

Supplementary Materials

Synthesis and Evaluation of ^{11}C -Labeled Triazolones as Probes for Imaging Fatty Acid Synthase Expression by Positron Emission Tomography

James M. Kelly*, Thomas M. Jeitner, Nicole Waterhouse, Wenchao Qu, Ethan J. Linstad, Banafshe Samani, Clarence Williams, Jr., Anastasia Nikolopoulou, Alejandro Amor-Coarasa, Stephen G. DiMagno, John W. Babich

* Corresponding author:

James M. Kelly, Ph.D.
Department of Radiology
Weill Cornell Medicine
Belfer Research Building
Room 1604
413 E 69th St
New York, NY 10021
jak2046@med.cornell.edu

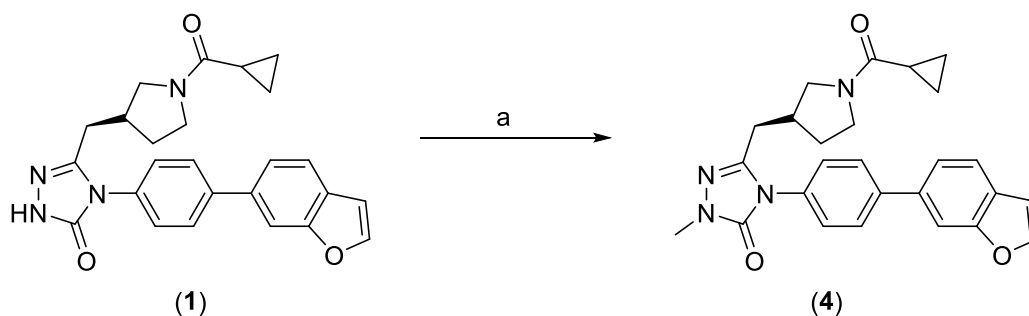
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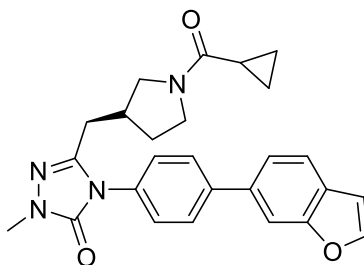
1. Synthesis of Precursor Compounds and Non-Radioactive Standards

General methods: All materials were obtained from commercial sources and used as received unless otherwise noted. Acetonitrile and d_3 -acetonitrile (Cambridge Isotope Laboratories) were stirred over P_2O_5 (12 h), distilled into flame-dried storage tubes, and transferred into an inert atmosphere glove box. All glassware and NMR tubes were oven dried (140 °C) for 24 h before they were transferred into the glove box for use. Glove box manipulations were performed under nitrogen in an MBraun Labmaster 130, equipped with a recirculating purifier, which removed oxygen and water. All NMR experiments reported were performed using a Bruker Avance 300 MHz, 400 MHz, 500 MHz, 600 MHz, or 700 MHz NMR spectrometers in the NMR laboratory at the University of Nebraska, the University of Illinois at Chicago, or Weill Cornell Medicine, and calibrated using residual protonated solvent. Yields from NMR scale reactions were determined by using the residual solvent peak as an internal standard. All J coupling values are given in Hz. Masses were determined by high resolution LC-MS or HR-MS using electrospray ionization (ESI). LC-MS mass determinations were performed on a Waters ACQUITY UPLC[®] coupled to a Waters ZSpray[™] ionizer and a Waters SQ Detector 2. The column used for the chromatography component was a Phenomenex Kinetex C18, 50 x 2.1 mm, 1.7 μ m and the mobile phase was a gradient of 5-95% MeCN + 0.1% TFA in H_2O + 0.1% TFA over 5 min. HR-MS mass determinations were performed on a Shimadzu LCMS IT-TOF[™]. The chromatography unit was bypassed for mass measurements. The optical rotation was determined using a JASCO P-1020 polarimeter. The samples were dissolved in CH_2Cl_2 to a concentration of 0.3% w/v and analyses were performed at 589 nm and 20 °C. Optical rotations are reported as the mean of 10 replicates \pm standard deviation.

Synthesis of 4:



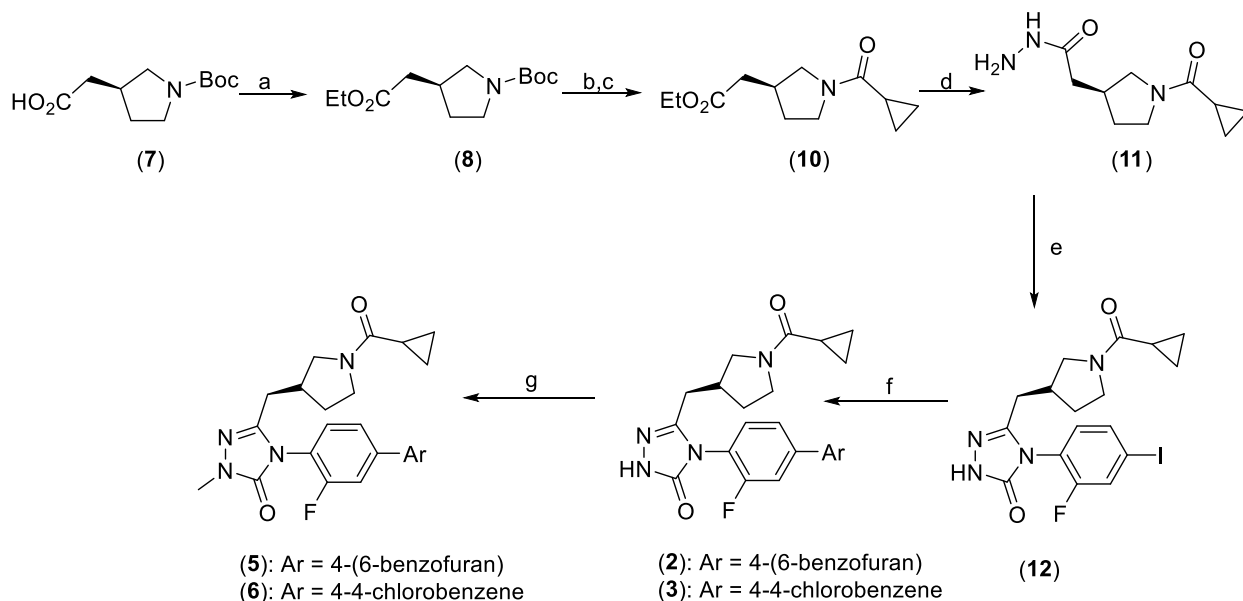
Scheme S1. Synthesis of **4** from GSK2194069. a. K_2CO_3 , CH_3I , DMF.



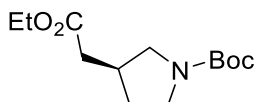
(S)-4-(4-(Benzofuran-6-yl)phenyl)-5-((1-(cyclopropanecarbonyl)pyrrolidin-3-yl)methyl)-2-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (4): To a solution of 3 mg (7 μ mol) GSK2194069 in 0.5 mL DMF was added 6.4 mg (46 μ mol) K_2CO_3 and 10 μ L (160 μ mol) methyl iodide. The reaction was stirred at room temperature for 24 h. The reaction was quenched with 2 mL 58% v/v 0.3 M $NH_4COOH/MeCN$, and the reaction was

purified in two fractions by semi-prep HPLC using a Phenomenex Luna C18(2) column, 250x10 mm and monitoring UV absorption at 254 nm. The mobile phase was 58% v/v 0.3 M NH₄COOH/MeCN, and the flow rate was 5 mL/min. Under these conditions, the product eluted with a retention time of 11.2 min. The fractions containing the product was collected, combined, and diluted with 200 mL H₂O. The diluted sample was passed through a pre-conditioned Sep-Pak C18 Plus Light cartridge (Waters). The cartridge was washed with 20 mL H₂O and eluted with 1 mL EtOH and 1 mL MeCN. The organic solvent was removed by heating to 75 °C under a stream of N₂ to give **4** as a white powder (2.8 mg, 90%). ¹H NMR (CDCl₃, 500 MHz, 25 °C): δ 7.83 (d, *J* = 1.5, 1H), 7.76 (d, *J* = 8.5, 2H), 7.71 (d, *J* = 2.5, 1H), 7.61 (d, *J* = 8.5, 1H), 7.55 (dd, *J* = 8.5, 2.0, 1H), 7.38 (d, *J* = 8.0, 2H), 6.86 (d, *J* = 2.0, 0.5, 1H), 3.85 (m, 1H), 3.70 (m, 1H), 3.53 (m, 1H), 3.18 (m, 1H), 3.55 (s, 3H), 2.64-2.42 (br m, 2.5H), 2.26-2.15 (br m, 1H), 2.10-2.02 (m, 0.5H), 1.77-1.68 (m, 0.5H), 1.61-1.56 (m, 1.5H), 1.02-0.96 (m, 2H), 0.79-0.74 (m, 2H). ¹³C NMR (CDCl₃, 150 MHz, 25 °C): δ 172.3, 154.8, 153.5, 145.9, 142.8, 137.2, 135.1, 131.5, 128.9, 128.8, 128.1, 127.4, 127.3, 123.9, 119.9, 111.8, 106.8, 51.8, 45.3, 42.8, 32.5, 29.7, 22.8, 12.2, 7.6, 7.5. LCMS calc. for C₂₆H₂₆N₄O₃ [M+H]⁺: 443.20. Found: 443.24. [α]_D²⁰ = -8.04 ± 0.59° (CH₂Cl₂).

Synthesis of **5** and **6**:

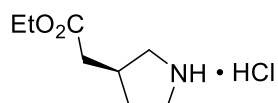


Scheme S2. Synthesis of non-radioactive standards **5** and **6** and their precursors **2** and **3**. a. EDC•HCl, DMAP, EtOH, CH₂Cl₂; b. HCl/dioxane; c. C₃H₅COCl, DIPEA, CH₂Cl₂; d. NH₂NH₂•H₂O, EtOH; e. 2-fluoro-4-iodoaniline, NEt₃, triphosgene, THF; f. ArB(OH)₂, Na₂CO₃, dioxane/H₂O, PdCl₂(dppf), *n*-BuLi, THF; .MeI, K₂CO₃, DMF.

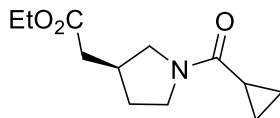


tert-Butyl (S)-3-(2-ethoxy-2-oxoethyl)pyrrolidine-1-carboxylate (8): (S)-2-(1-(tert-butoxycarbonyl)pyrrolidin-3-yl)acetic acid (**7**) (8 g, 35 mmol) was dissolved in dichloromethane (70 mL). Then EDC•HCl (7.4 g, 38.6 mmol), DMAP (0.4 g, 3.3 mmol), and EtOH (4.5 mL, 77 mmol) were added successively. The reaction was stirred for 16 h at room temperature. Then it was diluted with dichloromethane (70 mL), washed with

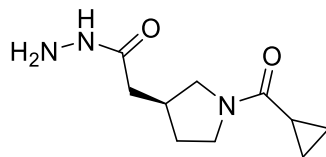
1 M HCl (10 mL) and saturated aqueous sodium carbonate (10 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure to give **8** as a pale yellow oil (9 g, 99%), which was used without further purification. ^1H NMR (CDCl_3 , 400 MHz, 25 $^\circ\text{C}$): δ 4.14 (br q, J = 7.1, 2H), 3.62-3.55 (m, 1H), 3.49-3.39 (m, 1H), 3.33-3.23 (m, 1H), 2.99-2.89 (m, 1H), 2.55 (sept J = 7.4, 1H), 2.42-2.31 (m, 2H), 2.06 (br s, 1H), 1.58-1.50 (m, 1H), 1.45 (s, 9H), 1.25 (t, J = 7.1, 3H)



Ethyl (S)-2-(pyrrolidin-3-yl)acetate hydrochloride (9): In a round bottom flask, pyrrolidine **8** (9 g, 35 mmol) was dissolved in 4 M HCl in dioxane (46 mL, 184 mmol), with evolution of gas, and stirred for 5 h at room temperature. The solvent was removed under high vacuum to give **9** as an off white solid (6.7 g, 99%) which was used without further purification. ^1H NMR (CDCl_3 , 400 MHz, 25 $^\circ\text{C}$): δ 9.80 (br s, 2H), 4.13 (q, J = 7.1, 2H), 3.62-3.55 (m, 1H), 3.49-3.39 (m, 1H), 3.33-3.23 (m, 1H), 3.02-2.93 (m, 1H), 2.72 (sept, J = 7.7, 1H), 2.50 (d, J = 7.2, 2H), 2.30-2.24 (m, 1H), 1.76-1.68 (m, 1H), 1.25 (t, J = 7.1, 3H).

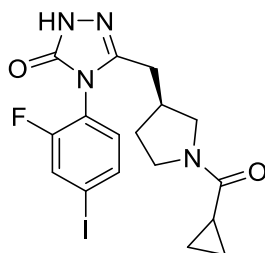


Ethyl (S)-2-(1-(cyclopropanecarbonyl)pyrrolidin-3-yl)acetate (10): Pyrrolidine **9** (6.5 g, 33.6 mmol) was dissolved in dichloromethane (65 mL) under nitrogen with stirring. DIPEA (11 mL, 66 mmol) was added slowly and the solution cooled in an ice bath. Cyclopropylcarbonyl chloride (3.7 mL, 40.1 mmol) was added slowly and the reaction allowed to warm to room temperature with stirring for 2 h. Then the reaction was poured into H_2O (50 mL), the organic layer separated, and the aqueous phase extracted with DCM (10 mL x 3). The organic layers were combined and washed with sat. NaHCO_3 (10 mL), dried over sodium sulfate and concentrated under reduced pressure. The resulting oil was placed under high vacuum for 1 d to yield **10** as an amber oil (7.46 g, 98%), which was used without further purification. ^1H NMR (CDCl_3 , 400 MHz, 25 $^\circ\text{C}$): δ 4.15 (q, J = 7.1, 1H), 4.13 (q, J = 7.1, 1H), 3.95-3.90 (m, 0.5H), 3.79-3.72 (m, 1H), 3.67-3.57 (m, 1H), 3.41-3.34 (m, 0.5H), 3.27-3.22 (m, 0.5H), 3.08-3.03 (m, 0.5H), 2.68 (sept, J = 7.6, 0.5H), 2.58 (sept, J = 7.6, 0.5H), 2.51-2.33 (m, 2H), 2.25-2.18 (m, 0.5H), 2.12-2.05 (m, 0.5H), 1.74-1.65 (m, 0.5H), 1.62-1.54 (m, 1.5H), 1.27 (t, J = 6.8, 1.5H), 1.25 (t, J = 6.8, 1.5H), 1.02-0.93 (m, 2H), 0.78-0.69 (m, 2H).

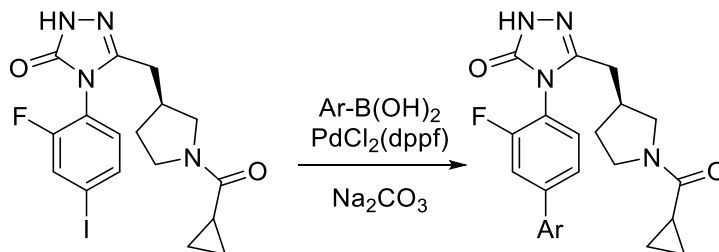


(S)-2-(1-(Cyclopropanecarbonyl)pyrrolidin-3-yl)acetohydrazide (11): Ester **10** (6.25 g, 27.7 mmol) and hydrazine hydrate (36 mL, 550 mmol) were dissolved in EtOH (60 mL) and refluxed for 16 h. The solvent was removed, H_2O azeotropically removed with isopropanol, and the resulting oil placed under high vacuum for 3 d to give **11** as a sticky, light amber oil (5.5 g, 94%). The compound was stored in the dark until further use. ^1H NMR (CDCl_3 , 400 MHz, 25 $^\circ\text{C}$): δ 6.80 (br s, 1H), 3.94-3.85 (br m, 2.5H), 3.78-3.71 (m, 1H), 3.66-3.58 (m, 1H), 3.44-3.36 (m, 0.5H), 3.31-3.27 (m, 0.5H), 3.09-3.04 (m, 0.5H), 2.74 (sept, J = 7.3, 0.5H), 2.64 (sept, J = 7.5, 0.5H), 2.31-2.20 (m, 2H), 2.12-2.05 (m, 0.5H), 1.77-1.67 (m, 0.5H), 1.66-1.52 (m,

2H), 1.03-0.92 (m, 2H), 0.80-0.71 (m, 2H). ^{13}C NMR (CDCl_3 , 100 MHz, 25 °C): δ 172.3, 171.9, 152.1, 51.8, 51.0, 46.0, 45.2, 39.2, 37.3, 36.0, 35.2, 34.4, 31.7, 30.2, 27.5, 16.5, 12.5, 12.2, 7.6, 7.5, 7.4, 7.3. HRMS (ESI) calculated for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_2$: 211.1321. Found: 211.1297. Δ_m = 11.4 ppm.

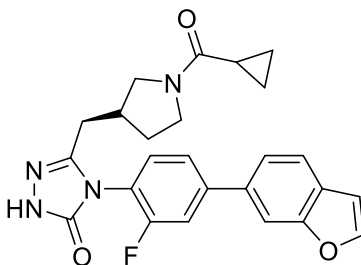


(S)-5-((1-(Cyclopropanecarbonyl)pyrrolidin-3-yl)methyl)-4-(2-fluoro-4-iodophenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (12): To a flame-dried Schlenk tube (solution A) was added a solution of 2-fluoro-4-iodoaniline (4.3 g, 18 mmol) in dry THF (90 mL). Dry triethylamine (20 mL, 144 mmol) was added, and the resulting mixture was stirred. To a separate flame-dried Schlenk flask (solution B), was added a stirred solution of triphosgene (5.4 g, 18 mmol) in dry THF (90 mL). Solution A was slowly added to solution B via cannula, and the resulting slurry was stirred for 16 h at room temperature. The reaction solution was filtered through an air-free filter funnel with a fine glass frit into a fresh, flame-dried Schlenk flask and the solvent removed under vacuum to give a red oil. Dry dichloromethane (60 mL) was added and the mixture was stirred. In a nitrogen atmosphere glove box, hydrazide **11** (1.9 g, 3.8 mmol) was dissolved in dry dichloromethane (25 mL) and added to the stirred reaction solution. The reaction was stirred for 16 h at room temperature, at which point a large amount of solid material (product) had precipitated. The product semi-carbazine was recovered by filtering the reaction solution through a 0.45 μm PTFE membrane filter to give a light amber solid (4.2 g, 94%). Then the semi-carbazine (1 g, 2.1 mmol) was suspended in 10% isopropanol/water (70 mL), K_2CO_3 (1.5 g, 10.5 mmol) added, and the mixture refluxed for 16 h. The solution was acidified to pH 5-5.5, extracted with ethyl acetate (10 mL x 5), dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (100% EtOAc \rightarrow 5% MeOH/EtOAc) to give **12** as a colorless solid (0.55 g, 57%). [Rotamers present in NMR spectra] ^1H NMR (CDCl_3 , 400 MHz, 25 °C): δ 10.45 (br s, 0.5H), 10.40 (br s, 0.5H), 7.68-7.64 (m, 2H), 7.09 (dd, J = 8.2, 7.9, 1H), 3.93-3.89 (m, 0.5H), 3.76-3.68 (m, 1H), 3.64-3.55 (m, 1H), 3.41-3.34 (m, 0.5H), 3.26-3.22 (m, 0.5H), 3.07-3.02 (m, 0.5H), 2.68-2.59 (m, 0.5H), 2.55-2.40 (m, 2.5H), 2.22-2.19 (m, 0.5H), 2.09-2.01 (m, 0.5H), 1.73-1.63 (m, 0.5H), 1.59-1.49 (m, 1.5H), 1.05-0.92 (m, 2H), 0.80-0.69 (m, 2H). ^{13}C NMR (CDCl_3 , 100 MHz, 25 °C): δ 172.42, 172.37, 158.5, 155.9, 154.75, 154.69, 146.0, 135.0, 131.2, 131.1, 126.9, 126.8, 126.7, 126.6, 120.3, 120.20, 120.18, 120.1, 95.4, 95.33, 95.29, 95.2, 77.4, 51.8, 51.1, 45.9, 45.3, 35.9, 34.2, 31.6, 30.4, 29.4, 29.3, 12.6, 12.4, 7.79, 7.73, 7.6. ^{19}F NMR (CDCl_3 , 376 MHz, 25 °C): δ -117.7 (br s, 1F). HRMS (ESI) calculated for $\text{C}_{17}\text{H}_{18}\text{FIN}_4\text{O}_2$: 456.0458. Found: 456.0457. Δ_m = 0.2 ppm.

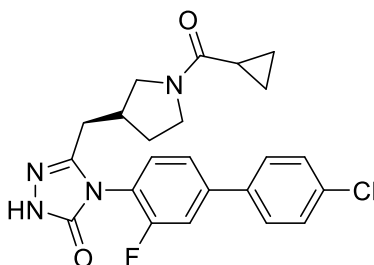


General cross-coupling procedure:

Triazolone **12** (100 mg, 0.22 mmol), arylboronic acid (0.26 mmol) and Na₂CO₃ (91 mg, 0.66 mmol) were weighed out in a nitrogen atmosphere glove box, transferred to a Schlenk tube, removed from the box and placed under nitrogen using a Schlenk line. Degassed 3:1 dioxane:water (1.5 mL) was added with stirring. In a nitrogen glove box, PdCl₂(dppf) (1 mg, cat.) was suspended in THF (0.5 mL), activated with a drop of *n*-BuLi (leading to a deep red solution), and added to the reaction mixture. The reaction vessels were sealed with a greased glass stopper and heated to 100 °C for 16 h. Then the reaction was acidified to pH 5-5.5 and extracted with ethyl acetate (2 mL x 4). The extracts were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude products were purified by column chromatography (typically 50% EtOAc/hexane → 10% MeOH/EtOAc) to give **2** and **3** as amber oils.



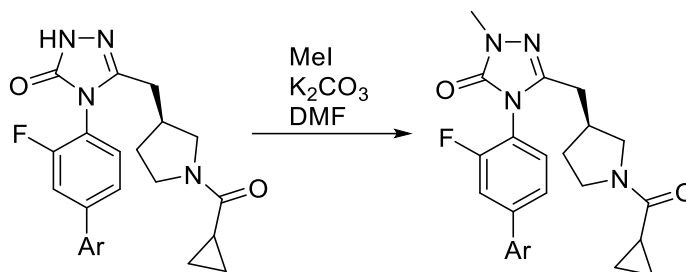
(R)-4-(4-(Benzofuran-6-yl)-2-fluorophenyl)-5-((1-(cyclopropanecarbonyl)pyrrolidin-3-yl)methyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (2): (95 mg, 97%). ¹H NMR (CDCl₃, 400 MHz, 25 °C): δ 10.24 (br s, 0.5H), 10.12 (br s, 0.5H), 7.80 (br s, 1H), 7.69 (br s, 1H), 7.60 (dd, J = 8.5, 2.8, 1H), 7.56-7.50 (m, 3H), 7.42 (dt, J = 11.7, 2.0, 1H), 6.84 (br s, 1H), 3.96-3.92 (m, 0.5H), 3.78-3.69 (m, 1H), 3.66-3.58 (m, 1H), 3.42-3.35 (m, 0.5H), 3.30-3.26 (m, 0.5H), 3.10-3.06 (m, 0.5H), 2.74-2.64 (m, 0.5H), 2.64-2.42 (br m, 2.5H), 2.26-2.15 (br m, 0.5H), 2.10-2.02 (m, 0.5H), 1.77-1.68 (m, 0.5H), 1.62-1.54 (m, 1.5H), 1.02-0.93 (m, 2H), 0.78-0.69 (m, 2H). ¹³C NMR (CDCl₃, 100 MHz, 25 °C): δ 172.4, 159.1, 156.6, 155.3, 155.2, 155.1, 146.6, 146.3, 146.2, 146.0, 145.93, 145.90, 145.8, 134.02, 133.95, 130.1, 130.0, 128.39, 128.37, 124.4, 123.9, 120.1, 118.6, 118.52, 118.48, 118.39, 116.1, 115.9, 112.13, 112.09, 106.9, 51.8, 51.2, 51.0, 45.9, 45.3, 36.0, 34.3, 31.6, 30.5, 29.5, 29.3, 12.6, 12.4, 7.8, 7.7, 7.6. ¹⁹F NMR (CDCl₃, 376 MHz, 25 °C): δ -120.2 (br s, 1F). HRMS (ESI) calculated for C₂₅H₂₃FN₄O₃: 446.1754. Found: 446.1685. Δ_m = 15.4 ppm. [α]_D²⁰ = -8.35 ± 0.30° (CH₂Cl₂).



(R)-4-(4'-Chloro-3-fluoro-[1,1'-biphenyl]-4-yl)-5-((1-(cyclopropanecarbonyl)pyrrolidin-3-yl)methyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (3): (95 mg, 98%). ¹H NMR (CDCl₃, 400 MHz, 25 °C): δ 10.94 (br s, 1H), 7.50 (dd, J = 8.5, 1.6, 2H), 7.47-7.39 (m, 5H), 3.93-3.89 (m, 0.5H), 3.75-3.67 (m, 1H), 3.63-3.53 (m, 1H), 3.40-3.33 (m, 0.5H), 3.27-3.23 (m, 0.5H), 3.07-3.02 (m, 0.5H), 2.70-2.63 (m, 0.5H), 2.63-2.40 (m, 2.5H), 2.23-2.14 (m, 0.5H), 2.08-2.00 (m, 0.5H), 1.73-1.64 (m, 0.5H), 1.59-1.49 (m, 1.5H), 1.01-0.91 (m, 2H), 0.78-0.69 (m, 2H). ¹³C NMR (CDCl₃, 100 MHz, 25 °C): δ 172.5, 159.1, 156.5, 155.15, 155.09, 146.2, 144.1, 144.0, 143.9, 137.2, 137.1, 135.0, 134.9, 130.3, 130.2, 129.41, 129.38, 128.5, 123.9, 119.31, 119.23, 119.18, 119.10, 115.7, 115.5, 51.8, 51.2, 45.9, 45.3, 35.9, 34.3, 31.5, 30.4, 29.4, 29.2, 12.6, 12.3, 7.74, 7.67, 7.57.

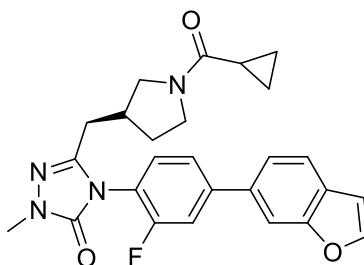
^{19}F NMR (CDCl_3 , 376 MHz, 25 °C): δ -119.7 (br s, 1F). HRMS (ESI) calculated for $\text{C}_{23}\text{H}_{22}\text{ClFN}_4\text{O}_2$: 440.1415. Found: 440.1339. $\Delta_m = 17.3$ ppm. $[\alpha]^{20}_{\text{D}} = -9.37 \pm 0.34^\circ$ (CH_2Cl_2).

Synthesis of 5 and 6:

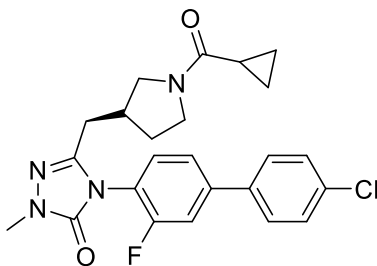


General triazolone methylation procedure:

Triazolone **2** or **3** (0.034 mmol) and K_2CO_3 (0.07 mmol) were combined in a storage tube with dry DMF (0.5 mL). Methyl iodide (3 μL , 0.048 mmol) was added, the tube was sealed, and the reaction was heated to 80 °C for 16 h. Then the reaction was poured into H_2O (5 mL) and extracted with ethyl acetate (2 mL x 3). The organic extracts were combined, washed successively with H_2O (2 mL) and brine (2 mL), dried over sodium sulfate, and concentrated under reduced pressure to afford *N*-methyltriazolones **5** and **6**.



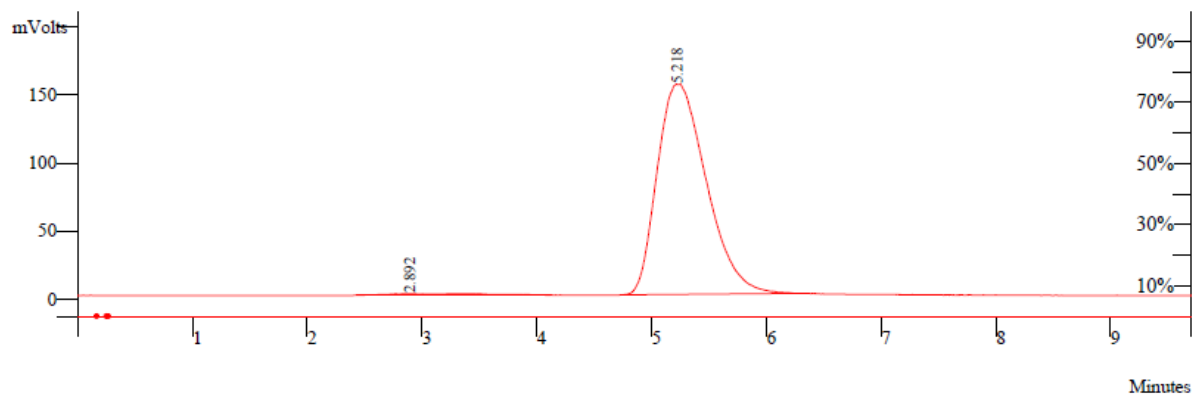
(R)-4-(4-(Benzofuran-6-yl)-2-fluorophenyl)-5-((1-(cyclopropanecarbonyl)pyrrolidin-3-yl)methyl)-2-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (5**):** (12 mg, 78%). ^1H NMR (CDCl_3 , 400 MHz, 25 °C): δ 7.80 (s, 1H), 7.69 (s, 1H), 7.59 (d, $J = 8.4$, 1H), 7.54-7.48 (m, 3H), 7.39 (dd, $J = 8.7$, 7.7, 1H), 6.84 (s, 1H), 3.94-3.90 (m, 0.5H), 3.79-3.69 (m, 1H), 3.65-3.56 (m, 1H), 3.52 (d, $J = 6.9$, 3H), 3.42-3.35 (m, 0.5H), 3.28-3.24 (m, 0.5H), 3.08-3.04 (m, 0.5H), 2.72-2.63 (m, 0.5H), 2.63-2.45 (br m, 2.5H), 2.26-2.15 (br m, 0.5H), 2.10-2.02 (m, 0.5H), 1.75-1.66 (m, 0.5H), 1.60-1.51 (m, 1.5H), 1.05-0.92 (m, 2H), 0.78-0.69 (m, 2H). ^{13}C NMR (CDCl_3 , 100 MHz, 25 °C): δ 172.3, 159.1, 156.6, 155.2, 146.24, 146.20, 145.84, 145.76, 145.73, 145.66, 144.5, 134.03, 133.98, 130.1, 130.0, 128.4, 124.3, 123.9, 120.1, 119.1, 119.02, 118.95, 118.89, 116.0, 115.9, 112.09, 112.07, 106.9, 51.8, 51.2, 51.0, 45.9, 45.3, 36.0, 34.3, 32.7, 31.6, 30.5, 29.5, 29.3, 12.6, 12.4, 7.8, 7.7, 7.6. ^{19}F NMR (CDCl_3 , 376 MHz, 25 °C): δ -120.3 (br s, 1F). HRMS (ESI) calculated for $\text{C}_{26}\text{H}_{25}\text{FN}_4\text{O}_3$: 460.1911. Found: 460.1846. $\Delta_m = 14.1$ ppm. $[\alpha]^{20}_{\text{D}} = -2.99 \pm 0.28^\circ$ (CH_2Cl_2).



(R)-4-(4'-Chloro-3-fluoro-[1,1'-biphenyl]-4-yl)-5-((1-(cyclopropanecarbonyl)pyrrolidin-3-yl)methyl)-2-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (6): (15 mg, 60%). ^1H NMR (CDCl_3 , 400 MHz, 25 °C): δ 10.94 (br s, 1H), 7.52 (br d, J = 8.4, 2H), 7.47-7.39 (m, 5H), 3.93-3.89 (m, 0.5H), 3.75-3.67 (m, 1H), 3.63-3.56 (m, 1H), 3.52 (s, 1.5H), 3.51 (s, 1.5H), 3.40-3.33 (m, 0.5H), 3.27-3.23 (m, 0.5H), 3.07-3.02 (m, 0.5H), 2.70-2.63 (m, 0.5H), 2.63-2.40 (m, 2.5H), 2.23-2.14 (m, 0.5H), 2.08-2.00 (m, 0.5H), 1.73-1.64 (m, 0.5H), 1.59-1.49 (m, 1.5H), 1.01-0.91 (m, 2H), 0.78-0.69 (m, 2H). ^{13}C NMR (CDCl_3 , 100 MHz, 25 °C): δ 172.3, 159.1, 156.6, 153.2, 144.3, 137.3, 137.2, 135.1, 135.0, 130.33, 130.28, 129.50, 129.48, 128.6, 123.9, 115.8, 115.6, 51.8, 51.2, 45.9, 45.3, 35.9, 34.3, 32.63, 32.60, 31.5, 30.4, 29.4, 29.2, 12.6, 12.3, 7.74, 7.67, 7.57. ^{19}F NMR (CDCl_3 , 376 MHz, 25 °C): δ -119.7 (br s, 1F). HRMS (ESI) calculated for $\text{C}_{24}\text{H}_{24}\text{ClFN}_4\text{O}_2$: 454.1572. Found: 454.1470. Δ_m = 22.5 ppm. $[\alpha]^{20}_D$ = $-8.74 \pm 0.16^\circ$ (CH_2Cl_2).

2. UV and Radiochromatograms of Purified Products

mVolts= RADIO



mAU = UV

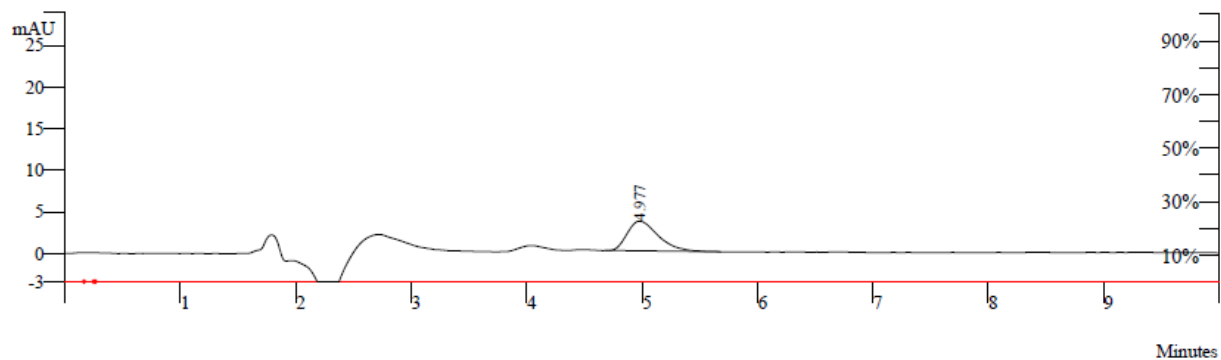
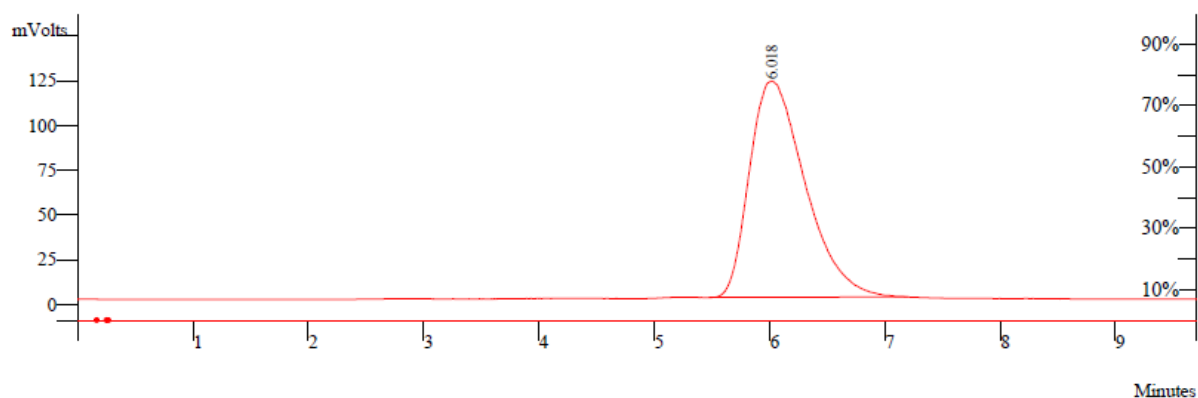


Figure S1. Radio (top) and UV (bottom) chromatograms of purified [^{11}C]**4**. Analysis was performed on a Phenomenex Luna C18(2) column (5 μm , 250 x 4.6 mm, 100 \AA) using a 50:50 0.3 M NH_4COOH (pH 4.2):MeCN mobile phase at a flow rate of 1.5 mL/min. The non-radioactive standard eluted with a retention time, t_R , of 4.94 min.

mVolts= RADIO



mAU = UV

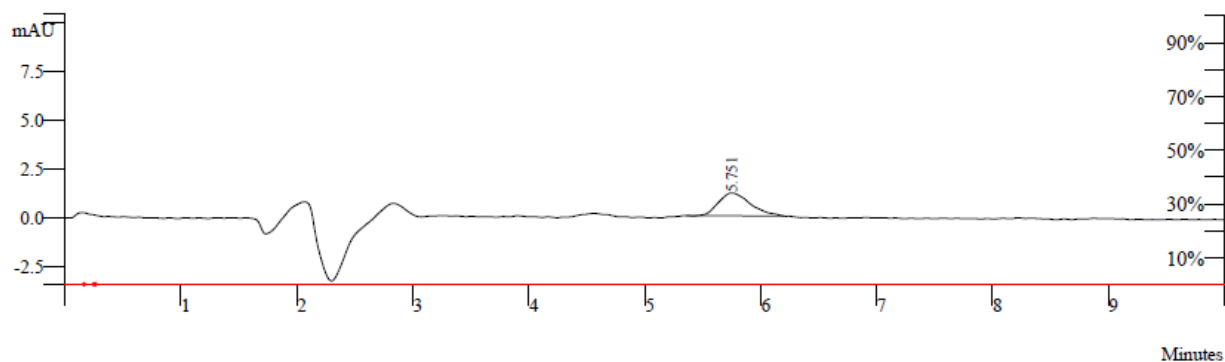


Figure S2. Radio (top) and UV (bottom) chromatograms of purified [^{11}C]5. Analysis was performed on a Phenomenex Luna C18(2) column (5 μm , 250 x 4.6 mm, 100 \AA) using a 50:50 0.3 M NH_4COOH (pH 4.2):MeCN mobile phase at a flow rate of 1.5 mL/min. The non-radioactive standard eluted with a retention time, t_R , of 5.77 min.

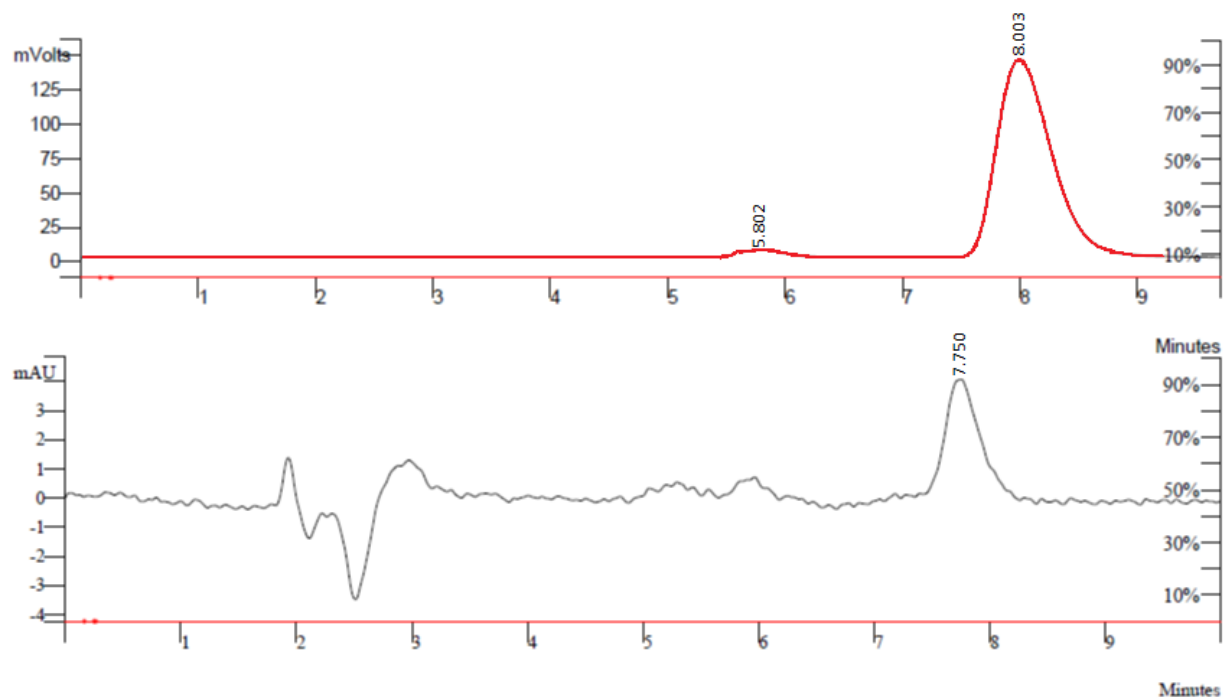


Figure S3. Radio (top) and UV (bottom) chromatograms of purified [^{11}C]6. Analysis was performed on a Phenomenex Luna C18(2) column (5 μm , 250 x 4.6 mm, 100 \AA) using a 50:50 0.3 M NH_4COOH (pH 4.2):MeCN mobile phase at a flow rate of 1.5 mL/min. The non-radioactive standard eluted with a retention time, t_R , of 7.69 min.

3. Determination of FASN Expression in LNCaP and PC3 cells

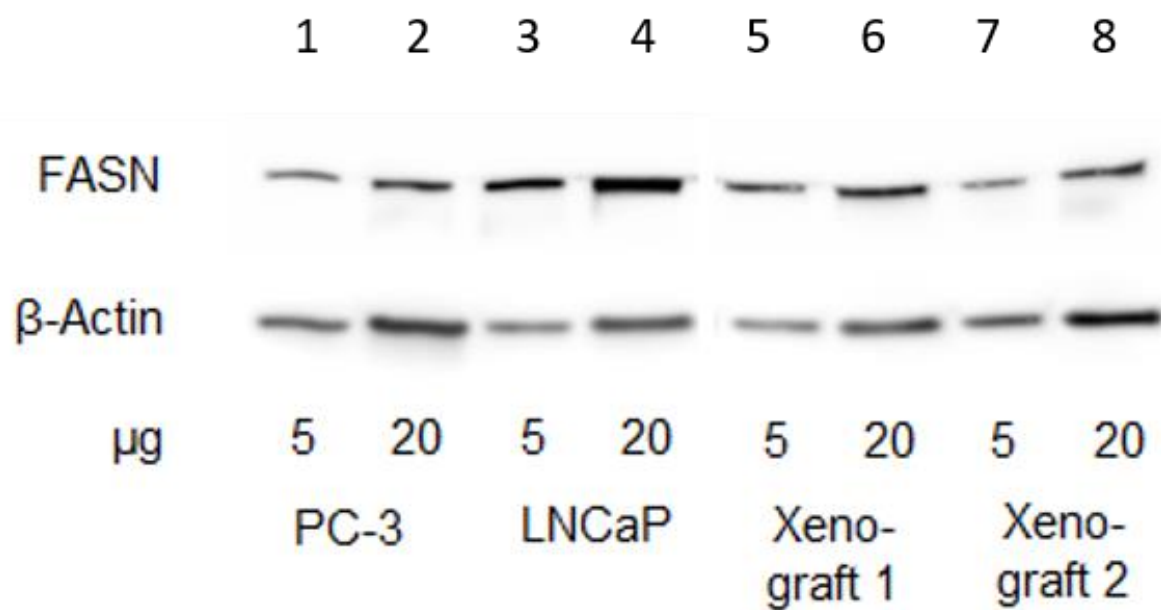


Figure S4. Shown are the FASN and β -actin staining due to 5 and 20 μ g PC-3 (lanes 1 and 2), 5 and 20 μ g LNCaP (lanes 3 and 4), 5 and 20 μ g excised LNCaP xenograft tumor #1 (lanes 5 and 6), and 5 and 20 μ g LNCaP xenograft tumor #2 (lanes 7 and 8).

4. Growth Inhibition Assay

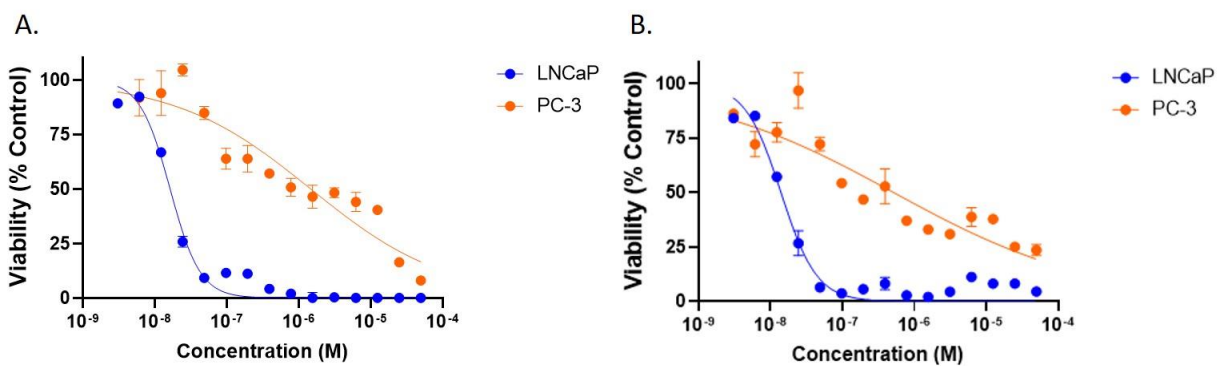


Figure S5. Comparison of viability of LNCaP and PC3 cells treated with **A.** GSK2194069 or **B.** **2** (right).

5. Comparison of *In vitro* Uptake in Aggregated versus Sub-Confluence LNCaP cells

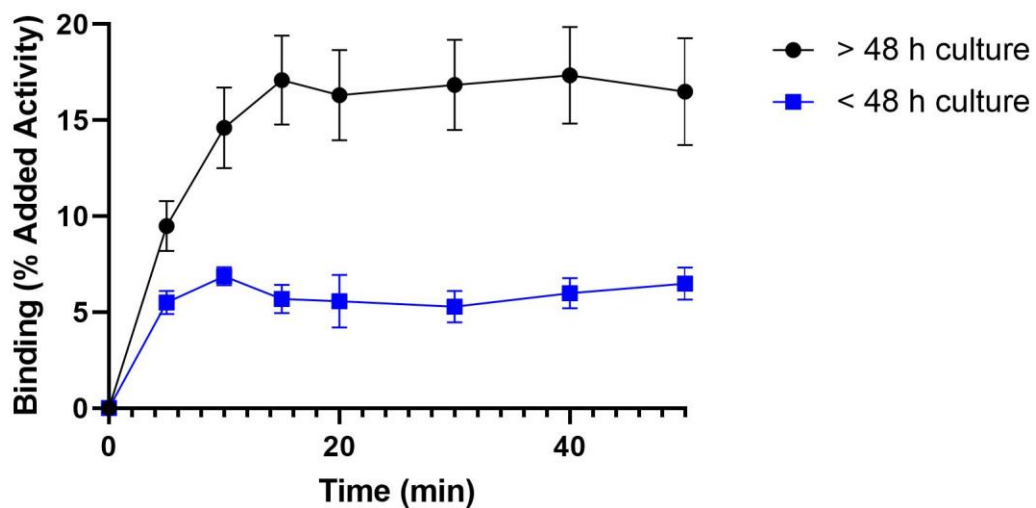
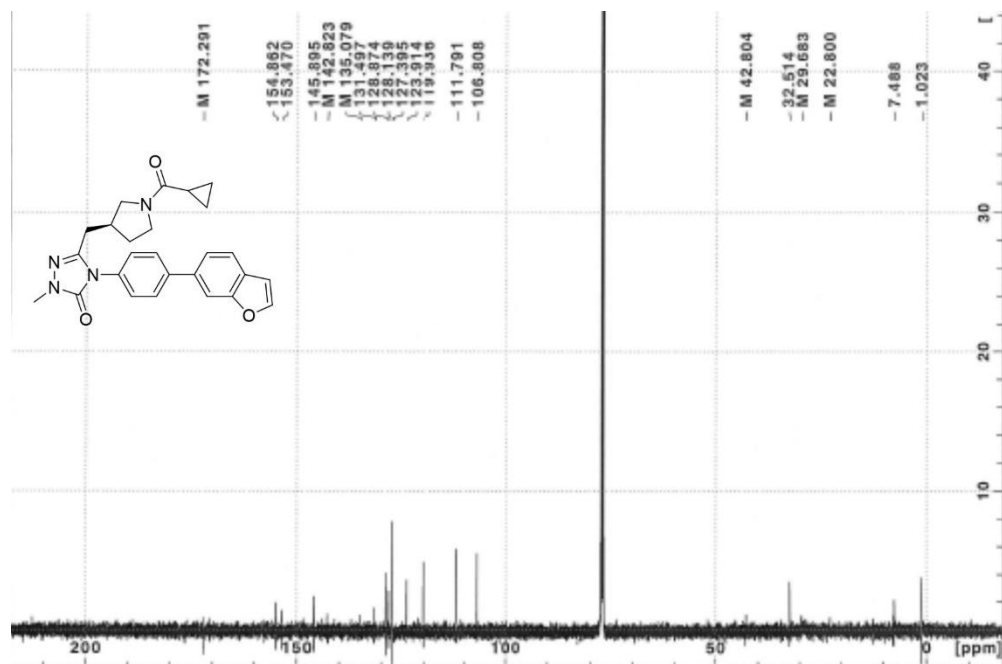


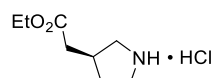
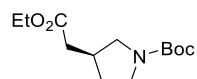
Figure S6. Comparison of $[^{11}\text{C}]\mathbf{5}$ binding to LNCaP cells cultured for > 48 h (black) and < 48 h (blue). The curves are corrected for decay and non-specific binding, but not corrected for protein content.

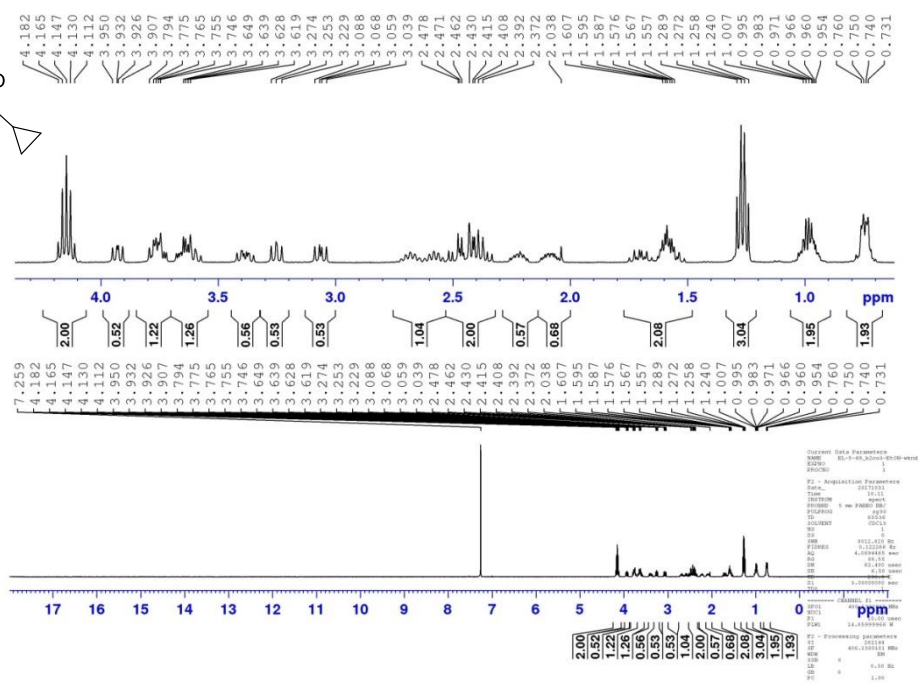
Chemical structure of compound 10: CN1C(=O)N(Cc2ccc(cc2-c3ccc4occc4c3)c5nc(=O)n(C)c5=O)CC1=O

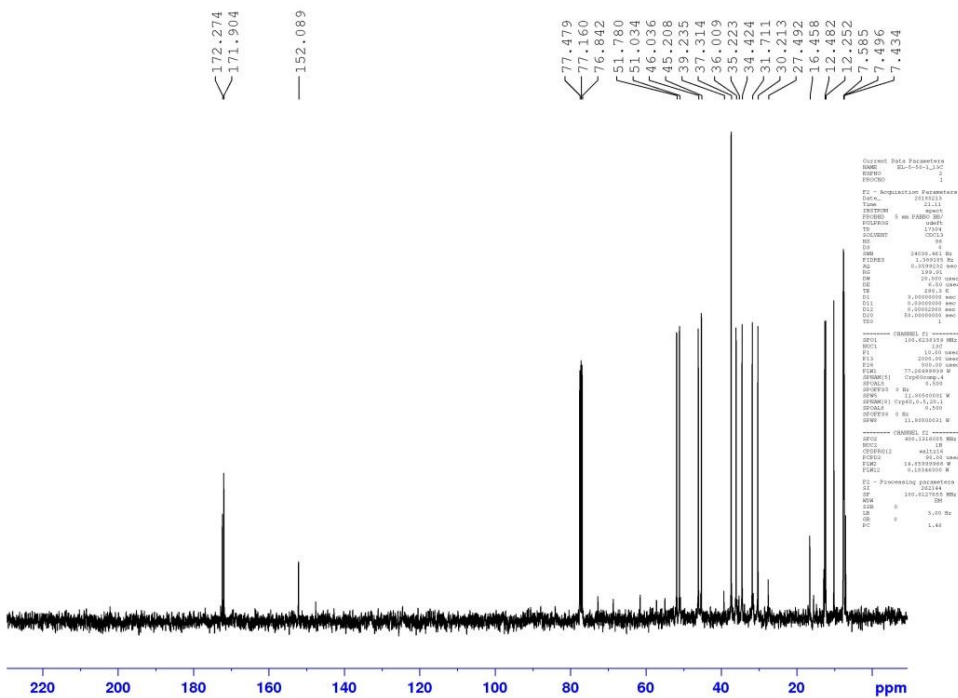
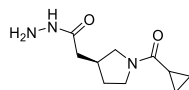
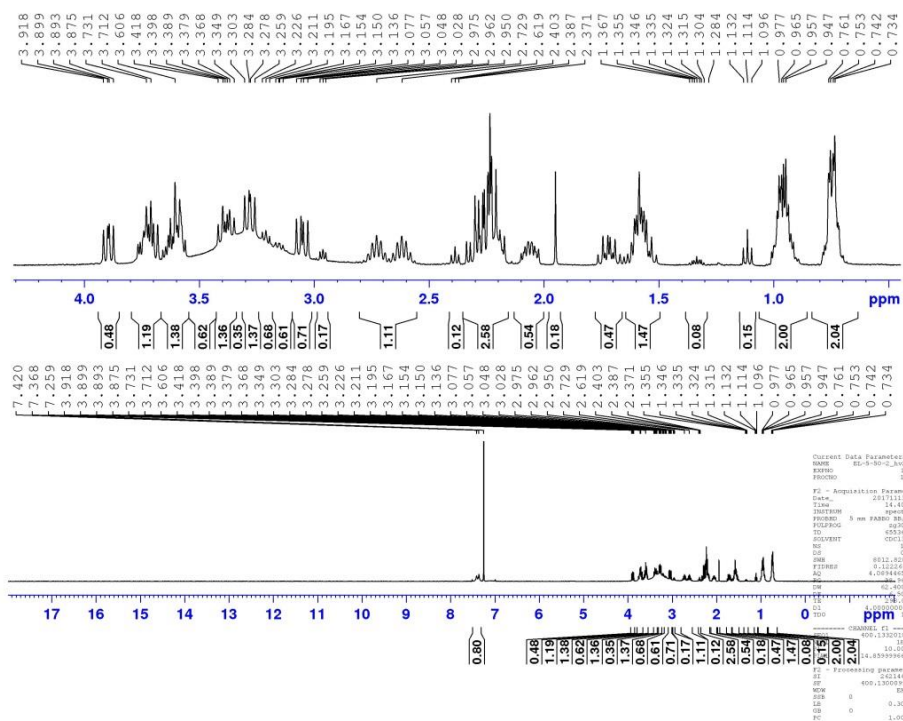
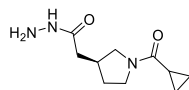
¹H NMR spectrum (CDCl₃) of compound 10. The spectrum shows peaks from 0 to 8 ppm. The chemical structure of compound 10 is shown above the spectrum. The spectrum includes integration values below the peaks and chemical shift values on the right side.

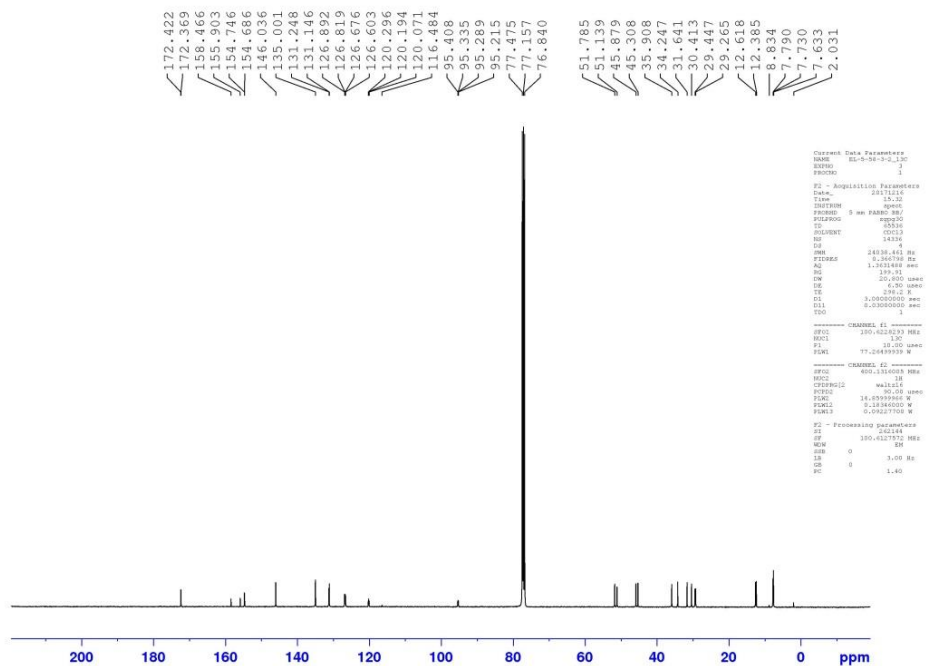
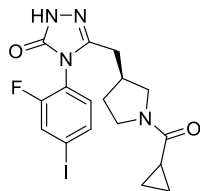
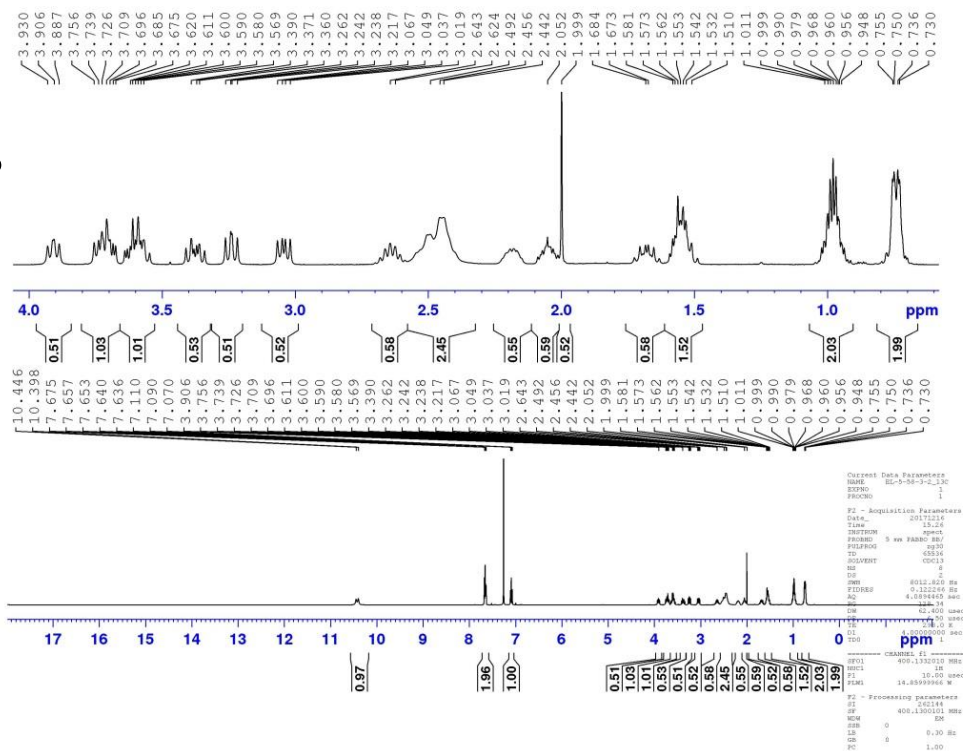
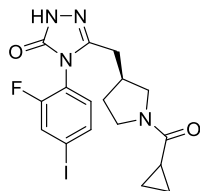
Chemical shift values (ppm): 7.834, 7.831, 7.772, 7.770, 7.769, 7.768, 7.767, 7.766, 7.765, 7.764, 7.763, 7.762, 7.761, 7.760, 7.759, 7.758, 7.757, 7.756, 7.755, 7.754, 7.753, 7.752, 7.751, 7.750, 7.749, 7.748, 7.747, 7.746, 7.745, 7.744, 7.743, 7.742, 7.741, 7.740, 7.739, 7.738, 7.737, 7.736, 7.735, 7.734, 7.733, 7.732, 7.731, 7.730, 7.729, 7.728, 7.727, 7.726, 7.725, 7.724, 7.723, 7.722, 7.721, 7.720, 7.719, 7.718, 7.717, 7.716, 7.715, 7.714, 7.713, 7.712, 7.711, 7.710, 7.709, 7.708, 7.707, 7.706, 7.705, 7.704, 7.703, 7.702, 7.701, 7.700, 7.699, 7.698, 7.697, 7.696, 7.695, 7.694, 7.693, 7.692, 7.691, 7.690, 7.689, 7.688, 7.687, 7.686, 7.685, 7.684, 7.683, 7.682, 7.681, 7.680, 7.679, 7.678, 7.677, 7.676, 7.675, 7.674, 7.673, 7.672, 7.671, 7.670, 7.669, 7.668, 7.667, 7.666, 7.665, 7.664, 7.663, 7.662, 7.661, 7.660, 7.659, 7.658, 7.657, 7.656, 7.655, 7.654, 7.653, 7.652, 7.651, 7.650, 7.649, 7.648, 7.647, 7.646, 7.645, 7.644, 7.643, 7.642, 7.641, 7.640, 7.639, 7.638, 7.637, 7.636, 7.635, 7.634, 7.633, 7.632, 7.631, 7.630, 7.629, 7.628, 7.627, 7.626, 7.625, 7.624, 7.623, 7.622, 7.621, 7.620, 7.619, 7.618, 7.617, 7.616, 7.615, 7.614, 7.613, 7.612, 7.611, 7.610, 7.609, 7.608, 7.607, 7.606, 7.605, 7.604, 7.603, 7.602, 7.601, 7.600, 7.599, 7.598, 7.597, 7.596, 7.595, 7.594, 7.593, 7.592, 7.591, 7.590, 7.589, 7.588, 7.587, 7.586, 7.585, 7.584, 7.583, 7.582, 7.581, 7.580, 7.579, 7.578, 7.577, 7.576, 7.575, 7.574, 7.573, 7.572, 7.571, 7.570, 7.569, 7.568, 7.567, 7.566, 7.565, 7.564, 7.563, 7.562, 7.561, 7.560, 7.559, 7.558, 7.557, 7.556, 7.555, 7.554, 7.553, 7.552, 7.551, 7.550, 7.549, 7.548, 7.547, 7.546, 7.545, 7.544, 7.543, 7.542, 7.541, 7.540, 7.539, 7.538, 7.537, 7.536, 7.535, 7.534, 7.533, 7.532, 7.531, 7.530, 7.529, 7.528, 7.527, 7.526, 7.525, 7.524, 7.523, 7.522, 7.521, 7.520, 7.519, 7.518, 7.517, 7.516, 7.515, 7.514, 7.513, 7.512, 7.511, 7.510, 7.509, 7.508, 7.507, 7.506, 7.505, 7.504, 7.503, 7.502, 7.501, 7.500, 7.499, 7.498, 7.497, 7.496, 7.495, 7.494, 7.493, 7.492, 7.491, 7.490, 7.489, 7.488, 7.487, 7.486, 7.485, 7.484, 7.483, 7.482, 7.481, 7.480, 7.479, 7.478, 7.477, 7.476, 7.475, 7.474, 7.473, 7.472, 7.471, 7.470, 7.469, 7.468, 7.467, 7.466, 7.465, 7.464, 7.463, 7.462, 7.461, 7.460, 7.459, 7.458, 7.457, 7.456, 7.455, 7.454, 7.453, 7.452, 7.451, 7.450, 7.449, 7.448, 7.447, 7.446, 7.445, 7.444, 7.443, 7.442, 7.441, 7.440, 7.439, 7.438, 7.437, 7.436, 7.435, 7.434, 7.433, 7.432, 7.431, 7.430, 7.429, 7.428, 7.427, 7.426, 7.425, 7.424, 7.423, 7.422, 7.421, 7.420, 7.419, 7.418, 7.417, 7.416, 7.415, 7.414, 7.413, 7.412, 7.411, 7.410, 7.409, 7.408, 7.407, 7.406, 7.405, 7.404, 7.403, 7.402, 7.401, 7.400, 7.399, 7.398, 7.397, 7.396, 7.395, 7.394, 7.393, 7.392, 7.391, 7.390, 7.389, 7.388, 7.387, 7.386, 7.385, 7.384, 7.383, 7.382, 7.381, 7.380, 7.379, 7.378, 7.377, 7.376, 7.375, 7.374, 7.373, 7.372, 7.371, 7.370, 7.369, 7.368, 7.367, 7.366, 7.365, 7.364, 7.363, 7.362, 7.361, 7.360, 7.359, 7.358, 7.357, 7.356, 7.355, 7.354, 7.353, 7.352, 7.351, 7.350, 7.349, 7.348, 7.347, 7.346, 7.345, 7.344, 7.343, 7.342, 7.341, 7.340, 7.339, 7.338, 7.337, 7.336, 7.335, 7.334, 7.333, 7.332, 7.331, 7.330, 7.329, 7.328, 7.327, 7.326, 7.325, 7.324, 7.323, 7.322, 7.321, 7.320, 7.319, 7.318, 7.317, 7.316, 7.315, 7.314, 7.313, 7.312, 7.311, 7.310, 7.309, 7.308, 7.307, 7.306, 7.305, 7.304, 7.303, 7.302, 7.301, 7.300, 7.299, 7.298, 7.297, 7.296, 7.295, 7.294, 7.293, 7.292, 7.291, 7.290, 7.289, 7.288, 7.287, 7.286, 7.285, 7.284, 7.283, 7.282, 7.281, 7.280, 7.279, 7.278, 7.277, 7.276, 7.275, 7.274, 7.273, 7.272, 7.271, 7.270, 7.269, 7.268, 7.267, 7.266, 7.265, 7.264, 7.263, 7.262, 7.261, 7.260, 7.259, 7.258, 7.257, 7.256, 7.255, 7.254, 7.253, 7.252, 7.251, 7.250, 7.249, 7.248, 7.247, 7.246, 7.245, 7.244, 7.243, 7.242, 7.241, 7.240, 7.239, 7.238, 7.237, 7.236, 7.235, 7.234, 7.233, 7.232, 7.231, 7.230, 7.229, 7.228,

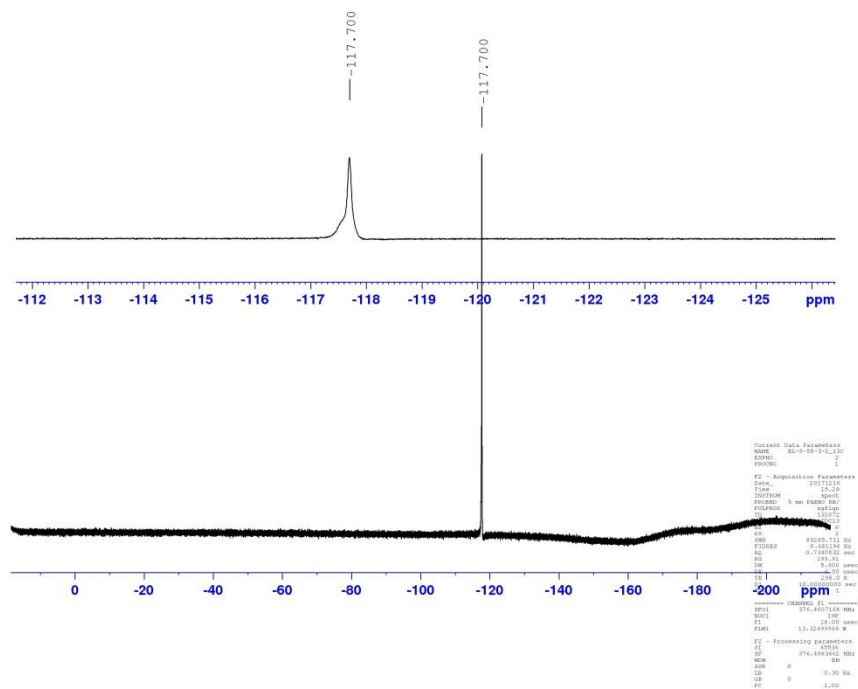
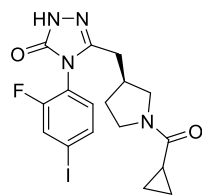


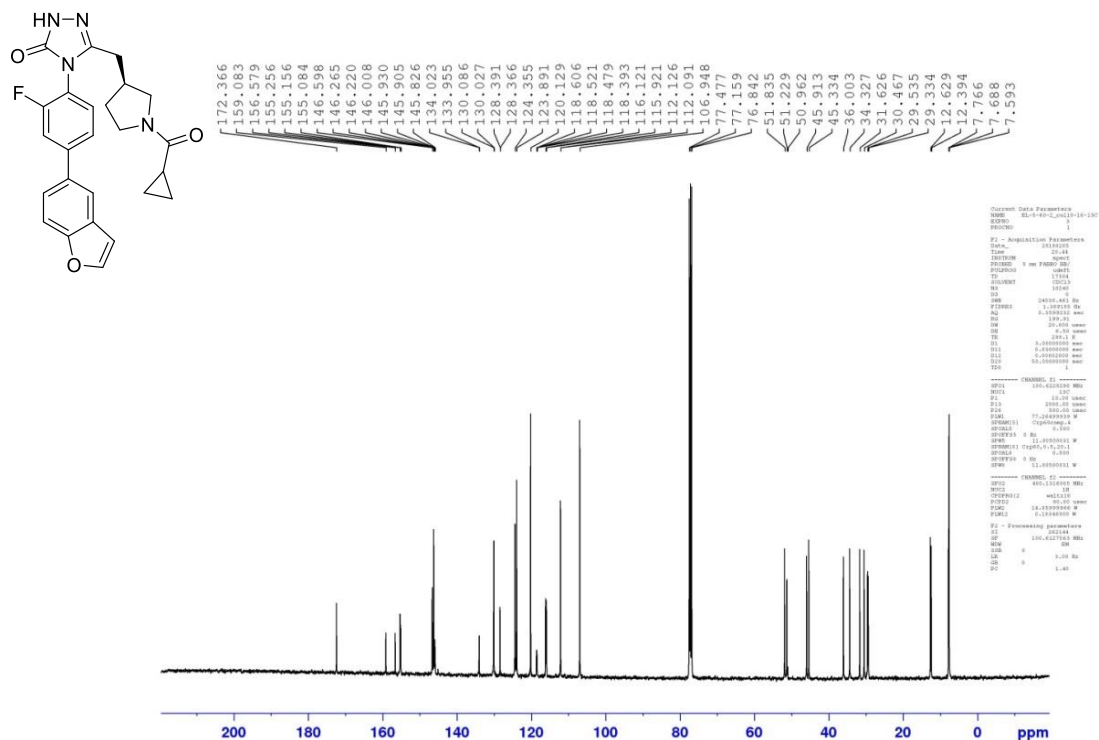


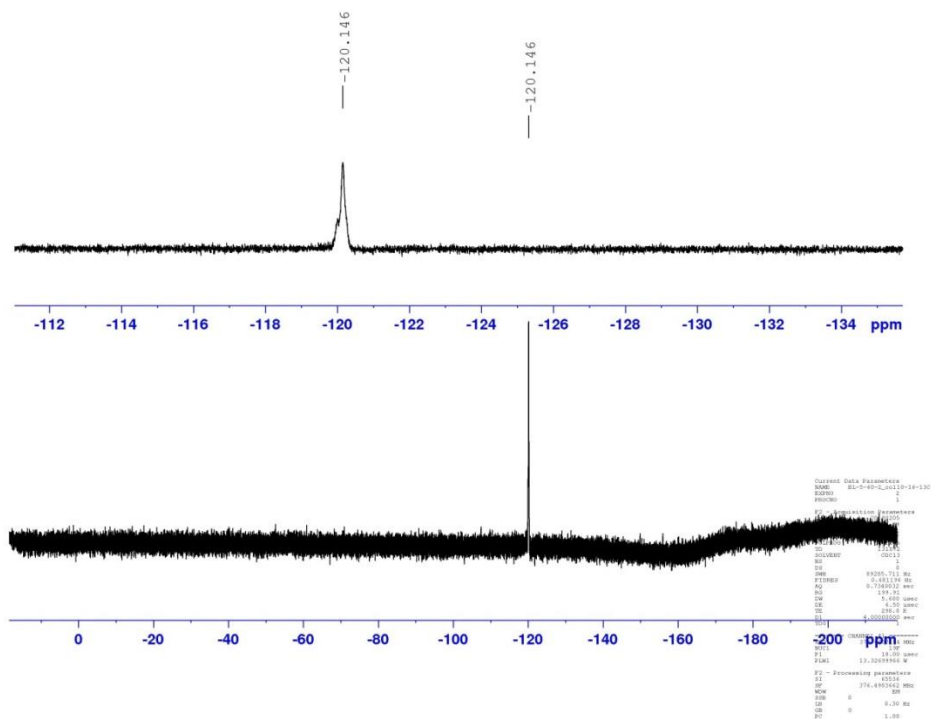
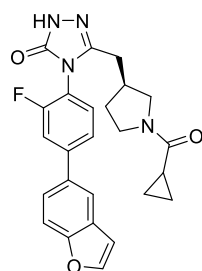


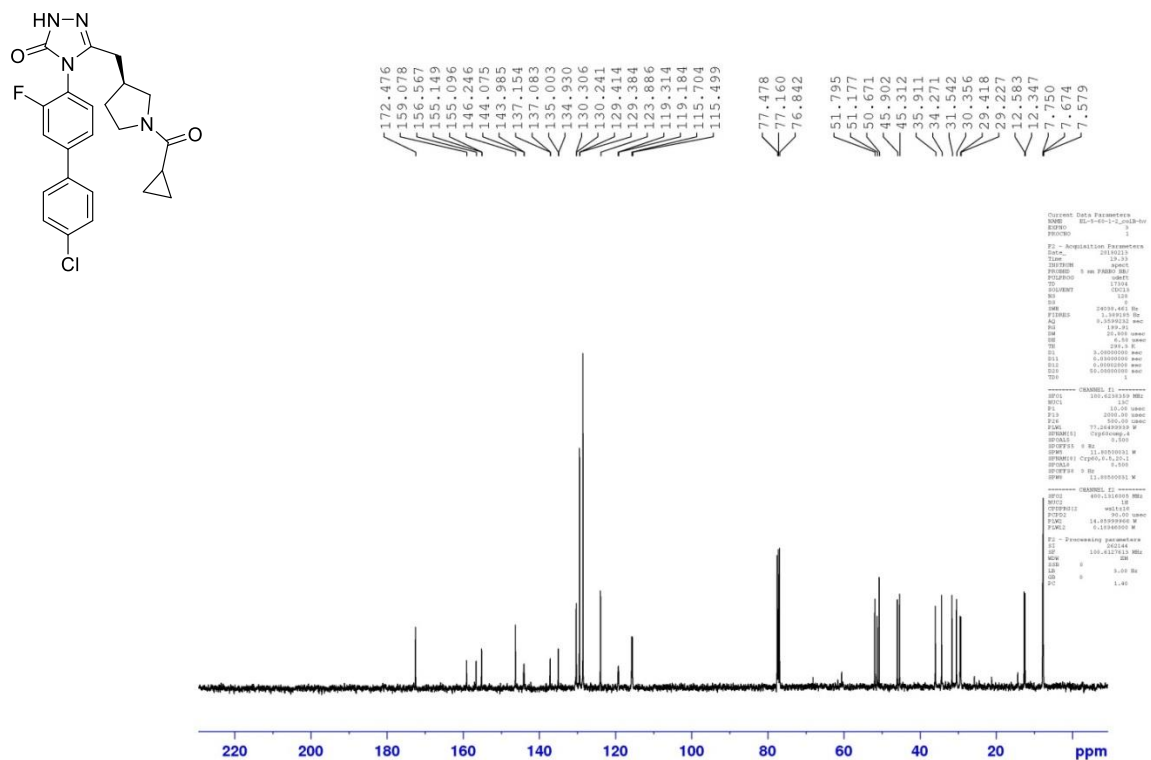
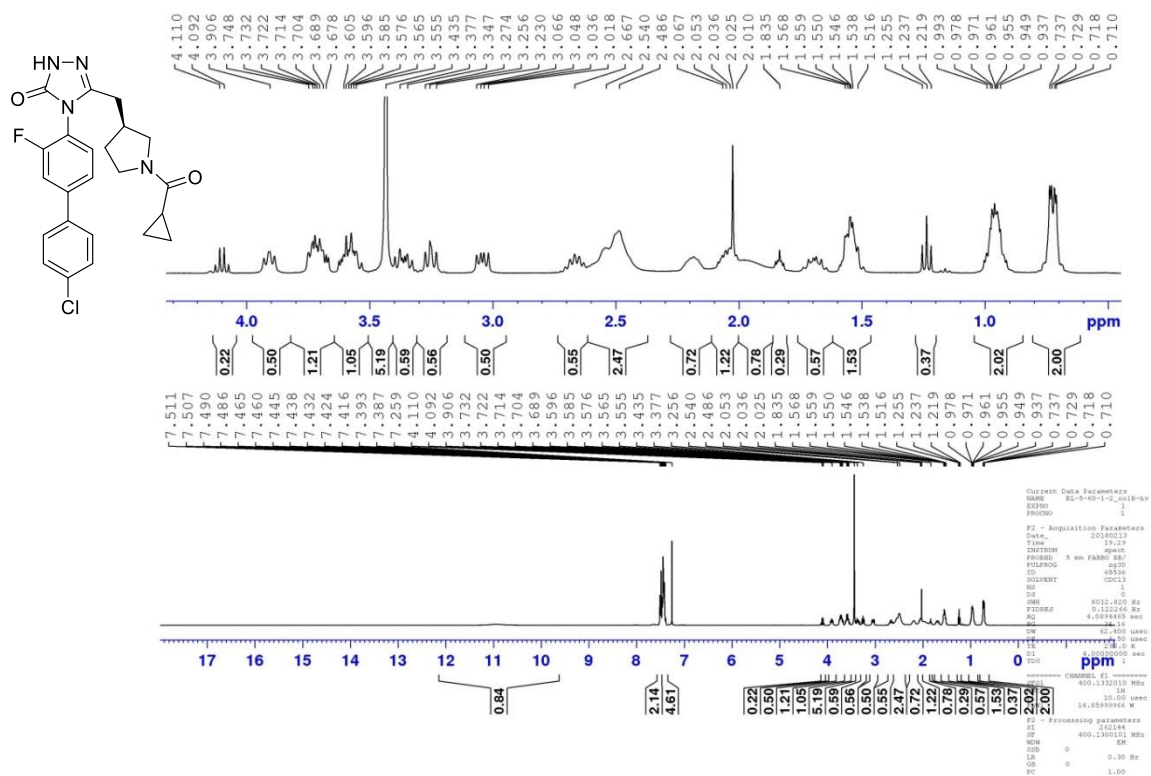


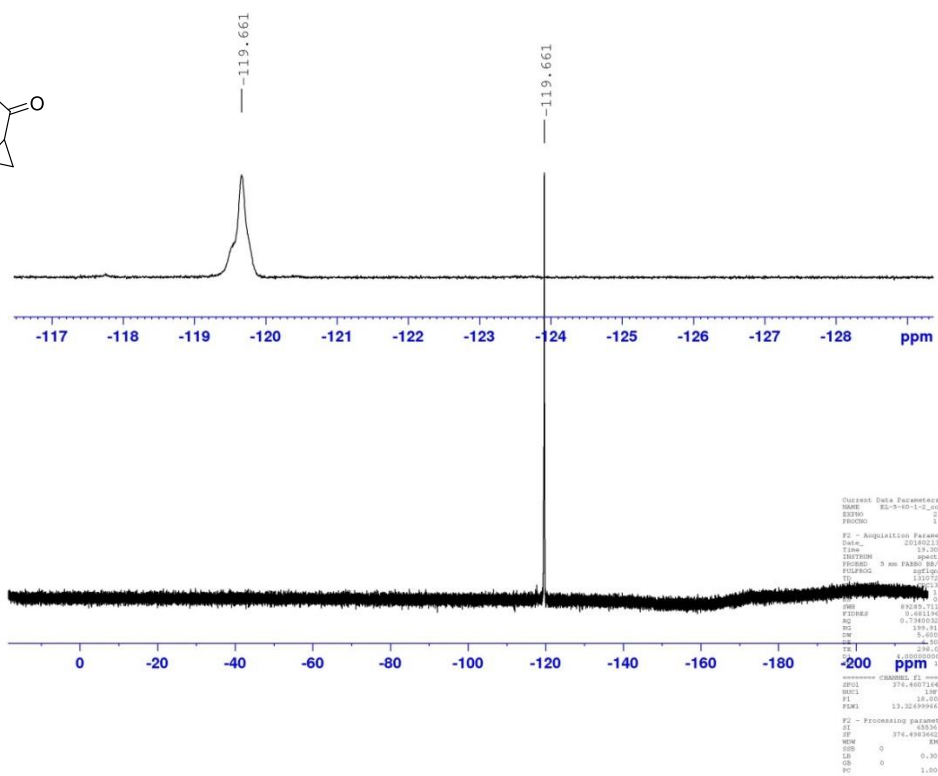
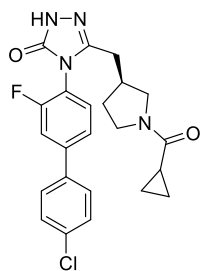


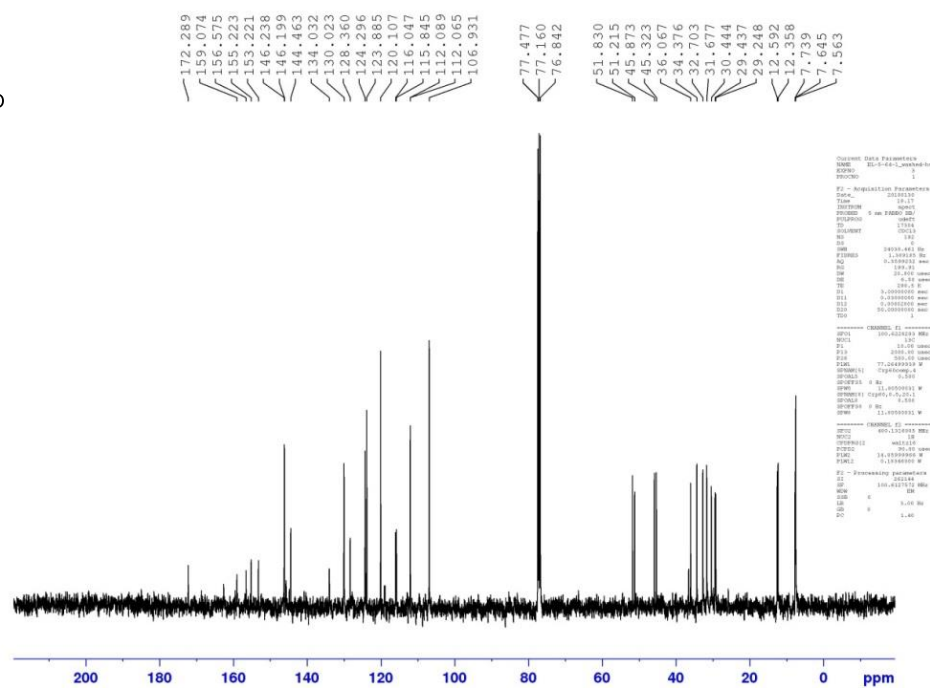
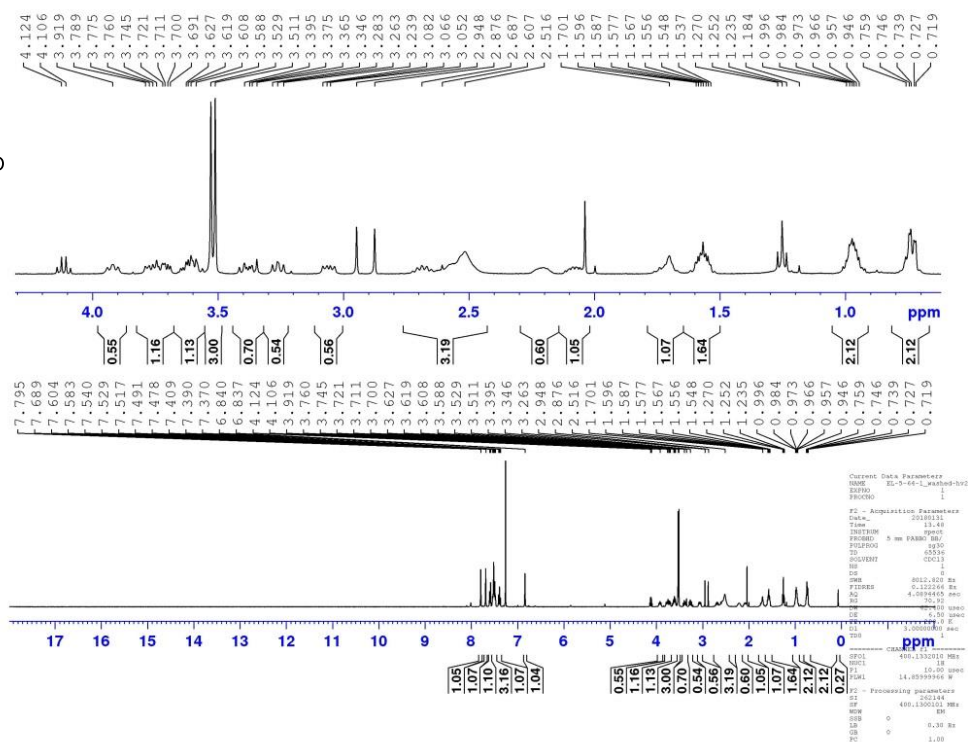


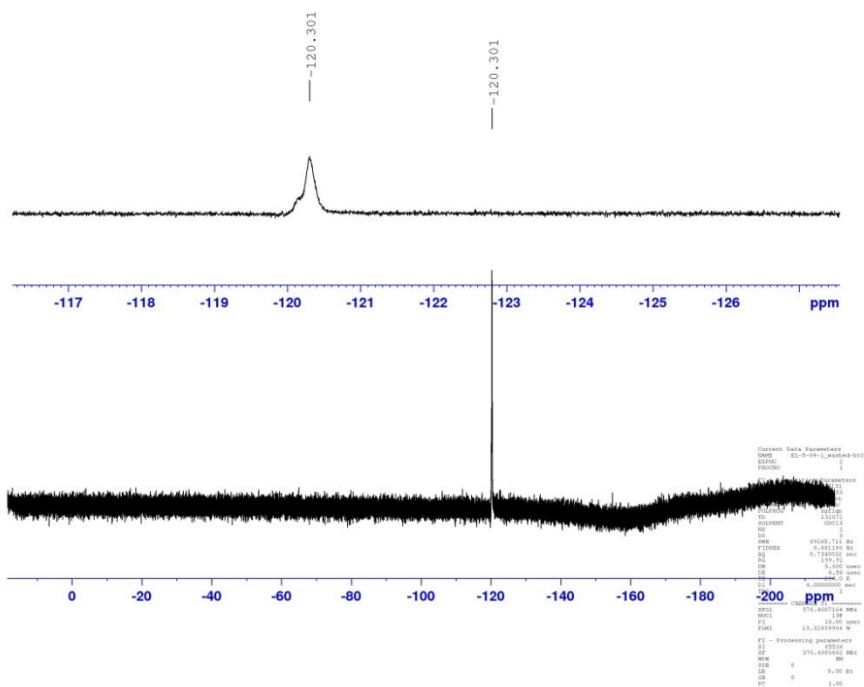












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