$; found: 333.1109.

(*S*)-Diethyl 2-(1-(4-chlorophenyl)-2-formylethyl)malonate (3c) Colorless oil (84% isolated yield). **$[\alpha]_D^{25.2}$** = +16.90 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.59 (t, *J* = 1.5 Hz, 1H), 7.31–7.08 (m, 4H), 4.28–4.11 (m, 2H), 4.07–3.87 (m, 3H), 3.66 (d, *J* = 9.9 Hz, 1H), 3.04–2.73 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 199.6, 167.9, 167.4, 138.6, 133.4, 129.7, 128.9, 62.0, 61.7, 57.3, 47.5, 38.8, 14.1, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{19}ClO_5$+Na^+: 349.0819 [*M*+Na]$^+$; found: 349.0814.

(*S*)-Diethyl 2-(1-(4-bromophenyl)-2-formylethyl)malonate (3d) Colorless oil (34% isolated yield). **$[\alpha]_D^{23.0}$** = +11.50 (*c* 0.2, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.60 (dd, *J* = 1.9, 1.2 Hz, 1H), 7.49–7.34 (m, 2H), 7.19–7.05 (m, 2H), 4.20 (qd, *J* = 7.1, 1.2 Hz, 2H), 3.98 (q, *J* = 7.1 Hz, 3H), 3.67 (d, *J* = 9.8 Hz, 1H), 3.05–2.77 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.05 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 199.6, 167.9, 167.4, 139.2, 131.9, 130.1, 121.5, 62.0, 61.8, 57.3, 47.5, 38.9, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{19}BrO_5$+Na^+: 393.0314 [*M*+Na]$^+$; found: 393.0307.

(*S*)-Diethyl 2-(1-(4-methylphenyl)-2-formylethyl)malonate (3e) Colorless oil (47% isolated yield). **$[\alpha]_D^{21.4}$** = +14.90 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.58 (t, *J* = 1.8 Hz, 1H), 7.10 (q, *J* = 8.2 Hz, 4H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.96 (q, *J* = 7.0 Hz, 3H), 3.68 (d, *J* = 10.0 Hz, 1H), 2.99–2.79 (m, 2H), 2.28 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.02 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 200.4, 168.2, 167.6, 137.2, 136.8, 129.5, 128.1, 61.9, 61.6, 57.8, 47.6, 39.3, 21.2, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{22}O_5$+Na^+: 329.1365 [*M*+Na]$^+$; found: 329.1360.

(*S*)-Diethyl 2-(1-(4-methoxyphenyl)-2-formylethyl)malonate (3f) Yellow oil (75% isolated yield). $[\alpha]_D^{21.9}$ = +15.90 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.58 (dd, *J* = 2.3, 1.4 Hz, 1H), 7.16 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 2H), 4.25–4.13 (m, 2H), 3.96 (q, *J* = 7.0 Hz, 3H), 3.76 (s, 3H), 3.66 (d, *J* = 10.0 Hz, 1H), 2.98–2.75 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 200.4, 168.2, 167.6, 158.9, 131.8, 129.3, 114.2, 61.9, 61.6, 57.9, 55.3, 47.7, 39.0, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{22}O_6$+Na^+: 345.1314 [*M*+Na]$^+$; found: 345.1309.

(*S*)-Diethyl 2-(1-(4-hydroxyphenyl)-2-formylethyl)malonate (3g) Yellow oil (65% isolated yield). $[\alpha]_D^{22.2}$ = +9.00 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.57 (dd, *J* = 2.5, 1.3 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 2H), 6.66 (d, *J* = 8.6 Hz, 2H), 5.87 (s, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.03–3.84 (m, 3H), 3.66 (d, *J* = 10.1 Hz, 1H), 2.98–2.75 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 201.0, 168.2, 167.9, 155.3, 131.3, 129.4, 115.7, 62.0, 61.8, 57.9, 47.7, 39.0, 14.1, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{20}O_6$+Na^+: 331.1158 [*M*+Na]$^+$; found: 331.1153.

(*S*)-Diethyl 2-(1-(4-cyanophenyl)-2-formylethyl)malonate (3h) Yellow oil (17% isolated yield). $[\alpha]_D^{21.1}$ = +8.00 (*c* 1.0, MeOH). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.61 (s, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.07 (td, *J* = 9.4, 4.6 Hz, 1H), 4.03–3.93 (m, 2H), 3.71 (d, *J* = 9.8 Hz, 1H), 3.08–2.89 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 198.8, 167.7, 167.2, 145.9, 132.5, 129.3, 118.6, 111.6, 62.2, 61.9, 56.8, 47.3, 39.2, 14.1, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{19}NO_5$+Na^+: 340.1161 [*M*+Na]$^+$; found: 340.1157.

(*S*)-Diethyl 2-(2-formyl-1-(2-methoxyphenyl)ethyl)malonate (3i) Yellow oil (73% isolated yield). $[\alpha]_D^{22.8}$ = +9.60 (*c* 0.5, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.60 (dd, *J* = 2.6, 1.6 Hz, 1H), 7.23–7.11 (m, 2H), 6.90–6.73 (m, 2H), 4.19 (dt, *J* = 7.2, 6.5 Hz, 3H), 4.06 (d, *J* = 10.2 Hz, 1H), 3.93 (q, *J* = 7.1 Hz, 2H), 3.85 (s, 3H), 3.07–2.80 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.99 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 201.3, 168.6, 168.0, 157.5, 130.3, 128.9, 127.4, 120.8, 111.1, 61.8, 61.4, 55.4, 55.2, 46.0, 36.5, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{22}O_6$+Na^+: 345.1314 [*M*+Na]$^+$; found: 345.1309.

(*S*)-Diethyl 2-(2-formyl-1-(2-chlorophenyl)ethyl)malonate (3j) Colorless oil (79% isolated yield). $[\alpha]_D^{22.6}$ = +7.00 (*c* 0.5, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.63 (t, *J* = 1.8 Hz, 1H), 7.45–7.04 (m, 4H), 4.66–4.38 (m, 1H), 4.33–4.14 (m, 2H), 4.14–3.88 (m, 3H), 3.12–2.90 (m, 2H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.07 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 200.0, 168.0, 167.5, 137.4, 134.1, 130.3, 129.4, 128.8, 127.2, 61.9, 61.8, 55.5, 46.3, 36.1, 14.1, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{19}ClO_5$+Na^+: 349.0819 [*M*+Na]$^+$; found: 349.0813.

(*S*)-Diethyl 2-(1-(3-chlorophenyl)-2-formylethyl)malonate (3k) Colorless oil (84% isolated yield). $[\alpha]_D^{22.4}$ = +14.80 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.60 (t, *J* = 1.5 Hz, 1H), 7.29–7.07 (m, 4H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.98 (q, *J* = 7.2 Hz, 3H), 3.67 (d, *J* = 9.8 Hz, 1H), 3.00–2.79 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 199.5, 167.9, 167.4, 142.2, 134.5, 130.1, 128.4, 127.8, 126.6,

62.0, 61.7, 57.2, 47.3, 39.1, 14.1, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{19}ClO_5$+Na^+: 349.0819 [*M*+Na]$^+$; found: 349.0813.

(*S*)-Diethyl 2-(1-(3-bromophenyl)-2-formylethyl)malonate (3l) Yellow oil (54% isolated yield). **$[\alpha]_D^{25.4}$** = +16.10 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.61 (t, *J* = 1.5 Hz, 1H), 7.44–7.31 (m, 2H), 7.23–7.12 (m, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.99 (q, *J* = 7.1 Hz, 3H), 3.68 (d, *J* = 9.8 Hz, 1H), 3.01–2.83 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.05 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 199.5, 167.9, 167.4, 142.5, 131.4, 130.8, 130.4, 127.1, 122.8, 62.1, 61.8, 57.3, 47.4, 39.1, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{19}BrO_5$+Na^+: 393.0314 [*M*+Na]$^+$; found: 393.0309.

(*S*)-Diethyl 2-(1-(3-methylphenyl)-2-formylethyl)malonate (3m) Yellow oil (66% isolated yield). **$[\alpha]_D^{21.5}$** = +14.20 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.58 (t, *J* = 1.9 Hz, 1H), 7.23–7.09 (m, 1H), 7.02 (d, *J* = 10.0 Hz, 3H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.95 (q, *J* = 7.0 Hz, 3H), 3.69 (d, *J* = 10.0 Hz, 1H), 3.00–2.74 (m, 2H), 2.30 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.01 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 200.4, 168.2, 167.6, 139.8, 138.4, 129.0, 128.7, 128.4, 125.2, 61.9, 61.5, 57.7, 47.5, 39.6, 21.5, 14.1, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{22}O_5$+Na^+: 329.1365 [*M*+Na]$^+$; found: 329.1360.

(*S*)-Diethyl 2-(1-(3-cyanophenyl)-2-formylethyl)malonate (3n) Yellow oil (29% isolated yield). **$[\alpha]_D^{25.4}$** = +14.80 (*c* 0.5, EtOAc). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 9.62 (s, 1H), 7.59–7.49 (m, 3H), 7.41 (t, *J* = 7.7 Hz, 1H), 4.21 (qd, *J* = 7.1, 1.1 Hz, 2H), 4.05 (td, *J* = 9.5, 4.5 Hz, 1H), 3.98 (qd, *J* = 7.1, 1.7 Hz, 2H), 3.70 (d, *J* = 9.7 Hz, 1H), 3.07–2.91 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.05 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 198.9, 167.7, 167.2, 142.0, 133.2, 132.0, 131.3, 129.6, 118.6, 112.9, 62.2, 61.9, 56.9, 47.3, 38.8, 14.14, 14.0. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{19}NO_5$+Na^+: 340.1161 [*M*+Na]$^+$; found: 340.1156.

(*S*)-Diethyl 2-(1-(3-methoxy-4-hydroxyphenyl)-2-formylethyl)malonate (3o) Yellow oil (53% isolated yield). **$[\alpha]_D^{25.3}$** = +10.80 (*c* 1.0, EtOAc). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 9.64–9.53 (m, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.76–6.70 (m, 2H), 5.57 (s, 1H), 4.23–4.15 (m, 2H), 4.00–3.91 (m, 3H), 3.86 (s, 3H), 3.67 (d, *J* = 10.0 Hz, 1H), 2.92–2.79 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 200.5, 168.2, 167.6, 146.6, 145.0, 131.7, 120.8, 114.6, 111.0, 61.9, 61.6, 57.9, 56.1, 47.7, 39.4, 14.2, 14.0. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{22}O_7$+Na^+: 361.1263 [*M*+Na]$^+$; found: 361.1257.

(*S*)-Diethyl 2-(1-formylpentan-2-yl)malonate (3p) Colorless oil (26% isolated yield). **$[\alpha]_D^{20.0}$** = +7.40 (*c* 1.0, EtOAc). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.74 (t, *J* = 1.5 Hz, 1H), 4.18 (q, *J* = 6.9 Hz, 4H), 3.50 (d, *J* = 6.0 Hz, 1H), 2.81–2.65 (m, 2H), 2.58–2.42 (m, 1H), 1.39–1.22 (m, 10H), 0.89 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 201.4, 168.9, 168.6, 61.6, 61.5, 54.6, 46.0, 34.8, 32.4, 20.2, 14.2, 14.1. **HRMS** (ESI): *m/z* calcd for $C_{13}H_{22}O_5$+Na^+: 281.1365 [*M*+Na]$^+$; found: 281.1360.

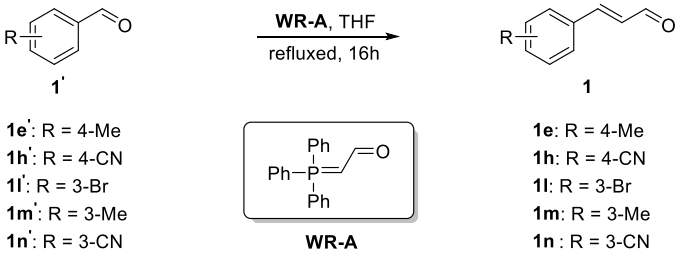
III. Chemical synthesis and analysis

General information

Chemicals were purchased from Bidepharm, Sigma Aldrich, Macklin, Merck or Fluka (unless noted otherwise) and were used without further purification. The *α,β*-unsaturated aldehydes **1e**, **1h**, **1l-n** were prepared using previously reported methods using the Wittig reaction.[7] The racemic reference compounds, required for chiral analysis of enzymatic products, was synthesized according to the published protocols.[8] Lithium 4-fluorobenzonate was prepared by the reaction of lithium methoxide and corresponding 4-fluorobenzoic acid.

NMR spectra were recorded on a Brucker 400 MHz spectrometer. Chiral HPLC analysis was performed on a Shimadzu LC-20AD instrument using Daicel Chiralcel columns at 35 °C and a mixture of HPLC-grade hexanes and isopropanol as eluent. For reactions that require heating, oil bath was used in all procedures. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Tsingdao silica gel plates (GF-254) and visualized under UV light at 254 nm. Staining was performed with an ethanolic solution of phosphomolybdic acid (PMA) and cerium sulfate, or by oxidative staining with an aqueous basic potassium permanganate ($KMnO_4$) solution and subsequent heating. Tsingdao silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. NMR spectra were recorded on either a Brüker Advance 400 (^{1}H: 400 MHz, ^{13}C: 101 MHz) or Brüker Advance 500 (^{1}H: 500 MHz, ^{13}C: 126 MHz) and were calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: ^{1}H NMR = 7.26 ppm, ^{13}C NMR = 77.16 ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were measured on ABI Q-star Elite. The ionization method is ESI and the mass analyzer type of TOF. Optical rotation values were recorded on a Rudolph Research Analytical Autopol I polarimeter (Rudolph Research Co.).

Chemical synthesis of α,β-unsaturated aldehydes



Compound **1e'/1h'/1l'/1m'/1n'** (12.5 mmol, 1.0 equiv) and **WR-A** (18.7 mmol, 1.5 equiv) were dissolved in THF (30 mL) under argon. The reaction mixture was heated at 70 °C for 16 h. After completion of the reaction, the mixture was cooled to room temperature, filtered, and concentrated under *vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate from 30:1 to 15:1) on silica gel to give the desired *α,β*-unsaturated aldehydes in 30%–71% yield.

(*E*)-3-(4-methylphenyl)acrylaldehyde (1e) Yellow oil. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 9.68 (d, *J* = 7.7 Hz, 1H), 7.49–7.41 (m, 3H), 7.23 (d, *J* = 7.9 Hz, 2H), 6.68 (dd, *J* = 15.9, 7.7 Hz, 1H), 2.39 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 193.9, 153.1, 142.1, 131.4, 130.0, 128.6, 127.8, 21.7. **HRMS** (ESI): *m/z* calcd for $C_{10}H_{10}O$+Na^+: 169.0629 [*M*+Na]$^+$; found: 169.0625.

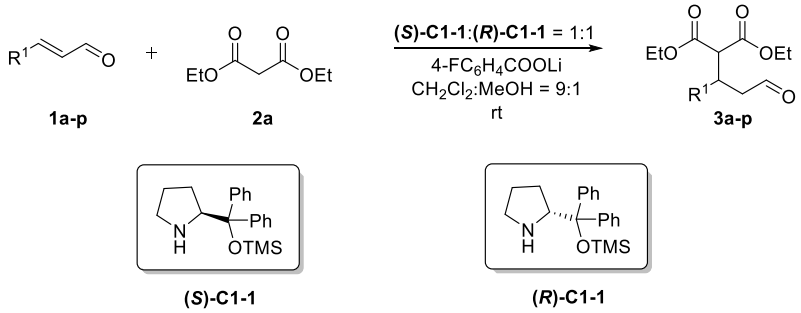
(*E*)-3-(4-cyanophenyl)acrylaldehyde (1h) Yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.76 (d, *J* = 7.5 Hz, 1H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 16.1 Hz, 1H), 6.77 (dd, *J* = 16.1, 7.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 193.0, 149.6, 138.3, 133.0, 131.3, 128.9, 118.3, 114.4. **HRMS** (ESI): *m/z* calcd for $C_{10}H_8NO^+$: 158.0606 [*M*+H]$^+$; found: 158.0603.

(*E*)-3-(3-bromophenyl)acrylaldehyde (1l) Yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.70 (d, *J* = 7.6 Hz, 1H), 7.69 (t, *J* = 1.8 Hz, 1H), 7.58–7.43 (m, 2H), 7.39 (d, *J* = 16.0 Hz, 1H), 7.30 (t, *J* = 7.8 Hz, 1H), 6.68 (dd, *J* = 16.0, 7.6 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 193.3, 150.7, 136.1, 134.0, 131.3, 130.7, 129.7, 127.0, 123.3. **HRMS** (ESI): *m/z* calcd for $C_9H_8BrO^+$: 210.9759 [*M*+H]$^+$; found: 210.9755.

(*E*)-3-(3-methylphenyl)acrylaldehyde (1m) Yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.70 (d, *J* = 7.7 Hz, 1H), 7.49–7.29 (m, 4H), 7.25 (s, 1H), 6.71 (dd, *J* = 15.9, 7.7 Hz, 1H), 2.40 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 193.9, 153.2, 139.0, 134.1, 132.3, 129.2, 129.1, 128.6, 125.9, 21.4. **HRMS** (ESI): *m/z* calcd for $C_{10}H_{11}O^+$: 147.0810 [*M*+H]$^+$; found: 147.0805.

(*E*)-3-(3-cyanophenyl)acrylaldehyde (1n) White solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.75 (d, *J* = 7.4 Hz, 1H), 7.89–7.76 (m, 2H), 7.76–7.69 (m, 1H), 7.58 (t, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 16.1 Hz, 1H), 6.75 (dd, *J* = 16.1, 7.5 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 193.0, 149.3, 135.4, 134.2, 132.1, 131.9, 130.7, 130.2, 118.0, 113.8. **HRMS** (ESI): *m/z* calcd for $C_{10}H_8NO^+$: 158.0606 [*M*+H]$^+$; found: 158.0602.

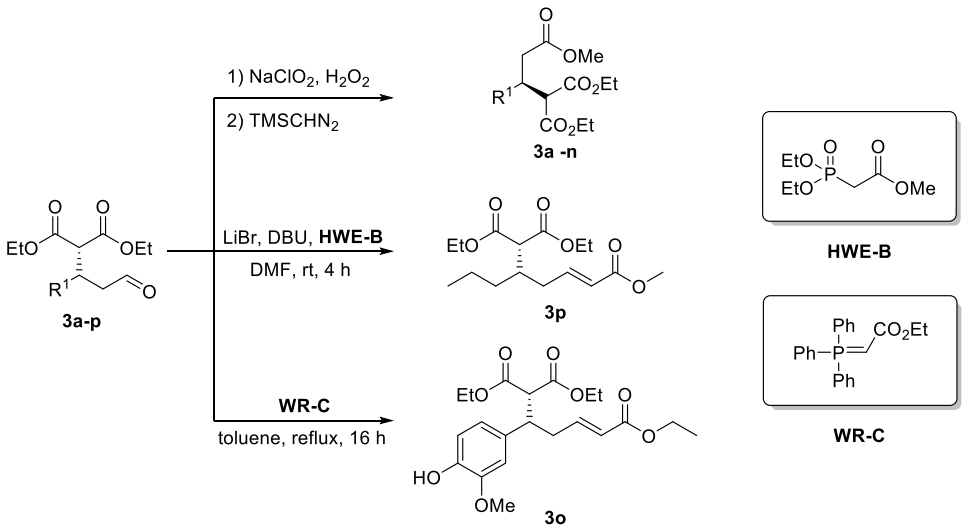
General Procedure for the Michael Addition



To a mixture of dichloromethane and methanol (2.0 mL, 9:1, v:v) was added *α,β*-unsaturated aldehyde **1a-p** (1.0 mmol), diethyl malonate **2a** (3.0 mmol), catalyst **(*R*)-C1-1** (2 mg, 0.005 mmol), **(*S*)-C1-1** (2 mg, 0.005 mmol) and lithium 4-fluorobenzonate (7.3 mg, 0.05 mmol). The reaction mixture was stirred at room temperature for

48–60 h until the α,β-unsaturated aldehyde was almost consumed. Water (5.0 mL) was added to the reaction mixture. Then the mixture was extracted with dichloromethane (3.0 mL) three times. The organic phases were combined, dried over anhydrous sodium sulfate, filtered, and evaporated under *vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate, 20:1 to 5:1) on silica gel to give the corresponding racemic products **3a-p**.

Oxidation of the aldehydes to the carboxylic esters



Method A: [9]

To a solution of **3a-n** (0.17 mmol, 1.0 equiv) in methanol/acetonitrile/water (1.0 mL/1.0 mL/1.0 mL) at 0 °C was added KH_2PO_4 (63 mg, 0.46 mmol, 2.7 equiv) and $NaClO_2$ (80%, 56 mg, 0.43 mmol, 2.5 equiv). After aqueous H_2O_2 (30%, 0.6 mL) was added, the mixture was warmed up to room temperature and stirred for 2 h. The pH was adjusted to 3.0 with 1 M HCl before saturated aqueous Na_2SO_3 (5 mL) was added slowly. The resulting mixture was extracted 3 times with 10 mL dichloromethane, the organic layers were combined, washed with 10 mL of water, dried over sodium sulfate, and concentrated under *vacuo*. The crude product was dissolved in toluene (2.0 mL) and MeOH (5.0 mL). Trimethylsilyldiazomethane (2.0 M in *n*-hexane) was added dropwise until the yellow color persisted. The solution was stirred for an additional 30 min and quenched with a drop of concentrated acetic acid. The solvent was evaporated under *vacuo*. The crude product was purified by column chromatography on silica gel to get the desired product **3a'-n'**.

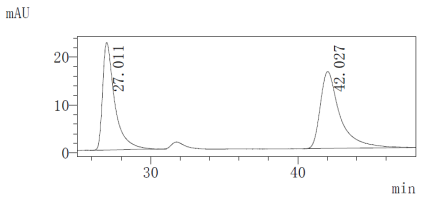
Method B: [8, 10]

To a solution of **3p** (0.1mmol, 1.0 equiv) in DMF (1 mL), lithium bromide (0.15 mmol, 1.5 equiv), **HWE-B** (0.15 mmol, 1.5 equiv), and DBU (0.15 mmol, 1.5 equiv) were added. The reaction mixture was stirred for 4 h at room temperature and passed directly through a silica gel column to give the desired product **3p'**.

Method C: [11]

To a solution of **3o** (5.0 mmol, 1.0 equiv) in toluene (25 mL) was added the Wittig reagent **WR-C** (7.5 mmol, 1.5 equiv) at room temperature. The reaction mixture was refluxed for 16 h. After completion of the reaction, the solvent was evaporated under *vacuo*. The residue was purified by column chromatography to give the desired product **3o'**.

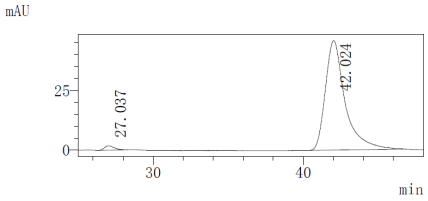
(*S*)-2-Ethyloxycarbonyl-3-phenylpetanedioic acid 5-ethyl ester 1-methyl ester (3a') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.31–7.16 (m, 5H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.93 (q, *J* = 7.2 Hz, 3H), 3.74 (d, *J* = 10.3 Hz, 1H), 3.53 (s, 3H), 2.92–2.64 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.99 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.7, 168.2, 167.6, 140.0, 128.6, 128.2, 127.5, 61.8, 61.5, 57.4, 51.7, 41.6, 38.7, 14.2, 13.8. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{22}O_6$+Na^+: 345.1314 [*M*+Na]$^+$; found: 345.1309. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (95/5), flow rate: 0.5 mL /min, ʎ = 254 nm, (τ_{minor} = 27.037 min, τ_{major}= 42.024 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	27.011	1279940	45.940
2	42.027	1506161	54.060
总计		2786102	100.000

racemic mixture of **3a'**

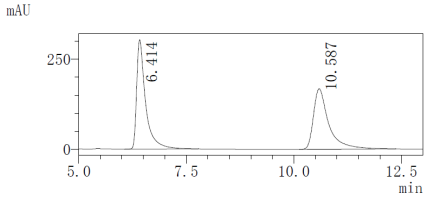


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	27.037	79445	1.860
2	42.024	4192731	98.140
总计		4272176	100.000

3a'

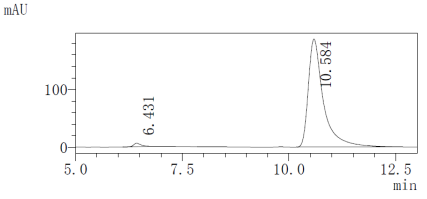
(*S*)-2-Ethyloxycarbonyl -3-(4-fluorophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3b') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.22 (dd, *J* = 8.6, 5.4 Hz, 2H), 6.96 (t, *J* = 8.7 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.00–3.86 (m, 3H), 3.69 (d, *J* = 10.2 Hz, 1H), 3.53 (s, 3H), 2.88–2.66 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.02 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.6, 168.0, 167.5, 163.3, 160.9, 135.7, 135.7, 129.9, 129.8, 115.6, 115.3, 61.9, 61.6, 57.4, 51.8, 40.8, 38.7, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{21}FO_6$+Na^+: 363.1220 [*M*+Na]$^+$; found: 363.1214. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, ʎ = 254 nm, (τ_{minor} = 6.431 min, τ_{major} = 10.584 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	6.414	4373875	51.408
2	10.587	4134228	48.592
总计		8508103	100.000

racemic mixture of **3b'**

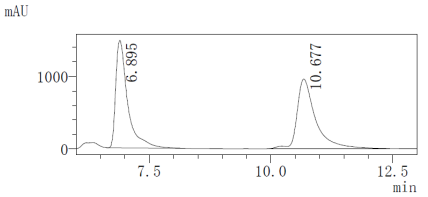


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	6.431	69190	1.479
2	10.584	4608360	98.521
总计		4677550	100.000

3b'

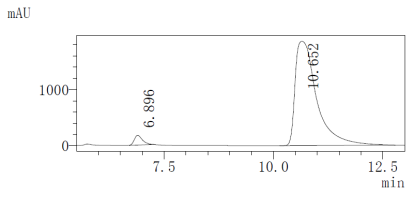
(*S*)-2-Ethyloxycarbonyl -3-(4-chlorophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3c') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.28–7.15 (m, 4H), 4.21 (q, *J* = 7.3 Hz, 2H), 4.01–3.84 (m, 3H), 3.69 (d, *J* = 10.2 Hz, 1H), 3.54 (s, 3H), 2.89–2.66 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.5, 167.9, 167.4, 138.6, 133.3, 129.7, 128.8, 62.0, 61.6, 57.2, 51.8, 40.9, 38.5, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{21}ClO_6$+Na^+: 379.0924 [*M*+Na]$^+$; found: 379.0919. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, ƛ = 211 nm, (τ_{minor} = 6.896 min, τ_{major} = 10.652 min).



PDA Ch1 211nm

Peak#	Ret. Time	Area	Area%
1	6.895	25143018	49.943
2	10.677	25200024	50.057
总计		50343042	100.000

racemic mixture of **3c'**

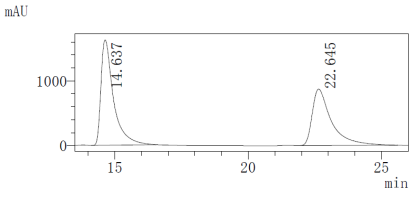


PDA Ch3 211nm

Peak#	Ret. Time	Area	Area%
1	6.896	2254616	3.416
2	10.652	63744597	96.584
总计		65999213	100.000

3c'

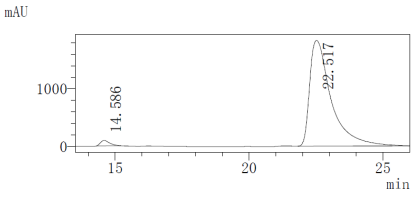
(*S*)-2-Ethyloxycarbonyl -3-(4-bromophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3d') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.40 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H), 4.21 (q, J = 6.9 Hz, 2H), 3.96 (q, J = 7.1 Hz, 2H), 3.89 (td, J = 10.1, 4.5 Hz, 1H), 3.69 (d, J = 10.1 Hz, 1H), 3.54 (s, 3H), 2.88 – 2.67 (m, 2H), 1.26 (d, J = 7.2 Hz, 3H), 1.04 (t, J = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.5, 167.9, 167.4, 139.1, 131.7, 130.1, 121.4, 62.0, 61.7, 57.1, 51.8, 41.0, 38.4, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{21}BrO_6$+Na^+: 423.0419 [*M*+Na]$^+$; found: 423.0414. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, ƛ = 210 nm, (τ_{minor} = 14.586 min, τ_{major} = 22.517 min).



PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	14.637	53176060	55.054
2	22.645	43413444	44.946
总计		96589503	100.000

racemic mixture of **3d'**



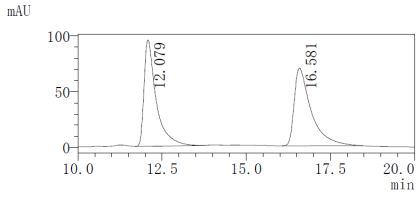
PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	14.586	2212396	2.111
2	22.517	102606789	97.889
总计		104819185	100.000

3d'

(*S*)-2-Ethyloxycarbonyl -3-(4-methylphenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3e') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.16–7.03 (m, 4H), 4.21 (q, *J* = 6.8 Hz, 2H), 3.98–3.83 (m, 3H), 3.71 (d, *J* = 10.2 Hz, 1H), 3.53 (s, 3H), 2.87–2.68 (m, 2H), 2.28 (s, 3H), 1.25 (d, *J* = 7.2 Hz, 3H), 1.01 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.8, 168.2, 167.7, 137.0, 136.9, 129.2, 128.0, 61.8, 61.5, 57.5, 51.71, 41.2, 38.7, 21.2, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{18}H_{24}O_6$+Na^+: 359.1471 [*M*+Na]$^+$; found: 359.1464. **HPLC**:

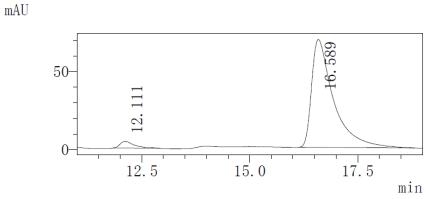
Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, λ = 254 nm, (τ_{minor} = 12.111 min, τ_{major} = 16.589 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	12.079	2377289	49.302
2	16.581	2444651	50.698
总计		4821940	100.000

racemic mixture of **3e'**

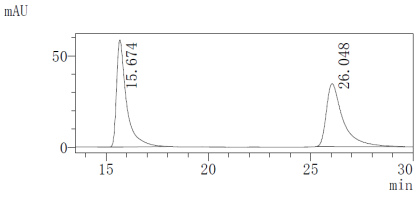


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	12.111	101066	3.965
2	16.589	2447984	96.035
总计		2549050	100.000

3e'

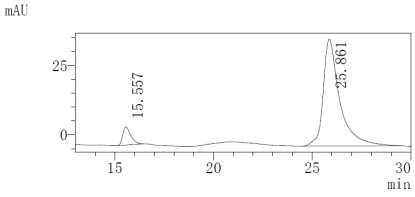
(*S*)-2-Ethyloxycarbonyl -3-(4-methoxyphenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3f') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.15 (d, *J* = 8.7 Hz, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.98–3.82 (m, 3H), 3.76 (s, 3H), 3.69 (d, *J* = 10.2 Hz, 1H), 3.53 (s, 3H), 2.92–2.55 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.02 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.8, 168.2, 167.7, 158.8, 131.9, 129.27, 113.9, 61.8, 61.4, 57.6, 55.3, 51.7, 40.8, 38.8, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{18}H_{24}O_7$+Na^+: 375.1420 [*M*+Na]$^+$; found: 375.1416. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, λ = 254 nm, (τ_{minor} = 15.557 min, τ_{major} = 25.861 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	15.674	1968620	50.531
2	26.048	1927268	49.469
总计		3895888	100.000

racemic mixture of **3f'**

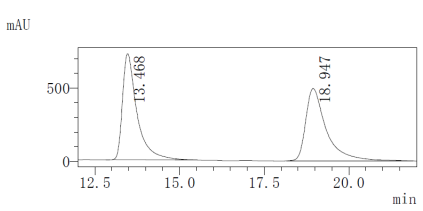


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	15.557	185483	7.596
2	25.861	2256372	92.404
总计		2441855	100.000

3f'

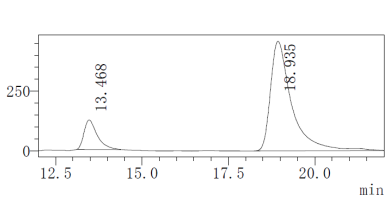
(*S*)-2-Ethyloxycarbonyl -3-(4-hydroxyphenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3g') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.08 (d, *J* = 8.6 Hz, 2H), 6.66 (d, *J* = 8.6 Hz, 2H), 4.25–4.17 (m, 2H), 3.95 (q, *J* = 7.1 Hz, 2H), 3.85 (td, *J* = 10.2, 4.5 Hz, 1H), 3.68 (d, *J* = 10.3 Hz, 1H), 3.54 (s, 3H), 2.87–2.65 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 172.1, 168.2, 167.9, 155.2, 131.5, 129.4, 115.5, 61.9, 61.6, 57. 7, 51.8, 40.9, 39.0, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{22}O_7$+Na^+: 361.1263 [*M*+Na]$^+$; found: 361.1256. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, λ = 210 nm, (τ_{minor} = 13.468 min, τ_{major} = 18.935 min).



PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	13.468	21505365	50.716
2	18.947	20898270	49.284
总计		42403635	100.000

racemic mixture of **3g'**

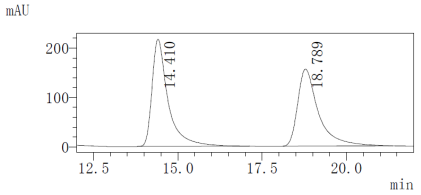


PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	13.468	3259132	14.314
2	18.935	19509476	85.686
总计		22768608	100.000

3g'

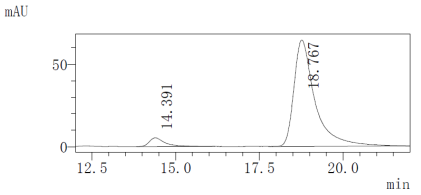
(*S*)-2-Ethyloxycarbonyl -3-(4-cyanophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3h') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 4.21 (q, *J* = 7.0 Hz, 2H), 4.02–3.89 (m, 3H), 3.73 (d, *J* = 10.0 Hz, 1H), 3.54 (s, 3H), 2.94–2.70 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.2, 167.6, 167.2, 145.8, 132.4, 129.3, 118.7, 111.5, 62.1, 61.8, 56.7, 51.9, 41.4, 38.0, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{18}H_{21}NO_6$+Na^+: 370.1267 [*M*+Na]$^+$; found: 370.1262. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, ƛ = 254 nm, (τ_{minor} = 14.391 min, τ_{major} = 18.767 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	14.410	7199938	51.830
2	18.789	6691609	48.170
总计		13891547	100.000

racemic mixture of **3h'**

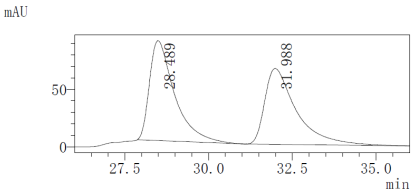


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	14.391	170707	5.703
2	18.767	2822786	94.297
总计		2993494	100.000

3h'

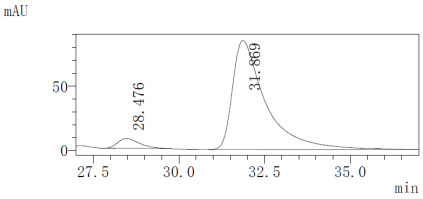
(*S*)-2-Ethyloxycarbonyl -3-(2-methoxyphenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3i') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.21–7.11 (m, 2H), 6.83 (t, *J* = 7.0 Hz, 2H), 4.23–4.15 (m, 2H), 4.12 (d, *J* = 10.3 Hz, 1H), 4.09–3.98 (m, 1H), 3.90 (q, *J* = 7.1 Hz, 2H), 3.84 (s, 3H), 3.52 (s, 3H), 3.04–2.74 (m, 2H), 1.24 (t, *J* = 7.2 Hz, 3H), 0.97 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 172.3, 168.6, 168.1, 157.7, 130.8, 128.6, 127.4, 120.5, 111.0, 61.6, 61.2, 55.4, 54.9, 51.6, 38.9, 36.4, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{18}H_{24}O_7$+Na^+: 375.1420 [*M*+Na]$^+$; found: 375.1414. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (95/5), flow rate: 0.5 mL /min, ƛ = 254 nm, (τ_{minor} = 28.476 min, τ_{major} = 31.869 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	28.489	4526264	51.107
2	31.988	4330222	48.893
总计		8856485	100.000

racemic mixture of **3i'**

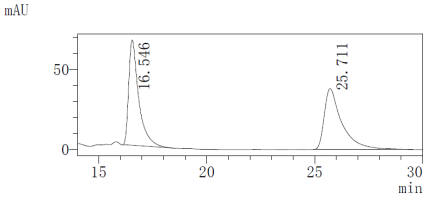


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	28.476	340623	5.643
2	31.869	5695979	94.357
总计		6036602	100.000

3i'

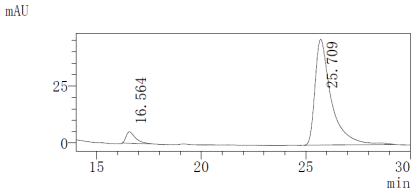
(*S*)-2-Ethyloxycarbonyl -3-(2-chlorophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3j') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.39–7.08 (m, 4H), 4.48–4.35 (m, 1H), 4.18 (qd, *J* = 7.1, 2.5 Hz, 2H), 4.01 (q, *J* = 7.2 Hz, 3H), 3.54 (s, 3H), 3.03–2.83 (m, 2H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.06 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.7, 168.0, 167.6, 137.5, 134.4, 130.2, 129.2, 128.6, 127.0, 61.8, 61.7, 55.3, 51.8, 37.9, 36.6, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{21}ClO_6$+Na^+: 379.0924 [*M*+Na]$^+$; found: 379.0919. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, ʎ = 254 nm, (τ_{minor} = 16.564 min, τ_{major} = 25.709 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	16.546	2112142	49.394
2	25.711	2163931	50.606
总计		4276073	100.000

racemic mixture of **3j'**

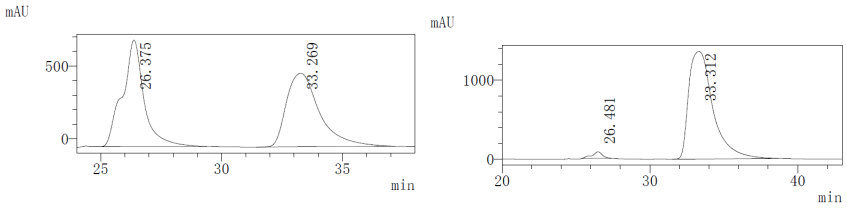


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	16.564	154394	5.797
2	25.709	2509137	94.203
总计		2663530	100.000

3j'

(*S*)-2-Ethyloxycarbonyl -3-(3-chlorophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3k') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.29–7.08 (m, 4H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.97 (q, *J* = 7.1 Hz, 2H), 3.89 (td, *J* = 10.0, 4.5 Hz, 1H), 3.70 (d, *J* = 10.0 Hz, 1H), 3.55 (s, 3H), 2.94–2.64 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.4, 167.9, 167.4, 142.2, 134.3, 129.8, 128.5, 127.7, 126.6, 62.0, 61.7, 57.1, 51.8, 41.2, 38.3, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{21}ClO_6$+Na^+: 379.0924 [*M*+Na]$^+$, found: 379.0919. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (95/5), flow rate: 0.5 mL /min, ʎ = 210 nm, (τ_{minor} = 26.481 min, τ_{major} = 33.312 min).



PDA Ch1 210nm

Peak#	Ret. Time	Area	Area%
1	26.375	45471344	48.646
2	33.269	48002239	51.354
总计		93473583	100.000

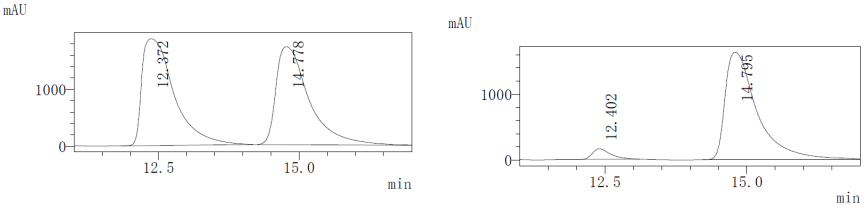
racemic mixture of **3k'**

PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	26.481	4089013	2.570
2	33.312	155022660	97.430
总计		159111674	100.000

3k'

(*S*)-2-Ethyloxycarbonyl -3-(3-bromophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3l') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.42–7.30 (m, 2H), 7.17 (dt, *J* = 15.4, 7.8 Hz, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 3.97 (q, *J* = 7.1 Hz, 2H), 3.88 (td, *J* = 9.9, 4.6 Hz, 1H), 3.70 (d, *J* = 10.0 Hz, 1H), 3.55 (s, 3H), 2.89–2.67 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.4, 167.9, 167.4, 142.5, 131.4, 130.6, 130.1, 127.1, 122.5, 62.0, 61.7, 57.1, 51.8, 41.1, 38.3, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{17}H_{21}BrO_6$+Na^+: 423.0419 [*M*+Na]$^+$; found: 423.0415. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, λ = 210 nm, (τ_{minor} = 12.402 min, τ_{major} = 14.795 min).



PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	12.372	74650416	51.411
2	14.778	70551570	48.589
总计		145201986	100.000

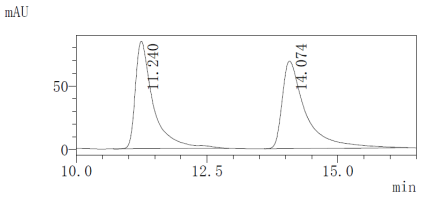
racemic mixture of **3l'**

PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	12.402	3629189	5.305
2	14.795	64780708	94.695
总计		68409897	100.000

3l'

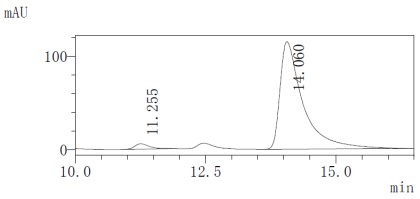
(*S*)-2-Ethyloxycarbonyl -3-(3-methylphenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3m') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.15 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.07–6.96 (m, 3H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.98–3.83 (m, 3H), 3.72 (d, *J* = 10.2 Hz, 1H), 3.54 (s, 3H), 2.89–2.69 (m, 2H), 2.30 (s, 3H), 1.25 (d, *J* = 7.1 Hz, 3H), 1.00 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.8, 168.2, 167.7, 140.0, 138.0, 129.0, 128.4, 128.2, 125.2, 61.8, 61.4, 57.5, 51.7, 41.5, 38.7, 21.6, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{18}H_{24}O_6$+Na^+: 359.1471 [*M*+Na]$^+$; found: 359.1465. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, λ = 254 nm, (τ_{minor} = 11. 255 min, τ_{major} = 14.060 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	11.240	2032208	48.509
2	14.074	2157169	51.491
总计		4189377	100.000

racemic mixture of **3m'**

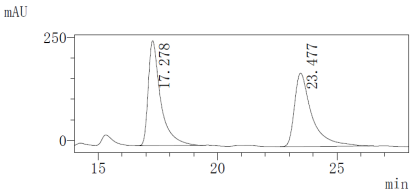


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	11.255	109481	3.004
2	14.060	3534671	96.996
总计		3644152	100.000

3m'

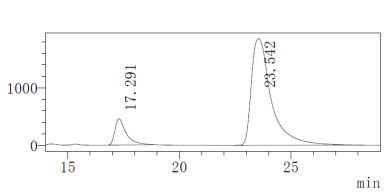
(*S*)-2-Ethyloxycarbonyl -3-(3-cyanophenyl)petanedioic acid 5-ethyl ester 1-methyl ester (3n') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.60–7.50 (m, 3H), 7.40 (dd, *J* = 8.3, 7.1 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.02–3.91 (m, 3H), 3.72 (d, *J* = 9.9 Hz, 1H), 3.55 (s, 3H), 2.89 (dd, *J* = 16.2, 4.4 Hz, 1H), 2.75 (dd, *J* = 16.1, 10.1 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 171.2, 167.6, 167.2, 141.8, 133.1, 132.0, 131.3, 129.4, 118.7, 112.7, 62.1, 61.8, 56.8, 52.0, 41.0, 38.1, 14.2, 13.9. **HRMS** (ESI): *m/z* calcd for $C_{18}H_{21}NO_6$+Na^+: 370.1267 [*M*+Na]$^+$; found: 370.1262. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, ƛ = 210 nm, (τ_{minor} = 17. 291 min, τ_{major} = 23.543 min).



PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	17.278	9165532	50.460
2	23.477	8998409	49.540
总计		18163941	100.000

racemic mixture of **3n'**

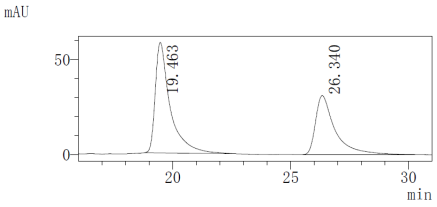


PDA Ch2 210nm

Peak#	Ret. Time	Area	Area%
1	17.291	16016582	12.239
2	23.542	114849416	87.761
总计		130865998	100.000

3n'

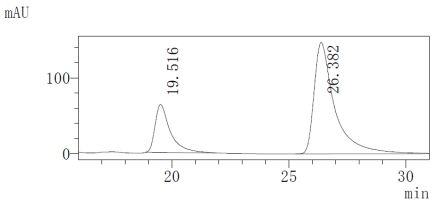
(*S*,*E*)-1,1,5-triethyl 2-(4-hydroxy-3-methoxyphenyl)pent-4-ene-1,1,5-tricarboxylate (3o') Yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.82 (d, *J* = 8.0 Hz, 1H), 6.68 (d, *J* = 7.7 Hz, 2H), 5.72 (d, *J* = 15.7 Hz, 1H), 5.53 (s, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.02–3.91 (m, 2H), 3.85 (s, 3H), 3.64 (d, *J* = 10.5 Hz, 1H), 3.48 (td, *J* = 10.0, 4.4 Hz, 1H), 2.71–2.45 (m, 2H), 1.26 (dt, *J* = 18.5, 7.1 Hz, 6H), 1.00 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 168.3, 167.7, 166.3, 146.5, 145.5, 144.8, 131.6, 123.6, 120.9, 114.5, 111.0, 61.8, 61.5, 60.4, 58.2, 56.1, 44.5, 36.9, 14.3, 14.2, 14.0. **HRMS** (ESI): *m/z* calcd for $C_{21}H_{28}O_8$+Na^+: 431.1682 [*M*+Na]$^+$; found: 431.1677. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (80/20), flow rate: 0.5 mL /min, ƛ = 254 nm, (τ_{minor} = 19.516 min, τ_{major} = 26.382 min).



PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	19.463	2541057	58.768
2	26.340	1782842	41.232
总计		4323899	100.000

racemic mixture of **3o'**

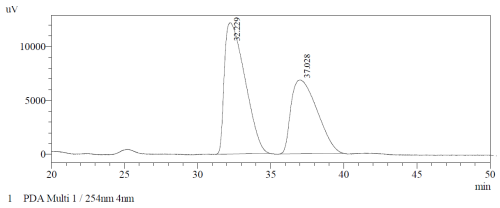


PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	19.516	2845826	23.933
2	26.382	9044927	76.067
总计		11890752	100.000

3o'

(*S,E*)-1,1-diethyl 5-methyl-2-propylpent-4-ene-1,1,5-tricarboxylate (3p') Colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.90 (dt, *J* = 15.6, 7.3 Hz, 1H), 5.85 (d, *J* = 15.6 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 4H), 3.73 (s, 3H), 3.39 (d, *J* = 6.5 Hz, 1H), 2.45–2.24 (m, 3H), 1.39–1.30 (m, 4H), 1.29–1.24 (m, 6H), 0.89 (q, *J* = 5.4, 4.5 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 168.8, 168.7, 166.9, 146.9, 123.3, 61.5, 61.4, 54.8, 51.6, 37.5, 34.0, 33.5, 20.1, 14.2, 14.2. **HRMS** (ESI): *m/z* calcd for $C_{16}H_{26}O_6$+Na^+: 337.1627 [*M*+Na]$^+$; found: 337.1621. **HPLC**: Daicel Chiralpak AD-H, hexane / 2-propanol (99.5/0.5), flow rate: 0.5 mL /min, ʎ = 254 nm, (τ_{minor} = 31.141 min, τ_{major} = 34.089 min).

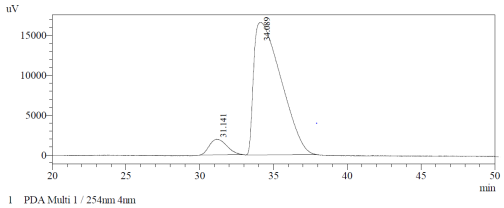


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	32.229	1183482	12194	58.217	64.038
2	37.028	849389	6848	41.783	35.962
Total		2032871	19043	100.000	100.000

racemic mixture of **3p'**



PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	31.141	161250	1964	7.019	10.561
2	34.089	2136147	16635	92.981	89.439
Total		2297397	18599	100.000	100.000

3p'

Chemoenzymatic synthesis of (+)-femoxetine

Optimized conditions were implemented for the bio-transformation on the preparative scale (200 mL) and performed starting from the product **3a** (10 mM), methylamine (200 mM, in buffer adjusted to pH 8.0 with HCl), purified IR361 (50 μM), D-glucose (50 mM), $NADP^+$ (0.5 mM), GDH (960 U) and Tris-HCl buffer (100 mM, pH 8.0) with 5% (v/v) DMSO. The reaction mixture was incubated at 30 °C with 180 r.p.m. shaking, and was monitored by GC–MS. After completion of the reaction, the mixture was extracted with ethyl acetate (3 × 50 mL) with centrifugation (6,000 × g, 10 min) to improve the separation of the two phases. The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under *vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate = 2:1) to provide **7** (177 mg, 34% yield) as colorless oil. **$[\alpha]_D^{24.5}$** = +12.20 (*c* 0.5, CH_2Cl_2). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.31 (t, *J* = 7.3 Hz, 2H), 7.25–7.16 (m,

3H), 4.05 (qd, *J* = 7.1, 2.8 Hz, 2H), 3.52 (t, *J* = 11.2 Hz, 2H), 3.48–3.37 (m, 1H), 3.33 (dd, *J* = 16.9, 3.8 Hz, 1H), 3.00 (s, 3H), 2.10 (dd, *J* = 8.8, 4.5 Hz, 2H), 1.06 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 170.2, 166.0, 141.5, 128.9, 127.4, 127.0, 61.2, 56.5, 49.1, 42.8, 35.0, 29.5, 14.1. **HRMS** (ESI): *m/z* calcd for $C_{15}H_{20}NO_3^+$: 262.1443 $[M+H]^+$; found: 262.1439.

Compound **7** (68 mg, 0.26 mmol) in THF (3 mL) was added dropwise to a stirred slurry of lithium aluminumhydride (237 mg, 6.25 mmol) in THF (5.5 mL) at 0 °C. The reaction mixture was warmed to room temperature and then heated to reflux overnight. After completion of the reaction, the mixture was cooled to room temperature, water (1 mL) was added dropwise. After stirring for 10 min, aqueous sodium hydroxide (2 M, 3 mL) was added to the mixture. After stirring for another 10 min, the mixture was poured into saturated Rochelle´s salt solution (30 mL). The mixture was extracted with ethyl acetate (4 × 20 mL) and dichloromethane (3 × 15 mL). The organic phases were combined, washed with 1 M NaOH and brine, dried over anhydrous sodium sulfate, filtered, and concentrated under *vacuo* to give the crude reduction product **5**. The crude product was dissolved in THF (2 mL), and triphenylphosphine (82 mg, 0.31 mmol) was added to the solution. The reaction mixture was cooled to 0 °C and DIAD (63 mg, 62 µL, 0.31 mmol) was added dropwise. The reaction mixture was left to stir for another 10 min at 0 °C before 4-methoxyphenol (65 mg, 0.52 mmol) in THF (1 mL) was added dropwise. The mixture was stirred for another 10 min, warmed to room temperature, and then heated at 50 °C for 2 h. After this time the reaction mixture was cooled to room temperature, concentrated, and redissolved in dichloromethane. The solution was washed with aqueous NaOH (2 M), and extracted with dichloromethane (3 × 15 mL). The organic extracts were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under *vacuo*. The residue was purified by column chromatography (MeOH / CH_2Cl_2 = 1:20, silica gel) to afford compound **4** (38 mg, 47% yield over two steps) as yellow oil. **$[\alpha]_D^{24.9}$** = +29.40 (*c* 0.5, MeOH). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.29-7.26 (m, 2H), 7.24–7.15 (m, 3H), 6.75 (d, *J* = 9.1 Hz, 2H), 6.67 (d, *J* = 9.1 Hz, 2H), 3.73 (s, 3H), 3.62 (dd, *J* = 9.4, 3.0 Hz, 1H), 3.53–3.45 (m, 1H), 3.25 (d, *J* = 11.4 Hz, 1H), 2.99 (d, *J* = 10.3 Hz, 1H), 2.45 (td, *J* = 11.6, 4.3 Hz, 1H), 2.37 (s, 3H), 2.34–2.27 (m, 1H), 2.12–2.00 (m, 2H), 1.94 (dd, *J* = 12.4, 3.8 Hz, 1H), 1.86 (d, *J* = 3.6 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 153.8, 153.2, 144.1, 128.7, 127.6, 126.7, 115.5, 114.6, 69.6, 59.8, 56.4, 55.8, 46.6, 44.4, 42.0, 34.4. **HRMS** (ESI): *m/z* calcd for $C_{20}H_{26}NO_2^+$: 312.1964 $[M+H]^+$; found: 312.1958.

IV. References

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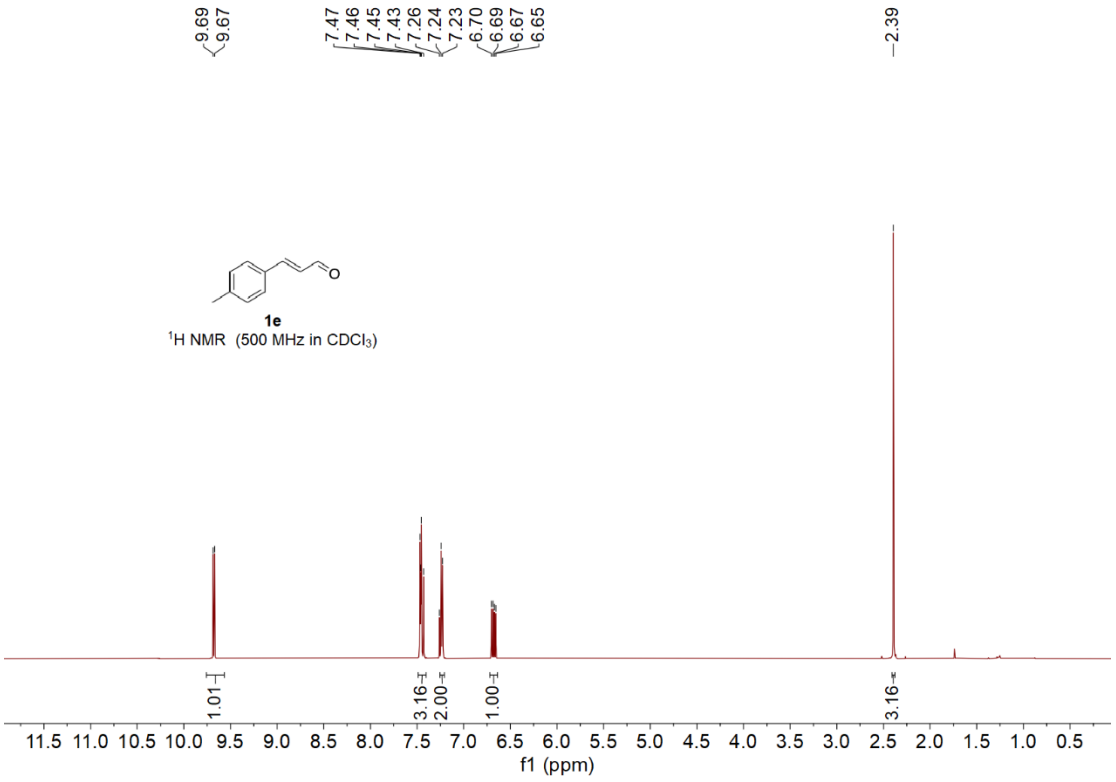
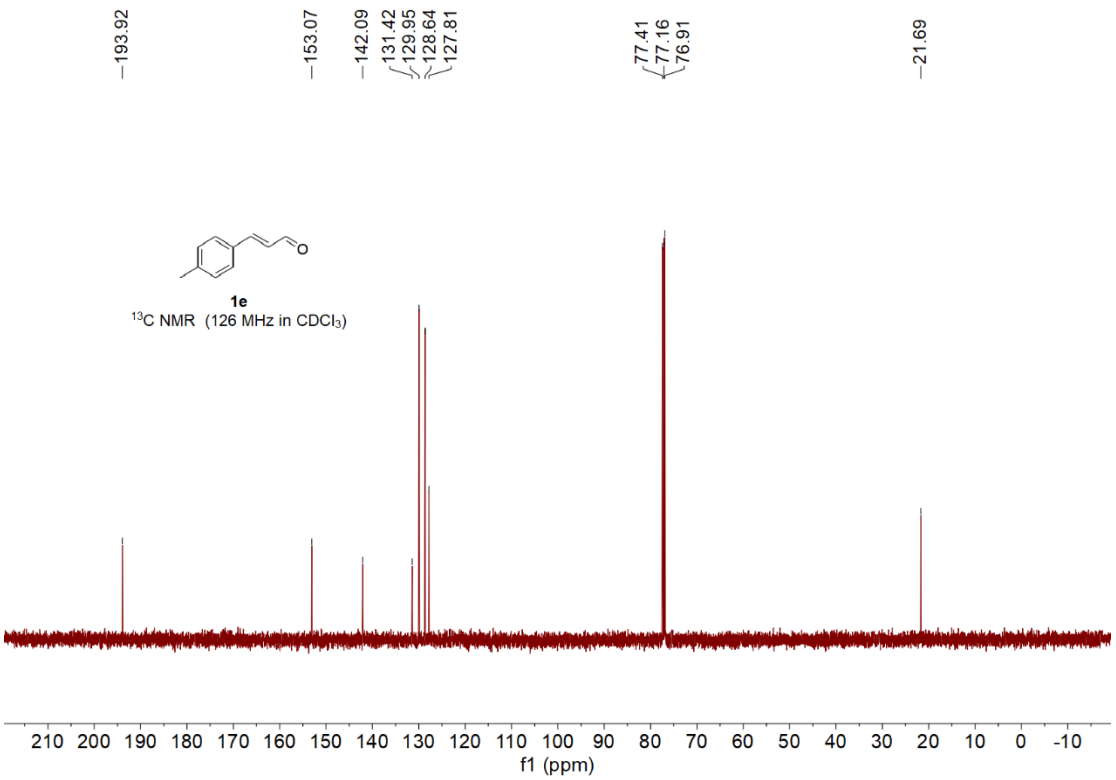
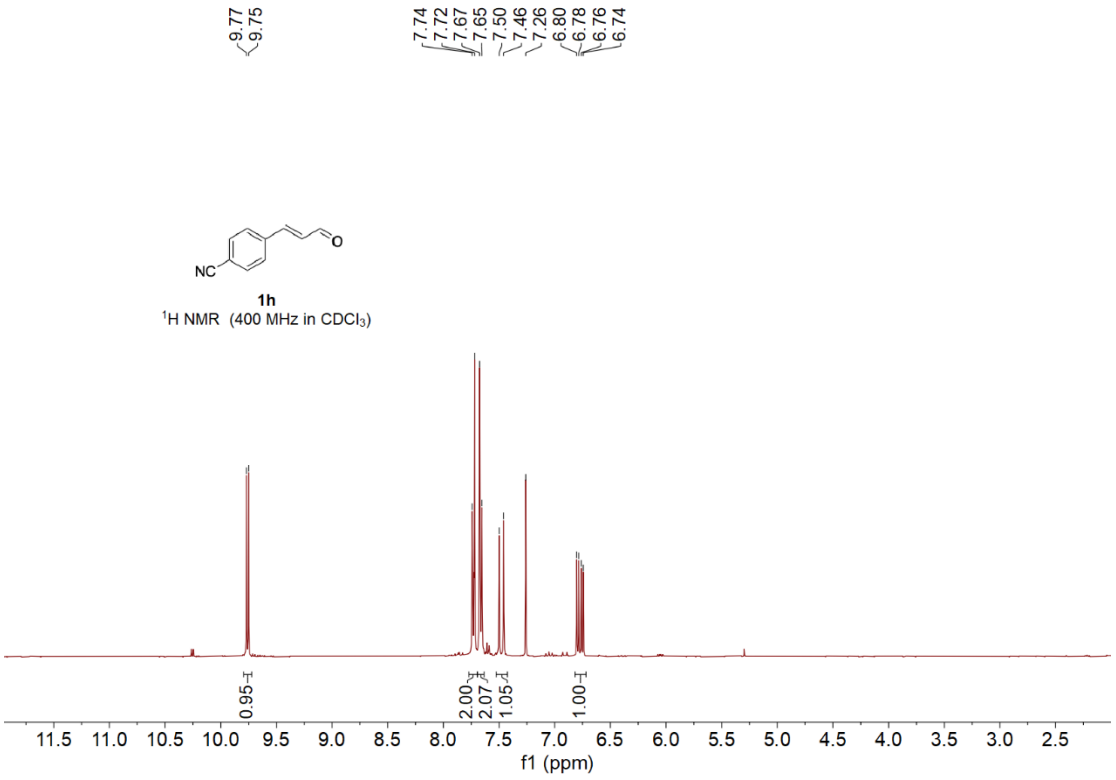
V. NMR spectra



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7.72
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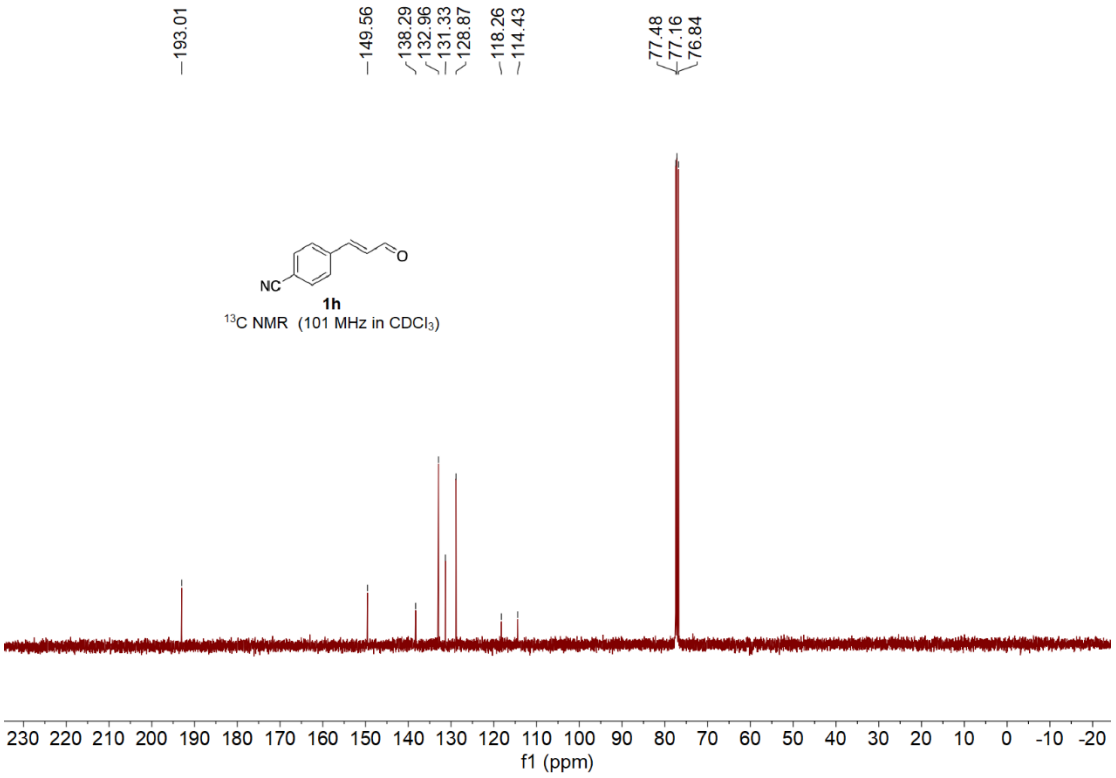
7.65
7.50
7.46
7.26
6.80
6.78
6.76
6.74
NC
O
1h
^{1}H NMR (400 MHz in CDCl$_3$)
0.95
2.00
2.07
1.05
1.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5
f1 (ppm)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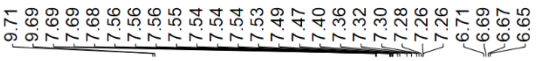
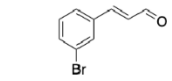
131.33
128.87
118.26
114.43
77.48
77.16
76.84
NC
O
1h
^{13}C NMR (101 MHz in CDCl$_3$)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)





1l

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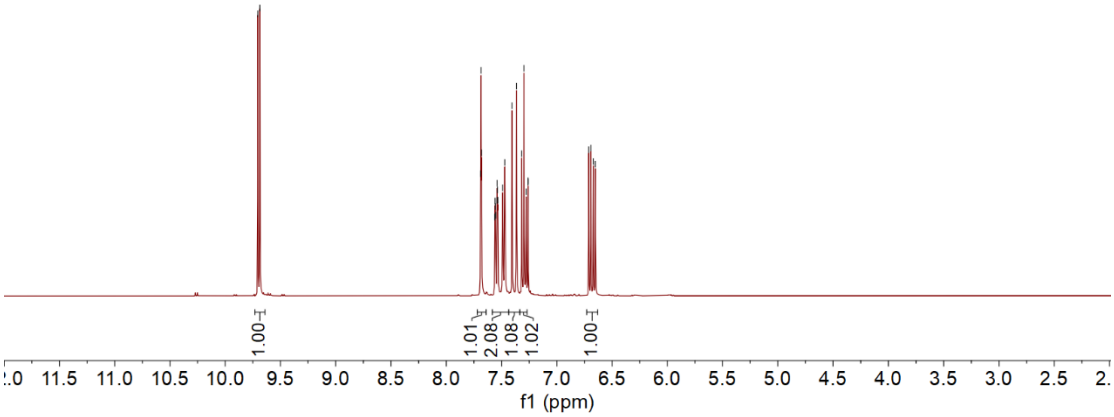

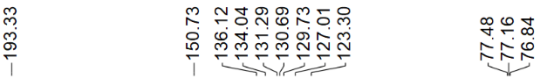

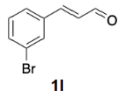

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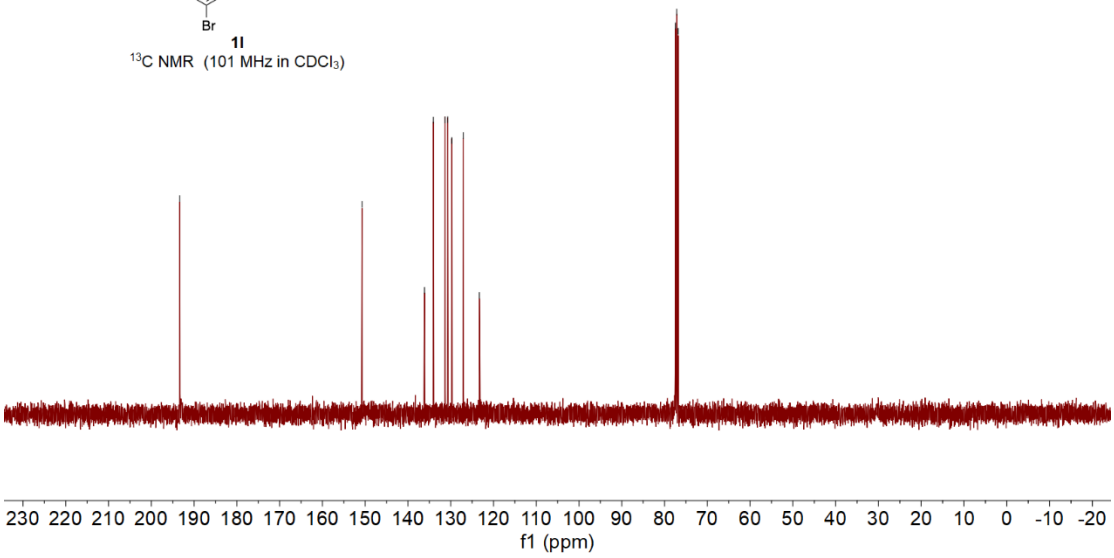

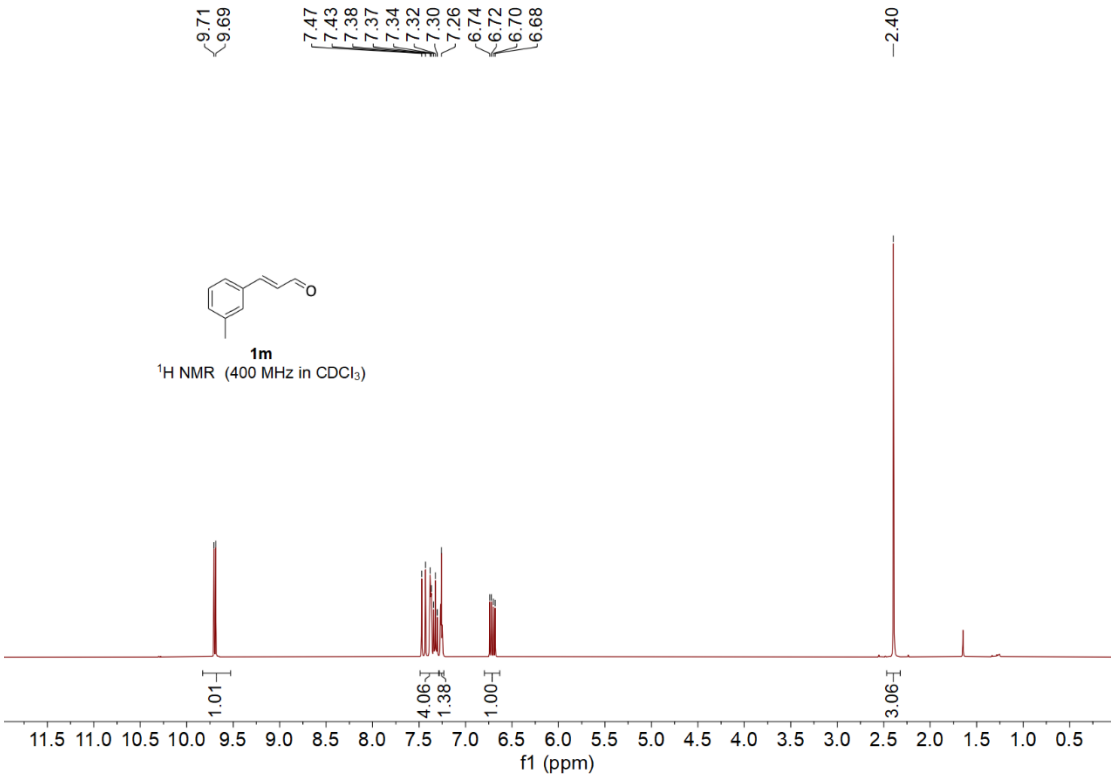
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7.32
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7.26
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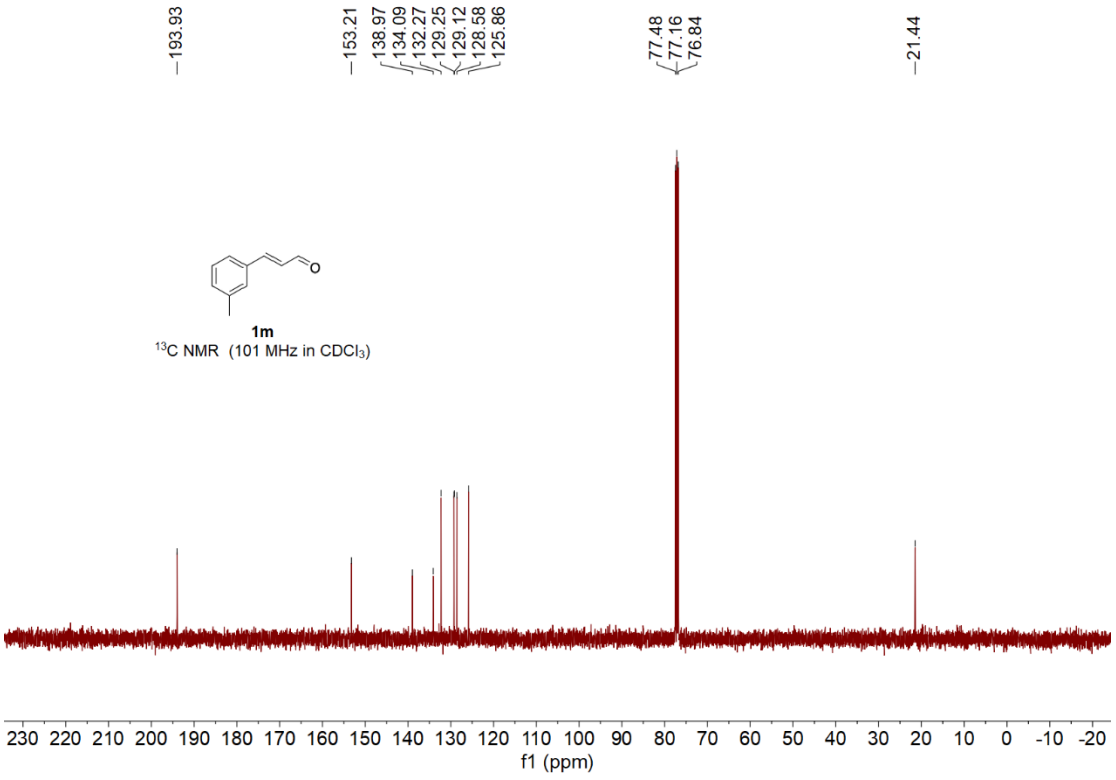
6.70
6.68
2.40
O
1m
1H NMR (400 MHz in CDCl3)
1.01
4.06
1.38
1.00
3.06
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)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129.25
129.12
128.58
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77.16
76.84
21.44
O
1m
13C NMR (101 MHz in CDCl3)
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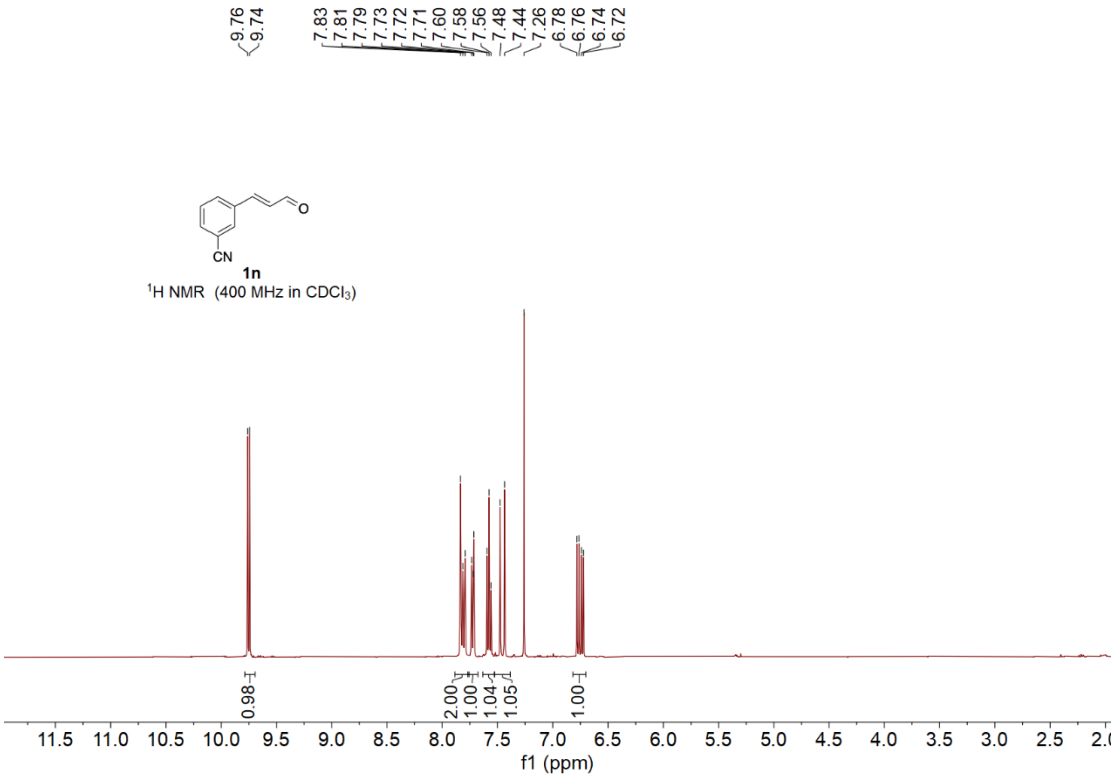
f1 (ppm)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7.58
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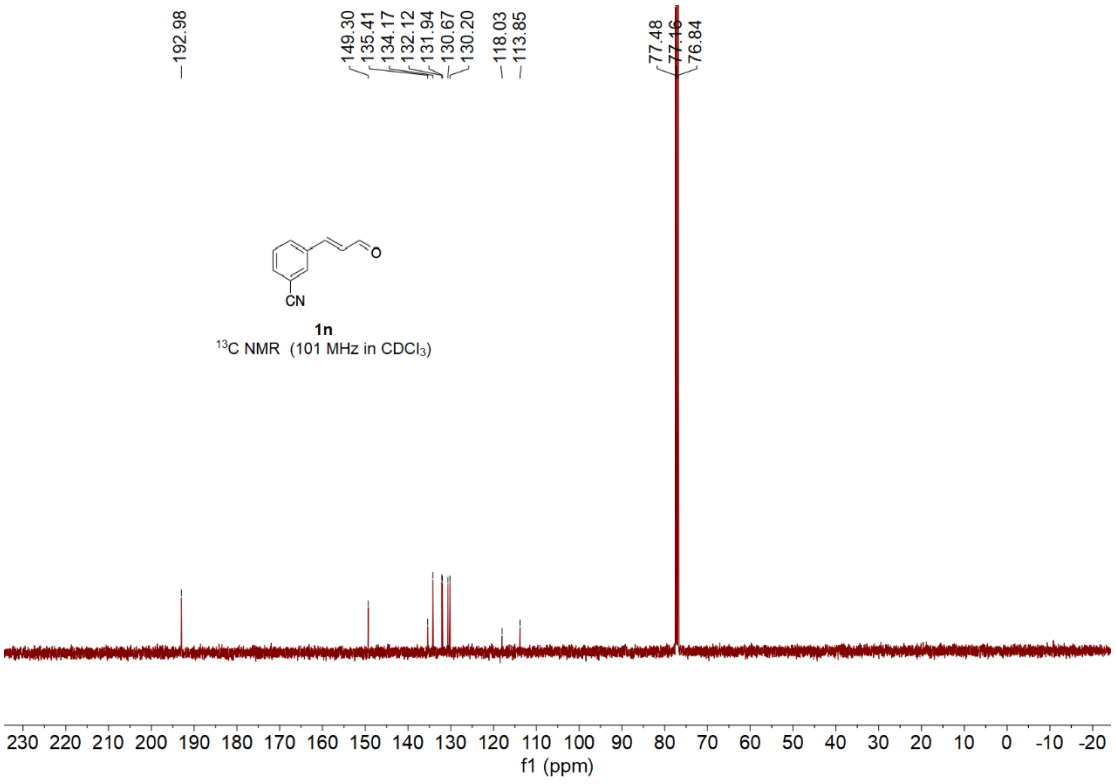
7.48
7.44
7.26
6.78
6.76
6.74
6.72
O
CN
1n
^{1}H NMR (400 MHz in CDCl$_3$)
0.98
2.00
1.00
1.04
1.05
1.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5
f1 (ppm)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130.20
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77.16
76.84
O
CN
1n
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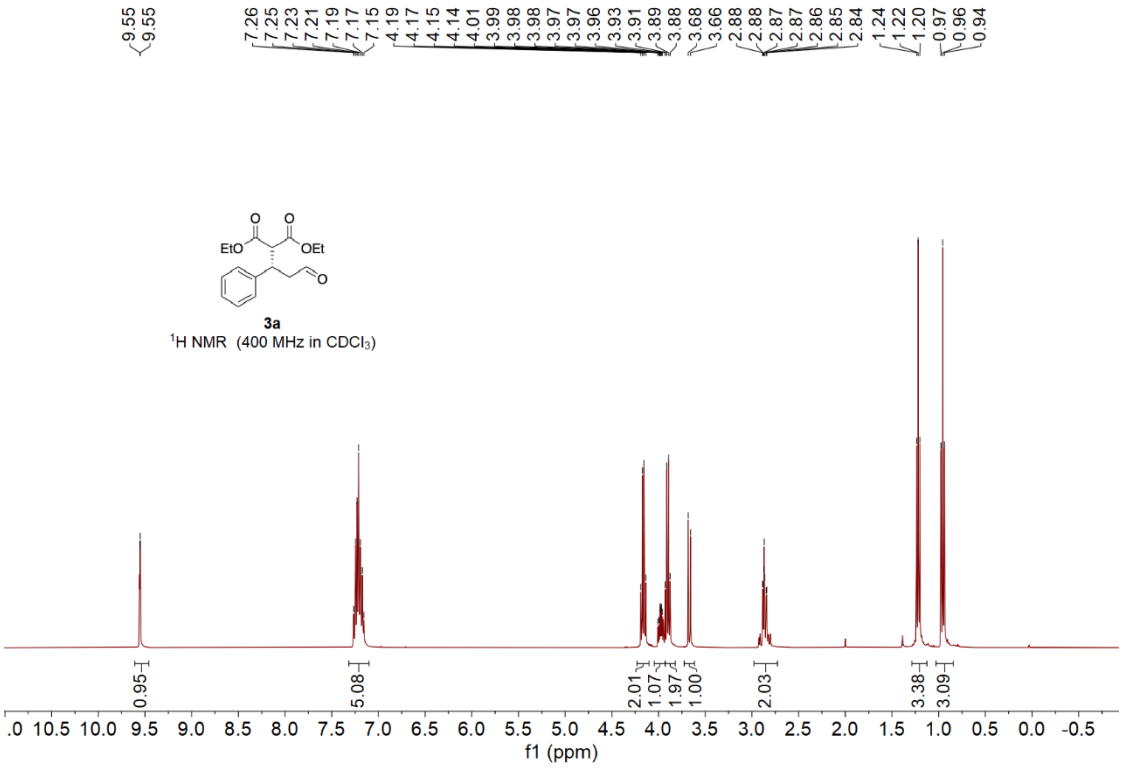
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)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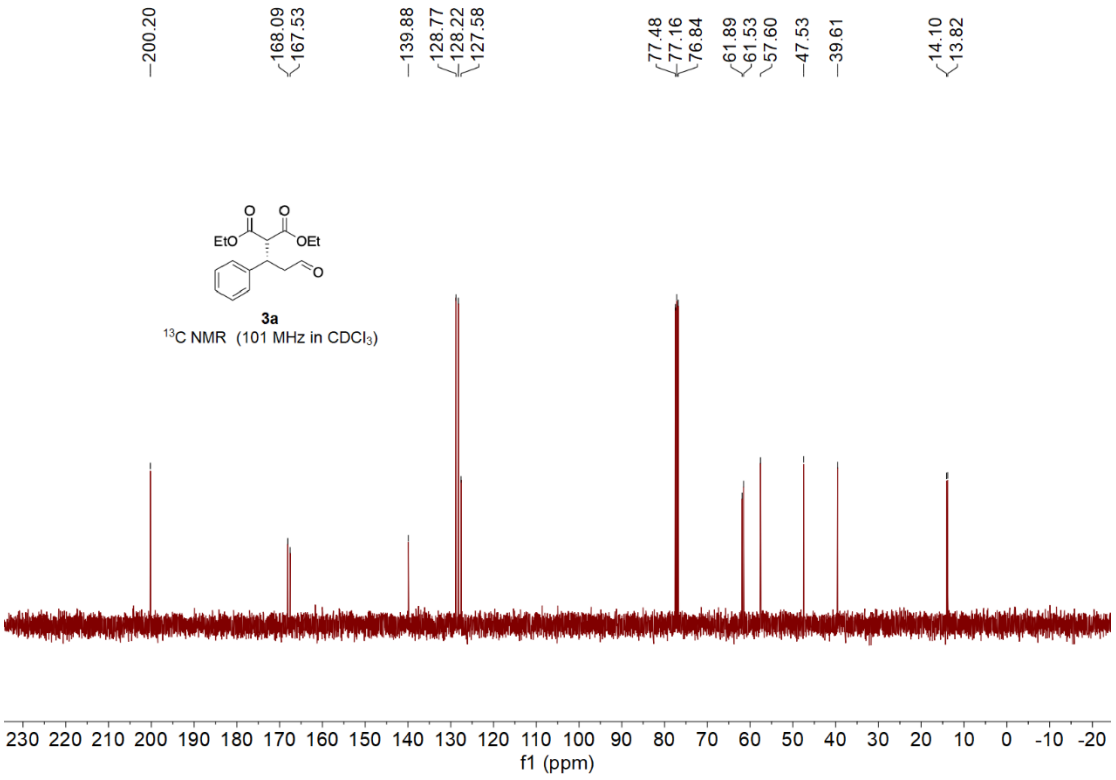
3.98
3.97
3.97
3.96
3.93
3.91
3.89
3.88
3.68
3.66
2.88
2.88
2.87
2.87
2.86
2.85
2.84
1.24
1.22
1.20
0.97
0.96
0.94
EtO
OEt
O
O
O
3a
1H NMR (400 MHz in CDCl3)
0.95
5.08
2.01
1.07
1.97
1.00
2.03
3.38
3.09
.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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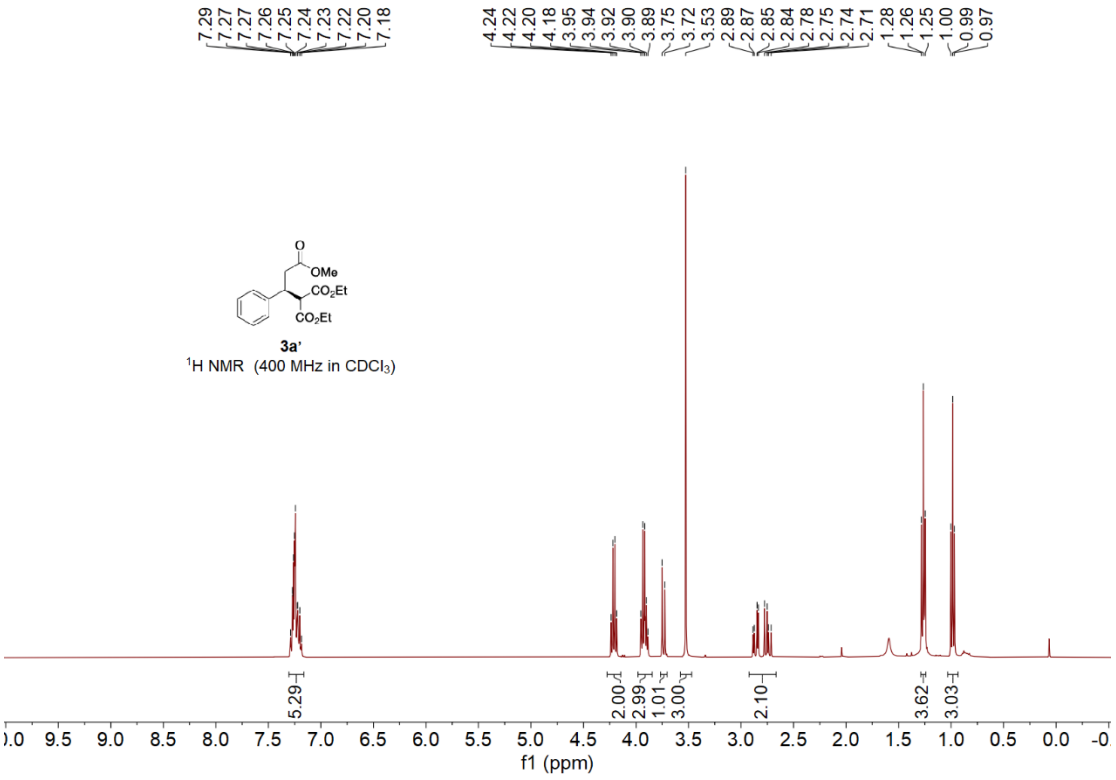
47.53
39.61
14.10
13.82
EtO
OEt
O
O
O
3a
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)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7.24
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7.18
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4.22
4.20
4.18
3.95
3.94
3.92
3.90
3.89
3.75
3.72
3.53
2.89
2.87
2.85
2.84
2.78
2.75
2.74
2.71
1.28
1.26
1.25
1.00
0.99
0.97
O
OMe
CO2Et
CO2Et
3a'
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3.00
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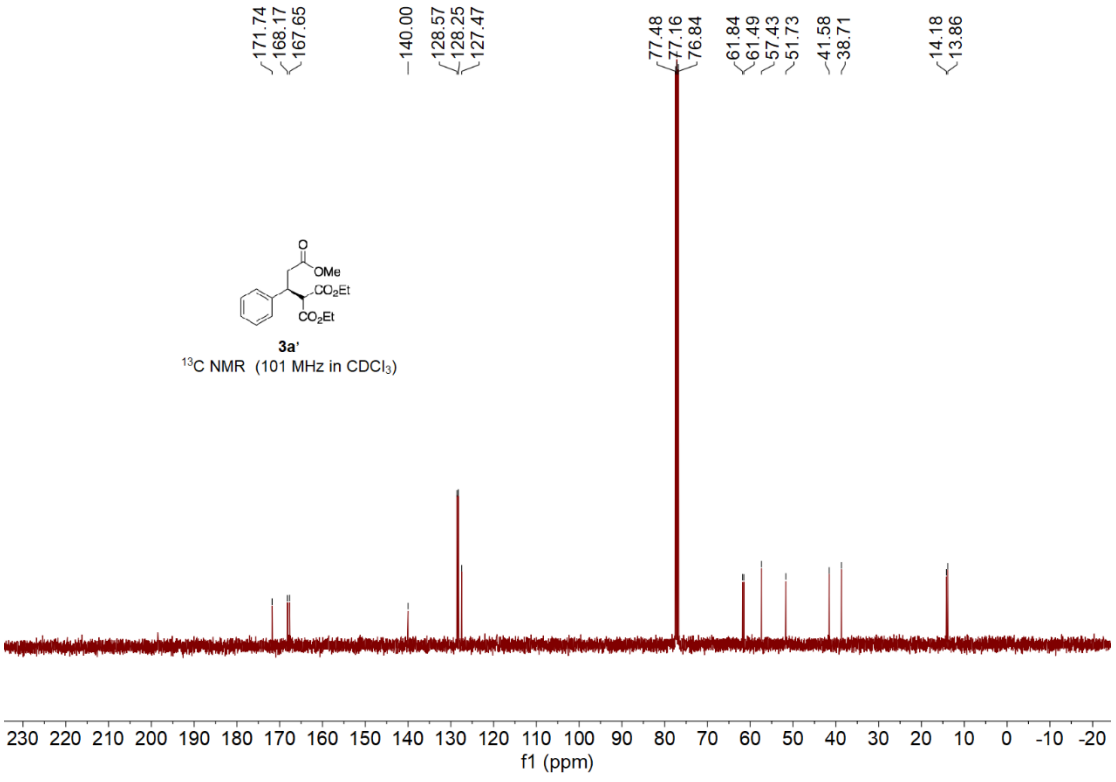
3.03
f1 (ppm)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76.84
61.84
61.49
57.43
51.73
41.58
38.71
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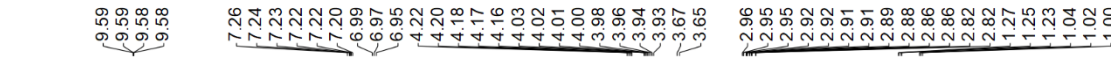
O
OMe
CO2Et
CO2Et
3a'
13C NMR (101 MHz in CDCl3)
f1 (ppm)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9.58
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6.95
4.22
4.20
4.18
4.17
4.16
4.03
4.02
4.01
4.00
3.98
3.96
3.94
3.93
3.67
3.65
2.96
2.95
2.95
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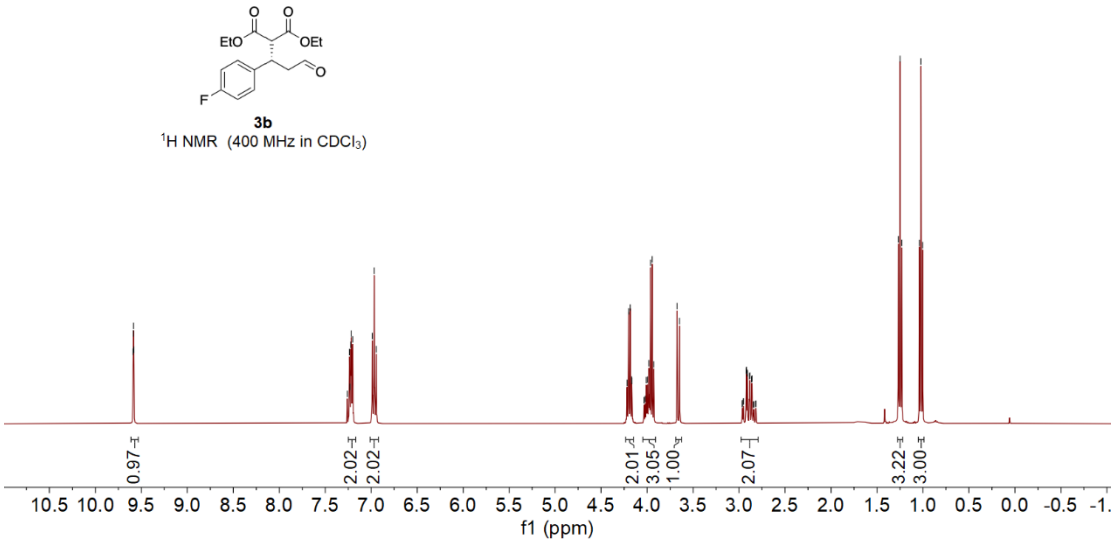
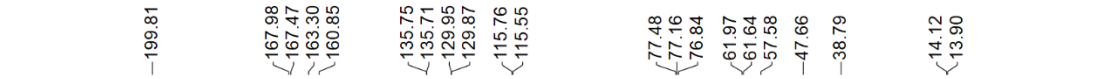
1.02


EtO
OEt
O
O
O
F
3b
1H NMR (400 MHz in CDCl3)
0.97
2.02
2.02
2.01
3.05
1.00
2.07
3.22
3.00
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.
f1 (ppm)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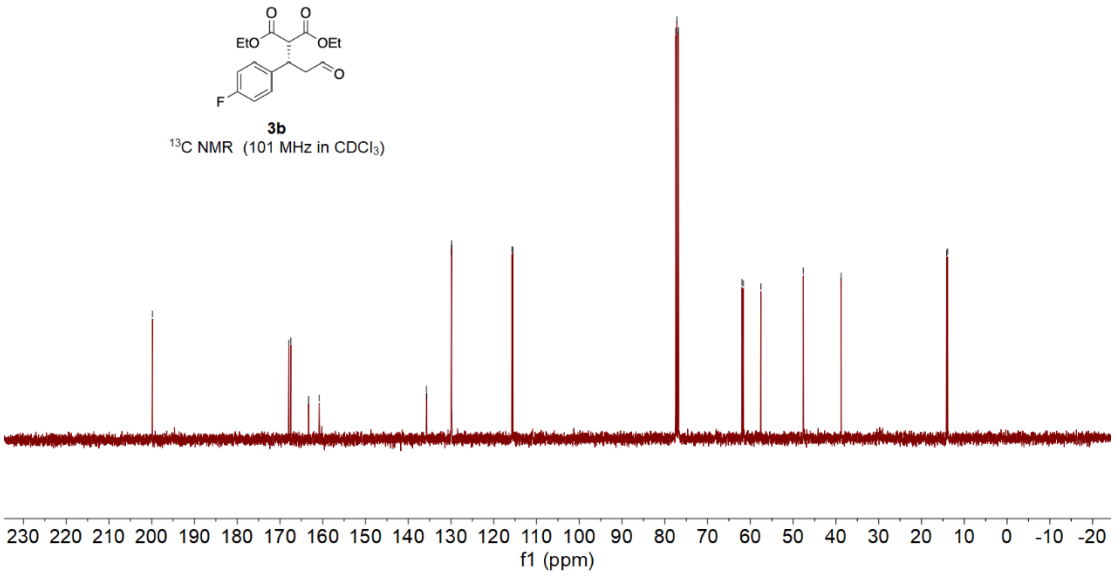
13.90


EtO
OEt
O
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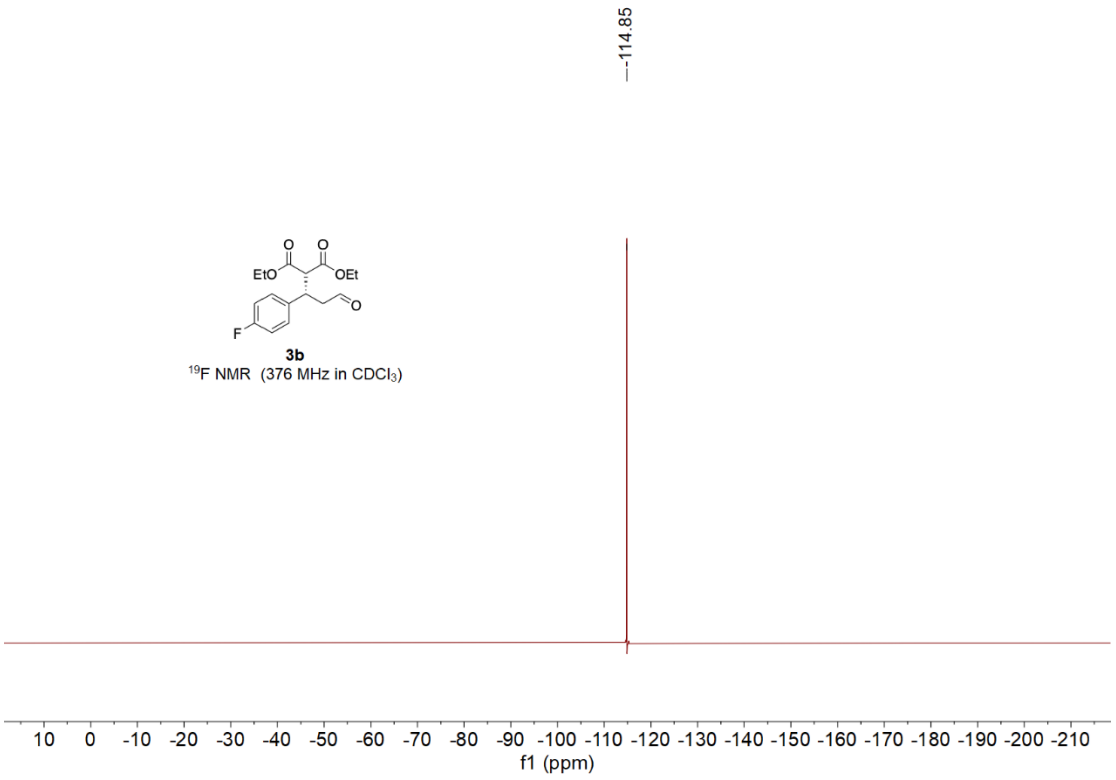
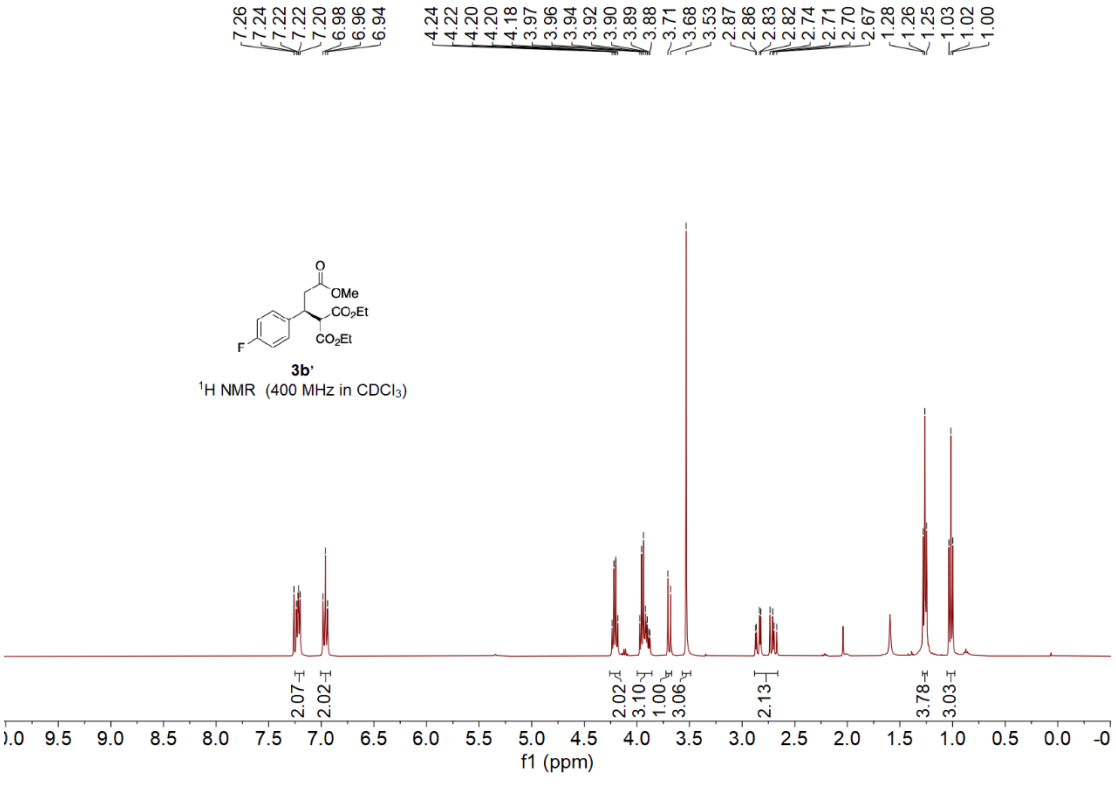
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)



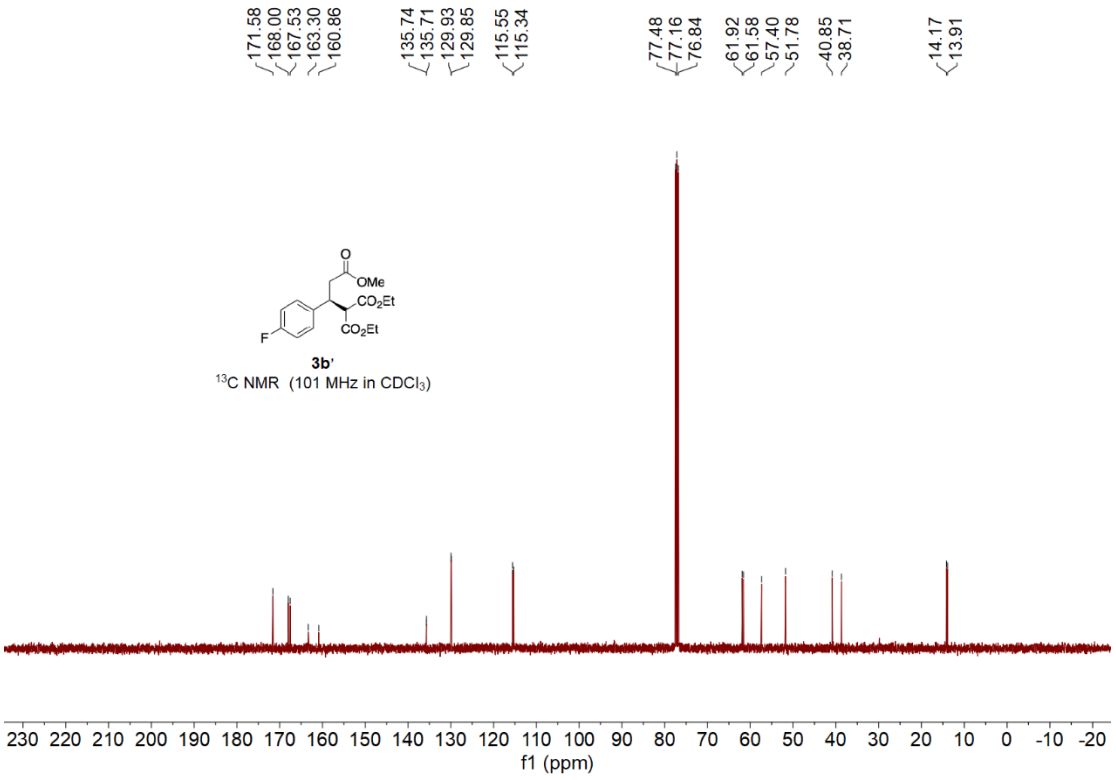
3b

^{19}F NMR (376 MHz in $CDCl_3$)



3b'

^{1}H NMR (400 MHz in $CDCl_3$)



3b'

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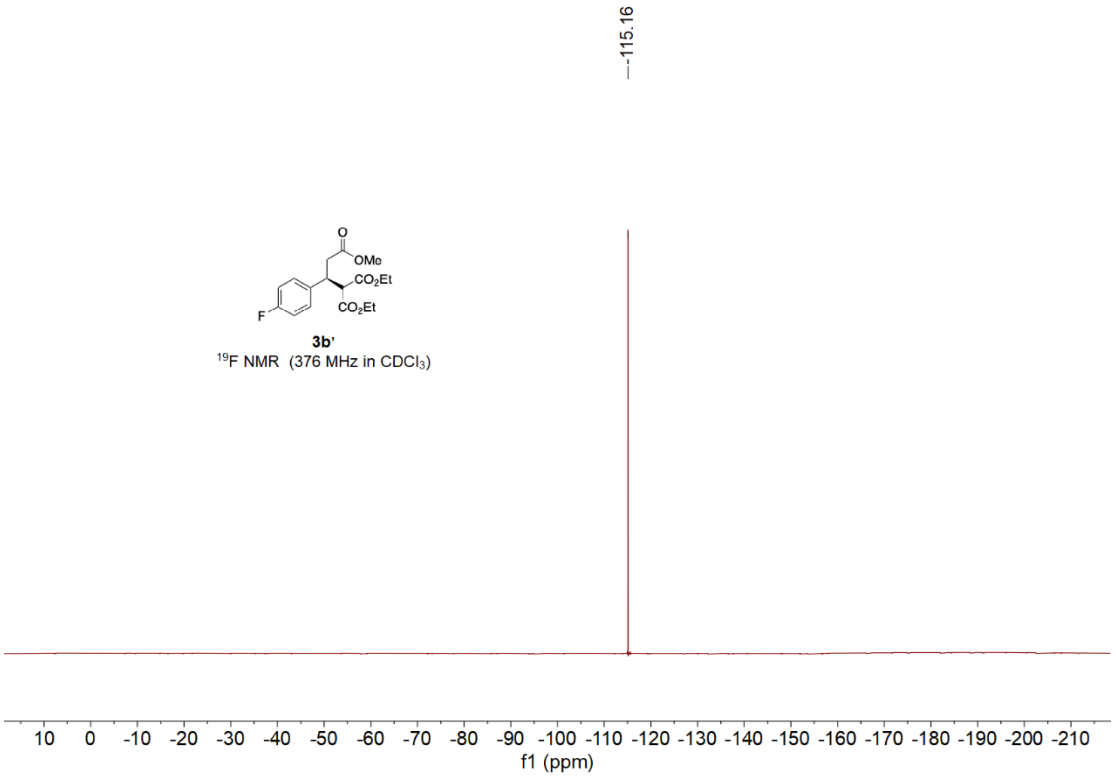
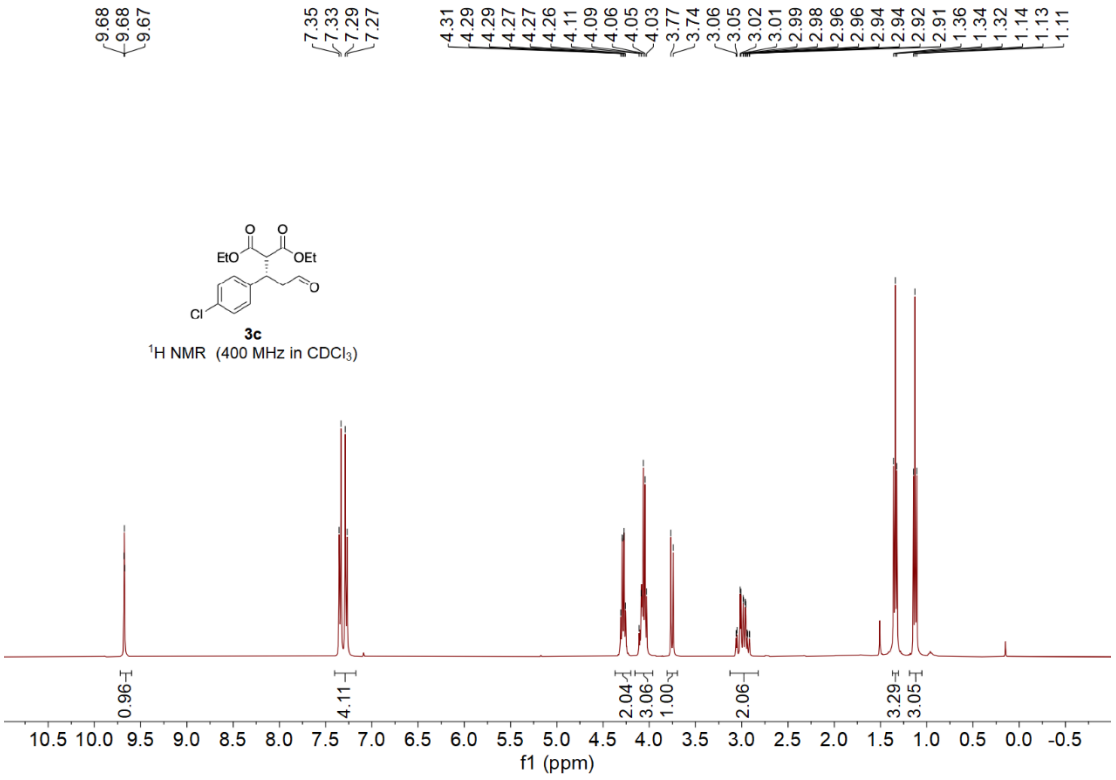
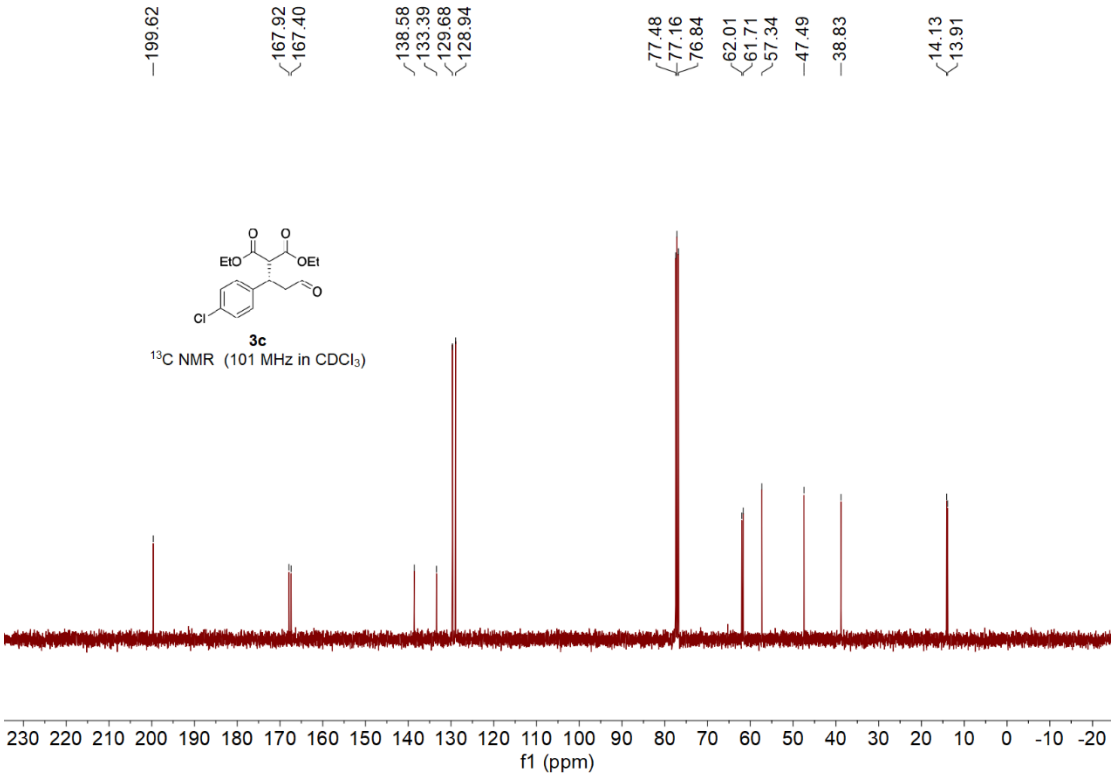
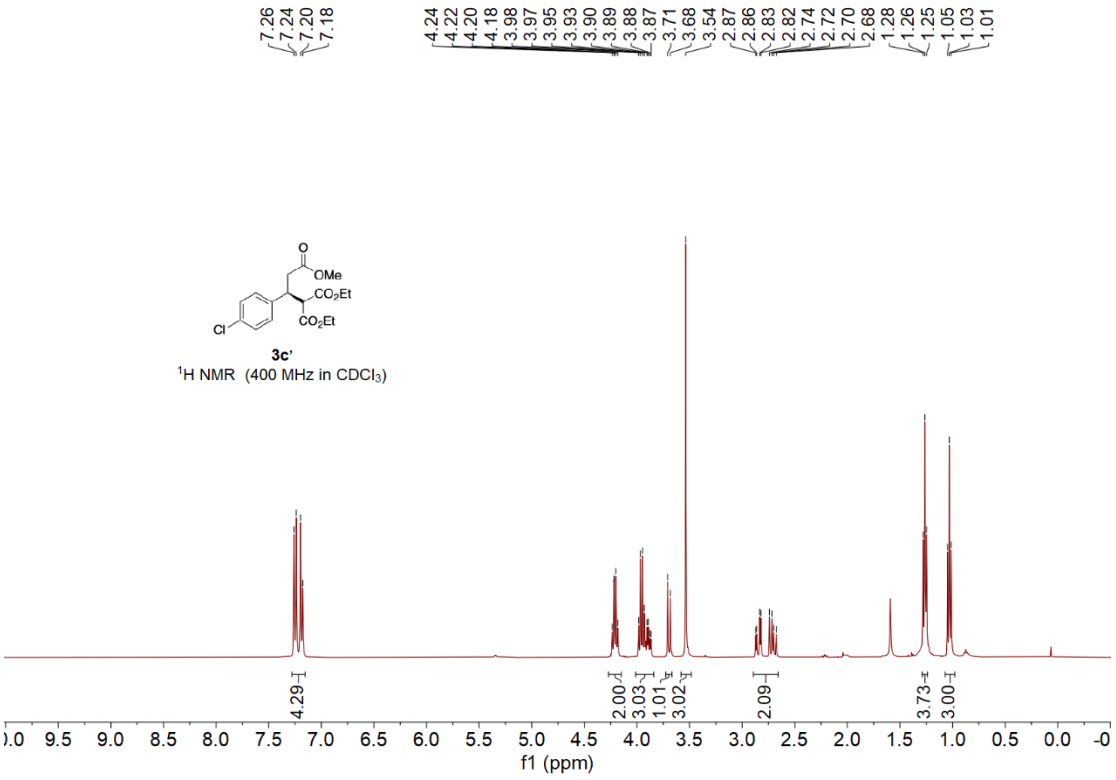


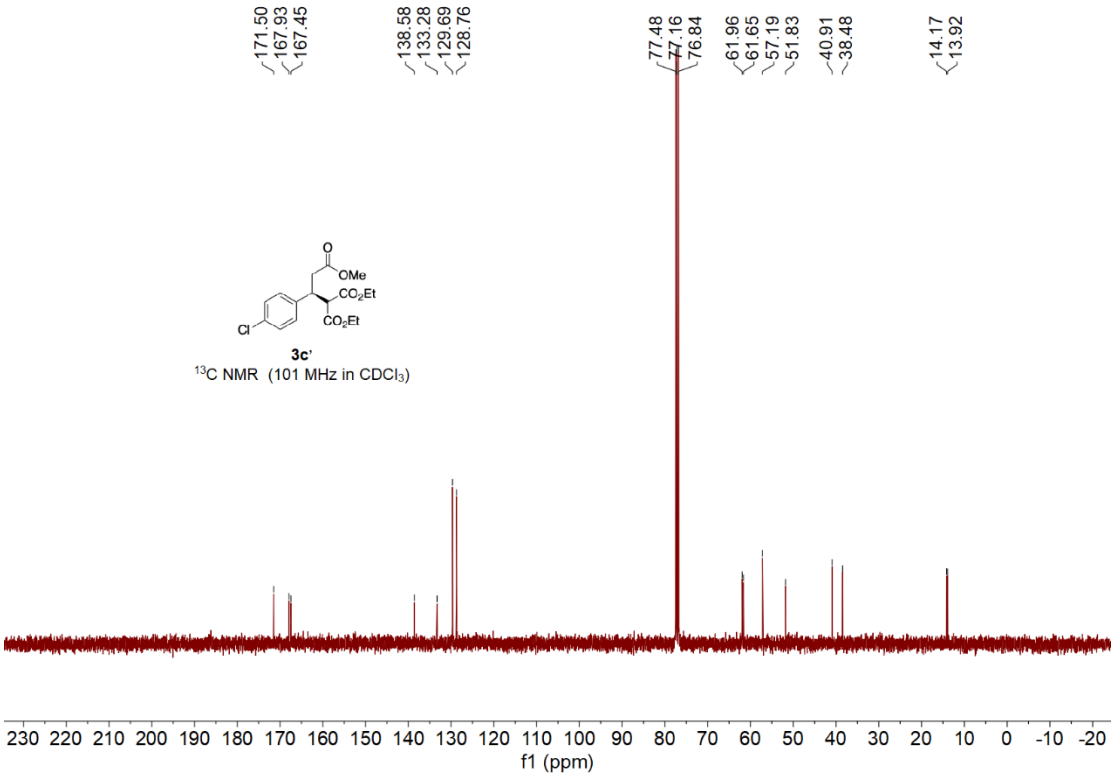
3b'

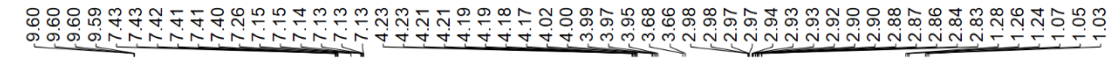
^{19}F NMR (376 MHz in $CDCl_3$)

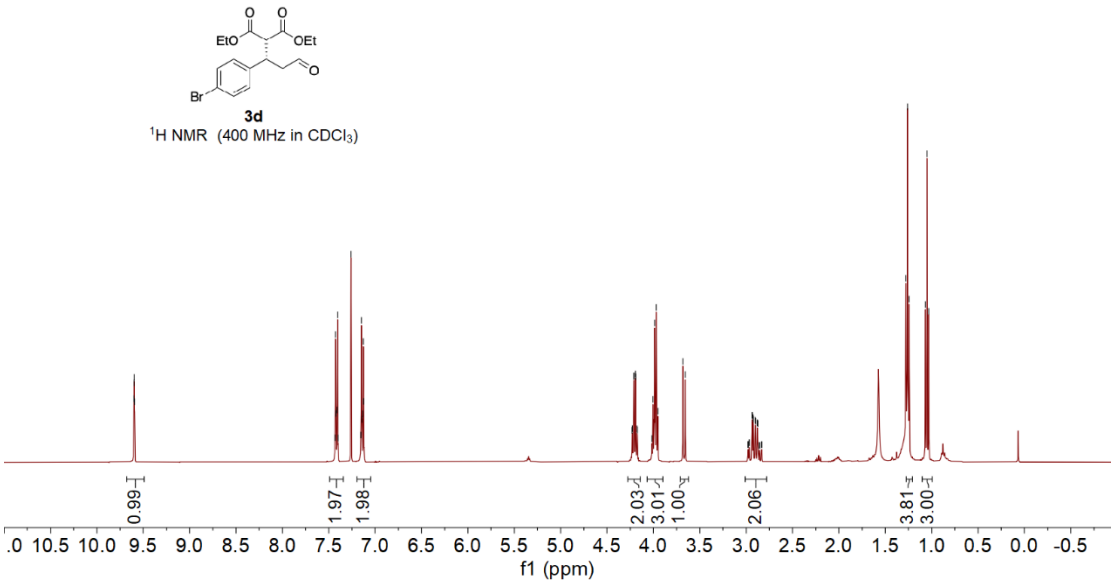

9.68
9.68
9.67
7.35
7.33
7.29
7.27
4.31
4.29
4.29
4.27
4.27
4.26
4.11
4.09
4.06
4.05
4.03
3.77
3.74
3.06
3.05
3.02
3.01
2.99
2.98
2.96
2.96
2.94
2.94
2.92
2.91
1.36
1.34
1.32
1.14
1.13
1.11
EtO
OEt
O
O
Cl
3c
1H NMR (400 MHz in CDCl3)
0.96
4.11
2.04
3.06
1.00
2.06
3.29
3.05
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

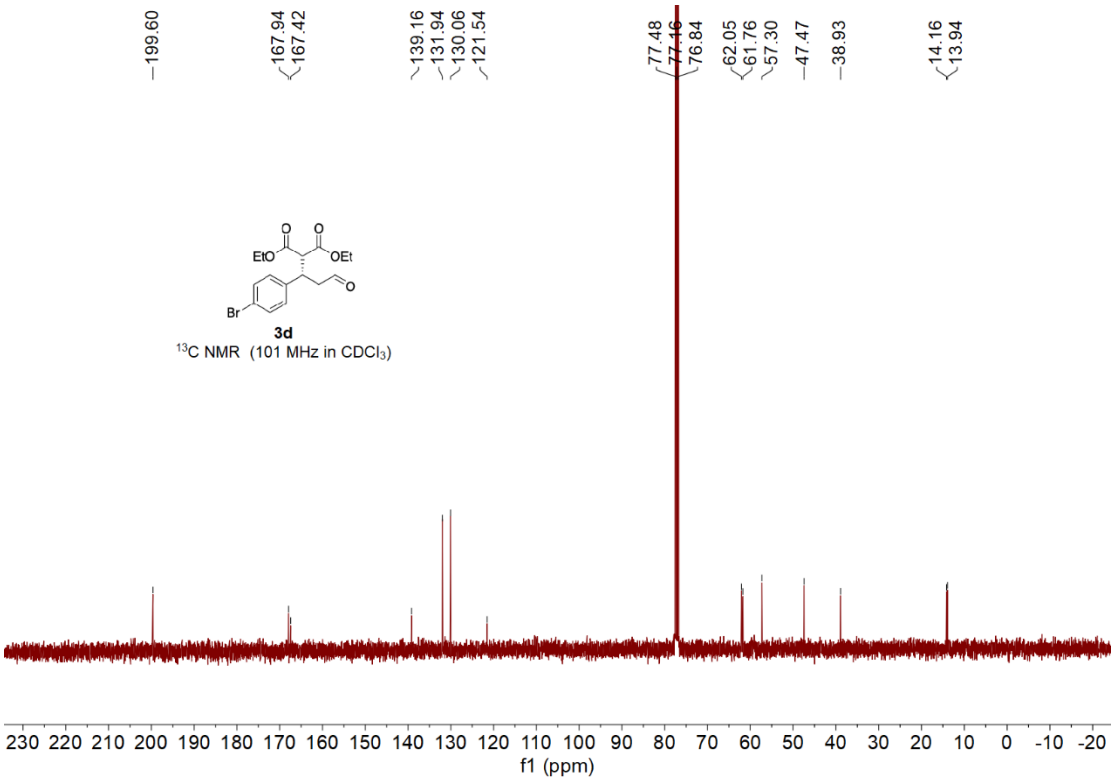

199.62
167.92
167.40
138.58
133.39
129.68
128.94
77.48
77.16
76.84
62.01
61.71
57.34
47.49
38.83
14.13
13.91
EtO
OEt
O
O
Cl
3c
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

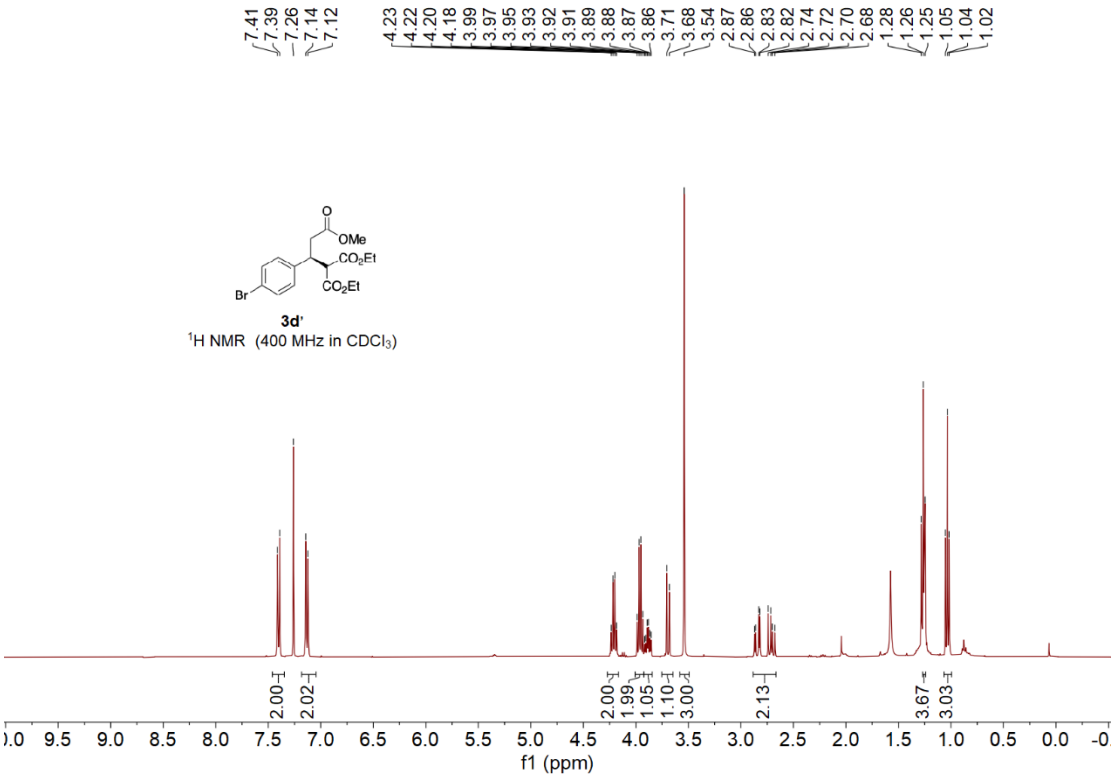

7.26
7.24
7.20
7.18
4.24
4.22
4.20
4.18
3.98
3.97
3.95
3.93
3.90
3.89
3.88
3.87
3.71
3.68
3.54
2.87
2.86
2.83
2.82
2.74
2.72
2.70
2.68
1.28
1.26
1.25
1.05
1.03
1.01
OMe
CO2Et
CO2Et
Cl
3c'
1H NMR (400 MHz in CDCl3)
4.29
2.00
3.03
1.01
3.02
2.09
3.73
3.00
f1 (ppm)

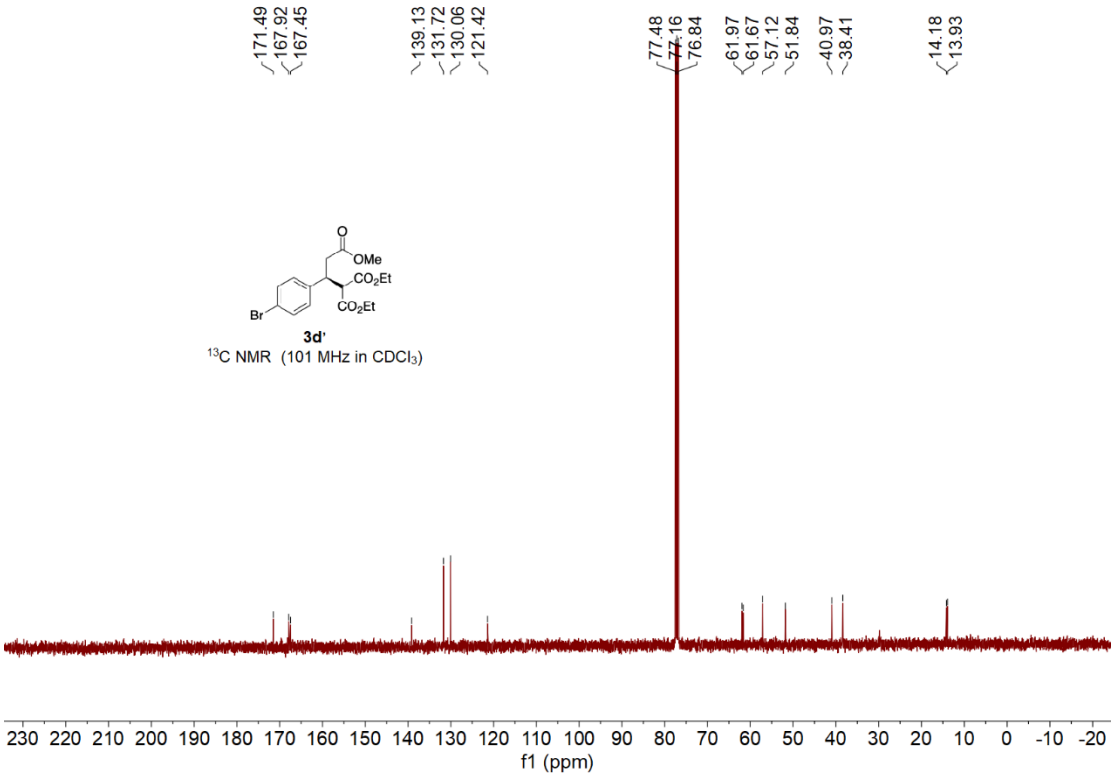

171.50
167.93
167.45
138.58
133.28
129.69
128.76
77.48
77.16
76.84
61.96
61.65
57.19
51.83
40.91
38.48
14.17
13.92
OMe
CO2Et
CO2Et
Cl
3c'
13C NMR (101 MHz in CDCl3)
f1 (ppm)

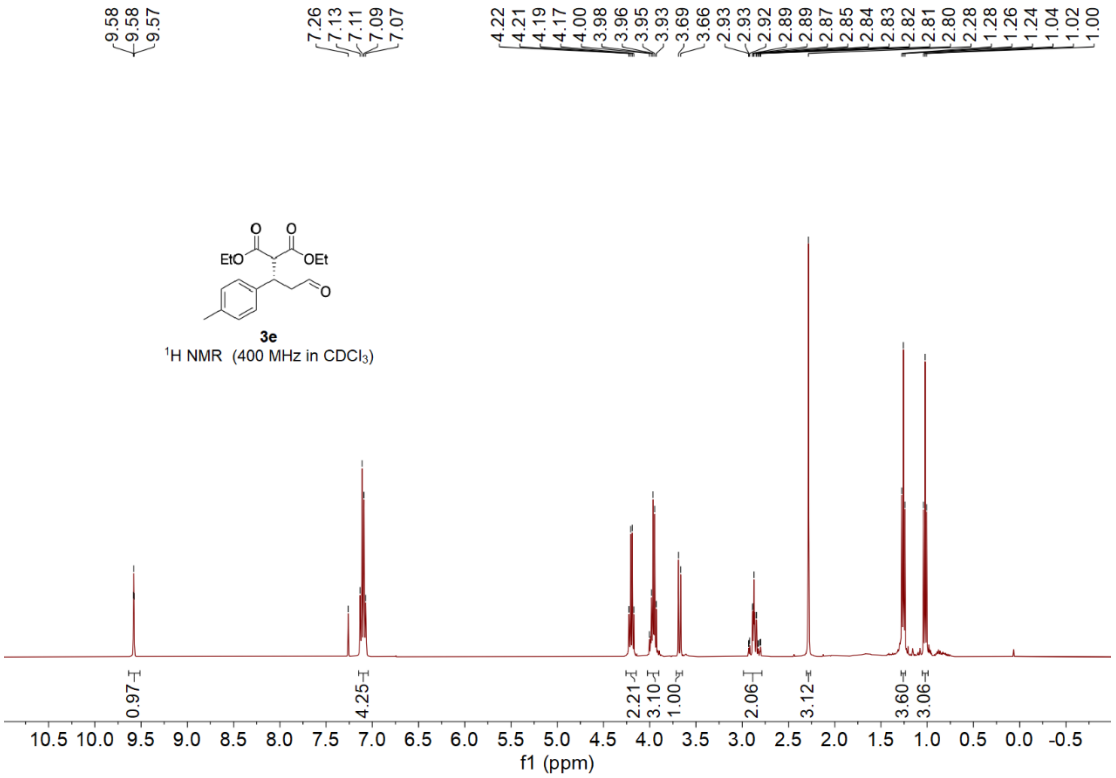

9.60 9.60 9.60 9.59 7.43 7.43 7.42 7.41 7.41 7.40 7.26 7.15 7.15 7.14 7.13 7.13 7.13 4.23 4.23 4.21 4.21 4.19 4.19 4.18 4.17 4.02 4.00 3.99 3.97 3.95 3.68 3.66 2.98 2.98 2.97 2.97 2.94 2.93 2.93 2.92 2.90 2.90 2.88 2.87 2.86 2.84 2.83 1.28 1.26 1.24 1.07 1.05 1.03

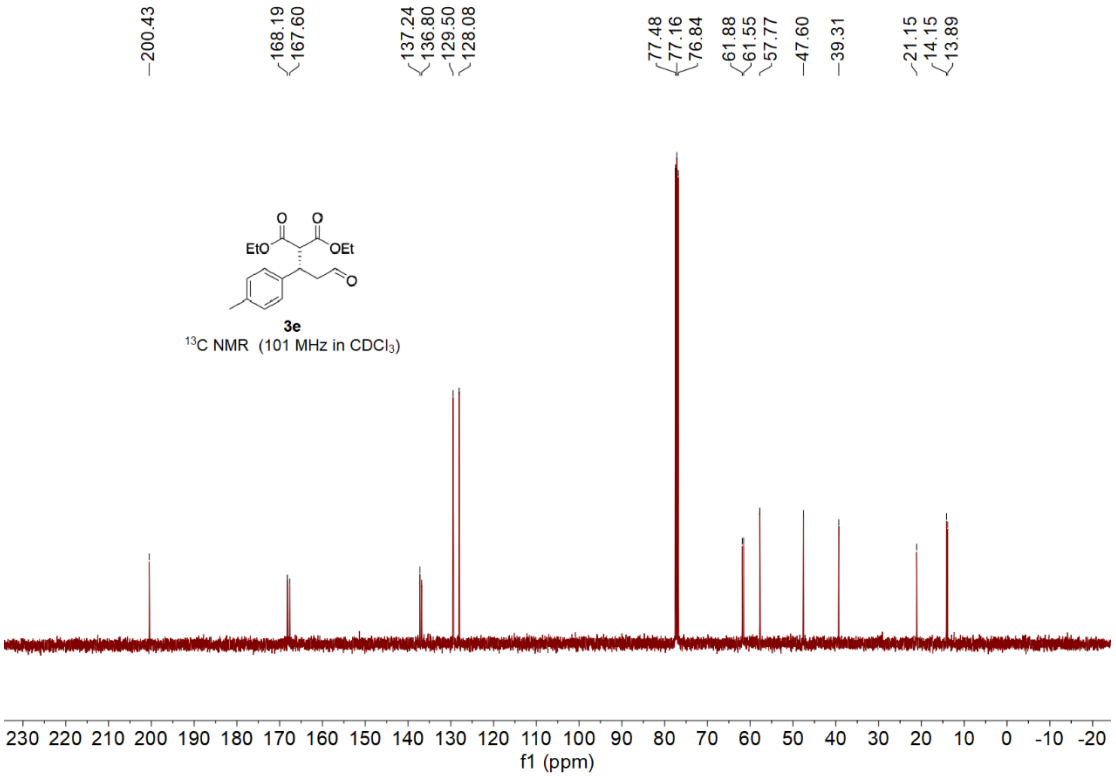

EtO
OEt
O
O
O
Br
3d
¹H NMR (400 MHz in CDCl₃)
0.99
1.97
1.98
2.03
3.01
1.00
2.06
3.81
3.00
.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

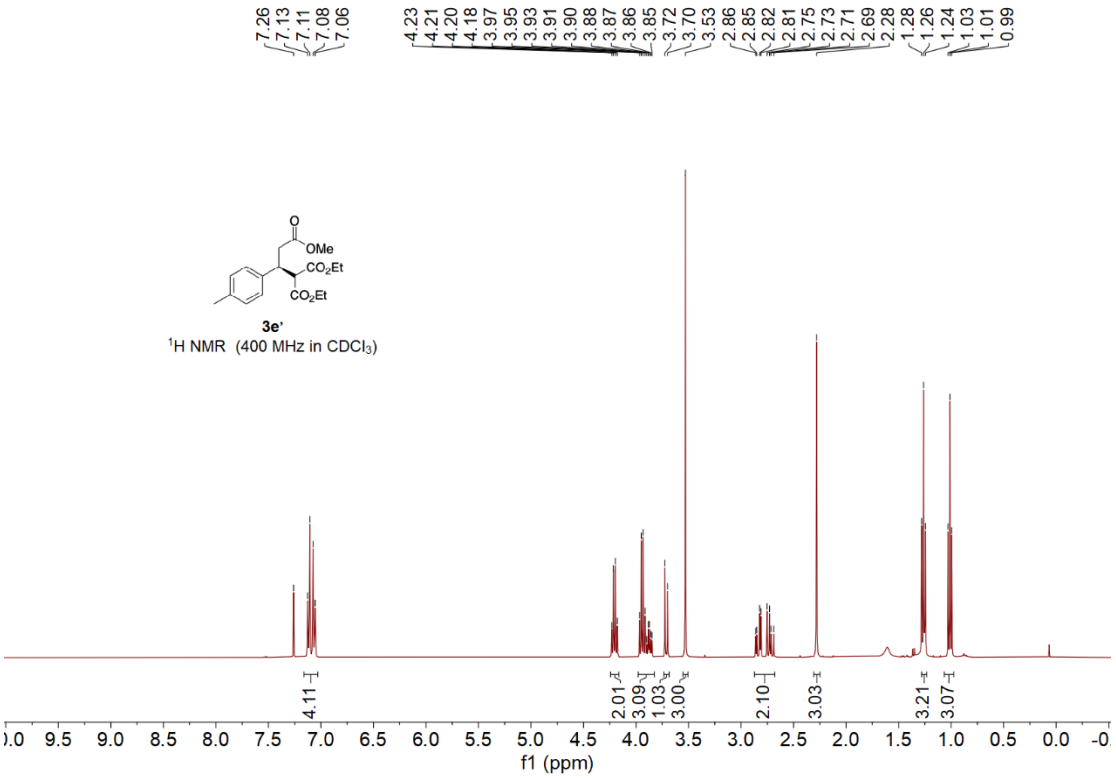

199.60
167.94
167.42
139.16
131.94
130.06
121.54
77.48
77.16
76.84
62.05
61.76
57.30
47.47
38.93
14.16
13.94
EtO
OEt
O
O
O
Br
3d
¹³C NMR (101 MHz in CDCl₃)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


7.41
7.39
7.26
7.14
7.12
4.23
4.22
4.20
4.18
3.99
3.97
3.95
3.93
3.92
3.91
3.89
3.88
3.87
3.86
3.71
3.68
3.54
2.87
2.86
2.83
2.82
2.74
2.72
2.70
2.68
1.28
1.26
1.25
1.05
1.04
1.02
OMe
CO2Et
CO2Et
Br
3d'
1H NMR (400 MHz in CDCl3)
2.00
2.02
2.00
1.99
1.05
1.10
3.00
2.13
3.67
3.03
f1 (ppm)


171.49
167.92
167.45
139.13
131.72
130.06
121.42
77.48
77.16
76.84
61.97
61.67
57.12
51.84
40.97
38.41
14.18
13.93
OMe
CO2Et
CO2Et
Br
3d'
13C NMR (101 MHz in CDCl3)
f1 (ppm)

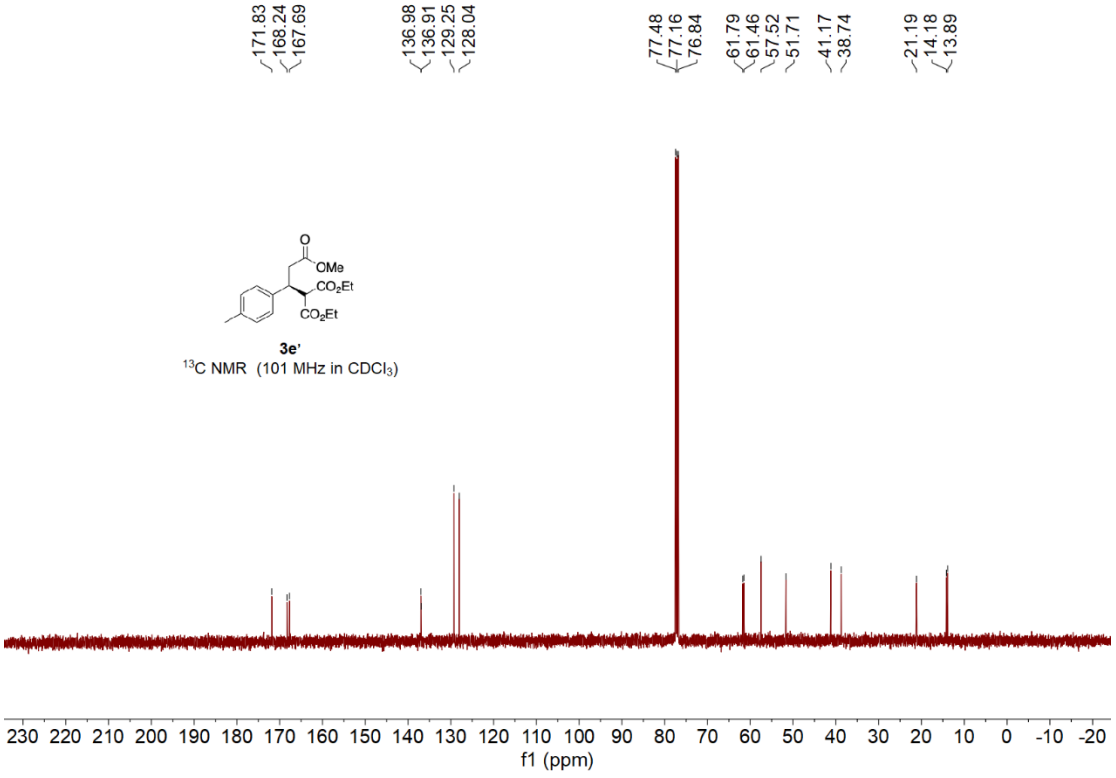

9.58
9.58
9.57
7.26
7.13
7.11
7.09
7.07
4.22
4.21
4.19
4.17
4.00
3.98
3.96
3.95
3.93
3.69
3.66
2.93
2.93
2.92
2.89
2.89
2.87
2.85
2.84
2.83
2.82
2.81
2.80
2.28
1.28
1.26
1.24
1.04
1.02
1.00
EtO
OEt
O
O
O
3e
1H NMR (400 MHz in CDCl3)
0.97
4.25
2.21
3.10
1.00
2.06
3.12
3.60
3.06
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


200.43
168.19
167.60
137.24
136.80
129.50
128.08
77.48
77.16
76.84
61.88
61.55
57.77
47.60
39.31
21.15
14.15
13.89
EtO
OEt
O
O
O
3e
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)



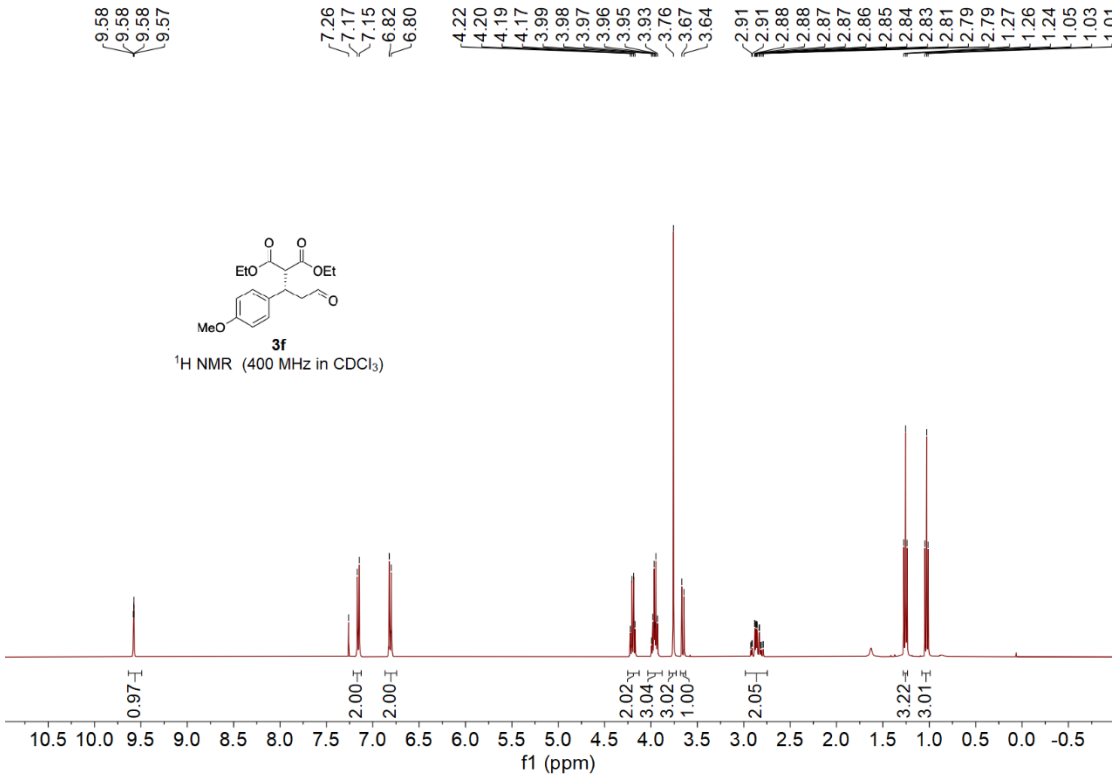
3e'

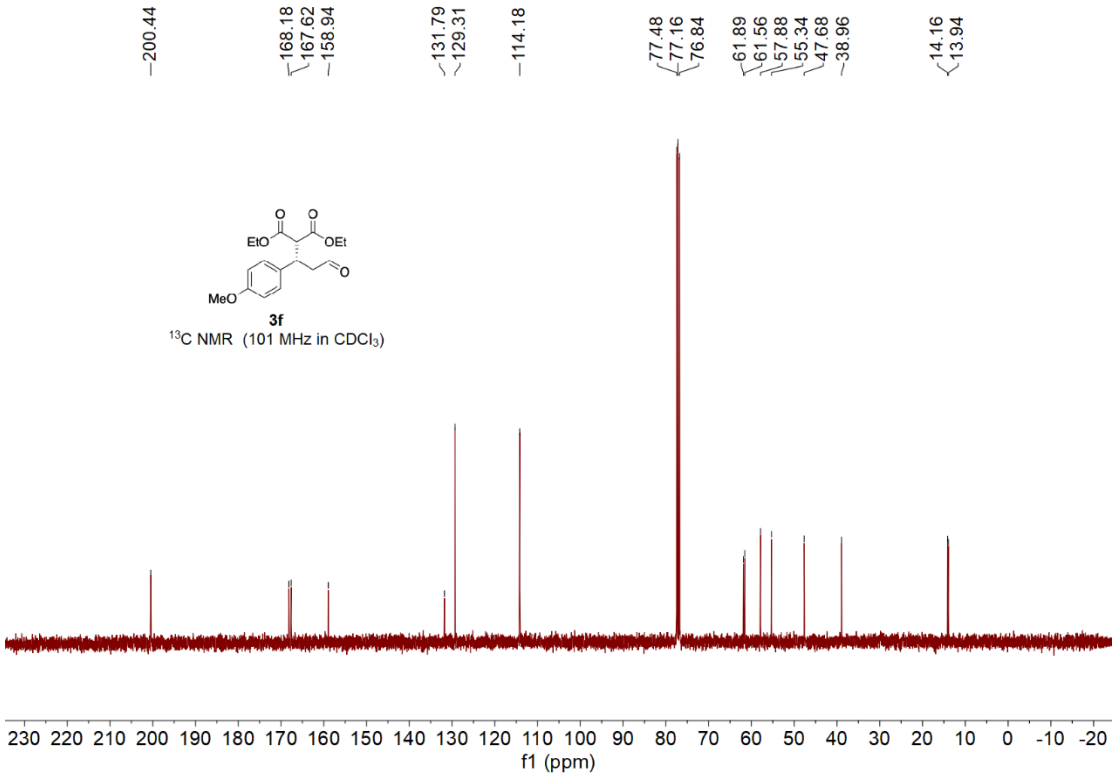
^{1}H NMR (400 MHz in $CDCl_3$)

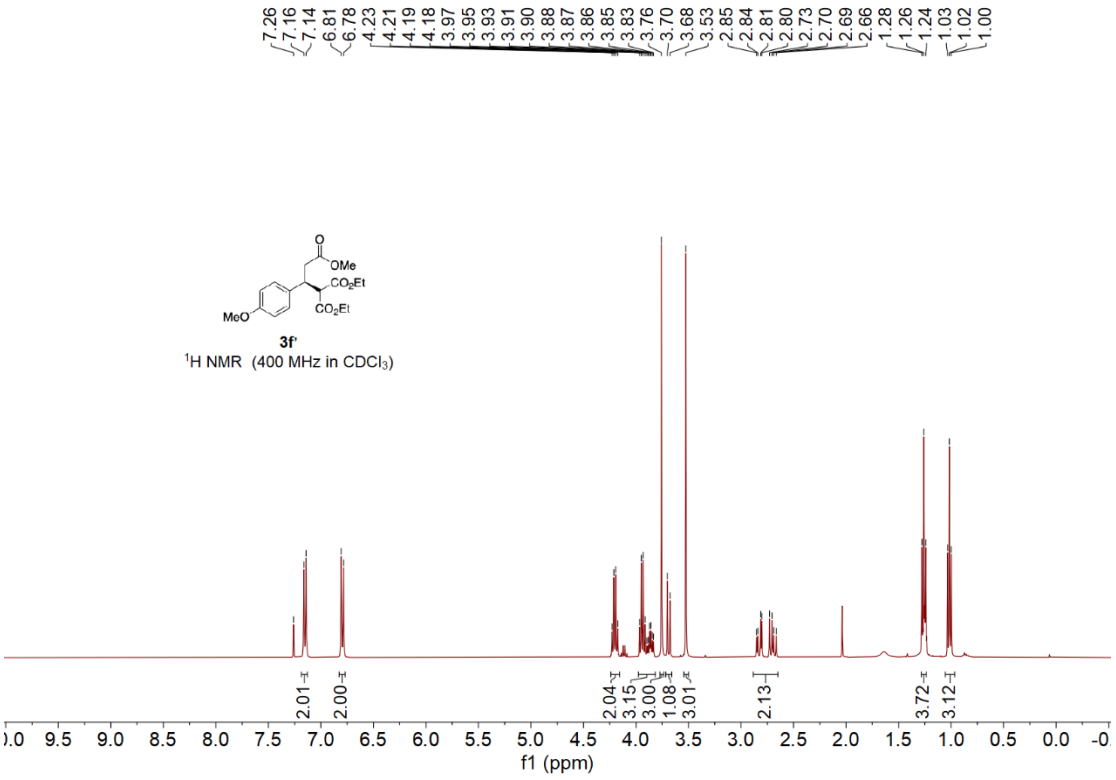


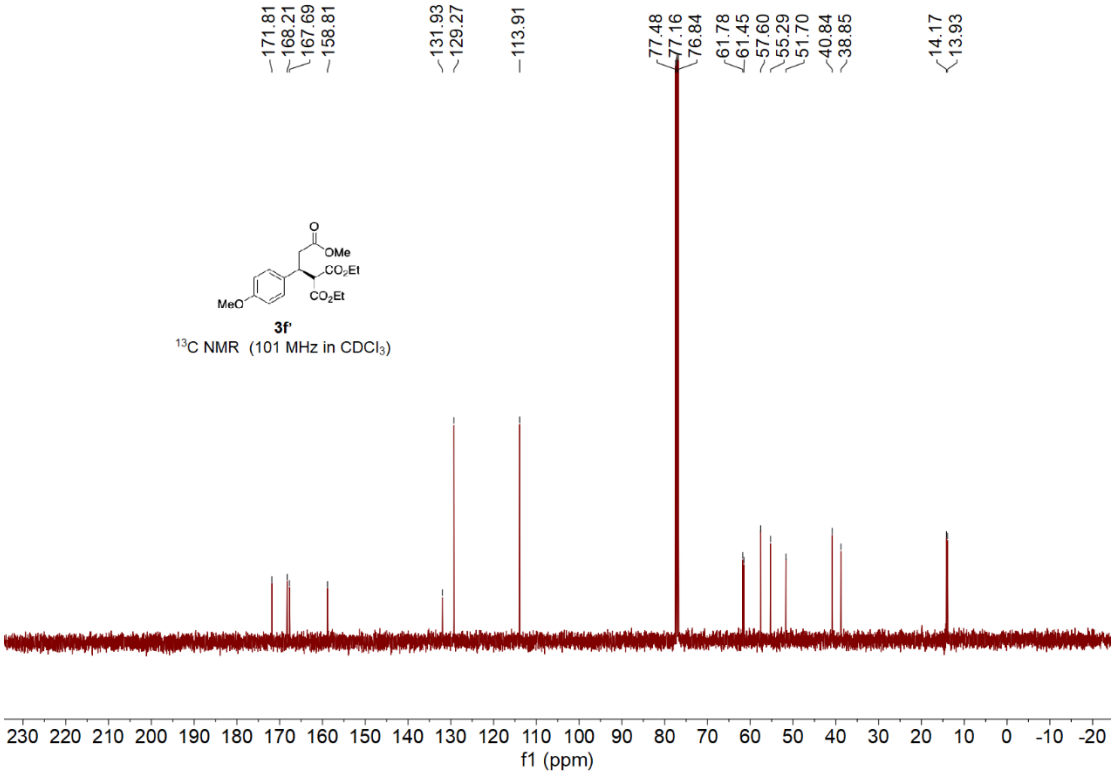
3e'

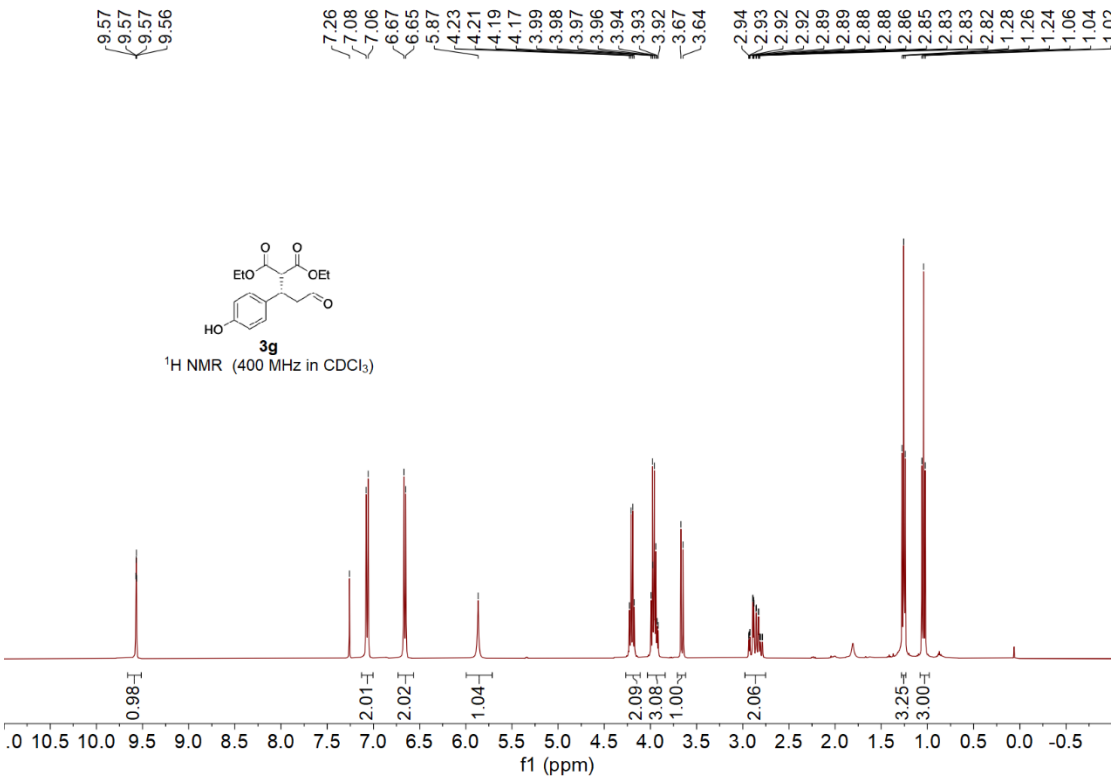
^{13}C NMR (101 MHz in $CDCl_3$)

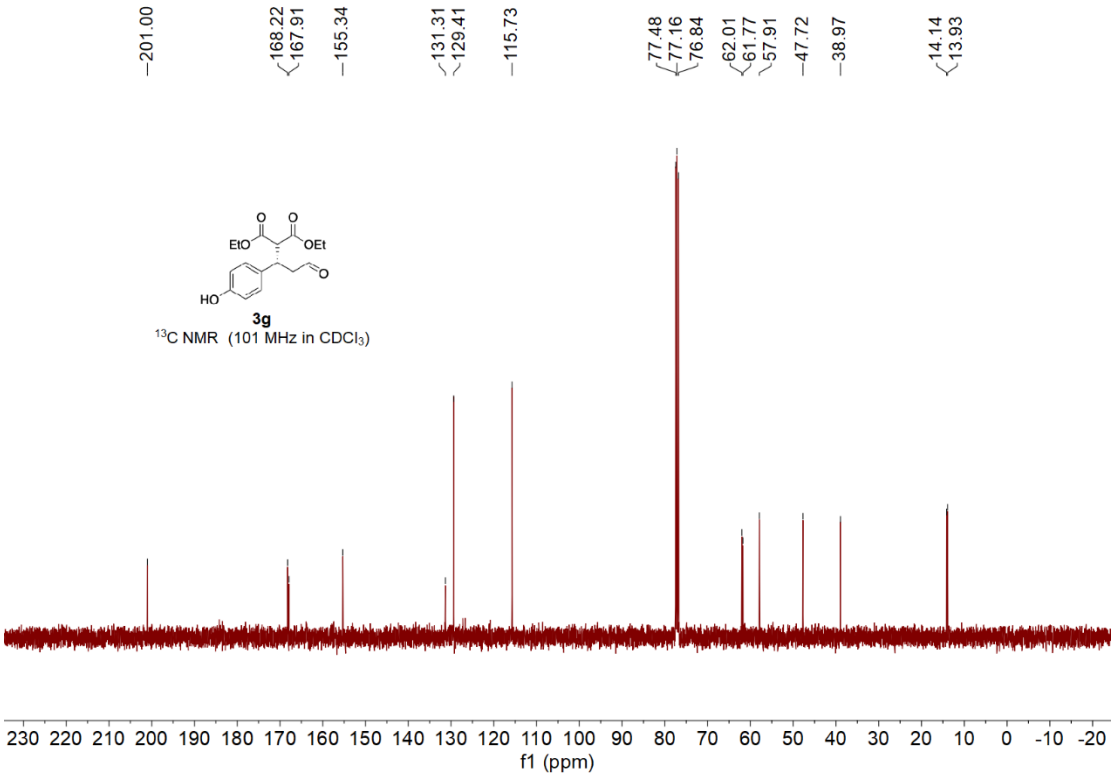

9.58
9.58
9.58
9.57
7.26
7.17
7.15
6.82
6.80
4.22
4.20
4.19
4.17
3.99
3.98
3.97
3.96
3.95
3.93
3.76
3.67
3.64
2.91
2.91
2.88
2.88
2.87
2.87
2.86
2.85
2.84
2.83
2.81
2.79
2.79
1.27
1.26
1.24
1.05
1.03
EtO
OEt
O
MeO
3f
^{1}H NMR (400 MHz in CDCl$_3$)
0.97
2.00
2.00
2.02
3.04
3.02
1.00
2.05
3.22
3.01
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

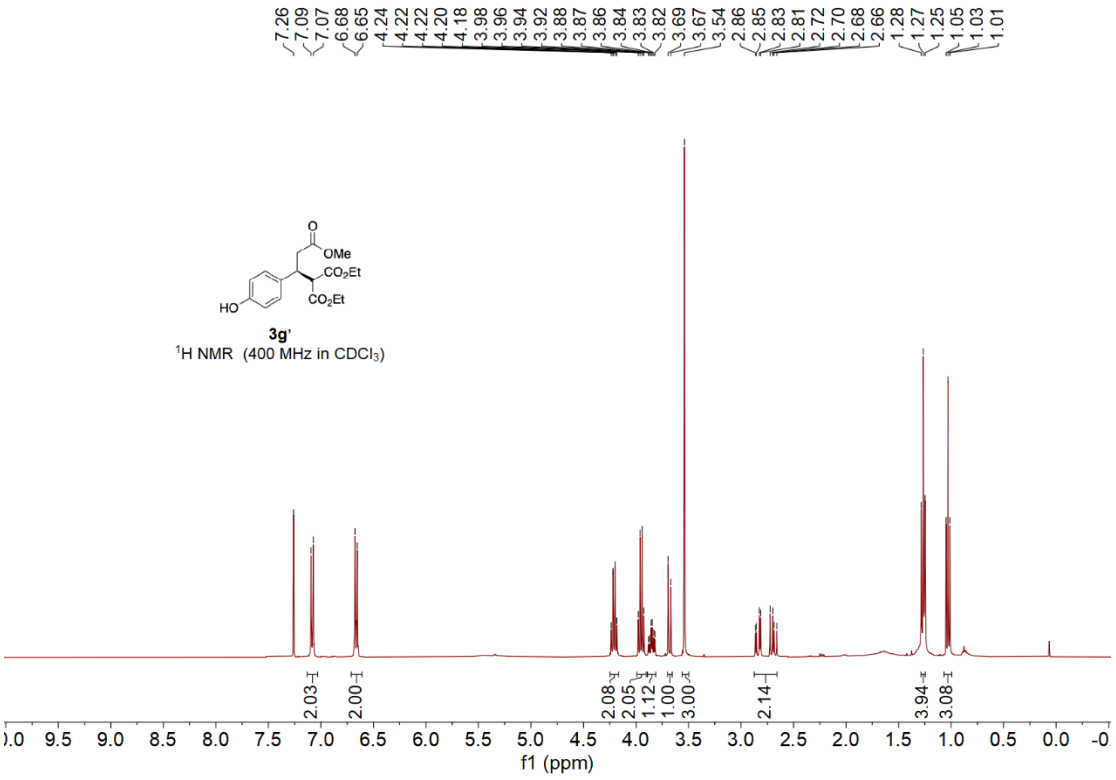

200.44
168.18
167.62
158.94
131.79
129.31
114.18
77.48
77.16
76.84
61.89
61.56
57.88
55.34
47.68
38.96
14.16
13.94
EtO
OEt
O
MeO
3f
^{13}C NMR (101 MHz in CDCl$_3$)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


7.26
7.16
7.14
6.81
6.78
4.23
4.21
4.19
4.18
3.97
3.95
3.93
3.91
3.90
3.88
3.87
3.86
3.85
3.83
3.76
3.70
3.68
3.53
2.85
2.84
2.81
2.80
2.73
2.70
2.69
2.66
1.28
1.26
1.24
1.03
1.02
1.00
OMe
CO2Et
CO2Et
MeO
3f'
1H NMR (400 MHz in CDCl3)
2.01
2.00
2.04
3.15
3.00
1.08
3.01
2.13
3.72
3.12
f1 (ppm)


171.81
168.21
167.69
158.81
131.93
129.27
113.91
77.48
77.16
76.84
61.78
61.45
57.60
55.29
51.70
40.84
38.85
14.17
13.93
OMe
CO2Et
CO2Et
MeO
3f'
13C NMR (101 MHz in CDCl3)
f1 (ppm)

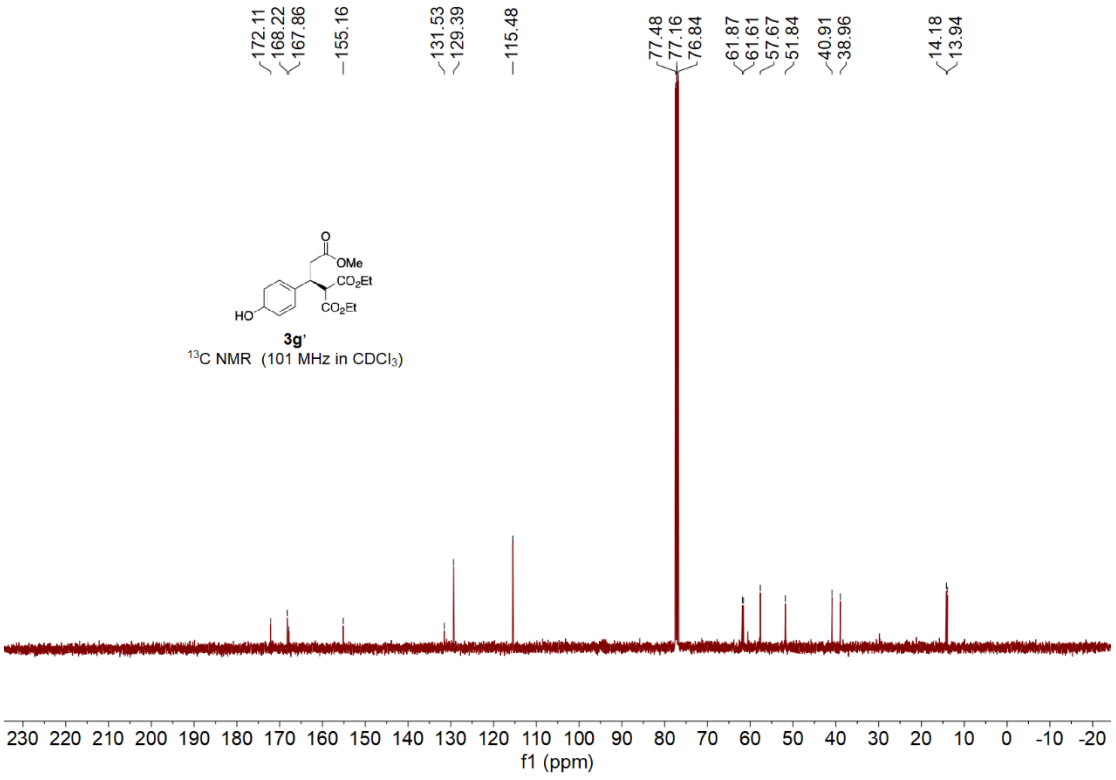

9.57
9.57
9.57
9.56
7.26
7.08
7.06
6.67
6.65
5.87
4.23
4.21
4.19
4.17
3.99
3.98
3.97
3.96
3.94
3.93
3.92
3.67
3.64
2.94
2.93
2.92
2.92
2.89
2.89
2.88
2.88
2.86
2.85
2.83
2.83
2.82
1.28
1.26
1.24
1.06
1.04
EtO
OEt
HO
3g
1H NMR (400 MHz in CDCl3)
0.98
2.01
2.02
1.04
2.09
3.08
1.00
2.06
3.25
3.00
f1 (ppm)


201.00
168.22
167.91
155.34
131.31
129.41
115.73
77.48
77.16
76.84
62.01
61.77
57.91
47.72
38.97
14.14
13.93
EtO
OEt
HO
3g
13C NMR (101 MHz in CDCl3)
f1 (ppm)



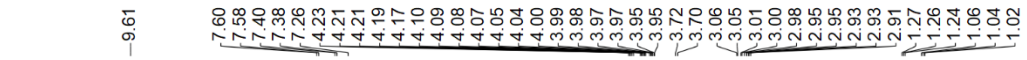
3g'

^{1}H NMR (400 MHz in $CDCl_3$)



3g'

^{13}C NMR (101 MHz in $CDCl_3$)


9.61
7.60
7.58
7.40
7.38
7.26
4.23
4.21
4.21
4.19
4.17
4.10
4.09
4.08
4.07
4.05
4.04
4.00
3.99
3.98
3.97
3.97
3.95
3.95
3.72
3.70
3.06
3.05
3.01
3.00
2.98
2.95
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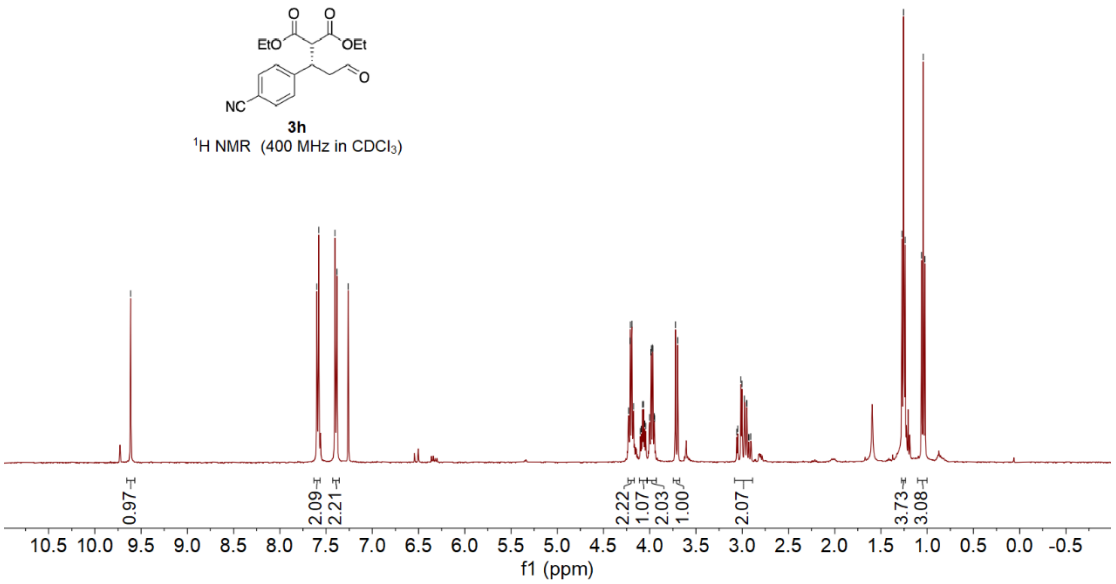
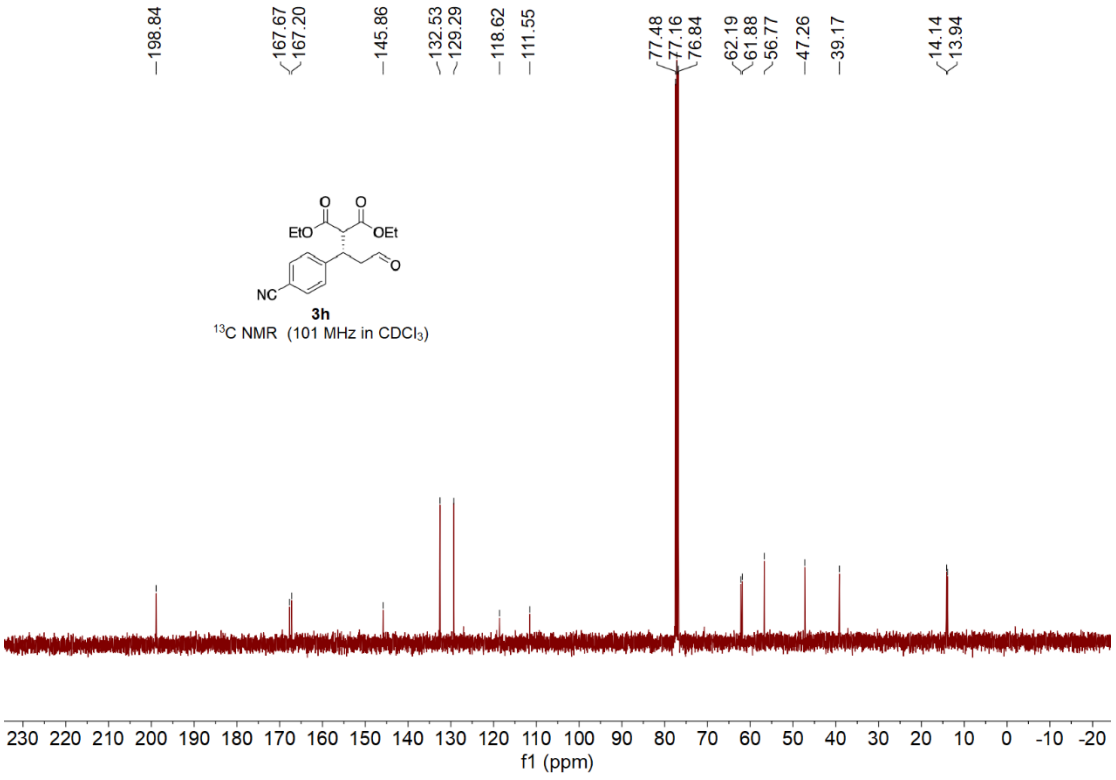
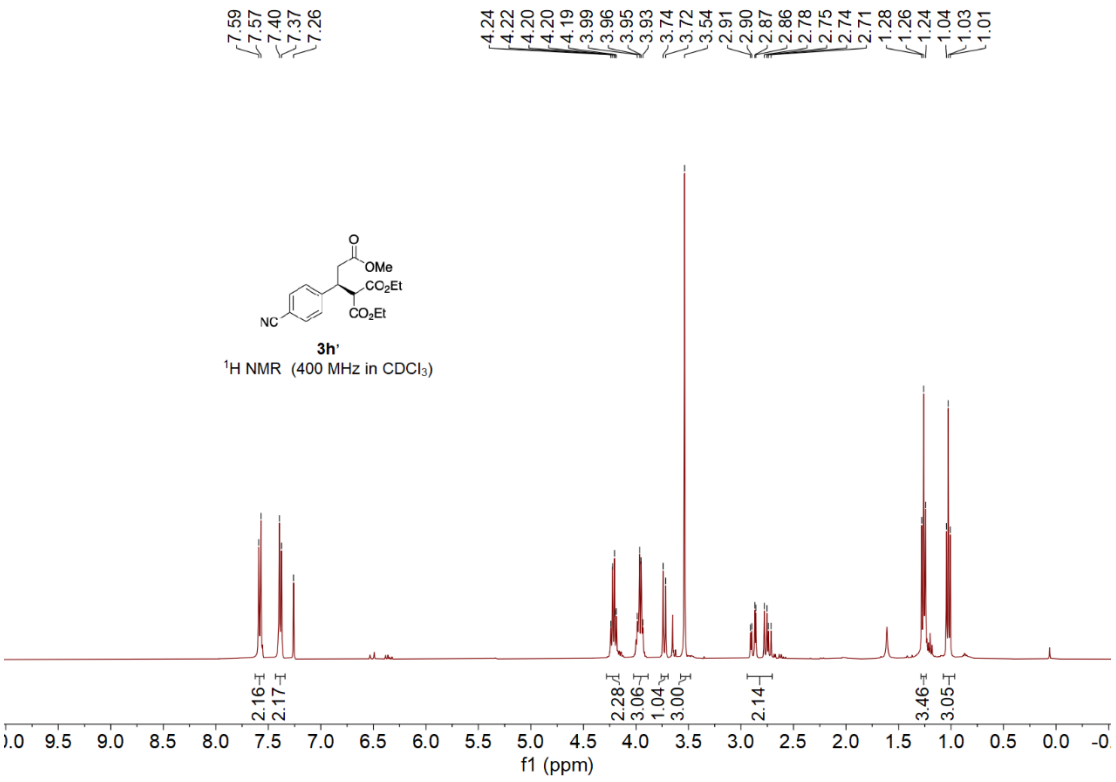
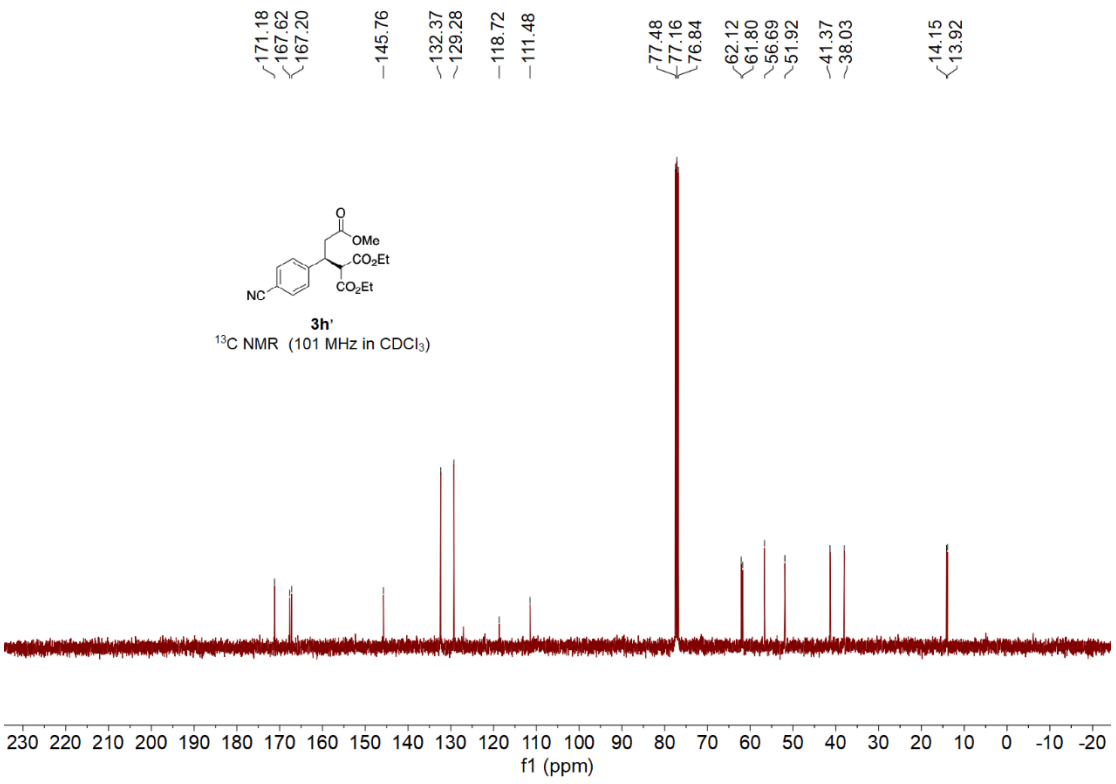
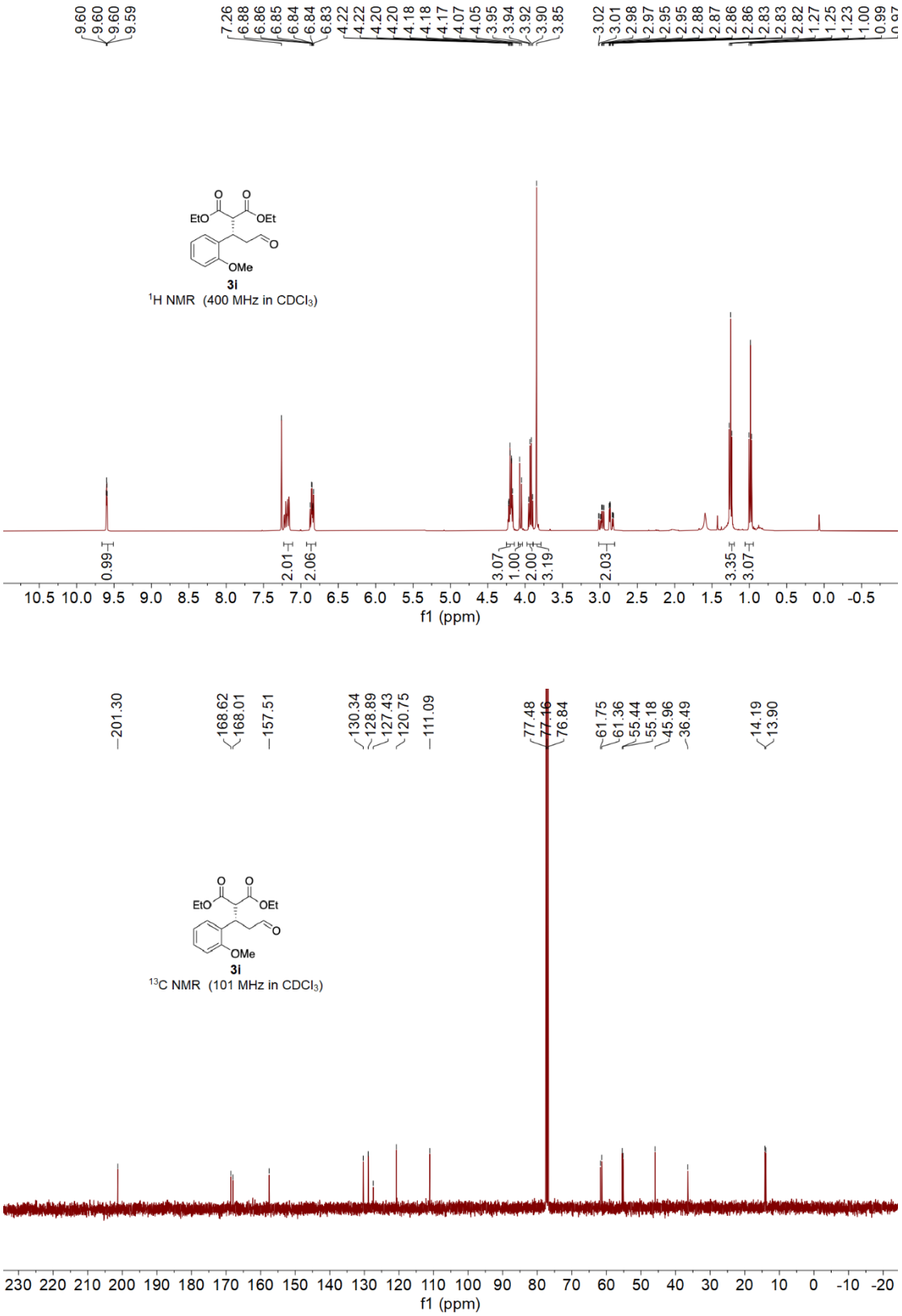
2.93
2.93
2.91
1.27
1.26
1.24
1.06
1.04
1.02


EtO
OEt
O
O
O
NC
3h
1H NMR (400 MHz in CDCl3)
0.97
2.09
2.21
2.22
1.07
2.03
1.00
2.07
3.73
3.08
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


198.84
167.67
167.20
145.86
132.53
129.29
118.62
111.55
77.48
77.16
76.84
62.19
61.88
56.77
47.26
39.17
14.14
13.94
EtO
OEt
O
O
O
NC
3h
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


7.59
7.57
7.40
7.37
7.26
4.24
4.22
4.20
4.20
4.19
3.99
3.96
3.95
3.93
3.74
3.72
3.54
2.91
2.90
2.87
2.86
2.78
2.75
2.74
2.71
1.28
1.26
1.24
1.04
1.03
1.01
OMe
CO2Et
CO2Et
NC
3h'
1H NMR (400 MHz in CDCl3)
2.16
2.17
2.28
3.06
1.04
3.00
2.14
3.46
3.05
f1 (ppm)


171.18
167.62
167.20
145.76
132.37
129.28
118.72
111.48
77.48
77.16
76.84
62.12
61.80
56.69
51.92
41.37
38.03
14.15
13.92
OMe
CO2Et
CO2Et
NC
3h'
13C NMR (101 MHz in CDCl3)
f1 (ppm)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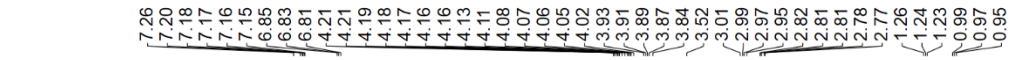
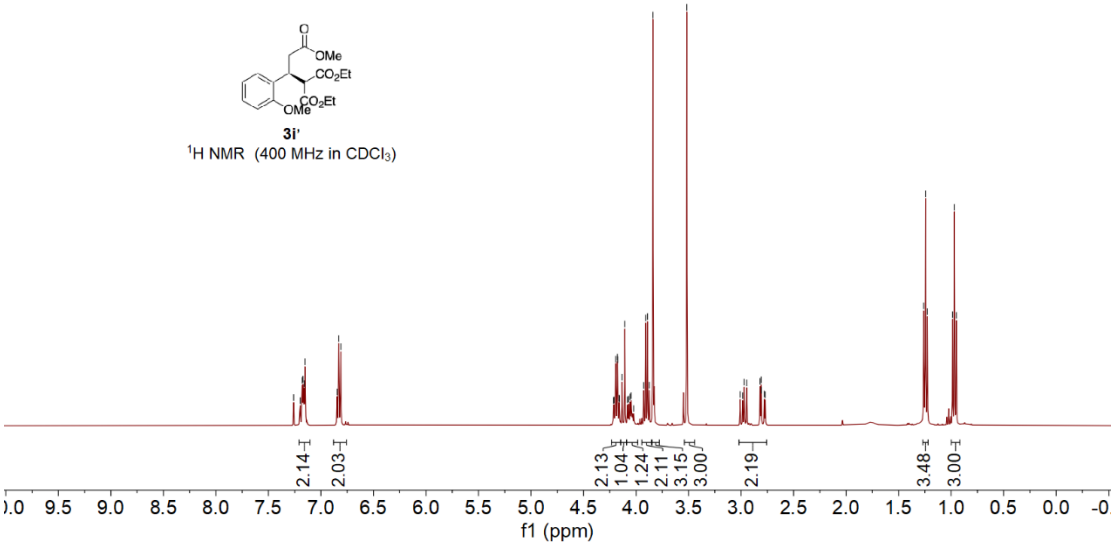
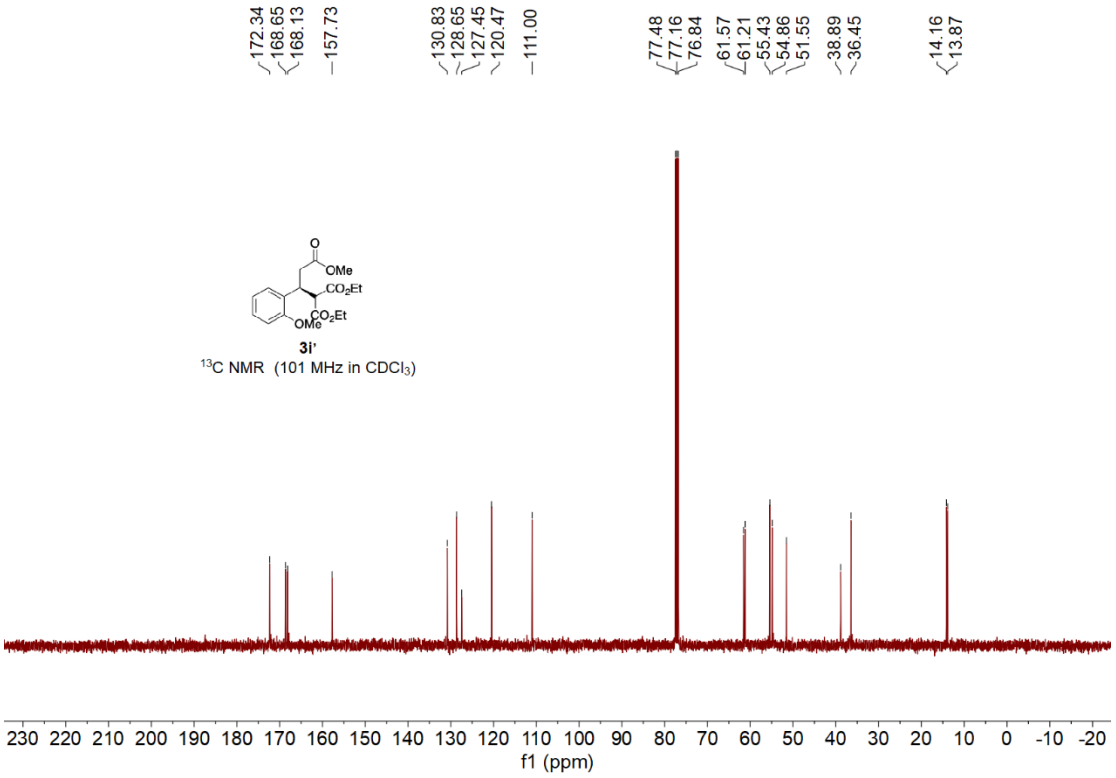
9.60
9.60
9.59
7.26
6.88
6.86
6.85
6.84
6.84
6.83
4.22
4.22
4.20
4.20
4.18
4.18
4.17
4.07
4.05
3.95
3.94
3.92
3.90
3.85
3.02
3.01
2.98
2.97
2.95
2.95
2.88
2.87
2.86
2.86
2.83
2.83
2.82
1.27
1.25
1.23
1.00
0.99
EtO
OEt
O
OMe
3l
1H NMR (400 MHz in CDCl3)
0.99
2.01
2.06
3.07
1.00
2.00
3.19
2.03
3.35
3.07
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
201.30
168.62
168.01
157.51
130.34
128.89
127.43
120.75
111.09
77.48
77.16
76.84
61.75
61.36
55.44
55.18
45.96
36.49
14.19
13.90
EtO
OEt
O
OMe
3l
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


7.26
7.20
7.18
7.17
7.16
7.15
6.85
6.83
6.81
4.21
4.21
4.19
4.18
4.17
4.16
4.16
4.13
4.11
4.08
4.07
4.06
4.05
4.02
3.93
3.91
3.89
3.87
3.84
3.52
3.01
2.99
2.97
2.95
2.82
2.81
2.81
2.78
2.77
1.26
1.24
1.23
0.99
0.97
0.95


O
OMe
CO2Et
CO2Et
OMe
3i'
1H NMR (400 MHz in CDCl3)
2.14
2.03
2.13
1.04
1.24
2.11
3.15
3.00
2.19
3.48
3.00
f1 (ppm)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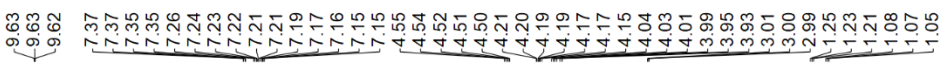
168.65
168.13
157.73
130.83
128.65
127.45
120.47
111.00
77.48
77.16
76.84
61.57
61.21
55.43
54.86
51.55
38.89
36.45
14.16
13.87
O
OMe
CO2Et
CO2Et
OMe
3i'
13C NMR (101 MHz in CDCl3)
f1 (ppm)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9.63
9.62
7.37
7.37
7.35
7.35
7.26
7.24
7.23
7.22
7.21
7.21
7.19
7.17
7.16
7.15
7.15
4.55
4.54
4.52
4.51
4.50
4.21
4.20
4.19
4.19
4.17
4.17
4.15
4.04
4.03
4.01
3.99
3.95
3.93
3.01
3.00
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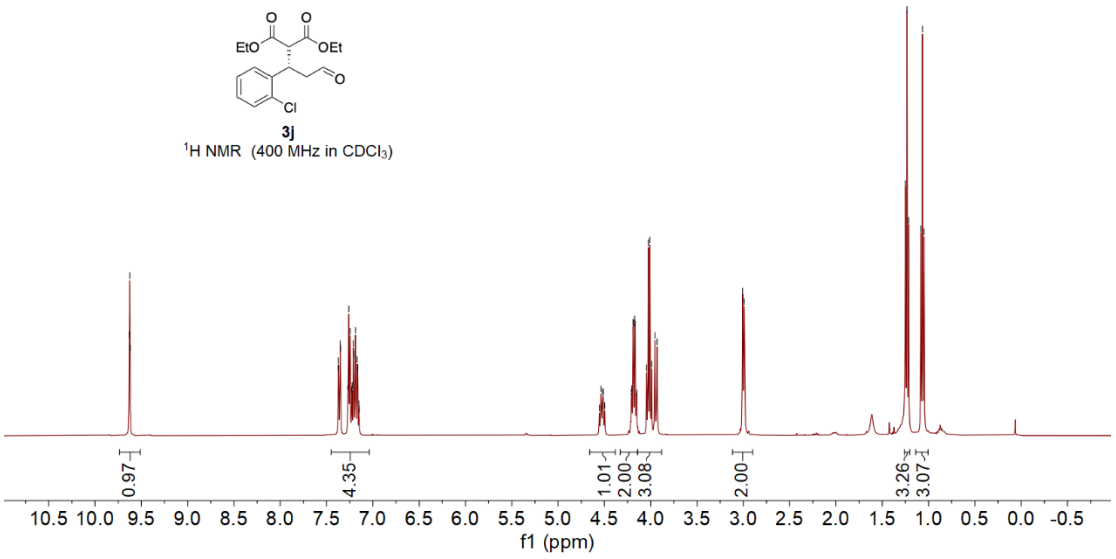
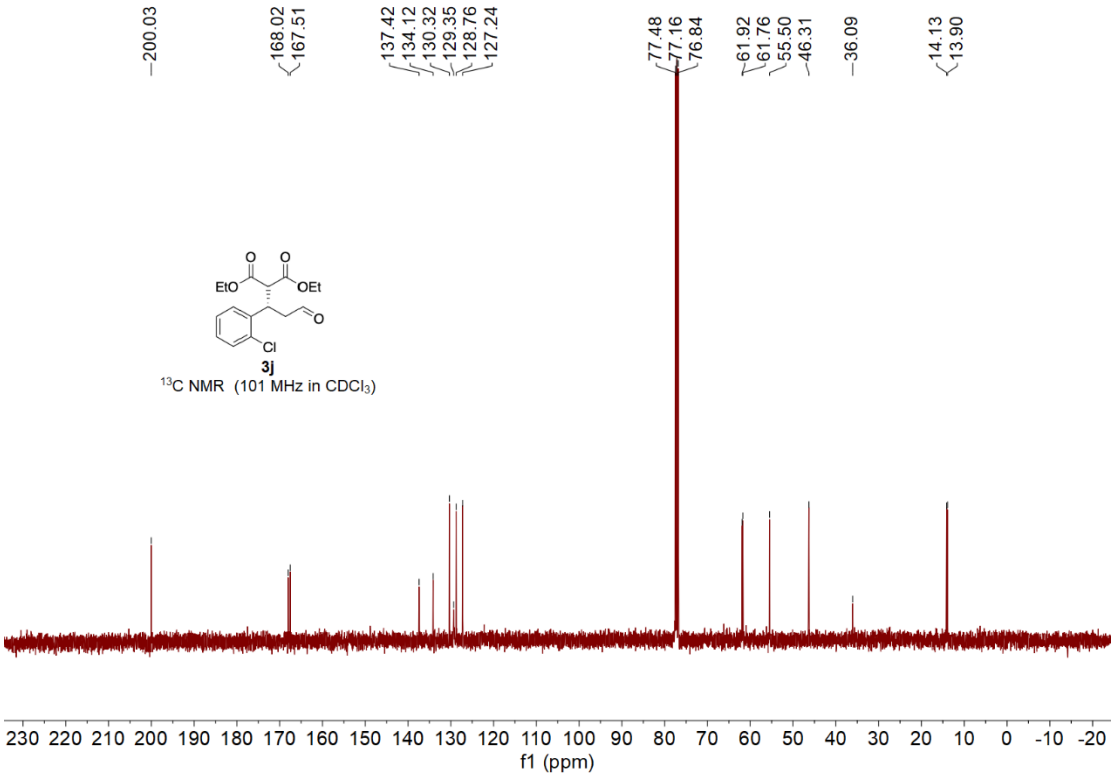
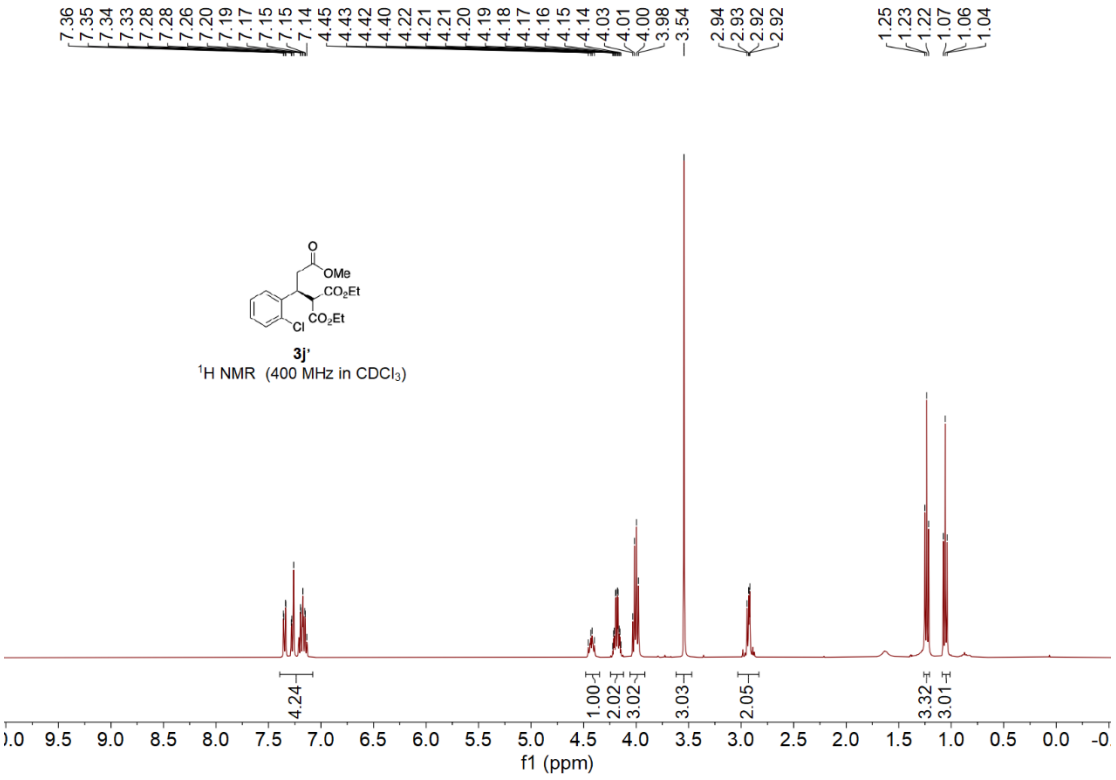
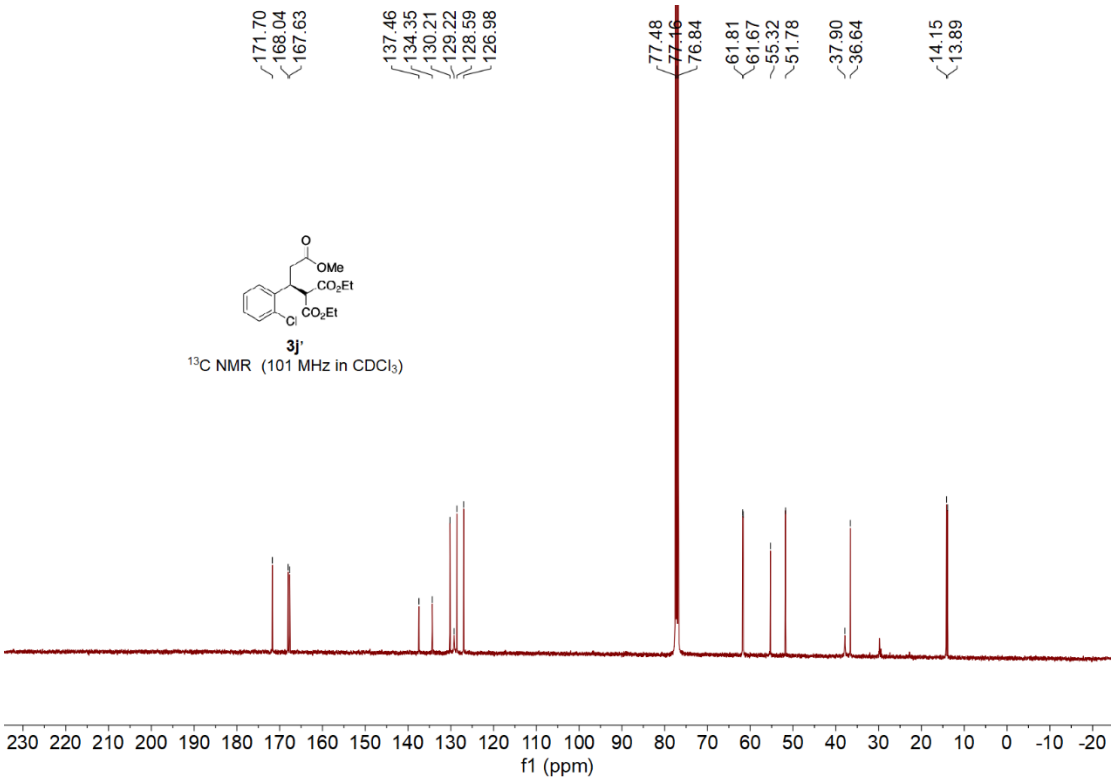
1.25
1.23
1.21
1.08
1.07
1.05


EtO
OEt
O
O
O
Cl
3j
^{1}H NMR (400 MHz in CDCl$_3$)
0.97
4.35
1.01
2.00
3.08
2.00
3.26
3.07
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


200.03
168.02
167.51
137.42
134.12
130.32
129.35
128.76
127.24
77.48
77.16
76.84
61.92
61.76
55.50
46.31
36.09
14.13
13.90
EtO
OEt
O
O
O
Cl
3j
^{13}C NMR (101 MHz in CDCl$_3$)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


7.36
7.35
7.34
7.33
7.28
7.28
7.26
7.20
7.19
7.17
7.15
7.15
7.14
4.45
4.43
4.42
4.40
4.22
4.21
4.21
4.20
4.19
4.18
4.17
4.16
4.15
4.14
4.03
4.01
4.00
3.98
3.54
2.94
2.93
2.92
2.92
1.25
1.23
1.22
1.07
1.06
1.04
OMe
CO2Et
CO2Et
Cl
3j'
1H NMR (400 MHz in CDCl3)
4.24
1.00
2.02
3.02
3.03
2.05
3.32
3.01
f1 (ppm)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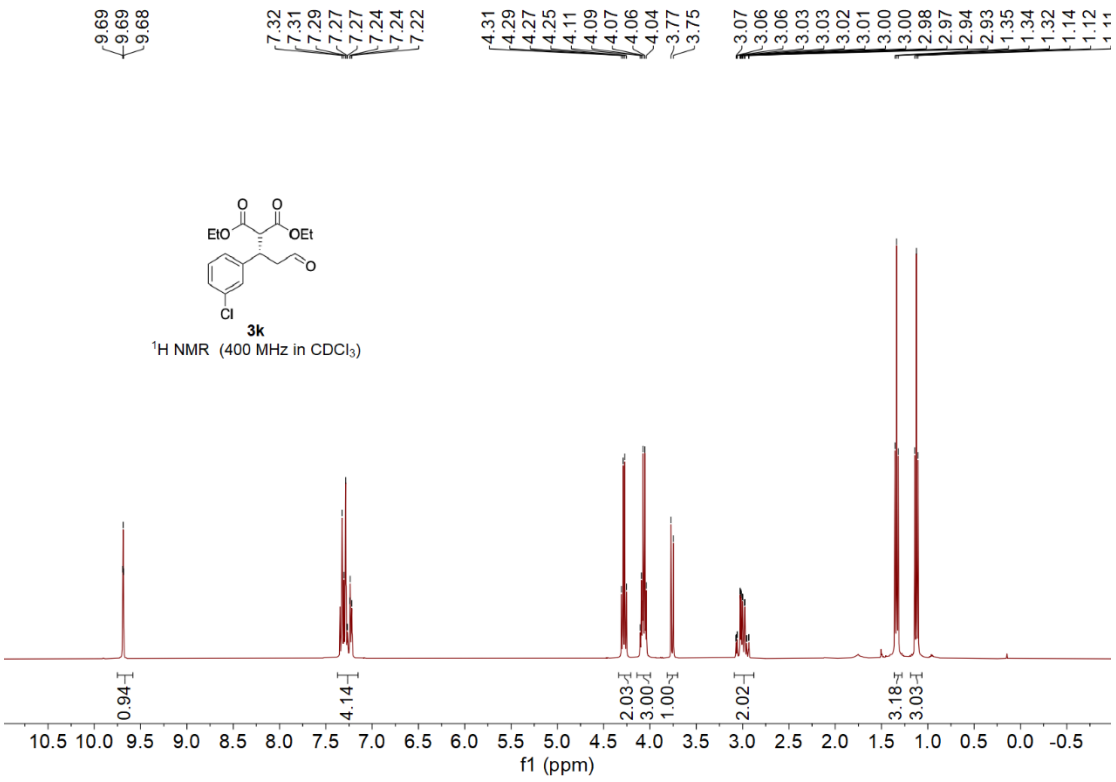
167.63
137.46
134.35
130.21
129.22
128.59
126.98
77.48
77.16
76.84
61.81
61.67
55.32
51.78
37.90
36.64
14.15
13.89
OMe
CO2Et
CO2Et
Cl
3j'
13C NMR (101 MHz in CDCl3)
f1 (ppm)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9.69
9.68
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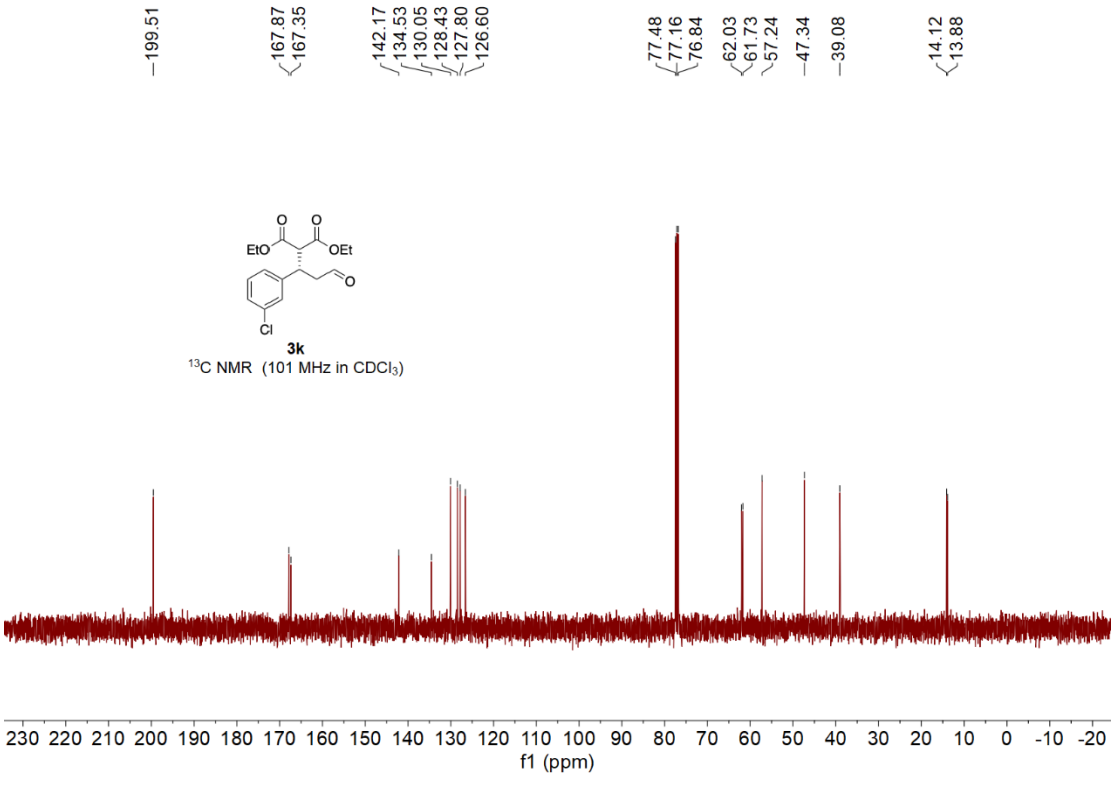
7.31
7.29
7.27
7.27
7.24
7.24
7.22
4.31
4.29
4.27
4.25
4.11
4.09
4.07
4.06
4.04
3.77
3.75
3.07
3.06
3.06
3.03
3.03
3.02
3.01
3.00
3.00
2.98
2.97
2.94
2.93
1.35
1.34
1.32
1.14
1.12
EtO
OEt
O
O
Cl
3k
1H NMR (400 MHz in CDCl3)
0.94
4.14
2.03
3.00
1.00
2.02
3.18
3.03
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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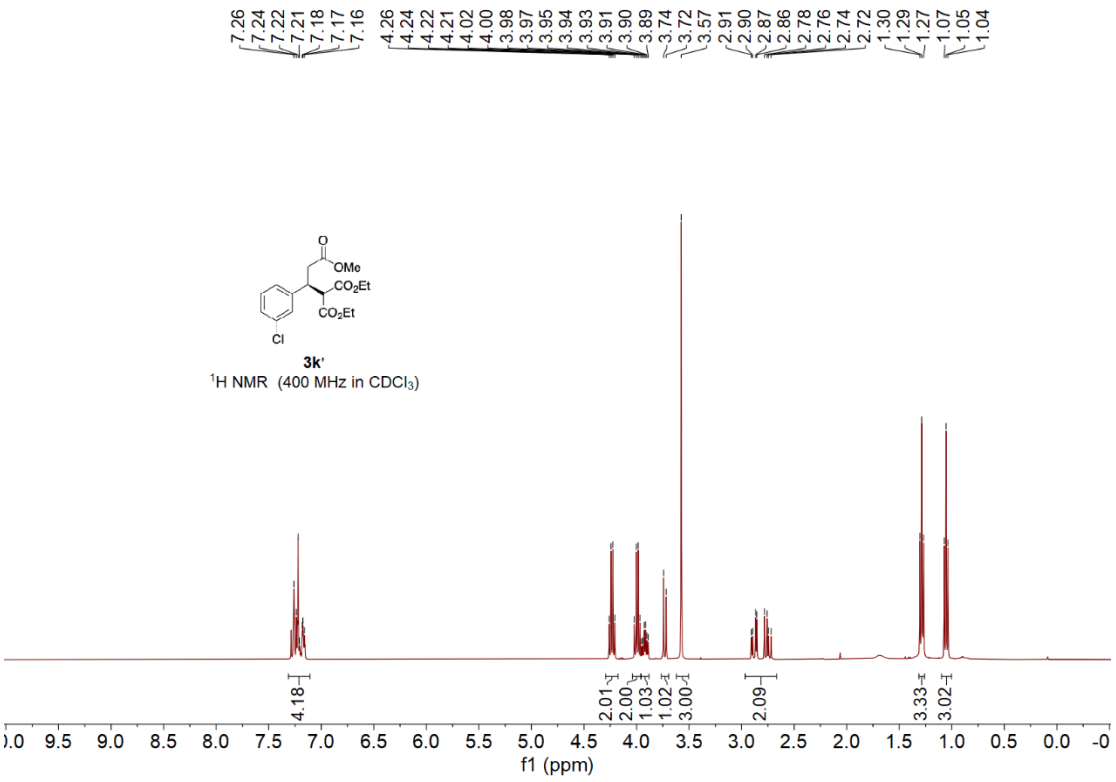
167.87
167.35
142.17
134.53
130.05
128.43
127.80
126.60
77.48
77.16
76.84
62.03
61.73
57.24
47.34
39.08
14.12
13.88
EtO
OEt
O
O
Cl
3k
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


7.26
7.24
7.22
7.21
7.18
7.17
7.16
4.26
4.24
4.22
4.21
4.02
4.00
3.98
3.97
3.95
3.94
3.93
3.91
3.90
3.89
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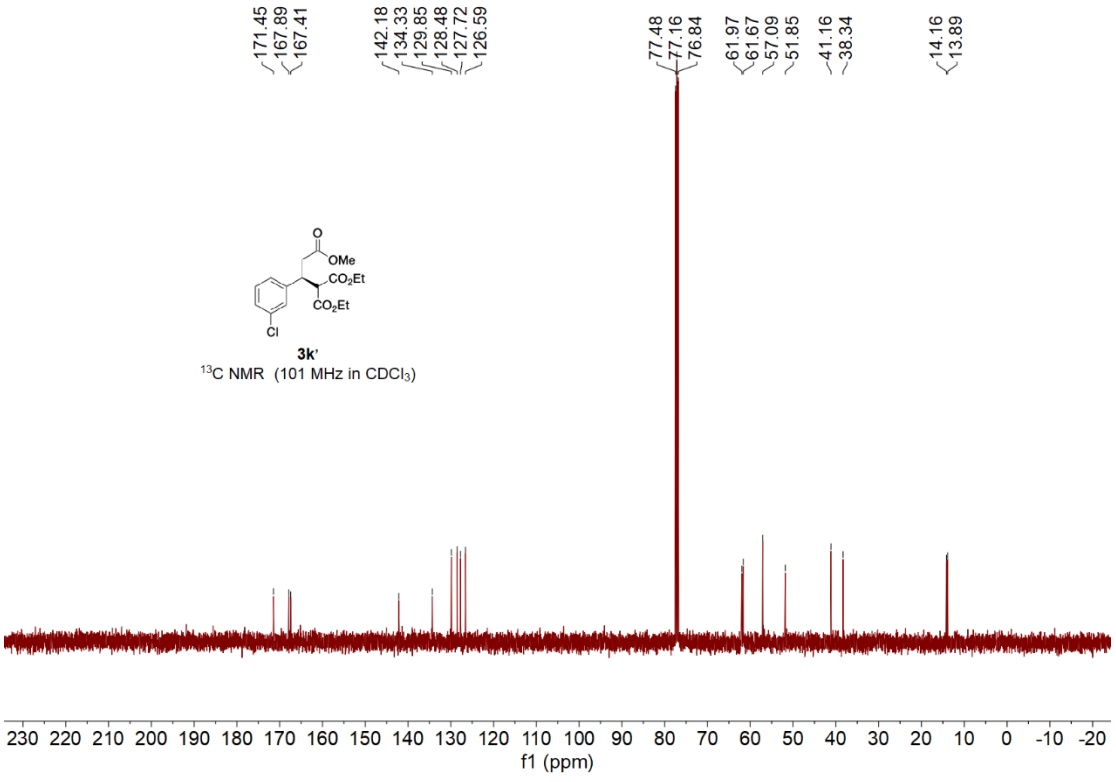
2.90
2.87
2.86
2.78
2.76
2.74
2.72
1.30
1.29
1.27
1.07
1.05
1.04
OMe
CO2Et
CO2Et
Cl
3k'
1H NMR (400 MHz in CDCl3)
4.18
2.01
2.00
1.03
1.02
3.00
2.09
3.33
3.02
f1 (ppm)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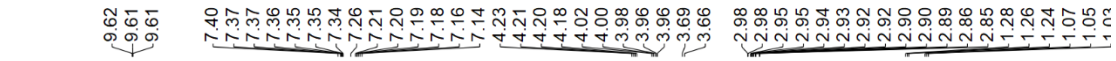
167.41
142.18
134.33
129.85
128.48
127.72
126.59
77.48
77.16
76.84
61.97
61.67
57.09
51.85
41.16
38.34
14.16
13.89
OMe
CO2Et
CO2Et
Cl
3k'
13C NMR (101 MHz in CDCl3)
f1 (ppm)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9.61
9.61
7.40
7.37
7.37
7.36
7.35
7.35
7.34
7.26
7.21
7.20
7.19
7.18
7.16
7.14
4.23
4.21
4.20
4.18
4.02
4.00
3.98
3.96
3.96
3.69
3.66
2.98
2.98
2.95
2.95
2.94
2.93
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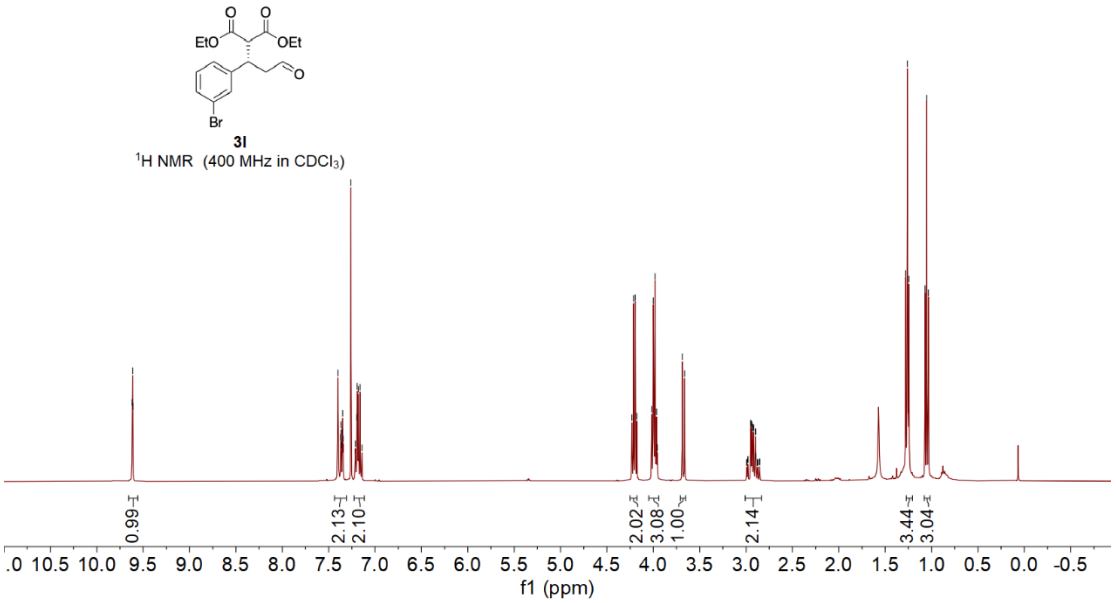
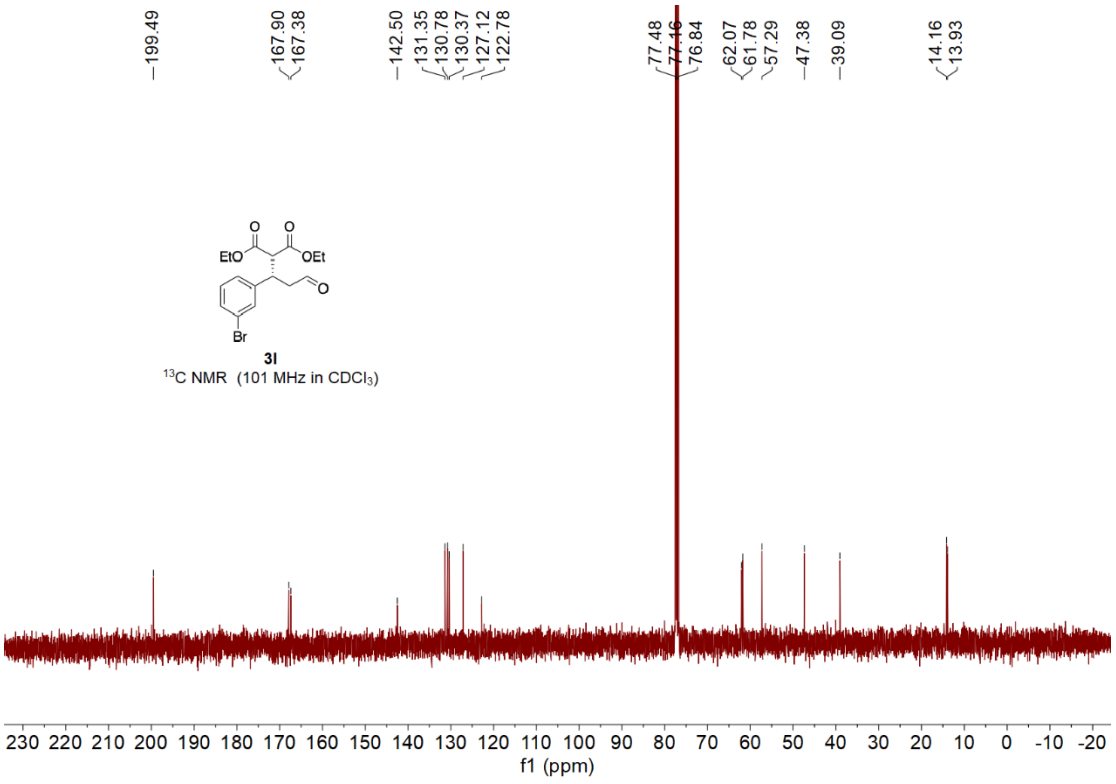
2.85
1.28
1.26
1.24
1.07
1.05


EtO
OEt
O
O
O
Br
3l
¹H NMR (400 MHz in CDCl₃)
0.99
2.13
2.10
2.02
3.08
1.00
2.14
3.44
3.04
.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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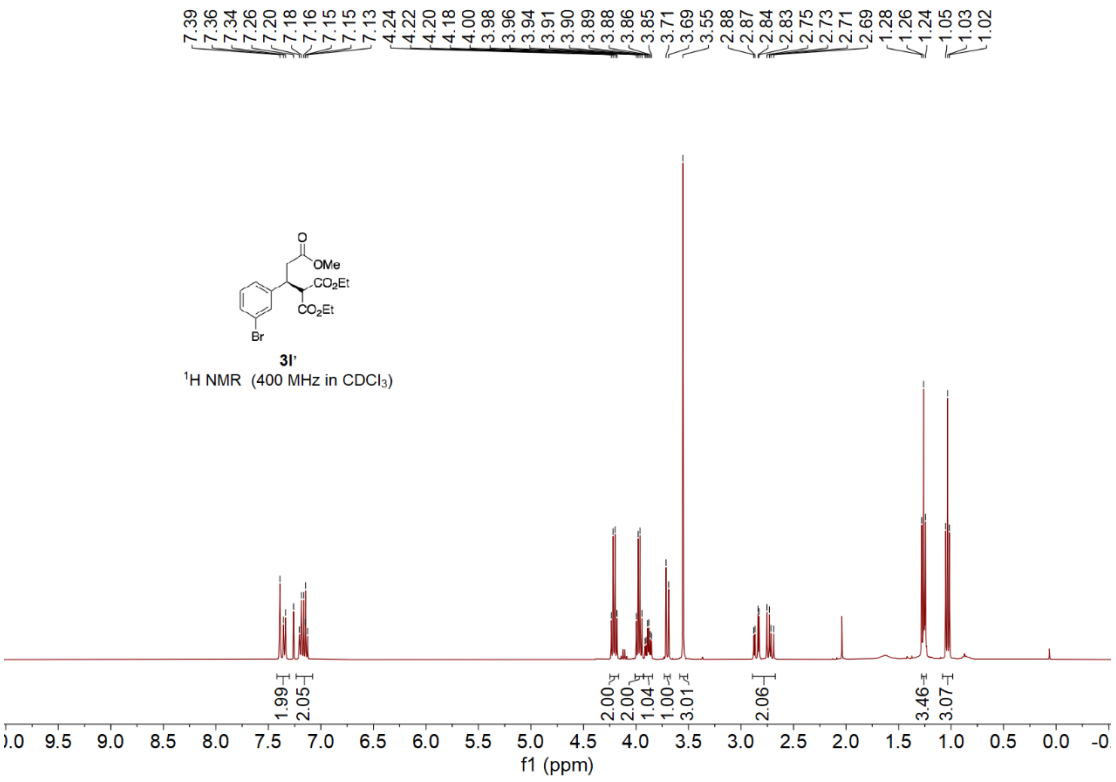
167.38
142.50
131.35
130.78
130.37
127.12
122.78
77.48
77.16
76.84
62.07
61.78
57.29
47.38
39.09
14.16
13.93
EtO
OEt
O
O
O
Br
3l
¹³C NMR (101 MHz in CDCl₃)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)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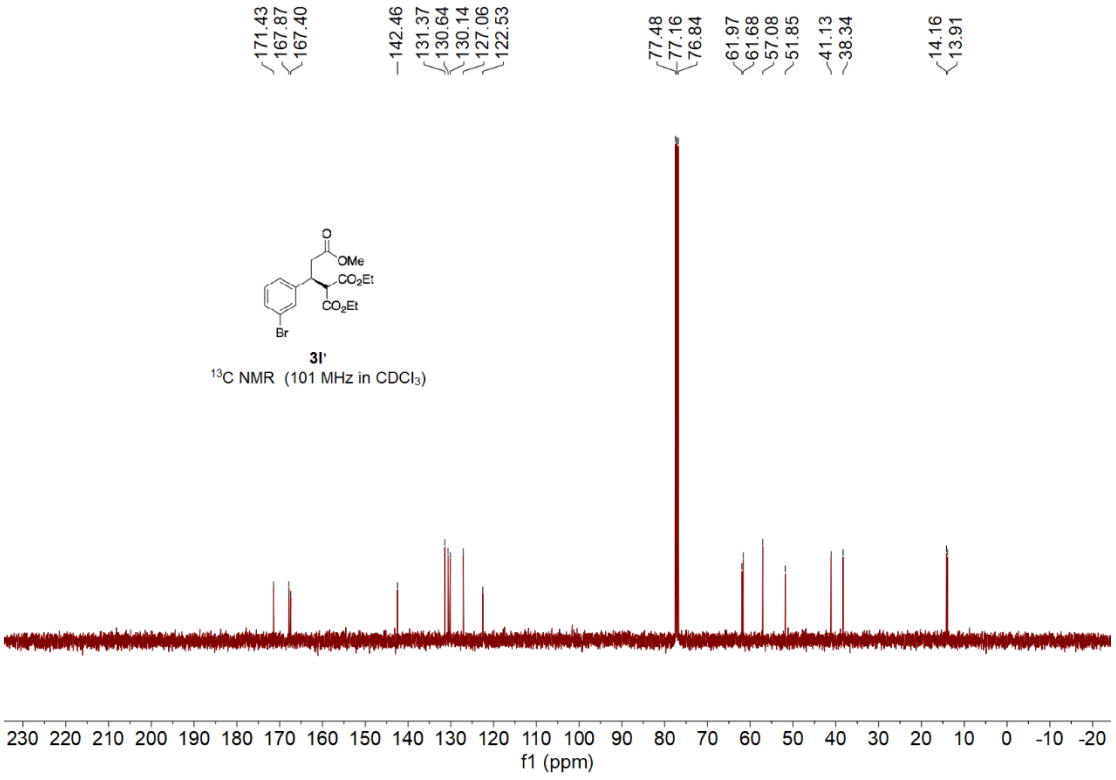
7.34
7.26
7.20
7.18
7.16
7.15
7.15
7.13
4.24
4.22
4.20
4.18
4.00
3.98
3.96
3.94
3.91
3.90
3.89
3.88
3.86
3.85
3.71
3.69
3.55
2.88
2.87
2.84
2.83
2.75
2.73
2.71
2.69
1.28
1.26
1.24
1.05
1.03
1.02
OMe
CO2Et
CO2Et
Br
3l'
1H NMR (400 MHz in CDCl3)
1.99
2.05
2.00
2.00
1.04
1.00
3.01
2.06
3.46
3.07
f1 (ppm)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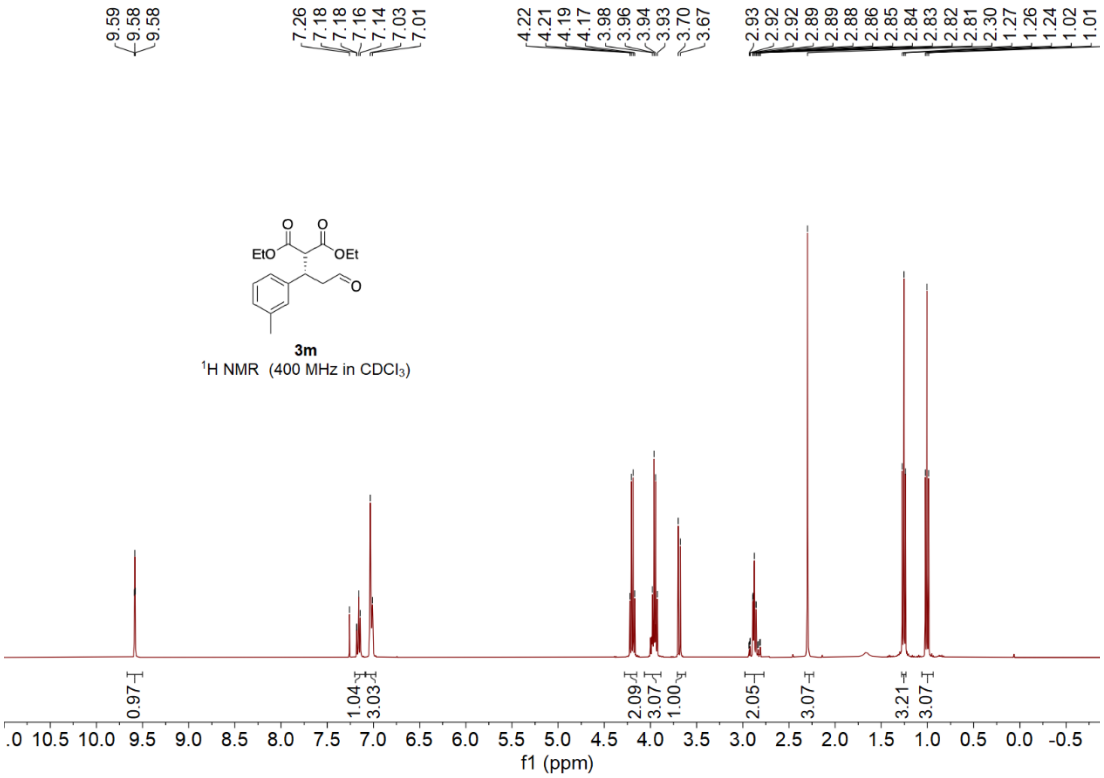
167.40
142.46
131.37
130.64
130.14
127.06
122.53
77.48
77.16
76.84
61.97
61.68
57.08
51.85
41.13
38.34
14.16
13.91
OMe
CO2Et
CO2Et
Br
3l'
13C NMR (101 MHz in CDCl3)
f1 (ppm)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9.58
9.58
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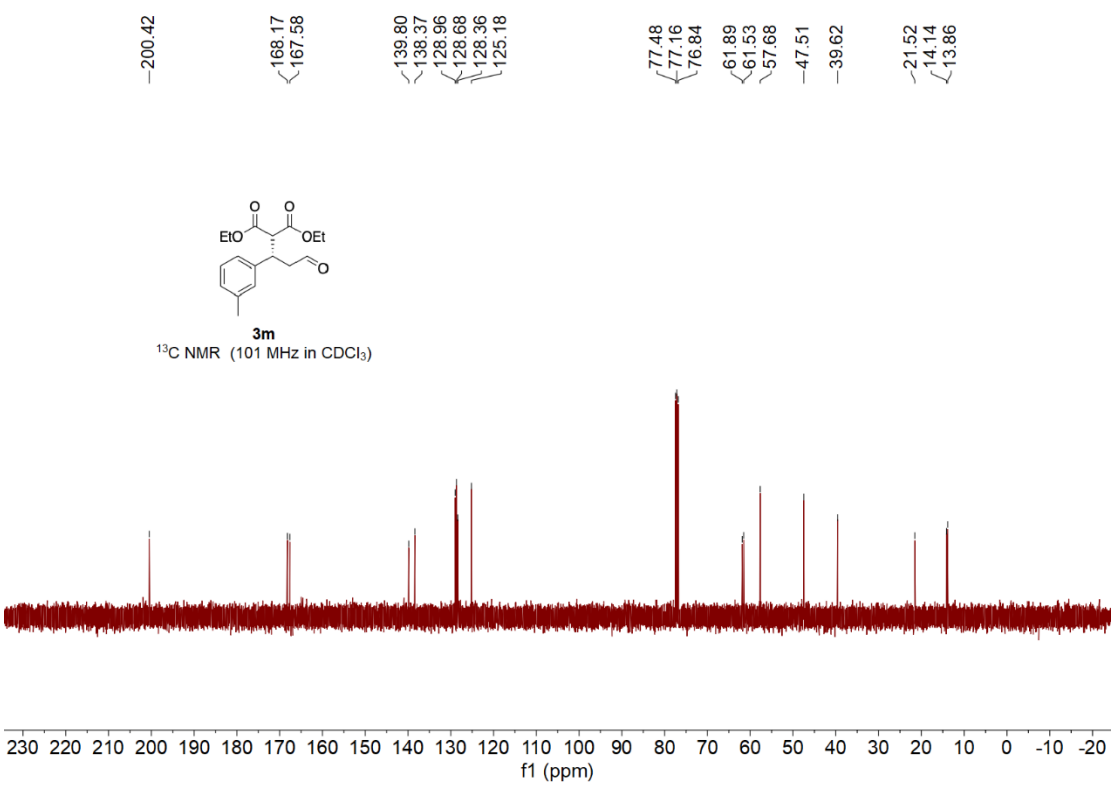
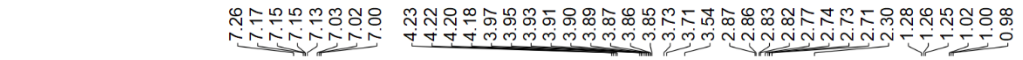
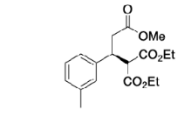
7.18
7.18
7.16
7.14
7.03
7.01
4.22
4.21
4.19
4.17
3.98
3.96
3.94
3.93
3.70
3.67
2.93
2.92
2.92
2.89
2.89
2.88
2.86
2.85
2.84
2.83
2.82
2.81
2.30
1.27
1.26
1.24
1.02
1.01
EtO
OEt
O
O
O
3m
1H NMR (400 MHz in CDCl3)
0.97
1.04
3.03
2.09
3.07
1.00
2.05
3.07
3.21
3.07
f1 (ppm)

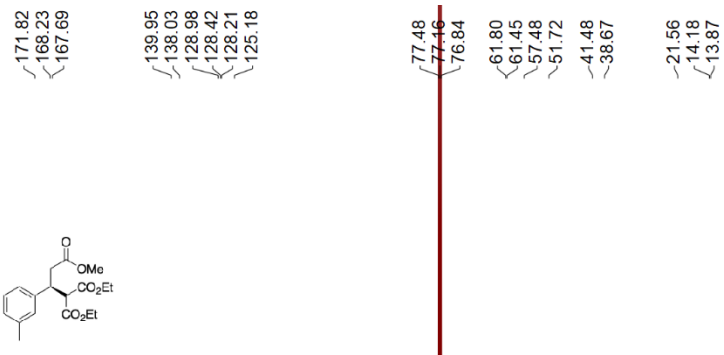

200.42
168.17
167.58
139.80
138.37
128.96
128.68
128.36
125.18
77.48
77.16
76.84
61.89
61.53
57.68
47.51
39.62
21.52
14.14
13.86
EtO
OEt
O
O
O
3m
13C NMR (101 MHz in CDCl3)
f1 (ppm)





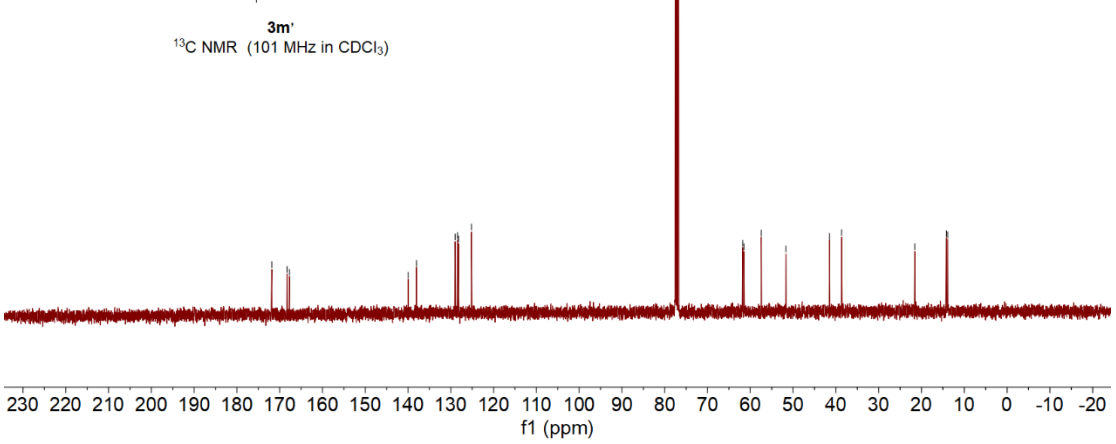
3m'

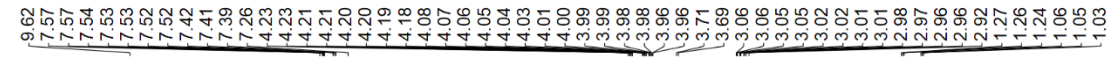
^{1}H NMR (400 MHz in $CDCl_3$)



3m'

^{13}C NMR (101 MHz in $CDCl_3$)




9.62
7.57
7.57
7.54
7.53
7.53
7.52
7.52
7.42
7.41
7.39
7.26
4.23
4.23
4.21
4.21
4.20
4.20
4.19
4.18
4.08
4.07
4.06
4.05
4.04
4.03
4.01
4.00
3.99
3.99
3.98
3.98
3.96
3.96
3.71
3.69
3.06
3.06
3.05
3.05
3.02
3.01
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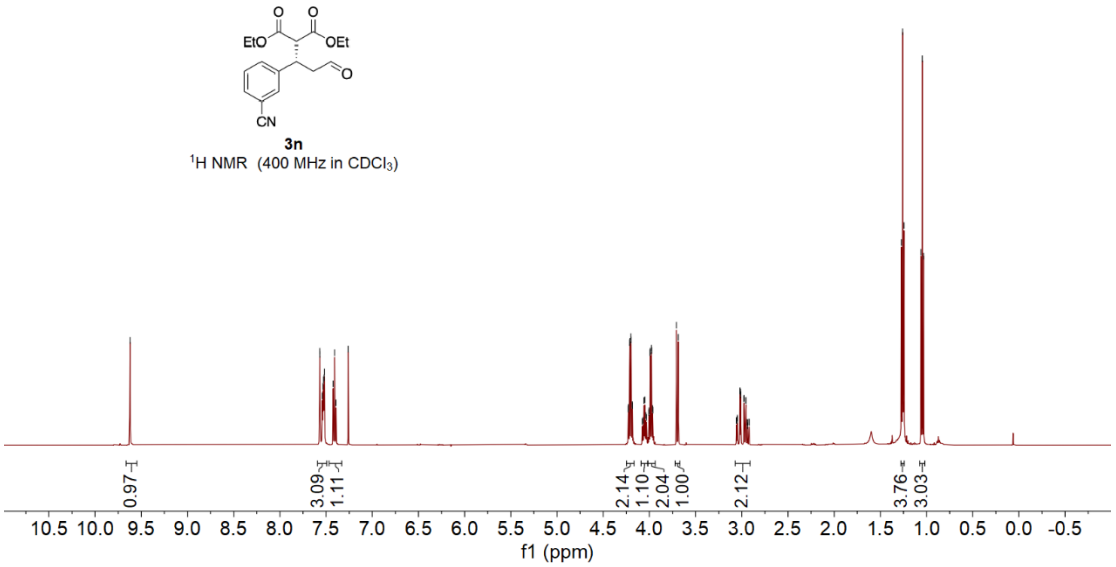
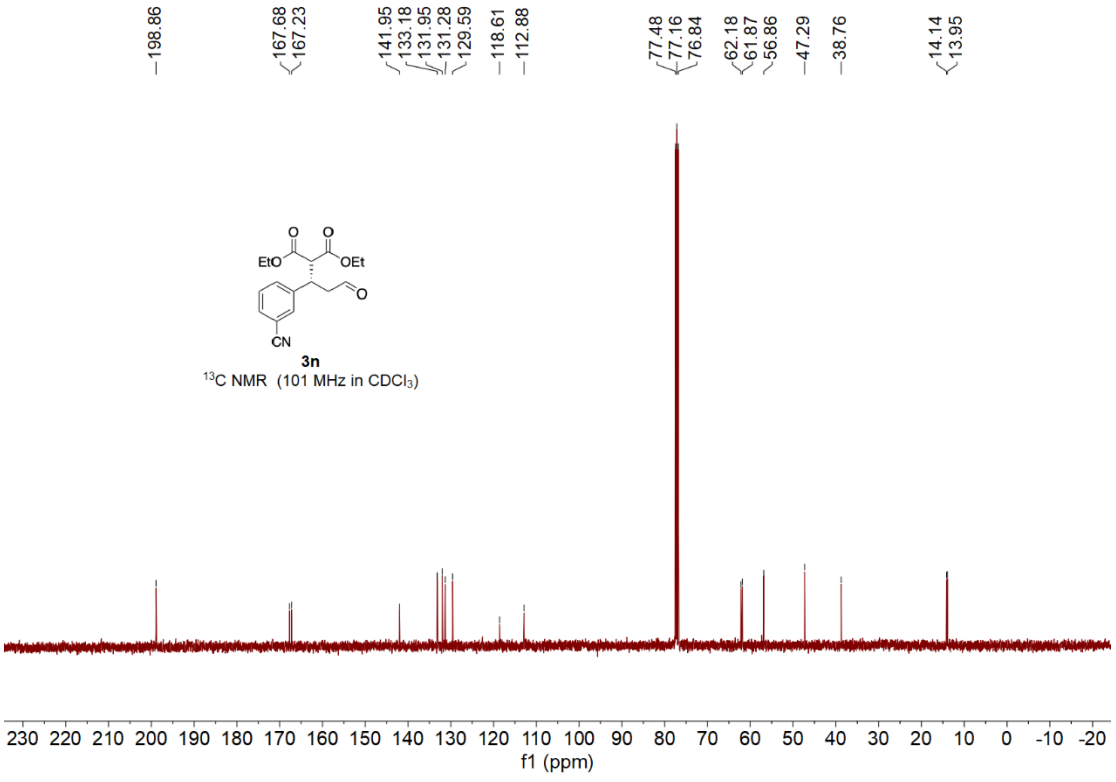
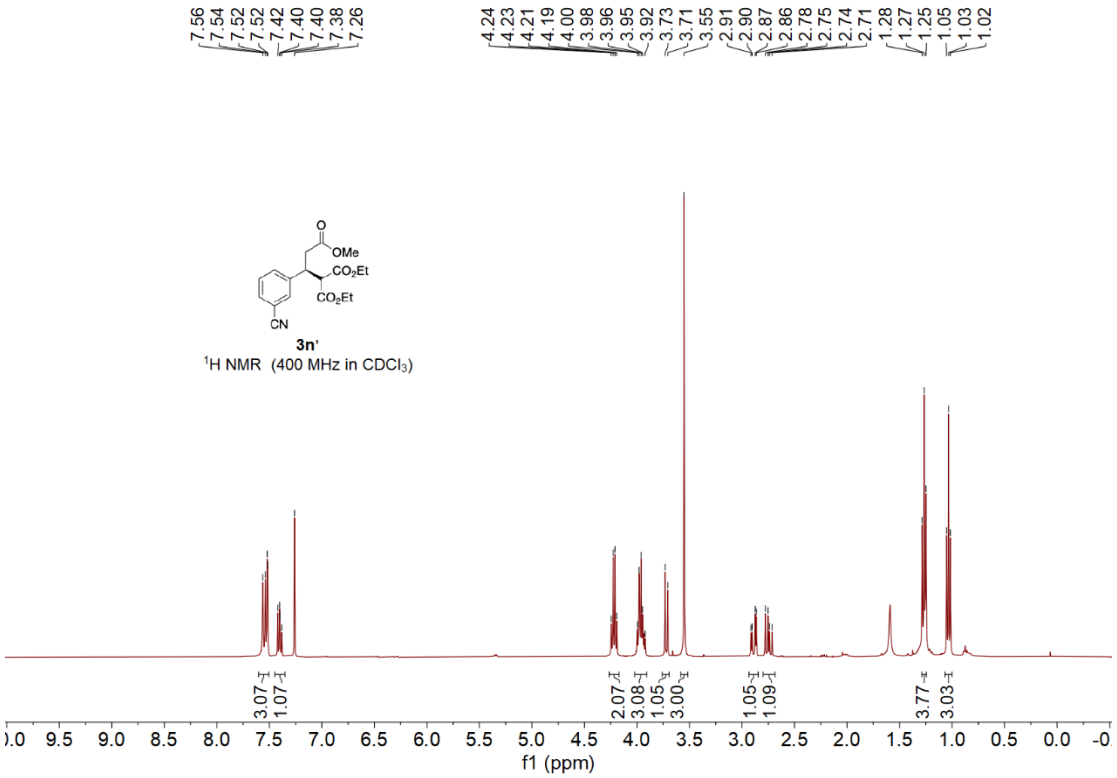
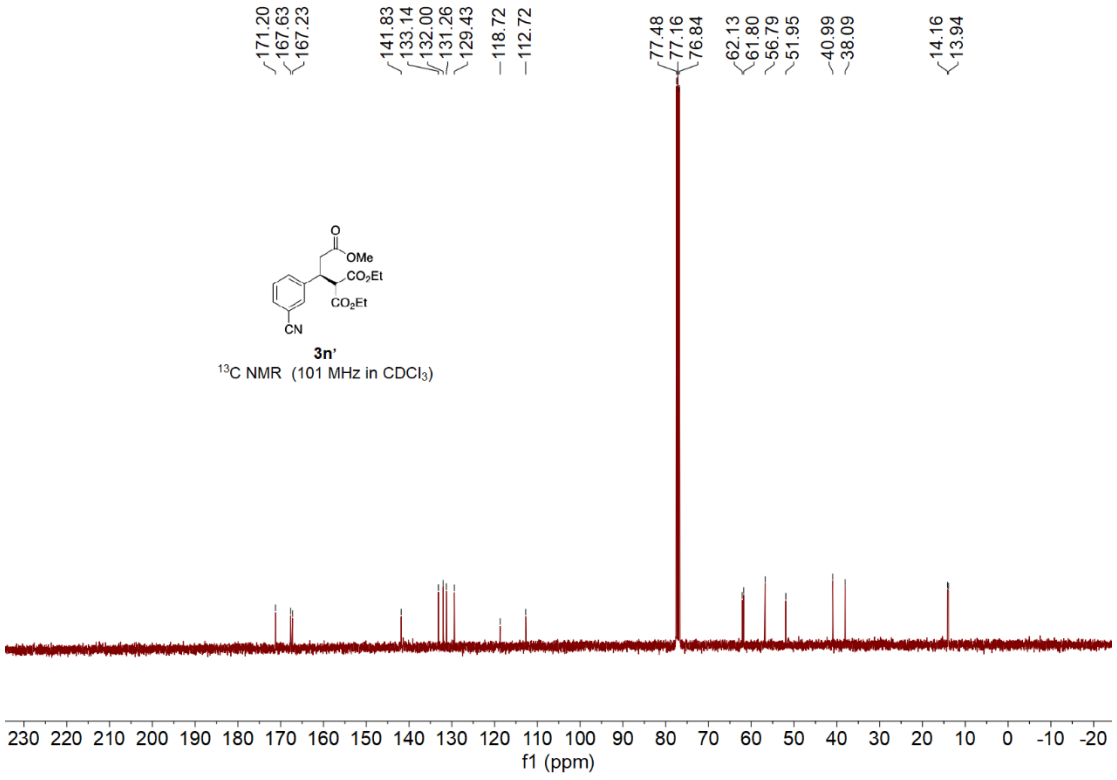
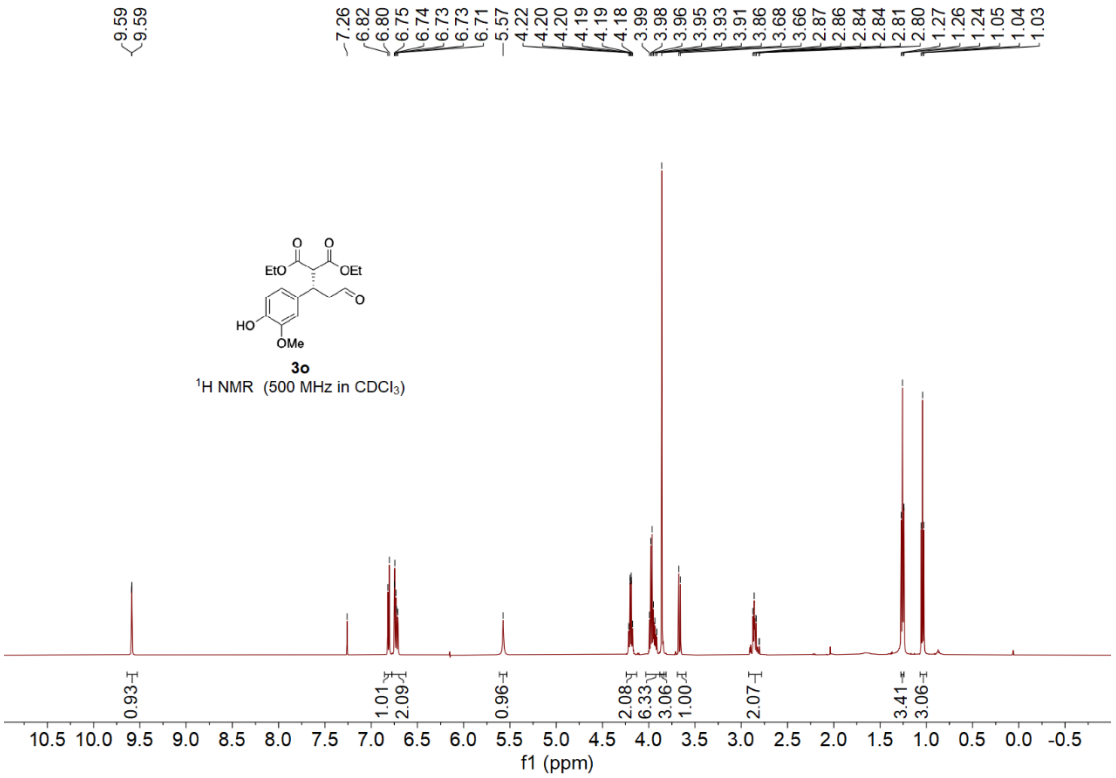
1.27
1.26
1.24
1.06
1.05
1.03

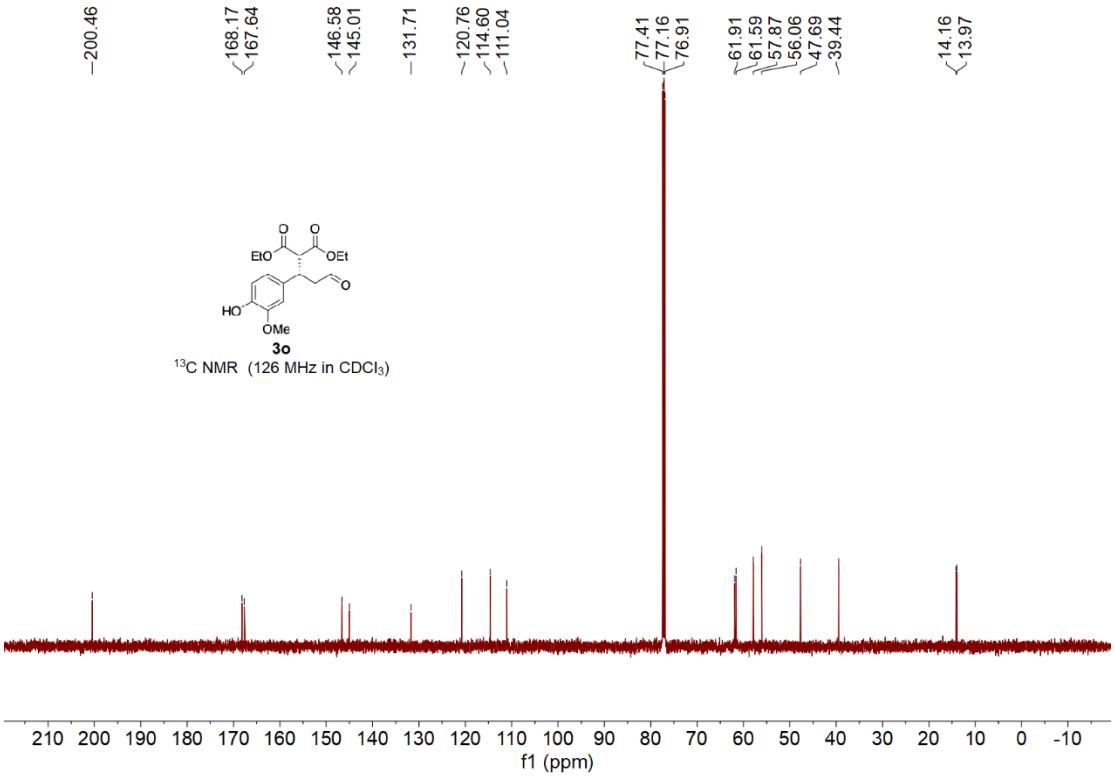

EtO
OEt
O
O
CN
3n
^{1}H NMR (400 MHz in CDCl$_3$)
0.97
3.09
1.11
2.14
1.10
2.04
1.00
2.12
3.76
3.03
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

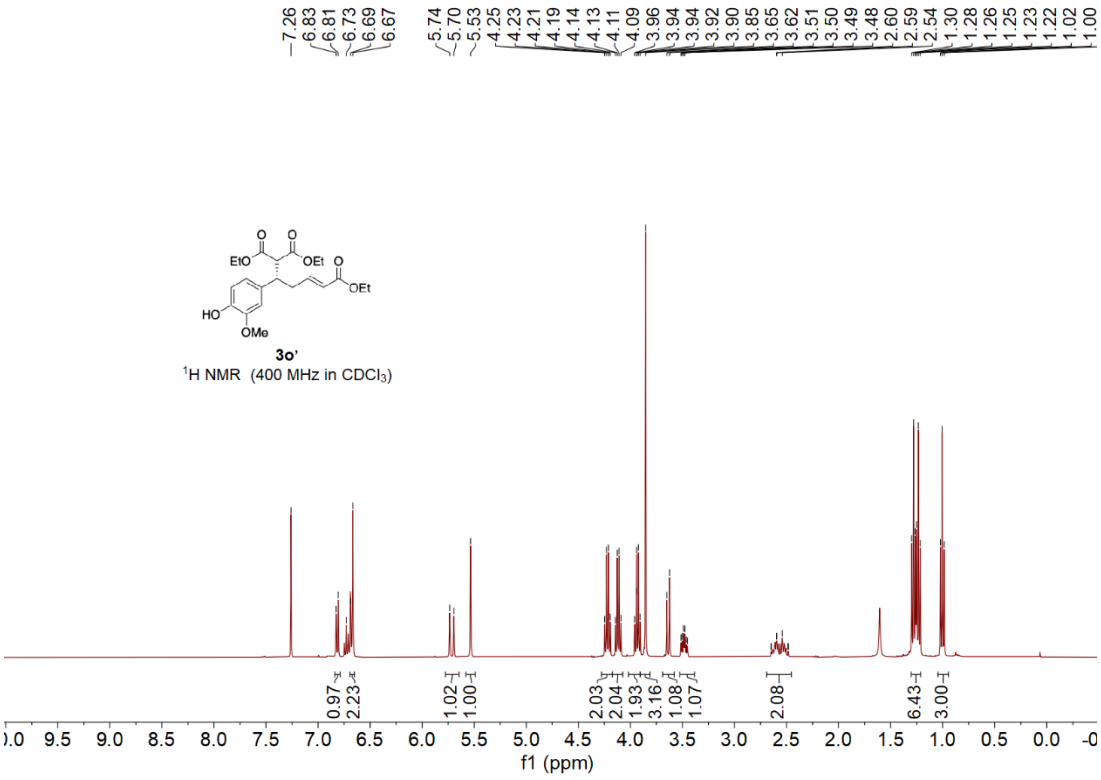

198.86
167.68
167.23
141.95
133.18
131.95
131.28
129.59
118.61
112.88
77.48
77.16
76.84
62.18
61.87
56.86
47.29
38.76
14.14
13.95
EtO
OEt
O
O
CN
3n
^{13}C NMR (101 MHz in CDCl$_3$)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

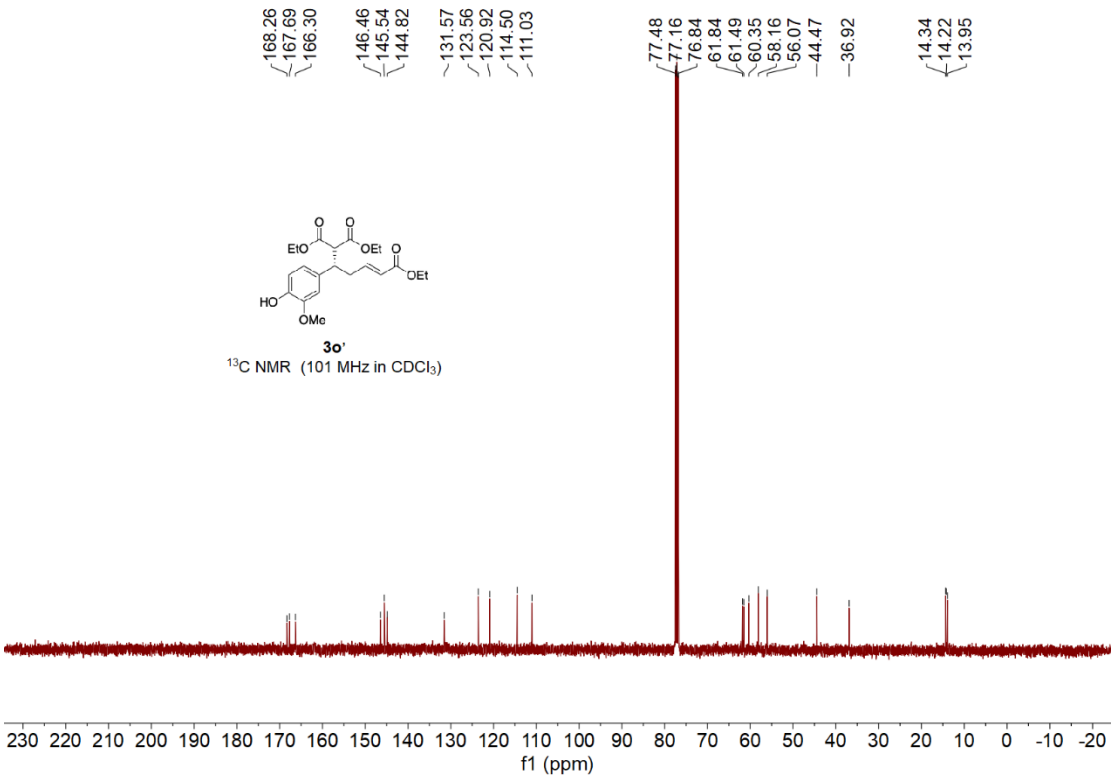

7.56
7.54
7.52
7.52
7.42
7.40
7.40
7.38
7.26
4.24
4.23
4.21
4.19
4.00
3.98
3.96
3.95
3.92
3.73
3.71
3.55
2.91
2.90
2.87
2.86
2.78
2.75
2.74
2.71
1.28
1.27
1.25
1.05
1.03
1.02
OMe
CO2Et
CO2Et
CN
3n'
1H NMR (400 MHz in CDCl3)
3.07
1.07
2.07
3.08
1.05
3.00
1.05
1.09
3.77
3.03
f1 (ppm)

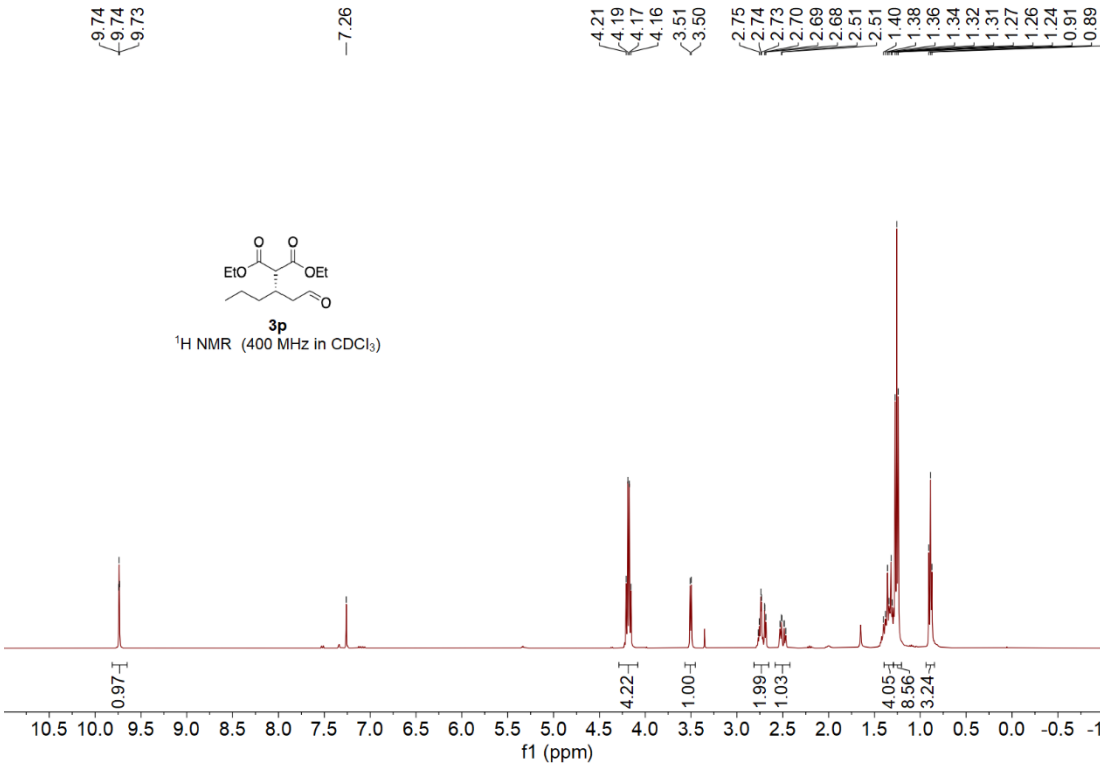

171.20
167.63
167.23
141.83
133.14
132.00
131.26
129.43
118.72
112.72
77.48
77.16
76.84
62.13
61.80
56.79
51.95
40.99
38.09
14.16
13.94
OMe
CO2Et
CO2Et
CN
3n'
13C NMR (101 MHz in CDCl3)
f1 (ppm)

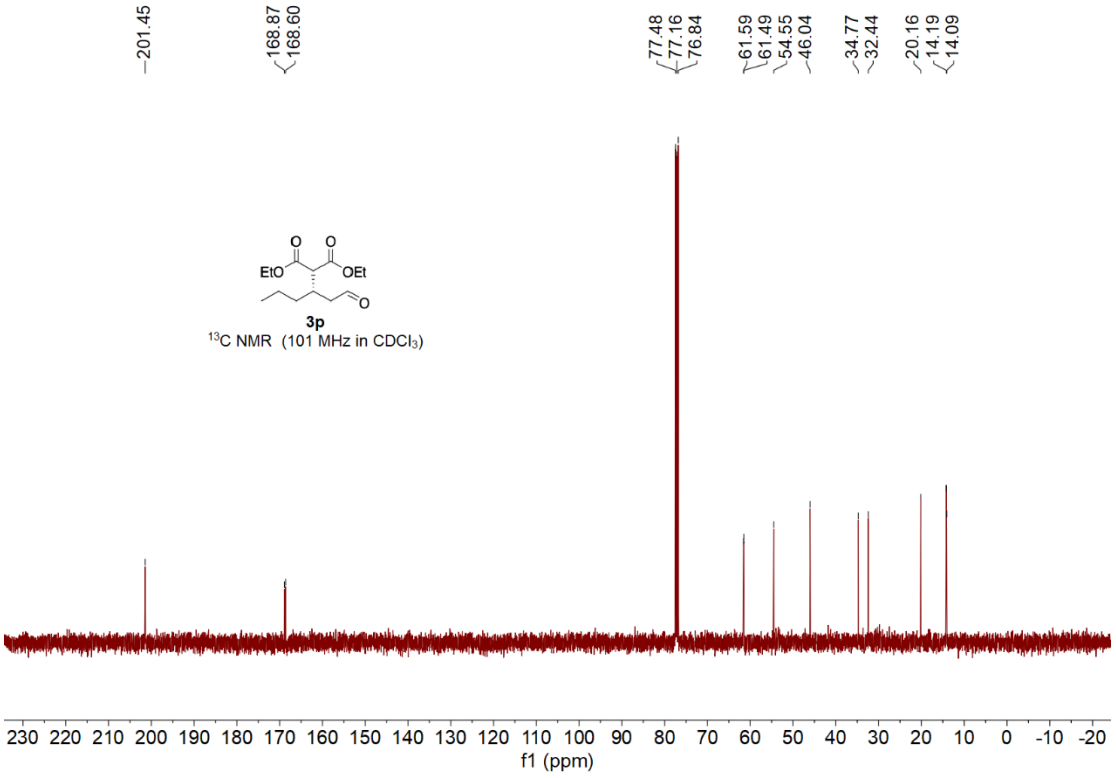

9.59
9.59
7.26
6.82
6.80
6.75
6.74
6.73
6.73
6.71
5.57
4.22
4.20
4.20
4.19
4.19
4.18
3.99
3.98
3.96
3.95
3.93
3.91
3.86
3.68
3.66
2.87
2.86
2.84
2.84
2.81
2.80
1.27
1.26
1.24
1.05
1.04
1.03
EtO
OEt
O
HO
OMe
3o
1H NMR (500 MHz in CDCl3)
0.93
1.01
2.09
0.96
2.08
6.33
3.06
1.00
2.07
3.41
3.06
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

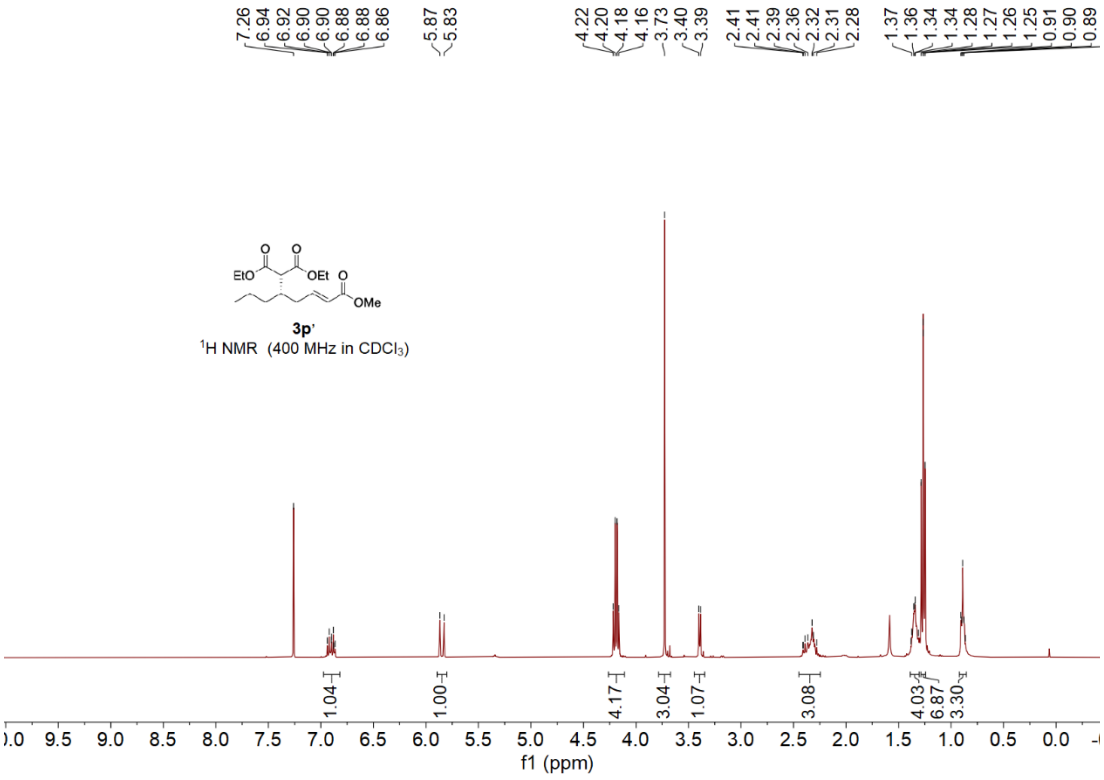

200.46
168.17
167.64
146.58
145.01
131.71
120.76
114.60
111.04
77.41
77.16
76.91
61.91
61.59
57.87
56.06
47.69
39.44
14.16
13.97
EtO
OEt
O
HO
OMe
3o
13C NMR (126 MHz in CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

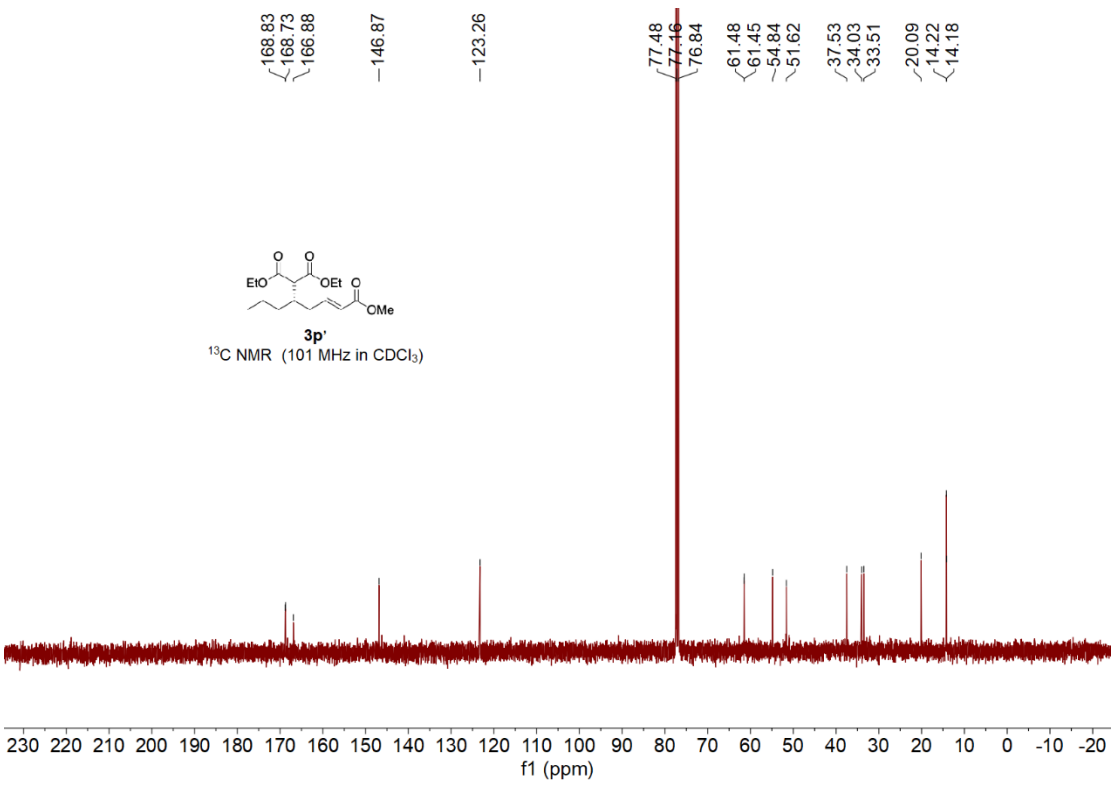

7.26
6.83
6.81
6.73
6.69
6.67
5.74
5.70
5.53
4.25
4.23
4.21
4.19
4.14
4.13
4.11
4.09
3.96
3.94
3.94
3.92
3.90
3.85
3.65
3.62
3.51
3.50
3.49
3.48
2.60
2.59
2.54
1.30
1.28
1.26
1.25
1.23
1.22
1.02
1.00
EtO
OEt
OEt
HO
OMe
3o'
1H NMR (400 MHz in CDCl3)
0.97
2.23
1.02
1.00
2.03
2.04
1.93
3.16
1.08
1.07
2.08
6.43
3.00
f1 (ppm)

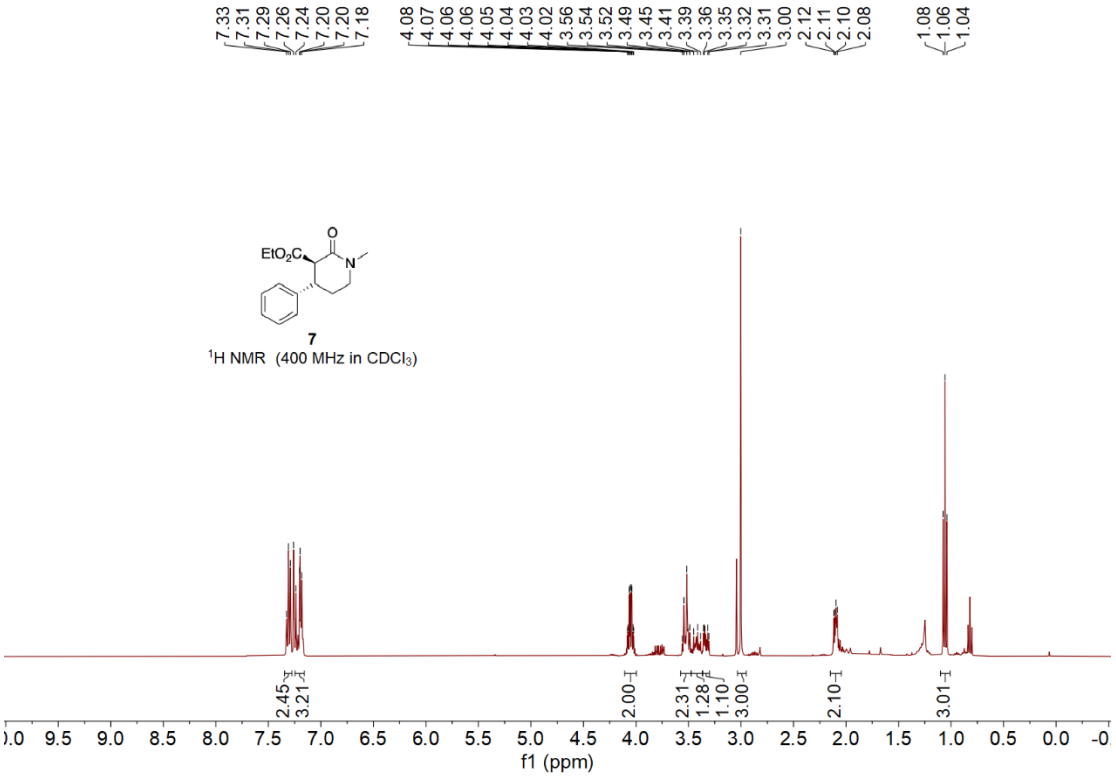

168.26
167.69
166.30
146.46
145.54
144.82
131.57
123.56
120.92
114.50
111.03
77.48
77.16
76.84
61.84
61.49
60.35
58.16
56.07
44.47
36.92
14.34
14.22
13.95
EtO
OEt
OEt
HO
OMe
3o'
13C NMR (101 MHz in CDCl3)
f1 (ppm)

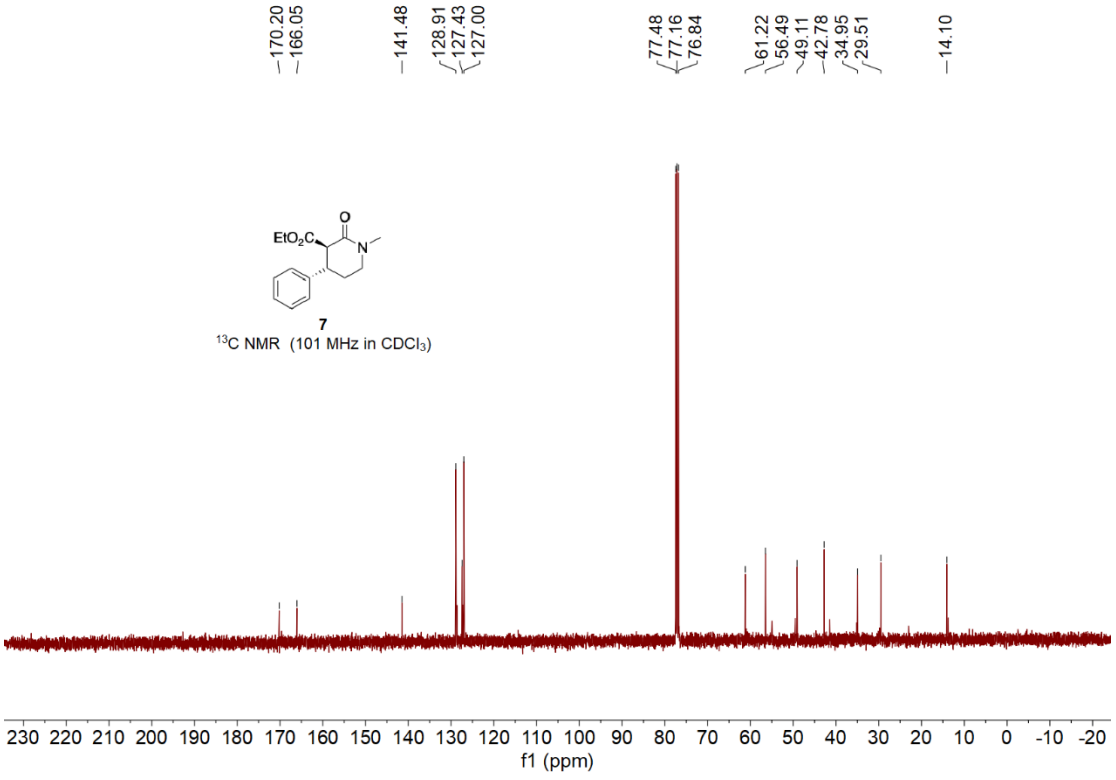

9.74
9.74
9.73
7.26
4.21
4.19
4.17
4.16
3.51
3.50
2.75
2.74
2.73
2.70
2.69
2.68
2.51
2.51
1.40
1.38
1.36
1.34
1.32
1.31
1.27
1.26
1.24
0.91
0.89
EtO
OEt
O
O
O
3p
1H NMR (400 MHz in CDCl3)
0.97
4.22
1.00
1.99
1.03
4.05
8.56
3.24
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
f1 (ppm)

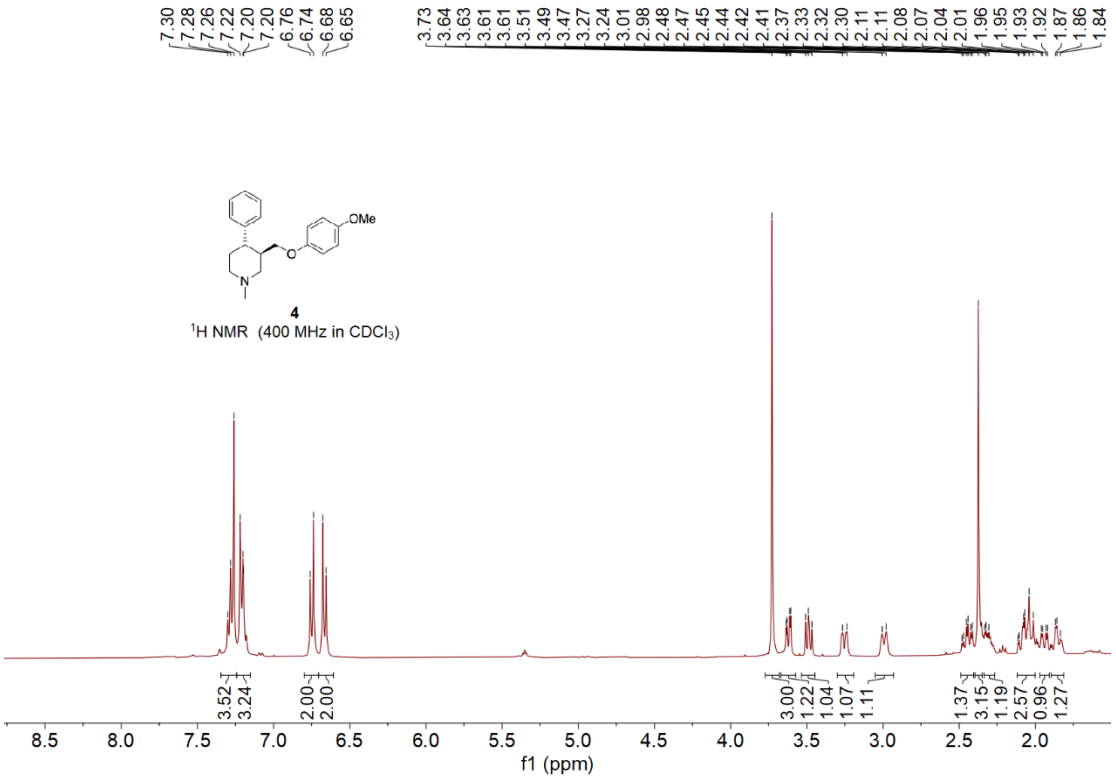

201.45
168.87
168.60
77.48
77.16
76.84
61.59
61.49
54.55
46.04
34.77
32.44
20.16
14.19
14.09
EtO
OEt
O
O
O
3p
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

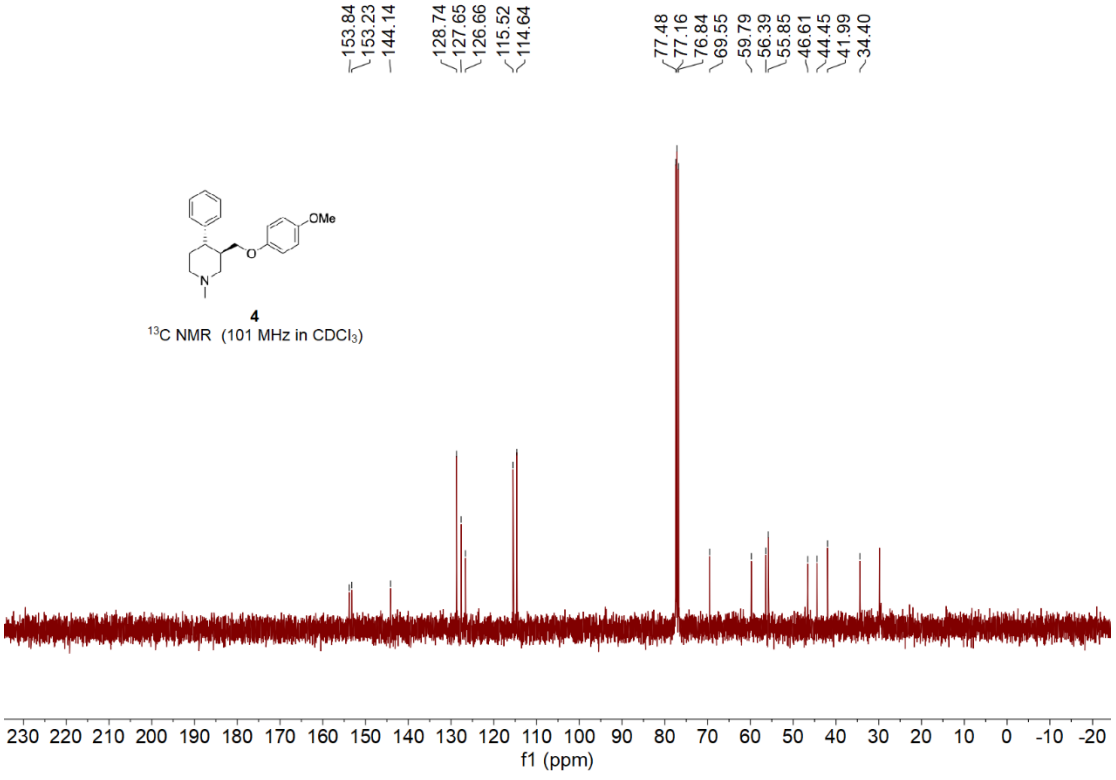

7.26
6.94
6.92
6.90
6.90
6.88
6.88
6.86
5.87
5.83
4.22
4.20
4.18
4.16
3.73
3.40
3.39
2.41
2.41
2.39
2.36
2.32
2.31
2.28
1.37
1.36
1.34
1.34
1.28
1.27
1.26
1.25
0.91
0.90
0.89
EtO
OEt
OMe
3p'
1H NMR (400 MHz in CDCl3)
1.04
1.00
4.17
3.04
1.07
3.08
4.03
6.87
3.30
f1 (ppm)


168.83
168.73
166.88
146.87
123.26
77.48
77.16
76.84
61.48
61.45
54.84
51.62
37.53
34.03
33.51
20.09
14.22
14.18
EtO
OEt
OMe
3p'
13C NMR (101 MHz in CDCl3)
f1 (ppm)


7.33
7.31
7.29
7.26
7.24
7.20
7.20
7.18
4.08
4.07
4.06
4.06
4.05
4.04
4.03
4.02
3.56
3.54
3.52
3.49
3.45
3.41
3.39
3.36
3.35
3.32
3.31
3.00
2.12
2.11
2.10
2.08
1.08
1.06
1.04
EtO2C
O
N
7
1H NMR (400 MHz in CDCl3)
2.45
3.21
2.00
2.31
1.28
1.10
3.00
2.10
3.01
f1 (ppm)


170.20
166.05
141.48
128.91
127.43
127.00
77.48
77.16
76.84
61.22
56.49
49.11
42.78
34.95
29.51
14.10
EtO2C
O
N
7
13C NMR (101 MHz in CDCl3)
f1 (ppm)


7.30
7.28
7.26
7.22
7.20
7.20
6.76
6.74
6.68
6.65
3.73
3.64
3.63
3.61
3.61
3.51
3.49
3.47
3.27
3.24
3.01
2.98
2.48
2.47
2.45
2.44
2.42
2.41
2.37
2.33
2.32
2.30
2.11
2.11
2.08
2.07
2.04
2.01
1.96
1.95
1.93
1.92
1.87
1.86
1.84
OMe
O
N
4
1H NMR (400 MHz in CDCl3)
3.52
3.24
2.00
2.00
3.00
1.22
1.04
1.07
1.11
1.37
3.15
1.19
2.57
0.96
1.27
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)


153.84
153.23
144.14
128.74
127.65
126.66
115.52
114.64
77.48
77.16
76.84
69.55
59.79
56.39
55.85
46.61
44.45
41.99
34.40
OMe
O
N
4
13C NMR (101 MHz in CDCl3)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)