A Click-addressable Cassette For Photoaffinity Labeling

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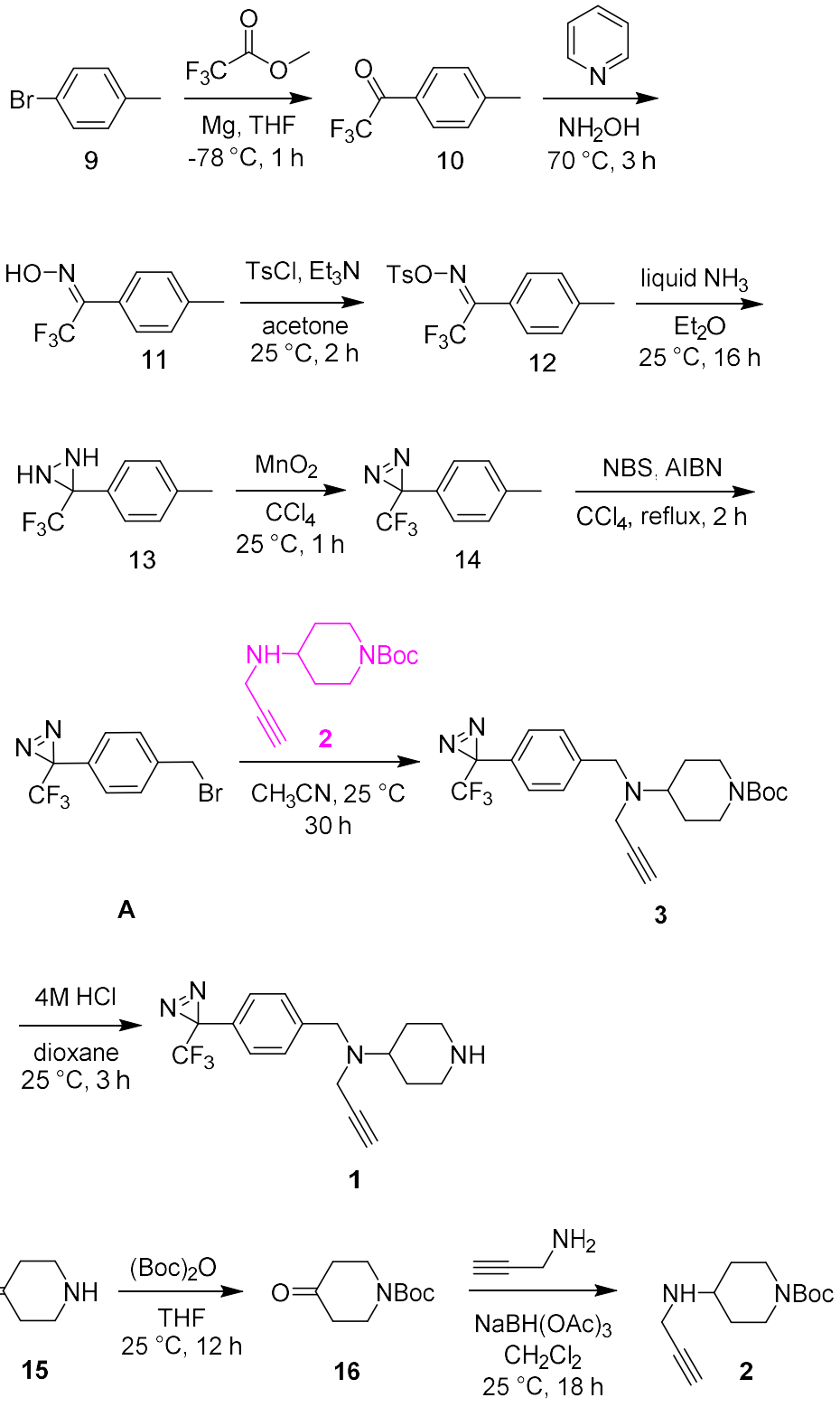
A. General Experimental Information

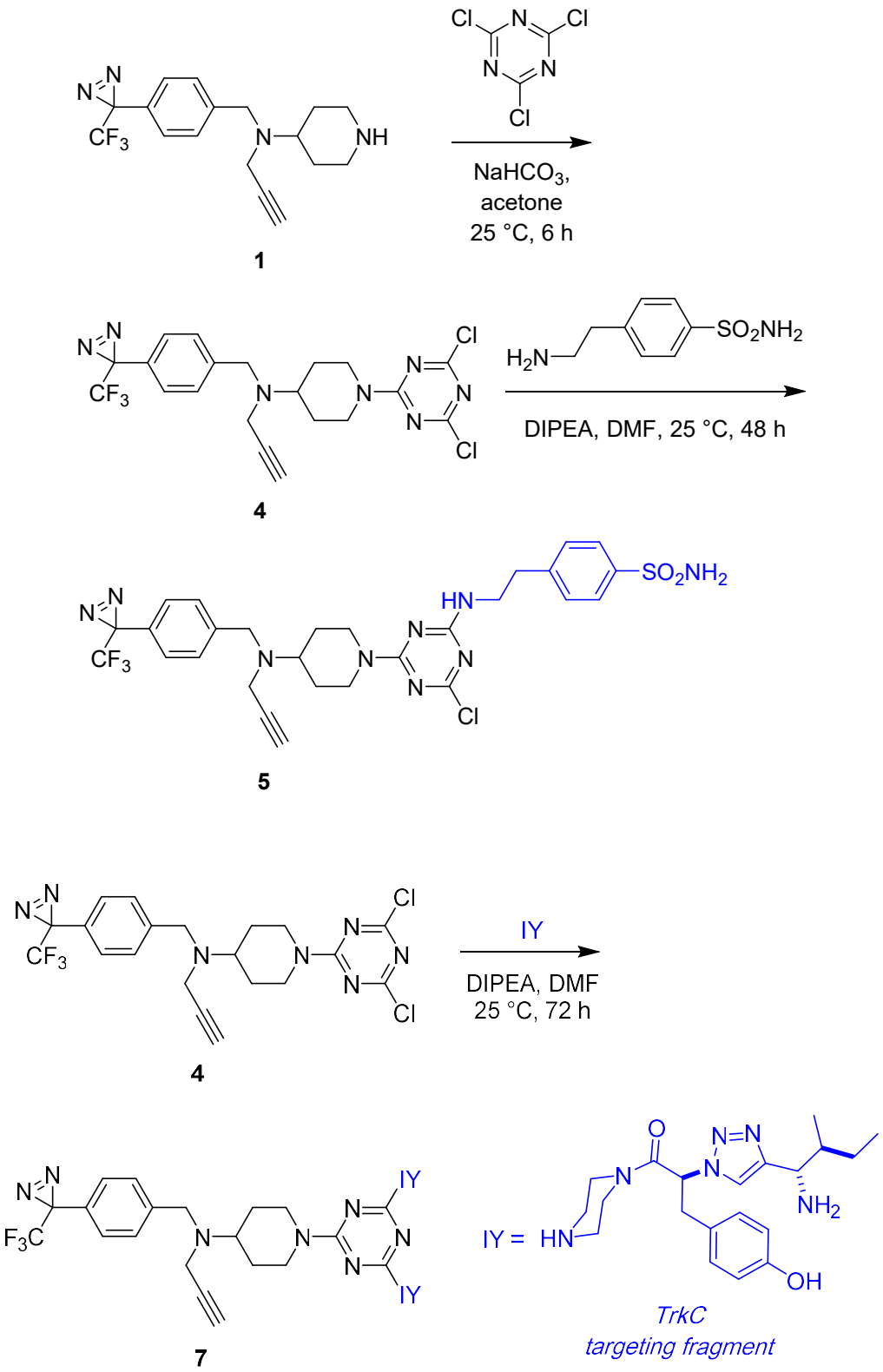
All reactions were carried out under an inert atmosphere (nitrogen or argon where stated) with dry solvents under anhydrous conditions. Glassware for anhydrous reactions was dried in an oven at 140 ºC for minimum 6 h prior to use. Dry solvents were obtained by passing the previously degassed solvents through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at a high commercial quality (typically 97 % or higher) and used without further purification, unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV. Flash column chromatography was performed using silica gel 60 (Silicycle, 230-400 mesh). ^{1}H and ^{13}C spectra were recorded on a 400 MHz spectrometer and were calibrated using residual non-deuterated solvent as an internal reference. The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, ddd = doublet of doublet of doublets.

Human Carbonic Anhydrase IX was purchased from Sino Biological Inc. (10107-H08H) as a lyophilized powder. Human TrkC was purchased from Sino Biological Inc. (10048-H08H-20) as a lyophilized powder. MDA-MB-231 cells (from American Type Culture Collection) were cultured on 75 cm^2 culture flasks in Dulbecco's Modified Eagle Medium/nutrient mixture F-12 (DMEM/F12, Sigma Chemical, St. Louis, MO) supplemented with 10 % FBS. NIH3T3-TrkC cells were obtained and cultured according to previous procedures[1]. Cells were cultured in a humidified incubator at 37 °C with 5 % CO_2 and 95 % air. Carbonic anhydrase IX polyclonal antibody and goat anti-rabbit (H+L) secondary antibody (HRP conjugated) were obtained from Thermo Fisher Scientific. ECL western blotting substrate was obtained from Pierce, Thermo Scientific.

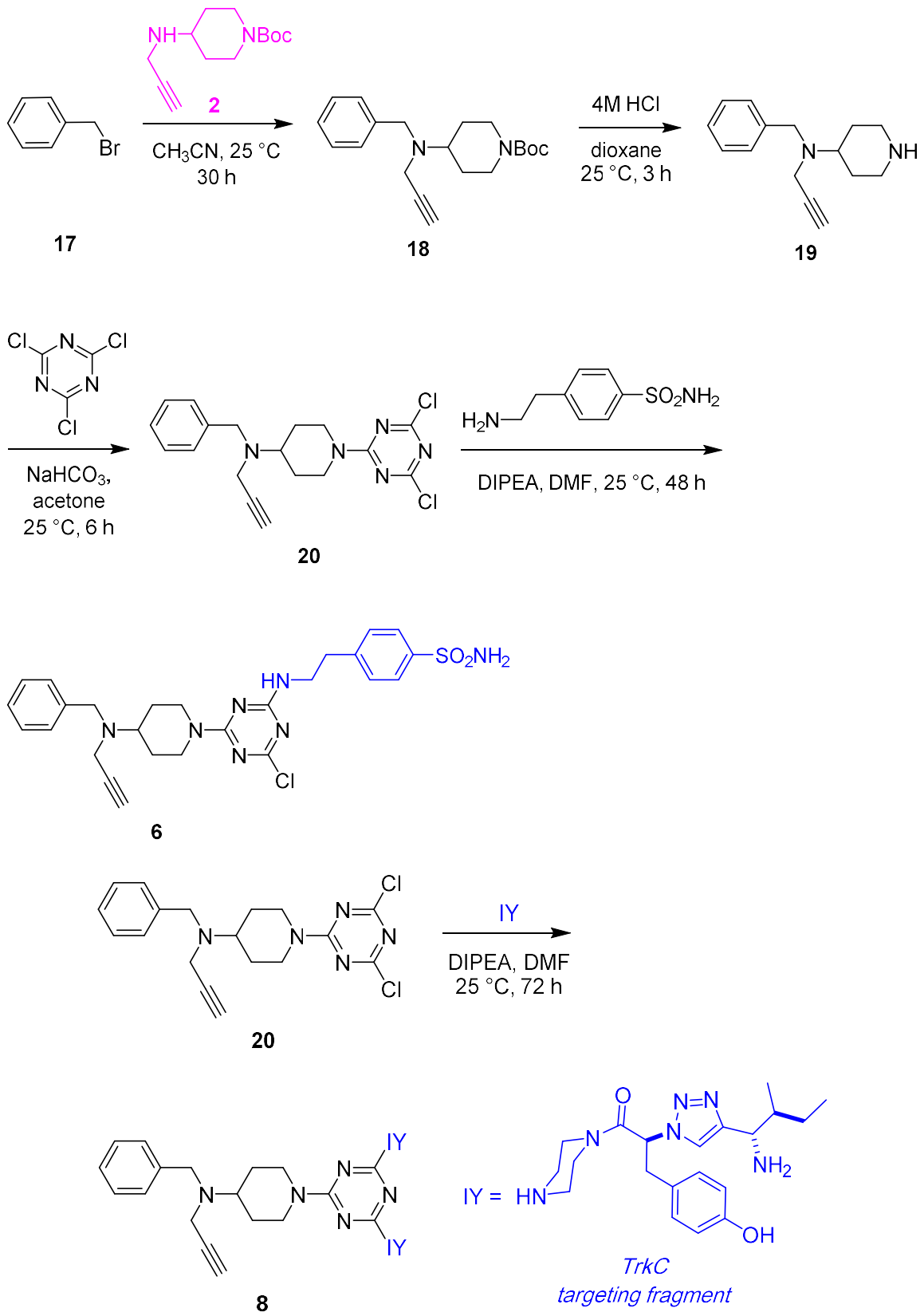
B. Synthesis of Photoaffinity Labeling Ligands



Scheme S1. Synthetic scheme of PAL cassette **1.**



Scheme S2. Synthetic scheme of PAL ligand for CAIX (**5**) and TrkC (**7**)**.**



Scheme S3. Synthetic scheme of PAL negative ligand **6** and **8.**

Synthesis of intermediate 2:

Synthesis of *tert*-butyl 4-oxopiperidine-1-carboxylate (**16**)

At 0 °C, Et_3N (70 mmol) was added dropwise to a stirred solution of 4-piperidone (10 mmol) in 20 mL DCM and 4 mL MeOH. Then $(Boc)_2O$ (12 mmol) was added dropwise at 0 °C. The reaction mixture was then stirred at room temperature overnight followed by the addition of 30 mL H_2O. The reaction mixture was extracted with DCM (3 x 60 mL). Organic phase was collected, dried over $MgSO_4$. The solvent was removed under vacuum, washed with hexane to give the product as a off-white solid (1.93 g, 95 %).

Synthesis of *tert*-butyl 4-(prop-2-yn-1-ylamino)piperidine-1-carboxylate (**2**)

Sodium triacetoxyborohydride (8.25 mmol) was added dropwise to a solution **16** (5.50 mmol) and propargylamine (6.25 mmol) in DCM and stirred at room temperature overnight. The reaction was quenched with 1 N HCl and basified with sat. Na_2CO_3 solution. The mixture was then extracted with DCM twice. Organic phase was collected, washed with Brine, dried over $MgSO_4$. The solvent was removed under vacuum to give the product as a yellow oil (1.09 g, 79 %). The crude material was used in the next step without further purification.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.01 (d, *J* = 12.5 Hz, 2H), 3.47 (d, *J* = 2.4 Hz, 2H), 2.86 (ddd, *J* = 12.7, 6.3, 3.1 Hz, 3H), 2.21 (t, *J* = 2.4 Hz, 1H), 1.85 – 1.76 (m, 2H), 1.46 (s, 9H), 1.28 (m, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 154.8, 82.1, 79.4, 71.3, 53.0, 42.3, 35.0, 32.0, 28.4.

Synthesis of PAL ligand:

Synthesis of *tert*-butyl 4-(prop-2-yn-1-yl(4-(3-(trifluoromethyl)-3H-diazirin-3-yl) benzyl)amino)piperidine-1-carboxylate (**3**)

Intermediate **A** was synthesized as reported[2]. (Note: for the synthesis of compound **13**, liquid NH_3 was trapped in dry ice-acetone bath, then warm up to r.t. in a sealed tube) Compound **2** (6 mmol) was added to a solution of **A** (3 mmol) in 15 mL MeCN and stirred at room temperature for 30 h. Solvent was removed under vacuum. The crude product was purified with flash chromatography (ethyl acetate : hexane = 1 : 5) to give compound **3** as a light yellow oil (0.86 g, 64 %).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.1 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 4.11 (s, 2H), 3.77 (s, 2H), 3.31 (d, *J* = 1.8 Hz, 2H), 2.78 (d, *J* = 14.2 Hz, 3H), 2.23 (s, 1H), 1.94 (d, *J* = 13.1 Hz, 2H), 1.56 (s, 2H), 1.49 (s, 9H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 154.7, 141.5, 129.0, 127.9, 127.0, 126.5, 79.7, 79.5, 77.2, 73.0, 58.7, 53.0, 43.0, 38.7, 29.7, 28.4.
(Noted: we could not find coupling of CF_3 in any ^{13}C NMRs; maybe they are too disperse)

^{19}F-NMR (376 MHz, $CDCl_3$) δ -65.33 (s)

LRMS (ESI+) m/z calcd for $C_{22}H_{27}F_3N_4NaO_2^+$ $(M+Na)^+$ 459.2; found 459.0.

Synthesis of N-(prop-2-yn-1-yl)-N-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl) piperidin-4-amine (**1**)

2.5 mL HCl (4M in dioxane) was added to **3** (0.5 mmol) and stirred at room temperature for 3 h. The solvent was removed under vacuum to give **1** as a light yellow oil (quantitative yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 3.78 (s, 2H), 3.31 (d, *J* = 2.3 Hz, 2H), 3.25 (d, *J* = 11.8 Hz, 2H), 2.79 – 2.68 (m, 3H), 2.40 (dd, *J* = 11.0, 3.9 Hz, 1H), 2.23 (d, *J* = 2.3 Hz, 1H), 2.03 (d, *J* = 12.3 Hz, 2H), 1.65 – 1.57 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 132.2, 131.2, 130.2, 127.1, 123.2, 82.2, 71.1, 60.0 , 50.5, 43.3, 39.0, 29.7, 28.0.

^{19}F-NMR (376 MHz, $CDCl_3$) δ -65.09 (s)

LRMS (ESI+) m/z calcd for $C_{17}H_{20}F_3N_4^+$ $(M+H)^+$ 337.2; found 336.9.

Synthesis of 1-(4,6-dichloro-1,3,5-triazin-2-yl)-N-(prop-2-yn-1-yl)-N-(4-(3-(trifluoro-methyl)-3H-diazirin-3-yl)benzyl)piperidin-4-amine (**4**)

Compound **1** (0.15 mmol) was dissolved in 2 mL acetone. $NaHCO_3$ (0.60 mmol) was added and stirred for 10 min. Afterwards, cyanuric chloride (0.15 mmol) was added and stirred at room temperature for another 6 h. The solvent was removed under vacuum. The mixture was partitioned between DCM and H_2O. The organic phase was collected, washed with H_2O and Brine, dried over $MgSO_4$. The solvent was removed under vacuum to give a light yellow oil. The crude material was used in next step without further purification.

Synthesis of 4-(2-((4-chloro-6-(4-(prop-2-yn-1-yl(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)amino)piperidin-1-yl)-1,3,5-triazin-2-yl)amino)ethyl)benzenesulfon-amide (**5**)

Compound **4** (0.2 mmol) and 4-(2-aminoethyl)benzenesulfonamide (0.2 mmol) was dissolved in 4 mL DMF. DIPEA (1.0 mmol) was added to the mixture and stirred at room temperature for 48 h. The reaction mixture was diluted with 10 mL H_2O, then extracted with ethyl acetate (3 x 10 mL). Organic phase was collected, washed with 10% w/v aqueous LiCl (10 mL) and Brine (10 mL), dried over $MgSO_4$. The solvent was removed under vacuum. The crude product was purified with flash chromatography (ethyl acetate : hexane = 1 : 1) to give **5** as a white solid (68 mg, 50 %).

^{1}H NMR (400 MHz, acetone-d_6) δ 7.83 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 6.86 (s, 1H), 6.47 (s, 2H), 4.76 (d, *J* = 12.6 Hz, 1H), 4.63 (d, *J* = 12.5 Hz, 1H), 3.87 (d, *J* = 7.4 Hz, 2H), 3.70 (d, *J* = 6.8 Hz, 2H), 3.37 (d, *J* = 2.0 Hz, 2H), 3.03 (d, *J* = 7.2 Hz, 5H), 2.71 (s, 1H), 2.07 (s, 2H), 1.57 (d, *J* = 12.9 Hz, 2H).

^{13}C NMR (101 MHz, acetone-d_6) δ 169.0, 166.0, 164.3, 144.1, 142.7, 142.3, 129.3 , 127.0, 126.4, 126.2, 121.0, 116.7, 79.8, 73.7, 58.6, 52.7, 42.5, 41.8, 38.5, 35.2, 35.0, 31.4.

^{19}F NMR (376 MHz, acetone-d_6) δ -66.32 (s).

HRMS (ESI+) m/z calcd for $C_{28}H_{30}ClF_3N_9O_2S$ $(M+H)^+$ 648.1878; found 648.1872.

Synthesis of (2S,2'S)-1,1'-((6-(4-(prop-2-yn-1-yl(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)amino)piperidin-1-yl)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(2-(4-((1S,2S)-1-amino-2-methylbutyl)-1H-1,2,3-triazol-1-yl)-3-(4-hydroxyphenyl)propan-1-one) (**7**)

To a solution of compound **4** (0.106 mmol) in DMF was added DIPEA (1.06 mmol) and IY[3] (0.212 mmol) in THF. The mixture was stirred at room temperature for 72 h. DMF was then removed via lyophilizer. The crude product was purified via prep-HPLC to give **7** as a white solid (28 mg, 22 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 2H), 8.21 (d, *J* = 9.1 Hz, 2H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.25 (d, *J* = 7.9 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 4H), 6.61 (d, *J* = 8.3 Hz, 4H), 6.14 – 6.04 (m, 2H), 4.59 (d, *J* = 12.2 Hz, 2H), 4.16 (dd, *J* = 23.4, 5.6 Hz, 2H), 3.75 (s, 2H), 3.65 – 3.23 (m, 26H), 3.13 (s, 2H), 2.81 (t, *J* = 11.3 Hz, 3H), 1.94 – 1.78 (m, 5H), 1.35 (dd, *J* = 12.7, 7.4 Hz, 4H), 1.05 – 0.96 (m, 2H), 0.90 – 0.82 (m, 6H), 0.76 (d, *J* = 6.7 Hz, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 166.4, 165.1, 164.7, 156.7, 143.0, 130.7, 129.6 , 126.8, 126.5, 126.1, 123.3, 115.5, 80.8, 76.0, 60.7, 59.0, 52.9, 51.8, 45.4, 43.1, 42.7, 42.4, 38.8, 37.6, 29.5, 25.4, 21.5, 15.0, 14.6, 11.7, 11.6.

^{19}F NMR (376 MHz, DMSO-d_6) δ -64.65 (s).

HRMS (MALDI+) m/z calcd for $C_{60}H_{77}F_3N_{19}O_4$ $(M+H)^+$ 1184.6353; found 1184.6298.

Synthesis of PAL negative ligand:

Synthesis of ligand **6** and **8** are similar to **5** and **7**, respectively, starting from benzyl bromide instead of compound **A**.

4-(2-((4-(4-(benzyl(prop-2-yn-1-yl)amino)piperidin-1-yl)-6-chloro-1,3,5-triazin-2-yl)amino)ethyl)benzenesulfonamide (**6**)

^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (s, 1H), 7.83 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.38 (t, *J* = 6.5 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.25 (t, *J* = 7.2 Hz, 1H), 6.88 (d, *J* = 5.6 Hz, 1H), 6.50 (s, 2H), 4.76 (d, *J* = 13.0 Hz, 1H), 4.63 (d, *J* = 12.3 Hz, 1H), 3.81 (d, *J* = 7.9 Hz, 2H), 3.70 (dd, *J* = 13.5, 6.7 Hz, 2H), 3.36 (dd, *J* = 7.6, 2.0 Hz, 2H), 3.12 – 2.94 (m, 6H), 2.70 (s, 1H), 1.59 (d, *J* = 12.4 Hz, 2H).

^{13}C NMR (101 MHz, acetone-d_6) δ 169.0, 166.0, 164.3, 144.1, 142.3, 139.8, 129.3, 128.6 (d, *J* = 4.6 Hz), 128.2, 126.9, 126.2, 80.0, 73.5, 58.3, 53.3, 42.5, 41.9, 38.2, 35.0.

LRMS (ESI+) m/z calcd for $C_{26}H_{31}ClN_7O_2S$ $(M+H)^+$ 540.2; found 540.2.

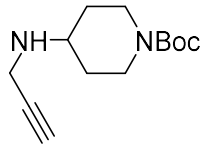
(2S,2'S)-1,1'-((6-(4-(benzyl(prop-2-yn-1-yl)amino)piperidin-1-yl)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(2-(4-((1S,2S)-1-amino-2-methylbutyl)-1H-1,2,3-triazol-1-yl)-3-(4-hydroxyphenyl)propan-1-one) (**8**)

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 2H), 8.21 (d, *J* = 6.2 Hz, 2H), 7.32 (d, *J* = 5.8 Hz, 4H), 7.27 – 7.22 (m, 1H), 6.98 (d, *J* = 8.4 Hz, 4H), 6.61 (d, *J* = 8.4 Hz, 4H), 6.13 – 6.05 (m, 2H), 4.59 (d, *J* = 12.7 Hz, 2H), 4.15 (dd, *J* = 23.7, 5.6 Hz, 2H), 3.71 (s, 2H), 3.60 – 3.45 (m, 14H), 3.41 (s, 4H), 3.32 – 3.22 (m, 8H), 3.12 (s, 1H), 2.83 (t, *J* = 11.5 Hz, 3H), 1.92 (d, *J* = 6.2 Hz, 2H), 1.84 (d, *J* = 6.0 Hz, 2H), 1.44 – 1.29 (m, 4H), 1.16 – 1.08 (m, 1H), 1.05 – 0.95 (m, 2H), 0.90 – 0.83 (m, 7H), 0.76 (d, *J* = 6.7 Hz, 4H).

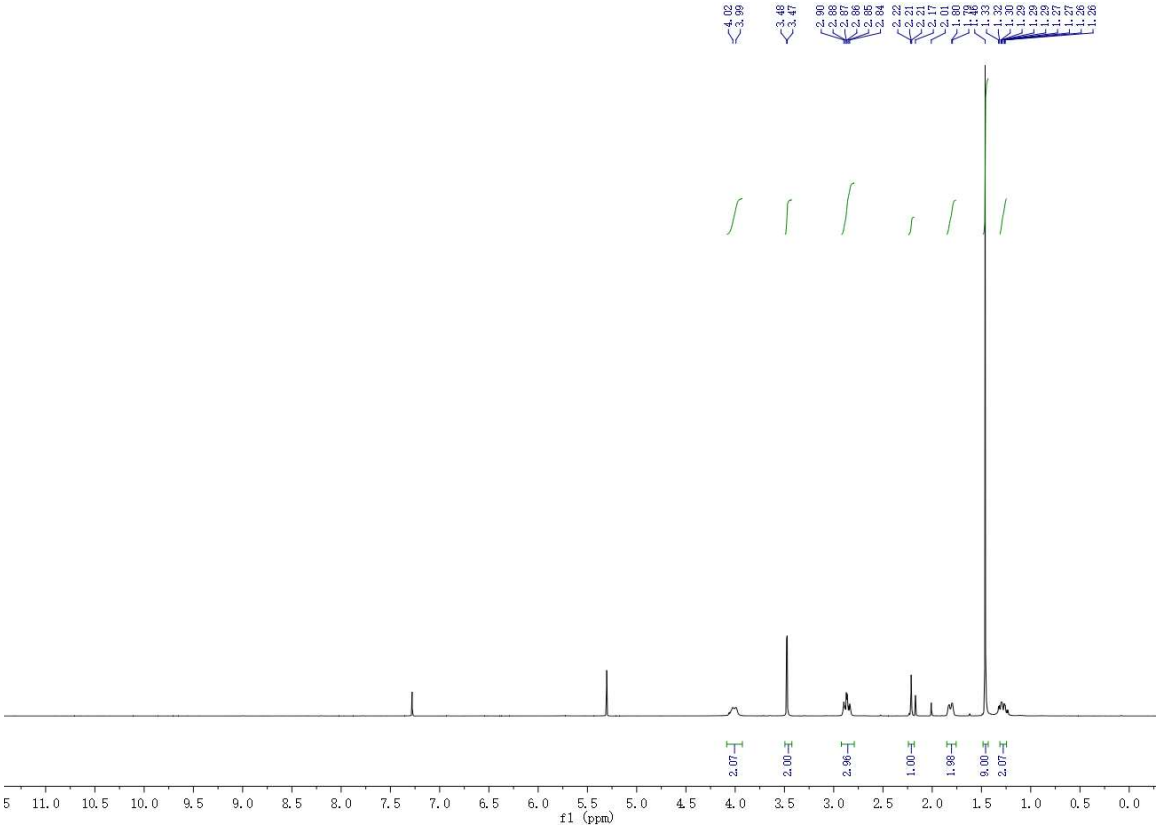
^{13}C NMR (101 MHz, DMSO-d_6) δ 166.4, 165.2, 164.7, 156.7, 140.0, 130.7, 128.9, 128.7, 127.3, 126.1, 123.3, 115.5, 80.9, 75.9, 60.7, 58.8, 53.4, 51.8, 45.5, 43.1, 42.4, 37.6, 29.6, 25.4, 21.5, 15.0, 14.56, 11.8, 11.6.

LRMS (MALDI+) m/z calcd for $C_{58}H_{78}N_{17}O_4$ $(M+H)^+$ 1076.6; found 1076.9.

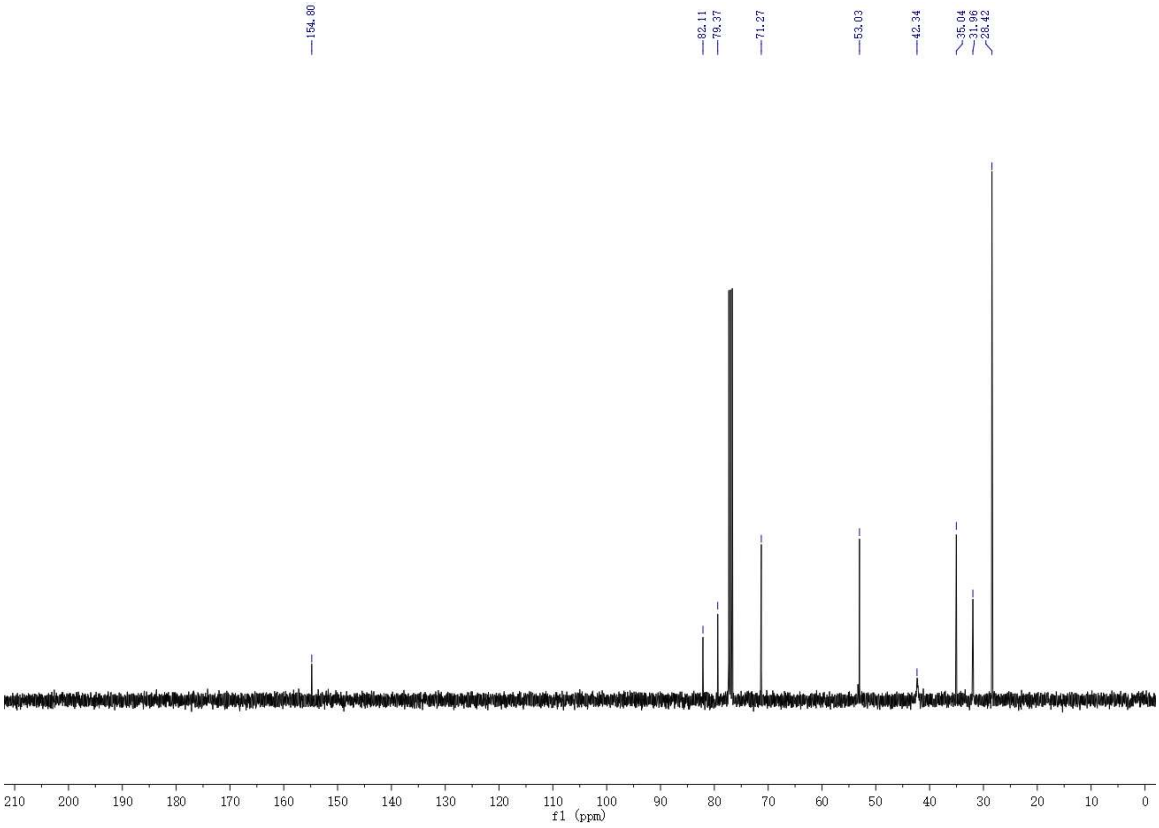
tert-butyl 4-(prop-2-yn-1-ylamino)piperidine-1-carboxylate (**2**)



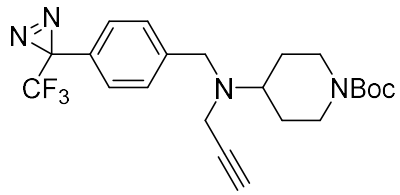
^{1}H-NMR ($CDCl_3$)



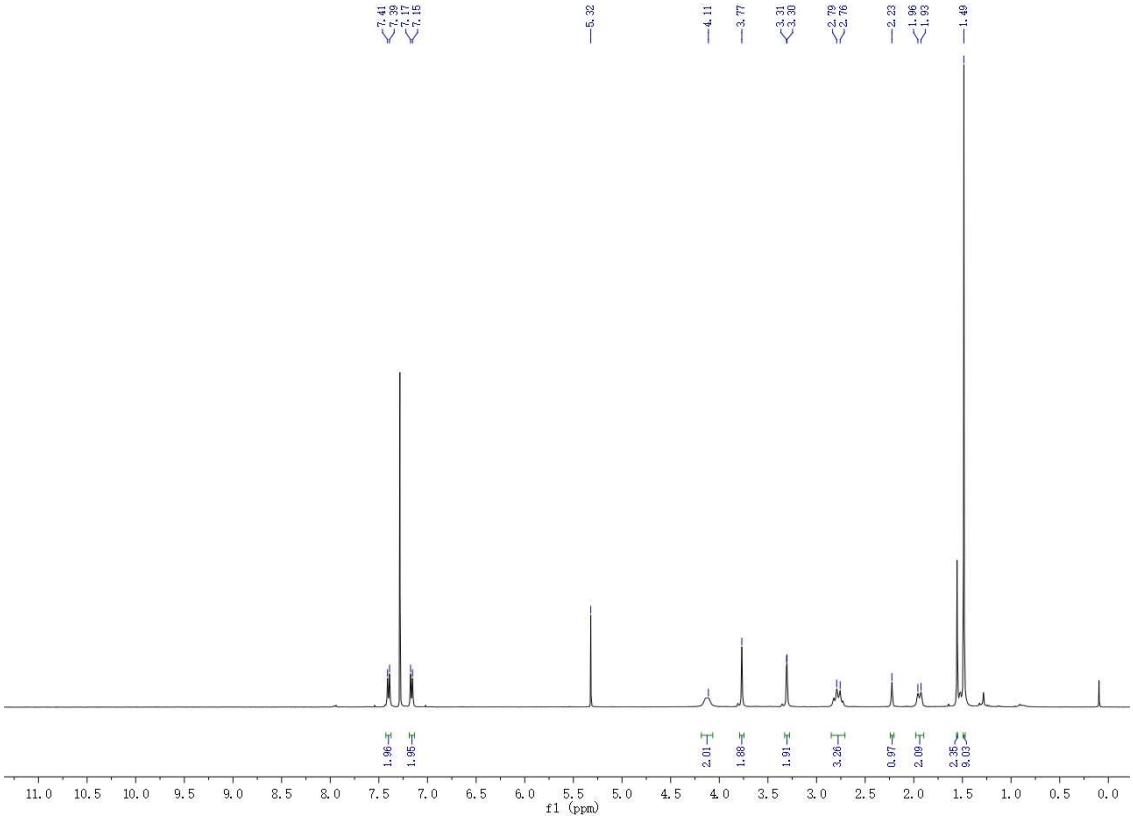
^{13}C-NMR ($CDCl_3$)



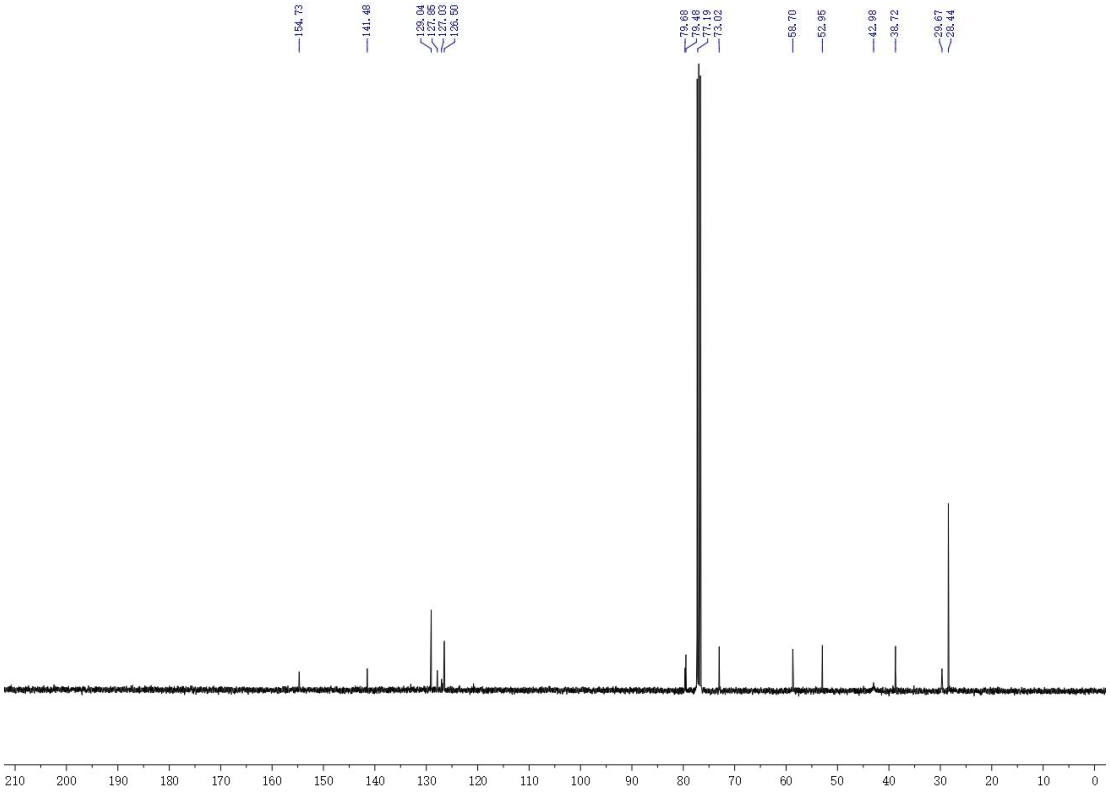
tert-butyl 4-(prop-2-yn-1-yl(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)amino) piperidine-1-carboxylate (**3**)



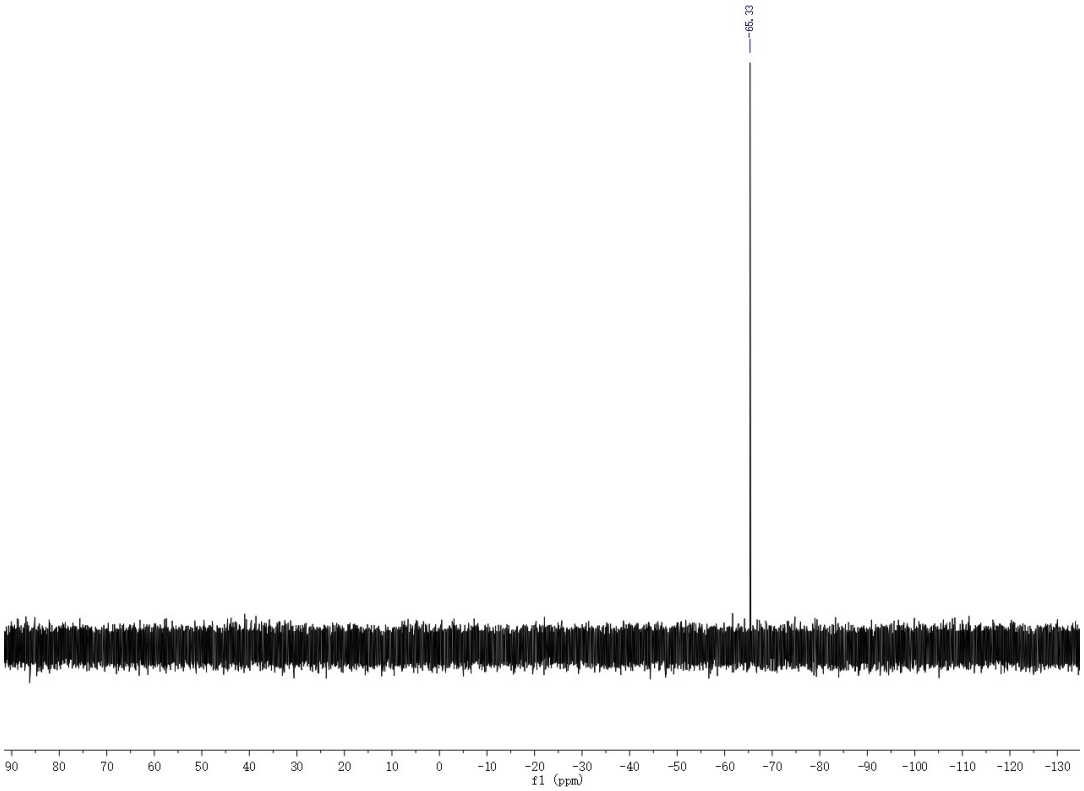
^{1}H-NMR ($CDCl_3$)



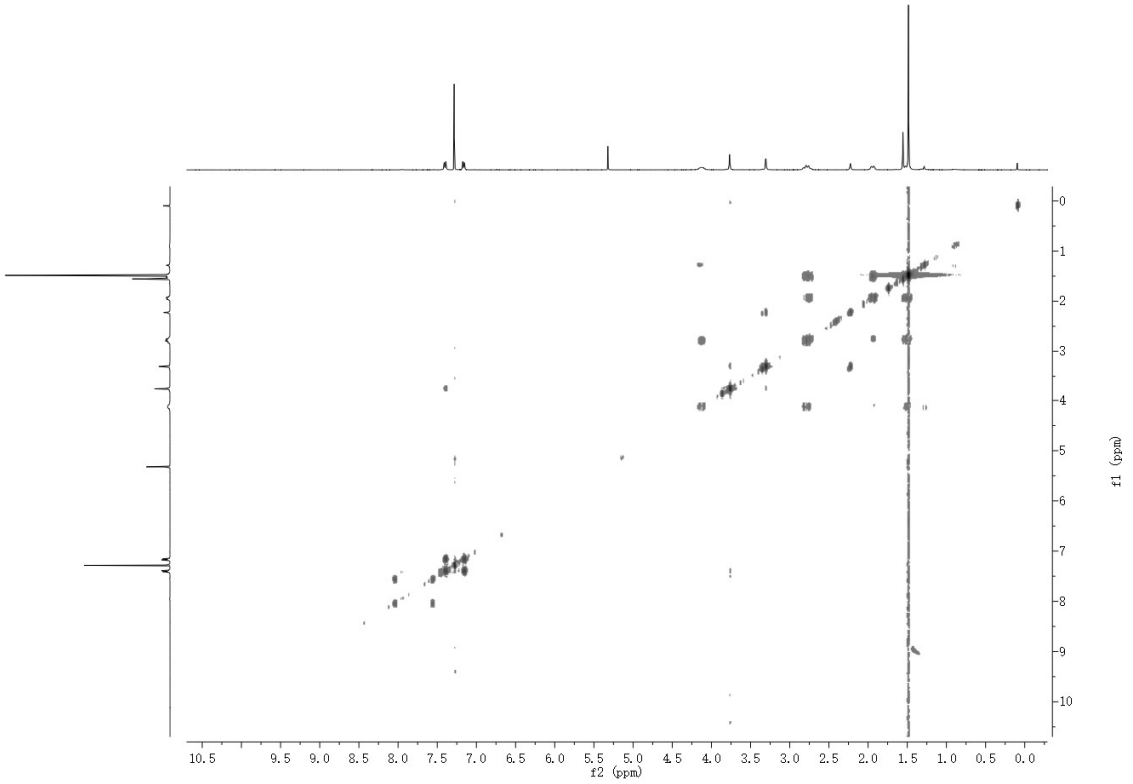
^{13}C-NMR ($CDCl_3$)



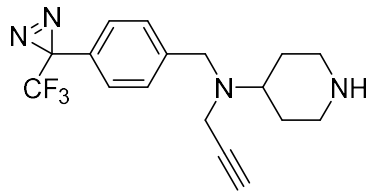
^{19}F-NMR ($CDCl_3$)



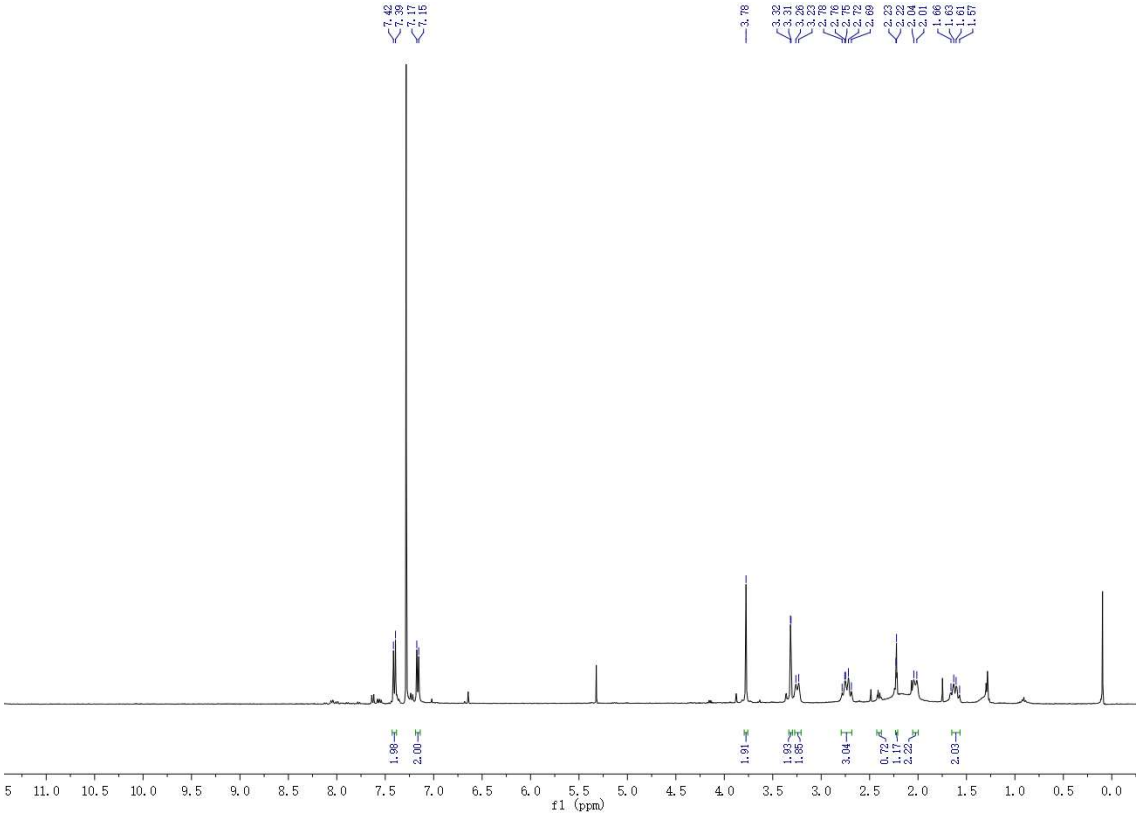
^{1}H-^{1}H COSY ($CDCl_3$)



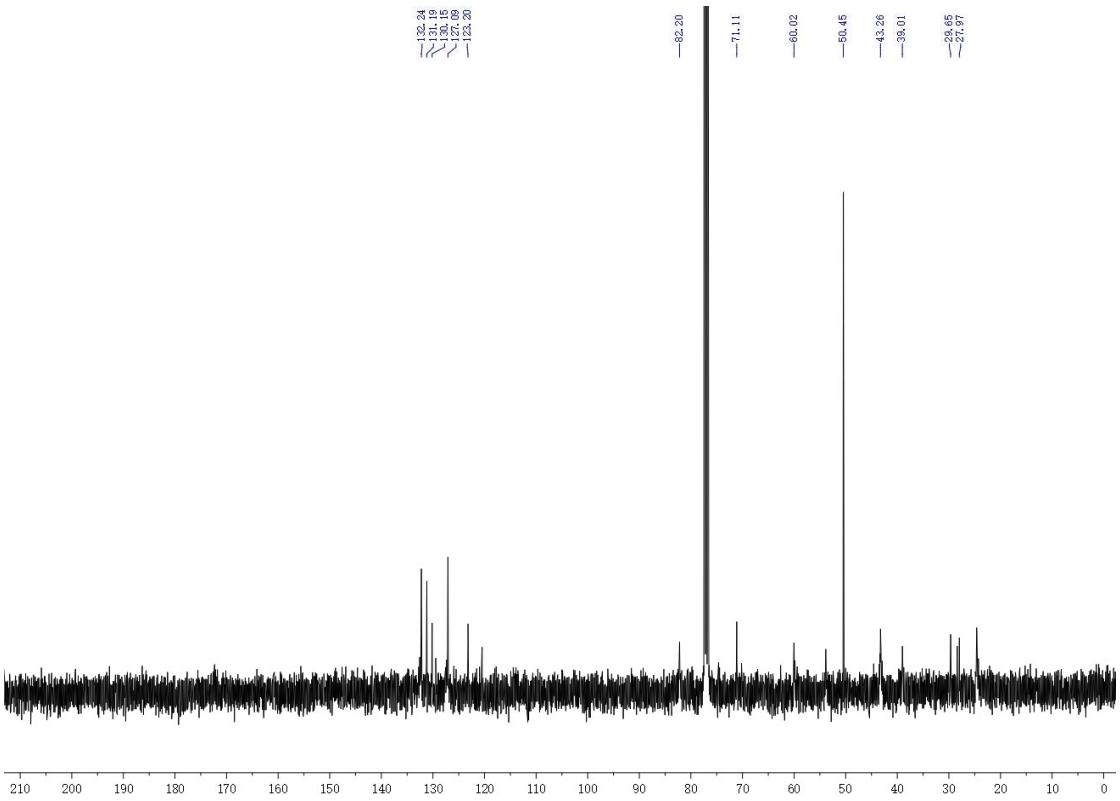
N-(prop-2-yn-1-yl)-N-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl) piperidin-4-amine (**1**)



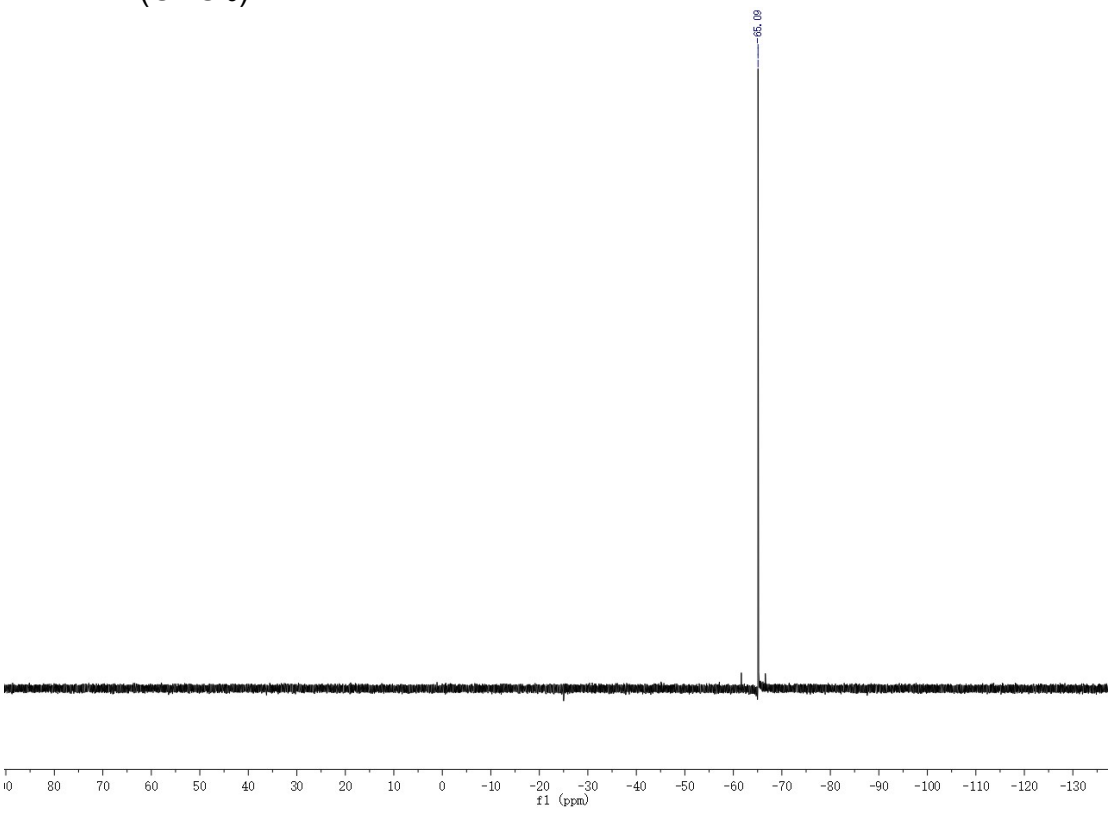
^{1}H-NMR ($CDCl_3$)



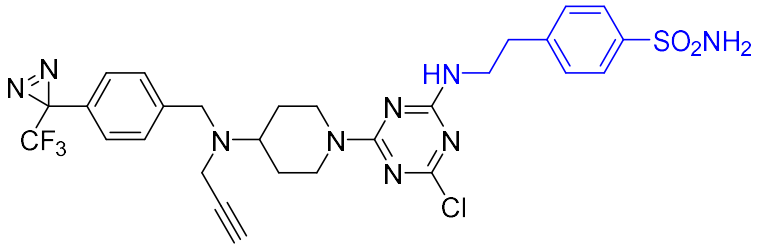
^{13}C-NMR ($CDCl_3$)



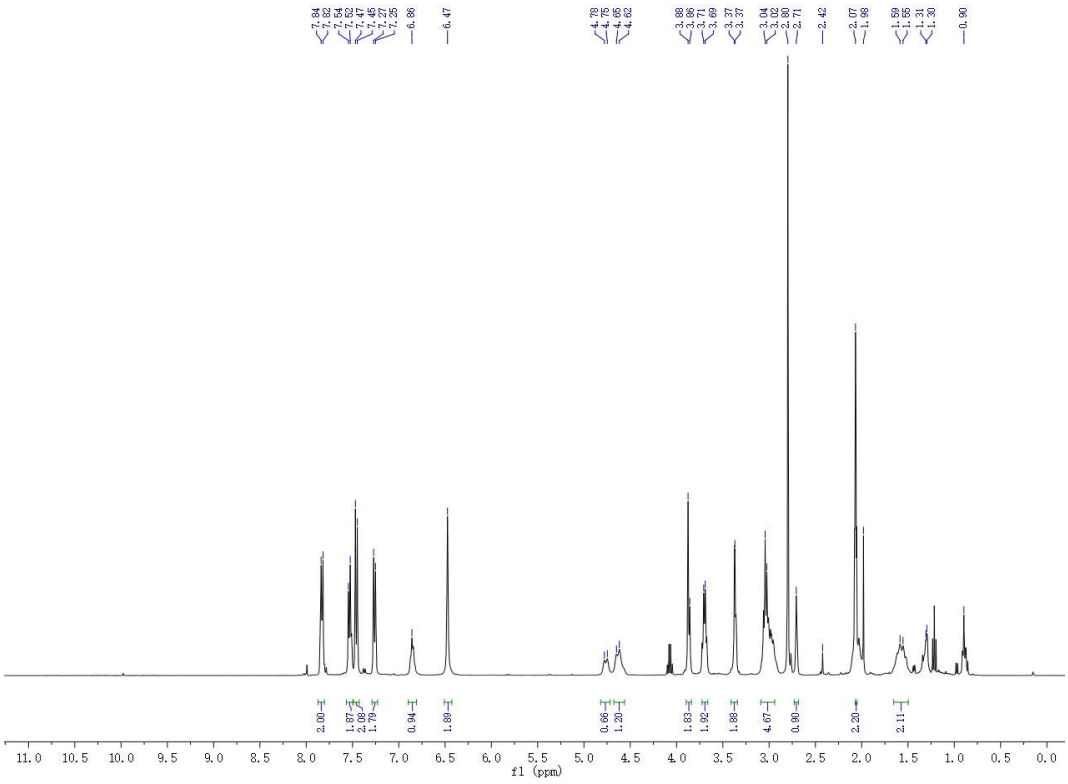
^{19}F-NMR ($CDCl_3$)



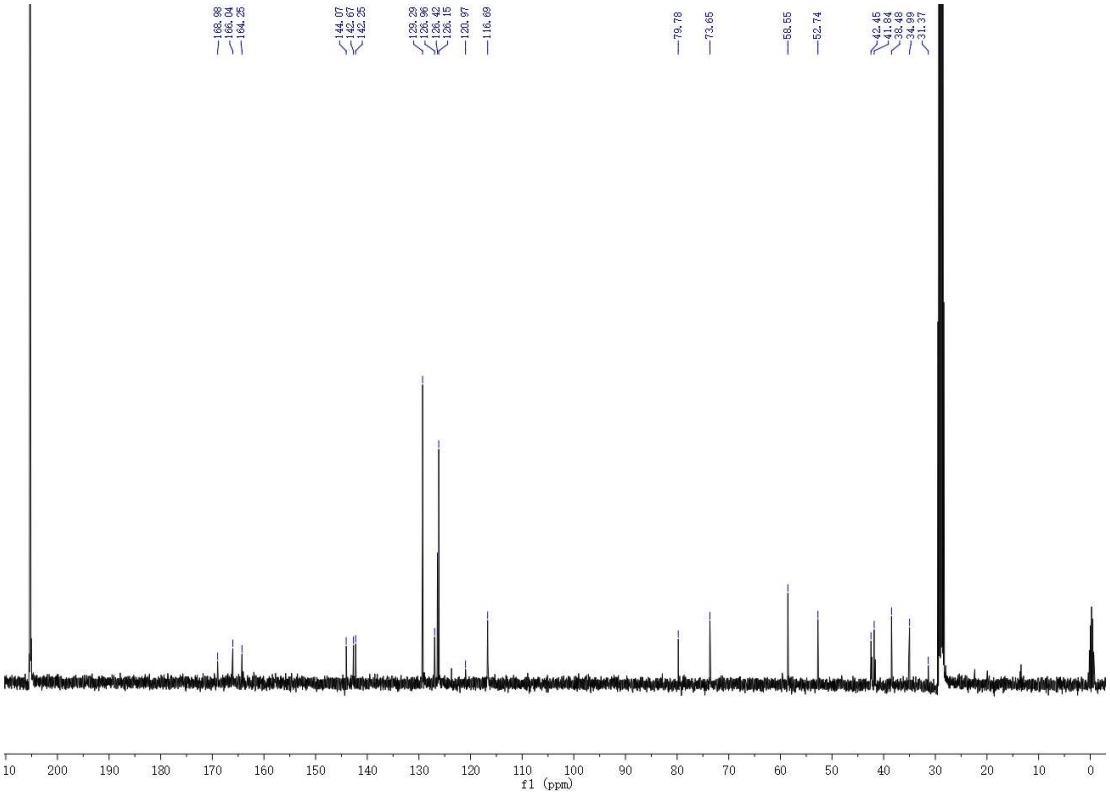
4-(2-((4-chloro-6-(4-(prop-2-yn-1-yl(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)amino)piperidin-1-yl)-1,3,5-triazin-2-yl)amino)ethyl)benzenesulfonamide (**5**)



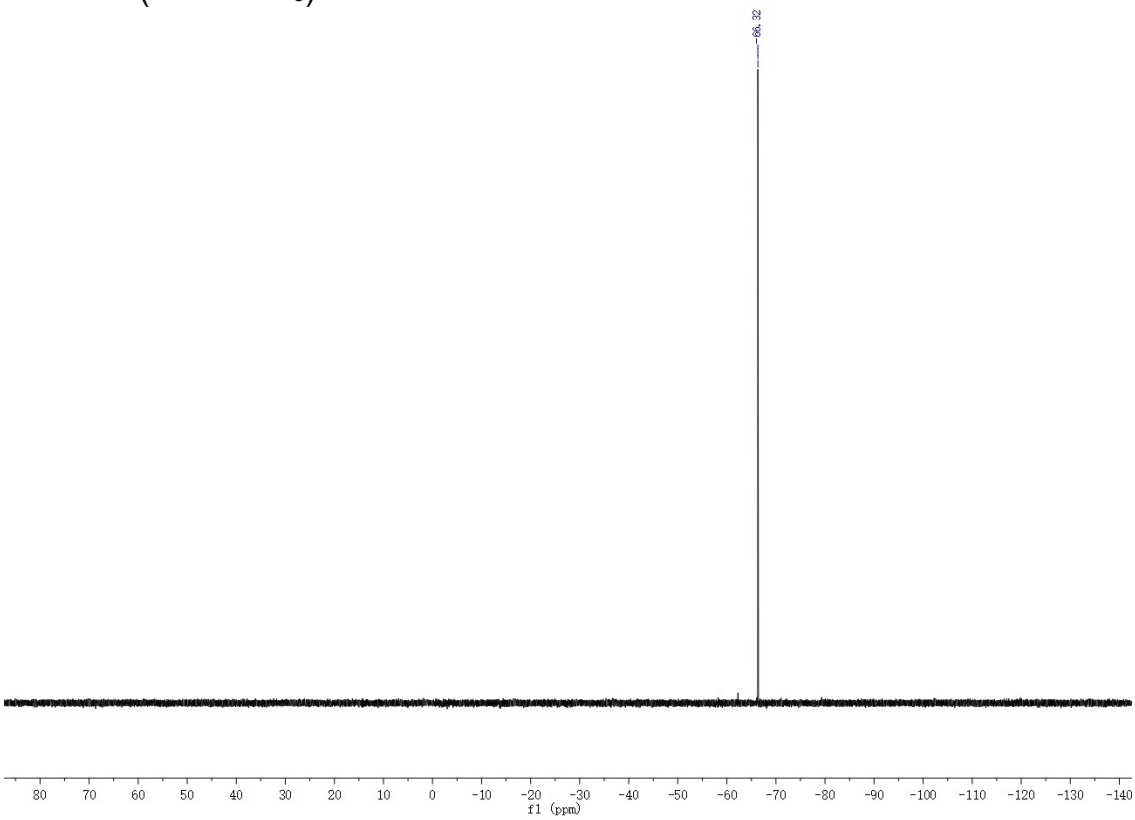
^{1}H-NMR (acetone-d$_6$)



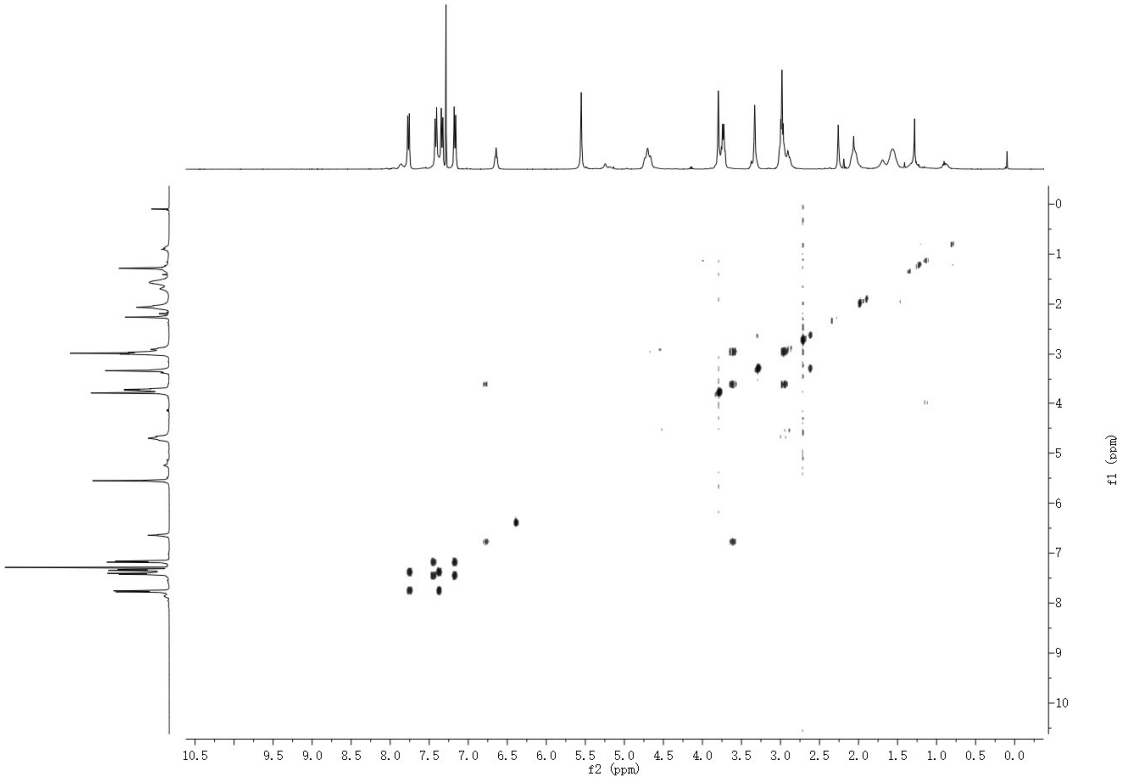
^{13}C-NMR (acetone-d$_6$)



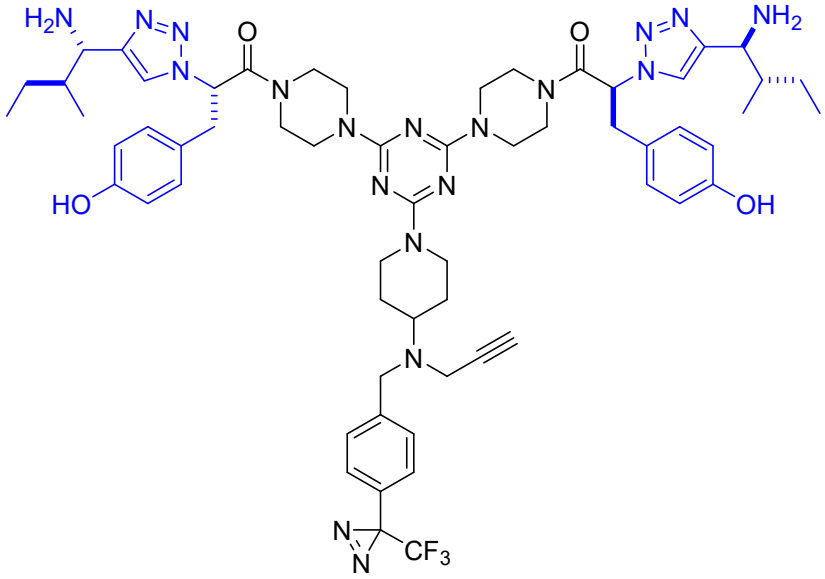
^{19}F-NMR (acetone-d$_6$)



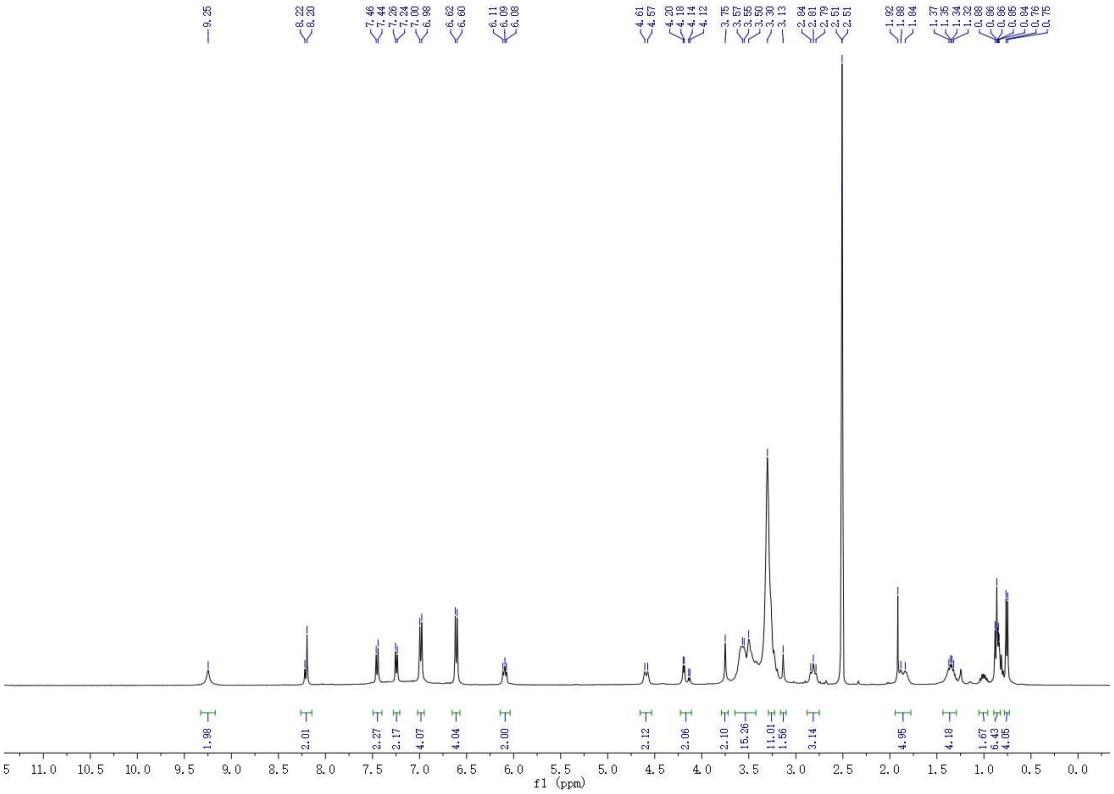
^{1}H-^{1}H COSY ($CDCl_3$)



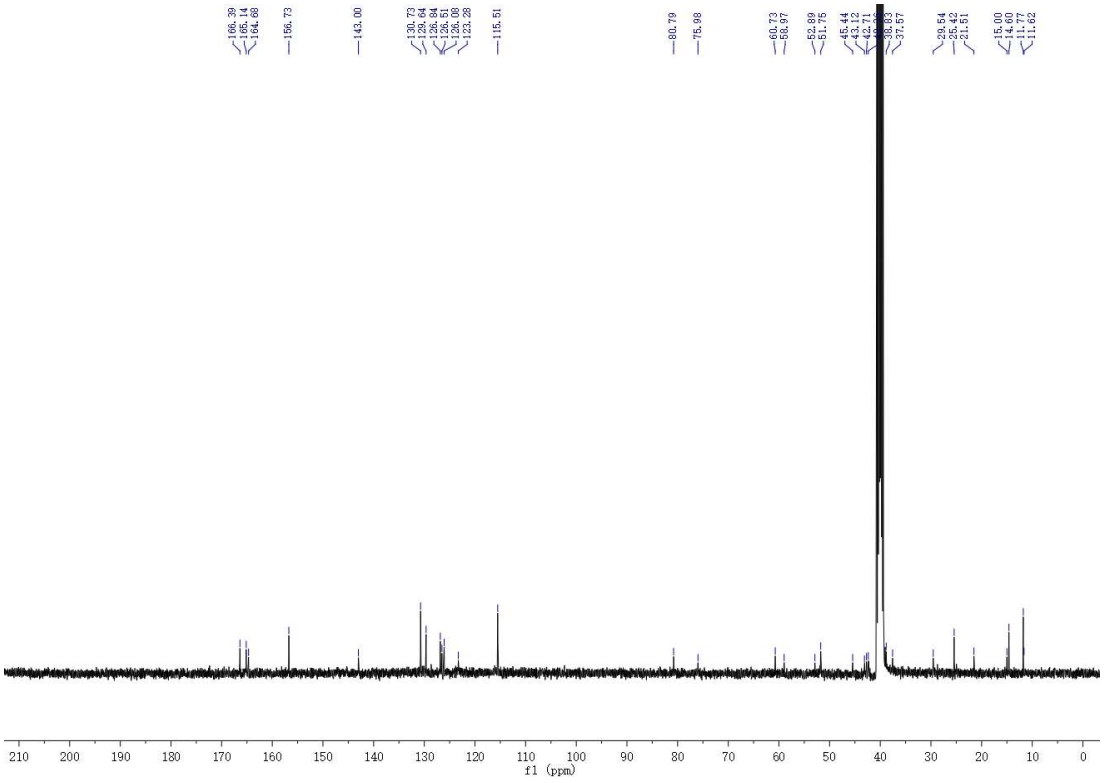
(2S,2'S)-1,1'-((6-(4-(prop-2-yn-1-yl(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)amino)piperidin-1-yl)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(2-(4-((1S,2S)-1-amino-2-methylbutyl)-1H-1,2,3-triazol-1-yl)-3-(4-hydroxyphenyl)propan-1-one) (**7**)



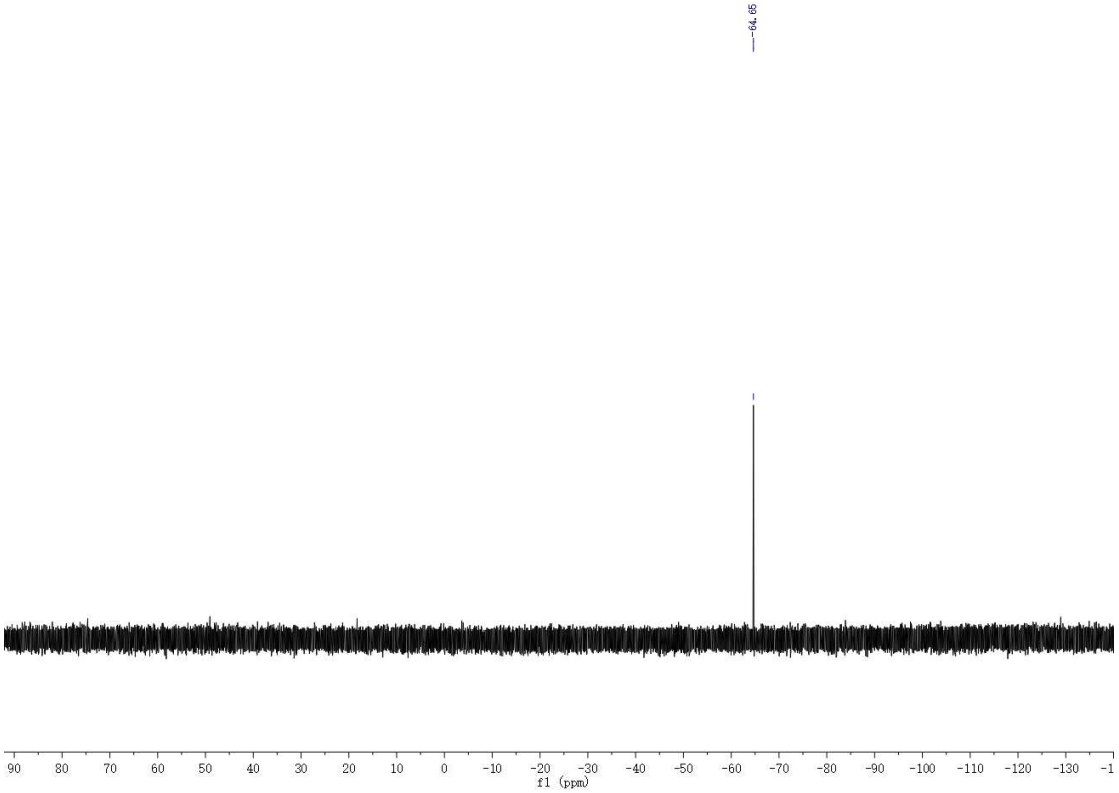
^{1}H-NMR (DMSO-d$_6$)



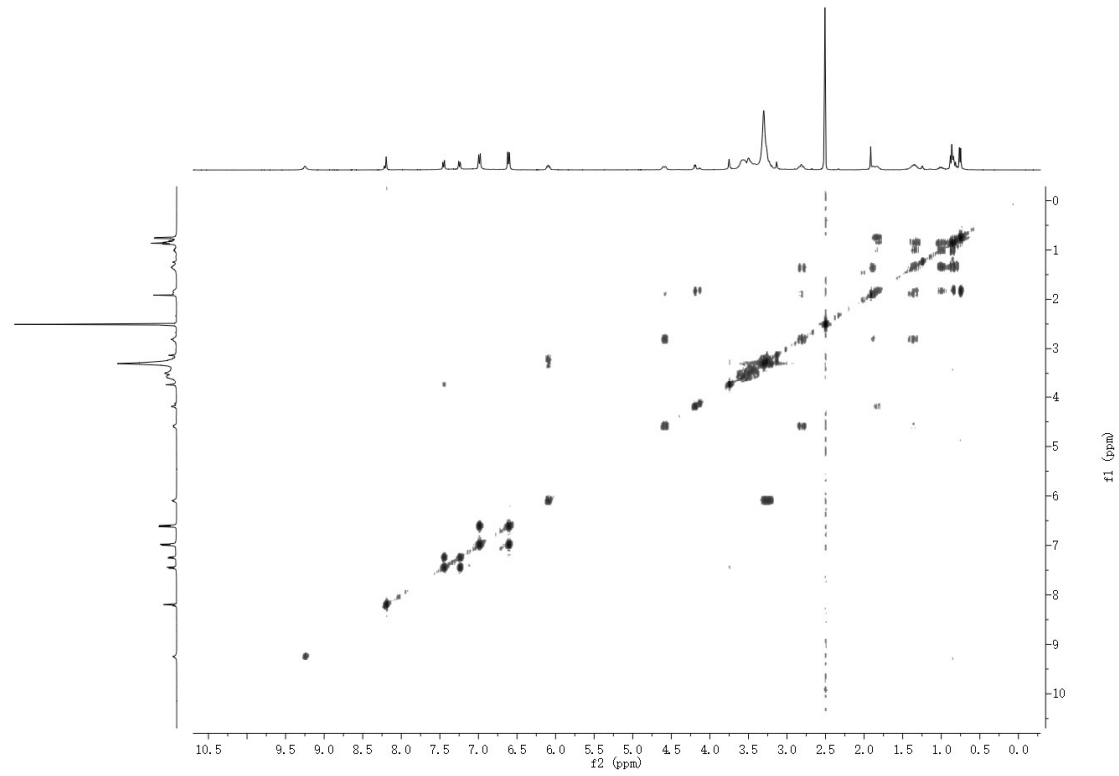
^{13}C-NMR (DMSO-d$_6$)



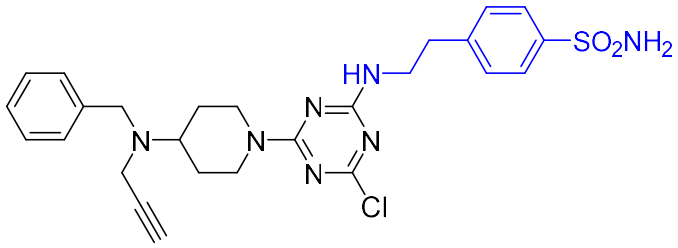
^{19}F-NMR (DMSO-d$_6$)



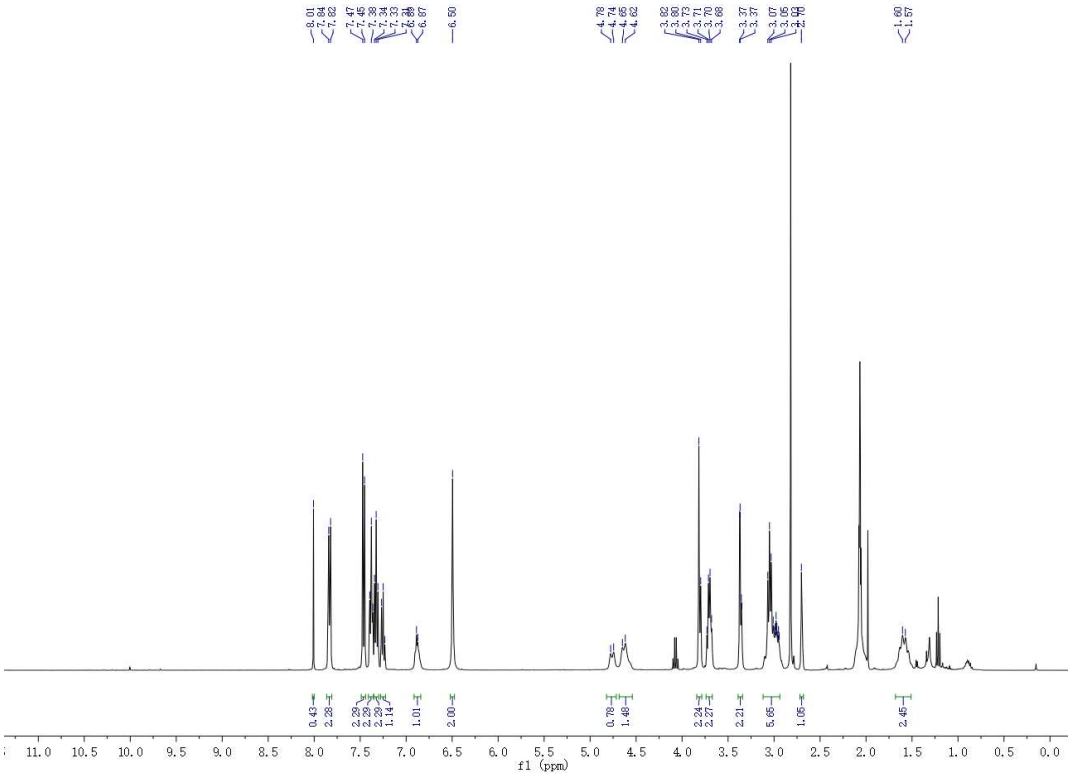
^{1}H-^{1}H COSY (DMSO-d$_6$)



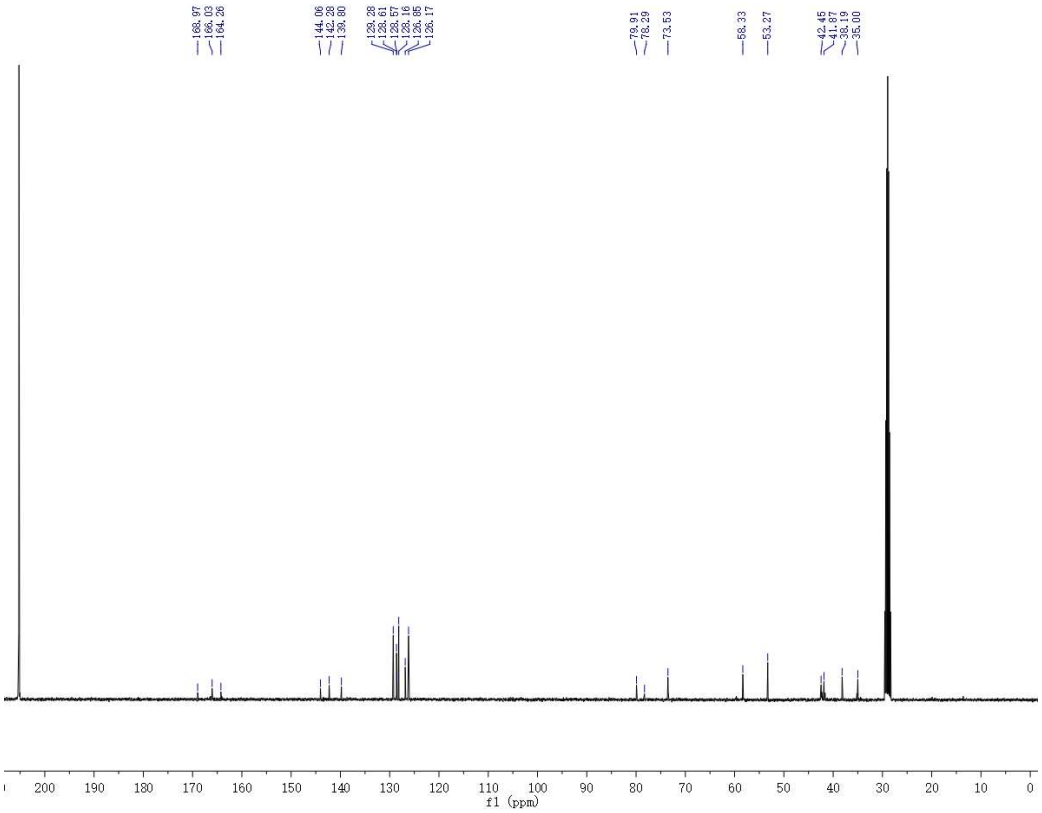
4-(2-((4-(4-(benzyl(prop-2-yn-1-yl)amino)piperidin-1-yl)-6-chloro-1,3,5-triazin-2-yl)amino)ethyl)benzenesulfonamide (**6**)



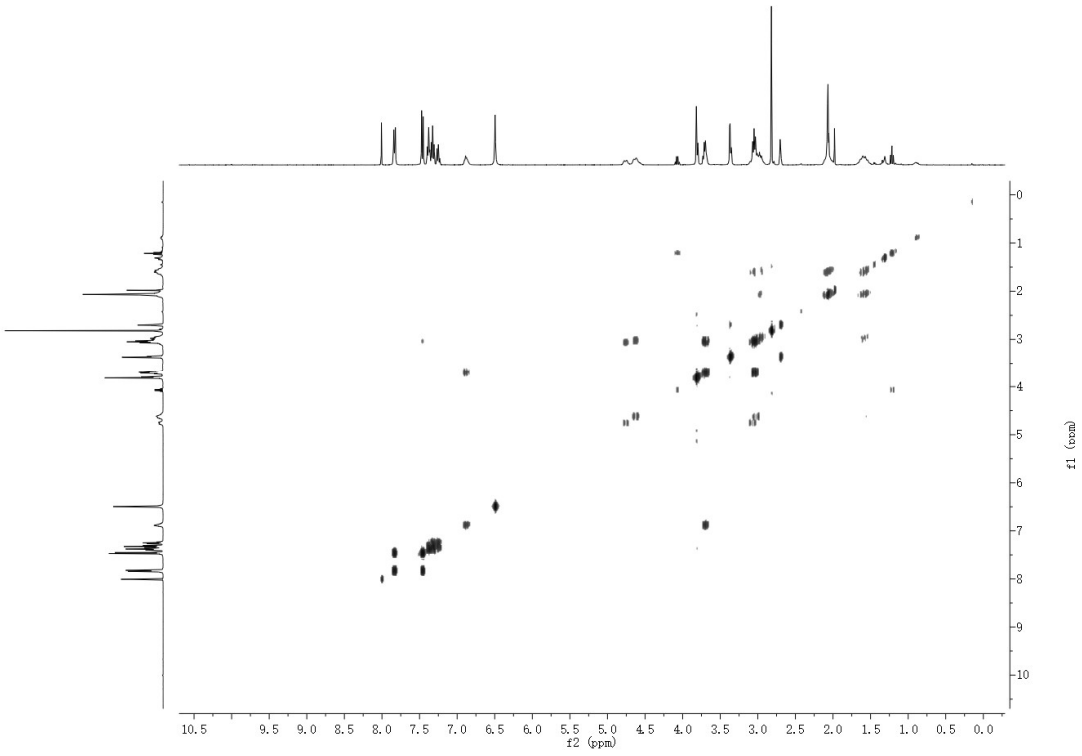
^{1}H NMR (acetone-d$_6$)



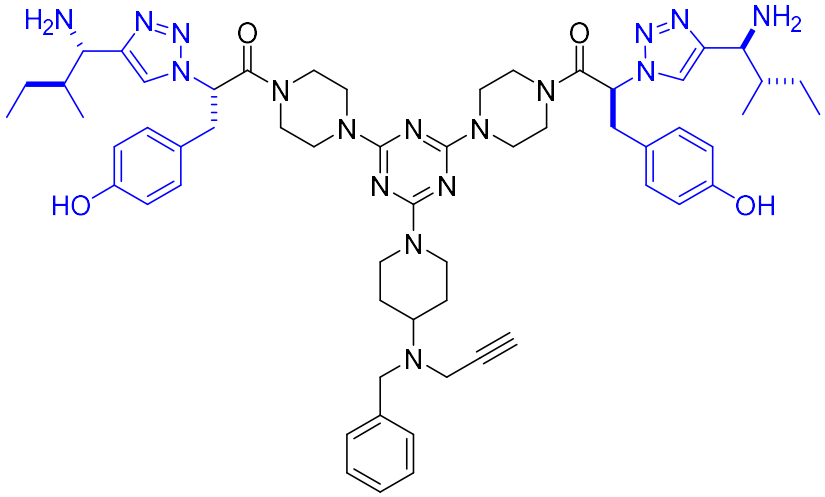
^{13}C NMR (acetone-d$_6$)



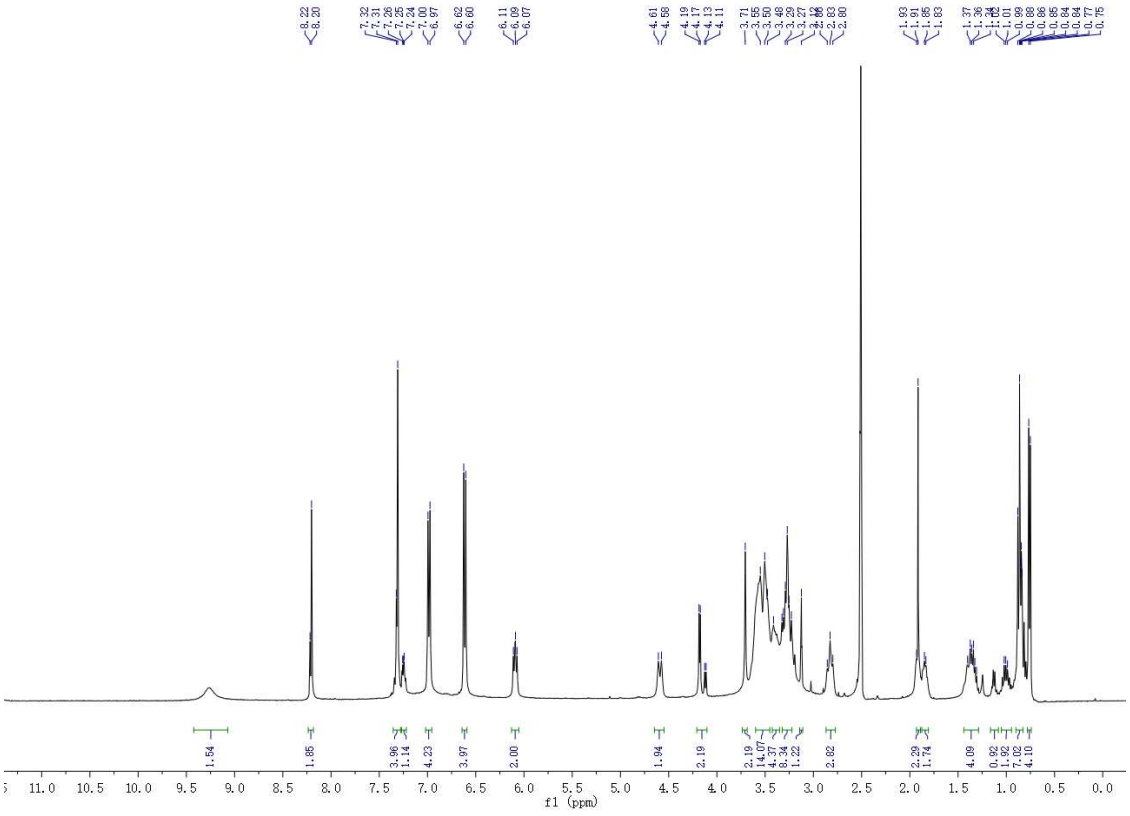
^{1}H-^{1}H COSY (acetone-d$_6$)



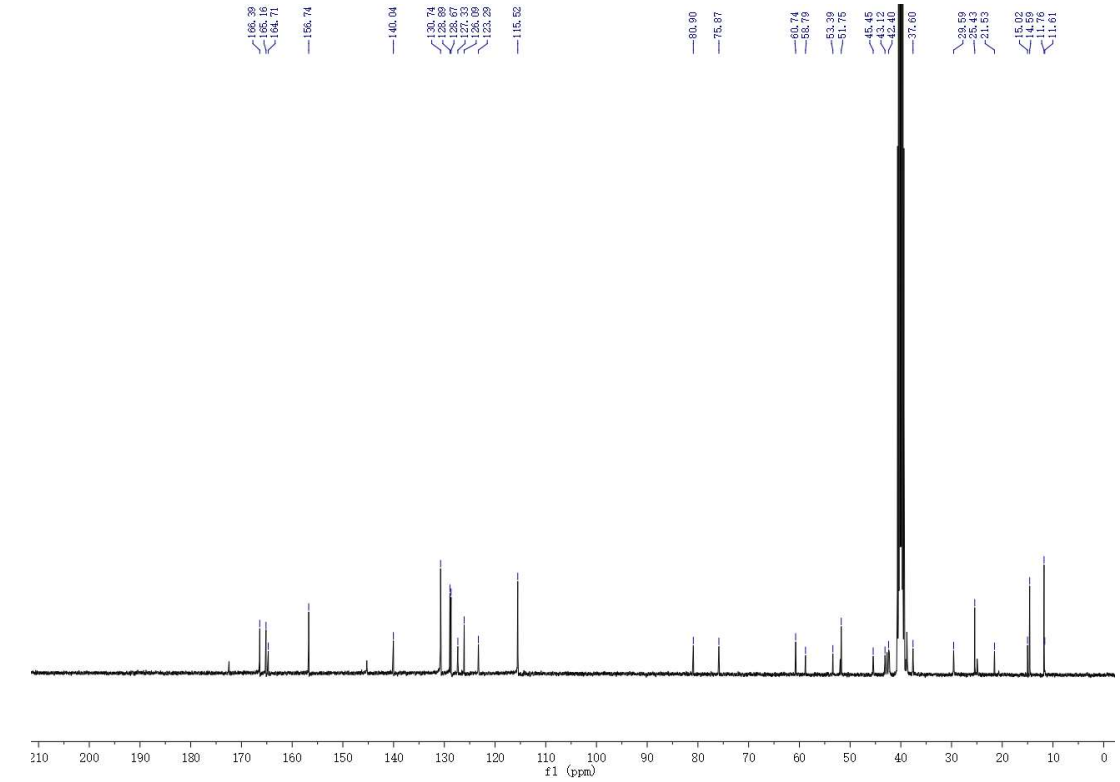
(2S,2'S)-1,1'-((6-(4-(benzyl(prop-2-yn-1-yl)amino)piperidin-1-yl)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(2-(4-((1S,2S)-1-amino-2-methylbutyl)-1H-1,2,3-triazol-1-yl)-3-(4-hydroxyphenyl)propan-1-one) (**8**)



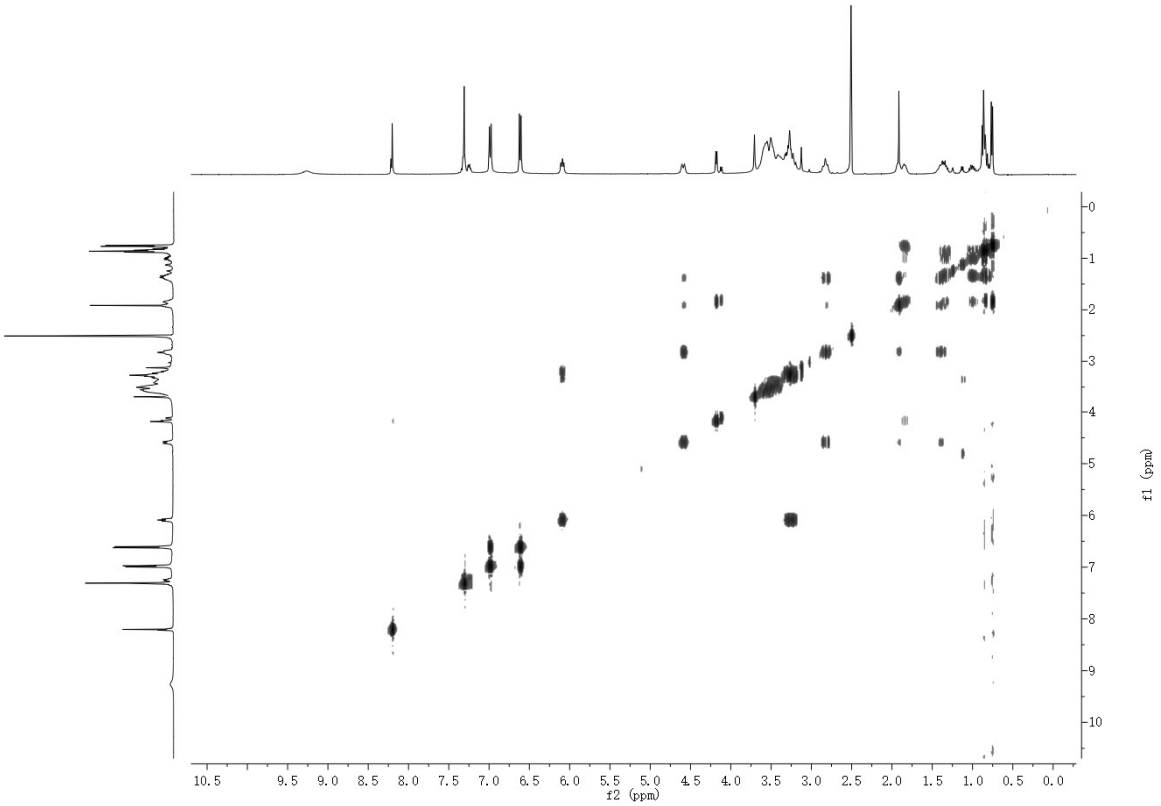
^{1}H NMR (DMSO-d$_6$)



^{13}C NMR (DMSO-d$_6$)



^{1}H-^{1}H COSY (DMSO-d$_6$)



C. Photoaffinity Labeling Experiments

Fluorescent photoaffinity labeling of recombinant CAIX and TrkC

Recombinant CAIX or TrkC protein (2 µg) was dissolved in 25 mM HEPES buffer (20 µL) and kept on ice. For competition experiments, PAL- ligand **6** or **8** was allowed to incubate with CAIX or TrkC recombinant protein at 4 °C for 1 h. Then, PAL+ ligand **5** or **7** was added to all protein samples and incubated in dark at 4 °C for 2 h. All samples except UV negative ones were transferred to a 96-well plate and irradiated by a LED UV flashlight (365 nm) at 4 °C for 30 min.

19.5 µL of each sample were transferred into new Eppendorf tubes. 2.5 µL 10% SDS solution in water was added to each tube and mixed by vortexing. Then 0.5 µL 5 mM azide-fluor 488 (Sigma-Aldrich) was added, followed by 2.5 µL of click reaction catalyst cocktail (prepared immediately before use: mix 1.5 volumes of 1.7 mM TBTA in 80% tert-butanol/20% DMSO, 0.5 volumes of 50 mM $CuSO_4$ and 0.5 volumes of 50 mM TCEP by vortexing). Samples were mixed by vortexing and incubated in dark at room temperature for 3 h. Click reaction was quenched by adding 5 µL of 6 x SDS sample buffer, then was boiled at 95 °C for 5-10 min. SDS-PAGE was performed by using a handcast 10% polyacrylamide gel. The gel was run for an additional 5 minutes after the dye font reached the end of the gel to ensure all of the excess unreacted dyes had completely exited the gel. The gel was then washed with distilled water (3 x 10 min) and scanned on a Typhoon FLA 9500 fluorescent gel scanner (Alexa-488).

Biotin photoaffinity labeling of target proteins (CAIX and TrkC) in cell lysates

Cells (MDA-MB-231 or NIH3T3-TrkC) were lysed by RIPA buffer (Pierce) according to manufacturer's instructions. The total protein concentration was ~ 2 mg/mL, determined by BCA protein assay (Pierce). 200 µL cell lysate was incubated with or without PAL+ ligand **5** or **7** in dark at 4 °C for 3 h. For competition experiments, cell lysate was preinhibited with 200-fold PAL- ligand **6** or **8** at 4 °C for 1 h. Samples were then transferred to a 96-well plate and irradiated by a LED UV flashlight (365nm) at 4 °C for 30 min. All samples were transferred into new Eppendorf tubes. 1 µL biotin azide (20 mM in DMSO) was added to each reaction, followed by 25 µL click reaction catalyst cocktail (prepare immediately before use: mix 1.5 volumes of 1.7 mM TBTA in 80% tert-butanol/20% DMSO, 0.5 volumes of 50 mM $CuSO_4$, and 0.5 mM volumes of 50 mM TCEP by vortexing). Samples were mixed by vortexing and incubated for 5 h at room temperature with constant shaking.

40 µL NeutrAvidin resin (Pierce) prewashed with 1x PBS was then added to the reaction mixture, incubated for 2.5 h with constant shaking at room temperature and then placed at 4 °C overnight. Samples were warmed up to room temperature on the next day and centrifuged to pellet beads. Supernatants were discarded and beads were washed with 400 µL PBS-T (PBS + 0.1% Tween 20) 5 x 5 min followed by PBS 1 x 5 min with shaking. The remaining liquid was completely removed. 40 µL 1 x SDS-buffer was added and boiled at 100 °C for 10 min to elute proteins on bead. SDS-PAGE gel was performed by using a handcast 10% polyacrylamide gel. The gel was washed with distilled water 3 x 5 min. Proteins were visualized either directly by CBB-G250 staining or by immunoblotting. For immunoblotting, proteins were transferred to PVDF membrane by Pierce Power Station according to manufacturer's instructions. Blots were detected using iBind Western Device according to manufacturer's instructions [polyclonal anti-CAIX antibody (1:1000), HRP-conjugated anti-rabbit IgG (H+L) (1:2000)]. Afterwards, blots were treated with ECL Western Blotting Substrate (Pierce) and scanned by ChemiDoc XRS (BioRad) imaging system.

D. Supporting Figures

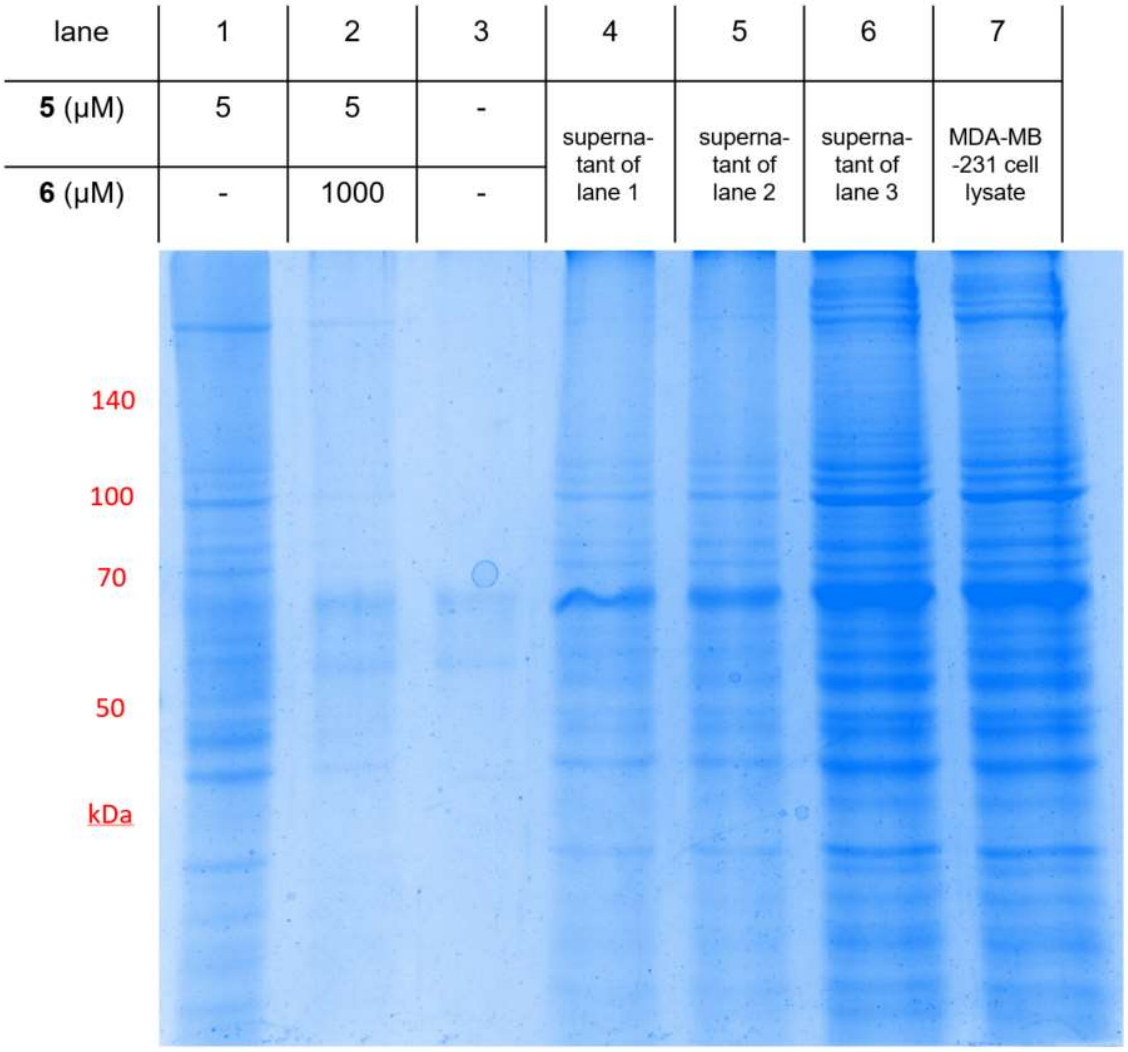


Figure S1. Biotin photoaffinity labeling of CAIX in MDA-MB-231 cell lysate followed by affinity pull-down assay. Eluted proteins (lane 1 - 3), supernatants (lane 4 - 6) and whole cell lysate (lane 7) stained with CBB-G250.

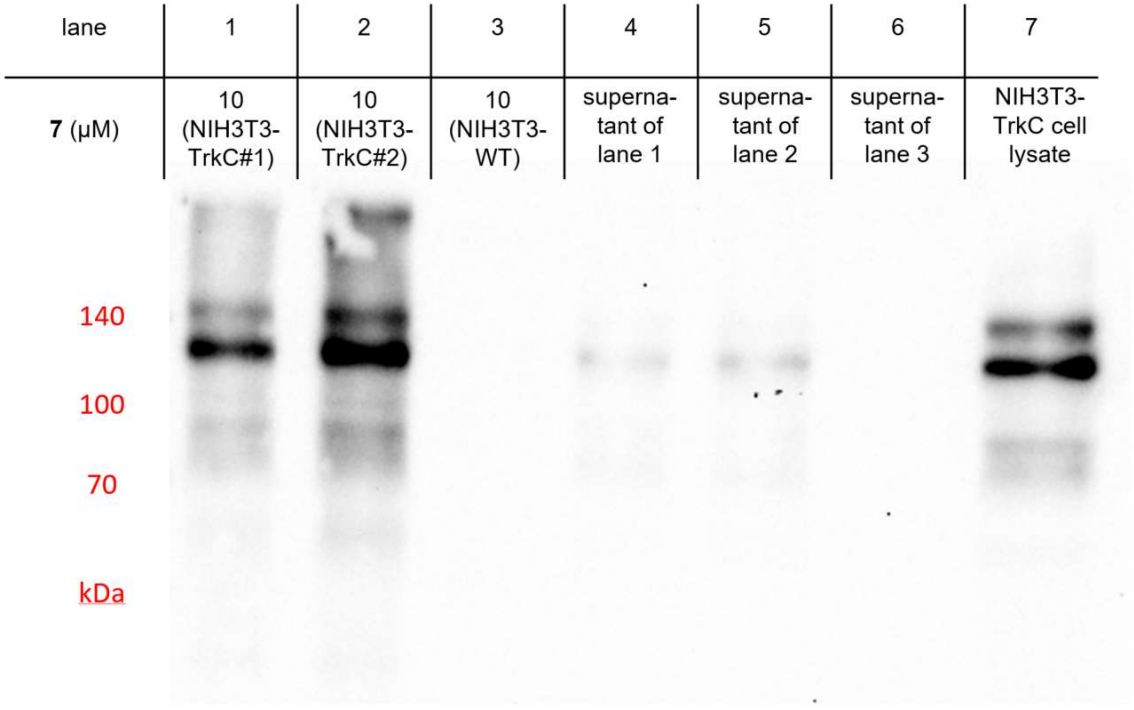


Figure S2. Biotin photoaffinity labeling of TrkC in NIH3T3-TrkC or NIH3T3-WT cell lysate followed by affinity pull-down assay. Eluted proteins (lane 1 - 2: NIH3T3-TrkC cell, and lane 3: NIH3T3-WT), corresponding supernatants (lane 4 - 6) and NIH3T3-TrkC whole cell lysate (lane 7) stained with CBB-G250.

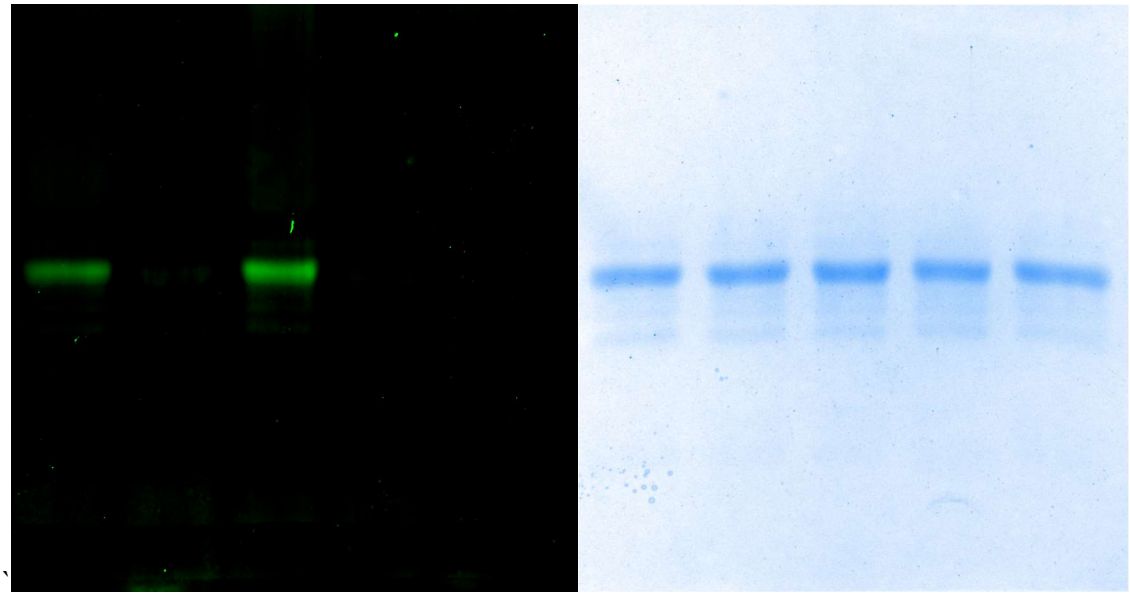


Figure S3. Original image of Figure 2a. Left: in-gel fluorescence (488 nm). Right: CBB-G250 staining image.

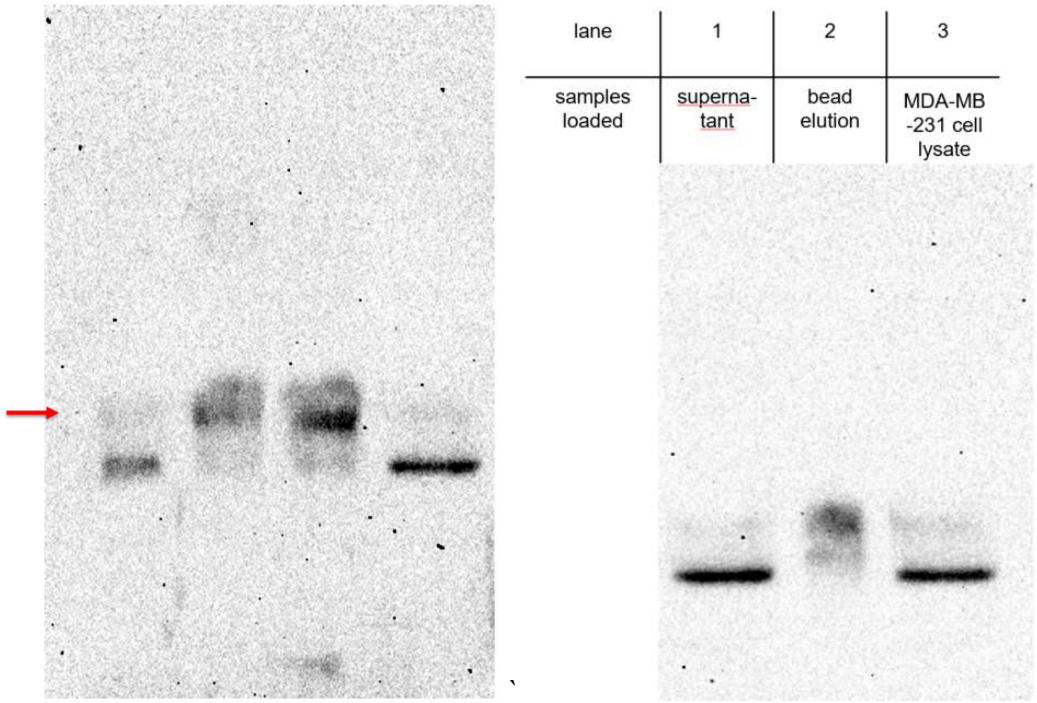


Figure S4. Original image of Figure 2b (left image). Band in red arrow proves to be a nonspecific band (right image): MDA-MB-231 whole cell lysates were directly treated with NeutrAvidin bead. The top band is present only in bead elution (lane 2) but not in supernatant (lane 1) suggesting it's a nonspecific band.

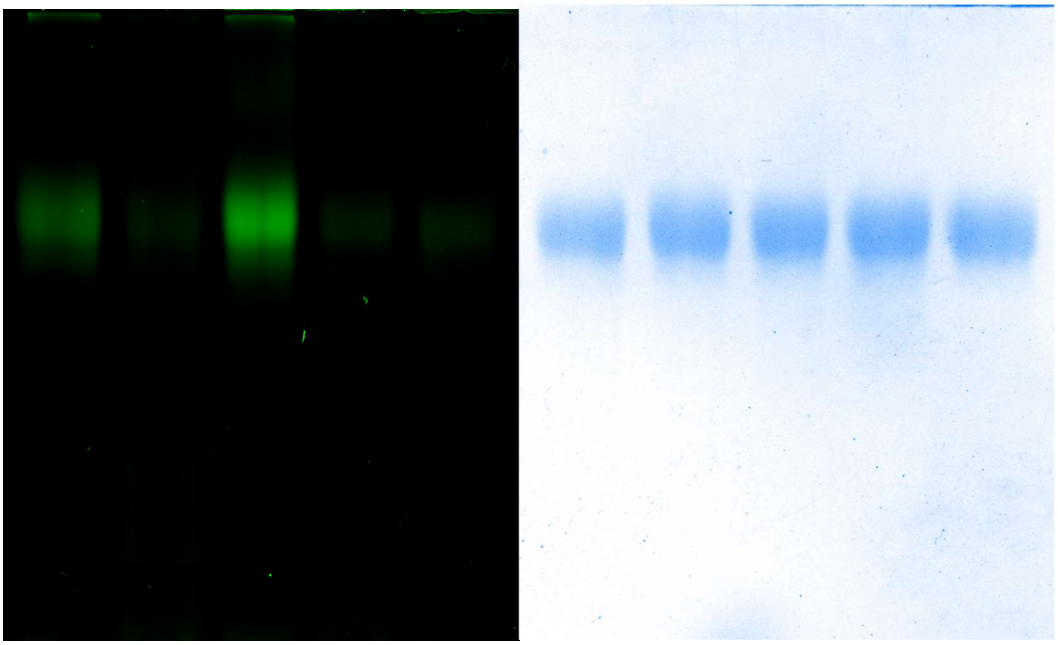


Figure S5. Original image of Figure 3a. Left: in-gel fluorescence (488 nm). Right: CBB-G250 staining image.

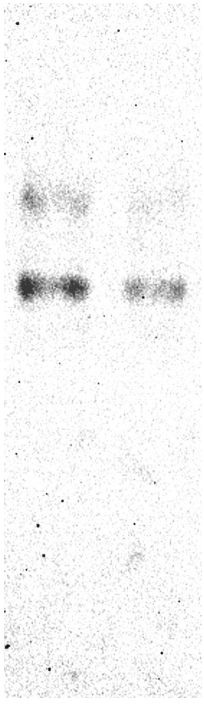


Figure S6. Original image of Figure 3b.

E. References

(1) Kamkaew, A. and Burgess, K. *J. Med. Chem.* **2013**, *56*, 7608-7614.
(2) Strømgaard, K.; Saito, D. R.; Shindou, H.; Ishii, S.; Shimizu, T. and Nakanish, K. *J. Med. Chem.* **2002**, *45*, 4038-4046.
(3) Chen, D.; Brahimi, F.; Angell, Y.; Li, Y.-C.; Moscowicz, J.; Saragovi, H. U.; Burgess, K. *ACS Chem. Biol.* **2009**, *4*, 769-781.