Supporting Information

Biocatalytic One-Carbon Ring Expansion of Aziridines to Azetidines via a Highly Enantioselective [1,2]-Stevens Rearrangement

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General Information and Protocols

Safety Statement. All chemical transformations were performed in a well-ventilated fume hood to avoid inhalation and exposure. For protein concentration determination experiments, carbon monoxide (CO) was used. CO is flammable, highly toxic, and can be lethal at high doses and must be used in a fume hood equipped with a CO detector to avoid accidental exposure. Other than that, no unexpected or unusually high safety concerns were raised with these methods. Safety notes for individual synthetic procedures will be documented alongside the procedure.

General Information. All chemical transformations were performed in a well-ventilated fume hood to avoid inhalation and exposure to chemicals. Reagents and solvents were obtained commercially (Sigma-Aldrich, Alfa Aesar, VWR, Fischer, Matrix Scientific, Oakwood Chemical, TCI America, and other suppliers) and used without prior purification unless otherwise stated. Organic solutions were concentrated under reduced pressure on an IKA RV 10 rotary evaporator. Thin-layer chromatography (TLC) was performed on commercial Millipore Silica Gel 60 plates containing the F254 fluorescent indicator. Visualization of the developed chromatographs was performed by irradiation with UV light, or treating with an appropriate TLC staining solution (e.g., Ceric Ammonium Molybdate, $KMnO_4$, or Bromocresol Green) followed by heating if necessary. Chromatographic purification was accomplished by flash chromatography on Silacycle F60 silica gel according to the method of Still[1] or using a Biotage Isolera One instrument.

Spectral Data. All NMR spectra were obtained at the Caltech Liquid NMR Facility. For azetidine products, ^{1}H and ^{13}C NMR were recorded on a Bruker Prodigy 400 MHz instrument (400 MHz and 101 MHz). ^{19}F NMR spectra were recorded on a Varian 300 MHz spectrometer (282 MHz). For intermediates, ^{1}H spectra were also recorded using a Varian 300 MHz spectrometer (300 MHz), a Varian 500 MHz spectrometer (500 MHz), and a Varian 600 MHz spectrometer (600 MHz). ^{1}H and ^{13}C spectra are referred to residual $CDCl_3$ solvent signals referenced at δ 7.26 and 77.0 ppm, respectively. For spectra taken in DMSO, the residual ^{1}H and ^{13}C solvent signals are referenced at δ 2.50 and 39.51 ppm, respectively. ^{19}F spectra are referenced by addition of the appropriate internal reference standard, using either fluorobenzene (referenced at δ –113.15 ppm) or hexafluorobenzene (referenced at δ –161.90 ppm) and are clearly labeled when shown. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, hept = heptet, m = multiplet, br s = broad singlet), and coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift, multiplicity, and coupling constant (Hz): no special nomenclature is used for equivalent carbons. Data for ^{19}F NMR are reported in terms of chemical shift, multiplicity, and coupling constant (Hz). High-resolution mass spectra (HRMS) were obtained at the Caltech Mass Spectrometry Facility.

Gas Chromatography Data. GC chromatography (GC) was performed on an Agilent Technologies 7820A GC system equipped with a split-mode capillary injection system and flame-ionization detectors. For achiral analyses, an Agilent J&W HP-5 Column was used as the stationary phase. For chiral analyses, the specific stationary phase is provided along with the chiral traces.

Cloning, Site-Saturation Mutagenesis (SSM), and Plasmid Isolation. Electrocompetent *Escherichia coli* (*E. coli*) cells were prepared following the protocol of Sambrook and Russell.[2]

Phusion polymerase and *Dpn*I were purchased from New England Biolabs (NEB, Ipswich, MA). SSM experiments were performed using primers bearing degenerate codons (NDT, VHG, TGG) as per the "22 codon trick" using a modified QuikChange™ protocol.[3] The PCR conditions were as follows (final concentrations): Phusion HF Buffer 1x, 0.2 mM dNTPs each, 0.5 μM of forward primers, 0.5 μM reverse primer, and 0.02 U/μL of Phusion polymerase. Upon completion of PCRs, the remaining template was digested with *Dpn*I. Gel purification was performed with a Zymoclean Gel DNA Recovery Kit (Zymo Research Corp, Irvine, CA). The purified PCR product was then assembled using the Gibson assembly protocol.[4]

Transformation of Cells and Plasmid Isolation. The assembly products obtained were used to transform electrocompetent *E. cloni*® EXPRESS BL21(DE3) cells (Lucigen, Middleton, WI) with a MicroPulser Electroporator (Bio-Rad, Hercules, CA). Luria-Bertani medium (LB; 0.6 mL) was added to electroporated cells, and they were incubated at 37 ºC with shaking at 220 rpm for 45 minutes before being plated on LB agar plates with 100 μg/mL ampicillin (LB-amp agar plates). Plates were incubated at 37 ºC overnight. Single colonies from these plates were used to inoculate flask cultures, prepare glycerol stocks, and isolate plasmids for sequencing. Plasmids were isolated using a QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany), and the genes were sequence-verified (Laragen, Inc.).

96-Well Plate Library Expression. 96-Well deep-well plates were shaken in an INFORS HT Multitron Shaker in all instances. Single colonies from LB-agar plates were picked using sterilized toothpicks, which were used to inoculate 400 μL of LB containing 100 μg/mL of ampicillin (LB-amp) in 2-mL 96-well deep-well plates. The plates were incubated at 37 ºC, 80% humidity, and 220 rpm overnight. For expression cultures, 50 μL of these precultures were used to inoculate 900 μL of Hyperbroth (AthenaES) with 100 μg/mL of ampicillin (HB-amp) per well in 96-well deep-well plates. The remaining overnight culture plates were sealed and stored in a 4 ºC refrigerator until needed again. The expression cultures were initially incubated at 37 ºC, 80% humidity, and 220 rpm for 2.5 hours, at which point they were allowed to chill on ice for 45 minutes. Expression of proteins was induced with isopropyl-β-D-thiogalactoside (IPTG) and cellular heme production was increased with 5-aminolevulinic acid (ALA). An induction mixture containing IPTG and ALA in HB-amp (50 μL) was added to each well such that the final concentrations of IPTG and ALA were 0.5 mM and 1.0 mM, respectively. The total culture volumes were 1 mL. The plates were then incubated at 22 ºC and 200 rpm overnight.

96-Well Plate Library Reactions and Screening. Expression cultures containing *E. coli* expressing hemoproteins of interest were centrifuged at 5000 × g for 5 minutes at 4 ºC. The supernatant was discarded, and nitrogen-free M9 minimal media (M9-N, 380 μL) was added to each well. The plates were then put into a vinyl Coy anaerobic chamber (0 – 30 ppm O_2) and the pellets were resuspended. To each well, 20 μL of a MeCN solution with 200 mM of the desired aziridine substrate and 300 mM of ethyl diazoacetate (EDA) were added. The final reaction volume was 400 μL, and the final concentrations of the desired aziridine and EDA were 10 mM and 15 mM, respectively. The plates were then sealed carefully with a foil cover, removed from the Coy chamber, and shaken at room temperature for 16 hours. Once complete, plates were worked up for processing by adding 600 μL of a 1:1 solution of ethyl acetate:cyclohexane with 1,3,5-trimethoxybenzene as an internal standard (10 mM concentration). A silicone sealing mat (AWSM1003S, ArcticWhite) was used to cover the plate, and the two layers were thoroughly

mixed. The plate was then centrifuged (5000 × g for 5 minutes at room temperature) to separate the phases. Afterwards, an aliquot of the organic layer was transferred to a GC vial insert in a GC vial and the samples were assayed by GC.

Wells with improved activity over control wells containing the parent variant were subjected to sequence identification and validation of activity. The corresponding wells in the overnight culture plate were streaked out on LB-Amp plates. A single parent control was streaked as well. Single colonies from these plates were then subjected to the small-scale protein expression conditions below, followed by the small-scale biocatalytic reaction protocol described below.

Small-Scale Protein Expression. Single colonies from LB-Agar plates were picked using a sterile pipette tip and were used to inoculate 6 mL of LB-amp in a 15-mL plastic culture tube. Cultures were incubated at 37 ºC with shaking at 220 rpm overnight in an Innova 4000 incubator. Two mL of these overnight cultures were used to inoculate 100 mL of HB-amp (1% v/v starter culture in expression culture) in 250-mL Erlenmeyer flasks. The remainder of the overnight culture was subjected to sequence identification (for new variants) and verification (for parent control wells). The expression cultures were incubated at 37 ºC and 220 rpm for 2.5 hours in an Innova 42 shaker, at which point they were held on ice for 45 minutes. Protein expression was then induced by direct addition of 100 μL of stock solutions containing 500 mM IPTG and 1.0 mM ALA such that the final concentrations were 0.5 mM and 1.0 mM, respectively. The cultures were shaken at 22 ºC and 140 rpm for 16 hours in an Innova 42 shaker.

Small-Scale Biocatalytic Reactions for Lineage Validation. The corresponding 100-mL expression cultures were pelleted (5000 × g for 5 minutes at 4 ºC) and resuspended in 6 mL of M9-N buffer. The protein concentration of this sample was determined by CO binding (*vide infra*), and 380-μL portions of whole-cell suspension (WCS) were prepared in GC vials such that the protein concentration was 5.25 μM. The whole-cell suspensions were put into a vinyl Coy anaerobic chamber, at which point 10 μL of a 400 mM solution of aziridine in MeCN followed by 10 μL of a 600 mM solution of EDA in MeCN were added such that the final reaction concentrations were 5.0 μM of the protein variant, 10 mM of the desired aziridine, and 15 mM of EDA. The GC vials were tightly capped with screwcaps with a septum, were brought out of the Coy chamber, and were allowed to shake at RT for 16 hours. Once complete, the reactions were transferred to a 1.7-mL Eppendorf tube and 600 μL of a 1:1 solution of ethyl acetate:cyclohexane with 1,3,5-trimethoxybenzene as an internal standard (10 mM concentration) were added. The layers were thoroughly mixed, and the sample was centrifuged (14,000 × g for 10 minutes at RT) to separate the phases. Afterwards, an aliquot of the organic layer was subjected to GC analysis and the samples were assayed by GC.

Large-Scale Protein Expression. Single colonies from LB-Agar plates were picked using a sterile pipette tip and were used to inoculate 25 mL of LB-amp in a 125-mL unbaffled Erlenmeyer flask. Cultures were incubated at 37 ºC with shaking at 220 rpm overnight in an Innova 4000 incubator. These overnight cultures (20 mL) were used to inoculate 1000 mL of HB-amp (1% v/v starter culture in expression culture) in a 2.8-L Erlenmeyer flasks. The remainder of the overnight culture was subjected to sequence identification. The expression cultures were incubated at 37 ºC and 220 rpm for 2.5 hours in an Innova 42 shaker, at which point they were held on ice for 45 minutes. Protein expression was then induced by direct addition of 1.0 mL of stock solutions containing 500 mM IPTG and 1.0 mM ALA such that the final concentrations were 0.5 mM and

1.0 mM, respectively. The cultures were shaken at 22 ºC and 140 rpm for 16 hours in an Innova 42 shaker.

Processing of Large Scale Expression Cultures for Preparative Biocatalytic Reactions. The corresponding 1-L cultures were pelleted (5000 × g for 5 minutes at 4 ºC) and resuspended in 40 mL of M9-N buffer. The whole-cell suspensions were held on ice until the protein concentration could be determined (*vide infra*) and reactions could be run.

Lysis of Whole-Cell Suspensions. Whole-cell suspensions from either small-scale protein expression or large-scale protein expression were lysed as follows. An aliquot of the whole-cell suspension (3 mL) was diluted in 3 mL of M9-N buffer, and the cells were lysed by sonication on ice for 2 minutes at 25% amplitude (1 second on, 2 second off) using a QSonica Q500 Sonicator and a ½-inch tip. The sonicated cell mixture was clarified by centrifugation (14,000 × g for 10 minutes at 4 ºC), removing the supernatant from the cellular debris into a fresh container.

Protein Concentration Determination via CO-Binding Assay. The CO-binding assay was performed with clarified lysate as described above using a modified literature procedure.[5] The extinction coefficient for $\varepsilon_{410\text{-}490}$ of 0.103 M^{-1} cm^{-1} as measured for $P411_{BM3}$-CIS was used to estimate the concentration of the P411 enzymes disclosed in this work. UV-Vis spectra were taken using a Tecan SPARK instrument using untreated, 96-well flat-bottom polystyrene microplates (Evergreen Scientific, 290-8115-01F).

Clarified lysate (90 μL) with the hemoprotein of interest was diluted with 90 μL of M9-N buffer prior to the addition of 20 μL of a 300 mM solution of sodium dithionite in M9-N. The solution was thoroughly mixed, and a UV-Vis absorbance measurement was taken at the peak maximum at 410 nm and at 490 nm as a baseline measurement. The plate was then transferred to a vacuum chamber, which was evacuated and backfilled with an atmosphere of CO. The plates were allowed to incubate under an atmosphere of CO for 30 minutes. Once incubation was complete, a second UV-Vis absorbance measurement was taken at 410 nm and at 490 nm. Beer's law was used to determine the hemoprotein concentration of the solution using the $\Delta A_{411\text{-}490}$ between the CO-bound and reduced samples, the $\varepsilon_{411\text{-}490}$ value above, and the pathlength.

Directed Evolution for Aziridine Ring Expansion

Table S1. Selected sequence differences between relevant P450/P411 variants and wild-type $P450_{BM3}$.

Select Connections Between $P450_{BM3}$ and Parent F2

Protein Variant	Mutations Relative to Wild-type $P450_{BM3}$
$P450_{BM3}$	None
$P450_{BM3}$-CIS	V78A F87V P142S T175I A184V S226R H236Q E252G T268A A290V L353V I366V E442K
$P411_{BM3}$-CIS	V78A F87V P142S T175I A184V S226R H236Q E252G T268A A290V L353V I366V C400S E442K
$P411_{BM3}$-CIS P248T I263G L437F (Parent F.2)	V78A F87V P142S T175I A184V S226R H236Q P248T E252G I263G T268A A290V L353V I366V C400S L437F E442K

Table S2. Evolutionary trajectory of P411 variants involved in this study.

Evolutionary Trajectory of P411 Variants Involved in This Study

Protein Variant	Mutations Relative to Parent (New Mutations in Bold)
Parent F2	None
Parent F2.1	**G263Y**
Parent F2.2	G263Y **T327V**
Parent F2.3	G263Y T327V **A330T**
Parent F2.4	G263Y **H266P** T327V A330T
Parent F2.5	**M177Q** G263Y H266P T327V A330T
Parent F2.6	M177Q G263Y H266P T327V A330T **T436G**
Parent F2.7	M177Q **L233F** G263Y H266P T327V A330T T436G
Parent F2.8	**T149M** M177Q L233F G263Y H266P T327V A330T T436G
Parent F2.9	**R47Q** T149M M177Q L233F G263Y H266P T327V A330T T436G
P411-AzetS	R47Q **M118K** T149M M177Q L233F G263Y H266P T327V A330T T436G

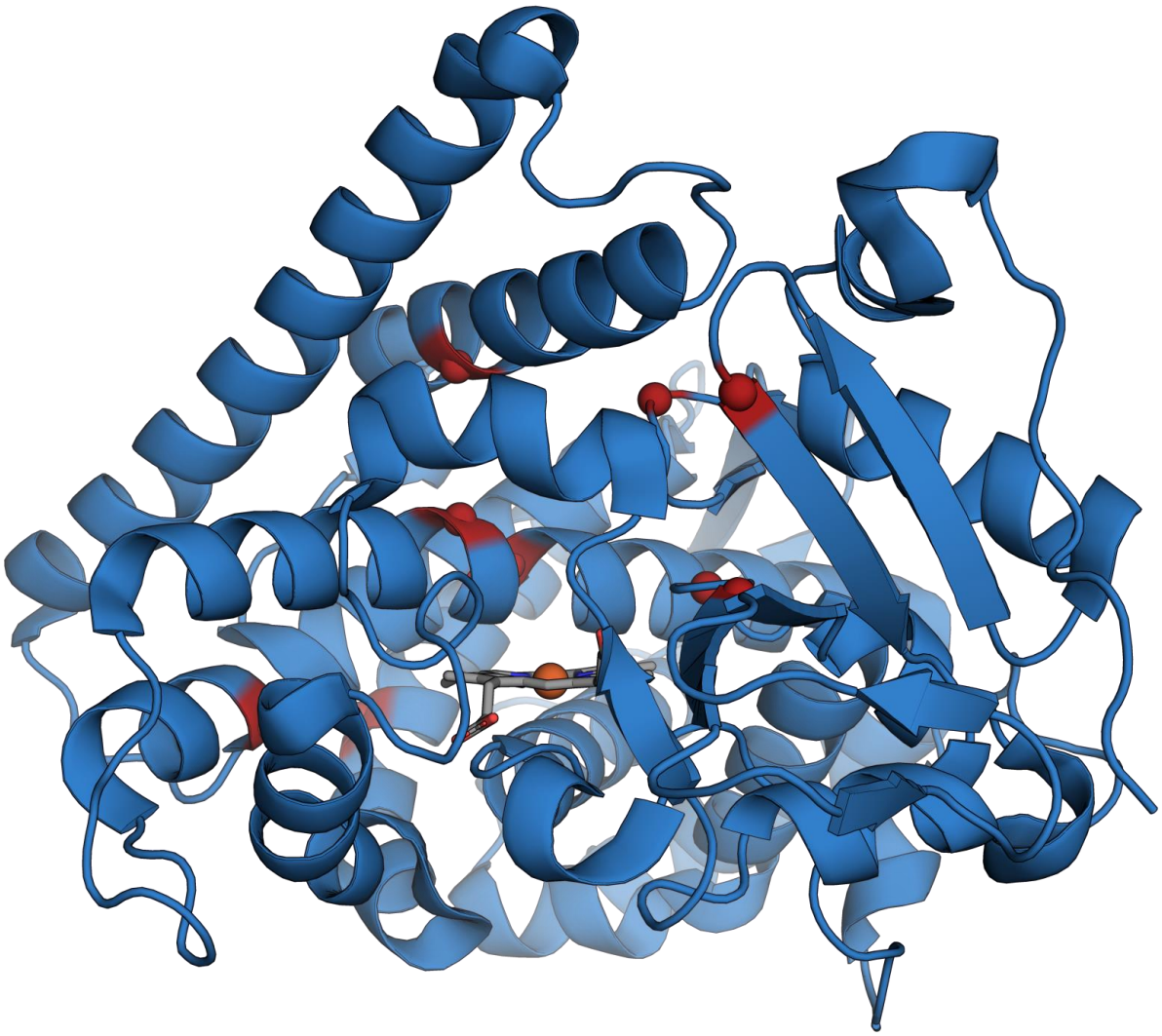


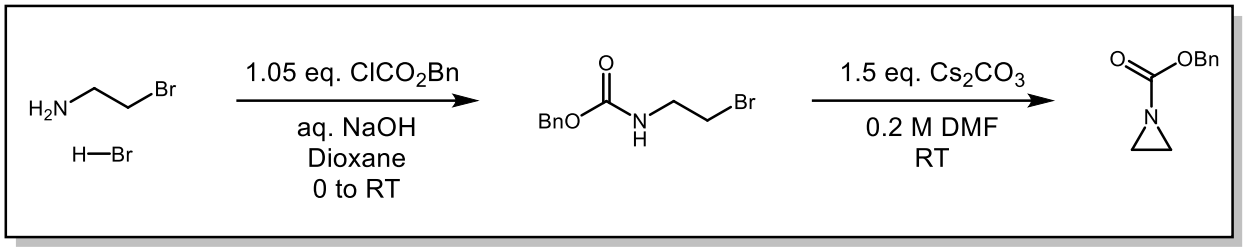
Figure S1: Homology model of Parent F2 with mutated sites shown in red. (PDB: 5UCW)[6]

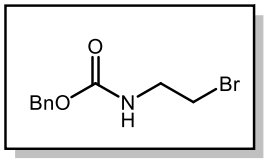
Control Experiments

Table S3. Control experiments performed to assess potential background reactivity. Reactions were performed on 4 μmol scale as outlined in the main body of the manuscript.

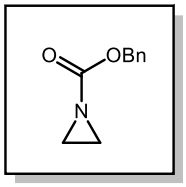
Entry	Changes to Conditions Above	Product Observed?
1	None	Yes
2	No Enzyme, 10 μM Hemin	No
3	No Enzyme, 10 μM Hemin, 300 mM sodium dithionite	No
4	No Enzyme, 1 mg/mL BSA	No
5	No Enzyme, 1 mg/mL BSA 300 mM sodium dithionite	No
6	No Enzyme, 10 μM Hemin, 1 mg/mL BSA	No
7	No Enzyme, 10 μM Hemin, 1 mg/mL BSA, 300 mM sodium dithionite	No
8	Buffer Only	No
9	Buffer with 300 mM sodium dithionite	No

Synthesis and Characterization of Substrates

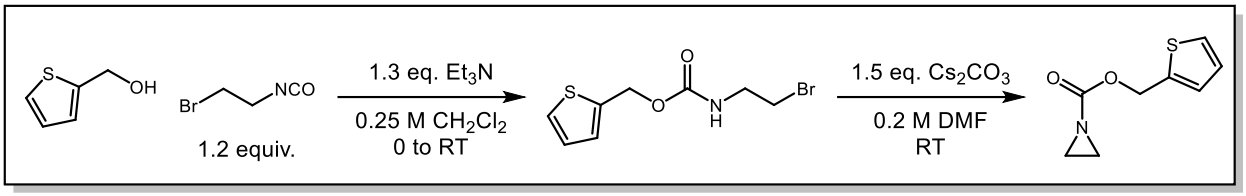


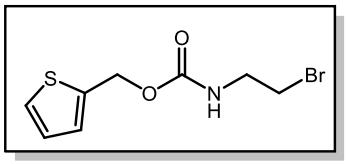


Benzyl (2-bromoethyl)carbamate. To a round-bottom flask equipped with a stir bar: 2-bromoethylamine hydrobromide (5.00 g, 24.4 mmol, 1.0 equiv.) was suspended in dioxane (24 mL). The suspension was chilled in an ice bath, and 1.0 M aq. NaOH solution (30 mL) was added slowly. Once addition was complete, benzyl chloroformate (4.37 g, 3.66 mL, 25.6 mmol, 1.05 equiv.) was added dropwise. The solution was allowed to ambiently warm to room temperature with stirring overnight. Once complete, the reaction was partitioned into 150 mL of diethyl ether. The aqueous layer was drained, and the organics were washed with an additional portion of water (30 mL) and brine (30 mL) before drying over sodium sulfate. Once dry, the organics were decanted from the drying agent and the volatiles were stripped under vacuum. The crude product was purified by silica gel column chromatography (gradient from 100% hexanes to 15% EtOAc in hexanes) to afford 4.65 g (74% yield) of the titled compound as a colorless oil that gradually solidifies upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.29 (m, 5H), 5.17 (br s, 1H), 5.12 (s, 2H), 3.62 (q, J = 5.9 Hz, 2H), 3.48 (t, J = 5.8 Hz, 2H). The spectrum obtained is in accord with prior literature reports.[7]

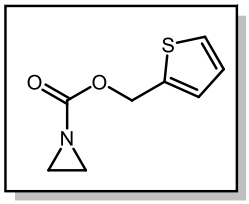


Benzyl aziridine-1-carboxylate. To a round-bottom flask equipped with a stir bar: benzyl (2-bromoethyl)carbamate (4.65 g, 18 mmol, 1 equiv.) was dissolved in DMF (90 mL). Cesium carbonate (8.80 g, 27 mmol, 1.5 equiv.) was added in a single portion. The reaction was allowed to stir vigorously at room temperature until judged complete by TLC analysis. Once complete, the reaction was partitioned into 180 mL of ethyl acetate and 90 mL of water. The phases were thoroughly mixed, and the aqueous layer was drained. The organics were washed with 5% aq. LiCl (w/w) solution (3x 30 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted from the drying agent and the volatiles were stripped under vacuum. The crude product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford 2.44 g (77% yield) of the titled compound as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 5H), 5.14 (s, 2H), 2.23 (s, 4H). The spectrum obtained is in accord with prior literature reports.[8]

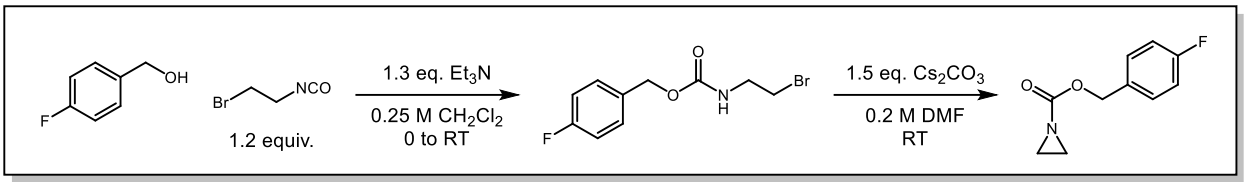


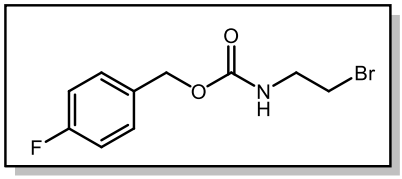


Thiophen-2-ylmethyl (2-bromoethyl)carbamate. Synthesized using the procedure of Marlin.[9] Thiophen-2-ylmethanol (457 mg, 0.38 mL, 4 mmol, 1.0 equiv.) and triethylamine (526 mg, 0.73 mL, 5.2 mmol, 1.3 equiv.) were dissolved in methylene chloride (16 mL). The flask was chilled in an ice bath, and 2-bromoethyl isocyanate (720 mg, 0.43 mL, 4.8 mmol, 1.2 equiv.) were added dropwise. The reaction was allowed to ambiently warm to RT with stirring and was allowed to stir until judged complete by TLC. Once complete, the volatiles were stripped under vacuum and the product was purified by silica gel column chromatography (gradient from 100% hexanes to 25% EtOAc in hexanes) to afford 1.02 g (97% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.12 – 7.06 (m, 1H), 6.99 (dd, *J* = 5.1, 3.5 Hz, 1H), 5.26 (s, 2H), 5.19 (s, 1H), 3.60 (q, *J* = 5.9 Hz, 2H), 3.46 (t, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.82, 138.21, 128.05, 126.83, 126.82, 61.15, 42.72, 32.35.

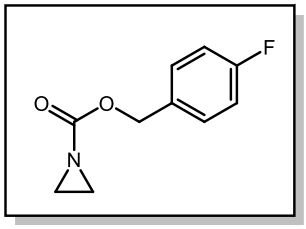


Thiophen-2-ylmethyl aziridine-1-carboxylate. Thiophen-2-ylmethyl (2-bromoethyl)carbamate (1.02 g, 3.86 mmol, 1 equiv.) was dissolved in DMF (19 mL). Cesium carbonate (1.89 g, 5.79 mmol, 1.5 equiv.) was added in a single portion, and the reaction was vigorously stirred at RT until judged complete by TLC. Once finished, the reaction was partitioned into 40 mL of EtOAc and 20 mL of water. The layers were thoroughly mixed and the aqueous layer was drained. The organics were further washed with 5% aq. LiCl solution (w/w) (3x 10 mL) prior to drying the organics over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped under vacuum. The crude product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford 707 mg (63% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.11 (ddt, *J* = 3.5, 1.3, 0.7 Hz, 1H), 6.98 (dd, *J* = 5.1, 3.5 Hz, 1H), 5.28 (d, *J* = 0.7 Hz, 2H), 2.22 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.35, 137.60, 128.33, 126.97, 126.81, 62.41, 25.88. HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_8H_{10}NO_2S$) requires *m/z* 183.0354, found *m/z* 183.0334.

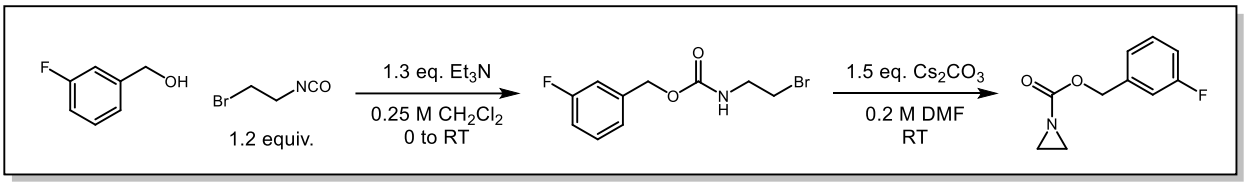


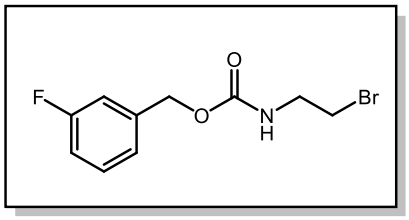


4-Fluorobenzyl (2-bromoethyl)carbamate. Synthesized using the procedure of Marlin.[9] 4-Fluorobenzyl alcohol (505 mg, 0.43 mL, 4 mmol, 1.0 equiv.) and triethylamine (526 mg, 0.73 mL, 5.2 mmol, 1.3 equiv.) were dissolved in methylene chloride (16 mL). The flask was chilled in an ice bath and 2-bromoethyl isocyanate (720 mg, 0.43 mL, 4.8 mmol, 1.2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring and was allowed to stir until judged complete by TLC. Once complete, the volatiles were stripped under vacuum and the product purified by silica gel column chromatography (gradient from 100% hexanes to 25% EtOAc in hexanes) to afford 830 mg (75% yield) of the titled compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.30 (m, 2H), 7.09 – 6.99 (m, 2H), 5.18 (br s, 1H), 5.07 (s, 2H), 3.61 (q, *J* = 5.9 Hz, 2H), 3.47 (t, *J* = 5.8 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 162.62 (d, *J* = 246.8 Hz), 156.00, 132.06 (d, *J* = 3.3 Hz), 130.13 (d, *J* = 8.2 Hz), 115.46 (d, *J* = 21.5 Hz), 66.24, 42.72, 32.45. ^{19}F NMR (282 MHz, $CDCl_3$) δ -113.76 (m). HRMS (ES+) exact mass calculated for $[M+H+CH_3CN]^+$ ($C_{12}H_{15}BrFN_2O_2$) requires *m/z* 317.0301, found *m/z* 317.0328.

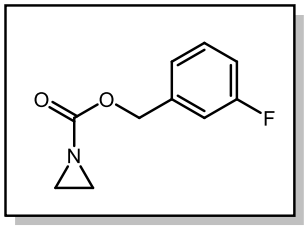


4-Fluorobenzyl aziridine-1-carboxylate. 4-Fluorobenzyl (2-bromoethyl)carbamate (830 mg, 3.00 mmol, 1 equiv.) was dissolved in DMF (15 mL). Cesium carbonate (1.47 g, 4.50 mmol, 1.5 equiv.) was added in a single portion, and the reaction was vigorously stirred at RT until judged complete by TLC. Once finished, the reaction was partitioned into 30 mL of EtOAc and 15 mL of water. The layers were thoroughly mixed, and the aqueous layer was drained. The organics were further washed with 5% aq. LiCl solution (w/w) (3x 10 mL) prior to drying the organics over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped under vacuum. The crude product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford 414 mg (71% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.32 (m, 2H), 7.13 – 6.94 (m, 2H), 5.09 (s, 2H), 2.22 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.50, 162.71 (d, *J* = 247.1 Hz), 131.54 (d, *J* = 3.3 Hz), 130.25 (d, *J* = 8.3 Hz), 115.49 (d, *J* = 21.6 Hz), 67.50, 25.86. ^{19}F NMR (282 MHz, $CDCl_3$) δ -112.79 (tt, *J* = 9.0, 5.7 Hz). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{10}H_{11}FNO_2$) requires *m/z* 196.0774, found *m/z* 196.0793.

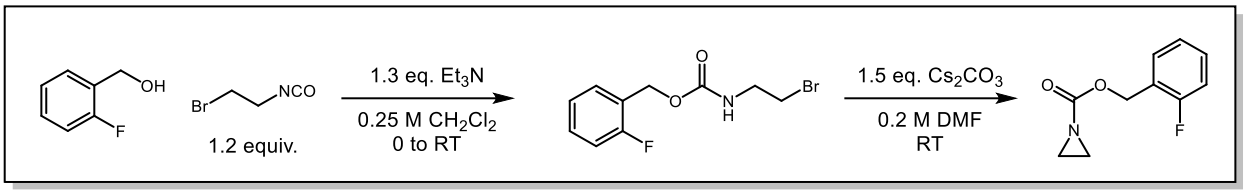


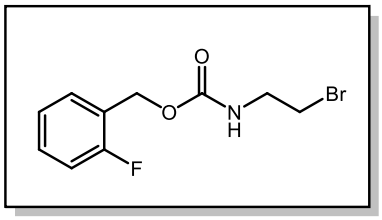


3-Fluorobenzyl (2-bromoethyl)carbamate. Synthesized using the procedure of Marlin.[9] 3-Fluorobenzyl alcohol (505 mg, 0.43 mL, 4 mmol, 1.0 equiv.) and triethylamine (526 mg, 0.73 mL, 5.2 mmol, 1.3 equiv.) were dissolved in methylene chloride (16 mL). The flask was chilled in an ice bath, and 2-bromoethyl isocyanate (720 mg, 0.43 mL, 4.8 mmol, 1.2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring and was allowed to stir until judged complete by TLC. Once complete, the volatiles were stripped under vacuum and the product purified by silica gel column chromatography (gradient from 100% hexanes to 25% EtOAc in hexanes) to afford 980 mg (89% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (td, *J* = 8.0, 5.8 Hz, 1H), 7.15 – 7.08 (m, 2H), 7.06 (dt, *J* = 9.5, 2.1 Hz, 1H), 7.04 – 6.97 (m, 1H), 5.24 (s, 1H), 5.10 (s, 2H), 3.61 (q, *J* = 5.9 Hz, 2H), 3.47 (t, *J* = 5.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.81 (d, *J* = 246.3 Hz), 155.89, 138.76 (d, *J* = 7.3 Hz), 130.09 (d, *J* = 8.1 Hz), 123.34 (d, *J* = 3.0 Hz), 115.04 (d, *J* = 21.1 Hz), 114.74 (d, *J* = 21.9 Hz), 66.03, 42.74, 32.38. ^{19}F NMR (282 MHz, $CDCl_3$) δ -113.02 (td, *J* = 9.0, 5.7 Hz). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{10}H_{12}BrFNO_2$) requires *m/z* 276.0035, found *m/z* 276.0047.

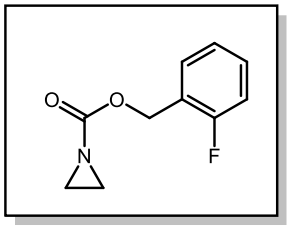


3-Fluorobenzyl aziridine-1-carboxylate. 3-Fluorobenzyl (2-bromoethyl)carbamate (980 mg, 3.55 mmol, 1 equiv.) was dissolved in DMF (18 mL). Cesium carbonate (1.73 g, 5.33 mmol, 1.5 equiv.) was added in a single portion, and the reaction was vigorously stirred at RT until judged complete by TLC. Once finished, the reaction was partitioned into 30 mL of EtOAc and 15 mL of water. The layers were thoroughly mixed, and the aqueous layer was drained. The organics were further washed with 5% aq. LiCl solution (w/w) (3x 10 mL) prior to drying the organics over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped under vacuum. The crude product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford 525 mg (76% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (td, *J* = 7.9, 5.8 Hz, 1H), 7.13 (ddt, *J* = 7.6, 1.6, 0.8 Hz, 1H), 7.08 (dt, *J* = 9.6, 2.1 Hz, 1H), 7.05 – 6.98 (m, 1H), 5.12 (s, 2H), 2.24 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.38, 162.80 (d, *J* = 246.5 Hz), 138.17 (d, *J* = 7.4 Hz), 130.13 (d, *J* = 8.1 Hz), 123.46 (d, *J* = 3.0 Hz), 115.20 (d, *J* = 21.0 Hz), 114.85 (d, *J* = 22.0 Hz), 67.26 (d, *J* = 1.9 Hz), 25.88. ^{19}F NMR (282 MHz, $CDCl_3$) δ -112.94 (td, *J* = 9.0, 5.8 Hz). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{10}H_{11}FNO_2$) requires *m/z* 196.0774, found *m/z* 196.0748.

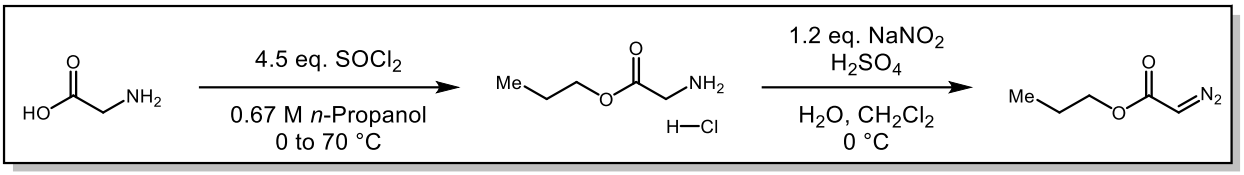


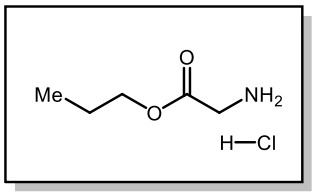


2-Fluorobenzyl (2-bromoethyl)carbamate. Synthesized using the procedure of Marlin.[9] 2-Fluorobenzyl alcohol (505 mg, 0.43 mL, 4 mmol, 1.0 equiv.) and triethylamine (526 mg, 0.73 mL, 5.2 mmol, 1.3 equiv.) were dissolved in methylene chloride (16 mL). The flask was chilled in an ice bath, and 2-bromoethyl isocyanate (720 mg, 0.43 mL, 4.8 mmol, 1.2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring and was allowed to stir until judged complete by TLC. Once complete, the volatiles were stripped under vacuum and the product purified by silica gel column chromatography (gradient from 100% hexanes to 25% EtOAc in hexanes) to afford 970 mg (88% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (td, *J* = 7.5, 1.8 Hz, 1H), 7.32 (tdd, *J* = 7.4, 5.3, 1.8 Hz, 1H), 7.14 (td, *J* = 7.5, 1.2 Hz, 1H), 7.07 (ddd, *J* = 9.7, 8.2, 1.2 Hz, 1H), 5.19 (s, 2H), 5.19 (br s, 1H -- overlaps with benzylic protons), 3.61 (q, *J* = 5.9 Hz, 2H), 3.47 (t, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.97 (d, *J* = 248.5 Hz), 155.95, 130.59 (d, *J* = 3.9 Hz), 130.21 (d, *J* = 8.2 Hz), 124.14 (d, *J* = 3.7 Hz), 123.38 (d, *J* = 14.4 Hz), 115.46 (d, *J* = 21.2 Hz), 60.86 (d, *J* = 4.3 Hz), 42.75, 32.42. ^{19}F NMR (282 MHz, $CDCl_3$) δ -118.37 (m). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{10}H_{12}BrFNO_2$) requires *m/z* 276.0035, found *m/z* 276.0042.

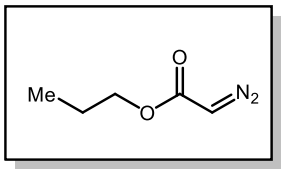


2-Fluorobenzyl aziridine-1-carboxylate. 2-Fluorobenzyl (2-bromoethyl)carbamate (970 mg, 3.51 mmol, 1 equiv.) was dissolved in DMF (18 mL). Cesium carbonate (1.72 g, 5.27 mmol, 1.5 equiv.) was added in a single portion, and the reaction was vigorously stirred at RT until judged complete by TLC. Once finished, the reaction was partitioned into 30 mL of EtOAc and 15 mL of water. The layers were thoroughly mixed, and the aqueous layer was drained. The organics were further washed with 5% aq. LiCl solution (w/w) (3x 10 mL) prior to drying the organics over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped under vacuum. The crude product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford 495 mg (72% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (td, *J* = 7.5, 1.9 Hz, 1H), 7.33 (tdd, *J* = 7.5, 5.3, 1.8 Hz, 1H), 7.14 (td, *J* = 7.5, 1.2 Hz, 1H), 7.07 (ddd, *J* = 9.6, 8.2, 1.1 Hz, 1H), 5.20 (s, 2H), 2.24 (s, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 163.47, 160.98 (d, *J* = 248.5 Hz), 130.57 (d, *J* = 3.7 Hz), 130.34 (d, *J* = 8.2 Hz), 124.15 (d, *J* = 3.7 Hz), 122.87 (d, *J* = 14.6 Hz), 115.48 (d, *J* = 21.2 Hz), 62.21 (d, *J* = 4.2 Hz), 25.90. ^{19}F NMR (282 MHz, $CDCl_3$) δ -118.17 (m). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{10}H_{11}FNO_2$) requires *m/z* 196.0774, found *m/z* 196.0750.



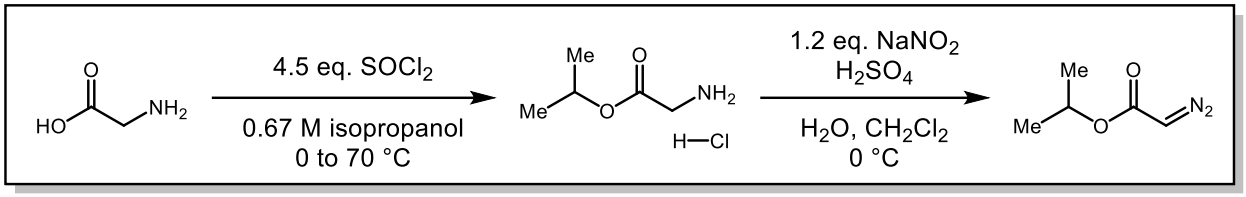


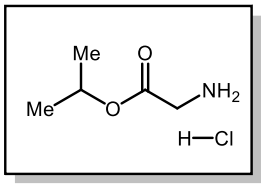
Propyl glycinate hydrochloride. To a 100-mL round-bottom flask equipped with a stir bar, septum, and drying tube: glycine (1.50 g, 20 mmol, 1.0 equiv.) was suspended in *n*-propanol (30 mL). The suspension was chilled in an ice bath with stirring prior to the dropwise addition of thionyl chloride (10.7 g, 6.56 mL, 90 mmol, 4.5 equiv.). Once addition was complete and the initial exotherm had subsided, the septum with drying tube was replaced with a reflux condenser with a drying tube and the solution was heated to 70 °C with stirring for 20 hours. When done, the reaction was allowed to cool to RT and the volatiles were stripped under vacuum. The product was precipitated by blanketing the crude oil with about 10 mL of diethyl ether and gently scratching to induce precipitation. The resultant solid was isolated by vacuum filtration, washing liberally with additional diethyl ether, and was thoroughly dried under vacuum to afford 2.42 g (79% isolated yield) of the titled compound as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 8.75 – 8.38 (br s, 3H), 4.10 (t, *J* = 6.6 Hz, 2H), 3.77 (s, 2H), 1.62 (hept, *J* = 7.4 Hz, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 167.65, 66.82, 39.10 (obscured by DMSO-*d*6; assigned via HSQC), 21.42, 10.17. HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_5H_{12}NO_2$) requires *m/z* 118.0868, found *m/z* 118.0898.



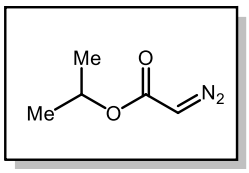
Propyl 2-diazoacetae (propyl diazoacetate). This procedure was adapted from that of Searle for the synthesis of ethyl diazoacetate.[10] *Safety note:* the procedure reported by Searle was performed on a 1-mole scale: our procedure is a hundred-fold reduction in scale as a matter of safety. Work should be performed in a well-ventilated fume hood.

To a 40-mL vial: propyl glycinate hydrochloride (1.54 g, 10 mmol, 1 equiv.) was added to a suspension of methylene chloride (2.5 mL) in water (6 mL). The solution was chilled in an ice bath prior to the addition of sodium nitrite (828 mg, 12 mmol, 1.2 equiv.) in a single portion. Once dissolved, a single drop of concentrated sulfuric acid was added, resulting in bubbling of the solution and the development of a yellow color. The solution was allowed to stir for 30 minutes on ice. Once complete, the reaction was transferred to a separatory funnel and diluted with 20 mL of methylene chloride. The aqueous layer was separated, and the organics were washed with saturated sodium bicarbonate (2x 5 mL) and brine (1x 5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles were stripped under vacuum, going no lower than 130 torr and spinning the flask in an ice-water bath. The resulting residue was purified by silica gel column chromatography (gradient from pentanes to 10% diethyl ether in pentanes, concentrating fractions as before) to afford 1.27 g (99% yield) of the titled compound as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.73 (s, 1H), 4.12 (t, *J* = 6.7 Hz, 2H), 1.67 (hept, *J* = 7.4 Hz, 1H), 0.94 (t, *J* = 7.4 Hz, 3H). The chemical shifts are in accord with prior literature reports.[11]





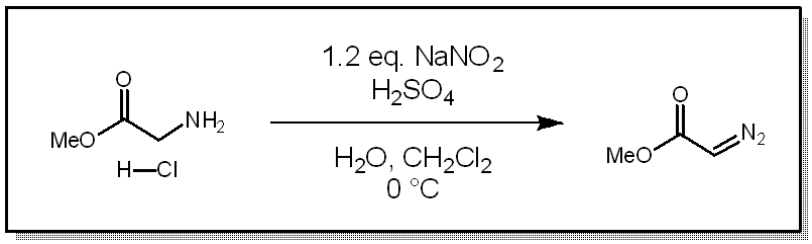
Isopropyl glycinate hydrochloride. To a 100-mL round-bottom flask equipped with a stir bar, septum, and drying tube: glycine (1.50 g, 20 mmol, 1.0 equiv.) was suspended in isopropanol (30 mL). The suspension was chilled in an ice bath with stirring prior to the dropwise addition of thionyl chloride (10.7 g, 6.56 mL, 90 mmol, 4.5 equiv.). Once addition was complete and the initial exotherm had subsided, the septum with drying tube was replaced with a reflux condenser with a drying tube and the solution was heated to 70 °C with stirring for 20 hours. When done, the reaction was allowed to cool to RT and the volatiles were stripped under vacuum. The product was precipitated by blanketing the crude oil with about 10 mL of diethyl ether and gently scratching to induce precipitation. The resultant solid was isolated by vacuum filtration, washing liberally with additional diethyl ether, and was thoroughly dried under vacuum to afford 2.33 g (76% isolated yield) of the titled compound as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 8.53 (br s, 3H), 4.99 (hept, *J* = 6.3 Hz, 1H), 3.71 (q, *J* = 6.2 Hz, 2H), 1.23 (d, *J* = 6.3 Hz, 6H). The chemical shifts are in accord with prior literature reports.[12]

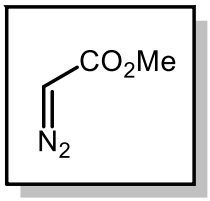


Isopropyl 2-diazoacetae (isopropyl diazoacetate). This procedure was adapted from that of Searle for the synthesis of ethyl diazoacetate.[9] *Safety note:* the procedure reported by Searle was performed on a 1-mole scale: our procedure is a hundred-fold reduction in scale as a matter of safety. Work should be performed in a well-ventilated fume hood.

To a 40-mL vial: isopropyl glycinate hydrochloride (1.54 g, 10 mmol, 1 equiv.) was added to a suspension of methylene chloride (2.5 mL) in water (6 mL). The solution was chilled in an ice bath prior to the addition of sodium nitrite (828 mg, 12 mmol, 1.2 equiv.) in a single portion. Once dissolved, a single drop of concentrated sulfuric acid was added, resulting in bubbling of the solution and the development of a yellow color. The solution was allowed to stir for 30 minutes on ice. Once complete, the reaction was transferred to a separatory funnel and diluted with 20 mL of methylene chloride. The aqueous layer was separated, and the organics were washed with saturated sodium bicarbonate (2x 5 mL) and brine (1x 5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles were stripped under vacuum, going no lower than 130 torr and spinning the flask in an ice-water bath. The resulting residue was purified

by silica gel column chromatography (gradient from pentanes to 10% diethyl ether in pentanes) to afford 738 mg (58% yield) of the titled compound as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.09 (hept, J = 6.3 Hz, 1H), 4.69 (s, 1H), 1.26 (d, J = 6.3 Hz, 6H). The chemical shifts are in accord with prior literature reports.[11]



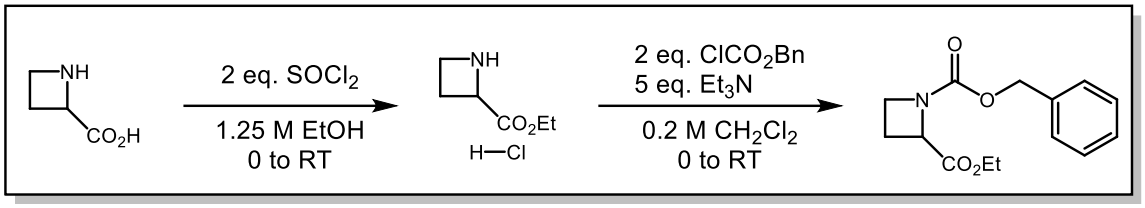


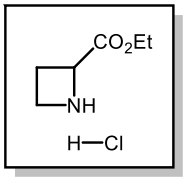
Methyl 2-diazoacetae (Methyl diazoacetate).

This procedure was adapted from that of Searle for the synthesis of ethyl diazoacetate.[10] *Safety note:* the procedure reported by Searle was performed on a 1-mole scale: our procedure is a hundred-fold reduction in scale as a matter of safety. Work should be performed in a well-ventilated fumehood.

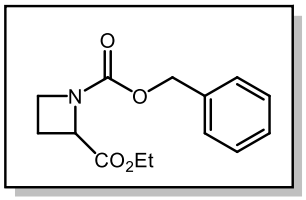
To a 40-mL vial: glycine methyl ester hydrochloride (1.26 g, 10 mmol, 1 equiv.) was added to a suspension of methylene chloride (2.5 mL) in water (6 mL). The solution was chilled on an ice bath prior to the addition of sodium nitrite (828 mg, 12 mmol, 1.2 equiv.) in a single portion. Once dissolved, a single drop of concentrated sulfuric acid was added, resulting in bubbling of the solution and the development of a yellow color. The solution was allowed to stir for 30 minutes on ice. Once complete, the reaction was transferred to a separatory funnel and diluted with 20 mL of methylene chloride. The aqueous layer was separated, and the organics were washed with saturated sodium bicarbonate (2x 5 mL) and brine (1x 5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles were stripped under vacuum, going no lower than 200 torr and spinning the flask in an ice-water bath. The resulting residue was purified by silica gel column chromatography (gradient from pentanes to 10% diethyl ether in pentanes) to afford 584 mg (58% yield) of the titled compound as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (s, 1H), 3.76 (s, 3). The chemical shifts are in accord with prior literature reports.[13]

Preparation of Authentic Standards

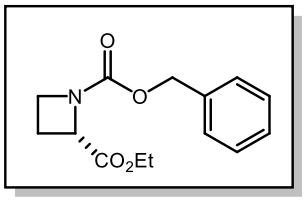




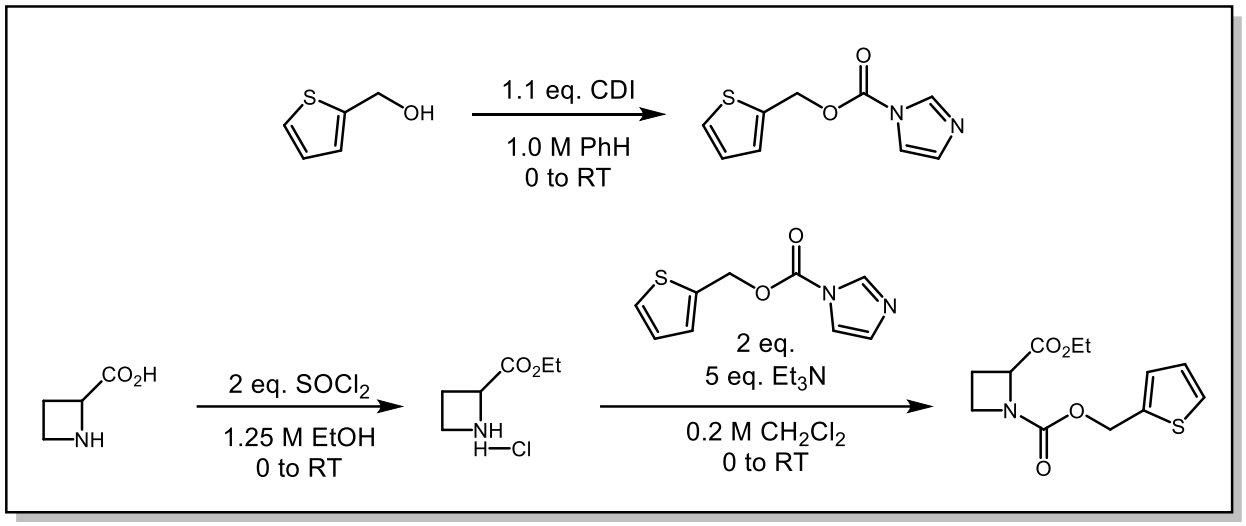
(±)-Ethyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (100 mg, 1 mmol, 1 equiv.) was suspended in ethanol (1.25 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.15 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring until judged complete by TLC. Once done, the solution was concentrated directly from this dram vial under vacuum to remove volatiles, and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.

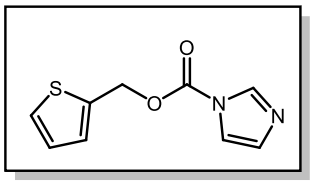


(±)-1-Benzyl 2-ethyl azetidine-1,2-dicarboxylate. The crude (±)-ethyl azetidine-2-carboxylate hydrochloride (165.62 mg, 1 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (5 mL), and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.69 mL, 5 mmol, 5 equiv.). Once addition was completed, benzyl chloroformate (0.29 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to warm to RT and stirring was continued until the reaction was judged complete by TLC analysis. Once complete, the reaction was transferred to a separatory funnel and quenched with 5 mL of saturated sodium bicarbonate solution. The layers were thoroughly mixed, and the aqueous layer was removed. The organic layer was washed with water (1x 5 mL) and brine (1x 5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles were removed under vacuum. The crude product was purified by silica gel column chromatography (gradient from hexanes to 33% EtOAc in hexanes) to afford 165 mg (63% yield) of the titled compound a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 5.10 (m, 2H), 4.68 (dd, J = 9.3, 5.3 Hz, 1H), 4.19 (br s, 2H), 4.12 (m, 1H), 3.97 (m, 1H), 2.57 (dtd, J = 11.5, 9.2, 6.3 Hz, 1H), 2.22 (ddt, J = 11.3, 9.0, 5.5 Hz, 1H), 1.30 – 1.17 (br m, 3H).

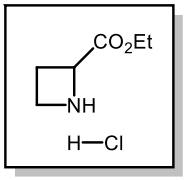


1-Benzyl 2-ethyl (*S*)-azetidine-1,2-dicarboxylate. This compound was synthesized using the analogous procedure to the racemate, except *(S)*-azetidine-2-carboxylic acid was used instead of racemic azetidine-2-carboxylic acid. This procedure yielded 82 mg (32% isolated yield) of the titled compound as a colorless oil over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 5.10 (m, 2H), 4.68 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.19 (br s, 2H), 4.12 (m, 1H), 3.97 (m, 1H), 2.57 (dtd, *J* = 11.5, 9.2, 6.3 Hz, 1H), 2.22 (ddt, *J* = 11.3, 9.0, 5.5 Hz, 1H), 1.30 – 1.17 (br m, 3H).

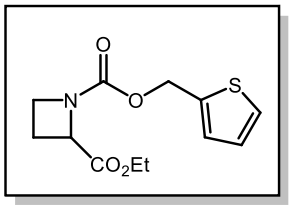




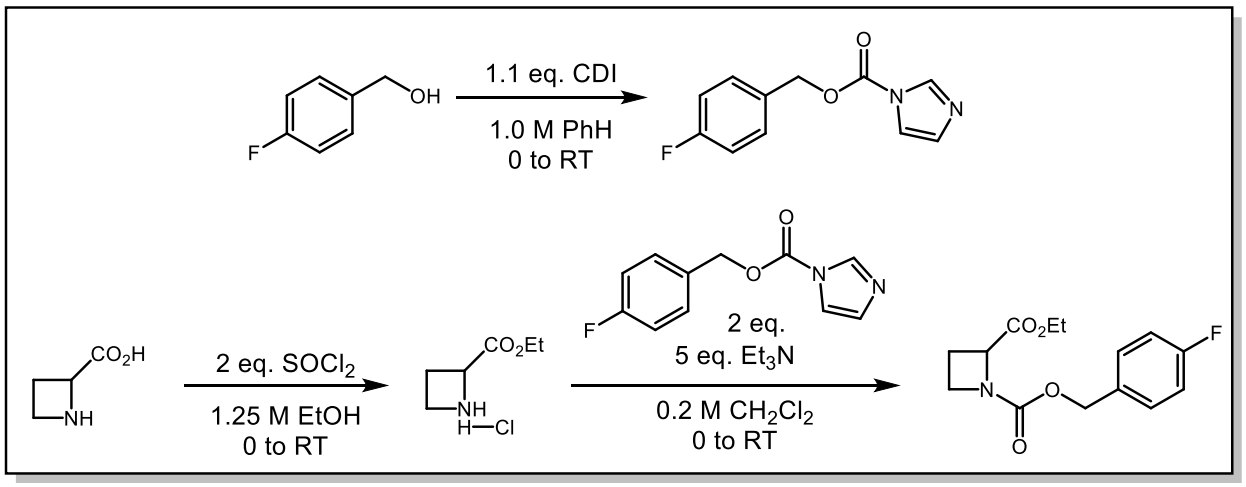
Thiophen-2-ylmethyl 1H-imidazole-1-carboxylate. Synthesized using the procedure reported by Snaddon.[14] To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: 2-thiophenemethanol (343 mg, 0.28 mL, 3 mmol, 1 equiv.) was dissolved in benzene (3 mL) and the solution was chilled in an ice bath. Carbonyldiimidazole (CDI) (503 mg, 3.3 mmol, 1.1 equiv.) was added by quickly removing the septum and pouring in the solid reagent in a single portion. Once the initial exotherm subsided, the reaction was allowed to stir at RT until judged complete by TLC. Once finished, a small amount of methylene chloride was added to fully dissolve all solids. The reaction mixture was washed with water (2x 1.5 mL) and brine (1.5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted, and the volatiles were removed under vacuum to afford 500 mg (80% yield) of the titled compound as a colorless oil. The purity of the crude was sufficient to carry forward without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 (2, 1H), 7.43 (t, *J* = 1.4 Hz, 1H), 7.41 (dd, *J* = 5.1, 1.3 Hz, 1H), 7.23 (dd, *J* = 3.3, 1.3 Hz, 1H), 7.06 (m, 1H), 7.04 (m, 1H), 5.58 (s, 2H). The spectral data are consistent with prior literature reports.[15]

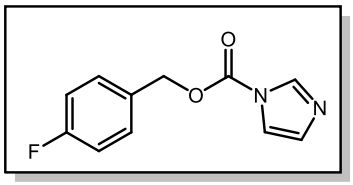


(±)-Ethyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (100 mg, 1 mmol, 1 equiv.) was suspended in ethanol (1.25 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.15 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring until judged complete by TLC. Once done, the solution was concentrated directly from this dram vial under vacuum to remove volatiles, and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.

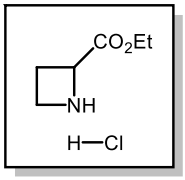


(±)-2-Ethyl-1-(thiophen-2-ylmethyl) azetidine-1,2-dicarboxylate. The crude **(±)**-ethyl azetidine-2-carboxylate hydrochloride (165.62 mg, 1 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (3 mL), and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.69 mL, 5 mmol, 5 equiv.). Once addition was complete, thiophen-2-ylmethyl 1H-imidazole-1-carboxylate (416 mg, 2 mmol, 2 equiv.) was added to the mixture in 2 mL of methylene chloride. The reaction was allowed to warm to RT, and stirring was continued until the reaction was judged complete by TLC analysis. When done, the reaction was transferred to a separatory funnel and diluted with 5 mL of methylene chloride. The reaction was washed with 1.0 M HCl (5 mL), sat. aq. $NaHCO_3$ (5 mL), and brine (5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped under vacuum. The crude residue was purified by silica gel column chromatography (gradient from 100% hexanes to 30% EtOAc in hexanes) to afford 93 mg (35% yield) of the titled compound as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (d, *J* = 5.1 Hz, 1H), 7.09 – 7.04 (m, 1H), 6.96 (t, *J* = 4.3 Hz, 1H), 5.21 (m, 2H), 4.66 (dd, *J* = 9.4, 5.3 Hz, 1H), 4.20 (br s, 2H), 4.16 – 4.06 (m, 1H), 3.96 (m, 1H), 2.63 – 2.48 (m, 1H), 2.20 (ddt, *J* = 10.8, 8.4, 5.5 Hz, 1H), 1.32 – 1.12 (m, 4H).

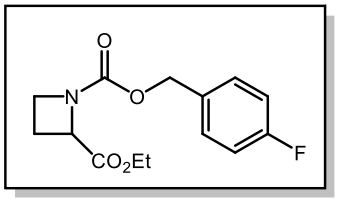




4-Fluorobenzyl 1H-imidazole-1-carboxylate. Synthesized using the procedure reported by Snaddon.[14] To an oven-dried 8 mL dram vial equipped with a stir bar, septum, and drying tube: 4-fluorobenzyl alcohol (378 mg, 0.33 mL, 3 mmol, 1 equiv.) was dissolved in benzene (3 mL), and the solution was chilled in an ice bath. Carbonyldiimidazole (CDI) (503 mg, 3.3 mmol, 1.1 equiv.) was added by quickly removing the septum and pouring in the solid reagent in a single portion. Once the initial exotherm subsided, the reaction was allowed to stir at RT until judged complete by TLC. Once finished, a small amount of methylene chloride was added to fully dissolve all solids. The reaction mixture was washed with water (2x 1.5 mL) and brine (1.5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles were removed under vacuum to afford 560 mg (85% yield) of the titled compound as a colorless oil. The purity of the crude was sufficient to carry forward without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.47 – 7.41 (m, 3H), 7.14 – 7.01 (m, 3H), 5.38 (s, 2H). The spectral data are consistent with prior literature reports.[16]

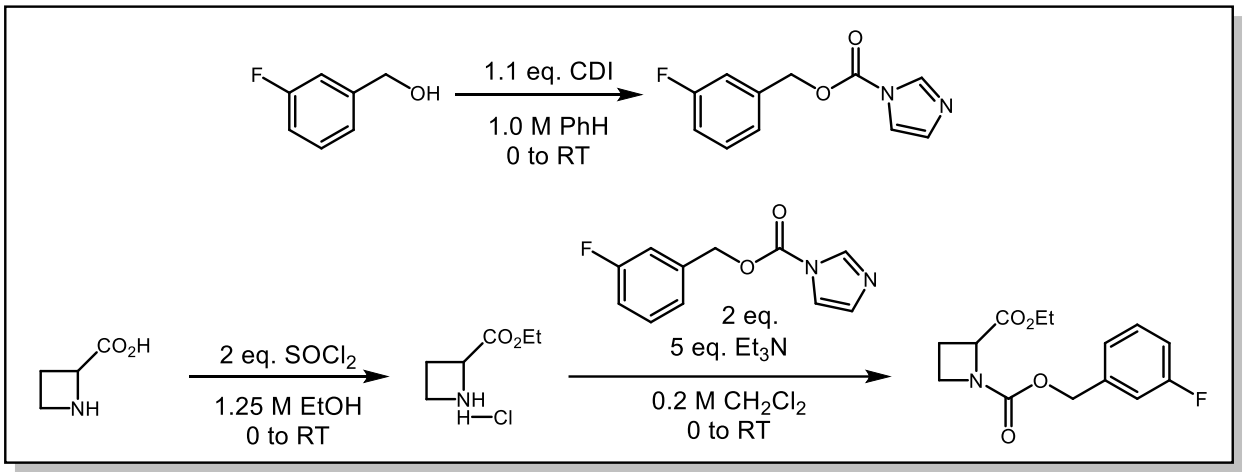


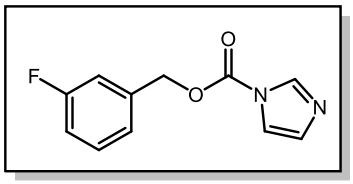
(±)-Ethyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (100 mg, 1 mmol, 1 equiv.) was suspended in ethanol (1.25 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.15 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring until judged complete by TLC. Once done, the solution was concentrated directly from this dram vial under vacuum to remove volatiles, and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.



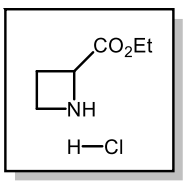
(±)-2-ethyl-1-(4-fluorobenzyl) azetidine-1,2-dicarboxylate. The crude **(±)-**ethyl azetidine-2-carboxylate hydrochloride (165.62 mg, 1 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (3 mL) and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.69 mL, 5 mmol, 5 equiv.). Once addition was complete, 4-fluorobenzyl 1H-imidazole-1-carboxylate (440 mg, 2 mmol, 2 equiv.) was added to the mixture in 2 mL of methylene chloride. The reaction was allowed to warm to RT, and stirring was continued until the reaction was judged complete by TLC analysis. When done, the reaction was transferred to a separatory funnel and diluted with 5 mL of methylene chloride. The reaction was washed with 1.0 M HCl (5 mL), sat. aq. $NaHCO_3$ (5 mL), and brine (5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped under vacuum. The crude residue was purified by silica gel column chromatography (gradient from

100% hexanes to 30% EtOAc in hexanes) to afford 71 mg (25% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 2H), 7.08 – 6.97 (m, 2H), 5.14 – 4.96 (m, 2H), 4.74 – 4.61 (m, 1H), 4.18 (br s, 2H), 4.10 (m, 1H), 4.02 – 3.86 (m, 1H), 2.56 (dddd, *J* = 11.5, 10.5, 8.5, 6.3 Hz, 1H), 2.29 – 2.13 (m, 1H), 1.33 – 1.13 (br s, 3H).



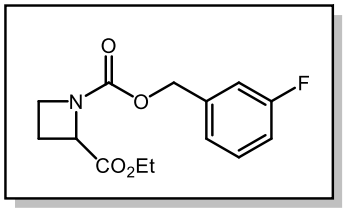


3-Fluorobenzyl 1H-imidazole-1-carboxylate. Synthesized using the procedure reported by Snaddon.[14] To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: 3-fluorobenzyl alcohol (378 mg, 0.33 mL, 3 mmol, 1 equiv.) was dissolved in benzene (3 mL) and the solution was chilled in an ice bath. Carbonyldiimidazole (CDI) (503 mg, 3.3 mmol, 1.1 equiv.) was added by quickly removing the septum and pouring in the solid reagent in a single portion. Once the initial exotherm subsided, the reaction was allowed to stir at RT until judged complete by TLC. Once finished, a small amount of methylene chloride was added to fully dissolve all solids and the mixture was diluted in 5 mL of EtOAc. The reaction mixture was washed with water (2x 1.5 mL) and brine (1.5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles removed under vacuum to afford 660 mg (quantitative yield) of the titled compound as a colorless oil. The purity of the crude was sufficient to carry forward without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (t, *J* = 1.1 Hz, 1H), 7.44 (t, *J* = 1.5 Hz, 1H), 7.39 (td, *J* = 8.0, 5.8 Hz, 1H), 7.32 (m, 1H), 7.22 (m, 1H), 7.18 – 7.05 (m, 3H), 5.40 (s, 2H). The spectral data are consistent with prior literature reports.[17]

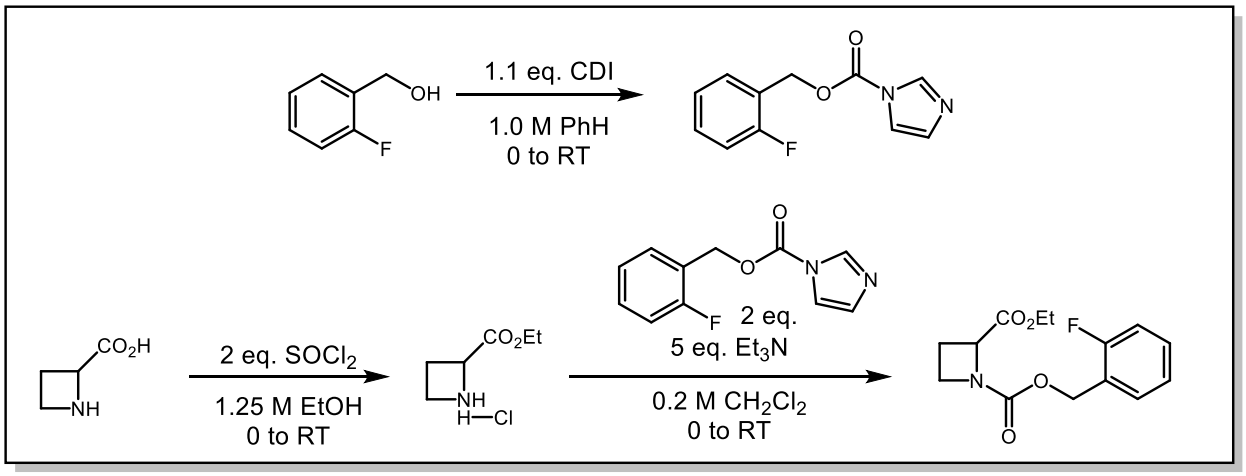


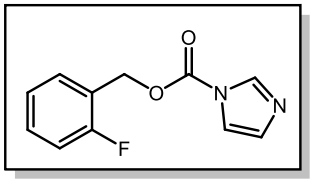
(±)-Ethyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (100 mg, 1 mmol, 1 equiv.) was suspended in ethanol (1.25 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.15 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring

until judged complete by TLC. Once done, the solution was concentrated directly from this dram vial under vacuum to remove volatiles, and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.

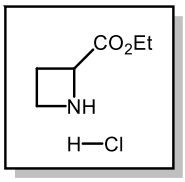


(±)-2-Ethyl-1-(3-fluorobenzyl) azetidine-1,2-dicarboxylate. The crude (±)-ethyl azetidine-2-carboxylate hydrochloride (165.62 mg, 1 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (3 mL) and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.69 mL, 5 mmol, 5 equiv.). Once addition was completed, 3-fluorobenzyl 1H-imidazole-1-carboxylate (440 mg, 2 mmol, 2 equiv.) was added to the mixture in 2 mL of methylene chloride. The reaction was allowed to warm to RT, and stirring was continued until the reaction was judged complete by TLC analysis. When done, the reaction was transferred to a separatory funnel and diluted with 5 mL of methylene chloride. The reaction was washed with 1.0 M HCl (5 mL), sat. aq. $NaHCO_3$ (5 mL), and brine (5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped under vacuum. The crude residue was purified by silica gel column chromatography (gradient from 100% hexanes to 30% EtOAc in hexanes) to afford 104 mg (37% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (td, *J* = 7.9, 5.8 Hz, 1H), 7.13 – 6.94 (m, 3H), 5.22 – 4.98 (m, 2H), 4.70 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.27 – 4.17 (m, 2H), 4.16 – 4.08 (m, 1H), 3.99 (ddd, *J* = 9.1, 8.1, 5.6 Hz, 1H), 2.59 (dtd, *J* = 11.6, 9.2, 6.3 Hz, 1H), 2.23 (ddt, *J* = 11.4, 9.1, 5.5 Hz, 1H), 1.30 – 1.18 (m, 3H).

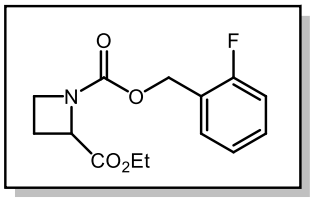




2-Fluorobenzyl 1H-imidazole-1-carboxylate. Synthesized using the procedure reported by Snaddon.[14] To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: 2-fluorobenzyl alcohol (378 mg, 0.32 mL, 3 mmol, 1 equiv.) was dissolved in benzene (3 mL), and the solution was chilled in an ice bath. Carbonyldiimidazole (CDI) (503 mg, 3.3 mmol, 1.1 equiv.) was added by quickly removing the septum and pouring in the solid reagent in a single portion. Once the initial exotherm subsided, the reaction was allowed to stir at RT until judged complete by TLC. Once finished, a small amount of methylene chloride was added to fully dissolve all solids and the mixture was diluted in 5 mL of EtOAc. The reaction mixture was washed with water (2x 1.5 mL) and brine (1.5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles were removed under vacuum to afford 580 mg (88% yield) of the titled compound as a colorless oil. The purity of the crude was sufficient to carry forward without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (t, *J* = 1.1 Hz, 1H), 7.50 – 7.37 (m, 3H), 7.23 – 7.09 (m, 2H), 7.06 (dd, *J* = 1.7, 0.8 Hz, 1H), 5.49 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.25 (d, *J* = 249.9 Hz), 148.49, 137.13, 131.39 (d, *J* = 8.3 Hz), 131.15 (d, *J* = 3.3 Hz), 130.63, 124.42 (d, *J* = 3.7 Hz), 121.22 (d, *J* = 14.5 Hz), 117.15, 115.83 (d, *J* = 20.9 Hz), 63.80 (d, *J* = 4.2 Hz). ^{19}F NMR (282 MHz, $CDCl_3$) δ -117.51 (m). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{11}H_{10}FN_2O_2$) requires *m/z* 211.0726, found *m/z* 211.0726.

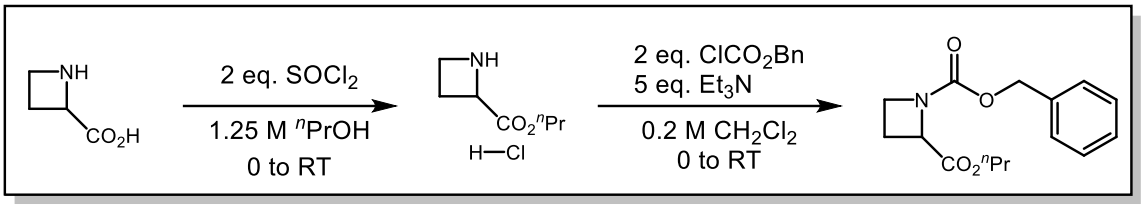


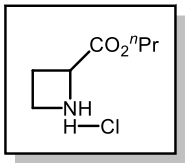
(±)-Ethyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (100 mg, 1 mmol, 1 equiv.) was suspended in ethanol (1.25 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.15 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring until judged complete by TLC. Once done, the solution was concentrated directly from this dram vial under vacuum to remove volatiles, and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.



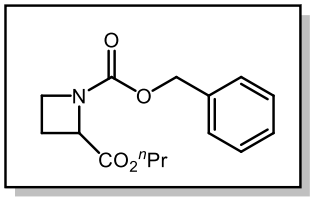
(±)-2-Ethyl-1-(2-fluorobenzyl) azetidine-1,2-dicarboxylate. The crude (±)-ethyl azetidine-2-carboxylate hydrochloride (165.62 mg, 1 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (3 mL), and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.69 mL, 5 mmol, 5 equiv.). Once addition was completed, 2-fluorobenzyl 1H-imidazole-1-carboxylate (440 mg, 2 mmol, 2 equiv.) was added to the mixture in 2 mL of methylene chloride. The reaction was allowed to warm to RT, and stirring was continued until the reaction was judged complete by TLC analysis. When done, the reaction was transferred to a separatory funnel and diluted with 5 mL of methylene chloride. The reaction was washed with 1.0 M HCl (5 mL), sat. aq. $NaHCO_3$ (5 mL), and brine (5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted and the volatiles stripped

under vacuum. The crude residue was purified by silica gel column chromatography (gradient from 100% hexanes to 30% EtOAc in hexanes) to afford 102 mg (36% yield) of the titled compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 1H), 7.29 (m, 1H), 7.12 (m, 1H), 7.04 (m, 1H), 5.17 (q, *J* = 12.7 Hz, 2H), 4.68 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.19 (m, 2H), 4.11 (td, *J* = 8.6, 6.3 Hz, 1H), 3.97 (td, *J* = 8.6, 8.2, 5.7 Hz, 1H), 2.57 (dtd, *J* = 11.6, 9.2, 6.3 Hz, 1H), 2.21 (ddt, *J* = 11.3, 9.0, 5.5 Hz, 1H), 1.24 (m, 3H).



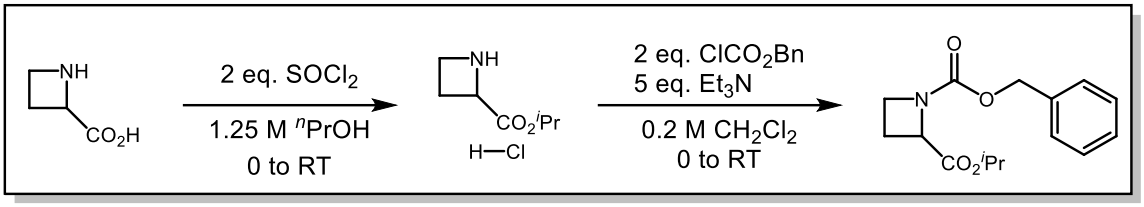


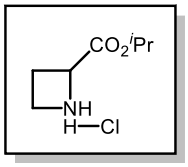
(±)-Propyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (50 mg, 1 mmol, 1 equiv.) was suspended in *n*-propanol (0.6 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.07 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring until judged complete by TLC. Once done, the brown solution was concentrated directly from this dram vial under vacuum to remove volatiles, and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.



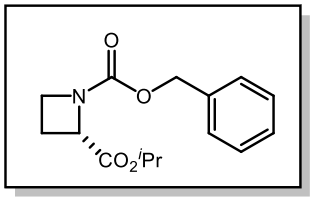
(±)-1-Benzyl 2-propyl azetidine-1,2-dicarboxylate. The crude (±)-propyl azetidine-2-carboxylate hydrochloride (89.9 mg, 0.5 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (2.5 mL) and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.35 mL, 2.5 mmol, 5 equiv.). Once addition was completed, benzyl chloroformate (0.14 mL, 1 mmol, 2 equiv.) was added dropwise. The reaction was allowed to warm to RT, and stirring was continued until the reaction was judged complete by TLC analysis. Once complete, the reaction was transferred to a separatory funnel and quenched with 5 mL of saturated sodium bicarbonate solution. The layers were thoroughly mixed, and the aqueous layer was removed. The organic layer was washed with water (1x 5 mL) and brine (1x 5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted, and the volatiles were removed under vacuum. The crude product was purified by silica gel column chromatography (gradient from hexanes to 33% EtOAc in hexanes) to afford 25 mg (18% yield) of the titled compound a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 5.17 – 5.04 (m, 2H), 4.69 (dd, *J* = 9.3, 5.2 Hz, 1H), 4.20 – 4.06 (m, 3H), 3.97 (ddd, *J* = 9.1, 8.1, 5.6 Hz,

1H), 2.57 (dtd, *J* = 11.5, 9.2, 6.3 Hz, 1H), 2.21 (ddt, *J* = 11.3, 8.9, 5.5 Hz, 1H), 1.64 (m, 2H – overlaps with HOD peak), 0.91 (m, 3H).

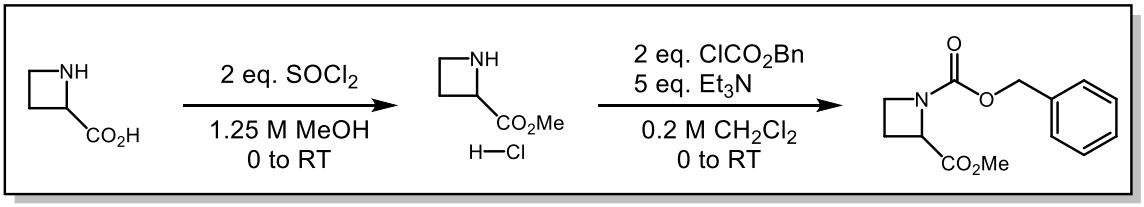


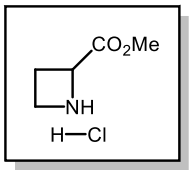


(±)-Isopropyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (50 mg, 1 mmol, 1 equiv.) was suspended in isopropanol (0.6 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.07 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring until judged complete by TLC. Once done, the brown solution was concentrated directly from this dram vial under vacuum to remove volatiles, and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.

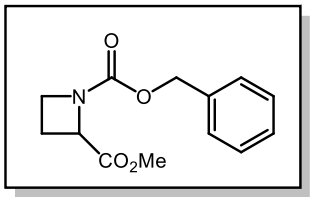


(±)-1-Benzyl 2-isopropyl azetidine-1,2-dicarboxylate. The crude (±)-isopropyl azetidine-2-carboxylate hydrochloride (89.9 mg, 0.5 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (2.5 mL) and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.35 mL, 2.5 mmol, 5 equiv.). Once addition was completed, benzyl chloroformate (0.14 mL, 1 mmol, 2 equiv.) was added dropwise. The reaction was allowed to warm to RT and stirring was continued until the reaction was judged complete by TLC analysis. Once complete, the reaction was transferred to a separatory funnel and quenched with 5 mL of saturated sodium bicarbonate solution. The layers were thoroughly mixed, and the aqueous layer was removed. The organic layer was washed with water (1x 5 mL) and brine (1x 5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted, and the volatiles removed under vacuum. The crude product was purified by silica gel column chromatography (gradient from hexanes to 33% EtOAc in hexanes) to afford 65 mg (47% yield) of the titled compound a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 5.18 – 4.98 (m, 3H), 4.64 (dd, *J* = 9.3, 5.2 Hz, 1H), 4.11 (m, 1H), 3.97 (m, 1H), 2.56 (dtd, *J* = 11.5, 9.2, 6.3 Hz, 1H), 2.18 (ddt, *J* = 11.2, 9.0, 5.5 Hz, 1H), 1.22 (m, 6H).





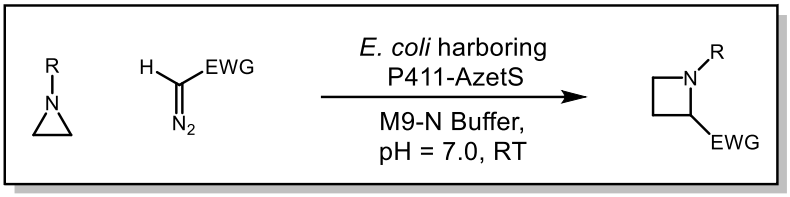
(±)-Methyl azetidine-2-carboxylate hydrochloride. To an oven-dried 8-mL dram vial equipped with a stir bar, septum, and drying tube: (±)-azetidine-2-carboxylic acid (100 mg, 1 mmol, 1 equiv.) was suspended in methanol (1.25 mL). The reaction was chilled in an ice bath, and thionyl chloride (0.15 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to ambiently warm to RT with stirring until judged complete by TLC. Once done, the solution was concentrated directly from this dram vial under vacuum to remove volatiles and the crude product was carried forward with no further purification. For the purposes of the subsequent reaction, the yield was presumed to be quantitative.



(±)-1-Benzyl 2-methyl azetidine-1,2-dicarboxylate. The crude methyl azetidine-2-carboxylate hydrochloride (151.59 mg, 1 mmol, 1 equiv.) synthesized above was suspended in methylene chloride (5 mL), and the dram vial was equipped with a stir bar, a septum, and a drying tube. The vial was chilled in an ice bath prior to the addition of triethylamine (0.69 mL, 5 mmol, 5 equiv.). Once addition was completed, benzyl chloroformate (0.29 mL, 2 mmol, 2 equiv.) was added dropwise. The reaction was allowed to warm to RT, and stirring was continued until the reaction was judged complete by TLC analysis. Once complete, the reaction was transferred to a separatory funnel and quenched with 5 mL of saturated sodium bicarbonate solution. The layers were thoroughly mixed, and the aqueous layer was removed. The organic layer was washed with water (1x 5 mL) and brine (1x 5 mL) prior to drying over sodium sulfate. Once dry, the organics were decanted, and the volatiles removed under vacuum. The crude product was purified by silica gel column chromatography (gradient from hexanes to 40% EtOAc in hexanes) to afford 123 mg (49% yield) of the titled compound a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.28 (m, 5H), 5.25 – 5.02 (m, 2H), 4.71 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.11 m, 1H), 3.98 (m, 1H), 3.76 (br s, 3H), 2.57 (dtd, *J* = 11.6, 9.2, 6.3 Hz, 1H), 2.23 (ddt, *J* = 11.4, 9.0, 5.5 Hz, 1H).

Enzymatic Reactions & Product Characterization

General Procedure for Preparative Scale Aziridine Ring Expansion:



To a 250-mL screw-cap Erlenmeyer flask: 47.5 mL of *E. coli* whole-cell suspension harboring P411-AzetS ([P411-AzetS] = 5.25 μM, final reaction concentration 5.0 μM, 0.25 μmol, $5 \cdot 10^{-4}$ equiv.) were added. The whole-cell suspension was degassed with nitrogen and put into an anerobic Coy chamber. Under inert atmosphere, 1.25 mL of an acetonitrile solution of the corresponding aziridine ([Aziridine] = 400 mM, final reaction concentration 10 mM, 0.5 mmol, 1 equiv.) and 1.25 mL of an acetonitrile solution of the corresponding diazo compound ([Diazo] = 600 mM, final reaction concentration 15 mM, 0.75 mmol, 1 equiv.) were added in sequence. The vial was securely capped to exclude oxygen, the flask was removed from the anaerobic chamber, and the reaction was allowed to shake at RT until judged complete (ca. 4–16 hours).

TTN and Analytical Yield Determination

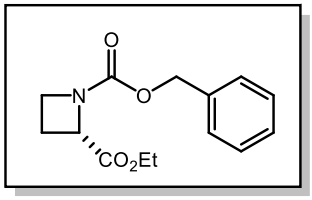
A 400 μL aliquot of the above reaction solution was mixed with 600 μL of a 1:1 solution of ethyl acetate:cyclohexane with 10 mM of the appropriate internal standard in a 1.7-mL Eppendorf tube. The layers were thoroughly mixed, and the phases were separated by centrifugation (14,000 *g*, 10 minutes, RT). Once separated, 100 μL of the organic layer was removed and diluted in 900 μL of ethyl acetate. This solution was subjected to GC-FID analysis to determine the analytical yield and TTN of the reaction.

Isolated Yield:

Once complete, the reaction was split into two 25-mL aliquots across two 50-mL falcon tubes. The suspensions were extracted three times with ethyl acetate as follows: 25 mL of ethyl acetate were added to each tube, and the phases were mixed by hand-shaking the tubes. Upon thorough mixing, the phases were separated by centrifugation (5,000 *g*, 5 minutes, RT) and the organic phase was removed. The combined organics were dried over sodium sulfate; once dry, the drying agent was decanted and the volatiles removed under vacuum. The crude residue was purified by silica gel column chromatography to yield the titled compounds.

Note About Stereochemical Assignments:

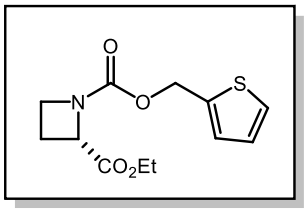
The absolute stereochemistry for the model substrate **2** was determined by synthesis of the *S*-enantiomer, which corresponds to the major product produced in the reaction. The stereochemistry of products **3–9** was assigned by analogy.



1-Benzyl 2-ethyl (*S*)-azetidine-1,2-dicarboxylate (2). Synthesized using the general procedure for preparative scale reactions starting with benzyl aziridine-1-carboxylate and ethyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 40% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	1560	1430	1490
%Yield (GC)	78%	72%	75%
Yield (Isolated)	91 mg (69%)	86 mg (65%)	67%
er (GC)	99:1	99:1	--/--

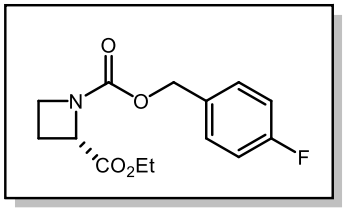
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 5.10 (m, 2H), 4.68 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.19 (br s, 2H), 4.12 (m, 1H), 3.97 (m, 1H), 2.57 (dtd, *J* = 11.5, 9.2, 6.3 Hz, 1H), 2.22 (ddt, *J* = 11.3, 9.0, 5.5 Hz, 1H), 1.30 – 1.17 (br m, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 171.12, 155.71, 136.41, 128.41, 128.00, 127.88, 66.78, 61.28, 60.57, 47.68, 20.70, 14.08. HRMS (FAB+) exact mass calculated for $[M+H]^+$ ($C_{14}H_{18}O_4N$) requires *m/z* 264.1236, found *m/z* 264.1249.



2-Ethyl 1-(thiophen-2-ylmethyl) (*S*)-azetidine-1,2-dicarboxylate (3). Synthesized using the general procedure for preparative scale reactions starting with thiophen-2-ylmethyl aziridine-1-carboxylate and ethyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	760	767	764
%Yield (GC)	38.0%	38.3%	38%
Yield (Isolated)	49.4 mg (37% yield)	51.4 mg (38% yield)	38%
er (GC)	99:1	99:1	--/--

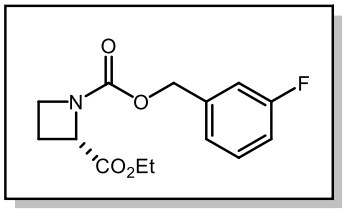
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.07 (d, *J* = 3.5 Hz, 1H), 6.96 (dd, *J* = 5.1, 3.5 Hz, 1H), 5.24 (m, 2H), 4.66 (dd, *J* = 9.4, 5.3 Hz, 1H), 4.20 (br s, 2H), 4.10 (m, 1H), 3.95 (m, 1H), 2.56 (dtd, *J* = 11.5, 9.2, 6.3 Hz, 1H), 2.20 (ddt, *J* = 11.3, 9.0, 5.5 Hz, 1H), 1.24 (br s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.02, 155.41, 138.48, 127.82, 126.68, 126.61, 61.29, 61.19, 60.57, 47.71, 20.70, 14.07. HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{12}H_{16}NO_4S$) requires *m/z* 270.0800, found *m/z* 270.0792.



2-Ethyl 1-(4-fluorobenzyl) (*S*)-azetidine-1,2-dicarboxylate (4). Synthesized using the general procedure for preparative scale reactions starting with 4-fluorobenzyl aziridine-1-carboxylate and ethyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 25% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	613	640	626
%Yield (GC)	30.6%	32.0%	31%
Yield (Isolated)	38 mg (27% yield)	38 mg (27% yield)	29%
er (GC)	99:1	99:1	--/--

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.02 (m, 2H), 5.06 (m, 2H), 4.67 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.19 (br s, 2H), 4.10 (m, 1H), 3.96 (m, 1H), 2.57 (dtd, *J* = 11.5, 9.2, 6.4 Hz, 1H), 2.21 (ddt, *J* = 11.3, 9.0, 5.5 Hz, 1H), 1.30 – 1.18 (br s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.05, 162.52 (d, *J* = 246.4 Hz), 155.58, 132.26 (d, *J* = 3.3 Hz), 129.90 (d, *J* = 8.4 Hz), 115.31 (d, *J* = 21.4 Hz), 66.07, 61.28, 60.37, 47.70, 20.69, 14.07. ^{19}F NMR (282 MHz, $CDCl_3$) δ -114.28 (br s). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{14}H_{17}FNO_4$) requires *m/z* 282.1142, found *m/z* 282.1148.

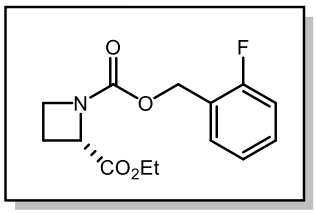


2-Ethyl 1-(3-fluorobenzyl) (*S*)-azetidine-1,2-dicarboxylate (5). Synthesized using the general procedure for preparative scale reactions using 3-fluorobenzyl aziridine-1-carboxylate and ethyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	650	712	681
%Yield (GC)	33%	36%	34%
Yield (Isolated)	41.7 mg (30% yield)	39.7 mg (29% yield)	29%
er (GC)	99:1	99:1	--/--

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (td, *J* = 7.9, 5.8 Hz, 1H), 7.13 – 6.94 (m, 3H), 5.22 – 4.98 (m, 2H), 4.70 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.27 – 4.17 (m, 2H), 4.16 – 4.08 (m, 1H), 3.99 (ddd, *J* = 9.1, 8.1, 5.6 Hz, 1H), 2.59 (dtd, *J* = 11.6, 9.2, 6.3 Hz, 1H), 2.23 (ddt, *J* = 11.4, 9.1, 5.5 Hz, 1H), 1.30 – 1.18 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.03, 162.79 (d, *J* = 246.1 Hz), 155.39, 138.96 (d, *J* = 7.4 Hz), 129.95 (d, *J* = 8.2 Hz), 123.09, 114.82 (d, *J* = 21.1 Hz), 114.48 (d, *J* = 22.0 Hz), 65.84, 61.35, 60.63, 47.59, 20.72, 14.06. ^{19}F NMR (282 MHz, $CDCl_3$) δ -113.22 (td, *J*

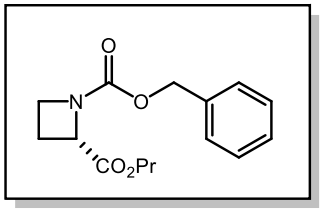
= 10.1, 6.5 Hz). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{14}H_{17}FNO_4$) requires *m/z* 282.1142, found *m/z* 282.1154.



2-Ethyl 1-(2-fluorobenzyl) (*S*)-azetidine-1,2-dicarboxylate (6). Synthesized using the general procedure for preparative scale reactions using 2-fluorobenzyl aziridine-1-carboxylate and ethyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	826	796	811
%Yield (GC)	41.3%	39.8%	41%
Yield (Isolated)	50.5 mg (36% yield)	50.2 mg (36% yield)	36%
er (GC)	99:1	99:1	--/--

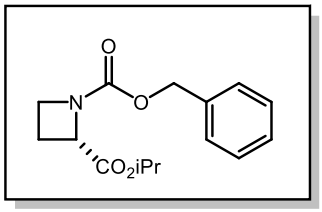
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 1H), 7.29 (m, 1H), 7.12 (m, 1H), 7.04 (m, 1H), 5.17 (q, *J* = 12.7 Hz, 2H), 4.68 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.19 (m, 2H), 4.11 (td, *J* = 8.6, 6.3 Hz, 1H), 3.97 (td, *J* = 8.6, 8.2, 5.7 Hz, 1H), 2.57 (dtd, *J* = 11.6, 9.2, 6.3 Hz, 1H), 2.21 (ddt, *J* = 11.3, 9.0, 5.5 Hz, 1H), 1.24 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.05, 160.81 (d, *J* = 248.3 Hz), 155.52, 130.29, 129.90 (d, *J* = 8.1 Hz), 124.02 (d, *J* = 3.7 Hz), 123.56 (d, *J* = 14.4 Hz), 115.28 (d, *J* = 21.2 Hz), 61.28, 60.76 (d, *J* = 4.4 Hz), 60.64 (br, obscured by doublet at 60.76), 47.70, 20.70, 14.05. ^{19}F NMR (282 MHz, $CDCl_3$) δ -118.40 (m). HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{14}H_{17}FNO_4$) requires *m/z* 282.1142, found *m/z* 282.1114.



1-Benzyl 2-propyl (*S*)-azetidine-1,2-dicarboxylate (7). Synthesized using the general procedure for preparative scale reactions using benzyl aziridine-1-carboxylate and propyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	314	411	363
%Yield (GC)	16%	21%	18%
Yield (Isolated)	19.6 mg (14% yield)	27.1 mg (20% yield)	17%
er (GC)	99:1	99:1	--/--

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 5.17 – 5.04 (m, 2H), 4.69 (dd, *J* = 9.3, 5.2 Hz, 1H), 4.20 – 4.06 (m, 3H), 3.97 (ddd, *J* = 9.1, 8.1, 5.6 Hz, 1H), 2.57 (dtd, *J* = 11.5, 9.2, 6.3 Hz, 1H), 2.21 (ddt, *J* = 11.3, 8.9, 5.5 Hz, 1H), 1.64 (m, 2H – overlaps with HOD peak), 0.91 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) 171.20, 155.70, 136.40, 128.40, 127.99, 127.89, 66.79, 66.78, 60.69, 47.62, 21.86, 20.74, 10.22. HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{15}H_{20}O_4N$) requires *m/z* 278.1392, found *m/z* 278.1400.

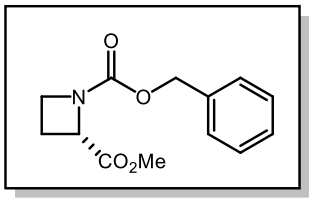


1-Benzyl 2-isopropyl (*S*)-azetidine-1,2-dicarboxylate (8).

Synthesized using the general procedure for preparative scale reactions using benzyl aziridine-1-carboxylate and propyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 20% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	511	617	564
%Yield (GC)	26%	31%	28%
Yield (Isolated)	34.8 mg (25% yield)	41.5 mg (30% yield)	28%
er (GC)	>99:1	>99:1	--/--

^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 5.18 – 4.98 (m, 3H), 4.64 (dd, *J* = 9.3, 5.2 Hz, 1H), 4.11 (m, 1H), 3.97 (m, 1H), 2.56 (dtd, *J* = 11.5, 9.2, 6.3 Hz, 1H), 2.18 (ddt, *J* = 11.2, 9.0, 5.5 Hz, 1H), 1.22 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.63, 155.70, 136.41, 128.40, 127.97, 127.86, 68.84, 66.75, 60.85, 47.62, 21.65, 21.60, 20.68. HRMS (ES+) exact mass calculated for $[M+H]^+$ ($C_{15}H_{20}O_4N$) requires *m/z* 278.1392, found *m/z* 278.1367.



1-Benzyl 2-methyl (*S*)-azetidine-1,2-dicarboxylate (9). Synthesized using the general procedure for preparative scale reactions starting with benzyl aziridine-1-carboxylate and methyl diazoacetate as substrates. The product was purified by silica gel column chromatography (gradient from 100% hexanes to 40% EtOAc in hexanes) to afford the titled compound as a colorless oil.

Value	Run 1	Run 2	Average
TTN (GC)	405	399	402
%Yield (GC)	20.2%	20.0%	20%
Yield (Isolated)	24 mg (19% yield)	21 mg (17% yield)	18%
er (GC)	81:19	81:19	

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.29 (m, 5H), 5.26 – 5.01 (m, 2H), 4.71 (dd, *J* = 9.1, 5.2 Hz, 1H), 4.11 (m, 1H), 3.98 (m, 1H), 3.73 (br s, 3H), 2.57 (dtd, *J* = 11.3, 9.2, 6.4 Hz, 1H), 2.23 (ddt, *J* = 11.4, 8.9, 5.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.58, 155.73, 136.40, 128.43, 128.03, 127.91, 66.82, 60.51, 52.27, 47.75, 20.69. HRMS (FAB+) exact mass calculated for $[M+H]^+$ ($C_{13}H_{16}O_4N$) requires *m/z* 250.1079, found *m/z* 250.1067.

Procedure for 10 mmol Scale Reaction

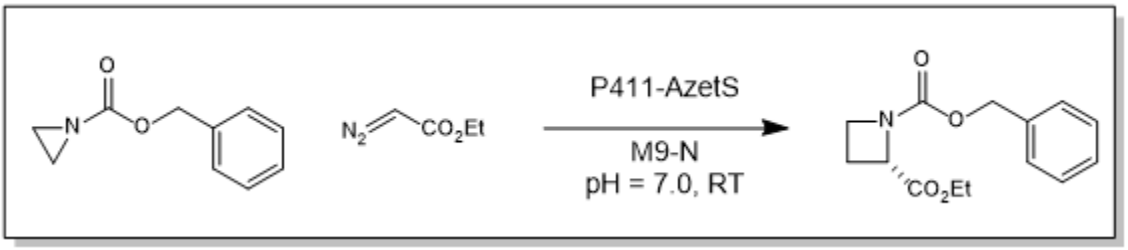
Starter Culture & Expression Culture:
A single colony of *E. coli* harboring plasmids encoding P411-AzetS was used to inoculate 200 mL of LB-amp. The culture was allowed to incubate overnight at 37 °C, with shaking at 240 RPM.

The following day: six expression cultures (1 L) were inoculated with 20 mL of the above overnight culture. The expression cultures were incubated at 37 °C, with shaking at 240 RPM, for 2.5 hours and the OD_{600} was measured to be 1.0. At this point, the six expression cultures were held on ice for 30 minutes wherein 1 mL of 1000x stocks of ALA and IPTG were added such that the final concentrations were 1.0 mM of ALA and 0.5 mM of IPTG, respectively. The cultures were allowed to incubate for 20 hours at 22 °C, with shaking at 140 RPM.

Preparation of Clarified Lysate for Protein Concentration Determination:

The expression cultures were pelleted by centrifugation (5,000 *g*, 5 minutes, 4 °C). The cell pellets were put into the anaerobic Coy chamber, wherein they were resuspended in 300 mL of rigorously degassed M9-N buffer adjusted to pH = 7.0 and combined in a screw-top Erlenmeyer. For protein concentration determination, 3 mL of the whole-cell suspension were diluted in 3 mL of additional M9-N, and the protein concentration was measured as described in the "General Information and Procedures" section of the SI. The Erlenmeyer with the whole-cell suspension was capped with an air-tight cap and was held on ice until the protein concentration can be accurately determined.

Reaction Setup:



Safety Note: 15 mmol of ethyl diazoacetate can be expected to liberate 15 mmol of N_2, or 336 mL of N_2. Use of a flask with excess headspace prevents overpressurization of the reaction vessel in this reaction.

Once the protein concentration was determined, the whole-cell suspension was diluted in a 2.0-L screw-top Erlenmeyer flask in the anaerobic Coy chamber with rigorously degassed M9-N buffer adjusted to pH = 7.0 such that 950 mL of whole-cell suspension had a protein concentration of 5.25 μM (final reaction concentration 5.0 μM, 5 μmol, $5 \cdot 10^{-4}$ equiv.). For this experiment, the OD_{600} was measured to be approximately 30. Once ready, 24 mL of a 417 mM solution of benzyl aziridine-1-carboxylate in MeCN (final reaction concentration, 10 mM, 10 mmol, 1.77 g, 1 equiv.)

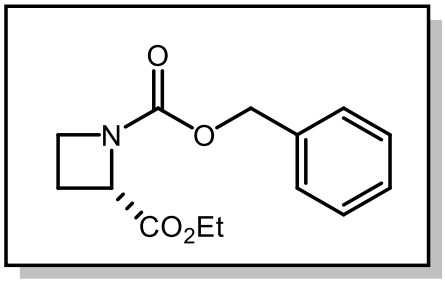
was added with agitation, followed by addition of 24 mL of a 625 mM solution of ethyl diazoacetate (final reaction concentration 15 mM, 1.71 g, 1.5 equiv.) with heavy agitation. The flask was tightly capped to exclude oxygen, pumped out of the anaerobic chamber, and was allowed to shake at 220 RPM until judged complete.

Workup and Isolation:

Once complete, three 400-μL aliquots were removed and used to determine the reaction TTN and yield by gas chromatography (1220 TTN, 61% yield). For preparative isolation, the reaction mixture was split into 2x 500-mL portions. Each portion was mixed with 500 mL of EtOAc; the layers were thoroughly mixed before the organic layer was removed (the layers may be separated by centrifugation, if necessary, at 5,000 *g* for 5 minutes at RT). The combined organics were dried over sodium sulfate; once dried, the supernatant was decanted from the drying agent and the volatiles removed under vacuum. The crude product was purified by silica gel column chromatography (gradient from 100% hexanes to 30% EtOAc in hexanes) to afford 1.44 g (55% isolated yield) of the titled compound as a colorless oil with 99:1 er. Characterization data was completely identical to other samples of **2** synthesized in this manuscript.

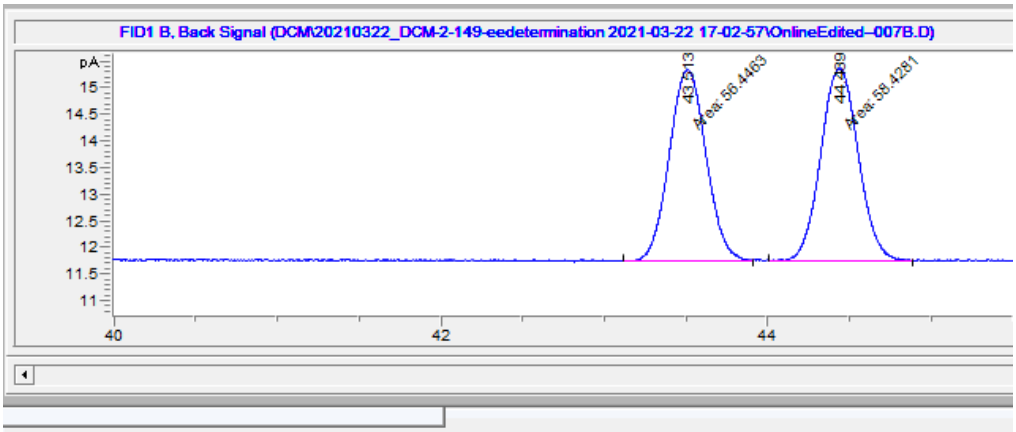
Chiral Traces for Determination of Enantiopurity: Preparative-Scale Reactions

The enantiopurity of all products was determined by gas chromatography using CP-Chirasil-Dex CB (Agilent) as a chiral stationary phase. All products could be separated using the same GC conditions, which were as follows: start at 50 °C, ramp at 10 °C/minute to 170 °C and hold for 38 minutes. Ramp at 30 °C/minute to 200 °C and hold for 9 minutes.



1-Benzyl 2-ethyl (*S*)-azetidine-1,2-dicarboxylate (2)

Racemic Standard:

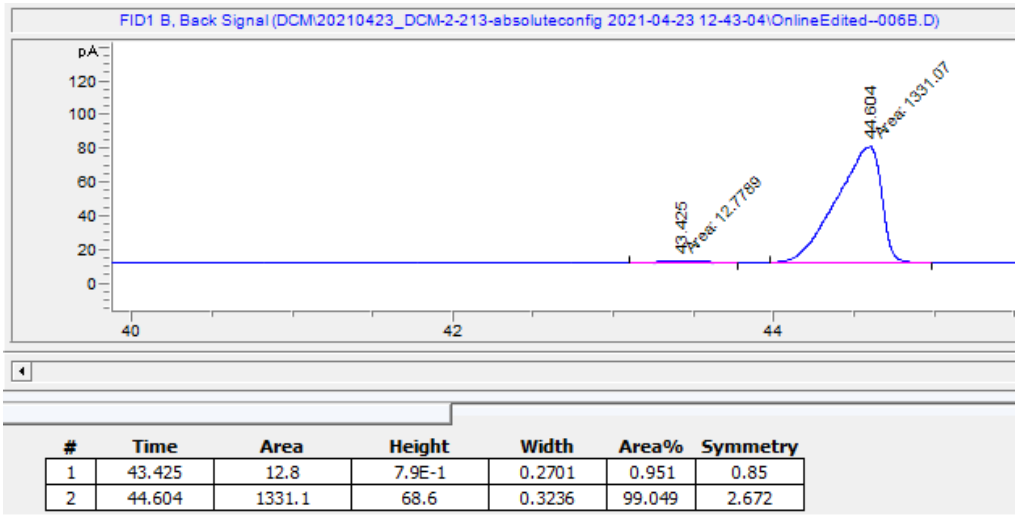


#	Time	Area	Height	Width	Area%	Symmetry
1	43.513	56.4	3.6	0.2618	49.137	0.954
2	44.439	58.4	3.6	0.2681	50.863	0.918

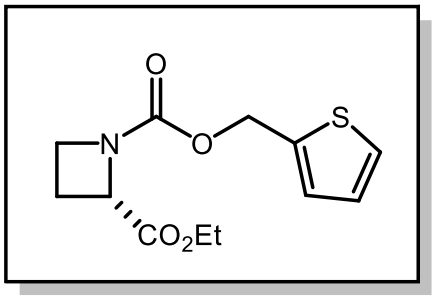
(*S*)-Enantiomer Standard:

#	Time	Area	Height	Width	Area%	Symmetry
1	44.721	1859.2	87.4	0.2682	100.000	4.229

Product from P411-AzetS:

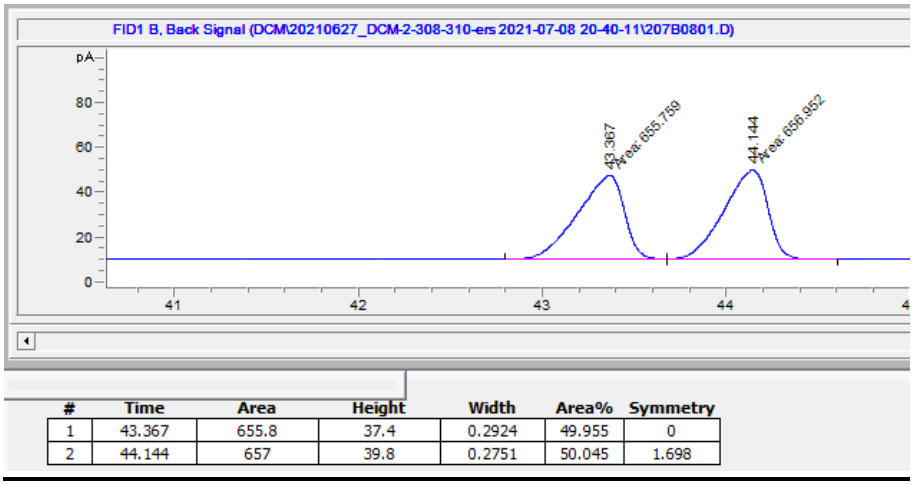


#	Time	Area	Height	Width	Area%	Symmetry
1	43.425	12.8	7.9E-1	0.2701	0.951	0.85
2	44.604	1331.1	68.6	0.3236	99.049	2.672



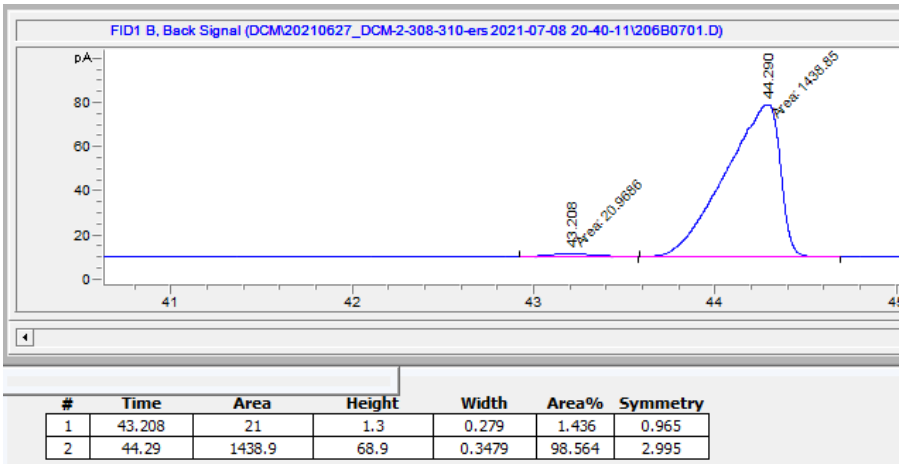
2-Ethyl 1-(thiophen-2-ylmethyl) (*S*)-azetidine-1,2-dicarboxylate (3)

Racemic Standard:

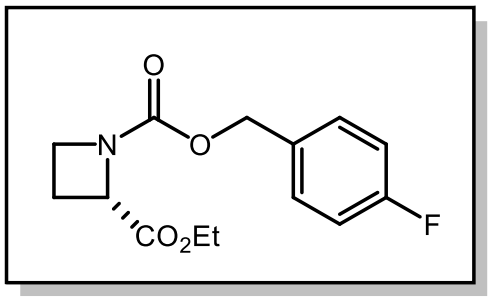


#	Time	Area	Height	Width	Area%	Symmetry
1	43.367	655.8	37.4	0.2924	49.955	0
2	44.144	657	39.8	0.2751	50.045	1.698

Product from P411-AzetS:

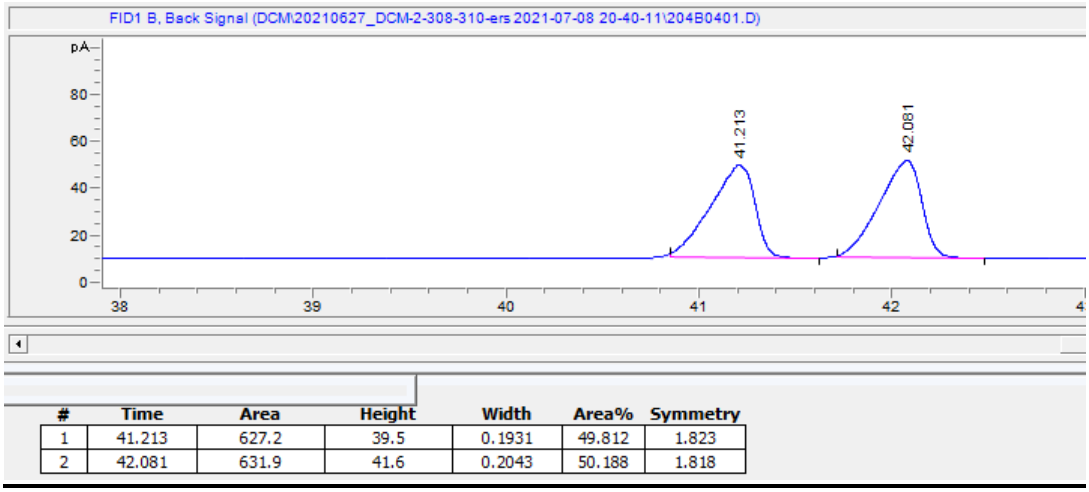


#	Time	Area	Height	Width	Area%	Symmetry
1	43.208	21	1.3	0.279	1.436	0.965
2	44.29	1438.9	68.9	0.3479	98.564	2.995



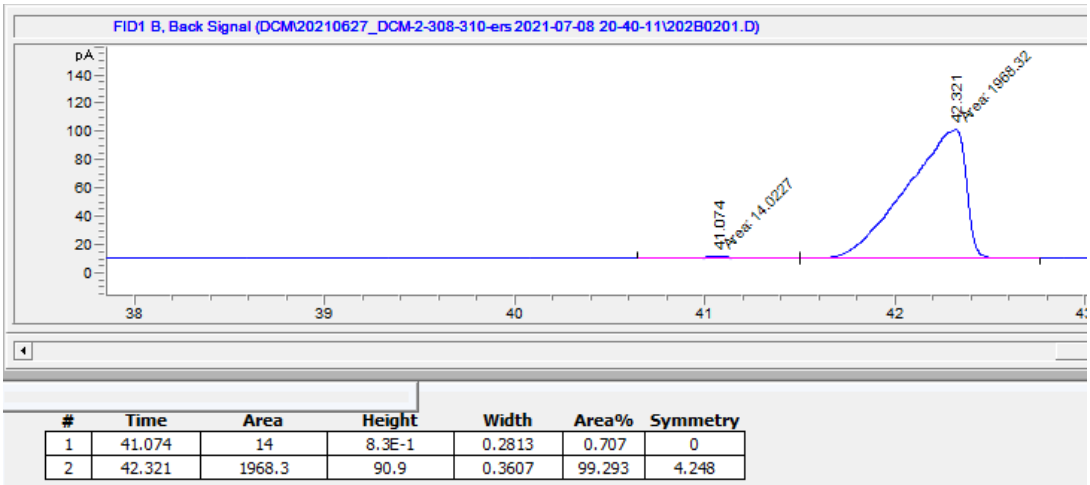
2-Ethyl 1-(4-fluorobenzyl) (*S*)-azetidine-1,2-dicarboxylate (4)

Racemic Standard:

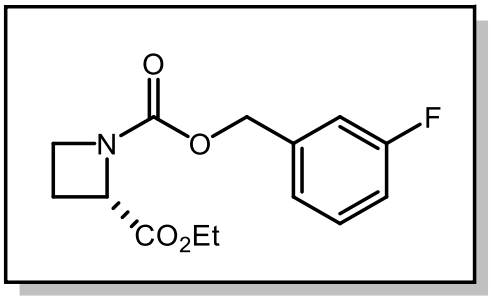


#	Time	Area	Height	Width	Area%	Symmetry
1	41.213	627.2	39.5	0.1931	49.812	1.823
2	42.081	631.9	41.6	0.2043	50.188	1.818

Product from P411-AzetS:

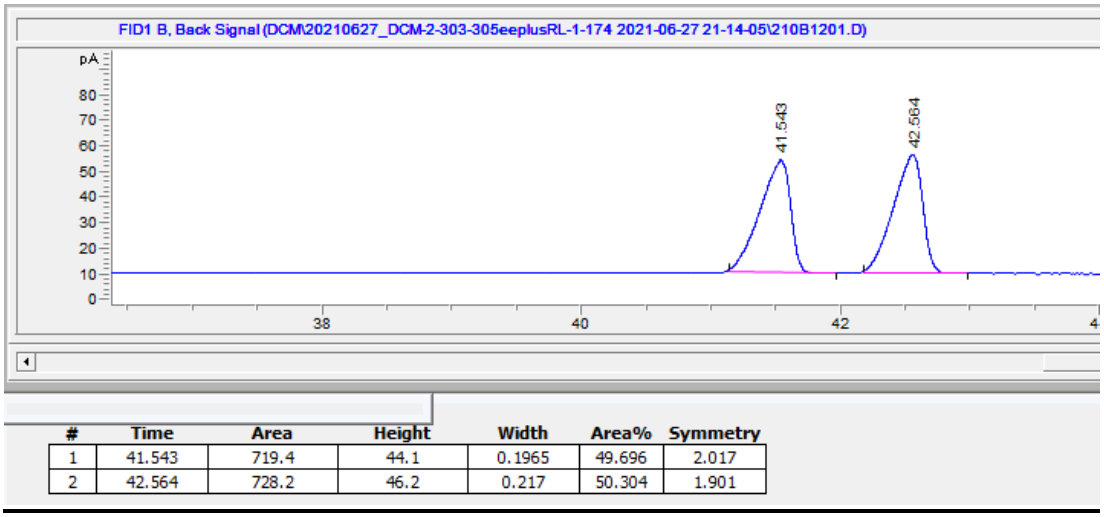


#	Time	Area	Height	Width	Area%	Symmetry
1	41.074	14	8.3E-1	0.2813	0.707	0
2	42.321	1968.3	90.9	0.3607	99.293	4.248



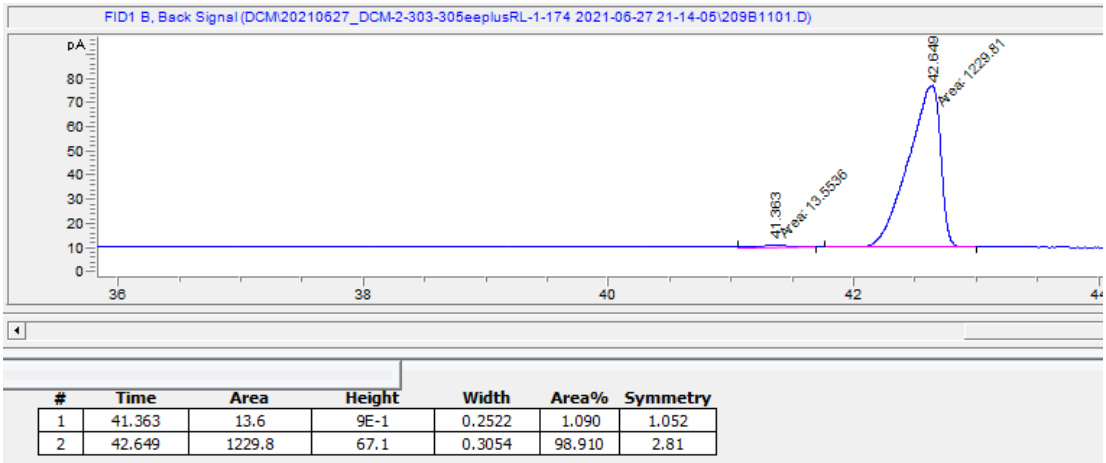
2-Ethyl 1-(3-fluorobenzyl) (*S*)-azetidine-1,2-dicarboxylate (5)

Racemic Standard:

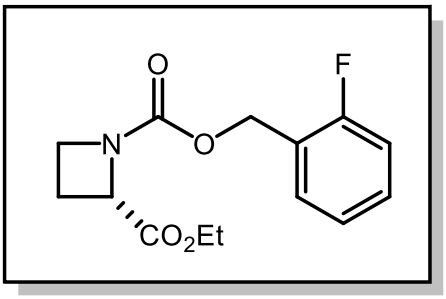


#	Time	Area	Height	Width	Area%	Symmetry
1	41.543	719.4	44.1	0.1965	49.696	2.017
2	42.564	728.2	46.2	0.217	50.304	1.901

Product from P411-AzetS:

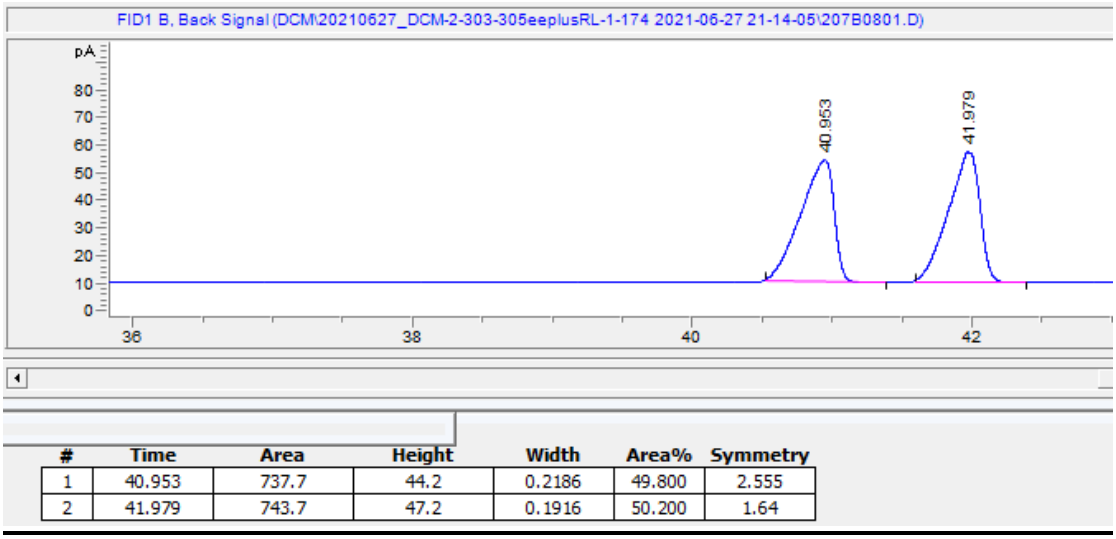


#	Time	Area	Height	Width	Area%	Symmetry
1	41.363	13.6	9E-1	0.2522	1.090	1.052
2	42.649	1229.8	67.1	0.3054	98.910	2.81



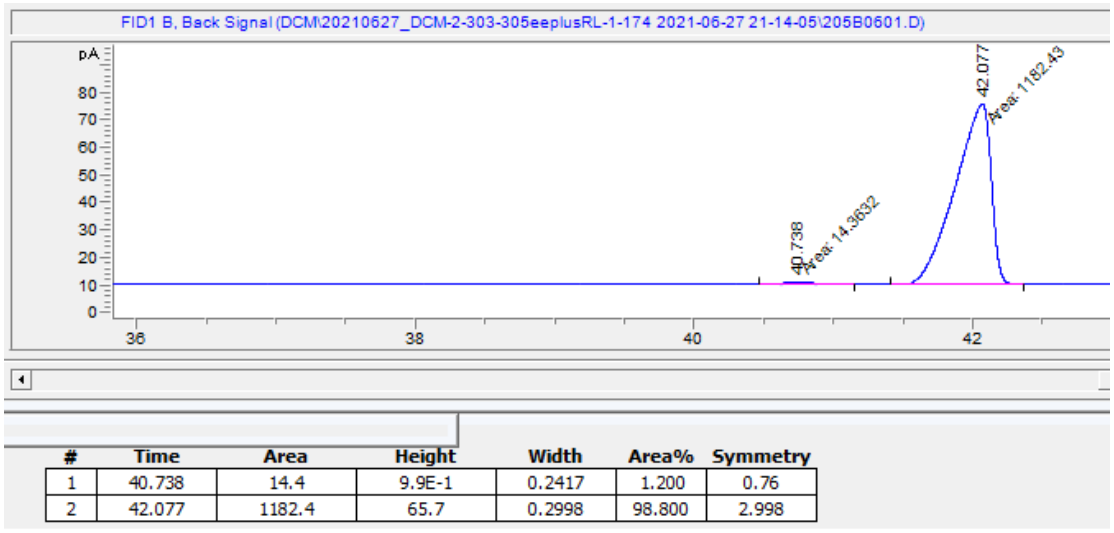
2-Ethyl 1-(2-fluorobenzyl) (*S*)-azetidine-1,2-dicarboxylate (6)

Racemic Standard:

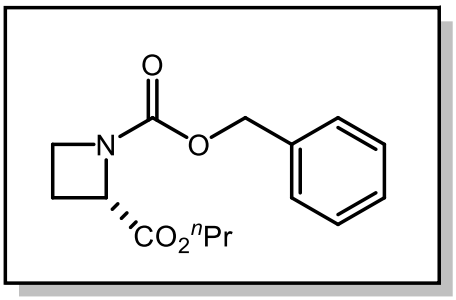


#	Time	Area	Height	Width	Area%	Symmetry
1	40.953	737.7	44.2	0.2186	49.800	2.555
2	41.979	743.7	47.2	0.1916	50.200	1.64

Product from P411-AzetS:

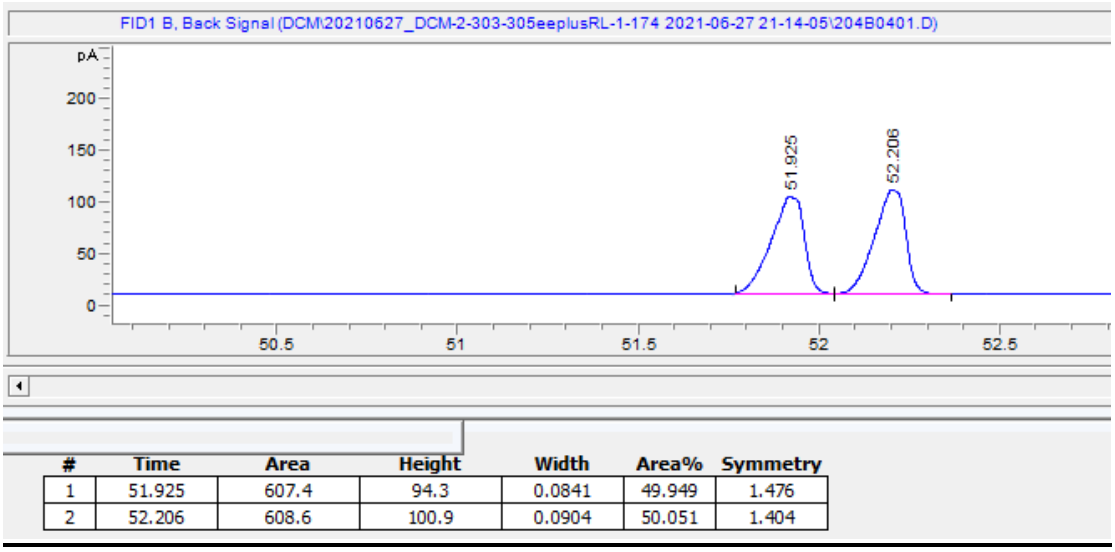


#	Time	Area	Height	Width	Area%	Symmetry
1	40.738	14.4	9.9E-1	0.2417	1.200	0.76
2	42.077	1182.4	65.7	0.2998	98.800	2.998



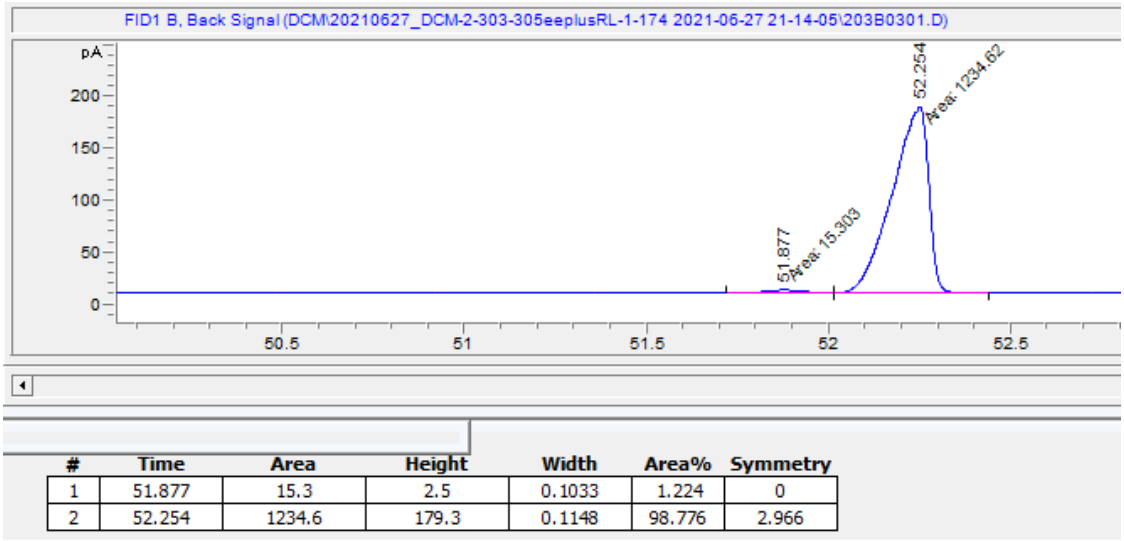
1-Benzyl 2-propyl azetidine-1,2-dicarboxylate (7)

Racemic Standard:

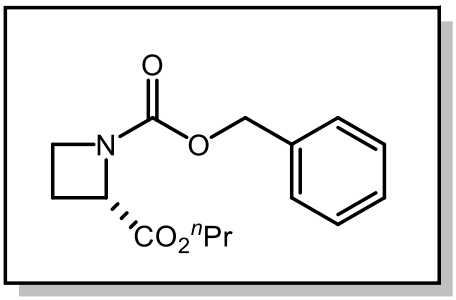


#	Time	Area	Height	Width	Area%	Symmetry
1	51.925	607.4	94.3	0.0841	49.949	1.476
2	52.206	608.6	100.9	0.0904	50.051	1.404

Product from P411-AzetS:

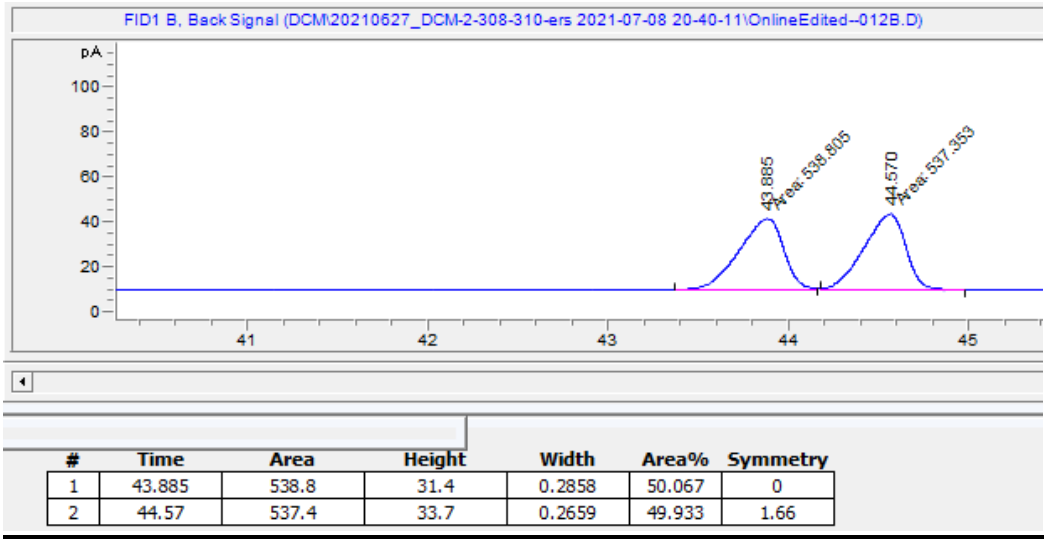


#	Time	Area	Height	Width	Area%	Symmetry
1	51.877	15.3	2.5	0.1033	1.224	0
2	52.254	1234.6	179.3	0.1148	98.776	2.966



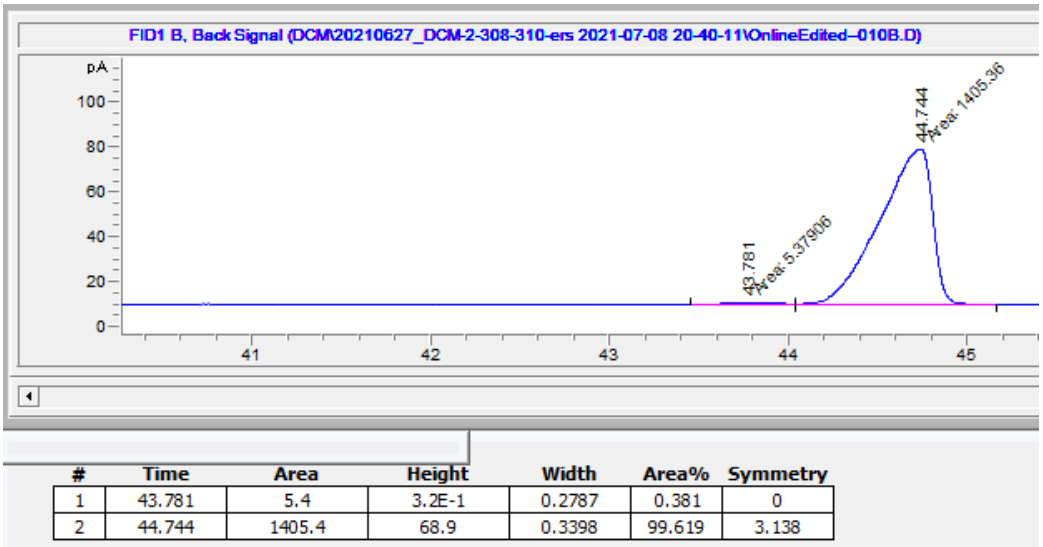
1-Benzyl 2-isopropyl azetidine-1,2-dicarboxylate (8)

Racemic Standard:

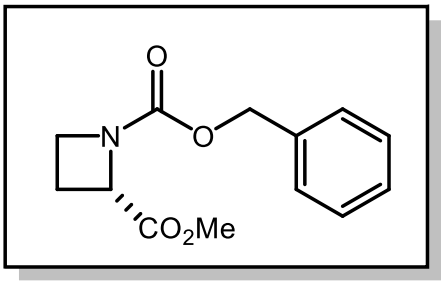


#	Time	Area	Height	Width	Area%	Symmetry
1	43.885	538.8	31.4	0.2858	50.067	0
2	44.57	537.4	33.7	0.2659	49.933	1.66

Product from P411-AzetS:

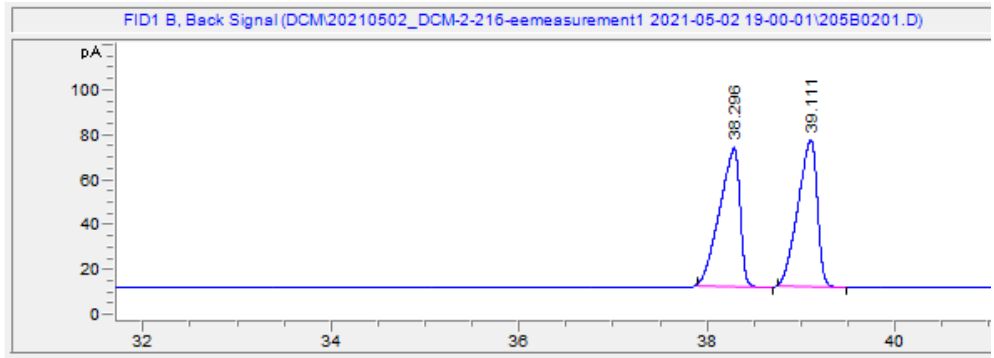


#	Time	Area	Height	Width	Area%	Symmetry
1	43.781	5.4	3.2E-1	0.2787	0.381	0
2	44.744	1405.4	68.9	0.3398	99.619	3.138



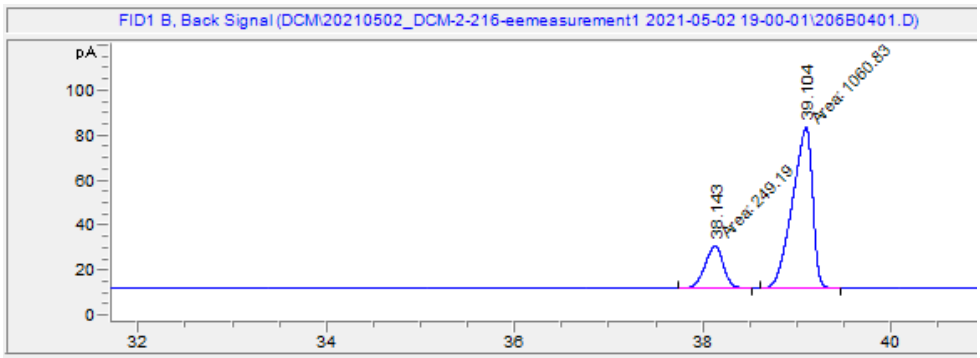
1-Benzyl 2-methyl azetidine-1,2-dicarboxylate (9)

Racemic Standard:



#	Time	Area	Height	Width	Area%	Symmetry
1	38.296	937.9	62	0.2005	50.010	2.45
2	39.111	937.6	65.5	0.177	49.990	1.84

Product from P411-AzetS:

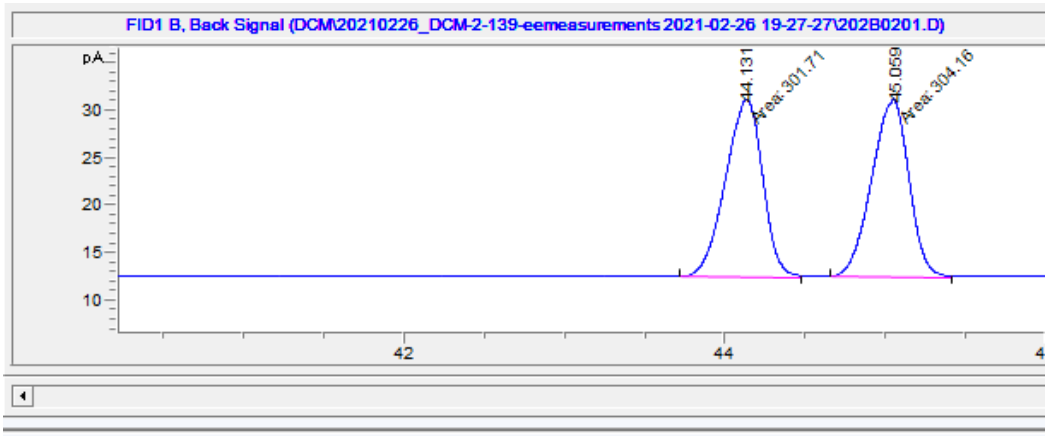


#	Time	Area	Height	Width	Area%	Symmetry
1	38.143	249.2	18.4	0.2263	19.022	1.262
2	39.104	1060.8	71.6	0.2469	80.978	1.946

Chiral Traces for Determination of Enantiopurity: Evolutionary Lineage

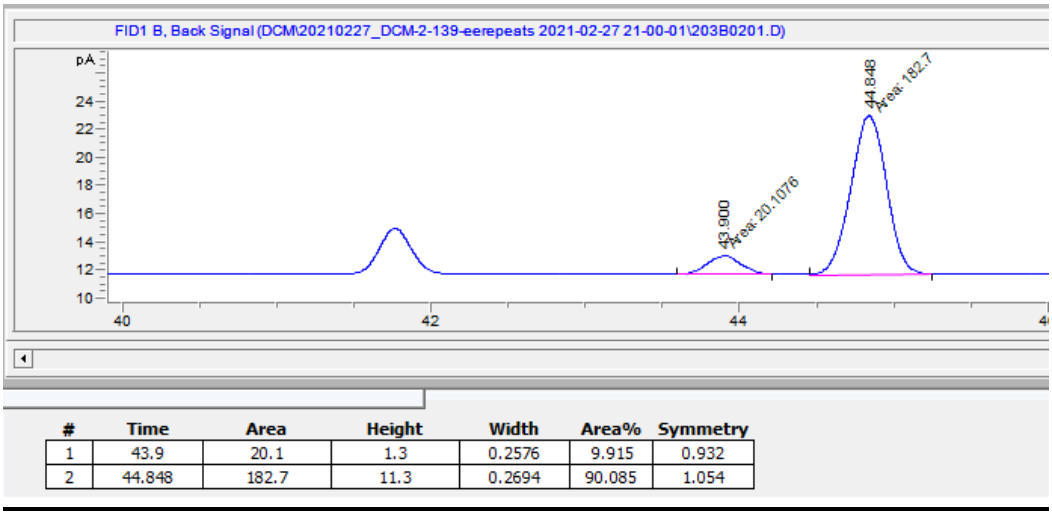
The enantiopurity was determined as outlined in the preceding section.

Racemic Standard:



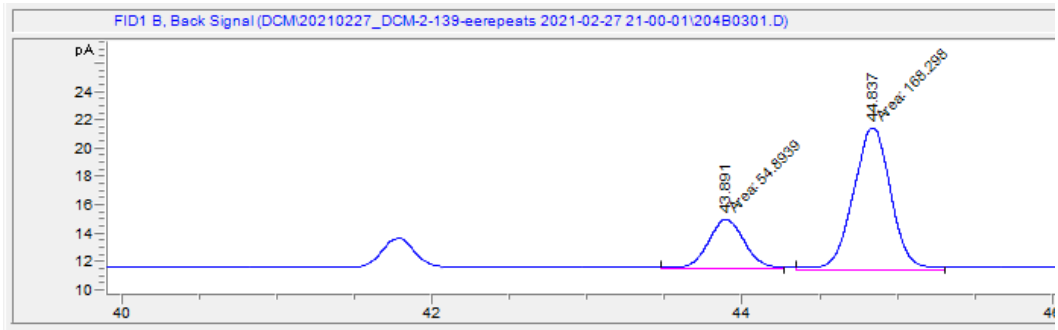
#	Time	Area	Height	Width	Area%	Symmetry
1	44.131	301.7	18.7	0.269	49.798	1.086
2	45.059	304.2	18.8	0.2695	50.202	1.399

Parent F2:



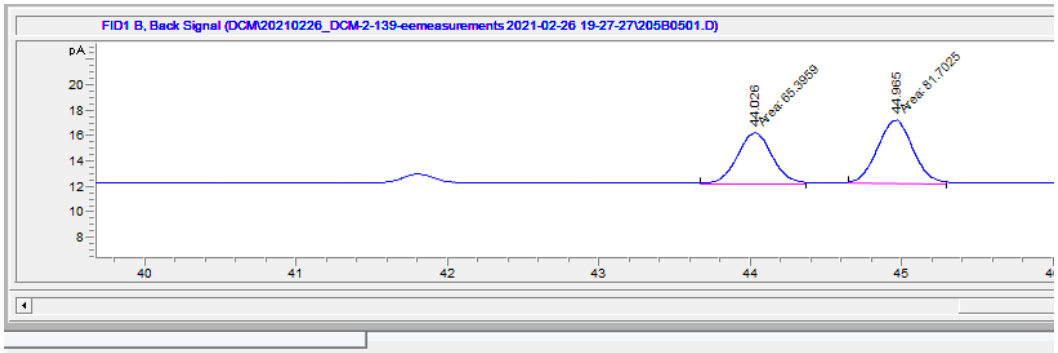
#	Time	Area	Height	Width	Area%	Symmetry
1	43.9	20.1	1.3	0.2576	9.915	0.932
2	44.848	182.7	11.3	0.2694	90.085	1.054

Parent F2.1:



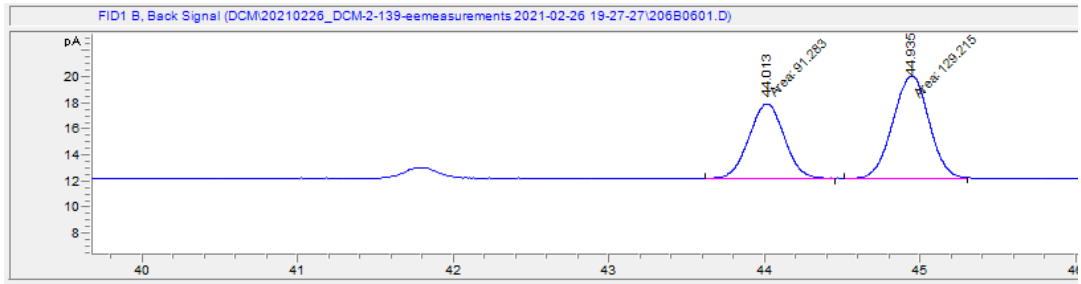
#	Time	Area	Height	Width	Area%	Symmetry
1	43.891	54.9	3.4	0.2693	24.595	0.814
2	44.837	168.3	10	0.2814	75.405	0.908

Parent F2.2:



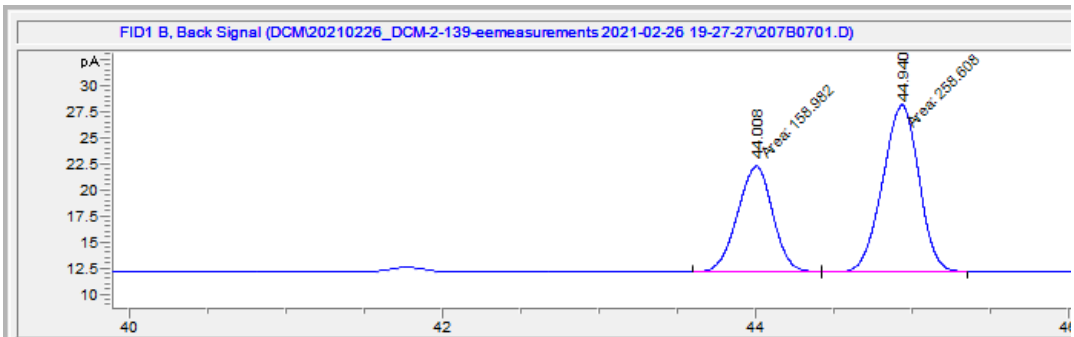
#	Time	Area	Height	Width	Area%	Symmetry
1	44.026	65.4	4	0.2704	44.457	1.02
2	44.965	81.7	5	0.2721	55.543	1.159

Parent F2.3:



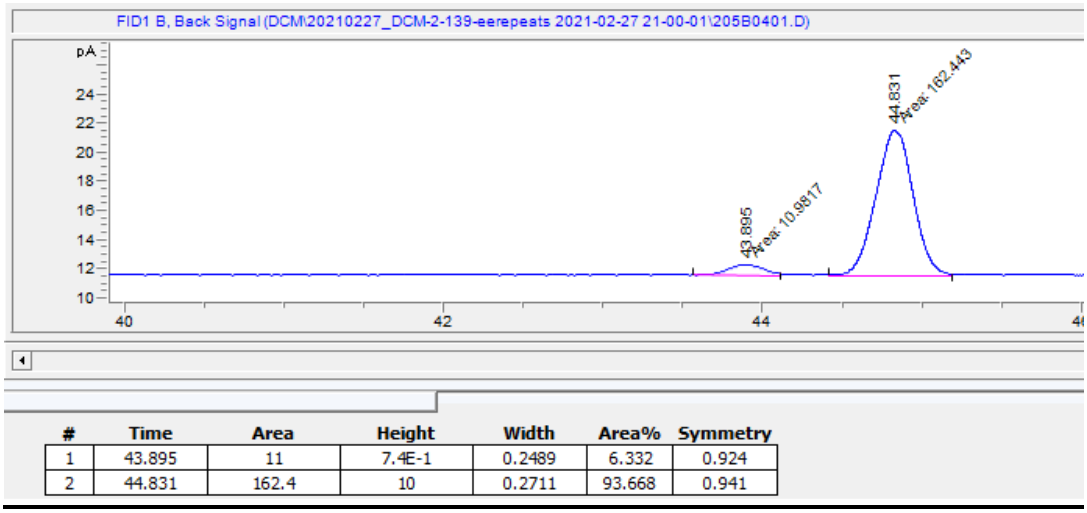
#	Time	Area	Height	Width	Area%	Symmetry
1	44.013	91.3	5.8	0.264	41.399	1.027
2	44.935	129.2	7.9	0.2724	58.601	0.983

Parent F2.4:



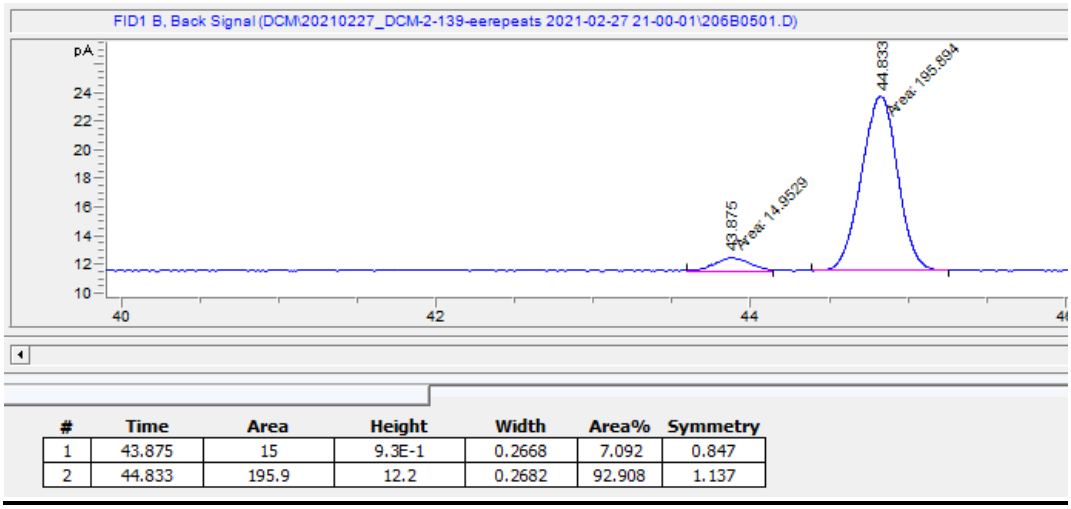
#	Time	Area	Height	Width	Area%	Symmetry
1	44.008	159	10.1	0.2617	38.071	1.079
2	44.94	258.6	16.1	0.2675	61.929	1.107

Parent F2.5:



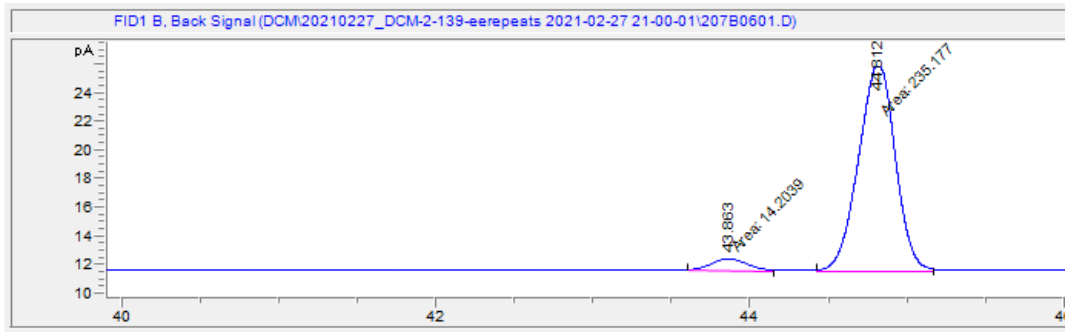
#	Time	Area	Height	Width	Area%	Symmetry
1	43.895	11	7.4E-1	0.2489	6.332	0.924
2	44.831	162.4	10	0.2711	93.668	0.941

Parent F2.6:



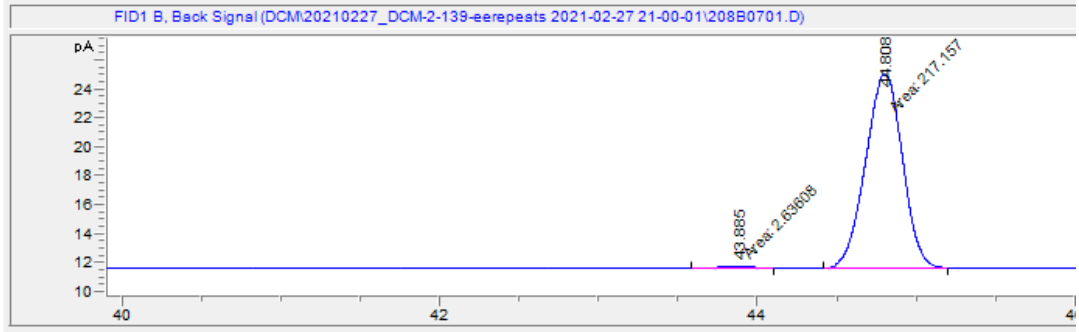
#	Time	Area	Height	Width	Area%	Symmetry
1	43.875	15	9.3E-1	0.2668	7.092	0.847
2	44.833	195.9	12.2	0.2682	92.908	1.137

Parent F2.7:



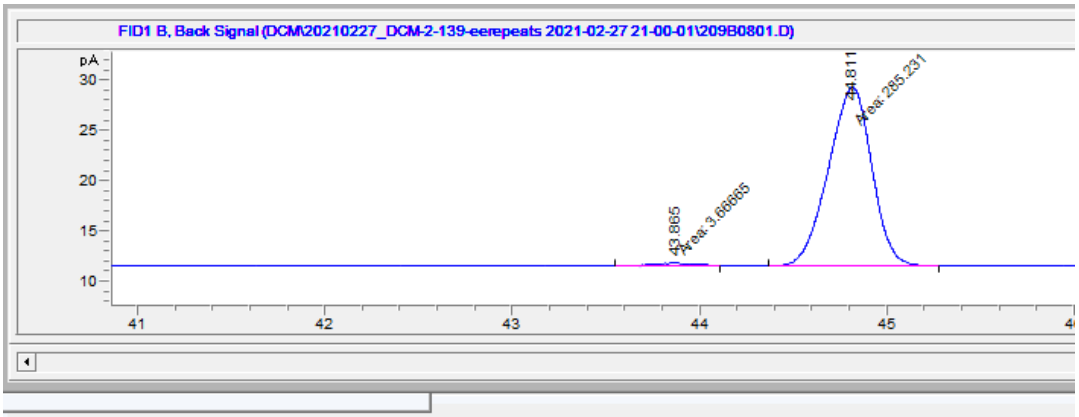
#	Time	Area	Height	Width	Area%	Symmetry
1	43.863	14.2	8.5E-1	0.2792	5.696	0.873
2	44.812	235.2	14.3	0.2739	94.304	0.956

Parent F2.8:



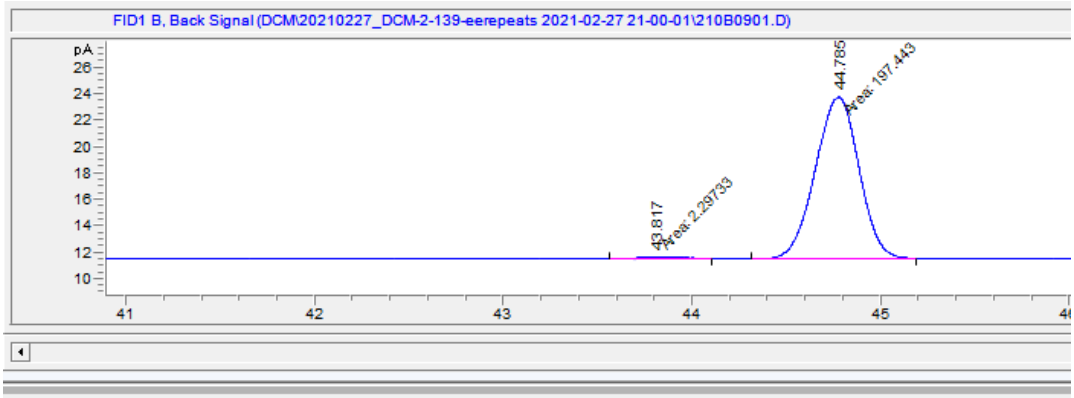
#	Time	Area	Height	Width	Area%	Symmetry
1	43.885	2.6	1.8E-1	0.2382	1.199	1.179
2	44.808	217.2	13.4	0.2702	98.801	1.068

Parent F2.9:



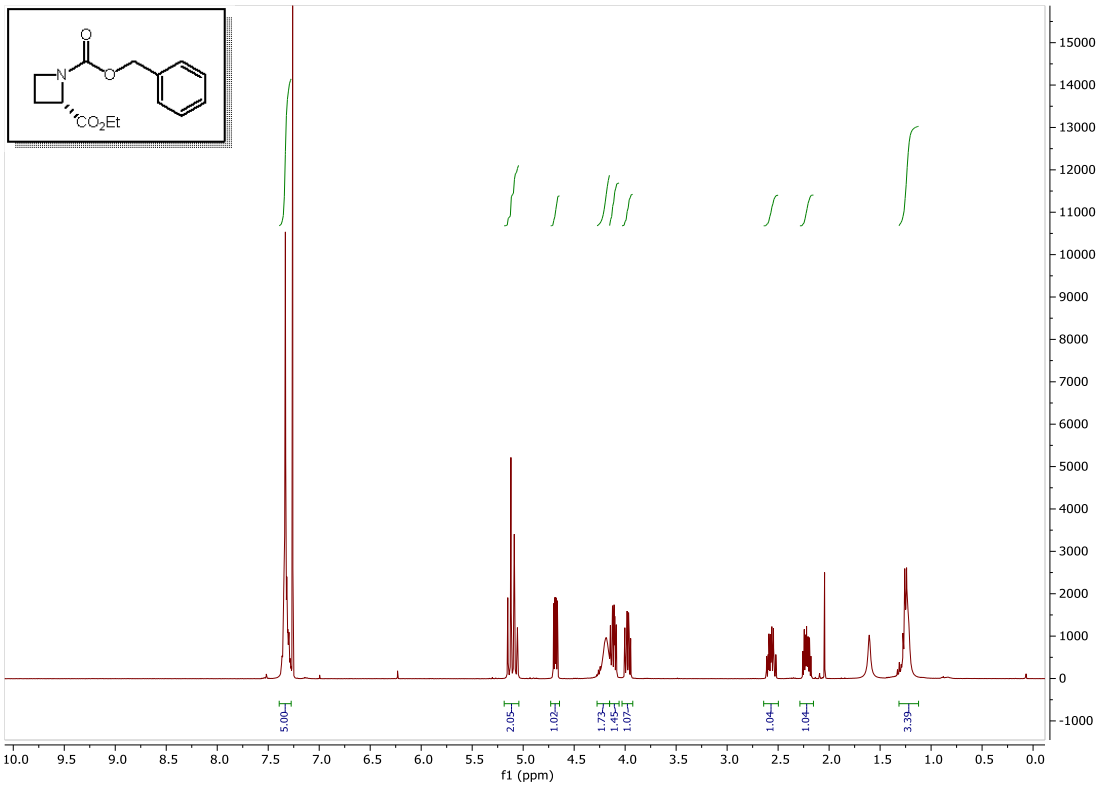
#	Time	Area	Height	Width	Area%	Symmetry
1	43.865	3.7	2.3E-1	0.2619	1.269	1.247
2	44.811	285.2	17.8	0.267	98.731	1.159

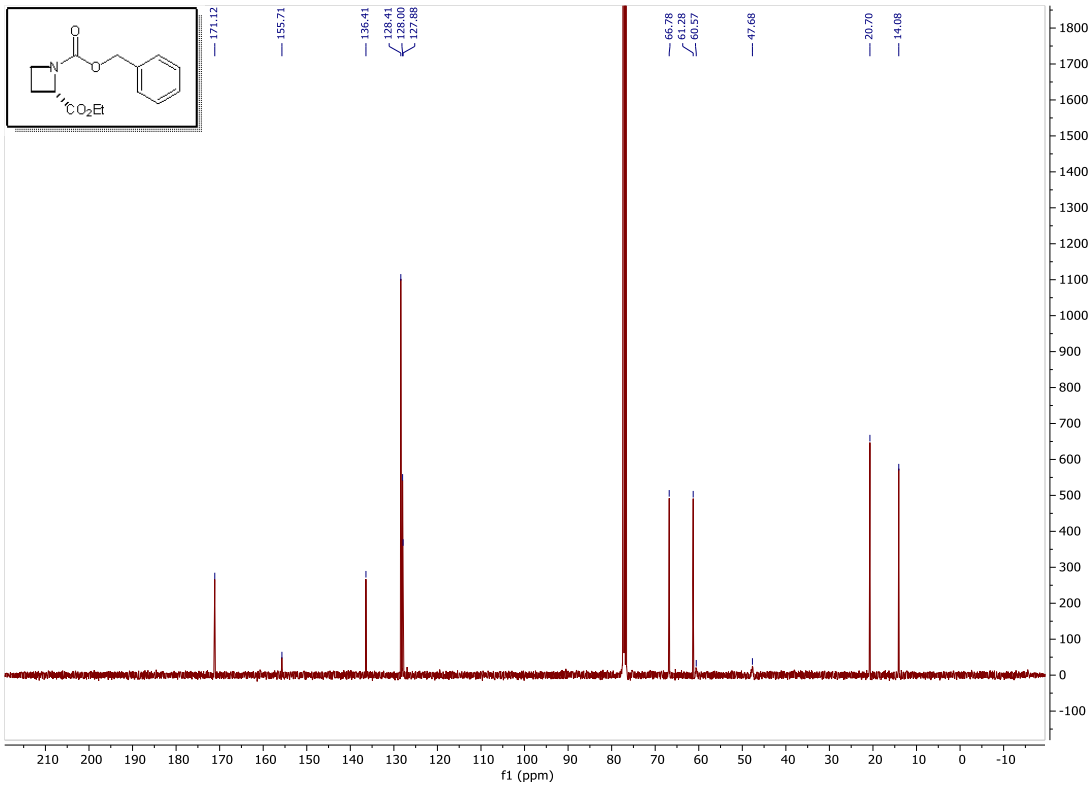
P411-AzetS:

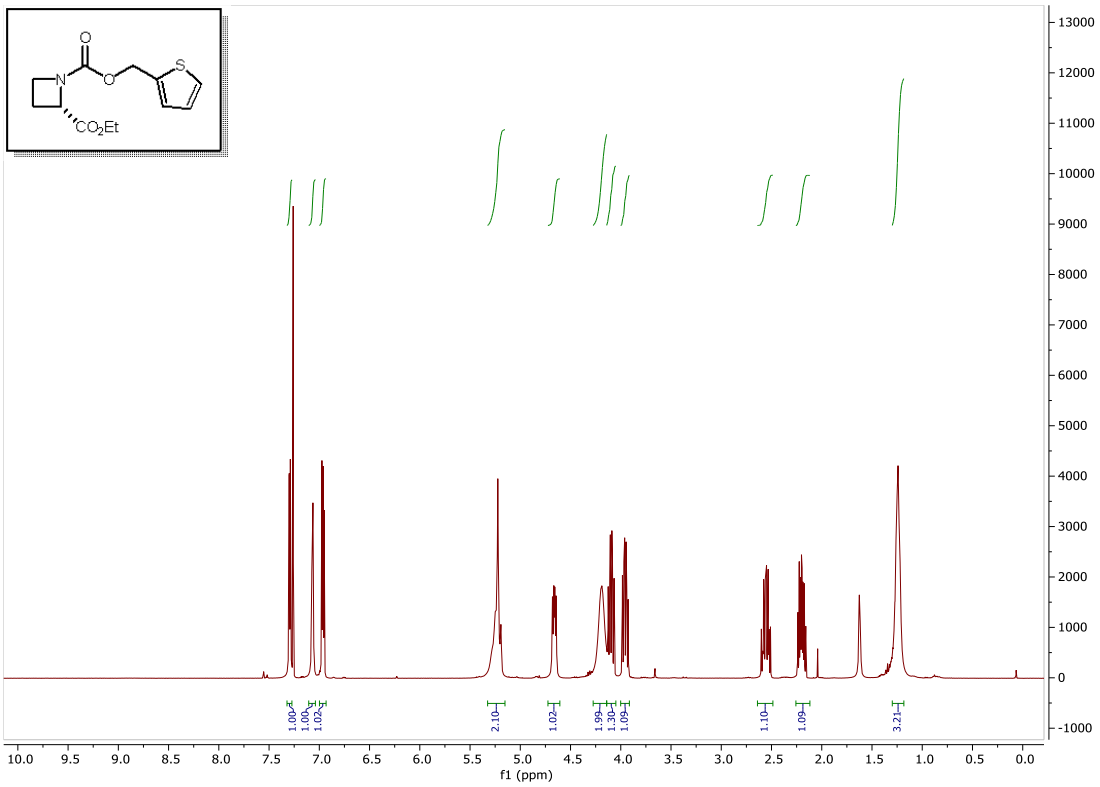


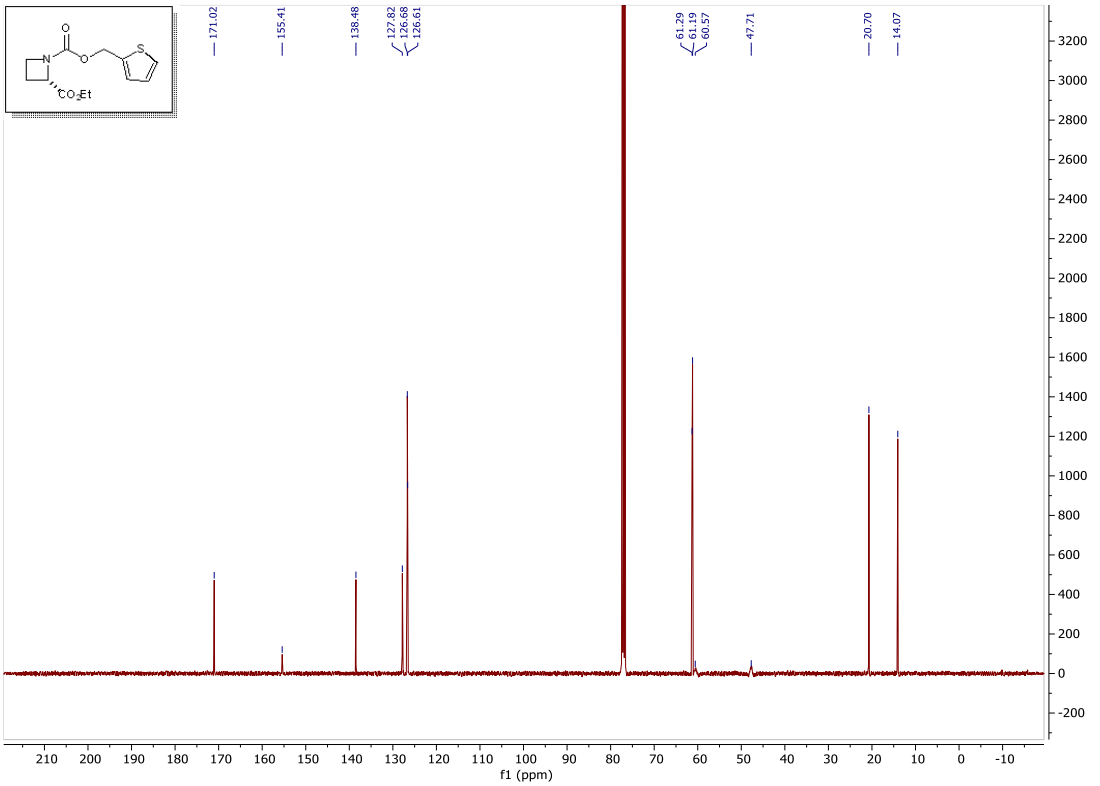
#	Time	Area	Height	Width	Area%	Symmetry
1	43.817	2.3	1.5E-1	0.2556	1.150	0.656
2	44.785	197.4	12.2	0.2688	98.850	1.147

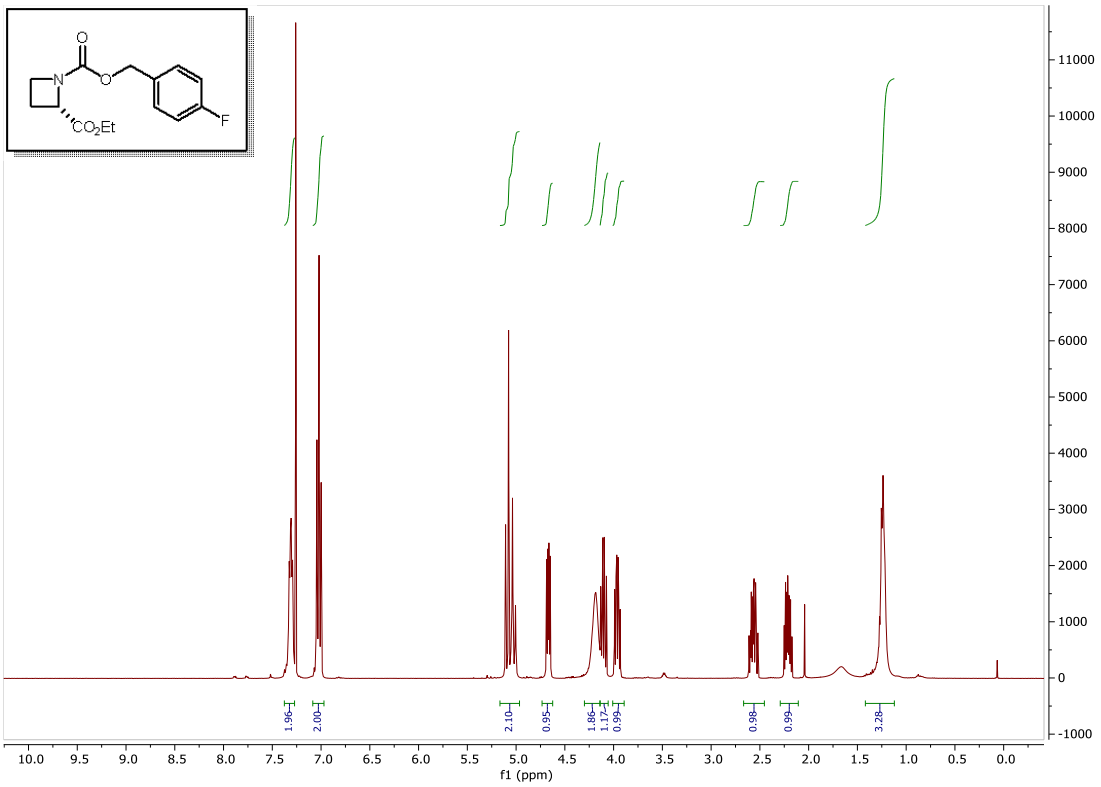
^{1}H, ^{13}C, and ^{19}F NMR Spectra of Enzymatic Reaction Products

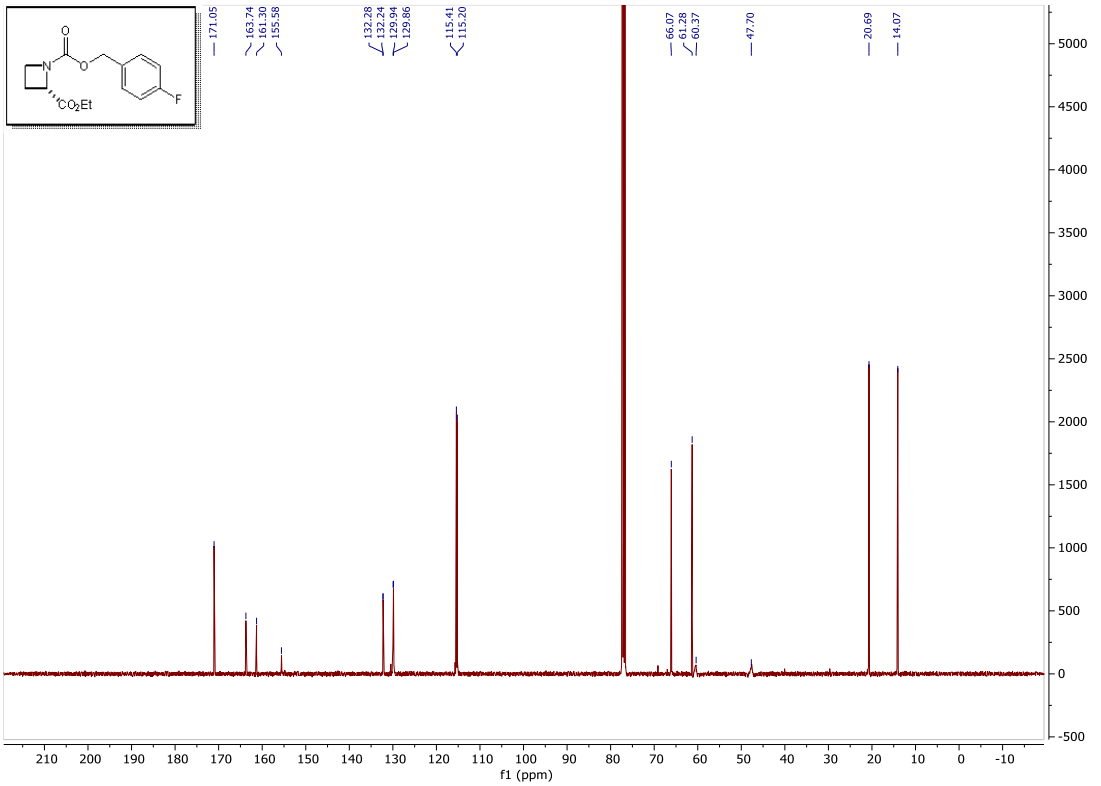


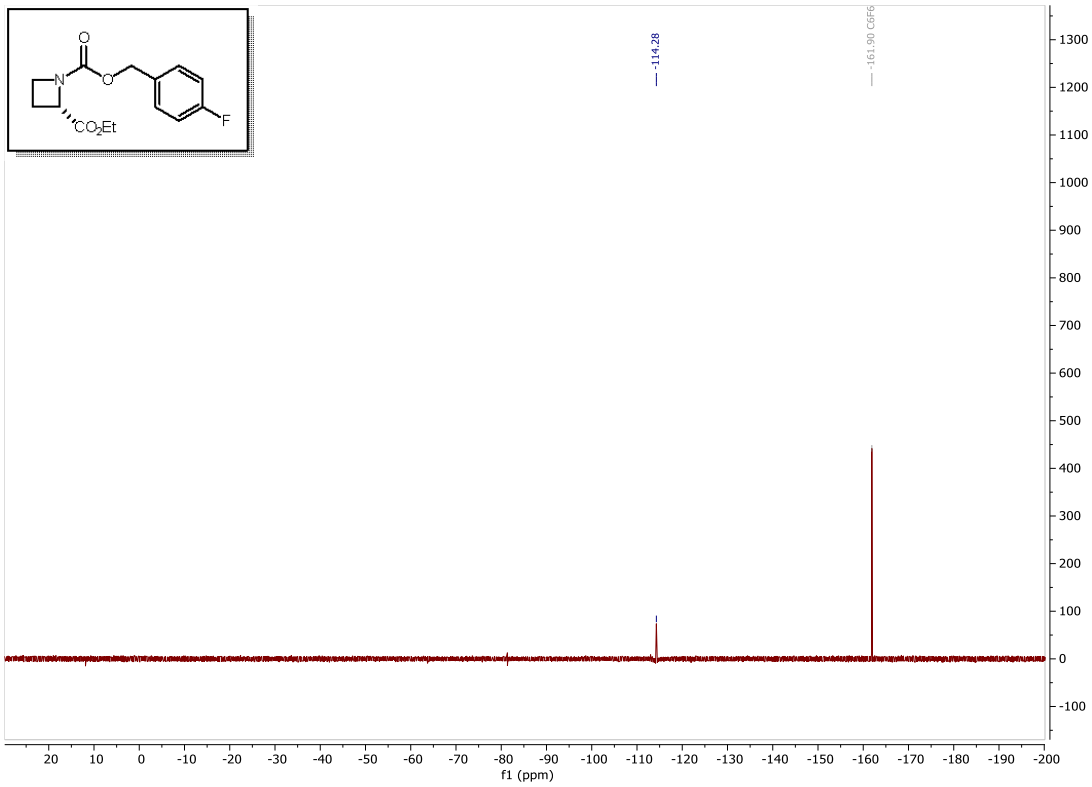


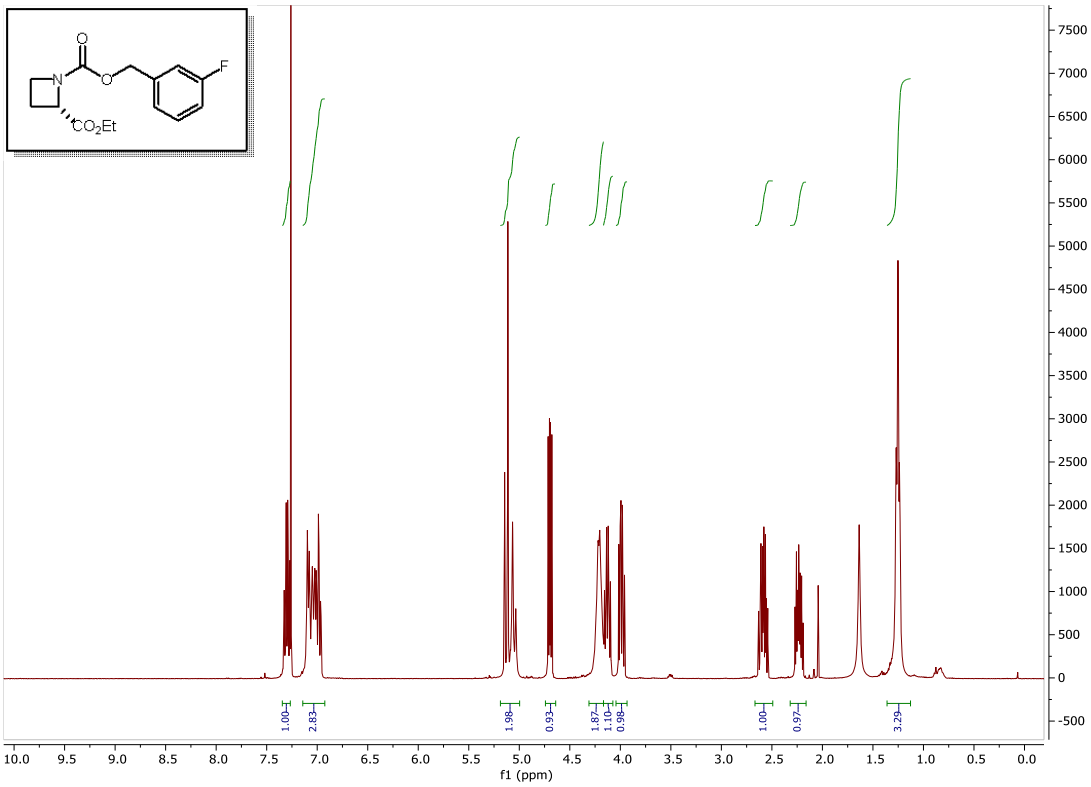

O
N
O
S
CO2Et
1.00
1.03
1.02
2.10
1.02
1.99
1.30
1.09
1.10
1.09
3.21
f1 (ppm)

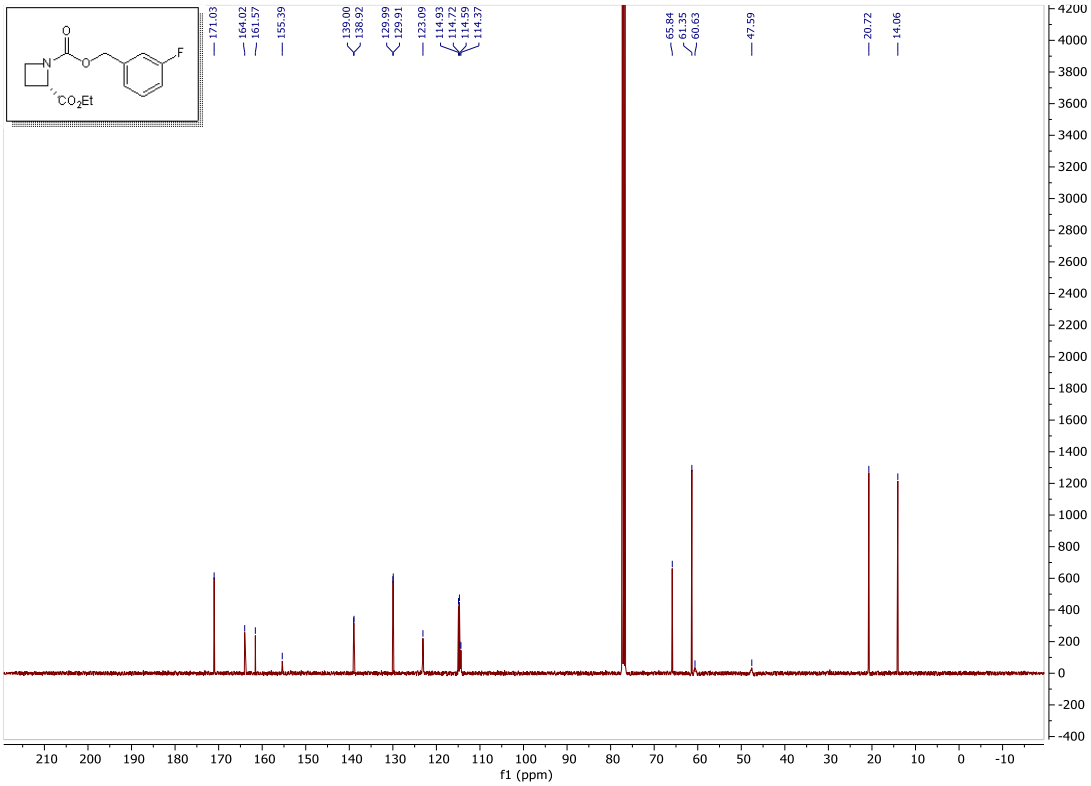

O
N
O
S
CO2Et
171.02
155.41
138.48
127.82
126.68
126.61
61.29
61.19
60.57
47.71
20.70
14.07
f1 (ppm)

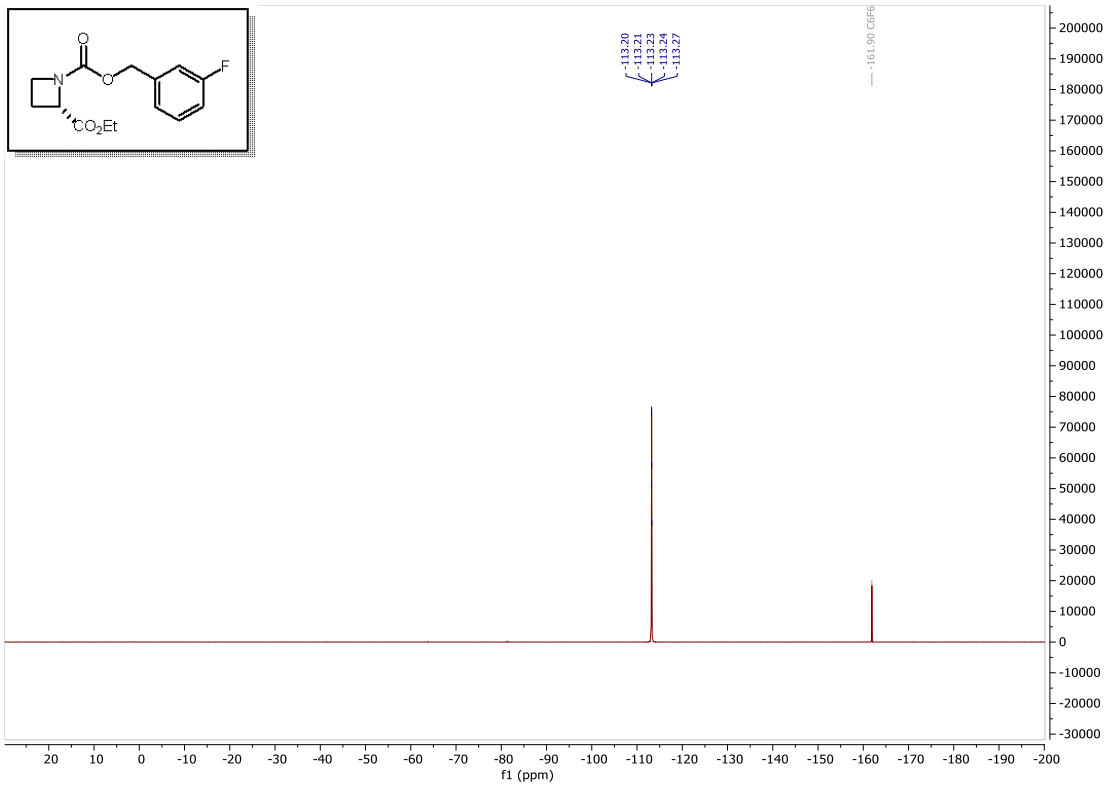

O
N
O
F
CO2Et
1.96
2.00
2.10
0.95
1.86
1.17
0.99
0.98
0.99
3.28
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

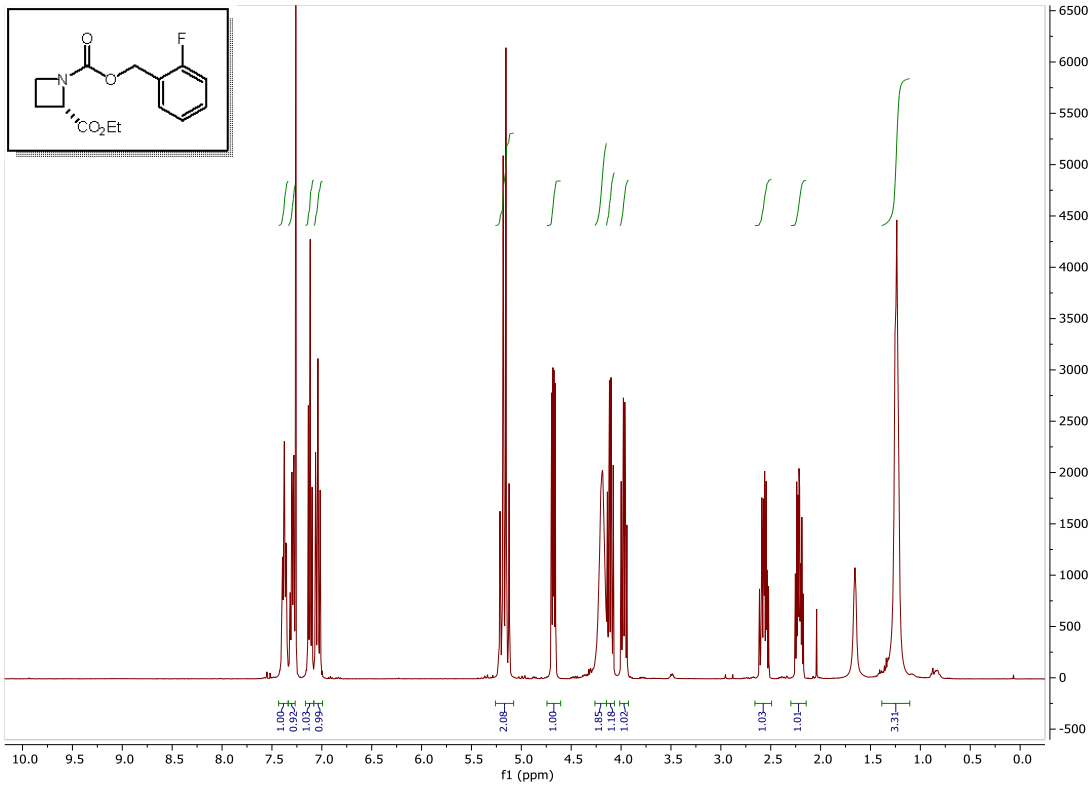

O
N
O
F
CO2Et
171.05
163.74
161.30
155.58
132.28
132.24
129.94
129.86
115.41
115.20
66.07
61.28
60.37
47.70
20.69
14.07
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

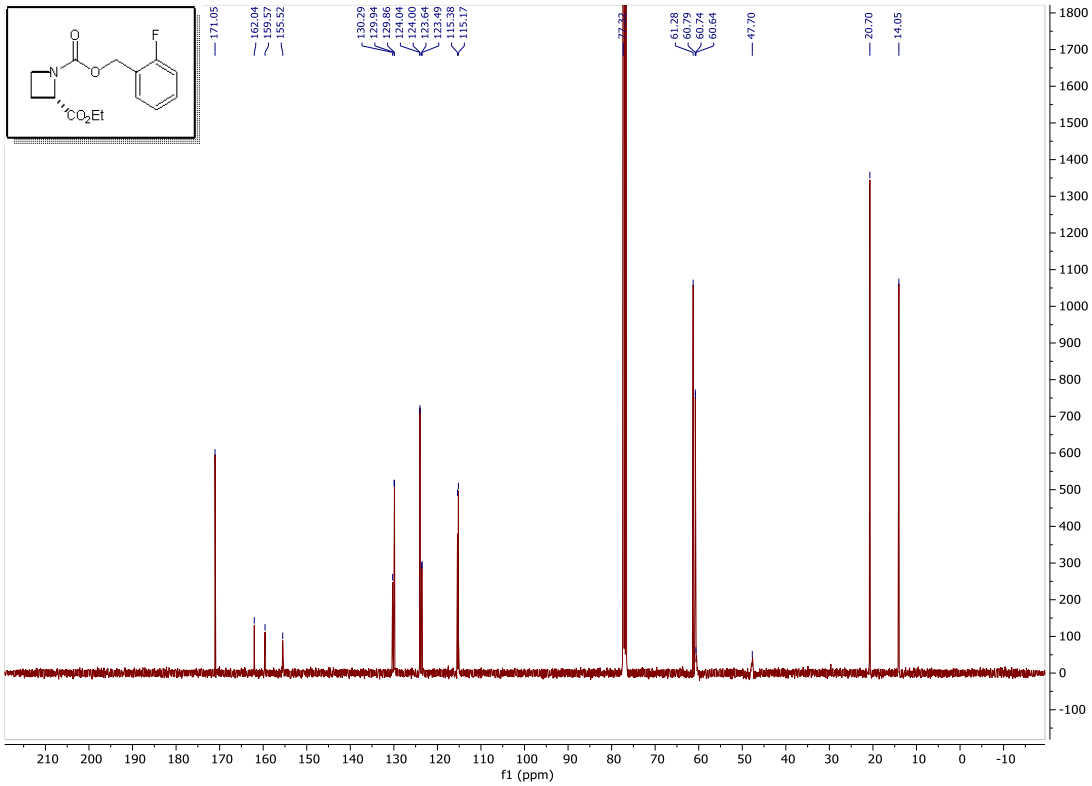

O
N
O
CO2Et
F
-114.28
-161.90 C6F6
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

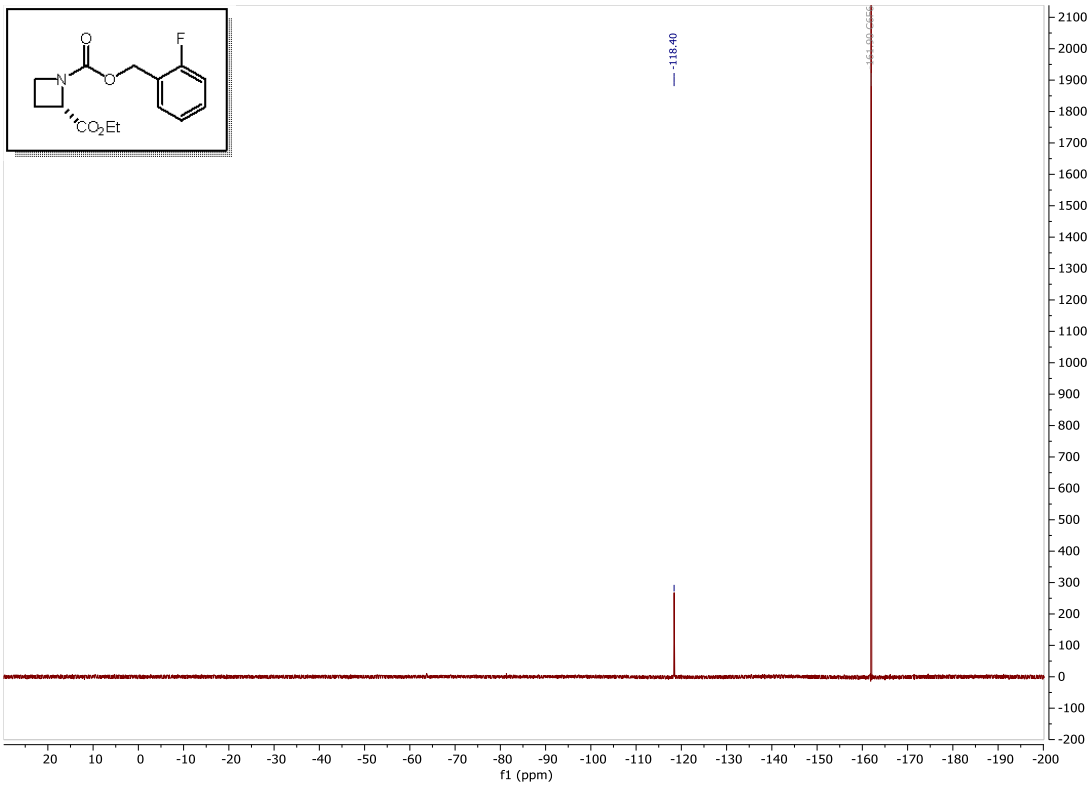

O
N
O
F
CO2Et
1.00
2.83
1.98
0.93
1.87
1.10
0.98
1.00
0.97
3.29
f1 (ppm)

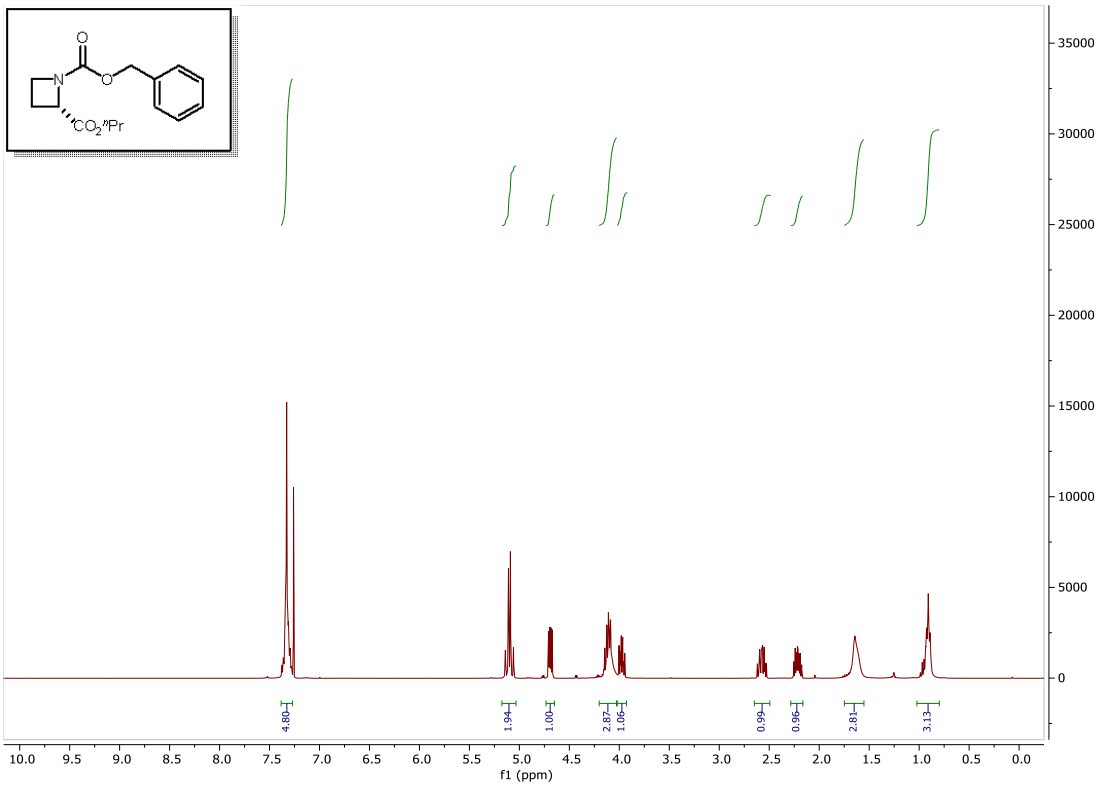

O
N
O
F
CO2Et
171.03
164.02
161.57
155.39
139.00
138.92
129.99
129.91
123.09
114.93
114.72
114.59
114.37
65.84
61.35
60.63
47.59
20.72
14.06
f1 (ppm)


-113.20
-113.21
-113.23
-113.24
-113.27
-161.90 C6F6
200000
190000
180000
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
-20000
-30000
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

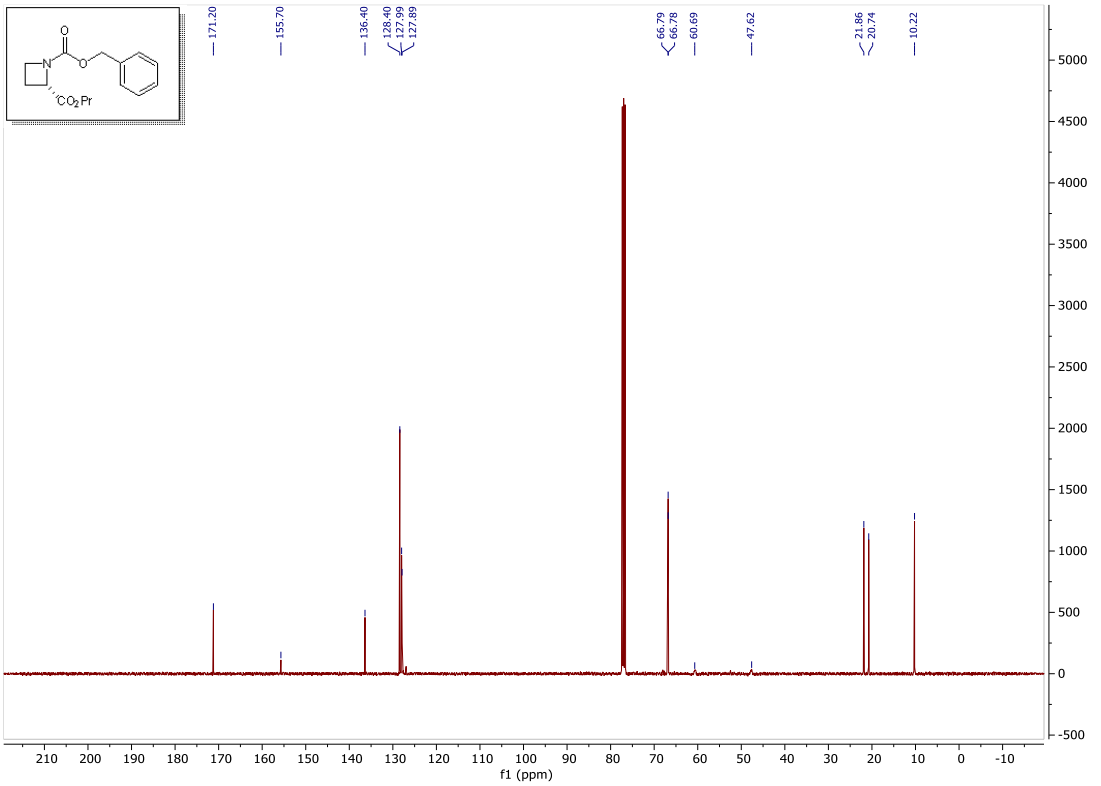

F
O
N
O
CO2Et
1.00
0.92
1.03
0.99
2.08
1.00
1.85
1.18
1.02
1.03
1.01
3.31
f1 (ppm)

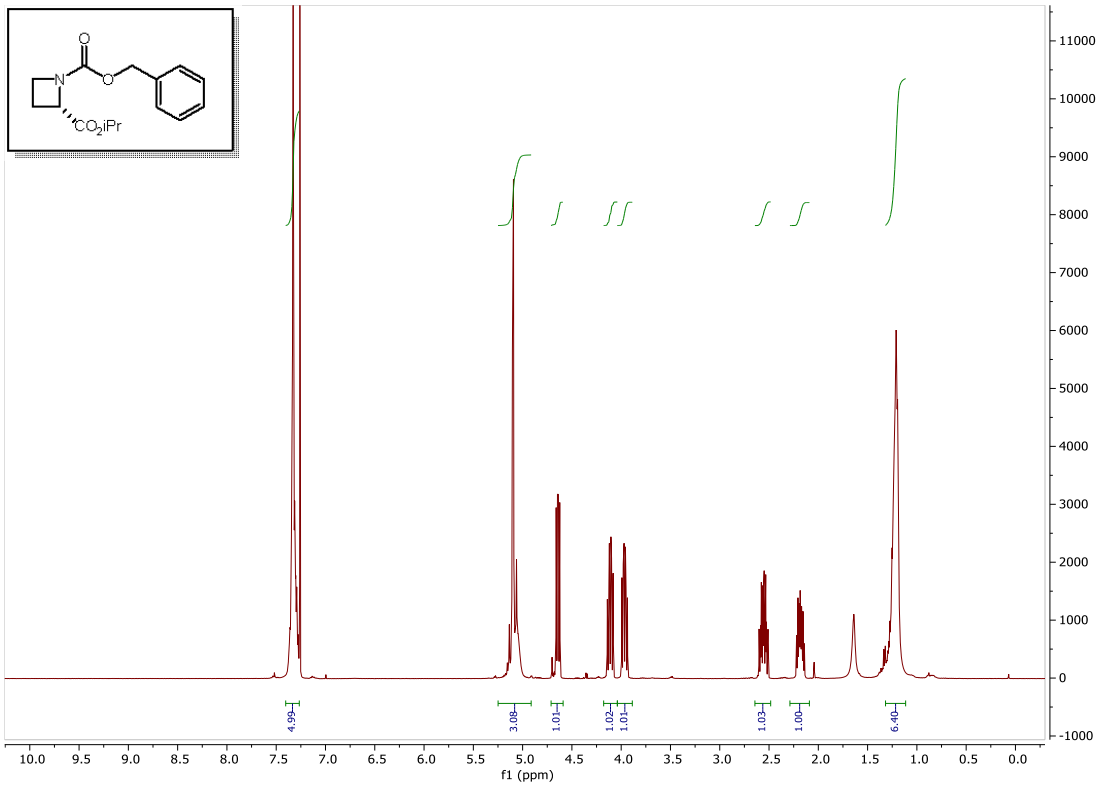

F
O
N
O
CO2Et
171.05
162.04
159.57
155.52
130.29
129.94
129.86
124.04
124.00
123.64
123.49
115.38
115.17
77.32
61.28
60.79
60.74
60.64
47.70
20.70
14.05
f1 (ppm)

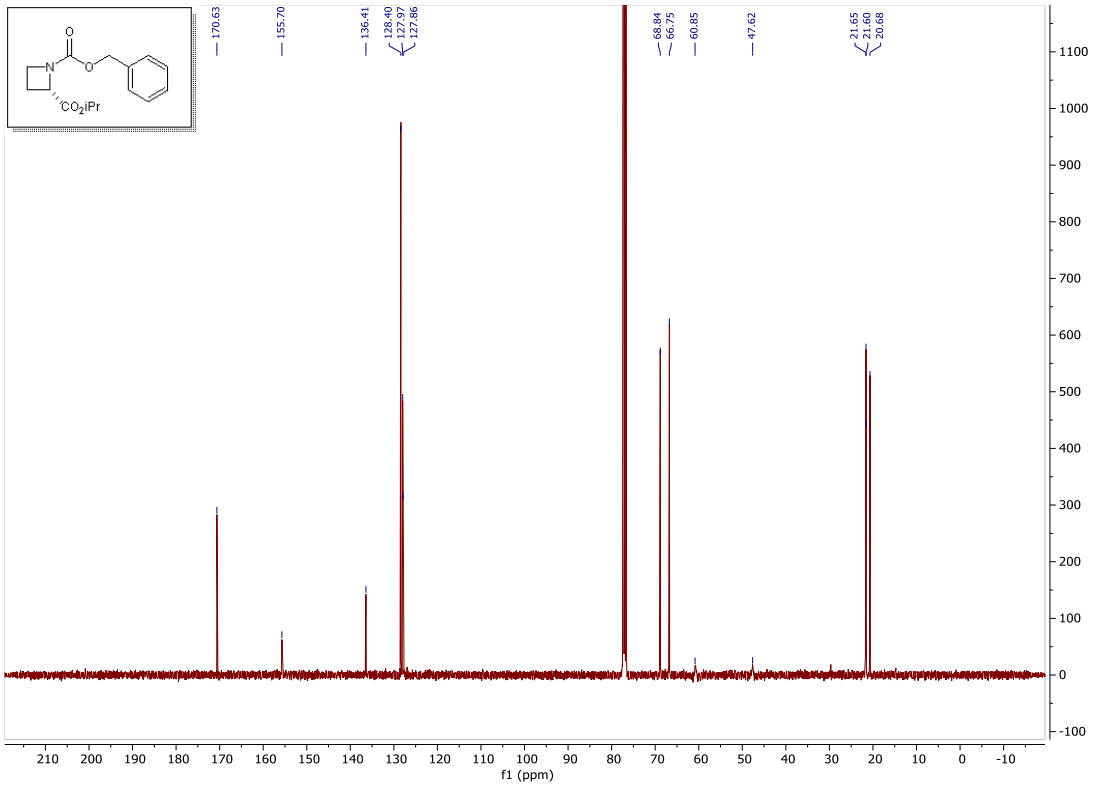

O
F
N
O
CO2Et
-118.40
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

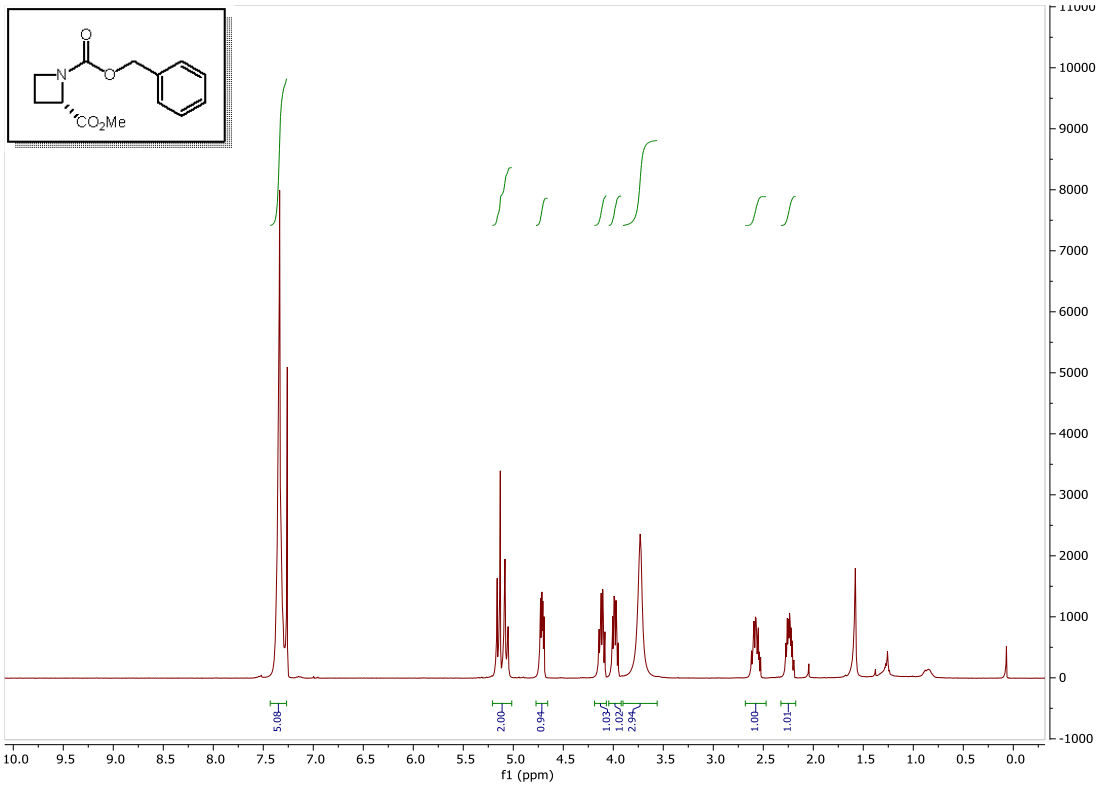


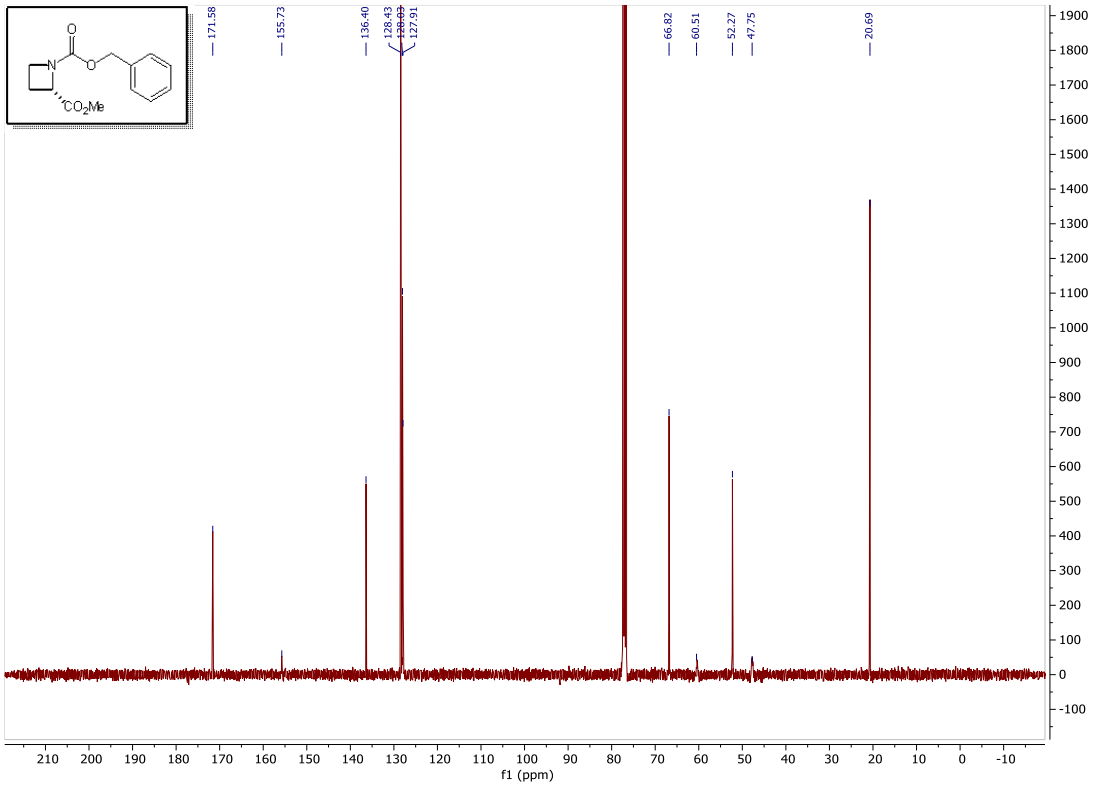
(N.B. methylene overlaps with HOD peak in 1H NMR, skewing integration)




CO2iPr
4.99
3.08
1.01
1.02
1.01
1.03
1.00
6.40
f1 (ppm)


CO2iPr
170.63
155.70
136.41
128.40
127.97
127.86
68.84
66.75
60.85
47.62
21.65
21.60
20.68
f1 (ppm)


CO2Me
5.08
2.00
0.94
1.03
1.02
2.94
1.00
1.01
f1 (ppm)


CO2Me
171.58
155.73
136.40
128.43
128.03
127.91
66.82
60.51
52.27
47.75
20.69
f1 (ppm)

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