SUPPORTING INFORMATION

Nitrene Transfer Catalyzed by a Non-Heme Iron Enzyme and Enhanced by Non-Native Small-Molecule Ligands

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MATERIALS AND METHODS

Synthetic chemistry

All manipulations were performed using oven-dried glassware (130 °C for a minimum of 12 hours) and standard Schlenk techniques under an atmosphere of argon, unless otherwise stated.

Solvents

ACS- and HPLC-grade solvents were purchased from Fisher Chemical. Anhydrous tetrahydrofuran was obtained by filtration through a drying column and a deoxygenation column on a Pure Process Technologies solvent system. High-purity water for PCR and HPLC was distilled after filtration through a deionizing column and organic removal column. Deuterated solvents were purchased from Cambridge Isotope Laboratories.

Chromatographic materials

Thin layer chromatography (TLC) was performed using EMD TLC plates pre-coated with 250 μm thickness silica gel 60 F_{254} and visualized by fluorescence quenching under UV light and staining with potassium permanganate or cerium ammonium molybdate. Preparative flash chromatography was performed using a Biotage Isolera automated chromatography instrument using columns hand-packed with silica gel (230–400 mesh, Silicyle Inc.).

Starting materials

All compounds were used as received from commercial suppliers, unless otherwise stated.

Analytical instrumentation

HPLC-MS analysis for initial activity determination was performed on an Agilent 1290 UPLC-MS equipped with a C18 silica column (1.8 μm packing, 2.1×50 mm). HPLC-MS analysis of site-saturation mutagenesis libraries was performed on an Agilent 1260 Infinity HPLC with an Agilent 6120 quadrupole mass spectrometer. Reverse-phase HPLC-UV analysis was performed with an Agilent 1200 series HPLC or an Agilent 1260 Series Infinity II HPLC using an Agilent Poroshell 120 EC-C18 column (4 μm packing, 2.1×50 mm) fitted with a Poroshell 120 guard column (1.7 μm packing, 2.1×5 mm). Normal-phase HPLC-UV analysis for chiral separations was performed with a Hewlett Packard Series 1100 HPLC instrument using a Daicel Chiralcel OJ-H column, (5 μm packing, 4.6×250 mm) or a Daicel Chiralpak IB column (5 μm packing, 4.6×250 mm).

NMR spectra were recorded on a Varian Unity/Inova 500 spectrometer operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C respectively, or a Bruker Avance 400 spectrometer operating at 400 MHz and 100 MHz for ^{1}H and ^{13}C respectively. NMR data were analyzed in MestReNova (MestreLab Research). Chemical shifts are reported in ppm with the solvent resonance as the internal standard. For ^{1}H NMR: $CDCl_3$, δ 7.26. For

^{13}C NMR: $CDCl_3$, δ 77.16. Data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, spt = septet, m = multiplet, br = broad; coupling constants in Hz; integration.

Biology and biocatalytic reactions

Materials

Oligonucleotides were purchased from IDT DNA. PCRs were run with Phusion® High-Fidelity PCR Kit (New England Biolabs). Gibson assembly mix[1] is prepared with isothermal master mix in-house and enzymes T5 exonuclease, Phusion® DNA polymerase, and Taq DNA ligase purchased from New England Biolabs. Oligonucleotide, DNA, and amino-acid sequences used in this work are given in the supplemental Excel document.

Cloning

Plasmids encoding *Pseudomonas savastanoi* ethylene-forming enzyme (Uniprot ID P32021), *Streptomyces sp*. 2-aminobutyric acid chlorinase (UniProt ID D0VX22), and *Arabidopsis thaliana* anthocyanidin synthase (UniProt ID Q96323), with the coding sequences codon-optimized for *Escherichia coli* were purchased from Twist Biosciences. Plasmids encoding *Gluconobacter oxydans* leucine dioxygenase (UniProt ID Q5FQD2), *Streptomyces vinaceus* arginine hydroxylase (UniProt ID Q6WZB0), and *Streptomyces muensis* leucine hydroxylase (UniProt ID A0A0E3URV8) were obtained from the laboratory of Prof. Hans Renata (Scripps Research Institute). The plasmid encoding *Escherichia coli* taurine dioxygenase (UniProt ID P37610) was obtained from the laboratory of Prof. Harry Gray (Caltech). All genes were encoded with a C-terminal His_6-tag for purification and inserted between the NdeI and XhoI cut sites in the pET-22b(+) vector (Novagen).

Plasmids were used to transform *E. cloni* BL21(DE3) cells (Lucigen) by electroporation. SOC medium (0.75 mL) was added and the cells were incubated at 37 °C for 45 minutes before being plated on Luria-Bertani medium (Research Products International) supplemented with ampicillin (100 μg mL^{-1}, LB-amp) agar plates.

Plasmids were isolated from stationary-phase cultures by miniprep (Qiagen) and Sanger sequencing was performed by Laragen, Inc. (Culver City, CA) using T7 promoter and T7 terminator primers.

Protein expression and purification

Starter cultures of LB-amp were inoculated from a single *E. coli* colony on an agar plate harboring a plasmid encoding the protein of interest and grown overnight to stationary phase at 37 °C. Expression cultures of Terrific Broth (Research Products International) supplemented with ampicillin (100 mg L^{-1}, TB-amp) were inoculated from the starter cultures (1% v/v) and shaken at 37 °C and 160 rpm in a Multitron Infors incubator. When the expression cultures reached OD_{600} ~ 0.8 (typically 2–3 hours), they were cooled on ice for 20 minutes. Protein expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG,

0.5 mM). Cultures were incubated at 22 °C and 110 rpm overnight (16–24 hours). Cells were pelleted by centrifugation (5000×*g*, 10 minutes).

For reactions with whole cells, cell pellets were resuspended in MOPS buffer (20 mM pH 7.0) to OD_{600} 30. For reactions with cell lysate, the whole cell suspensions were lysed by sonication (QSonica Q500 sonicator, 25% amplitude, 33% duty cycle, 3 minutes). The lysate was clarified by centrifugation (20,817×*g*, 10 minutes).

For purification, cell pellets were frozen at −20 °C for at least 24 hours. Cells were resuspended in binding buffer (20 mM Tris·HCl, 100 mM sodium chloride, 20 mM imidazole, pH 7.0, ~5 mL/g wet cells) and lysed by sonication (QSonica Q500 sonicator, 25% amplitude, 33% duty cycle, 4 minutes). The lysate was clarified by centrifugation (20,817 *g*, 10 minutes) followed by filtration (0.45 µm syringe filter). The protein was purified using an Äkta Purifier with a HisTrap HP column (GE Healthcare), eluting with a gradient of 20–500 mM imidazole. Fractions containing the protein of interest were pooled and dialyzed at 4 °C against MOPS buffer (20 mM pH 7.0) containing 1 mM EDTA (>100:1 v/v) (Spectrum Laboratories Spectra/Por 12–14 kD membrane) for four hours, then against MOPS buffer (20 mM pH 7.0) overnight (12–16 hours). The dialyzed protein was concentrated by centrifugal filtration (Amicon Ultra-15 10 kD MWCO) to a final concentration of 40–100 mg mL^{-1}. The concentrated protein was divided into aliquots (50–100 µL), flash-frozen on powdered dry ice, and stored at −80 °C. Protein concentration was determined by Bradford assay (Bio-Rad Quick Start Bradford).

Site-saturation mutagenesis and library screening

Site-saturation mutagenesis was performed using the 22-codon method[2]. Oligonucleotides including the three 22-codon trick codons (NDT, VHG, TGG) and oligonucleotides within the ampicillin resistance cassette were used to amplify the plasmid in two pieces, with an overlap for Gibson assembly in the gene encoding the protein of interest (and where the mutation is introduced) and an overlap for Gibson assembly in the gene encoding β-lactamase (which confers ampicillin resistance). This two-piece assembly can prevent mis-assembled constructs from conferring antibiotic resistance. The oligonucleotide sequences which generated the variants reported in in this work are listed in Table S1. All other oligonucleotides used during protein engineering (Protein Engineering Strategies) are available in the supplemental workbook 1, which also contains the DNA and amino-acid sequences of the variants reported in this work. PCR products were loaded on 1% agarose gels with loading dye containing SYBR Gold nucleic acid gel stain (Thermo Fisher) and visualized on a blue transilluminator. The DNA bands at the expected size were excised and the DNA was extracted with a Zymoclean Gel DNA recovery kit. The two linear PCR products for a given site-saturation mutagenesis library were assembled *via* isothermal Gibson assembly (50 °C, 1 hour). *E. cloni* BL21(DE3) cells (Lucigen) were transformed by electroporation with the Gibson assembly product without further purification. SOC medium (0.75 mL) was added to the electroporated cells and the cells were incubated at 37 °C for 45 minutes before being plated on LB-amp agar plates. The LB-amp agar plates

with the plated cells were incubated at 37 °C for 12–18 hours and stored at 4 °C until the libraries were picked. Single colonies from the agar plates were picked with sterile toothpicks and used to inoculate starter cultures (0.5 mL LB-amp) in 96 deep-well plates. The starter culture plates were grown at 37 °C, 250 rpm, and 80% humidity in a Multitron Infors shaker overnight (14–16 hours). The starter cultures (50 µL) were used to inoculate expression cultures (1 mL TB-amp) in 96 deep-well plates. In parallel, glycerol stock plates were prepared for long-term storage by mixing starter culture (50 µL) with sterile glycerol (50% v/v, 50 µL) and frozen at −80 °C. The expression cultures were grown at 37 °C, 250 rpm, and 80% humidity for three hours, then cooled on ice for 20 minutes. Protein expression was induced by addition of IPTG (0.5 mM). Cultures were incubated at 22 °C and 220 rpm overnight (18–20 hours). Cells were pelleted (5000×*g*, 5 minutes) and the cell pellets were frozen at −20 °C for at least 24 hours prior to use.

In site-saturation mutagenesis round 1, cells were resuspended in MOPS buffer (20 mM pH 7.0) and brought into the Coy anaerobic chamber. Ferrous ammonium sulfate (40 mM in water, 10 µL, 1 mM final concentration, prepared immediately before use), disodium α-ketoglutarate (40 mM in water, 10 µL, 1 mM final concentration), L-ascorbic acid (40 mM in water, 10 µL, 1 mM final concentration, prepared immediately before use), styrene (400 mM in ethanol, 10 µL, 10 mM final concentration), and *p*-toluenesulfonyl azide (400 mM in ethanol, 10 µL, 10 mM final concentration) were added to each well. The plates were sealed with foil covers and shaken at room temperature for two hours. To quench the reactions, acetonitrile (400 µL) was added and the reaction plate was shaken for an additional 30 minutes. Insoluble material was pelleted by centrifugation (6000×*g*, 10 minutes) and 200 µL of the supernate was filtered through a 0.2 µm PTFE 96-well filter plate into a 96-well microplate (3000×*g*, 2 minutes). The microplate was sealed with a pierceable cover and analyzed via HPLC-MS (Analytical instrumentation).

After site-saturation mutagenesis round 1, cells were resuspended in MOPS buffer (20 mM pH 7.0) containing 1 mM sodium acetate. Under air, ferrous ammonium sulfate (40 mM in water, 10 µL, 1 mM final concentration, prepared immediately before use), L-ascorbic acid (40 mM in water, 10 µL, 1 mM final concentration, prepared immediately before use), styrene (400 mM in ethanol, 10 µL, 10 mM final concentration), and *p*-toluenesulfonyl azide (400 mM in ethanol, 10 µL, 10 mM final concentration) were added to each well. The plates were sealed with foil covers and shaken at room temperature for two hours. To quench the reactions, acetonitrile (400 µL) was added and the reaction plate was shaken for an additional 30 minutes. Insoluble material was pelleted by centrifugation (6000×*g*, 10 minutes) and 200 µL of the supernate was filtered through a 0.2 µm PTFE 96-well filter plate into a 96-well microplate (3000×*g*, 2 minutes). The microplate was sealed with a pierceable cover and analyzed via HPLC-MS (Analytical instrumentation).

Wells which showed an apparent enhancement in aziridine product formation were streaked out (from the glycerol stock plates prepared in parallel) onto LB-amp agar plates and incubated at 37 °C. Starter cultures (5 mL LB-amp) were inoculated from single colonies and incubated overnight at 37 °C. Plasmids were isolated from these cultures and sequenced as described above (Cloning). Unique variants were then

regrown in Erlenmeyer flasks as described above (Protein expression and purification) and assayed in clarified cell lysate for nitrene-transfer activity.

Table S1. Oligonucleotides used for mutagenesis. Mutated codons are denoted here as NNN for simplicity; in practice they are a 12:9:1 ratio of NDT:VHG:TGG for site saturation or the appropriate single codon for site-directed mutagenesis. ampR forward and reverse oligonucleotides are used together with the mutagenesis oligonucleotides to amplify the plasmid in two pieces. ampR forward is used with reverse mutagenesis primers and ampR reverse is used with forward mutagenesis primers. Additional oligonucleotide sequences used in the protein engineering (Protein Engineering Strategies) are given in the supplemental Excel document.

Mutations relative to wild type	Direction	Sequence
T97X	Forward	CCGACTTCCCCGAAATTTTC*NNN*GTCTGCAAAGATCTTTC
T97X	Reverse	GAAAATTTCGGGGAAGTCGGGCTTTCCAGCAGTCACCTC
R171X	Forward	GATGGATGGCACCACATG*NNN*GTGTTGCGTTTTCCGCC
R171X	Reverse	CATGTGGTGCCATCCATCGCGGGTCAAATCTG
R277X	Forward	GGTGAAACTTAATACACGTGAG*NNN*TTTGCTTGCGCGTACTTCCATGAGCCG
R277X	Reverse	CACGTGTATTAAGTTTCACCTTATGCGGAGTGCTAAGTAACTGTCCCCCG
F314X C317M	Forward	CACTATGGGGAACATTTCACGAACATG*NNN*ATGCGTATGTATCCTGACCG
F314X	Reverse	CATGTTCGTGAAATGTTCCCCATAGTGAATGCGCTCATTGGCC
C317X	Forward	TCACGAACATGTTCATGCGT*NNN*TATCCTGACCGCATTACCACACAGC
C317X	Reverse	CATGAACATGTTCGTGAAATGTTCCCCATAGTGAATGCGCTC
ampR	Forward	CCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGC
ampR	Reverse	CGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAG

Analytical-scale biocatalytic aziridination reactions

Biocatalytic reactions were set up in 2-mL screw-cap vials (Agilent). Purified apoprotein (350 µL, 22.9 µM in 20 mM MOPS pH 7.0, final concentration 20 µM) was added to the vial. Solutions of ferrous ammonium sulfate and L-ascorbic acid were prepared immediately prior to use. Reactions to be set up anaerobically were brought into a Coy vinyl anaerobic chamber (nitrogen atmosphere, 0–10 ppm oxygen). To each reaction was added in order ferrous ammonium sulfate (40 mM in water, 10 µL, 1 mM final concentration), sodium acetate or other additive (40 mM in water, 10 µL, 1 mM final concentration), and L-ascorbic acid (40 mM in water, 10 µL, 1 mM final concentration). Each reaction was then charged with styrene (400 mM in ethanol, 10 µL, 10 mM final concentration) immediately followed by *p*-toluenesulfonyl azide (400 mM in

ethanol, 10 µL, 10 mM final concentration, 500 max. TTN). The reactions were sealed and shaken at room temperature for three hours unless otherwise noted. To quench the reactions, acetonitrile (350 µL) was added to each vial, followed by internal standard propiophenone (0.1% v/v in acetonitrile, 50 µL). The sample was transferred to a 1.7-mL Eppendorf tube, vortexed, and then centrifuged (20817×*g*, 5 minutes). 250 µL of the supernate was transferred to HPLC vial inserts for reverse-phase HPLC analysis. The remaining supernate was partially concentrated *in vacuo* to remove acetonitrile and ethanol. Cyclohexane (500 µL) was added to the resulting aqueous suspension. The mixture was thoroughly shaken and then centrifuged (20817×*g*, 5 minutes). 250 µL of the organic layer was transferred to HPLC vial inserts for normal-phase chiral HPLC analysis.

Analytical-scale biocatalytic C–H insertion reactions

Biocatalytic reactions were set up in 2-mL screw-cap vials (Agilent). Purified apoprotein (360 µL, 22.2 µM in 20 mM MOPS pH 7.0, final concentration 20 µM) was added to the vial. Solutions of ferrous ammonium sulfate and L-ascorbic acid were prepared immediately prior to use. Reactions to be set up anaerobically were brought into a Coy vinyl anaerobic chamber (nitrogen atmosphere, 0–10 ppm oxygen). To each reaction was added in order ferrous ammonium sulfate (40 mM in water, 10 µL, 1 mM final concentration), sodium acetate or other additive (40 mM in water, 10 µL, 1 mM final concentration), L-ascorbic acid (40 mM in water, 10 µL, 1 mM final concentration). Each reaction was then charged with 2-ethylbenzenesulfonyl azide (400 mM in ethanol, 10 µL, 10 mM final concentration, 500 max. TTN). The reactions were sealed and shaken at room temperature for six hours unless otherwise noted. To quench the reactions, acetonitrile (350 µL) was added to each vial, followed by internal standard propiophenone (0.5% v/v in acetonitrile, 50 µL). The sample was transferred to a 1.7-mL Eppendorf tube, vortexed, and then centrifuged (20817×*g*, 5 minutes). 250 µL of the supernate was transferred to HPLC vial inserts for reverse-phase HPLC analysis. The remaining supernate was partially concentrated *in vacuo* to remove acetonitrile and ethanol. Hexanes (250 µL, HPLC grade) and ethyl acetate (250 µL, HPLC grade) were added. The resulting mixture was thoroughly shaken and then centrifuged (20817×*g*, 5 minutes). 250 µL of the organic layer was transferred to HPLC vial inserts for normal-phase chiral HPLC analysis.

PROTEIN ENGINEERING STRATEGIES

Site-saturation mutagenesis round 1

When designing our protein engineering strategy, we considered our expected screening throughput (maximum number of samples screened per day) and what we hypothesized about what factors affect *Ps*EFE's aziridination activity. As our primary screen would be via LC-MS (Analytical instrumentation), the screen would be approximately 3–4 minutes per sample (approximately 400 clones per day at maximum). With this medium-throughput screening capability we opted for site-saturation mutagenesis at positions which might reasonably modulate enzymatic activity. As we hypothesize that the nitrene-transfer reaction proceeds within the arginine substrate binding pocket, we primarily focused on mutations within that region of the protein. We ordered primers for 24 site-saturation libraries targeting residues in the first shell of the arginine and α-ketoglutarate binding sites (amino acid positions 84, 86, 87, 91, 171, 173, 175, 186, 189, 191, 192, 198, 206, 228, 268, 270, 277, 279, 281, 283, 314, 316, 317, 318). Out of these 24 libraries, 15 were successfully cloned, expressed, and screened on the first pass (84, 86, 87, 91, 171, 173, 186, 189, 191, 192, 277, 283, 314, 316, 317). Libraries which were not generated on the first pass could be revisited in future rounds of mutagenesis.

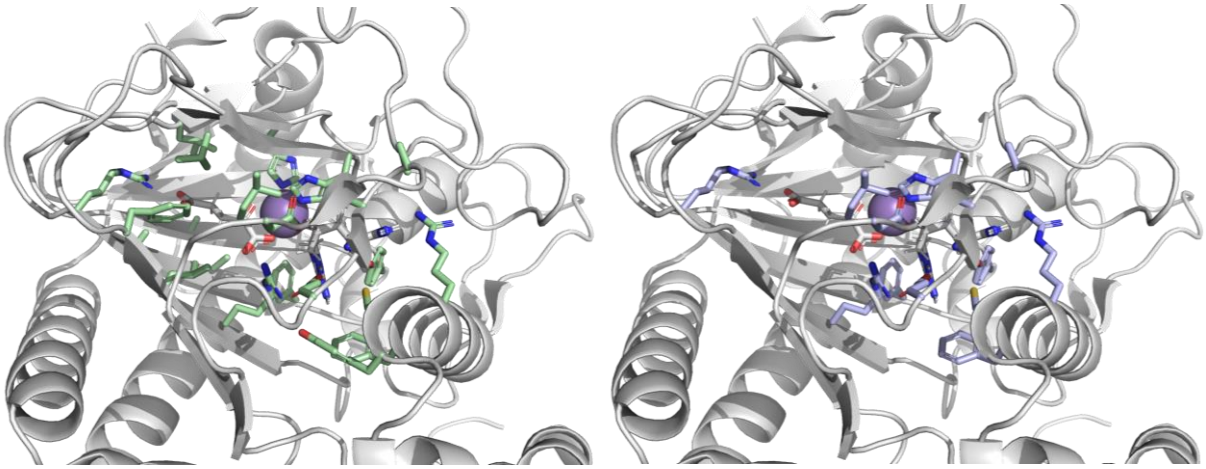


Figure S1. Structural representation of *Ps*EFE complexed with α-ketoglutarate (represented in sticks) and Mn (the metal with which the protein was crystallized, purple sphere) (PDB ID: 5VKB). Left: 24 positions for which primers for site-saturation mutagenesis were designed and ordered; side chains are represented as sticks and shown in pale green. Right: 15 positions for which site-saturation mutagenesis libraries were cloned, expressed, and screened for aziridination activity; side chains are represented as sticks and shown in light blue.

Site-saturation mutagenesis libraries were screened at a rate of 80 clones per library, as well as eight clones of the parent and eight sterile controls. The screening was performed on the LC-MS (Analytical instrumentation) using the MSD signal at m/z = +274 ($[M+H]^+$) to quantify aziridine product formation.

In parallel to screening the libraries from round 1, we determined that purified wild-type *Ps*EFE has

enhanced activity when adding acetate in place of α-ketoglutarate and that the reaction also works under aerobic conditions (

Experimental Data). Rather than modifying the screening conditions partway through a single round of directed evolution, the remaining libraries were screened under anaerobic conditions with α-ketoglutarate as the supplemented ligand. Variants with enhanced aziridination activity relative to the parent protein (*Ps*EFE wild type) were then sequenced and expressed at 40-mL scale (Protein expression and purification). The validation reactions were then performed with clarified cell lysate, testing the variants under anaerobic and aerobic conditions and supplementing with either acetate or α-ketoglutarate.

While there were variants with modest enhancement from other site-saturation libraries, *Ps*EFE C317M was by far the highest enhancement in aziridination activity, including under aerobic, acetate-supplemented conditions. We therefore fixed *Ps*EFE C317M as the parent for the second round.

Site-saturation mutagenesis round 2

In the second round of evolution we targeted 10 positions for site-saturation mutagenesis (84, 86, 91, 171, 186, 192, 277, 283, 314, 316). The specific libraries chosen were ones which either failed in cloning or expression during round 1 or showed some variants with modest improvements in activity during round 1 screening. Most of these positions could be targeted using the primers purchased in the first round of mutagenesis. For positions 314 and 316 new primers were purchased to include the C317M mutation (otherwise the mutation would be overwritten in PCR amplification). These libraries were prepared as described above (Site-saturation mutagenesis and library screening). These libraries were screened under aerobic conditions and supplemented with acetate in place of α-ketoglutarate. Of these libraries, the largest enhancements in aziridination activity in the screening data were found at the libraries targeting positions 171, 277, and 314. In sequencing the variants with beneficial mutations we also found a non-programmed single nucleotide substitution (C290T) which introduced the amino-acid substitution T97M.

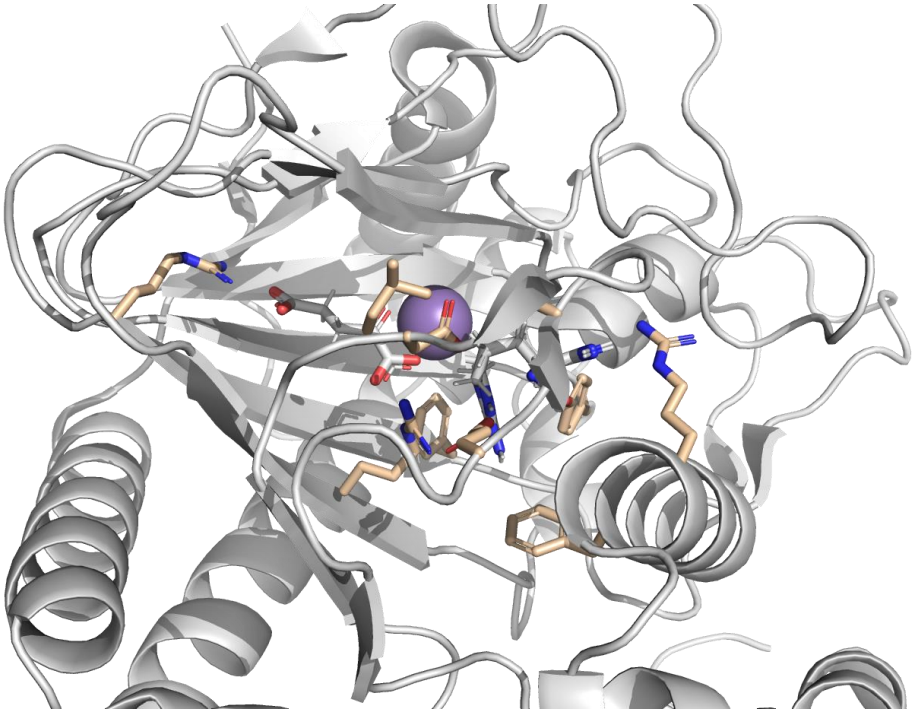


Figure S2. Residues targeted in the second round of mutagenesis. 10 residues for which site-saturation mutagenesis libraries were cloned, expressed, and screened for aziridination activity; side chains are represented as sticks and shown in tan.

Recombination of round 2 beneficial mutations

As the second round of evolution had multiple modest improvements rather than one variant which was clearly the best, we prepared a recombination library of the mutations found in the best-performing variants in the round 2 validation reactions. In preparing a recombination library we introduce the mutations via PCR similar to the site-saturation mutagenesis cloning strategies. The mutations included in the recombination library were T97M, R171A, R171V, R171L, R277H, F314M, F314Q, F314L; at each position we also include the parent residue in the library, resulting in 64 possible variants.

As the four amino-acid residues were spread out across the gene, we were able to generate three PCR fragments which included the programmed mutations at positions 97, 171, and 314 (Figure S3). To introduce the mutation at position 277, we used an equimolar mixture of plasmids *Ps*EFE C317M and *Ps*EFE R277H C317M as the template for the PCR. These three PCR products were gel extracted and assembled via Gibson assembly. The remaining expression and screening was as described above (Site-saturation mutagenesis and library screening).

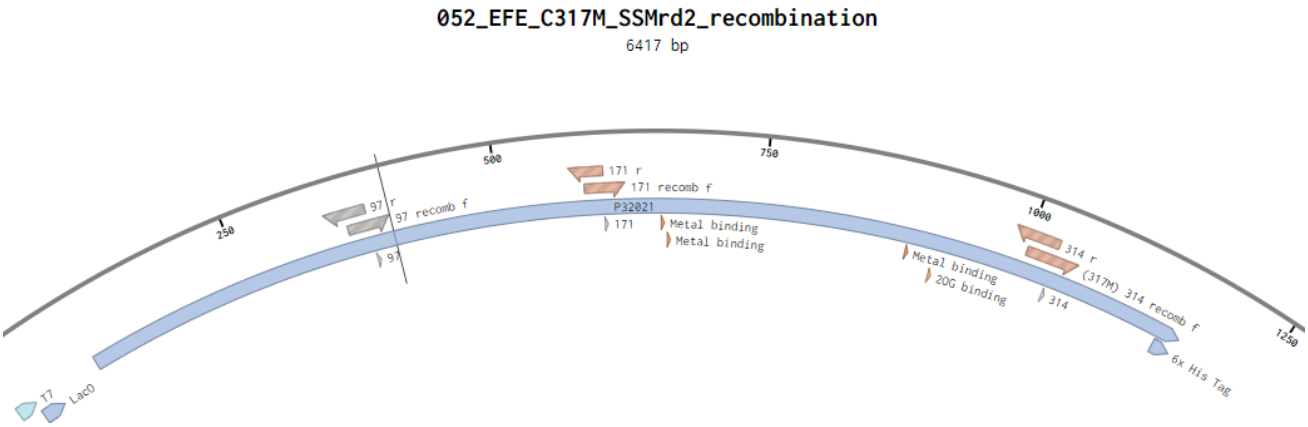
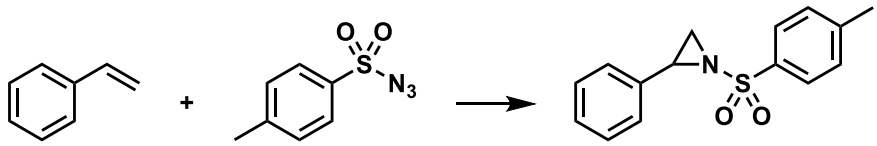


Figure S3. Recombination library primer design. The promoter (T7lac), gene (P32021, *Ps*EFE), and primer binding sites are annotated. The plasmid map was generated via Benchling.

To get high coverage and redundancy of this recombination library, four 96-well plates of this recombination library were screened (320 clones). The *Ps*EFE VMM variant reported in the main text and its R277H variant were found in the screening and validation of this recombination library.

EXPERIMENTAL DATA

Initial evaluation of α-ketoglutarate-dependent iron dioxygenases



1 **2** **3**

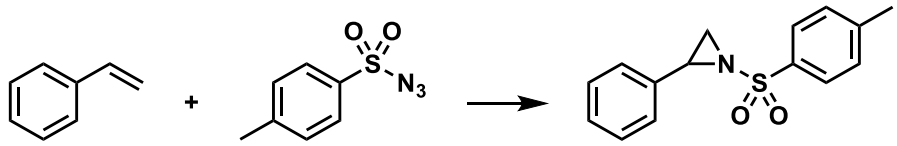
Reactions were performed as described above (Analytical-scale biocatalytic aziridination reactions) except enzyme concentrations were 50 μM, disodium α-ketoglutarate was used as the additive, and acetonitrile was used as the co-solvent. Activity was assayed by HPLC-MS (Analytical instrumentation). Activities were measured by integrating the extracted chromatogram for m/z = +274 ($[M+H]^+$) and normalized to the negative control, bovine serum albumin.

Table S2. Activities of α-KG-dependent iron enzyme towards aziridination to form **3**.

Enzyme	Relative activity
***P. savastanoi* ethylene-forming enzyme**	**12.0**
Streptomyces sp. 2-aminobutyric acid chlorinase	0.93
A. thaliana anthocyanidin synthase	0.54
G. oxydans leucine dioxygenase	1.11
E. coli taurine dioxygenase	0.61
S. vinaceus arginine hydroxylase	0.57
S. muensis leucine hydroxylase	0.61
Bovine serum albumin (negative control)	1.00

Reaction condition controls

Aziridination reaction



1 **2** **3**

Reactions were performed in triplicate as described above with acetate as additive (Analytical-scale biocatalytic aziridination reactions) except where noted. Yields given are the average of the triplicates.

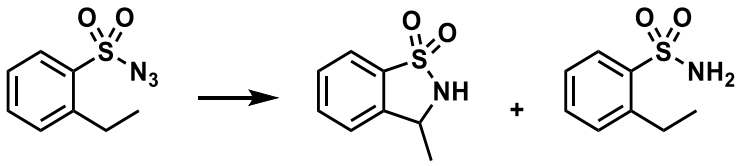
Table S3. Aziridination reaction controls with wild-type *Ps*EFE.

Deviation from standard conditions	Aziridine yield (%)
None	0.56%
No iron	0.01%
No ascorbate	0.50%
No acetate	0.04%
αKG instead of acetate	0.08%
Succinate instead of acetate	0.11%
N-oxalylglycine instead of acetate	0.64%
H189A D191A mutant (disrupted iron binding)	<0.01%

Table S4. Aziridination reaction controls with *Ps*EFE T97M R171L R277H F314M C317M (MLHMM)

Deviation from standard conditions	Aziridine yield (%)
None	23.8
No iron	0.1
No ascorbate	15.2
No acetate	2.1
α-Ketoglutarate instead of acetate	2.6
N-oxalylglycine instead of acetate	2.4
Aerobic	4.7
Aerobic, no ascorbate	2.9

C–H insertion reaction



4 **5** **6**

Reactions were performed in triplicate as described above (Analytical-scale biocatalytic C–H insertion reactions) with acetate as the additive, except where otherwise noted. Data shown are the average of the triplicates.

Table S5. C–H insertion reaction controls (max. 500 TTN)

Variant	Additive	TTN (**5**)	**5/6**
Wild type	Acetate	12.3	1.6
R171V F314M C317M (VMM)	Acetate	313	24
R171V F314M C317M (VMM)	None	24.6	0.9
R171V F314M C317M (VMM)	α-Ketoglutarate	130	9.0
R171V F314M C317M (VMM)	*N*-Oxalylglycine	447	105
R171V R277H F314M C317M (VHMM)	Acetate	243	32
R171V R277H F314M C317M (VHMM)	None	27.0	3.4
R171V R277H F314M C317M (VHMM)	α-Ketoglutarate	30.8	3.8
R171V R277H F314M C317M (VHMM)	*N*-Oxalylglycine	33.3	4.1

C–H insertion reaction with whole cells and cell lysate

Reactions were performed in triplicate as described above (Analytical-scale biocatalytic C–H insertion reactions), except whole cell suspensions and cell lysates were used instead of purified protein solutions. Data shown are the average of the triplicates. Note that the chemoselectivities are generally much lower than with purified protein; this is presumably due to increased reduction of the nitrene by the cellular background.

Table S6. C–H insertion with whole cells and lysate

Variant	Formulation	Additive	% Yield (**5**)	**5/6**
VMM	Whole cells	None	36%	1.6
VMM	Whole cells	Acetate	37%	1.7
VMM	Whole cells	αKG	36%	1.6
VMM	Whole cells	NOG	30%	1.6
VMM	Lysate	None	76%	7.7
VMM	Lysate	Acetate	72%	7.0
VMM	Lysate	αKG	69%	6.3
VMM	Lysate	NOG	90%	12.7
VHMM	Whole cells	None	33%	3.5
VHMM	Whole cells	Acetate	34%	3.7
VHMM	Whole cells	αKG	33%	3.4
VHMM	Whole cells	NOG	27%	3.1
VHMM	Lysate	None	73%	15.8
VHMM	Lysate	Acetate	75%	16.9
VHMM	Lysate	αKG	73%	14.3
VHMM	Lysate	NOG	77%	17.0

SDS-PAGE of *Ps*EFE variants

Large-scale protein expression, lysis, and purification was carried out as described in the methods section (Protein expression and purification). Whole-cell samples were taken after resuspension, clarified lysate samples were taken following sonication and centrifugation, and purified protein samples were taken after

HisTrap purification. Samples were mixed 1:1 with 2X Laemmli loading buffer (Bio-Rad Laboratories, Inc.) with added 2-mercaptoethanol. Samples were heated to 95 °C in a thermomixer block, briefly centrifuged, and loaded on an Any kD™ Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad Laboratories, Inc.). Gels were run at 150 V for 45 minutes. The gel was washed with water, then stained by microwaving with Coomassie solution. Gels were destained with successive rounds of microwaving with water, followed by gentle shaking overnight in water.

Large-scale protein expression, lysis, and purification was carried out as described in the methods section (Protein expression and purification). Whole-cell samples were taken after resuspension, clarified lysate samples were taken following sonication and centrifugation, and purified protein samples were taken after HisTrap purification. These samples were mixed 1:1 with 2X Laemmli loading buffer (Bio-Rad Laboratories, Inc.) with added 2-mercaptoethanol. Samples were heated to 95 °C in a thermomixer block, briefly centrifuged, and loaded on an Any kD™ Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad Laboratories, Inc.). Gels were run at 150 V for 30–45 minutes. Gels were washed with water, then stained by microwaving the gels with Coomassie solution. Gels were destained with successive rounds of microwaving with water, followed by gentle shaking overnight in water.

Aziridination variant protein purification

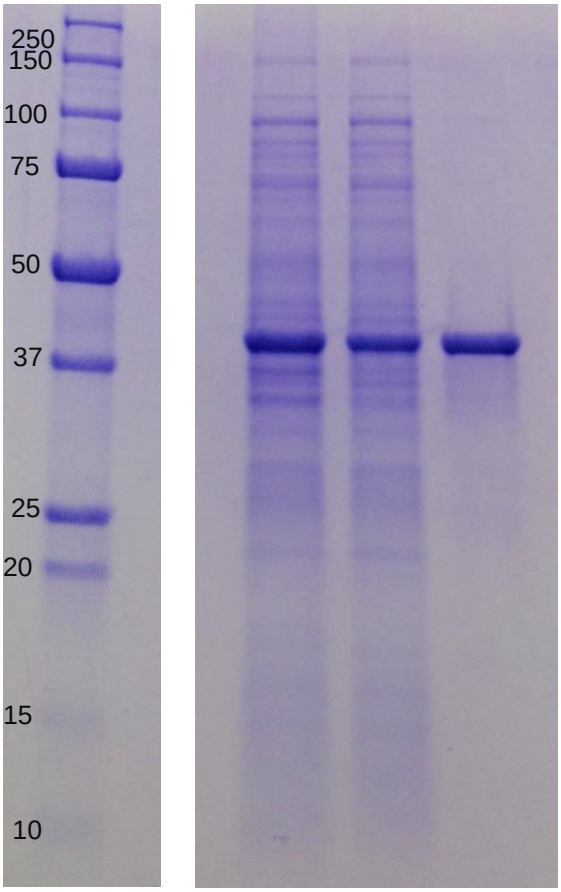


Figure S4. SDS-PAGE of *Ps*EFE aziridination variant T97M R171L R277H F314M C317M (PsEFE MLHMM). The protein is shown from left to right as whole-cell sample, clarified lysate sample, and purified protein sample. Whole cells and lysates were diluted 25-fold; purified protein was diluted 50-fold (each dilution is prior to addition of 2X Laemmli loading dye). The ladder and sample were run on the same gel; unrelated protein samples were cropped out for image clarity. The SDS-PAGE image brightness was increased in Microsoft Word for image clarity and is not being used for quantitation.

C–H insertion variant protein purification

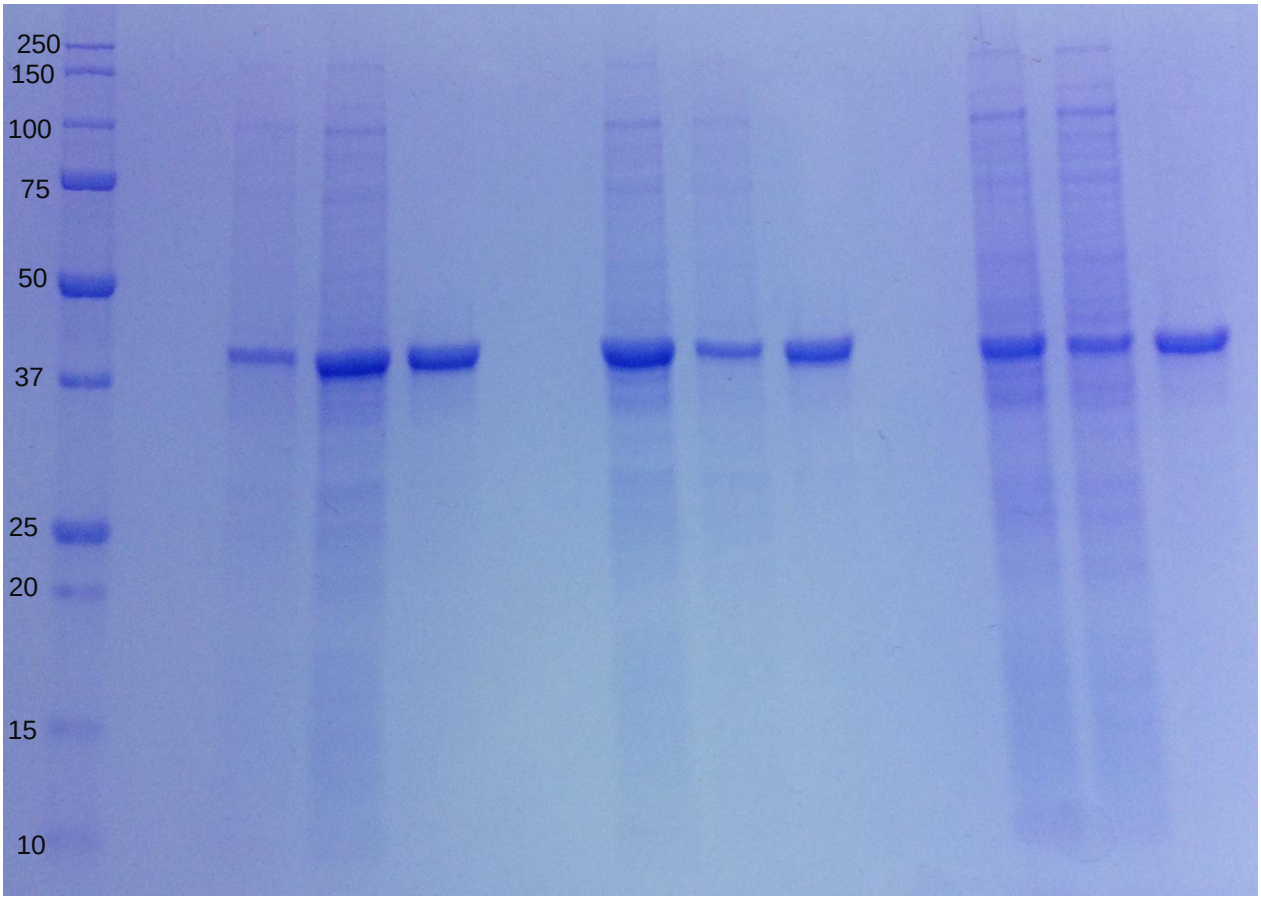


Figure S5. SDS-PAGE of *Ps*EFE C–H insertion variants. Each protein is shown from left to right as whole-cell sample, clarified lysate sample, and purified protein sample. Whole cells and lysates were diluted 25-fold; purified protein was diluted 50-fold (each dilution is prior to addition of 2X Laemmli loading dye). Protein 1: R171V F314M C317M, Protein 2: R171V R277H F314M C317M. Protein 3: T97M R171V R277H F314L C317M. The SDS-PAGE image brightness was increased in Microsoft Word for image clarity and is not being used for quantitation.

SDS-PAGE of all purified variants

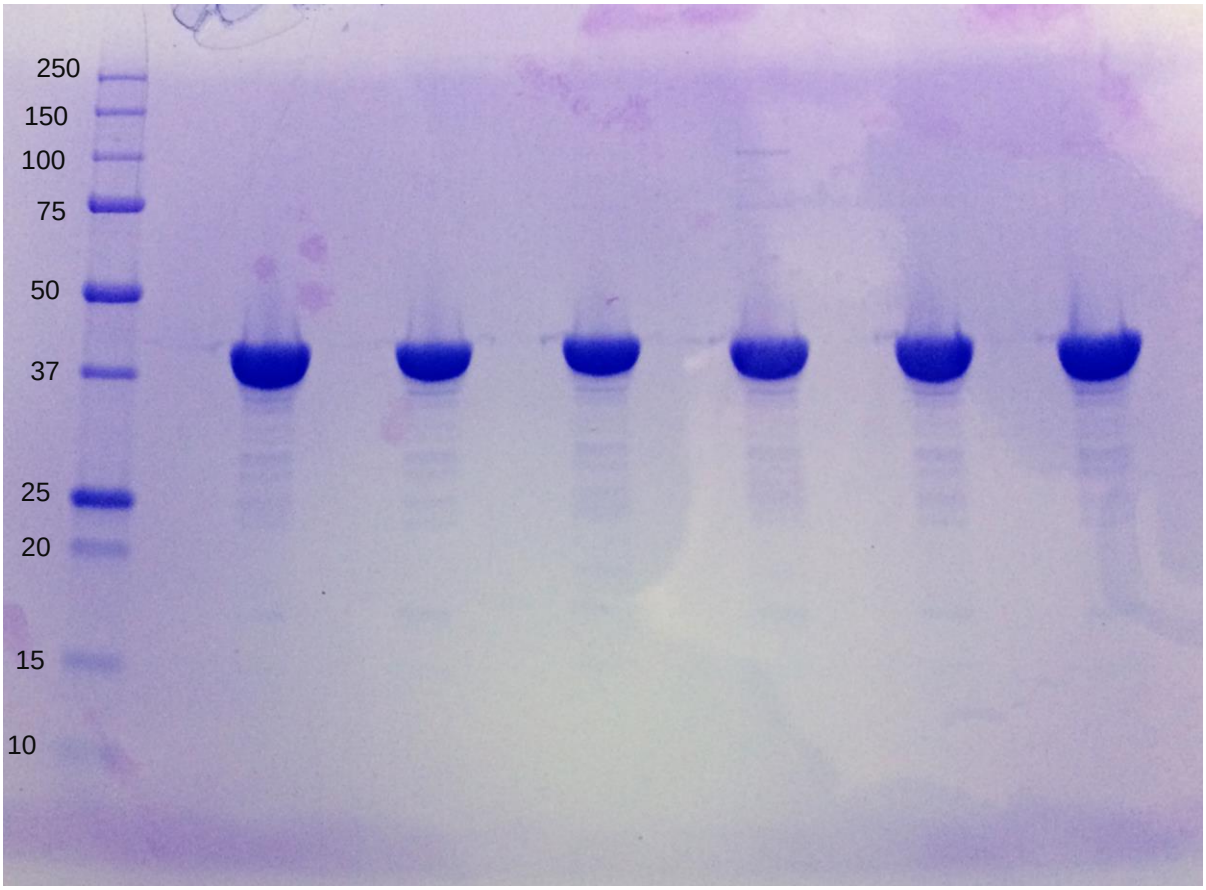


Figure S6. SDS-PAGE of all purified *Ps*EFE variants, diluted to approximately 1 mg mL^{-1} before addition of 2X loading buffer. From left to right: molecular weight ladder; wild type; H189A D191A; C317M; T97M R171L R277H F314M C317M; R171V F314M C317M; R171V R277H F314M C317M. The SDS-PAGE image brightness was increased in Microsoft Word for image clarity and is not being used for quantitation.

Reaction time courses

Aziridination time course

A time course was run with *Ps*EFE WT and *Ps*EFE MLLHMM, a variant with one additional mutation (I186L) relative to *Ps*EFE MLHMM with decreased activity and slightly increased enantioselectivity. Purified protein reactions were set up in triplicate both anaerobically and aerobically, as described in the methods section above (Analytical-scale biocatalytic aziridination reactions). Time points were taken at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 8 hours, at which point those reactions were quenched by addition of acetonitrile (350 µL) and the internal standard propiophenone (1 µL mL^{-1} in acetonitrile, 50 µL).

As we see in Figure S7, the reaction with wild-type *Ps*EFE is essentially done by the 15 minute mark, while the yield with MLLHMM continues to increase until approximately 2 hours. The reaction appears to proceed for a longer time anaerobically than aerobically for *Ps*EFE MLLHMM; the low activity and fast reaction

completion for wild-type *Ps*EFE makes such comparisons challenging.

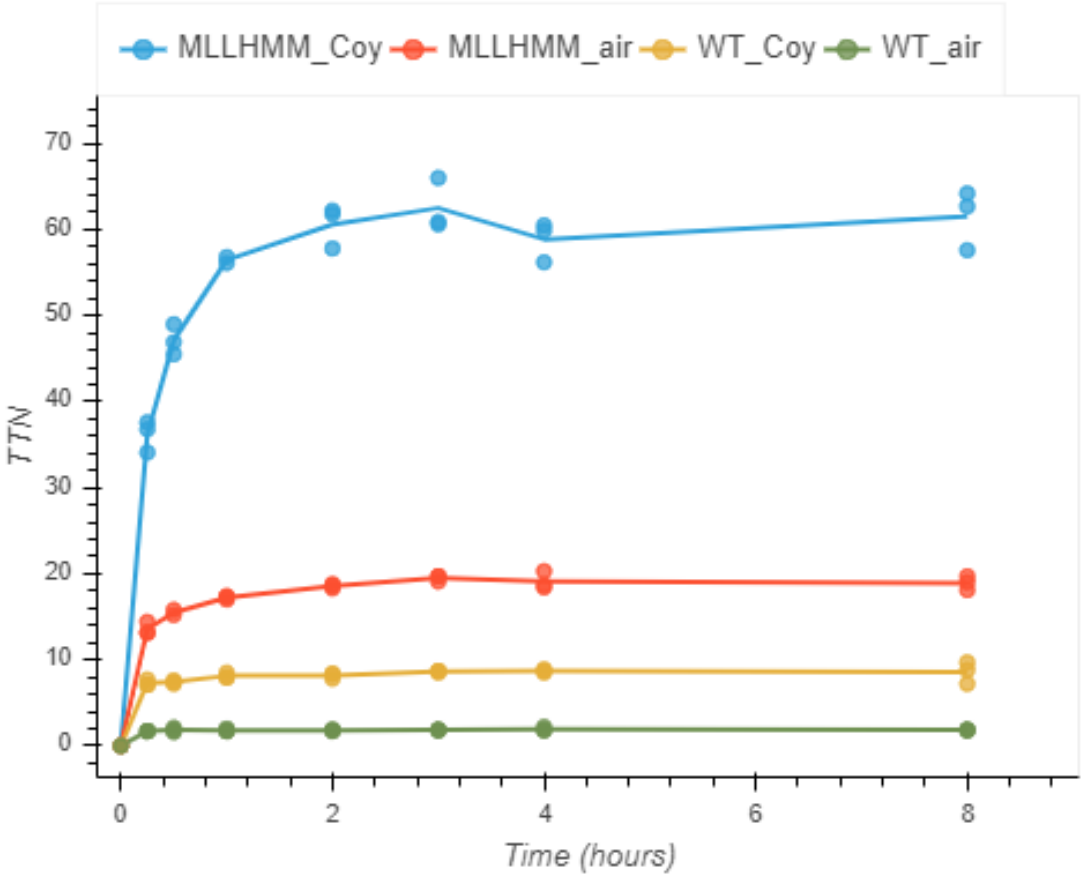


Figure S7. Aziridination time course. Time courses denoted “air” were set up aerobically; time courses denoted “Coy” were set up anaerobically.

Evolved variant thermostability

During our purified protein reactions, we do observe what is ostensibly protein precipitation over time. This visual observation, together with our time-course data, indicate that one possible reason for limited activity might be the protein’s stability to the reaction conditions over time. The thermostability of wild-type *Ps*EFE has been measured with ITC[3]; not surprisingly, the protein is reported to have increased stability in the presence of iron and α-ketoglutarate. We used the thermal shift assay[4] using SYPRO orange (Thermo Fisher Scientific).

Thermal shift assay samples were prepared in triplicate anaerobically under similar conditions as described above (Reaction condition controls). To a PCR tube with purified *Ps*EFE wild type or a *Ps*EFE variant (stripped and dialyzed, 10–15 µM final concentration) was added (to a final concentration of 1.25 mM each) either:

- ferrous ammonium sulfate

- ferrous ammonium sulfate, L-ascorbic acid, and α-ketoglutarate
- ferrous ammonium sulfate, L-ascorbic acid, and sodium acetate

Following these additions, to each tube was added 5 µL SYPRO orange (25-fold diluted in water). The PCR tubes were sealed, brought out of the anaerobic chamber, and analyzed on an Stratagene Mx3005P qPCR machine (Agilent Technologies, Inc.). The temperature program ran from 25 °C to 99 °C, holding for 30 seconds per degree before measuring fluorescence on the SYPRO channel and increasing temperature. The melting temperature for a given temperature was taken as the maximum of the numerical first derivative, representing the inflection point of the protein's melt curve.

The data are presented in Figure S8. We can see that, even though beneficial mutations were only chosen based on activity and stereoselectivity, the protein's stability improved from wild type to the final variants. We also see a significant enhancement in thermostability for early variants upon addition of α-ketoglutarate (noted as 2OG for 2-oxoglutarate), which is not observed in the later evolved variants.

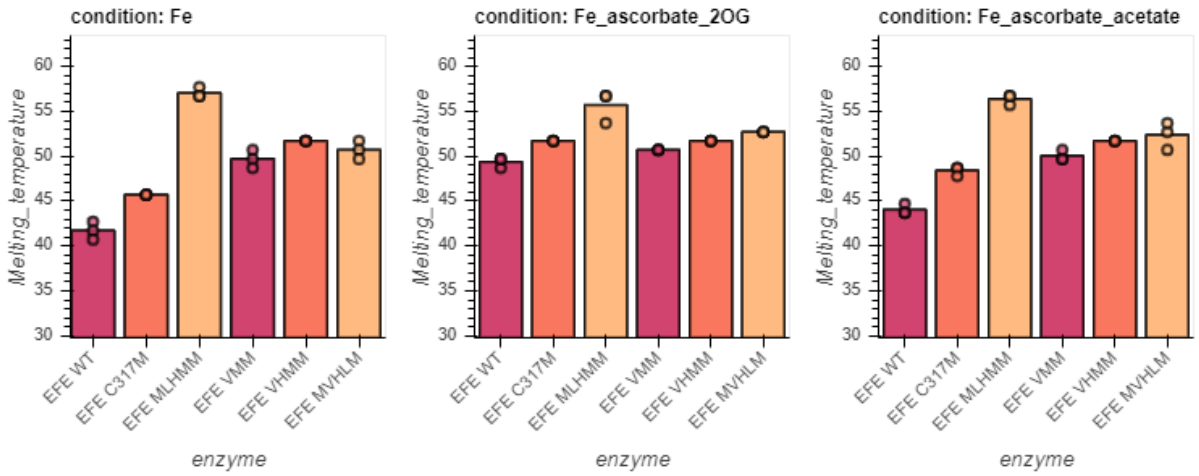


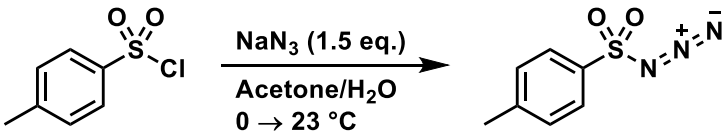
Figure S8. Thermostability of wild-type and evolved *Ps*EFE variants for aziridination and intramolecular C-H insertion.

Synthesis of sulfonyl azide substrates

Safety statement

Organic azides are potentially explosive compounds. We have not observed any problems in our handling of the compounds described, but care should be taken, especially on large scales.

p-Toluenesulfonyl azide (2)



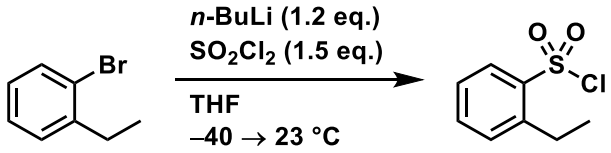
Under air, *p*-toluenesulfonyl chloride (19.1 g, 100 mmol, 1.00 equiv.) was dissolved in acetone (200 mL) in a 500 mL round-bottomed flask with a magnetic stir bar and cooled to 0 °C. A solution of sodium azide (9.75 g, 150 mmol, 1.50 equiv.) in water (60 mL) was added dropwise over one hour with stirring. Once addition was complete, the reaction was allowed to warm to room temperature and stirred for 16 hours. The acetone was removed *in vacuo*, and the resulting mixture was extracted with diethyl ether (2×100 mL). The combined organic layers were washed with water (2×100 mL), saturated aqueous sodium bicarbonate (100 mL), and brine (100 mL), then dried over magnesium sulfate and concentrated *in vacuo* to afford the title compound as a colorless oil that solidified upon storage at −20 °C (19.1 g, 97%).

NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.80 (d, *J* = 8.5 Hz, 2 H), 7.38 (d, *J* = 8.5 Hz, 2 H), 2.45 (s, 3 H)

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 146.3, 135.3, 130.3, 127.4, 21.7

2-Ethylbenzenesulfonyl chloride (S1)[5]

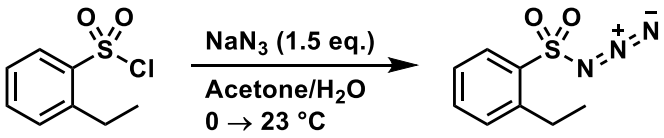


Under argon, 1-bromo-2-ethylbenzene (2.07 mL, 2.78 g, 15.0 mmol, 1.00 equiv.) was dissolved in anhydrous tetrahydrofuran (30 mL) in a 100 mL round-bottomed flask with magnetic stirring and cooled to −40 °C. *n*-Butyllithium (2.5 M solution in hexanes, 7.20 mL, 18.0 mmol, 1.20 equiv.) was added dropwise by syringe over two minutes. The reaction was stirred at −40 °C for thirty minutes, then sulfuryl chloride (1.82 mL, 3.04 g, 22.5 mmol, 1.5 equiv.) was added dropwise by syringe over two minutes. The reaction was allowed to warm to room temperature and stirred for 16 hours. The reaction was cooled to 0 °C, then carefully quenched by the addition of ice-cold water (50 mL). The resulting mixture was extracted with diethyl ether (2×50 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica (50 g), eluting with a gradient of 0 to 20% diethyl ether/hexanes, to afford the title compound as a slightly yellow oil (1.10 g, 36%).

NMR Spectroscopy:

^{1}H NMR (400 MHz, $CDCl_3$, 23 ºC, δ): 8.07 (d, *J* = 8.1 Hz, 1 H), 7.66 (t, *J* = 7.5 Hz, 1 H), 7.49 (d, *J* = 7.6 Hz, 1 H), 7.41 (t, *J* = 7.9 Hz, 1 H), 3.20 (q, *J* = 7.5 Hz, 2 H), 1.37 (t, *J* = 7.5 Hz, 3 H)

2-Ethylbenzenesulfonyl azide (4)[5]



Under air, 2-ethylbenzenesulfonyl chloride **S1** (1.00 g, 4.89 mmol, 1.00 equiv.) was dissolved in acetone (8 mL) in a 20 mL scintillation vial with magnetic stirring and cooled to 0 °C. A solution of sodium azide (476 mg, 7.33 mmol, 1.50 equiv.) in water (2.5 mL) was added dropwise over two minutes with stirring. Once addition was complete, the reaction was allowed to warm to room temperature and stirred for six hours. The acetone was removed *in vacuo*, and the resulting mixture was extracted with diethyl ether (2×15 mL). The combined organic layers were washed with water, saturated aqueous sodium bicarbonate, and brine (15 mL each), then dried over magnesium sulfate and concentrated *in vacuo* to afford the title compound as a yellow oil (1.01 g, 98%).

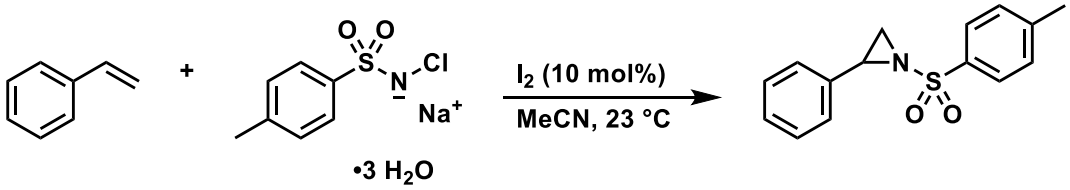
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 8.01 (dd, *J* = 8.0, 1.3 Hz, 1 H), 7.62 (td, *J* = 7.6, 1.3 Hz, 1 H), 7.46 (dd, *J* = 7.7, 0.7 Hz, 1 H), 7.37 (td, *J* = 8.0, 1.2 Hz, 1 H), 3.03 (q, *J* = 7.5 Hz, 2 H), 1.31 (t, *J* = 7.5 Hz, 3 H)

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 144.5, 136.3, 134.9, 131.4, 129.4, 126.4, 26.1, 15.2

Synthesis of authentic product standards

2-Phenyl-1-(*p*-toluenesulfonyl)aziridine (*rac*-3)



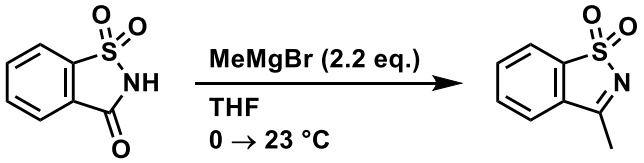
Under argon, chloramine-T trihydrate (4.23 g, 15.0 mmol, 1.00 equiv.) and iodine (381 mg, 1.50 mmol, 0.100 equiv.) were dissolved in acetonitrile (100 mL). Styrene (3.45 mL, 3.13 g, 30.0 mmol, 2.00 equiv.) was added dropwise, and the reaction was stirred at room temperature for 18 hours. The reaction mixture was partitioned between water (50 mL) and dichloromethane (100 mL), and the layers were separated. The aqueous layer was extracted with dichloromethane (2×100 mL). The combined organic layers were concentrated *in vacuo* and the residue was purified by flash column chromatography on silica (100 g) eluting with hexanes/ethyl acetate (6:1 v/v) containing 1% triethylamine to afford the title compound as a colorless solid (3.55 g, 87%).

NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.87 (d, *J* = 8.3 Hz, 2 H), 7.33 (d, *J* = 8.3 Hz, 2 H), 7.30–7.25 (m, 3 H), 7.24–7.20 (m, 2 H), 3.78 (dd, *J* = 7.2, 4.5 Hz, 1 H), 2.99 (d, *J* = 7.2 Hz, 1 H), 2.44 (s, 3 H), 2.40 (d, *J* = 4.4 Hz, 1 H)

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 144.8, 135.1, 135.0, 129.9, 128.7, 128.4, 128.1, 126.7, 41.2, 36.1, 21.8

3-Methylbenzo[*d*]isothiazole 1,1-dioxide (S2)[6]

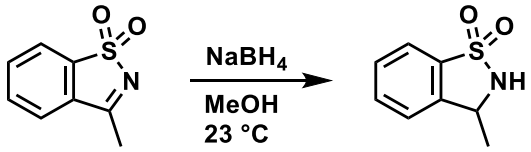


Under argon, saccharin (1.00 g, 5.46 mmol, 1.00 equiv.) was dissolved in anhydrous tetrahydrofuran (25 mL) in a 100 mL round-bottomed flask with magnetic stirring and cooled to 0 °C. Methylmagnesium bromide (3 M solution in diethyl ether, 4.00 mL, 12.0 mmol, 2.20 equiv.) was added dropwise by syringe over 10 minutes. After addition, the reaction was stirred at 0 °C for five minutes, then slowly warmed to room temperature and stirred at room temperature for 16 hours. The reaction was then cooled to 0 °C and carefully poured into ice-cold hydrochloric acid (1 M, 30 mL). The resulting mixture was extracted with dichloromethane (2×50 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo* to afford the title compound as a colorless solid (crude yield 1.04 g, 105%) which was used in the next step without further purification.

NMR Spectroscopy:

^{1}H NMR (400 MHz, $CDCl_3$, 23 ºC, δ): 7.94–7.90 (m, 1 H), 7.77–7.73 (m, 2 H), 7.71–7.67 (m, 1 H), 2.67 (s, 3 H)

3-Methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (*rac*-5)



Under air, 3-methyl-[*d*]isothiazole 1,1-dioxide **S2** (500 mg, 2.76 mmol, 1.00 equiv.) was dissolved in methanol (20 mL) in a 50 mL round-bottomed flask with magnetic stirring. Sodium borohydride (522 mg, 13.8 mmol, 5.00 equiv.) was slowly added over two minutes. The reaction mixture bubbled vigorously and became warm to the touch. The reaction was stirred at room temperature for thirty minutes to ensure complete reaction. The reaction was cooled to 0 °C, then poured carefully into cold hydrochloric acid (2.5 M, 40 mL). The methanol was removed *in vacuo* and the resulting mixture was extracted with dichloromethane

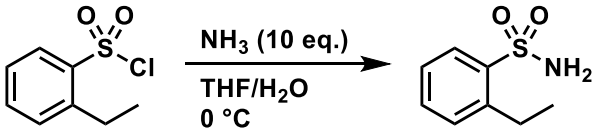
(3×25 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica (25 g), eluting with a gradient of 10 to 60% ethyl acetate/hexanes to afford the title compound as a colorless solid (378 mg, 75%).

NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.74 (d, *J* = 8.0 Hz, 1 H), 7.61 (td, *J* = 7.6, 1.0 Hz, 1 H), 7.50 (t, *J* = 7.6 Hz, 1 H), 7.38 (d, *J* = 7.7 Hz, 1 H), 5.15 (br d, *J* = 4.8 Hz, 1 H), 4.78 (qd, *J* = 6.7, 4.8 Hz, 1 H), 1.59 (d, *J* = 6.7 Hz, 3 H)

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 141.8, 135.4, 133.3, 129.2, 124.0, 121.2, 53.5, 21.5

2-Ethylbenzenesulfonamide (6)



Under air, 2-ethylbenzenesulfonyl chloride **S1** (50.0 mg, 244 µmol, 1.00 equiv.) was dissolved in tetrahydrofuran (1 mL) in a 4 mL vial with magnetic stirring and cooled to 0 °C. Ammonia (28% w/v in water, 149 µL, 2.44 mmol, 10.0 equiv.) was added dropwise over one minute. After stirring for five minutes, the reaction mixture was partitioned between water and ethyl acetate (10 mL each). The layers were separated and the aqueous layer was extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with brine (10 mL), dried over sodium sulfate, and concentrated *in vacuo* to afford the title compound as a colorless solid (41.8 mg, 92%).

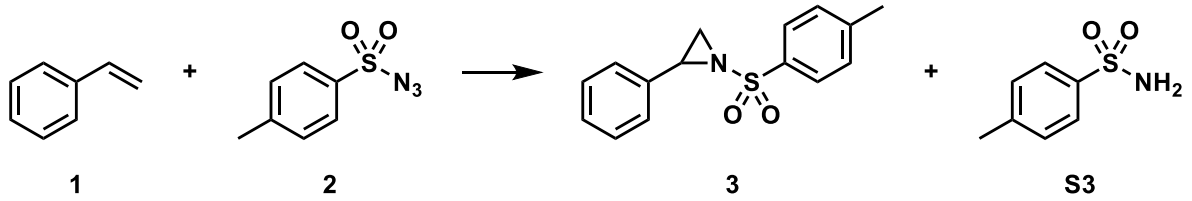
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.99 (d, *J* = 8.0 Hz, 1 H), 7.51 (t, *J* = 7.5 Hz, 1 H), 7.39 (d, *J* = 7.6 Hz, 1 H), 7.29 (t, *J* = 7.7 Hz, 1 H), 5.02 (br s, 2 H), 3.07 (q, *J* = 7.5 Hz, 2 H), 1.33 (t, *J* = 7.5 Hz, 3 H)

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ):143.0, 139.6, 133.1, 130.7, 128.3, 126.2, 26.1, 15.3

HPLC analytical methods and calibration curves

Aziridination reaction



1 2 3 S3

Samples for HPLC calibration curves were prepared as simulated reaction samples. To MOPS buffer (20 mM pH 7.0, 380 µL) was added a solution of the appropriate reaction product in acetonitrile (0–100 µM, 20 µL, final concentration 0–5 mM). To this sample was added the internal standard propiophenone (0.1% v/v in acetonitrile, 50 µL) and acetonitrile (350 µL). The product concentration in the curves below corresponds to the concentration in the reaction mixture; the final analytical sample is two-fold diluted.

Analysis was performed on an Agilent 1200 series HPLC with water/acetonitrile mobile phase (1 mL min^{-1} flow), with an Agilent Poroshell 120 EC-C18 column (4 µm packing, 2.1×50 mm) fitted with a Poroshell 120 guard column (1.7 µm packing, 2.1×5 mm), injecting 5 µL. Detection was at 230 nm (16 nm bandwidth). The gradient program and retention times are given in Table S7 and Table S8, respectively.

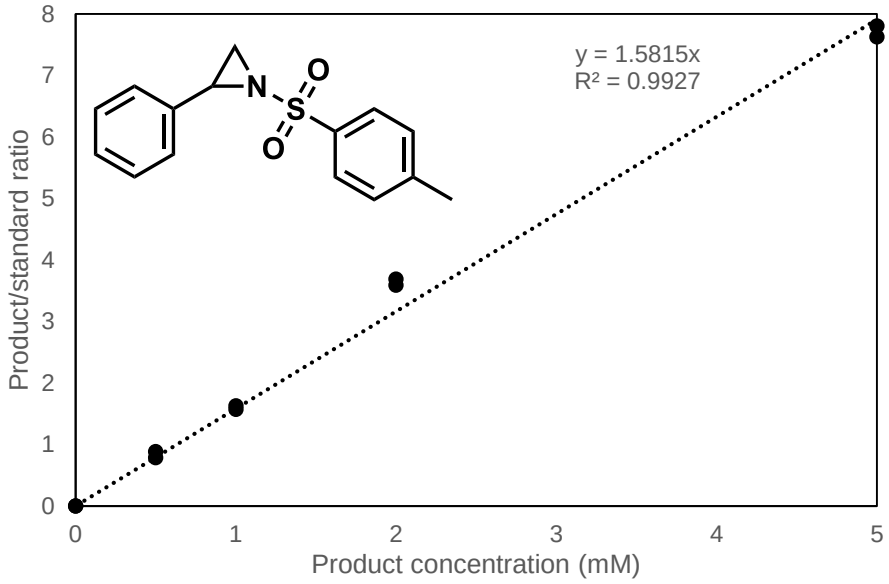
Table S7. HLPC gradient program for aziridination analysis

Time (minutes)	% Acetonitrile
0.00	20
0.50	20
1.00	40
5.00	65
5.50	95
6.00	95
6.01	20
7.00	20

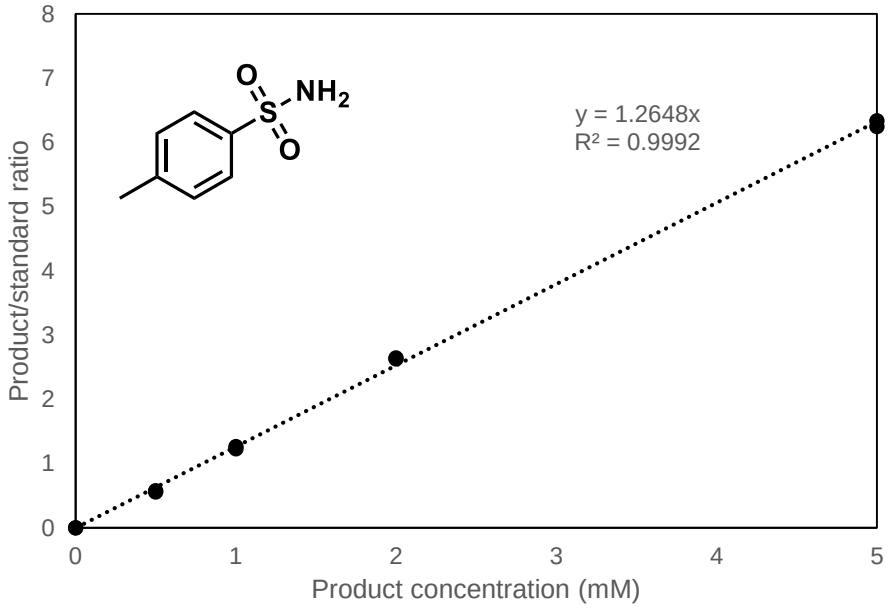
Table S8. HPLC retention times for aziridination analysis

Compound	Retention time (minutes)
p-Toluenesulfonamide	0.58
Propiophenone	2.24
p-Toluenesulfonyl azide	2.81
Styrene	3.01
2-Phenyl-1-(*p*-toluenesulfonyl)aziridine	3.45

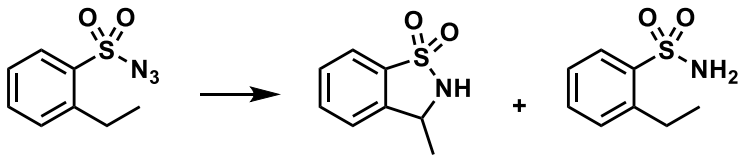
2-Phenyl-1-(*p*-toluenesulfonyl)aziridine (3) calibration curve



***p*-Toluenesulfonamide (S3) calibration curve**



C–H insertion reaction



4 **5** **6**

Calibration curve samples were prepared as described above for the aziridination reaction, except the internal standard used was propiophenone (0.5% v/v in acetonitrile, 50 µL) and the final product concentrations ranged from 1–10 mM. The product concentration shown corresponds to the concentration in the reaction mixture; the final analytical sample is twofold diluted.

Analysis was performed on an Agilent 1260 Infinity II HPLC instrument with water/acetonitrile mobile phase (1 mL min^{-1} flow), with an Agilent Poroshell 120 EC-C18 column (4 µm packing, 2.1×50 mm) fitted with a Poroshell 120 guard column (1.7 µm packing, 2.1×5 mm), injecting 5 µL. Detection was at 220 nm (4 nm bandwidth). The gradient program and retention times are given in Table S9 and Table S10, respectively.

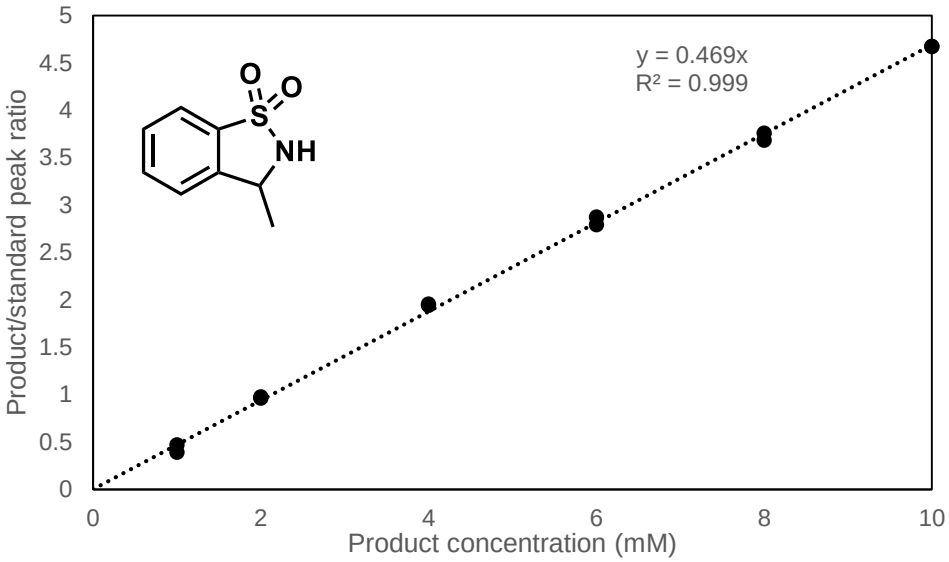
Table S9. HPLC gradient program for C–H insertion analysis

Time (minutes)	% Acetonitrile
0.00	12
1.00	12
3.50	95
4.00	95
4.01	12
5.00	12

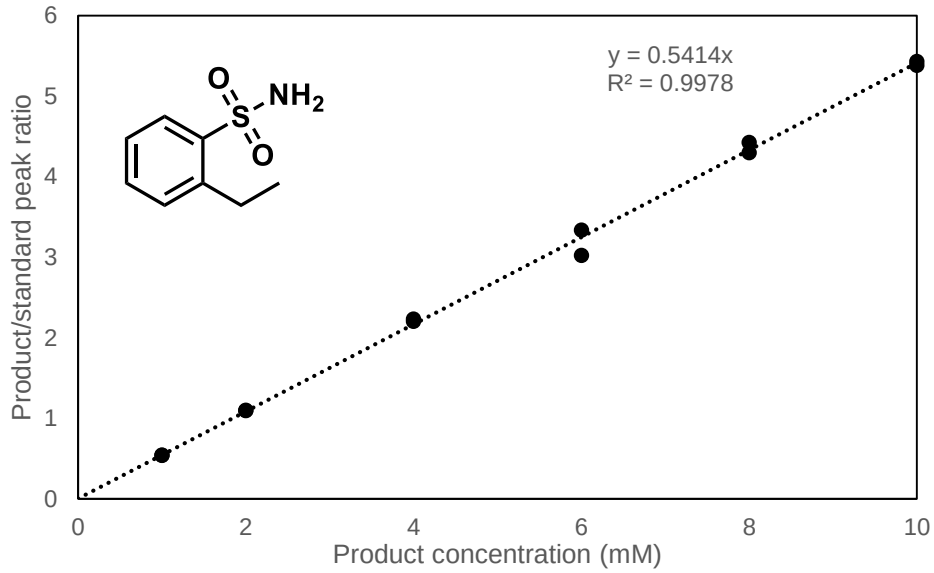
Table S10. HPLC retention times for C–H insertion analysis

Compound	Retention time (minutes)
3-Methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide	1.05
2-Ethylbenzenesulfonamide	2.38
Propiophenone	3.00
2-Ethylbenzenesulfonyl azide	3.48

3-Methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (5) calibration curve



2-Ethylbenzenesulfonamide (6) calibration curve



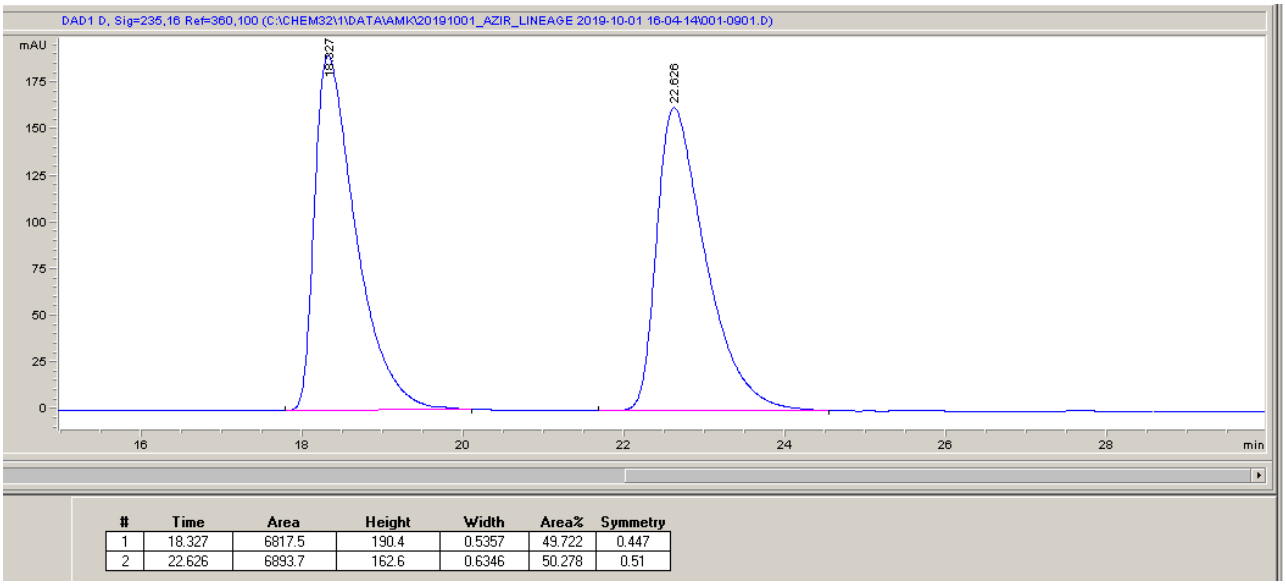
Chiral analysis

Chiral analysis was performed by HPLC with a chiral stationary phase, using a Hewlett Packard Series 1100 HPLC instrument with hexanes/2-propanol mobile phase (1 mL min^{-1} flow).

2-Phenyl-1-(*p*-toluenesulfonyl)aziridine (3)

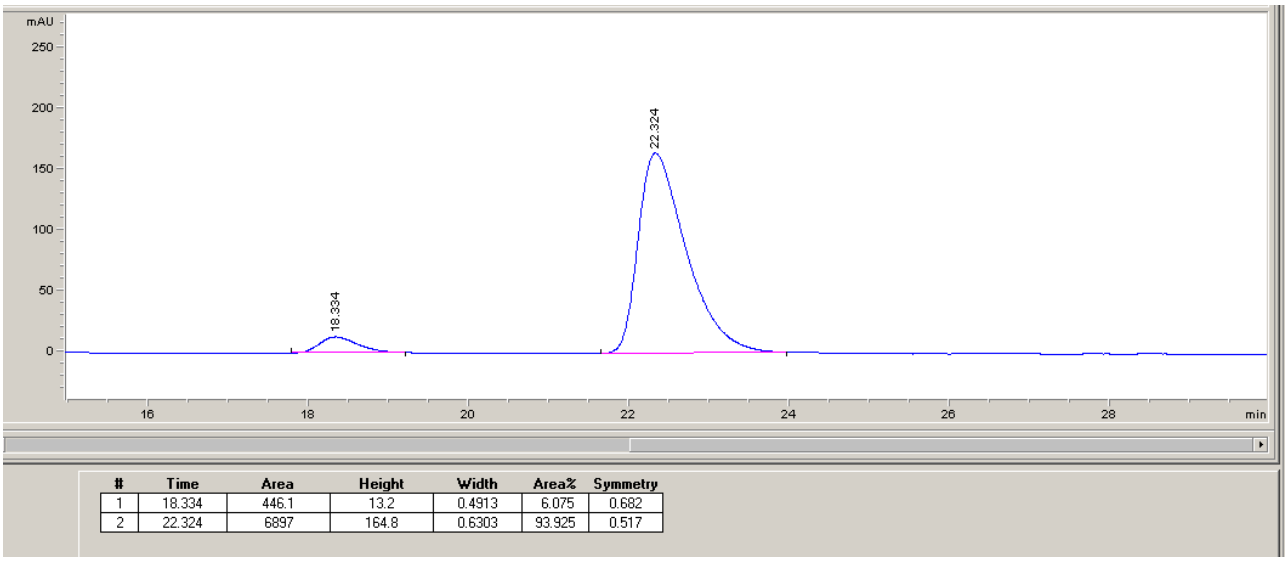
Analysis was performed with a Daicel Chiralcel OJ-H column, (5 µm packing, 4.6×250 mm), with an isocratic 30% 2-propanol/70% hexanes mobile phase. The peak areas were analyzed at 235 nm (16 nm bandwidth).

Absolute configuration was assigned by reference to the literature[7]. The enantiomers elute in the order (*S*), (*R*).



#	Time	Area	Height	Width	Area%	Symmetry
1	18.327	6817.5	190.4	0.5357	49.722	0.447
2	22.626	6893.7	162.6	0.6346	50.278	0.51

Figure S9. Chiral HPLC trace of *rac*-**3**.

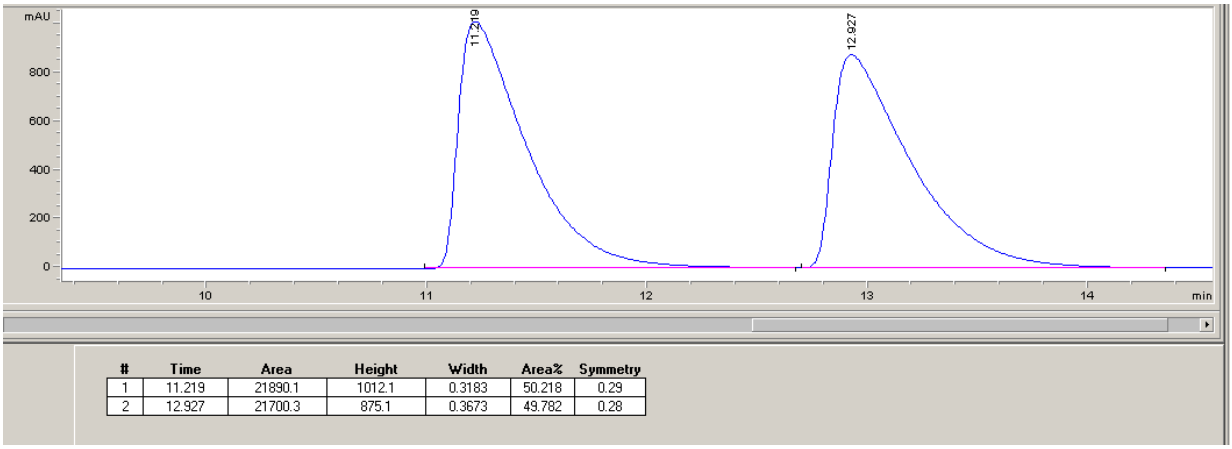


#	Time	Area	Height	Width	Area%	Symmetry
1	18.334	446.1	13.2	0.4913	6.075	0.682
2	22.324	6897	164.8	0.6303	93.925	0.517

Figure S10. Chiral HPLC trace of *Ps*EFE MLHMM-catalyzed product **3**.

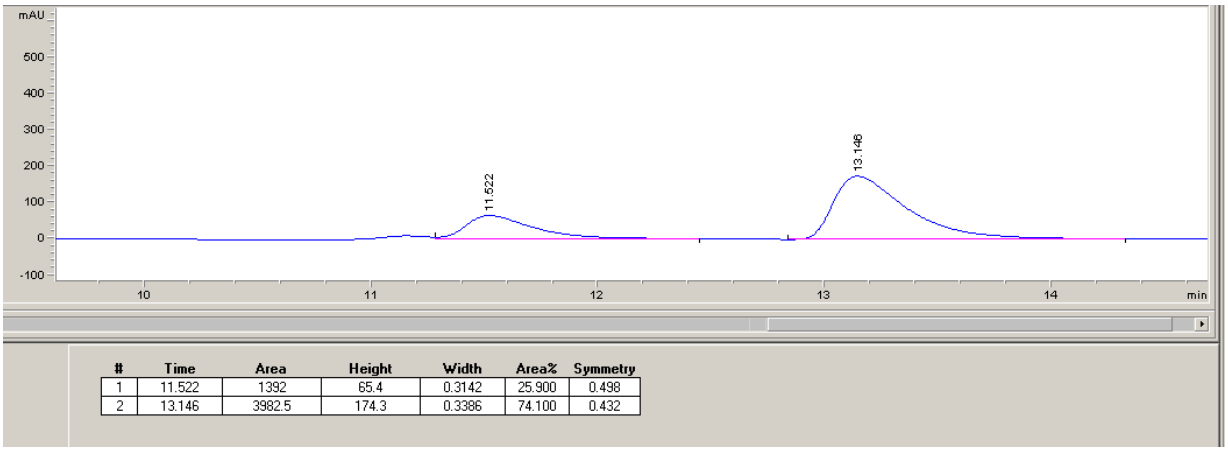
3-Methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (5)

Analysis was performed with a Daicel Chiralpak IB column (5 µm packing, 4.6×250 mm), with an isocratic 25% 2-propanol/75% hexanes mobile phase. The peak areas were analyzed at 220 nm (16 nm bandwidth).



#	Time	Area	Height	Width	Area%	Symmetry
1	11.219	21890.1	1012.1	0.3183	50.218	0.29
2	12.927	21700.3	875.1	0.3673	49.782	0.28

Figure S11. Chiral HPLC trace of *rac*-**5**.



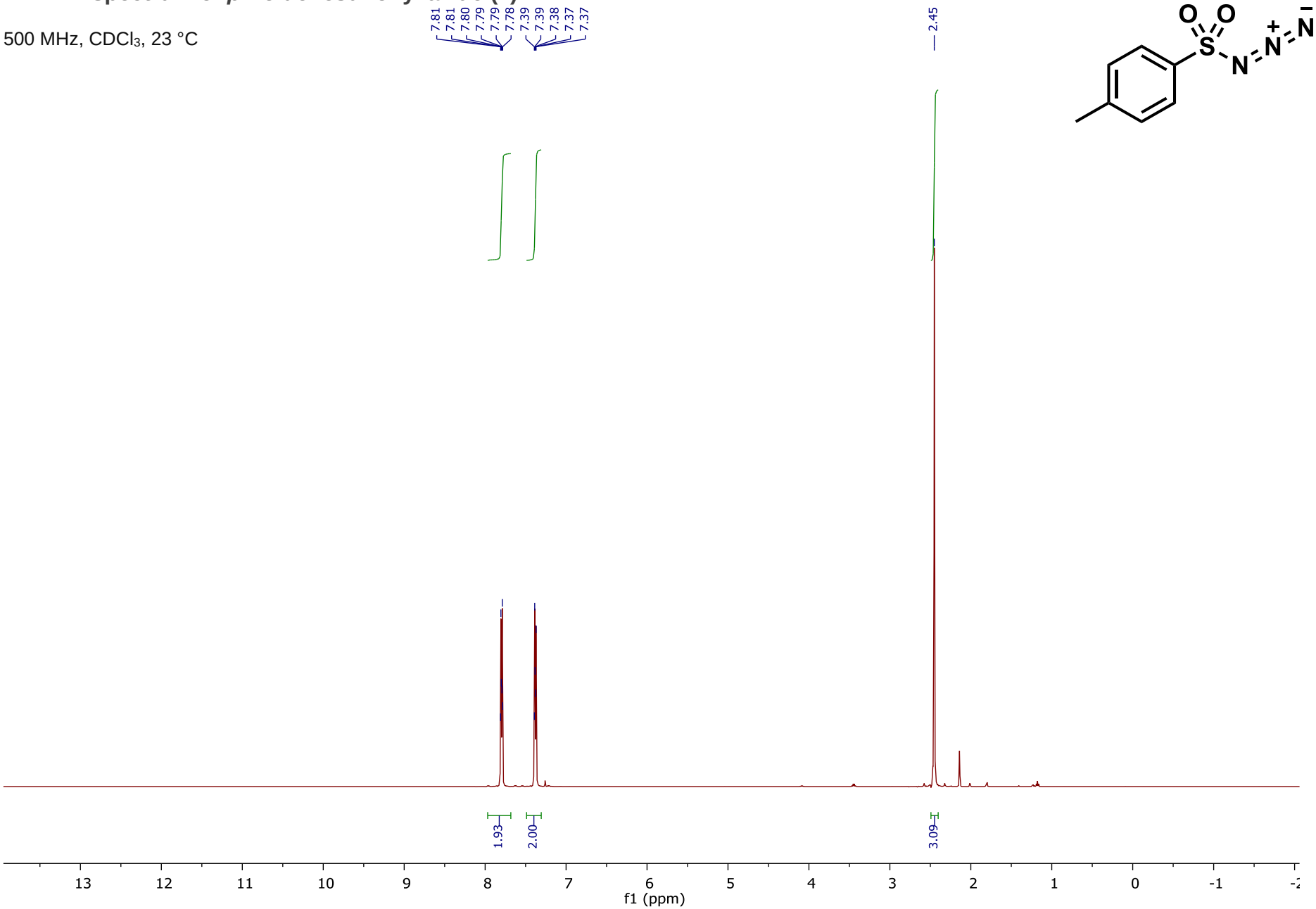
#	Time	Area	Height	Width	Area%	Symmetry
1	11.522	1392	65.4	0.3142	25.900	0.498
2	13.146	3982.5	174.3	0.3386	74.100	0.432

Figure S12. Chiral HPLC trace of *Ps*EFE VMM-catalyzed product **5**.

SPECTROSCOPIC DATA

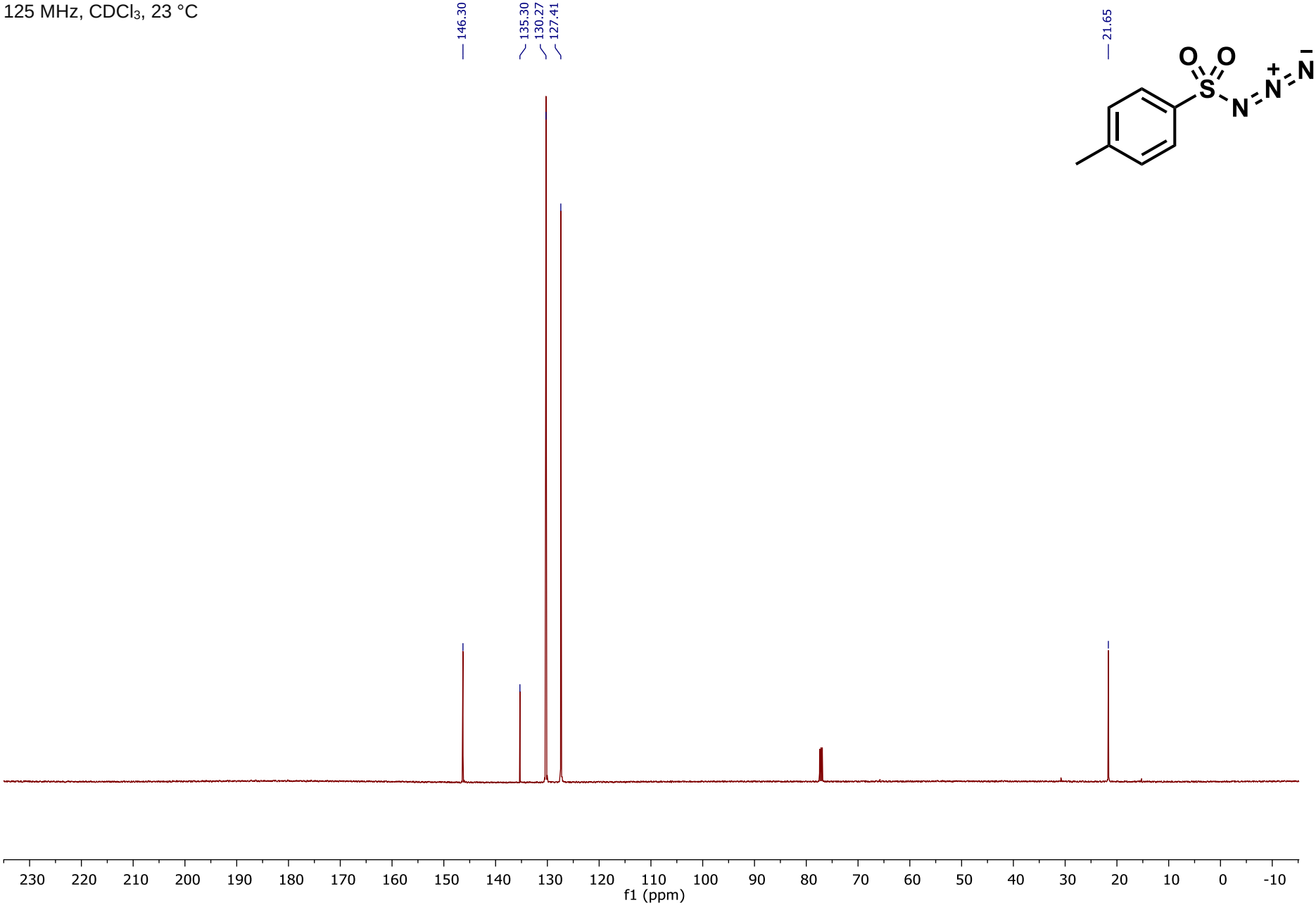
^{1}H NMR spectrum of *p*-Toluenesulfonyl azide (2)

500 MHz, $CDCl_3$, 23 °C



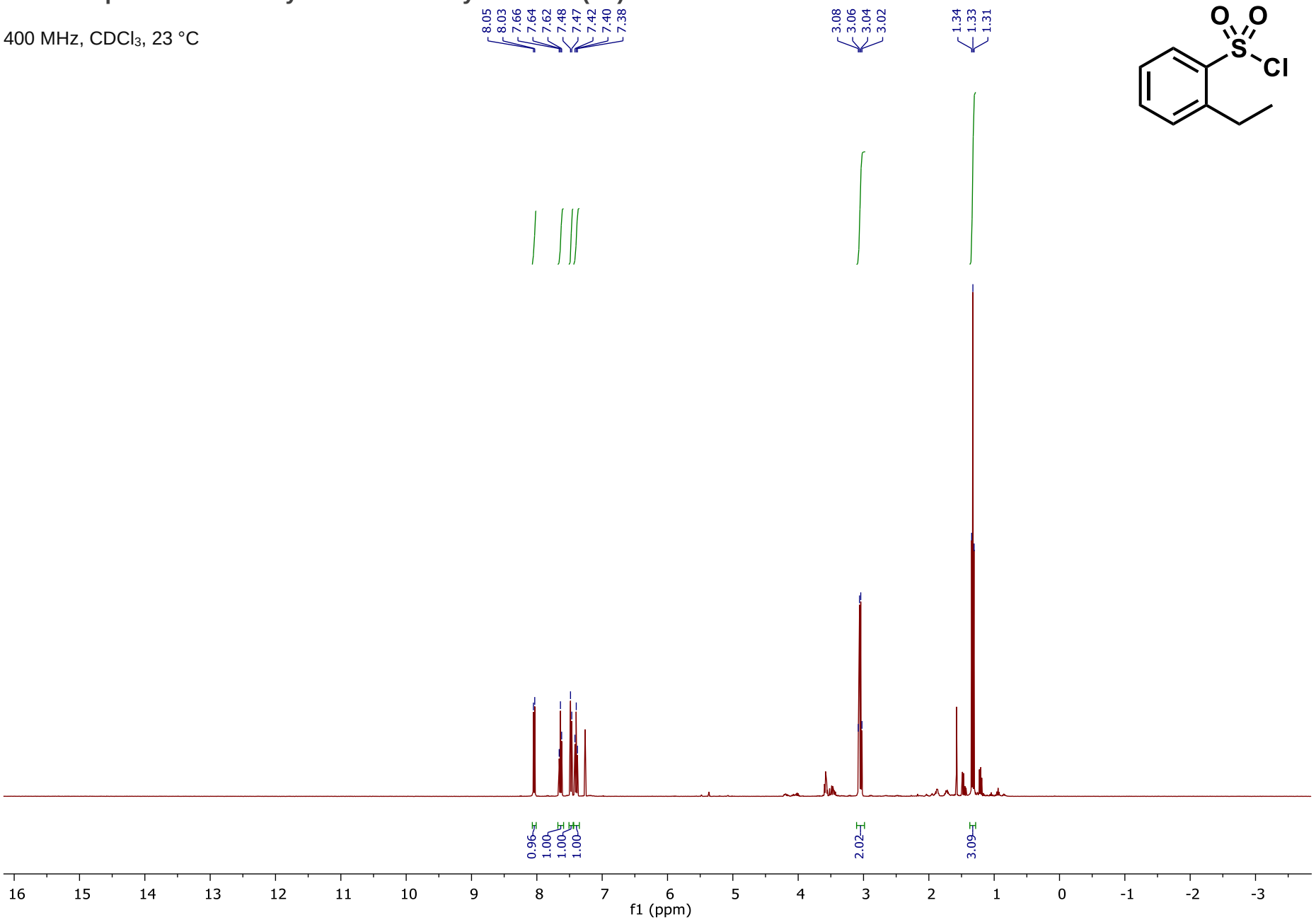
^{13}C NMR spectrum of *p*-Toluenesulfonyl azide (2)

125 MHz, $CDCl_3$, 23 °C



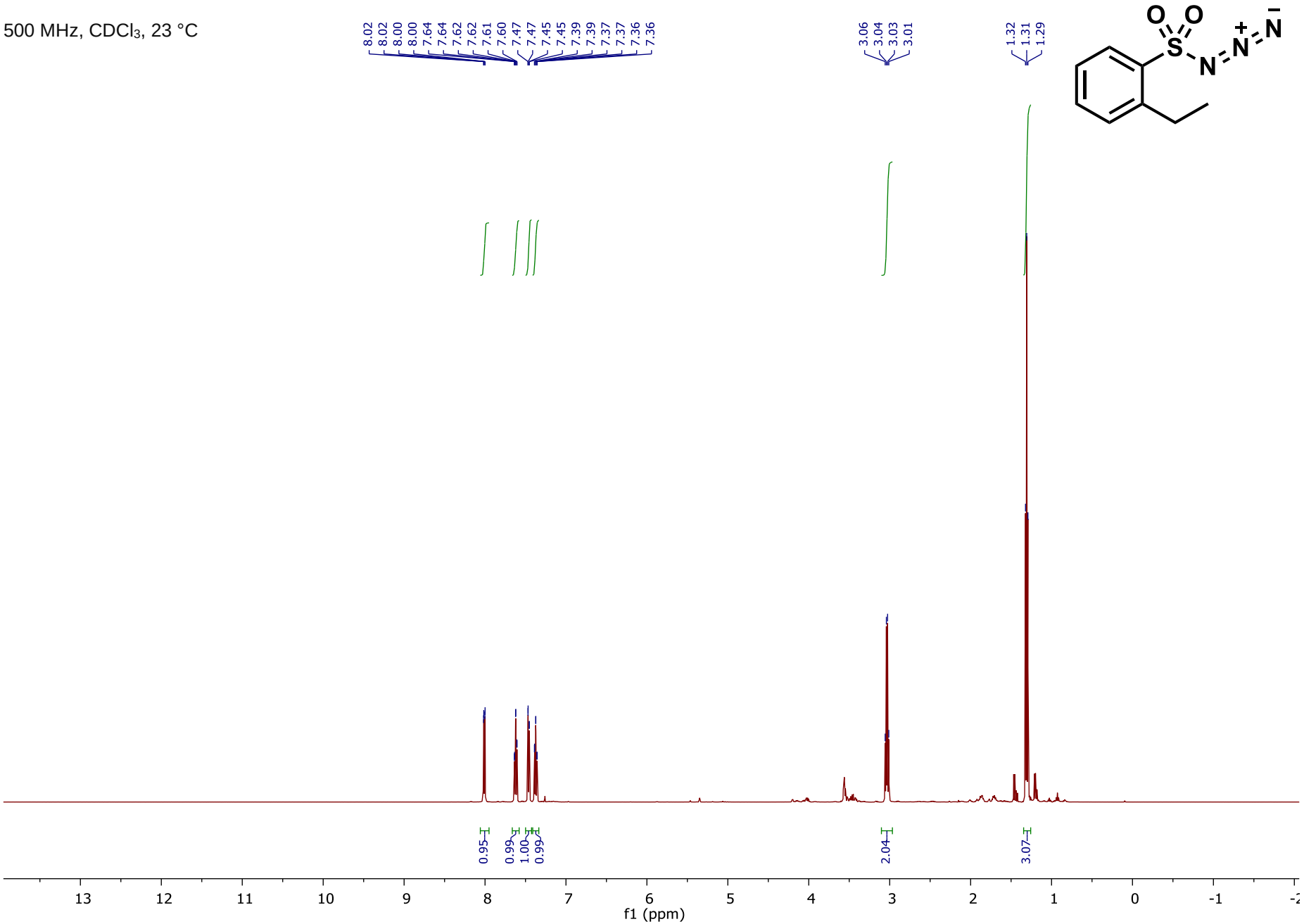
^{1}H NMR spectrum of 2-Ethylbenzenesulfonyl chloride (S1)

400 MHz, $CDCl_3$, 23 °C



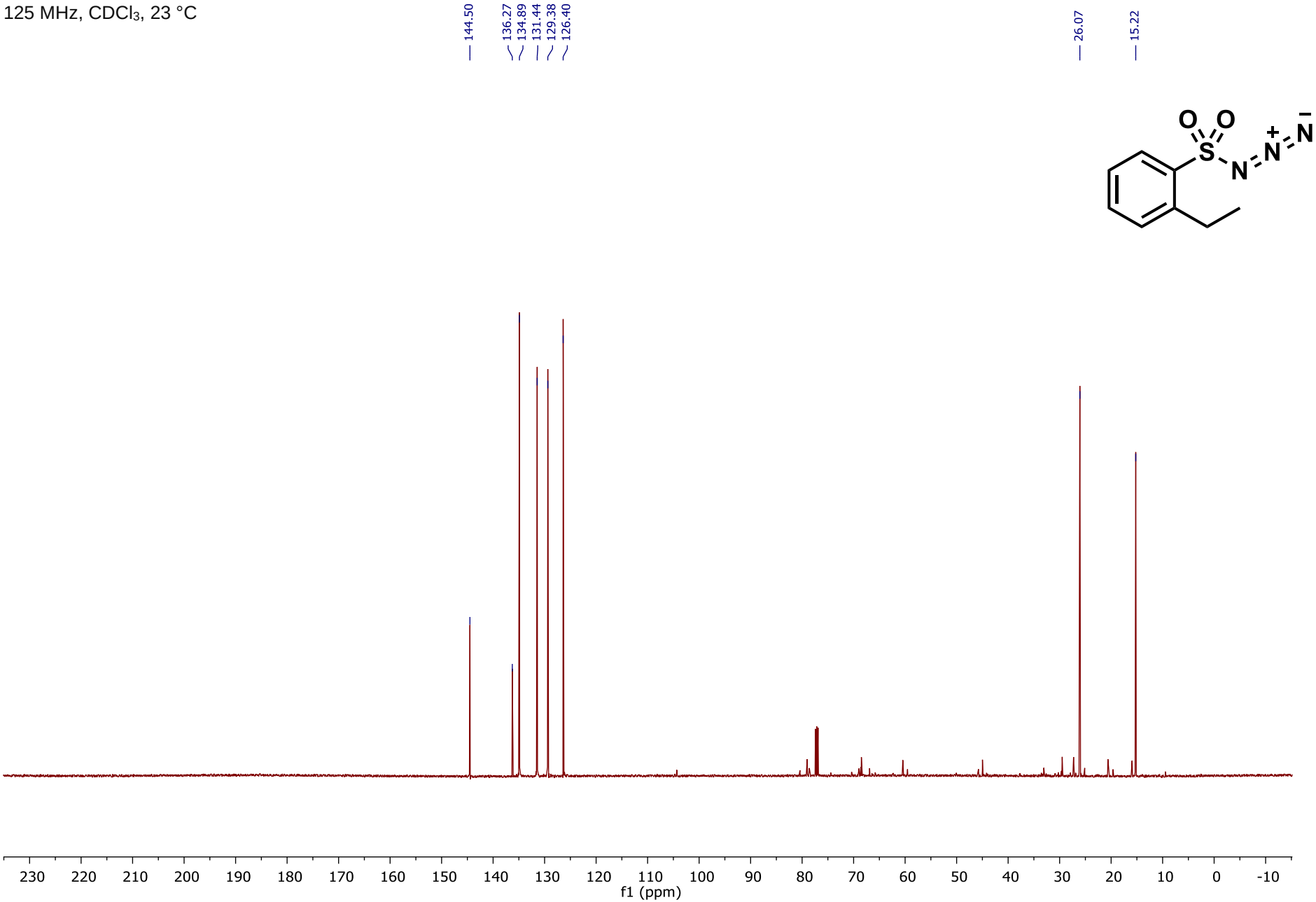
^{1}H NMR spectrum of 2-Ethylbenzenesulfonyl azide (4)

500 MHz, $CDCl_3$, 23 °C



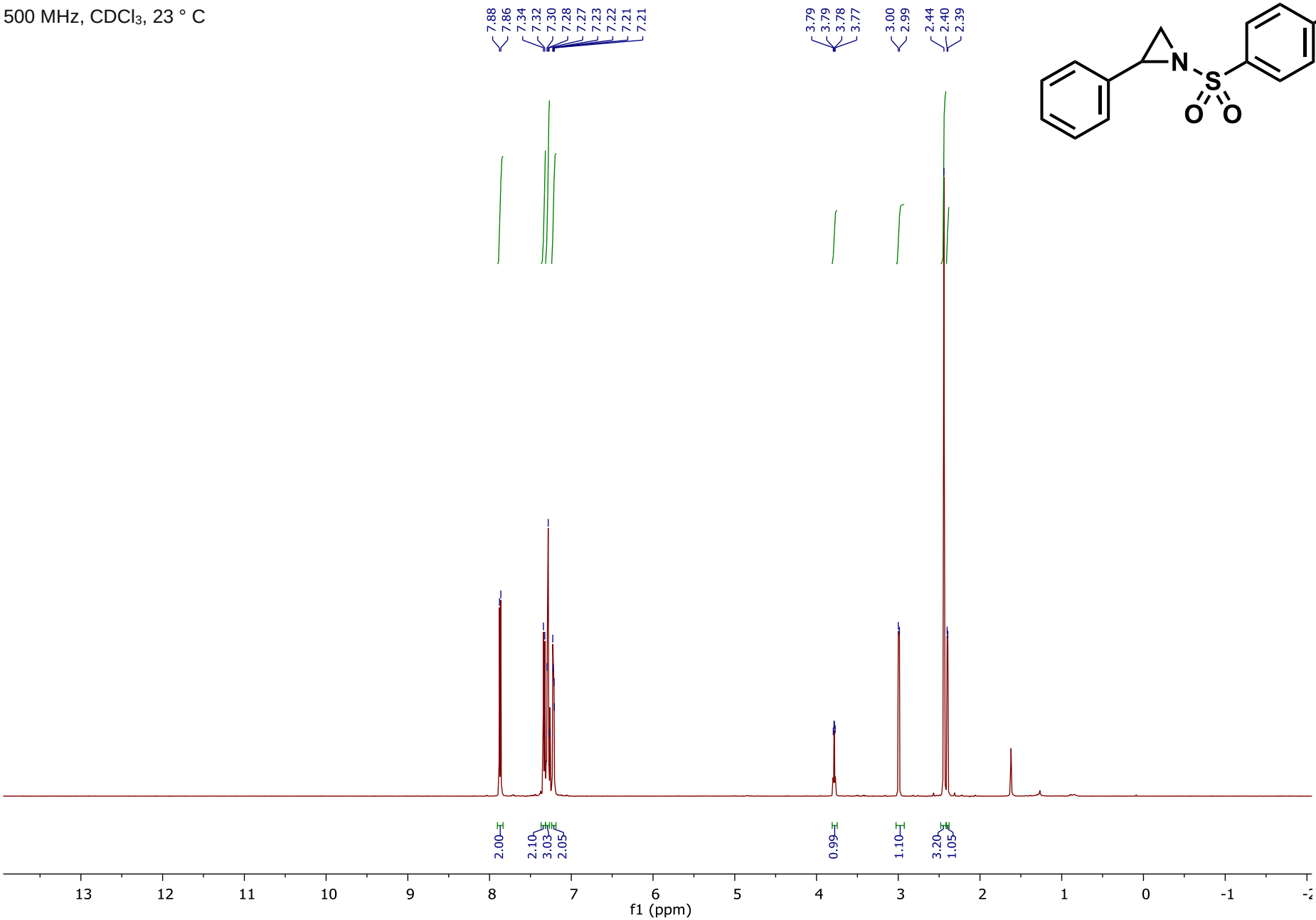
^{13}C NMR spectrum of 2-Ethylbenzenesulfonyl azide (4)

125 MHz, $CDCl_3$, 23 °C



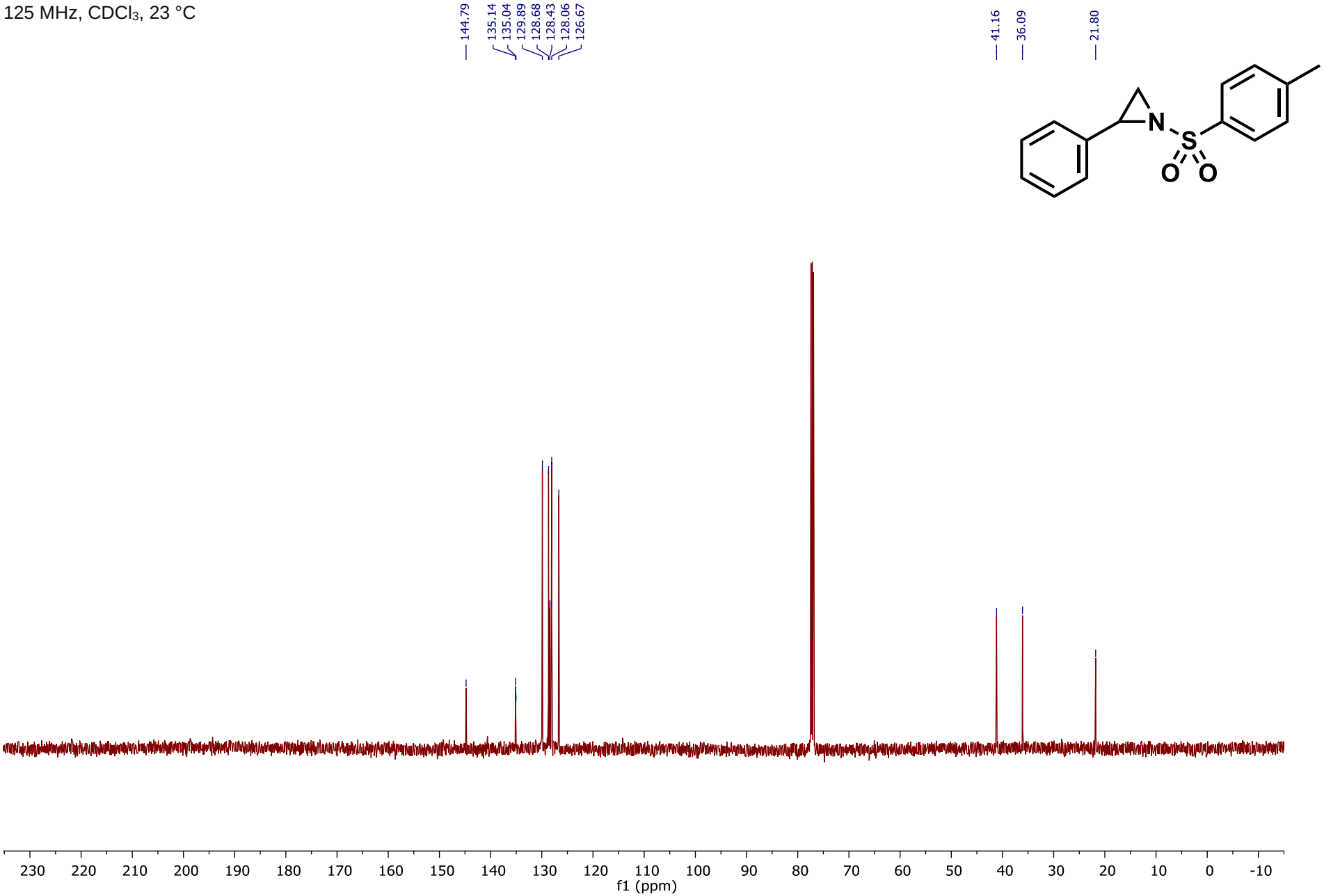
^{1}H NMR spectrum of 2-Phenyl-1-(*p*-toluenesulfonyl)aziridine (*rac*-3)

500 MHz, $CDCl_3$, 23 ° C



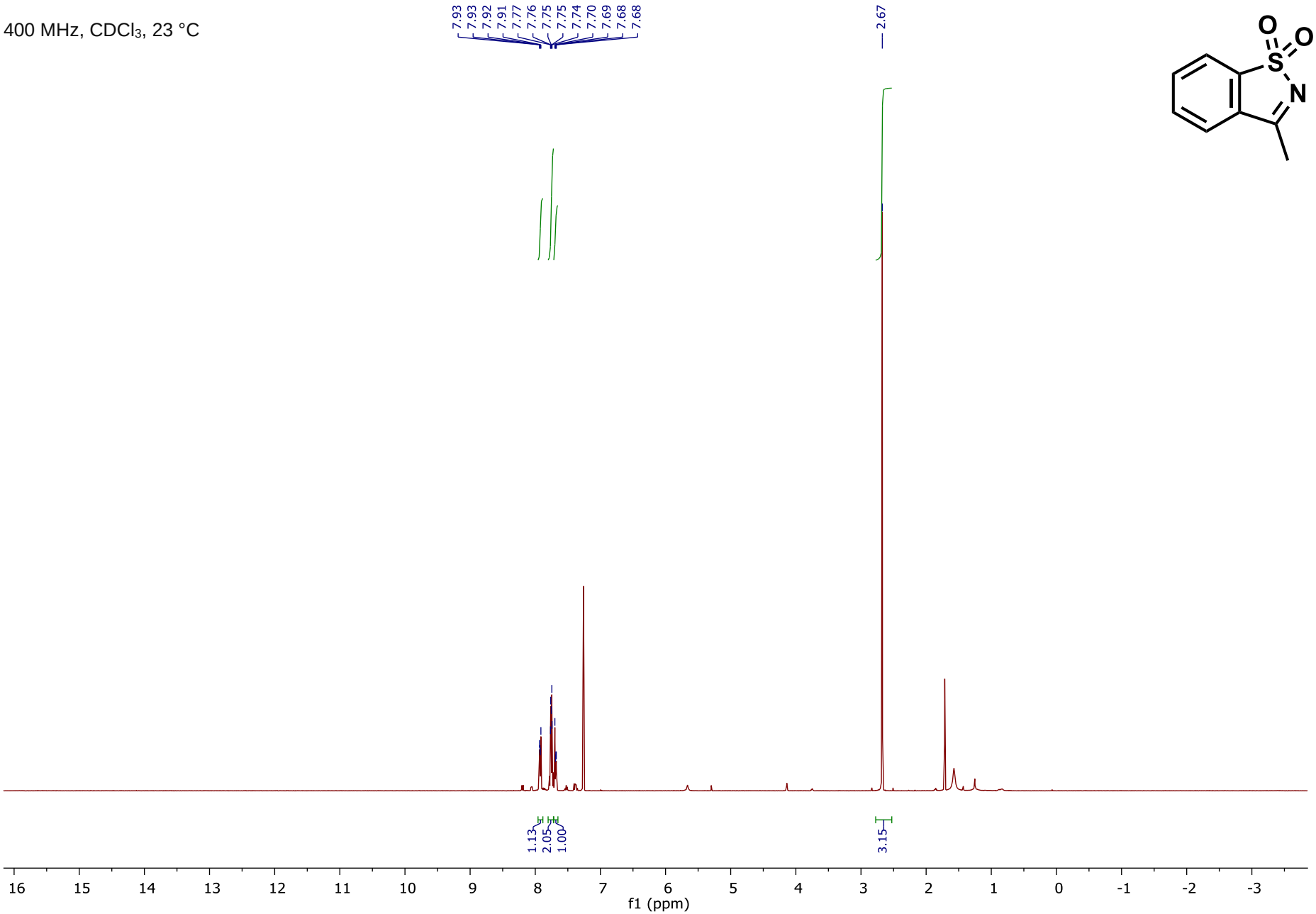
^{13}C NMR spectrum of 2-Phenyl-1-(*p*-toluenesulfonyl)aziridine (*rac*-3)

125 MHz, $CDCl_3$, 23 °C



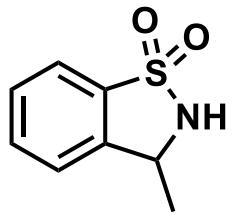
^{1}H NMR spectrum of 3-Methylbenzo[*d*]isothiazole 1,1-dioxide (S2)

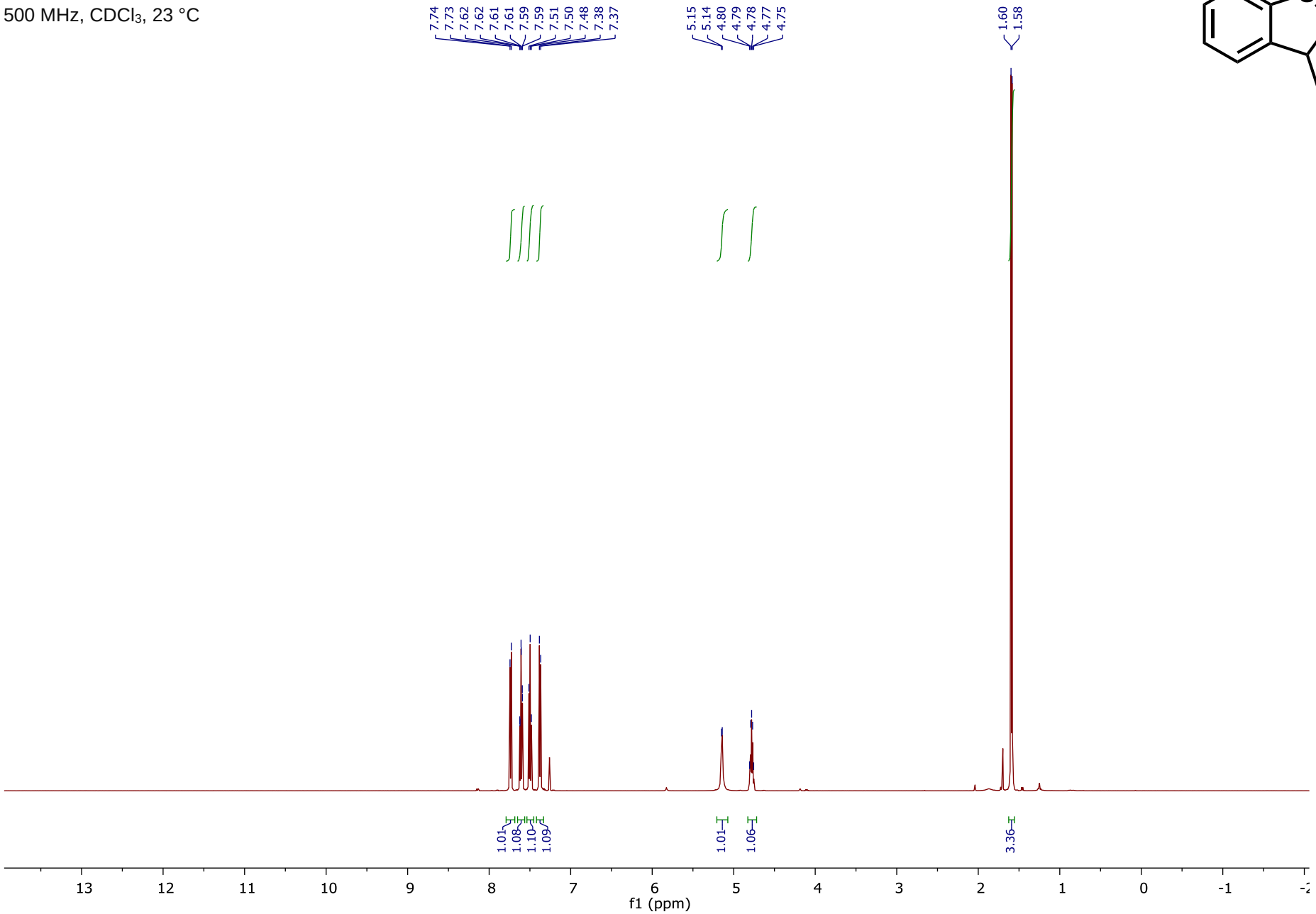
400 MHz, $CDCl_3$, 23 °C



^{1}H NMR spectrum of 3-Methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (*rac*-5)

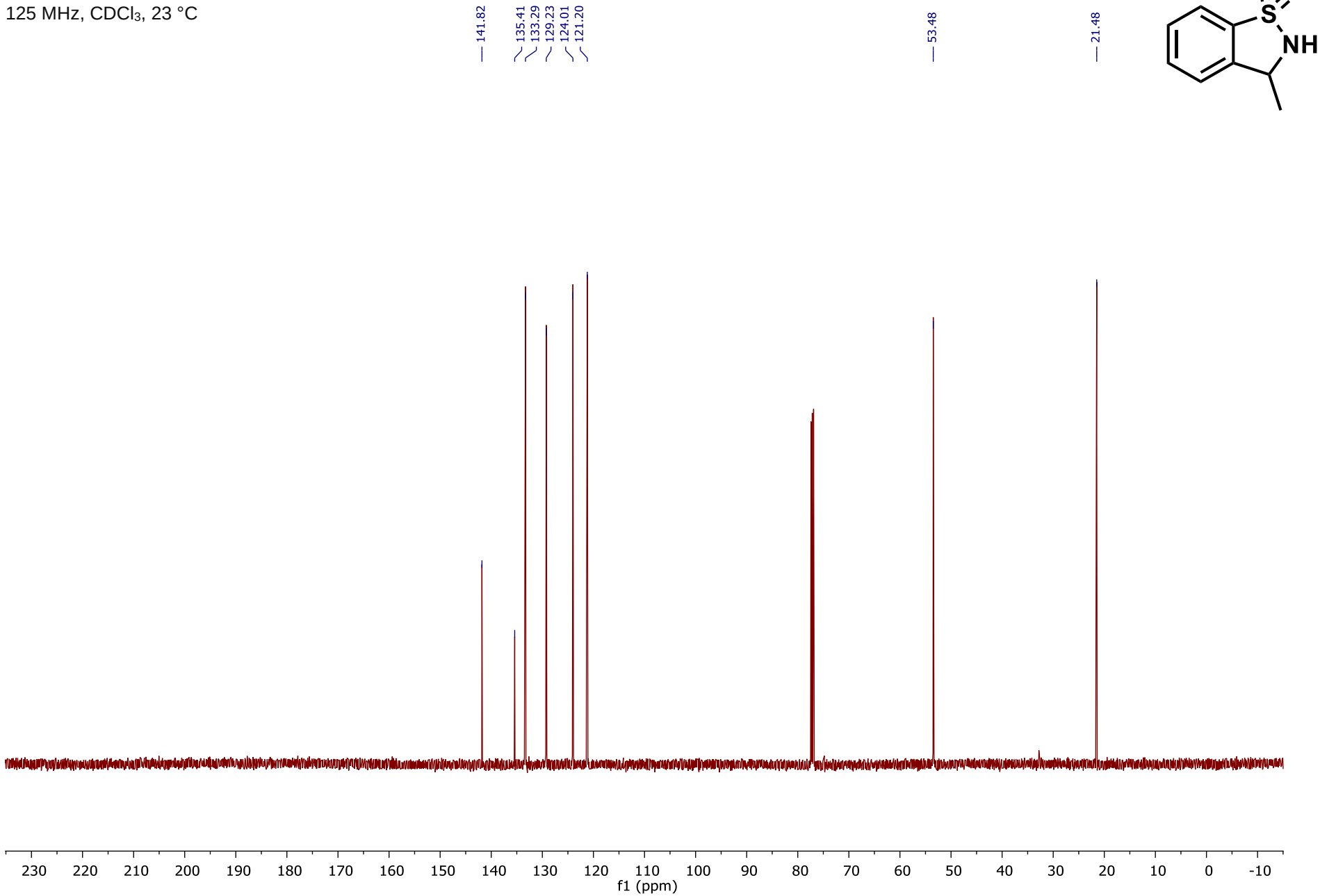
500 MHz, $CDCl_3$, 23 °C





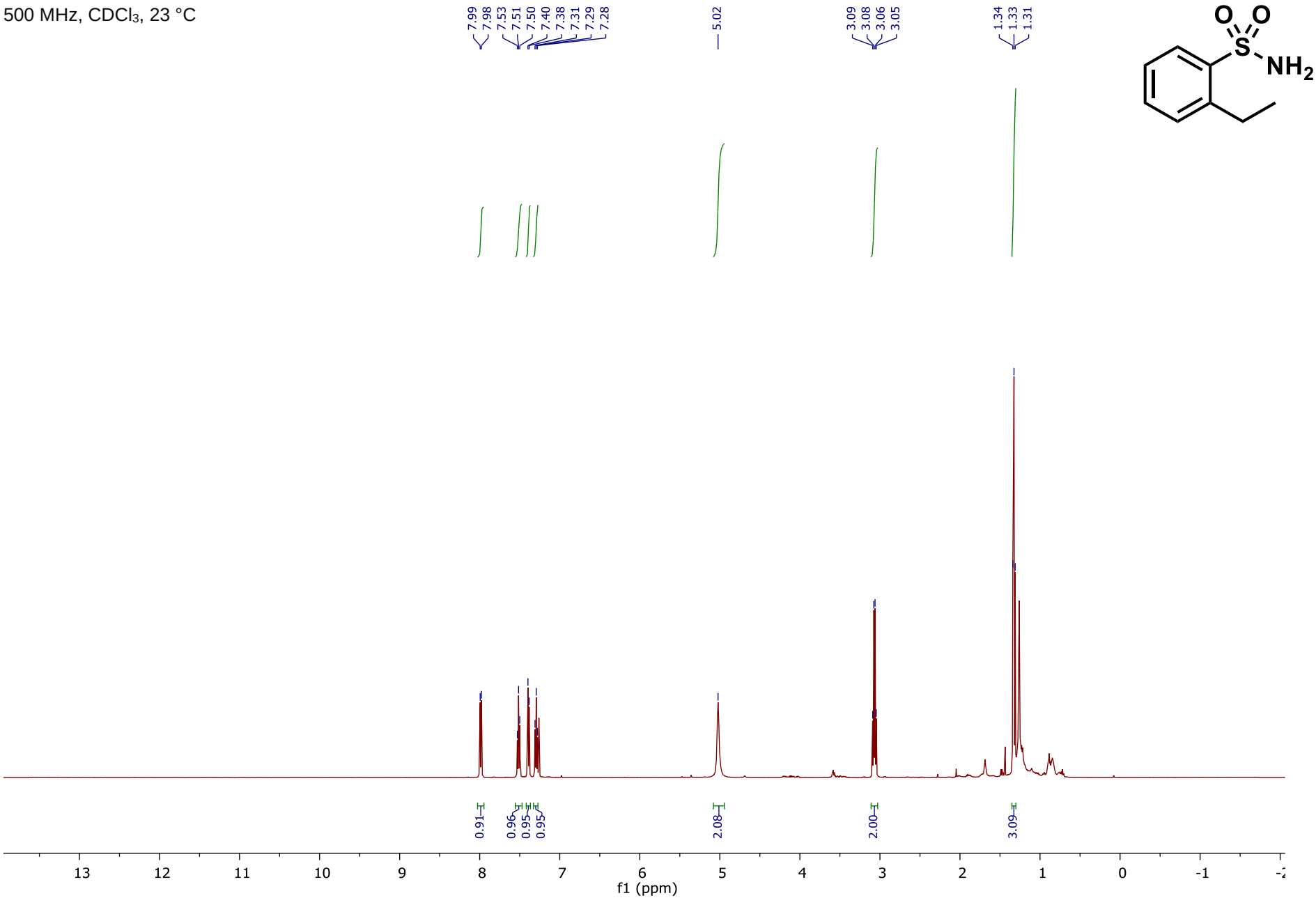
^{13}C NMR spectrum of 3-Methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (*rac*-5)

125 MHz, $CDCl_3$, 23 °C



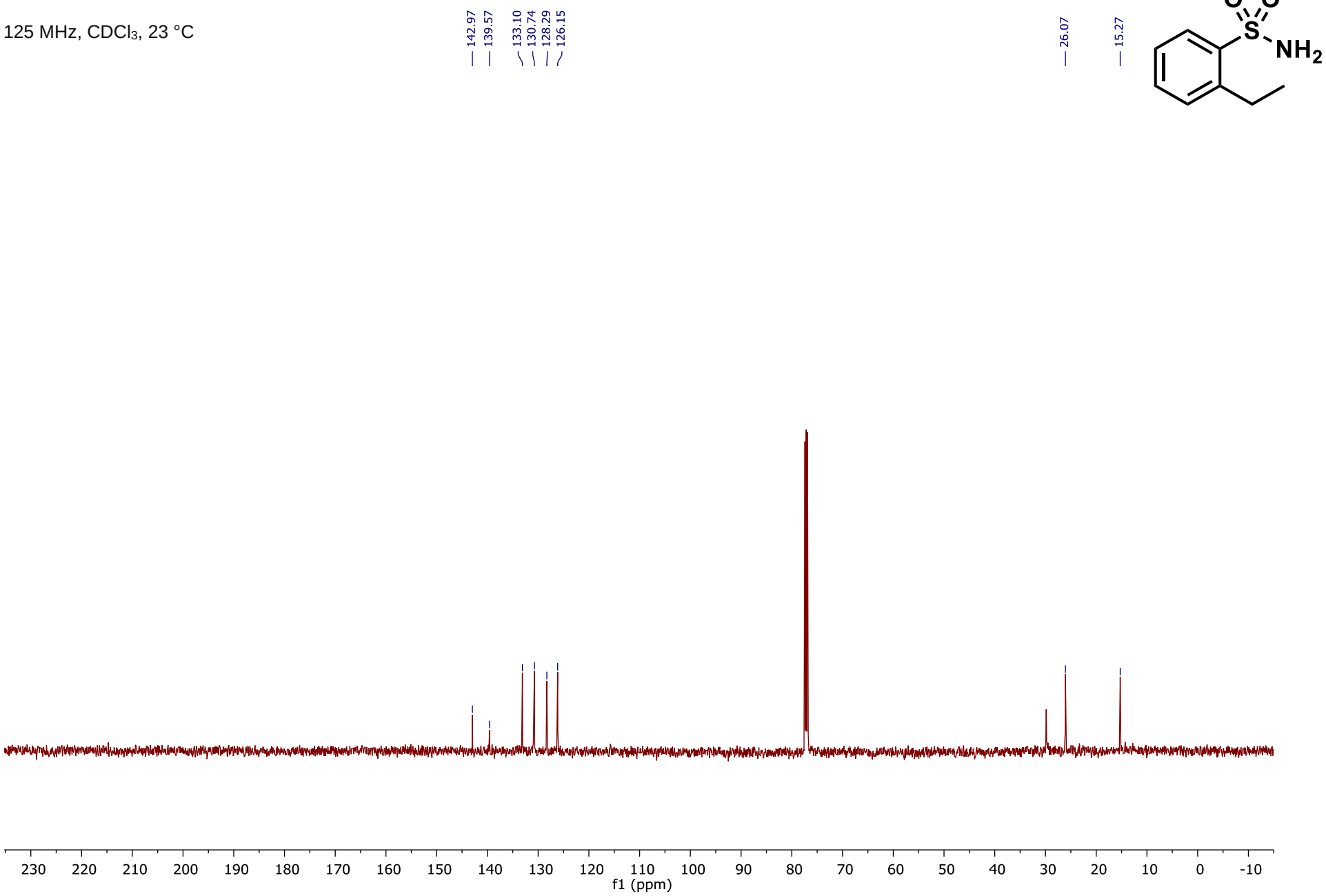
^{1}H NMR spectrum of 2-Ethylbenzenesulfonamide (6)

500 MHz, $CDCl_3$, 23 °C



^{13}C NMR spectrum of 2-Ethylbenzenesulfonamide (6)

125 MHz, $CDCl_3$, 23 °C



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