**Supplementary Information**

# Enzymatic assembly of carbon–carbon bonds via iron-catalysed *sp*$^3$ C–H functionalization

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**This PDF file includes:**

## I. General methods

**(A) General.** Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, VWR, Alfa Aesar, Combi-Blocks) and used without further purification. Silica gel chromatography was carried out using AMD Silica Gel 60, 230-400 mesh. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian Inova 300 MHz, Varian Inova 500 MHz, or Bruker Prodigy 400 MHz instrument in $CDCl_3$ and are referenced to residual protio solvent signals. Data for $^{1}H$ NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets), coupling constant (Hz), integration. Sonication was performed using a Qsonica Q500 sonicator. High-resolution mass spectra were obtained at the California Institute of Technology Mass Spectral Facility. Synthetic reactions were monitored by thin layer chromatography (TLC, Merck 60 gel plates) using a UV-lamp or an appropriate TLC stain for visualization.

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

**(B) Chromatography.** Analytical reversed-phase high-performance liquid chromatography (HPLC) was carried out using an Agilent 1200 series instrument and a Kromasil 100 C18 column (4.6 × 50 mm, 5 μm) or an Eclipse XDB C18 column (4.6 x 150 mm, 3 μm) with water and acetonitrile as the mobile phase. Analytical chiral HPLC was conducted using either an Agilent 1200 series instrument with *n*-hexane and isopropanol as the mobile phase or JACSO 2000 series supercritical fluid chromatography (SFC) system with supercritical $CO_2$ and isopropanol as the mobile phase. Enantiomers were separated using one of the following chiral columns: Chiralpak AD-H, Chiralpak IC (4.6 mm × 25 cm), Chiralcel OB-H (4.6 mm × 25 cm), Chiralcel OD-H (4.6 mm × 25 cm), Chiralcel OJ-H (4.6 mm × 25 cm). Gas chromatography (GC) analysis was carried out using an Agilent 7820A or Shimadzu GC-17A GC system, both equipped with an FID detector and with a J&W HP-5 column (30 m × 0.32 mm, 0.25 μm film). Chiral GC was conducted using either an Agilent 7820A instrument (FID) and an Agilent CycloSil-B column (30 m × 0.32 mm, 0.25 μm film) or an Agilent 6850 GC (FID) with a Chiraldex G-TA column (30 m × 0.25 mm, 0.12 μm film). Gas chromatography-mass spectrometry (GC-MS) analyses were carried out using a Shimadzu GCMS-QP2010SE system and J&W HP-5ms column (30 m × 0.25 mm, 0.25 μm film).

**(C) Cloning and site-saturation mutagenesis.** pET22b(+) was used as a cloning and expression vector for all enzymes described in this study. All enzymes described in this study were expressed with a *C*-terminal 6xHis-tag. Site-saturation mutagenesis was performed using the "22c-trick" method[1]. The PCR products were digested with *Dpn*I, gel purified, and ligated using Gibson Mix™ (ref. 2). The ligation mixture was used to directly transform electrocompetent *E. coli* BL21 *E. cloni* (Lucigen) cells.

**(D) Expression of P450 and P411 variants in 96-well plates.** Single colonies from $LB_{amp}$ agar plates were picked using sterile toothpicks and cultured in deep-well 96-well plates containing

$LB_{amp}$ (300 μL/well) at 37 ºC, 220 rpm shaking, and 80% relative humidity overnight. After, $HB_{amp}$ (1000 μL/well) in a deep-well 96-well plate was inoculated with an aliquot (50 μL/ well) of these overnight cultures and allowed to shake for 3 hours at 37 ºC, 220 rpm, and 80% relative humidity. The plates were cooled on ice for 30 minutes and the cultures were induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 20 ºC, 150 rpm for 16–20 hours.

**(E) Reaction screening in 96-well plate format.** Cells in deep-well 96-well plates were pelleted (3,000 × g, 5 min, RT) and resuspended in M9-N buffer (20 μL/well) by gentle vortexing. A GOX oxygen depletion system was added (20 μL/well of a stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0) and the 96-well plate was transferred into an anaerobic chamber. In the anaerobic chamber, argon-sparged reaction buffer (50 mM glucose in M9-N or 33 mM glucose in M9-N, 300 μL/well) was added, followed by alkane substrate (10 μL/well, 400 mM in EtOH) and ethyl diazoacetate (10 μL/well, 400 mM in EtOH). In some cases, the substrates and reaction buffer were mixed together prior to addition to the plate. The plate was sealed with an aluminum foil and shaken at room temperature and 500 rpm in the anaerobic chamber. After 5–20 hours, the seal was removed and the reactions were worked up following the appropriate method below.

*Product formation screening using GC and GC-MS.* After 5–20 hours, a solution of 0.4 mM 1,3,5-trimethoxybenzene (internal standard) in a mixed solvent system (cyclohexane/ ethyl acetate = 1:1, 510 μL) was added. The plate was tightly sealed with a reusable silicone mat, vortexed (15 s × 3) and centrifuged (3,000 × g, 5 min) to completely separate the organic and aqueous layers. The organic layers (180 μL/well) were transferred to 300 μL vial inserts, which were then placed in 2 mL vials and analyzed by GC.

*Product formation screening using HPLC.* After 5–20 hours, the reaction mixtures, or an aliquot thereof (150 μL/well), were quenched by the addition of an equal or greater volume of acetonitrile (400 μL/well or 150–200 μL/well). This step is kept consistent within each round of directed evolution. The plate containing the resulting mixture was tightly sealed with a reusable silicone mat, vortexed (15 s × 3) and centrifuged (3,000 × g, 5 min) to pellet the cells. The supernatant was filtered through an AcroPrep 96-well filter plate (0.2 μm) into a shallow-well plate and analyzed by reverse-phase HPLC.

*Enantioselectivity screening.* After 5–24 hours, mixed solvent (cyclohexane/ ethyl acetate = 1:1, 250–500 μL/ well) was added to the reaction mixtures or aliquots thereof (250 μL). The plate containing the resulting mixture was tightly sealed with a reusable silicone mat, vortexed (15 s × 3) and centrifuged (3,000 × g, 5 min) to completely separate the organic and aqueous layers. When smaller volumes of mixed solvent were used for the extraction (< 400 μL), the extraction mixture was transferred to a 1.6 mL Eppendorf tube, vortexed (15 s × 3), and centrifuged (20,000 × g, 1 min). The organic layers (180 μL/well) were transferred to 300 μL vial inserts, which were then placed in 2 mL vials and analyzed by chiral HPLC (IC column, 2% *i*-PrOH in *n*-hexane).

**(F) Expression of P411 variants.** *E. coli* cells expressing the appropriate P411 variants were grown overnight in 5 mL $LB_{amp}$. Preculture (2 mL) was used to inoculate 48 mL of $HB_{amp}$ in a 125 mL Erlenmeyer flask; this culture was incubated at 37 ºC, 230 rpm for 2.5 hours. Cultures were then cooled on ice (20–30 min) and induced with 0.5 mM IPTG and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 20 ºC, 130 rpm, for 16–18 hours. Cultures were then centrifuged (2,600 × g, 10 min, 4 ºC) and the pellets were resuspended in M9-N buffer to $OD_{600}$ = 60. An aliquot of each cell suspension (3 mL) was used to determine the P411 expression level using the hemochrome assay after lysis by sonication.

**(G) Hemochrome assay for the determination of heme protein concentration.** *E. coli* cells expressing heme protein and resuspended in M9-N buffer were lysed by sonication using a Qsonica Q500 sonicator equipped with a microtip (2 mins, 1 second on, 1 second off, 25% amplitude); samples were kept on wet ice for this process. The resulting lysed solution was centrifuged (20,000 × g, 10 min, 4 °C) to remove cell debris. The supernatant (clarified lysate) was separated from the pellet and kept on ice until use. In a falcon tube, a solution of 0.2 M NaOH, 40% (v/v) pyridine, 0.5 mM $K_3Fe(CN)_6$ was prepared (pyridine-NaOH-$K_3Fe(CN)_6$ solution). Separately, a solution of 0.5 M $Na_2S_2O_4$ (sodium dithionite) was prepared in 0.1 M NaOH. To an Eppendorf tube containing 500 µL of clarified lysate in M9-N buffer was added 500 µL of the pyridine-NaOH-$K_3Fe(CN)_6$ solution, mixed, and transferred to a cuvette; the UV-Vis spectrum of the oxidized $Fe^{III}$ state was recorded immediately. To the cuvette was then added 10 µL of the sodium dithionite solution. The cuvette was sealed with parafilm and the UV-Vis spectrum of the reduced $Fe^{II}$ state was recorded immediately. A cuvette containing 500 µL of M9-N, 100 µL 1 M NaOH, 200 µL pyridine, and 200 µL water (complete mixture without protein and $K_3Fe(CN)_6$) was used as a reference for all absorbance measurements. Concentrations of cytochromes P450, cytochromes P411, and globins were determined using a published extinction coefficient for heme *b*, $\varepsilon_{556(reduced)-540(oxidized)}$ = 23.98 $mM^{-1}cm^{-1}$ (ref. 3). Cytochrome *c* concentration was measured using a modified procedure, reported previously[4].

**(H) Biotransformations using whole *E. coli* cells.** Suspensions of *E. coli* expressing the appropriate heme protein variant in M9-N buffer (typically $OD_{600}$ = 30) were degassed by bubbling with argon in sealed vials for at least 40 minutes; the cells were kept on ice during this time. Separately, a solution of *D*-glucose (250 mM in M9-N) was degassed by sparging with argon for at least 30 minutes. All solutions were then transferred into an anaerobic chamber for reaction set up. To a 2 mL vial were added a GOX oxygen depletion solution (20 µL of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0), *D*-glucose (40 µL of 250 mM stock solution in M9-N buffer), degassed suspension of *E. coli* expressing P411 (typically $OD_{600}$ = 30, 320 µL), alkane substrate (10 µL of 400 mM stock solution in EtOH), and diazo compound (10 µL of 400 mM stock solution in EtOH) in the listed order. Final reaction volume was 400 µL; final concentrations were 10 mM alkane substrate, 10 mM diazo compound, and 25 mM *D*-glucose. ***Note:*** reaction performed at $OD_{600}$ = 30 indicates that 320 µL of $OD_{600}$ = 30 cells were added, and likewise for other reaction $OD_{600}$ descriptions. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. A modified procedure was used for reactions conducted at 4 °C. Reactions were set up in the same manner, except kept on ice. Reactions were shaken in a cold room (4 °C) and 500 rpm for 18 hours. The reactions were worked up and analyzed by HPLC or GC as appropriate; the reaction workup procedures are outlined in detail in **Section VIII**. Preparative scale enzymatic reactions were performed using a different procedure which is described in detail in **Section IX**. Protein concentration was determined using the hemochrome assay (**Section I (G)**).

**(I) Enzymatic reactions using clarified *E. coli* lysate.** Lysates for biocatalytic reactions were prepared as follows: *E. coli* cells expressing the appropriate heme protein variant were resuspended in M9-N buffer and adjusted to $OD_{600}$ = 60. The cell suspension, in 3 mL portions, was lysed by sonication using a Qsonica Q500 sonicator equipped with a microtip (2 mins, 1 second on, 1 second off, 25% amplitude); samples were kept on wet ice for this process. The resulting lysed solution was centrifuged (20,000 × g, 10 min, 4 °C) to remove cell debris. Protein concentration of the supernatant (clarified lysate) was determined using the hemochrome assay (see **Section I (G)**); the protein concentration in lysate was adjusted to the desired amount by addition of M9-N buffer. Lysate was placed in a sealed vial and the headspace of the vial was purged with a stream of argon for at least 40 minutes. The lysate was kept on ice during all parts of this procedure. Separately, *D*-glucose solution (500 mM in M9-N buffer) and $Na_2S_2O_4$ (20 mM in M9-N) were degassed by bubbling the solutions with argon for at least 40 minutes. All solutions were then transferred into an anaerobic chamber for reaction set up. To a 2 mL vial were added a GOX oxygen depletion solution (20 μL of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0), *D*-glucose (20 μL of 500 mM stock solution in M9-N buffer), lysate (320 μL), $Na_2S_2O_4$ (20 μL of 20 mM solution in M9-N), alkane substrate (10 μL of 400 mM stock solution in EtOH), and ethyl diazoacetate (10 μL of 400 mM stock solution in EtOH) in the listed order. Final reaction volume was 400 μL; final concentrations were typically 2.0 μM heme protein, 1 mM $Na_2S_2O_4$, 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. Reactions were analyzed following the same methods as described for biotransformations using whole *E. coli* cells (see **Section VIII**).

**(J) Protein purification.** *E. coli* BL21 *E. cloni* cells carrying a plasmid encoding a P411 variant were grown overnight in 19 mL $LB_{amp}$ (37 ºC, 250 rpm). $HB_{amp}$ (450 mL) in a 1 liter flask was inoculated with 19 mL of the preculture and shaken for 2.5 hours at 37 °C and 220 rpm. Cultures were cooled on ice (30 min) and induced with 0.5 mM IPTG and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 22 °C, 130 rpm, for 16–20 hours. Cultures were then centrifuged (5,000 × g, 10 min, 4 ºC) and the cell pellets frozen at -20 ºC. For purification, frozen cells from two such cultures were resuspended in His-trap buffer A (25 mM tris, 100 mM NaCl, 20 mM imidazole, pH 7.5, 4 mL/g of cell wet weight), loaded with hemin (1 mg/g wet cell weight) and powdered DNaseI, and lysed by sonication. To pellet cell debris, lysates were centrifuged (20,000 × g, 20 min, 4 °C). The protein containing a *C*-terminal 6xHis-tag was purified with a nickel NTA column (1 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTA purified FPLC system (GE healthcare). Proteins were eluted on a linear gradient from His-trap buffer A to His-trap buffer B (25 mM tris, 300 mM imidazole, 100 mM NaCl, pH 7.5) over 10 column volumes. Fractions containing the desired heme protein were combined, concentrated, and subjected to three exchanges of phosphate buffer (0.1 M potassium phosphate, pH 8.0) using centrifugal filters (10 kDa molecular weight cut-off, Amicon Ultra, Merck Millipore) to remove excess salt and imidazole. Concentrated proteins were aliquoted, flash-frozen on powdered dry ice, and stored at -80 ºC.

**(K) Enzymatic reactions using purified heme protein.** A solution of NADPH or $Na_2S_2O_4$ in phosphate buffer (0.1 M potassium phosphate, pH 8.0) was degassed by sparging with argon for at least 30 minutes (reaction solution). Separately, a solution of *D*-glucose (250 mM in 0.1 M potassium phosphate, pH 8.0) was also degassed in the same manner. Crimp vials (2 mL) were each charged with the GOX oxygen depletion system (20 μL of a stock solution containing 14,000

U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate, pH 8.0). After degassing was complete, all solutions, 2 mL crimp vials, and purified protein (in 0.1 M potassium phosphate, pH 8.0), kept on ice, were brought into the anaerobic chamber. *D*-Glucose (40 μL of 250 mM solution), reaction buffer containing reductant (300 μL, reductant is either NADPH or $Na_2S_2O_4$), and purified protein (20 μL) were added to the 2 mL vial. The mixture was shaken for 5 min to ensure even distribution of the proteins; then, reaction vials were charged with alkane substrate (10 μL of 400 mM stock solution in EtOH) and ethyl diazoacetate (10 μL of 400 mM stock solution in EtOH). Final reaction volume was 400 μL. Final concentrations were 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose; concentrations of protein and reductant are variable and described with the individual experiment. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. The reactions were quenched by the addition of acetonitrile (400 μL) and internal standard (10 μL). This mixture was then transferred to a microcentrifuge tube and centrifuged (20,000 × g, 10 min). The supernatant was transferred to a vial and analyzed by HPLC.

## II. Supplementary Figures S1–S11 and Tables S1–S5

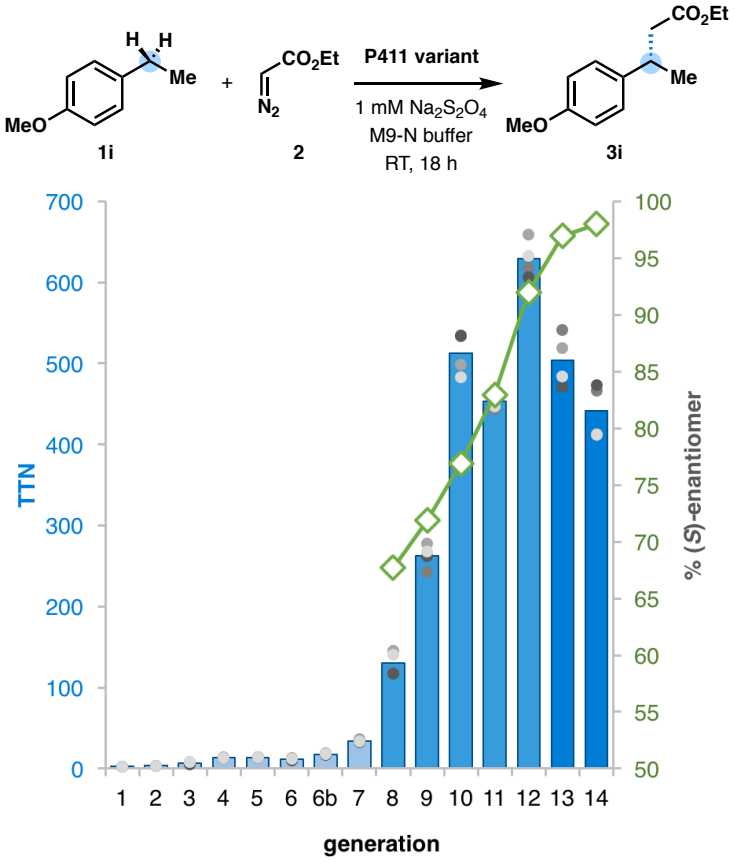
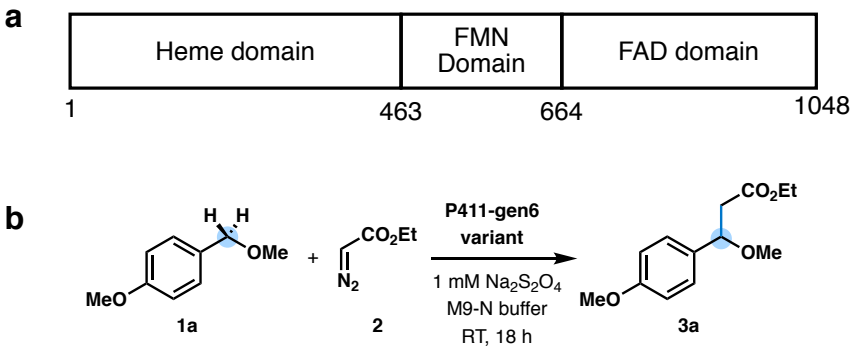



**Fig. S1.** Evolutionary lineage from P-4 A82L to P411-CHF evaluated for C–H alkylation of 4-ethylanisole (**1i**).

**Notes:** Standard reaction conditions: clarified lysate of *E. coli* expressing the indicated heme protein variant (see **Section IV**), 10 mM substrate **1i**, 10 mM ethyl diazoacetate (**2**), 1 mM $Na_2S_2O_4$. Reactions with generations 1, 2, and 3 variants employed 4.0 μM heme protein; all other reactions used 2.0 μM heme protein. Bars represent average TTNs from reactions performed in quadruplicate; each TTN data point is shown as a grey dot. Enantioselectivity results are represented by green diamonds. See Table S13 in **Section IV** for the data presented here. TTN, total turnover number; RT, room temperature.




| P411-gen6 variant | TTN to 3a |
|---|---|
| Full-length (P411-gen6) | 59 |
| ΔFAD (P411-gen6b) | 98 |
| Heme domain only | 73 (100[†]) |

**Fig. S2.** Truncation of a full-length P411 protein delivers active C–H alkylation enzymes.

**Notes: a,** Domain architecture of cytochrome $P450_{BM3}$. For its native monooxygenase activity, the FMN and FAD domains, collectively called the reductase domain, are responsible for delivering the necessary reducing equivalents from NADPH to the heme domain. The end of the FMN domain and the fragment of the polypeptide chain included in the ΔFAD complex were chosen based on a report by S. Govindaraj and T. L. Poulos[5]. **b,** Systematic truncation of the P411-gen6 full-length protein was performed to deliver P411-gen6b (P411ΔFAD-gen6, amino acids 1–664) and P411-gen6 heme-domain only (amino acids 1–463). Under the stated reaction conditions, both P411-gen6b (ΔFAD) and the heme domain only protein delivered **3a** with higher total turnover compared with the full-length protein. Standard reaction conditions: lysate of *E. coli* with 2.0 μM heme protein, 10 mM **1a**, 10 mM **2**, and 1 mM $Na_2S_2O_4$ (unless otherwise indicated). TTN results are an average of at least duplicate reactions. RT, room temperature; TTN, total turnover number. See **Section IV** for more information on P411-gen6 and P411-gen6b. [†]5 mM dithionite was used in these reactions.

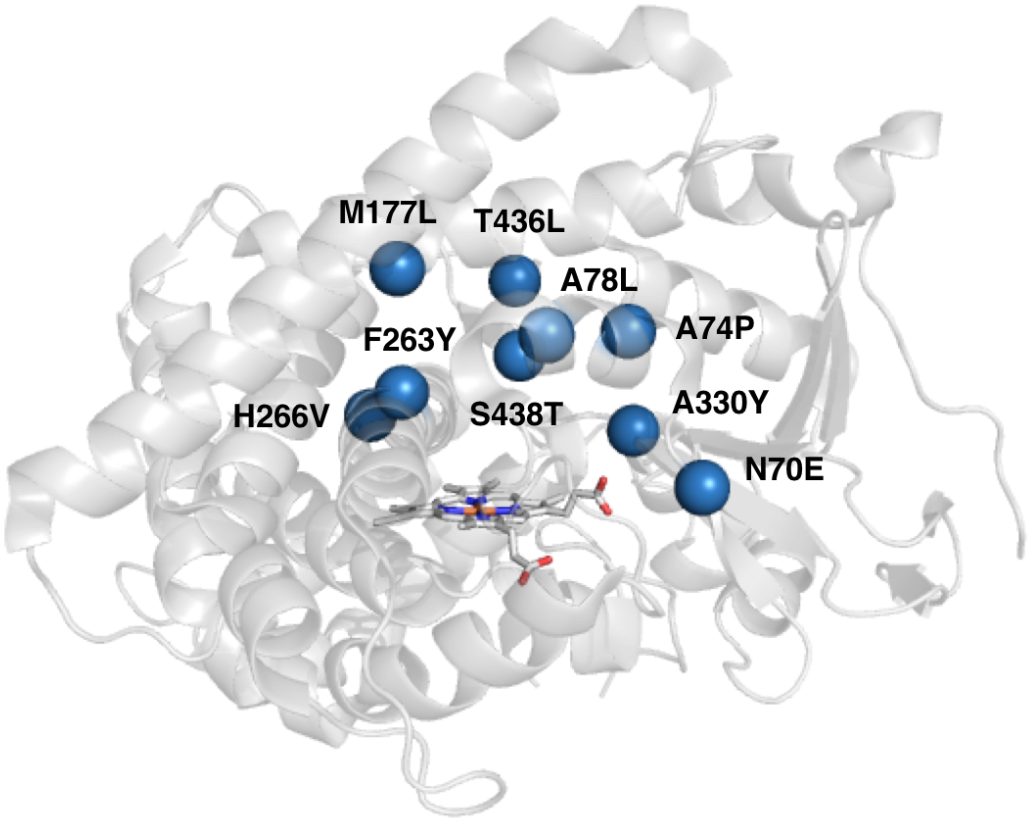



**Fig. S3.** Structural visualization of amino acids mutated during directed evolution of P-4 A82L to P411-CHF.

**Notes:** The structure of P-4 A82L (heme domain) was modeled using the crystal structure of a related P411 variant (PDB: 5UCW), which contains two additional mutations. Considering only the changes incurred in the heme domain, the following mutations were accumulated in going from P-4 A82L to P411-CHF: N70E, A74P, A78L, M177L, F263Y, H266V, A330Y, T436L, S438T (shown as blue spheres, residues 327 and 437 were not included in this analysis because P-4 A82L and P411-CHF contain the same amino acid residues at those positions). Most of the mutations are at positions that line the distal heme pocket and all of the mutated residues are within 15 Å of the iron atom in the heme cofactor.

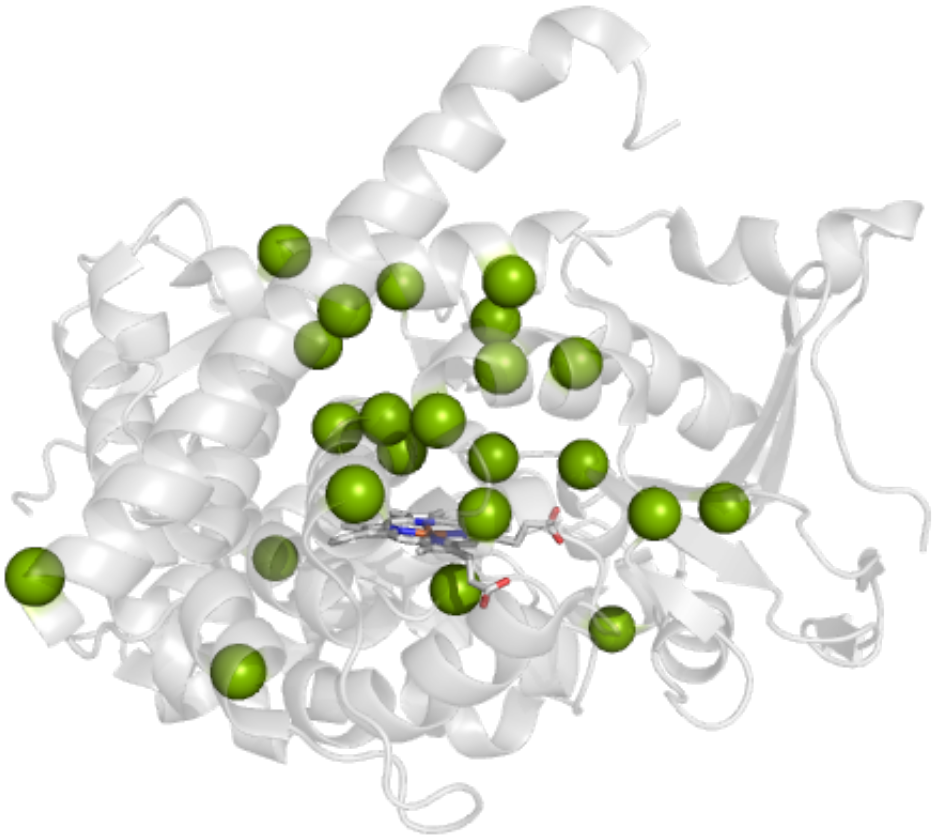


**Fig. S4.** Structural visualization of amino acid differences between $P450_{BM3}$ wild-type and P411-CHF.

**Notes:** The structure of $P450_{BM3}$ wild-type (heme domain) was modeled using the crystal structure of H. M. Girvan *et al.* (PDB: 2IJ2). Comparing only the heme domains of the two proteins, the following 23 amino acids are changed in P411-CHF relative to $P450_{BM3}$ wild-type: N70E, A74P, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, T436L, E442K (shown as green spheres). Overall, 5% of the amino acids in the heme domain of $P450_{BM3}$ have been substituted in P411-CHF.

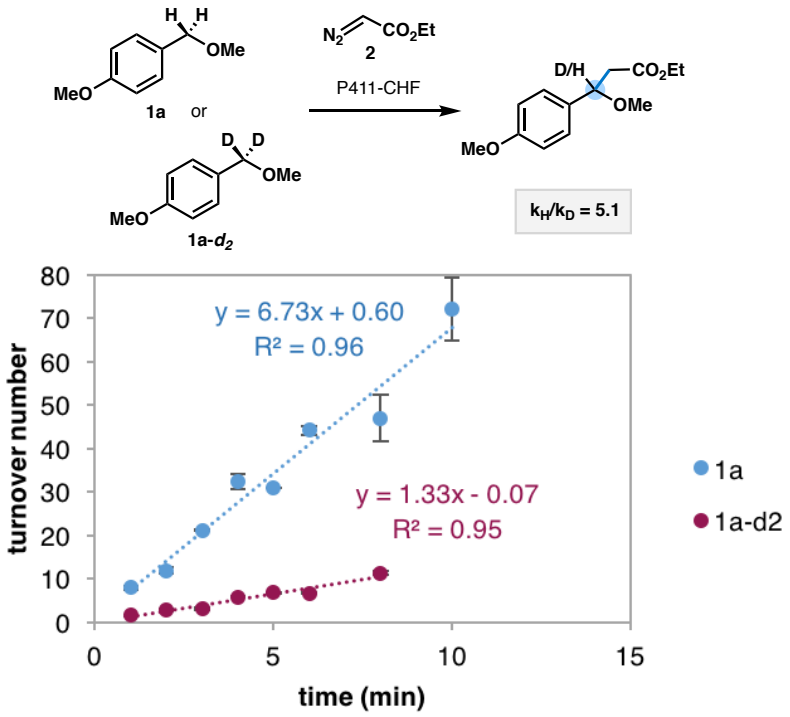



**Fig. S5.** Kinetic isotope effect of C–H alkylation catalyzed by P411-CHF.

**Notes:** Data points represent an average of duplicate measurements; error bars represent one standard deviation. Data collected at the 10-minute time point using substrate **1a-*d*$_2$** were excluded due to non-linear behavior. Detailed experimental methods are described below.

Independent rate experiments with P411-CHF show an intermolecular kinetic isotope effect (KIE, $k_H/k_D$) of 5.1. This suggests that C–H insertion is rate-determining and could possibly involve a linear transition state[6]. In contrast, kinetic isotope effects for rhodium catalysts with carboxylate ligands are significantly less (KIE = 1.55–3.2)[7,8]; this has been invoked as evidence to support a widely accepted three-centered transition state for C–H insertion with these systems[9]. The difference in KIE between P411-CHF and the rhodium-carboxylate catalysts suggests that these systems may have different transitions states or different mechanisms for the C–H insertion step. Since the nature of the C–H insertion step could influence the substrate and product profiles of the catalyst, this is one strong motivation to develop diverse systems for this chemistry.

**Determination of initial rates.** Initial rates were measured from independent reactions set up in parallel using clarified lysate of *E. coli* cells overexpressing P411-CHF. The concentration of P411-CHF was normalized to be 2.0 μM in each reaction. A modified version of the procedure for reactions with lysate (**Section I (I)**) was followed. The modification is as follows: after combining all components of the reaction mixture except the alkane and diazo substrates, the 2 mL reaction vial was allowed to shake in the anaerobic chamber at 500 rpm for at least 10 minutes to ensure even mixing. Reaction vials were then charged with alkane substrate (10 μL, 400 mM in EtOH) and ethyl diazoacetate (10 μL, 400 mM in EtOH) and shaken at 500 rpm, room temp. Final concentrations were 2.0 μM P411-CHF, 1 mM $Na_2S_2O_4$, 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose. Reactions were set up in duplicate and products quantified at 1-minute intervals by quenching with acetonitrile (400 μL) and internal standard (10 μL, 60 mM ethyl phenoxyacetate in MeCN). This mixture was then removed from the anaerobic chamber,

transferred to a microcentrifuge tube, and centrifuged (20,000 × g, 10 minutes). The supernatant was transferred to a vial and analyzed by HPLC (see **Section VIII**). Turnover number was calculated by dividing the concentration of product (mM) by concentration of P411-CHF (0.002 mM).

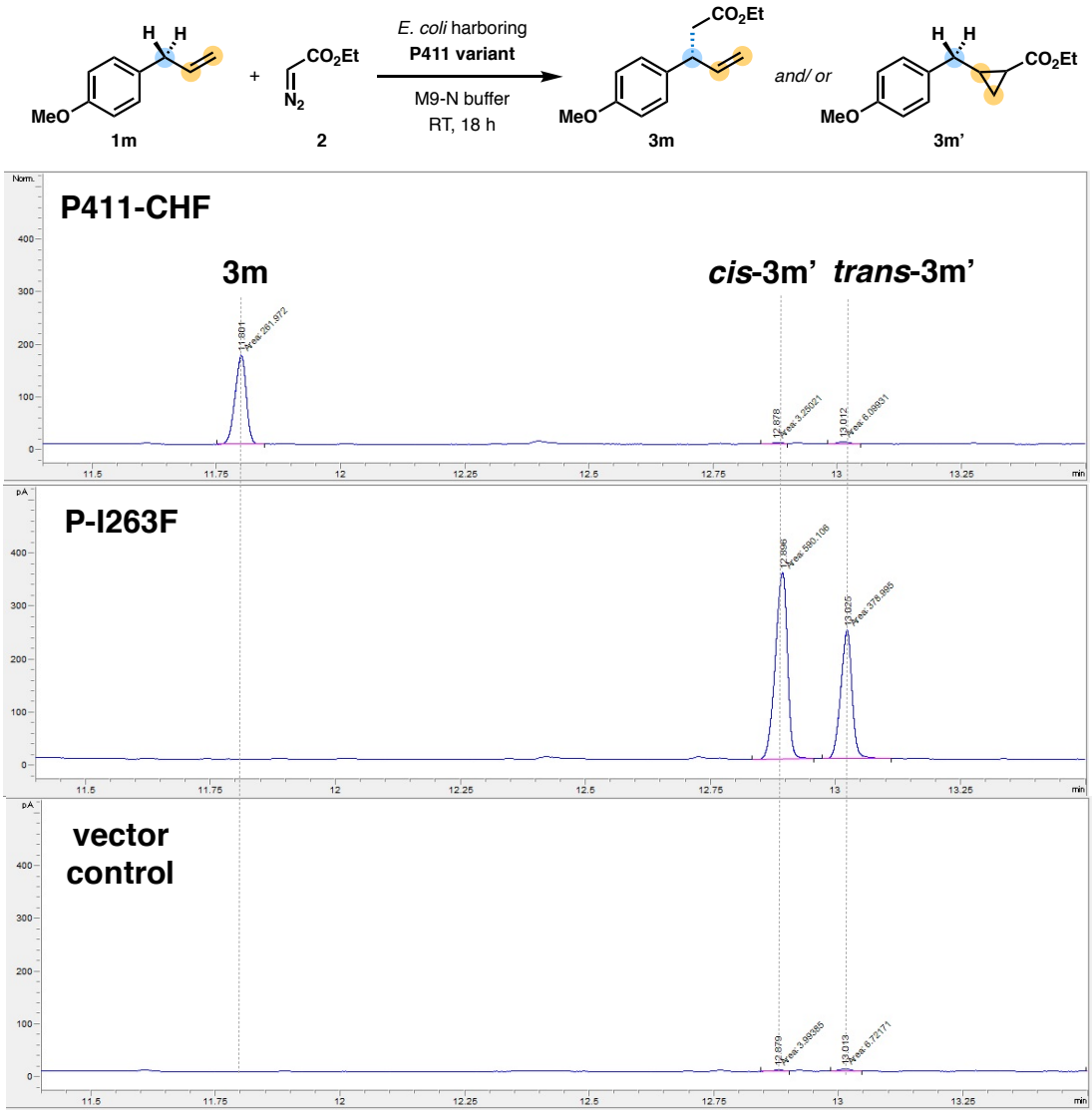



**Fig. S6.** Product profiles for the reaction of 4-allylanisole (**1m**) and ethyl diazoacetate (**2**) with P411-CHF and with P-I263F.

**Notes:** Representative GC traces of reactions performed with P411-CHF (top), P-I263F (middle), or a vector control (bottom) in *E. coli* cells. Reactions were performed with 10 mM substrate **1m** and 10 mM **2**, following the general procedure detailed in **Section I (H)**. Vector control indicates that *E. coli* harboring pET22b(+) encoding a protein that does not contain a transition metal cofactor (halohydrin dehalogenase, UniProt ID: Q93D82) was employed in the reaction.

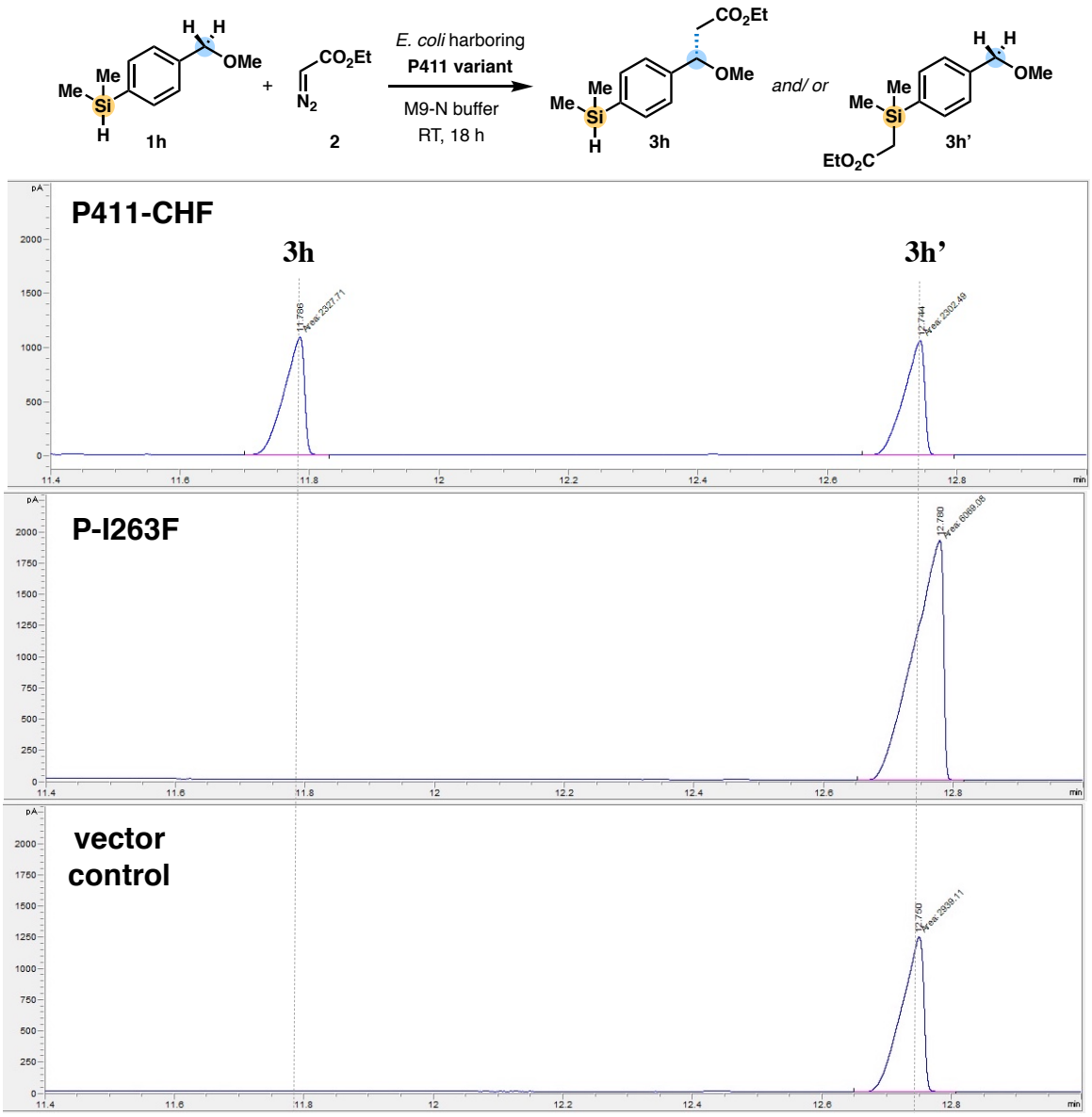



**Fig. S7.** P411-CHF catalyzes C–H alkylation of **1h** with ethyl diazoacetate (**2**).

**Notes:** Representative GC traces of reactions performed with P411-CHF (top), P-I263F (middle), or a vector control (bottom) in *E. coli* cells. Reactions were performed as in **Fig. S6**, with substrates **1h** and **2**.

While P411-CHF was able to access C–H alkylation product **3h** (930 TTN to **3h**, 1:1 **3h**:**3h'**) in the presence of a reactive Si–H bond, reaction with *E. coli* harboring P-I263F only afforded Si–H insertion product **3h'**. Additionally, compound **3h'** was also observed in vector control reactions employing whole *E. coli* cells. Neither **3h** nor **3h'** were detected in a control reaction where **1h** and **2** were combined in M9-N buffer in the absence of cells. See **Section VIII** for detailed analysis of product concentrations.

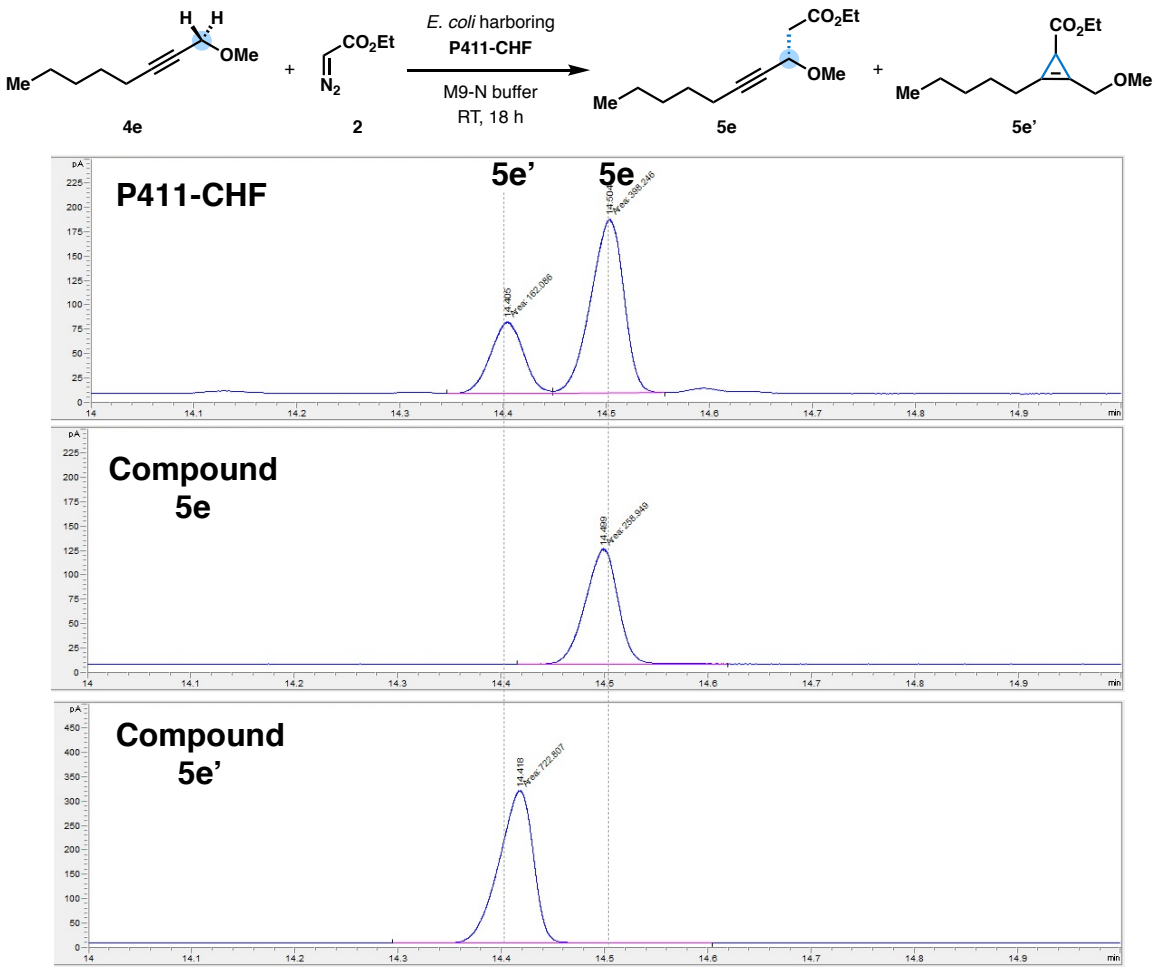



**Fig. S8.** Enzymatic reaction of P411-CHF with substrate **4e** and ethyl diazoacetate (**2**) produces cyclopropene **5e'** as a side product.

**Notes:** Representative GC trace of a reaction performed using P411-CHF in *E. coli* cells (top) with 10 mM substrate **4e** and 10 mM ethyl diazoacetate (**2**), following the general procedure detailed in **Section I (H)**. Also shown are GC traces for reference compounds **5e** (middle) and **5e'** (bottom), used for the identification of P411-CHF synthesized products.

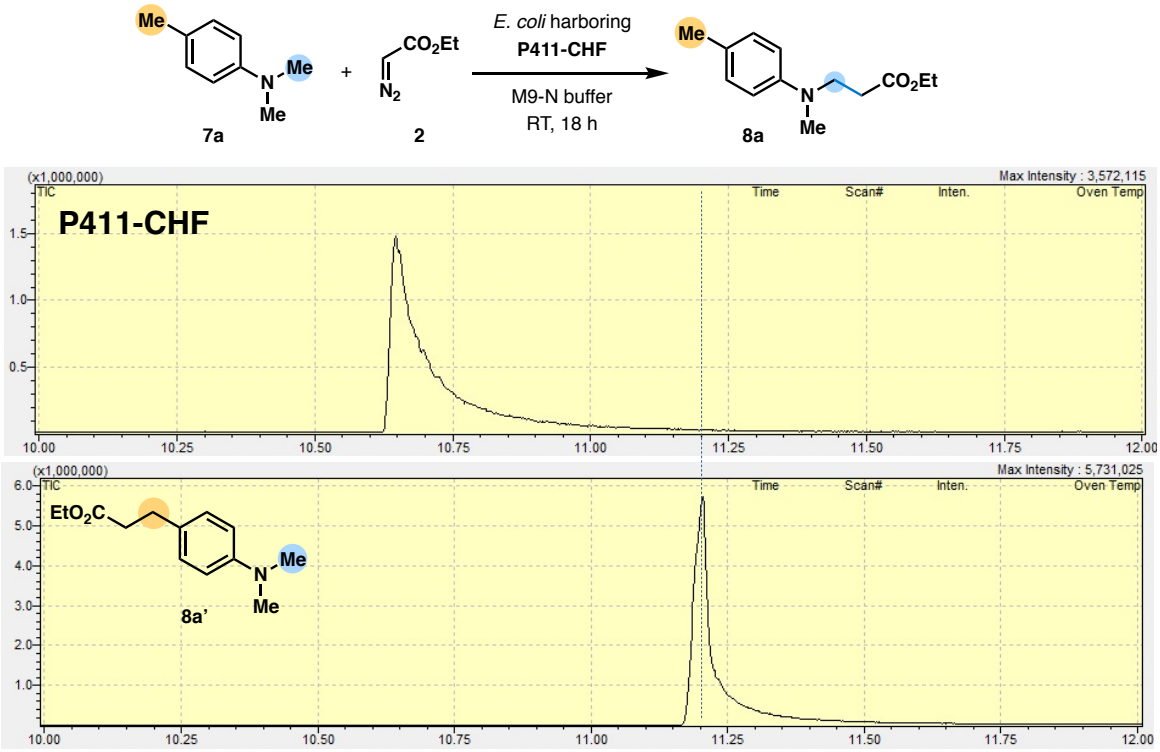



**Fig. S9.** Enzymatic C–H alkylation of 4,*N*,*N*-trimethylaniline (**7a**) with P411-CHF is selective for the α-amino C–H bond.

**Notes:** Representative GC-MS trace of a reaction performed using P411-CHF in *E. coli* cells (top) with 10 mM substrate **7a** and 10 mM ethyl diazoacetate (**2),** following the general procedure detailed in **Section I (H)**. Also shown is a trace for reference compound **8a'** (bottom), which is not observed in the reaction with P411-CHF. This demonstrates that P411-CHF has exquisite selectivity for the alkylation of α-amino C–H bonds in the presence of primary benzylic C–H bonds.

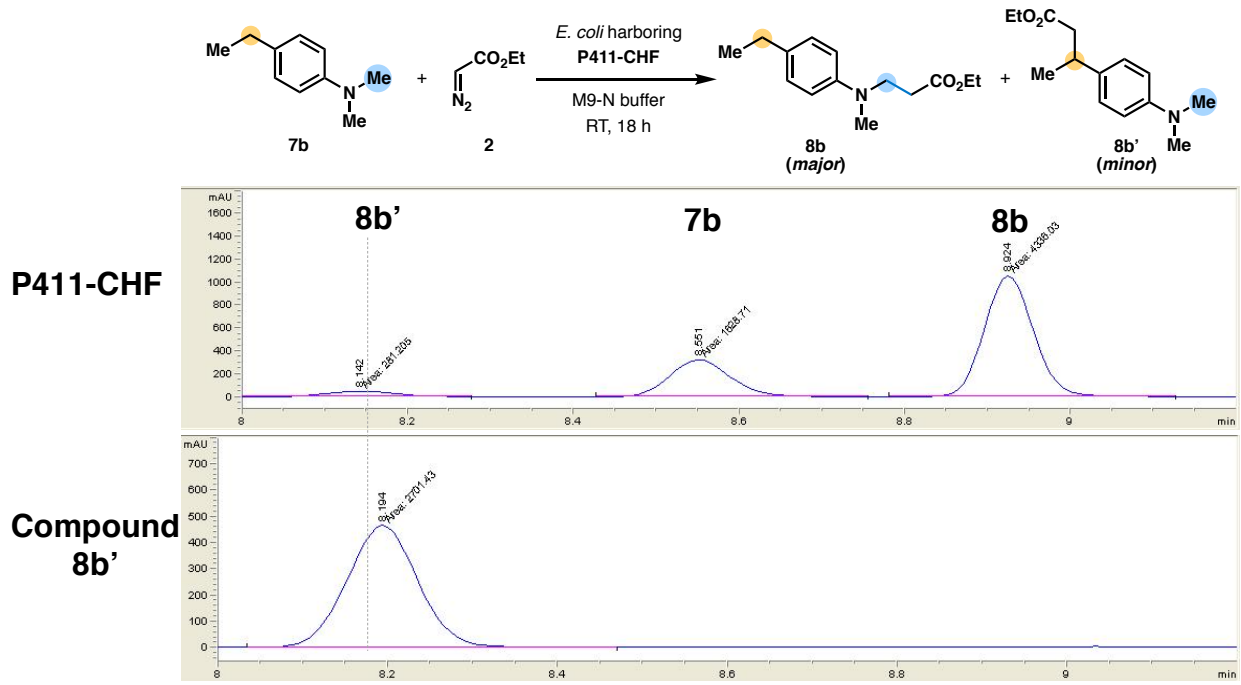



**Fig. S10.** Product profile of P411-CHF catalyzed C–H alkylation of 4-ethyl-*N*,*N*-dimethylaniline (**7b**) with ethyl diazoacetate (**2**).

**Notes:** Representative HPLC trace of a reaction performed using P411-CHF in *E. coli* cells (top) with 10 mM substrate **7b** and 10 mM ethyl diazoacetate (**2**)**,** following the general procedure detailed in **Section I (H)**. Also shown is a trace for reference compound **8b'** (bottom), which was identified as a minor side product in the P411-CHF catalyzed reaction. Major product **8b** was assigned by performing a preparative scale enzymatic reaction (see **Section IX**) and isolation and characterization of the major product.

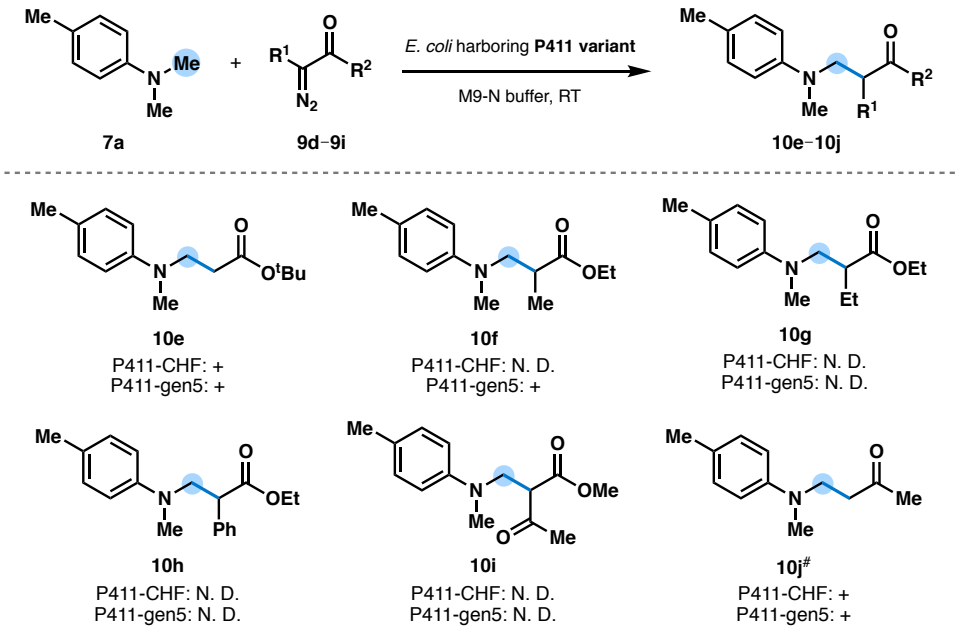


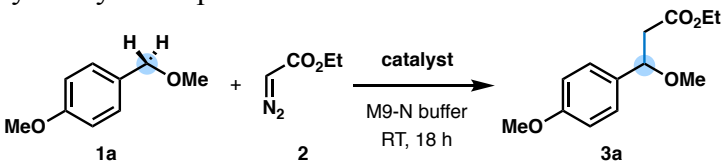

**Fig. S11.** Additional diazo substrates tested for C–H alkylation with P411-CHF and P411-gen5. '+' indicates product was detected; N. D., not detected. #Other products derived from compound **7a** were also observed by GC-MS.

**Notes:** Reactions were performed using P411-CHF ($OD_{600}$ = 59) or P411-gen5 ($OD_{600}$ = 49) in *E. coli* cells with 10 mM coupling partner **7a** and 10 mM diazo compound**.** Product formation was analyzed by GC-MS only. The identity of the product was not confirmed by comparison with chemically synthesized reference compounds or through isolation and characterization. These preliminary results are noteworthy, but should not be used alone for drawing conclusions.

Diazo compounds **9a**, **9b**, and **9c**, for the formation of products **10a**, **10b**, and **10c**, were also investigated with P411-CHF (+, +, +, respective preliminary results) and P411-gen5 (+, N. D., +). Additional variants were tested for these transformations and subsequent preparative scale reactions for product isolation and characterization were pursued; these results are described in Fig. 4c.

Because P411-CHF and its related variants are fully genetically encoded and can be readily optimized by directed evolution, these enzymes can serve as starting points for the evolution of future variants. Some of these future variants will certainly surpass P411-CHF in C–H alkylation activity with alternative diazo reagents.

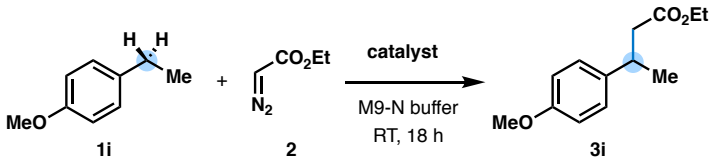
**Table S1.** Initial results for C–H alkylation of *p*-methoxybenzyl methyl ether (**1a**) with ethyl diazoacetate (**2**) catalyzed by heme proteins and control reactions.




| Catalyst | Catalyst concentration | TTN to 3a |
|---|---|---|
| P-4 A82L (in *E. coli* cells, $OD_{600}$=29) | 4.0 μM | 13 |
| *Rma* NOD Y32G (in *E. coli* cells, $OD_{600}$=30) | 11.6 μM | 7 (12[†]) |
| Vector control (in *E. coli* cells, $OD_{600}$=30)[*] | N. A. | n. d. |
| Hemin | 400 μM | n. d.[†, #] |
| Hemin + BSA | 400 μM (hemin), 0.6 mg/mL (BSA) | n. d.[†, #] |

**Notes:** Reactions performed with 10 mM **1a** and 10 mM **2**; results are the average of duplicate reactions. BSA, bovine serum albumin; TTN, total turnover number; n. d., not detected. [†]These reactions contain 1 mM $Na_2S_2O_4$, used as reductant. [*]Vector control indicates that *E. coli* harboring pET22b(+) encoding a protein which does not have a transition metal cofactor (halohydrin dehalogenase, UniProt ID: Q93D82) was employed in the reaction. [#]For reactions with hemin ± BSA, a 0.5 mM solution of hemin (± BSA, 0.75 mg/mL) in M9-N (320 μL) was used instead of a cell suspension; no *D*-glucose was added to these reactions.

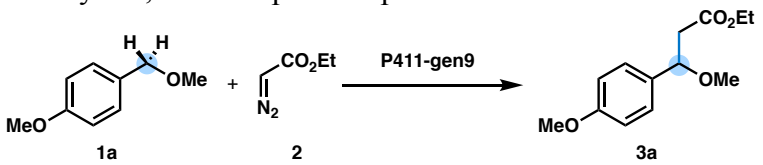
**Table S2.** Initial results for C–H alkylation of 4-ethylanisole (**1i**) with ethyl diazoacetate (**2**) catalyzed by heme proteins and control reactions.




| Catalyst | Catalyst concentration | TTN to 3i |
|---|---|---|
| P-4 A82L (in *E. coli* cells, $OD_{600}$=21) | 4.0 μM | 3 |
| *Rma* NOD Y32G (in *E. coli* cells, $OD_{600}$=30) | 11.6 μM | < 1 (2[†]) |
| Vector control (in *E. coli* cells, $OD_{600}$=30) | N. A. | n. d. |
| Hemin | 400 μM | n. d.[†, #] |
| Hemin + BSA | 400 μM (hemin), 0.6 mg/mL (BSA) | n. d.[†, #] |

**Notes:** Reactions performed with 10 mM **1i** and 10 mM **2**; results are the average of duplicate reactions. Other notes are the same as in **Table S1**.

**Table S3.** P411-gen9, an evolved P411ΔFAD C–H alkylation enzyme, is active in whole *E. coli* cells, in clarified *E. coli* lysate, and as a purified protein.

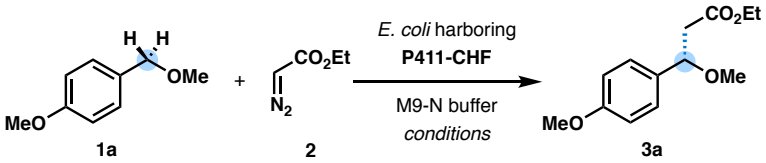



| Form | [P411], μM | Exogenous reductant | TTN to 3a |
|---|---|---|---|
| whole *E. coli* cells ($OD_{600}$ = 15–17) | 2.0 μM | None | 900 |
| Lysate | 2.0 μM | $Na_2S_2O_4$, 1 mM | 940 |
| Purified protein | 11.9 μM | $Na_2S_2O_4$, 1 mM | 150 |
| Purified protein | 11.9 μM | $Na_2S_2O_4$, 5 mM | 210 |
| Purified protein | 10.0 μM–11.9 μM | NADPH, 10 mM | 250 |

**Notes:** Reactions were performed with 10 mM **1a** and 10 mM **2** for 18–20 hours at room temperature, following the procedures detailed in **Section I** (**H, I, and K**). Results are the average of at least duplicate reactions. See **Section IV** for more information on P411-gen9.

These experiments demonstrate that C–H alkylation can be catalyzed using an evolved P411ΔFAD enzyme in whole *E. coli* cells, in clarified *E. coli* lysate, and as a purified protein. The results using purified P411-gen9 are preliminary and the TTN values should be interpreted with caution as no studies regarding the effect of purification conditions on the activity of the enzyme nor studies to optimize the reaction conditions were pursued.

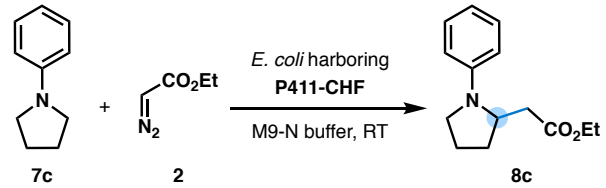
**Table S4.** Enzymatic C–H alkylation reactions performed using *E. coli* cells harboring P411-CHF under non-standard conditions.




| Conditions | TTN to 3a | e.r. |
|---|---|---|
| Anaerobic, full system, room temp. | 2150 | 96.7 : 3.3 |
| Anaerobic, full system, 4 °C | 2090 | 98.0 : 2.0 |
| Anaerobic, no GOX, room temp. | 2100 | 96.7 : 3.3 |
| Anaerobic, no GOX, no *D*-glucose, room temp. | 1770 | 96.7 : 3.3 |
| Aerobic, no GOX, no *D*-glucose, room temp. | 30 | not measured |

**Notes:** Standard reaction conditions are *E. coli* cells ($OD_{600}$ = 30) expressing P411-CHF with 10 mM **1a** and 10 mM ethyl diazoacetate (**2**). Reactions were allowed to proceed at the indicated temperature for 18 h. For reactions without the GOX oxygen depletion solution, 20 μL of M9-N buffer was added instead; for reactions without *D*-glucose, 40 μL of M9-N buffer was added. The aerobic reaction was set up under air, without prior degassing of cells or reagents with argon. Results are the average of duplicate reactions.

**Table S5.** Reaction progress analysis of enantioselectivity.




| Time | Relative amount of 8c formed[†] | e.r. |
|---|---|---|
| 15 min | 0.045 | 83.6 : 16.4 |
| 30 min | 0.062 | 83.2 : 16.8 |
| 1 h | 0.093 | 83.5 : 16.5 |
| 20 h | 1 | 83.9 : 16.1 |
| 18 h (*isolated*) [ξ] | not applicable | 82.8 : 17.2 |

**Notes:** Standard reaction conditions are *E. coli* cells ($OD_{600}$ = 30) expressing P411-CHF with 10 mM **7c** and 10 mM ethyl diazoacetate (**2**), following the general procedure detailed in **Section I (H)**. Reactions were quenched after the indicated time by the addition of cyclohexane (500 μL) and internal standard (10 μL of 40 mM ethyl benzoate). The mixture was transferred to a 1.5 mL microcentrifuge tube, vortexed (10 seconds, 3 times), and centrifuged (20,000 × g, 5 minutes) to completely separate the organic and aqueous layers. The organic layer was taken for chiral HPLC analysis (see **Section XI**). [†]This refers to the ratio of the HPLC peak areas for **8c** (sum of enantiomers) over internal standard in a reaction quenched at the indicated time to that of a reaction quenched at 20 h. [ξ]Product was isolated after an enzymatic reaction for 18 h; preparative scale reaction conditions were employed and full details are provided in **Section IX**.

This experiment demonstrates that enantioselectivity of enzymatic C–H alkylation does not change with time and substrate conversion. While it is possible that the protein is altered during the course of the reaction[10], this does not affect the enantioselectivity of the product.

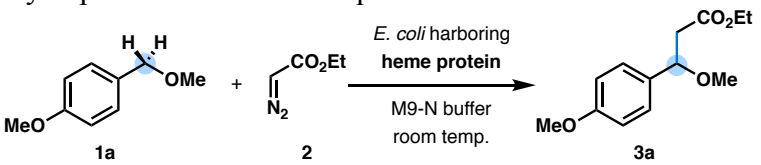
## III. Screening of enzymes for C–H alkylation activity

### Testing diverse heme proteins for reaction discovery

A composite plate of 40 heme proteins and their variants from various organisms were screened for formation of product **3a** from substrates **1a** and **2**. These proteins were cloned and used in other studies, including carbene Si–H insertion[4] and alkene cyclopropanation[11,12]. Expression of these proteins followed the procedures as described by the prior studies and testing for initial activity was carried out with whole *E. coli* cells. The general procedure for reaction screening in 96-well plate format (**Section I (E)**) was employed and the reactions were analyzed by GC-MS. *Note:* Since this was an initial test, proper expression of the indicated proteins was not verified. Consequently, negative results should be interpreted with caution. From this experiment, it was observed that *Rma* NOD Y32G had a low level of C–H alkylation activity (Table S6).

In addition, eight diverse heme proteins, including *Rma* NOD Y32G, were chosen and tested for C–H alkylation activity using substrates **1a** and **2** following the general procedure for small scale enzymatic reactions using whole *E. coli* cells (**Section I (H)**) (Table S7). Product formation by variant *Rma* NOD Y32G was characterized by HPLC (see **Section VIII**). Other reactions were analyzed by GC-MS for formation of product **3a** and the expression of all proteins were measured using the hemochrome assay. Conditions with no exogenous reductant as well as with the addition of 1 mM $Na_2S_2O_4$ (final concentration) were tested.

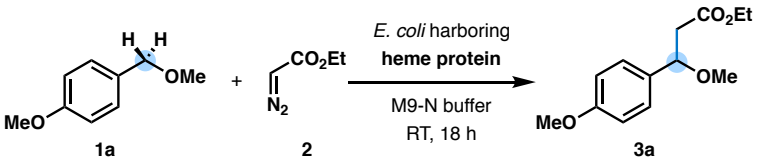
**Table S6.** Preliminary experiments with heme proteins.




| UniProt ID | Organism | Annotation | Abbrev. name | No. of variants | Ref. | Formation of 3a |
|---|---|---|---|---|---|---|
| B3FQS5 | *Rhodothermus marinus* | Cytochrome *c* | *Rma* cyt *c* | 9 | *4* | n. d. |
| P15452 | *Hydrogenobacter thermophilus* | Cytochrome *c*-552 | *Hth* cyt *c* | 2 | *4* | n. d. |
| P00080 | *Rhodopila globiformis* | Cytochrome *c*2 | - | 2 | *4* | n. d. |
| D0MGT2 | *Rhodothermus marinus* | Nitric oxide dioxygenase | *Rma* NOD | 10 | *11* | detected[†] |
| P02185 | *Physeter catodon (Sperm whale)* | Myoglobin | Mb | 1 | *11*[*] | n. d. |
| B3DUZ7 | *Methylacidiphilum infernorum* | Hemoglobin-like flavoprotein | HGG[#] | 2 | *11*[*] | n. d. |
| O66586 | *Aquifex aeolicus* | Thermoglobin | - | 1 | *11* | n. d. |
| G7VHJ7 | *Pyrobaculum ferrireducens* | Protoglobin | - | 1 | *11* | n. d. |
| Q0PB48 | *Campylobacter jejuni* | Truncated hemoglobin | - | 1 | *11* | n. d. |
| Q9YFF4 | *Aeropyrum pernix* | Protoglobin | - | 10 | *11* | n. d. |
| O31607 | *Bacillus subtilis* | Truncated hemoglobin | - | 1 | *12* | n. d. |

**Notes:** Reactions performed with 10 mM **1a** and 10 mM **2**. No., number; Abbrev., abbreviated; Ref., reference.; n. d., not detected. [†]Small amount of **3a** was detected in the reaction with variant *Rma* NOD Y32G. No product **3a** was detected in the reaction mixtures of other *Rma* NOD variants. [*]While these heme proteins were not reported in this reference, the expression conditions employed for these variants followed those given in the reference. [#]This protein is abbreviated as Hell's Gate Globin (HGG) on the basis of a prior literature report which uses this name[13].

**Table S7.** Heme proteins tested for C–H alkylation activity.



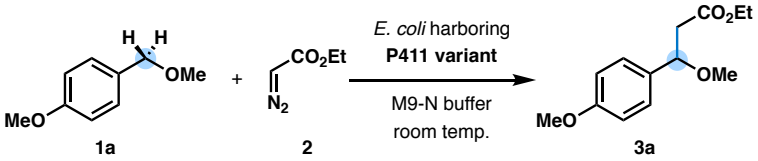

| UniProt ID | Organism | Annotation | Abbrev. Name | Mutation(s) from WT | Formation of 3a |
|---|---|---|---|---|---|
| P14779 | *Bacillus megaterium* | cytochrome P450/NADPH-P450 reductase | $P450_{BM3}$ | none[*] | n. d. |
| Q55080 | *Sulfolobus acidocaldarius* | Cytochrome P450 | CYP119 | none[*] | n. d. |
| D0MGT2 | *Rhodothermus marinus* | Nitric oxide dioxygenase | *Rma* NOD | Y32G[+, *] | 7 TTN[†] (12 TTN[ξ]) |
| B3DUZ7 | *Methylacidiphilum infernorum* | Hemoglobin-like flavoprotein | HGG | none[+, *] | n. d. |
| P02185 | *Physeter catodon (Sperm whale)* | Myoglobin | Mb | H64V V68A D122N[+, *] | n. d. |
| B3FQS5 | *Rhodothermus marinus* | Cytochrome *c* | *Rma* cyt *c* | none[+, #] | n. d. |
| B3FQS5 | *Rhodothermus marinus* | Cytochrome *c* | *Rma* cyt *c* | V75T M100D M103E[+, #] | n. d. |
| P15452 | *Hydrogenobacter thermophilus* | Cytochrome *c* | *Hth* cyt *c* | none[+, #] | n. d. |

**Notes:** Heme proteins were tested in whole *E. coli* cells both with and without the addition of 1 mM $Na_2S_2O_4$ (final concentration). Reactions were performed with 10 mM **1a** and 10 mM **2**; results are the average of at least duplicate reactions. Abbrev., abbreviated; WT, wild-type; n. d., not detected; TTN, total turnover number. [†]This data is also included in Table S1. [ξ]Reaction includes 1 mM $Na_2S_2O_4$. [+]These variants are also included in Table S6. [*]These proteins were expressed following the procedure of A. M. Knight *et al.*[11]. [#]Cytochrome *c* variants were expressed following the procedure of S. B. J. Kan *et al.*[4].

**Screening previously engineered cytochrome $P450_{BM3}$ variants**

A composite plate of 36 cytochrome $P450_{BM3}$ variants from lineages engineered for non-natural reactions including C–H amination[14], olefin aziridination[15], and olefin cyclopropanation[16] was screened for C–H alkylation activity using substrates **1a** and **2**. The general procedure for reaction screening in 96-well plate format (**Section I (E)**) was employed and the reactions were analyzed by HPLC. Most variants showed no activity (17 variants) or trace activity (15 variants) for **3a** formation. The four highest performing variants, all which contain an axial cysteine-to-serine mutation (P411), are shown in Table S8. While P-4 A82L was chosen as the parent protein for the directed evolution of a C–H alkylation enzyme, the information gained from this experiment was used to guide the first several rounds of evolution.

**Table S8.** Engineered cytochrome P411 variants show promiscuous C–H alkylation activity.




| Name | Mutations from $P450_{BM3}$ WT | Relative amount of 3a formed[†] |
|---|---|---|
| P-4 A82L | V78A, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K | 1 |
| P-4 A82L A78V F263Y | A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K | 1.4 |
| P-4 A82L A78V F263L | A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263L, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K | 0.5 |
| P-4 A82L A78V F263L E267D (a.k.a. $P411_{CHA}$) | A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263L, E267D, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K | 0.7 |

**Notes:** Reactions were performed with 10 mM **1a** and 10 mM **2** following the general procedure for reaction screening in 96-well plate format (**Section I (E)**). Results are the average of duplicate reactions. All variants are reported in ref. *14*. [†]This refers to the ratio of the HPLC peak area for **3a** in reactions with the indicated variant to that in reactions with P-4 A82L.

## IV. Directed evolution of C–H alkylation enzymes

### Construction of site-saturation libraries

Site-saturation libraries were generated employing the "22c-trick" method[1] and screened in one 96-well plate; double site-saturation libraries were generated using the same method to target two different sites and these were screened in three 96-well plates. Following the general screening in 96-well plate procedure (**Section I (E)**), variants which exhibited higher formation of C–H alkylation product (**3a** or **3i**) or improved enantioselectivity for product **3a** were identified. A summary of the amino acid residues targeted for mutagenesis is presented in Table S9, as well as the beneficial mutation(s) selected for each round of mutagenesis. The locations of the selected beneficial mutations are displayed on a structural model of the P411 enzyme (**Section II**, Fig. S3).

Variants which were identified to show higher activity and/ or enantioselectivity during screening were streaked out on $LB_{amp}$ agar plates. A single colony was selected, sequenced, and the TTN measured for both products **3a** and **3i** using clarified lysate of *E. coli* cells overexpressing the desired protein (unless otherwise indicated, the concentration of P411 variant was normalized such that each reaction contained 2.0 μM enzyme, general procedure **Section I (I)**). Enantiomeric ratios of the enzymatic products produced by P411-gen6 and further evolved variants were also characterized. The results are summarized in Tables S12 and S13.

**Table S9.** Summary of directed evolution for C–H alkylation.

| Round | Parent | Diversification strategy[†] | Screening substrate (selection criteria) | Changes made[*] |
|---|---|---|---|---|
| 1 | **P-4 A82L** | Individual variants identified as active for C–H amination[14] | 4-ethylanisole (**1i**) (activity) | F263Y |
| 2 | **P-4 A82L F263Y** | Site-saturation mutagenesis A78X | 4-ethylanisole (**1i**) (activity) | A78L |
| 3 | **P-4 A82L F263Y A78L** | Site-saturation mutagenesis T327X | 4-ethylanisole (**1i**) (activity) | T327I |
| 4 | **P411-gen4** (P-4 A82L F263Y A78L T327I) | Site-saturation mutagenesis A74X, E267X | 4-ethylanisole (**1i**) (activity) | A74G |
| 5 | **P411-gen5** (P411-gen4 A74G) | Site-saturation mutagenesis A328X, H92X, R255X, A264X, H100X, F393X, L437X | *p*-methoxybenzyl methyl ether (**1a**) (activity) | L437Q |
| 6 | **P411-gen6** (P411-gen5 L437Q) | Protein truncations: full-length P411, ΔFAD domain, heme-domain only | *p*-methoxybenzyl methyl ether (**1a**) (activity) | ΔFAD domain |
| 6b | **P411-gen6b** (P411ΔFAD-gen6) | Site-saturation mutagenesis A78X, F87X, I263X, T438X | *p*-methoxybenzyl methyl ether (**1a**) | S438T |

| | | | | |
|---|---|---|---|---|
| | | | (activity) | |
| 7 | **P411-gen7** (P411-gen6b S438T) | Site-saturation mutagenesis[Ψ] A330X, F331X, T436X, A82X, L181X, L188X | *p*-methoxybenzyl methyl ether (**1a**) (activity) | T436L |
| 8 | **P411-gen8** (P411-gen7 T436L) | Site-saturation mutagenesis D63X, F162X, M177X, V178X, L439X | 4-ethylanisole (**1i**) (activity) | M177L |
| 9 | **P411-gen9** (P411-gen8 M177L) | Site-saturation mutagenesis F87X, E267X, M118X, L437X, H266X, S332X, T260X, T365X | 4-ethylanisole (**1i**) (activity) | H266V |
| 10 | **P411-gen10** (P411-gen9 H266V) | Site-saturation mutagenesis N70X, T88X, H171X, H361X, P329X, T269X, L75X, L52X | 4-ethylanisole (**1i**) (activity) & *p*-methoxybenzyl methyl ether (**1a**) (enantioselectivity) | N70E |
| 11 | **P411-gen11** (P411-gen10 N70E) | Site-saturation mutagenesis L71X, S72X, F261X, G265X, L86X, I401X, A330X, C400X | *p*-methoxybenzyl methyl ether (**1a**) (activity & enantioselectivity) | A330Y |
| 12 | **P411-gen12** (P411-gen11 A330Y) | Double site-saturation mutagenesis P329X-F331X[#], A328X-F331X[#], T327X-T268X, A74X-L437X | *p*-methoxybenzyl methyl ether (**1a**) (activity & enantioselectivity) | I327T |
| 13a | **P411-gen13** (P411-gen12 I327T) | Double site-saturation mutagenesis L181X-L437X, V178X-E267X, M118X-I401X | *p*-methoxybenzyl methyl ether (**1a**) (activity & enantioselectivity | None |
| 13b | **P411-gen13** (P411-gen12 I327T) | Testing previously identified beneficial and neutral mutations[ξ]. | *p*-methoxybenzyl methyl ether (**1a**) (activity & enantioselectivity | G74P, Q437L |
| 14 | **P411-CHF** (P411-gen13 G74P Q437L) | N. A. | N. A. | N. A. |

**Notes:** Some residues were saturated more than once, in different parents. Gen, generation; N. A., not applicable. [†]Residues for site-saturation mutagenesis libraries are listed relative to the amino acid at that position in wild-type $P450_{BM3}$. *Beneficial mutations are listed relative to the amino acid at that position in the parent protein. [Ψ]Random mutagenesis by error-prone PCR on this parent enzyme and screening for C–H alkylation activity was also performed (unpublished results).

However, no mutations from this study were carried forward to the next enzyme generation; the F162L mutation, identified in this context, was included in the diversification strategy of round 13b. [#]Only NDT libraries were constructed and screened for this double-site saturation experiment. [ξ]Select mutations identified from previous rounds of mutagenesis and screening were introduced in various combinations to P411-gen13. Twenty-seven variants were attempted and screened.

**Table S10.** Mutations and truncations relative to $P450_{BM3}$ wild-type for the P411 variants described in Table S9.

| Gen. | Name | Mutations relative to $P450_{BM3}$ WT | Domain composition[†] |
|---|---|---|---|
| 1 | P-4 A82L | V78A, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K | Full-length |
| 2 | P-4 A82L F263Y | V78A, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K | Full-length |
| 3 | P-4 A82L F263Y A78L | V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K | Full-length |
| 4 | **P411-gen4** (P-4 A82L F263Y A78L T327I) | V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T438S, E442K | Full-length |
| 5 | **P411-gen5** (P411-gen4 A74G) | A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T438S, E442K | Full-length |
| 6 | **P411-gen6** (P411-gen5 L437Q) | A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, L437Q, T438S, E442K | Full-length |
| 6b | **P411-gen6b** (P411ΔFAD–gen6) | A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, L437Q, T438S, E442K | ΔFAD domain |
| 7 | **P411-gen7** (P411-gen6b S438T) | A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, L437Q, E442K | ΔFAD domain |
| 8 | **P411-gen8** (P411-gen7 T436L) | A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K | ΔFAD domain |
| 9 | **P411-gen9** | A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, T268G, | ΔFAD domain |

| | | | |
|---|---|---|---|
| | (P411-gen8 M177L) | A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K | |
| 10 | **P411-gen10** (P411-gen9 H266V) | A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K | ΔFAD domain |
| 11 | **P411-gen11** (P411-gen10 N70E) | N70E, A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K | ΔFAD domain |
| 12 | **P411-gen12** (P411-gen11 A330Y) | N70E, A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, A330Y, L353V, I366V, C400S, T436L, L437Q, E442K | ΔFAD domain |
| 13 | **P411-gen13** (P411-gen12 I327T) | N70E, A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, T436L, L437Q, E442K | ΔFAD domain |
| 14 | **P411-CHF** (P411-gen13 G74P Q437L) | N70E, A74P, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, T436L, E442K | ΔFAD domain |

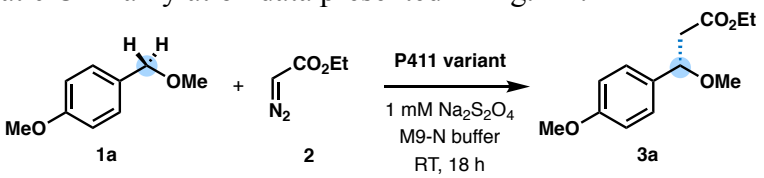
[†]Full-length cytochrome P411 variants contain amino acids 1–1048. Cytochrome P411 variants containing the FAD truncation (ΔFAD domain) contain amino acids 1–664.

**Table S11. Other P411 variants described in this study.**

| Name | Mutations from $P450_{BM3}$ WT | Reference |
|---|---|---|
| P-I263F | V78A, F87V, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268A, A290V, L353V, I366V, C400S, T438S, E442K | *17* |
| P411-IY T327I | N70E, A74G, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, A330Y, L353V, I366V, C400S, I401L, T436L, L437Q, E442K | *N. A.* [†] |

[†]N. A., not applicable. The parent of this variant, P411-IY, was made for round 13b of directed evolution (see Table S9). The P411-IY T327I variant, which contains an additional T327I mutation, was identified in the context of a separate carbene transfer project (Chen, K., Zhou, A. Z. & Arnold, F. H., unpublished results).

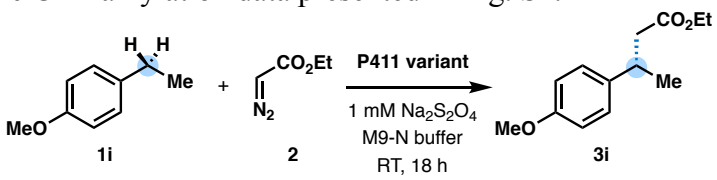
**Table S12.** Enzymatic C–H alkylation data presented in Fig. 2B.




| Variant | [P411], μM | TTN ± SD | e.r. |
|---|---|---|---|
| P-4 A82L | 4.0 | 14 ± 2 | N. A. |
| P-4 A82L F263Y | 4.0 | 29 ± 1 | N. A. |
| P-4 A82L F263Y A78L | 4.0 | 23 ± 5 | N. A. |
| P411-gen4 | 2.0 | 48 ± 3 | N. A. |
| P411-gen5 | 2.0 | 68 ± 3 | N. A. |
| P411-gen6 | 2.0 | 59 ± 4 | *rac* |
| P411-gen6b | 2.0 | 98 ± 2 | *rac* |
| P411-gen7 | 2.0 | 200 ± 4 | 55.0 : 45.0 |
| P411-gen8 | 2.0 | 560 ± 50 | 59.4 : 40.6 |
| P411-gen9 | 2.0 | 940 ± 30 | 64.7 : 35.3 |
| P411-gen10 | 2.0 | 1480 ± 50 | 68.1 : 31.9 |
| P411-gen11 | 2.0 | 1240 ± 30 | 77.9 : 22.1 |
| P411-gen12 | 2.0 | 1490 ± 40 | 88.5 : 11.5 |
| P411-gen13 | 2.0 | 1960 ± 40 | 94.0 : 6.0 |
| P411-CHF | 2.0 | 2020 ± 40 | 96.7 : 3.3 |

**Notes:** Reactions were performed using clarified *E. coli* lysate with 10 mM **1a** and 10 mM **2**, following the general procedure detailed in **Section I (I)**. Each reported TTN is the average of quadruplicate reactions. TTN, total turnover number, refers to TTN to **3a**; SD, standard deviation; N. A., not applicable, indicates that this value was not measured; *rac,* racemic. Parts of this data are also included in Fig. S2 and Table S3.

**Table S13.** Enzymatic C–H alkylation data presented in Fig. S1.




| Variant | [P411], μM | TTN ± SD | e.r. |
|---|---|---|---|
| P-4 A82L | 4.0 | 2 ± 0 | N. A. |
| P-4 A82L F263Y | 4.0 | 4 ± 0 | N. A. |
| P-4 A82L F263Y A78L | 4.0 | 7 ± 2 | N. A. |
| P411-gen4 | 2.0 | 13 ± 1 | N. A. |
| P411-gen5 | 2.0 | 14 ± 0 | N. A. |
| P411-gen6 | 2.0 | 12 ± 1 | N. A. |
| P411-gen6b | 2.0 | 18 ± 1 | N. A. |
| P411-gen7 | 2.0 | 34 ± 1 | N. A. |
| P411-gen8 | 2.0 | 130 ± 20 | 67.7 : 32.3 |
| P411-gen9 | 2.0 | 260 ± 20 | 71.9 : 28.1 |
| P411-gen10 | 2.0 | 510 ± 30 | 76.9 : 23.1 |
| P411-gen11 | 2.0 | 450 ± 10 | 83.0 : 17.0 |
| P411-gen12 | 2.0 | 630 ± 20 | 92.0 : 8.0 |
| P411-gen13 | 2.0 | 500 ± 30 | 96.9 : 3.1 |
| P411-CHF | 2.0 | 440 ± 30 | 98.0 : 2.0 |

**Notes:** Reactions were performed using clarified *E. coli* lysate with 10 mM **1i** and 10 mM **2**, following the general procedure detailed in **Section I (I)**. Each reported TTN is the average of quadruplicate reactions. TTN, total turnover number, refers to TTN to **3i**; SD, standard deviation; N. A., not applicable, indicates that this value was not measured.

## V. Nucleotide and amino acid sequences

All heme proteins disclosed below were cloned into a pET22b(+) vector and contain a *C*-terminal 6xHis-tag.

DNA and amino acid sequence of **P-4 A82L**, a previously reported cytochrome P411 variant[14] which was used as the starting point for directed evolution:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAACACAGATAA
ACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCGAGGCGCCTGGTCGTGTAA
CGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAATCACGCTTTGATAAAAACTTAAGTCAA
GCGCTGAAATTTGCACGTGATTTTCTTGGAGACGGGTTAGCCACAAGCTGGACGCATGAAAAAAATTGGAAAAA
AGCGCATAATATCTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAGGCTATCATGCGATGATGGTCGATATCG
CCGTGCAGCTTGTTCAAAAGTGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGT
TTAACGCTTGATACAATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGAGATCAGCCTCATCC
ATTTATTATAAGTATGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGACGACCCAG
CTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGTAGATAAAATTATTGCA
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CAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAATTAGCTCATTTGCCA
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VVASKELQQPGSARSTRHLEIELPKEASYQEGDHLGVIPRNYEGIVNRVTARFGLDASQQIRLEAEEEKLAHLP
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EEKGRYAKDVWAGLEHHHHHH.

DNA and amino acid sequence of **P411-gen5**, which was employed for the synthesis of key intermediate (+)-**8f** in the total synthesis of (+)-cuspareine:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAACACAGATAA
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FTLPKDPETPLIMVGPGTGVAPFRGFVQARKQLKEQGQSLGEAHLYFGCRSPHEDYLYQEELENAQSEGIITLH
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EEKGRYAKDVWAGLEHHHHHH.

DNA and amino acid sequence of **P411-CHF**, an evolved C–H alkylation enzyme:

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DNA and amino acid sequence of **P411-IY T327I**, a C–H alkylation enzyme derived from P411-gen13.

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DNA and amino acid sequence of ***Rma*** **NOD Y32G**, a heme protein which exhibits trace C–H alkylation activity.

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## VI. Substrate Synthesis and Characterization

Commercially available alkane and diazo substrates were used as received: **1a**, **1d**, **1f**, **1g**, **1i**–**1k**, **1m**, **7a**, **7c**–**7f**, **9a**, **9e**, **9f** (custom synthesis, Arch Bioscience). Compound **1c** was also commercial (Combi-Blocks), though the commercial product was used only for synthesis. Ethyl diazoacetate (**2**, Sigma-Aldrich) was concentrated under reduced pressure and its concentration relative to residual dichloromethane was determined by $^1$H NMR. Diazo compounds **9h** (ref. 18) and **9i** (ref. 19) are known and were prepared according to literature procedures. Caution: although no safety issues were encountered, diazo compounds are reactive and should be used with caution.

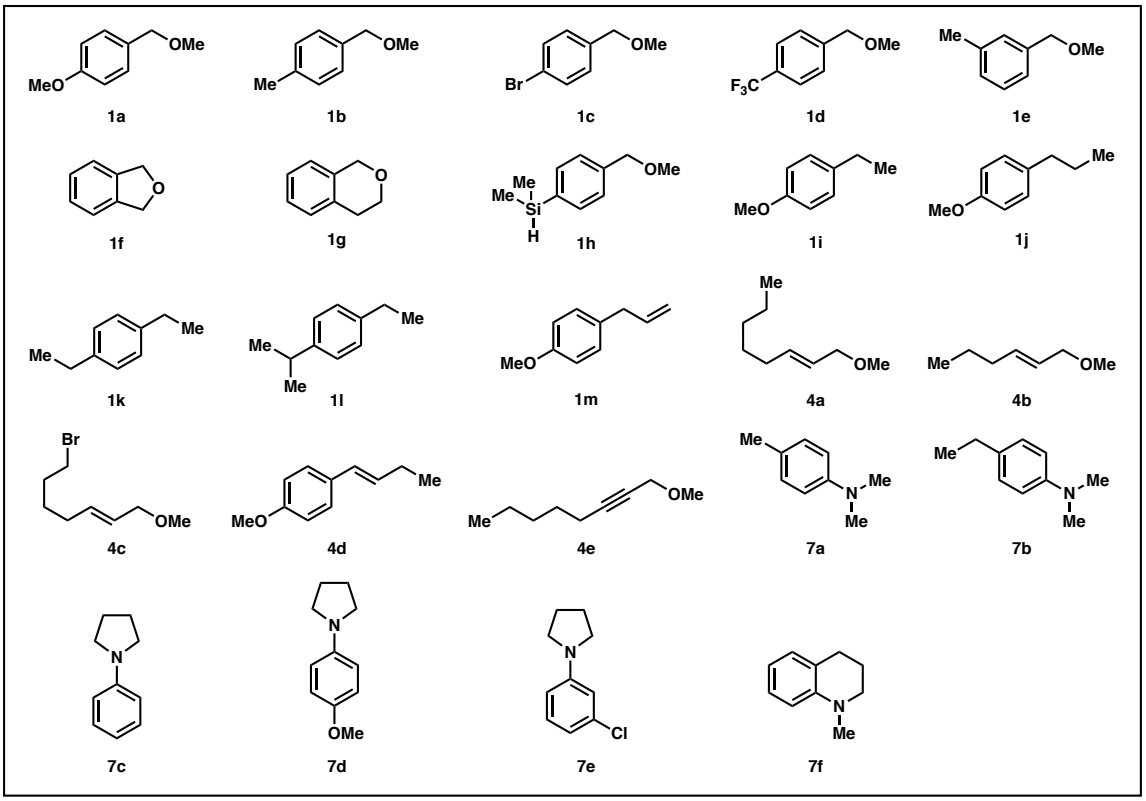



**Fig. S12**. Alkane substrates. This terminology is used to emphasize that the reaction reported here acts on a $sp^3$-hybridized C–H bond in the alkane portion of the substrate.

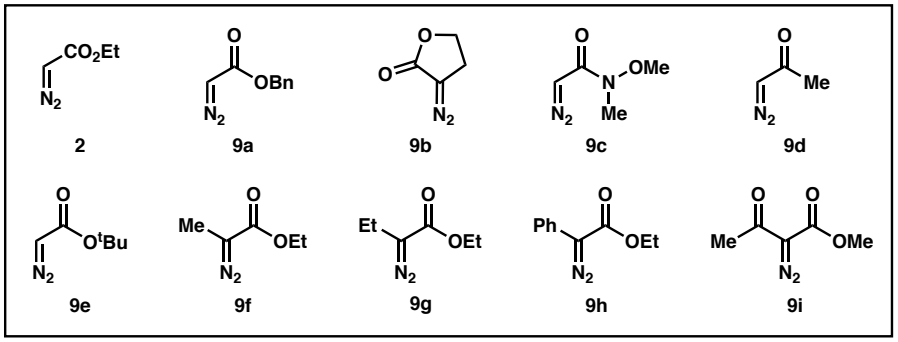


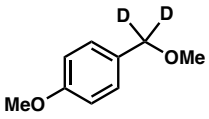

**Fig. S13.** Diazo compounds.

**General Procedure A: Methylation of alcohols**

To a 250 mL round bottom flask was added NaH (60% dispersion in mineral oil, 15–30 mmol, 1.2–1.5 equiv.). The flask was evacuated and filled with argon (3 times). Anhydrous THF (45–80 mL) was added by syringe and the reaction mixture was cooled to 0 °C in an ice bath. Alcohol (10–20 mmol, 1.0 equiv.) in THF (5–10 mL) was added dropwise and the reaction mixture was allowed to warm to room temperature and stirred for 30 minutes. Following, iodomethane (20–40 mmol, 2.0 equiv.) in THF (10 mL) was added and the reaction was stirred at room temperature (8–15 hours). The reaction was quenched by the addition of brine (60 mL) or $NH_4Cl$ (sat. aq., 60 mL) and the phases were separated. The aqueous layer was extracted with diethyl ether (3 × 60 mL); the combined organics were washed with aq. sodium thiosulfate (10% w/v, 50 mL, when necessary), dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate or pentane / diethyl ether afforded compounds the desired products in 37–99% yield.

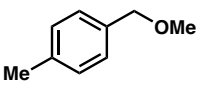
**1-Methoxy-4-(methoxymethyl-*d*$_2$)benzene (1a-*d*$_2$)**

Labeled substrate **1a-*d*$_2$** was prepared from methyl 4-methoxybenzoate using a two-step sequence to 98% deuterium incorporation at the benzylic position. First, to a dry round bottom flask, under argon, was added $LiAlD_4$ (0.23 g, 5.5 mmol, 1.1 equiv.) and anhydrous $Et_2O$ (10 mL). A solution of methyl 4-methoxybenzoate (0.83 g, 5 mmol, 1.0 equiv.) in dry $Et_2O$ (5 mL) was added dropwise and the reaction was allowed to stir at room temperature for 12 hours. Following, the reaction mixture was cooled to 0 °C and diluted with $Et_2O$. The reaction was quenched by the addition of 0.2 mL $H_2O$, 0.2 mL NaOH (aq., 1M), and 0.6 mL $H_2O$. The mixture was allowed to warm to room temperature and stirred for 15 minutes. $MgSO_4$ was added and the mixture was stirred for a further 15 minutes, filtered, and concentrated under reduced pressure. The crude product was purified by silica column chromatography with hexanes / ethyl acetate to give (4-methoxyphenyl)methanol-*d*$_2$ (0.43 g, 61% yield, 98% deuterium incorporation), with spectral data in agreement with literature report[20]. Methylation of this compound was performed following **General Procedure A** (note: reaction performed on 3.0 mmol scale) to afford **1a-*d*$_2$** (0.43 g, 61% yield, 98% deuterium incorporation).



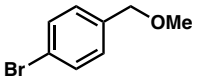

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 3.81 (s, 3H), 3.36 (s, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 159.3, 130.3, 129.5, 113.9, 73.7 (m, labeled), 57.8, 55.4. **HRMS** (EI) m/z: 154.0964 ($M^{+\cdot}$); calc. for $C_9H_{10}O_2{}^2H_2$: 154.0963.

**1-(Methoxymethyl)-4-methylbenzene (1b)**



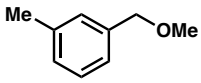

Prepared from *p*-tolylmethanol using **General Procedure A**. This compound is known in the literature[21]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 7.9 Hz, 2H), 4.43 (s, 2H), 3.37 (s, 3H), 2.35 (s, 3H).

**1-Bromo-4-(methoxymethyl)benzene (1c)**




Prepared from (4-bromophenyl)methanol using **General Procedure A**. This compound is known[21]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.47 (d, $J$ = 8.4 Hz, 2H), 7.21 (d, $J$ = 8.6 Hz, 2H), 4.41 (s, 2H), 3.38 (s, 3H).

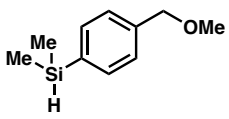
**1-(Methoxymethyl)-3-methylbenzene (1e)**




Prepared from *m*-tolylmethanol using **General Procedure A**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.28 – 7.21 (m, 1H), 7.19 – 7.08 (m, 3H), 4.43 (s, 2H), 3.40 (s, 3H), 2.36 (s, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 138.2, 128.6, 128.5, 128.4, 124.9, 74.9, 58.3, 21.5. **HRMS** (FAB) m/z: 135.0810 [$(M + H^+)–H_2$]; calc. for $C_9H_{11}O$: 135.0810.

**(4-(Methoxymethyl)phenyl)dimethylsilane (1h)**

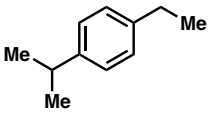
In a 250 mL round bottom flask, under argon, 1-bromo-4-(methoxymethyl)benzene (3.0 g, 15 mmol, 1.0 equiv.) in anhydrous THF (60 mL) was cooled to -78 °C. A solution of *n*-butyllithium (9 mL, 2.5 M in hexanes, 22.5 mmol, 1.5 equiv.) was added dropwise. The resulting mixture was stirred at -78 °C for 2 hours before the dropwise addition of chlorodimethylsilane (2.4 mL, 22.5 mmol, 1.5 equiv.). The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was cooled to 0 °C and quenched with $NH_4Cl$ (sat. aq., 20 mL). The aqueous layer was extracted with diethyl ether (3 × 30 mL); the combined organics were washed with brine (30 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude reaction mixture was purified by silica column chromatography with hexanes / ethyl acetate to afford **1h** (2.14 g, 79% yield). A second round of purification by silica column chromatography with hexanes / ether was performed on a portion of the product.




**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.54 (d, $J$ = 8.1 Hz, 2H), 7.35 (d, $J$ = 8.1 Hz, 2H), 4.47 (s, 2H), 4.43 (hept, $J$ = 3.7 Hz, 1H), 3.40 (s, 3H), 0.35 (d, $J$ = 3.7 Hz, 6H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 139.3, 136.9, 134.3, 127.3, 74.7, 58.3, -3.6. **HRMS** (FAB) m/z: 179.0894 [$(M + H^+)–H_2$]; calc. for $C_{10}H_{15}OSi$: 179.0892.

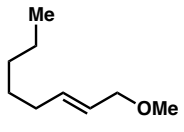
**1-Ethyl-4-isopropylbenzene (1l)**

The following procedure was modified from the literature[22]. To a 250 mL round bottom flask were added Pd/C (10% Pd on activated charcoal, 486 mg, 20% w/w), 4-isopropylacetophenone (2.43 g, 15 mmol), and methanol (60 mL). The solution was sparged with $H_2$ and stirred under 1 atm $H_2$ for 48 hours; monitoring the mixture by TLC showed that that the reaction did not go to completion under these conditions. The crude reaction mixture was filtered through a pad of Celite, dried over dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification by silica column chromatography with hexanes afforded product **1l** (218 mg, 1.47 mmol, 10% yield).



**$^1$H NMR** (500 MHz, $CDCl_3$) δ 7.19 – 7.13 (m, 4H), 2.90 (hept, *J* = 6.9 Hz, 1H), 2.65 (q, *J* = 7.6 Hz, 2H), 1.29 – 1.24 (m, 9H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 146.2, 141.7, 127.9, 126.5, 33.8, 28.5, 24.2, 15.7. **HRMS** (FAB) m/z: 149.1327 ($M + H^+$); calc. for $C_{11}H_{17}$: 149.1330.

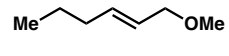
**(*E*)-1-Methoxyoct-2-ene (4a)**



Prepared from (*E*)-oct-2-en-1-ol using **General Procedure A**. This compound is known in the literature[23]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.70 (dtt, *J* = 15.6, 6.6, 1.2 Hz, 1H), 5.54 (dtt, *J* = 15.3, 6.2, 1.4 Hz, 1H), 3.86 (dq, *J* = 6.2, 1.0 Hz, 2H), 3.31 (s, 3H), 2.08 – 1.99 (m, 2H), 1.43 – 1.34 (m, 2H), 1.34 – 1.21 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 135.2, 126.1, 73.5, 57.8, 32.4, 31.5, 28.9, 22.7, 14.2.

**(*E*)-1-Methoxyhex-2-ene (4b)**

Prepared from (*E*)-hex-2-en-1-ol using a modified version of **General Procedure A**. To a 100 mL dry round bottom flask, cooled under argon, were added (*E*)-hex-2-en-1-ol (2.0 g, 20 mmol, 1.0 equiv.), DMF (35 mL), and iodomethane (5.7 g, 40 mmol, 2.0 equiv.). The resulting solution was cooled to 0 °C and NaH (60% dispersion in mineral oil, 960 mg, 24 mmol, 1.2 equiv.) was added portion-wise. The mixture was stirred at 0 °C for 30 minutes, then allowed to warm to room temperature and stirred for an additional 3 hours. The reaction mixture was cooled to 0 °C, quenched with the addition of $NH_4Cl$ (sat. aq., 30 mL), and diluted with diethyl ether (50 mL). Phases were separated and the aqueous layer was extracted with diethyl ether (3 × 50 mL). The combined organics were washed with $H_2O$ (2 × 25 mL) and brine (25 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure (≥ 200 mbar). Purification by silica column chromatography with pentane / diethyl ether afforded compound **4b** (746 mg, 6.5 mmol, 33% yield).

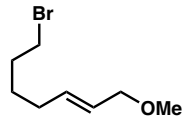


This compound is known in the literature[24]. **$^1$H NMR** (500 MHz, $CDCl_3$) δ 5.70 (dtt, *J* = 15.4, 6.6, 1.2 Hz, 1H), 5.55 (dtt, *J* = 15.4, 6.3, 1.4 Hz, 1H), 3.87 (dq, *J* = 6.3, 1.1 Hz, 2H), 3.32 (s, 3H), 2.06 – 2.00 (m, 2H), 1.42 (*app.* sext, *J* = 7.4 Hz, 2H), 0.91 (t, *J* = 7.3 Hz, 3H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 134.9, 126.3, 73.4, 57.8, 34.5, 22.4, 13.8.

**(*E*)-7-Bromo-1-methoxyhept-2-ene (4c)**

To a 100 mL flamed dried flask was added Grubbs' catalyst 2$^{nd}$ generation (85 mg, 1 mol%). The flask was then evacuated and backfilled with argon for three times. Under argon, a dry $CH_2Cl_2$ solution containing 6-bromo-1-hexene (1.63 g, 10 mmol, 1.0 equiv.) and crotonaldehyde (3.50 g, 50 mmol, 5.0 equiv.) was added to the flask. The mixture was stirred under reflux for 20 hours and then cooled to room temperature and filtered through a silica plug. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (hexanes / ethyl acetate) to give (*E*)-7-bromohept-2-enal (1.6 g, 84% yield). This product was then dissolved in 10 mL dry THF and then added slowly to a suspension of $NaBH_4$ (375 mg, 10 mmol, 1.0 equiv.) in dry THF (10 mL) at 0 °C. To this reaction mixture, iodine (1.27 g, 5 mmol, 0.5 equiv.) in 10 mL of THF was slowly added at 0 °C. Reaction was stirred until the

aldehyde was fully reduced as indicated by TLC. The reaction was quenched with $NH_4Cl$ (sat. aq.), the phases were separated, and the aqueous phase was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the crude alcohol product was used directly without purification. **General Procedure A** was used for the methylation step and the final product **4c** was obtained with 50% overall yield (1.03g, 5 mmol).

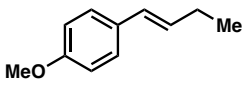



This compound is known in the literature[25]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.68 (dtt, *J* = 15.3, 6.4, 1.1 Hz, 1H), 5.57 (dtt, *J* = 15.4, 6.0, 1.2 Hz, 1H), 3.86 (dq, *J* = 5.9, 1.0 Hz, 2H), 3.41 (t, *J* = 6.8 Hz, 2H), 3.32 (s, 3H), 2.14 – 2.05 (m, 2H), 1.92 – 1.82 (m, 2H), 1.57 – 1.49 (m, 2H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 133.9, 127.0, 73.3, 57.9, 33.8, 32.3, 31.5, 27.7. **HRMS** (EI) m/z: 205.0216 ($M - H^+$); calc. for $C_8H_{14}{}^{79}BrO$: 205.0228.

**(*E*)-1-(But-1-en-1-yl)-4-methoxybenzene (4d)**

This compound was accessed in a two-step sequence. First, to propyltriphenylphosphonium bromide (7.6 g, 19.7 mmol, 1.0 equiv.) suspended in anhydrous THF (70 mL) and cooled to 0 °C was added *n*-butyllithium (2.5 M in hexanes, 7.9 mL, 19.7 mmol, 1.0 equiv.) dropwise over 10 min to form a bright orange solution. After stirring for 1 hour, 4-methoxybenzaldehyde (2.7 g, 19.7 mmol, 1.0 equiv.) was added dropwise over 5 min. The reaction mixture was allowed to slowly warm to room temperature and stirred at room temperature overnight. The reaction mixture was diluted with pentane (50 mL) and the resulting solution was washed with HCl (aq., 0.1 M, 50 mL), $H_2O$ (50 mL), $NaHCO_3$ (sat. aq., 50 mL), and brine (50 mL). The organics were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with pentane / diethyl ether to afford (*E*:*Z*)-**4d** (2:1 *E*:*Z*, 2.50 g, 15.4 mmol, 78% yield).

Next, (*E*:*Z*)-**4d** was isomerized following a literature method[26]. To a dry 25 mL round bottom flask, under argon, were added (*E*:*Z*)-**4d** (300 mg, 1.85 mmol), $(MeCN)_2PdCl_2$ (235 mg, 50 mol%), and 4 mL anhydrous dichloromethane. The resulting mixture was stirred at room temperature for 24 hours. The crude reaction mixture was filtered through Celite and concentrated under reduced pressure. Purification by silica column chromatography using hexanes / diethyl ether delivered **4d** (> 20:1 *E*:*Z*, 279 mg, 1.72 mmol, 93% yield).

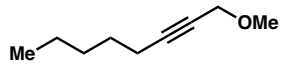



This compound is known in the literature[26]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.33 (dt, *J* = 15.7, 1.6 Hz, 1H), 6.13 (dt, *J* = 15.8, 6.5 Hz, 1H), 3.80 (s, 3H), 2.26 – 2.16 (m, 2H), 1.08 (t, *J* = 7.5 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 158.7, 130.9, 130.7, 128.2, 127.1, 114.0, 55.4, 26.2, 14.0.

**1-Methoxyoct-2-yne (4e)**

To a solution of 3-methoxyprop-1-yne (845 µL, 10 mmol, 1.0 equiv.) in anhydrous THF (50 mL) at -20 °C, was added *n*-butyllithium (2 M in THF, 6 mL, 12 mmol, 1.2 equiv.) and HMPA (869 µL, 5 mmol, 0.5 equiv.) dropwise over 5 min. The resulting mixture was stirred at -20 °C for 3 hours before the addition of 1-iodopentane (1.96 mL, 15 mmol, 1.5 equiv.). The reaction was allowed to slowly warm to room temperature in 2 hours and stirred for additional 18 hours. The

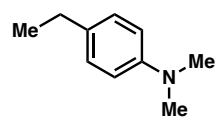
reaction was then quenched by $NH_4Cl$ (sat. aq., 20 mL) and $H_2O$ (30 mL), and extracted by diethyl ether (40 mL × 3). The combined organic layer was washed by $H_2O$ (50 mL) and brine (50 mL), and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with pentane / diethyl ether to afford **4e** (1.04 g, 7.4 mmol, 74% yield).




This compound is known in the literature[27]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 4.07 (t, $J$ = 2.2 Hz, 2H), 3.37 (s, 3H), 2.22 (tt, $J$ = 7.2, 2.2 Hz, 2H), 1.56 – 1.47 (m, 2H), 1.41 – 1.26 (m, 4H), 0.89 (t, $J$ = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 87.4, 75.8, 60.4, 57.5, 31.2, 28.5, 22.3, 18.9, 14.1. **HRMS** (EI) m/z: 139.1128 $[(M - H^{\bullet})^+]$; calc. for $C_9H_{15}O$: 139.1123.

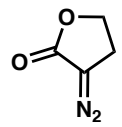
**4-Ethyl-*N*,*N*-dimethylaniline (7b)**

4-Ethylaniline (0.605 g, 5 mmol, 1.0 equiv.) and formaldehyde (1.8 mL, 50 mmol, 10.0 equiv.) were mixed in acetic acid (30 mL). The solution was stirred for 30 min at 0 °C before portionwise addition of $NaBH_3CN$ (1.57 g, 25 mmol, 5.0 equiv.). After the reaction was stirred overnight, NaOH (aq., 2M) was used to neutralize the reaction at 0 °C until pH 8-10. The crude product was extracted with diethyl ether (30 mL × 3). The combined organic layer was washed with $H_2O$ (50 mL) and brine (50 mL), and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with hexanes / ethyl acetate to afford **7b** (635 mg, 4.25 mmol, 85% yield).




This compound is known in the literature[28]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.09 (d, $J$ = 8.8 Hz, 2H), 6.72 (d, $J$ = 8.7 Hz, 2H), 2.92 (s, 6H), 2.57 (q, $J$ = 7.6 Hz, 2H), 1.21 (t, $J$ = 7.6 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 149.1, 132.8, 128.5, 113.3, 41.2, 27.9, 16.1.

**3-Diazodihydrofuran-2(3*H*)-one (9b)**



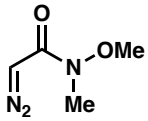

The preparation of the title compound **9b** followed a modified procedure reported by Sattely *et al*[29]. Sodium azide (4.83 g, 74.3 mmol, 4 equiv.), sodium hydroxide (80 mL of 2 M in water, 160 mmol), tetrabutylammonium bromide (60.0 mg, 0.190 mmol, 0.01 equiv.), and hexane (80 mL) were combined in a 500-mL flask with magnetic stir bar open to the air and cooled to 0 °C. With vigorous stirring, triflic anhydride (6.20 mL, 37.1 mmol, 2 equiv.) was added dropwise. After 15 min, a solution of 2-acetyl-butyrolactone (2.00 mL, 18.6 mmol) in acetonitrile (70 mL) was poured into the vessel through a funnel, followed by an additional 10 mL of acetonitrile to complete the transfer. The initially colorless reaction mixture immediately turned yellow. After stirring for 20 min at 0 °C, the mixture was diluted with ice water (50 mL) and chilled EtOAc (50 mL) and transferred to a separatory funnel. After phase separation and removal of the organic fraction, the aqueous layer was washed with chilled EtOAc (50 mL × 5). The combined organic layer was dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. The resulting crude product was purified by silica column chromatography with hexanes / ethyl acetate as eluents. The yellow-colored fractions were concentrated to afford the product as a bright yellow crystalline solid (1.2–1.6 g, 60–75% yield). Spectral data are consistent with Sattely *et al*[29].

**2-Diazo-*N*-methoxy-*N*-methylacetamide (9c)**

4-Methylbenzenesulfonohydrazide (9.31 g, 50 mmol, 1.0 equiv.) was dissolved in aqueous hydrochloric acid (2 M, 30 mL) and warmed to 50 °C (solution 1). 2-Oxoacetic acid (7.40 g of 50% in water, 50 mmol, 1.0 equiv.) was dissolved in water (100 mL) and heated to 50 °C (solution 2). Pre-warmed solution 1 was slowly transferred to solution 2. The reaction mixture was then stirred at 60 °C for 4 h until all the hydrozone product crashed out. The mixture was cooled to 0 °C and kept for 2 h. The product 2-(2-tosylhydrazineylidene)acetic acid (9.88 g, 82% yield) was collected by filtration, washed with hexane: ethyl acetate (10:1) and dried under vaccum.

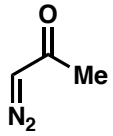
2-(2-Tosylhydrazineylidene)acetic acid (4.84 g, 20 mmol, 1.0 equiv.) was dissolved in dry dichloromethane (30 mL). Thionyl chloride (16 mL) and *N*,*N*-dimethyl formaldehyde (3 drops, *cat*.) were added to the solution. The reaction mixture was stirred at room temperature for 1 h and then heated to reflux (~ 50 °C) for 5 h until the starting material was completely dissolved and the reaction turned clear and light yellow. After the reaction was cooled to room temperature, organic solvent and the excess thionyl chloride was removed under reduced pressure. The resulting mixture (solid) was then dissolved in dry dichloromethane (20 mL) and used for the next step.

*N*,*O*-Dimethylhydroxylamine hydrochloride (3.91 g, 40 mmol, 2.0 equiv.) and triethylamine (11.2 mL, 80 mmol, 4.0 equiv.) were mixed in dry dichloromethane (80 mL) and stirred for 30 min. The solution of acyl chloride was added dropwise over 20 min to the reaction mixture at 0 °C. The reaction was then stirred at room temperature for 5 h before water (80 mL) was added to quench the reaction. The liquid phases were transferred to a separatory funnel, and the aqueous phase was extracted with dichloromethane (50 mL× 4). The combined organic phase was washed with water (40 mL) and brine (40 mL), dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. The resulting crude product was purified by silica column chromatography with hexanes / ethyl acetate to afford **9c** as a yellow liquid (0.82 g, 32% yield).




This compound is known in the literature[30]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.33 (s, 1H), 3.66 (s, 3H), 3.19 (s, 3H).

**1-Diazopropan-2-one (9d)**

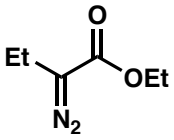



The preparation of the title compound **9d** followed a modified procedure reported by Zhang *et al*[31]. To a solution of acetylacetone (3.4 mL, 33.0 mmol, 1.10 equiv.) and triethylamine (5.04 mL, 36.4 mmol, 1.21 equiv.) in dry acetonitrile (25 mL), a solution of *p*-acetamidobenzenesulfonyl azide (7.20 g, 30.0 mmol, 1.0 equiv.) in dry acetonitrile (25 mL) was added dropwise. The reaction mixture was stirred at room temperature for 4 h. Then, the solvent was removed under reduced pressure and the resulting mixture was then purified by silica column chromatography with hexanes / ethyl acetate to give 3-diazopentane-2,4-dione (3.65 g, 96% yield) as a pale yellow liquid.

3-Diazopentane-2,4-dione (1.89 g, 15 mmol, 1.0 equiv.) was dissolved in diethyl ether (25 mL). An aqueous solution (25 mL) of NaOH (3.00 g, 75 mmol, 5.0 equiv.) was added dropwise over 10 min to the ether layer with vigorous stirring at 0 °C. The reaction mixture turned dark brown within 20 min and was then stirred at room temperature for 4 h. The liquid phases were transferred to a separatory funnel, and the aqueous phase was extracted with diethyl ether (30 mL × 5). The combined organic phase was dried over $Na_2SO_4$, filtered, and concentrated under reduced

pressure (T = 24 °C, P ≥ 20 kPa) to give product **9d** as a volatile yellow liquid (0.892 g, 71% yield). Spectral data is consistent with Zhang *et al*[31].

**Ethyl 2-diazobutanoate** (**9g**)




The preparation of the title compound **9g** followed a modified procedure reported by Huang *et al*[32]. To a solution of ethyl 2-ethylacetoacetate (3.16 g, 20.0 mmol, 1.0 equiv.) and *p*-acetamidobenzenesulfonyl azide (7.21 g, 30.0 mmol, 1.5 equiv.) in dry acetonitrile (50 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 4.5 mL, 30.0 mmol, 1.5 equiv.) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. Water (50 mL) was added to quench the reaction. Acetonitrile was removed under reduced pressure (T = 24 °C, P ≥ 20 kPa). The mixture was extracted with diethyl ether (25 mL × 4). The combined ether layer extract was washed with water (30 mL) and brine (30 mL), dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure (T = 24 °C, P ≥ 30 kPa). The crude product was purified by silica column chromatography with hexanes / ethyl acetate to give product **9g** as a volatile yellow liquid (2.40 g, 84% yield). Spectral data is consistent with Huang *et al*[32].

## VII. Synthesis and characterization of reference compounds

Racemic reference compounds corresponding to enzymatic products and side-products were prepared according to the following procedures. Reference compounds are characterized below.

### General Procedure B: Aldol reaction and methylation synthetic sequence

In a dry 100 or 250 mL round bottom flask, under argon, a solution of diisopropylamine (6–24 mmol, 1.1–1.2 equiv.) in THF (15–30 mL) was cooled to 0 °C (**General Procedure B-1**) or -78 °C (**General Procedure B-2**). *n*-Butyllithium (6–25 mmol, 1.1–1.2 equiv., 1.6 or 2.5 M in hexanes) was added dropwise and the resulting mixture was stirred at the appropriate temperature for 15-30 min. The mixture was cooled to -78 °C and kept at this temperature for the remainder of the reaction. Ethyl acetate (14–28 mmol, 1.4 equiv., **General Procedure B-1** or 6–10 mmol, 1.0 equiv., **General Procedure B-2**) was added dropwise and the mixture was stirred for an additional 30-45 min. Then, aldehyde (10–20 mmol, 1.0 equiv., **General Procedure B-1** or 9–11 mmol, 1.1–1.5 equiv., **General Procedure B-2**) as a solution in THF (15–30 mL, **General Procedure B-1**) or neat (**General Procedure B-2**) was added slowly and the solution was stirred for a further 0.5–3 hours. The reaction mixture was quenched at -78 °C by the addition of $NH_4Cl$ (sat. aq., 10–30 mL) and allowed to thaw to room temperature. For **General Procedure B-1** only, HCl (1 M aq., 1.5–3.0 mL) was also added. Phases were separated and the aqueous phase was extracted with ethyl acetate or diethyl ether (3 × 20–30 mL). The combined organics were washed with $NH_4Cl$ (sat. aq., 2 × 10–15 mL), brine (10 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded the desired aldol adducts in 56–95% yield.

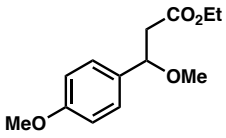
In the appropriate reaction vessel, aldol adduct (3–4 mmol, 1.0 equiv.), $Ag_2O$ (9–10 mmol, 2.5–3.0 equiv.), and solvent (10–15 mL) were combined, followed by iodomethane (40–60 mmol, 10–15 equiv., **General Procedure B-1** or 9 mmol, 3.0 equiv., **General Procedure B-2**). The reaction was then stirred at the specified temperature for 24–48 hours, with additional equivalents of iodomethane (10–20 mmol, 2.5–5 equiv., **General Procedure B-1**) added as necessary. For **General Procedure B-1**, the reaction was performed in a vial equipped with a pressure release cap, toluene was employed as the solvent, and the reaction mixture was stirred at 70 °C. For **General Procedure B-2**, diethyl ether was employed as solvent and the reaction mixture was stirred at room temperature; the reaction vessel was covered in aluminum foil to protect its contents from light. The crude mixture was filtered through a pad of Celite and concentrated under reduced pressure. Purification was performed by silica column chromatography with hexanes / ethyl acetate; if necessary, a second purification by reverse phase chromatography was performed (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / acetonitrile eluent system). The desired products were obtained in 25–57% yield.

**General Procedure C: Horner-Wadsworth-Emmons reaction and Pd/C alkene reduction synthetic sequence**

In a dry round bottom flask, under argon, NaH (60% dispersion in mineral oil, 7.4–12 mmol, 1.1–2.0 equiv.) in anhydrous THF (8–23 mL) was cooled to 0 °C. Triethyl phosphonoacetate (7.4–18 mmol, 1.1–3.0 equiv.) was added dropwise and the mixture was allowed to warm to room temperature and stirred for 1 hour. Ketone (5–6.7 mmol, 1.0 equiv.) in THF (2–4 mL) was added and the reaction was stirred at room temperature for 12–18 hours (for the preparation of **3j** and **3l**) or heated to reflux (for the preparation of **3i, 3k, 8a'**, and **8b'**). The reaction was quenched with $NH_4Cl$ (sat. aq., 20 mL). Phases were separated and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organics were washed with brine (10–20 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. When necessary, the crude product was purified by silica column chromatography with hexanes / ethyl acetate to afforded the desired alkene compounds in 23% to quantitative yield.

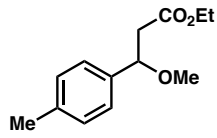
To a round bottom flask were added Pd/C (10% Pd on activated charcoal, 24–30% w/w of alkene), methanol (5–6 mL), and alkene (1.2–2.3 mmol). $H_2$ was bubbled through the solution for ~30 minutes. The reaction was stirred at room temperature under 1 atm $H_2$ until complete reduction of the alkene was observed by TLC (typically 3–8 hours). The crude product was filtered through a pad of Celite and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded the desired products in quantitative yield.

**Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (3a)**



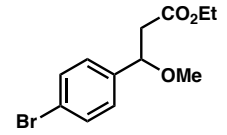

This compound was prepared from 4-methoxybenzaldehyde using **General Procedure B-1**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.58 (dd, *J* = 9.0, 4.9 Hz, 1H), 4.14 (qd, *J* = 7.1, 1.2 Hz, 2H), 3.81 (s, 3H), 3.19 (s, 3H), 2.80 (dd, *J* = 15.2, 9.0 Hz, 1H), 2.55 (dd, *J* = 15.2, 4.9 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

**Ethyl 3-methoxy-3-(*p*-tolyl)propanoate (3b)**



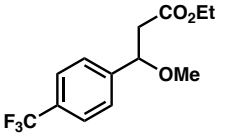

This compound was prepared from 4-methylbenzaldehyde using **General Procedure B-1**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 7.9 Hz, 2H), 4.60 (dd, *J* = 9.2, 4.7 Hz, 1H), 4.14 (qd, *J* = 7.1, 1.2 Hz, 2H), 3.21 (s, 3H), 2.79 (dd, *J* = 15.3, 9.2 Hz, 1H), 2.55 (dd, *J* = 15.3, 4.7 Hz, 1H), 2.35 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.2, 137.9, 137.6, 129.4, 126.7, 80.0, 60.7, 56.9, 43.7, 21.3, 14.3. **HRMS** (FAB) m/z: 221.1169 $[(M + H^+)–H_2]$; calc. for $C_{13}H_{17}O_3$: 221.1178

**Ethyl 3-(4-bromophenyl)-3-methoxypropanoate (3c)**



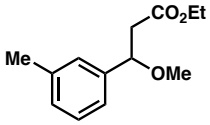

This compound was prepared from 4-bromobenzaldehyde using **General Procedure B-1**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.3 Hz, 2H), 4.59 (dd, *J* = 8.9, 5.0 Hz, 1H), 4.14 (qd, *J* = 7.1, 0.7 Hz, 2H), 3.21 (s, 3H), 2.77 (dd, *J* = 15.4, 8.9 Hz, 1H), 2.53 (dd, *J* = 15.4, 5.0 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.8, 139.8, 131.9, 128.5, 122.0, 79.6, 60.8, 57.1, 43.5, 14.3. **HRMS** (FAB) m/z: 287.0282 ($M + H^+$); calc. for $C_{12}H_{16}{}^{79}BrO_3$: 287.0283

**Ethyl 3-methoxy-3-(4-(trifluoromethyl)phenyl)propanoate (3d)**




This compound was prepared from 4-(trifluoromethyl)benzaldehyde using **General Procedure B-1**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 4.70 (dd, *J* = 8.8, 4.9 Hz, 1H), 4.15 (qd, *J* = 7.2, 0.7 Hz, 2H), 3.24 (s, 3H), 2.79 (dd, *J* = 15.5, 8.9 Hz, 1H), 2.56 (dd, *J* = 15.4, 4.9 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7, 145.0, 130.4 (q, *J* = 32.4 Hz), 127.1, 125.7 (q, *J* = 3.8 Hz), 124.2 (q, *J* = 272.1 Hz), 79.7, 60.9, 57.3, 43.5, 14.3. **HRMS** (FAB) m/z: 277.1041 ($M + H^+$); calc. for $C_{13}H_{16}F_3O_3$: 277.1052

**Ethyl 3-methoxy-3-(*m*-tolyl)propanoate (3e)**



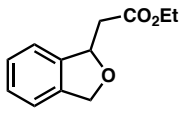

This compound was prepared from 3-methylbenzaldehyde using **General Procedure B-1**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.27 – 7.21 (m, 1H), 7.16 – 7.08 (m, 3H), 4.60 (dd, *J* = 9.2, 4.6 Hz, 1H), 4.15 (qd, *J* = 7.1, 1.3 Hz, 2H), 3.22 (s, 3H), 2.79 (dd, *J* = 15.3, 9.3 Hz, 1H), 2.56 (dd, *J* = 15.3, 4.6 Hz, 1H), 2.36 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.2, 140.7, 138.3, 128.9, 128.6, 127.4, 123.8, 80.2, 60.7, 57.0, 43.7, 21.6, 14.3. **HRMS** (FAB) m/z: 223.1338 ($M + H^+$); calc. for $C_{13}H_{19}O_3$: 223.1334.

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

This compound was prepared by the method of U. S. Dakarapu *et al*.[33]. To a flame-dried Schlenk flask under argon was added $[Ir(coe)_2Cl]_2$ (5 mg, 0.0056 mmol, 0.11 mol%), phthalide (671 mg, 5 mmol, 1.0 equiv.), anhydrous dichloromethane (1.6 mL), and $H_2SiEt_2$ (1.3 mL, 10 mmol, 2 equiv.). The reaction mixture was stirred for 14 hours at room temperature. The reaction mixture was concentrated under reduced pressure to afford the crude silyl acetal, which was used without purification.

In a dry round bottom flask, the crude silyl acetal (5 mmol, 1.0 equiv.) was combined with THF (5 mL) and the resulting mixture cooled to 0 °C. To the mixture were added triethyl phosphonoacetate (1.23 g, 5.5 mmol, 1.1 equiv.) and $KOSiMe_3$ (713 mg, 5 mmol, 1.0 equiv.) in THF (7.5 mL). The reaction was allowed to warm to room temperature and stirred for 1.5 hours. The reaction was quenched with the addition of $NH_4Cl$ (sat. aq., 12 mL) and the aqueous phase was extracted with diethyl ether (3 × 15 mL). The combined organics were washed with brine (15 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded desired product **3f** with impurities (667 mg,

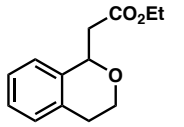
3.2 mmol, 65% yield). A portion of the product was taken for a second purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / acetonitrile eluent system).




Spectral data are in agreement with literature report[33]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 2H), 7.25 – 7.16 (m, 2H), 5.71 – 5.63 (m, 1H), 5.19 – 5.13 (m, 1H), 5.11 – 5.04 (m, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.80 (dd, *J* = 15.6, 4.9 Hz, 1H), 2.73 (dd, *J* = 15.6, 7.9 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.0, 140.8, 139.3, 128.0, 127.6, 121.3, 121.2, 80.5, 72.9, 60.8, 41.8, 14.3.

**Ethyl 2-(isochroman-1-yl)acetate (3g)**

This compound was prepared by the method of R. E. TenBrink *et al*.[34]. To a 100 mL dry round bottom flask, under argon, were added 2-phenylethanol (1.47 g, 12 mmol, 1.0 equiv.), ethyl 3,3-diethoxypropionate (90% technical grade, 2.51 g, 13.2 mmol, 1.1 equiv.), and anhydrous dichloromethane (5 mL). The resulting mixture was cooled to 0 °C and $TiCl_4$ (1 M in dichloromethane, 26.4 mL, 26.4 mmol, 2.2 equiv.) was added slowly. The reaction was stirred for 2 hours at 0 °C and a second portion of ethyl 3,3-diethoxypropionate (90% technical grade, 0.12 g, 0.6 mmol, 0.05 equiv.) was added. The reaction was stirred for an additional 2 hours at 0 °C. The mixture was poured into ice cold HCl (aq., 1 M, 20 mL) and the aqueous phase was extracted with dichloromethane (2 x 20 mL). The combined organics were washed with brine (30 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded desired product **3g** with minor impurities (2.59 g, ~11.8 mmol, ~98% yield). A portion of the product was taken for a second purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / acetonitrile eluent system).

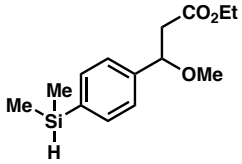



Spectral data are in agreement with literature report[34]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.22 – 7.15 (m, 2H), 7.15 – 7.09 (m, 1H), 7.08 – 7.02 (m, 1H), 5.25 (dd, *J* = 9.6, 3.5 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.13 (ddd, *J* = 11.4, 5.2, 4.2 Hz, 1H), 3.82 (ddd, *J* = 11.4, 9.0, 3.9 Hz, 1H), 3.04 – 2.93 (m, 1H), 2.88 (dd, *J* = 15.2, 3.6 Hz, 1H), 2.80 – 2.68 (m, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.4, 136.9, 134.1, 129.2, 126.8, 126.4, 124.6, 73.1, 63.2, 60.8, 41.9, 28.9, 14.3.

**Ethyl 3-(4-(dimethylsilyl)phenyl)-3-methoxypropanoate (3h)**

This compound was prepared from ethyl 3-(4-bromophenyl)-3-methoxypropanoate (**3c**). The following procedure was modified from the literature[35]. To a 25 mL round bottom flask was added Mg turnings* (48 mg, 2.0 mmol, 2.0 equiv.), flame dried, and cooled under positive argon pressure. (*Mg turnings were prepared by washing with 0.1 M HCl, sonication, then washing with $H_2O$ and acetone.) THF (3 mL), LiCl (64 mg, 1.5 mmol, 1.5 equiv.), and $Me_2SiHCl$ (170 mg, 1.8 mmol, 1.8 equiv.) were added and the resulting mixture was stirred for 30 minutes at room temperature under positive argon pressure. Aryl bromide **3c** (287 mg, 1.0 mmol, 1.0 equiv.) was added dropwise via syringe and the reaction was stirred for an additional 2 hours. The crude reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded desired

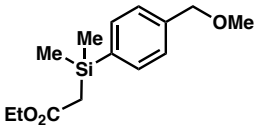
product **3h** (145 mg, 0.54 mmol, 54% yield).



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 4.63 (dd, *J* = 9.3, 4.5 Hz, 1H), 4.42 (hept, *J* = 3.8 Hz, 1H), 4.15 (qd, *J* = 7.1, 1.6 Hz, 2H), 3.23 (s, 3H), 2.79 (dd, *J* = 15.3, 9.3 Hz, 1H), 2.56 (dd, *J* = 15.3, 4.5 Hz, 1H), 1.24 (t, *J* = 7.2 Hz, 3H), 0.35 (d, *J* = 3.8 Hz, 6H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1, 141.8, 137.4, 134.4, 126.2, 80.2, 60.7, 57.1, 43.7, 14.3, -3.6. **HRMS** (FAB) m/z: 265.1253 [(M + $H^+$)–$H_2$]; calc. for $C_{14}H_{21}SiO_3$: 265.1260.

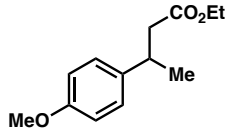
**Ethyl 2-((4-(methoxymethyl)phenyl)dimethylsilyl)acetate (3h')**

This compound was prepared by rhodium-catalyzed Si–H insertion. To a dry 50 mL round bottom flask, under argon, was added (4-(methoxymethyl)phenyl)dimethylsilane (**1h**) (541 mg, 3 mmol, 1.0 equiv.), $Rh_2(OAc)_4$ (13.3 mg, ~1 mol%), and anhydrous dichloromethane (12 mL). The mixture was cooled to -78 °C, after which ethyl diazoacetate (393 mg, 3.0 mmol, 1.0 equiv.) in dichloromethane (3 mL) was added dropwise to the solution over 2 hours. The reaction was allowed to slowly warm to room temperature and stirred for a total of 12 hours. The crude reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude mixture was purified by silica column chromatography using hexanes / ethyl acetate to deliver **3h'** with impurities. A second purification by silica column chromatography using hexanes / diethyl ether/ dichloromethane afforded **3h'** (92.6 mg, 0.35 mmol, 12% yield).



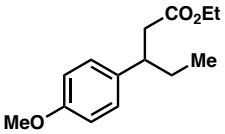
**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 4.46 (s, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 3.39 (s, 3H), 2.11 (s, 2H), 1.16 (t, *J* = 7.1 Hz, 3H), 0.40 (s, 6H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.7, 139.7, 136.4, 133.8, 127.2, 74.6, 60.1, 58.3, 26.4, 14.5, -2.6. **HRMS** (FAB) m/z: 265.1260 [(M + $H^+$)–$H_2$]; calc. for $C_{14}H_{21}SiO_3$: 265.1260.

**Ethyl 3-(4-methoxyphenyl)butanoate (3i)**



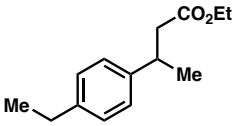
This compound was prepared from 1-(4-methoxyphenyl)ethan-1-one using **General Procedure C**. **$^1$H NMR** (500 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.08 (qd, *J* = 7.2, 1.2 Hz, 2H), 3.79 (s, 3H), 3.24 (h, *J* = 7.1 Hz, 1H), 2.57 (dd, *J* = 14.9, 7.2 Hz, 1H), 2.51 (dd, *J* = 14.9, 8.0 Hz, 1H), 1.28 (d, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

**Ethyl 3-(4-methoxyphenyl)pentanoate (3j)**



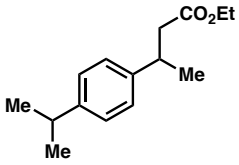
This compound was prepared from 1-(4-methoxyphenyl)propan-1-one using **General Procedure C**. Spectral data are in agreement with literature report[36]; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.09 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 4.03 (qd, *J* = 7.2, 1.3 Hz, 2H), 3.78 (s, 3H), 2.95 (tdd, *J* = 9.0, 7.0, 5.3 Hz, 1H), 2.60 (dd, *J* = 15.0, 7.0 Hz, 1H), 2.51 (dd, *J* = 14.9, 8.3 Hz, 1H), 1.68 (ddq, *J* = 13.3, 7.4, 5.4 Hz, 1H), 1.56 (ddq, *J* = 13.5, 9.4, 7.3 Hz, 1H), 1.14 (t, *J* = 7.1 Hz, 3H), 0.78 (t, *J* = 7.4 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.7, 158.2, 136.1, 128.5, 113.8, 60.3, 55.3, 43.3, 41.9, 29.4, 14.3, 12.1.

**Ethyl 3-(4-ethylphenyl)butanoate (3k)**




This compound was prepared from 1-(4-ethylphenyl)ethan-1-one using **General Procedure C**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.14 (*app.* s, 4H), 4.08 (q, *J* = 7.1 Hz, 2H), 3.25 (dp, *J* = 8.3, 7.0 Hz, 1H), 2.66 – 2.48 (m, 4H), 1.29 (d, *J* = 6.9 Hz, 3H), 1.26 – 1.15 (m, 6H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.7, 143.1, 142.3, 128.0, 126.8, 60.4, 43.2, 36.2, 28.5, 22.0, 15.7, 14.3. **HRMS** (FAB) m/z: 221.1532 ($M + H^+$); calc. for $C_{14}H_{21}O_2$: 221.1542

**Ethyl 3-(4-isopropylphenyl)butanoate (3l)**

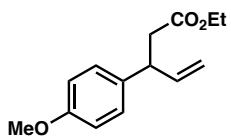



This compound was prepared from 1-(4-isopropylphenyl)ethan-1-one using **General Procedure C**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.15 (*app.* s, 4H), 4.08 (q, *J* = 7.1 Hz, 2H), 3.25 (dp, *J* = 8.5, 6.9 Hz, 1H), 2.87 (hept, *J* = 6.9 Hz, 1H), 2.60 (dd, *J* = 14.9, 6.7 Hz, 1H), 2.51 (dd, *J* = 14.9, 8.4 Hz, 1H), 1.29 (d, *J* = 7.0 Hz, 3H), 1.23 (d, *J* = 6.9 Hz, 6H), 1.18 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.7, 147.0, 143.2, 126.8, 126.6, 60.4, 43.3, 36.2, 33.8, 24.2, 21.9, 14.3. **HRMS** (FAB) m/z: 235.1696 ($M + H^+$); calc. for $C_{15}H_{23}O_2$: 235.1698.

**Ethyl 3-(4-methoxyphenyl)pent-4-enoate (3m)**

This compound was accessed in a two-step sequence. First, *p*-methoxycinnamaldehyde (811 mg, 5 mmol, 1.0 equiv.) was reduced using $NaBH_4$ (227 mg, 6 mmol, 1.1 equiv.) in methanol (15 mL) under standard reaction conditions (0 °C for 2 hours). The reaction mixture was quenched with $NH_4Cl$ (sat. aq., 10 mL) and diluted with dichloromethane (15 mL). Phases were separated and the aqueous layer was extracted with dichloromethane (4 × 15 mL). The combined organics were washed with brine (25 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate delivered *p*-methoxycinnamyl alcohol (752 mg, 4.6 mmol, 92% yield), with spectral data that match literature report[37].

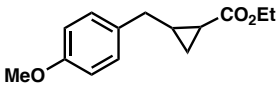
Next, to a 50 mL round bottom flask equipped with short-path condenser were added *p*-methoxycinnamyl alcohol (740 mg, 4.5 mmol, 1.0 equiv.), triethyl orthoacetate (7.3 g, 45 mmol, 10 equiv.), and propionic acid (52 mg, 0.7 mmol, 0.15 equiv.). Following standard Johnson-Claisen rearrangement conditions, this mixture was heated to 140 °C until complete conversion of *p*-methoxycinnamyl alcohol was observed by TLC (~23 hours). Additional propionic acid (2 × 52 mg) was added after 6 hours and 9 hours reaction time. The reaction mixture was removed from heat, concentrated under reduced pressure, and purified using silica gel chromatography with hexanes / ethyl acetate as eluents. A second purification by silica gel chromatography with hexanes / ether afforded **3m** (357 mg, 1.6 mmol, 36% yield).




Spectral data for **3m** are in agreement with literature report[38]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.96 (ddd, *J* = 17.5, 9.9, 6.9 Hz, 1H), 5.09 – 5.05 (m, 1H), 5.03 (dt, *J* = 5.4, 1.3 Hz, 1H), 4.07 (qd, *J* = 7.1, 1.0 Hz, 2H), 3.86 – 3.80 (m, 1H), 3.78 (s, 3H), 2.73 (dd, *J* = 15.0, 8.0 Hz, 1H), 2.65 (dd, *J* = 15.0, 7.6 Hz, 1H), 1.18 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.1, 158.4, 140.7, 134.6, 128.6, 114.6, 114.0, 60.5, 55.4, 44.9, 40.6, 14.3.

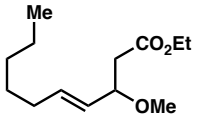
**Ethyl 2-(4-methoxybenzyl)cyclopropane-1-carboxylate (3m')**

This compound was prepared by rhodium-catalyzed alkene cyclopropanation. To a dry 100 mL round bottom flask, under argon, were added 4-allylanisole (3.0 g, 20 mmol, 10 equiv.), $Rh_2(OAc)_4$ (8.8 mg, ~1 mol%), and anhydrous dichloromethane (10 mL). Ethyl diazoacetate (262 mg, 2 mmol, 1.0 equiv.) in dichloromethane (10 mL) was added over ~8 hours using a syringe pump; the reaction mixture was allowed to stir for a total of 20 hours at room temperature. The reaction mixture was diluted with diethyl ether (20 mL), filtered through a pad of Celite, and concentrated under reduced pressure. Several rounds of purification by silica column chromatography with hexanes / ethyl acetate or hexanes / diethyl ether eluent systems afforded *cis*-**3m'** and *trans*-**3m'** as individual isomers (combined mass 148.1 mg, 0.632 mmol, 32% yield).



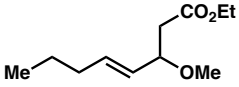

Spectral data are in agreement with literature report[39]. Characterization data for *cis*-**3m'**: **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 2.86 (dd, *J* = 14.9, 6.9 Hz, 1H), 2.77 (dd, *J* = 15.0, 7.6 Hz, 1H), 1.77 (ddd, *J* = 8.8, 7.6, 5.9 Hz, 1H), 1.56 – 1.44 (m, 1H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.14 – 1.06 (m, 2H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 173.1, 158.0, 133.7, 129.3, 113.9, 60.5, 55.4, 32.1, 23.1, 18.7, 14.5, 13.7. Characterization data for *trans*-**3m'**: **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.12 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.11 (qd, *J* = 7.1, 1.1 Hz, 2H), 3.79 (s, 3H), 2.71 (dd, *J* = 14.7, 6.3 Hz, 1H), 2.52 (dd, *J* = 14.8, 7.1 Hz, 1H), 1.65 (ddtd, *J* = 8.7, 7.1, 6.4, 4.1 Hz, 1H), 1.52 – 1.46 (m, 1H), 1.27 – 1.20 (m, 4H), 0.81 (ddd, *J* = 8.2, 6.3, 4.2 Hz, 1H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 174.4, 158.2, 132.3, 129.5, 113.9, 60.5, 55.4, 37.6, 23.4, 20.3, 15.3, 14.4.

**Ethyl (*E*)-3-methoxydec-4-enoate (5a)**



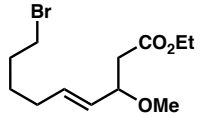

This compound was prepared from (*E*)-oct-2-enal using **General Procedure B-2**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.69 (dt, *J* = 15.4, 6.8 Hz, 1H), 5.28 (ddt, *J* = 15.4, 8.3, 1.5 Hz, 1H), 4.14 (qd, *J* = 7.2, 0.8 Hz, 2H), 3.97 (td, *J* = 8.2, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, *J* = 14.9, 8.1 Hz, 1H), 2.42 (dd, *J* = 14.9, 5.5 Hz, 1H), 2.10 – 1.97 (m, 2H), 1.43 – 1.20 (m, 9H), 0.88 (t, *J* = 6.9 Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

**Ethyl (*E*)-3-methoxyoct-4-enoate (5b)**




This compound was prepared from (*E*)-hex-2-enal using **General Procedure B-2**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.69 (dt, *J* = 15.4, 6.8 Hz, 1H), 5.29 (ddt, *J* = 15.4, 8.2, 1.5 Hz, 1H), 4.14 (qd, *J* = 7.1, 0.8 Hz, 2H), 3.97 (td, *J* = 8.1, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, *J* = 14.9, 8.1 Hz, 1H), 2.42 (dd, *J* = 14.9, 5.6 Hz, 1H), 2.06 – 1.99 (m, 2H), 1.40 (sext, *J* = 7.3 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.2, 135.3, 128.9, 79.0, 60.6, 56.2, 41.5, 34.3, 22.4, 14.4, 13.7. **HRMS** (FAB) m/z: 199.1320 [$(M + H^+)–H_2$]; calc. for $C_{11}H_{19}O_3$: 199.1334.

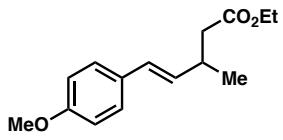
**Ethyl (*E*)-9-bromo-3-methoxynon-4-enoate (5c)**




This compound as prepared from (*E*)-7-bromohept-2-enal using **General Procedure B-2**. The synthesis of (*E*)-7-bromohept-2-enal was described in the synthesis of compound **4c** in **Section VI**. **$^1$H NMR** (400 MHz, Chloroform-*d*) δ 5.67 (dt, *J* = 15.4, 6.7 Hz, 1H), 5.31 (dd, *J* = 15.4, 8.1 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.97 (td, *J* = 8.0, 5.5 Hz, 1H), 3.40 (t, *J* = 6.7 Hz, 2H), 3.25 (s, 3H), 2.59 (dd, *J* = 15.0, 8.0 Hz, 1H), 2.41 (dd, *J* = 15.0, 5.6 Hz, 1H), 2.08 (q, *J* = 7.2 Hz, 2H), 1.91 – 1.79 (m, 2H), 1.53 (p, *J* = 7.5 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1, 134.3, 129.5, 78.8, 60.6, 56.3, 41.4, 33.7, 32.2, 31.4, 27.7, 14.4. **HRMS** (FAB) m/z: 293.0764 ($M + H^+$); calc. for $C_{12}H_{22}O_3{}^{79}Br$ : 293.0752.

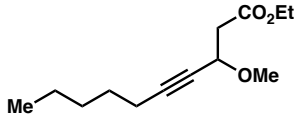
**Ethyl (*E*)-5-(4-methoxyphenyl)-3-methylpent-4-enoate (5d)**

To a 6 mL vial equipped with a stir bar was added Grubbs' catalyst 2$^{nd}$ generation (10 mg, 2 mol%). The vial was then evacuated and backfilled with argon for three times. Under argon, a dry $CH_2Cl_2$ solution (2 mL) containing 4-vinylanisole (100 mg, 0.75 mmol) and ethyl 3-methylpent-4-enoate (503 mg, 3.75 mmol) was added to the vial via syringe. The mixture was stirred at 40 °C for 24 hours and then cooled to room temperature and filtered through a silica plug. The solvent was removed under reduced pressure and the crude product was purified using silica column chromatography with hexanes / ethyl acetate to give **5d** (37 mg, 20% yield).




**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.30 – 7.24 (m, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.34 (d, *J* = 15.9 Hz, 1H), 5.99 (dd, *J* = 15.9, 7.6 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.80 (s, 3H), 2.90 – 2.75 (m, 1H), 2.41 (dd, *J* = 14.7, 7.3 Hz, 1H), 2.34 (dd, *J* = 14.7, 7.3 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.14 (d, *J* = 6.7 Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

**Ethyl 3-methoxydec-4-ynoate (5e)**

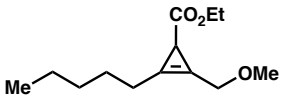



This compound was prepared from oct-2-ynal using **General Procedure B-2**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 4.39 (ddt, *J* = 8.3, 5.4, 2.0 Hz, 1H), 4.16 (qd, *J* = 7.2, 1.0 Hz, 2H), 3.39 (s, 3H), 2.73 (dd, *J* = 15.5, 8.4 Hz, 1H), 2.63 (dd, *J* = 15.5, 5.4 Hz, 1H), 2.20 (td, *J* = 7.1, 2.0 Hz, 2H), 1.50 (p, *J* = 7.2 Hz, 2H), 1.41 – 1.29 (m, 4H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.89 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.4, 87.3, 67.7, 60.8, 56.6, 41.7, 31.1, 28.4, 22.3, 18.8, 14.3, 14.1 (one carbon may be overlapping with the solvent peaks). **HRMS** (FAB) m/z: 227.1638 ($M + H^+$); calc. for $C_{13}H_{23}O_3$: 227.1647.

**Ethyl 2-(methoxymethyl)-3-pentylcycloprop-2-ene-1-carboxylate (5e')**

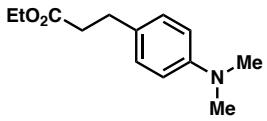
This compound was prepared by rhodium-catalyzed cyclopropenation. To a dry 50 mL round bottom flask was added 1-methoxyoct-2-yne (**4e**) (280 mg, 2.0 mmol, 1.0 equiv.), $Rh_2(OAc)_4$ (9.0 mg, 1 mol%), and anhydrous dichloromethane (6 mL). The mixture was cooled to -78 °C, after which ethyl diazoacetate (87%, 525 mg, 4.0 mmol, 2.0 equiv.) in dichloromethane (5 mL) was added dropwise to the solution over 6 hours. The reaction was allowed to slowly warm to room temperature and stirred for a total of 18 hours. The reaction mixture was concentrated

under reduced pressure. The crude product was purified by silica column chromatography using hexanes / ethyl acetate, followed by C18 column using methanol / water, to afford **5e'** (26 mg, 0.11 mmol, 6% yield).



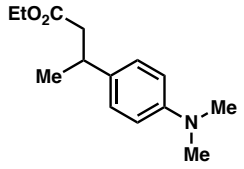

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 4.37 (t, *J* = 1.6 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.39 (s, 3H), 2.47 (tt, *J* = 7.5, 1.6 Hz, 2H), 2.20 (s, 1H), 1.64 – 1.52 (m, 2H), 1.37 – 1.28 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 3H), 0.93 – 0.86 (m, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 176.2, 110.3, 102.5, 65.8, 60.2, 58.6, 31.5, 26.7, 24.7, 22.7, 22.5, 14.5, 14.1. **HRMS** (EI) m/z: 226.1573 ($M^{+\bullet}$); calc. for $C_{13}H_{22}O_3$: 226.1569.

**Ethyl 3-(4-(dimethylamino)phenyl)propanoate (8a')**




This compound was prepared from 4-(dimethylamino)benzaldehyde using **General Procedure C**. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.09 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 8.7 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.92 (s, 6H), 2.89 – 2.82 (m, 2H), 2.61 – 2.54 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 173.4, 149.4, 129.0, 128.8, 113.1, 60.4, 41.0, 36.6, 30.2, 14.4. **HRMS** (EI) m/z: 221.1430 ($M^{+\bullet}$); calc. for $C_{13}H_{19}NO_2$: 221.1416.

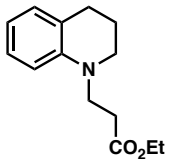
**Ethyl 3-(4-(dimethylamino)phenyl)butanoate (8b')**




This compound was prepared from 1-(4-(dimethylamino)phenyl)ethan-1-one using **General Procedure C**. Spectral data are in agreement with literature report[40]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.10 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 8.7 Hz, 2H), 4.08 (qd, *J* = 7.1, 1.1 Hz, 2H), 3.20 (dt, *J* = 8.4, 6.8 Hz, 1H), 2.92 (s, 6H), 2.57 (dd, *J* = 14.8, 6.8 Hz, 1H), 2.49 (dd, *J* = 14.8, 8.4 Hz, 1H), 1.27 (d, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.8, 149.4, 134.0, 127.4, 113.0, 60.3, 43.5, 41.0, 35.7, 22.0, 14.4.

**Ethyl 3-(3,4-dihydroquinolin-1(2*H*)-yl)propanoate (8f')**

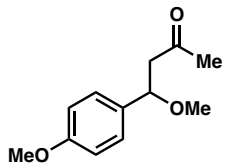
To a 100-mL round-bottom flask were added 1,2,3,4-tetrahydroquinoline (266.4 mg, 2.0 mmol, 1.0 equiv.), ethyl 3-bromopropanoate (0.97 mL, 6.0 mmol, 3.0 equiv.), $K_2CO_3$ (0.552 g, 4.0 mmol, 2.0 equiv.), KI (66.0 mg, 0.4 mmol, 0.2 equiv.) and *N*,*N*-dimethylformamide (30 mL). The reaction mixture was heated at 120 °C for 4 hours. After the reaction was cooled to room temperature and quenched by $H_2O$ (40 mL), the crude product was extracted by diethyl ether (20 mL × 3). The combined organic layer was washed by $H_2O$ (40 mL) and brine (40 mL), and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with pentane / diethyl ether, followed by C18 column with methanol / water, to afford **8f'** (350 mg, 1.5 mmol, 75% yield).




This compound is known in the literature[41]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.11 – 7.01 (m, 1H), 6.95 (dq, *J* = 7.1, 1.1 Hz, 1H), 6.66 – 6.54 (m, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.65 – 3.57 (m, 2H), 3.33 – 3.25 (m, 2H), 2.75 (t, *J* = 6.4 Hz, 2H), 2.64 – 2.54 (m, 2H), 1.99 – 1.89 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.6, 144.6, 129.5, 127.3, 122.8, 116.2, 110.7, 60.7, 49.5, 47.4, 31.5, 28.1, 22.3, 14.4.

**4-Methoxy-4-(4-methoxyphenyl)butan-2-one (10d)**

This compound was prepared according to the procedure of Yadav *et al*[42]. Briefly, a mixture of 4-anisaldehyde (10 mmol), 2,2-dimethoxypropane (20 mmol) and iodine (0.2 mmol) in dry methylene chloride (20 mmol) was stirred under $N_2$ for 30 min. After the reaction was complete as indicated by TLC, the reaction mixture was diluted with water and extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with sodium thiosulfate (aq., 15% w/v) and brine, and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with hexanes / ethyl acetate.




**$^1$H NMR** (300 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 2H), 6.88 (d, $J$ = 8.8 Hz, 2H), 4.58 (dd, $J$ = 8.8, 4.5 Hz, 1H), 3.79 (s, 3H), 3.16 (s, 3H), 3.05 – 2.88 (m, 1H), 2.57 (dd, $J$ = 15.8, 4.5 Hz, 1H), 2.14 (s, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

## VIII. Small scale enzymatic reactions and product calibration curves

Enzymatic reactions performed on analytical scale were conducted following the general procedure described below, also described in **Section I (H)** and **Section I (I)**. Product formation was quantified by HPLC or GC based on the calibration curve of the corresponding racemic reference compound (**Section VII**). TTN is defined as the amount of product divided by total heme protein as measured by the hemochrome assay (**Section I (G)**). Analysis data presented in this section are for results shown in Fig. 3 and Fig. 4.

### General procedure for biotransformations using whole *E. coli* cells

Suspensions of *E. coli* expressing the appropriate heme protein variant in M9-N buffer (generally $OD_{600}$ = 30) were degassed by bubbling with argon in sealed vials for at least 40 minutes; the cells were kept on ice during this time. Separately, a solution of *D*-glucose (250 mM in M9-N) was degassed by sparging with argon for at least 30 minutes. All solutions were then transferred into an anaerobic chamber for reaction set up. To a 2 mL vial were added a GOX oxygen depletion solution (20 μL of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0), *D*-glucose (40 μL of 250 mM stock solution in M9-N buffer), degassed suspension of *E. coli* expressing heme protein (generally $OD_{600}$ = 30, 320 μL), alkane substrate (10 μL of 400 mM stock solution in EtOH), and ethyl diazoacetate (10 μL of 400 mM stock solution in EtOH) in the listed order. Final reaction volume was 400 μL; final concentrations were 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose. ***Note:*** reaction performed at $OD_{600}$ = 30 indicates that 320 μL of $OD_{600}$ = 30 cells were added, and likewise for other reaction $OD_{600}$ descriptions. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. A modified procedure was used for reactions conducted at 4 °C: reactions were set up in the same manner, except kept on ice and were shaken in a cold room (4 °C) at 500 rpm for 18 hours. Protein concentration was determined using the hemochrome assay as described in **Section I (G)**.

*Reaction workup for quantitative HPLC analysis.* The reactions were quenched by addition of acetonitrile (400 μL) and internal standard (10 μL of 60 mM ethyl phenoxyacetate or 40 mM ethyl benzoate in acetonitrile, as appropriate). This mixture was then transferred to a microcentrifuge tube, vortexed (10 seconds, 3 times) and centrifuged (20,000 × g, 10 minutes). The supernatant was taken for HPLC analysis.

*Reaction workup for quantitative GC analysis.* Internal standard 1,3,5-trimethoxybenzene (10 μL of 40 mM stock solution in cyclohexane) was added to the reaction vial followed by mixed solvent (cyclohexane / ethyl acetate = 1 : 1, 800 μL). The mixture was transferred to a 1.5 mL microcentrifuge tube, vortexed (10 seconds, 3 times), and centrifuged (20,000 × g, 5 minutes) to completely separate the organic and aqueous layers. The organic layer was taken for GC analysis.

### HPLC calibration curve preparation

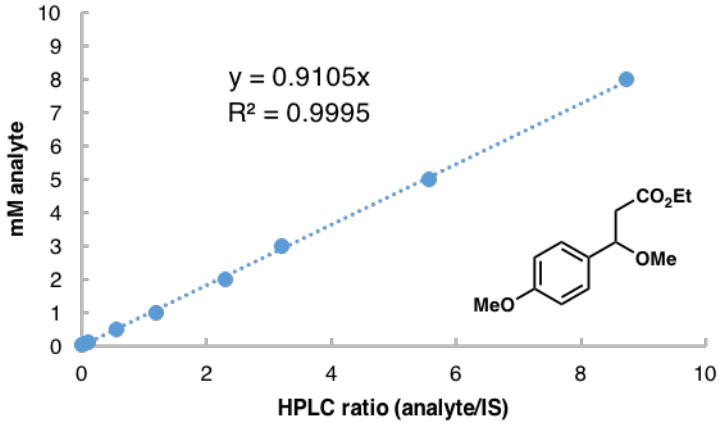
Stock solutions of chemically synthesized products at various concentrations (1 to 200 mM in EtOH) were prepared. To a 2 mL vial were added 380 μL water, 20 μL product stock solution, 10 μL internal standard (60 mM ethyl phenoxyacetate or 40 mM ethyl benzoate in acetonitrile, as appropriate) and 400 μL acetonitrile. The mixture was vortexed and analyzed by HPLC. Data points represent the average of duplicate runs. The standard curves plot product concentration in

mM (y-axis) against the ratio of product area to internal standard area on the HPLC (x-axis).

**GC calibration curve preparation**

Stock solutions of chemically synthesized products at various concentrations (0.2 to 200 mM in EtOH) were prepared. To a microcentrifuge tube were added 380 µL M9-N buffer, 20 µL product stock solution, 10 µL internal standard (40 mM 1,3,5-trimethoxybenzene in cyclohexane), and 800 µL mixed solvent system (cyclohexane : ethyl acetate = 1:1). The mixture was vortexed (10 seconds, 3 times) then centrifuged (20,000 × g, 5 min) to completely separate the organic and aqueous layers. The organic layer was removed for GC analysis. All data points represent the average of at least duplicate runs. The standard curves plot product concentration in mM (y-axis) against the ratio of product area to internal standard area on the GC (x-axis).

**Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (3a)**
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm



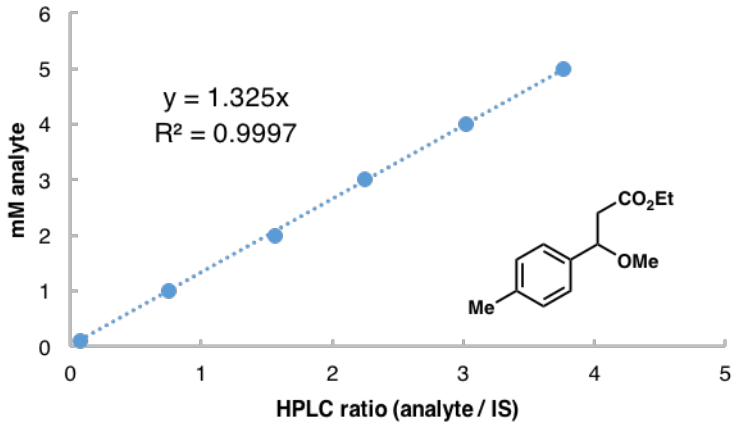

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1a** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/µM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3a**-(1) | 5457.7 | 1066.1 | 5.119 | 4.66 | 2.19 | 2133 | | |
| **3a**-(2) | 5469.8 | 1137.5 | 4.809 | 4.38 | 2.19 | 2004 | | |
| **3a**-(3) | 5740.7 | 1129.0 | 5.085 | 4.63 | 2.07 | 2238 | | |
| **3a**-(4) | 5684.8 | 1133.4 | 5.016 | 4.57 | 2.07 | 2208 | 2150 | 100 |

**Notes:** Pdt = product area, IS = internal standard area, [Pdt] = product concentration in reaction, [PC] = protein concentration in reaction, Avg. TTN = average total turnover number, SD TTN = standard deviation of TTN. These notes apply for the following tables.

**Ethyl 3-methoxy-3-(*p*-tolyl)propanoate (3b)**

HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

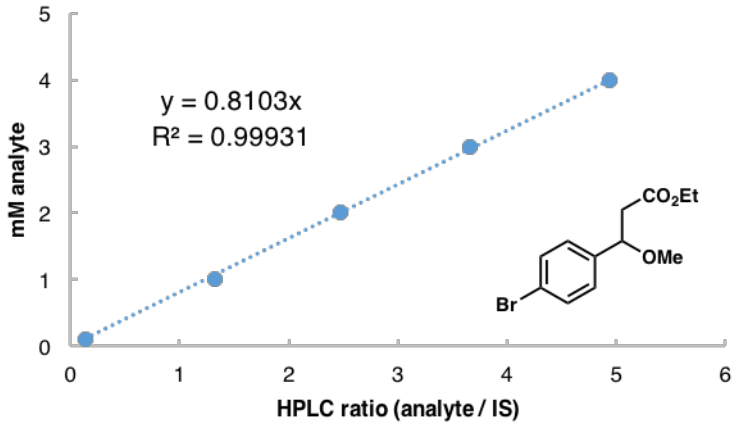



Analysis data for **P411-CHF** catalyzed C–H alkylation of **1b** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3b**-(1) | 2719.0 | 2242.6 | 1.212 | 1.61 | 2.19 | 735 | | |
| **3b**-(2) | 2760.9 | 2233.2 | 1.236 | 1.64 | 2.19 | 750 | | |
| **3b**-(3) | 3257.6 | 2244.6 | 1.451 | 1.92 | 2.07 | 930 | | |
| **3b**-(4) | 3275.5 | 2253.5 | 1.454 | 1.93 | 2.07 | 931 | 840 | 110 |

**Ethyl 3-(4-bromophenyl)-3-methoxypropanoate (3c)**

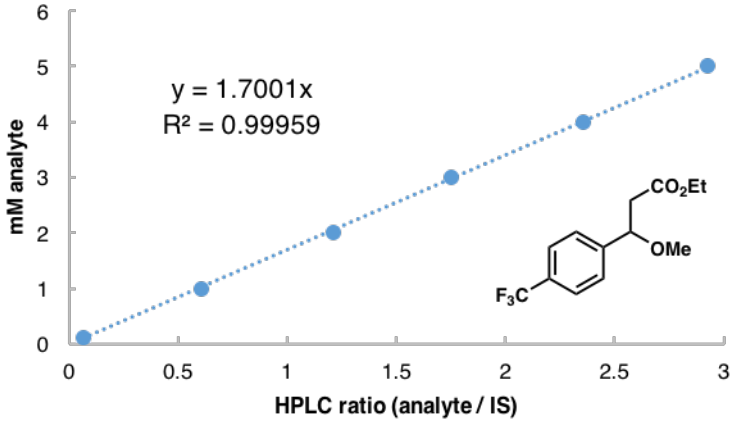
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1c** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3c**-(1) | 2433.4 | 2269.5 | 1.072 | 0.87 | 2.19 | 398 | | |
| **3c**-(2) | 2583.9 | 2204.0 | 1.172 | 0.95 | 2.19 | 435 | | |
| **3c**-(3) | 2728.2 | 2237.2 | 1.219 | 0.99 | 2.07 | 478 | | |
| **3c**-(4) | 2024.0 | 2324.4 | 0.871 | 0.71 | 2.07 | 341 | 410 | 60 |

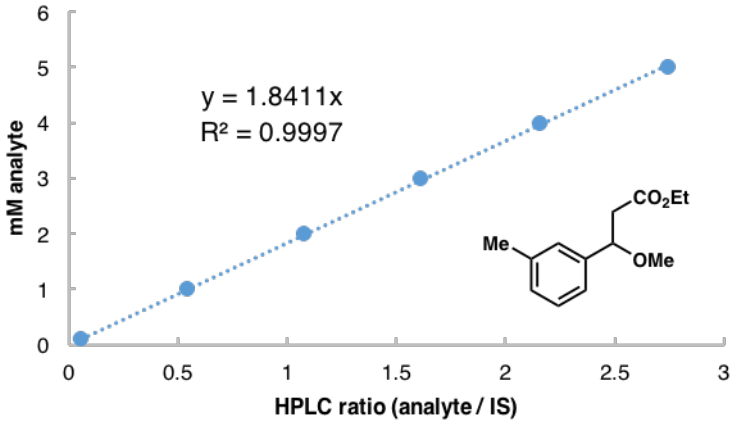
**Ethyl 3-methoxy-3-(4-(trifluoromethyl)phenyl)propanoate (3d)**
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1d** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3d**-(1) | 1689.4 | 2249.3 | 0.751 | 1.28 | 2.19 | 584 | | |
| **3d**-(2) | 1691.5 | 2250.5 | 0.752 | 1.28 | 2.19 | 585 | | |
| **3d**-(3) | 1858.5 | 2192.0 | 0.848 | 1.44 | 2.07 | 697 | | |
| **3d**-(4) | 1848.4 | 2229.5 | 0.829 | 1.41 | 2.07 | 681 | 640 | 60 |

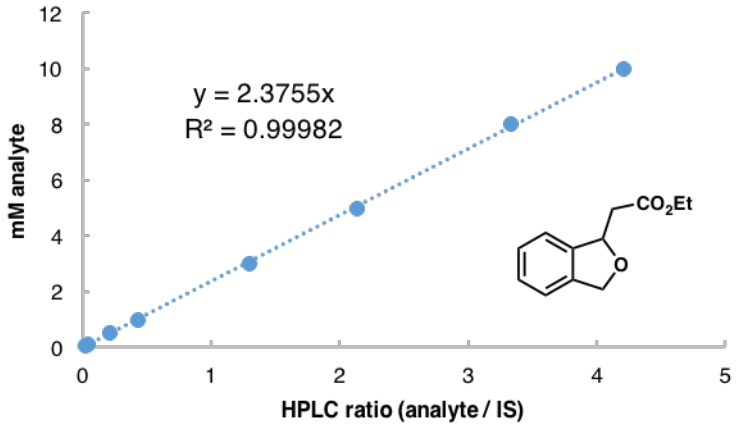
**Ethyl 3-methoxy-3-(*m*-tolyl)propanoate (3e)**
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1e** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3e**-(1) | 853.7 | 2313.9 | 0.369 | 0.68 | 1.84 | 370 | | |
| **3e**-(2) | 832.9 | 2311.4 | 0.360 | 0.66 | 1.84 | 361 | | |
| **3e**-(3) | 1083.7 | 2335.0 | 0.464 | 0.85 | 2.06 | 416 | | |
| **3e**-(4) | 1102.1 | 2327.5 | 0.474 | 0.87 | 2.06 | 424 | 390 | 30 |

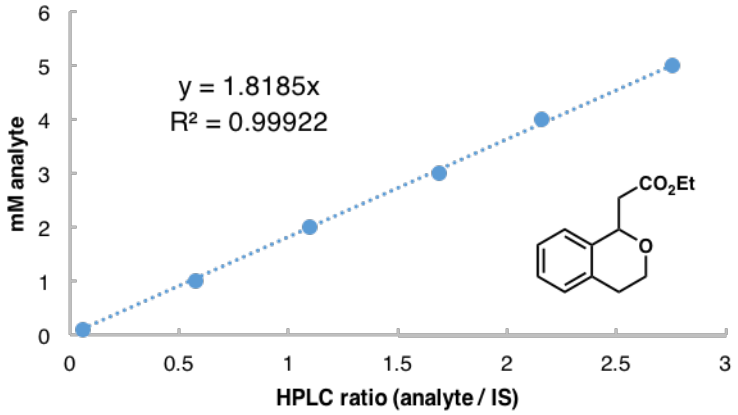
**Ethyl 2-(1,3-dihydroisobenzofuran-1-yl)acetate (3f)**
HPLC calibration curve with ethyl benzoate as internal standard (IS), at 220 nm




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1f** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3f**-(1) | 1548.2 | 2209.2 | 0.701 | 1.66 | 1.84 | 906 | | |
| **3f**-(2) | 1530.2 | 2212.9 | 0.691 | 1.64 | 1.84 | 894 | | |
| **3f**-(3) | 1737.5 | 2216.2 | 0.784 | 1.86 | 2.06 | 906 | | |
| **3f**-(4) | 1716.1 | 2212.9 | 0.775 | 1.84 | 2.06 | 896 | 900 | 10 |

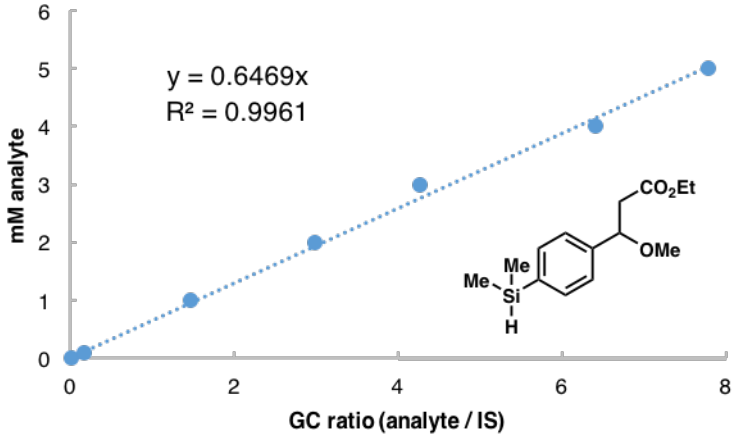
**Ethyl 2-(isochroman-1-yl)acetate (3g)**
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1g** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3g**-(1) | 2681.7 | 2293.4 | 1.169 | 2.13 | 1.84 | 1158 | | |
| **3g**-(2) | 2646.0 | 2329.7 | 1.136 | 2.07 | 1.84 | 1125 | | |
| **3g**-(3) | 3344.6 | 2355.0 | 1.420 | 2.58 | 2.06 | 1257 | | |
| **3g**-(4) | 3388.4 | 2318.8 | 1.461 | 2.66 | 2.06 | 1293 | 1210 | 80 |

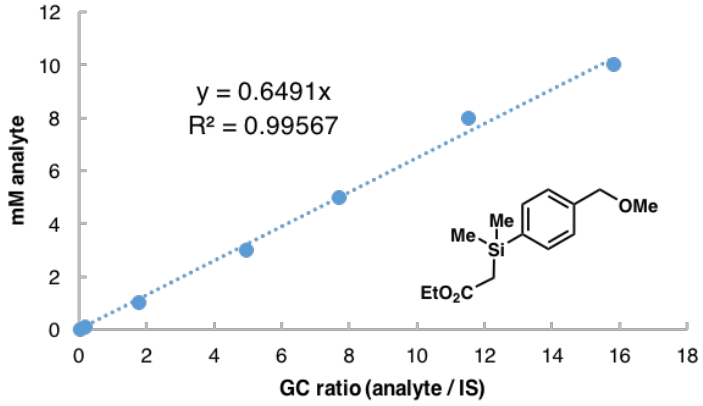
**Ethyl 3-(4-(dimethylsilyl)phenyl)-3-methoxypropanoate (3h)**
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1h** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3h**-(1) | 2327.7 | 772.6 | 3.013 | 1.95 | 1.99 | 978 | | |
| **3h**-(2) | 2193.5 | 729.1 | 3.009 | 1.95 | 1.99 | 977 | | |
| **3h**-(3) | 2222.0 | 752.2 | 2.954 | 1.91 | 2.21 | 865 | | |
| **3h**-(4) | 2288.8 | 755.6 | 3.029 | 1.96 | 2.21 | 887 | 930 | 60 |

**Ethyl 2-((4-(methoxymethyl)phenyl)dimethylsilyl)acetate (3h')**
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)




Analysis data for formation of **3h'** promoted by *E. coli* with **P411-CHF** (room temp.):[†]

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | Avg. [Pdt]/mM | SD [Pdt]/mM |
|---|---|---|---|---|---|---|---|
| **3h'**-(1) | 2302.5 | 772.6 | 2.980 | 1.93 | 1.99 | | |
| **3h'**-(2) | 2154.0 | 729.1 | 2.954 | 1.92 | 1.99 | | |
| **3h'**-(3) | 2123.4 | 752.2 | 2.823 | 1.83 | 2.21 | | |
| **3h'**-(4) | 2165.5 | 755.6 | 2.866 | 1.86 | 2.21 | 1.89 | 0.05 |

[†]Entries are from the same reactions as those used for analysis of **3h**. TTN is not calculated.

Analysis data for formation of **3h'** promoted by *E. coli* with **P-I263F** (room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | Avg. [Pdt]/mM | SD [Pdt]/mM |
|---|---|---|---|---|---|---|---|
| **3h'**-(5) | 5975.7 | 826.8 | 7.228 | 4.69 | 2.47 | | |
| **3h'**-(6) | 6069.1 | 848.6 | 7.152 | 4.64 | 2.47 | | |
| **3h'**-(7) | 5927.5 | 842.4 | 7.036 | 4.57 | 2.50 | | |
| **3h'**-(8) | 5974.8 | 823 | 7.260 | 4.71 | 2.50 | 4.65 | 0.06 |

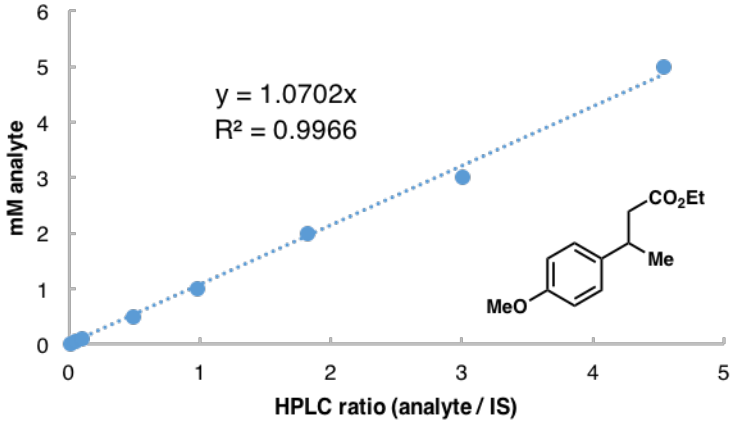
Analysis data for formation of **3h'** promoted by **vector control** *E. coli* (room temp.):[†]

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM[#] | Avg. [Pdt]/mM | SD [Pdt]/mM |
|---|---|---|---|---|---|---|---|
| **3h'**-(9) | 2939.1 | 847.9 | 3.466 | 2.25 | 0.77 | | |
| **3h'**-(10) | 2704.2 | 823.7 | 3.283 | 2.13 | 0.77 | | |
| **3h'**-(11) | 2933.7 | 865.3 | 3.390 | 2.20 | 0.87 | | |
| **3h'**-(12) | 2893.1 | 823.2 | 3.514 | 2.28 | 0.87 | 2.22 | 0.07 |

**Notes:** [†]Vector control reactions employ *E. coli* cells that express a protein which does not contain a transition metal cofactor (halohydrin dehalogenase, UniProt ID: Q93D82). [#][PC] refers to the concentration of heme *b* measured by the hemochrome assay. Since a heme protein is not over-expressed in these cells, this is a measurement of free hemin and/or native heme *b*-containing proteins found in the *E. coli*.

**Ethyl 3-(4-methoxyphenyl)butanoate (3i)**

HPLC calibration curve with ethyl benzoate as internal standard (IS), at 220 nm

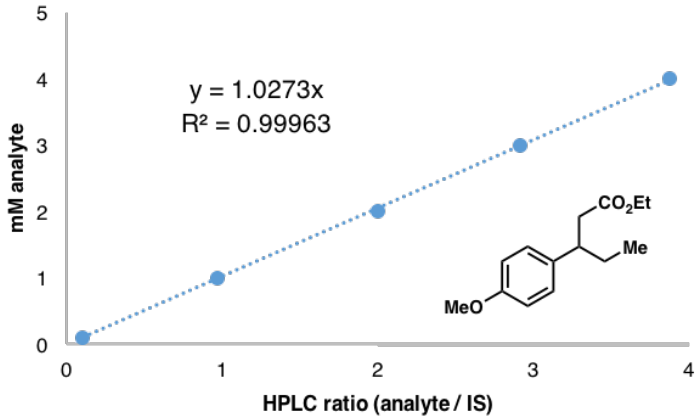



Analysis data for **P411-CHF** catalyzed C–H alkylation of **1i** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3i**-(1) | 2267.1 | 2277.4 | 0.995 | 1.07 | 2.02 | 527 | | |
| **3i**-(2) | 2224.4 | 2284.4 | 0.974 | 1.04 | 2.02 | 515 | | |
| **3i**-(3) | 2533.9 | 2294.2 | 1.104 | 1.18 | 2.24 | 528 | | |
| **3i**-(4) | 2592.3 | 2297.6 | 1.128 | 1.21 | 2.24 | 540 | 530 | 10 |

**Ethyl 3-(4-methoxyphenyl)pentanoate (3j)**

HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

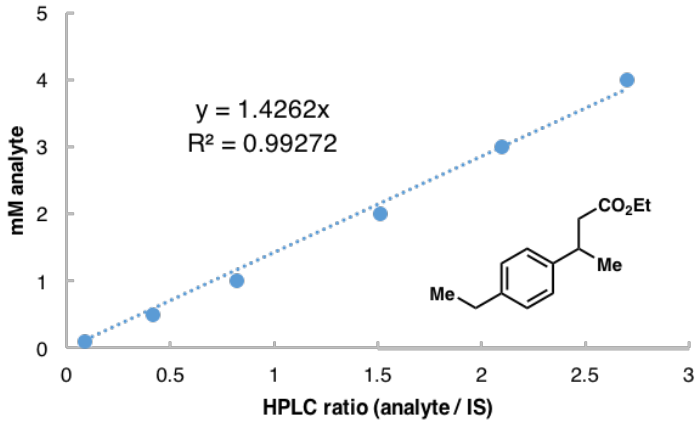



Analysis data for **P411-CHF** catalyzed C–H alkylation of **1j** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3j**-(1) | 192.6 | 2319.1 | 0.083 | 0.09 | 2.02 | 42 | | |
| **3j**-(2) | 200.1 | 2285.5 | 0.088 | 0.09 | 2.02 | 44 | | |
| **3j**-(3) | 236.0 | 2277.5 | 0.104 | 0.11 | 2.24 | 48 | | |
| **3j**-(4) | 245.6 | 2308.5 | 0.106 | 0.11 | 2.24 | 49 | 46 | 3 |

**Ethyl 3-(4-ethylphenyl)butanoate (3k)**

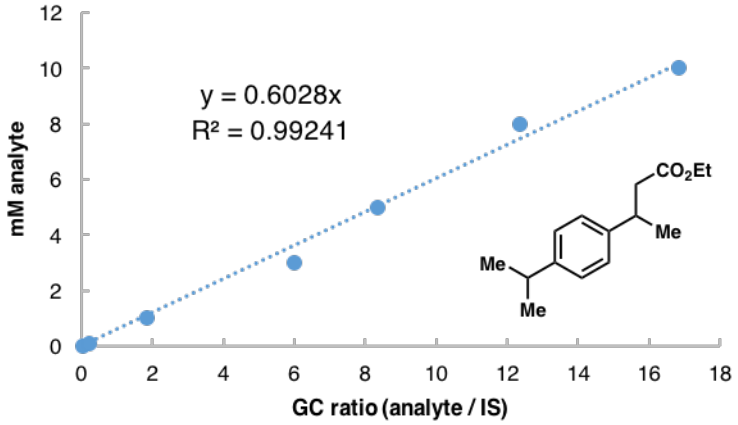
HPLC calibration curve with ethyl benzoate as internal standard (IS), at 220 nm




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1k** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3k**-(1) | 970.6 | 2320.8 | 0.418 | 0.60 | 1.80 | 331 | | |
| **3k**-(2) | 953.2 | 2327.4 | 0.410 | 0.58 | 1.80 | 324 | | |
| **3k**-(3) | 1034.1 | 2412.6 | 0.429 | 0.61 | 1.78 | 343 | | |
| **3k**-(4) | 1056.0 | 2426.4 | 0.435 | 0.62 | 1.78 | 348 | 340 | 10 |

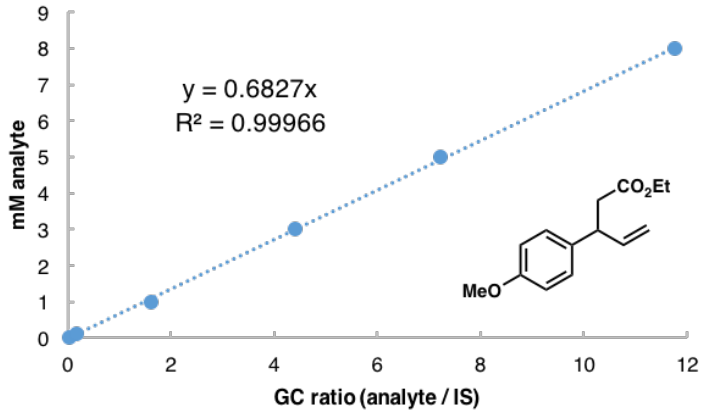
**Ethyl 3-(4-isopropylphenyl)butanoate (3l)**
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)




Analysis data for **P411-CHF** catalyzed C–H alkylation of **1l** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3l**-(1) | 354.6 | 765.1 | 0.463 | 0.28 | 1.99 | 140 | | |
| **3l**-(2) | 369.9 | 755.0 | 0.490 | 0.30 | 1.99 | 148 | | |
| **3l**-(3) | 414.3 | 761.5 | 0.544 | 0.33 | 2.21 | 149 | | |
| **3l**-(4) | 392.5 | 767.8 | 0.511 | 0.31 | 2.21 | 140 | 140 | 5 |

**Ethyl 3-(4-methoxyphenyl)pent-4-enoate (3m)**
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

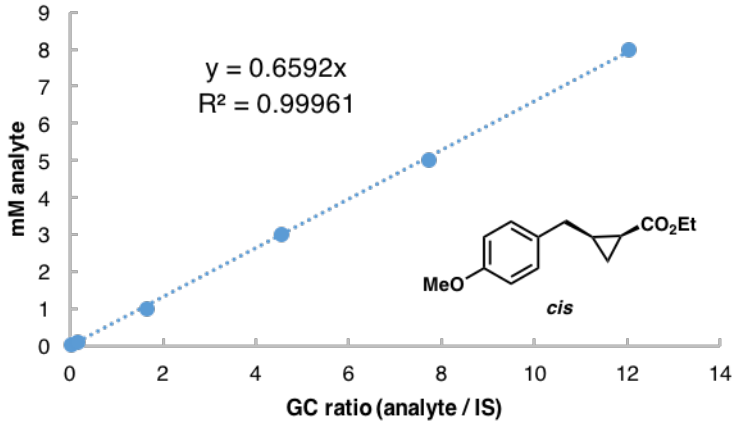


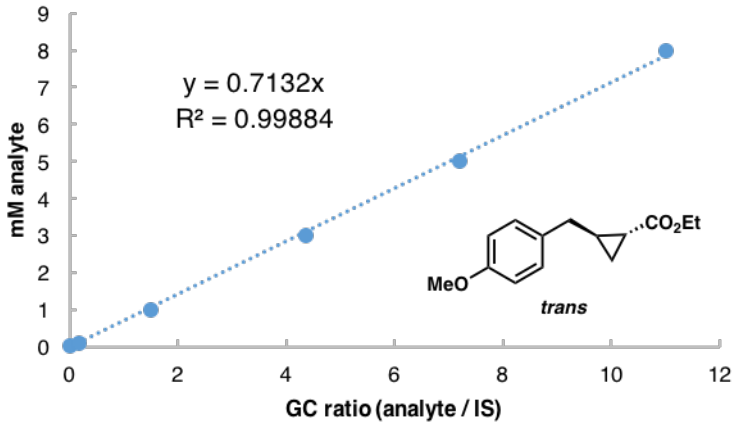

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1m** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **3m**-(1) | 238.1 | 794.4 | 0.300 | 0.20 | 1.99 | 103 | | |
| **3m**-(2) | 252.2 | 797.3 | 0.316 | 0.22 | 1.99 | 108 | | |
| **3m**-(3) | 262.0 | 784.7 | 0.334 | 0.23 | 2.21 | 103 | | |
| **3m**-(4) | 254.9 | 753.3 | 0.338 | 0.23 | 2.21 | 105 | 100 | 3 |

**Ethyl 2-(4-methoxybenzyl)cyclopropane-1-carboxylate (3m')**

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)



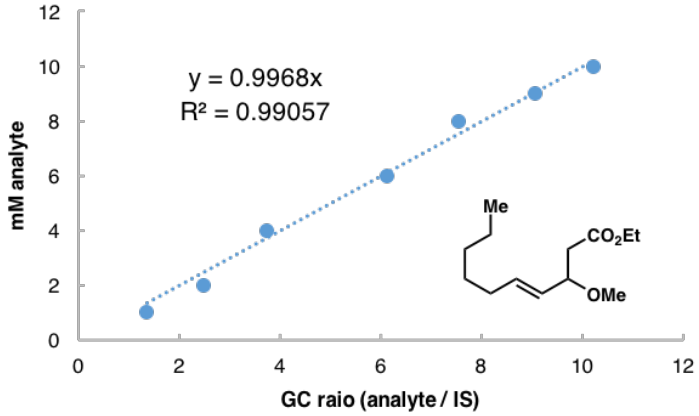

Analysis data for **P-I263F** catalyzed cyclopropanation of **1m** (whole cells, room temp.):

| Pdt-Entries | Pdt (*cis*) | Pdt (*trans*) | IS | [Pdt]/ mM* | [PC]/ μM | TTN | d.r.† | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|---|
| **3m'**-(5) | 556.9 | 359.4 | 887.9 | 0.702 | 2.47 | 284 | 59:41 | | |
| **3m'**-(6) | 424.4 | 281.6 | 847.1 | 0.567 | 2.47 | 230 | 58:42 | | |
| **3m'**-(7) | 590.1 | 379.0 | 826.2 | 0.798 | 2.50 | 319 | 59:41 | | |
| **3m'**-(8) | 528.7 | 344.7 | 818.2 | 0.726 | 2.50 | 290 | 59:41 | 280 | 40 |

**Notes:** *Refers to the total concentration of cyclopropane products (*cis*-**3m'** + *trans*-**3m'**). †Diastereomeric ratio (d.r.) is given as *cis* : *trans*.

**Ethyl (*E*)-3-methoxydec-4-enoate (5a)**

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

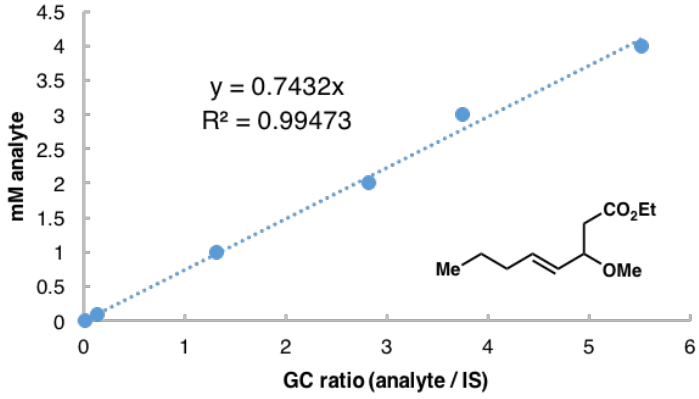



Analysis data for **P411-CHF** catalyzed C–H alkylation of **4a** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **5a**-(1) | 1710.3 | 200.0 | 8.552 | 8.52 | 2.25 | 3789 | | |
| **5a**-(2) | 1765.1 | 217.4 | 8.119 | 8.09 | 2.25 | 3597 | | |
| **5a**-(3) | 1623.6 | 215.6 | 7.531 | 7.51 | 1.94 | 3879 | | |
| **5a**-(4) | 1482.7 | 204.9 | 7.236 | 7.21 | 1.94 | 3727 | 3750 | 120 |

**Ethyl (*E*)-3-methoxyoct-4-enoate (5b)**

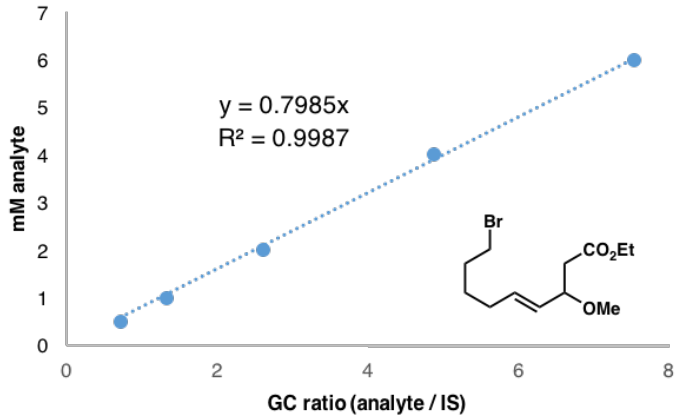
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)




Analysis data for **P411-CHF** catalyzed C–H alkylation of **4b** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **5b**-(1) | 2065.4 | 812.0 | 2.544 | 1.89 | 1.99 | 949 | | |
| **5b**-(2) | 2036.8 | 795.5 | 2.560 | 1.90 | 1.99 | 955 | | |
| **5b**-(3) | 2250.2 | 793.6 | 2.835 | 2.11 | 2.21 | 954 | | |
| **5b**-(4) | 2217.2 | 790.8 | 2.804 | 2.08 | 2.21 | 944 | 950 | 5 |

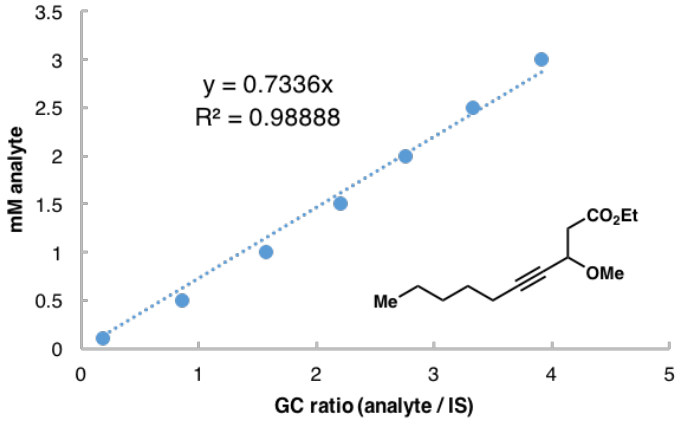
**Ethyl (*E*)-9-bromo-3-methoxynon-4-enoate (5c)**
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)




Analysis data for **P411-CHF** catalyzed C–H alkylation of **4c** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **5c**-(1) | 1071.0 | 179.5 | 5.967 | 4.76 | 1.80 | 2645 | | |
| **5c**-(2) | 1062.7 | 178.6 | 5.950 | 4.75 | 1.80 | 2637 | | |
| **5c**-(3) | 1142.9 | 175.3 | 6.520 | 5.21 | 1.78 | 2917 | | |
| **5c**-(4) | 1140.9 | 180.4 | 6.324 | 5.05 | 1.78 | 2829 | 2760 | 140 |

**Ethyl 3-methoxydec-4-ynoate (5e)**
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)




Analysis data for **P411-CHF** catalyzed C–H alkylation of **4e** (whole cells, room temp.):

| Pdt-Entries | Pdt | IS | Pdt/IS | [Pdt]/mM | [PC]/μM | TTN | Avg. TTN | SD TTN |
|---|---|---|---|---|---|---|---|---|
| **5e**-(1) | 405.4 | 903.5 | 0.449 | 0.33 | 1.80 | 183 | | |
| **5e**-(2) | 370.3 | 856.6 | 0.432 | 0.32 | 1.80 | 176 | | |
| **5e**-(3) | 388.3 | 843.5 | 0.460 | 0.34 | 1.78 | 189 | | |
| **5e**-(4) | 407.2 | 828.8 | 0.491 | 0.36 | 1.78 | 202 | 190 | 10 |

## IX. Enzymatic C–H alkylation reactions on preparative scale

### Preparation of *E. coli* cells expressing P411 variants for preparative-scale reactions

$HB_{amp}$ (480 mL) in a 1 L flask was inoculated with an overnight culture (20 mL, $LB_{amp}$) of *E. cloni* BL21 (DE3) (*E. coli*) cells containing a pET22b(+) plasmid encoding the desired P411 variant. The culture was shaken at 37 °C and 220 rpm (no humidity control) for 2.5 hours. The culture was placed on ice for 40 minutes, and 5-aminolevulinic acid (1.0 mM final concentration) and IPTG (0.5 mM final concentration) were added. The incubator temperature was reduced to 20 °C, and the culture was allowed to shake for 16–18 hours at 130 rpm. When greater amounts of cells were required, additional *E. coli* cultures were prepared in this manner. Cells were pelleted by centrifugation (3,000 × g, 5 min, 4 °C), resuspended in M9-N buffer and adjusted to $OD_{600}$ = 60. An aliquot of cells at $OD_{600}$ = 60 (3 mL) was taken for the hemochrome assay (see **Section I (G)**) to determine protein concentration. Unless otherwise indicated, the cell suspension was then diluted 1 : 1 with M9-N buffer to achieve $OD_{600}$ = 30, which was used for the reaction. Cell suspensions in M9-N buffer were kept on ice until use.

### General Procedure D: Enzymatic C–H alkylation reactions on preparative scale

*Procedure D-I (alkane substrate is limiting reagent).* To an Erlenmeyer flask equipped with a screw cap (reaction vessel, Chemglass CG-1543: 250 mL, 500 mL, or 1000 mL) was added a suspension of *E. coli* expressing the indicated P411 variant (generally $OD_{600}$ = 30). The headspace of the reaction vessel was degassed with argon (at least 1 hour for volumes less than 200 mL, at least 2 hours for volumes greater than 200 mL) while kept on ice. To degas the headspace of a flask containing *E. coli* cells, the flask is covered with aluminum foil and a stream of argon is flowed through the flask just above the cell suspension. Separately, a solution of *D*-glucose (250 mM in M9-N) was bubbled with argon and the headspace of a flask containing GOX oxygen depletion system (a solution of 14,000 U/mL catalase and 1,000 U/mL glucose oxidase, kept on ice) was degassed for at least 1 hour. In an anaerobic chamber, GOX, *D*-glucose, alkane substrate (1.0 equiv.), and diazo compound (1.0 equiv.) were added to the reaction vessel in this order. The vessel was capped, sealed with parafilm, and shaken (150–200 rpm) at room temperature. After one hour, the reaction vessel was transferred again to the anaerobic chamber where a second portion of *E. coli* cells expressing the P411 variant (the headspace of the flask containing these cells was degassed with argon following same procedure) and additional diazo compound (1.0 equiv.) were added. The vessel was capped, sealed with parafilm, and shaken (150–200 rpm) at room temperature for 14–17 additional hours. Final conditions were *E. coli* expressing P411 variant, alkane substrate (0.2–0.5 mmol, 1.0 equiv., larger scales for **3a**, **5a**, and **8f,** see **Section X**), diazo compound (2.0 equiv.), *D*-glucose (25 mM), GOX oxygen depletion system (700 U/mL catalase, 50 U/mL glucose oxidase), 2 vol% EtOH in M9-N buffer under anaerobic conditions; total reaction time 15–18 hours.

*Procedure D-II (diazo compound is limiting reagent).* To an Erlenmeyer flask equipped with a screw cap (reaction vessel, Chemglass CG-1543: 250 mL, 500 mL, or 1000 mL) was added a suspension of *E. coli* expressing the indicated P411 variant. The headspace of the reaction vessel was degassed with argon (at least 1 hour) while kept on ice. To degas the headspace of a flask containing *E. coli* cells, the flask is covered with aluminum foil and a stream of argon is flowed through the flask just above the cell suspension. Separately, a solution of *D*-glucose (250 mM in M9-N) was bubbled with argon. In an anaerobic chamber, *D*-glucose, alkane substrate (2.0 equiv.),

and diazo compound (1.0 equiv.) were added to the reaction vessel in this order. The vessel was capped, sealed with parafilm, and shaken (150–200 rpm) at room temperature. Final conditions were *E. coli* expressing P411 variant, alkane substrate (1.0 mmol, 2.0 equiv.), diazo compound (0.5 mmol, 1.0 equiv.), *D*-glucose (25 mM), 2 vol% EtOH in M9-N buffer under anaerobic conditions; total reaction time 15–18 hours.

*Workup Procedure D-i.* Every 35 mL portion of the preparative scale reaction mixture was transferred to a 50 mL Eppendorf conical tube (catalog no. 0030122178). To the reaction mixture in every tube was added 15 mL mixed organic solvent (1 : 1 hexanes : ethyl acetate); the solution was shaken vigorously and centrifuged (10,000 × g, 5 minutes, Beckman-Coulter Avanti J-25 centrifuge equipped with JA-12 rotor) to separate the organic and aqueous layers. The organic layer was collected and the aqueous layer was subject to three additional rounds of extraction. The organic layers were combined, dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification was performed by silica column chromatography with either hexanes / ethyl acetate or hexanes / dichloromethane / diethyl ether as eluent systems to afford the desired product. Additional purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / methanol eluent system) was utilized if necessary. TTNs were calculated based on measured protein concentration and the isolated yield of the product.

*Workup Procedure D-ii.* Every ~100–125 mL portion of preparative scale reaction mixture was transferred to a centrifuge bottle. To the reaction mixture in every bottle was added equal volume ethyl acetate; the solution was shaken vigorously and centrifuged (14,000 × g, 10 minutes, Beckman-Coulter Avanti J-25 centrifuge equipped with JA-10 rotor) to separate the organic and aqueous layers. The organic layer was collected and the aqueous layer was subject to three additional rounds of extraction. The combined organics were dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification was performed by silica column chromatography with either hexanes / ethyl acetate or hexanes / dichloromethane / diethyl ether as eluent systems to afford the desired product. Additional purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / methanol eluent system) was utilized if necessary. TTNs were calculated based on measured protein concentration and the isolated yield of the product.

**Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (3a)**

This compound was prepared using a modified version of **General Procedure D-I** carried out at 4 °C. The reaction mixture was kept on ice during the addition of all reagents and shaken in an incubator set to 4 °C at 150 rpm. In addition, a solution of $Na_2S_2O_4$ (5 mL, 40 mM in M9-N, bubbled with argon for 1 hour) was added following the second addition of *E. coli* cells expressing P411-CHF. Final conditions were *E. coli* expressing P411-CHF ($OD_{600}$ = 44), alkane substrate (1.0 mmol, 1.0 equiv.), ethyl diazoacetate (2.0 mmol, 2.0 equiv.), *D*-glucose (25 mM), GOX oxygen depletion system (700 U/mL catalase, 50 U/mL glucose oxidase), $Na_2S_2O_4$ (1 mM), 2 vol% EtOH in M9-N buffer under anaerobic conditions at 4 °C; total reaction time 18 hours.

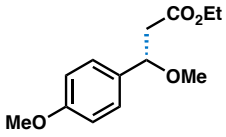
The reaction was quenched with the addition of 100 mL acetonitrile. The crude reaction mixture was transferred to a centrifuge tube, shaken vigorously, and centrifuged to pellet the cells (14,000 × g, 10 minutes). The supernatant was decanted and the acetonitrile was removed under reduced pressure. Following, the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The cell pellet was resuspended in $H_2O$ and this suspension was also extracted using ethyl acetate (3 ×

20 mL; centrifugation (3,000 × g, 5 min) was used to help separate the organic and aqueous layers. The combined organics were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification by reverse phase preparative HPLC (column: Eclipse XDB-C8, 5 um, 9.4 × 250 mm) using water / acetonitrile eluent system afforded **3a.**

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ = 44, 4 °C) | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 110.0 | 51.0* | 4.77 | 0.768 | 10.0 | 20.0 |
| **Alkane substrate (1a) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.50 | 2.0 | 1.0 | 1.0 | 1.0; 1.0 | 2.0 |
| **Purification eluent** | **Product** | | | | |
| **1:** water / MeCN (reverse phase preparative HPLC) | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN | |
| | 194.7 | 0.817 | 82% | 1060 | |

*****A solution of $Na_2S_2O_4$ (5 mL, 40 mM in M9-N, bubbled with argon for 1 hour) was added following the second addition of *E. coli* cells expressing P411-CHF. Final concentration of $Na_2S_2O_4$ in the reaction is 1 mM.

**Notes:** [PC] = protein concentration in original cell suspension, n_pro = amount of protein in the reaction, n_1 = amount of alkane substrate in the reaction, n_2 = total amount of diazo compound in the reaction, add. = addition, m[Pdt] = mass of product isolated, n[Pdt] = amount of product. [a] GOX oxygen depletion system is 14,000 U/mL catalase and 1,000 U/ mL glucose oxidase in 0.1 M potassium phosphate buffer (pH=8.0); the final reaction mixture contains 700 U/mL catalase and 50 U/mL glucose oxidase. [b] *D*-glucose stock solution is 250 mM in M9-N buffer; final concentration of *D*-glucose in the reaction is 25 mM. These notes apply for all tables in this section.

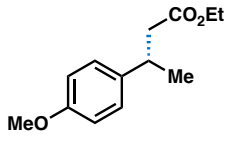



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.58 (dd, *J* = 9.0, 4.9 Hz, 1H), 4.14 (qd, *J* = 7.1, 1.2 Hz, 2H), 3.81 (s, 3H), 3.19 (s, 3H), 2.80 (dd, *J* = 15.2, 9.0 Hz, 1H), 2.55 (dd, *J* = 15.2, 4.9 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.2, 159.5, 132.7, 128.0, 114.0, 79.7, 60.7, 56.7, 55.4, 43.7, 14.3. **HRMS** (EI) m/z: 238.1213 ($M^{+\cdot}$); calc. for $C_{13}H_{18}O_4$: 238.1205. **$[\alpha]^{23}_D$** = –46.354 ± 0.411° (*c* 0.5, $CHCl_3$). **SFC Chiralpak AD-H column** (3% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C), $t_r$ = 9.02 min (major), 10.50 min (minor), 98.0 : 2.0 e.r.

**Ethyl 3-(4-methoxyphenyl)butanoate (3i)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-CHF, $OD_{600}$ = 29)** | | | | **GOX solution[a]** | ***D*-glucose in M9-N[b]** |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 110.0 | 56.0 | 2.25 | 0.374 | 10.0 | 20.0 |
| **Alkane substrate (1i) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.10 | 2.0 | 0.2 | 0.20 | 1.0; 1.0 | 0.4 |
| **Purification eluent** | **Product** | | | | |
| **1:** Hex / EtOAc (normal phase) | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN | |
| **2:** water / MeOH (reverse phase) | 21.3 | 0.096 | 48% | 260 | |

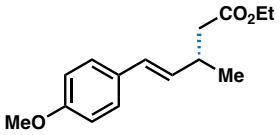



Spectral data is in agreement with literature report[40]. **$^1$H NMR** (500 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.08 (qd, *J* = 7.2, 1.2 Hz, 2H), 3.79 (s, 3H), 3.24 (h, *J* = 7.1 Hz, 1H), 2.57 (dd, *J* = 14.9, 7.2 Hz, 1H), 2.51 (dd, *J* = 14.9, 8.0 Hz, 1H), 1.28 (d, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 172.6, 158.2, 138.0, 127.8, 113.9, 60.4, 55.4, 43.4, 35.9, 22.1, 14.3. **$[\alpha]^{23}_D$** = +26.334 ± 0.676° (*c* 0.5, $CHCl_3$). **SFC Chiralcel OB-H column** (supercritical $CO_2$, 2.5 mL/min, 40 °C), $t_r$ = 5.50 min (minor), 6.24 min (major), 97.9 : 2.1 e.r.

**Ethyl (*E*)-5-(4-methoxyphenyl)-3-methylpent-4-enoate (5d)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*ii*.

| ***E. coli* suspension in M9-N (variant: P411-CHF, $OD_{600}$ = 32)** | | | | **GOX solution[a]** | ***D*-glucose in M9-N[b]** |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 137.5 | 70.0 | 2.59 | 0.536 | 12.5 | 25.0 |
| **Alkane substrate (4d) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.10 | 2.5 | 0.25 | 0.20 | 1.25; 1.25 | 0.50 |
| **Purification eluent** | **Product** | | | | |
| Hex / (4 : 1 DCM : $Et_2O$) (normal phase) | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN | |
| | 9.7 | 0.039 | 15.6% | 70 | |

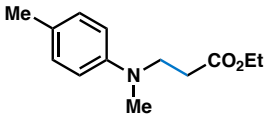



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.30 – 7.24 (m, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.34 (d, *J* = 15.9 Hz, 1H), 5.99 (dd, *J* = 15.9, 7.6 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.80 (s, 3H), 2.90 – 2.75 (m, 1H), 2.41 (dd, *J* = 14.7, 7.3 Hz, 1H), 2.34 (dd, *J* = 14.7, 7.3 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.14 (d, *J* = 6.7 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.6, 159.0, 132.3, 130.4, 128.3, 127.3, 114.0, 60.4, 55.4, 42.1, 34.3, 20.5, 14.5. **HRMS** (FAB) m/z: 248.1417 ($M^{+\cdot}$); calc. for $C_{15}H_{20}O_3$: 248.1413. **SFC Chiralcel OB-H column** (3% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C), $t_r$ = 6.62 min (minor), 7.66 min (major), 97.0 : 3.0 e.r.

**Ethyl 3-(methyl(*p*-tolyl)amino)propanoate (8a)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ = 29) | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 55.0 | 28.0 | 2.13 | 0.177 | 5.0 | 10.0 |
| **Alkane substrate (7a) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.50 | 1.0 | 0.50 | 1.00 | 0.50; 0.50 | 1.0 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / EtOAc (normal phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 91.2 | 0.412 | 82% | 2330 |

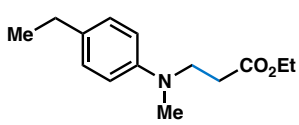



Spectral data is in agreement with literature report[43]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.05 (d, *J* = 8.3 Hz, 2H), 6.67 (d, *J* = 8.6 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.64 (t, *J* = 7.2 Hz, 2H), 2.90 (s, 3H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.25 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.6, 146.8, 129.9, 126.3, 113.1, 60.7, 49.1, 38.5, 31.8, 20.4, 14.3.

**Ethyl 3-((4-ethylphenyl)(methyl)amino)propanoate (8b)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*ii*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ **= 30)** | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[a] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 55.0 | 28.0 | 2.02 | 0.168 | 5.0 | 10.0 |
| **Alkane substrate (7b) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.50 | 1.0 | 0.50 | 1.00 | 0.50; 0.50 | 1.0 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / (4 : 1 DCM : $Et_2O$) (normal phase) **2:** water / MeOH (reverse phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 88.0 | 0.374 | 75% | 2230 |

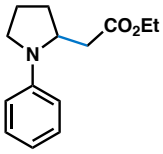



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.08 (d, *J* = 8.7 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.65 (t, *J* = 7.2 Hz, 2H), 2.91 (s, 3H), 2.60 – 2.51 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.20 (t, *J* = 7.6 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.6, 146.9, 132.8, 128.7, 113.0, 60.7, 49.1, 38.5, 31.9, 27.9, 16.1, 14.3. **HRMS** (ESI-TOF) m/z: 236.1673 ($M + H^+$); calc. for $C_{14}H_{22}NO_2$: 236.1651.

**Ethyl 2-(1-phenylpyrrolidin-2-yl)acetate (8c)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*ii*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ **= 31)** | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 55.0 | 28.0 | 2.65 | 0.220 | 5.0 | 10.0 |
| **Alkane substrate (7c) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.50 | 1.0 | 0.50 | 1.00 | 0.50; 0.50 | 1.0 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / (4 : 1 DCM : $Et_2O$) (normal phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 104.1 | 0.446 | 89% | 2030 |

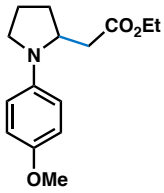



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.29 – 7.20 (m, 2H), 6.69 (tt, *J* = 7.2, 1.1 Hz, 1H), 6.62 (d, *J* = 7.9 Hz, 2H), 4.21 – 4.14 (m, 3H), 3.48 – 3.37 (m, 1H), 3.23 – 3.14 (m, 1H), 2.79 (dd, *J* = 15.0, 2.9 Hz, 1H), 2.22 (dd, *J* = 15.0, 10.5 Hz, 1H), 2.12 – 1.98 (m, 3H), 1.97 – 1.82 (m, 1H), 1.29 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.2, 146.6, 129.5, 116.0, 112.0, 60.6, 55.5, 48.0, 37.8, 31.1, 23.1, 14.4. **HRMS** (ESI-TOF) m/z: 234.1491 ($M + H^+$); calc. for $C_{14}H_{20}NO_2$: 234.1494. **$[\alpha]^{23}_D$** = +2.056 ± 0.834° (*c* 0.5, $CHCl_3$). **HPLC Chiralcel OD-H column** (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, room temperature), $t_r$ = 6.20 min (minor), 8.58 min (major), 82.8 : 17.2 e.r.

**Ethyl 2-(1-(4-methoxyphenyl)pyrrolidin-2-yl)acetate (8d)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ = 31**)** | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 55.0 | 28.0 | 2.17 | 0.180 | 5.0 | 10.0 |
| **Alkane substrate (7d) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.50 | 1.0 | 0.50 | 1.0 | 0.50; 0.50 | 1.0 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / EtOAc (normal phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| **2:** water / MeOH (reverse phase) | | 102.1 | 0.388 | 78% | 2150 |

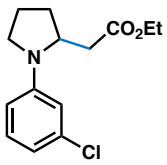



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.86 (d, *J* = 9.1 Hz, 2H), 6.58 (d, *J* = 9.1 Hz, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.13 – 4.05 (m, 1H), 3.76 (s, 3H), 3.42 – 3.35 (m, 1H), 3.17 – 3.09 (m, 1H), 2.76 (dd, *J* = 14.9, 3.0 Hz, 1H), 2.20 (dd, *J* = 14.9, 10.4 Hz, 1H), 2.13 – 1.96 (m, 3H), 1.92 – 1.82 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.3, 151.1, 141.5, 115.3, 112.9, 60.6, 56.1, 56.0, 48.6, 38.1, 31.1, 23.3, 14.4. **HRMS** (FAB) m/z: 263.1518 ($M^{+\cdot}$); calc. for $C_{15}H_{21}NO_3$: 263.1521. **$[\alpha]^{23}_D$** = +7.310 ± 0.478° (*c* 0.5, $CHCl_3$). **HPLC Chiralcel OD-H column** (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, room temperature), $t_r$ = 7.25 min (minor), 8.12 min (major), 83.7 : 16.3 e.r.

**Ethyl 2-(1-(3-chlorophenyl)pyrrolidin-2-yl)acetate (8e)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ **= 29)** | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 137.5 | 70.0 | 3.24 | 0.673 | 12.5 | 25.0 |
| **Alkane substrate (7e) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.20 | 2.5 | 0.50 | 0.40 | 1.25; 1.25 | 1.0 |
| **Purification eluent** | **Product** | | | | |
| **1:** Hex / EtOAc (normal phase) | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN | |
| **2:** water / MeOH (reverse phase) | 89.6 | 0.335 | 67% | 500 | |



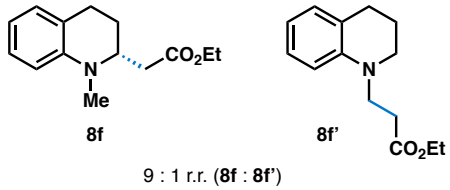

**$^1$H NMR** (500 MHz, $CDCl_3$) δ 7.13 (t, *J* = 8.1 Hz, 1H), 6.65 (dd, *J* = 7.9, 1.8 Hz, 1H), 6.57 (t, *J* = 2.2 Hz, 1H), 6.48 (dd, *J* = 8.3, 2.3 Hz, 1H), 4.23 – 4.12 (m, 3H), 3.44 – 3.36 (m, 1H), 3.22 – 3.12 (m, 1H), 2.74 (dd, *J* = 15.1, 3.0 Hz, 1H), 2.23 (dd, *J* = 15.1, 10.4 Hz, 1H), 2.13 – 2.01 (m, 3H), 1.97 – 1.85 (m, 1H), 1.30 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 171.9, 147.6, 135.3, 130.4, 115.9, 111.9, 110.2, 60.7, 55.5, 48.1, 37.7, 31.1, 23.0, 14.4. **HRMS** (ESI-TOF) m/z: 268.1094 ($M + H^+$); calc. for $C_{14}H_{19}NO_2{}^{35}Cl$: 268.1104. **$[\alpha]^{23}_D$** = +7.144 ± 0.875° (*c* 0.5, $CHCl_3$). **HPLC Chiralcel OD-H column** (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, room temperature), $t_r$ = 6.29 min (minor), 6.87 min (major), 90.3 : 9.7 e.r.

**Ethyl (*R*)-2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate ((–)-8f)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ **= 31)** | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 55.0 | 28.0 | 2.40 | 0.199 | 5.0 | 10.0 |
| **Alkane substrate (7f) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.50 | 1.0 | 0.50 | 1.00 | 0.50; 0.50 | 1.0 |
| **Purification eluent** | **Product** | | | | |
| **1:** Hex / (4 : 1 DCM : $Et_2O$) (normal phase) | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN | |
| **2:** water / acetonitrile (reverse phase preparative HPLC) | 48.9[c] | 0.210 | 42% | 1050 | |

[c]Regioisomer ratio 9 : 1 for **8f** : **8f'** by $^1$H NMR.



9 : 1 r.r. (**8f** : **8f'**)

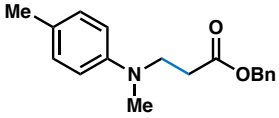
**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.14 – 7.02 (m, 1H, both isomers), 6.99 (d, *J* = 7.3 Hz, 0.9H, isomer **8f**), 6.97 – 6.93 (m, 0.1H, isomer **8f'**), 6.68 – 6.51 (m, 2H, both isomers), 4.15 (q, *J* = 7.1 Hz, 2H, both isomers), 3.86 – 3.78 (m, 0.9H, isomer **8f**), 3.65 – 3.57 (m, 0.2H, isomer **8f'**), 3.32 – 3.25 (m, 0.2H, isomer **8f'**), 2.93 (s, 2.7H, isomer **8f**), 2.91 – 2.79 (m, 0.9H, isomer **8f**), 2.77 – 2.67 (m, 1.1H, both isomers), 2.65 – 2.54 (m, 1.1H, both isomers), 2.39 (dd, *J* = 14.7, 8.6 Hz, 0.9H, isomer **8f**), 2.08 – 1.84 (m, 2H, both isomers), 1.30 – 1.24 (m, 3H, both isomers). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.6, 172.3, 144.8, 144.6, 129.5, 129.0, 127.3, 127.3, 122.8, 121.6, 116.2, 116.2, 111.0, 110.7, 60.7, 60.7, 56.1, 49.6, 47.3, 37.8, 36.7, 31.5, 28.2, 25.5, 23.3, 22.3, 14.4. With variant **P411-CHF**: **$[\alpha]^{23}_D$** = –2.980 ± 0.898° (*c* 1.0, $CHCl_3$). With variant **P411-CHF**: **SFC Chiralcel OB-H column** (3% *i*-PrOH supercritical $CO_2$, 2.5 mL/min, 40 °C), $t_r$ = 6.47 min (major), 7.38 min (minor), 73.0 : 27.0 e.r.

**Benzyl 3-(methyl(*p*-tolyl)amino)propanoate (10a)**

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ = 30) | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 55.0 | 28.0 | 2.04 | 0.170 | 5.0 | 10.0 |
| **Alkane substrate (7a) stock in EtOH** | | | **Diazo (9a) stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.50 | 1.0 | 0.50 | 1.00 | 0.50; 0.50 | 1.0 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / EtOAc (normal phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 19.0[c] | 0.061[d] | 12%[d] | 360[d] |

[c] Isolated with 10% diazo dimer.
[d] Corrected for diazo dimer.

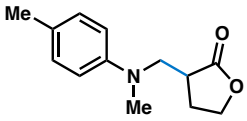



This compound is known in the literature.[44] **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 5H), 7.04 (d, *J* = 8.7 Hz, 2H), 6.66 (d, *J* = 8.8 Hz, 2H), 5.11 (s, 2H), 3.66 (t, *J* = 7.2 Hz, 2H), 2.87 (s, 3H), 2.61 (t, *J* = 7.2 Hz, 2H), 2.25 (s, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.4, 146.7, 135.9, 133.9, 129.9, 128.7, 128.4, 126.3, 113.2, 66.5, 49.1, 38.5, 31.8, 20.4. **HRMS** (ESI-TOF) m/z: 284.1635 ($M + H^+$); calc. for $C_{18}H_{22}NO_2$: 284.1651.

**3-((Methyl(*p*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (10b)**

This compound was prepared using **General Procedure D-II** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-IY T327I,** $OD_{600}$ = 55) | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Volume/ mL | | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 88.0 | | 6.77 | 0.595 | 0.0 | 10.0 |
| **Alkane substrate (7a) stock in EtOH** | | | **Diazo (9b) stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL | n_2/mmol |
| 1.0 | 1.0 | 1.0 | 0.50 | 1.0 | 0.50 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / EtOAc (normal phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 37.2 | 0.170 | 34% | 280 |

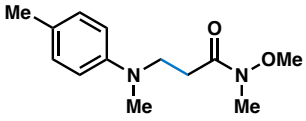



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.3 Hz, 2H), 6.68 (d, *J* = 8.6 Hz, 2H), 4.35 (td, *J* = 8.8, 2.6 Hz, 1H), 4.15 (ddd, *J* = 10.0, 9.1, 6.6 Hz, 1H), 3.94 (dd, *J* = 15.1, 4.5 Hz, 1H), 3.43 (dd, *J* = 15.1, 8.0 Hz, 1H), 2.96 (s, 3H), 2.91 (dddd, *J* = 10.6, 8.6, 8.0, 4.5 Hz, 1H), 2.34 (dddd, *J* = 12.7, 8.9, 6.6, 2.6 Hz, 1H), 2.26 (s, 3H), 2.11 (dtd, *J* = 12.8, 10.2, 8.5 Hz, 1H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 178.5, 146.8, 130.0, 126.7, 113.1, 66.7, 53.3, 39.6, 38.7, 28.1, 20.4. **HRMS** (FAB) m/z: 218.1172 ([(M + $H^+$)–$H_2$); calc. for $C_{13}H_{16}NO_2$: 218.1181. With variant **P411-IY T327I**: **HPLC Chiralcel OD-H column** (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, 32 °C), $t_r$ = 22.826 min (major), 24.286 min (minor), 78.0 : 22.0 e.r.

***N*-Methoxy-*N*-methyl-3-(methyl(*p*-tolyl)amino)propanamide (10c)**

This compound was prepared using **General Procedure D-II** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-IY T327I,** $OD_{600}$ = 55) | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Volume/ mL | | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 88.0 | | 7.00 | 0.616 | 0.0 | 10.0 |
| **Alkane substrate (7a) stock in EtOH** | | | **Diazo (9c) stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL | n_2/mmol |
| 1.0 | 1.0 | 1.0 | 0.50 | 1.0 | 0.50 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / EtOAc (normal phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 72.3 | 0.306 | 61% | 500 |



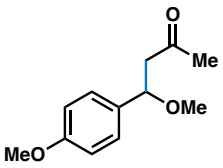

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.04 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.7 Hz, 2H), 3.70 – 3.64 (m, 2H), 3.62 (s, 3H), 3.17 (s, 3H), 2.92 (s, 3H), 2.66 (t, *J* = 7.2 Hz, 2H), 2.25 (s, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 173.2, 146.8, 129.9, 125.9, 112.8, 61.5, 48.8, 38.6, 32.2, 29.0, 20.4. **HRMS** (ESI-TOF) m/z: 237.1616 (M + $H^+$); calc. for $C_{13}H_{21}N_2O_2$: 237.1603.

**4-Methoxy-4-(4-methoxyphenyl)butan-2-one (10d)**

This compound was prepared using **General Procedure D-II** and Workup Procedure D-*i*.

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ = 60)[c] | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Volume/ mL | | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 88.0 | | 8.17 | 0.719 | 0.0 | 10.0 |
| **Alkane substrate (1a) stock in EtOH** | | | **Diazo (9d) stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL | n_2/mmol |
| 1.0 | 1.0 | 1.0 | 0.50 | 1.0 | 0.50 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / EtOAc (normal phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 21.8 | 0.105 | 21% | 150 |

[c] Enzyme was expressed using a modified procedure. After addition of 5-aminolevulinic acid (1.0 mM final concentration) and IPTG (0.5 mM final concentration), the incubator temperature was set to 24 °C, and the culture was allowed to shake for 20 hours at 140 rpm. Product formation was also observed when cultures were expressed following the typical protocol.




**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.58 (dd, *J* = 8.8, 4.5 Hz, 1H), 3.81 (s, 3H), 3.17 (s, 3H), 2.97 (dd, *J* = 15.8, 8.8 Hz, 1H), 2.58 (dd, *J* = 15.8, 4.5 Hz, 1H), 2.15 (s, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 206.9, 159.4, 133.0, 127.9, 114.1, 79.3, 56.6, 55.4, 52.0, 31.2. **HRMS** (ESI-TOF) m/z: 231.0999 ($M + Na^+$); calc. for $C_{12}H_{16}O_3Na$: 231.0997. **HPLC Chiralcel OJ-H column** (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, 28 °C), $t_r$ = 20.152 min (major), 21.760 min (minor), 71.0 : 29.0 e.r.

## X. Syntheses of (+)-lyngbic acid and (+)-cuspareine

Experimental details for the formal synthesis of (*R*)-(+)-lyngbic acid and the total synthesis of (*R*)-(+)-cuspareine can be found below. Enzymatic C–H alkylation is the key stereo-defining step in the routes to both molecules.

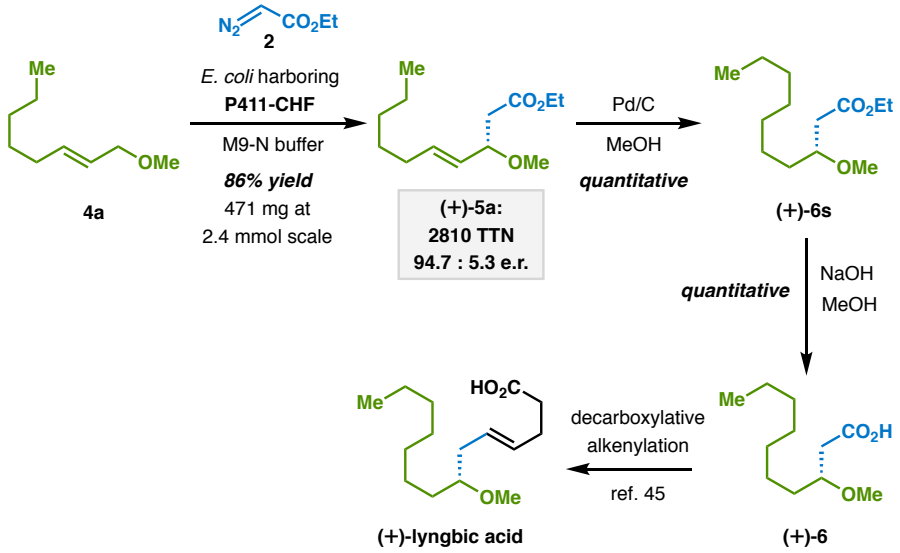



**Fig. S14.** Detailed scheme for the formal synthesis of (+)-lyngbic acid.

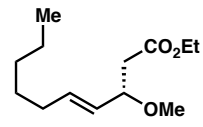
### Ethyl (*E*)-3-methoxydec-4-enoate (5a)

Prepared following **General Procedure D-I** and Workup Procedure D-*ii* (see **Section IX**).

| ***E. coli* suspension in M9-N (variant: P411-CHF,** $OD_{600}$ **= 29)** | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 220.0 | 112.0 | 2.21 | 0.734 | 20.0 | 40.0 |
| **Alkane substrate (4a) stock in EtOH** | | | **Ethyl diazoacetate (2) stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.60 | 4.0 | 2.4 | 1.2 | 2.0; 2.0 | 4.8 |
| **Purification eluent** | | **Product** | | | |
| **1:** Hex / (4 : 1 DCM : $Et_2O$) (normal phase) **2:** water / MeOH (reverse phase) | | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN |
| | | 470.6 | 2.061 | 86% | 2810 |

**Notes:** [PC] = protein concentration in original cell suspension, n_pro = amount of protein in the reaction, n_1 = amount of alkane substrate in the reaction, n_2 = total amount of diazo compound in the reaction, add. = addition, m[Pdt] = mass of product isolated, n[Pdt] = amount of product. [a] GOX oxygen depletion system is 14,000 U/mL catalase and 1,000 U/ mL glucose oxidase in 0.1 M potassium phosphate buffer (pH=8.0); the final reaction mixture contains 700 U/mL catalase and 50 U/mL glucose oxidase. [b] *D*-glucose stock solution is 250 mM; final concentration of *D*-

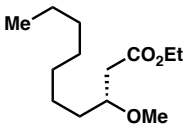
glucose in the reaction is 25 mM. These notes apply for all tables in this section.




**$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.69 (dt, *J* = 15.4, 6.8 Hz, 1H), 5.28 (ddt, *J* = 15.4, 8.3, 1.5 Hz, 1H), 4.14 (qd, *J* = 7.2, 0.8 Hz, 2H), 3.97 (td, *J* = 8.2, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, *J* = 14.9, 8.1 Hz, 1H), 2.42 (dd, *J* = 14.9, 5.5 Hz, 1H), 2.10 – 1.97 (m, 2H), 1.43 – 1.20 (m, 9H), 0.88 (t, *J* = 6.9 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.2, 135.6, 128.7, 79.0, 60.6, 56.2, 41.5, 32.3, 31.5, 28.9, 22.6, 14.4, 14.2. **HRMS** (EI) m/z: 228.1717 ($M^{+\bullet}$); calc. for $C_{13}H_{24}O_3$: 228.1726. **$[\alpha]^{23}_D$** = +7.504 ± 0.733° (*c* 0.5, $CHCl_3$). **GC CycloSil–B column** (110 °C), $t_r$ = 53.72 min (minor), 55.35 min (major), 94.7 : 5.3 e.r. Absolute stereochemistry was assigned after elaboration to (+)-**6**.

**Ethyl 3-methoxydecanoate** (**6s**)

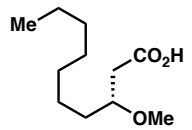
To a solution of ethyl (*E*)-3-methoxydec-4-enoate (**5a**, 114.2 mg, 0.5 mmol, 1.0 equiv.) in MeOH (5 mL) was added Pd/C (10% Pd on activated charcoal, 13.3 mg, 2.5 mol%). $H_2$ was bubbled through the solution for ~30 minutes. The reaction was stirred at room temperature under 1 atm $H_2$ for 16 hours. The crude product was filtered through a pad of Celite and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded **6s** (116.9 mg, 0.5 mmol, quantitative yield).




**$^1$H NMR** (400 MHz, $CDCl_3$) δ 4.15 (q, *J* = 7.1 Hz, 2H), 3.63 (ddt, *J* = 7.3, 6.3, 5.5 Hz, 1H), 3.35 (s, 3H), 2.52 (dd, *J* = 15.0, 7.3 Hz, 1H), 2.39 (dd, *J* = 15.0, 5.4 Hz, 1H), 1.57 – 1.41 (m, 2H), 1.40 – 1.20 (m, 13H), 0.88 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.0, 78.0, 60.5, 57.1, 39.7, 34.1, 31.9, 29.8, 29.4, 25.2, 22.8, 14.4, 14.2. **HRMS** (FAB) m/z: 231.1965 ($M + H^+$); calc. for $C_{13}H_{27}O_3$: 231.1960. **$[\alpha]^{23}_D$** = +4.293 ± 0.136° (*c* 1.0, MeOH). Absolute stereochemistry was assigned after derivatization to (+)-**6**.

**3-Methoxydecanoic acid** (**6**)

To a solution of ethyl 3-methoxydecanoate (**6s**, 46.1 mg, 0.2 mmol, 1.0 equiv.) in MeOH (2 mL) was added NaOH (aq., 15%, 2 mL). The reaction mixture was stirred at room temperature for 1 hour and then slowly acidified with HCl (aq., 1 M) at 0 °C until pH 2–3. Extraction by dichloromethane (15 mL × 3), drying over magnesium sulfate, followed by concentration under reduced pressure afforded product **6** (40.8 mg, 0.2 mmol, quantitative yield) without further purification.




This compound is known in the literature[46]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 3.63 (p, *J* = 6.0 Hz, 1H), 3.39 (s, 3H), 2.55 (dd, *J* = 15.5, 6.9 Hz, 1H), 2.50 (dd, *J* = 15.5, 5.3 Hz, 1H), 1.68 – 1.55 (m, 1H), 1.55 – 1.42 (m, 1H), 1.38 – 1.20 (m, 10H), 0.88 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 176.2, 77.7, 57.1, 39.1, 33.6, 31.9, 29.7, 29.4, 25.1, 22.8, 14.2. **$[\alpha]^{23}_D$** = +4.021 ± 1.649° (*c* 1.0, MeOH). The absolute configuration of (+)-**6** was assigned to be (*R*) by comparing the measured optical rotation value with the literature reported value for (*R*)-(+)-**6** (lit. $[\alpha]^{27}_D$ = +3.0°, *c* 0.67, MeOH)[46]. **HRMS** (EI) m/z: 202.1598 ($M^{\bullet+}$); calc. for $C_{11}H_{22}O_3$: 202.1569.

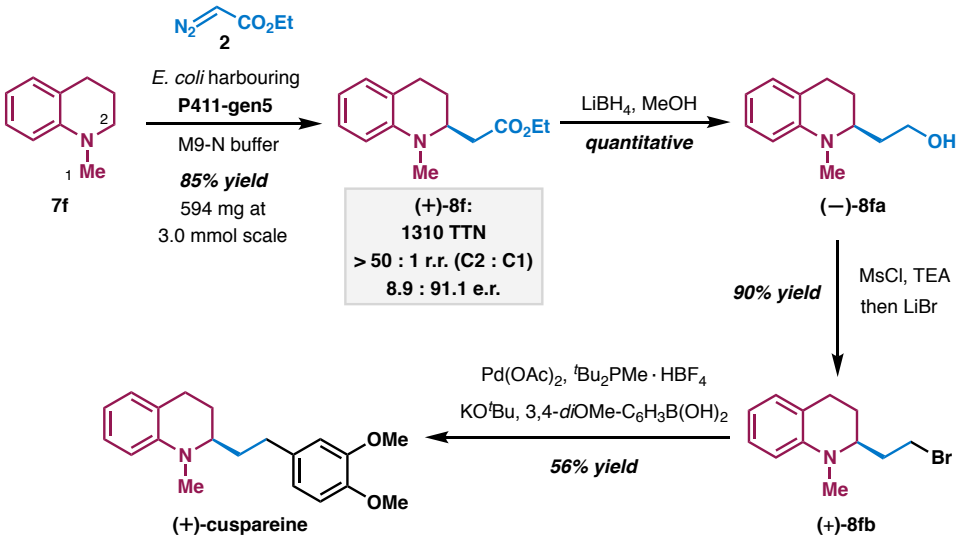


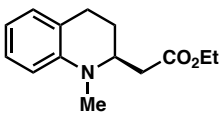

**Fig. S15.** Detailed scheme for the synthesis of (+)-cuspareine.

**Ethyl (*S*)-2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate ((+)-8f)**

Prepared following **General Procedure D-I** and Workup Procedure D-*ii* (see **Section IX**).

| ***E. coli* suspension in M9-N (variant: P411-gen5,** $OD_{600}$ = 31) | | | | **GOX solution**[a] | ***D*-glucose in M9-N**[b] |
|---|---|---|---|---|---|
| Addition 1, volume/ mL | Addition 2, volume/mL | [PC]/μM | n_pro/μmol | volume/mL | volume/mL |
| 275.0 | 140.0 | 4.67 | 1.938 | 25.0 | 50.0 |
| **Alkane substrate (7f) stock in EtOH** | | | **Ethyl diazoacetate stock in EtOH** | | |
| stock/M | volume/mL | n_1/mmol | stock/M | volume/mL add. 1; add. 2 | n_2/mmol |
| 0.60 | 5.0 | 3.0 | 1.20 | 2.5; 2.5 | 6.0 |
| **Purification eluent** | **Product** | | | | |
| **1:** Hex / (4 : 1 DCM : $Et_2O$) **2:** water / MeOH (reverse phase) | m[Pdt]/mg | n[Pdt]/mmol | yield | TTN | |
| | 594.1[c] | 2.546 | 85% | 1310 | |

[c]Regioisomer ratio > 50 : 1 for **8f** : **8f'** by $^1$H NMR

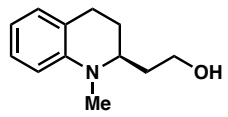



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.14 – 7.04 (m, 1H), 6.99 (d, *J* = 7.3 Hz, 1H), 6.63 (td, *J* = 7.3, 1.1 Hz, 1H), 6.55 (d, *J* = 8.2 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.86 – 3.78 (m, 1H), 2.93 (s, 3H), 2.91 – 2.79 (m, 1H), 2.71 (ddd, *J* = 16.6, 5.5, 2.8 Hz, 1H), 2.60 (ddd, *J* = 14.7, 5.4, 0.8 Hz, 1H), 2.39 (dd, *J* = 14.7, 8.6 Hz, 1H), 2.07 – 1.95 (m, 1H), 1.89 (ddt, *J* = 13.4, 5.8, 2.9 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 172.3, 144.8, 129.0, 127.3, 121.6, 116.2, 111.0, 60.7, 56.1, 37.8, 36.6, 25.5, 23.3, 14.4. **HRMS** (FAB) m/z: 233.1405 ($M^{+\cdot}$); calc. for $C_{14}H_{19}NO_2$: 233.1416. With variant **P411-gen5**: **$[\alpha]^{23}_D$** = +9.440 ± 0.292° (*c* 0.5, $CHCl_3$). With variant **P411-gen5**: **SFC Chiralcel OB-H column** (supercritical $CO_2$/isopropanol = 97:3, 2.5 mL/min, 40 °C), $t_r$ = 6.44 min (minor), 7.25 min (major), 8.9 : 91.1 e.r. Absolute stereochemistry was assigned after elaboration

to (+)-cuspareine.

**2-(1-Methyl-1,2,3,4-tetrahydroquinolin-2-yl)ethan-1-ol (8fa)**

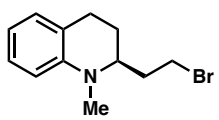
To a solution of ethyl 2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate (**8f**, 233.3 mg, 1.0 mmol, 1.0 equiv.) and MeOH (60.8 μL, 1.5 mmol, 1.5 equiv.) in dry diethyl ether (20 mL) was added $LiBH_4$ (2 M in THF, 1.0 mL, 2.0 mmol, 2.0 equiv.) dropwise. The reaction mixture was heated to reflux (~45 °C) for 4 hours (monitored by TLC). Upon completion, the reaction was quenched by ethyl acetate (2 mL) and stirred for 30 min at room temperature, quenched by MeOH (2 mL) and stirred for another 20 min at 0 °C, and finally quenched by $NH_4Cl$ (sat. aq., 5 mL) at 0 °C. The crude product was then extracted by diethyl ether (30 mL × 3). The combined organic layer was washed by brine (30 mL), dried over sodium sulfate and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded **8fa** (192.1 mg, 1.0 mmol, quantitative yield).




This compound is known in the literature[47]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.12 – 7.06 (m, 1H), 7.01 – 6.96 (m, 1H), 6.62 (td, *J* = 7.3, 1.2 Hz, 1H), 6.59 (dd, *J* = 8.2, 1.1 Hz, 1H), 3.82 – 3.71 (m, 2H), 3.51 – 3.44 (m, 1H), 2.97 (s, 3H), 2.88 – 2.76 (m, 1H), 2.70 (ddd, *J* = 16.4, 5.3, 3.4 Hz, 1H), 2.00 – 1.81 (m, 3H), 1.72 – 1.61 (m, 2H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 145.4, 129.0, 127.3, 122.2, 116.2, 112.0, 60.6, 56.2, 39.1, 34.6, 24.8, 23.8. **$[\alpha]^{23}_D$** = –28.096 ± 2.416° (*c* 0.5, $CHCl_3$). Absolute stereochemistry was assigned after derivatization to (+)-cuspareine.

**2-(2-Bromoethyl)-1-methyl-1,2,3,4-tetrahydroquinoline (8fb)**

To a solution of (1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)methanol (**8fa**, 114.8 mg, 0.6 mmol, 1.0 equiv.) and triethylamine (133.8 μL, 0.96 mmol, 1.6 equiv.) in dry THF (12 mL) at 0 °C was added methanesulfonyl chloride (MsCl, 70 μL, 1.5 equiv.). The reaction mixture was warmed to room temperature over 30 min and stirred for additional 30 min. Upon completion, the reaction was quenched by $NaHCO_3$ (sat. aq., 10 mL) and $H_2O$ (10 mL). The mesylated product was then extracted by diethyl ether (30 mL × 3). The combined organic layer was washed by brine (30 mL), dried over sodium sulfate and concentrated under reduced pressure. The mesylated product was then dissolved in DMF (6 mL) and LiBr (259.5 mg, 3.0 mmol, 5.0 equiv.) was added. The reaction was heated to 70 °C and stirred for 2 hours. Upon completion, the reaction was quenched by $H_2O$ (30 mL). The crude product was then extracted by diethyl ether (30 mL × 3). The combined organic layer was washed by $H_2O$ (30 mL) and brine (30 mL), dried over sodium sulfate and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded **8fb** (136.8 mg, 0.54 mmol, 90% yield).

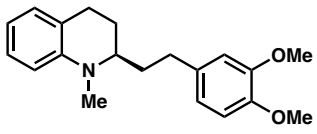



**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.12 – 7.06 (m, 1H), 7.01 – 6.95 (m, 1H), 6.61 (td, *J* = 7.3, 1.2 Hz, 1H), 6.56 (dd, *J* = 8.2, 1.1 Hz, 1H), 3.58 – 3.47 (m, 2H), 3.43 (ddd, *J* = 10.1, 7.7, 6.3 Hz, 1H), 2.99 (s, 3H), 2.84 – 2.66 (m, 2H), 2.22 – 2.11 (m, 1H), 2.01 – 1.90 (m, 2H), 1.90 – 1.81 (m, 1H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 145.1, 129.0, 127.4, 121.5, 116.0, 111.2, 57.0, 38.7, 34.9, 31.0, 24.3, 23.6. **HRMS** (FAB) m/z: 254.0548 ($M + H^+$); calc. for $C_{12}H_{17}{}^{79}BrN$: 254.0544. **$[\alpha]^{23}_D$** = +33.638 ± 2.022° (*c* 0.5, $CHCl_3$). Absolute stereochemistry was assigned after derivatization to (+)-cuspareine.

(+)-**Cuspareine**

(+)-Cuspareine was synthesized through Suzuki-Miyaura cross-coupling between an alkyl bromide and an aryl boronic acid. The reaction conditions for this cross-coupling are derived from those described by Fu *et al.*[48].

To a 50-mL resealable Schlenk tube were added $Pd(OAc)_2$ (4.04 mg, 0.018 mmol, 15 mol%), di-*tert*-butyl(methyl)phosphonium tetrafluoroborate ($t$Bu$_2$PMe·HBF$_4$, 8.93 mg, 0.036 mmol, 30 mol%), KO*t*Bu (40.4 mg, 0.36 mmol, 3.0 equiv.) and *t*Amyl-OH (0.6 mL). The tube was charged with Ar and sealed. The mixture was stirred at 60 °C for 20 min until the color of mixture turned pale yellow. After the mixture was cooled to room temperature, (3,4-dimethoxyphenyl)boronic acid (54.6 mg, 0.30 mmol, 2.5 equiv.) and a solution of 2-(2-bromoethyl)-1-methyl-1,2,3,4-tetrahydroquinoline (**8fb**, 30.5 mg, 0.12 mmol, 1.0 equiv.) in *t*Amyl-OH (0.6 mL) were added to the tube. Another portion of *t*Amyl-OH (0.8 mL) was used to wash the solution residue of **8fb** and then transferred to the tube. The tube was charged with Ar and sealed again. After the reaction mixture was stirred at 60 °C for 15 hours, it was then cooled to room temperature, diluted with diethyl ether (6 mL), filtrated through a pad of Celite, washed by diethyl ether (30 mL) and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded (+)-cuspareine (20.8 mg, 0.067 mmol, 56% yield).




Spectral data is in agreement with literature report[49]. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.09 (td, $J$ = 7.6, 1.4 Hz, 1H), 6.99 (d, $J$ = 7.0 Hz, 1H), 6.80 (d, $J$ = 8.0 Hz, 1H), 6.75 – 6.70 (m, 2H), 6.60 (td, $J$ = 7.3, 1.1 Hz, 1H), 6.54 (d, $J$ = 8.2 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.33 – 3.26 (m, 1H), 2.92 (s, 3H), 2.91 – 2.80 (m, 1H), 2.74 – 2.63 (m, 2H), 2.54 (ddd, $J$ = 13.9, 10.1, 6.4 Hz, 1H), 2.01 – 1.87 (m, 3H), 1.74 (dddd, $J$ = 13.6, 10.1, 8.8, 5.4 Hz, 1H). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 149.0, 147.3, 145.4, 134.8, 128.8, 127.2, 121.8, 120.2, 115.5, 111.7, 111.4, 110.7, 58.5, 56.1, 56.0, 38.2, 33.2, 32.1, 24.5, 23.7. **$[\alpha]^{23}_D$** = +23.404 ± 0.723° (*c* 0.5, $CHCl_3$). The absolute configuration of (+)-cuspareine was assigned to be (*R*) by comparing the measured optical rotation value with the literature reported value for (*R*)-(+)-cuspareine (lit. $[\alpha]^{27}_D$ = +23.516°, *c* 0.8, $CHCl_3$)[49].

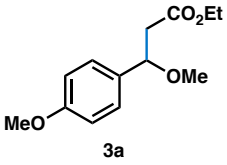
## XI. Determination of enantioselectivity

Enantioselectivity of enzymatic C–H alkylation products were determined by chiral HPLC, chiral SFC, or chiral GC analysis. Representative traces, and their conditions, are shown below.

The absolute configuration of P411-CHF synthesized **3i** was assigned to be (*S*) by comparing the chiral HPLC separation of *rac*-**3i** and P411-CHF synthesized **3i** with that reported in the literature[50]. The absolute configuration was further confirmed by comparing the optical rotation values for P411-CHF synthesized **3i** and (*S*)-(+)-**3i** as reported in the literature[50]. The absolute configurations of **3a**–**3h** and **3j**–**3m** were inferred by analogy, assuming the selectivity with P411-CHF remains the same in the enzymatic syntheses of those compounds.

The absolute configuration of P411-CHF synthesized **5a** was determined to be (*S*) after elaboration to (+)-**6** (see **Section X** for more details). The absolute configurations of **5b**–**5e** were inferred by analogy, assuming the selectivity with P411-CHF remains the same in the enzymatic syntheses of those compounds.

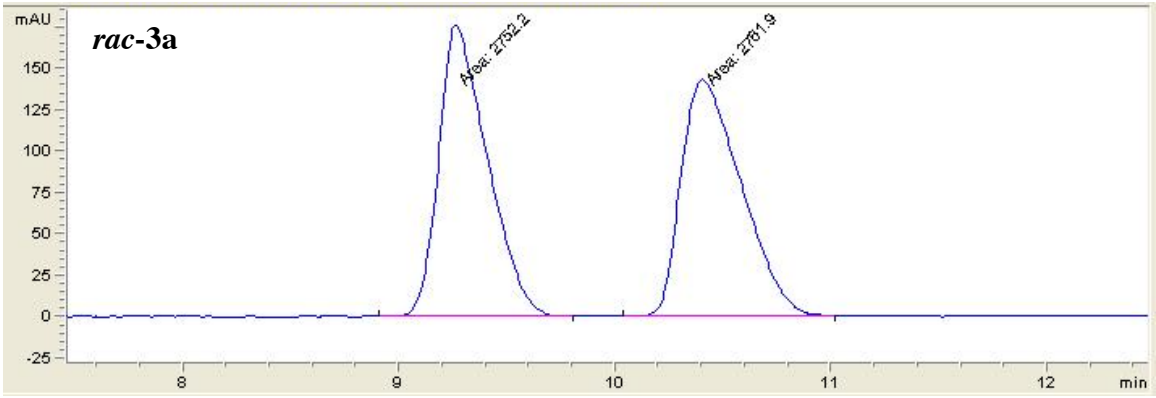
The absolute configuration of P411-gen5 synthesized **8f** was determined to be (*S*) after elaboration to (+)-cuspareine (see **Section X** for more details). As P411-CHF shows the opposite selectivity for the synthesis of **8f**, P411-CHF produces (*R*)-**8f** in excess.

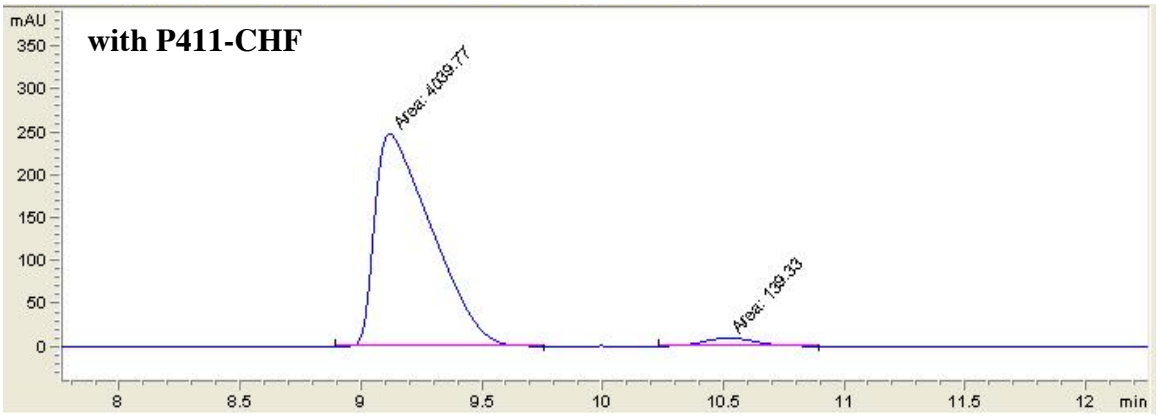


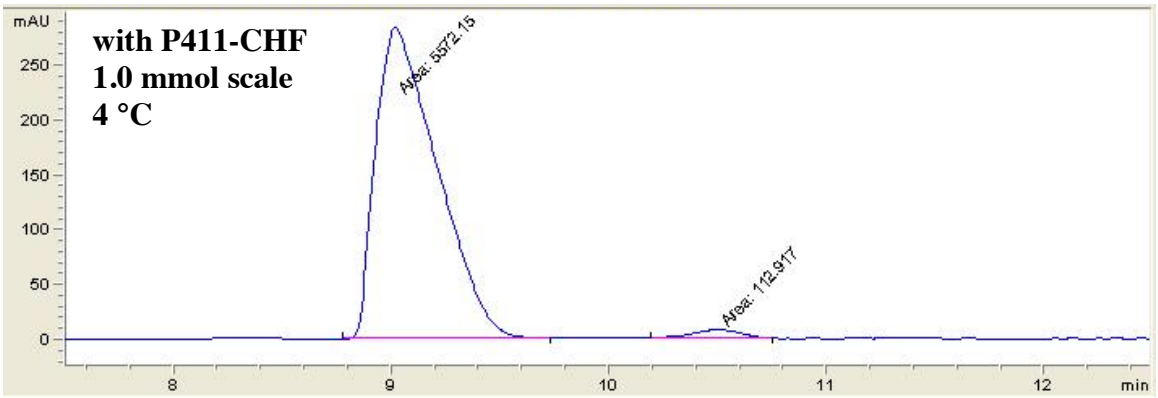

3a

**Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (3a)**

SFC (Chiralpak AD-H column): 3% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 235 nm.

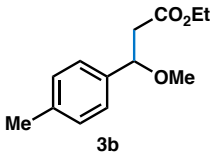




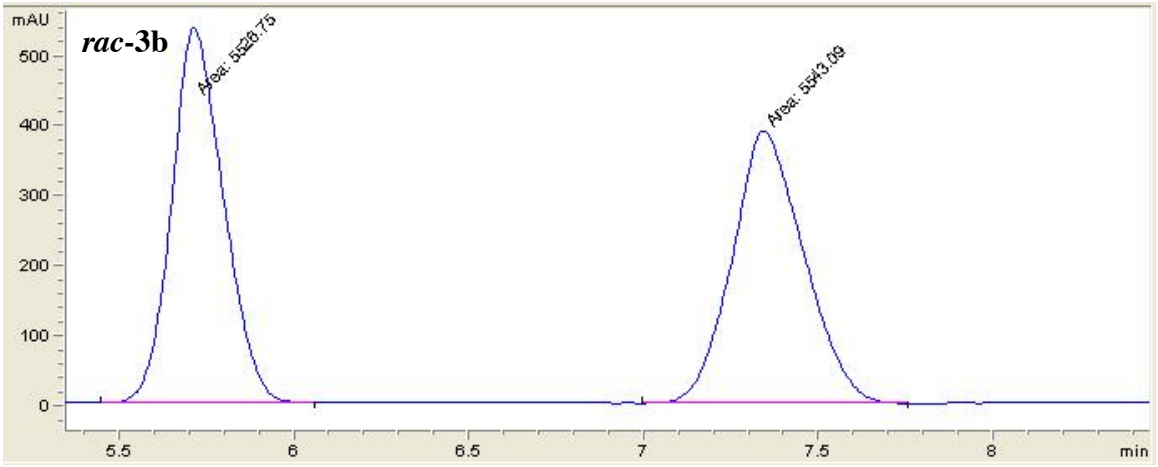

| ***rac*-3a** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 9.262<br>10.404 | 2752.2<br>2761.9 | 49.91<br>50.09 | 9.119<br>10.524 | 4039.8<br>139.3 | 96.67<br>3.33 |
| Total | 5514.1 | 100.00 | Total | 4179.1 | 100.00 |

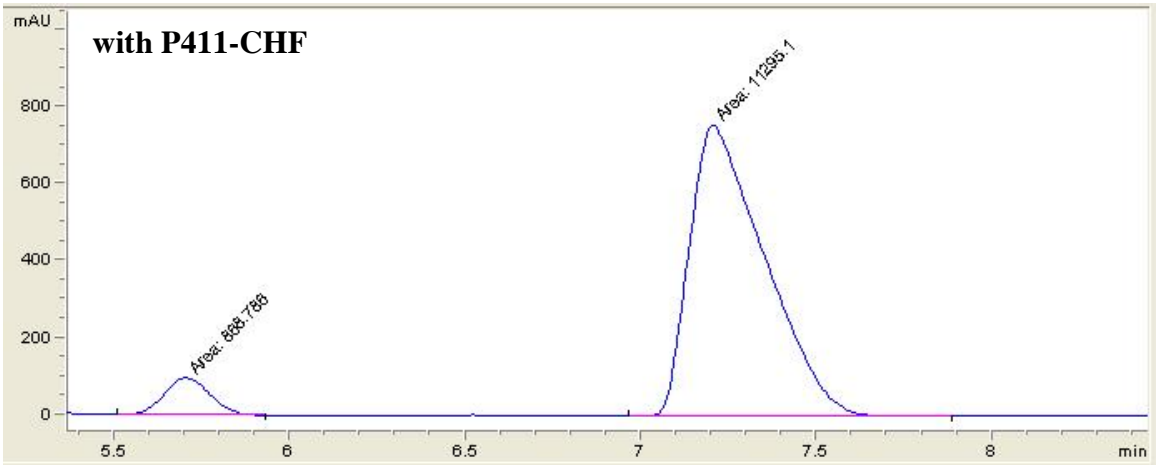
| **with P411-CHF, 1.0 mmol scale, 4 °C** | | |
|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % |
| 9.016<br>10.501 | 5572.2<br>112.9 | 98.01<br>1.99 |
| Total | 5685.1 | 100.00 |



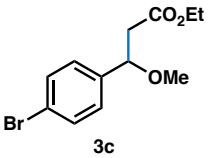

**Ethyl 3-methoxy-3-(*p*-tolyl)propanoate (3b)**

SFC (Chiralpak AD-H column): 2% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm.



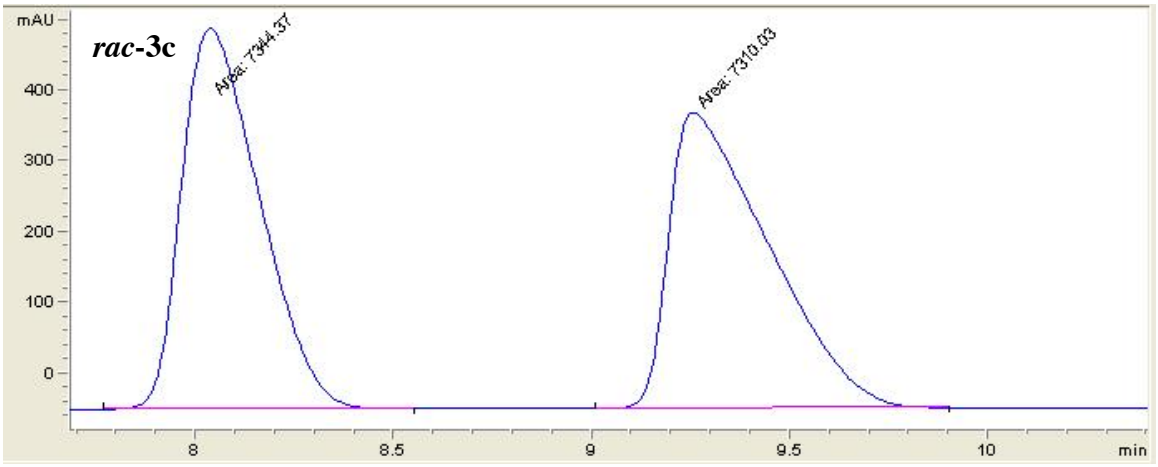



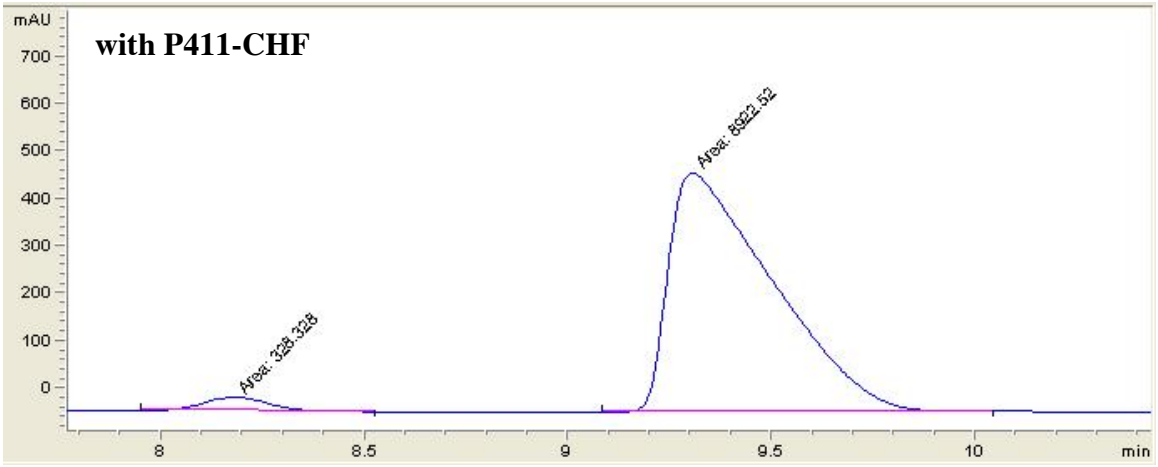

| ***rac*-3b** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 5.712<br>7.342 | 5526.8<br>5543.1 | 49.93<br>50.07 | 5.705<br>7.205 | 868.8<br>11295.1 | 7.14<br>92.86 |
| Total | 11069.9 | 100.00 | Total | 12163.9 | 100.00 |



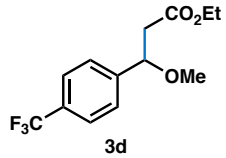

**Ethyl 3-(4-bromophenyl)-3-methoxypropanoate (3c)**

SFC (Chiralpak AD-H column): 3% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm.



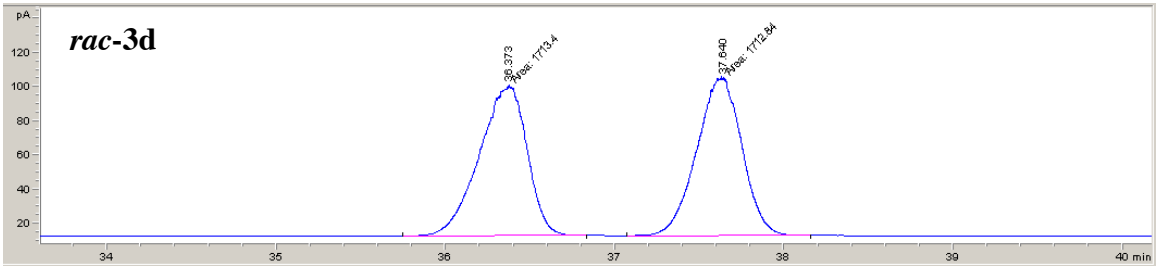



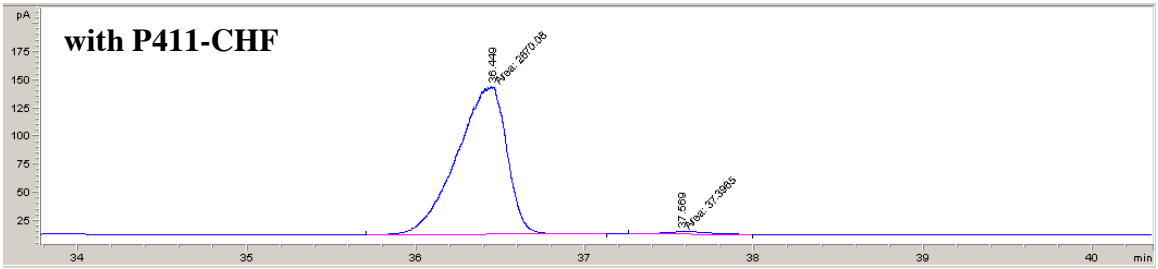

| ***rac*-3c** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 8.037<br>9.256 | 7344.4<br>7310.0 | 50.12<br>49.88 | 8.181<br>9.306 | 328.3<br>8922.5 | 3.55<br>96.45 |
| Total | 14654.4 | 100.00 | Total | 9250.8 | 100.00 |



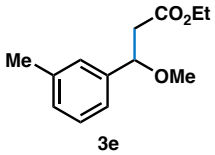

**Ethyl 3-methoxy-3-(4-(trifluoromethyl)phenyl)propanoate (3d)**

GC (Chiraldex G-TA column): 110 °C



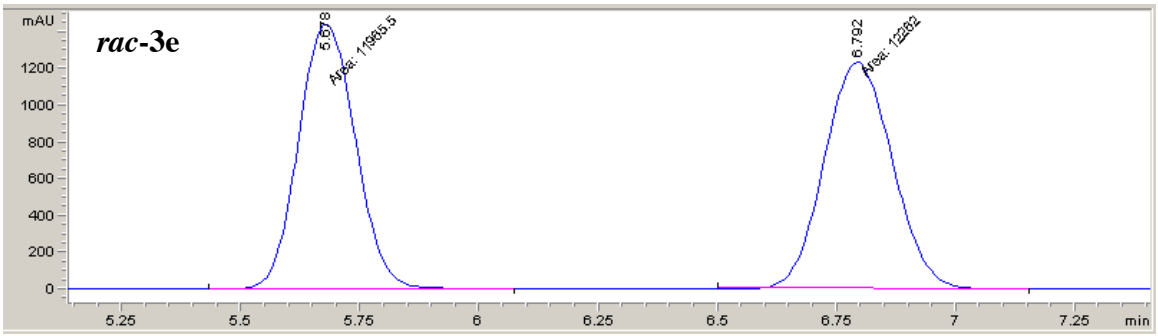

| ***rac*-3d** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 36.373<br>37.640 | 1713.4<br>1712.8 | 50.01<br>49.99 | 36.449<br>37.569 | 2670.1<br>37.4 | 98.62<br>1.38 |
| Total | 3426.2 | 100.00 | Total | 2707.5 | 100.00 |

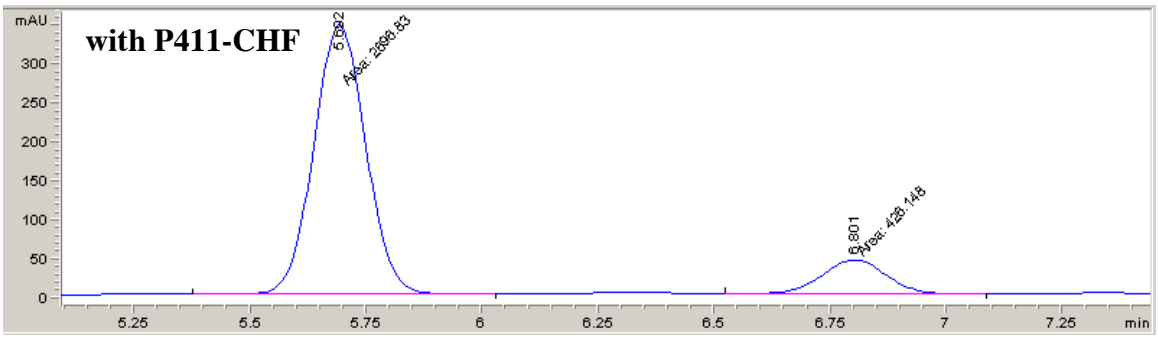



3e

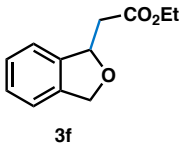
**Ethyl 3-methoxy-3-(*m*-tolyl)propanoate (3e)**

HPLC (Chiralpak IC column): 4% *i*-PrOH in hexane, 1.5 mL/min, 23 °C, 210 nm



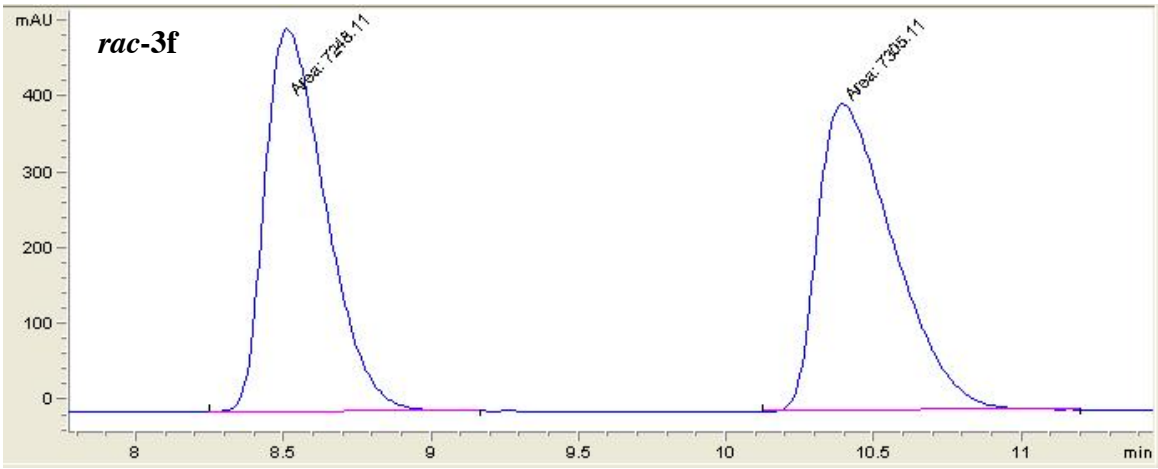

| ***rac*-3e** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 5.678 | 11965.5 | 49.39 | 5.692 | 2696.8 | 86.35 |
| 6.792 | 12262.0 | 50.61 | 6.801 | 426.1 | 13.65 |
| Total | 24227.5 | 100.00 | Total | 3122.9 | 100.00 |

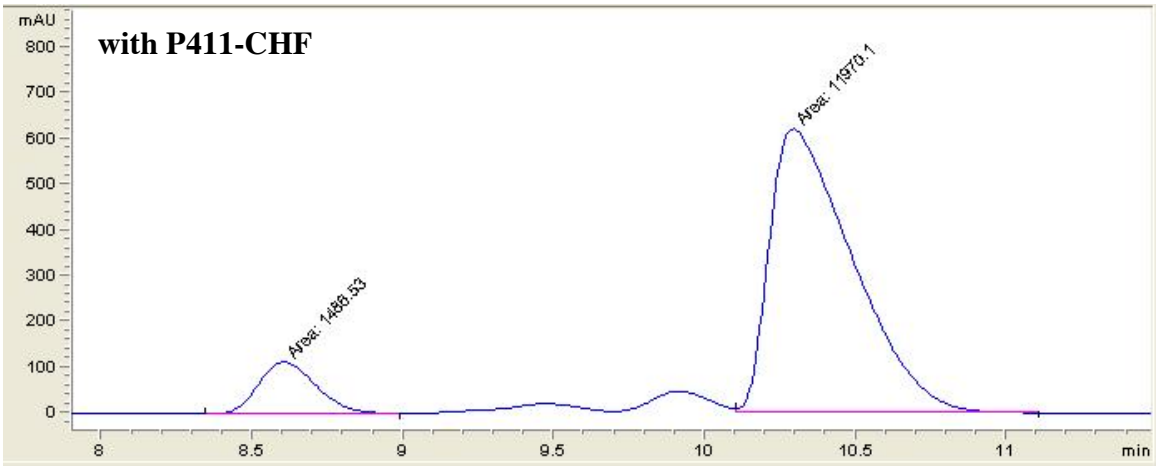



3f

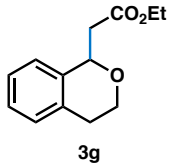
**Ethyl 2-(1,3-dihydroisobenzofuran-1-yl)acetate (3f)**

SFC (Chiralpak AD-H column): 3% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm



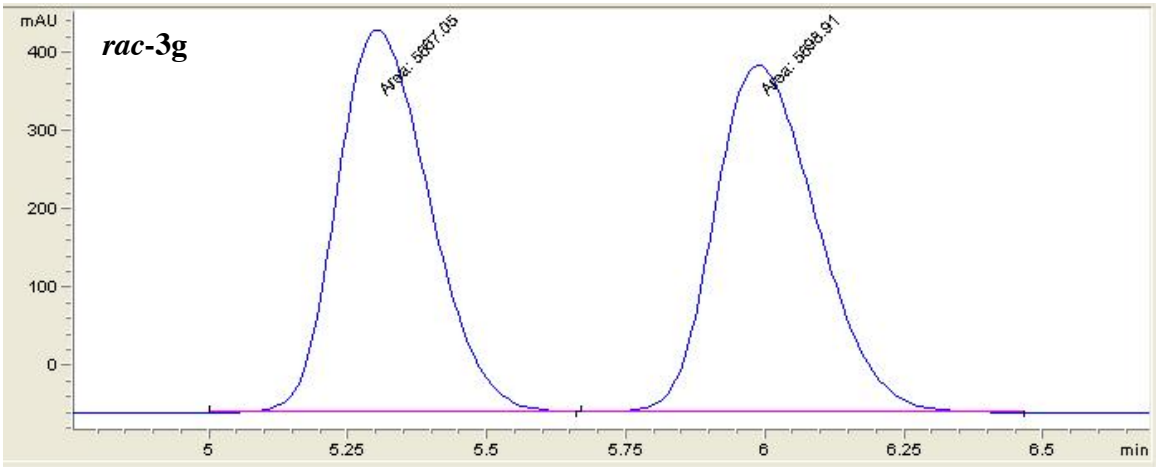

| ***rac*-3f** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 8.511<br>10.392 | 7248.1<br>7305.1 | 49.80<br>50.20 | 8.604<br>10.294 | 1486.5<br>11970.1 | 11.05<br>88.95 |
| Total | 14553.2 | 100.00 | Total | 13456.6 | 100.00 |

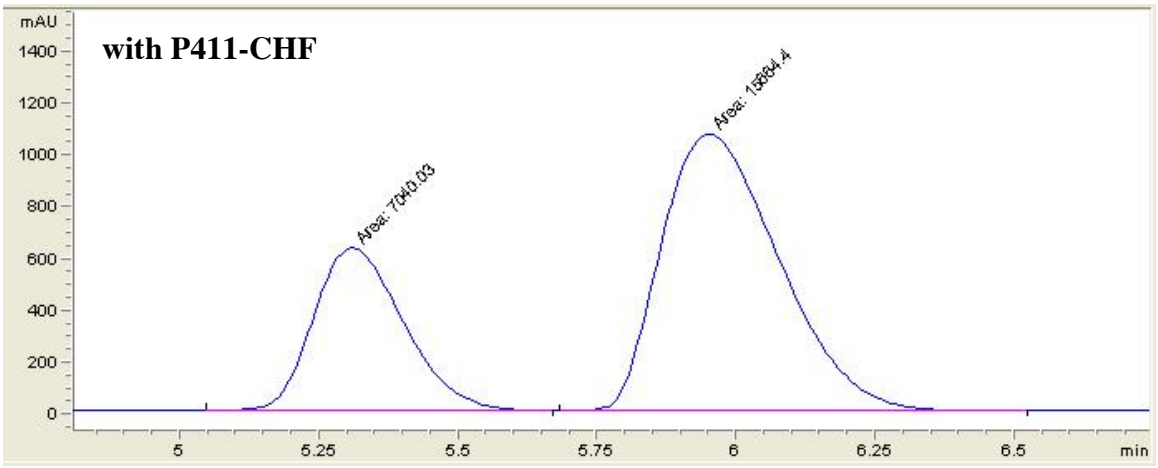



3g

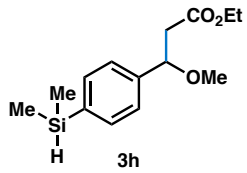
**Ethyl 2-(isochroman-1-yl)acetate (3g)**

SFC (Chiralcel OJ-H column): 0.2% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm



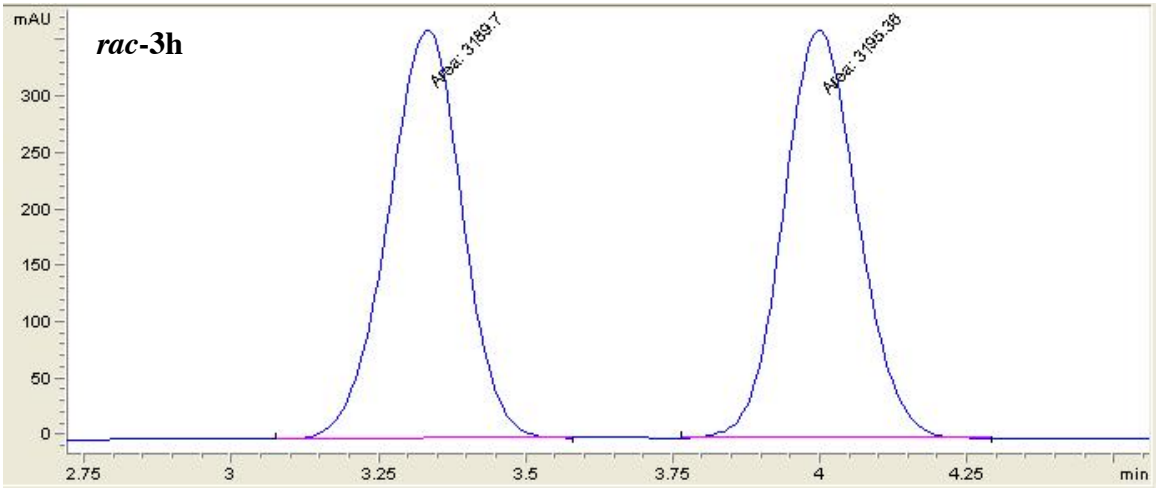



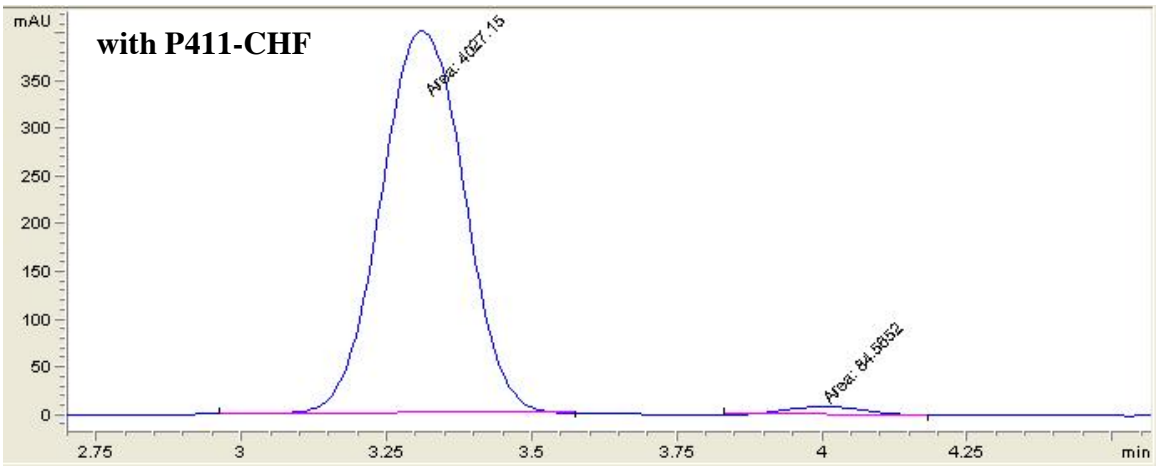

| ***rac*-3g** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 5.301 | 5667.0 | 49.86 | 5.309 | 7040.0 | 31.01 |
| 5.987 | 5698.9 | 50.14 | 5.951 | 15664.4 | 68.99 |
| Total | 11365.9 | 100.00 | Total | 22704.4 | 100.00 |



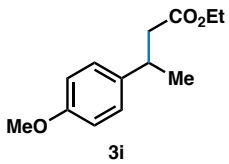

**Ethyl 3-(4-(dimethylsilyl)phenyl)-3-methoxypropanoate (3h)**

SFC (Chiralcel OD-H column): 2% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm



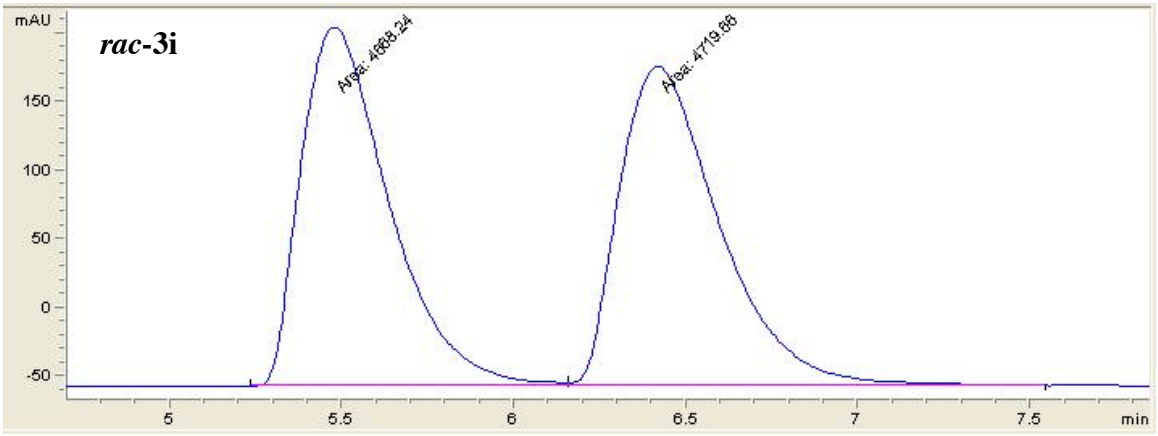

| ***rac*-3h** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 3.332 | 3189.7 | 49.96 | 3.309 | 4027.1 | 97.94 |
| 3.998 | 3195.4 | 50.04 | 3.997 | 84.6 | 2.06 |
| Total | 6385.1 | 100.00 | Total | 4111.7 | 100.00 |

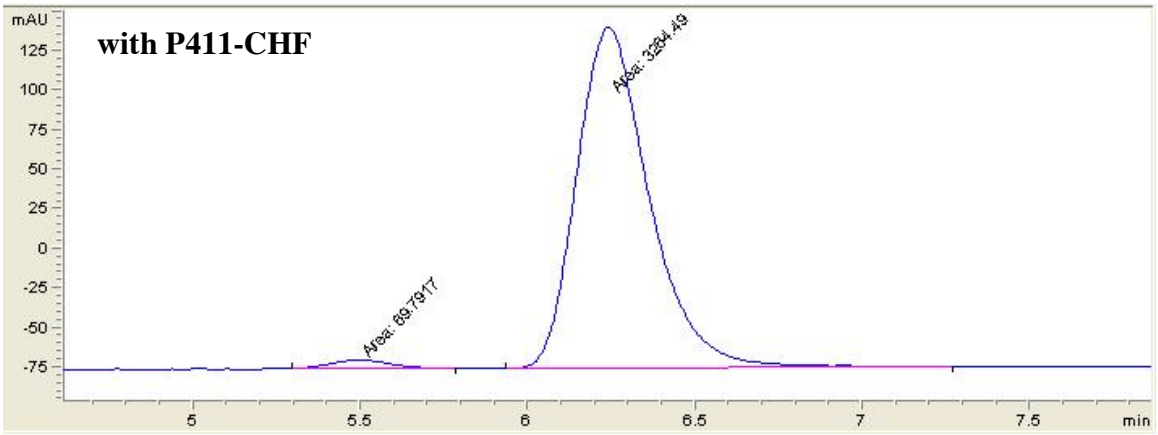



3i

**Ethyl 3-(4-methoxyphenyl)butanoate (3i)**

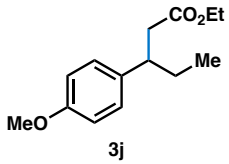
SFC (Chiralcel OB-H column): supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm.




| ***rac*-3i** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 5.478<br>6.417 | 4668.2<br>4719.7 | 49.73<br>50.27 | 5.496<br>6.240 | 69.8<br>3264.5 | 2.09<br>97.91 |
| Total | 9387.9 | 100.00 | Total | 3334.3 | 100.00 |

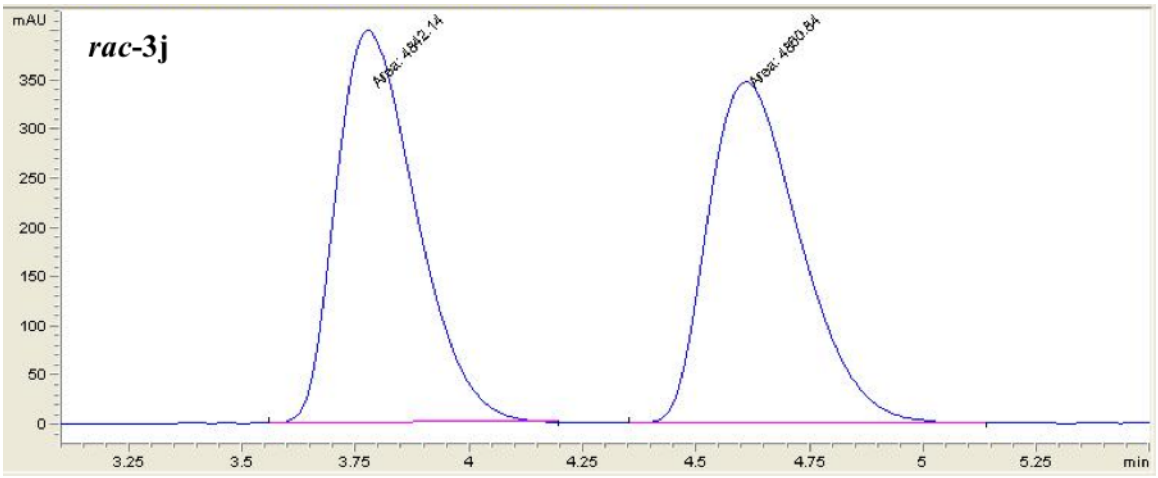
The absolute configuration of P411-CHF synthesized **3i** was determined to be (*S*) by comparing the chiral HPLC separation (Chiralcel OJ-H column, 1% *i*-PrOH in hexanes, traces not shown) of *rac*-**3i** and P411-CHF synthesized **3i** with that reported in the literature[50]. The absolute configuration was further confirmed by comparing the optical rotation value for P411-CHF synthesized **3i**, $[\alpha]^{23}_D$ = +26.3° (*c* 0.5, $CHCl_3$, see **Section XI**), and (*S*)-(+)-**3i** as reported in the literature ($[\alpha]^{23}_D$ = +26.2°, *c* 0.59, $CHCl_3$)[50].

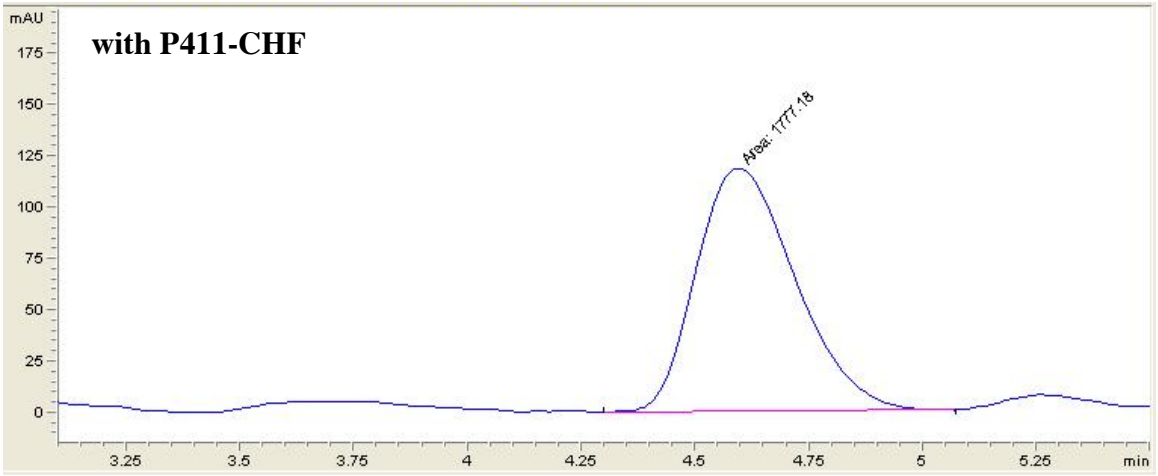



3j

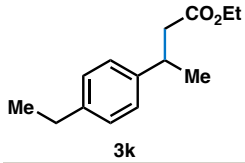
**Ethyl 3-(4-methoxyphenyl)pentanoate (3j)**

SFC (Chiralcel OB-H column): 1% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm.



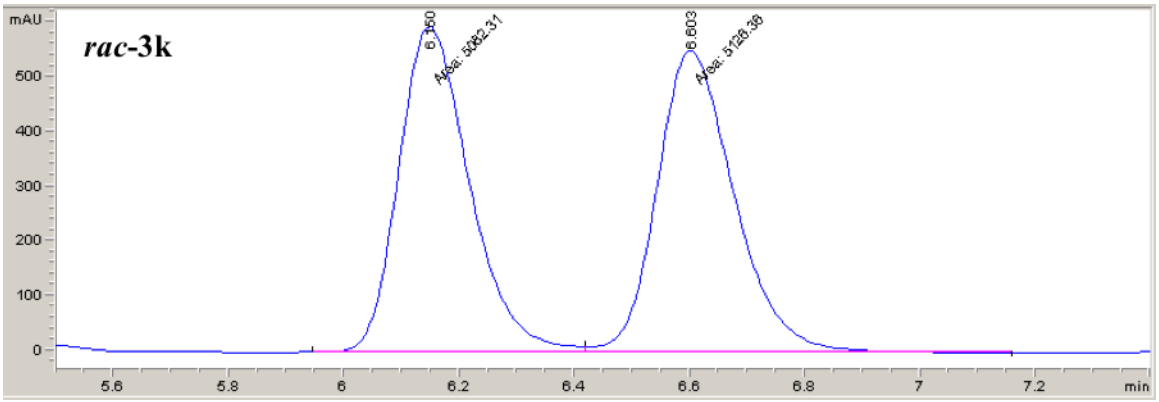

| ***rac*-3j** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 3.778<br>4.609 | 4842.1<br>4860.8 | 49.90<br>50.10 | 3.788<br>4.595 | n. d.<br>1777.2 | -<br>100.00 |
| Total | 9702.9 | 100.00 | Total | 1777.2 | 100.00 |

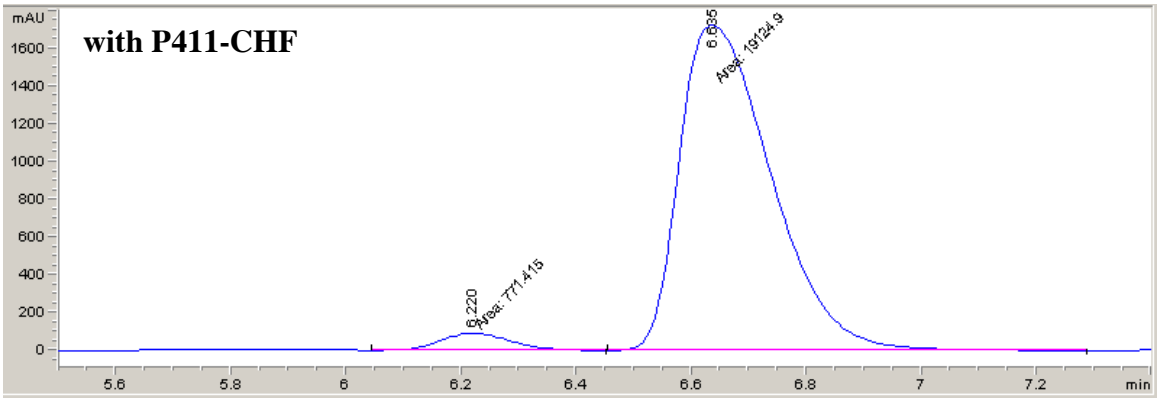



3k

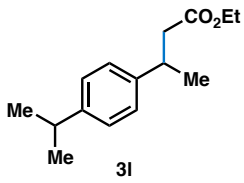
**Ethyl 3-(4-ethylphenyl)butanoate (3k)**

HPLC (Chiralcel OD-H column): 3% *i*-PrOH in hexane, 1.0 mL/min, 32 °C, 210 nm.



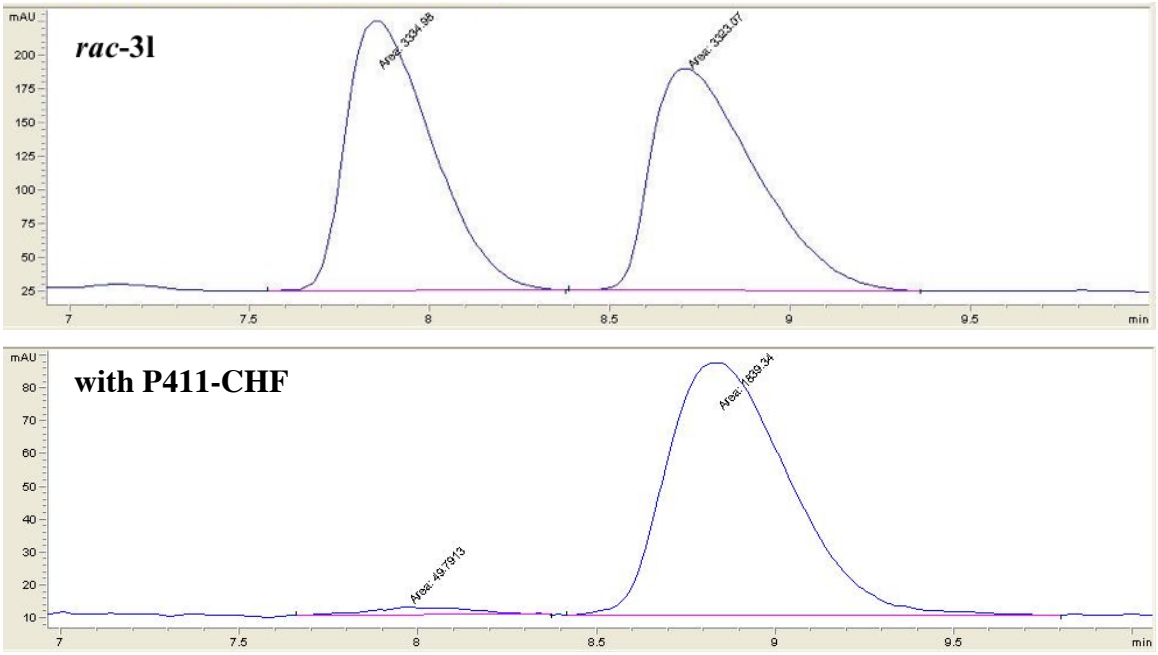

| ***rac*-3k** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 6.150 | 5082.3 | 49.78 | 6.220 | 771.4 | 3.88 |
| 6.603 | 5126.4 | 50.22 | 6.635 | 19124.9 | 96.12 |
| Total | 10208.7 | 100.00 | Total | 19896.3 | 100.00 |



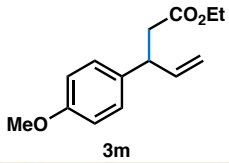

3l

**Ethyl 3-(4-isopropylphenyl)butanoate (3l)**

SFC (Chiralpak IC column): 0.5% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm.



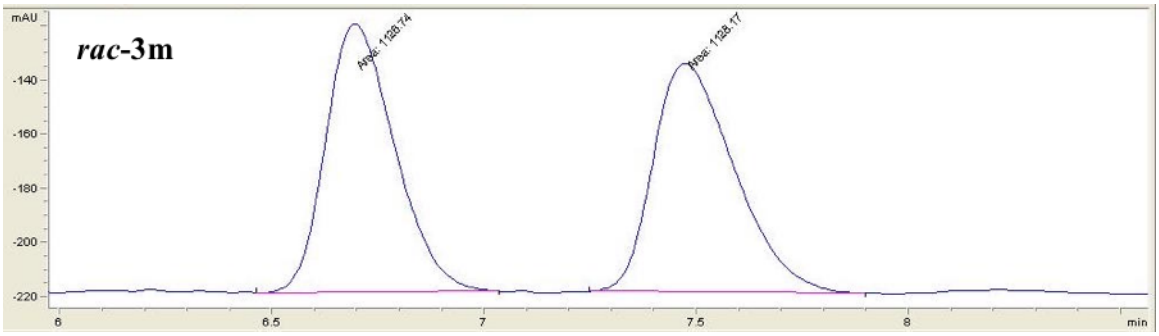

| ***rac*-3l** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 7.850<br>8.707 | 3335.0<br>3323.1 | 50.09<br>49.91 | 7.970<br>8.836 | 49.8<br>1839.3 | 2.64<br>97.36 |
| Total | 6658.1 | 100.00 | Total | 1889.1 | 100.00 |

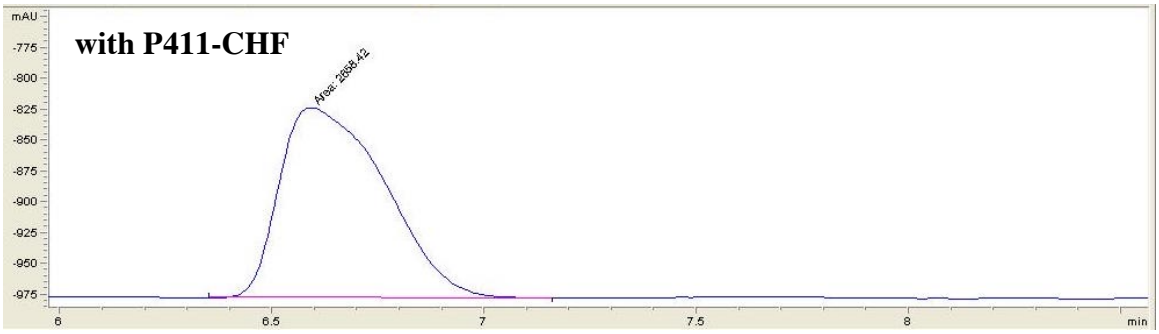



3m

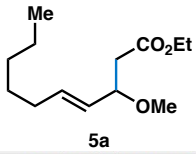
**Ethyl 3-(4-methoxyphenyl)pent-4-enoate (3m)**

SFC (Chiralpak AD-H column): 1% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 210 nm.



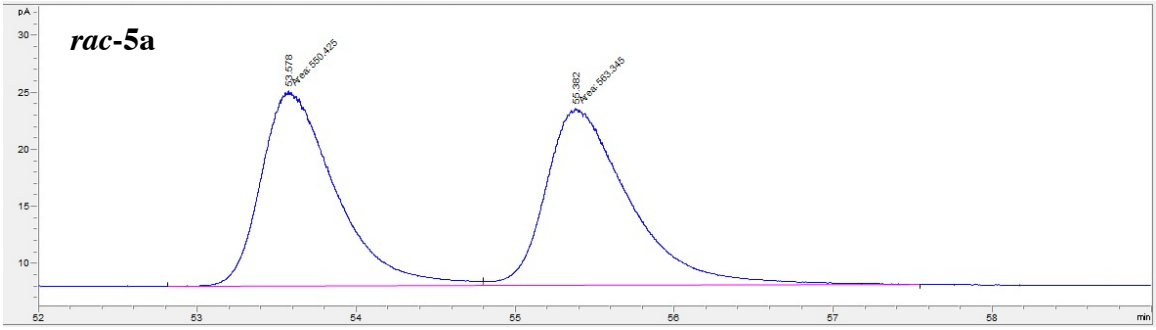

| ***rac*-3m** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 6.694<br>7.472 | 1126.7<br>1128.2 | 49.97<br>50.03 | 6.591<br>7.472 | 2658.4<br>n. d. | 100.00<br>- |
| Total | 2254.9 | 100.00 | Total | 2658.4 | 100.00 |

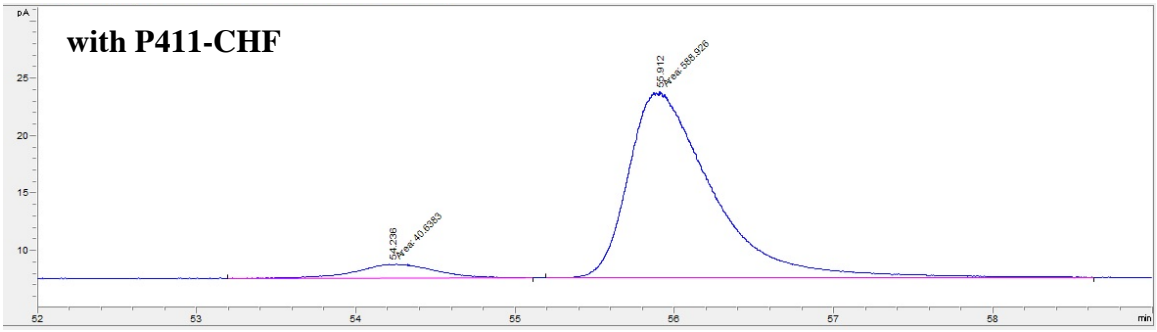


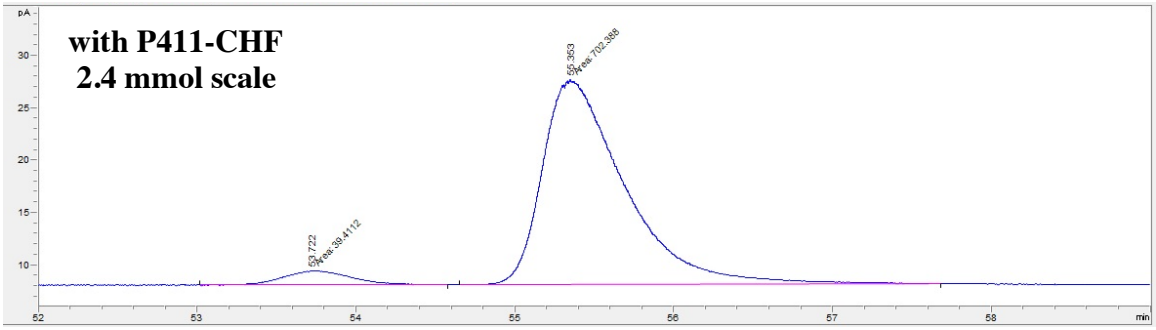

5a

**Ethyl (*E*)-3-methoxydec-4-enoate (5a)**

GC (CycloSil–B column): 110 °C



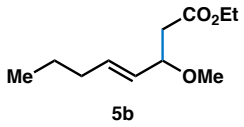




| ***rac*-5a** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 53.578 | 550.4 | 49.42 | 54.236 | 40.6 | 6.45 |
| 55.382 | 563.3 | 50.58 | 55.912 | 588.9 | 93.55 |
| Total | 1113.7 | 100.00 | Total | 629.5 | 100.00 |

| **with P411-CHF, 2.4 mmol scale** | | |
|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % |
| 53.722 | 39.4 | 5.31 |
| 55.353 | 702.4 | 94.69 |
| Total | 741.8 | 100.00 |

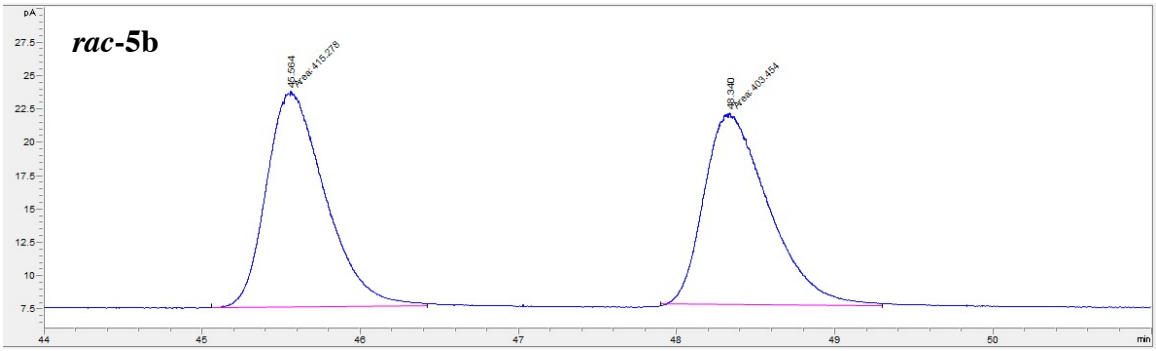
The absolute configuration of P411-CHF synthesized **5a** was determined to be (*S*) after derivatization to (+)-**6** (see **Section X** for more details).

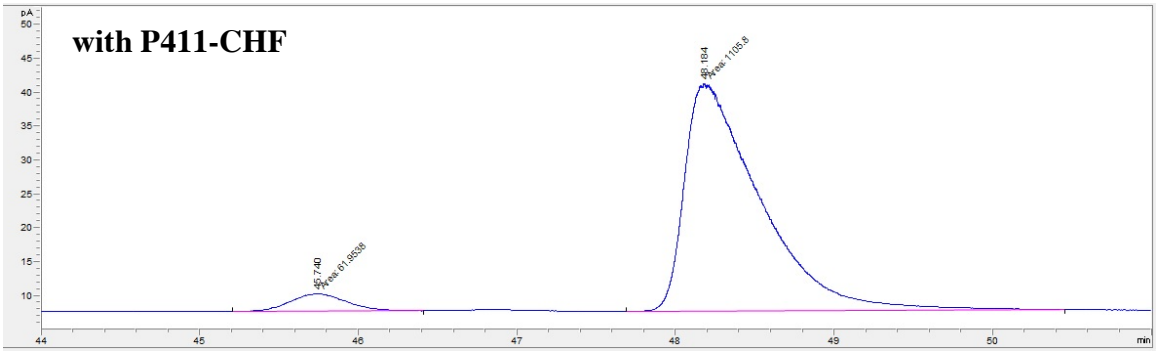



5b

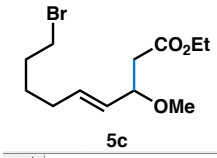
**Ethyl (*E*)-3-methoxyoct-4-enoate (5b)**

GC (CycloSil–B column): 90 °C



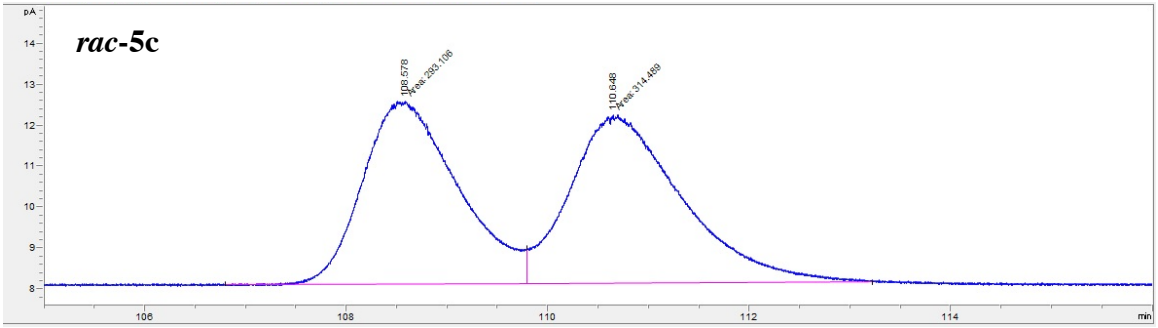

| ***rac*-5b** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 45.564<br>48.340 | 415.3<br>403.5 | 50.72<br>49.28 | 45.740<br>48.184 | 62.0<br>1105.8 | 5.31<br>94.69 |
| Total | 818.8 | 100.00 | Total | 1167.8 | 100.00 |

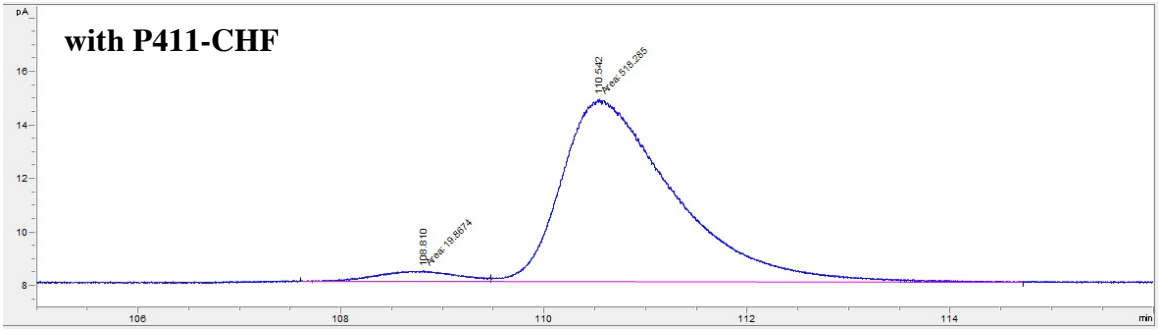



5c

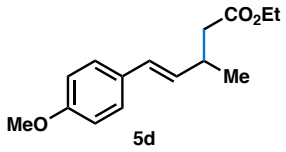
**Ethyl (*E*)-9-bromo-3-methoxynon-4-enoate (5c)**

GC (CycloSil–B column): 130 °C



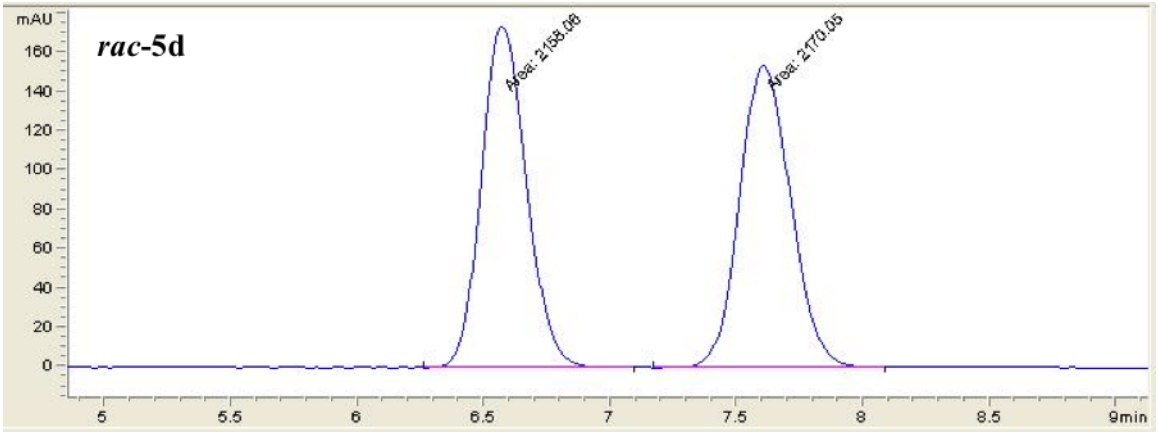



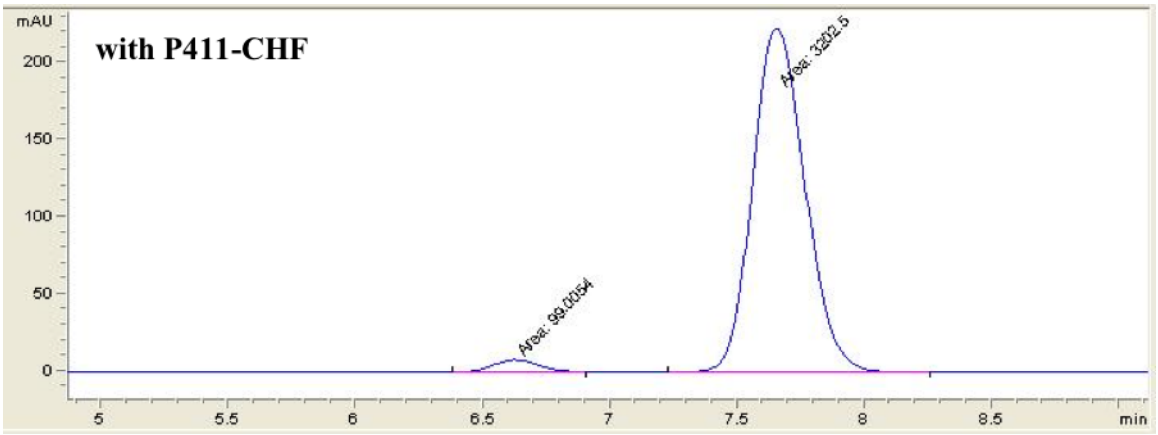

| ***rac*-5c** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 108.58<br>110.65 | 293.1<br>314.5 | 48.24<br>51.76 | 108.81<br>110.54 | 19.90<br>518.30 | 3.70<br>96.30 |
| Total | 607.6 | 100.00 | Total | 538.2 | 100.00 |



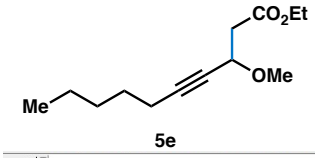

**Ethyl (*E*)-5-(4-methoxyphenyl)-3-methylpent-4-enoate (5d)**

SFC (Chiralcel OB-H column): 3% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 254 nm.



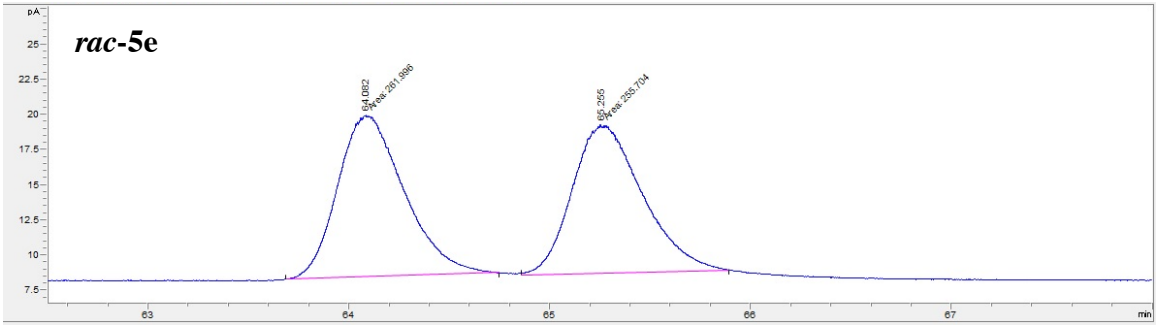

| ***rac*-5d** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 6.571<br>7.605 | 2158.1<br>2170.0 | 49.86<br>50.14 | 6.624<br>7.655 | 99.0<br>3202.5 | 3.00<br>97.00 |
| Total | 4328.1 | 100.00 | Total | 3301.5 | 100.00 |

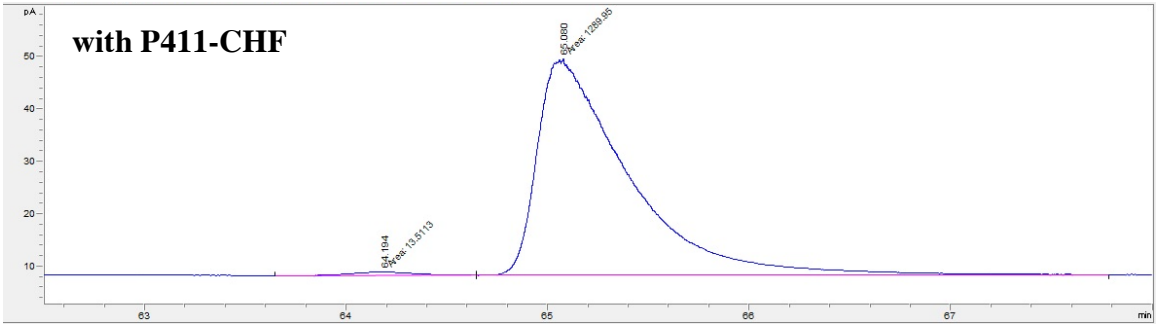



5e

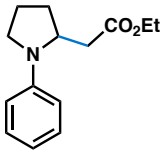
**Ethyl 3-methoxydec-4-ynoate (5e)**

GC (CycloSil–B column): 110 °C for 50 min., ramp 1 °C per minute for 10 min. (to 120 °C), 120 °C for 10 min.



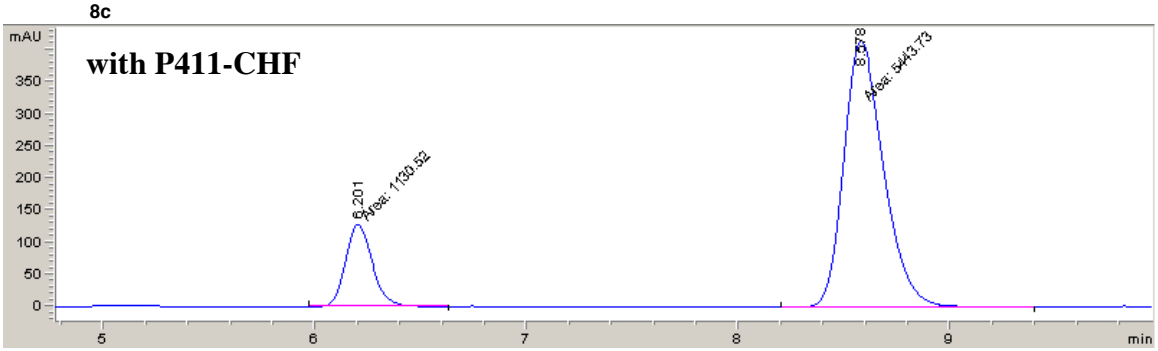




| ***rac*-5e** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 64.08<br>65.26 | 262.0<br>255.7 | 50.61<br>49.39 | 64.19<br>65.08 | 13.5<br>1289.9 | 1.04<br>98.96 |
| Total | 517.7 | 100.00 | Total | 1303.4 | 100.00 |



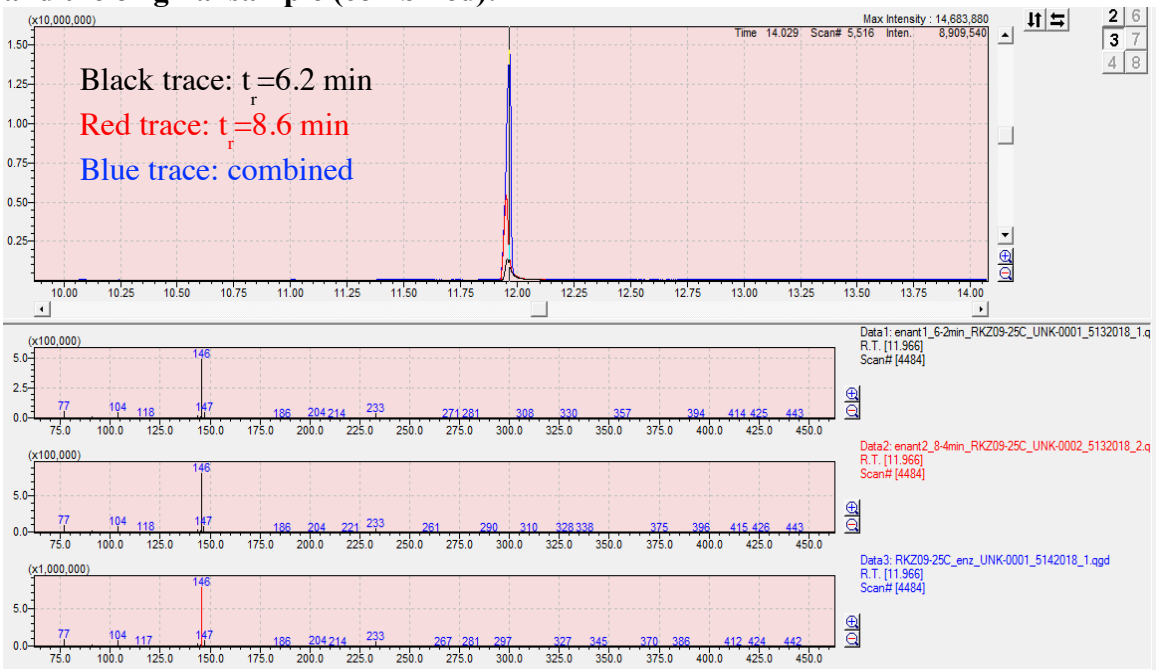

**Ethyl 2-(1-phenylpyrrolidin-2-yl)acetate (8c)**

HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, room temperature, 235 nm.

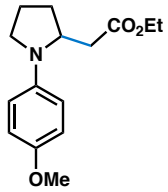



| 8c with P411-CHF | | |
|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % |
| 6.201 | 1130.5 | 17.20 |
| 8.578 | 5443.7 | 82.80 |
| Total | 6574.2 | 100.00 |

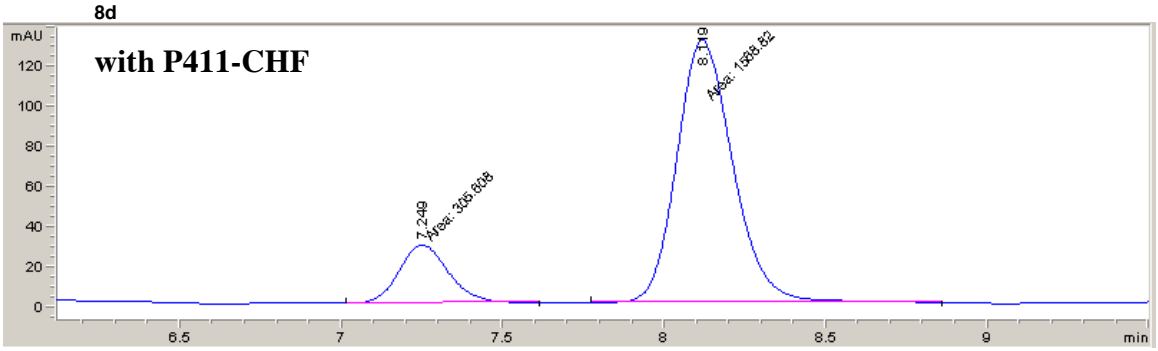
**GC-MS traces for separated enantiomers ($t_r$ = 6.2 min, $t_r$ = 8.6 min) individually analyzed and the original sample (combined):**




GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.

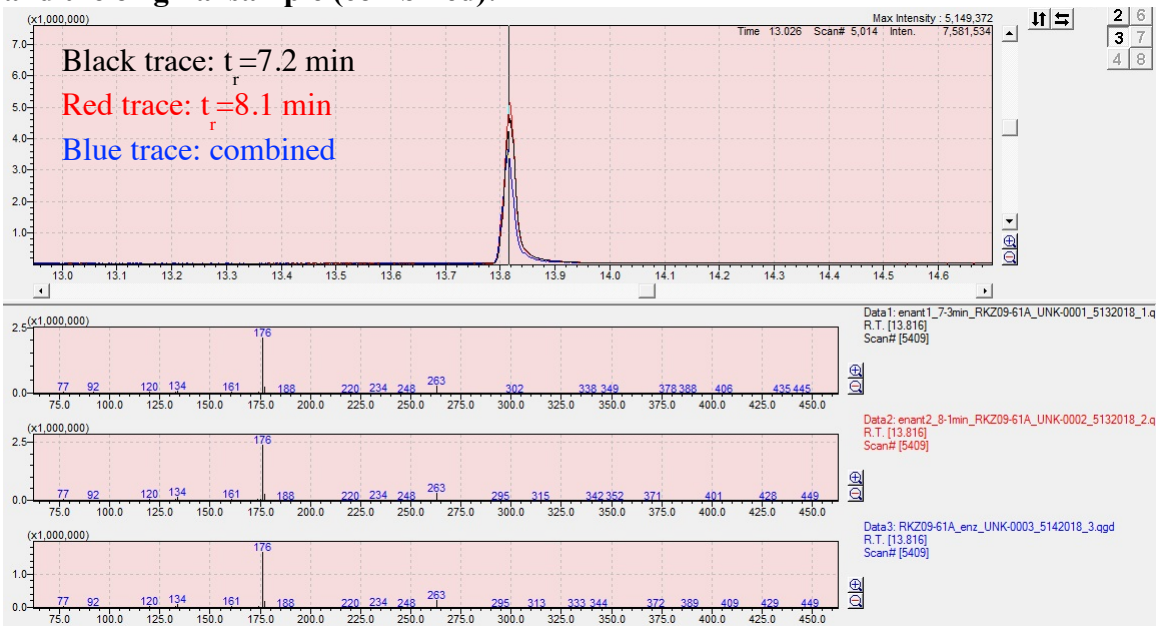



**Ethyl 2-(1-(4-methoxyphenyl)pyrrolidin-2-yl)acetate (8d)**
HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, room temperature, 235 nm.

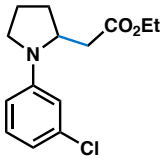



| 8d with P411-CHF | | |
|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % |
| 7.249<br>8.119 | 305.6<br>1568.8 | 16.30<br>83.70 |
| Total | 1874.4 | 100.00 |

**GC-MS traces for separated enantiomers ($t_r$ = 7.2 min, $t_r$ = 8.1 min) individually analyzed and the original sample (combined):**



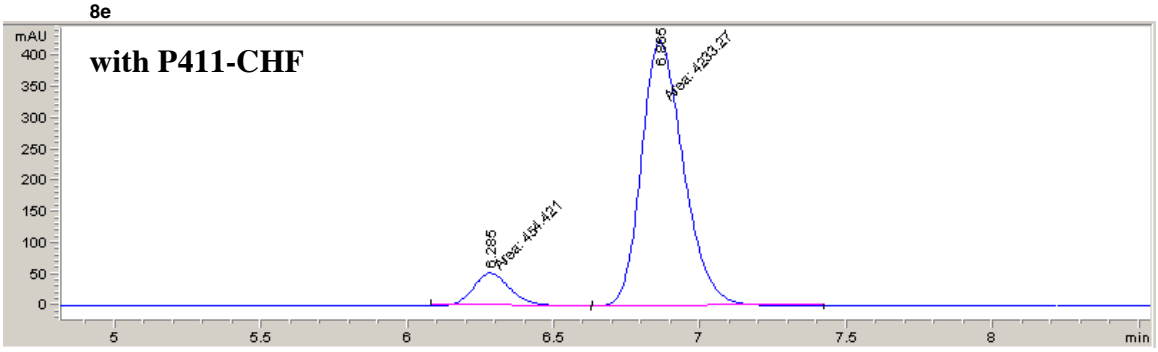

GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.




8e

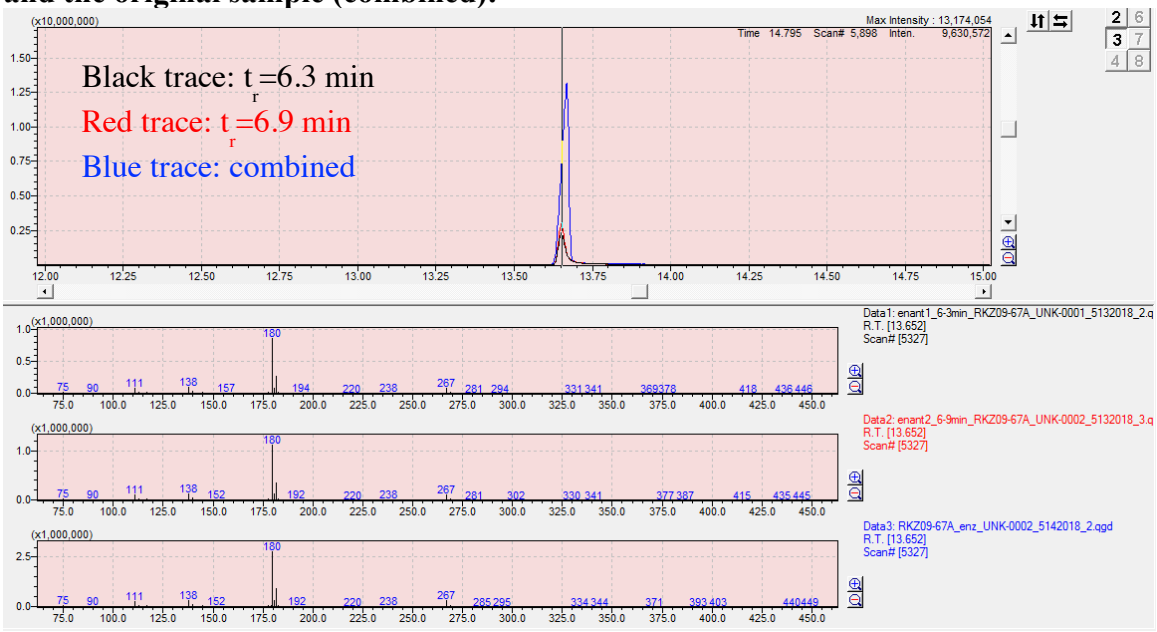
**Ethyl 2-(1-(3-chlorophenyl)pyrrolidin-2-yl)acetate (8e)**

HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, room temperature, 235 nm.

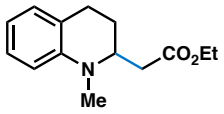



| 8e with P411-CHF | | |
|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % |
| 6.285 | 454.4 | 9.69 |
| 6.865 | 4233.3 | 90.31 |
| Total | 4687.7 | 100.00 |

**GC-MS traces for separated enantiomers ($t_r$ = 6.3 min, $t_r$ = 6.9 min) individually analyzed and the original sample (combined):**

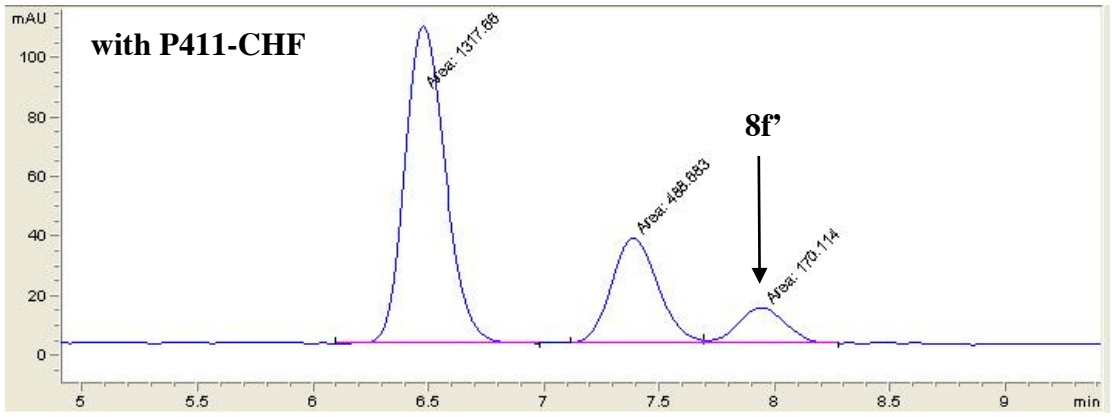


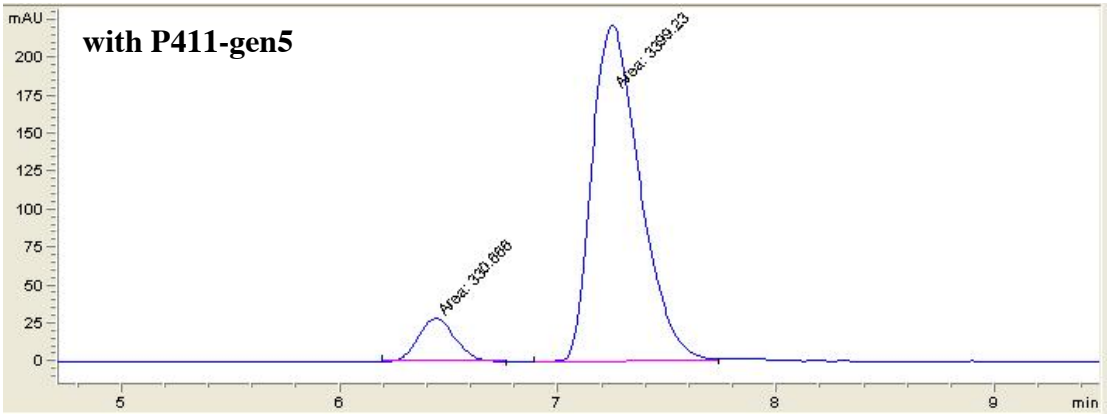

GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.




8f

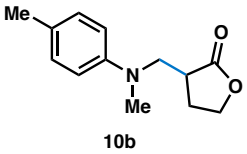
**Ethyl 2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate (8f)**
SFC (Chiralcel OB-H column): 3% *i*-PrOH in supercritical $CO_2$, 2.5 mL/min, 40 °C, 235 nm.




| **8f with P411-CHF** | | | **8f with P411-gen5** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 6.473<br>7.384 | 1317.7<br>488.7 | 72.95<br>27.05 | 6.437<br>7.246 | 330.7<br>3399.2 | 8.86<br>91.14 |
| Total | 1806.4 | 100.00 | Total | 3729.9 | 100.00 |

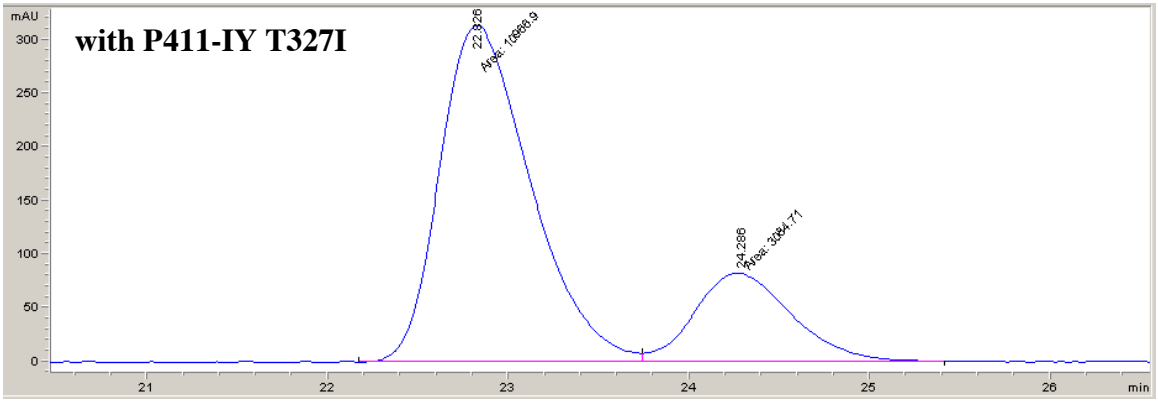
The absolute configuration of P411-gen5 synthesized **8f** was determined to be (*S*) after derivatization to (+)-cuspareine (see **Section X** for more details). As P411-CHF shows the opposite selectivity for the synthesis of **8f**, P411-CHF produces (*R*)-**8f** in excess.




10b

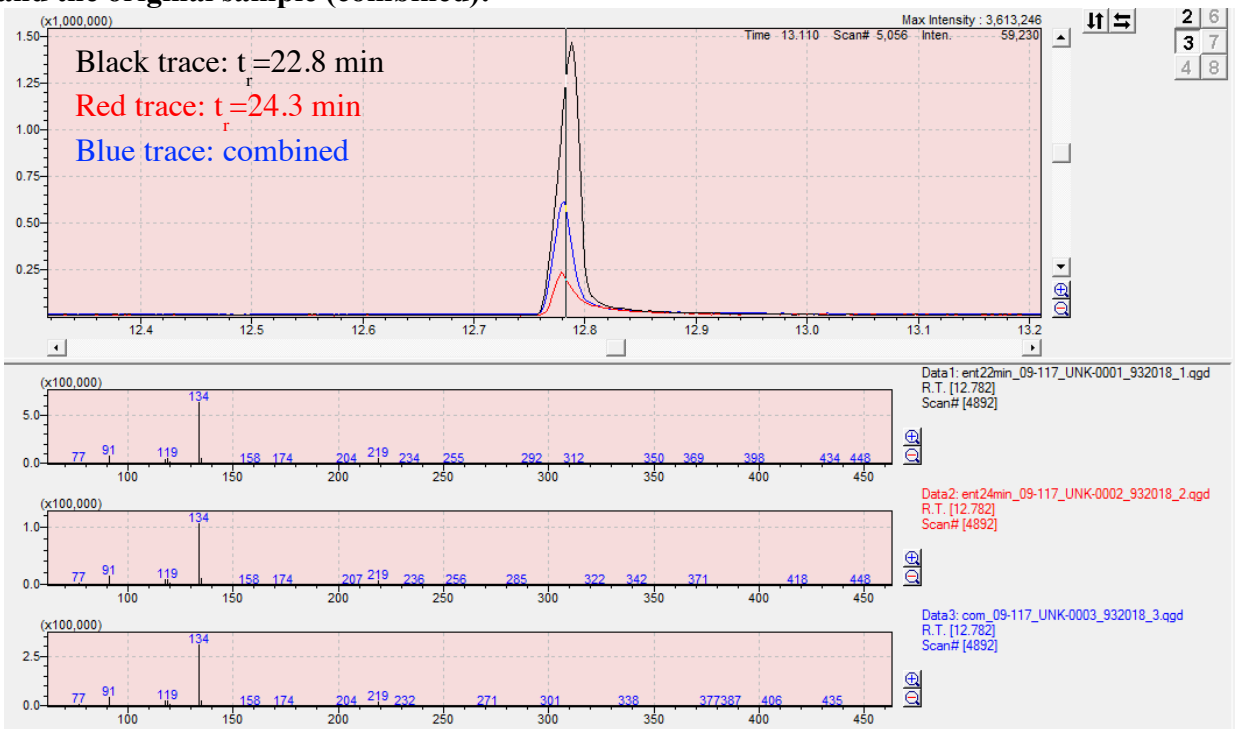
**3-((Methyl(*p*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (10b)**

HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, 32 °C, 254 nm.

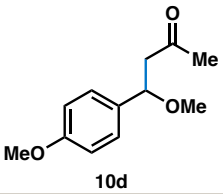



| **10b with P411-IY T327I** | | |
|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % |
| 22.826 | 10966.9 | 78.05 |
| 24.286 | 3084.7 | 21.95 |
| Total | 14051.6 | 100.00 |

**GC-MS traces for separated enantiomers ($t_r$ = 22.8 min, $t_r$ = 24.3 min) individually analyzed and the original sample (combined):**



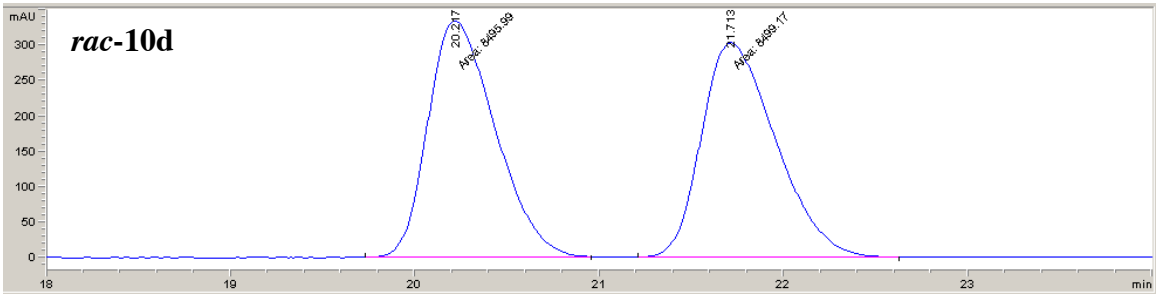

GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.

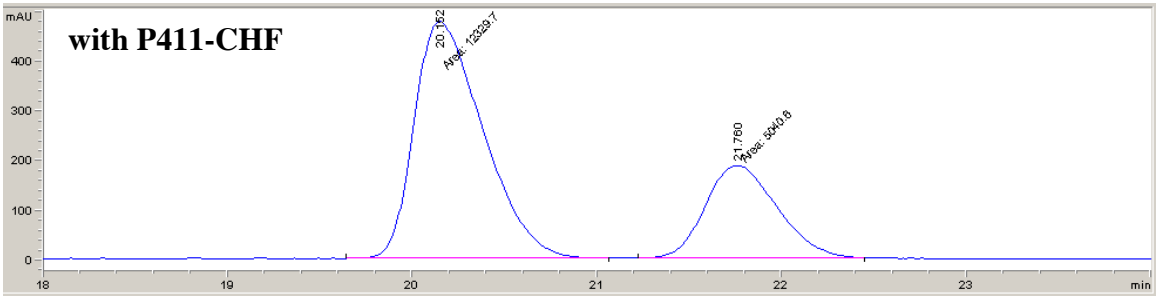



10d

**4-Methoxy-4-(4-methoxyphenyl)butan-2-one (10d)**

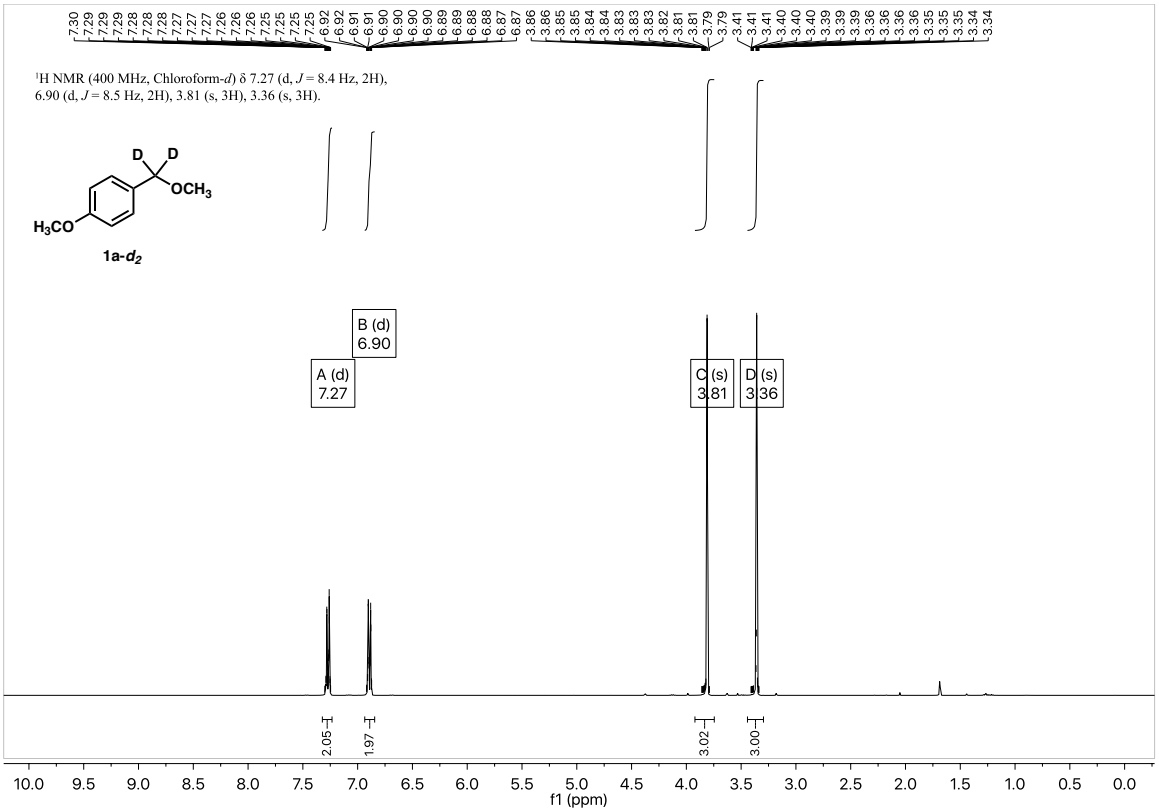
HPLC (Chiralcel OJ-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, 28 °C, 220 nm.

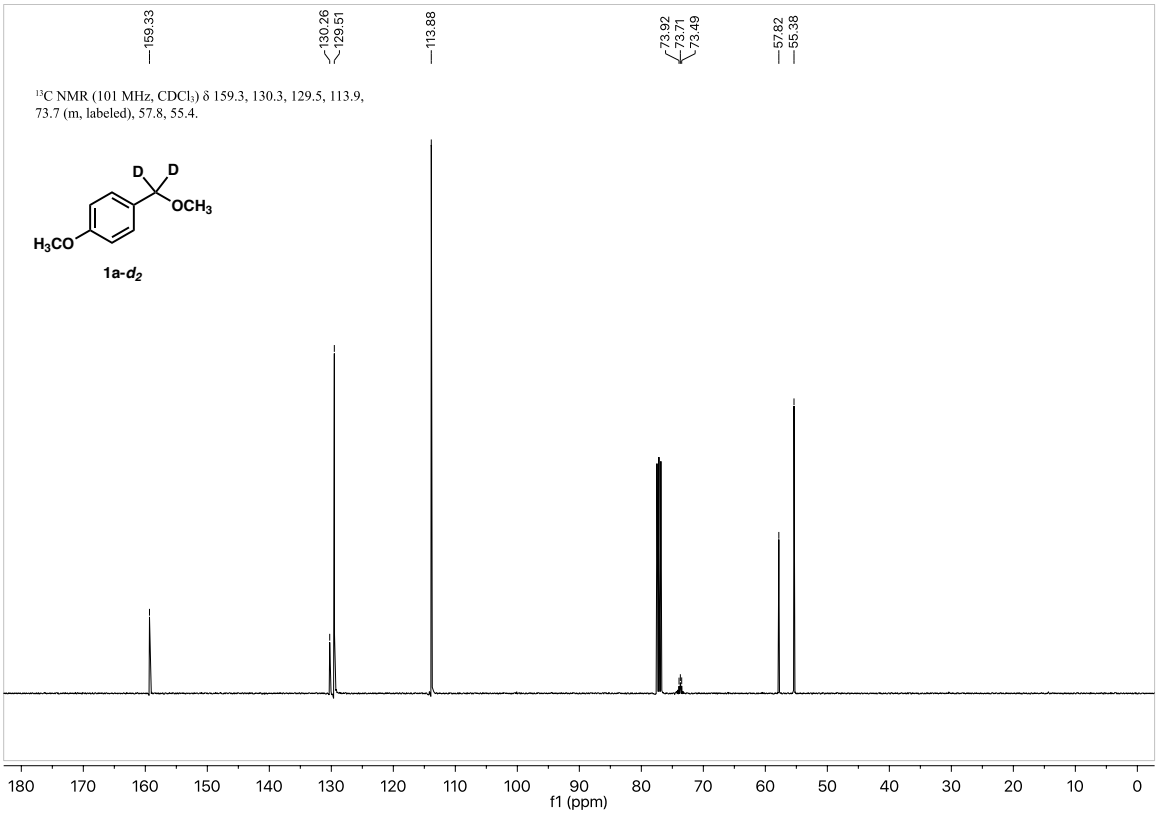


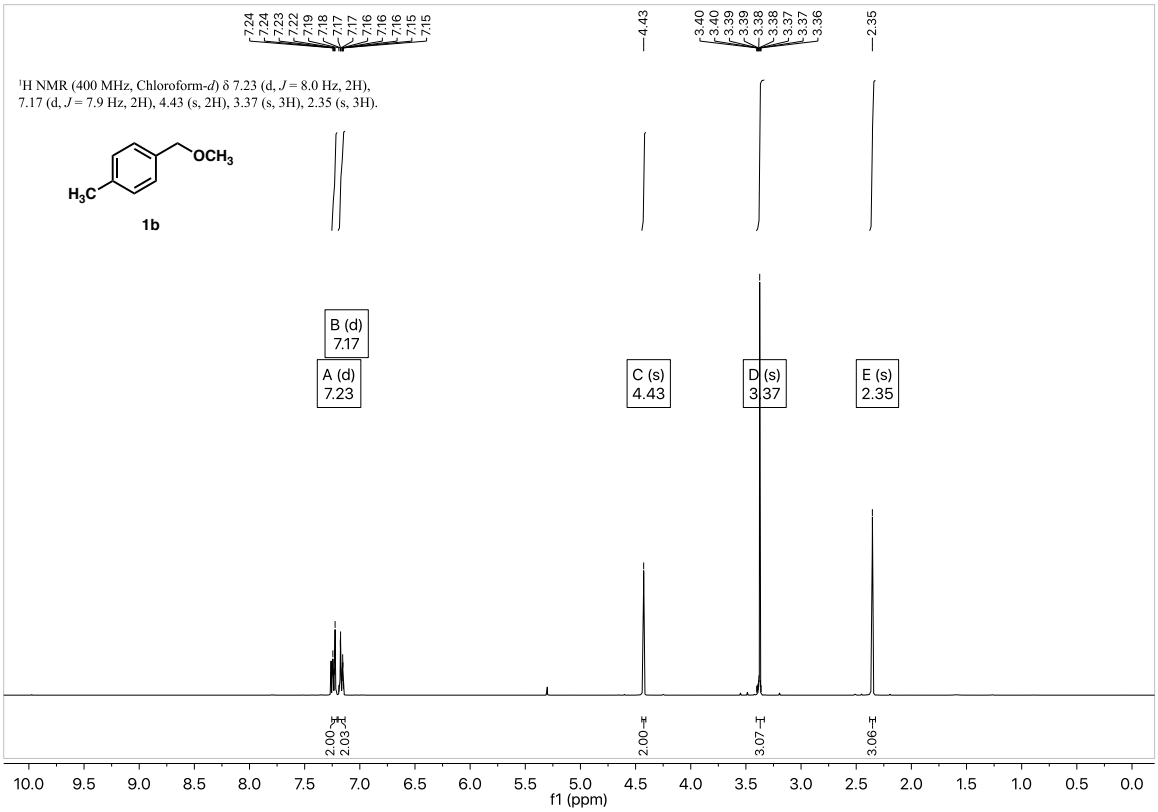



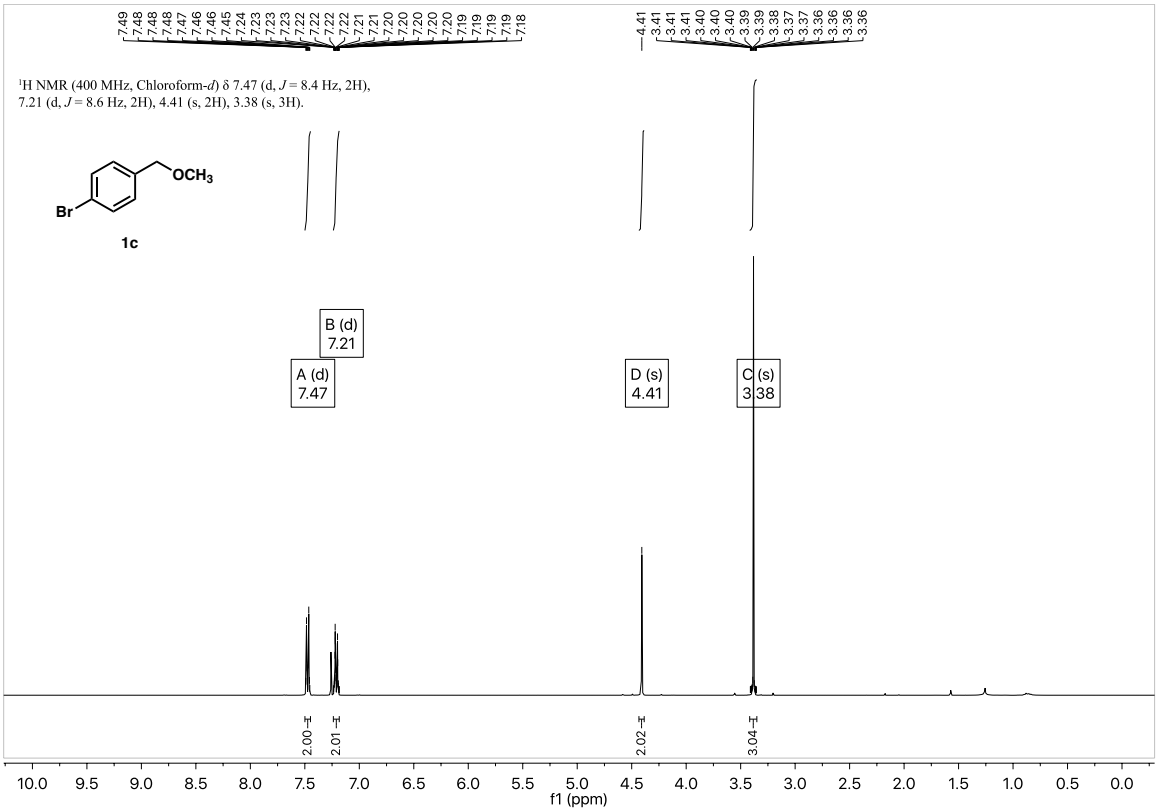

| ***rac*-10d** | | | **with P411-CHF** | | |
|---|---|---|---|---|---|
| Retention Time (min) | Area (mAU*s) | Area % | Retention Time (min) | Area (mAU*s) | Area % |
| 20.217 | 8495.99 | 49.99 | 20.152 | 12329.7 | 70.98 |
| 21.713 | 8499.17 | 50.01 | 21.760 | 5040.6 | 29.02 |
| Total | 16995.16 | 100.00 | Total | 17370.3 | 100.00 |

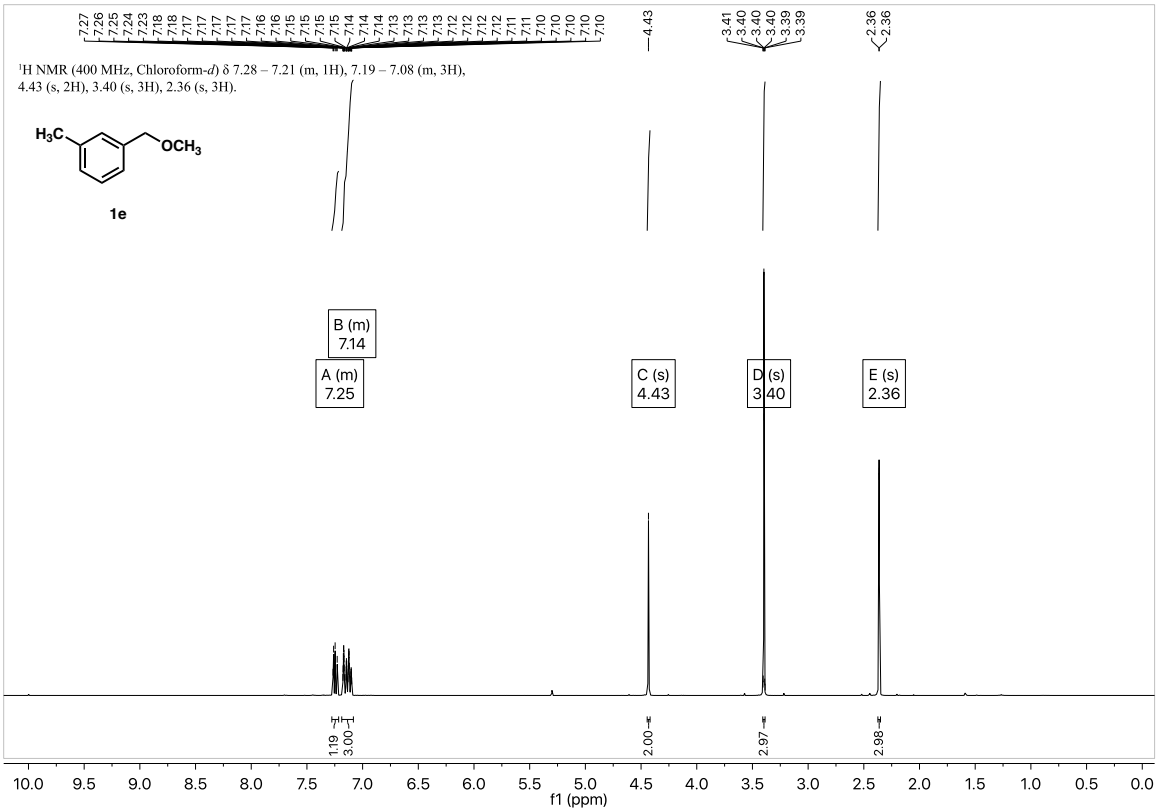
# XII. $^{1}H$ and $^{13}C$ NMR Spectra

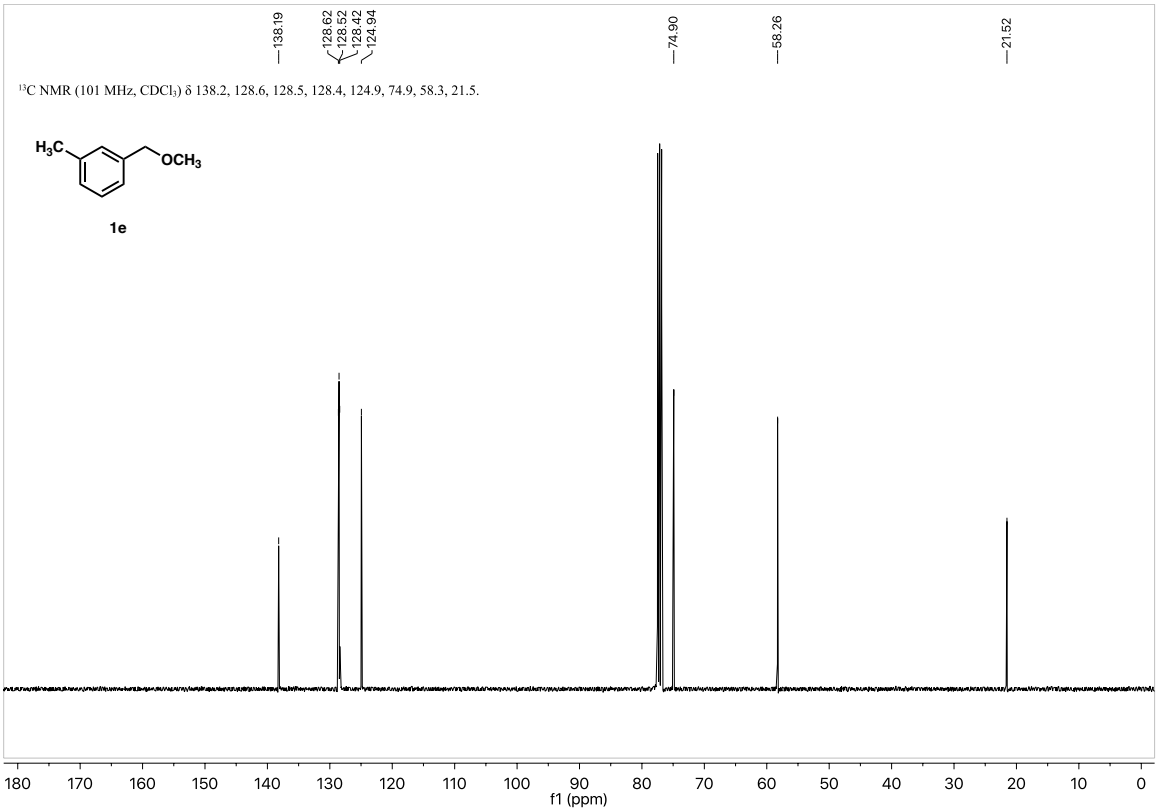


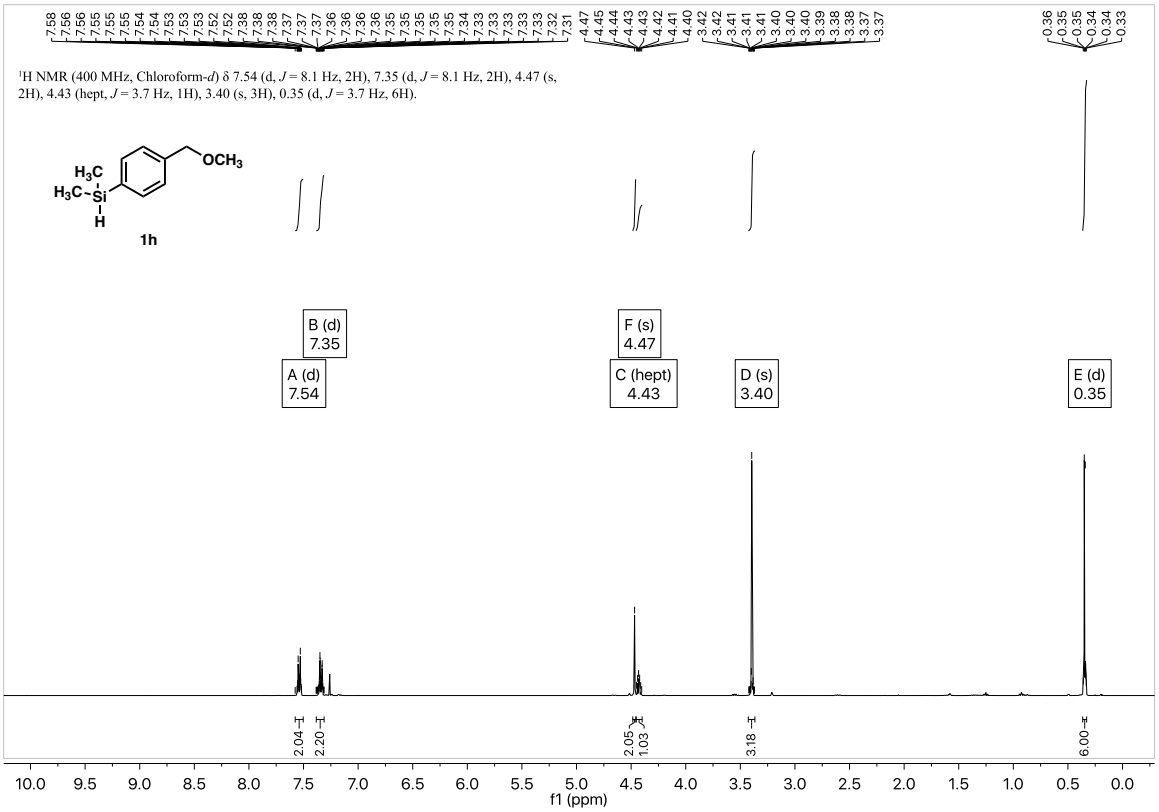



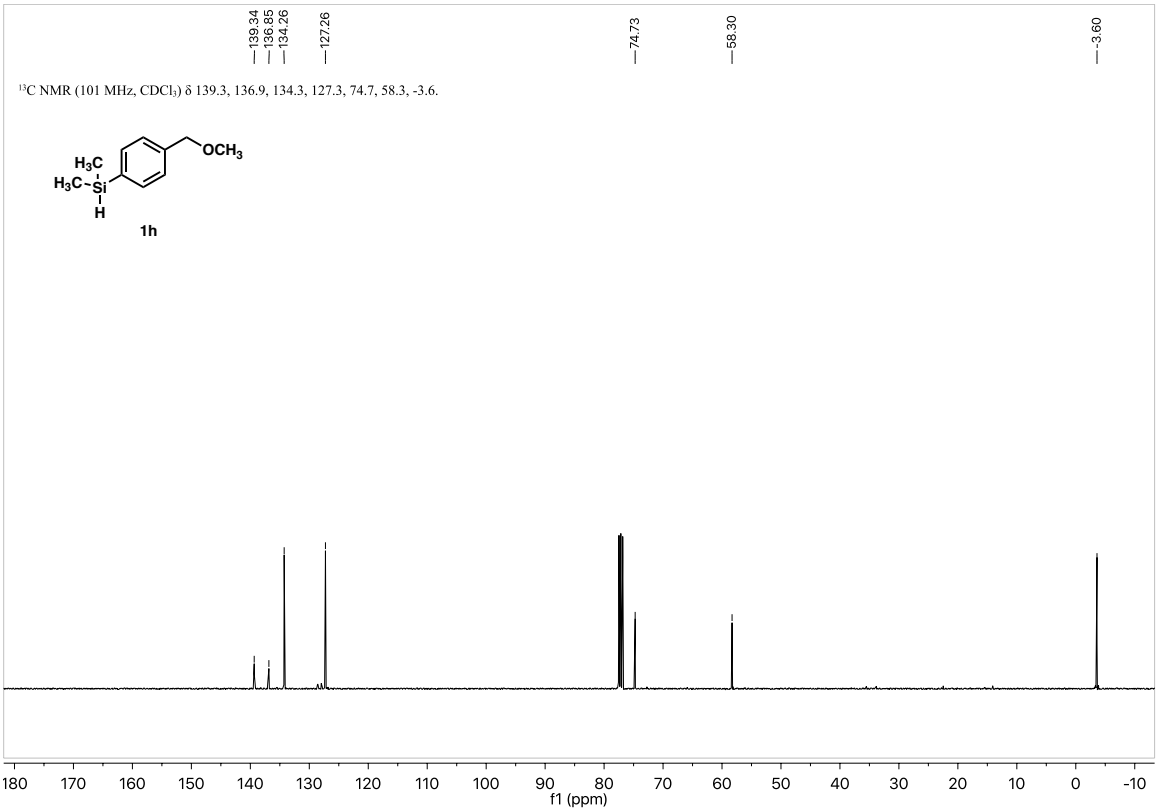

7.24
7.24
7.23
7.22
7.19
7.18
7.17
7.17
7.16
7.16
7.16
7.15
7.15
4.43
3.40
3.40
3.39
3.39
3.38
3.38
3.37
3.37
3.36
2.35
1H NMR (400 MHz, Chloroform-d) δ 7.23 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 7.9 Hz, 2H), 4.43 (s, 2H), 3.37 (s, 3H), 2.35 (s, 3H).
OCH3
H3C
1b
B (d) 7.17
A (d) 7.23
C (s) 4.43
D (s) 3.37
E (s) 2.35
2.00
2.03
2.00
3.07
3.06
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
f1 (ppm)

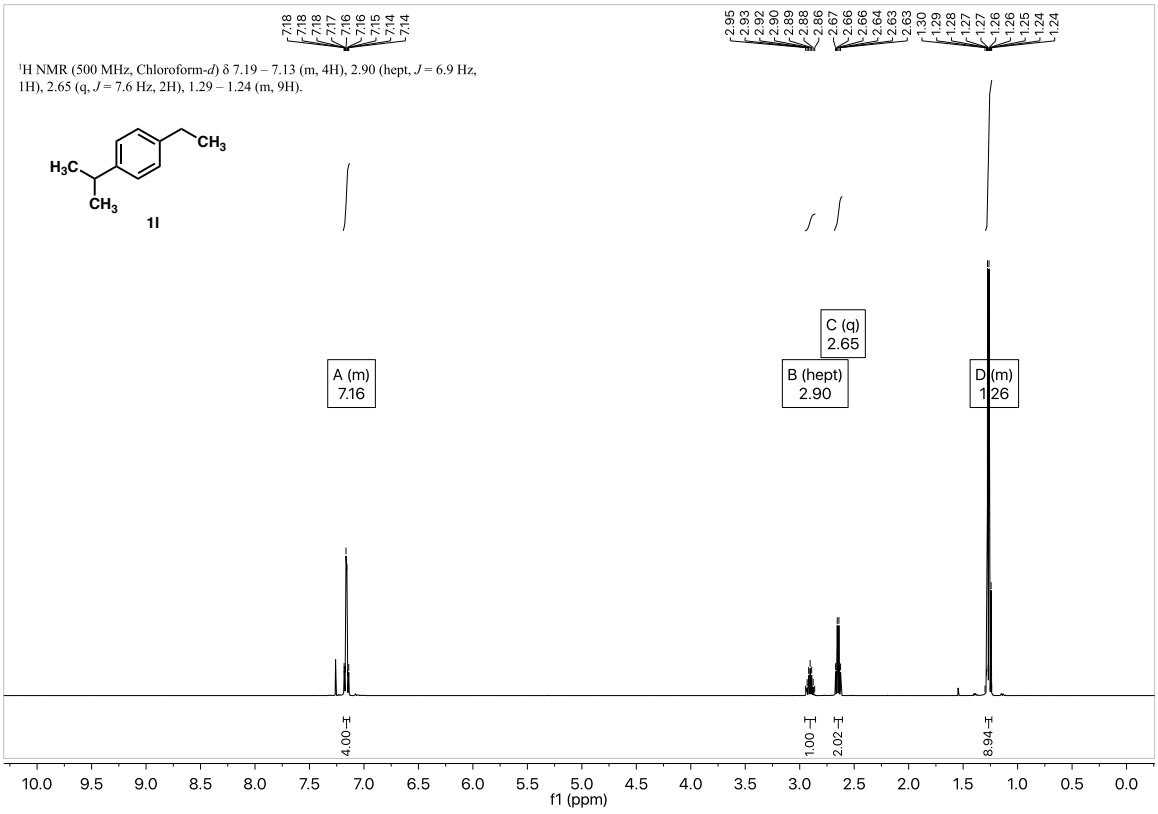

7.49
7.48
7.48
7.48
7.47
7.46
7.46
7.45
7.24
7.23
7.23
7.23
7.22
7.22
7.22
7.22
7.21
7.21
7.20
7.20
7.20
7.20
7.20
7.19
7.19
7.19
7.19
7.18
4.41
3.41
3.41
3.41
3.40
3.40
3.40
3.39
3.39
3.38
3.37
3.37
3.36
3.36
3.36
3.36
1H NMR (400 MHz, Chloroform-d) δ 7.47 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 4.41 (s, 2H), 3.38 (s, 3H).
OCH3
Br
1c
B (d) 7.21
A (d) 7.47
D (s) 4.41
C (s) 3.38
2.00
2.01
2.02
3.04
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
f1 (ppm)

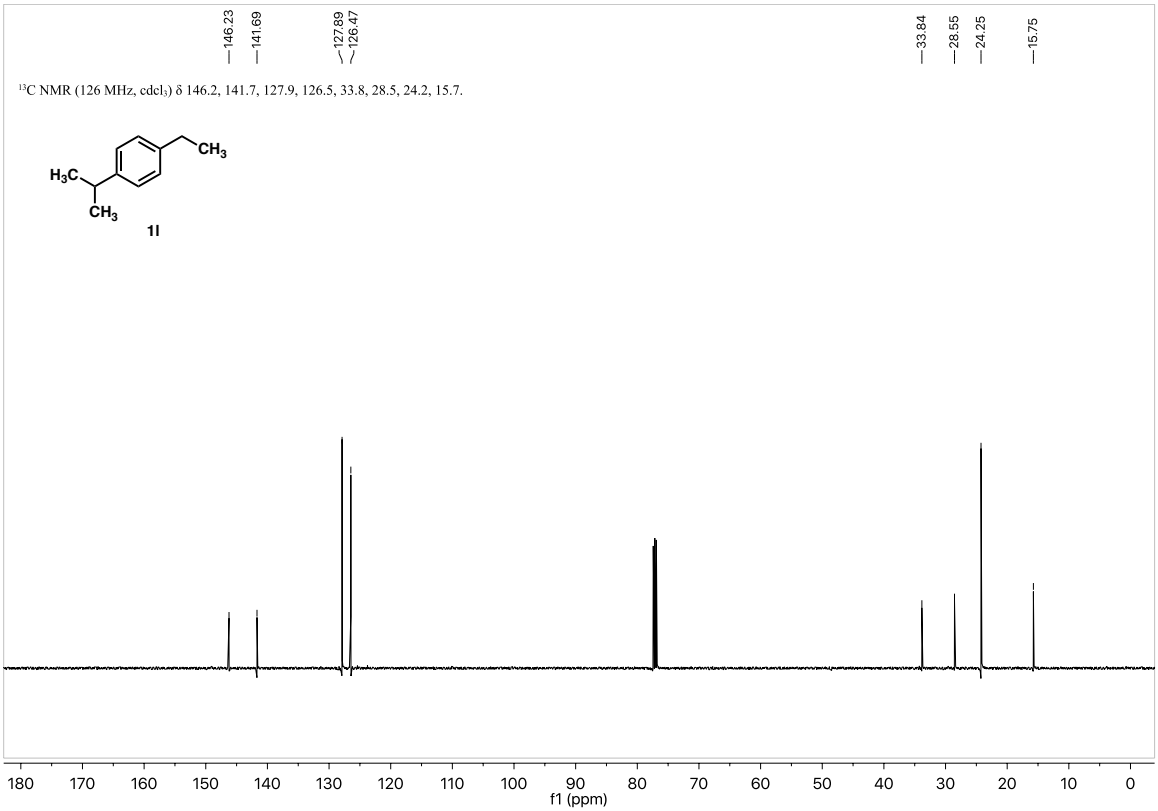

7.27 7.26 7.25 7.24 7.23 7.18 7.18 7.17 7.17 7.17 7.17 7.17 7.16 7.16 7.15 7.15 7.15 7.15 7.14 7.14 7.14 7.13 7.13 7.13 7.13 7.12 7.12 7.12 7.12 7.11 7.11 7.10 7.10 7.10 7.10 7.10 7.10
4.43
3.41 3.40 3.40 3.40 3.39 3.39
2.36 2.36
1H NMR (400 MHz, Chloroform-d) δ 7.28 – 7.21 (m, 1H), 7.19 – 7.08 (m, 3H), 4.43 (s, 2H), 3.40 (s, 3H), 2.36 (s, 3H).
H3C
OCH3
1e
B (m) 7.14
A (m) 7.25
C (s) 4.43
D (s) 3.40
E (s) 2.36
1.19
3.00
2.00
2.97
2.98
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
f1 (ppm)

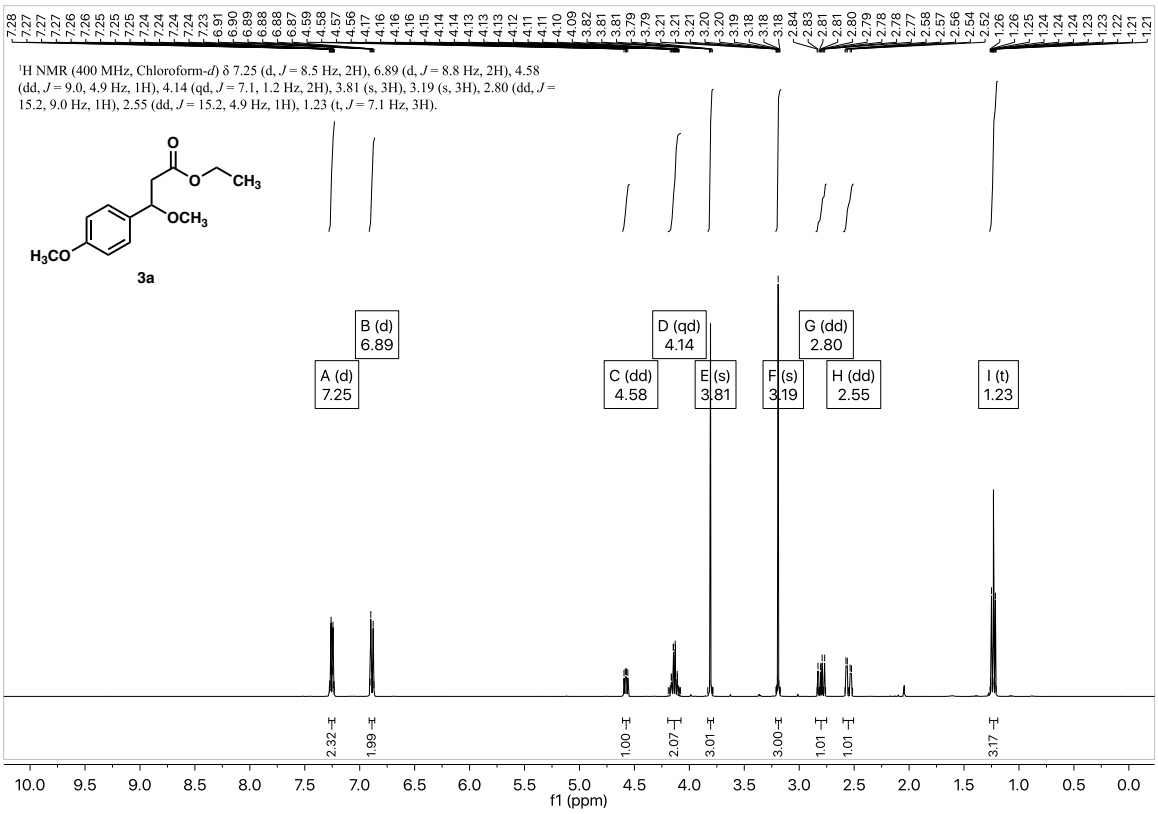

138.19
128.62
128.52
128.42
124.94
74.90
58.26
21.52
13C NMR (101 MHz, CDCl3) δ 138.2, 128.6, 128.5, 128.4, 124.9, 74.9, 58.3, 21.5.
H3C
OCH3
1e
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

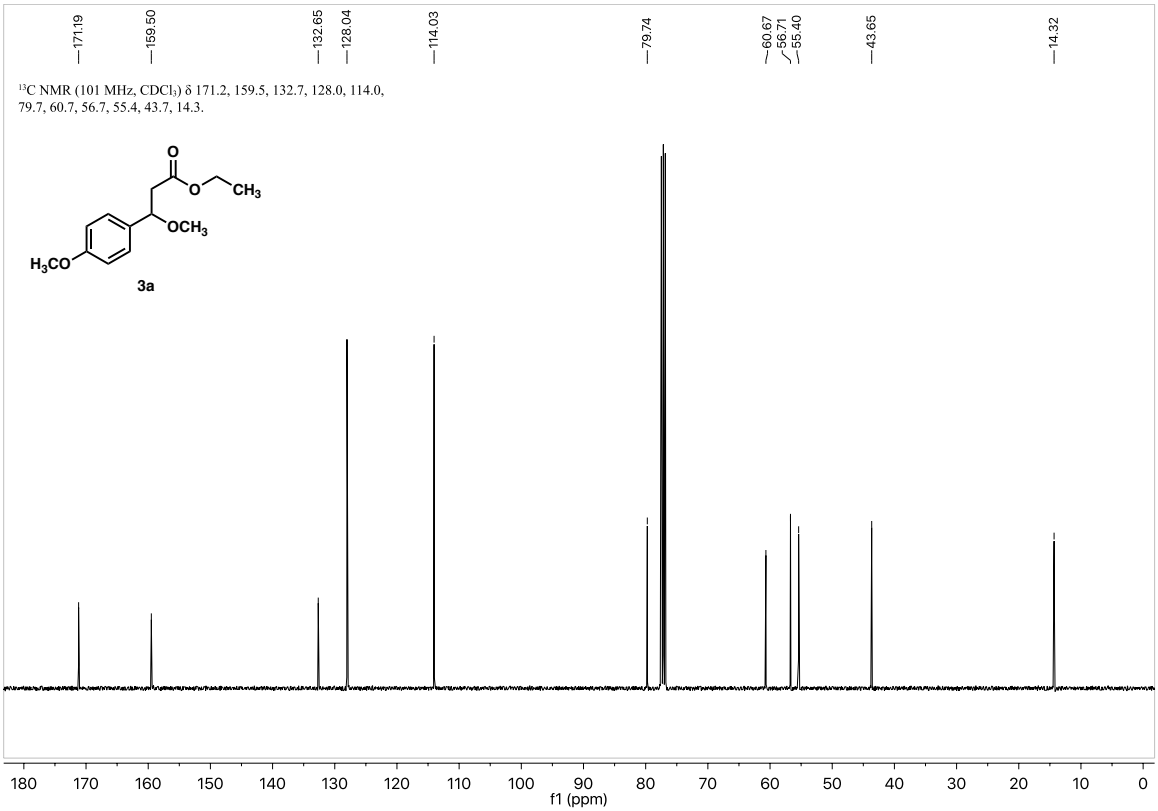

7.58 7.56 7.56 7.55 7.55 7.55 7.54 7.54 7.53 7.53 7.53 7.52 7.52 7.52 7.38 7.38 7.38 7.37 7.37 7.37 7.36 7.36 7.36 7.36 7.35 7.35 7.35 7.35 7.35 7.34 7.33 7.33 7.33 7.33 7.33 7.32 7.31
4.47 4.45 4.44 4.43 4.43 4.42 4.41 4.40
3.42 3.42 3.41 3.41 3.41 3.40 3.40 3.40 3.39 3.38 3.38 3.37 3.37
0.36 0.35 0.35 0.34 0.34 0.33
[1]H NMR (400 MHz, Chloroform-d) δ 7.54 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 4.47 (s, 2H), 4.43 (hept, J = 3.7 Hz, 1H), 3.40 (s, 3H), 0.35 (d, J = 3.7 Hz, 6H).
H3C
H3C-Si
H
OCH3
1h
B (d) 7.35
A (d) 7.54
F (s) 4.47
C (hept) 4.43
D (s) 3.40
E (d) 0.35
2.04
2.20
2.05
1.03
3.18
6.00
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
f1 (ppm)

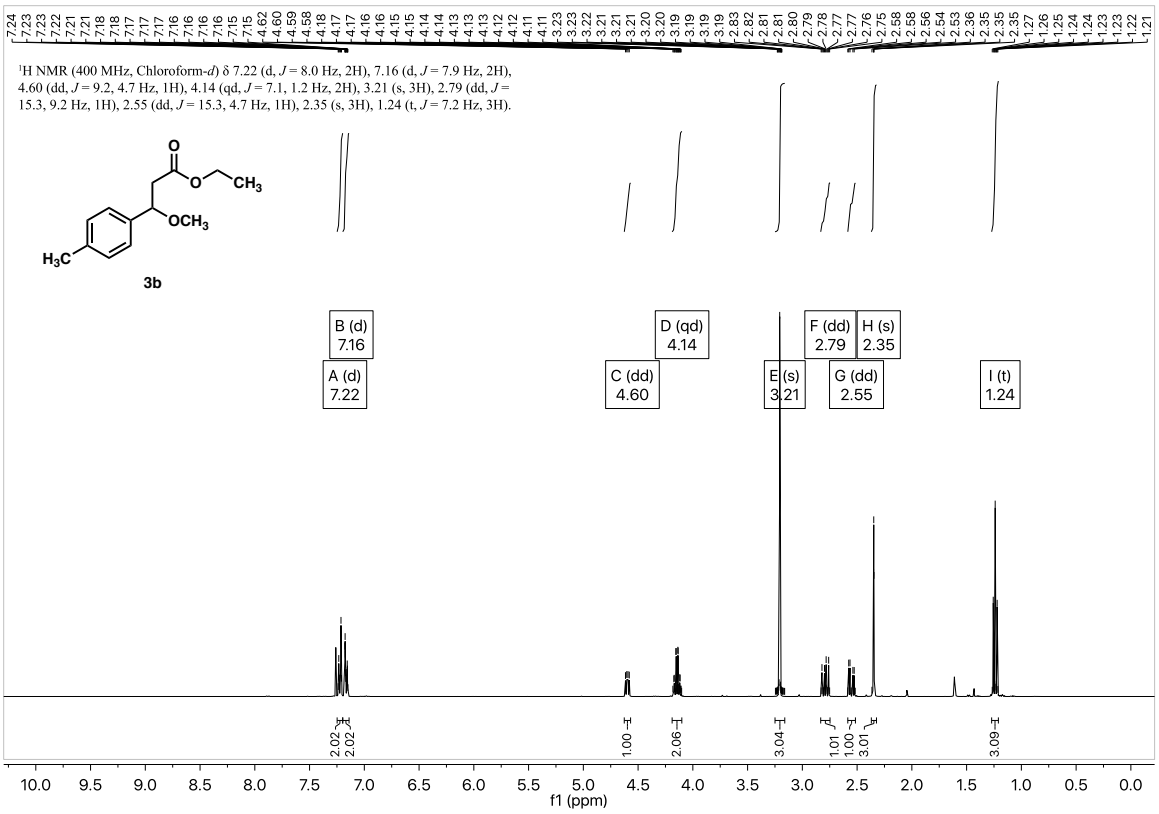

139.34
136.85
134.26
127.26
74.73
58.30
-3.60
[13]C NMR (101 MHz, CDCl3) δ 139.3, 136.9, 134.3, 127.3, 74.7, 58.3, -3.6.
H3C
H3C-Si
H
OCH3
1h
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

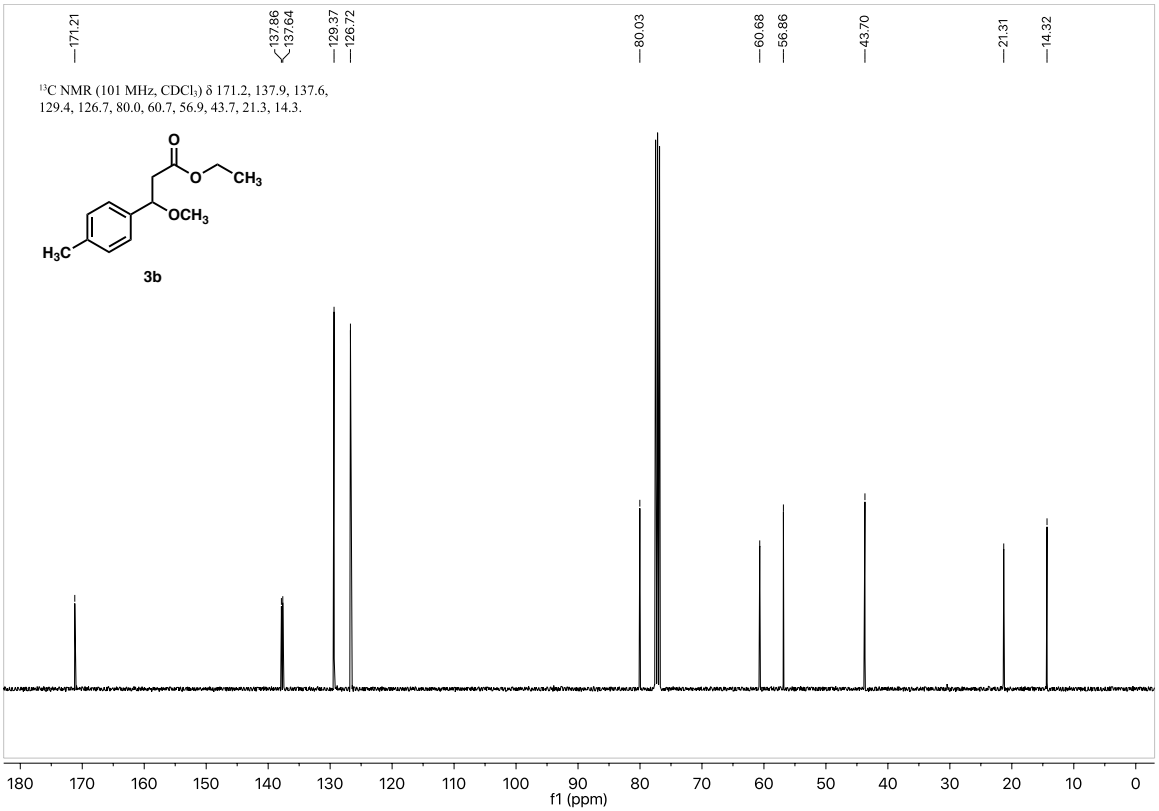

7.18
7.18
7.18
7.17
7.16
7.16
7.15
7.14
7.14
2.95
2.93
2.92
2.90
2.89
2.88
2.86
2.67
2.66
2.66
2.64
2.63
2.63
1.30
1.29
1.28
1.27
1.27
1.26
1.26
1.25
1.24
1.24
[1]H NMR (500 MHz, Chloroform-d) δ 7.19 – 7.13 (m, 4H), 2.90 (hept, J = 6.9 Hz, 1H), 2.65 (q, J = 7.6 Hz, 2H), 1.29 – 1.24 (m, 9H).
H3C
CH3
CH3
1l
A (m)
7.16
B (hept)
2.90
C (q)
2.65
D (m)
1.26
4.00
1.00
2.02
8.94
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
f1 (ppm)

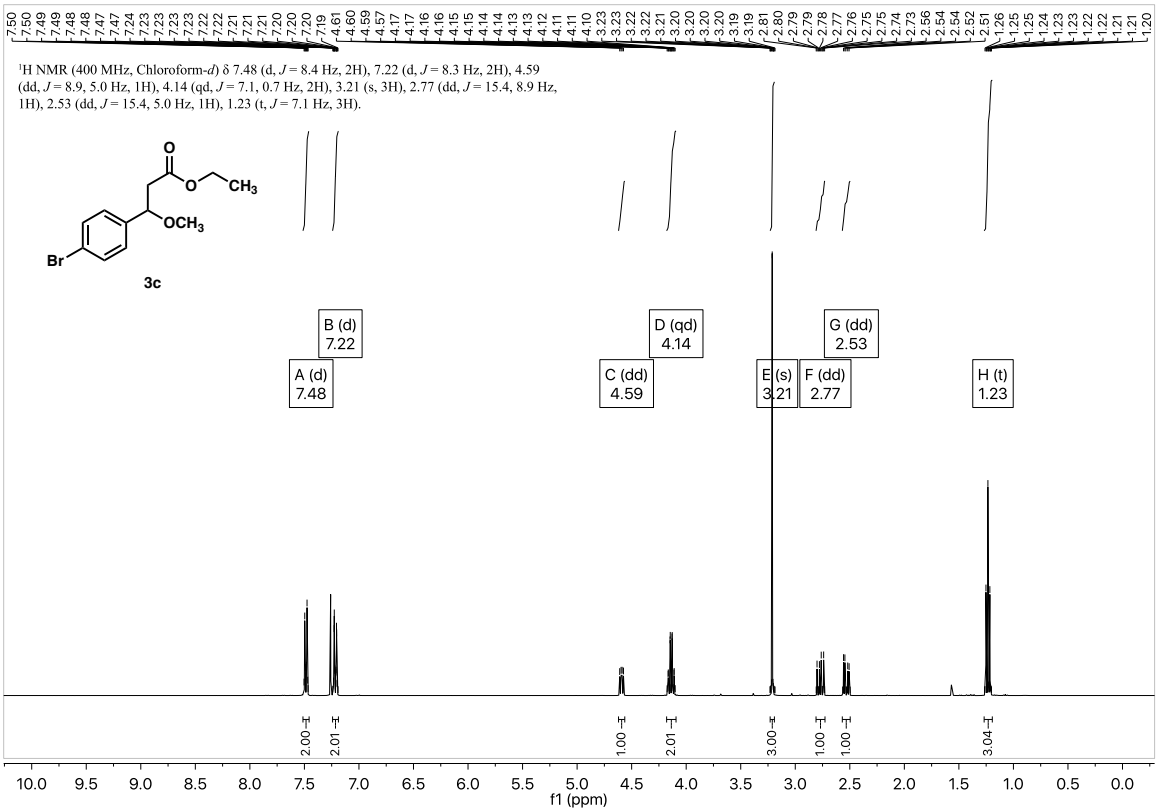

146.23
141.69
127.89
126.47
33.84
28.55
24.25
15.75
[13]C NMR (126 MHz, cdcl3) δ 146.2, 141.7, 127.9, 126.5, 33.8, 28.5, 24.2, 15.7.
H3C
CH3
CH3
1l
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

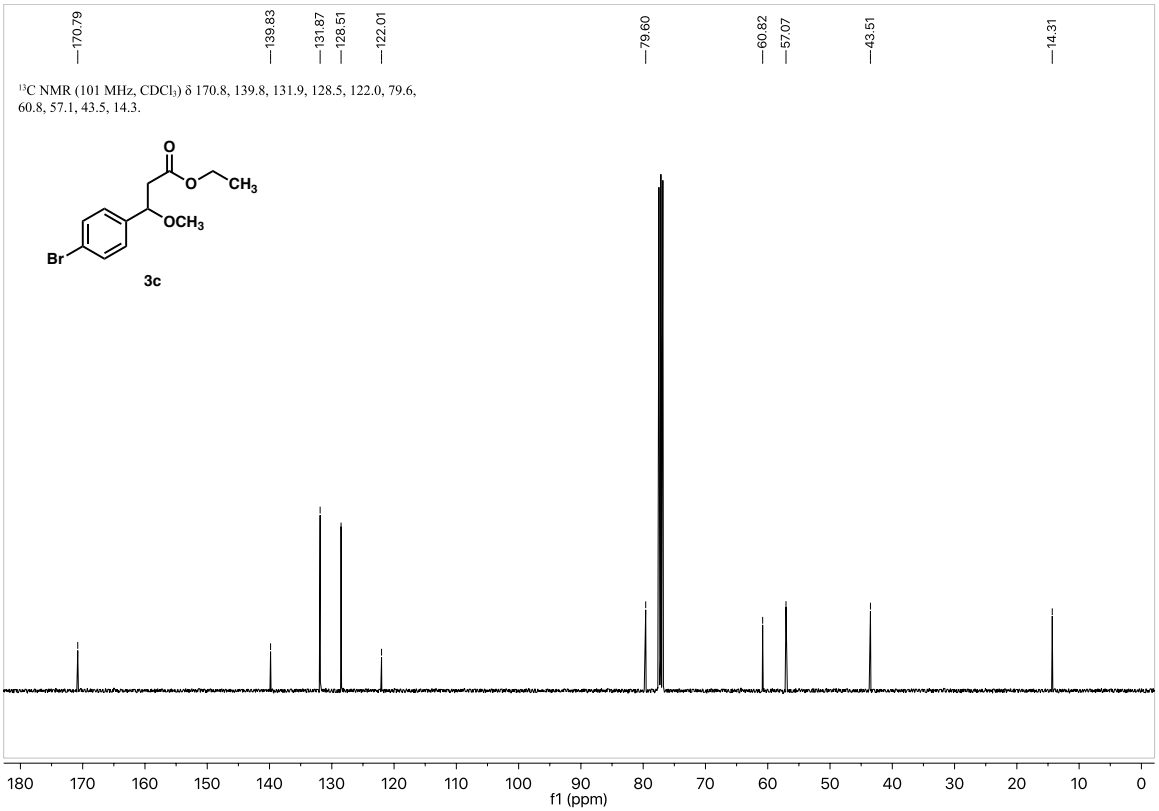

[1]H NMR (400 MHz, Chloroform-d) δ 7.25 (d, J = 8.5 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H), 4.58 (dd, J = 9.0, 4.9 Hz, 1H), 4.14 (qd, J = 7.1, 1.2 Hz, 2H), 3.81 (s, 3H), 3.19 (s, 3H), 2.80 (dd, J = 15.2, 9.0 Hz, 1H), 2.55 (dd, J = 15.2, 4.9 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H).
3a
A (d) 7.25
B (d) 6.89
C (dd) 4.58
D (qd) 4.14
E (s) 3.81
F (s) 3.19
G (dd) 2.80
H (dd) 2.55
I (t) 1.23
2.32
1.99
1.00
2.07
3.01
3.00
1.01
1.01
3.17
f1 (ppm)

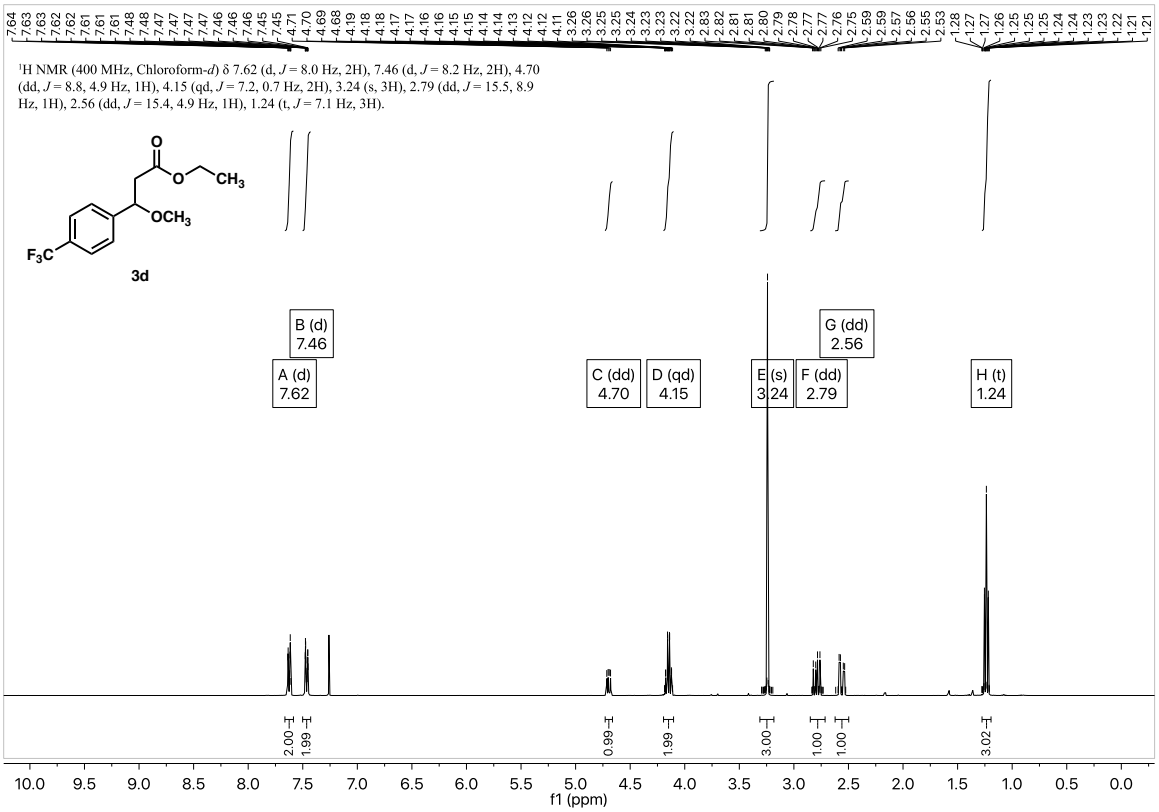

[13]C NMR (101 MHz, CDCl3) δ 171.2, 159.5, 132.7, 128.0, 114.0, 79.7, 60.7, 56.7, 55.4, 43.7, 14.3.
3a
171.19
159.50
132.65
128.04
114.03
79.74
60.67
56.71
55.40
43.65
14.32
f1 (ppm)

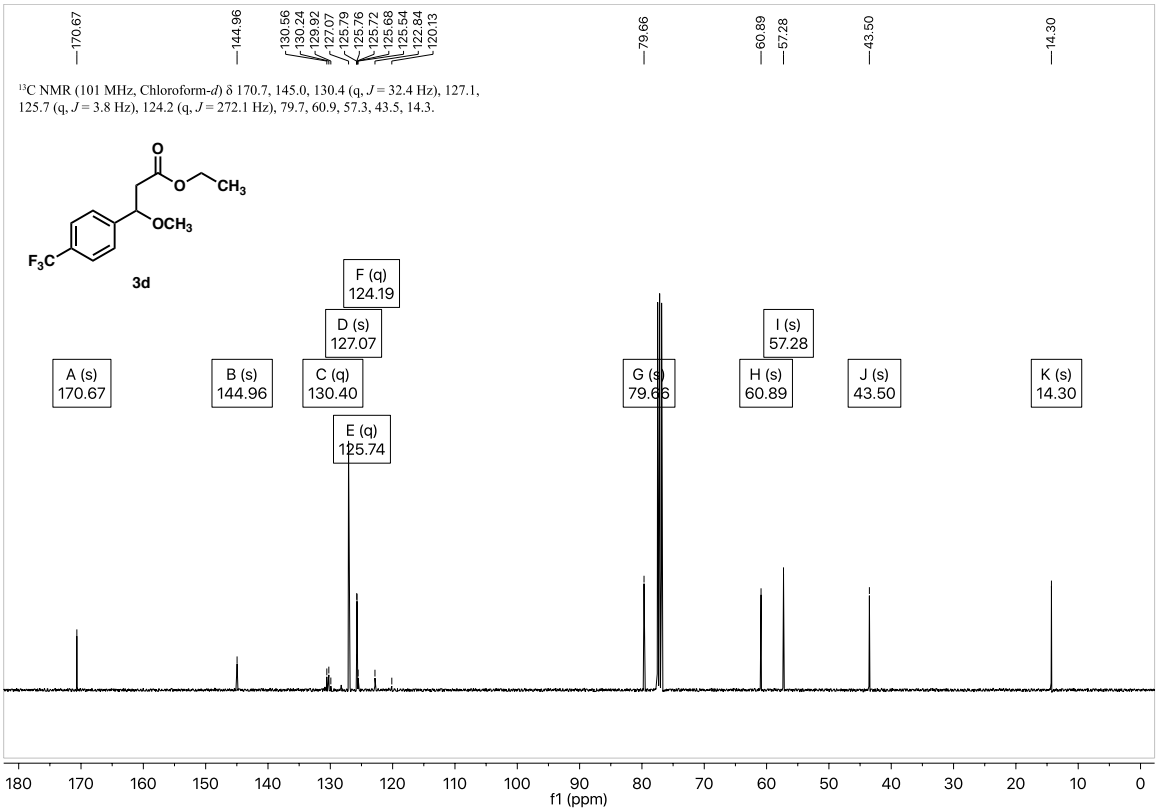

7.24 7.23 7.23 7.23 7.22 7.21 7.21 7.18 7.18 7.17 7.17 7.17 7.16 7.16 7.16 7.16 7.15 7.15 4.62 4.60 4.59 4.58 4.18 4.17 4.17 4.16 4.16 4.15 4.15 4.14 4.14 4.13 4.13 4.12 4.12 4.11 4.11 3.23 3.23 3.22 3.21 3.21 3.21 3.20 3.20 3.19 3.19 3.19 2.83 2.82 2.81 2.81 2.80 2.79 2.78 2.77 2.77 2.76 2.75 2.58 2.58 2.56 2.54 2.53 2.36 2.35 2.35 2.35 1.27 1.26 1.25 1.24 1.24 1.23 1.23 1.22 1.21
1H NMR (400 MHz, Chloroform-d) δ 7.22 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 7.9 Hz, 2H), 4.60 (dd, J = 9.2, 4.7 Hz, 1H), 4.14 (qd, J = 7.1, 1.2 Hz, 2H), 3.21 (s, 3H), 2.79 (dd, J = 15.3, 9.2 Hz, 1H), 2.55 (dd, J = 15.3, 4.7 Hz, 1H), 2.35 (s, 3H), 1.24 (t, J = 7.2 Hz, 3H).
O
O
CH3
OCH3
H3C
3b
B (d) 7.16
A (d) 7.22
D (qd) 4.14
C (dd) 4.60
E (s) 3.21
F (dd) 2.79
H (s) 2.35
G (dd) 2.55
I (t) 1.24
2.02
2.02
1.00
2.06
3.04
1.01
1.00
3.01
3.09
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
f1 (ppm)

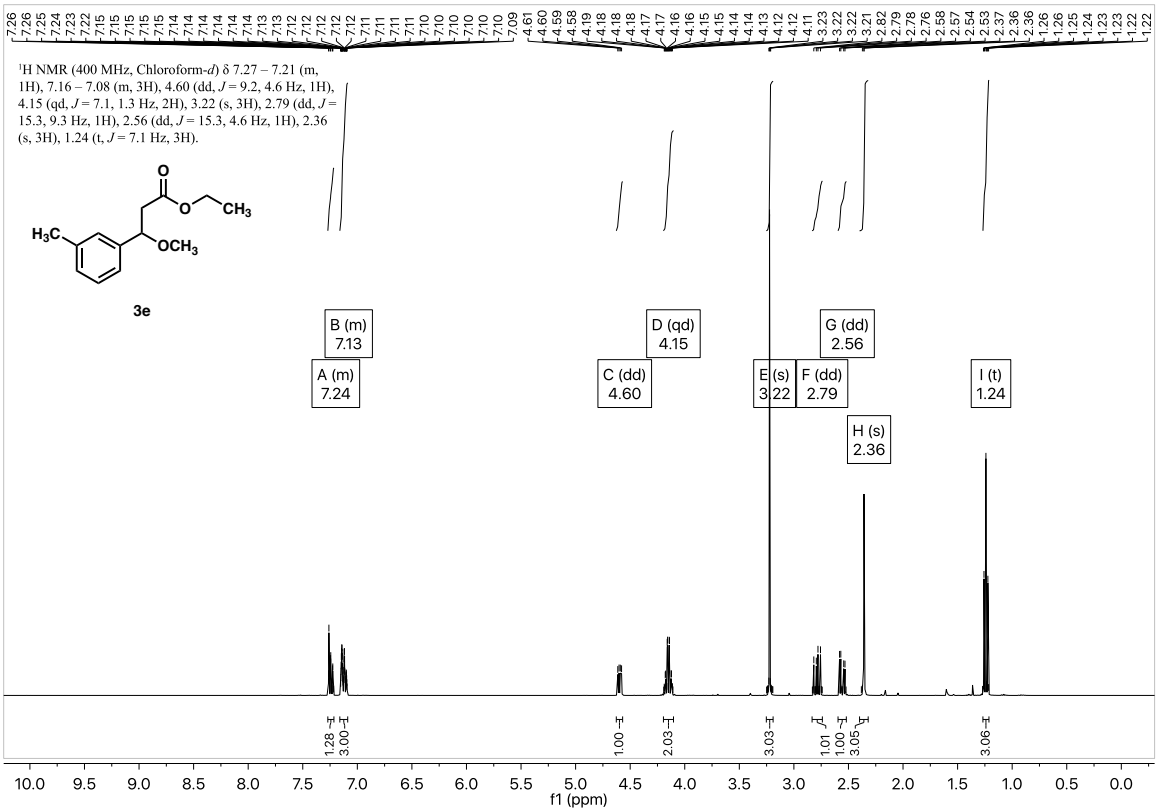

171.21
137.86
137.64
129.37
126.72
80.03
60.68
56.86
43.70
21.31
14.32
13C NMR (101 MHz, CDCl3) δ 171.2, 137.9, 137.6, 129.4, 126.7, 80.0, 60.7, 56.9, 43.7, 21.3, 14.3.
O
O
CH3
OCH3
H3C
3b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

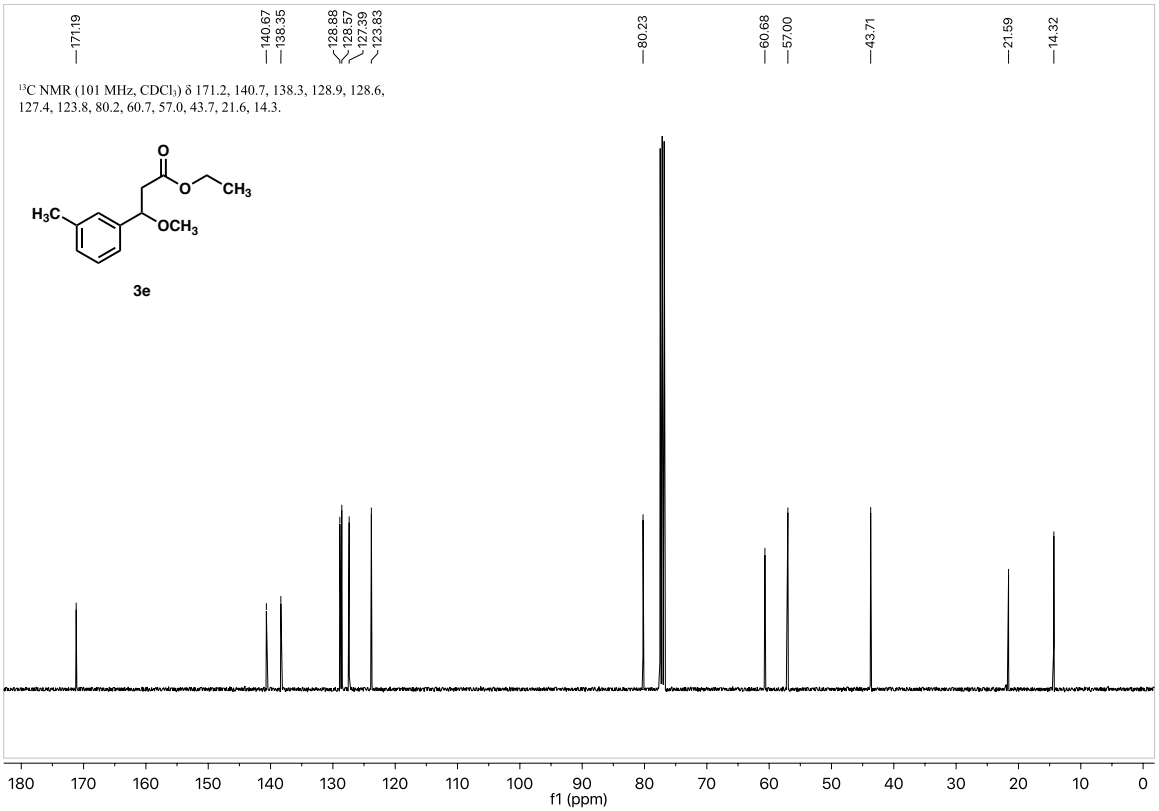

7.50 7.50 7.49 7.49 7.48 7.48 7.47 7.47 7.24 7.23 7.23 7.23 7.23 7.22 7.22 7.21 7.21 7.21 7.20 7.20 7.20 7.19 4.61 4.60 4.59 4.57 4.17 4.16 4.16 4.15 4.15 4.14 4.14 4.13 4.13 4.12 4.11 4.11 4.10 3.23 3.23 3.22 3.22 3.21 3.20 3.20 3.20 3.20 3.19 3.19 2.81 2.80 2.79 2.79 2.78 2.77 2.76 2.75 2.75 2.74 2.73 2.56 2.54 2.54 2.52 2.51 1.26 1.25 1.25 1.24 1.23 1.23 1.22 1.22 1.21 1.21 1.20
1H NMR (400 MHz, Chloroform-d) δ 7.48 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.3 Hz, 2H), 4.59 (dd, J = 8.9, 5.0 Hz, 1H), 4.14 (qd, J = 7.1, 0.7 Hz, 2H), 3.21 (s, 3H), 2.77 (dd, J = 15.4, 8.9 Hz, 1H), 2.53 (dd, J = 15.4, 5.0 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H).
O
O
CH3
OCH3
Br
3c
A (d) 7.48
B (d) 7.22
C (dd) 4.59
D (qd) 4.14
E (s) 3.21
F (dd) 2.77
G (dd) 2.53
H (t) 1.23
2.00
2.01
1.00
2.01
3.00
1.00
1.00
3.04
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
f1 (ppm)

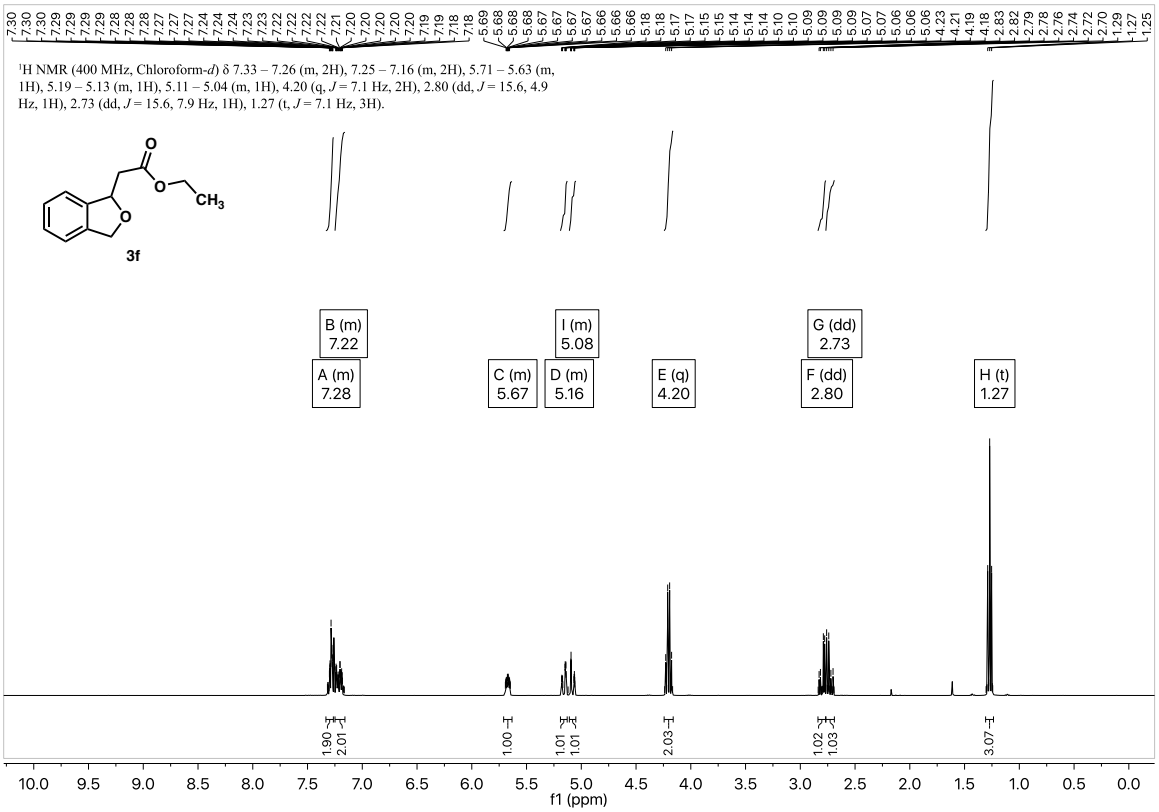

170.79
139.83
131.87
128.51
122.01
79.60
60.82
57.07
43.51
14.31
13C NMR (101 MHz, CDCl3) δ 170.8, 139.8, 131.9, 128.5, 122.0, 79.6, 60.8, 57.1, 43.5, 14.3.
O
O
CH3
OCH3
Br
3c
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

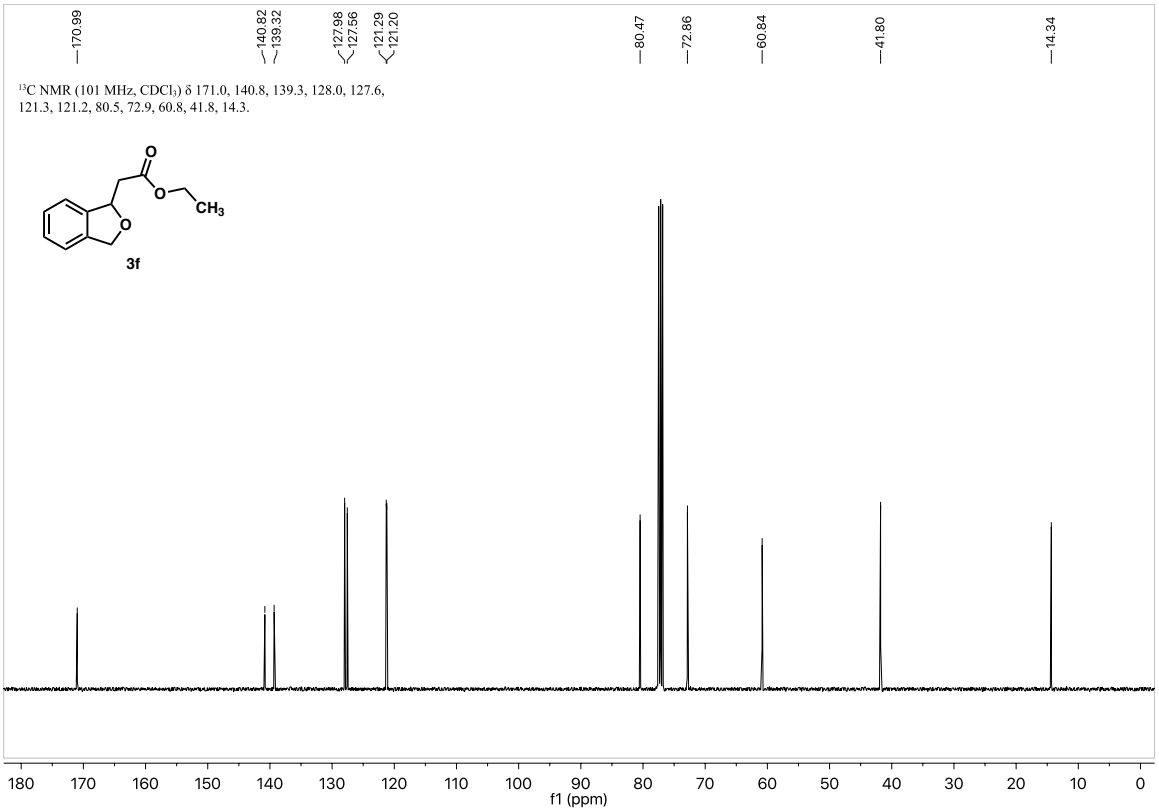

7.64 7.63 7.63 7.62 7.62 7.61 7.61 7.61 7.48 7.48 7.47 7.47 7.47 7.47 7.47 7.46 7.46 7.46 7.45 7.45 4.71 4.70 4.69 4.68 4.19 4.18 4.18 4.17 4.17 4.16 4.16 4.15 4.15 4.14 4.14 4.13 4.12 4.12 4.11 3.26 3.26 3.25 3.25 3.24 3.23 3.23 3.22 3.22 2.83 2.82 2.81 2.81 2.80 2.79 2.78 2.77 2.77 2.76 2.75 2.59 2.59 2.57 2.56 2.55 2.53 1.28 1.27 1.27 1.26 1.25 1.25 1.25 1.24 1.24 1.23 1.23 1.22 1.21 1.21
1H NMR (400 MHz, Chloroform-d) δ 7.62 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 4.70 (dd, J = 8.8, 4.9 Hz, 1H), 4.15 (qd, J = 7.2, 0.7 Hz, 2H), 3.24 (s, 3H), 2.79 (dd, J = 15.5, 8.9 Hz, 1H), 2.56 (dd, J = 15.4, 4.9 Hz, 1H), 1.24 (t, J = 7.1 Hz, 3H).
O
O
CH3
OCH3
F3C
3d
B (d) 7.46
A (d) 7.62
C (dd) 4.70
D (qd) 4.15
E (s) 3.24
F (dd) 2.79
G (dd) 2.56
H (t) 1.24
2.00
1.99
0.99
1.99
3.00
1.00
1.00
3.02
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
f1 (ppm)

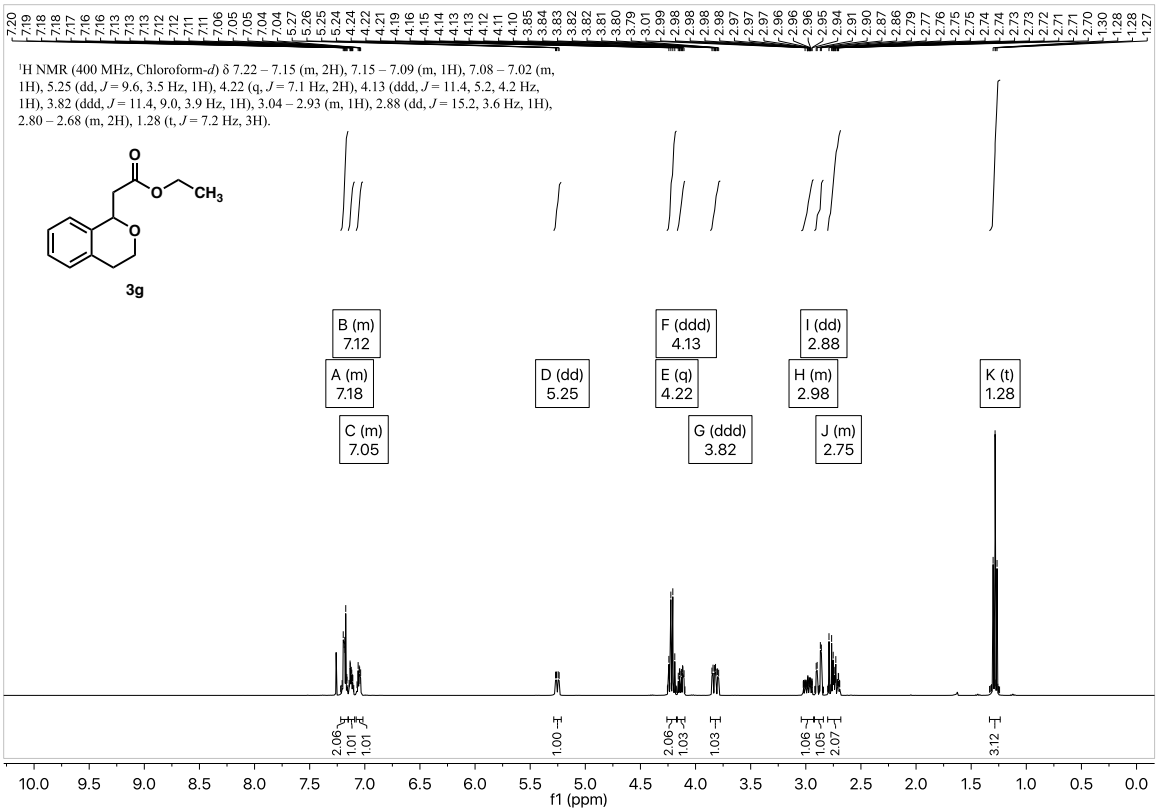

170.67
144.96
130.56
130.24
129.92
127.07
125.79
125.76
125.72
125.68
125.54
122.84
120.13
79.66
60.89
57.28
43.50
14.30
13C NMR (101 MHz, Chloroform-d) δ 170.7, 145.0, 130.4 (q, J = 32.4 Hz), 127.1, 125.7 (q, J = 3.8 Hz), 124.2 (q, J = 272.1 Hz), 79.7, 60.9, 57.3, 43.5, 14.3.
O
O
CH3
OCH3
F3C
3d
F (q) 124.19
D (s) 127.07
I (s) 57.28
A (s) 170.67
B (s) 144.96
C (q) 130.40
G (s) 79.66
H (s) 60.89
J (s) 43.50
K (s) 14.30
E (q) 125.74
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

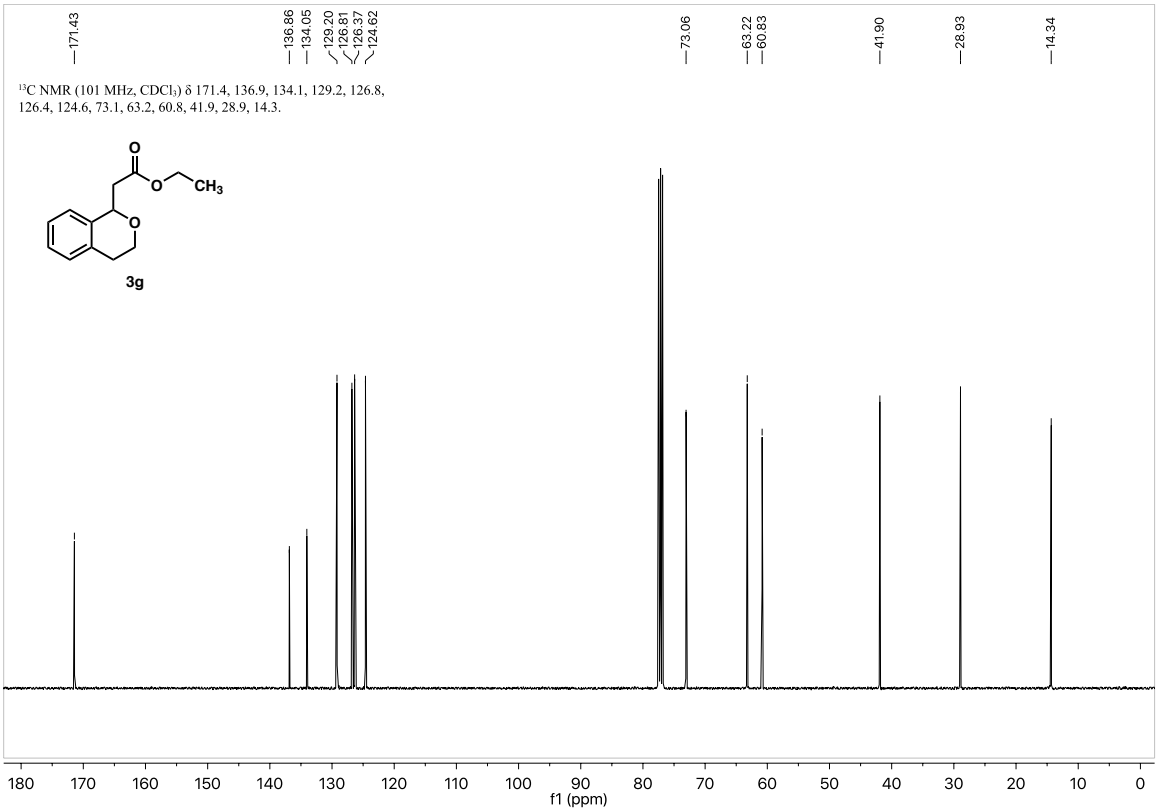

7.26 7.26 7.25 7.24 7.23 7.22 7.15 7.15 7.15 7.15 7.15 7.14 7.14 7.14 7.14 7.14 7.13 7.12 7.12 7.12 7.12 7.11 7.11 7.11 7.10 7.10 7.10 7.10 7.10 7.09 4.61 4.60 4.59 4.58 4.19 4.18 4.18 4.17 4.17 4.16 4.16 4.15 4.14 4.14 4.13 4.12 4.12 4.11 3.23 3.22 3.22 3.21 2.82 2.79 2.78 2.76 2.58 2.57 2.54 2.53 2.37 2.36 2.36 1.26 1.25 1.24 1.23 1.22 1.22
[1]H NMR (400 MHz, Chloroform-d) δ 7.27 – 7.21 (m, 1H), 7.16 – 7.08 (m, 3H), 4.60 (dd, J = 9.2, 4.6 Hz, 1H), 4.15 (qd, J = 7.1, 1.3 Hz, 2H), 3.22 (s, 3H), 2.79 (dd, J = 15.3, 9.3 Hz, 1H), 2.56 (dd, J = 15.3, 4.6 Hz, 1H), 2.36 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H).
O
O
CH3
H3C
OCH3
3e
B (m) 7.13
A (m) 7.24
C (dd) 4.60
D (qd) 4.15
E (s) 3.22
F (dd) 2.79
G (dd) 2.56
H (s) 2.36
I (t) 1.24
1.28
3.00
1.00
2.03
3.03
1.01
1.00
3.05
3.06
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
f1 (ppm)

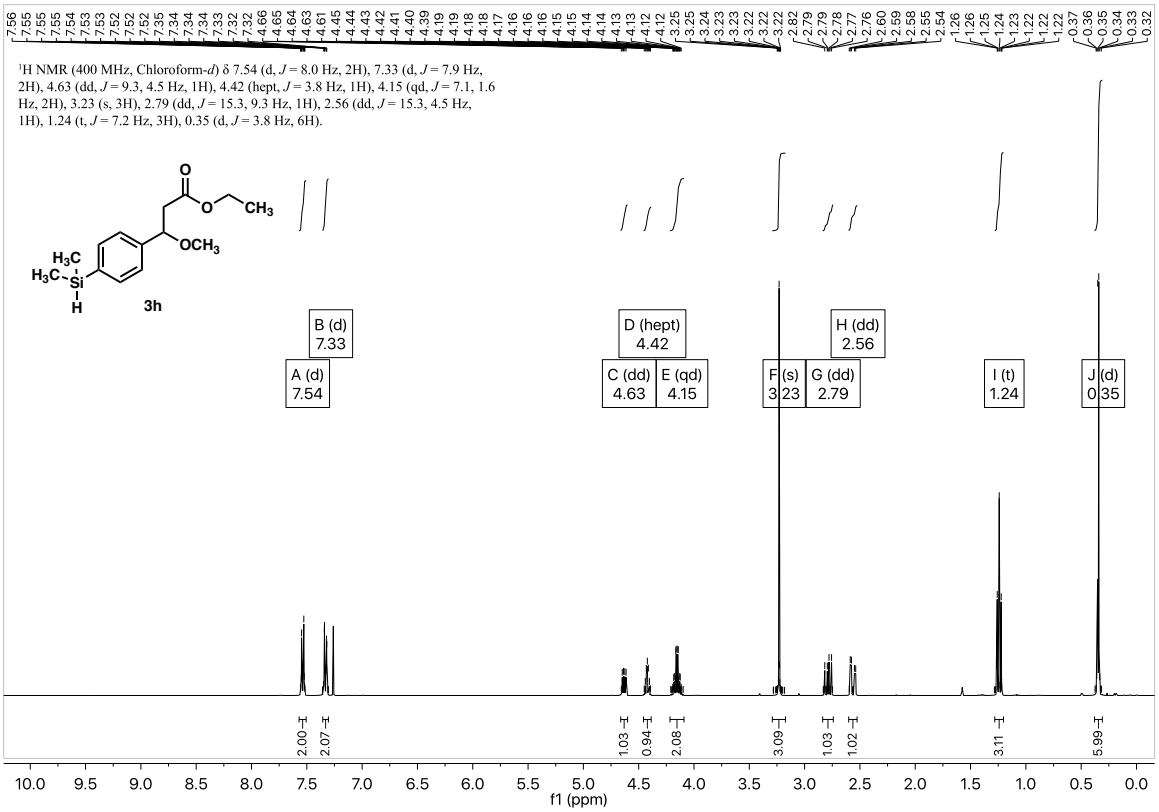

171.19
140.67
138.35
128.88
128.57
127.39
123.83
80.23
60.68
57.00
43.71
21.59
14.32
[13]C NMR (101 MHz, CDCl3) δ 171.2, 140.7, 138.3, 128.9, 128.6, 127.4, 123.8, 80.2, 60.7, 57.0, 43.7, 21.6, 14.3.
O
O
CH3
H3C
OCH3
3e
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

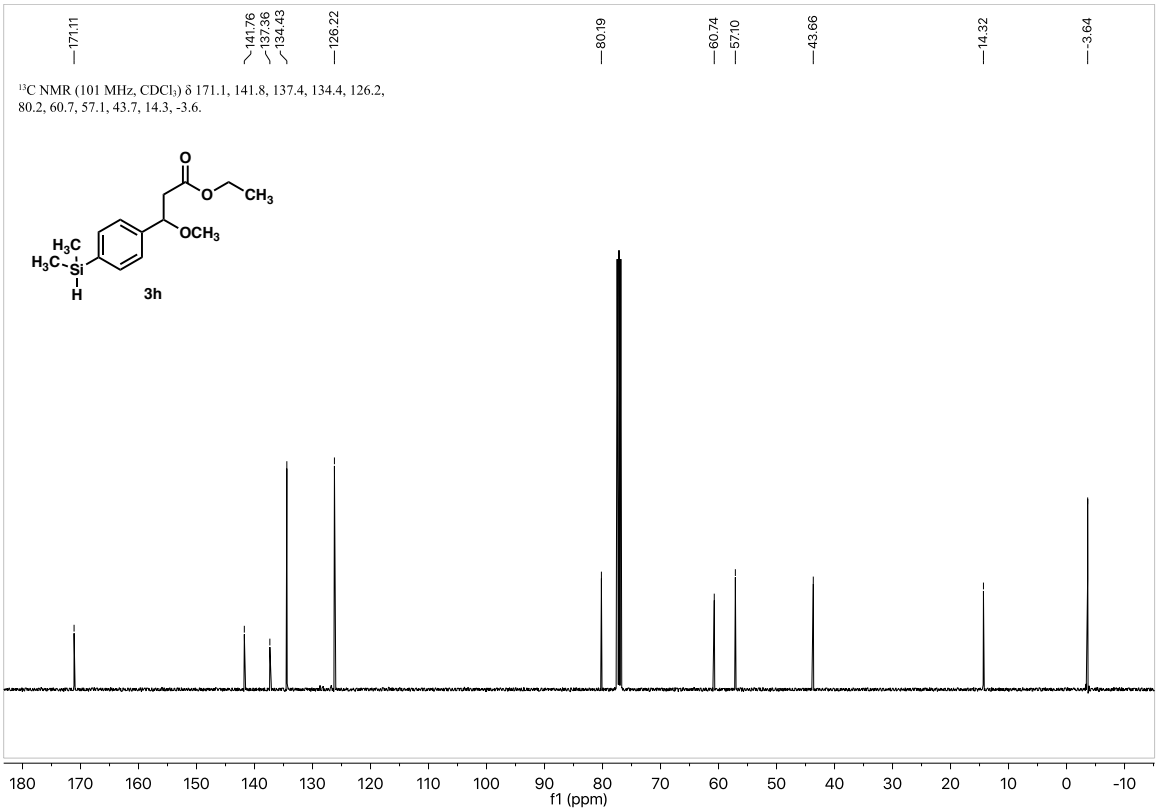

7.30 7.30 7.30 7.29 7.29 7.29 7.29 7.28 7.28 7.28 7.27 7.27 7.27 7.24 7.24 7.24 7.23 7.23 7.22 7.22 7.22 7.22 7.21 7.20 7.20 7.20 7.20 7.20 7.19 7.19 7.18 7.18 5.69 5.68 5.68 5.68 5.67 5.67 5.67 5.67 5.66 5.66 5.66 5.18 5.17 5.17 5.15 5.15 5.14 5.14 5.14 5.10 5.10 5.09 5.09 5.09 5.09 5.09 5.07 5.07 5.06 5.06 5.06 4.23 4.21 4.19 4.18 2.83 2.82 2.79 2.78 2.76 2.74 2.72 2.70 1.29 1.27 1.25
[1]H NMR (400 MHz, Chloroform-d) δ 7.33 – 7.26 (m, 2H), 7.25 – 7.16 (m, 2H), 5.71 – 5.63 (m, 1H), 5.19 – 5.13 (m, 1H), 5.11 – 5.04 (m, 1H), 4.20 (q, J = 7.1 Hz, 2H), 2.80 (dd, J = 15.6, 4.9 Hz, 1H), 2.73 (dd, J = 15.6, 7.9 Hz, 1H), 1.27 (t, J = 7.1 Hz, 3H).
O
O
CH3
O
3f
B (m) 7.22
A (m) 7.28
C (m) 5.67
I (m) 5.08
D (m) 5.16
E (q) 4.20
G (dd) 2.73
F (dd) 2.80
H (t) 1.27
1.90
2.01
1.00
1.01
1.01
2.03
1.02
1.03
3.07
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
f1 (ppm)

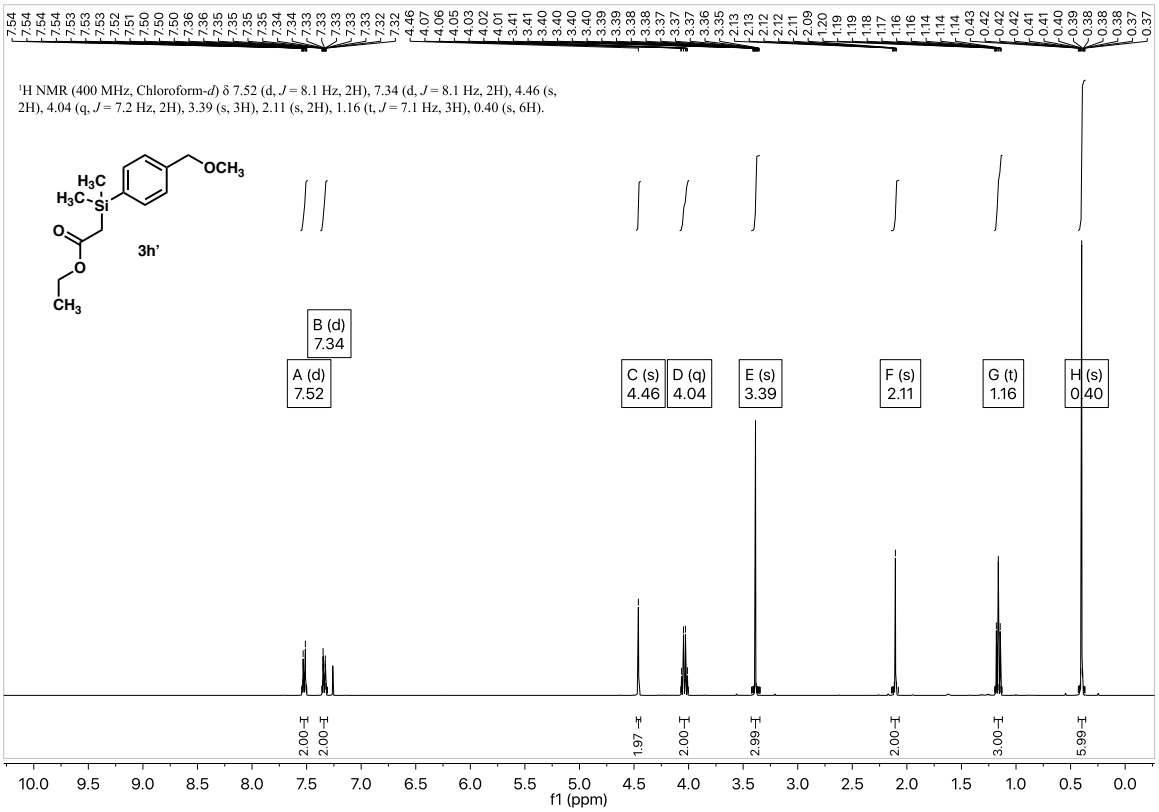

170.99
140.82
139.32
127.98
127.56
121.29
121.20
80.47
72.86
60.84
41.80
14.34
[13]C NMR (101 MHz, CDCl3) δ 171.0, 140.8, 139.3, 128.0, 127.6, 121.3, 121.2, 80.5, 72.9, 60.8, 41.8, 14.3.
O
O
CH3
O
3f
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

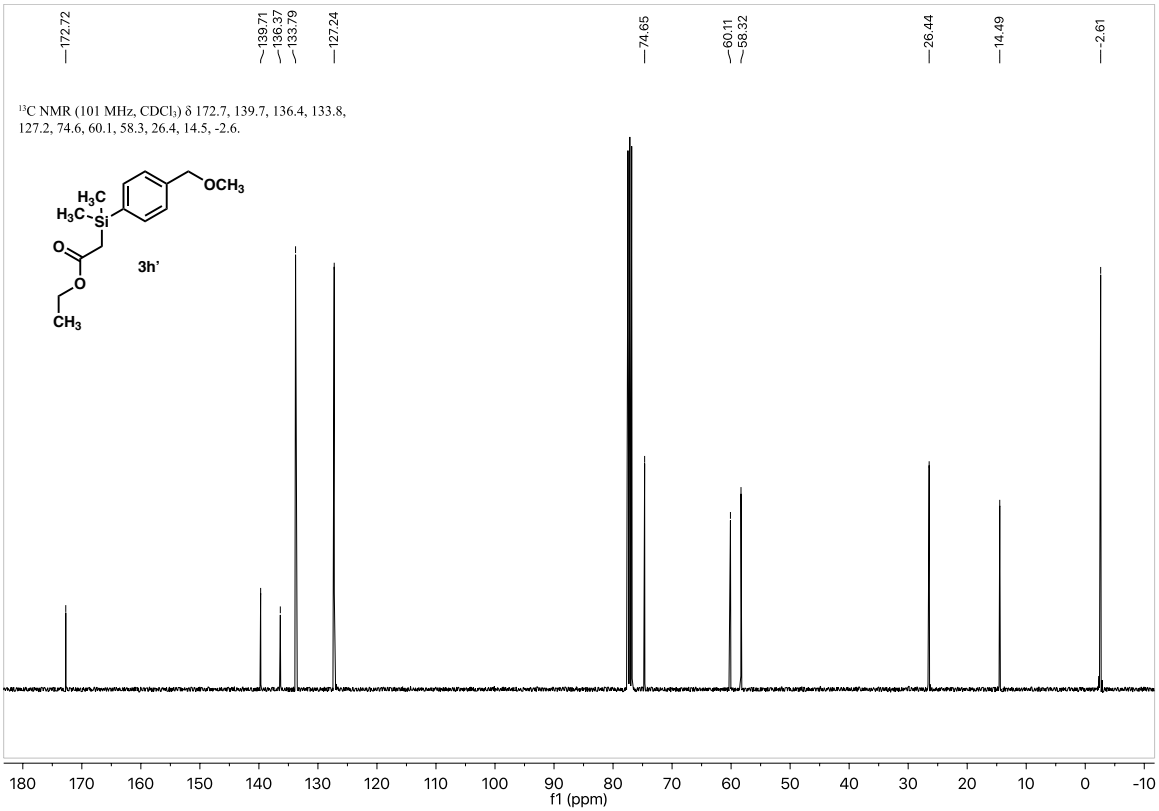

7.20 7.19 7.18 7.18 7.17 7.16 7.16 7.13 7.13 7.13 7.12 7.12 7.11 7.11 7.06 7.05 7.05 7.04 7.04 5.27 5.26 5.25 5.24 4.24 4.22 4.21 4.19 4.16 4.15 4.14 4.13 4.12 4.11 4.10 3.85 3.84 3.83 3.82 3.82 3.81 3.80 3.79 3.01 2.99 2.98 2.98 2.98 2.97 2.97 2.97 2.96 2.96 2.96 2.95 2.94 2.91 2.90 2.87 2.86 2.79 2.77 2.76 2.75 2.75 2.74 2.74 2.73 2.72 2.71 2.71 2.70 1.30 1.28 1.28 1.27
1H NMR (400 MHz, Chloroform-d) δ 7.22 – 7.15 (m, 2H), 7.15 – 7.09 (m, 1H), 7.08 – 7.02 (m, 1H), 5.25 (dd, J = 9.6, 3.5 Hz, 1H), 4.22 (q, J = 7.1 Hz, 2H), 4.13 (ddd, J = 11.4, 5.2, 4.2 Hz, 1H), 3.82 (ddd, J = 11.4, 9.0, 3.9 Hz, 1H), 3.04 – 2.93 (m, 1H), 2.88 (dd, J = 15.2, 3.6 Hz, 1H), 2.80 – 2.68 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H).
3g
B (m) 7.12
A (m) 7.18
C (m) 7.05
D (dd) 5.25
F (ddd) 4.13
E (q) 4.22
G (ddd) 3.82
I (dd) 2.88
H (m) 2.98
J (m) 2.75
K (t) 1.28
2.06 1.01 1.01 1.00 2.06 1.03 1.03 1.06 1.05 2.07 3.12
f1 (ppm)

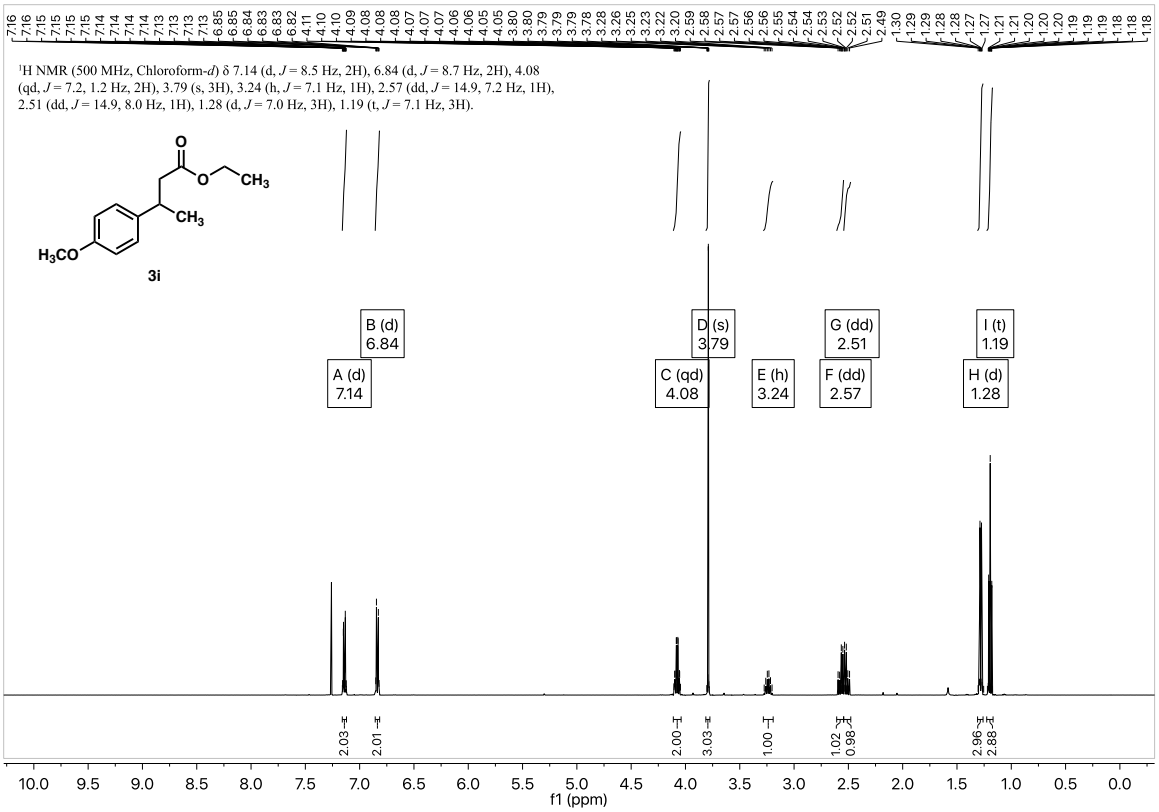

171.43 136.86 134.05 129.20 126.81 126.37 124.62 73.06 63.22 60.83 41.90 28.93 14.34
13C NMR (101 MHz, CDCl3) δ 171.4, 136.9, 134.1, 129.2, 126.8, 126.4, 124.6, 73.1, 63.2, 60.8, 41.9, 28.9, 14.3.
3g
f1 (ppm)

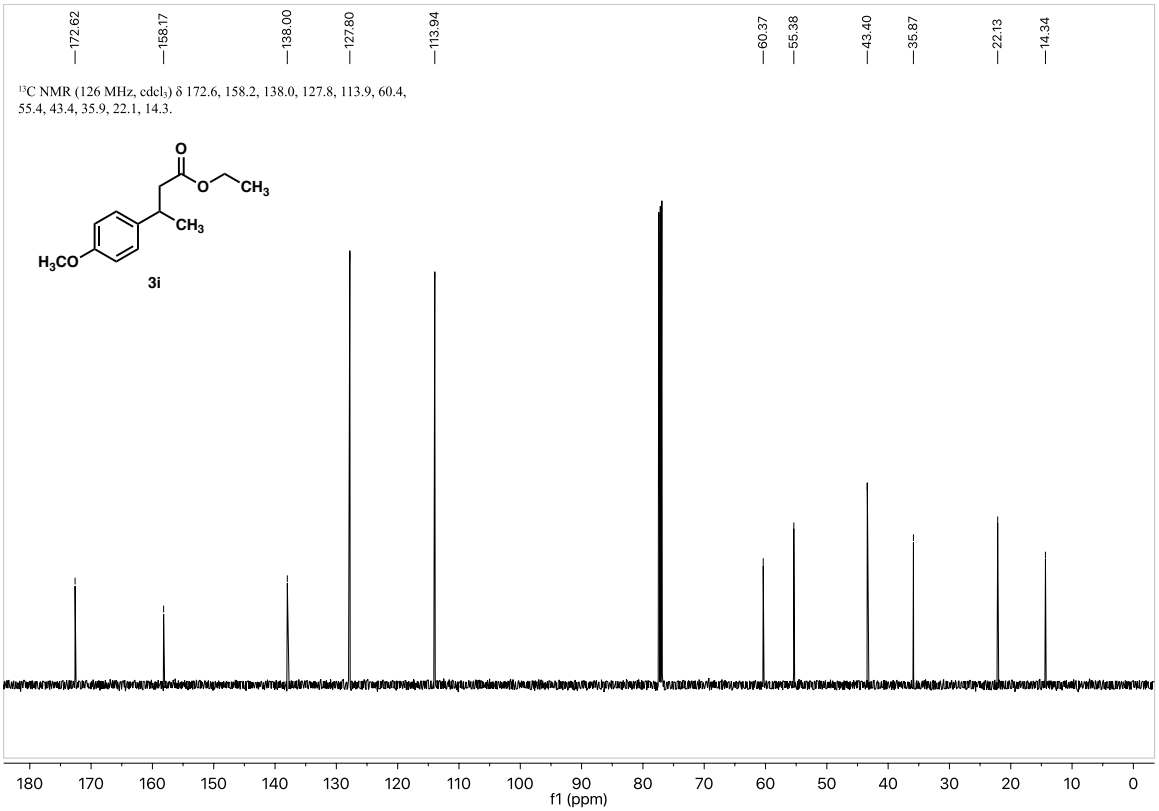

7.56 7.55 7.55 7.55 7.54 7.53 7.53 7.52 7.52 7.52 7.52 7.35 7.34 7.34 7.34 7.33 7.32 7.32 4.66 4.65 4.64 4.63 4.61 4.45 4.44 4.43 4.42 4.41 4.40 4.39 4.19 4.19 4.18 4.18 4.17 4.16 4.16 4.16 4.15 4.15 4.14 4.14 4.13 4.12 4.12 3.25 3.25 3.24 3.23 3.23 3.22 3.22 3.22 2.82 2.79 2.79 2.78 2.77 2.76 2.60 2.59 2.58 2.55 2.54 1.26 1.26 1.25 1.24 1.23 1.22 1.22 1.22 0.37 0.36 0.35 0.34 0.33 0.32
1H NMR (400 MHz, Chloroform-d) δ 7.54 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 4.63 (dd, J = 9.3, 4.5 Hz, 1H), 4.42 (hept, J = 3.8 Hz, 1H), 4.15 (qd, J = 7.1, 1.6 Hz, 2H), 3.23 (s, 3H), 2.79 (dd, J = 15.3, 9.3 Hz, 1H), 2.56 (dd, J = 15.3, 4.5 Hz, 1H), 1.24 (t, J = 7.2 Hz, 3H), 0.35 (d, J = 3.8 Hz, 6H).
O
O
CH3
OCH3
H3C
H3C
Si
H
3h
B (d) 7.33
A (d) 7.54
D (hept) 4.42
C (dd) 4.63
E (qd) 4.15
F (s) 3.23
H (dd) 2.56
G (dd) 2.79
I (t) 1.24
J (d) 0.35
2.00
2.07
1.03
0.94
2.08
3.09
1.03
1.02
3.11
5.99
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
f1 (ppm)

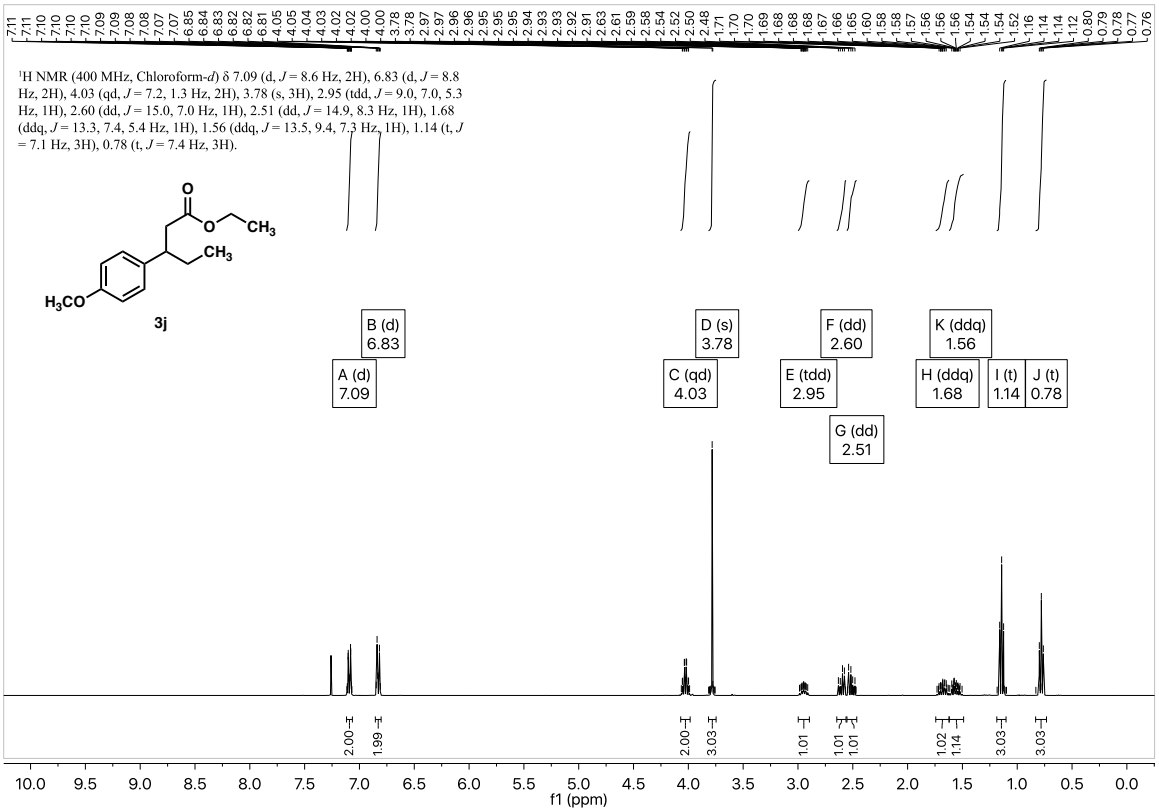

171.11
141.76
137.36
134.43
126.22
80.19
60.74
57.10
43.66
14.32
-3.64
13C NMR (101 MHz, CDCl3) δ 171.1, 141.8, 137.4, 134.4, 126.2, 80.2, 60.7, 57.1, 43.7, 14.3, -3.6.
O
O
CH3
OCH3
H3C
H3C
Si
H
3h
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

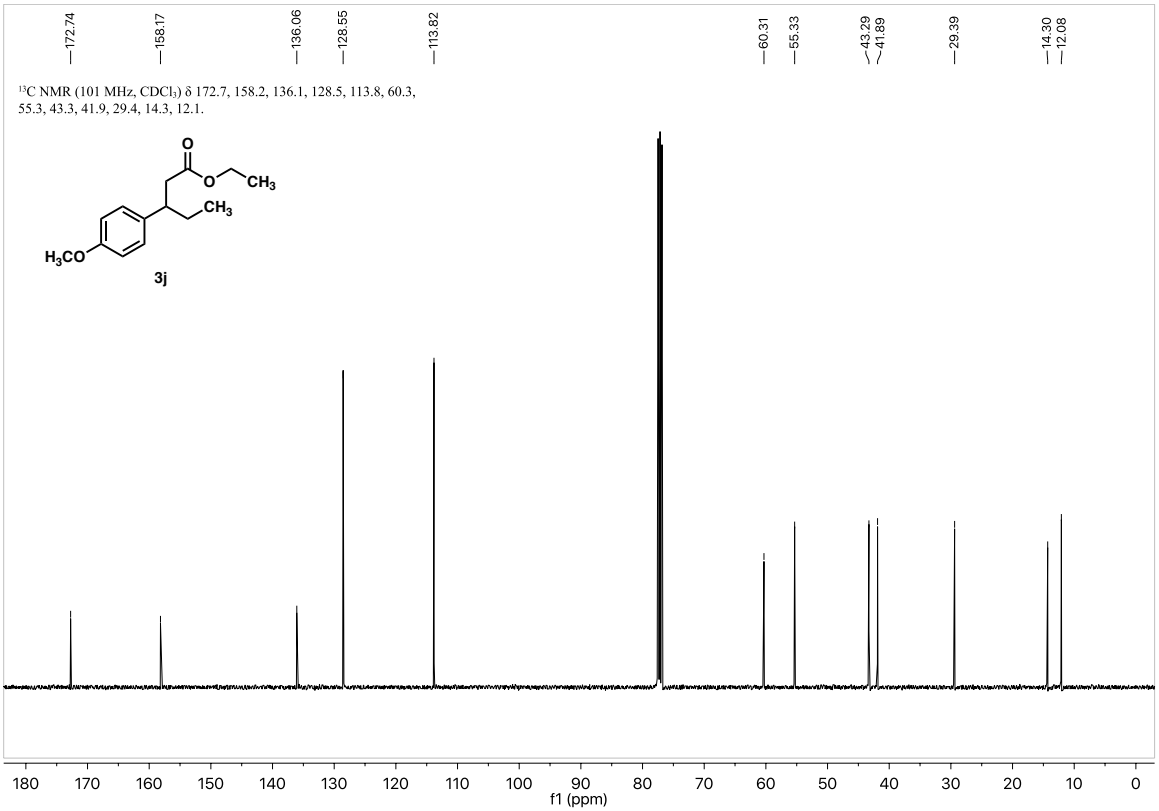

7.54 7.54 7.54 7.54 7.53 7.53 7.53 7.52 7.51 7.50 7.50 7.50 7.36 7.35 7.35 7.35 7.34 7.34 7.33 7.33 7.33 7.33 7.32 7.32
4.46 4.07 4.06 4.05 4.03 4.02 4.01 3.41 3.41 3.40 3.40 3.40 3.39 3.39 3.38 3.38 3.37 3.37 3.36 3.35 2.13 2.12 2.12 2.11 2.09 1.20 1.19 1.19 1.18 1.17 1.16 1.16 1.14 1.14 1.14 0.43 0.42 0.42 0.42 0.41 0.41 0.40 0.39 0.38 0.38 0.38 0.37 0.37
[1]H NMR (400 MHz, Chloroform-d) δ 7.52 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 4.46 (s, 2H), 4.04 (q, J = 7.2 Hz, 2H), 3.39 (s, 3H), 2.11 (s, 2H), 1.16 (t, J = 7.1 Hz, 3H), 0.40 (s, 6H).
H3C
H3C
Si
OCH3
O
O
CH3
3h'
B (d) 7.34
A (d) 7.52
C (s) 4.46
D (q) 4.04
E (s) 3.39
F (s) 2.11
G (t) 1.16
H (s) 0.40
2.00
2.00
1.97
2.00
2.99
2.00
3.00
5.99
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
f1 (ppm)

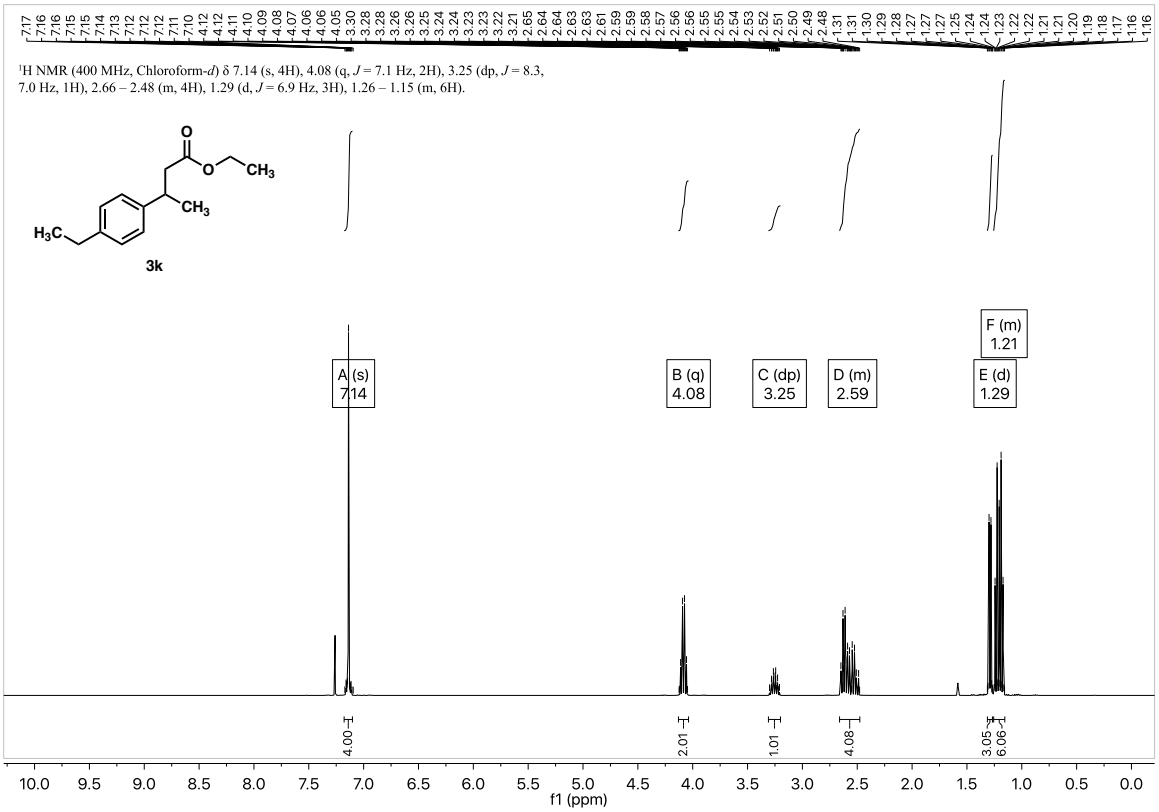

172.72
139.71
136.37
133.79
127.24
74.65
60.11
58.32
26.44
14.49
-2.61
[13]C NMR (101 MHz, CDCl3) δ 172.7, 139.7, 136.4, 133.8, 127.2, 74.6, 60.1, 58.3, 26.4, 14.5, -2.6.
H3C
H3C
Si
OCH3
O
O
CH3
3h'
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

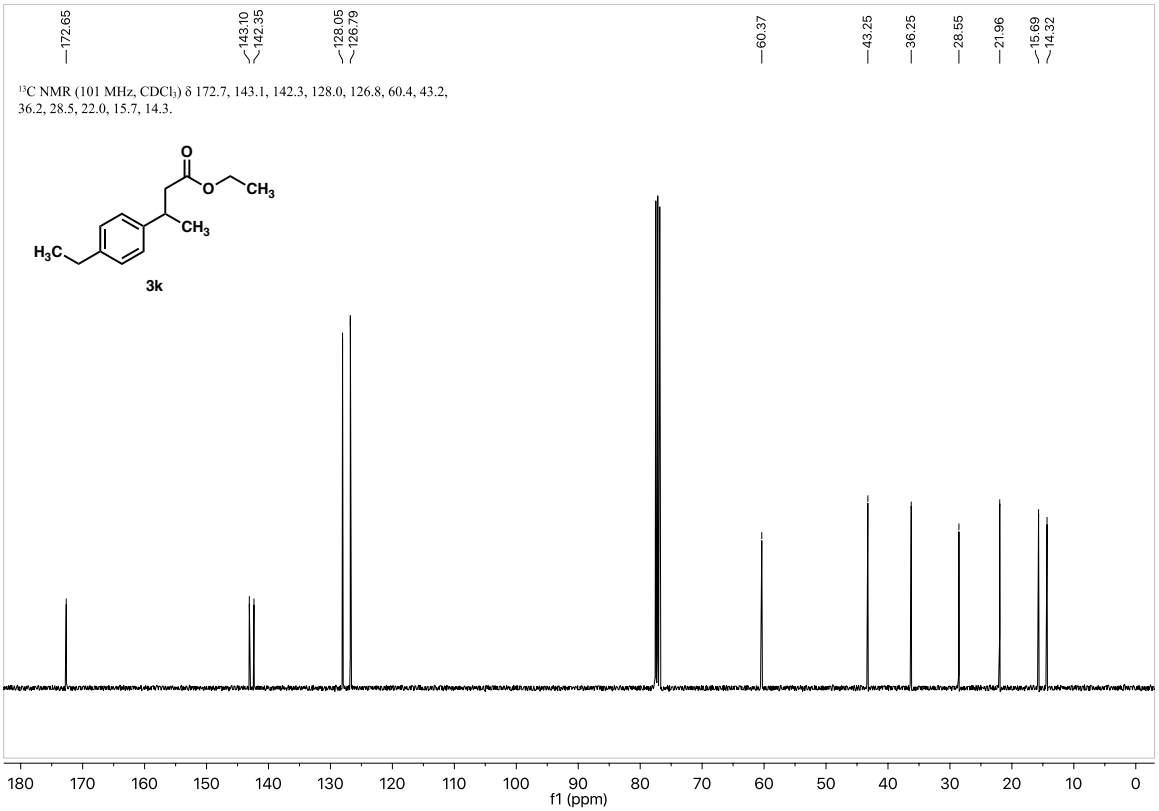

7.16 7.16 7.15 7.15 7.15 7.15 7.14 7.14 7.14 7.14 7.13 7.13 7.13 7.13 6.85 6.85 6.84 6.83 6.83 6.82 4.11 4.10 4.10 4.09 4.08 4.08 4.08 4.07 4.07 4.07 4.06 4.06 4.05 4.05 3.80 3.80 3.79 3.79 3.79 3.78 3.28 3.26 3.25 3.23 3.22 3.20 2.59 2.58 2.57 2.57 2.56 2.56 2.55 2.54 2.54 2.53 2.52 2.52 2.51 2.49 1.30 1.29 1.29 1.28 1.28 1.27 1.27 1.21 1.21 1.20 1.20 1.20 1.19 1.19 1.19 1.18 1.18 1.18
1H NMR (500 MHz, Chloroform-d) δ 7.14 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 4.08 (qd, J = 7.2, 1.2 Hz, 2H), 3.79 (s, 3H), 3.24 (h, J = 7.1 Hz, 1H), 2.57 (dd, J = 14.9, 7.2 Hz, 1H), 2.51 (dd, J = 14.9, 8.0 Hz, 1H), 1.28 (d, J = 7.0 Hz, 3H), 1.19 (t, J = 7.1 Hz, 3H).
O
O
CH3
CH3
H3CO
3i
A (d) 7.14
B (d) 6.84
C (qd) 4.08
D (s) 3.79
E (h) 3.24
F (dd) 2.57
G (dd) 2.51
H (d) 1.28
I (t) 1.19
2.03
2.01
2.00
3.03
1.00
1.02
0.98
2.96
2.88
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
f1 (ppm)

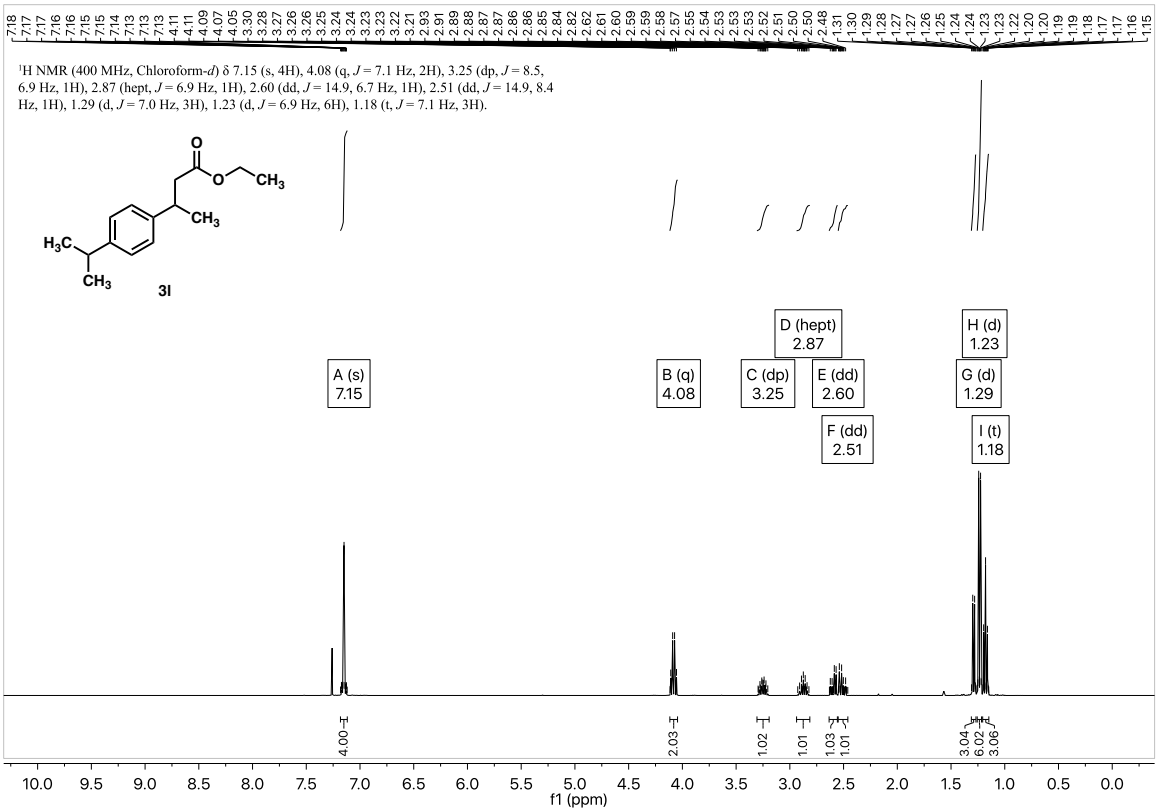

172.62
158.17
138.00
127.80
113.94
60.37
55.38
43.40
35.87
22.13
14.34
13C NMR (126 MHz, cdcl3) δ 172.6, 158.2, 138.0, 127.8, 113.9, 60.4, 55.4, 43.4, 35.9, 22.1, 14.3.
O
O
CH3
CH3
H3CO
3i
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

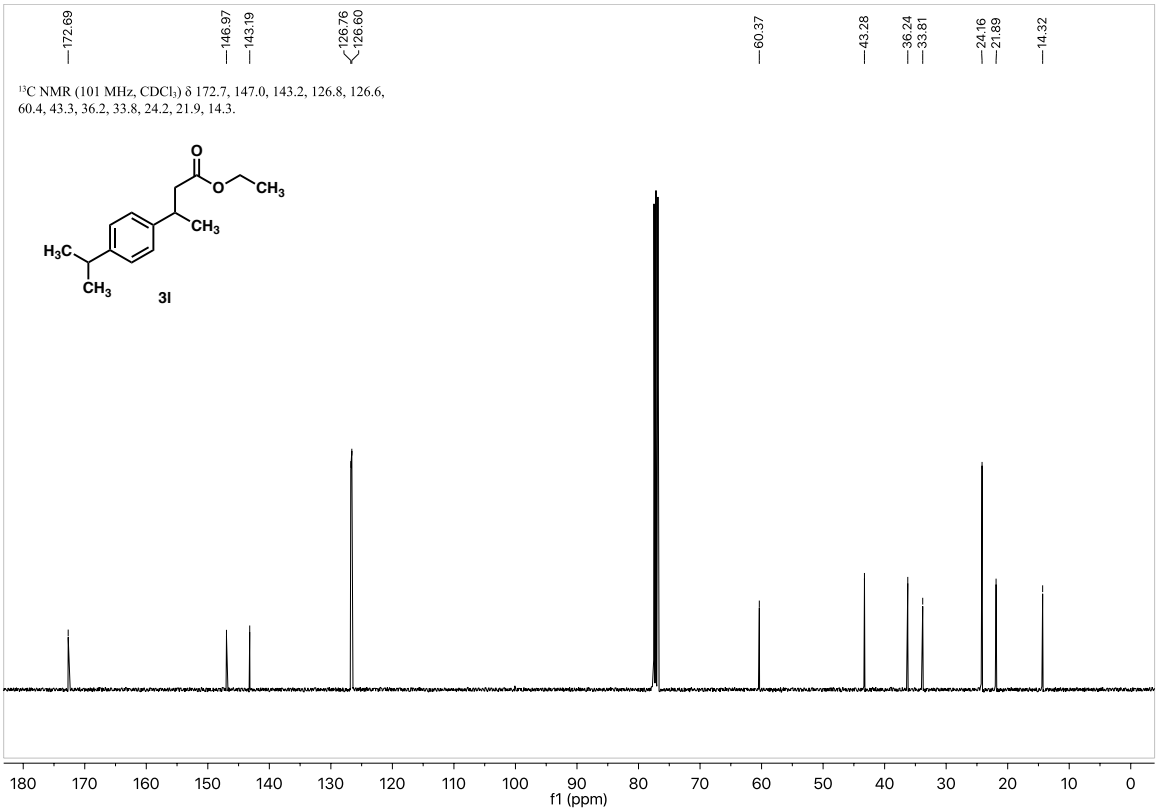

7.11 7.11 7.10 7.10 7.10 7.10 7.09 7.09 7.09 7.08 7.08 7.08 7.07 7.07 7.07 6.85 6.84 6.83 6.82 6.82 6.81 4.05 4.04 4.03 4.02 4.02 4.00 4.00 3.78 3.78 2.97 2.97 2.96 2.96 2.95 2.95 2.95 2.94 2.93 2.93 2.92 2.91 2.63 2.61 2.59 2.58 2.54 2.52 2.50 2.48 1.71 1.70 1.69 1.68 1.68 1.67 1.66 1.65 1.60 1.58 1.58 1.57 1.56 1.56 1.56 1.54 1.54 1.54 1.52 1.16 1.14 1.12 0.80 0.79 0.78 0.77 0.76
1H NMR (400 MHz, Chloroform-d) δ 7.09 (d, J = 8.6 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 4.03 (qd, J = 7.2, 1.3 Hz, 2H), 3.78 (s, 3H), 2.95 (tdd, J = 9.0, 7.0, 5.3 Hz, 1H), 2.60 (dd, J = 15.0, 7.0 Hz, 1H), 2.51 (dd, J = 14.9, 8.3 Hz, 1H), 1.68 (ddq, J = 13.3, 7.4, 5.4 Hz, 1H), 1.56 (ddq, J = 13.5, 9.4, 7.3 Hz, 1H), 1.14 (t, J = 7.1 Hz, 3H), 0.78 (t, J = 7.4 Hz, 3H).
O
O
CH3
CH3
H3CO
3j
A (d) 7.09
B (d) 6.83
C (qd) 4.03
D (s) 3.78
E (tdd) 2.95
F (dd) 2.60
G (dd) 2.51
H (ddq) 1.68
I (t) 1.14
J (t) 0.78
K (ddq) 1.56
2.00 1.99 2.00 3.03 1.01 1.01 1.01 1.02 1.14 3.03 3.03
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
f1 (ppm)

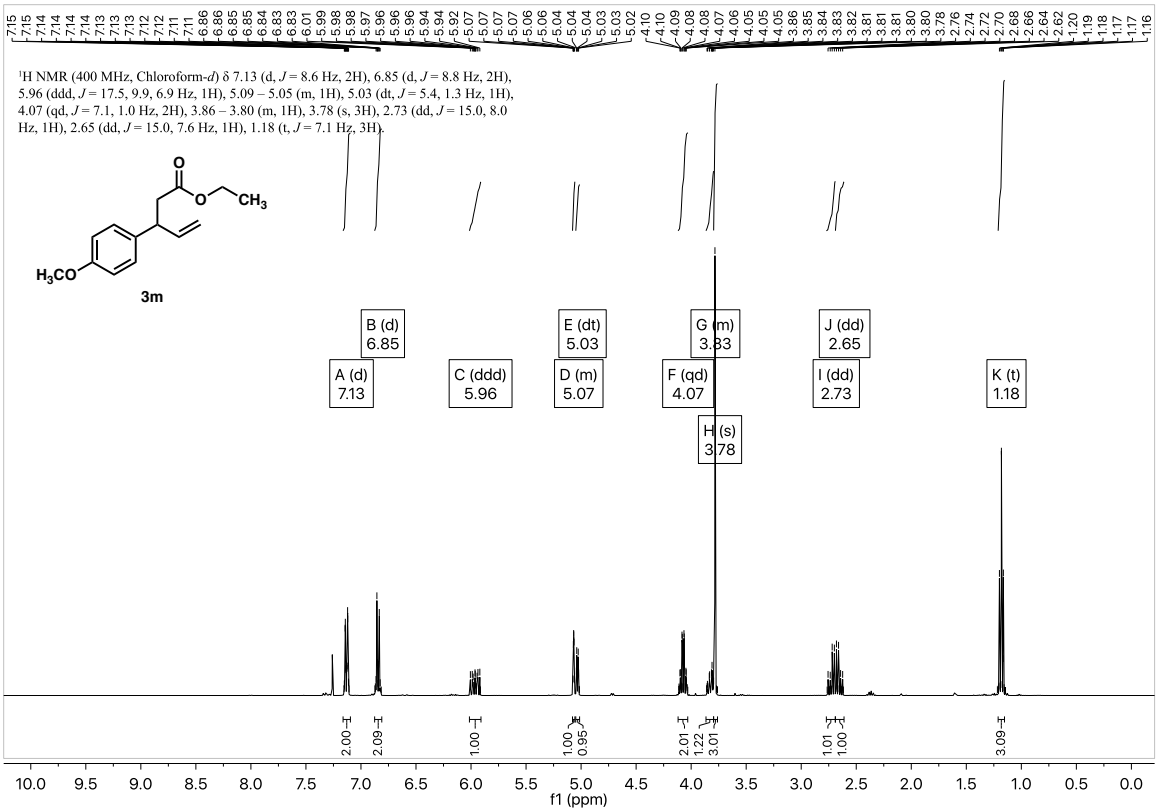

172.74 158.17 136.06 128.55 113.82 60.31 55.33 43.29 41.89 29.39 14.30 12.08
13C NMR (101 MHz, CDCl3) δ 172.7, 158.2, 136.1, 128.5, 113.8, 60.3, 55.3, 43.3, 41.9, 29.4, 14.3, 12.1.
O
O
CH3
CH3
H3CO
3j
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

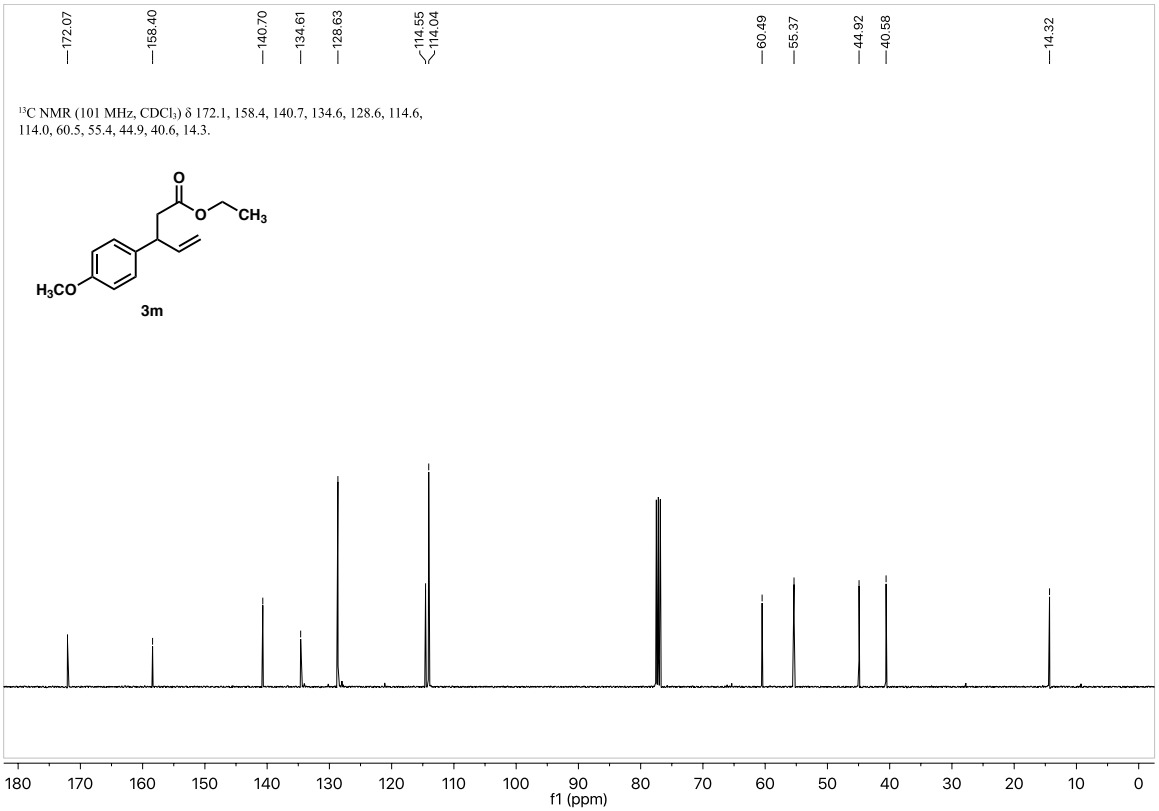

7.17 7.16 7.16 7.15 7.15 7.14 7.13 7.12 7.12 7.12 7.11 7.10 4.12 4.11 4.10 4.09 4.08 4.07 4.06 4.06 4.05 3.30 3.28 3.28 3.26 3.26 3.25 3.24 3.24 3.23 3.23 3.22 3.21 2.65 2.64 2.64 2.63 2.61 2.59 2.59 2.58 2.57 2.56 2.56 2.55 2.55 2.54 2.53 2.52 2.51 2.50 2.49 2.48 1.31 1.31 1.30 1.29 1.28 1.27 1.27 1.25 1.24 1.24 1.23 1.22 1.21 1.21 1.20 1.19 1.18 1.17 1.16 1.16
1H NMR (400 MHz, Chloroform-d) δ 7.14 (s, 4H), 4.08 (q, J = 7.1 Hz, 2H), 3.25 (dp, J = 8.3, 7.0 Hz, 1H), 2.66 – 2.48 (m, 4H), 1.29 (d, J = 6.9 Hz, 3H), 1.26 – 1.15 (m, 6H).
O
O
CH3
CH3
H3C
3k
A (s) 7.14
B (q) 4.08
C (dp) 3.25
D (m) 2.59
F (m) 1.21
E (d) 1.29
4.00
2.01
1.01
4.08
3.05
6.06
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
f1 (ppm)

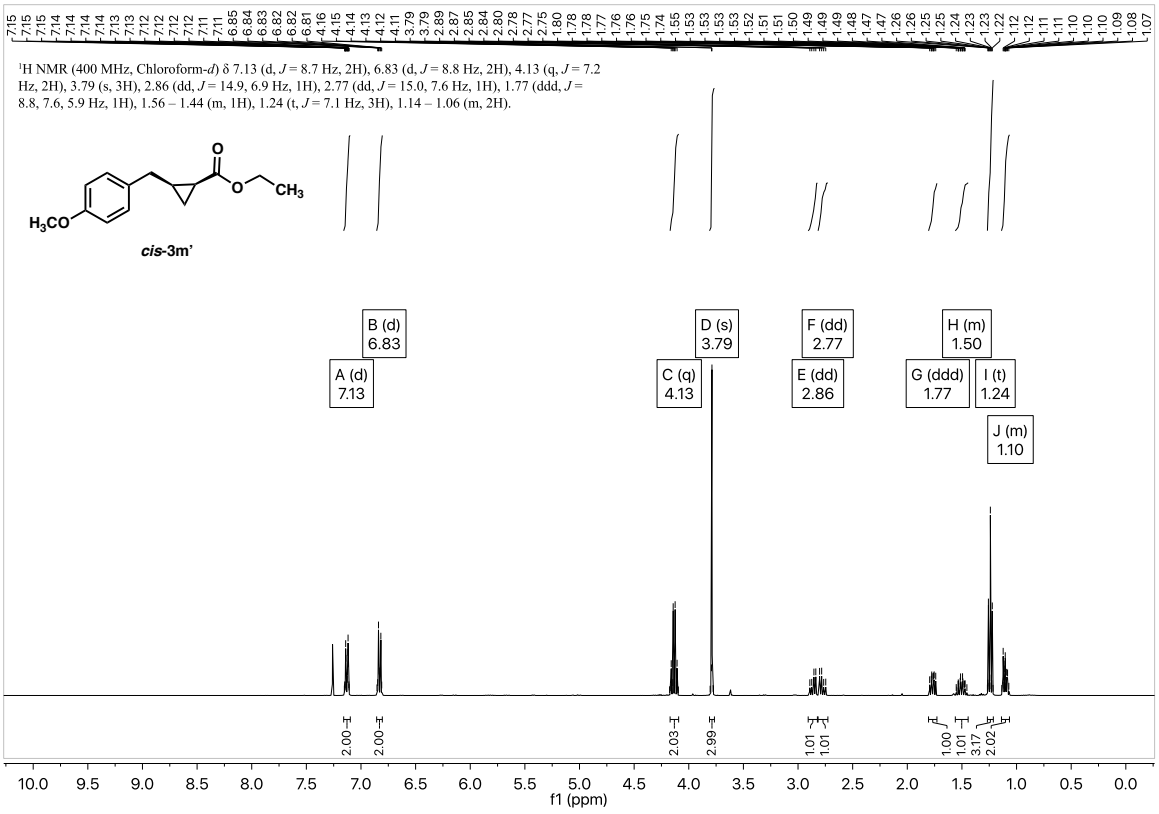

172.65
143.10 142.35
128.05 126.79
60.37
43.25
36.25
28.55
21.96
15.69 14.32
13C NMR (101 MHz, CDCl3) δ 172.7, 143.1, 142.3, 128.0, 126.8, 60.4, 43.2, 36.2, 28.5, 22.0, 15.7, 14.3.
O
O
CH3
CH3
H3C
3k
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

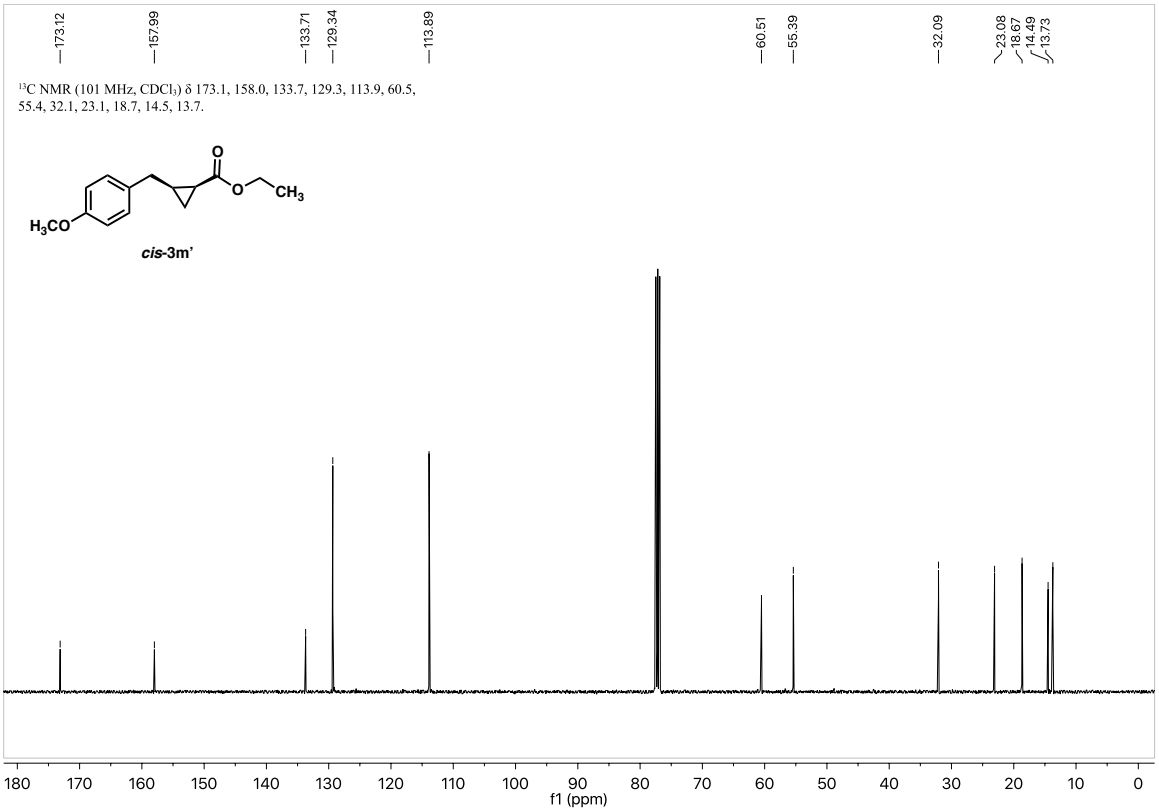

7.18 7.17 7.17 7.16 7.16 7.15 7.15 7.14 7.13 7.13 7.13 4.11 4.11 4.09 4.07 4.05 3.30 3.28 3.27 3.26 3.26 3.25 3.24 3.24 3.23 3.23 3.22 3.21 2.93 2.91 2.89 2.88 2.87 2.87 2.86 2.86 2.85 2.84 2.82 2.62 2.61 2.60 2.59 2.59 2.58 2.57 2.55 2.54 2.53 2.53 2.52 2.51 2.50 2.50 2.48 1.31 1.30 1.29 1.28 1.27 1.27 1.26 1.25 1.24 1.24 1.23 1.23 1.22 1.20 1.20 1.19 1.19 1.18 1.17 1.17 1.16 1.15
1H NMR (400 MHz, Chloroform-d) δ 7.15 (s, 4H), 4.08 (q, J = 7.1 Hz, 2H), 3.25 (dp, J = 8.5, 6.9 Hz, 1H), 2.87 (hept, J = 6.9 Hz, 1H), 2.60 (dd, J = 14.9, 6.7 Hz, 1H), 2.51 (dd, J = 14.9, 8.4 Hz, 1H), 1.29 (d, J = 7.0 Hz, 3H), 1.23 (d, J = 6.9 Hz, 6H), 1.18 (t, J = 7.1 Hz, 3H).
O
O
CH3
CH3
H3C
CH3
3l
A (s) 7.15
B (q) 4.08
C (dp) 3.25
D (hept) 2.87
E (dd) 2.60
F (dd) 2.51
G (d) 1.29
H (d) 1.23
I (t) 1.18
4.00
2.03
1.02
1.01
1.03
1.01
3.04
6.02
3.06
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
f1 (ppm)

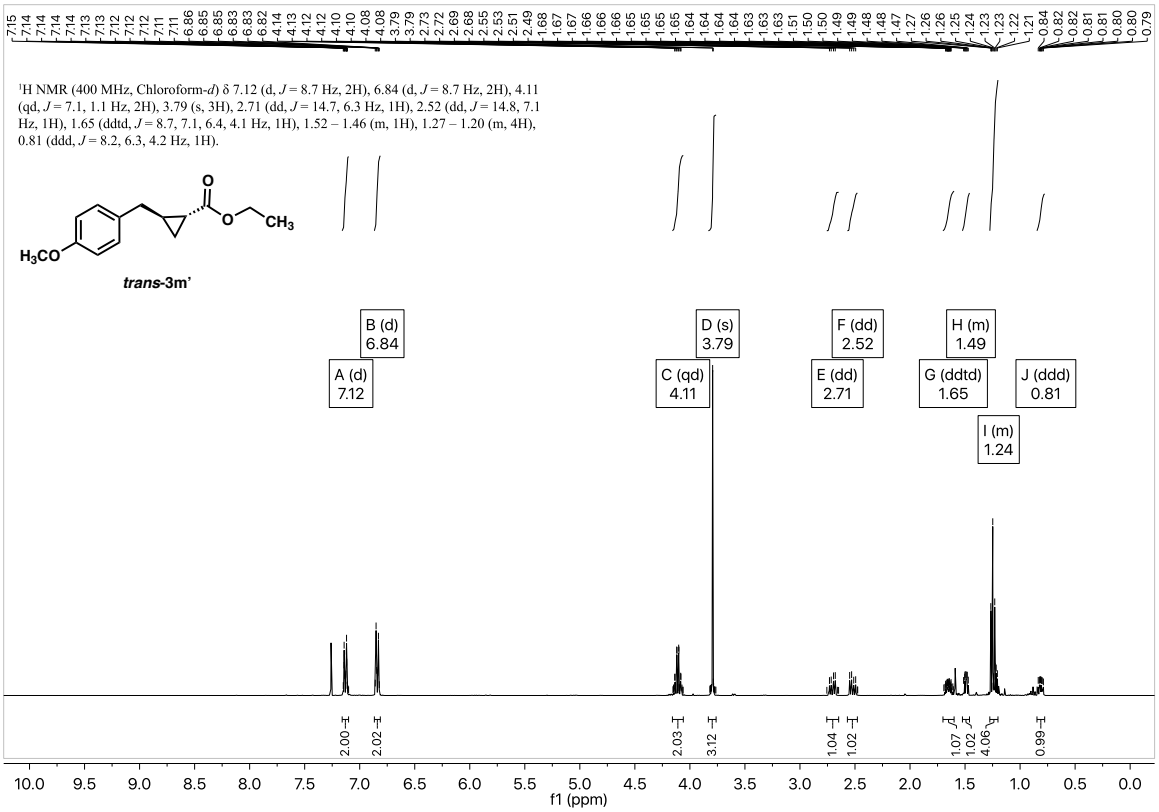

172.69
146.97
143.19
126.76
126.60
60.37
43.28
36.24
33.81
24.16
21.89
14.32
13C NMR (101 MHz, CDCl3) δ 172.7, 147.0, 143.2, 126.8, 126.6, 60.4, 43.3, 36.2, 33.8, 24.2, 21.9, 14.3.
O
O
CH3
CH3
H3C
CH3
3l
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

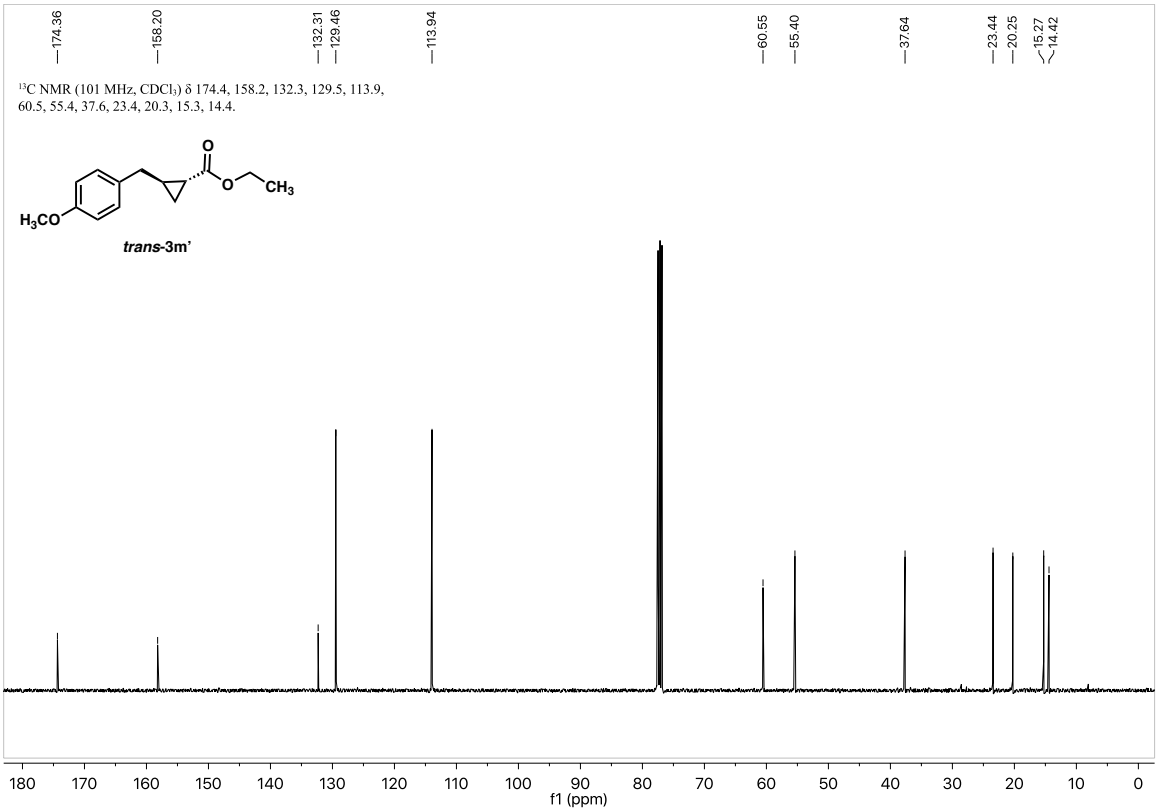

$^1$H NMR (400 MHz, Chloroform-*d*) δ 7.13 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.96 (ddd, *J* = 17.5, 9.9, 6.9 Hz, 1H), 5.09 – 5.05 (m, 1H), 5.03 (dt, *J* = 5.4, 1.3 Hz, 1H), 4.07 (qd, *J* = 7.1, 1.0 Hz, 2H), 3.86 – 3.80 (m, 1H), 3.78 (s, 3H), 2.73 (dd, *J* = 15.0, 8.0 Hz, 1H), 2.65 (dd, *J* = 15.0, 7.6 Hz, 1H), 1.18 (t, *J* = 7.1 Hz, 3H).
3m
A (d) 7.13
B (d) 6.85
C (ddd) 5.96
D (m) 5.07
E (dt) 5.03
F (qd) 4.07
G (m) 3.83
H (s) 3.78
I (dd) 2.73
J (dd) 2.65
K (t) 1.18
f1 (ppm)

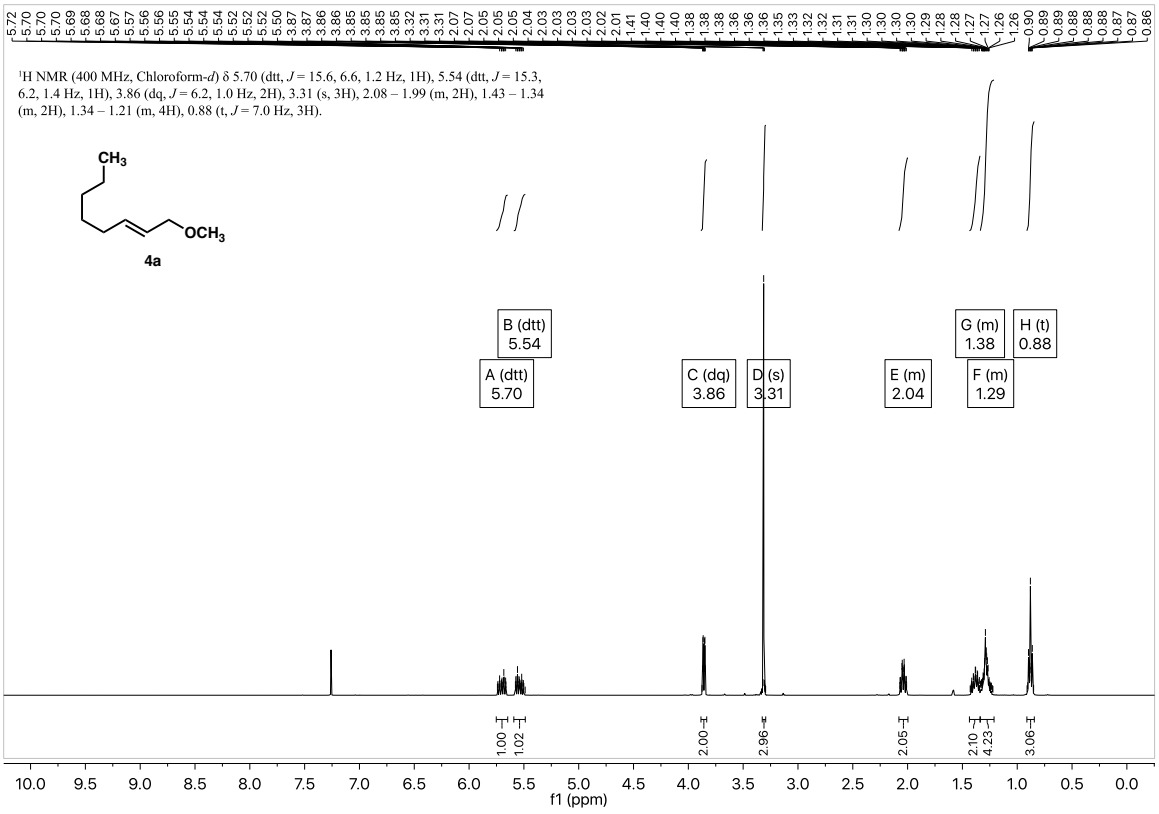

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.1, 158.4, 140.7, 134.6, 128.6, 114.6, 114.0, 60.5, 55.4, 44.9, 40.6, 14.3.
3m
172.07
158.40
140.70
134.61
128.63
114.55
114.04
60.49
55.37
44.92
40.58
14.32
f1 (ppm)

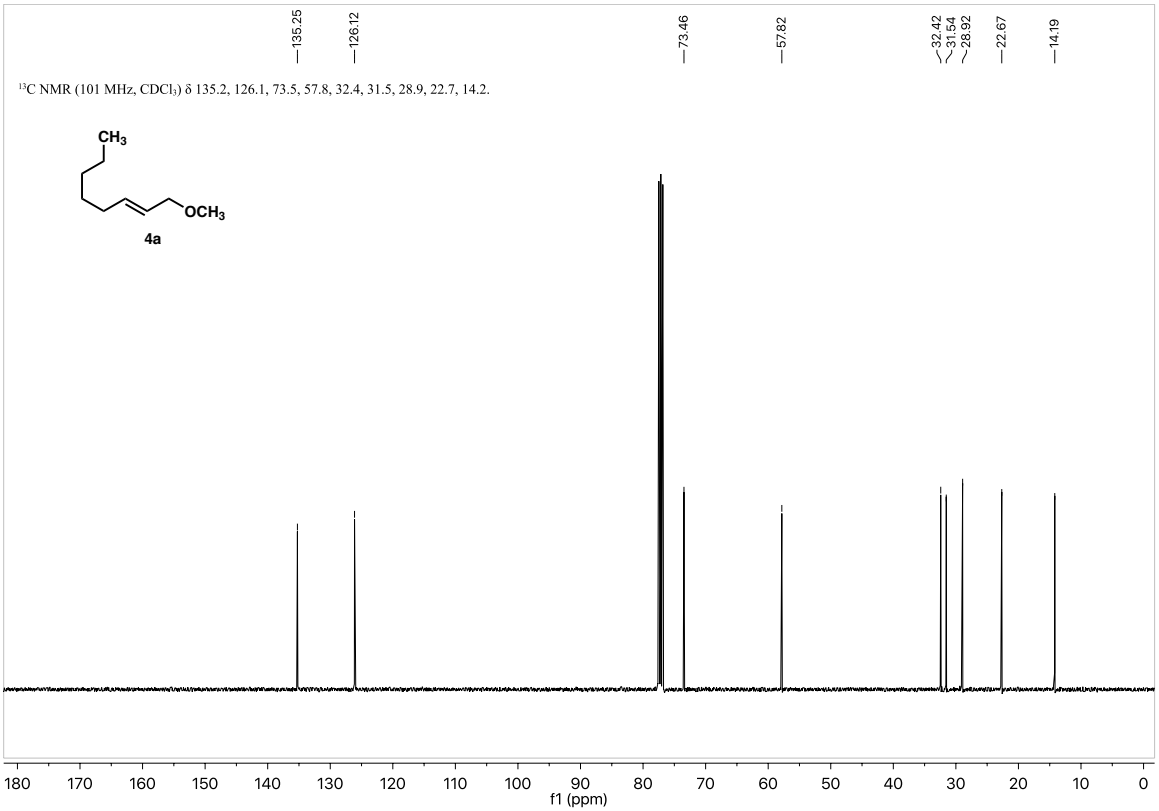

7.15 7.15 7.15 7.14 7.14 7.14 7.14 7.13 7.12 7.12 7.12 7.12 7.11 7.11 6.85 6.84 6.83 6.82 6.82 6.81 4.16 4.15 4.14 4.13 4.12 4.11 3.79 3.79 2.89 2.87 2.85 2.84 2.80 2.78 2.77 2.75 1.80 1.78 1.78 1.77 1.76 1.76 1.75 1.74 1.55 1.53 1.53 1.53 1.52 1.51 1.51 1.50 1.49 1.49 1.49 1.48 1.47 1.47 1.26 1.26 1.25 1.25 1.24 1.23 1.23 1.22 1.12 1.12 1.11 1.11 1.10 1.10 1.10 1.09 1.08 1.07
[1]H NMR (400 MHz, Chloroform-d) δ 7.13 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.79 (s, 3H), 2.86 (dd, J = 14.9, 6.9 Hz, 1H), 2.77 (dd, J = 15.0, 7.6 Hz, 1H), 1.77 (ddd, J = 8.8, 7.6, 5.9 Hz, 1H), 1.56 – 1.44 (m, 1H), 1.24 (t, J = 7.1 Hz, 3H), 1.14 – 1.06 (m, 2H).
O
O
CH3
H3CO
cis-3m'
A (d) 7.13
B (d) 6.83
C (q) 4.13
D (s) 3.79
E (dd) 2.86
F (dd) 2.77
G (ddd) 1.77
H (m) 1.50
I (t) 1.24
J (m) 1.10
2.00
2.00
2.03
2.99
1.01
1.01
1.00
1.01
3.17
2.02
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
f1 (ppm)

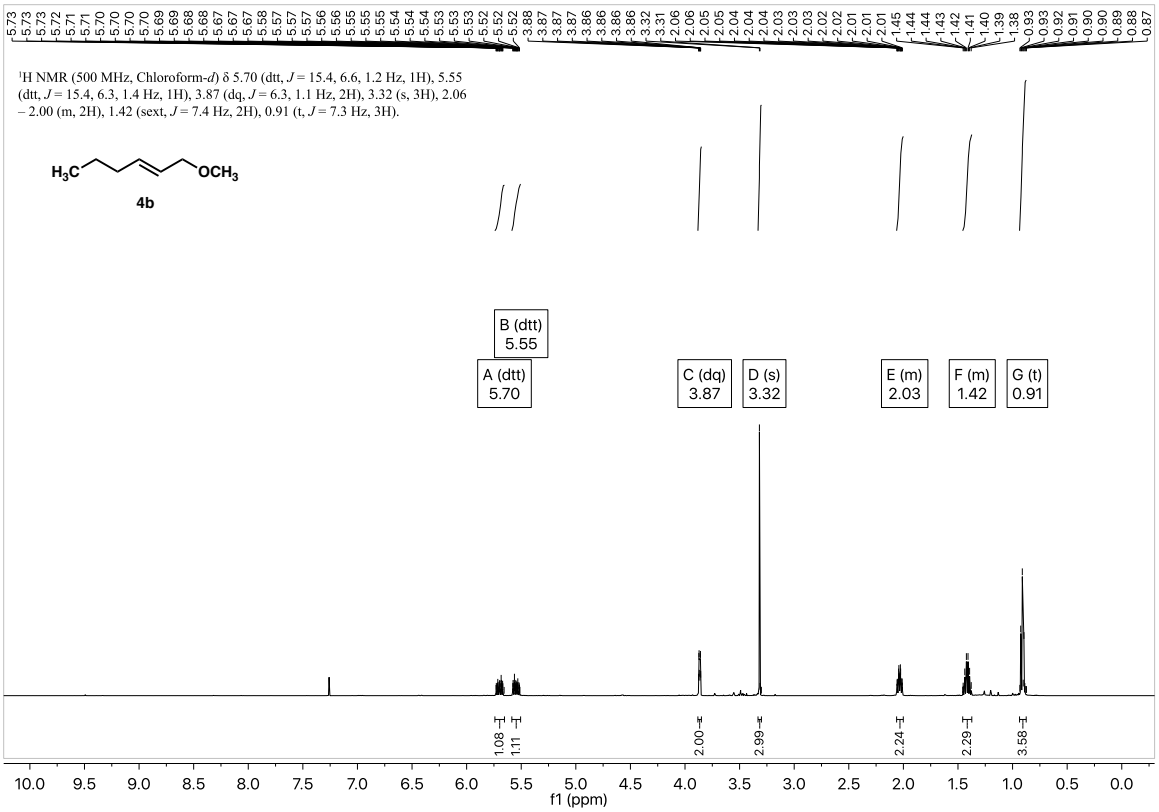

173.12 157.99 133.71 129.34 113.89 60.51 55.39 32.09 23.08 18.67 14.49 13.73
[13]C NMR (101 MHz, CDCl3) δ 173.1, 158.0, 133.7, 129.3, 113.9, 60.5, 55.4, 32.1, 23.1, 18.7, 14.5, 13.7.
O
O
CH3
H3CO
cis-3m'
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

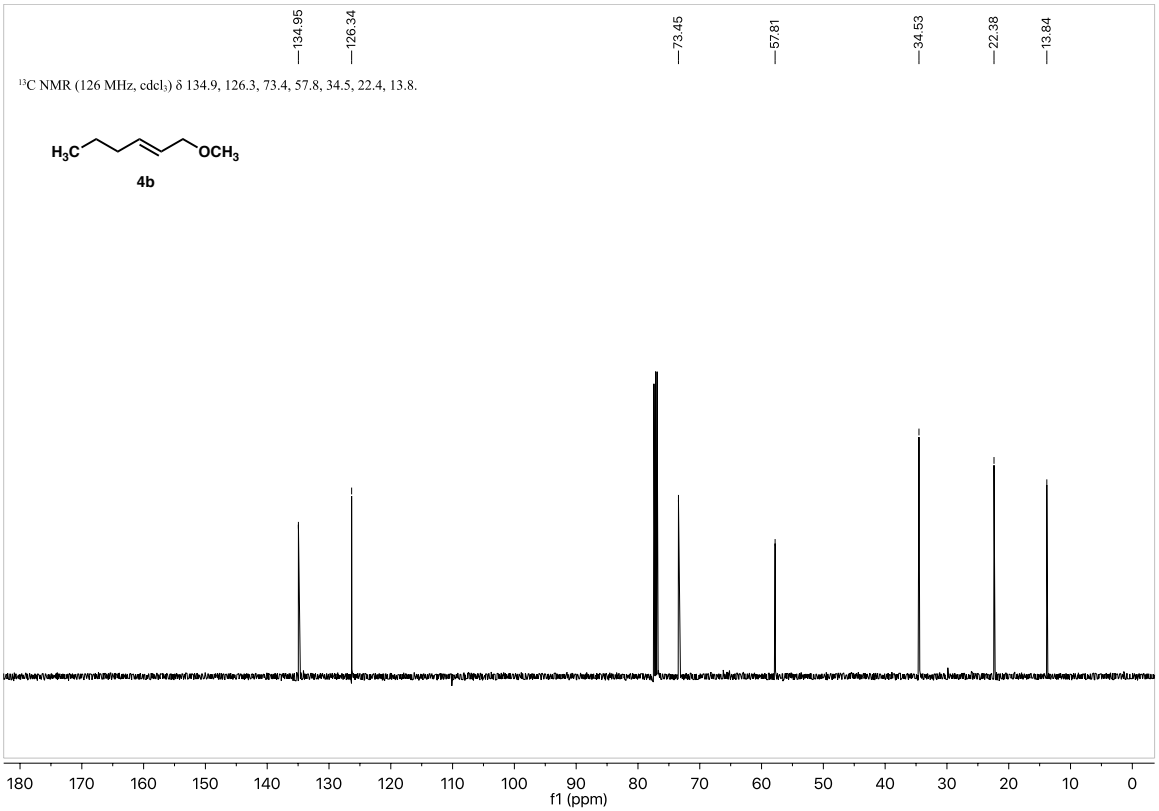

7.15 7.14 7.14 7.14 7.14 7.13 7.13 7.12 7.12 7.12 7.11 7.11 6.86 6.85 6.85 6.83 6.83 6.82 4.14 4.13 4.12 4.12 4.10 4.10 4.08 4.08 3.79 3.79 2.73 2.72 2.69 2.68 2.55 2.53 2.51 2.49 1.68 1.67 1.67 1.66 1.66 1.66 1.65 1.65 1.65 1.65 1.64 1.64 1.64 1.64 1.63 1.63 1.63 1.51 1.50 1.50 1.49 1.49 1.48 1.48 1.47 1.27 1.26 1.26 1.25 1.24 1.23 1.23 1.22 1.21 0.84 0.82 0.82 0.81 0.81 0.80 0.80 0.79
¹H NMR (400 MHz, Chloroform-d) δ 7.12 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 4.11 (qd, J = 7.1, 1.1 Hz, 2H), 3.79 (s, 3H), 2.71 (dd, J = 14.7, 6.3 Hz, 1H), 2.52 (dd, J = 14.8, 7.1 Hz, 1H), 1.65 (ddtd, J = 8.7, 7.1, 6.4, 4.1 Hz, 1H), 1.52 – 1.46 (m, 1H), 1.27 – 1.20 (m, 4H), 0.81 (ddd, J = 8.2, 6.3, 4.2 Hz, 1H).
O
O
CH3
H3CO
trans-3m'
A (d) 7.12
B (d) 6.84
C (qd) 4.11
D (s) 3.79
E (dd) 2.71
F (dd) 2.52
G (ddtd) 1.65
H (m) 1.49
I (m) 1.24
J (ddd) 0.81
2.00
2.02
2.03
3.12
1.04
1.02
1.07
1.02
4.06
0.99
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
f1 (ppm)

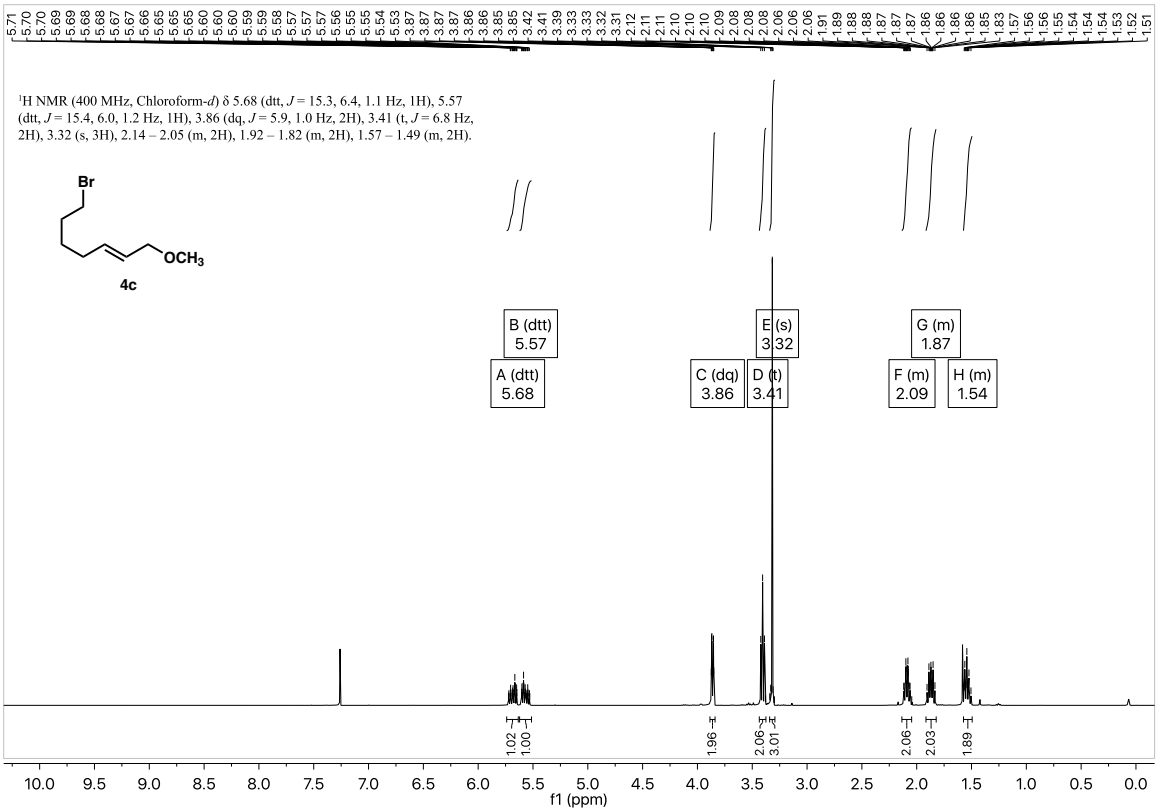

174.36
158.20
132.31
129.46
113.94
60.55
55.40
37.64
23.44
20.25
15.27
14.42
¹³C NMR (101 MHz, CDCl3) δ 174.4, 158.2, 132.3, 129.5, 113.9, 60.5, 55.4, 37.6, 23.4, 20.3, 15.3, 14.4.
O
O
CH3
H3CO
trans-3m'
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

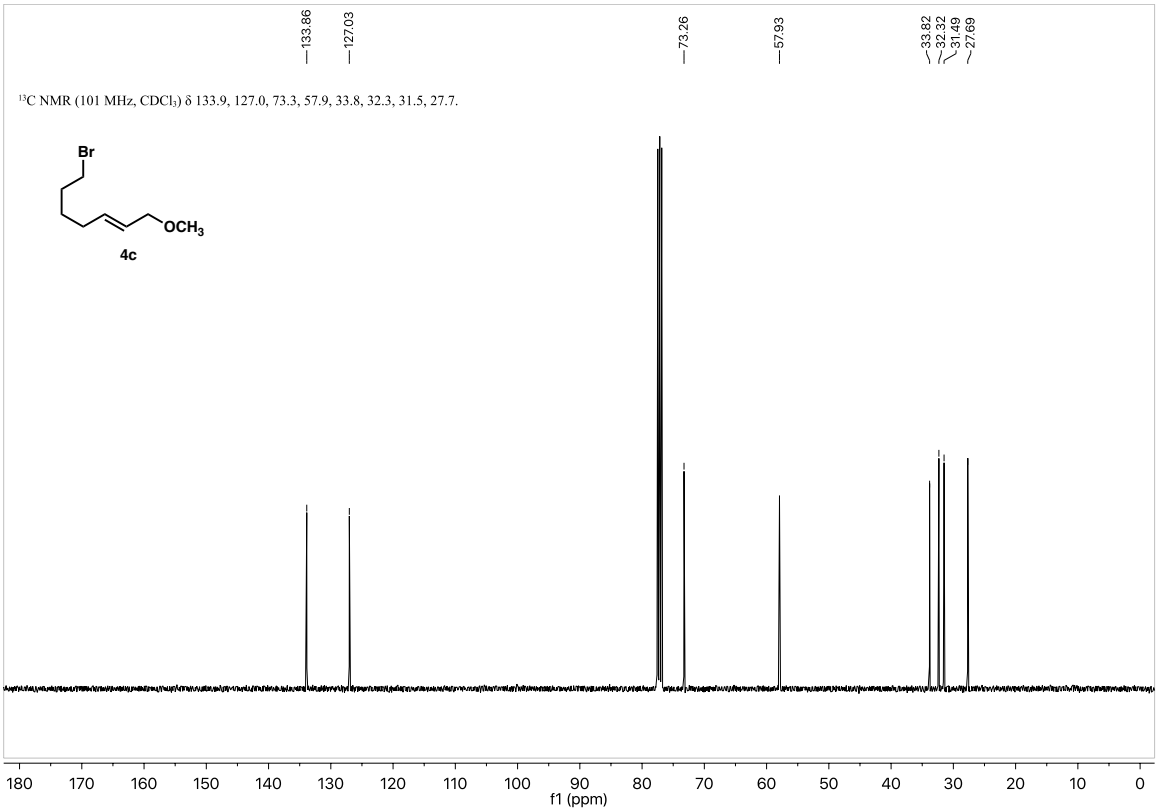

5.72 5.70 5.70 5.70 5.69 5.68 5.68 5.67 5.57 5.56 5.56 5.55 5.54 5.54 5.54 5.52 5.52 5.52 5.50 3.87 3.87 3.86 3.86 3.85 3.85 3.85 3.85 3.32 3.31 3.31 2.07 2.07 2.05 2.05 2.05 2.04 2.03 2.03 2.03 2.02 2.01 1.41 1.40 1.40 1.38 1.38 1.36 1.36 1.36 1.35 1.33 1.32 1.32 1.31 1.31 1.30 1.30 1.30 1.29 1.28 1.28 1.27 1.27 1.26 1.26 0.90 0.89 0.89 0.88 0.88 0.88 0.88 0.87 0.87 0.86
[1]H NMR (400 MHz, Chloroform-d) δ 5.70 (dtt, J = 15.6, 6.6, 1.2 Hz, 1H), 5.54 (dtt, J = 15.3, 6.2, 1.4 Hz, 1H), 3.86 (dq, J = 6.2, 1.0 Hz, 2H), 3.31 (s, 3H), 2.08 – 1.99 (m, 2H), 1.43 – 1.34 (m, 2H), 1.34 – 1.21 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H).
CH3
OCH3
4a
B (dtt) 5.54
A (dtt) 5.70
C (dq) 3.86
D (s) 3.31
E (m) 2.04
G (m) 1.38
F (m) 1.29
H (t) 0.88
1.00
1.02
2.00
2.96
2.05
2.10
4.23
3.06
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
f1 (ppm)

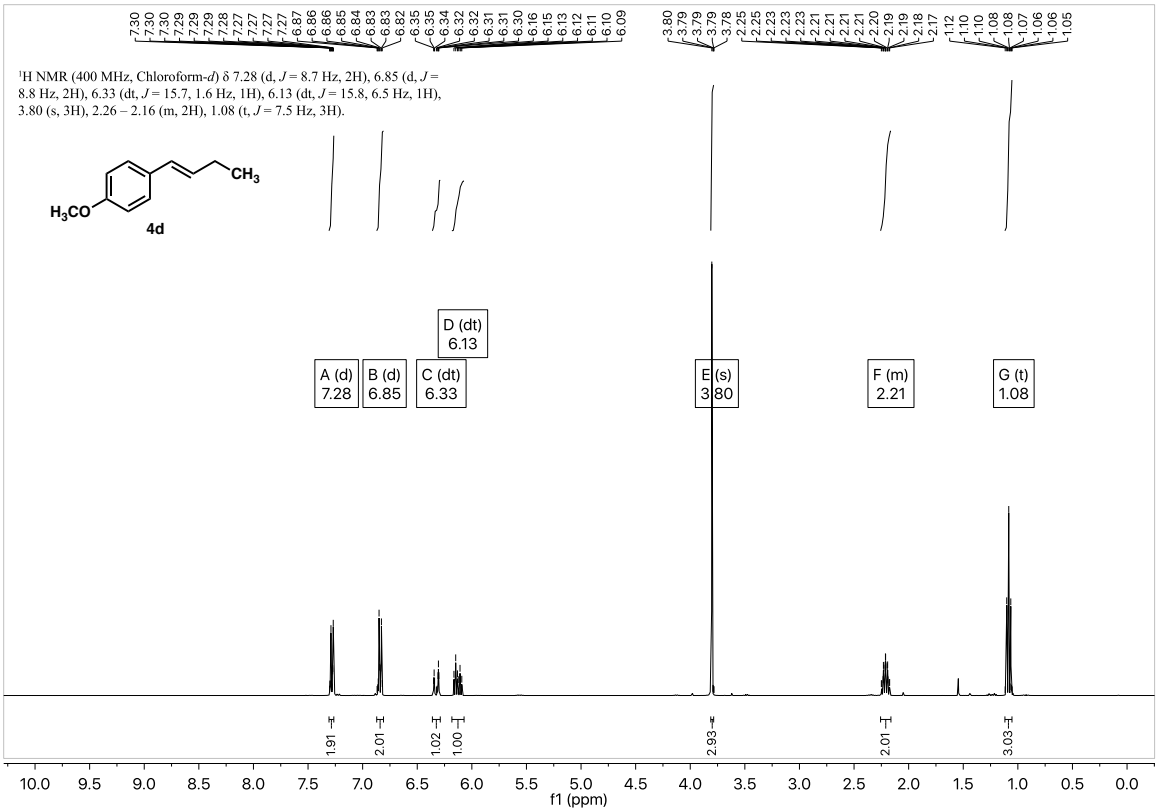

135.25
126.12
73.46
57.82
32.42
31.54
28.92
22.67
14.19
[13]C NMR (101 MHz, CDCl3) δ 135.2, 126.1, 73.5, 57.8, 32.4, 31.5, 28.9, 22.7, 14.2.
CH3
OCH3
4a
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

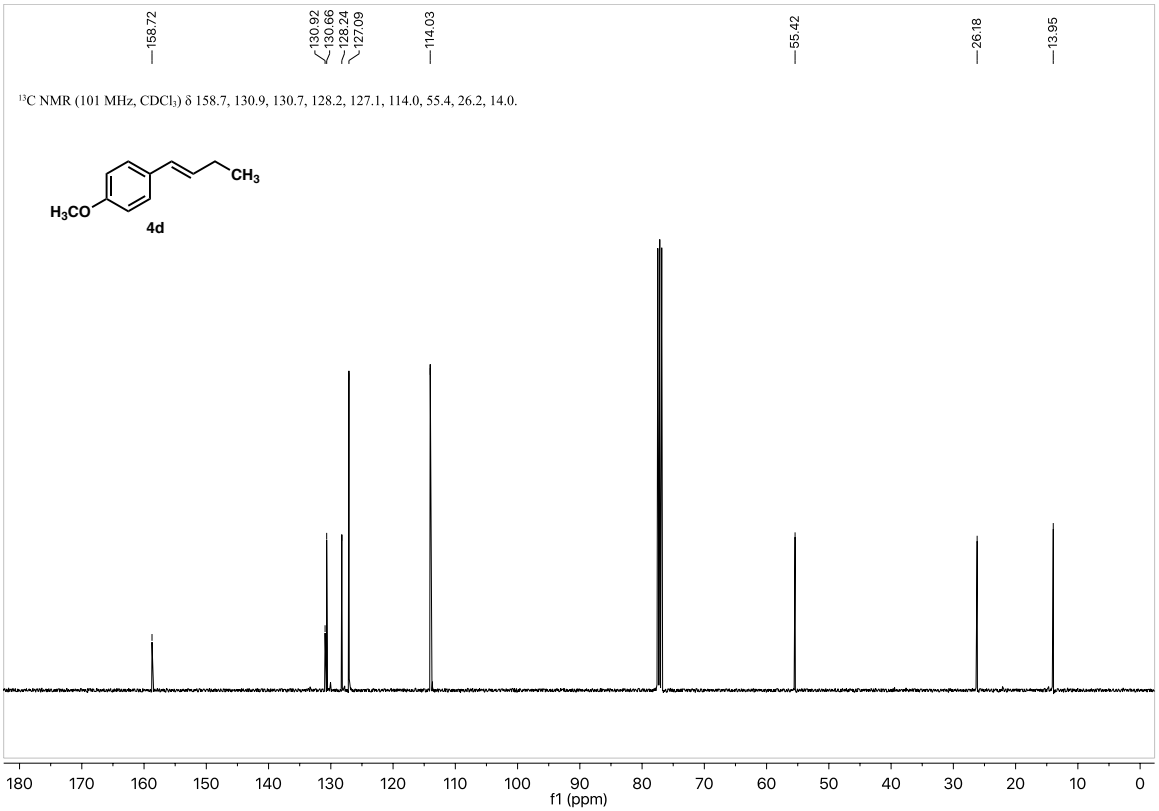

5.73 5.73 5.73 5.72 5.71 5.71 5.70 5.70 5.70 5.70 5.70 5.69 5.69 5.68 5.68 5.67 5.67 5.67 5.67 5.58 5.57 5.57 5.57 5.56 5.56 5.55 5.55 5.55 5.55 5.54 5.54 5.54 5.53 5.53 5.53 5.52 5.52 5.52 5.52
3.88 3.87 3.87 3.87 3.86 3.86 3.86 3.86 3.32 3.31 2.06 2.06 2.05 2.05 2.04 2.04 2.04 2.03 2.03 2.03 2.02 2.02 2.01 2.01 2.01 1.45 1.44 1.44 1.43 1.42 1.41 1.40 1.39 1.38 0.93 0.93 0.92 0.91 0.90 0.90 0.89 0.88 0.87
[1]H NMR (500 MHz, Chloroform-d) δ 5.70 (dtt, J = 15.4, 6.6, 1.2 Hz, 1H), 5.55 (dtt, J = 15.4, 6.3, 1.4 Hz, 1H), 3.87 (dq, J = 6.3, 1.1 Hz, 2H), 3.32 (s, 3H), 2.06 – 2.00 (m, 2H), 1.42 (sext, J = 7.4 Hz, 2H), 0.91 (t, J = 7.3 Hz, 3H).
H3C
OCH3
4b
B (dtt) 5.55
A (dtt) 5.70
C (dq) 3.87
D (s) 3.32
E (m) 2.03
F (m) 1.42
G (t) 0.91
1.08
1.11
2.00
2.99
2.24
2.29
3.58
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
f1 (ppm)

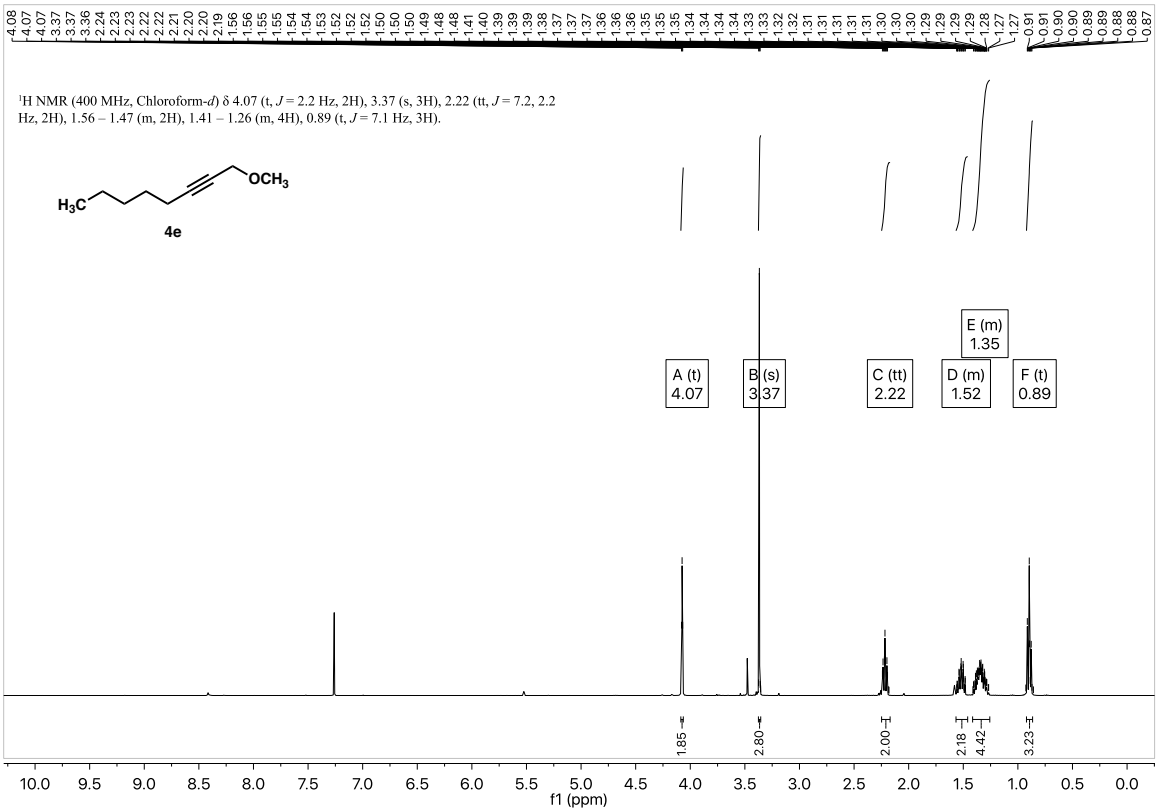

134.95
126.34
73.45
57.81
34.53
22.38
13.84
[13]C NMR (126 MHz, cdcl3) δ 134.9, 126.3, 73.4, 57.8, 34.5, 22.4, 13.8.
H3C
OCH3
4b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

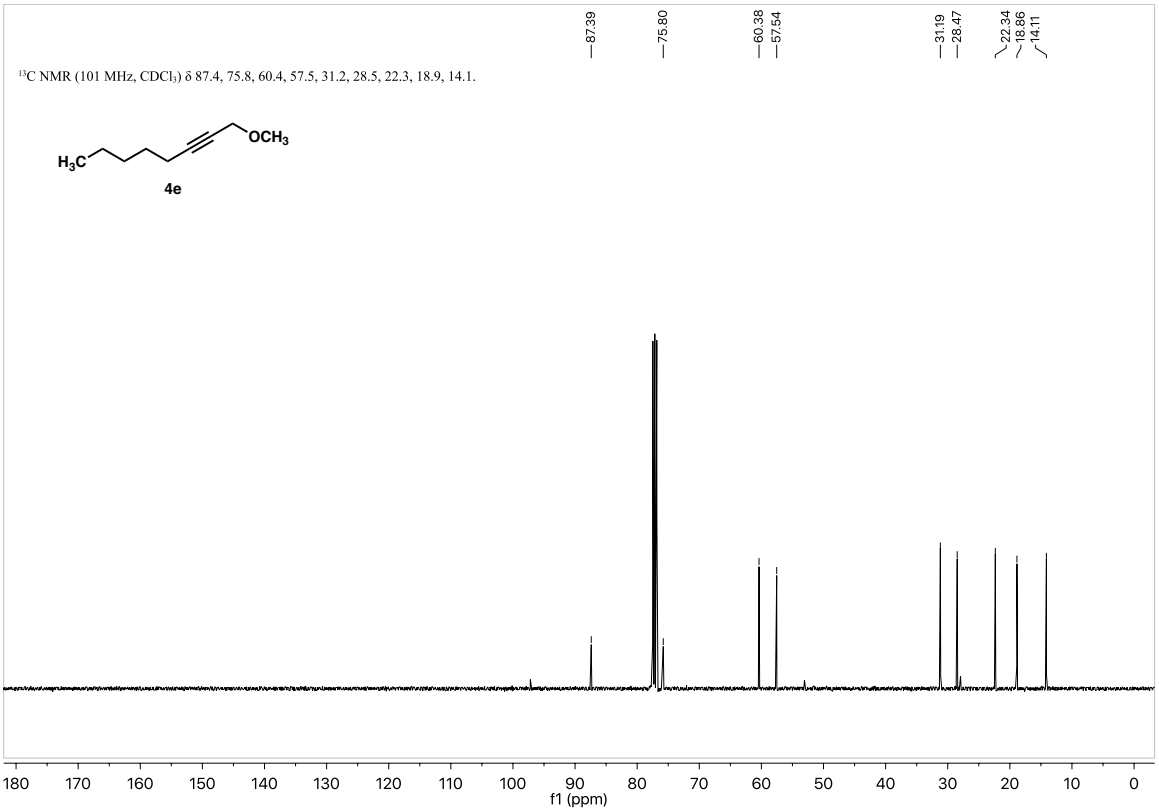

5.71 5.70 5.70 5.69 5.69 5.68 5.68 5.68 5.67 5.67 5.66 5.65 5.65 5.65 5.60 5.60 5.60 5.59 5.59 5.58 5.57 5.57 5.57 5.56 5.55 5.55 5.54 5.53 3.87 3.87 3.87 3.87 3.86 3.86 3.85 3.85 3.42 3.41 3.39 3.33 3.32 3.31 2.12 2.11 2.11 2.10 2.10 2.10 2.09 2.08 2.08 2.08 2.06 2.06 2.06 1.91 1.89 1.88 1.88 1.87 1.87 1.87 1.86 1.86 1.86 1.86 1.85 1.83 1.57 1.56 1.56 1.55 1.54 1.54 1.54 1.53 1.52 1.51
1H NMR (400 MHz, Chloroform-d) δ 5.68 (dtt, J = 15.3, 6.4, 1.1 Hz, 1H), 5.57 (dtt, J = 15.4, 6.0, 1.2 Hz, 1H), 3.86 (dq, J = 5.9, 1.0 Hz, 2H), 3.41 (t, J = 6.8 Hz, 2H), 3.32 (s, 3H), 2.14 – 2.05 (m, 2H), 1.92 – 1.82 (m, 2H), 1.57 – 1.49 (m, 2H).
Br
OCH3
4c
B (dtt) 5.57
A (dtt) 5.68
C (dq) 3.86
D (t) 3.41
E (s) 3.32
G (m) 1.87
F (m) 2.09
H (m) 1.54
1.02
1.00
1.96
2.06
3.01
2.06
2.03
1.89
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
f1 (ppm)

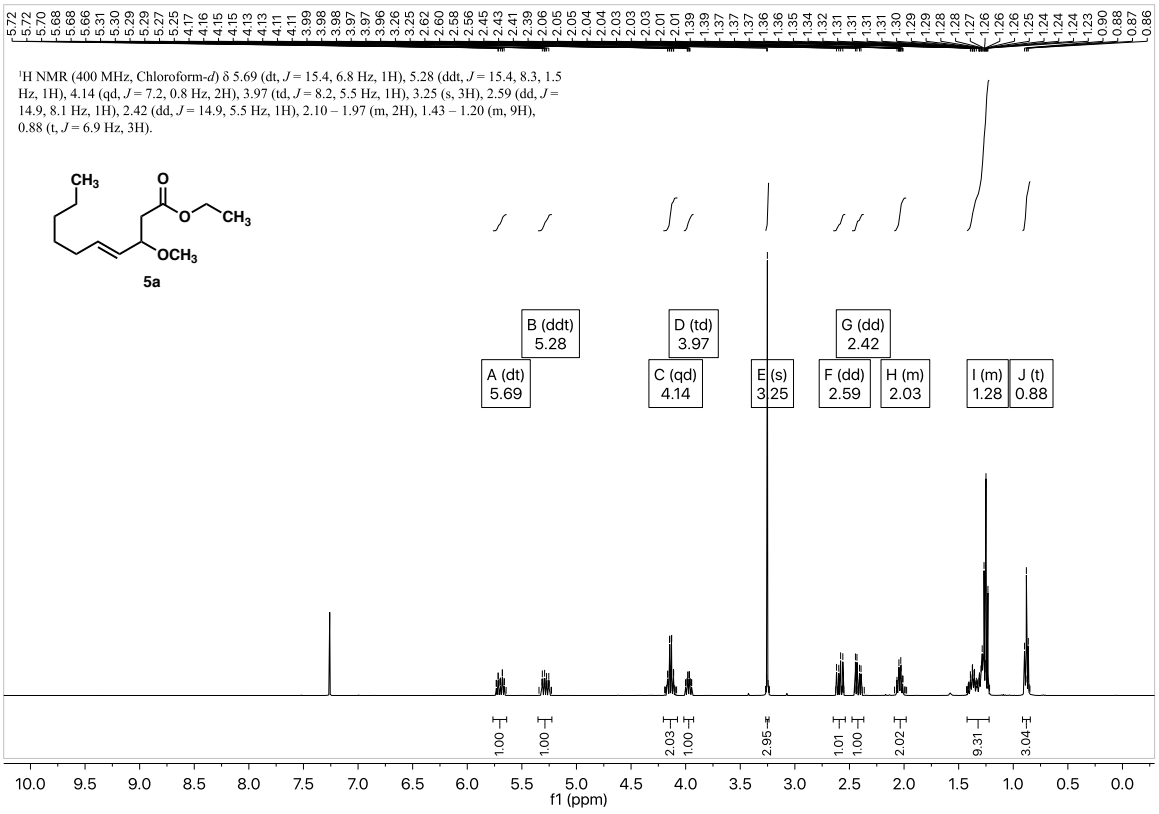

133.86
127.03
73.26
57.93
33.82
32.32
31.49
27.69
13C NMR (101 MHz, CDCl3) δ 133.9, 127.0, 73.3, 57.9, 33.8, 32.3, 31.5, 27.7.
Br
OCH3
4c
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

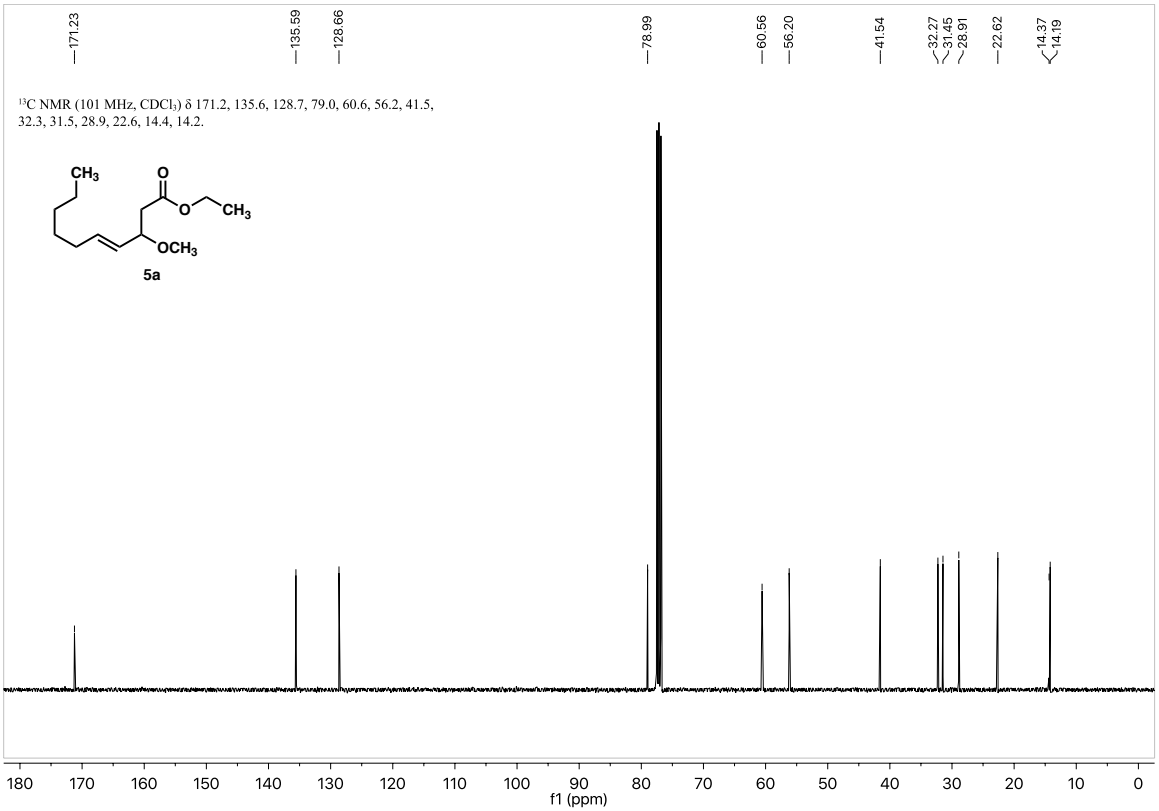

7.30 7.30 7.30 7.29 7.29 7.29 7.28 7.27 7.27 7.27 7.27 6.87 6.86 6.86 6.85 6.84 6.83 6.83 6.82 6.35 6.35 6.34 6.32 6.32 6.31 6.31 6.30 6.16 6.15 6.13 6.12 6.11 6.10 6.09
3.80 3.79 3.79 3.79 3.78 2.25 2.25 2.23 2.23 2.23 2.21 2.21 2.21 2.21 2.20 2.19 2.19 2.18 2.17 1.12 1.10 1.10 1.08 1.08 1.07 1.06 1.06 1.05
1H NMR (400 MHz, Chloroform-d) δ 7.28 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.33 (dt, J = 15.7, 1.6 Hz, 1H), 6.13 (dt, J = 15.8, 6.5 Hz, 1H), 3.80 (s, 3H), 2.26 – 2.16 (m, 2H), 1.08 (t, J = 7.5 Hz, 3H).
H3CO
CH3
4d
A (d) 7.28
B (d) 6.85
C (dt) 6.33
D (dt) 6.13
E (s) 3.80
F (m) 2.21
G (t) 1.08
1.91
2.01
1.02
1.00
2.93
2.01
3.03
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
f1 (ppm)

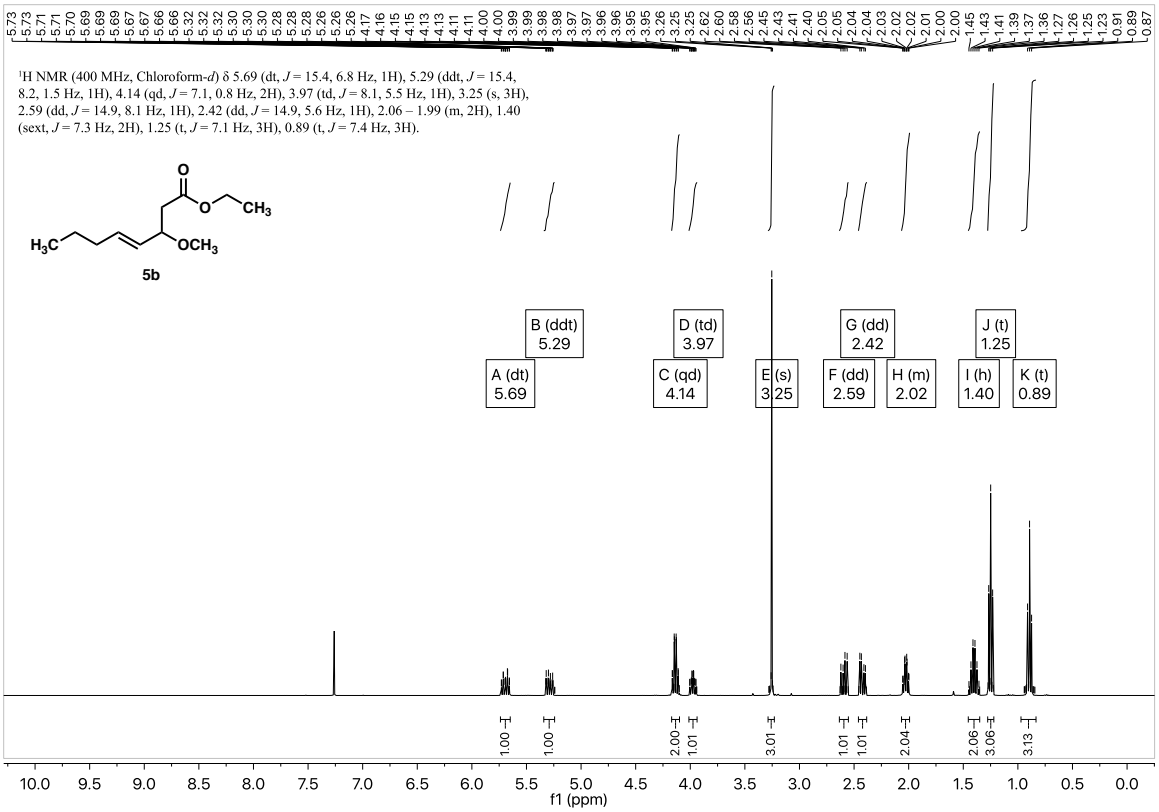

158.72
130.92
130.66
128.24
127.09
114.03
55.42
26.18
13.95
13C NMR (101 MHz, CDCl3) δ 158.7, 130.9, 130.7, 128.2, 127.1, 114.0, 55.4, 26.2, 14.0.
H3CO
CH3
4d
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

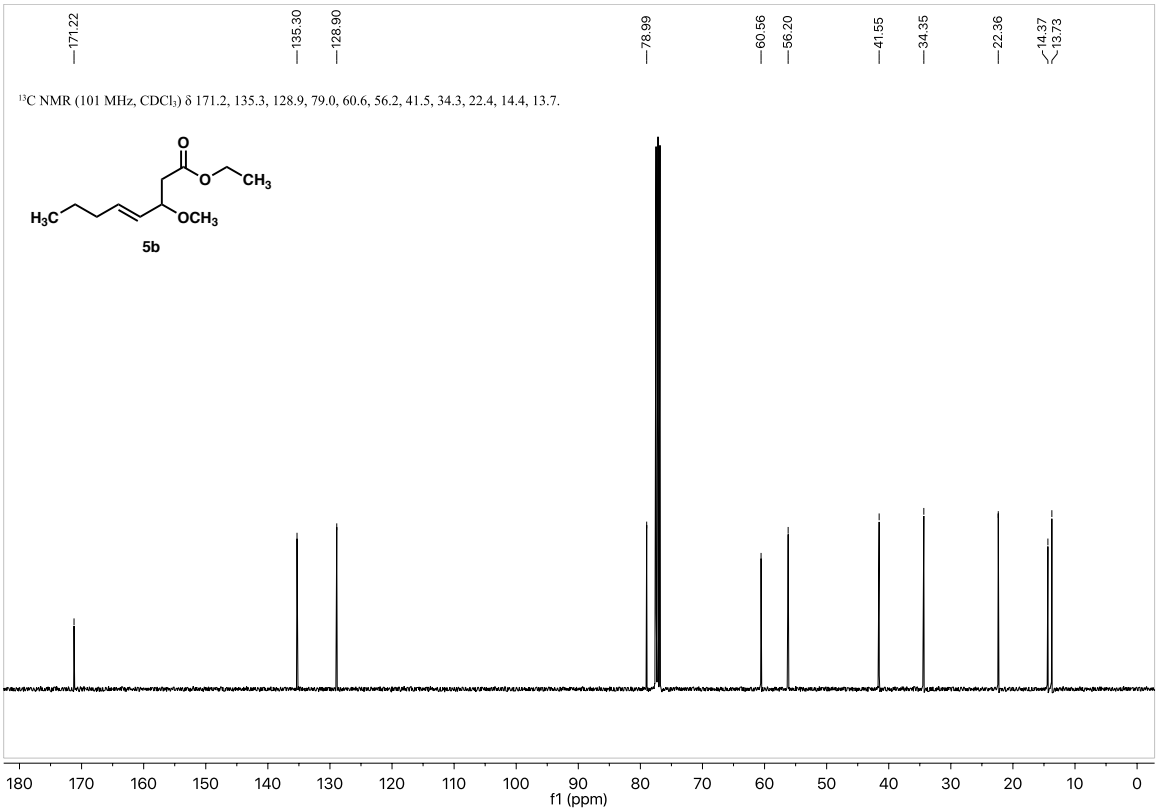

4.08 4.07 4.07 3.37 3.37 3.36 2.24 2.23 2.23 2.22 2.22 2.21 2.20 2.20 2.19 1.56 1.56 1.55 1.55 1.54 1.54 1.53 1.52 1.52 1.52 1.50 1.50 1.50 1.49 1.48 1.48 1.41 1.40 1.39 1.39 1.39 1.38 1.37 1.37 1.37 1.36 1.36 1.36 1.35 1.35 1.35 1.34 1.34 1.34 1.34 1.33 1.33 1.32 1.32 1.31 1.31 1.31 1.31 1.31 1.30 1.30 1.30 1.29 1.29 1.29 1.29 1.28 1.27 1.27 0.91 0.91 0.90 0.90 0.89 0.89 0.89 0.88 0.88 0.87
[1]H NMR (400 MHz, Chloroform-d) δ 4.07 (t, J = 2.2 Hz, 2H), 3.37 (s, 3H), 2.22 (tt, J = 7.2, 2.2 Hz, 2H), 1.56 – 1.47 (m, 2H), 1.41 – 1.26 (m, 4H), 0.89 (t, J = 7.1 Hz, 3H).
H3C
OCH3
4e
A (t) 4.07
B (s) 3.37
C (tt) 2.22
D (m) 1.52
E (m) 1.35
F (t) 0.89
1.85
2.80
2.00
2.18
4.42
3.23
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
f1 (ppm)

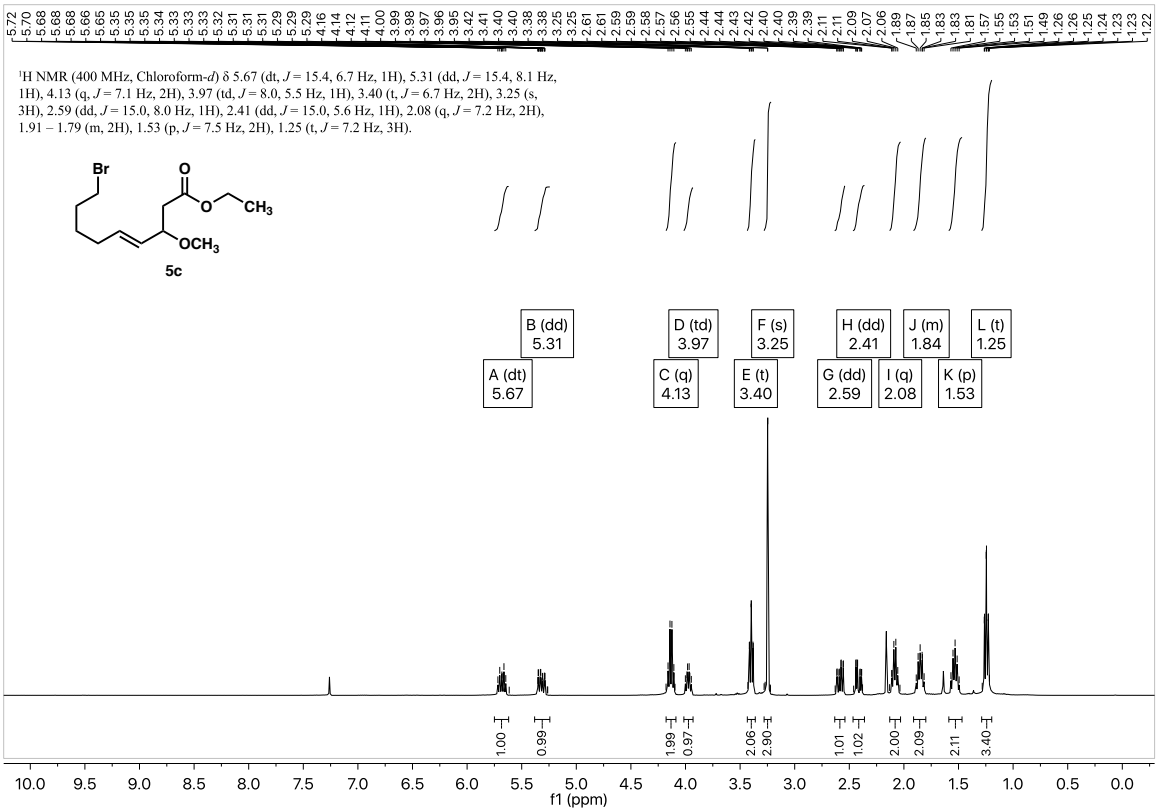

87.39 75.80 60.38 57.54 31.19 28.47 22.34 18.86 14.11
[13]C NMR (101 MHz, CDCl3) δ 87.4, 75.8, 60.4, 57.5, 31.2, 28.5, 22.3, 18.9, 14.1.
H3C
OCH3
4e
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

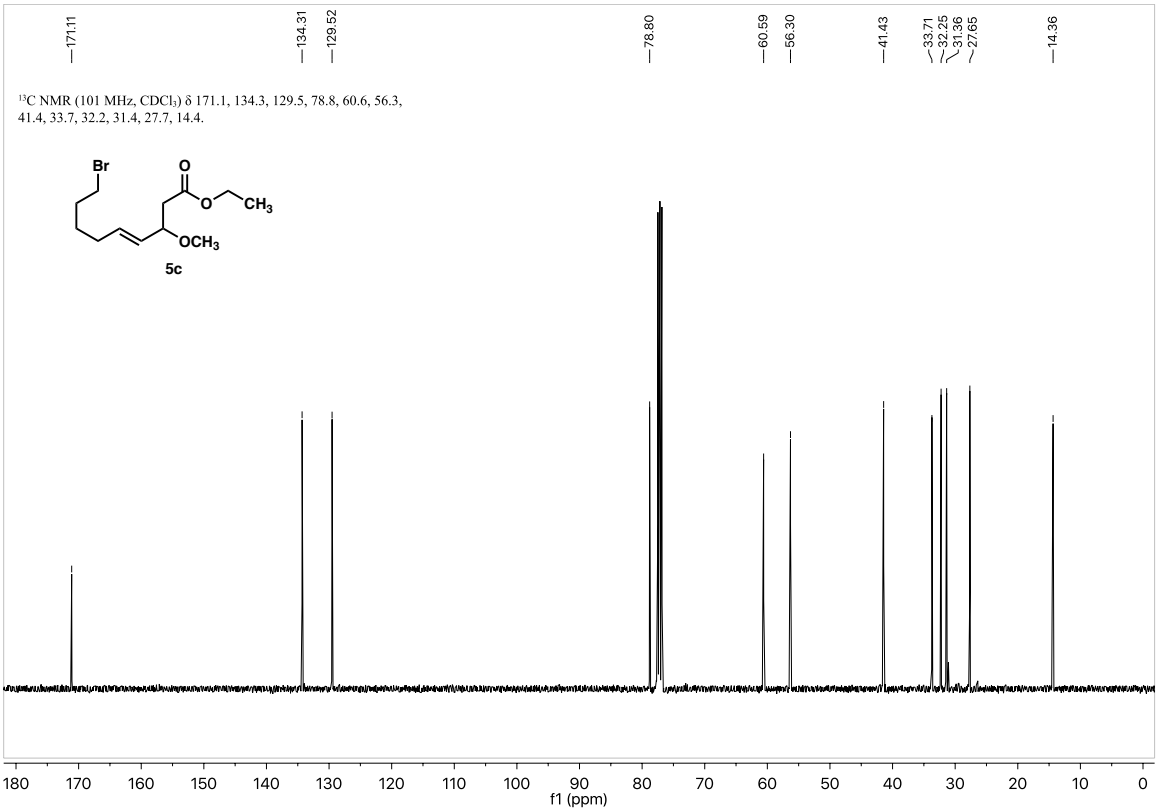

5.72 5.72 5.70 5.68 5.68 5.66 5.31 5.30 5.29 5.29 5.27 5.25 4.17 4.16 4.15 4.15 4.13 4.13 4.11 4.11 3.99 3.98 3.98 3.97 3.97 3.96 3.26 3.25 2.62 2.60 2.58 2.56 2.45 2.43 2.41 2.39 2.06 2.05 2.05 2.04 2.04 2.03 2.03 2.03 2.01 2.01 1.39 1.37 1.37 1.36 1.36 1.35 1.34 1.32 1.31 1.31 1.31 1.30 1.29 1.29 1.28 1.28 1.27 1.26 1.26 1.26 1.25 1.24 1.24 1.24 1.23 0.90 0.88 0.87 0.86
[1]H NMR (400 MHz, Chloroform-d) δ 5.69 (dt, J = 15.4, 6.8 Hz, 1H), 5.28 (ddt, J = 15.4, 8.3, 1.5 Hz, 1H), 4.14 (qd, J = 7.2, 0.8 Hz, 2H), 3.97 (td, J = 8.2, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, J = 14.9, 8.1 Hz, 1H), 2.42 (dd, J = 14.9, 5.5 Hz, 1H), 2.10 – 1.97 (m, 2H), 1.43 – 1.20 (m, 9H), 0.88 (t, J = 6.9 Hz, 3H).
CH3
O
O
CH3
OCH3
5a
A (dt) 5.69
B (ddt) 5.28
C (qd) 4.14
D (td) 3.97
E (s) 3.25
F (dd) 2.59
G (dd) 2.42
H (m) 2.03
I (m) 1.28
J (t) 0.88
1.00
1.00
2.03
1.00
2.95
1.01
1.00
2.02
9.31
3.04
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
f1 (ppm)

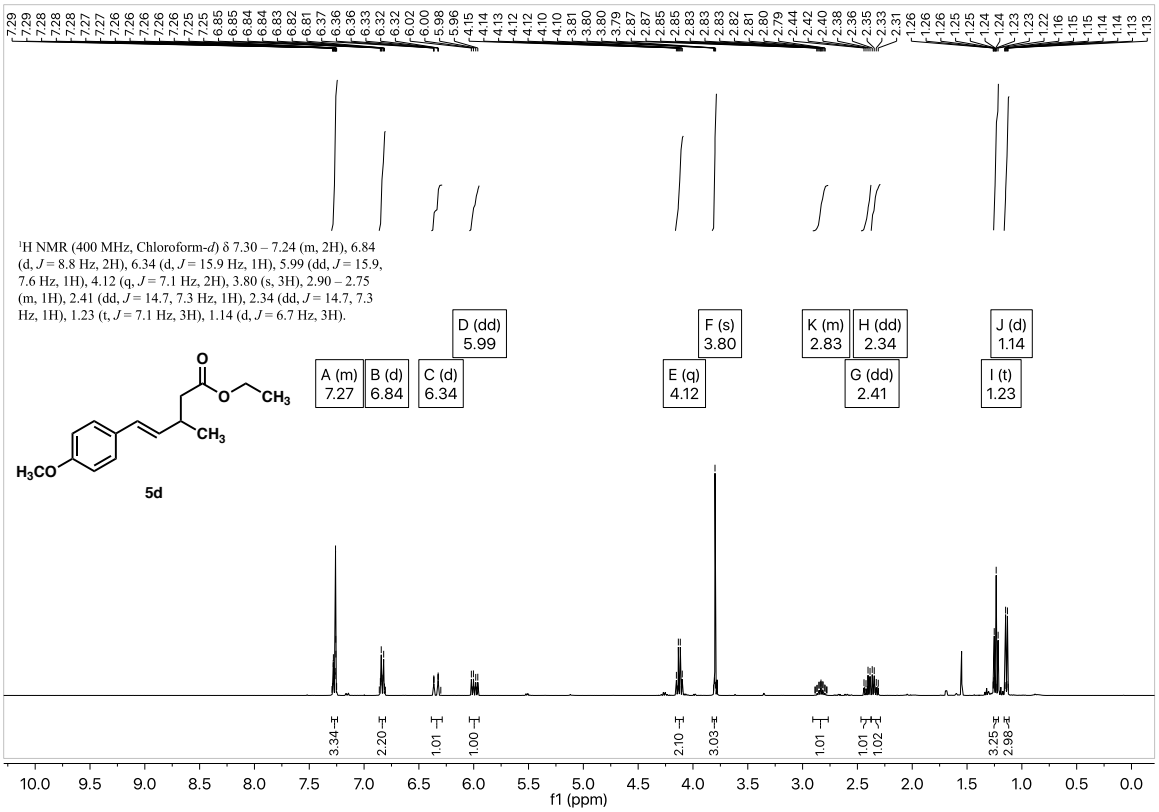

171.23
135.59
128.66
78.99
60.56
56.20
41.54
32.27
31.45
28.91
22.62
14.37
14.19
[13]C NMR (101 MHz, CDCl3) δ 171.2, 135.6, 128.7, 79.0, 60.6, 56.2, 41.5, 32.3, 31.5, 28.9, 22.6, 14.4, 14.2.
CH3
O
O
CH3
OCH3
5a
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

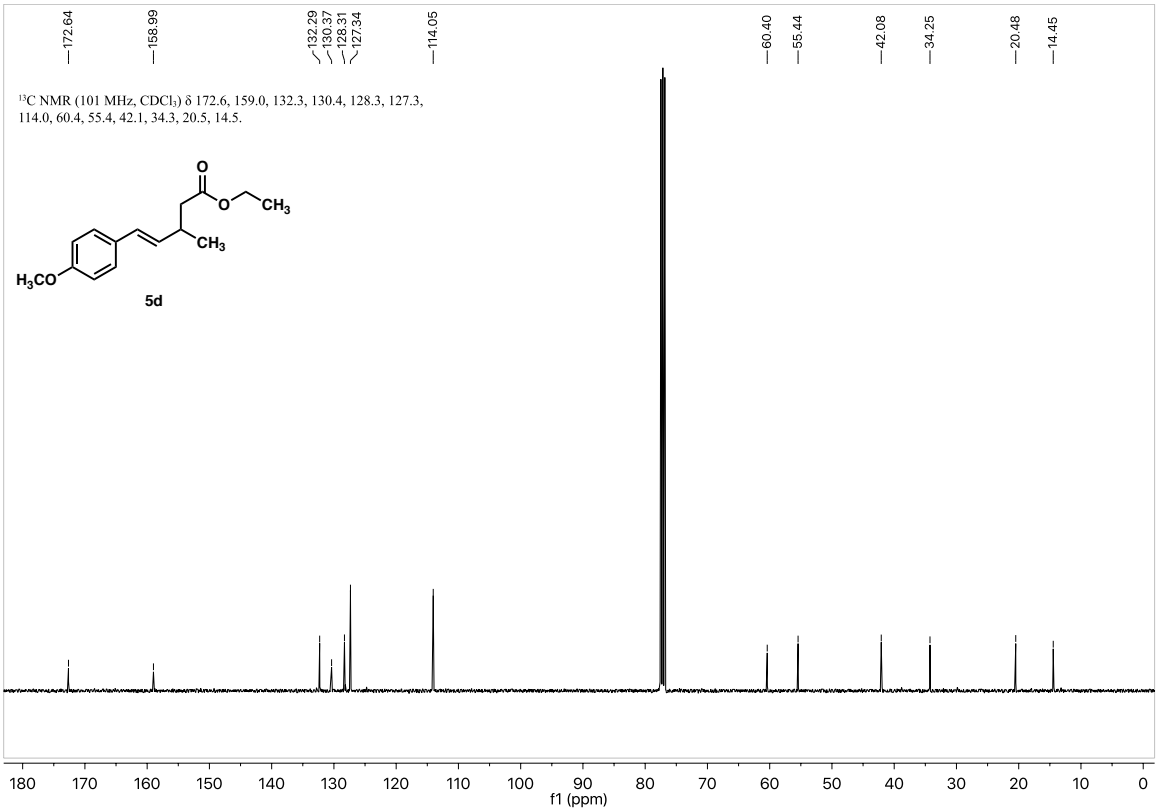

5.73 5.73 5.71 5.71 5.70 5.69 5.69 5.69 5.67 5.67 5.66 5.66 5.32 5.32 5.32 5.30 5.30 5.30 5.28 5.28 5.28 5.26 5.26 5.26 4.17 4.16 4.15 4.13 4.11 4.11 4.00 4.00 3.99 3.99 3.98 3.98 3.97 3.96 3.96 3.95 3.95 3.26 3.25 2.62 2.60 2.58 2.56 2.45 2.43 2.41 2.40 2.05 2.05 2.04 2.04 2.03 2.02 2.02 2.01 2.00 2.00 1.45 1.43 1.41 1.39 1.37 1.36 1.27 1.26 1.25 1.23 0.91 0.89 0.87
1H NMR (400 MHz, Chloroform-d) δ 5.69 (dt, J = 15.4, 6.8 Hz, 1H), 5.29 (ddt, J = 15.4, 8.2, 1.5 Hz, 1H), 4.14 (qd, J = 7.1, 0.8 Hz, 2H), 3.97 (td, J = 8.1, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, J = 14.9, 8.1 Hz, 1H), 2.42 (dd, J = 14.9, 5.6 Hz, 1H), 2.06 – 1.99 (m, 2H), 1.40 (sext, J = 7.3 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H).
O
O
CH3
H3C
OCH3
5b
A (dt) 5.69
B (ddt) 5.29
C (qd) 4.14
D (td) 3.97
E (s) 3.25
F (dd) 2.59
G (dd) 2.42
H (m) 2.02
I (h) 1.40
J (t) 1.25
K (t) 0.89
1.00
1.00
2.00
1.01
3.01
1.01
1.01
2.04
2.06
3.06
3.13
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
f1 (ppm)

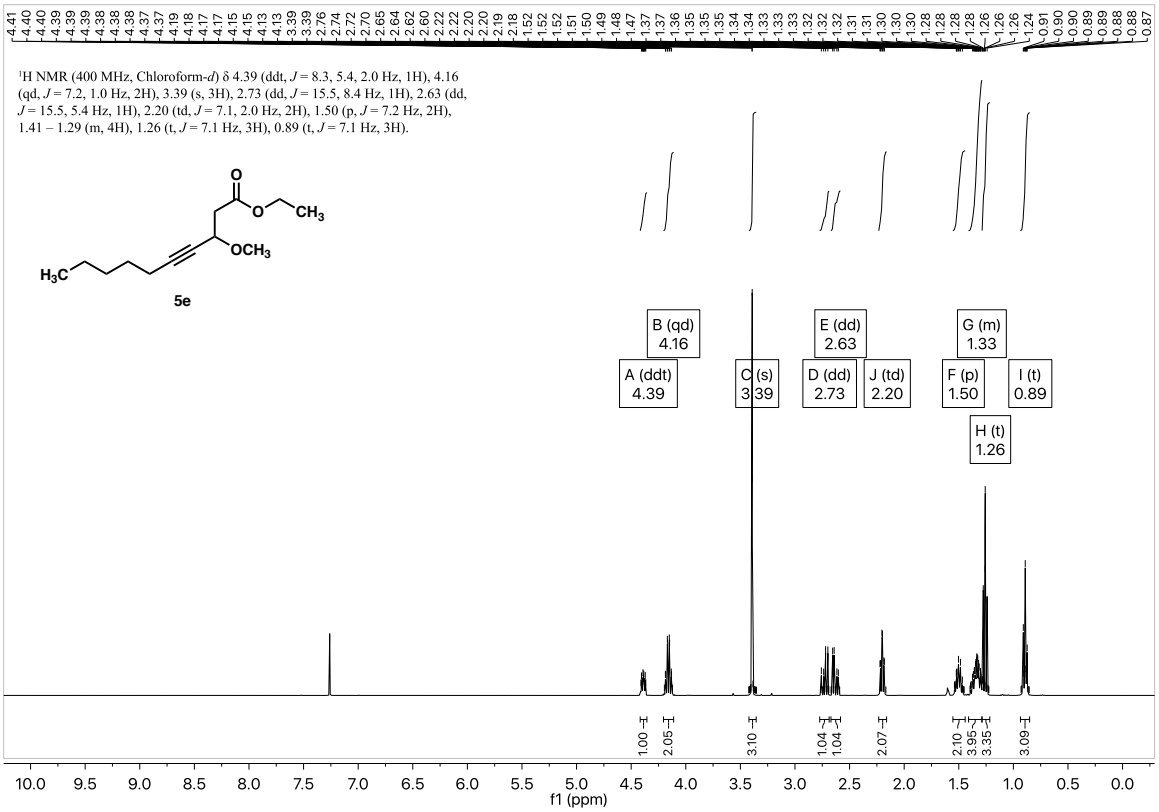

171.22
135.30
128.90
78.99
60.56
56.20
41.55
34.35
22.36
14.37
13.73
13C NMR (101 MHz, CDCl3) δ 171.2, 135.3, 128.9, 79.0, 60.6, 56.2, 41.5, 34.3, 22.4, 14.4, 13.7.
O
O
CH3
H3C
OCH3
5b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

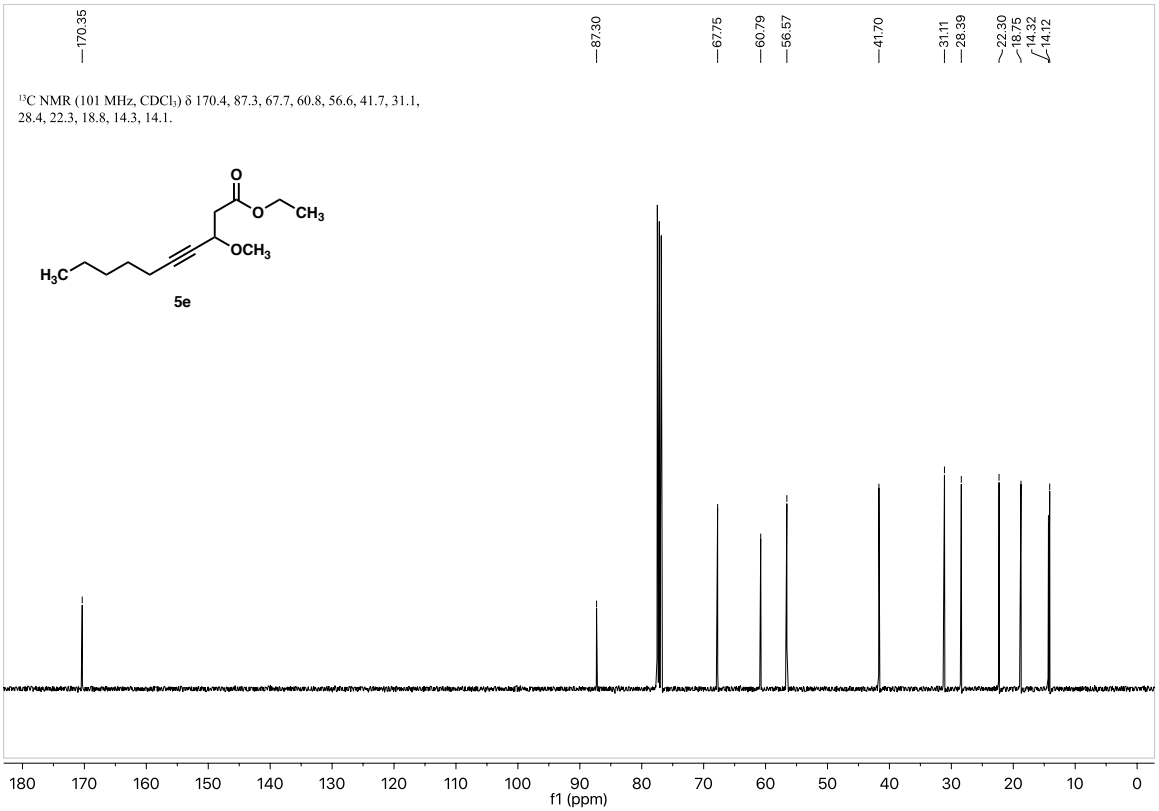

5.72 5.70 5.68 5.68 5.68 5.68 5.66 5.66 5.65 5.35 5.35 5.35 5.34 5.33 5.33 5.33 5.32 5.31 5.31 5.31 5.31 5.29 5.29 5.29 4.16 4.14 4.12 4.11 4.00 3.99 3.98 3.97 3.96 3.95 3.42 3.41 3.40 3.40 3.38 3.38 3.25 3.25 2.61 2.61 2.59 2.59 2.58 2.57 2.56 2.55 2.44 2.43 2.42 2.40 2.40 2.39 2.39 2.11 2.11 2.09 2.07 2.06 1.89 1.87 1.85 1.83 1.83 1.81 1.57 1.55 1.53 1.51 1.49 1.26 1.26 1.25 1.24 1.23 1.23 1.22
1H NMR (400 MHz, Chloroform-d) δ 5.67 (dt, J = 15.4, 6.7 Hz, 1H), 5.31 (dd, J = 15.4, 8.1 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 3.97 (td, J = 8.0, 5.5 Hz, 1H), 3.40 (t, J = 6.7 Hz, 2H), 3.25 (s, 3H), 2.59 (dd, J = 15.0, 8.0 Hz, 1H), 2.41 (dd, J = 15.0, 5.6 Hz, 1H), 2.08 (q, J = 7.2 Hz, 2H), 1.91 – 1.79 (m, 2H), 1.53 (p, J = 7.5 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H).
Br
O
O
CH3
OCH3
5c
A (dt) 5.67
B (dd) 5.31
C (q) 4.13
D (td) 3.97
E (t) 3.40
F (s) 3.25
G (dd) 2.59
H (dd) 2.41
I (q) 2.08
J (m) 1.84
K (p) 1.53
L (t) 1.25
1.00
0.99
1.99
0.97
2.06
2.90
1.01
1.02
2.00
2.09
2.11
3.40
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
f1 (ppm)

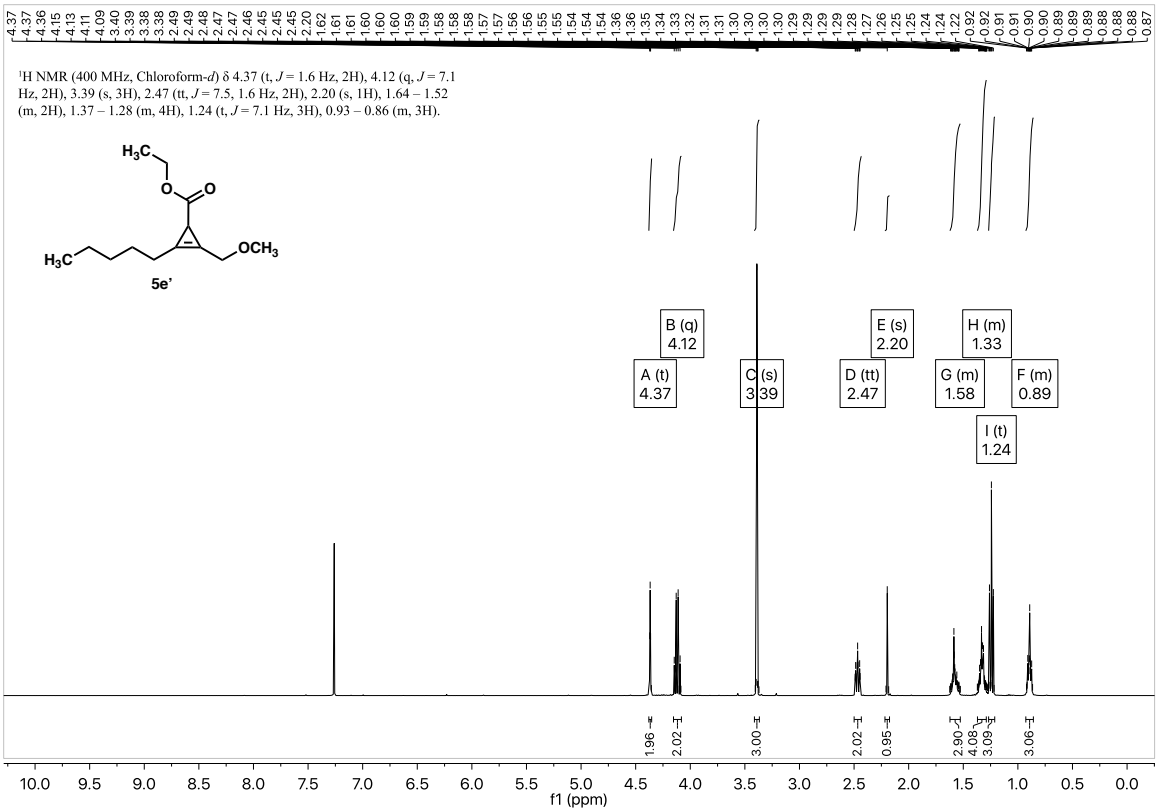

171.11
134.31
129.52
78.80
60.59
56.30
41.43
33.71
32.25
31.36
27.65
14.36
13C NMR (101 MHz, CDCl3) δ 171.1, 134.3, 129.5, 78.8, 60.6, 56.3, 41.4, 33.7, 32.2, 31.4, 27.7, 14.4.
Br
O
O
CH3
OCH3
5c
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

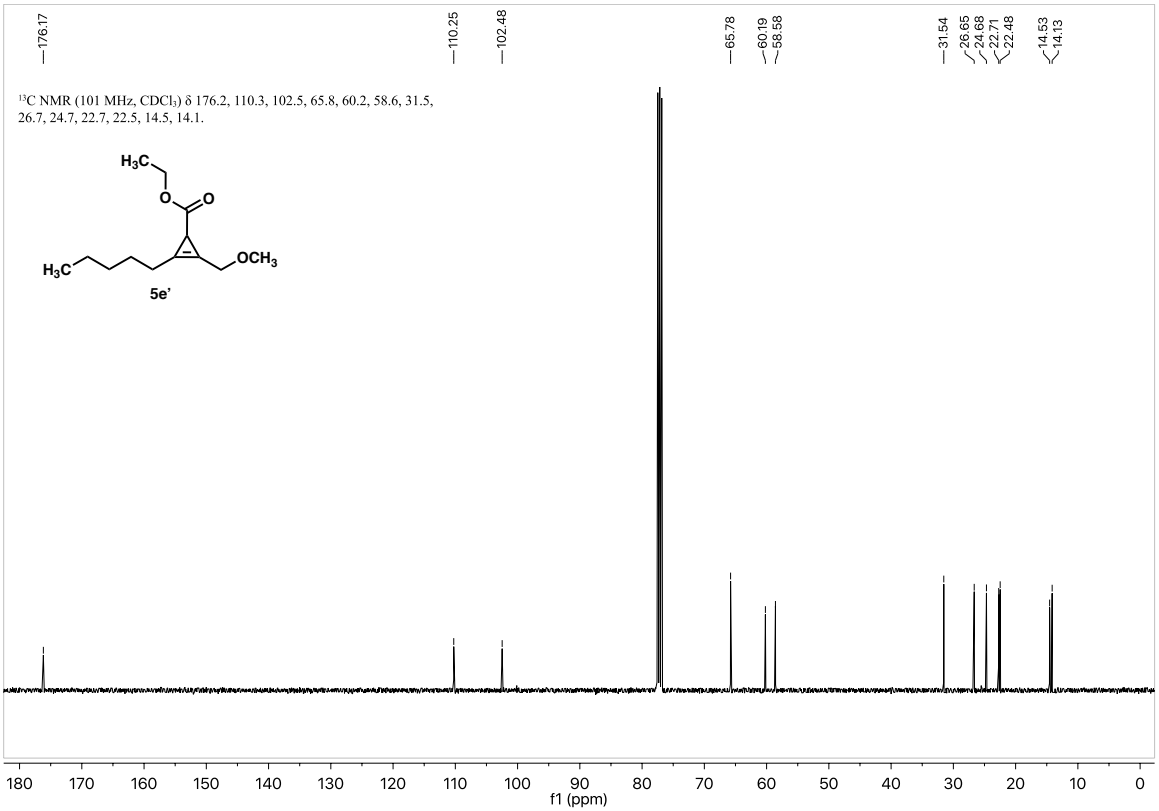

7.29 7.29 7.28 7.28 7.28 7.27 7.27 7.26 7.26 7.26 7.26 7.26 7.25 7.25 6.85 6.85 6.84 6.84 6.83 6.82 6.81 6.37 6.36 6.36 6.33 6.32 6.32 6.02 6.00 5.98 5.96 4.15 4.14 4.13 4.12 4.12 4.10 4.10 3.81 3.80 3.80 3.79 2.87 2.85 2.85 2.83 2.83 2.82 2.81 2.80 2.79 2.44 2.42 2.40 2.38 2.36 2.35 2.33 2.31 1.26 1.26 1.26 1.25 1.25 1.24 1.24 1.23 1.23 1.22 1.16 1.15 1.15 1.14 1.14 1.13 1.13
[1]H NMR (400 MHz, Chloroform-d) δ 7.30 – 7.24 (m, 2H), 6.84 (d, J = 8.8 Hz, 2H), 6.34 (d, J = 15.9 Hz, 1H), 5.99 (dd, J = 15.9, 7.6 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.80 (s, 3H), 2.90 – 2.75 (m, 1H), 2.41 (dd, J = 14.7, 7.3 Hz, 1H), 2.34 (dd, J = 14.7, 7.3 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H), 1.14 (d, J = 6.7 Hz, 3H).
A (m) 7.27
B (d) 6.84
C (d) 6.34
D (dd) 5.99
E (q) 4.12
F (s) 3.80
G (dd) 2.41
H (dd) 2.34
I (t) 1.23
J (d) 1.14
K (m) 2.83
O
O
CH3
CH3
H3CO
5d
3.34 2.20 1.01 1.00 2.10 3.03 1.01 1.01 1.02 3.25 2.98
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
f1 (ppm)

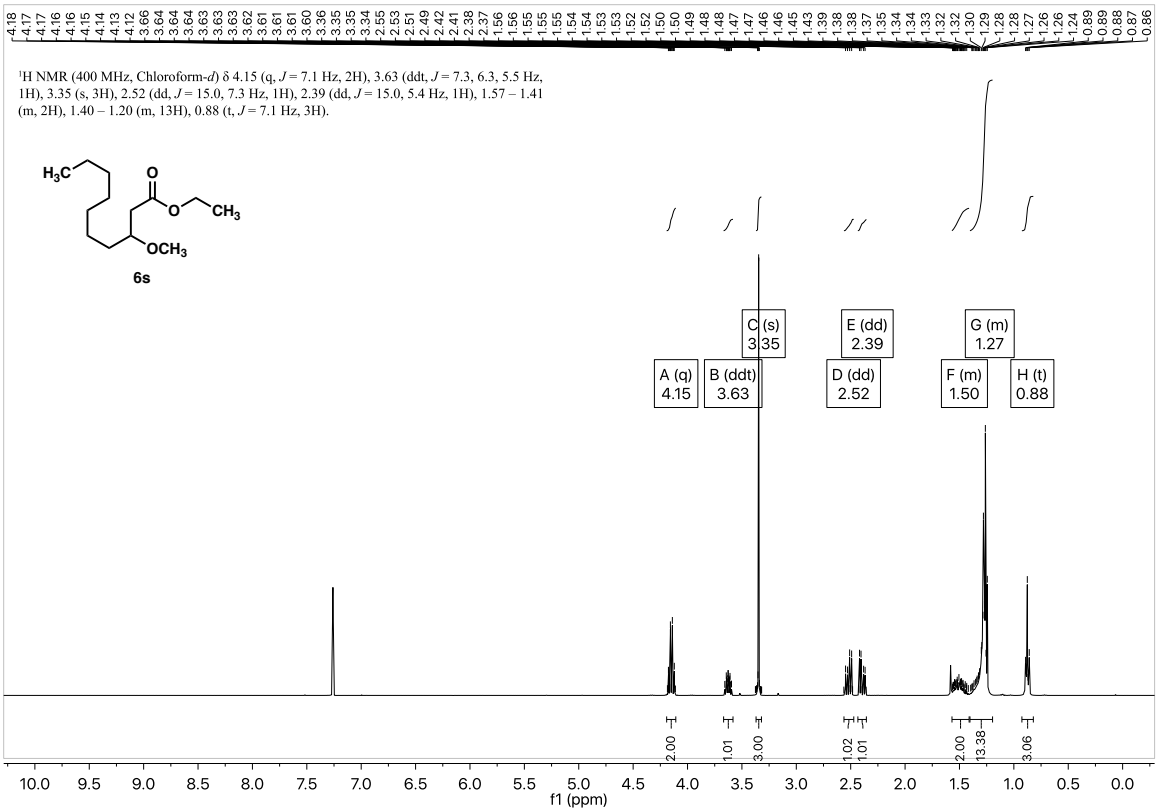

172.64 158.99 132.29 130.37 128.31 127.34 114.05 60.40 55.44 42.08 34.25 20.48 14.45
[13]C NMR (101 MHz, CDCl3) δ 172.6, 159.0, 132.3, 130.4, 128.3, 127.3, 114.0, 60.4, 55.4, 42.1, 34.3, 20.5, 14.5.
O
O
CH3
CH3
H3CO
5d
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

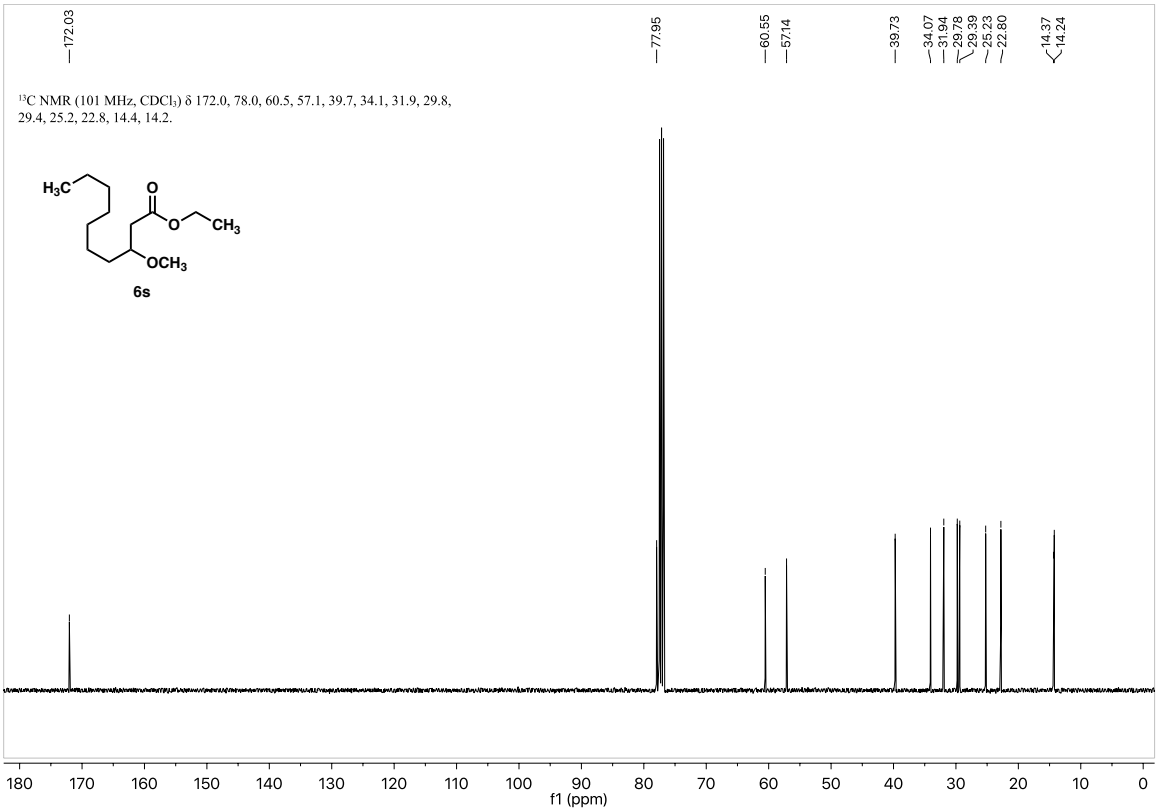

4.41 4.40 4.40 4.39 4.39 4.39 4.38 4.38 4.38 4.38 4.37 4.37 4.19 4.18 4.17 4.17 4.15 4.15 4.13 3.39 3.39 2.76 2.74 2.72 2.70 2.65 2.64 2.62 2.60 2.22 2.22 2.20 2.20 2.19 2.18 1.52 1.52 1.52 1.51 1.50 1.49 1.48 1.47 1.37 1.37 1.36 1.35 1.35 1.35 1.34 1.34 1.33 1.33 1.33 1.32 1.32 1.32 1.31 1.31 1.30 1.30 1.30 1.28 1.28 1.28 1.28 1.26 1.26 1.26 1.24 0.91 0.90 0.90 0.90 0.89 0.89 0.89 0.88 0.88 0.87
[1]H NMR (400 MHz, Chloroform-d) δ 4.39 (ddt, J = 8.3, 5.4, 2.0 Hz, 1H), 4.16 (qd, J = 7.2, 1.0 Hz, 2H), 3.39 (s, 3H), 2.73 (dd, J = 15.5, 8.4 Hz, 1H), 2.63 (dd, J = 15.5, 5.4 Hz, 1H), 2.20 (td, J = 7.1, 2.0 Hz, 2H), 1.50 (p, J = 7.2 Hz, 2H), 1.41 – 1.29 (m, 4H), 1.26 (t, J = 7.1 Hz, 3H), 0.89 (t, J = 7.1 Hz, 3H).
O
O
CH3
OCH3
H3C
5e
B (qd) 4.16
A (ddt) 4.39
C (s) 3.39
E (dd) 2.63
D (dd) 2.73
J (td) 2.20
G (m) 1.33
F (p) 1.50
I (t) 0.89
H (t) 1.26
1.00
2.05
3.10
1.04
1.04
2.07
2.10
3.95
3.35
3.09
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
f1 (ppm)

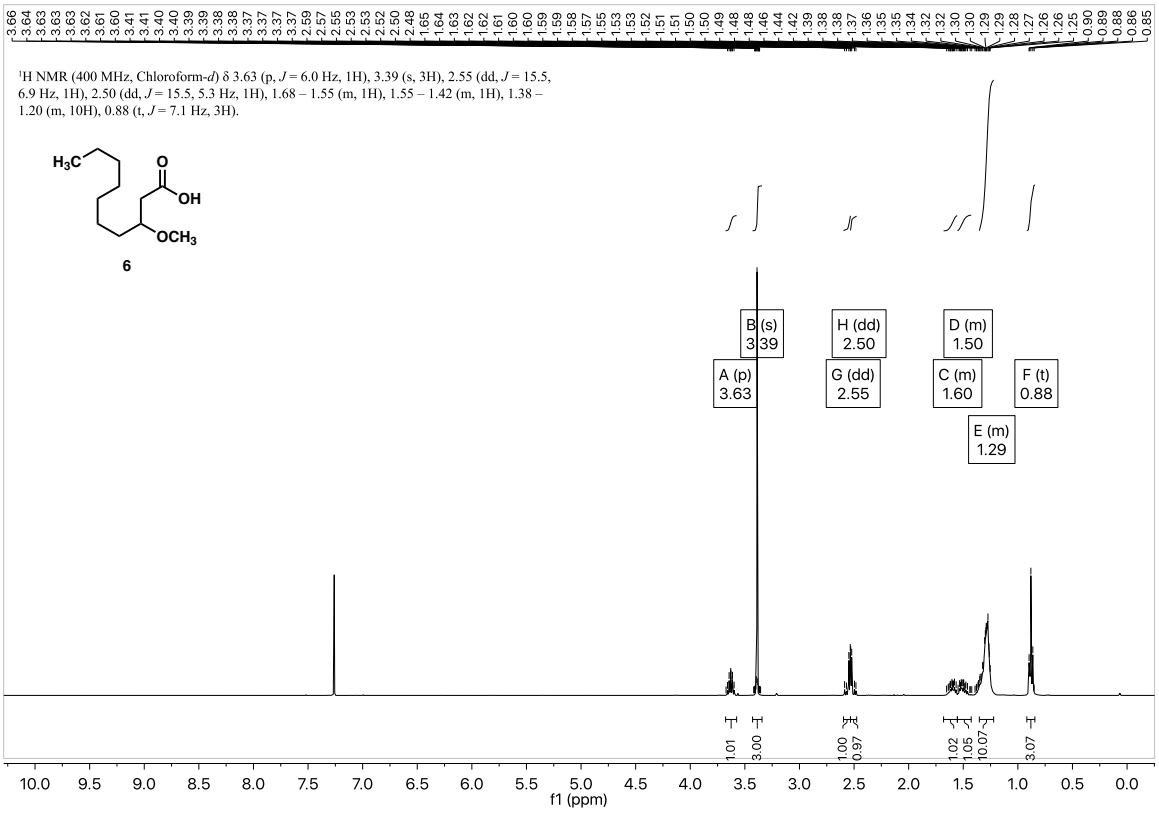

170.35
87.30
67.75
60.79
56.57
41.70
31.11
28.39
22.30
18.75
14.32
14.12
[13]C NMR (101 MHz, CDCl3) δ 170.4, 87.3, 67.7, 60.8, 56.6, 41.7, 31.1, 28.4, 22.3, 18.8, 14.3, 14.1.
O
O
CH3
OCH3
H3C
5e
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

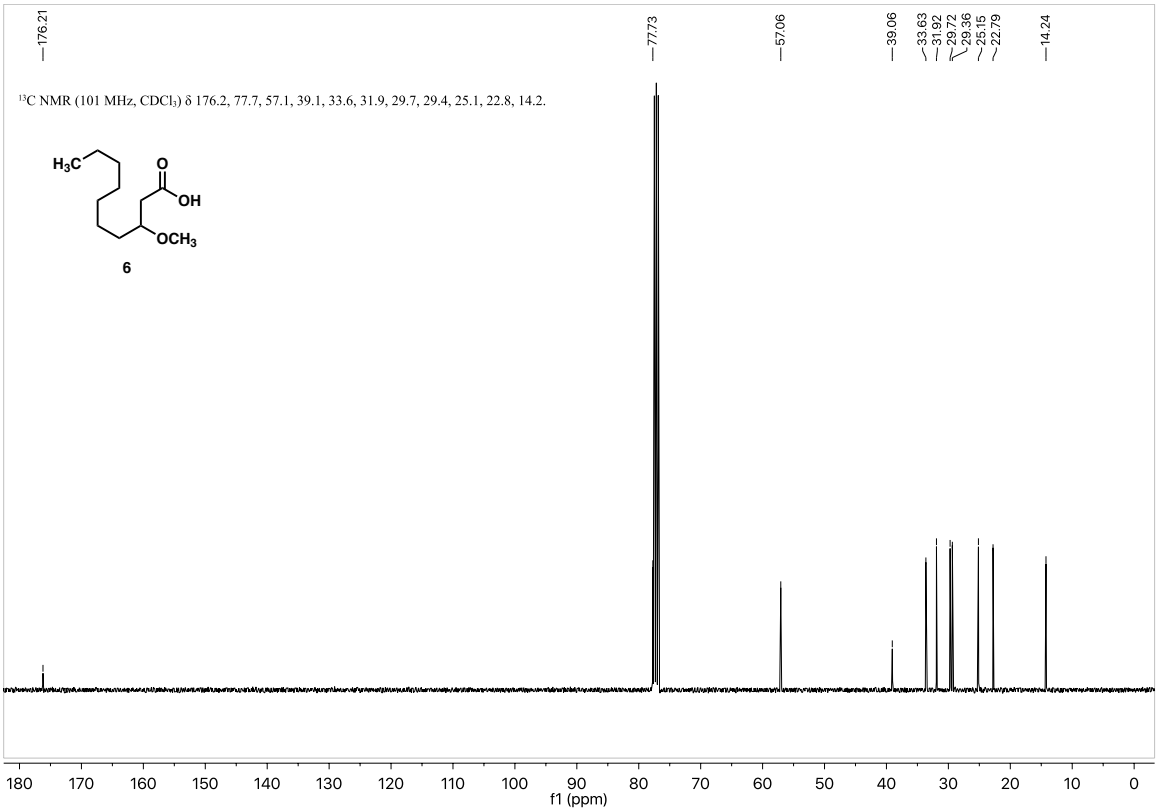

4.37 4.37 4.36 4.15 4.13 4.11 4.09 3.40 3.39 3.38 3.38 2.49 2.49 2.48 2.47 2.47 2.46 2.45 2.45 2.45 2.20 1.62 1.61 1.61 1.60 1.60 1.60 1.59 1.59 1.58 1.58 1.58 1.57 1.57 1.56 1.56 1.55 1.55 1.54 1.54 1.36 1.36 1.35 1.34 1.33 1.32 1.31 1.31 1.30 1.30 1.30 1.30 1.29 1.29 1.29 1.29 1.28 1.27 1.26 1.25 1.25 1.24 1.24 1.22 0.92 0.92 0.92 0.91 0.91 0.90 0.90 0.90 0.89 0.89 0.89 0.89 0.88 0.88 0.88 0.88 0.87
1H NMR (400 MHz, Chloroform-d) δ 4.37 (t, J = 1.6 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 3.39 (s, 3H), 2.47 (tt, J = 7.5, 1.6 Hz, 2H), 2.20 (s, 1H), 1.64 – 1.52 (m, 2H), 1.37 – 1.28 (m, 4H), 1.24 (t, J = 7.1 Hz, 3H), 0.93 – 0.86 (m, 3H).
H3C
O
O
H3C
OCH3
5e'
A (t) 4.37
B (q) 4.12
C (s) 3.39
D (tt) 2.47
E (s) 2.20
G (m) 1.58
H (m) 1.33
F (m) 0.89
I (t) 1.24
1.96
2.02
3.00
2.02
0.95
2.90
4.08
3.09
3.06
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
f1 (ppm)

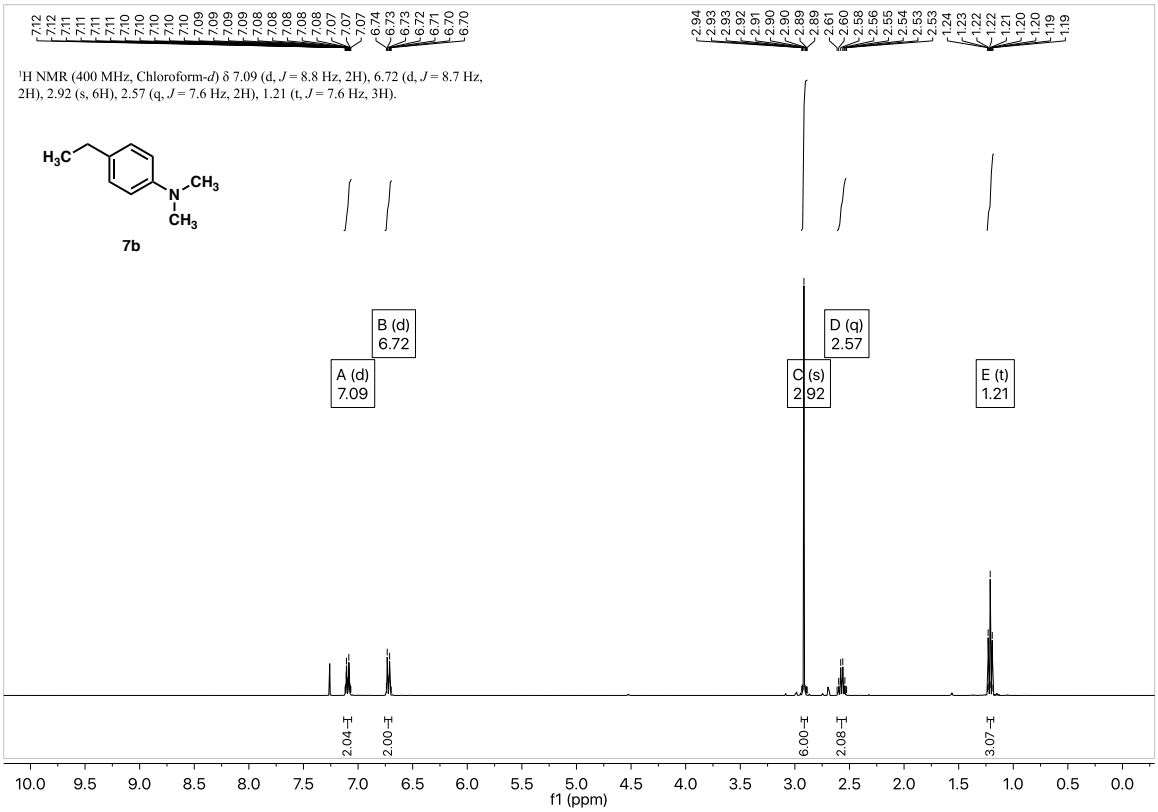

176.17
110.25
102.48
65.78
60.19
58.58
31.54
26.65
24.68
22.71
22.48
14.53
14.13
13C NMR (101 MHz, CDCl3) δ 176.2, 110.3, 102.5, 65.8, 60.2, 58.6, 31.5, 26.7, 24.7, 22.7, 22.5, 14.5, 14.1.
H3C
O
O
H3C
OCH3
5e'
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

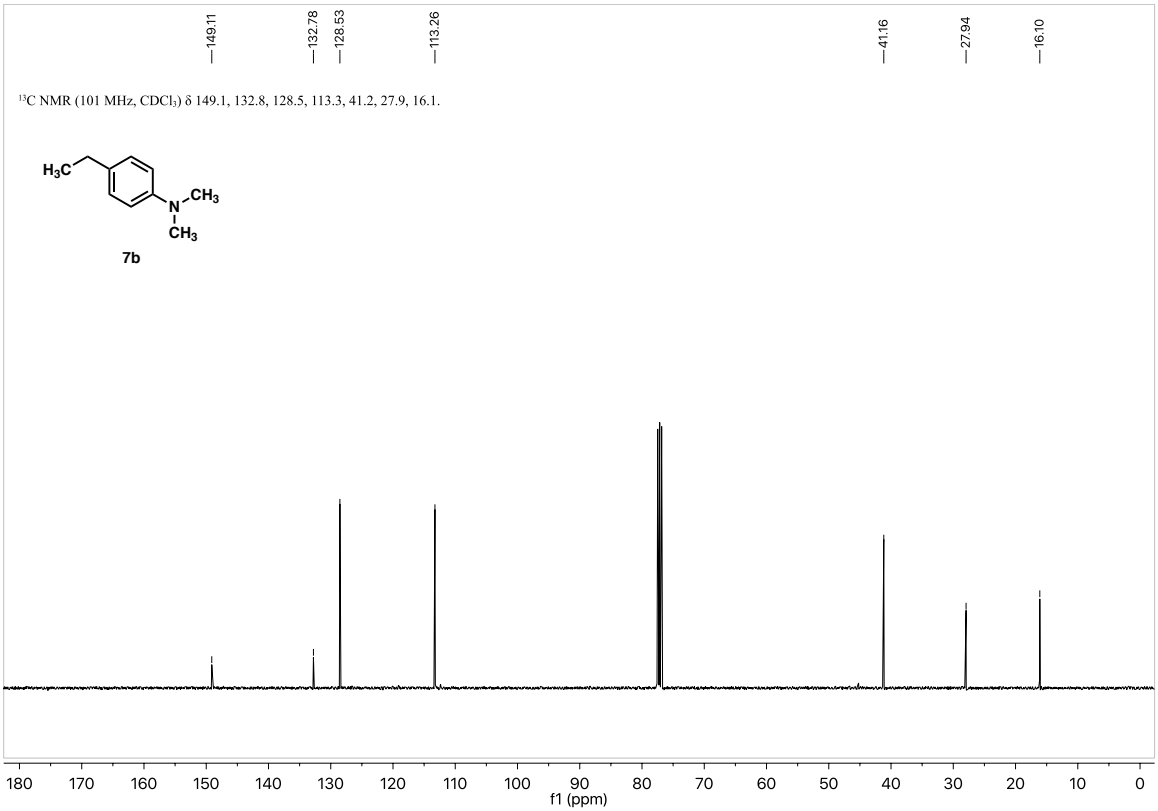

4.18 4.17 4.17 4.16 4.16 4.15 4.14 4.13 4.12 3.66 3.64 3.64 3.64 3.63 3.63 3.63 3.62 3.61 3.61 3.61 3.60 3.36 3.35 3.35 3.34 2.55 2.53 2.51 2.49 2.42 2.41 2.38 2.37 1.56 1.56 1.55 1.55 1.55 1.54 1.54 1.53 1.53 1.52 1.52 1.50 1.50 1.49 1.48 1.48 1.47 1.47 1.46 1.46 1.45 1.43 1.39 1.38 1.38 1.37 1.35 1.34 1.34 1.33 1.32 1.32 1.30 1.29 1.28 1.28 1.27 1.26 1.26 1.24 0.89 0.89 0.89 0.88 0.87 0.86
[1]H NMR (400 MHz, Chloroform-d) δ 4.15 (q, J = 7.1 Hz, 2H), 3.63 (ddt, J = 7.3, 6.3, 5.5 Hz, 1H), 3.35 (s, 3H), 2.52 (dd, J = 15.0, 7.3 Hz, 1H), 2.39 (dd, J = 15.0, 5.4 Hz, 1H), 1.57 – 1.41 (m, 2H), 1.40 – 1.20 (m, 13H), 0.88 (t, J = 7.1 Hz, 3H).
H3C
O
O
CH3
OCH3
6s
A (q) 4.15
B (ddt) 3.63
C (s) 3.35
D (dd) 2.52
E (dd) 2.39
F (m) 1.50
G (m) 1.27
H (t) 0.88
2.00
1.01
3.00
1.02
1.01
2.00
13.38
3.06
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
f1 (ppm)

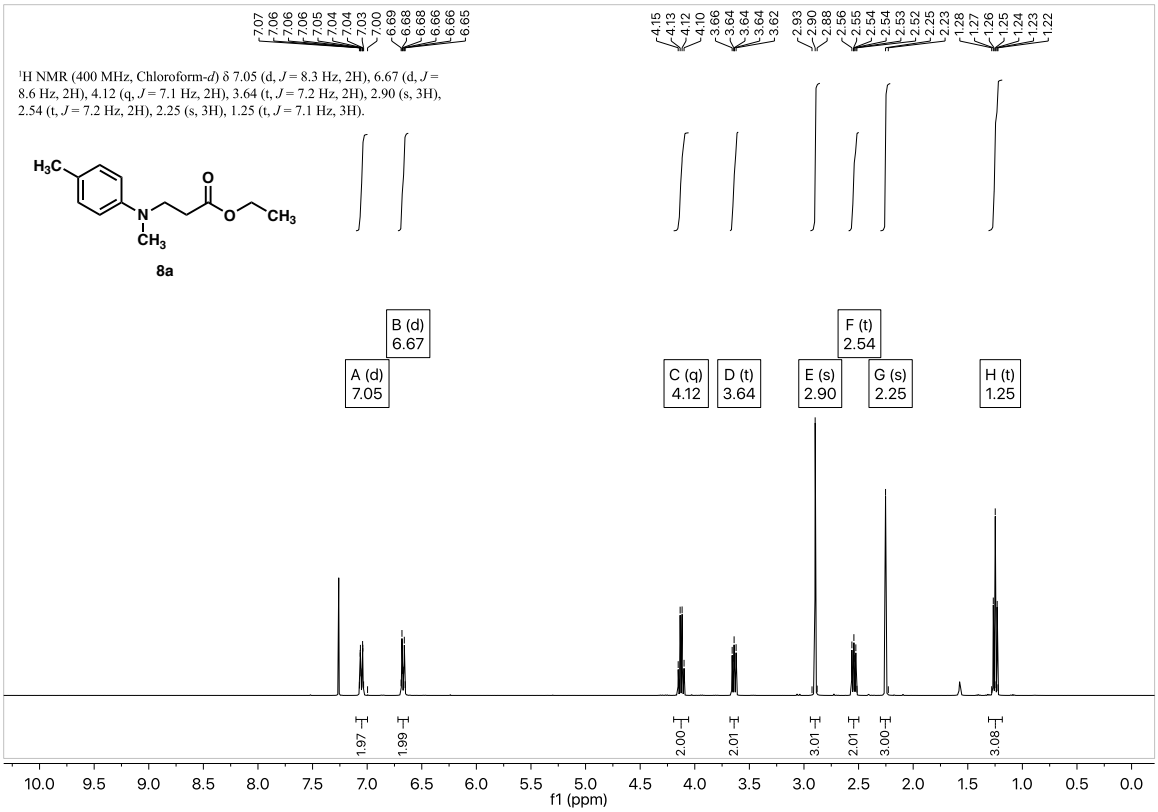

172.03
77.95
60.55
57.14
39.73
34.07
31.94
29.78
29.39
25.23
22.80
14.37
14.24
[13]C NMR (101 MHz, CDCl3) δ 172.0, 78.0, 60.5, 57.1, 39.7, 34.1, 31.9, 29.8, 29.4, 25.2, 22.8, 14.4, 14.2.
H3C
O
O
CH3
OCH3
6s
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

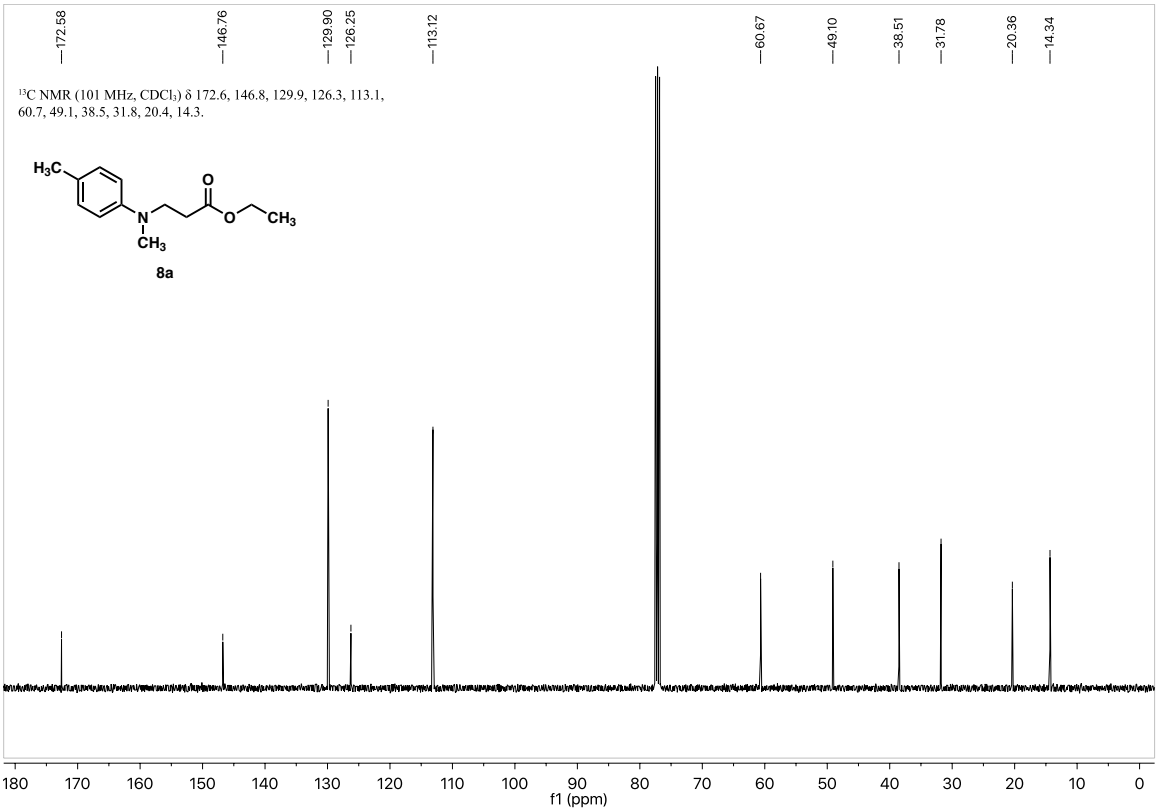

3.66 3.64 3.63 3.63 3.63 3.62 3.61 3.60 3.41 3.41 3.40 3.40 3.39 3.39 3.38 3.38 3.37 3.37 3.37 3.37 2.59 2.57 2.55 2.53 2.53 2.52 2.50 2.48 1.65 1.64 1.63 1.62 1.62 1.61 1.60 1.60 1.59 1.59 1.58 1.57 1.55 1.53 1.53 1.52 1.51 1.51 1.50 1.50 1.49 1.48 1.48 1.46 1.44 1.42 1.39 1.38 1.38 1.37 1.36 1.35 1.35 1.34 1.32 1.32 1.30 1.30 1.29 1.29 1.28 1.27 1.26 1.26 1.25 0.90 0.89 0.88 0.86 0.85
¹H NMR (400 MHz, Chloroform-d) δ 3.63 (p, J = 6.0 Hz, 1H), 3.39 (s, 3H), 2.55 (dd, J = 15.5, 6.9 Hz, 1H), 2.50 (dd, J = 15.5, 5.3 Hz, 1H), 1.68 – 1.55 (m, 1H), 1.55 – 1.42 (m, 1H), 1.38 – 1.20 (m, 10H), 0.88 (t, J = 7.1 Hz, 3H).
H3C
O
OH
OCH3
6
A (p) 3.63
B (s) 3.39
G (dd) 2.55
H (dd) 2.50
C (m) 1.60
D (m) 1.50
E (m) 1.29
F (t) 0.88
1.01
3.00
1.00
0.97
1.02
1.05
10.07
3.07
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
f1 (ppm)

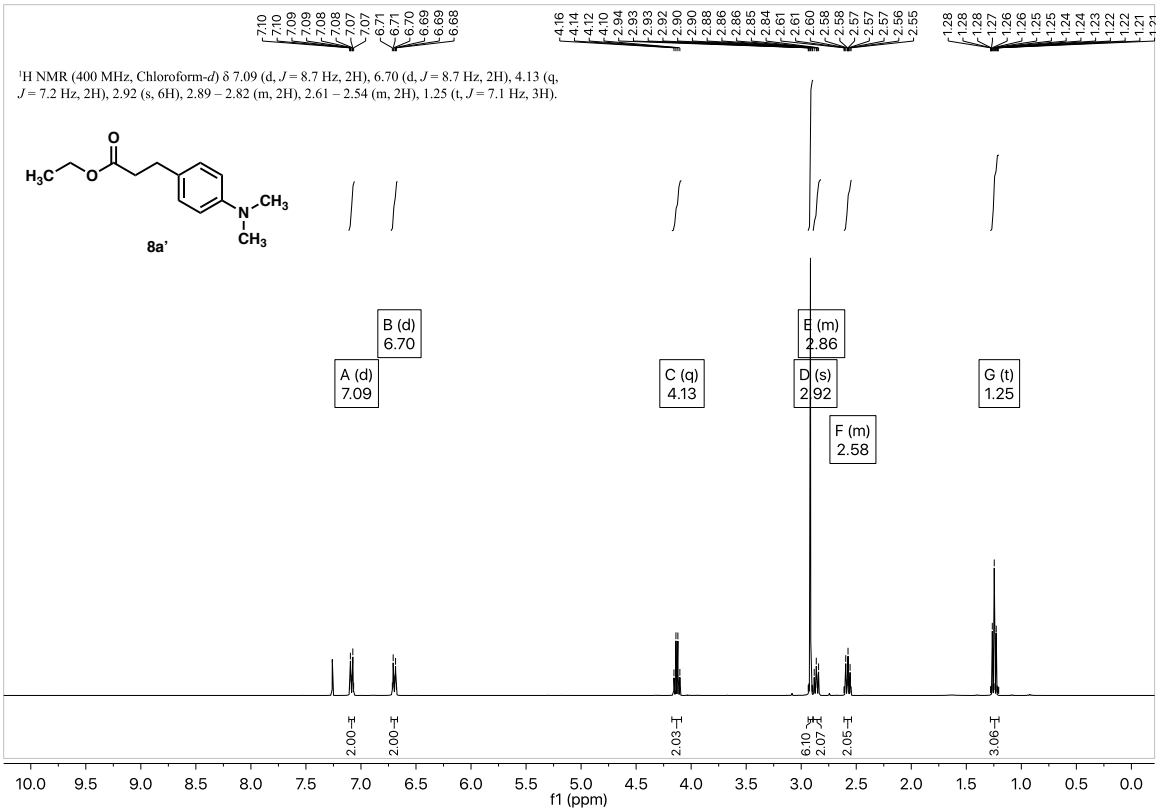

176.21
77.73
57.06
39.06
33.63
31.92
29.72
29.36
25.15
22.79
14.24
¹³C NMR (101 MHz, CDCl3) δ 176.2, 77.7, 57.1, 39.1, 33.6, 31.9, 29.7, 29.4, 25.1, 22.8, 14.2.
H3C
O
OH
OCH3
6
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

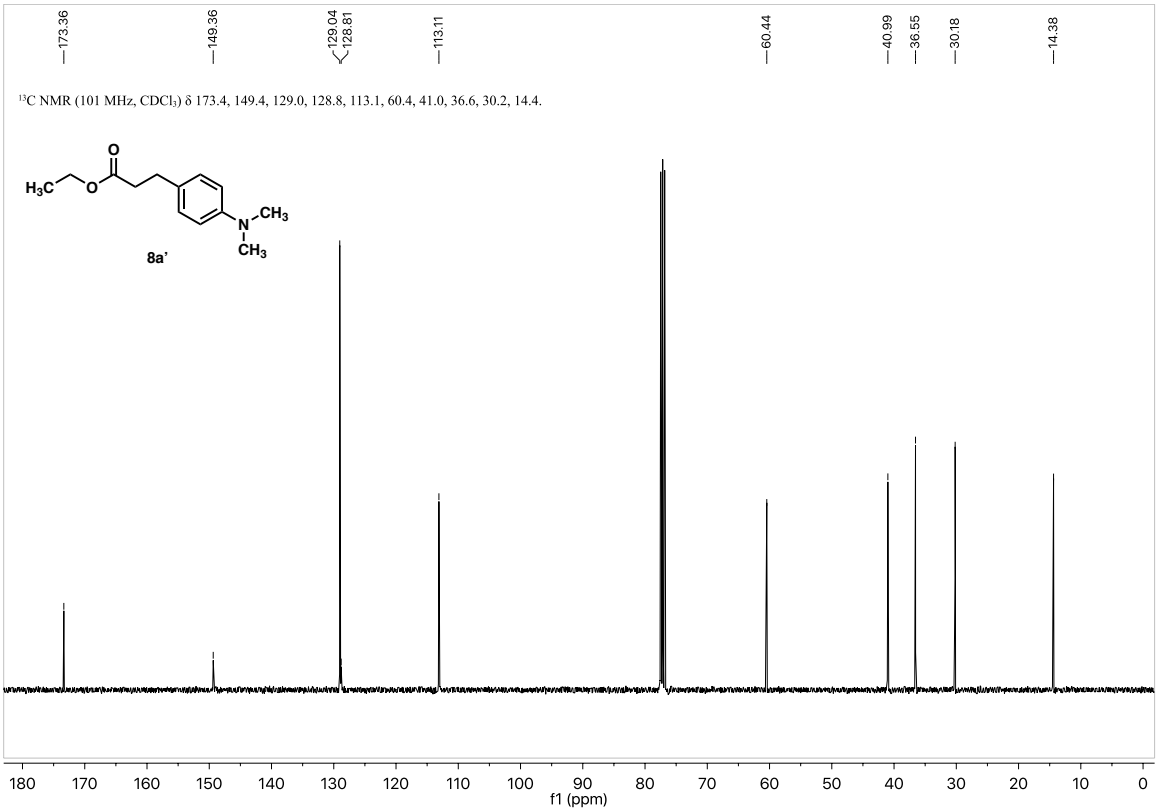

7.12 7.12 7.11 7.11 7.11 7.11 7.10 7.10 7.10 7.10 7.10 7.09 7.09 7.09 7.09 7.08 7.08 7.08 7.08 7.08 7.07 7.07 7.07 6.74 6.73 6.73 6.72 6.71 6.70 6.70
2.94 2.93 2.93 2.92 2.91 2.90 2.90 2.89 2.89 2.61 2.60 2.58 2.56 2.55 2.54 2.53 2.53 1.24 1.23 1.22 1.22 1.21 1.20 1.20 1.19 1.19
¹H NMR (400 MHz, Chloroform-*d*) δ 7.09 (d, *J* = 8.8 Hz, 2H), 6.72 (d, *J* = 8.7 Hz, 2H), 2.92 (s, 6H), 2.57 (q, *J* = 7.6 Hz, 2H), 1.21 (t, *J* = 7.6 Hz, 3H).
H3C
N
CH3
CH3
7b
A (d) 7.09
B (d) 6.72
C (s) 2.92
D (q) 2.57
E (t) 1.21
2.04
2.00
6.00
2.08
3.07
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
f1 (ppm)

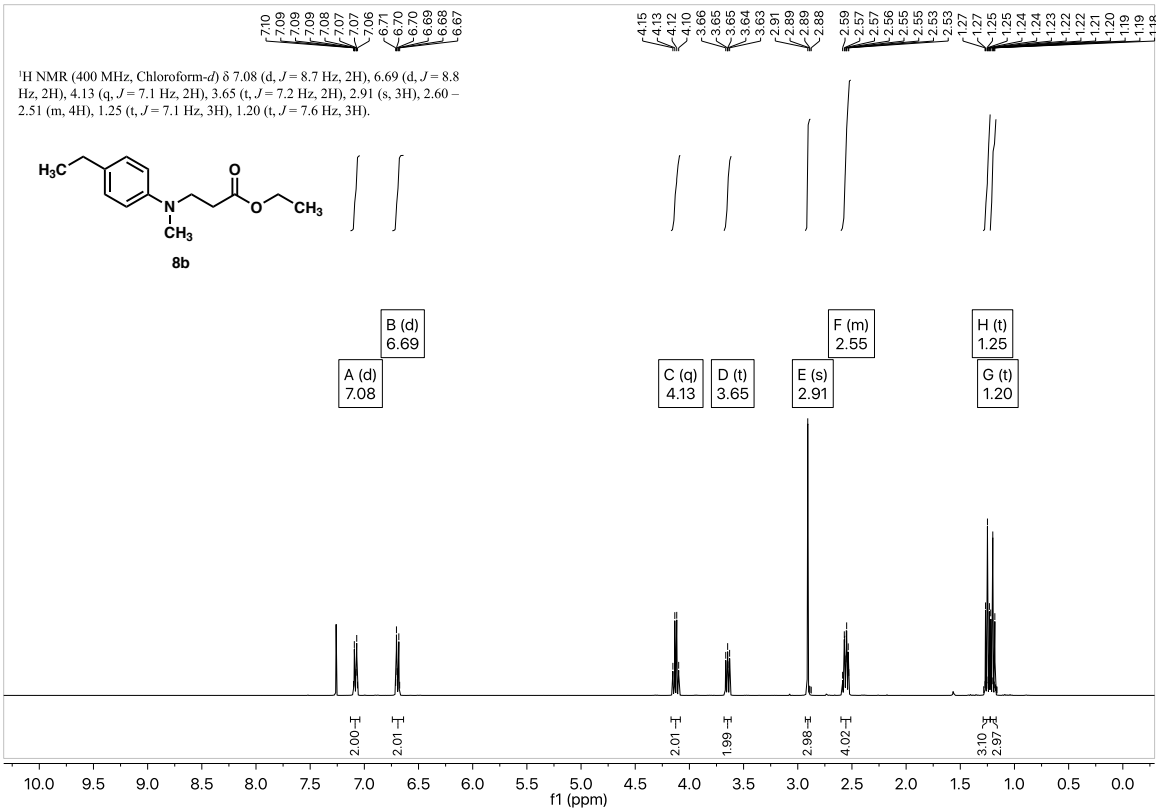

149.11
132.78
128.53
113.26
41.16
27.94
16.10
¹³C NMR (101 MHz, CDCl3) δ 149.1, 132.8, 128.5, 113.3, 41.2, 27.9, 16.1.
H3C
N
CH3
CH3
7b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

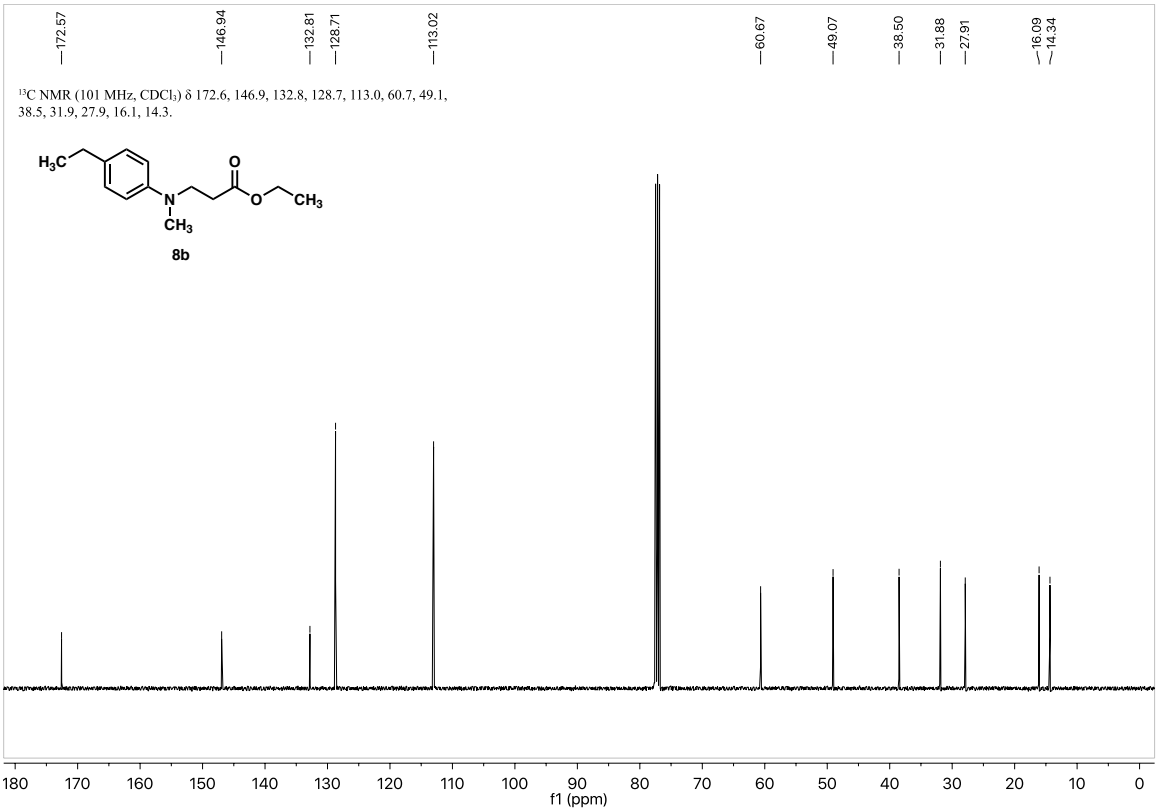

7.07 7.06 7.06 7.06 7.05 7.04 7.04 7.03 7.00 6.69 6.68 6.68 6.66 6.66 6.65
4.15 4.13 4.12 4.10 3.66 3.64 3.64 3.64 3.62 2.93 2.90 2.88 2.56 2.55 2.54 2.54 2.53 2.52 2.25 2.23 1.28 1.27 1.26 1.25 1.24 1.23 1.22
1H NMR (400 MHz, Chloroform-d) δ 7.05 (d, J = 8.3 Hz, 2H), 6.67 (d, J = 8.6 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 3.64 (t, J = 7.2 Hz, 2H), 2.90 (s, 3H), 2.54 (t, J = 7.2 Hz, 2H), 2.25 (s, 3H), 1.25 (t, J = 7.1 Hz, 3H).
H3C
N
CH3
O
O
CH3
8a
A (d) 7.05
B (d) 6.67
C (q) 4.12
D (t) 3.64
E (s) 2.90
F (t) 2.54
G (s) 2.25
H (t) 1.25
1.97
1.99
2.00
2.01
3.01
2.01
3.00
3.08
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
f1 (ppm)

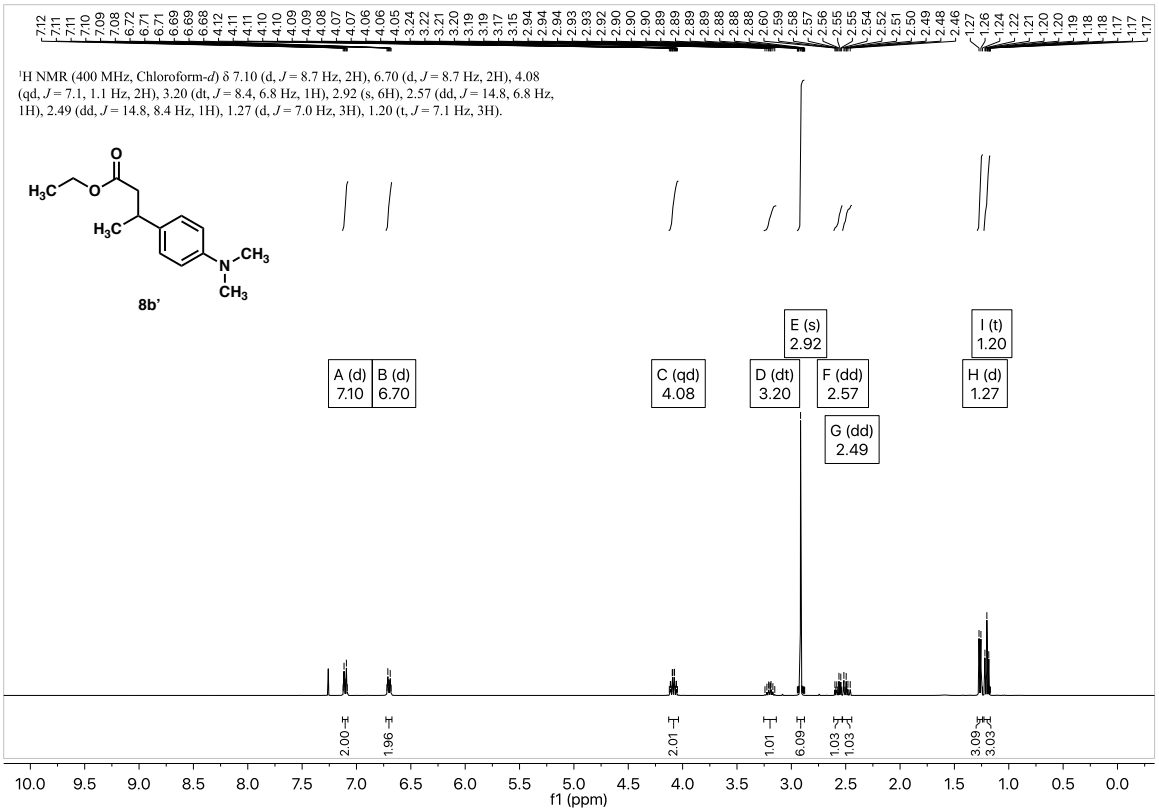

172.58 146.76 129.90 126.25 113.12 60.67 49.10 38.51 31.78 20.36 14.34
13C NMR (101 MHz, CDCl3) δ 172.6, 146.8, 129.9, 126.3, 113.1, 60.7, 49.1, 38.5, 31.8, 20.4, 14.3.
H3C
N
CH3
O
O
CH3
8a
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

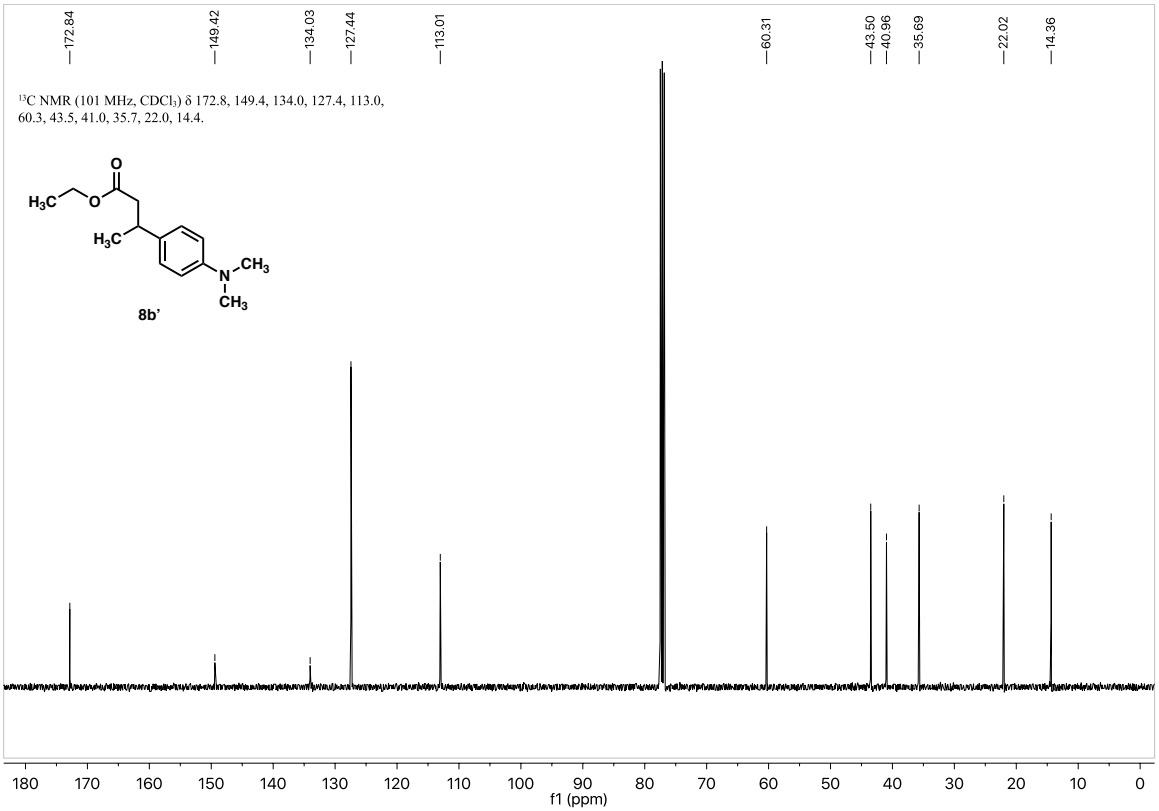

7.10 7.10 7.09 7.09 7.08 7.08 7.07 7.07 6.71 6.71 6.70 6.69 6.69 6.68
4.16 4.14 4.12 4.10 2.94 2.93 2.93 2.92 2.90 2.90 2.88 2.86 2.86 2.85 2.84 2.61 2.61 2.60 2.58 2.58 2.57 2.57 2.57 2.56 2.55
1.28 1.28 1.28 1.27 1.26 1.26 1.25 1.25 1.24 1.24 1.23 1.22 1.22 1.21
1H NMR (400 MHz, Chloroform-d) δ 7.09 (d, J = 8.7 Hz, 2H), 6.70 (d, J = 8.7 Hz, 2H), 4.13 (q, J = 7.2 Hz, 2H), 2.92 (s, 6H), 2.89 – 2.82 (m, 2H), 2.61 – 2.54 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H).
8a'
A (d) 7.09
B (d) 6.70
C (q) 4.13
D (s) 2.92
E (m) 2.86
F (m) 2.58
G (t) 1.25
2.00 2.00 2.03 6.10 2.07 2.05 3.06
f1 (ppm)

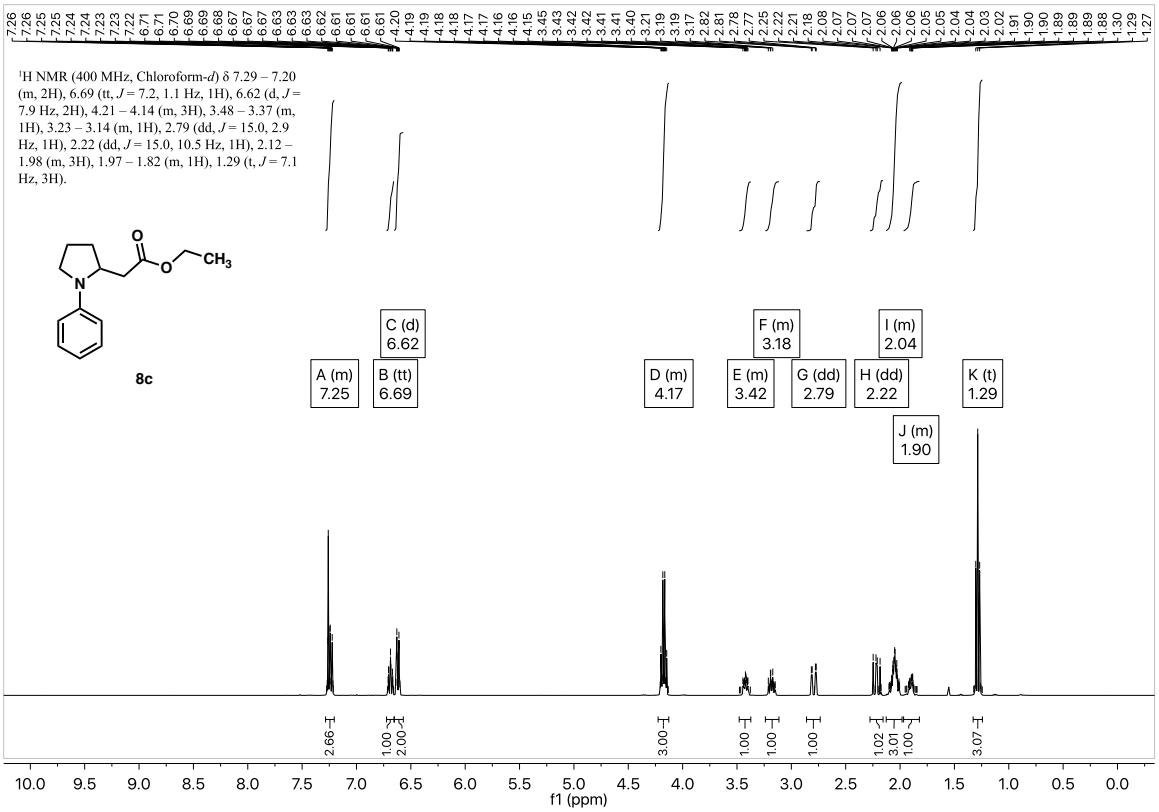

173.36 149.36 129.04 128.81 113.11 60.44 40.99 36.55 30.18 14.38
13C NMR (101 MHz, CDCl3) δ 173.4, 149.4, 129.0, 128.8, 113.1, 60.4, 41.0, 36.6, 30.2, 14.4.
8a'
f1 (ppm)


7.10
7.09
7.09
7.09
7.08
7.08
7.07
7.07
7.06
6.71
6.70
6.70
6.69
6.68
6.67
4.15
4.13
4.12
4.10
3.66
3.65
3.65
3.64
3.63
2.91
2.89
2.89
2.88
2.59
2.57
2.57
2.56
2.55
2.55
2.53
2.53
1.27
1.25
1.25
1.24
1.24
1.23
1.22
1.22
1.21
1.20
1.19
1.19
1.18
1H NMR (400 MHz, Chloroform-d) δ 7.08 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.8 Hz, 2H), 4.13 (q, J = 7.1 Hz, 2H), 3.65 (t, J = 7.2 Hz, 2H), 2.91 (s, 3H), 2.60 – 2.51 (m, 4H), 1.25 (t, J = 7.1 Hz, 3H), 1.20 (t, J = 7.6 Hz, 3H).
H3C
O
N
CH3
O
CH3
8b
A (d) 7.08
B (d) 6.69
C (q) 4.13
D (t) 3.65
E (s) 2.91
F (m) 2.55
G (t) 1.20
H (t) 1.25
2.00
2.01
2.01
1.99
2.98
4.02
3.10
2.97
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
f1 (ppm)


172.57
146.94
132.81
128.71
113.02
60.67
49.07
38.50
31.88
27.91
16.09
14.34
13C NMR (101 MHz, CDCl3) δ 172.6, 146.9, 132.8, 128.7, 113.0, 60.7, 49.1, 38.5, 31.9, 27.9, 16.1, 14.3.
H3C
O
N
CH3
O
CH3
8b
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.12 7.11 7.11 7.10 7.09 7.08 6.72 6.71 6.71 6.69 6.69 6.68 4.12 4.11 4.11 4.10 4.10 4.09 4.09 4.08 4.07 4.07 4.06 4.06 4.05 3.24 3.22 3.21 3.20 3.19 3.19 3.17 3.15 2.94 2.94 2.93 2.92 2.90 2.90 2.90 2.89 2.89 2.89 2.88 2.88 2.88 2.60 2.59 2.58 2.57 2.56 2.55 2.55 2.54 2.52 2.51 2.50 2.49 2.48 2.46 1.27 1.26 1.24 1.22 1.21 1.20 1.20 1.19 1.18 1.18 1.17 1.17 1.17
[1]H NMR (400 MHz, Chloroform-d) δ 7.10 (d, J = 8.7 Hz, 2H), 6.70 (d, J = 8.7 Hz, 2H), 4.08 (qd, J = 7.1, 1.1 Hz, 2H), 3.20 (dt, J = 8.4, 6.8 Hz, 1H), 2.92 (s, 6H), 2.57 (dd, J = 14.8, 6.8 Hz, 1H), 2.49 (dd, J = 14.8, 8.4 Hz, 1H), 1.27 (d, J = 7.0 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H).
8b'
A (d) 7.10
B (d) 6.70
C (qd) 4.08
D (dt) 3.20
E (s) 2.92
F (dd) 2.57
G (dd) 2.49
H (d) 1.27
I (t) 1.20
2.00
1.96
2.01
1.01
6.09
1.03
1.03
3.09
3.03
f1 (ppm)

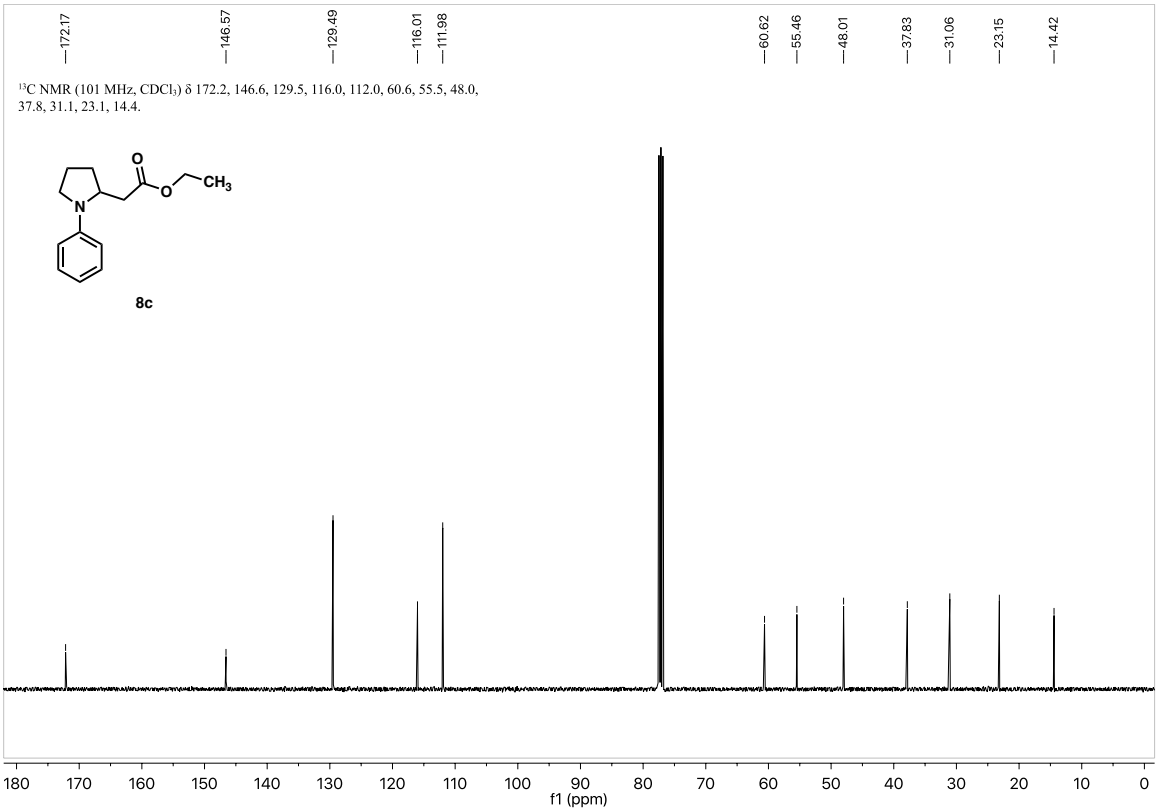

172.84 149.42 134.03 127.44 113.01 60.31 43.50 40.96 35.69 22.02 14.36
[13]C NMR (101 MHz, CDCl3) δ 172.8, 149.4, 134.0, 127.4, 113.0, 60.3, 43.5, 41.0, 35.7, 22.0, 14.4.
8b'
f1 (ppm)




$^{1}$H NMR (400 MHz, Chloroform-*d*) δ 7.29 – 7.20 (m, 2H), 6.69 (tt, *J* = 7.2, 1.1 Hz, 1H), 6.62 (d, *J* = 7.9 Hz, 2H), 4.21 – 4.14 (m, 3H), 3.48 – 3.37 (m, 1H), 3.23 – 3.14 (m, 1H), 2.79 (dd, *J* = 15.0, 2.9 Hz, 1H), 2.22 (dd, *J* = 15.0, 10.5 Hz, 1H), 2.12 – 1.98 (m, 3H), 1.97 – 1.82 (m, 1H), 1.29 (t, *J* = 7.1 Hz, 3H).



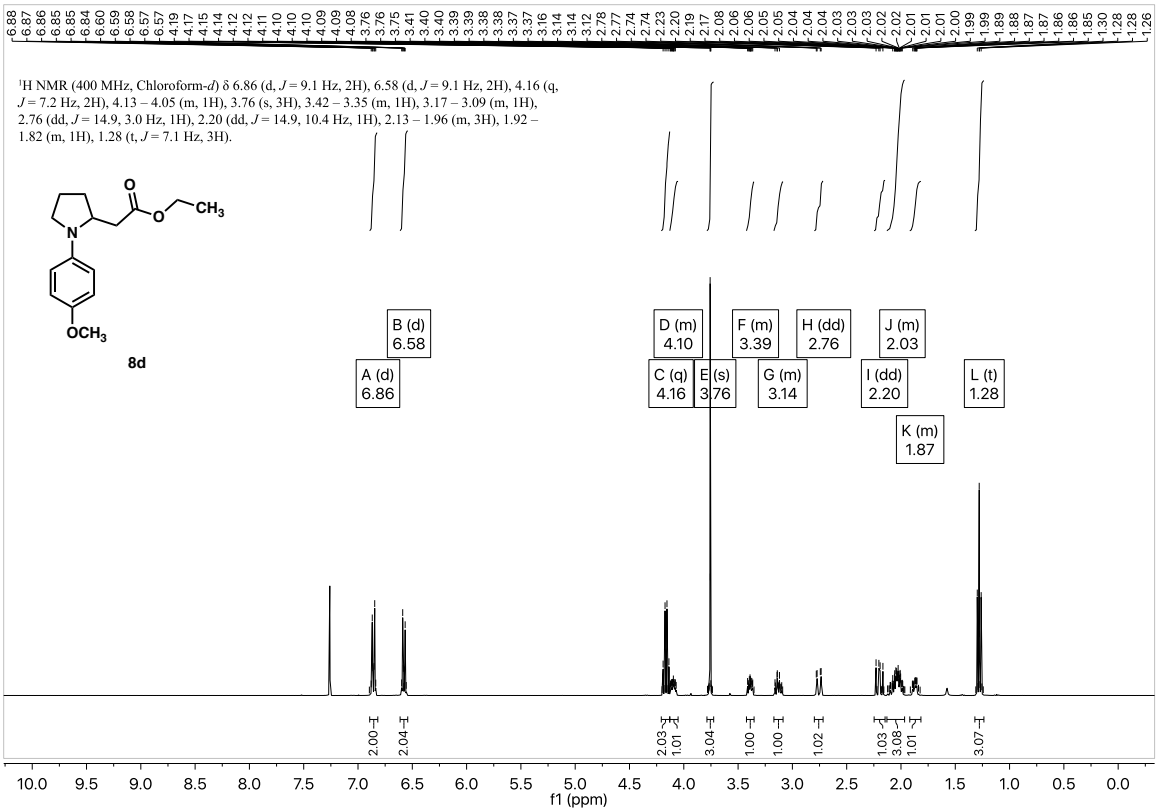
**8c**

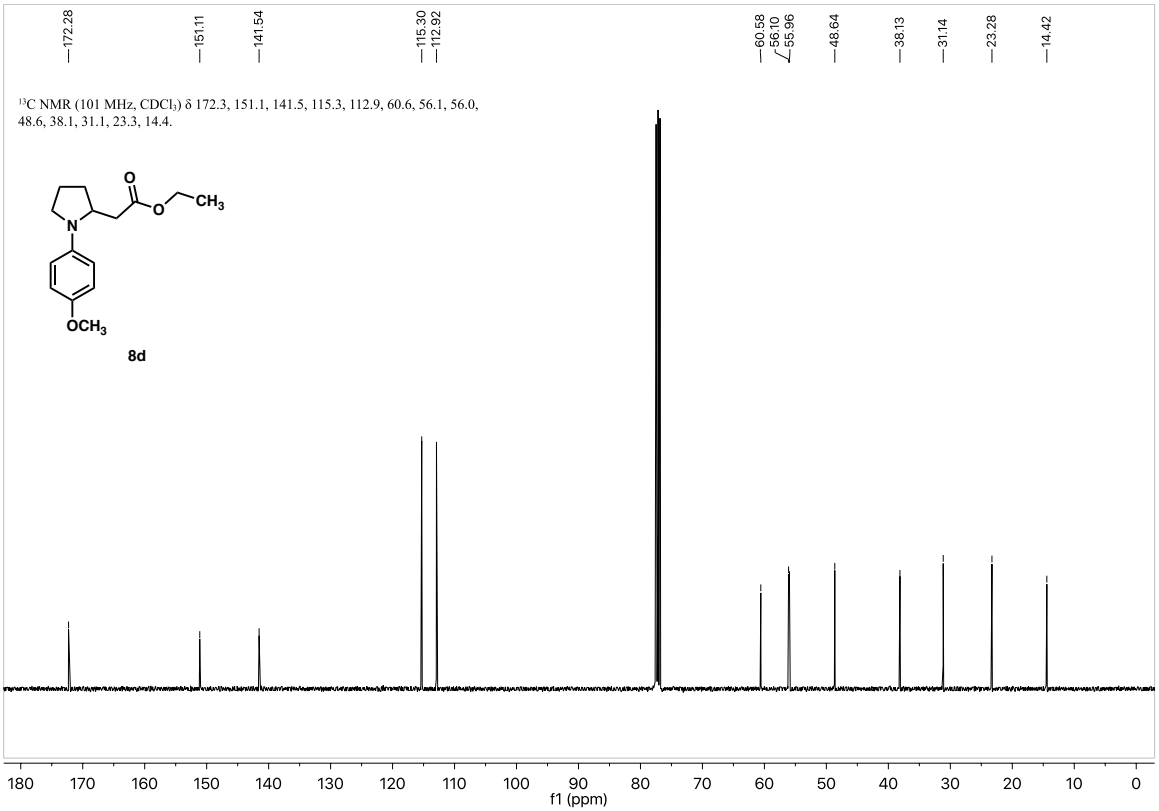


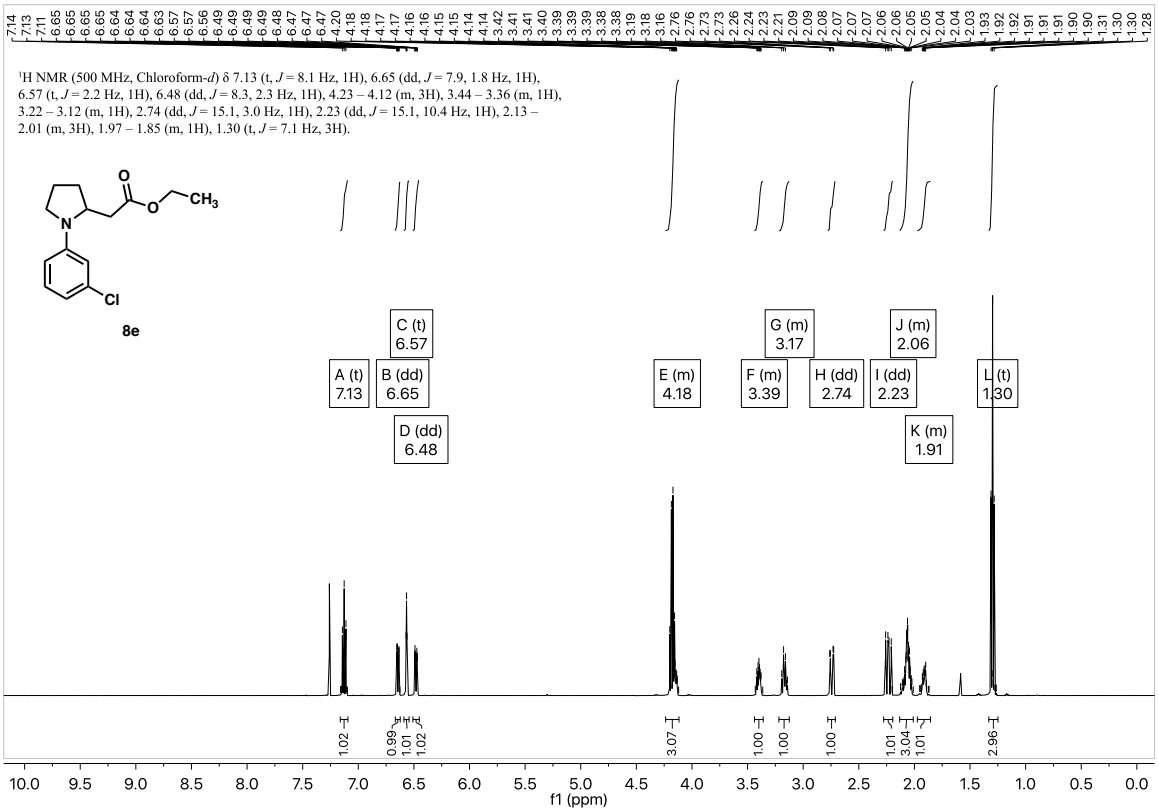

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.2, 146.6, 129.5, 116.0, 112.0, 60.6, 55.5, 48.0, 37.8, 31.1, 23.1, 14.4.

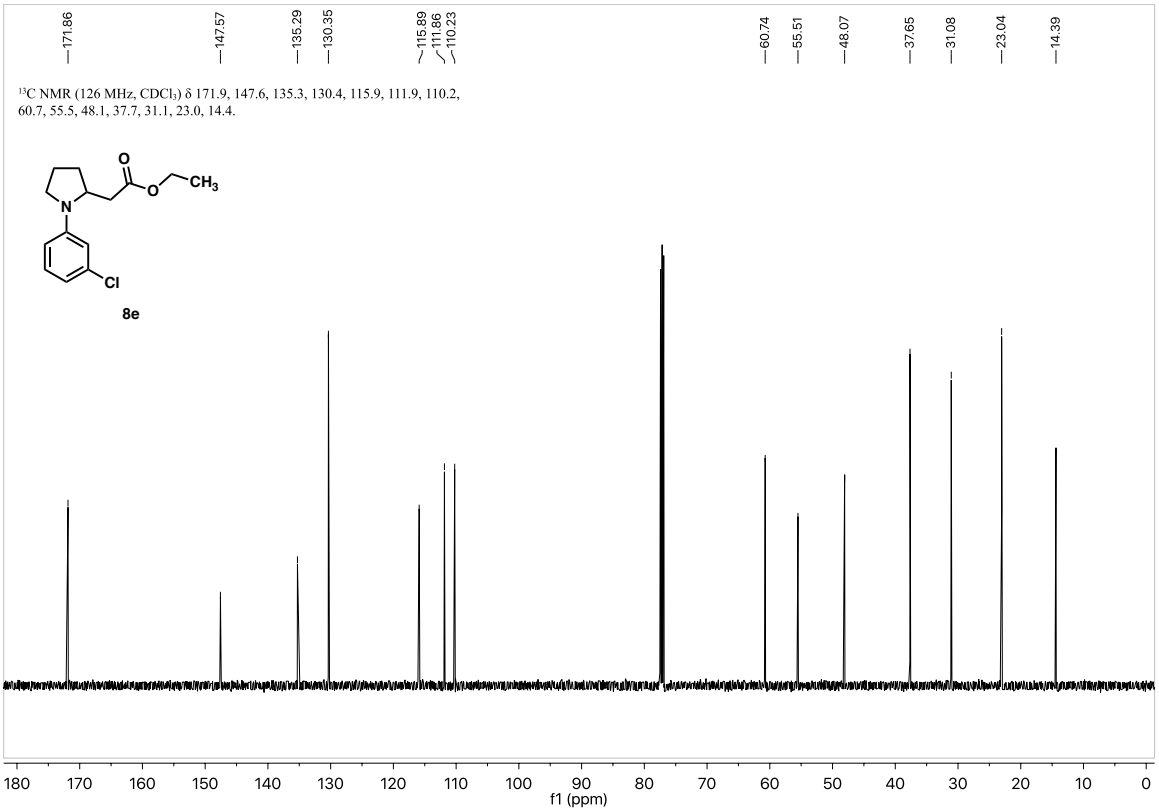


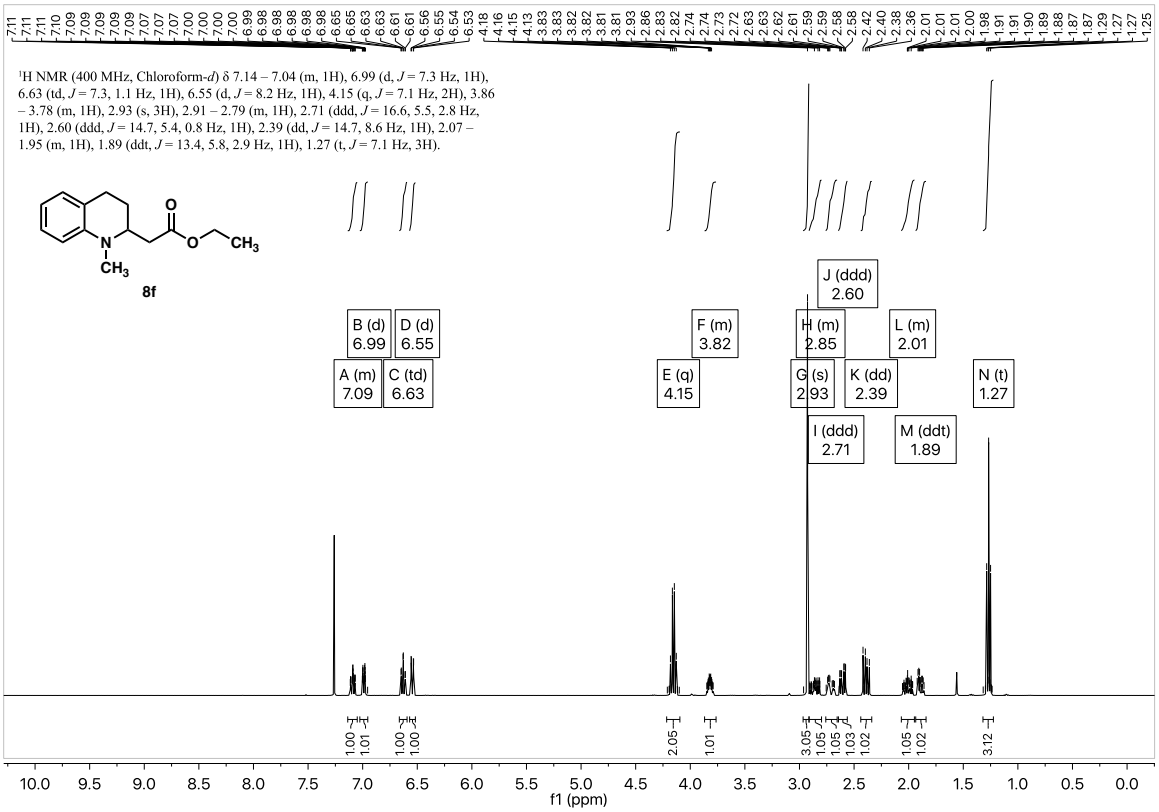
**8c**

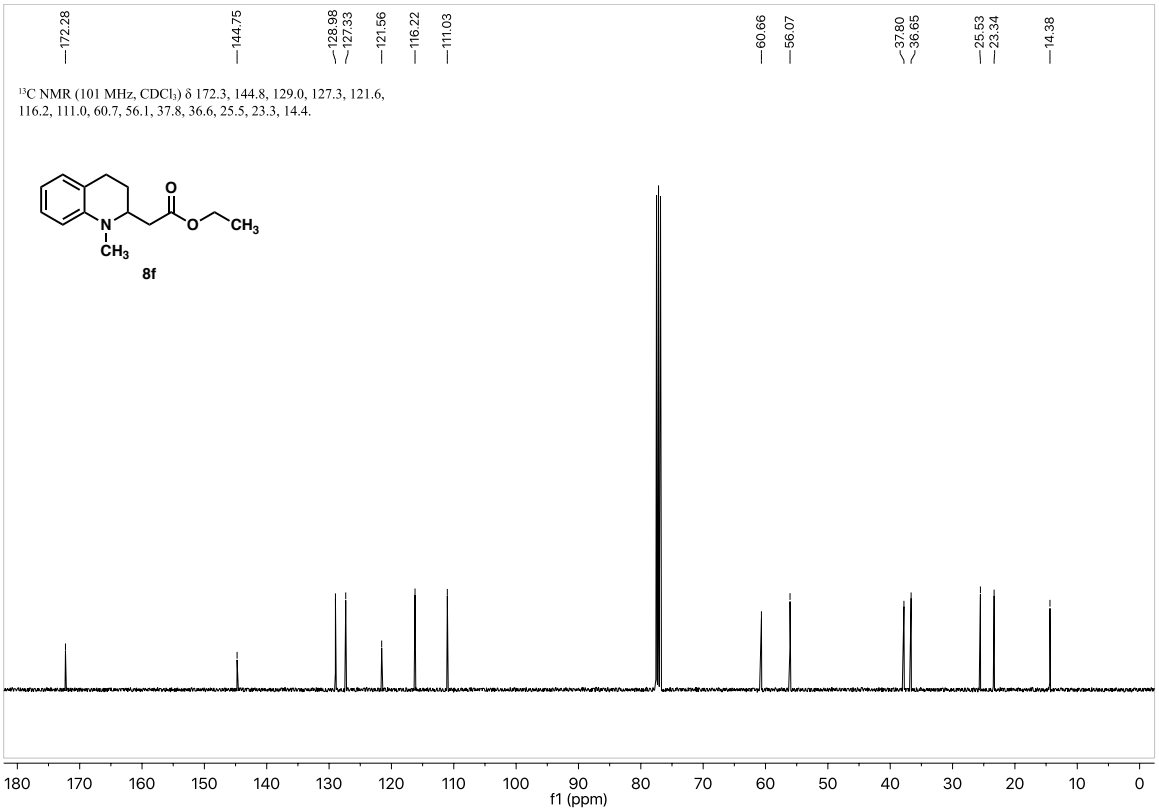

6.88 6.87 6.86 6.85 6.84 6.60 6.59 6.58 6.57 6.57 4.19 4.17 4.15 4.14 4.12 4.12 4.11 4.10 4.10 4.10 4.09 4.09 4.08 3.76 3.76 3.75 3.41 3.40 3.39 3.38 3.38 3.37 3.37 3.16 3.14 3.14 3.12 2.78 2.77 2.74 2.74 2.23 2.20 2.19 2.17 2.08 2.06 2.06 2.05 2.05 2.04 2.04 2.04 2.03 2.03 2.02 2.02 2.02 2.01 2.01 2.01 2.00 1.99 1.99 1.89 1.88 1.87 1.87 1.86 1.86 1.85 1.30 1.28 1.28 1.26
[1]H NMR (400 MHz, Chloroform-d) δ 6.86 (d, J = 9.1 Hz, 2H), 6.58 (d, J = 9.1 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 4.13 – 4.05 (m, 1H), 3.76 (s, 3H), 3.42 – 3.35 (m, 1H), 3.17 – 3.09 (m, 1H), 2.76 (dd, J = 14.9, 3.0 Hz, 1H), 2.20 (dd, J = 14.9, 10.4 Hz, 1H), 2.13 – 1.96 (m, 3H), 1.92 – 1.82 (m, 1H), 1.28 (t, J = 7.1 Hz, 3H).
O
O
CH3
N
OCH3
8d
A (d) 6.86
B (d) 6.58
C (q) 4.16
D (m) 4.10
E (s) 3.76
F (m) 3.39
G (m) 3.14
H (dd) 2.76
I (dd) 2.20
J (m) 2.03
K (m) 1.87
L (t) 1.28
2.00
2.04
2.03
1.01
3.04
1.00
1.00
1.02
1.03
3.08
1.01
3.07
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
f1 (ppm)

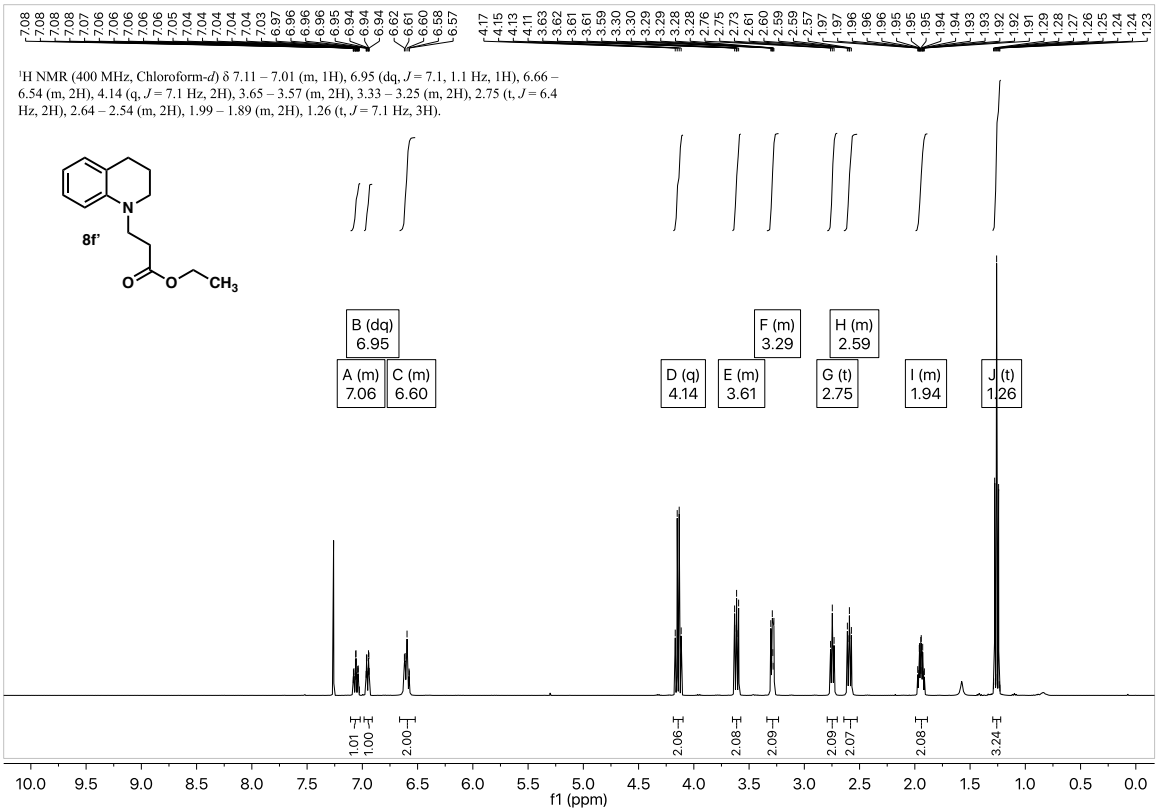

172.28
151.11
141.54
115.30
112.92
60.58
56.10
55.96
48.64
38.13
31.14
23.28
14.42
[13]C NMR (101 MHz, CDCl3) δ 172.3, 151.1, 141.5, 115.3, 112.9, 60.6, 56.1, 56.0, 48.6, 38.1, 31.1, 23.3, 14.4.
O
O
CH3
N
OCH3
8d
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

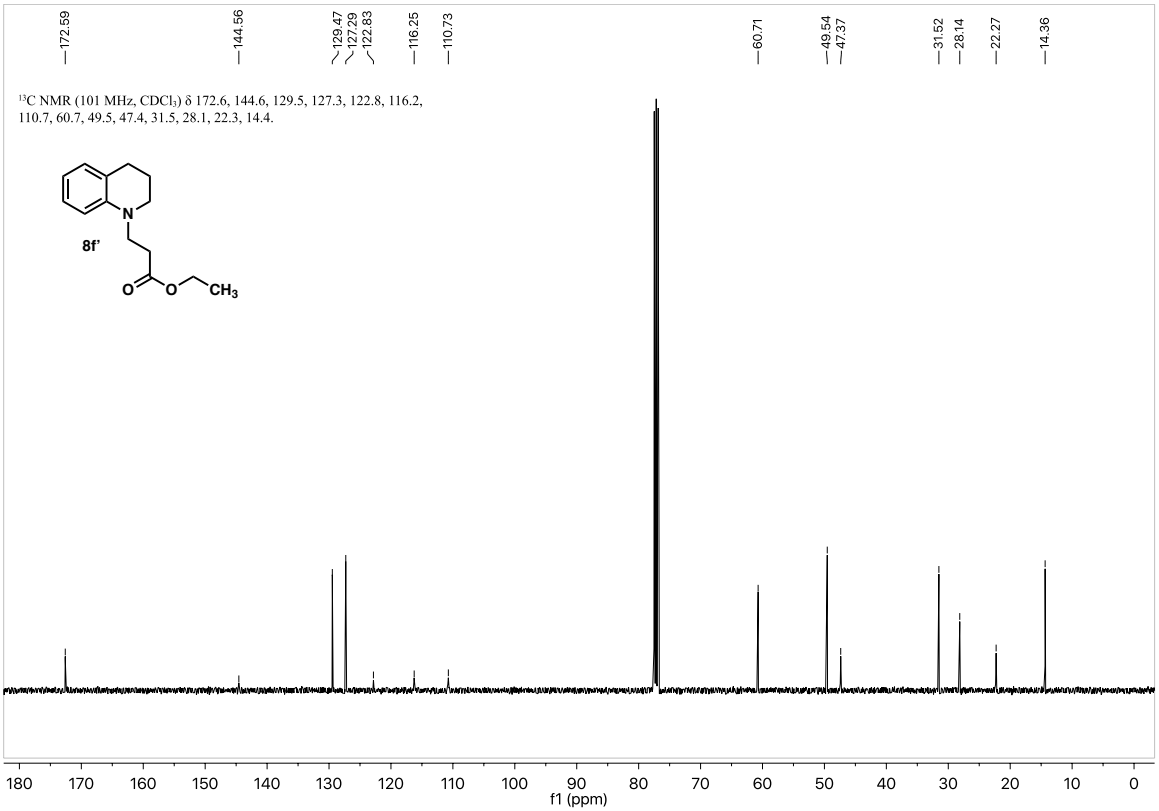

7.14 7.13 7.11 6.65 6.65 6.65 6.65 6.64 6.64 6.64 6.63 6.57 6.57 6.56 6.49 6.49 6.49 6.48 6.47 6.47 6.47 4.20 4.18 4.17 4.17 4.16 4.16 4.15 4.15 4.14 4.14 3.42 3.41 3.41 3.40 3.39 3.39 3.39 3.38 3.38 3.19 3.18 3.16 2.76 2.73 2.26 2.24 2.23 2.21 2.09 2.09 2.08 2.07 2.07 2.07 2.06 2.06 2.05 2.05 2.04 2.04 2.03 1.93 1.92 1.92 1.91 1.91 1.90 1.90 1.31 1.30 1.30 1.28
[1]H NMR (500 MHz, Chloroform-d) δ 7.13 (t, J = 8.1 Hz, 1H), 6.65 (dd, J = 7.9, 1.8 Hz, 1H), 6.57 (t, J = 2.2 Hz, 1H), 6.48 (dd, J = 8.3, 2.3 Hz, 1H), 4.23 – 4.12 (m, 3H), 3.44 – 3.36 (m, 1H), 3.22 – 3.12 (m, 1H), 2.74 (dd, J = 15.1, 3.0 Hz, 1H), 2.23 (dd, J = 15.1, 10.4 Hz, 1H), 2.13 – 2.01 (m, 3H), 1.97 – 1.85 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H).
O
O
CH3
N
Cl
8e
A (t) 7.13
B (dd) 6.65
C (t) 6.57
D (dd) 6.48
E (m) 4.18
F (m) 3.39
G (m) 3.17
H (dd) 2.74
I (dd) 2.23
J (m) 2.06
K (m) 1.91
L (t) 1.30
1.02
0.99
1.01
1.02
3.07
1.00
1.00
1.00
1.01
3.04
1.01
2.96
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
f1 (ppm)

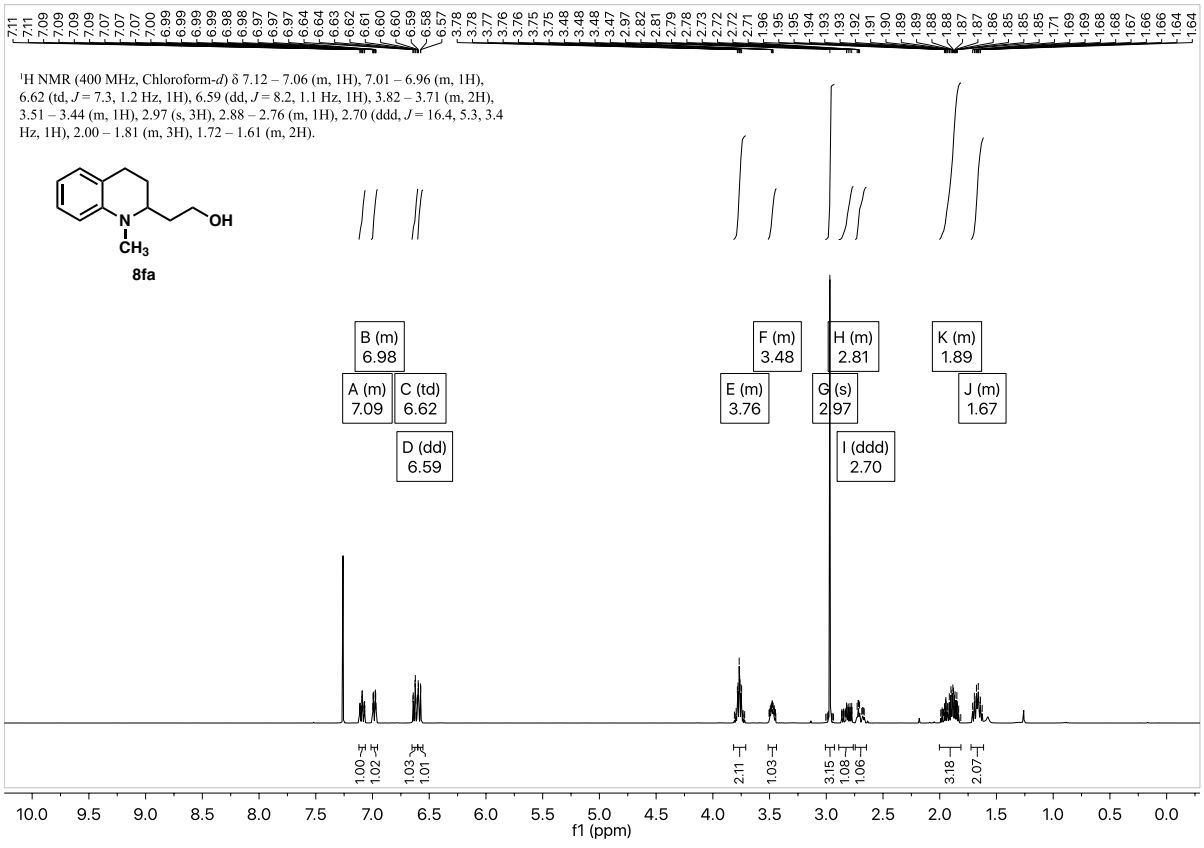

171.86
147.57
135.29
130.35
115.89
111.86
110.23
60.74
55.51
48.07
37.65
31.08
23.04
14.39
[13]C NMR (126 MHz, CDCl3) δ 171.9, 147.6, 135.3, 130.4, 115.9, 111.9, 110.2, 60.7, 55.5, 48.1, 37.7, 31.1, 23.0, 14.4.
O
O
CH3
N
Cl
8e
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

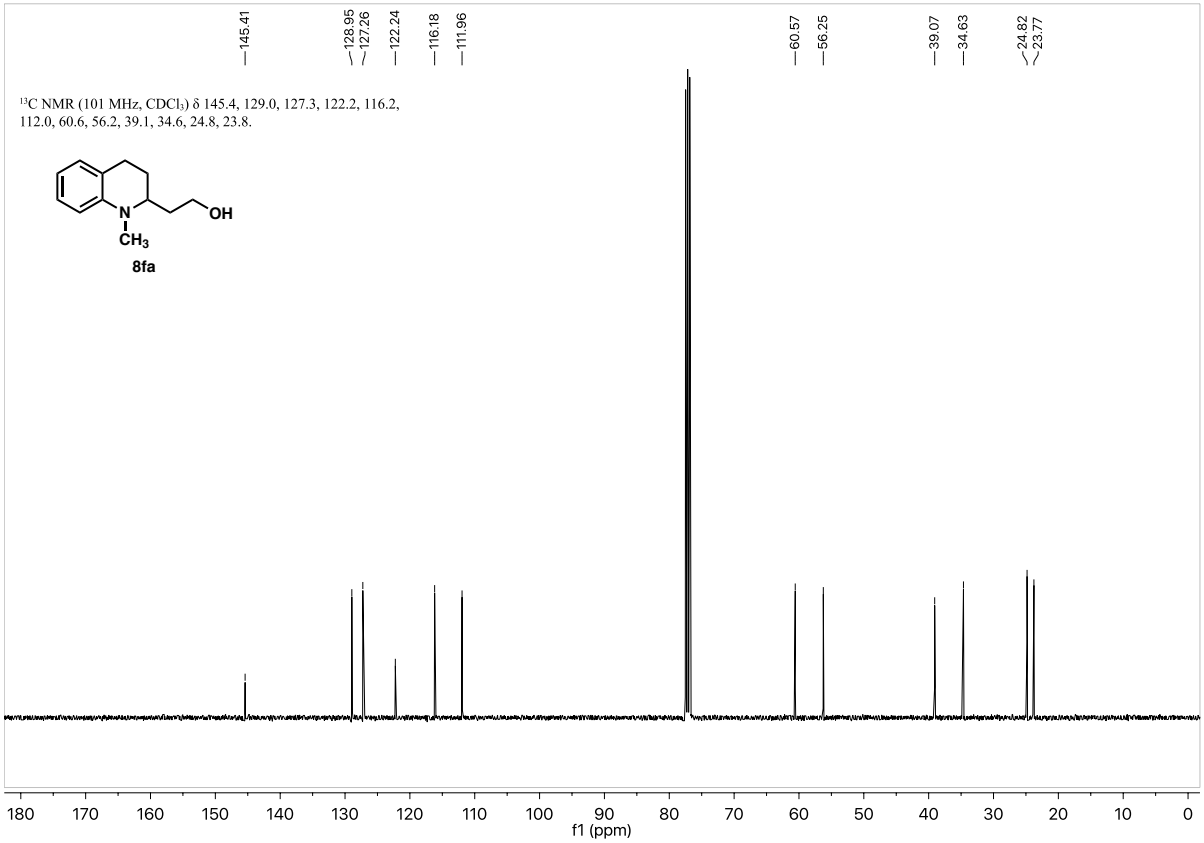

7.11 7.11 7.11 7.10 7.09 7.09 7.09 7.09 7.09 7.09 7.07 7.07 7.07 7.07 7.00 7.00 7.00 7.00 6.99 6.98 6.98 6.98 6.98 6.98 6.65 6.65 6.63 6.63 6.61 6.61 6.56 6.55 6.54 6.53 4.18 4.16 4.15 4.13 3.83 3.83 3.82 3.82 3.81 3.81 2.93 2.86 2.83 2.82 2.74 2.74 2.73 2.72 2.63 2.63 2.62 2.61 2.59 2.59 2.59 2.58 2.58 2.42 2.40 2.38 2.36 2.01 2.01 2.01 2.00 1.98 1.91 1.91 1.90 1.89 1.88 1.87 1.87 1.29 1.27 1.27 1.25
1H NMR (400 MHz, Chloroform-d) δ 7.14 – 7.04 (m, 1H), 6.99 (d, J = 7.3 Hz, 1H), 6.63 (td, J = 7.3, 1.1 Hz, 1H), 6.55 (d, J = 8.2 Hz, 1H), 4.15 (q, J = 7.1 Hz, 2H), 3.86 – 3.78 (m, 1H), 2.93 (s, 3H), 2.91 – 2.79 (m, 1H), 2.71 (ddd, J = 16.6, 5.5, 2.8 Hz, 1H), 2.60 (ddd, J = 14.7, 5.4, 0.8 Hz, 1H), 2.39 (dd, J = 14.7, 8.6 Hz, 1H), 2.07 – 1.95 (m, 1H), 1.89 (ddt, J = 13.4, 5.8, 2.9 Hz, 1H), 1.27 (t, J = 7.1 Hz, 3H).
8f
A (m) 7.09
B (d) 6.99
C (td) 6.63
D (d) 6.55
E (q) 4.15
F (m) 3.82
G (s) 2.93
H (m) 2.85
I (ddd) 2.71
J (ddd) 2.60
K (dd) 2.39
L (m) 2.01
M (ddt) 1.89
N (t) 1.27
1.00 1.01 1.00 1.00 2.05 1.01 3.05 1.05 1.05 1.03 1.02 1.05 1.02 3.12
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
f1 (ppm)

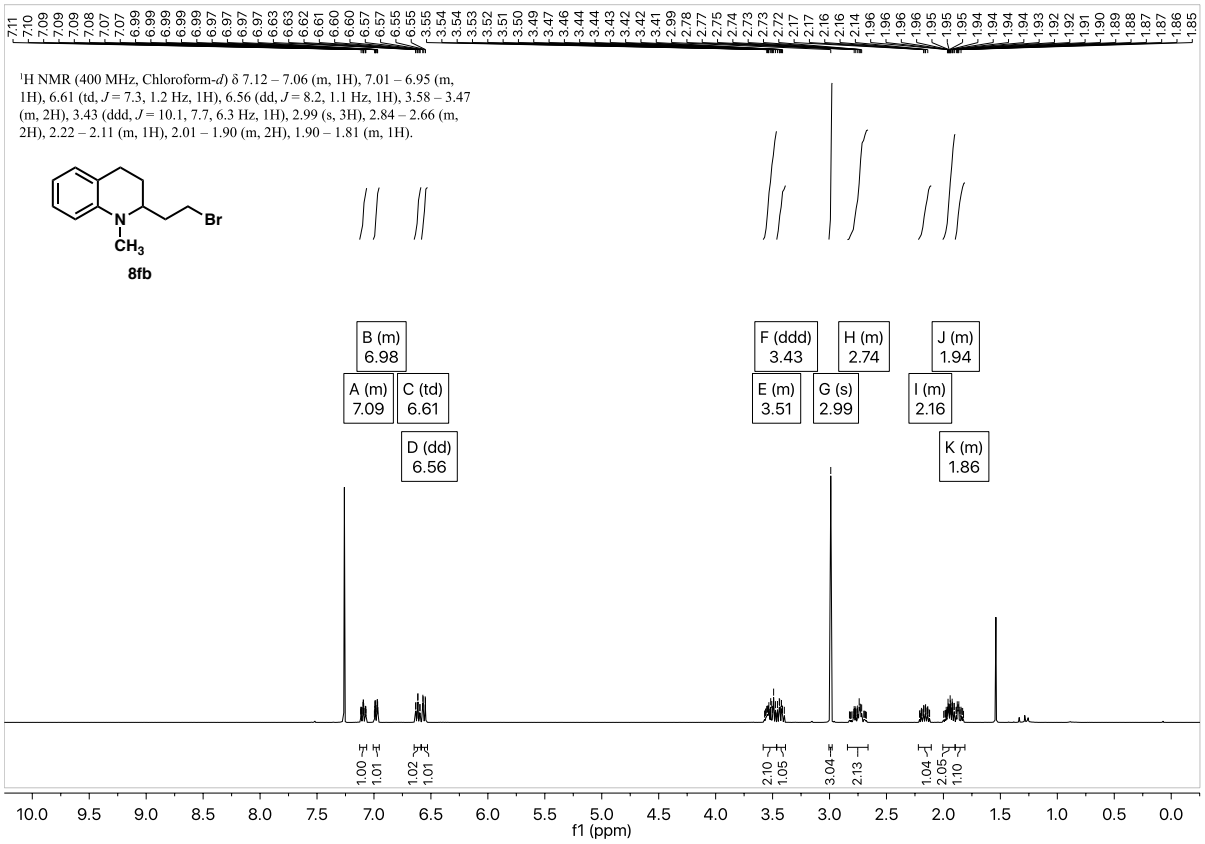

172.28 144.75 128.98 127.33 121.56 116.22 111.03 60.66 56.07 37.80 36.65 25.53 23.34 14.38
13C NMR (101 MHz, CDCl3) δ 172.3, 144.8, 129.0, 127.3, 121.6, 116.2, 111.0, 60.7, 56.1, 37.8, 36.6, 25.5, 23.3, 14.4.
8f
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

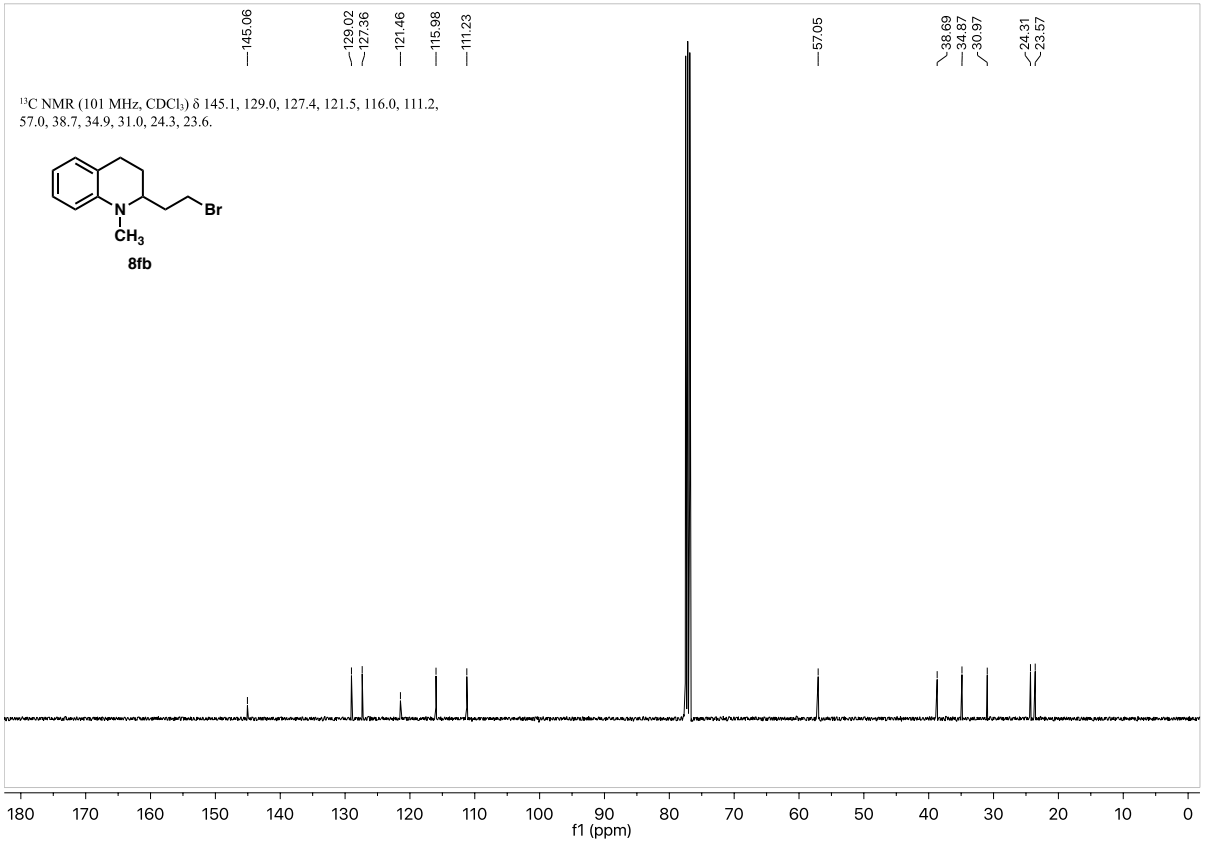

7.08 7.08 7.08 7.08 7.07 7.06 7.06 7.06 7.06 7.06 7.06 7.05 7.04 7.04 7.04 7.04 7.03 6.97 6.96 6.96 6.96 6.95 6.94 6.94 6.94 6.62 6.61 6.60 6.58 6.57
4.17 4.15 4.13 4.11 3.63 3.62 3.61 3.61 3.59 3.30 3.30 3.29 3.29 3.28 3.28 2.76 2.75 2.73 2.61 2.60 2.59 2.59 2.57 1.97 1.97 1.96 1.96 1.95 1.95 1.95 1.94 1.94 1.93 1.92 1.92 1.91 1.29 1.28 1.27 1.26 1.25 1.24 1.24 1.23
1H NMR (400 MHz, Chloroform-d) δ 7.11 – 7.01 (m, 1H), 6.95 (dq, J = 7.1, 1.1 Hz, 1H), 6.66 – 6.54 (m, 2H), 4.14 (q, J = 7.1 Hz, 2H), 3.65 – 3.57 (m, 2H), 3.33 – 3.25 (m, 2H), 2.75 (t, J = 6.4 Hz, 2H), 2.64 – 2.54 (m, 2H), 1.99 – 1.89 (m, 2H), 1.26 (t, J = 7.1 Hz, 3H).
8f'
B (dq) 6.95
A (m) 7.06
C (m) 6.60
D (q) 4.14
F (m) 3.29
E (m) 3.61
H (m) 2.59
G (t) 2.75
I (m) 1.94
J (t) 1.26
1.01
1.00
2.00
2.06
2.08
2.09
2.09
2.07
2.08
3.24
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
f1 (ppm)

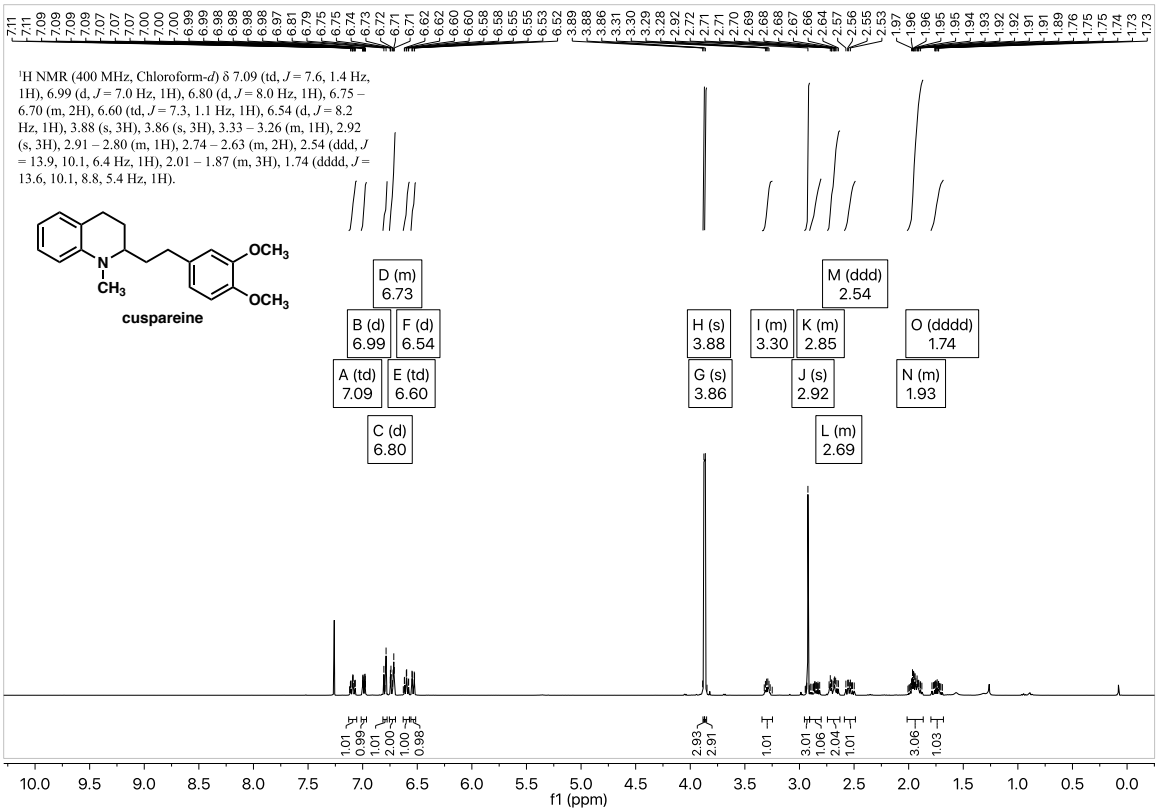

172.59
144.56
129.47
127.29
122.83
116.25
110.73
60.71
49.54
47.37
31.52
28.14
22.27
14.36
13C NMR (101 MHz, CDCl3) δ 172.6, 144.6, 129.5, 127.3, 122.8, 116.2, 110.7, 60.7, 49.5, 47.4, 31.5, 28.1, 22.3, 14.4.
8f'
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

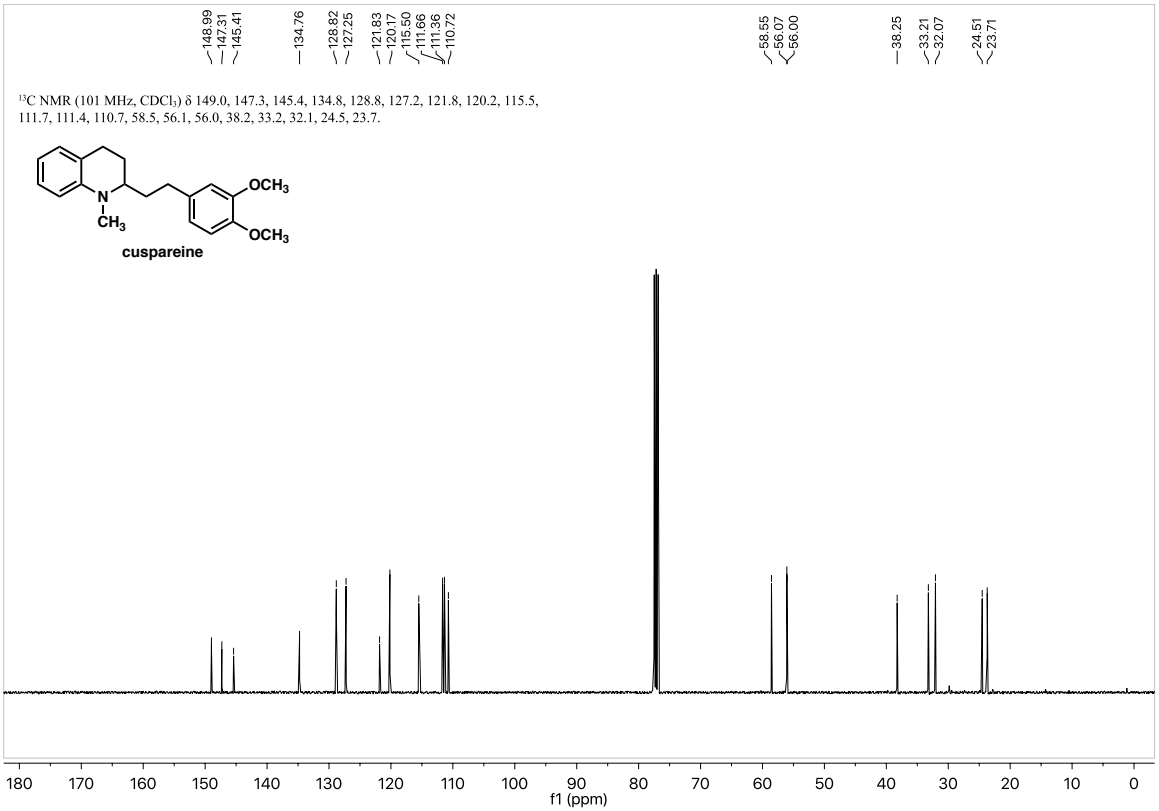

7.11 7.11 7.09 7.09 7.09 7.09 7.07 7.07 7.07 7.07 7.00 6.99 6.99 6.99 6.99 6.98 6.98 6.97 6.97 6.97 6.64 6.64 6.63 6.62 6.61 6.60 6.60 6.59 6.58 6.57
3.78 3.78 3.77 3.77 3.76 3.76 3.75 3.75 3.48 3.48 3.47 2.97 2.82 2.81 2.79 2.78 2.73 2.72 2.72 2.71 1.96 1.95 1.95 1.94 1.93 1.93 1.92 1.91 1.90 1.89 1.89 1.88 1.88 1.87 1.87 1.86 1.85 1.85 1.85 1.71 1.69 1.69 1.68 1.68 1.67 1.66 1.66 1.64 1.64
[1]H NMR (400 MHz, Chloroform-d) δ 7.12 – 7.06 (m, 1H), 7.01 – 6.96 (m, 1H), 6.62 (td, J = 7.3, 1.2 Hz, 1H), 6.59 (dd, J = 8.2, 1.1 Hz, 1H), 3.82 – 3.71 (m, 2H), 3.51 – 3.44 (m, 1H), 2.97 (s, 3H), 2.88 – 2.76 (m, 1H), 2.70 (ddd, J = 16.4, 5.3, 3.4 Hz, 1H), 2.00 – 1.81 (m, 3H), 1.72 – 1.61 (m, 2H).
N
CH3
OH
8fa
B (m) 6.98
A (m) 7.09
C (td) 6.62
D (dd) 6.59
F (m) 3.48
E (m) 3.76
H (m) 2.81
G (s) 2.97
I (ddd) 2.70
K (m) 1.89
J (m) 1.67
1.00
1.02
1.03
1.01
2.11
1.03
3.15
1.08
1.06
3.18
2.07
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
f1 (ppm)

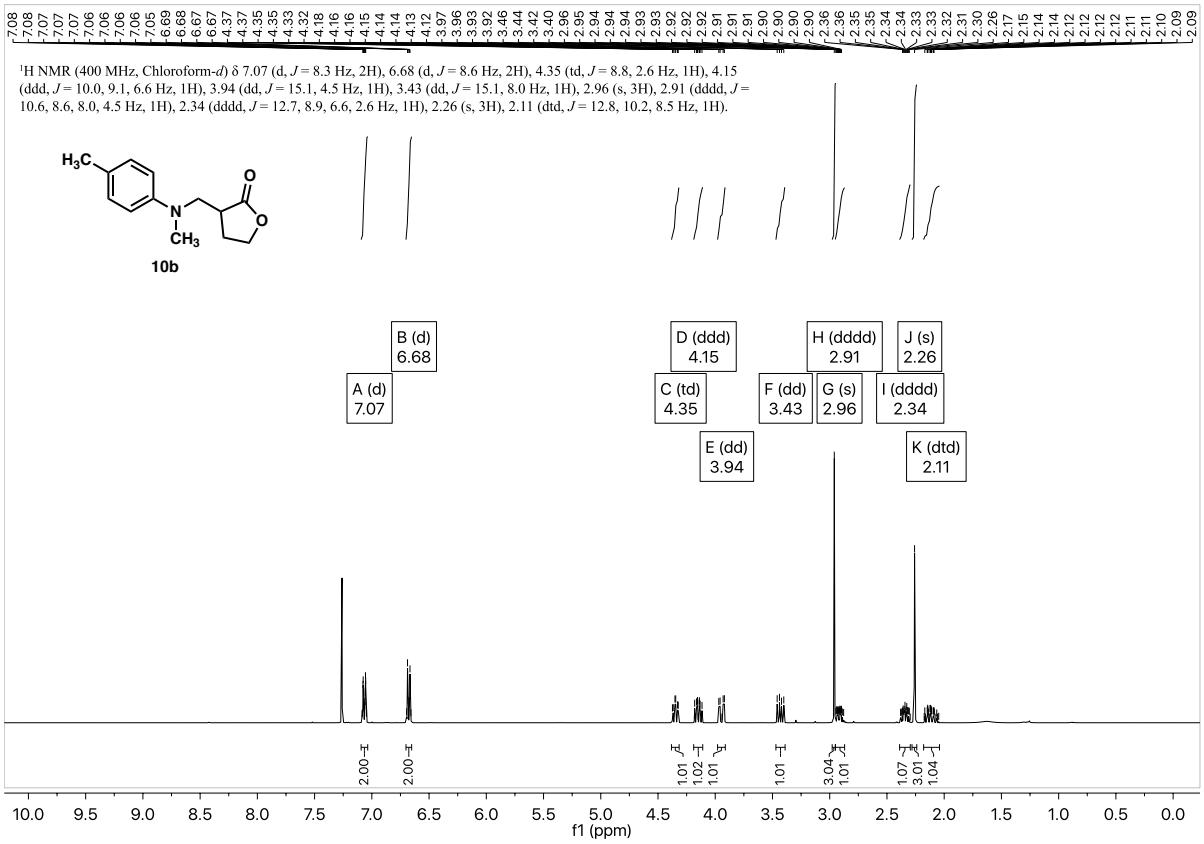

145.41
128.95
127.26
122.24
116.18
111.96
60.57
56.25
39.07
34.63
24.82
23.77
[13]C NMR (101 MHz, CDCl3) δ 145.4, 129.0, 127.3, 122.2, 116.2, 112.0, 60.6, 56.2, 39.1, 34.6, 24.8, 23.8.
N
CH3
OH
8fa
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

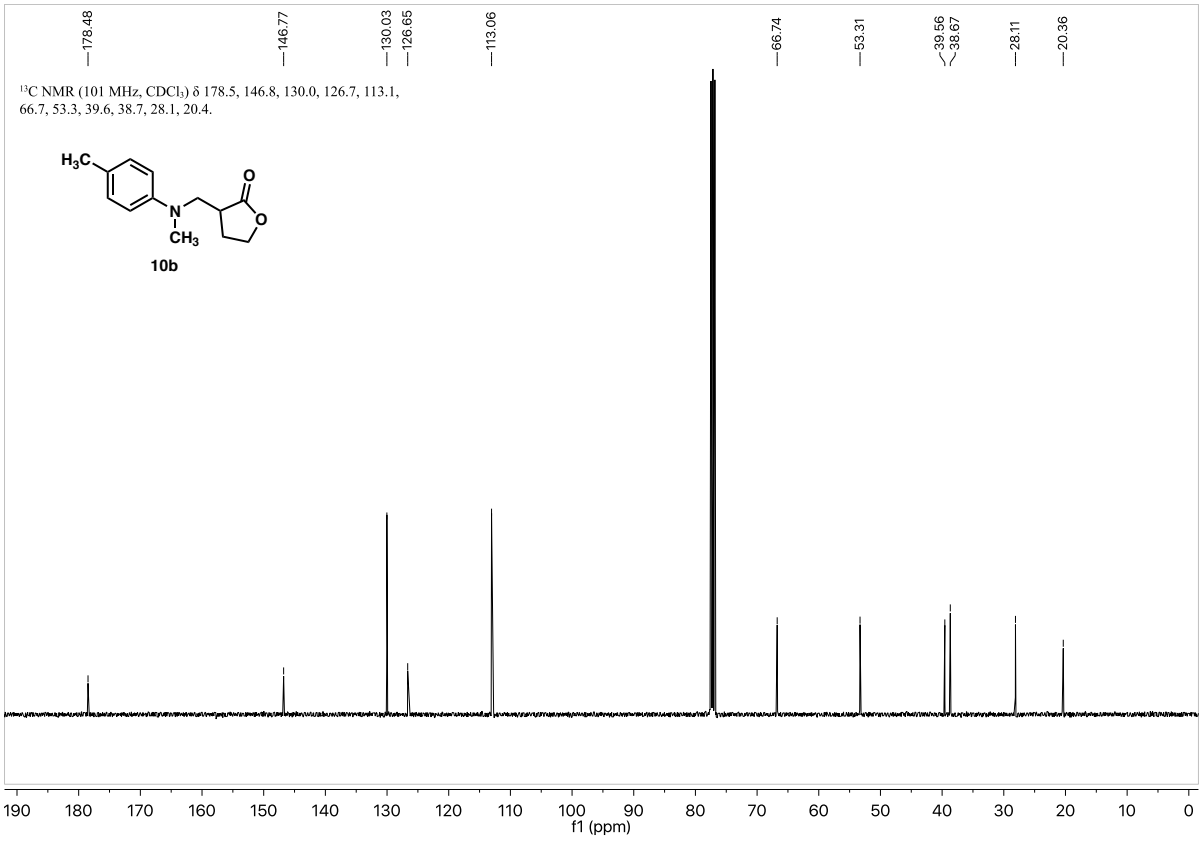

[1]H NMR (400 MHz, Chloroform-d) δ 7.12 – 7.06 (m, 1H), 7.01 – 6.95 (m, 1H), 6.61 (td, J = 7.3, 1.2 Hz, 1H), 6.56 (dd, J = 8.2, 1.1 Hz, 1H), 3.58 – 3.47 (m, 2H), 3.43 (ddd, J = 10.1, 7.7, 6.3 Hz, 1H), 2.99 (s, 3H), 2.84 – 2.66 (m, 2H), 2.22 – 2.11 (m, 1H), 2.01 – 1.90 (m, 2H), 1.90 – 1.81 (m, 1H).
N
CH3
Br
8fb
B (m) 6.98
A (m) 7.09
C (td) 6.61
D (dd) 6.56
F (ddd) 3.43
E (m) 3.51
G (s) 2.99
H (m) 2.74
J (m) 1.94
I (m) 2.16
K (m) 1.86
1.00
1.01
1.02
1.01
2.10
1.05
3.04
2.13
1.04
2.05
1.10
f1 (ppm)

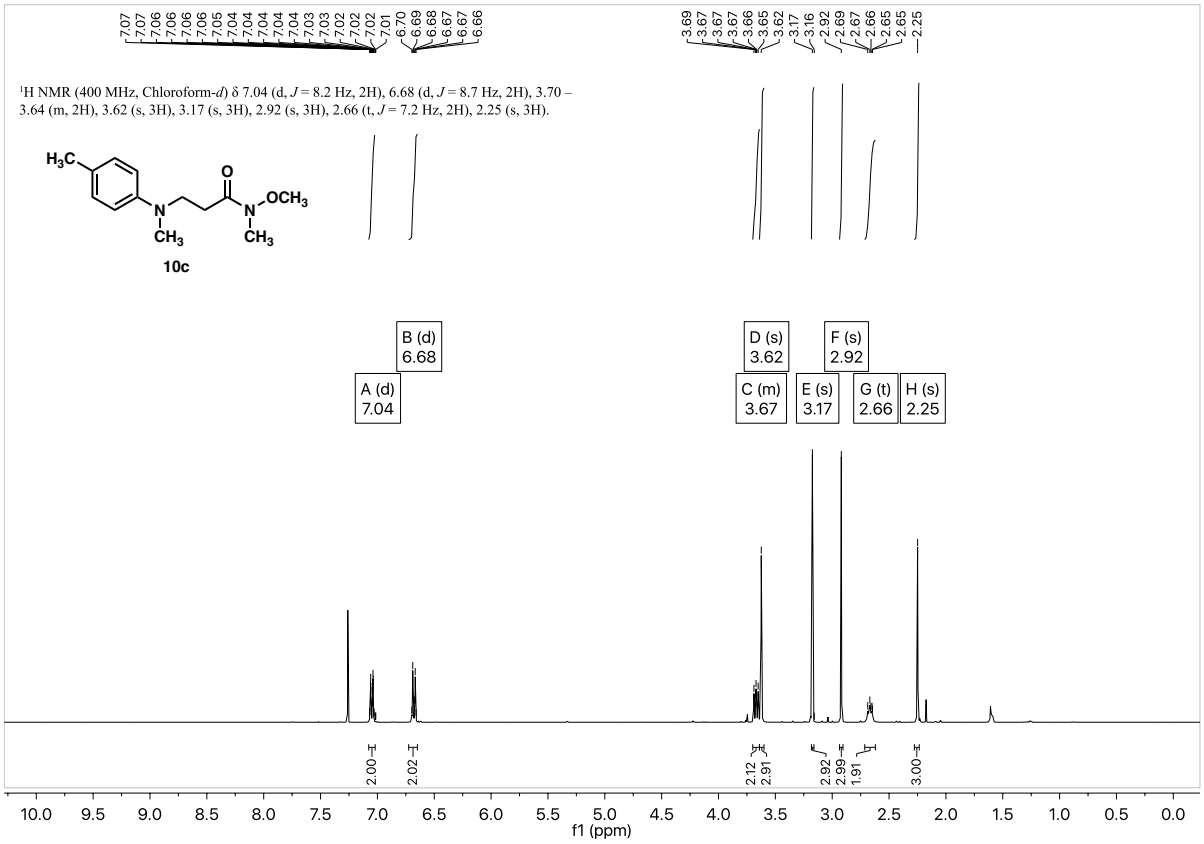

145.06
129.02
127.36
121.46
115.98
111.23
57.05
38.69
34.87
30.97
24.31
23.57
[13]C NMR (101 MHz, CDCl3) δ 145.1, 129.0, 127.4, 121.5, 116.0, 111.2, 57.0, 38.7, 34.9, 31.0, 24.3, 23.6.
N
CH3
Br
8fb
f1 (ppm)

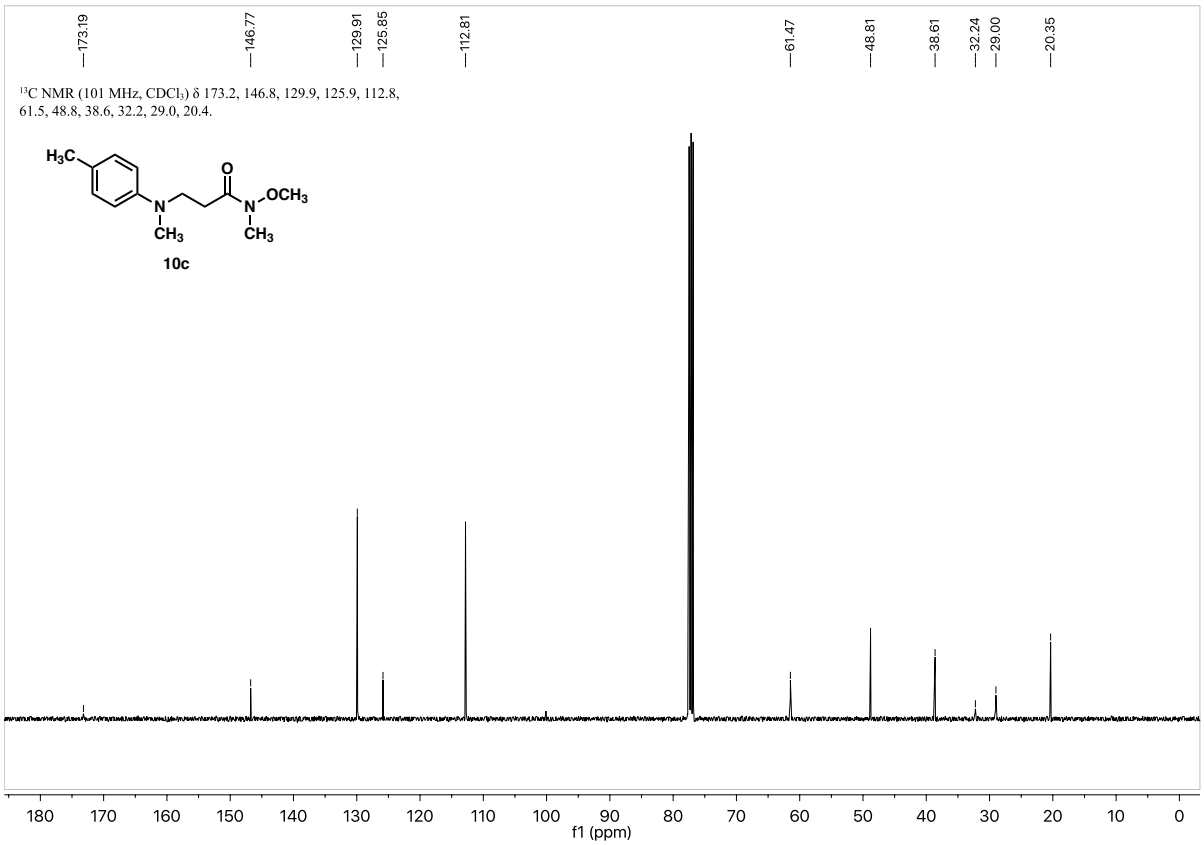

[1]H NMR (400 MHz, Chloroform-d) δ 7.09 (td, J = 7.6, 1.4 Hz, 1H), 6.99 (d, J = 7.0 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.75 – 6.70 (m, 2H), 6.60 (td, J = 7.3, 1.1 Hz, 1H), 6.54 (d, J = 8.2 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.33 – 3.26 (m, 1H), 2.92 (s, 3H), 2.91 – 2.80 (m, 1H), 2.74 – 2.63 (m, 2H), 2.54 (ddd, J = 13.9, 10.1, 6.4 Hz, 1H), 2.01 – 1.87 (m, 3H), 1.74 (dddd, J = 13.6, 10.1, 8.8, 5.4 Hz, 1H).
cuspareine
D (m) 6.73
B (d) 6.99
F (d) 6.54
A (td) 7.09
E (td) 6.60
C (d) 6.80
H (s) 3.88
G (s) 3.86
I (m) 3.30
M (ddd) 2.54
K (m) 2.85
J (s) 2.92
L (m) 2.69
O (dddd) 1.74
N (m) 1.93
f1 (ppm)

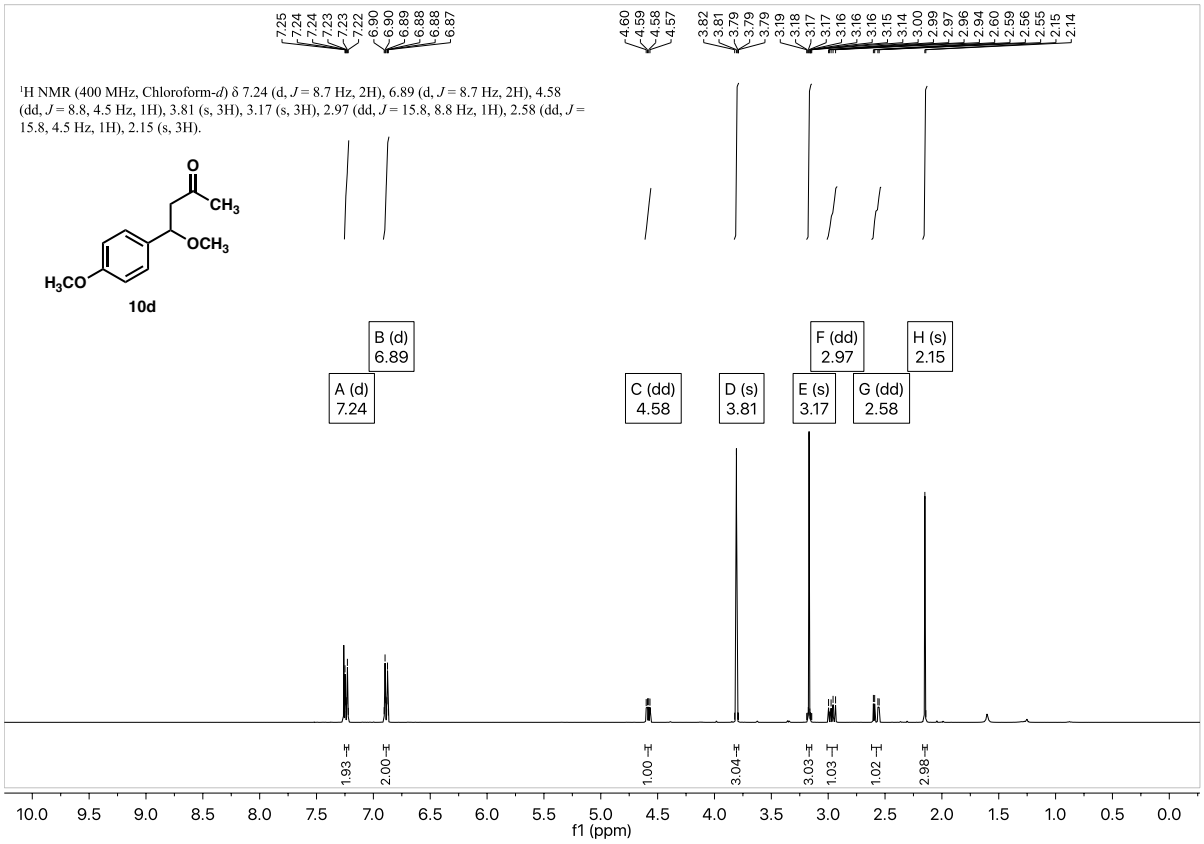

[13]C NMR (101 MHz, CDCl3) δ 149.0, 147.3, 145.4, 134.8, 128.8, 127.2, 121.8, 120.2, 115.5, 111.7, 111.4, 110.7, 58.5, 56.1, 56.0, 38.2, 33.2, 32.1, 24.5, 23.7.
cuspareine
f1 (ppm)

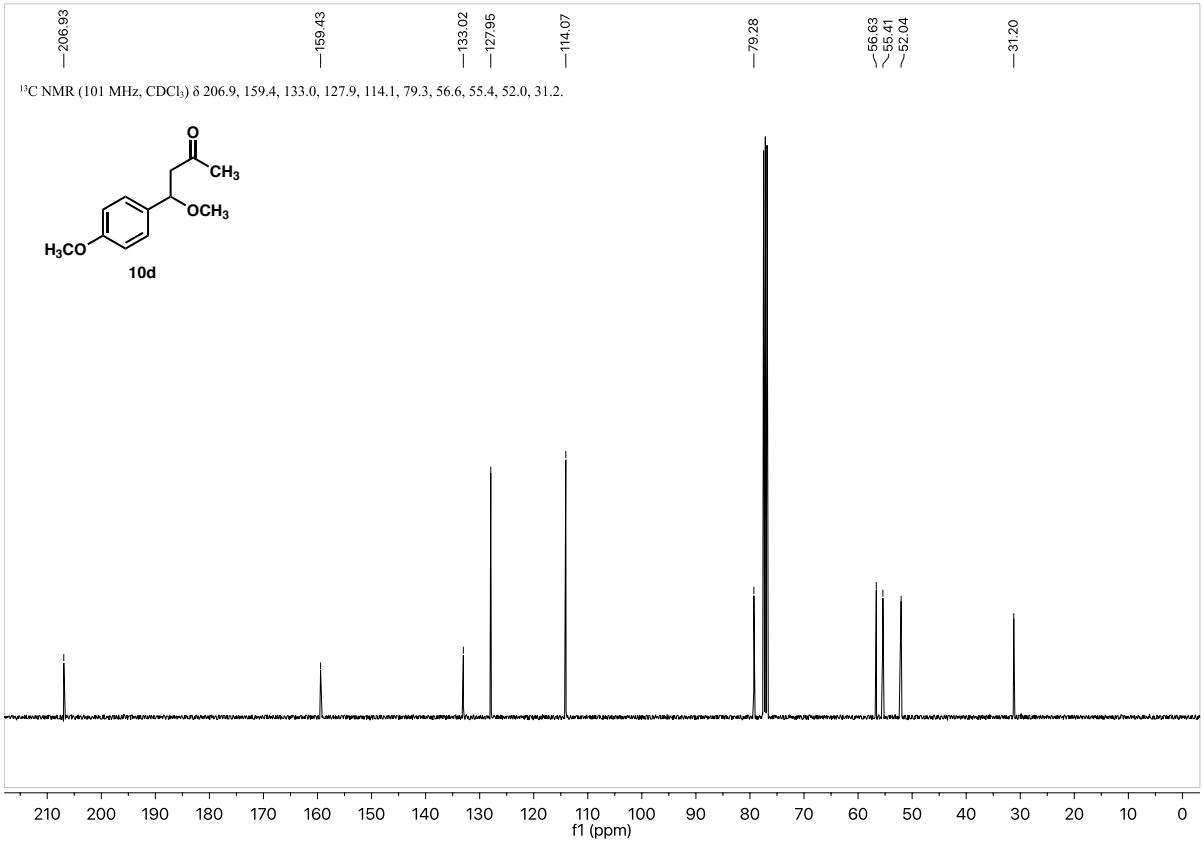

[1]H NMR (400 MHz, Chloroform-d) δ 7.07 (d, J = 8.3 Hz, 2H), 6.68 (d, J = 8.6 Hz, 2H), 4.35 (td, J = 8.8, 2.6 Hz, 1H), 4.15 (ddd, J = 10.0, 9.1, 6.6 Hz, 1H), 3.94 (dd, J = 15.1, 4.5 Hz, 1H), 3.43 (dd, J = 15.1, 8.0 Hz, 1H), 2.96 (s, 3H), 2.91 (dddd, J = 10.6, 8.6, 8.0, 4.5 Hz, 1H), 2.34 (dddd, J = 12.7, 8.9, 6.6, 2.6 Hz, 1H), 2.26 (s, 3H), 2.11 (dtd, J = 12.8, 10.2, 8.5 Hz, 1H).
10b
A (d) 7.07
B (d) 6.68
C (td) 4.35
D (ddd) 4.15
E (dd) 3.94
F (dd) 3.43
G (s) 2.96
H (dddd) 2.91
I (dddd) 2.34
J (s) 2.26
K (dtd) 2.11
2.00
2.00
1.01
1.02
1.01
1.01
3.04
1.01
1.07
3.01
1.04
f1 (ppm)


178.48
146.77
130.03
126.65
113.06
66.74
53.31
39.56
38.67
28.11
20.36
[13]C NMR (101 MHz, CDCl3) δ 178.5, 146.8, 130.0, 126.7, 113.1, 66.7, 53.3, 39.6, 38.7, 28.1, 20.4.
10b
f1 (ppm)


7.07 7.07 7.06 7.06 7.06 7.06 7.05 7.04 7.04 7.04 7.04 7.03 7.03 7.02 7.02 7.02 7.01 6.70 6.69 6.68 6.67 6.67 6.66
3.69 3.67 3.67 3.67 3.66 3.65 3.62 3.17 3.16 2.92 2.69 2.67 2.66 2.65 2.65 2.25
[1]H NMR (400 MHz, Chloroform-*d*) δ 7.04 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.7 Hz, 2H), 3.70 – 3.64 (m, 2H), 3.62 (s, 3H), 3.17 (s, 3H), 2.92 (s, 3H), 2.66 (t, *J* = 7.2 Hz, 2H), 2.25 (s, 3H).
H3C
O
N
OCH3
CH3
N
CH3
10c
B (d) 6.68
A (d) 7.04
D (s) 3.62
F (s) 2.92
C (m) 3.67
E (s) 3.17
G (t) 2.66
H (s) 2.25
2.00
2.02
2.12
2.91
2.92
2.99
1.91
3.00
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
f1 (ppm)


173.19 146.77 129.91 125.85 112.81 61.47 48.81 38.61 32.24 29.00 20.35
[13]C NMR (101 MHz, $CDCl_3$) δ 173.2, 146.8, 129.9, 125.9, 112.8, 61.5, 48.8, 38.6, 32.2, 29.0, 20.4.
H3C
O
N
OCH3
CH3
N
CH3
10c
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.25 7.24 7.24 7.23 7.23 7.22 6.90 6.90 6.89 6.88 6.88 6.87
4.60 4.59 4.58 4.57
3.82 3.81 3.79 3.79 3.79 3.19 3.18 3.17 3.17 3.16 3.16 3.16 3.15 3.14 3.00 2.99 2.97 2.96 2.94 2.60 2.59 2.56 2.55 2.15 2.14
[1]H NMR (400 MHz, Chloroform-*d*) δ 7.24 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.58 (dd, *J* = 8.8, 4.5 Hz, 1H), 3.81 (s, 3H), 3.17 (s, 3H), 2.97 (dd, *J* = 15.8, 8.8 Hz, 1H), 2.58 (dd, *J* = 15.8, 4.5 Hz, 1H), 2.15 (s, 3H).
O
CH3
OCH3
H3CO
10d
A (d) 7.24
B (d) 6.89
C (dd) 4.58
D (s) 3.81
E (s) 3.17
F (dd) 2.97
G (dd) 2.58
H (s) 2.15
1.93
2.00
1.00
3.04
3.03
1.03
1.02
2.98
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
f1 (ppm)


206.93
159.43
133.02
127.95
114.07
79.28
56.63
55.41
52.04
31.20
[13]C NMR (101 MHz, $CDCl_3$) δ 206.9, 159.4, 133.0, 127.9, 114.1, 79.3, 56.6, 55.4, 52.0, 31.2.
O
CH3
OCH3
H3CO
10d
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

## XIII. Supplemental References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