Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2015

Supporting Information

Controlled proliferation and screening of mammalian cells on a hydrogel-functionalized small molecule microarray

Biwei Zhu, Bo Jiang, Zhenkun Na and Shao Q. Yao*

Department of Chemistry, National University of Singapore, Singapore 117543.
**Correspondence should be addressed to SQY (chmyaosq@nus.edu.sg)*

1. General Information:

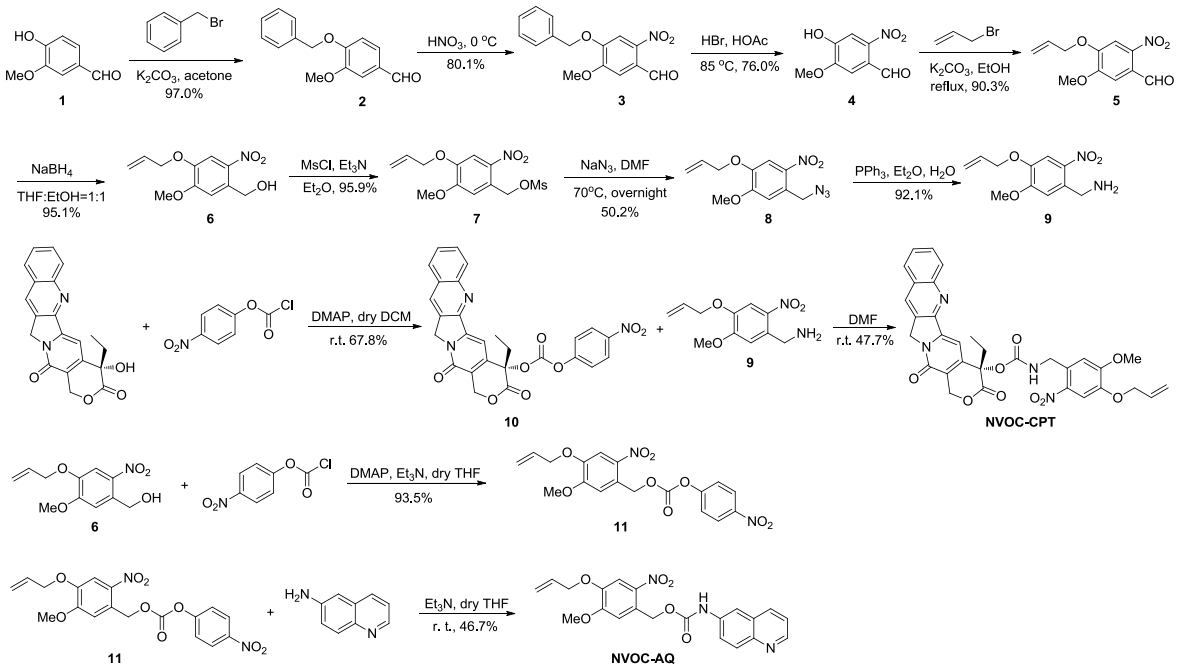
All chemicals were purchased from commercial vendors and used without further purification, unless otherwise noted. *N*,*N*-Dimethylformamide (DMF) and Dichloromethane (CH_2Cl_2, DCM) were distilled over CaH_2. All non-aqueous reactions were carried out under nitrogen atmosphere in oven-dried glasswares. Reaction progress was monitored by TLC on pre-coated silica plates (Merck 60 F254, 250 μm thicknesses) and spots were visualized by ceric ammonium molybdate, ninhydrin, basic $KMnO_4$, UV light or iodine. Flash column chromatography was carried out using Merck silica gel (0.040-0.063). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker model Avance 300 MHz or DPX-500 MHz NMR spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks ($CDCl_3$ = 7.26 ppm, MeOD = 3.31 ppm). ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz) and multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet), dt (doublet of triplet). MS spectra were recorded on a Finnigan LCQ mass spectrometer, a Shimadzu LC-IT-TOF spectrometer or a Shimadzu LC-ESI spectrometer. Analytical HPLC was carried out on Shimadzu LC-IT-TOF or LC-ESI systems equipped with an autosampler, using reverse-phase Phenomenex Luna 5 μm C_{18} 100 Å 50 × 3.0 mm columns. Preparative HPLC was carried out on Gilson preparative HPLC system using Trilution software and reverse-phase Phenomenex Luna 5 μm $C_{18\ (2)}$ 100 Å 50 × 30.00 mm column. 0.1% TFA/H2O and 0.1% TFA/acetonitrile were used as eluents for all HPLC experiments. The flow rate was 0.6 mL/min for analytical HPLC and 8 mL/min for preparative HPLC. UV-vis absorption and fluorescence spectra were measured by using a Shimadzu UV-vis spectrophotometer and a Perkin Elmer LS50 spectrofluorometer, respectively. All the measurements were performed at room temperature.

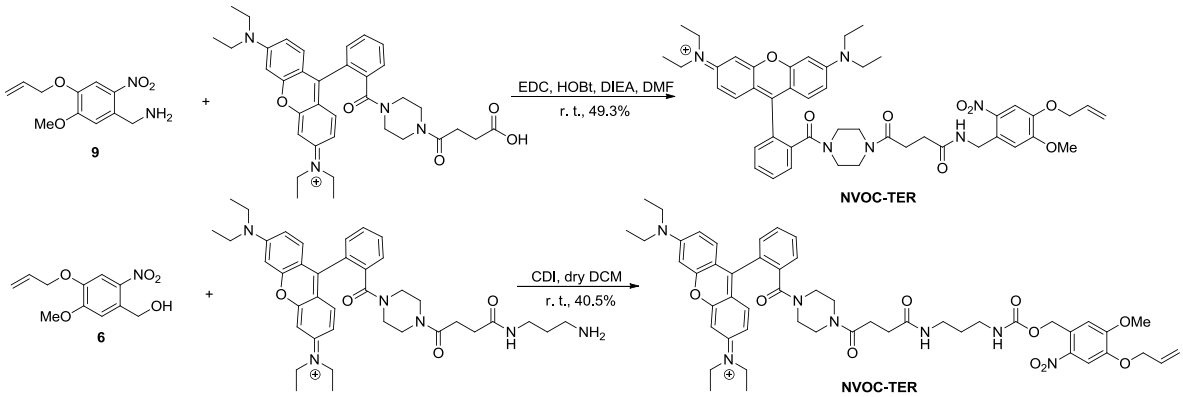
Antibodies were purchased from the following vendors: anti-cleaved caspase 3 (#9661, Cell Signaling) Rabbit, HRP-conjugated secondary antibodies: Goat anti-Rabbit IgG (#31210, Pierce). Nuclear staining reagent Hochest 33342, trihydrochloride, trihydrate (10 mg/mL solution in water) were purchased from Invitrogen. Western blot was done by using the SuperSignal® West Dura Extended Duration Substrate Kit (200 mL Kit, Thermal Scientific). FACS experiments were carried out on a BD Accuri™ C6 cell analyzer. Imaging was done by In Cell Analyzer 2200 (GE). Cell lines were obtained

from the National Cancer Institute Developmental Therapeutics Program (NCI-60). Prostate cancer (PC-3) cells, Chinese hamster ovary (CHO-9) cells, Mouse embryonic fibroblast cell line (NIH 3T3) cells and human epithelioid carcinoma (HeLa) cells were cultured in Dulbecco's modified Eagle medum (DMEM; Invitrogen) containing 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), 100 units/mL penicillin, and 100 μg/mL streptomycin (Thermo Scientific) and maintained in a humidified 37 °C incubator with 5% CO_2. Protein concentration was determined by Bradford protein assay. Recombinant PSA protease was obtained from Sigma-Aldrich (USA).

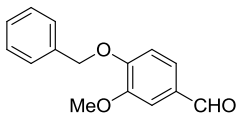
2. Synthesis and Characterization

NVOC-CPT was synthesized, as shown in Fig. 1b, from the commercially available vanillin through intermediate **4**. Briefly, introduction of an allyl group in **4**, which served as an immobilization handle for **NVOC-CPT** in the SMM construction/thiol-ene reaction, gave **5** (90.3% yield). Subsequently, $NaBH_4$ reduction gave **6** (95.1%). The OH group in **6** was next converted to NH_2 in 3 steps to give **9** (44% overall), which was coupled to the tertiary OH of CPT via a carbamate linkage to deliver the final compound (47.7% in 2 steps). Another caged small molecule, **NVOC-AQ** was similarly synthesized by coupling a highly fluorescent dye, 6-aminoquinoline (AQ), to the NVOC-containing intermediate **11**. The carbamate linkage in **NVOC-AQ** effectively quenched the fluorescence of the compound, but upon UV irradiation and "uncaging", its fluorescence would increase significantly due to the release of free AQ. The **Caged-RGD peptide** was synthesized by using solid-phase peptide synthesis (Fig 1b and Scheme S2 in ESI); starting from a resin-bound RGD peptide, (CGRGDSP), the self-immolative **Linker** was coupled to the Asp side-chain of the RGD peptide via an ester linkage. Subsequent introduction of the PSA peptide substrate, followed by TER coupling and finally TFA cleavage of the final peptide, gave the desired product. A positive RGD control (**CGRGDSP peptide**) was synthesized as well.

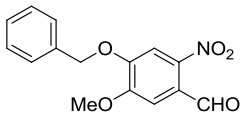




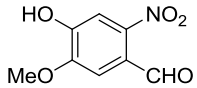
Scheme S1. Synthetic scheme of **NVOC-CPT**, **NVOC-AQ**, **NVOC-TER** and **NVOCL-TER**.



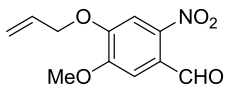
4-(benzyloxy)-3-methoxybenzaldehyde (2). A mixture of vanillin (11 g, 0.072 mol), benzyl bromide (13.5 g, 0.079 mol), potassium carbonate (5 g, 0.036 mol) and acetone (165 mL) was refluxed for 12 h. After the completion of the reaction, water (165 mL) was added. The precipitated product was recrystallized from ethanol to yield compound **2** as a white solid (16.8 g, 97.0%). ^{1}H NMR (300 MHz, Acetone) δ 9.83 (s, 1H), 7.51-7.45 (m, 3H), 7.37 (ddd, *J* = 19.7, 8.2, 4.4 Hz, 4H), 7.20 (d, *J* = 8.2 Hz, 1H), 5.21 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 191.28, 154.61, 151.07, 137.64, 131.44, 129.35, 128.87, 128.60, 126.59, 113.50, 110.58, 71.24, 56.16. MS (IT-TOF): [M+H^{+}] calcd for $C_{15}H_{14}O_3$ 243.09, found 243.10



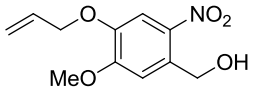
4-(benzyloxy)-5-methoxy-2-nitrobenzaldehyde (3). Compound **2** (13.5 g, 0.056 mol) was added to nitric acid (65 mL) and the reaction was stirred at 0 ^{o}C for 1 h. The solid product was collected by filtration and recrystallized from ethyl acetate to provide compound **3** as yellow needles (12.8 g, 80.1%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.43 (d, *J* = 1.1 Hz, 1H), 7.67 (s, 1H), 7.4-7.40 (m, 6H), 5.27 (s, 2H), 4.02 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.74, 153.70, 151.39, 143.59, 134.81, 128.88, 128.69, 127.56, 127.18, 125.70, 110.00, 108.88, 71.54, 56.70. MS (IT-TOF): [M+H^{+}] calcd for $C_{15}H_{13}NO_5$ 288.08, found 288.08.



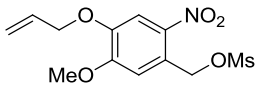
4-hydroxy-5-methoxy-2-nitrobenzaldehyde (4). A mixture of **3** (5.2 g, 0.018 mol) and acetic acid (36.2 mL) was kept at 85 ^{o}C while 48% hydrobromic acid (11 mL) was added dropwise. The reaction mixture was kept at 85 ^{o}C for 1 h. The solid product was collected by filtration and recrystallized from hot ethanol to obtain compound **4** as yellow needles (2.74 g, 76.0%). ^{1}H NMR (300 MHz, MeOD) δ 10.24 (s, 1H), 7.51 (s, 1H), 7.44 (s, 1H), 4.01 (s, 3H); MS (IT-TOF): [M+H^{+}] calcd for $C_8H_7NO_5$ 198.03, found 198.08.



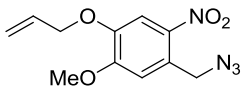
4-(allyloxy)-5-methoxy-2-nitrobenzaldehyde (5). Compound **4** (1.6 g, 8.1 mmol), potassium carbonate (1.7 g, 12.3 mmol) and allyl bromide (1.17 g, 9.7 mmol) were mixed in dry ethanol (16.2 mL), and the reaction was refluxed overnight. Subsequently, the mixture was poured into water, extracted into ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate and concentrated. The crude product was further purified by column chromatography (10% ethyl acetate/90% hexane) to obtain compound **5** as a yellow solid (1.74 g, 90.3%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.45 (s, 1H), 7.62 (s, 1H), 7.42 (s, 1H), 6.07 (ddd, *J* = 16.0, 10.6, 5.3 Hz, 1H), 5.49 (d, *J* = 17.2 Hz, 1H), 5.41 (d, *J* = 10.6 Hz, 1H), 4.76 (d, *J* = 5.4 Hz, 2H), 4.02 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.72, 153.52, 151.31, 131.19, 125.60, 119.74, 109.98, 108.59, 70.44, 56.72. MS (IT-TOF): [M+H$^+$] calcd for $C_{11}H_{11}NO_5$ 238.06, found 238.09



(4-(allyloxy)-5-methoxy-2-nitrophenyl)methanol (6). To a solution of **5** (1.6 g, 6.7 mmol) in THF-ethanol (1:1, 36.2 mL) at 0 ^{o}C was added $NaBH_4$ (506 mg, 13.4 mmol) slowly. The mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layers were combined, washed with water and brine, dried over anhydrous sodium sulfate and concentrated to afford the desired product **6** as a pale white solid which can be used in the next step without further purification (1.53 g, 95.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.17 (s, 1H), 6.07 (ddd, *J* = 22.7, 10.7, 5.4 Hz, 1H), 5.41 (dd, *J* = 30.2, 13.2 Hz, 2H), 4.96 (s, 2H), 4.68 (d, *J* = 5.5 Hz, 2H), 4.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.22, 154.28, 139.65, 132.33, 131.92, 119.15, 111.27, 109.91, 70.16, 62.88, 56.45. MS (IT-TOF): [M+H$^+$] calcd for $C_{11}H_{13}NO_5$ 240.08, found 240.10

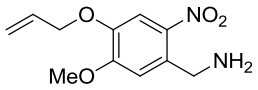


4-(allyloxy)-5-methoxy-2-nitrobenzyl methanesulfonate (7). Methanesulfonyl chloride (481.2 mg, 4.2 mmol) was added dropwise to a stirred solution of compound **6** (830 mg, 3.5 mmol) and triethylamine (425 mg, 4.2 mmol) in anhydrous diethyl ether (5.25 mL) at 0 ^{o}C. After 3 h, water was added to the reaction mixture. The organic layer was separated, washed with water and brine, dried over anhydrous sodium sulfate and concentrated to afford the desired product **7** as a yellow solid which can be used in the next step without further purification (1.06 g, 95.9%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.15 (s, 1H), 6.11-6.02 (m, 1H), 5.65 (s, 2H), 5.47 (dd, *J* = 17.3, 1.3 Hz, 1H), 5.37 (dd, *J* = 10.5, 1.0 Hz, 1H), 4.69 (d, *J* = 5.5 Hz, 2H), 4.01 (s, 3H), 3.14 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.27, 147.68, 139.32, 131.69, 125.08, 119.32, 110.85, 109.93, 70.21, 68.80, 56.61, 37.81. MS (IT-TOF): [M+H$^+$] calcd for $C_{12}H_{15}NO_7S$ 318.06, found 318.09

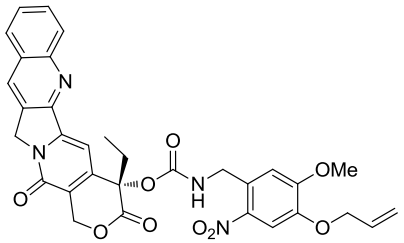


1-(allyloxy)-4-(azidomethyl)-2-methoxy-5-nitrobenzene (8). Sodium azide (221 mg, 3.4 mmol) was added to a solution of compound **7** (430 mg, 1.36 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 70 ^{o}C for 3 h. The resulting mixture was poured into water and the crude product was extracted with ethyl acetate. The organic phase was combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to afford the desired product **8** as a yellow solid (347.36 mg, 50.2%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.07 (s, 1H), 6.16-5.98 (m, 1H), 5.41 (dd, *J* = 29.9, 13.9 Hz, 2H), 4.87

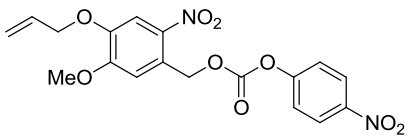
(s, 2H), 4.68 (d, J = 5.4 Hz, 2H), 4.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0, 147.15, 139.63, 131.83, 126.74, 119.20, 111.25, 110.04, 70.19, 56.51, 52.24. MS (IT-TOF): [M+H$^+$] calcd for $C_{11}H_{12}N_4O_4$ 265.09, found 265.05.



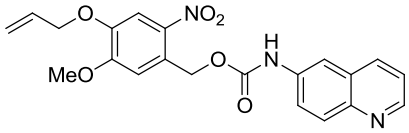
(4-(allyloxy)-5-methoxy-2-nitrophenyl)methanamine (9). To a solution of **8** (200 mg, 0.76 mmol) in anhydrous diethyl ether (1 mL) at 0 ^{o}C was added triphenylphosphine (299 mg, 1.14 mmol), and the mixture was allowed to stir at room temperature for 5 h. Water was then added and the reaction was further stirred for another 20 h. The resulting mixture was poured into 10% aq. HCl, and extracted with diethyl ether. The aqueous layer was then adjusted to basic form with sodium carbonate prior to extraction with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The crude product was further purified by column chromatography (3% MeOH/97% DCM) to afford the desired product **9** as a yellow oil (166.10 mg, 92.1%). ^{1}H NMR (500 MHz, DMSO) δ 7.82 (s, 1H), 7.22 (s, 1H), 6.13-6.02 (m, 1H), 5.45 (dd, J = 17.3, 1.6 Hz, 1H), 5.31 (dd, J = 10.6, 1.4 Hz, 1H), 4.70 (d, J = 5.3 Hz, 2H), 4.34 (s, 2H), 4.01 (s, 3H). MS (IT-TOF): [M+H$^+$] calcd for $C_{11}H_{14}N_2O_4$ 239.10, found 239.12.



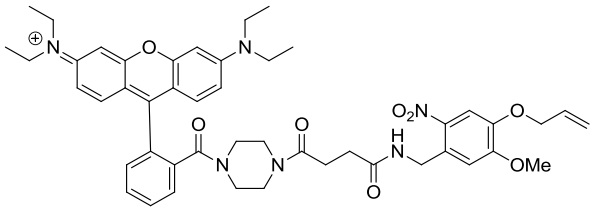
NVOC-CPT. Camptothecin (CPT, 20 mg, 0.06 mmol) and 4-nitrophenyl chloroformate (36.3 mg, 0.18 mmol) were dissolved in HPLC-grade DCM (1 mL) at 0 ^{o}C, followed by the addition of DMAP (44 mg, 0.36 mmol). The resulting solution was stirred at room temperature for 1 h. The resulting mixture was diluted with DCM and washed with 0.1 M HCl solution. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to ~ 0.5 mL. The crude product was precipitated by ether, filtered and dried to give compound **10** as a yellow powder which was used directly in the next step (20 mg, 67.8%).[1] Compound **10** (20 mg) dissolved in DMF (1 mL) was added compound **9**, and the resulting mixture was stirred at room temperature for 3 h. DMF was subsequently removed under reduced pressure, and the resulting residue was purified by column chromatography (100% ethyl acetate) followed by preparative TLC to yield the titled compound (11.4 mg, 47.7%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (d, J = 88.2 Hz, 2H), 7.95 (d, J = 7.9 Hz, 1H), 7.86 (s, 1H), 7.65 (dd, J = 26.7, 16.7 Hz, 2H), 7.34 (s, 1H), 6.93 (s, 1H), 6.03-5.92 (m, 1H), 5.69 (d, J = 17.1 Hz, 1H), 5.35 (dd, J = 41.6, 13.8 Hz, 5H), 4.74-4.37 (m, 4H), 3.87 (s, 3H), 2.17 (d, J = 65.2 Hz, 2H), 0.96 (d, J = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.16, 157.39, 154.86, 154.20, 152.40, 148.83, 146.98, 146.76, 146.09, 140.06, 131.89, 131.17, 130.71, 129.59, 128.98, 128.04, 127.70 (d, J = 9.0 Hz), 126.88, 119.88, 119.25, 119.01, 112.60, 96.29, 76.01, 70.02, 67.06, 56.63, 50.60, 43.12, 31.98, 7.60. HRMS: [M+Na$^+$] calcd for $C_{32}H_{28}N_4O_9$ 635.1856, found 635.1762.



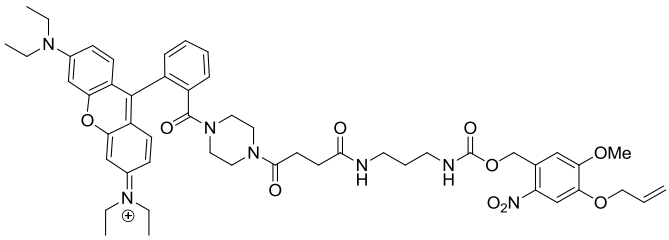
4-(allyloxy)-5-methoxy-2-nitrobenzyl (4-nitrophenyl) carbonate (11). To a stirred solution of compound **6** (50 mg, 0.21 mmol) in dry THF, DMAP (4 mg, 0.03 mmol) and triethylamine (48 μL) was added 4-nitrophenyl chloroformate (63.5 mg, 0.32 mmol). The resulting mixture was stirred at room temperature for 1.5 h. Subsequently, the mixture was concentrated *in vacuo* and the residue was partitioned between ethyl acetate and water. The organic fraction was washed with water and brine, dried over anhydrous sodium sulfate and concentrated. The crude product was further purified by column chromatography (17% ~ 20% ethyl acetate in hexane) to afford the desired product **11** as a yellow oil (84.5 mg, 93.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, *J* = 9.1 Hz, 2H), 7.76 (s, 1H), 7.40 (d, *J* = 9.1 Hz, 2H), 7.11 (s, 2H), 6.05 (ddd, *J* = 16.0, 10.6, 5.3 Hz, 1H), 5.69 (s, 2H), 5.52-5.29 (m, 2H), 4.68 (d, *J* = 5.4 Hz, 2H), 4.00 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.90, 154.02, 152.07, 147.56, 145.41, 139.67, 131.59, 126.13, 125.32, 121.70, 119.31, 110.86, 110.00, 70.17, 67.67, 56.50. MS (IT-TOF): [M+H$^+$] calcd for $C_{11}H_{14}N_2O_4$ 239.10, found 239.12.



NVOC-AQ. To a solution of 6-aminoquinoline (15 mg, 0.04 mmol) and triethylamine (7 μL, 0.048 mmol) in anhydrous THF (3 mL) was added a solution of compound **11** (dissolved in anhydrous THF). The mixture was stirred at room temperature overnight. Upon solvent evaporation, the residue was purified by column chromatography (8% ethyl acetate in DCM) to afford the desired product as a light yellow solid (7.1 mg, 46.7%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (d, *J* = 9.1 Hz, 2H), 7.78 (s, 1H), 7.42 (d, *J* = 9.1 Hz, 2H), 7.26 (s, 2H), 7.11 (s, 1H), 6.08 (ddd, *J* = 22.6, 10.7, 5.4 Hz, 1H), 5.71 (s, 2H), 5.48 (d, *J* = 17.3 Hz, 1H), 5.38 (d, *J* = 10.5 Hz, 1H), 4.70 (d, *J* = 5.5 Hz, 2H), 4.02 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.36, 154.06, 152.08, 147.70, 145.57, 139.89, 139.86, 131.72, 125.38, 125.17, 121.71, 119.33, 110.88, 110.86, 110.11, 110.08, 70.23, 67.72, 56.56. HRMS: [M+Na$^+$] calcd for $C_{21}H_{19}N_3O_6$ 432.1274, found 432.1263.



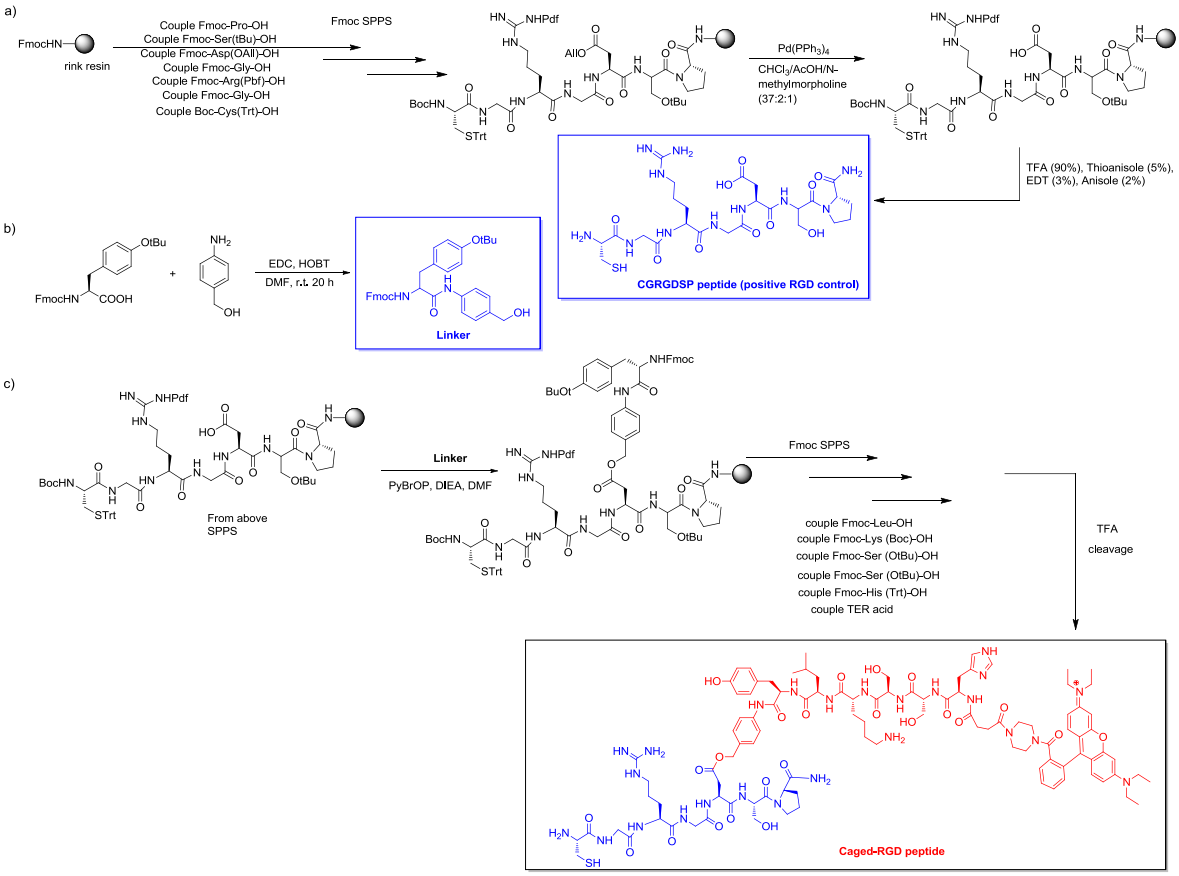
NVOC-TER. Compound **9** (12 mg, 0.05 mmol) dissolved in dry DMF (1 mL) was added EDC•HCl (8.8 mg, 0.046 mmol), HOBt (6.2 mg, 0.046 mmol), DIEA (17 mg, 0.132 mmol) and TER acid[2] (20 mg, 0.033 mmol). The reaction mixture was allowed to stir at room temperature overnight in the dark. The crude product was further purified by prep-HPLC to afford the desired product as a dark red solid (13.4 mg, 49.3%). HRMS: [M$^+$] calcd for $C_{47}H_{55}N_6O_8^+$ 831.4076, found 831.4076.



NVOCL-TER. To a solution of TER-acid (50 mg, 0.082 mmol) in DMF (25 ml), HBTU (37 mg,

0.098 mmol) and HOBt (13.2 mg, 0.098 mmol) was added and stirred at room temperature for 10 min. *tert*-Butyl 3-aminopropylcarbamate[3] (21.4 mg, 0.12 mmol) and DIEA (21 mg, 0.16 mmol) was added and the pH should be kept around 8. The mixture was allowed to stir at room temperature overnight. After the reaction was completed, The reaction solution was transferred to the separation funnel and saturated sodium chloride solution was added. The crude product was extracted with isopropyl alcohol/DCM =2:1 co-solvent. The organic layer was collected, dried over Na_2SO4 and concentrated (keep the temperature below 40℃). The residue was redissolved in chloroform until no more salt precipitate out. Then filtered, collected the filtrate and concentrate again to obtain product Boc-protected **TER-amine** as a dark red solid which was used in the next step without further purification (62.8 mg, 87.6%).

The Boc-protected **TER-amine** (30 mg, 0.039 mmol) was dissolved in HPLC DCM (3 ml), then trifluoroacetic acid (3 ml) was added dropwise and the mixture was stirred at room temperature for 30 min. when the reaction was completed, solvent was removed under reduced pressure to afford free **TER-amine**. Simultaneously, a solution of *N*,*N'*-Carbonyldiimidazole (CDI, 5.84 mg, 0.036 mmol) in dry DCM (0.5 mL) at 0 ^{0}C was added compound **6** (8.6 mg, 0.036 mmol). The resulting mixture was stirred at room temperature for 8 h, **TER-amine** (20 mg, 0.03 mmol) was next added and the solution was stirred at room temperature for another 72 h. The resulting crude product was further purified by prep-HPLC to provide the desired product as a dark red solid (11.3 mg, 40.5%). HRMS: [M^+] calcd for $C_{51}H_{62}N_7O_{10}^+$ 932.4553, found 932.4553.



Scheme S2. Solid-phase synthesis of CGRGDSP peptide and **Caged-RGD peptide**.

CGRGDSP peptide (positive RGD control). As shown in Scheme S2a, Rink amide resin (500 mg, 0.25 mmol) was swelled in DMF for approximately 30 min and then filtered. The resin was then treated with 20% piperidine in DMF for 1 h at room temperature to remove the Fmoc-protective group and washed with DMF for 3 times, DCM for three times, MeOH for three times and DMF for three times. After that, Fmoc-Pro-OH (4.0 eq., 337.37 mg, 1 mmol), and standard coupling reagents, HOBT (4.0 eq., 135.13 mg, 1 mmol), HBTU (4.0 eq., 379.25 mg, 1 mmol) and DIEA (8.0 eq., 330 μL, 2 mmol) were dissolved in 150 mL of DMF. The mixture was added to the resin and the reaction was stirred overnight. The resin was then washed with DMF for 3 times, DCM for three times, MeOH for three times and DMF for three times. Fmoc-Ser-(tBu)-OH, Fmoc-Asp (OAll)-OH, Fmoc-Gly-OH, Fmoc-Arg(pdf)-OH, Fmoc-Gly-OH and Boc-Cys (Trt)-OH were sequentially coupled to the resin similarly. The allyl group was removed using the following procedure. First, the resin was dried at 40 ^{o}C under reduced pressure in a glass vessel sealed with a rubber septum. Next, the vessel was flushed with argon gas before introduction of $Pd(PPh_3)_4$ (3 eq.) and a mixture of $CHCl_3$/AcOH/*N*-methylmorpholine (37:2:1) (15 mL/g of resin). The reaction was continued under Ar for 2 h with occasional gentle agitation. Subsequently, the allyl-deprotected resin was transferred to a sintered glass funnel and washed consecutively with 0.5% DIPEA (in DMF) and sodium diethyldithiocarbomate (0.5% w/w in DMF) to remove the catalyst thoroughly. The resin was further washed with DMF for 3 times, DCM for three times, MeOH for three times and DMF for three times. Finally, the resin was washed with DCM for three times and dried thoroughly under reduced pressure, the cleavage solution (TFA/Thioanisole/EDT/Anisole = 90:5:3:2) was added to the resin and the mixture was stirred at room temperature for 1 h. The resin was filtered off. The solvent was removed *in vacuo*. The synthesized CGRGDSP peptide was purified by preparative HPLC (80% yield). MS (IT-TOF): $[M+H^+]$ calcd for $C_{25}H_{43}N_{11}O_{10}S$ 690.29, found 690.10.

Fmoc-*L*-tyrosyl-4-(hydroxymethyl)anilide (Linker). Fmoc-*L*-tyrosyl-4-(hydroxymethyl)anilide was synthesized using a previously published procedure (Scheme S2b).[4] To a solution of *p*-aminobenzyl alcohol (323 mg, 2.62 mmol) in anhydrous THF (54.5 mL) cooled to 0 ^{o}C, HOBt (354 mg, 2.62 mmol), Fmoc-tyrosine (1 g 2.18 mmol), and EDC•HCl (503 mg, 2.62 mmol) were added. The mixture was stirred at 0 ^{o}C for 2 h and at room temperature overnight. Upon solvent removal, the residue was taken up in ethyl acetate and washed with saturated NH_4Cl, $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash chromatography (EtOAc:Hexane = 1:1) to obtain compound **Linker** as a brown solid (1.11 g, 90.2%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1H), 8.02 (s, 1H), 7.65 (d, *J* = 7.3 Hz, 2H), 7.43 (t, *J* = 8.5 Hz, 2H), 7.28 (t, *J* = 7.1 Hz, 2H), 7.10 (dd, *J* = 62.3, 18.9 Hz, 8H), 6.78 (d, *J* = 8.1 Hz, 2H), 5.81 (s, 1H), 4.55-4.00 (m, 6H), 3.00 (s, 2H), 1.18 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.08, 156.52, 154.24, 143.54 (d, *J* = 25.2 Hz), 141.14, 136.96, 136.46, 131.09, 129.72, 127.66, 127.49, 127.03, 124.96, 124.21, 120.23, 119.88, 78.36, 67.32, 64.49, 57.16, 46.84, 38.23, 28.66. MS (IT-TOF): $[M+H^+]$ calcd for $C_{35}H_{36}N_2O_5$ 565.26; found, 565.26.

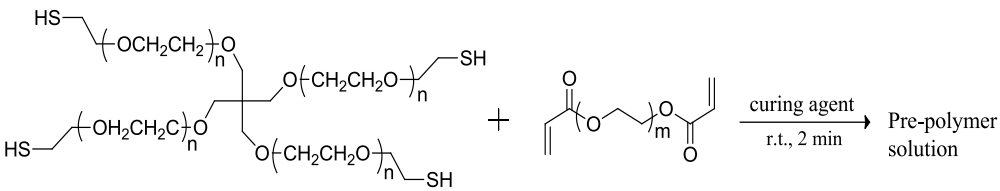
Caged-RGD peptide. Scheme S2c illustrates the synthesis of **Caged-RGD peptide**, from the above ally-deprotected resin (100 mg, with loading ~0.6 mmol/g). Upon DMF washes of the resin, a mixture of compound **Linker** (4 eq.), PyBOP (4 eq.) and DIEA (8 eq.) dissolved in DMF (10 mL) and pre-activated for 20 min, was added. The reaction was shaken at room temperature overnight. The resin was washed with DMF for 3 times, DCM for three times, MeOH for three times and DMF for three times. Next, the resin was treated with 20% piperidine in DMF for 1 h at room temperature to remove the Fmoc-protective group. The resin was then washed with DMF for 3 times, DCM for three times, MeOH for three times and DMF for three times. After that, Fmoc-Leu-OH (4 eq.) pre-activated with HOBT (4

equiv.), HBTU (4 equiv.) and DIEA (8 equiv.) in DMF for 10 min, was added. The coupling was continued at room temperature overnight. The cycle of Fmoc deprotection/coupling was repeated with Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-His(Trt)-OH. Finally, the resin was coupled with TER acid (2 eq.) similarly at room temperature with continuous shaking for 2 d. After washing with DMF for 3 times, DCM for three times, MeOH for three times and DCM for three times and dried thoroughly under reduced pressure, the resin was cleaved (TFA/Thioanisole/EDT/Anisole = 90:5:3:2) at room temperature for 1 h. The resin was filtered off. The solvent was removed *in vacuo*. The synthesized **Caged-RGD peptide** was purified by preparative HPLC. MS (IT-TOF): $[M+H^+]$ calcd for $C_{101}H_{140}N_{25}O_{23}S^+$ 2103, 1/2 $[M+H]^+$ 1051.5, found, 1052.

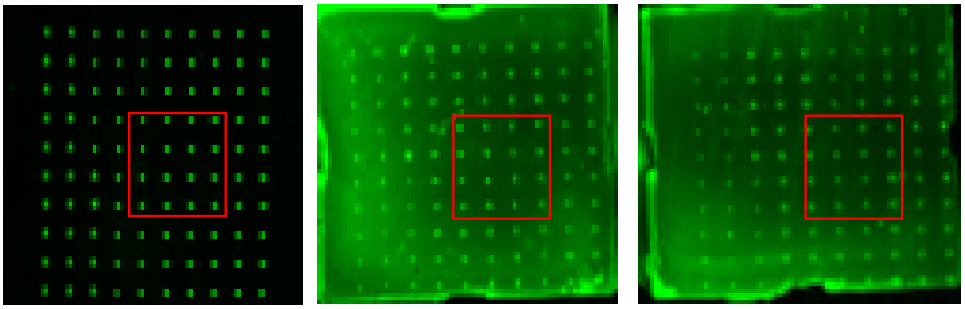
3. SMM and Hydrogel-Functionalized SMM Preparations.

The compound spotting and SMM fabrication were carried out based on previously published protocols with necessary changes.[5] Briefly, a mixture of **Caged-RGD peptide** (5 mM) and **NVOC-CPT** (10 μM) were dissolved in a DMSO/H_2O cosolvent (1:1) and directly printed on standard glass slides (10 x 10 grids), with a spotting diameter and spot-to-spot spacing of 500 ± 5 μm and 1500 ± 10 μm, respectively. The spotting pattern was shown in Fig. 2a, and positive control spots where the **Positive RGD control** (CGRGDSP peptide) was used in place of **Caged-RGD peptide**, were spotted on alternating rows, where applicable, **NVOC-TER** and **NVOCL-TER** were also spotted (final concentration: 20 μM) directly as positive control dye spots, or co-spotted with the the non-fluorescent peptide (i.e. CGRGDSP). The resulting SMM prior to hydrogel functionalization was imaged on a Typhoon 9410 fluorescence scanner (GE Amersham) to ensure the quality of the resulting SMM. Representative microarray images of these SMMs are shown in Fig. 2a (left) and Fig. S1 (left).

Next, the above SMM was hydrogel-functionalized based on a previously published protocol by Hawker et al.[6] First, a pre-polymer solution containing 10% (m/v) 4-arm-PEG-SH (dissolved in deionized water at a concentration of 0.1 g/mL), PEGDA700, deionized water, 10% ammonium persulfate solution (APS, aq.) and N,N,N,N,-Tetramethyl-ethylenediamine (TEMED) at a ratio of 125:62.5:62.5:12.5:1, was prepared (Scheme S3). The pre-polymer solution was then slowly poured on top of the SMM covered with a gel mold. The hydrogel formed within 2 min at room temperature. To ensure the quality of the hydrogel-functioanlized SMM, it was imaged again on the Typhoon 9410 fluorescence scanner (Fig. 2a central image, and Fig. S1b); no obvious spot diffusion or enlargement was observed, indicating successful transfer/immobilization of spotted small molecules/peptides of the hydrogel-functionalized SMM. After the compound mixtures were transferred from glass slides to hydrogels, nearly no decrease/variation in the fluorescence intensity of spots was observed (RFU of compound spots' on glass slides: 35.5 ± 4.0; RFU of compound spots' after washing: 35.4 ± 6.3). To grow mammalian cells on these hydrogel-functionalized SMMs, they were first immersed in a 10% P/S (100 units/mL penicillin/streptomycin) solution in PBS overnight, and then immersed in 1x PBS (sterilized) overnight before being using for cell culture. After washing, the fluorescence intensity didn't decrease much (RFU: 31.7 ± 5.1). During the hrdrogel preparation processes, the spot size didn't change much as well (500 ± 10 μm after washing). Figure S1c shows a representative image of the same hydrogel-functionalized SMM (from Fig. 1a/b) upon extensive washes with water and PBS overnight. No significant changes in fluorescent spots and intensities were detected, indicating again complete immobilization of small molecules/peptides on the hydrogel-functionalized SMM.



Scheme S3. Sketch of hydrogel preparation by using thiol-ene reaction. The terminal cysteine of **Caged-RGD peptide** and the terminal alkene handle of **NVOC-CPT** were used as parts of the handles needed in the thiol-ene reaction, and to attach these compounds covalently to hydrogel polymer matrix.



a) b) c)

Figure S1. Fluorescence images of a representative SMM a) prior to hydrogel functionalization, b) after hydrogel-functionalization, and 3) after overnight washes with H_2O and PBS. For spotting pattern, see Fig. 2a in the maintext.

The physical properties especially the surface properties of the hydrogel were characterized by Fourier-transformed infrared (FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS), scanning electron microscope (SEM) as well as energy dispersive spectroscopy (EDX) as shown in Fig. S2. The main molecular structures of hydrogel are O-H, C-H, S-H, C-C, C-O and C-S bonds. Based on the FT-IR spectrum, all characteristic peaks that belong to caged-RGD peptide were observed: C-O (C-S) bond was observed at 1730 cm $^{-1}$, O-H was at 3500 cm $^{-1}$ and C-H vibration peaks was at 2931 and 2864 cm $^{-1}$. The specific bands belonging to the functionalized hydrogel including C-N (1100cm^{-1}), N-H (3420 cm^{-1}, 947 cm^{-1}) were also found. In addition, the peak of the specific amide bond of the peptide at 1640 cm $^{-1}$ was observed only in the functionalized hydrogels, indicating the successful incorporation of the peptides in the hydrogels.[7] XPS analyzed the binding energy of the specific chemical bonds of functionalized hydrogel structures, mainly including the C-N band, C-C, C-O and C-S bands. SEM shows the surface information of the hydrogel and EDX gives the surface element contents of the hydrogels. All these data proved the successful formation of the small molecule-peptide contained hydrogels.

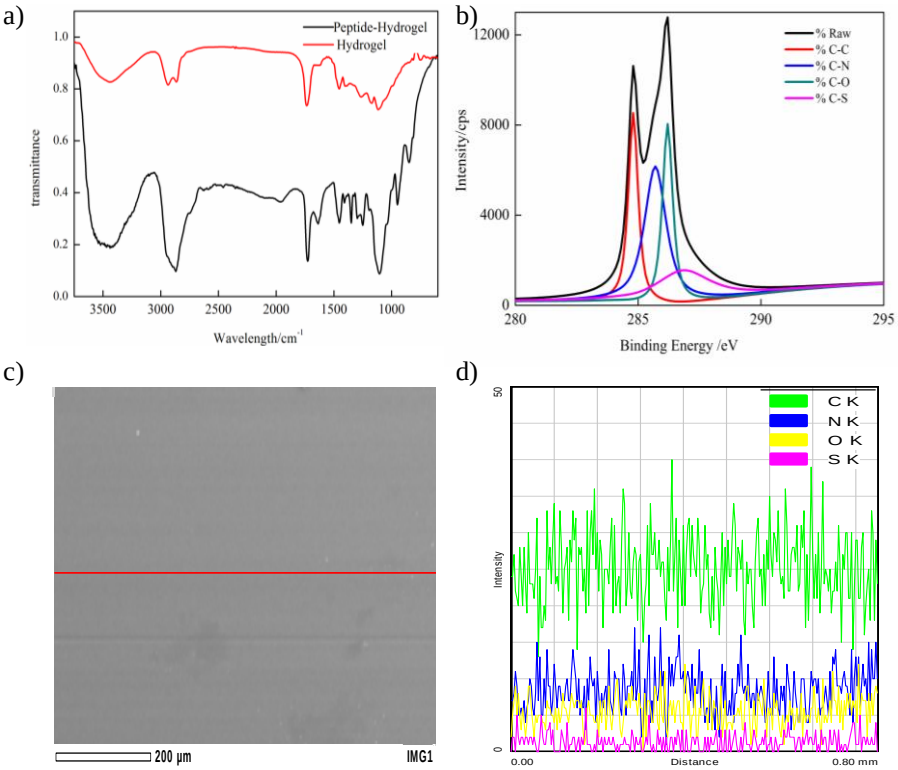
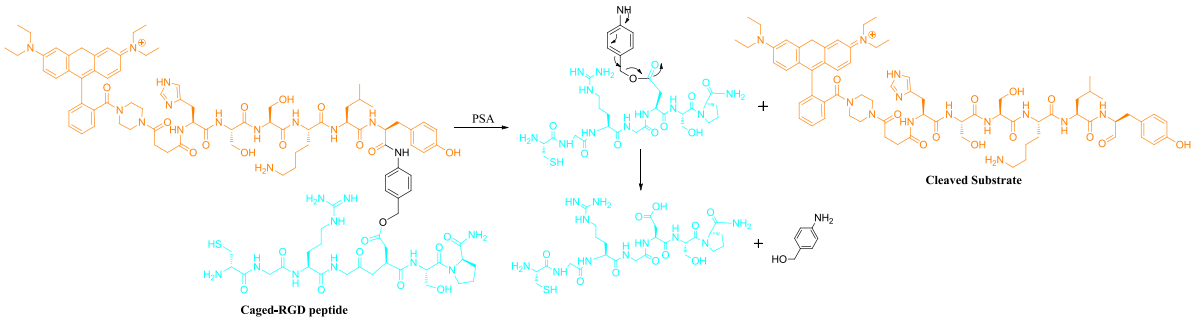


Figure S2. Physical parameter determination of the functionalized hydrogels. a) FT-IR; b) XPS; c) SEM; d) EDX.

4. Other Assays and Biology.

The cleavage efficiency of the **Caged-RGD peptide** by PSA protease was determined *in vitro* as shown below, and summarized in Fig. 3a in the maintext. The peptide (1 mg/ml) was incubated with PSA protease (0.1 mg/mL) in 1×PBS (*p*H 7.4) for 4 h at 37 °C and the cleavage processes was monitored by using LC-MS at different time points (0, 0.5, 1, 2, 4 h; see Fig. S3).



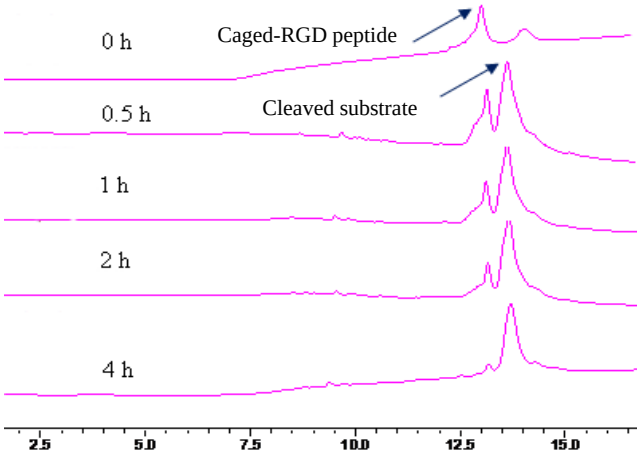


Figure S3. (A) Schematic showing PSA-catalyzed enzymatic cleavage of **Caged-RGD peptide** (20 μM in PBS), and subsequent spontaneous fragmentation of **Linker** followed by release of the fully functional RGD peptide. (B) Time-dependent HPLC profiles to monitor PSA cleavage of **Caged-RGD peptide**. Recombinant PSA protease (Sigma) was used and the reaction was done in PBS buffer (*p*H 7.4) over a course of 4 h. The cleaved, fully functional RGD peptide was confirmed by MS.

Functionalized hydrogels, as earlier described, were placed in 6-well cell culture plates and immersed in phosphate buffered saline (1× PBS) containing 1% penicillin-streptomycin overnight at room temperature. Different cell lines (PC-3, CHO-9, NIH3T3 and HeLa) were seeded at 30K cells cm^{-2} on the hydrogels using serum-free DMEM medium with 1% penicillin-streptomycin. After 3 h, the medium was removed gently and replaced with normal DMEM growth medium. After three days of culturing, the cells grew as a round shape cluster (Fig. S4). After cell grew full on the peptide containing spots, the total live cell count was summarized also in Table S1. The fluorescent spots indicate the position of peptides, which were shown as an array like SMM array on glass slides. The resulting spot diameters were around 0.5 mm (close to that expected for hemispherical spots), and the center-to-center distance between two spots was around 1.5 mm. As shown in Fig. S5, after one day culturing on hydrogels, only PC-3 cells grew on the spots containing **Caged-RGD peptide** or CGRGDSP peptide, other types of cells grew on spots containing CGRGDSP peptide, but grew only sparingly on **Caged-RGD peptide**. Only PC-3 cells were known to overexpress and secrete PSA protease, thus were capable of cleaving **Caged-RGD peptide** to restore its activity. The cell type-specific growth results were also summarized in Table S1. Subsequently, the SMM was visualized upon cell staining with Hochest nuclear stain. Three channels were employed including BF: (bright-field), PEP (to detect fluorescence peptides/dyes; in red), and NU (to detect nuclear stained mammalian cells; in blue). Images were processed with Photoshop CS6, and merged where shown (Fig. S5 & Fig. 2b).

Table S1. Summary of cell growth/adhesion on the hydrogel-functionalized SMMs with different types of mammalian cells.

Peptide / Cell lines (total live cell count)	**Positive RGD control** (CGRGDSP peptide)	**Caged-RGD peptide**
PC-3 (prostate cancer cell line)	++++ (300 ± 50 cells)	++++ (310 ± 50 cells)

HeLa (cancer cell line)	++++ (260 ± 50 cells)	+ (0)
NIH 3T3 (normal cell line)	++++ (330 ± 50 cells)	+ (0)
CHO-9 (normal cell line)	++++ (280 ± 50 cells)	+ (0)

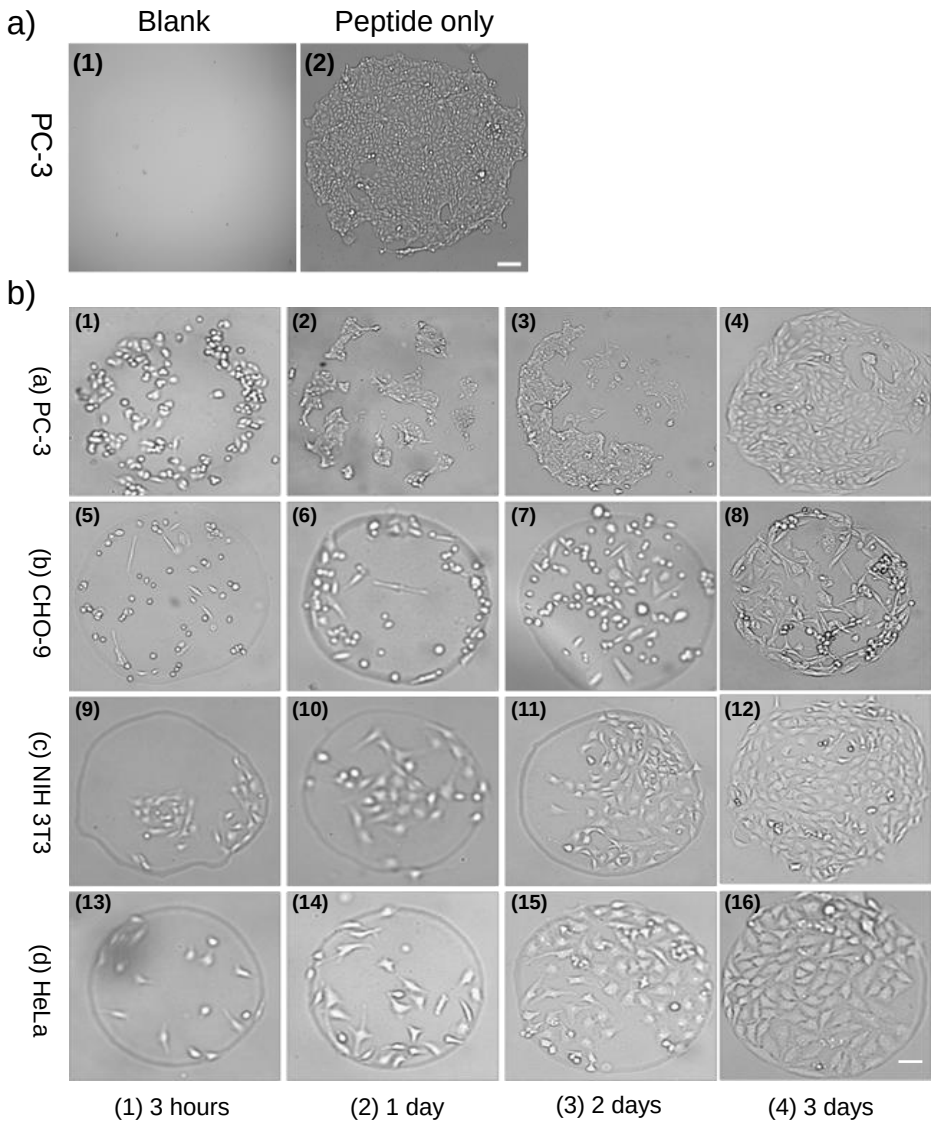


Figure S4. a) Control images of cell growth on functionalized hydrogels. Panel 1: Hydrogel surface only, without cell seeding; panel 2: cell grew on hydrogels which were only modified with CGRGDSP peptide. Here PC-3 was used as model cell line. b) Time-dependent cell growth/adhesion on hydrogel-functionalized SMMs spotted with the **Positive RGD control** (CGRGDSP peptide). Panels (1~4): PC-3 cells; panels (5~8): CHO-9 cells; panels (9~12): NIH 3T3 cells; panels (13~16): HeLa cells. In the first 3 h, the cells were cultured in 10% FBS-free DMEM medium. After that, the medium was changed to normal DMEM growth medium. All cells were visualized under DIC (bright-field). Scale Bar = 50 µm.

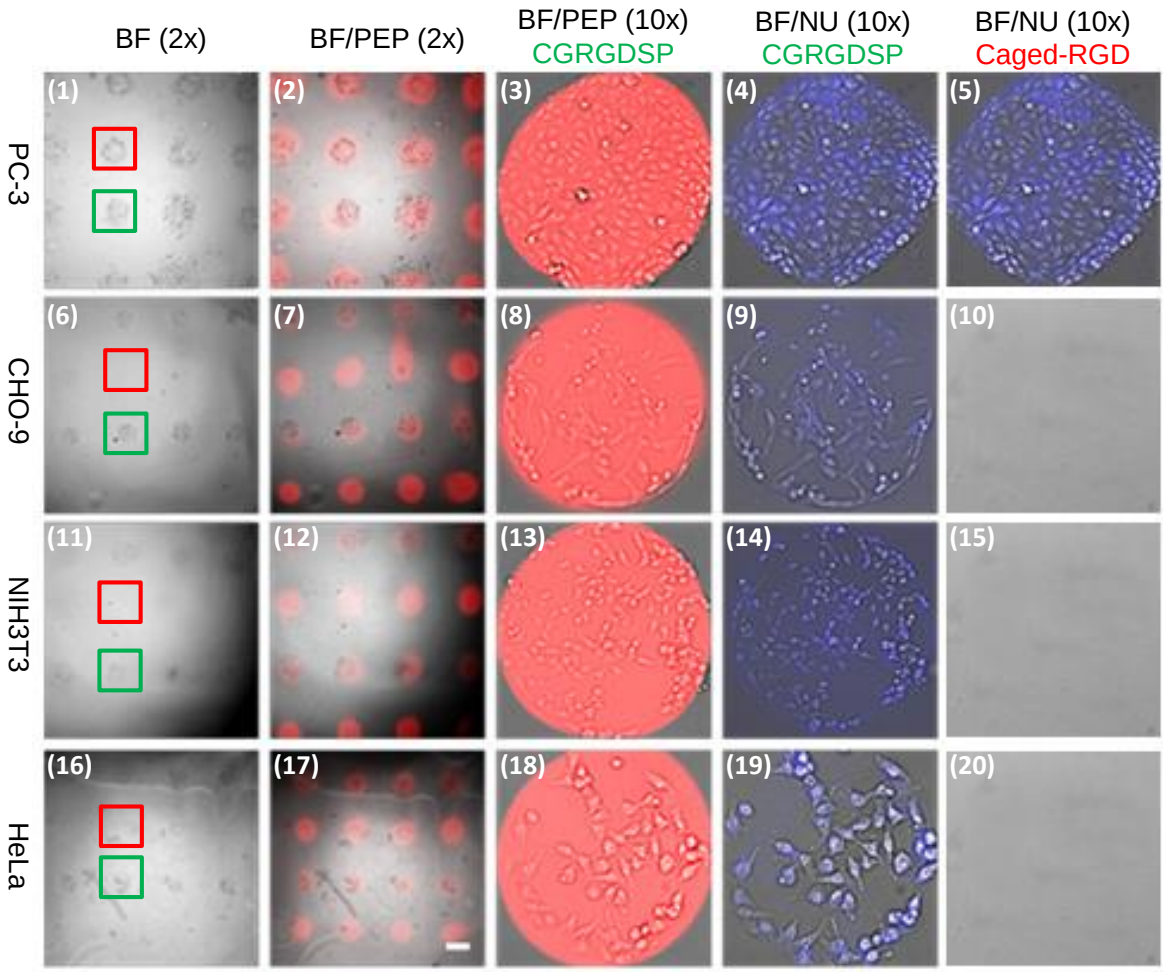


Figure S5. Fluorescence microarray images of different cell lines (PC-3. CHO-9, NIH 3T3 and HeLa) grown on a 10 x 10 grid SMM spotted with **NVOC-CPT**/**Caged-RGD peptide** and **NVOC-CPT**/CGRGDSP peptide on alternating rows. The fluorescence was either from TER of the fluorescently labelled peptide, or co-spotting with **NVOC-TER/NVOCL-TER**. Upon one day growth of different mammalian cells on the hydrogel-functionalized SMM, the arrays were imaged using fluorescence microscopy (at 2x & 10x magnifications). The red boxes highlighted the cells grown in **Caged-RGD peptide** modified regions, while the green boxes highlighted the cells grown in **CGRGDSP peptide** (positive control peptide) modified regions, the magnifications of cell images were shown also (the three columns of 10x magnification images at right, titled in red and green color respectively) The images were acquired under BF (bright-field), PEP (fluorescent peptide; in red) and NU (Hochest nuclear stain; in blue) channels, and merged where indicated. Scale bar: 500 μm.

To ensure the success of the light-induced release of Camptothecin (CPT), a solution-phase test was applied. The photo-labile small molecule **NVOC-CPT** was dissolved in PBS buffer (20 μM, 20 μL) and irradiated with 1000 μJ/cm^2 UV exposure, then a UV time-dependent experiment was carried out (0, 10, 20, 30, 40, 40, 50, 60 min). During the whole irradiation processes, the reaction solution was cooled using ice. The recovered fluorescence intensity of the reaction solution was detected using Tecan microplate reader (Multimode Reader, Infinite®200; λ_{ex} = 350 nm, λ_{em} = 442 nm) (Fig. 3a). **NVOC-AQ** was next used for on-chip UV-induced compound release experiments, due to its more favorable fluorescence properties. Upon SMM fabrication and hydrogel functionalization as earlier described, with

different spotting concentrations of **NVOC-AQ** (0 to 100 μM), UV irradiation (1000 μJ/cm^2, ice cooling) was initiated for indicated periods of time. The on-gel fluorescence signals (showing as fluorescent spots on hydrogels) at different UV time points and **NVOC-AQ** concentrations were captured by In-Cell Analyzer 2200, using DAPI channel (excitation filter: 390 ± 18 nm; emission filter: 432.5 ± 48 nm) to quantify the fluorescent spots. The data was processed by the software ImageJ to measure the exact fluorescence intensity and plotted graphically (Fig. 3b). The fluorescence image was also shown in Figure S6. Based on the image, it can be confirmed that even after UV irradiation, the spot size became slightly bigger, but there were still enough space between two spots, which helped avoid passive diffusion effect between neighbouring spots.

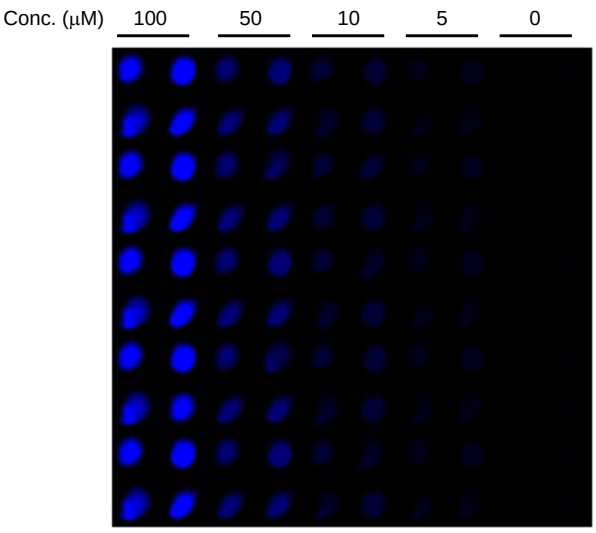


Figure S6. Fluorescence images of on-gel drug release assay after 1 h UV irradiation. Most of the fluorescence is released. At gradual drug concentrations, the fluorescence intensity increases gradually.

In order to confirm whether the released CPT was taken up by mammalian cells, an *in vitro* experiment was performed. Cells (PC-3, with HeLa as controls) were grown for three days on hydrogel-functionalized SMMs containing either the positive control peptide (CGRGDSP) or **Caged-RGD peptide** (5 mM), and co-spotted with **NVOC-CPT** (0 to 100 μM; NC = no compound). After photo-release (1000 μJ/cm^2, ice cooling) for 20 min, the cells were further incubated at 37 ^{o}C overnight. Subsequently, cells were stained with Hochest stain and microscopically visualized at different magnifications. Morphological changes of cells as a result of drug uptakes were apparent for PC-3 at this stage (see Fig. 3c), and much more with the HeLa cells (see Fig. S7a); by comparing panels 1 and 5. One could easily detect significant changes in HeLa cell morphology - cells became round and some were even dead. Cellular effects due to drug uptakes were further confirmed by collection of affected cells, lysis followed by fluorescence analysis of the resulting cell lystes (Fig. 3d & S7b) and Western blotting analysis (Fig. 3e & S7c). The fluorescence intensity of the total cell lysates were recorded using the Tecan microplate reader (λ_{ex} = 350 nm, λ_{em} = 442 nm). To confirm cell apoptosis caused by released CPT, the cleaved Caspases-3 expression levels of affected cells were determined. WB experiments were carried out by referring to the general procedure using anti-cleaved Caspase 3 (1: 1000). The molecular weight of target protein cleaved-Caspase 3 is around 17 kDa. The band intensity was determined by Image J. Before UV irradiation, there was nearly no caspase-3 activity, however,

after UV irradiation, with the increase of immobilized **NVOC-CPT** concentration, the expression level of cleaved Caspase-3 increased as well, and reached balance at the concentration of 50 μM. These results further indicate that the UV-induced release of CPT was successful; the free CPT went into the cells and cause cell apoptosis.

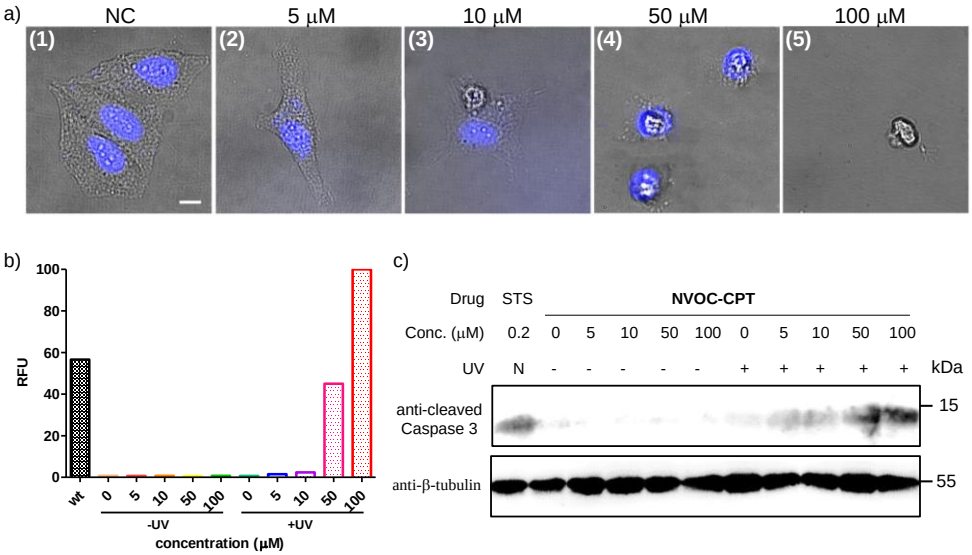
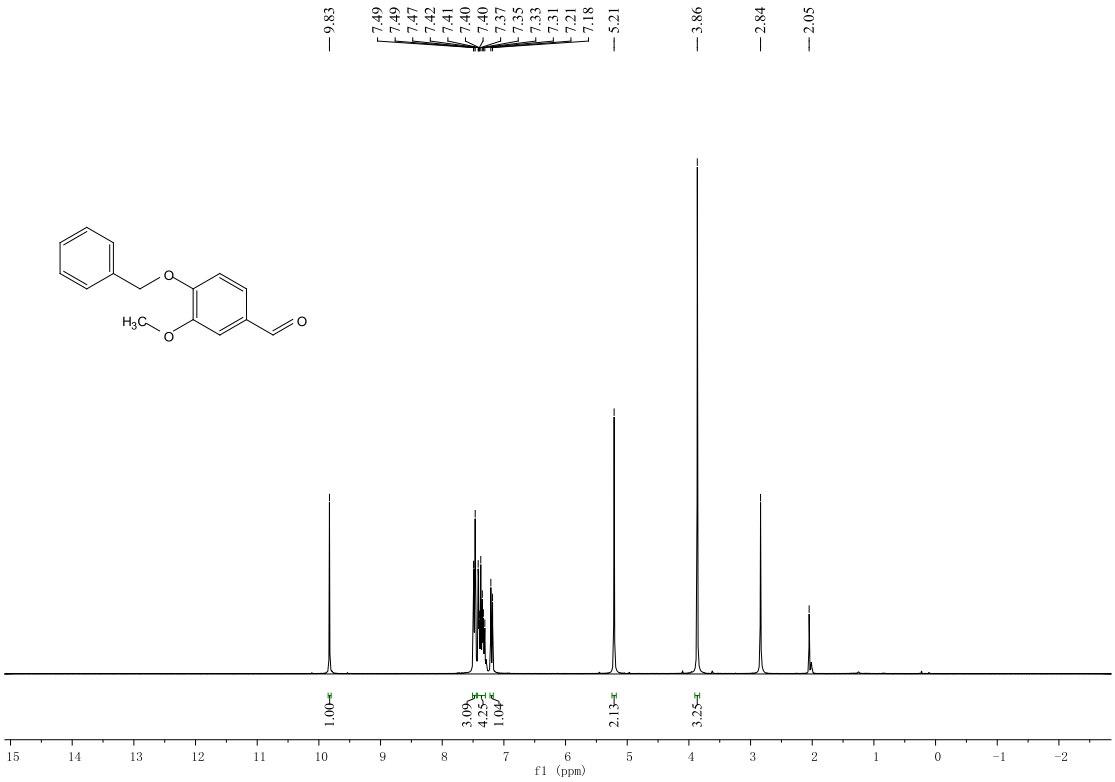
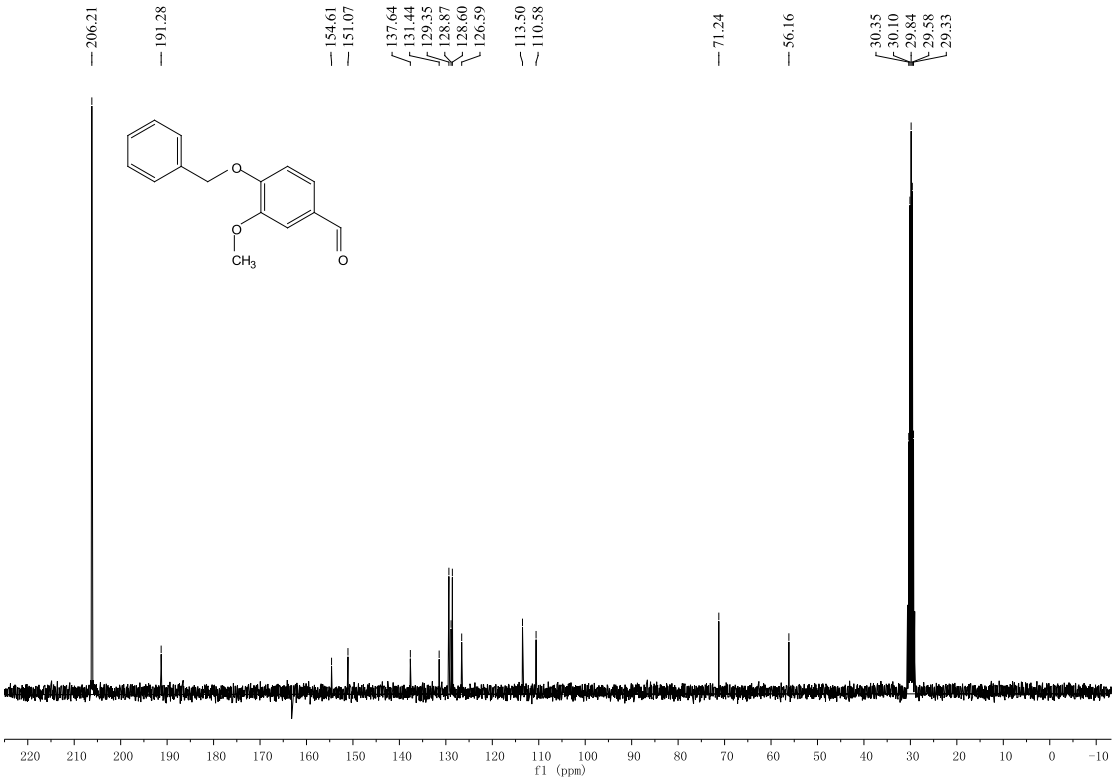


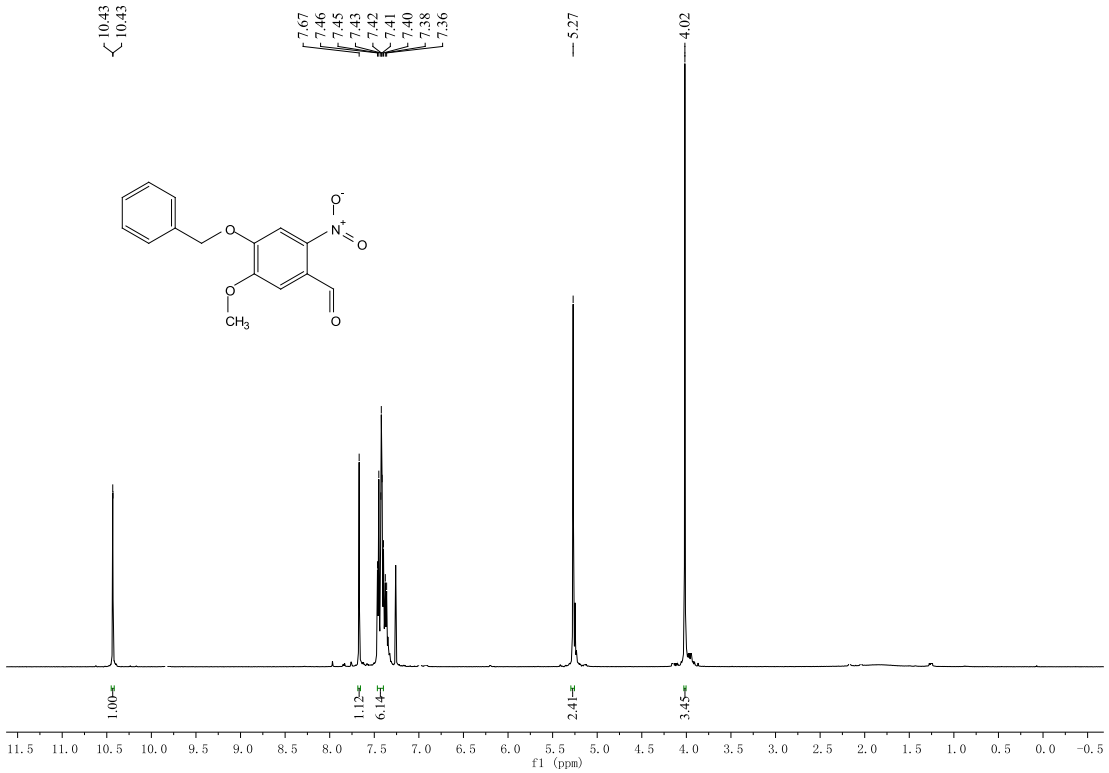
Figure S7. a) Apoptosis effect caused by released CPT on HeLa cells grown on positive control peptide (**CGRGDSP peptide**) after overnight incubation in 37 ^{o}C, Panels (1~5): cells' morphology change at different concentrations of immobilized CPT. NC: Negative Control (no CPT): cells' morphology before UV irradiation. Scale bar: 10 μM. b) Relative fluorescence intensity of lysates of HeLa cells obtained from (a). c) Western Blot determination of cleaved Caspase-3 expression level of HeLa cells after UV-induced CPT release and subsequent overnight incubation in 37 ^{o}C incubator before cell harvest and lysis.

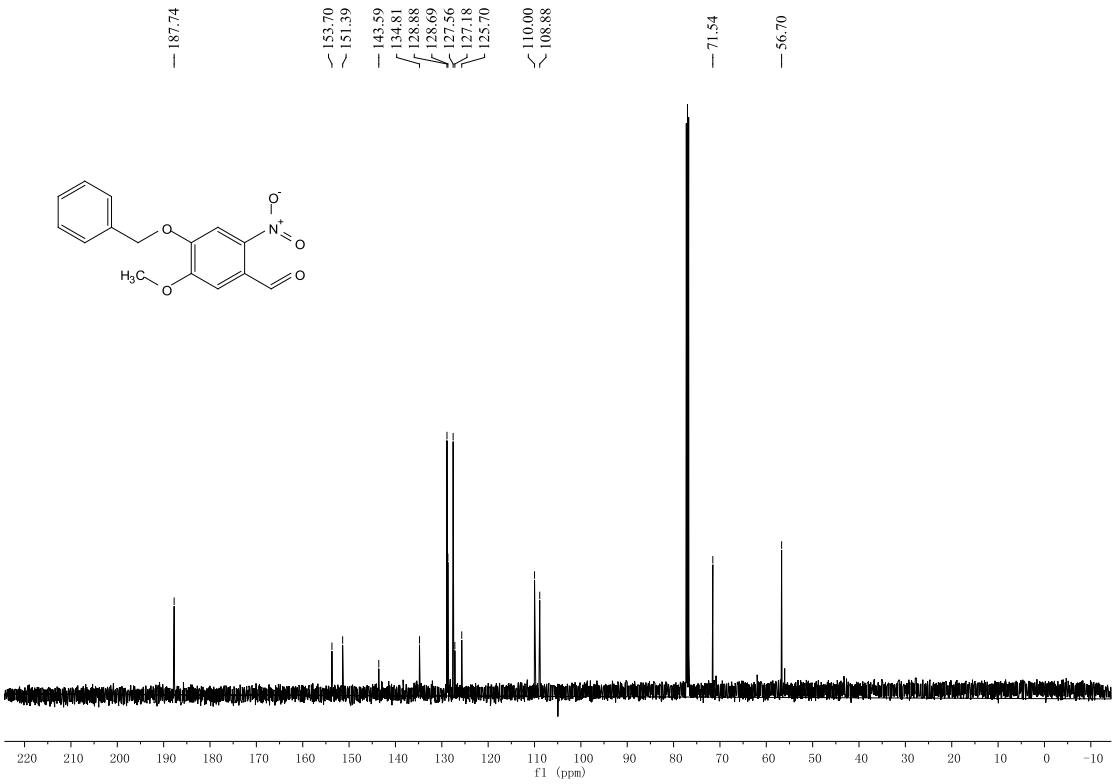
References:

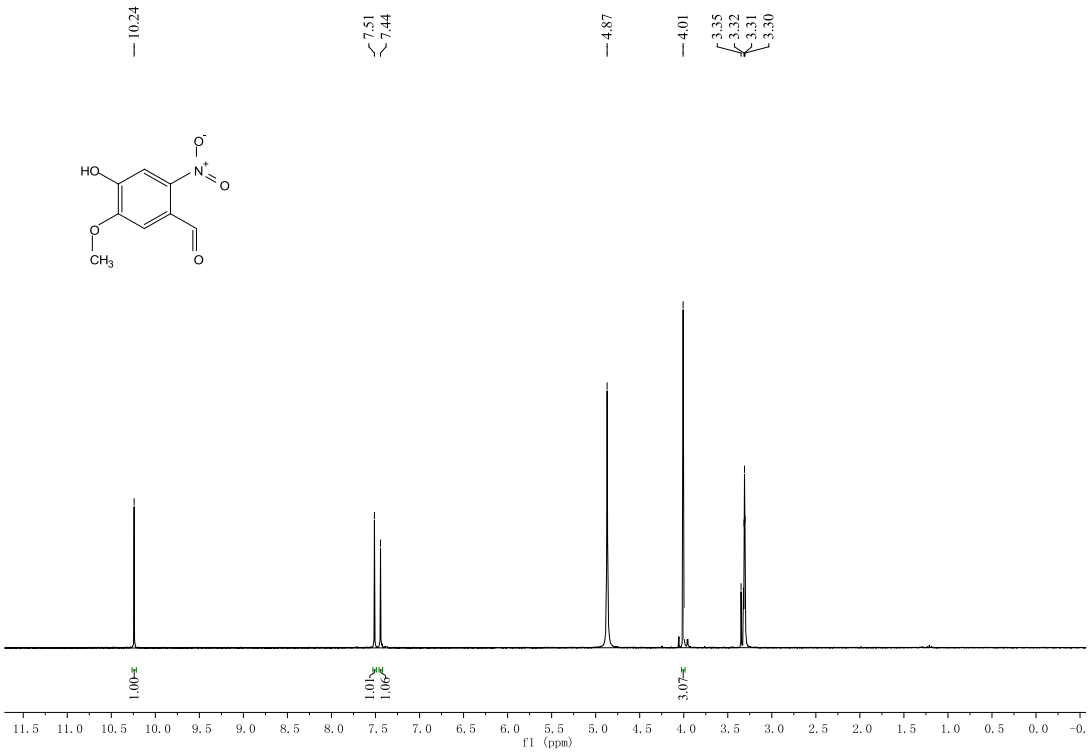
1. N. Pessah; M. Reznik; M. Shamis; F. Yantiri; H. Xin; K. Bowdish; N. Shomron; G. Astb and D. Shabata., *Bioorg. Med. Chem*,. 2004, **12,** 1859.
2. M. B. Francis, T. Nguyen, *Org. Lett*., 2003, **5**, 3245.
3. M. Hu; L. Li; H. Wu; Y. Su; P.Y. Yang; M. Uttamchandani; Q. H. Xu; and S. Q. Yao., *J. Am. Chem. Soc.*, 2011, **133**, 12009.
4. G. B. Jones; C. F. Crasto; J. E. Mathews; L. F. Xie; M. O. Mitchell; A. E. Shafey; A. V. Damico; G. J. Bubley., *Bioorgan Med Chem*, 2006, **14**, 418.
5. H. Wu; J. Ge; P. Yang; M. Uttamchandani. and S. Q. Yao., *J. Am. Chem. Soc*., 2011, **133,** 1946.
6. N. Gupta, B. F. Lin, L. M. Campos, M. D. Dimitriou, S. T. Hikita, N. D. Treat, M. V. Tirrell, D. O. Clegg, E. J. Kramer and C. J. Hawker, *Nat. Chem.* 2010, **2**, 138.
7. K. Liang; J. J. Richardson; H. Ejima; G. K. Such; J. W. Cui; F. Caruso., *Adv. Mater.*, 2014, **26,** 2398.

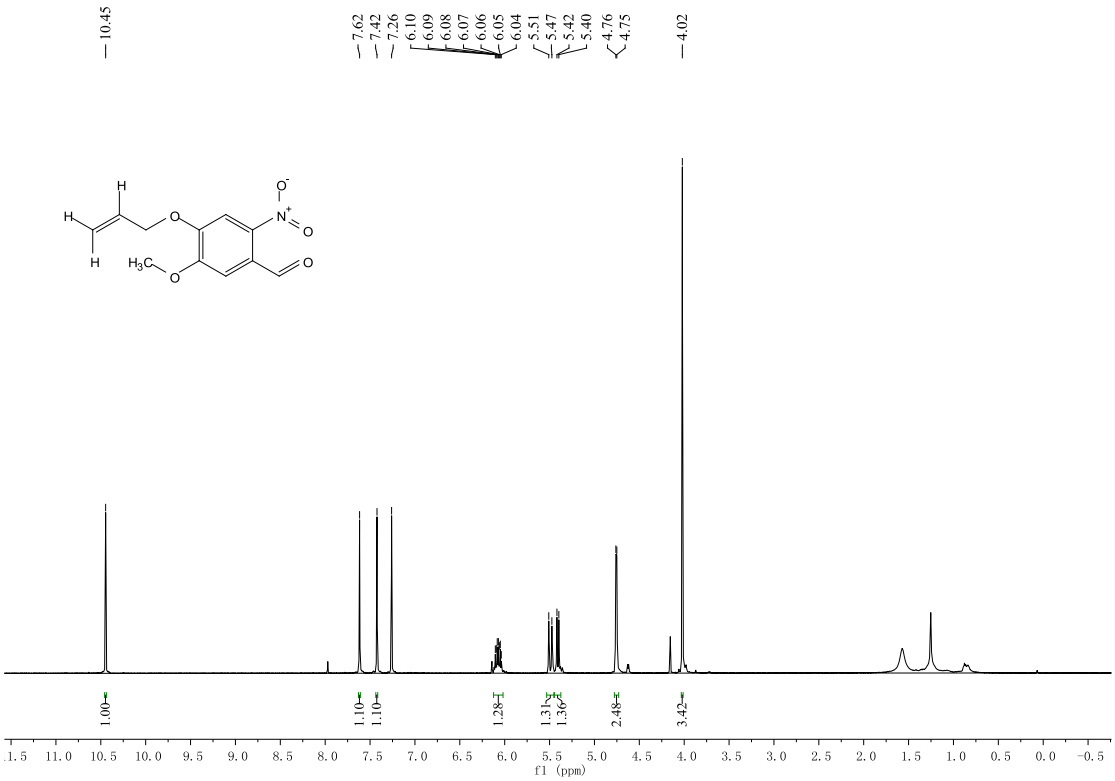

9.83
7.49
7.49
7.47
7.42
7.41
7.40
7.40
7.37
7.35
7.33
7.31
7.21
7.18
5.21
3.86
2.84
2.05
1.00
3.09
4.25
1.04
2.13
3.25
f1 (ppm)

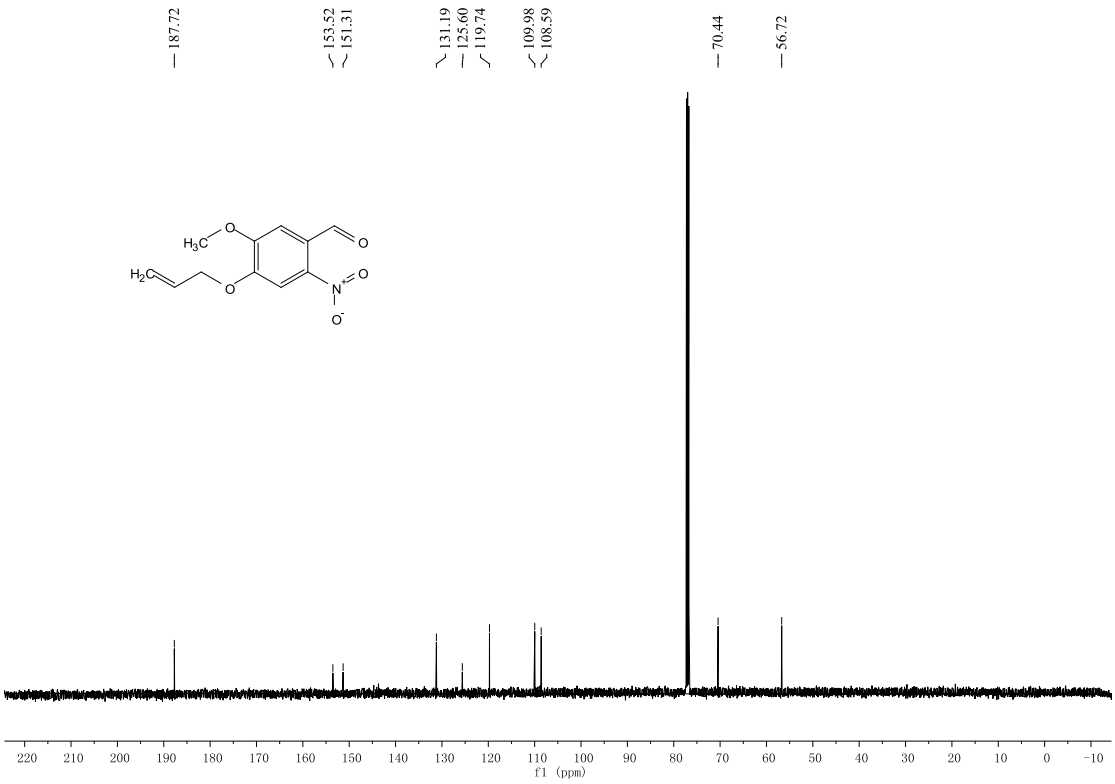

206.21
191.28
154.61
151.07
137.64
131.44
129.35
128.87
128.60
126.59
113.50
110.58
71.24
56.16
30.35
30.10
29.84
29.58
29.33
f1 (ppm)

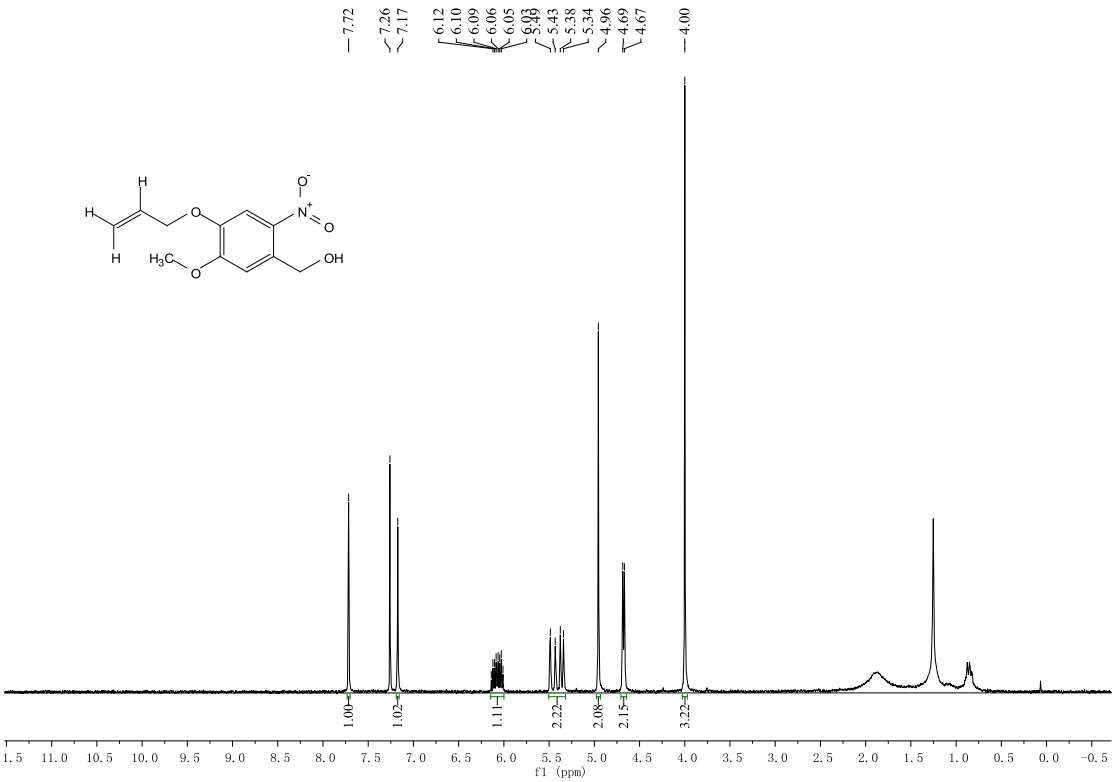

10.43
10.43
7.67
7.46
7.45
7.43
7.42
7.41
7.40
7.38
7.36
5.27
4.02
1.00
1.12
6.14
2.41
3.45
f1 (ppm)

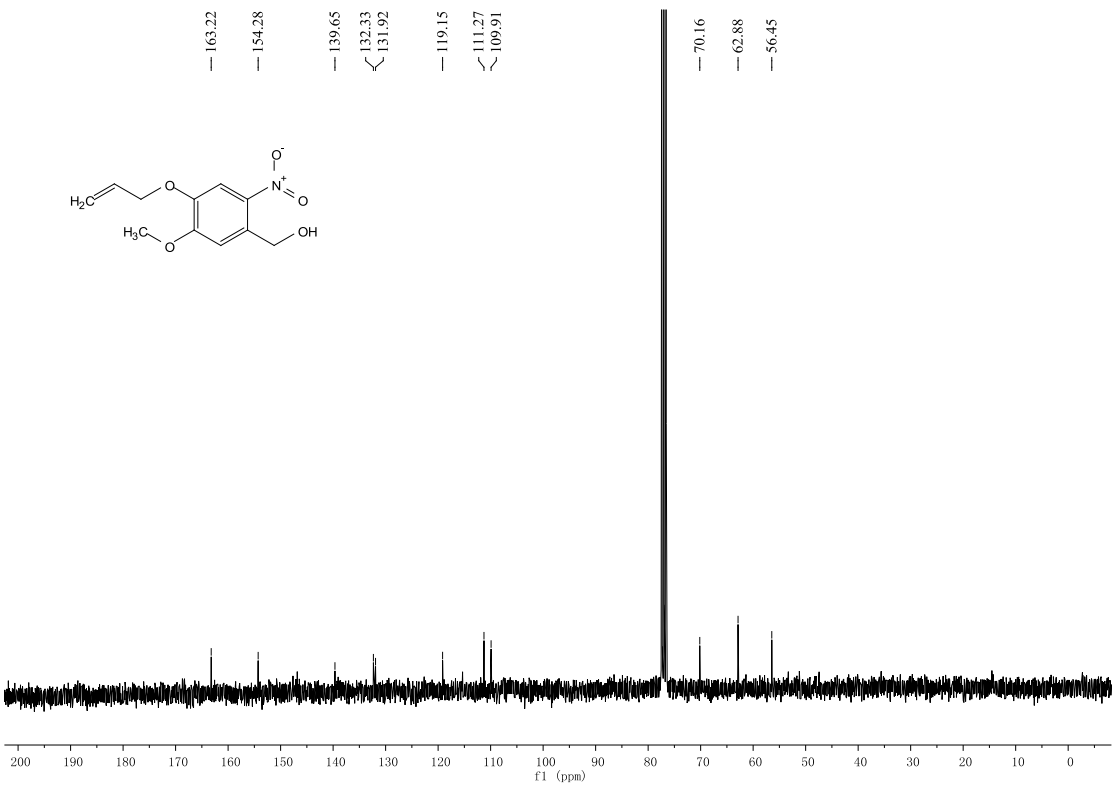

187.74
153.70
151.39
143.59
134.81
128.88
128.69
127.56
127.18
125.70
110.00
108.88
71.54
56.70
f1 (ppm)

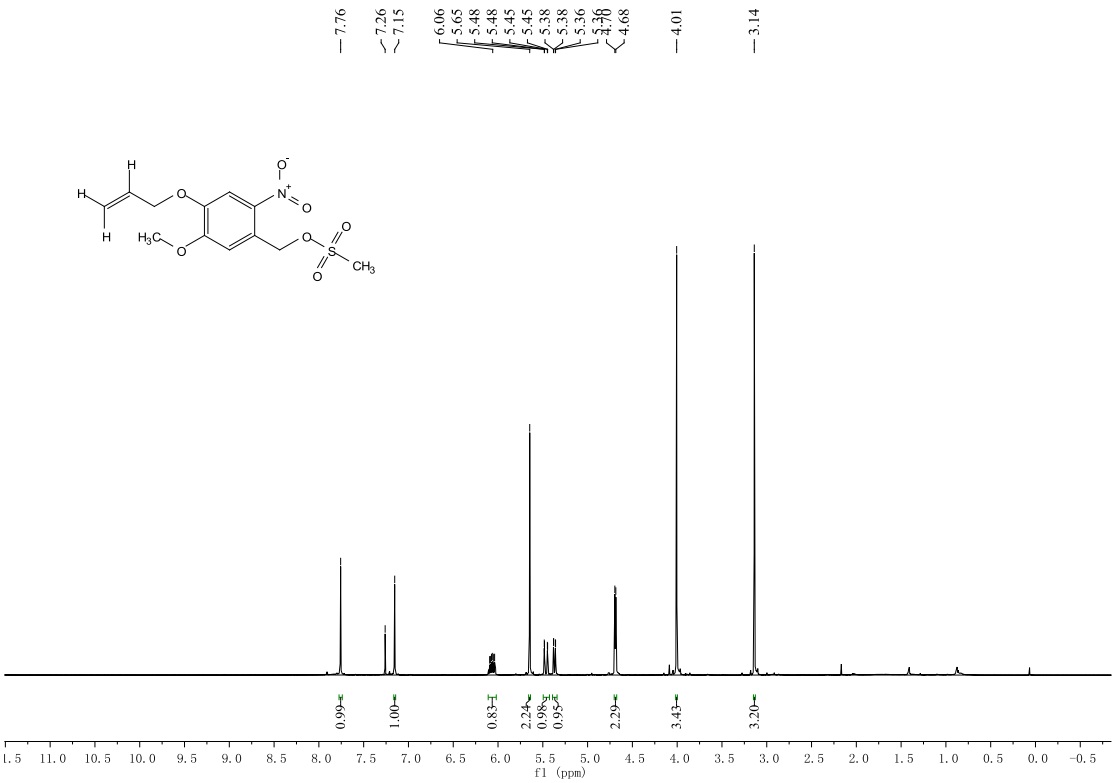

10.24
7.51
7.44
4.87
4.01
3.35
3.32
3.31
3.30
HO
O
N
O
O
CH3
O
1.00
1.01
1.06
3.07
f1 (ppm)

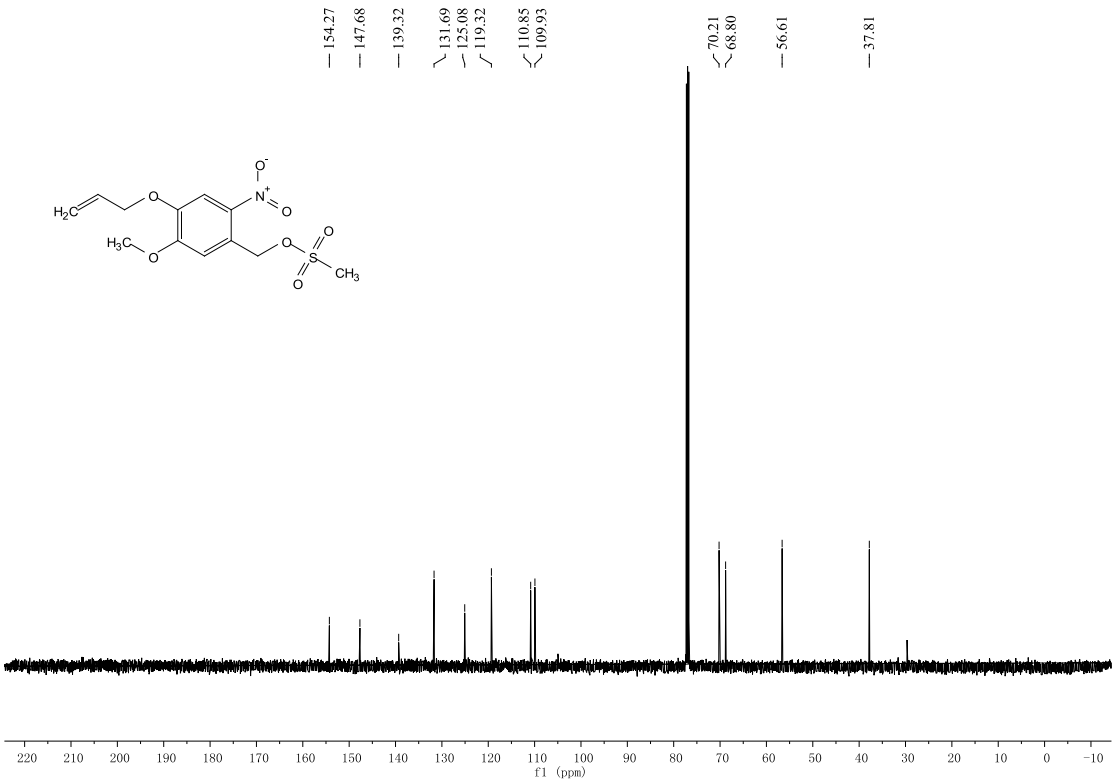

10.45
7.62
7.42
7.26
6.10
6.09
6.08
6.07
6.06
6.05
6.04
5.51
5.47
5.42
5.40
4.76
4.75
4.02
H
H
H
O
O
N
O
H3C
O
O
1.00
1.10
1.10
1.28
1.31
1.36
2.48
3.42
f1 (ppm)

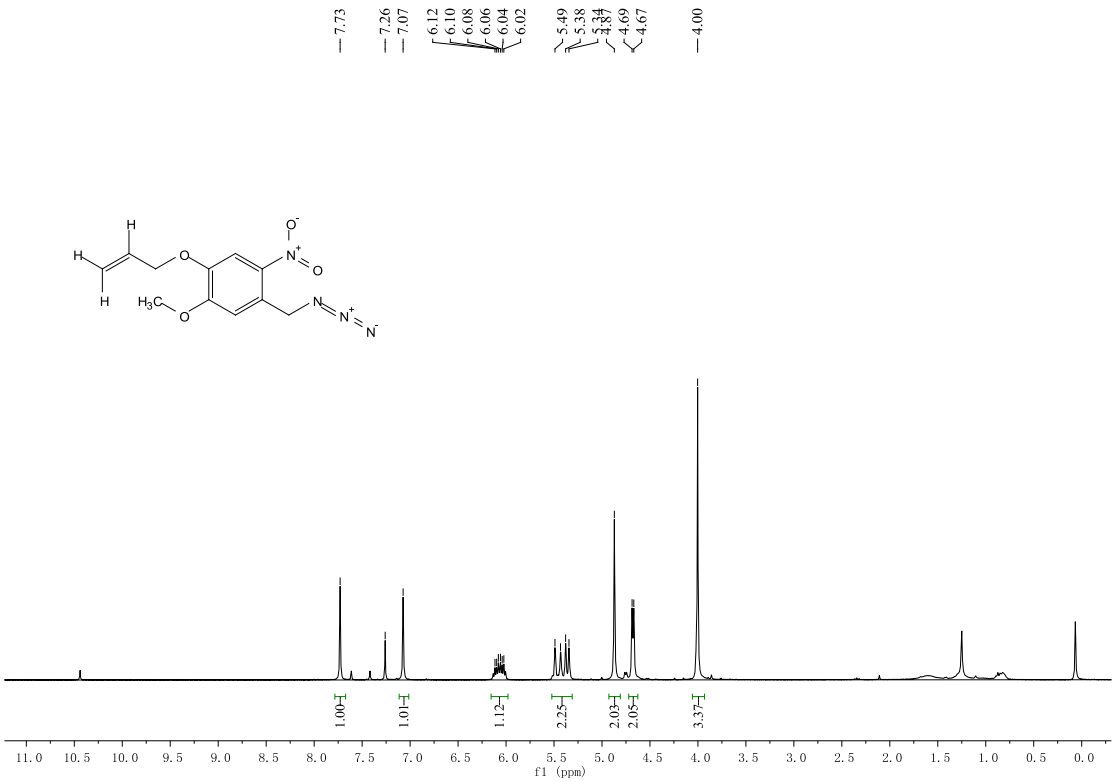

187.72
153.52
151.31
131.19
125.60
119.74
109.98
108.59
70.44
56.72
f1 (ppm)

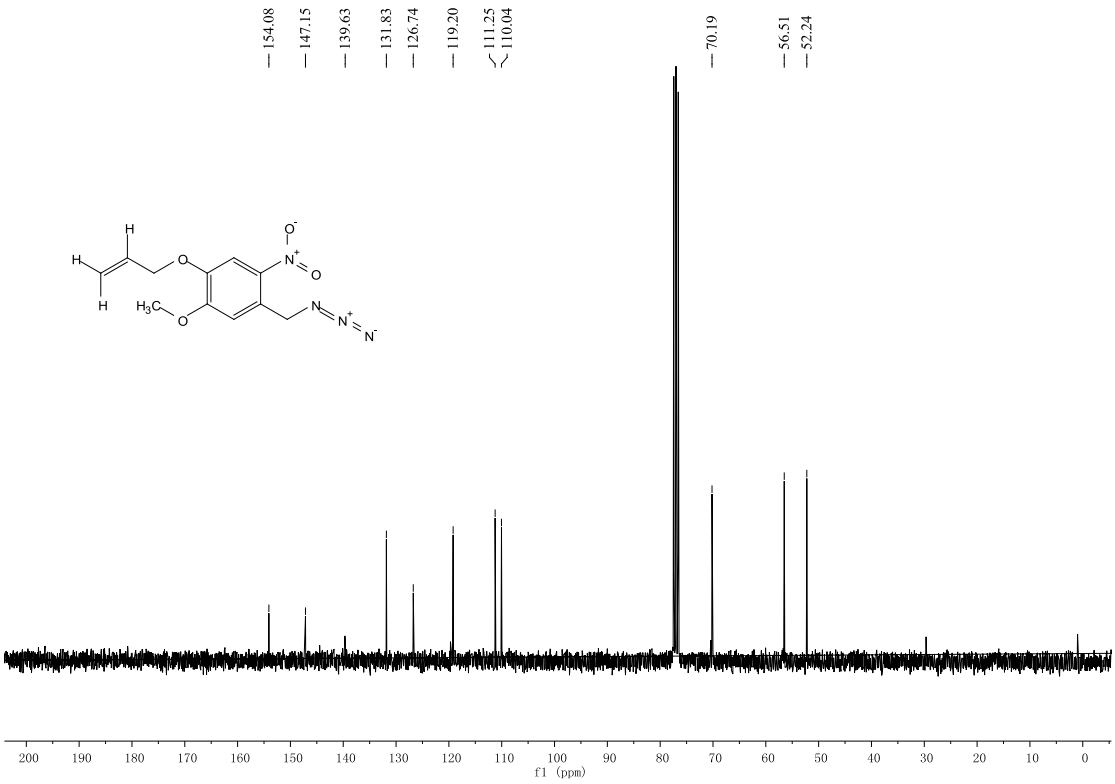

7.72
7.26
7.17
6.12
6.10
6.09
6.06
6.05
6.03
5.49
5.43
5.38
5.34
4.96
4.69
4.67
4.00
1.00
1.02
1.11
2.22
2.08
2.15
3.22
f1 (ppm)

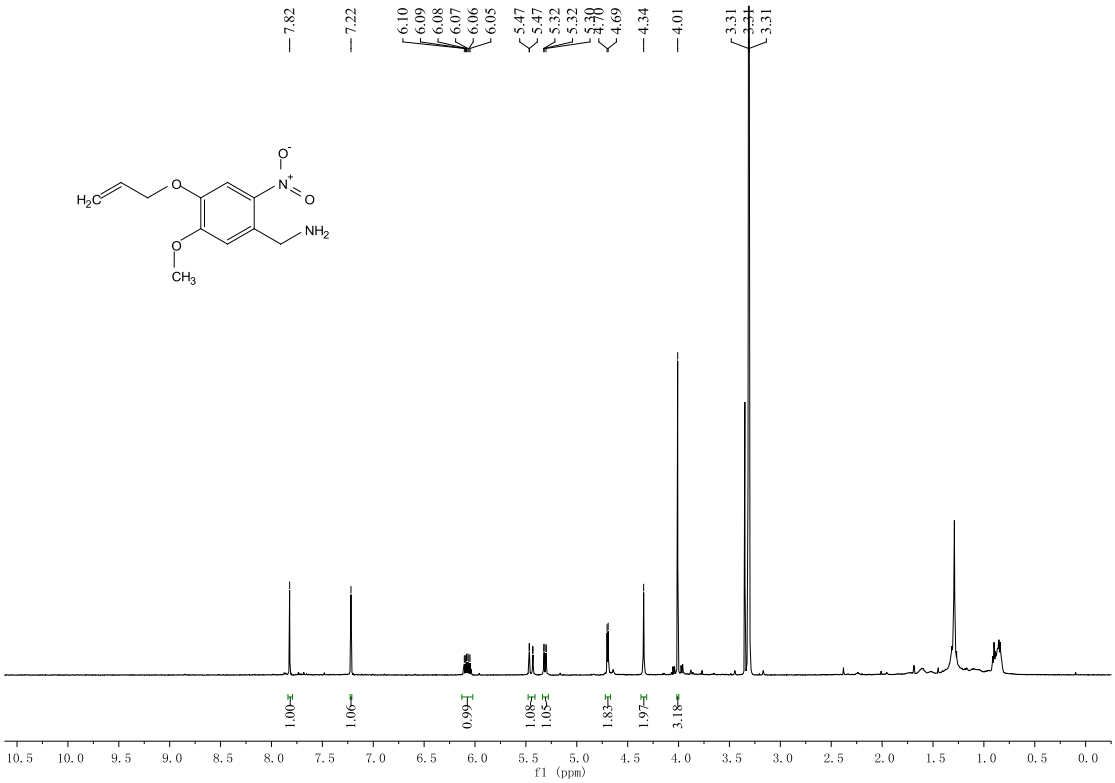

163.22
154.28
139.65
132.33
131.92
119.15
111.27
109.91
70.16
62.88
56.45
f1 (ppm)

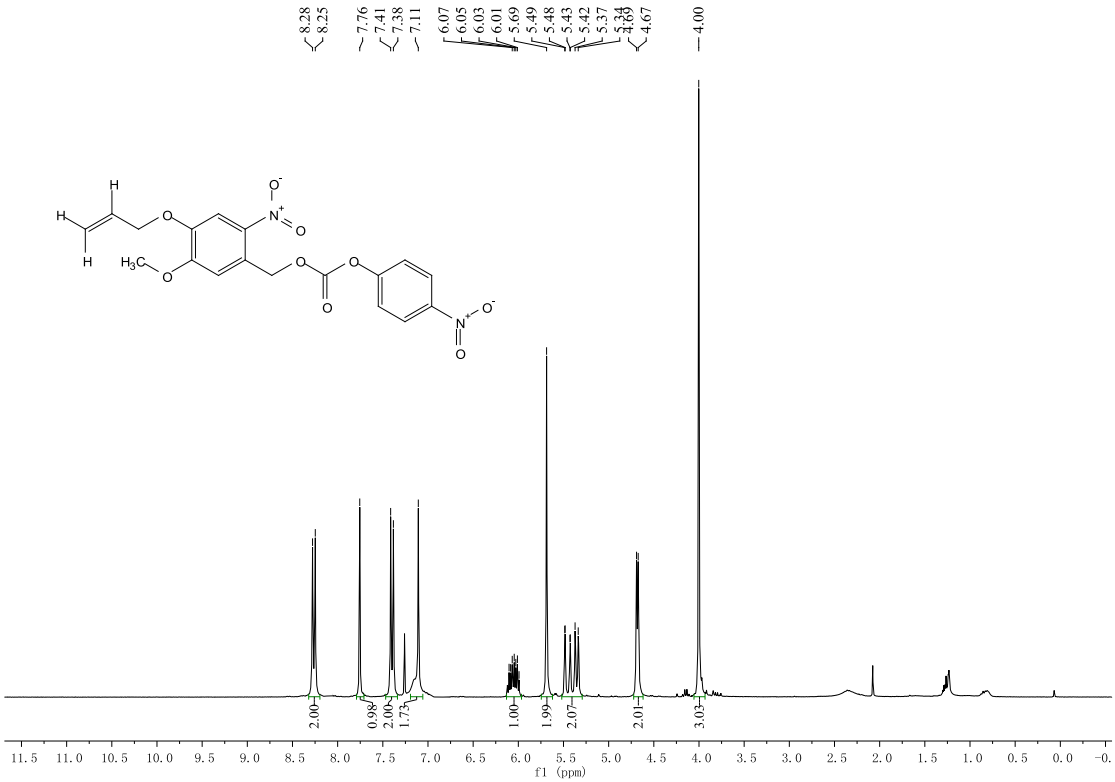

7.76
7.26
7.15
6.06
5.65
5.48
5.48
5.45
5.45
5.38
5.38
5.36
5.36
4.70
4.68
4.01
3.14
0.99
1.00
0.83
2.24
0.98
0.95
2.29
3.43
3.20
f1 (ppm)

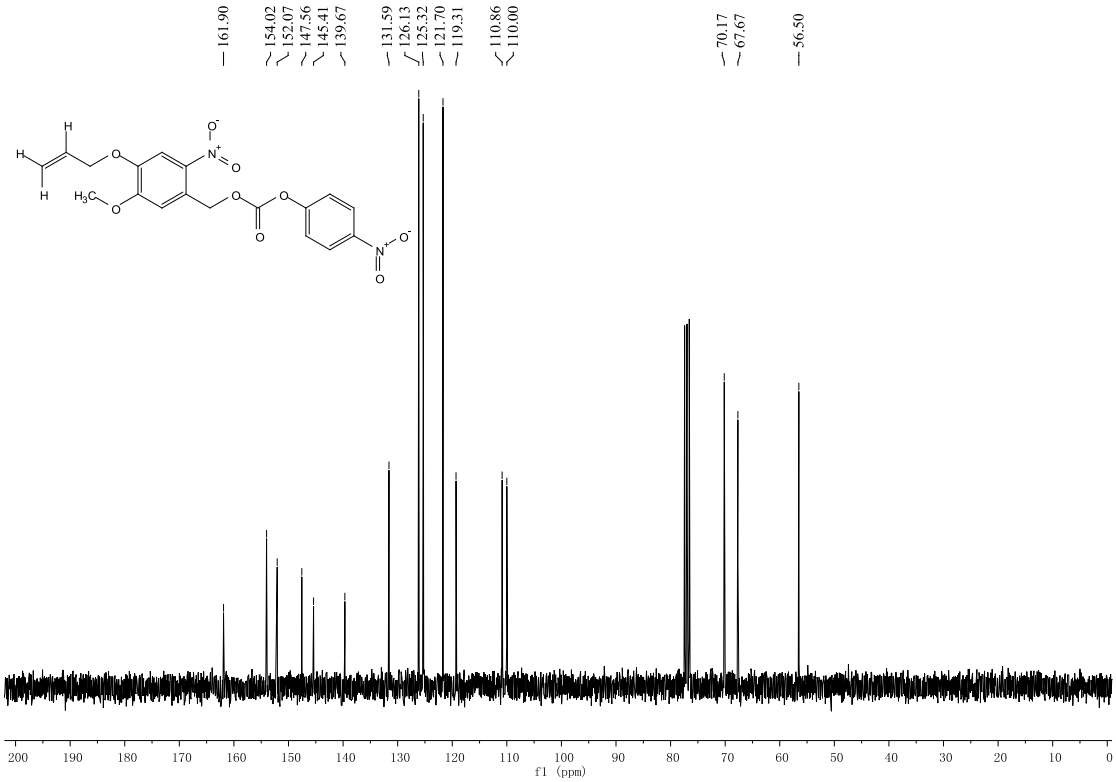

154.27
147.68
139.32
131.69
125.08
119.32
110.85
109.93
70.21
68.80
56.61
37.81
f1 (ppm)

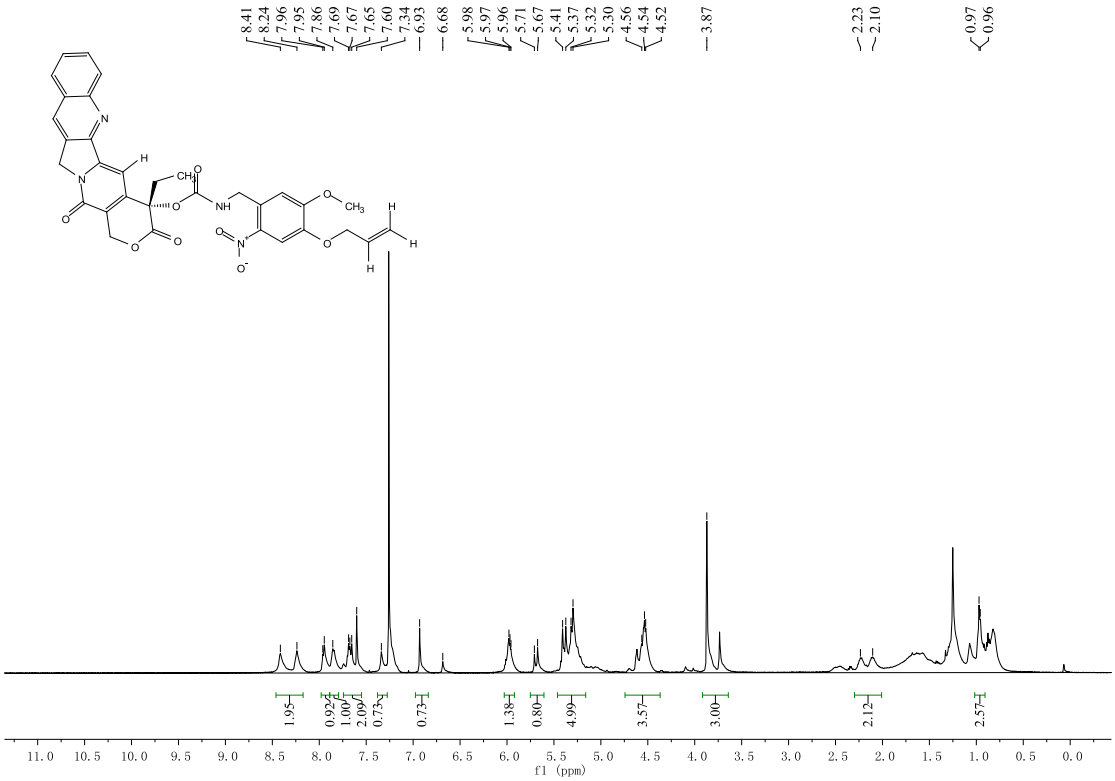

7.73
7.26
7.07
6.12
6.10
6.08
6.06
6.04
6.02
5.49
5.38
5.34
5.31
4.87
4.69
4.67
4.00
1.00
1.01
1.12
2.25
2.03
2.05
3.37
f1 (ppm)

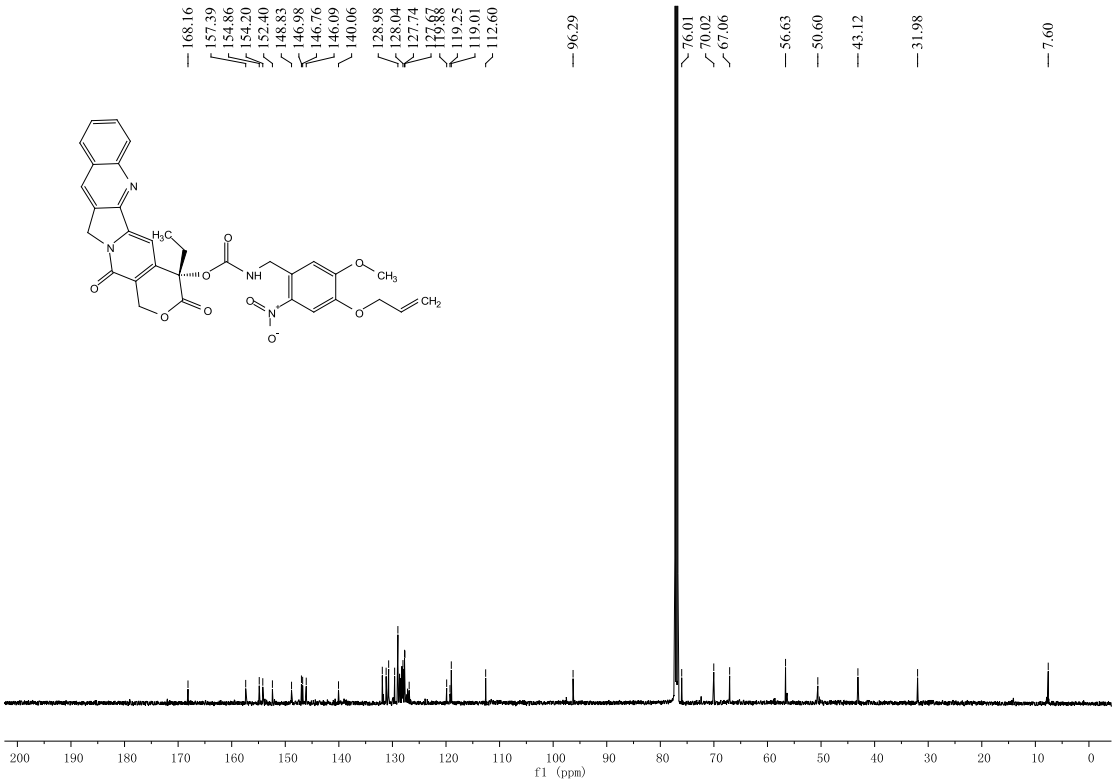

154.08
147.15
139.63
131.83
126.74
119.20
111.25
110.04
70.19
56.51
52.24
f1 (ppm)

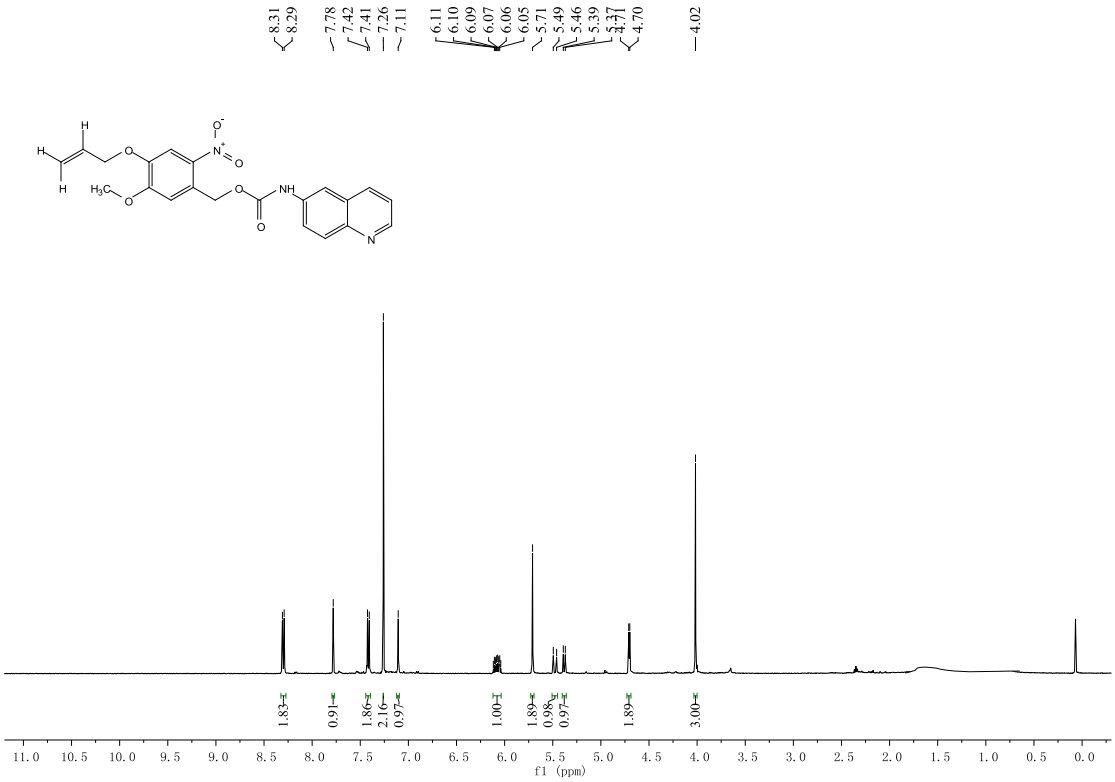

7.82
7.22
6.10
6.09
6.08
6.07
6.06
6.05
5.47
5.47
5.32
5.32
4.69
4.34
4.01
3.31
1.00
1.06
0.99
1.08
1.05
1.83
1.97
3.18
f1 (ppm)

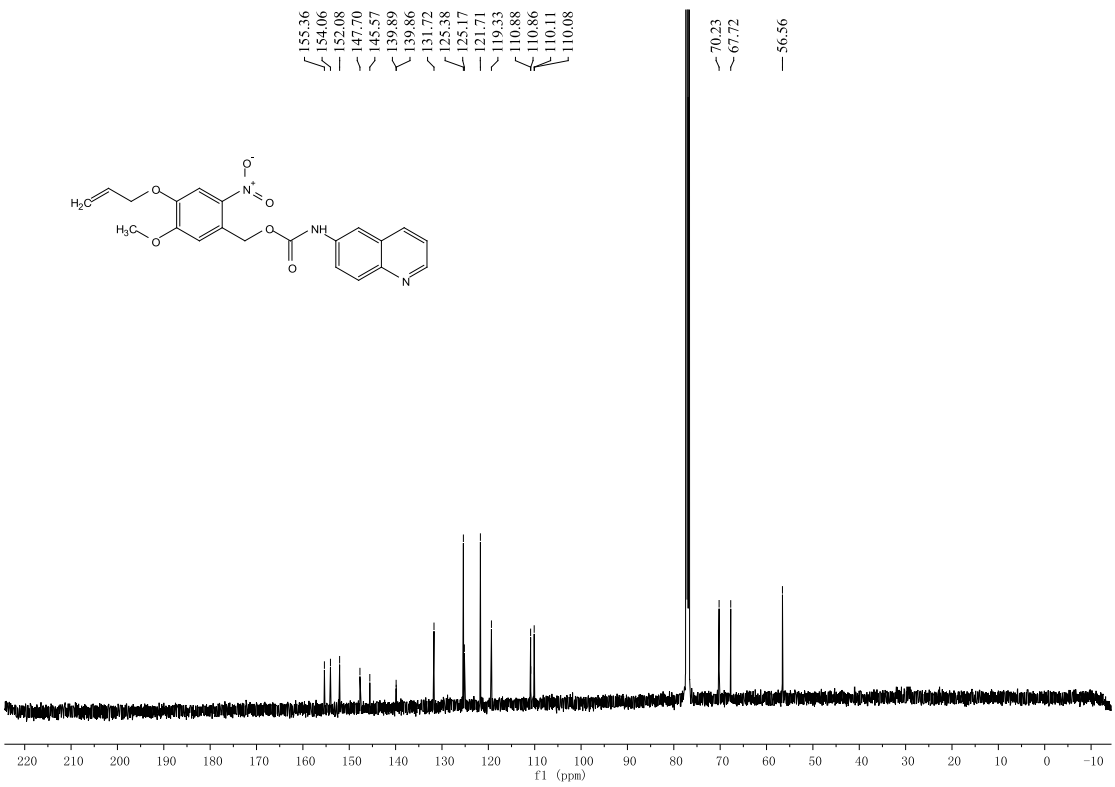

8.28
8.25
7.76
7.41
7.38
7.11
6.07
6.05
6.03
6.01
5.69
5.49
5.48
5.43
5.42
5.37
5.34
5.33
4.67
4.00
2.00
0.98
2.00
1.73
1.00
1.99
2.07
2.01
3.03
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.
f1 (ppm)

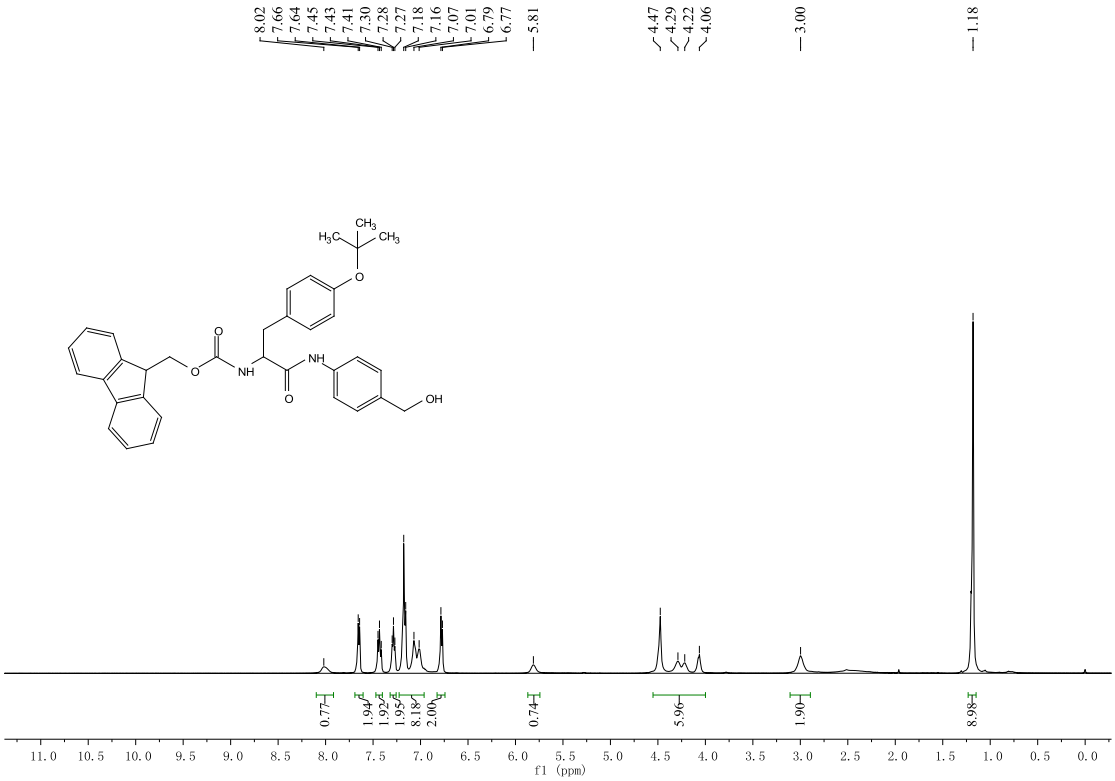

161.90
154.02
152.07
147.56
145.41
139.67
131.59
126.13
125.32
121.70
119.31
110.86
110.00
70.17
67.67
56.50
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

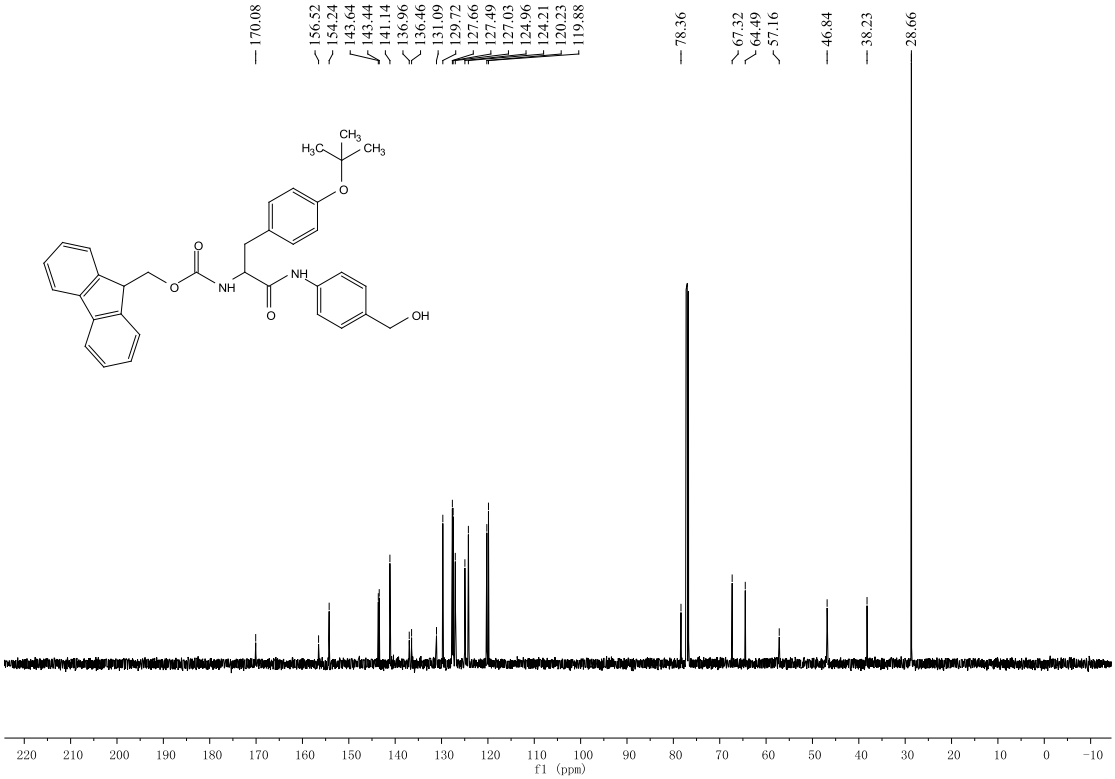

8.41
8.24
7.96
7.95
7.86
7.69
7.67
7.65
7.60
7.34
6.93
6.68
5.98
5.97
5.96
5.71
5.67
5.41
5.37
5.32
5.30
4.56
4.54
4.52
3.87
2.23
2.10
0.97
0.96
1.95
0.92
1.00
2.09
0.73
0.73
1.38
0.80
4.99
3.57
3.00
2.12
2.57
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


168.16
157.39
154.86
154.20
152.40
148.83
146.98
146.76
146.09
140.06
128.98
128.04
127.74
127.67
119.88
119.25
119.01
112.60
96.29
76.01
70.02
67.06
56.63
50.60
43.12
31.98
7.60
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


8.31
8.29
7.78
7.42
7.41
7.26
7.11
6.11
6.10
6.09
6.07
6.06
6.05
5.71
5.49
5.46
5.39
5.37
4.71
4.70
4.02
1.83
0.91
1.86
2.16
0.97
1.00
1.89
0.98
0.97
1.89
3.00
f1 (ppm)


155.36
154.06
152.08
147.70
145.57
139.89
139.86
131.72
125.38
125.17
121.71
119.33
110.88
110.86
110.11
110.08
70.23
67.72
56.56
f1 (ppm)


8.02
7.66
7.64
7.45
7.43
7.41
7.30
7.28
7.27
7.18
7.16
7.07
7.01
6.79
6.77
5.81
4.47
4.29
4.22
4.06
3.00
1.18
0.77
1.94
1.92
1.95
8.18
2.00
0.74
5.96
1.90
8.98
f1 (ppm)


170.08
156.52
154.24
143.64
143.44
141.14
136.96
136.46
131.09
129.72
127.66
127.49
127.03
124.96
124.21
120.23
119.88
78.36
67.32
64.49
57.16
46.84
38.23
28.66
f1 (ppm)