Supporting Information

Synthesis of (*S*)-3-amino-4-(difluoromethylenyl)cyclopent-1-ene-1-carboxylic acid (OV329), a potent inactivator of γ-aminobutyric acid aminotransferase

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General Methods

(1*R*,4*S*)-2-Azabicyclo[2.2.1]hept-5-en-3-one was purchased from Acella Chembio. **12** was either purchased from Enamine Chemicals or synthesized from diethyl bromodifluoromethyl-phosphonate and 2-mercaptopyridine.[1] All other reagents were purchased from Sigma-Aldrich or Acros Organics and used without further purification. Anhydrous solvents (THF, CH_2Cl_2, DMF) were purified before use by passing through a column composed of activated alumina and a supported copper redox catalyst. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogeneous material. Analytical thin-layer chromatography (TLC) was performed using Merck Silica Gel 60 Å F-254 precoated plates (0.25 mm thickness), and components were visualized by ultraviolet light (254 nm) and/or ceric ammonium molybdate stain. Flash column chromatography was performed on a Teledyne Combiflash Rf Plus automated flash purification system with various Taledyne cartridges (4-80 g, 40-63 µm, 60 Å). Purifications were preformed with hexanes and ethyl acetate unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-III NMR spectrometer at 500 MHz and 126 MHz, respectively, in $CDCl_3$ or D_2O. Chemical shifts were reported in ppm, multiplicities are indicated by s = singlet, d = doublet, t = triplet, q = quartet, sep = septet, dd = doublet of doublet, dt = doublet of triplet, m = multiplet, br = broad resonance. Coupling constants 'J' were reported in Hz. High resolution mass spectral data were obtained on an Agilent 6210 LC-TOF spectrometer in the positive ion mode using electrospray ionization with an Agilent G1312A HPLC pump and an Agilent G1367B autoinjector at the Integrated Molecular Structure Education and Research Center (IMSERC), Northwestern University. Analytical HPLC to determine purity was performed using an Agilent Infinity 1260 HPLC with a reversed-phase Phenomenex Kintex C-18 column (50 x 2.1 mm, 2.6 µm), detecting with UV absorbance at 254 nm.

Synthesis of Compounds

(1R,4S)-2-(4-Methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one. 4-Methoxybenzyl alcohol (35.80 mL, 0.29 mol, 1.5 equiv) was added dropwise to concentrated HCl (300 mL) and stirred for 1 h. Water was added, and the liquid was extracted (2 x 100 mL) with diethyl ether. The diethyl ether was dried over Na_2SO_4 and concentrated to a volume of about 50 mL. To a 2 L flask, equipped with an addition funnel, was added (1*R*,4*S*)-2-azabicyclo[2.2.1]hept-5-en-3-one (21.00 g, 0.19 mol), DMF (600 mL) , and THF (600 mL), and the flask was cooled to 0 °C. NaH (8.45 g, 0.21 mol, 1.1 equiv, 60% dispersion in mineral oil) was added portionwise. The flask was placed under N_2 and stirred for 30 min. The Et_2O/PMBCl solution was transferred to the addition funnel and added dropwise at 0 °C. The reaction mixture was stirred for 6 h at room temp. Upon completion, THF was removed *in vacuo,* and diethyl ether and water were added. Any solids were filtered, and the layers were separated. The aqueous layer was extracted (3 x 100 mL) with diethyl ether, and the organic layers were combined and washed with brine (2 x 200 mL). After drying over Na_2SO_4 and concentration, a yellow oil was obtained. The crude oil was purified by flash chromatography to yield 32.2 g (0.14 mol, 73% yield) of protected material. Spectra matched those in the literature.[2]

*(1R,4R,6S,7R)-7-Bromo-2-(4-methoxybenzyl)-3-oxo-2-azabicyclo[2.2.1]heptan-6-yl acetate (***11**). To a solution of the above compound (10.00 g, 43.62 mmol) in AcOH (110.0 mL) was added 1,3-dibromo-5,5-dimethylhydantoin (7.48 g, 26.17 mmol, 0.6 equiv). The reaction was stirred for 6 h, and upon completion, water was added. The aqueous layer was extracted (3 x 200 mL) with diethyl ether, and the organic layers were combined, washed with 1 M NaOH, dried over Na_2SO_4, and concentrated. Purification by flash chromatography yielded **11** (14.40 g, 39.25 mmol, 90% yield) as a thick oil. Spectra matched those in the literature.[2]

(1R,4R,7R)-7-Bromo-2-(4-methoxybenzyl)-2-azabicyclo[2.2.1]heptane-3,6-dione. Method A: **11** (12.8 g, 34.7 mmol) was dissolved in MeOH (270 mL) and K_2CO_3 (14.40 g, 104.28 mmol, 3.0 equiv) was added. The reaction was stirred for 1 h, filtered, and then concentrated. Ethyl acetate and water were added, and the layers were separated. The organic layer was dried over Na_2SO_4 and concentrated to yield an off-white colored solid, which was used directly in the next step.

The compound above was placed in a 500 mL flask and purged with argon. Dichloromethane (170 mL) was added followed by 4 Å molecular sieves (10 g). TPAP (122.2 mg, 0.35 mmol. 0.01 equiv) and NMO (8.14 g, 69.52 mmol, 2.0 equiv) were then added, and the reaction mixture was stirred overnight, which was then filtered and concentrated to a volume of 20 mL and loaded directly onto a flash column. The resulting yellowish solid can be recrystallized from hexanes/ethyl acetate to obtain a white powder (5.96 g, 18.38 mmol, 52% yield). Spectra matched those in the literature. [2]

Method B: **11** (25.5 g, 69.2 mmol) was dissolved in MeOH (300 mL) and K_2CO_3 (30 g, 0.23 mol, 3 equiv) was added. The reaction was stirred for 1 h, filtered, and then concentrated. Ethyl acetate and water were added, and the layers were separated. The organic layer was dried over Na_2SO_4 and concentrated to yield an off-white colored solid, which was used directly in the next step.

A three-neck flask was equipped with a vent line to a bubble, dropping funnel with nitrogen inlet, and septum. Dichloromethane (160 mL) was added, and the flask was purged with nitrogen. Oxalyl chloride (8.40 mL, 98.0 mmol, 1.4 equiv) was added, and the reaction was cooled to -78 °C. DMSO (11.60 mL, 0.16 mol, 2.3 equiv) was added to the addition funnel and then added dropwise slowly at a rate to control the vigorous gas evolution. After addition, the reaction was stirred at -78 °C for 10 min. The deacylated material was dissolved in dichloromethane (160 mL) and added slowly to the reaction via addition funnel. The reaction was stirred for 10 min at -78 °C. Triethylamine (68.3 mL, 0.49 mol, 7 equiv) was then added dropwise via addition funnel. Upon completion, the reaction was stirred at -78 °C for 10 min, warmed to room temp, and quenched with 1 M HCl. After separation, the organic layer was dried over Na_2SO_4 and concentrated in a fume hood. Purification via flash chromatography yielded **11** as a beige solid (13.5 g, 41.7 mmol, 60% yield).

(1R,4R,7R)-7-Bromo-6-(difluoromethylene)-2-(4-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (**13**). **11** (1.00 g, 3.09 mmol) and 2-((difluoromethyl)sulfinyl)pyridine (**12**, 715.10 mg, 3.70 mmol, 1.2 equiv) were added to a round bottom flask and purged with argon. DMF (15 mL) was then added, and the reaction was cooled to between -55 and -65 °C. KO^{t}Bu (623.0 mg, 5.55 mmol, 1.8 equiv, 0.5 M in DMF) was added via syringe pump over 1 h. The temperature was maintained between -55 and -65 °C. After addition, the reaction mixture was further stirred for 30 min at -60 °C. Saturated NH_4Cl (5.00 mL) was then added, and the reaction mixture was stirred for 5 min at -60 °C before 6 M HCl (5.00 mL) was added. After 5 min of stirring at -60 °C, the reaction mixture was warmed to room temp and then to 65 °C for 1 h. After being cooled, the reaction mixture was diluted with brine, extracted (2 x 20 mL) with ethyl acetate, and washed with brine (10 mL). Upon drying over Na_2SO_4 and concentrating a yellow oil was obtained, which was purified via flash chromatography to yield a white solid (620.0 mg, 1.73 mmol, 58% yield). $[\alpha]_D^{23\,°C}$= -46.6 4 (*c* 0.80, $CHCl_3$); **m.p.** 85-87 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 4.60 (d, *J* = 14.6 Hz, 1H), 4.19 (s, 0H), 4.14 (s, 0H), 3.90 (d, *J* = 14.7 Hz, 1H), 3.79 (s, 1H), 3.00 (s, 0H), 2.83 (dq, *J* = 14.6, 3.0 Hz, 1H), 2.27 (d, *J* = 15.2 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.0, 159.5, 153.9 (dd, *J* = 287.5, 283.8 Hz), 129.7, 127.5, 114.3, 87.1 (dd, *J* = 24.9, 23.5 Hz), 63.4, 63.3, 55.3, 50.8 (d, *J* = 17.1 Hz), 44.6, 24.8. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -88.15 (dp, *J* = 55.1, 2.7 Hz), -88.88 (dq, *J* = 54.8, 2.8 Hz). **IR** (film, cm^{-1}) 3013, 1785, 1683, 1551; **HMRS (ESI$^+$)** calc'd for $C_{15}H_{14}BrF_2NO_2$+Na^+: 380.0074; found 380.0075.

(1R,4R,7R)-7-Bromo-6-(difluoromethylene)-2-azabicyclo[2.2.1]heptan-3-one (**14**): **13** (140 mg, 0.39 mmol) was added to MeCN (2.0 mL) and cooled to 0 °C. Ceric ammonium nitrate (643.5 mg, 1.17 mmol, 3 equiv) in 0.75 mL of H_2O was added dropwise. The reaction mixture was allowed to warm to room temp and stirred for 1 h. After completion, water was added, and the solution was extracted (2 x 15 mL) with ethyl acetate. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography yielded **14** as a white solid (75 mg, 0.315 mmol, 80% yield). $[\alpha]_D^{23\,°C}$= +38.5 (*c* 0.90, $CHCl_3$); **m.p.** 139-141 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.87 (s, 1H), 4.41 (s, 1H), 4.32 (s, 1H), 2.96 (s, 1H), 2.87 (dq, *J* = 15.4, 3.4 Hz, 1H), 2.32 (d, *J* = 15.2 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.9, 153.5 (t, *J* = 287.5 Hz), 88.7 (t, *J* = 24.1 Hz), 60.6, 51.5, 50.3, 24.3. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -88.60 (dq, *J* = 55.1, 2.8 Hz), -88.88 (dp, *J* = 54.6, 2.5 Hz). **IR** (film, cm^{-1}) 3249. 1788, 1678, 1397; **HMRS (ESI$^+$)** calc'd for $C_7H_6BrF_2NO$+H^+: 237.9679; found 237.9678

Methyl (S)-3-((tert-butoxycarbonyl)amino)-4-(difluoromethylene)cyclopent-1-ene-1-carboxylate (**16**): **14** (890.0 mg, 3.74 mmol) was added to dichloromethane (18.0 mL) followed by the sequential addition of Boc_2O (978.8 mg, 4.49 mmol, 1.2 equiv), DMAP (45.7 mg, 0.37 mmol, 0.1 equiv), and Et_3N (0.78 mL, 5.61 mmol, 1.5 equiv). The reaction mixture was stirred for 1 h and then was washed with 1 M HCl (10 mL), dried over Na_2SO_4, and concentrated. The resulting oil was dissolved in methanol (18.0 mL), then K_2CO_3 (1.55 g, 11.21 mmol, 3.0 equiv) was added, and the reaction mixture was stirred for 6 h. After completion, as indicated by LC/MS (methanolysis of the lactam occurs in the first 10 min), the reaction mixture was diluted with brine and extracted (3 x 200 mL) with ethyl acetate. Upon drying over Na_2SO_4, concentrating,

and purifying by flash chromatography **16** was obtained as a white solid (570 mg, 1.97 mmol, 52 % yield)**.** $[\alpha]_D^{23\,^\circ C}$**=** +104.8 (*c* 0.50, $CHCl_3$); **m.p.** 95-97 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.58 (s, 1H), 5.50 (d, *J* = 9.1 Hz, 1H), 4.63 (d, *J* = 8.7 Hz, 1H), 3.75 (s, 3H), 3.33 (d, *J* = 20.4 Hz, 1H), 3.21 (dd, *J* = 20.3, 2.7 Hz, 1H), 1.42 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.3, 154.7, 154.6, 152.4 (t, *J* = 288.5 Hz), 150.1, 140.6, 135.5, 88.9 (dd, *J* = 21.8, 20.2 Hz), 80.1, 55.3, 51.9, 31.1, 28.3. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -84.49 (d, *J* = 43.6 Hz), -85.91 (d, *J* = 43.4 Hz). **IR** (film, cm^{-1}) 3347, 2987, 1773, 1681; **HMRS (ESI$^+$)** calc'd for $C_{13}H_{17}F_2NO_4$+Na^+: 312.1023; found 312.1018.

(S)-3-Amino-4-(difluoromethylene)cyclopent-1-ene-1-carboxylic acid hydrochloride (**1**): **15** (570.0 mg, 1.97 mmol) was dissolved in dioxane (1.00 mL), and 6 M HCl (9 mL) was added. After being heated at 80 °C for 2 h, the reaction mixture was concentrated to yield **1** as a light brown powder (403.0 mg, 1.90 mmol, 97% yield). Crystallization from ethanol/diethyl ether increased purification to >99%. $[\alpha]_D^{23\,^\circ C}$**=** +67.2 (*c* 0.90, H_2O); **m.p.** 207 °C (decomp.); **^{1}H NMR** (500 MHz, D_2O) δ 6.59 (s, 1H), 4.70 (s, 1H), 3.39 (d, *J* = 20.5 Hz, 1H), 3.33 (d, *J* = 20.8 Hz, 1H). **^{13}C NMR** (126 MHz, D_2O) δ 167.2, 153.0 (dd, *J* = 290.1, 288.4 Hz), 141.8, 134.3, 86.1 (dd, *J* = 26.6, 21.2 Hz), 54.8 (d, *J* = 5.7 Hz), 31.1. **^{19}F NMR** (470 MHz, D_2O) δ -83.1 (dq, *J* = 40.8, 2.8 Hz), -83.5 (dq, *J* = 40.4, 2.1 Hz). **IR** (film, cm^{-1}) 3348, 3075, 2981, 2883, 2829, 2600, 2434, 1771, 1686; **HMRS (ESI$^-$)** calc'd for $C_7H_7F_2NO_2$-H: 174.0372; found 174.0369.

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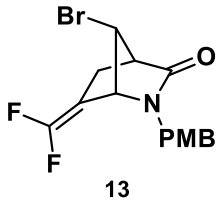
(1) Zhou, Q.; Ruffoni, A.; Gianatassio, R.; Fujiwara, Y.; Sella, E.; Shabat, D.; Baran, P. S. *Angew Chem Int Ed Engl* **2013**, 52, 3949-3952.

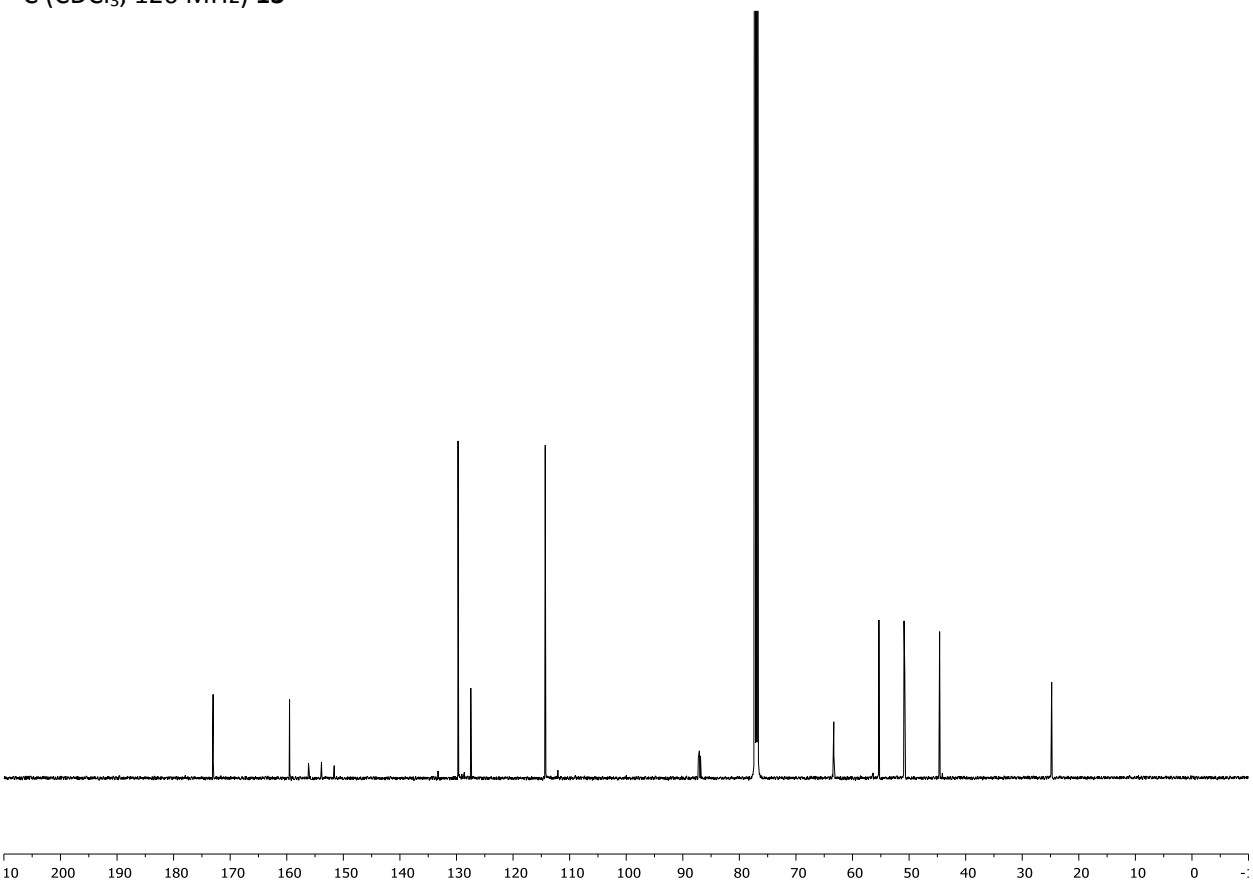
(2) Qiu, J.; Silverman, R. B. *J. Med. Chem.* **2000**, 43, 706-720

^{1}H ($CDCl_3$, 500 MHz) **13**



13

^{13}C ($CDCl_3$; 126 MHz) **13**



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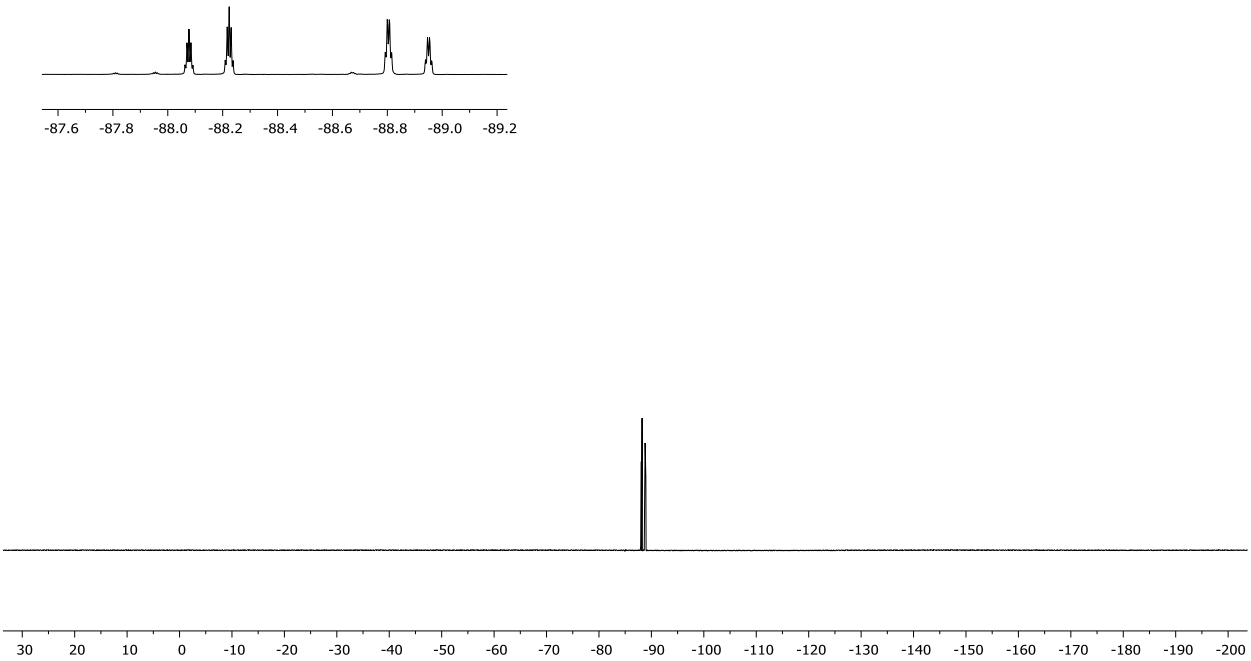

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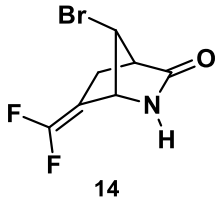

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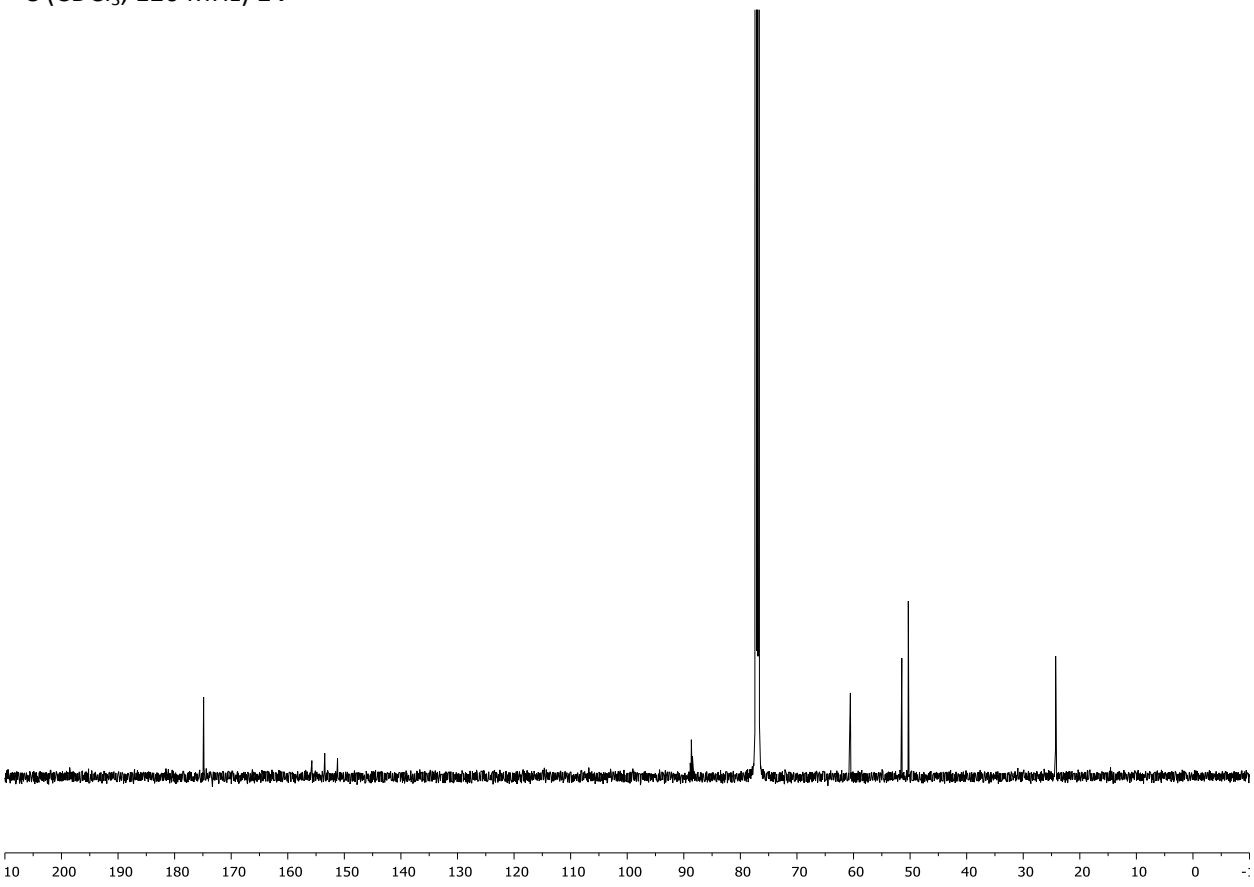

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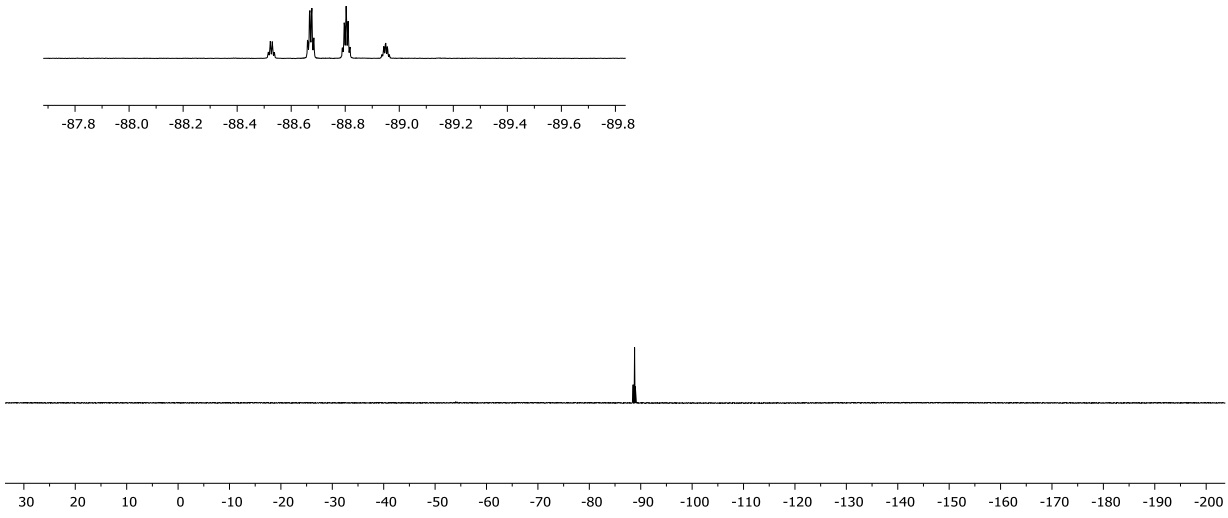

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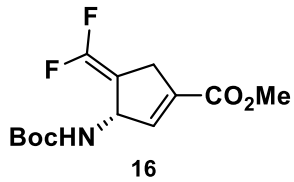
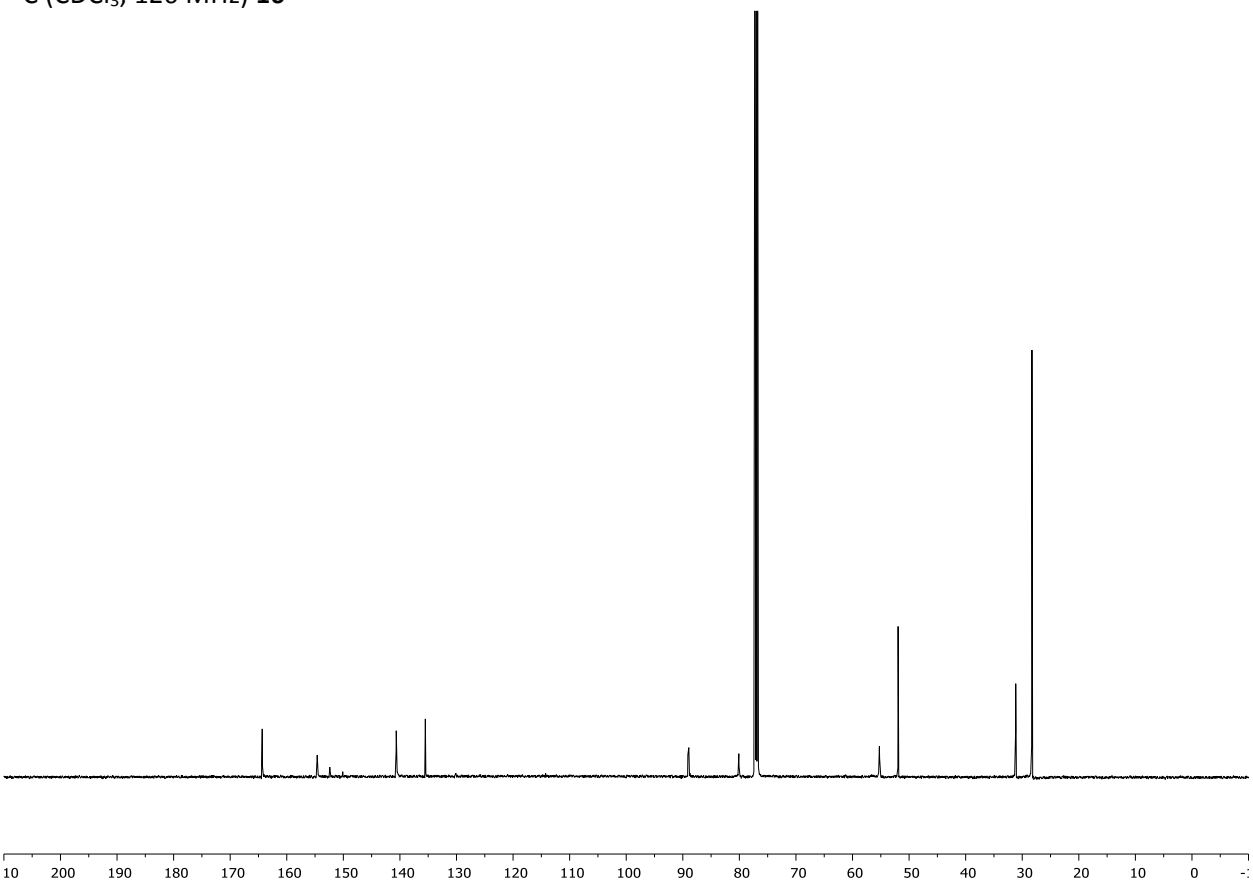
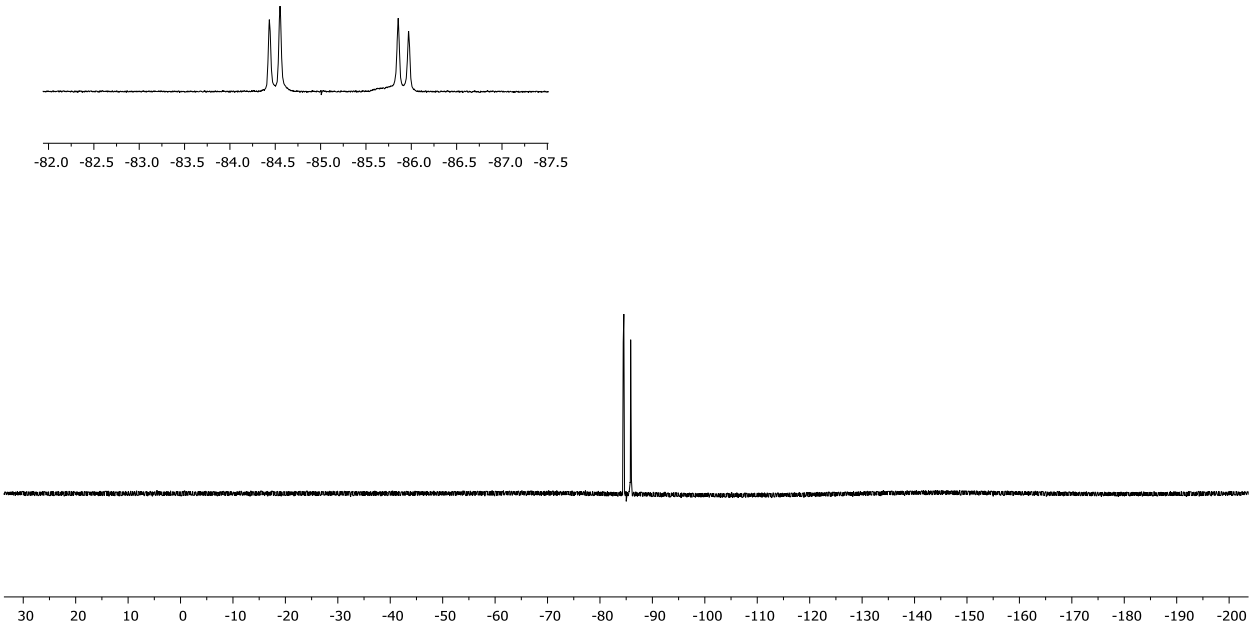


16

^{13}C ($CDCl_3$; 126 MHz) **16**



^{19}F ($CDCl_3$; 127.5 MHz) **16**



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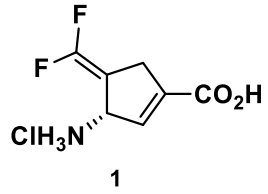
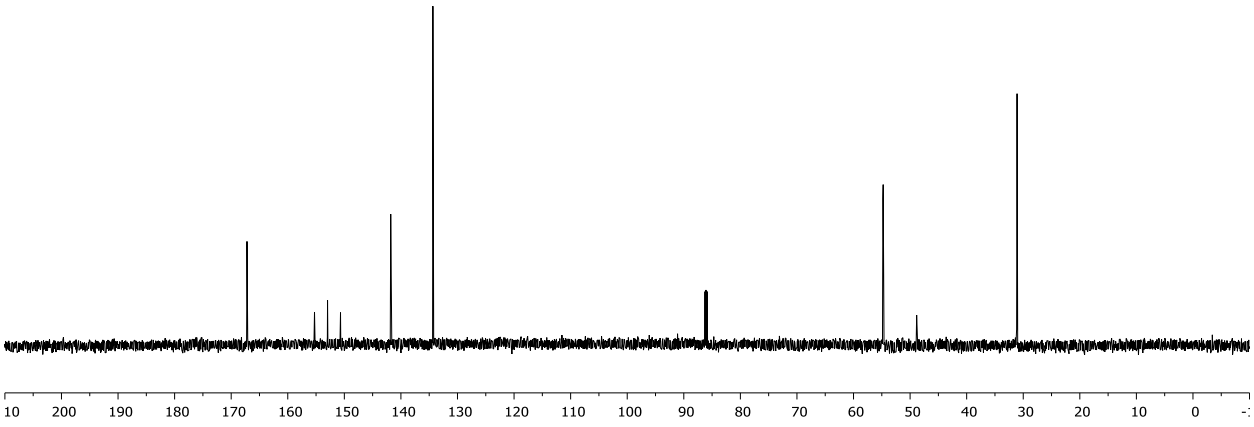


1

^{13}C ($CDCl_3$; 126 MHz) **1**



^{19}F ($CDCl_3$; 127.5 MHz) **1**