Electronic Supplementary Material (ESI) for ChemComm.


## Supporting Information for

# The efficiency of $^{18}F$ labelling of prostate specific membrane antigen ligand via strain-promoted azide-alkyne reaction: reaction speed versus hydrophilicity.

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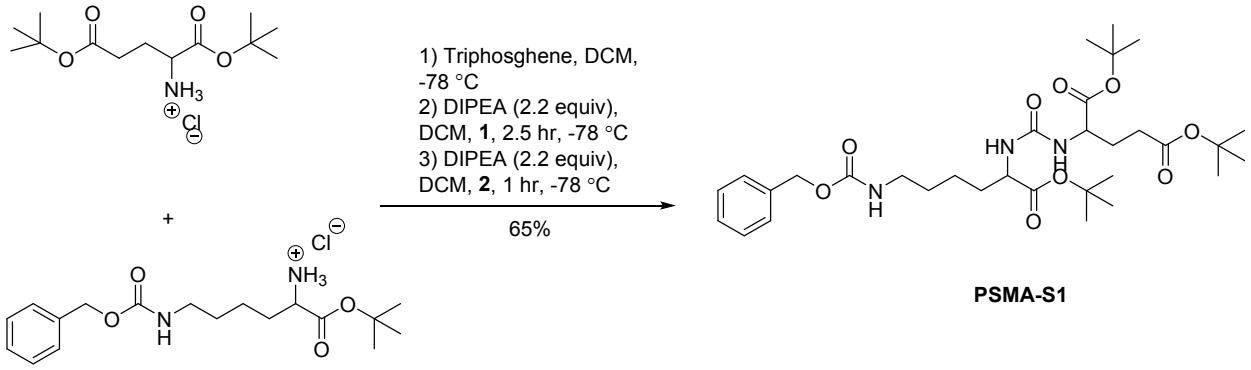
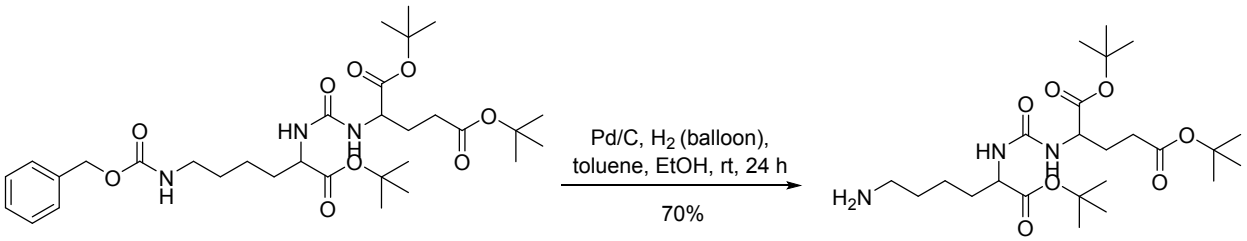
**General Methods and Materials.** All organic solvents were dried and freshly distilled before use; tetrahydrofuran was distilled from sodium/benzophenone ketyl and dichloromethane was distilled from $CaH_2$. Azido-PEG2-NHS ester was purchased from BroadPharm (San Diego, CA). Other reagents were obtained from Aldrich or VWR and used as received. Photo-ODIBO-$EG_4$-Tos[1] and BCN 4-nitrophenyl chloroformate[2] were prepared as reported previously. Thin-layer chromatography (TLC) was preformed using commercial silica gel 60 $F_{254}$ coated aluminum-backed sheets. Visualization was accomplished with UV light (254 nm) and $KMnO_4$ stain by heating. Purification was carried out on an automated flash chromatography/medium-pressure liquid chromatography (MPLC) system using normal-phase silica flash columns (4, 12, 24 or 40 g) or reverse-phase C-18 columns (15, 50, 150 g). Flash chromatography was performed using 40-63 μm silica gel. NMR spectra were recorded in $CDCl_3$, $D_2O$ and DMSO-$d_6$ using 400 MHz instrument. Chemical shifts are reported in parts per million (ppm) and are referenced to the center line of the solvent (for $CDCl_3$, δ 7.26 ppm for $^1H$ NMR and 77.2 for $^{13}C$ NMR; for $D_2O$, δ 4.79 ppm for $^1H$ NMR; for DMSO-$d_6$, δ 2.50 ppm for $^1H$ NMR and 39.5 for $^{13}C$ NMR). Coupling constants are given in hertz (Hz). HRMS data were collected with a hybrid linear trap quadrupole Fourier Transform (LTQ FT) and an electrospray ion source (ESI). Spectroscopic data for the known compounds prepared according to the methodology described in the paper match with those reported in the literature. The preparative photolyses were conducted using a Rayonet photoreactor equipped with sixteen 4W 350 nm fluorescent lamps. PSMA-$NH_2$ was synthesized according to the procedures adapted from previously reported methods[4,5].




**Tri-tert-butyl-(9*S*,13*S*)-3,11-dioxo-1-phenyl-2-oxa-4,10,12-triazapentadecane-9,13,15-tricarboxylate (PSMA-S1).**[1,2] In a flame-dried and argon purged round-bottom flask was added triphosghene (0.132 g, 0.444 mmol, 0.37 equiv) followed by anhydrous DCM (2.24 mL). The mixture was cooled to -78 °C for 10 mins. In a 20 mL flame-dried and argon-purged scintillation vial was added 2-amino-6-benzyloxycarbonylamino-hexanoic acid tert-butyl ester hydrochloride (447 mg, 1.19 mmol, 1.0 equiv) to this was added DCM (2.24 mL) and DIPEA (0.459 mL, 2.64 mmol, 2.2 equiv) this mixture was stirred at rt for 10 mins. After 10 mins stirring at rt, this solution was then slowly added in small aliquots to the triphosghene solution at -78 °C over 3 h. After 3 h, a solution of L-glutamic acid di-tert-butyl ester hydrochloride (355 mg, 1.19 mmol, 1.0 equiv) in

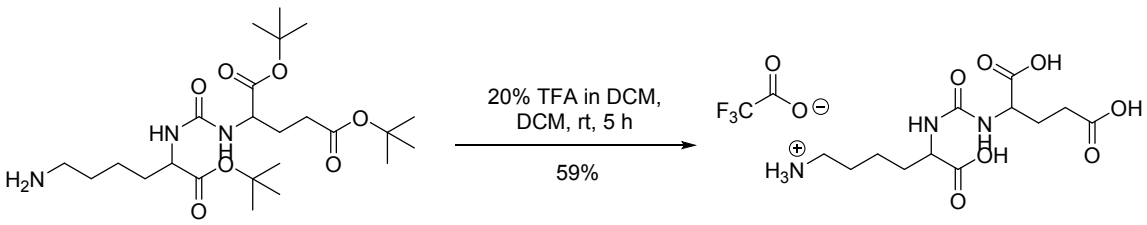
DCM (2.24 mL) and DIPEA (0.459 mL, 2.64 mmol, 2.2 equiv) this mixture was stirred at rt for 10 mins. After 10 mins, this solution was added to the reaction mixture containing triphosghene in one portion at -78 °C. The reaction was stirred for 1 h at -78 °C. After stirring for 45 mins the mixture was warmed to rt and the reaction was concentrated to dryness, diluted with 10 mL EtOAc, washed with 2 N aqueous sodium bisulfate ($NaHSO_4$) (2 x 10 mL), brine (2 x 10 mL), and dried over $Na_2SO_4$. The organic layer was filtered and concentrated by rotary evaporation to yield crude product as a pale yellow oil. The crude oil was purified by normal phase MPLC eluting from 100% hexanes to 70:30 EtOAc:hexanes. Like fractions were combined and concentrated to give **PSMA-S1** as a colorless sticky oil (491 mg, 65%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H), 5.22 – 5.04 (m, 4H), 4.33 (qd, *J* = 7.8, 4.8 Hz, 2H), 3.18 (q, *J* = 9.9, 8.2 Hz, 2H), 2.38 – 2.19 (m, 2H), 2.12 – 1.99 (m, 1H), 1.91 – 1.70 (m, 2H), 1.71 – 1.20 (m, 33H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.5, 172.5, 157.0, 156.7, 136.8, 128.6, 128.2, 128.1, 82.2, 81.9, 80.7, 66.7, 53.4, 53.1, 40.8, 32.8, 31.7, 29.5, 28.5, 28.2, 28.2, 28.1, 22.4; HRMS (LTQ FT) calcd for $C_{32}H_{51}N_3O_9Na$ [M + Na]$^+$: 644.3553, found 644.3541.




**PSMA-S1** **PSMA-S2**

**Di-tert-butyl (((*S*)-6-amino-1-(tert-butoxy)-1-oxohexan-2-yl)carbamoyl)-L-glutamate (PSMA-S2)**. [1,2] In a flame-dried and argon purged round-bottom flask was added argon was added 10% Pd/C (332 mg, 0.303 mmol, 0.4 equiv) the reaction vessel was slowly purged with argon to this was then added toluene (4.84 mL) to make a slurry of the Pd/C mixture and wash the walls of the round-bottom flask. To this mixture was then added EtOH (13.3 mL) and the flask was purged again with argon. To the reaction flask was then added tri-tert-butyl(9*S*,13*S*)-3,11-dioxo-1-phenyl-2-oxa-4,10,12-triazapentadecane-9,13,15-tricarboxylate **PSMA-S1** (470 mg, 0.757 mmol, 1.0 equiv) as a solution in EtOH (8.84 mL) and toluene (4.03 mL). The reaction mixture was purged with argon for about 5 mins. After purging with argon, the reaction round bottom flask was fitted with a $H_2$ gas balloon and purged. The mixture was stirred at rt under $H_2$ and monitored by TLC (10:90 MeOH:DCM and 70:30 EtOAc:hexanes, staining with $KMnO_4$). The reaction mixture was stirred overnight at rt. After stirring for about 24 h, the flask was purged with argon to remove the $H_2$. The

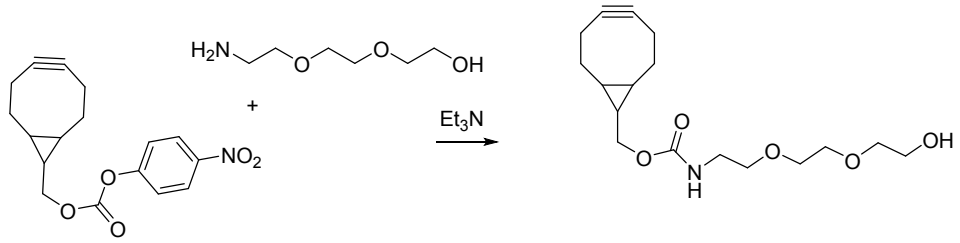
reaction mixture was filtered through a pad of celite and the celite was washed with fresh EtOH. The filtrate was concentrated by rotary evaporation to give crude product as a thick pale yellow oil. The crude oil was purified by reverse-phase chromatography eluting with 100% water to 60:40 MeCN:$H_2O$. Like fractions were combined and concentrated (the organic solvent was removed by rotary evaporation and the aqueous was removed by lyophilization) to give **PSMA-S2** as a pale pink thick oil (260 mg, 70%). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.29 (t, *J* = 7.4 Hz, 2H), 4.37 – 4.27 (m, 2H), 2.74 (s, 2H), 2.54 (s, 1H), 2.39 – 2.21 (m, 2H), 2.13 – 2.01 (m, 1H), 1.92 – 1.72 (m, 2H), 1.71 – 1.57 (m, 1H), 1.57 – 1.33 (m, 33H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.4, 156.9, 82.1, 81.7, 80.5, 53.4, 53.0, 32.6, 31.6, 28.3, 28.1, 28.0, 28.0, 22.3; HRMS (LTQ FT) calcd for $C_{24}H_{46}N_3O_7$ $[M + H]^+$: 488.3330, found 488.3326.



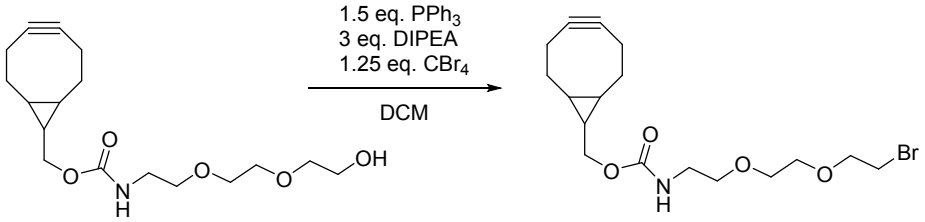

**PSMA-S2** **PSMA-$NH_2$**

**(((*S*)-5-Amino-1-carboxypentyl)carbamoyl)-L-glutamic acid (TFA salt) (PSMA-$NH_2$).** [1,2] In a 20 mL flame-dried and argon purged reaction vial was added di-tert-butyl (((*S*)-6-amino-1-(tert-butoxy)-1-oxohexan-2-yl)carbamoyl)-L-glutamate **PSMA-S2** (210 mg, 0.432 mL) was added using 3.0 mL of anhydrous DCM. The mixture was cooled using an ice bath and to the mixture was added 20% TFA in DCM (5.0 mL, 64.8 mmol, 150 equiv). The reaction mixture was warmed to rt and monitored by TLC (10:90 MeOH:DCM and 70:30 EtOAc:hexanes, staining with $KMnO_4$) and LC-MS analysis. After 5 h, the starting material was consumed and the reaction mixture was concentrated by rotary evaporation to give crude product as a pale yellow thick oil. The crude oil was purified by reverse phase chromatography eluting with 100% water to 90:10 MeCN:$H_2O$. Like fractions were combined and concentrated (the organic solvent was removed by rotary evaporation and the water was removed by lyophilization) to give isolated product as an off-white solid. This solid was then dissolved in MeOH (0.50 mL) and ether (5.0 mL) and product was precipitated from the mixture. The precipitated product was then washed with fresh $Et_2O$ (4 x 10 mL) and dried under high vacuum. It was observed in the $^1$H NMR that the final product had residual ether remaining (<10%) even after multiple cycles of re-dissolving product in $H_2O$ or $D_2O$ and freeze drying overnight to remove the $Et_2O$ (4 cycles of re-dissolving and lyophilizing) or drying the solid under high vacuum with mild heating (50°C for 6 h). **PSMA-$NH_2$** was afforded as a fluffy cotton-like white solid with <10% ether remaining in the sample (91 mg, 59% yield). $^1$H NMR (400 MHz, $D_2O$) δ 4.20 (td, *J* = 9.2, 4.9 Hz, 2H), 3.01 (t, *J* = 7.5 Hz, 2H), 2.51 (t, *J* = 7.4 Hz, 2H), 2.26 – 2.08 (m, 1H), 2.01 – 1.82 (m, 1H), 1.78 – 1.63 (m, 3H), 1.53 – 1.41 (m, 2H). $^{19}$F NMR (376 MHz, $D_2O$) δ -75.51; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.72 (s, 3H), 6.41 – 6.26 (m, 2H), 4.14

– 4.02 (m, 2H), 2.81 – 2.71 (m, 2H), 2.30 – 2.17 (m, 2H), 1.98 – 1.86 (m, 1H), 1.77 – 1.57 (m, 2H), 1.59 – 1.45 (m, 3H), 1.40 – 1.27 (m, 2H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 174.4, 174.1, 173.7, 157.3, 52.1, 51.7, 38.7, 31.7, 29.9, 27.5, 26.7, 22.1.; HRMS (LTQ FT) calcd for $C_{12}H_{23}N_3O_7$ $[M + H]^+$: 320.1452, found 320.1449.




**BCN-EG$_3$**. Triethylene glycol amine (1.53 g, 10.3 mmol) was added to a mixture of BCN 4-nitrophenyl chloroformate (2.50 g, 7.93 mmol) and triethylamine (2.41 g, 23.8 mmol) in DMF (240 mL). The mixture was stirred for 24 h, solvent evaporated *in vacuo*, and purified by flash chromatography (1:9 EtOAc/hexanes to 100% EtOAc) to provide 2.56 g (87%) of BCN- EG$_3$ as a clear oil, which was used in the next step without further purification.

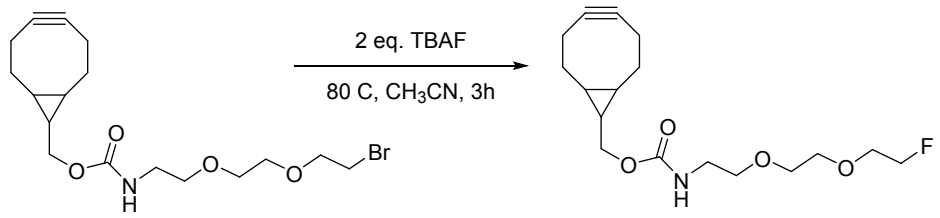



**BCN-EG$_3$-Br**. Carbon tetrabromide (3.92g, 11.83 mmol) was added to a solution of BCN-EG$_3$ (3.08g, 9.47 mmol), triphenyl phosphine (3.72g, 14.20 mmol), and DIPEA (3.67 g, 28.4 mmol) in DCM (65 mL). The mixture was stirred at r.t. for 3 h and concentrated *in vacuo*. The crude mixture was purified by flash chromatography (1:1 EtOAc/hexanes) to give 1.51 g (41%) of BCN-EG$_3$-Br as a clear oil.

**$^1$H-NMR**: 5.16 (br s, 1H), 3.95 (d, J = 7.6 Hz, 2H), 3.79 (t, J = 7.2 Hz, 2H), 3.61 (m, 4H), 3.55 (t, J = 6.1 Hz, 2H), 3.46 (t, J= 6.4 Hz, 2H), 3.35 (m, 2H), 2.38 (m, 2H), 2.24 (m, 2H), 2.13 (m, 2H), 1.34 (m, 2H), 0.69 (m, 3H) .

**$^{13}$C-NMR**: 156.8, 98.8, 71.2, 70.4, 70.20, 70.16, 69.09, 40.8, 33.3, 30.2, 23.8, 22.8, 21.4.

**HRMS**: $C_{17}H_{26}BrNO_4$, calc. [M+H] 388.1123, found 388.1118

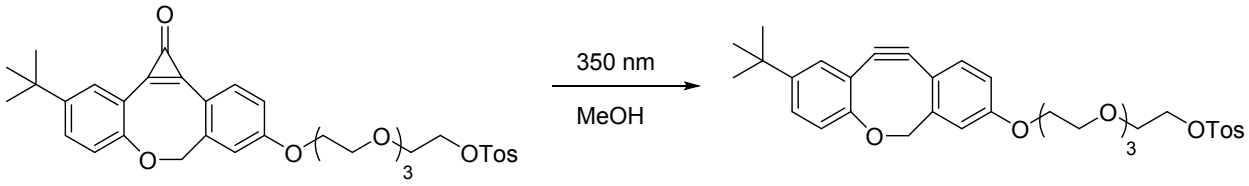



**BCN-EG$_3$-F**. TBAF (1.1mL, 1 M solution, 1.08 mmol) was added to a solution of BCN-EG$_3$-Br (350 mg, 0.9 mmol) in acetonitrile (10 mL); the mixture was refluxed for 2.5 h, and cooled to r.t. DCM (15 mL) and water (10 mL) were added to the mixture, the organic layer was separated, washed with water and brine. BCN-EG$_3$-F was purified by column chromatography (EtOAc/Hexanes, 1: 20, then 1:10), and was isolated as a colorless oil (212 mg, 72 %). The product is a mixture of exo-/endo- isomers in 1:3 ratio.

**$^1$H-NMR**: 5.17 (br s, 1H), 4.58 (dt, $J_d$ = 32 Hz, $J_t$ = 4.2 Hz, 2H), 3.96 (d, J = 6.7 Hz, 2H), 3.75 (dt, $J_d$ = 25 Hz, $J_t$ = 4.1 Hz, 2H), 3.70-3.60 (m, 4H), 3.56 (t, J = 5.1 Hz, 2H), 3.36 (m, 2H), 2.39 (m, 2H), 2.26 (m, 2H), 2.14 (m, 2H), 1.36 (m, 2H), 0.72 (m, 3H).

**$^{13}$C-NMR**: 156.8, 98.8, 83.1 (d, J = 168 Hz), 70.7, 70.4 (d, J= 19 Hz), 70.2, 70.2, 69.0, 40.7, 33.25, 29.0, 22.8, 21.4.

**HRMS**: $C_{17}H_{26}FNO_4$, calc. [M+H] 328.1919, found 328.1924

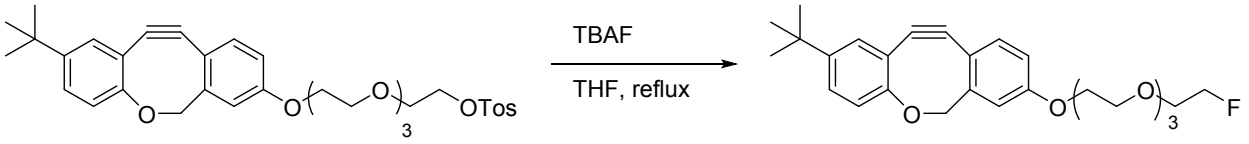



**ODIBO-EG$_4$-Tos**. Photo-ODIBO-EG$_4$-Tos (0.390 g, 0.61 mmol) in methanol (600 mL) was irradiated for 20 minutes at 350 nm. The reaction mixture was then concentrated *in vacuo* and purified via flash chromatography (1:1 hexanes: acetone) to afford ODIBO-EG$_4$-Tos (0.290 g, 78%) as a faint yellow oil.

**$^1$H-NMR**: 7.79-7.81 (d, J = 8.2 Hz, 2H), 7.33-7.35 (d, J = 8.1 Hz, 2H), 7.21-7.27 (m, 3H), 7.10-7.12 (dd, J =7.2, 2.1 Hz, 1H), 7.03 (d, 2.5 Hz, 1H), 6.89-6.92 (dd, J = 8.4, 2.5 Hz, 1H), 5.16-5.19 (d, J = 12.0 Hz, 1H), 4.52-4.55 (d, J = 12.0 Hz, 1H), 4.15-4.17 (m, 4H), 3.86-3.88 (t, J = 4.7 Hz, 2H), 3.65-3.73 (m, 6H), 3.60 (s, 4H), 2.44 (s, 3H), 1.32 (s, 9H).

**$^{13}$C-NMR**: 167.35, 158.78, 149.15, 147.00, 144.99, 133.22, 130.03, 128.19, 126.92, 125.61, 123.75, 121.41, 118.36, 117.92, 117.70, 114.66, 114.22, 110.76, 78.08, 71.07, 70.98, 70.90, 70.79, 69.84, 69.45, 68.91, 67.93, 34.60, 31.60, 21.86.

**HRMS**: $C_{34}H_{41}O_8S^+$, calc. [M+H] 609.2517, found 609.2499.

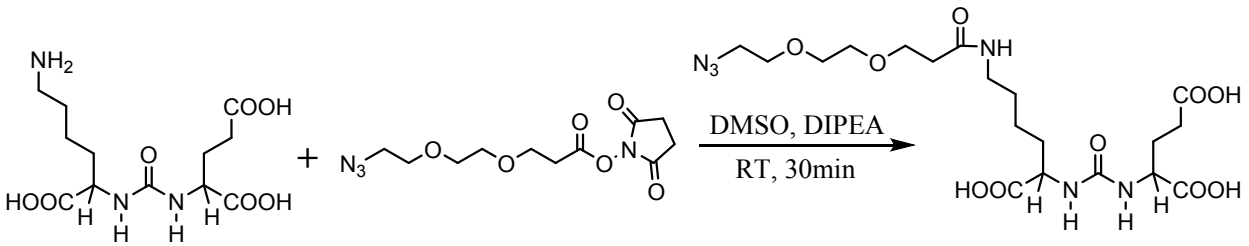



**ODIBO-EG$_4$-F**: TBAF (0.424 mL, 1M THF) was added to a solution of ODIBO-EG$_4$-Tos (0.129 g, 0.212 mmol) in THF (5 mL) and refluxed for 30 minutes. The reaction mixture was then concentrated in vacuo, re-dissolved into ethyl acetate (150 mL), washed with saturated ammonium chloride (2x 50 mL), brine (1x 50 mL), and dried over $MgSO_4$. The organic layer was then filtered, concentrated in vacuo, and purified via flash chromatography (2:1 hexanes: acetone) to afford (0.071 g, 73%) of ODIBO-EG$_4$-F as a faint yellow oil.

**$^1$H-NMR**: 7.22-7.26 (m, 3H), 7.10-7.12 (m, 1H), 7.03-7.04 (d, J = 2.5 Hz, 1H), 6.90-6.93 (dd, J = 8.4, 2.6 Hz, 1H), 5.17-5.20 (d, J = 12.0 Hz, 1H), 4.62-4.64 (t, J = 4.2 Hz, 1H), 4.53-4.56 (d, J = 12.0 Hz, 1H), 4.50-4.52 (t, J = 4.2 Hz, 2H), 4.16-.4.18 (t, 4.8 Hz, 2H), 3.87-3.89 (t, J = 4.8 Hz, 2H), 3.78-3.80 (t, J = 4.2 Hz, 1H), 3.70-3.76 (m, 9H), 1.32 (s, 9H).

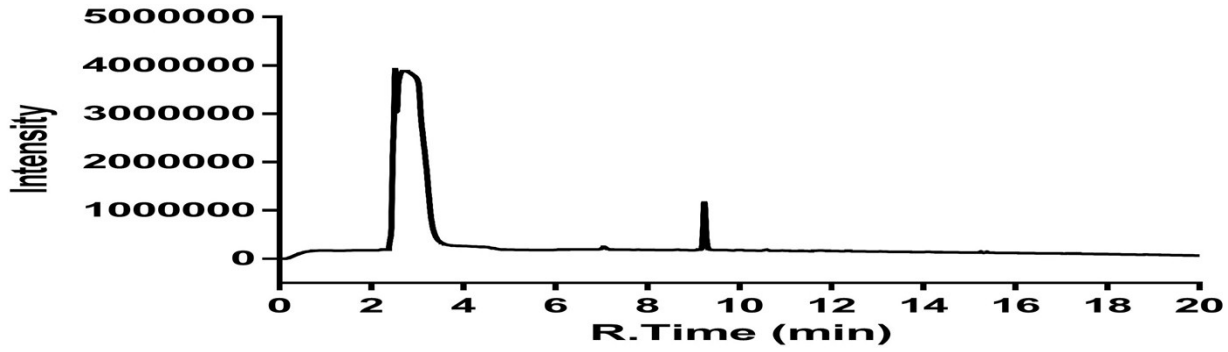
**$^{13}$C-NMR**: 167.34, 158.79, 149.14, 146.99, 126.92, 125.61, 123.75, 121.40, 118.37, 117.92, 117.70, 114.66, 114.22, 110.75, 83.37 (d, $^1$JCF = 168 Hz), 78.08, 71.09, 71.04, 70.92, 70.88, 70.62 (d, $^2$JCF = 20 Hz), 69.83, 67.93, 34.60, 31.60.

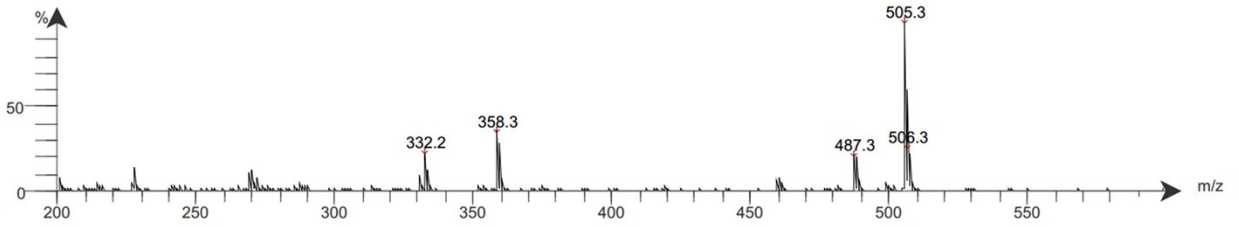
**HRMS**: $C_{27}H_{34}FO_5^+$, calc. [M+H] 457.2385, found 457.2389.

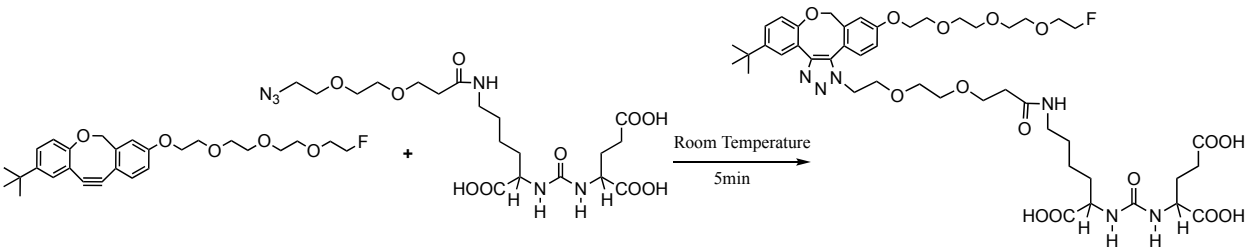



**PSMA-N$_3$**: PSMA-NH$_2$ (1 mg, 3.13 µmol) was dissolved in 30 µL of anhydrous DMSO and mixed with another 30 µL of DMSO containing $N_3$-NHS (1.4 mg, 4.66 µmol). 10 µL of DIPEA was added to the mixture and incubated at room temperature for 30min. The reaction was loaded on HPLC for analysis and further purification to yield 1.4 mg of PSMA-N$_3$

**ESI-MS**: $C_{19}H_{32}N_6O_{10}^+$, Calc. [M+H] 504.50, found 505.30

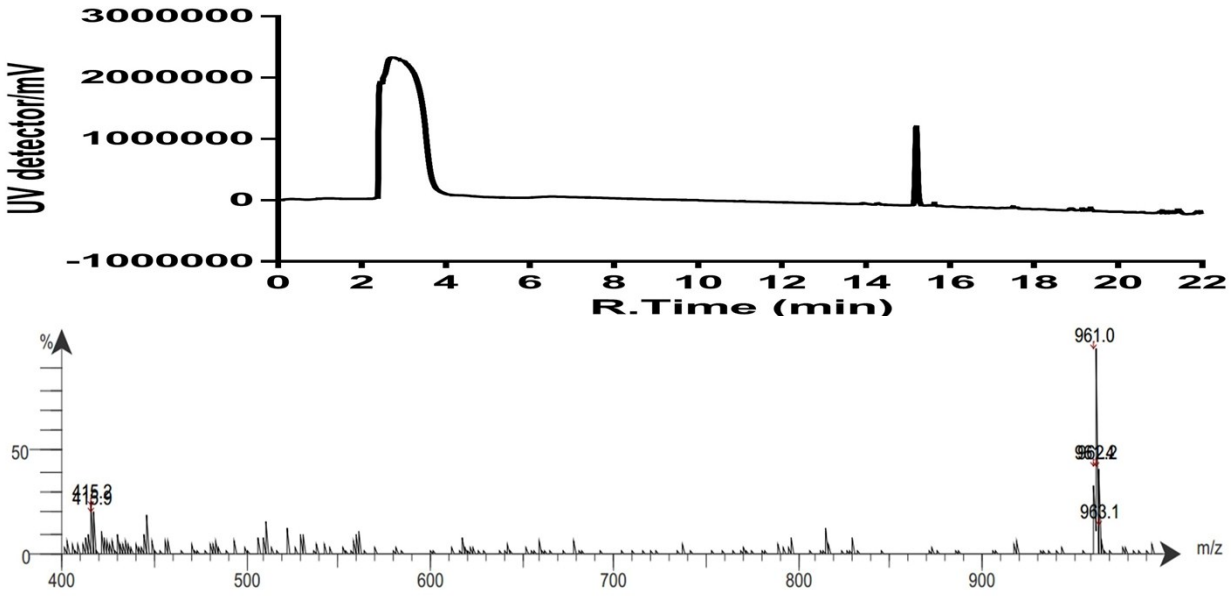


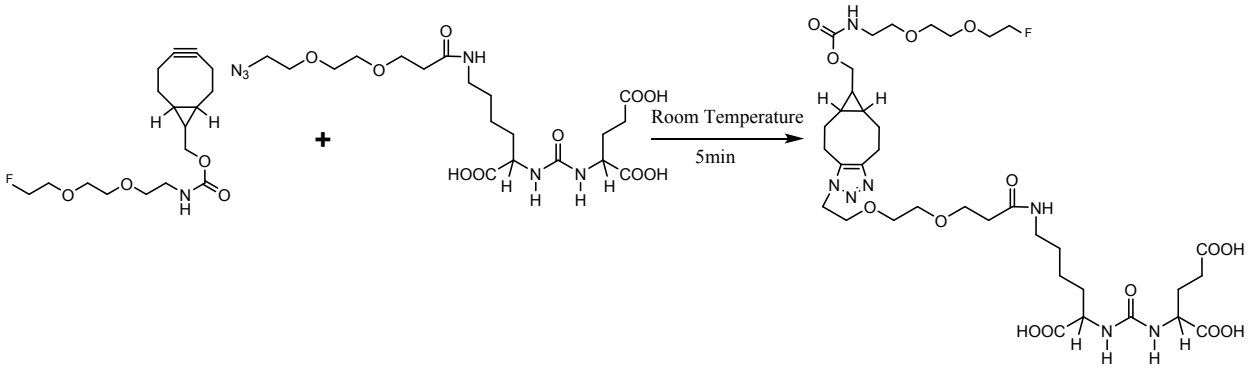



**ODIBO-EG$_4$-F-PSMA-N$_3$**: PSMA-N$_3$ (10 µg, 0.02 µmol) was dissolved in 10 µL of DMSO. ODIBO-EG$_4$-F (15 µg, 0.03 µmol) was dissolved in 10 µL of DMSO and added to the previous solution. The mixture was incubated at room temperature for 5min before loading on HPLC for analysis.

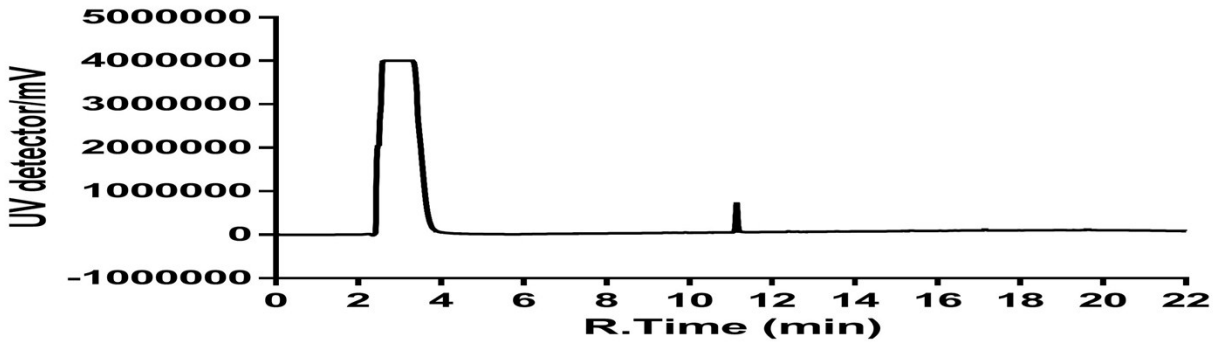
**ESI-MS:** $C_{46}H_{65}FN_6O_{15}^+$, Calc. [M+H] 961.05, found 961.00

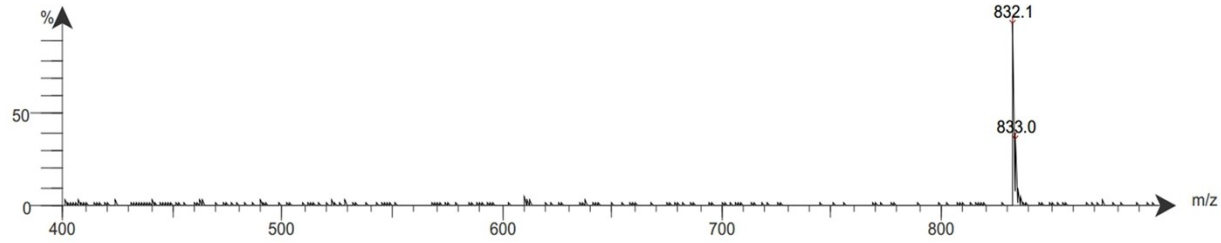





**BCN-EG$_3$-F-PSMA-N$_3$**: PSMA-N$_3$ (10 µg, 0.02 µmol) was dissolved in 10 µL of DMSO. BCN-EG$_3$-F (65 µg, 0.2 µmol) was dissolved in 10 µL of DMSO and added to the previous solution. The mixture was incubated at room temperature for 5 min before loading on HPLC for analysis.

**ESI-MS**: $C_{36}H_{58}FN_7O_{14}^+$, Calc. [M+H] 832.89, found 832.10




**Kinetic experiments.**

The kinetics of the reactions of ODIBO-EG$_4$ and BCN-EG$_3$ with water-soluble azide NH$_2$-EG$_4$-N$_3$ was studied in methanol or PBS (with 5% of MeOH for solubility, pH=7.4) at 25.0±0.1°C. The accurate rate measurements were conducted under pseudo-first order conditions, using variable concentrations of the azide at 10-fold or higher excess. Consumption of ODIBO-EG$_4$ was followed by the decay of the characteristic alkyne peak at 321 nm, while the progress of the reaction BCN-

$EG_3$ was monitored by the formation of triazole band at 231 nm. The rates were measured in triplicate at each azide concentration. The second order rate constants have been calculated by the least squares analysis of the observed rate constant dependence on the azide concentration.

| | $k$ ($M^{-1}s^{-1}$) in PBS | $k$ ($M^{-1}s^{-1}$) in MeOH |
|---|---|---|
| ODIBO-$EG_4$ | 7.9±0.3 | 1.40±0.04 |
| BCN-$EG_3$ | 0.29±0.01 | 0.051±0.004 |

**Radiochemistry Experiment**

Analytical reverse-phase HPLC was performed on a SPD-M30A photodiode array detector (Shimadzu) and model 105S single-channel radiation detector (Carroll & Ramsey Associates) using Gemini 5μ C18 column (250 x 4.6 mm). The flow was 1 mL/min. Solvent A is 0.1% TFA in water and solvent B is 0.1% TFA in acetonitrile. The mobile phase was 5% solvent B and 95% solvent A from 0 to 2min and ramped to 95% solvent B and 5% solvent A in 20min.

**Radiochemistry**

The radiolabeling of $^{18}$F-**2** was based on the following protocol. 2mg of **1** was dissolved in 20 μL of anhydrous acetonitrile and added with 7.4 GBq of TBA$^{18}$F. The reaction was sealed and heated at 85$^{o}$C for 10min. The reaction mixture was then quenched with 1mL of 5% acetic acid and passed through aluminum column (Sep-Pak) to remove unreacted $^{18}$F. The crude was loaded on HPLC for purification and the fraction containing $^{18}$F-**2** was collected.

For $^{18}$F-**4**, 2mg of **3** was dissolved in 100 μL in DMSO, THF or acetonitrile and added with 370 MBq of TBA$^{18}$F. The reaction was sealed and heated and room temperature, 60 $^{o}$C, 80 $^{o}$C and 100 $^{o}$C for 10, 20 or 30min. The reaction was quenched with 1mL of 5% acetic acid and passed through aluminum column (Sep-Pak). The crude was loaded on HPLC for purification and the fraction containing $^{18}$F-**4** was collected. For large scale reaction, 2mg of **3** was dissolved in 20 μL of acetonitrile and added with 7.4 GBq of TBA$^{18}$F. The reaction was sealed and heated at 80 $^{o}$C for 10min. The following procedures are the same as previously described.

For $^{18}$F-**6**, the pH of 185 MBq of $^{18}$F-**2** was adjusted to 7 with 0.1N NaOH followed by adding 10 μg of **5**. The mixture was incubated at room temperature for 5min before loading on HPLC for analysis and purification.

For $^{18}$F-**7**, the pH of 185 MBq of $^{18}$F-**4** was adjusted to 5.5, 7 or 8.5 with 0.25M of ammonium acetate buffer (pH 5.5), 0.1N NaOH or 1M borate buffer (pH 8.5). 10 or 50 μg of **5** was added to the solution and incubated at room temperature, 40$^{o}$C, 60$^{o}$C and 80$^{o}$C for 15min. The mixture was loaded on HPLC for analysis and purification.

Both $^{18}$F-**6** and $^{18}$F-**7** collected from HPLC was reconstituted in 1X PBS and adjusted to pH 7 with 0.1N NaOH. The solution was then subjected to rotary evaporation to remove the acetonitrile. The final solution was used for further in vivo experiments.

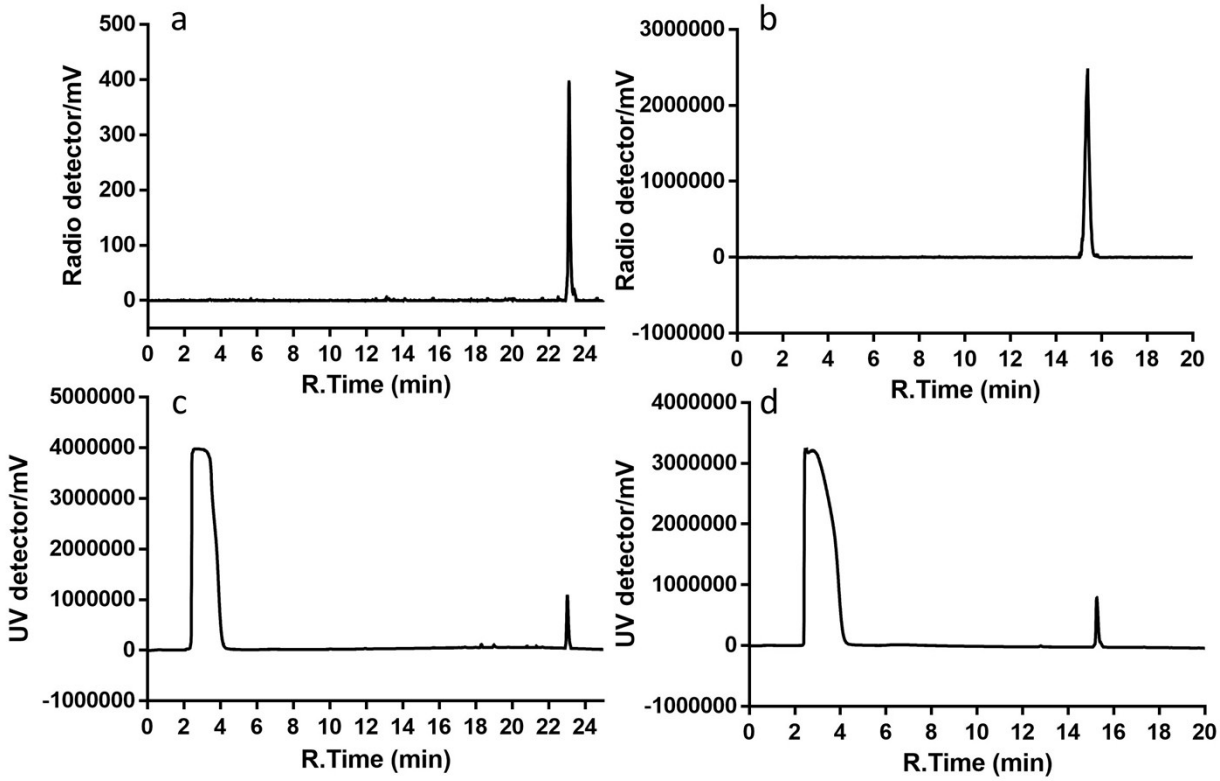



Figure S1. Radio HPLC profile of (a) $^{18}$F-**2** and (b) $^{18}$F-**4**. UV HPLC profile of (c) $^{19}$F-**2** and (d) $^{19}$F-**4**

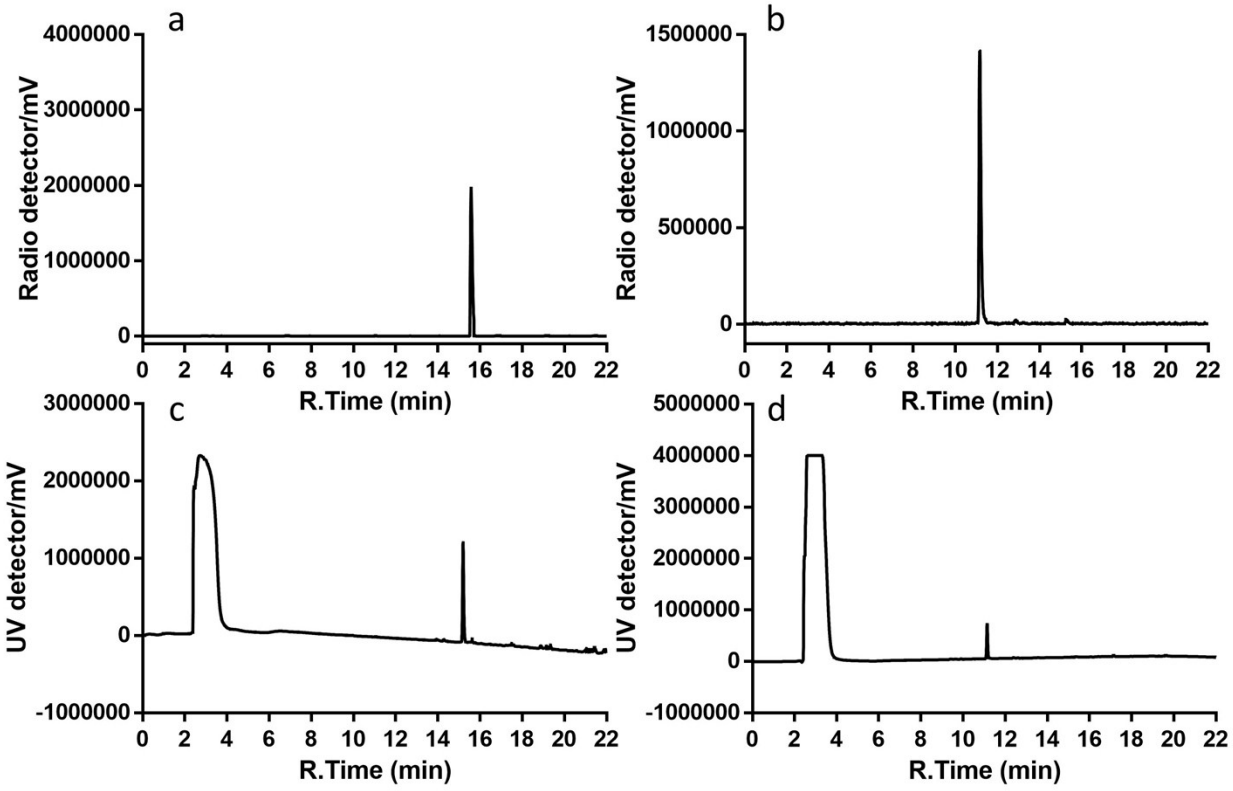



Figure S2. Radio HPLC profile of (a) $^{18}$F-**6** and (b) $^{18}$F-**7**. UV HPLC profile of (c) $^{19}$F-**6** and (d) $^{19}$F-**7**

**In vitro cell binding assay**

The PSMA specificity of substrates PSMA-617, $^{19}$F-**6** and $^{19}$F-**7** were evaluated using NAALADase enzyme activity of PSMA as described with modification.[3] In brief, rhPSMA (R&D system) was diluted to 0.4 µg/mL in assay buffer containing 50 mM HEPES and 0.1 M NaOH (pH 7.5). The substrate was diluted to 40 µM in assay buffer. Then 125µL of diluted rhPSMA and 125 µL of diluted substrate were combined. For positive control, 125µL of Ac-Asp--Glu (Sigma Aldrich) was used. For negative control, inactivate 125 µL of rhPSMA by heating it at 95°C for 5 min, then combined with 125 µL of Ac-Asp-Glu substrate. The mixture was incubated at 37°C for 1 hour. The reaction was stopped by heating at 95°C for 5 min, then cooled down to room temperature. Orthophthaldiadehyde (OPA) was prepared in OPA buffer to concentration of 15 mM with 0.2 M NaOH and 0.1% β-Mercaptoethanol (v/v), and 250 µL of OPA solution was added to all vials, vortexed, incubated at room temperature for 10 min. Then 200 µL aliquot was transferred to a F16 black Maxisorp plate (Nunc). The signal was read at excitation and emistion wavelength of 330 nm and 450 nm (top read), respectively in endpoint mode. The NAALADase specific activity was calculated according to manufacturer's instruction.

**Small animal PET imaging**

Animal procedures were performed according to a protocol approved by the UNC Institutional Animal Care and Use Committee. LNCap tumor bearing mice were intravenously injected with

3.7 MBq (~100 μCi) of $^{18}$F-**6** or $^{18}$F-**7**. At 40 min and 120 min post injection, a 10-min static emission scan was acquired with a small animal PET scanner (GE eXplore, Vista). In the blocking study, 100 μg of the unlabeled **5** was coinjected with $^{18}$F-**7** and imaged at 120min post injection. The region of interests (ROIs) were calculated as percentage of injected dose per gram of tissue based on the assumption of 1g/mL of tissue density.

**Synthesis of MSA-azide**

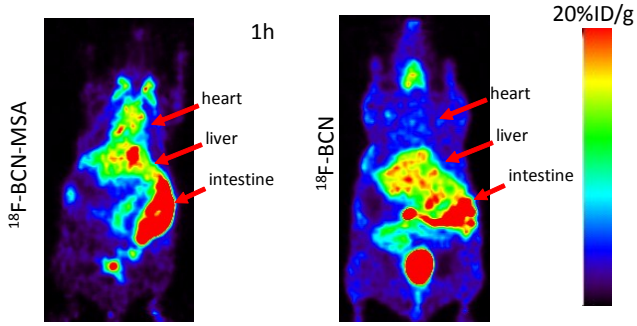
1 mg of mouse serum albumin (Sigma) was dissolved in 100uL water. 50 eq of azide-NHS (broadpharm) was dissolved in 20uL DMSO and added to the MSA solution. 6uL of 20x borate buffer was added to adjust the pH to 8.5. The mixture was incubated at room temperature for 3h and purified with PD-10 column.

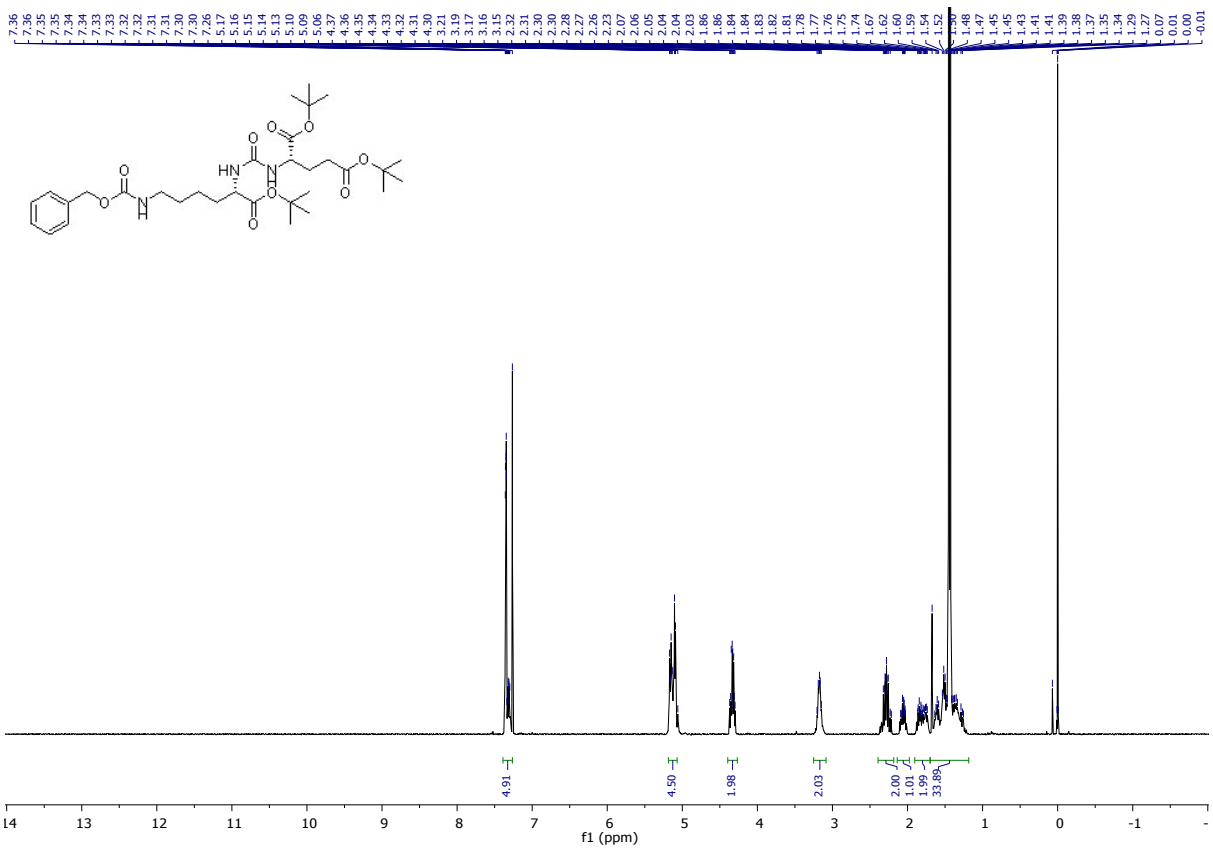
**Synthesis of $^{18}$F-BCN-MSA**

111MBq of $^{18}$F-BCN was adjusted to pH 7.0 using 0.1N NaOH followed by adding 100ug of the MSA-azide. The reaction was purified by PD-10 column and desired fraction containing $^{18}$F-BCN-MSA was collected.

**$^{18}$F-BCN-HSA imaging**

3.7MBq of $^{18}$F-BCN and $^{18}$F-BCN-HSA was intravenously injected into non-tumor bearing animals. At 1h post injection, animals were subjected to 10min static emission scan. $^{18}$F-BCN-HSA demonstrated prominent blood activity in heart region at 1 h post injection. In contrast, $^{18}$F-BCN did not have significant uptake in heart region. Overall, radiolabeling of large molecules is more complicated. The hydrophilicity of final agents, position of modification, degree of modification, and charge change could all affect the distribution of the final agents.

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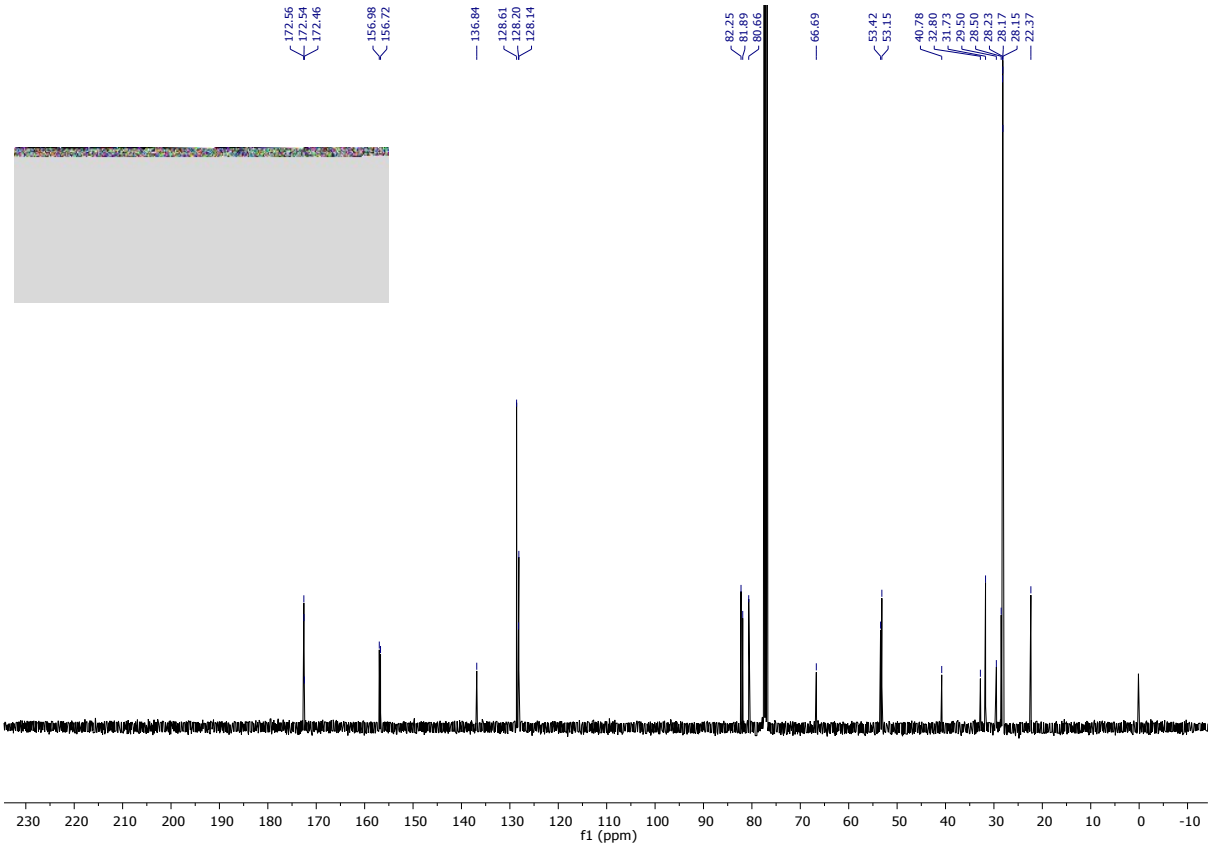
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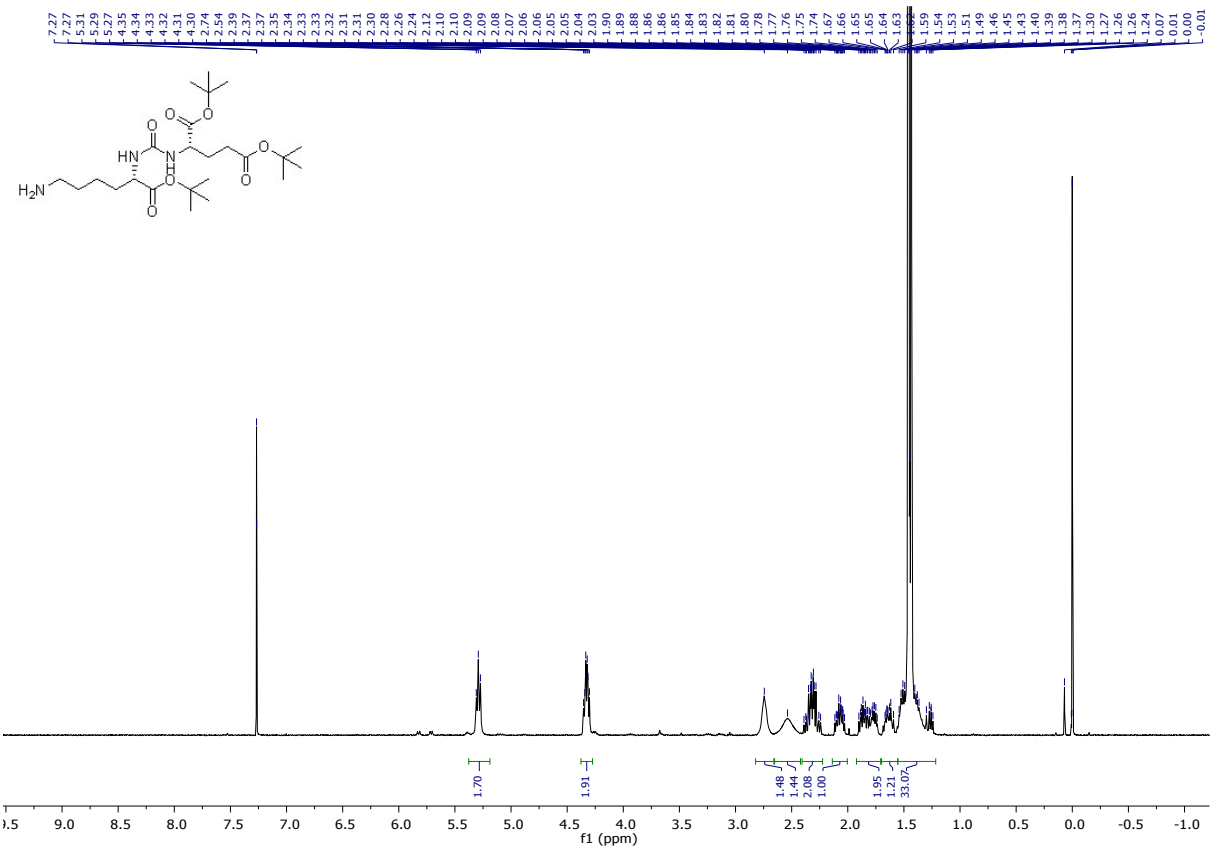
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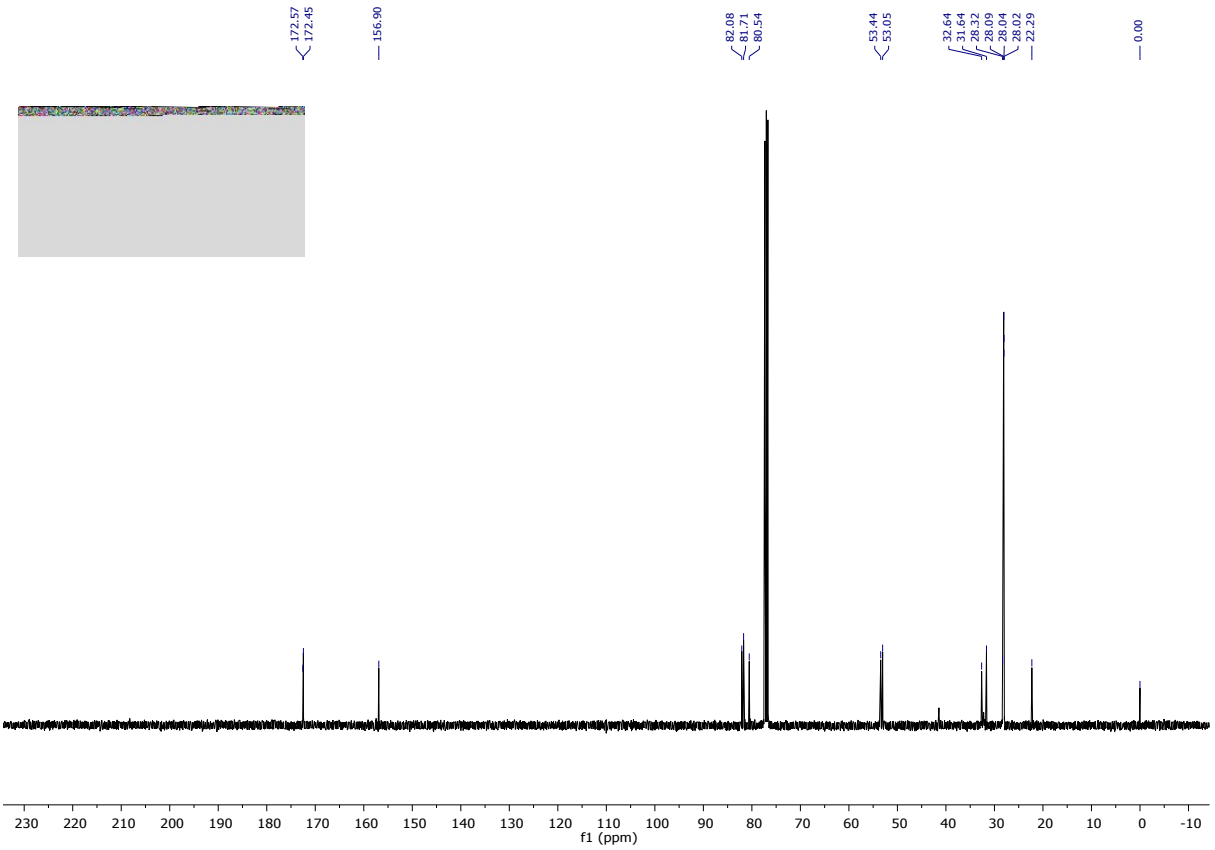
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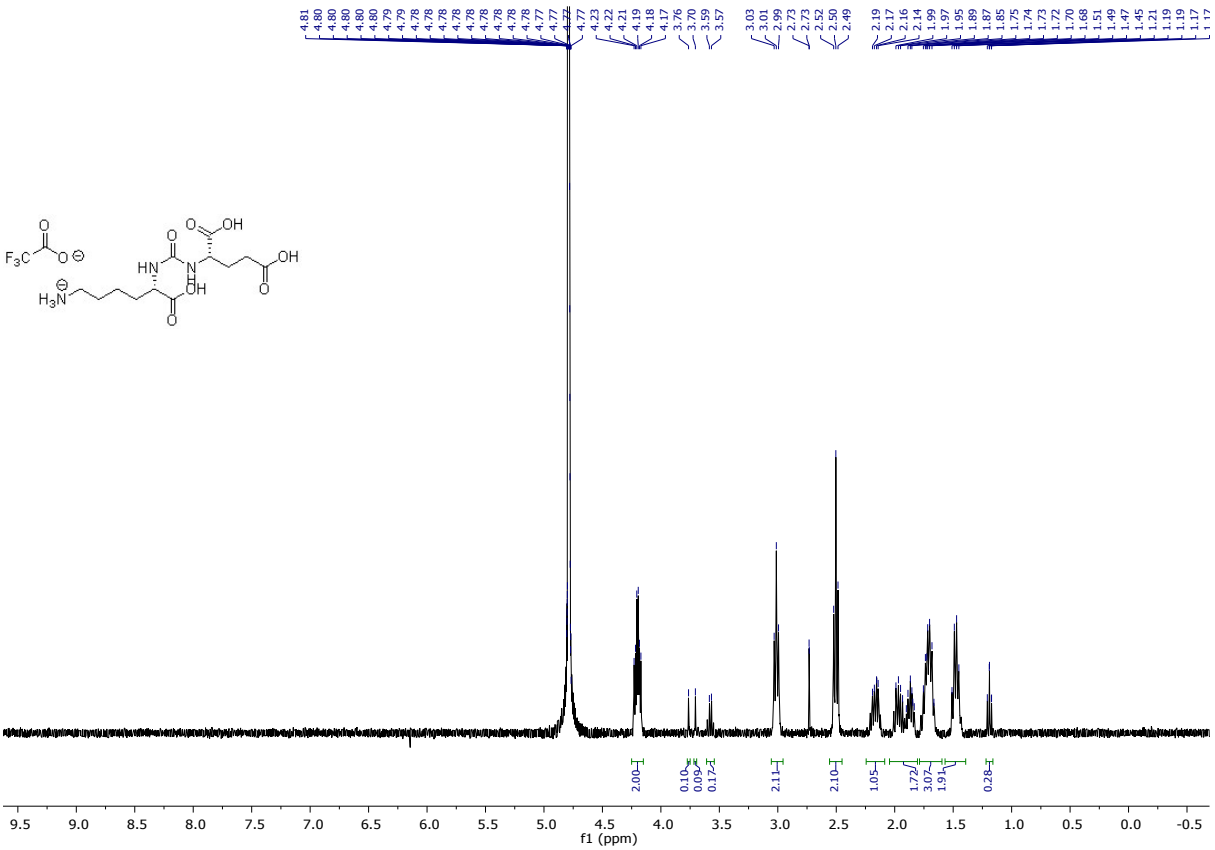
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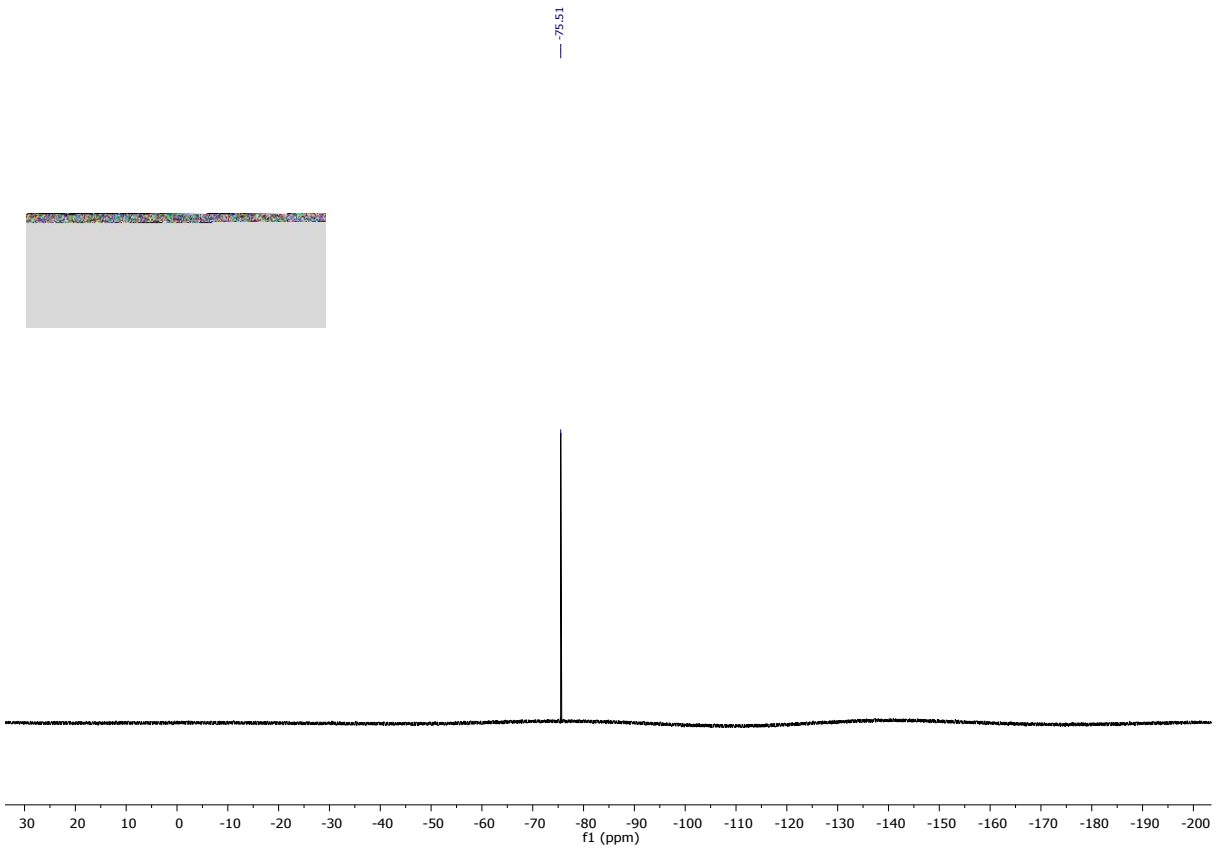
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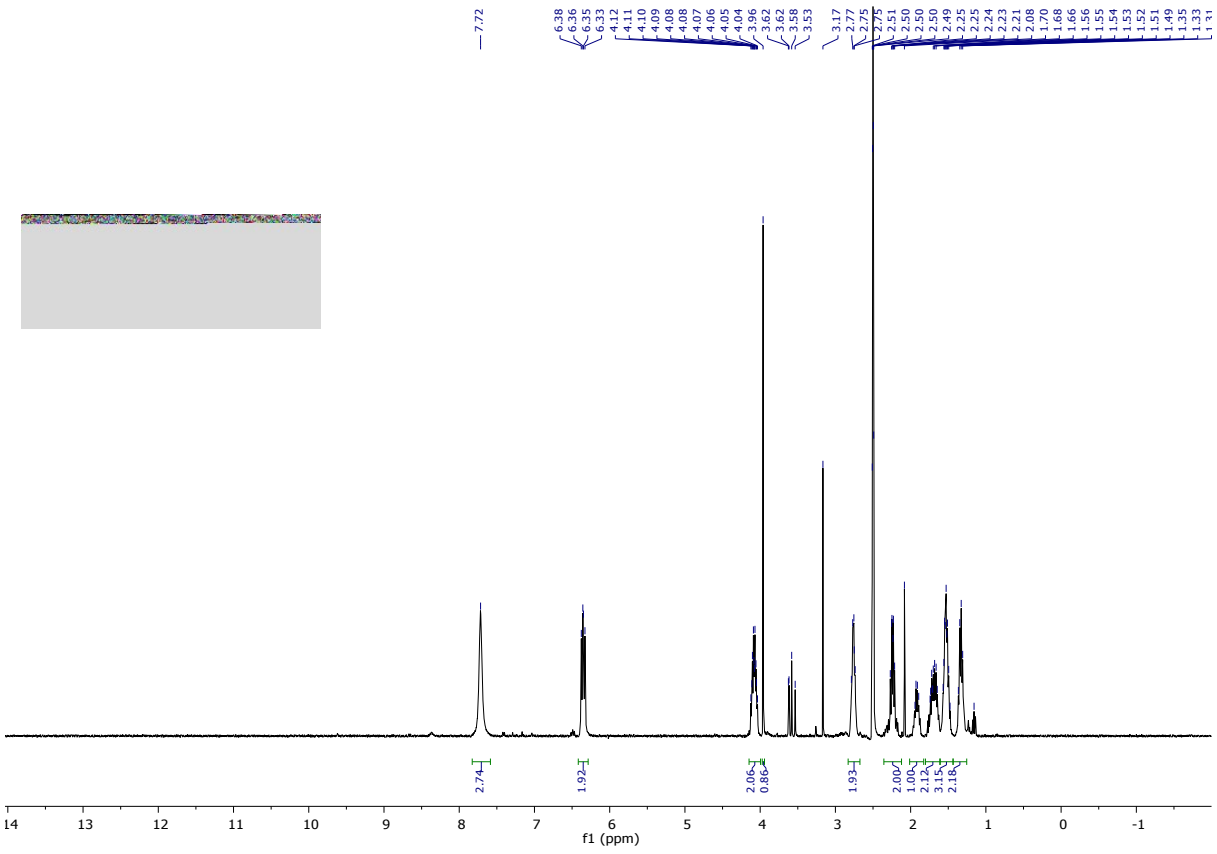
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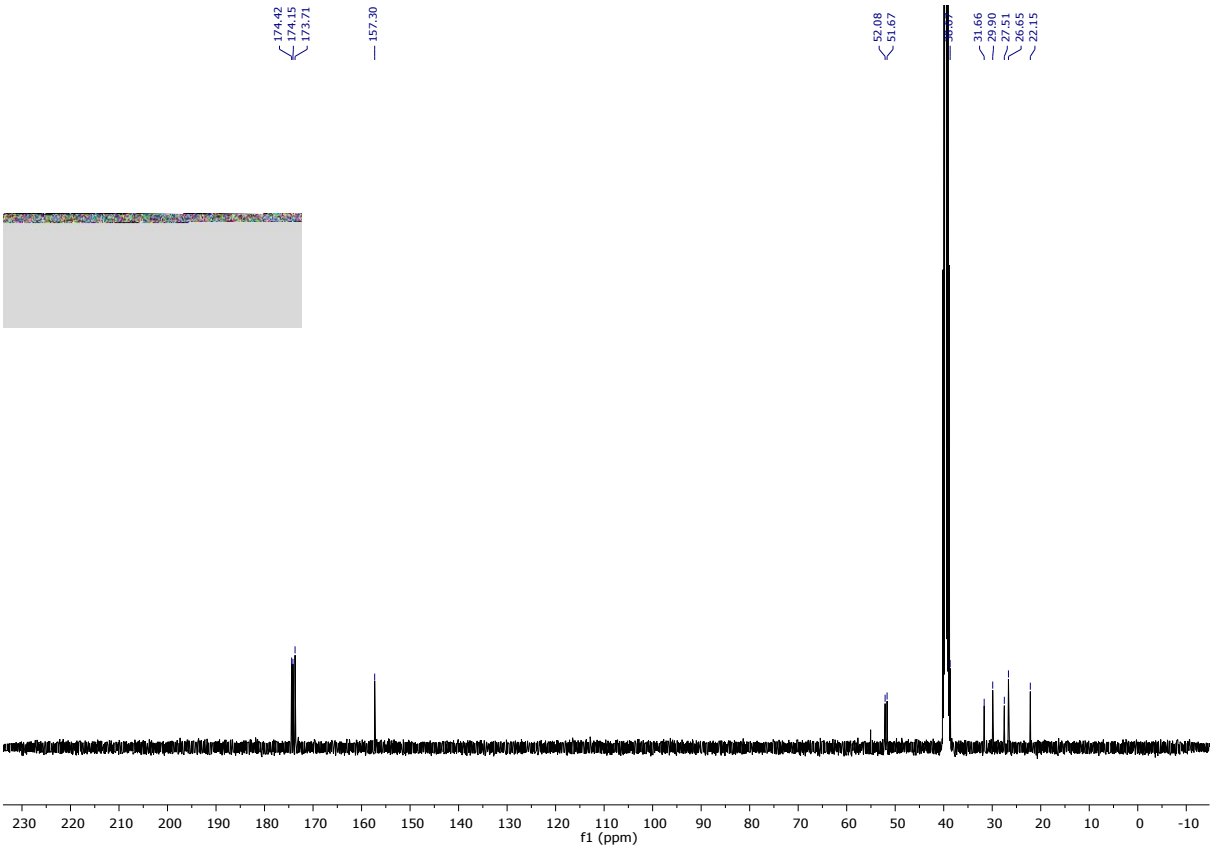


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