

Chemoselective, Enzymatic C-H Bond Amination Catalyzed by a Cytochrome P450 Containing an Ir(Me)- PIX Cofactor

Paweł Dydio*, Hanna M. Key*, Hiroki Hayashi, Douglas S. Clark, and John F.
Hartwig†

†correspondence to: jhartwig@berkeley.edu

*Authors contributed equally

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I. Protein Expression, Purification, and Characterization

a. General Methods

Unless otherwise noted, the chemicals, salts, and solvents used were reagent grade and used as received from commercial suppliers without further purification. All expression media and buffers were prepared using ddH₂O (MilliQ A10 Advantage purification system, Millipore). All expression media were sterilized using either an autoclave (45 min, 121°C) or a sterile syringe filter (0.22 µm). To maintain sterile conditions, sterile materials and *E. coli* cells were manipulated near a lit Bunsen burner.

b. Genes and Cloning

The WT CYP119 gene cloned into the vector 2BT (6xHis-TEV-ORF; AddGene #29666) was purchased from GenScript with codon optimization for *E. coli* (Table S1). The genes for WT P450 BM3 and P450-CAM were obtained as a gift from Prof. Humin Zhao (University of Illinois) and cloned to the vector 2BT at the QB3 Macrolab at UC Berkeley.

c. Media Preparation

Preparation of optimized minimal expression media: Salts (15 g Na₂HPO₄, 7.5 g K₂HPO₄, 0.3 g NaH₂PO₄, 0.3 g KH₂PO₄, 1.5 g NaCl, 5 g NH₄Cl) were dissolved in 2 L ddH₂O and autoclaved to give a media with pH ~8.0 - 8.2. Solutions of glucose (20%), casamino acids (BD Company, low Fe, 20%), and MgSO₄ (1 M), were autoclaved separately. Solutions of ampicillin (100 mg/ mL) and CaCl₂ (1 M) were sterilized by syringe filter. The following amounts of the listed solutions were added per 2 L of sterile salt solution: 40 mL glucose, 20 mL casamino acids, 4 mL MgSO₄, 100 µL CaCl₂, 2 mL ampicillin. Stock solutions were stored for several weeks (at room temperature, except for the stock solution of ampicillin, which was stored at – 20 °C); prepared media was stored for less than 1 day. Minimal media plates were prepared from the same media with the addition of 17 g agar/L media. In this case, agar was autoclaved in 1 L ddH₂O, and salts were autoclaved separately as a 20X solution, after which they were added to the agar solution.

d. Mutagenesis

Site-directed mutagenesis was performed using the QuickChange Lightning mutagenesis kit (Agilent); requisite double stranded DNA primers were designed according to the Agilent Primer Design Program and purchased from Integrated DNA Technology. PCR reactions were performed according to the manufacturer's directions. PCR reactions contained: 5 µL reaction buffer, 34 µL ddH₂O, 1.5 µL QuickSolution, 1 µL plamids (50 ng/µL), 1.25 µL sense primer (100 ng/µL), 1.25 µL antisense primer (100 ng/ µL), 5 µL dNTPs (2 mM/base), and 1 µL polymerase.

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

DNA Isolation and Storage: Following the completion of the above set of PCR procedures, 1.5 uL DPN 1 was added to each reaction, and the reactions were further incubated (3 h, 37°C). The crude PCR mixture was used to transform XL-10 Gold Ultracompetent cells (45 uL cells, 2 uL PCR reactions). The mixture was incubated on ice (30 min), heat shocked (30 s, 42 °C), recovered with SOC media (1 h, 37 °C, 275 rpm), and plated on LB plates. Plates were grown (18 h, 37 °C), and individual colonies were used to inoculate 1 mL rich media cultures, which were grown in 96-well plates (13 h, 37 °C, 300 rpm). DNA was isolated from the 96-well cultures using magnetic bead technology at the UC Berkeley DNA Sequencing Facility. Alternatively, individual colonies were used to inoculate 4 mL rich media cultures and grown overnight (13 h, 37 °C, 300 rpm), and the plasmids were purified using a Qiagen DNA Miniprep kit according to the manufacturer's instructions.

e. **Protein Expression**

Optimized Expression of Apo CYP119 for production of purified enzyme: BL21 Star competent *E. coli* cells (25 uL, QB3 Macrolab, UC Berkeley) were thawed on ice, transferred to PCR tubes, and transformed with the desired plasmid solution (1 uL, 50-250 ng/ uL). The cells were incubated on ice (30 min), heat shocked (30 sec, 42°C), re-cooled on ice (2 min), and recovered by incubation with SOC media (100 uL, 37 °C, 1 h, 250 rpm). Aliquots of the cultures (20uL) were plated on minimal media agar (expression media supplemented with 17 g agar/L) poured in a standard 24-well array plate and incubated (20 h, 37 °C) to produce approximately 4-40 colonies per plate. Single colonies were used to inoculate starter cultures (1 mL, expression media), which were grown in 24 well plates (4-8 hours, 37°C, 250 rpm) and used to inoculate 100 mL overnight cultures (minimal media, 37° C, 250 rpm). Each overnight culture was used to inoculate 750 mL of expression media, which was further grown (9 h, 37°C, 275 rpm). Expression was induced with IPTG (800 uL, 1M), and expression was allowed to continue for 15 h (30° C, 250 rpm). Cells were harvested by centrifugation (5000 rpm, 15 min, 4° C), and the pellets were resuspended in 20 mL Ni-NTA lysis buffer (50 mM NaPi, 250 mM NaCl, 10 mM Imidazole, pH = 8.0) and stored at -80° C until purification.

Protein Purification: Cell suspensions were thawed in a room temperature ice bath, decanted to 50 mL glass beakers, and lysed on ice by sonication (4x30 sec on, 2x2 min off, 65% power). Cell debris was removed by centrifugation (10 000 rpm, 30 min, 4° C), and Ni-NTA (5 mL, 50% suspension per 850 mL cell culture) was added. The lysates were briefly incubated with Ni-NTA on an end-over-end mixer (30 min, rt, 20 rpm) and poured into glass frits (coarse, 50 mL). The resin was washed with Ni-NTA lysis buffer (3 x 35 mL), and the wash fractions were monitored using Bradford assay dye. The desired protein was eluted with 18 mL Ni-NTA elution buffer (50 mM NaPi, 250 mM NaCl, 250 mM Imidazole, pH = 8.0), dialyzed against Tris buffer (10 mM, pH = 8.0, 12 h, 4° C), concentrated to the desired concentration using a spin concentrator, and metallated within several hours as described subsequently. Apo protein was not stored for more than 8 hours.

Optimized Expression of Apo CYP119 for reaction analysis in lysate: BL21 Star competent *E. coli* cells (25 uL, QB3 Macrolab, UC Berkeley) were thawed on ice, transferred to standard PCR tubes fused in a 96-well array, and transformed with the

desired plasmid solution (1 uL, 50-250 ng/ uL). The array of PCR tubes was incubated on ice (30 min), heat shocked (30 sec, 42°C), re-cooled on ice (2 min), and recovered by incubation addition of 100 uL SOC media. The cell cultures were transferred to a standard 96-well tissue culture plate and incubated at 37 °C for 1 h (250 rpm). Aliquots of the cultures (20uL) were plated on minimal media agar (expression media supplemented with 17 g agar/L) prepared in a standard 24-well array plate and incubated (20 h, 37 °C) to produce approximately 4-40 colonies per plate well. Single colonies were used to inoculate starter cultures (1 mL, expression media), which were grown in 24 well plates (3 hours, 37°C, 250 rpm) and used to inoculate 45 mL cultures (minimal media, 37° C, 250 rpm, 250 mL flask) that were further grown for 7 hours under the same conditions. Each culture was used induced with IPTG (50 uL, 1M), and expression was allowed to continue for 15 h (30° C, 250 rpm). Cells were harvested by centrifugation (10000 rpm, 10 min, 4° C), and the pellets were resuspended in 0.5 mL lysis buffer (100 mM NaPi, 250 mM NaCl, pH = 8.0) and stored at -80° C until purification.

Protein Purification: Cell suspensions were thawed in a room temperature ice bath, transferred to a deep well, 96-well plate, and lysed on ice by sonication (5x2 sec on, 4x2 sec off, 65% power). Cell debris was removed by centrifugation (4 000 rpm, 30 min, 4° C), and the crude lysate was transferred to the upper chamber of the Slide-A-Lyzer™ MINI Dialysis Devices (ThermoFisher, part # 88405). The lysate was dialyzed for a total of 4 hours against Tris buffer (10 mM, pH = 8), during which time the buffer in the lower chamber was replaced 4 times. During dialysis, the tubes were gently agitated on an orbital shaker operating at rt, 150 rpm. Ir(Me)-PIX CYP119 was generated in situ in the lysate as described in section III.

f. Protein Characterization

Gel Electrophoresis: Protein purity was analyzed by sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis using precast gels (polyacrylamide, 10-20% linear gradient, Biorad).

Mass Spectrometry: Apo-proteins were analyzed with an Agilent 1200 series liquid chromatograph connected in-line with an Agilent 6224 time-of-flight (TOF) LC/MS system using a Turbospray ion source.

II. Organic Synthesis and Characterization

a. General methods and materials

Unless stated otherwise, all reactions and manipulations were conducted on the laboratory bench in air with reagent grade solvents. Reactions under inert gas atmosphere were carried out in the oven dried glassware in a nitrogen-filled glovebox or by standard Schlenk techniques under nitrogen.

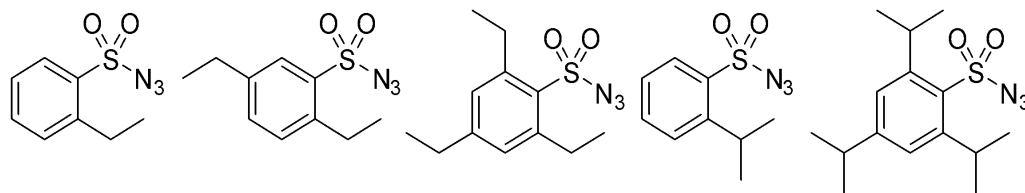
NMR spectra were acquired on 400 MHz, 500 MHz, 600 MHz, or 900 MHz Bruker instruments at the University of California, Berkeley. NMR spectra were processed with MestReNova 9.0 (Mestrelab Research SL). Chemical shifts are reported in ppm and referenced to residual solvent peaks¹. Coupling constants are reported in hertz. Chiral SFC analysis was conducted on a JASCO SF-2000 integrated analytical SFC system. GC analyses were obtained on an Agilent 6890 GC equipped with an HP-5 column (25 m x

0.20 mm ID x 0.33 m film) and an FID detector. High-resolution mass spectra were obtained via the Micro-Mass/Analytical Facility operated by the College of Chemistry, University of California, Berkeley.

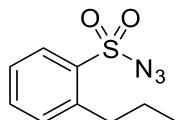
Unless noted otherwise, all reagents and solvents were purchased from commercial suppliers and used without further purification. If required, dichloromethane (DCM) and tetrahydrofuran (THF) were degassed by purging with argon for 15 minutes and dried with a solvent purification system containing a one-meter column of activated alumina; dried and degassed acetonitrile, 1,2-xylene, toluene, N,N-dimethylformamide (DMF), ethanol and methanol were purchased from commercial suppliers and used as received.

b. Substrates

The synthetic procedures and characterization of 2-ethylbenzenesulfonyl azide (**2**),² 2,5-diethylbenzenesulfonyl azide (**15**),³ 2,4,6-triethylbenzenesulfonyl azide (**16**)³, and 2-isopropylbenzenesulfonyl azide (**1**)⁴ were reported previously. 2,4,6-triisopropylbenzenesulfonyl azide (**17**) was purchased from a commercial supplier.



2-Propylbenzenesulfonyl azide (**9**):



2-Propylbenzenesulfonyl azide was prepared following a synthetic procedure developed for analogous compounds:⁴ to a stirred solution of 1-bromo-2-propylbenzene⁵ (5 g, 25 mmol) in 50 mL of dry THF was added *n*-butyllithium (12 mL, 2.5 M in hexanes, 30 mmol) dropwise at -78 °C, and the reaction mixture was stirred 1 h. Sulfuryl chloride (2.5 mL, 31 mmol) was added at -78 °C, the cooling bath was removed and the reaction mixture was stirred overnight at room temperature. Then the reaction was quenched with water (30 mL). The product was extracted with diethyl ether (3 x 50 mL), and the combined organic layers were washed with brine (30 mL), dried over MgSO₄ and evaporated. The crude product was purified by column chromatography on silica gel, with a mixture of hexanes and ethyl acetate (100:0 → 95:5 gradient) as the eluent. Fractions of the pure 2-propylbenzenesulfonyl chloride were combined, and the solvent evaporated, yielding 500 mg of product as colorless liquid, which was subjected to the next step without further purification.

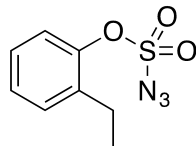
To a solution of 2-propylbenzenesulfonyl chloride in an acetone : water mixture (40 mL, 1:1 v/v) was added sodium azide (500 mg, 7.7 mmol) at 0 °C. The cooling bath was removed and the reaction mixture was let to stir for 24 h. Then the reaction mixture was concentrated to ca. 20 mL. The product was extracted with diethyl ether (3 x 30 mL), and the combined organic layers were washed with brine (30 mL), dried over MgSO₄ and evaporated. The crude product was purified by column chromatography on silica gel,

with a mixture of hexanes and ethyl acetate (100:0 → 95:5 gradient) as the eluent. Fractions of the pure product were combined, and the solvent evaporated, yielding 400 mg (7% yield, 2 steps) of product as colorless liquid.

¹H NMR (500 MHz, CDCl₃): d = 8.01 (d, J = 8.0 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 2.99 – 2.89 (m, 2H), 1.69 (dq, J = 15.0, 7.4 Hz, 2H), 1.00 (t, J = 7.3 Hz, 3H);

¹³C NMR (151 MHz, CDCl₃): d = 143.38, 136.73, 134.74, 134.71, 132.20, 129.69, 126.56, 35.15, 24.65, 14.26;

2-Ethylphenyl sulfurazidate (12):



To a stirred solution of 1-(azidosulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate⁶ (351 mg, 1.00 mmol) in CH₃CN (2.00 ml) was added the solution of 2-ethylphenol (122 mg, 1.00 mmol) and DBU (152 mg, 1.00 mmol) in CH₃CN (1.00 ml) slowly at 0 °C. The reaction mixture was warmed to 25 °C. After stirring for 1 h at ambient temperature, the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel, with a mixture of hexanes and ethyl acetate (100:0 → 95:5 gradient) as the eluent. Fractions of the pure product were combined, and the solvent evaporated, yielding benzenesulfonamide product **12** (114 mg, 50% yield).

¹H NMR (400 MHz, CDCl₃): d = 7.29 – 7.14 (m, 4H), 2.68 (q, J = 8.0 Hz, 2H), 1.17 (t, J = 8.0 Hz, 3H);

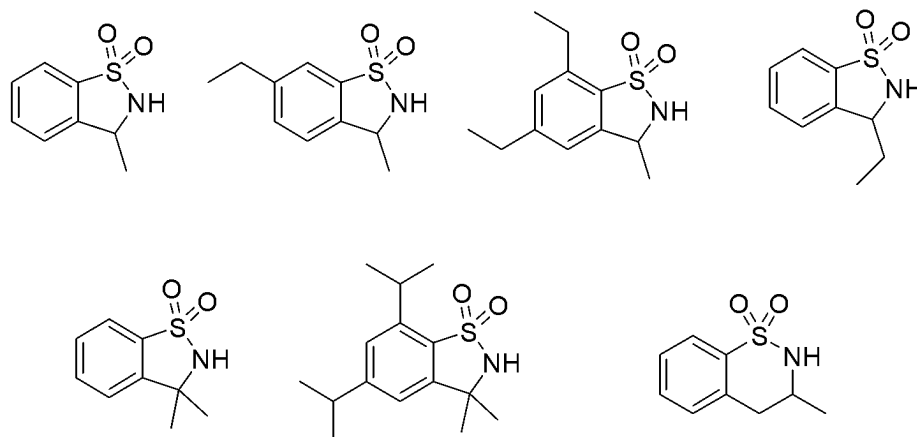
¹³C NMR (151 MHz, CDCl₃): d = 148.22, 136.68, 130.34, 128.26, 127.39, 121.05, 22.80, 14.01;

HR MS (EI): calcd. for C₈H₉N₃O₃S [M]⁺: 227.0359, found: 227.0366.

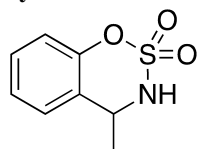
c. Authentic Products

General procedure for synthesis of sultams

In a closed vial, a solution of benzenesulfonyl azide (100 mg) and Ir(Me)-PIX (~4 mg) in toluene (6 ml) was stirred at 80 °C. The reaction progress was monitored by TLC. Upon completion (~16 h), the volatile materials were evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel, with a mixture of hexanes and ethyl acetate (100:0 → 70:30 gradient) as eluent. Fractions of the pure product were combined, and the solvent evaporated, yielding sultam products. The NMR data match those of the reported molecules: 3-methyl-2,3-dihydrobenzo[d]isothiazole 1,1-dioxide (**4**),² 6-ethyl-3-methyl-2,3-dihydrobenzo[d]isothiazole 1,1-dioxide (**7**),³ 5,7-diethyl-3-methyl-2,3-dihydrobenzo[d]isothiazole 1,1-dioxide (**8**),³ 3,3-dimethyl-2,3-dihydrobenzo[d]isothiazole 1,1-dioxide (**3**),⁴ 5,7-diisopropyl-3,3-dimethyl-2,3-dihydrobenzo[d]isothiazole 1,1-dioxide (**18**),³ 3-ethyl-2,3-dihydrobenzo[d]isothiazole 1,1-dioxide (**10**),⁷ and 3-methyl-3,4-dihydro-2*H*-benzo[e][1,2]thiazine 1,1-dioxide (**11**).⁸



Synthesis of Compound 13:



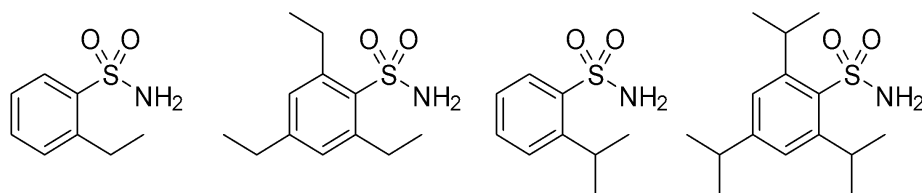
In a closed vial, a solution of benzenesulfonyl azide (16.8 mg, 0.0740 mmol) and Ir(Me)-PIX (1 mg) in benzene-*d*₆ (0.75 ml) and DMF (0.05 ml) was stirred at 50 °C. The reaction progress was monitored by ¹H NMR spectroscopy. After 48 h, the volatile materials were evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel, with a mixture of hexanes and ethyl acetate (100:0 → 90:10 gradient) as eluent. Fractions of the pure product were combined, and the solvent evaporated, yielding product (1.1 mg, 0.0055 mmol, 7.4% yield). The NMR data match those of the reported molecule.⁹

¹H NMR (400 MHz, CDCl₃): δ = 7.34 – 7.30 (m, 1H), 7.23 – 7.18 (m, 2H), 7.01 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 4.96 – 4.88 (m, 1H), 4.51 (brs, 1H) 1.72 (d, J = 8.0 Hz, 3H);

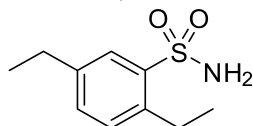
¹³C NMR (151 MHz, CDCl₃): δ = 150.98, 129.56, 126.40, 125.53, 123.68, 118.77, 53.09, 20.19;

General procedure for synthesis of benzenesulfonamide

To a solution of benzenesulfonyl azide (100 mg, 0.324-0.441 mmol) in THF (5 ml) sodium borohydride (100 mg, 2.64 mmol) was added at room temperature. The reaction progress was monitored by TLC. Upon completion (~1 h), the reaction was quenched with water (10 ml). The reaction mixture was concentrated to ca. 10 ml. The product was extracted with ethyl acetate (3 x 30 mL), and the combined organic layers were washed with brine (30 mL), dried over MgSO₄ and evaporated. The crude product was purified by column chromatography on silica gel, with a mixture of hexanes and ethyl acetate (100:0 → 70:30 gradient) as the eluent. Fractions of the pure product were combined, and the solvent evaporated, yielding benzenesulfonamide products. The NMR data match those of the reported molecules: 2,4,6-triethylbenzenesulfonamide (**19**),⁴ 2-ethylbenzenesulfonamide (**6**),¹⁰ 2,4,6-triisopropylbenzenesulfonamide (**20**),⁴ and 2-isopropylbenzenesulfonamide (**5**).¹¹



2,5-Diethylbenzenesulfonamide (21):



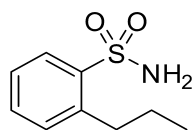
¹H NMR (600 MHz, CDCl₃): δ = 1H NMR (400 MHz, Chloroform-d) δ 7.82 (s, 1H), 7.36 – 7.25 (m, 2H), 4.81 (bs, 2H), 3.00 (q, J = 7.5 Hz, 2H), 2.64 (q, J = 7.6 Hz, 2H), 1.28 (t, J = 7.5 Hz, 3H), 1.21 (t, J = 7.6 Hz, 3H);

¹H NMR (600 MHz, DMSO-*d*₆): δ = 7.70 (s, 1H), 7.40-7.30 (m, 2 C_{Ar}-H + 2 NH), 2.96 (q, J = 7.5 Hz, 2H), 2.63 (q, J = 7.6 Hz, 2H), 1.19 (q, J = 7.2 Hz, 6H);

¹³C NMR (151 MHz, DMSO-*d*₆): δ = 141.70, 141.30, 139.11, 131.25, 130.55, 126.26, 27.62, 24.89, 15.48, 15.40;

HR MS (EI): calcd. for C₉H₁₃NO₂S [M]⁺: 239.0728, found: 239.0731.

2-Propylbenzenesulfonamide (22):

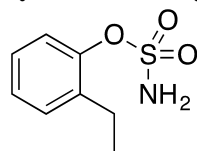


¹H NMR (600 MHz, CDCl₃): δ = 7.96 (d, J = 8.0 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.26 (t, J = 7.7 Hz, 1H), 4.94 (s, 2H), 2.98 – 2.92 (m, 2H), 1.70 (h, J = 7.4 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H);

¹³C NMR (151 MHz, CDCl₃): δ = 141.68, 139.87, 132.89, 131.46, 128.40, 126.23, 35.15, 24.48, 14.410;

HR MS (EI): calcd. for C₉H₁₃NO₂S [M]⁺: 199.0667, found: 199.0668.

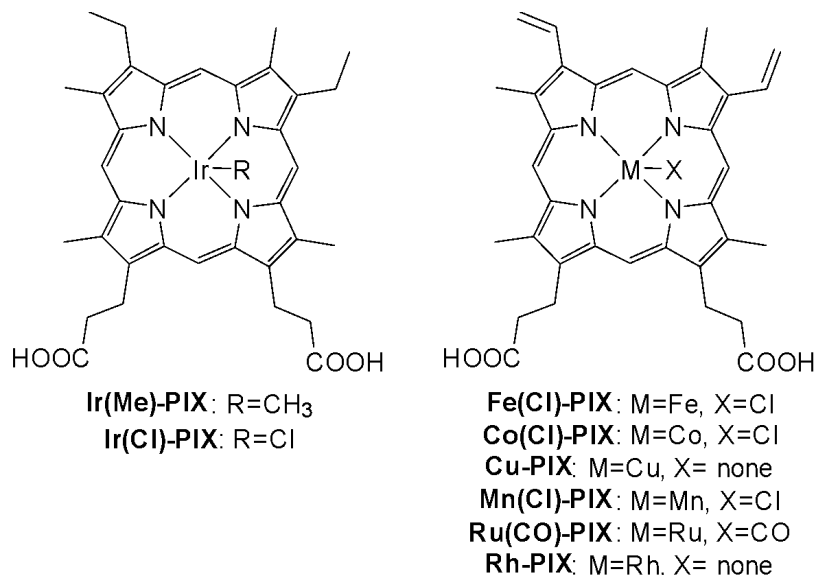
Synthesis of Aryloxysulfonamide (14):



To a stirred solution of ClSO₂NCO (1.74 ml, 20.0 mmol) in CH₃CN (10 ml) was added formic acid (0.755 ml, 20.0 mmol) dropwise at 0 °C. After stirring at 0 °C for 5 min, the reaction mixture was allowed to warm up to room temperature and stirred overnight. The resulting solution of ClSO₂NH₂ was used next step. To the solution of ClSO₂NH₂ (2.5 ml) was added a solution of 2-ethylphenol (403 mg, 3.30 mmol) in DMA (2 ml) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction was poured into water and extracted with Et₂O (3 x 20 mL), and the combined organic layers were washed with brine (30 mL), dried over MgSO₄ and evaporated. The crude product was purified by column chromatography on silica gel, with a mixture of hexanes and ethyl acetate (100:0 @91:9 gradient) as the eluent. Fractions of the pure product were combined, and the solvent evaporated, yielding aryloxysulfonamide product **14**. The NMR data match that of the reported molecule.¹²

- d. **Metallo-mesoporphyrin IX (Ir(Me)-PIX and Ir(Cl)-PIX) and metallo-protoporphyrin IX (Fe(Cl)-PIX, Co(Cl)-PIX, Cu-PIX, Mn(Cl)-PIX, Ru(CO)-PIX, Rh-PIX) cofactors used in the studies:**

e.



The synthetic procedures and characterization of Ir(Me)-PIX and Ir(Cl)-PIX were reported previously.¹³ The other cofactors (Fe(Cl)-PIX, Co(Cl)-PIX, Cu-PIX, Mn(Cl)-PIX, Ru(CO)-PIX, Rh-PIX) were purchased from commercial suppliers (Aldrich and Frontier Scientific).

III. Catalytic Experiments

- a. *General Methods:* Unless otherwise noted, catalytic reactions were performed in 4 mL individually-capped vials. Reactions were assembled in a nitrogen atmosphere glove box. Solutions of catalyst (either crude lysate supplemented with Ir(Me)-PIX-CYP119 or purified Ir(Me)-PIX-CYP119) were gently degassed on a Schlenk line (3 cycles vacuum/refill) before being brought into a glove box in sealed vials. Organic reagents were added as stock solutions in DMF, such that the final amount of DMF in the reaction was approximately 2% by volume. If necessary, protein catalysts were diluted to reaction concentrations in sodium phosphate buffer (100 mM, pH = 6.0) before being added to reaction vials. Unless otherwise noted, all reactions with purified enzymes were performed with catalysts generated from a 1:2 ratio of Ir(Me)-cofactor : apo protein, with 0.34 mol% catalyst loading with respect to metal-cofactor and limiting reagent. All reactions with lysate contained 5.8 μ M catalyst. Unless otherwise noted, all reactions were conducted in a shaking incubator (37 °C, 275 rpm).

- b. Procedure for catalytic experiments

The catalyst stock solution generated using purified protein: The Ir(Me)-CYP119 catalyst was prepared by addition of a stock solution of Ir(Me)-PIX (3.1 mM in DMF) to a solution of the apo-protein (0.13mM in 10 mM Tris buffer, pH = 8), such that the resulting solution had a 1 : 2 ratio of Ir(Me)-PIX : CYP119. This ratio was used to ensure that all Ir-PIX was bound. The mixture was incubated for 5 min, and desalted with the

NAP-10 desalting column equilibrated with reaction buffer (100 mM NaPi, 100 mM NaCl, pH = 6.0). The protein mixture was diluted to the required reaction concentration with the same reaction buffer.

The catalyst stock solution using lysate: 0.5 mL of cell lysate was prepared as described in Section Ie. Upon the last dialysis of the lysate using the Slide-A-Lyzer™ MINI Dialysis Devices (ThermoFisher, part # 88405) the bottom chamber was emptied, and 10 uL of the stock solution of Ir(Me)-PIX cofactor (0.0029 umol, 0.29 mM Ir(Me)-PIX solution in DMF) was added to the cell lysate in the upper chamber. (To ensure that all Ir-PIX was bound, the amount of the cofactor added was lower than the lowest expected amount of the protein. The content of apo protein in the lysate was estimated based on the average yield of purified apo protein (5-25 mg/L culture, depending on the mutant) during isolation and purification protocol described in Section Ie). The mixture of the lysate and Ir(Me)-PIX cofactor was incubated for 5 min. Then, the bottom chamber of the dialyzer was refilled with the reaction buffer (100 mM NaPi, 100 mM NaCl, pH = 6.0) and the tube was incubated on an orbital shaking platform (1 h, 150 rpm, room temp.). The catalyst solution from the upper chamber was transferred to a 4 mL glass vial, the catalyst solution was degassed and brought into a nitrogen atmosphere glovebox as described in Section IIIa.

The C-H Amination reaction set-up: 500 ul of the catalyst stock solution was added to a vial, followed by addition of a stock solution of the appropriate sulfone azide substrate (2.5 umol in 10 uL DMF). The vial was sealed with a cap, removed from a glovebox and incubated in a shaker (37 °C, 275 rpm, 66 h). Upon completion, the reaction mixture was analyzed as described in section IV.

IV. Analysis of Yield and Enantiomeric Ratio (er)

Yields of products and side products, as well as, enantiomeric ratios were determined by chiral SFC using acetanilide as an internal standard (Figure S2, S3, Table S4, S5). The methods used to analyze reaction mixtures for each substrate are summarized in Table S4. Representative traces can be found in Figure S3. Samples for analysis were prepared as follows: Saturated NaCl (500 uL) was added to each reaction vial, followed by a solution of acetanilide (1 ml, 0.05 ug/ml) in EtOAc. Each vial was capped and the contents of the vial were mixed by shaking. The phases separation was achieved by centrifugation (2 500 rpm, 5 min, rt). The organic layer was removed from the top of the vial by pipet, transferred to a new vial. The aqueous phase was extracted twice with EtOAc (2 x 500 ul). The organic extract of each vial were combined, evaporated and redissolved in MeOH (200 ul) for analysis by SFC.

Control extraction studies: To 500 ul of either a catalyst stock solution of Ir(Me)-CYP119 – prepared as in section IIIb – or phosphate buffer (100 mM, pH = 6), a stock solution of product **4** (2.5 umol in 7.5 uL MeOH) was added. Saturated NaCl (500 uL) was added to each vial, followed by a solution of acetanilide (1 ml, 0.05 ug/ml) in EtOAc. Each vial was capped and the contents of the vial were mixed by shaking. The separation of phases was achieved by centrifugation (2 500 rpm, 5 min, rt). The organic layer was removed from the top of the vial by pipet, transferred to a new vial. The aqueous phase was extracted twice with EtOAc (2 x 500 ul). The organic extract of each vial were combined, evaporated and redissolved in MeOH (200 ul) for analysis by SFC. These experiments showed that the product was extracted nearly quantitatively from the

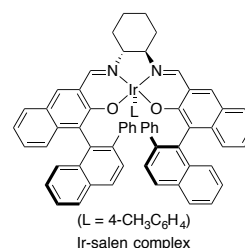
catalyst solution mixture – Table S5. These experiments confirmed the accuracy of the method.

V. Determination of Absolute Configuration

To determine the absolute configuration of the sultam products **4**, **7** and **11**, enantioenriched samples of the authentic products were prepared using substrates **2**, **9**, and **15** with a chiral Ir-salen complex as catalyst. The optical rotations of these samples were then compared to those previously reported in a study that determined the absolute configuration of these products. Then, the retention time of these products in SFC were compared with those of the enzymatic reactions reported here to determine the absolute configuration of products formed by the enzyme catalysts.

General procedure for synthesis of sultams with chiral Ir-salen complex

In a closed vial, a solution of benzenesulfonyl azide (0.3 mmol) and Ir-salen complex² (10 mg, 9.0 μ mol) in toluene (0.75 ml) was stirred at room temperature. After stirring for 24 h, the volatile materials were evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel, with a mixture of hexane and ethyl acetate (100:0 to 80:20) as eluent. The fractions containing the pure product were combined, and the solvent evaporated, yielding sultam products. The NMR data match those of the reported molecules. The enantiomeric excess was determined by SFC analysis with the same condition reported in Table S4.



Compound	ee and Optical Rotation	Reported Data ^{2,8}	Retention Times
4	48% ee, $[\alpha]_D^{25} = -12.2$ (c = 1.0 CHCl ₃)	92% ee (S), $[\alpha]_D^{25} = -26.9$ (c = 1.03 CHCl ₃)	t(prd-) = 2.25 min t(prd+) = 2.65 min
7	42% ee, $[\alpha]_D^{25} = -10.2$ (c = 1.1 CHCl ₃)	91% ee (S), $[\alpha]_D^{25} = -24.2$ (c = 1.03 CHCl ₃)	t(prd-) = 2.1 min t(prd+) = 2.7 min
11	99% ee, $[\alpha]_D^{25} = +67.2$ (c = 0.59 CHCl ₃)	98% ee (R), $[\alpha]_D^{25} = -69.4$ (c = 1.0 CHCl ₃)	t(prd-) = 4.3 min t(prd+) = 5.8 min

Synthesis of optically pure (R)-13.

To determine the absolute configuration of the product **13**, optically pure (R)-**13** was prepared from 2-[(1R)-1-aminoethyl]-phenol and sulfonyl chloride: To a solution of 2-[(1R)-1-aminoethyl]-phenol (27.4 mg, 0.200 mmol, Sigma-Aldrich®) and Et₃N (16 μ l, 0.20 mmol) in CH₂Cl₂ (1.2 ml) was added a solution of SO₂Cl₂ (55 μ l, 0.40 mmol), CH₂Cl₂ (0.5 ml) at -78 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The resulting mixture was purified by column chromatography on silica gel with a mixture of hexane and ethyl acetate (100:0 to 80:20) as eluent. The fractions containing the pure product were combined, and the solvent evaporated, yielding sultam products. The NMR data match those of the reported molecules. The enantiomeric excess was determined by SFC analysis with the same condition of Table S4; 99% ee, retention times of 2.6 min (minor), 2.8 min (major).

These experiments and previous reports show that the absolute configurations of **4**, **7**, **11** and **13** are (R).

I. Supporting Figures

Figure S1. Deconvoluted mass spectrum of CYP119-C317G acquired by LC-MS

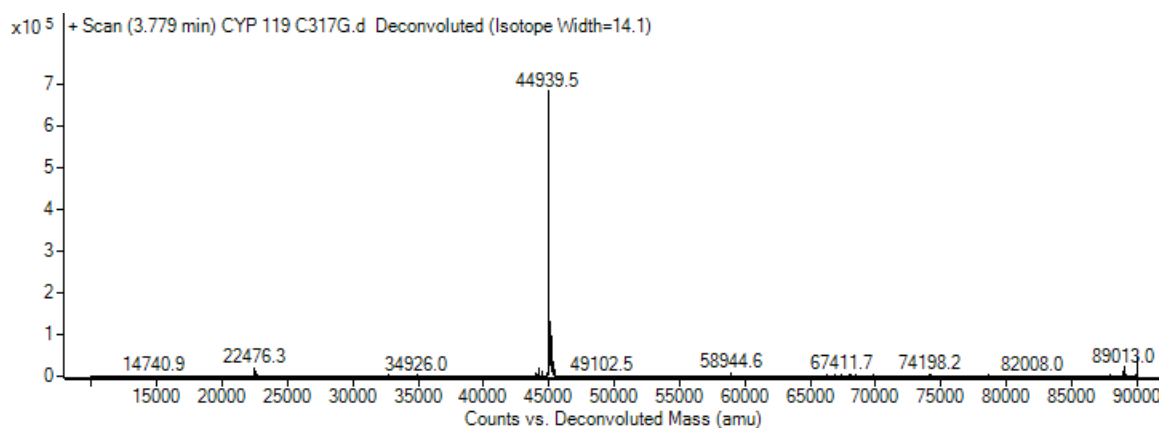
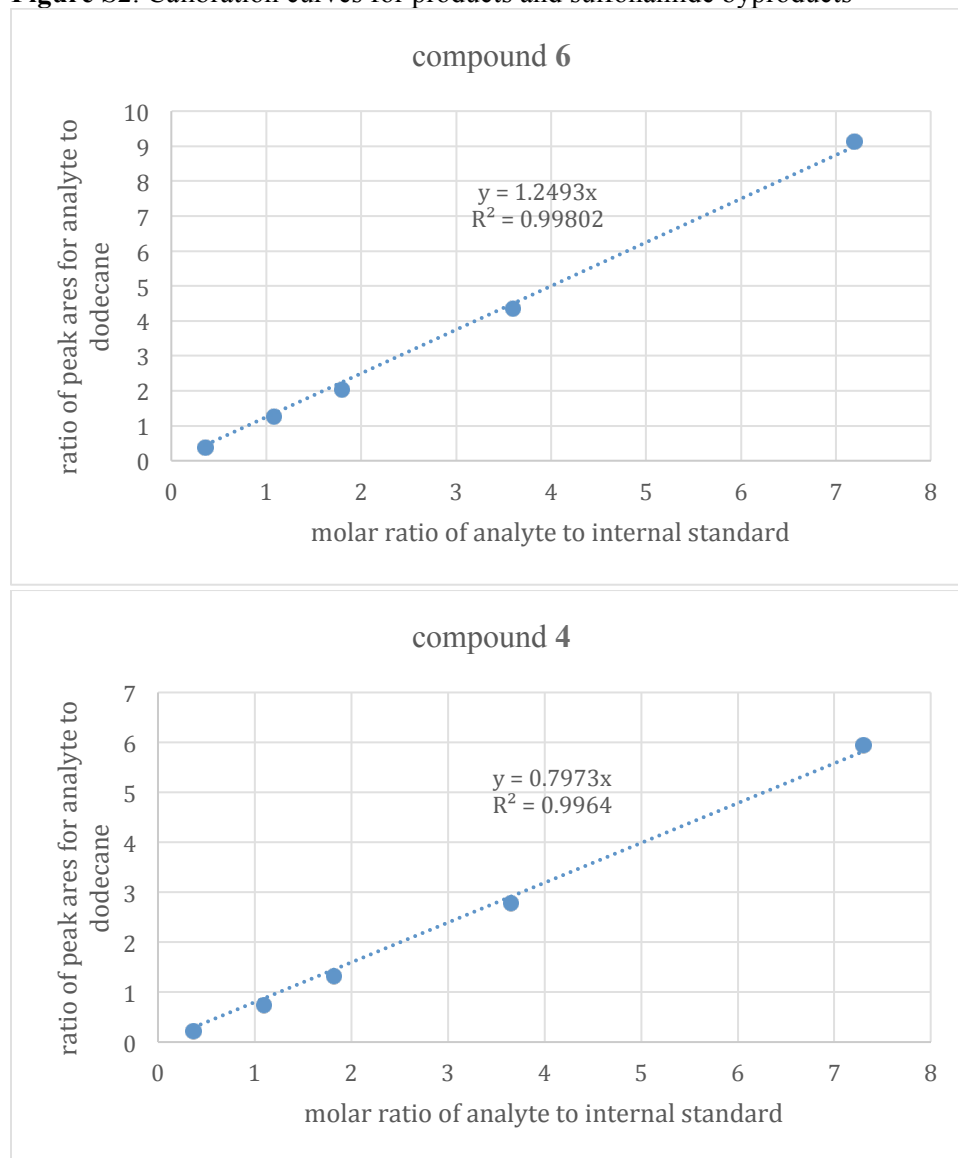
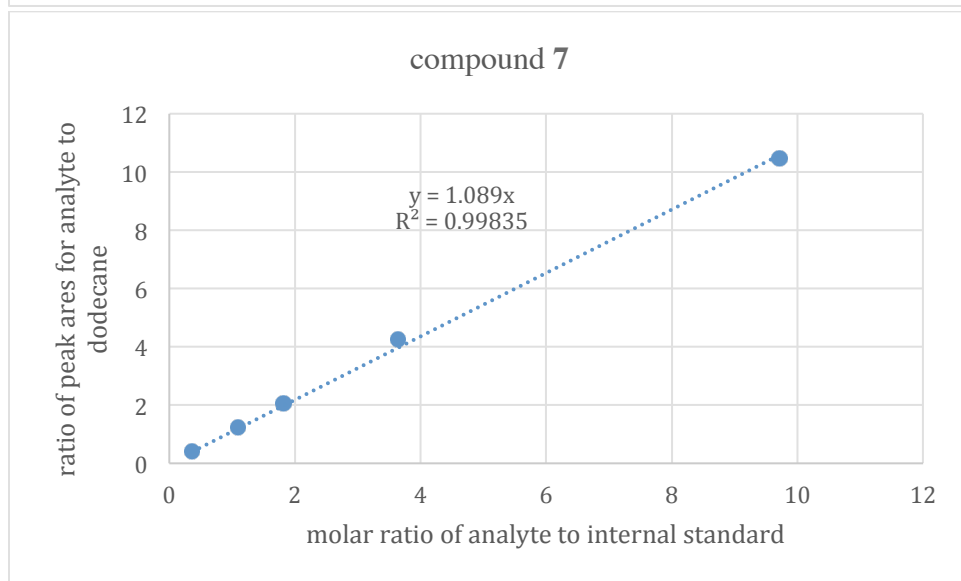
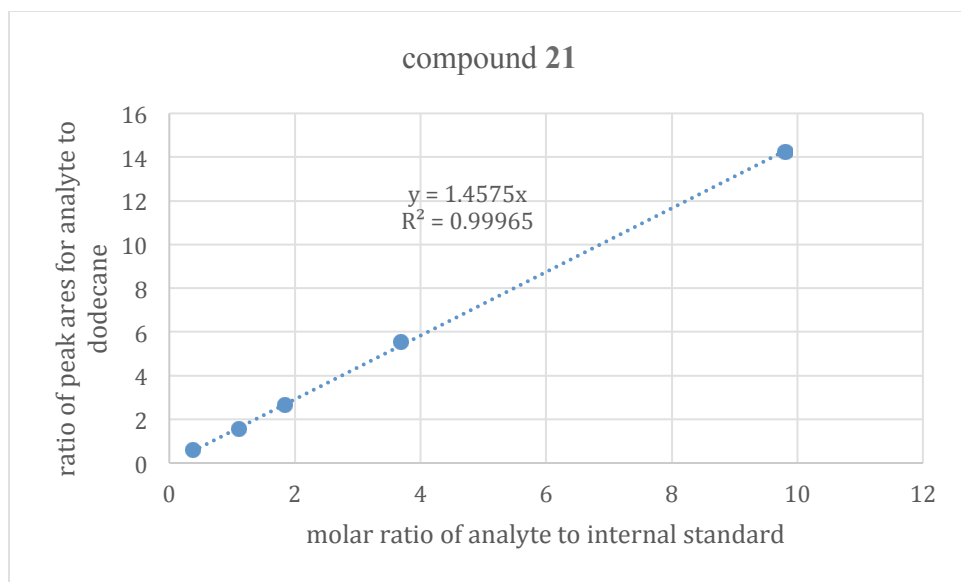
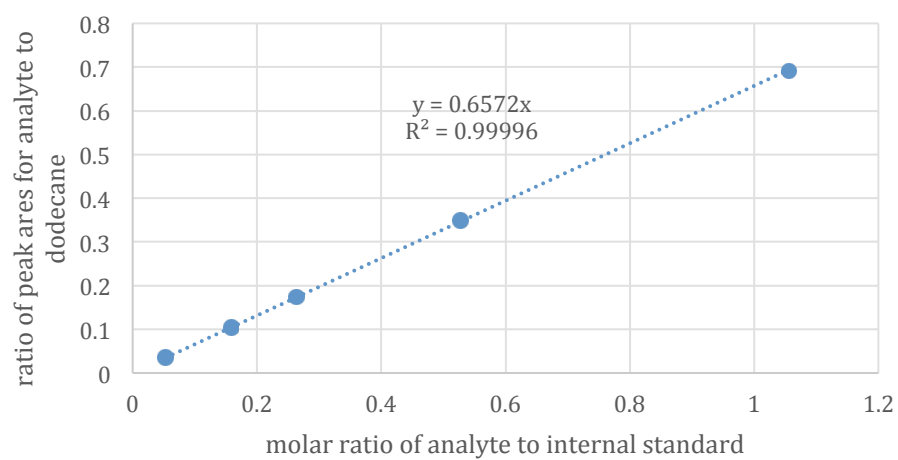


Figure S2. Calibration curves for products and sulfonamide byproducts

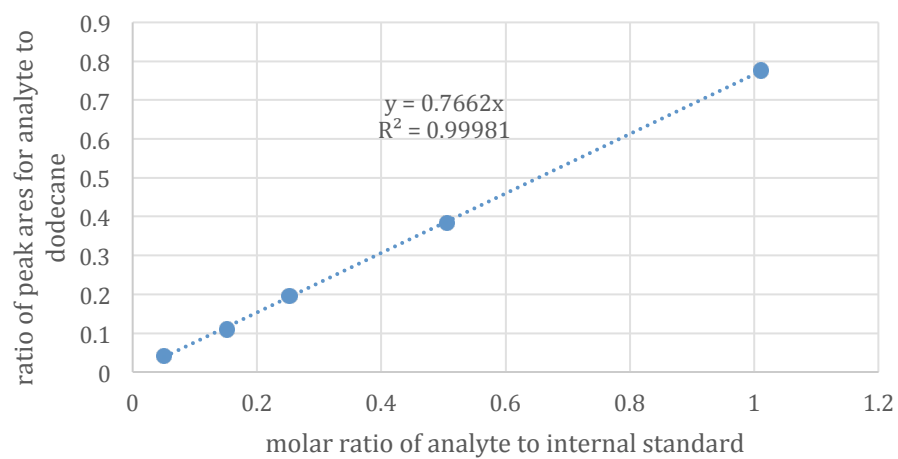


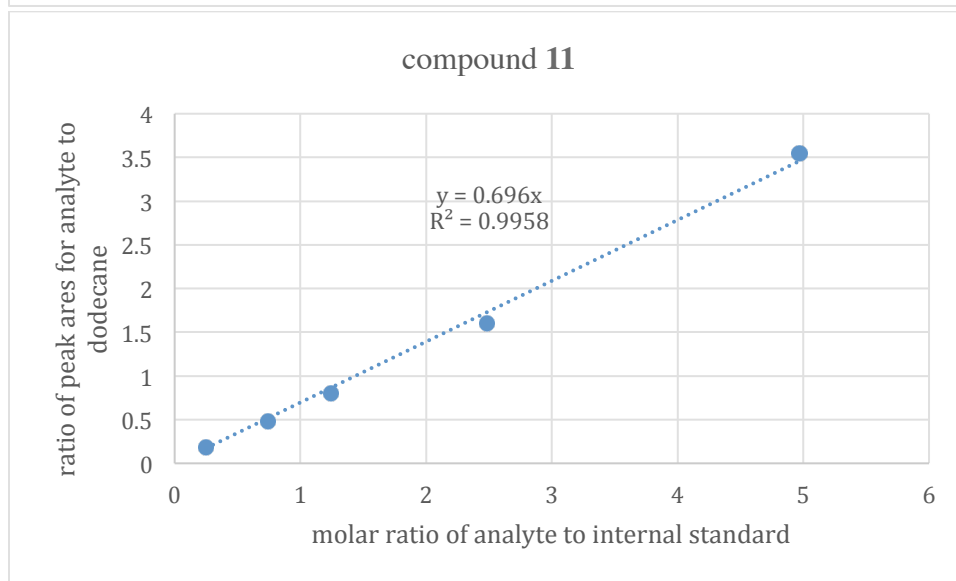
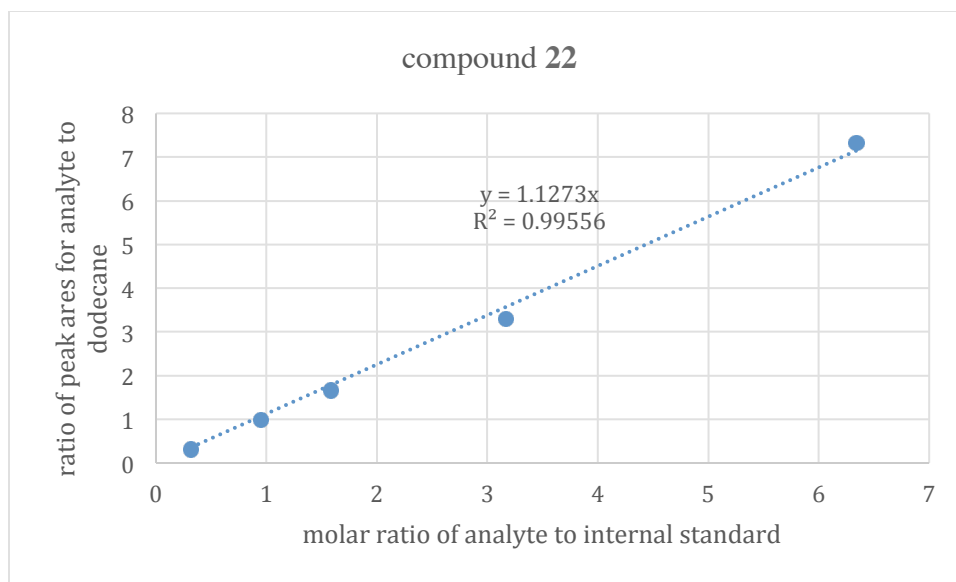


compound 19

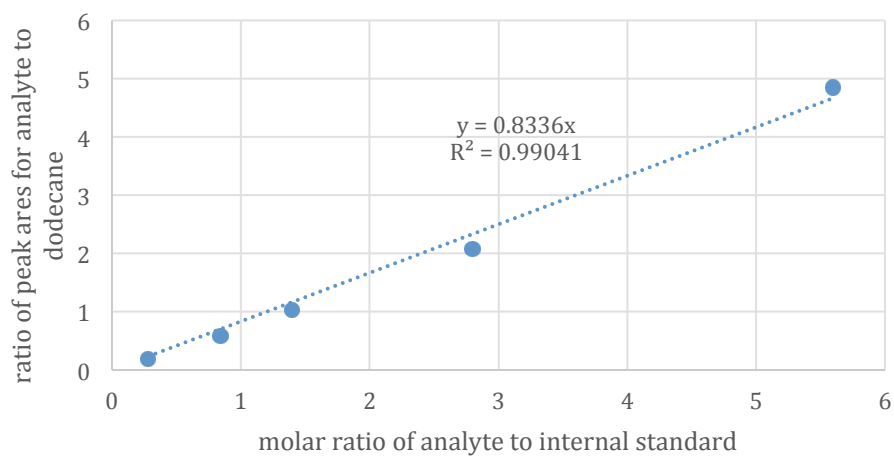


compound 8

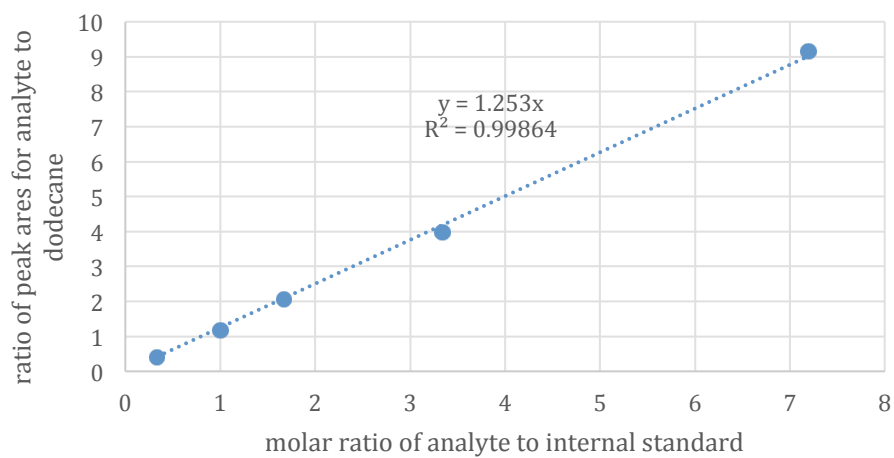




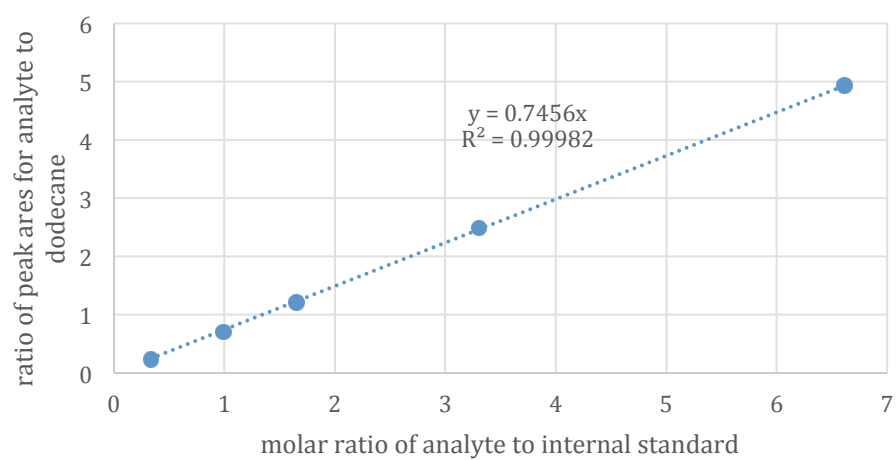
compound 10



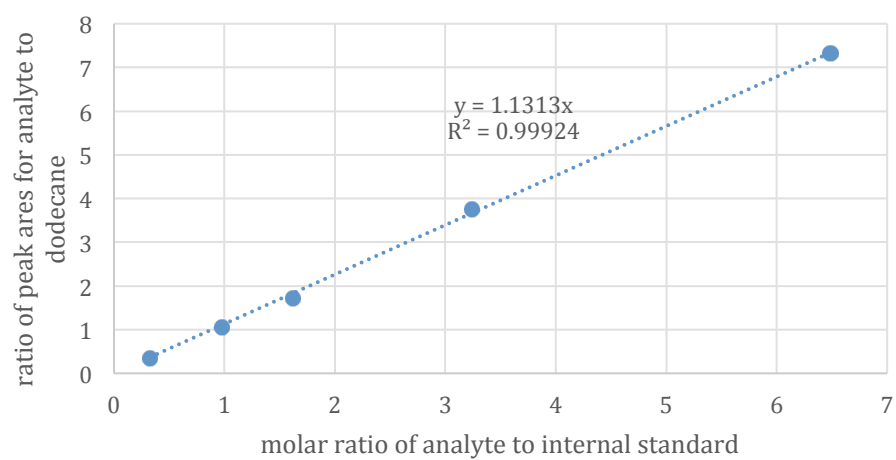
compound 5



compound 3



compound 20



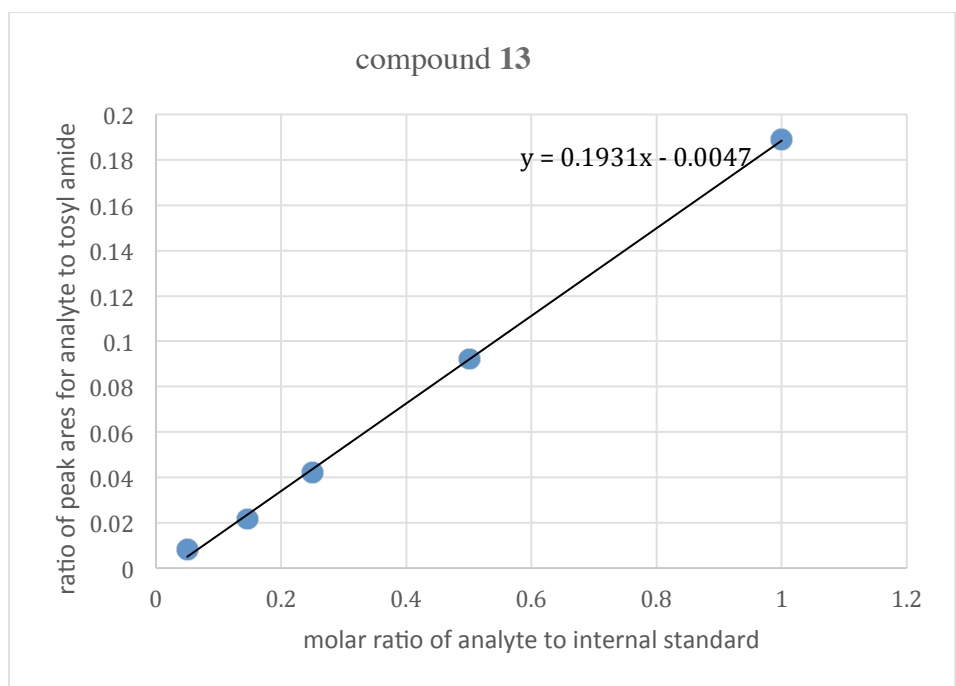
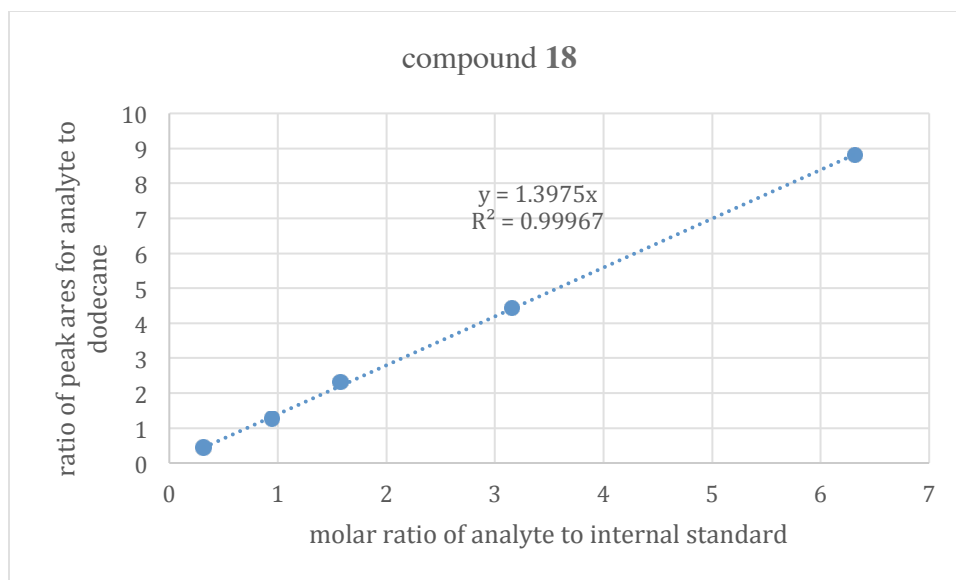
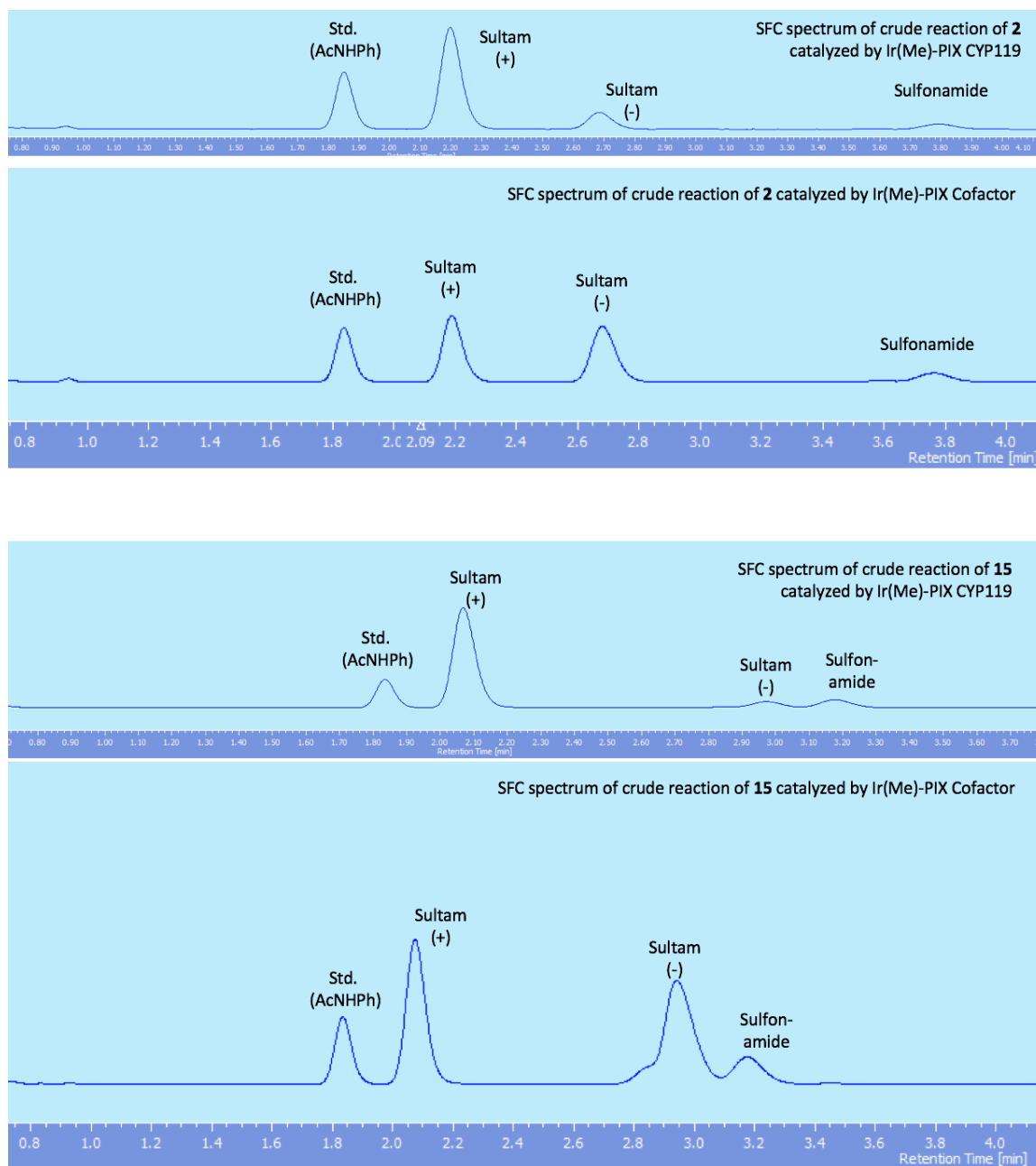
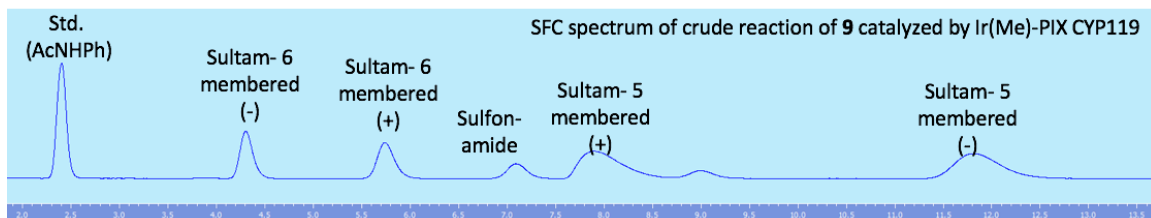
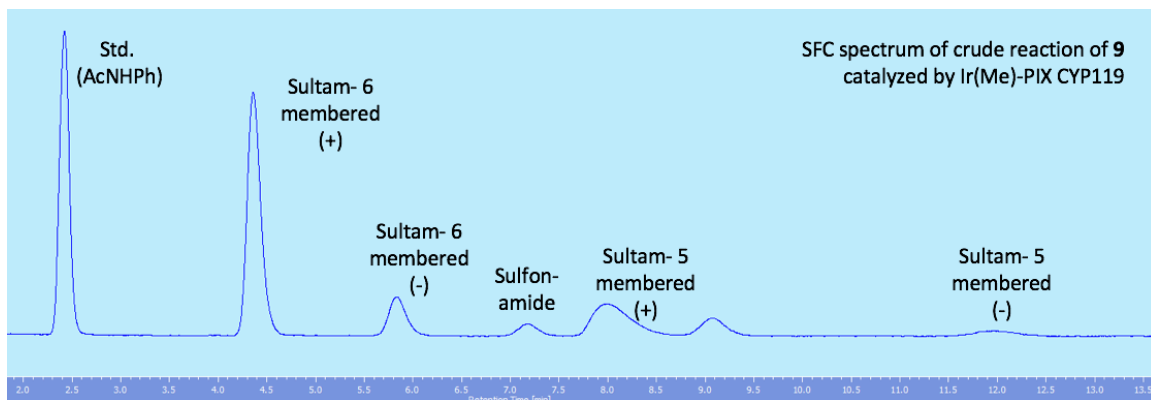
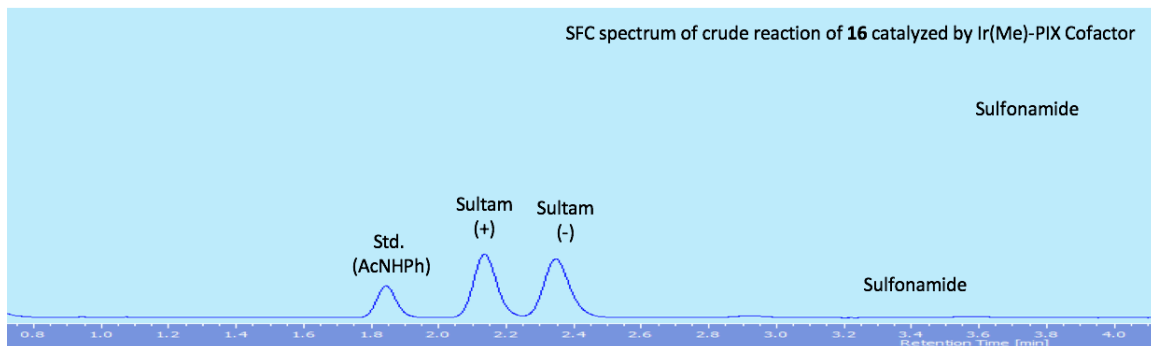
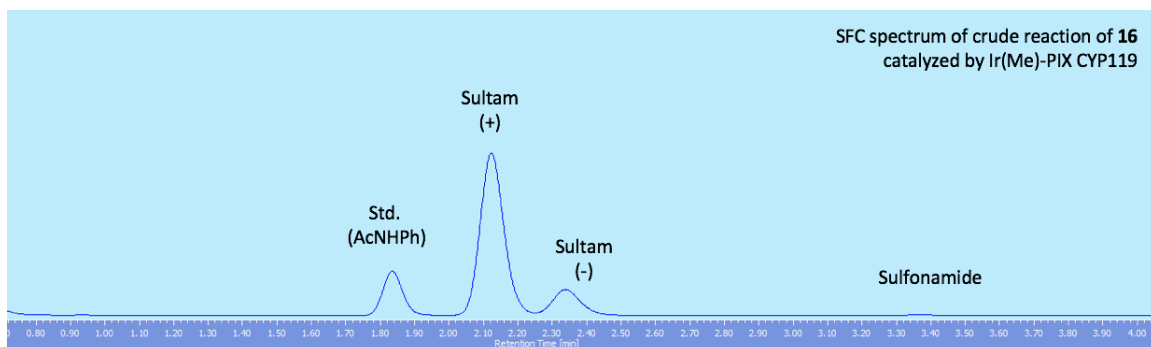
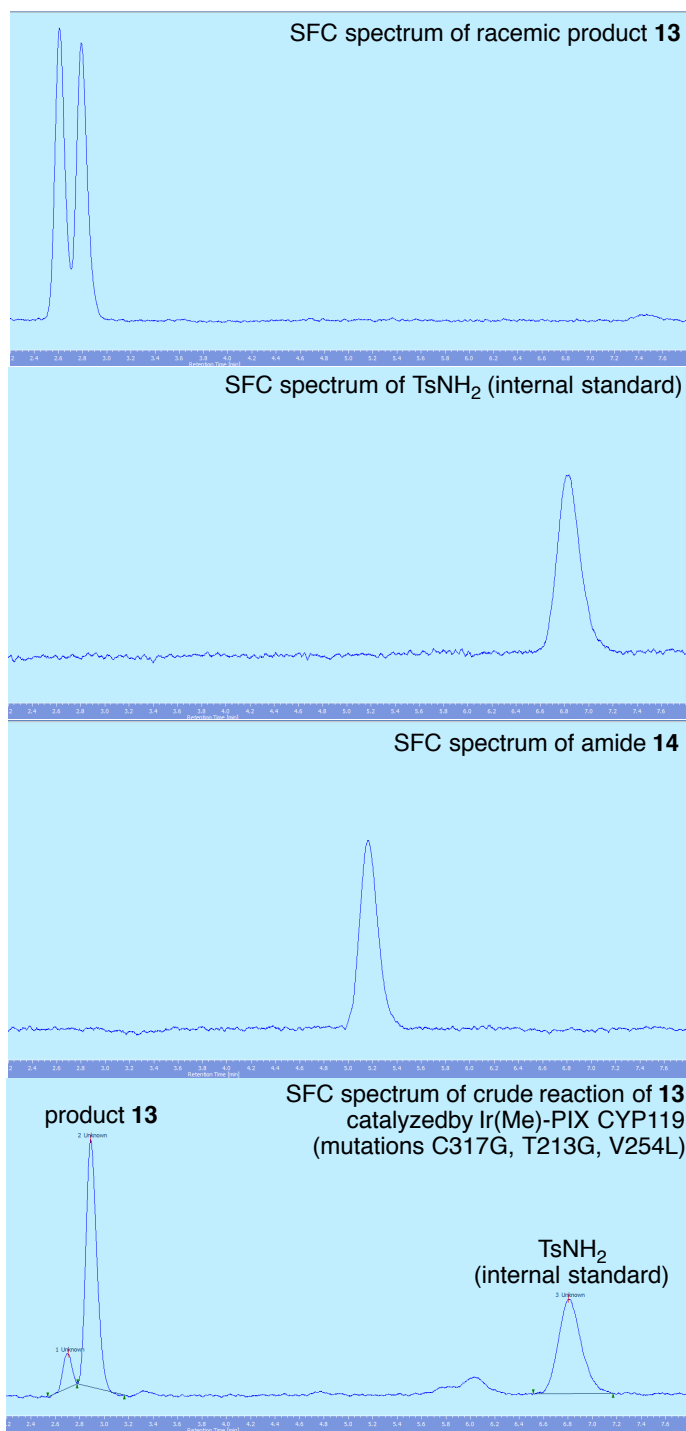


Figure S3. Chiral SFC traces for products (racemates and enantioenriched samples)







II. Supporting Tables

Table S1. Sequence information for the heme protein used in this study

Amino acid sequence of protein used in this study

Protein	Organism	Construction	Vector	Sequence
CYP 119	<i>Sulfolobus solfataricus</i>	6xHis-TEV-CYP119	2BT	EGDIHMKSSHHHHHHENLYFQSNAMYDWFSEMRKKDPVYYDGNIW QVFSYRYTKEVLNNFSKFSSDLTGYHERLEDLRNGKIRFDIPTRYTML TSDPPLHDELRMSADIFSPQKLQTLET FIRETTTSLSDSIDPREDDIVK KLAVPLPIIVISKILGLPIEDKEKFKEWSDLVAFRLGKPGEIFELGKKYL ELIGYVKDHLNSGTEVVS RVVNSNLSDIEKLG YIILLIAGNETTTNLIS NSVIDFTRFNLWQRIREENLYLKAIEEALRYSPVMRTVRKTKERVKL GDQTIEEGEYVRVWIASANRDEEVFHDGEKFIPDRNPNPHLSFGSGIH LCLGAPLARLEARIAIEEFKRFRHIEILDTEKVPNEVLNGYKRLVVRL KSNE

Table S2. Plasmid library obtained from site directed mutagenesis of the CYP119 gene within the 2B plasmid in the course of directed evolution.

	69X	209X	213X	254X
L		115-C10	116-G2	115-B12
V	115-D1	115-C2	116-F7	
A			116-H3	115-B6
G	115-G4	115-C8	115-F2	115-E8
Y	115-D3		116-D1	115-B8
W		115-C12	116-F1	115-B10
F	115-D10		115-H8	115-B11
T	115-D2			117-A1

T213G + ...

	69X	209X	254X	310X	155X	152X
L			115-E3			
V	116-B1					
A			117-E4			
G	115-G2		117-E7			
Y	115-G5		116-E6			140-H7
W	116-A12		115-E2	140-B9		
F	115-H1		115-E7		141-C1	
T	115-G10	115-F7	115-E4		141-B1	

	69L	69V	69A	69G	69Y	69W	69F	69T
213L						116-B8		115-H9
213V		116-B10		116-A11	116-A10		116-B11	
213A		116-B3	116-B6			115-H11	116-B5	
213G								
213Y			116-B7		116-A9			115-H4
213W					116-B4			116-A5
213F			115-H5			115-H2	115-H7	
213T								

	254L	254V	254A	254G	254Y	254W	254F	254T
213L	116-D3					116-C10		116-C4
213V				116-C12				
213A	116-C3			116-E8			116-C6	
213G								
213Y			116-D7					
213W	116-E12				116-D12			116-E3
213F					116-E4	116-E10		
213T								

	209L	209V	209A	209G	209Y	209W	209F	209T
213L		116-F2				116-G6		
213V								
213A								
213G								
213Y								
213W							116-D2	
213F								
213T								

	209L	209V	209A	209G	209Y	209W	209F	209T
254L	117-C3					117-A10		
254V								
254A						117-A3		
254G								
254Y		117-A4				117-B3		
254W	117-C5	117-B11						
254F		117-C2		117-B7				
254T								

	69L	69V	69A	69G	69Y	69W	69F	69T
209L		117-F6		117-G8	117-F5	117-G6		117-H12
209V			117-F9			117-H3		117-H7
209A								
209G		117-H8	117-G4				117-G9	
209Y								
209W		117-H2		117-H6			117-F7	117-F11
209F								
209T			117-G5	117-H4	117-H5	117-H10		117-G1

213G, 254L (115-E3) + Additional Mutation

	69X	310X	318X
L		140-B10	
V	140-B6		
A	140-A3	140-D2	
G	140-B3	140-B8	
Y	140-A4	140-C5	
W	140-B2		
F	140-A8		
T		140-C1	

213G, 254L (115-E3) +

	152X	155X	
L	140-H1		
V	141-A5	141-C10	
A		141-B3	
G	141-A9	141-C3	
Y	140-H2	141-B11	
W	141-A11	141-B2	
F	140-H11	141-D3	
T	140-G9	141-G4	

213G, 69Y (115-G5) +

	69X	310X	318X
L	142-H4		
V			
A			
G		140-C11	
Y	142-H11		
W	142-G7	140-D9	
F			
T	142-H12		

213G, 69Y (115-G5) +

	152X	155X	
L			
V	140-G8		
A			
G			
Y	140-H10		
W	140-H6		
F			
T			

T213A (116-H3) +

	69X	254X	310X
L			166-A12
V			
A			
G		165-H1	
Y		165-H3	166-A6
W			
F		165-H6	166-A9
T		165-H7	166-A7

T213A (116-H3) +

	318X	152X	155X
L		166-C7	
V	166-B11		166-D2
A			
G			
Y	166-B1	166-C4	166-D7
W	166-B6		166-D5
F	166-B7	166-C3	166-D4
T	166-B3		166-D1

254X

	69F, 213G	69V, 213G	
L	141-F6	141-E9	
V			
A		141-E1	
G			
Y	141-D7	141-D9	
W	141-F1	141-E3	
F		141-E7	
T			

69Y, 254L, 213G +

	310X	155X	
L			
V			
A			
G			
Y			
W	140-B9	141-B12	
F			
T		141-B7	

69W, 213G (116-A12) +

	209X	254X	310X
L	165-A4	165-B6	
V	165-A5		165-C11
A		165-B2	165-C10
G	165-A11		165-C1
Y	165-A7	165-B8	
W		165-B3	165-C5
F	165-A2		
T		165-B7	165-C2

69W, 213G (116-A12) +

	318X	152X	155X
L		165-E8	165-F11
V	165-D9		
A			
G	165-D3		
Y	165-D7	165-E5	165-F5
W	165-D2	165-E7	165-F2
F		165-E1	165-F4
T	165-D1		165-F1

Table S3. Complete, tabulated results for the directed evolution of Ir(Me)-CYP119 for intramolecular C-H amination reaction of substrate **2**. The identity of the mutants can be found in Table S2. In instances of “trace” or no TON, the enantioselectivity was not determined (-)

#	Mutant	ee	TON
1	XI-93-G4	-	0
2	XI-93-H5	-	0
3	XI-93-H4	-	0
4	XI-93-E2	11	5
5	XI-93-E3	32	1
6	XI-193-F1	-	Trace
7	XI-121-D4	-	0
8	XI-121-F5	-	0
9	XI-121-E5	-	0
10	XI-121-B5	-	Trace
11	XI-121-D5	-	0
12	XI-121-A5	-	0
13	XI-121-A6	-	0
14	XI-121-G6	-	0
15	XI-121-B6	-	0
16	XI-121-F7	-	0
17	XI-121-A7	24	13
18	XI-121-D7	-	0
19	XI-121-C7	-	0
20	XI-121-F9	-	0
21	XI-121-E9	-	0
22	XI-121-D9	-	0
23	XI-121-A9	-	0
24	XI-143-F8	-	0
25	XI-143-C9	36	22
26	XI-143-G8	42	18
27	XI-143-B9	56	8
28	XI-143-H9	39	3
29	XI-143-E9	35	20
30	XI-143-B8	49	47
31	XI-143-A7	38	7
32	XI-143-H7	36	9
33	XI-143-E7	-	0
34	XI-116-B11	-13	2
35	XI-193-A6	-	0
36	XI-193-D6	-	0
37	XI-193-F5	-	0
38	XI-193-E5	-	0
39	XI-193-B4	40	3
40	XI-193-D4	-	0
41	XI-193-A4	-	0
42	XI-193-G4	-	0
43	XI-193-C4	-	0
44	XI-193-B5	-	0
45	XI-193-C11	-	0
46	XI-93-H4	22	16
47	XI-93-F1	-	0
48	X-115-G10	-	0
49	X-116-B2	-33	10
50	X-117-H3	-	0
51	X-142-H4	-	0
52	X-140-C11	-	0
53	X-140-H6	-	0
54	X-140-B6	1	6
55	X-140-B2	-	0
56	X-140-B8	66	10
57	X-140-H11	10	27
58	X-141-A11	-31	5
59	X-141-B11	-	Trace
60	X-115-G2	-	0
61	X-117-F5	-	0
62	X-117-H10	-	0
63	X-142-H11	-	0
64	X-140-D9	-	0
65	X-141-F6	-	0
66	X-140-A3	-	Trace
67	X-140-A8	-	Trace
68	X-140-C5	-	0
69	X-141-A5	-	0
70	X-141-C10	-	0
71	X-141-B2	-	0
72	X-115-G5	-	0
73	X-117-H5	-	0
74	X-117-G9	-7	4
75	X-117-G7	-	0
76	X-117-G8	-	0
77	X-117-D7	-	0
78	X-140-B3	-	0
79	X-140-B10	-	0
80	X-140-H1	-	Trace
81	X-141-A9	-7	19
82	X-141-B3	-5	25
83	X-141-DC	-11	27
84	X-115-H1	-48	4
85	X-117-G6	-	0
86	X-117-F7	-	0
87	X-142-H2	-	0
88	X140-H10	-	0
89	X-141-F1	-	0
90	X-140-A4	-	0
91	X-140-D2	-	0
92	X-140-G9	-	0
93	X-141-A3	20	66
94	X-141-C3	-4	24
95	X-141-G4	-	0
96	X-166-C4	-	0
97	X-166-D4	-	0
98	X-167-G7	-	Trace
99	X-167-H9	-	0
100	X-167-D8	-	0
101	X-165-H1	-	0
102	X-165-E2	-	Trace
103	X-165-A7	-	0
104	X-165-B8	-	0
105	X-165-E5	-	Trace
106	X-165-F5	-	Trace
107	X-165-C1	-	0
108	X-166-C3	-	0
109	X-166-D1	-	Trace
110	X-167-G9	-	0
111	X-167-H7	-	Trace
112	X-167-D5	-	0
113	X-165-H3	-	0
114	X-165-A4	-9	2
115	X-165-A2	-	0
116	X-165-B3	20	0.5
117	X-165-E7	-	0
118	X-165-F2	-	0
119	X-165-C5	-	0
120	X-166-D5	-42	5
121	X-166-C7	-44	6
122	X-167-H5	-19	3
123	X-167-D1	-	0
124	X-167-D10	31	1
125	X-165-H6	-	Trace
126	X-165-A5	-	Trace
127	X-165-B6	59	5
128	X-165-B7	-10	2
129	X-165-E1	-3	13
130	X-165-F4	-	19
131	X-165-C2	-	0
132	X-166-D7	-43	4
133	X-166-D2	-28	5
134	X-167-H4	-26	5
135	X-167-D6	-	0
136	X-167-A8	-	0
137	X-165-H7	-4	2
138	X-165-A11	26	8
139	X-165-B2	49	1
140	X-165-E8	14	10
141	X-165-F1	7	9
142	X-165-D7	-	Trace

Table S4. The SFC methods used to separate enantiomers of the sultam products and sulfonamides.

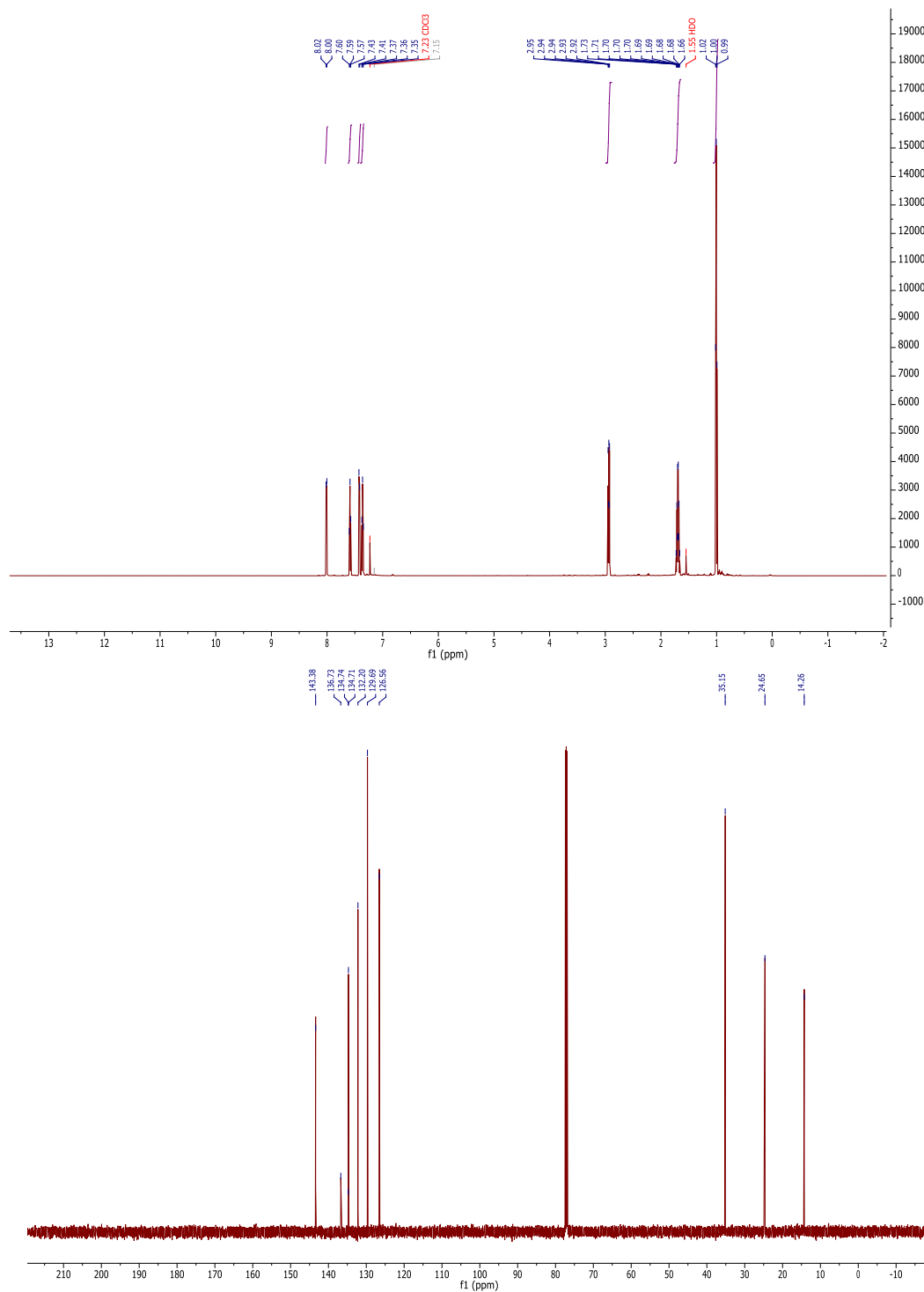
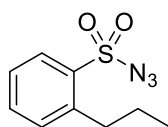
Substrate	Column	Method	Compound	Retention Times
1	Chiracel OD-H (Diacel)	Isocratic: 7% MeOH, 4 ml/min flow	AcNHPh Substrate Sultam Sulfonamide	t(ref)=1.9 min t(sub)=0.7 min t(prd+)=2.25 min t(prd-)=2.65 min t(by-prd)=3.8 min
1	Chiracel OZ-H (Diacel)	Isocratic: 7% MeOH, 4 ml/min flow	AcNHPh Substrate Sultam Sulfonamide	t(ref)=1.5 min t(sub)=0.8 min t(prd+)=2.6 min t(prd-)=2.9 min t(by-prd)=3.6 min
2	Chiracel OD-H (Diacel)	Isocratic: 4% MeOH, 4 ml/min flow	AcNHPh Substrate Sultam Sulfonamide	t(ref)=4.0 min t(sub)=0.8 min t(prd)=3.3 min t(by-prd)=5.2 min
2	Chiracel OZ-H (Diacel)	Isocratic: 7% MeOH, 4 ml/min flow	AcNHPh Substrate Sultam Sulfonamide	t(ref)=1.5 min t(sub)=0.8 min t(prd)=2.5 min t(by-prd)=3.3 min
15	Chiracel OD-H (Diacel)	Isocratic: 7% MeOH, 4 ml/min flow	AcNHPh Substrate Sultam Sulfonamide	t(ref)=1.9 min t(sub)=0.7 min t(prd+)=2.1 min t(prd-)=2.7 min t(by-prd)=2.9 min
15	Chiracel OZ-H (Diacel)	Isocratic: 7% MeOH, 4 ml/min flow	AcNHPh Substrate Sultam Sulfonamide	t(ref)=1.5 min t(sub)=1.3 min t(prd+)=3.0 min t(prd-)=3.6 min t(by-prd)=3.8 min
16	Chiracel OD-H (Diacel)	Isocratic: 7% MeOH, 4 ml/min flow	AcNHPh Substrate Sultam Sulfonamide	t(ref)=1.9 min t(sub)=0.7 min t(prd+)=2.2 min t(prd-)=2.4 min t(by-prd)=4.6 min
16	Chiracel OJ-H (Diacel)	Isocratic: 4% MeOH, 4 ml/min flow	TsNH2 Substrate Sultam Sulfonamide	t(ref)=4.4 min t(sub)=0.55 min t(prd+)=1.8 min t(prd-)=2.25 min t(by-prd)=3.6 min
9	Chiracel AS-H (Diacel)	Isocratic: 5% MeOH, 2.5 ml/min flow	AcNHPh Substrate 5-Membered sultam (A) 6-Membered sultam (B) Sulfonamide	t(ref)=2.4 min t(sub)=0.55 min t(prdA+)=8.1 min t(prdA-)=12.5 min t(prdB+)=4.3 min t(prdB-)=5.8 min t(by-prd)=7.2 min
17	Chiracel OJ-H (Diacel)	Isocratic: 1.5% MeOH, 4 ml/min flow	AcNHPh Substrate	t(ref)=5.6 min t(sub)=0.8 min

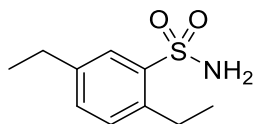
			Sultam Sulfonamide	t(prd)=1.6 min t(by-prd)=3.6 min
12	Chiralcel OD-H (Diacel)	Isocratic: 5% MeOH, 4 ml/min flow	Substrate Sultam Sulfonamide TsNH ₂	t(sub)=0.6 min t(prd+)=2.6 min t(prd-)=2.8 min t(by-prd)=5.2 min t(ref)=6.8 min

Table S5. Validation of SFC yields: Control studies of the extraction of the known amount of the product **4** from the pseudo-reaction mixture.

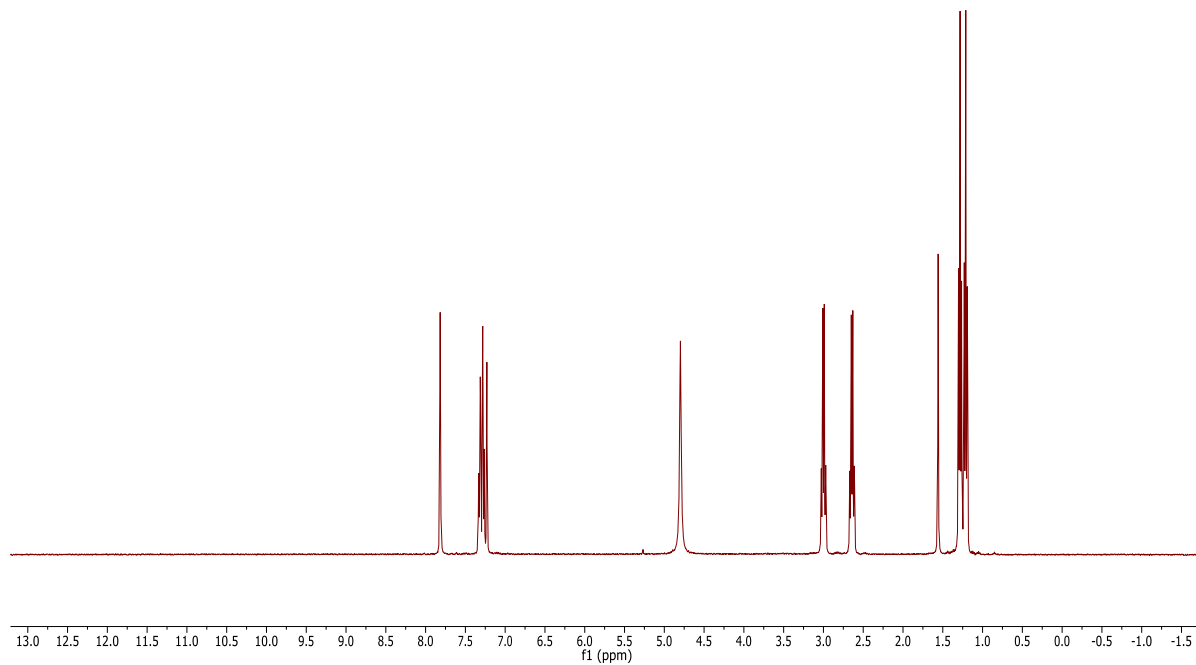
Experiment	1	2	3	4	5	6
extracted product	97.9%	96.3%	101.4%	107.7%	113.0%	101.8%

NMR spectra:

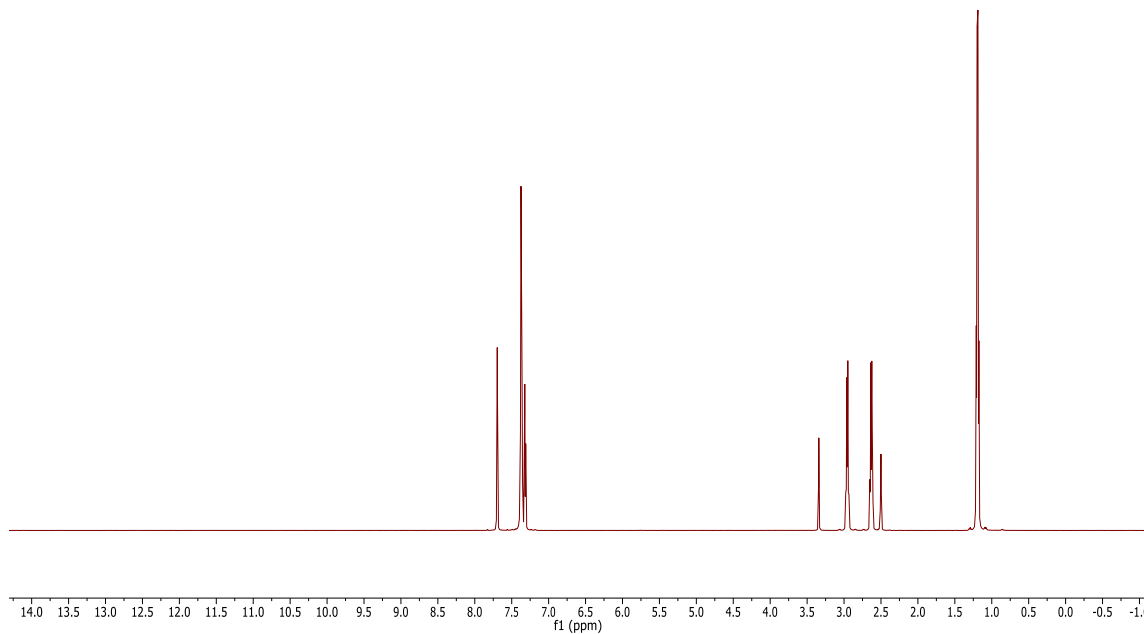


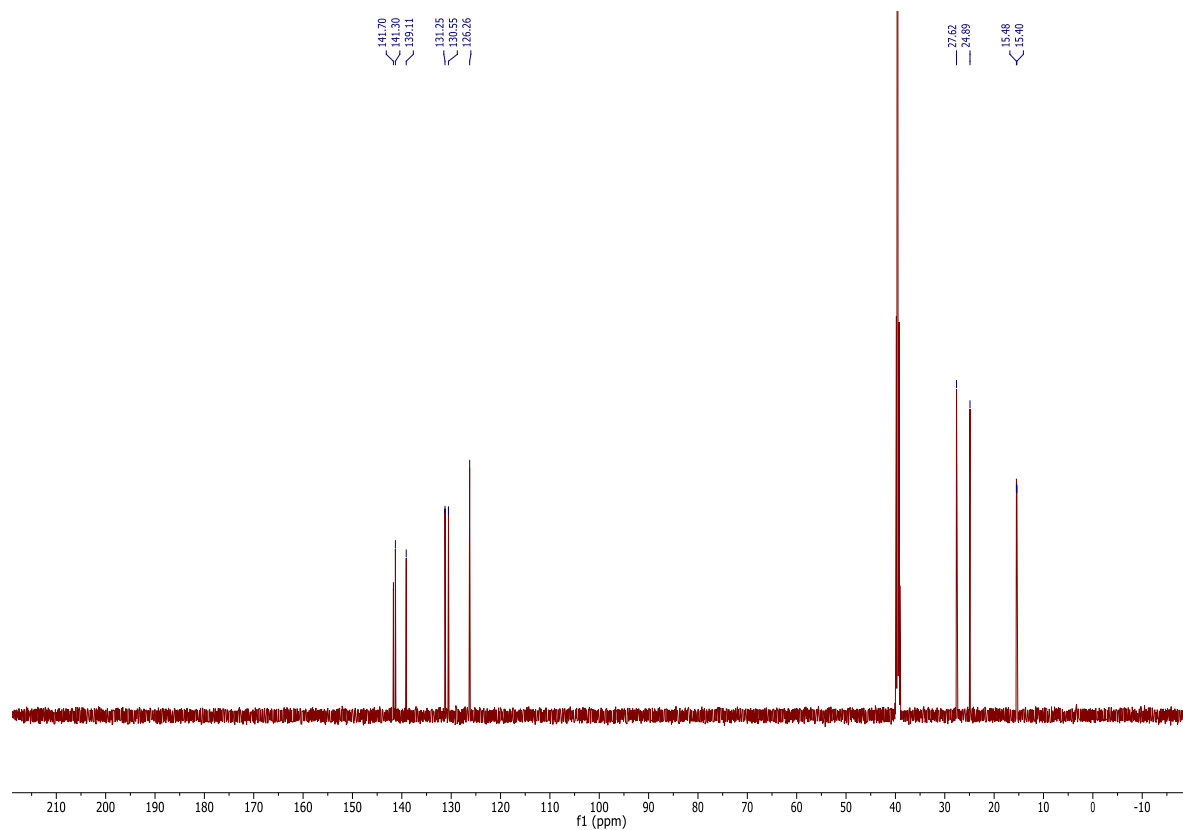


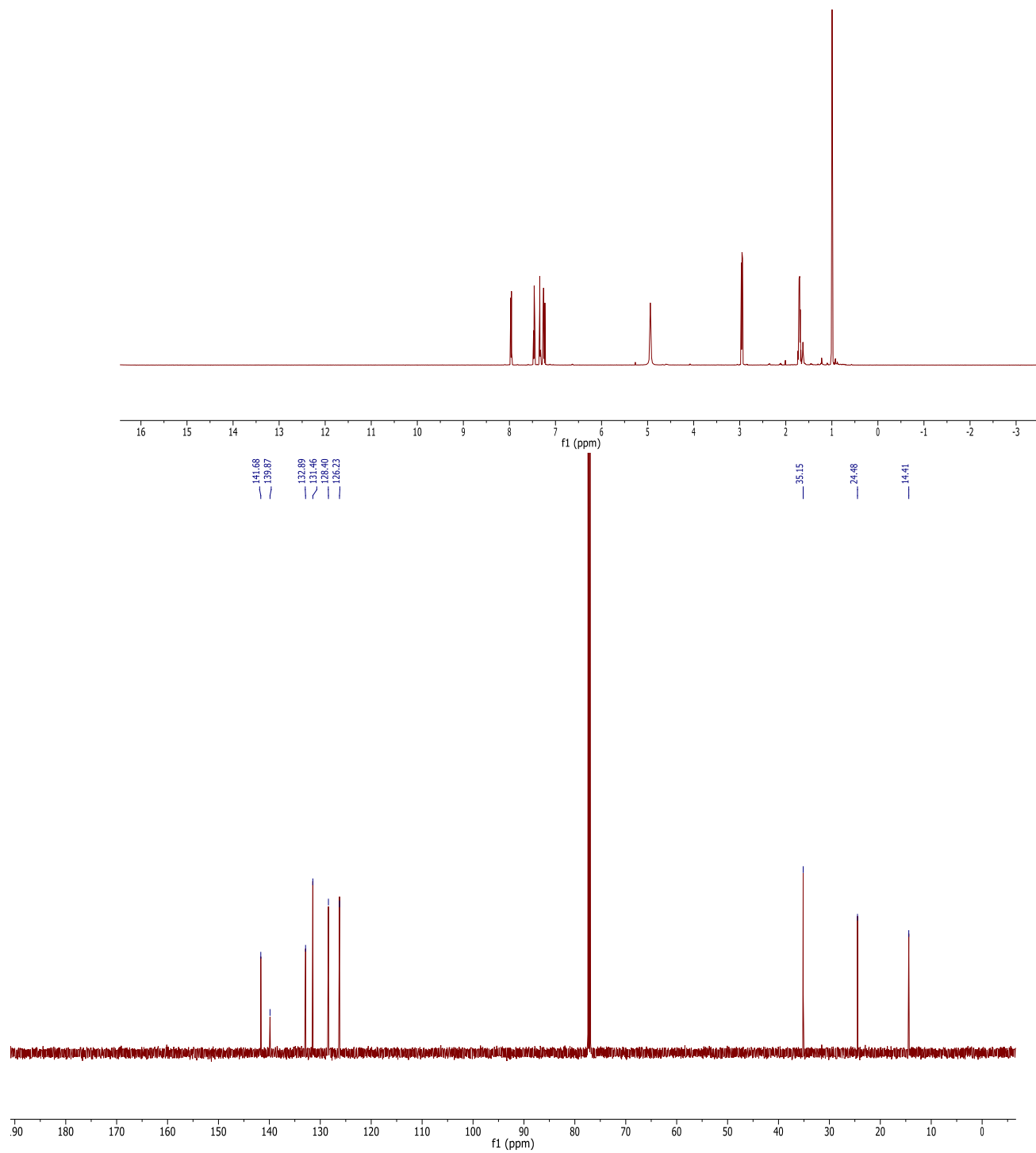
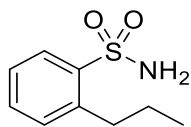
In CDCl₃:



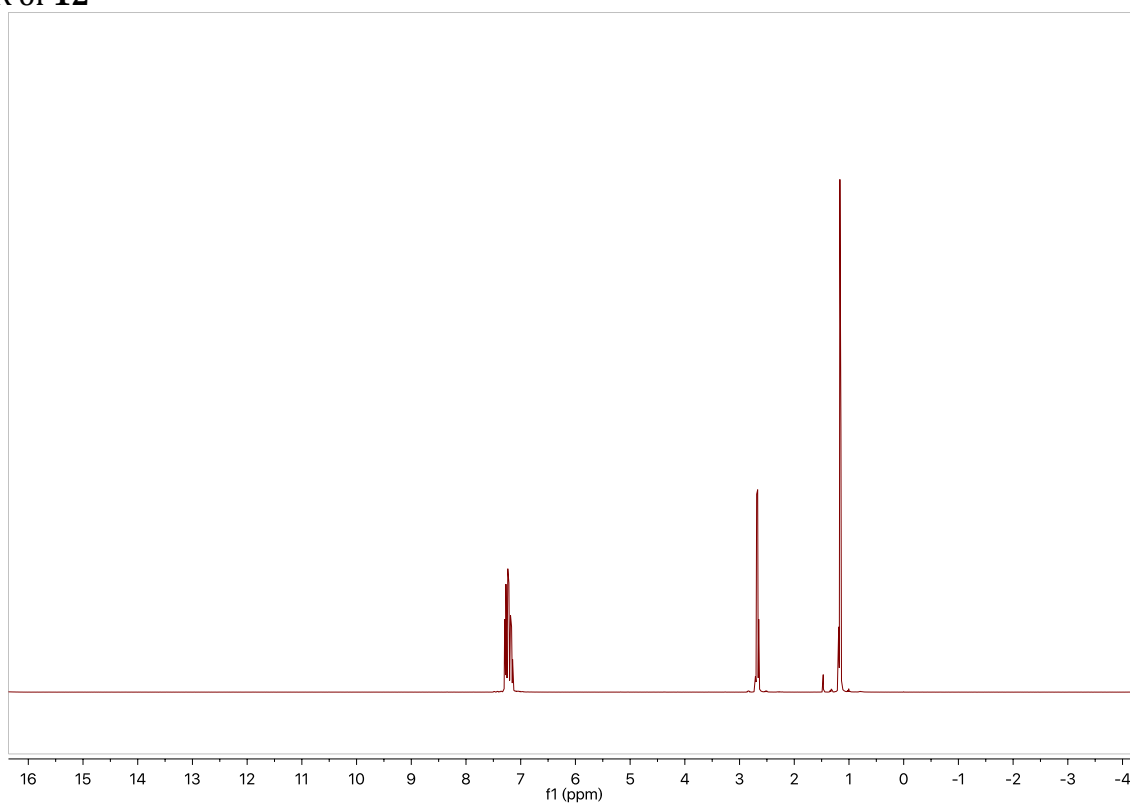
In DMSO-d₆:



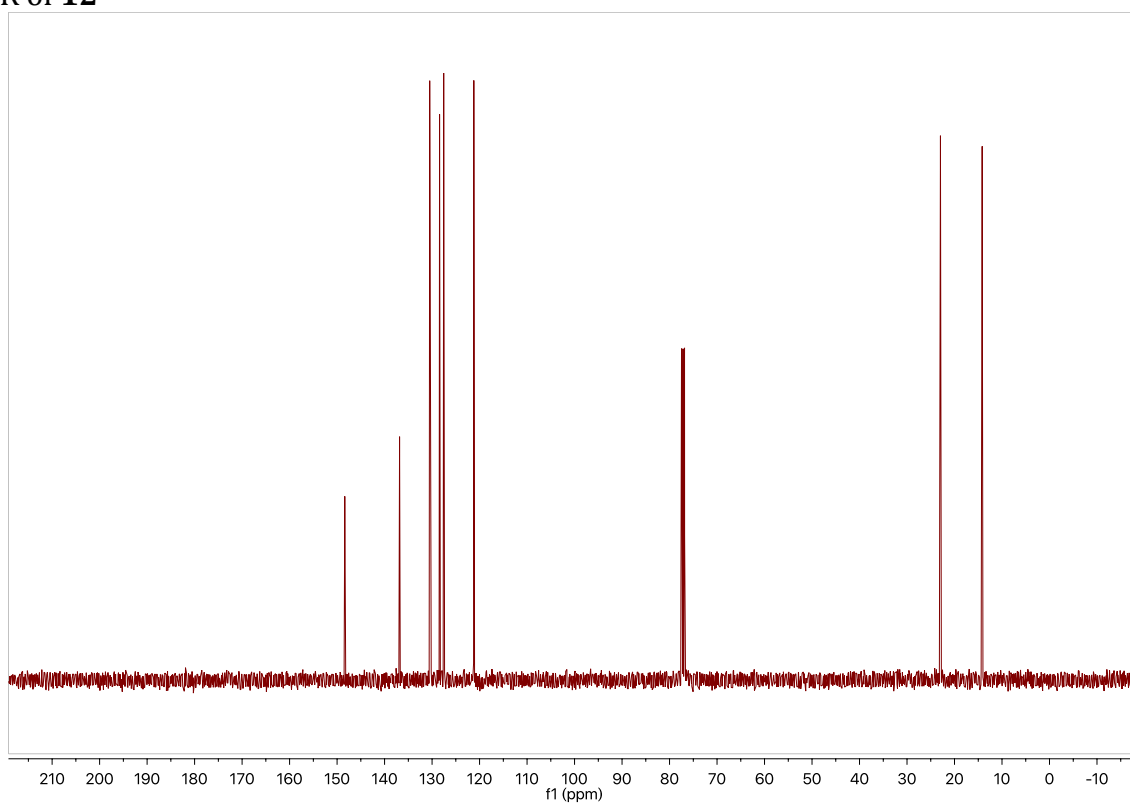




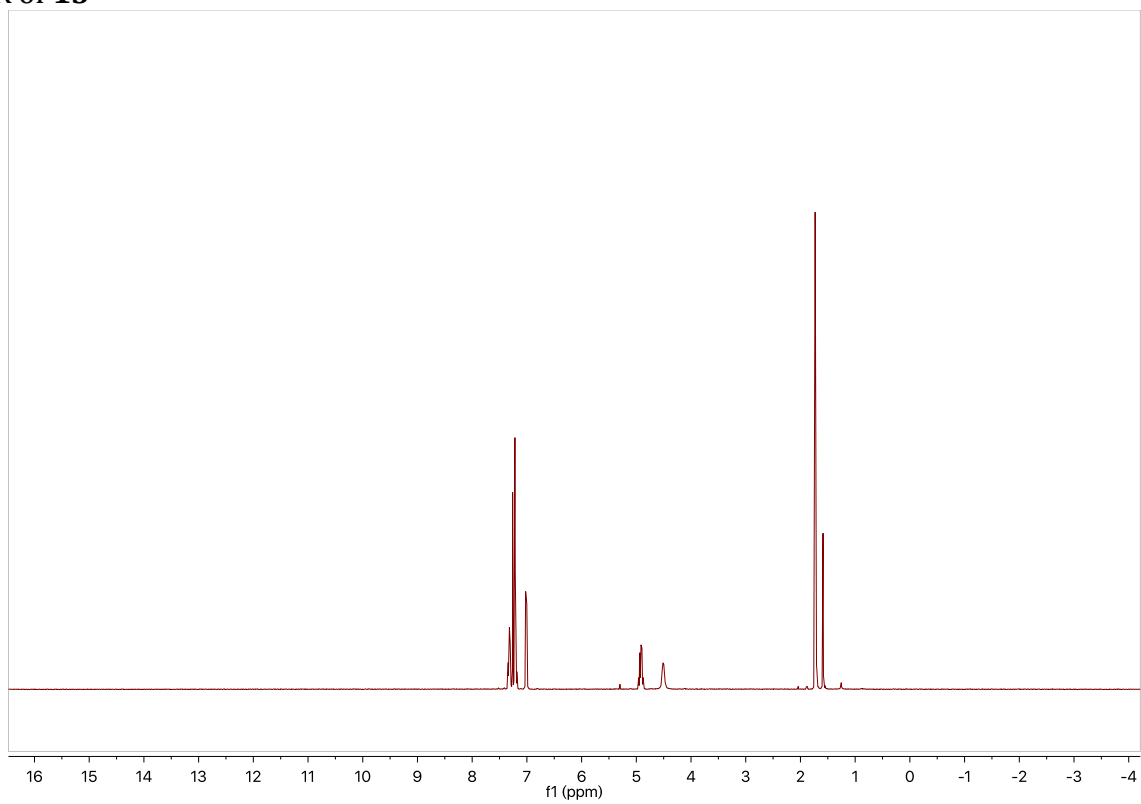
^1H NMR of **12**



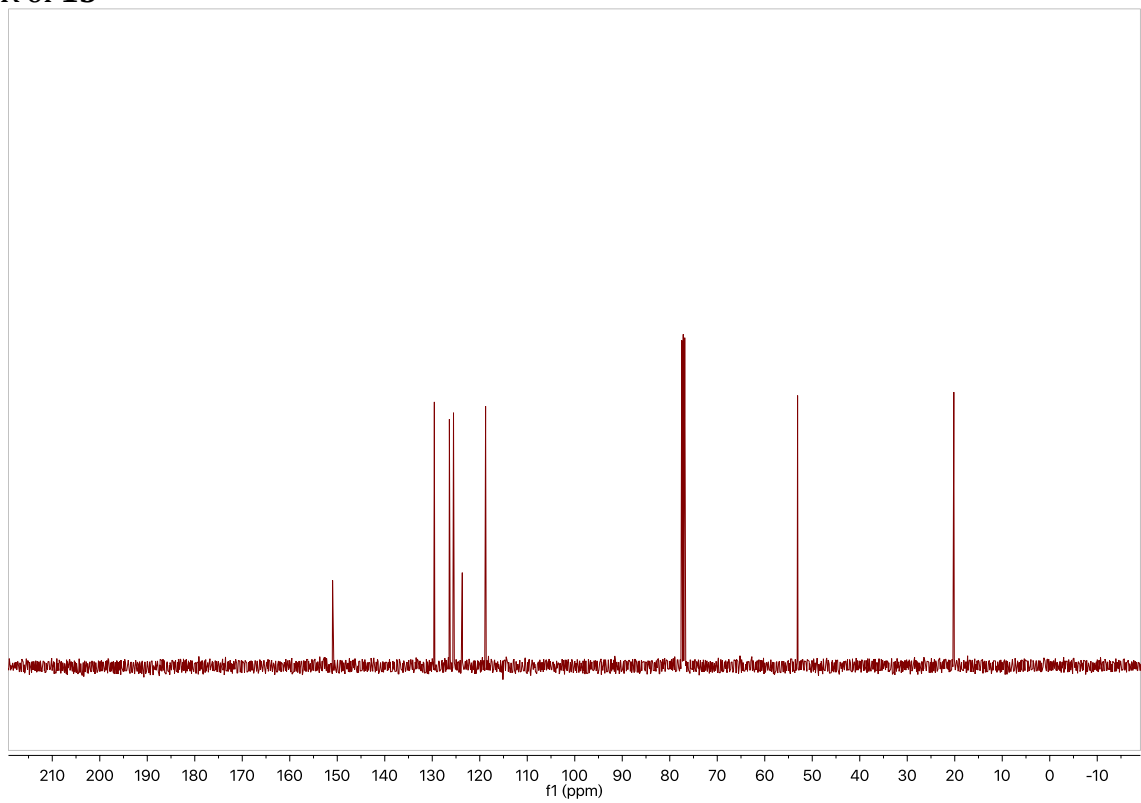
^{13}C NMR of **12**



^1H NMR of **13**



^{13}C NMR of **13**



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