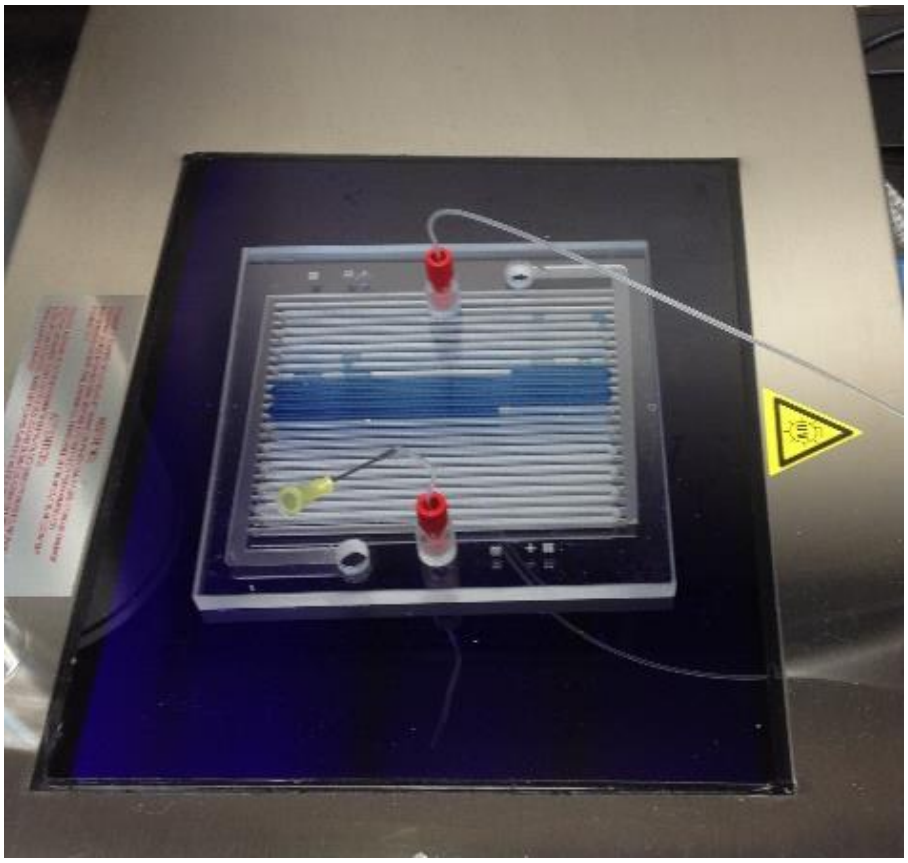
General

All reactions were carried out with commercial solvents and reagents that were used as received. Concentration and removal of trace solvents was done via a Büchi rotary evaporator equipped with a dry ice/acetone condenser, and vacuum applied from an aspirator or Büchi V-500 pump. Nuclear magnetic resonance (NMR) spectra were recorded using deuterium oxide. Signal positions (δ) are given in parts per million from tetramethylsilane ($\delta = 0$) and were measured relative to the signal of the solvent (^{1}H NMR: CD_3CN: δ = 1.94, D_2O: δ = 4.79; ^{13}C NMR: CD_3CN: δ = 118.26). ^{19}F NMR spectra are referenced to trifluoroacetic acid in D_2O δ = -76.6. Coupling constants (J values) are given in Hertz (Hz) and are reported to the nearest 0.1 Hz. ^{1}H NMR spectral data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet), coupling constants, number of protons. NMR spectra were recorded on a Bruker Avance 600 equipped with a QNP or TCI cryoprobe (600 MHz), a Bruker 400 (400 MHz) or a Bruker 500 (500 MHz). Where necessary, *N*,*N*-dimethylformamide (DMF) or 1,3,5-tris(trifluoromethyl)benzene was added to the crude reaction mixtures and used as an internal standard. Yields were then calculated following analysis of ^{1}H NMR spectra. Preparative RP-HPLC was performed on an Agilent 1200 series instrument with a SiliCycle SiliaChrom dtC18 semipreparative column (5 um, 100 Å, 10 x 250 mm) with a flow rate of 5 mL/min, or a Phenomenex Gemini-NX C18 preparative column (5um, 110Å, 50 x 30 mm) with a flow rate of 15 mL/min. Analytical HPLC was carried out on an Agilent 1200 series HPLC system equipped with a diode array detector (DAD) and Raytest GABI Star scintillation detector.



Supplemental Figure 1. Reactor configuration used in this study: Transilluminator (Jena Analytik UVP Transilluminator PL TFL-40V) with glass microreactor (Little Things Factory, reactor XXL-KOE), (total volume = 5mL) used in the fluorination and radiofluorination described in this work.

Measured light intensity at reaction site >6000 $\mu W/cm^2$

Synthesis

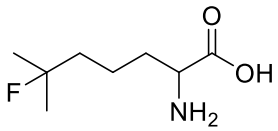
Most of the parent amino acids used in this study were commercially available as enantiomerically pure compounds. Leucine (L), homoleucine (HL), β-amino homoleucine (BAHL), α-methyl leucine (αML) were purchased in both (*S*)- and (*R*)- configurations. The anticonvulsant drug pregabalin is commercially available as its (*S*)-isomer. In the case of racemic compounds, "homohomoleucine" (HHL) and α-methylhomoleucine (αMHL), an enzymatic resolution was carried out to access the (*S*)-isomer according to Chenault et al(*1*). As these compounds were not ideal substrates for this enzymatic resolution, only the (*S*)-isomer was isolated, as incomplete deacylation would result in enantiomeric contamination of the (*R*)-configured amino acid.

Radical fluorination of the BCAAS were carried out as reported by us previously(*2*). Briefly, a TFA or HCl salt of the substrate (0.1 M final concentration), sodium decatungstate (NaDT, 5 mol%) and *N*-fluorobenzenesulfonimide (NFSI, 3 eq.) were dissolved in 3:1 CH_3CN:H_2O with sonication. The resulting

solution was filtered and loaded onto a photoreactor as depicted in Supporting Figure 1. Irradiation was carried out for 40 min, and then the solution was removed via syringe, and the PTFE reaction tube washed with 2 mL CH_3CN. The solution was diluted with CH_3CN (5 mL) and loaded onto a strong cation exchange cartridge (Silicycle SCX resin, 500 mg). After loading, the cartridge was washed with CH_3CN (10 mL), followed by H_2O (10 mL). The fluorinated product/starting material mixture was eluted with ~4 mL 150 mM $NaHCO_3$ (1 mL/min approximate flow rate) acidified with HOAc to pH = 5 and lyophilized. Pure samples of all fluorinated BCAAs suitable for characterization could be obtained by preparative RP-HPLC as described above eluting with solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in CH_3CN) on a gradient of 2% →30% solvent B over 30 min (5 mL/min flow rate) to yield the FBCAAs as TFA salts

Characterization data

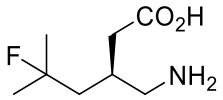
6-FHHL



^{1}H NMR: (D_2O, 600 MHz): δ 1.30 (overlapping doublets, *J* = 23.0 Hz, 6H), 1.45 (m, 2H), 1.65 (m, 2H), 1.87 (m, 2H), 3.91 (t, J = 6.5 Hz, 1H); ^{13}C NMR (D_2O, 150 MHz): δ 19.0 (d, J = 5.0 Hz), 25.5 (d, J = 6.6 Hz), 25.7 (d, J = 6.6 Hz), 30.1, 39.8 (d, J = 23.5 Hz), 53.4, 98.7 (d, J = 160 Hz), 172.9; ^{19}F NMR (D_2O, 470 MHz): -134.2

HRMS (ESI$^+$) calcd for $C_8H_{16}FNO_2H^+$ 178.1234, found 178.1246

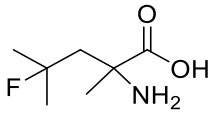
5-FPregab



^{1}H NMR: (D_2O, 600 MHz): δ 1.34 (overlapping doublets, J = 22.7 Hz, 6H), 1.73 (m, 2H), 2.40 (m, 1H), 2.47 (m, 2H), 3.02 (m, 2H); ^{13}C NMR (D_2O, 150 MHz): δ 25.4 (d, J = 24.7 Hz), 26.6 (d, J = 23.8 Hz), 29.4, 37.5, 41.6 (d, J = 21.8 Hz), 43.4, 98.0 (d, J = 159.9 Hz), 176.3; ^{19}F NMR (D_2O, 470 MHz): -136.5

HRMS (ESI$^+$) calcd for $C_8H_{16}FNO_2H^+$ 178.1234, found 178.1250

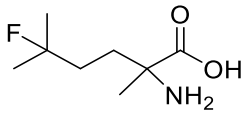
4-FαML



^{1}H NMR: (D_2O, 600 MHz): δ 1.34 (d, J = 22.3 Hz, 3H), 1.36 (d, J = 22.9 Hz, 3H), 1.51 (s, 3H), 2.21 (dd, J = 15.6, 11.2 Hz, 1H), 2.43 (dd, J = 34.3, 15.6 Hz, 1H); ^{13}C NMR (D_2O, 150 MHz): δ 24.4 (d, J = 24.2 Hz), 24.8, 28.8 (d, J = 23.0 Hz), 45.8 (d, J = 20.6 Hz), 58.6, 97.1 (d, J = 160.4 Hz), 174.8; ^{19}F NMR (D_2O, 470 MHz): -138.6

HRMS (ESI$^+$) calcd for $C_7H_{14}FNO_2H^+$ 164.1087, found 164.1091

5-FαMHL



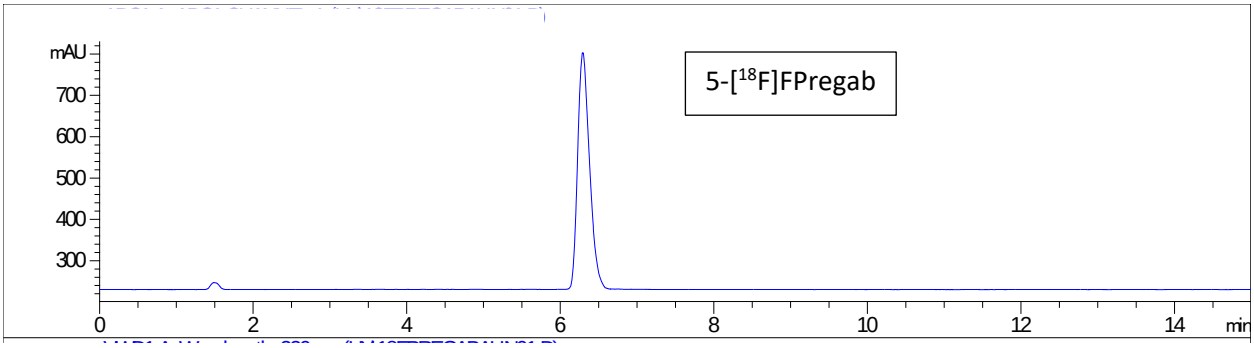
^{1}H NMR: (D_2O, 600 MHz): δ 1.30 (overlapping doublets, J = 22.6 Hz, 6H), 1.43 (s, 3H), 1.52 (m, 1H), 1.69 (m, 1H), 1.79 (td, J = 13.2, 3.7 Hz, 1H), 1.92 (td, J = 13.6, 4.3 Hz, 1H); ^{13}C NMR (D_2O, 150 MHz): δ 22.4, 25.4 (d, J = 23.7 Hz), 25.7 (d, J = 24.4 Hz), 31.5 (d, J = 5.5 Hz), 34.6 (d, J = 23.2 Hz), 61.1, 97.5 (d, J = 160.0 Hz), 176.3; ^{19}F NMR (D_2O, 470 MHz): -135.8

HRMS (ESI$^+$) calcd for $C_8H_{16}FNO_2H^+$ 178.1234, found 178.1239

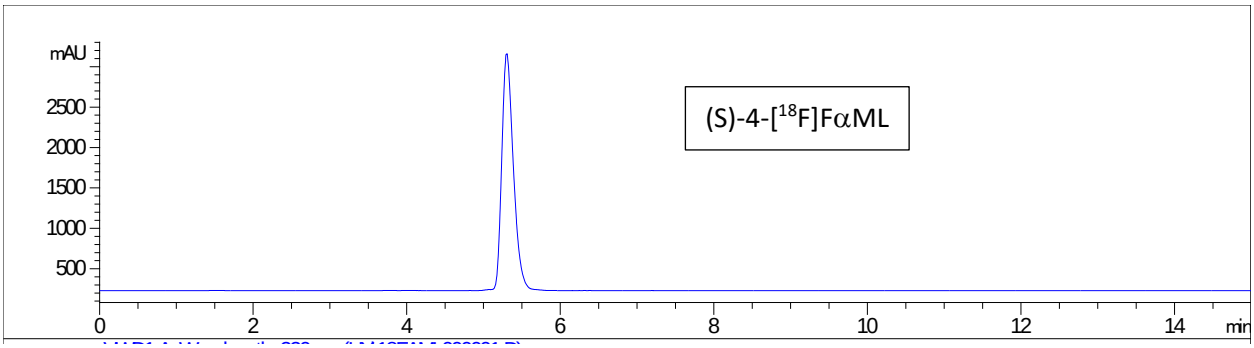
Determination of BCAA pKa values

The pKa values were determined by SiriusT3 pH-metric pKa method. For the titration approx. 1.0 mg of solid sample was weighed into a vial and dissolved in 1.5 mL of water containing 0.15 M KCl as background electrolyte. The pH of the dilute sample solution was adjusted to pH 2.0 by addition of 0.5 M HCl and then titrated with standardized base (0.5 M KOH) to pH 12.2 at 25°C under argon atmosphere. During the titration more than 25 pH readings (volume of titrant vs. pH) were collected and used for the evaluation of the pKa values. A standard assay template provided in the SiriusT3 software was used for the experiment (SiriusT3Control Version 1.1.1.1).

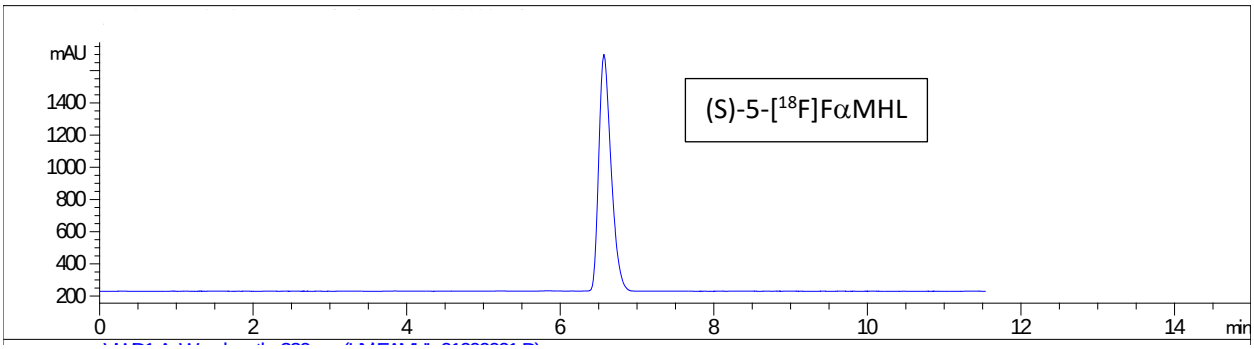
Radiotraces of [^{18}F]FBCAAs



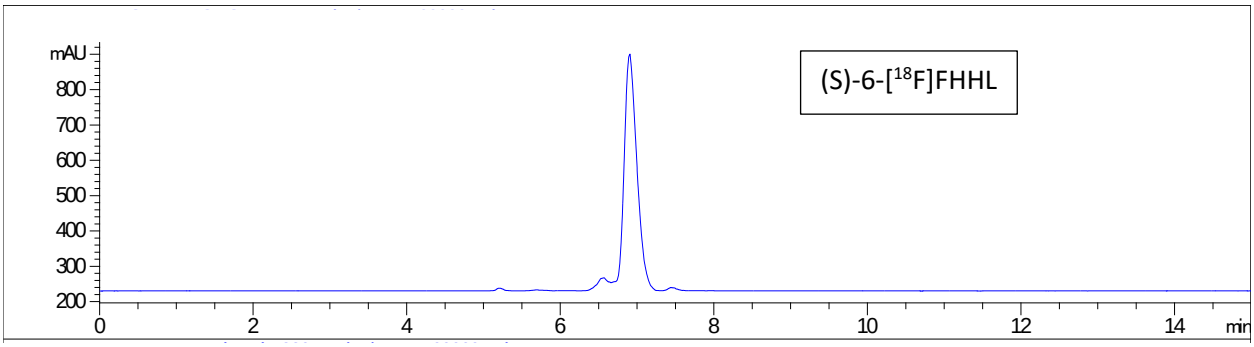
Supplemental Figure 2. Radiodetected HPLC trace of purified 5-[^{18}F]FPregab



Supplemental Figure 3. Radiodetected HPLC trace of purified (S)-4-[^{18}F]FαML



Supplemental Figure 4. Radiodetected HPLC trace of purified (S)-5-[^{18}F]FαMHL



Supplemental Figure 5. Radiodetected HPLC trace of purified (S)-6-[^{18}F]FHHL

Determination of Molar Activity

To determine the molar activity (MA) of the radiotracers in reported in this study, the purified product mixtures were eluted from the ion exchange column in 1 mL fractions. Each fraction was counted, then

the whole sample was allowed to decay at –20 °C. After ~100 h, the fractions were then acidified with HOAc and lyophilized to dryness. Each entire dried fraction was then taken up in D_2O and *N*,*N*-dimethylformamide (5 μl, 65 μmol) was added as an internal standard. After thorough mixing the ^{1}H and ^{19}F NMR spectra were recorded. Amounts of fluorinated compound were determined by analysis of the ^{1}H NMR spectra. Molar activity was then determined by correlating the amount of fluorinated product in each fraction to its activity.

***In vitro* cell experiments**

LAT uptake assay

CHO-K1 cells were a gift from Dr. Michael Silverman (Simon Fraser University, Canada). Cells were maintained in T175 flasks in a humidified incubator (37 °C and 5% CO_2) using minimal essential alpha medium (ThermoFisher # 32571036) supplemented with 5% FCS (ThermoFisher # 10082139), and 1% penicillin/streptomycin (ThermoFisher 15140122). They were routinely passaged at ~90 % confluence. The day before the assay, cells were trypsinized, diluted and plated into clear-bottom 96-well tissue culture plates (Perkin-Elmer, ViewPlate 96 TC). 100 μL of a cell suspension with a density of 3 x 10^5 cells added to each well resulted in a 90-100% confluent cell monolayer in each well 18 h later. The next day, the cells were washed with Na^+-free buffer (10 mM HEPES, 5 mM Tris, 140 mM choline chloride, 2.5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM K_2HPO_4, 10 mM glucose pH = 7.4 with KOH) two times for 20 minutes at 37 °C to deplete endogenous amino acids and wash out sodium. Meanwhile, a compound plate was made which consisted of 8 final concentrations (~1% DMSO) of the BCAA in the above Na^+- free buffer containing 92.5 kBq/mL [^{3}H]leucine (PerkinElmer, 4.64 TBq/mmol, ~20 nM leucine). The drug plate also had two wells for each test compound – one with no compound, and one with saturating leucine (10 mM). To start the assay, the cell plates were inverted and shaken out, and 150 μL from each of the wells on the compound plate was added to the corresponding wells of the cell plate simultaneously. After 1 min at room temperature, the solutions were removed, and the cells were washed with 3 times with ice-cold Na^+ - free buffer. The cell plates were then dried at 80 °C for 1 h, and 50 μL of scintillation cocktail (PerkinElmer, MicroScint-20) was added. The plate was then counted on a PerkinElmer MicroBeta^2 2450 microplate reader. Data was plotted in GraphPad Prism 6 and is the result of at least three independent experiments.

Competition assay

The PC-3 cell line was obtained from ATCC. All cell lines were authenticated by short tandem repeat (STR) profiling. The cells were seeded in 24-well plates until approximately 90% confluence, and a fixed amount (74 kBq) of radioactive tracer was added to each well. Blocking experiments were performed using 10 mM of competitive substrate. Following a 60 min incubation, each well was washed with ice-cold HEPES buffer. Replicate wells were used for cell counting. The cells were lysed with 1 M NaOH. The activity in supernatant, washes and cell lysates was measured using a PerkinElmer Wizard 2480 gamma counter. The activity is reported as the percentage of incubated activity, and cellular uptake was normalized to cell number.

***In vivo* biodistribution and PET experiments**

All animal experiments were conducted in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC) and approved by the Animal Care Committee of the University of British

Columbia (ACC UBC). *In vivo* biodistribution studies were performed in healthy mice (n = 4 each) to evaluate normal organ uptake of the [^{18}F]FBCAAs. Biodistribution studies were also obtained in immunocompromised mice (NOD.Cg-Rag1^{tm1Mom} Il2rg^{tm1Wjl}/SzJ) bearing human glioma (U-87 MG, obtained from ATCC) to evaluate tumor accumulation of the [^{18}F]FBCAA radiotracers. For tumor bearing mice, ~5 x 10^6 cells were injected subcutaneously in the dorsal flank of the mice. The tumors were grown to a diameter of approximately 7 - 10 mm prior to the biodistribution study. The animals were lightly sedated with isoflurane, and 1 - 2 MBq of ^{18}F-labeled radiotracer was administered intravenously *via* the caudal lateral tail vein. The radioactivity in the syringe was measured before and after injection to ensure accurate determination of the amount injected. The animals quickly recovered from sedation and were allowed to roam free in the cage during the uptake period. At 60 min, the animals were sedated with isoflurane, sacrificed by CO_2 asphyxiation and their blood was collected by cardiac puncture. The organs were harvested, rinsed with saline, blotted dry, and weighed. Radioactivity in each organ was measured using a PerkinElmer Wizard 2480 gamma counter, calibrated using a standard curve of ^{18}F. Organ uptake data is reported as the percentage of injected dose per gram of tissue (%ID/g).

Dynamic and static PET/CT acquisitions were performed in a distinct set of mice to obtain representative images and follow the kinetics of uptake in normal organs and U-87 tumor. The mice were sedated with isoflurane inhalation, a catheter was placed in the caudal lateral tail vein for dynamic scan, and the mice were placed in a preclinical PET/CT scanner (Siemens Inveon). For static scans, mice were injected similarly as for other biodistribution studies. A low-dose CT scan was performed using 40 kV X-rays at 500 µA for attenuation correction and co-registration. Following CT imaging, the list-mode dynamic acquisition was started, and the radiotracer was injected (3.2 – 3.4 MBq). Dynamic scanning was continued for 60 min. In addition to dynamic images, static images were reconstructed at 50-60 minutes using an iterative reconstruction algorithm (3D-OSEM/MAP). For data analysis, the Inveon Research Workplace (IRW) software was used. Regions of interest (ROIs) were drawn using the same volume for tumor, brain and muscle for each study animal. Values using different parameters such as SUV_{mean} (mean standard uptake values) and %ID/g for uptake in tumor, brain and muscle were measured. The time activity curves of %ID/g and SUV for each ROI from each mouse were also obtained from the IRW. All parameters for each group were averaged and expressed as mean ± SD.

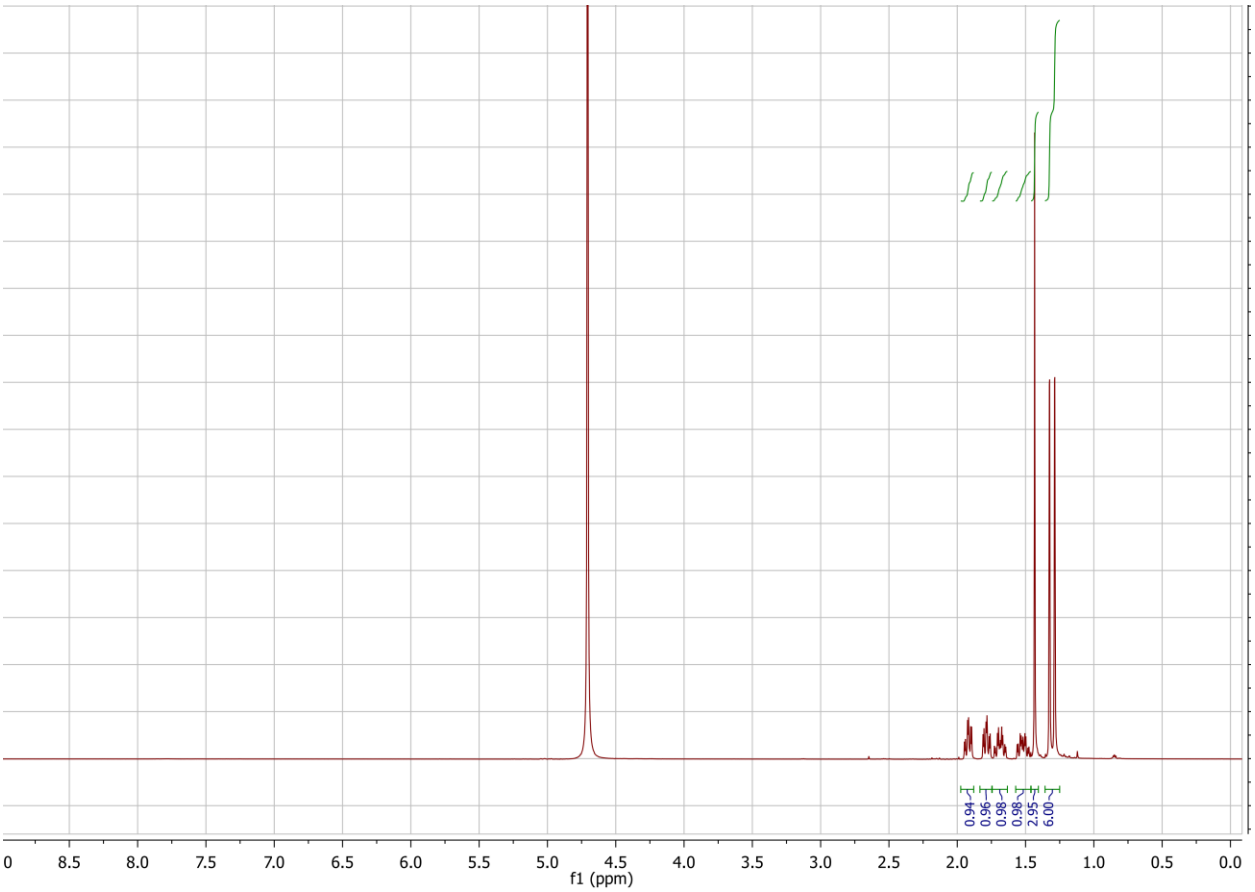
Statistical Analysis

Test statistics and p-values were calculated using GraphPad software. The descriptive statistics are reported as mean ± standard deviation when applicable. Comparison of means was conducted using a two-sample t-test, assuming equal variance. A p value of <0.05 was considered significant.

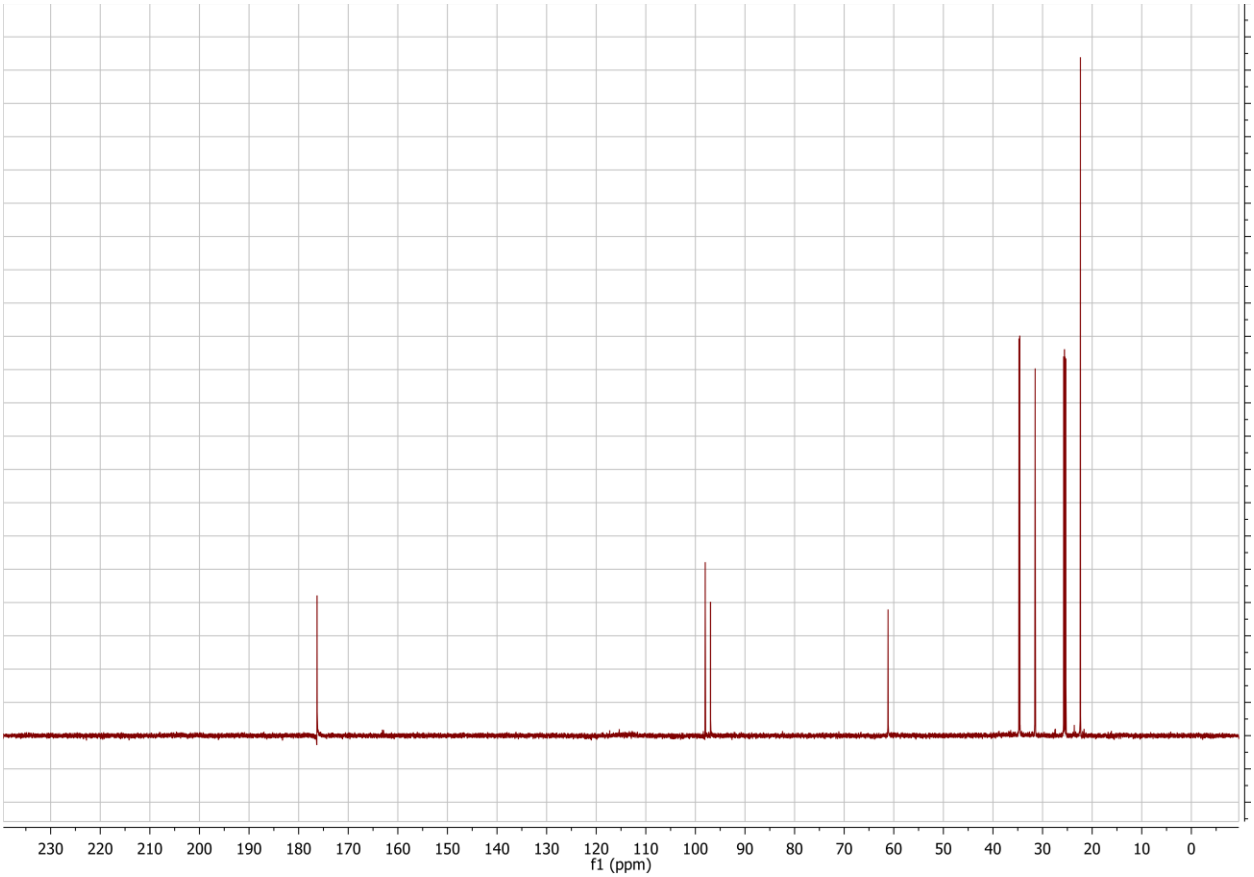
Tissue	Tracer						
	(S)-5-[^{18}F]FHL	(S)-6-[^{18}F]FHHL	(R)-5-[^{18}F]FHL	(S)-5-[^{18}F]FBAHL	5-[^{18}F]FPregab	(S)-4-[^{18}F]FαML	(S)-5-[^{18}F]FαMHL
Blood	2.74 ± 0.36	1.96 ± 0.11	2.45 ± 0.12	2.54 ± 0.36	0.59 ± 0.09	1.24 ± 0.12	1.29 ± 0.21
Fat	0.24 ± 0.07	0.14 ± 0.01	0.24 ± 0.05	0.22 ± 0.05	0.07 ± 0.03	0.12 ± 0.05	0.14 ± 0.05
Uterus	3.04 ± 0.98	2.34 ± 0.46	2.98 ± 0.23	2.53 ± 0.43	0.95 ± 0.41	1.54 ± 0.28	1.35 ± 0.21
Ovaries	4.89 ± 1.76	3.46 ± 0.48	3.58 ± 0.57	3.83 ± 0.82	1.19 ± 0.15	2.37 ± 1.61	1.92 ± 0.49
Intestine	2.49 ± 0.29	1.70 ± 0.3	2.29 ± 0.66	2.31 ± 0.6	1.01 ± 0.23	1.24 ± 0.28	1.29 ± 0.18
Spleen	3.28 ± 0.84	2.47 ± 0.31	3.04 ± 0.7	2.77 ± 0.35	0.94 ± 0.18	1.68 ± 0.38	1.68 ± 0.3
Pancreas	15.59 ± 1.45	10.47 ± 0.75	16.11 ± 3.82	15.2 ± 5.56	6.07 ± 1.14	13.59 ± 3.78	9.34 ± 1.43
Stomach	1.16 ± 0.27	0.58 ± 0.14	0.94 ± 0.3	0.95 ± 0.35	0.35 ± 0.11	0.54 ± 0.21	0.54 ± 0.22
Liver	2.50 ± 0.37	1.74 ± 0.14	2.27 ± 0.2	2.25 ± 0.19	0.81 ± 0.07	1.47 ± 0.02	1.69 ± 0.3
Adrenal	2.19 ± 0.86	2.07 ± 0.67	1.54 ± 0.33	2.06 ± 0.58	0.52 ± 0.06	0.93 ± 0.68	0.97 ± 0.35
Kidney	8.26 ± 1.43	4.37 ± 0.86	6.15 ± 0.34	4.19 ± 0.49	1.96 ± 0.25	3.79 ± 0.53	4.66 ± 0.81
Heart	2.94 ± 0.45	2.2 ± 0.29	2.71 ± 0.14	2.14 ± 0.17	0.77 ± 0.09	1.41 ± 0.12	1.43 ± 0.16
Lungs	2.69 ± 0.44	2.19 ± 0.18	2.35 ± 0.1	2.21 ± 0.17	0.64 ± 0.09	1.27 ± 0.2	1.19 ± 0.16
Tumour	5.29 ± 0.84	4.85 ± 1.12	4.66 ± 0.32	3.04 ± 0.22	0.58 ± 0.27	1.55 ± 0.32	1.21 ± 0.2
Bone	1.25 ± 0.35	2.61 ± 0.4	1.03 ± 0.19	1.40 ± 0.25	3.36 ± 0.55	0.93 ± 0.14	1.04 ± 0.21
Muscle	2.43 ± 0.34	2.12 ± 0.24	2.33 ± 0.1	2.26 ± 0.18	0.88 ± 0.09	1.11 ± 0.09	1.09 ± 0.11
Brain	1.63 ± 0.3	1.60 ± 0.33	1.59 ± 0.15	0.37 ± 0.02	0.12 ± 0.03	0.37 ± 0.12	0.10 ± 0.01
Tail	2.16 ± 0.32	3.05 ± 1.64	1.72 ± 0.12	2.25 ± 0.77	1.72 ± 0.51	1.33 ± 0.39	1.28 ± 0.44

Supplemental Table 1. Biodistribution of [^{18}F]FBCAAs in U-87 tumor bearing mice at 60 min post injection. All values are % ID/g of tissue

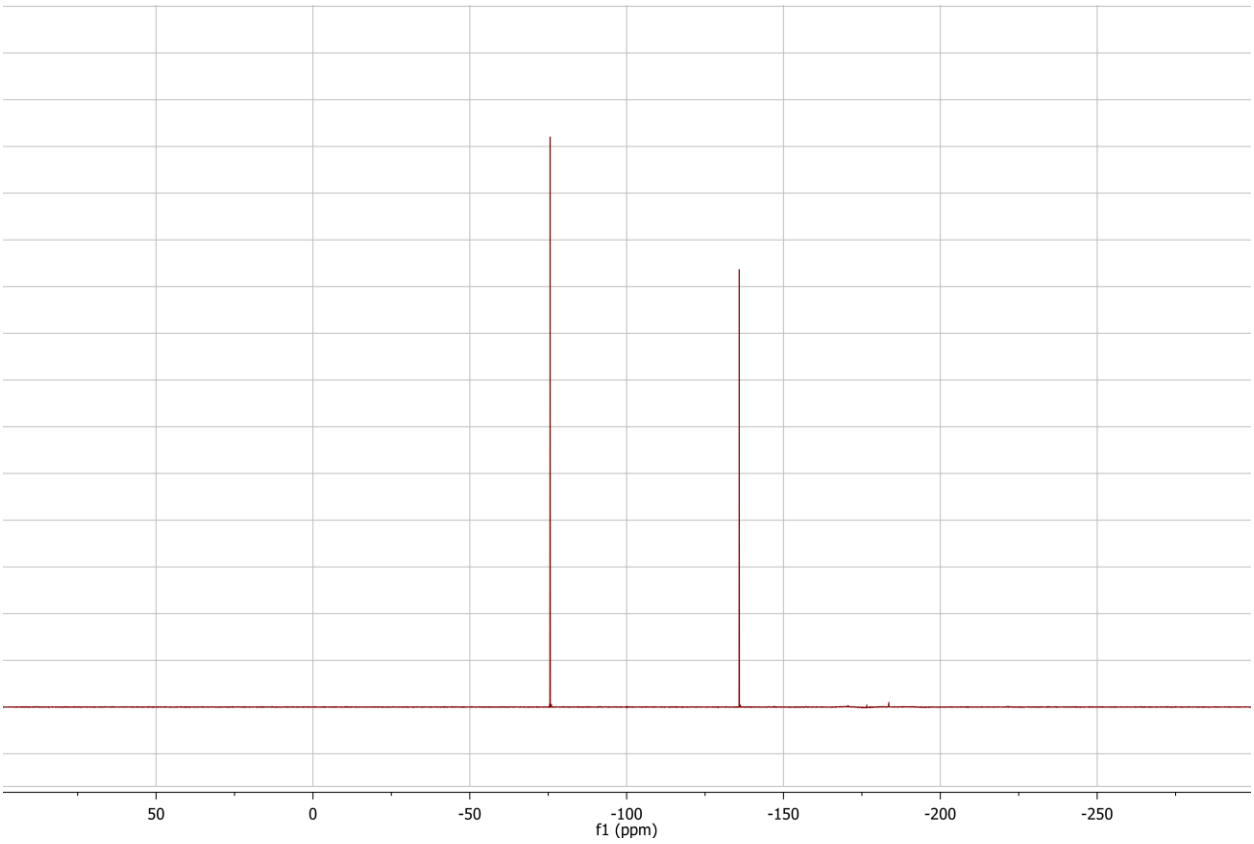
NMR spectra



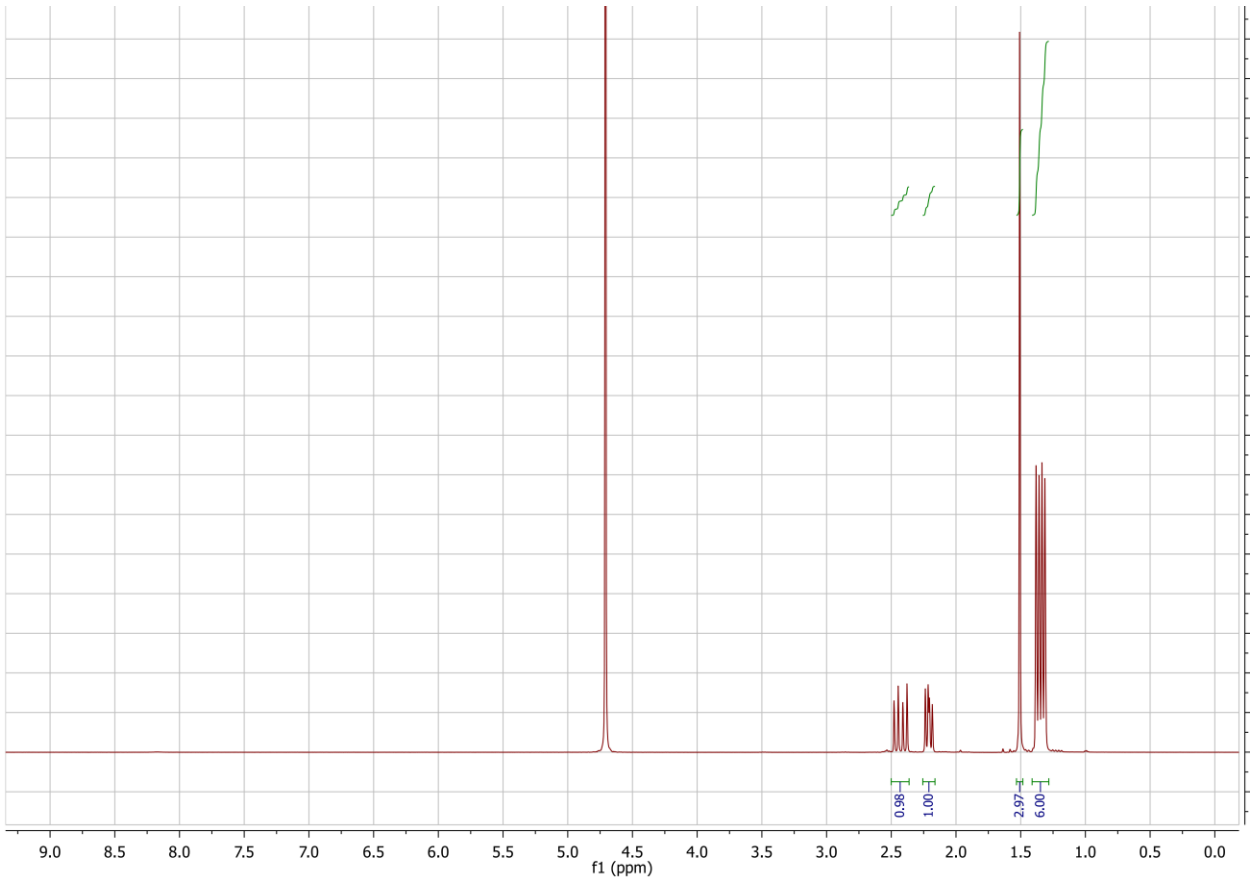
Supplemental Figure 6. ^{1}H NMR spectrum (600 MHz) of 5-FαMHL recorded in D_2O



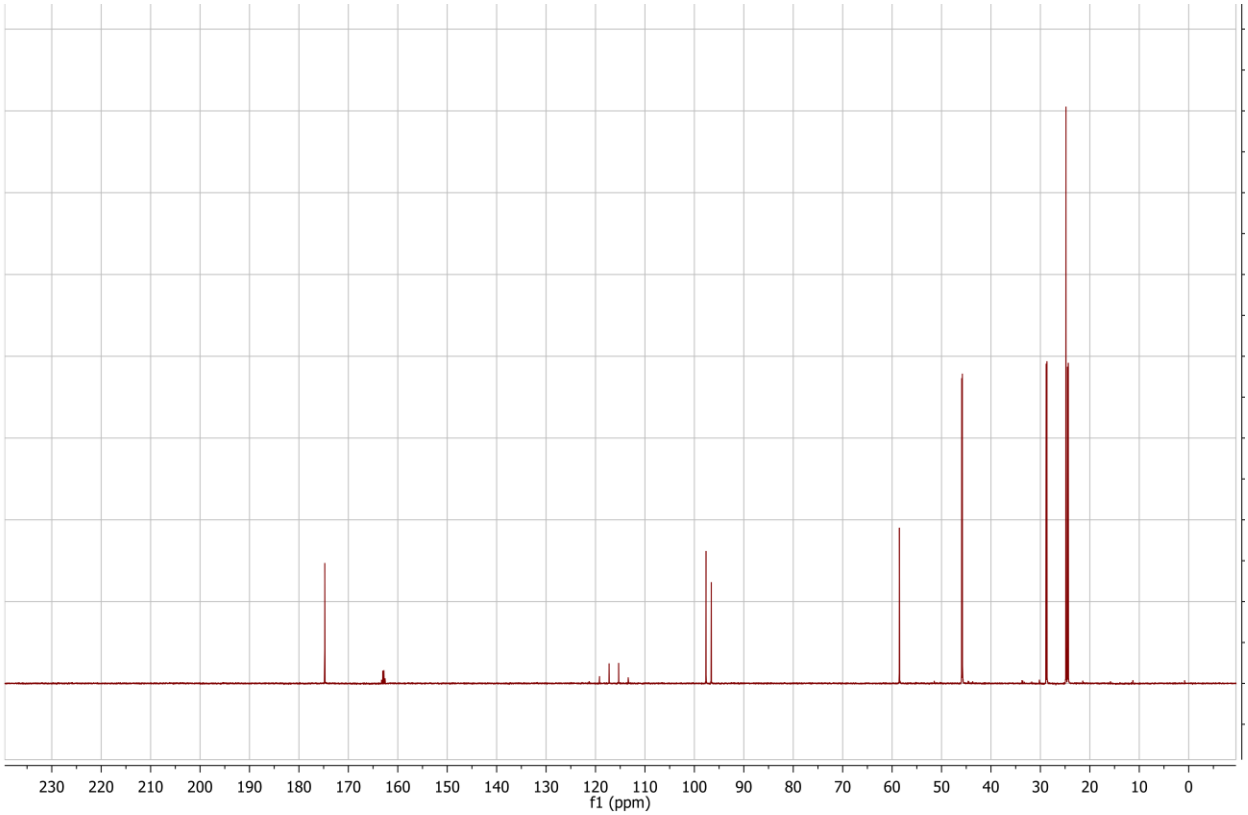
Supplemental Figure 7. ^{13}C NMR spectrum (150 MHz) of 5-FαMHL recorded in D_2O



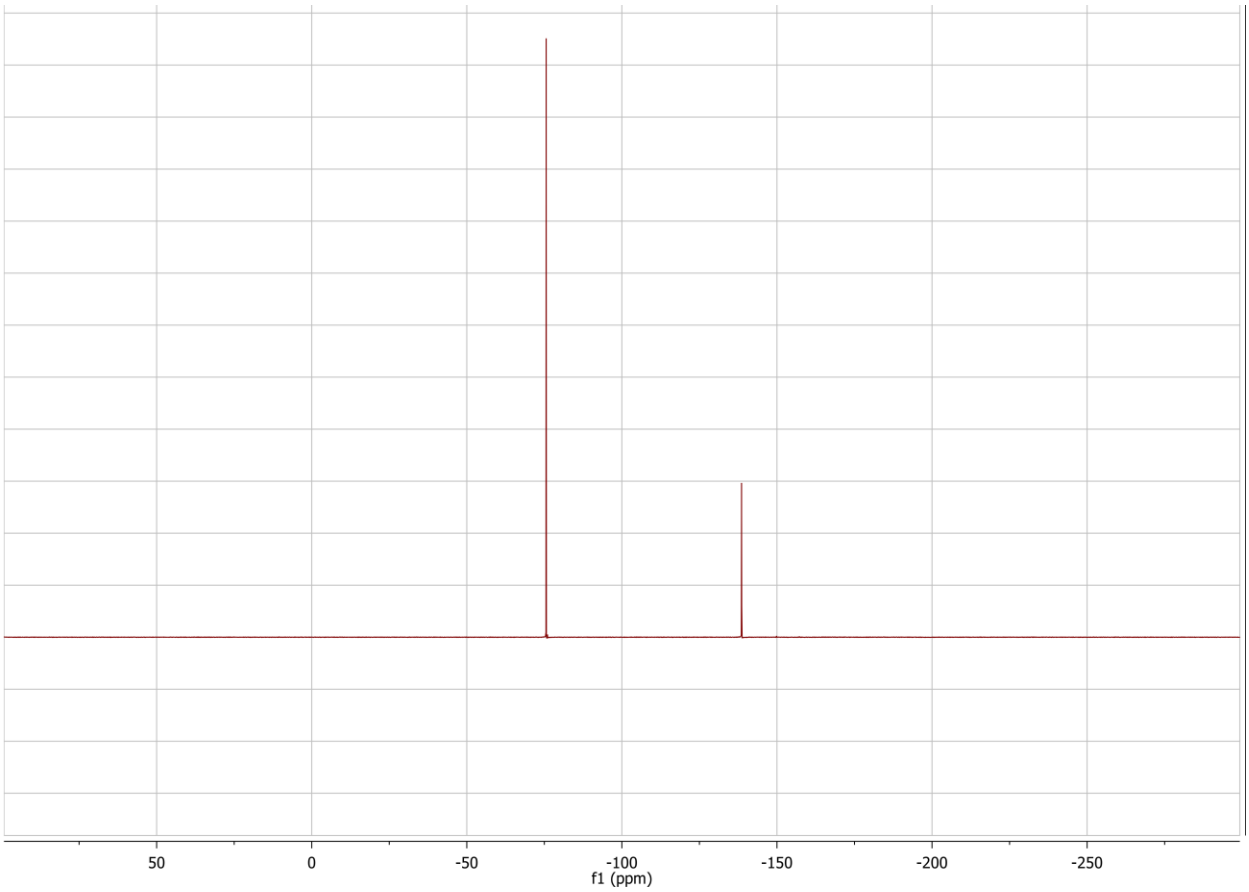
Supplemental Figure 8. ^{19}F NMR spectrum (470 MHz) of 5-FαMHL recorded in D_2O



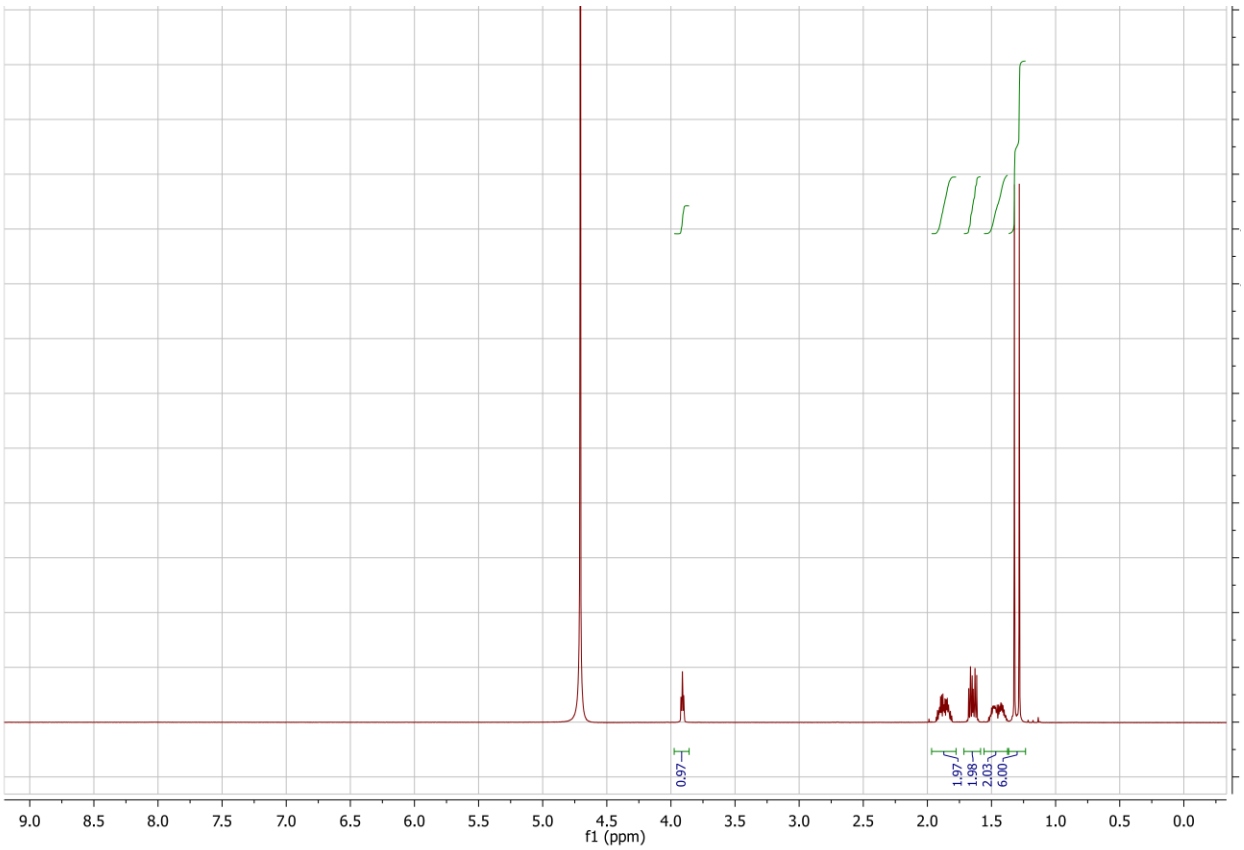
Supplemental Figure 9. ^{1}H NMR spectrum (600 MHz) of 5-FαML recorded in D_2O



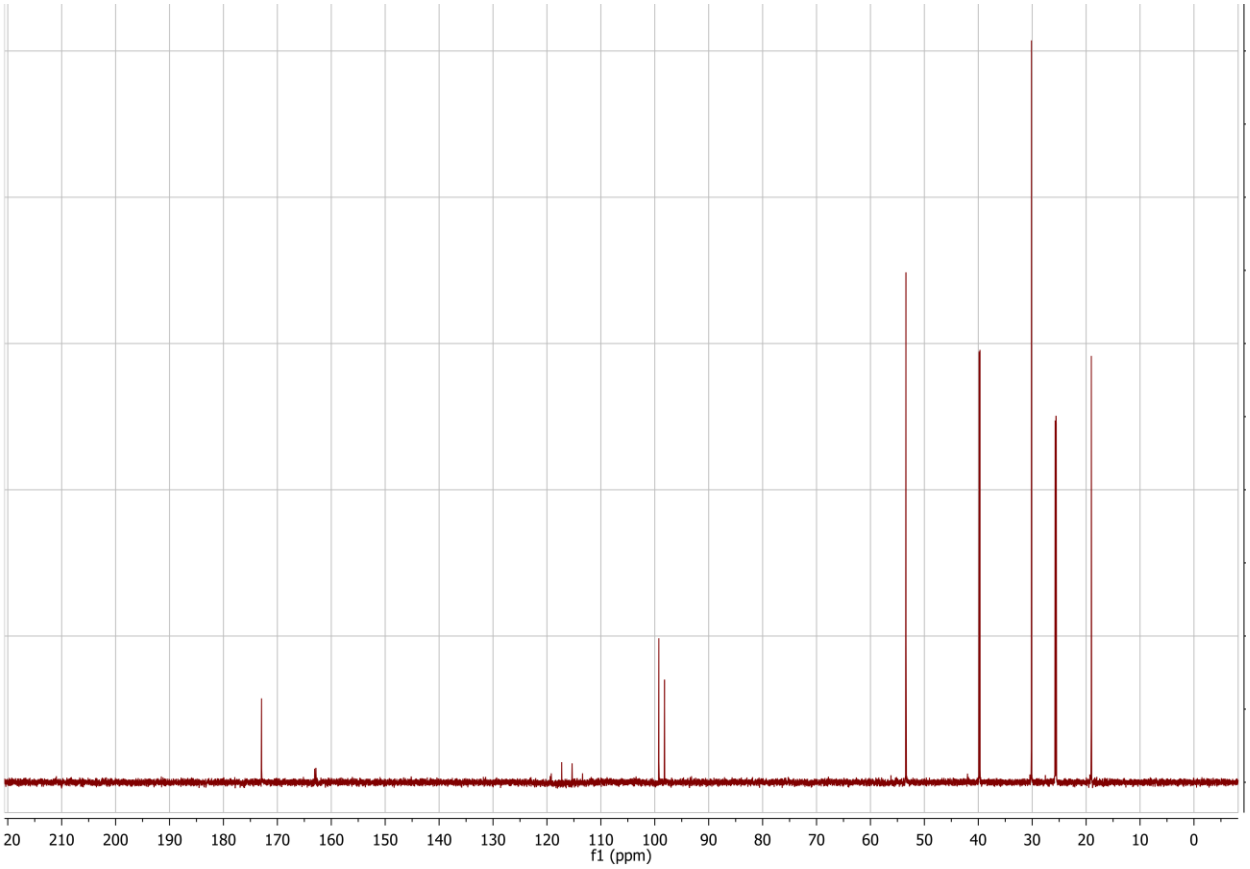
Supplemental Figure 10. ^{13}C NMR spectrum (150 MHz) of 5-FαML recorded in D_2O



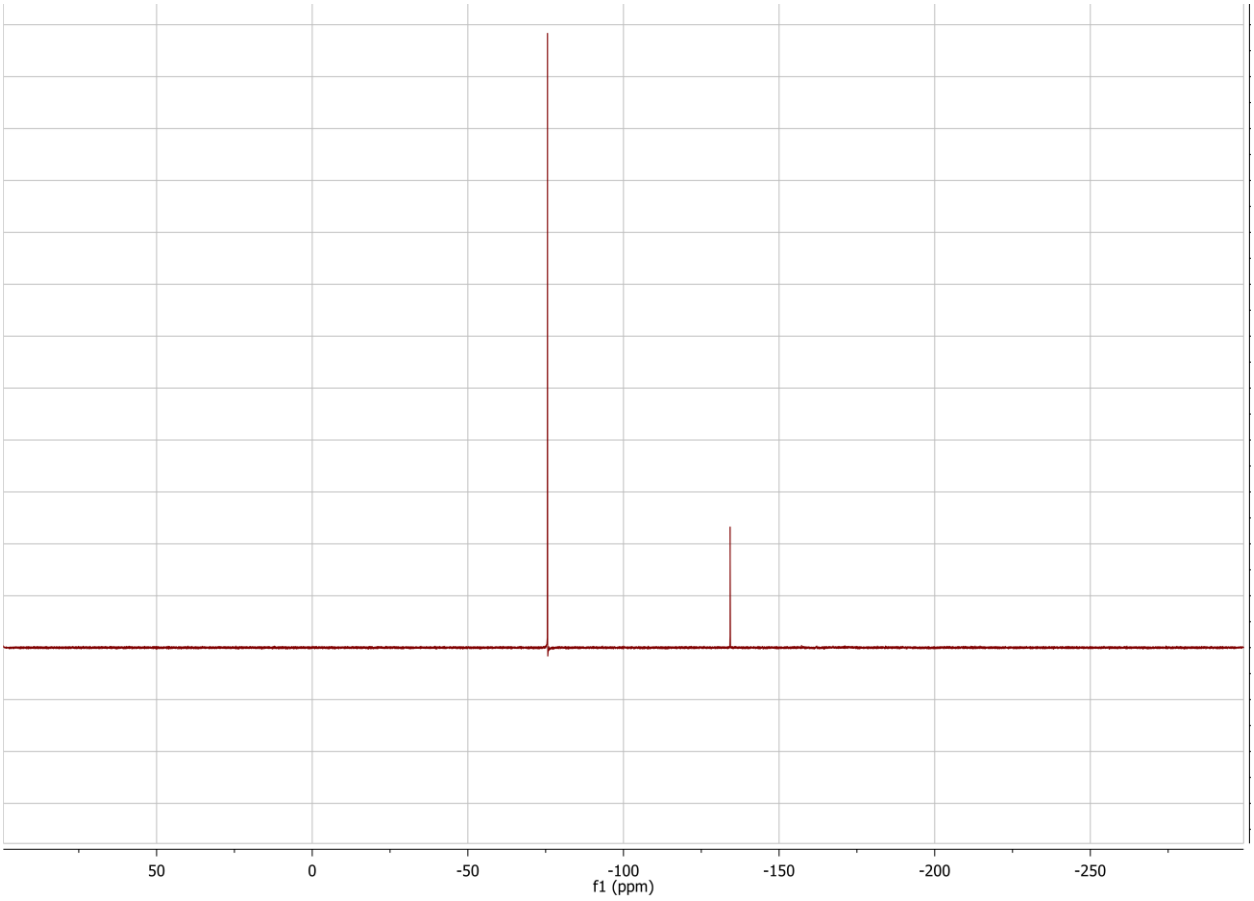
Supplemental Figure 11. ^{19}F NMR spectrum (470 MHz) of 5-FαML recorded in D_2O



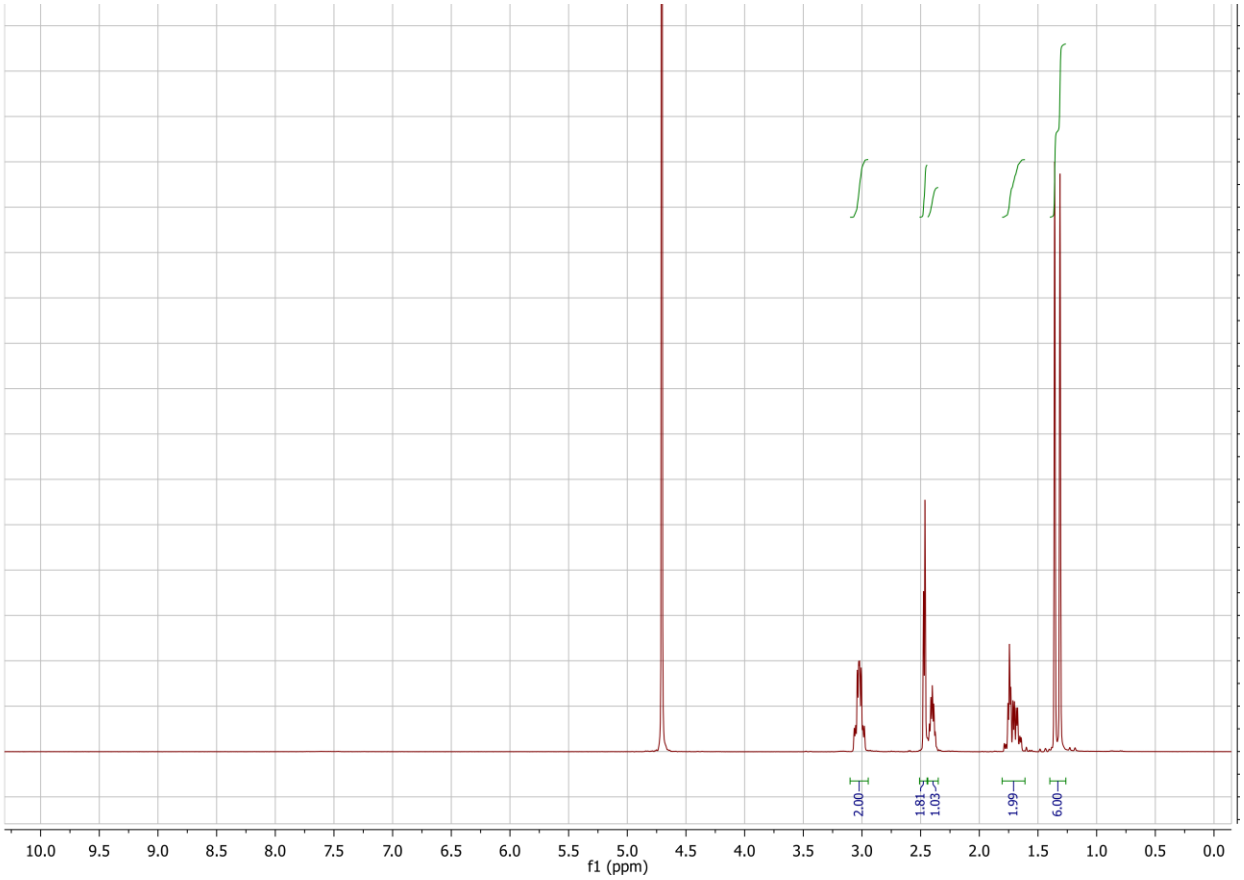
Supplemental Figure 12. ^{1}H NMR spectrum (600 MHz) of 6-FHHL recorded in D_2O



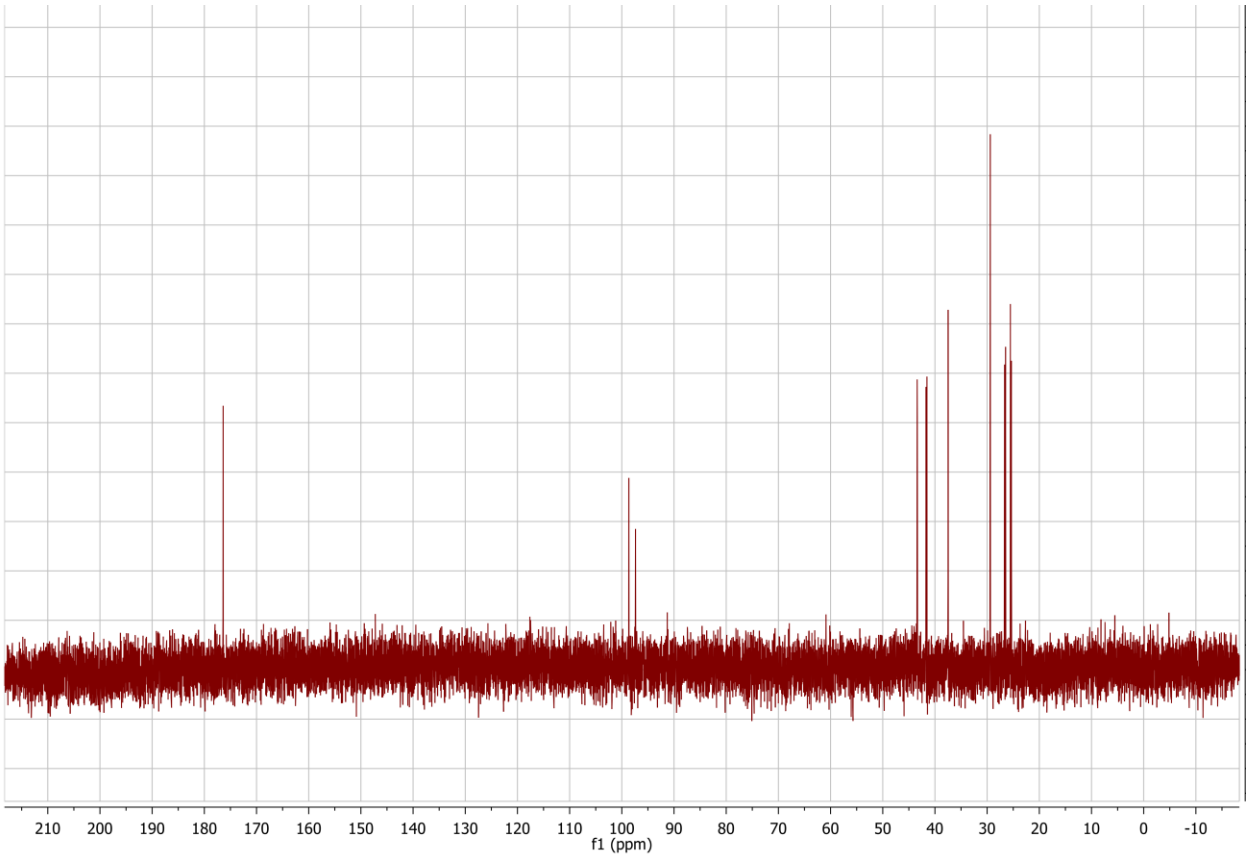
Supplemental Figure 13. ^{13}C NMR spectrum (150 MHz) of 6-FHHL recorded in D_2O



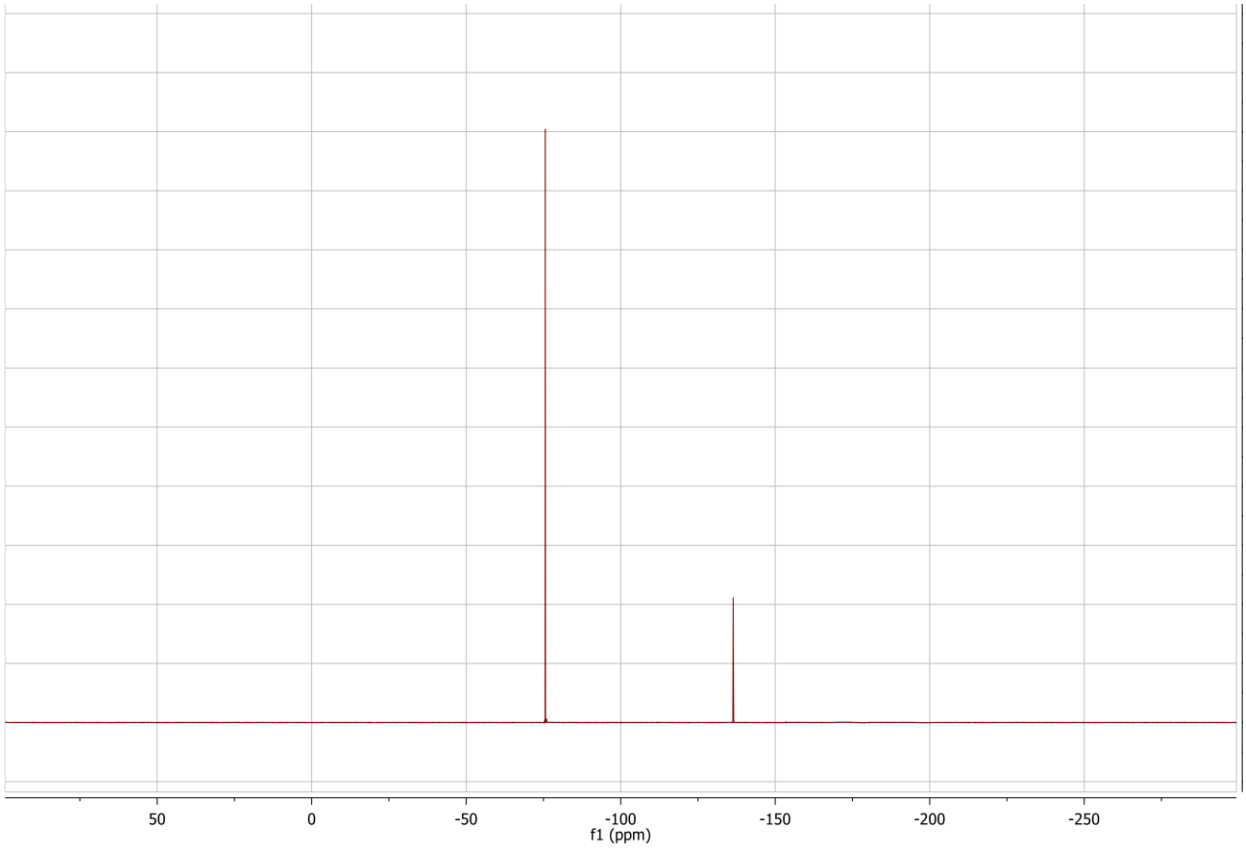
Supplemental Figure 14. ^{19}F NMR spectrum (470 MHz) of 6-FHHL recorded in D_2O



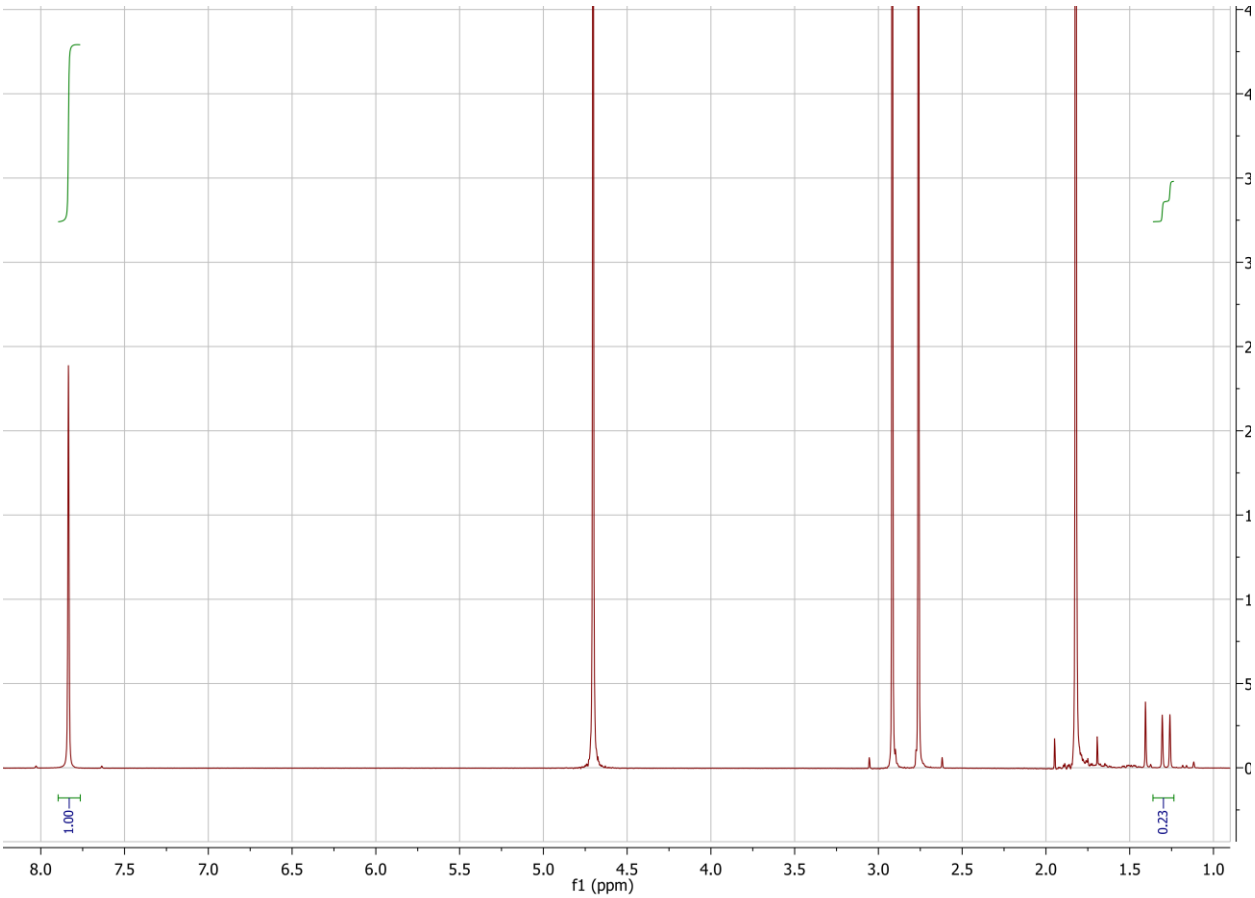
Supplemental Figure 15. ^{1}H NMR spectrum (600 MHz) of 5-FPregab recorded in D_2O



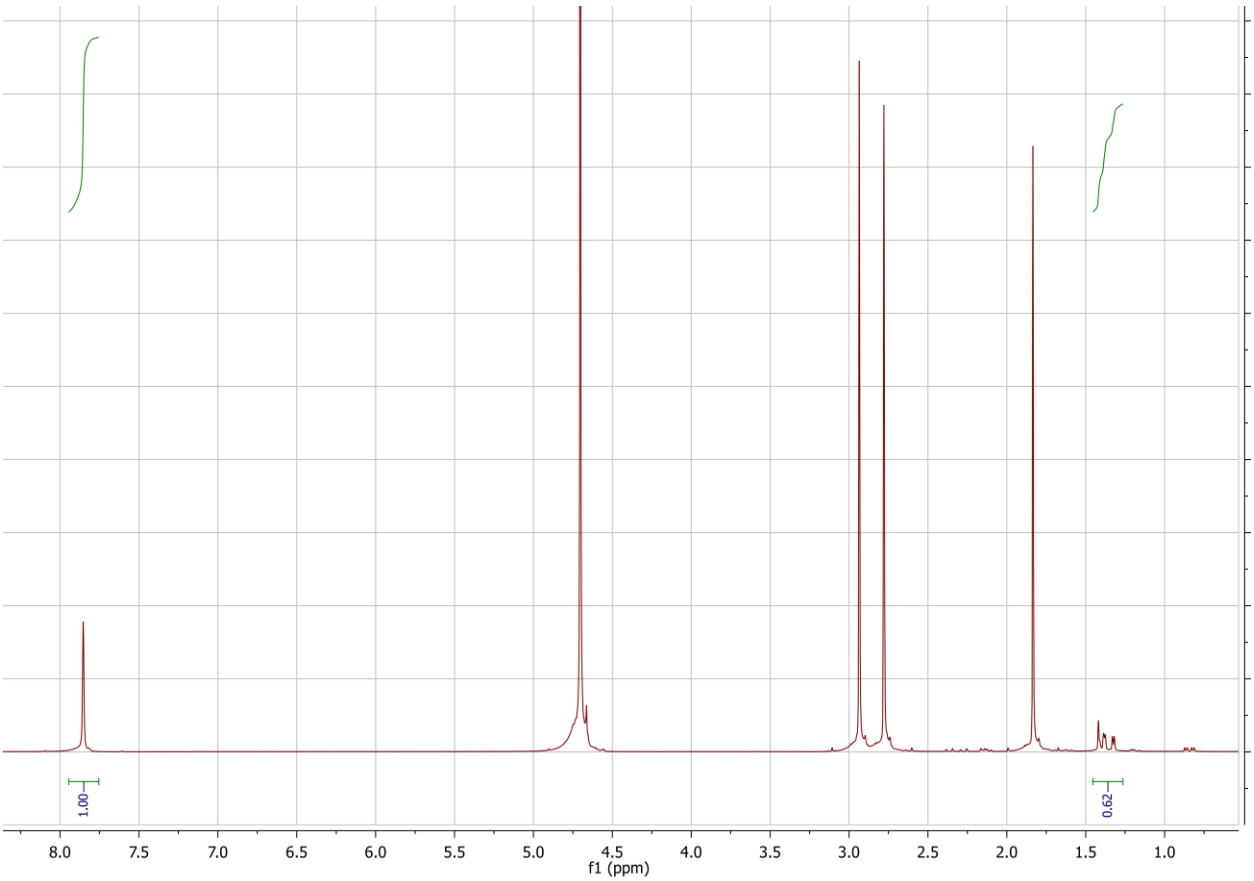
Supplemental Figure 16. ^{13}C NMR spectrum (150 MHz) of 5-FPregab recorded in D_2O



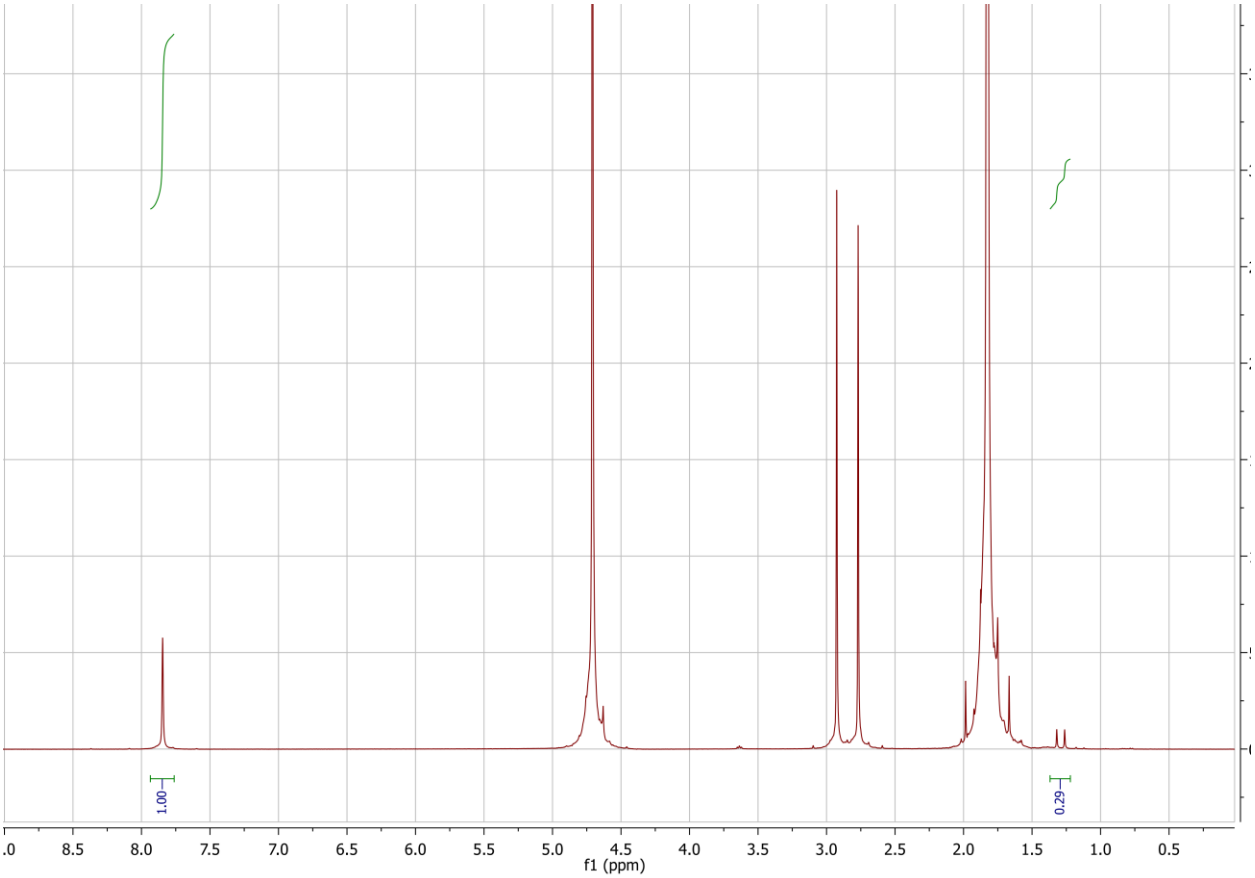
Supplemental Figure 17. ^{19}F NMR spectrum (470 MHz) of 5-FPregab recorded in D_2O



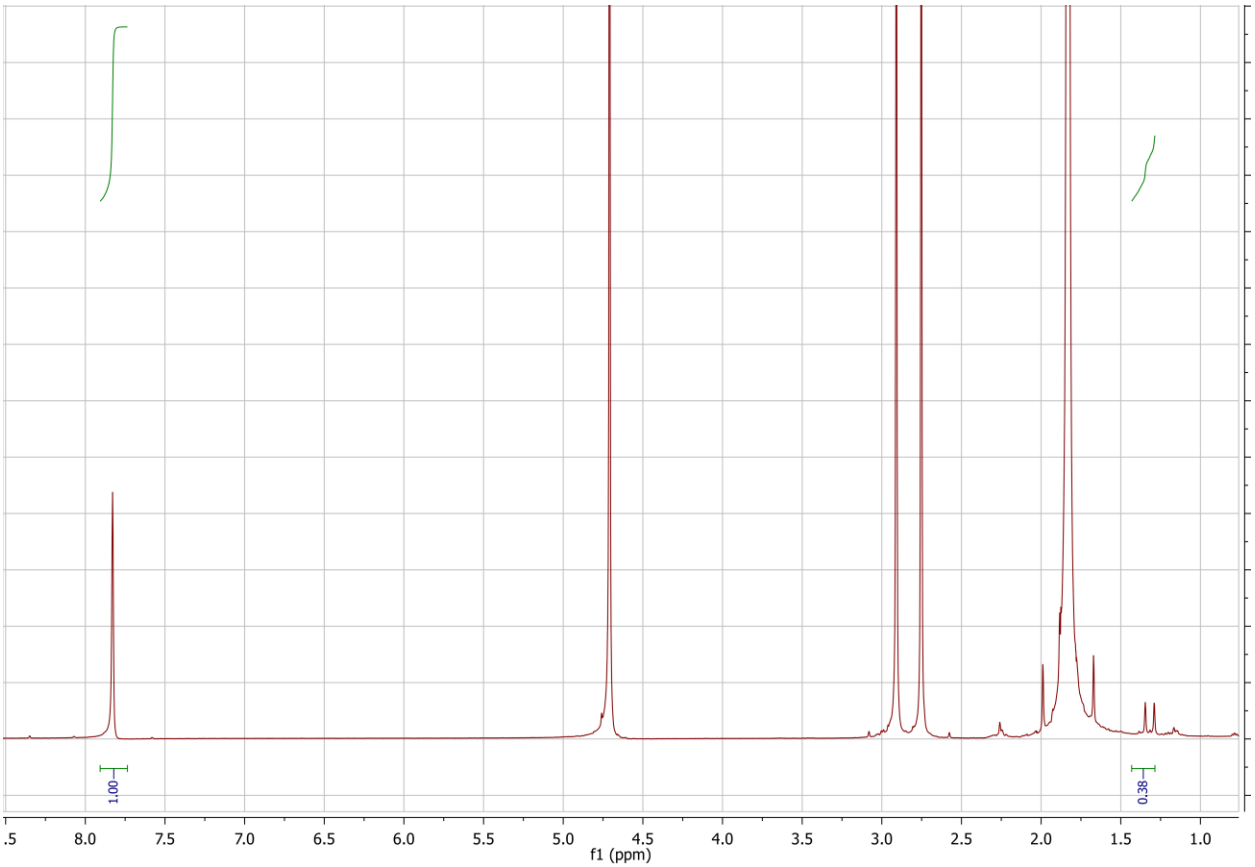
Supplemental Figure 18. Typical ^{1}H NMR spectrum (500 MHz, D_2O, 5 μL DMF as internal standard) of purified (*S*)-5-[^{18}F]FαMHL after ~100 h decay at –20 °C



Supplemental Figure 19. Typical ^{1}H NMR spectrum (500 MHz, D_2O, 5 μL DMF as internal standard) of purified (*S*)-4-[^{18}F]FαML after ~100 h decay at –20 °C



Supplemental Figure 20. Typical ^{1}H NMR spectrum (500 MHz, D_2O, 5 μL DMF as internal standard) of purified (*S*)-6-[^{18}F]FHHL after ~100 h decay at –20 °C



Supplemental Figure 21. Typical ^{1}H NMR spectrum (500 MHz, D_2O, 5 μL DMF as internal standard) of purified 5-[^{18}F]FPregab after ~100 h decay at –20 °C

Supplemental references

1. Chenault H, Dahmer J, Whitesides G. Kinetic Resolution of Unnatural and Rarely Occurring Amino-Acids - Enantioselective Hydrolysis of N-acyl Amino-Acids Catalyzed by acylase-I. *Journal of the American Chemical Society.* 1989;111:6354-6364.

2. Nodwell MB, Yang H, Čolović M, et al. (18)F-Fluorination of Unactivated C-H Bonds in Branched Aliphatic Amino Acids: Direct Synthesis of Oncological Positron Emission Tomography Imaging Agents. *J Am Chem Soc.* 2017;139:3595-3598.