Supplementary Information

for

A region-confined PROTAC nanoplatform for spatiotemporally tunable protein degradation and enhanced cancer therapy

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Materials.

4-Dimethylaminopyridine (DMAP, CAS: 1122-58-3), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, CAS: 25952-53-8), Trifluoroacetic acid (TFA, CAS: 76-05-1), N, N-Diisopropylethylamine (DIEA, CAS: 7087-68-5), 1-Hydroxybenzotriazole (HOBT, CAS: 2592-95-2), 2,2'-Azobis (2-methylpropionitrile) (AIBN, CAS: 78-67-1), Sodium Hydrosulfite ($Na_2S_2SO_4$, CAS: 7775-14-6), 1-(bromomethyl)-4-nitrobenzene (CAS: 100-11-8), (bromomethyl)benzene (CAS: 100-39-0) and methanol (HPLC, MeOH, CAS: 67-56-1) were purchased from J&K Chemicals (Shanghai, China). 4-Cyano-4-(dodecylsulfanylthiocarbonyl) sulfanylpentanoic acid (CDP, CAS: 870196-80-8), 2-(diisopropylamino) ethyl methacrylate (DPA, CAS: 16715-83-6) and 2-hydroxyethyl methacrylate (HEMA, CAS: 868-77-9) were obtained from Sigma-Aldrich (Shanghai, China). Collagenase IV (40510ES60), Ribonuclease A (10407ES60) and Hyaluronidase (20426ES60) were purchased from Yeasen Biotechnology (Shanghai) Co., Ltd. 2,2'-(propane-2,2-diylbis(sulfanediyl))bis(ethan-1-ol) (CAS: 1644545-52-7), 2,2'-(ethane-1,2-diylbis(oxy))bis(ethan-1-ol) (CAS: 112-27-6), Methacryloyl chloride (CAS: 920-46-7) were both purchased from TCI (Shanghai, China). Tert-butyl 2-(3-(2-aminoethoxy)propoxy)acetate (CAS: 1948273-09-3) was purchased from Shanghai Tebo Chemical Technology Co., LTD. Fmoc-protected heptapeptide Gly-Pro-Leu-Gly-Leu-Ala-Gly (Fmoc-GPLGLAG, 470303) was synthesized by GL Biochem. Co., Ltd (Shanghai, China). Methoxy poly(ethylene glycol) amine ($mPEG_{113}$-NH_2, 280023) was purchased from Seebio Biotech. Co., Ltd (Shanghai, China). Tert-butyl ((2S)-1-((4R)-4-hydroxy-2-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl 1oxobutan -2-yl) carbamate (CAS: 1997302-16-5) and triethylene glycol and tert-butyl (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate (JQ1,

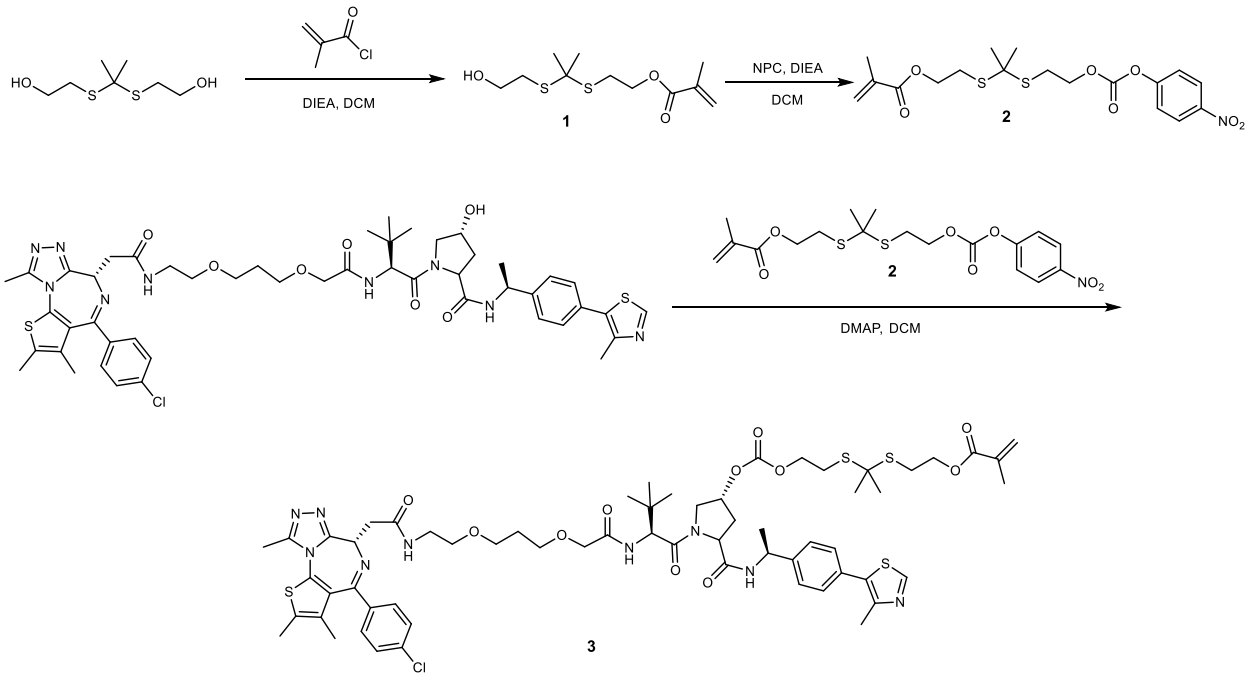
CAS: 1268524-70-4) were obtained from Ruozhi Chemical Technology Co., LTD. (Shanghai, China). Bis(4-nitrophenyl) carbonate (NPC, CAS: 5070-13-3) and Pyropheophorbide a (PPa, CAS: 24533-72-0) and were purchased from Dibai biotechnology CO., LTD. (Shanghai, China).

Dulbecco's modified eagle medium (DMEM, MA0212), penicillin-streptomycin solution (MA0110), PBS buffer solution (1 ×, MA0015), 0.25% trypsin-EDTA (MA0233), bovine serum albumin (BSA, fraction V, MB0094), BCA protein quantification kit (MA0082), cell counting kit-8 (CCK-8, MA0218), loading buffer (5×, MA0003-D), protein marker (10-190 kDa, MA0342), TBST buffer solution (10×, MA0091), ECL luminescence reagent (MA0186), 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI, MA0128), LysoTracker-green DND-26 (MB6042), singlet oxygen fluorescent probe SOSG (MA0326), reactive oxygen species fluorescent probe DCFH-DA (MB4682), MG132 proteasome inhibitor (MB5137) were purchased from Meilun Biotech Co., Ltd (Dalian, China). B27™ (50 ×, Gibco™, 17504044) and Fetal bovine serum (FBS, Gibco™, 10099141C) were purchased from Thermo Fisher Scientific. Peroxidase-Conjugated Goat Anti-Rabbit IgG (H+L) (33101ES60), Peroxidase-Conjugated Goat Anti-Mouse IgG (H+L) (33201ES60) and TRIzol Reagent (10606ES60) were purchased from YEASEN (Shanghai, China). Human EGF (PeproTech, AF-100-15) and Human FGF (PeproTech, AF-100-17A) cytokines were obtained from Neobioscience. The anti-BRD4 antibody (ab128874), anti-CDK4 antibody (ab199728), anti-CDK6 antibody (ab241554), anti-p21 antibody (ab109199), anti-caspase-3 antibody (ab124787), anti-OCT4 antibody (ab200834), anti-SOX2-Alexa Fluor®647 antibody (ab279687), anti-NANOG antibody (ab109250), anti-CD133-Alexa Fluor®647 antibody (ab252127), anti-GAPDH antibody (ab8245), anti-β-actin antibody (ab8226), anti-β-tubulin antibody (ab78078) were all purchased from Abcam (Shanghai, China). Anti-CD44-APC antibody (70-AH04405-100) was purchased from MultiSciences Biotech Co., Ltd. Anti-CD24-

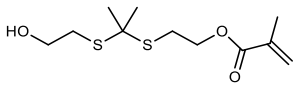
PerCP-Cy5.5 antibody (11-0247-42) was purchased from eBioscience Inc. Hypoxyprobe^{TM} kit (MA 01803) was acquired from Hypoxyprobe, Inc. PrimeScript^{TM}RT reagent Kit (RR047A) and TB Green® Premix Ex Taq^{TM} II (RR820L) were purchased from Takara Bio, Inc. All other reagents and solvents were analytical grade and obtained from SinoPharm Chemical Reagent Co., Ltd. (Shanghai, China).

Supplementary Methods

Synthesis of ARV771-TK



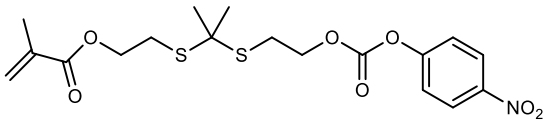
Synthesis of 2-((2-((2-hydroxyethyl)thio)propan-2-yl)thio)ethyl methacrylate (1)



2,2'-(propane-2,2-diylbis(sulfanediyl))bis(ethan-1-ol) (commercially obtained, 392.6 mg, 2.0 mmol, 1.0 eq) and DIEA (775.4 mg, 6.0 mmol, 3.0 eq) were dissolved in DCM. Next, the solution of methacryloyl chloride (commercially available, 208.0 mg, 2.0 mmol, 1.0 eq) in DCM was dropped into above mixture, and continuous stirred for 24 h. At the end of reaction, the mixture was washed

with water and saturated NH_4Cl solution, the organic solvent was collected and dried by anhydrous Na_2SO_4. The initial product was depurated by silica gel column chromatography (n-hexane: ethyl acetate = 4:1) to gain compound **1** as light-yellow oily liquid (380.2 mg, 72% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.16 (s, 1H), 5.61 (s, 1H), 4.34 (t, *J* = 7.1 Hz, 2H), 3.81 (t, *J* = 6.3 Hz, 2H), 2.96 (t, *J* = 7.1 Hz, 2H), 2.88 (t, *J* = 6.1 Hz, 2H), 1.97 (s, 3H), 1.66 (s, 6H).

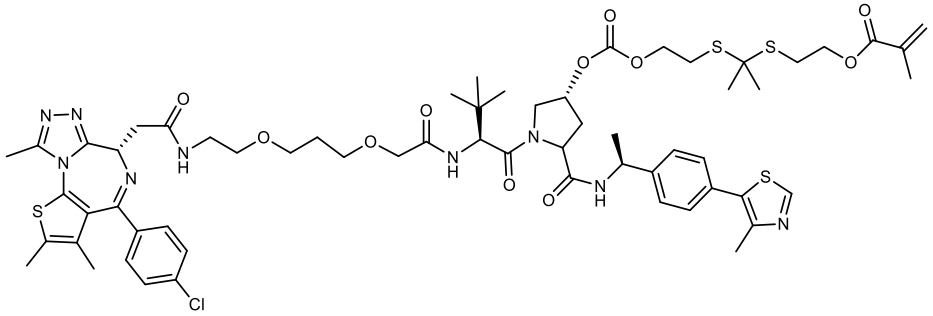
Synthesis of 2-((2-((2-(((4-nitrophenoxy)carbonyl)oxy)ethyl)thio)propan-2 yl)thio)ethyl methacrylate (2)



Bis(4-nitrophenyl) carbonate (NPC, commercially available, 851.3 mg, 2.8 mmol, 1.2 eq) and DIEA (1085.6 mg, 8.4 mmol, 3.0 eq) were dissolved in DCM, subsequently, the solution of compound **1** (380.2 mg, 1.4 mmol, 1.0 eq) in DCM was added dropwise into the above mixture. The reaction liquid was stirred under argon protection at room temperature for 6 h. Then, the mixture was diluted by DCM, and washed by water and saturated NH_4Cl solution orderly. The raw production was obtained after the organic solvent system was filtered and dried by anhydrous Na_2SO_4. The compound **2** was finally gained through silica gel column chromatography to depurate (n-hexane: ethyl acetate = 4:1) as light-yellow oily liquid (510.6 mg, 85% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (d, *J* = 9.1 Hz, 2H), 7.43 (d, *J* = 9.0 Hz, 2H), 6.15 (s, 1H), 5.62 (s, 1H), 4.47 (t, *J* = 6.9 Hz, 2H), 4.35 (t, *J* = 7.0 Hz, 2H), 3.03 (t, *J* = 6.9 Hz, 2H), 2.96 (t, *J* = 7.0 Hz, 2H), 1.97 (s, 3H), 1.70 -1.65 (m, 6H).

Synthesis of 2-((2-((2-(((((3R)-1-((S)-2-(tert-butyl)-15-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-

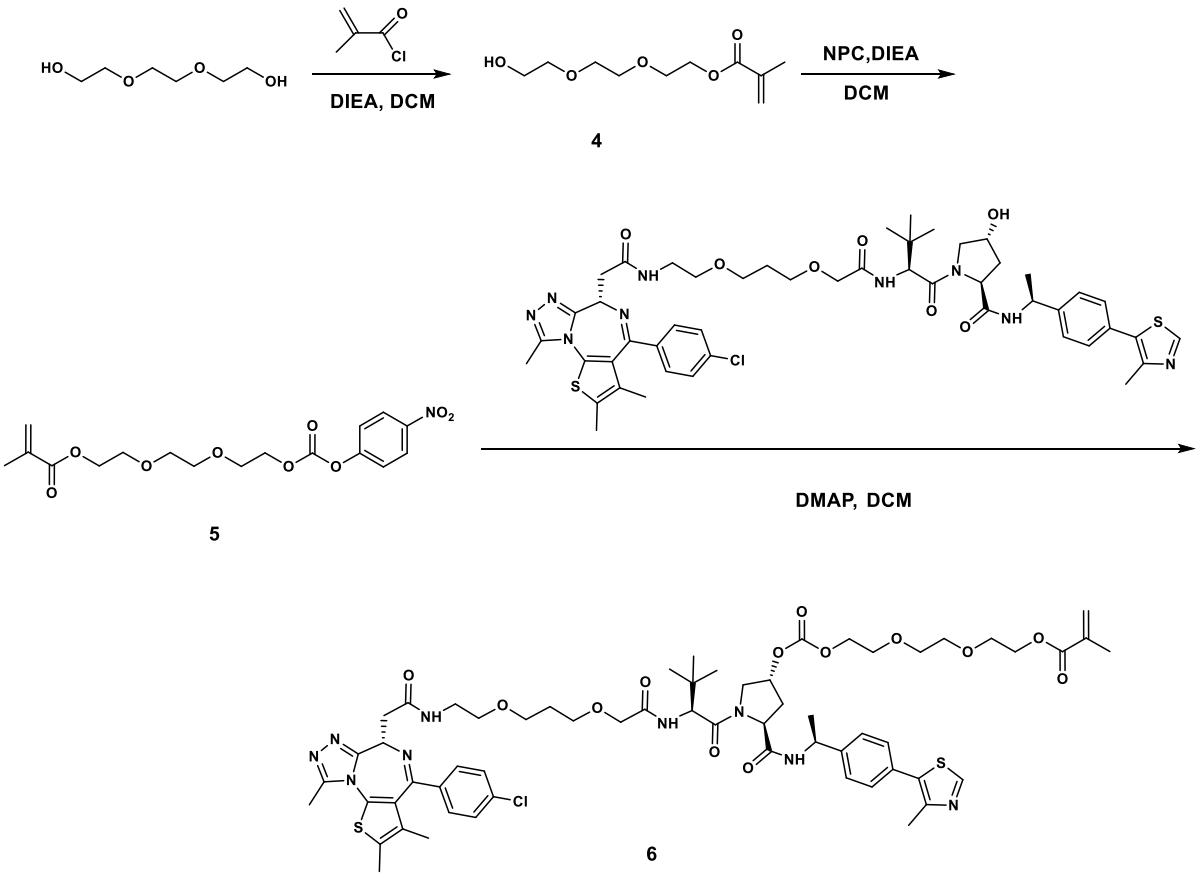
diazapentadecanoyl)-5-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)carbonyl)oxy)ethyl)thio)propan-2-yl)thio)ethyl methacrylate (3, ARV771-TK)



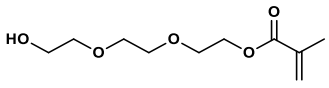
ARV771 (synthesized by previous study[1], 788.0 mg, 0.8 mmol, 1.0 eq), compound **2** (510.6 mg, 1.2 mmol, 1.5 eq), DIEA (206.4 mg, 1.6 mmol, 2.0 eq) and DMAP (97.6 mg, 1.2 mmol, 1.5 eq) were dissolved in DCM, and stirred overnight at room temperature. At the end of reaction, the solution was washed with water and saturated NH_4Cl solution, filtered and dried over anhydrous Na_2SO_4. The crude product was further purified by silica gel column chromatography (DCM:MeOH = 80:1~15:1) to obtain compound **3** as white solid (612.2 mg, 60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.30 (s, 3H), 7.27 (s, 1H), 6.14 (s, 1H), 5.63-5.58 (m, 1H), 5.32-5.27 (m, 1H), 5.06 (p, *J* = 7.1 Hz, 1H), 4.88 (t, *J* = 7.6 Hz, 1H), 4.71-4.64 (m, 2H), 4.33 (d, *J* = 3.1 Hz, 1H), 4.32 (d, *J* = 3.3 Hz, 2H), 4.31 (d, *J* = 3.3 Hz, 2H), 4.28 (s, 1H), 4.00 (q, *J* = 15.5 Hz, 2H), 3.95-3.89 (m, 1H), 3.71-3.64 (m, 4H), 3.64-3.58 (m, 3H), 3.55 (ddd, *J* = 15.1, 9.4, 5.3 Hz, 3H), 3.50-3.44 (m, 1H), 2.96-2.90 (m, 4H), 2.64 (s, 3H), 2.62-2.56 (m, 1H), 2.52 (s, 3H), 2.43 (s, 3H), 2.41-2.36 (m, 1H), 1.96 (s, 3H), 1.94-1.83 (m, 2H), 1.71 (s, 3H), 1.64 (s, 6H), 1.40 (d, *J* = 7.0 Hz, 3H), 1.10 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.48, 170.20, 169.27, 169.18, 166.69, 153.73, 149.77, 142.82, 136.35, 135.60, 130.53, 129.40, 128.87, 128.21, 126.05, 125.46, 76.81, 76.56, 76.30, 76.02, 69.77, 68.99, 68.62, 67.50, 66.62, 63.26, 57.89, 56.16, 55.96, 53.73, 53.31, 48.43, 39.21, 38.15, 35.12, 32.96, 30.51, 28.92, 28.65, 28.33, 26.02, 21.44, 17.84, 15.54, 13.94, 12.65, 11.29.

ESI m/z Calcd. for $C_{61}H_{78}ClN_9O_{11}S_4$ $[M+H]^+$ 1276.4, Found 1276.4. Calcd. for $C_{61}H_{78}ClN_9O_{11}S_4$ $[M+Na]^+$ 1298.4, Found 1298.3.

Synthesis of 2-(2-(2-(((((3R,5S)-1-((S)-2-(tert-butyl)-15-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-diazapentadecanoyl)-5-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)carbonyl)oxy)ethoxy)ethoxy)ethyl methacrylate (6, ARV771-Et)



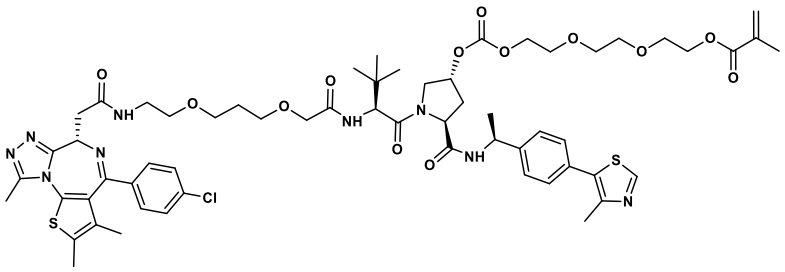
Synthesis of 2-(2-(2-hydroxyethoxy)ethoxy)ethyl methacrylate (4)



The 2,2'-(ethane-1,2-diylbis(oxy))bis(ethan-1-ol) (commercially available, 300.2 mg, 2.0 mmol, 1.0 eq) and DIEA (775.4 mg, 6.0 mmol, 3.0 eq) were dissolved in DCM. Next, the mixture of methacryloyl chlodide (commercially obtainded, 208.0 mg, 2.0 mmol, 1.0 eq) in DCM was dropped into above

solution. The reactive solution was stirred continuously for 24 h, and then the mixture was washed by water and saturated NH_4Cl solution one by one. The raw production was next obtained after above organic solvent system was filtered and then dried through Na_2SO_4. Silica gel column chromatography (n-hexane: ethyl acetate = 4:1) was used to depurate the obtained raw product, thus the compound **4** was gained as colorless oily liquid (301.0 mg, 69% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 6.14 (s, 1H), 5.61-5.56 (m, 1H), 4.34-4.29 (m, 2H), 3.78-3.74 (m, 2H), 3.74-3.71 (m, 2H), 3.69-3.60 (m, 6H), 1.95 (s, 3H).

Synthesis of 2-(2-(2-(((((3R,5S)-1-((S)-2-(tert-butyl)-15-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6Hthieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-diazapentadecanoyl)-5-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)carbonyl)oxy)ethoxy)ethoxy)ethyl methacrylate (6, ARV771-Et)

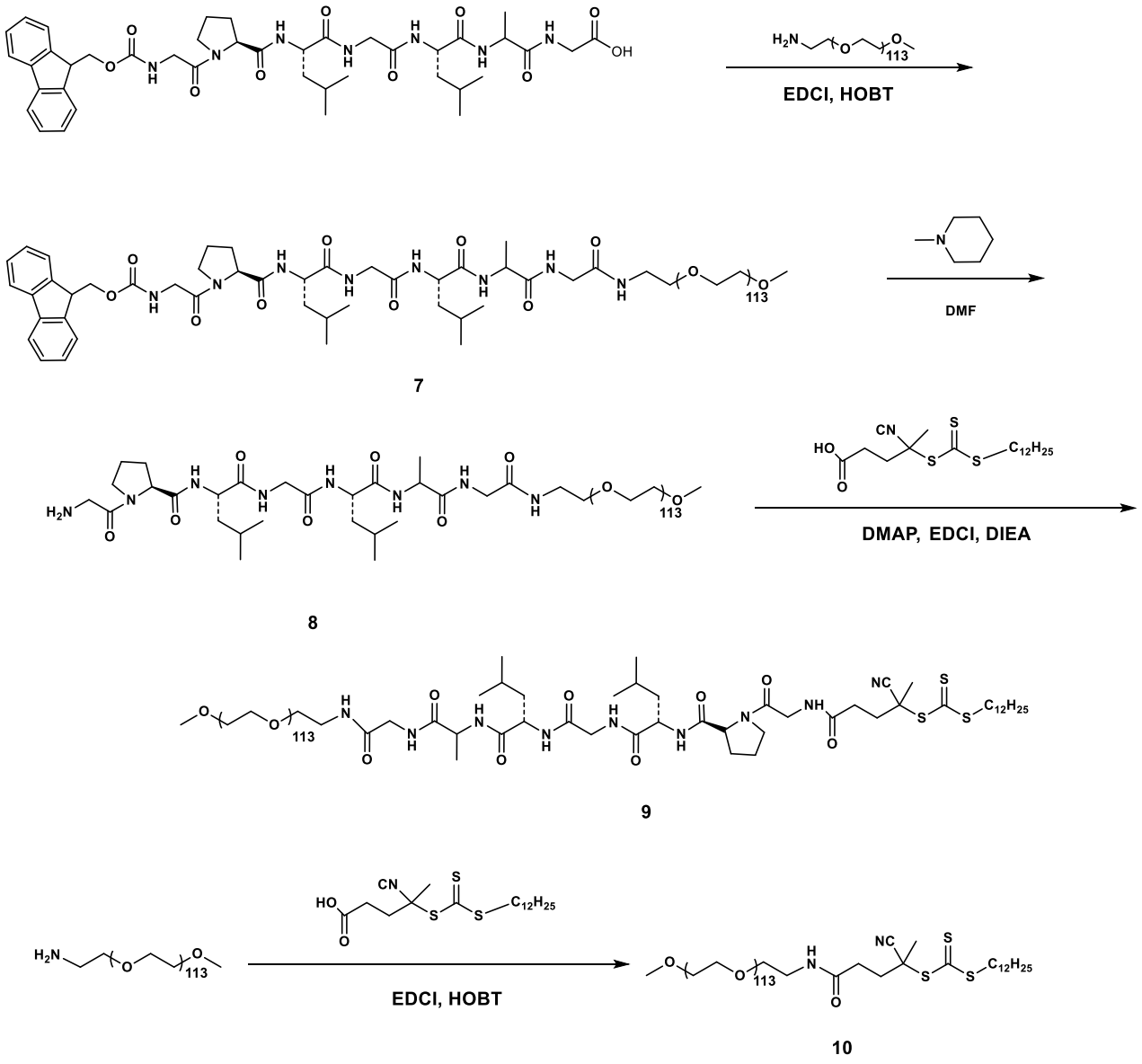


The 2-(2-(2-(((4-nitrophenoxy) carbonyl)oxy)ethoxy)ethoxy)ethyl methacrylate (**5**) was firstly synthesized. The bis(4-nitrophenyl) carbonate (NPC, commercially available, 182.4 mg, 0.6 mmol, 1.2 eq) and DIEA (193.6 mg, 1.5 mmol, 3.0 eq) were dissolved in DCM, subsequently, the liquid of compound **4** (109.1 mg, 0.5 mmol, 1.0 eq) in DCM was dropped into the solution, stirred for 6 h with the protection of argon at room temperature. Then, the reactive mixture was diluted by DCM and washed by water and saturated NH_4Cl solution, further dried over anhydrous Na_2SO_4.

Subsequently, ARV771 (98.5 mg, 0.1 mmol, 1.0 eq), above compound **5** (58.1 mg, 0.15 mmol, 1.5 eq), DIEA (25.8 mg, 0.2 mmol, 2.0 eq) and DMAP (18.3 mg, 0.15 mmol, 1.5 eq) were together dissolved

in DCM. The reaction continued overnight at room temperature. After completed, the solution was washed by water and saturated NH_4Cl solution, further dried over anhydrous Na_2SO_4. Silica gel column chromatography (DCM:MeOH = 80:1~15:1) was utilized to purified the crude product. The finally compound **6** was gained as white solid (73.7 mg, 60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.87 (dd, *J* = 13.2, 7.7 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.36-7.27 (m, 7H), 6.12 (s, 1H), 5.57 (s, 1H), 5.26 (s, 1H), 5.08-4.99 (m, 1H), 4.84 (q, *J* = 7.5 Hz, 1H), 4.69-4.62 (m, 2H), 4.35-4.21 (m, 4H), 4.03-3.44 (m, 21H), 2.62 (s, 3H), 2.59-2.50 (m, 1H), 2.49 (s, 3H), 2.40 (s, 3H), 2.33 (dd, *J* = 21.0, 13.8 Hz, 2H), 1.94 (s, 3H), 1.69 (s, 3H), 1.67 (s, 1H), 1.53 (s, 1H), 1.38 (d, *J* = 6.9 Hz, 3H), 1.25 (s, 3H), 1.10-1.05 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.21, 170.74, 170.66, 169.97, 163.67, 155.82, 150.22, 149.99, 148.37, 143.30, 136.88, 136.39, 131.66, 131.17, 131.04, 130.88, 130.53, 129.86, 129.30, 128.68, 126.41, 77.30, 77.04, 76.79, 73.28, 70.25, 69.35, 69.23, 67.88, 58.66, 56.70, 54.16, 48.81, 39.63, 38.41, 35.81, 33.97, 30.14, 29.70, 29.24, 26.92, 26.51, 21.93, 16.08, 14.39, 13.11, 11.66, 9.14. ESI m/z Calcd. for $C_{60}H_{76}ClN_9O_{13}S_2$ $[M+Na]^+$ 1252.5, Found 1252.8.

Synthesis of macromolecular chain transfer agents $mPEG_{113}$-CTA (9), $mPEG_{113}$-GALGLPG-CTA (10)



Synthesis of mPEG$_{113}$-GALGLPG-CTA (9)

Fmoc-GPLGLPG (commercially available, 250.0 mg, 0.3 mmol, 3.0 eq), EDCI (58.7 mg, 0.3 mmol, 3.0 eq), HOBT (41.4 mg, 0.3 mmol, 3.0 eq) and DIEA (77.5 mg, 0.6 mmol, 6.0 eq) were dissolved in anhydrous DMF. The mixture was stirred for 90 min to activate the carboxyl group firstly. Next, the solution of mPEG$_{113}$-NH$_2$ (commercially available, 500.0 mg, 0.1 mmol, 1 eq) in anhydrous DMF was dropwise added into above mixture, stirred for 24 h under room temperature. Subsequently, 20% (v/v) 4-Methylpiperidine was added into the reactive solution and stirred overnight to remove the Fmoc group. Then, the raw product was purified by dialyzing against ethanol and DI water, the final product

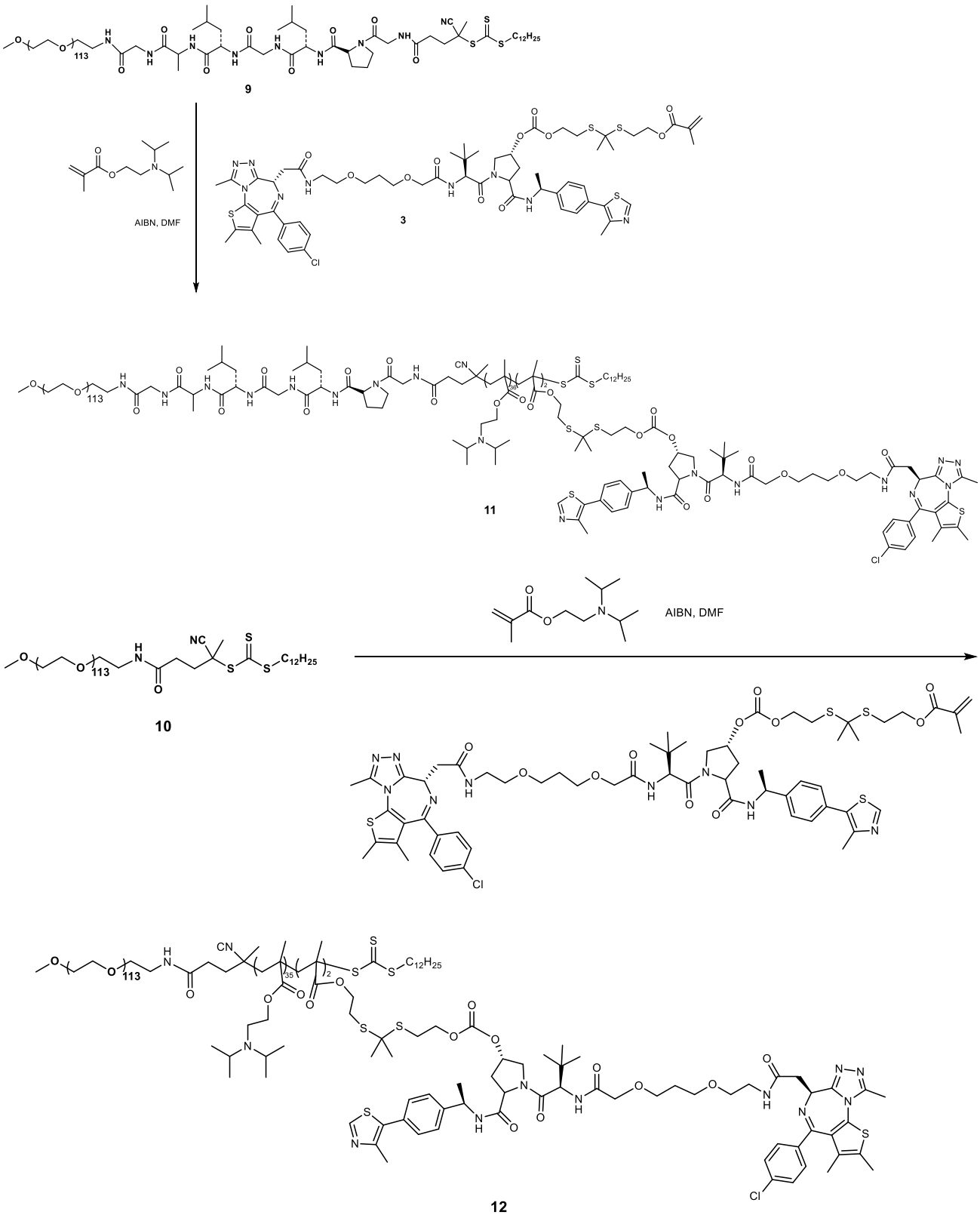
(mPEG$_{113}$-GALGLPG) was gained as off-white powder after lyophilized.

Then, CDP (commercially obtained, 121.1 mg, 0.3 mmol, 3.0 eq), EDCI (115.1 mg, 0.6 mmol, 6.0 eq), HOBT (81.1 mg, 0.6 mmol, 6.0 eq) and DIEA (77.5 mg, 0.6 mmol, 6.0 eq) were together dissolved in anhydrous DMF, stirred for 90 min. Next, the solution of mPEG$_{113}$-GALGLPG (566.0 mg, 0.1 mmol, 1 eq) in DMF was dropped into above mixture, stirred for 24 h under room temperature. The product as yellowish solid was obtained after dialyzed against ethanol and DI water, further lyophilized.

Synthesis of mPEG$_{113}$-CTA (10)

CDP (commercially obtained, 121.1 mg, 0.3 mmol, 3.0 eq), HOBT (81.1 mg, 0.6 mmol, 6.0 eq), EDCI (115.1 mg, 0.6 mmol, 6.0 eq), and DIEA (77.5 mg, 0.6 mmol, 6.0 eq) were together dissolved in anhydrous DMF, reacted for 90 min firstly. Next, the mPEG$_{113}$-NH_2 (commercially available, 500.0 mg, 0.1 mmol, 1.0 eq) was dissolved in anhydrous DMF and then dropped into above mixture, stirred for 24 h. After the reaction completed, the reactive solution was dialyzed against ethanol and DI water. And further lyophilized to give the final yellowish product.

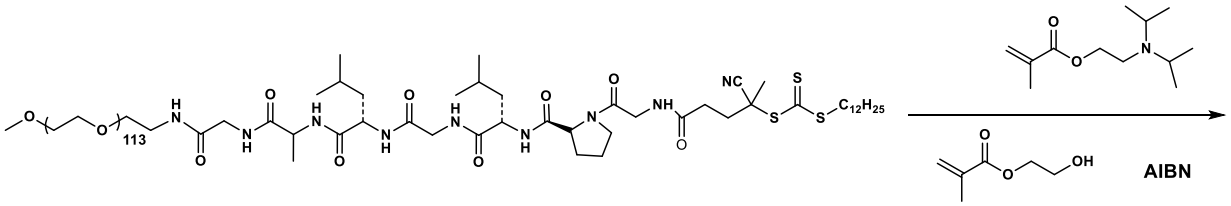
Synthesis of mPEG$_{113}$-GALGLPG-*b*-P(DPAm-*r*-ARV771-TKn) (11) and mPEG$_{113}$-*b*-P(DPAm-*r*-ARV771-TKn) (12)



The reversible addition fragmentation chain transfer (RAFT) polymerization was used to prepared the ROS-activatable PROTAC nanoparticle. mPEG$_{113}$-GALGLPG-CTA (200.0 mg, 0.033 mmol, 1.0 eq) or mPEG$_{113}$-CTA (200.0 mg, 0.037, 1.0 eq), 2-(diisopropylamino)ethyl methacrylate (DPA,

commercially available, 466.2 mg, 2.2 mmol, 60 eq), ARV771-Tk (168.3 mg, 0.13 mmol, 4.0 eq) and AIBN (0.5 mg, 0.0033 mmol, 0.1 eq) were dissolved in 1.5 mL of anhydrous DMF, stirred for 24 h under the temperature of 70 ℃. Then, the reaction solution was dialyzed against ethanol and DI water orderly, further lyophilized to give final product.

Synthesis of mPEG$_{113}$-GALGLPG-*b*-P(DPAm-*r*-HEMAn) (13) and mPEG$_{113}$-*b*-P(DPAm-*r*-HEMAn) (14)



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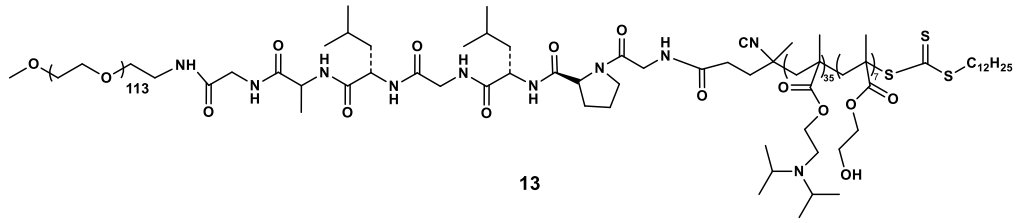

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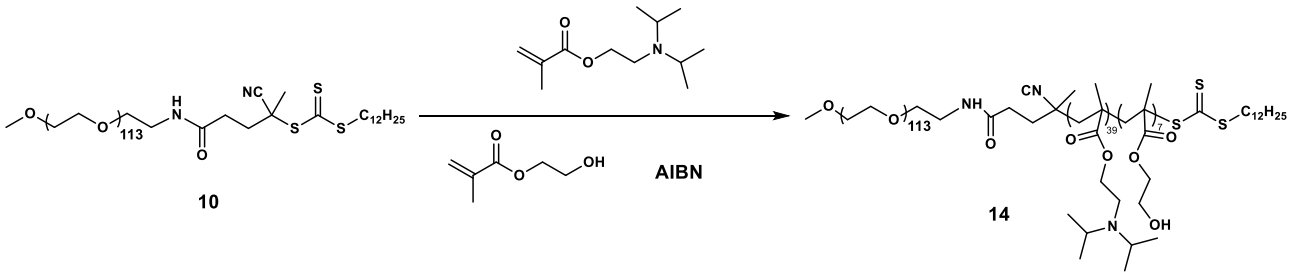
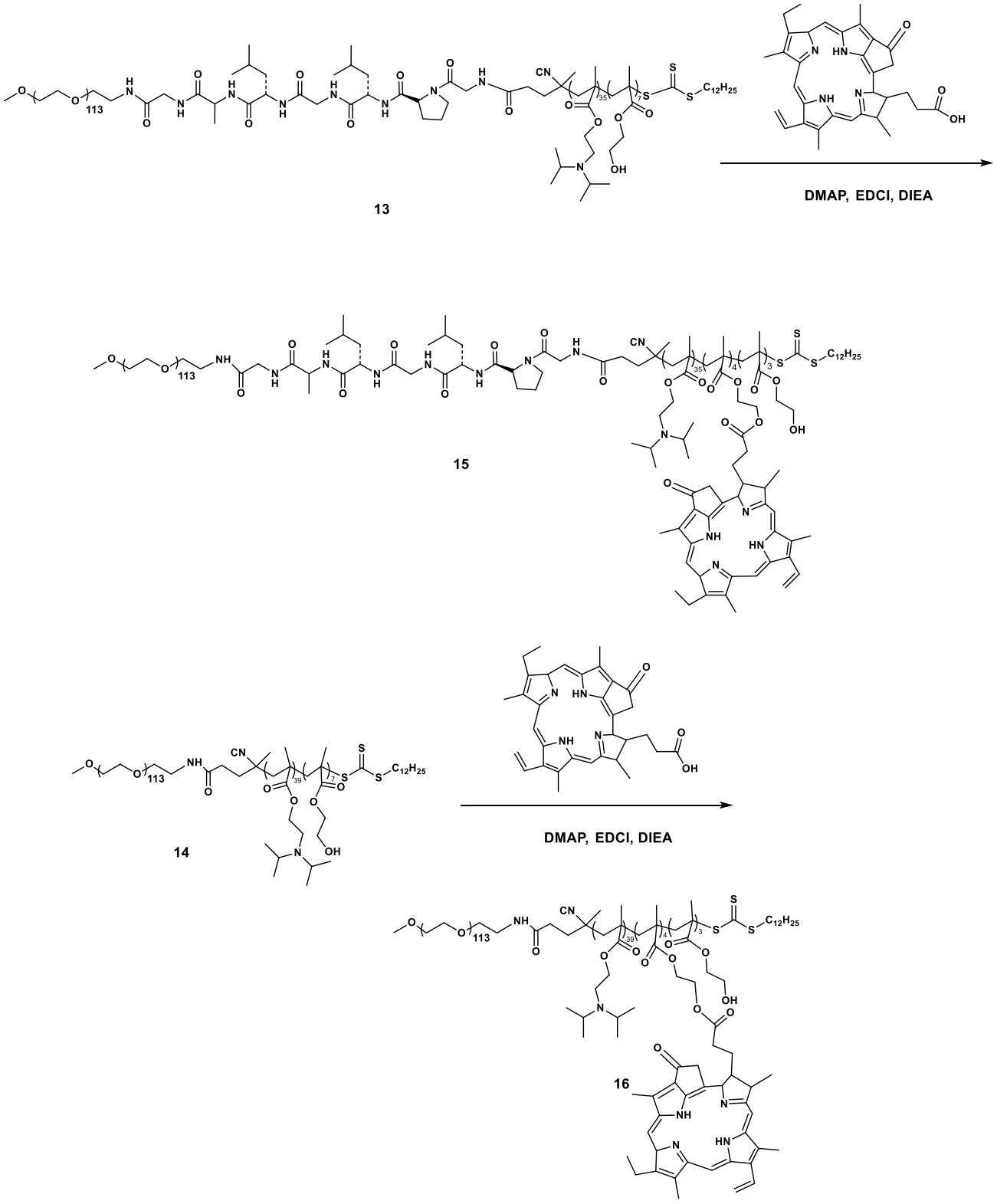


10

14

mPEG$_{113}$-GALGLPG-CTA (200.0 mg, 0.033 mmol, 1.0 eq) or mPEG$_{113}$-CTA (200.0 mg, 0.037, 1.0 eq), 2-(diisopropylamino)ethyl methacrylate (DPA, commercially available, 466.2 mg, 2.2 mmol, 60 eq), 2-hydroxyethyl methacrylate (HEMA, commercially available, 42.9 mg, 0.33 mmol, 10 eq) and AIBN (0.5 mg, 0.0033 mmol, 0.1 eq) were together dissolved in 1.5 mL of anhydrous DMF. The RAFT polymerization was continued for 24 h under the temperature of 70 ℃. The reactive mixture was dialyzed against DI water and further lyophilized to obtain the resultative product.

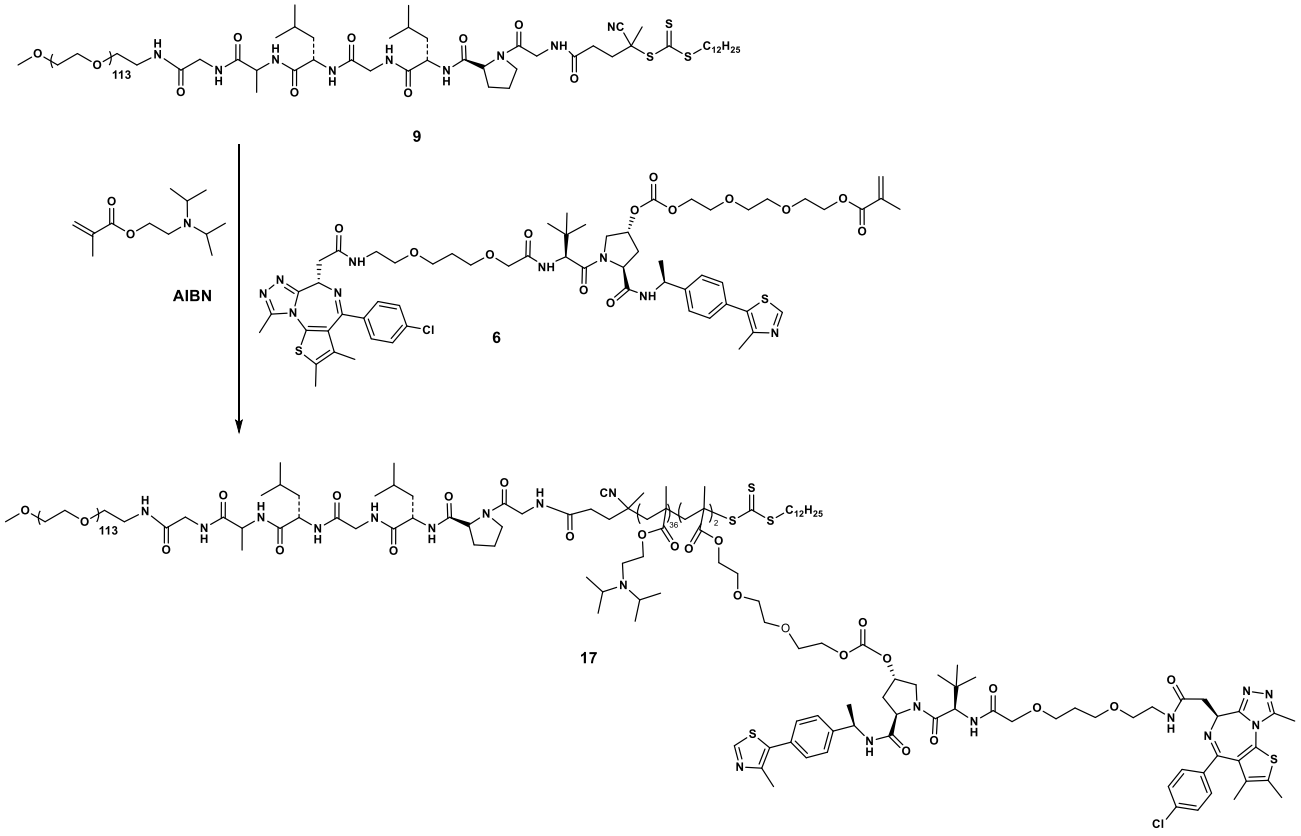
Synthesis of mPEG$_{113}$-GALGLPG-P(DPAm-*r*-PPan) (15) and mPEG$_{113}$-P(DPAm-*r*-PPan) (16)



Briefly, pyropheophorbide a (PPa, commercially acquired, 93.2 mg, 0.18 mmol, 14 eq), EDCI (67.1 mg, 0.36 mmol, 28 eq), DMAP (42.9 mg, 0.36 mmol, 28 eq) and DIEA (45.3 mg, 0.36 mmol, 28 eq)

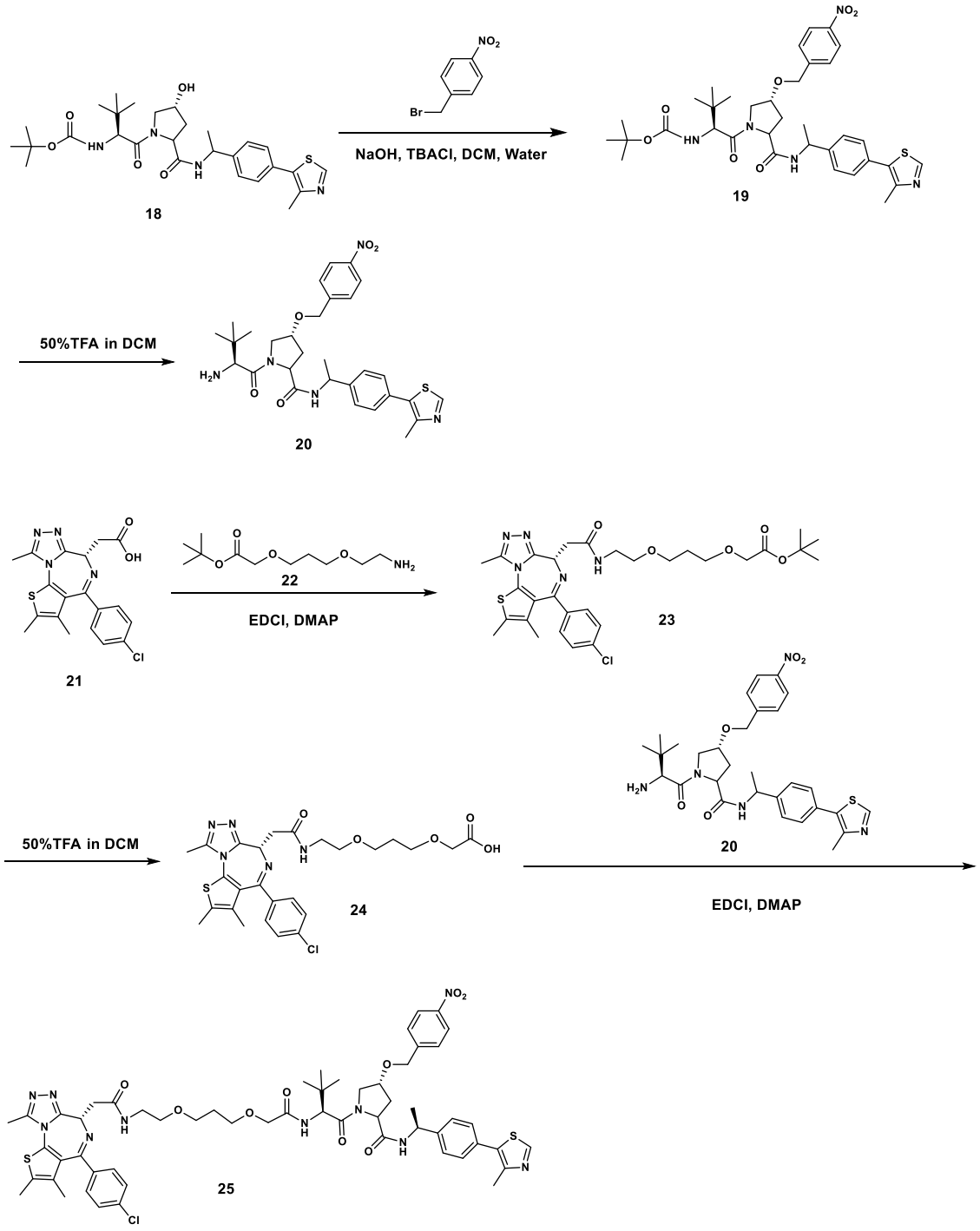
were together dissolved in anhydrous DMF, stirred for 90 min. Then, the solution of $mPEG_{113}$-GALGLPG-*b*-P(DPAm-*r*-HEMAn) (201.4 mg, 0.012 mmol, 1.0 eq) or mPEG113-b-P(DPAm-*r*-HEMAn) (200.0 mg, 0.012 mmol, 1.0 eq) in anhydrous DMF was added into above mixture dropwise. The reaction sustained for 24 h under room temperature. Next, the reactive mixture was dialyzed against DMSO and DI water orderly, and further lyophilized to give final PPa-labled deblock copolymers.

Synthesis of ROS-insensitive PROTAC of $mPEG_{113}$-GALGLPG-P(DPAm-*r*-ARV771-Etn) (17)

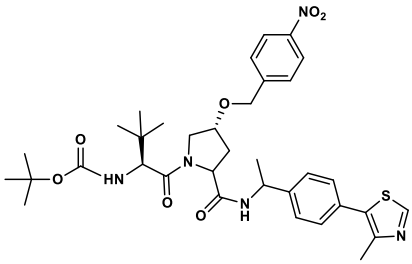


The $mPEG_{113}$-GALGLPG-CTA (60.0 mg, 0.0098 mmol, 1.0 eq), DPA (124.6 mg, 0.59 mmol, 60 eq), ARV771-Et (50.0 mg, 0.039 mmol, 4.0 eq) and AIBN (0.14 mg, 0.000098 mmol, 0.1 eq) were dissolved in the 1.0 mL of anhydrous DMF, stirred for 24 h under the temperature of 70 ℃. The mixture was further purified through dialyzing against ethanol and DI water seriatim, further lyophilized to gain the final product.

Synthesis of ARV771-Nb (25)

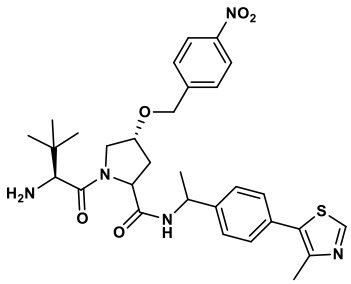


Synthesis of tert-butyl ((2S)-3,3-dimethyl-1-((4R)-2-((1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)-4-((4-nitrobenzyl)oxy)pyrrolidin-1-yl)-1-oxobutan-2-yl)carbamate (19)



The compound **18** (commercially acquired, 300.0 mg, 0.57 mmol, 1.0 eq) and tetrabutylammonium chloride (TBACl, commercially acquired, 31.4 mg, 0.11 mmol, 0.2 eq) were dissolved in the DCM, and then the 20% NaOH solution was added into above mixture. Next, the 1-(bromomethyl)-4-nitrobenzene (commercially acquired, 215.0 mg, 0.62 mmol, 1.1 eq) was added into the solution, stirred for 3 h under room temperature. Next, the solution was washed by water, dried via Na_2SO_4, filtered to gain the raw product. Afterward, the crude product was further purified through silica gel column chromatography (DCM:MeOH = 80:1~15:1). The finally compound **19** was gained as white solid (172.1 mg, 44.7% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.21 (d, *J* = 8.6 Hz, 2H), 7.55-7.47 (m, 2H), 7.44-7.31 (m, 5H), 5.21 (d, *J* = 9.9 Hz, 1H), 4.66-4.53 (m, 2H), 4.38 (s, 1H), 4.35-4.32 (m, 1H), 3.83-3.73 (m, 1H), 3.67 (dt, *J* = 11.2, 5.7 Hz, 1H), 2.79-2.70 (m, 1H), 2.55 (s, 3H), 2.30-2.18 (m, 1H), 1.41-1.35 (m, 9H), 1.27 (d, *J* = 11.8 Hz, 3H), 0.92 (d, *J* = 10.7 Hz, 9H).

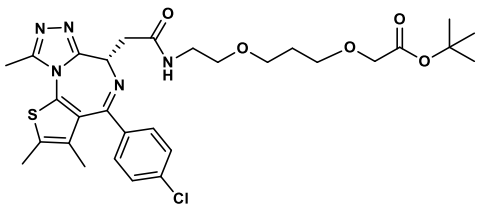
Synthesis of (4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-N-(1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)-4-((4-nitrobenzyl)oxy)pyrrolidine-2-carboxamide (20)



Compound **19** (150 mg, 0.22 mmol, 1.0 eq) was dissolved in DCM solution containing 50% TFA (v/v), stirred at room temperature for 3 h. Then, the solvent was removed under pressure 3 h post-reaction.

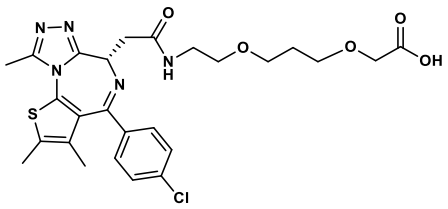
Afterward, the raw product was redissolved in DCM, washed with water, dried over Na_2SO_4, and further purified by silica gel chromatography (DCM:MeOH = 80:1~15:1) to give final compound **20** (76.3 mg, 60.1% yield) as white solid. ^{1}H NMR (500 MHz, MeOD) δ 8.88 (s, 1H), 8.22 (d, *J* = 8.7 Hz, 2H), 7.60 (d, *J* = 8.7 Hz, 2H), 7.46-7.40 (m, 5H), 7.35 (dd, *J* = 8.0, 6.6 Hz, 2H), 5.04-4.97 (m, 1H), 4.60 (s, 2H), 4.05 (d, *J* = 11.6 Hz, 1H), 3.95-3.81 (m, 1H), 3.71 (dt, *J* = 40.4, 20.3 Hz, 2H), 3.45 (s, 1H), 2.48 (d, *J* = 2.3 Hz, 4H), 2.00 (ddd, *J* = 13.6, 9.5, 4.8 Hz, 1H), 1.51 (d, *J* = 7.0 Hz, 3H), 1.08 (s, 9H).

Synthesis of tert-butyl (S)-2-(3-(2-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6 yl) acetamido)ethoxy)propoxy) acetate (23)



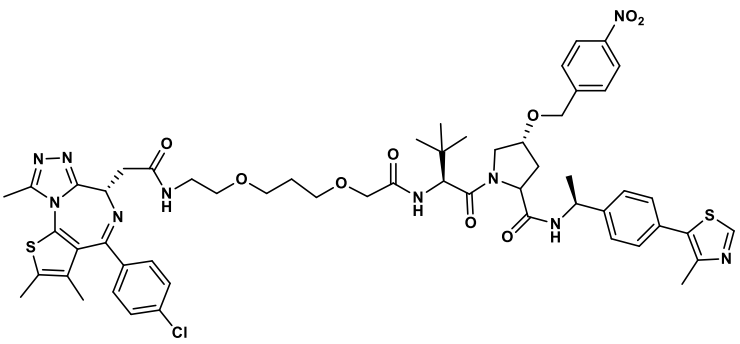
Compound **21** (200.4 mg, 0.50 mmol, 1.0 eq), compound **22** (commercially available, 128.2 mg, 0.55 mmol, 1.1 eq), DIEA (322.5 mg, 2.5 mmol, 5.0 eq) and HATU (239.3 mg, 0.63 mmol, 1.3 eq) were together dissolved in DCM. The reactive mixture was stirred contineously for 12 h at room temperature. Next, the solution was washed by water and saturated NH_4Cl solution, dried over Na_2SO_4. The silica gel column chromatography (DCM:MeOH = 80:1~15:1) was utilized to purified the crude product to give the final compound **23** as light-yellow oily liquid (175.4 mg, 57% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.7 Hz, 2H), 4.69-4.58 (m, 1H), 3.97 (d, *J* = 7.9 Hz, 2H), 3.58 (ddt, *J* = 18.6, 10.4, 6.3 Hz, 8H), 3.48-3.36 (m, 2H), 2.67 (d, *J* = 2.8 Hz, 3H), 2.66 (s, 3H), 2.40 (s, 3H), 1.90 (p, *J* = 6.2 Hz, 2H), 1.48 (s, 9H).

Synthesis of (S)-2-(3-(2-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)ethoxy)propoxy)acetic acid (24)



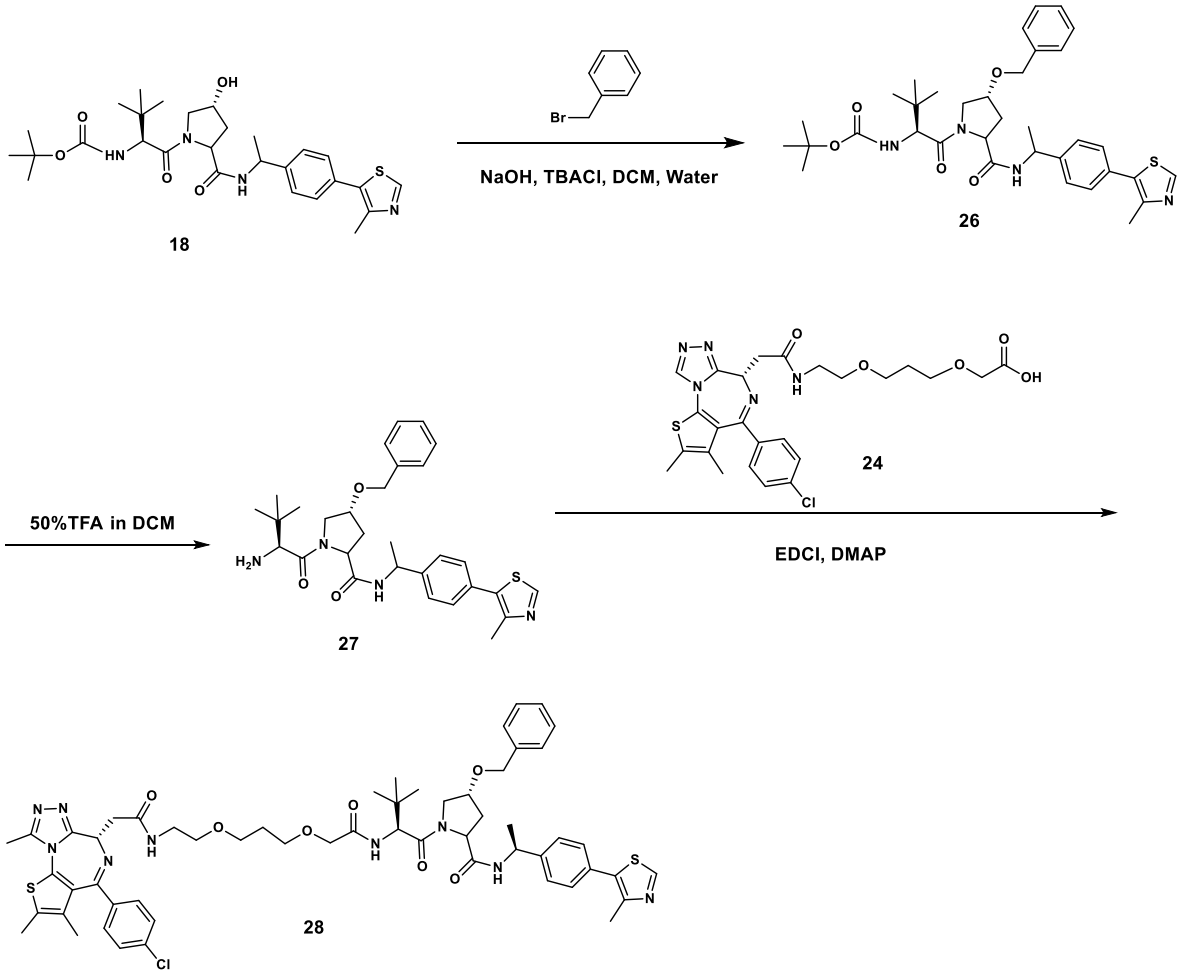
Compound **23** (150 mg, 0.24 mmol, 1.0 eq) was dissolved in the solution of 50% TFA (v/v) in DCM, stirred for 3 h under room temperature. Next, the TFA and solvent was removed by vacuum evaporation. Then, the crude product was dissolved in DCM, and washed by water, dried via anhydrous Na2SO4, purified by silica gel column chromatography (DCM:MeOH = 80:1~20:1) to obtained compound **24** as slight yellow solid (102.2 mg, 75% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 8.8 Hz, 2H), 7.30 (s, 1H), 4.73 (dd, *J* = 8.6, 5.4 Hz, 1H), 4.12 (dd, *J* = 40.6, 16.6 Hz, 2H), 3.84-3.57 (m, 7H), 3.53-3.39 (m, 3H), 2.67 (d, *J* = 7.7 Hz, 3H), 2.40 (d, *J* = 9.2 Hz, 3H), 1.90-1.83 (m, 2H), 1.68 (d, *J* = 6.0 Hz, 3H).

Synthesis of (4R)-1-((S)-2-(tert-butyl)-15-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-diazapentadecanoyl)-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)-4-((4-nitrobenzyl)oxy)pyrrolidine-2-carboxamide (25, ARV771-Nb)

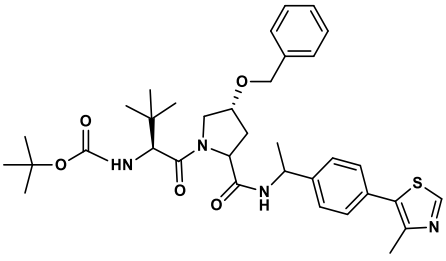


Compound **19** (60.0 mg, 0.1 mmol, 1.0 eq), compound **24** (67.1 mg, 0.12 mmol, 1.2 eq), HUAT (56.3 mg, 0.15 mmol, 1.5 eq) and DIEA (64.5 mg, 0.5 mmol, 5.0 eq) were together dissolved in DCM, stirred overnight. After the reaction completed, the mixture was washed by water and saturated NH_4Cl solution orderly, and then dried via anhydrous Na_2SO_4 solution, filtered to give raw product. Silica gel column chromatography (DCM:MeOH = 80:1 ~20:1) was utilized to purified the crude product to gain the final compound **25** as white solid (68.3 mg, 61% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.16 (t, *J* = 9.0 Hz, 2H), 7.86 (dd, *J* = 23.9, 7.8 Hz, 1H), 7.42 (dt, *J* = 26.0, 13.1 Hz, 4H), 7.35-7.27 (m, 5H), 5.30 (s, 2H), 5.03 (p, *J* = 7.0 Hz, 1H), 4.88 (dd, *J* = 8.0, 6.7 Hz, 1H), 4.78 (d, *J* = 9.8 Hz, 1H), 4.72-4.63 (m, 2H), 4.59-4.49 (m, 1H), 4.36-4.30 (m, 1H), 4.24 (d, *J* = 11.2 Hz, 1H), 4.00 (d, *J* = 15.5 Hz, 1H), 3.90 (dd, *J* = 15.4, 7.5 Hz, 1H), 3.82-3.72 (m, 1H), 3.70-3.38 (m, 10H), 2.64 (d, *J* = 3.3 Hz, 1H), 2.62 (d, *J* = 6.7 Hz, 3H), 2.54-2.47 (m, 4H), 2.40 (s, 3H), 2.33-2.26 (m, 1H), 1.95-1.83 (m, 2H), 1.68 (d, *J* = 5.4 Hz, 3H), 1.36 (t, *J* = 7.3 Hz, 3H), 1.10 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 170.72, 170.18, 169.47, 169.35, 162.94, 155.39, 149.78, 149.39, 147.91, 146.89, 144.96, 142.76, 136.29, 136.04, 131.34, 131.18, 130.50, 130.31, 130.11, 129.36, 128.85, 128.18, 127.30, 126.02, 123.12, 77.27, 76.82, 76.56, 76.31, 69.71, 69.25, 69.00, 68.75, 67.56, 58.07, 55.81, 53.69, 52.96, 52.53, 48.41, 39.20, 38.14, 36.37, 35.15, 33.46, 28.89, 26.01, 21.46, 15.61, 13.92, 12.64, 11.28. ESI m/z Calcd. for $C_{56}H_{65}ClN_{10}O_9S_2$ $[M+H]^+$ 1121.4, Found 1121.6.

Synthesis of ARV771-Ph (28)



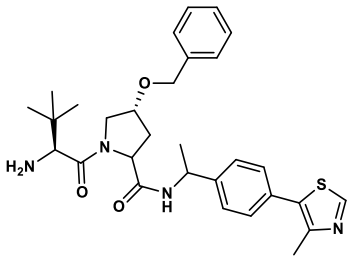
Synthesis of tert-butyl ((2S)-1-((4R)-4-(benzyloxy)-2-((1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (26)



The compound **18** (commercially available, 150.0 mg, 0.28 mmol, 1.0 eq) and tetrabutylammonium chloride (TBACl, commercially acquired, 16.0 mg, 0.056 mmol, 0.2 eq) were dissolved in the DCM, and then added 20% NaOH solution. Next, (bromomethyl)benzene (commercially acquired, 52.7 mg, 0.31 mmol, 1.1 eq) was added into above mixture, stirred for 3 h under room temperature. The solution was then washed with water, dried by Na_2SO_4, filtered. Afterward, the raw product was further purified

by silica gel column chromatography (DCM:MeOH = 80:1~15:1) to give the compound **26** as white solid (85.3 mg, 48% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H), 7.51-7.27 (m, 8H), 5.28-5.19 (m, 2H), 5.12-5.00 (m, 1H), 4.73 (q, *J* = 8.0 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.47 (d, *J* = 11.5 Hz, 1H), 4.41-4.24 (m, 1H), 4.07 (d, *J* = 10.9 Hz, 1H), 3.79 (dd, *J* = 11.7, 4.5 Hz, 1H), 3.62 (dd, *J* = 10.9, 4.4 Hz, 1H), 2.81-2.61 (m, 1H), 2.57 (s, 3H), 2.21-2.00 (m, 1H), 1.51-1.45 (m, 9H), 1.41 (s, 3H), 1.02 (d, *J* = 6.7 Hz, 9H).

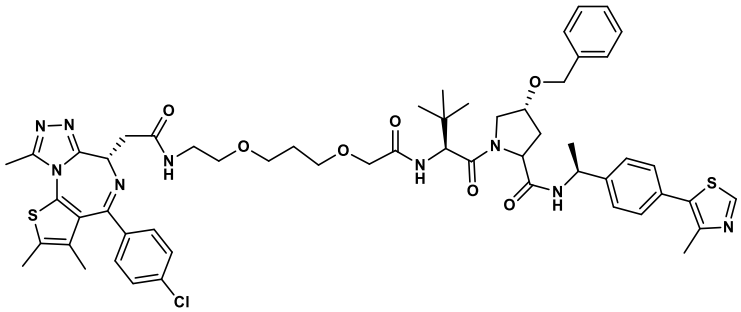
Synthesis of (4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-(benzyloxy)-N-(1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (27)



Compound **25** (70 mg, 0.11 mmol, 1.0 eq) was dissolved in DCM containing 50% of TFA (v/v), stirred at room temperature for 3 h. Next, the solvent and TFA were removed by vacuum evaporation to give the crude product. The raw product was then diluted with DCM, washed by water, dried with anhydrous Na_2SO_4, purified by silica gel column chromatography (DCM:MeOH = 80:1~15:1). The final compound **27** was obtained as white solid (44.1 mg, 73% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.74 (s, 1H), 7.45-7.27 (m, 9H), 5.17-4.93 (m, 1H), 4.74 (s, 1H), 4.66-4.40 (m, 2H), 4.33 (s, 1H), 3.76-3.56 (m, 2H), 3.41 (s, 1H), 2.53 (s, 4H), 2.08 (s, 1H), 1.48 (t, *J* = 10.2 Hz, 3H), 1.04 (s, 9H).

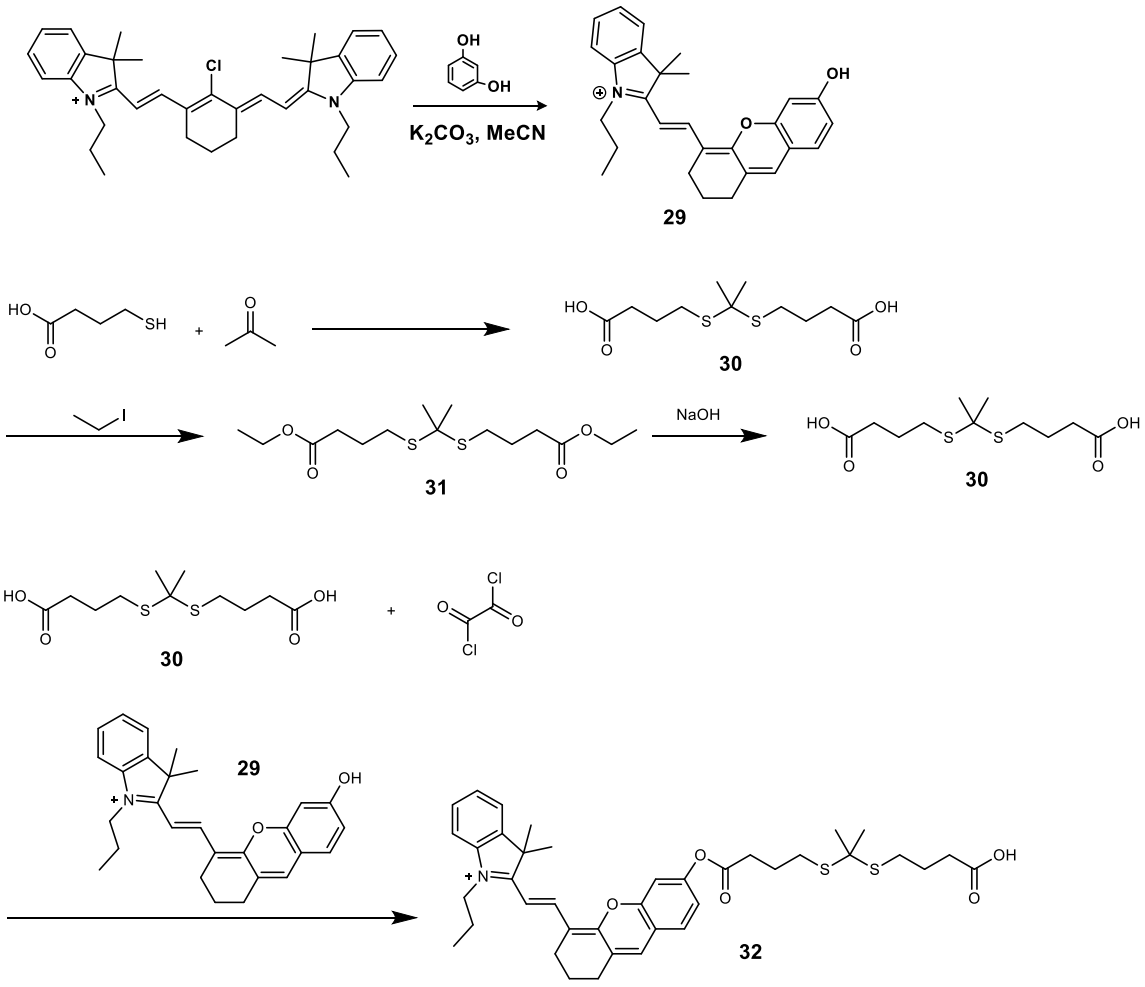
Synthesis of (4R)-4-(benzyloxy)-1-((S)-2-(tert-butyl)-15-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,14-dioxo-6,10-dioxa-3,13-diazapentadecanoyl)-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide

(28)

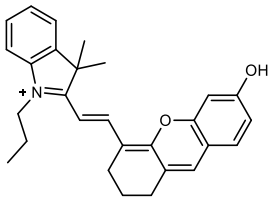


Compound **27** (30.0 mg, 0.056 mmol, 1.0 eq), compound **24** (34.5 mg, 0.062 mmol, 1.1 eq), HATU (31.5 mg, 0.084 mmol, 1.5 eq) and DIEA (36.1 mg, 0.28 mmol, 5.0 eq) were together dissolved in DCM, stirred overnight. After the reaction completed, the solution was washed with water and saturated NH_4Cl solution, dried over anhydrous Na_2SO_4 solution, and further filtered to give the raw product. The silica gel column chromatography (DCM:MeOH = 80:1~15:1) was used to purify the crude product to obtain final compound **28** as white solid (31.9 mg, 53% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.48-7.40 (m, 3H), 7.37-7.27 (m, 12H), 5.09-4.98 (m, 1H), 4.85-4.74 (m, 2H), 4.68 (t, *J* = 6.8 Hz, 1H), 4.58-4.43 (m, 2H), 4.29 (d, *J* = 3.5 Hz, 1H), 4.12 (d, *J* = 11.1 Hz, 1H), 3.96 (dd, *J* = 44.0, 15.5 Hz, 2H), 3.76-3.39 (m, 12H), 2.92 (d, *J* = 10.6 Hz, 1H), 2.65 (d, *J* = 9.3 Hz, 7H), 2.52 (s, 3H), 2.46-2.37 (m, 4H), 2.30-2.19 (m, 1H), 1.94-1.85 (m, 2H), 1.69 (s, 3H), 1.08 (s, 9H). ESI m/z Calcd. for $C_{56}H_{66}ClN_9O_7S_2$ $[M+H]^+$ 1075.4, Found 1076.6.

Synthesis of CY-TK



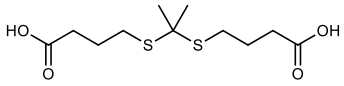
Synthesis of (*E*)-2-(2-(6-hydroxy-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3,3-dimethyl-1-propyl-3*H*-indol-1-ium (29)



Grinded potassium carbonate (128 mg, 1.14 mmol) and resorcinol (168 mg, 1.14 mmol) were stirred in 6 mL MeCN at 55 °C under N_2 atmosphere for 15 minutes. Then, the IR-780 (commercially obtained, 200 mg, 0.38 mmol) dissolved in 4 mL MeCN was added dropwise into above mixture, stirring for 3 h. The reaction mixture was then diluted with DCM and washed with water for three times. The organic phase was collected, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was then purified with silica gel chromatography (DCM:MeOH = 25:1) to obtain pure compound **29**

as black solid (110 mg, 73% yield). ^{1}H NMR (500 MHz, CDCl3) δ 8.42 (d, *J* = 13.8 Hz, 1H), 7.41-7.29 (m, 3H), 7.09-6.92 (m, 3H), 6.68 (s, 1H), 6.42 (d, *J* = 51.5 Hz, 1H), 5.95 (d, *J* = 14.1 Hz, 1H), 5.23 (s, 1H), 3.98 (s, 2H), 2.63 (d, *J* = 39.8 Hz, 5H), 1.85 (s, 4H), 1.68 (s, 6H), 1.01 (s, 3H).

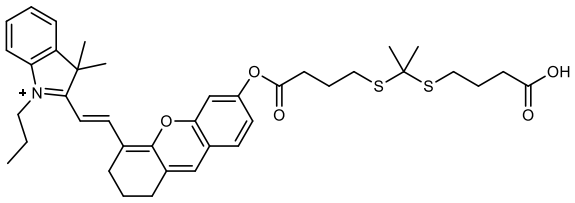
Synthesis of 4,4'-(propane-2,2-diylbis(sulfanediyl))dibutyric acid (30)



Aim to obtained compound **30**, compound **31** was firstly synthesized. Briefly, 4-mercaptobutanoic acid (commercially obtained, 300 mg, 2.5 mmol) was dissolved in 5 mL acetone with 300 uL TFA. The reaction mixture was stirred for 24 h, and then acetone and TFA were removed under reduced pressure. The crude product was dissolved in anhydrous DMF, iodoethane (409.5 mg, 2.65 mmol) and Cs_2CO_3 (1018.19 mg, 3.13 mmol) were added into above mixture, stirring for 12 h. the reaction was monitored by TLC, and then the raw product was obtained through extracted with ethyl acetate, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was then purified with silica gel chromatography (petroleum ether:ethyl acetate = 60:1 ~ 30:1) to obtain pure compound **31** as oily liquid (240 mg, 57% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.07 (q, *J* = 7.2 Hz, 4H), 2.57 (t, *J* = 7.3 Hz, 4H), 2.35 (t, *J* = 7.4 Hz, 4H), 1.84 (p, *J* = 7.3 Hz, 4H), 1.51 (s, 6H), 1.19 (t, *J* = 7.2 Hz, 6H).

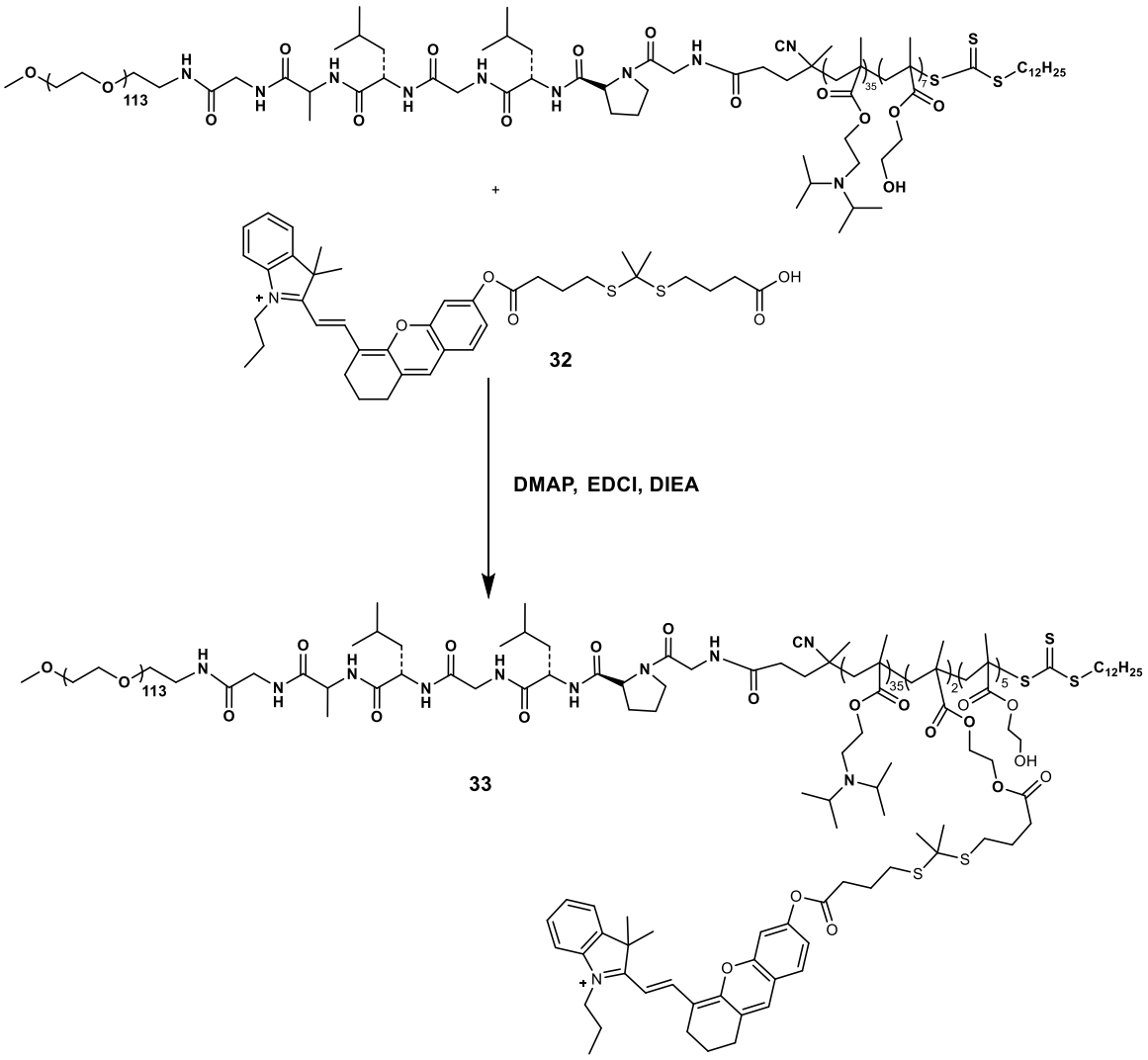
The compound **31** was stirred in saturated NaOH solution for 1 h, then the pH of the mixture was adjusted to acid with HCl, the aim product was extracted through ethyl acetate, dried over Na_2SO_4 filtered and concentrated under reduced pressure (160 mg, 80% yield). ^{1}H NMR (500 MHz, CDCl3) δ 2.74-2.65 (m, 4H), 2.52 (t, *J* = 7.2 Hz, 4H), 2.00-1.90 (m, 4H), 1.60 (s, 6H).

Synthesis of (*E*)-2-(2-(6-((4-((2-((3-carboxypropyl)thio)propan-2-yl)thio)butanoyl)oxy)-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3,3-dimethyl-1-propyl-3*H*-indol-1-ium (32)



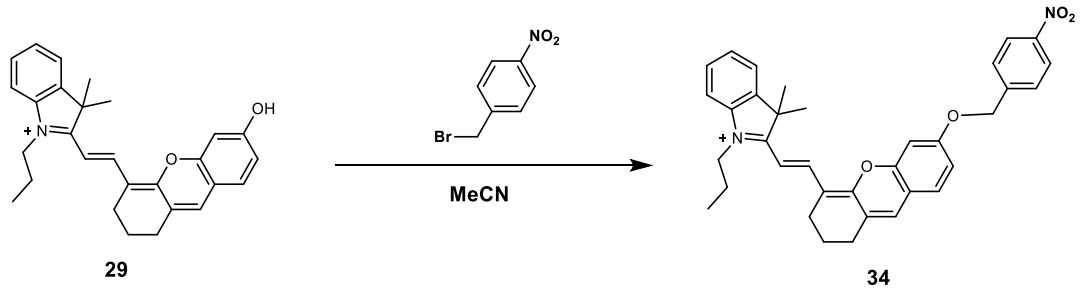
Compound **30** (20 mg, 0.156 mmol) was dissolved in anhydrous DCM, and then oxalyl dichloride was diluted by anhydrous DCM and dropped into above solution under ice bath. The mixture was stirred for 30 min, and then concentrated under reduced pressure. The obtained product was dissolved into anhydrous DCM and dropped into the mixture of compound **29** (24 mg, 0.059 mmol) and TEA. After 2 h of reaction, the crude was then purified with silica gel chromatography (DCM:MeOH = 15:1) to obtain pure compound **32** as black solid (23.8 mg, 60% yield). ^{1}H NMR (500 MHz, CDCl3) δ 8.44 (d, J = 14.0 Hz, 1H), 7.38-7.21 (m, 4H), 7.04 (d, J = 7.9 Hz, 2H), 6.29 (d, J = 8.0 Hz, 1H), 5.96 (d, J = 14.2 Hz, 1H), 5.23 (s, 1H), 3.98 (t, J = 6.9 Hz, 2H), 3.14-2.92 (m, 3H), 2.80-2.43 (m, 7H), 1.84 (dd, J = 11.8, 5.9 Hz, 4H), 1.67 (s, 6H), 1.41-1.10 (m, 9H), 1.00 (dd, J = 13.1, 5.7 Hz, 3H). ESI m/z Calcd. for $C_{39}H_{48}NO_5S_2$+ $[M]^+$ 674.3, Found 674.5.

Synthesis of mPEG$_{113}$-GALGLPG-*b*-P(DPAm-*r*-CY-TKn) (33)



Compound **32** (10 mg, 0.015 mmol), EDCI (8.2 mg, 0.045 mmol), DMAP (5.3 mg, 0.045 mmol) and DIEA (5.6 mg, 0.36 mmol) were together dissolved in anhydrous DMF, stirred for 90 min. Then, the solution of $mPEG_{113}$-GALGLPG-*b*-P(DPAm-*r*-HEMAn) (20 mg, 0.0012 mmol) in anhydrous DMF was added into above solution. The reaction stirred for 24 h under room temperature. Next, the solution was dialyzed against EtOH and DI water orderly, and further lyophilized to give final CY-labled deblock copolymers.

Synthesis of (*E*)-3,3-dimethyl-2-(2-(6-((4-nitrobenzyl)oxy)-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-1-propyl-3*H*-indol-1-ium (CY-Nb)



Compound **29** (30 mg, 0.073 mmol) and 1-(bromomethyl)-4-nitrobenzene (23.5 mg,0.109 mmol) were dissolved in anhydrous MeCN, stirred for 5 h under 50 ℃. The mixture was concentrated under reduced pressure and further purified with silica gel chromatography (DCM:MeOH = 30:1) to obtain pure compound **34** as black solid (22.7 mg, 56% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (d, *J* = 14.9 Hz, 1H), 8.19 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 7.4 Hz, 1H), 7.42- 7.38 (m, 1H), 7.36-7.26 (m, 3H), 7.17 (d, *J* = 1.6 Hz, 2H), 6.91 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.44 (d, *J* = 14.8 Hz, 1H), 5.45 (s, 1H), 5.23 (s, 2H), 4.37 (t, *J* = 7.2 Hz, 2H), 2.68 (dd, *J* = 12.4, 6.3 Hz, 3H), 1.98-1.73 (m, 10H), 1.03 (t, *J* = 7.4 Hz, 3H). ESI m/z Calcd. for $C_{35}H_{35}N_2O_4$+ $[M]^+$ 547.26, Found 547.4.

Supplementary Table 1. List of abbreviations

Abbreviation	Full name
Abl1	abelson murine leukemia viral oncogene homolog 1
BRD4	bromodomain and extraterminal protein 4
Cdk1	cyclin-dependent kinase 1
CDK4/*Cdk4*	cyclin-dependent kinase 4
CDK6/*Cdk6*	cyclin-dependent kinase 6
Cdk7	cyclin-dependent kinase 7
CLSM	confocal laser scanning microscopy
CY	hemicyanine
CSCs	cancer stem-like cells
DEGs	differentially expressed genes
DLS	dynamic light scattering
DPA	2-(diisopropylamino)ethyl methacrylate
GPC	gel permeability chromatography
H&E	hematoxylin and eosin stain
HEMA	2-hydroxyethyl methacrylate
HNSCC	head-neck squamous cell carcinoma
HIF	hypoxia inducible factor
HPLC	high-performance liquid chromatography
^{1}H-NMR	proton nuclear magnetic resonance
KEGG	kyoto encyclopedia of genes and genomes
Klf4	kruppel like factor 4
Kras	kirsten rat sarcoma viral oncogene
LC-MS	liquid chromatography-mass spectrometry
Mdm2	murine double minute 2
Met	c-Mesenchymal-epithelial transition factor
MMP-2	matrix metalloproteinase 2
MS	mass spectrometry
OCT4	octamer-binding transcription factor 4
p21/*Cdkn1a*	cyclin-dependent kinase inhibitor 1A
PAI	photoacoustic imaging
PDT	photodynamic therapy
PDI	polydispersity index
PEG	poly(ethylene glycol)
POI	protein of interest
PROTAC	PROteolysis TArgeting Chimeras
PPa	pyropheophorbide a
qPCR	quantitative polymerase chain reaction
Raf1	murine leukemia viral oncogene homolog 1
RAFT	reversible addition fragmentation chain transfer
ROS	reactive oxygen species

SOX2	SRY-box transcription factor 2
Sox4	SRY-box transcription factor 4
TEM	transmission electron microscopy
TK	thioketal
TNBC	triple negative breast cancer
TNF	tumor necrosis factor
TUNEL	TdT-mediated dUTP nick end labeling
VHL	Von Hippel-Lindau disease
Wnt7b	wingless-type MMTV integration site family member 7B

Supplementary Table 2. List of real-time PCR primers

Gene name	Forward primer	Reward primer
Gapdh	CAAGGCTGTGGGCAAGGTCATC	GTGTCGCTGTTGAAGTCAGAGGAG
Rif1	CGGAAGACTGTGGTATGGCTGAAC	TGCCTCCGACTTGTAGGGTATGG
Wnt7b	CAACGAGTGCCAGTACCAGTTCC	TCTTGATCTCCCGAGCGTCCAC
Sox4	CAAGCACCTGGCGGAGAAGAAG	AGGAGGAGGAAGAGGAGGAGTGG
Klf4	AGAGGAGCCCAAGCCAAAGAGG	GTGTTTACGGTAGTGCCTGGTCAG
Cdc73	AGCGTCAACATCGGCAAGTATAGAC	ATCTCGGGTCACATCTACCTCAGC
Cdkn1a	GCCCGTGAGCGATGGAACTTC	CCTGCCTCCTCCCAACTCATCC
Mdm2	AGGCAGGGGAGAGTGATACAGATTC	CAGGAAGCCAATTCTCACGAAGGG
Cdk6	GTGACCAGCAGCGGACAAATAAAAC	ACGACCACTGAGGTTAGAGCCATC
Cdk4	TTGCCAGCCGAAACGATCAAGG	TCCACCACTTGTCACCAGAATGTTC

Supplementary Table 3. ^{1}H-NMR spectrum and GPC-determined molecular weights of the diblock copolymers synthesized in this research.

Copolymer	^{1}H-NMR	GPC (Da)		PDI*
	Mn (kDa)	*Mw* (kDa)	*Mn* (kDa)	(*Mw/Mn*)
PGDT	16.2	18.9	15.3	1.22
PDT	15.0	18.4	15.0	1.23
PGDH	14.3	18.2	14.0	1.30
PDH	14.2	19.0	12.2	1.56
PGDA	16.7	21.7	15.2	1.43
PDA	15.9	24.9	15.6	1.60
PGDE	16.2	17.3	14.0	1.23

* GPC examination was achieved with a mobile phase of tetrahydrofuran (THF) at a flow rate of 1.0 mL/min and temperature of 35 ℃.

PGDT: $mPEG_{113}$-GALGLPG-*b*-P(DPA_{36}-*r*-ARV771-TK_2);

PDT: $mPEG_{113}$-*b*-P(DPA_{35}-*r*-ARV771-TK_2);

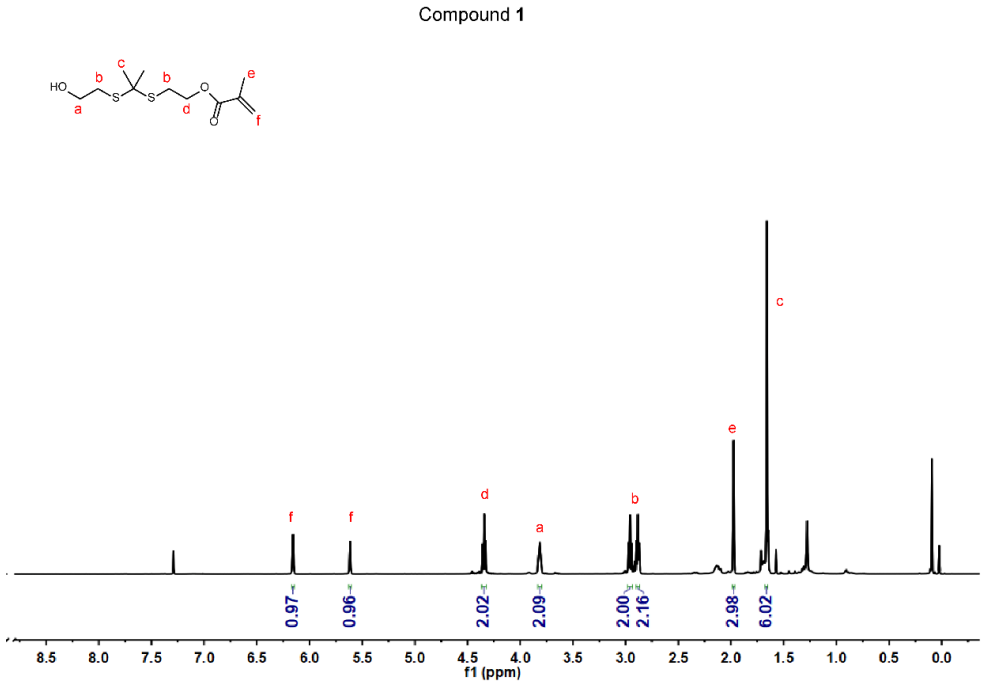
PGDH: $mPEG_{113}$-GALGLPG-*b*-P(DPA_{35}-*r*-$HEMA_7$);

PDH: $mPEG_{113}$-*b*-P(DPA_{39}-*r*-$HEMA_7$);

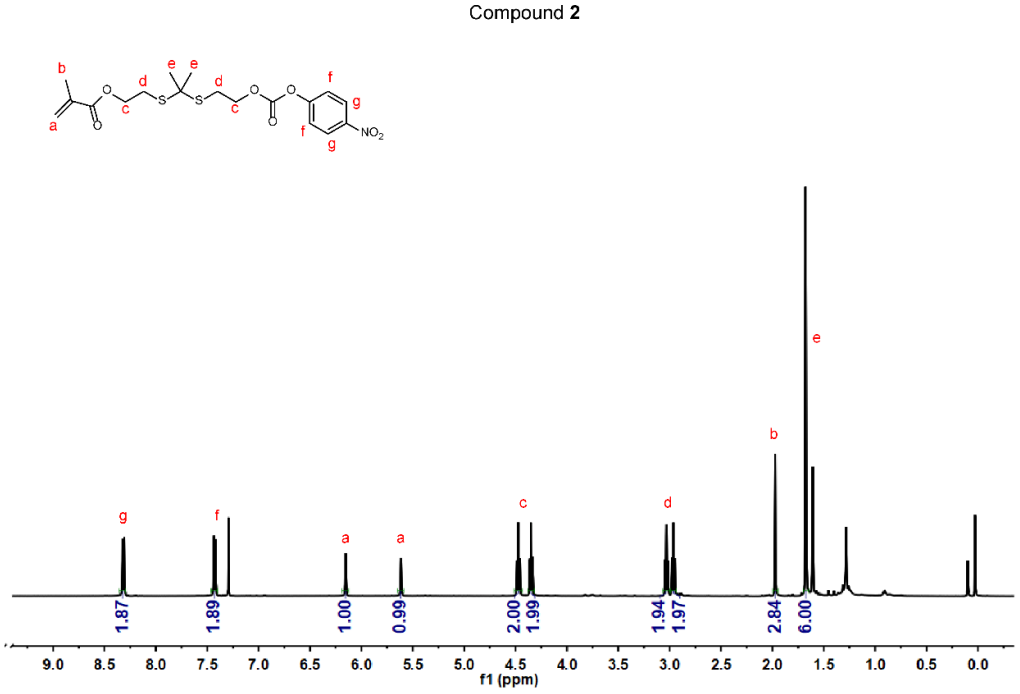
PGDA: $mPEG_{113}$-GALGLPG-*b*-P(DPA_{35}-*r*-$HEMA_7$-PPa_4);

PDA: $mPEG_{113}$-GALGLPG-*b*-P(DPA_{39}-*r*-$HEMA_7$-PPa_4);

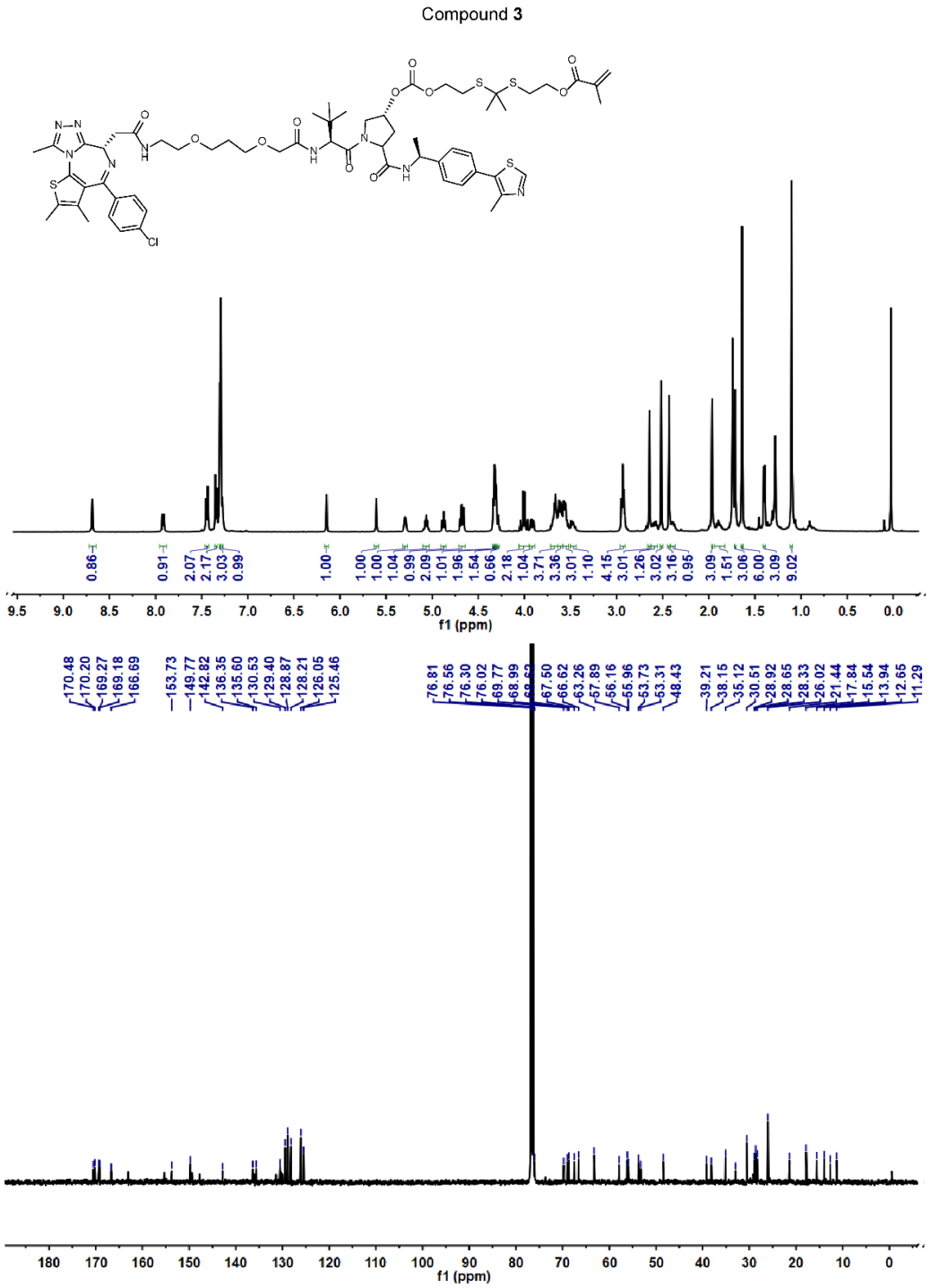
PGDE: $mPEG_{113}$-GALGLPG-*b*-P(DPA_{36}-*r*-ARV771-Et_2).



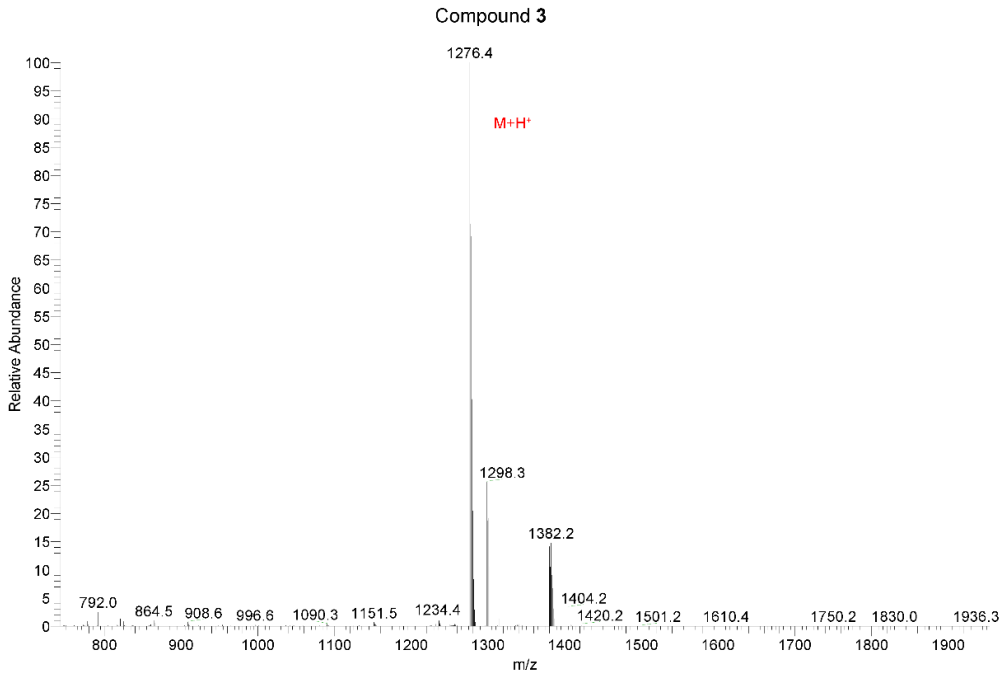
Supplementary Figure 1. ^{1}H-NMR spectrum of compound **1** ($CDCl_3$).



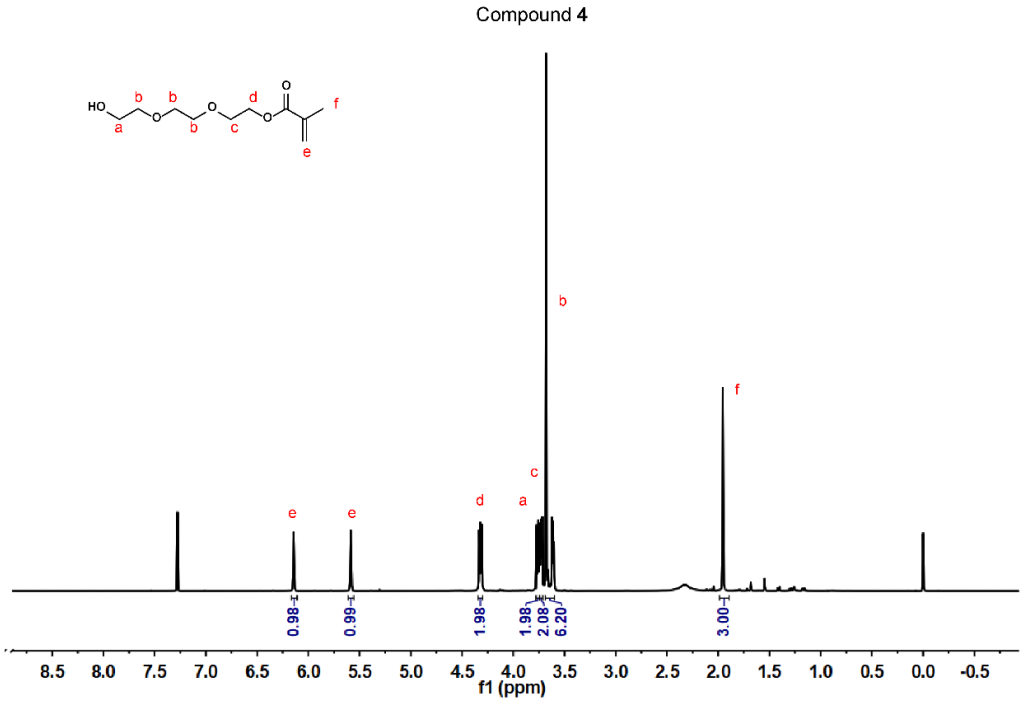
Supplementary Figure 2. ^{1}H-NMR spectrum of compound **2** ($CDCl_3$).



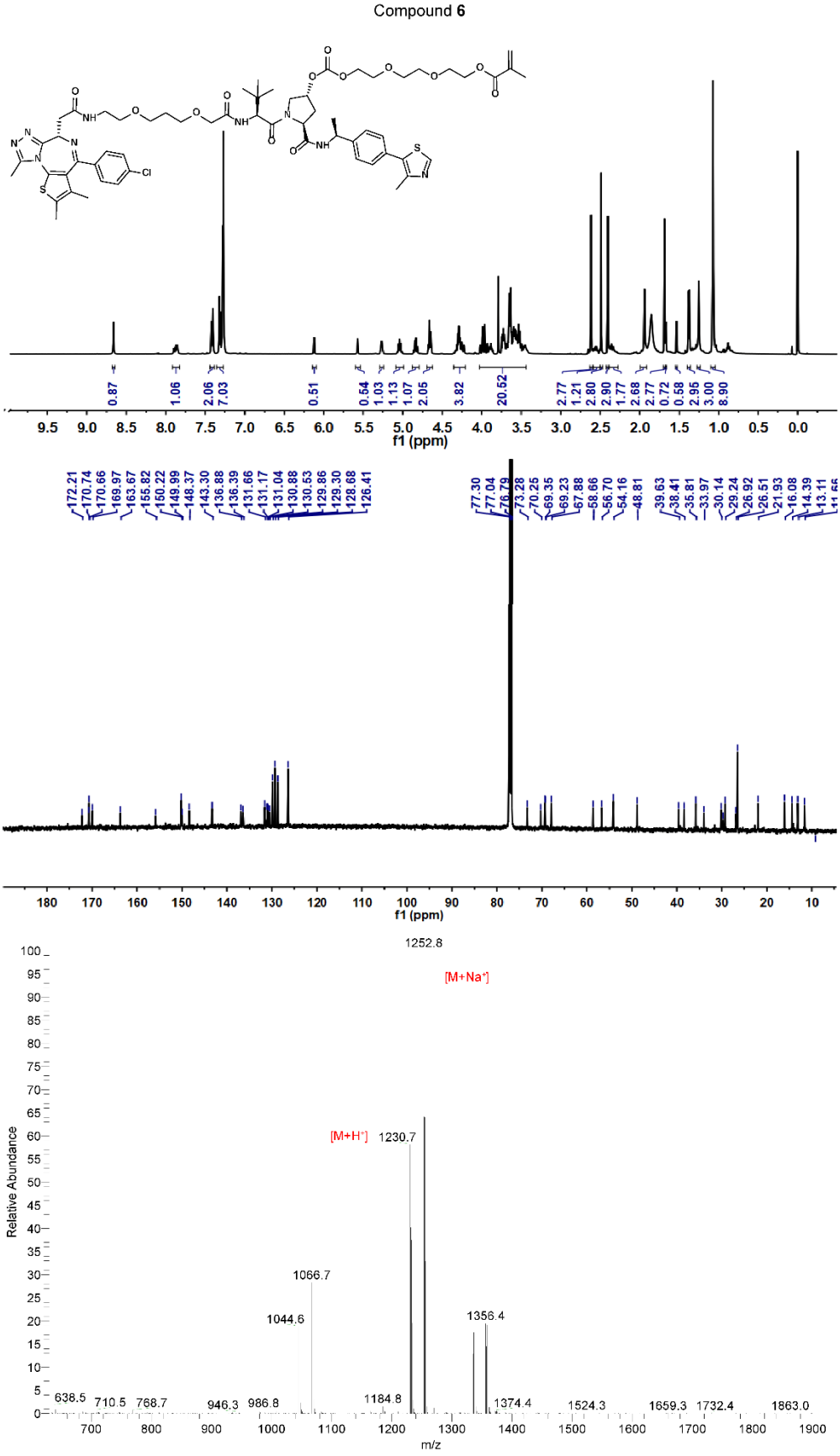
Supplementary Figure 3. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **3** ($CDCl_3$).



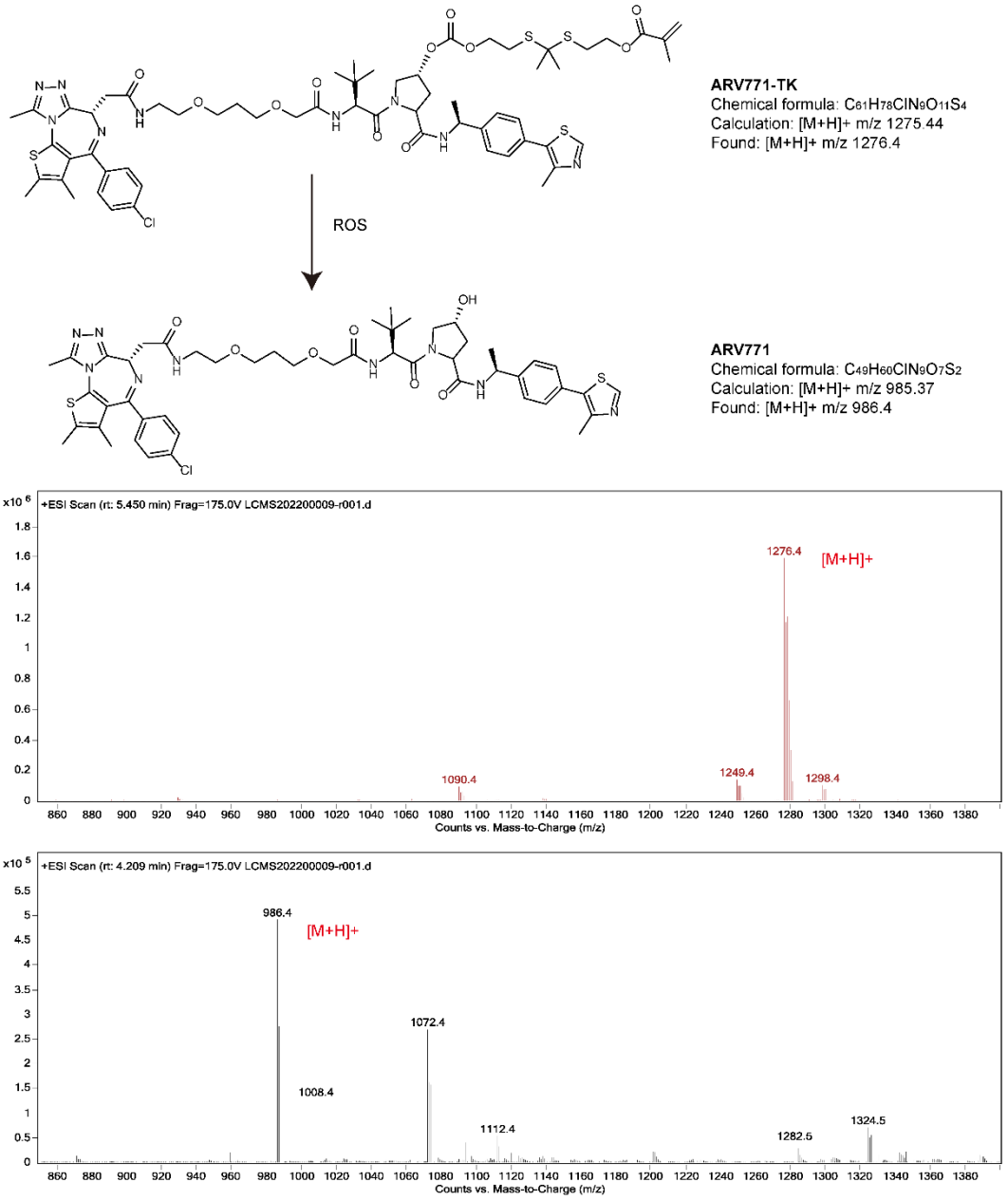
Supplementary Figure 4. Mass spectrum of compound **3**.



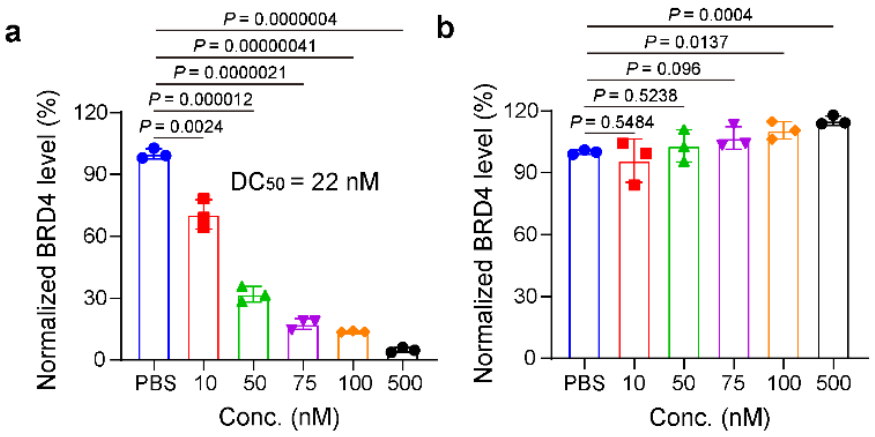
Supplementary Figure 5. ^{1}H-NMR spectrum of compound **4** ($CDCl_3$).



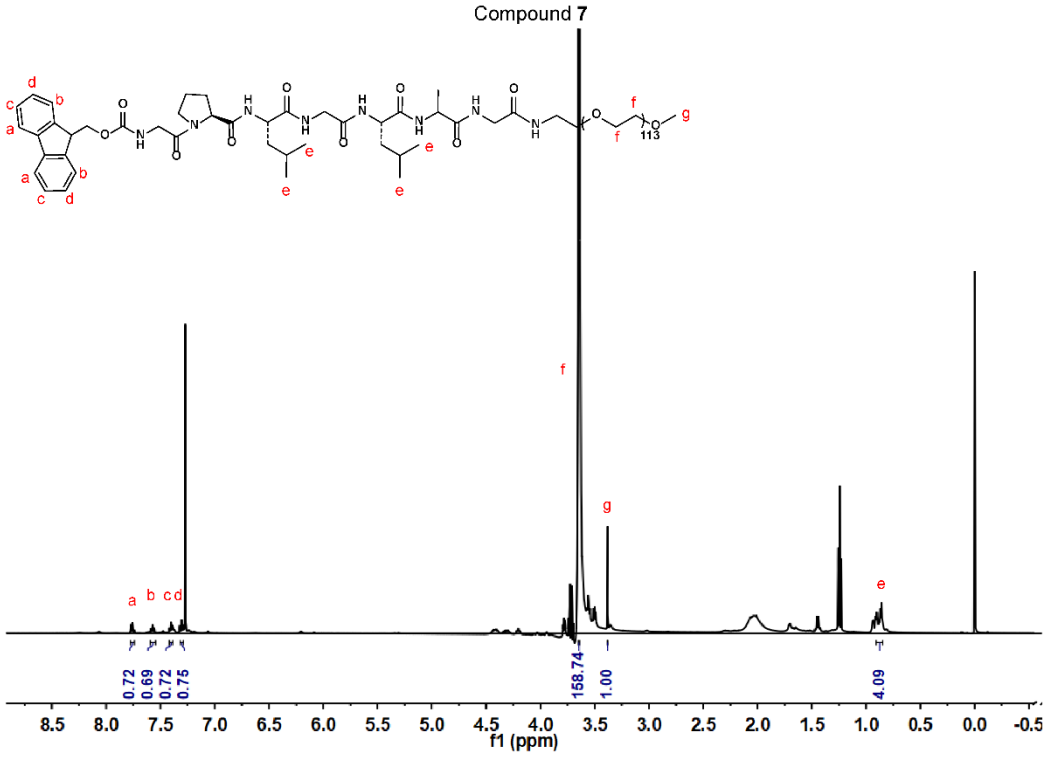
Supplementary Figure 6. ^{1}H-NMR, ^{13}C-NMR spectrum ($CDCl_3$) and mass spectrum of compound **6**.



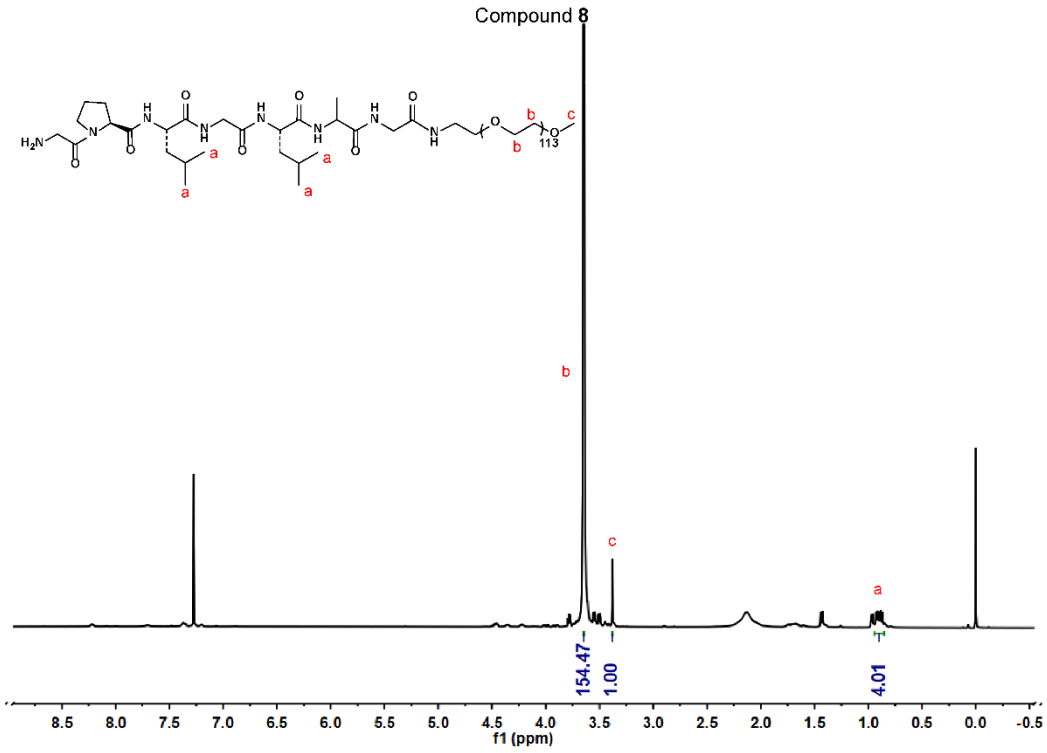
Supplementary Figure 7. Liquid chromatography-mass spectrometry of the ARV771-TK suspension after being treated with PPa and 671 nm laser irradiation.



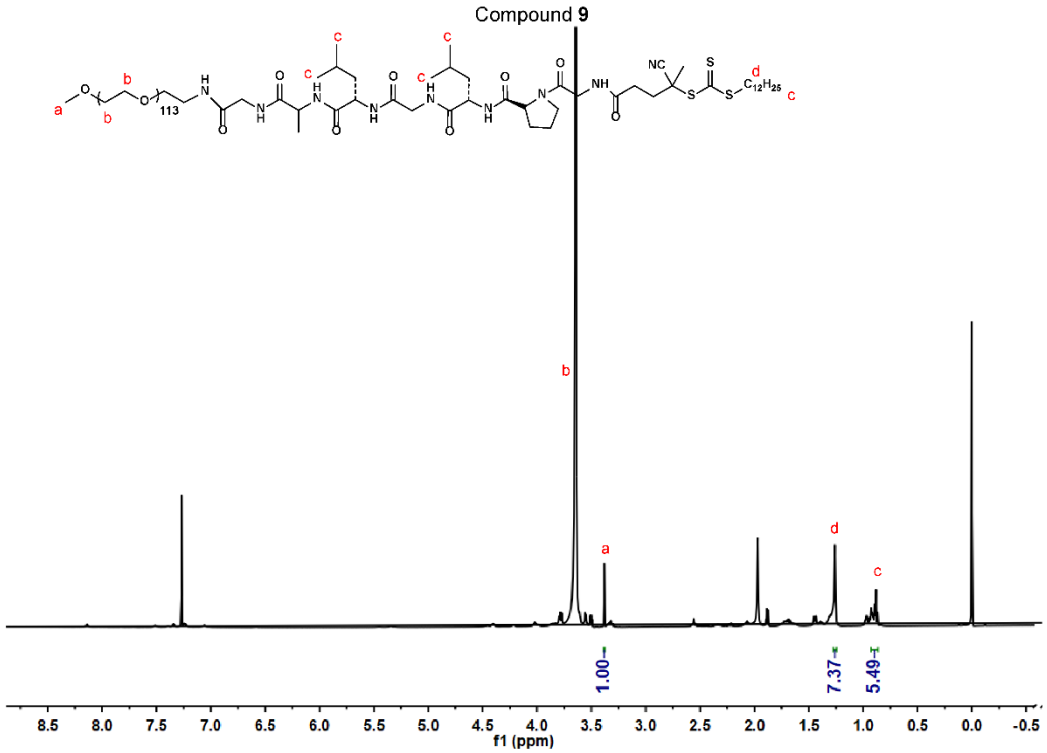
Supplementary Figure 8. Normalized BRD4 expression in MDA-MB-231 cells with the treatment of (a) ARV771, (b) ARV771-TK. (n = 3 independent experimental cell lines). Statistical analysis was performed by two-sided unpaired t-test. All data are presented as mean ± SD.



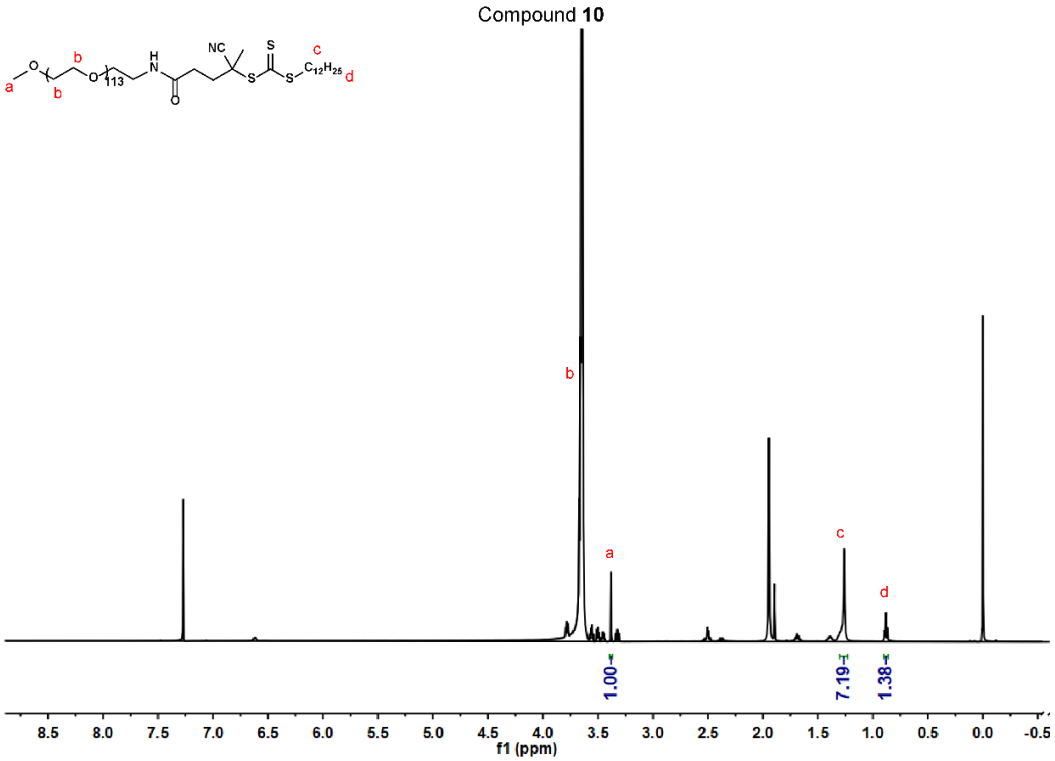
Supplementary Figure 9. ^{1}H-NMR spectrum of compound **7** ($CDCl_3$).



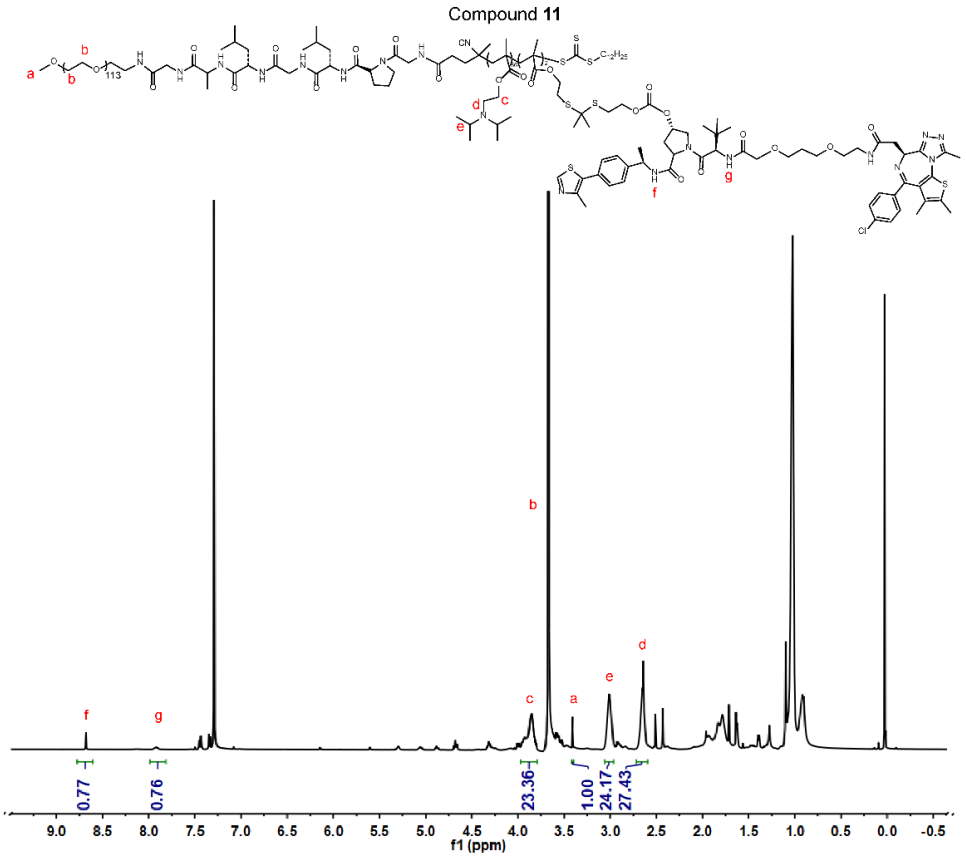
Supplementary Figure 10. ^{1}H-NMR spectrum of compound **8** ($CDCl_3$).



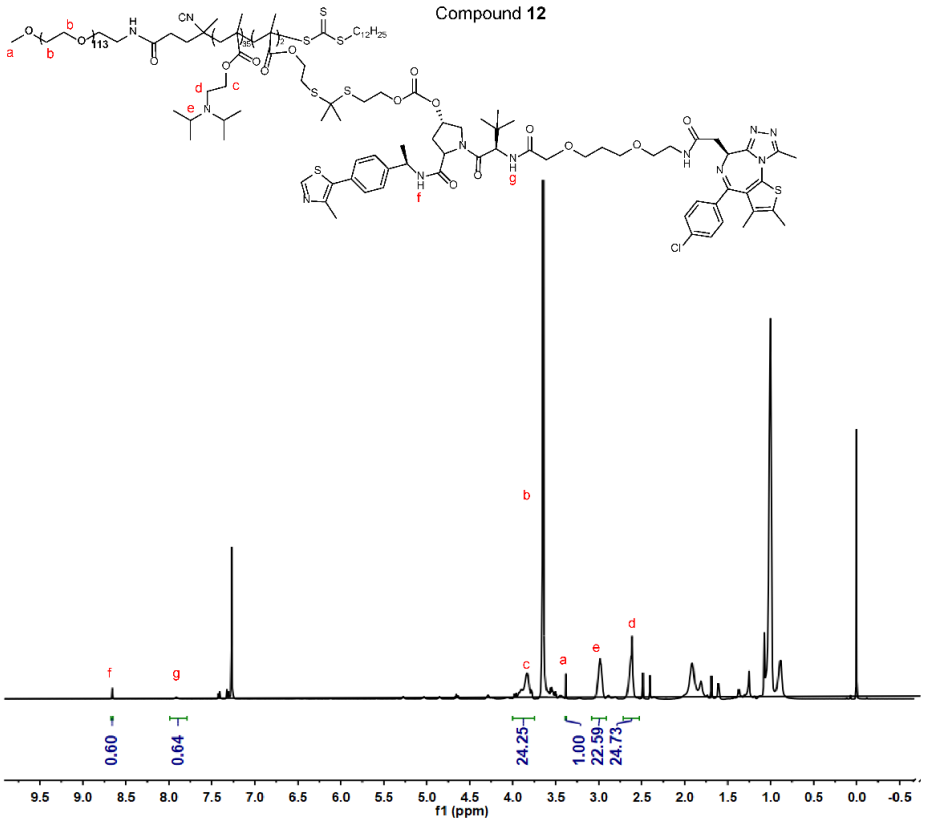
Supplementary Figure 11. ^{1}H-NMR spectrum of compound **9** ($CDCl_3$).



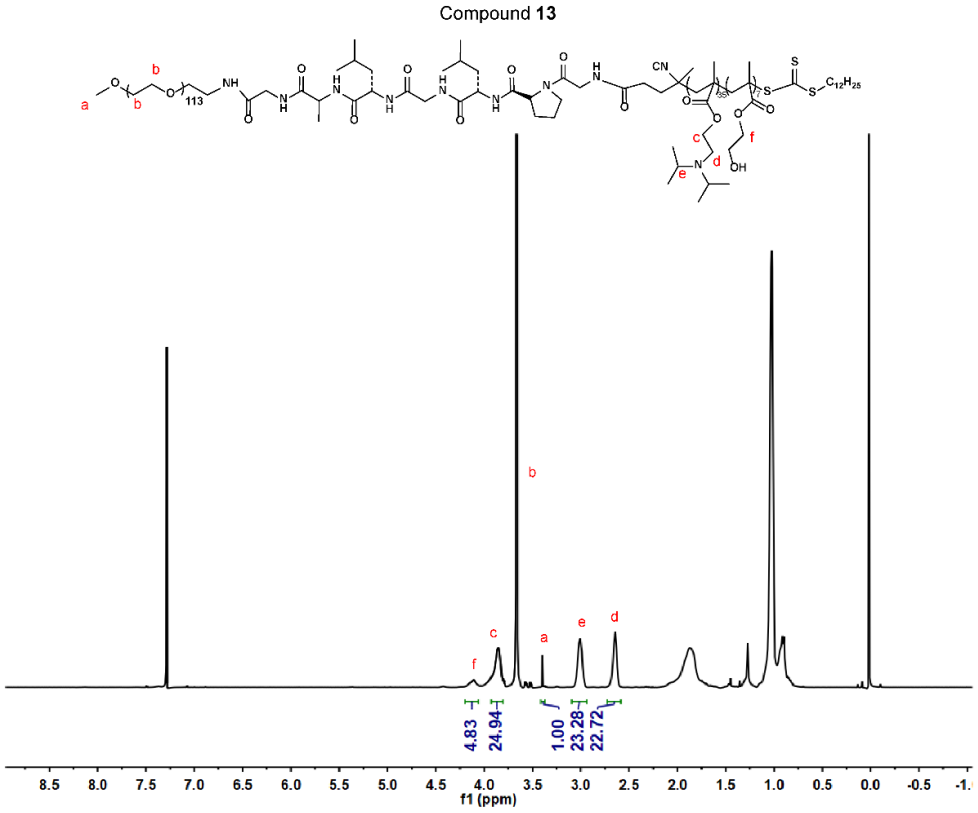
Supplementary Figure 12. ^{1}H-NMR spectrum of compound **10** ($CDCl_3$).



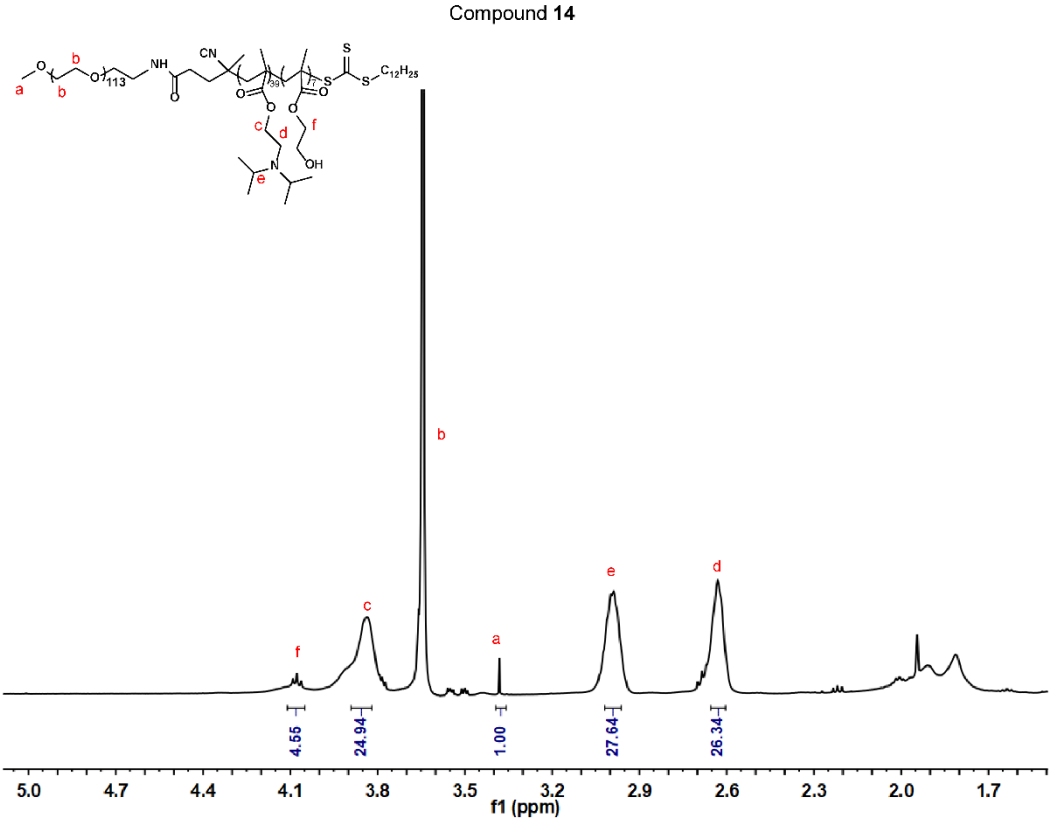
Supplementary Figure 13. ^{1}H-NMR spectrum of compound **11** ($CDCl_3$).



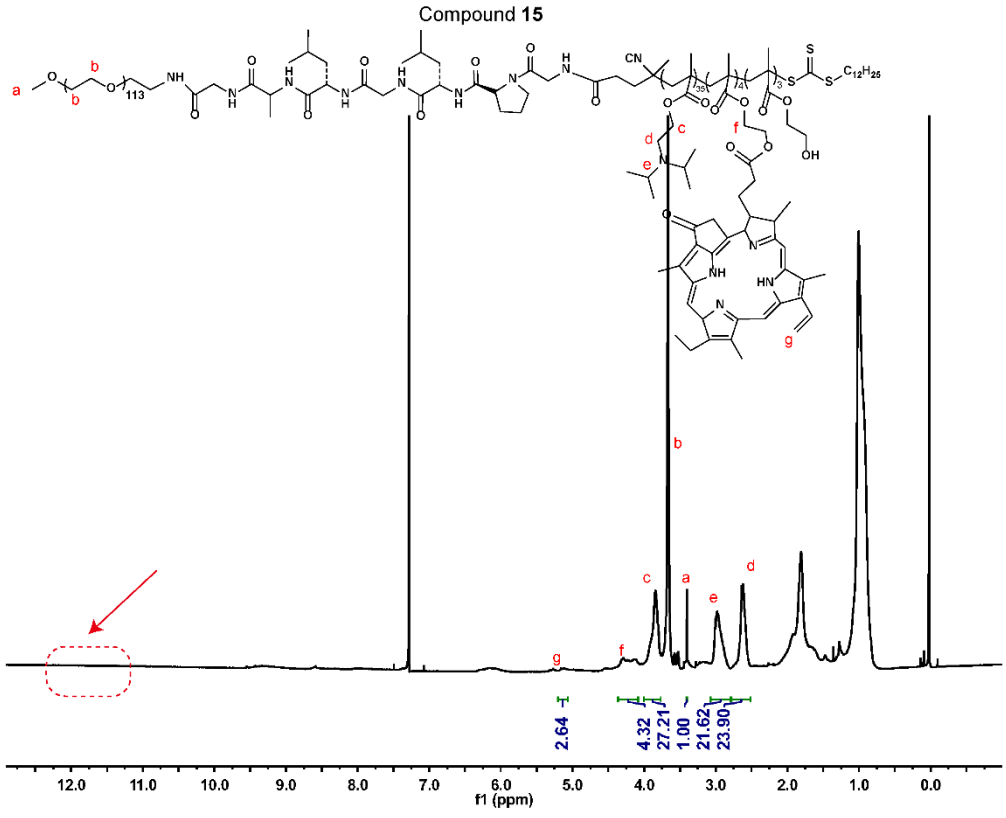
Supplementary Figure 14. ^{1}H-NMR spectrum of compound **12** ($CDCl_3$).



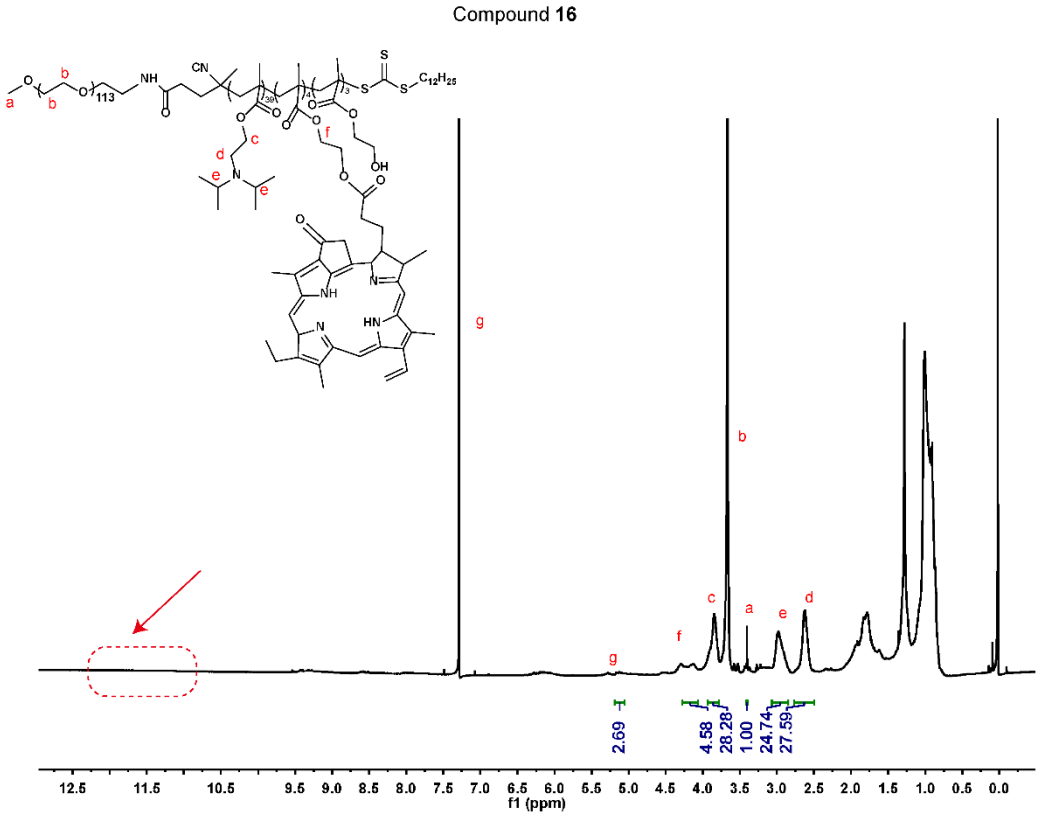
Supplementary Figure 15. ^{1}H-NMR spectrum of compound **13** ($CDCl_3$).



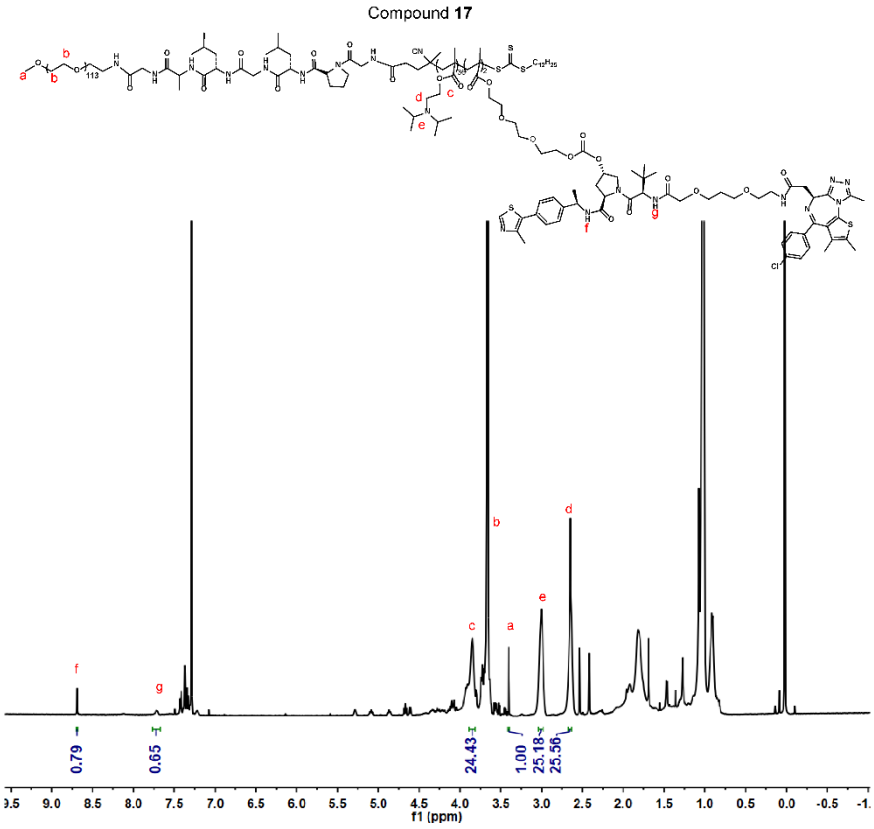
Supplementary Figure 16. ^{1}H-NMR spectrum of compound **14** ($CDCl_3$).



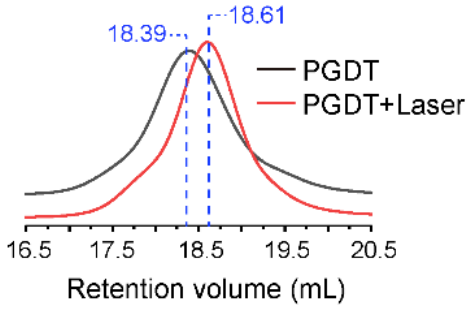
Supplementary Figure 17. ^{1}H-NMR spectrum of compound **15** ($CDCl_3$).



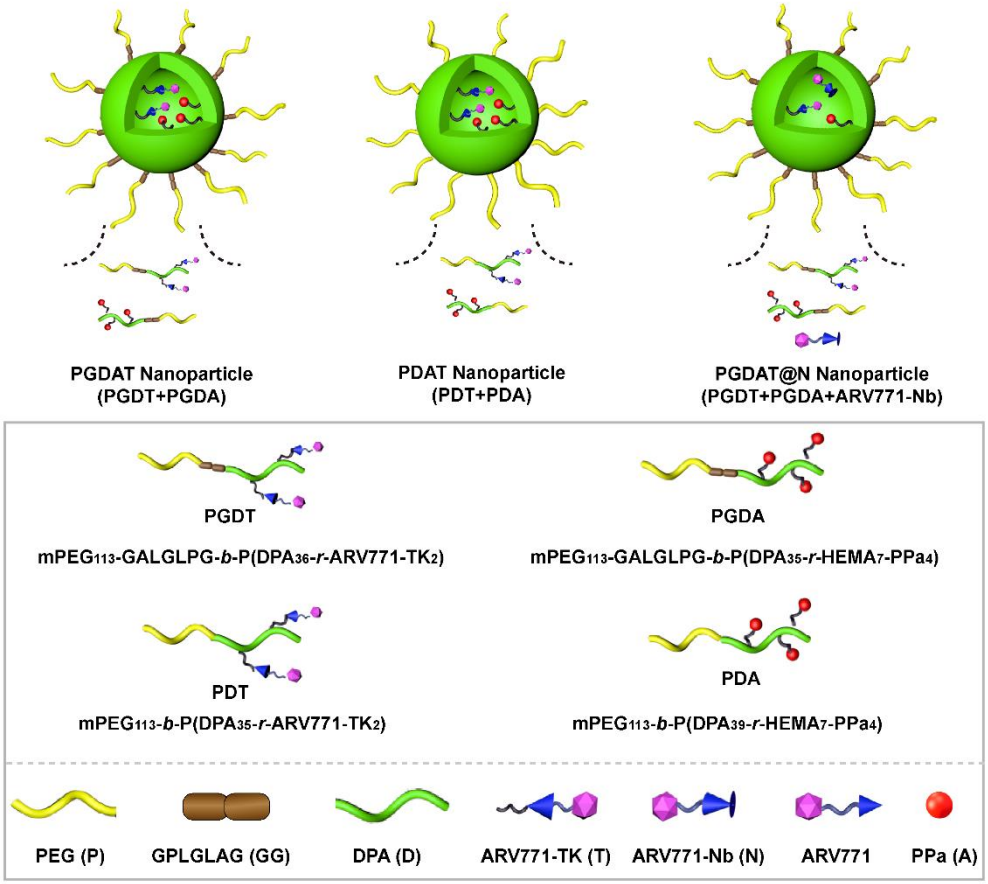
Supplementary Figure 18. ^{1}H-NMR spectrum of compound **16** ($CDCl_3$).



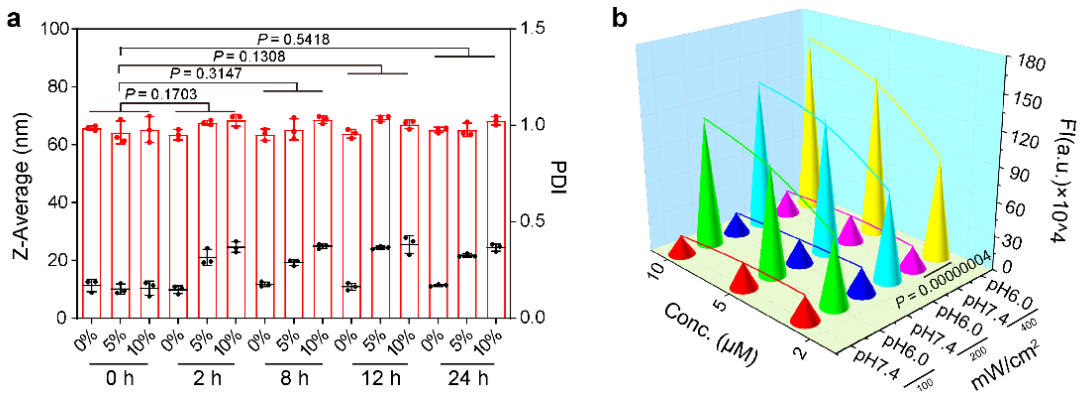
Supplementary Figure 19. ^{1}H-NMR spectrum of compound **17** ($CDCl_3$).



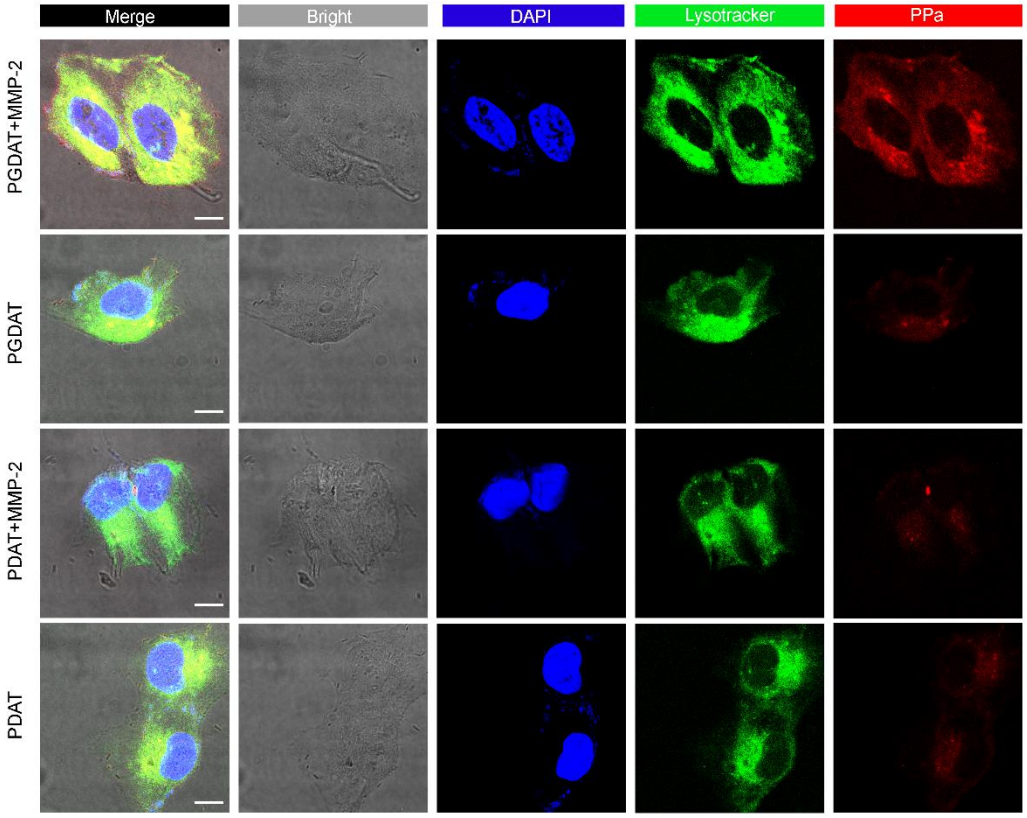
Supplementary Figure 20. The representative GPC plots of PDT-induced molecular weight change of PGDT polymer.



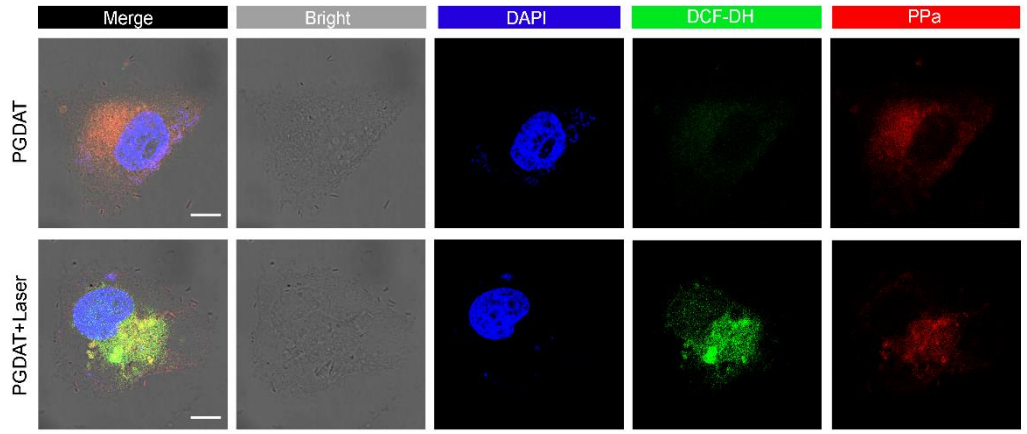
Supplementary Figure 21. Cartoon illustration of nanoparticle construction and acronyms used throughout the study.



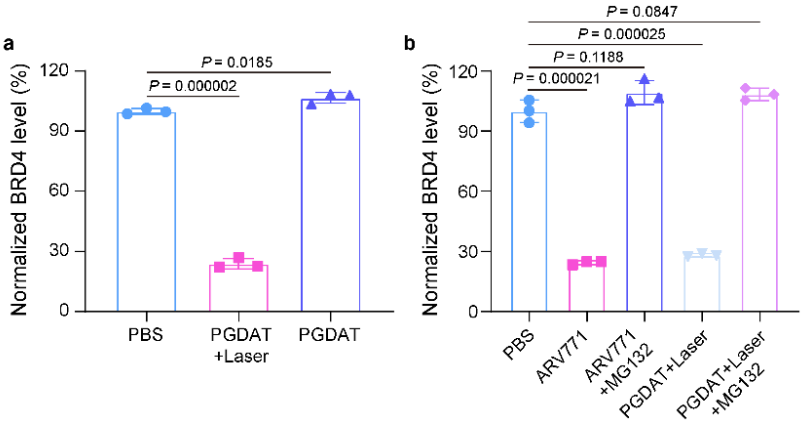
Supplementary Figure 22. a, Averaged hydrodynamic diameter and PDI of PGDAT nanoparticle as a function of FBS concentrations and incubation time (n = 3 independent experimental units). The p values represent the averaged hydrodynamic diameter. Statistical analysis was performed by two-way ANOVA (or mixed model). **b,** ROS generation property of PGDAT nanoparticle with different dosages and photodensity at the neutral pH 7.4 and acidic pH 6.0 condition. SOSG probe was added into the PGDAT nanoparticle suspensions before laser irradiation and then the fluorescence intensity was detected immediately post the laser treatment. Statistical analysis was performed by two-sided unpaired t-test.



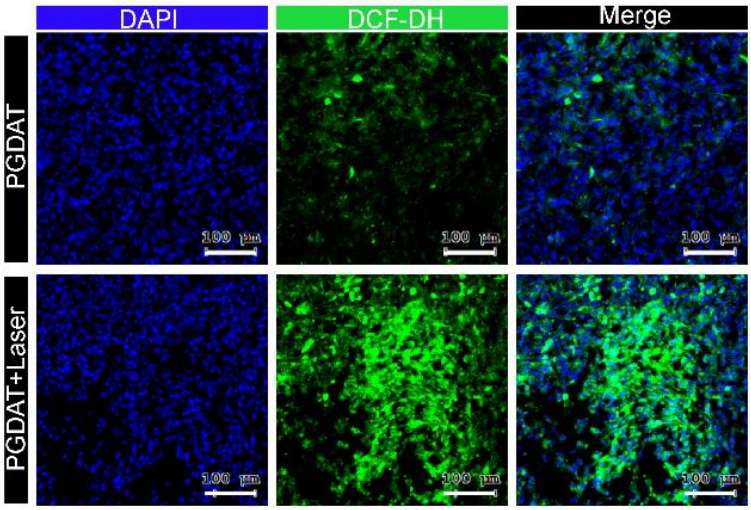
Supplementary Figure 23. Representative CLSM images of the intracellular distribution of the PROTAC nanoparticle post 12 h incubation (scale bar = 10 μm, the blue represents DAPI, the green represents Lysotracker and the red represents PPa).



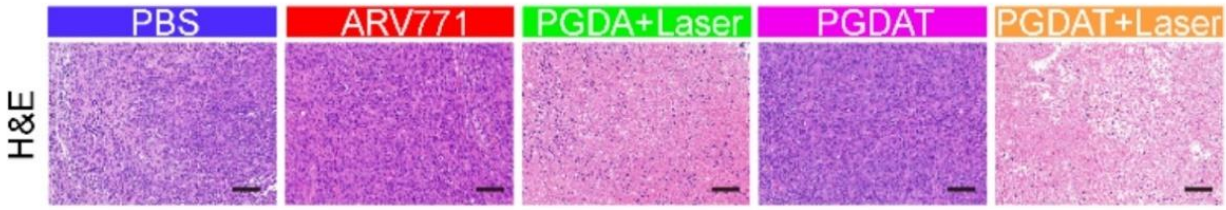
Supplementary Figure 24. CLSM measurement of PDT-mediated ROS generation, the MDA-MB-231 cells were treated with PGDAT nanoparticle for 12 h, and next irradiated with 671 nm laser (scale bar = 10 μm, the blue represents DAPI, the green represents DCF-DH and the red represents PPa).



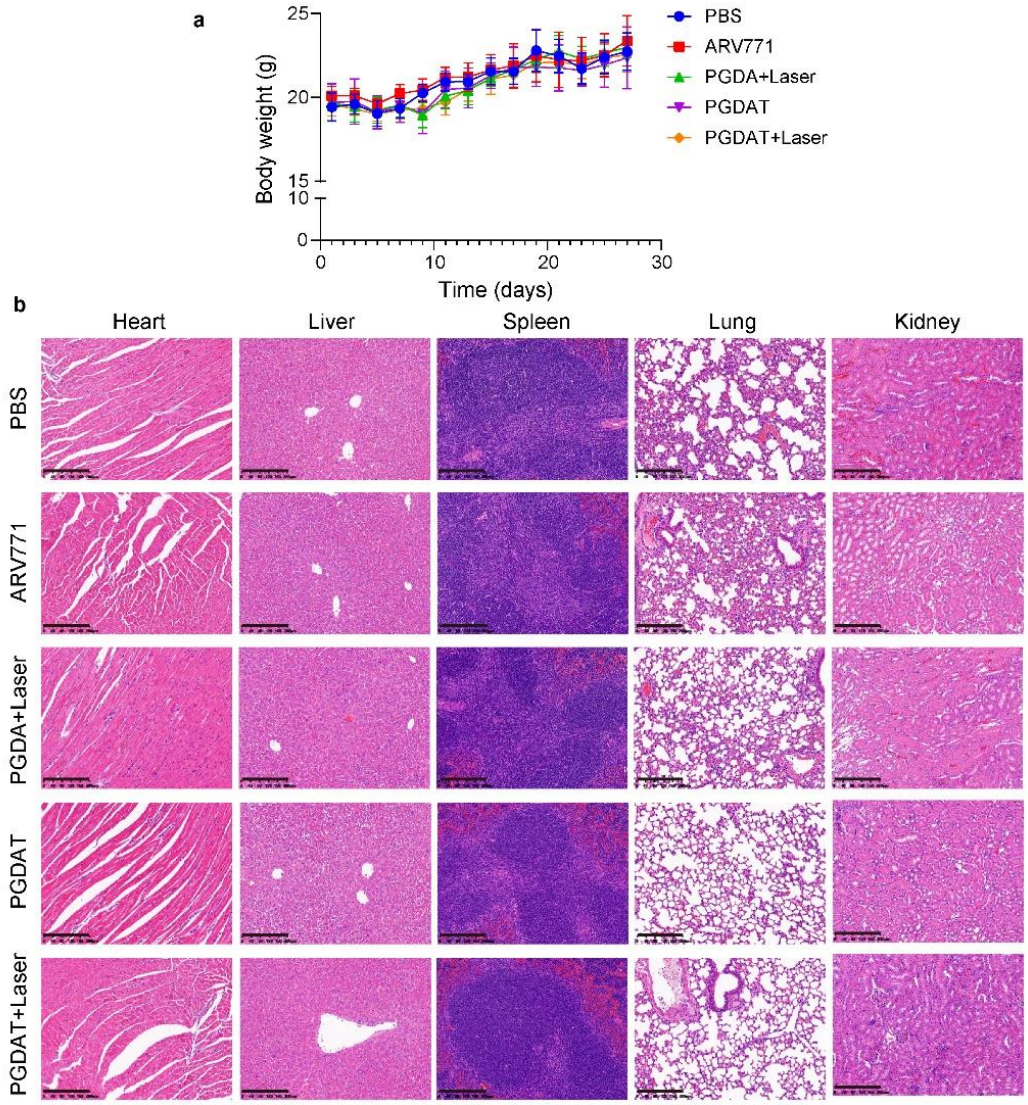
Supplementary Figure 25. a & b, Normalized BRD4 expression in MDA-MB-231 cells subjected to various treatments for 24 h (the identified ARV771 concentration of 1.0 μM, and MG132 concentration of 5 mM, the photodensity was 400 mW/cm^2 and laser time was 5 min (n = 3 independent experimental cell lines). Statistical analysis was performed by two-sided unpaired t-test. All data are presented as mean ± SD.



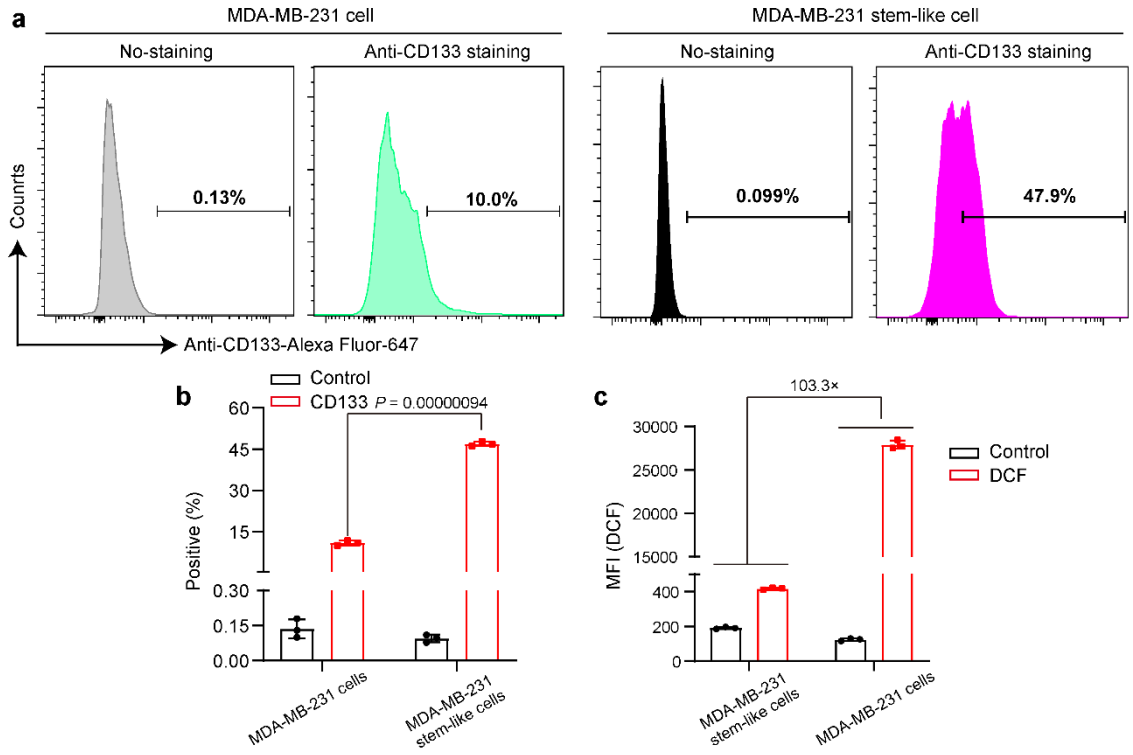
Supplementary Figure 26. CLSM examination of PDT-induced intratumoral ROS production (scale bar = 100 μm, the blue represents DAPI, the green represents DCF-DH). The ROS probe DCFH-DA was intratumorally injected after tumor-bearing mice were intravenously injected with PGDAT nanoparticle for 36 h, and then the tumor tissues were harvested immediately for further analysis after 671 nm laser irradiation for 5 min with the photodensity of 400 mW/cm^2.



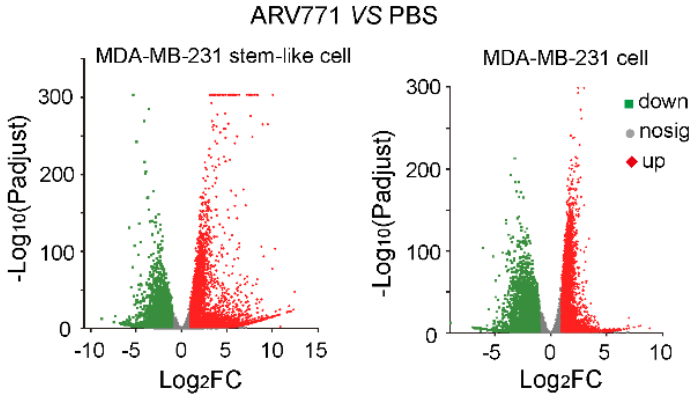
Supplementary Figure 27. H&E staining of the tumor sections at end of treatments (scale bar = 100 μm).



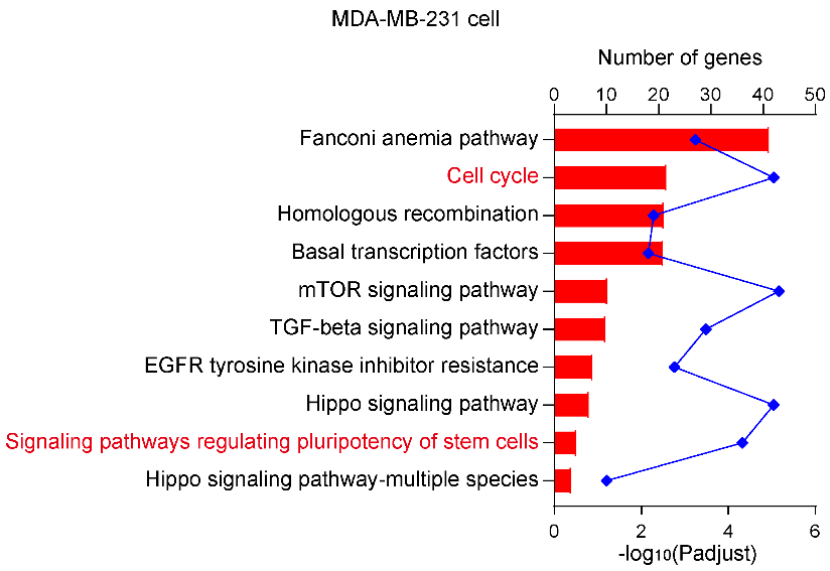
Supplementary Figure 28. a, Averaged body weight change of the tumor-bearing mice during the experimental period (n = 6 mice). **b**, H&E staining of the major organs (heart, liver, spleen, lung, kidney) of tumor-bearing mice at the end of antitumor experiment (scale bar = 200 μm).



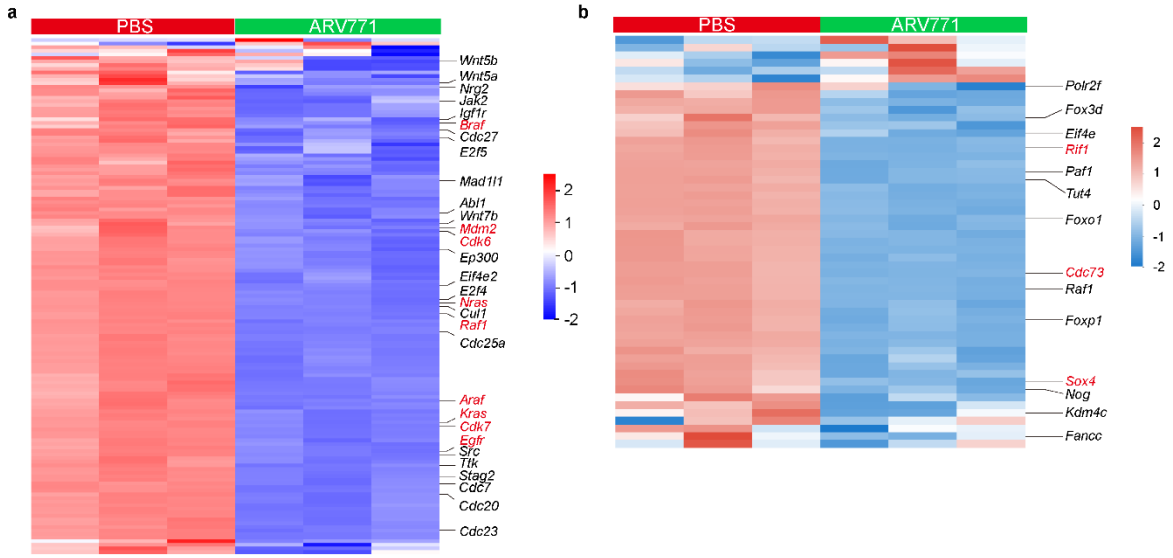
Supplementary Figure 29. Flow cytometric plots **(a)** and the averaged positive ratio **(b)** of the $CD133^+$ cell population ratio in MDA-MB-231 cells and enriched tumor spheroids (n = 3 independent experimental cell lines). Statistical analysis was performed by two-sided unpaired t-test. **c**, Flow cytometric measurement of averaged fluorescence signals of intracellular DCF in MDA-MB-231 cells and enriched tumor spheroids (n = 3 independent experimental cell lines). All data are presented as mean ± SD.



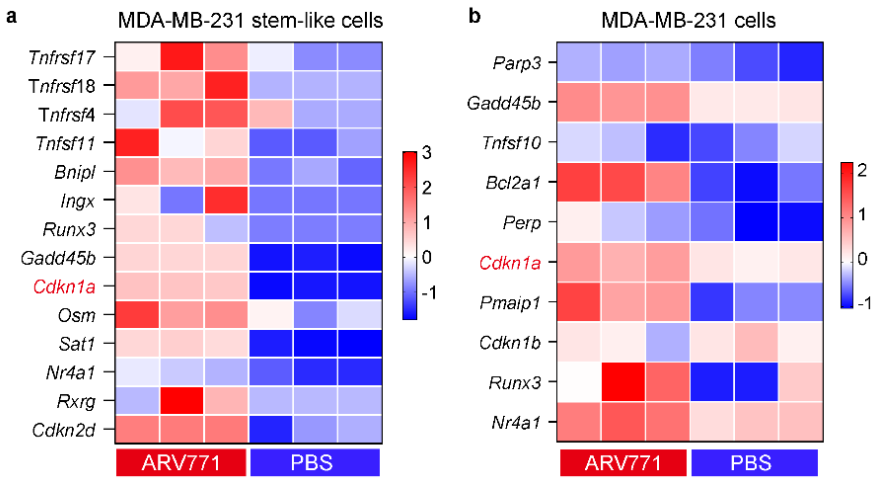
Supplementary Figure 30. Volcano plots of the differential expressed genes in the RNA-seq analysis, MDA-MB-231stem-like cells or MDA-MB-231 cells were treated with 1 μM ARV771 for 24 h (n = 3 independent experimental cell lines).



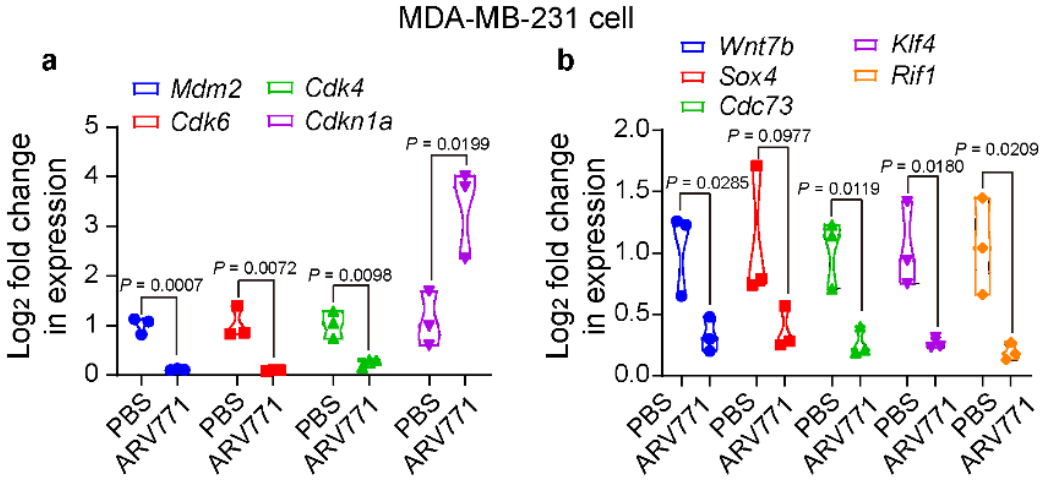
Supplementary Figure 31. KEGG enrichment analysis of the pathways involved in the biological effect of the ARV771 on the MDA-MB-231 cells (statistical difference was calculated using two-sided Fisher's exact test, n = 3 independent experimental cell lines).



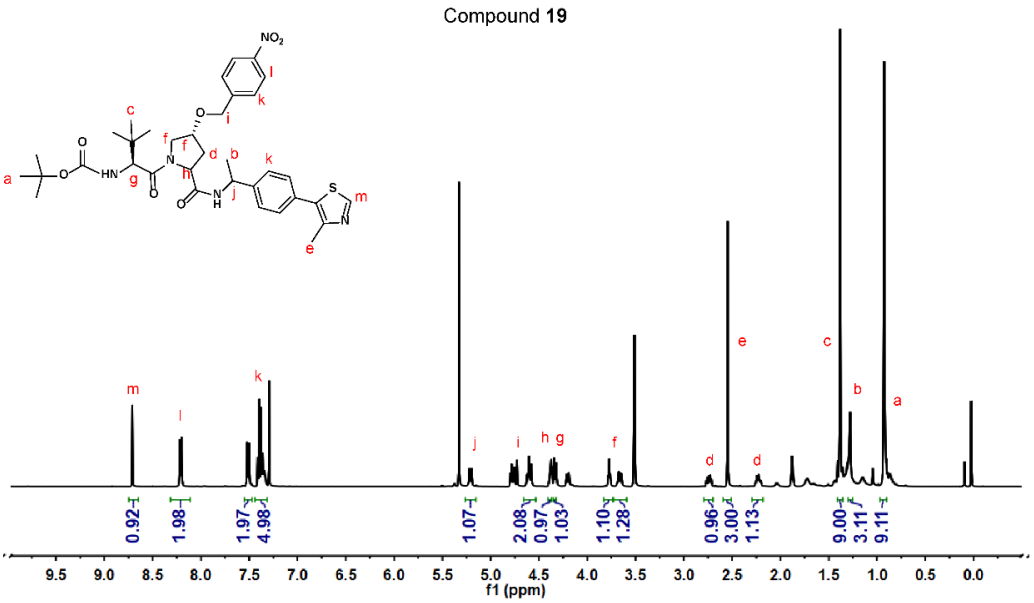
Supplementary Figure 32. Heat map of differential expressed genes associated with cell cycle (**a**) and cell stemness (**b**) in the RNA-seq analysis on the MDA-MB-231 cells post treated with ARV771 (n = 3 independent experimental cell lines).



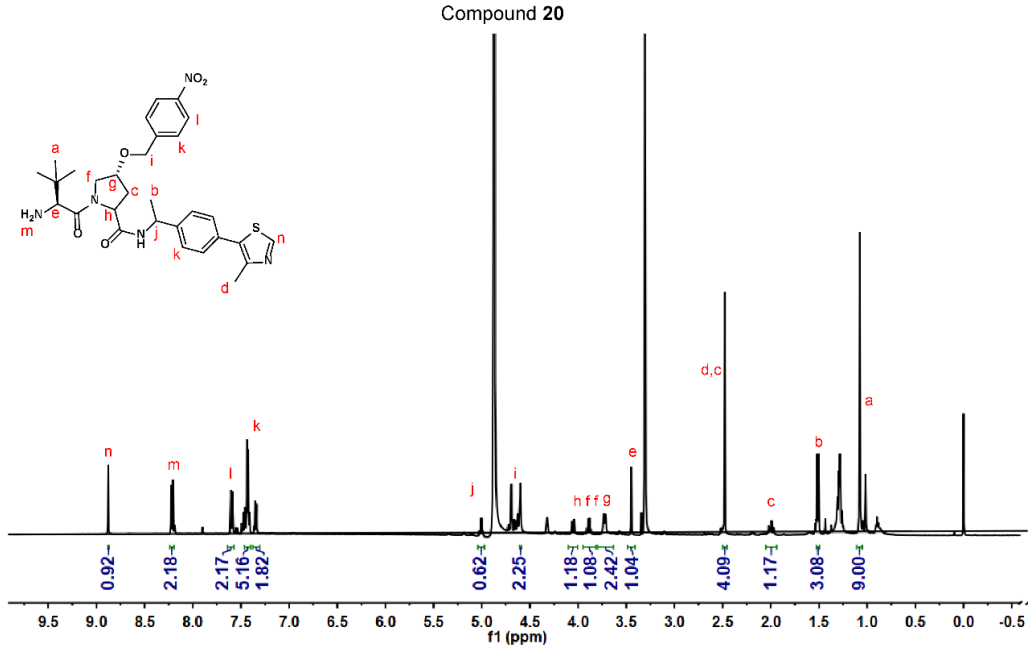
Supplementary Figure 33. Heat map of differential expressed genes related with cell apoptosis in the RNA-seq analysis on the MDA-MB-231 stem-like cells (**a**) and MDA-MB-231 cells (**b**) post treated with ARV771 (n = 3 independent experimental cell lines).



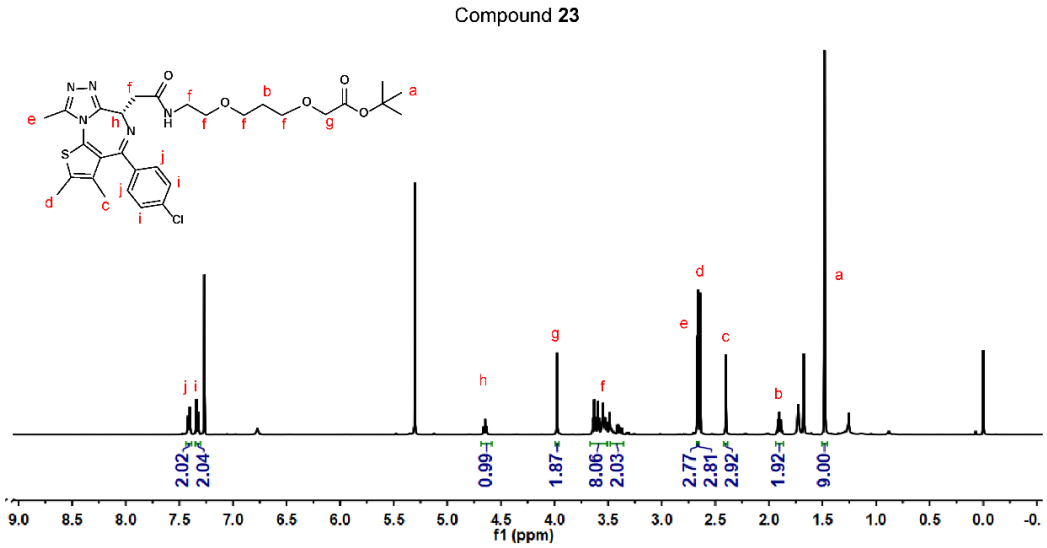
Supplementary Figure 34. Quantitative PCR assay of the **(a)** cell cycle- and **(b)** cell stemness-related mRNA levels in MDA-MB-231 cells after being treated with 1.0 μM of ARV771 (n = 3 independent experimental cell lines). Statistical analysis was performed by two-sided unpaired t-test. All data are presented as mean ± SD.



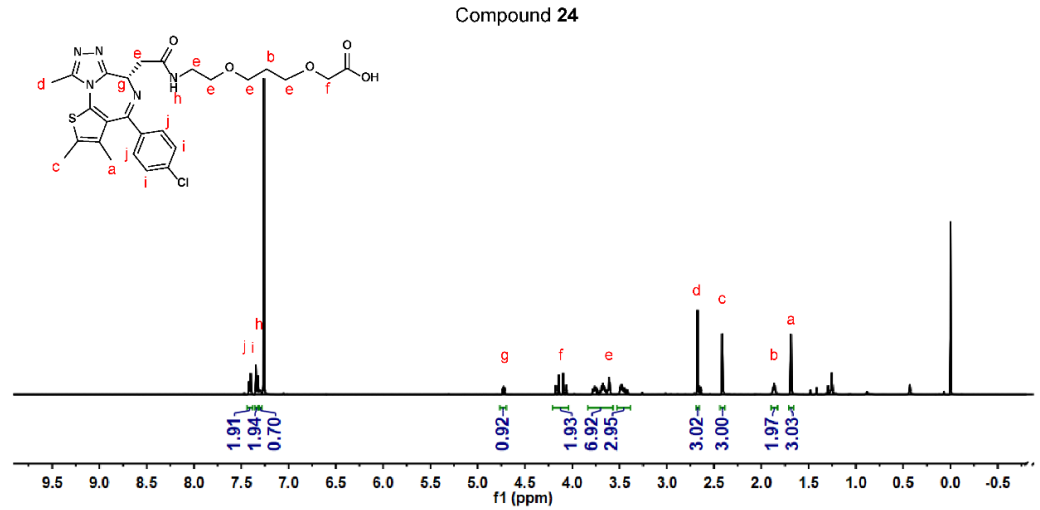
Supplementary Figure 35. ^{1}H-NMR spectrum of compound **19** ($CDCl_3$).



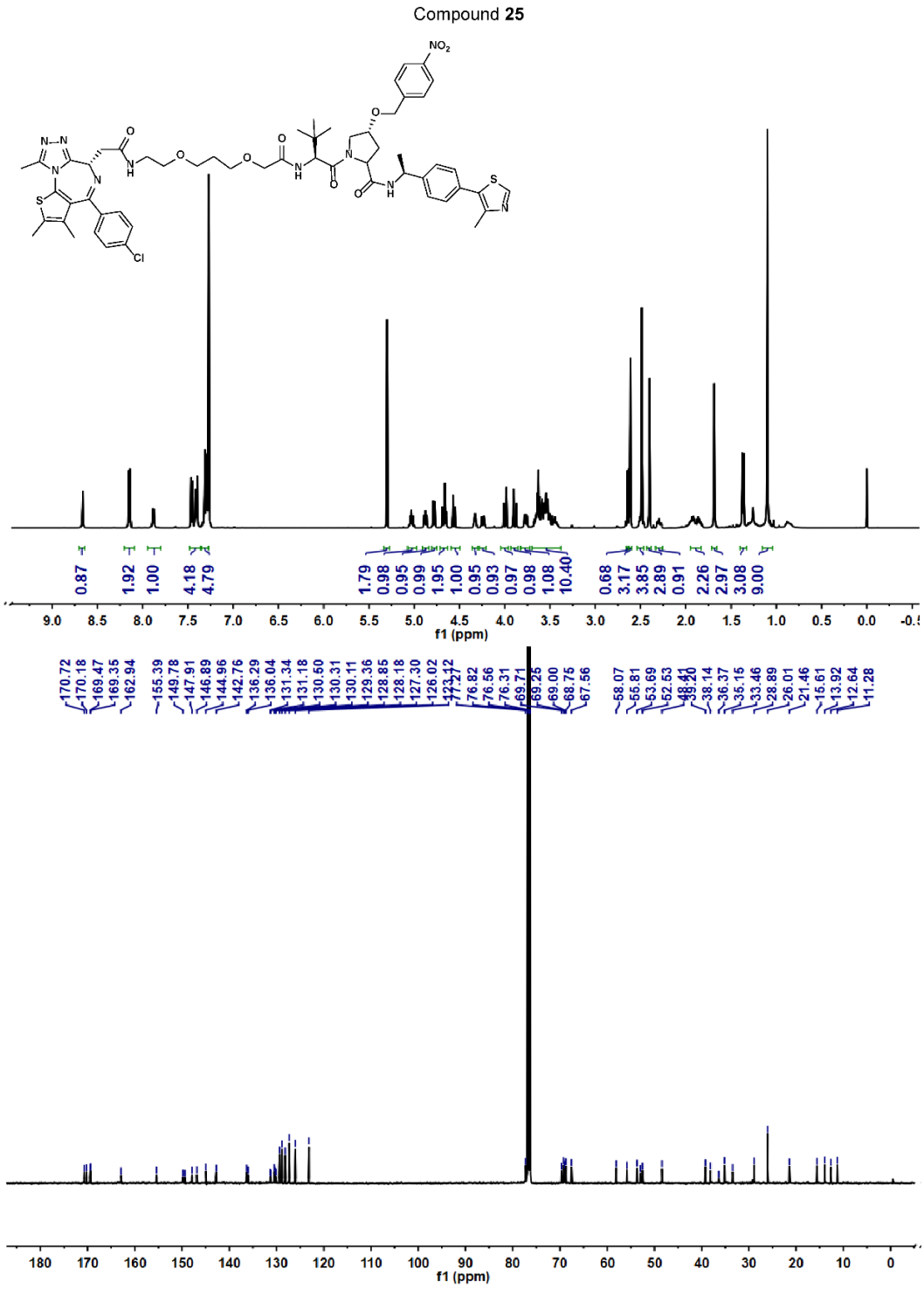
Supplementary Figure 36. ^{1}H-NMR spectrum of compound **20** ($CDCl_3$).



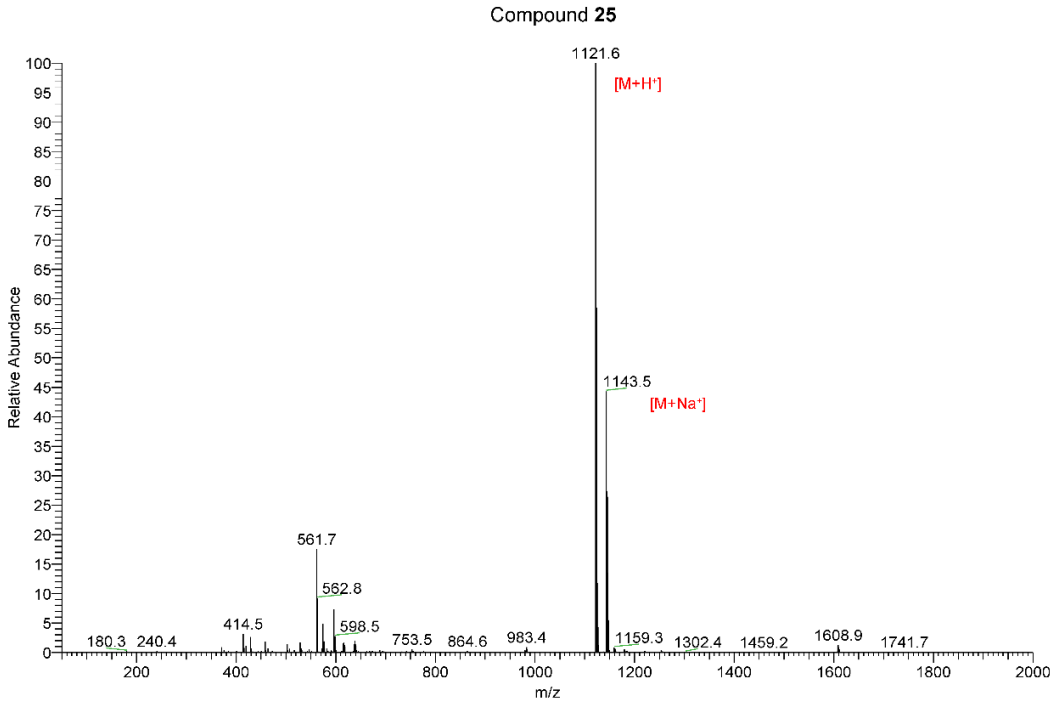
Supplementary Figure 37. ^{1}H-NMR spectrum of compound **23** ($CDCl_3$).



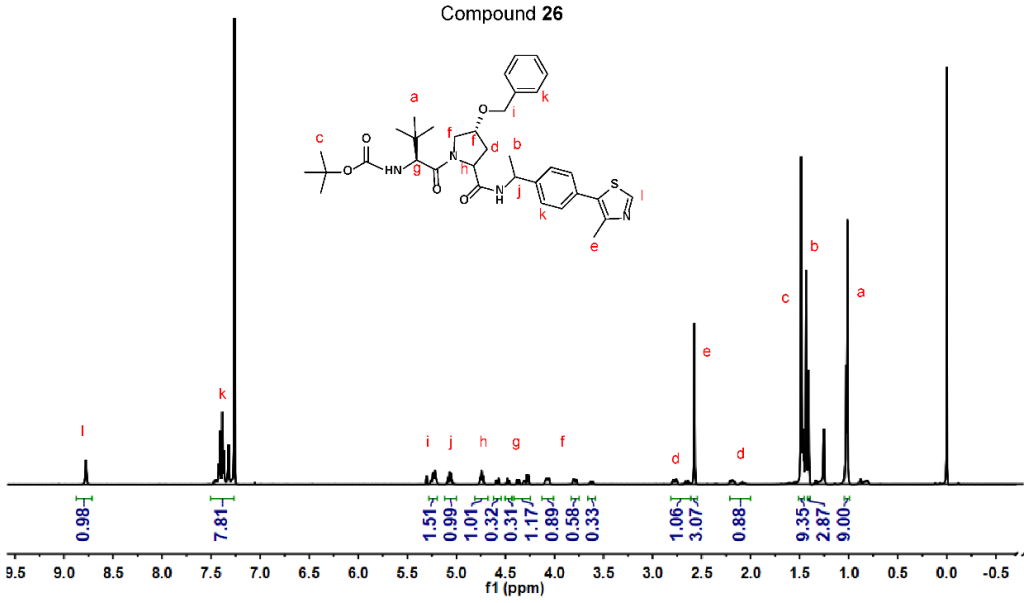
Supplementary Figure 38. ^{1}H-NMR spectrum of compound **24** ($CDCl_3$).



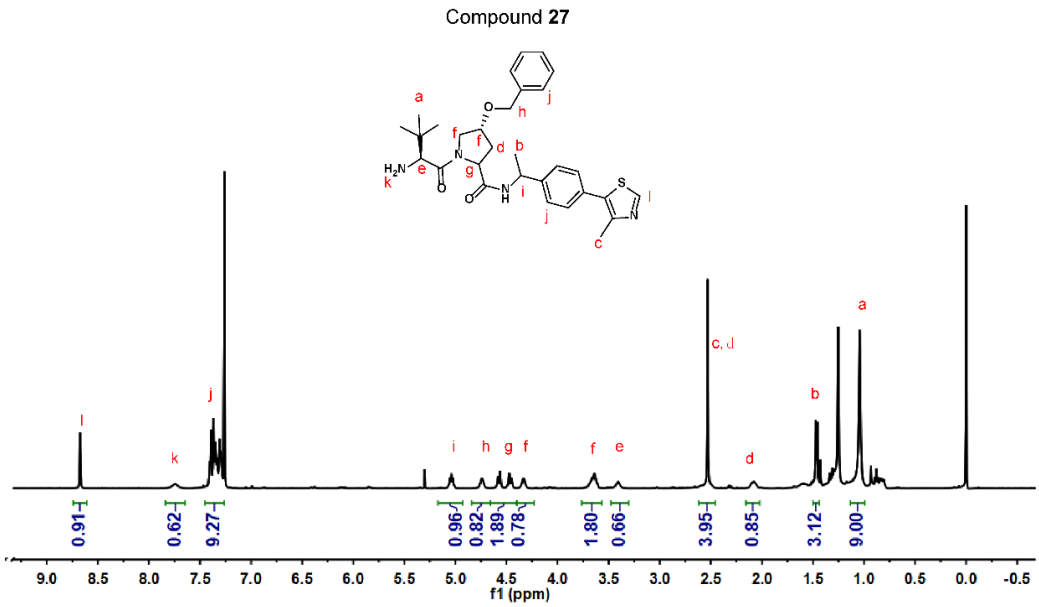
Supplementary Figure 39. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **25** ($CDCl_3$).



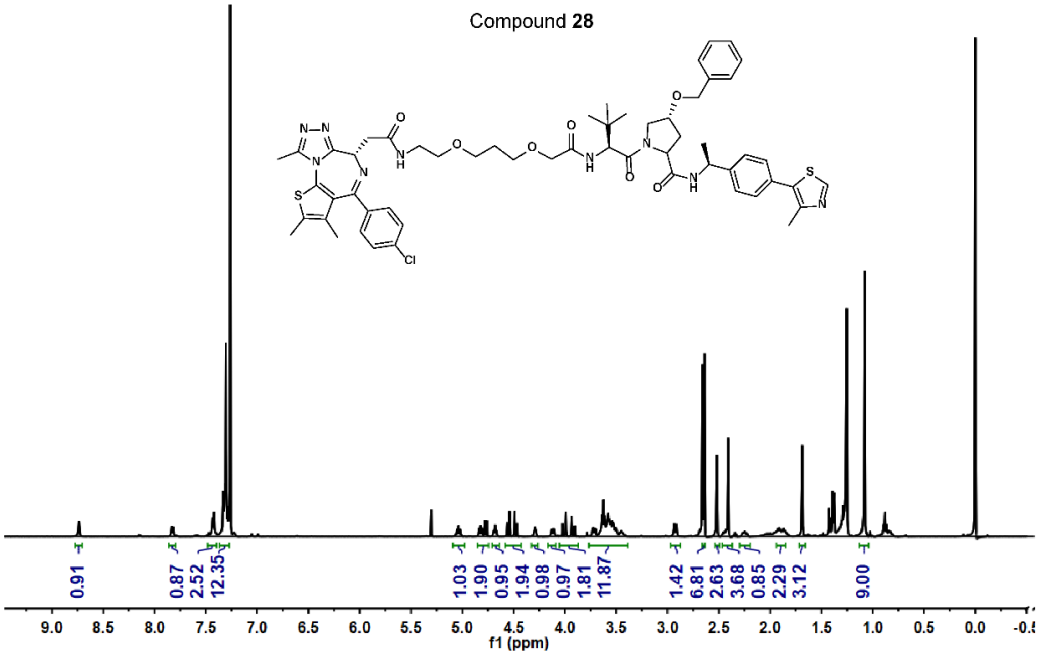
Supplementary Figure 40. Mass spectrum of compound **25**.



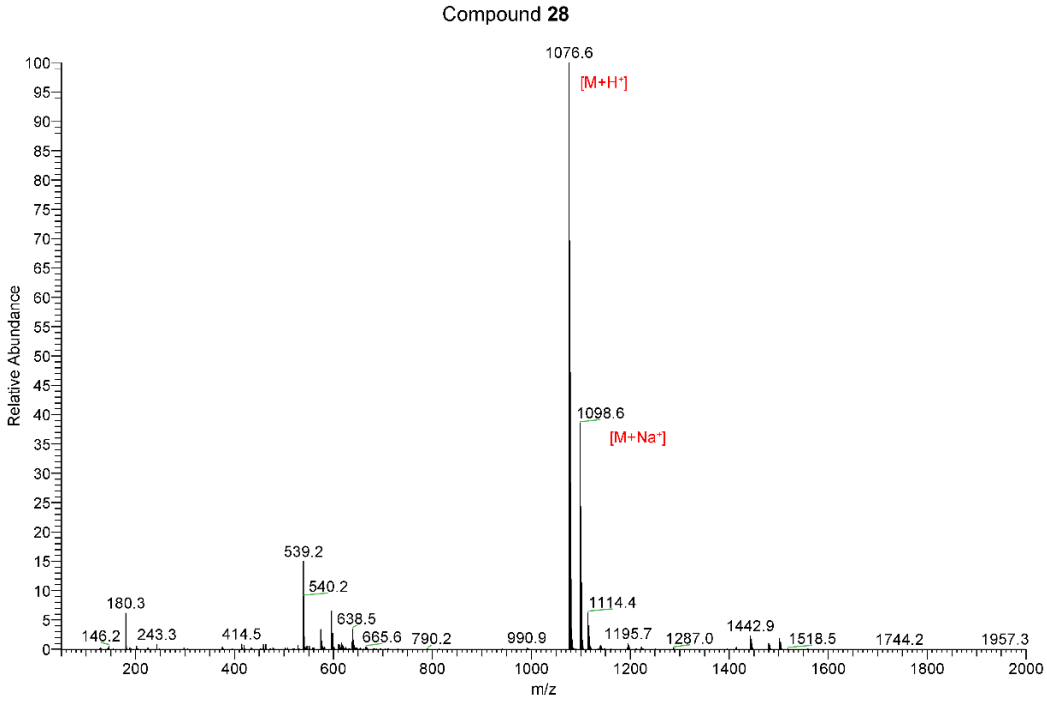
Supplementary Figure 41. ^{1}H-NMR spectrum of compound **26** ($CDCl_3$).



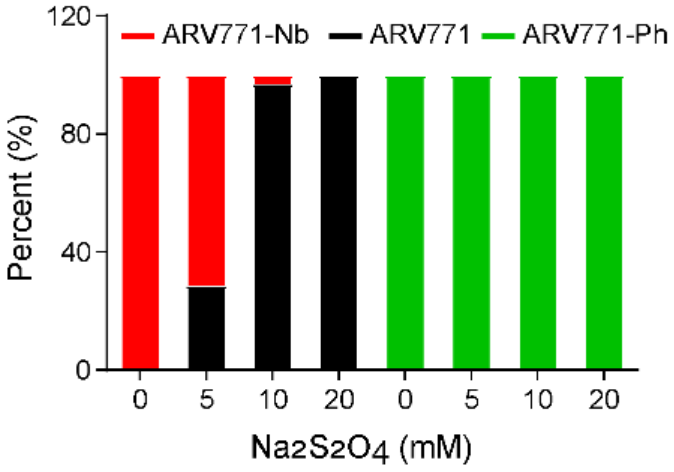
Supplementary Figure 42. ^{1}H-NMR spectrum of compound **27** ($CDCl_3$).



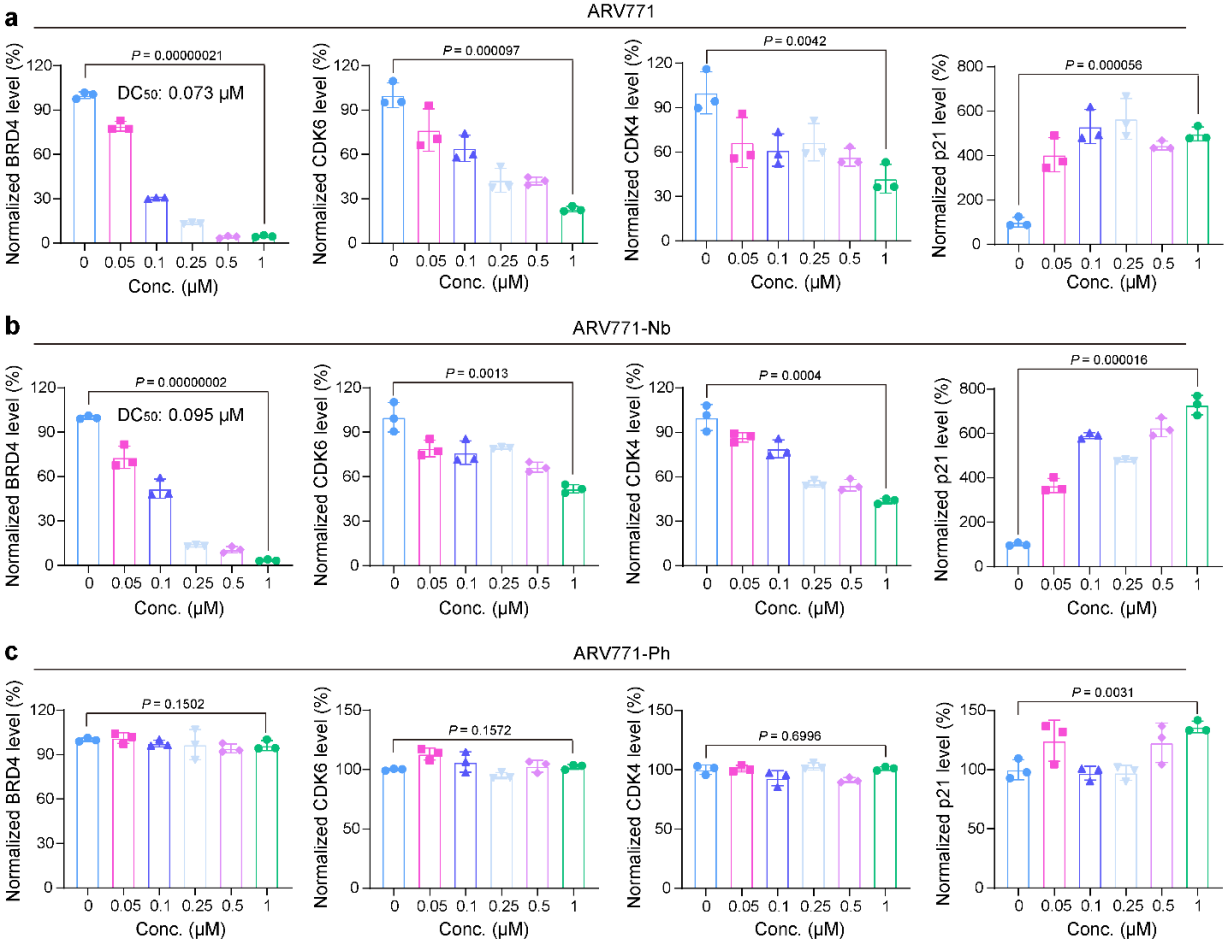
Supplementary Figure 43. ^{1}H-NMR spectrum of compound **28** ($CDCl_3$).



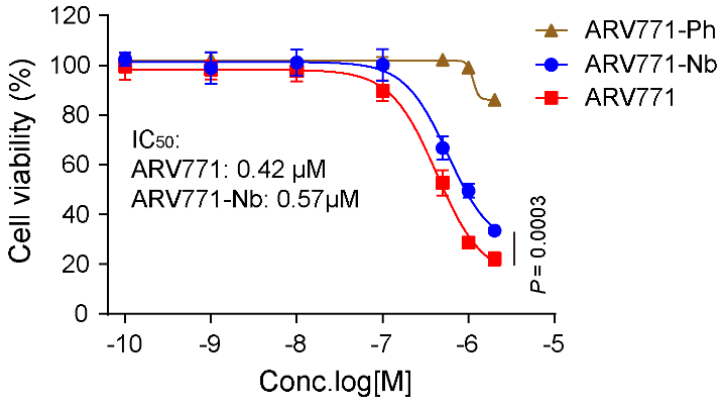
Supplementary Figure 44. Mass spectrum of compound **28**.



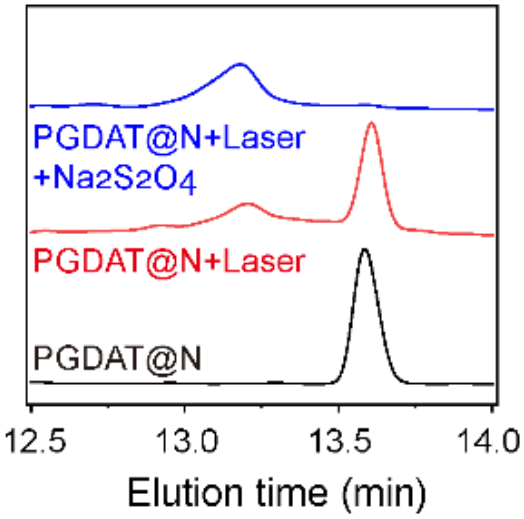
Supplementary Figure 45. Quantitative ARV771 release percentages after ARV771-Nb and ARV771-Ph treated with different concentrations of $Na_2S_2O_4$ using HPLC analysis.



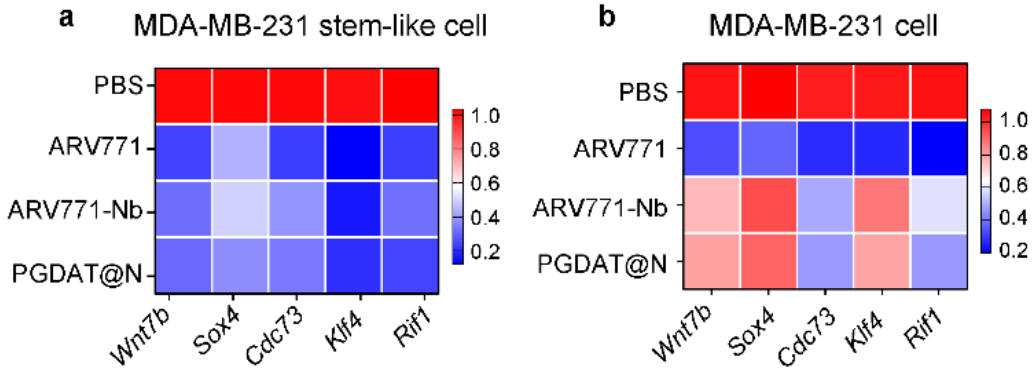
Supplementary Figure 46. Semi-quantitation of the western blot band of MDA-MB-231 stem-like cells after being treated with (**a**) ARV771, (**b**)ARV771-Nb and (**c**) ARV771-Ph for 24 h (n = 3 independent experimental cell lines). Statistical analysis was performed by two-sided unpaired t-test. All data are presented as mean ± SD.



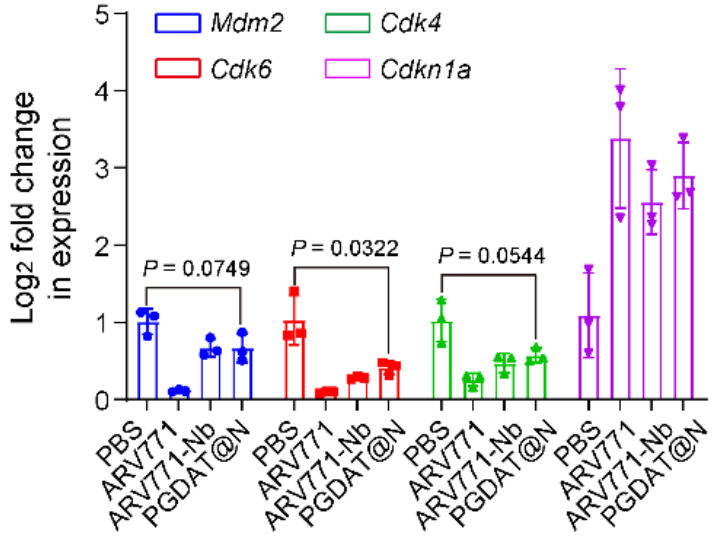
Supplementary Figure 47. Cytotoxicity (CCK-8 assay) of PROTAC molecule and its derivatives to MDA-MB-231 stem-like cells (n = 4 independent experimental cell lines). Statistical analysis was performed by two-way ANOVA (or mixed model). All data are presented as mean ± SD.



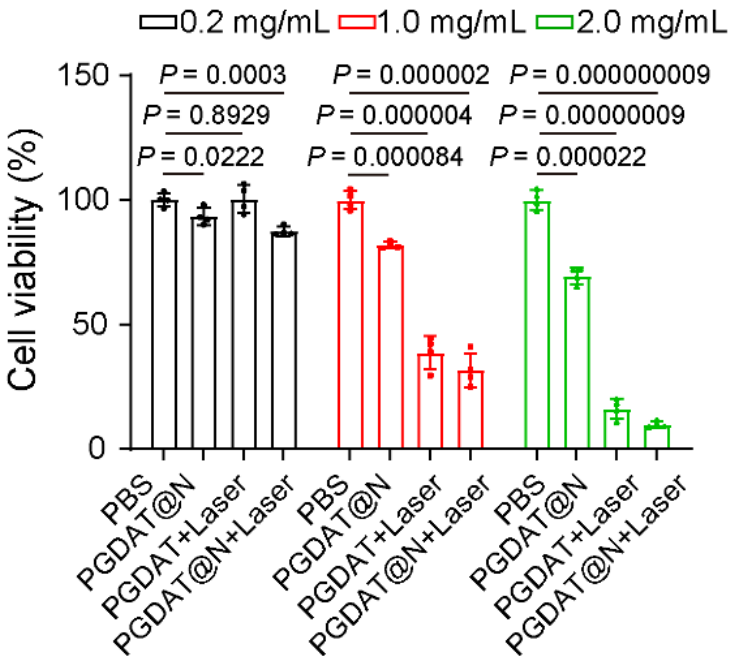
Supplementary Figure 48. HPLC profiles of the photoactivity- and reduction-mediated ARV771 recovery from PGDAT@N nanoparticle.



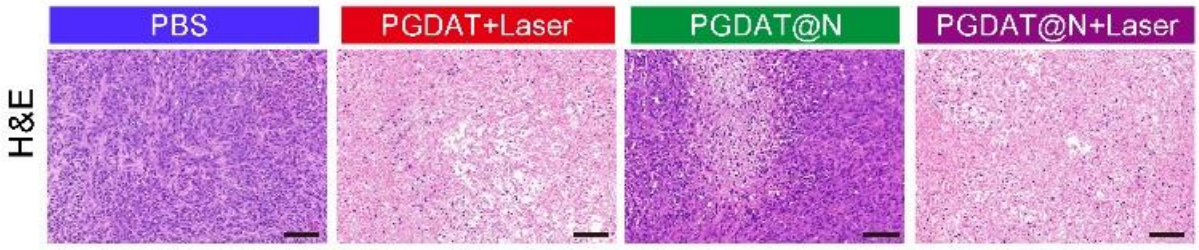
Supplementary Figure 49. Quantitative PCR analysis of mRNA expression change on the (**a**) MDA-MB-231 stem-like cell and (**b**) MDA-MB-231 cell subjected to various treatments (n = 3 independent experimental cell lines).



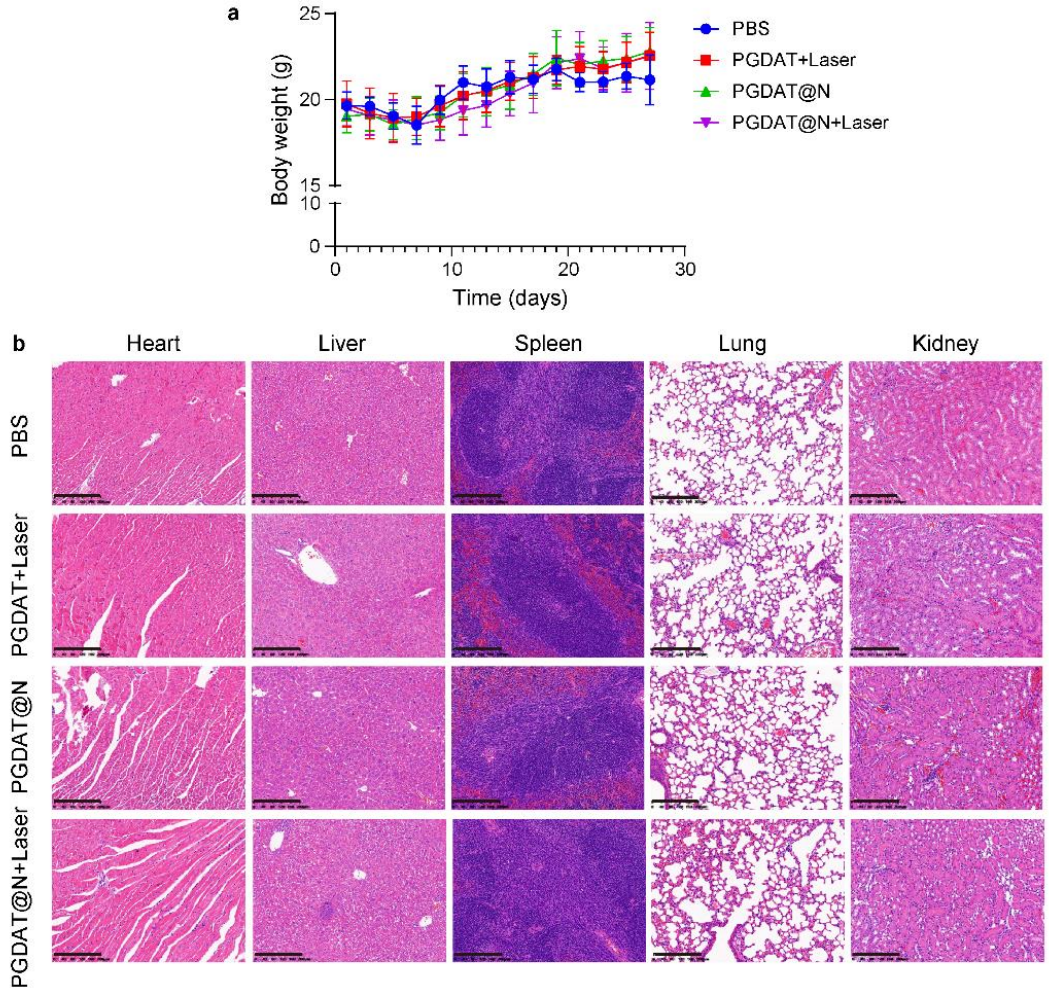
Supplementary Figure 50. Quantitative PCR analysis of relative mRNA level on the MDA-MB-231 cells after being treated with diverse formulations (n = 3 independent experimental cell lines). Statistical analysis was performed by two-sided unpaired t-test. All data are presented as mean ± SD.



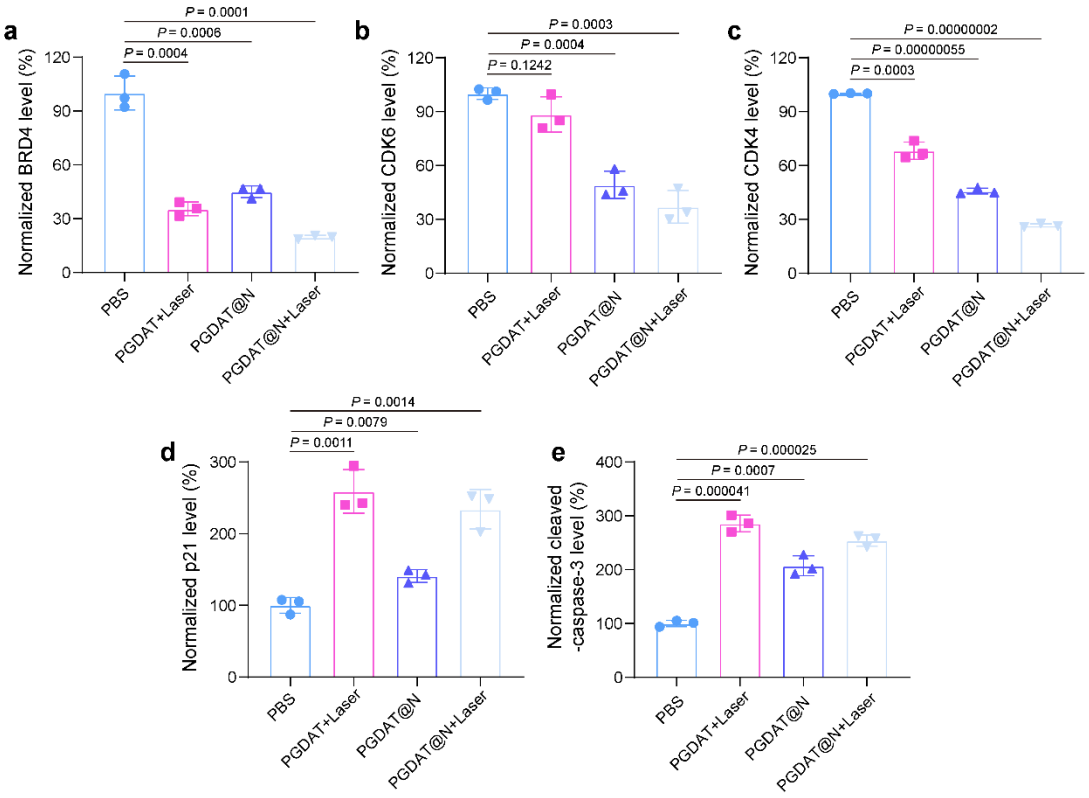
Supplementary Figure 51. CCK-8 analysis of the cell viability of MDA-MB-231 cells treated with diverse patterns (n = 3 independent experimental cell lines). Statistical analysis was performed by two-sided unpaired t-test. All data are presented as mean ± SD.



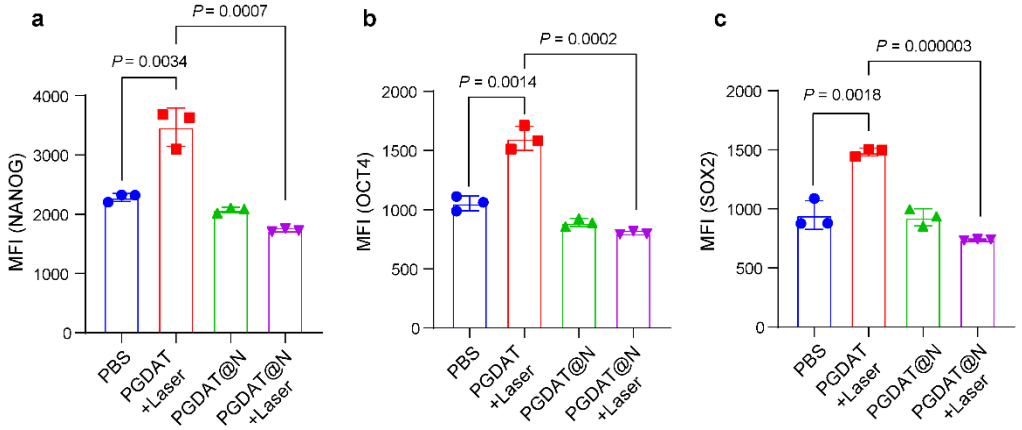
Supplementary Figure 52. H&E staining of the tumor sections at the end of treatments (scale bar = 100 μm).



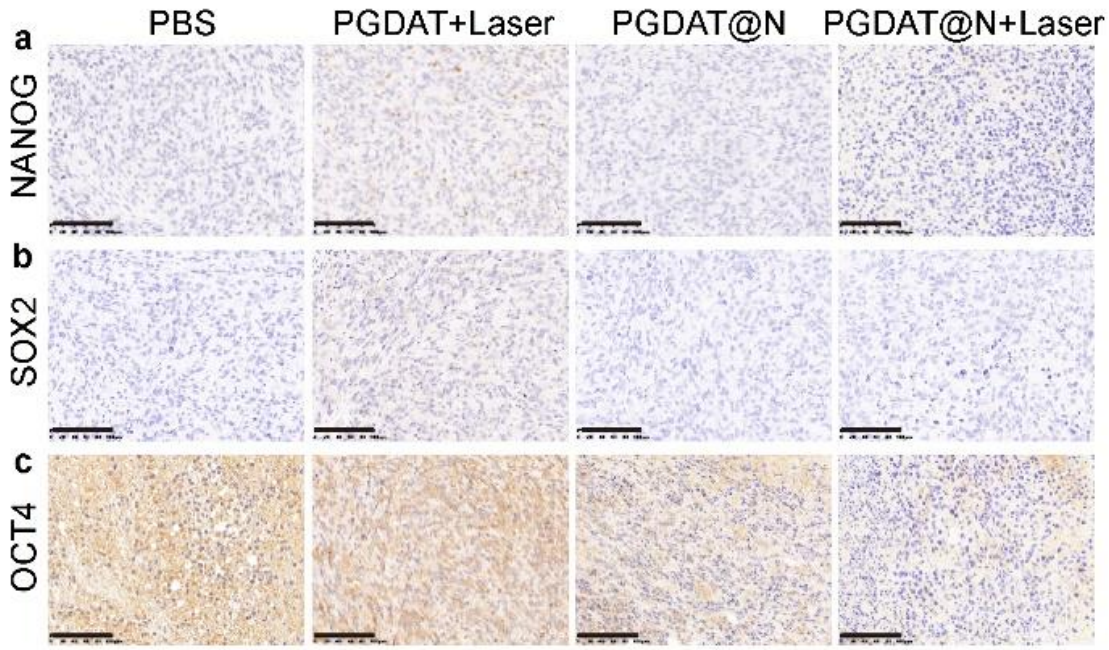
Supplementary Figure 53. a, Body weight curves of tumor-bearing mice during the experimental period (n = 6 mice). **b**, H&E staining of the major organs of tumor-bearing mice at the end of antitumor study (heart, liver, spleen, lung and kidney, scale bar = 200 μm). All data are presented as mean ± SD.



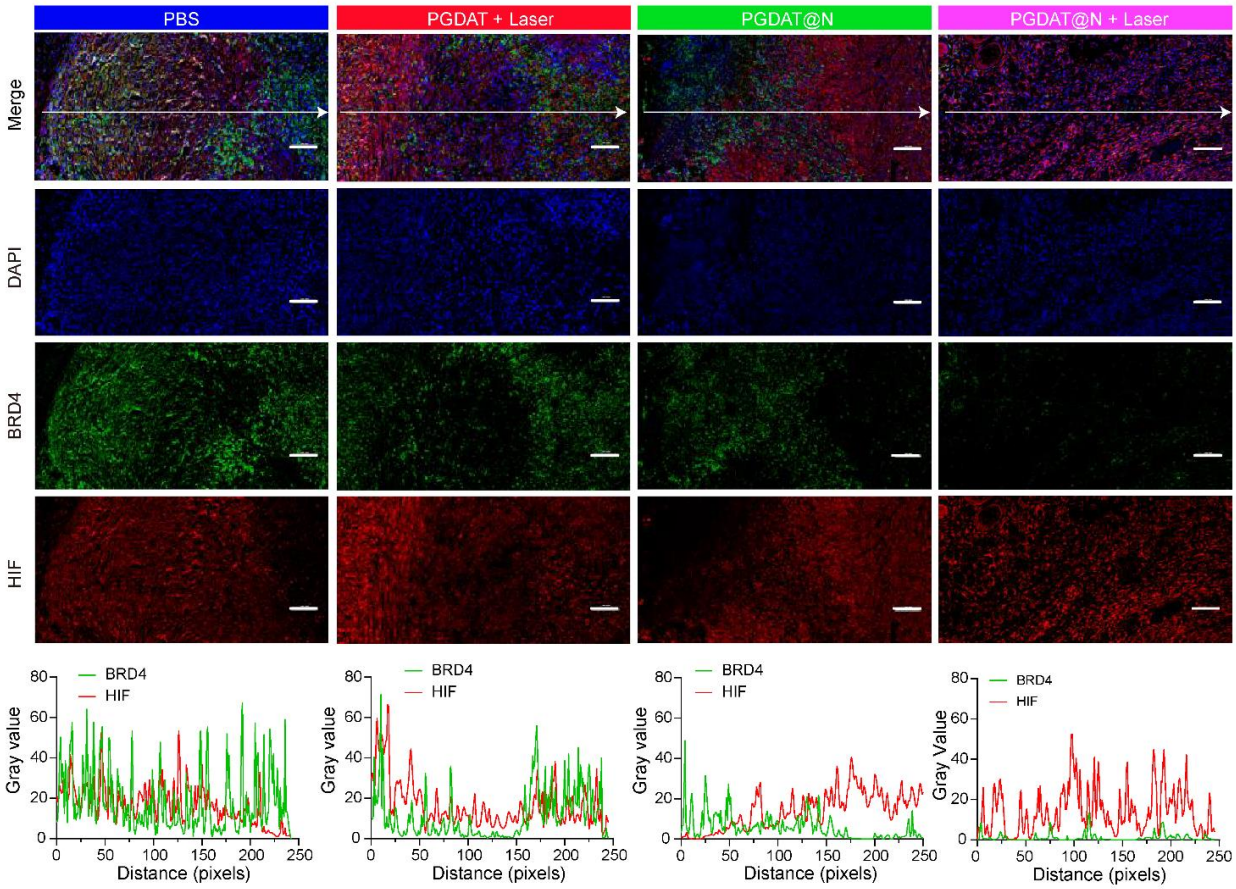
Supplementary Figure 54. Semi-quantitation of the western blot band of different protein (**a**) BRD4, (**b**) CDK6, (**c**) CDK4, **(d)** p21 and **(e)** cleaved-caspase-3 in the tumor tissues after the MDA-MB-231 tumor-bearing mice were treated with various methods (n = 3 mice). Statistical analysis was performance by two-sided unpaired t-test. All data are presented as mean ± SD.



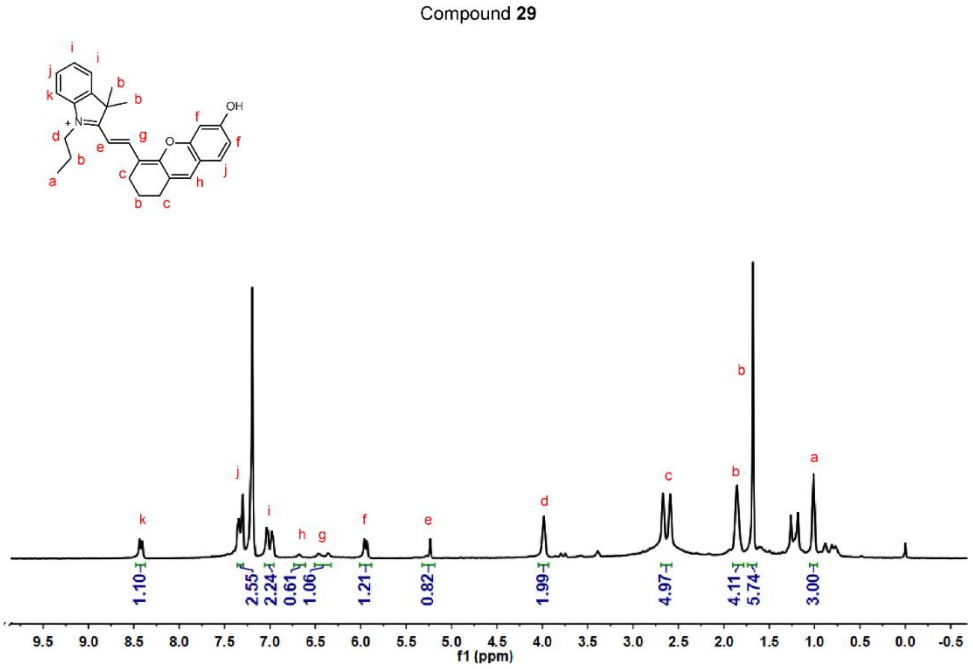
Supplementary Figure 55. Flow cytometric assay of intratumoral (**a**) NANOG, (**b**) OCT4 and (**c**) SOX2 expression after the tumor-bearing mice subjected to predetermined treatments (n = 3 mice). Statistical analysis was performance by two-sided unpaired t-test. All data are presented as mean ± SD.



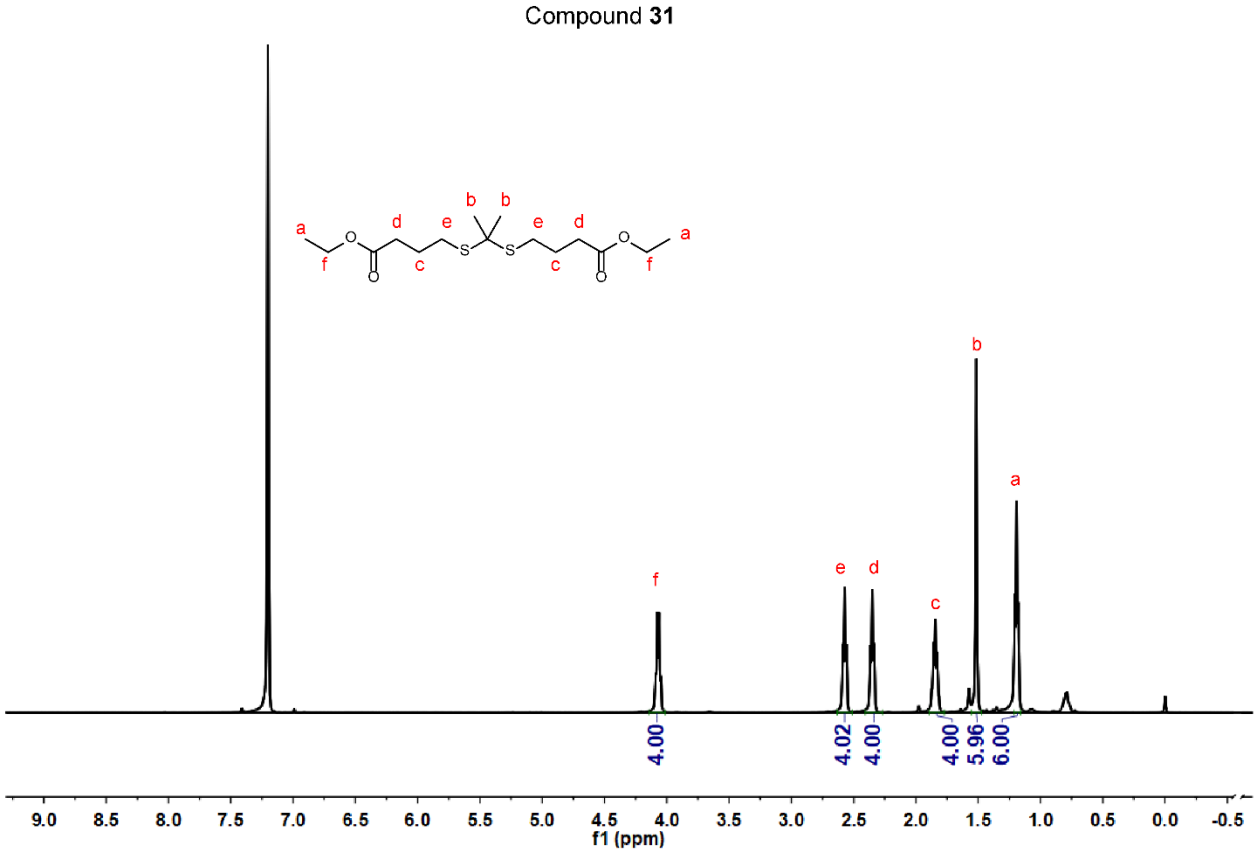
Supplementary Figure 56. IHC examination of (**a**) NANOG, (**b**) SOX2 and (**c**) OCT4 expression in the tumor sections after the tumor-bearing mice subjected to predetermined treatments (scale bar = 100 μm).



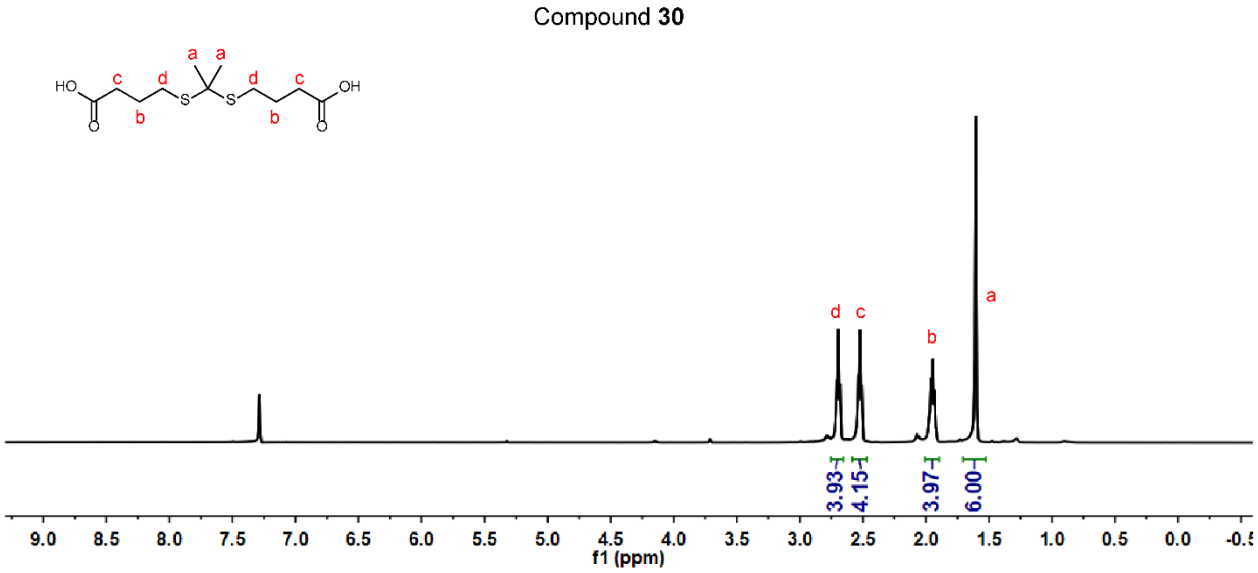
Supplementary Figure 57. Ex-vivo CLSM images of tumor section post subjected to different treatments (scale bar = 200 μm, the blue represents DAPI, the green represents BRD4 and the red represents HIF) and the fluorescence value as the arrow indicated area.



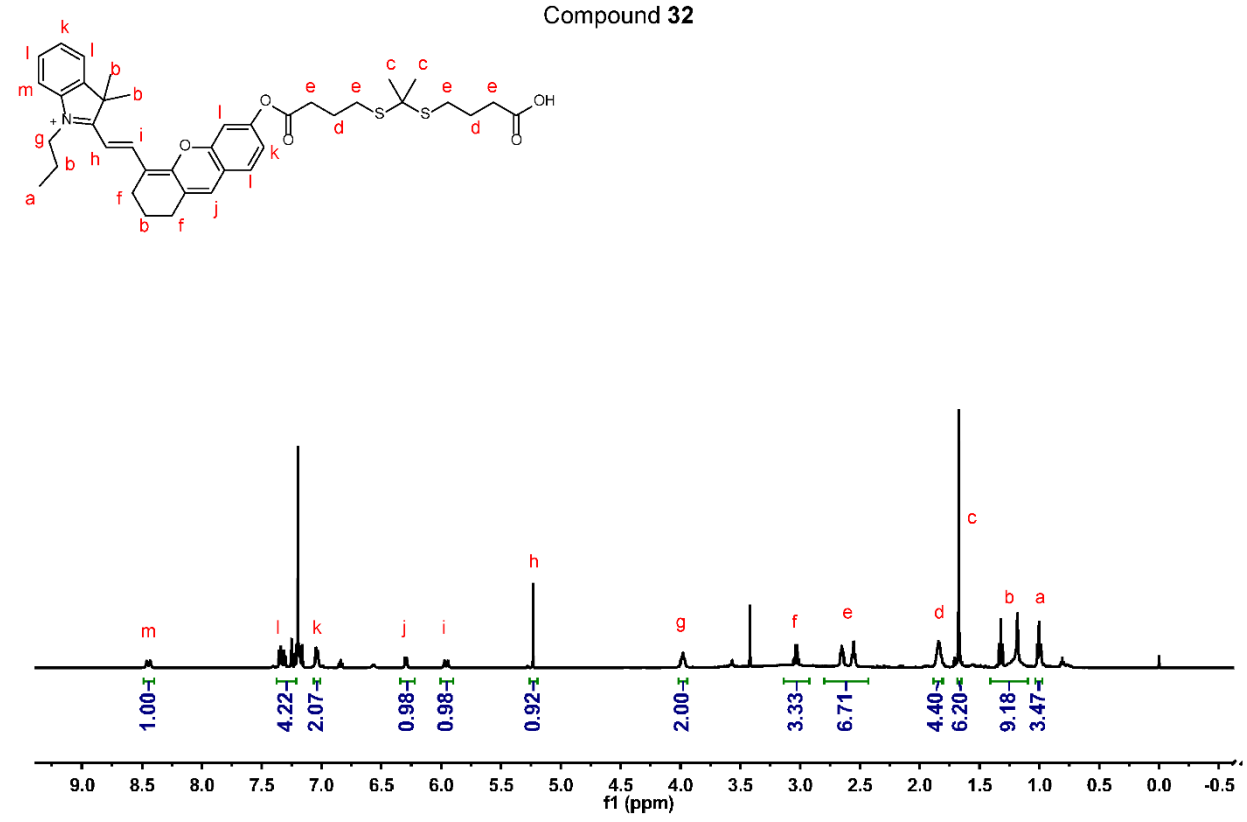
Supplementary Figure 58. ^{1}H-NMR spectrum of compound **29** ($CDCl_3$).



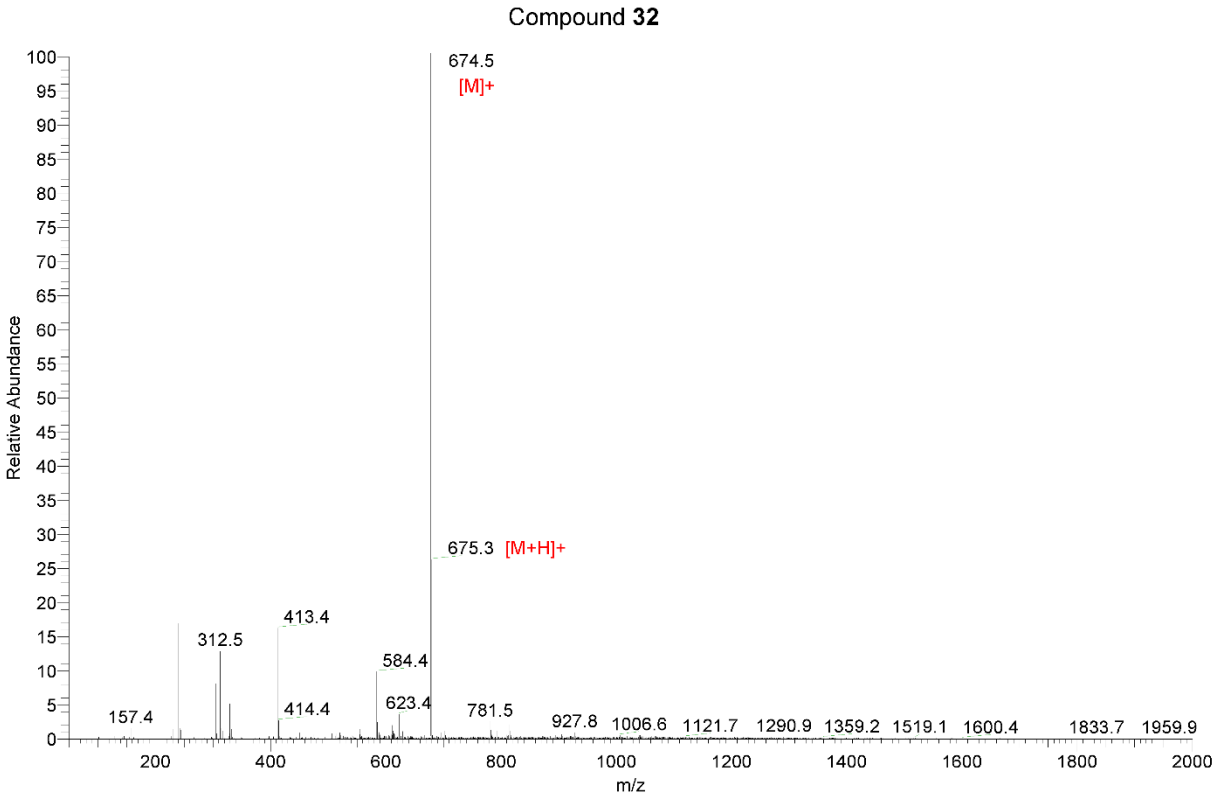
Supplementary Figure 59. ^{1}H-NMR spectrum of compound **31** ($CDCl_3$).



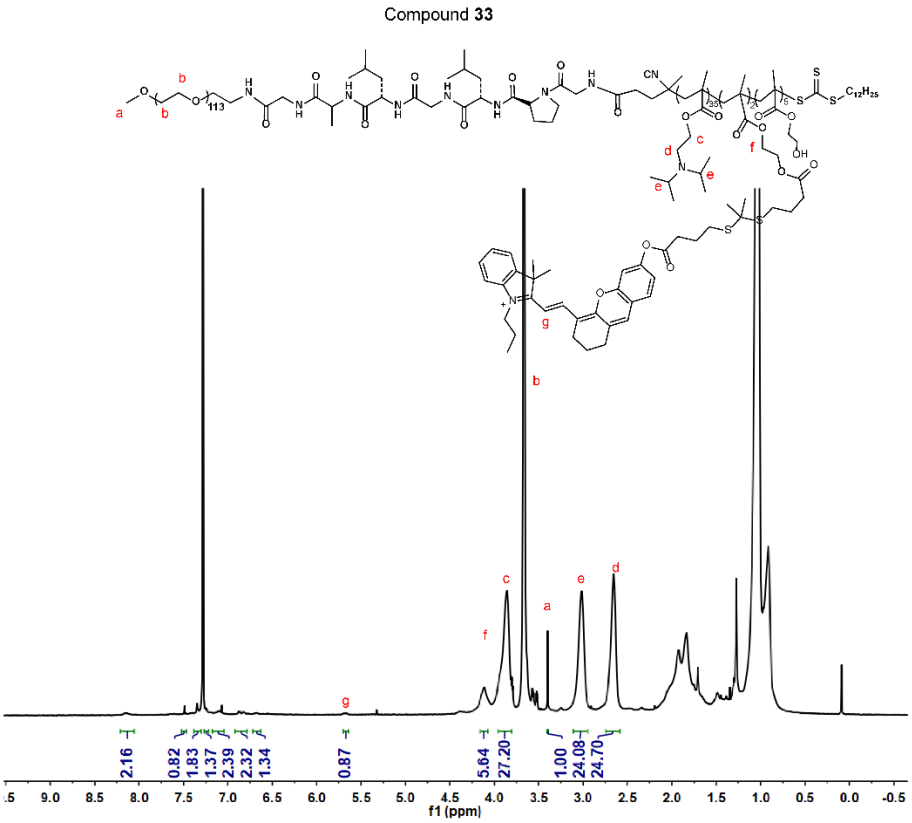
Supplementary Figure 60. ^{1}H-NMR spectrum of compound **30** ($CDCl_3$).



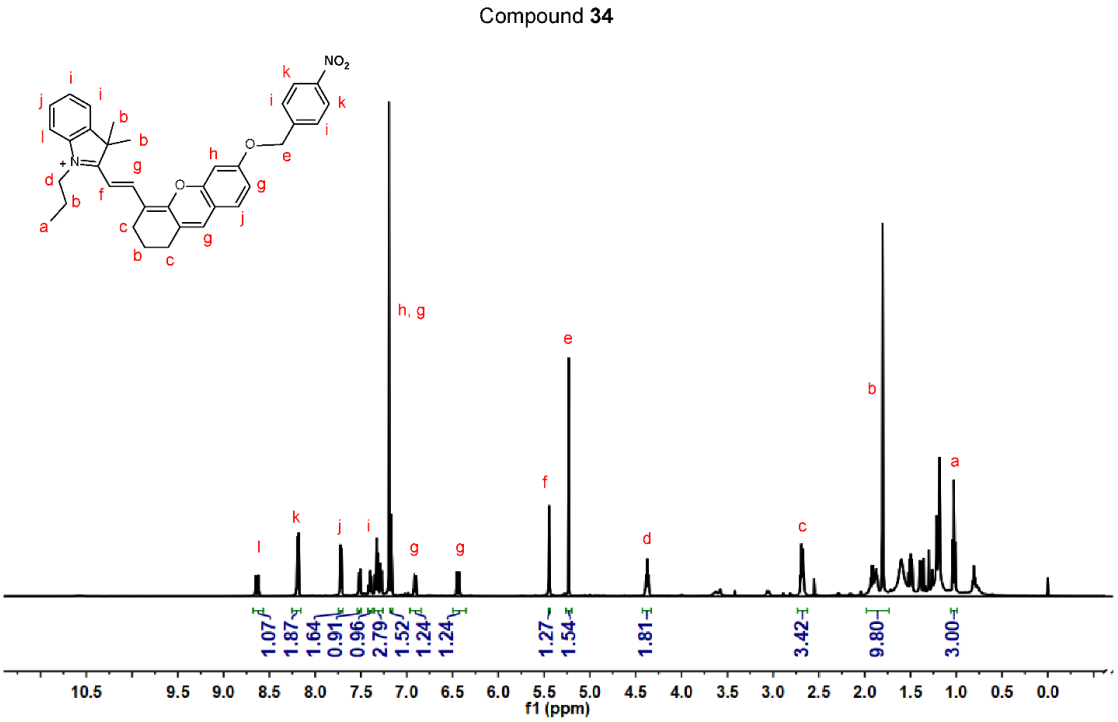
Supplementary Figure 61. ^{1}H-NMR spectrum of compound **32** ($CDCl_3$).



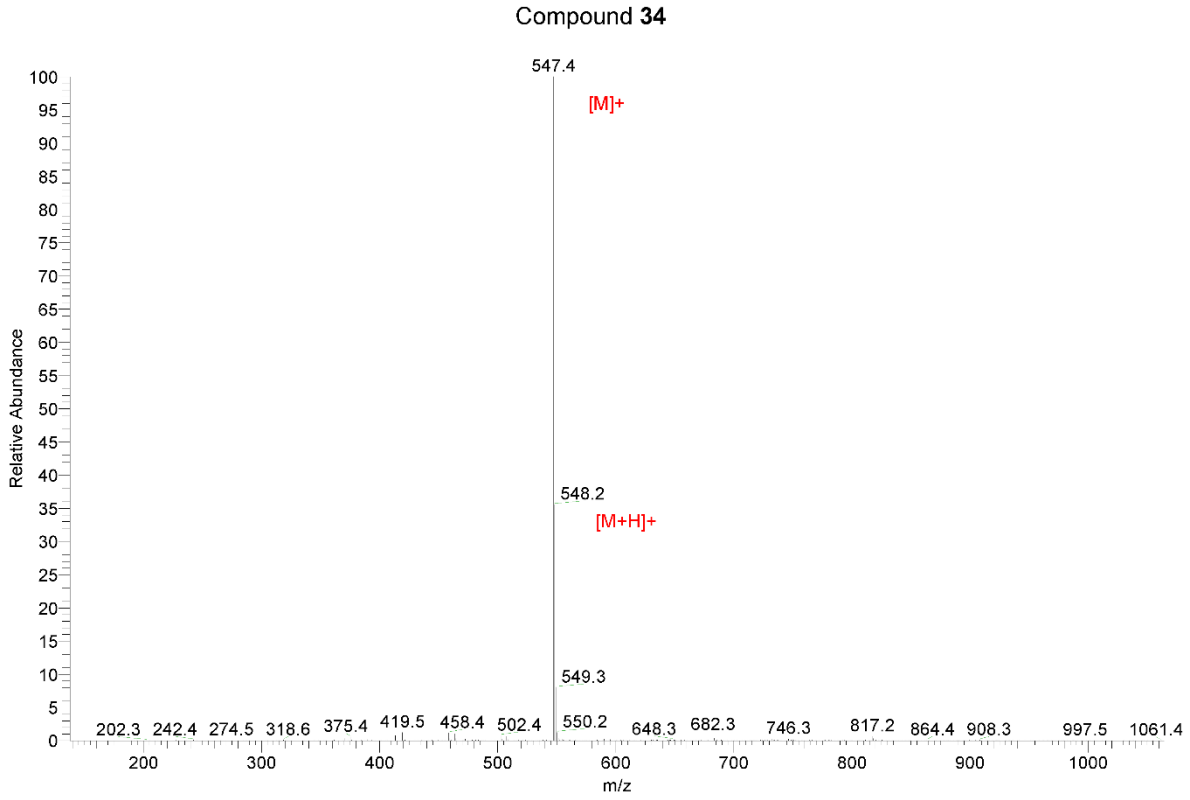
Supplementary Figure 62. Mass spectrum of compound **32**.



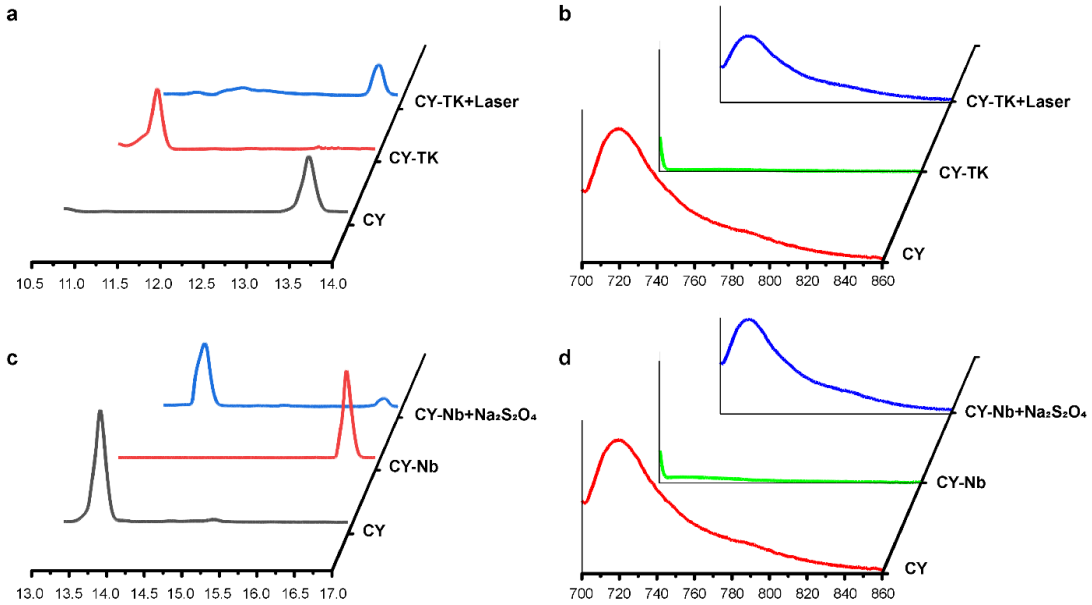
Supplementary Figure 63. ^{1}H-NMR spectrum of compound **33** ($CDCl_3$).



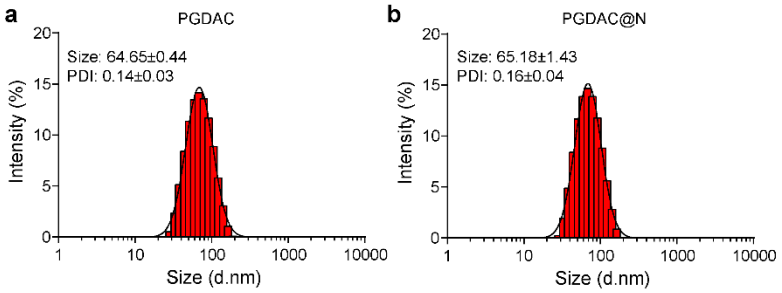
Supplementary Figure 64. ^{1}H-NMR spectrum of compound **34** ($CDCl_3$).



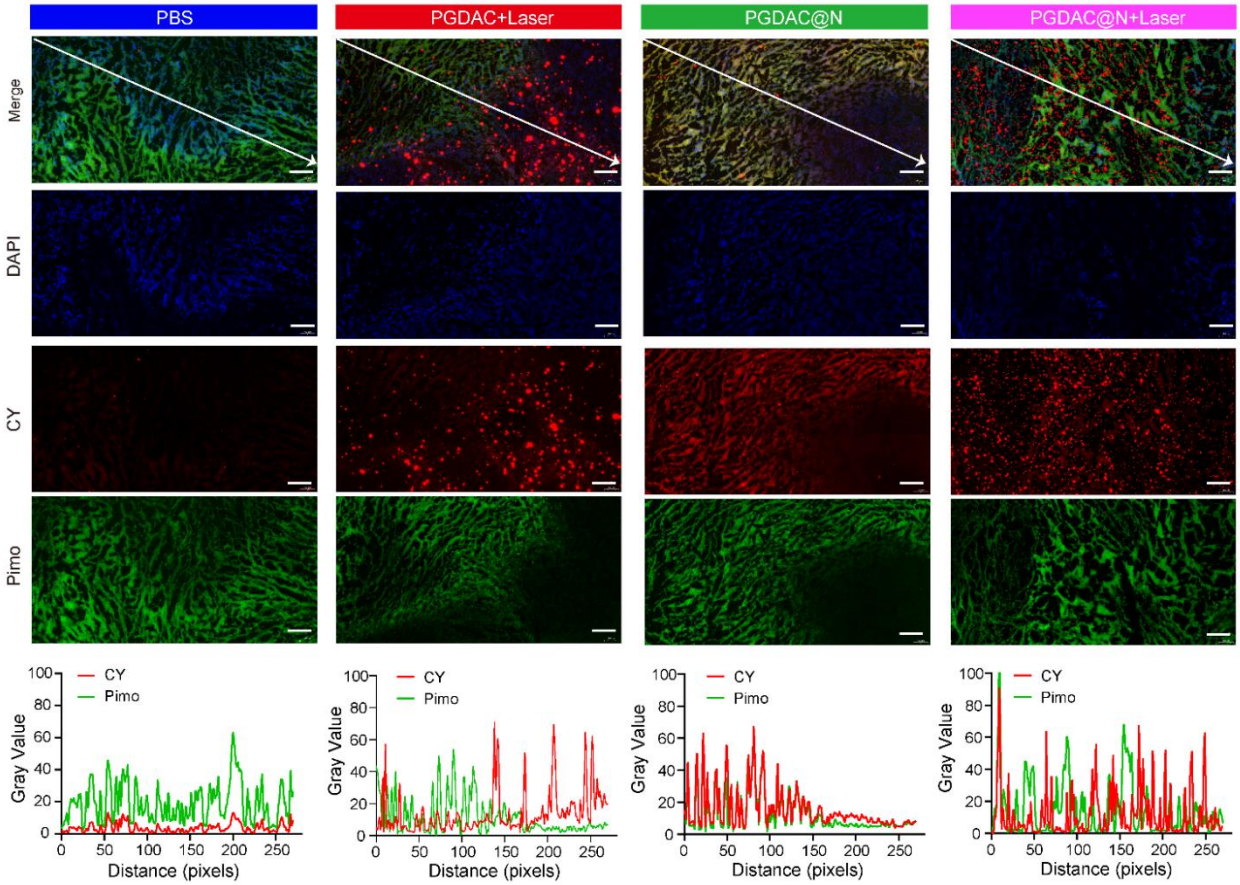
Supplementary Figure 65. Mass spectrum of compound **34**.



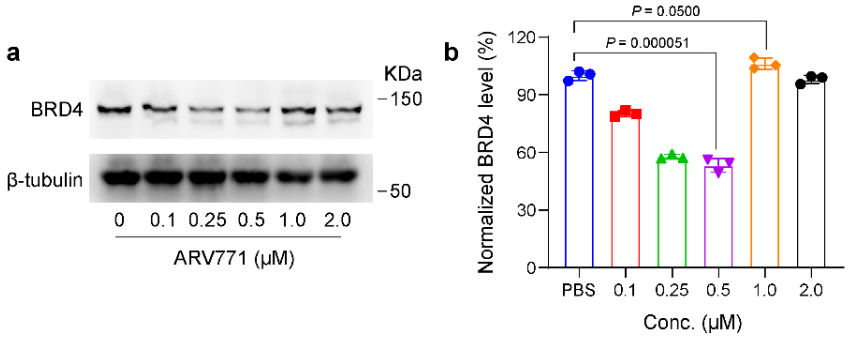
Supplementary Figure 66. HPLC profiles and fluorescence spectrum of the photoactivity- and reduction-mediated CY recovery from CY-TK (**a & b**) and CY-Nb (**c & d**).



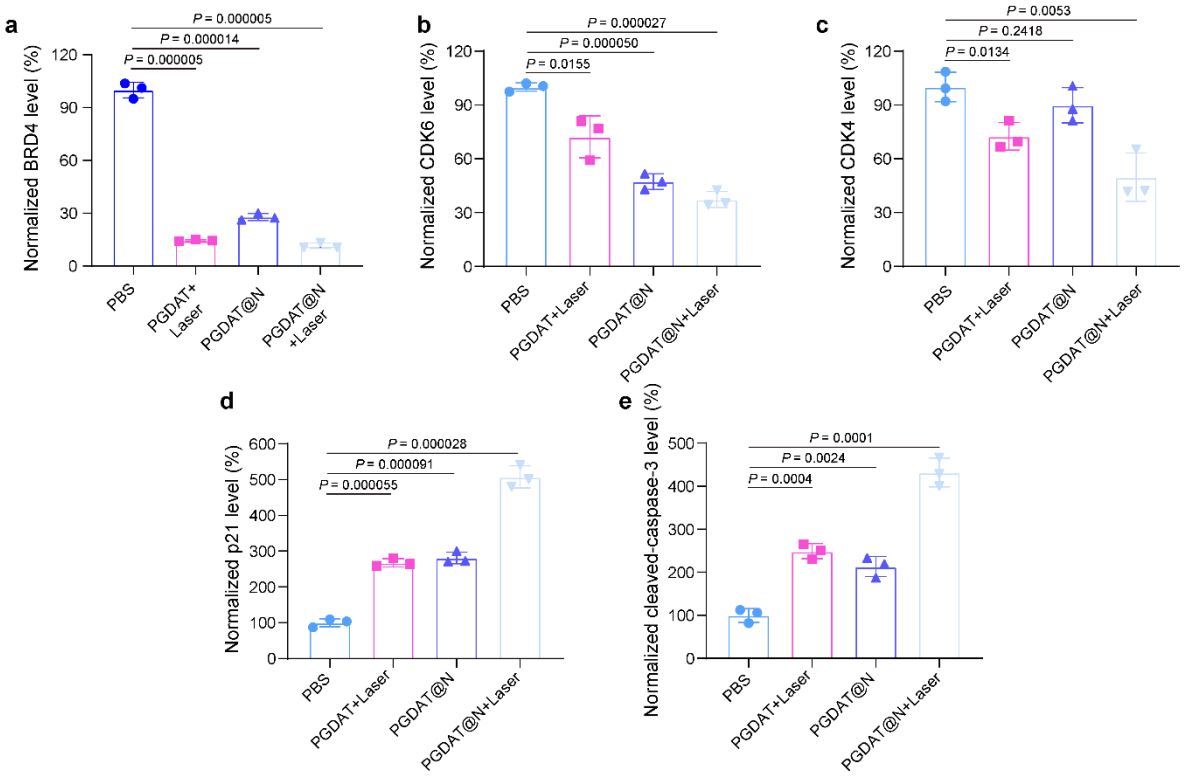
Supplementary Figure 67. DLS determined hydrodynamic diameter data of PGDAC **(a)** and PGDAC@N **(b)** nanoparticle.



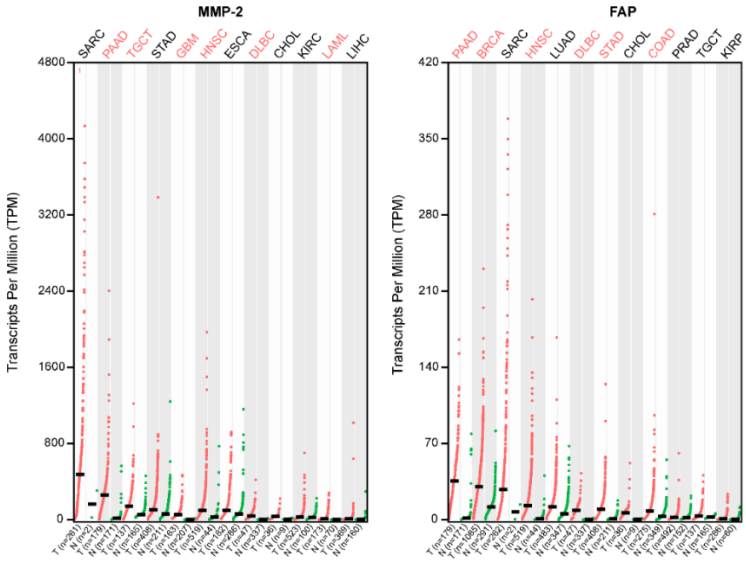
Supplementary Figure 68. Ex-vivo CLSM images of tumor section post subjected to different treatments (scale bar = 200 μm, the blue represents DAPI, the green represents pimo and the red represents CY) and the fluorescence value as the arrow indicated area.



Supplementary Figure 69. Western blot assay of BRD4 expression of HN30 cell after being subjected to ARV771 **(a)** and the semi-quantitative result **(b)** (n = 3 independent experimental cell lines). Statistical analysis was performance by two-sided unpaired t-test. All data are presented as mean ± SD.



Supplementary Figure 70. Semi-quantitation of the western blot band of different protein (**a**) BRD4, (**b**) CDK6, (**c**) CDK4, **(d)** p21 and **(e)** cleaved-caspase-3 in the tumor tissues after the HN30 tumor-bearing mice were treated with various methods (n = 3 mice). Statistical analysis was performance by two-sided unpaired t-test. All data are presented as mean ± SD.



Supplementary Figure 71. The MMP-2 and FAP gene expression profiles in the different tumor types and normal tissue of human patients (http://gepia.cancer-pku.cn/).

Supplementary Figure 72. Uncropped western blot source data for Supplementary Figure 69.

Supplementery reference

[1] Gao, J. Hou, B. Zhu, Q. Yang, L. Jiang X., Zou, Z. Li, X. Xu, T. Zheng, M. Chen, Y.H. Xu, Z. Xu, H. Yu, H. Engineered bioorthogonal POLY-PROTAC nanoparticles for tumour-specific protein degradation and precise cancer therapy, Nat. Commun. **13**, 4318 (2022).