SUPPORTING INFORMATION

Development of a Fluorinated Class-I HDAC Radiotracer Reveals Key Chemical Determinants of Brain Penetrance

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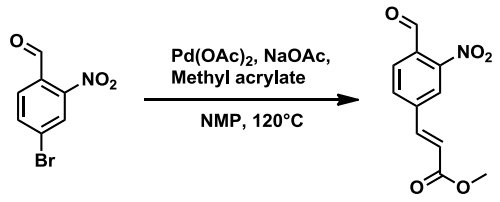
MATERIALS AND METHODS

All air-and moisture-insensitive reactions were carried out under an ambient atmosphere and magnetically stirred. All air-and moisture-sensitive manipulations were performed using oven-dried glassware, under nitrogen atmosphere. Flash chromatography was performed on Dynamic Adsorbents Silica Gel 40–63 µm particle size on a Biotage Isolera One instrument, with an automatically generated gradient of hexanes and ethyl acetate. Anhydrous acetonitrile was purchased from VWR and used as received. All deuterated solvents were purchased from Cambridge Isotope Laboratories. NMR spectra were recorded on either a Varian Unity/Inova 600 spectrometer operating at 600 MHz for ^{1}H acquisitions, a Varian Unity/Inova 500 spectrometer operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C acquisitions, respectively, or a Varian Mercury 400 spectrometer operating at 375 MHz for ^{19}F acquisitions. Chemical shifts are reported in ppm with the solvent resonance as the internal standard. For ^{1}H NMR: $CDCl_3$, δ 7.26; d_6-DMSO, δ 2.05; For ^{13}C NMR: $CDCl_3$, δ 77.16; d_6-DMSO, δ 39.52. Data is reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants in Hz; carbon signals are singlets unless otherwise noted. All substrates were used as received from commercial suppliers, unless otherwise stated.

EXPERIMENTAL DATA

Synthesis of Reagents, Precursors, Standards and Blocking Agents

4-Formyl-3-Nitro Methyl Cinnamate (2)



1 2

To 4-bromo-2-nitrobenzaldehyde (5.70 g, 24.5 mmol, 1 eq), oven-dried sodium acetate (2.24 g, 27.3 mmol, 1.10 eq) and palladium acetate (11 mg, 50 µmol, 2.0 mol%) under nitrogen atmosphere was added 60 mL NMP. The mixture was heated to 120 °C and methyl acrylate (3.20 g, 37.2 mmol, 1.50 eq) were added as soon as the temperature was reached. After TLC showed complete consumption of starting material, the mixture was cooled to room temperature, partitioned between ethyl acetate and 5% LiCl (aq) and the aqueous layer was extracted twice more with ethyl acetate. The combined organic layers were washed twice with 5% LiCl (aq) and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography. 5.70 g (24.2 mmol, 98.8 %) of **(2)** was obtained as an off-white solid.

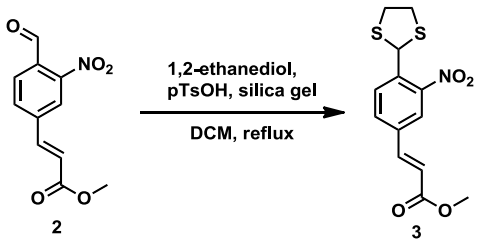
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 10.42 (s, 1 H), 8.23 (d, *J*=1.0 Hz, 1 H), 7.99 (d, *J*=7.8 Hz, 1 H), 7.88 (d, *J*=8.3 Hz, 1 H), 7.72 (d, *J*=16.1 Hz, 1 H), 6.63 (d, *J*=15.7 Hz, 1 H), 3.85 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 187.3, 166.0, 150.0, 140.6, 140.2, 132.7, 131.4, 130.4, 123.4, 123.3, 52.2.

HRMS-FIA(m/z) calc'd for $C_{11}H_9NO_5$ $[M+H]^+$, 236.0559; found, 236.0563.

4-(1,3-dithiolan-2-yl)-3-nitro methyl cinnamate (3)



2 3

To aldehyde **(2)** (200 mg, 851 µmol, 1 eq) was added 1,2-ethanedithiol (86 µl, 1.0 mmol, 1.2 eq), pTsOH monohydrate (20 mg, 0.12 mmol, 14 mol%), 4 g silica gel and 25 mL DCM. The mixture was stirred at reflux for 2.5 h, cooled to room temperature and filtered. The silica gel was washed with three lots of DCM and the combined filtrates were concentrated *in vacuo*. The residue was purified by flash chromatography. 260 mg (835 µmol, 98.1%) of **(3)** was obtained as a light yellow solid.

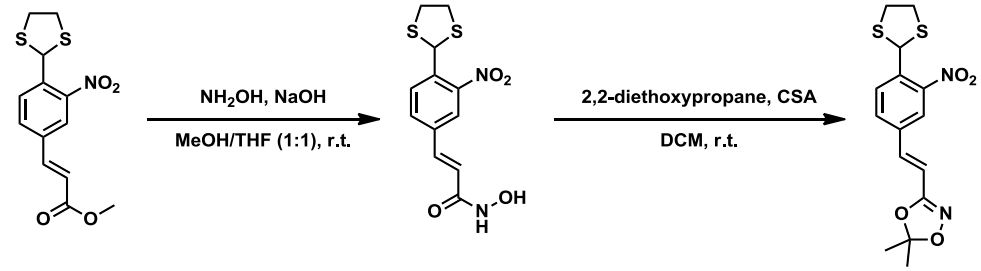
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 8.08 (d, *J*=8.3 Hz, 1 H), 7.98 (s, 1 H), 7.70 (d, *J*=7.8 Hz, 1 H), 7.63 (d, *J*=16.1 Hz, 1 H), 6.49 (d, *J*=16.1 Hz, 1 H), 6.16 (s, 1 H), 3.81 (s, 3 H), 3.32 - 3.58 ppm (m, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 166.5, 148.5, 141.4, 138.7, 135.0, 131.9, 131.1, 123.7, 120.9, 52.0, 50.4, 40.0.

HRMS-FIA(m/z) calc'd for $C_{13}H_{13}S_2NO_4$ $[M+H]^+$, 312.0359; found, 312.0363.

3-(4-(1,3-dithiolan-2-yl)-2-nitrostyryl)-5,5-dimethyl-1,4,2-dioxazole (4)



3 4a 4

Ester **(3)** (200 mg, 640 µmol, 1 eq) was dissolved in 3 mL MeOH/THF (1:1). At room temperature, 0.5 mL hydroxylamine solution (50% in water) and 0.5 mL 5M NaOH were added. After 2 minutes, the mixture was poured onto 10 mL of an ice/water mixture and 2 mL conc. HCl were added. The mixture was kept at 0°C for several minutes, then the precipitate was isolated by filtration and washed with water. 102mg (327 µmol, 51.1%) of hydroxamic acid were obtained and used without further purification after drying under high vacuum for 24 h.

To a suspension of hydroxamate (50 mg, 0.16 mmol, 1 eq) in 4 mL DCM was added 2,2-diethoxypropane (63

mg, 0.48 mmol, 3 eq) and camphorsulfonic acid (37 mg, 0.16 mmol, 1 eq). The solution became clear within a few minutes, stirring was continued for four hours at room temperature until no hydroxamic acid was remaining. The solution was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by flash chromatography. **(4)** was obtained as a light yellow oil (39 mg, 0.11 mmol, 69%).

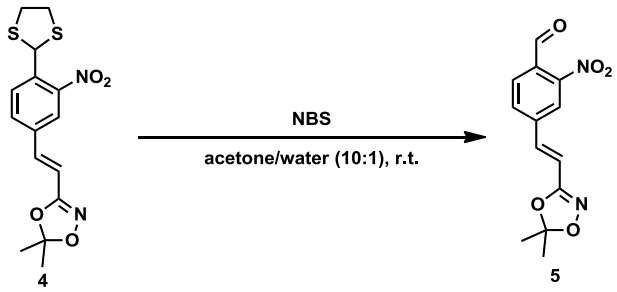
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 8.08 (d, *J*=8.3 Hz, 1 H), 7.91 (d, *J*=1.5 Hz, 1 H), 7.67 (dd, *J*=8.3, 1.5 Hz, 1 H), 7.13 (d, *J*=16.1 Hz, 1 H), 6.67 (d, *J*=16.1 Hz, 1 H), 6.17 (s, 1 H), 3.31 - 3.51 (m, 4 H), 1.65 ppm (s, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 157.8, 148.6, 137.8, 135.7, 134.2, 131.1, 130.9, 123.2, 116.1, 112.6, 50.4, 39.6, 24.9.

HRMS-FIA(m/z) calc'd for $C_{15}H_{16}S_2N_2O_4$ $[M+H]^+$, 353.0624; found, 353.0621.

3-(4-formyl-2-nitrostyryl)-5,5-dimethyl-1,4,2-dioxazole (5)



To an emulsion of dithiolane **(4)** (42 mg, 0.12 mmol) in 1 mL of a 10:1 mixture of acetone and water was added freshly recrystallized NBS and stirred at room temperature for 30 min. The reaction mixture was then diluted with ethyl acetate, washed with water, dried over sodium sulfate and filtered. The filtrate was concentrated *in vacuo* and purified by preparative thin layer chromatography with DCM as mobile phase. 5.0 mg (18 µmol, 15%) of **(5)** was obtained as a yellow oil which crystallized upon standing to give a light yellow solid.

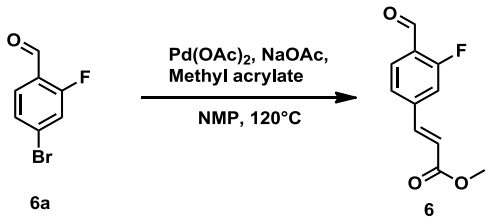
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 10.42 (s, 1 H), 8.16 (d, *J*=1.76 Hz, 1 H), 7.99 (d, *J*=8.22 Hz, 1 H), 7.85 (dd, *J*=8.80, 1.80 Hz, 1 H), 7.21 (d, *J*=15.80 Hz, 1 H), 6.82 (d, *J*=15.85 Hz, 1 H), 1.67 (s, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 187.3, 157.6, 147.2*, 140.9, 133.5, 131.6, 130.6, 130.4, 123.1, 116.6, 115.1, 24.6. *peak very weak

HRMS-FIA(m/z) calc'd for $C_{13}H_{12}N_2O_5$ $[M+H]^+$, 276.0746; found, 276.0755.

3-fluoro-4-formyl methyl cinnamate (6)



6a 6

To 4-bromo-2-fluorobenzaldehyde (10.0 g, 49.3 mmol, 1.00 eq), oven-dried sodium acetate (4.44 g, 5.42 mmol, 1.10 eq) and palladium acetate (14 mg, 0.20 mmol, 4.0 mol%) under nitrogen atmosphere was added 80 mL NMP. The mixture was heated to 120 °C and methyl acrylate (6.60 mg, 76.4 mmol, 1.55 eq) were added as soon as the temperature was reached. After TLC showed complete consumption of starting material, the mixture was cooled to room temperature, partitioned between ethyl acetate and 5% LiCl (aq) and the aqueous layer was extracted twice more with ethyl acetate. The combined organic layers were washed twice with 5% LiCl (aq) and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography. 8.42g (40.0 mmol, 82.0%) of **(6)** were obtained as an off-white solid.

NMR Spectroscopy:

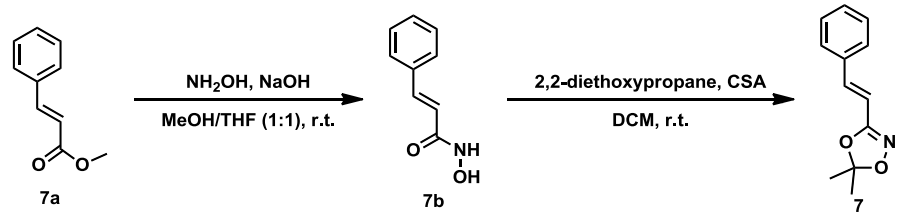
^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 10.34 (s, 1 H), 7.88 (t, *J*=7.3 Hz, 1 H), 7.64 (d, *J*=15.7 Hz, 1 H), 7.40 (d, *J*=8.3 Hz, 1 H), 7.30 (d, *J*=10.8 Hz, 1 H), 6.53 (d, *J*=16.1 Hz, 1 H), 3.82 ppm (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 186.36, 166.05 (d, *J*=93.5 Hz), 163.63, 142.36, 141.88, 129.22, 124.74, 124.15, 122.12, 115.47 (d, *J*=20.0 Hz), 52.06.

^{19}F NMR (470 MHz, $CDCl_3$, 23 ºC, δ): –124.36.

HRMS-FIA(m/z) calc'd for $C_{11}H_9FO_3$ $[M+H]^+$, 208.0536; found, 208.0546.

5,5-dimethyl-3-styryl-1,4,2-dioxazole (7)



To ethyl cinnamate (5.00 g, 28.4 mmol, 1.00 eq) in 20 mL MeOH/THF (1:1) was added 20 mL 50% aqueous hydroxylamine and stirred at room temperature for 12 h. The reaction mixture was then poured onto ice/6M HCl (50 mL) and extracted with DCM and twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate and filtered. The filtrate was concentrated *in vacuo* to afford a colorless oil, which was triturated with ether until crystallization occurred. The white solid was isolated by filtration, washed with water and ether and dried under vacuum. 3.20 g (19.6 mmol, 69.0 %) of cinnamyl hydroxamate were obtained and used without further purification.

To cinnamyl hydroxamate (3.00 g, 18.4 mmol, 1.00 eq) in 50 mL DCM were added camphorsulfonic acid (4.27 g, 18.4 mmol, 1.00 eq) and 2,2-diethoxypropane (7.30 g, 55.2 mmol, 3.00 eq). The mixture was stirred for 12 hours at room temperature. The solution was then washed with water and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The light yellow oil was subjected to flash column chromatography to afford 2.78 g (13.7 mmol, 74.0%) of **(7)** as a white solid.

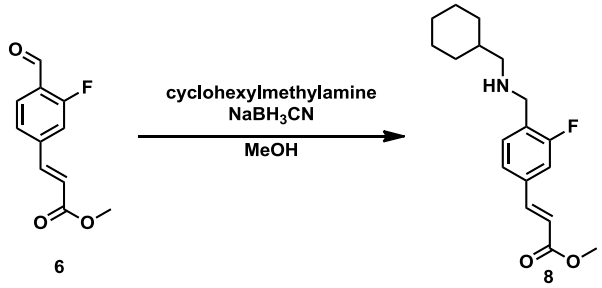
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.47 (d, *J*=6.6 Hz, 2 H), 7.29 - 7.42 (m, 3 H), 7.18 (d, *J*=16.4 Hz, 1 H), 6.61 (d, *J*=16.4 Hz, 1 H), 1.66 ppm (s, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 158.5, 137.7, 135.0, 129.5, 128.9, 127.3, 115.4, 109.5, 24.8.

HRMS-FIA(m/z) calc'd for $C_{12}H_{13}NO_2$ $[M+H]^+$, 204.1019; found, 204.1026.

Methyl 4-(((cyclohexylmethyl)amino)methyl-3-fluorocinnamate (8)



To a solution of cyclohexylmethylamine (652 mg, 5.76 mmol, 1.20 eq) and **(6)** (1.00 g, 4.80 mmol, 1.00 eq) in 10 mL methanol was added sodium cyanoborohydride (452 mg, 7.20 mmol, 1.50 eq) and stirred at room temperature for 24 h. The reaction mixture was then partitioned between ethyl acetate and water, the

aqueous layer was extracted two more times with ethyl acetate, the combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The oily residue was redissolved in dichloromethane and concentrated HCl was added until no further precipitation was observed. The solid was isolated by filtration, washed with isopropanol, ether and dried under vacuum. 1.45 g (4.75 mmol, 82.4 %) of **(8)** was obtained as a white solid.

NMR Spectroscopy:

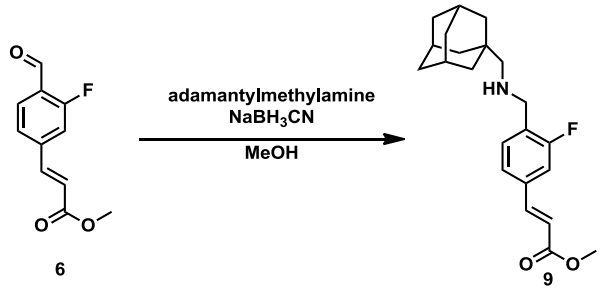
^{1}H NMR (500 MHz, d_6-DMSO, 23 ºC, δ): 9.54 (br. s., 2 H), 7.84 (s, 1 H), 7.72 (d, *J*=10.8 Hz, 1 H), 7.58 - 7.68 (m, 2 H), 6.77 (d, *J*=16.1 Hz, 1 H), 4.13 (s, 2 H), 3.34 (s, 2 H), 2.73 (d, *J*=6.4 Hz, 2 H), 1.51 - 1.90 (m, 6 H), 1.02 - 1.27 (m, 3 H), 0.90 ppm (d, *J*=12.2 Hz, 2 H).

^{13}C NMR (125 MHz, d_6-DMSO, 23 ºC, δ): 166.84, 161.21 (d, *J*=232.7 Hz), 143.06, 137.70, 133.39, 125.06, 121.50, 120.55, 115.10, 52.86, 52.10, 52.07, 43.54, 34.60, 30.55, 26.00, 25.49.

^{19}F NMR (470 MHz, d_6-DMSO, 23 ºC, δ): -115.51.

HRMS-FIA(m/z) calc'd for $C_{18}H_{24}FNO_2$ $[M+H]^+$, 306.1869; found, 306.1937.

Methyl 4-(((adamantylmethyl)amino)methyl-3-fluorocinnamate (9)



6 9

A solution of adamantylmethylamine (95.2 mg, 0.576 mmol, 1.20 eq) and **(6)** (100 mg, 0.480 mmol, 1.00 eq) in 2 mL methanol was stirred for 30 min, then sodium borohydride (27.2 mg, 0.720 mmol, 1.50 eq) and stirred at room temperature for 12 h. The reaction mixture was then partitioned between ethyl acetate and water, the aqueous layer was extracted two more times with ethyl acetate, the combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The oily residue was purified by flash column chromatography. 135 mg (0.378mmol, 78.8%) of **(9)** was obtained as a white solid.

NMR Spectroscopy:

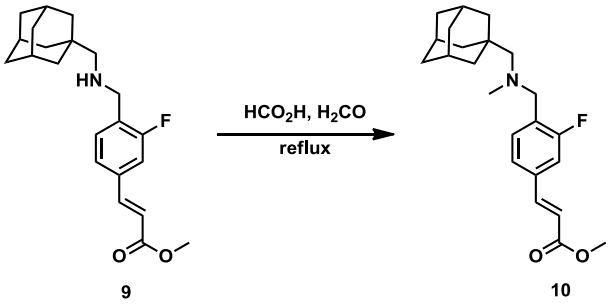
^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.60 (d, *J*=16.1 Hz, 1 H), 7.37 (t, *J*=7.6 Hz, 1 H), 7.24 (d, *J*=7.8 Hz, 1 H), 7.16 (d, *J*=10.8 Hz, 1 H), 6.38 (d, *J*=15.7 Hz, 1 H), 3.81 (s, 2 H), 3.78 (s, 3 H), 2.20 (s, 2 H), 1.93 (br. s., 3 H), 1.65 - 1.72 (m, 3 H), 1.57 - 1.64 (m, 3 H), 1.49 ppm (s, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 167.12, 161.20 (d, *J*=246.1 Hz), 143.47, 134.91 (d, *J*=7.6 Hz), 130.41 (d, *J*=5.7 Hz), 130.23 (d, *J*=15.3 Hz),124.01, 118.47, 114.03 (d, *J*=25.8 Hz), 61.97, 51.74, 47.73 (d, *J*=1.9 Hz), 40.79, 37.18, 33.40, 28.42.

^{19}F NMR (470 MHz, $CDCl_3$, 23 ºC, δ): -118.81.

HRMS-FIA(m/z) calc'd for $C_{22}H_{28}FNO_2$ $[M+H]^+$, 358.2182; found, 358.2240.

Methyl 4-(((adamantylmethyl)(methyl)amino)methyl-3-fluorocinnamate (10)



9 10

(9) (150 mg, 420 µmol) was refluxed in 2 mL formic acid and 2 mL formalin for 24 h. The reaction mixture was neutralized with saturated sodium bicarbonate solution and partitioned between water and ethyl acetate. The aqueous layer was extracted twice more with ethyl acetate, the combined organic layers were washed with water and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography to yield **(10)** (101 mg, 273 µmol, 65%) as a white solid.

NMR Spectroscopy:

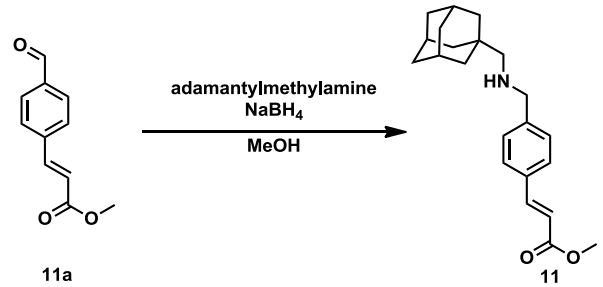
^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.69 (d, *J*=16.1 Hz, 1H), 7.61 (s, 1H), 7.27 - 7.39 (m, 2H), 7.23 (d, *J*=10.3 Hz, 1H), 6.47 (d, *J*=16.1 Hz, 1H), 3.87 (br. s., 3H), 3.66 (br. s., 2H), 2.29 (s, 3H), 2.18 (s, 2H), 2.00 (s, 3H), 1.62 - 1.81 (m, 6H), 1.56 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 167.21, 161.31 (d, *J*=237.5 Hz), 143.61, 134.72, 131.39, 129.56, 123.84, 118.38, 114.15, 113.96, 71.02, 57.23, 51.76, 45.65, 41.02, 37.23, 35.18, 28.50.

^{19}F NMR (470 MHz, $CDCl_3$, 23 ºC, δ): -117.62.

HRMS-FIA(m/z) calc'd for $C_{23}H_{30}FNO_2$ $[M+H]^+$, 372.2339; found, 372.2388.

Methyl 4-(((adamantylmethyl)amino)methyl)-cinnamate (11)



11a 11

Adamantylmethylamine (1g, 6.0 mmol) and (*E*)-methyl 4-formylcinnamate (1g, 5.3 mmol) was dissolved in

MeOH (30 mL) and the mixture was stirred at room temperature for 2 h. Sodium borohydride (0.61g, 16 mmol) was then added, and the suspension was stirred overnight at room temperature. The white precipitate was filtered and dried to obtain the product **(11)** (1.35 g, yield: 75%).

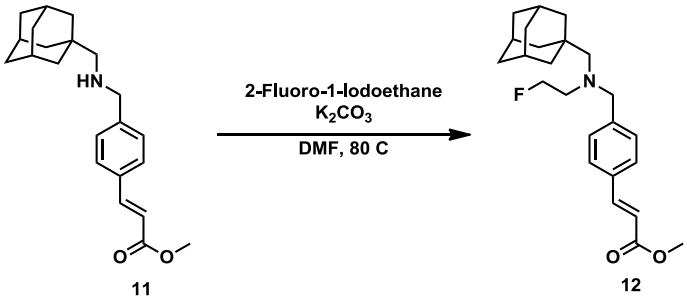
NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.69 (d, *J*=15.7 Hz, 1 H), 7.48 (d, *J*=8.3 Hz, 2 H), 7.35 (d, *J*=7.8 Hz, 2 H), 6.42 (d, *J*=15.7 Hz, 1 H), 3.76 - 3.85 (m, 5 H), 2.23 (s, 2 H), 1.96 (br. s., 3 H), 1.59 - 1.76 (m, 6 H), 1.47 - 1.56 ppm (m, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 167.6, 144.8, 143.7, 132.9, 128.4, 128.1, 117.1, 62.1, 54.3, 51.7, 40.9, 37.2, 33.5, 28.5.

HRMS-FIA(m/z) calc'd for $C_{22}H_{29}NO_2$ $[M+H]^+$, 340.2277; found, 340.2242.

Methyl 4-(((adamantylmethyl)(2-fluoroethyl)amino)methyl)-cinnamate (12)



11 12

To a solution of **(11)** (220 mg, 0.647 mmol, 1.00 eq) in 3 mL DMF was added 2-fluoro-1-iodoethane (118 mg, 0.679 mmol, 1.05 eq) and sodium hydride (19 mg, 0.78 mmol, 1.20 eq) and stirred at 80°C under nitrogen atmosphere for 48 h. The reaction mixture was cooled to room temperature, partitioned between water and ethyl acetate, the aqueous layer extracted twice with ethyl acetate and the combined organic layers washed with water and brine. The organic phases were then dried over magnesium sulfate, filtered and concentrated *in vacuo*. The product was purified by flash chromatography. 78 mg (0.202 mmol, 31%) of **(12)** were obtained as a clear oil.

NMR Spectroscopy:

^{1}H NMR (500 MHz, $CDCl_3$, 23 ºC, δ): 7.69 (d, *J*=15.7 Hz, 1 H), 7.37 - 7.53 (m, 4 H), 6.42 (d, *J*=16.1 Hz, 1 H), 4.34 - 4.54 (m, 2 H), 3.81 (s, 3 H), 3.71 (s, 2 H), 2.62 - 2.78 (m, 2 H), 2.26 (s, 2 H), 1.95 (br. s., 3 H), 1.59 - 1.79 (m, 6 H), 1.52 ppm (br. s., 6 H).

^{13}C NMR (125 MHz, $CDCl_3$, 23 ºC, δ): 167.6, 144.8, 143.0, 133.0, 128.9, 128.0, 117.1, 82.8, 68.6, 55.8, 51.7, 41.2, 37.2, 35.1, 31.6, 28.5.

^{19}F NMR (470 MHz, $CDCl_3$, 23 ºC, δ): -220.52 .

HRMS-FIA(m/z) calc'd for $C_{24}H_{32}FNO_2$ $[M+H]^+$, 386.2495; found, 386.2510.

General Procedure for Synthesis of Hydroxamates

To a solution of the respective ester (1 mmol) in 5 ml MeOH/THF (1:1) was added sequentially 1.5 mL hydroxylamine solution (50% in water) and 1 mL 1 M NaOH. The reaction mixture was stirred at room temperature until TLC showed complete consumption of starting material. Then, the reaction mixture was cooled to 0°C and brought to pH 7 with 1M HCl, when precipitation occurred. The solid was isolated by filtration, washed with water and dried under high vacuum.

Characterization MGS1

NMR Spectroscopy:

^{1}H NMR (500 MHz, d_6-DMSO, 23 ºC, δ): 10.93 (br. s., 1H), 9.00 - 9.48 (br. s., 1H), 7.74 (d, *J*=7.3 Hz, 1H), 7.31 - 7.57 (m, 3H), 6.58 (dd, *J*=15.9, 3.7 Hz, 1H), 4.14 (s, 2H), 2.76 (s, 2H), 1.48 - 1.88 (m, 6H), 1.02 - 1.27 (m, 3H), 0.80 - 0.99 (m, 2H).

^{13}C NMR (125 MHz, d_6-DMSO, 23 ºC, δ): 163.3, 162.0 (d, J=246.5 Hz), 139.5, 137.4, 134.1, 124.6, 122.9, 120.9, 115.2, 53.6, 44.3, 35.3, 31.2, 26.7, 26.2.

^{19}F NMR (470 MHz, d_6-DMSO, 23 ºC, δ): -115.58.

HRMS-FIA(m/z) calc'd for $C_{17}H_{23}FN_2O_2$ $[M+H]^+$, 307.1781; found, 307.1822.

Characterization MGS2

NMR Spectroscopy:

^{1}H NMR (500 MHz, d_6-DMSO, 23 ºC, δ): 7.46 (t, *J*=7.8 Hz, 1H), 7.25 - 7.40 (m, 3H), 6.47 (d, *J*=16.1 Hz, 1H), 3.67 (s, 2H), 2.10 (s, 3H), 1.89 (s, 3H), 1.51 - 1.72 (m, 6H), 1.45 (s, 6H).

^{13}C NMR (125 MHz, d_6-DMSO, 23 ºC, δ): 163.6, 161.7 (d, J=236 Hz), 137.2, 136.7, 131.4, 130.0, 124.3, 121.6, 114.6, 62.5, 47.7, 41.4, 37.8, 34.3, 28.9.

^{19}F NMR (470 MHz, d_6-DMSO, 23 ºC, δ): -119.45.

HRMS-FIA(m/z) calc'd for $C_{21}H_{27}FN_2O_2$ $[M+H]^+$, 359.2135; found, 359.2155.

Characterization MGS3

NMR Spectroscopy:

^{1}H NMR (500 MHz, d_6-DMSO, 23 ºC, δ): d = 7.30 - 7.55 (m, 4 H), 6.45 (d, *J*=15.6 Hz, 1 H), 3.53 (s, 2 H), 2.15 (s, 3 H), 2.02 - 2.10 (m, 2 H), 1.88 (br. s., 3 H), 1.50 - 1.70 (m, 6 H), 1.43 ppm (br. s., 6 H) .

^{13}C NMR (125 MHz, d_6-DMSO, 23 ºC, δ): 162.92, 161.32 (d, *J*=243.6 Hz), 137.41, 136.36, 132.08, 127.88 (d, *J*=24.9 Hz), 123.13, 120.53, 114.32 (d, *J*=27.8 Hz), 70.49, 57.27, 45.87, 40.88, 37.19, 35.18, 28.29.

^{19}F NMR (470 MHz, d_6-DMSO, 23 ºC, δ): -117.22.

HRMS-FIA(m/z) calc'd for $C_{21}H_{27}FN_2O_2$ $[M+H]^+$, 373.2291; found, 373.2256.

Characterization CN146

NMR Spectroscopy:

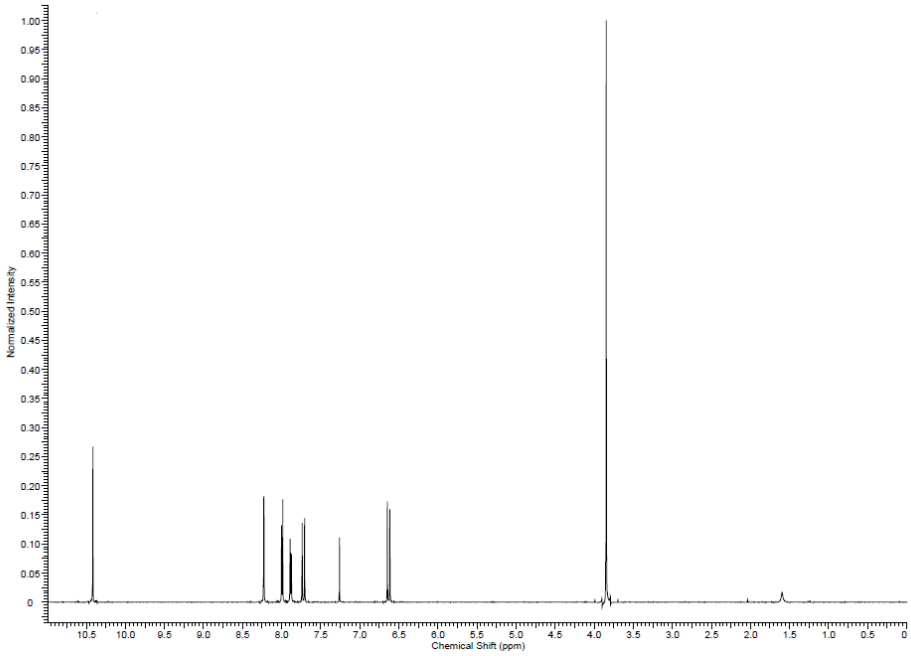
^{1}H NMR (500 MHz, d_6-DMSO, 23 ºC, δ): 10.74 (br. s., 1 H), 9.01 (br. s., 1 H), 7.50 (d, *J*=8.3 Hz, 2 H), 7.36 - 7.45 (m, 3 H), 6.43 (d, *J*=14.7 Hz, 1 H), 4.43 (dt, *J*=47.4, 4.9 Hz, 2 H), 3.64 (s, 2 H), 2.62 (dt, *J*=25.9, 4.9 Hz, 2 H), 2.22 (s, 2 H), 1.89 (br. s., 3 H), 1.64 (d, *J*=11.7 Hz, 3 H), 1.57 (d, *J*=11.2 Hz, 3 H), 1.47 ppm (br. s., 6 H).

^{13}C NMR (125 MHz, d_6-DMSO, 23 ºC, δ): 163.31, 142.11, 138.59, 133.75, 129.22, 127.85, 118.92, 82.95 (d, *J*=170.6 Hz), 67.94, 61.54, 55.95 (d, *J*=26.8 Hz), 40.94, 37.17, 35.10, 28.27.

^{19}F NMR (470 MHz, d_6-DMSO, 23 ºC, δ): -219.41.

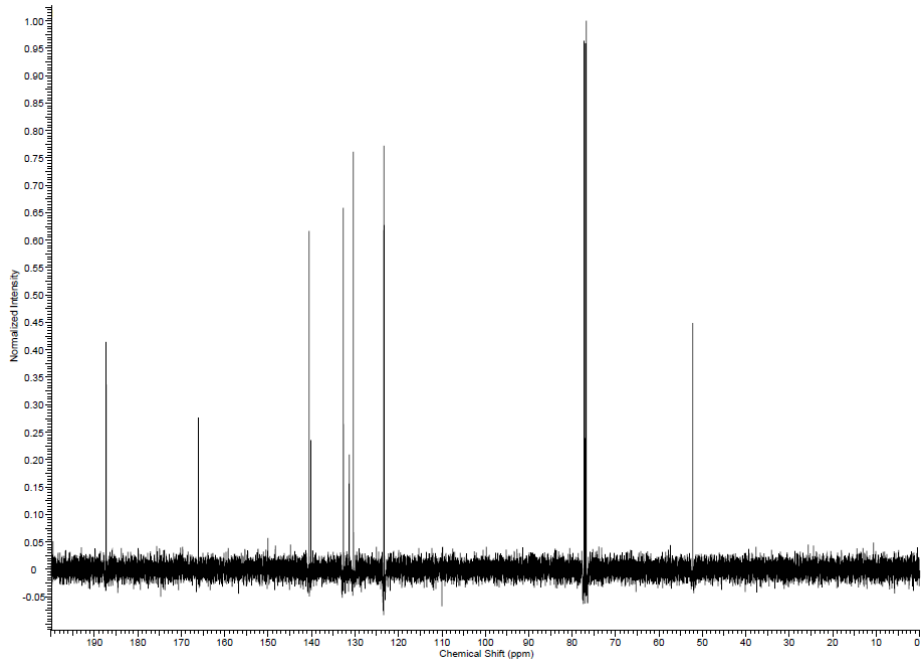
HRMS-FIA(m/z) calc'd for $C_{21}H_{27}FN_2O_2$ $[M+H]^+$, 387.2448; found, 387.2518.

NMR Spectra of Prepared Compounds

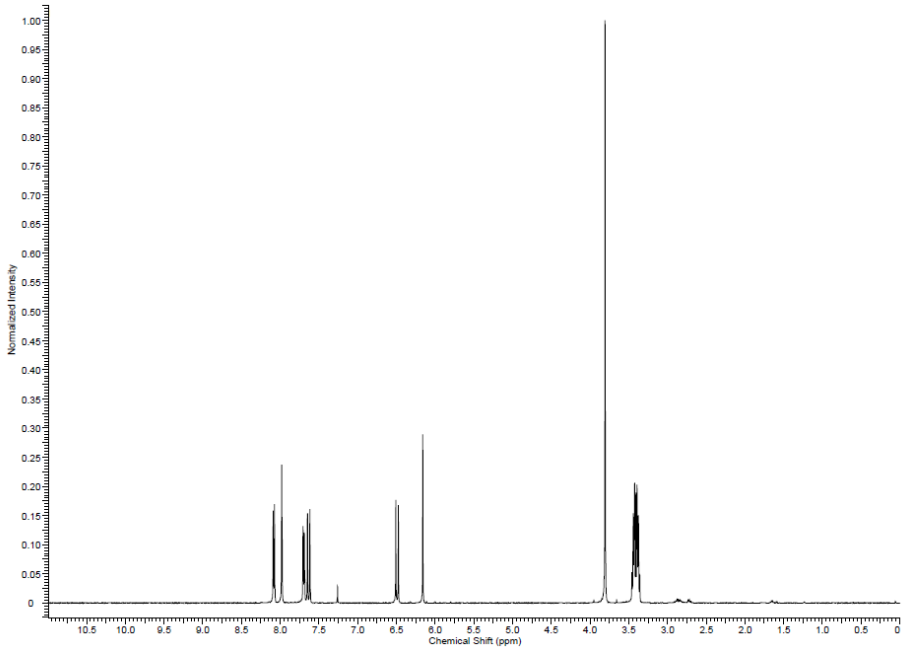


^{1}H-NMR Spectrum of (2)

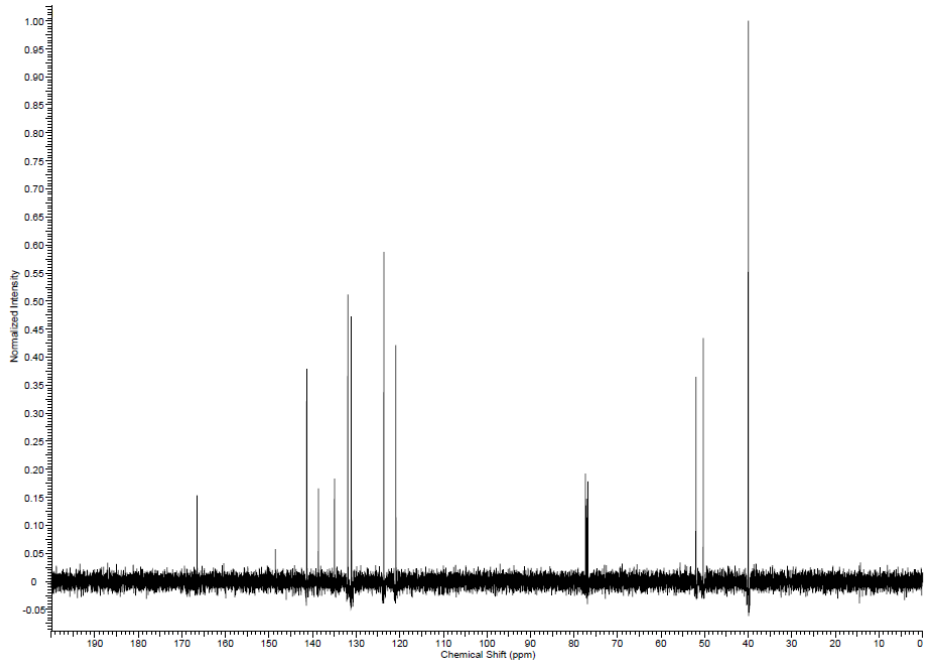
^{13}C-NMR spectrum of (2)



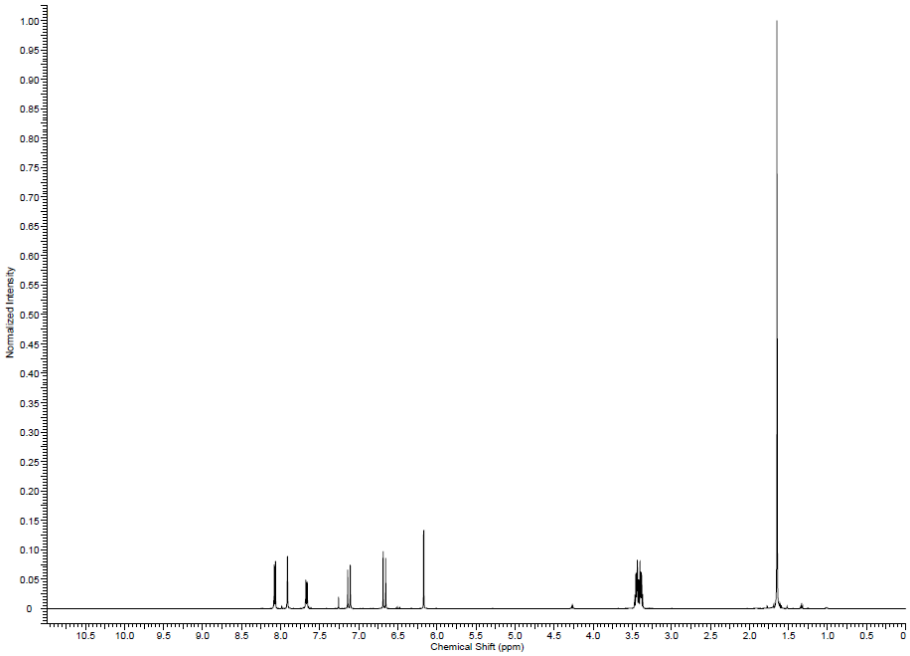
^{1}H-NMR spectrum of (3)



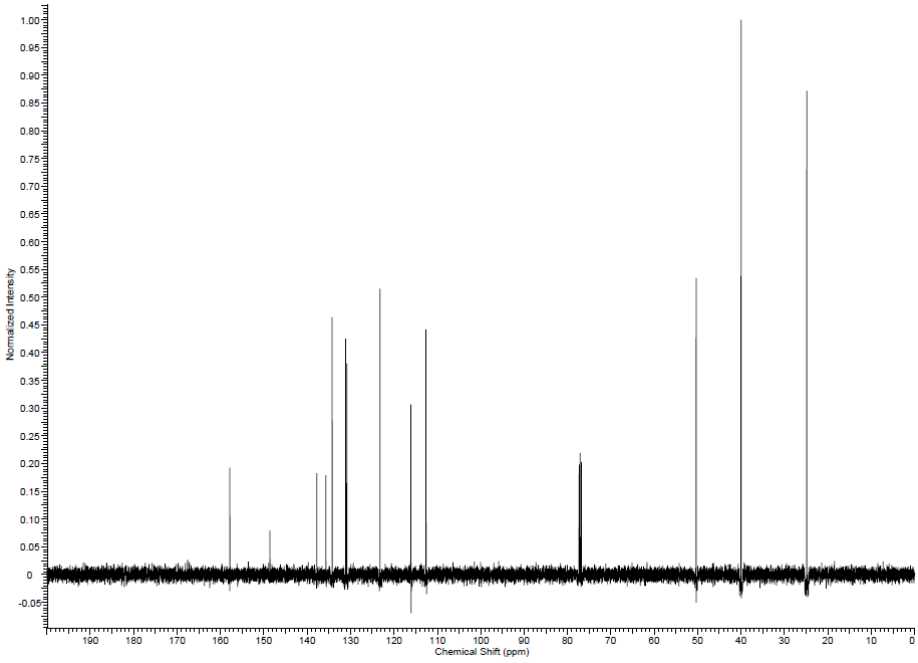
^{13}C-NMR spectrum of (3)



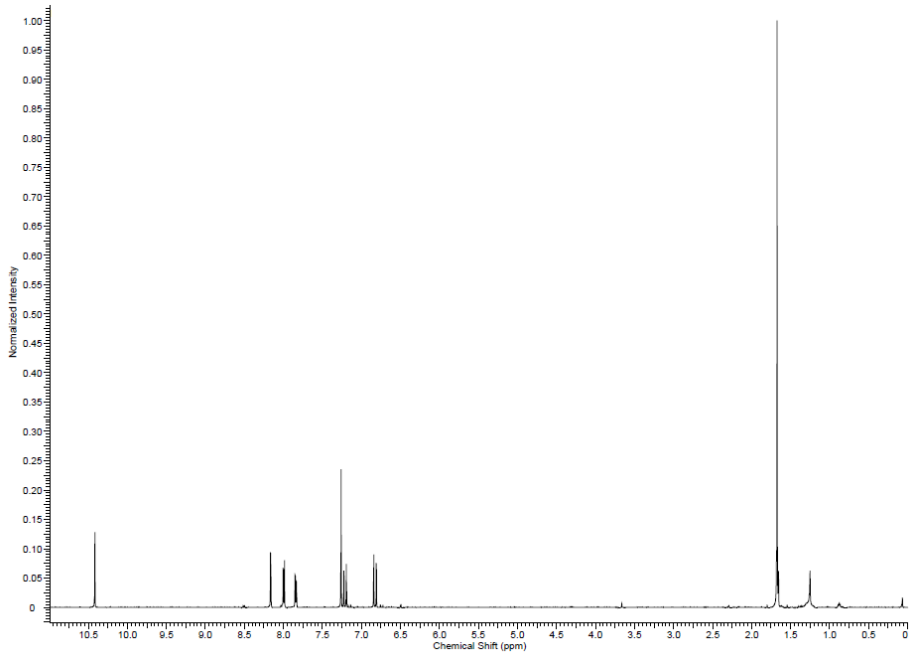
^{1}H-NMR spectrum of (4)



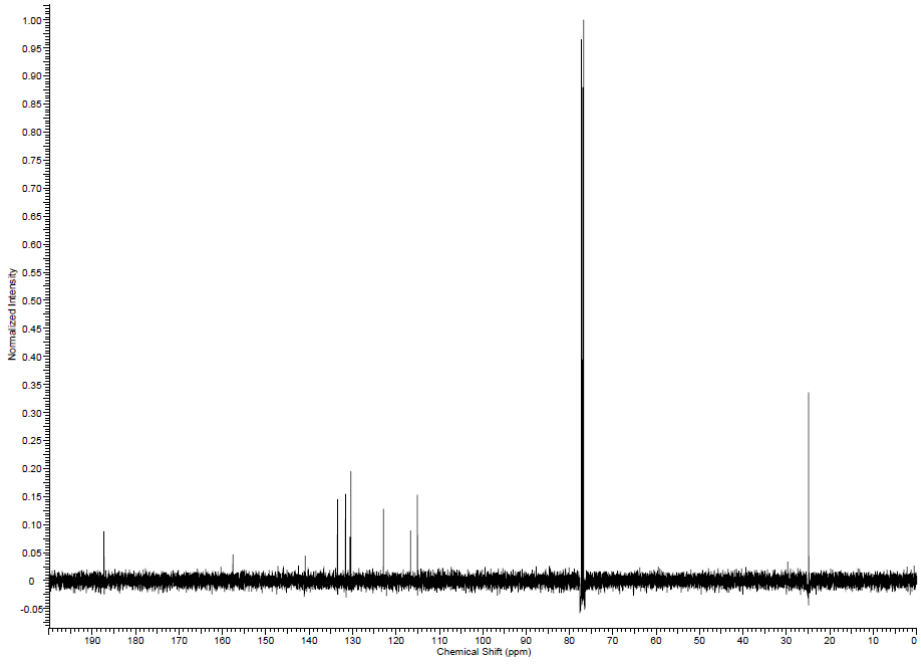
^{13}C-NMR spectrum of (4)



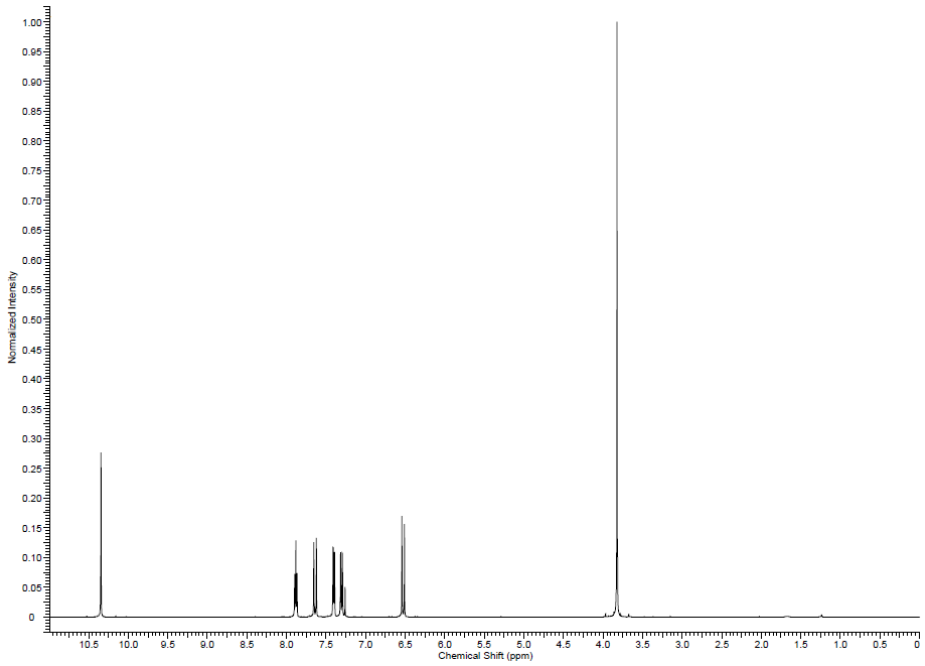
^{1}H-NMR spectrum of (5)



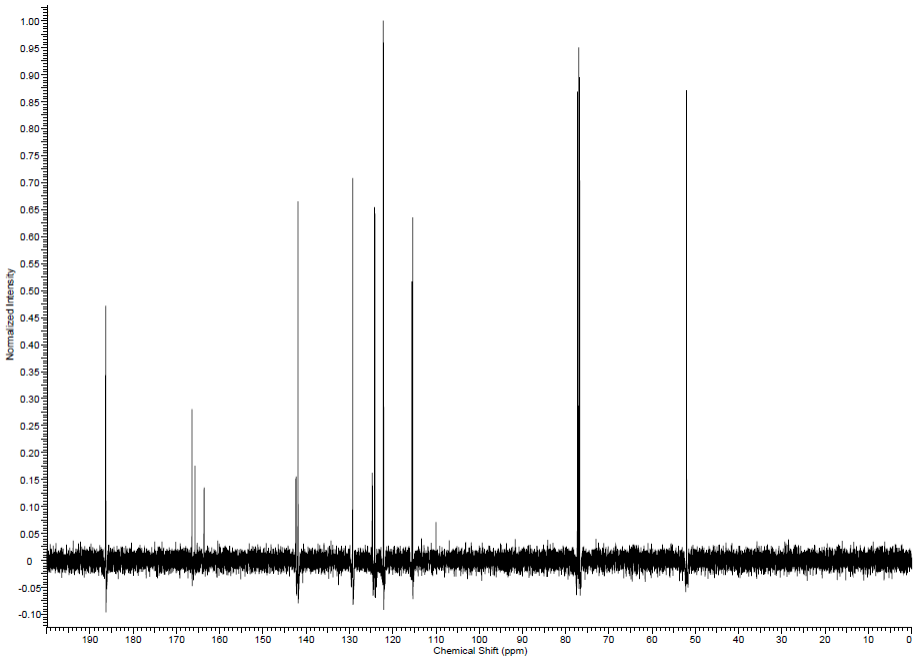
^{13}C-NMR spectrum of (5)



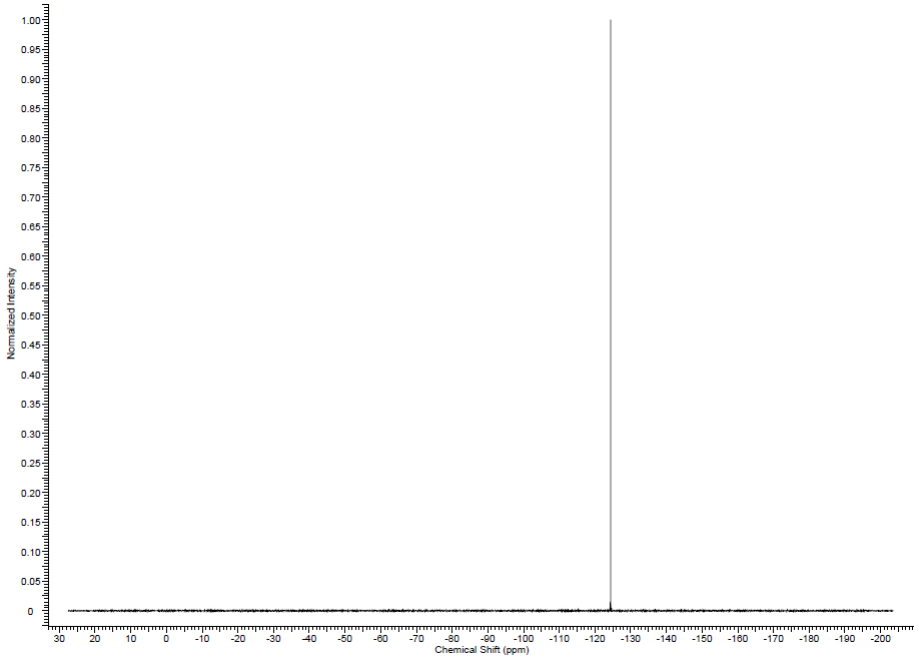
^{1}H-NMR spectrum of (6)



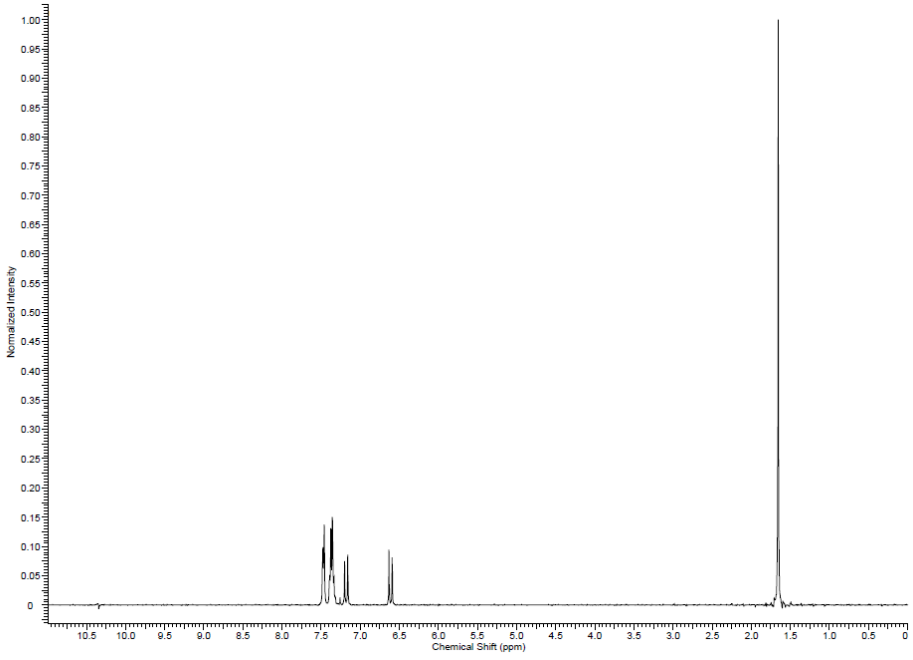
^{13}C-NMR spectrum of (6)



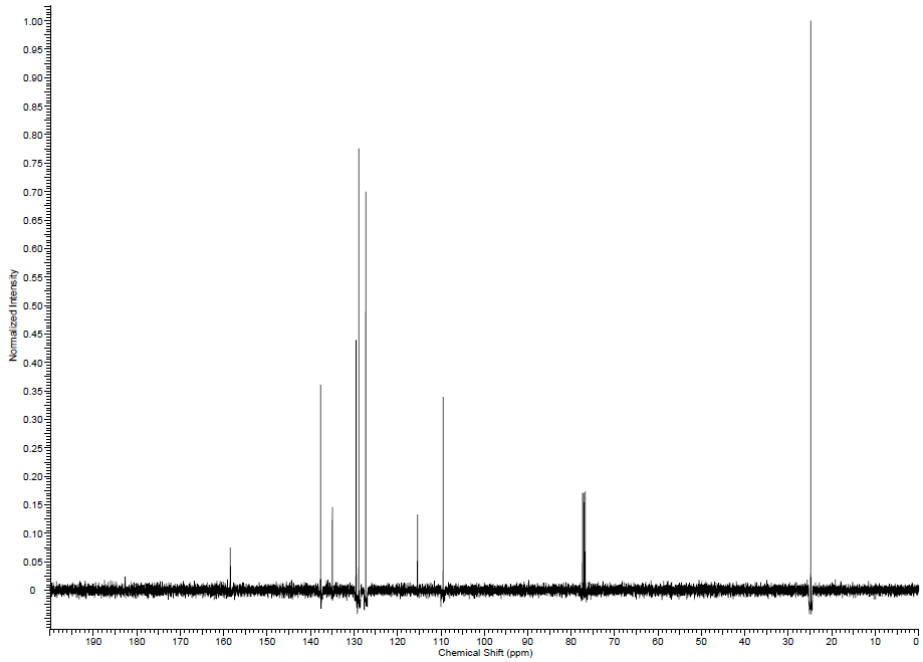
^{19}F-NMR spectrum of (6)



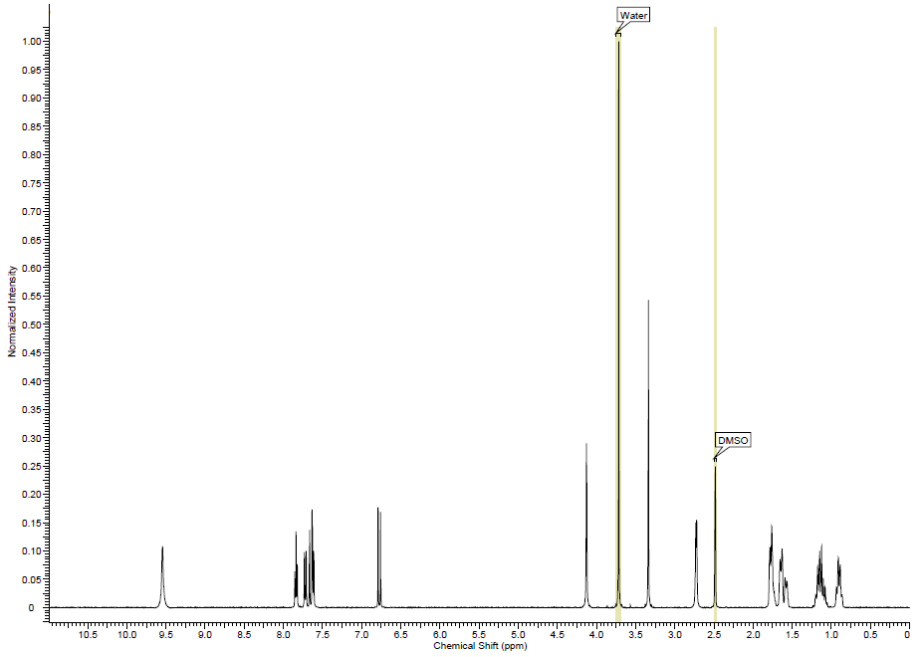
^{1}H-NMR spectrum of (7)



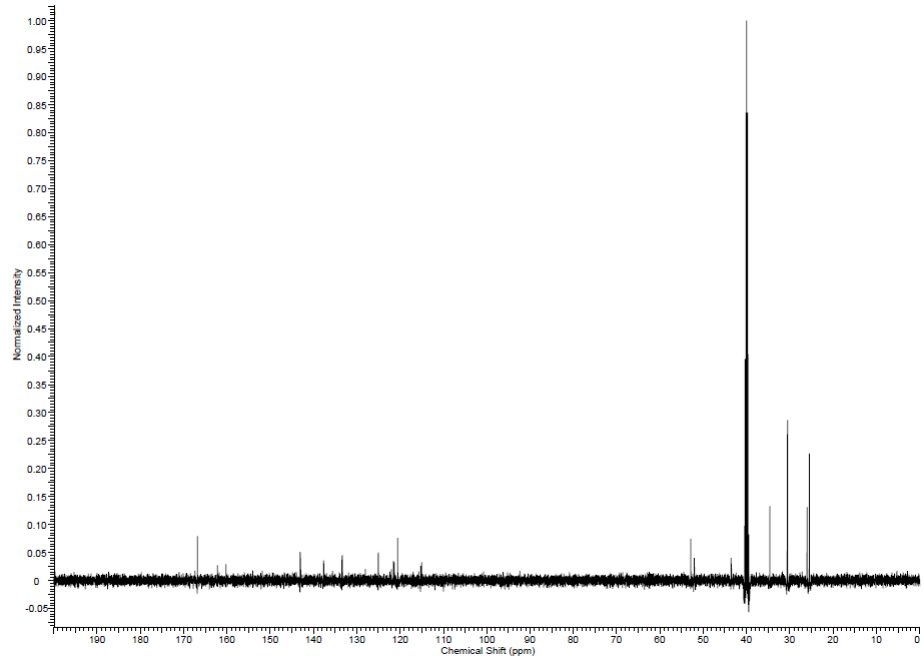
^{13}C-NMR spectrum of (7)



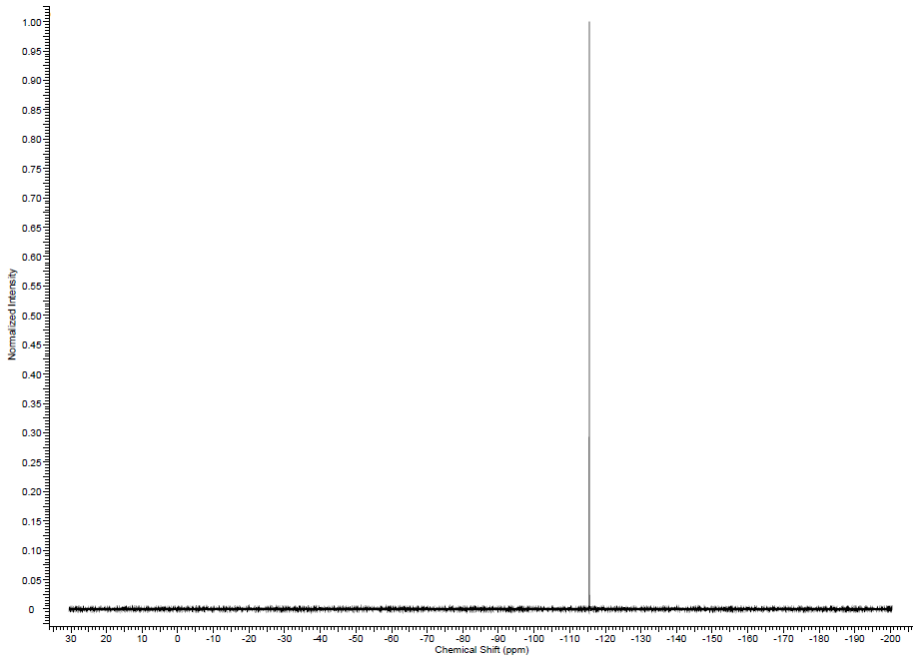
^{1}H-NMR spectrum of (8)



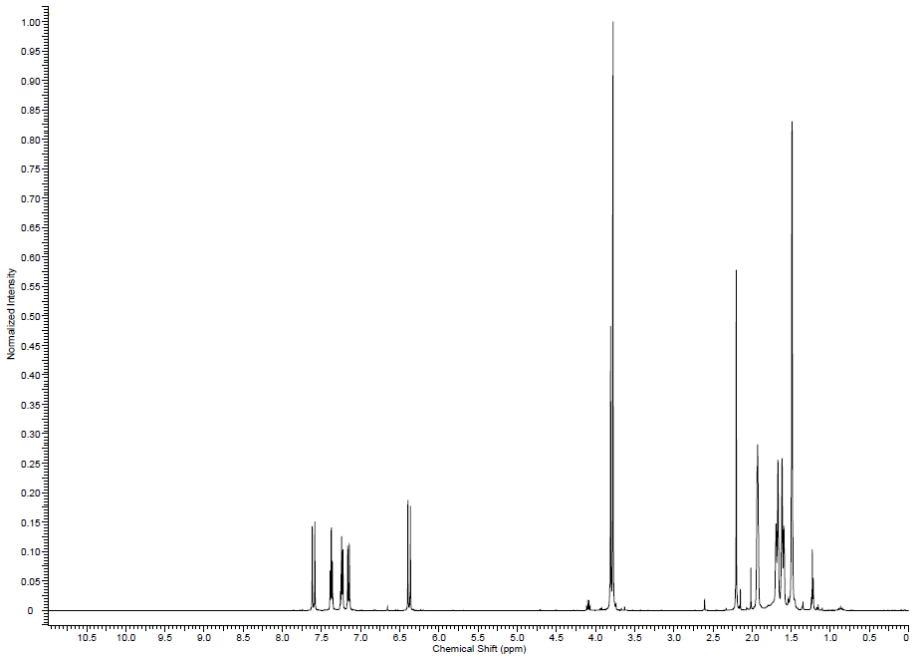
^{13}C-NMR spectrum of (8)



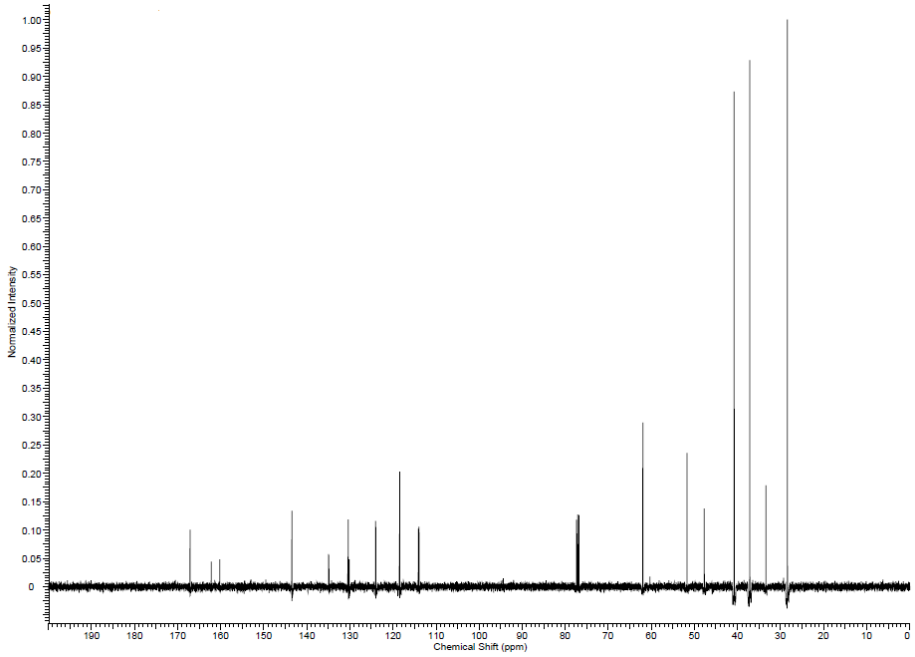
^{19}F-NMR spectrum of (8)



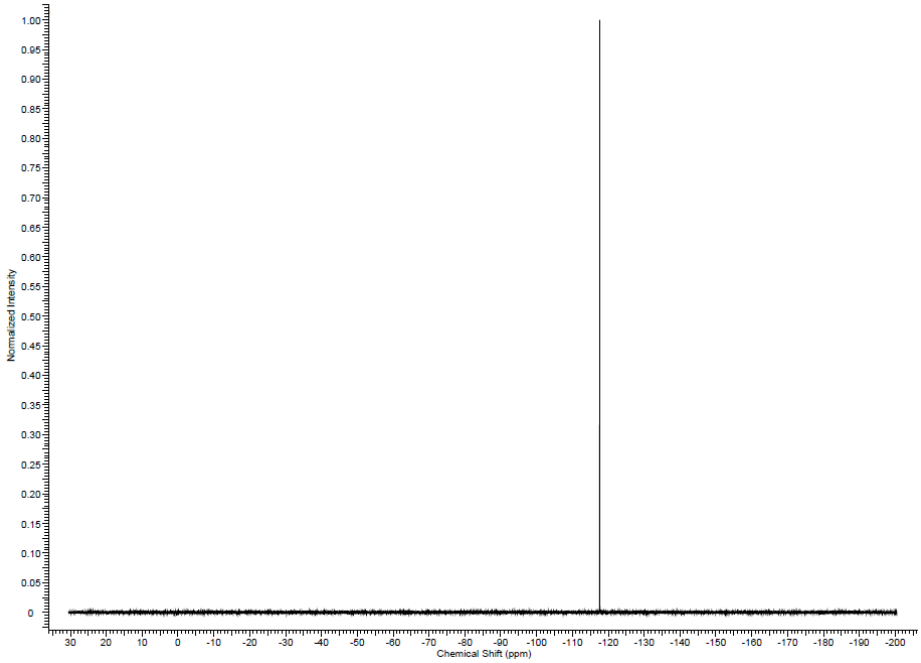
^{1}H-NMR spectrum of (9)



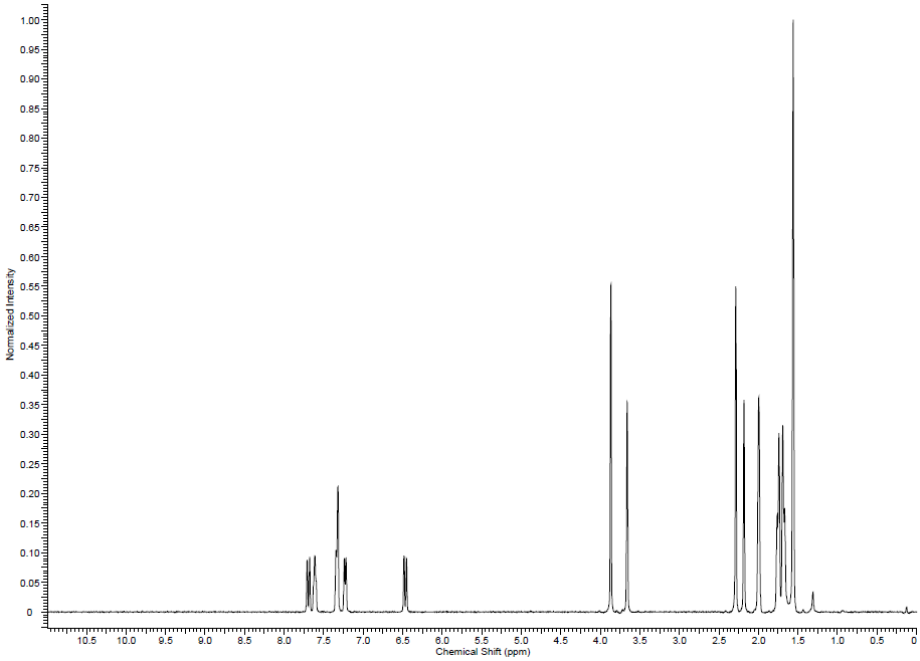
^{13}C-NMR spectrum of (9)



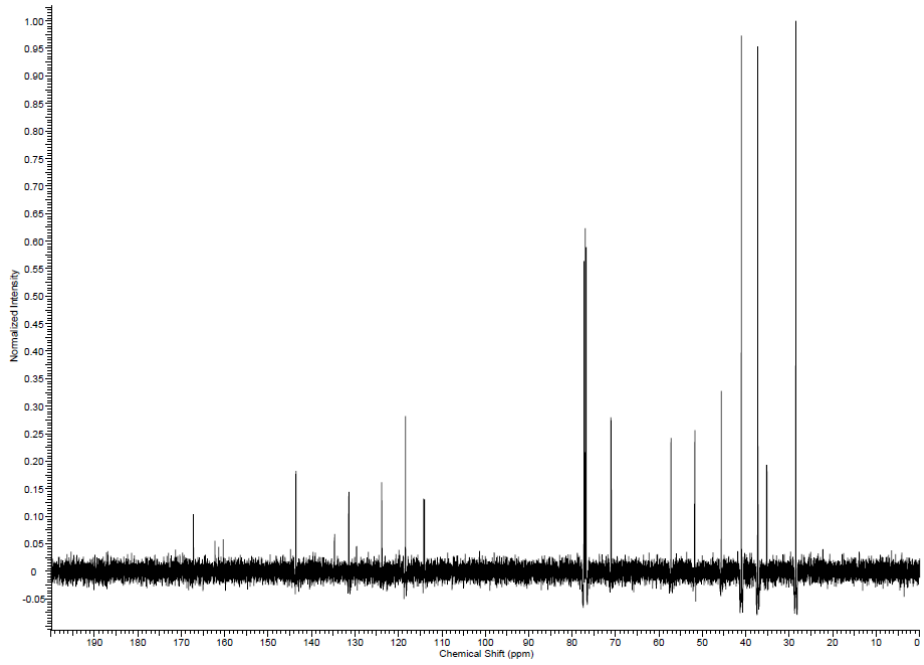
^{19}F-NMR spectrum of (9)



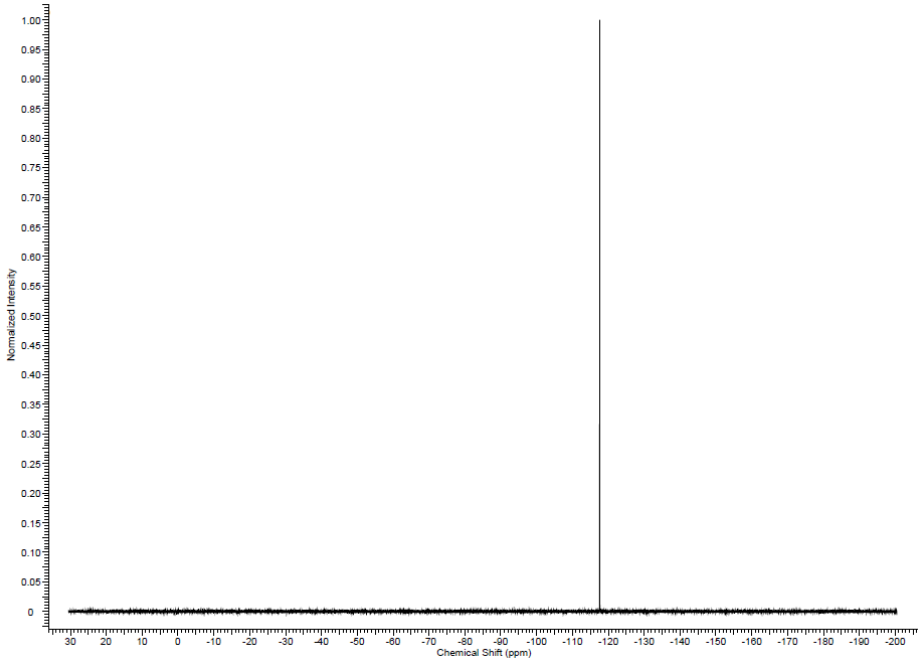
^{1}H-NMR spectrum of (10)



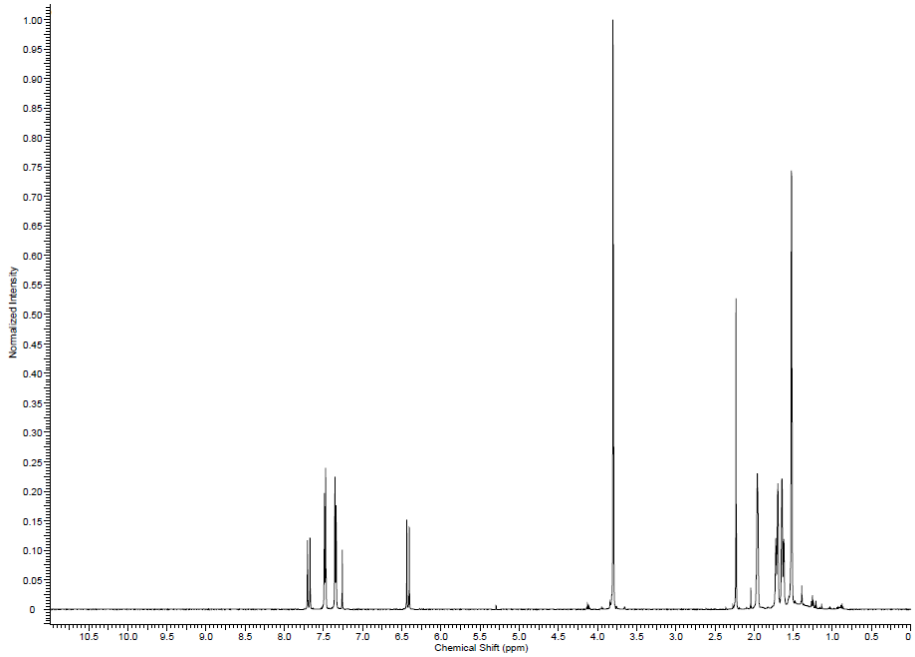
^{13}C-NMR spectrum of (10)



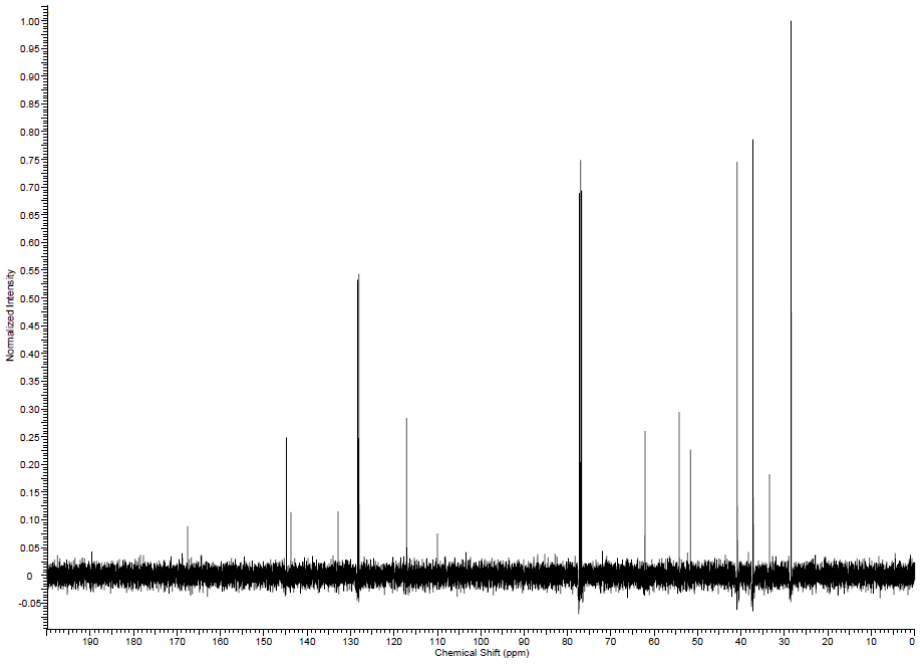
^{19}F-NMR spectrum of (10)



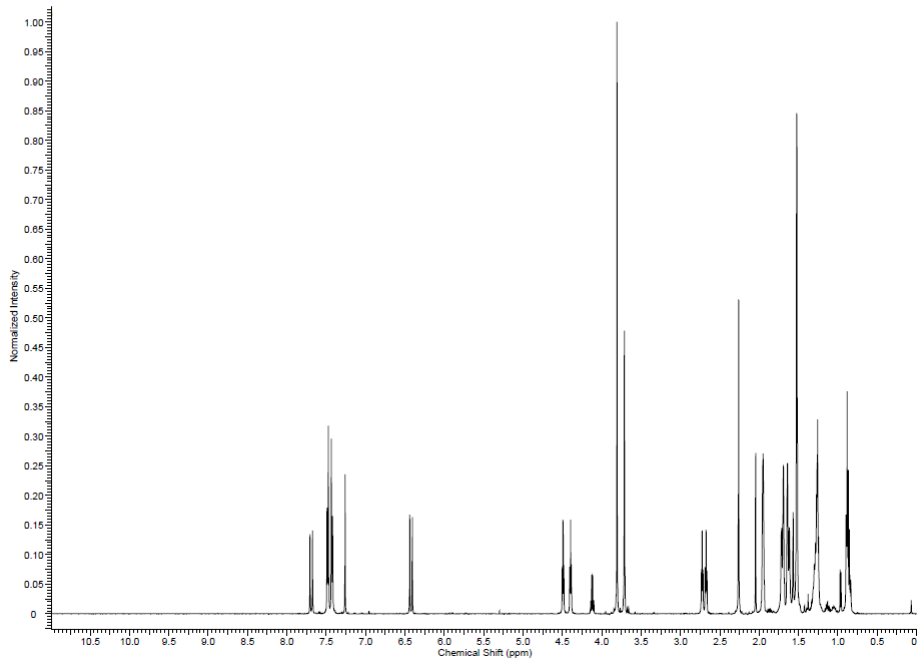
^{1}H-NMR spectrum of (11)



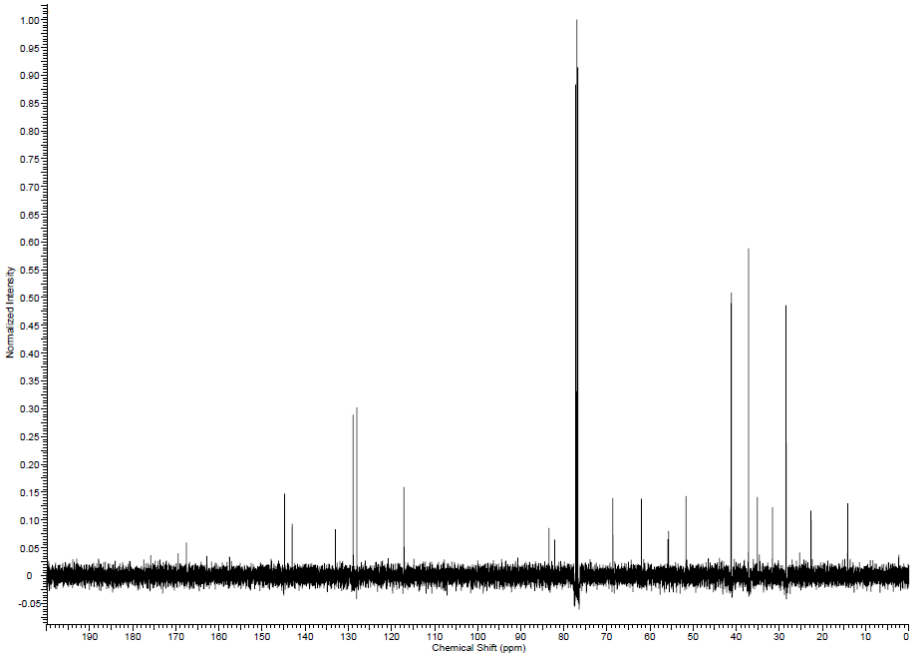
^{13}C-NMR spectrum of (11)



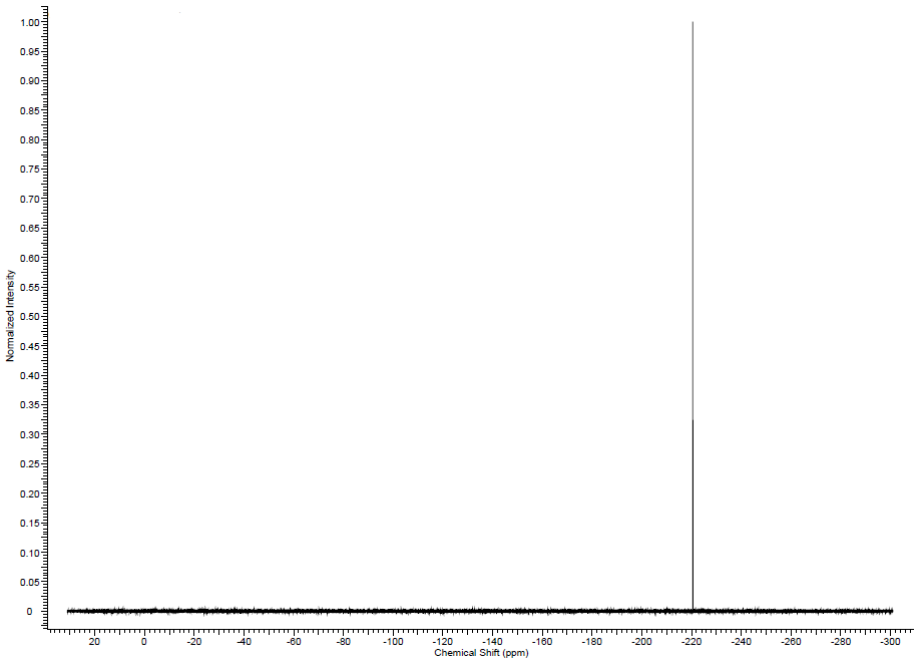
^{1}H-NMR spectrum of (12)



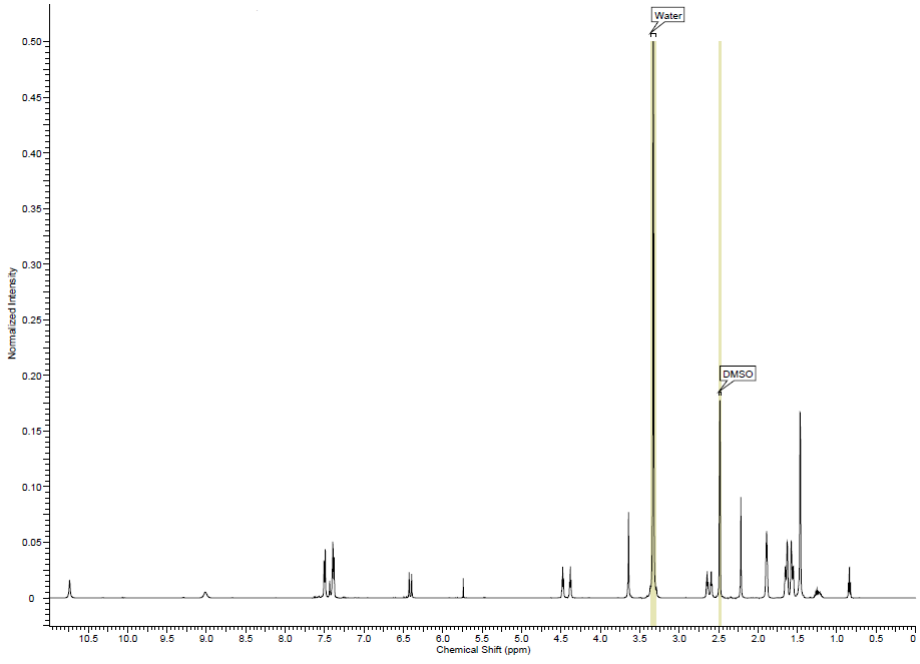
^{13}C-NMR spectrum of (12)



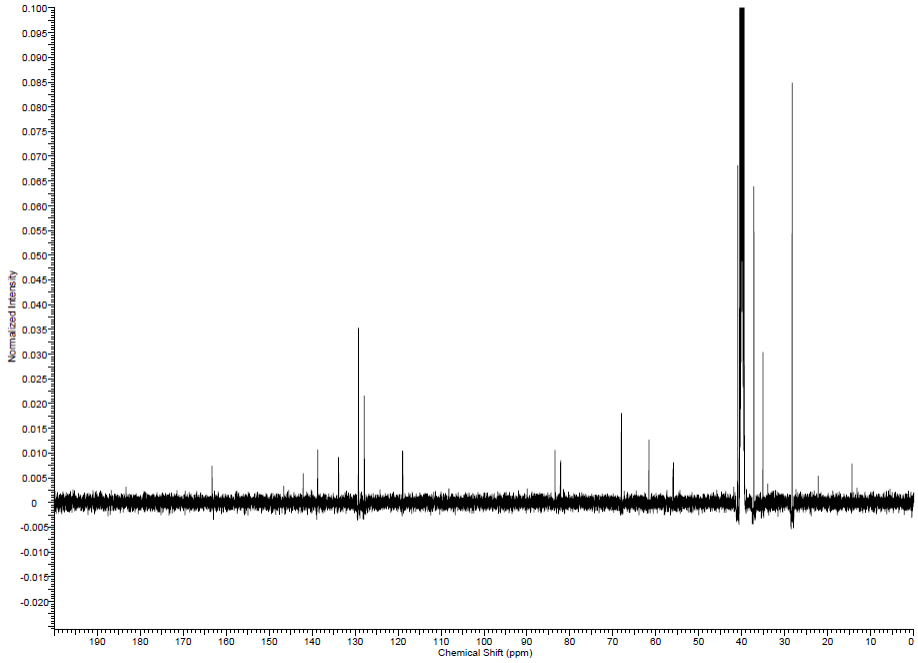
^{19}F-NMR spectrum of (12)



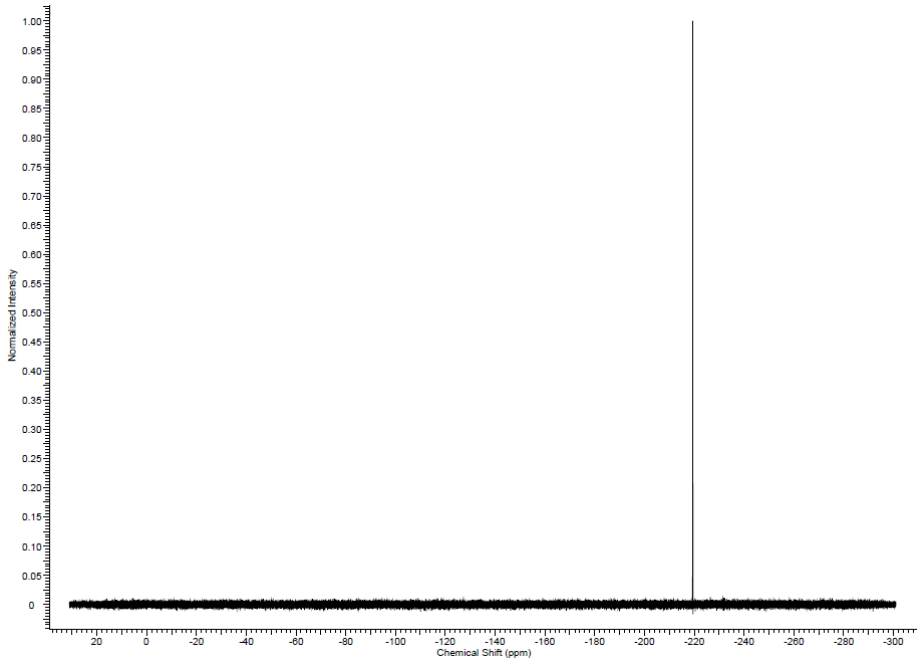
^{1}H-NMR spectrum of CN146



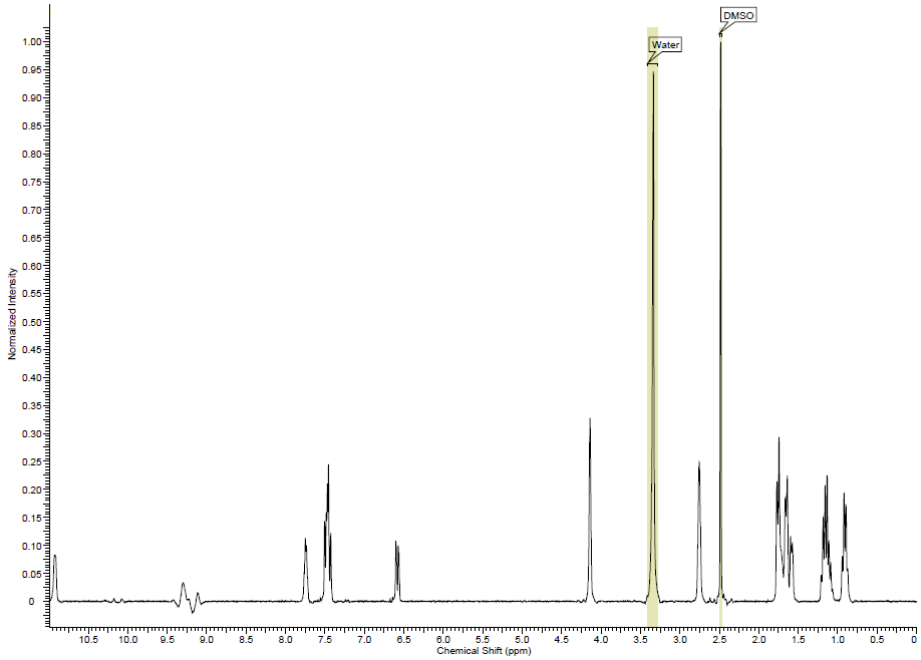
^{13}C-NMR spectrum of CN146



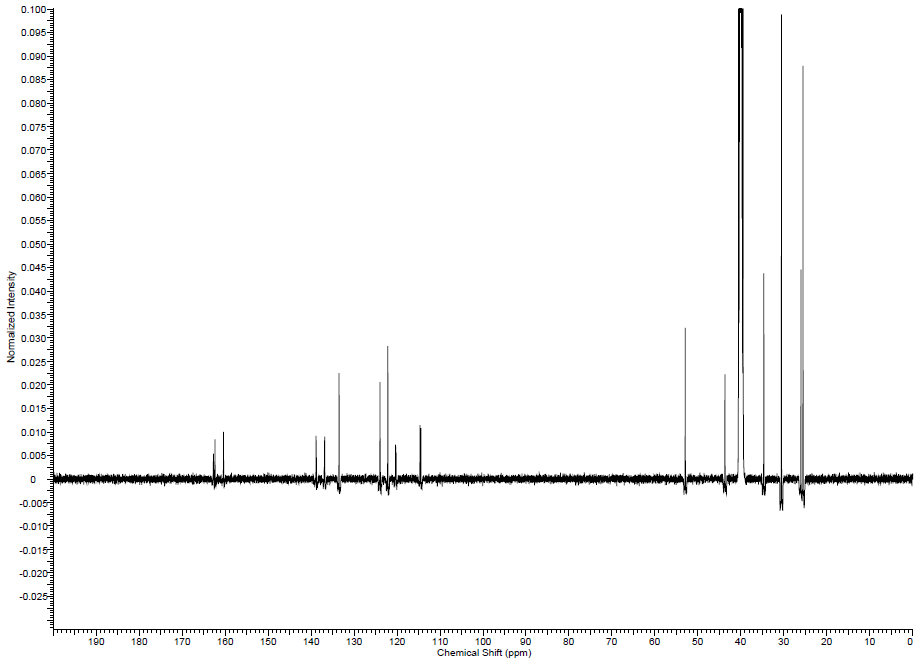
^{19}F-NMR of CN146



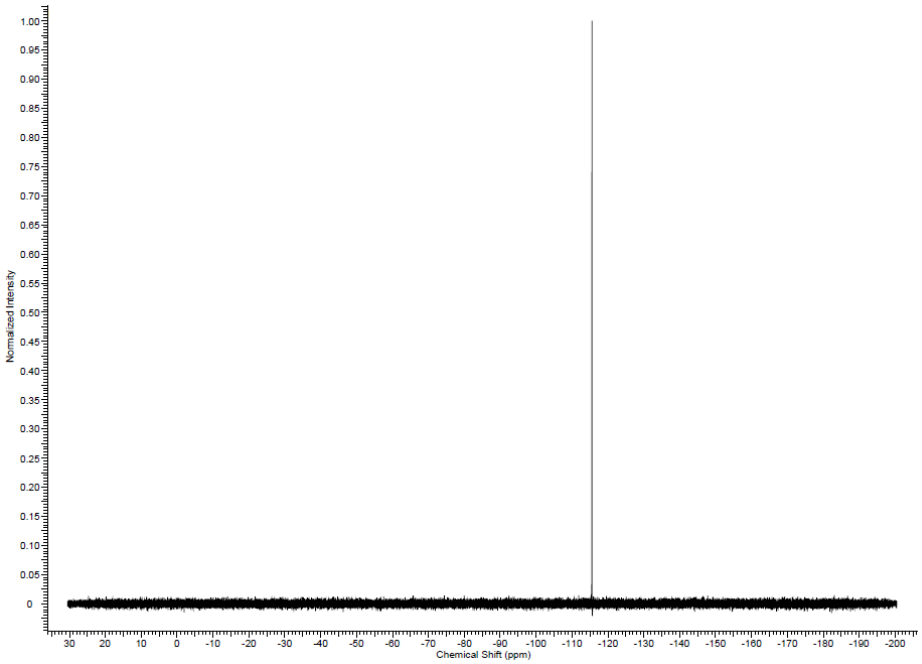
^{1}H-NMR spectrum of MGS1



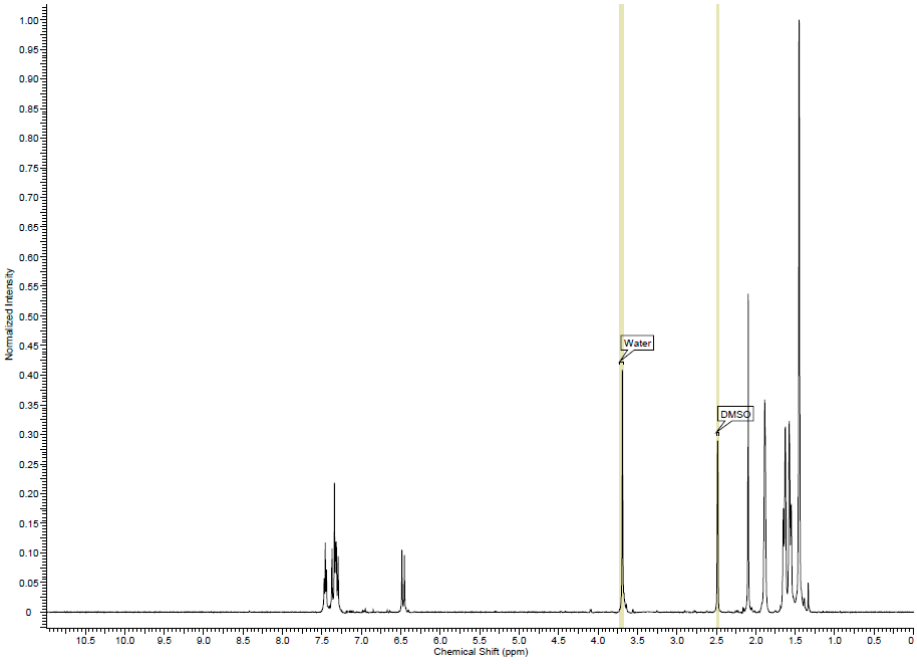
^{13}C-NMR spectrum of MGS1



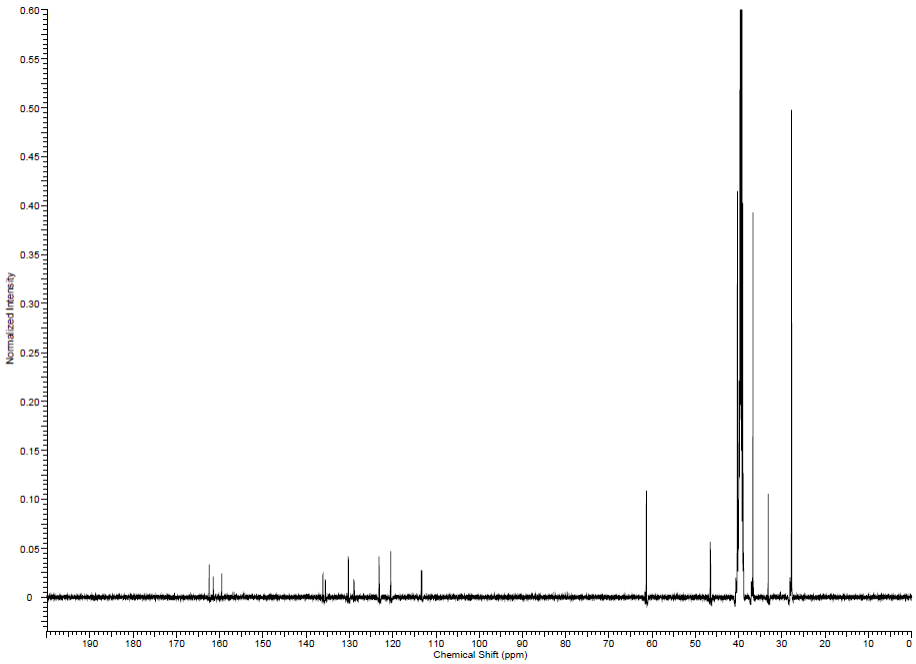
^{19}F-NMR spectrum of MGS1



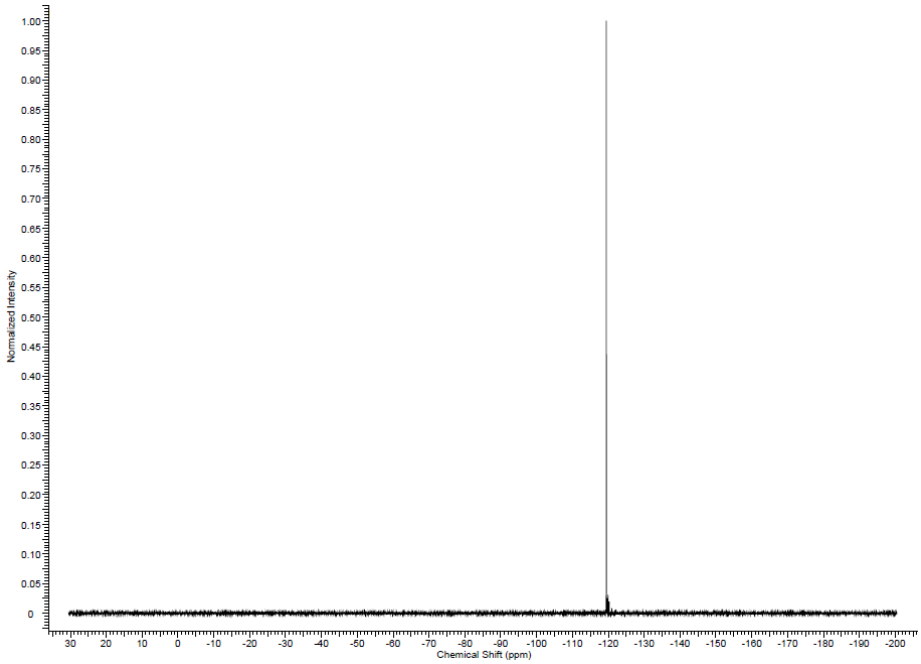
^{1}H-NMR spectrum of MGS2



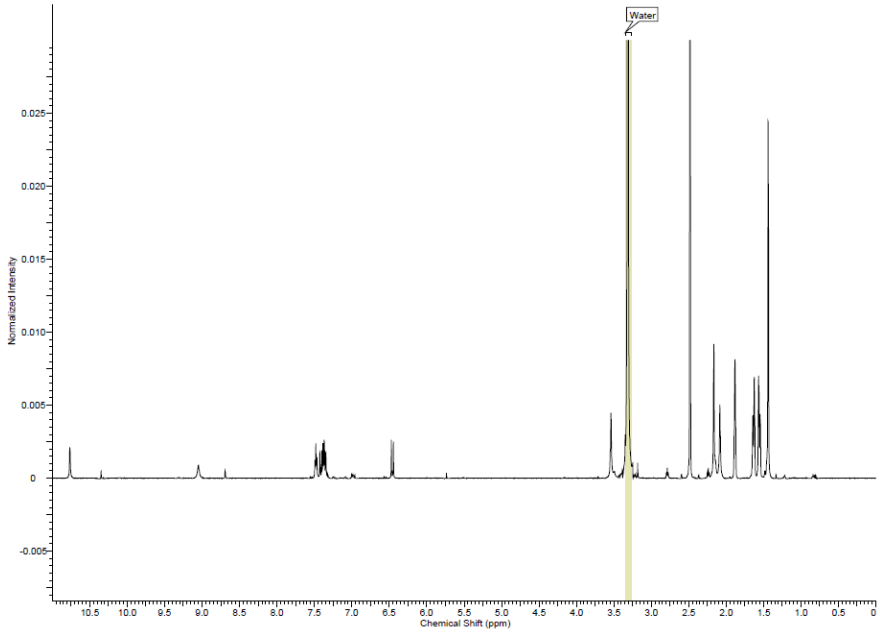
^{13}C-NMR spectrum of MGS2



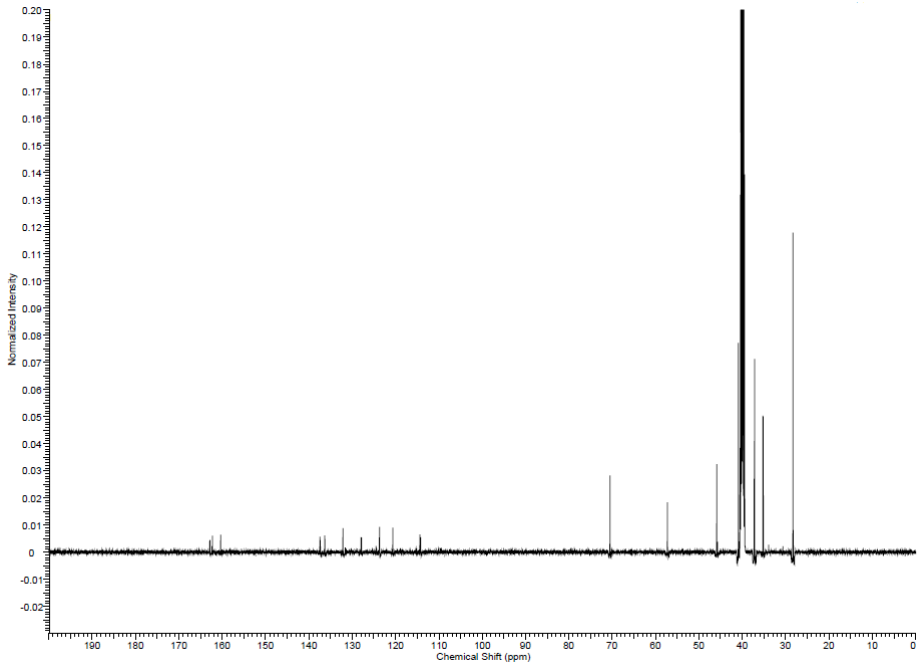
^{19}F-NMR spectrum of MGS2



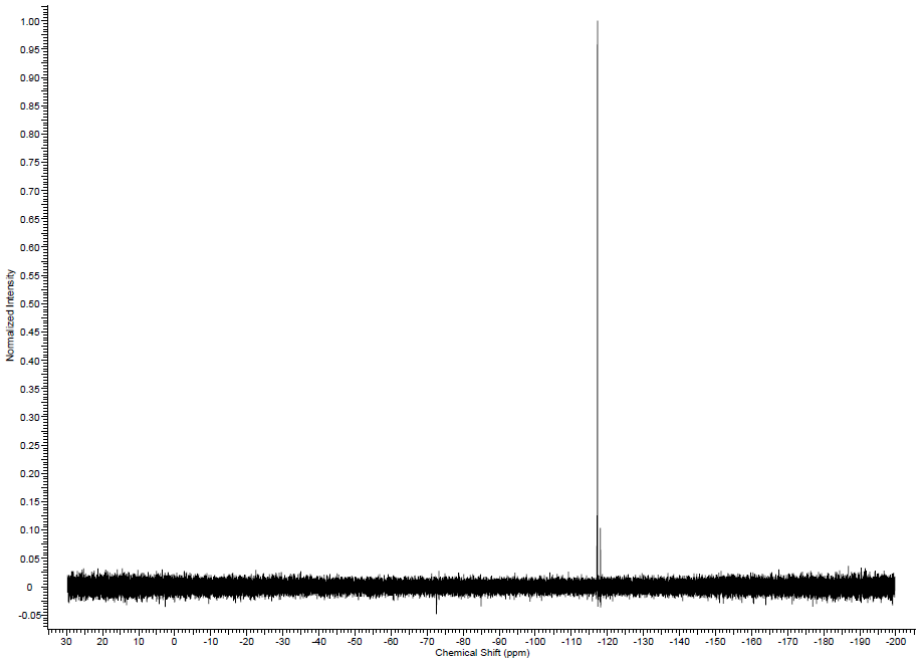
^{1}H-NMR spectrum of MGS3



^{13}C-NMR spectrum of MGS3



^{19}F-NMR spectrum of MGS3



Radiosyntheses of MGS1-3

Preparation of [^{18}F] Fluoride

Aqueous [18F]fluoride obtained from a cyclotron was passed through a SPE Chromafix 30-PS-HCO3 cartridge that had been previously conditioned with 5.0 mg/mL aqueous potassium carbonate and then washed with 18 mL of Millipore Milli-Q water. The captured [^{18}F]fluoride was washed by passing 2 mL of Millipore Milli-Q water through the cartridge. [^{18}F]Fluoride was eluted from the cartridge into a conical vial using 5.0 mg/mL of base in Millipore Milli-Q water/acetonitrile solution 1:4 (v/v)(2.0 mL, 6.6 µmol).

Note: for all semi preparative HPLC purifications in the following radiochemical procedures, a reverse phase column (9.4 x 250mm Agilent Eclipse XDB-C18, 5 µm) was used.

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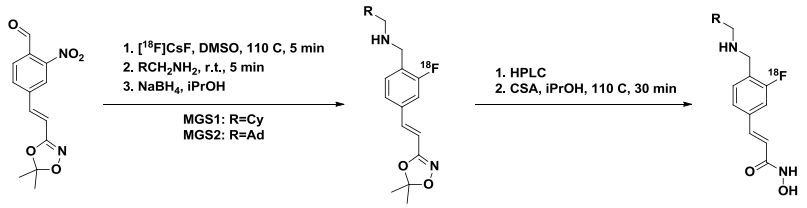
[^{18}F]MGS1 and 2

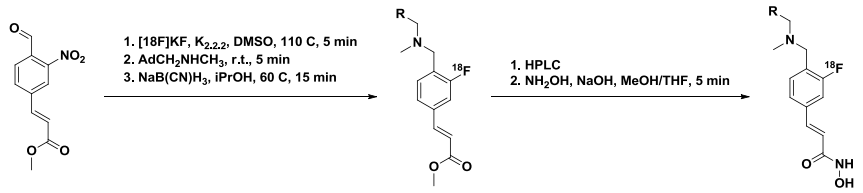


[^{18}F]Fluoride (94.23 mCi for **MGS1**, 523.6 mCi for **MGS2**) was eluted from the cartridge into a 4 mL glass vial using 0.7 mL of 5.0 mg/mL aqueous cesium carbonate. Azeotropic dry down at 110 °C was performed by adding 1 mL of dry acetonitrile to the eluted fluoride and evaporating the mixture under a stream of nitrogen gas. After complete evaporation, 1 ml of acetonitrile was added and evaporated in the same way, the process was repeated another time. After dry down, 10 µL Millipore Milli-Q water were carefully added to the solid, resolubilizing as much as possible. Then 0.8 mg of precursor **4** in 0.3 mL DMSO were added and heated for 5 min at 110 °C. The solution was transferred into another 4 mL glass vial containing 20 µL of the respective amine and stirred for 5 min at room temperature. Then 1 mL of a saturated solution of sodium borohydride in isopropanol was added. After stirring for 10 min at 60 °C, the mixture was diluted to 2 mL with water and purifed by semi-preparative HPLC (gradient water/MeCN with 0.1% TFA each at 5 ml/min; **MGS1**: 55:45 (v:v), R_T 8-10 min; **MGS2**: 5 min 35:65 (v:v), then ramp to 60:40 (v:v) over 20 min, R_T 17min). The isolated fractions containing the product were diluted with water, loaded onto a C-18 Sep-Pak solid phase extraction cartridge (conditioned with 1mL ethanol and 20 mL water), washed with 10 mL of water and eluted with 1.5 mL isopropanol. 5 mg of **6** and 5 mg camphor sulfonic acid were added and the mixture was heated to 110 °C for 30 min. The vessel was opened for the last five minutes to reduce the volume. Then the mixture was diluted to 2 mL with water and purified by semi-preparative HPLC to isolate the product.

For **MGS1**, a gradient 0.1%TFA (aq)/MeCN (90:10 (v:v) for 5min, then ramp to 35:65 (v:v) over 20 min, R_T about 11 min) was employed for separation and reformulation via a C-18 SepPak solid phase extraction cartridge was successful. 2.1 mCi of **MGS1** was obtained (7% radiochemical yield, decay corrected at TOI, 180 min synthesis time).

For **MGS2**, a direct cut method using EtOH/0.01M NaOH (40:60 (v:v), R_T 40 min) was used to purify the final product and the eluted radiolabeled compound formulated by neutralizing the solution with acetic acid and dilution with saline to 10% ethanol content for injection. 1.5 mCi of **MGS2** was obtained (0.9% radiochemical yield, decay corrected at TOI, 180 min synthesis time).

[^{18}F]MGS3



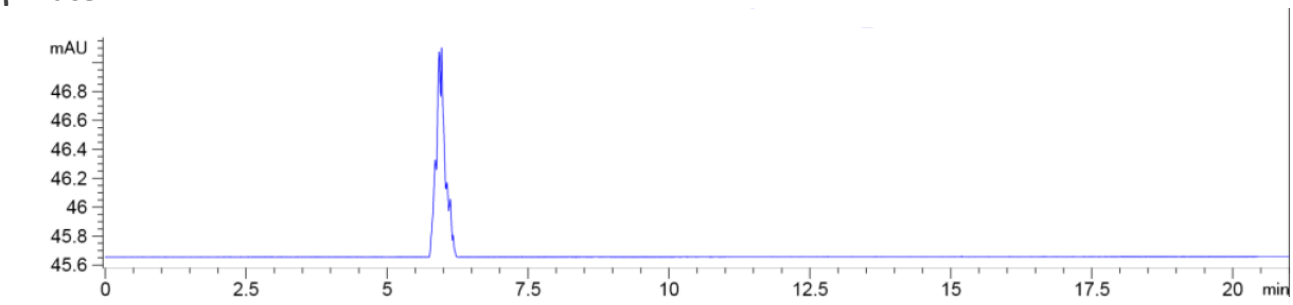
[^{18}F]Fluoride (610 mCi) was eluted from the cartridge into a 4 mL glass vial using 0.4 mL of 5.0 mg/mL aqueous potassium carbonate. Azeotropic dry down at 110 °C was performed by adding 0.7 mL of 5 mg/mL $K_{2.2.2}$ in dry acetonitrile to the eluted fluoride and evaporating the mixture under a stream of nitrogen gas. After complete evaporation, 1 ml of acetonitrile was added and evaporated in the same way, the process was repeated another time. 1 mg of 4-formyl-3-nitro methyl cinnamate in 0.3 mL DMSO was added to the vial and heated to 110 °C for 5 minutes. The resulting brown liquid was diluted to 20 mL with water, loaded onto a Sep-Pak C-18 solid phase extraction cartridge and elute with 1 mL EtOH into a vial containing adamantylmethyl methylamine and stirred for 5 min at room temperature. Then 65 mg $NaB(CN)H_3$ were added at once and the mixture stirred for 10 min at 60 °C, diluted to 2 mL with water and purifed by HPLC (gradient water/MeCN with 0.1% TFA each, 75:25 (v:v) for 5 min, then ramp to 40:60 (v:v) over 20 min, R_T 17 min). The isolated fractions containing the product were diluted with water, loaded onto a C-18 Sep-Pak solid phase extraction cartridge, washed with 10 mL of water and eluted with 1.5 mL MeOH/TFA 1:1. To the solution were added 200 µL each 50% aq. NH_2OH and 6M NaOH and stirred at room temperature for 5 min. a gradient of water containing 0.1% TFA and acetonitrile was employed for separation (25:75 (v:v) for 5 min, then ramp to 65:35 (v:v) over 20 min, R_T about 16 min, broad peak). The fractions containing the product were diluted with water, loaded onto an Oasis HLB solid phase extraction cartride and washed with 10 mL of water. The product was eluted with ethanol diluted with saline to 10% ethanol content for injection. 0.80 mCi **MGS3** was obtained (0.3% radiochemical yield, decay corrected at TOI, 130 min synthesis time).

Quality control of injected doses

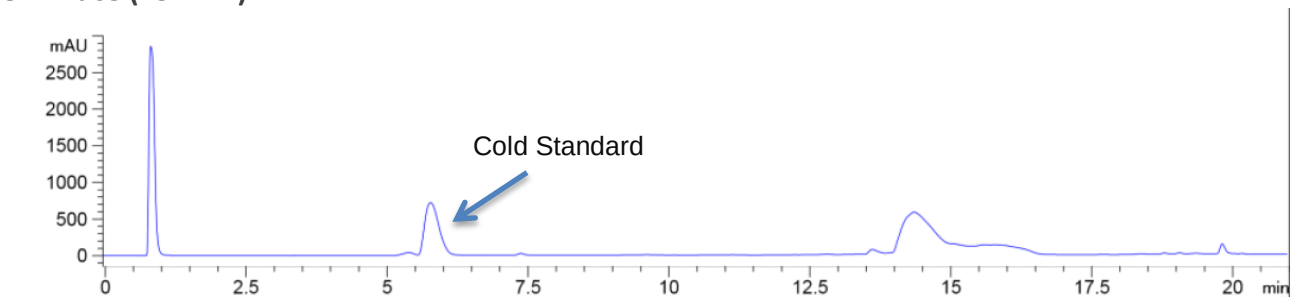
Quality control was performed by analytical HPLC (reverse phase 4.6 x 150mm Agilent Eclipse XDB-C18, 5 µm). Aliquots of the injected dose were loaded onto an HPLC system to control their purity. In a second run, the radiolabeled product was coinjected with a non-radioactive standard and retention times were compared. After completion of the chromatograph, peaks on UV and radioactivity detector were integrated and the radiochemical and chemical purity were determined by the area of integration. All tracers matched the retention time of the respective standards and exceeded 95% chemical purity and 95% radiochemical purity. Shown below are γ-traces of purified radiotracer spiked with cold standard aligned with the UV-trace at 254 nm.

[^{18}F]MGS1

γ-Trace

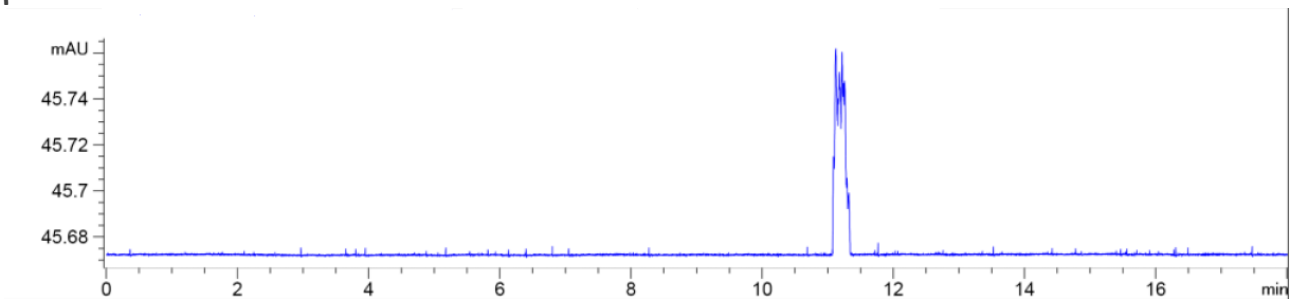


UV-Trace (254 nm)

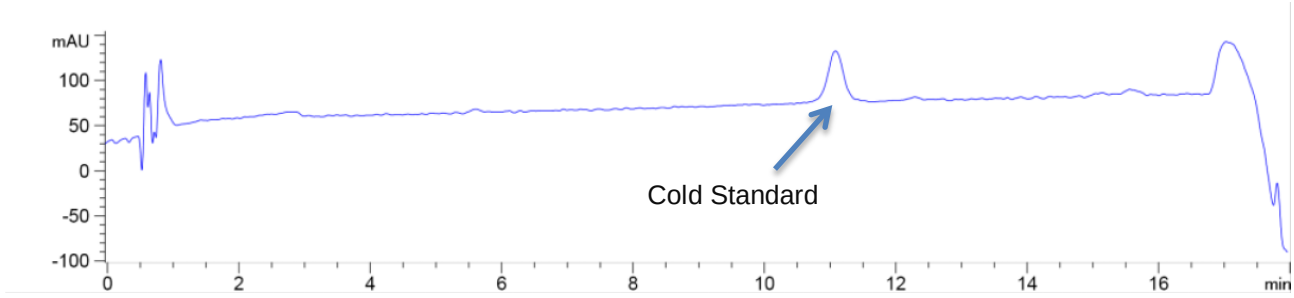


[^{18}F]MGS2

γ-Trace

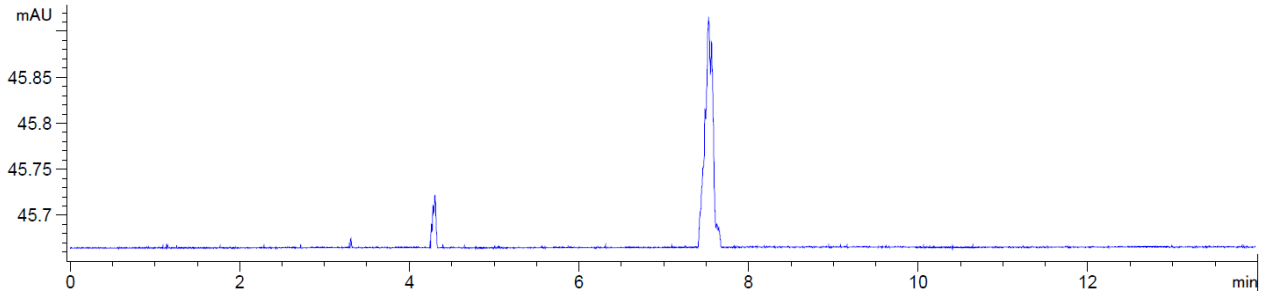


UV-Trace (254 nm)

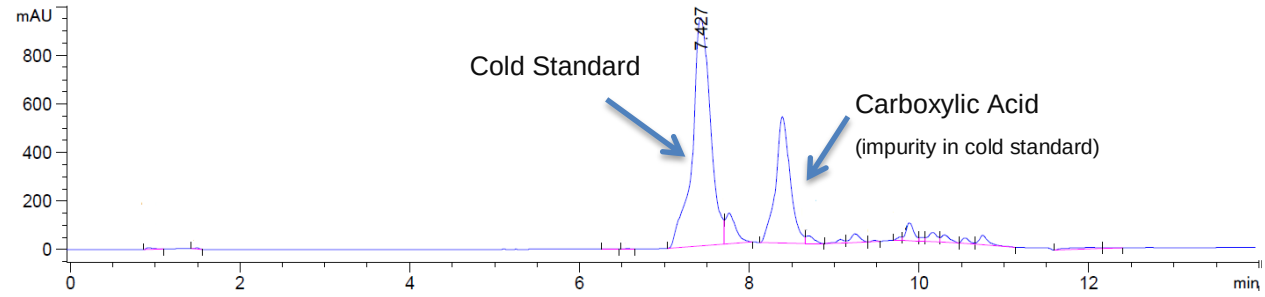


[^{18}F]MGS 3

γ-Trace



UV-Trace (254 nm)



Blocking Experiments to Confirm Brain Penetrance

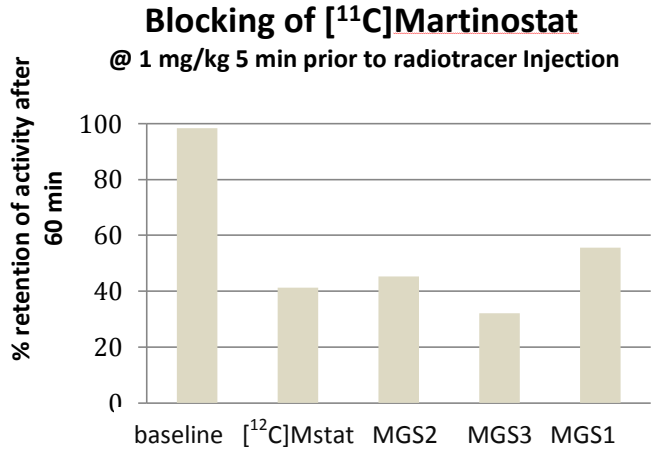


Figure 1-SI. [^{11}C]Martinostat blocking experiments confirm brain penetrance of the MGS series. Rat preparation and imaging was performed as stated in the Materials and Methods section. [^{11}C]Martinostat was synthesized as cited. Data analysis after reconstruction was performed as follows: The PET image was coregistered to a CT scan of the same or age matched animal. An ellipsoid whole brain ROI was applied and the TAC extracted. The data was normalized to 100% at 10 minutes. The % signal at 60 minutes is represented in the graph below. Administration of blocking agent was performed 5 min prior to radiotracer administration as a 1 mg/mL solution in DMSO/Tween80/Saline (1:1:8). The administered dose was 1 mg/kg body weight of the animal.

HDAC Inhibition Assay

The assays were carried out at the Broad Institute. All recombinant human HDACs were purchased from BPS Bioscience. The substrates, Broad Substrates A and B, were synthesized in house. All other reagents were purchased from Sigma-Aldrich. Caliper EZ reader II system was used to collect all data. HDAC inhibition assays: Compounds were tested in a 12-point dose curve with 3-fold serial dilution starting from 33.33 μM. Purified HDACs were incubated with 2 μM (the concentration is kept the same for all the HDACs, below Km of substrate) carboxyfluorescein (FAM)-labeled acetylated or trifluoroacetylated peptide substrate (Broad Substrates A and B, respectively) and test compound for 60 min at room temperature, in HDAC assay buffer that contained 50 mM HEPES (pH 7.4), 100 mM KCl, 0.01% BSA, and 0.001% Tween-20. Reactions were terminated by the addition of the known pan HDAC inhibitor LBH -589 (panobinostat) with a final concentration of 1.5 μM. Substrate and product were separated electrophoretically, and fluorescence intensity in the substrate and product peaks were determined and analyzed by Labchip EZ Reader. The reactions were performed in duplicate for each sample. IC_{50} values were automatically calculated by Origin8 using 4 Parameter Logistic Model. The percent inhibition was plotted against the compound concentration, and the IC_{50} value was determined from the logistic dose−response curve fitting by Origin 8.0 software.

Regional Analysis of SUV in Baboon Brain

Table 1-SI. SUV values for different brain regions in baboon brain for different radiotracers. Regions are based on Black baboon atlas and averaged if they appear bilaterally. Treatment of data as described in Marterials and Methods section. Graphic comparison of Martinostat with each [^{18}F]-radiotracer is depicted in Figure 4.

Region	*[^{11}C]Martinostat*	*[^{18}F]MGS1*	*[^{18}F]MGS2*	*[^{18}F]MGS3*
Orbitofrontal Ctx	2.690704	0.507060804	1.333912833	1.864945153
DLPFC	2.909065	0.602183629	1.440652667	2.434923242
Cingulate-anterior	2.780695333	0.72408169	1.534795667	2.262442948
Cingulate-posterior	2.830093667	0.718239117	1.477809667	2.270359299
Amygdala	2.528690833	0.566712904	1.3340095	1.959587828
Hippocampus	2.6695835	0.758395532	1.418172	1.945038455
ventral caudate	3.2318105	0.62588607	1.311393667	2.155984595
body caudate	2.969039	0.542714031	1.459730667	2.218534396
Putamen	3.413536167	0.592210811	1.606991	2.730515097
Accumbens	3.01345	0.610874903	1.4572765	2.246417015
MD thalamus	3.2610665	0.794245541	1.752823167	2.649003028
VPL thalamus	2.916255667	0.61728678	1.5919275	2.187538624
Habenula lateral	3.092857	0.842391553	1.778956833	2.040610527
Midline CB nuclei	3.888948667	0.939019177	1.786383	2.752854972
Cerebellum	3.254168	0.73952541	1.692338167	2.614775322
Corpus Callosum genu	2.376623	0.62442936	1.342047667	1.732081632
striate cortex	3.275043333	0.841550035	1.447194667	2.514682906
motor upper extremity	2.601000833	0.468627295	1.410959	2.097487895
motor face	2.916494833	0.493315436	1.661703667	2.355384385
SMA	2.721712333	0.785505823	1.620005	2.472368228
Corpus callosum splenium	2.081931333	0.543213847	1.081502	1.880822032
centrum semiovale	1.8385955	0.417439746	0.989116667	1.435889579